IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Index of Documents

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Particulars</th>
<th>Page Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Representation u/s 25(1) by Opponent</td>
<td>1-26</td>
</tr>
<tr>
<td>3.</td>
<td>Exhibit 2 - Statement on a Nonproprietary Name Adopted by the USAN Council for Sofosbuvir</td>
<td>29-30</td>
</tr>
<tr>
<td>4.</td>
<td>Exhibit 3 - Valentino Stella, Prodrugs as Therapeutics, Opinion on Therapeutic Patents, March 2004, Vol 14 No.3</td>
<td>31-37</td>
</tr>
<tr>
<td>5.</td>
<td>Exhibit 4 - Amended claims for 3658/KOLNP/2009</td>
<td>38-41</td>
</tr>
<tr>
<td>8.</td>
<td>Exhibit 7 - Christopher McGuigan, et al, Certain Phosphoramidate Derivatives of Dideoxy Uridine (ddU) are Active Against HIV and successfully By-pass Thymidine Kinase,</td>
<td>276-280</td>
</tr>
<tr>
<td>Exhibit</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>10. Exhibit 8 - Dominique Cahard et al, <em>Aryloxy Phosphoramidate Triesters as Pro-Tides</em>, Mini-Reviews in Medicinal Chemistry, 2004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>281-293</td>
<td></td>
</tr>
<tr>
<td></td>
<td>294-300</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>12. Exhibit 10 - WO 2006/067606</td>
<td></td>
</tr>
<tr>
<td></td>
<td>301-347</td>
<td></td>
</tr>
<tr>
<td></td>
<td>348-407</td>
<td></td>
</tr>
<tr>
<td></td>
<td>408-532</td>
<td></td>
</tr>
</tbody>
</table>
STATEMENT OF CASE OF THE OPPONENT

1. The Opponent is a not-for-profit public service organisation having its registered address at 16192 Coastal Highway, Lewes, Delaware, 19958-9776, U.S.A. I-MAK consists of lawyers and scientists working to protect the public domain against undeserved patents to ensure that patents do not act as a barrier to research and restrict the public’s access to affordable medicines.

2. One such application of concern is Application No. 3658/KOLNP/2009 (‘3658) titled ‘Nucleoside Phosphoramidate Prodrugs’ filed in India on 20 October 2009. ‘3658 is a national phase application stemming from International Application WO 2008/121634, filed on 26 March 2008 and

3. Based on the information provided by the Patent Office, 3658 is currently awaiting examination. Accordingly, as permitted under s25(1) of the Act and Rule 55(1), any person may file a representation by way of opposition at the appropriate office (Kolkata) before the grant of a patent. The Opponent submits its opposition and supporting evidence to 3658 based on the grounds set out below. The Opponent, as is allowed under s25(1) of the Act and Rule 55(1), also requests a hearing before this matter is finally decided.

**Background to 3658**

4. The hepatitis C virus (HCV) presents a serious global health problem. The virus is transmitted through direct contact with an infected person's blood. Persons with needle-stick injury, health care workers with exposure to blood/blood products, transfusion/blood product recipients, organ transplant recipients and intravenous drug users are some of the populations at risk from HCV. Globally, the World Health Organization estimates that over 170 million people are chronic carriers of HCV and are likely to develop liver cancer and/or cirrhosis. India alone has an estimated 12 million people who
are chronically infected with HCV, with 96,000 deaths annually due to the infection. India is also home to 2.1 million people living with HIV (PLHIV) and applying the global co-infection rate of 13% implies that approximately 273,000 people in this community may be co-infected with HCV.

Given the public health crisis around HCV, it is imperative that people living with HCV are able to access the latest and most effective treatments without unmerited patents standing in the way. Undeserved patents of the nature applied for in ‘3658 affords a company, such as the Applicant, artificial exclusive rights, which then allows it to price a medicine beyond the reach of not only Indian patients, but also many in need in other developing and even developed countries.

5. ‘3658 claims an invention for a phosphoramidate prodrug and its stereoisomers of a nucleoside derivative for treating viral diseases, including HCV. The compounds claimed in ‘3658 are inhibitors of RNA-dependent RNA viral replication and the HCV NS5B polymerase. The phosphoramidate prodrug claimed in ‘3658 is of the 5’ monophosphate derivative of the β-D-2’-deoxy-2’-α-flouro-2’-β-C methyluridine nucleoside, also known as sofosbuvir under the International Non-Proprietary (INN) nomenclature (Exhibit 2).

Nucleoside compounds and their derivatives, in particular uridine derivatives, have a long history of use for antiviral drugs. The first marketed antiviral nucleoside was idoxuridine in 1959 for treating herpes simplex keratitis.
Since then, uses for nucleosides have been researched and marketed in relation to cancer, hepatitis B, HCV and HIV, including but not limited to zidovudine (first synthesised as a potential anti-cancer medicine but marketed for HIV), acyclovir (herpes virus), lamivudine (HIV), ribavirine (HCV) and ganciclovir (cytomegalovirus).

The invention claimed in ‘3658 is for a prodrug of a uridine nucleoside derivative for inhibiting NS5B polymerase. Pages 6-7 of ‘3658 describe the problem the Applicant is seeking to resolve:

“Nucleoside inhibitors of NS5B polymerase can act either as a non-natural substrate that results in chain termination or as a competitive inhibitor which competes with nucleotide binding to the polymerase. To function as a chain terminator the nucleoside analog must be taken up by the cell and converted in vivo to a triphosphate to compete for the polymerase nucleotide binding site. This conversion to the triphosphate is commonly mediated by cellular kinases which imparts additional structural requirements on a potential nucleoside polymerase inhibitor. Unfortunately, this limits the direct evaluation of nucleosides as inhibitors of HCV replication to cell-based assays capable of in situ phosphorylation. In some cases the biological activity of a nucleoside is hampered by its poor substrate characteristics for one or more kinases needed to convert it into the active phosphate form. Formation of the monophosphate by a nucleoside kinase is generally viewed as the rate limiting step of the three phosphorylation events. To circumvent the need for the initial phosphorylation step in the metabolism of a nucleoside
to the active triphosphate analog, the preparation of stable phosphate prodrugs has been reported”. Page 7 of ‘3658 then goes on to provide additional reasons for using prodrugs to improve the physicochemical and pharmacokinetics properties of nucleosides, including systemic absorption.

Indeed, numerous marketed medicines based on nucleosides have been based on the prodrug approach, including but not limited to valganciclovir, tenofovir disoproxil and adefovir dipivoxil. One particular prodrug strategy that has been successfully adopted since the early 1990s to deliver nucleoside monophosphate drugs into the cell by circumventing the first and inefficient rate-limiting phosphorylation step of nucleosides and improving the cellular penetration of nucleotides has been the ProTide (pronucleotide) approach. The prodrug strategy, including the ProTide approach, has been applied time and time again over the past 20 years to the extent that in the recent study by an expert in the field, Valentino Stella stated that the vast majority “contain little true novelty either in a chemical or biological sense”. Exhibit 3 Prodrugs as Therapeutics, Opinion on Therapeutic Patents, March 2004, Vol 14 No.3 page 279, 2nd column.

The ProTide prodrug strategy is the invention for which the Applicant seeks a patent in ‘3658. Should a patent be granted for ‘3658 it will provide the Applicant with unmerited additional exclusivity over the base active ingredient by another 4-5 years, thereby causing further obstruction and delay to more affordable versions of the medicine.
6. As the Background above describes, the invention claimed in ‘3658 relates to a nucleoside phosphoramidate prodrug for the compound known as sofosbuvir. According to documents made available on the Patent Office website, the initial filing of ‘3658 on 20 October 2009 comprised of 80 claims covering various prodrugs, their stereoisomers and processes for making the various compounds. On the same date, the Applicant submitted a more specific set of claims, attached as Exhibit 4. The Opponent believes that it is the set of claims as set out in Exhibit 4 that the Applicant wishes to be examined and accordingly sets out its grounds of opposition against the same. However, should the Applicant amend any of its claims during examination, as is necessary for a fair opposition proceeding and hearing, it is respectfully requested that the Controller keep the Opponent informed.

7. The current set of claims for ‘3658 as set out in Exhibit 4 may be summarised as follows:

a) Claim 1 covers (S)-2-[(2R,3R,4R,5R)-5-(2,4-Dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydro-furan-2-ylmethoxy]-phenoxy-phosphorylamino-propionic acid isopropyl ester or a stereoisomer thereof. The claim covers the methylester of L-alanine as part of the prodrug moiety of the phosphonate group. The ester specifically used is an isopropyl ester.
b) Claim 2 is a dependent claim covering a composition comprising the compound or a stereoisomer in Claim 1 and a pharmaceutically acceptable medium.

c) Claims 3-5 are dependent on Claim 1 and cover methods of treatment, whether in the form of a composition and a pharmaceutically acceptable medium or as a method of treating, by using an effective amount of the compound or stereoisomer in Claim 1 for treating HCV and various other viruses.

d) Claim 6 covers a process for preparing the compound as claimed in Claim 1.

e) Claim 7 is a product-by-process claim comprising the compound or stereoisomer thereof as claimed in Claim 1 using the process as claimed in Claim 6.

f) Claim 8 covers the diastereomer of Claim 1.

g) Claim 9 is a dependent claim covering the compound as claimed in Claim 8 and a pharmaceutically acceptable medium.

h) Claims 10-12 are dependent on Claim 8 and cover methods of treatment, whether in the form of a composition and a pharmaceutically acceptable medium or as a method of treating, by
using an effective amount of the compound or stereoisomer in Claim 1 for treating HCV and various other viruses.

i) Claim 13 covers a process for preparing the compound as claimed in Claim 8.

j) Claim 14 is a product-by-process claim comprising the compound or stereoisomer thereof as claimed in Claim 8 using the process as claimed in Claim 13.

8. Based on the claims set out above, the Opponent believes that Claims 1-14 are not patentable under the following grounds of s25(1) of the Act:

a) S25(1)(b) – that the invention so far as claimed in any claim of the complete specification has been published before the priority date of the claim.

b) s25(1)(e) – that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step having regard to the matter published as mentioned in clause (b) or having regard to what was used in India before the priority date of the applicant’s claim.
c) s25(1)(f) – that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act, in particular under sections 3(d).

d) S25(1)(h) – that the applicant has failed to disclose to the Controller the information required by s8 or has furnished the information that in any material particular was false to his knowledge.

Claims 1-14 of ‘3658 are not patentable under sections 25(1)(b) and 2(1)(l) of the Act

9. Section 2(1)(l) states that a new invention means any invention or technology which has not been anticipated by publication in any document or used in the country or elsewhere in the world before the date of filing of the patent application with complete specification i.e. the subject matter has not fallen in the public domain or that it does not form part of the state of the art.

10. Under the above definition and the published matter/existing knowledge in the field prior to the priority dates of ‘3658 (30 March 2007, 24 October 2007 and 21 March 2008), the Opponent is of the view that the subject matter of claims 1-14 does not amount to a new invention.

11. As discussed in the Background section above and on pages 6 and 7 of ‘3658, the Applicant sets out the problem it intended to resolve, namely a suitable phosphoramidate prodrug of the 5’monophosphate derivative of the \( \beta-D-2'-\)
deoxy-2’-α-flouro-2’-β-C methyluridine nucleoside in order to activate its phosphate as well as to improve the physicochemical and pharmacokinetics properties of the said compound.

12. The Opponent first draws the Controller’s attention to patent WO 2005/003147 (‘147) published on 13 January 2005 and attached as Exhibit 5. The applicant for the ‘147 patent is the same as for the ‘3658 patent. ‘147 covers an invention for “(2'R)-2’-deoxy-2’-flouro-2’-C-methyl nucleoside (β-D or β-L), or its pharmaceutically salt or prodrug thereof, and the use of such compounds for the treatment of a host infected with a virus belonging to the flaviviridae family, including HCV (page 16, lines 3-8)”. Page 16 at lines 16-24 of ‘147 adds that the 2’substitutions of β-D or β-L nucleosides of the invention claimed impart greater specificity for HCV and include a method for treating various viruses included HCV, or its pharmaceutically acceptable salt or prodrug.

More specifically, the compound set out in Claim 6 of ‘147 (as shown below) covers the structure of the base compound for sofosbuvir, including its monophosphate, diphosphate, triphosphate or a stabilised phosphate prodrug:
6. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) or its pharmaceutically acceptable salt or prodrug thereof of the structure:

```
    R^1O
   /   \
  /     \
 X     Base
 /       \
/         \
R^2O      CH_3
```

wherein Base is a purine or pyrimidine base;

X is O, S, CH_2, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)_2,
wherein W is F, Cl, Br, or I; and,

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group.

*(Claim 6 of WO 2005/03147)*
To illustrate:

Claim 6 of ‘147 provides:

“A (2’R)-2’-deoxy-2’-fluoro-C-methyl nucleoside (β-D or β-L) or its pharmaceutically acceptable salt or prodrug thereof of the structure”.

Sofosbuvir is a (2’R)-2’-deoxy-2’-fluoro-2’-C-methyl nucleoside as specified:

“wherein Base is a purine or pyrimidine base”

Uracil is the “base” of sofosbuvir. Uracil is a well known pyrimidine base:

“X is O, S, CH2, Se, NH, N-alkyl, CHW (R,S, or racemic), C (W)2, wherein W is F, Cl, Br, or I;”

In the compound sofosbuvir, X=O (oxygen), which is the first atom mentioned for X in claim 6:

“R1 and R7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate or a stabilized phosphate prodrug”.

In the compound sofosbuvir, R1=H and R7=H. In the prodrug compound of sofosbuvir which is delivered to the patient, sofosbuvir is a stabilised phosphate prodrug, specifically a phosphoramidate.
Claim 6, therefore, covers the actual structure of sofosbuvir in its base form, but also in its complete form as administered to patients by way of a phosphoramidine-stabilised prodrug.

Further confirmation of this can be found through the disclosures made throughout ‘147. Under the heading “Definitions”, on page 31, lines 7-22 of ‘147, the patent document states: “The term “pharmaceutically acceptable salt or prodrug” is used throughout the specification to describe any acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of a compound which upon administration to a patient, provides the active compound…..Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolysed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated to produce the active compound”.

Pages 42-43 of ‘147 further provides that the invention contemplates and includes the 5’-triphosphate triphosphoric acid ester derivatives of a nucleoside compound and pharmaceutically acceptable salts of the triphosphate ester, as well as pharmaceutically acceptable salts of 5’disphosphate and 5’monophosphate ester derivatives of the formulas claimed.
Pages 45-48 and 57-61 of ‘147 provide further discussion of prodrugs and pharmaceutical compositions for the compounds claimed. Page 46, lines 16-18 of ‘147 state: “Any of the nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside”. Pages 47-48 disclose how pharmaceutical compositions based upon the β-D or β-L compound or its pharmaceutically acceptable salt or prodrug can be prepared in a therapeutically effective amount for treating a flaviviridae infection, including HCV. Page 59, lines 4-23 of ‘147, discusses similar phosphoramidates as claimed in the present patent ‘3658.

Pages 51-54 of ‘147, under the heading ‘Stereoisomerism and Polymorphism’ discusses how the nucleoside compounds covered have several chiral centres and may exist in and be isolated in optically active and racemic forms, as do most amino acids which can exist as separate enantiomers. Pages 52-53 then set out commonly known techniques in the art for obtaining optically active materials.

13. Based on the disclosures and claims made in ‘147, Claims 1-14 of ‘3658 are already covered or at least anticipated. ‘147, by stating ‘any acceptable form’ of a prodrug can be adopted for the compounds, clearly anticipates that a phosphoramidate prodrug could be used to increase the activity bioavailability, stability or otherwise alter the properties of the claimed nucleosides, including sofosbuvir as claimed in Claim 6 of ‘147. The disclosures and Claims in ‘147 also anticipate the stereoisomers of suitable
prodrugs, their pharmaceutical compositions and use as methods of treatment. In view of the disclosures and claims made in ‘147, ‘3658 is not a new invention.

Claims 1-14 of ‘3658 are not patentable under sections 25(1)(e) and 2(1)(j) and 2(1)(ja) of the Act

14. In the alternative and without prejudice to the aforesaid, Claims 1-14 of ‘3658 lack any inventive step. Section 2(1)(j) states that an invention means a new product or process involving an inventive step. Section 2(1)(ja) qualifies the meaning of ‘inventive step’ as being a “feature of an invention that involves a technical advance compared to existing knowledge and that makes the invention not obvious to a person skilled in the art”. Section 25(1)(e) defines the abovementioned sections for the purpose of an opposition as “an invention which is obvious and clearly does not involve any inventive step having regard to matter published as mentioned in s25(1)(b) or having regard to what was used in India before the priority date of the applicant’s claim”.

15. Under the above definitions and the published matter/existing knowledge in the field prior to the priority dates of ‘3658, the Opponent is of the view that the subject matter of Claims 1-14 do not amount to a technical advance and would have been obvious to a person skilled in the art.

16. As the discussion above already demonstrates, the disclosure and claims made in ‘147 (Exhibit 5) cover the structure of the base compound
sofosbuvir as well as the phosphoramide-stabilised prodrug. As such, ‘147 would have made Claims 1-14 of ‘3658 obvious to a person skilled in the art.

17. To further substantiate the above, the Opponent refers the Controller to the following prior art:


*Exhibit 6* demonstrates how the use of an aryloxy phosphoramidate ProTide prodrug approach for a ribonucleoside 4’ azidouridine derivative (a uridine based nucleoside) was able to deliver the monophosphates to HCV replicon cells and unleash the antiviral potential of the triphosphate in a manner that vastly improved the antiviral activity over the parent compound. Moreover, the ProTide prodrugs tested by the authors included the alanine isopropyl ester as the phosphoramidate (Table 1, compound 15 on page 4), the exact same prodrug as claimed by the Applicant in ‘3658.

**Exhibit 7** demonstrates the success of using the ProTide prodrug strategy to activate the active triphosphates of an inactive HIV compound ddU. On page 13, column 2 of Exhibit 7, the authors state how the aryloxy phosphoramidate (the prodrug as used in ‘3658) is a potent agent being 50 times more active than the parent nucleoside.

c) Dominique Cahard et al., *Aryloxy Phosphoramidate Triesters as Pro-Tides*, Mini-Reviews in Medicinal Chemistry, 2004 (Exhibit 8).

**Exhibit 8** reiterates the benefits of the aryloxy phosphoramidate prodrug strategy, including the identical prodrug used by the Applicant in ‘3658. On pages 376-378 of Exhibit 8, the authors also discuss the benefits of amino acids (including the L-alanine ester) and phosphate stereochemistry, which the Applicant also claims as an invention in ‘3658.


Exhibit 9 reinforces the common knowledge in the field of how to activate nucleosides by intracellular phosphorylation drug activation and how the ProTide prodrug approach was known to be advantageous by providing the drug in a form that is already mono-phosphorylated.

Exhibit 10 covers uridine nucleoside derivatives as antiviral drugs against flaviviridae, especially HCV. On page 5, lines 31-37, ‘606 discusses amino acid esters e.g. alanine esters as potential prodrugs.


18. Taking into the consideration the prior art provided in Exhibits 5-11 and the existing common general knowledge of aryloxy phosphoramidate prodrugs, Claims 1-14 of ‘3658 would have been obvious to one skilled in the art. It would have been obvious for the Applicant to pursue the phosphoramidate prodrug approach adopted for the compounds in ‘3658 including their stereoisomers, pharmaceutical compositions, processes for preparing the compounds and methods of treatment. As already discussed in the Background section above and admitted by the Applicant on page 7 of ‘3658, it is well known to a skilled person in the art that a prodrug of the compounds claimed in ‘3658 would have advantages such as activating the phosphate and improving the pharmacokinetic characteristics of the parent compounds discussed in ‘147. As such, claims 1-14 of ‘3658 would have been obvious to
try and do not amount to a technical advance over the art and lack any inventive step.

Claims 1-5, 7-12 and 14 of ‘3658 are not patentable under sections 25(1)(f) and 3(d) of the Act.

‘3658 is a new form of a known substance that does not result in an enhancement of the known efficacy of the known form:

19. In the alternative and without prejudice to the grounds raised above Claims 1-5, 7-12 and 14 are not patentable inventions as set out under s3d. Section 3d of the Act provides that the mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance. For the purpose of s3d, substances such as esters, metabolites and other derivatives of a known substance shall be considered the same substance unless they differ significantly with regard to efficacy.

20. The recent decision of Supreme Court of India in Novartis AG v Union of India & Others, Civil Appeal Nos. 2706-2716, 2728 and 2717-2727 of 2013 (Novartis) at page 90, paragraph 179 confirmed that the test of efficacy can only be therapeutic efficacy. Pages 90-91, paragraph 180 of Novartis states that not all advantageous or beneficial properties are relevant but only such properties that directly relate to efficacy, being therapeutic efficacy in the case of medicine.
In view of s3d, as already set out above, ‘147 (Exhibit 5) disclosed and claimed the base compound of sofosbuvir. ‘3658 seeks a prodrug form of the compound sofosbuvir as disclosed in ‘147 and is, therefore, a new form of a known substance. In order for the ‘3658 to meet the requirements of s3d of the Act it must show that the prodrug form enhances the therapeutic efficacy of the already known form. However, the Applicant in the specification as filed for ‘3658 has failed to submit any comparative data that shows an enhancement of therapeutic efficacy over the known form disclosed and claimed in ‘147. As such, ‘3658 should be prima facie refused under this ground.

Even if the Applicant was to submit data, it should be noted that according to the recent Supreme Court ruling in Novartis, physico-chemical properties such as pharmacokinetic improvements would not be considered to amount to therapeutic efficacy. Given that the phosphoramidate prodrug claimed in ‘3658 is designed to improve the pharmacokinetic properties of the base compound disclosed in ‘147, and once delivered intracellularly cleaves back to the known form without any additional therapeutic efficacy, the Opponent believes that the ‘3658 does not amount to a patentable invention as set out under s3d.

‘3658 is the mere discovery of any new property or new use for a known substance:

In the alternative and without prejudice to the above, the subject matter claimed in ‘3658 is a new use for a known substance. WO 2005/012327
(‘012327) published on 10 February 2005 (Exhibit 12) claims various phosphoramidate derivatives of nucleotides for use in the treatment of cancer.

The base moieties of, for example, each of the nucleosides deoxyuridine, cytabrine, gemcitabine and citidine may be substituted at the 5’-position.

24. Claim 1 of ‘012327 provides the following broad Markush formula:

![Chemical structure diagram]

1. A chemical compound having formula I:

   R-O-C-Ph-N-P-O

wherein:
- R is selected from the group comprising alkyl, aryl and alkylaryl;
- R’ and R” are independently selected from the group comprising H, alkyl and alkylaryl, or R’ and R” together form an alkylene chain so as to provide, together with the C atom to which they are attached, a cyclic system;
- Q is selected from the group comprising -O- and -CH₂-;
- X and Y are independently selected from the group comprising H, F, Cl, Br, I, OH and methyl (-CH₃);
- Ar is a monocyclic aromatic ring moiety or a fused bicyclic aromatic ring moiety, either of which said ring moieties are carbocyclic or heterocyclic and is optionally substituted;
- Z is selected from the group comprising H, alkyl and halogen; and
- n is 0 or 1,

   wherein when n is 0, Z’ is -NH₂ and a double bond exists between position 3 and position 4, and
   when n is 1, Z’ is =O₂;

or a pharmaceutically acceptable derivative or metabolite of a compound of formula I;

with the proviso that, except where R is 2-Bu (-CH₂-CH(CH₃)₂) and one of R’ and R” is H and one of R’ and R” is methyl (-CH₃), when n is 1 and X and Y are both H, then Ar is not unsubstituted phenyl (-C₆H₅).

Claim 1 WO 2005/012327
Claim 1 of 012327 clearly covers the molecule sofosbuvir and its prodrug as claimed by ‘3658 in the following manner:

"R is selected from the group comprising alkyl, aryl and alkyaryl".

In sofosbuvir as claimed in ‘3658 R=isopropyl which is a common alkyl group:

"R' and R” are independently selected from the group comprising H, alkyl and alkyaryl or R’ and R” together form an alkylene chain so as to provide, together with the C atom to which they are attached, a cyclic system”.

In sofosbuvir R’ and R” are respectively –H and the common alkyl group – CH3 (methyl):

"Q is selected from the group comprising –O- and –CH2-”.

In sofosbuvir Q= -O-:

"X and Y are independently selected from the group comprising H, F, Cl, Br, I, OH and methyl (-CH3)”.

In sofosbuvir X and Y are methyl (-CH3) and F:
“Ar is a monocyclic aromatic ring moiety or a fused bicyclic aromatic ring moiety, either of which said ring moieties is carbocyclic or heterocyclic and is optionally substituted”.

In sofosbuvir Ar is an unsubstituted phenyl (-C6H5) group:

“Z is selected from the group comprising H, alkyl and halogen and n is 0 or 1, wherein n is 0, Z’ is –NH2 and a double bond exists between position 3 and position 4 and when n is 1, Z’ is =O”.

In sofosbuvir n is 1, and Z’ is =O:

“Or a pharmaceutically acceptable derivative or metabolite of formula I; with the proviso that, except where R is 2-Bu (-CH2-CH(CH3)2) and one of R’ and R” is H and one of R’ and R” is methyl (-CH3), when n is 1 and X and Y are both H, then Ar is not unsubstituted phenyl (-C6H5)”.

Ar may be an unsubstituted phenyl (-C6H5).

25. As described above, ‘012327 claims the prodrug of sofosbuvir claimed in ‘3658. Page 73, line 5 provides one example of many showing use of phosphoramidate esters containing alanine as the amino-acid, and unsubstituted phenyl (-C6H5) is clearly exemplified by the preparation and use of the following phosphonyl chloride derivative to incorporate such phosphoramidate prodrug moiety into the base compound:
26. In view of the subject matter claimed in ‘012327, the subject matter in ‘3658 amounts to a new use for a known substance. As such all the claims in ‘3658 should be not be considered an invention.

Claims 3-5 and 10-12 of ‘3658 are not patentable under sections 25(1)(f) and 3(i) of the Act.

27. Section 3(i) of the Act provides that therapeutic or other treatment of human beings are not inventions that are patentable. As Claims 3-5 and 10-12 are merely methods of administration for treating human beings using the compounds claimed in ‘3658, they should be rejected.

Claims 1-14 are not patentable under sections 25(1)(h) and s8 of the Act.

28. As required under s8 of the Act, the Applicant is required to provide all information regarding the prosecution of patent applications for the same or substantially the same invention as ‘3658 in other countries. Based on the information accessible via the Patent Office’s website, it appears the
Applicant has failed to comply with this requirement. Accordingly, the ‘3658 application should be refused on this ground.

**PRAYER:**

Based on the grounds and evidence presented above the Opponent requests:

a) that Indian Application No. 3658/KOLNP/2009 in the name of Pharmasset, Inc. be refused in its entirety;

b) the Opponent be allowed to make further submissions in the event the Applicant makes any amendments to its claims;

c) the Opponent be permitted to file further evidence if necessary to support its case;

d) the Opponent be granted an opportunity of being heard in the matter before any final orders are passed.

Dated 16 day of November 2013,

For and behalf of the Initiative for Medicines, Access & Knowledge (I-MAK)
Address for service in connection with these proceedings is:
Rajeshwari & Associates
Amsoft Business Centre
UNITECH Trade Centre
Sector 43
Gurgaon
122 002 Haryana
India

To:
The Controller of Patents
The Patent Office, Kolkata

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 1

**Title of the invention:** NUCLEOSIDE PHOSPHORAMIDATE PRODRUGS

| (51) International classification | :C07H19/10; C07H19/20 |
| (31) Priority Document No | :60/909,315 |
| (32) Priority Date | :30/03/2007 |
| (33) Name of priority country | :U.S.A. |
| (86) International Application No | :PCT/US2008/058183 |
| Filing Date | :26/03/2008 |
| (87) International Publication No | :WO2008/121634 |

| (61) Patent of Addition to Application Number | NA |
| Filing Date | :NA |
| (62) Divisional to Application Number | NA |
| Filing Date | :NA |

**Abstract:**

Disclosed herein are phosphoramidate prodrugs of nucleoside derivatives for the treatment of viral infections in mammals, which is a compound, its stereoisomer, salt (acid or basic addition salt), hydrate, solvate, or crystalline form thereof, represented by the following structure: Also disclosed are methods of treatment, uses, and processes for preparing each of which utilize the compound represented by formula I.

Number of Pages = 744

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 2

Statement on a Nonproprietary Name Adopted by the USAN Council for Sofosbuvir
STATEMENT ON A NONPROPRIETARY NAME ADOPTED BY THE USAN COUNCIL

USAN

SOFOSBUVIR

PRONUNCIATION

soe fos' bue vir

THERAPEUTIC CLAIM

Treatment of chronic HCV infection

CHEMICAL NAMES

1. L-Alanine, \( N-[[P(S),2'R]-2'-\text{deoxy}-2'-\text{fluoro}-2'-\text{methyl}-P-\text{phenyl}-5'-\text{uridylyl}]-, 1\)-methylethyl ester

2. 1-methylethyl \( N-[[S]-\{(2R,3R,4R,5R)-5-(2,4-\text{dioxo}-3,4-\text{dihydropyrimidin}-1(2H)-yl)-4-\text{fluoro}-3-\text{hydroxy}-4\text{-methyltetrahydrofuran}-2-y1\text{methoxy}\text{phenoxyphosphoryl}]-L\)-alaninate

STRUCTURAL FORMULA

\[
\begin{align*}
\text{H}_3\text{C} & \hspace{1cm} \text{O} \\
\text{H}_3\text{C} & \hspace{1cm} \text{H} \\
\text{O} & \hspace{1cm} \text{P} \\
\text{N} & \hspace{1cm} \text{H} \\
\text{O} & \hspace{1cm} \text{H}_3\text{C} \\
\text{O} & \hspace{1cm} \text{O} \\
\text{O} & \hspace{1cm} \text{O} \\
\text{O} & \hspace{1cm} \text{O} \\
\text{F} & \hspace{1cm} \text{CH}_3 \\
\text{CH}_3 & \hspace{1cm} \text{H} \\
\text{H} & \hspace{1cm} \text{H}_3\text{C} \\
\end{align*}
\]

MOLECULAR FORMULA

\( C_{22}H_{29}FN_3O_9P \)

MOLECULAR WEIGHT

529.5

TRADEMARK

None as yet

SPONSOR

Gilead Sciences

CODE DESIGNATION

PSI-7977

CAS REGISTRY NUMBER

1190307-88-0

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 3

Valentino Stella, Prodrugs as Therapeutics, Opinion on Therapeutic Patents, March 2004, Vol 14 No.3
Opinion on Therapeutic Patents

Editorial
- Prodrugs as therapeutics

Reviews
- IL-10-related cellular cytokines and their receptors
- Cathepsin S inhibitors
- Melanin-concentrating hormone antagonists: antiobesity agents
- Ligands of the melanocortin receptors, 2002 – 2003 update
- Selective oestrogen receptor modulators
- Compounds for the treatment of varicella-zoster virus infections
- Management of idiopathic achalasia: drugs, balloon or knife?

Biotechnology Patent Focus
- High-throughput analysis of mRNA expression

Patent Evaluations
- Novel EP; receptor antagonists
- Vertex inhibitors of Aurora-2, GSK-3 and Src kinase
Expert Opinion on Therapeutic Patents

Aims and scope
Expert Opinion on Therapeutic Patents aims to provide an evaluated guide to developments in the recent patent literature. The journal is divided into 12 therapeutic sections. Each section is reviewed every six months, allowing systematic and comprehensive coverage of the most important topics:

- Pulmonary-Allergy, Dermatological, Gastrointestinal & Arthritis (Jan & Jul)
- Anti-infectives (Feb & Aug)
- Biologics, Immunologicals & Drug Delivery (Mar & Sep)
- Central & Peripheral Nervous Systems (Apr & Oct)
- Cardiovascular, Renal, Endocrine & Metabolic (May & Nov)
- Oncologic (Jun & Dec)

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Prodrugs as therapeutics

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Prodrugs are bio-reversible derivatives of drug molecules used to overcome some barriers to the utility of the parent drug molecule. These barriers include, but are not limited to, solubility, permeability, stability, presystemic metabolism and targeting limitations. The patent literature shows a dramatic increase in numbers of prodrug patents (a 2000% increase in 2002 compared to 1993), with claims for cancer treatment comprising 37% of these. This increase is largely due to the rise from North American-based multinationals and smaller drug delivery companies mirroring the overall trend. In 2001 and 2002, 14% of all new approved chemical drugs were prodrugs. It appears that prodrugs are becoming an integral part of the drug discovery paradigm.

Keywords: bio-reversible derivatives, prodrug


1. Introduction

What are prodrugs? The prodrug concept can be best explained by Scheme 1. When a new chemical entity has some barrier or limitation to utility, it may not be developable as a therapeutic agent. For example, the drug may be water-insoluble, making it difficult, if not impossible, for a safe injectable form of the drug to be developed for human use. Another limitation might be that the drug, although effective if given by injection, cannot be absorbed from the gastrointestinal tract (GIT). This may be because it is too polar to cross the cells lining the GIT or because the chemical is metabolised (destroyed) by enzymes present in these cells or in the liver, thus preventing the drug from reaching systemic circulation. A goal of many researchers is to achieve Ehrlich's "magic bullet." That is, the design of a drug that has only the drug target while minimizing drug exposure to other sites in the body, thus minimizing toxicity. This "targeting" idea has been the goal of numerous researchers via the use of prodrugs. Thus, the identified barrier is the lack of "drug targeting." Another barrier might be an "economic" barrier. What if this new entity were a natural product or a chemical previously reported and therefore in the public domain? Commercialisation would be discouraged by the lack of protection. Thus, bio-reversible prodrug derivatives with sufficient utility/new/obviousness to warrant patentability can be used to overcome these barriers and justify commercialisation, market issues willing.

2. Evaluation of patents and research to date

Approximately 5–7% of all commercialised drugs worldwide are prodrugs. Bernardelli et al. reported that there were 25 new therapeutic chemical (11) and biological (7), entities approved worldwide in 2001 (12). Of the new chemical entities, three were clearly prodrugs, and two other compounds, although not labelled as such, may behave as prodrugs. Doherty reported that during 2002, 33 new entities were approved worldwide, 31 being clearly chemical and 2 biological (13). Of the 31 new chemical entities (NCEs), 7 were clearly prodrugs. 1 was probably acting as a prodrug and one compound was a soft drug. Therefore, over the last 2 years, of the 49 NCEs, 7 were clearly prodrugs.
Prodrugs as therapeutics

Scheme 1. A scheme illustrating the prodrug concept.

(14%), with that number rising to possibly 11 (22%) if the 'possible' and the soft drug candidates are included.

When asked to write this editorial, a patent search was initiated with the following keywords: prodrug(s), derivates, bioreversible, derivatives, antibody-directed enzyme prod- rug therapy (ADEPT) using a database compiled from Thompson Current Drugs Patent Fast Alert®. The search for patents issued since 1993 resulted in 1,396 hits. A separate search initiated using just the word prodrug at the US patent office website yielded 6,590 hits (1976 to present). Since many bioreversible derivatives are not necessarily labelled as prodrugs per se, but may in fact be acting as prodrugs, this number probably underestimates the total number of prodrug-related patents. Nevertheless, both searches presumably identified a cross-section of prodrug patents. For convenience, the smallest number identified in the Patent Fast Alert search was further analysed. A plot of the percentage of patents per year relative to the number in 1993 (19, i.e. at 100%) from the smaller of the two searches is shown in Figure 1. A significant increase in the numbers over time can be seen, indicating an increased interest in this area. When an analysis of each patent was undertaken, however, 605 of the 1396 patents (43%) claiming prodrugs could not be assigned a reason, or the literature that was over- come. That is, as of 1994, many patents reporting NCEs began including terms such as 'and prodrug thereof' or similar language. Some drugs found to have these encouraged researchers in other companies or academia to file patents on prodrug improvements of such drugs while still under patent.

Although such prodrug patents might be allowed, their use was not, because the active form of the prodrug, the parent drug, was covered by the composition patent on the parent drug. Nevertheless, companies then found themselves having to negotiate the right to the prodrug or engineer around it if the prodrug was, in fact, superior. By including and justifying 'and prodrugs thereof' in the original patent and/or its extensions, this concern was alleviated somewhat. That is, the chain of the drug substance and 'and, prodrugs' appeared to be a defensive posture on the part of the patentee. This was particularly the case for patents from large pharmaceutical drug companies from Europe, North America and Asia.

Further analysis of the individual patent showed some interesting trends. One variable considered was region of origin broken down into the following: North America (small companies and multinationals), Asia (including Australia and India), Europe/Africa (including Eastern Europe, the Middle East and South Africa), worldwide research institutes (including foundations and universities), individuals, and others. Over the past 10 years, the number of patents issued to North American and mainly US-based small and multinational companies represented 31%–54% of all patents per year, with numbers following the general trend of increasing numbers of patents with time. The most aggressive company was Pfizer. If Pharma/d-Ujinh, Warner-Lambert and Agouron patents are included with the Pfizer numbers, as these companies are now a part of Pfizer, clearly Pfizer holds the dominant number of patents that can be identified as including the term prodrug. However, as mentioned above, many of these patents were part of a larger chain of the active drug substance. A few smaller companies seem to have a focus on prodrugs. Three examples are MetaPhasis, Xenosport and Nobex.

The percentage of all prodrug patents issued to institutes varied from 9%–32% (peaking in 1996, but since decreasing as a percentage), from 0%–4% to individuals and 0%–4% to patents of unknown country. The only interesting aspect in these numbers is that, since 1997, the number of patents issued to research institutes, foundations and universities has remained in the range 22%–30% per year.

An interesting trend is seen with Asian-based company patents. Prior to 1999, Asian-based companies were not that active, 0%–10% (except for 1995, 29%). Starting in 2000, however, the numbers of approved patents jumped from 11 (1999) to 36 (33%), 55 (24%) and 83 (24%) for 2000 through 2002, and 45 or 26% for patents issued through to mid-year 2003. The most active companies during this time were Shionogi and Takeda. Unlike the North American data, interest in prodrug solutions to drug design and delivery
problems by our Asian colleagues is a fairly recent phenomenon. Now, however, that much of the jump in patents from the two Japanese companies would fit under the category of defensive patents discussed above. In many cases a look at the structure of the active drug with the additional claim of produgs suggested that produgs would serve very little advantage.

The numbers for European companies (broadly defined) show some similarities to the North American company data. As a percentage of issued patents, the numbers varied from 15 – 26%, with the high mark for numbers being 49 (2002) and percentage at 26% in 1995. The major players were Boehringer-Ingelheim, Aventis, Novartis, GlaxoSmith-Kline, AstraZeneca and Hoffmann-La Roche but no one company stood out from the others either in numbers or percentage. Clearly, the number of patents per year in this sampling is increasing with time. This was also seen from PubMed searches for the number of published papers per year identified by the word produgs(s).

Why the renewed interest in produgs? Many potential modern drug candidates are initially identified using purified or semipurified receptors or enzymes thought to represent the ‘active’ site for the chemical. High throughput screens (HTS) are used to sift through the large numbers of molecules in a company’s library to identify what has been termed as structural leads. Further refinement then leads to chemicals being classified as drug product candidates. Unfortunately, this approach to drug discovery often results in what has been called the high affinity trap, meaning that chemicals identified in this manner result in poor clinical candidates because it is difficult to build in drug-like characteristics after the receptor interaction is optimised. That is, these molecules have physical/chemical and or pharmacokinetic properties that make them unsuitable as drugs. The more recent paradigm, whereby HTS for pharmaceutical properties also identifies drugable properties, has led to greater success in identifying drug leads and ultimately better drug candidates. However, as Lipinski and colleagues (34) have shown, modern drug molecules are becoming larger, and more complex, resulting in a larger number of these possible clinical candidates not being deliverable. This is where the renewed interest in produgs appears to originate. That is, when drug-like properties cannot be built in, without degrading the intrinsic activity, temporary modification of those properties through produrg manipulation can solve the barrier limiting the use of the drug. Produgs can be used to help build in the drug-like properties. Currently, the pharmaceutical industry and academia have rediscovered the produg strategy and appear to be making it an integral part of the drug design paradigm.

Is there any use therapeutic area that seems to require the use of produgs to solve design/ delivery issues? In the 1,396 patents, the following observations were made. The application of produgs to anticancer agents makes up 36.8% of all pending patents. More effective targeting of drugs makes up 14.9% of patents (out of 1,396). A high percentage of these relate to tumour or metastable site targeting. The use in carbogranular drugs is the most addressed therapeutic area at 25.6%, while antimicrobials are similar at 22.6%. This number can be further broken down in antibiotics (12.6%) and other antimicrobials (10.0%). Two other prominent areas are CNS drugs at 18.7% and anti-inflammatory drugs at 17.6%. Smaller numbers were seen for hormones (11.1%) and drugs used to treat immunological diseases (10.3%). These numbers mirror the prevailing research emphasis in many companies. Because of the toxic nature of many anticancer drugs and the goal of discovering more selective agents in an effort to minimise toxicity, it was not surprising to see the large number of produg patents in this area.

As with many non-produg patents, many patents do not show a high degree of creativity but build upon the initial creativity of others. This was clearly seen with the introduction of the novel ADEPT and gene-directed enzyme produrg therapy (GDEPT) produg concept in 1993, with a large percentage of the 1993 – 2003 anticancer produg patents claiming variations on this approach. In analyzing the 1,396 patents, what percentage could be considered truly novel and unique? This is a very subjective judgement. Although not all the 1,396 patents were read in detail and the abstracts often provide inadequate detail to judge, it is my opinion that the vast majority of produg patents contain little true novelty either in the chemical or biological sense. Researchers are still making and claiming essers of carboxylic acids and alcohols. In my opinion, <5 – 10% of the 1,396 patents represent true creativity.

The barriers to drug delivery proposed to be overcome was not easy to identify in many patents (605 or 43% of the 1,396) and, in many cases, more than one barrier to drug delivery might have been mentioned. Of the remaining 791 patents, 208 claimed to target drugs to their site of action after parenteral (injectable) administration. Many of these were ADEPT, GDEPT-based produg combinations or variants thereof. These numbers showed to drop in 2002/2003, perhaps reflecting the lack of commercial success in this area. The predominant drugs used in many of these patents were the anthracycline glycoside family of anticancer drugs. For drugs intended for parenteral use, 53 examples of produgs with increased aqueous solubility were reported. Twenty-three of these patents included the anticancer drug paclitaxel and eight included camptothecins. Thirty patents attempted to claim sustained release of drugs after injectable dosing. Produgs for the delivery of nitric oxide (NO) and various produgs useful in the treatment of ischemic diseases were quite novel. There were at least 32 produg-related patents claiming improved transdermal, ophthalmic or intranasal advantages, while a number of patents just claimed better activity (9) or greater safety (37).

Oral drug delivery is the most popular form of drug delivery worldwide and the poor oral availability of many experimental drugs due to limited aqueous solubility or poor
transcellular permeability is reflected in the patent literature. These include 150 patents where prodrugs solved permeability limitations (many of these were for the newer antiviral drugs), 46 solubility limitations (an increasing trend is the use of phosphate ester-based prodrugs) and 49 patents that claimed improved oral bioavailability. There were 21 patents that reported improved oral sustained release whilst 26 patents included formulation advantages. A number in this latter category included the delivery of gases NO and CS2, and the liquid buccal acid. Targeting transporters in the GIT for improved oral drug delivery were claimed in 21 patents. Surprisingly, only two patents claimed prodrugs specifically for the prevention of pre-systemic metabolism. There was a number of patents claiming improved delivery of CNS drugs, with many of these reflecting a more recent trend. There were also a number of methods patents.

Examples of recent, commercially introduced prodrugs include Hepsera™ (adefovir dipivoxil) and Victrelis™ (tenofovir disoproxil fumarate), antivirals from Gilead Sciences used in the treatment of hepatitis and AIDs, respectively; Vlerecal™ (valganciclovir), another antiviral (Roche Holding AG); Benicar® (olmesartan, Sanofi Co. Ltd), an antihypertensive drug; and Dynastat® (paracetamol), an analgesic (Pharmacia, now Pfizer). Prodrugs in development include Aquavan™ (GPI-15715), a water-soluble prodrug of the anaesthetic agent propofol [8] from Guilford/ProQuest and zinclageran (Esara™) and BIBR-1048 (dibigaran etexilate), oral anticoagulant drugs being developed by AstraZeneca [9].

3. Future prospects

In conclusion, it appears that the prodrug or chemical approach to solving drug delivery problems is becoming an integral part of the drug design and discovery paradigm. To effect a prodrug programme clearly takes a team approach involving synthetic medicinal chemists, biologists and toxicologists, drug metabolism specialists, pharmacokineticists and formulators. The frustrations that drug discovery teams encounter with the new bigger, more complex drug molecule candidates have renewed the interest in this novel problemsolving technique and have led to some significant recent commercial successes. As in any area of research, there are many needs, such as bypassing drug efflux mechanisms, achieving true drug targeting and preventing pre-systemic metabolism that could use some creative prodrug solutions. In one of my current capacities as a consultant to the pharmaceutical industry, I have probably been asked to talk about and consult on the application of prodrugs as a problem-solving tool more in the last two years than in my previous 30 years in that capacity. I see a bright future for this old drug delivery tool.

Bibliography


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IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 4

Amended claims for 3658/KOLNP/2009
We claim:

1. (S)-2-[[2R,3R,4R,5R]-5-(2,4-Dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydro-furan-2-ylmethoxy-phenoxy-phosphorylamo]-propionic acid isopropyl ester or a stereoisomer thereof.

2. A composition comprising the compound or a stereoisomer thereof as claimed in claim 1 and a pharmaceutically acceptable medium.

3. A composition for treating a hepatitis C virus, which comprises an effective amount of the compound or a stereoisomer thereof as claimed in claim 1 and a pharmaceutically acceptable medium.

4. A method of treating a subject infected by a virus, which comprises: administering to the subject an effective amount of the compound or a stereoisomer thereof as claimed in claim 1; wherein the virus is selected from among hepatitis C virus, West Nile virus, a yellow fever virus, a dengue virus, a rhinovirus, a polio virus, a hepatitis A virus, a bovine viral diarrhea virus, and a Japanese encephalitis virus.

5. A method of treating a hepatitis C virus infection in a subject in need thereof, which comprises: administering to the subject an effective amount of the compound or a stereoisomer thereof as claimed in claim 1.

6. A process for preparing the compound or a stereoisomer thereof as claimed in claim 1, said process comprising:

reacting a compound 4" with a nucleoside analog 5'

wherein X' is a leaving group.
7. A product comprising the compound or a stereoisomer thereof as claimed in claim 1 obtained by a process comprising:
reacting a compound 4'' with a nucleoside analog 5'

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{N} & \quad \text{P} \quad \text{X}' \\
\text{O} & \quad \text{N} \\
\text{HO} & \quad \text{F} \quad \text{CH}_3
\end{align*}
\]

wherein X' is a leaving group.

8. (S)-isopropyl 2-(((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate.

9. A composition comprising the compound as claimed in claim 8 and a pharmaceutically acceptable medium.

10. A composition for treating a hepatitis C virus, which comprises an effective amount of the compound as claimed in claim 8 and a pharmaceutically acceptable medium.

11. A method of treating a subject infected by a virus, which comprises:
administering to the subject an effective amount of the compound as claimed in claim 8; wherein the virus is selected from among hepatitis C virus, West Nile virus, a yellow fever virus, a dengue virus, a rhinovirus, a polio virus, a hepatitis A virus, a bovine viral diarrhea virus, and a Japanese encephalitis virus.

12. A method of treating a hepatitis C virus infection in a subject in need thereof, which comprises: administering to the subject an effective amount of the compound as claimed in claim 8.
13. A process for preparing the compound as claimed in claim 8, said process comprising:
reacting a compound 4'' with a nucleoside analog 5'

wherein X' is a leaving group.

14. A product comprising the compound as claimed in claim 8 obtained by a process comprising:
reacting a compound 4'' with a nucleoside analog 5'

wherein X' is a leaving group.

Dated this 20th day of October 2009.

Abhishek Sen
of S. MAJUMDAR & CO.
Applicants' Agent

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 5

WO 2005/003147
Title: MODIFIED FLUORINATED NUCLEOSIDE ANALOGUES

Abstract: The disclosed invention provides compositions and methods of treating a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection in a host, including animals, and especially humans, using a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides, or a pharmaceutically acceptable salt or prodrug thereof.
MODIFIED FLUORINATED NUCLEOSIDE ANALOGUES

This application is being filed on 21 April 2004 as a PCT International Patent application in the name of PHARMASSET LTD. a US resident, applicants for all designations except the US.

FIELD OF THE INVENTION

The present invention includes (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides having the natural β-D configuration and methods for the treatment of Flaviviridae infections, especially hepatitis C virus (HCV).

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial number of infected individuals, estimated to be 2-15% of the world's population. There are an estimated 4.5 million infected people in the United States alone, according to the U.S. Center for Disease Control. According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and vertically from infected mothers or carrier mothers to their offspring. Current treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon-α alone or in combination with the nucleoside analog ribavirin, are of limited clinical benefit as resistance develops rapidly. Moreover, there is no established vaccine for HCV. Consequently, there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.

The HCV virion is an enveloped positive-strand RNA virus with a single oligoribonucleotide genomic sequence of about 9600 bases which encodes a
polyprotein of about 3,010 amino acids. The protein products of the HCV gene consist of the structural proteins C, E1, and E2, and the non-structural proteins NS2, NS3, NS4A and NS4B, and NS5A and NS5B. The nonstructural (NS) proteins are believed to provide the catalytic machinery for viral replication. The NS3 protease releases NS5B, the RNA-dependent RNA polymerase from the polyprotein chain. HCV NS5B polymerase is required for the synthesis of a double-stranded RNA from a single-stranded viral RNA that serves as a template in the replication cycle of HCV. Therefore, NS5B polymerase is considered to be an essential component in the HCV replication complex (K. Ishi, et al., "Expression of Hepatitis C Virus NS5B Protein: Characterization of Its RNA Polymerase Activity and RNA Binding," *Heptology*, 29: 1227-1235 (1999); V. Lohmann, et al., "Biochemical and Kinetic Analysis of NS5B RNA-Dependent RNA Polymerase of the Hepatitis C Virus," *Virology*, 249: 108-118 (1998)). Inhibition of HCV NS5B polymerase prevents formation of the double-stranded HCV RNA and therefore constitutes an attractive approach to the development of HCV-specific antiviral therapies.

HCV belongs to a much larger family of viruses that share many common features.

*Flaviviridae Viruses*


Pestiviruses and hepaciviruses are closely related virus groups within the Flaviviridae family. Other closely related viruses in this family include the GB virus A, GB virus A-like agents, GB virus-B and GB virus-C (also called hepatitis G virus, HGV). The hepacivirus group (hepatitis C virus; HCV) consists of a number of closely related but genotypically distinguishable viruses that infect humans. There are at least 6 HCV genotypes and more than 50 subtypes. Due to the similarities between pestiviruses and hepaciviruses, combined with the poor ability of hepaciviruses to grow efficiently in cell culture, bovine viral diarrhea virus (BVDV) is often used as a surrogate to study the HCV virus.

The genetic organization of pestiviruses and hepaciviruses is very similar. These positive stranded RNA viruses possess a single large open reading frame (ORF) encoding all the viral proteins necessary for virus replication. These proteins are expressed as a polyprotein that is co- and post-translationally processed by both cellular and virus-encoded proteinases to yield the mature viral proteins. The viral proteins responsible for the replication of the viral genome RNA are located within approximately the carboxy-terminal. Two-thirds of the ORF are termed nonstructural (NS) proteins. The genetic organization and polyprotein processing of the nonstructural protein portion of the ORF for pestiviruses and hepaciviruses is very similar. For both the pestiviruses and hepaciviruses, the mature nonstructural (NS) proteins, in sequential order from the amino-terminus of the nonstructural protein coding region to the carboxy-terminus of the ORF, consist of p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.


*Treatment of HCV Infection with Interferon*

Interferons (IFNs) have been commercially available for the treatment of chronic hepatitis for nearly a decade. IFNs are glycoproteins produced by immune cells in response to viral infection. IFNs inhibit replication of a number of viruses,
including HCV, and when used as the sole treatment for hepatitis C infection, IFN can in certain cases suppress serum HCV-RNA to undetectable levels. Additionally, IFN can normalize serum amino transferase levels. Unfortunately, the effect of IFN is temporary and a sustained response occurs in only 8%-9% of patients chronically infected with HCV (Gary L. Davis. Gastroenterology 18:S104-S114, 2000). Most patients, however, have difficulty tolerating interferon treatment, which causes severe flu-like symptoms, weight loss, and lack of energy and stamina.


Interferon alpha-2a and interferon alpha-2b are currently approved as monotherapy for the treatment of HCV. ROFERON®-A (Roche) is the recombinant form of interferon alpha-2a. PEGASYS® (Roche) is the pegylated (i.e. polyethylene glycol modified) form of interferon alpha-2a. INTRON®A (Schering Corporation) is the recombinant form of Interferon alpha-2b, and PEG-INTRON® (Schering Corporation) is the pegylated form of interferon alpha-2b.
Other forms of interferon alpha, as well as interferon beta, gamma, tau and omega are currently in clinical development for the treatment of HCV. For example, INFERGEN (interferon alphancon-1) by InterMune, OMNIFERON (natural interferon) by Viragen, ALBUFERON by Human Genome Sciences, REBIF (interferon beta-la) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, and interferon gamma, interferon tau, and interferon gamma-lb by InterMune are in development.

Ribavirin


Ribavirin reduces serum amino transferase levels to normal in 40% of patients, but it does not lower serum levels of HCV-RNA (Gary L. Davis, 2000). Thus, ribavirin alone is not effective in reducing viral RNA levels. Additionally, ribavirin has significant toxicity and is known to induce anemia. Ribavirin is not approved for monotherapy against HCV. It has been approved in combination with interferon alpha-2a or interferon alpha-2b for the treatment of HCV.

Ribavirin is a known inosine monophosphate dehydrogenase inhibitor that does not have specific anti-HCV activity in the HCV replicon system (Stuyver et al. Journal of Virology, 2003, 77, 10689-10694).

Combination of Interferon and Ribavirin

The current standard of care for chronic hepatitis C is combination therapy with an alpha interferon and ribavirin. The combination of interferon and ribavirin for the treatment of HCV infection has been reported to be effective in the treatment of interferon naïve patients (Battaglia, A.M. et al., Ann. Pharmacother. 34:487-494, 2000), as well as for treatment of patients when histological disease is present.
(Berenguer, M. et al. *Antivir. Ther.* 3(Suppl. 3):125-136, 1998). Studies have shown that more patients with hepatitis C respond to pegylated interferon-alpha/ribavirin combination therapy than to combination therapy with unpegylated interferon alpha. However, as with monotherapy, significant side effects develop during combination therapy, including hemolysis, flu-like symptoms, anemia, and fatigue. (Gary L. Davis, 2000). Combination therapy with PEG-INTRON® (peginterferon alpha-2b) and REBETOL® (Ribavirin, USP) capsules are available from Schering Corporation. REBETOL® (Schering Corporation) has also been approved in combination with INTRON® A (Interferon alpha-2b, recombinant, Schering Corporation). Roche's PEGASYS® (pegylated interferon alpha-2a) and COPEGUS® (ribavirin), as well as Three River Pharmaceutical's Ribosphere® are also approved for the treatment of HCV.

PCT Publication Nos. WO 99/59621, WO 00/37110, WO 01/81359, WO 02/32414 and WO 03/02446 1 by Schering Corporation disclose the use of pegylated interferon alpha and ribavirin combination therapy for the treatment of HCV. PCT Publication Nos. WO 99/15 194, WO 99/64016, and WO 00/24355 by Hoffmann-La Roche Inc. also disclose the use of pegylated interferon alpha and ribavirin combination therapy for the treatment of HCV.

*Additional Methods to Treat Flaviviridae Infections*

The development of new antiviral agents for *Flaviviridae* infections, especially hepatitis C, is currently underway. Specific inhibitors of HCV-derived enzymes such as protease, helicase, and polymerase inhibitors are being developed. Drugs that inhibit other steps in HCV replication are also in development, for example, drugs that block production of HCV antigens from the RNA (IRES inhibitors), drugs that prevent the normal processing of HCV proteins (inhibitors of glycosylation), drugs that block entry of HCV into cells (by blocking its receptor) and nonspecific cytoprotective agents that block cell injury caused by the virus infection. Further, molecular approaches are also being developed to treat hepatitis C, for example, ribozymes, which are enzymes that break down specific viral RNA molecules, antisense oligonucleotides, which are small complementary segments of DNA that bind to viral RNA and inhibit viral replication, and RNA interference
techniques are under investigation (Bymock et al. *Antiviral Chemistry & Chemotherapy*, 11:2; 79-95 (2000); De Francesco et al. in *Antiviral Research*, 58: 1-16 (2003); and Kronke et al., *J. Virol.*, 78:3436-3446 (2004)).

Bovine viral diarrhea virus (BVDV) is a pestivirus belonging to the family *Flaviviridae* and has been used as a surrogate for *in vitro* testing of potential antiviral agents. While activity against BVDV may suggest activity against other flaviviruses, often a compound can be inactive against BVDV and active against another flavivirus. Sommadossi and La Colla have revealed (“Methods and compositions for treating flaviviruses and pestiviruses”, PCT WO 01/92282) that ribonucleosides containing a methyl group at the 2’ “up” position have activity against BVDV. However, it is unclear whether these compounds can inhibit other flaviviruses, including HCV in cell culture or at the HCV NS5B level. Interestingly while this publication discloses a large number of compounds that are 2’-methyl-2’-X-ribonucleosides, where X is a halogen, fluorine is not considered. Furthermore, a synthetic pathway leading to nucleosides halogenated at the 2’ “down” position is not shown by these inventors.

Dengue virus (DENV) is the causative agent of Dengue hemorrhagic fever (DHF). According to the world Health Organization (WHO), two fifths of the world population are now at risk for infection with this virus. An estimated 500,000 cases of DHF require hospitalization each year with a mortality rate of 5% in children.

West Nile virus (WNV), a flavivirus previously known to exist only in intertropical regions, has emerged in recent years in temperate areas of Europe and North America, presenting a threat to public health. The most serious manifestation of WNV infection is fatal encephalitis in humans. Outbreaks in New York City and sporadic occurrences in the Southern United States have been reported since 1999.

There is currently no preventive treatment of HCV, Dengue virus (DENV) or West Nile virus infection. Currently approved therapies, which exist only against HCV, are limited. Examples of antiviral agents that have been identified as active against the hepatitis C flavivirus include:

1) Protease inhibitors:
Substrate-based NS3 protease inhibitors (Attwood et al., PCT WO 98/22496, 1998; Attwood et al., *Antiviral Chemistry and Chemotherapy* 1999, 10, 259-273; Attwood et al., Preparation and use of amino acid derivatives as anti-viral agents, German Patent Pub. DE 19914474; Tung et al. Inhibitors of serine proteases, particularly hepatitis C virus NS3 protease, PCT WO 98/17679), including alphaketoamides and hydrazinoureas, and inhibitors that terminate in an electrophile such as a boronic acid or phosphonate (Llinas-Brunet et al, Hepatitis C inhibitor peptide analogues, PCT WO 99/07734) are being investigated.

Non-substrate-based NS3 protease inhibitors such as 2,4,6-trihydroxy-3-nitro-benzamide derivatives (Sudo K. et al., *Biochemical and Biophysical Research Communications*, 1997, 238, 643-647; Sudo K. et al. *Antiviral Chemistry and Chemotherapy*, 1998, 9, 186), including RD3-4082 and RD3-4078, the former substituted on the amide with a 14 carbon chain and the latter processing a para-phenoxypyphenyl group are also being investigated.

SCH 68631, a phenanthrenequinone, is an HCV protease inhibitor (Chu M. et al., Tetrahedron Letters 3 7:7229-7232, 1996). In another example by the same authors, SCH 351633, isolated from the fungus *Penicillium griseofulvum*, was identified as a protease inhibitor (Chu M. et al., *Bioorganic and Medicinal Chemistry Letters* 9:1949-1952). Nanomolar potency against the HCV NS3 protease enzyme has been achieved by the design of selective inhibitors based on the macromolecule eglin c. Eglin c, isolated from leech, is a potent inhibitor of several serine proteases such as S. griseus proteases A and B, α-chymotrypsin, chymase and subtilisin (Qasim M.A. et al., *Biochemistry* 36:1598-1607, 1997).

Several U.S. patents disclose protease inhibitors for the treatment of HCV. For example, U.S. Patent No. 6,004,933 to Spruce et al. discloses a class of cysteine protease inhibitors for inhibiting HCV endopeptidase 2. U.S. Patent No. 5,990,276 to Zhang et al. discloses synthetic inhibitors of hepatitis C virus NS3 protease. The inhibitor is a subsequence of a substrate of the NS3 protease or a substrate of the NS4A cofactor. The use of restriction enzymes to treat HCV is disclosed in U.S. Patent No. 5,538,865 to Reyes et al. Peptides as NS3 serine protease inhibitors of HCV are disclosed in WO 02/008251 to Corvas International, Inc. and WO
02/08187 and WO 02/008256 to Schering Corporation. HCV inhibitor tripeptides are disclosed in U.S. Patent Nos. 6,534,523, 6,410,531, and 6,420,380 to Boehringer Ingelheim and WO 02/060926 to Bristol Myers Squibb. Diaryl peptides as NS3 serine protease inhibitors of HCV are disclosed in WO 02/48172 to Schering Corporation. Imidazoleindinones as NS3 serine protease inhibitors of HCV are disclosed in WO 02/08198 to Schering Corporation and WO 02/48157 to Bristol Myers Squibb. WO 98/17679 to Vertex Pharmaceuticals and WO 02/48116 to Bristol Myers Squibb also disclose HCV protease inhibitors.

2) Thiazolidine derivatives which show relevant inhibition in a reverse-phase HPLC assay with an NS3/4A fusion protein and NS5A/5B substrate (Sudo K. et al., Antiviral Research, 1996, 32, 9-18), especially compound RD-1-6250, possessing a fused cinnamoyl moiety substituted with a long alkyl chain, RD4 6205 and RD4 6193;


4) A phenanthrenequinone possessing activity against protease in a SDS-PAGE and autoradiography assay isolated from the fermentation culture broth of Streptomyces sp., Sch 68631 (Chu M. et al., Tetrahedron Letters, 1996, 37, 7229-7232), and Sch 351633, isolated from the fungus Penicillium griseofulvum, which demonstrates activity in a scintillation proximity assay (Chu M. et al., Bioorganic and Medicinal Chemistry Letters 9, 1949-1952);


7) Antisense phosphorothioate oligodeoxynucleotides (S-ODN) complementary to sequence stretches in the 5' non-coding region (NCR) of the virus (Alt M. et al.,


9) Ribozymes, such as nuclease-resistant ribozymes (Maccjak, D. J. et al., Hepatology 1999, 30, abstract 995) and those disclosed in U.S. Patent No. 6,043,077 to Barber et al., and U.S. Patent Nos. 5,869,253 and 5,610,054 to Draper et al.;

10) Nucleoside analogs have also been developed for the treatment of Flaviviridae infections.

Idenix Pharmaceuticals discloses the use of certain branched nucleosides in the treatment of flaviviruses (including HCV) and pestiviruses in International Publication Nos. WO 01/90121 and WO 01/92282. Specifically, a method for the treatment of hepatitis C virus infection (and flaviviruses and pestiviruses) in humans and other host animals is disclosed in the Idenix publications that includes administering an effective amount of a biologically active 1', 2', 3' or 4'-branched β-D or β-L nucleosides or a pharmaceutically acceptable salt or derivative thereof, administered either alone or in combination with another antiviral agent, optionally in a pharmaceutically acceptable carrier.


Other patent applications disclosing the use of certain nucleoside analogs to treat hepatitis C virus infection include: PCT/CAOO/01316 (WO 01/32153; filed November 3, 2000) and PCT/CAOI/00197 (WO 01/60315; filed February 19, 2001) filed by BioChem Pharma, Inc. (now Shire Biochem, Inc.); PCT/USO2/01531 (WO

PCT Publication No. WO 99/43691 to Emory University, entitled "$2$'-Fluoronucleosides" discloses the use of certain 2'-fluoronucleosides to treat HCV. U.S. Patent No. 6,348,587 to Emory University entitled "2'$'$-fluoronucleosides" discloses a family of 2'-fluoronucleosides useful for the treatment of hepatitis B, HCV, HIV and abnormal cellular proliferation. The 2' substituent is disclosed to be in either the "up" or "down" position.

Eldrup et al. (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (April 27, 2003, Savannah, Ga.)) described the structure activity relationship of 2'-modified nucleosides for inhibition of HCV.

Bhat et al. (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (April 27, 2003, Savannah, Ga.); p A75) describe the synthesis and pharmacokinetic properties of nucleoside analogues as possible inhibitors of HCV RNA replication. The authors report that 2'-modified nucleosides demonstrate potent inhibitory activity in cell-based replicon assays.
Olsen et al. (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (April 27, 2003, Savannah, Ga.) p A76) also described the effects of the 2'-modified nucleosides on HCV RNA replication.


12) Other compounds currently in preclinical or clinical development for treatment of hepatitis C virus infection include: Interleukin-10 by Schering-Plough, IP-SO1 by Intemeron, Merimebodib (VX-497) by Vertex, AMANTADINE® (Symmetrel) by Endo Labs Solvay, HEPTAZYME® by RPI, IDN-6556 by Idun Pharma., XTL-002 by XTL., HCV/MFS9 by Chiron, CIVACIR® (hepatitis C Immune Globulin) by NABI, LEVOVIRIN® by ICN/Ribapharm, VIRAMIDINE® by ICN/Ribapharm, ZADAXIN® (thymosin alpha-l) by SciClone, thymosin plus pegylated interferon by Sci Clone, CEPLENE® (histamine dihydrochloride) by Maxim, VX 950 / LY 570310 by Vertex/Eli Lilly, ISIS 14803 by Isis Pharmaceutical/Elan, IDN-6556 by Idun Pharmaceuticals, Inc., JTK 003 by AKROS Pharma, BILN-2061 by Boehringer Ingelheim, CellCept (mycophenolate mofetil) by Roche, T67, a β-tubulin inhibitor, by Tularik, a therapeutic vaccine directed to E2 by Immgenetics, FK788 by Fujisawa Healthcare, Inc., 1dB 1016 (Siliphos, oral silybin-phosphatidylcholine phytosome), RNA replication inhibitors (VP50406) by ViroPharma/Wyeth, therapeutic vaccine by Intercell, therapeutic vaccine by Epimmune/Genencor, IRES inhibitor by Anadys, ANA 245 and ANA 246 by Anadys, immunotherapy (Therapore) by Avant, protease inhibitor by Corvas/SChering, helicase inhibitor by Vertex, fusion inhibitor
by Trimeris, T cell therapy by CellExSys, polymerase inhibitor by Biocryst, targeted RNA chemistry by PTC Therapeutics, Dicatation by Immtech, Int., protease inhibitor by Agouron, protease inhibitor by Chiron/Medivir, antisense therapy by AVI BioPharma, antisense therapy by Hybridon, hemopurifier by Aethlon Medical, therapeutic vaccine by Merix, protease inhibitor by Bristol-Myers Squibb/Axys, Chron-VacC, a therapeutic vaccine, by Tripep, UT 231 B by United Therapeutics, protease, helicase and polymerase inhibitors by Genelabs Technologies, IRES inhibitors by Immusol, R803 by Rigel Pharmaceuticals, INFERGEN® (interferon alphancon-1) by InterMune, OMNIFERON® (natural interferon) by Viragen, ALBUFERON® by Human Genome Sciences, REBIF® (interferon beta-la) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, interferon gamma, interferon tau, and Interferon gamma-1b by InterMune. Rigel Pharmaceuticals is developing a non-nucleoside HCV polymerase inhibitor, R803, that shows promise as being synergistic with IFN and ribavirin.

13) A summary of several investigational drugs, including several discussed above, that are currently in various phases of development for the treatment of HCV, are summarized below:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism / Target</th>
<th>Company</th>
<th>U.S. Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>BILN-2061</td>
<td>NS3 Serine-protease inhibitor</td>
<td>Boehringer Ingelheim</td>
<td>Phase II</td>
</tr>
<tr>
<td>ISIS 14803</td>
<td>Antisense / Prevent Translation of RNA</td>
<td>ISIS / Elan</td>
<td>Phase II</td>
</tr>
<tr>
<td>Viramidine</td>
<td>Prodrug of Ribavirin</td>
<td>Ribapharm</td>
<td>Phase II</td>
</tr>
<tr>
<td>NM 283</td>
<td>Inhibitor of HCV RNA Polymerase</td>
<td>Idenix</td>
<td>Phase II / III</td>
</tr>
<tr>
<td>VX-497</td>
<td>IMPDH Inhibitor</td>
<td>Vertex</td>
<td>Phase I / II</td>
</tr>
<tr>
<td>JKT-003</td>
<td>Inhibitor of HCV RNA Polymerase</td>
<td>Japan Tobacco / Akros</td>
<td>Phase I / II</td>
</tr>
<tr>
<td>Levovirin</td>
<td>L-Ribavirin analog</td>
<td>Ribapharm / Roche</td>
<td>Phase I / II</td>
</tr>
<tr>
<td>Isatoribine; ANA245</td>
<td>Nucleoside analog Interact with TLR7 receptor</td>
<td>Anadys</td>
<td>Phase I</td>
</tr>
<tr>
<td>Albuferon</td>
<td>Immune modulator</td>
<td>Human Genome Sciences</td>
<td>Phase I</td>
</tr>
<tr>
<td>Peg-Interven</td>
<td>Immune modulator</td>
<td>Intermune</td>
<td>Phase</td>
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</tr>
<tr>
<td>VX-950</td>
<td>Inhibitor of HCV</td>
<td>Vertex</td>
<td>Preclinical</td>
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<tr>
<td></td>
<td>NS3-4A protease</td>
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<td></td>
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<td>SCH 6</td>
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<td>Schering Plough</td>
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<tr>
<td></td>
<td>NS3-4A protease</td>
<td></td>
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<tr>
<td>R803</td>
<td>Inhibitor of HCV RNA polymerase</td>
<td>Rigel</td>
<td>Phase I</td>
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<tr>
<td>HCV-086</td>
<td>--</td>
<td>ViroPharma/Wyeth</td>
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<tr>
<td>R1479</td>
<td>Inhibitor of HCV RNA polymerase</td>
<td>Roche</td>
<td>Phase I</td>
</tr>
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</table>

Nucleoside prodrugs have been previously described for the treatment of other forms of hepatitis. WO 00/09531 and WO 01/96353 to Idenix Pharmaceuticals, discloses 2'-deoxy-β-L-nucleosides and their 3’-prodrugs for the treatment of HBV. U.S. Patent No. 4,957,924 to Beau champ discloses various therapeutic esters of acyclovir.

In light of the fact that HCV infection has reached epidemic levels worldwide, and has tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat hepatitis C that have low toxicity to the host.

Further, given the rising threat of other flaviviridae infections, there remains a strong need to provide new effective pharmaceutical agents that have low toxicity to the host.

**SUMMARY OF THE INVENTION**

There is currently no preventive treatment of Hepatitis C virus (HCV), Dengue virus (DENV) or West Nile virus (WNV) infection, and currently approved therapies, which exist only against HCV, are limited. Design and development of pharmaceutical compounds is essential, especially those that are synergistic with other approved and investigational Flaviviridae, and in particular HCV, therapeutics.
for the evolution of treatment standards, including more effective combination therapies.

The present invention provides a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L), or its pharmaceutically acceptable salt or prodrug thereof, and the use of such compounds for the treatment of a host infected with a virus belonging to the *Flaviviridae* family, including hepatitis C, West Nile Virus and yellow fever virus. In addition, the nucleosides of the present invention show actively against rhinovirus. Rhinoviruses (RVs) are small (30 nm), nonenveloped viruses that contain a single-strand ribonucleic acid (RNA) genome within an icosahedral (20-sided) capsid. RVs belong to the *Picornaviridae* family, which includes the genera Enterovirus (polioviruses, coxsackieviruses groups A and B, echoviruses, numbered enteroviruses) and Hepatovirus (hepatitis A virus). Approximately 101 serotypes are identified currently. Rhinoviruses are most frequently associated with the common cold, nasopharyngitis, croup, pneumonia, otitis media and asthma exacerbations.

The inventor has made the unexpected discovery that the 2' substitutions on the β-D or β-L nucleosides of the present invention impart greater specificity for hepatitis C virus as well as exhibiting lower toxicity following administration to a host. The invention also includes a method for treating a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection, that includes the administration of an anti-virally effective amount of a β-D or β-L nucleoside disclosed herein, or its pharmaceutically acceptable salt or prodrug, optionally in a pharmaceutically acceptable carrier or diluent, optionally in combination or alternation with another effective antiviral agent.

The nucleosides of the present invention, possess the unique properties of having greater specificity for the hepatitis C virus and lower toxicity in culture or when administered into an animal. One potential, but non-limiting reason for this is the presence of the 2'-fluoro substitution on the ribose ring. For example, U.S. Patent No. 6,348,587 to Schinazi et al., discloses a family of 2'-fluoro nucleoside compounds that are useful in the treatment of hepatitis C virus infection. In contrast, are 2'-methyl substitutions such as found in 2'-C-methylcytidine as shown in WO
2004/02999 to Idenix wherein the 2'-methyl substitution on the nucleoside ring at the 2' position is not specific to hepatitis C.

Thus, in one aspect, the antivirally effective nucleoside is a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) or its pharmaceutically acceptable salt or prodrug thereof of the general formula:

![Chemical Structure](image)

wherein

(a) Base is a naturally occurring or modified purine or pyrimidine base;

(b) X is O, S, CH₂, Se, NH, N-alkyl, CHW (R,S, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

(c) R¹ and R⁷ are independently H, phosphate, including 5'-monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is OH or phosphate; R¹ and R² or R² can also be linked with cyclic phosphate group; and

(d) R² and R²' are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
SO₂(C₄₋₅ alkyl), SO₂(C₄₋₅ alkynyl), SO₂(C₄₋₅ alkenyl), O₃S(C₄₋₅ acyl),
O₃S(C₄₋₅ alkyl), O₃S(C₄₋₅ alkenyl), NH₂, NH(C₄₋₅ alkyl), NH(C₄₋₅ alkenyl),
NH(C₄₋₅ alkynyl), NH(C₄₋₅ acyl), N(C₄₋₅ alkyl), N(C₄₋₅ alkyl)₂, N(C₁₋₈ acyl)₂, wherein
alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to
three halogen (Cl, Br, F, I), NO₂, C(O)O(C₄₋₅ alkyl), C(O)O(C₄₋₅ alkyl),
C(O)O(C₄₋₅ alkynyl), C(O)O(C₄₋₅ alkenyl), O(C₄₋₅ acyl), O(C₄₋₅ alkyl), O(C₄₋₅
alkynyl), S(C₄₋₅ acyl), S(C₄₋₅ alkyl), S(C₄₋₅ alkenyl), S(C₄₋₅ alkynyl), SO(C₄₋₅
acyl), SO(C₄₋₅ alkyl), SO(C₄₋₅ alkynyl), SO(C₄₋₅ alkenyl), SO₂(C₄₋₅ acyl),
SO₂(C₄₋₅ alkyl), SO₂(C₄₋₅ alkynyl), SO₂(C₄₋₅ alkenyl), O₃S(C₄₋₅ acyl),
O₃S(C₄₋₅ alkyl), O₃S(C₄₋₅ alkenyl), NH₂, NH(C₄₋₅ alkyl), NH(C₄₋₅ alkenyl),
NH(C₄₋₅ alkynyl), NH(C₄₋₅ acyl), N(C₄₋₅ alkyl)₂, N(C₄₋₅ acyl)₂, R² and R²'
can be together to form a vinyl optionally substituted by one or two of N₃,
CN, Cl, Br, F, I, NO₂; OR' and

(e) R⁶ is an optionally substituted alkyl (including lower alkyl), cyano
(CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl
(CH₂F), azido (N₃), CHCN, CH₂N₂, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂,
alkyne (optionally substituted), or fluoro.

In various aspects of the invention, the Base can be selected from

wherein

(a) Y is N or CH.

(b) R³, R⁴ and R⁵ are independently H, halogen (including F, Cl, Br, I), OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of
C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆ such as
CF₃ and CH₂CH₂F, lower alkenyl of C₂₋₆ such as CH=CH₂,
halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as 
CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ 
such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-
C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, 
halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, 
CO₂R’, CONH₂, CONHR’, CONR₂, CH=CHCO₂H, 
CH=CHCO₂R’;

wherein R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly 
when the alkyl is an amino acid residue), cycloalkyl, 
optionally substituted alkynyl of C₂-C₆, optionally substituted 
lower alkenyl of C₂-C₆, or optionally substituted acyl.

In still another aspect, the (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof can be of the formula:

![Chemical Structure Image]

wherein

(a) Base, Y, R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R’ are as described above.

Various aspects of the present invention also include pharmaceutical compositions comprising any of the (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) described herein or their pharmaceutically acceptable salts or prodrugs thereof and a pharmaceutically acceptable carrier.

The present invention also provides in various aspects, methods for the treatment or prophylaxis of hepatitis C virus infection, West Nile virus infection, a yellow fever viral infection or a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside disclosed herein. The invention also includes methods for treating or preventing Flaviviridae infection, including all members of the Hepacivirus genus.
(HCV), Pestivirus genus (BVDV, CSFV, BDV), or Flavivirus genus (Dengue virus, Japanese encephalitis virus group (including West Nile Virus), and Yellow Fever virus).

In various aspects, the (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl β-D-nucleoside
has an EC₅₀ (effective concentration to achieve 50% inhibition) when tested in an
appropriate cell-based assay, of less than 15 micromolar, and more particularly, less
than 10 or 5 micromolar. In other aspects, the nucleoside is enantiomerically
enriched.

The present invention also provides methods for the treatment or prophylaxis
of a hepatitis C virus infection, West Nile virus infection, a yellow fever viral
infection or a rhinovirus infection in a host comprising administering an effective
amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides (β-D or β-L) disclosed
herein, or its pharmaceutically acceptable salt or prodrug thereof, in combination or
alternation with one or more other effective antiviral agent(s), optionally in a
pharmaceutically acceptable carrier or diluent thereof, as described herein. Nonlimiting examples of the types of antiviral agents or their prodrugs that can be
used in combination with the compounds disclosed herein include, but are not
limited to: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated
interferon, interferon beta, interferon gamma, interferon tau and interferon omega;
an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon in
combination with ribavirin; a protease inhibitor including NS3 inhibitor; a helicase
inhibitor; a polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense
oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another
nucleoside, nucleoside prodrug or nucleoside derivative; a 1-aminoo-
alkeycyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile
acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadnucleic
acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine;
silybin-phosphatidlycholine phytosome; and mycophenolate.

The following non-limiting aspects illustrate some general methodology to
obtain the nucleosides of the present invention. Specifically, the synthesis of the
present nucleosides can be achieved by either of two general means:
1) alkylation of the appropriately modified carbohydrate building block, subsequent fluorination, followed by coupling to form the nucleosides of the present invention (Scheme 1) or

2) glycosylation to form the nucleoside followed by alkylation and fluorination of the pre-formed nucleosides of the present invention (Scheme 2).

In addition, the L-enantiomers corresponding to the compounds of the invention can be prepared following the same general methods (Schemes 1 or 2), beginning with the corresponding L-carbohydrate building block or nucleoside L-enantiomer as the starting material.

Thus, the present invention includes at least the following general features:

(a) β-D and β-L nucleosides of the general formulas disclosed, or their pharmaceutically acceptable salts or prodrugs thereof, as described herein;

(b) processes for the preparation of the β-D and β-L nucleosides of the general formula disclosed, or their pharmaceutically acceptable salts or prodrugs thereof, as described herein;

(c) pharmaceutical compositions comprising a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier or diluent thereof, as described herein, for the treatment or prophylaxis of a viral infection in a host;

(d) pharmaceutical compositions comprising a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein, for the treatment or prophylaxis of a viral infection in a host;

(e) methods for the treatment or prophylaxis of a Flaviviridae infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host comprising administering an effective
amount of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein;

(f) methods for the treatment or prophylaxis of a Flaviviridae infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host comprising administering an effective amount of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination or alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein;

(g) use of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, as described herein, for the treatment or prophylaxis of a Flaviviridae infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;

(h) use of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination or alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier, as described herein, for the treatment or prophylaxis of a Flaviviridae infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;

(i) use of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, as described herein, in the manufacture of a medicament for the treatment or prophylaxis of a Flaviviridae infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;
(j) use of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination or alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier, as described herein, in the manufacture of a medicament for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;

(k) use of a β-D or β-L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier or diluent, as described herein, in a medical therapy, i.e. as antiviral for example for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection;

(l) use of a β-D or β-L nucleoside of the general formulas disclosed, as described herein, or its pharmaceutically acceptable salt or prodrug thereof, i.e. as antiviral agent, in combination or alternation with one or more other effective therapeutic agent(s), i.e. another antiviral agent, optionally in a pharmaceutically acceptable carrier or diluent, as described herein, in a medical therapy, for example for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 is a graphical depiction of the dose-dependant reduction of the replicon HCV RNA based on the treatment with β-D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. **(A):** The viral reduction was compared to the reduction of cellular RNA levels (ribosomal RNA) to obtain therapeutic index values. EC\textsubscript{90} which represents the effective concentration 90% at 96 hours following the dose dependant administration of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine was determined to be 5 μM. **(B):** HCV RNA was significantly reduced in a dose-dependent manner for 7 days following treatment with 25 μM.
**Figure 2** depicts the average weight change (%) of female Swiss mice in the toxicity study of β-D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine at various doses. Intraperitoneal injections were given on days 0 to day 5 of the 0, 3.3, 10, 33, 100 mg/kg. Each dosing group contained 5 mice and no mice died during the 30-day study.

**Figure 3** depicts the pharmacokinetics of β-D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in Rhesus monkeys given a single dose (33.3 mg/kg) oral or intravenous dose of β-D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine.

**Detailed Description of the Invention**

Various embodiments of the invention are now described in detail. As used in the description herein and throughout the claims that follow, the meaning of “a,” “an,” and “the” includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein and throughout the claims that follow, the meaning of “in” includes “in” and “on” unless the context clearly dictates otherwise.

The terms used in this specification generally have their ordinary meanings in the art, within the context of the invention, and in the specific context where each term is used. Certain terms that are used to describe the invention are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. For convenience, certain terms may be highlighted, for example using italics and/or quotation marks. The use of highlighting has no influence on the scope and meaning of a term; the scope and meaning of a term is the same, in the same context, whether or not it is highlighted. It will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the
invention or of any exemplified term. Likewise, the invention is not limited to various embodiments given in this specification.

As used herein, "about" or "approximately" shall generally mean within 20 percent, preferably within 10 percent, and more preferably within 5 percent of a given value or range. Numerical quantities given herein are approximate, meaning that the term "about" or "approximately" can be inferred if not expressly stated.

The present invention provides (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides and their pharmaceutically acceptable salts and prodrugs for the treatment of hepatitis C virus infection, West Nile virus infection, a yellow fever viral infection or a rhinovirus infection in a host.

The disclosed compounds or their pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HCV infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HCV antigen positive or who have been exposed to HCV.

The compounds disclosed herein can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. The compound or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of the compound can be converted into the parent compound, for example, by hydrolysis.
Definitions

The term "independently" is used herein to indicate that the variable, which
is independently applied, varies independently from application to application.
Thus, in a compound such as R³XYR⁴, wherein R³ is "independently carbon or
nitrogen", both R³ can be carbon, both R⁴ can be nitrogen, or one R³ can be carbon
and the other R⁴ nitrogen.

As used herein, the terms "enantiomerically pure" or "enantiomerically
enriched" refers to a nucleoside composition that comprises at least approximately
95%, and preferably approximately 97%, 98%, 99% or 100% of a single enantiomer
of that nucleoside.

As used herein, the term "substantially free of" or "substantially in the
absence of" refers to a nucleoside composition that includes at least 85 or 90% by
weight, preferably 95% to 98% by weight, and even more preferably 99% to 100%
by weight, of the designated enantiomer of that nucleoside. In a preferred
embodiment, in the methods and compounds of this invention, the compounds are
substantially free of enantiomers.

Similarly, the term "isolated" refers to a nucleoside composition that
includes at least 85 or 90% by weight, preferably 95% to 98% by weight, and even
more preferably 99% to 100% by weight, of the nucleoside, the remainder
comprising other chemical species or enantiomers.

The term "alkyl," as used herein, unless otherwise specified, refers to a
saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon
of typically C₁ to C₁₀, and specifically includes methyl, trifluoromethyl, ethyl,
propyl, isopropyl, cyclopropyl, butyl, isobutyl, t-butyl, pentyl, cyclopentyl,
isopentyl, neopentyl, hexyl, iso hexyl, cyclohexyl, cyclohexymethyl, 3-
methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The term includes both
substituted and unsubstituted alkyl groups. Alkyl groups can be optionally
substituted with one or more moieties selected from the group consisting of
hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic
acid, sulfate, phosphonic acid, phosphate, or phosphonate, or any other viable
functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected, as necessary, as known to those skilled in the art, for example, as taught in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999, hereby incorporated by reference.

The term “lower alkyl,” as used herein, and unless otherwise specified, refers to a C₁ to C₄ saturated straight, branched, or if appropriate, a cyclic (for example, cyclopropyl) alkyl group, including both substituted and unsubstituted forms. Unless otherwise specifically stated in this application, when alkyl is a suitable moiety, lower alkyl is preferred. Similarly, when alkyl or lower alkyl is a suitable moiety, unsubstituted alkyl or lower alkyl is preferred.

The terms “alkylamino” or “arylamino” refer to an amino group that has one or two alkyl or aryl substituents, respectively.

The term “protected,” as used herein and unless otherwise defined, refers to a group that is added to an oxygen, nitrogen, or phosphorus atom to prevent its further reaction or for other purposes. A wide variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis. Non-limiting examples include: C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₃Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, tert-butylidemethylsilyl, tert-butylidiphenylsilyl, and 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

The term “aryl,” as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The term includes both substituted and unsubstituted moieties. The aryl group can be substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in T.W. Greene and P.G.M. Wuts, “Protective Groups in Organic Synthesis,” 3rd ed., John Wiley & Sons, 1999.
The terms “alkaryl” or “alkylaryl” refer to an alkyl group with an aryl substituent. The terms “aralkyl” or “arylalkyl” refer to an aryl group with an alkyl substituent.

The term “halo,” as used herein, includes chloro, bromo, iodo and fluoro.

The term “acyl” refers to a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl or lower alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxyethyl, aryl including phenyl optionally substituted with halogen (F, Cl, Br, I), C1 to C4 alkyl or C6 to C4 alkoxy, sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-t-butyldisilyl) or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group.

The term “lower acyl” refers to an acyl group in which the non-carbonyl moiety is lower alkyl.

The term "purine" or "pyrimidine" base includes, but is not limited to, adenine, N6-alkylpurines, N6-acylpurines (wherein acyl is C(O)(alkyl, aryl, alkylaryl, or arylalkyl), N6-benzylpurine, N6-halopurine, N6-vinylpurine, N6-acetylenic purine, N6-acyl purine, N6-hydroxyalkyl purine, N6-allylaminopurine, N6-thioallyl purine, N2-alkylpurines, N2-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azapyrimidine, including 6-azacytosine, 2- and/or 4-mercaptopurymidine, uracil, 5-halouracil, including 5-fluouracil, C5-alkylpyrimidines, C5-benzylpyrimidines, C5-halopyrimidines, C5-vinylpyrimidine, C5-acetylenic pyrimidine, C5-acyl pyrimidine, C5-hydroxyalkyl purine, C5-amidopyrimidine, C5-cyanopyrimidine, C5-iodopyrimidine, C6-lodo-pyrimidine, C5-Br-vinyl pyrimidine, C5-Br-vinyl pyrimidine, C5-nitropyrimidine, C5-amino- pyrimidine, N2-alkylpurines, N2-alkyl-6-thiopurines, 5-azacytidinyl, 5-azaauracil, triazolopyrimidine, imidazolopyrimidine, pyrrolopyrimidinyl, and pyrazolopyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 2,6-diaminopurine, and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well
known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, and t-butyldiphenylsilyl, trityl, alkyl groups, and acyl groups such as acetyl and propionyl, methanesulfonfonyl, and p-toluenesulfonfyl.

The term "acyl" or "O-linked ester" refers to a group of the formula C(O)R', where R' is an straight, branched, or cyclic alkyl (including lower alkyl), amino acid, aryl including phenyl, alicyl, aralkyl including benzyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxyethyl; or substituted alicyl (including lower alkyl), aryl including phenyl optionally substituted with chloro, bromo, fluoro, iodo, C_1 to C_4 alkyl or C_1 to C_4 alkoxy, sulfonate esters such as alkyl or aralkyl sulfononyl including methanesulfonfyl, the mono, di or triphosphate ester, trityl or monomethoxy-trityl, substituted benzyl, alkaryl, aralkyl including benzyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxyethyl. Aryl groups in the esters optimally comprise a phenyl group. In particular, acyl groups include acetyl, trifluoroacetyl, methylacetyl, cyclopropylacetyl, cyclopropylcarboxy, propionyl, butyryl, hexanoyl, heptanoyl, octanoyl, neo-heptanoyl, phenylacetyl, 2-acetoxy-2-phenylacetyl, diphenylacetyl, α-methoxy-α-trifluoromethyl-phenylacetyl, bromoacetyl, 2-nitro-benzeneacetyl, 4-chlorobenzenecacetyl, 2-chloro-2,2-diphenylacetyl, 2-chloro-2-phenylacetyl, trimethylacetyl, chlorodifluoroacetyl, perfluoroacetyl, fluoroacetyl, bromodifluoroacetyl, methoxyacetyl, 2-thiopheneacetyl, chlorosulfonfylacetyl, 3-methoxyphenylacetyl, phenoxyacetyl, tert-butylacetyl, trichloroacetyl, monochloroacetyl, dichloroacetyl, 7H-dodecafluoro-heptanoyl, perfluoro-heptanoyl, 7H-dodecafluoroheptanoyl, 7-chlorododecafluoro-heptanoyl, 7-chloro-dodecafluoro-heptanoyl, 7H-dodecafluorohexanoyl, 7H-dodecafluoroheptanoyl, nonafluoro-3,6-dioxahexanoyl, nonafluoro-3,6-dioxahaheptanoyl, perfluoroheptanoyl, methoxybenzoyl, methyl 3-amino-5-phenyliothiophene-2-carboxyl, 3,6-dichloro-2-methoxy-benzoyl, 4-(1,1,2,2-tetrafluoro-ethoxy)-benzoyl, 2-bromo-propionyl, omega-aminocapryl, decanoyl, n-pentadecanoyl, stearyl, 3-cyclopentyl-propionyl, 1 -benzene-carboxyl, O-acetylimandelyl, pivaloyl acetyl, 1-adamantane-carboxyl, cyclohexane-carboxyl, 2,6-pyridinedicarboxyl, cyclopropane-carboxyl, cyclobutane-carboxyl, perfluorocyclohexyl carboxyl, 4-methylbenzoyl, chloromethyl isoxazolyl carbonyl, perfluorocyclohexyl carboxyl, crotonyl, 1-methyl-1H-indazole-3-carboxyl, 2-
propanoyl, isovaleryl, 1-pyrrolidinocarbonyl, 4-phenylbenzoyl. When the term acyl is used, it is meant to be a specific and independent disclosure of acetyl, trifluoroacetyl, methylacetyl, cyclopropanecarbonyl, propionyl, butyryl, hexanoyl, heptanoyl, octanoyl, nonanoyl, phenylacetyl, diphenylacetyl, trifluoromethylphenylacetyl, bromoacetetyl, 2-chloro-2,2-diphenylacetyl, 2-chloro-2-phenylacetyl, trimethylacetyl, chlorodifluoroacetyl, perfluorooctanoyl, fluoroacetetyl, bromodifluoroacetyl, 2-thiopheneacetetyl, tert-butylacetetyl, trichloroacetetyl, monochloroacetetyl, dichloroacetetyl, methoxybenzoyl, 2-bromo-propionyl, decanoyl, n-pentadecanoyl, stearoyl, 3-cyclopentyl-propionyl, 1-benzene-carboxyl, pivaloyl acetyl, 1-adamantane-carboxyl, cyclohexane-carboxyl, 2,6-pyridinedicarboxyl, cyclopropane-carboxyl, cyclobutane-carboxyl, 4-methylbenzoyl, crotonyl, 1-methyl-1H-indazole-3-carboxyl, 2-propenyl, isovaleryl, 4-phenylbenzoyl.

The term "amino acid" includes naturally occurring and synthetic α, β, γ or δ amino acids, and includes but is not limited to, amino acids found in proteins, i.e. glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine. In a preferred embodiment, the amino acid is in the L-configuration. Alternatively, the amino acid can be a derivative of alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, arginyl, histidinyl, β-alanyl, β-valinyl, β-leucinyl, β-isoleucinyl, β-prolinyl, β-phenylalaninyl, β-tryptophanyl, β-methioninyl, β-glycinyl, β-serinyl, β-threoninyl, β-cysteinyl, β-tyrosinyl, β-asparaginyl, β-glutaminyl, β-aspartoyl, β-glutaroyl, β-lysyl, β-arginyl or β-histidinyl. When the term amino acid is used, it is considered to be a specific and independent disclosure of each of the esters of α, β, γ or δ glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine in the D and L-configurations.

The term "host," as used herein, refers to a unicellular or multicellular organism in which the virus can replicate, including cell lines and animals, and preferably a human. Alternatively, the host can be carrying a part of the viral
genome, whose replication or functions can be altered by the compounds of the present invention. The term host specifically refers to infected cells, cells transfected with all or part of the viral genome, and animals, in particular, primates and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention.

The term "pharmaceutically acceptable salt or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of a compound which, upon administration to a patient, provides the active compound. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated to produce the active compound.

I. Active Compound, and Physiologically Acceptable Derivatives and Salts Thereof

A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:
wherein Base refers to a naturally occurring or modified purine or pyrimidine base; X is O, S, CH₂, Se, NH, N-alkyl, CHW, C(W)₂, wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is OH or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group; and

R² and R² are independently H, C₁-₄ alkyl, C₁-₄ alkenyl, C₁-₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), O(C₁-₄ alkyl), O(C₁-₄ alkyl), O(C₁-₄ alkyl), S(C₁-₄ alkyl), S(C₁-₄ alkyl), S(C₁-₄ alkyl), S(C₁-₄ alkyl), S(C₁-₄ alkyl), SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkyl), O₂S(C₁-₄ alkyl), O₂S(C₁-₄ alkyl), O₂S(C₁-₄ alkyl), NH₂, NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alkyl), NH(C₁-₄ alky}
alkyl), SO(C\textsubscript{1-4} alkynyl), SO(C\textsubscript{1-4} alkenyl), SO\textsubscript{2}(C\textsubscript{1-4} acyl), SO\textsubscript{2}(C\textsubscript{1-4} alkyl), SO\textsubscript{2}(C\textsubscript{1-4} alkenyl), O\textsubscript{3}S(C\textsubscript{1-4} acyl), O\textsubscript{3}S(C\textsubscript{1-4} alkyl), O\textsubscript{3}S(C\textsubscript{1-4} alkenyl), NH\textsubscript{2}, NH(C\textsubscript{1-4} alkyl), NH(C\textsubscript{1-4} alkenyl), NH(C\textsubscript{1-4} alkylnyl), NH(C\textsubscript{1-4} acyl), N(C\textsubscript{1-4} alkyl)\textsubscript{2}, N(C\textsubscript{1-4} acyl)\textsubscript{2}, OR\textsuperscript{7}, R\textsuperscript{2} and R\textsuperscript{2} can be linked together to form a vinyl optionally substituted by one or two of N\textsubscript{3}, CN, Cl, Br, F, I, NO\textsubscript{2} and R\textsuperscript{6} is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH\textsubscript{3}, OCH\textsubscript{3}, OCH\textsubscript{2}CH\textsubscript{3}, hydroxy methyl (CH\textsubscript{2}OH), fluoromethyl (CH\textsubscript{2}F), azido (N\textsubscript{3}), CHCN, CH\textsubscript{2}N\textsubscript{3}, CH\textsubscript{2}NH\textsubscript{2}, CH\textsubscript{2}NHCH\textsubscript{3}, CH\textsubscript{2}N(CH\textsubscript{3})\textsubscript{2}, alkyne (optionally substituted), or fluoro.

In a second embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

![Nucleoside Structure](image)

wherein Base, R\textsuperscript{1}, R\textsuperscript{2}, R\textsuperscript{2}, R\textsuperscript{6} and R\textsuperscript{7} are as defined above.

A third embodiment provides a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

![Nucleoside Structure](image)

wherein X, R\textsuperscript{1}, R\textsuperscript{2}, R\textsuperscript{2}, R\textsuperscript{6} and R\textsuperscript{7} are as defined above, and Base is selected from
Y is N or CH;

R³, R⁴ and R⁵ are independently H, halogen (including F, Cl, Br, I), OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

In a fourth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

wherein Base is selected from
and, wherein R¹, R², R²', R₃, R⁴, R⁵, R⁶ and Y are as defined above.

A fifth embodiment provides a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

wherein Base refers to a naturally occurring or modified purine or pyrimidine base;

R⁷ is independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R² is independently H or phosphate; R¹ and R² can also be linked with cyclic phosphate group; and

wherein X and R¹ are as defined above.
In a sixth embodiment, a \((2'R)-2'-\text{deoxy}-2'-\text{fluoro-2'}-\text{C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:}\)

![Chemical Structure](image)

wherein Base refers to a naturally occurring or modified purine or pyrimidine base; and

wherein \(R^1\) and \(R^7\) are as defined above.

A seventh embodiment provides a \((2'R)-2'-\text{deoxy-2'}-\text{fluoro-2'}-\text{C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:}\)

![Chemical Structure](image)

wherein Base is selected from

(a) \[
\begin{array}{c}
\text{Y} \\
\text{N} \\
\text{N} \\
\text{R}^4 \\
\text{R}^5
\end{array}
\]

(b) \[
\begin{array}{c}
\text{R}^3 \\
\text{R}^4
\end{array}
\]

and wherein \(X, Y, R^1, R^3, R^4, R^5, R^7\) and \(R'\) are as defined above.
In an eighth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

\[
\begin{align*}
R^1 & \quad \text{Base} \\
& \quad \text{R}^7 \\
& \quad \text{F} \\
& \quad \text{CH}_3
\end{align*}
\]

wherein Base is selected from

(a) 
\[
\begin{align*}
Y & \quad R^4 \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{R}^5 \\
\end{align*}
\]

(b) 
\[
\begin{align*}
R^3 & \quad R^4 \\
\text{N} & \quad \text{O}
\end{align*}
\]

and, wherein Y, R^1, R^3, R^4, R^5, R^7 and R' are as defined above.

A ninth embodiment provides a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

\[
\begin{align*}
R^1 & \quad \text{Base} \\
& \quad \text{R}^6 \\
& \quad \text{X} \\
& \quad \text{R}^2 \\
& \quad \text{R}^2 \\
& \quad \text{F} \\
& \quad \text{CH}_3
\end{align*}
\]

wherein Base is:

\[
\begin{align*}
R^3 & \quad \text{R}^4 \\
\text{N} & \quad \text{O}
\end{align*}
\]
and wherein X is defined as above, R\(^1\) is H, R\(^2\) is OH, R\(^3\) is H, R\(^3\) is H, R\(^4\) is NH\(_2\) or OH, and R\(^6\) is H.

In a tenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

![Structure](image)

wherein Base is:

![Base Structure](image)

and wherein R\(^1\) is H, R\(^2\) is OH, R\(^2\) is H, R\(^3\) is H, R\(^4\) is NH\(_2\) or OH, and R\(^6\) is H.

An eleventh embodiment provides a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

![Structure](image)

wherein Base is:

![Base Structure](image)
and wherein X is defined as above, \( R^1 \) is H, \( R^3 \) is H, \( R^4 \) is NH\(_2\) or OH, \( R^6 \) is H, and \( R^7 \) is H.

In a twelfth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

![Structure](image)

wherein Base is:

![Base Structure](image)

and wherein \( R^1 \) is H, \( R^3 \) is H, \( R^4 \) is NH\(_2\) or OH, and \( R^7 \) is H.

A thirteenth embodiment provides a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

![Structure](image)

In a fourteenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:
wherein X, R₁, R⁶ and R⁷ are as defined above.

In a fifteenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:

wherein R₁, R⁶ and R⁷ are as defined above.

In a sixteenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:
In a seventeenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:

wherein X and R¹ are as defined above.

In an eighteenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:

In a nineteenth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:
wherein X and R\(^1\) are as defined above.

In a twentieth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:

![Chemical structure](image)

The present invention also contemplates 5'-triphosphate triphosphoric acid ester derivates of the 5'-hydroxyl group of a nucleoside compound of the present invention having the following general structural formula:

![Chemical structure](image)

wherein Base, X, R\(^2\), R\(^2'\), and R\(^6\) are as defined as above.

The compounds of the present invention are also intended to include pharmaceutically acceptable salts of the triphosphate ester as well as pharmaceutically acceptable salts of 5'-diphosphate and 5'-monophosphate ester derivatives of the following structural formulas, respectively.

![Chemical structure](image)
wherein Base, $X$, $R^2$, $R'^2$ and $R^6$ are as defined above.

Further non-limiting examples of phosphoric acid derivatives are the nucleosides of the present invention are shown below:
The present invention also contemplates that any phosphate nucleoside derivative can include a 5'- (S-acyl-2-thioethyl)phosphate or "SATE" mono or diester derivative of the 5'-monophosphates.

Alternative embodiments are also contemplated wherein the N-4 amino group on a phosphate nucleoside derivative can be replaced with H, F, Cl, Br or I.

Additional embodiments include 3' and/or 5' produrgs as described in more detail herein.

In the various embodiments, the fluorinated derivatives are preferred. Fluorine is viewed as "isosteric" with hydrogen because of its size (Van der Waals radii for H is 1.20Å and for F 1.35Å). However, the atomic weight (18.998) and electronegativity of fluorine (4.0 [Pauling's scale], 4.000 [Sanderson's scale]) are more similar to oxygen (3.5 [Pauling], 3.654 [Sanderson]) than hydrogen (2.1 [Pauling], 2.592 [Sanderson]) (March, J., "Advances in Organic Chemistry: Reactions, Mechanisms, and Structure" Third edition, 1985, p. 14., Wiley Interscience, New York). Fluorine is known to be capable of forming a hydrogen bond, but unlike a hydroxyl group (which can act both as proton acceptor and proton donor) fluorine acts only as a proton acceptor. On the other hand, 2'-fluoro-ribonucleosides can be viewed as analogues of both ribonucleosides and deoxynucleosides. They may be better recognized by viral RNA polymerase at the triphosphate level than by the host RNA polymerase thus selectively inhibiting the viral enzyme.

II. Pharmaceutically Acceptable Salts and Prodrugs

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically
acceptable salt may be appropriate. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. In particular, examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

Any of the nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, Antiviral Research, 27 (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

The active nucleoside can also be provided as a 5'-phosphoether lipid or a 5'-ether lipid, as disclosed in the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C. Piantadosi. 1990. “Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation.” AIDS Res. Hum. Retro Viruses. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-

Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at the 5'-OH position of the nucleoside or lipophilic preparations, include U.S. Patent Nos. 5,149,794; 5,194,654; 5,223,263; 5,256,641; 5,411,947; 5,463,092; 5,543,389; 5,543,390; 5,543,391; and 5,554,728, all of which are incorporated herein by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to the nucleosides of the present invention, or lipophilic preparations, include WO 89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910, WO 94/26273, WO 96/15132, EP 0 350 287, EP 93917054.4, and WO 91/19721.

III. Pharmaceutical Compositions

Pharmaceutical compositions based upon a β-D or β-L compound disclosed herein or its pharmaceutically acceptable salt or prodrug can be prepared in a therapeutically effective amount for treating a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection, optionally in combination with a pharmaceutically acceptable additive, carrier or excipient. The therapeutically effective amount may vary with the infection or condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient treated.

In one aspect according to the present invention, the compound according to the present invention is formulated preferably in a mixture with a pharmaceutically
acceptable carrier. In general, it is preferable to administer the pharmaceutical composition in orally administrable form, but formulations may be administered via parenteral, intravenous, intramuscular, transdermal, buccal, subcutaneous, suppository or other route. Intravenous and intramuscular formulations are preferably administered in sterile saline. One of ordinary skill in the art may modify the formulation within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising its therapeutic activity. In particular, a modification of a desired compound to render it more soluble in water or other vehicle, for example, may be easily accomplished by routine modification (salt formulation, esterification, etc.).

In certain pharmaceutical dosage forms, the prodrug form of the compound, especially including acylated (acylated or other) and ether derivatives, phosphate esters and various salt forms of the present compounds, is preferred. One of ordinary skill in the art will recognize how to readily modify the present compound to a prodrug form to facilitate delivery of active compound to a targeted site within the host organism or patient. The artisan also will take advantage of favorable pharmacokinetic parameters of the prodrug form, where applicable, in delivering the desired compound to a targeted site within the host organism or patient to maximize the intended effect of the compound in the treatment of a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection.

The amount of compound included within therapeutically active formulations, according to the present invention, is an effective amount for treating the infection or condition, in preferred embodiments, a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. In general, a therapeutically effective amount of the present compound in pharmaceutical dosage form usually ranges from about 50 mg to about 2,000 mg or more, depending upon the compound used, the condition or infection treated and the route of administration. For purposes of the present invention, a prophylactically or preventively effective amount of the compositions, according to the present invention, falls within the same concentration range as set forth above for
therapeutically effective amount and is usually the same as a therapeutically effective amount.

Administration of the active compound may range from continuous (intravenous drip) to several oral administrations per day (for example, Q.I.D., B.I.D., etc.) and may include oral, topical, parenteral, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration, among other routes of administration. Enteric-coated oral tablets may also be used to enhance bioavailability and stability of the compounds from an oral route of administration. The most effective dosage form will depend upon the pharmacokinetics of the particular agent chosen, as well as the severity of disease in the patient. Oral dosage forms are particularly preferred, because of ease of administration and prospective favorable patient compliance.

To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of one or more of the compounds according to the present invention is preferably mixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carriers, such as dextrose, mannitol, lactose and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, the tablets or capsules may be enteric-coated for sustained release by standard techniques. The use of these dosage forms may significantly impact the bioavailability of the compounds in the patient.
For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients, including those that aid dispersion, also may be included. Where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

Liposomal suspensions (including liposomes targeted to viral antigens) may also be prepared by conventional methods to produce pharmaceutically acceptable carriers. This may be appropriate for the delivery of free nucleosides, acyl nucleosides or phosphate ester prodrug forms of the nucleoside compounds according to the present invention.

In particularly preferred embodiments according to the present invention, the compounds and compositions are used to treat, prevent or delay the onset of a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. The present compounds are preferably administered orally, but may be administered parenterally, topically or in suppository form.

The compounds according to the present invention, because of their low toxicity to host cells in certain instances, may be advantageously employed prophylactically to prevent a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection or to prevent the occurrence of clinical symptoms associated with the viral infection or condition. Thus, the present invention also encompasses methods for the prophylactic treatment of viral infection, and in particular a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. In this aspect, according to the present invention, the present compositions are used to prevent or delay the onset of a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. This prophylactic method comprises administration to a patient in need of such treatment, or who is at risk for the development of the virus or condition, an amount of a compound according to the present invention effective for alleviating, preventing or delaying
the onset of the viral infection or condition. In the prophylactic treatment according to the present invention, it is preferred that the antiviral compound utilized should be low in toxicity and preferably non-toxic to the patient. It is particularly preferred in this aspect of the present invention that the compound that is used should be maximally effective against the virus or condition and should exhibit a minimum of toxicity to the patient. In the case of a Flaviviridae infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection, compounds according to the present invention, which may be used to treat these disease states, may be administered within the same dosage range for therapeutic treatment (i.e., about 250 micrograms up to 1 gram or more from one to four times per day for an oral dosage form) as a prophylactic agent to prevent the proliferation of the viral infection, or alternatively, to prolong the onset of the viral infection, which manifests itself in clinical symptoms.

In addition, compounds according to the present invention can be administered in combination or alternation with one or more antiviral agents, including other compounds of the present invention. Certain compounds according to the present invention may be effective for enhancing the biological activity of certain agents according to the present invention by reducing the metabolism, catabolism or inactivation of other compounds and as such, are co-administered for this intended effect.

IV. Stereoisomerism and Polymorphism

It is appreciated that nucleosides of the present invention have several chiral centers and may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically active, diastereomeric, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein. It being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).
Carbons of the nucleoside are chiral, their nonhydrogen substituents (the base and the CHOR groups, respectively) can be either cis (on the same side) or trans (on opposite sides) with respect to the sugar ring system. The four optical isomers therefore are represented by the following configurations (when orienting the sugar moiety in a horizontal plane such that the oxygen atom is in the back): cis (with both groups "up", which corresponds to the configuration of naturally occurring β-D nucleosides), cis (with both groups "down", which is a nonnaturally occurring β-L configuration), trans (with the C2' substituent "up" and the C4' substituent "down"), and trans (with the C2' substituent "down" and the C4' substituent "up"). The "D-nucleosides" are cis nucleosides in a natural configuration and the "L-nucleosides" are cis nucleosides in the nonnaturally occurring configuration.

Likewise, most amino acids are chiral (designated as L or D, wherein the L enantiomer is the naturally occurring configuration) and can exist as separate enantiomers.

Examples of methods to obtain optically active materials are known in the art, and include at least the following.

i) physical separation of crystals - a technique whereby macroscopic crystals of the individual enantiomers are manually separated. This technique can be used if crystals of the separate enantiomers exist, i.e., the material is a conglomerate, and the crystals are visually distinct;

ii) simultaneous crystallization - a technique whereby the individual enantiomers are separately crystallized from a solution of the racemate, possible only if the latter is a conglomerate in the solid state;

iii) enzymatic resolutions - a technique whereby partial or complete separation of a racemate by virtue of differing rates of reaction for the enantiomers with an enzyme;

iv) enzymatic asymmetric synthesis - a synthetic technique whereby at least one step of the synthesis uses an enzymatic reaction to obtain an
enantiomerically pure or enriched synthetic precursor of the desired enantiomer;

v) **chemical asymmetric synthesis** - a synthetic technique whereby the desired enantiomer is synthesized from an achiral precursor under conditions that produce asymmetry (i.e., chirality) in the product, which may be achieved using chiral catalysts or chiral auxiliaries;

vi) **diastereomer separations** - a technique whereby a racemic compound is reacted with an enantiomerically pure reagent (the chiral auxiliary) that converts the individual enantiomers to diastereomers. The resulting diastereomers are then separated by chromatography or crystallization by virtue of their now more distinct structural differences and the chiral auxiliary later removed to obtain the desired enantiomer;

vii) **first- and second-order asymmetric transformations** - a technique whereby diastereomers from the raceniate equilibrate to yield a preponderance in solution of the diastereomer from the desired enantiomer or where preferential crystallization of the diastereomer from the desired enantiomer perturbs the equilibrium such that eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the diastereomer;

viii) **kinetic resolutions** - this technique refers to the achievement of partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the enantiomers with a chiral, non-racemic reagent or catalyst under kinetic conditions;

ix) **enantiospecific synthesis from non-racemic precursors** - a synthetic technique whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;
x) **chiral liquid chromatography** - a technique whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase. The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;

xi) **chiral gas chromatography** - a technique whereby the racemate is volatilized and enantiomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral adsorbent phase;

xii) **extraction with chiral solvents** - a technique whereby the enantiomers are separated by virtue of preferential dissolution of one enantiomer into a particular chiral solvent;

xiii) **transport across chiral membranes** - a technique whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane which allows only one enantiomer of the racemate to pass through.

Chiral chromatography, including simulated moving bed chromatography, is used in one embodiment. A wide variety of chiral stationary phases are commercially available.

Some of the compounds described herein contain olefinic double bonds and unless otherwise specified, are meant to include both E and Z geometric isomers.

In addition, some of the nucleosides described herein, may exist as tautomers, such as, keto-enol tautomers. The individual tautomers as well as mixtures thereof are intended to be encompassed within the compounds of the present invention as illustrated below.

A (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine:
A (2'R)-2'-deoxy-2'-fluoro-2'-C-methylguanosine:

A (2'R)-2-amino-2'-deoxy-2'-fluoro-2'-C-methyladenosine:
In each example above, the first drawn structure is the preferred form.

V. Prodrugs and Derivatives

The active compound can be administered as any salt or prodrug that upon administration to the recipient is capable of providing directly or indirectly the parent compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and a compound, which has been alkylated, acylated, or otherwise modified at the 5'-position, or on the purine or pyrimidine base (a type of "pharmaceutically acceptable prodrug"). Further, the modifications can affect the biological activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the salt or prodrug and testing its antiviral activity according to the methods described herein, or other methods known to those skilled in the art.

Pharmaceutically Acceptable Salts

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically acceptable salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed by addition of acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorate, a-ketoglutarate, a-
glycerophosphate, formate, fumarate, propionate, glycolate, lactate, pyruvate, oxalate, maleate, and salicylate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate, carbonate salts, hydrobromate and phosphoric acid. In a preferred embodiment, the salt is a mono- or di- hydrochloride salt.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made. In one embodiment, the salt is a hydrochloride, hydrobromide, or mesylate salt of the compound. In another embodiment, the pharmaceutically acceptable salt is a dihydrochloride, dihydrobromide, or dimesylate salt.

**Nucleotide Prodrug Formulations**

The nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono-, di- or triphosphate of the nucleoside reduces polarity and allows passage into cells.

Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alicyc, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischoferger, Antiviral Research, 1995, 27:1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

In an alternative embodiment, the nucleoside is delivered as a phosphonate or a SATE derivative.

The active nucleoside can also be provided as a 2'-, 3'- and/or 5'-phosphoether lipid or a 2'-, 3'- and/or 5'-ether lipid. Non-limiting examples are described include the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C.


Aryl esters, especially phenyl esters, are also provided. Nonlimiting examples are disclosed in DeLambert et al., J. Med. Chem. 37: 498 (1994). Phenyl esters containing a carboxylic ester ortho to the phosphate are also provided. Khaninei and Torrence, J. Med. Chem.; 39:41094115 (1996). In particular, benzyl esters, which generate the parent compound, in some cases using substituents at the
ortho- or para-position to accelerate hydrolysis, are provided. Examples of this class of prodrugs are described by Mitchell et al., J. Chem. Soc. Perkin Trans. I 2345 (1992); Brook, et al. WO 91/19721; and Glazier et al. WO 91/1 9721.

Cyclic and noncyclic phosphonate esters are also provided. Nonlimiting examples are disclosed in Hunston et al., J. Med. Chem. 27: 440-444 (1984) and Starrett et al. J. Med. Chem. 37: 1857-1864 (1994). Additionally, cyclic 3',5'-phosphate esters are provided. Nonlimiting examples are disclosed in Meier et al. J. Med. Chem. 22: 811-815 (1979). Cyclic 1',3'-propanyl phosphonate and phosphate esters, such as ones containing a fused aryl ring, i.e. the cyclosaligenyl ester, are also provided (Meier et al., Bioorg. Med. Chem. Lett. 7: 99-104 (1997)). Unsubstituted cyclic 1',3'-propanyl esters of the monophosphates are also provided (Farquhar et al., J. Med. Chem. 26: 1153 (1983); Farquhar et al., J. Med. Chem. 28: 1358 (1985)) were prepared. In addition, cyclic 1',3'-propanyl esters substituted with a pivaloyloxy methoxy group at C-1' are provided (Freed et al., Biochem. Pharmac. 38: 3193 (1989); Biller et al., U.S. Pat. No. 5,157,027).

Cyclic phosphoramidates are known to cleave in vivo by an oxidative mechanism. Therefore, in one embodiment of the present invention, a variety of substituted 1',3' propanyl cyclic phosphoramidates are provided. Non-limiting examples are disclosed by Zon, Progress in Med. Chem. 19, 1205 (1982). Additionally, a number of 2'- and 3'- substituted proesters are provided. 2'-Substituents include methyl, dimethyl, bromo, trifluoromethyl, chloro, hydroxy, and methoxy; 3'-substituents including phenyl, methyl, trifluoromethyl, ethyl, propyl, isopropyl, and cyclohexyl. A variety of 1'-substituted analogs are also provided.

Cyclic esters of phosphorus-containing compounds are also provided. Non-limiting examples are described in the following:

- di and tri esters of phosphoric acids as reported in Nifantyev et al., Phosphorus, Sulfur Silicon and Related Elements, 113: 1 (1996); Wijnberg et al., EP-180276 A1;
the asymmetric synthesis of L-Dopa precursors. Sylvain et al., DE3S 12781 Al;


**N^4-acyl Prodrugs**

The invention also provides N^4- acyl prodrugs. A non-limiting example of an N^4-acyl derivative of (2'R)-2'-F-2'-C-methylcytidine is shown below:

![Reaction Diagram]

wherein R can be any acyl group as described herein.

The invention also contemplates other embodiments, wherein the prodrug of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) includes biologically cleavable moieties at the 3' and/or 5' positions. Preferred moieties are natural of synthetic D or L amino acid esters, including D or L -valyl, though preferably L -amino acids esters, such as L -valyl, and alkyl esters including acetyl. Therefore, this invention specifically includes 3'-L or D - amino acid ester and 3', 5'-L or D -diamino acid ester of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) nucleosides, preferably L -amino acid, with any desired purine or pyrimidine base, wherein the parent drug optionally has an EC_{50} of less than 15 micromolar, and even more preferably less than 10 micromolar; 3'-(alkyl or aryl) ester or 3',5'-L -di(alkyl or aryl) ester of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) with any desired purine or pyrimidine base, wherein the parent drug optionally has an EC_{50} of less than10 or 15 micromolar; and prodrugs of 3',5'-diesters of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides (β-D or β-L) wherein (i) the 3' ester is an amino acid ester and the 5'-ester is an alkyl or aryl ester; (ii) both esters are amino acid esters; (iii) both esters are independently alkyl or aryl esters; and (iv) the 3'
ester is independently an alkyl or aryl ester and the 5'-ester is an amino acid ester, wherein the parent drug optionally has an EC$_{50}$ of less than 10 or 15 micromolar.

Non-limiting examples of prodrugs falling within the invention are:

VI. Combination or Alternation Therapy

In another embodiment, for the treatment, inhibition, prevention and/or prophylaxis of any viral infection described herein, the active compound or its derivative or salt can be administered in combination or alternation with another antiviral agent. In general, in combination therapy, effective dosages of two or more agents are administered together, whereas during alternation therapy, an effective dosage of each agent is administered serially. The dosage will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

It has been recognized that drug-resistant variants of flaviviruses, pestiviruses or HCV can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in viral replication. The efficacy of a drug against the viral infection can be prolonged, augmented, or restored by administering the compound in
combination or alternation with a second, and perhaps third, antiviral compound that induces a different mutation from that caused by the principle drug. Alternatively, the pharmacokinetics, biodistribution or other parameter of the drug can be altered by such combination or alternation therapy. In general, combination therapy is typically preferred over alternation therapy because it induces multiple simultaneous stresses on the virus.

For example, one skilled in the art will recognize that any antiviral drug or therapy can be used in combination or alternation with any nucleoside of the present invention. Any of the viral treatments described in the Background of the Invention can be used in combination or alternation with the compounds described in this specification. Nonlimiting examples of the types of antiviral agents or their prodrugs that can be used in combination with the compounds disclosed herein include: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-aminoalkycyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenyllic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

Further nonlimiting examples of the types of drugs or their prodrugs described above include: acyclovir (ACV), ganciclovir (GCV or DHPG) and its prodrugs (e.g. valyl-ganciclovir), E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), (E)-5-vinyl-1-β-D-arabinosyluracil (VaraU), (E)-5-(2-bromovinyl)-1-β-D-arabinosyluracil (BV-araU), 1-(2-deoxy-2-fluoro-β-D-arabinosyl)-5-iodocytosine (D-FLAC), 1-(2-deoxy-2-fluoro-β-L-arabinosyl)-5-methyluracil (L-FMAU, or
clevudine), (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA], (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-2,6-diaminopurine [(S)-HPMPDAP], (S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine [(S)-HPMPC, or cidofovir], and (2S,4S)-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]-5-iodouracil (L-5-IoddU), entecavir, lamivudine (3TC), LdT, LdC, tenofovir, and adefovir, the (-)-enantiomer of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ((-)-FTC); the (-)-enantiomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (3TC); carbovir, acyclovir, famciclovir, penciclovir, AZT, DDI, DDC, L(-)-FMAU, D4T, amdoxovir, Reverset, Racivir, abacavir, L-DDA phosphate prodrugs, and β-D-dioxolanyl-6-chloropurine (ACP), non-nucleoside RT inhibitors such as nevirapine, MKC-442, DMP-226 (sustiva), protease inhibitors such as indinavir, saquinavir, Kaletra, atazanavir; and anti-HIV compounds such as BILN-2061, ISIS 14803; viramidine, NM 283, VX-497, JKT-003, levovirin, isatoribine, albuferon, Peg-infergen, VX-950, R803, HCV-086, R1479 and DMP45.

Pharmaceutical Compositions

Hosts, including humans, infected with pestivirus, flavivirus, HCV infection, or any other condition described herein, or another organism replicating through a RNA-dependent RNA viral polymerase, or for treating any other disorder described herein, can be treated by administering to the patient an effective amount of the active compound or a pharmaceutically acceptable prodrug or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

A preferred dose of the compound for a Flaviviridae infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection will be in the range from about 50 to about 2000 mg one to four times per day. Lower doses may be useful, and thus ranges can include from 50 – 1,000 mg one to four times per day. The effective dosage range of the pharmaceutically acceptable salts and prodrugs can be calculated based on the weight of the parent nucleoside to be delivered. If the salt or prodrug exhibits activity in itself the effective dosage can
be estimated as above using the weight of the salt or prodrug, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 25 to 3000 mg, preferably 50 to 2000 mg of active ingredient per unit dosage form. An oral dosage of 50-1000 mg is usually convenient, including in one or multiple dosage forms of 50, 100, 200, 250, 300, 400, 500, 600, 700, 800, 900 or 1000 mgs. Also contemplated are doses of 0.1-50 mg, or 0.1-20 mg or 0.1-10.0 mg. Furthermore, lower doses may be utilized in the case of administration by a non-oral route, as, for example, by injection or inhalation.

Ideally the active ingredient should be administered to achieve peak plasma concentrations ($C_{\text{max}}$) of the active compound of from about 5.0 to 70 $\mu$M, preferably about 5.0 to 15 $\mu$M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients
and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The compound can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The compound or a pharmaceutically acceptable prodrug or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, anti-inflammatory agents, anti-inflammatories, or other antivirals, including other nucleoside compounds. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.
If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polyylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

**VII. Biological Methods**

**Antiviral Testing Of Candidate Compounds With HCV Replicon System In Huh7 Cells.**

Huh7 cells harboring the HCV replicon can be cultivated in DMEM media (high glucose, no pyruvate) containing 10% fetal bovine serum, 1X non-essential Amino Acids, Pen-Strep-Glu (100 units/liter, 100 microgram/liter, and 2.92 mg/liter, respectively) and 500 to 1000 microgram/milliliter G418. Antiviral screening assays can be done in the same media without G418 as follows: in order to keep cells in logarithmic growth phase, cells are seeded in a 96-well plate at low density,
for example 1000 cells per well. The test compound is added immediately after seeding the cells and incubate for a period of 3 to 7 days at 37°C in an incubator. Media is then removed, and the cells are prepared for total nucleic acid extraction (including replicon RNA and host RNA). Replicon RNA can then be amplified in a Q-RT-PCR protocol, and quantified accordingly. The observed differences in replicon HCV RNA levels compared to the untreated control is one way to express the antiviral potency of the test compound.

In another typical setting, a compound might reduce the viral RNA polymerase activity, but not the host RNA polymerase activity. Therefore, quantification of rRNA or beta-actin mRNA (or any other host RNA fragment) and comparison with RNA levels of the no-drug control is a relative measurement of the inhibitory effect of the test compound on cellular RNA polymerases.

Phosphorylation Assay of Nucleoside to Active Triphosphate

To determine the cellular metabolism of the compounds, Huh-7 cells are obtained from the American Type Culture Collection (Rockville, MD), and are grown in 225 cm² tissue culture flasks in minimal essential medium supplemented with non-essential amino acids, 1% penicillin-streptomycin. The medium is renewed every three days, and the cells are sub cultured once a week. After detachment of the adherent monolayer with a 10 minute exposure to 30 mL of trypsin-EDTA and three consecutive washes with medium, confluent Huh-7 cells are seeded at a density of 2.5 x 10⁶ cells per well in a 6-well plate and exposed to 10 μM of [³H] labeled active compound (500 dpm/pmol) for the specified time periods. The cells are maintained at 37 °C under a 5% CO₂ atmosphere. At the selected time points, the cells are washed three times with ice-cold phosphate-buffered saline (PBS). Intracellular active compound and its respective metabolites are extracted by incubating the cell pellet overnight at −20 °C with 60% methanol followed by extraction with an additional 20 μL of cold methanol for one hour in an ice bath. The extracts are then combined, dried under gentle filtered air flow and stored at −20 °C until HPLC analysis.

Bioavailability Assay in Cynomolgus Monkeys

67
Within 1 week prior to the study initiation, the cynomolgus monkey is surgically implanted with a chronic venous catheter and subcutaneous venous access port (VAP) to facilitate blood collection and underwent a physical examination including hematology and serum chemistry evaluations and the body weight was recorded. Each monkey (six total) receives approximately 250 μCi of ³H-labeled compound combined with each dose of active compound at a dose level of 10 mg/kg at a dose concentration of 5 mg/mL, either via an intravenous bolus (3 monkeys, IV), or via oral gavage (3 monkeys, PO). Each dosing syringe is weighed before dosing to gravimetrically determine the quantity of formulation administered. Urine samples are collected via pan catch at the designated intervals (approximately 18-0 hours pre-dose, 0-4, 4-8 and 8-12 hours post-dosage) and processed. Blood samples are collected as well (pre-dose, 0.25, 0.5, 1, 2, 3, 6, 8, 12 and 24 hours post-dosage) via the chronic venous catheter and VAP or from a peripheral vessel if the chronic venous catheter procedure should not be possible. The blood and urine samples are analyzed for the maximum concentration (Cmax), time when the maximum concentration is achieved (Tmax), area under the curve (AUC), half life of the dosage concentration (T1/2), clearance (CL), steady state volume and distribution (Vss) and bioavailability (F).

**Bone Marrow Toxicity Assay**

Human bone marrow cells are collected from normal healthy volunteers and the mononuclear population are separated by Ficoll-Hypaque gradient centrifugation as described previously by Sommadossi J-P, Carlisle R. "Toxicity of 3'-azido-3'-deoxythymidine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine for normal human hematopoietic progenitor cells in vitro" Antimicrobial Agents and Chemotherapy 1987; 31:452-454; and Sommadossi J-P, Schinazi RF, Chu CK, Xie M-Y. "Comparison of cytotoxicity of the (-) and (+)-enantiomer of 2',3'-dideoxy-3'-thiaeytidine in normal human bone marrow progenitor cells" Biochemical Pharmacology 1992; 44:1921-1925. The culture assays for CFU-GM and BFU-E are performed using a bilayer soft agar or methylcellulose method. Drugs are diluted in tissue culture medium and filtered. After 14 to 18 days at 37 °C in a humidified atmosphere of 5% CO₂ in air, colonies of greater than 50 cells are counted using an
inverted microscope. The results are presented as the percent inhibition of colony
formation in the presence of drug compared to solvent control cultures.

**Mitochondria Toxicity Assay**

Fifty microliters of 2X drug dilutions were added per well in a 96 well plate. A “no drug” (media only) control was used to determine maximum amount of mitochondrial DNA produced and ribosomal DNA. 3TC @ 10 μM was used as a negative control, and ddC @ 10 μM was used as a toxic control. Ribosomal DNA levels were used to determine specific toxicity to mitochondria or generally cytotoxicity. HepG2 cells (5,000 cells/well at 50 μl) were added to the plate. The plate was incubated at 37°C in a humidified 5% CO₂ atmosphere for 7 days. After incubation, the supernatant was removed and stored for lactic acid quantification, and total DNA was extracted from cells as described in the RNeasy 96 handbook (February 1999), pages 22-23. No DNA digestions were performed, therefore total RNA and DNA were extracted.

The extracted DNA was amplified and the change in mitochondrial DNA and ribosomal DNA for each sample was determined. The fold difference in mitochondrial DNA normalized for ribosomal DNA relative to control was calculated.

Lactic acid quantification was performed by the D-Lactic Acid/ L-Lactic acid test kit (Boehringer Mannheim/ R-Biopharm/ Roche). The total amount of lactic acid produced for each sample was found as well as the fold change in lactic acid production (% of lactic acid / % of rDNA) as described in the manufacturers instructions.

**Cytotoxicity Assay**

50 μl of 2X drug dilutions were added per well in a 96 well plate. Final concentrations of drug ranged from 1 to 100 μM. A “no drug” (media only) control was used to determine the minimum absorbance values and a “cells + media only” control was used for maximum absorbance value. A solvent control was also used. Cells were then added (PBM: 5 x 10⁴ cells/well; CEM : 2.5 x 10³ cells/well; Vero, HepG2, Huh-7, and Clone A: 5 x 10³ cells/well) and incubated at 37°C in a
humidified 5% CO₂ atmosphere for 3-5 days (PBM :5 days; CEM : 3 days, all others :4 days). After incubation, 20 μl of MTS dye was added from Cell Titer Aqueous One Solution Cell Proliferation Assay to each well and the plate was re-incubated for 2-4 hours. The absorbance (490 nm) was then read on an ELISA plate reader using the media only/ no cell wells as blanks. Percent inhibition was found and used to calculate the CC₅₀.

**In vivo Toxicity in Mice**

*In vivo* toxicity was also determined following injections into female Swiss mice of the various nucleosides disclosed in the present invention. Intraperitoneal injections were given on days 0, day 1, day 2, day 3, and day 5 of varying doses of the particular nucleoside. Separate animals were injected with vehicle as control groups. In these studies, each dosing group contained 5-10 mice. The average weight change in each of the mice was measured as a sign of toxicity of the compound.

**(BVDV) Yield Reduction Assay**

Madin-Darby Bovine Kidney (MDBK) cells were grown in Dulbecco’s modified eagle medium supplemented with 10% horse serum and 100μg/ml penicillin-streptomycin. Cells were seeded in a 96-well plate at 5 x 10⁵ cells/well and incubated for 72h at 37°C in a humidified 5% CO₂ atmosphere. Cells were infected with either cytopathic (NADL strain) or noncytopathic (SD-1 strain) BVDV at a virus dilution of 10⁻² and incubated for 45 min. Cell monolayers were washed three times with medium. Fresh medium-containing test compounds in dose response concentrations or ribavirin, as a positive control, were added to cultures and medium containing no drug was added to the no-drug controls. After 72h incubation, supernatant was collected and viral RNA was extracted using the QI Amp Viral RNA Mini Kit (Qiagen, CA). Viral load was determined by Q-RT-PCR using primers specific for either NADL or SD-1 (1).

**VIII. Synthetic Protocol**
The following non-limiting embodiments illustrate some general methodologies to obtain the nucleosides of the present invention. Two representative general methods for the preparation of compounds of the present invention are outlined in Schemes 1 and 2 while more specific examples of these general methods are provided in Scheme 3 (Example 1), Scheme 4 (Example 2), Scheme 5 (Example 3), and Scheme 6 (Example 4). Scheme 1 represents a generalized process starting from a (2R) 2-deoxy-2-methyl-2-fluoro-carbohydrate and forms the nucleosides of the present invention by condensing with a nucleobase. Scheme 2 starts from a pre-formed, purine or pyrimidine nucleoside, optionally substituted at C-4' and constructs the C-2' (R) methyl, fluoro nucleosides of the present invention. While these schemes illustrate the syntheses of compounds of the present invention of general formulas (I) and (II) wherein there is a furanose ring in the β-D-ribo configuration, this is not intended to be a limitation on the scope of the process invention in any way, and they should not be so construed. Those skilled in the art of nucleoside and nucleotide synthesis will readily appreciate that known variations of the conditions and processes of the following preparative procedures and known manipulations of the nucleobase can be used to prepare these and other compounds of the present invention. Additionally, the L-enantiomers corresponding to the compounds of the invention can be prepared following the same methods, beginning with the corresponding L-carbohydrate building block or nucleoside L-enantiomer as the starting material.
1. Glycosylation of the nucleobase with an appropriately modified sugar

Scheme 1

Step 1: 

\[ \text{PGO} \text{O} \text{C} \text{OR} \rightarrow \text{PGO} \text{O} \text{H} \rightarrow \text{PGO} \text{O} \text{F} \]

**1-1** \rightarrow **1-2** \rightarrow **1-3**

Step 2:

**1-4** \rightarrow **1-5**

Step 3:

PGO = Protecting group

R = Lower alkyl, acyl, mesyl, benzoyl.

Base = as defined herein.

**1-6**

Step 1 in Scheme 1 introduces the 2-methyl group by using an appropriate alkylating agent such as methylolithium, trimethylaluminum, or methylmagnesium bromide in an anhydrous solvent such as tetrahydrofuran (THF), chloroform, or diethyl ether. Compounds 1-1 through 1-4 can be purely α or β or they may exist as an anomic mixture containing both α and β anomers in any ratio. However, the preferred anomic configuration of structure 1-1 is β.

Step 2 introduces the fluorine atom at the 2-position of the alkyl furanoside. This can be achieved by treatment of the tertiary alcohol, 1-2, with a commercially available fluorinating reagent such as (diethylamino)sulfur trifluoride (DAST) or Deoxofluor in an anhydrous, aprotic solvent such as tetrahydrofuran, chloroform, dichloromethane, or toluene. Preferably the stereochemistry proceeds with inversion of configuration at C-2. That is, starting from a C-2 hydroxyl “up” (or
arabinofuranoside) in structure 1-2, the C-2 fluorine is “down” in the intermediate ribofuranoside 1-3.

In step 3, the optional protecting groups (Pg) can be deprotected and reprotected to groups more suitable for the remaining manipulations (T.W. Greene and P.G.M. Wuts, “Protective Groups in Organic Synthesis,” 3rd ed., John Wiley & Sons, 1999). For example, benzyl ethers (Bn) may be difficult to remove in the protected nucleoside, 1-5 and may be deprotected and replaced with a group more facile to remove from the nucleoside of structural type 1-5. Furthermore, the anomeric position (C-1) can also be optionally manipulated to a suitable group for the coupling reaction with the nucleobase (step 4). Several methods for anomic manipulations are established to those skilled in the art of nucleoside synthesis. Some non-limiting examples by treatment of the alkyl furyanoside (1-3, R = alkyl) with a mixture of acetic anhydride, acetic acid, and a catalytic amount of sulfuric acid (acetolysis) to provide structure 1-4 where R = Ac, with optional protecting groups. Also, the alkyl group in 1-3 may be converted to an acetate, benzoate, mesylate, tosylate, triflate, or tosylate, for example, by first hydrolyzing the 1-Oalkyl group to a 1-hydroxyl group by using a mineral acid consisting of but not limited to sulfuric acid, hydrochloric acid, and hydrobromic acid or an organic acid consisting of but not limited to trifluoroacetic acid, acetic acid, and formic acid (at ambient temperature or elevated temperature). The reducing sugar could then be converted to the desired carbohydrate by treatment with acetyl chloride, acetic anhydride, benzyol chloride, benzoic anhydride, methanesulfonyl chloride, triflic anhydride, trifyl chloride, or tosyl chloride in the presence of a suitable base such as triethylamine, pyridine, or dimethylaminopyridine.

The nucleosidic linkage is constructed by treatment of intermediate 1-3 or 1-4 with the appropriate persilylated nucleobase in the presence of a lewis acid such as tin tetrachloride, titanium tetrachloride, trimethylsilyltriflate, or a mercury (II) reagent (HgO/HgBr₂) usually at an elevated temperature in an aprotic solvent such as toluene, acetonitrile, benzene, or a mixture of any or all of these solvents.

The optional protecting groups in the protected nucleosides or structural formula 1-5 can be cleaved following established deprotection methodologies (T.W.

73

2. Modification of a pre-formed nucleoside

Scheme 2

Pre-formed nucleoside

Step 1

Step 2

Step 3

Step 4

Step 5

Step 6

Pg = Protecting group

Base = as defined herein (optionally protected)

X = as defined herein

R^6 = as defined herein

The starting material for this process is an appropriately substituted purine or pyrimidine nucleoside with a 2’-OH and 2’-H. The nucleoside can be purchased or
can be prepared by any known means including standard coupling techniques. The nucleoside can be optionally protected with suitable protecting groups, preferably with acyl or silyl groups, by methods well known to those skilled in the art, as taught by T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999.

The purine or pyrimidine nucleoside can then be oxidized at the 2'-position with the appropriate oxidizing agent in a compatible solvent at a suitable temperature to yield the 2'-modified nucleoside. Possible oxidizing agents are a mixture of dimethylsulfoxide, trifluoroacetic anhydride or acetic anhydride (a Swern/Moffat oxidation), chromium trioxide or other chromate reagent, Dess-Martin periodinane, or by ruthenium tetroxide/sodium periodate.

The optionally protected nucleoside 2'-ketone is then alkylated using such alkylating agents methyl lithium, trimethylaluminum, methylmagnesium bromide, or similar reagents in an anhydrous solvent such tetrahydrofuran (THF), chloroform, or diethyl ether usually at temperatures below 0 °C. Compounds of the structural formula 2-3 are preferred to have the 2'(5) or 2'-methyl “down”, 2'-OH “up” configuration.

The nucleoside of structure 2-3 can be deprotected and reprotected with a number of protecting groups such as an O-acyl (alkyl or aryl), O-sulfonyl, or an N-acyl (alkyl or aryl) for the base. This optional reprotection step need not be limited to protecting groups that function as chemical protecting groups. Other protecting groups such as long chain acyl groups of between 6 and 18 carbon units or amino acids can be introduced independently on the nucleobase or the sugar. The protecting groups can serve as prodrugs of the active substance.

Step 5 introduces the fluorine atom at the 2' position of the pre-formed nucleoside. This can be achieved by treatment of the tertiary alcohol, 2-4, with a commercially available fluorinating reagent such as (diethylamino)sulfur trifluoride (DAST) or Deoxofluor in an anhydrous, aprotic solvent such as tetrahydrofuran, chloroform, dichloromethane, or toluene. Preferably the stereochemistry proceeds with inversion of configuration at the 2' position. That is, starting from a C-2' hydroxyl “up” (or arabinonucleoside) in structure 2-4, the C-2' fluorine is “down” in
the intermediate nucleoside 2-5. The absolute configuration of a nucleoside of structure 2-4 is (2′S) while the absolute configuration of a nucleoside of structure 2-5 is (2′R).


The following working examples provide a further understanding of the method of the present invention and further exemplify the general examples in Schemes 1 and 2 above. These examples are of illustrative purposes, and are not meant to limit the scope of the invention. Equivalent, similar or suitable solvents, reagents or reaction conditions may be substituted for those particular solvents, reagents or reaction conditions described without departing from the general scope of the method.

EXAMPLES

Example 1

Synthesis of (2′R)-2′-Deoxy-2′-Fluoro-2′-C-Methylcytidine Starting from a Carbohydrate
Scheme 3

Step 1: Compound 3-1 (7.7 g, 0.022 mmol) was dissolved in anhydrous diethyl ether and cooled to -78 °C. To this solution was added MeLi (30 mL, 1.6 M in diethyl ether). After the reaction was complete, the mixture was treated with ammonium chloride (1 M, 65 mL) and the organic phase was separated, dried (Na₂SO₄), filtered, and concentrated to dryness. Silica gel chromatography followed by crystallization from diethyl ether-hexanes afforded pure compound 3-2 (6.31 g).

1H NMR (400 MHz, CDCl₃): δ 1.40 (s, 3H), 3.41 (s, 3H), 3.49 (dd, 1H, J = 10.3, 6.89 Hz), 3.57 (dd, 1H, J = 10.3, 3.88 Hz), 3.84 (d, 1H, J = 7.3 Hz), 4.03 (m, 1H), 4.48 (s,1H), 4.58 (m, 3H), 4.83 (d, 1H, J = 11.6 Hz), 7.31–7.36 (m, 10H); 13C NMR (100 MHz, CDCl₃): δ 18.4, 55.4, 72.2, 73.4, 79.5, 80.2, 84.7, 107.4, 127.7, 127.8, 127.83, 128.5, 138.2, 138.3.
**Step 2**: Compound 3-2 was dissolved in CH₂Cl₂ and was treated with DAST (4.0 mL, 30.3 mmol) at room temperature. The solution was stirred at room temp overnight. The so-obtained mixture was poured into sat NaHCO₃ (100 mL) and washed with sat NaHCO₃ (1 x 15 mL). The organic layer was further worked up in the usual manner. Silica gel chromatography (1:5 EtOAc-hexanes) gave crude compound 3-3 (0.671 g) that was sufficiently pure for the next step. ¹H NMR (400 MHz, CDCl₃): δ 1.43 (d, 3H, J = 22.8 Hz), 3.35 (s, 3H), 3.49 (dd, 1H, J = 10.5, 5.4 Hz), 3.55 (dd, 1H, J = 10.5, 4.1 Hz), 3.87 (dd, 1H, J = 23.5, 7.5 Hz), 4.26 (m, 1H), 4.56 (d, 2H, J = 6.9 Hz), 4.66 (d, 2H, J = 8.2 Hz), 4.72 (d, 1H, J = 10.8 Hz), 7.29–7.36 (m, 10H); ¹³C NMR (100 MHz, CDCl₃): δ 17.0 (d, J = 24.4 Hz), 55.2, 77.1, 73.4, 73.8, 77.3, 80.3, 81.2 (d, J = 16 Hz), 99.7 (d, J = 178.9 Hz), 106.8 (d, J = 32.0 Hz), 127.7, 127.8, 128.1, 128.3, 128.5, 128.6, 137.8, 138.3; ¹⁹F NMR (100 MHz, CDCl₃): δ -8.2 (m, 1F).

**Step 3**: Compound 3-3 (0.39 g, 1.1 mmol) was dissolved in 1:2 EtOH-EtOAc and treated with Pd/C (~0.1 g) and cyclohexene (~1 mL). The mixture was heated to reflux overnight and then filtered through celite. The solvent was removed in vacuo and the residue was dissolved in pyridine (~5 mL). To this solution was added benzoyl chloride (0.22 mL, 1.83 mmol) and the mixture was stirred at room temp overnight. The pyridine was removed in vacuo and the residue was partitioned between CH₂Cl₂ and sat NaHCO₃ (10.0 mL). The organic phase was dried (Na₂SO₄), filtered, and the solution was concentrated to dryness. Column chromatography provided 0.350 g of pure compound 3-4. ¹H NMR (400 MHz, CDCl₃): δ 1.53 (d, 3H, J = 22.4 Hz), 3.39 (s, 3H), 4.46 (dd, 1H, J = 11.6, 4.7 Hz), 4.58 (m, 1H), 4.65 (dd, 1H, J = 11.6, 3.9 Hz), 4.87 (d, 1H, J = 9.9 Hz), 5.64 (dd, 2H, J = 24.1, 7.8 Hz), 7.29–7.36 (m, 10H); ¹⁹F NMR (100 MHz, CDCl₃): δ -7.5 (m, 1F).

**Step 4**: A solution of bis(trimethylsilyl)-N-benzyolcytosine (0.28 g, 0.77 mmol) and compound 3-4 (0.20 g, 0.5 mmol) in 1,2 dichloroethane (2 mL) and toluene (2 mL) was treated with TMSCOTF (0.15 mL, 0.77 mmol). After most of the starting material disappeared as judged by TLC, the solution was cooled to room temp, washed with water (1 x 5 mL), brine (1 x 5 mL), dried (Na₂SO₄), filtered, and concentrated to dryness. Flash chromatography followed by crystallization from CH₂Cl₂-hexanes afforded compound 3-5 (68 mg). mp 241 °C; ¹H NMR (400 MHz, CDCl₃): δ 1.53 (d, 3H, J = 22.4 Hz), 3.39 (s, 3H), 4.46 (dd, 1H, J = 11.6, 4.7 Hz), 4.58 (m, 1H), 4.65 (dd, 1H, J = 11.6, 3.9 Hz), 4.87 (d, 1H, J = 9.9 Hz), 5.64 (dd, 2H, J = 24.1, 7.8 Hz), 7.29–7.36 (m, 10H); ¹⁹F NMR (100 MHz, CDCl₃): δ -7.5 (m, 1F).
CDCl$_3$): δ 1.49 (d, 3H, $J = 22.4$ Hz), 4.64 (dd, 1H, $J = 12.9$, 3.4 Hz), 4.73 (app d, 1H, $J = 9.5$ Hz), 4.89 (dd, 1H, $J = 12.7$, 2.2 Hz), 5.56 (dd, 1H, $J = 20.7$, 8.6 Hz), 6.52 (d, 1H, $J = 15.9$ Hz), 7.38–7.67 (m, 10H), 7.89 (d, 2H, $J = 6.9$ Hz), 8.07–8.11 (m, 5H), 8.67 (s, 1H); $^{19}$F NMR (100 MHz, CDCl$_3$): δ 2.85 (m, 1F).

**Step 5:** Compound 3-5 (40 mg, 0.05 mmol) was dissolved in methanolic ammonia and stirred at room temp for 48 h. The solution was concentrated to dryness and chromatographed (SiO$_2$) eluting with 1:4 EtOH-CH$_2$Cl$_2$. The yield was about 12 mg of pure (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine, 3-6. $^1$H NMR (400 MHz, DMSO-d$_6$): δ 1.16 (d, 3H, $J = 22.0$ Hz), 3.61 (dd, 1H, $J = 11.6$, 5.2 Hz), 3.60–3.83 (m, 3H, $J = 10.5$, 5.4 Hz), 5.24 (s, 1H, exchangeable with D$_2$O), 5.59 (s, 1H, exchangeable with D$_2$O), 5.71 (d, 1H, $J = 7.3$ Hz), 6.08 (d, 1H, $J = 19.0$ Hz), 7.24 (d, 1H, $J = 17.7$ Hz, exchangeable with D$_2$O), 7.87 (d, 1H); $^{19}$F NMR (100 MHz, DMSO-d$_6$): δ 4.13 (m, 1F).

**Example 2**

*Synthesis of (2'R)-2'-Deoxy-2'-Fluoro-2'-C-Methylcytidine Starting from Cytidine*
Scheme 4

TIDPS = 1,3-(1,1,3,3-Tetraisopropyldisiloxanylidene)

**Step 1:** To a suspension of cytidine (100 g, 0.411 mol) in DMF (2.06 L) is added benzoic anhydride (102.4 g, 0.452 mol). The mixture was stirred at room temperature for 20 h. The DMF was removed *in vacuo* and the residue was triturated with diethyl ether. The resulting solid was collected by suction filtration and washed with diethyl ether (2 x 200 mL). Further drying *in vacuo* at room temperature gave the N\(^4\) benzamide (140.6 g, 98.3%). A portion of this material
(139.3 g, 0.401 mol) was dissolved in anhydrous pyridine (1.2 L) and was treated with 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane (141.4 mL, 0.441 mol) at room temp. The solution was stirred at room temperature overnight. The mixture was concentrated to near dryness in vacuo and coevaporated with toluene (3 x 200 mL). The residue was treated with EtOAc (1.8 L) and washed with HCl (2 x 200 mL, 0.05 N), NaHCO₃ (5 %, 2 x 400 mL). The organic layer was washed dried (Na₂SO₄), filtered, and evaporated to dryness. Compound 4-1 (256.5 g, >100%) was isolated as a white foam and used without further purification.

**Step 2:** Compound 4-1 (236.5 g, 0.40 mol) was dissolved in dry THF (1.22 L). Anhydrous dmso (180.8 mL, 2.1 mol) was added and the resulting solution was cooled to between -20 °C and -15 °C. Trifluoroacetic anhydride (90.6 mL, 0.64 mol) was added dropwise over 45 minutes and the solution was stirred between -20 °C and -15 °C for 2 hrs after which anhydrous triethylamine (223.5 mL, 1.6 mol) was added over 20 min. The crude reaction containing ketone 4-2 was dissolved in EtOAc (500 mL), and the resulting solution was washed with H₂O (3 x 400 mL), dried (Na₂SO₄) and the solvents were removed in vacuo to give a yellow solid that was purified on a silica gel column eluting with a stepwise gradient of Et₂O (0–60%) in hexanes followed by a stepwise gradient of EtOAc (50–100%) in hexanes. The crude ketone so-obtained (~192 g) was crystallized from petroleum ether to give ketone 4-2 (138.91 g, 57.5% from cytidine) as a white solid and 22 g of unreacted starting material, 4-1, as a yellow solid.

**Step 3:** Compound 4-2 (48.57 g, 8.26 mmol) was dissolved in anhydrous toluene (~400 mL) and the solvent was removed in vacuo with exclusion of moisture. The residue was then further dried in vacuo (oil pump) for another 2 h. With strict exclusion of moisture, the residual foam was dissolved in anhydrous diethyl ether (1.03 L) under argon. The resulting solution was cooled to -78 °C under argon and MeLi (1.6 M, 258.0 mL, 0.413 mol) was added dropwise via additional funnel. After the addition was complete, the mixture was stirred for 2 h at -78 °C. Aqueous 1 M NH₄Cl (500 mL) was added slowly. After warming to room temperature, the mixture was washed with H₂O (2 x 500 mL), dried (Na₂SO₄), and then concentrated to dryness to give a brown foam (~60 g, >100%).
The reaction was performed two more times using 37.62 g and 56.4 g of compound 4-2. The combined crude products (128.0 g, 0.212 mol) were dissolved in THF (1.28 L) and treated with concd HOAc (23 mL, 0.402 mol). To the solution was added TBAF (384.0 mL, 1 M in THF). The solution was stirred at room temp for 0.75 h and the mixture was treated with silica gel (750 g) and concentrated to dryness. The powder was placed on a silica gel column packed in CH2Cl2. Elution with 1:7 EtOH-CH2Cl2 afforded a dark waxy solid that was pre-adsorbed on silica gel (300 g) and chromatographed as before. Compound 4-3 (46.4 g, 53.0 % from 4-2) was isolated as an off-white solid. 1H NMR (DMSO-d6): δ 1.20 (s, 3H, CH3), 3.62–3.69 (m, 2H), 3.73–3.78 (m, 2H), 5.19 (t, 1H, J = 5.4 Hz, OH-5'), 5.25 (s, 1H, OH-2'), 5.52 (d, 1H, J = 5.0 Hz, OH-3'), 5.99 (s, 1H, H-1'), 7.32 (d, 1H, J = 5.8 Hz), 7.50 (Ψt, 2H, J = 7.7 Hz), 7.62 (Ψt, 1H, J = 7.3 Hz), 8.00 (d, 2H, J = 7.3 Hz), 8.14 (d, 1H, J = 6.9 Hz), 11.22 (s, 1H, NH). Anal. Calcd for C17H19N3O6 • 0.5 H2O: C, 55.13; H, 5.44; N, 11.35. Found: C, 55.21; H, 5.47; N, 11.33.

Step 4: Compound 4-3 (46.0 g, 0.13 mol) was dissolved in anhydrous pyridine and concentrated to dryness in vacuo. The resulting syrup was dissolved in anhydrous pyridine under argon and cooled to 0 °C with stirring. The brown solution was treated with benzoyl chloride (30 mL, 0.250 mol) dropwise over 10 min. The ice bath was removed and stirring continued for 1.5 h whereby TLC showed no remaining starting material. The mixture was quenched by the addition of water (5 mL) and concentrated to dryness. The residue was dissolved in a minimal amount of CH2Cl2 and washed with satd NaHCO3 (1 x 500 mL) and H2O (1 x 500 mL). The organic phase was dried (Na2SO4) and filtered, concentrated to dryness and chromatographed on silica gel eluting with a stepwise gradient of EtOAc-hexanes (25-60%) to provide compound 4-4 as yellow foam (48.5 g, 67%). 1H NMR (CDCl3): δ 1.64 (s, 3H, CH3), 4.50 (m, 1H, H-4), 4.78–4.85 (m, 2H, H-5',5a'), 5.50 (d, 1H, J = 3.4 Hz, H-3'), 6.42 (s, 1H, H-1'), 7.44–7.54 (m, 7H, Ar), 7.57–7.66 (m, 3H, Ar), 7.94 (d, 2H, J = 7.8 Hz), 8.05–8.09 (m, 4H, Ar), 8.21 (d, 1H, J = 7.3 Hz). Anal. Calcd for C31H27N3O8: C, 65.37; H, 4.78; N, 7.38. Found: C, 65.59; H, 4.79; N, 7.16.

Step 5: Compound 4-4 (7.50 g, 0.013 mol) was dissolved in anhydrous toluene (150 mL) under argon and cooled to -20 °C. DAST (2.5 mL, 18.9 mmol)
was added slowly and the cooling bath was removed after the addition was complete. Stirring was continued for 1 h and the mixture was poured into satd NaHCO₃ (100 mL) and washed until gas evolution ceased. The organic phase was dried (Na₂SO₄), concentrated, and purified by silica gel chromatography eluting with 1:1 EtOAc-hexanes. Yield was 1.22 g (16.3%) of pure 4-5 as a white solid. mp 241 °C (CH₂Cl₂-hexanes); ¹H NMR (CDCl₃): δ 1.49 (d, 3H, J = 22.4 Hz, CH₃), 4.64 (dd, 1H, J = 3.44, 12.9 Hz, H-5'), 4.73 (d, 1H, J = 9.5 Hz, H-4'), 4.90 (dd, 1H, J = 2.4, 12.7 Hz, H-5a'), 5.56 (dd, 1H, J = 8.6, 20.7 Hz, H-3'), 6.52 (d, 1H, J = 18.0 Hz, H-1'), 7.47-7.57 (m, 7H, Ar), 7.62-7.71 (m, 3H, Ar), 7.89 (d, 2H, J = 6.9 Hz), 8.07-8.11 (m, 5H, Ar), 8.67 (bs, 1H, NH). ¹⁹F NMR (CDCl₃): δ 3.3 (m). Anal. Calcd for C₃₁H₂₄FN₃O₇ • 0.7 H₂O: C, 63.74; H, 4.72; N, 7.20. Found: C, 63.71; H, 4.54; N, 7.20.

**Step 6:** Compound 4-5 (6.30 g, 0.011 mol) was suspended in methanolic ammonia (ca 7 N, 150 mL) and stirred at room temperature overnight. The solvent was removed in vacuo, co-evaporated with methanol (1 x 20 mL), and pre-adsorbed onto silica gel. The white powder was placed onto a silica gel column (packed in CHCl₃) and the column was eluted with 9% EtOH in CHCl₃, then 17% EtOH and finally 25% EtOH in CHCl₃. Concentration of the fractions containing the product, filtration through a 0.4 µm disk, and lyophilization from water afforded compound 4-6, 2.18 g (76%). ¹H NMR (DMSO-d₆): δ 1.17 (d, 3H, J = 22.3 Hz, CH₃), 3.63 (dd, 1H, J = 2.7, 13.7 Hz, H-5'), 3.70-3.84 (m, 3H, H-3', H-4', H-5a'), 5.24 (app s, 1H, OH-3'), 5.60 (d, 1H, J = 5.4 Hz, H-5'), 5.74 (d, 1H, J = 7.71 Hz, H-5), 6.07 (d, 1H, J = 18.9 Hz, H-1'), 7.31 (s, 1H, NH₂), 7.42 (s, 1H, NH₂), 7.90 (d, 1H, J = 7.3 Hz, H-6). ¹⁹F NMR (DMSO-d₆): δ 2.60 (m). Anal. Calcd for C₁₆H₁₄FN₃O₄ • 1.4 H₂O: C, 44.22; H, 5.95; N, 14.77. Found: C, 42.24; H, 5.63; N, 14.54. Compound 4-6 (0.10 g, 0.386 mmol) was converted to the hydrochloride salt by dissolving in water (2 mL) and adjusting the pH to approximately 3.0 with 1 M HCl. The water was removed in vacuo and the residue was crystallized from aqueous EtOH to give 4-6 as the hydrochloride salt (71.0 mg). mp 243 °C (dec); ¹H NMR (DMSO-d₆): δ 1.29 (d, 3H, J = 22.6 Hz, CH₃), 3.65 (dd, 1H, J = 2.3, 12.7 Hz, H-5'), 3.76-3.90 (m, 3H, H-3', H-4', H-5a'), 5.96 (d, 1H, J = 17.3 Hz, H-1'), 6.15 (d, 1H, J = 7.9 Hz, H-5), 8.33 (d, 1H, J = 7.9 Hz, H-6), 8.69 (s, 1.5H, NH), 9.78 (s, 1.5H, NH). ¹⁹F

**Example 3**

*Synthesis of (2'R)-6-Chloro-2'-Deoxy-2'-Fluoro-2'-C-Methylpurine Starting from 6-Chloropurine Riboside.*

**Scheme 5**

6-Chloropurine riboside

[Diagram showing the reaction steps from 6-Chloropurine riboside to 5-6]

**TIDPS** = 1,3-(1,1,3,3-Tetraisopropyldisiloxanylidene)

84
**Step 1:** The nucleoside, 6-chloropurine riboside, (3.18 g, 11.09 mmol) was dissolved in anhydrous pyridine (300 mL) and was treated dropwise with 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane (4.08 mL, 12.75 mmol) at 0 °C under an argon atmosphere. The solution was brought to room temp and stirred overnight. The mixture was concentrated to near dryness in vacuo, dissolved in a minimal amount of chloroform, and washed with HCl (100 mL, 0.05 N) and NaHCO₃ (5%, 100 mL). The organic layer was dried (Na₂SO₄), filtered, and evaporated to dryness to afford compound 5-1 as an amber glass (6.10 g, >100%) that was used without further purification. ¹H NMR (CDCl₃): δ 1.01–1.13 (m, 24H), 4.03–4.18 (m, 3H), 4.58 (d, 1H, J = 5.2 Hz), 5.01 (m, 1H), 6.07 (s, 1H), 8.31 (s, 1H), 8.71 (s, 1H).

**Step 2:** Compound 5-1 (7.13 g, 13.47 mmol) was dissolved in dry THF (35 mL). Anhydrous DMSO (5.11 mL, 72.06 mmol) was added and the resulting solution was cooled to between -20 °C and -15 °C. Trifluoroacetic anhydride (3.06 mL, 21.69 mmol) was added dropwise over 45 minutes and the solution was stirred between −20 °C and −15 °C for 2 hrs after which anhydrous triethylamine (8.08 mL, 57.92 mmol) was added over 20 min. The crude reaction containing ketone 5-2 was dissolved in Et₂O (25 mL), and the resulting solution was washed with H₂O (2 x 50 mL), dried (Na₂SO₄) and the solvents were removed in vacuo to give a yellow solid that was purified on a silica gel column eluting with a stepwise stepwise gradient of 0–50% petroleum ether-diethyl ether afforded compound 5-2 as a mixture with the corresponding geminal diol. The glass was dissolved in CH₂Cl₂ and stirred over an excess of MgSO₄ for 36 h. The mixture, free from the geminal diol, was filtered, and evaporated to dryness to afford compound 5-2 as an amber glass (7.0 g, 97%). ¹H NMR (CDCl₃): δ 1.01–1.13 (m, 24H), 4.09–4.22 (m, 3H), 5.55 (d, 1H, J = 9.6 Hz), 5.80 (s, 1H), 8.19 (s, 1H), 8.61 (s, 1H).

**Step 3:** A solution of compound 5-2 (7.0 g, 13.26 mmol) in anhydrous tetrahydrofuran (45 mL) was cooled to -78 °C with stirring under an argon atmosphere. To the solution was added methylmagnesium bromide (15.85 mL, 3.0 M in ethyl ether) dropwise over a 30 min period. After stirring for an additional 3 h at -78 °C, the reaction was quenched by the careful addition of aqueous 1 M NH₄Cl (50.0 mL). After warming to room temperature, the mixture was washed with H₂O
(2 x 500 mL), dried (Na₂SO₄), and concentrated to dryness to give a brown foam (3.8 g) that was dissolved in tetrahydrofuran (50 mL) and treated with a solution of TBAF (18.9 mL, 1 M solution in THF) and glacial acetic acid (0.85 mL) at room temp. The solution was stirred at room temp for 2h, concentrated to dryness, and purified by silica gel chromatography to give compound 5-3 (2.0 g, 50%).

**Step 4:** Compound 5-3 (0.491 g, 1.63 mmol) was dissolved in pyridine (3 mL) and treated with acetic anhydride (0.38 mL, 4.08 mL) at room temp. The solution was stirred at room temp for 2 h after which time, the solution was concentrated to dryness and treated with diethyl ether (10 mL) and water (5 mL). The organic layer was further washed with water (2 x 10 mL), dried (Na₂SO₄), filtered, and evaporated to dryness to give compound 5-4 as a foam (0.450 g, 91.0%).

1H NMR (CDCl₃): δ 1.39 (s, 3H), 2.15 (s, 3H), 2.21 (s, 3H), 4.27 (m, 1H), 4.49 (dd, 1H, J = 4.2, 11.9 Hz ), 4.57 (dd, 1H, J = 6.16, 11.9 Hz), 5.14 (d, 1H, J = 3.1 Hz), 6.25 (s, 1H), 8.54 (s, 1H), 8.75 (s, 1H).

**Step 5:** Compound 5-4 (0.100 g, 0.259 mmol) was dissolved in anhydrous toluene (3.0 mL) under argon and cooled to -20 °C. DAST (0.2 mL, 1.55 mmol) was added slowly and the cooling bath was removed after the addition was complete. Stirring was continued for 1 h and the mixture was poured into satd NaHCO₃ (100 mL) and washed until gas evolution ceased. The organic phase was dried (Na₂SO₄), concentrated, and purified by silica gel chromatography eluting with 30% Et₂O-petroleum ether gave pure 5-5 (0.028 g, 27.9%).

1H NMR (CDCl₃): δ 1.24 (d, 3H, J = 22.8 Hz), 2.20 (s, 3H), 2.22 (s, 3H), 4.41–4.55 (m, 3H), 4.47 (dd, 1H, J = 9.2, 22.0 Hz), 6.37 (d, 1H, J = 17.6 Hz), 8.45 (s, 1H), 8.82 (s, 1H).

**Step 6:** Compound 5-5 (0.018 g, 0.047 mmol) was dissolved in methanol (5 mL) and treated with a solution of sodium methoxide (3.6 mg, 0.67 mmol) in methanol (5 mL). The solution was stirred at room temp for 1 h, neutralized with concd acetic acid and chromatographed on silica gel eluting with a stepwise gradient of Et₂O/methanol (0-5%) to afford compound 5-6 (0.010 g, 70.9%).

1H NMR (CDCl₃): δ 1.23 (d, 3H, J = 22.4 Hz), 4.04 (dd, 1H, J = 2.11, 12.5 Hz), 4.17 (dd, 1H, J = 1.5, 9.2 Hz), 4.25 (dd, 1H, J = 1.9, 12.3 Hz), 4.61(dd, 1H, J = 9.2, 22.3 Hz), 6.37 (d, 1H, J = 17.3 Hz), 8.70 (s, 1H), 8.78 (s, 1H).
Example 4

Synthesis of (2'R)-2'-Deoxy-2'-Fluoro-2'-C-Methyladenosine Starting from (2'R)-6-Chloro-2'-Deoxy-2'-Fluoro-2'-C-Methylpurine

Scheme 6

\[
\begin{align*}
\text{Step 1: } & \text{ Compound 5-5 (0.100 g, 0.26 mmol) was heated in a pressure tube} \\
& \text{with methanolic ammonia (ca. 7 N, 25 mL) at 80 °C for 12 h. The crude reaction} \\
& \text{was pre-adsorbed onto silica gel and purified by column chromatography eluting} \\
& \text{with a stepwise gradient of Et}_2\text{O-MeOH (0-5%). The impure product was converted} \\
& \text{to the hydrochloride salt by dissolving the compound in a minimal amount of} \\
& \text{ethanol and treating the solution with 0.5 mL of a 0.6 M HCl solution.} \\
& \text{Concentration to near dryness gave compound 6-1 as off-white cystals (0.020 g,} \\
& \text{24.2%). } ^1\text{H NMR (CD}_3\text{OD): } \delta \text{ 1.19 (d, 3H, } J = 22.3 \text{ Hz), 3.88 (dd, 1H, } J = 2.7, \\
& \text{12.7 Hz), 4.06 (dd, 1H, } J = 2.1, 12.5 \text{ Hz), 4.11 (app d, 1H, } J = 9.2 \text{ Hz), 4.35 (dd,} \\
& \text{1H, } J = 9.4, 24.5 \text{ Hz), 6.35 (d, 1H, } J = 16.5 \text{ Hz), 8.43 (s, 1H), 8.85 (s, 1H).}
\end{align*}
\]

Example 5

Antiviral Activity of (2'R)-2'-Deoxy-2'-Fluoro-2'-C-Methylcytidine

HCV Replicon Assay

The anti-flavivirus activity of the compounds was determined as described by Stuyver, et al. (“Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture”, Antimicrobial Agents and Chemotherapy 47:244-245 (2003)). The compound was dissolved in DMSO and
added to the culture media at final concentrations ranging from 3 to 100 µM. A 4-
days incubation resulted in dose-dependant reduction of the replicon HCV RNA
(Figure 1A). A 1-log reduction of replicon RNA (or EC₉₀ value) was reached at
approximately 2.5 µM. Measurement of the reduction of rRNA gave an indication
of the inhibitory effect on cellular polymerases. Subtraction of this cellular toxicity
value from the antiviral values resulted in the therapeutic index line and EC₉₀ value.
Based on these calculations, an average EC₉₀ value, corrected for cellular toxicity, of
approximately 2.5 µM was obtained. Figure 1A shows the dose-dependant
reduction of the replicon HCV RNA based on the treatement with (2'R)-2'-deoxy-2'-
fluoro-2'-C-methylcytidine. The viral reduction was compared to the reduction of
cellular RNA levels (ribosomal RNA) to obtain therapeuric index values. EC₉₀
represents the effective concentration 90% at 96 hours following the dose dependant
administration of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. Figure 1B shows the
prolonged reduction in replicon HCV RNA up to 7 days following treatment with 5
and 25 µM.

The activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in the replicon
system is summarized in Table 1. The EC₉₀ values for (2'R)-2'-deoxy-2'-fluoro-2'-C-
methylcytidine as well as 2'-C-methylcytidine and 2'-C-methyladenosine are shown
for three separate replicon clones (HCV-WT (Wild Type), 9-13 and 21-5) as well as
two other clones (S282T and rRNA). The EC₉₀ values for (2'R)-2'-deoxy-2'-fluoro-
2'-C-methylcytidine were in the range of 1.6 to 4.6µM for the replicon clones.
In contrast the EC₉₀ values for 2'-C-methylcytidine were in the range of 6.6-37.4µM.
Interestingly, the EC₉₀ values for 2'-C-methyladenosine were comparable to those of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. The activity of (2'R)-2'-deoxy-2'-
fluoro-2'-C-methylcytidine and 2'-C-methylcytidine in other replicons tested is
shown in Table 2.

Polymerase Assay

Table 3 shows the potencty of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine-
5'-triphosphate (TP) in the NSSB polymerase assay. The inhibitory concentration
50% was determined to be in the range of 1.7 to 7.7µM.
Toxicity

A summary of the toxicity data for (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine using the mitochondrial toxicity assay is shown in Tables 6 and 7. Table 7 shows the lack of effects of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine and 2'-C-methylcytidine on mitochondrial DNA synthesis and lack of effects on lactic acid increase in this assay. Results shows the relative lack of toxicity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. Table 6 shows a cytotoxicity analysis in various cell lines (Clone A, Huh7, HepG2, MDBK, PBM, CEM, Vero, MRC-5). Cytotoxic concentration 50% (CC50) was greater than 75-100μM in all clones tested for (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine as well as 2'-C-methylcytidine. In contrast is the relative toxicity of 2'-C-methyladenosine.

The effects the nucleoside analogs tested on human bone marrow cells is depicted in Table 9. As shown, the IC50 values for 2'-methyl-2'-fluorocytidine were significantly higher (98.2, BFU-E) and 93.9 (CFU-GM) as compared to 2'-methylcytadine or AZT. Results show that 2'-methyl-2'-fluorocytidine was significantly less toxic than compared to the other nucleoside compounds.

Animal Studies

Figure 2 depicts the average weight change (%) of female Swiss mice in vivo the toxicity analysis of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine at various doses. Intraperitoneal injections were given on days 0 to day 5 of the 0, 3.3, 10, 33, 100 mg/kg. Each dosing group contained 5 mice and no mice died during the 30-day study. No significant toxicity was observed in the mice.

Figure 3 and Table 6 summarize the pharmacokinetic parameters of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in Rhesus monkeys given a single dose (33.3 mg/kg) oral (Table 6, Figure 3) or intravenous dose (Figure 3) of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine.

Other Antiviral Activity
Summary of the range of antiviral activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine is shown in Table 4. Table shows that in addition to HCV virus (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine shows activity against Rhinovirus, West Nile virus, Yellow Fever virus, and Dengue virus.

Table 5 shows the lack of activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine on HCV surrogate models BVDV as well as other viruses including HIV, HBV and Corona virus. In contrast, 2'-C-methylcytidine and 2'-C-methyladenosine show greater activity in the HCV surrogate model, BVDV. These results show the necessity for screening this series of compounds against the HCV replicon system versus surrogate HCV systems.

Table 1: Summary of the Anti-HCV Replicon Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine*

<table>
<thead>
<tr>
<th>Replicon</th>
<th>(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine</th>
<th>2'-C-methylcytidine</th>
<th>2'-C-methyladenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV-WT 1b</td>
<td>4.6 ± 2.0</td>
<td>21.9 ± 4.3</td>
<td>2.1 ± 0.27</td>
</tr>
<tr>
<td>S282T mut. 1b</td>
<td>30.7 ± 11.7</td>
<td>37.4 ± 12.1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>9-13 (subgenomic)</td>
<td>4.6 ± 2.3</td>
<td>13.0</td>
<td>0.7</td>
</tr>
<tr>
<td>21-5 (full-length)</td>
<td>1.6 ± 0.7</td>
<td>6.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Values represent EC₉₀ (µM)
**Table 2: Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine and 2'-C-methylcytidine in other Replicons**

<table>
<thead>
<tr>
<th>Replicon</th>
<th>(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine</th>
<th>2'-C-methylcytidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC_{90} (µM)</td>
<td>IC_{90} (µM)</td>
</tr>
<tr>
<td>1b (Ntat)</td>
<td>3.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1b (Btat)</td>
<td>11.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1a (pp1aSI-7)</td>
<td>34.7</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

**Table 3: HCV 1b NS5B Polymerase Assay (IC_{50}, µM)**

<table>
<thead>
<tr>
<th></th>
<th>(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine TP</th>
<th>2'-C-methylcytidine TP</th>
<th>2'-C-methyladenosine TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-Type NS5B</td>
<td>1.7 ± 0.4^{a}</td>
<td>6.0 ± 0.5</td>
<td>20.6 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>7.7 ± 1.2^{b}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S282T</td>
<td>2.0^{a}</td>
<td>26.9 ± 5.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td>8.3 ± 2.4^{c}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^{a} Values determined using batch 1; ^{b} Value determined using batch 2 and 3; and ^{c} Value determined using batch 2.
### Table 4: Summary of Antiviral Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell</th>
<th>EC_{50}, CPE (µM)</th>
<th>EC_{50}, CPE (µM)</th>
<th>NR (^a)</th>
<th>CC_{50}, CPE (µM)</th>
<th>CC_{50}, CPE (µM)</th>
<th>NR (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Nile</td>
<td>Vero</td>
<td>32</td>
<td>12</td>
<td>&gt;100</td>
<td>32</td>
<td>&gt;100</td>
<td>32</td>
</tr>
<tr>
<td>Dengue Type 2</td>
<td>Vero</td>
<td>32/55</td>
<td>&gt;100/&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Yellow Fever</td>
<td>Vero</td>
<td>19/3.2</td>
<td>32/12</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Influenza A (H1N1)</td>
<td>MDCK</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Influenza A (H3N2)</td>
<td>MDCK</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Influenza B</td>
<td>MDCK</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Rhinovirus Type 2</td>
<td>KB</td>
<td>25</td>
<td>20</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>VEE</td>
<td>Vero</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td>SARS-CoV</td>
<td>Vero</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

\(^a\)NR = Neutral Red.

### Table 5: Summary of Antiviral Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine

<table>
<thead>
<tr>
<th>Virus</th>
<th>(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine (EC_{90}, µM)</th>
<th>2'-C-methylcytidine (EC_{90}, µM)</th>
<th>2'-C-methyladenosine (EC_{90}, µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDVncp</td>
<td>&gt;22</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>BVDVcp</td>
<td>&gt;100</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>RSV</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>HIV(^a)</td>
<td>&gt;100</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HBV</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>ND</td>
</tr>
<tr>
<td>Coronavirus 229E</td>
<td>&gt;100</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not determined.
### Table 6: Cytotoxicity Studies

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine CC&lt;sub&gt;50&lt;/sub&gt;, µM</th>
<th>2'-C-methylyctidine CC&lt;sub&gt;50&lt;/sub&gt;, µM</th>
<th>2'-C-methyladenosine CC&lt;sub&gt;50&lt;/sub&gt;, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CloneA</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>37</td>
</tr>
<tr>
<td>Huh7</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>30</td>
</tr>
<tr>
<td>HepG2</td>
<td>75</td>
<td>&gt;100</td>
<td>58</td>
</tr>
<tr>
<td>MDBK</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>PBM</td>
<td>&gt;100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEM</td>
<td>&gt;100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vero</td>
<td>&gt;100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRC-5</td>
<td>&gt;100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Results determined using MTS assay.

### Table 7: Mitochondrial Toxicity Study

<table>
<thead>
<tr>
<th>Compound</th>
<th>mtDNA Synthesis (IC&lt;sub&gt;50&lt;/sub&gt;, µM)</th>
<th>Lactic Acid Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine</td>
<td>&gt;25</td>
<td>No effect ≥ 33µM</td>
</tr>
<tr>
<td>2'-C-methylyctidine</td>
<td>&gt;25</td>
<td>No effect ≥ 33µM</td>
</tr>
</tbody>
</table>

### Table 8: Preliminary PK Parameters in Rhesus Monkeys Following a Single Oral Dose of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine at 33.3 mg/kg
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>μM</td>
<td>9.6 ± 2.7</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>hours</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt;</td>
<td>μMxh</td>
<td>44.2 ± 22.2</td>
</tr>
<tr>
<td>T 1/2</td>
<td>hours</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>F%</td>
<td>21 ± 11</td>
</tr>
</tbody>
</table>

Table 9: Effect of Nucleoside Analogs on Human Bone Marrow Cells

<table>
<thead>
<tr>
<th>Compound (β-D-analog)</th>
<th>BFU-E</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td></td>
</tr>
<tr>
<td>2'-fluoro-2'-C-methylcytidine</td>
<td>98.2</td>
<td>93.9</td>
</tr>
<tr>
<td>2'-C-methylcytidine</td>
<td>20.1</td>
<td>13.2</td>
</tr>
<tr>
<td>AZT</td>
<td>0.08</td>
<td>0.95</td>
</tr>
</tbody>
</table>
WE CLAIM:

1. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

   \[
   \text{Base} \quad \begin{array}{c}
   R^1 O \\
   R^6 \\
   R^2 \\
   R^3 \\
   R^2 \quad \text{CH}_3 \\
   \end{array}
   \]

   wherein

   Base is a purine or pyrimidine base;

   X is O, S, CH\_2, Se, NH, N-alkyl, CH\_W (R, S, or racemic), C(W)\_2,
   wherein W is F, Cl, Br, or I;

   R\^1 and R\^7 are independently H, phosphate, including monophosphate,
   diphosphate, triphosphate, or a stabilized phosphate prodrug,
   H-phosphonate, including stabilized H-phosphonates, acyl,
   including optionally substituted phenyl and lower acyl, alkyl,
   including lower alkyl, O-substituted carboxyalkylamino or its
   peptide derivatives, sulfonate ester, including alkyl or
   arylalkyl sulfonyl, including methanesulfonyl and benzyl,
   wherein the phenyl group is optionally substituted, a lipid,
   including a phospholipid, an L or D-amino acid (or racemic
   mixture), a carbohydrate, a peptide, a cholesterol, or other
   pharmacologically acceptable leaving group which when
   administered \textit{in vivo} is capable of providing a compound
   wherein R\^1 is H or phosphate; R\^2 is H or phosphate; R\^1 and
   R\^2 or R\^7 can also be linked with cyclic phosphate group;

   R\^2 and R\^2 are independently H, C\_{1-4} alkyl, C\_{1-4} alkenyl, C\_{1-4} alkynyl,
   vinyl, N\_3, CN, Cl, Br, F, I, NO\_2, C(O)O(C\_{1-4} alkyl), C(O)O(C\_{1-4}
   alkyl), C(O)O(C\_{1-4} alkenyl), C(O)O(C\_{1-4}
   alkynyl), O(C\_{1-4}
   acyl), O(C\_{1-4} alkyl), O(C\_{1-4} alkenyl), S(C\_{1-4}
   acyl), S(C\_{1-4}
   alkyl), S(C\_{1-4} alkenyl), S(C\_{1-4} alkynyl), SO(C\_{1-4}
   acyl), SO(C\_{1-4}
   acyl), SO(C\_{1-4}
   acyl),
alkyl), SO(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) acyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) alkynyl), O\(_2\)S(C\(_{1-4}\) acyl), O\(_2\)S(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) alkenyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) acyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-18}\) acyl), wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N\(_3\), CN, one to three halogen (Cl, Br, F, I), NO\(_2\), CO(O)O(C\(_{1-4}\) alkyl), CO(O)O(C\(_{1-4}\) alkynyl), O(C\(_{1-4}\) acyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) alkyl), S(C\(_{1-4}\) alkynyl), S(C\(_{1-4}\) alkenyl), SO(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) acyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) alkynyl), O\(_2\)S(C\(_{1-4}\) acyl), O\(_2\)S(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) alkenyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) acyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-4}\) acyl), OR\(^7\); R\(^2\) and R\(^2\)' can be linked together to form a vinyl optionally substituted by one or two of N\(_3\), CN, Cl, Br, F, I, NO\(_2\); and

R\(^6\) is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH\(_3\), OCH\(_3\), OCH\(_2\)CH\(_3\), hydroxy methyl (CH\(_2\)OH), fluoromethyl (CH\(_2\)F), azido (N\(_3\)), CHCN, CH\(_2\)N\(_3\), CH\(_2\)NH\(_2\), CH\(_2\)NHCH\(_3\), CH\(_2\)N(CH\(_3\))\(_2\), alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof.

2. The (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of claim 1 or its pharmaceutically acceptable salt or prodrug thereof, wherein Base is selected from the group consisting of:
(a) 

wherein

Y is N or CH.

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂, CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

3. The (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of claim 1 or its pharmaceutically acceptable salt or prodrug thereof,

wherein Base is selected from the group consisting of (a) or (b):

![Diagram](image-url)
and wherein R¹ is H, R² is OH, R²' is H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

4. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

\[ \text{Base} \]

\[ \text{R}^1 \text{O} \]

\[ \text{R}^2 \text{O} \]

\[ \text{R}^3 \text{R}^4 \]

\[ \text{R}^5 \text{CH}_3 \]

wherein

Base is selected from the group consisting of

(a)

(b)

Y is N or CH;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein $R^1$ is H or phosphate; $R^2$ is H or phosphate; $R^1$ and $R^2$ or $R^7$ can also be linked with cyclic phosphate group;

$R^2$ and $R^2$ are independently H, C$_{1-4}$ alkyl, C$_{1-4}$ alkenyl, C$_{1-4}$ alkynyl, vinyl, N$_3$, CN, Cl, Br, F, I, NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ alkenyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ acyl), SO$_2$(C$_{1-4}$ alkyl), OS$_2$(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ acyl), O$_2$S(C$_{1-4}$ alkyl), O$_2$S(C$_{1-4}$ alkenyl), NH$_2$, NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-8}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ alkenyl), C(O)O(C$_{1-4}$ alkynyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ acyl), SO$_2$(C$_{1-4}$ alkyl), OS$_2$(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ acyl), O$_2$S(C$_{1-4}$ alkyl), O$_2$S(C$_{1-4}$ alkenyl), NH$_2$, NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-4}$ acyl)$_2$, OR$^7$; $R^2$ and $R^2$ can be linked together to form a vinyl optionally substituted by one or two of N$_3$, CN, Cl, Br, F, I, NO$_2$;

$R^3$, $R^4$ and $R^5$ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH$_2$, NHR', NR$_2$; lower alkyl of C$_{1-6}$, halogenated (F, Cl, Br, I) lower alkyl of C$_{1-6}$ such as CF$_3$ and CH$_2$CH$_2$F, lower alkenyl of C$_{2-6}$ such as CH=CH$_2$, halogenated (F, Cl, Br, I) lower alkenyl of C$_{2-6}$ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C$_{2-6}$
such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof.

5. The (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of claim 4 or its pharmaceutically acceptable salt or prodrug thereof, wherein

Base is

and R¹ is H, R² is OH, R²' is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.
6. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) or its pharmaceutically acceptable salt or prodrug thereof of the structure:

![Chemical Structure](image)

wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,

wherein W is F, Cl, Br, or I; and,

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group.

7. The (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of claim 6 or its pharmaceutically acceptable salt or prodrug thereof,

wherein Base is selected from the group consisting of:
Y is N or CH;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁–C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁–C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂–C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂–C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂–C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂–C₆, lower alkoxy of C₁–C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁–C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’; and,

R’ is an optionally substituted alkyl of C₁–C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂–C₆, optionally substituted lower alkenyl of C₂–C₆, or optionally substituted acyl.

8. The (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of claim 6 or its pharmaceutically acceptable salt or prodrug thereof,

wherein Base is selected from the group consisting of (a) or (b):
and wherein \( R^1 \) and \( R^2 \) are H, \( R^3 \) is H, and \( R^4 \) is NH\(_2\) or OH, and \( R^5 \) is NH\(_2\).

9. A (2'\(R\))-2'-deoxy-2'-fluoro-2'-\(C\)-methyl nucleoside (\(\beta\)-D or \(\beta\)-L) of the formula:

\[
\begin{align*}
\text{Base} & \quad \text{X} \\
\text{CH}_3 & \quad \text{R}\stackrel{O}{\longrightarrow}
\end{align*}
\]

wherein Base is

\[
\begin{align*}
\text{R}^3 & \quad \text{R}^4 \\
\text{R}^3 & \quad \text{R}^4
\end{align*}
\]

\( X \) is O, S, CH\(_2\), Se, NH, N-alkyl, CHW (\( R \), S, or racemic), C(W)\(_2\), wherein W is F, Cl, Br, or I;

\( R^1 \) and \( R^2 \) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonfyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R³ can also be linked with cyclic phosphate group;

R² and R³ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkynyl), S(C₁₋₄ acyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₈ acyl)₂, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkynyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₈ acyl)₂, OR²; R² and R³ can be linked
together to form a vinyl optionally substituted by one or two of N₂, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR², CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof.

10. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula
R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆
such as C=CH, halogenated (F, Cl, Br, I) lower alkynyl of C_2-C_6, lower alkoxy of C_1-C_6 such as CH_2OH and CH_3CH_2OH, halogenated (F, Cl, Br, I) lower alkoxy of C_1-C_6, CO_2H, CO_2R', CONH_2, CONHR', CONR'_2, CH=CHCO_2H, CH=CHCO_2R';

R' is an optionally substituted alkyl of C_1-C_12 (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C_2-C_6, optionally substituted lower alkenyl of C_2-C_6, or optionally substituted acyl;

or its pharmaceutically acceptable salt or prodrug thereof.

11. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

![Nucleoside Structure]

12. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

![Nucleoside Structure]
wherein

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalky lamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein $R^1$ or $R^7$ is independently H or phosphate; $R^1$ and $R^7$ can also be linked with cyclic phosphate group; and,

$R^6$ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH$_3$, OCH$_3$, OCH$_2$CH$_3$, hydroxy methyl (CH$_2$OH), fluoromethyl (CH$_2$F), azido (N$_3$), CHCN, CH$_2$N$_3$, CH$_2$NH$_2$, CH$_2$NHCH$_3$, CH$_2$N(CH$_3$)$_2$, alkyne (optionally substituted), or fluoro.

13. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

![Chemical Structure](attachment:formula.png)
14. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

\[
\begin{align*}
\text{O} & \\
\text{N} & \\
\text{N} & \\
\text{NH} & \\
\text{NH}_2 & \\
\text{R}^1 & \\
\text{X} & \\
\text{CH}_3 & \\
\text{HO} & \\
\text{F} & \\
\end{align*}
\]

wherein

X is O, S, CH$_2$, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)$_2$, wherein W is F, Cl, Br, or I; and

R$^1$ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein R$^1$ is H or phosphate.

15. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:
16. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxylalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound
wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R³ can also be linked with cyclic phosphate group;

R² and R² are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₂S(C₁₋₄ acyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ alkenyl)₂, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₂S(C₁₋₄ acyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ alkenyl)₂, OR⁷; R² and R² can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₃N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, a pharmaceutically acceptable carrier.
17. The composition of claim 16, wherein Base is selected from the group consisting of:

![Chemical Structures]

(a) ![Chemical Structure] (b) ![Chemical Structure]

wherein

Y is N or CH.

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₃CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂', CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

18. The composition of claim 16, wherein

Base is selected from the group consisting of (a) or (b):
and wherein $R^1$ is H, $R^2$ is OH, $R^3$ is H, $R^3$ is H, and $R^4$ is NH$_2$ or OH, and $R^5$ is NH$_2$.

19. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) of the formula:

wherein

Base is selected from the group consisting of

$Y$ is N or CH;

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R³ can also be linked with cyclic phosphate group;

R² and R³ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)(O(C₁₋₄ alkyl), C(O)(O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkynyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkenyl), S(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkenyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkenyl), O₂S(C₁₋₄ acyl), O₂S(C₁₋₄ alkynyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)(O(C₁₋₄ alkyl), C(O)(O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkenyl), S(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkenyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkenyl), O₂S(C₁₋₄ acyl), O₂S(C₁₋₄ alkynyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR; R² and R³ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;
R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR₂’, CH=CHCO₂H, CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof in a pharmaceutically acceptable carrier.

20. The composition of claim 19, wherein

25 Base is

![Chemical Structure](attachment:image.png)
and R¹ is H, R² is OH, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

21. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the structure:

![Chemical Structure](image)

wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,

wherein W is F, Cl, Br, or I; and,

R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when
administered in vivo is capable of providing a compound
wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷
can also be linked with cyclic phosphate group.
22. The composition of claim 21, wherein

Base is selected from the group consisting of:

(a) 

(b) 

Y is N or CH;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₃CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

23. The composition of claim 21, wherein

Base is selected from the group consisting of (a) or (b):
(a) \[
\begin{array}{c}
\text{Y} \\
\text{N} \\
\text{R}^4 \\
\text{R}^5
\end{array}
\]

and wherein \( R^1 \) and \( R^7 \) are H, \( R^3 \) is H, and \( R^4 \) is NH\(_2\) or OH, and \( R^5 \) is NH\(_2\).

5

24. A pharmaceutical composition comprising a \((2'R)-2'-\text{deoxy}-2'-\text{fluoro}-2'-\text{C-methyl nucleoside (}\beta-\text{D or } \beta-\text{L) of the formula:}\)

![Chemical structure](image)

wherein

Base is

![Chemical structure](image)

\( X \) is O, S, CH\(_2\), Se, NH, N-alkyl, CHW (\( R, S \), or racemic), C(W)\(_2\), wherein W is F, Cl, Br, or I;

\( R^1 \) and \( R^7 \) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R² can also be linked with cyclic phosphate group;

R² and R² are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkynyl), O(C₁₋₄ alkenyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkynyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkenyl), N(C₁₋₄ alkyl)₂, N(C₁₋₈ alkyl)₂, wherein alkyl, alkenyl, alkynyl and vinyl are optically substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkenyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkynyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkenyl), N(C₁₋₄ alkyl)₂, N(C₁₋₈ alkyl)₂, OR²; R² and R² can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR², lower alkyl of C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆ such as CF₃
and \( \text{CH}_2\text{CH}_2\text{F} \), lower alkenyl of \( \text{C}_2\text{-C}_6 \) such as \( \text{CH}=\text{CH}_2 \), halogenated (F, Cl, Br, I) lower alkenyl of \( \text{C}_2\text{-C}_6 \) such as \( \text{CH}=\text{CHCl}, \text{CH}=\text{CHBr} \) and \( \text{CH}=\text{CHI} \), lower alkynyl of \( \text{C}_2\text{-C}_6 \) such as \( \text{C}=\text{CH} \), halogenated (F, Cl, Br, I) lower alkynyl of \( \text{C}_2\text{-C}_6 \), lower alkoxy of \( \text{C}_1\text{-C}_6 \) such as \( \text{CH}_2\text{OH} \) and \( \text{CH}_2\text{CH}_2\text{OH} \), halogenated (F, Cl, Br, I) lower alkoxy of \( \text{C}_1\text{-C}_6 \), \( \text{CO}_2\text{H} \), \( \text{CO}_2\text{R} \), \( \text{CONH}_2 \), \( \text{CONHR} \), \( \text{CONR}^2 \), \( \text{CH}=\text{CHCO}_2\text{H} \), \( \text{CH}=\text{CHCO}_2\text{R} \);

\( \text{R}^1 \) is an optionally substituted alkyl of \( \text{C}_1\text{-C}_{12} \) (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of \( \text{C}_2\text{-C}_6 \), optionally substituted lower alkenyl of \( \text{C}_2\text{-C}_6 \), or optionally substituted acyl; and

\( \text{R}^6 \) is an optionally substituted alkyl (including lower alkyl), cyano (CN), \( \text{CH}_3 \), \( \text{OCH}_3 \), \( \text{OCH}_2\text{CH}_3 \), hydroxy methyl (\( \text{CH}_2\text{OH} \)), fluoromethyl (\( \text{CH}_2\text{F} \)), azido (\( \text{N}_3 \)), \( \text{CHCN} \), \( \text{CH}_2\text{N}_3 \), \( \text{CH}_2\text{NH}_2 \), \( \text{CH}_2\text{NHCH}_3 \), \( \text{CH}_2\text{N(CH}_3)_2 \), alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising a \((2'\text{R})\)-2'-deoxy-2'-fluoro-2'-\( \text{C} \)-methyl nucleoside (\( \beta\)-D or \( \beta\)-L) of the formula:

```
\[
\begin{array}{c}
\text{Base} \\
\text{CH}_3 \\
\text{R}^1 \text{O} \\
\text{R}^2 \text{O} \\
\text{F}
\end{array}
\]
```

25. Wherein

Base is
R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂, CH=CHCO₂H, CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally
substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier of the formula:

![Chemical Structure Image]

27. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L), or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the formula:

![Chemical Structure Image]

wherein

R¹ and R² are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein $R^1$ or $R^7$ is independently H or phosphate; $R^1$ and $R^7$ can also be linked with cyclic phosphate group; and,

$R^6$ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH$_3$, OCH$_3$, OCH$_2$CH$_3$, hydroxy methyl (CH$_2$OH), fluoromethyl (CH$_2$F), azido (N$_3$), CHCN, CH$_2$N$_3$, CH$_2$NH$_2$, CH$_2$NHCH$_3$, CH$_2$N(CH$_3$)$_2$, alkyne (optionally substituted), or fluoro.

28. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the formula:
29. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-(beta-D)-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof in a pharmaceutically acceptable carrier of the formula:

wherein

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,

wherein W is F, Cl, Br, or I; and

R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl,

wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound

wherein R¹ is H or phosphate;
30. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the structure:

```
\begin{align*}
    \text{O} & \\
    \text{N} & \\
    \text{N} & \\
    \text{NH} & \\
    \text{NH}_2 & \\
    \text{O} & \\
    \text{F} & \\
    \text{CH}_3 & \\
    \text{HO} & \\
    \text{HO} & \\
    \text{CH}_3 & \\
\end{align*}
```

31. Use of an an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

```
\begin{align*}
    \text{Base} & \\
    \text{R}^1 & \\
    \text{R}^2 & \\
    \text{R}^3 & \\
    \text{R}^4 & \\
    \text{R}^5 & \\
    \text{R}^6 & \\
    \text{R}^7 & \\
\end{align*}
```

wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein R\textsuperscript{1} is H or phosphate; R\textsuperscript{2} is H or phosphate; R\textsuperscript{1} and R\textsuperscript{2} or R\textsuperscript{3} can also be linked with cyclic phosphate group;

R\textsuperscript{2} and R\textsuperscript{2i} are independently H, C\textsubscript{1-4} alkyl, C\textsubscript{1-4} alkenyl, C\textsubscript{1-4} alkynyl, vinyl, N\textsubscript{3}, CN, Cl, Br, F, I, NO\textsubscript{2}, C(O)O(C\textsubscript{1-4} alkyl), C(O)O(C\textsubscript{1-4} alkyl), C(O)O(C\textsubscript{1-4} alkynyl), C(O)O(C\textsubscript{1-4} alkenyl), O(C\textsubscript{1-4} acyl), O(C\textsubscript{1-4} alkyl), O(C\textsubscript{1-4} alkenyl), S(C\textsubscript{1-4} acyl), S(C\textsubscript{1-4} alkyl), S(C\textsubscript{1-4} alkynyl), S(C\textsubscript{1-4} alkenyl), SO(C\textsubscript{1-4} acyl), SO(C\textsubscript{1-4} alkyl), SO(C\textsubscript{1-4} alkynyl), SO(C\textsubscript{1-4} alkenyl), SO\textsubscript{2}(C\textsubscript{1-4} acyl), SO\textsubscript{2}(C\textsubscript{1-4} alkyl), SO\textsubscript{2}(C\textsubscript{1-4} alkynyl), SO\textsubscript{2}(C\textsubscript{1-4} alkenyl), O\textsubscript{2}S(C\textsubscript{1-4} acyl), O\textsubscript{2}S(C\textsubscript{1-4} alkyl), O\textsubscript{2}S(C\textsubscript{1-4} alkynyl), O\textsubscript{2}S(C\textsubscript{1-4} alkenyl), NH\textsubscript{2}, NH(C\textsubscript{1-4} alkyl), NH(C\textsubscript{1-4} alkenyl), NH(C\textsubscript{1-4} alkynyl), NH(C\textsubscript{1-4} acyl), N(C\textsubscript{1-4} alkyl)\textsubscript{2}, N(C\textsubscript{1-18} acyl)\textsubscript{2}, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N\textsubscript{3}, CN, one to three halogen (Cl, Br, F, I), NO\textsubscript{2}, C(O)O(C\textsubscript{1-4} alkyl), C(O)O(C\textsubscript{1-4} alkyl), C(O)O(C\textsubscript{1-4} alkynyl), C(O)O(C\textsubscript{1-4} alkenyl), O(C\textsubscript{1-4} acyl), O(C\textsubscript{1-4} alkyl), O(C\textsubscript{1-4} alkenyl), S(C\textsubscript{1-4} acyl), S(C\textsubscript{1-4} alkyl), S(C\textsubscript{1-4} alkynyl), S(C\textsubscript{1-4} alkenyl), SO(C\textsubscript{1-4} acyl), SO(C\textsubscript{1-4} alkyl), SO(C\textsubscript{1-4} alkynyl), SO(C\textsubscript{1-4} alkenyl), SO\textsubscript{2}(C\textsubscript{1-4} acyl), SO\textsubscript{2}(C\textsubscript{1-4} alkyl), SO\textsubscript{2}(C\textsubscript{1-4} alkynyl), SO\textsubscript{2}(C\textsubscript{1-4} alkenyl), O\textsubscript{2}S(C\textsubscript{1-4} acyl), O\textsubscript{2}S(C\textsubscript{1-4} alkyl), O\textsubscript{2}S(C\textsubscript{1-4} alkynyl), O\textsubscript{2}S(C\textsubscript{1-4} alkenyl), NH\textsubscript{2}, NH(C\textsubscript{1-4} alkyl), NH(C\textsubscript{1-4} alkenyl), NH(C\textsubscript{1-4} alkynyl), NH(C\textsubscript{1-4} acyl), N(C\textsubscript{1-4} alkyl)\textsubscript{2}, N(C\textsubscript{1-4} acyl)\textsubscript{2}, OR\textsuperscript{7}; R\textsuperscript{2} and R\textsuperscript{2i} can be linked together to form a vinyl optionally substituted by one or two of N\textsubscript{3}, CN, Cl, Br, F, I, NO\textsubscript{2};

R\textsuperscript{6} is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH\textsubscript{3}, OCH\textsubscript{3}, OCH\textsubscript{2}CH\textsubscript{3}, hydroxy methyl (CH\textsubscript{2}OH), fluoromethyl (CH\textsubscript{2}F), azido (N\textsubscript{3}), CH\textsubscript{2}CN, CH\textsubscript{2}N\textsubscript{3}, CH\textsubscript{2}NH\textsubscript{2},
CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

32. The use of claim 31,

wherein Base is selected from the group consisting of:

![Diagram](a)

![Diagram](b)

Y is N or CH.

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₃CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’; and,
R' is an optionally substituted alkyl of C₁–C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkenyl of C₂–C₆, optionally substituted lower alkenyl of C₂–C₆, or optionally substituted acyl.

33. The use of claim 31, wherein

Base is selected from the group consisting of (a) or (b):

(a)  
(b)  

and wherein R¹ is H, R² is OH, R³ is H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

34. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

wherein

Base is selected from the group consisting of
and wherein \( Y \) is N or CH;

\( R^1 \) and \( R^7 \) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein \( R^1 \) is H or phosphate; \( R^2 \) is H or phosphate; \( R^1 \) and \( R^2 \) or \( R^7 \) can also be linked with cyclic phosphate group;

\( R^2 \) and \( R^3 \) are independently H, C\(_{1-4}\) alkyl, C\(_{1-4}\) alkenyl, C\(_{1-4}\) alkynyl, vinyl, N\(_3\), CN, Cl, Br, F, I, NO\(_2\), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) alkynyl), S(C\(_{1-4}\) alkynyl), S(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) acyl), SO(C\(_{1-4}\) acyl), SO(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) alkynyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) acyl), O\(_2\)S(C\(_{1-4}\) acyl), O\(_2\)S(C\(_{1-4}\) acyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-8}\) alkyl), wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N\(_3\), CN, one to three
halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkenyl), SO(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkynyl), O₂S(C₁₋₄ acyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR²; R² and R²⁻ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR⁻, SH, SR⁻, NH₂, NHR⁻, NR²⁻, lower alkyl of C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂₋₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂₋₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂₋₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂₋₆, lower alkoxy of C₁₋₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁₋₆, CO₂H, CO₂R⁻, CONH₂, CONHR⁻, CONR²⁻, CH=CHCO₂H, CH=CHCO₂R⁻;

R’ is an optionally substituted alkyl of C₁₋₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂₋₆, optionally substituted lower alkenyl of C₂₋₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

35. The use of claim 34, wherein

Base is

\[ \begin{align*}
\text{and } R^1 & \text{ is H, } R^2 \text{ is OH, } R^{2'} \text{ is H, } R^3 \text{ is H, } R^4 \text{ is } \\
\text{NH}_2 \text{ or OH, and } R^6 \text{ is H.} 
\end{align*} \]

36. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the structure:

\[ \begin{align*}
\text{wherein Base is a purine or pyrimidine base; } \\
X & \text{ is O, S, CH}_2, \text{ Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)}_2, \text{ wherein W is F, Cl, Br, or I; and, } \\
R^1 \text{ and } R^7 & \text{ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, } \\
\text{H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, }
\end{align*} \]
including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

37. The use of claim 36, wherein

Base is selected from the group consisting of:

![Chemical structures](image)

(a)  
(b)  

Y is N or CH;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'_2, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as
CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂, CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

38. The use of claim 36, wherein

Base is selected from the group consisting of (a) or (b):

![Diagram](attachment:image.png)

(a) and (b)

and wherein R¹ and R⁷ are H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

39. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:
wherein

Base is

\[
\begin{array}{c}
\text{R}^3 \\
\text{R}^4
\end{array}
\]

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,

wherein W is F, Cl, Br, or I;

R¹ and R² are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid (or racemic
mixture), a carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when
administered in vivo is capable of providing a compound
wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and
R² or R² can also be linked with cyclic phosphate group;

R² and R³ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl,
viny, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
alkyl), C(O)O(C₁₋₄ alkenyl), C(O)O(C₁₋₄ alkynyl), O(C₁₋₄
acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄
alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄
alkyl), SO(C₁₋₄ alkenyl), SO(C₁₋₄ alkynyl), SO₂(C₁₋₄ acyl),
SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkynyl), O₃S(C₁₋₄
acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄
alkyl), NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl),
N(C_{1-4} alkyl), N(C_{1-18} acyl), wherein alkyl, alkenyl, alkynyl and vinyl are optimally substituted by N_{3}, CN, one to three halogen (Cl, Br, F, I), NO_{2}, C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkenyl), C(O)O(C_{1-4} alkynyl), O(C_{1-4} acyl), O(C_{1-4} alkyl), O(C_{1-4} alkenyl), S(C_{1-4} acyl), S(C_{1-4} alkyl), S(C_{1-4} alkenyl), S(C_{1-4} alkynyl), SO(C_{1-4} acyl), SO(C_{1-4} alkyl), SO(C_{1-4} alkenyl), SO(C_{1-4} alkynyl), SO_{2}(C_{1-4} acyl), SO_{2}(C_{1-4} alkyl), SO_{2}(C_{1-4} alkenyl), SO_{2}(C_{1-4} alkynyl), O_{3}S(C_{1-4} acyl), O_{3}S(C_{1-4} alkyl), O_{3}S(C_{1-4} alkenyl), NH_{2}, NH(C_{1-4} acyl), NH(C_{1-4} alkyl), NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl), N(C_{1-4} acyl), OR^{7}, R^{2} and R^{2i} can be linked together to form a vinyl optionally substituted by one or two of N_{3}, CN, Cl, Br, F, I, NO_{2};

R^{3} and R^{4} are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH_{2}, NHR', NR'_{2}, lower alkyl of C_{1-6}, halogenated (F, Cl, Br, I) lower alkyl of C_{1-6} such as CF_{3} and CH_{2}CH_{2}F, lower alkenyl of C_{2-6} such as CH=CH_{2}, halogenated (F, Cl, Br, I) lower alkenyl of C_{2-6} such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C_{2-6} such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C_{2-6}, lower alkoxy of C_{1-6} such as CH_{2}OH and CH_{2}CH_{2}OH, halogenated (F, Cl, Br, I) lower alkoxy of C_{1-6}, CO_{2}H, CO_{2}R', CONH_{2}, CONHR', CONR'_{2}, CH=CHCO_{2}H, CH=CHCO_{2}R';

R' is an optionally substituted alkyl of C_{1-12} (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C_{2-6}, optionally substituted lower alkenyl of C_{2-6}, or optionally substituted acyl;

R^{5} is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_{3}, OCH_{3}, OCH_{2}CH_{3}, hydroxy methyl (CH_{2}OH),
fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier for the treatment or prophylaxis of hepatitis C infection in a host.

40. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

![Chemical structure](image)

wherein

Base is

![Base structure](image)

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R² is independently H or phosphate; R¹ and R² can also be linked with cyclic phosphate group;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkylnyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkylnyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂, CH=CHCO₂H, CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkylnyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host

41. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier for the treatment or prophylaxis of hepatitis C infection in a host.

42. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

\[
\text{Cl} \quad \text{N} \quad \text{N} \\
\text{R}^1 \text{O} \quad \text{O} \quad \text{CH}_3 \\
\text{R}^6 \text{R}^7 \text{O} \\
\text{F}
\]

wherein

\[
R^1 \text{ and } R^7 \text{ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when}
\]
administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and,

\[ R^6 \] is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier for the treatment or prophylaxis of hepatitis C infection in a host

43. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

![Chemical Structure](image)

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host

44. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:
wherein

\( X \) is O, S, CH\(_2\), Se, NH, N-alkyl, CHW (R, S, or racemic), C(W),
wherein W is F, Cl, Br, or I; and

\[ R^1 \]

is H, phosphate, including monophosphate, diphosphate,
triphosphate, or a stabilized phosphate prodrug, H-
phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when
administered \textit{in vivo} is capable of providing a compound
wherein \( R^1 \) is H or phosphate;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C
infection in a host.

45. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-
2'-C-methyl nucleoside (\( \beta -D \)) of the formula:
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

46. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

![Structure Diagram]

wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,

wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other
pharmacologically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R<sup>1</sup> is H or phosphate; R<sup>2</sup> is H or phosphate; R<sup>1</sup> and R<sup>2</sup> or R<sup>7</sup> can also be linked with cyclic phosphate group;

R<sup>2</sup> and R<sup>21</sup> are independently H, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkenyl, C<sub>1-4</sub> alkynyl, vinyl, N<sub>3</sub>, CN, Cl, Br, F, I, NO<sub>2</sub>, C(O)O(C<sub>1-4</sub> alkyl), C(O)O(C<sub>1-4</sub> alkyl), C(O)O(C<sub>1-4</sub> alkynyl), C(O)O(C<sub>1-4</sub> alkenyl), O(C<sub>1-4</sub> acyl), O(C<sub>1-4</sub> alkyl), O(C<sub>1-4</sub> alkenyl), S(C<sub>1-4</sub> acyl), S(C<sub>1-4</sub> alkyl), S(C<sub>1-4</sub> alkenyl), S(C<sub>1-4</sub> alkynyl), SO(C<sub>1-4</sub> acyl), SO(C<sub>1-4</sub> alkyl), SO(C<sub>1-4</sub> alkenyl), SO(C<sub>1-4</sub> alkynyl), SO(C<sub>1-4</sub> acyl), SO<sub>2</sub>(C<sub>1-4</sub> acyl), SO<sub>2</sub>(C<sub>1-4</sub> alkyl), SO<sub>2</sub>(C<sub>1-4</sub> alkenyl), SO<sub>2</sub>(C<sub>1-4</sub> alkynyl), O<sub>2</sub>S(C<sub>1-4</sub> acyl), O<sub>2</sub>S(C<sub>1-4</sub> alkyl), O<sub>2</sub>S(C<sub>1-4</sub> alkenyl), NH<sub>2</sub>, NH(C<sub>1-4</sub> acyl), NH(C<sub>1-4</sub> alkenyl), NH(C<sub>1-4</sub> alkynyl), NH(C<sub>1-4</sub> acyl), N(C<sub>1-4</sub> alkyl)<sub>2</sub>, N(C<sub>1-8</sub> acyl)<sub>2</sub>, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N<sub>3</sub>, CN, one to three halogen (Cl, Br, F, I), NO<sub>2</sub>, C(O)O(C<sub>1-4</sub> alkyl), C(O)O(C<sub>1-4</sub> alkyl), C(O)O(C<sub>1-4</sub> alkenyl), C(O)O(C<sub>1-4</sub> alkynyl), O(C<sub>1-4</sub> acyl), O(C<sub>1-4</sub> alkyl), O(C<sub>1-4</sub> alkenyl), S(C<sub>1-4</sub> acyl), S(C<sub>1-4</sub> alkyl), S(C<sub>1-4</sub> alkenyl), S(C<sub>1-4</sub> alkynyl), SO(C<sub>1-4</sub> acyl), SO(C<sub>1-4</sub> alkyl), SO(C<sub>1-4</sub> alkenyl), SO(C<sub>1-4</sub> alkynyl), SO<sub>2</sub>(C<sub>1-4</sub> acyl), SO<sub>2</sub>(C<sub>1-4</sub> alkyl), SO<sub>2</sub>(C<sub>1-4</sub> alkenyl), SO<sub>2</sub>(C<sub>1-4</sub> alkynyl), O<sub>2</sub>S(C<sub>1-4</sub> acyl), O<sub>2</sub>S(C<sub>1-4</sub> alkyl), O<sub>2</sub>S(C<sub>1-4</sub> alkenyl), NH<sub>2</sub>, NH(C<sub>1-4</sub> acyl), NH(C<sub>1-4</sub> alkenyl), NH(C<sub>1-4</sub> alkynyl), NH(C<sub>1-4</sub> acyl), N(C<sub>1-4</sub> alkyl)<sub>2</sub>, N(C<sub>1-4</sub> acyl)<sub>2</sub>, OR<sup>7</sup>; R<sup>2</sup> and R<sup>21</sup> can be linked together to form a vinyl optionally substituted by one or two of N<sub>3</sub>, CN, Cl, Br, F, I, NO<sub>2</sub>;

R<sup>6</sup> is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH<sub>3</sub>, OCH<sub>3</sub>, OCH<sub>2</sub>CH<sub>3</sub>, hydroxy methyl (CH<sub>2</sub>OH), fluoromethyl (CH<sub>2</sub>F), azido (N<sub>3</sub>), CHCN, CH<sub>2</sub>N<sub>3</sub>, CH<sub>2</sub>NH<sub>2</sub>, CH<sub>2</sub>NHCH<sub>3</sub>, CH<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub>, alkyne (optionally substituted), or fluoro;
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

47. The method of claim 46,

wherein Base is selected from the group consisting of:

(a) 

(b) 

\( Y = \text{N or CH.} \)

\( R^3, R^4 \text{ and } R^5 \) are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

wherein R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.
48. The method of claim 46, wherein

Base is selected from the group consisting of (a) or (b):

(a) \[ \begin{array}{c}
R^4 \\
R^5 \\
Y \\
N \\
N \\
R^4 \\
\end{array} \]

(b) \[ \begin{array}{c}
R^3 \\
R^4 \\
\end{array} \]

and wherein \( R^1 \) is H, \( R^2 \) is OH, \( R^3 \) is H, \( R^3 \) is H,
and \( R^4 \) is NH\(_2\) or OH, and \( R^5 \) is NH\(_2\).

49. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a \( (2'R)-2'-\) deoxy-2'-fluoro-2'-C-methyl nucleoside (\( \beta\)-D or \( \beta\)-L) of the formula:

\[ \begin{array}{c}
R^1 \end{array} \]
\[ \begin{array}{c}
O \\
R^5 \\
R^6 \\
R^2 \\
\end{array} \]
\[ \begin{array}{c}
Base \\
\end{array} \]
\[ \begin{array}{c}
CH_3 \\
R^2 \\
F \\
\end{array} \]

wherein

Base is selected from the group consisting of

(a) \[ \begin{array}{c}
R^4 \\
R^5 \\
Y \\
N \\
N \\
R^5 \\
\end{array} \]

(b) \[ \begin{array}{c}
R^3 \\
R^4 \\
\end{array} \]
and wherein Y is N or CH;

R₁ and R² are independently H, phosphate, including monophosphate, diphasphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R² can also be linked with cyclic phosphate group;

R² and R²⁽²⁾ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ acyl), C(O)O(C₁₋₄ alkynyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), O(C₁₋₄ alkynyl), S(C₁₋₄ acyl), S(C₁₋₄ alkenyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkenyl), SO(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkynyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkynyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ alkyl)₂, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkenyl), SO(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkynyl), O₂S(C₁₋₄ alkyl)
4 acyl), O₃S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ acyl)₂, N(C₁₋₄ alkyl)₂, OR⁷; R² and R⁴ can be linked together to form a vinyl optionally substituted by one or two of N₂, CN, Cl, Br, F, I, NO₂;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₃CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

50. The method of claim 49, wherein
Base is

and $R^1$ is H, $R^2$ is OH, $R^{21}$ is H, $R^3$ is H, $R^4$ is NH$_2$ or OH, and $R^6$ is H.

51. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) or its pharmaceutically acceptable salt or prodrug thereof of the structure:

wherein Base is a purine or pyrimidine base;

$X$ is O, S, CH$_2$, Se, NH, N-alkyl, CHW ($R$, $S$, or racemic), C(W)$_2$, wherein W is F, Cl, Br, or I; and,

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,
pharmacologically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R\(^1\) is H or phosphate; R\(^2\) is H or phosphate; R\(^1\) and R\(^2\) or R\(^7\) can also be linked with cyclic phosphate group;

R\(^2\) and R\(^{2*}\) are independently H, C\(_{1-4}\) alkyl, C\(_{1-4}\) alkenyl, C\(_{1-4}\) alkynyl, vinyl, N\(_3\), CN, Cl, Br, F, I, NO\(_2\), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkynyl), C(O)O(C\(_{1-4}\) alkenyl), O(C\(_{1-4}\) acyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) alkyl), S(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) acyl), SO(C\(_{1-4}\) alkyl), SO(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) acyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkenyl), O\(_2\)S(C\(_{1-4}\) acyl), O\(_2\)S(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) alkenyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) acyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-4}\) alkenyl), N(C\(_{1-4}\) alkynyl), N(C\(_{1-4}\) acyl), and vinyl are optionally substituted by N\(_3\), CN, one to three halogen (Cl, Br, F, I), NO\(_2\), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) acyl), C(O)O(C\(_{1-4}\) alkynyl), C(O)O(C\(_{1-4}\) alkenyl), O(C\(_{1-4}\) acyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) alkyl), S(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) acyl), SO(C\(_{1-4}\) alkyl), SO(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) acyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkenyl), O\(_2\)S(C\(_{1-4}\) acyl), O\(_2\)S(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) alkenyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) acyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-4}\) alkenyl), N(C\(_{1-4}\) alkynyl), OR\(^7\); R\(^2\) and R\(^{2*}\) can be linked together to form a vinyl optionally substituted by one or two of N\(_3\), CN, Cl, Br, F, I, NO\(_2\);

R\(^6\) is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH\(_3\), OCH\(_3\), OCH\(_2\)CH\(_3\), hydroxy methyl (CH\(_2\)OH), fluoromethyl (CH\(_2\)F), azido (N\(_3\)), CHCN, CH\(_2\)N\(_3\), CH\(_2\)NH\(_2\), CH\(_2\)NHCH\(_3\), CH\(_2\)N(CH\(_3\))\(_2\), alkyne (optionally substituted), or fluoro;
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

47. The method of claim 46,

wherein Base is selected from the group consisting of:

(a)

(b)

Y is N or CH.

R³, R⁴, and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’; and,

wherein R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

143
48. The method of claim 46, wherein
Base is selected from the group consisting of (a) or (b):

(a) ![Formula Image]
(b) ![Formula Image]

and wherein $R^1$ is H, $R^{2}$ is OH, $R^{2'}$ is H, $R^3$ is H, and $R^4$ is NH$_2$ or OH, and $R^5$ is NH$_2$.

49. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'C-methyl nucleoside ($\beta$-D or $\beta$-L) of the formula:

![Formula Image]

wherein
Base is selected from the group consisting of

(a) ![Formula Image]
(b) ![Formula Image]
and wherein Y is N or CH;

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphasate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein $R^1$ is H or phosphate; $R^2$ is H or phosphate; $R^1$ and $R^2$ or $R^7$ can also be linked with cyclic phosphate group;

$R^2$ and $R^4$ are independently H, C$_{1-4}$ alkyl, C$_{1-4}$ alkenyl, C$_{1-4}$ alkynyl, vinyl, N$_3$, CN, Cl, Br, F, I, NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ alkenyl), C(O)O(C$_{1-4}$ alkynyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl), O$_2$S(C$_{1-4}$ alkyl), O$_2$S(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ alkynyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-18}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl), O$_2$S(C$_{1-4}$ acyl), O$_2$S(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ alkynyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-18}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl), O$_2$S(C$_{1-4}$ acyl), O$_2$S(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ alkynyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-18}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl), O$_2$S(C$_{1-4}$ acyl), O$_2$S(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ alkynyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-18}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl), O$_2$S(C$_{1-4}$ acyl), O$_2$S(C$_{1-4}$ alkenyl), O$_2$S(C$_{1-4}$ alkynyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-18}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkeny
4 acyl), O$_2$S(C$_{1-4}$ alkyl), O$_3$S(C$_{1-4}$ alkenyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-4}$ acyl)$_2$, OR$^2$; R$^2$ and R$^{2i}$ can be linked together to form a vinyl optionally substituted by one or two of N$_3$, CN, Cl, Br, F, I, NO$_2$;

R$^3$, R$^4$ and R$^5$ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH$_2$, NHR', NR'$^2$, lower alkyl of C$_{1-6}$, halogenated (F, Cl, Br, I) lower alkyl of C$_{1-6}$ such as CF$_3$ and CH$_2$CH$_2$F, lower alkenyl of C$_{2-6}$ such as CH=CH$_2$, halogenated (F, Cl, Br, I) lower alkenyl of C$_{2-6}$ such as CH=CHCH$_2$Cl, CH=CHBr and CH=CHI, lower alkynyl of C$_{2-6}$ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C$_{2-6}$, lower alkoxy of C$_{1-6}$ such as CH$_2$OH and CH$_2$CH$_2$OH, halogenated (F, Cl, Br, I) lower alkoxy of C$_{1-6}$, CO$_2$H, CO$_2$R', CONH$_2$, CONHR', CONR'$^2$, CH=CHCO$_2$H, CH=CHCO$_2$R';

R' is an optionally substituted alkyl of C$_{1-12}$ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkenyl of C$_{2-6}$, optionally substituted lower alkenyl of C$_{2-6}$, or optionally substituted acyl;

R$^6$ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH$_3$, OCH$_3$, OCH$_2$CH$_3$, hydroxy methyl (CH$_2$OH), fluoromethyl (CH$_2$F), azido (N$_3$), CHCN, CH$_2$N$_3$, CH$_2$NH$_2$, CH$_2$NHCH$_3$, CH$_2$N(CH$_3$)$_2$, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

50. The method of claim 49, wherein
Base is

and $R^1$ is H, $R^2$ is OH, $R^{2\prime}$ is H, $R^3$ is H, $R^4$ is

$\text{NH}_2$ or OH, and $R^6$ is H.

51. A method for the treatment or prophylaxis of a rhinovirus infection
comprising administering to a host an antivirally effective amount of a (2'R)-2'-
deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) or its pharmaceutically
acceptable salt or prodrug thereof of the structure:

wherein Base is a purine or pyrimidine base;

$X$ is O, S, CH$_2$, Se, NH, N-alkyl, CHW ($R$, $S$, or racemic), C(W)$_2$,
wherein W is F, Cl, Br, or I; and,

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein \( R^1 \) or \( R^2 \) is independently H or phosphate; \( R^1 \) and \( R^2 \) can also be linked with cyclic phosphate group;

optionally in a pharmaceutically acceptable carrier.

52. The method of claim 51, wherein

Base is selected from the group consisting of:

(a)

\[
\begin{align*}
Y \quad R^4 \\
R^5 \\
\end{align*}
\]

(b)

\[
\begin{align*}
R^3 \\
R^4 \\
\end{align*}
\]

\( Y \) is N or CH;

\( R^3, R^4 \) and \( R^5 \) are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₃CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

53. The method of claim 51, wherein
Base is selected from the group consisting of (a) or (b):

(a) \[ \text{structure} \]
(b) \[ \text{structure} \]

and wherein R¹ and R⁷ are H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

54. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'C-methyl nucleoside (β-D or β-L) of the formula:

[structure]

wherein
Base is
X is O, S, CH$_2$, Se, NH, N-alkyl, CHW ($R$, $S$, or racemic), C(W)$_2$, wherein W is F, Cl, Br, or I;

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein $R^1$ is H or phosphate; $R^7$ is H or phosphate; $R^1$ and $R^2$ or $R^7$ can also be linked with cyclic phosphate group;

$R^2$ and $R^4$ are independently H, C$_{1-4}$ alkyl, C$_{1-4}$ alkenyl, C$_{1-4}$ alkynyl, vinyl, N$_3$, CN, Cl, Br, F, I, NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ alkynyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), O(C$_{1-4}$ alkynyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl), O$_3$S(C$_{1-4}$ alkyl), O$_3$S(C$_{1-4}$ alkenyl), O$_3$S(C$_{1-4}$ alkynyl), NH$_2$, NH(C$_{1-4}$ alkyl), NH(C$_{1-4}$ alkenyl), NH(C$_{1-4}$ alkynyl), NH(C$_{1-4}$ acyl), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-18}$ acyl)$_2$, wherein alkyl, alkenyl, alkynyl, and vinyl are optionally substituted by N$_3$, CN, one to three halogen (Cl, Br, F, I), NO$_2$, C(O)O(C$_{1-4}$ alkyl), C(O)O(C$_{1-4}$ acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkenyl), S(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ acyl), SO$_2$(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ alkynyl).
SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₅S(C₁₋₄ acyl), O₅S(C₁₋₄ alkyl), O₅S(C₁₋₄ alkenyl), NH₃, NH(NH(C₁₋₄ alkyl), NH(NH(C₁₋₄ alkynyl), NH(NH(C₁₋₄ alkenyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁻, R² and R⁴ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkenyl of C₂-C₆ such as C=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkenyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.
A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

\[
\begin{array}{c}
\text{Base} \\
\text{R}^1O \\
\text{R}^7O \\
\text{CH}_3 \\
\text{F} \\
\end{array}
\]

wherein

Base is

\[
\begin{array}{c}
\text{R}^3 \\
\text{R}^4 \\
\text{R}^5 \\
\text{R}^6 \\
\text{R}^7 \\
\text{O} \\
\text{N} \\
\text{C} \\
\end{array}
\]

\(\text{R}^1\) and \(\text{R}^7\) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein \(\text{R}^1\) or \(\text{R}^7\) is independently H or phosphate; \(\text{R}^1\) and \(\text{R}^7\) can also be linked with cyclic phosphate group;

\(\text{R}^3\) and \(\text{R}^4\) are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆,
halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃
and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂,
halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as
CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆
such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-
C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH,
halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H,
CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H,
CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the
alkyl is an amino acid residue), cycloalkyl, optionally
substituted alkynyl of C₂-C₆, optionally substituted lower
alkenyl of C₂-C₆, or optionally substituted acyl;
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier.

56. A method for the treatment or prophylaxis of a rhinovirus infection
comprising administering to a host an antivirally effective amount of a (2'R)-2'-
deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt
or prodrug thereof of the formula:

![Chemical Structure](image)

optionally in a pharmaceutically acceptable carrier.

57. A method for the treatment or prophylaxis of a rhinovirus infection
comprising administering to a host an antivirally effective amount of a (2'R)-2'-
deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

\[
\begin{align*}
\text{Cl} \\
\text{R}^1 \backslash \text{O} \\
\text{R}^5 \\
\text{R}^6 \backslash \text{O} \\
\text{R}^7 \\
\text{CH}_3
\end{align*}
\]

wherein

\( R^1 \) and \( R^7 \) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein \( R^1 \) or \( R^7 \) is independently H or phosphate; \( R^1 \) and \( R^7 \) can also be linked with cyclic phosphate group; and,

\( R^6 \) is an optionally substituted alkyl (including lower alkyl), cyano (CN), \( \text{CH}_3 \), \( \text{OCH}_3 \), \( \text{OCH}_2\text{CH}_3 \), hydroxy methyl (CH\(_2\)OH), fluoromethyl (CH\(_2\)F), azido (N\(_3\)), CHCN, CH\(_2\)N\(_3\), CH\(_2\)NH\(_2\), CH\(_2\)NHCH\(_3\), CH\(_2\)N(CH\(_3\))\(_2\), alkyne (optionally substituted), or fluoro; and

optionally in a pharmaceutically acceptable carrier.
58. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

![Chemical Structure](image)

optionally in a pharmaceutically acceptable carrier.

59. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

![Chemical Structure](image)

wherein

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)_2,

wherein W is F, Cl, Br, or I; and

R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate;

optionally in a pharmaceutically acceptable carrier.

60. A method for the treatment or prophylaxis of a a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-β) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

\[
\begin{align*}
\text{HO-} & \quad \text{O} \\
\text{HO-} & \quad \text{CH}_3 \\
\text{F} & \\
\text{NH}_2
\end{align*}
\]

optionally in a pharmaceutically acceptable carrier.

61. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-β or β-L) of the formula:

\[
\begin{align*}
\text{R}^1 & \quad \text{O} \\
\text{R}^2 & \\
\text{X} & \\
\text{R}^6 & \\
\text{F} & \\
\text{CH}_3
\end{align*}
\]
wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid (or racemic
mixture), a carbohydrate, a peptide, a cholesterol, or other
pharmacologically acceptable leaving group which when
administered in vivo is capable of providing a compound
wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and
R² or R⁷ can also be linked with cyclic phosphate group;

R² and R⁴ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl,
viny, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkyl), O(C₁₋₄
acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄
alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄
alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkenyl), SO₂(C₁₋₄ alkynyl), O₂S(C₁₋₄
acyl), O₂S(C₁₋₄ alkyl), O₂S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄
alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),
N(C₁₋₄ alkyl)₂, N(C₁₋₈ acyl)₂, wherein alkyl, alkenyl, alkynyl
and vinyl are optionally substituted by N₃, CN, one to three
halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄
acyl), O(C$_{1-4}$ alkyl), O(C$_{1-4}$ alkenyl), S(C$_{1-4}$ acyl), S(C$_{1-4}$ alkyl), S(C$_{1-4}$ alkynyl), S(C$_{1-4}$ alkenyl), SO(C$_{1-4}$ acyl), SO(C$_{1-4}$ alkyl), SO(C$_{1-4}$ alkynyl), SO(C$_{1-4}$ alkenyl), SO$_2$(C$_{1-4}$ acyl), SO$_2$(C$_{1-4}$ alkyl), SO$_2$(C$_{1-4}$ alkynyl), SO$_2$(C$_{1-4}$ alkenyl), O$_3$S(C$_{1-4}$ acyl), O$_3$S(C$_{1-4}$ alkyl), O$_3$S(C$_{1-4}$ alkenyl), NH$_2$, NH(NH$_3$ alkyl), NH(NH$_3$ alkynyl), NH(NH$_3$ alkenyl), NH(NH$_3$ alkene), N(C$_{1-4}$ alkyl)$_2$, N(C$_{1-4}$ acyl)$_2$, OR$^7$; R$^2$ and R$^3$ can be linked together to form a vinyl optionally substituted by one or two of N$_2$, CN, Cl, Br, F, I, NO$_2$;

R$^6$ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH$_3$, OCH$_3$, OCH$_2$CH$_3$, hydroxyl methyl (CH$_2$OH), fluoromethyl (CH$_2$F), azido (N$_3$), CHCN, CH$_2$N$_3$, CH$_2$NH$_2$, CH$_2$NHCH$_3$, CH$_2$N(CH$_3$)$_2$, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

62. The use of claim 61,

wherein Base is selected from the group consisting of:

![Diagram](a) ![Diagram](b)

Y is N or CH.
$R^3$, $R^4$ and $R^5$ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH$_2$, NHR', NR'=, lower alkyl of C$_1$-C$_6$, halogenated (F, Cl, Br, I) lower alkyl of C$_1$-C$_6$ such as CF$_3$ and CH$_2$CH$_2$F, lower alkenyl of C$_2$-C$_6$ such as CH=CH$_2$, halogenated (F, Cl, Br, I) lower alkenyl of C$_2$-C$_6$ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C$_2$-C$_6$ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C$_2$-C$_6$, lower alkoxy of C$_1$-C$_6$ such as CH$_2$OH and CH$_2$CH$_2$OH, halogenated (F, Cl, Br, I) lower alkoxy of C$_1$-C$_6$, CO$_2$H, CO$_2$R', CONH$_2$, CONHR', CONR'=, CH=CHCO$_2$H, CH=CHCO$_2$R'; and,

$R'$ is an optionally substituted alkyl of C$_1$-C$_{12}$ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C$_2$-C$_6$, optionally substituted lower alkenyl of C$_2$-C$_6$, or optionally substituted acyl.

63. The use of claim 61, wherein

Base is selected from the group consisting of (a) or (b):

\[
\begin{align*}
&\begin{array}{c}
\text{(a)} \\
\text{(b)}
\end{array}
\end{align*}
\]

and wherein $R^1$ is H, $R^2$ is OH, $R^{2''}$ is H, $R^3$ is H, and $R^4$ is NH$_2$ or OH, and $R^5$ is NH$_2$.

64. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) of the formula:
wherein

Base is selected from the group consisting of

(a) \[
\begin{array}{c}
\text{Y} \\
\text{N} \\
\text{N} \\
\text{R}^5
\end{array}
\]

(b) \[
\begin{array}{c}
\text{R}^4 \\
\text{R}^3
\end{array}
\]

Y is N or CH;

R\text{I} and R\text{II} are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R\text{I} is H or phosphate; R\text{II} is H or phosphate; R\text{I} and R\text{II} or R\text{III} can also be linked with cyclic phosphate group;

R\text{II} and R\text{III} are independently H, C\text{I} to C\text{IV} alkyl, C\text{I} to C\text{IV} alkenyl, C\text{I} to C\text{IV} alkynyl, vinyl, N\text{III}, CN, Cl, Br, F, I, NO\text{II}, C(O)O(C\text{I} to C\text{IV} alkyl), C(O)O(C\text{I} to C\text{IV} alkyl)
4 alkyl), C(O)O(C_{1-4} alkynyl), C(O)O(C_{1-4} alkenyl), O(C_{1-4} acyl), O(C_{1-4} alkyl), O(C_{1-4} alkenyl), S(C_{1-4} acyl), S(C_{1-4} alkyl), S(C_{1-4} alkynyl), S(C_{1-4} alkenyl), SO(C_{1-4} acyl), SO(C_{1-4} alkyl), SO(C_{1-4} alkynyl), SO(C_{1-4} alkenyl), SO_{2}(C_{1-4} acyl), SO_{2}(C_{1-4} alkyl), SO_{2}(C_{1-4} alkenyl), O_{2}S(C_{1-4} acyl), O_{2}S(C_{1-4} alkyl), O_{2}S(C_{1-4} alkenyl), NH_{2}, NH(C_{1-4} alkyl), NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl)_{2}, N(C_{1-8} acyl)_{2}, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N_{3}, CN, one to three halogen (Cl, Br, F, I), NO_{2}, C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} acyl), O(C_{1-4} alkyl), O(C_{1-4} alkenyl), O(C_{1-4} alkynyl), O(C_{1-4} acyl), S(C_{1-4} alkyl), S(C_{1-4} alkenyl), S(C_{1-4} alkynyl), S(C_{1-4} acyl), SO(C_{1-4} alkyl), SO(C_{1-4} alkenyl), SO(C_{1-4} alkynyl), SO(C_{1-4} acyl), SO_{2}(C_{1-4} alkyl), SO_{2}(C_{1-4} alkenyl), SO_{2}(C_{1-4} alkynyl), O_{2}S(C_{1-4} acyl), O_{2}S(C_{1-4} alkyl), O_{2}S(C_{1-4} alkenyl), NH_{2}, NH(C_{1-4} alkyl), NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl)_{2}, N(C_{1-4} acyl)_{2}, OR^{7}; R^{2} and R^{4} can be linked together to form a vinyl optionally substituted by one or two of N_{3}, CN, Cl, Br, F, I, NO_{2};

R^{3}, R^{4} and R^{5} are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH_{2}, NR', NR^{2}, lower alkyl of C_{1-6}, halogenated (F, Cl, Br, I) lower alkyl of C_{1-6} such as CHF, CH_{2}F, lower alkyl of C_{2-6} such as CH=CH_{2}, halogenated (F, Cl, Br, I) lower alkyl of C_{2-6} such as CH=CHCl, CH=CHBr and CH=CHI, lower alkyl of C_{2-6} such as C≡CH, halogenated (F, Cl, Br, I) lower alkyl of C_{2-6}, lower alkoxy of C_{1-6} such as CH_{2}OH and CH_{2}CH_{2}OH, halogenated (F, Cl, Br, I) lower alkoxy of C_{1-6}, CO_{2}H, CO_{2}R', CONH_{2}, CONHR', CONR'_{2}, CH=CHCO_{2}H, CH=CHCO_{2}R';
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

65. The use of claim 64, wherein

Base is

and R¹ is H, R² is OH, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

66. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the structure:
wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and,

R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
pharmacologically acceptable leaving group which when
administered in vivo is capable of providing a compound
wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷
can also be linked with cyclic phosphate group and

or its pharmacologically acceptable salt or prodrug thereof, optionally in a
pharmacologically acceptable carrier, for the treatment or prophylaxis of a yellow
fever virus infection in a host

67. The use of claim 66, wherein

Base is selected from the group consisting of:
Y is N or CH;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C=CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’; and,

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

The use of claim 66, wherein

Base is selected from the group consisting of (a) or (b):
and wherein \( R^1 \) and \( R^7 \) are H, \( R^3 \) is H, and \( R^4 \) is
NH\(_2\) or OH, and \( R^5 \) is NH\(_2\).

69. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-
2'-C-methyl nucleoside (\( \beta-D \) or \( \beta-L \)) of the formula:

\[
\begin{align*}
\text{Base} & \quad X = O, S, \text{CH}_2, \text{Se}, \text{NH}, \text{N-alkyl}, \text{CHW} (R, S, \text{or racemic}), \text{C(W)}_2, \\
\text{wherein } W & \text{ is F, Cl, Br, or I;}
\end{align*}
\]

\( X \) is O, S, CH\(_2\), Se, NH, N-alkyl, CHW (\( R, S, \text{or racemic} \)), C(W)\(_2\),
wherein \( W \) is F, Cl, Br, or I;

\( R^1 \) and \( R^7 \) are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R² can also be linked with cyclic phosphate group;

R² and R⁴ are independently H, C₁-₄ alkyl, C₁-₄ alkenyl, C₁-₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkynyl), C(O)O(C₁-₄ alkenyl), O(C₁-₄ acyl), O(C₁-₄ alkyl), O(C₁-₄ alkenyl), S(C₁-₄ acyl), S(C₁-₄ alkyl), S(C₁-₄ alkynyl), S(C₁-₄ alkenyl), SO(C₁-₄ acyl), SO(C₁-₄ alkyl), SO(C₁-₄ alkynyl), SO(C₁-₄ alkenyl), SO₂(C₁-₄ acyl), SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkynyl), SO₂(C₁-₄ alkenyl), O₂S(C₁-₄ acyl), O₂S(C₁-₄ alkyl), O₂S(C₁-₄ alkynyl), O₂S(C₁-₄ alkenyl), NH₂, NH(C₁-₄ alkyl), NH(C₁-₄ alkenyl), NH(C₁-₄ alkynyl), NH(C₁-₄ acyl), N(C₁-₄ alkyl)₂, N(C₁-₄ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optinally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkynyl), C(O)O(C₁-₄ alkenyl), O(C₁-₄ acyl), O(C₁-₄ alkyl), O(C₁-₄ alkenyl), S(C₁-₄ acyl), S(C₁-₄ alkyl), S(C₁-₄ alkynyl), S(C₁-₄ alkenyl), SO(C₁-₄ acyl), SO(C₁-₄ alkyl), SO(C₁-₄ alkynyl), SO(C₁-₄ alkenyl), SO₂(C₁-₄ acyl), SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkynyl), SO₂(C₁-₄ alkenyl), O₂S(C₁-₄ acyl), O₂S(C₁-₄ alkyl), O₂S(C₁-₄ alkynyl), O₂S(C₁-₄ alkenyl), NH₂, NH(C₁-₄ alkyl), NH(C₁-₄ alkenyl), NH(C₁-₄ alkynyl), NH(C₁-₄ acyl), N(C₁-₄ alkyl)₂, N(C₁-₄ acyl)₂, OR⁷; R² and R⁴ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆,
halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂', CH=CHCO₂H, CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R₆ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host

70. Use of antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:
R^1 and R^7 are independently H, phosphate, including monophosphate, diprophosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group;

R^3 and R^4 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NH₂', NR₂', lower alkyl of C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂₋₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂₋₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkenyl of C₂₋₆ such as C=CH, halogenated (F, Cl, Br, I) lower alkenyl of C₂₋₆, lower alkoxy of C₁₋₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁₋₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂', CH=CHCO₂H, CH=CHCO₂R';
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆₅, optionally substituted lower alkenyl of C₂-C₆₅, or optionally substituted acyl.

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

71. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

![Chemical Structure](image1)

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

72. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

![Chemical Structure](image2)
wherein

\( R^1 \) and \( R^2 \) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein \( R^1 \) or \( R^2 \) is independently H or phosphate; \( R^1 \) and \( R^2 \) can also be linked with cyclic phosphate group; and,

\( R^6 \) is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH\(_3\), OCH\(_3\), OCH\(_2\)CH\(_3\), hydroxy methyl (CH\(_2\)OH), fluoromethyl (CH\(_2\)F), azido (N\(_3\)), CH\(_2\)CN, CH\(_2\)N\(_3\), CH\(_2\)NH\(_2\), CH\(_2\)NHCH\(_3\), CH\(_2\)N(CH\(_3\))\(_2\), alkyne (optionally substituted), or fluoro, and

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

73. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (\( \beta\)-D) of the formula:
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

74. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

wherein

\[ X = O, S, CH_2, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)_2, \]

wherein W is F, Cl, Br, or I; and

\[ R^1 \text{ and } R^2 \text{ are independently } H, \text{ phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, } \]
\[ H\text{-phosphonate, including stabilized } H\text{-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonyl ester, including alkyl or aryalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an } L \text{ or } D\text{-amino acid, a carbohydrate, a peptide, a cholesterol, or other } \]
pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein $R^1$ is H or phosphate;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

75. Use of an antivirally effective amount of a $(2'R)$-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D) of the formula:

\[
\begin{array}{c}
\text{O} \\
\text{HO} \quad \text{O} \\
\text{HO} \\
\text{F} \\
\text{CH}_3
\end{array}
\]

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

76. Use an antivirally effective amount of a $(2'R)$-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) of the formula:

\[
\begin{array}{c}
\text{R}_1^0 \\
\text{R}_6^0 \\
\text{R}_2^0 \\
\text{F} \\
\text{CH}_3
\end{array}
\]

wherein

Base is a purine or pyrimidine base;
X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid (or racemic
mixture), a carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when
administered in vivo is capable of providing a compound
wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and
R² or R⁷ can also be linked with cyclic phosphate group;

R² and R³ are independently H, C₁-₄ alkyl, C₁-₄ alkenyl, C₁-₄ alkynyl,
vinyln, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄
alkyl), C(O)O(C₁-₄ alkynyl), C(O)O(C₁-₄ alkenyl), O(C₁-₄
acyl), O(C₁-₄ alkyl), O(C₁-₄ alkenyl), S(C₁-₄ acyl), S(C₁-₄
alkyl), S(C₁-₄ alkynyl), S(C₁-₄ alkenyl), SO(C₁-₄ acyl), SO(C₁-₄
alkyl), SO(C₁-₄ alkynyl), SO(C₁-₄ alkenyl), SO₂(C₁-₄ acyl),
SO₂(C₁-₄ alkyl), SO₂(C₁-₄ alkenyl), SO₂(C₁-₄ alkynyl), O₃S(C₁-₄
acyl), O₃S(C₁-₄ alkyl), O₃S(C₁-₄ alkenyl), NH₂, NH(C₁-₄
alkyl), NH(C₁-₄ alkenyl), NH(C₁-₄ alkynyl), NH(C₁-₄ acyl),
N(C₁-₄ alkyl)₂, N(C₁-₁₈ acyl)₂, wherein alkyl, alkenyl, alkynyl
and vinyl are optionally substituted by N₃, CN, one to three
halogen (Cl, Br, F, I), NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄
alkyl), C(O)O(C₁-₄ alkynyl), C(O)O(C₁-₄ alkenyl), O(C₁-₄
acyl), O(C₁-₄ alkyl), O(C₁-₄ alkenyl), S(C₁-₄ acyl), S(C₁-₄
alkyl), S(C₁-₄ alkynyl), S(C₁-₄ alkenyl), SO(C₁-₄ acyl), SO(C₁-₄
alkyl), SO(C₁-₄ alkynyl), SO(C₁-₄ alkenyl), SO₂(C₁-₄ acyl),
SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkynyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R²ⁱ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

The use of claim 76,

wherein Base is selected from the group consisting of:

(a)

R⁴

(b)

Y is N or CH.

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR², lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂,
halogenated (F, Cl, Br, I) lower alkenyl of C<sub>2</sub>-C<sub>6</sub> such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C<sub>2</sub>-C<sub>6</sub> such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C<sub>2</sub>-C<sub>6</sub>, lower alkoxy of C<sub>1</sub>-C<sub>6</sub> such as CH<sub>2</sub>OH and CH<sub>2</sub>CH<sub>2</sub>OH, halogenated (F, Cl, Br, I) lower alkoxy of C<sub>1</sub>-C<sub>6</sub>, CO<sub>2</sub>H, CO<sub>2</sub>R', CONH<sub>2</sub>, CONHR', CONR'<sub>2</sub>, CH=CHCO<sub>2</sub>H, CH=CHCO<sub>2</sub>R'; and,

R' is an optionally substituted alkyl of C<sub>1</sub>-C<sub>12</sub> (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C<sub>2</sub>-C<sub>6</sub>, optionally substituted lower alkenyl of C<sub>2</sub>-C<sub>6</sub>, or optionally substituted acyl.

78. The use of claim 76, wherein

Base is selected from the group consisting of (a) or (b):

(a) ![Chemical Structure](https://example.com/structure_a.png)

(b) ![Chemical Structure](https://example.com/structure_b.png)

and wherein R<sup>1</sup> is H, R<sup>2</sup> is OH, R<sup>2'</sup> is H, R<sup>3</sup> is H, and R<sup>4</sup> is NH<sub>2</sub> or OH, and R<sup>5</sup> is NH<sub>2</sub>.

79. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:
wherein

Base is selected from the group consisting of

(a) 

(b) 

Y is N or CH;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyle and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R²' are independently H, C₁-₄ alkyl, C₁-₄ alkenyl, C₁-₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkynyl), C(O)O(C₁-₄ alkenyl), O(C₁-₄
acyl), O(C_{1-4} alkyl), O(C_{1-4} alkenyl), S(C_{1-4} acyl), S(C_{1-4} alkyl), S(C_{1-4} alkenyl), S(C_{1-4} alkynyl), SO(C_{1-4} acyl), SO(C_{1-4} alkyl), SO(C_{1-4} alkenyl), SO(C_{1-4} alkynyl), SO_{2}(C_{1-4} acyl), SO_{2}(C_{1-4} alkyl), SO_{2}(C_{1-4} alkenyl), SO_{2}(C_{1-4} alkynyl), O_{2}S(C_{1-4} acyl), O_{2}S(C_{1-4} alkyl), O_{2}S(C_{1-4} alkenyl), NH_{2}, NH(C_{1-4} alkyl), NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl)_{2}, N(C_{1-18} acyl)_{2}, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N_{3}, CN, one to three halogen (Cl, Br, F, I), NO_{2}, C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} acyl), C(O)O(C_{1-4} alkenyl), C(O)O(C_{1-4} alkynyl), O(C_{1-4} alkyl), O(C_{1-4} acyl), O(C_{1-4} alkenyl), O(C_{1-4} alkynyl), S(C_{1-4} acyl), S(C_{1-4} alkyl), S(C_{1-4} alkenyl), S(C_{1-4} alkynyl), SO(C_{1-4} acyl), SO(C_{1-4} alkenyl), SO(C_{1-4} alkynyl), SO_{2}(C_{1-4} acyl), SO_{2}(C_{1-4} alkyl), SO_{2}(C_{1-4} alkenyl), SO_{2}(C_{1-4} alkynyl), O_{2}S(C_{1-4} acyl), O_{2}S(C_{1-4} alkyl), O_{2}S(C_{1-4} alkenyl), NH_{2}, NH(C_{1-4} alkyl), NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl)_{2}, N(C_{1-4} acyl)_{2}, OR^{7}; R^{2} and R^{2^{'}} can be linked together to form a vinyl optionally substituted by one or two of N_{3}, CN, Cl, Br, F, I, NO_{2};

R^{3}, R^{4} and R^{5} are independently H, halogen including F, Cl, Br, I, OH, OR^{',} SH, SR^{',} NH_{2}, NHR^{',} NR^{2}, lower alkyl of C_{1-6}, halogenated (F, Cl, Br, I) lower alkyl of C_{1-6} such as CF_{3} and CH_{2}CH_{2}F, lower alkyl of C_{2-6} such as CH=CH_{2}, halogenated (F, Cl, Br, I) lower alkyl of C_{2-6} such as CH=CHCl, CH=CHBr and CH=CHI, lower alkyl of C_{2-6} such as C≡CH, halogenated (F, Cl, Br, I) lower alkyl of C_{2-6}, lower alkoxy of C_{1-6} such as CH_{2}OH and CH_{2}CH_{2}OH, halogenated (F, Cl, Br, I) lower alkoxy of C_{1-6}, CO_{2}H, CO_{2}R^{'}, CONH_{2}, CONHR^{',} CONR^{2}, CH=CHCO_{2}H, CH=CHCO_{2}R^{'};
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R₆ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

80. The use of claim 79, wherein

Base is

![Chemical Structure](image)

and R¹ is H, R² is OH, R²' is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

81. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the structure:
wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and,

R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when
administered in vivo is capable of providing a compound
wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷
can also be linked with cyclic phosphate group;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile
virus infection in a host.

82. The use of claim 81, wherein

Base is selected from the group consisting of:
Y is N or CH;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR¹, SH, SR¹, NH₂, NHR¹, NR², lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R¹, CONH₂, CONHR¹, CONR², CH=CHCO₂H, CH=CHCO₂R¹; and,

R¹ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

The use of claim 81, wherein

Base is selected from the group consisting of (a) or (b):
and wherein $R^1$ and $R^7$ are $H$, $R^3$ is $H$, and $R^4$ is NH$_2$ or OH, and $R^5$ is NH$_2$.

84. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-
2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) of the formula:

\[
\text{Base}
\]

wherein

Base is

\[
\text{X is O, S, CH}_2, \text{ Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)$_2$,}
\]

wherein W is F, Cl, Br, or I;

$R^1$ and $R^7$ are independently $H$, phosphate, including monophosphate, 
diphosphate, triphosphate, or a stabilized phosphate prodrug, 
H-phosphonate, including stabilized H-phosphonates, acyl, 
including optionally substituted phenyl and lower acyl, alkyl, 
including lower alkyl, O-substituted carboxyalkylamino or its 
peptide derivatives, sulfonate ester, including alkyl or 
arlylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R² are independently H, C₁-₄ alkyl, C₁-₄ alkenyl, C₁-₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), O(C₁-₄ acyl), O(C₁-₄ alkyl), O(C₁-₄ alkyl), S(C₁-₄ acyl), S(C₁-₄ alkyl), S(C₁-₄ alkynyl), S(C₁-₄ alkenyl), SO(C₁-₄ acyl), SO(C₁-₄ alkyl), SO(C₁-₄ alkynyl), SO(C₁-₄ alkenyl), SO₂(C₁-₄ acyl), SO₂(C₁-₄ alkynyl), SO₂(C₁-₄ alkenyl), O₃S(C₁-₄ acyl), O₃S(C₁-₄ alkenyl), NH₂, NH(C₁-₄ alkyl), NH(C₁-₄ alkenyl), NH(C₁-₄ alkynyl), NH(C₁-₄ acyl), N(C₁-₄ alkyl)₂, N(C₁-₄ alkenyl)₂, wherein alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkyl), C(O)O(C₁-₄ alkynyl), C(O)O(C₁-₄ alkenyl), O(C₁-₄ acyl), O(C₁-₄ alkyl), O(C₁-₄ alkenyl), S(C₁-₄ acyl), S(C₁-₄ alkyl), S(C₁-₄ alkynyl), S(C₁-₄ alkenyl), SO(C₁-₄ acyl), SO(C₁-₄ alkyl), SO(C₁-₄ alkynyl), SO(C₁-₄ alkenyl), SO₂(C₁-₄ acyl), SO₂(C₁-₄ alkynyl), SO₂(C₁-₄ alkenyl), O₃S(C₁-₄ acyl), O₃S(C₁-₄ alkenyl), NH₂, NH(C₁-₄ alkyl), NH(C₁-₄ alkenyl), NH(C₁-₄ alkynyl), NH(C₁-₄ acyl), N(C₁-₄ alkyl)₂, N(C₁-₄ alkenyl)₂, OR²; R² and R² can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R² and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR’₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃
and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R⁺, CONH₂, CONHR⁻, CONR⁻₂, CH=CHCO₂H, CH=CHCO₂R⁺;

R⁺ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

85. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:
$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered \textit{in vivo} is capable of providing a compound wherein $R^1$ or $R^7$ is independently H or phosphate; $R^1$ and $R^7$ can also be linked with cyclic phosphate group;

$R^3$ and $R^4$ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH$_2$, NHR', NR$_2$, lower alkyl of C$_1$-C$_6$, halogenated (F, Cl, Br, I) lower alkyl of C$_1$-C$_6$ such as CF$_3$ and CH$_2$CH$_2$F, lower alkenyl of C$_2$-C$_6$ such as CH=CH$_2$, halogenated (F, Cl, Br, I) lower alkenyl of C$_2$-C$_6$ such as
CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl; or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

86. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

![Chemical structure](image)

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.
87. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

wherein

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and,

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro and,

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.
88. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-
2'-C-methyl nucleoside (β-D) of the formula:

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile
virus infection in a host.

89. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-
2'-C-methyl nucleoside (β-D) of the formula:

wherein

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and

R¹ is H, phosphate, including monophosphate, diphosphate,
triphosphate, or a stabilized phosphate prodrug, H-
phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

90. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

\[
\text{\includegraphics{image.png}}
\]
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

91. Use an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

![Chemical Structure](image)

wherein

- Base is a purine or pyrimidine base;
- X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂,
- wherein W is F, Cl, Br, or I;
- R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;
- R² and R² are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ alkyl).
acyl), O(C₁₄ alkyl), O(C₁₄ alkenyl), S(C₁₄ acyl), S(C₁₄ alkyl), S(C₁₄ alkenyl), SO(C₁₄ acyl), SO(C₁₄ alkyl), SO(C₁₄ alkenyl), SO₂(C₁₄ acyl), SO₂(C₁₄ alkyl), SO₂(C₁₄ alkenyl), O₂S(C₁₄ acyl), O₂S(C₁₄ alkyl), O₂S(C₁₄ alkenyl), NH₂, NH(C₁₄ alkyl), NH(C₁₄ alkenyl), NH(C₁₄ acyl), N(C₁₄ alkyl), N(C₁₄ acyl), N(C₁₄ acyl), wherein alkyl, alkenyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₄ alkyl), C(O)O(C₁₄ alkyl), C(O)O(C₁₄ alkenyl), C(O)O(C₁₄ acyl), O(C₁₄ alkyl), O(C₁₄ alkenyl), O(C₁₄ acyl), S(C₁₄ alkyl), S(C₁₄ alkenyl), SO(C₁₄ alkyl), SO(C₁₄ alkenyl), SO₂(C₁₄ alkyl), SO₂(C₁₄ alkenyl), O₂S(C₁₄ alkyl), O₂S(C₁₄ alkenyl), O₂S(C₁₄ acyl), NH₂, NH(C₁₄ alkyl), NH(C₁₄ alkenyl), NH(C₁₄ acyl), N(C₁₄ alkyl), N(C₁₄ acyl), N(C₁₄ acyl), wherein R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

92. The use of claim 91,

wherein Base is selected from the group consisting of:
Y is N or CH.

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR₂', CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

The use of claim 91, wherein

Base is selected from the group consisting of (a) or (b):
and wherein \( R^1 \) is H, \( R^2 \) is OH, \( R^{2\prime} \) is H, \( R^3 \) is H, and \( R^4 \) is NH$_2$ or OH, and \( R^5 \) is NH$_2$.

94. Use of an antivirally effective amount of a (2\(^{\prime}R\))-2\(^{\prime}\)-deoxy-2\(^{\prime}\)-fluoro-2\(^{\prime}\)-C-methyl nucleoside (\( \beta-D \)) of the formula:

\[
\text{Base}
\]

wherein

Base is selected from the group consisting of

\[
\text{(a)}
\]

\[
\text{(b)}
\]

\( Y \) is N or CH;

\( R^1 \) and \( R^7 \) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R^1 is H or phosphate; R^2 is H or phosphate; R^1 and R^2 or R^7 can also be linked with cyclic phosphate group;

R^2 and R^2^ are independently H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, vinyl, N_3, CN, Cl, Br, F, I, NO_2, C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkyl), O(C_{1-4} alkyl), O(C_{1-4} alkyl), O(C_{1-4} alkyl), O(C_{1-4} alkenyl), O(C_{1-4} alkenyl), O(C_{1-4} alkenyl), O(C_{1-4} alkenyl), S(C_{1-4} acyl), S(C_{1-4} alkyl), S(C_{1-4} alkynyl), S(C_{1-4} alkenyl), SO(C_{1-4} acyl), SO(C_{1-4} acyl), SO(C_{1-4} alkenyl), SO(C_{1-4} alkenyl), SO_2(C_{1-4} acyl), SO_2(C_{1-4} acyl), SO_2(C_{1-4} alkenyl), SO_2(C_{1-4} alkenyl), O_2S(C_{1-4} acyl), O_2S(S(C_{1-4} alkyl), O_2S(S(C_{1-4} alkenyl), NH_2, NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl), N(C_{1-4} alkyl), N(C_{1-4} alkyl), wherein alkyl, alkenyl, alkenyl and vinyl are optimally substituted by N_3, CN, Cl, one to three halogen (Cl, Br, F, I), NO_2, C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkyl), C(O)O(C_{1-4} alkenyl), O(C_{1-4} alkyl), O(C_{1-4} alkyl), O(C_{1-4} alkyl), O(C_{1-4} alkenyl), O(C_{1-4} alkenyl), S(C_{1-4} acyl), S(C_{1-4} alkalyl), S(C_{1-4} alkynyl), S(C_{1-4} alkenyl), SO(C_{1-4} acyl), SO(C_{1-4} acyl), SO(C_{1-4} alkenyl), SO(C_{1-4} alkenyl), SO_2(C_{1-4} acyl), SO_2(C_{1-4} acyl), SO_2(C_{1-4} alkenyl), SO_2(C_{1-4} alkenyl), O_2S(C_{1-4} acyl), O_2S(S(C_{1-4} alkyl), O_2S(S(C_{1-4} alkenyl), NH_2, NH(C_{1-4} alkenyl), NH(C_{1-4} alkynyl), NH(C_{1-4} acyl), N(C_{1-4} alkyl), N(C_{1-4} alkyl), N(C_{1-4} alkyl), OR^2; R^2 and R^2^ can be linked together to form a vinyl optionally substituted by one or two of N_3, CN, Cl, Br, F, I, NO_2;
$R^3$, $R^4$ and $R^5$ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH$_2$, NHR', NR''$_2$, lower alkyl of C$_1$-C$_6$, halogenated (F, Cl, Br, I) lower alkyl of C$_1$-C$_6$ such as CF$_3$ and CH$_2$CH$_2$F, lower alkenyl of C$_2$-C$_6$ such as CH=CH$_2$, halogenated (F, Cl, Br, I) lower alkenyl of C$_2$-C$_6$ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C$_2$-C$_6$ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C$_2$-C$_6$, lower alkoxy of C$_1$-C$_6$ such as CH$_2$OH and CH$_2$CH$_2$OH, halogenated (F, Cl, Br, I) lower alkoxy of C$_1$-C$_6$, CO$_2$H, CO$_2$R', CONH$_2$, CONHR', CONR''$_2$, CH=CHCO$_2$H, CH=CHCO$_2$R';

$R'$ is an optionally substituted alkyl of C$_1$-C$_{12}$ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C$_2$-C$_6$, optionally substituted lower alkenyl of C$_2$-C$_6$, or optionally substituted acyl;

$R^6$ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH$_3$, OCH$_3$, OCH$_2$CH$_3$, hydroxy methyl (CH$_2$OH), fluoromethyl (CH$_2$F), azido (N$_3$), CHCN, CH$_2$N$_3$, CH$_2$NH$_2$, CH$_2$NHCH$_3$, CH$_2$N(CH$_3$)$_2$, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

95. The use of claim 94, wherein

Base is
and $R^1$ is H, $R^2$ is OH, $R^{2'}$ is H, $R^3$ is H, $R^4$ is NH$_2$ or OH, and $R^6$ is H.

96. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta$-D or $\beta$-L) of the structure:

```
  R^1O
  /\  
 R^2O------X
  |     \  F
  |     CH_3
  \      /
   \    /  
    \  /   
     \/
```

wherein Base is a purine or pyrimidine base;

$X$ is O, S, CH$_2$, Se, NH, N-alkyl, CHW ($R$, $S$, or racemic), C(W)$_2$, wherein W is F, Cl, Br, or I; and,

$R^1$ and $R^7$ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound
wherein R\(^1\) or R\(^7\) is independently H or phosphate; R\(^1\) and R\(^7\) can also be linked with cyclic phosphate group;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

97. The use of claim 96, wherein

Base is selected from the group consisting of:

![Chemical Structures](image)

Y is N or CH;

R\(^3\), R\(^4\) and R\(^5\) are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH\(_2\), NHR', NR'\(_2\), lower alkyl of C\(_1\)-C\(_6\), halogenated (F, Cl, Br, I) lower alkyl of C\(_1\)-C\(_5\) such as CF\(_3\) and CH\(_2\)CH\(_2\)F, lower alkenyl of C\(_2\)-C\(_6\) such as CH=CH\(_2\), halogenated (F, Cl, Br, I) lower alkenyl of C\(_2\)-C\(_6\) such as CH=CHCl, CH=CHBr and CH=CHI, lower alkenyl of C\(_2\)-C\(_6\) such as C=CH, halogenated (F, Cl, Br, I) lower alkynyl of C\(_2\)-C\(_6\), lower alkoxy of C\(_1\)-C\(_6\) such as CH\(_2\)OH and CH\(_2\)CH\(_2\)OH, halogenated (F, Cl, Br, I) lower alkoxy of C\(_1\)-C\(_6\), CO\(_2\)H, CO\(_2\)R', CONH\(_2\), CONHR', CONR'\(_2\), CH=CHCO\(_2\)H, CH=CHCO\(_2\)R'; and,

R' is an optionally substituted alkyl of C\(_1\)-C\(_{12}\) (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally
substituted alkynyl of C_2-C_6, optionally substituted lower alkenyl of C_2-C_6, or optionally substituted acyl.

98. The use of claim 96, wherein

Base is selected from the group consisting of (a) or (b):

(a) \[
\begin{align*}
\text{N} & \text{N} \\
R^4 & \text{Y} \\
R^5 & \text{X} \\
\text{R}^3 & \text{R}^4 \\
\end{align*}
\]

(b) \[
\begin{align*}
\text{R}^3 & \text{R}^4 \\
\text{R}^5 & \text{R}^4 \\
\end{align*}
\]

and wherein R^1 and R^7 are H, R^3 is H, and R^4 is NH2 or OH, and R^5 is NH2.

99. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

\[
\begin{align*}
\text{R}^1 & \text{O} \\
\text{R}^6 & \text{R}^2 \\
\text{R}^2 & \text{CH}_3 \\
\text{R}^3 & \text{R}^4 \\
\text{Base} & \text{X} \\
\end{align*}
\]

wherein

Base is

\[
\begin{align*}
\text{R}^3 & \text{R}^4 \\
\text{R}^5 & \text{R}^4 \\
\end{align*}
\]

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;
R\(^1\) and R\(^7\) are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfanyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R\(^1\) is H or phosphate; R\(^2\) is H or phosphate; R\(^1\) and R\(^2\) or R\(^7\) can also be linked with cyclic phosphate group;

R\(^2\) and R\(^2\)\(^1\) are independently H, C\(_{1-4}\) alkyl, C\(_{1-4}\) alkenyl, C\(_{1-4}\) alkynyl, vinyl, N\(_3\), CN, Ci, Br, F, I, NO\(_2\), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkynyl), C(O)O(C\(_{1-4}\) alkenyl), O(C\(_{1-4}\) acyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) alkyl), S(C\(_{1-4}\) alkynyl), S(C\(_{1-4}\) alkenyl), SO(C\(_{1-4}\) acyl), SO(C\(_{1-4}\) alkyl), SO(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkynyl), SO\(_2\)(C\(_{1-4}\) alkenyl), O\(_2\)S(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) alkenyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) acyl), N(C\(_{1-4}\) alkyl), N(C\(_{1-4}\) alkenyl), N(C\(_{1-4}\) alkynyl), where alkyl, alkenyl, alkynyl and vinyl are optionally substituted by N\(_3\), CN, one to three halogen (Cl, Br, F, I), NO\(_2\), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkyl), C(O)O(C\(_{1-4}\) alkenyl), C(O)O(C\(_{1-4}\) alkynyl), O(C\(_{1-4}\) acyl), O(C\(_{1-4}\) alkyl), O(C\(_{1-4}\) alkenyl), S(C\(_{1-4}\) acyl), S(C\(_{1-4}\) alkyl), S(C\(_{1-4}\) alkynyl), S(C\(_{1-4}\) alkenyl), SO(C\(_{1-4}\) acyl), SO(C\(_{1-4}\) alkyl), SO(C\(_{1-4}\) alkynyl), SO(C\(_{1-4}\) alkenyl), SO\(_2\)(C\(_{1-4}\) alkyl), SO\(_2\)(C\(_{1-4}\) alkynyl), SO\(_2\)(C\(_{1-4}\) alkenyl), O\(_2\)S(C\(_{1-4}\) alkyl), O\(_2\)S(C\(_{1-4}\) alkenyl), NH\(_2\), NH(C\(_{1-4}\) alkyl), NH(C\(_{1-4}\) alkenyl), NH(C\(_{1-4}\) alkynyl), NH(C\(_{1-4}\) acyl),
N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR²; R² and R²⁺ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR’, SH, SR’, NH₂, NHR’, NR₂, lower alkyl of C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂₋₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂₋₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂₋₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂₋₆, lower alkoxy of C₁₋₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁₋₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR₂, CH=CHCO₂H, CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁₋₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂₋₆, optionally substituted lower alkenyl of C₂₋₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

100. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:
R₁ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as
CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R’, CONH₂, CONHR’, CONR’₂, CH=CHCO₂H, CH=CHCO₂R’;

R’ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

101. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

102. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:
wherein

R\textsuperscript{1} and R\textsuperscript{7} are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein R\textsuperscript{1} or R\textsuperscript{7} is independently H or phosphate; R\textsuperscript{1} and R\textsuperscript{7} can also be linked with cyclic phosphate group; and,

R\textsuperscript{6} is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH\textsubscript{3}, OCH\textsubscript{3}, OCH\textsubscript{2}CH\textsubscript{3}, hydroxy methyl (CH\textsubscript{2}OH), fluoromethyl (CH\textsubscript{2}F), azido (N\textsubscript{3}), CHCN, CH\textsubscript{2}N\textsubscript{3}, CH\textsubscript{2}NH\textsubscript{2}, CH\textsubscript{2}NHCH\textsubscript{3}, CH\textsubscript{2}N(CH\textsubscript{3})\textsubscript{2}, alkyne (optionally substituted), or fluoro and,

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

103. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

104. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

![Chemical Structure](image)

wherein

X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and

R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,

203
including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered in vivo is capable of providing a compound wherein $R^1$ is H or phosphate;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

105. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

\[
\begin{align*}
\text{HO-} & \quad \text{O-} \\
\text{HO-} & \quad \text{F-} \\
\text{HO-} & \quad \text{CH}_3 \\
\end{align*}
\]

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

106. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

\[
\begin{align*}
\text{HO-} & \quad \text{O-} \\
\text{HO-} & \quad \text{F-} \\
\text{HO-} & \quad \text{CH}_3 \\
\end{align*}
\]
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

107. The use claim 31, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

108. The use claim 41, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
thiazolidine derivative; a benzamidine, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

109. The use of claim 43, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzamidine, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

110. The use of claim 45, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanihe, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

111. The use of claim 46, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanihe, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

112. The use of claim 56, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

113. The use of claim 58, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.
114. The use of claim 60, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanimide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-aminodalkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

115. The use of claim 61, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanimide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-aminodalkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

116. The use of claim 71, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amo-alkylocyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylethanolamine phytosome; and mycophenolate.

117. The use of claim 73, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amo-alkylocyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylethanolamine phytosome; and mycophenolate.
prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

118. The use of claim 75, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

119. The use of claim 76, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

120. The use of claim 86, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

121. The use of claim 88, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

122. The use of claim 90, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

123. The use of claim 91, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

124. The use of claim 101, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

125. The use of claim 103, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
 alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-alkylocyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

126. The use of claim 105, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylecxohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.
127. A method of synthesizing a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) comprising glycosylation of a nucleobase with an intermediate structure:

![Structure 1-4]

wherein R is lower alkyl, acyl, benzyol, or mesyl; and Pg is any acceptable protecting group consisting of but not limited to C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, tert-butylidemethylsilyl, tert-butylidiphenylsilyl, or both Pg's may come together to for a 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene).

128. A method of synthesizing a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) comprising selective deprotection of either Pg in an intermediate of the structure:

![Structure 2-5]

wherein, X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and Pg is independently any pharmaceutically acceptable protecting group selected from the group consisting of C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, tert-butylidemethylsilyl, tert-butylidiphenylsilyl, or both Pg's may come together to for a 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene).
129. An intermediate in the synthesis of a (2'R)-2'-deoxy-2'-fluoro-2'-C-
meethyl nucleoside (β-D or β-L), wherein the intermediate is of the structure:

wherein R is lower alkyl, acyl, benzoyl, or mesyl; and Pg is any acceptable
protecting group consisting of but not limited to C(O)-alkyl, C(O)Ph, C(O)aryl,
CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-
alkeky, SO₂-aryl, tert-butyldimethylsilyl, tert-butyldiphenylsilyl, or both Pg's may
come together to for a 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene).

130. An intermediate in the synthesis of a (2'R)-2'-deoxy-2'-fluoro-2'-C-
meethyl nucleoside (β-D or β-L), wherein the intermediate is of the structure:

wherein, X is O, S, CH₂, Se, NH, N-alkyl, CHW (R, S, or racemic),
C(W)₂, wherein W is F, Cl, Br, or I; and Pg is independently any
pharmacologically acceptable protecting group selected from the group
consisting of C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-
alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-
aryl, tert-butyldimethylsilyl, tert-butyldiphenylsilyl, or both Pg's may
come together to for a 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene).
Figure 3

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 6

Application of the Phosphoramidate ProTide Approach to 4'-Azidouridine Confers Sub-micromolar Potency versus Hepatitis C Virus on an Inactive Nucleoside

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We report the application of our phosphoramide ProTide technology to the ribonucleoside analogue 4'-azidouridine to generate novel antiviral agents for the inhibition of hepatitis C virus (HCV). 4'-Azidouridine did not inhibit HCV, although 4'-azidocytidine was a potent inhibitor of HCV replication under similar assay conditions. However, 4'-azidouridine triphosphate was a potent inhibitor of RNA synthesis by HCV polymerase, raising the question as to whether our phosphoramide ProTide approach could effectivly deliver 4'-azidouridine monophosphate to HCV replicon cells and unleash the antiviral potential of the triphosphate. Twenty-two phosphoramidates were prepared, including variations in the aryl, ester, and amino acid regions. A number of compounds showed sub-micromolar inhibition of HCV in cell culture without detectable cytotoxicity. These results confirm that phosphoramide ProTides can deliver monophosphates of ribonucleoside analogues and suggest a potential path to the generation of novel antiviral agents against HCV infection. The generic message is that ProTide synthesis from inactive parent nucleosides may be a warranted drug discovery strategy.

Introduction

The hepatitis C virus (HCV) was identified for the first time in 1989 as a single-stranded positive sense RNA virus of the Flaviviridae family. According to the World Health Organization (WHO), more than 170 million people are estimated to be chronically infected by this virus, which is a major cause of severe liver disease.

At present, treatment options comprise immunotherapy using recombinant interferon (often pegylated) in combination with ribavirin. The clinical benefit of this treatment is limited, and a vaccine has not yet been developed. The development of selective inhibitors of essential viral enzymes such as the serine protease NS3 or the RNA-dependent RNA polymerase NS5b are expected to improve the potency and tolerability of future treatment options for HCV infected patients.

Nucleoside analogues have already been validated as an important class of polymerase inhibitors of other viral targets, such as HCMV, HSV, HIV, and HBV. All antiviral agents acting via a nucleoside analogue mode of action need to be phosphorylated, most of them to their corresponding 5'-triphosphates, by cellular and/or viral enzymes. The nucleotide triphosphate analogues will then inhibit the requisite polymerase and/or compete with natural nucleoside triphosphates as substrates for incorporation into viral nucleic acid during viral replication.

Recently, 4'-azidocytidine was discovered to be a potent inhibitor of HCV replication in cell culture. The corresponding 5'-triphosphate was described as a competitive inhibitor of cytidylate incorporation by HCV polymerase and a potent inhibitor of native, membrane-associated HCV replicase in vitro.

Interestingly, the corresponding uridine analogue, 4'-azidouridine (I), was inactive as an inhibitor of HCV replication in the cell-based replicon system.

It was hypothesized that (I) (I, Figure 1) may be a poor substrate for phosphorylation by cellular enzymes. The first phosphorylation step to produce the 5'-monophosphate has often been found to be the rate-limiting step in the pathway to intracellular nucleotide triphosphate formation, suggesting that nucleoside monophosphate analogues could be useful antiviral agents. However, as unmodified agents, nucleoside monophosphates are unstable in biological media and they also show poor membrane permeation because of the associated negative charges at physiological pH.

Our arylxoy phosphoramide ProTide approach allows bypass of the initial kinase dependence by intracellular delivery of the monophosphorylated nucleoside analogue as a membrane permeable "ProTide" form. This technology greatly increases the lipophilicity of the nucleoside monophosphate analogue with a consequent increase of membrane permeation and intracellular availability. Previously we have demonstrated the success of our approach with the arylxoy phosphoramide derivatives of dA, dT, LdC, and dA. These nucleoside monophosphate analogues were shown to exhibit greatly enhanced activity against HIV compared to the parent nucleoside analogues in vitro. In contrast to the parent nucleosides, full antiviral activity of the phosphonate analogues was retained in kinase deficient cell lines, which was consistent with an efficient bypass of the first phosphorylation step in HIV infected cells. Arylxy phosphoramidates are considered to be efficient lipophilic prodrugs of the corresponding 5'-monophosphates species in which the two masking groups are represented by an amino acid ester and an aryl moiety. After passive diffusion...
Figure 1. Structure of AZU and its corresponding phenyl-phosphoramidate ProvTide.

through cell membranes, the suggested activation pathway involves initial enzyme-catalyzed cleavage of the carboxylic ester, followed by the internal nucleophile attack of the acid residue on the phosphorus center, displacing the arylxy group. The putative transient, cyclic mixed-anhydride is then rapidly hydrolyzed to the corresponding amino acid phosphononester. Last, a suggested phosphoramidate activity catalyzes the cleavage of the P-N bond in free the nucleoside monophosphate intracellularly. In the current study we tested the possibility to apply our ProvTide approach to the inactive 4'-azidouridine (1) in order to achieve bypass of the first phosphorylation step and thereby generate novel antiviral agents (2) with potent activity against HCV.

Results and Discussion

Chemistry. The synthesis of 1 has been previously described.15 To prepare monophosphate produgs (2) of 1 we initially followed the previously described phosphoramidate chemistry for the synthesis of ProvTides developed in our laboratory, using 1-methylimidazole (NMI) as the coupling agent.15,17,18 Several attempts were performed using different conditions (different amino acid esters, different reaction conditions) without successful isolation of the corresponding arylxy-phosphoramidate. These initial unsuccessful attempts might be explained considering the presence of a bulky group (arlyxy) at the 4'-position adjacent to the coupling site at the 5'-position; in our previously published ProvTide examples the 4'-position was unsubstituted.

The method of Uchiyama was investigated next.19 This approach is based on the treatment of a nucleoside with 1 equiv of a strong organic base, such as a solution of tert-butyl magnesium chloride (tBuMgCl), to form the corresponding metal alkoxide. In the case of (1), this reaction was observed to be very rapid and gave yields between 3% and 20% of desired products. In the first instance, we synthesized 4'-azidouridine phosphoramidates starting from an unprotected nucleoside. The apparent reactivity at the 2'- and 3'-positions was low, suggesting high regioselectivity for the reaction at the 5'-position. In this way it was also possible to synthesize compounds 13, 21, and 26. In order to achieve higher solubility in the reaction solvent (dichloromethane) and increase reactivity at the 5'-position, the 2'- and 3'-positions of 1 were protected with a cyclopentyl group.20 The final synthetic pathway (Scheme 1) involves the coupling of phenyl dichlorophosphate with different amino acid ester salts (5) to give the corresponding phenolxy-phosphorochloridates (6), which were purified by flash chromatography and then coupled with the 2',3'-O,C,C,cyclopentylidine derivative 7 of (1) in the presence of tBuMgCl (1 M solution in THF).

The deprotection step was performed with a solution of 80% formic acid in water for 4 h at room temperature (Scheme 2). Due to the stereochemistry at the phosphorus center, the final compounds were always isolated as mixtures of two diastereomers. The presence of these diastereoisomers in the final preparations was confirmed by 31P (two peaks), 1H, and 13C NMR. A total of 22 phenyl phosphoramidates were synthesized as reported in Table 1.

We have previously reported extensive structure-activity relationship (SAR) studies of anti-HIV phosphonamides exploring the amino acid region, including natural amino acid variation,22 unnatural α,ω-dialkyls,23 stereochemical variation,24 and amino acid extensions25 and replacements.26 In general, L-alanine and the unnatural amino acid α,ω-dimethylglycine showed the best activity for the +4T parent molecule versus HIV.11,12,15

Using the previously described method (Scheme 1), we synthesized the L-alanine (12), α,ω-dimethylglycine (18), cyclopentylglycine (20), L-phenylalanine (22), L-leucine (27), L-methionine (29), ethyl L-glutamate (31), and L-proline (28) phosphoramidates of 1, each bearing an ethyl group. Further investigations on the amino acid variation were conducted on a series of benzyl esters: L-alanine (17), α,ω-dimethylglycine (19), cyclopentylglycine (21), L-phenylalanine (23), L-valine (24), and glycine (25). We further compared the importance of the stereochemistry at the amino acid position by preparing a p-alanine benzyl ester phosphoramidate (26). On the basis of the L-alanine phenyl phosphoramidate backbone, we also explored the SAR of different esters including methyl (11), ethyl (12), butyl (13), 2-butyl (14), isopropyl (15), tert-butyll (16), and benzyl (17). In order to have an indirect proof of phenyl phosphoramidate metabolism, we synthesized the N-methylidine (30) and β-alanine (32) analogues, which were considered unfavorable substrates according to the postulated mechanism of activation.15

Recently we noted an increase of in vitro potency of a 1-naphthyl phosphoramidate analogue compared to the corresponding phenyl derivative while investigating the antiviral activity of BVDU phosphonamides.27 Therefore, similar phosphoramidate analogues were also generated for (1). The synthesis of the 1-naphthyl phosphoramidate (33) was performed by reacting 1-naphthyl phosphonochloridate with the corresponding phosphoramidate (Scheme 3), which was then coupled to an amino acid ester and the nucleoside analogue according to our standard procedures. In this case, the separation of the two phosphate diastereoisomers (34 and 35) was achieved by using a semi-preparative HPLC purification with elution conditions of 75% water/25% acetonitrile. The 31P NMR spectrum showed the presence of only one peak for the first of the two fractions separated, and the 1H NMR spectrum supported the suggestion of a single diastereisomer in this case. The second fraction contained an excess of the second diastereisomer together with a minor proportion (estimated at 7% by 31P NMR integration) of the first diastereosomer (see Supporting Information for data).

Antiviral Activity. The phenyl phosphoramidates described above (11–32) were characterized in vitro as inhibitors of HCV replication in a cell culture system as previously reported.6,8 Data are presented in Table 1 as EC50 values (representing the concentration of compounds reducing HCV replication by 50%) and CC50 values (representing the concentration of compounds reducing cell viability by 50%) as determined using the WST-1 assay. All compounds showed CC50 values greater than 100 μM. The parent compound 1 did not inhibit HCV replication significantly in the replicon system (EC50 > 100 μM). In contrast, a number of phosphoramidate derivatives showed
potent inhibition of HCV replication. Assuming that 4'-azidouridine-5'-triphosphate is the active HCV polymerase inhibitor, these results support the notion that the active phosphoramidates successfully delivered 4'-azidouridine monophosphate intracellularly, that 4'-azidouridine (1) is inefficiently phosphorylated to the monophosphate in replicon cells, and that 4'-azidouridine monophosphate can be phosphorylated to the 5'-triphosphate in replicon cells. As shown in Table 2, 4'-azidouridine triphosphate notably inhibited recombinant HCV polymerase NS5b in vitro, and did so with similar sub-micromolar potency, like that of the previously described NS5b inhibitor R1479-7P (4'-azidocytidine triphosphate). Therefore, the application of our phosphoramidate approach showed to be a successful tool in overcoming the phosphorylation block of 1 and converting an inactive nucleoside analogue to a potent inhibitor of HCV replication, thus accessing the full potential of the 4'-azidouridine triphosphate.

As shown in Table 1, l-alanine derivatives represented a series of active antiviral phosphoramidates (11-17). Low or sub-micromolar activity was noted in marked contrast to the inactive nucleoside parent (1). The tert-butyl ester (10) was the least active of the series, which was in agreement with the SARs previously obtained in the d4T series25 and may relate to the relative stability of tertiary esters to enzyme-mediated hydrolysis. The isopropyl ester (15) showed high potency and represented one of the most active phosphoramidates prepared. Similarly, the 2-butyl ester (14) was highly active in our assay in contrast to previous observations with other nucleoside analogues. Together with the benzyl analogue (17), these three esters provided the most potent compounds of HCV replication.
inhibitors in the L-alanine series, all having μM inhibition of HCV. The antiviral activity of these three phosphorodiamidates was exceptional compared to the parent compound 1 (EC_{50} > 100 μM), providing strong support for the notion of ProTide-mediated kinase bypass.

In the benzyl ester family, L-alanine (17) provided the most active compound with p-alanine (26) and glycine (25) being only slightly less potent. These results were striking when compared to the 60-70 fold reduction in anti-HIV potency for d4T ProTides with an L-alanine to glycine replacement and a 20-30 fold reduction for the corresponding abacavir ProTides.21,22 This reinforces our earlier conclusion that a separate ProTide motif optimization process is needed for each nucleoside analogue versus a given target. It may be that cell line dependent enzyme expression may determine different phosphorodiamidate SARs.

The presence of a methyl (β- and γ-alanine, 26 and 17) or β,β-dimethyl (19) enhanced the activity compared to larger and hydrophobic amino acid side chain residues such as L-valine (24), L-phenylalanine (23), and cyclopropylglycine (21), which were weakly active in the replicon assay.

An unexpected correlation was found between amino acid and ester function. While the L-phenylalanine derivative was substantially inactive as a benzyl ester (23), the corresponding ethyl ester (22) showed a significantly increased antiviral activity, displaying an EC_{50} value of 3.4 μM. Therefore, matrix-based optimization of amino acid and ester functions may be preferential over stepwise approaches.

The inactivity of the β-alanine (23) and of the N-methyl glycine (30) compounds might underscore the presence of an α-amino acid and a free NH as a minimum requirement in the amino acid structure to enable the metabolic activation of aryloxy-phosphorodiamidates. However, the proline compound (with a blocked NH) did show modest (28) activity, pointing to a complex amino acid SAR.

In conclusion, ester variation was widely tolerated except for the tert-butyl group which gave a slight reduction in potency in the L-alanine series (16) and the benzyl in the case of the L-phenylalanine derivative (23). L-Alanine remained the most effective amino acid, with glycine and β-alanine showing only slightly reduced potency. Dimethylglycine, L-lysine, and L-proline also provided compounds with antiviral potencies in a low micromolar range. It therefore appears that the amino acid core could be considerably varied to give antiviral agents with potencies within a 10-fold range in replicon cells. Importantly, potency optimization requires consideration of both amino acid and ester moieties as most clearly shown for the ethyl and benzyl esters of the L-phenylalanine analogues. Moreover, quite distinct SARs emerged from this family versus HCV as compared to our prior studies in other families.

We also explored the possibility to replace the phenyl substituent on the phosphate with a more hydrophobic moiety, 1-naphthyl. Previously, we noted an increase in ₪ NTP potency of 1-naphthyl-phosphorodiamidates compared to the corresponding phenyl phosphorodiamidates when investigating 3'BU-phosphoramidates in an antiviral assay.20 We synthesized 33, the 1-naphthyl analogue of 17 (β-alanine benzyl ester). As shown in Table 3, compound 33 inhibited HCV replication with an EC_{50} of 0.22 μM, leading to a further increase in antiviral activity (450-fold) in comparison to 2'-azidothymidine (Table 3). One of the two phosphorus diastereomers could be purified using a C18 reverse phase semi-preparative HPLC. One of the two main fractions obtained showed only one {31P} NMR peak. The second fraction was less pure, although the second diastereoisomer appeared as the major component of the mixture. We have previously reported a method for the prediction of the phosphorus configuration of such diastereoisomers based on a different 31P NMR profile of the methylphospho-proton of the benzyl ester.20 Applying this concept to compounds 34 and 35, we noticed that in one case (more polar, 35), a clear 31P-system was observed while, for the other diastereoisomer (less polar, 34), the two protons displayed an apparent doublet. Conformational studies were performed using the Sybyl 7.0 software package. The lowest energy conformation found for each diastereoisomer is shown in Figure 2. These differences in proton profiles can be explained by the ability of one, but not the other, diastereoisomer to form π-π interactions between the naphthyl and the phenyl group of the benzyl ester resulting in a constrained conformation. This interaction can only occur with the 3-phosphorus configuration (35) with the two methylene...
protons becoming non-magnetically equivalent (AB system). For the diastereoisomer with R phospheho configuration (34), this interaction does not occur and the higher degree of flexibility around the methylene renders its protons even more magnetically similar (apparent doublet). The biological activities of the separated diastereoisomers (34 and 35) were comparable to each other and to the mixture (33) (Table 3).

Interestingly, application of similar ProTide methods to the active 4'-azidocytidine gave little or no boost in anti-HCV activity (data not shown), implying a rather efficient phosphorylation of this nucleoside analogue, with which ProTide methods presumably cannot compete.

Conclusion

A series of phosphoramide ProTides of 4'-azidouridine were prepared and evaluated as inhibitors of HCV replication in vitro. The phosphoramide approach provided novel compounds with highly increased potency in the replicon assay when compared to the inactive parent compound, corresponding to boosts in anti-HCV potency of >450-fold. All phosphoramidates tested were nontoxic in the replicon assay (CC50 > 100 μM). The most active compound prepared in the series was the 1-naphthylalanine benzyl ester phosphoramide with an EC50 of 0.22 μM in the replicon assay. The diastereoisomers of this compound were separated by HPLC and their absolute phosphorus configurations predicted by modeling and NMR. However, they did not show any differences in biological activity. This report demonstrates the ability of the ProTide approach to successfully bypass the rate limiting initial phosphorylation of a ribonucleoside analogue and thus confer significant antiviral activity on an inactive parent nucleoside.

Experimental Section

Biology. HCV replicon assay was performed in the stable replicon cell line 2259-23 derived from Huh-7 cells stably transfected with a bicistronic HCV replicon (genotype 1b) expressing the renilla luciferase reporter gene, as described. The RNA synthesis activity of recombinant HCV polyprotein was measured in incorporation of radiolabeled UMP into acid-insoluble RNA products using HCV genotype derived cDNAs as a template and in a primer-independent RNA synthesis assay.1 Recombinant proteins were translated at amide acid position 570 and derived from genotype 1b strain BKK (NS5B570-BK) or Cen (NS5B570-Cen).

Chemistry. General Procedures. All experiments involving water-sensitive compounds were conducted under siccophorously dry conditions. Anhydrous tetrahydrofuran and dichloromethane were purchased from Aldrich, Proton, carbon, and phosphorus NMR spectra were recorded on a Bruker Avance spectrometer operating at 500, 125, and 202 MHz, respectively. All 1H and 31P spectra were recorded proton-decoupled. All NMR spectra were recorded in CD3OD at room temperature (20 °C ± 3 °C). Chemical shifts for 1H and 13C spectra are quoted in parts per million downfield from tetramethylsilane. Coupling constants are quoted to 1J values. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), broad signal (br), doublet of doublet (DD), doublet of triplet (dt), or multiplet (m). Chemical shifts for 31P spectra are quoted in parts per million relative to an external phosphoric acid standard. Many proton and carbon NMR signals were split due to the presence of phosphoramide) diastereoisomers in the samples. The mobile liquid chromatography was fast atom bombardment (FAB) using MNOA as matrix. Column chromatography refers to flash columns carried out using Merck silica gel 60 (40–60 μM) as stationary phase.

For convenience, standard procedures have been given, as similar procedures were employed for reactions concerning the synthesis of precursors and derivatives of ProTides. Variations from these procedures and individual purification methods are given in the main text. Preparative and spectroscopic data on individual precursors, blocked nucleosides are given as Supporting Information only (see below), excluding only the first example.

Standard Procedure 1: Preparation of 2',3'-O-4'-Cyclopentylidene-4'-azidouridine Phosphoramidates. To a solution of 2',3'-O-4'-cyclopentylidene-4'-azidouridine (1.0 mol equiv) in dry THF (15 ml) was added a 1 M solution of the appropriate phosphoramide (2.0 mol equiv) in dry THF (15 ml) and stirred for 15 min. A 1 M solution of the appropriate phosphoramide (2.0 mol equiv) in dry THF was added dropwise and then stirred overnight. A saturated solution of NH4Cl was added, and the solvent was removed under reduced pressure to give a yellow solid, which was subsequently purified by chromatography.

Standard Procedure 2: Deprotection of 2',3'-O-4'-Cyclopentylidene-4'-azidouridine Phosphoramidates. The appropriate 2',3'-O-4'-cyclopentylidene-4'-azidouridine phosphoramide was added to a solution 80% of formic acid in water. The reaction was stirred at room temperature for 4 h. The solvent was removed under reduced pressure, and the obtained oil was purified by chromatography.

Standard Procedure 3: Preparation of 4'-Azidouridine Phosphoramidates via Free Nucleoside Synthesis. To a solution of 4'-azidouridine (1.0 mol equiv) and 31P NMR spectra (CD3OD) were recorded. The reaction was then diluted with the appropriate phosphoramide (2.0 mol equiv) in dry THF was added dropwise and then stirred overnight. A saturated solution of NH4Cl was added, and the solvent was removed under reduced pressure to give a yellow solid, which was purified by chromatography.

HPLC Method Used for the Separation of Compound 34 and 35. A Varian Prostar instrument using a Polaris C18-A 100 μm column, elution was performed using a mobile phase consisting of water/acetonitrile 70/30 H2O/ACN 17 min elution time with a flow of 20 ml/min. Minimal leaching on column: 8 mg of phosphoramide per run.

Synthesis of 2',3'-O-4'-Cyclopentylidene-4'-azidouridine S'-s-(Phenylmethylthiol)-alanil amine) Phosphate (Methyl N-[(1-[31P])piperidinyl-2-yi]-3-4'-azido-4'-(4-hydroxymethyl)-2,2'-cyclopentylidene-3-dioxo-6-methylpryridine-2,4'-dione (Phenyl)-3-phosphoryl)-s-(alanil amine)]. Prepared according to the standard procedure 1. from 2',3'-O-4'-cyclopentylidene-4'-azidouridine (150 mg, 0.427 mmol), 9H-MeCl (0.85 mL, 1 M solution in THF, 0.854 mmol), and phenylmethylthioalaninamidine) phosphorochloridate (0.85 mL of solution 1 M in THF, 0.854 mmol). The crude product was purified by column chromatography, using as eluent CH2Cl2/MeOH (65/5). The pure product was a white solid (84.5 mg, 0.263 mmol, 61%). δ (2H-CH2-OH): 3.14, 3.04, 3.0, δ (4H-CH2-OH): 7.69 (1H, t, CH=O-CH2-OMe, 8.35 (2H, t, 2 CH-phenyl), 7.26–7.19 (3H, m, 3 CH-phenyl), 5.97 (1H, s, N=CH-c=O-Me), 5.70 (1H, t, 1H-CH=O), 5.12–5.02 (2H, m, CH2=CH-CH=CH2), 4.01 (1H, m, CH2=CH-Me), 3.70 (3H, d, CH3-phenyl), 2.21–2.12 (2H, m, CH2-cyclohexyl), 1.79–1.73 (6H, 3 CH3-cyclohexyl), 1.37 (3H, s, CH3-alanine), J = 9.5 Hz).

Synthesis of 4'-Azidouridine 5'-s-(Phenylmethylthio)-alanil amine) Phosphate (Methyl N-[(1-[31P])-4-s-(phenylmethylthio)-4'-azido-4'-(4-hydroxymethyl)-2,2'-cyclopentylidene-3-dioxo-6-methylpryridine-2,4'-dione (Phenyl)-3-phosphoryl)-s-(alanil amine)]. Prepared according to the standard procedure 2, from 2',3'-O-4'-cyclopentylidene-4'-azidouridine 5'-s-(phenylmethylthio)-alaninamidine) phosphate (135 mg, 0.222 mmol), and a solution 80% of HOCH3 in water (10 mL). The crude was purified by column chromatography.

270
using as eluent CHCl₃:MeOH (82). The obtained pure product was a white solid (65 mg, 0.116 mmol, 54%). δ (d₆-CDCl₃): 3.50, 3.31; δₗ (d₆-CDCl₃): 7.65 (1H, d, H₂'-uridine), 7.38 (2H, m, 2 CH₂-phenyl), 7.28–7.30 (3H, m, 3 CH₂-phenyl), 6.15 (1H, m, H₁'-uridine), 5.72 (1H, m, H₅'-uridine), 4.42–4.36 (2H, m, H₂'-uridine), 4.13–4.15 (2H, m, 1H, H₅'-uridine), 4.00 (1H, q, H₃'-uridine), 3.70 (1H, m, H₂'-uridine), 3.35 (3H, m, CH₂=O), 2.86 (4H, m, 2 CH₂-phenyl), 1.35 (3H, m, CH₃-CH₂-CH₂-OH), MS (EI): m/z 549,112 (M⁺Na⁺), C₂₃H₂₂N₂O₁₂Na⁺ requires 549,118 (M⁺Na⁺).

Synthesis of 2',3'-O-4-Cyclopentidene-4'-azouridine 5'-O-[Phenyethyloxy-phenacyl]-y-Phosphite (Ethyl N-(1-[(3-Ar,AR,AR,AR,AR,AR)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentidinyl][3,4-d]-4-Methyl-6-yl]pyrimidine-2,4(1H,3H)-dione) (Phenoy-phosphityl-[alaminyl]). See Supporting Information for preparative and spectroscopic data.

Synthesis of 5'-O-[Phenyloxy-yl-phenacyl]-y-Phosphite (Phenyl N-(1-[(3-Ar,AR,AR,AR,AR,AR,AR)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentidinyl][3,4-d]-4-Methyl-6-yl]pyrimidine-2,4(1H,3H)-dione) (Phenoy-phosphityl-[alaminyl]). See Supporting Information for preparative and spectroscopic data.

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Synthesis of 2',3',5'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxydimethyl)glycyl] Phosphate (Ethyl N-[(3aR,4R,6aS)-4-Azidotetrahydro-4-(hydroxymethyl)-2-cyclopentylidene-3,4-diol-6-yl]pyrimidine-2,4(1H,3H)-dione) (Phenyloxyl)phosphoryl-dimethylglycylate. See Supporting Information for preparative and spectroscopic data.

Synthesis of 2',3',5'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenylbenzoxyl-dimethylglycyl] Phosphate (Benzyl N-[(3aR,4R,6aS)-4-Azidotetrahydro-3,4-dihydrorxy-5-(hydroxymethyl)furarn-2-y1pyrimidine-2,4(1H,3H)-dione) (Phenyloxyl)phosphoryl-dimethylglycylate. See Supporting Information for preparative and spectroscopic data.

Synthesis of 2',3',5'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenylbenzoxyl-dimethylglycyl] Phosphate (Ethyl N-[(3aR,4R,6aS)-4-Azidotetrahydro-4-(hydroxymethyl)-2-cyclopentylidene-3,4-diol-6-yl]pyrimidine-2,4(1H,3H)-dione) (Phenyloxyl)phosphoryl-cyclopentylglycinate. See Supporting Information for preparative and spectroscopic data.

Synthesis of 2',3',5'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenylbenzoxyl-d-phenylalaninyl] Phosphate (Ethyl N-[(3aR,4R,6aS)-4-Azidotetrahydro-4-(hydroxymethyl)-2-cyclopentylidene-3,4-diol-6-yl]pyrimidine-2,4(1H,3H)-dione) (Phenyloxyl)phosphoryl-cyclopentylglycinate. See Supporting Information for preparative and spectroscopic data.
Azido-tetrahydro-3,4-dihydroxy-5-hydroxymethylfuran-2-y1-
pyrimidine-2,4(1H,3H)-dione (Phenyloxy)-phosphorl-1,1-
benzylxyctalanilinate (23). Prepared according to the standard procedure 2, from 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-[phenylbenzylxyctalanilinate]. The crude product was purified by column chromatography, using as eluent CHCl3/MeOH (8/2). The obtained pure product was a white solid (130 mg, 0.192 mmol, 87%). βδ₂ (d, CH₃OH): 3.21, 3.02; δ₂ (d, CH₂OH): 7.51 (1H, dd, H₆-uridine), 7.32, 7.23 (6H, m, 2 CH₂-phenyl, 2 CH₂-lateral chain, 2 CH=benzyl), 7.18, 7.09 (3H, m, 3 CH₁-phenyl, 3 CH-lateral chain, 3 CH benzyl), 0.14 (1H, dd, H₁'-uridine), 5.64 (1H, dd, H₅'-uridine), 5.16, 5.09 (4H, m, H₂'-uridine, H₂'-uridine), 4.23 (2H, m, H₃'-uridine), 4.05 (1H, m, CH₂C₆H₆), 1.36 (3H, m, CH₁-phenyl), 4.38 (2H, m, H₂'-uridine), 1.61 (3H, m, CH₂-phenyl, 1.60 (3H, m, H₂'-uridine), 1.73, 1.50, 1.49 (12H, s, CH₂, CH₃, CH₂, CH₃, C=O). 3.99 (1H, m, CH₂-lateral chain), 2.91, 2.87 (2H, m, CH₂-lateral chain, MS (EI) 710.1732 (M⁺), C₹H₁₃NO₆Na₃ requires 710.1737. Anal. (C₹H₁₃NO₆Na₃) C, H, N.

Synthesis of 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-(Phenyloxy)-benzylxyctalanilinate. Phosphate (Ethyl N-[1-(3aR,4R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-
cyanoethylfuran-3,4-dy]-1-methoxy-6-ethylpyrimidine-2,4(1H,3H)
dione) (Phenyloxy)-phosphoryl-1,1-benzylxyctalanilinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyloxy]-benzylxyctalanilinate. Phosphate (Benzyl N-[1-(3aR,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-hydroxymethyl-2-furan-2-y1pyrimidine-2,4(1H,3H)
dione) (Phenyloxy)-phosphoryl-1,1-benzylxyctalanilinate). Prepared according to the standard procedure 2, from 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-[phenylbenzylxyctalanilinate] (72 mg, 0.248 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (70 mg, 0.116 mmol, 55%). βδ₂ (d, CH₂OH): 4.45, 4.14; δ₂ (d, CH₂OH): 7.02 (11, m, H₁'-uridine), 6.8 Hz, 7.39, 7.32, 7.0 (2H, m, CH₂-phenyl), 7.24, 7.18 (1M, m, CH₂-lateral chain, CH₂-phenyl), 6.14 (1M, m, H₁'-uridine), 5.68 (1H, m, H₁'-uridine, J = 6.8 Hz), 5.18, 5.10 (2H, m, CH₂, CH₂-phenyl), 4.37, 4.30 (2H, m, H₂'-uridine, H₂'-uridine), 4.22, 4.14 (2H, m, H₂'-uridine), 3.76 (2H, m, CH₂OH), 2.07 (1H, m, CH₂C₆H₆), 0.90 (3H, t, CH₃-value, J = 8.6 Hz), 0.84 (3H, t, 1 CH₃-value, J = 7.8 Hz) MS (EI) 633.1737 (M⁺), C₹H₁₃NO₆Na₃ requires 633.1732. Anal. (C₹H₁₃NO₆Na₃) C, H, N.

Synthesis of 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-[Phenyloxy]-benzylxyctalanilinate (Phosphoryl) (Benzyl N-[1-(3aR,4R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-
cyanoethylfuran-3,4-dy]-1-methoxy-6-ethylpyrimidine-2,4(1H,3H)
dione) (Phenyloxy)-phosphoryl-1,1-benzylxyctalanilinate). Prepared according to the standard procedure 2, from 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-[phenylbenzylxyctalanilinate] (135 mg, 0.208 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (111 mg, 0.190 mmol, 91%). βδ₂ (d, CH₂OH): 3.83, 3.47; δ₂ (d, CH₂OH): 7.61 (1H, dd, H₂'-uridine), 7.36 (2H, t, 2 CH₂-phenyl), 7.25, 7.30 (2H, m, 3 CH₂-phenyl), 6.16 (1H, d, H₃'-uridine), 3.72 (1H, dd, H₅'-uridine), 4.40, 4.35 (2H, m, H₂'-uridine, H₂'-uridine), 4.22 (2H, br, H₃'-uridine), 4.15, 4.18 (2H, m, CH⁺, CH⁺-ethyl), 3.90 (1H, br, CH₃), 1.54 (3H, m CH₂-lateral chain, CH₂-lateral chain), 1.27, 1.19 (3H, m, CH₂-ethyl), 0.65; 0.36 (3H, t, CH₂-lateral chain, CH₂-lateral chain), 0.05 (3H, t, CH₂-lateral chain). MS (EI) 595.1733 (M⁺), C₹H₁₃NO₆Na₃ requires 595.1731. Anal. (C₹H₁₃NO₆Na₃) C, H, N.

Synthesis of 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-[Phenyloxy]-benzylxyctalanilinate (Phosphoryl) (Ethyl N-[1-(3aR,4R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-
cyanoethylfuran-3,4-dy]-1-methoxy-6-ethylpyrimidine-2,4(1H,3H)
dione) (Phenyloxy)-phosphoryl-1,1-benzylxyctalanilinate). Prepared according to the standard procedure 2, from 2',3'-O-Cyclpentylidene-4'-azidouridine 5'-O-[phenylbenzylxyctalanilinate] (121 mg, 0.190 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (101 mg, 0.178 mmol, 94%). βδ₂ (d, CH₂OH): 6.89, 4.29; δ₂ (d, CH₂OH): 7.65 (1H, dd, H₂'-uridine), 7.39 (2H, t, 2 CH₂-phenyl), 7.30, 7.23 (3H, m, 3 CH₂-phenyl), 6.12 (1H, dd, H₃'-uridine), 5.71 (1H, dd, H₃'-uridine), 4.39, 4.29 (2H, m, H₂'-uridine), 1.63 (3H, m, H₂'-uridine), 4.25, 4.13 (2H, m, H₂'-uridine, CH₂-ethyl), 3.42 (1H, m, CH₂-ethyl), 2.25, 2.17 (2H, m, CH₂-phenyl), 2.02, 1.84 (4H, m, 2

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273
Synthesis of 2',3'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenylthioethyl]-methionolyl] Phosphate (Ethyl N-[(1-[2RS,3SR,5SR]-5-Azido-tetrabutyroxy-3,4-dihydroxy-5-(hydroxymethyl)furarn-2'-yrimidilinde (Phenoxy)-phosphoryl]-methionyl)] (29). Prepared according to the standard procedure 2, from 2',3'-O-cyclopentylidene-4'-azidouridine 5'-O-[phenylthioethyl]-methionolyl) phosphate (187 mg, 0.230 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl3/MeOH (8:2). The purified product was a white solid (135 mg, 0.216 mmol, 88%). Δ2 (dCHCl3): 5.12, 4.93; δ2 (dCHCl3): 7.66 (Hh, H-6'uridine), 7.42-7.38 (2H, H-2'Phenyl), 7.29, 7.23 (3H, 2'H-uridine), 6.15 (Hh, 2'H-uridine, J = 2.1 Hz), 5.74 (2'H, H-3'H-uridine), 4.42-4.29 (2H, H-3'H-uridine, H-5'S-uridine), 2.46-2.26 (7H, H-5'S-uridine, C6H15-ethyl), 1.90 (Hh, CH3-siato), 3.80 (Hh, CH3-siato), 2.83 (Hh, CH3-siato), 1.38 (2H, H-2'H-ethyl), J = 7.2 Hz. MS (EI) 673.15 (M+N+1). Δ2 (dCHCl3) 5.12, 4.93; δ2 (dCHCl3) 7.66 (Hh, H-6'uridine), 7.42-7.38 (2H, H-2'Phenyl), 7.29, 7.23 (3H, 2'H-uridine), 6.15 (Hh, 2'H-uridine, J = 2.1 Hz), 5.74 (2'H, H-3'H-uridine), 4.42-4.29 (2H, H-3'H-uridine, H-5'S-uridine), 2.46-2.26 (7H, H-5'S-uridine, C6H15-ethyl), 1.90 (Hh, CH3-siato), 3.80 (Hh, CH3-siato), 2.83 (Hh, CH3-siato), 1.38 (2H, H-2'H-ethyl), J = 7.2 Hz. MS (EI) 673.15 (M+N+1).
Acknowledgment. We thank Helen Murphy for secretarial assistance.

Supporting Information Available: Analytical data on target compounds, preparative and spectroscopic data on blocked nucleoside intermediates, and figures of HPLCs of separated chromatographic isomers. This material is available free of charge via the Internet at http://pubs.acs.org.

References

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 7

Christopher McGuigan, et al, Certain Phosphoramidate Derivatives of Dideoxy Uridine (ddU) are Active Against HIV and successfully By-pass Thymidine Kinase, FEBS Letter 351, 1994
Certain phosphoramidate derivatives of dideoxy uridine (ddU) are active against HIV and successfully by-pass thymidine kinase

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Received 27 June 1994

Abstract

As part of our effort to deliver masked phosphates inside living cells we have discovered that certain phosphate triester derivatives of the inactive nucleoside analogue, dideoxy uridine (ddU) are inhibitors of HIV replication at μM levels. Moreover, we note that certain phosphoramidate derivatives retain their activity in thymidine kinase-deficient cells, which indicates that they do indeed act by intracellular release of the free nucleotide, and that they successfully by-pass the nucleoside kinase. The increased structural freedom in drug design which this allows may have implications for dealing with the emergence of resistance and may stimulate the discovery of improved therapeutic agents.

Key words: Nucleoside; Nucleotide; Anti-HIV

1. Introduction

Although nucleoside analogues, such as 3'-azidothymidine (AZT, 1, Fig. 1) continue to dominate anti-HIV drug therapy they have a number of major limitations, such as their inherent toxicity, a dependence on kinase-mediated activation to generate the bio-active (tri)phosphate forms, and the emergence of resistance [1-2].

We [3-6] and others [7-9] have pursued a masked phosphate approach in an attempt to improve on the therapeutic potential of the parent nucleoside analogues. In this approach, inactive phosphate derivatives of the nucleoside analogue are designed to penetrate the cell membrane and liberate the bio-active nucleotides intracellularly. Masking of the phosphate group is necessary on account of the extremely poor membrane penetration by the polar (charged) free nucleotide. One mechanism by which masked phosphates may lead to enhanced selectivity of action arises from what we have termed 'kinase bypass' [3]. Thus, the complete dependence of administered (anti-HIV) nucleoside analogues on host nucleoside kinase-mediated activation places constraints upon the structures of nucleoside analogues which might be active. Nucleoside analogues which fall outside these strict constraints will be inactive, even if their 5'-triphosphates (the bio-active form) are potent and selective inhibitors of a viral target, such as reverse transcriptase (RT). Several such cases are known. Dideoxythymidine, and 3'-O-methylthymidine are examples of nucleoside analogues which are inactive against HIV, whilst their triphosphates are exceptionally potent inhibitors of HIV RT [10]; the inactivity of the nucleoside being attributed to poor phosphorylation by host kinases. If the masked phosphate strategy were able to deliver nucleotides intracellularly, the nucleoside kinase would be by-passed and the structural constraints such host enzymes impose would be obviated. In this way, wider structural variation of the nucleoside analogue would be permitted, and more specific (less toxic) inhibitors of viral function may arise. We have recently reported on the success of this 'kinase by-pass' strategy with several highly modified 3'-substituted nucleosides [11-12].

We now report the success of this approach with the simple nucleoside analogue dideoxy uridine (ddU, 2).

This is essentially inactive against HIV, but judicious phosphorylation leads to the introduction of a significant, selective antiviral effect. Moreover, this effect is retained in thymidine kinase-deficient cells, indicating a successful by-pass of this enzyme, and strongly supporting the suggested intracellular delivery of free nucleotides by this strategy. Whilst other researchers have recently reported the failure of the by-pass approach with phosphoramidates derived from ddu [13], we herein clearly demonstrate the success of this strategy with our previously reported aryloxy phosphoramidates [6]

2. Materials and methods

2.1. Chemistry

General synthetic procedures were similar to those we have described [5]. All nucleotides were pure by high-field multi-nuclear NMR and reverse phase High Performance Liquid Chromatography (HPLC:...
AC5 system, 50 × 250 mm × 4.6 mm, Spherisorb ODS2 5 μ column, gradient elution using 5% acetonitrile in water (A), and 5% water in acetonitrile (B), with 20% B for 0-10 min, then a linear gradient to 80% B at 30 min, with a flow rate of 1 mL/min.

2.1.1. 2',3'-dideoxy uridine (d4U, 2).

(a) 5'-Trityl uridine. Trityl chloride (4.1 g, 14.7 mmol) was added to a solution of uridine (3 g, 12.3 mmol) in pyridine (50 mL) and the reaction mixture heated at 50°C for 24 h. The solvent was removed under high vacuum and the residue purified by chromatography on silica using 5% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions gave the product as a white solid (5.32 g, 89%). 

(b) 5'-Trityl-2',3'-thiouridine. A solution of 5'-trityl uridine (1 g, 2.05 mmol) and thiocarbonyldiimidazole (0.04 g, 2.24 mmol) in THF (20 mL) was stirred at ambient temperature for 17 h. After evaporation of the solvent under reduced pressure the residue was purified by chromatography on silica using 5% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions gave the product as a white solid (990 mg, 91%).

2.1.2. 2',3'-dideoxy uridine 5'-bis[2,2,3-trichlorophenyl]phosphate (3a).

DAU (2) (0.08 g, 0.38 mmol) and N-methyl imidazole (0.155 g, 1.89 mmol) were mixed in THF (10 mL) and bis(2,2,3-trichlorophenyl) phosphorochloridate (0.186 g, 0.49 mmol) was added slowly at ambient temperature. After 1 h the solvent was evaporated and the residue dissolved in CHCl3 (30 mL) washed with HCl (1 M, 10 mL), sodium bicarbonate solution (15 mL) and water (15 mL). The organic phase was dried (MgSO4) and evaporated under reduced pressure. The crude product was purified by chromatography on silica eluting with 4% methanol in chloroform. Pooling and evaporation of appropriate fractions under reduced pressure gave the product (0.20 g, 95%). 

2.1.3. 2',3'-dideoxy uridine 5'-[(ethyl methoxylaminyl)phosphorochloridate (0.11 g, 0.47 mmol) was added slowly at ambient temperature. After 4 h the solvent was evaporated and the crude product purified entirely as described for (3a) above, except that a second chromatographic column was necessary, using an eluant of 15% methanol in ethyl ether, in order to obtain pure (3b) (0.051 g, 54%).

2.2. Virology

2.2.1. Antiviral assays.

The anti-HIV-1 activities and toxicities of compounds were assessed in two cell lines [14] CR166 (a normal T-cell transformed by co-cultivation with leukaemia lymphocytes harbouring HTLV-I) were infected with the HIV-IIIB strain of HIV-1, which gave the T-cell line derived from a patient with lymphoblastic leukaemia, were infected with HIV-1 strains GB8 and HIB. JM cells are relatively resistant to the antiviral effects of AZT and a number of its derivatives.

Cells were grown in RPMI 1640 with 10% calf serum. 4 x 10^6 cells per microtiter plate well were mixed with 5-fold dilutions of compound prior to addition of 10 CCID50 units of virus and incubated for 5-7 days. Formation of syncytia was examined from 2 days post-infection. Culture fluid was collected at 5-7 days and gp120 antigen production was measured by ELISA. Cell viability of infected cells and cytotoxicity to uninfected cell controls were assessed by the MTT-Formazan method [19].

2.2.2. gp120 antigen assay.

A microtiter antigen capture ELISA was developed [16] using a lectin (GNA) from Galanthus nivalis (Vector Labs., Peterborough, UK) and anti-HIV antibodies in human serum. The plates were coated with lectin (0.5 μg), and after blocking with 10% calf serum, dilutions of virus supernatants in 0.2% Emeprin solution (Albertson and Wilson Ltd., Whitehaven, UK) were added to the wells and incubated at 4°C for 12-16 h. Bound antigen was detected using an anti-human IgG antibodies coupled to horseradish peroxidase.

3. Results and Discussion

Dideoxy uridine (d4U, 2) was prepared via three independent routes, all involving a final hydrogenation of the dideoxy dideoxy compound d4U. Thus, 5'-silylation of uridine, followed by thiocarbonate formation, elimination with triethylphosphite and deprotection with fluoride, gave 2',3'-dideoxy 2',3'-didehydro uridine (d4U) [17] in an overall yield of 50%. Alternatively, a 5'-trityl protecting group could be used in this synthesis to give d4U, again via the 2',3'-thiouracil, in an overall yield of 58%. Alternatively, by the procedures of Horwitz [18] 2'-deoxyuridine could be converted to its 3',5'-dimesylate, which gave the 3',5'-oxetane, and which could be converted to d4U on treatment with strong base. The
confirmed by 'H, 13C NMR, mass spectrometry and (3b) was prepared by methods we have reported [4] and was allowed to react with ddU to give the target compound (3a) in good yield. Full carbon-13 and (where appropriate) phosphorus-31 NMR data for the nucleoside (2) and the phosphates (3a-c) are given in Table 1.

The parent nucleoside (2) and the corresponding masked phosphates (3a-c) were tested for their ability to inhibit the replication of HIV-1 in C8166 cells, and in thymidine kinase-deficient JM cells, data being presented in Table 2. As expected, the parent nucleoside (2) is active only at the highest concentrations tested, and is essentially inactive in JM cells. The bis(trichloroethyl) phosphate (3a) is approximately 5–10 times more active in each assay. On the other hand, the simple phosphoramidate (3b) is devoid of antiviral activity in this assay, as has been recently noted by other researchers in a different assay [13]. However, the arylx phosphoramidate (3c) is a potent agent, being approximately 50-times more active than the parent nucleoside analogue. This confirms the importance of data derived from assays in kinase deficient cells for the interpretation of the activities of blocked nucleotides. As we have noted [20] only

Table 1 Carbon-13 and phosphorus-31 NMR data for compounds (2) and (3a-c)

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<th>3a</th>
<th>3b</th>
<th>3c</th>
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*Multiple. All spectra were recorded in CDC13, except for (2) which were recorded in CDCl3 plus CD3OD. Data are presented as δ in ppm. All spectra were recorded using proton decoupling. In the case of carbon data phosphorus coupling constants in Hz are superscripted. Many carbon peaks for (3b-c) display diastereomic splitting arising from mixed stereochemistry at the phosphate centre.
Table 2

<table>
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<td>CC&lt;sub&gt;50&lt;/sub&gt;</td>
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<td>2</td>
<td>200</td>
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<td>&gt; 1,000</td>
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<tr>
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The antiviral activity and cytotoxicity of test compounds in two different cell lines. ED<sub>50</sub> represents the concentration of compound (in µM) that decreases viral antigen production in infected cells to 50% of control. CC<sub>50</sub> represents the concentration of compound (in µM) which causes 50% cytotoxicity to uninfected cells.

such data will allow a clear understanding of the likely mechanism of action of blocked nucleotides, and discriminate between the release of nucleosides and nucleotides.

In conclusion, we report the antiviral activity of certain masked phosphate derivatives of the inactive nucleoside ddU. We note that aryloxy phosphoramidates are particularly efficacious, and attribute the introduction of activity to kinase by-pass. Finally we stress the importance of data in kinase-deficient cells for the clear interpretation of results on blocked phosphates.

Acknowledgements: We thank the AIDS Directed Programme of the Medical Research Council and the Biomedical research Programme of the European Community for financial support.

References


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A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 8

Dominique Cahard et al, Aryloxy Phosphoramidate Triesters as Pro-Tides, Mini-Reviews in Medicinal Chemistry, 2004
Aryloxy Phosphoramidate Triesters as Pro-Tides

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Abstract: We herein describe the development of aryloxy phosphoramidate triesters as an effective pro-tide motif for the intracellular delivery of charged bio-active antiviral nucleoside monophosphates. The review covers the discovery of such aryl phosphoramidates, their mechanism of action and structure-activity relationships. The application of this strategy to a range of antiviral nucleosides is highlighted.

Keywords: Nucleotide, Pro-drug, Phosphoramidate.

SCOPE OF THIS WORK

We will describe the discovery, in vitro evaluation, Structure-Activity Relationships (SARs) and Mechanism of Action (MoA) of phosphoramidate triesters of a range of antiviral nucleosides. We describe these compounds as triesters to emphasise the fact that all of the charges on the phosphate nucleus are blocked and to distinguish our compounds from the phosphoramidate diesters described by Wagner and Coworkers [1]. These latter compounds have been well reviewed [1,2], operated by a quite separate mechanism, and displayed distinct SARs to those compounds we will describe here. Therefore we will not include them below. We will thus describe the development of fully blocked phosphoramidates, culminating in our lead series, the aryloxy phosphoramidates.

ALKYL AND HALOALKYL PHOSPHATE TRIESTERS

Early work from our laboratories indicated that simple alkyl triesters of the antiviral agent araA (vidarabine) and the anti-neoplastic agent araC (cytarabine), of general formulas 1 and 2, (Fig. 1) respectively displayed significant biological activity in tissue culture [3,4]. However, analogous dialkyl phosphate triesters of AZT (3) were devoid of significant anti-HIV activity, in marked contrast to the parent nucleoside analogue [5]. Similarly, whilst haloalkyl phosphate triesters of araA and araC (4, 5) had enhanced biological activity [6], the corresponding AZT derivatives (6) and also those of 2',3'-dideoxycytidine (ddC) (7) were in general poorly active [7].

Thus, although the bis(trifluoroethyl) analogue (6) was active at 0.4µM, and thus >200 times more active than compound (3), it was still 100-fold less potent than AZT itself [7]. Attempts to boost the potency of these haloalkyl phosphate triesters by changing the degree of halogenation were in general not successful [8].

ALKYLOXY PHOSPHORAMIDATES

The original rationale for preparing phosphoramidate-based pro-tides was the possibility that HIV aspartate protease [9] might cleave a suitable oligo-peptide from the phosphate moiety of a blocked nucleotide phosphoramidate. Simple model mono-amino acyl analogues were prepared and evaluated in the first instance and were of sufficient interest to pursue in their own right. Thus, a series of simple alkyl phosphate triesters of AZT were prepared with a small family of methyl esterified aminoacids (8) [10]. By comparison to earlier dialkyl phosphates of AZT (3, 6) the alkyl phosphate triesters (8) showed significant anti-HIV activity. A notable dependence of the antiviral activity on the aminoacid side-chain began to emerge; with alanine being most efficacious, and with leucine and, particularly, isoleucine being less active [10,11]. By contrast, the alkyl phosphate chain could be varied from C1 to C6 with no significant change in activity [11].

In a subsequent study [12], α- aminoacids were compared to their β,γ derivatives etc (9). Anti-HIV activity was maximal for the parent α system (glycine) and diminished with increasing alkyl spacer length, being 10-fold less active for n=3 as compared to n=1 [12].

Given the earlier improvements in antiviral activity noted for the haloalkyl phosphate parents, we wondered whether haloalkyl phosphoramidates might also be more potent. Therefore, a small series of compounds (10) was prepared [13]. For each of the aminoacids glycine, alanine and valine, the alkyl chain either was ethyl, trifluoroethyl or trichloroethyl. However, by contrast to earlier observations, we herein noted no enhancement in antiviral potency compared with the haloalkyl compounds, with one striking exception. The trichloroethyl alanine compound (10, X=Cl, R=Me) was active at 0.08µM and thus 50 times more potent than either the ethyl or trifluoroethyl analogues. Interestingly, this enhancement was only seen for the alanine series, and not for the glycine and valine systems [13]. Thus, alanine emerged as a preferred aminoacid, although the mechanistic origins of this preference were, and still largely remain, unknown. Much of the preceding literature from our labs and others has utilized alanine as the empirical aminoacid of choice, although as we will note below there are other aminoacids, which may usefully substitute for it.
PHOSPHORODIAMIDATES

Given the promising activity of alkyl phosphoramidates, particularly those related to alanine, we wondered whether diaminates might also be efficacious. Thus, several methyl esterified amino acyl phosphoramidates (11) were prepared and tested [14]. Non-aryl acyl phosphoramidates derived from simple primary and secondary amines were also prepared. Structure-activity relationships were noted that indicated a strong preference for aminoacids such as phenylalanine [14]. Thus a different aminoacid SAR emerged for these diaminates as compared to the earlier alkyl phosphoramidates. It is intriguing to note that this preference for aromatic side-chains was also seen by Wagner for the rather un-related phosphoramide diesters [1]. However, in general the diaminates appeared to offer no biological advantage over the amidates, and the chemical yields of the diaminates were significantly lower; hence they were not further pursued.

LACTYL DERIVED SYSTEMS

In an effort to establish the importance of the bridging aminoacid nitrogen atom for the biological activity of the phosphoramidates a small family of isosteric O-linked analogues derived from lactic and glycolic acid was prepared (12) [15]. In each case, lengthening of the alkyl phosphate chain (R”) leads to a reduction in potency. It was also notable that glycolyl systems (R”=H) were more active than lactyl (R”=Me) by a factor of ca. 20. This is in contrast to the earlier work on phosphoramidates noted above where alanine was preferred over glycine [10,11]. A brief hydrolytic stability study was undertaken on compounds 12, which revealed liberation of polar compounds and traces of AZT in biological media, but not in DMSO/water. Thus, enzyme-mediated activation was possible. However, since the anti-HIV activity of even the most active compound in the series was significantly (>10-fold) lower than AZT itself, these compounds were not further pursued.

DIARYL PHOSPHATES OF AZT

One of our major breakthroughs in phosphoramidate pro-tide research was made in 1992, when we noted the efficacy of arylxy phosphates and phosphoramidates [16]. Thus, diaryl phosphates (13, Fig. 2) were prepared from AZT using simple phosphorochloridate chemistry. For the first time, the anti-HIV activity of these phosphate derivatives of AZT exceeded that of the parent nucleoside in some cases. Thus, the bis (p-nitrophenyl) phosphate was ca. 3-fold more potent than AZT vs. HIV-1 in C8166 cells, with an EC₅₀ of 3nM [16]. Moreover, whilst AZT was almost inactive (EC₅₀ 100µM) in the JM cell line, the substituted diaryl phosphate was 10-times more active (EC₅₀ 10µM). At the time, it was considered that JM was AZT – insensitive due to poor phosphorylation [17]. It later emerged that an AZT-efflux pump was the source of this poor AZT sensitivity [18]. However, the conclusion remains valid that the diaryl phosphate was more able to retain activity in the JM cell line, and that this may imply a (small) degree of intracellular phosphate delivery. The nitro group was implicated as vital to this activity, as the parent diphenyl phosphate was ca. 100-fold less active (C8166 cells). The electron-withdrawing power of the p-nitro groups and putative enhancements in aryl leaving group ability were suggested as the major driving force of this SAR [16].

Thus, a series of analogues of (13) were prepared, with various alternative para substituents (CN, SMe, CF₃, 1, OMe, H) [19]. A very clear correlation emerged between electron-withdrawing power of the para substituent and antiviral potency; the nitro and cyano substituted compounds being the most potent, the parent phenyl substituted compound intermediate in activity and the methoxy analogue least active, being 500-fold less active than the nitro compound. The effect of location of the electron withdrawing nitro group on the aryl rings was also briefly pursued, with symmetrical bis-ortho nitro and bis-meta nitro analogues being prepared [20]. In a study of both HIV-1 and HIV-2 in several cell lines it was found that the location of the nitro group had little effect on activity. However, for the first time we were able to assess the activity of the phosphate pro-drugs in the ‘true’ kinase-deficient cell line CEM-TK-. This was a clear but disappointing result, with all of the diaryl phosphates losing almost all their activity, alongside AZT, in the TK- cell line. This most likely implied poor intracellular phosphate delivery and that the diaryl phosphates were acting largely, if not entirely, as AZT pro-drugs, not as AZTMP pro-drugs as intended [20]. However, the earlier work using JM cells on phosphoramidates [16] had indicated that aryloxy phosphoramides may offer a chance for true phosphate delivery, and this became the main focus of our work.

ARYLOXY PHOSPHORAMIDATES OF AZT

Thus, a series of aryloxy phosphoramidates of AZT was prepared (14) with various p-aryl substituents and several aminoacids [21]. Compounds were only studied in the AZT-resistant JM cell line to probe potential (implied) AZTMP release, and the alanine phosphoramidate emerged as strikingly effective. In HIV-1 infected JM all cultures, AZT was inhibitory at 100µM, whilst the phenyl methoxy alaninyl phosphoramidate (14, R=Me, A=Ph) was active at 0.8µM. This was taken as the first evidence of a successful nucleotide delivery. As had been noted by us previously in other series there was a marked preference for alanine over leucine (10-fold) and glycine (>100-fold). Moreover, whilst electron-withdrawing aryl substitution had been noted to be very effective in the diaryl systems [19], it was detrimental here. Para fluoro substitution had a slight adventitious effect, but not significantly so, whilst para-nitro substitution led to a 100-fold loss of activity. In a subsequent study [22] the range of aryl substituents was extended and compounds studied in true TK⁺ and TK⁻ cell lines. None of the phosphoramidates retained the high (2-4 nM) potency of AZT in TK competent cell lines (CEM and MT-4) against either HIV-1 or HIV-2 [22]. However, whilst AZT lost all of its activity in the TK⁻ deficient cell line CEM/TK⁻, most of the phosphoramidates retained antiviral activity, thus being ca >10-35-fold more active than AZT in this assay. Again, alanine emerged as an important component, with the glycine analogue being inactive in HIV-infected CEM/TK⁺ all cultures. In this assay, leucine and phenylalanine were as effective as alanine, although they were less so in CEM/TK⁺ assays. Thus, the parent phenyl methoxy alaninyl phosphoramidate emerged as an important lead compound [22].
Fig. (1). Structures of some nucleosides and nucleotides. All nucleotides are 5’-linked.
APPLICATION TO OTHER NUCLEOSIDES

As with other research groups reported in this compilation, we had sought to find a universal phosphate delivery motif that could be applied to a range of nucleosides. Indeed as early as 1993 we suggested that the phenyl alanyl phosphoramidate approach might be successful on a range of nucleosides (ddC, d4T) and phosphonates (PMEA) [22]. This has subsequently been confirmed to be the case with extensive application of the technology by others and us.

Stavudine (d4T) was an early application of ours [23]. This was a rational choice based on the known kinetics of phosphorylation of d4T. Thus, whilst the 2nd phosphorylation (AZTMP to AZTDTP) but not the first phosphorylation (AZT to AZTMP) is regarded as rate limiting for AZT, the first step (d4T to d4TMP) is thought in general to be the slow step for d4T [24]. Thus, an intracellular (mono) nucleotide delivery should have maximal impact for d4T and similar nucleosides. In the first instance (halo)alkyloxy phosphoramidates of d4T (15) were prepared [23] and found to retain activity in d4T-resistant JM cells. The activity was dependent on the haloalkyl group; the parent propyl system being poorly active. Subsequent studies in HIV-infected CEM/TK- cell cultures [25] revealed the aryloxy phosphoramidates of d4T (16) to be highly effective and, notably, to retain their full activity in CEM/TK- cells. In this study the benzyl ester emerged as slightly more potent than the parent methyl compound, being almost 10-times more active than d4T in CEM/TK- assays and thus ca 300-500 fold more active than d4T, in CEM/TK- assays. Extensive studies followed on these promising d4T derivatives [26,27] which we will discuss later.

In 1994 Franchetti and coworkers [28] applied the aryl phosphoramidate technology to 8-aza-isoddA (17) and isoddA (18). Very significant boosts in the antiviral potency of the parent nucleosides were noted; >25-fold for (17) and 350-800 fold for (18). This was important an work, which demonstrated the power of the arylloxy phosphoramidate approach to greatly improve the biological profiles of poorly active nucleosides. Thus, (18) was transformed from 32µM activity versus HIV-2, to 40nM activity, on phosphoramidate formation. Subsequent analysis of these compounds by the Montpellier team [29] leads to the clear conclusion that they function as efficient intracellular phosphate delivery forms.

To a large extent this could be regarded as an example of what in 1990 we termed ‘kinase bypass’, wherein an inactive, or moderately active and poorly phosphorylated nucleoside could be ‘activated’ or potentiaded by suitable pro-tide modification [5,30]. A further example of this has emerged in our labs on application of the technology to ddU [31]. Thus, whilst dideoxuridine (ddU) is almost inactive (EC50 200µM) vs. HIV-1 in C8166 cells, the phosphoramidate (19) was noted to be active at low µM levels and to retain potency in the AZT-resistant JM cell line. This activity was specific to the arylloxy phosphoramidate both with our lab [31] and the Montpellier group [32] noting poor activity for the alkyloxy phosphoramidates.

Given the success of the phosphoramidate approach by the Franchetti lab when applied to iso nucleosides [28], we were interested to pursue other sugar modifications. Therefore, we applied the approach to 2',3'-dideoxy-3'-thiacytidine (3TC) [33]. In fact, compound 20 was found to be less effective than 3-TC in deoxycytidine (dCyd) kinase competent HIV-1 and -2 infected cell assays, but assay in dCK deficient cells indicated far less of an impact on potency for the phosphoramidate than the parent CEM cells 3TC (ca. 20-fold vs 2000-fold). Interestingly, both compounds were equally effective versus hepatitis B virus in hepatoma G2 cells indicating efficient pro-tide activation in these cells but not in the CEM cells used for the HIV assay [33]. This was amongst the first indications that the (relative) efficacy of phosphoramidates might be cell-line dependent.

One of the most remarkable demonstrations of the effectiveness of the arylloxy phosphoramidate approach came from our application of the technology to the dideoxydidehydro purine d4A [34]. Compounds of the type 350-800 fold for (18) (ca. 30-fold), their extraordinary potency enhancements still leave them with enhanced selectivities (50-150 fold) [34], and they are taken as a good example of nucleoside'kinase' (in this case adenosine) bypass. Subsequent application of the technology to dideoxyadenosine ddA (22) revealed a similar outcome; a >100-fold potency boost, with some increase in cytotoxicity [35].

The Detroit-based lab a Jiri Zemlicka has pioneered the synthesis of highly modified nucleosides with alkene, alkyne, alkene, methyleneacyclop propane, methyleneacyclo-butane and spiropentane modifications and successfully applied our phosphoramidate technology. Indeed, they have recently reviewed these efforts [36].

Thus, phenyl methoxy alaninyl phosphoramidates of the anti-HIV active adenallene (23) and the inactive hypoxallene (24) were prepared [37].

A 10-20 fold boost in anti-HIV potency was noted on phosphoramidate formation from (23). Alkenyl adenine nucleosides such as (25) and (26) were similarly studied [38,39]. In these cases, both the Z (25) and E (26) nucleosides were inactive, whilst the phosphoramidate of (25) was active in the 1-10 µM range and non-toxic; the isomeric phosphoramidate (26) remained inactive. The hypoxanthine analogue of (25) was also poorly active [38,39].

A study of methyleneacyclopropane nucleoside phosphoramidates (27) was conducted by the Zemlicka group [40-43]. Besides these active Z-isomers, the inactive E-series were also phosphorylated and compounds evaluated against a very wide range of viruses (HCMV, HSV-1, HSV-2, HHV-6, EBV, VZV, HBV, HIV-1 and HIV-2). Amongst the conclusions were the following:

- The Z-adenine compound is a potent inhibitor of a variety of viruses, but is cytotoxic.
- The Z-guanine analogue is active against HCMV, HBV, EBV and VZV and is non-cytotoxic.
- The Z-diaminopurine is highly active against HBV and HIV-1, with lower activity against other viruses and is non-cytotoxic. This compound emerged as the best candidate for further development. Again, whilst the E-isomers of the parent nucleosides were either inactive or very poorly active, their phosphoramidates emerged as potent and selective antivirals, particularly the adenine compound.

The Zemlicka group has also applied the phosphoramidate technology to methylene cyclobutane (28) [44] and spiropentane (29) [45] nucleosides with varying degrees of efficacy.

Further pursuing the kinase bypass approach we prepared some inactive novel d4T derivatives with 5-halo substituents in place of the 5-methyl group (30) and converted them to their phosphoramidates [46]. Whilst all compounds (30) were poorly active/inactive at >10-50 µM, the phosphoramidates were all active. The phenyl methoxy alaninyl phosphoramidate of the 5-chloro compound (30, X = Cl) was active at sub µM concentrations, being >100-fold more potent than its nucleoside parent, and was also non-cytotoxic [46].

Inspired by Zemlicka, who had shown that phosphoramidate formation could broaden the spectrum of activity of nucleoside analogues, we sought its application to a variety of nucleosides with different therapeutic targets. Thus, the anti-herpetic agents acyclovir (31), BVDU (32) and netivudine (33) were all converted to their phosphoramidates [47-49].

In general terms, the approach failed for acyclovir (31), where the phosphoramidate was significantly less active than the parent versus HSV-2, and slightly more active versus where the phosphoramidate was significantly less active than the parent versus HSV-2, and slightly more active versus netivudine (33) [49] was again disappointing, with little activity being noted.

Finally, our application of the technology to the potent anti-VZV agent netivudine (33) [49] was again disappointing, with little activity being noted.

Thus, in conclusion others and we have demonstrated that the phenol methoxyalaninyl group may significantly enhance the potency, selectivity and activity of spectrum of a range of nucleosides and by-pass their dependence on nucleoside-kinase mediated activation. The approach is very successful for the Zemlicka agents with highly modified sugar regions, and for ddA and d4A. It is also effective for d4T and AZT in nucleoside kinase deficient cells. However, it is clearly dependent on the nature of the parent nucleoside and the cell line/target studied. The example of BVDU highlights this final point [48,50].

**APPLICATION TO ACYCLIC NUCLEOSIDE PHOSPHONATES**

Until recently all of the published work on phosphoramidate pro-tides was on nucleoside analogues, as noted above. However, recently there have been a few reports of application of the technology to phosphonates, and in particular acyclic nucleoside phosphonates (ANPs). The Gilead group who has been active in the commercialization of ANPs have reported [52] that aryloxy phosphonamidates (34, Fig. 2) of PMPA (tenofovir) are highly active anti-retrovirals. We reached the same conclusion for PMPA and the closely related PMEA [53]. Boosts in antiviral potency of 30-100 fold were noted for PMPA and PMEA, with the usual preference for alanine as the aminoacid component.

**Fig. (2).** Structures of some nucleotides.
Interestingly, we noted a significant preference for L-alanine over D-alanine (5-60 fold), whereas Gilead observed a preference for one phosphonate diastereoisomer over the other, observations we will further discuss later under ‘stereochemistry’ matters. Gilead commenced clinical trials on their amide GS7340 in 2002, most notably progressing with one phosphate diastereoisomer, afforded by an efficient large-scale synthesis and isomer separation [54].

**D4T Aryloxy Phosphoramidate SARs**

We have conducted fairly extensive structure-activity relationship studies of various regions of the phosphoramidate unit when attached to d4T, with over 250 analogues prepared to date [26,27]. These will be discussed by the respective region of the phosphoramidate:

**Ester Region**

Some early work on AZT alkyloxy phosphoramidates revealed the importance of the carboxyl ester region for anti-HIV activity [55], which was subsequently confirmed for d4T aryloxy analogues [56]. Thus, a range of primary, secondary, tertiary, alkyl, benzyl and linear and branched esters related to (16) were prepared and evaluated against HIV-1 and –2 in thymidine kinase – competent and –deficient cell lines. Data are presented in Graph 1 as plot of potency (1/EC50) for a range of esters, versus HIV-1 in CEM TK+ cells, with d4T as control. A number of esters lead to potent activity, comparable with, or slightly more potent than, the methyl parent with compound. The benzyl, and naphthyl esters in particular were noted to be highly potent. The t-butyl ester analogue on the other hand was >10-times less potent than the methyl ester parent compound. As we will note below under Mechanism of Action studies, this correlates well with the poor esterase susceptibility of this particular ester. We later conducted a QSAR analysis using calculated physical properties (TSARTM) for 15 esters related to (16), which showed a good degree of correlation between predicted and measured activity, with a clear dependence on the shape and electronic distribution of the ester [57]. In particular, there emerged a strong dependence on the directional component of the ester group lipophilicity (the ‘lipole moment’), indicating that electron withdrawing groups in the ester, but removed from the ester bond, should boost potency.

**Amino Acid**

As noted above, alanine had arisen as the aminoacid of choice based on limited studies with alkyloxy phosphoramidates of AZT. We now compared 13 aminoacids related to (16, R=Me) [58]. Very clear SARs emerged from this study as presented in Graph 2, along with other highly amino-modified systems will be described later. Thus, versus HIV-1 in CEM TK+ cells, alanine remained the most effective aminoacid. However, several other aminoacids led to potencies, which were not significantly reduced, notably the un-natural, achiral α,α-dimethylglycine compound (35), which was slightly more potent than the alanine compound in CEM / TK+ cells. In fact, all of the phosphoramidates retained full potency in the nucleoside

![Graph 1](image_url)  
**Graph 1.** The effect of ester changes on antiviral potency.
The effect of amino acid changes on antiviral potency.

kinase deficient CEM cell assay, indicating action via intracellular d4TMP release. However, some amino acids were less effective; proline in particular, leads to a compound 20-100 times less potent than (16); whether this relates to the importance of a free amino acid NH, or steric or conformational issues relating particularly to proline is not entirely clear. However, valine and isoleucine were also poorly effective, indicating some steric restriction issues related to the side-chain. On the contrary, however, glycine is a striking example; the simple loss of the alanine methyl group resulting in a ca 60-70 fold reduction in potency.

The potency of (35) was an important discovery, since for the first time it indicated that 'un-natural' (or less common) amino acids could be utilized in the phosphoramidate approach. Indeed, we subsequently studied the \( \alpha,\alpha \)-diethyl- and -dipropyl analogues of (35), but found that these were poorly active (≥100-fold less potent than the dimethyl parent) [59]. On the other hand, considerable tolerance was allowed for un-natural, non-alkyl glycines, of the type (36). Thus, whilst \( \alpha \)-ethylglycine was 10-fold less effective than alanine, the n-propyl and n-butyl analogues showed no subsequent losses in potency [59].

These compounds are therefore all substantially more potent than the 'natural' glycine system. Replacement of the side-chain in (36) by a phenyl did however lead to a further loss of activity, to yield a material similar in potency to the glycine compound [58,59].

**Amino Acid Stereochemistry**

Given the importance of the amino acid side-chain it became interesting to probe its stereochemical requirements. Thus, we prepared the isomeric, D- compound related to (16, R=Me) and found it to be 20-30 fold less effective than the L-parent [60]. Despite this reduction in potency, the D-compound did retain full potency in TK-deficient CEM assays, indicating its functioning entirely as a d4TMP delivery form, with little or no free d4T release. It is further interesting to note that the D-alanine compound is of similar (slightly higher) potency as the glycine analogue [58,60]. This implies that a methyl group on the L-face of the amino acid (as in L-alanine and \( \alpha,\alpha \)-dimethylglycine) contributes about a log in potency to the baseline of glycine, that the D-face-methyl group of D-alanine cannot substitute, and that the Pro-D-methyl group of \( \alpha,\alpha \)-dimethylglycine is neither advantageous nor detrimental to potency.

**Amino Acid Replacement**

In this section we briefly describe some large-scale amino acid changes, which have a significant (largely negative) impact on potency. Thus, replacement of the amino acid in (16) by a family of non-aminoacyl simple n-
alkylamines (C3 to C12) leads to a complete removal of activity [61]. The same result was observed for simple alkylamine analogues of the AZT phosphoramidates (14) [61]. Thus, an aminoacid appears a pre-requisite for a successful aryloxy phosphoramidate approach. This is in marked contrast to recent data from the Montpellier group, who has prepared phosphoramidate - SATE hybrids of AZT, and found them to be highly potent and not to depend on an aminoacid type structure for potency [62]. As noted by Peyrottes et al. in this Compilation, this indicates a different mechanism of action for SATE - phosphoramidates as compared to aryloxy phosphoramidates (see below).

We had previously noted that chain extended aminoacid-related alklyoxy phosphoramidates of type (9) were poorly effective [12]. It was of interest to extend this study to analogous beta- and other aminoacids, when applied to aryloxy derivatives of d4T. Thus, compounds (37) were prepared and found to be very poorly active; all extended compounds were ca 40-times less potent than the glycine parent compound [63]. Interestingly the β-alanine compound (37, n=2) was thus ca 2500 times less potent than its structural isomer, the alanine lead compound (16, R=Me). It is striking therefore, that the beta- and further extended aminoacids are no more effective as aryloxy phosphoramidate motifs than simple n-alkylamines [61,63] (Graph 2) and that the substantial potency benefit offered by aminoacids such as alanine does not extend to their beta- and longer isomers. We will discuss this SAR further below under Mechanism of Action.

Finally in this section, we note that the bridging aminoacid nitrogen atom is vital for the antiviral potency, since its replacement by a bridging oxygen atom, as in (12), but now for aryloxy phosphoramidates of d4T (38), leads to a very significant loss (ca.600-fold) of activity [64].

Aryl Substitution

We had previously noted the effect of aryl substitution on aryloxy phosphoramidates of AZT (14) [21,22]. We now extended this study to d4T with a variety of substituents ranging from electron donating to electron withdrawing (39) [65]. It was notable that strongly electron withdrawing groups (p-CN, p-NO2) lead to slight reductions in potency, whilst several substituents, such as the p-COOEt and, particularly p-Cl groups showed significant potency boosts. The p-chloro compound emerges as a new lead structure, being 14-times more potent than the phenyl parent compound, with an EC50 versus HIV-1 in wild-type nucleoside kinase competent CEM cells of 5nM, and being fully active in the CEM/TK- assays. The chloro compound was also the most lipophilic studied, and logP measurements indicated a correlation (r=0.9) between measured logP and antiviral activity. However, this may be an artefact of the small sample size, and logP may not in itself correlate with activity in wider compound series. In general, this study concluded that lipophilic substituents which were mildly electron-withdrawing (σ 0.15 - 0.48) were preferred [65]. Indeed, a subsequent more rigorous QSAR analysis of 21 compounds of this type confirmed lipophilicity as an important factor, with steric and electronic factors of more secondary importance [66].

Uckun et al. also noted the efficacy of compounds of the type (39), and in particular with a para-bromo substituent [67-69]. This group later went on to suggest these agents as virucidals [70,71].

Phosphate Stereochemistry

One of the notable structural features of all of the phosphoramidate triesters, excluding some phosphorodiamidates [14], is the presence of a chiral centre at the phosphate. Due to the chirality of the nucleoside all of the compounds prepared are thus isolated as a pair of diastereoisomers. In this regard the phosphoramidate approach is similar to the cycloSAL approach of Meier reported in this compilation. As early as 1990 we had started to partly separate the diastereoisomers [10]. Thus, some allyloxy phosphoramidates of AZT (8) were partly separated by flash silica chromatography and fractions enriched in the more lipophilic (‘fast’) and less lipophilic (‘slow’) isomer were separately evaluated. We found a small, ca 3-fold, difference in potency, with the ‘fast’ isomer being less potent. A subsequent study on some mixed haloalkyl triesters of the type (6) indicated a 10-fold difference, with the ‘fast’ isomer being more potent [8]. In neither case was any link made between the absolute chirality at the phosphorus atom and the relative lipophilicity/potency; such work was only to emerge later. Thus, working with alanine phosphonamidates of PMEA (34), researchers at Gilead found a 10-fold difference in potency, with the S-phosphate isomer being more potent [52]. Their recent disclosure of a large scale synthesis and purification of the most active isomer clears the way towards clinical evaluation of single isomers of phosphoramidate triesters [54].

MECHANISM OF ACTION STUDIES

The putative first activation step for these phosphoramidates is esterase mediated carboxyl ester cleavage. In an effort to model this in a predictive sense, we exposed various esters of type (16) to pig liver esterase, and followed the P-31 NMR signal [56]. The parent compound always shows two closely spaced signals, due to the phosphate diastereoisomers. Upon esterase treatment these signals collapsed to give a downfield singlet (δP 8.2ppm). This was characterised as the amino acyl phosphate monoester (Fig. 3, A). Whilst the rate of cleavage of various esters did not readily correlate with antiviral activity, we did note that esterase lability was a necessary (but not sufficient) condition for high biological potency; the t-butyl ester in particular was not hydrolysed, and that phosphoramidate was the least potent assayed in cell culture [56].

As noted above, subsequent studies on compounds with chain-extended aminoacids of type (37) [63] showed the importance of the α-aminoacid for activity. Indeed, the esterase study on these compounds, each of which had a methyl ester, showed that they were all well processed. However, only the α-compound, derived from glycine, proceeded to form the intermediate of type A (Fig. 3). The beta- and longer homologues were processed only to the carboxylate B (Fig. 3). It appears that for entropic, or steric, reasons the loss of the phenyl group from these systems does not proceed under the conditions of the assay, diesters of type A do not arise, and the antiviral activity is poor. We surmise that an alpha aminoacid (or similar) is required for neighboring group assistance to displace the phenyl group,
and that in its absence phenyl loss does not take place, and moreover, that in the absence of such a displacement the phenyl group is not lost in vitro, and thus the antiviral action is limited.

Similar mechanistic conclusions were derived by the Montpellier group [72] on the isoddA compounds of Franchetti (18) using an elegant online ISRP-cleaning HPLC method they had earlier reported for POM esters [73]. However, in contrast to our work, under some conditions this group noted an alternative pathway involving loss of the aryl group prior to ester hydrolysis, to give compounds of the type C (Fig. 3) [72]. We believe this to be specific to compounds with strong electron withdrawing aryl substituents (e.g. nitro) and/or under conditions of reduced esterase activity, and may be of limited in vitro significance for un-substituted phenyl systems.

We confirmed our general view using radio labelled compound (16, R=Me) incubated with a range of cell lines used in the antiviral assays and then studied by HPLC [74]. In these studies high levels of metabolite A were noted, which varied with the cell line, but in general corresponded with high anti-retroviral activity. Thus, compound ‘A’ was suggested as an important intracellular depot form for the free nucleotide [74]. This study was subsequently extended to include a wide range of compounds of the general type (36), with varying aminoacids and esters, and also chain extended compounds (37), and AZT analogues (14) [75]. Again, the generation of intermediates of the general type ‘A’ was seen as a necessary condition for high antiviral potency. For the first time, this study rationalised many of the SARs noted previously, such as the strong aminoacid preferences we had seen [58]. For example, valine had been noted to be a poorly effective aminoacid, and its phosphoramidate was now of to be poorly processed by carboxy esterase, human serum, and CEM cell extracts to the type ‘A’ compound [75].

In this study, the final step in conversion of metabolites ‘A’ to the free nucleotide was also studied, namely the cleavage of the aminoacid moiety. This represents a phosphoramidase activity [EC 3.9.1.1] as originally defined by Dixon and Webb [76]. However, a variety of phosphoramidase preparations have been reported from a range of sources, and “pure” enzyme has proved elusive [77-81]. Using a rat liver cytosolic preparation we were able to partially purify a fraction with the ability to hydrolyse compounds of type A to generate d4TMP. The fraction was distinct from creatine kinase, alkaline phosphatase and phosphodiesterase on the basis of its lack of inhibition by known reagents (enzyme inhibitions), and may truly be described as a phosphoramidase [81]. It appears to have a molecular weight in the range of 50-100KDa, has a pH optimum of 7.4, and is inhibited by the phosphoramidase inhibitor iodobenzene [82].

Lastly, when exposed to carboxyl esterase hydrolysis the poorly active lactate and glycolate compounds (38) [64] gave a rapid hydrolysis to give amino acyl intermediates related to ‘A’ (but O-bridged). Thus, we surmise that such lactates are poorly effective as antivirals due to poor onward processing of this intermediate by phosphodiesterase under the conditions of the assay, and that such analogues are not substrates for the putative phosphoramidase that hydrolyses ‘A’.

**CONCLUSIONS**

The aryloxy phosphoramidate approach has emerged, along with SATE, CycloSAL, aryl phosphoramidate diester and others [1,2] as a viable method for the intracellular
delivery of free monophosphates of a range of nucleoside analogues. The approach works poorly for AZT, where the second phosphorylation is rate limiting [24] but well for d4T and a range of dd and d4 nucleosides. D4A is a particularly dramatic example with >1,000 fold boosts in potency on phosphoramidate formation. Indeed, recent work from our labs in collaboration with researchers at GlaxoSmithKline on the carbocyclic L- d4A (Fig. 4) has shown potency enhancements of almost 10,000 on phosphoramidate formation [83]. We believe that this has reached the levels that we originally described over 10 years ago as ‘kinase bypass’ [5], wherein an ‘inactive’ nucleoside is activated by phosphate pro-drug formation. This suggests the prudency of pro-tide synthesis on a range of nucleoside analogues, and particularly not just those selected as active in initial screens; the inactivity of other structures may simply correspond to poor initial phosphorylation, which may now be by-passed with pro-tides. The activity of the phosphoramidates of the highly modified Zemlicka nucleosides highlights this point well [36].

In terms of SARs, it has emerged that an alpha aminoacid is essential for this approach, by contrast to phosphoramidate-SATE hybrids of the Montpellier group. Alanine remains a good choice of aminoacid, although the achiral α,α-dimethylglycine is a good alternative. The ester and aryl moieties can be varied considerably, provided the ester can be cleaved by esterase, and the aryl is a reasonable leaving group. A P-N bond is also essential for activity, with lactate isosteres being poorly active. This appears to correlate with putative phosphoramidase mediated cleavage of the key amino acyl intermediate (A, Fig. 3). Summary SARs and their mechanistic origins are collected together in (Fig. 4).

Finally, the issue of phosphate stereochemistry is worth considering. All of the current syntheses of phosphoramidate triesters currently give mixtures, often approximately 1:1, of the phosphate diastereoisomers. These can be separated by chromatography, and often have 5-10 fold potency differences. Whilst it may be possible for mixtures to be progressed towards the clinic, such progression faces substantial regulatory hurdles, and separation and clinical evaluation of pure isomers seem more likely. The recent example of Gilead’s GS-7340 supports this view [54]. On the Kilo scale, Gilead is using moving bed chromatography to separate the unwanted diastereomer. On a smaller scale, we have reported the novel use of molecular imprinted polymers (MIPs), which may be a useful tool in the research lab in this regard [84].

As in vivo data begin to emerge on arylxy phosphoramidates [85] and with clinical evaluation proceeding, the next few years will confirm whether or not they have a role to play in future drug development for viruses and beyond.

REFERENCES

Aryloxy Phosphoramidate Triesters as Pro-Tides

Mini-Reviews in Medicinal Chemistry, 2004, Vol. 4, No. 4 381


IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 9

Aryl Phosphoramildate Derivatives of d4T Have Improved Anti-HIV Efficacy in Tissue Culture and May Act by the Generation of a Novel Intracellular Metabolite

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New phosphate derivatives of the anti-HIV nucleoside analogue d4T were prepared as potential, membrane-soluble prodrugs of the biactive free nucleotide. The enhanced antiviral potency and/or reduced cytotoxicity of the derivatives leads to an increase in selectivity relative to the parent nucleoside analogue. Moreover, the derivatives appear to bypass the dependence of the nucleoside on thymidine kinase-mediated activation, retaining full activity in thymidine kinase-deficient cells. This strongly suggests the successful intracellular delivery of free nucleotides by the masked phosphate prodrug produgs. This is further confirmed by studies using radiolabeled compound which clearly demonstrate the generation of d4T mono-, di- and triphosphates from the prodrug, even in thymidine kinase-deficient cells. Moreover, we herein report the generation of a new metabolite, a partially hydrolyzed phosphate ester, alaninyl d4T monophosphate. We suggest that at least part of the antiviral action of the produgs derives from the intracellular generation of such novel diesters which may add considerable weight to the suggested further preclinical development of the phosphate produgs.

Introduction

Several members of the 2',3'-dideoxynucleoside (ddN) series and potent inhibitors of human immunodeficiency virus (HIV) in cell culture. The 5'-triphosphates of these nucleoside analogues are potent inhibitors of HIV reverse transcriptase. As a rule, the activation (phosphorylation) of these nucleosides is in most cases accomplished by cellular nucleoside and nucleotide kinases. Thus, in contrast to other antiviral agents (e.g., acyclovir) where (in cases simpler) virus-specific thymidine kinase mediates the first step of the conversion of the drug to the intracellular active species, the 2',3'-dideoxynucleoside analogues depend on cellular nucleotide kinases for their phosphorylation. However, in many cases the ddN derivatives have a poor affinity for nucleoside kinases (e.g., 2',3'-dideoxyuridine for deoxy uridine kinase, 2',3'-dideoxyhydroxy-2',3'-dideoxythymidine and 2',3'-dideoxyuridine for thymidine kinase, 2',3'- dideoxyadenosine for adenosine kinase and deoxy uridine kinase, and 2',3'-dideoxyinosine for 5'-nucleotidase). Moreover, the dependence on phosphorylation for activation of the particular nucleoside analogue may be a particular problem in cells where the nucleoside kinase activity is known to be low or even lacking (i.e., monocytes/macrophages). Therefore, we have sought to overcome this dependence on nucleoside kinase activation by the development of a suitable nucleotide delivery strategy. The viability of such an approach is entirely based on the ability to suitably modify the phosphate structure of a membrane-soluble masked nucleotide to enable intracellular delivery and release of the free phosphate form. We have previously noted the success of this strategy with (aryloxy)phosphoramidates (2) derived from AZT (1). These phosphoramidates retained good activity in thymidine kinase-deficient (TK) cells, by comparison to thymidine kinase-competent cells, indicating a (partial) independence of thymidine kinase activation. We were particularly keen to apply this technology to the 2',3'-dideoxy-2',3'-dideoxa analogue of thymidine (d4T) (3) for several reasons. This nucleoside analogue has been noted to be a potent inhibitor of HIV, but displays reduced cytotoxicity in certain cells (e.g., bone marrow progenitor cells) compared to AZT (1,2). Furthermore, it is known that the kinetics of the three phosphorylation steps from the nucleoside analogue to the (biactive) triphosphate differ in the case of d4T, by comparison to AZT and other 3'-modified nucleoside analogues. In particular, the rate-limiting step for AZT appears to be the conversion of mono- to di-phosphate, whereas the conversion of nucleoside to monophosphate may well be rate-limiting for d4T. It could follow that the intracellular delivery of preformed d4T monophosphate (d4TMP) may be more useful than the delivery of AZT monophosphate. From a series of blocked phosphate esters of d4T we have noted significant and selective anti-HIV activity for a phosphoramidate derivative S0324 which we herein describe. Particularly interesting is the observation that this material may act by an additional mechanism of action.

Results and Discussion

Chemistry. D4T (3) was prepared from thymidine essentially by the method of Herwitz et al., noting the later commentaries of Mansuri et al. Then, phenyl metoxyalanylaminophosphorochloridate was allowed to react with d4T using THF/N-methylimidazole to give compound 4a in good yield (88%). As anticipated, this material displayed two closely spaced signals in the 31P NMR (δ, ca. 3.3 ppm) corresponding to the presence of diastereoisomers, resulting from mixed stereochemistry at the phosphate center. Similar diastereomic splitting, and phosphorus coupling where appropriate,
were also noted in the H-decoupled 13C spectrum. The presence of diastereoisomers was also apparent from 1H NMR spectroscopy, and analytical HPLC studies on 4a.

Similarly prepared were the analogues of 4a with other amino acids: glycine 4b, valine 4c, leucine 4d, phenylalanine 4e, and methionine 4f. Each of the analogues was isolated as a mixture of [phosphate] diastereoisomers, as evidenced from spectroscopic and chromatographic data.

We were also interested in studying the structure-activity relationships operating in the aryl region of the phosphoramide. In particular, we had previously noted that, for diaryl phosphates of AZT, the introduction of electron-withdrawing groups within the aryl moieties leads to a substantial increase in antiviral activity. This was taken as corresponding to the increased ability of such systems to undergo intracellular liberation of the aryl moiety and release of the phosphate onto the free nucleosides. Thus, we prepared the 2,4-diformyl-5a, 3-trifluoromethyl 5b, tert-butyl 5c, and 3,5-dichloro 5d analogues, each with some degree of electron withdrawal. For purposes of comparison, we also prepared the 4-ethyl compound 5e which lacks any electron-withdrawing nature.

Finally, we prepared the analogues lacking the aryl moiety entirely, and instead carrying a methyl group (6a) or an ethyl group (6b). These were designed to simply probe the necessity for antiviral action of an aryl group.

Antiviral Activity. The parent nucleoside d4T (3) and phosphoramidates (4a-f, 5a-e, and 6a,b) were tested for their ability to inhibit the replication of HIV, as previously described, and the results obtained using HIV-1- or HIV-2-infected CEM and MT-4 cells are displayed in Table 1. The clinically used nucleoside analogue AZT (1) was included as reference material, and tests were also conducted in thymidine kinase-deficient CEM cells.

It is notable that the phosphoramidate derivative 4a is approximately 4-10-fold more potent than d4T itself against HIV-1 and HIV-2 in MT-4 and CEM cells. Moreover, the >5-fold reduced cytotoxicity of 4a by comparison to d4T 3 in MT-4 cells leads to a 50-fold improved selectivity index for the phosphoramidate. Furthermore, it is particularly striking that, whereas d4T is virtually inactive in thymidine kinase-deficient CEM cells, the phosphoramidate 4a retains full activity, being ca. 100-fold more potent than d4T in these cells. Similarly, while AZT is inherently more potent than either d4T or the d4T phosphate in thymidine kinase-competent CEM cells, the phosphoramidate derivative 4a is ≥1000 times more active than AZT in the kinase-deficient CEM cell line.

In terms of structure-activity relationships in operation, it is apparent that relatively small changes in the amino acid region lead to significant changes in activity. Thus, of the series 4a-f, the alanine compound 4a is the most potent, with leucine 4d, phenylalanine 4e, and methionine 4f analogues being approximately 10-fold less active, depending on the virus and cell line in question. The glycine compound 4b is less active still, and the valine analogue 4c is the least active of the series, being approximately 100-fold less potent than the lead alanine compound 4a. However, throughout this series, it is notable that full activity is always retained in TK- cells by comparison to the activity shown in TK+ cells.

Similarly, there is some variation in activity with varying substitution in the arylxoy moiety, studying the series 4a, and 5a-e. The dibromophenyl analogue 5a is perhaps slightly more active than the parent phenyl compound 4a, although this is at the cost of some cytotoxicity in CEM cells. The (trifluoromethyl)phenyl (5b), dichlorophenyl (5d), and the ethylphenyl (5e) compounds are approximately equipotent with the parent phenyl system, while the pentfluorophenyl analogue 5c is approximately 50-fold less potent. Again, unlike the parent phenyl compound, some of the substituted aryl analogues appear to exhibit significant cytotoxicity. As a result, the highest selectivity index is displayed by the parent phenyl compound 4a.

Finally, the importance of an aryl moiety for antiviral activity is most evident noting the data for the methyl (6a) and ethyl (6b) phosphates. These show very little antiviral activity, being approximately 10,000-fold less potent than the lead compound 4a.

Data in TK- versus TK+ cells clearly demonstrate that the antiviral activity of the phosphoramidates 4a-f and 5a-e is independent of thymidine kinase-mediated phosphorylation. The most obvious mechanism of action consistent with this observation is of intracellular delivery of the free nucleotide d4TP from the phosphoramidates, and further phosphorylation to generate the active metabolite d4T triphosphate (d4TTP). In order to test this hypothesis, we incubated CEM cells with 3H-labeled compound 4a and subsequently studied the formation of radioactively labeled intracellular metabolites by HPLC. Markers of authentic d4T mono- and di-, and triphosphate were included, along with d4T (3) and blocked phosphoramide 4a. The study was compared with labeled d4T (3), and both experiments were also performed using thymidine kinase-deficient CEM cells. Measurable levels of d4T mono-, di-, and triphosphate were formed from either d4T incubation or incubation with 4a using kinase-competent cells. However, with kinase-deficient cells there was no detectable phospho-
Figure 1.

On the other hand, levels of d4T triphosphate generated by 4a incubation were entirely maintained in the thymidine kinase-deficient cells. Indeed, CEM (wild type) and CEM-11 cells generate 0.25 and 0.48 nmol/mL cells of d4TTP respectively after 20 h of incubation with 0.2 μM 4a. There was thus clear evidence that 4a could give rise to significant levels of the bioactive metabolite d4T triphosphate, by a mechanism which was entirely independent of thymidine kinase, unlike d4T which could give similar levels of the triphosphate, but by an entirely thymidine kinase-dependent process. This is consistent with the suggested intracellular hydrolysis of phosphoramidates such as 4a to give d4T monophosphate and the thymidylate kinase-mediated phosphorylation of this material to the higher phosphates. The alternative hydrolysis of 4a to give d4T could not play a major role in the activation pathway, otherwise the generation of d4T triphosphate (and the antiviral activity) would have been significantly reduced in thymidine kinase-deficient cells. However, we noted in the HPLC chromatogram arising from incubation with radiolabeled 4a, using either kinase-competent or kinase-deficient cells, the formation of high levels of a new metabolite, of apparent polarity intermediary between d4T mono- and diphosphate. The concentration of this material, as estimated from the radiolabel, was approximately 10-200 times the levels of d4T triphosphate generated, depending on the initial concentration of the phosphoramidate 4a. At its EC50 (ca. 0.2 μM) 4a generates levels of the new metabolite 10-15-fold higher than the levels of d4T triphosphate (d4TTP) generated from d4T administration to the cell cultures over a comparable incubation time (24 h). At longer incubation times (72 h) the new metabolite was still present at ca. 10-fold higher levels than those of d4TTP. Eventually we were able to prepare a synthetic material identical to this metabolite, on the basis of HPLC retention time in two different systems, by the regioslective base-catalyzed hydrolysis of compound 4a. This product was then identified as the novel phosphate diester 7 arising from cleavage of both the phenyl and methyl ester groups. We noted that this material could also be generated from 4a using hog liver esterase, incubated for 24 h at 37°C. Further evidence for the structure of the metabolite, identified as 7, came from the observation that compounds 4c, 4e, and 4f gave products with hog liver esterase with similar, but nonidentical HPLC retention times. This is entirely consistent with the suggestion that the amino acid moiety is retained in the metabolite. Furthermore, the metabolite and the synthetic material 7 were both stable to alkaline phosphatase, as anticipated for the proposed structure. We wondered whether the metabolite 7 could exert antiviral action via release of d4T (at least in TK+ cells) or d4T monophosphate, and subsequent phosphorylation to the triphosphate act in an entirely novel way. Indeed, we suggest that the much greater concentrations of 7 generated, by comparison to the intracellular levels of d4T triphosphate,
Indicates that 7 could contribute to the antiviral efficacy of phosphonamidates such as 4a via intracellular release of d4TMP. However, it is likely that the contribution of each mechanism to the overall antiviral effect will vary with the exact structure of the phosphonamidate, the initial drug concentration, and the cell type studied. It is quite feasible that some of the structure—activity relationships we noted above, particularly involving variations in the amino group/methyl portion, may relate to the efficiency of the intracellular generation of d4TP monophosphate and/or the formation of the phosphate diester metabolite 7. Alternatively, and particularly in the case of the amino acid variations, some of the apparent preference for certain amino acids may also relate either to the relative efficiency of formation of the analogues of (7) to or the efficiency of their subsequent activation to d4TTP. This is currently under active investigation in our laboratory.

In conclusion, the phosphonamidate derivatives of d4T herein described showed advantage over d4T itself, particularly in thymidine kinase-deficient cells. This leads to enhanced antiviral selectivity relative to the parent drug. Metabolic studies indicate that the phosphonamidates and/or the d4TTP levels in TK- cells. We also note the novel discovery of a new metabolite, a phosphate diester retaining the amino acid. We thus report on a new mechanism of inhibition of HIV; the d4T phosphonamidates can act via a d4T or d4TMP depot form, which may be entirely independent of thymidine kinase. We suggest that this new discovery offers great promise for the development of new improved therapies for HIV infection and AIDS.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine and dichloromethane were dried by heating under reflux over calcium hydride for several hours followed by distillation. Dichloromethane was further dried over activated 4 Å molecular sieves. Tetrahydrofuran was dried by heating under reflux over sodium and benzophenone followed by distillation. N,N-Dimethylformamide was purified by distillation. Nucleosides were dried by storage at elevated temperature in vacuo over P2O5. Proton, carbon, and phosphorus Nuclear Magnetic Resonance (1H, 13C, 31P NMR) spectra were recorded on a Bruker Avance DPX spectrometer operating at 300.75, and 121.5 MHz, respectively, with 1H and 31P spectra being recorded proton-decoupled. All NMR spectra were recorded in CDC13 at room temperature (20 ± 3 °C). 1H and 13C chemical shifts are quoted in parts per million downfield from tetramethylsilane. Values refer to coupling constants, and signal splitting patterns are described as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m), or combinations thereof. 31P chemical shifts are quoted in parts per million relative to an external phosphoric acid standard. Many proton and carbon NMR signals were split due to the presence of phosphonate diastereoisomers in the samples. The mode of localization for mass spectroscopy unless stated was fast atom bombardment (FAB) with MNOBA as matrix. Chromatography refers to flash column chromatography and was carried out using Merck silica gel 60 (40-63 μm) as stationary phase. Thin layer chromatography was performed using Alugram SIL G/UV254 aluminum-backed silica gel plates. HPLC was conducted on an AAS quaternary system, using an ODS column and an eluant of water/acetonitrile with 82% water 0–10 min, then a linear gradient to 20% of water at 30 min, with a flow rate of 1 mL/min and detection by UV at 255 nm. Final products showed purities exceeding 98% with undetectable levels (<0.02%) of parent nucleosides in every case. (Aryl)phosphonamidines, and (aryl)phosphorothioamidates were prepared entirely as previously noted.

General Procedure. Phenyl methoxazolinyl phosphonoester (240 mg, 0.96 mmol, 2.0 eq) was added to a stirred solution of d4T (300 mg, 0.45 mmol, 1 eq) and V-methylimidazole (143.5 μL, 1.8 equiv) in tetrahydrofuran (121 mL) at ambient temperature. After 4 h, the solvent was removed under reduced pressure. The gum was dissolved in chloroform (10 mL) and washed with 1 M HCl (10 mL), sodium bicarbonate solution (10 mL), and water (5 × 15 mL). The organic phase was dried (MgSO4), and the volatiles were removed in vacuo. The residue was purified by column chromatography on silica with elution by chloroform–methanol (97:3). Pcoiling and evaporation of appropriate fractions gave the product as a white foam.

2,3′-Dideoxy-2,3′-didehydroxydymine 5′-(phenyl methylxazolinyl) phosphate (2a); yield 93%; λmax 250 (3.12), 265 (3.10), 310 (3.11), 333 (3.18), 350 (3.18), 400 (3.06), 450 (3.04), 500 (3.06), 530 (3.03), 550 (3.02) (in 95% MeOH/H2O).

3′-Dideoxy-2,3′-didehydroxydymine 5′-(phenyl methylxazolinyl) phosphate (2b); yield 96%; λmax 250 (3.28), 265 (3.27), 305 (3.26), 325 (3.25), 355 (3.23), 405 (3.20), 465 (3.17), 500 (3.14), 550 (3.12) (in 95% MeOH/H2O).

2,3′-Dideoxy-2,3′-didehydroxydymine 5′-(phenyl methoxazolinyl) phosphate (2c); yield 96%; λmax 250 (3.29), 265 (3.27), 305 (3.26), 335 (3.25), 385 (3.23), 425 (3.20), 465 (3.17), 515 (3.12) (in 95% MeOH/H2O).

2,3′-Dideoxy-2,3′-didehydroxydymine 5′-(phenyl methoxyxalyl) phosphate (2d); yield 96%; λmax 250 (3.30), 265 (3.28), 305 (3.26), 355 (3.24), 405 (3.20), 445 (3.15), 495 (3.11), 535 (3.07) (in 95% MeOH/H2O).

Medical and Dental University School of Medicine, Tokyo, Japan) CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD) and CEM/TK cells were a gift from Prof. S. Eriksson and Dr. A. Karlsson (Karolinska Institute, Stockholm, Sweden). MT-1 and CEM cells were infected with HIV-1 as previously described. 9 Briefly, 5 x 10^6 MT-1 or CEM cells/mL were infected with HIV-1 or HIV-2 at approximately 100 CCD50 (50% cell culture infective dose/mL) per cell suspension. Then, 100 µL of the cell suspension was transferred to round bottom 48-well plates and mixed with 100 µL of the appropriate dilutions of the test compounds. After 4 days, cell growth was recorded microscopically in the HIV-infected CEM cell cultures, and after 5 days, the number of viable cells was determined by trypan blue staining of the HIV-infected MT-1 cell cultures. The 50% effective concentration (EC50) and 50% cytotoxic concentration (CC50) were defined as the concentration required to reduce by 50% the number of viable cells (MT-1) or giant cells (CEM) in the virus-infected and mock-infected cell cultures, respectively.

Cells used to establish CEM and MT-1 cultures were incubated in 24-well tissue culture plates (Costar, Cambridge, MA), each containing 1200 cells/mL. Each plate contained 100 µL of fresh cell culture medium containing 10% fetal bovine serum. After 6 days the transformation of the test cultures was examined microscopically. The EC50 was defined as the compound concentration that was required to inhibit MSV-induced transformation by 50%.

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References


IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 10

WO 2006/067606
Title: URIDINE DERIVATIVES AS ANTIVIRAL DRUGS AGAINST A FLAVIVIRIDAE, ESPECIALLY HCV

Abstract: The invention relates to the use of an uridine derivative of formula (I): wherein R1 represents monohalogenated alkynyl or dihalogenated alkenyl; R2 is chosen from among hydrogen, hydroxyl, O-alkyl, O-CO alkyl and halogen; R3 is chosen from among hydrogen, hydroxyl, O-alkyl, O-CO alkyl, halogen, SH, S-alkyl and N2; and R4 is chosen from among hydroxyl, O-alkyl, O-CO alkyl, O-phosphate, O-diphosphate, O-triphosphate and O-phosphonate, as well as its possible tautomers, its possible pharmaceutically acceptable addition salts with an acid or a base, and its N-oxide forms, for the preparation of a drug having antiviral activity against a Flaviviridae.
URIDINE DERIVATIVES AS ANTIVIRAL DRUGS AGAINST A FLAVIVIRIDAE, ESPECIALLY HCV

The present invention relates to the use of uridine derivative(s) for the preparation of a drug having antiviral activity against a Flaviviridae, e.g. against hepatitis C virus (HCV). It also relates to new compounds having such antiviral activity, to drugs incorporating them and to a method of treating a subject (a human or an animal) with such a drug.

Hepatitis C Virus (HCV) infection remains a main public health preoccupation with 175 million people infected in the world. Currently, the best treatment available consists in the association of pegylated interferon (IFN) alpha 2b and ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide). 20% of patients infected with HCV genotype 2 or 3 and 50% of patients infected with HCV genotype 1 are responders to this treatment. Therefore new treatment regimens based on more potent and specific inhibitors of HCV replication are required to improve the current antiviral strategies against chronic HCV infection.

The absence of a cellular system able to allow a complete multiplication cycle of HCV in vitro is a limiting factor for the search of new drugs. Recently, major knowledge has been gained about the mechanism of HCV replication. Study models have been developed such as the replicon system described in Ju-Tao Guo et al. (J. Virol. 2001, 75, 18: 8516-8523), allowing replication of HCV subgenome in cell culture.


The pharmaceutical importance of nucleoside analogues against viral DNA polymerases such as HIV-RT, HBV and HSV polymerases, has prompted the design and synthesis of analogous viral RNA polymerase. While numerous of non-nucleoside inhibitors of NS5B-

Providing the art with new efficient and acceptable anti-Flaviridae, in particular anti-HCV, drugs or treatments would be of great benefit.

An objective of the invention is to provide the art with new treatments effective against a Flaviviridae infection, e.g. against FCV, say new treatments having a strong antiviral activity with no or acceptable side effects.

Another objective of the invention is to provide a treatment that is efficient against the major HCV genotypes, including genotypes 1, 2 and 3.

Another objective of the invention is to provide a treatment that is efficient against any HCV genotype.

These objectives are attained by the use of some uridine derivatives that will be described in details below.

A first object of the invention is the use of a uridine derivative of formula (I):

![Chemical Structure](image)

wherein

- $R^1$ represents monohalogenated alkyl or dihalogenated alkenyl;
- $R^2$ is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- $R^3$ is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH, -S-alkyl and N$^3$; and
- $R^4$ is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -O-phosphate,
  - O-diphosphate, -O-triphosphate and -O-phosphonate,

as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its N-oxide forms, for the preparation of a drug having antiviral activity against a Flaviviridae.

In the above formula (I):
"monohalogenated alkynyl" is $-\text{C} \equiv \text{C} = \text{X}$ or $-\text{C} \equiv \text{C} = \text{Ak}(\text{X})$, where $\text{Ak}(\text{X})$ denotes a linear or branched $\text{C}_1$-$\text{C}_{10}$ alkyl radical, one hydrogen atom of which being substituted by one halogen atom $\text{X}$;

"dihalogenated alkenyl" is $-\text{C}(\text{Y})=\text{CH}(\text{X})$, $-\text{C}(\text{Y})=\text{C}(\text{X})$alkyl or $-\text{C}(\text{Y})=\text{C} = \text{Ak}(\text{X})$, where $\text{Y}$ is halogen and $\text{Ak}(\text{X})$ denotes a linear or branched $\text{C}_1$-$\text{C}_{10}$ alkyl radical, one hydrogen atom of which being substituted by one halogen atom $\text{X}$;

"alkyl", unless otherwise indicated, represents a linear or branched $\text{C}_1$-$\text{C}_{10}$ alkyl radical;

"halogen" is fluorine, chlorine, bromine or iodine, preferably chlorine, bromine or iodine.

The dihalogenated alkenyl may present a $Z$ or $E$ configuration, the $E$ configuration being preferred, i.e. the two halogen atoms $\text{X}$ and $\text{Y}$ are not on the same side of the planar double bond.

Preferred configuration for $-\text{C}(\text{Y})=\text{CH}(\text{X})$ or $-\text{C}(\text{Y})=\text{C}(\text{X})$alkyl is therefore respectively:

\[\text{H} - \text{X} \quad \text{or} \quad \text{Alkyl} - \text{X} \quad \text{Y}\]

According to a first embodiment, a preferred uridine derivative of formula (I) useful for the preparation of a drug having antiviral activity against a Flaviviridae, presents the following characteristics:

- $\text{R}^1$ represents $-\text{C} \equiv \text{C} = \text{X}$ or $-\text{C}(\text{Y})=\text{CH}(\text{X})$;
- $\text{X}$ and $\text{Y}$ each independently represents halogen;
- $\text{R}^2$ is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- $\text{R}^3$ is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH, -S-alkyl and N$^3$; and
- $\text{R}^4$ is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -phosphate, -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate,

as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its N-oxide forms.

Preferred compounds of formula (I) are those where

- $\text{R}^1$ represents chloroethyl, bromoethyl, iodoethyl or $-\text{C}(\text{Y})=\text{CH}(\text{X})$, where $\text{Y}$ is chosen from among chlorine, bromine and iodine, and $\text{X}$ is bromine or iodine.
- $\text{R}^2$, $\text{R}^3$ and $\text{R}^4$ being as defined above,

as well as their possible tautomers, their possible pharmaceutically acceptable additions salts with an acid or a base, and their N-oxide forms.
Compounds where R² and R³ do not simultaneously represent hydrogen form another sub-
group of even more preferred compounds.

Particularly preferred compounds for the intended above-mentioned use are those wherein

- R¹ represents chloroethyl, bromoethyl, iodoethyl or \( -\text{C}(Y)=\text{CH}(X) \), where Y is
  chosen from among chlorine, bromine and iodine, and X is bromine or iodine.
- R² is chosen from among hydrogen, hydroxyl, and -O-alkyl;
- R³ is chosen from among hydrogen, hydroxyl, -O-alkyl, -SH, and -S-alkyl; and
- R² and R³ not simultaneously being each hydrogen; and
- R⁴ is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl -O-phosphate,
  -O-diphosphate, -O-triphosphate and -O-phosphonate,
as well as their possible tautomers, their possible pharmaceutically acceptable additions
salts with an acid or a base, and their N-oxide forms.

A most preferred uridine derivative of formula (I) is chosen from among the following
compounds:

- \( (E)\text{-}5\text{-}(1\text{-Chloro}-2\text{-iodovinyl})\text{-}2\text{'}\text{-deoxyuridine} \),
- \( (E)\text{-}5\text{-}(1,2\text{-Diodovinyl})\text{-}2\text{'}\text{-deoxyuridine} \),
- \( 5\text{-}(2\text{-Iodoethynyl})\text{-}2\text{'}\text{-deoxyuridine} \),
- \( (E)\text{-}5\text{-}(1\text{-Bromo}-2\text{-iodovinyl})\text{-}2\text{'}\text{-deoxyuridine} \),
- \( (E)\text{-}5\text{-}(1,2\text{-Dibromovinyl})\text{-}2\text{'}\text{-deoxyuridine} \),
- \( 5\text{-}(2\text{-Bromoethyl})\text{-}2\text{'}\text{-deoxyuridine} \),
- \( (E)\text{-}5\text{-}(1\text{-Chloro}-2\text{-iodovinyl})\text{-}uridine \),
- \( (E)\text{-}5\text{-}(1,2\text{-Diodovinyl})\text{-}uridine \),
- \( 5\text{-}(2\text{-Iodoethynyl})\text{-}uridine \),
- \( (E)\text{-}5\text{-}(1\text{-Bromo}-2\text{-iodovinyl})\text{-}uridine \),
- \( (E)\text{-}5\text{-}(1,2\text{-Dibromovinyl})\text{-}uridine \), and
- \( 5\text{-}(2\text{-bromoethyl})\text{-}uridine \),
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts
with an acid or a base, and its N-oxide forms.

Compounds of formula (I) wherein R⁴ represents -O-phosphate, -O-diphosphate,
-O-triphosphate and -O-phosphonate are deemed to represents pro-drugs of the
Corresponding compounds wherein R⁴ represents -OH. Other pro-drugs leading to
compounds wherein R⁴ is -OH are also encompassed within the present invention. Such
other pro-drugs include for instance esters, such as aminoacid esters, e.g. alanine esters
and glycine esters, quaternary salts of phosphate, and the like.
Acids that may be used to form pharmaceutically acceptable addition salts of the compound of formula (I) are organic or inorganic acids. Such salts include for example hydrochlorides, hydrobromides, sulfates, hydrogensulfates, dihydrogene-phosphates, citrates, maleates, fumarates, trifluoroacetates, 2-naphthalenesulfonate and para-toluenesulfonate.

Similarly, bases that may be used to form pharmaceutically acceptable addition salts of the compound of formula (I) are organic or inorganic bases. Such salts include for example metallic salts from alkali metals, alkaline-earth metals or transition metals, such as sodium, potassium, calcium, magnesium, aluminum. Other suitable bases include ammoniac or secondary or tertiary amines (diethylamine, triethylamine, piperidine, piperazone, morpholine) or basic amino-acids, or alternatively osamines (such as meglumine), or aminoalcohols (such as 3-aminobutanol and 2-aminoethanol).

The present invention also encompasses salts that are useful for suitable separation or crystallization of the compounds of formula (I), such as salts obtained from chiral amines or acids.

Example of chiral amines include for example quinine, brucine, (S)-1-(benzyloxy-methyl)propylamine, (-)-ephedrine, (4S,5R)-(+)-1,2,2,3,4-tetramethyl-5-phenyl-1,3-oxazolidine, (R)-1-phenyl-2-p-tolylethylamine, (S)-phenylglycinol, (-)-N-methylephedrine, (+)-(2S,3R)-4-dimethyamino-3-methyl-1,2-diphenyl-2-butanol, (S)-phenylglycinol, (S)-α-methylbenzylamine, alone or in mixtures.

Example of chiral acids include for example (+)-d-dio-O-benzoyltartaric acid, (-)-l-dio-O-benzoyltartaric acid, (-)-di-O,O'-p-toluy1-l-tartaric acid, (+)-di-O,O'-p-toluy1-d-tartaric acid, (R)-(+)malic acid, (S)-(+)malic acid, (+)-campanic acid, (-)-campanic acid, (+)-camphoric acid, (-)-camphoric acid, (S)-(++)-2-phenylpropionic acid, (R)-(++)-2-phenylpropionic acid, d-(++)-mandelic acid, l-(++)-mandelic acid, d-tartaric acid, l-tartaric acid, alone or mixtures.

In an embodiment, the invention more specifically relates to the use of such an uridine derivative and an efficient amount of an interferon (IFN), for the preparation of a drug having antiviral activity against a Flaviviridae. It also relates to the use of such an uridine derivative for the preparation of a drug having antiviral activity against a Flaviviridae, that is intended to be used in an recipient that is treated with an efficient amount of IFN. The recipient may have been treated recently with the IFN (for instance less than 30 days before), or received IFN at
approximately the same time or later, for instance less than 30 days. The IFN may be chosen among those that are described infra.

In another embodiment, the IFN is replaced or accompanied (concomitant or successive administration) by the use of another drug such as one of those described infra.

According to a preferred aspect, the Flaviviridae is HCV and the use of a uridine derivative of formula (I) is for the preparation of a drug having antiviral activity against HCV. There exists up to 6 HCV genotypes classified 1-6. Any of them is included in the present invention. The major genotypes are genotypes 1, 2 and 3, and a "use" according to the invention may be, for example, directed against HCV genotype 1, HCV genotype 2, HCV genotype 3, HCV genotypes 1 and 2, HCV genotypes 2 and 3, HCV genotypes 1 and 3, HCV genotypes 1, 2 and 3. The use may also be against any and all HCV genotypes, including quasispecies.

In another aspect of the invention, the Flaviviridae member may be a member of the Japanese encephalitis virus group, including Japanese encephalitis virus and West Nile Virus.

Alternatively it may be a member of the Yellow fever virus group.

Alternatively it may be a member of the Pestivirus group, such as Bovine viral diarrhea virus (BVDV-1 and/or BVDV-2), Classical swine fever virus, Border disease virus.

It may be appreciated that the recipient may be a human or an animal, depending on the Flaviviridae concerned.

Among the compounds of formula (I), 5-bromoethyl-2'-deoxyuridine is known and reported by Eger K. et al. (Tetrahedron, 1994, 50, 8371-8380), whereas compounds of formula (I), wherein R¹ represents dihalogenated alkenyl are new.

Therefore, a further object of the present invention is a compound of formula (I):
wherein
- \( R^1 \) represents dihalogenated alkenyl;
- \( R^2, R^3 \) and \( R^4 \) have the same definitions as \( R^2, R^3 \) and \( R^4 \), respectively, in the above formula (I);
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its N-oxide forms.

Compounds of formula (I) may be used for the preparation of a drug having an antiviral activity against a Flaviviridae.

In the above formula (I), "dihalogenated alkenyl" has the same meaning as defined above, i.e. \(-C(Y)=CH(X), -C(Y)=C(X)alkyl\) or \(-C(Y)=C-Ak(X)\), where \( Y \) is halogen and \( Ak(X) \) denotes a linear or branched \( C_1-C_{10} \) alkyl radical, one hydrogen atom of which being substituted by one halogen atom \( X \);

All definitions and preferred embodiments described hereinbefore for compounds of formula (I) also apply to compounds of formula (I'), \( R^1 \) not possibly representing however a monohalogenated alkynyl radical.

Accordingly, a preferred compound of formula (I') presents the following characteristics:
- \( R^1 \) represents \(-C(Y)=CH(X)\);
- \( X \) and \( Y \) each independently represents halogen;
- \( R^2 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- \( R^3 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH, -S-alkyl and \( N^2 \); and
- \( R^4 \) is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate,
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its N-oxide forms.
Preferred compounds of formula (I') are those where:
- $R^1$ represents $\text{-C(Y)=CH(X)}$, where $Y$ is chosen from among chlorine, bromine and iodine, and $X$ is bromine or iodine.
- $R^2$, $R^3$ and $R^4$ being as defined above,
as well as their possible tautomers, their possible pharmaceutically acceptable additions salts with an acid or a base, and their N-oxide forms.

 Particularly preferred compounds for the intended above-mentioned use are those wherein
- $R^1$ represents $\text{-C(Y)=CH(X)}$, where $Y$ is chosen from among chlorine, bromine and iodine, and $X$ is bromine or iodine.
- $R^2$ is chosen from among hydrogen, hydroxyl, and $\text{-O-alkyl}$;
- $R^3$ is chosen from among hydrogen, hydroxyl, $\text{-O-alkyl, -SH, and -S-alkyl}$; and
- $R^2$ and $R^3$ not simultaneously being each hydrogen; and
- $R^4$ is chosen from among hydroxyl, $\text{-O-alkyl, -O-CO-alkyl, -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate,}$
as well as their possible tautomers, their possible pharmaceutically acceptable additions salts with an acid or a base, and their N-oxide forms.

The present invention is further related to the process for the preparation of compounds of formula (I) and formula (I').

Halogenated pyrimidine 4a-c in Scheme 1 below are prepared by direct reaction with halogens following the procedure reported by Asakura et al. (J. Org. Chem., 1990, 55, 4928-4933) via a cerium(IV)-mediated halogenation at the C-5 of uracil derivatives. Thus, treatment of the known acetylated nucleosides 3a-c, according to a process well known by the one skilled in the art, with elemental iodine and ceric ammonium nitrate (CAN) at 80°C, gives the corresponding protected 5-iodouracil nucleosides 4a-c in excellent yields. This CAN-mediated halogenation has advantages over other available methods as mild reaction conditions are employed with short reaction times.

![Scheme 1](image_url)
In the above scheme 1, P and P', which may be identical or different, stand for protective groups of hydroxyl groups, and are well-known to the person skilled in the art. Preferably, protective groups P and P' are chosen from among acyl groups such as CH₃C=O, trityl, monomethoxytrityl, mesyl, and the like.

In scheme 1 above, protective groups P and P' each preferably an acyl group, specifically CH₃C=O.

The introduction of a C-5-alkynyl group is performed by a Pd(0)-mediated reaction, using the Sonogashira conditions (Sonogashira K., Comprehensive Organic Synthesis, Trost/Fleming (Eds), Pergamon Press, New York, 1991, vol 3, p. 521; Sonogashira K. et al.; Tetrahedron Lett., 1975, 16, 4467; Takahashi S. et al., Synthesis, 1980, 627; Ratovelomana V. et al., Synth. Commun., 1981, 11, 917). Thus, reaction of the resulting pure acetylated 5-iodouracil 4a-c with trimethylsilylacetylene in the presence of triethylamine (Et₃N, base), cuprous iodide (CuI, co-catalyst) and PdCl₂(PPh₃)₂ (catalyst) in anhydrous dimethyl formamide (DMF) at room temperature, followed by a removal of the trimethylsilyl (TMS) group with TBAF (tetrabutyl ammonium fluoride) affords the corresponding 5-ethynyluracil nucleosides 5a-c. In most cases, it is observed that slight elevations in the reaction temperature lead to increase in the rate of coupling. In all cases, a by-product, furanopyrimidine derivatives is either isolated with a < 7% yield, or just detected by TLC; nevertheless, the using of DMF reduces the amount of this cyclic by-product; this base-catalyzed cyclization was previously reported by Bleackley et al. (Tetrahedron, 1976, 32, 2795) by base treatment of (E)-5-(2-bromovinyl)uracil.

The halogenation of alkynes 5a-c is realized using halogenating agents (such as iodonium di-syn-collidine perchlorate (IDCP), N-iodosuccinimide (NIS) or chloroiodine (ICL) for iodination, bromonium di-syn-collidine perchlorate (Br(coll)₂ClO₄) for bromination, or, for chlorination, sulfuryl chloride, Cl₂, CCl₄, N-chlorophtallimide, dichlorine monoxide or POCl₃, in anhydrous polar solvent, for example methane nitrile (CH₃CN) according to reaction Scheme 2, where P and P' are as defined above for Scheme 1. These halogenating reagents are known to act as very reactive electrophiles (such as I⁺ and Br⁺, respectively).
The final deprotection of protected nucleosides 6a-c and 7a-c is realized under smooth basic conditions (for example 1M sodium hydroxide in a mixture of H₂O/EtOH/pyridine) when P = P' = Ac) to afford final 5-haloethynyluracil nucleosides 12a-c and 13a-c, respectively, in good to moderate yield.

Dihalogenation of the above 5-ethynyl nucleosides (Scheme 3) leads to hitherto unknown 5-(1,2-dihalogenated)ethynyluracil nucleosides 14a-c to 17a-c. Several halogenating reagents (i.e. I₂, IBr, ICl or Br₂) are used to reach the corresponding 1,2-dihaloderivatives. In all cases of halo-iodination, the formation of vicinal halo-iodoalkenes (8a-c to 10a-c) occurs with high anti-stereospecificity, implicating the intermediary of an iodonium ion in the reaction sequence, and a high and unique regioselectivity, implicating a predominantly Markovnikov addition. The formation of 11a-c using Br₂ occurs also with a E-stereochemistry.
The final deprotection of protected nucleosides 8a-c to 11a-c is realized as previously reported (for example 1M NaOH in a mixture of H₂O/EtOH/pyridine when P = P'Ac) and final 5-(1,2-dihaloethyl)uracil nucleosides 14a-c (for X = Y = I), 15a-c (for X = I, Y = Br), 16a-c (for X = I, Y = Cl), 17a-c (for X = Y = Br) are obtained, respectively.

All other compounds encompassed by formulae (I) and (I') are easily prepared from the above described synthetic route with easily conceivable modifications and/or adaptations well known by the one skilled in the art.

For illustrative purposes only, compounds of formula (I) or of formula (I') may be obtained according to scheme 4 below, wherein P preferably represents mesyl and P' preferably represents monomethoxytrityl.

![Chemical Structure](image)

Scheme 4

In scheme 4 above, compound 19 is obtained after specific deprotection, in mild basic conditions, the free resulting hydroxyl group being substituted by methods well known in the art, for example using diethylaminosulfur trifluoride, affording compound 21.

Compounds 21 and 22 are obtained from compound 20 under conditions similar as those described above for the preparation of compounds 12 and 13.

Compound 23 results from the deprotection of the P' protecting group of compound 20.
Compounds 24a-c and 25a-c are respectively obtained using a similar process as the one disclosed above for the preparation of compounds 8-11a-c and 14-17a-c.

As further illustrative purpose only, compounds of formula (I) and of formula ('I') wherein R³ (and R'^³ respectively) represents N₃ may be obtained according the following scheme 5.

Scheme 5

In the above scheme 5, P and P' each denote protective groups defined as defined above and advantageously P represents mesyl and P' represents monomethoxytrityl.

Azidation of compound 18 may be carried out using conventional techniques of azidation and for example using sodium azide in polar aprotic solvent, such as for example dimethylformamide under reflux, resulting in compound 26.

Compounds 27-31 are obtained through similar methods as those disclosed above for the preparation of compounds 21-25 respectively.

Compound 18 in scheme 4 and scheme 5 above may be obtained according to a process similar to the process for the preparation of compound 5 in the above scheme 1.

Alternatively, compound of formula 18 may advantageously be obtained according to the preparation process of scheme 6 below:
In scheme 6 above, P and P' are preferably different, each representing a protective group as defined above. Particularly preferred are compounds wherein P' represents monomethoxytrityl and P represents mesyl.

The introduction of the P' protective group is carried out using conventional techniques, and for example, where P' represents monomethoxytrityl using para-anisylchlorodiphenylmethane in mild basic conditions, such as dry pyridine at reflux.

The selective introduction of protective group I is also achieved using conventional techniques, and, for example, when P represents mesyl, using methanesulfonylchloride in mild basic conditions, such as for example dry pyridine at 0°C.

In scheme 6 above, the introduction of a C-5-alkynyl group is performed as stated above in scheme 1 for the preparation of compound 5.

Compounds 14-31 a-c are purified, when necessary, using conventional methods known in the art.

Compounds of formulae (I) and (I') may be obtained in pure enantiomeric form and/or isomeric form, or in mixture, in all proportions, of enantiomers and/or isomers. Separations of such enantiomers and/or isomers is run according to techniques well known in the art.
The above compounds are also optionally converted into pharmaceutically acceptable addition salts with an acid or a base, and optionally into their N-oxide form, using appropriate and suitable methods as known by the one skilled in the art.

All starting materials are known and either commercially available or prepared, directly or with slight variations easily conceivable by the one skilled in the art, from one or more processes disclosed in the scientific literature, patents and patents application, the "Chemical Abstracts", on-line data bases and the Internet.

Another object of the invention is a drug or drug formulation having an antiviral activity against a Flaviviridae, in particular HCV, comprising at least one compound of formulae (I) (the active ingredient) with a pharmaceutically acceptable vehicle, carrier or excipient. "Acceptable" means that this further ingredient is compatible with the active ingredient included in the formulation and is not deleterious to the recipient thereof. The pharmaceutically acceptable vehicle, carrier or excipient are well known to the one skilled in the art. For example, for parenteral administration, it can be a 0.9 % NaCl saline solution for a phosphate buffer. The pharmaceutical acceptable vehicles, carriers or excipients also cover any compound or combination of compounds facilitating the administration of the active ingredient and/or improving preservation.

In the whole specification and Claims, the words "comprise/comprising" are synonymous with (means the same thing as) "include/including", "contain/containing", are inclusive or open-ended and do not exclude additional, unrecited elements.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal or sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. These various formulations are well known, as well as their methods of preparation. The choice of the formulation may vary depending upon the identity of the recipient, and will generally be chosen according to the best practice for a human or for the animal considered. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Formulations for oral administration may be presented as discrete units such as, for example: capsules, cachets or tablets each containing a predetermined amount of active ingredient; a powder or granules; a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or an emulsion, e.g. a water-in-oil or an oil-in-water emulsion. The active ingredient may also be presented as a bolus, electuary or paste. The solid forms may optionally be coated and may be formulated so as to provide slow or controlled release of the
active ingredient. For infections of the eye or other external tissues (e.g. skin or mouth), the formulations are preferably presented as ointment or cream for topical application, containing an efficient amount of active ingredient. Formulations suitable for administration to the eye also include eye drops where the active ingredient is dissolved or suspended in a suitable carrier, such as an aqueous solvent. Formulations for rectal administration may be presented as a suppository with a suitable base. Liquid or solid carriers may be used for nasal administration. Pessaries, tampons, creams, gels, pastes, foams or spray formulations may be used for vaginal administration. Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions, suspensions or emulsions.

The amount of active ingredient in a single dosage form will depend upon a number of factors including the identity of the recipient and the condition to be treated. In general, however, for a human, the amount of active ingredient in a single dose form will be in the range, for example, of 1 mg to 10 g, preferably in the range of 100 mg to 5 g, most preferably in the range of 500 to 2000 mg.

For multiple dosage form, for example a cream or ointment, the concentration of active ingredient will depend upon a number of factors including the identity of the recipient and the condition to be treated. In general, however, for a human, the concentration of active ingredient in a multiple dose form will be in the range, for example, of 0.01 to 200 mg of active ingredient per g of formulation, preferably in the range of 0.1 to 100 mg/g, most preferably in the range of 1 to 50 mg/g.

In one embodiment, the drug formulation according to the invention further comprises an additional compound having an antiviral activity against a Flaviviridae or that is useful in the treatment of the infection or its symptoms. The use according to the invention thus encompasses the use of at least two different active ingredients, at least one of them being an uridine derivative as described herein.

In a first aspect, this additional compound is at least one formula (I) compound. Thus the invention formulation does comprise at least two formula (I) compounds or derivatives.

In a second aspect, the drug formulation having an antiviral activity against a Flaviviridae comprises an additional compound which is not an uridine derivative according to the invention. The drug formulation may be then represented as comprising:

(1) an uridine derivative of formula (I):
wherein
- \( R^1 \) represents monohalogenated alkynyl or dihalogenated alkenyl;
- \( R^2 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- \( R^3 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH,
  -S-alkyl and \( N^3 \); and
- \( R^4 \) is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -O-phosphate,
  -O-diphosphate, -O-triphosphate and -O-phosphonate,
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts
with an acid or a base, and its N-oxide forms,
(2) an immunomodulator or another active ingredient having an antiviral activity against a
Flaviviridae or being useful in treating the infection by a Flaviviridae, and
(3) a pharmaceutically acceptable vehicle, carrier or excipient.

The additional compound is advantageously an immunomodulator, especially an interferon
(IFN) or a derivative of an interferon such as the so-called pegylated forms of interferon
(pegylated interferon), such as any those currently used in the medical field. It may be in
particular IFN-\( \alpha \), e.g. IFN-\( \alpha \) 2b, such as pegylated IFN-\( \alpha \) 2b Peg-Intron\( ^{\circledR} \) (Schering Plough),
IFN-\( \alpha \) 2a, such as pegylated IFN-\( \alpha \) 2a Pegasys\( ^{\circledR} \) (Roche), Albuferon-a\( ^{\circledR} \) (Human Genome
Sciences), Multiferon\( ^{\circledR} \) (Viragen) ; IFN-\( \beta \), e.g. IFN-\( \beta \) 1a, such as Rebif\( ^{\circledR} \) IFN-\( \beta \) 1a (Serono) ;
IFN-\( \gamma \), e.g. IFN-\( \gamma \) 1b, such as IFN-\( \gamma \) 1b (from Interimmune Pharmaceuticals) ; IFN-\( \omega \), e.g. IFN-
\( \omega \) from Biomedines.

Other immunomodulators may be associated to the invention compounds, such as histamine
dihydrochloride (e.g. Ceplene\( ^{\circledR} \), Maxim Pharmaceuticals), Thymosine-a (SciClone
Pharmaceuticals), Interleukines such as IL-12 and IL-10, therapeutical vaccines (e.g. based
on the E1 protein), preparation for passive immunity transfer (e.g. Cicavir\( ^{\circledR} \), NABI).

More generally, the invention compounds may find an interest in the association to any
available drug used in the treatment of the infection by a Flaviviridae, especially HCV. Thus,
the drug formulation may include in addition an active principle chosen among antisens
molecules, protease inhibitors, helicase inhibitors, polymerase inhibitors, nucleoside or nucleotide analogs (chain terminators, ribonucleoside analogs, sugar modified nucleoside analogs), ribavirine or ribavirine pro-drug, inosine monophosphate dehydrogenase inhibitors.

The invention encompasses of course combinations of at least two different additional compounds with an uridine derivative according to the invention.

In a third aspect, the drug or drug formulation according to the invention comprises at least two formula (I) compounds and at least one additional compound as described above, in particular an IFN or derivative thereof.

Another object of the invention is thus a drug formulation comprising at least one formula (I) compound, at least one additional compound as described supra, in particular an IFN or derivative thereof, and a pharmaceutically acceptable vehicle, carrier or excipient, as described supra.

Another object of the invention is a method for antiviral treatment of a recipient (a human or an animal), wherein the recipient is administered with an efficient amount of a drug or drug formulation according to the invention.

In an embodiment, the method includes the administration of an efficient amount of uridine derivative(s) and an efficient amount of interferon(s) (IFN). The uridine derivative and the IFN may be in admixture, or may be separately formulated and are either co-injected after extemporaneous mixture, or administered separately to the recipient. As an alternative, the method provides for the administration of an efficient amount of uridine derivative(s), where this administration is intended to be made in a recipient that is treated with an efficient amount of IFN(s). The recipient may have been treated recently with the IFN(s) (for instance less than 30 days before), or he receives IFN(s) at approximately the same time or later, for instance less than 30 days. In another embodiment, the IFN is replaced or accompanied (concomitant or successive administration) by the administration of another drug such as one of those described supra. Any and all characteristics recited with respect to the use according to the invention apply to the method.

As mentioned above, the compounds or derivatives, and the formulations or compositions comprising them may be administered by any route appropriate to the condition to be treated. Suitable routes including oral, rectal, nasal, topical (including buccal or sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal,
intrathecal and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient. Various suitable formulation types have been described earlier and may be used in the method.

For each of the above-indicated infectious events or indications the amount required of the active ingredient will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician. In general, however, for each of these indications, a suitable, effective dose will be in the range 0.01 to 200 mg per kilogram body weight of recipient per day, preferably in the range of 2 to 100 mg, most preferably 10 to 40 mg per kilogram body weight of recipient per day. The dose may if desired be presented as two, three, four or more sub-doses administered at appropriate intervals throughout the day.

The invention is described further in details with the following description of non-limitative examples.

**EXAMPLES**

Commercially available chemicals and solvents are reagent grade and used as received. Dry tetrahydrofuran, pyridine and dichloromethane are obtained from distillation over calcium hydride (CaH₂) or sodium (Na), N,N-dimethylformamide and ethanol over barium oxide (BaO) over calcium hydride (CaH₂). Triethylamine is dried over potassium hydroxide (KOH). The reactions are monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F₂₅₄, E. Merck). Compounds are visualized by UV irradiation and/or spraying with 20% sulphuric acid (H₂SO₄) in ethanol (EtOH), followed by charring at 150 °C. Column chromatography is performed on Silica Gel 60 M (0.040-0.063 mm, E. Merck). Melting points (mp) are recorded on a Büchi (Dr. Tottoli) and are uncorrected. ¹H and ¹³C NMR spectra are recorded on a Bruker AVANCE DPX 250 Fourier Transform spectrometer at 250 MHz for ¹H and 62.9 MHz for ¹³C, respectively, in (D)-chloroform, (D₆)-methanol and (D₆)-DMSO, shift values in ppm relative to SiMe₄ as internal reference, unless otherwise stated; signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), J in Hz. [α]D were performed with a Perkin-Elmer (model 41) polarimeter. UV spectra were recorded on a Beckman DU-640 spectrophotometer. Mass spectra are recorded on a Perkin-Elmer SCIEX API-300 (heated nebulizer) spectrometer. High-resolution Mass spectra (HRMS) were performed by the Centre Regional de Mesures Physiques de l’Ouest, University of Rennes 1 (France) using the FAB (Fast Atom Bombardment) or ESI (Electron Spray Ionization) mode. The nomenclature of the obtained compounds is in accordance with the IUPAC rules. The
numbering and assignment of the chemical shifts for all described compounds are related to the corresponding ribose derivatives.

Preparation of compounds of formula (I)

General procedure for iodination of acetylated nucleosides

A solution of acetylated nucleoside 3a-c (synthetic route described by L. B. Townsend and R. S. Tipson in "Nucleic acid chemistry", Wiley Eds., 1978) (15 mmol) in dry methane nitrile (CH₃CN, 150 mL), ceric ammonium nitrate (CAN, 4.94 g, 0.6 eq.) and iodine (I₂, 2.28 g; 0.6 eq.) is refluxed until completion (typically 1 h, checked by TLC). After cooling to room temperature (rt), solvents are evaporated under reduced pressure and the dark oily residue is dissolved in ethyl acetate (AcOEt, 300 mL) and water (H₂O, 50 mL). The biphasic mixture is cooled in an ice bath and a saturated Na₂S₂O₃ solution is smoothly added until complete decolouration. The organic layer is washed with water (2 x 50 mL) and brine (50 mL), dried over magnesium sulfate (MgSO₄) and concentrated under reduced pressure. The white foam is triturated with pentane (50 mL), filtrated and dried under reduced pressure to afford pure iodinated compounds 4a-c, respectively.

General procedure for Sonogashira cross-coupling and subsequent desilylation

Iodinated nucleoside 4a-c (5 mmol) was dissolved in a mixture of dry dimethyl formamide (DMF; 15 mL), dry triethyl amine (Et₃N; 2.06 mL, 3 eq.) and trimethyl silyl acetylene (2.06 mL, 3 eq.). Cuprous iodide (CuI; 190 mg, 0.2 eq.) and PdCl₂(PPh₃)$_2$ (350 mg, 0.1 eq.) were then added and the reaction mixture was stirred at room temperature until completion (typically 5-20 h, checked by TLC). Solvents were evaporated under reduced pressure. The oily residue was dissolved in AcOEt (250 mL) then washed with water (5 x 40 mL) and brine (40 mL). The organic layer was dried over MgSO₄ and the solvents were evaporated under reduced pressure to a dark oil. A first purification using a short path flash chromatography (eluent: methylene chloride (CH₂Cl₂) then MeOH/CH₂Cl₂ 5%) afforded the desired compound contaminated with coloured reaction co-products. Pure silylated alkynes (2 mmol), obtained after a second flash chromatography (eluent: hexanes/AcOEt 7/3 then 1/1), were dissolved in dry CH₃CN (20 mL). Tetrabutyl ammonium fluoride (TBAF) monohydrate (583 mg, 1.05 eq.) was added and the resulting solution was stirred at room temperature until completion (typically 30 min. to 2 h, checked by TLC). Solvents were evaporated under reduced pressure at room temperature and the oily residue was submitted to a flash column chromatography (eluent: hexanes/AcOEt 1/1 then AcOEt then MeOH/AcOEt 2% then 5%) to afford pure 5-ethylidyne nucleosides 5a-c, respectively.

General procedure for mono-halogenation of 5-ethylidyne nucleosides
5-Ethynyl nucleoside 5a-c (0.5 mmol) is dissolved in 5 mL dry CH$_2$CN and the mixture is cooled in an ice bath. 0.7 mmol (1.4 eq.) of the halogenating reagent (i.e. IDCP (iodonium di-syn-collidine perchlorate) or Br(coll)$_2$ClO$_4$) and 11 mg Ag(coll)$_2$ClO$_4$ (0.05 eq.) are added and the mixture is stirred in the dark at rt (typically 2 h-20 h, checked by TLC). The reaction mixture is cooled to 0°C then quenched by a saturated Na$_2$S$_2$O$_3$ solution (2 mL) and extracted with AcOEt (4 x 10 mL). The organic layer is washed with water (10 mL), a 1M HCl (hydrochloric acid) solution (3 x 5 mL), water (2 x 5 mL) and brine (10 mL) then dried over MgSO$_4$ and concentrated in vacuo. The solid residue is purified by flash chromatography (eluent: hexanes/AcOEt, 1/1, v/v) to yield the desired mono-halogenated compound 6-7a-c.

**Example 1:** 3',5'-Di-O-acetyl-5-(2-iodoethynyl)-2'-deoxyuridine (6a)

The title compound is obtained according to the above described general procedures from 3',5'-Di-O-acetyl-2'-deoxy-5-iodouridine (4a) (see Asakura, J. et al., J. Org. Chem., 1990, 55, 4928-4933; and Asakura, J. et al., Tetrahedron Lett., 1988, 29, 2855-2858) or from 3',5'-di-O-acetyl-2'-deoxy-5-ethyluridine (5a) (see US 5,028,596).

**Yield:** 93%.

mp: 175-177 (dec) °C

$[\alpha]_D^{20}$ -32 (c 0.5, CHCl$_3$)

$^1$H NMR (CDCl$_3$) δ 11.68 (br s, 1H, NH), 8.02 (s, 1H, H-6), 6.12 (t, J = 7.1 Hz, 1H, H-1'), 5.20 (m, 1H, H-3'), 4.33-4.15 (m, 3H, H-4',5'), 2.55 (m, 1H, H-2'a), 2.31 (m, 1H, H-2'b), 2.07 (s, 3H, OAc), 2.05 (s, 3H, OAc).

HRMS m/z 486.1982 calculated for C$_{16}$H$_{18}$N$_2$O$_7$Na, found m/z 486.1986.

**Example 2:** 3',5'-Di-O-acetyl-5-(2-bromoethynyl)-2'-deoxyuridine (7a)

According to a procedure similar to that of Example 1, the title compound is obtained as an oil.

**Yield:** 21%.

$[\alpha]_D^{20}$ -68 (c 1.0, CHCl$_3$)

$^1$H NMR (CDCl$_3$) δ 8.88 (br s, 1H, NH), 7.95 (s, 1H, H-6), 6.31 (dd, J = 7.3, 6.4 Hz, 1H, H-1'), 5.25 (m, 1H, H-3'), 4.38-4.30 (m, 3H, H-4',5'), 2.55 (m, 1H, H-2'a), 2.31 (m, 1H, H-2'b), 2.18 (s, 3H, OAc), 2.12 (s, 3H, OAc).

HRMS m/z 439.1978 calculated for C$_{14}$H$_{15}$BrN$_2$O$_7$Na, found m/z 439.1981.

Examples 3 to 6 below are obtained according to similar processes.

**Example 3:** 2',3',5'-Tri-O-acetyl-5-(2-iodoethynyl)-uridine (6b)

**Yield:** 68%
mp: 100-102 °C
$[\alpha]_D^{20} -71$ (c 1.0, CHCl$_3$)

$^1$H NMR (CDCl$_3$) δ 9.41 (br s, 1H, NH), 7.91 (s, 1H, H-6), 6.07 (d, $J = 4.6$ Hz, 1H, H-1’), 5.35 (m, 2H, H-2’,3’), 4.38 (m, 3H, H-4’,5’), 2.25 (s, 3H, OAc), 2.12 (s, 2 x 3H, 2 x OAc).

HRMS m/z 544.2352 calculated for C$_{17}$H$_{17}$N$_2$O$_6$Na, found m/z 544.2349.

Example 4: 2’,3’,5’-Tri-O-acetyl-5-(2-bromoethyl)-uridine (7b)

Yield: 58% as a colorless oil
$[\alpha]_D^{20} -59$ (c 0.4, CHCl$_3$)

$^1$H NMR (CDCl$_3$) δ 9.75 (br s, 1H, NH), 7.92 (s, 1H, H-6), 6.07 (d, $J = 4.4$ Hz, 1H, H-1’), 5.35 (m, 2H, H-2’,3’), 4.37 (m, 3H, H-4’,5’), 2.22 (s, 3H, OAc), 2.12 (s, 2 x 3H, 2 x OAc).

HRMS m/z 497.2348 calculated for C$_{17}$H$_{17}$BrN$_2$O$_6$Na, found m/z 497.2351.

Example 5: 2’,5’-Di-O-acetyl-5-(2-iodoethyl)-3’-deoxyuridine (6c)

Step a): 2’,5’-Di-O-acetyl-3’-deoxy-5-iodouridine (4c)

$^1$H NMR (CDCl$_3$) δ 9.53 (br s, 1H, NH), 7.83 (s, 1H, H-6), 5.82 (d, $J = 1.6$ Hz, 1H, H-1’), 5.30 (m, 1H, H-2’), 4.55 (m, 1H, H-4’), 4.41 (dd, $J = 12.8$, 2.8 Hz, 1H, H-5’a), 4.33 (dd, $J = 12.8$, 3.8 Hz, 1H, H-5’b), 2.31-1.95 (m, 2H, H-3’), 2.23 (s, 3H, OAc), 2.13 (s, 3H, OAc).

Step b): 2’,5’-Di-O-acetyl-3’-deoxy-5-ethynyluridine (5c)

$^1$H NMR (CDCl$_3$) δ 9.8 (br s, 1H, NH), 7.88 (s, 1H, H-6), 5.78 (s, 1H, H-1’), 5.31 (d, $J = 7.5$ Hz, 1H, H-2’), 4.48 (m, 1H, H-4’), 4.35 (dd, $J = 12.5$, 1.8 Hz, 1H, H-5’a), 4.25 (dd, $J = 12.5$, 3.4 Hz, 1H, H-5’b), 3.1 (s, 1H, H-Csp), 2.31-1.95 (m, 2H, H-3’), 2.12 (s, 3H, OAc), 2.05 (s, 3H, OAc).

HRMS m/z 360.2968 calculated for C$_{13}$H$_{16}$N$_2$O$_7$Na, found m/z 360.2971.

Step c): 2’,5’-Di-O-acetyl-5-(2-iodoethyl)-3’-deoxyuridine (6c)

Yield: 76% as an oil
$[\alpha]_D^{20} -49$ (c 0.8, CHCl$_3$)

$^1$H NMR (CDCl$_3$) δ 11.66 (br s, 1H, NH), 7.98 (s, 1H, H-6), 5.74 (s, 1H, H-1’), 5.30 (d, $J = 7.5$ Hz, 1H, H-2’), 4.43 (m, 1H, H-4’), 4.28 (br s, 2H, H-5’), 2.31-1.95 (m, 2H, H-3’), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc).

HRMS m/z 486.1982 calculated for C$_{15}$H$_{15}$N$_2$O$_7$Na, found m/z 486.1986.

Example 6: 2’,5’-Di-O-acetyl-5-(2-bromoethyl)-3’-deoxyuridine (7c)

Yield: 25% as an oil
$[\alpha]_D^{20} -52$ (c 0.4, CHCl$_3$)
1H NMR (DMSO-d6) δ 11.75 (br s, 1H, NH), 8.07 (s, 1H, H-6), 5.85 (s, 1H, H-1'), 5.31 (d, J = 7.5 Hz, 1H, H-2'), 4.43 (m, 1H, H-4'), 4.28 (br s, 2H, H-5'), 2.31-1.95 (m, 2H, H-3'), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc). HRMS m/z 439.1978 calculated for C15H15BrN2O7Na, found m/z 439.1975.

General procedure for di-halogenation of 5-ethynyl nucleosides
5-ethynyl nucleoside 5a-c (0.5 mmol) is dissolved in 5 mL dry CH3CN and the mixture is cooled in an ice bath. 0.6 mmol (1.2 eq.) of a solution of the halogenating reagent (i.e. I2, IBr, ICl or Br2) in 1 mL dry CH3CN is added dropwise and the mixture is stirred at 0°C until completion (typically 15 min.-2 h, checked by TLC). The reaction mixture is quenched at 0°C by a saturated Na2S2O3 solution (2 mL) then extracted with AcOEt (3 x 10 mL). The organic layer is washed with water (2 x 5 mL) and brine (10 mL) then dried over MgSO4 and concentrated in vacuo to yield the desired di-halogenated compounds of examples 7-18 (8-11a-c) in a reasonably pure form. Pure analytical samples were obtained using flash chromatography (eluent: hexanes/ACOEt, 1/1, v/v).

Example 7:  \((E)-3',5'-Di-O-acetyl-5-(1,2-diiodovinyl)-2'-deoxyuridine\) (8a)
94% yield; mp 76-78 °C; \([\alpha]_D^{20} -19\) (c 0.3, CHCl3); 1H NMR (CDCl3) δ 9.72 (br s, 1H, NH), 7.67 (s, 1H, H-6), 7.45 (s, 1H, =CHI), 6.36 (dd, J = 8.1, 5.6 Hz, 1H, H-1'), 5.25 (m, 1H, H-3'), 4.50-4.28 (m, 3H, H-4',5'), 2.60 (m, 1H, H-2'a), 2.30 (m, 1H, H-2'b), 2.17 (s, 3H, OAc), 2.08 (s, 3H, OAc). HRMS m/z 614.1106 calculated for C15H16I2N2O7Na, found m/z 614.1110.

Example 8:  \((E)-2',3',5'-Tri-O-acetyl-5-(1,2-diiodovinyl)-uridine\) (8b)
98% yield; mp 83-85 °C; \([\alpha]_D^{20} -41\) (c 1.0, CHCl3); 1H NMR (CDCl3) δ 9.56 (br s, 1H, NH), 7.58 (s, 1H, H-6), 7.40 (s, 1H, =CHI), 6.15 (d, J = 4.6 Hz, 1H, H-1'), 5.36 (m, 2H, H-2',3'), 4.38 (br s, 3H, H-4',5'), 2.19 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.11 (s, 3H, OAc). HRMS m/z 672.1476 calculated for C17H18I2N2O9Na, found m/z 672.1480.

Example 9:  \((E)-2',5'-Di-O-acetyl-5-(1,2-diiodovinyl)-3'-deoxyuridine\) (8c)
87% yield; mp 75-77 °C; \([\alpha]_D^{20} -23\) (c 0.3, CHCl3); 1H NMR (CDCl3) δ 9.38 (br s, 1H, NH), 7.64 (s, 1H, H-6), 7.39 (s, 1H, =CHI), 5.92 (d, J = 1.6 Hz, 1H, H-1'), 5.38 (m, 1H, H-4'), 4.58 (m, 1H, H-2'), 4.44 (dd, J = 12.5, 4.4 Hz, 1H, H-5'a), 4.33 (dd, 1H, H-5'b), 2.31-1.95 (m, 2H, H-3'), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS m/z 614.1106 calculated for C15H16I2N2O7Na, found m/z 614.1101.

Example 10:  \((E)-3',5'-Di-O-acetyl-5-(1-bromo-2-iodovinyl)-2'-deoxyuridine\) (9a)
96% yield; mp 82-84 °C; [α]D²⁰ -14 (c 4.0, CHCl₃); ¹H NMR (CDCl₃) δ 9.78 (br s, 1H, NH), 7.71 (s, 1H, H-6), 7.08 (s, 1H, =CHI), 6.25 (dd, J = 8.1, 5.6 Hz, 1H, H-1’), 5.28 (m, 1H, H-3’), 4.50-4.05 (m, 3H, H-4’, 5’), 2.58 (m, 1H, H-2’a), 2.20 (m, 1H, H-2’b), 2.12 (s, 3H, OAc), 1.96 (s, 3H, OAc). HRMS m/z 567.1102 calculated for C₁₅H₁₈BrN₂O₃Na, found m/z 567.1107.

**Example 11:** *(E)-2’,3’,5’-Tri-O-acetyl-5-(1-bromo-2-iodovinyl)-uridine (9b)*

92% yield; mp 86-88 °C; [α]D²⁰ -39 (c 2.0, CHCl₃); ¹H NMR (CDCl₃) δ 9.57 (br s, 1H, NH), 7.66 (s, 1H, H-6), 7.15 (s, 1H, =CHI), 6.15 (d, J = 4.7 Hz, 1H, H-1’), 5.38 (m, 2H, H-2’,3’), 4.38 (m, 3H, H-4’,5’), 2.18 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.13 (s, 3H, OAc). HRMS m/z 625.1469 calculated for C₁₇H₁₈BrN₂O₄Na, found m/z 625.1473.

**Example 12:** *(E)-2’,5’-Di-O-acetyl-5-(1-bromo-2-iodovinyl)-3’-deoxyuridine (9c)*

89% yield as an oil; [α]D²⁰ -19 (c 0.5, CHCl₃); ¹H NMR (CDCl₃) δ 9.63 (br s, 1H, NH), 7.61 (s, 1H, H-6), 7.13 (s, 1H, =CHI), 5.92 (s, 1H, H-1’), 5.38 (m, 1H, H-4’), 4.58 (m, 1H, H-2’), 4.44 (dd, J = 12.4, 2.6 Hz, 1H, H-5’a), 4.33 (dd, J = 12.4, 4.4 Hz, 1H, H-5’b), 2.31-1.95 (m, 2H, H-3’), 2.16 (s, 3H, OAc), 2.14 (s, 3H, OAc). HRMS m/z 567.1102 calculated for C₁₅H₁₈BrN₂O₄Na, found m/z 567.1105.

**Example 13:** *(E)-3’,5’-Di-O-acetyl-5-(1-chloro-2-iodovinyl)-2’-deoxyuridine (10a)*

91% yield; mp 81-83 °C; [α]D²⁰ -14 (c 0.7, CHCl₃); ¹H NMR (CDCl₃) δ 9.28 (br s, 1H, NH), 7.75 (s, 1H, H-6), 6.91 (s, 1H, =CHI), 6.32 (m, 1H, H-1’), 5.24 (m, 1H, H-3’), 4.50-4.30 (m, 3H, H-4’,5’), 2.75 (m, 1H, H-2’a), 2.18 (m, 1H, H-2’b), 2.13 (s, 2 x 3H, 2 x OAc). HRMS m/z 522.6592 calculated for C₁₅H₁₈ClN₂O₄Na, found m/z 522.6597.

**Example 14:** *(E)-2’,3’,5’-Tri-O-acetyl-5-(1-chloro-2-iodovinyl)-uridine (10b)*

97% yield; mp 85-87 °C; [α]D²⁰ -27 (c 0.8, CHCl₃); ¹H NMR (CDCl₃) δ 9.43 (br s, 1H, NH), 7.68 (s, 1H, H-6), 6.92 (s, 1H, =CHI), 6.15 (br s, 1H, H-1’), 5.36 (m, 2H, H-2’,3’), 4.38 (m, 3H, H-4’,5’), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS m/z 580.6962 calculated for C₁₅H₁₈ClN₂O₄Na, found m/z 580.6958.

**Example 15:** *(E)-2’,5’-Di-O-acetyl-5-(1-chloro-2-iodovinyl)-3’-deoxyuridine (10c)*

83% yield; mp 87-89 °C; [α]D²⁰ -13 (c 1.0, CHCl₃); ¹H NMR (CDCl₃) δ 9.75 (br s, 1H, NH), 7.74 (s, 1H, H-6), 6.90 (s, 1H, =CHI), 5.91 (d, J = 1.7 Hz, 1H, H-1’), 5.38 (m, 1H, H-4’), 4.58 (m, 1H, H-2’), 4.43 (dd, J = 12.5, 2.8 Hz, 1H, H-5’a), 4.33 (dd, J = 12.5, 4.1 Hz, 1H, H-5’b), 2.35-1.95 (m, 2H, H-3’), 2.14 (s, 2 x 3H, 2 x OAc). HRMS m/z 522.6592 calculated for C₁₅H₁₈ClN₂O₄Na, found m/z 522.6593.
Example 16: 3',5'-Di-O-acetyl-5-(1,2-dibromovinyl)-2'-deoxyuridine (11a)

97% yield; mp 79-81 °C; [α]_D^20 -15 (c 1.0, CHCl₃); ¹H NMR (CDCl₃) δ 8.85 (br s, 1H, NH), 7.75 (s, 1H, H-6), 6.89 (s, 1H, =CHBr), 6.33 (dd, J = 8.1, 5.6 Hz, 1H, H-1'), 5.24 (m, 1H, H-3'), 4.45-4.25 (m, 3H, H-4',5'), 2.65 (m, 1H, H-2'a), 2.18 (m, 1H, H-2'b), 2.14 (s, 2 x 3H, 2 x OAc). HRMS m/z 520.1098 calculated for C₁₅H₁₆Br₂N₂O₇Na, found m/z 520.1099.

Example 17: (E)-2',3',5'-Tri-O-acetyl-5-(1,2-dibromovinyl)-uridine (11b)

92% yield; mp 83-85 °C; [α]_D^20 -43 (c 0.5, CHCl₃); ¹H NMR (CDCl₃) δ 9.38 (br s, 1H, NH), 7.66 (s, 1H, H-6), 6.90 (s, 1H, =CHBr), 6.12 (dd, J = 4.8 Hz, 1H, H-1'), 5.36 (m, 2H, H-2',3'), 4.38 (m, 3H, H-4',5'), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS m/z 578.1488 calculated for C₁₇H₁₈Br₂N₂O₇Na, found m/z 578.1473.

Example 18: 2',5'-Di-O-acetyl-5-(1,2-dibromovinyl)-3'-deoxyuridine (11c)

82% yield; mp 78-80 °C; [α]_D^20 -16 (c 0.8, CHCl₃); ¹H NMR (CDCl₃) δ 9.61 (br s, 1H, NH), 7.72 (s, 1H, H-6), 6.88 (s, 1H, =CHI), 5.88 (dd, J = 1.5 Hz, 1H, H-1'), 5.38 (m, 1H, H-4'), 4.58 (m, 1H, H-2'), 4.43 (dd, J = 12.5, 2.8 Hz, 1H, H-5'a), 4.33 (dd, J = 12.5, 4.1 Hz, 1H, H-5'b), 2.35-1.95 (m, 2H, H-3'), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc). HRMS m/z 520.1098 calculated for C₁₅H₁₆Br₂N₂O₇Na, found m/z 520.1103.

General procedure for deacetylation

Acetylated nucleoside analogue 6-11a-c (1 mmol) is dissolved in 10 mL pyridine and 5 mL EtOH. The reaction mixture is cooled to -10°C and 5 mL of a 1M NaOH (sodium hydroxide) aqueous solution is added. The resulting solution is stirred at this temperature until completion (typically 1-4 h, checked by TLC). The reaction mixture is neutralized with Dowex and filtered through a fritted glass funnel. Solvents are evaporated in vacuo and the oily residue is submitted to a flash column chromatography using an appropriate eluent (typically hexanes/AcOEt 25/75 then AcOEt then MeOH/AcOEt 1%) to yield pure compounds 12-17a-c (Examples 19-36).

Example 19: 5-(2-iodoethyl)-2'-deoxyuridine (12a)

68% yield; mp 135-137 °C; [α]_D^20 +14 (c 0.8, MeOH); ¹H NMR (CD₃OD) δ 8.32 (s, 1H, H-6), 6.22 (td, J = 6.6 Hz, 1H, H-1'), 4.41 (m, 1H, H-3'), 3.95 (m, 1H, H-4'), 3.83 (dd, J = 12.1, 3.0 Hz, 1H, H-5'a), 3.73 (dd, J = 12.1, 3.4 Hz, 1H, H-5'b), 2.40-2.12 (m, 2H, H-2'). HRMS m/z 402.1229 calculated for C₁₁H₁₁I₂N₂O₃Na, found m/z 402.1231.

Example 20: 5-(2-iodoethyl)-uridine (12b)
70% yield as an oil; \([\alpha]_D^{20}\) -12 (c 0.2, MeOH); \(^1\)H NMR (DMSO-d6) \(\delta\) 11.65 (br s, 1H, NH), 8.29 (s, 1H, H-6), 5.72 (m, 1H, H-1'), 5.05-5.12 (3 x br s, 3 x OH), 4.05-3.95 (m, 2H, H-2',3'), 3.85 (m, 1H, H-4'), 3.70-3.55 (m, 2H, H-5'). HRMS m/z 418.1223 calculated for C\(_{11}\)H\(_{11}\)N\(_2\)O\(_6\)Na, found m/z 418.1219.

**Example 21:** 5-(2-Iodoethynyl)-3'-deoxyuridine (12c)

66% yield as an oil; \([\alpha]_D^{20}\) +6 (c 1.0, MeOH); \(^1\)H NMR (DMSO-d6) \(\delta\) 11.65 (s, 1H, NH), 8.48 (s, 1H, H-6), 5.59 (s, 1H, H-1'), 5.54 (d, \(J = 4.1\) Hz, 1H, OH), 5.23 (t, \(J = 5.1\) Hz, 1H, OH), 4.32 (m, 1H, H-4'), 4.21 (m, 1H, H-2'), 3.75 (m, 1H, H-5'a), 3.52 (m, 1H, H-5'b), 1.98 (m, 1H, H-3'a), 1.76 (m, 1H, H-3'b). HRMS m/z 402.1229 calculated for C\(_{11}\)H\(_{11}\)N\(_2\)O\(_6\)Na, found m/z 402.1233.

**Example 22:** 5-(2-Bromoethynyl)-2'-deoxyuridine (13a)

80% yield as an oil; \([\alpha]_D^{20}\) +3 (c 0.3, MeOH); \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 8.30 (s, 1H, H-6), 6.05 (t, \(J = 6.3\) Hz, 1H, H-1'), 4.21 (m, 1H, H-3'), 3.78 (m, 1H, H-4'), 3.65-3.50 (m, 2H, H-5'), 2.15 (dd, \(J = 5.6, 5.0\) Hz, 2H, H-2'). HRMS m/z 355.1225 calculated for C\(_{11}\)H\(_{11}\)BrN\(_2\)O\(_6\)Na, found m/z 355.1227.

**Example 23:** 5-(2-bromoethynyl)-uridine (13b)

74% yield as an oil; \([\alpha]_D^{20}\) -23 (c 0.5, MeOH); \(^1\)H NMR (DMSO-d6) \(\delta\) 11.69 (br s, 1H, NH), 8.39 (s, 1H, H-6), 5.73 (d, \(J = 4.7\) Hz, 1H, H-1'), 5.41 (d, \(J = 5.3\) Hz, OH), 5.24 (t, \(J = 4.7\) Hz, OH), 5.08 (d, \(J = 5.3\) Hz, OH), 4.10-3.91 (m, 2H, H-2',3'), 3.85 (m, 1H, H-4'), 3.75-350 (m, 2H, H-5'). HRMS m/z 371.1219 calculated for C\(_{11}\)H\(_{11}\)BrN\(_2\)O\(_6\)Na, found m/z 371.1224.

**Example 24:** 5-(2-Bromoethynyl)-3'-deoxyuridine (13c)

72% yield as an oil; \([\alpha]_D^{20}\) -29 (c 1.0, MeOH); \(^1\)H NMR (DMSO-d6) \(\delta\) 11.63 (s, 1H, NH), 8.53 (s, 1H, H-6), 5.59 (s, 1H, H-1'), 5.56 (d, \(J = 4.1\) Hz, 1H, OH), 5.26 (t, \(J = 5.1\) Hz, 1H, OH), 4.32 (m, 1H, H-4'), 4.25 (m, 1H, H-2'), 3.81 (m, 1H, H-5'a), 3.61 (m, 1H, H-5'b), 1.95 (m, 1H, H-3'a), 1.72 (m, 1H, H-3'b). HRMS m/z 355.1225 calculated for C\(_{11}\)H\(_{11}\)BrN\(_2\)O\(_6\)Na, found m/z 355.1223.

**Example 25:** (E)-5-(1,2-Diiodovinyl)-2'-deoxyuridine (14a)

72% yield; mp 103-105 °C; \([\alpha]_D^{20}\) +28 (c 0.3, MeOH); \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 8.27 (s, 1H, H-6), 7.52 (s, 1H, =CHI), 6.28 (t, \(J = 6.3\) Hz, 1H, H-1'), 4.43 (m, 1H, H-3'), 3.97 (m, 1H, H-4'), 3.83 (dd, \(J = 11.9, 3.2\) Hz, 1H, H-5'a), 3.73 (m, \(J = 11.9, 3.4\) Hz, 1H, H-5'b), 2.43-2.12 (m, 2H, H-2'). HRMS m/z 530.0353 calculated for C\(_{11}\)H\(_{12}\)I\(_2\)N\(_2\)O\(_6\)Na, found m/z 530.0558.
Example 26: (E)-5-(1,2-Diodovinyl)-uridine (14b)
82% yield; mp 107-109 °C; [α]_D^20 +5 (c 1.0, MeOH); ^1H NMR (DMSO-d_6) δ 11.65 (br s, 1H, NH), 8.05 (s, 1H, H-6), 7.05 (s, IH, =CHI), 5.80 (d, J = 4.7 Hz, 1H, H-1'), 5.50-5.10 (3 x br s, 3 x OH), 4.12-3.95 (m, 2H, H-2',3'), 3.87 (m, 1H, H-4'), 3.75-355 (m, 2H, H-5'). HRMS m/z 546.0347 calculated for C_{11}H_{12}I_2N_2O_8Na, found m/z 546.0351.

Example 27: (E)-5-(1,2-Diodovinyl)-3'-deoxyuridine (14c)
76% yield as an oil; [α]_D^20 +1.0 (c 0.8, MeOH); ^1H NMR (DMSO-d_6 + D_2O) δ 8.21 (s, 1H, H-6), 7.55 (s, 1H, =CHI), 5.61 (s, 1H, H-1'), 4.32 (m, 1H, H-4'), 4.25 (m, 1H, H-2'), 3.81 (m, 1H, H-5'a), 3.52 (m, 1H, H-5'b), 1.87 (m, 1H, H-3'a), 1.74 (m, 1H, H-3'b). HRMS m/z 530.0553 calculated for C_{11}H_{12}I_2N_2O_8Na, found m/z 530.0554.

Example 28: (E)-5-(1-Bromo-2-iodovinyl)-2'-deoxyuridine (15a)
78% yield as an oil; [α]_D^20 +26 (c 2.0, MeOH); ^1H NMR (CD_3OD) δ 8.35 (s, 1H, H-6), 7.39 (s, 1H, =CHI), 6.25 (t, J = 6.5 Hz, 1H, H-1'), 4.45 (m, 1H, H-3'), 3.94 (m, 1H, H-4'), 3.86 (dd, J = 11.9, 3.2 Hz, 1H, H-5'a), 3.72 (dd, J = 11.9, 3.4 Hz, 1H, H-5'b), 2.45-2.15 (m, 2H, H-2'). HRMS m/z 483.0349 calculated for C_{11}H_{13}Br_2I_2N_2O_8Na, found m/z 483.0352.

Example 29: (E)-5-(1-Bromo-2-iodovinyl)-uridine (15b)
76% yield as an oil; [α]_D^20 -1 (c 0.3, MeOH); ^1H NMR (D_2O) δ 8.27 (s, 1H, H-6), 7.45 (s, 1H, =CHI), 5.79 (d, J = 4.7 Hz, 1H, H-1'), 4.15-3.92 (m, 2H, H-2',3'), 3.88 (m, 1H, H-4'), 3.67 (dd, J = 11.8, 2.5 Hz, 1H, H-5'a), 3.56 (dd, 1H, H-5'b). HRMS m/z 499.0343 calculated for C_{11}H_{13}Br_2I_2N_2O_8Na, found m/z 499.0348.

Example 30: (E)-5-(1-Bromo-2-iodovinyl)-3'-deoxyuridine (15c)
68% yield as an oil; [α]_D^20 +3 (c 1.0, MeOH); ^1H NMR (DMSO-d_6 + D_2O) δ 8.35 (s, 1H, H-6), 7.39 (s, 1H, =CHI), 5.61 (d, J = 1.3 Hz, 1H, H-1'), 4.32 (m, 1H, H-4'), 4.25 (m, 1H, H-2'), 3.76 (dd, J = 12.2, 2.4 Hz, 1H, H-5'a), 3.52 (dd, J = 12.2, 2.8 Hz, 1H, H-5'b), 1.97 (m, 1H, H-3'a), 1.75 (m, 1H, H-3'b). HRMS m/z 483.0349 calculated for C_{11}H_{13}Br_2I_2N_2O_8Na, found m/z 483.0344.

Example 31: (E)-5-(1-Chloro-2-iodovinyl)-2'-deoxyuridine (16a)
71% yield; mp 109-111 °C; [α]_D^20 +34 (c 0.5, MeOH); ^1H NMR (CD_3OD) δ 8.38 (s, 1H, H-6), 7.06 (s, 1H, =CHI), 6.30 (t, J = 6.5 Hz, 1H, H-1'), 4.43 (ddd, J = 6.5, 3.8, 3.6 Hz, 1H, H-3'), 3.96 (ddd, J = 3.6, 3.4, 3.0 Hz, 1H, H-4'), 3.83 (ddd, J = 11.9, 3.1 Hz, 1H, H-5'a), 3.73 (dd, J = 11.9, 3.4 Hz, 1H, H-5'b), 2.45-2.10 (m, 2H, H-2'). HRMS m/z 438.5839 calculated for C_{11}H_{12}Cl_2I_2N_2O_8Na, found m/z 438.5843.
Example 32: (E)-5-(1-Chloro-2-iodovinyl)-uridine (16b)
78% yield; mp 109-111 °C; $\left[\alpha\right]_D^{20} +5$ (c 0.4, MeOH); $^1$H NMR (D$_2$O) $\delta$ 8.45 (s, 1H, H-6), 7.05 (s, 1H, =CHI), 5.89 (br s, 1H, H-1'), 4.30 (br s, 2H, H-2',3'), 4.10 (m, 1H, H-4'), 3.85 (dd, $J = 11.5, 2.2$ Hz, 1H, H-5'a), 3.75 (dd, 1H, H-5'b). HRMS m/z 454.5833 calculated for C$_{11}$H$_{12}$ClIN$_2$O$_5$Na, found m/z 454.5835.

Example 33: (E)-5-(1-Chloro-2-iodovinyl)-3'-deoxyuridine (16c)
70% yield as an oil; $\left[\alpha\right]_D^{20} +2$ (c 2.0, MeOH); $^1$H NMR (DMSO-d$_6$ + D$_2$O) $\delta$ 8.38 (s, 1H, H-6), 7.18 (s, 1H, =CHI), 5.65 (d, $J = 1.2$ Hz, 1H, H-1'), 4.32 (m, 1H, H-4'), 4.25 (m, 1H, H-2'), 3.76 (dd, $J = 12.3, 2.6$ Hz, 1H, H-5'a), 3.52 (dd, $J = 12.3, 2.8$ Hz, 1H, H-5'b), 2.01 (m, 1H, H-3'a), 1.75 (m, 1H, H-3'b). HRMS m/z 438.5839 calculated for C$_{11}$H$_{12}$ClIN$_2$O$_5$Na, found m/z 438.5837.

Example 34: (E)-5-(1,2-Dibromovinyl)-2'-deoxyuridine (17a)
85% yield as an oil; $\left[\alpha\right]_D^{20} +26$ (c 0.5, MeOH); $^1$H NMR (CD$_2$OD) $\delta$ 8.39 (s, 1H, H-6), 7.06 (s, 1H, =CHBr), 6.28 (t, $J = 6.5$ Hz, 1H, H-1'), 4.41 (m, 1H, H-3'), 3.95 (m, 1H, H-4'), 3.82 (dd, $J = 11.9, 2.9$ Hz, 1H, H-5'a), 3.73 (dd, $J = 11.9, 3.4$ Hz, 1H, H-5'b), 2.45-2.10 (m, 2H, H-2'). HRMS m/z 436.0345 calculated for C$_{11}$H$_{12}$Br$_2$N$_2$O$_5$Na, found m/z 436.0348.

Example 35: (E)-5-(1,2-Dibromovinyl)-uridine (17b)
72% yield as an oil; $\left[\alpha\right]_D^{20} -14$ (c 0.5, MeOH); $^1$H NMR (D$_2$O) $\delta$ 8.45 (s, 1H, H-6), 7.07 (s, 1H, =CHBr), 5.93 (d, $J = 5.2$ Hz, 1H, H-1'), 4.18 (m, 2H, H-2',3'), 4.05 (m, 1H, H-4'), 3.88 (dd, $J = 12.5, 2.5$ Hz, 1H, H-5'a), 3.74 (dd, $J = 12.5, 2.8$ Hz, 1H, H-5'b). HRMS m/z 452.0339 calculated for C$_{11}$H$_{12}$Br$_2$N$_2$O$_5$Na, found m/z 452.0342.

Example 36: (E)-5-(1,2-Dibromovinyl)-3'-deoxyuridine (17c)
74% yield as an oil; $\left[\alpha\right]_D^{20} +5$ (c 1.0, MeOH); $^1$H NMR (DMSO-d$_6$ + D$_2$O) $\delta$ 8.42 (s, 1H, H-6), 7.25 (s, 1H, =CHI), 5.64 (br s, 1H, H-1'), 4.35 (m, 1H, H-4'), 4.25 (m, 1H, H-2'), 3.78 (m, H-5'a), 3.54 (m, H-5'b), 1.95 (m, 1H, H-3'a), 1.75 (m, 1H, H-3'b). HRMS m/z 436.0345 calculated for C$_{11}$H$_{12}$Br$_2$N$_2$O$_5$Na, found m/z 436.0340.

Preparation of the key intermediate 18 (for both 3'-F and 3'-N$_3$ series)

1-(4-Hydroxy-5-trityloxymethyl-tetrahydro-furan-2-yl)-1H-pyrimidine-2,4-dione (32)
A solution of 2'-deoxyuridine (10.0 g, 43.9 mmol) and para-anisylchlorodiphenylmethane (16.3 g, 52.6 mmol) in dry pyridine (235 mL) is refluxed for 2 hours. After cooling at room
temperature (rt), pyridine is evaporated under reduced pressure, then dichloromethane (400mL) is added and the solution is washed with water (50 mL x3), dried over MgSO₄, filtered through a fritted glass funnel and concentrated under reduced pressure to afford the crude product, which is submitted to a flash silica gel column chromatography (eluuent: hexanes/EtOAc: 5/5 to EtOAc/MeOH: 9/1) to give the pure protected nucleoside 32 (21.9 g, 99 %) as a white solid. The physico-chemical data of the compound are fully related with those previously published.

1-(4-Hydroxy-5-trityloxymethyltetrahydrofuran-2-yl)-5-iodo-1H-pyrimidine-2,4-dione (33)

A solution of 32 (21.9 g, 43.8 mmol), I₂ (17.0 g, 66.9 mmol), CAN (17.7 g, 32.3 mmol), NaHCO₃ (1.0 g) in dry acetonitrile (480 mL) is refluxed for 4 days. After cooling at rt, a solution of saturated Na₂SO₃ (800 mL) and EtOAc (200 mL) are added: aqueous layer is extracted with EtOAc (300 mL x2), then organic layer is washed with saturated Na₂SO₃ (300mL), dried over MgSO₄, filtered through a fritted glass funnel and concentrated under reduced pressure to afford the crude product, which is submitted to a flash silica gel column chromatography (eluuent: hexanes/EtOAc: 6/4 to EtOAc) to give the pure iodinated nucleoside 33 (12.8 g, 57 %) as a white solid. The physico-chemical data of the compound are fully related with those previously published.

1-(4-Hydroxy-5-trityloxymethyl-tetrahydro-furan-2-yl)-5-trimethylsilanylethynyl-1H-pyrimidine-2,4-dione (34)

33 (5.00 g, 8.0mmol) is dissolved in a mixture of dry DMF (62.5 mL), dry Et₃N (3.3 mL, 24.0mmol), and trimethylsilylacetylene (3.4 mL, 24.0 mmol). Cul (300.0 mg, 1.6 mmol) and PdCl₂(PPh₃)₂ (563 mg) are then added and the reaction mixture is stirred at rt under argon for 16 hours. Solvents are evaporated under reduced pressure. The crude product is submitted to a flash silica gel column chromatography (eluuent: hexanes/EtOAc: 5/5 to 2/8) to give 34 (4.10 g, 86 %) as a pale yellow solid. [α]D²⁰ +27.5 (c 0.5, CHCl₃); mp 104 °C; ¹H NMR (CDCl₃) δ : 2.08-2.33 (m, 1H, H-2'a), 2.42-2.64 (m, 1H, H-2'b), 3.20-3.52 (m, 2H, H-5'), 3.78 (s, 3H, OCH₃), 4.05-4.25 (m, 1H, H-4'), 4.40-4.53 (m, 1H, H-3'), 6.31 (dd, J = 5.6 Hz, 8.1 Hz, 1H, H-1'), 6.78-7.55 (m, 14H, trityl), 8.05 (s, 1H, H-6); ¹³C NMR (CDCl₃) δ : 41.5 (C-2'), 55.3 (OCH₃), 63.7 (C-5'), 72.5 (C-3'), 85.9 (C-1'), 86.6 (C-4'), 87.3 (C), 94.9 (C), 99.9 (C), 100.7 (C), 113.5 (CH), 127.2 (CH), 128.2 (CH), 128.3 (C), 130.5 (CH), 135.1 (C), 142.8 (C-6), 144.0 (C), 144.1 (C), 149.5 (C=O), 158.8 (C), 161.5 (C);

5-Ethynyl-1-(4-hydroxy-5-trityloxymethyl-tetrahydro-furan-2-yl)-1H-pyrimidine-2,4-dione (35)
A solution of **34** (616.3 mg, 1.03 mmol), TBAF (303.4 mg, 1.16 mmol) in dry acetonitrile (24.0 mL) is stirred at rt for 2 hours. Solvent is removed under reduced pressure, the crude product is submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 5/5 to 2/8) to give **35** (516.8 mg, 95%) as a pale yellow solid. \([\alpha]_D^{20} +14.5\) (c 1.0, CHCl₃); mp 110°C; \(^1\)H NMR (CDCl₃) δ: 2.22-2.40 (m, 1H, H-2′a), 2.45-2.60 (m, 1H, H-2′b), 2.90 (s, 1H, CH alkyne), 3.41 (t, \(J=3.6\) Hz, 2H, H-5′), 3.81 (s, 3H, OCH₃), 4.15-4.20 (m, 1H, H-4′), 4.51-4.62 (m, 1H, H-3′), 6.30 (t, \(J=8.2\) Hz, 1H, H-1′), 6.70-7.50 (m, 14H, trityl), 8.10 (s, 1H, H-6); \(^13\)C NMR (CDCl₃) δ: 41.6 (C-2′), 55.4 (OCH₃), 63.7 (C-5′), 72.4 (C-3′), 74.2 (C), 82.2 (C), 85.9 (C-1′), 86.6 (C-4′), 87.5 (C), 99.4 (C), 113.5 (CH), 127.3 (CH), 128.2 (CH), 128.3 (C), 128.4 (C), 130.5 (CH), 135.1 (C), 143.8 (C-6), 143.9 (C), 144.1 (C), 149.4 (C=O), 158.9 (C), 161.7 (C);

**Methanesulfonic acid 5-(5-ethynyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2-trityloxy-methyltetrahydrofuran-3-yl ester (18)**

A solution of **35** (500.0 mg, 0.95 mmol) and methanesulfonylchloride (0.73 mL, 9.50 mmol) in dry pyridine at 0°C is allowed to warm to rt and is stirred for 16 hours. After cooling at 0°C, ice, water (100 mL) and EtOAc (100 mL) are added, the aqueous layer is extracted with EtOAc (50 mL x3), the organic layers are washed with water (50 mL x3), dried over MgSO₄, filtered through a fritted glass funnel and concentrated under reduced pressure to afford the crude product, which is submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 5/5) to give **18** (587.0 mg, 99%) as an orange solid. \([\alpha]_D^{20} +28.5\) (c 1.0, CHCl₃); mp 99°C; \(^1\)H NMR (CDCl₃) δ: 2.30-2.50 (m, 1H, H-2′a), 2.70-2.82 (m, 1H, H-2′b), 2.91 (s, 1H, CH alkyne), 3.02 (s, 3H, OCH₃), 3.33-3.57 (m, 2H, H-5′), 3.80 (s, 3H, OCH₃), 4.35 (se, 1H, H-4′), 5.31 (se, 1H, H-3′), 6.29 (t, \(J=8.2\) Hz, 1H, H-1′), 6.75-7.50 (m, 14H, trityl), 8.07 (s, 1H, H-6), 8.58 (brs, 1H, NH); \(^13\)C NMR (CDCl₃) δ: 38.8 (SCH₃), 39.3 (C-2′), 55.4 (OCH₃), 63.0 (C-5′), 74.0 (C-3′), 79.8 (C), 82.4 (C), 84.5 (C-1′), 85.4 (C-4′), 88.0 (C), 99.9 (C), 113.6 (CH), 127.5 (CH), 128.3 (CH), 128.3 (CH), 129.2 (C), 130.5 (CH), 134.6 (C), 143.2 (C-6), 143.6 (C), 143.7 (C), 149.2 (C=O), 159.0 (C), 161.2 (C);

**Example 37**: 5-(1,2-Di-iodovinyl)-1-(4-fluoro-5-hydroxymethyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (25a)

Step a): 5-Ethynyl-1-(4-hydroxy-5-trityloxyethyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (19)

A solution of **18** (50.0 mg, 0.08 mmol) in dioxan (2.0 mL) and aqueous 1M NaOH (168 µL, 0.16 mmol) is refluxed for 2 hours. After cooling at rt, solvents are removed in vacuo and the crude product is submitted to a flash silica gel column chromatography (eluent:
hexanes/EtOAc: 5/5 to EtOAc) to give 19 (41.0 mg, 93 %) as a pale yellow solid. \([\alpha]_D^{20} -13.0 (c 1.0, CHCl_3); \) mp 124 °C; \(^1H\) NMR (CDCl_3) \(\delta: 2.08-2.25 (m, 1H, H-2'), 2.47-2.66 (m, 1H, H-2'), 3.07 (s, 1H, CH alkyn), 3.43-3.72 (m, 2H, H-5'), 3.79 (s, 3H, OCH_3), 4.00-4.12 (m, 1H, H-4'), 4.38-4.50 (m, 1H, H-3'), 6.15 (t, J = 6.1 Hz, 1H, H-1'), 6.80-7.55 (m, 14H, trityl), 8.15 (s, 1H, H-6); \(^13C\) NMR (CDCl_3) \(\delta: 41.1 (C-2'), 55.4 (OCH_3), 61.9 (C-5'), 70.9 (C-3'), 75.0 (C), 81.7 (CH alkyn), 83.4 (C-1'), 85.9 (C-4'), 87.5 (C), 98.5 (C), 113.6 (CH), 127.4 (CH), 128.2 (CH), 130.4 (CH), 134.9 (C), 143.8 (C), 145.3 (C-6), 149.5 (C=O), 159.0 (C), 161.8 (C);

Step b): 5-Ethynyl-1-(4-fluoro-5-trityloxymethyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (20)

To a solution of the product Step (a) (1.26 g, 2.4 mmol) in dry dichloromethane (77.0 mL) at 0°C is added dropwise diethylaminosulfur trifluoride (0.6mL, 4.8mmol), and the reaction mixture is stirred for 15 minutes. Then a saturated NaHCO_3 solution (50.0 mL) is added, the aqueous layer is extracted with CH_2Cl_2 (50 mL x 3), the organic layers are washed with water (50 mL), dried over MgSO_4, filtered through a fritted glass funnel and concentrated under reduced pressure to afford the crude product, which is submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 5/5) to give 20 (805.0 mg, 64 %) as a pale yellow solid. \([\alpha]_D^{20} +12.0 (c 1.0, CHCl_3); \) mp 54 °C; \(^1H\) NMR (CDCl_3) \(\delta: 2.12-2.46 (m, 1H, H-2'), 2.68-2.90 (m, 1H, H-2'b), 2.88 (s, 1H, CH alkyn), 3.35-3.48 (m, 2H, H-5'), 3.80 (s, 3H, OCH_3), 4.37 (d, J = 27.4 Hz, 1H, H-4'), 5.28 (dd, J = 4.6 Hz, 53.6 Hz, 1H, H-3'), 6.34 (dd, J = 5.3 Hz, 9.0 Hz, 1H, H-1'), 6.80-7.50 (m, 14H, trityl), 8.10 (s, 1H, H-6); \(^13C\) NMR (CDCl_3) \(\delta: 39.7 (d, J = 84.2 Hz, C-2'), 55.4 (OCH_3), 63.4 (d, J = 40.9 Hz, C-5'), 82.2 (CH alkyn), 84.8 (d, J = 101.1 Hz, C-4'), 85.7 (C-1'), 87.8 (C), 94.5 (d, J = 710.0 Hz, C-3'), 99.7 (C), 113.6 (CH), 127.4 (CH), 128.3 (CH), 128.4 (CH), 130.5 (CH), 134.6 (C), 143.4 (C-6), 143.6 (C), 143.7 (C), 149.2 (C=O), 159.0 (C x 2);

General procedure for di-halogenation of 5- ethynyl compound

5-ethynyl nucleoside of example 37, Step b) (0.5 mmol) is dissolved in dry acetonitrile (5.0 mL) and the reaction mixture is cooled to an ice bath. A solution of the halogenating reagent (i.e. I_2, IBr or ICl; 0.6 mmol) in dry CH_2CN (1.0 mL) is added dropwise at 0°C and the reaction mixture is stirred until completion (typically 15 min.-2 hours, checked by TLC). The reaction mixture is quenched at 0°C by a saturated Na_2SO_3 solution (2 mL) then extracted with EtOAc (10 mL x 3). The organic layer is washed with water (5 mL x 2) and brine (10 mL), then dried over MgSO_4, filtered through a fritted glass funnel and concentrated under reduced pressure
to afford the crude product, which is submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 8/2 to 3/7) to give the desired di-halogenated compound.

Step c): 5-(1,2-Diiodovinyl)-1-(4-fluoro-5-trityloxy)methyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (24a)

Prepared from compound 20 of step b) using I₂ with the typical procedure described before to give 24a (32%) as an orange solid. [α]D²⁰ +4.5 (c 1.0, CHCl₃); mp 58 °C; ¹H NMR (CDCl₃) δ: 2.05-2.35 (m, 1H, H-2'a), 2.67-2.86 (m, 1H, H-2'b), 3.43 (d, J = 3.8 Hz, 2H, H-5'), 3.81 (s, 3H, OCH₃), 4.34 (td, J = 3.5 Hz, 27.0 Hz, 1H, H-4'), 5.23 (dd, J = 4.7 Hz, 53.7 Hz, 1H, H-3'), 6.33 (dd, J = 5.0 Hz, 9.1 Hz, 1H, H-1'), 6.75-7.50 (m, 15H, CH alkene, trityl), 7.63 (s, 1H, H-6), 9.00 (se, 1H, NH); ¹³C NMR (CDCl₃) δ: 39.4 (d, J = 84.2 Hz, C-2'), 55.5 (OCH₃), 63.1 (d, J = 38.5 Hz, C-5'), 84.5 (d, J = 101.0 Hz, C-4'), 85.3 (C), 85.5 (CH alkene), 87.5 (C), 87.7 (C-1'), 94.1 (d, J = 710.0 Hz, C-3'), 113.6 (CH), 118.1 (C), 127.4 (CH), 128.3 (CH), 128.4 (CH), 130.5 (CH), 134.8 (C), 139.2 (C-6), 143.7 (C), 143.8 (C), 149.5 (C=O), 158.9 (C), 159.0 (C);

Step d): 5-(1,2-Diiodovinyl)-1-(4-fluoro-5-hydroxymethyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (25a)

Prepared from compound 24a, using the typical procedure described before to give 25a (51%) as a yellow solid. [α]D²⁰ +16.0 (c 1.0, CH₂OH); mp 112 °C; UV (MeOH) λₘₐₓ 232 nm, 274 nm; ¹H NMR (CD₃OD) δ: 2.13-2.47 (m, 1H, H-2'a), 2.50-2.77 (m, 1H, H-2'b), 3.80 (d, J = 2.8 Hz, 2H, H-5'), 4.32 (td, J = 2.9 Hz, 27.0 Hz, 1H, H-4'), 5.30 (dd, J = 4.6 Hz, 53.7 Hz, 1H, H-3'), 6.36 (dd, J = 5.6 Hz, 9.0 Hz, 1H, H-1'), 7.53 (s, 1H, CH alkene), 8.28 (s, 1H, H-6); ¹³C NMR (CD₃OD) δ: 40.3 (d, J = 81.9 Hz, C-2'), 62.7 (d, J = 43.3 Hz, C-5'), 87.0 (C-1'), 87.3 (d, J = 96.3 Hz, C-4'), 87.3 (C), 88.1 (CH alkene), 96.0 (d, J = 698.0 Hz, C-3'), 119.2 (C), 142.2 (C-6), 151.5 (C=O), 161.8 (C=O);

Compounds of examples 38 and 39 are obtained in a similar way starting from compounds 24b and 24c respectively.

Example 38: 5-(1-Bromo-2-iodovinyl)-1-(4-fluoro-5-hydroxymethyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (25b).

Prepared from compound 24b, using the typical procedure described before to give the title compound (52 %) as a pale yellow solid. [α]D²⁰ +13.0 (c 0.6, CH₂OH); mp 107 °C; UV (MeOH) λₘₐₓ 298 nm; ¹H NMR (CD₃OD) δ: 2.14-2.47 (m, 1H, H-2'a), 2.52-2.78 (m, 1H, H-2'b), 3.80 (d, J = 3.0 Hz, 2H, H-5'), 4.31 (td, J = 2.9 Hz, 27.0 Hz, 1H, H-4'), 5.30 (dd, J = 4.7 Hz, 53.7 Hz, 1H, H-3'), 6.36 (dd, J = 5.6 Hz, 9.0 Hz, 1H, H-1'), 7.30 (s, 1H, CH alkene), 8.36 (s, 1H, H-6); ¹³C NMR (CD₃OD) δ: 40.3 (d,C-2'), 62.7 (d, C-5'), 83.2 (CH alkene), 87.0 (C-1'),
87.3 (d, C-4'), 87.3 (C), 96.0 (d, C-3'), 115.5 (C), 116.0 (C), 143.9 (C-6), 151.5 (C=O), 161.8 (C=O);

**Example 39**: (1-Chloro-2-iodovinyl)-1-(4-fluoro-5-hydroxymethyltetrahydrofuran-2-yl)-1H-pyrimidine-2,4-dione (25e)

Prepared from compound 24c, using the typical procedure described before to give the title compound (76%) as an orange solid. \([\sigma]_D^{20} +17.0 (c 0.7, CH_3OH); mp 87^\circ C; UV (MeOH)\) \(\lambda_{max} 274 \text{ nm}; ^1H NMR (CD_3OD) \delta : 2.13-2.48 (m, 1H, H-2'a), 2.52-2.75 (m, 1H, H-2'b), 3.80 (d, J = 2.8 Hz, 2H, H-5'), 4.30 (td, J = 2.8 Hz, 26.8 Hz, 1H, H-4'), 5.30 (dd, J = 4.6 Hz, 5.35 Hz, 1H, H-3'), 6.37 (dd, J = 5.7 Hz, 8.8 Hz, 1H, H-1'), 7.06 (s, 1H, CH alkene), 8.38 (s, 1H, H-6); \(^{13}C\) NMR (CD_3OD) \delta : 40.2 (d, J = 84.2 Hz, C-2'), 62.7 (d, J = 43.3 Hz, C-5'), 81.1 (CH alkene), 87.0 (C-1'), 87.4 (d, J = 93.9 Hz, C-4'), 96.2 (d, J = 698.0 Hz, C-3'), 114.5 (C), 128.9 (C), 146.0 (C-6), 151.2 (C=O), 164.2 (C=O);

**Example 40**: 1-(4-Fluoro-5-hydroxymethyltetrahydrofuran-2-yl)-5-iodoethynyl-1H-pyrimidine-2,4-dione (22)

Step a): 1-(4-Fluoro-5-trityloxyethyl-tetrahydro-furan-2-yl)-5-iodoethynyl-1H-pyrimidine-2,4-dione (21)

5-ethynyl nucleoside 20 (0.5 mmol) was dissolved in dry acetonitrile (5.0 mL) and the reaction mixture is cooled to an ice bath. A solution of the halogenating reagent (i.e. IDCP; 0.7 mmol) and Ag(coll)_2ClO_4 (11.0 mg) are added and the reaction mixture is stirred in the dark at rt (typically 2 h-20 h, checked by TLC). The reaction mixture is quenched at 0°C by a saturated Na_2SO_4 solution (2 mL) then extracted with EtOAc (10 mL x4). The organic layer is washed with water (10 mL), a 1M HCl solution (5 mL x3), water (5 mL x2), and brine (10 mL), then dried over MgSO_4, filtered through a fluted glass funnel and concentrated under reduced pressure to afford the crude product, which is submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 8/2 to 3/7) to give 21 (26%) as yellow solid. \([\sigma]_D^{20} +122.0 (c 1.0, CHCl_3); mp 73 \text{ ^\circ C}; ^1H NMR (CDCl_3) \delta : 2.15-2.48 (m, 1H, H-2'a), 2.68-2.93 (m, 1H, H-2'b), 3.30-3.56 (m, 2H, H-5'), 3.81 (s, 3H, OCH_3), 4.38 (d, J = 27.0 Hz, 1H, H-4'), 5.32 (dd, J = 4.4 Hz, 53.4 Hz, 1H, H-3'), 6.33 (dd, J = 5.3 Hz, 9.1 Hz, 1H, H-1'), 6.75-7.50 (m, 14H, trityl), 8.01 (s, 1H, H-6), 9.20 (se, 1H, NH); \(^{13}C\) NMR (CDCl_3) \delta : 12.1 (CCl), 39.8 (d, J = 86.6 Hz, C-2'), 55.4 (OCH_3), 63.4 (d, J = 40.9 Hz, C-5'), 84.2 (C), 84.9 (d, J = 101.1 Hz, C-4'), 86.0 (C-1'), 87.8 (C), 94.5 (d, J = 707.5 Hz, C-3'), 101.0 (C), 113.6 (CH), 127.5 (CH), 127.9 (C), 128.1 (CH), 128.2 (CH), 130.4 (CH), 134.6 (C), 143.5 (C), 143.7 (C-6), 143.8 (C), 149.2 (C=O), 159.0 (C), 161.6 (C);
Step b): 1-(4-Fluoro-5-hydroxymethyltetrahydrofuran-2-yl)-5-iodoethynyl-1H-pyrimidine-2,4-dione (22)
A solution of the protected nucleoside 21 (0.3 mmol) in acetic acid 80 % (30 mL) is stirred until completion (checked by TLC, typically 18 hours). After evaporation of the solvent, the crude product was submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 2/8) to give 22 (47 %) as a yellow solid. \([\alpha]_D^{20} -1.5 \ (c 1.0, \ CH_3OH)\); mp 140°C; UV (MeOH) \(\lambda_{\text{max}}\) 233 nm, 298 nm; \(^1\)H NMR (CD\(_3\)OD) \(\delta\): 2.11-2.45 (m, 1H, H-2'a), 2.46-2.75 (m, 1H, H-2'b), 3.79 (d, \(J = 3.2 \) Hz, 2H, H-5'), 4.29 (td, \(J = 2.9 \) Hz, 27.0 Hz, 1H, H-4'), 5.27 (dd, \(J = 4.9 \) Hz, 53.9 Hz, 1H, H-3'), 6.28 (dd, \(J = 5.5 \) Hz, 8.9 Hz, 1H, H-1'), 8.30 (s, 1H, H-6); \(^1^3\)C NMR (CD\(_3\)OD) \(\delta\): 15.8 (CH), 39.8 (d, \(J = 81.8 \) Hz, C-2'), 62.5 (d, \(J = 43.3 \) Hz, C-5'), 85.2 (C), 87.0 (C-1'), 87.3 (d, \(J = 96.3 \) Hz, C-4'), 96.0 (d, \(J = 700.4 \) Hz, C-3'), 101.5 (C), 146.0 (C-6), 151.1 (C=O), 164.4 (C=O).

Example 41: 1-(4-Azido-5-hydroxymethyltetrahydrofuran-2-yl)-5-(1,2-diiodovinyl)-1H-pyrimidine-2,4-dione (31a)

Step a): 1-(4-Azido-5-trityloxytetrahydrofuran-2-yl)-5-ethynyl-1H-pyrimidine-2,4-dione (26)
A solution of 18 (100 mg, 0.16 mmol) and NaN\(_3\) (35 mg, 0.54 mmol) in DMF (5 mL) is refluxed overnight. After evaporation of volatiles, the crude product is submitted to a flash silica gel column chromatography (eluent: hexanes/EtOAc: 2/8) to give 26 (61.0 mg, 70%) as a pale yellow solid. \([\alpha]_D^{20} +13 \ (c 0.8, \ CH_3OH)\); mp 110°C; \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 2.35-2.46 (m, 1H, H-2'a), 2.52-2.62 (m, 1H, H-2'b), 2.69 (s, 1H, CH alkyne), 3.37-3.52 (m, 2H, H-5'), 3.82 (s, 3H, OCH\(_3\)), 4.00-4.04 (m, 1H, H-4'), 4.28-4.34 (m, 1H, H-3'), 6.15 (t, \(J = 6.1 \) Hz, 1H, H-1'), 6.88 (d, \(J = 8.9 \) Hz, 2H, trityl), 7.21-7.51 (m, 14H, trityl), 8.08 (s, 1H, H-6); \(^1^3\)C NMR (CDCl\(_3\)) \(\delta\): 38.6 (C-2'), 55.3 (OCH\(_3\)), 60.4 (C-3'), 62.7 (C-5'), 82.3 (CH alkyne), 84.0 (C-4'), 85.5 (C-1'), 87.6 (C), 99.4 (C), 113.6 (CH x2), 127.3 (CH), 128.1 (CH), 128.2 (CH), 130.4 (CH), 134.7 (C), 143.7 (C), 149.2 (C), 158.9 (C), 161.6 (C);

Step b): 1-(4-Azido-5-trityloxytetrahydrofuran-2-yl)-5-(1,2-diido-vinyl)-1H-pyrimidine-2,4-dione (30a)
Prepared from compound of Step a), using \(\text{I}_2\) with the typical procedure described before for the preparation of 24a to give 30a (52 %) as a pale yellow solid. \([\alpha]_D^{20} +16 \ (c 1.0, \ CH_3OH)\); mp 139°C; \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 2.23-2.34 (m, 1H, H-2'a), 2.51-2.61 (m, 1H, H-2'b); 3.36-3.49 (m, 2H, H-5'), 3.81 (s, 3H, OCH\(_3\)), 3.98-4.05 (m, 1H, H-4'), 4.16-4.26 (m, 1H, H-3'), 6.16 (t, \(J = 6.6 \) Hz, 1H, H-1'), 6.87 (d, \(J = 8.8 \) Hz, 2H, trityl), 7.21-7.43 (m, 13H, trityl, CH alkyne), 7.58 (s, 1H, H-6); \(^1^3\)C NMR (CDCl\(_3\)) \(\delta\): 38.1 (C-2'), 55.2 (OCH\(_3\)), 60.4 (C-3'), 62.8 (C-5'), 83.5 (C-
4'), 85.1 (C-1'), 87.1 (C), 87.2 (CH alkene), 113.3 (CH), 117.6 (C), 127.1 (CH), 128.0 (CH), 128.1 (CH), 130.2 (CH), 135.6 (C), 139.0 (C-6), 143.5 (C), 143.6 (C), 149.1 (C=O), 158.6 (C), 158.7 (C);

Step c: 1-(4-Azido-5-hydroxymethyltetrahydrofuran-2-yl)-5-(1,2-diodo-vinyl)-1H-pyrimidine-2,4-dione (31a)
Prepared from compound 30a using the typical procedure described before for the preparation of 24a to give 31a (78%) as a pale yellow gum. [α]_D^20 +17 (c 1.0, CH₃OH); UV (MeOH) _λ_max_ 225 nm, 284 nm; ^1^H NMR (DMSO-d₆) δ : 2.34-2.43 (m, 2H, H-2'), 3.56-3.71 (m, 2H, H-5'), 3.85-3.90 (m, 1H, H-4'), 4.35-4.41 (m, 1H, H-3'), 5.26 (t, J = 4.9 Hz, 1H, OH), 6.09 (t, J = 6.3 Hz, 1H, H-1'), 7.61 (s, 1H, CH alkene), 8.06 (s, 1H, H-6'), 11.68 (s, 1H, NH); ^1^C NMR (DMSO-d₆) δ : 37.5 (C-2'), 60.4 (C-3'), 61.4 (C-5'), 85.0 (C-1', C-4'), 88.9 (C-I), 90.2 (CH alkene), 117.7 (C-5), 140.5 (C-6), 150.1 (C=O), 159.9 (C=O).

Compounds of examples 42 and 43 are obtained in a similar way, starting from compounds 30b and 30c respectively.

**Example 42: 1-(4-Azido-5-hydroxymethyltetrahydrofuran-2-yl)-5-(1-bromo-2-iodo-vinyl)-1H-pyrimidine-2,4-dione (31b)**
Prepared from compound 30b using the typical procedure described before to give 31b (53%) as a pale yellow gum. [α]_D^20 +11 (c 1.0, CH₃OH); UV (MeOH) _λ_max_ 229 nm, 276 nm; ^1^H NMR (DMSO-d₆) δ : 2.36-2.43 (m, 2H, H-2'), 3.57-3.69 (m, 2H, H-5'), 3.86-3.91 (m, 1H, H-4'), 4.35-4.42 (m, H-3'), 5.28 (brs, 1H, OH), 6.09 (t, J = 5.9 Hz, 1H, H-1'), 7.45 (s, 1H, CH alkene), 8.18 (s, 1H, H-6'), 11.7 (s, 1H, NH); ^1^C NMR (DMSO-d₆) δ : 37.0 (C-2'), 60.0 (C-3'), 60.7 (C-5'), 84.6 (C-1', C-4'), 85.6 (CH alkene), 113.9 (C), 114.2 (C), 141.9 (C-6), 149.6 (C=O), 159.4 (C=O);

**Example 43: 1-(4-Azido-5-hydroxymethyltetrahydrofuran-2-yl)-5-(1-chloro-2-iodo-vinyl)-1H-pyrimidine-2,4-dione (31c)**
Prepared from compound 30c using the typical procedure described before to give 31c (57%) as a pale yellow gum. [α]_D^20 +28 (c 1.0, CH₃OH); UV (MeOH) _λ_max_ 214 nm, 273 nm; ^1^H NMR (DMSO-d₆) δ : 2.33-2.44 (m, 2H, H-2'), 3.33-3.71 (m, 1H, H-5'), 3.86-3.91 (m, 1H, H-4'), 4.35-4.42 (m, 1H, H-3'), 5.28 (t, J = 4.9 Hz, 1H, OH), 6.09 (t, J = 6.2 Hz, 1H, H-1'), 7.23 (s, 1H, CH alkene), 8.22 (s, 1H, H-6'), 11.73 (s, 1H, NH); ^1^C NMR (DMSO-d₆) δ : 37.7 (C-2'), 60.7 (C-3'), 61.3 (C-5'), 84.5 (CH), 85.2 (CH x2), 113.0 (C), 127.4 (C), 143.1 (C-6), 150.3 (C=O), 160.1 (C=O);
Example 44: 1-(4-Azido-5-hydroxymethyl-tetrahydro-furan-2-yl)-5-idoethynyl-1H-pyrimidine-2,4-dione (28)

Prepared from compound 26 using a similar procedure as the one disclose above for Example 40, to give the title compound 60 % as a pale yellow gum, $[\alpha]_D^{20} +15$ (c 1.0, CH$_3$OH); UV (MeOH) $\lambda_{max}$ 236 nm, 295 nm; $^1$H NMR (DMSO-d$_6$) $\delta$: 2.24-2.34 (m, 1H, H-2'a), 2.39-2.46 (m, 1H, H-2'b), 3.54-3.72 (m, 2H, H-5'), 3.79-3.84 (m, 1H, H-4'), 4.34-4.41 (m, 1H, H-3'), 5.30 (t, $J$ = 5.3 Hz, 1H, OH), 6.01 (t, $J$ = 6.0 Hz, 1H, H-1'), 8.22 (s, 1H, H-6), 11.66 (s, 1H, NH); $^{13}$C NMR (DMSO-d$_6$) $\delta$: 19.8 (C), 36.8 (C-2'), 59.2 (C-3'), 60.2 (C-5'), 84.3 (C-1', C-4'), 84.8 (C), 99.0 (C), 144.6 (C-6), 149.3 (C=O), 161.7 (C=O).

Biological evaluation

Antiviral and Cytotoxicity assays for HCV. Huh7 cells harbouring the subgenomic HCV replicon BM4-5 (J.T. Guo et al., J. Virol. 2001, 75 : 8516-8523) were used in this study. Cells were maintained in Dulbecco's modified Eagle's medium high glucose 4.5 g/l (LifeTechnologies) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin and streptomycin, 1% L-Pyruvate and 500 g/ml of genetin (G418, Invitrogen). Genetin was used to select cells permitting the HCV RNA replication. Cells were passaged every four days with a 1:4 ratio.

Cells were seeded in 6-wells plates at a density of 2.5 x 10$^5$ cells per well, sixteen hours before the beginning of treatment. Cells were treated with the molecules administered at different concentrations in complete medium that did not contain genetin. The administration of each drug was renewed every day for three consecutive days. Ribavirin (ICN Pharmaceuticals), mycophenolic acid (MPA, Sigma), and interferon alpha-2b (IntronA®) were used in the same conditions as positive controls. The concentrations used for these drugs were respectively: 0; 0.5; 1; 2; 4; 8; 16; 32; 64; 128; 256 and 512 $\mu$M for ribavirin, 0; 2.5; 5; 10; 20; 40 $\mu$M for mycophenolic acid, and 0; 0.1; 1; 10; 100 and 1000 U/l/mL for IFN alpha-2b. Total RNA was extracted at the end of the treatment (24 hours after the last day of treatment) with the "Extract all " reagent (Eurobio), which is a mix of phenol and guanidium thiocyanate. Northern blot analysis was then performed using the NorthernMax™-Gly kit (Ambion), following manufacturer's instructions. Five micrograms of total RNA were denatured in glyoxal buffer at 50°C for 30 minutes, separated by 1.1% agarose gel electrophoresis, and then transferred for 12 hours onto a charged nylon membrane (HybondN+, Amersham). Hybridization was carried out with three different $[^{32}P]$CTP-labelled riboprobes obtained by in vitro transcription (Riboprobe in vitro transcription system, Promega). Two probes were complementary to the NS5A region of the HCV genome of
negative polarity and positive polarity. A third probe was complementary to the beta-actin mRNA and obtained by in vitro transcription from a specific plasmid (pTRI beta actin human, reference 7424, Ambion). First, the blot was hybridized with the riboprobes directed against the negative strand of HCV RNA and beta-actin mRNA, respectively. After one night of hybridization at 68°C, the membrane was washed, then exposed to X-ray film and a phosphor screen (phosphorimager). This screen was then scanned and quantitative analysis was achieved using ImageQuant software. The amount of beta-actin mRNA was used as an internal loading control to standardize the amount of HCV RNA detected. The same membrane was subsequently hybridized with the negative sense riboprobe to determine the level of positive strand HCV RNA, using the same approach.

For cell viability assays, cells were seeded in 96-well plates at a density of 12,500 cells per well. They were treated by the different molecules with the same concentrations and conditions than those used for the antiviral assays. Then, cell viability was measured by neutral red assay. Neutral red specifically colors lysosomes and its accumulation depends on cellular membrane integrity. The yield of neutral red incorporated in cells is proportional to the number of living cells. At the end of treatment, culture medium was removed; cells were washed by PBS, then coloured with neutral red at 0.005% for 3 hours at 37°C. Cells were then fixed 1 minute by formol calcium and lysed by a treatment with a vol/vol mixture of acetic acid and ethanol. After 15 minutes incubation, absorbance was read at 490nm.

**Antiviral and cytotoxicity assays for BVDV.** Noncytopathic-BVDV-free MDBK cells (European Collection of Animal Cell Cultures, Porton Down, U.K.) were kindly provided by Dr N. Zitzmann (Oxford University). Cells were propagated in DMEM F12 (Eurobio) supplemented with 10% horse serum (Life Technologies), 1% L-glutamin (Gibco), 1% penicillin and streptomycin (Gibco). The NADL cytopathic (CP) BVDV strain was obtained from ATCC: VR-504.

MDBK cells were seeded in microwell plates (96 wells) at a density of $1.10^5$ cells per well, then infected with BVDV (strain NADL) (at dilution inducing 100% cytopathic effect three days post inoculation, i.e. approximately 10 plaque forming units (pfu) per well at three days post infection) for one hour, at 37°C. After a wash with DMEM, infected cells were incubated for three days in the presence of absence of drug; each concentration of drug was added in 12 consecutive wells. The appearance of CPE was visually checked the third day post inoculation to evaluate the IC50 on BVDV (strain NADL), according to the different conditions of treatment. By definition, the IC50 is the concentration of drug that inhibits 50% of the CPE in comparison to cells inoculated but untreated presenting 100% of CPE.
Uninfected MDBK cells were grown in the absence or presence of varying concentrations of drug tested. After 3 days, toxicity was evaluated by neutral red coloration as described above.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Anti-HCV activity and cytotoxicity on Replicon System (+)-RNA</th>
<th>Cytotoxicity (CC₅₀)</th>
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<tr>
<td></td>
<td>CC₅₀</td>
<td>IC₅₀</td>
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<tr>
<td>IFNα-2b</td>
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</tr>
<tr>
<td>17c</td>
<td>&gt; 200</td>
<td>≥ 200</td>
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Table 1: Evaluation of the antiviral activity and cells viability (obtained with neutral red test) against Hepatitis C Virus (HCV) replication using cell line harboring HCV subgenomic replicon, of the antiviral activity against HIV-1 in PBM cells and of the cytotoxicity against PBM, CEM and VERO cells in vitro. Results are expressed in μM, except for IFN which is expressed as International Units/ml. Selectivity Index (SI) is the ratio between CC$_{50}$ and IC$_{50}$.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>CC$_{50}$ (Neutral Red)</th>
<th>EC$_{50}$ ECP inhibition (3rd day p.i.)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribavirin</td>
<td>&gt; 100</td>
<td>36 μM</td>
<td>&gt; 2.77</td>
</tr>
<tr>
<td>12a-c, 13b, 14a-c, 15c, 16a-c</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Table 2: Neutral red testing and antiviral assay on BVDV strain NADL expressed in μM.

It should be clearly understood that the invention defined by the attached claims is not limited to the particular embodiments indicated in the above description, but encompasses the variants which depart neither from the context nor from the spirit of the present invention.
1. Use of an uridine derivative of formula (I):

\[
\begin{array}{c}
\text{H} \\
\text{O} \\
\text{R'} \quad \text{R'} \\
\text{O} \\
\text{O} \\
\text{R}^1 \quad \text{R}^2 \\
\text{R}^3 \quad \text{R}^4
\end{array}
\]

wherein
- \( R^1 \) represents monohalogenated alkynyl or dihalogenated alkenyl;
- \( R^2 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- \( R^3 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH, -S-alkyl and \( N^3 \); and
- \( R^4 \) is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate,
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its \( N \)-oxide forms,
for the preparation of a drug having antiviral activity against a Flaviviridae.

2. The use according to claim 1, wherein the uridine derivative of formula (I)
presents the following characteristics:
- \( R^1 \) represents \(-C=\text{C}-\text{X}\) or \(-C(\text{Y})=\text{CH}(\text{X})\);
- \( X \) and \( Y \) each independently represents halogen;
- \( R^2 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- \( R^3 \) is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH, -S-alkyl and \( N^3 \); and
- \( R^4 \) is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -phosphate, -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate,
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its \( N \)-oxide forms.

3. The use according to claim 2, wherein \( R^1 \) represents chloroethynyl, bromoethynyl, iodoethynyl or \(-C(\text{Y})=\text{CH}(\text{X})\), where \( Y \) is chosen from among chlorine, bromine and iodine, and \( X \) is bromine or iodine.
4. The use according to claim 2, wherein
- R\(^1\) represents chloroethynyl, bromoethynyl, iodoethynyl or -C(Y)=CH(X), where Y is chosen from among chlorine, bromine and iodine, and X is bromine or iodine;
- R\(^2\) is chosen from among hydrogen, hydroxyl, and -O-alkyl;
- R\(^3\) is chosen from among hydrogen, hydroxyl, -O-alkyl, -SH, and -S-alkyl;
- R\(^2\) and R\(^3\) not simultaneously being each hydrogen; and
- R\(^4\) is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate, as well as their possible tautomers, their possible pharmaceutically acceptable addition salts with an acid or a base, and their N-oxide forms.

5. The use according to claim 1, wherein the uridine derivatives is chosen from among the following compounds:
   - (E)-5-(1-Chloro-2-iodovinyl)-2'-deoxyuridine,
   - (E)-5-(1,2-Diodovinyl)-2'-deoxyuridine,
   - 5-(2-Iodoethynyl)-2'-deoxyuridine,
   - (E)-5-(1-Bromo-2-iodovinyl)-2'-deoxyuridine,
   - (E)-5-(1,2-Dibromovinyl)-2'-deoxyuridine,
   - 5-(2-Bromoethynyl)-2'-deoxyuridine,
   - (E)-5-(1-Chloro-2-iodovinyl)-uridine,
   - (E)-5-(1,2-Diodovinyl)-uridine,
   - 5-(2-Iodoethynyl)-uridine,
   - (E)-5-(1-Bromo-2-iodovinyl)-uridine,
   - (E)-5-(1,2-Dibromovinyl)-uridine, and
   - 5-(2-bromoethynyl)-uridine,
as well as its possible tautomers, its possible pharmaceutically acceptable additions salts with an acid or a base, and its N-oxide forms.

6. The use according to any one of claims 1 to 5, wherein an uridine derivative of formula (I) as well as its possible tautomers, its possible pharmaceutically acceptable addition salts with an acid or a base, and its N-oxide forms, and an interferon are used for the preparation of a drug having antiviral activity against a Flaviviridae.

7. The use according to any one of claims 1 to 6, for the preparation of a drug having antiviral activity against hepatitis C virus (HCV).
8. A compound of formula (I'):

\[ \text{Diagram of compound (I')} \]

wherein
- \( R' \) represents \(-\text{C}(Y) = \text{CH}(X), -\text{C}(Y) = \text{C}(X)\text{-alkyl} \) or \(-\text{C}(Y) = \text{C}-\text{Ak}(X) \), where \( Y \) is halogen and \( \text{Ak}(X) \) denotes a linear or branched \( \text{C}_1\text{-C}_{10} \) alkyl radical, one hydrogen atom of which being substituted by one halogen atom \( X \);
- \( R^2, R^3 \) and \( R^4 \) have the same definitions as \( R^2, R^3 \) and \( R^4 \), respectively, in the above formula (I);

as well as its possible tautomers, its possible pharmaceutically acceptable addition salts with an acid or a base, and its N-oxide forms.

9. The compound according to claim 8, wherein:
- \( R' \) represents \(-\text{C}(Y) = \text{CH}(X) \);
- \( X \) and \( Y \) each independently represents halogen;
- \( R^2 \) is chosen from among hydrogen, hydroxyl, \(-\text{O}-\text{alkyl} \) and halogen;
- \( R^3 \) is chosen from among hydrogen, hydroxyl, \(-\text{O}-\text{alkyl} \), \(-\text{O}-\text{CO}-\text{alkyl} \), halogen, \(-\text{SH} \), \(-\text{S}-\text{-alkyl} \) and \( N^3 \); and
- \( R^4 \) is chosen from among hydroxyl, \(-\text{O}-\text{alkyl} \), \(-\text{O}-\text{CO}-\text{alkyl} \), \(-\text{O}-\text{phosphate} \), \(-\text{O}-\text{di-phosphate} \), \(-\text{O}-\text{tri-phosphate} \) and \(-\text{O}-\text{phosphonate} \),

as well as its possible tautomers, its possible pharmaceutically acceptable addition salts with an acid or a base, and its N-oxide forms.

10. The compound according to claim 9, wherein \( R' \) represents \(-\text{C}(Y) = \text{CH}(X) \), where \( Y \) is chosen from among chlorine, bromine and iodine, and \( X \) is bromine or iodine.

11. The compound according to claim 9, wherein
- \( R' \) represents \(-\text{C}(Y) = \text{CH}(X) \), where \( Y \) is chosen from among chlorine, bromine and iodine, and \( X \) is bromine or iodine.
- \( R^2 \) is chosen from among hydrogen, hydroxyl, and \(-\text{O}-\text{alkyl} \);
- \( R^3 \) is chosen from among hydrogen, hydroxyl, \(-\text{O}-\text{alkyl} \), \(-\text{SH} \), and \(-\text{S}-\text{alkyl} \); and
12. A drug formulation having an antiviral activity against a Flaviviridae comprising

(1) an uridine derivative of formula (I):

![Chemical Structure](image)

wherein

- R₁ represents monohalogenated alkynyl or dihalogenated alkenyl;
- R₂ is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl and halogen;
- R₃ is chosen from among hydrogen, hydroxyl, -O-alkyl, -O-CO-alkyl, halogen, -SH, -S-alkyl and N₃; and
- R₄ is chosen from among hydroxyl, -O-alkyl, -O-CO-alkyl, -O-phosphate, -O-diphosphate, -O-triphosphate and -O-phosphonate,

as well as its possible tautomers, its possible pharmaceutically acceptable addition salts with an acid or a base, and its N-oxide forms.

(2) an immunomodulator or another active ingredient having an antiviral activity against a Flaviviridae, and

(3) a pharmaceutically acceptable vehicle, carrier or excipient.

13. The drug formulation according to claim 12, wherein ingredient (2) comprises an interferon.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

A61K53/70     A61P53/12

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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</table>

**X** Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
- **"A"** document defining the general state of the art which is not considered to be of particular relevance
- **"E"** earlier document published on or after the international filing date
- **"C"** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- **"O"** document referring to an oral disclosure, use, exhibition or other means
- **"P"** document published prior to the international filing date but later than the priority date claimed
- **"T"** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- **"X"** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- **"Y"** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other documents, such combination being obvious to a person skilled in the art
- **"A"** document member of the same patent family

**Date of the actual completion of the international search**

13 March 2006

**Date of mailing of the international search report**

25/04/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5018 Patentlaan 2 NL - 2280 HU Rijswijk
Tel. (+31-70) 340-0340, Tx. 31 651 apn nl, Fax. (+31-70) 340-3016

Authorized officer

Cattell, James
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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</thead>
</table>

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 11

WO 2002/08241
Title: PRODRUGS OF PHOSPHONATE NUCLEOTIDE ANALOGUES AND METHODS FOR SELECTING AND MAKING SAME

Abstract: A novel method is provided for screening prodrugs of methoxyporphosphate nucleotide analogues to identify prodrugs selectively targeting desired tissues with antiviral or antitumor activity. This method has led to the identification of novel mixed ester-amidates of PMPA for retroviral or hepadnaviral therapy, including compounds of structure (5a) having substituent groups as defined herein. Compositions of these novel compounds in pharmaceutically acceptable excipients and their use in therapy and prophylaxis are provided. Also provided is an improved method for the use of magnesium alkoxide for the preparation of starting materials and compounds for use herein.

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Agents: HENSLEY, Max, D. et al.; Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404 (US).


Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published: — without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
Prodrugs of Phosphonate Nucleotide Analogues and Methods for Selecting and Making Same

This application relates to prodrugs of methoxyphosphonate nucleotide analogues. In particular it relates to improved methods for making and identifying such prodrugs.

Summary of the Invention

Prodrugs of methoxyphosphonate nucleotide analogues intended for antiviral or antitumor therapy, while known, traditionally have been selected for their systemic effect. For example, such prodrugs have been selected for enhanced bioavailability, i.e., ability to be absorbed from the gastrointestinal tract and converted rapidly to parent drug to ensure that the parent drug is available to all tissues. However, applicants now have found that it is possible to select prodrugs that become enriched at therapeutic sites, as illustrated by the studies described herein where the analogues are enriched at localized focal sites of HIV infection. The objective of this invention is, among other advantages, to produce less toxicity to bystander tissues and greater potency of the parental drug in tissues which are the targets of therapy with the parent methoxyphosphonate nucleotide analogue.

Accordingly, pursuant to these observations, a screening method is provided for identifying a methoxyphosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue comprising:

(a) providing at least one of said prodrugs;
(b) selecting at least one therapeutic target tissue and at least one non-target tissue;
(c) administering the prodrug to the target tissue and to said at least one non-target tissue; and
(d) determining the relative antiviral activity conferred by the prodrug in the tissues in step (c).

In preferred embodiments, the target tissue are sites where HIV is actively replicated and/or which serve as an HIV reservoir, and the non-target tissue is an intact animal. Unexpectedly, we found that selecting lymphoid tissue as the target tissue for the practice of this method for HIV led to identification of prodrugs that enhance the delivery of active drug to such tissues.

A preferred compound of this invention, which has been identified by this method has the structure (1),
where Ra is H or methyl, and chirally enriched compositions thereof, salts, their free base and solvates thereof.

A preferred compound of this invention has the structure (2)

and its enriched diasteromers, salts, free base and solvates.

In addition, we unexpectedly found that the chirality of substituents on the phosphorous atom and/or the amidate substituent are influential in the enrichment observed in the practice of this invention. Thus, in another embodiment of this
invention, we provide diastereomERICally enriched compounds of this invention having the structure (3)

\[
\begin{align*}
\text{B} & \quad \text{E} \quad \text{PO} \\
\quad & \quad \quad \text{R}^1 \\
\quad & \quad \quad \quad \text{R}^2
\end{align*}
\]

(3)

which are substantially free of the diastereomer (4)

\[
\begin{align*}
\text{B} & \quad \text{E} \quad \text{PO} \\
\quad & \quad \quad \text{R}^1 \\
\quad & \quad \quad \quad \text{R}^2
\end{align*}
\]

(4)

wherein

- \( R^1 \) is an oxyester which is hydrolyzable \textit{in vivo}, or hydroxyl;
- \( B \) is a heterocyclic base;
- \( R^2 \) is hydroxyl, or the residue of an amino acid bonded to the P atom through an amino group of the amino acid and having each carboxy substituent of the amino acid optionally esterified, but not both of \( R^1 \) and \( R^2 \) are hydroxyl;
- \( E \) is \(-(\text{CH}_2)_2-, -\text{CH(\text{CH}_3})\text{CH}_2-, -\text{CH(\text{CH}_2F})\text{CH}_2-, -\text{CH(\text{CH}_2\text{OH})\text{CH}_2-,}
-\text{CH(\text{CH=CH}_2})\text{CH}_2-, -\text{CH(C=CH})\text{CH}_2-, -\text{CH(\text{CH}_2\text{N}_3})\text{CH}_2-,
-\text{CH(\text{R}_6\text{O})\text{CH(\text{R}_6})-, -\text{CH(\text{R}_9})\text{CH}_2\text{O- or -\text{CH(\text{R}_8})O-, wherein the right hand bond is linked to the heterocyclic base;}
- the broken line represents an optional double bond;
- \( R^4 \) and \( R^5 \) are independently hydrogen, hydroxy, halo, amino or a substituent having 1-5 carbon atoms selected from acyloxy, alkoxy, alkylthio, alkylamino and dialkylamino;
R⁶ and R'' are independently H, C₁-C₆ alkyl, C₁-C₆ hydroxyalkyl, or C₂-C₇ alkanoyl;
R⁷ is independently H, C₁-C₆ alkyl, or are taken together to form -O- or -CH₂-;
R⁸ is H, C₁-C₆ alkyl, C₁-C₆ hydroxyalkyl or C₁-C₆ haloalkyl; and
R⁹ is H, hydroxymethyl or acyloxymethyl;
and their salts, free base, and solvates.
The diastereomers of structure (3) are designated the (5) isomers at the phosphorus chiral center.
Preferred embodiments of this invention are the diastereomerically enriched compounds having the structure (5a)

which is substantially free of diastereomer (5b)
wherein

$R^5$ is methyl or hydrogen;

$R^6$ independently is H, alkyl, alkenyl, alkynyl, aryl or arylalkyl, or $R^6$ independently is alkyl, alkenyl, alkynyl, aryl or arylalkyl which is substituted with from 1 to 3 substituents selected from alkylamino, alkylaminoalkyl, dialkylaminoalkyl, dialkylamino, hydroxy, oxo, halo, amino, alkylthio, alkoxy, alkoxyalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylalkoxyalkyl, haloalkyl, nitro, nitroalkyl, azido, azidoalkyl, alkylacyl, alkylacylalkyl, carboxyl, or alkylacylamino;

$R^7$ is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group;

$R^{11}$ is amino, alkylamino, oxo, or dialkylamino; and

$R^{12}$ is amino or H;

and its salts, tautomers, free base and solvates.

A preferred embodiment of this invention is the compound of structure (6), 9-[(R)-2-[[((S)-[[(S)-1-]
(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl)methoxy]propyl]adenine, also designated herein GS-7340

Another preferred embodiment of this invention is the fumarate salt of structure (5) (structure (7)), 9-[(R)-2-[[((S)-[[(S)-1-}
(isopropoxycarbonyl)ethylamino|phenoxyphosphinyl|methoxy|propyl|adenine fumarate (1:1), also designated herein GS-7340-2

\[
\text{\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}}
\]

The compounds of structures (1)-(7) optionally are formulated into compositions containing pharmaceutically acceptable excipients. Such compositions are used in effective doses in the therapy or prophylaxis of viral (particularly HIV or hepadnaviral) infections.

In a further embodiment, a method is provided for the facile manufacture of 9-[2-(phosphonomethoxy)propyl]adenine (hereinafter "PMPA") or 9-[2-(phosphonomethoxy)ethyl] adenine (hereinafter "PMEA") using magnesium alkoxide, which comprises combining 9-(2-hydroxypropyl)adenine or 9-(2-hydroxyethyl)adenine, protected p-toluenesulfonyloxymethylphosphonate and magnesium alkoxide, and recovering PMPA or PMEA, respectively.

**Detailed Description of the Invention**

The methoxyphosphonate nucleotide analogue parent drugs for use in this screening method are compounds having the structure A-OH₂P(O)(OH)₂ wherein A is the residue of a nucleoside analogue. These compounds are known per se and are not part of this invention. More particularly, the parent compounds comprise a heterocyclic base B and an aglycon E, in general having the structure

\[
\begin{align*}
\text{B} & \quad \text{E} \quad \text{P} \quad \text{OH} \\
\text{OH} & 
\end{align*}
\]
wherein the group B is defined below and group E is defined above. Examples are described in U.S. Patent Nos. 4,659,825, 4,808,716, 4,724,233, 5,142,051, 5,130,427, 5,650,510, 5,663,159, 5,302,585, 5,476,938, 5,696,263, 5,744,600, 5,688,778, 5,386,030, 5,733,896, 5,352,786, and 5,798,340, and EP 821,690 and 654,037.

The prodrugs for use in the screening method of this invention are covalently modified analogues of the parent methoxyphosphonate nucleotide analogues described in the preceding paragraph. In general, the phosphorus atom of the parent drug is the preferred site for prodrug modification, but other sites are found on the heterocyclic base B or the aglycon E. Many such prodrugs are already known. Primarily, they are esters or amidates of the phosphorus atom, but also include substitutions on the base and aglycon. None of these modifications per se is part of this invention and none are to be considered limiting on the scope of the invention herein.

The phosphorus atom of the methoxyphosphonate nucleotide analogues contains two valences for covalent modification such as amidation or esterification (unless one phosphoryl hydroxyl is esterified to an aglycon E hydroxyl substituent, whereupon only one phosphorus valence is free for substitution). The esters typically are aryloxy. The amidates ordinarily are naturally occurring monoamino acids having free carboxyl group(s) esterified with an alkyl or aryl group, usually phenyl, cycloalkyl, or t-, n- or s- alkyl groups. Suitable prodrugs for use in the screening method of this invention are disclosed for example in U.S. Patent No. 5,798,340. However, any prodrug which is potentially believed to be capable of being converted in vivo within target tissue cells to the free methoxyphosphonate nucleotide analogue parent drug, e.g., whether by hydrolysis, oxidation, or other covalent transformation resulting from exposure to biological tissues, is suitable for use in the method of this invention. Such prodrugs may not be known at this time but are identified in the future and thus become suitable candidates available for testing in the method of this invention. Since the prodrugs are simply candidates for screening in the methods their structures are not relevant to practicing or enabling the screening method, although of course their structures ultimately are dispositive of whether or not a prodrug will be shown to be selective in the assay.
The pro-moieties bound to the parent drug may be the same or different. However, each prodrug to be used in the screening assay will differ structurally from the other prodrugs to be tested. Distinct, i.e. structurally different, prodrugs generally are selected on the basis of either their stereochemistry or their covalent structure, or these features are varied in combination. Each prodrug tested, however, desirably is structurally and stereochemically substantially pure, else the output of the screening assay will be less useful. It is of course within the scope of this invention to test only a single prodrug in an individual embodiment of the method of this invention, although typically then one would compare the results with prior studies with other prodrugs.

We have found that the stereochemistry of the prodrugs is capable of influencing the enrichment in target tissues. Chiral sites are at the phosphorus atom and are also found in its substituents. For example, amino acid used in preparing amidates may be D or L forms, and the phosphonate esters or the amino acid esters can contain chiral centers as well. Chiral sites also are found on the nucleoside analogue portion of the molecules, but these typically are already dictated by the stereochemistry of the parent drug and will not be varied as part of the screen. For example the R isomer of PMPA is preferred as it is more active than the corresponding S isomer. Typically these diasteromers or enantiomers will be chirally enriched if not pure at each site so that the results of the screen will be more meaningful. As noted, distinctiveness of stereoisomers is conferred by enriching or purifying the stereoisomer (typically this will be a diastereomer rather than an enantiomer in the case of most methoxyphosphonate nucleotide analogues) free of other stereoisomers at the chiral center in question, so that each test compound is substantially homogeneous. By substantially homogeneous or chirally enriched, we mean that the desired stereoisomer constitutes greater than about 60% by weight of the compound, ordinarily greater than about 80% and preferably greater than about 95%.
Novel Screening Method

Once at least one candidate prodrug has been selected, the remaining steps of the screening method of this invention are used to identify a prodrug possessing the required selectivity for the target tissue. Most conveniently the prodrugs are labeled with a detectable group, e.g. radiolabeled, in order to facilitate detection later in tissues or cells. However, a label is not required since other suitable assays for the prodrug or its metabolites (including the parent drug) can also be employed. These assays could include mass spectrometry, HPLC, bioassays or immunoassays for instance. The assay may detect the prodrug and any one or more of its metabolites, but preferably the assay is conducted to detect only the generation of the parent drug. This is based on the assumption (which may not be warranted in all cases) that the degree and rate of conversion of prodrug to antivirally active parent diphosphate is the same across all tissues tested. Otherwise, one can test for the diphosphate.

The target tissue preferably will be lymphoid tissue when screening for prodrugs useful in the treatment of HIV infection. Lymphoid tissue will be known to the artisan and includes CD4 cells, lymphocytes, lymph nodes, macrophages and macrophage-like cells including monocytes such as peripheral blood monocytic cells (PBMCs) and glial cells. Lymphoid tissue also includes non-lymphoid tissues that are enriched in lymphoid tissues or cells, e.g. lung, skin and spleen. Other targets for other antiviral drugs of course will be the primary sites of replication or latency for the particular virus concerned, e.g., liver for hepatitis and peripheral nerves for HSV. Similarly, target tissues for tumors will in fact be the tumors themselves. These tissues are all well-known to the artisan and would not require undue experimentation to select. When screening for antiviral compounds, target tissue can be infected by the virus.

Non-target tissues or cells also are screened as part of the method herein. Any number or identity of such tissues or cells can be employed in this regard. In general, tissues for which the parent drug is expected to be toxic will be used as non-target tissues. The selection of a non-target tissue is entirely dependent upon
the nature of the prodrug and the activity of the parent. For example, non-hepatic tissues would be selected for prodrugs against hepatitis, and untransformed cells of the same tissue as the tumor will suffice for the antitumor-selective prodrug screen.

It should be noted that the method of this invention is distinct from studies typically undertaken to determine oral bioavailability of prodrugs. In oral bioavailability studies, the objective is to identify a prodrug which passes into the systemic circulation substantially converted to parent drug. In the present invention, the objective is to find prodrugs that are not metabolized in the gastrointestinal tract or circulation. Thus, target tissues to be evaluated in the method of this invention generally do not include the small intestines or, if the intestines are included, then the tissues also include additional tissues other than the small intestines.

The target and non-target tissues used in the screening method of this invention typically will be in an intact living animal. Prodrugs containing esters are more desirably tested in dogs, monkeys or other animals than rodents; mice and rat plasma contains high circulating levels of esterases that may produce a misleading result if the desired therapeutic subject is a human or higher mammal.

It is not necessary to practice this method with intact animals. It also is within the scope of this invention to employ perfused organs, in vitro culture of organs (e.g. skin grafts) or cell lines maintained in various forms of cell culture, e.g. roller bottles or zero gravity suspension systems. For example, MT-2 cells can be used as a target tissue for selecting HIV prodrugs. Thus, the term "tissue" shall not be construed to require organized cellular structures, or the structures of tissues as they may be found in nature, although such would be preferred. Rather, the term "tissue" shall be construed to be synonymous with cells of a particular source, origin or differentiation stage.

The target and non-target tissue may in fact be the same tissue, but the tissues will be in different biological status. For example, the method herein could be used to select for prodrugs that confer activity in virally-infected tissue (target tissue) but which remain substantially inactive in virally-uninfected cells (corresponding non-target tissue). The same strategy would be employed to select
prophylactic prodrugs, i.e., prodrugs metabolized to antivirally active forms incidental to viral infection but which remain substantially unmetabolized in uninfected cells. Similarly, prodrugs could be screened in transformed cells and the untransformed counterpart tissue. This would be particularly useful in comparative testing to select prodrugs for the treatment of hematological malignancies, e.g. leukemias.

Without being limited by any particular theory of operation, tissue selective prodrugs are thought to be selectively taken up by target cells and/or selectively metabolized within the cell, as compared to other tissues or cells. The unique advantage of the methoxyphosphonate prodrugs herein is that their metabolism to the dianion at physiological pH ensures that they will be unable to diffuse back out of the cell. They therefore remain effective for lengthy periods of time and are maintained at elevated intracellular concentrations, thereby exhibiting increased potency. The mechanisms for enhanced activity in the target tissue are believed to include enhanced uptake by the target cells, enhanced intracellular retention, or both mechanisms working together. However, the manner in which selectivity or enhanced delivery occurs in the target tissue is not important. It also is not important that all of the metabolic conversion of the prodrug to the parent compound occurs within the target tissue. Only the final drug activity-conferring conversion need occur in the target tissue; metabolism in other tissues may provide intermediates finally converted to antiviral forms in the target tissue.

The degree of selectivity or enhanced delivery that is desired will vary with the parent compound and the manner in which it is measured (% dose distribution or parent drug concentration). In general, if the parent drug already possess a generous therapeutic window, a low degree of selectivity may be sufficient for the desired prodrug. On the other hand, toxic compounds may require more extensive screening to identify selective prodrugs. The relative expense of the method of this invention can be reduced by screening only in the target tissue and tissues against which the parent compound is known to be relatively toxic, e.g. for PMEA, which is nephrotoxic at higher doses, the primary focus will be on kidney and lymphoid tissues.
The step of determining the relative antiviral activity of a prodrug in the selected tissues ordinarily is accomplished by assaying target and non-target tissues for the relative presence or activity of a metabolite of the prodrug, which metabolite is known to have, or is converted to, a metabolite having antiviral or antitumor activity. Thus, typically one would determine the relative amount of the parent drug in the tissues over substantially the same time course in order to identify prodrugs that are preferentially metabolized in the target tissue to an antivirally or antitumor active metabolite or precursor thereof which in the target tissue ultimately produces the active metabolite. In the case of antiviral compounds, the active metabolite is the diphosphate of the phosphonate parent compounds. It is this metabolite that is incorporated into the viral nucleic acid, thereby truncating the elongating nucleic acid strand and halting viral replication. Metabolites of the prodrug can be anabolic metabolites, catabolic metabolites, or the product of anabolism and catabolism together. The manner in which the metabolite is produced is not important in the practice of the method of this invention.

The method of this invention is not limited to assaying a metabolite which per se possesses antiviral or antitumor activity. Instead, one can assay inactive precursors of the active metabolites. Precursors of the antivirally active diphosphate metabolite include the monophosphate of the parent drug, monophosphates of other metabolites of the parent drug (e.g., an intermediate modification of a substituent on the heterocyclic base), the parent itself and metabolites generated by the cell in converting the prodrug to the parent prior to phosphorylation. The precursor structures may vary considerably as they are the result of cellular metabolism. However, this information is already known or could be readily determined by one skilled in the art.

If the prodrug being assayed does not exhibit antitumor or antiviral activity per se then adjustments to the raw assay results may be required. For example, if the intracellular processing of the inactive metabolite to an active metabolite occurs at different rates among the tissues being tested, the raw assay results with the inactive metabolite would need to be adjusted to take account of the differences
among the cell types because the relevant parameter is the generation of activity in
the target tissue, not accumulation of inactive metabolites. However, determining
the proper adjustments would be within the ordinary skill. Thus, when step (d) of
the method herein calls for determining the activity, activity can be either
measured directly or extrapolated. It does not mean that the method herein is
limited to only assaying intermediates that are active per se. For instance, the
absence or decline of the prodrug in the test tissues also could be assayed. Step (d)
only requires assessment of the activity conferred by the prodrug as it interacts
with the tissue concerned, and this may be based on extrapolation or other indirect
measurement.

Step (d) of the method of this invention calls for determining the "relative"
activity of the prodrug. It will be understood that this does not require that each
and every assay or series of assays necessarily must also contain runs with the
selected non-target tissue. On the contrary, it is within the scope of this invention
to employ historical controls of the non-target tissue or tissues, or algorithms
representing results to be expected from such non-target tissues, in order to
provide the benchmark non-target activity.

The results obtained in step (d) are then used optimally to select or identify a
prodrug which produces greater antiviral activity in the target tissue than in the
non-target tissue. It is this prodrug that is selected for further development.

It will be appreciated that some preassessment of prodrug candidates can be
undertaken before the practice of the method of this invention. For example, the
prodrug will need to be capable of passing largely unmetabolized through the
gastrointestinal tract, it will need to be substantially stable in blood, and it should
be able to permeate cells at least to some degree. In most cases it also will need to
complete a first pass of the hepatic circulation without substantial metabolism.
Such prestudies are optional, and are well-known to those skilled in the art.

The same reasoning as is described above for antiviral activity is applicable
to antitumor prodrugs of methoxyphosphonate nucleotide analogues as well.
These include, for example, prodrugs of PMEG, the guanyl analogue of PMEA. In
this case, cytotoxic phosphonates such as PMEG are worthwhile candidates to pursue as their cytotoxicity in fact confers their antitumor activity.

A compound identified by this novel screening method then can be entered into a traditional preclinical or clinical program to confirm that the desired objectives have been met. Typically, a prodrug is considered to be selective if the activity or concentration of parent drug in the target tissue (% dose distribution) is greater than 2x, and preferably 5x, that of the parent compound in non-target tissue. Alternatively, a prodrug candidate can be compared against a benchmark prodrug. In this case, selectivity is relative rather than absolute. Selective prodrugs will be those resulting in greater than about 10x concentration or activity in the target tissue as compared with the prototype, although the degree of selectivity is a matter of discretion.

**Novel Method for Preparation of Starting Materials or Intermediates**

Also included herein is an improved method for manufacture of preferred starting materials (parent drugs) of this invention, PMEA and (R)-PMPA. Typically, this method comprises reacting 9-(2-hydroxypropyl)adenine (HPA) or 9-(2-hydroxyethyl)adenine (HEA) with a magnesium alkoxide, thereafter adding the protected aglycon synthon p-toluene-sulfonyloxymethylphosphonate (tosylate) to the reaction mixture, and recovering PMPA or PMEA, respectively.

Preferably, HPA is the enriched or isolated R enantiomer. If a chiral HPA mixture is used, R-PMPA can be isolated from the chiral PMPA mixture after the synthesis is completed.

Typically the tosylate is protected by lower alkyl groups, but other suitable groups will be apparent to the artisan. It may be convenient to employ the tosylate presubstituted with the prodrug phosphonate substituents which are capable of acting as protecting groups in the tosylation reaction, thereby allowing one to bypass the deprotection step and directly recover prodrug or an intermediate therefore.

The alkyl group of the magnesium alkoxide is not critical and can be any C1-

C6 branched or normal alkyl, but is preferably t-butyl (for PMPA) or isopropyl (for
PMEA). The reaction conditions also are not critical, but preferably comprise heating the reaction mixture at about 70-75°C with stirring or other moderate agitation.

If there is no interest in retaining the phosphonate substituents, the product is deprotected (usually with bromotrimethylsilane where the tosylate protecting group is alkyl), and the product then recovered by crystallization or other conventional method as will be apparent to the artisan.

Heterocyclic Base

In the compounds of this invention depicted in structures (3) and (4), the heterocyclic base B is selected from the structures

wherein

- $R^{15}$ is H, OH, F, Cl, Br, I, OR, SH, SR, NH$_2$, or NHR;
- $R^{16}$ is C$_1$-C$_6$ alkyl or C$_2$-C$_6$ alkenyl including CH$_3$, CH$_2$CH$_3$, CH$_2$CCH, CH$_2$CHCH$_2$ and C$_3$H$_7$;
- $R^{17}$ is C$_1$-C$_6$ alkyl or C$_2$-C$_6$ alkenyl including CH$_3$, CH$_2$CH$_3$, CH$_2$CCH, CH$_2$CHCH$_2$, and C$_3$H$_7$;
- $R^{18}$ is N, CF, CCl, CBr, CI, CR, CSR, or COR;
- $R^{19}$ is H, C$_1$-C$_9$ alkyl, C$_2$-C$_9$ alkenyl, C$_2$ - C$_9$ alkynyl, C$_1$-C$_9$ alkyl-C$_1$-C$_9$ alkoxy, or C$_7$-C$_9$ aryl-alkyl unsubstituted or substituted by OH, F, Cl, Br or I, therefore including -CH$_3$, -CH$_2$CH$_3$, -CHCH$_2$, -CHCHBr, -CH$_2$CH$_2$Cl, -CH$_2$CH$_2$F, -CH$_2$CCH, -CH$_2$CHCH$_2$, -C$_3$H$_7$, -CH$_2$OH, -CH$_2$OCH$_3$, -CH$_2$OC$_2$H$_5$, -CH$_2$OCCH, -CH$_2$OCH$_2$CH$_2$H, -CH$_2$C$_3$H$_7$, -CH$_2$CH$_2$OH, -CH$_2$CH$_2$OCH$_3$,
-CH₂CH₂OC₂H₅, -CH₂CH₂OCCH₃, -CH₂CH₂OCH₂CH₂CH₂, and
-CH₂CH₂OC₃H₇;

R²⁰ is N or CH;

R²¹ is N, CH, CCN, CCF₃, CC=CH or CC(O)NH₂;

R²² is H, OH, NH₂, SH, SCH₃, SCH₂CH₃, SCH₂CCH, SCH₂CHCH₂, SC₃H₇,
NH(CH₃), N(CH₃)₂, NH(CH₂CH₃), N(CH₂CH₃)₂, NH(CH₂CCH),
NH(CH₂CHCH₂), NH(C₃H₇), halogen (F, Cl, Br or I) or X wherein X is
-(CH₂)ₘ(O)ₙ(CH₂)ₘN(R¹⁰)₂ wherein each m is independently 0-2, n is 0-1, and
R¹⁰ independently is

H,

C₁-C₁₅ alkyl, C₂-C₁₅ alkenyl, C₆-C₁₅ arylalkenyl, C₆-C₁₅
arylalkynyl, C₂-C₁₅ alkynyl, C₁-C₆-alkylamino-C₁-C₆ alkyl, C₅-C₁₅ aralkyl, C₆-
C₁₅ heteroaralkyl, C₅-C₆ ary1, C₂-C₆ heterocycloalkyl,

C₂-C₁₅ alkyl, C₃-C₁₅ alkenyl, C₆-C₁₅ arylalkenyl, C₃-C₁₅ alkynyl,
C₇-C₁₅ arylalkynyl, C₁-C₆-alkylamino-C₁-C₆ alkyl, C₅-C₁₅ aralkyl, C₆-C₁₅
heteroalkyl or C₃-C₆ heterocycloalkyl wherein methylene in the alkyl moiety not
adjacent to N⁶ has been replaced by –O–,

optionally both R¹⁰ are joined together with N to form a saturated or
unsaturated C₂-C₅ heterocycle containing one or two N heteroatoms and
optionally an additional O or S heteroatom,

or one of the foregoing R¹⁰ groups which is substituted with 1 to 3
halo, CN or N₃; but optionally at least one R¹⁰ group is not H;

R²³ is H, OH, F, Cl, Br, I, SCH₃, SCH₂CH₃, SCH₂CCH, SCH₂CHCH₂,
SC₃H₇, OR¹⁶, NH₂, NHR¹⁷ or R²²; and

R²⁴ is O, S or Se.

B also includes both protected and unprotected heterocyclic bases,
particularly purine and pyrimidine bases. Protecting groups for exocyclic amines
and other labile groups are known (Greene et al. "Protective Groups in Organic
Synthesis") and include N-benzoyl, isobutryryl, 4,4'-dimethoxytrityl (DMT) and the
like. The selection of protecting group will be apparent to the ordinary artisan and will depend upon the nature of the labile group and the chemistry which the protecting group is expected to encounter, e.g. acidic, basic, oxidative, reductive or other conditions. Exemplary protected species are N^4^-benzoylcytosine, N^6^-benzoyladenine, N^2^-isobutyrylguanine and the like.

Protected bases have the formulas Xa.1, Xla.1, Xlb.1, XIIa.1 or XIIIa.1

\[
\begin{align*}
\text{(Xa.1)} & \quad \text{(Xla.1)} & \quad \text{(Xlb.1)} & \quad \text{(XIIa.1)} & \quad \text{(XIIIa.1)} \\
\end{align*}
\]

wherein R^{39}, R^{40}, R^{21}, R^{24} have the meanings previously defined; R^{22A} is R^{39} or R^{22} provided that R^{22} is not NH2; R^{23A} is R^{39} or R^{23} provided that R^{23} is not NH2; R^{39} is NHR^{40}, NHC(O)R^{38} or CR^{46}N(R^{38})_{2} wherein R^{36} is C1-C19 alkyl, C1-C19 alkenyl, C3-C10 aryl, adamantoyl, alkylaryl, or C3-C10 aryl substituted with 1 or 2 atoms or groups selected from halogen, methyl, ethyl, methoxy, ethoxy, hydroxy and cyano; R^{38} is C1-C10 alkyl, or both R^{38} together are 1-morpholino, 1-piperidine or 1-pyrrolidine; R^{40} is C1-C14 alkyl, including methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentylyl, hexyl, octyl and decanyl; and R^{46} is hydrogen or CH3.

For bases of structures Xla.1 and Xlb.1, if R^{39} is present at R^{22A} or R^{23A}, both R^{39} groups on the same base will generally be the same. Exemplary R^{36} are phenyl, phenyl substituted with one of the foregoing R^{36} ary1 substituents, -C10H15 (where C10H15 is 2-adamantoyl), -CH2-C6H5, -C6H5, -CH(CH3)2, -CH2CH3, methyl, butyl, t-butyl, heptanoyl, nonanoyl, undecanoyl, or undecenyl.

Specific bases include hypoxanthine, guanine, adenine, cytosine, inosine, thymine, uracil, xanthine, 8-aza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 7-deaza-8-aza
derivatives of adenine, guanine, 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 1-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 7-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 3-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 6-azacytosine; 5-fluorocytosine; 5-chlorocytosine; 5-iodocytosine; 5-bromocytosine; 5-methylcytosine; 5-bromovinyluracil; 5-fluorouracil; 5-chlorouracil; 5-iodouracil; 5-bromouracil; 5-trifluoromethyluracil; 5-methoxymethyluracil; 5-ethynyluracil and 5-propynyluracil.

Preferably, B is a 9-purinyl residue selected from guany1, 3-deazaguan1, 1-deazaguan1, 8-azaquany1, 7-deazaguan1, adenyl, 3-deazaadenyl, 1-deazadenyl, 8-azaadenyl, 7-deazaadenyl, 2,6-diaminopurinyl, 2-aminopurinyl, 6-chloro-2-aminopurinyl and 6-thio-2-aminopurinyl, or a B’ is a 1-pyrimidinyl residue selected from cytosinyl, 5-halocytosinyl, and 5-(C1-C3-alkyl)cytosinyl.

Preferred B groups have the formula

\[
\begin{align*}
\text{R}\text{Z} & \text{R}^\text{Z} & \text{R}\text{Z} & \text{R}^\text{Z} & \text{R}^\text{Z} & \text{R}\text{Z} & \text{R}^\text{Z} & \text{R}^\text{Z} & \text{R}^\text{Z} \\
\end{align*}
\]

wherein

\[\text{R}^{22}\text{ is X; }\]

\[\text{X is } -(\text{CH}_2)_m(\text{O})_n(\text{CH}_2)_m\text{N(R}^{10})_2 \text{ wherein } m=0-2, n=0-1, \text{ and } \]

\[\text{R}^{10}\text{ independently is }\]

\[\text{H, }\text{C}_1-\text{C}_6 \text{ alkyl, C}_2-\text{C}_6 \text{ alkenyl, C}_6-\text{C}_15 \text{ arylalkenyl, C}_6-\text{C}_15 \text{ arylalkynyl, C}_2-\text{C}_15 \text{ alkynyl, C}_1-\text{C}_6-\text{alkylamino-C}_1-\text{C}_6 \text{ alkyl, C}_5-\text{C}_15 \text{ aralkyl, C}_6-\text{C}_15 \text{ heteroaralkyl, C}_5-\text{C}_6 \text{ aryl, C}_2-\text{C}_6 \text{ heterocycloalkyl,}\]
C2-C15 alkyl, C3-C15 alkenyl, C6-C15 aryalkenyl, C3-C15 alkynyl, C7-C15 aryalkynyl, C1-C6-alkylamino-C1-C6 alkyl, C5-C15 aralkyl, C6-C15 heteroalkyl or C3-C6 heterocycloalkyl wherein methylene in the alkyl moiety not adjacent to N6 has been replaced by -O-, optionally both R10 are joined together with N to form a saturated or unsaturated C2-C5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom, or one of the foregoing R10 groups is substituted with 1 to 3 halo, CN or N3; but optionally at least one R10 group is not H; and Z is N or CH, provided that the heterocyclic nucleus varies from purine by no more than one Z.

E groups represent the aglycons employed in the methoxyphosphonate nucleotide analogues. Preferably, the E group is -CH(CH3)CH2- or -CH2CH2-. Also, it is preferred that the side groups at chiral centers in the aglycon be substantially solely in the (R) configuration (except for hydroxymethyl, which is the enriched (S) enantiomer).

R1 is an in vivo hydrolyzable oxyester having the structure -OR35 or -OR6 wherein R35 is defined in column 64, line 49 of U.S. Patent No. 5,798,340, herein incorporated by reference, and R6 is defined above. Preferably R1 is aryloxy, ordinarily unsubstituted or para-substituted (as defined in R6) phenoxy.

R2 is an amino acid residue, optionally provided that any carboxy group linked by less than about 5 atoms to the amidate N is esterified. R2 typically has the structure

\[
\begin{align*}
\text{R}^{11} & \quad \text{O} \\
\text{R}^{12} & \quad \text{R}^{13} \\
\text{R}^{14} & \quad \text{N}
\end{align*}
\]

wherein
n is 1 or 2;

R^{11} is R^4 or H; preferably R^3 = C_3-C_9 alkyl; C_3-C_9 alkyl substituted independently with OH, halogen, O or N; C_3-C_9 aryl; C_3-C_9 aryl which is independently substituted with OH, halogen, O or N; or C_3-C_9 arylalkyl which is independently substituted with OH, halogen, O or N;

R^{12} independently is H or C_1-C_9 alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, COOR^{11} and halogen; C_3-C_9 aryl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, COOR^{11} and halogen; or C_3-C_9 aryl-alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, COOR^{11} and halogen;

R^{13} independently is C(O)-OR^{11}; amino; amide; guanidinyl; imidazolyl; indolyl; sulfoxide; phosphoryl; C_1-C_9 alkylamino; C_1-C_9 alkylidamino; C_1-C_6 alkenylamino; hydroxy; thiol; C_1-C_9 alkoxy; C_1-C_9 alkthiol; (CH_2)_n COOR^{11}; C_1-C_6 alkyl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_10 alkoxyphenyl; C_2-C_6 alkenyl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_10 alkoxyphenyl; and C_6-C_{12} aryl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl; and

R^{14} is H or C_1-C_9 alkyl or C_1-C_9 alkyl independently substituted with OH, halogen, COOR^{11}, O or N; C_3-C_9 aryl; C_3-C_9 aryl which is independently substituted with OH, halogen, COOR^{11}, O or N; or C_3-C_9 arylalkyl which is independently substituted with OH, halogen, COOR^{11}, O or N.

Preferably, R^{11} is C_1-C_9 alkyl, most preferably isopropyl, R^{13} is the side chain of a naturally occurring amino acid, n = 1, R^{12} is H and R^{14} is H. In the compound of structure (2), the invention includes metabolites in which the phenoxy and isopropyl esters have been hydrolyzed to -OH. Similarly, the de-esterified enriched phosphonoamidate metabolites of compounds (5a), 5(b) and (6) are included within the scope of this invention.

Aryl and "O" or "N" substitution are defined in column 16, lines 42-58, of

Typically, the amino acids are in the natural or l amino acids. Suitable specific examples are set forth in U. S. Patent No. 5,798,340, for instance Table 4 and col. 8-10 therein.

Alkyl as used herein, unless stated to the contrary, is a normal, secondary, tertiary or cyclic hydrocarbon. Unless stated to the contrary alkyl is C₁₋₁₂.

Examples are -CH₃, -CH₂CH₃, -CH₂CH₂CH₃, -CH(CH₃)₂, -CH₂CH₂CH₂CH₃, -CH₂CH(CH₃)₂, -CH(CH₃)CH₂CH₃, -CH₂CH₂CH₂CH₃, -CH(CH₃)CH₂CH₂CH₃, -CH(CH₃)₂CH₂CH₃, and -CH(CH₃)C(CH₃)₃. Alkenyl and alkynyl are defined in the same fashion, but contain at least one double or triple bond, respectively.

Where enol or keto groups are disclosed, the corresponding tautomers are to be construed as taught as well.

The prodrug compounds of this invention are provided in the form of free base or the various salts enumerated in U. S. Patent No. 5,798,340, and are formulated with pharmaceutically acceptable excipients or solvating diluents for use as pharmaceutical products also as set forth in U. S. Patent No. 5,798,340. These prodrugs have the antiviral and utilities already established for the parent drugs (see U. S. Patent 5,798,340 and other citations relating to the methoxyphosphonate nucleotide analogues). It will be understood that the diastereomer of structure (4) at least is useful as an intermediate in the chemical production of the parent drug by hydrolysis in vitro, regardless of its relatively unselective character as revealed in the studies herein.

The invention will be more fully understood by reference to the following examples:
Adenine to PMEA using Magnesium Isopropoxide. To a suspension of adenine (16.8g, 0.124 mol) in DMF (41.9 ml) was added ethylene carbonate (12.1g, 0.137 mol) and sodium hydroxide (.100g, 0.0025 mol). The mixture was heated at 130°C overnight. The reaction was cooled to below 50°C and toluene (62.1 ml) was added. The slurry was further cooled to 5°C for 2 hours, filtered, and rinsed with toluene (2x). The wet solid was dried in vacuo at 65°C to yield 20.0g (90%) of 9-(2-hydroxyethyl)adenine as an off-white solid. Mp: 238-240°C.

9-(2-Hydroxyethyl)adenine (HEA) (20.0g, 0.112 mol) was suspended in DMF (125 ml) and heated to 80°C. Magnesium isopropoxide (11.2g, 0.0784 mol), or alternatively magnesium t-butoxide, was added to the mixture followed by diethyl p-toluenesulfonyloxymethylphosphonate (66.0g, 0.162 mol) over one hour. The mixture was stirred at 80°C for 7 hours. 30 ml of volatiles were removed via vacuum distillation and the reaction was recharged with 30 ml of fresh DMF. After cooling to room temperature, bromotrimethylsilane (69.6g, 0.450 mol) was added and the mixture heated to 80°C for 6 hours. The reaction was concentrated to yield a thick gum. The gum was dissolved into 360 ml water, extracted with 120 ml dichloromethane, adjusted to pH 3.2 with sodium hydroxide, and the resulting slurry stirred at room temperature overnight. The slurry was cooled to 4°C for one hour. The solids were isolated by filtration, washed with water (2x), and dried in
vacuo at 56°C to yield 20 g (65.4%) of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) as a white solid. Mp: > 200°C dec. ¹H NMR (D₂O) • 3.49 (t, 2H); 3.94 (t, 2H); 4.39 (t, 2H); 8.13 (s, 1H); 8.22 (s, 1H).

Example 1b

Adenine to PMPA using Magnesium t-Butoxide. To a suspension of adenine (40 g, 0.296 mol) in DMF (41.9 ml) was added (R)-propylene carbonate (34.5 g, 0.338 mol) and sodium hydroxide (4.80 g, 0.012 mol). The mixture was heated at 130°C overnight. The reaction was cooled to 100°C and toluene (138 ml) was added followed by methanesulfonic acid (4.7 g, 0.049 mol) while maintaining the reaction temperature between 100-110°C. Additional toluene (114 ml) was added to create a homogeneous solution. The solution was cooled to 3°C over 7 hours and then held at 3°C for one hour. The resulting solid was isolated by filtration and rinsed with acetone (2x). The wet solid was dried in vacuo at 80°C to yield 42.6 g (75%) of (R)-9-[2-(hydroxy)propyl]adenine (HPA) as an off-white solid. Mp: 188-190°C.

(R)-9-[2-(hydroxy)propyl]adenine (HPA) (20.0 g, 0.104 mol) was suspended in DMF (44.5 ml) and heated to 65°C. Magnesium t-butoxide (14.2 g, 0.083 mol), or alternatively magnesium isopropoxoide, was added to the mixture over one hour followed by diethyl p-toluenesulfonyloxyethylphosphonate (66.0 g, 0.205 mol)
over two hours while the temperature was kept at 78°C. The mixture was stirred at 75°C for 4 hours. After cooling to below 50°C, bromotrimethylsilane (73.9 g, 0.478 mol) was added and the mixture heated to 77°C for 3 hours. When complete, the reaction was heated to 80°C and volatiles were removed via atmospheric distillation. The residue was dissolved into water (120 ml) at 50°C and then extracted with ethyl acetate (101 ml). The pH of the aqueous phase was adjusted to pH 1.1 with sodium hydroxide, seeded with authentic (R)-PMPA, and the pH of the aqueous layer was readjusted to pH 2.1 with sodium hydroxide. The resulting slurry was stirred at room temperature overnight. The slurry was cooled to 4°C for three hours. The solid was isolated by filtration, washed with water (60 ml), and dried in vacuo at 50°C to yield 18.9 g (63.5%) of crude(R)-9-[2-(phosphonomethoxy)propyl]adenine (PMPA) as an off-white solid.

The crude(R)-9-[2-(phosphonomethoxy)propyl]adenine was heated at reflux in water (255 ml) until all solids dissolved. The solution was cooled to room temperature over 4 hours. The resulting slurry was cooled at 4°C for three hours. The solid was isolated by filtration, washed with water (56 ml) and acetone (56 ml), and dried in vacuo at 50°C to yield 15.0 g (50.4%) of (R)-9-[2-(phosphonomethoxy)propyl]adenine (PMPA) as a white solid. Mp: 278-280°C.
Example 2
Preparation of GS-7171 (III)

Scheme 1

I

(anhydrous)

OH

Et$_3$N

NMP

II

NH$_2$

OH

SOCl$_2$

CH$_2$Cl$_2$

Et$_2$N

III

GS-7171

NH$_2$

OH

IV

GS-7340

NH$_2$

OH

HO-CH$_2$COOH

CH$_3$CN

V

GS-7340-02
A glass-lined reactor was charged with anhydrous PMPA, (I) (14.6 kg, 50.8 mol), phenol (9.6 kg, 102 mol), and 1-methyl-2-pyrrolidinone (39 kg). The mixture was heated to 85°C and triethylamine (6.3 kg, 62.3 mol) added. A solution of 1,3-dicyclohexylcarbodiimide (17.1 kg, 82.9 mol) in 1-methyl-2-pyrrolidinone (1.6 kg) was then added over 6 hours at 100°C. Heating was continued for 16 hours. The reaction was cooled to 45°C, water (29 kg) added, and cooled to 25°C. Solids were removed from the reaction by filtration and rinsed with water (15.3 kg). The combined filtrate and rinse was concentrated to a tan slurry under reduced pressure, water (24.6 kg) added, and adjusted to pH = 11 with NaOH (25% in water). Fines were removed by filtration through diatomaceous earth (2 kg) followed by a water (4.4 kg) rinse. The combined filtrate and rinse was extracted with ethyl acetate (28 kg). The aqueous solution was adjusted to pH = 3.1 with HCl (37% in water) (4 kg). Crude II was isolated by filtration and washed with methanol (12.7 kg). The crude II wet cake was slurried in methanol (58 kg). Solids were isolated by filtration, washed with methanol (8.5 kg), and dried under reduced pressure to yield 9.33 kg II as a white powder: ^1H NMR (D_2O) δ 1.2 (d, 3H), 3.45 (q, 2H), 3.7 (q, 2H), 4 (m, 2H), 4.2 (q, 2H), 4.35 (dd, 2H), 6.6 (d, 2H), 7 (t, 1H), 7.15 (t, 2H), 8.15 (s, 1H), 8.2 (s, 1H); ^31P NMR (D_2O) δ 15.0 (decoupled).

GS-7171 (III). (Scheme 1) A glass-lined reactor was charged with monophenyl PMPA, (II), (9.12 kg, 25.1 mol) and acetonitrile (30.7 kg). Thionyl chloride (6.57 kg, 56.7 mol) was added below 50°C. The mixture was heated at 75°C until solids dissolved. Reaction temperature was increased to 80°C and volatiles (11.4 kg) collected by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, dichloromethane (41 kg) added, and cooled to −29°C. A solution of (L)-alanine isopropyl ester (7.1 kg, 54.4 mol) in dichloromethane (36 kg) was added over 60 minutes at −18°C followed by triethylamine (7.66 kg, 75.7 mol) over 30 minutes at −18 to −11°C. The reaction mixture was warmed to room temperature and washed five times with sodium dihydrogen phosphate solution (10% in water, 15.7 kg each wash). The organic solution was dried with anhydrous sodium sulfate (18.2 kg), filtered, rinsed with dichloromethane (28 kg), and concentrated to an oil
under reduced pressure. Acetone (20 kg) was charged to the oil and the mixture concentrated under reduced pressure. Acetone (18.8 kg) was charged to the resulting oil. Half the product solution was purified by chromatography over a 38 x 38 cm bed of 22 kg silica gel 60, 230 to 400 mesh. The column was eluted with 480 kg acetone. The purification was repeated on the second half of the oil using fresh silica gel and acetone. Clean product bearing fractions were concentrated under reduced pressure to an oil. Acetonitrile (19.6 kg) was charged to the oil and the mixture concentrated under reduced pressure. Acetonitrile (66.4 kg) was charged and the solution chilled to 0 to -5°C for 16 hours. Solids were removed by filtration and the filtrate concentrated under reduced pressure to 5.6 kg III as a dark oil: $^1$H NMR (CDCl$_3$) δ 1.1 (m 12H), 3.7 (m, 1H), 4.0 (m, 5H), 4.2 (m, 1H), 5.0 (m, 1H), 6.2 (s, 2H), 7.05 (m, 5H), 8.0 (s, 1H), 8.25 (d, 1H); $^{31}$P NMR (CDCl$_3$) δ 21.0, 22.5 (decoupled).

Alternate Method for GS-7171(III)

**Scheme 2**

![Chemical Structures]

1. (anhydrous)

2. $\text{SOCl}_2$ → I → II

3. $\text{SOCl}_2$ → III

GS-71
Monophenyl PMPA (II). A round-bottom flask with reflux condenser and nitrogen inlet was placed in a 70°C oil bath. The flask was charged with anhydrous PMPA (I) (19.2 g, 67 mmol), N,N-dimethylformamide (0.29 g, 3.3 mmol), and tetramethylene sulfone (40 mL). Thionyl chloride (14.2 g, 119 mmol) was added over 4 hours. Heating was increased to 100°C over the same time. A homogeneous solution resulted. Phenoxymethylsilane (11.7 g, 70 mmol) was added to the solution over 5 minutes. Heating in the 100°C oil bath continued for two hours more. The reaction was poured into rapidly stirring acetone (400 mL) with cooling at 0°C. Solids were isolated by filtration, dried under reduced pressure, and dissolved in methanol (75 mL). The solution pH was adjusted to 3.0 with potassium hydroxide solution (45% aq.) with cooling in ice/water. The resulting solids were isolated by filtration, rinsed with methanol, and dried under reduced pressure to 20.4 g II (Scheme 2) as a white powder.

GS-7171 (III). Monophenyl PMPA (II) (3 g, 8.3 mmol), tetramethylene sulfone (5 mL), and N,N-dimethylformamide (1 drop) were combined in a round bottom flask in a 40°C oil bath. Thionyl chloride (1.96 g, 16.5 mmol) was added. After 20 minutes the clear solution was removed from heat, diluted with dichloromethane (10 mL), and added to a solution of (L)-alanine isopropyl ester (5 g, 33 mmol) and diisopropylethylamine (5.33 g, 41 mmol) in dichloromethane (20 mL) at -10°C. The reaction mixture was warmed to room temperature and washed three times with sodium dihydrogenphosphate solution (10% aq., 10 mL each wash). The organic solution was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a oil. The oil was combined with fumaric acid (0.77 g, 6.6 mmol) and acetonitrile (40 mL) and heated to reflux to give a homogeneous solution. The solution was cooled in an ice bath and solids isolated by filtration. The solid GS-7171 fumarate salt was dried under reduced pressure to 3.7 g. The salt (3.16 g, 5.3 mmol) was suspended in dichloromethane (30 mL) and stirred with potassium carbonate solution (5 mL, 2.5 M in water) until the solid dissolved. The organic layer was isolated, then washed with water (5 mL), dried over anhydrous
sodium sulfate, and concentrated under reduced pressure to afford 2.4 g III as a tan foam.

Example 3

A. Diastereomer Separation by Batch Elution Chromatography

The diastereomers of GS-7171 (III) were resolved by batch elution chromatography using a commercially available Chiralpak AS, 20 μm, 21 x 250 mm semi-preparative HPLC column with a Chiralpak AS, 20 μm, 21 x 50 mm guard column. Chiralpak® AS is a proprietary packing material manufactured by Diacel and sold in North America by Chiral Technologies, Inc. (U. S. Patent Nos. 5,202,433, RE 35,919, 5,434,298, 5,434,299 and 5,498,752). Chiralpak AS is a chiral stationary phase (CSP) comprised of amylosetris[(6)-α-methylbenzyl carbamate] coated onto a silica gel support.

The GS-7171 diastereomeric mixture was dissolved in mobile phase, and approximately 1 g aliquots of GS-7171 were pumped onto the chromatographic system. The undesired diastereomer, designated GS-7339, was the first major broad (approx. 15 min. duration) peak to elute from the column. When the GS-7339 peak had finished eluting, the mobile phase was immediately switched to 100% methyl alcohol, which caused the desired diastereomer, designated GS-7340 (IV), to elute as a sharp peak from the column with the methyl alcohol solvent front. The methyl alcohol was used to reduce the over-all cycle time. After the first couple of injections, both diastereomers were collected as a single large fractions containing one of the purified diastereomers (>99.0% single diastereomer). The mobile phase solvents were removed *in vacuo* to yield the purified diastereomer as a friable foam.

About 95% of the starting GS-7171 mass was recovered in the two diastereomer fractions. The GS-7340 fraction comprised about 50% of the total recovered mass.
The chromatographic conditions were as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>GS-7171 – Acetonitrile : Isopropyl Alcohol (90:10)</td>
</tr>
<tr>
<td></td>
<td>(Final) 100% Methyl Alcohol</td>
</tr>
<tr>
<td>Flow</td>
<td>10 mL/minute</td>
</tr>
<tr>
<td>Run Time</td>
<td>About 45 minute</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 275 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Elution Profile</td>
<td>GS-7339 (diastereomer B)</td>
</tr>
<tr>
<td></td>
<td>GS-7340 (diastereomer A; IV)</td>
</tr>
</tbody>
</table>

B. Diastereomer Separation of GS-7171 by SMB Chromatography

For a general description of simulated moving bed (SMB) chromatography, see Strube et al., "Organic Process Research and Development" 2:305-319 (1998).

**GS-7340 (IV).** GS-7171 (III), 2.8 kg, was purified by simulated moving bed chromatography over 10 cm by 5 cm beds of packing (Chiral Technologies Inc., 20 micron Chiralpak AS coated on silica gel) (1.2 kg). The columns were eluted with 30% methanol in acetonitrile. Product bearing fractions were concentrated to a solution of IV in acetonitrile (2.48 kg). The solution solidified to a crystalline mass wet with acetonitrile on standing. The crystalline mass was dried under reduced pressure to a tan crystalline powder, 1.301 kg IV, 98.7% diastereomeric purity: mp 117 – 120°C; $^1$H NMR (CDCl$_3$) δ 1.15 (m 12H), 3.7 (t, 1H), 4.0 (m, 5H), 4.2 (dd, 1H), 5.0 (m, 1H), 6.05 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.2 (s, 1H); $^{31}$P NMR (CDCl$_3$) δ 21.0 (decoupled).

C. Diastereomer Separation by C18 RP-HPLC

GS-7171 (III) was chromatographed by reverse phase HPLC to separate the diastereomers using the following summary protocol.
Chromatographic column: Phenomenex Luna™ C18(2), 5 μm, 100 Å pore size, (Phenomenex, Torrance, CA), or equivalent
Guard column: Pellicular C18 (Alltech, Deerfield, IL), or equivalent
Mobile Phase: A — 0.02% (85%) H₃PO₄ in water : acetonitrile (95:5)
B — 0.02% (85%) H₃PO₄ in water : acetonitrile (50:50)

Mobile Phase Gradient:

<table>
<thead>
<tr>
<th>Time</th>
<th>% Mobile Phase A</th>
<th>% Mobile Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>32</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Run Time: 50 minutes
Equilibration Delay: 10 min at 100% mobile phase A
Flow Rate: 1.2 mL/min
Temperature: Ambient
Detection: UV at 260 nm
Sample Solution: 20 mM sodium phosphate buffer, pH 6
Retention Times: GS-7339, about 25 minutes
GS-7340, about 27 minutes

D. Diastereomer Separation by Crystallization

GS-7340 (IV). A solution of GS-7171 (III) in acetonitrile was concentrated to an amber foam (14.9g) under reduced pressure. The foam was dissolved in acetonitrile (20 mL) and seeded with a crystal of IV. The mixture was stirred overnight, cooled to 5°C, and solids isolated by filtration. The solids were dried to 2.3 g IV as white crystals, 98% diastereomeric purity (³¹P NMR): ¹H NMR (CDCl₃) δ 1.15 (m 12H), 3.7 (t, 1H), 3.95 (m, 2H), 4.05 (m, 2H), 4.2 (m, 2H), 5.0 (m, 1H), 6.4 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.2 (s, 1H); ³¹P NMR (CDCl₃) δ 19.5 (decoupled). X-ray crystal analysis of a single crystal selected from this product yielded the following data:
Crystal Color, Habit: colorless, column
Crystal Dimensions: 0.25 X 0.12 X 0.08 mm
Crystal System: orthorhombic
Lattice Type: Primitive
Lattice Parameters:
- \( a = 8.352(1) \, \text{Å} \)
- \( b = 15.574(2) \, \text{Å} \)
- \( c = 18.253(2) \, \text{Å} \)
- \( V = 2374.2(5) \, \text{Å}^3 \)

Space Group: \( P2_12_12_1 \) (#19)
Z value: 4
\( D_{\text{cal}} \): 1.333 g/cm\(^3\)
\( F_{000} \): 1008.00
\( \mu(\text{MoK}\alpha) \): 1.60 cm\(^{-1}\)

Example 4
Preparation of Fumarate Salt of GS-7340

GS-7340-02 (V). (Scheme 1) A glass-lined reactor was charged with GS-7340 (IV), (1.294 kg, 2.71 mol), fumaric acid (284 g, 2.44 mol), and acetonitrile (24.6 kg). The mixture was heated to reflux to dissolve the solids, filtered while hot and cooled to 5°C for 16 hours. The product was isolated by filtration, rinsed with acetonitrile (9.2 kg), and dried to 1329 g (V) as a white powder: mp 119.7 - 121.1°C; [\( \alpha \)]\(D\) -41.7° (c 1.0, acetic acid).
Example 5
Preparation of GS-7120 (VI)

Scheme 3

A 5 L round bottom flask was charged with monophenyl PMPA, (II), (200 g, 0.55 mol) and acetonitrile (0.629 kg). Thionyl chloride (0.144 kg, 1.21 mol) was added below 27°C. The mixture was heated at 70°C until solids dissolved. Volatiles (0.45 L) were removed by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, dichloromethane (1.6 kg) was added and the mixture was cooled to -20°C. A solution of (L)-α-aminobutyric acid ethyl ester (0.144 kg, 1.1 mol) in dichloromethane (1.33 kg) was added over 18 minutes at -20 to -10°C followed by triethylamine (0.17 kg, 1.65 mol) over 15 minutes at -8 to -15°C. The reaction mixture was warmed to room temperature and washed four times with sodium dihydrogenphosphate solution (10% aq., 0.3 L each wash). The organic solution was dried with anhydrous sodium sulfate (0.5 kg) and filtered. The solids were rinsed with dichloromethane (0.6 kg) and the combined filtrate and rinse was concentrated to an oil under reduced pressure. The oil was purified by chromatography over a 15 x 13 cm bed of 1.2 kg silica gel 60, 230 to 400 mesh. The column was eluted with a gradient of dichloromethane and methanol. Product bearing fractions were concentrated under reduced pressure to afford 211 g VI (Scheme 3) as a tan foam.
Example 5a
Diastereomer Separation of GS-7120 by Batch Elution Chromatography

The diastereomeric mixture was purified using the conditions described for GS-7171 in Example 3A except for the following:

Mobile Phase
   (Initial) : GS-7120 – Acetonitrile : Isopropyl Alcohol (98:2)
   (Final) : 100% Methyl Alcohol
Elution Profile
   : GS-7341 (diastereomer B)
   : GS-7342 (diastereomer A)

Example 6
Diastereomer Separation of GS-7120 by Crystallization

A 1 L round bottom flask was charged with monophenyl PMPA, (II), (50 g, 0.137 mol) and acetonitrile (0.2 L). Thionyl chloride (0.036 kg, 0.303 mol) was added with a 10°C exotherm. The mixture was heated to reflux until solids dissolved. Volatiles (0.1 L) were removed by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, dichloromethane (0.2 kg) was added, and the mixture was cooled to −20°C. A solution of (L)-α aminobutyric acid ethyl ester (0.036 kg, 0.275 mol) in dichloromethane (0.67 kg) was added over 30 minutes at −20 to −8°C followed by triethylamine (0.042 kg, 0.41 mol) over 10 minutes at up to −6°C. The reaction mixture was warmed to room temperature and washed four times with sodium dihydrogenphosphate solution (10% aq., 0.075 L each wash). The organic solution was dried with anhydrous sodium sulfate (0.1 kg) and filtered. The solids were rinsed with ethyl acetate (0.25 L, and the combined filtrate and rinse was concentrated to an oil under reduced pressure. The oil was diluted with ethyl acetate (0.25 L), seeded, stirred overnight, and chilled to −15°C. The solids were isolated by filtration and dried under reduced pressure to afford 17.7 g of GS-7342 (Table 5) as a tan powder: \(^1^H\) NMR (CDCl\(_3\)) \(\delta\) 0.95 (t, 3H), 1.3 (m, 6H), 1.7, (m, 2H),
3.7 (m, 2H), 4.1 (m, 6H), 4.4 (dd, 1H), 5.8 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.4 (s, 1H); 
\[^{31}\text{P NMR} (\text{CDCl}_3) \delta 21\) (decoupled).

**Example 7**

Diastereomer Separation of GS-7097

The diastereomeric mixture was purified using the conditions described for GS-7171 (Example 3A) except for the following:

Mobile Phase (Initial) : GS-7120 – Acetonitrile : Isopropyl Alcohol (95:5)
Mobile Phase (Final) : 100% Methyl Alcohol

Elution Profile

: GS-7115 (diastereomer B)
: GS-7114 (diastereomer A)

**Example 8**

Alternative Procedure for Preparation of GS-7097

**GS-7097**: Phenyl PMPA, Ethyl L-Alanyl Amidate. Phenyl PMPA (15.0 g, 41.3 mmol), L-alanine ethyl ester hydrochloride (12.6 g, 83 mmol) and triethylamine (11.5 mL, 83 mmol) were slurried together in 500 mL pyridine under dry N\(_2\). This suspension was combined with a solution of triphenylphosphine (37.9 g, 145 mmol), Aldrithiol 2 (2,2'-dipyridyl disulfide) (31.8 g, 145 mmol), and 120 mL pyridine. The mixture was heated at an internal temperature of 57°C for 15 hours. The complete reaction was concentrated under vacuum to a yellow paste, 100 g.

The paste was purified by column chromatography over a 25 x 11 cm bed of 1.1 kg silica gel 60, 230 to 400 mesh. The column was eluted with 8 liters of 2% methanol in dichloromethane followed by a linear gradient over a course of 26 liters eluent up to a final composition of 13% methanol. Clean product bearing fractions were concentrated to yield 12.4 g crude (5), 65% theory. This material was contaminated with about 15% (weight) triethylamine hydrochloride by \(^1\text{H NMR}\). The contamination was removed by dissolving the product in 350 mL ethyl acetate, extracting with 20 mL water, drying the organic solution over anhydrous sodium...
sulfate, and concentrating to yield 11.1 g pure GS-7097 as a white solid, 58% yield. The process also is employed to synthesize the diastereomeric mixture of GS-7003a and GS-7003b (the phenylalanyl amidate) and the mixture GS-7119 and GS-7335 (the glycyl amidate). These diastereomers are separated using a batch elution procedure such as shown in Example 3A, 6 and 7.

Example 9

*In Vitro* Studies of Prodrug Diastereomers

The *in vitro* anti-HIV-1 activity and cytotoxicity in MT-2 cells and stability in human plasma and MT-2 cell extracts of GS-7340 (freebase) and tenofovir disoproxil fumarate (TDF), are shown in Table 1. GS-7340 shows a 10-fold increase in antiviral activity relative to TDF and a 200-fold increase in plasma stability. This greater plasma stability is expected to result in higher circulating levels of GS-7340 than TDF after oral administration.

Table 1. *In Vitro* Activity and Stability

<table>
<thead>
<tr>
<th>HIV-1 Activity</th>
<th>Cytotoxicity</th>
<th>Stability T 1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50µM&lt;/sub&gt;</td>
<td>CC&lt;sub&gt;50µM&lt;/sub&gt;</td>
</tr>
<tr>
<td>GS 7340</td>
<td>0.005</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>TDF</td>
<td>0.05</td>
<td>70</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>5</td>
<td>6000</td>
</tr>
</tbody>
</table>

In order to estimate the relative intracellular PMPA resulting from the intracellular metabolism of TDF as compared to that from GS-7340, both prodrugs and PMPA were radiolabeled and spiked into intact human whole blood at equimolar concentrations. After 1 hour, plasma, red blood cells (RBCs) and peripheral blood mononuclear cells (PBMCs) were isolated and analyzed by HPLC with radiometric detection. The results are shown in Table 2.
After 1 hour, GS-7340 results in 10x and 30x the total intracellular concentration of PMPA species in PBMCs as compared to TDF and PMPA, respectively. In plasma after 1 hour, 84% of the radioactivity is due to intact GS-7340, whereas no TDF is detected at 1 hour. Since no intact TDF is detected in plasma, the 10x difference at 1 hour between TDF and GS-7340 is the minimum difference expected in vivo. The HPLC chromatogram for all three compounds in PBMCs is shown in Figure 1.

Table 2. PMPA Metabolites in Plasma, PBMCs and RBCs After 1 h Incubation of PMPA Prodrugs or PMPA in Human Blood.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Matrix</th>
<th>Total C-14 Recovered, µg-eq</th>
<th>Metabolites (% of Total Peak Area)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PMPA %</td>
<td>PMPA %</td>
</tr>
<tr>
<td>GS-7340 (60 µg-eq)</td>
<td>Plasma/FP</td>
<td>43.0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>1.25</td>
<td>45</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>RBC/FP</td>
<td>12.6</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>GS-4331 (TDF) (60 µg-eq)</td>
<td>Plasma/FP</td>
<td>48.1</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>0.133</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>RBC/FP</td>
<td>10.5</td>
<td>93</td>
<td>7.0</td>
</tr>
<tr>
<td>PMPA (60 µg-eq)</td>
<td>Plasma/FP</td>
<td>55.7</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>0.033</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>RBC/FP</td>
<td>3.72</td>
<td>74</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 1. HPLC/C-14 Traces of PBMC Extracts from Human Blood Incubated for 1 h at 37°C with TDF, GS-7340 or PMPA.

Met. X and Met Y (metabolites X and Y) are shown in Table 5. Lower case "p" designates phosphorylation. These results were obtained after 1 hour in human blood. With increasing time, the in vitro differences are expected to increase, since 84% of GS-7340 is still intact in plasma after one hour. Because intact GS-7340 is present in plasma after oral administration, the relative clinical efficacy should be related to the IC_{50} values seen in vitro.

In Table 3 below, IC_{50} values of tenofovir, TDF, GS-7340, several nucleosides and the protease inhibitor nelfinavir are listed. As shown, nelfinavir and GS-7340 are 2-3 orders of magnitude more potent than all other nucleotides or nucleosides.
Table 3. *In Vitro* Anti-HIV-1 Activities of Antiretroviral Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adefovir (PMEA)</td>
<td>$13.4 \pm 4.2^1$</td>
</tr>
<tr>
<td>Tenofovir (PMPA)</td>
<td>$6.3 \pm 3.3^1$</td>
</tr>
<tr>
<td>AZT</td>
<td>$0.17 \pm 0.08^1$</td>
</tr>
<tr>
<td>3TC</td>
<td>$1.8 \pm 0.25^1$</td>
</tr>
<tr>
<td>d4T</td>
<td>$8 \pm 2.5^1$</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>$0.006 \pm 0.002^1$</td>
</tr>
<tr>
<td>TDF</td>
<td>0.05</td>
</tr>
<tr>
<td>GS 7340</td>
<td>0.005</td>
</tr>
</tbody>
</table>


Additional studies of the *in vitro* cell culture anti-HIV-1 activity and CC$_{50}$ of separated diastereomers of this invention were conducted and the results tabulated below.
Table 4. Effect of Diastereomer

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diastereomer</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Fold change</th>
<th>A/B activity</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMPA</td>
<td>-</td>
<td>5</td>
<td>1x</td>
<td>-</td>
<td>6000</td>
</tr>
<tr>
<td>Ala-methylester</td>
<td>Mixture 1:1</td>
<td>0.025</td>
<td>200x</td>
<td>20x</td>
<td>80</td>
</tr>
<tr>
<td>GS-6957a</td>
<td>A</td>
<td>0.0075</td>
<td>670x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS-6957b</td>
<td></td>
<td>0.15</td>
<td>33x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe-methylester</td>
<td>Mixture 1:1</td>
<td>0.03</td>
<td>170x</td>
<td>10x</td>
<td>60</td>
</tr>
<tr>
<td>GS-7003a</td>
<td>A</td>
<td>0.01</td>
<td>500x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS-7003b</td>
<td>B</td>
<td>0.1</td>
<td>50x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly-ethylester</td>
<td>Mixture 1:1</td>
<td>0.5</td>
<td>10x</td>
<td>20x</td>
<td></td>
</tr>
<tr>
<td>GS-7119</td>
<td>A</td>
<td>0.05</td>
<td>100x</td>
<td></td>
<td>&gt;100</td>
</tr>
<tr>
<td>GS-7335</td>
<td>B</td>
<td>1.0</td>
<td>5x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala-isopropyl</td>
<td>Mixture 1:1</td>
<td>0.01</td>
<td>500x</td>
<td>12x</td>
<td></td>
</tr>
<tr>
<td>GS-7340</td>
<td>A</td>
<td>0.005</td>
<td>1,000x</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>GS-7339</td>
<td>B</td>
<td>0.06</td>
<td>83x</td>
<td></td>
<td>&gt;100</td>
</tr>
<tr>
<td>ABA-ethyl</td>
<td>Mixture 1:1</td>
<td>0.008</td>
<td>625x</td>
<td>7.5x</td>
<td>&gt;100</td>
</tr>
<tr>
<td>GS-7342</td>
<td>A</td>
<td>0.004</td>
<td>1,250x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS-7341</td>
<td>B</td>
<td>0.03</td>
<td>170x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala-ethyl</td>
<td>Mixture 1:1</td>
<td>0.02</td>
<td>250x</td>
<td>10x</td>
<td>60</td>
</tr>
<tr>
<td>GS-7114</td>
<td>A</td>
<td>0.005</td>
<td>1,000x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS-7115</td>
<td>B</td>
<td>0.05</td>
<td>100x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


"Phe-methylester" is the methylphenylalaninyl monoamidate, phenyl monoester of tenofovir; "gly-methylester" is the methylglycyl monoamidate, phenyl monoester of tenofovir.

In each instance above, isomer A is believed to have the same absolute stereochemistry as GS-7340 (S), and isomer B is believed to have the same absolute stereochemistry that of GS-7339.
The *in vitro* metabolism and stability of separated diastereomers were determined in PLCE, MT-2 extract and human plasma. A biological sample listed below, 80 μL, was transferred into a screw-capped centrifuge tube and incubated at 37°C for 5 min. A solution containing 0.2 mg/mL of the test compound in a suitable buffer, 20 μL, was added to the biological sample and mixed. The reaction mixture, 20 μL, was immediately sampled and mixed with 60 μL of methanol containing 0.015 mg/mL of 2-hydroxymethylnaphthalene as an internal standard for HPLC analysis. The sample was taken as the time-zero sample. Then, at specific time points, the reaction mixture, 20 μL, was sampled and mixed with 60 μL of methanol containing the internal standard. The mixture thus obtained was centrifuged at 15,000 G for 5 min and the supernatant was analyzed with HPLC under the conditions described below.

The biological samples evaluated are as follows.

1. PLCE (porcine liver carboxyesterase from Sigma, 160 u/mg protein, 21 mg protein/mL) diluted 20 fold with PBS (phosphated-buffered saline).
2. MT-2 cell extract was prepared from MT-2 cells according to the published procedure [A. Pompon, I. Lefebvre, J.-L. Imbach, S. Kahn, and D. Farquhar, "Antiviral Chemistry & Chemotherapy", 5:91–98 (1994)] except for using HEPES buffer described below as the medium.
3. Human plasma (pooled normal human plasma from George King Biomedical Systems, Inc.)

The buffer systems used in the studies are as follows.

In the study for PLCE, the test compound was dissolved in PBS. PBS (phosphate-buffered saline, Sigma) contains 0.01 M phosphate, 0.0027 M potassium chloride, and 0.137 M sodium chloride. pH 7.4 at 37°C.

In the study for MT-2 cell extracts, the test compound was dissolved in HEPES buffer. HEPES buffer contains 0.010 M HEPES, 0.05 M potassium chloride, 0.005 M magnesium chloride, and 0.005 M dl-dithiothreitol. pH 7.4 at 37°C.
In the study for human plasma, the test compound was dissolved in TBS. TBS (tris-buffered saline, Sigma) contains 0.05 M Tris, 0.0027 M potassium chloride, and 0.138 M sodium chloride. pH 7.5 at 37°C.

The HPLC analysis was carried out under the following conditions:

- **Column:** Zorbax RX-C8, 4.6 x 250 mm, 5 μ
  (MAC-MOD Analytical, Inc. Chadds Ford, PA)
- **Detection:** UV at 260 nm
- **Flow Rate:** 1.0 mL/min
- **Run Time:** 30 min
- **Injection Volume:** 20 μL
- **Column Temperature:** Ambient temperature

**Mobile Phase A:** 50 mM potassium phosphate (pH 6.0)/CH3CN = 95/5 (v/v)

**Mobile Phase B:** 50 mM Potassium phosphate (pH 6.0)/CH3CN = 50/50 (v/v)

**Gradient Run:**
- 0 min 100% Mobile Phase A
- 25 min 100% Mobile Phase B
- 30 min 100% Mobile Phase B

The results are shown below in Table 5 (also including selected IC₅₀ data from Table 4).
Table 5. In Vitro Metabolism of Isomers A and B of PMPA monoamidate at 37°C

<table>
<thead>
<tr>
<th>No.</th>
<th>PMPA monoamidate structure</th>
<th>HIV IC₅₀ (µM)</th>
<th>PLCE hydrolysis rate and product</th>
<th>MT-2 extract hydrolysis rate and product</th>
<th>Human Plasma Stability (HP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A O R - NH-CH₂COOEt</td>
<td>0.005</td>
<td>( t_{1/2} = 2.9 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 2.9 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 148 ) min Met. Y</td>
</tr>
<tr>
<td></td>
<td>Isomer A GS7114</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A O R - NH-CH₂COOEt</td>
<td>0.05</td>
<td>( t_{1/2} = 8.0 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 150.6 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 495 ) min Met. Y</td>
</tr>
<tr>
<td></td>
<td>Isomer B GS7115</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>A O R - NH-CH₂COOEtPr</td>
<td>0.005</td>
<td>( t_{1/2} = 3.3 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 28.3 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 90.0 ) min Met. Y</td>
</tr>
<tr>
<td></td>
<td>Isomer A GS7340</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A O R - NH-CH₂COOEtPr</td>
<td>0.06</td>
<td>( t_{1/2} = 10.1 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} &gt; 1000 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 231 ) min Met. Y</td>
</tr>
<tr>
<td></td>
<td>Isomer B GS7339</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>A O R - NH-CH₂COOEt</td>
<td>0.004</td>
<td>( t_{1/2} = 3.9 ) min Met. X</td>
<td>( t_{1/2} = 49.2 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 103 ) min Met. Y</td>
</tr>
<tr>
<td></td>
<td>Isomer A GS7342</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>A O R - NH-CH₂COOEt</td>
<td>0.03</td>
<td>( t_{1/2} = 11.3 ) min Met. X</td>
<td>( t_{1/2} &gt; 1000 ) min Met. X &amp; PMPA</td>
<td>( t_{1/2} = 257 ) min Met. Y</td>
</tr>
<tr>
<td></td>
<td>Isomer B GS7341</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>A O R - CH₂COOEt</td>
<td>0.05</td>
<td>( t_{1/2} &lt; 0.14 ) min MonoPOC PMPA</td>
<td>( t_{1/2} = 70.7 ) min monoPOC PMPA</td>
<td>( t_{1/2} = 0.41 ) min monoPOC PMPA</td>
</tr>
</tbody>
</table>

Met. X:  
\[ A \]

Met. Y:  
\[ A \]

A =  

\[ \text{NH}_2 \]

\[ \text{R}^{13} \]

\[ \text{OH} \]

\[ \text{R}^{11} \]
Example 10
Plasma and PBMC Exposures Following Oral Administration
Of Prodrug Diastereomers to Beagle Dogs

The pharmacokinetics of GS 7340 were studied in dogs after oral
administration of a 10 mg-eq/kg dose.

Formulations. The prodrugs were formulated as solutions in 50 mM citric acid
within 0.5 hour prior to dose. All compounds used in the studies were synthesized
by Gilead Sciences. The following lots were used:

<table>
<thead>
<tr>
<th>GSI</th>
<th>Amidate Amino acid</th>
<th>AA Ester</th>
<th>Diastereoisomer</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-7340-2</td>
<td>Alanine</td>
<td>i-Propyl</td>
<td>Isomer A</td>
<td>1504-187-19</td>
</tr>
<tr>
<td>GS-7339</td>
<td>Alanine</td>
<td>i-Propyl</td>
<td>Isomer B</td>
<td>1509-185-31</td>
</tr>
<tr>
<td>GS7114</td>
<td>Alanine</td>
<td>Ethyl</td>
<td>Isomer A</td>
<td>1509-181-26</td>
</tr>
<tr>
<td>GS7115</td>
<td>Alanine</td>
<td>Ethyl</td>
<td>Isomer B</td>
<td>1509-181-22</td>
</tr>
<tr>
<td>GS7119</td>
<td>Glycine</td>
<td>Ethyl</td>
<td>Isomer A</td>
<td>1428-163-28</td>
</tr>
<tr>
<td>GS7342</td>
<td>α-Aminobutyric Acid</td>
<td>Ethyl</td>
<td>Isomer A</td>
<td>1509-191-12</td>
</tr>
<tr>
<td>GS7341</td>
<td>α-Aminobutyric Acid</td>
<td>Ethyl</td>
<td>Isomer B</td>
<td>1509-191-7</td>
</tr>
</tbody>
</table>

Dose Administration and Sample Collection. The in-life phase of this study was
conducted in accordance with the recommendations of the "Guide for the Care and
Use of Laboratory Animals" (National Institutes of Health publication 86-23) and
was approved by an Institutional Animal Care and Use Committee. Fasted male
beagle dogs (10 ± 2 kg) were used for the studies. Each drug was administered as a
single dose by oral gavage (1.5–2 ml/kg). The dose was 10 mg-equivalent of
PMPA/kg. For PBMCs, blood samples were collected at 0 (pre-dose), 2, 8, and 24 h
post-dose. For plasma, blood samples were collected at 0 (pre-dose), 5, 15, and 30
min, and 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose. Blood (1.0 ml) was processed
immediately for plasma by centrifugation at 2,000 rpm for 10 min. Plasma samples
were frozen and maintained at 70°C until analyzed.

Peripheral Blood Mononuclear Cell (PBMC) preparation. Whole blood (8 ml)
drawn at specified time points was mixed in equal proportion with phosphate
buffered saline (PBS), layered onto 15 ml of Ficoll-Paque solution (Pharmacia
Biotech,) and centrifuged at 400 x g for 40 min. PBMC layer was removed and
washed once with PBS. Formed PMBC pellet was reconstituted in 0.5 ml of PBS, cells were resuspended, counted using hemocytometer and maintained at 70°C until analyzed. The number of cells multiplied by the mean single-cell volume was used in calculation of intracellular concentrations. A reported value of 200 femtoliters/cell was used as the resting PBMC volume (B. L. Robins, R.V. Srinivas, C. Kim, N. Bischofberger, and A. Fridland, Antimicrob. Agents Chemother. 42, 612 (1998).

**Determination of PMPA and Prodrugs in plasma and PBMCs.** The concentration of PMPA in dog plasma samples was determined by derivatizing PMPA with chloroacetaldehyde to yield a highly fluorescent N¹, N⁶-ethenoadenine derivative (L. Naesens, J. Balzarini, and E. De Clercq, Clin. Chem. 38, 480 (1992). Briefly, plasma (100 μl) was mixed with 200 μl acetonitrile to precipitate protein. Samples were then evaporated to dryness under reduced pressure at room temperature. Dried samples were reconstituted in 200 μl derivatization cocktail (0.34% chloroacetaldehyde in 100 mM sodium acetate, pH 4.5), vortexed, and centrifuged. Supernatant was then transferred to a clean screw-cap tube and incubated at 95°C for 40 min. Derivatized samples were then evaporated to dryness and reconstituted in 100 μl of water for HPLC analysis.

Before intracellular PMPA could be determined by HPLC, the large amounts of adenine related ribonucleotides present in the PBMC extracts had to be removed by selective oxidation. We used a modified procedure of Tanaka et al (K. Tanaka, A. Yoshioka, S. Tanaka, and Y. Wataya, Anal. Biochem., 139, 35 (1984). Briefly, PBMC samples were mixed 1:2 with methanol and evaporated to dryness under reduced pressure. The dried samples were derivatized as described in the plasma assay. The derivatized samples were mixed with 20 μL of 1M rhamnose and 30 μL of 0.1M sodium periodate and incubated at 37°C for 5 min. Following incubation, 40 μL of 4M methylamine and 20 μL of 0.5M inosine were added. After incubation at 37°C for 30 min, samples were evaporated to dryness under reduced pressure and reconstituted in water for HPLC analysis.
No intact prodrug was detected in any PBMC samples. For plasma samples potentially containing intact prodrugs, experiments were performed to verify that no further conversion to PMPA occurred during derivatization. Prodrug standards were added to drug-free plasma and derivatized as described. There were no detectable levels of PMPA present in any of the plasma samples, and the projected % of conversion was less than 1%

The HPLC system was comprised of a P4000 solvent delivery system with AS3000 autoinjector and F2000 fluorescence detector (Thermo Separation, San Jose, CA). The column was an Inertsil ODS-2 column (4.6 x 150 mm). The mobile phases used were: A, 5% acetonitrile in 25 mM potassium phosphate buffer with 5 mM tetrabutyl ammonium bromide (TBABr), pH 6.0; B, 60% acetonitrile in 25 mM potassium phosphate buffer with 5 mM TBABr, pH 6.0. The flow rate was 2 ml/min and the column temperature was maintained at 35°C by a column oven. The gradient profile was 90% A/10% B for 10 min for PMPA and 65%A/35%B for 10 min for the prodrug. Detection was by fluorescence with excitation at 236 nm and emission at 420 nm, and the injection volume was 10 μl. Data was acquired and stored by a laboratory data acquisition system (PeakPro, Beckman, Allendale, NJ).

Pharmacokinetic Calculations. PMPA and prodrug exposures were expressed as areas under concentration curves in plasma or PBMC from zero to 24 hours (AUC). The AUC values were calculated using the trapezoidal rule.

Plasma and PBMC Concentrations. The results of this study is shown in Figures 2 and 3. Figure 2 shows the time course of GS 7340-2 metabolism summary of plasma and PBMC exposures following oral administration of pure diastereoisomers of the PMPA prodrugs.
Figure 2. PMPA and Prodrug Concentration in Plasma and PBMCs Following Oral Administration of GS 7340-2 to Dogs at 10 mg-eq/kg.

The bar graph in Figure 2 shows the AUC (0-24h) for tenofovir in dog PBMCs and plasma after administration of PMPA s.c., TDF and amidate ester prodrugs. All of the amidate prodrugs exhibited increases in PBMC exposure. For example, GS 7340 results in a ~21-fold increase in PBMC exposure as compared to PMPA s.c. and TDF; and a 6.25-fold and 1.29-fold decrease in plasma exposure, respectively.
Figure 3. Depicts Tenofovir Exposure in PBMCs and Plasma Upon Administration of 10 mg-eq/kg in dogs

AUC(0-24h) for PMPA in PBMC and Plasma Following an Oral Dose of 10 mg-eq/kg PMPA Prodrugs to Dogs.

These data establish in vivo that GS 7340 can be delivered orally, minimizes systemic exposure to PMPA and greatly enhances the intracellular concentration of PMPA in the cells primarily responsible for HIV replication.
Table 6

**PMPA Exposure in PBMC and Plasma from Oral Prodrugs of PMPA in Dogs**

<table>
<thead>
<tr>
<th>GS#</th>
<th>Molety</th>
<th>PMPA AUC in Plasma</th>
<th>PMPA AUC in PBMC</th>
<th>Prodrug in Plasma</th>
<th>PBMC/Plasma Exposure Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-7114</td>
<td>Mono-Ala-Et-A</td>
<td>5.8 0.9 2</td>
<td>708 331 5</td>
<td>YES</td>
<td>122</td>
</tr>
<tr>
<td>GS-7115</td>
<td>Mono-Ala-Et-B</td>
<td>6.6 1.5 2</td>
<td>284 94 5</td>
<td>YES</td>
<td>43</td>
</tr>
<tr>
<td>GS-7340-2</td>
<td>Mono-Ala-IPr-A</td>
<td>5.0 1.1 5</td>
<td>805 222 5</td>
<td>YES</td>
<td>161</td>
</tr>
<tr>
<td>GS-7339</td>
<td>Mono-Ala-IPr-A</td>
<td>6.4 1.3 2</td>
<td>200 57 5</td>
<td>YES</td>
<td>31</td>
</tr>
<tr>
<td>GS-7119</td>
<td>Mono-Gly-Et-A</td>
<td>6.11 1.86 2</td>
<td>530 304 5</td>
<td>YES</td>
<td>87</td>
</tr>
<tr>
<td>GS-7342</td>
<td>Mono-ABA-Et-A</td>
<td>4.6 1.2 2</td>
<td>1060 511 5</td>
<td>YES</td>
<td>230</td>
</tr>
<tr>
<td>GS7341</td>
<td>Mono-ABA-Et-B</td>
<td>5.8 1.4 2</td>
<td>199 86 5</td>
<td>YES</td>
<td>34</td>
</tr>
</tbody>
</table>

**Example 11**

**Biodistribution of GS-7340**

As part of the preclinical characterization of GS-7340, its biodistribution in dogs was determined. The tissue distribution of GS-7340 (isopropyl alaninyl monoamidate, phenyl monoester of tenofovir) was examined following oral administration to beagle dogs. Two male animals were dosed orally with $^{14}C$=GS-7340 (8.85 mg-equiv. of PMPA/kg, 33.2 μCi/kg; the 8-carbon of adenine is labeled) in an aqueous solution (50 mM citric acid, pH 2.2). Plasma and peripheral blood mononuclear cells (PBMCs) were obtained over the 24-hr period. Urine and feces were cage collected over 24 hr. At 24 h after the dose, the animals were sacrificed and tissues removed for analysis. Total radioactivity in tissues was determined by oxidation and liquid scintillation counting.

The biodistribution of PMPA after 24 hours after a single oral dose of radiolabelled GS 7340 is shown in Table 4 along with the data from a previous study with TDF (GS-4331). In the case of TDF, the prodrug concentration in the plasma is below the level of assay detection, and the main species observed in plasma is the parent drug. Levels of PMPA in the lymphatic tissues, bone marrow, and skeletal muscle are increased 10-fold after administration of GS-7340.
Accumulation in lymphatic tissues is consistent with the data observed from the PBMC analyses, since these tissues are composed primarily of lymphocytes. Likewise, accumulation in bone marrow is probably due to the high percentage of lymphocytes (70%) in this tissue.

Table 7. Excretion and Tissue Distribution of Radiolabelled GS-7340 in Dogs (Mean, N=2) Following an Oral Dose at 10 mg-eq. PMPA/kg.

<table>
<thead>
<tr>
<th>Tissue/Fluid</th>
<th>GS-4331 % Dose</th>
<th>GS-7340 % Dose</th>
<th>GS-7340 Conc. (ug-eq/g)</th>
<th>GS-4331 Conc. (ug-eq/g)</th>
<th>Tissue Conc. Ratio of GS 7340 to GS-4331</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>12.40</td>
<td>16.45</td>
<td>38.30</td>
<td>52.94</td>
<td>1.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.58</td>
<td>3.78</td>
<td>87.90</td>
<td>80.21</td>
<td>0.9</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.03</td>
<td>0.34</td>
<td>0.53</td>
<td>4.33</td>
<td>8.2</td>
</tr>
<tr>
<td>Iliac Lymph Nodes</td>
<td>0.00</td>
<td>0.00</td>
<td>0.51</td>
<td>0.41</td>
<td>10.6</td>
</tr>
<tr>
<td>Axillary Lymph Nodes</td>
<td>0.00</td>
<td>0.00</td>
<td>0.37</td>
<td>0.54</td>
<td>14.8</td>
</tr>
<tr>
<td>Inguinal Lymph Nodes</td>
<td>0.00</td>
<td>0.00</td>
<td>0.28</td>
<td>0.41</td>
<td>15.0</td>
</tr>
<tr>
<td>Mesenteric Lymph Nodes</td>
<td>0.00</td>
<td>0.04</td>
<td>1.20</td>
<td>6.88</td>
<td>5.7</td>
</tr>
<tr>
<td>Thyroid Gland</td>
<td>0.00</td>
<td>0.00</td>
<td>0.30</td>
<td>4.78</td>
<td>15.8</td>
</tr>
<tr>
<td>Pituitary Gland</td>
<td>0.00</td>
<td>0.00</td>
<td>0.23</td>
<td>1.80</td>
<td>7.8</td>
</tr>
<tr>
<td>Salivary Gland (L+R)</td>
<td>0.00</td>
<td>0.03</td>
<td>0.45</td>
<td>5.54</td>
<td>12.3</td>
</tr>
<tr>
<td>Adrenal Gland</td>
<td>0.00</td>
<td>0.00</td>
<td>1.90</td>
<td>3.47</td>
<td>1.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.00</td>
<td>0.17</td>
<td>0.63</td>
<td>8.13</td>
<td>12.8</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.00</td>
<td>0.01</td>
<td>0.57</td>
<td>3.51</td>
<td>6.2</td>
</tr>
<tr>
<td>Prostate</td>
<td>0.00</td>
<td>0.00</td>
<td>0.23</td>
<td>2.14</td>
<td>9.1</td>
</tr>
<tr>
<td>Testes (L+R)</td>
<td>0.02</td>
<td>0.02</td>
<td>1.95</td>
<td>2.01</td>
<td>1.0</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>0.00</td>
<td>0.01</td>
<td>0.11</td>
<td>1.12</td>
<td>10.1</td>
</tr>
<tr>
<td>Heart</td>
<td>0.03</td>
<td>0.15</td>
<td>0.46</td>
<td>1.97</td>
<td>4.3</td>
</tr>
<tr>
<td>Femoral Bone</td>
<td>0.00</td>
<td>0.00</td>
<td>0.08</td>
<td>0.28</td>
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<td>0.20</td>
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<td>Abdominal fat</td>
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<td>0.16</td>
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<td>Eye (L+R)</td>
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<td>&lt;LOD</td>
<td>&lt;LOD</td>
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<tr>
<td>Cerebrospinal Fluid</td>
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<td>&lt;LOD</td>
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<td>n.d.</td>
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<tr>
<td>Spinal Cord</td>
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<td>&lt;LOD</td>
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<td>Stomach</td>
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<td>Feces</td>
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<td>Total GI Tract Contents</td>
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<td>Plasma at 24 h</td>
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<td>0.20</td>
<td>1.0</td>
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<tr>
<td>Plasma at 0.25 h</td>
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<td>n.a.</td>
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<td>PBMC*</td>
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<td>n.d.</td>
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<td>n.d.</td>
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<td>Whole Blood</td>
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<td>0.20</td>
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<tr>
<td>Total Recovery</td>
<td>81.10</td>
<td>68.96</td>
<td></td>
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<td></td>
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</table>

* Calculated using typical recovery of 15 x 10^9 cells total, and mean PBMC volume of 0.2 picoliters/cell
n.s. = no sample, n.a. = not applicable, n.d. = not determined.
CLAIMS:

1. A screening method for identifying a methoxyphosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue comprising:
   (a) providing at least one of said prodrugs;
   (b) selecting at least one therapeutic target tissue and at least one non-target tissue;
   (c) administering the prodrug to the target tissue and to said at least one non-target tissue; and
   (d) determining the relative activity conferred by the prodrug in the tissues in step (c).

2. The method of claim 1 wherein the activity is antiviral activity or antitumor activity.

3. The method of claim 2 wherein the activity is antiviral activity.

4. The method of claim 3 wherein the activity is anti-HIV or anti-HBV activity.

5. The method of claim 1 wherein the prodrug is a prodrug of PMPA or PMEA.

6. The method of claim 5 wherein the prodrug is a phosphonoamide, phosphonoester or mixed phosphonoamidate/phosphonoester.

7. The method of claim 6 wherein the amidate is an amino acid amidate.

8. The method of claim 6 wherein the ester is an aryl ester.

9. The method of claim 1 further comprising selecting a prodrug having a relative activity in the target tissue that is greater than 10 times that of the non-target tissue.
10. The method of claim 1 wherein the target and non-target tissue are in an animal, the prodrug is administered to the animal and the relative activity is determined by analysis of the animal tissues after administration of the prodrug.

11. The method of claim 1 wherein activity in the target and non-target tissues is determined by assaying the amount of at least one metabolite of the prodrug in the tissues.

12. The method of claim 12 wherein the metabolite is the parental drug.

13. The method of claim 12 wherein the metabolite is the diphosphate of the parental drug.

14. The method of claim 1 wherein the target tissue is virally infected tissue and the non-target tissue is the same tissue which is not virally infected.

15. The method of claim 1 wherein the target tissue is lymphoid tissue and the activity is anti-HIV activity.

16. The method of claim 1 wherein the target tissue is liver and the activity is anti-HBV activity.

17. The method of claim 1 wherein the target tissue is hematological and the activity is antitumor activity.

18. The method of claim 1 wherein the target tissue is malignant and the non-target tissue is the same tissue but non-malignant.
19. A compound having the structure (1)

![Chemical Structure (1)]

where Ra is H or methyl,
and chirally enriched compositions thereof, salts, their free base and solvates thereof.

20. A compound having the structure (2)

![Chemical Structure (2)]

and its enriched diasteromers, salts, free base and solvates.

21. A diastereomerically enriched compound having the structure (3)

![Chemical Structure (3)]
which is substantially free of the diastereomer (4)

$$
\begin{align*}
 & B \quad E \quad R^1 \\
 & R^2
\end{align*}
$$

wherein

$R^1$ is an oxyester which is hydrolyzable in vivo, or hydroxyl;

$B$ is a heterocyclic base;

$R^2$ is hydroxyl, or the residue of an amino acid bonded to the P atom through an amino group of the amino acid and having each carboxy substituent of the amino acid optionally esterified, but not both of $R^1$ and $R^2$ are hydroxyl;

$E$ is -(CH$_2$)$_2$-, -(CH(CH$_3$)CH$_2$)-, -CH(CH$_2$F)CH$_2$-, -CH(CH$_2$OH)CH$_2$-, -CH(CH=CH$_2$)CH$_2$-, -CH(C=CH)CH$_2$-, -CH(CH$_2$N$_3$)CH$_2$-, -CH(R$_6^6$)OCH(R$_6^6$)-, -CH(R$_9^9$)CH$_2$O- or -CH(R$_8^8$)O-, wherein the right hand bond is linked to the heterocyclic base;

the broken line represents an optional double bond;

$R^4$ and $R^5$ are independently hydrogen, hydroxy, halo, amino or a substituent having 1-5 carbon atoms selected from acyloxy, alkoxy, alkylthio, alkylamino and dialkylamino;

$R^6$ and $R^9$ are independently H, C$_1$-C$_6$ alkyl, C$_1$-C$_6$ hydroxyalkyl, or C$_2$-C$_7$ alkanoyl;

$R^7$ is independently H, C$_1$-C$_6$ alkyl, or are taken together to form -O- or -CH$_2$-;
R^8 is H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl; and
R^9 is H, hydroxymethyl or acyloxymethyl;
and their salts, free base, and solvates.

22. A diastereomERICally enriched compound having the structure (5a)

![Chemical structure](5a)

which is substantially free of diastereomer (5b)

![Chemical structure](5b)

wherein
R^5 is methyl or hydrogen;
R^6 independently is H, alkyl, alkenyl, alkynyl, aryl or arylalkyl, or R^6 independently is alkyl, alkenyl, alkynyl, aryl or arylalkyl which is substituted with from 1 to 3 substituents selected from alkylamino, alkylaminoalkyl, dialkylaminoalkyl, dialkylamino, hydroxyl, oxo, halo, amino, alkylthio, alkoxy,
alkoxyalkyl, aryl oxy, aryloxyalkyl, arylalkoxy, arylalkoxyalkyl, haloalkyl, nitro, nitroalkyl, azido, azidoalkyl, alkylacyl, alkylacylalkyl, carboxyl, or alkylacylamino;

R⁷ is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group;

R¹¹ is amino, alkylamino, oxo, or dialkylamino; and

R¹² is amino or H;

and it salts, tautomers, free base and solvates.

23. A compound of structure (6)

\[
\text{\includegraphics{image6.png}}
\]

and its salts and solvates.

24. A compound of structure (7)

\[
\text{\includegraphics{image7.png}}
\]

26. The composition of claim 25 wherein the excipient is a gel.

27. The composition of claim 25 which is suitable for topical administration.

28. A method for antiviral therapy or prophylaxis comprising administering a compound of any of claims 19-24 in a therapeutically or prophylactically effective amount to a subject in need of such therapy or prophylaxis.

29. A method for use of magnesium alkoxide comprising reacting 9-(2-hydroxypropyl)adenine (HPA) or 9-(2-hydroxyethyl)adenine (HEA), magnesium alkoxide, and protected p-toluenesulfonyloxyethylphosphonate.

30. The method of claim 29 further comprising recovering PMPA or PMEA, respectively.

31. The method of claim 29 wherein the phosphonate of the p-toluenesulfonyloxyethylphosphonate is protected by ethyl ester.

32. The method of claim 29 wherein the alkoxide is a C_1-C_6 alkoxide.

33. The method of claim 32 wherein the alkoxide is t-butyl or isopropyl oxide.

IN THE MATTER OF:

A representation by way of opposition under s25(1) of The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005 (“the Act”) and Rule 55 of The Patents Rules, 2003 as amended by the Patents Rules, 2006 (“the Rules”) by the Initiative for Medicines, Access & Knowledge (I-MAK) (“the OPPONENT”)

And

Indian Application No. 3658/KOLNP/2009 by Pharmasset, Inc (“the APPLICANT”)

Exhibit 12

WO 2005/012327
Abstract: Phosphorominate derivatives of nucleotides and their use in the treatment of cancer are described. The base moieties of, for example, each of deoxyuridine, cytarabine, gemicitabine and cidofovir may be substituted at the 5-position. The phosphoraminate moiety has attached to the P atom an aryl-O moiety and an α-amino acid moiety. The α-amino acid moiety may correspond to or be derived from either a naturally occurring or a non-naturally occurring amino acid.
Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
Chemical Compounds

The present invention relates to nucleotide derivatives and their use in the treatment of cancer.

Nucleoside analogues such as fluorodeoxyuridine (1), cytarabine (2) and gemcitabine (3) are well established as anticancer agents. They function as inhibitors of DNA synthesis after activation to their 5′-phosphate form.


In general the phosphate prodrugs have biological properties and therapeutic activities that are similar to, or somewhat lower than, the parent nucleoside analogue.

We have carried out extensive work in this area from an antiviral perspective, largely on dideoxy nucleosides, and have reported a phosphoramidate approach which has been widely adopted for the delivery of bio-active phosphates of antiviral nucleosides.

An example is the phosphoramidate (4) derived from anti-HIV d4T (5).

This work has lead to the optimal description of phenyl methoxycalaninyl phosphoramidate as the prototype pro-moiety for the intracellular delivery of bioactive nucleotides [Balzarini et al, PNAS, 1996, 93, 7295-9; McGuigan et al, J. Med. Chem., 1996, 39, 1748-53].

Lackey et al [Biochem Pharmacol., 2001, 61, 179-89] have reported the application of our phosphoramidate pro-drug method for antiviral nucleosides to the anti-herpetic agent bromovinyl-2'-deoxyuridine (BVDU) (6). In particular, they have found that the phenyl methoxycalaninyl phosphoramidate (7) has significant anti-cancer activity. This is in marked contrast to the parent (antiviral) nucleoside (6).
Limited SAR has been presented by this group, although in their patent applications [WO0239952, EP1200455, CA2317505, US6339151, EP116797, AU2451601] they claim a series of general variations in the base, and phosphate regions. However, based on our prior art, the phenyl methoxyalaninyl phosphoramide (7) would be anticipated to be amongst the most optimal of structures.

Surprisingly, it has now been found that other derivatives of oxyamino acid-phosphoramide nucleoside analogues are significantly more potent in the treatment of cancer than the phenyl methoxyalaninyl phosphoramide (7).

According to a first aspect of the present invention there is provided a compound of formula I:

![Chemical structure](image)

wherein:

R is selected from the group comprising alkyl, aryl and alkylaryl;
R' and R'' are, independently, selected from the group comprising H, alkyl and alkylaryl, or R' and R'' together form an alkylene chain so as to provide, together with the C atom to which they are attached, a cyclic system;
Q is selected from the group comprising –O- and –CH₂–;
X and Y are independently selected from the group comprising H, F, Cl, Br, I, OH and methyl (–CH₃);
Ar is a monocyclic aromatic ring moiety or a fused bicyclic aromatic ring moiety, either of which ring moieties is carbocyclic or heterocyclic and is optionally substituted;
Z is selected from the group comprising H, alkyl and halogen; and
n is 0 or 1,
wherein
when n is 0, Z' is -NH$_2$ and a double bond exists between position 3 and position 4, and
when n is 1, Z' is =O;

or a pharmaceutically acceptable derivative or metabolite of a compound of formula I;

with the proviso that when n is 1, X and Y are both H, R is methyl (-CH$_3$), one of R' and R'' is H and one of R' and R'' is methyl (-CH$_3$), then Ar is not phenyl (-C$_6$H$_5$).

By “a pharmaceutically acceptable derivative” is meant any pharmaceutically acceptable salt, ester or salt of such ester or any other compound which upon administration to a recipient is capable of providing (directly or indirectly) a compound of formula (I).

Suitably, except where R is 2-Bu (-CH$_2$-CH(CH$_3$)$_2$) and one of R' and R'' is H and one of R' and R'' is methyl (-CH$_3$), when n is 1 and X and Y are both H, then Ar is not unsubstituted phenyl (-C$_6$H$_5$).

By “pharmaceutically acceptable metabolite” is meant a metabolite or residue of a compound of formula (I) which gives rise in use to a compound of formula (II):

wherein n, Q, R, R', R'', X, Y, Z and Z' have the meanings described above and below for formula I, and additionally R can be H, with the proviso that when n is 1, X and Y are both
H, R is methyl (−CH₃), one of R' and R'' is H and one of R' and R'' is methyl (−CH₃), then Z is not −CH=CHBr.

Suitably, with respect to compounds of formula II, when n is 1 and Z either is or is not −CH=CHBr, the moiety ROCOCR'R''NH- corresponds neither to alanine (i.e., as above, R is not methyl (−CH₃), one of R' and R'' is not H and one of R' and R'' is not methyl (−CH₃)) nor to tryptophan (i.e., α-amino-β-indolylpropionic acid).

More suitably with respect to compounds of formula II, when n is 1 and Z either is or is not −CH=CHBr, the moiety ROCOR'R''NH is neither derived from nor corresponds to any naturally occurring amino acid.

Even more suitably, with respect to compounds of formula II, when n is 1 or 0, the moiety ROCOCR'R''NH- does not correspond to alanine (i.e., R is not methyl (−CH₃), one of R' and R'' is not H and one of R' and R'' is not methyl (−CH₃)), does not preferably correspond to tryptophan, and even more preferably the said moiety does not correspond to any naturally occurring amino acid.

Most preferably the moiety ROCOCR'R''NH- in compounds of formula II corresponds to a non-naturally occurring amino acid.

Reference in the present specification to an alkyl group means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g., alkenyl or alkynyl) hydrocarbyl radical. Where cyclic, the alkylene group is preferably C₃ to C₁₂, more preferably C₅ to C₁₀, more preferably C₅ to C₇. Where acyclic, the alkyl group is preferably C₁ to C₁₆, more preferably C₁ to C₆.

Reference in the present specification to an aryl group means an aromatic group containing 5 to 14 ring atoms, for example phenyl or naphthyl. The aromatic group may be a heteroaromatic group containing one, two, three or four, preferably one, heteroatoms selected, independently, from the group consisting of O, N and S. Examples of such heteroaromatic groups include pyridyl, pyrrolyl, furanyl and thiophenyl. Preferably, the aryl group comprises phenyl or substituted phenyl.
The alkyl and aryl groups may be substituted or unsubstituted. Where substituted, there will generally be one to three substituents present, preferably one substituent. Substituents may include halogen atoms, by which is meant F, Cl, Br and I atoms, and halomethyl groups such as CF₃ and CCl₃; oxygen containing groups such as oxo, hydroxy, carboxy, carboxyC₁₋₁₆alkyl, alkoxy, alkyl, alkoyloxy, arylxy, arylolyl and arylloyloxy; nitrogen containing groups such as amino, C₁₋₆alkylamino, diC₁₋₆alkylamino, cyano, azide and nitro; sulphur containing groups such as thiol, C₁₋₆alkylthiol, sulphonyl and sulphoxide; heterocyclic groups which may themselves be substituted; alkyl groups as defined above, which may themselves be substituted; and aryl groups as defined above, which may themselves be substituted, such as phenyl and substituted phenyl. Substituents on said heterocyclic, alkyl and aryl groups are as defined immediately above.

Reference in the present specification to alkoxy and aryloxy groups means, respectively, alkyl-O- (for example where alkyl is C₁ to C₁₆, preferably C₁ to C₆) and aryl-O- (for example where aryl is a 5 to 14 membered aromatic mono- or bifused ring moiety, optionally containing 1, 2, 3 or 4 heteroatoms selected, independently, from O, S and N, preferably aryl is phenyl).

Reference in the present specification to alkoyl and aryloyl groups means, respectively, alkyl-CO- (for example where alkyl is C₁ to C₁₆, preferably C₁ to C₆) and aryl-CO- (for example where aryl is a 5 to 14 membered aromatic mono or bifused ring moiety, optionally containing 1, 2, 3 or 4 heteroatoms selected, independently, from O, S and N, preferably aryl is phenyl).

Reference in the present specification to alkoyloxy and aryloyloxy means, respectively, alkyl-CO-O (for example where alkyl is C₁ to C₁₆, preferably C₁ to C₆) and aryl-CO-O (for example where aryl is a 5 to 14 membered mono- or bifused aromatic ring system, optionally containing 1, 2, 3 or 4 heteroatoms selected, independently, from O, S and N, preferably aryl is phenyl).

Reference in the present specification to heterocyclic groups means groups containing one or more, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, pyrrolidinyl,
pyrrolinyl, imidazolidinyl, imidazoliny1, pyrazolidinyl, tetrahydrofuranyl, pyranyl, pyronyl, pyridyl, pyrazinyl, pyridazinyl, piperidyl, piperazinyl, morpholinyl, thionaphthyl, benzofuranyl, isobenzofuranyl, indolyl, oxyindolyl, isoindolyl, indazolyl, indolinyl, 7-azaindolyl, isoindazolyl, benzopyranyl, coumarinyl, isocoumarinyl, quinolyl, isoquinolyl, naphthidinyl, cinnolinyl, quinazolinyl, pyridopyridyl, benzoxazinyl, quinoxadiny1, chromenyl, chromanyl, isochromanyl and carbolinyl.

The group Ar comprises a substituted or unsubstituted aryl group, wherein the term “aryl group” and the possible substitution of said group is as defined herein. Preferably, Ar is a substituted or unsubstituted phenyl group. Particularly preferred substituents are electron withdrawing groups such as halogen (preferably chlorine or fluorine), trihalomethyl (preferably trifluoromethyl), cyano and nitro groups. For example, Ar can be phenyl, 3,5-dichloro-phenyl, p-trifluoromethyl-phenyl, p-cyano-phenyl, or p-nitro-phenyl. When Ar is a heteroaromatic group, preferably it is optionally substituted pyridyl.

Suitably, R is a C_{1-16} primary or secondary alkyl group, a C_{5-7} carbocyclic aryl group or a C_{1-4}alkylC_{5-11}aryl group. More suitably, R is a C_{1-10} alkyl group, a phenyl group or C_{1-3} alkylC_{5-7} aryl group. Preferably R is unsubstituted.

Preferably, R is methyl (-CH_{3}), ethyl (-C_{2}H_{5}), n- or i- propyl (-C_{3}H_{7}), n- or i- butyl (-C_{4}H_{9}) or benzyl (-CH_{2}C_{6}H_{5}). Most preferably, R is benzyl. Particularly, R is preferably benzyl when one of R’ and R” is H and one of R’ and R” is methyl (-CH_{3}), especially when Ar is unsubstituted phenyl, n is 0 and each of X and Y is F.

Suitably, R’ and R” are each independently selected from the group comprising H, C_{1-6} primary, secondary or tertiary alkyl, C_{1-3}alkylC_{5-7}aryl, or, when together they form an alkylene chain, they provide, together the C atom to which they are attached, a C_{3-8} carbocyclic aliphatic ring.

Preferably, R’ and R” are the same and are alkyl, more preferably they are both methyl, ethyl or n- or i- propyl.
Alternatively, preferably, R' and R" are, independently, H, methyl (-CH₃), secondary butyl (-CH₂-CH-(CH₃)₂), benzyl (-CH₂C₆H₅), or, together with the C atom to which they are attached, provide a C₅-₆ ring.

Preferred compounds include those where R' and R" are both methyl, one of R' and R" is H and one of R' and R" is methyl, and R' and R", together with the C atom to which they are attached, provide a pentyl ring.

When R' and R" are different, the C atom to which they are attached is chiral. The present compounds can be L or D or a mixture of stereoisomers. Preferably they are L.

It will be appreciated that the moiety -O-C(O)-CR'R"-NH- corresponds to a carboxy-protected α-amino acid. R' and R" can thus correspond to the side chains of a naturally occurring amino acid.

For example, when one of R' and R" is H and one of R' and R" is Me or PhCH₂, the moiety corresponds to alanine or phenylalanine, respectively.

Preferably, the stereochemistry at the asymmetric centre -CR'R" corresponds to an L-amino acid. The stereochemistry at the asymmetric centre -CR'R" can, however, correspond to a D-amino acid. Alternatively, mixtures of of compounds can be employed having asymmetric centres corresponding to L and D amino acids.

In the present specification by "naturally occurring amino acid" we mean Alanine, Arginine, Asparagine, Aspartic Acid, Cysteine, Cystine, Glycine, Glutamic Acid, Glutamine, Histidine, Hydroxylysine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine and Valine.

The present invention is not, however, limited to compounds having a moiety corresponding to a naturally occurring amino acid. The present invention specifically includes compounds having a moiety which corresponds to a non-naturally occurring amino acid, such as, for example, those where R'=R''=alkyl, or, where together with the C atom to which they are attached, R' and R" provide a cyclic moiety. Preferably with
respect to the compound of formula I, the moiety ROCOCR'R’"NH- corresponds to or is
derived from a non-naturally occurring amino acid.

With respect to compounds of formula I when n is 1, the moiety ROCOCR'R’"NH-
preferably neither corresponds to nor is derived from alanine, more preferably neither
corresponds to nor is derived from either of alanine or tryptophan, even more preferably
neither corresponds to nor is derived from any naturally occurring amino acid.

With respect to compounds of formula I when n is 0, the moiety ROCOCR'R’"NH-
preferably neither corresponds to nor is derived from alanine, more preferably neither
corresponds to nor is derived from either of alanine or tryptophan, even more preferably
neither corresponds to nor is derived from any naturally occurring amino acid.

Preferably Q is O.

Preferably, X and Y are, independently, selected from the group comprising F, H and OH.

When n is 1, preferably each of X and Y is H.

When n is 0, preferably each of X and Y is F, or X is OH and Y is H, or X is H and Y is
OH.

When Z is F, Q is O, n is 1 and X and Y are each H, the base moiety of the compound of
formula I corresponds to that of fluorodeoxyuridine i.e. compound (1) above.

When Z is H, Q is O, n is 0 and X is OH and Y is H, the base moiety of the compound of
formula I corresponds to that of cytarabine i.e. compound (2) above.

When Z is H, Q is O, n is 0 and X and Y are each F, the base moiety of the compound of
formula I corresponds to that of gemcitabine i.e. compound (3) above.

When Z is H, Q is O, n is 0 and X is H and Y is OH, the base moiety of the compound of
formula I corresponds to that of cytidine.
Compounds of formula I wherein n is 0 and X and Y are F are preferred. Particularly preferred are compounds of formula I wherein n is 0, X and Y are F, Q is O and Z is H, corresponding to phosphoramidated gemcitabine.

Also preferred are compounds of formula I wherein n is 0 and X is OH and Y is H. Particularly preferred are compounds of formula I wherein n is 0, X is OH, Y is H, Q is O and Z is H, corresponding to phosphoramidated cytarabine.

Also preferred are compounds of formula I wherein n is 0 and X is H and Y is OH. Particularly preferred are compounds of formula I wherein n is 0, X is H, Y is OH, Q is O and Z is H, corresponding to phosphoramidated cytidine.

Suitably, Ar is a 5 to 14 membered aromatic ring moiety. The one or two rings may include 1, 2, 3 or 4 heteroatoms, preferably 1, selected, independently, from O, S and N.

Preferably, Ar is a carbomonocyclic aromatic ring moiety. More preferably, Ar is a C₆ monocyclic aromatic ring moiety, ie is optionally substituted phenyl.

One, two, three or four substituents, which may be the same or different, may be present on Ar and are selected from the group comprising halogen, which may be -F, -Cl, -Br or -I; -NO₂; -NH₂; optionally substituted -C₁₋₃alkyl; optionally substituted -C₁₋₃alkoxy, preferably methoxy (-OCH₃); optionally substituted –SC₁₋₃alkyl; -CN; optionally substituted -COC₁₋₃alkyl; and optionally substituted -CO₂C₁₋₃alkyl. The optional substituents are one or more up to six, preferably three, members selected from the group comprising halogen which may be F, Cl, Br and I and NO₂. Preferred substituents on Ar include F, Cl, CF₃, and NO₂.

The substituents may be at any position on the ring moiety. Where the ring moiety is C₆ ie phenyl, a single substituent at the 2 (ortho) or 4 (para) position is preferred. Where Ar is phenyl, a single substituent at the 4 position is more preferred.
Preferably, Ar is an optionally substituted phenyl moiety. More preferably, Ar is selected from the group comprising: Ph-, pCF₃C₆H₄-, pFC₆H₄-, pNO₂C₆H₄-, pClC₆H₄- and oClC₆H₄-.

Suitably, Z is selected from the group comprising H, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₁₋₆ alkenyl, substituted C₁₋₆ alkenyl, C₁₋₆ alkynyl, substituted C₁₋₆ alkynyl and halogen, where halogen is F, Cl, Br or I. Substituents that may be present on the alkenyl or alkynyl moiety are selected from the group comprising F, Cl, Br, I, and –CO₂Me. One, two or three substituents may be present. The alkenyl and alkynyl groups may contain one or more sites of unsaturation.

Where Z is substituted alkenyl or alkynyl, the substituent is preferably on the terminal C atom.

Preferably Z is selected from the group comprising H, F, optionally substituted C₁₋₆ alkyl particularly Me (-CH₃), optionally substituted C₁₋₆ alkenyl and optionally substituted C₁₋₆ alkynyl, the optional substituents being as recited immediately above.

When n is 1, Z' is O, Q is O and X and Y are each H, preferably Z is a substituted C₂ alkenyl (i.e. ethenyl or vinyl) moiety (-CH=CH-); more preferably, Z is bromovinyl (-CH=CHBr) or methylpropenoate (-CH=CHCO₂Me); and most preferably, Z is -CH=CHBr.

With respect to compounds of formula II, preferably when n is 1 and X and Y are both H, then Z is not F.

With respect to compounds of formula II, when n is 0, preferably X is not H and Y is not OH, more preferably X is OH and Y is H or X and Y are both F.

With respect to compounds of formula II, when n is 0, X is OH and Y is H, preferably neither R' nor R" is phenylmethyl (ie benzyl) or 3-methylindolyl (ie 3-CH₂indolyl).
Surprisingly, modifying the ester moiety in compound (7) has been found to show a marked increase in potency with respect to cancer cell lines. A preferred compound embodying the present invention is the benzyl ester (8). It has surprisingly been found that the benzyl ester (8) is very significantly more potent against several cancer cell lines than the methyl ester (7):

![Chemical structure of compound 8]

Compound (8) inhibits the growth of colon cancer cell line HT115 by 50% at 1.4 μM, whilst (7) requires a concentration of 244 μM; (8) is thus 174 times more potent. Compound (8) is also 8 times more potent than (7) versus prostate cancer cell line PC-3 (19 μM vs. 155 μM).

The degree of potency enhancement for (8) vs. (7) is surprising based on the prior art. Thus, comparing the equivalent phosphoramidates of d4T reveals a ca 4-fold potency boost of (10) over (9) [McGuigan et al, AVCC, 1998, 9, 473-9].

![Chemical structures of compounds 9 and 10]

This would imply that the benzyl phosphoramidate motif in (10) is ca 4-fold more efficient at the intracellular delivery of the bio-active free phosphate forms of d4T than is the methyl ester (9). A person skilled in the art would anticipate a similar degree of
enhancement for the benzyl phosphoramidate of BVDU (8) over the methyl ester (7) whilst we observed an almost 200-fold enhancement for colon cancer as noted above.

Surprising efficacy of modifications in the amino acid and aryl moieties of the BVDU phosphoramidate has also been found in compounds embodying the present invention.

Thus, compound (11) has simultaneous modification in these two regions, being the p-trifluoromethylphenyl benzyl [α,α-dimethylglycyl] phosphoramidate.

Compound 11 shows high potency against a range of cancer cell types and is significantly and surprisingly more potent than (7). Thus, for breast cancer (11) is 60-fold more active (1.3 μM vs 79 μM), and for prostate cancer (11) is 254-fold more potent (0.61 μM vs. 155 μM). Against colon cancer, (11) is 35-fold more potent (7 μM vs 244 μM). Again, the degree of enhancement of the analogue (11) vs. (7) is surprising based on prior art. Thus, comparing (12) [dimethyl glycine modification] and (13) [p-CF₃ phenyl modification] to (9) shows no significant difference in potency.
Thus 50% effective doses vs HIV-1 for (9), (12) and (13) are: 0.075, 0.29, and 0.01 μM respectively; within experimental error, (12) and (13) are identical in potency to (9). Thus a person skilled in the art would have predicted that (11) would show little enhancement over (7) as opposed to the 35 to 254-fold enhancements noted above.

Thus, compounds embodying the present invention and having variations in one or more of the ester (R), amino acid (R', R'') and aryl (Ar) region of the phosphoramidate structure compared to phenyl methoxyalaninyl phosphoramide can give surprising and substantial potency boosts of pro-tides derived from BVDU against a range of cancer cell types.

According to a further aspect of the present invention there is provided a compound having formula I according to the present invention for use in a method of treatment, preferably in the prophylaxis or treatment of cancer.

According to a further aspect of the present invention there is provided a method of prophylaxis or treatment of cancer comprising administration to a patient in need of such treatment an effective dose of a compound having formula I according to the present invention.

According to a further aspect of the present invention there is provided use of a compound having formula I of the present invention in the manufacture of a medicament for use in the treatment or prophylaxis of cancer.

According to a further aspect of the present invention there is provided a pharmaceutical composition comprising a compound having formula I of the present invention in combination with a pharmaceutically acceptable excipient, carrier or diluent.

According to a further aspect of the present invention there is provided a method of preparing a pharmaceutical composition comprising the step of combining a compound having formula I of the present invention with a pharmaceutically acceptable excipient, carrier or diluent.
The present invention is particularly applicable for the treatment of a patient having breast cancer, colon cancer or prostate cancer. Examples of such cancers include breast MDA MB231, colon HT115 and prostate PC-3.

The compound having formula I or pharmaceutical composition according to the present invention can be administered to a patient, which may be human or animal, by any suitable means.

The medications employed in the present invention can be administered by oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration.

For oral administration, the compounds of the invention will generally be provided in the form of tablets or capsules, as a powder or granules, or as an aqueous solution or suspension.

Tablets for oral use may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while cornstarch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

Capsules for oral use include hard gelatin capsules in which the active ingredient is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.
Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinylpyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

The compounds of the invention may also be presented as liposome formulations.

In general a suitable dose will be in the range of 0.1 to 300 mg per kilogram body weight of the recipient per day. A preferred lower dose is 0.5 mg per kilogram body weight of recipient per day, a more preferred lower dose is 6 mg per kilogram body weight of recipient per day, an even more preferred lower dose is 10 mg per kilogram body weight per recipient per day. A suitable dose is preferably in the range of 6 to 150 mg per kilogram body weight per day, and most preferably in the range of 15 to 100 mg per kilogram body weight per day. The desired dose is preferably presented as two, three, four, five or six or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 10 to 1500 mg, preferably 20 to 1000 mg, and most preferably 50 to 700 mg of active ingredient per unit dosage form.

According to a further aspect of the present invention there is provided a process for the preparation of a compound having formula I according to the present invention, the process comprising reacting of a compound of formula (III):
with a compound of formula (IV):

![Formula IV](image_url)

wherein Ar, n Q, R, R', R'', X, Y, Z', and Z have the meanings described above with respect to formula (I).

Embodiments of the present invention will now be described, by way of example only, with reference to the following examples, experimental procedures and experimental data.

Data are presented for a range of structures against tumour cell types representing a range of common cancers in man with un-met clinical need: breast MDA MB231, colon HT115, prostate PC-3. Data from these assays are presented as Table I.

**Experimental Procedure**

**General methods**

The following anhydrous solvents and reagents were bought from Aldrich with sure stopper: dichloromethane (DCM), diethyl ether (Et2O), tetrahydrofuran THF), N-methylimidazole (NMI), methanol (MeOH), dimethylformamide (DMF), 1,4-dioxane. Triethylamine was dried on molecular sieves of 4 Angstrom.
Thin Layer Chromatography

Thin layer chromatography (TLC) was performed on commercially available Merck Kieselgel 60 F254 plates and separated components were visualized using ultraviolet light (254 nm and 366 nm).

Column Chromatography

Columns were performed using (Kieselgel 60, 35-70µm, Fluka) as the stationary phase. Samples were applied as a concentrated solution in the same eluent, or pre-adsorbed onto silica gel.

NMR Spectroscopy

$^1$H, $^{13}$C and $^{31}$P-NMR were recorded on a Bruker Avance DPX300 spectrometer with operating frequencies of 300MHz, 75MHz and 121MHz respectively. $^{31}$P-NMR spectra are reported in units of δ relative to 85% phosphoric acid as external standard, positive shifts are downfield. The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad signal), dd (doublet of doublet), dt (doublet of triplet). Starred signal signal are splitted due to stereoisomeric mixtures.

Standard procedures

For practical purposes, standard procedures are given where applicable.


To a stirring solution of anhydrous alcohol (10 mol eq.) was added thionyl chloride (2 mol eq.) at 0°C, and the resulting solution stirred for 1 hr. After warming to room temperature, the appropriate amino acid (1 mol eq) was added and the reaction heated at reflux for 6-16 hrs. Removal of solvent and recrystallisation from methanol/ether gave the amino ester hydrochloride salts.


The appropriate amino acid (1.0 mol eq.), $p$-toluene sulfonic acid (1.0 mol eq.) and anhydrous benzyl alcohol (4.1 mol eq.) were heated at reflux in toluene (10 mol eq.) with Dean-Stark trap for 24 hrs. On cooling to room temperature, Et$_2$O was added and the
mixture was left in ice bath for 1hr then filtrated and washed with Et₂O. The solid was dissolved in DCM and washed with 10% K₂CO₃ and water. The organic layer was dried over MgSO₄, filtered and the solvent removed under reduced pressure to give an oil. This was solubilized in acetone and neutralized with 1 M HCl. Et₂O was added and the solid was filtered and washed with Et₂O to give a white solid.

**Standard procedure 3: Synthesis of Phosphorodichloridate species.**

Phosphorus oxychloride (1.0 mol eq.) and the appropriate substituted phenol (1.0 mol) were stirred with anhydrous diethylether (31 mol eq.). To this was added anhydrous triethylamine (1.0 mol eq.) at −80 °C and left to rise to room temperature over 16 hrs. the triethylamine hydrochloride salt was filtered off, and the filtrate reduced to dryness to give the crude product as a clear liquid.

**Standard procedure 4: Synthesis of Phosphochloridate species.**

Phosphochloridate (1.0 mol eq.) and the appropriate amino ester hydrochloric salt (1.0 mol eq.) were suspended in anhydrous DCM. Anhydrous triethylamine was added dropwise at −80 °C and after 1hr the reaction was left to rise to room temperature. The formation of phosphochloridate was monitored by ³¹P-NMR. After 2-5 hrs the solvent was removed under reduced pressure and the solid obtained washed with anhydrous ether (2x20 ml), filtered, and the filtrate reduced to dryness to give the products as crude oil. These oils were usually used without further purification.

**Standard procedure 5: Synthesis of Phosphoroamidate derivatives.**

To a stirring solution of (E)-5-(2-bromovinyl)-2'-deoxyuridine (1.0 mol eq.) and the appropriate phosphochloridate (2.0-3.0 mol eq) in anhydrous THF at −80°C was added dropwise over 1 min NMI (5.0 mol eq.). After 15 mins the reaction was left to rise to room temperature and stirred at room temperature for 2-19 hrs. The solvent was removed under reduced pressure and the yellow oil obtained was dissolved in DCM, washed with 0.5 M HCl, and water. The organic layer is dried over MgSO₄, filtered, reduced to dryness and purified by flash chromatography (Chloroform/Methanol 97/3, Dichloromethane/Methanol 97/3).
Synthesis of Methyl-1-amino-1-cyclopentanoate hydrochloride salt.
C₆H₁₄ClNO₃, MW=179.68.

This was synthesised according to *Standard Procedure 1*, using 1-amino-1-cyclopentanecarboxylic acid (3.876 g, 30 mmol) with thionyl chloride (4.44 mL, 45 mmol,) and anhydrous methanol (15.5 mL). The product was isolated as a white solid (4.81 g, yield 89%).

1H-NMR (CDCl₃; 300 MHz): δ 9.1 (3H, bs, NH₃⁺Cl⁻), 3.85 (3H, s, OCH₃), 2.3-2.2 (4H, m, 4H cyclopentane), 2.15 (2H, 2H cyclopentane), 1.95 (2H, m, 2H cyclopentane).

13C-NMR (CDCl₃; 75 MHz): δ 26.6 (2CH₂ cyclopentane), 38.1 (2CH₂ cyclopentane), 54.8 (CH₃O), 66.6 (C₄ cyclopentane), 174.1 (COOMe).

Synthesis of Ethyl-1-amino-1-cyclopentanoate hydrochloride salt.
C₈H₁₆ClNO₂, MW=193.71.

This was synthesised according to *Standard Procedure 1*, using 1-amino-1-cyclopentanecarboxylic acid (5.0 g, 38.6 mmol) with thionyl chloride (5.72 mL, 58 mmol) and anhydrous ethanol (29 mL). The product was isolated as a white solid (6.98 g, yield 93%).

1H-NMR (CDCl₃; 300 MHz): δ 9.0 (3H, bs, NH₃⁺Cl⁻), 4.3 (2H, q, 3J=8, OCH₂CH₃), 2.3-2.2 (4H, m, 4H cyclopentane), 2.15 (2H, 2H cyclopentane), 1.95 (2H, m, 2H cyclopentane), 1.4 (3H, t, 3J=8, OCH₂CH₃).

13C-NMR (CDCl₃; 75 MHz): δ 14.5 (CH₃CH₂), 25.8 (2CH₃ cyclopentane), 37.4 (2CH₂ cyclopentane), 63.0 (CH₃CH₂), 66.2 (C₄ cyclopentane), 172.1 (COOEt).
Synthesis of Benzyl-1-amino-1-cyclopentanoate hydrochloride salt.

\[ C_{14}H_{18}CINO_2, \text{ MW}=255.78. \]

This was synthesised according to \textit{Standard Procedure 2}, using 1-amino-1-cyclopentanecarboxylic acid (3.682 g, 28.5 mmol) with \( p \)-toluene sulfonic acid monohydrate (5.625 g, 29.55 mmol) and anhydrous benzyl alcohol (12 mL, 116 mmol), in Toluene (20 mL). The product was isolated as a white solid (6.441 g, yield 88.5%).

\textbf{Hydrochloride salt.} \( ^1 \)H-NMR (CDCl\(_3\); 300 MHz): \( \delta \) 9.05 (3H, bs, NH\(_2^+\)Cl), 7.4-7.25 (5H, m, Ph), 5.15 (2H, s, CH\(_2\)Ph), 2.3 (4H, m, 4H cyclopentane), 2.15 (2H, 2H cyclopentane), 1.95 (2H, m, 2H cyclopentane).

\( ^{13} \)C-NMR (CDCl\(_3\); 75 MHz): \( \delta \) 25.9 (2CH\(_2\) cyclopenten), 37.3 (2CH\(_2\) cyclopenten), 66.3 (C\(_g\) cyclopentane), 68.3 (CH\(_2\)Ph), 129.2, 129.0, 128.8 (‘o’, ‘m’, CH\(_2\)Ph), 135.5 (‘p’, CH\(_2\)Ph), 172.1 (COOBn).

Synthesis of methyl-2-amino-2-methylpropanoate hydrochloride salt

\[ C_5H_{12}CINO_3, \text{ MW 153.61.} \]

This was synthesised according to \textit{Standard Procedure 1}, using 2-amino-isobutyric acid (5.102 g, 48.49 mmol) with thionyl chloride (11.538 g, 96.98 mmol, 7.04 mL) and anhydrous methanol (19.6 mL). The product was isolated as a white solid (6.636 g, yield 89.2%).

\( ^1 \)H-NMR (CDCl\(_3\); 300 MHz): \( \delta \) 8.81 (3H, bs, NH\(_2\)Cl), 3.83 (3H, s, OCH\(_3\)), 1.74 (6H, s, [CH\(_3\)]\(_2\)C).

\( ^{13} \)C-NMR (CDCl\(_3\); 75 MHz): \( \delta \) 24.1, 24.3 ([CH\(_3\)]\(_2\)C), 57.9 (C[CH\(_3\)]\(_2\)), 172.4 (COOCH\(_3\)).
Synthesis of ethyl-2-amino-2-methylpropanoate hydrochloride salt.
C₆H₁₄ClNO₂, MW 167.63.

\[
\begin{align*}
\text{HCH₂N} & \quad \text{O} \\
\text{O} & \quad \text{O}
\end{align*}
\]

This was synthesised according to *Standard Procedure 1*, using 2-amino-isobutyric acid (5.102 g, 48.49 mmol) with thionyl chloride (11.772 g, 98.95 mmol, 7.2 mL) and anhydrous ethanol (29 mL). The product was isolated as a white solid (7.159 g, yield 86.3%).

\(^1\)H-NMR (CDCl₃; 300 MHz): δ 8.93 (3H, bs, NH₂Cl), 4.3 (2H, q, \(J=7.1\) Hz, OCH₃), 1.75 (6H, s, [CH₃]₂C), 1.33 (3H, t, \(J=7.1\) Hz, OCH₂CH₃).

\(^1^3\)C-NMR (CDCl₃; 75 MHz): δ 14.4 (CH₃CH₂O), 24.3 ([CH₃]₂C), 57.9 ([CH₃]₂C), 63.1 (OCH₂CH₃), 171.6 (COOCH₂CH₃).

Synthesis of benzyl-2-amino-2-methylpropanoate hydrochloride salt.
C₁₁H₁₆ClNO₂, MW 229.70.

\[
\begin{align*}
\text{HCH₂N} & \quad \text{O} \\
\text{O} & \quad \text{O} \quad \text{Ph}
\end{align*}
\]

This was synthesised according to *Standard Procedure 2*, using 2-amino-isobutyric acid (1.960 g, 19.00 mmol) with \(p\)-toluene sulfonic acid monohydrate (3.750g, 19.7 mmol) and benzylic alcohol (8.360 g, 77.30 mmol, 8 mL), in toluene (20 mL). The product was isolated as a white solid (2.556 g, yield 87.4%)

\(p\)-toluenesulfonate salt: \(^1\)H-NMR (CDCl₃, 300 MHz): δ 8.40 (3H, bs, NH₂Cl), 7.79 (2H, d, \(J=8.0\) Hz, \('m'\) \(p\)-TSA), 7.34 (5H, m, CH₂Ph), 7.14 (2H, d, \(J=8.0\) Hz, \('o'\) \(p\)-TSA), 5.16 (2H, s, CH₂Ph), 2.38 (3H, s, CH₃ \(p\)-TSA), 1.57 (6H, s, [CH₃]₂C)
$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 21.8 (CH$_3$, p-TSA), 23.9 ([CH$_3$]$_2$C), 57.8 ([CH$_3$]$_2$), 68.3 (CH$_2$Ph), 126.55, 128.5, 128.8, 129.0, 129.3 (CH$_2$Ph+p-TSA), 135.4 (‘ipso’, CH$_2$Ph), 140.8 (‘p’, p-TSA), 141.9 (‘ipso’, p-TSA), 171.9 (COOCH$_2$Ph).

**Hydrochloride salt:** $^1$H-NMR (CDCl$_3$; 300 MHz): δ 9.10 (3H, bs, NH$_3$Cl), 7.41-7.31 (5H, m, CH$_2$Ph), 5.27 (2H, s, CH$_2$Ph), 1.77 ([CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 24.2 ([CH$_3$]$_2$C), 58.0 ([CH$_3$]$_2$), 68.5 (CH$_2$Ph), 128.62, 129.0, 129.1 (‘o’, ‘m’, ‘p’, CH$_2$Ph), 135.2 (‘ipso’, CH$_2$Ph), 171.8 (COOCH$_2$Ph).

10 **Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine**

(E)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine

![Chemical structure of (E)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine](image)

15 A mixture of Pd(OAc)$_2$ (0.316 g, 1.41 mmol), PPh$_3$ (0.741 g, 2.82 mmol), and triethylamine (4.9 mL) in 1,4-dioxane (50 mL) was stirred at 70°C until an intense red colour had developed. To this 5-iodo-2'-deoxyuridine (10 g, 28.24 mmol) and methylacrylate (4.862 g, 56.48 mmol, 5.1 mL) in 1,4-dioxane (20 mL) were added and the mixture stirred at refluxed for 30 mins. The reaction was filtered while still hot and the filtrate cooled over night at 4°C. The resulting pale yellow precipitate was filtered, washed with DCM and dried in vacuo to give the product as white solid (6.2 g, yield 70.7%).

$^1$H-NMR (DMSO-$d_6$; 300 MHz) δ 11.64 (1H, bs, NH-3), 8.42 (1H, s, H-6), 7.37 (1H, d, $^3$J=15.8 Hz, H vinylic), 6.86 (1H, d, $^3$J=15.8 Hz, H vinylic), 6.13 (1H, t, $^3$J=6.5 Hz, H-1'), 5.27-5.20 (2H, 2bs, OH-3', OH-5'), 4.27 (1H, m, H-3'), 3.81 (1H, m, H-4'), 3.68 (3H, s, CH$_3$), 3.60 (2H, m, H-5'), 2.18 (2H, m, H-2').

$^{13}$C-NMR (DMSO-$d_6$; 75 MHz): δ 40.4 (C-2'), 51.6 (CH$_3$), 66.7 (C-5'), 70.0 (C-3'), 85.2 (C-4'), 88.0 (C-1'), 108.5 (C-5), 116.5 (C-5b), 138.5 (C-5a), 144.4 (C-6), 149.6, 162.1 (C-2, C-4), 167.6 (COO).
(E)-5-(2-Carboxyvinyl)-2'-deoxyuridine

(E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine (6.0 g, 19.33 mmol) was dissolved in 300 mL of 1 M NaOH and the mixture stirred at room temperature for 3 hrs, filtered and the filtrate adjusted to pH 2 with 1M HCl. On cooling at 4°C a white precipitate formed. This was filtered off and washed with cold water (2x 20 ml) and acetone (2x20 mL) and dried to give a white solid (4.441 g, yield 77.1%).

1H-NMR (DMSO-d6; 300 MHz): δ 12.18 (1H, bs, CO₂H), 11.64 (1H, s, NH-3), 8.40 (1H, s, H-6), 7.30 (1H, d, 3J=15.6 Hz, H vinylic), 6.78 (1H, d, 3J=15.8 Hz, H vinylic), 6.14 (1H, t, 3J=6.4 Hz, H-1'), 5.38 -5.08 (2H, bs, OH-3', OH-5'), 4.26 (1H, m, H-3'), 3.80 (1H, m, H-4'), 3.64 (2H, m, H-5'), 2.18 (2H, m, H-2').

13C-NMR (DMSO-d6; 75 MHz): δ 40.1 (C-2'), 61.2 (C-5'), 70.1 (C-3'), 85.1 (C-4'), 88.0 (C-1'), 108.7 (C-5), 118.0 (C-5b), 137.9 (C-5a), 143.9 (C-6), 149.6, 162.1 (C-2, C-4), 168.4 (COOH).

(E)-5-(2-bromovinyl)-2'-deoxyuridine

To a solution of (E)-5-(2-carboxyvinyl)-2'-deoxyuridine (5.777 g, 19.37 mmol) in dimethylformamide (29 mL) was added K₂CO₃ (5.890 g, 42.61 mmol) and the suspension stirred at room temperature for 15 mins. A solution of N-bromosuccinimide (3.655 g,
20.53 mmol) was added dropwise over 30 mins at 20°C. The resulting suspension was filtered and the solid washed with DMF. The combined filtrate and washings were evaporated to dryness in vacuo and the residue dissolved in MeOH. To this silica gel was added and the suspension evaporated to dryness and the solid applied to the top of chromatographic column. The column was eluted with chloroform/methanol 92:8 to give a white solid (5787g, 71.9%). Crystallisation from water gave a white powder.

$^1$H-NMR (DMSO-d$_6$: 300 MHz) $\delta$ 11.59 (1H, bs, NH-3), 8.08 (1H, s, H-6), 7.25 (1H, d, $^3$J=13.6 Hz, H-5b), 6.85 (1H, d, $^3$J=13.6 Hz, H-5a), 6.13 (1H, t, $^3$J=6.5 Hz, H-1’), 5.29 (1H, bs, OH-3’), 5.13 (1H, bs, OH-5’), 4.24 (1H, m, H-3’), 3.79 (1H, m, H-4’), 3.66 (2H, m, H-5’), 2.51 (1H, m, H-2’), 2.14 (1H, m, H-2’).

$^{13}$C-NMR (DMSO-d$_6$: 75 MHz): $\delta$ 40.2 (C-2’), 61.3 (C-5), 70.3 (C-4’), 84.8 (C-3’), 87.8 (C-1’), 108.9 (C-5b), 110.0 (C-5), 130.3 (C-5a), 149.6, 162.1 (C-2, C4).

15 Synthesis of (E)-5-(2-Bromovinyl)-2’-deoxyuridine-5’-[phenyl-(methoxy-L-alaninyl)]-phosphate (CPF 1).

$C_{21}H_{25}BrN_{3}O_{8}P$, MW 574.32.

![Chemical Structure Image]

This was synthesised according to Standard procedure 5, using BVdU (300 mg, 0.90 mmol), Phenyl-(methoxy-L-alaninyl)-phosphorochloridate (472 mg, 1.7 mmol), NMI (4.5 mmol, 378 µL) in THF (9 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH$_2$Cl$_2$/Methanol 97:3 to give the pure product as a white foamy solid (356 mg, yield 69%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 4.72, 4.40.
Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 3).

C_{22}H_{27}BrN_{3}O_{5}P, MW=588.34.

This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), Phenyl-(ethoxy-L-alaninyl)-phosphorochloridate (249 mg, 0.9 mmol), NMI (2.8 mmol, 190 μL) in THF (4 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH_{2}Cl_{2}/Methanol 97:3 to give the pure product as a white foamy solid (145 mg, yield 55%).
of H-2'), 2.10-2.00 (1H, m, one of H-2'), 1.40 (3H, d, \(^{3}J=7\) Hz, CH\(_2\)ala), 1.25 (3H, 2t, \(^{3}J=7\) Hz, CH\(_2\)CH\(_2\)O).

\(^{13}\)C-NMR (CDCl\(_3\), 75 MHz): \(\delta\) 14.5 (CH\(_3\)CH\(_2\)O) 21.2, 21.1 (CH\(_3\)ala), 40.9,40.7 (C-2'), 50.8, 50.7 (CHala), 62.2, 62.1 (CH\(_3\)CH\(_2\)O), 66.5, 66.3 (C-5'), 70.9, 70.6 (C-3'), 86.0, 85.6 (C-1', C-4'), 110.1 (C-5b), 111.8 (C-5), 120.6 ('o', OPh), 125.0 ('p', OPh), 129.0 (C-5a), 130.2 ('m', OPh), 138.2 (C-6), 149.9 (C-4), 150.7 ('ipso', OPh), 162.3 (C-2), 174.2,174.1 (COOCH\(_2\)CH\(_3\)).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoyl-L-alaninyl)]-phosphate (CPF 2).

C\(_{27}\)H\(_{29}\)BrN\(_{3}\)O\(_{5}\)P, MW=649.08.

This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), Phenyl-(benzylxoy-L-alaninyl)-phosphorochloridate (249 mg, 0.9 mmol), NMI (2.8 mmol, 190 \(\mu\)L) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH\(_2\)Cl\(_2\)/Methanol 97:3 to give the pure product as a white foamy solid (228 mg, yield 78%).

\(^{31}\)P-NMR (CDCl\(_3\), 121 MHz): \(\delta\) 4.74, 4.44.

\(^{1}\)H-NMR (CDCl\(_3\), 300 MHz): \(\delta\) 10.31 (1H, bs, H-3), 7.63 (1H, 2xs, H-6), 7.45-7.14 (11H, m, OPh+CH\(_2\)Ph, H-5b), 6.75-6.66 (1H, 2d, \(^{3}J=14\) Hz, H-5a), 6.30-6.25 (1H, m, H-1'), 5.18-50.9 (1H, s, CH\(_2\)Ph), 4.70-4.04 (6H, m, H-3', H-5', H-4', N\(^{\text{H}}\), CHala), 2.42 (1H, m, one of H-2'), 2.02 (1H, m, one of H-2'), 1.40 (3H, d, \(^{3}J=7\) Hz, CH\(_3\)ala).

\(^{13}\)C-NMR (CDCl\(_3\), 75 MHz): \(\delta\) 20.7, 20.8 (CH\(_3\)ala), 40.4 (C-2'), 50.4 (CHala), 66.0 (C-5'), 67.4 (CH\(_2\)Ph), 70.6 (C-3'), 85.4, 85.5, 85.6, 85.8 (C-1', C-4'), 109.9 (C-5b), 111.5 (C-5b),
120.2 ('o', OPh), 125.4 ('p', OPh), 128.5, 128.6, 129.9 ('m' OPh, Bn, C-5a), 135.1 ('ipso', CH₂Ph) 137.8 (C-6), 149.8 (C-4) 150.2 ('ipso', OPh), 161.8 (C-2), 173.6 (COOBn).

5 Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(methoxy-L-alaninyl)]-phosphate (CPF 5).

C₂₁H₂₄BrF₃O₅P, MW=592.31.

This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), para-fluorophenyl-(methoxy-L-alaninyl)-phosphorochloridate (442 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (177 mg, yield 50%).

³¹P-NMR (CDCl₃, 121 MHz): δ 5.10, 4.81.

¹H-NMR (CDCl₃; 300 MHz): δ 10.1 (1H, bs, H-3), 7.60 (1H, 2xs, H-6), 7.39-7.32 (1H, 2d, ³J=14 Hz, H-5b), 7.20-6.95 (4H, m, OPh), 6.70-6.60 (1H, 2d, ³J=14 Hz, H-5a), 6.30-6.15 (1H, 2t, ³J=6 Hz, H1’), 4.55-4.29 (3H, m, H-5’+H-3’), 4.15 (1H, NH), 4.05-3.85 (2H, H-4’, CHala), 3.72 (3H, 2s, CH₃O), 2.49-2.32 (1H, m, one of H-2’), 2.15-2.05 (1H, m, one of H-2’), 1.35 (3H, 2d, ³J=6 Hz, CH₃ ala).

¹³C-NMR (DMSO; 75 MHz): δ 21.2 (CH₃ ala), 40.8 (C-2’), 50.8, 50.6 (CH(CH₃)), 53.2 (CH₃O), 66.7, 66.3 (C-5’), 71.9, 71.8 (C-3’), 86.1, 85.7, 85.8 (C-1’, C-4’), 110.3 (C-5b), 111.9 (C-5), 117.0, 116.7 ('o', OPh), 122.0 ('m', OPh), 128.2 (C-5a), 138.2 (C-6), 149.0 ('ipso', OPh) 149.9 (C-4), 158.5 ('p', OPh), 163.2(C-2), 175.1 (COOCH₃).
Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 6).

C$_{22}$H$_{28}$BrF$_{2}$N$_{3}$O$_{9}$P, MW=606.33.

This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), para-fluorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate (464 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH$_{2}$Cl$_{2}$/Methanol 97:3 to give the pure product as a white foamy solid (240 mg, yield 66%).

$^{31}$P-NMR (CDCl$_{3}$, 121 MHz): δ 5.14, 4.88.

$^{1}$H-NMR (CDCl$_{3}$, 300 MHz): δ 10.25 (1H, bs, H-3), 7.85 (1H, 2xs, H-6), 7.44-7.39 (1H, 2d, $^3$J=14 Hz, H-5b), 7.3-7.0 (4H, m, OPh), 6.8-6.65 (1H, 2d, $^3$J=14 Hz, H-5a), 6.35-6.25 (1H, 2t, $^3$J=6 Hz, H1'), 4.6-4.1 (6H, m, H-5', H-3', CHala, NH, CH$_{2}$CH$_{2}$O), 4.02 (1H, m, H-4'), 2.55-2.45 (1H, m, one of H-2'), 2.20-2.10 (1H, m, one of H-2'), 1.40 (3H, d, $^3$J=8 Hz, CH$_{2}$ala), 1.25 (3H, 2t, $^3$J=7 Hz, CH$_{2}$CH$_{2}$O).

$^{13}$C-NMR (CDCl$_{3}$, 75 MHz): δ 14.5 (CH$_{3}$CH$_{2}$O) 21.3 (CH$_{3}$ala), 40.8,40.7 (C-2'), 50.8, 50.7 (CHala), 62.3 (CH$_{3}$CH$_{2}$O), 66.7, 66.3 (C-5'), 71.1, 70.7 (C-3'), 86.1, 85.8, 85.6, 85.4 (C-1', C-4'), 110.4 (C-5b), 111.9 (C-5), 117.0 ('o', OPh), 122.2 ('m', OPh), 128.9 (C-5a), 138.2 (C-6), 146.4 ('ipso', OPh), 149.9 (C-4), 158.5 ('p', OPh), 162.2, 161.8 (C-2), 174.2 (COOCH$_{2}$CH$_{3}$).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(benzoxyl-L-alaninyl)]-phosphate (CPF 7).

C$_{27}$H$_{28}$BrF$_{2}$N$_{3}$O$_{9}$P, MW=668.40.
This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), para-fluorophenyl-(benzylxoy-L-alaninyl)-phosphorochloridate (556 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH2Cl2/Methanol 97:3 to give the pure product as a white foamy solid (256 mg, yield 64%).

$^{31}$P-NMR (CDCl3, 121 MHz): δ 4.74, 4.44.

$^1$H-NMR (CDCl3, 300 MHz): δ 7.69 (1H, 2xs, H-6), 7.45-7.39 (1H, 2d, $^3$J=14 Hz, H-5b), 7.37-7.00 (9H, m, OPh+CH2Ph), 6.75-6.65 (1H, 2d, $^3$J=14 Hz, H-5a), 6.30-6.2 (1H, 2t, $^3$J=6Hz, H-1'), 5.2 (1H, 2s, CH2Ph), 4.85-4.00 (6H, m, H-3',H-5',H-4', NH2, CHala), 2.47 (1H, m, one of H-2'), 2.0-2.15 (1H, m, one of H-2'), 1.38 (3H, d, $^3$J=7 Hz, CH3ala).

$^{13}$C-NMR (CDCl3, 75 MHz): δ 21.2, 21.1 (CH3ala), 40.7 (C-2'), 50.4 (CHala), 66.7, 66.4 (C-5'), 67.8 (CH2Ph), 71.1, 70.7 (C-3'), 86.0, 85.7, 85.4, 85.3 (C-1', C-4'), 110.4 (C-5b), 111.9 (C-5), 117.0 ('o', OPh), 122.0 ('m', OPh), 128.7, 128.6 (Bn, C-5a), 135.4 ('ipso', CH2Ph) 138.2 (C-6), 146.5 ('ipso', OPh), 149.9 (C-4), 158.5 ('p' OPh), 162.2 (C-2), 173.9 (COOBn).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-nitrophenyl-(methoxy-L-alaninyl))-phosphate (CPF 10).

C$_{21}$H$_{24}$BrN$_4$O$_{11}$P, MW=619.31.
This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), para-nitrophenyl-(methoxy-L-alaninyl)-phosphorochloridate (483 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (211 mg, yield 57%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.95.

¹H-NMR (MeOD; 300 MHz): δ 8.3-8.2 (2H, m, OPh) 7.8-7.75 (1H, 2xs, H-6), 7.35-7.30, 7.55-7.4 (2H, m, OPh), 7.35-7.30 (1H, 2d, ³J=14 Hz, H-5b), 6.80-6.70 (1H, 2d, ³J=14 Hz, H-5a), 6.30-6.2 (1H, 2t, ³J=6 Hz, H1'), 4.5-4.3 (3H, m, H-5',H-3'), 4.2-4.0 (2H, m, H-4', CHala), 3.72 (3H, 2s, CH₃O), 2.35-2.15 (2H, m, 2 H-2'), 1.35 (3H, 2d, ³J=7Hz, CH₃_ab).

¹³C-NMR (DMSO; 75 MHz): δ 20.9 (CH₃_ab), 41.6, 41.5 (C-2'), 52.0, 51.9 (CH[CH₃]), 53.4 (CH₃O), 68.5 (C-5'), 72.4, 72.3 (C-3'), 87.7, 87.4, 87.0, 86.9 (C-1', C-4'), 109.8 (C-5b), 112.8 (C-5), 122.6 ('o', OPh), 127.1 ('m', OPh), 130.8 (C-5a), 140.3 (C-6), 146.5 ('ipso', OPh), 151.4 (C-4), 157.2 ('p', OPh), 163.9 (C-2), 175.8,175.5 (COOCH₃).

*Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-nitrophenyl-(ethoxy-L-alaninyl))-phosphate (CPF 9).*

C₂₂H₂₆BrN₄O₁₁P, MW=633.34.
This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), para-nitrophenyl-(ethoxy-L-alaniny1)-phosphorochloridate (504 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (5 mL) for 1 hr. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (232 mg, yield: 61%).

1³P-NMR (CDCl₃, 121 MHz): δ 4.28.

1H-NMR (CDCl₃, 300 MHz): δ 10.25 (1H, bs, H-3), 8.25-8.2 (2H, 2d, J=9Hz OPh), 7.7 (1H, 2xs, H-6), 7.5-7.45 (2H, 2d, J=9Hz, OPh), 7.4-7.35 (1H, 2d, J=14 Hz, H-5b), 6.7-6.65 (1H, 2d, J=14 Hz, H-5a), 6.3-6.2 (1H, 2t, J=6 Hz, H1’), 4.8-4.1 (7H, m, H-5’, H-4’ H-3’, CHala, NH, CH₃CH₂O), 2.45-2.4 (1H, m, one of H-2’), 2.20-2.10 (1H, m, one of H-2’), 1.40 (3H, d, J=8 Hz, CH₂ab), 1.3 (3H, 2t, J=7 Hz, CH₂CH₂O).

13C-NMR (CDCl₃, 75 MHz): δ 14.5 (CH₃CH₂O) 21.1 (CHala), 40.6 (C-2’), 50.8, 50.7 (CHala), 62.5 (CH₃CH₂O), 66.9, 66.8 (C-5’), 71.2, 70.9 (C-3’), 86.3, 85.9, 85.4, 85.3 (C-1’, C-4’), 110.3 (C-5b), 111.8 (C-5), 121.3 (‘o’, OPh), 126.1 (‘m’, OPh), 128.8 (C-5a), 138.4 (C-6), 145.1 (‘ipso’, OPh), 149.9 (C-4), 155.5 (‘p’, OPh), 162.3 (C-2), 174.0, 173.9 (COOCH₂CH₃).

Synthesis of (E)-5-(2-Bromovinyl)-2’-deoxyuridine-5’-[para-nitrophenyl-(benzoyxy-L-alaniny1)]-phosphate (CPF 8).

C₂₇H₂₈BrN₄O₁₁P, MW=695.41.
This was synthesised according to \textit{Standard procedure 5}, using BVdU (200 mg, 0.60 mmol), para-nitrophenyl-(benzyloxy-L-alaninyl)-phosphorochloridate (597 mg, 1.5 mmol), NMI (4.98 mmol, 332 \(\mu\)L) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with \(\text{CH}_2\text{Cl}_2\)/Methanol 97:3 to give the pure product as a white foamy solid (228 mg, yield 55%).

\(^{31}\text{P}\)-NMR (CDCl\(_3\), 121 MHz): \(\delta\) 4.74, 4.44.

\(^1\text{H}\)-NMR (CDCl\(_3\), 300 MHz): \(\delta\) 10.4-10.3 (1H, bs, H-3), 8.2-8.1 (2H, m, OPh), 7.69 (1H, 2xs, H-6), 7.4-7.2 (1H, 2d, \(^3\)J=14 Hz, H-5b), 7.37-7.00 (7H, m, OPh+CH\(_2\)Ph), 6.75-6.65 (1H, 2d, \(^3\)J=14 Hz, H-5a), 6.25-6.15 (1H, 2t, \(^3\)J=6Hz, H-1'), 5.2 (1H, d, CH\(_2\)Ph), 4.87 (1H, m, H-3'), 4.6-4.2 (3H, m, H-5', CHala) 4.2-4.00 (2H, m, H-4', NH\(_3\)), 2.55-2.45 (1H, m, one of H-2'), 2.2-2.05 (1H, m, one of H-2'), 1.38 (3H, d, \(^3\)J=7 Hz, CH\(_3\)ala).

\(^1\text{C}\)-NMR (CDCl\(_3\), 75 MHz): \(\delta\) 21.2, 21.1 (CH\(_3\)ala), 40.6 (C-2'), 50.9 (CHala), 67.1, 67.0 (C-5'), 68.0 (CH\(_2\)Ph), 71.3, 70.9 (C-3'), 86.3, 86.0, 85.3, 85.2 (C-1', C-4'), 110.4 (C-5b), 111.9, 111.8 (C-5), 121.3 ('o', OPh), 126.2-126.1 ('m', OPh), 129.1, 128.7, 128.6 (Bn, C-5a), 135.4 ('ipso', CH\(_2\)Ph), 138.3 (C-6), 145.1 ('ipso', OPh), 149.9 (C-4), 155.6 ('p' OPh), 162.2 (C-2), 173.8,173.7 (COOBn).

\(\text{Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'}-[\text{para-(trifluoromethyl)phenyl-}(\text{methoxy-L-alaninyl})]-\text{phosphate (CPF 15).}

\text{C}_{22}\text{H}_{24}\text{BrF}_3\text{N}_3\text{O}_9, \text{MW}=642.31.}
This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl-(methoxy-L-alaninyl)-phosphorochloridate (518.8 mg, 1.5 mmol), NMI (246.3 mg, 3.0 mmol, 239 µL) in THF (5 mL) for 4 hrs. The crude product was purified by column chromatography, eluting with chloroform/methanol 97:3 to give the pure product as a white foamy solid (211.1 mg, yield 54.7%).

$^{31}$P-NMR (MeOD, 121 MHz): δ 5.23, 5.07.

$^1$H-NMR (MeOD, 300 MHz): δ 7.80 (1H, s, H-6), 7.70 (2H, d, $^3$J=8.7 Hz, OPh), 7.47-7.42 (2H, m, OPh), 7.37 (1H, d, $^3$J=13.6 Hz, H-5b), 6.82-6.78 (1H, d, $^3$J=13.6 Hz, H-5a), 6.30-6.23 (1H, m, H-1'), 4.52-4.29 (3H, m, H-3'+H-5'), 4.17-4.13 (1H, m, H-4'), 4.05-3.91 (1H, m, CHCH$_3$), 3.67 (3H, s, OCH$_3$), 2.35-2.32 (1H, m, one of H-2'), 2.23-2.16 (1H, m, one of H-2'), 1.37-1.34 (3H, d, $^3$J=7.1 Hz, CHCH$_3$).

$^{13}$C-NMR (MeOD, 75 MHz): δ 20.6, 20.7, 20.8, 20.9 (CHCH$_3$), 41.5, 41.7 (C-2'), 51.9, 52.0 (CHCH$_3$), 68.2, 68.3 (C-5'), 72.4, 72.5 (C-3'), 87.1, 87.2, 87.4, 87.6 (C-1', C-4'), 109.7 (C-5b), 112.6 (C-5), 122.5, 122.7 ('o', OPh), 125.8 (CF$_3$, $^3$J=269 Hz), 128.7 ('m', OPh), 128.8 ('p', $^3$J=33 Hz, OPh), 130.9 (C-5a), 140.3 (C-6), 151.4, 151.5 ('ipso', OPh), 155.1, 155.2 (C-4), 164.0 (C-2), 175.6, 175.9, (COOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[para-(trifluoromethyl)-phenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 25).

C$_{23}$H$_{26}$BrF$_3$N$_3$O$_9$P, MW=656.34
This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), phenyl-(ethoxy-L-alaninyl)-phosphorochloridate (539.5 mg, 1.5 mmol), NMI (246.3 mg, 3.0 mmol, 239 µL) in THF (5 mL) for 20 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 95:5 to give the pure product as a white foamy solid (172.6 mg, yield 43.8%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 4.65, 4.35.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 10.05 (1H, s, H-3), 7.69-7.64 (3H, m, H-6+OPh), 7.46-7.39 (3H, m, OPPh+ H-5b), 6.76-6.68 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.34-6.25 (1H, m, H-1'), 4.57-4.35 (4H, m, H-3'+H-5'+NH$_2$), 4.27-4.13 (4H, m, H-4'+OCH$_2$CH$_3$+OH-3'), 4.12-3.98 (1H, m, CHCH$_3$), 2.53-2.47 (1H, m, one of H-2'), 2.21-2.12 (1H, m, one of H-2'), 1.43-1.40 (3H, d, $^3$J=7.0 Hz, CHCH$_3$), 1.28, 1.27 (3H, 2t, $^3$J=7.0 Hz, OCH$_2$CH$_3$)

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 14.5 (CH$_3$CH$_2$O), 21.2, 21.3 (CHCH$_3$), 40.7 (C-2'), 50.8, 50.9 (CHCH$_3$), 62.4 (CH$_2$CH$_2$O), 66.3, 66.7 (C-5'), 70.7, 71.1 (C-3'), 85.3, 85.4, 85.8, 86.1 (C-1', C-4'), 110.5 (C-5b), 112.0 (C-5), 122.0 ('O', OPPh), 124.2 (CF$_3$, $^3$J=271 Hz), 127.7, 127.8, 128.7 ('m', 'p', OPPh), 128.8 (C-5a), 138.0 (C6), 149.7 ('ipso', OPPh), 153.2 (C-4), 161.9 (C-2), 174.0, 174.1 (COOCH$_2$CH$_3$).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-trifluorophenyl-(benzoxyl-L-alaninyl))-phosphate (CPF 4).

$C_{28}H_{26}BrF_{3}N_{3}O_{9}$P, MW=718.41.
This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), para-trifluorophenyl-(benzoxyl-L-alaninyl)-phosphorochloridate (632 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (6 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH$_2$Cl$_2$/Methanol 97:3 to give the pure product as a white foamy solid (308 mg, yield 71%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 5.31, 4.87.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 10.05 (1H, bs, H-3), 7.7, 7.25 (11H, m, H-5b, H-6 OPh+CH$_2$Ph), 6.75-6.65 (1H, 2d, $^3$J=14 Hz, H-5a), 6.35-6.2 (1H, 2t, $^3$J=6Hz, H-1'), 5.15 (1H, 2s, CH$_2$Ph), 4.6-4.25 (4H, m, H-5', H-3', CHala) 4.2-4.00 (2H, m, H-4', NH$_2$), 2.55-2.4 (1H, m, one of H-2'), 2.2-2.05 (1H, m, one of H-2'), 1.38 (3H, d, $^3$J=7 Hz, CH$_3$ala).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 21.2, 21.1 (CH$_3$ala), 40.7 (C-2'), 50.9, 50.8 (CHala), 67.1, 67.0 (C-5'), 68.0 (CH$_2$Ph), 71.2, 70.9 (C-3'), 86.1, 85.8, 85.5, 85.4 (C-1', C-4'), 110.2 (C-5b), 111.9, 111.8 (C-5), 121.1 ('o', OPh), 125.1 (d, J=270Hz, CF$_3$), 127.6 ('m', OPh), 129.1, 128.7, 128.6 (Bn, C-5a), 130.1 ('p', q, J=32Hz, OPh) 135.4 ('ipso', CH$_2$Ph) 138.2 (C-6), 150.2, 150.1 (C-4), 153.6 ('ipso' OPh), 162.7 (C-2), 173.9, 173.6 (COOBn).

**Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(methoxy-L-alaninyl)]-phosphate (CPF 13).**

C$_{21}$H$_{24}$BrClN$_3$O$_9$P, MW=608.76.
This was synthesised according to **Standard procedure 5**, using BVdU (200 mg, 0.60 mmol), 4-chlorophenyl-(methoxy-L-alaninyl)-phosphorochloridate (374.5 mg, 1.2 mmol), NMI (246.3 mg, 3.0 mmol, 239 μL) in THF (8 mL) for 5 hrs. The crude product was purified by column chromatography, eluting with Chloroform/Methanol 97:3 to give the pure product as a white foamy solid (139.0 mg, yield 38.0%).

^{31}P-NMR (CDCl₃, 121 MHz): δ 4.81, 4.54.

^{1}H-NMR (CDCl₃, 300 MHz): δ 10.11 (1H, bs, H-3), 7.68 (1H, s, H-6), 7.46-7.40 (1H, d, J=13.6 Hz, H-5b), 7.35-7.20 (4H, m, OPh), 6.76-6.67 (1H, 2d, J=13.6 Hz, H-5a), 6.34-6.24 (1H, m, H-1'), 4.58-4.40 (5H, m, H-3'+H-5'+NHz), 4.36-4.19 (1H, m, H-4'), 4.07-3.99 (1H, m, CH(CH₃)), 3.75 (3H, s, OCH₃), 2.49-2.48 (1H, m, one of H-2'), 2.17-2.15 (1H, m, one of H-2'), 1.42-1.39 (3H, d, J=7.0 Hz, CHCH₃).

^{13}C-NMR (CDCl₃, 75 MHz): δ 21.2 (CH(CH₃)), 40.7, 40.8 (C-2'), 50.6, 50.8 (CHCH₃), 53.2, 53.3 (OCH₃), 66.4, 66.7 (C-5'), 70.8, 71.2 (C-3'), 85.4, 85.5, 85.8, 86.2 (C-1', C-4'), 110.5 (C-5b), 111.9, 112.0 (C-5), 122.0 (‘o’, OPh), 128.9 (C-5a), 130.3 (‘m’, OPh), 131.1 (‘p’, OPh), 138.2 (C-6), 149.1, 149.2 (‘ipsos’, OPh), 149.8 (C-4), 162.1, 162.2 (C-2), 174.5, 174.6 (COOCH₃).

**Synthesis of (E)-5-(2-bromovinyl)-2’-deoxyuridine-5’-[4-chlorophenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 11).**

C_{22}H_{26}BrN_{3}O_{9}P, MW=622.79.
This was synthesised according to *Standard procedure 5*, using BVdU (300 mg, 0.90 mmol), 4-chlorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate (557.7 mg, 1.71 mmol), NMl (221.7 mg, 2.7 mmol, 215 μL) in THF (10 mL) for 16 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (168.4 mg, yield 30.0%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 4.88, 4.65.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 9.51 (1H, bs, H-3), 7.69-7.68 (1H, 2s, H-6), 7.49-7.43 (1H, 2d, $^3$J=13.6 Hz, H-5b), 7.37-7.22 (4H, m, OPh), 6.79-6.71 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.33-6.24 (1H, m, H-1'), 4.62-4.34 (3H, m, H-3'+H-5'), 4.28-3.89 (5H, m, H-4'+OCH$_2$CH$_3$+CH$_3$+NH), 2.59-2.45 (1H, m, one of H-2'), 2.22-2.14 (1H, m, one of H-2'), 1.43-1.41 (3H, d, $^3$J=7.0 Hz, CH$_2$OH), 1.33-1.28 (3H, 2t, $^3$J=7.2 Hz, OCH$_2$CH$_3$)

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 14.5 (CH$_3$CH$_2$O), 21.2, 21.3 (CH$_2$CH$_3$), 40.7 (C-2'), 50.7, 50.8 (CHCH$_3$), 62.4 (CH$_3$CH$_2$O), 66.7 (C-5'), 70.8, 71.2 (C-3'), 85.4, 85.8, 86.1 (C-1', C-4'), 110.4 (C-5b), 112.0 (C-5), 122.0, 122.1 ('o', OPh), 128.9 (C-5a), 130.3 ('m', OPh), 131.1 ('p', OPh), 138.2 (C-6), 149.2 ('ipso', OPh), 150.0 (C-4), 162.2 (C-2), 174.1, 174.2 (COOCH$_2$CH$_3$).

**Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-(4-chlorophenyl-(benzoxyl-L-alaninyl)]-phosphate (CPF 12).**

$C_{22}H_{26}BrN_{3}O_{9}P$, MW=622.79.
This was synthesised according to Standard procedure 5, using BVdU (300 mg, 0.90 mmol), 4-chlorophenyl-(benzoxyl-L-alaninyl)-phosphorochloridate (698.7 mg, 1.80 mmol), NMI (369.5 mg, 4.5 mmol, 358.7 μL) in THF (10 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 95:5 to give the pure product as a white foamy solid (310.0 mg, yield 50.3%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 4.81, 4.53.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 10.10 (1H, bs, H-3), 7.65-7.63 (1H, 2s, H-6), 7.69-7.68 (1H, 2s, H-6), 7.46, 7.41 (1H, 2d, $^3$J=13.6 Hz, H-5b), 7.40-7.17 (9H, m, OPh), 6.75-6.66 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.33-6.23 (1H, 2t, $^3$J=6.0 Hz, H-1'), 5.17 (2H, s, CH$_2$Ph), 4.60-4.23 (4H, m, H-3'+H-5'+NH), 4.20-3.97 (2H, m, H-4'+CH$_2$H), 2.48-2.44 (1H, m, one of H-2'), 2.15-2.05 (1H, m, one of H-2') 1.43-1.40 (3H, d, $^3$J=7.0 Hz, CH$_2$H$_3$).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 21.2 (CH$_2$H$_3$), 40.7 (C-2'), 50.8, 50.9 (CH$_2$H$_3$), 66.6 (C-5'), 67.9 (CH$_2$Ph), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.8, 86.1 (C-1', C-4'), 110.5 (C-5b), 111.9, 112.0 (C-5), 122.0 (O', OPh), 128.7, 129.0, 129.1, 130.3 ('m', OPh+C-5a), 131.1 ('ipso', CH$_2$Ph), 135.4 ('p', OPh), 138.2 (C-6), 149.1 ('ipso', OPh), 150.0 (C-4), 162.1 (C-2), 173.9, 174.0 (COOCH$_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-(phenyl-(methoxyl-α,α-dimethylglycineyl)]-phosphate (CPF 26).

C$_{22}$H$_{27}$BrN$_3$O$_9$P, MW 588.34
This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate (437.5 mg, 1.5 mmol), NMI (246.3 mg, 3.0 mmol, 239.1 μL) in THF (5 mL) for 4 hrs. The crude product was purified by column chromatography, eluting with chloroform/methanol 97:3 to give the pure product as a white foamy solid (117 mg, yield 33.1%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 3.36, 3.14

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 9.91 (1H, bs, H-3), 7.73, 7.65 (1H, 2s, H-6), 7.50-7.43 (1H, 2d, $^3J$=13.6 Hz, H-5b), 7.41-7.02 (5H, m, OPh), 6.81-6.71 (1H, 2d, $^3J$=13.6 Hz, H-5a), 6.34-6.28 (1H, m, H1'), 4.55-4.17 (6H, m, H-5'+H-4'+H3', NH, OH-3'), 3.78 (3H, s, CH$_3$O), 2.53-2.39 (1H, m, one of H-2'), 2.25-1.99 (1H, m, one of H-2'), 1.60 (6H, s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 27.5, 27.4, 27.2 ([CH$_3$]$_2$C), 40.7, 40.6 (C-2'), 53.5 ([CH$_3$]O), 57.6 ([CH$_3$]$_2$), 66.5, 66.2 (C-5'), 70.7, 71.1 (C-3'), 85.4, 85.6, 85.5, 85.9 (C-1', C-4'), 110.4 (C-5b), 111.9 (C-5), 120.5, 120.6 ('o', OPh), 125.7 ('p', OPh), 128.9 (C-5a), 130.3 ('m', OPh), 138.0, 138.3 (C-6), 149.8 ('ipso', OPh) 150.9, 150.8 (C-4), 162.0, 162.1 (C-2), 176.4, 176.2 (COOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(ethoxy-α,α-dimethylglycycinyl)]-phosphate (CPF 27).

C$_{23}$H$_{25}$BrN$_3$O$_9$P, MW=602.37
This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), phenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate (458.0 mg, 1.5 mmol), NMI (246.3 mg, 3.0 mmol, 239.1 μL) in THF (5 mL) for 5 hrs. The crude product was purified by column chromatography, eluting with chloroform/methanol 97:3 to give the pure product as a white foamy solid (106 mg, yield 29.3%).

$^{31}$P-NMR (MeOD, 121 MHz): δ 3.91, 3.85

$^1$H-NMR (MeOD, 300 MHz): δ 7.84, 7.81 (1H, 2s, H-6), 7.44-7.20 (6H, m, OPh+H-5b), 6.88-6.81 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.34-6.28 (1H, m, H-1’), 4.50-4.34 (3H, m, H-5’+H-3’), 4.23-4.15 (3H, m, H-4’+CH$_3$CH$_2$O), 2.38-2.28 (1H, m, one of H-2’), 2.22-2.09 (1H, m, one of H-2’), 1.51 (6H, s, [CH$_3$]$_2$C), 1.29 (3H, t, $^3$J=7 Hz, CH$_3$CH$_2$O)

$^{13}$C-NMR (MeOD, 75 MHz): δ 14.9 (CH$_3$CH$_2$O) 27.9, 28.3 ([CH$_3$]$_2$C), 41.5 (C-2’), 58.51 ([CH$_3$]$_2$), 63.1 (CH$_3$CH$_2$O), 68.2 (C-5’), 72.6 (C-3’), 87.1, 87.4 (C-1’, C-4’), 109.6 (C-5b), 112.7 (C-5b), 122.0, 122.1, 122.2, (‘ο’, OPh), 126.7 (‘p’, OPh), 131.0, 131.2 (C-5a, ‘m’ OPh), 140.4 (C-6), 151.4 (‘ipso’, OPh) 152.5 (C-4), 164.0 (C-2), 177.2 (COOCH$_2$CH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5’-[phenyl-(benzoyl-α,α-dimethylglycinyl)]-phosphate (CPF 14).

C$_{28}$H$_{31}$BrN$_3$O$_9$P, MW=664.44.
This was synthesised according to Standard procedure 5, using BVdU (242 mg, 0.73 mmol), phenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate (533.0 mg, 2.0 mmol), NMI (298.0 mg, 3.63 mmol, 289 μL) in THF (5 mL) for 4 hrs. The crude product was purified by column chromatography, eluting with chloroform/methanol 97:3 to give the pure product as a white foamy solid (129.0 mg, yield 26.7%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 3.39, 3.12.
$^1$H-NMR (CDCl$_3$, 300 MHz): δ 9.92 (1H, bs, H-3), 7.67-7.60 (1H, 2s, H-6), 7.48-7.41 (1H, 2d, $^3$J=13.6 Hz, H-5b), 7.40-7.16 (10H, m, OPh+CH$_2$Ph), 6.78-6.67 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.31-6.25 (1H, m, H-1'), 5.18 (1H, s, CH$_2$Ph), 4.50-4.09 (6H, m, H-3'+H-5'+H-4', NH, OH-3'), 2.48-2.25 (1H, m, one of H-2'), 2.16-1.82 (1H, m, one of H-2'), 1.60 (6H, s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 27.3, 27.4, 28.5 ([CH$_3$]$_2$C), 40.6, 40.7 (C-2'), 57.6, 57.6 (C[CH$_3$]$_2$), 66.2, 66.5 (C-5'), 68.1 (CH$_2$Ph), 70.6, 71.1 (C-3'), 85.4, 85.5, 85.6, 85.8 (C-1', C-4'), 110.4 (C-5b), 112.0 (C-5), 120.4, 120.5, 120.6, 125.7, 128.4, 128.5, 128.8, 128.9, 130.3 (OPh, C-5a), 135.7 ('ipso', CH$_2$Ph) 138.1, 138.3 (C-6), 149.8, 150.8, 150.9 ('ipso' OPh, C-4), 162.1 (C-2), 177.5, 175.7 (COOCH$_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(methoxy-α,α-dimethylglycyl)]-phosphate (CPF 45).

C$_{22}$H$_{26}$BrN$_4$O$_{11}$P, MW=633.34.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), 4-nitrophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate (378.8 mg, 1.13 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 µL) in THF (5 mL) for 3 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (145.7 mg, yield 50.9 %).

$^{31}$P-NMR (MeOD, 121 MHz): δ 3.61, 3.56.

$^1$H-NMR (MeOD, 300 MHz): δ 8.30-8.25 (2H, 2d, $^3$J=9.0 Hz, OPh), 7.79-7.78 (1H, 2s, H-6), 7.49-7.46 (2H, d, $^3$J=9.0 Hz, OPh), 7.37-7.32 (1H, 2d, $^3$J=13.6 Hz, H-5b), 6.79-6.72 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.32-6.25 (1H, m, H-1’), 4.48-4.35 (3H, m, H-3’+H-5’), 4.15-4.14 (1H, m, H-4’), 3.71 (3H, s, CH$_3$O), 2.41-2.17 (2H, m, H-2’), 1.51 (6H, s, [CH$_3$)$_2$C].

$^3$C-NMR (CDCl$_3$, 75 MHz): δ 28.0, 28.1, 28.2, 28.3 ([CH$_3$)$_2$C], 41.4, 41.5 (C-2’), 53.6 (CH$_3$O), 58.7 ([CH$_3$)$_2$C], 68.5 (C-5’), 72.3, 72.4 (C-3’), 86.9, 87.0, 87.4, 87.5 (C-1’, C-4’), 109.7 (C-5b), 112.6 (C-5), 122.8, 122.9 (‘o’, OPh), 127.0 (‘m’, OPh), 130.9 (C-5a), 140.5 (C-6), 146.5 (‘p’, OPh), 151.5 (‘ipso’, OPh), 157.3 (C-4), 164.0 (C-2), 177.5 (COOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(ethoxy-α,α-dimethylglycylinyl)]-phosphate (CPF 46).

C$_{23}$H$_{28}$BrN$_4$O$_{11}$P, MW=647.3.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), 4-nitrophenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate (442.1 mg, 1.26 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 µL) in THF (5 mL) for 4 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (152.9 mg, yield 52.5 %).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 3.00, 2.96.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 10.28 (1H, bs, H-3), 8.25-8.12 (2H, 2d, $^3$J=9.0 Hz, OPh), 7.68-7.67 (1H, 2s, H-6), 7.46-7.32 (3H, m, OPh+H-5b), 6.69-6.67 (1H, 2d, $^3$J=13.5 Hz, H-5a), 6.32-6.26 (1H, m, H-1'), 4.75-4.36 (5H, m, H-3'+H-5'+OH-3'+NH$_2$), 4.25-4.17 (3H, m, OCH$_2$CH$_3$, H-4'), 2.60-2.98 (1H, m, one of H-2'), 2.31-2.10 (1H, m, one of H-2'), 1.58 (6H, s, [CH$_3$]$_2$C), 1.30-1.28 (3H, 2t, $^3$J=7.1 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 14.5 (CH$_3$CH$_2$O), 27.1, 27.2, 27.3, 27.4 ([CH$_3$]$_2$C), 40.6 (C-2'), 57.7 ([CH$_3$]$_2$), 62.7 (CH$_3$CH$_2$O), 67.0 (C-5'), 71.0, 71.2 (C-3'), 85.4, 85.9, 86.1 (C-1', C-4'), 110.3 (C-5b), 111.9 (C-5), 121.2, 121.3 ('o', OPh), 126.2 ('m', OPh), 128.8 (C-5a), 138.4 (C-6), 145.0 ('p', OPh), 150.0 (C-4), 155.7-155.9 ('ipso', OPh), 162.2 (C-2), 175.0-175.1 (COOCH$_2$CH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-(4-nitrophenyl-(benzoxyl-$\alpha$,-$\alpha$-dimethylglycinyl)]-phosphate (CPF 47).

C$_{28}$H$_{50}$Br$_4$N$_4$O$_{11}$P, MW=709.44
This was synthesised according to **Standard procedure 5**, using BVdU (100 mg, 0.30 mmol), 4-nitrophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate (309.6 mg, 1.07 mmol), NMI (123.7 mg, 1.5 mmol, 120.1 μL) in THF (5 mL) for 5 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (160.2 mg, yield 50.2 %).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 2.95, 2.89.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 10.16 (1H, bs, H-3), 8.26-8.24 (2H, 2d, $^3J=9.1$ Hz, OPh), 7.71-7.69 (1H, 2s, H-6), 7.48-7.37 (8H, m, OPh+CH$_2$Ph, H-5b), 6.75-6.72 (1H, 2d, $^3J=13.5$ Hz, H-5a), 6.36-6.29 (1H, m, H-1'), 5.24 (2H, s, CH$_3$Ph), 4.81-4.40 (5H, m, H-3'+H-5'+OH-3', NH), 4.22-4.21 (1H, m, H-4'), 2.57-2.36 (1H, m, one of H-2'), 2.27-2.22 (1H, m, one of H-2'), 1.64 (6H, s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 27.4 ([CH$_3$]$_2$C), 40.6 (C-2'), 57.8 (C[CH$_3$]$_2$), 67.0 (C-5'), 68.2 (CH$_2$Ph), 71.1, 71.2 (C-3'), 85.3, 86.2 (C-1', C-4'), 110.5 (C-5b), 111.9 (C-5), 121.2, 126.2, 128.5, 128.8, 129.0, 129.1 ('o', 'm', 'p', CH$_2$Ph+OPh+C-5a), 135.5 ('ipso', CH$_2$Ph), (C-5a), 138.4 (C-6), 145.0 ('p', OPh), 150.0 (C-4), 155.7 ('ipso', OPh), 162.2 (C-2), 175.4-175.5 (COOCH$_2$Ph).

*Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(methoxy-α,α-dimethylglycincyl)]-phosphate (CPF 42).*

C$_{22}$H$_{26}$BrClN$_3$O$_6$P, MW=622.79.
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate (440.2 mg, 1.35 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 µL) in THF (5 mL) for 6 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (146.7 mg, yield 56.5%).

$^{31}$P-NMR (MeOD, 121 MHz): δ 3.98 (s).

$^1$H-NMR (MeOD, 300 MHz): δ), 7.71-7.69 (1H, 2s, H-6), 7.31-7.13 (5H, m, OPH+H-5b), 6.73-6.66 (1H, 2d, 3J=13.6 Hz, H-5a), 6.23-6.16 (1H, m, H-1’), 4.39-4.22 (3H, m, H-3’+H-5’), 4.05-4.03 (1H, m, H-4’), 3.61 (3H, s, CH$_3$O), 2.29-2.19 (1H, m, one of H-2’), 2.15-2.05 (1H, m, one of H-2’), 1.38 (6H, s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 28.0, 28.2, 28.3, 28.4 ([CH$_3$]$_2$C), 41.5, 41.6 (C-2’), 53.5, 53.6 (CH$_3$O), 58.6 ([CH$_3$]$_2$), 68.2 (C-5’), 72.4, 72.5 (C-3’), 87.1, 87.2, 87.3, 87.4 (C-1’, C-4’), 109.7 (C-5b), 112.7 (C-5), 123.7, 123.8 (’o’, OPPh), 130.9, 131.1 (’m’, OPPh+C-5a), 131.9 (’p’, OPPh), 140.4 (C-6), 151.1, 151.2, 151.4 (’ipsso’, OPPh+C-4), 164.0 (C-2), 177.6, 177.7 (COOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2’-deoxyuridine-5’-[4-chlorophenyl-(ethoxy-α,α-dimethylglycinyl)]-phosphate (CPF 43).

C$_{23}$H$_{28}$BrClN$_3$O$_9$P, MW=636.81.
This was synthesised according to **Standard procedure 5**, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate (413.3 mg, 1.22 mmol), NMI (184.7 mg, 2.25 mmol, 179.3 μL) in THF (5 mL) for 16 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (74 mg, yield 25.8 %).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 3.47, 3.33.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 10.03-9.99 (1H, 2bs, H-3), 7.70-7.67 (1H, 2s, H-6), 7.47-7.43 (1H, 2d, $^3$J=13.6 Hz, H-5b), 7.35-7.20 (4H, m, OPh), 6.77-6.68 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.33-6.27 (1H, m, H-1’), 4.55-4.29 (5H, m, H-3’+H-5’+ OH-3’+NH), 4.22-4.17 (2H, q, $^3$J=7.1 Hz, OCH$_2$CH$_3$+H-4’), 2.53-2.42 (1H, m, one of H-2’), 2.22-2.08 (1H, m, one of H-2’), 1.57-1.54 (6H, 2s, [CH$_3$]$_2$C), 1.31-1.30 (3H, 2t, $^3$J=7.1 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 14.5 ([CH$_3$]$_2$O), 27.2, 27.3, 27.4 ([CH$_3$]$_2$C), 40.7 (C-2’), 57.6 ([CH$_3$]$_2$O), 62.6 (CH$_2$CH$_2$O), 66.5, 66.6 (C-5’), 70.8, 71.1 (C-3’), 85.5, 85.74, 86.0 (C-1’, C-4’), 110.4 (C-5b), 112.0 (C-5), 121.9, 122.0, 122.1 (‘ω’, OPh), 128.9, 130.2 (‘m’, OPh+C-5a), 130.9 (‘p’, OPh), 138.3 (C-6), 149.4 (‘ipso’, OPh), 149.9 (C-4), 162.1, 162.2 (C-2), 175.7-175.9 (COOCH$_2$CH$_3$).

**Synthesis of** (E)-5-(2-bromovinyl)-2’-deoxyuridine-5’-[4-chlorophenyl-(benzoyl-α,α-dimethylglycinyl)]-phosphate (CPF 44).

C$_{28}$H$_{36}$BrClN$_3$O$_9$P, MW=698.88.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate (505.0 mg, 1.25 mmol), NMI (184.7 mg, 2.25 mmol, 179.3 µL) in THF (5 mL) for 16 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (134.8 mg, yield 42.9%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 3.44, 3.26.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 9.96-9.93 (1H, 2bs, H-3), 7.66-7.65 (1H, 2s, H-6), 7.47-7.41 (1H, 2d, $^3$J=13.5 Hz, H-5b), 7.39-7.18 (9H, m, OPh+CH$_2$Ph) 6.74-6.69 (1H, 2d, $^3$J=13.5 Hz, H-5a), 6.31-6.25 (1H, m, H-1'), 5.19 (2H, CH$_2$Ph), 4.51-4.29 (4H, m, H-3'+H-5'+NH), 4.15-4.12 (2H, m, H-4'+OH-3'), 2.48-2.40 (1H, m, one of H-2'), 2.18-2.05 (1H, m, one of H-2'), 1.60-1.59 (6H, 2s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 27.1, 27.5 ([C(CH$_3$)$_2$C], 40.7 (C-2'), 57.7 (C[CH$_3$]$_2$C), 66.4, 66.6 (C-5'), 68.2 (CH$_2$Ph), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.7, 86.0 (C-1', C-4'), 110.5 (C-5b), 112.0 (C-5), 121.9, 122.0, 128.4, 128.5, 128.9, 129.1 ('o', 'm', 'p', CH$_2$Ph+OPh+C-5a), 131.0 ('ipso', CH$_2$Ph), 135.6 ('p', OPh), 138.1 (C-6), 149.3 ('ipso', OPh), 149.8 (C-4), 162.1 (C-2), 175.6 (COOCH$_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[para-(trifluoromethyl)-phenyl-(benzoxyl-$\alpha$,$\alpha$-dimethylglucosyl)]-phosphate (CPF 48).

C$_{29}$H$_{36}$BrF$_3$N$_3$O$_9$P, MW=732.44.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), 4-(trifluoromethyl)-phenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate (529.45 mg, 1.22 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 µL) in THF (5 mL) for 4 hrs. The crude product was purified by column chromatography, eluting with dichloromethane/methanol 97:3 to give the pure product as a white foamy solid (142.1 mg, yield 43.1%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 3.16, 3.01.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 10.06-10.02 (1H, 2bs, H-3), 7.67-7.66 (1H, s, H-6), 7.64-7.60 (2H, 2d, $^3$J=8.8 Hz, OPh), 7.46-7.32 (8H, m, OPh+ CH$_2$Ph +H-5b), 6.77-6.68 (1H, 2d, $^3$J=13.6 Hz, H-5a), 6.31-6.26 (1H, m, H-1'), 5.18 (2H, s, CH$_2$Ph), 4.61-4.32 (4H, m, H-3'+H-5'+NH), 4.16-4.15 (2H, m, H-4'+OH-3'), 2.48-2.41 (1H, m, one of H-2'), 2.23-2.09 (1H, m, one of H-2'), 1.60-1.58 (6H, 2s, C(CH$_3$)$_2$)

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 27.0, 27.4, 27.5 (C(CH$_3$)$_2$), 40.6 (C-2'), 57.7, 57.8 (C(CH$_3$)$_2$), 66.8, 66.5 (C-5'), 68.2 (CH$_2$Ph), 70.8, 71.1 (C-3'), 85.4, 85.7, 86.0 (C-1', C-4'), 110.4 (C-5b), 111.9 (C-5), 120.8, 120.9, 121.0, 127.6, 127.7, 128.0, 128.5, 128.8, 129.0 ('o', 'm', 'p', OPh+ CH$_2$Ph+ C-5a), 124.2 (CF$_3$, $^3$J=267 Hz), 135.6 ('ipso', CH$_2$Ph), 138.2 (C-6), 149.9 (C-4), 153.3 ('ipso', OPh), 162.1 (C-2), 175.4 (COOCH$_2$Ph).

20 Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 16).

C$_{24}$H$_{29}$BrN$_3$O$_9$P, MW=614.38.
This was synthesised according to **Standard procedure 5**, using BVdU (250 mg, 0.75 mmol), Phenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate (589 mg, 1.87 mmol), NMI (6.2 mmol, 415 µL) in THF (7 mL) for 3 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (234 mg, yield 51%).

³¹P-NMR (CDCl₃, 121 MHz): δ 3.87, 3.82.

¹H-NMR (CDCl₃; 300 MHz): δ 10.35-10.2 (1H, bs, H-3), 7.65 (1H, 2xs, H-6), 7.44-7.39 (1H, 2d, J=13 Hz, H-5b), 7.37-7.15 (5H, m, OPH), 6.8 (1H, 2d, J=13 Hz, H-5a), 6.30 (1H, 2t, J=6 Hz, H1'), 4.4-4.2 (4H, m, H-5', H-3', NH), 4.1 (1H, H-4'), 3.72 (3H, 2s, CH₃O), 2.49-2.40 (1H, m, one of H-2'), 2.35-2.01 (5H, m, one of H-2'+4H cyclopentane), 1.8-1.6 (4H, m, 4H cyclopentane).

¹³C-NMR (DMSO; 75 MHz): δ 24.4, 24.3, 24.2 (2CH₂ cyclopent), 39.2, 38.6, 38.5 (2CH₂ cyclopent), 40.0 (C-2'), 53.2 (CH₃O), 66.4 (Ca cyclopentane), 66.6 (C-5'), 70.9 (C-3'), 85.8, 85.6, 85.4, 85.3 (C-1', C-4'), 110.2 (C-5b), 111.9 (C-5), 120.7-120.6 (α', OPH), 125.7 (p', OPH), 129.0 (C-5a), 130.2 (m', OPh), 138.5 (C-6), 149.9 (C-4), 150.9, 150.8 (ipso', OPH), 162.3(C-2), 176.3, 176.2 (COOCH₃).

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**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(ethoxy-α,α-cycloleucinyl)]-phosphate (CPF 17).**

C₂₅H₃₁BrN₅O₉P, MW=628.41.
This was synthesised according to **Standard procedure 5**, using BVdU (250 mg, 0.75 mmol), Phenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate (642 mg, 1.87 mmol), NMI (6.2 mmol, 415 µL) in THF (7 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (258 mg, yield 55%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.23, 4.1.

¹H-NMR (CDCl₃, 300 MHz): δ 10.3-10.1 (1H, bs, H-3), 7.8-7.75 (1H, 2xs, H-6), 7.51 (1H, 2d, J=14 Hz, H-5b), 7.45-7.10 (5H, m, OPh), 6.8 (1H, 2d, J=14 Hz, H-5a), 6.22 (1H, 2t, J=4 Hz, H1'), 4.55-4.05 (7H, m, H-5', H-3', H-4', NH, CH₃CH₂O), 2.50-2.40 (1H, m, one of H-2'), 2.35-1.95 (5H, m, one of H-2'+4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane), 1.25 (3H, 2t, J=7 Hz, CH₃CH₂O).

¹³C-NMR (CDCl₃, 75 MHz): δ 14.5 (CH₃CH₂O), 24.5, 24.4 (2CH₂ cyclopentane), 39.2, 38.9 38.8, 38.4 (2CH₂ cyclopentane), 40.6 (C-2'), 62.2, 62.1 (CH₃CH₂O), 66.2 (CH₃ cyclopentane), 66.6 (C-5'), 70.8 (C-3'), 85.7, 85.5 (C-1', C-4'), 110.2 (C-5b), 111.5 (C-5), 120.7, 120.6 (‘o’, OPh), 125.6 (‘p’, OPh), 129.7 (C-5a), 130.2 (‘m’, OPh), 138.5, 138.3 (C-6), 149.7 (C-4), 150.9, 150.8 (‘ipso’, OPh), 162.3 (C-2), 176.3 (COOCH₂CH₃).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxo-α,α-cycloleucinyl)]-phosphate (CPF 18).**

C₃₀H₃₃BrN₃O₇P, MW=690.48.
This was synthesised according to **Standard procedure 5**, using BVdU (200 mg, 0.6 mmol), Phenyl-(benzyloxy-α,α-cyclocucinyl)-phosphorochloridate (589 mg, 1.5 mmol), NMI (4.98 mmol, 332 µl) in THF (5 mL) for 10 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (127 mg, yield 31%).

**³¹P-NMR (CDCl₃, 121 MHz):** δ 4.11, 4.01.

**¹H-NMR (CDCl₃, 300 MHz):** δ 10.2 (1H, bs, H-3), 7.8-7.6 (1H, 2xs, H-6), 7.45-7.4 (1H, 2d, J=14 Hz, H-5b), 7.40-7.10 (10H, m, OPh+CH₂Ph), 6.85 (1H, 2d, J=14 Hz, H-5a), 6.20 (1H, m, H-1'), 5.15 (1H, s, CH₃Ph), 4.4-4.2 (3H, m, H-3',H-4', N(H)), 4.1 (2H, m, H-5'), 2.45-2.35 (1H, m, one of H-2'), 2.35-1.95 (5H, m, one of H-2'+4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

**¹³C-NMR (CDCl₃, 75 MHz):** δ 24.4, 24.3, 24.2 (2CH₂ cyclopentane), 39.9, 39.7 38.6, 38.5 (2CH₂ cyclopentane), 40.5 (C-2'), 66.2 (C₃ cyclopentane), 66.5 (C-5'), 67.8 (CH₃Ph), 70.8, 70.7 (C-3'), 85.7, 85.6, 85.5, 85.4 (C-1', C-4'), 110.2 (C-5b), 111.8, 118.7 (C-5b), 120.7, 120.5 ('o', OPh), 125.7 ('p', OPh), 130.2, 129.0, 128.8, 128.7, 128.5 ('m' OPh, Bn, C-5a), 135.8 ('ipso', CH₂Ph) 138.4, 138.2 (C-6), 149.8 (C-4), 150.9, 150.8 ('ipso', OPh), 162.2 (C-2), 175.7, 175.5 (COOBn).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridyine-5'-[para-nitrophenyl-(methoxy-α,α-cyclocucinyl)]-phosphate (CPF 19).**

C₂₄H₂₅BrN₄O₁₁P, MW=659.38.
This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), para-nitrophenyl-(methoxy-\(\alpha,\alpha\)-cycloleucinyl)-phosphorochloridate (543 mg, 1.5 mmol), NMI (4.98 mmol, 332 \(\mu\)L) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with \(\text{CH}_2\text{Cl}_2/\text{methanol}\) 97:3 to give the pure product as a white foamy solid (239 mg, yield 60%).

\(^{31}\text{P}-\text{NMR (CDCl}_3, 121 \text{MHz)}: \delta 3.73.\)

\(^1\text{H-NMR (CDCl}_3; 300 \text{ MHz)}: \delta 10.5-10.2 (1\text{H, bs, H-3}), 8.35-8.25 (2\text{H, 2d, } ^3J=6 \text{ Hz OPh}), 7.8-7.75 (1\text{H, 2xs, H-6}), 7.47 (2\text{H ,2d}, ^3J=6 \text{ Hz , OPh}), 7.45-7.35 (1\text{H, 2d, } ^3J=14 \text{ Hz, H-5b}), 6.75-6.67 (1\text{H, 2d, } ^3J=14 \text{ Hz, H-5a}), 6.30 (1\text{H, 2t, } ^3J=6 \text{ Hz, H1'}), 4.65-4.4 (3\text{H, m, H-5',H-3'}), 4.25-4.20 (1\text{H, m, H-4'}), 3.79 (3\text{H, s, CH}_3\text{O}), 2.6-2.4 (1\text{H, m, one of H-2'}), 2.3-1.98 (5\text{H, m, one of H-2'+4H cyclopentane}), 1.9-1.76 (4\text{H, m, 4H cyclopentane}).\)

\(^{13}\text{C-NMR (CDCl}_3; 75 \text{ MHz)}: \delta 24.4, 24.3, 24.2 (2\text{CH}_2 \text{ cyclopent}), 39.2, 39.1 (2\text{CH}_2 \text{ cyclopent}), 40.5 (C-2'), 53.4, 53.3 (\text{CH}_3\text{O}), 66.8 (\text{Cq cyclopentane}), 67.1 (C-5'), 70.9 (C-3'), 86.1, 86.0, 85.5, 85.4 (C-1', C-4'), 110.2 (C-5b), 111.8 (C-5), 121.3, 121.2 ('o', OPh), 126.2 ('m', OPh), 128.9 (C-5a), 138.6 (C-6), 144.9 ('ipso', OPh) 149.9 (C-4), 155.9, 155.8 ('p', OPh), 162.3 (C-2'), 176.3 (\text{COOCH}_3).\)

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-nitrophenyl-(ethoxy-\(\alpha,\alpha\)-cycloleucinyl)]-phosphate (CPF 20).

\(\text{C}_{25}\text{H}_{30}\text{BrN}_{3}\text{O}_{11}\text{P, MW=673.4.}\)
This was synthesised according to Standard procedure 5, using BVdU (200 mg, 0.60 mmol), para-nitrophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate (563 mg, 1.5 mmol), NMI (4.98 mmol, 332 μL) in THF (5 mL) for 1 hr. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (240 mg, yield: 59%).

³¹P-NMR (CDCl₃, 121 MHz): δ 3.83, 3.79.

¹H-NMR (CDCl₃, 300 MHz): δ 8.25-8.2 (2H, 2d, ³J=9Hz OPh), 7.66 (1H, s, H-6), 7.4 (2H, 2d, ³J=9Hz, OPh), 7.3 (1H, 2d, ³J=14 Hz, H-5b), 6.85 (1H, 2d, ³J=14 Hz, H-5a), 6.3-6.2 (1H, m, H1'), 4.7-4.45 (4H, m, H-5', H-3', NH), 4.2-4.05 (3H, m, H-4', CH₃CH₂O), 2.55-2.4 (1H, m, one of H-2'), 2.2-1.95 (5H, m, one of H-2'+4H cyclopentane), 1.95-1.8 (4H, m, 4H cyclopentane), 1.2 (3H, 2t, ³J=8 Hz, CH₃CH₂O).

¹³C-NMR (CDCl₃, 75 MHz): δ 14.9 (CH₃CH₂O), 24.5, 24.4 (2CH₂ cyclopent), 39.1, 39.0, 38.8 (2CH₂ cyclopent), 40.7 (C-2'), 62.4 (CH₃CH₂O), 66.5 (C₆ cyclopentane), 67.0 (C-5'), 70.9 (C-3'), 85.9, 85.4 (C-1', C-4'), 110.2 (C-5b), 111.8 (C-5), 121.3 ('o', OPh), 126.2 ('m', OPh), 128.8 (C-5a), 138.5 (C-6), 144.9 (′ipso′, OPh), 149.9 (C-4), 155.5 (′p′, OPh), 162.3 (C-2), 175.8, 175.7 (COOCH₂CH₃).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-nitrophenyl-(benzoxyl-α,α-cycloleucinyl)]-phosphate (CPF 21).

C₃₀H₃₂BrN₄O₁₁P, MW=735.47.
This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), para-nitrophenyl-(benzoyloxy-α,α-cycloleucinyl)-phosphorochloridate (656 mg, 1.5 mmol), NMI (4.98 mmol, 332 µL) in THF (5 mL) for 3 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (269 mg, yield 61%).

³¹P-NMR (CDCl₃, 121 MHz): δ 3.72.

¹H-NMR (CDCl₃, 300 MHz): δ 10.3 (1H, bs, H-3), 8.22-8.12 (2H, 2d, J=7 Hz, OPh), 7.65 (1H, 2xs, H-6), 7.45-7.30 (8H, m, H-5b+OPh+CH₂Ph), 6.72-6.65 (1H, 2d, J=14 Hz, H-5a), 6.28 (1H, 2t, J=6Hz, H-1'), 5.15 (1H, d, CH₂Ph), 4.6-4.35 (4H, m, H-3', H-5', H-4', NH₅), 2.55-2.4 (1H, m, one of H-2'), 2.3-1.92 (5H, m, one of H-2'+4H cyclopentane), 1.85-1.6 (4H, m, 4H cyclopentane).

¹³C-NMR (CDCl₃, 75 MHz): δ 24.4, 24.3, 24.2 (2CH₂ cyclopentane), 39.1, 38.9, 38.7 (2CH₂ cyclopentane), 40.5 (C-2'), 66.9 (Cq cyclopentane), 67.1 (C-5'), 68.0 (CH₂Ph), 70.9 (C-3'), 85.3, 85.0 (C-1', C-4'), 110.3 (C-5b), 111.8 (C-5), 121.2 (‘o’, OPh), 126.1 (‘m’, OPh), 129.0, 128.8 (Bn, C-5a), 135.7 (‘ipso’, CH₂Ph), 138.5 (C-6), 144.9 (‘ipso’, OPh), 149.9 (C-4), 155.8 (‘p’ OPh), 162.3 (C-2), 175.6 (COOBn).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 22).

C₂₄H₂₉BrFN₃O₉P, MW=632.37.
This was synthesised according to **Standard procedure 5**, using BVdU (200 mg, 0.60 mmol), para-fluorophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate (503 mg, 1.5 mmol), NMI (4.98 mmol, 332 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (251 mg, yield 66%).

**³¹P-NMR (CDCl₃, 121 MHz):** δ 4.22.

**¹H-NMR (CDCl₃; 300 MHz):** δ 10.3 (1H, bs, H-3), 7.70 (1H, 2xs, H-6), 7.4 (1H, 2d, ³J=14 Hz, H-5b), 7.25-7.15 (2H, m, OPh), 7.1-6.95 (2H, m, OPh), 6.70 (1H, 2d, ³J=14 Hz, H-5a), 6.30-6.15 (1H, 2t, ³J=5 Hz, H1'), 4.55-4.05 (5H, m, H-5'+H-3', NH, H-4'), 3.72 (3H, 2s, CH₃O), 2.55-2.35 (1H, m, one of H-2'), 2.25-1.92 (5H, m, one of H-2'+4H cyclopentane), 1.85-1.6 (4H, m, 4H cyclopentane).

**¹³C-NMR (DMSO; 75 MHz):** δ 24.4, 24.3, 24.2 (2CH₂ cyclopentane), 39.3, 39.2, 38.9, 38.5 (2CH₂ cyclopentane), 40.6 (C-2'), 53.3, 53.2 (CH₃O), 66.5 (Cq cyclopentane), 66.7 (C-5'), 70.9 (C-3'), 85.8, 85.7, 85.4 (C-1', C-4'), 110.2 (C-5b), 111.9 (C-5), 116.9, 116.6 ('o', OPh), 122.2, 122.0 ('m', OPh), 128.5 (C-5a), 138.5 (C-6), 146.7 ('ipso', OPh) 149.9 (C-4), 158.5 ('p', OPh), 162.3(C-2), 176.4, 176.3 (COOCH₃).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-fluorophenyl-(ethoxy-α,α-cycloleucinyl))-phosphate (CPF 23).**

C₂₅H₃₀BrF₅N₃O₇P, MW=646.4.
This was synthesised according to **Standard procedure 5**, using BVdU (200 mg, 0.60 mmol), para-fluorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate (524 mg, 1.5 mmol), NMI (4.98 mmol, 332 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH$_2$Cl$_2$/Methanol 97:3 to give the pure product as a white foamy solid (274 mg, yield 71%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 5.30.

1H-NMR (CDCl$_3$, 300 MHz): δ 10.35 (1H, bs, H-3), 7.7 (1H, 2xs, H-6), 7.44 (1H, 2d, J=14 Hz, H-5b), 7.25-7.15 (2H, m, OPh), 7.1-6.95 (2H, m, OPh), 6.7 (1H, 2d, J=14 Hz, H-5a), 6.30 (1H, 2t, J=6 Hz, H1'), 4.55,4.3 (3H, m, H-5', H-3'), 4.2-4.1 (4H, m, NH, H-4', CH$_3$CH$_2$O), 2.55-2.4 (1H, m, one of H-2'), 2.22-1.90 (5H, m, one of H-2'+4H cyclopentane), 1.8-1.6 (4H, m, 4H cyclopentane), 1.3-1.2 (3H, 2t, J=7 Hz, CH$_3$CH$_2$O).

13C-NMR (CDCl$_3$, 75 MHz): δ 14.5 (CH$_3$CH$_2$O), 24.6, 24.4, 24.3 (2CH$_2$ cyclopent), 39.3, 39.2, 38.9, 38.6 (2CH$_2$ cyclopent), 40.6 (C-2'), 62.2 (CH$_3$CH$_2$O), 66.5 (Cg cyclopentane), 66.7 (C-5'), 71.0 (C-3'), 85.8, 85.7, 85.5, 85.4 (C-1', C-4'), 110.2 (C-5b), 111.9 (C-5), 116.9, 116.5 ('o', OPh), 122.2, 122.1 ('m', OPh), 129.0 (C-5a), 138.5 (C-6), 146.8,146.7 ('ipso', OPh), 149.9 (C-4), 158.5 ('p', OPh), 162.3 (C-2), 175.9, 175.8 (COOCH$_2$CH$_3$).

**Synthesis of** (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-fluorophenyl-(benzoxyl-α,α-cycloleucinyl)]-phosphate (CPF 24).

C$_{30}$H$_{32}$BrN$_3$O$_9$P, MW=708.47.
This was synthesised according to **Standard procedure 5**, using BVDu (200 mg, 0.60 mmol), para-fluorophenyl-(benzoxo-α,α-cycloleucinyl)-phosphorochloridate (616 mg, 1.5 mmol), NMI (4.98 mmol, 332 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (283 mg, yield 67%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.27.

¹H-NMR (CDCl₃, 300 MHz): δ 10.3-9.85 (1H, bs, H-3), 7.65 (1H, 2xs, H-6), 7.45-7.35 (1H, 2d, ²J=14 Hz, H-5b), 7.40-7.30 (5H, m, CH₂Ph), 7.25-7.15 (2H, m, OPh), 7.05-6.95 (2H, m, OPh), 6.71 (1H, 2d, ²J=14 Hz, H-5a), 6.27 (1H, 2t, ³J=6Hz, H-1’), 5.15 (1H, s, CH₂Ph), 4.45 (1H, m, H-3’), 4.40-4.30 (2H, m, H-5’-H-1’), 4.20-4.05 (2H, m, H-4’, NH), 2.5-2.4 (1H, m, one of H-2’), 2.25-1.9 (5H, m, one of H-2’+4H cyclopentane), 1.8-1.6 (4H, m, 4H cyclopentane).

¹³C-NMR (CDCl₃, 75 MHz): δ 24.5, 24.3, 24.2 (2CH₂ cyclopent), 39.7, 39.6, 39.3, 39.2 (2CH₂ cyclopent), 40.5, 40.0 (C-2’), 66.6 (C₉ cyclopentane), 67.2, 66.7 (C-5’), 69.0 (CH₂Ph), 70.8, 70.7 (C-3’), 85.8, 85.7, 85.4, 85.3 (C-1’, C-4’), 110.3 (C-5b), 111.8 (C-5), 116.9, 116.6 (‘o’, OPh), 122.2, 122.1 (‘m’, OPh), 129.0, 128.9, 128.6, 128.5 (Bn, C-5a), 135.8(‘ipsa’, CH₂Ph) 138.5 (C-6), 146.8, 146.7 (‘ipsa’, OPh), 149.9 (C-4), 158.5 (‘p’ OPh), 162.2 (C-2), 175.7, 175.0 (COOBn).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-chlorophenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 32).**

C₂₄H₂₈BrClN₃O₅P, MW=648.82.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), para-chlorophenyl-(methoxy-α,α-cycloeleuciny1)-phosphorochloridate (475 mg, 1.35 mmol), NMI (4.5 mmol, 300 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (187 mg, yield 64%).

³¹P-NMR (MeOD, 121 MHz): δ 4.64.

¹H-NMR (MeOD; 300 MHz): δ 7.75 (1H, 2xs, H-6), 7.32 (1H, 2d, ³J=14 Hz, H-5b), 7.32-7.27 (2H, m, OPh), 7.20-7.11 (2H, m, OPh), 6.72 (1H, 2d, ³J=14 Hz, H-5a), 6.27-6.20 (1H, 2t, ³J=6 Hz, H1'), 4.35 (1H, m, H-3'), 4.30 (2H, m, H-5') 4.1 (2H, m, H-4'), 3.72 (3H, 2s, CH₃O), 2.32-2.20 (1H, m, one of H-2'), 2.20-1.92 (5H, m, one of H-2'+4H cyclopentane), 1.8-1.6 (4H, m, 4H cyclopentane).

¹³C-NMR (MeOD; 75 MHz): δ 25.7, 25.6 (2CH₂ cyclopent), 41.7, 41.6, 41.4, 41.3 (2CH₂ cyclopent), 42.7 (C'-2'), 54.1, 53.9 (CH₃O), 67.8 (C₆ cyclopentane), 69.1, 69.0 (C-5'), 73.8 (C-3'), 88.4, 88.3, 88.2 (C-1', C-4'), 110.2 (C-5b), 111.8 (C-5), 122.1, 121.9 ('o', OPh), 128.9 (C-5a), 130.6 ('m', OPh), 130.8 ('p', OPh), 138.5 (C-6), 149.5, 149.4 ('ipso', OPh), 149.9 (C-4), 162.2(C-2), 175.6 (COOCH₃).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-chlorophenyl-(ethoxy-α,α-cycloeleuciny1))-phosphate (CPF 33).

C₂₅H₃₀BrClN₃O₇P, MW=662.85.
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), para-chlorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate (495 mg, 1.35 mmol), NMI (4.5 mmol, 300 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (240 mg, yield 66%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.15.

¹H-NMR (CDCl₃, 300 MHz): δ 10.25-10.1 (1H, bs, H-3), 7.65 (1H, 2xs, H-6), 7.4-7.3 (1H, 2d, J=14 Hz, H-5b), 7.25-7.20 (2H, m, O₆Ph), 7.20-7.10 (2H, m, O₆Ph), 6.75 (1H, 2d, J=14 Hz, H-5a), 6.20 (1H, m, H1'), 4.35 (3H, m, H-3', H-5'), 4.2-4.0 (4H, m, H-4', NH, CH₃CH₂O), 2.45-2.25 (1H, m, one of H-2'), 2.25-1.85 (5H, m, one of H-2'+4H cyclopentane), 1.75-1.55 (4H, m, 4H cyclopentane), 1.2 (3H, 2t, J=7 Hz, CH₂CH₂O).

¹³C-NMR (CDCl₃, 75 MHz): δ 14.5 (CH₃CH₂O), 24.5, 24.4 (2CH₂ cyclopentene), 39.3, 39.2, 38.8, 38.6 (2CH₂ cyclopentene), 40.5 (C-2'), 62.3 (CH₃CH₂O), 66.1 (C₆ cyclopentene), 66.7 (C-5'), 70.8 (C-3'), 85.8, 85.4 (C-1', C-4'), 110.3 (C-5b), 111.9 (C-5), 122.1, 121.9 (C', O₆Ph), 129.0 (C-5a), 130.2 ('m', O₆Ph), 130.8 ('p', O₆Ph), 138.5 (C-6), 149.5, 149.4 ('ipso', O₆Ph), 149.9 (C-4), 162.3 (C-2), 175.9 (COOCH₂CH₃).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(para-chlorophenyl-(benzoxyl-α,α-cycloleucinyl))-phosphate (CPF 34).

C₇₀H₃₂BrClN₃O₉P, MW=724.92.
This was synthesised according to **Standard procedure 5**, using BVdU (150 mg, 0.45 mmol), para-chlorophenyl-(benzylxyo-α,α-cycloleucinyl)-phosphorochloridate (578 mg, 1.35 mmol), NMI (4.5 mmol, 300 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (222 mg, yield 68%).

**⁴¹P-NMR (CDCl₃, 121 MHz):** δ 4.11, 4.05.

**¹H-NMR (CDCl₃, 300 MHz):** δ 7.65 (1H, 2xs, H-6), 7.45-7.29 (10H, m, H-5b, 2H OPh+CH₂Ph), 7.20-7.15 (2H, m, OPh), 6.75-6.67 (1H, 2d, J=14 Hz, H-5a), 6.28 (1H, 2t, J=6Hz, H-1'), 5.15 (1H, 2s, CH₂Ph), 4.5 (1H, m, H-3'), 4.35 (2H, m, H-5') 4.1 (H, m, H-4'), 4.00 (1H, m, NPh), 2.48-2.35 (1H, m, one of H-2'), 2.3-1.92 (5H, m, one of H-2'+4H cyclopentane), 1.8-1.6 (4H, m, 4H cyclopentane).

**¹³C-NMR (CDCl₃, 75 MHz):** δ 24.5, 24.4, 24.3, 24.2 (2CH₂ cyclopentane), 39.3, 38.8, 38.6 (2CH₂ cyclopentane), 40.5 (C-2'), 66.7 (C₉ cyclopentane), 67.9 (CH₂Ph), 68.4 (C-5'), 70.7 (C-3'), 85.7, 85.7, 85.4, 85.3 (C-1', C-4'), 110.3 (C-5b), 111.8 (C-5), 122.0, 121.9 ('α', OPh), 129.1, 128.3, 128.2 (Bn, 'm', OPh), 130.2 (C-5a), 135.8 ('ipso', CH₂Ph), 136.3 ('p' OPh), 138.2 (C-6), 149.5, 149.3 ('ipso', OPh), 149.9 (C-4), 162.2 (C-2), 175.7, 175.5 (COOBn).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-trifluorophenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 28).**

**C₂₅H₂₉BrF₃N₃O₉P, MW=682.38.**
This was synthesised according to **Standard procedure 5**, using BVdU (150 mg, 0.45 mmol), para-trifluorophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate (521 mg, 1.35 mmol), NMI (4.5 mmol, 300 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (199 mg, yield 65%).

³¹P-NMR (CDCl₃, 121 MHz): δ 3.80.

¹H-NMR (CDCl₃; 300 MHz): δ 7.70 (1H, 2s, H-6), 7.55 (1H, 2d, ³J = 14 Hz, H-5b), 7.45-7.32 (4H, m, OPh), 6.72 (1H, 2d, ³J = 14 Hz, H-5a), 6.28 (1H, 2t, ³J = 6 Hz, H1'), 4.55 (1H, m, H-3'), 4.45 (2H, m, H-5'), 4.25 (1H, H-4'), 4.15 (1H, NH), 3.71 (3H, 2s, CH₃O), 2.6-2.4 (1H, m, one of H-2'), 2.3-1.9 (5H, m, one of H-2'+4H cyclopentane), 1.85-1.6 (4H, m, 4H cyclopentane).

¹³C-NMR (CDCl₃; 75 MHz): δ 24.4, 24.3, 24.2 (2CH₂ cyclopent), 39.2, 39.1, 38.8, 38.6 (2CH₂ cyclopent), 40.5 (C-2'), 53.9 (CH₃O), 66.3 (C₉ cyclopentane), 66.8 (C-5'), 70.9 (C-3'), 85.8, 85.4 (C-1', C-4'), 110.3 (C-5b), 111.9 (C-5), 125.1 (d, ³J = 270Hz, CF₃), 127.1, 127.0 ('o', OPh), 127.8 ('m', OPh), 128.9 (C-5a), 129.0 ('p', q, ³J = 32Hz, OPh), 138.5 (C-6), 149.9 (C-4), 153.5 ('ipso', OPh), 162.2 (C-2), 176.3, 176.2 (COOCH₃).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-trifluorophenyl-(ethoxy-α,α-cycloleucinyl)]-phosphate (CPF 29).**

C₂₆H₃₈BrF₃N₃O₅P, MW=696.40.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), para-trifluorophenyl-(ethoxy-α,α-cyclopeucinyl)-phosphorochloridate (540 mg, 1.35 mmol), NMI (4.50 mmol, 300 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (185 mg, yield 59%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.30.

¹H-NMR (CDCl₃, 300 MHz): δ 10.35 (1H, bs, H-3), 7.70 (1H, 2xs, H-6), 7.40 (1H, 2d, J=14 Hz, H-5b), 7.28-7.14 (2H, m, OPh), 7.05-6.95 (2H, m, OPh), 6.70 (1H, 2d, J=14 Hz, H-5a), 6.3 (1H, m, H1'), 4.55-4.3 (3H, m, H-5', H-3'), 4.2-4.1 (3H, m, H-4', CH₃CH₂O), 2.5-2.35 (1H, m, one of H-2'), 2.20-1.9 (5H, m, one of H-2'+4H cyclopentane), 1.85-1.6 (4H, m, 4H cyclopentane), 1.25 (3H, 2t, J=7 Hz, CH₂CH₂O).

¹³C-NMR (CDCl₃, 75 MHz): δ 14.5 (CH₃CH₂O), 24.5, 24.4 (2CH₂ cyclopent), 39.3, 39.2, 38.9, 38.5 (2CH₂ cyclopent), 40.6 (C-2'), 62.2 (CH₂CH₂O), 66.7 (Cq cyclopentane), 67.4, 67.3 (C-5'), 70.9 (C-3'), 85.8, 85.7 (C-1', C-4'), 110.2 (C-5b), 111.9 (C-5), 116.8, 116.5 ('o', OPh), 122.2, 122.1 ('m', OPh), 125.1 (d, J=270Hz, CF₃), 129.0 (C-5a), 131.1 ('p', q, J=32Hz, OPh), 138.5 (C-6), 146.8, 146.7 ('ipso', OPh), 149.9 (C-4), 162.3 (C-2), 175.9,175.8 (COOCH₂CH₂).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5’-[para-trifluorophenyl-(benzoxyl-α,α-cyclopeucinyl)]-phosphate (CPF 30).

C₃₁H₃₂BrF₃N₃O₉P, MW=758.47.
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), para-trifluorophenyl-(benzylxy-α,α-cycloleucinyl)-phosphorochloridate (623 mg, 1.35 mmol), NMI (4.5 mmol, 300 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (218 mg, yield 64%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.30.

¹H-NMR (CDCl₃, 300 MHz): δ 10.35 (1H, bs, H-3), 7.65 (1H, 2xs, H-6), 7.55 (2H, m, 2H OPPh), 7.45-7.25 (8H, m, 2H OPPh+CH₂Ph+ H-5b), 6.7 (1H, 2d, 3J=14 Hz, H-5a), 6.30 (1H, 2t, 3J=6Hz, H-1’), 5.15 (1H, 2s, CH₂Ph), 4.55-4.35 (3H, m, H-3’+ H-5’), 4.25 (1H, H-4’), 4.10 (1H, NH), 2.55-2.35 (1H, m, one of H-2’), 2.30-1.92 (5H, m, one of H-2’+4H cyclopentane), 1.8-1.6 (4H, m, 4H cyclopentane).

¹³C-NMR (CDCl₃, 75 MHz): δ 25.5, 24.4, 24.3, 24.2 (2CH₂ cyclopentan), 39.2,39.1, 38.7, 38.6 (2CH₂ cyclopentan), 40.5, 40.0 (C-2’), 66.4 (Cq cyclopentane), 66.8 (C-5’), 68.0 (CH₂Ph), 70.9 (C-3’), 86.0, 85.8, 85.4, 85.3 (C-1’, C-4’), 110.3 (C-5b), 111.9 (C-5), 121.8, 120.8 (’o, m’, OPPh), 125.2 (d, J=270Hz, CF₃), 128.5, 127.7, 127.5 (Bn, C-5a), 129,2 (’p’,q , J=32Hz, OPPh), 135.4 (’ipso’, CH₂Ph), 138.5 (C-6), 149.9 (C-4), 153.5 (’ipso’ OPPh), 162.2 (C-2), 175.6,175.5 (COOBn).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-L-phenylalaninyl)]-phosphate (CPF 36).

C₂₇H₂₉BrN₃O₇P, MW=650.41.
This was synthesised according to **Standard procedure 5**, using BVdU (150 mg, 0.45 mmol), Phenyl-(methoxy-L-phenylalaninyl)-phosphorochloridate (477 mg, 1.35 mmol), NMI (4.42 mmol, 190 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (169 mg, yield 58%).

³¹P-NMR (CDCl₃, 121 MHz): δ 4.79, 4.71.

¹H-NMR (CDCl₃; 300 MHz): δ 9.95 (1H, bs, H-3), 7.60-7.55 (1H, 2xs, H-6), 7.48-7.4 (1H, 2d, J=14 Hz, H-5b), 7.3-7.1 (10H, m, CH₂Ph+ OPh), 6.75-6.65 (1H, 2d, J=14 Hz, H-5a), 6.27-6.18 (1H, m, H1’), 4.57-4.29 (6H, m, H-5’,H-3’,H-4’, NH, CHphenylala), 3.70 (3H, 2s, CH₃O), 3.01 (2H, m, CH₂Ph), 2.35-2.20 (1H, m, one of H-2’), 2.07-1.95 (1H, m, one of H-2’).

¹³C-NMR (CDCl₃; 75 MHz): δ 36.3 (CH₂phenylalanine), 41.9, 41.8 (C-2’), 53.0 (CH₃O), 56.6, 56.1 (CHphenylala), 67.1 (C-5’), 71.3, 70.7 (C-3’), 85.7, 85.6, 85.5, 85.4 (C-1’, C-4’), 110.4 (C-5b), 111.9 (C-5), 120.6,120.5 (’a’, OPh), 127.8 (’p’, OPh), 130.1, 129.9, 129.8, 129.1 (CH₂Ph, C-5a, ’m’ OPh), 138.0, 137.9 (C-6), 149.8 (C-4), 150.7,150.6 (’ipso’, OPh), 162.1, 162.0 (C-2), 173.5 (COOCH₃).

**Synthesis of (E)-5-(2-Bromovinyl)-2’-deoxyuridine-5’-[phenyl-(methoxy-L-leucinyl)]-phosphate (CPF 35).**

C₂₄H₃₁BrN₃O₉P, MW=616.40.
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), Phenyl-(methoxy-L-leucinyl)-phosphorochloridate (432 mg, 1.35 mmol), NMI (4.42 mmol, 190 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (167 mg, yield 60%).

³¹P-NMR (CDCl₃, 121 MHz): δ 5.14, 4.60.

¹H-NMR (CDCl₃; 300 MHz): δ 10.1 (1H, bs, H-3), 7.75 (1H, 2xs, H-6), 7.45 (1H, 2d, ³J=14 Hz, H-5b), 7.4-7.2 (5H, m, OPh), 6.85 (1H, 2d, ³J=14 Hz, H-5a), 6.27-6.18 (1H, 2t, ³J=6 Hz, H1'), 4.5-4.2 (4H, m, H-5',H-3', NH), 4.1 (1H, m,H-4'), 3.95 (1H, m, CHCH₂CH(CH₃)₂), 3.70 (3H, 2s, CH₃O), 2.40-2.20 (1H, m, one of H-2'), 2.05-1.95 (1H, m, one of H-2'), 1.8 (1H, m, CHCH₂CH(CH₃)₂), 1.8-1.5 (2H, m, CHCH₂CH(CH₃)₂), 1.0-0.9 (6H, m, CHCH₂CH(CH₃)₂).

¹³C-NMR (CDCl₃; 75 MHz): δ 23.2, 23.1, 22.0, 21.9 (2C, CHCH₂CH(CH₃)₂), 24.9, 24.7 (CHCH₂CH(CH₃)₂), 40.6 (C-2'), 43.7, 43.6 (CHCH₂CH(CH₃)₂), 53.0 (CH₃O), 53.7, 53.6 (CHCH₂CH(CH₃)₂), 66.6, 66.3 (C-5'), 71.1, 70.8 (C-3'), 86.0, 85.7, 85.6, 85.5 (C-1', C-4'), 110.4 (C-5b), 111.9 (C-5), 120.6, 120.5, 120.4 ('o', OPh), 125.8, 125.7 ('p', OPh), 128.9 (C-5a), 130.2 ('m' OPh), 138.1 (C-6), 149.9 (C-4), 150.8, 150.7 ('ipso', OPh), 162.2 (C-2), 175.1, 174.9 (COOCH₃).

**Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-(phenyl-(benzoxyl-L-leucinyl))-phosphate (CPF 37).**

C₃₀H₅₂BrN₅O₉P, MW=692.49.
This was synthesised according to Standard procedure 5, using BVdU (150 mg, 0.45 mmol), Phenyl-(benzoxyl-L-leucinyl)-phosphorochloridate (534 mg, 1.35 mmol), NMI (4.42 mmol, 190 \mu L) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH\textsubscript{2}Cl\textsubscript{2}/Methanol 97:3 to give the pure product as a white foamy solid (199 mg, yield 64%).

\textsuperscript{31}P-NMR (CDCl\textsubscript{3}, 121 MHz): \(\delta\) 5.18, 4.54.

1\textsuperscript{H}-NMR (CDCl\textsubscript{3}; 300 MHz): \(\delta\) 9.95-9.85 (1H, bs, H-3), 7.55 (1H, 2xs, H-6), 7.38 (1H, 2d, \(J=14\) Hz, H-5b), 7.3-7.1 (5H, m, CH\textsubscript{2}Ph+ O\textsubscript{Ph}), 6.65 (1H, 2d, \(J=14\) Hz, H-5a), 6.26-6.14 (1H, 2t, \(J=6\) Hz, H1'), 5.1 (2H, 2s, CH\textsubscript{2}Ph) 4.4-3.8 (6H, m, H-5',H-3', NH, H-4', CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 2.35-2.25 (1H, m, one of H-2'), 1.95-1.85 (1H, m, one of H-2'), 1.6-1.4 (3H, m, CH\textsubscript{2}CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 0.8 (6H, m, CH\textsubscript{2}CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}).

1\textsuperscript{3}C-NMR (CDCl\textsubscript{3}; 75 MHz): \(\delta\) 23.2, 23.1, 22.0, 21.9 (2C, CH\textsubscript{2}CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 24.9, 24.7 (CH\textsubscript{2}CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 40.7 (C-2'), 43.9, 43.8 (CH\textsubscript{2}CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 53.9, 53.7 (CH\textsubscript{2}CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 66.4, 66.2 (C-5'), 67.8, 67.7 (CH\textsubscript{2}Ph), 71.1, 70.7 (C-3'), 85.9, 85.6, 85.4, 85.3 (C-1', C-4'), 110.4 (C-5b), 111.9 (C-5), 120.6, 120.5 ('o', O\textsubscript{Ph}), 125.8, 125.7 ('p', O\textsubscript{Ph}), 130.2, 129.1, 128.9 (C-5a, CH\textsubscript{2}Ph, 'm' O\textsubscript{Ph}), 135.4 ('ipso', CH\textsubscript{2}Ph), 138.1 (C-6), 149.8 (C-4), 150.2 ('ipso', O\textsubscript{Ph}), 162.1 (C-2), 175.7, 174.6 (C\textsubscript{OOBn}).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-nitrophenyl-(benzoxyl-L-leucinyl)]-phosphate (CPF 38).

C\textsubscript{30}H\textsubscript{34}BrN\textsubscript{4}O\textsubscript{11}P, MW=737.49.

25
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), para-nitrophenyl-(benzoyx-L-leucinyl)-phosphorochloridate (595 mg, 1.35 mmol), NMI (4.42 mmol, 190 µL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH$_2$Cl$_2$/Methanol 97:3 to give the pure product as a white foamy solid (176 mg, yield 53%).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 5.72, 4.35.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 10.2 (1H, bs, H-3), 8.1 (2H, m, 2H OPh), 7.65 (1H, 2xs, H-6), 7.45-7.2 (8H, m, H-5b, CH$_2$Ph+ 2H OPh), 6.65 (1H, 2d, $^3$J=14 Hz, H-5a), 6.35-6.2 (1H, 2t, $^3$J=6 Hz, H1), 5.15 (2H, 2s, CH$_2$Ph) 4.7-3.9 (6H, m, H-5',H-3', NH, H-4', CHCH$_2$CH(CH$_3$)$_2$), 2.55-2.4 (1H, m, one of H-2'), 2.15-2.05 (1H, m, one of H-2'), 1.7-1.5 (3H, m, CHCH$_2$CH(CH$_3$)$_2$), 0.95-0.8 (6H, m, CHCH$_2$CH(CH$_3$)$_2$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 23.2, 23.1, 22.0, 21.9 (2C, CHCH$_2$CH(CH$_3$)$_2$), 24.9, 24.8 (CHCH$_2$CH(CH$_3$)$_2$), 40.6 (C-2'), 43.7, 43.6 (CHCH$_2$CH(CH$_3$)$_2$), 53.9, 53.7 (CHCH$_2$CH(CH$_3$)$_2$), 66.9 (C-5'), 67.9 (CH$_2$Ph), 71.2, 70.8 (C-3'), 85.8, 85.3, 85.2 (C-1', C-4'), 110.6 (C-5b), 111.9 (C-5), 121.3 ('o', OPh), 129.2, 129.1, 128.8, 126.2 (C-5a, CH$_2$Ph, 'm' OPh), 135.4, 135.3 ('ipso', CH$_2$Ph), 138.2 (C-6), 145.2, 145.1 ('ipso', OPh), 149.9 (C-4), 155.5 ('p', OPh), 162.1 (C-2), 174.2 (COOBn).

Synthesis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-chlorophenyl-(benzoxyl-L-leucinyl)]-phosphate (CPF 39).

C$_{30}$H$_{34}$BrClN$_3$O$_9$P, MW=726.94.
This was synthesised according to **Standard procedure 5**, using BVdU (150 mg, 0.45 mmol), para-chlorophenyl-(benzoyxy-L-leucinyl)-phosphorochloridate (581 mg, 1.35 mmol), NMI (4.42 mmol, 190 μL) in THF (5 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 97:3 to give the pure product as a white foamy solid (221 mg, yield 68%).

³¹P-NMR (CDCl₃, 121 MHz): δ 5.27, 4.76.

¹H-NMR (CDCl₃; 300 MHz): δ 10.25-10.15 (1H, bs, H-3), 7.65 (1H, 2xs, H-6), 7.45 (1H, 2d, J=14 Hz, H-5b), 7.4-7.15 (9H, m, CH₂Ph+ OPh), 6.7 (1H, 2d, J=14 Hz, H-5a), 6.35-6.2 (1H, 2t, J=6 Hz, H1'), 5.15 (2H, 2s, CH₂Ph) 4.55-3.9 (6H, m, H-5', H-3', NH, H-4', CHCH₂CH(CH₃)₂), 2.5-2.4 (1H, m, one of H-2'), 2.15-2.0 (1H, m, one of H-2'), 1.7-1.45 (3H, m, CHCH₂CH(CH₃)₂), 0.94-0.82 (6H, m, CHCH₂CH(CH₃)₂).

¹³C-NMR (CDCl₃; 75 MHz): δ 23.1, 23.0, 22.2, 22.0 (2C, CHCH₂CH(CH₃)₂), 24.9, 24.7 (CHCH₂CH(CH₃)₂), 40.7 (C-2'), 43.9, 43.8 (CHCH₂CH(CH₃)₂), 53.9, 53.7 (CHCH₂CH(CH₃)₂), 66.7, 66.3 (C-5'), 67.8 (CH₂Ph), 71.1, 70.7 (C-3'), 85.8, 85.7, 85.4 (C-1', C-4'), 110.5 (C-5b), 111.9 (C-5), 122.1, 122.0 ('α', OPh), 130.2, 129.1, 129.0 (C-5a, CH₂Ph, 'm' OPh), 131.1, 130.9 ('p', OPh), 135.5, 135.4 ('ipso', CH₂Ph), 138.2 (C-6), 149.2, 149.1 ('ipso', OPh), 149.2, 149.1 (C-4), 162.2 (C-2), 174.2, 174.2 (COOBn).
Synthesis of Gemcitabine-[phenyl-(benzoxyl-L-alaninyl)]-phosphate.

C_{25}H_{27}F_{2}N_{4}O_{8}P, MW=580.47 (CPF 31).

This was synthesised according to Standard procedure 5, using gemcitabine (131 mg, 0.5 mmol), Phenyl-(benzoxyl-L-alaninyl)-phosphorochloridate (529 mg, 1.5 mmol), NMI (4.42 mmol, 300 µL) in THF/pyridine (4/2 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH_{2}Cl_{2}/Methanol 95:5 to give the pure product as a white foamy solid (46 mg, yield 16%).

{sup}^{31}P-NMR (MeOD, 121 MHz): δ 5.05, 4.94.

{sup}^{1}H-NMR (MeOD, 300 MHz): δ 7.6-7.5 (1H, 2d, {sup}^{3}J=7Hz H-6), 7.4-7.2 (10H. m, OP_{Ph}+CH_{2}Ph), 6.25 (1H, m, H-1'), 5.95 (1H, 2d, {sup}^{3}J=7Hz, H-5), 5.19 (1H, 2s, CH_{2}Ph), 4.55-4.1 (3H, m, H-3', H-4', CHala), 4.05 (2H, m, H-5'), 1.20 (3H, 2t, {sup}^{3}J=6 Hz, CH_{3}ala).

{sup}^{13}C-NMR (MeOD, 75 MHz): δ 20.8, 20.7 (CH_{3}ala), 52.2, 52.0 (CHala), 66.1 (C-5'), 68.4 (CH_{2}Ph), 71.9, 71.3 (C-3'), 80.6 (C-4'), 85.9 (C-1'), 97.1 (C-5), 121.8, 121.6 ('o', OP_{Ph}), 123 (C-2'), 126.2 ('p', OP_{Ph}), 131.8, 130.0, 129.7 ('m' OP_{Ph}, Bn), 137.9 ('ipso', CH_{2}Ph), 142.7, 142.6 (C-6), 152.5, 152.4 ('ipso', OP_{Ph}), 158.2 (C-2), 168.0 (C-4), 175.3, 174.9 (COOBn).
\[ C_{25}H_{26}ClF_2N_4O_8P, \text{MW}=614.92 (CPF 40). \]

This was synthesised according to **Standard procedure 5**, using gemcitabine (131 mg, 0.5 mmol), para-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate (582 mg, 1.5 mmol), NMI (4.42 mmol, 300 µL) in THF/pyridine (4/2 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH$_2$Cl$_2$/Methanol 95:5 to give the pure product as a white foamy solid (76 mg, yield 25%).

$^{31}$P-NMR (MeOD, 121 MHz): \( \delta \) 5.08.

$^1$H-NMR (MeOD, 300 MHz): \( \delta \) 7.65 (1H, 2d, $^3J=7$Hz H-6), 7.5-7.2 (9H, m, O\textit{Ph}+CH$_2$\textit{Ph}), 6.2 (1H, m, H-1’), 5.9 (1H, 2d, $^3J=7$Hz, H-5), 5.12 (1H, 2s, CH$_2$Ph), 4.6-4.1 (3H, m, H-3’, H-4’, CHala), 4.05 (2H, m, H-5’), 1.45-1.35 (3H, 2t, $^3J=6$Hz, CH$_3$ala).

$^{13}$C-NMR (MeOD, 75 MHz): \( \delta \) 20.9, 20.7 (CH$_3$ala), 52.2, 52.0 (CHala), 66.4, 66.2 (C-5’), 68.5 (CH$_2$Ph), 71.5 (C-3’), 80.7 (C-4’), 86.4 (C-1’), 97.2 (C-5), 123.5 (‘o’, O\textit{Ph}), 126.9 (C-2’), 131.2, 130.6, 130.3 (‘m’ O\textit{Ph}, Bn), 131.9 (‘p’, O\textit{Ph}) 137.5 (‘ipso’, CH$_2$Ph), 142.8, 142.7 (C-6), 151.4, 151.0 (‘ipso’, O\textit{Ph}), 158.2 (C-2), 166.9 (C-4), 175.1, 174.9 (COOBn).
Synthesis of Gemcitabine-[para-chlorophenyl-(benzoxyl-α,α-dimethylglycynyl)]-phosphate (CPF 41).

C₂₆H₂₈ClF₂N₄O₈P, MW=628.95.

This was synthesised according to Standard procedure 5, using gemcitabine (131 mg, 0.5 mmol), para-chlorophenyl-(benzoxyl-α,α-dimethylglycynyl)-phosphorochloridate (603 mg, 1.5 mmol), NMI (4.42 mmol, 300 µL) in THF/pyridine (4/3 mL) for 2 hrs. The crude product was purified by column chromatography, eluting with CH₂Cl₂/Methanol 95:5 to give the pure product as a white foamy solid (163 mg, yield 52%).

³¹P-NMR (MeOD, 121 MHz): δ 3.56, 3.52.

¹H-NMR (MeOD, 300 MHz): δ 7.55 (1H, 2d, ³J=7Hz, H-6), 7.4-7.15 (9H, m, OPh+CH₂Ph), 6.25 (1H, m, H-1’), 5.85 (1H, 2d, ³J=7Hz, H-5), 5.15 (1H, 2s, CH₂Ph), 4.55-4.1(3H, m, H-3’, H-4’), 4.05 (2H, m, H-5’), 1.50 (6H, m, ³J=6 Hz, 2CH₃dimethylgly).

¹³C-NMR (MeOD, 75 MHz): δ 28.2, 28.0 (CH₃ dimethylgly), 58.6 (Cq dimethylgly), 66.2, 66.1 (C-5’), 66.7 (CH₂Ph), 71.5 (C-3’), 80.6 (C-4’), 86.4 (C-1’), 97.0 (C-5), 123.9, 123.6 (’o’, OPh), 127.3 (C-2’), 130.0, 129.7 (’m’ OPh, Bn), 131.8 (’p’, OPh), 137.6 (’ipso’, CH₂Ph), 142.8, 142.7 (C-6), 151.2, 151.1 (’ipso’, OPh), 158.1 (C-2), 167.9 (C-4), 176.8, 176.7 (COOBn).
Synthesis of Phenyl-(methoxy-L-alaninyl)-phosphorochloridate.

C₁₀H₁₃ClNO₄P, MW=277.64.

This is synthesised according to *Standard procedure 4*, using L-alanine methyl ester hydrochloride (2 g, 14.3 mmol), phenyldichlorophosphate (3.02 g, 2.14 ml, 14.3 mmol), and TEA (2.9 g, 4.0 ml, 28.7 mmol) in DCM (60 mL), to yield 3.91 g (98%) of crude product used without further purification.

³¹P-NMR (CDCl₃, 121 MHz): δ 9.28, 8.97.

¹H-NMR (CDCl₃; 300 MHz): δ 7.39-7.34 (2H, m , ’o’ OPh), 7.29-7.20 (2H, m , ’m+p’ OPh), 4.98 (1H, bs, NH₂), 4.27-4.09 (1H, m, CHala), 3.78 (3H, s, OCH₃), 1.52-1.49 (3H, 2xd, 3J=7Hz, CH₃ala).

¹³C-NMR (CDCl₃; 75 MHz): δ 20.9 (CH₃ala), 51.0 (CHala), 53.6 (OCH₃), 120.9 (’o’ OPh), 126.4 (’p’, OPh), 130.2 (’m’, OPh), 150.1 (‘ipso’, OPh), 173.6 (COOCH₃).

Synthesis of Phenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

C₁₁H₁₅ClNO₄P, MW=291.67.

This is synthesised according to *Standard procedure 4*, using L-alanine ethyl ester hydrochloride (770 mg, 5.01 mmol), phenyldichlorophosphate (1.12g, 5.01 mmol, 749
µL), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.02 (69%) of oil.

\( ^{31}\text{P-NMR (CDCl}_3, 121 \text{ MHz): } \delta 9.49, 9.07. \)

\( ^{1}\text{H-NMR (CDCl}_3; 300 \text{ MHz): } \delta 7.39-7.34 \text{ (2H, m,'o' OPh), 7.29-7.20 \text{ (2H, m, 'm+p' OPh)}, 4.95 \text{ (1H, bs, NH)])}, \text{ 4.3-4.1 (3H, m, OCH}_3\text{CH}_3, \text{CHala), 1.50 (3H, 2x, } ^{3}J=7\text{Hz, CH}_3\text{ala), 1.30 (3H, t, } ^{3}J=7.1 \text{ Hz, OCH}_2\text{CH}_3). \)

\( ^{13}\text{C-NMR (CDCl}_3; 75 \text{ MHz): } \delta 14.5 \text{ (CH}_3\text{CH}_2), 20.9 \text{ (CH}_3\text{ala), 51.0 (CHala), 62.6 CH}_3\text{CH}_2), 120.9 (\text{'o' OPh), 126.5 ('} p', \text{ OPh), 130.1 ('} m', \text{ OPh), 150.1 ('} ipso', \text{ OPh), 175.1 (COOCH}_2\text{CH}_3). \)

Synthesis of Phenyl-(benzoyl-L-alaninyl)-phosphorochloridate.

C\(_{16}\)H\(_{17}\)ClNO\(_4\)P, MW= 353.74.

\[
\text{\includegraphics[width=0.5\textwidth]{synthesis.png}}
\]

This is synthesised according to Standard procedure 4, using L-alanine benzyl ester hydrochloride (1.0 g, 4.64 mmol), phenyl-dichlorophosphate (980 mg, 0.69 ml, 4.64 mmol), and TEA (0.94 g, 1290 µL, 9.27 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 6:4) affording 1.61 (98%) of oil.

\( ^{31}\text{P-NMR (CDCl}_3, 121 \text{ MHz): } \delta 9.41, 9.23. \)

\( ^{1}\text{H-NMR (CDCl}_3; 300 \text{ MHz): } \delta 7.41-7.21 \text{ (10H, m, OPh+CH}_2\text{Ph), 5.24 (2H, s, CH}_2\text{Ph), 4.95-4.88 (1H, bs, NH)], 4.36-4.15 (1H, m, CHala), 1.52-1.49 (3H, 2x, } ^{3}J=7\text{Hz, CH}_3\text{ala).} \)

\( ^{13}\text{C-NMR (CDCl}_3; 75 \text{ MHz): } \delta 20.8 \text{ (CH}_3\text{ala), 51.1 (CHala), 68.0 (CH}_2\text{Ph), 121.0 ('} \text{o'} \text{ OPh), 126.4 ('} p', \text{ OPh), 130.3, 129.0, 128.7 ('} m'OPh, \text{CH}_2\text{Ph), 135.5 ('} ipso', \text{CH}_2\text{Ph), 150.2 ('} ipso', \text{OPh), 172.9 (COOCH}_2\text{Ph).} \)

Synthesis of p-nitrophenyl-(methoxy-L-alaninyl)-phosphorochloridate.

C\(_{10}\)H\(_{12}\)ClN\(_2\)O\(_4\)P, MW=322.64.
This is synthesised according to Standard procedure 4, using L-alanine methyl ester hydrochloride (0.70 g, 5.01 mmol), p-nitrophenyldichlorophosphate (1.362 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.60 g (99%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.13, 9.03.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 8.1 (2H, 2d, $^3$J=8Hz, OPh), 7.3 (2H, 2d, $^3$J=8Hz, OPh), 5.0 (1H, bs, NH), 4.1 (1H, m, CHala), 3.75 (3H, s, OCH$_3$), 1.5-1.45 (3H, m, CH$_3$ala).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 20.8, 20.7 (CH$_3$ala), 51.1, 50.9 (CHala), 53.2, 53.2 (OCH$_3$), 121.8, 121.6 ('o’ OPh), 126.5 ('m’, OPh), 145.7 ('ipso’, OPh), 154.7, 154.6 ('p’, OPh), 173.4, 173.2 (COOCH$_3$).

Synthesis of p-nitrophenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

C$_{11}$H$_{14}$ClN$_2$O$_5$P, MW=336.67.

This is synthesised according to Standard procedure 4, using L-alanine ethyl ester hydrochloride (770 mg, 5.01 mmol), p-nitrophenyldichlorophosphate (1.362g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL), to yield 1.64 g (98%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.06, 8.81.
$^1$H-NMR (CDCl$_3$; 300 MHz): δ 8.1 (2H, m, OPh), 7.4 (2H, m, OPh), 4.9-4.7 (1H, bs, NH), 4.3-4.1 (3H, m, OCH$_2$CH$_3$, CHala), 1.55-1.45 (3H, 2xd, $^3$J=7Hz, CH$_2$ala), 1.40 (3H, t, $^3$J=7Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 14.5 (CH$_3$CH$_2$), 21.1, 20.9 (CH$_2$ala), 51.2, 51.0 (CHala), 62.6, CH$_3$CH$_2$, 121.7, 121.3 (‘o’ OPh), 126.2, 126.0 (‘m’, OPh), 145.7 (‘ipso’, OPh), 154.5 (‘p’, OPh), 173.4, 173.3 (COOCH$_2$CH$_3$).

**Synthesis of p-nitrophenyl-(benzoxy-L-alaninyl)-phosphorochloridate.**

C$_{16}$H$_{16}$ClN$_2$O$_6$P, MW= 398.04.

![Chemical Structure](image)

This is synthesised according to *Standard procedure 4*, using L-alanine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-nitrophenyl-dichloro phosphate (1.362 g, 5.01 mmol), and TEA (1.4 mL, 1.4 mmol) in DCM (40 mL), to yield 1.85 g (93%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 9.15, 9.06.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 8.15 (2H, m, OPh), 7.45 (2H, m, OPh), 7.35-7.25 (5H, m, CH$_2$Ph), 5.2 (2H, 2s, CH$_2$Ph), 5.00 (1H, bs, NH), 4.2 (1H, m, CHala), 1.64 (3H, 2xd, $^3$J=7Hz, CH$_2$ala).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 20.8 (CH$_3$ala), 51.1 (CHala), 68.0 (CH$_2$Ph), 121.4 (‘o’ OPh), 126.1 (‘m’ OPh), 130.3, 129.0 (CH$_2$Ph), 145.7 (‘ipso’, CH$_2$Ph), 150.2 (‘ipso’, OPh), 154.6 (‘p’, OPh), 172.9 (COOCH$_2$Ph).

**Synthesis of p-fluorophenyl-(methoxy-L-alaninyl)-phosphorochloridate.**

C$_{10}$H$_{12}$ClFNO$_4$P, MW=295.63.
This is synthesised according to Standard procedure 4, using L-alanine methyl ester hydrochloride (0.70 g, 5.01 mmol), p-fluorophenyl dichlorophosphate (1.210 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.11 g (75%) of oil.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.98, 9.96.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.1 (2H, m, OPh), 6.95 (2H, m, OPh), 5.0 (1H, bs, NH), 4.25-4.1 (1H, m, CHala), 3.78 (3H, 2s, OCH$_3$), 1.55 (3H, m, CH$_3$ala).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 20.8 (CH$_3$ala), 51.1, 50.9 (CHala), 53.3 (OCH$_3$), 117.1, 117.0 (‘o’ OPh), 122.6, 122.5 (‘m’, OPh), 146.0 (‘ipso’, OPh), 159.1, 159.0 (‘p’, OPh), 173.4, 173.2 (COOCH$_3$).

Synthesis of p-fluorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

C$_{11}$H$_{14}$ClFNO$_3$P, MW=309.66.

This is synthesised according to Standard procedure 4, using L-alanine ethyl ester hydrochloride (770 mg, 5.01 mmol), p-fluorophenyl dichlorophosphate (1.210g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.07 (69%) of oil.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 10.04, 9.95.
\(^1\)H-NMR (CDCl\(_3\); 300 MHz): \(\delta\) 7.1 (2H, m, OPh), 6.95 (2H, m, OPh), 5.0 (1H, bs, NH), 4.25-4.1 (3H, m, OCH\(_2\)CH\(_3\), CHala), 1.55 (3H, m, CH\(_3\)ala), 1.40 (3H, t, \(^3J=7\)Hz, OCH\(_2\)CH\(_3\)).
\(^13\)C-NMR (CDCl\(_3\); 75 MHz): \(\delta\) 14.5 (CH\(_3\)CH\(_2\)), 21.1, 21.0 (CH\(_3\)ala), 51.2, 51.1 (CHala), 62.6 (CH\(_3\)CH\(_2\)), 117.3 ('o' OPh), 122.2, 122.0 ('m', OPh), 145.9, 145.8 ('ipso', OPh), 159.0 ('p', OPh), 173.6, 173.5 (COOCH\(_2\)CH\(_3\)).

**Synthesis of p-fluorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate.**

C\(_{16}\)H\(_{16}\)ClFNO\(_4\)P, MW= 371.73.

![Chemical Structure](image)

This is synthesised according to **Standard procedure 4**, using L-alanine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-fluorophenyl-dichloro phosphate (1.210 mg, 5.01 mmol), and TEA (1.4mL, 1.4 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.599 (86%) of oil.

\(^31\)P-NMR (CDCl\(_3\), 121 MHz): \(\delta\) 9.15, 9.06.
\(^1\)H-NMR (CDCl\(_3\); 300 MHz): \(\delta\) 7.35-7.25 (5H, m, CH\(_2\)Ph), 7.1 (2H, m, OPh), 6.95 (2H, m, OPh), 5.2 (2H, 2s, CH\(_2\)Ph), 5.00 (1H, bs, NH), 4.25-4.1 (1H, m, CHala), 1.55 (3H, m, CH\(_3\)ala).
\(^13\)C-NMR (CDCl\(_3\); 75 MHz): \(\delta\) 20.8 (CH\(_3\)ala), 51.1, 51.0 (CHala), 68.1 (CH\(_2\)Ph), 117.0, 116.9 ('o' OPh), 122.6 ('m' OPh), 130.3, 129.0 (CH\(_2\)Ph), 135.7 ('ipso', CH\(_2\)Ph), 146.1, 146.0 ('ipso', OPh), 158.9 ('p' OPh), 173.1 (COOCH\(_2\)Ph).

**Synthesis of 4-(trifluoromethyl)-phenyl-(methoxy-L-alaninyl)-phosphorochloridate.**

C\(_{11}\)H\(_{12}\)ClF\(_3\)NO\(_4\)P, MW=345.64.
This is synthesised according to Standard procedure 4, using L-alanine methyl ester hydrochloride (1.0 g, 7.16 mmol), 4-(trifluoromethyl)-phenyl-phosphodichloridate (1.998 g, 7.16 mmol), and TEA (1.449 g, 14.32 mmol, 1916 μL) in DCM (30 mL), to yield 2.202 g (89.0%) of crude product used without further purification.

$^3$P-NMR (CDCl$_3$, 121 MHz): δ 9.36, 9.22.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.66 (2H, d, $^3$J=8.1 Hz, OPh), 7.44-7.33 (2H, m, OPh), 5.10 (1H, bs, NH), 3.81-3.78 (3H, 2s, CH$_2$O), 3.77-3.68 (1H, m, CH$_3$CH), 1.56-1.52 (3H, m, CHCH$_2$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 20.6, 20.7 (CH$_3$CH), 50.9, 51.1 (CHCH$_3$), 53.2 (CH$_3$O), 121.4 ('o', OPh), 124.1 (CF$_3$, J=270 Hz), 128.0 ('m', OPh), 128.6 ('p', J=34 Hz), 152.4, 152.6 ('ipso', OPh), 173.4, 173.5 (COOCH$_3$).

Synthesis of 4-(trifluoromethyl)-phenyl-(ethoxy-L-alaninyl)-phosphorochloridate.
C$_{12}$H$_{14}$ClF$_3$NO$_4$, MW=359.67.

This is synthesised according to Standard procedure 4, using L-alanine ethyl ester hydrochloride (1.0 g, 6.50 mmol), 4-(trifluoromethyl)-phenyl-phosphodichloridate (1.813 g, 6.50 mmol), and TEA (1.316 g, 13.00 mmol, 1740 μL) in DCM (30 mL), to yield 2.150 g (92.2%) of crude product used without further purification.

$^3$P-NMR (CDCl$_3$, 121 MHz): δ 9.33, 9.28.
\(^1\)H-NMR (CDCl\(_3\); 300 MHz): \(\delta\) 7.70 (2H, d, \(^3\)J=8.2 Hz, OPh), 7.46-7.39 (2H, m, OPh), 4.78 (1H, bs, NH), 4.33-4.17 (3H, m, CH\(_3\)CH\(_2\)O+ CH(CH\(_3\))\(_2\)), 1.59-1.55 (1H, m, CHCH\(_3\)), 1.56-1.52 (3H, m, CH\(_2\)CH\(_3\)).

\(^{13}\)C-NMR (CDCl\(_3\); 75 MHz): \(\delta\) 14.5 (CH\(_3\)CH\(_2\)O), 20.8, 20.9 (CH\(_3\)CH), 50.3, 50.9 (CH(CH\(_3\))), 62.3, 62.5 (CH\(_3\)CH\(_2\)O), 121.4 ('o', OPh), 124.1 (CF\(_3\), \(J=270\) Hz), 127.7 ('m', OPh), 128.7 ('p', \(J=33\) Hz), 152.4 ('ipso', OPh), 172.9 (COOCH\(_2\)CH\(_3\)).

Synthesis of \(p\)-trifluorophenyl-(benzoxyl-L-alaninyl)-phosphorochloridate.

\(\text{C}_{17}\text{H}_{16}\text{ClF}_{3}\text{NO}_{4}\text{P}, \text{MW}=421.73\).

\[
\text{F}_3\text{C}\quad \text{O} \quad \text{P} \quad \text{Cl} \\
\text{BnO} \quad \text{NH} \\
\text{O} \quad \text{O}
\]

This is synthesised according to \textit{Standard procedure 4}, using L-alanine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-trifluorophenyl-dichloro phosphate (1.490 mg, 5.01 mmol), and TEA (1.4 mL, 1.4 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 6:4) affording 1.80 (85%) of oil.

\(^{31}\)P-NMR (CDCl\(_3\), 121 MHz): \(\delta\) 9.11, 8.84.

\(^1\)H-NMR (CDCl\(_3\); 300 MHz): \(\delta\) 7.65 (2H, m, OPh), 7.4-7.2 (7H, m, CH\(_2\)Ph + 2H OPh), 5.25 (2H, 2s, CH\(_2\)Ph), 4.75-4.55 (1H, bs, NH), 4.25-4.1 (1H, m, CHala), 1.60-1.55 (3H, 2d, \(J=7\)Hz, CH\(_3\)ala).

\(^{13}\)C-NMR (CDCl\(_3\); 75 MHz): \(\delta\) 20.9 (CH\(_3\)ala), 51.3, 51.0 (CHala), 68.2, 68.1 (CH\(_2\)Ph), 121.4, 120.9 ('o', OPh), 125.2 (d, \(J=270\)Hz, CF\(_3\)), 126.6 ('m', OPh), 129.1, 128.8, 127.8 (Bn), 130.0 ('p', \(J=32\)Hz, OPh), 135.4 ('ipso', CH\(_2\)Ph), 153.0 ('ipso', OPh), 172.8 (COOCH\(_2\)Ph).

Synthesis of 4-chlorophenyl-(methoxy-L-alaninyl)-phosphorochloridate.

\(\text{C}_{10}\text{H}_{12}\text{Cl}_{2}\text{NO}_{4}\text{P}, \text{MW}=312.09\).
This is synthesised according to Standard procedure 4, using L-alanine methyl ester hydrochloride (1.0 g, 7.16 mmol), 4-chlorophenylphosphorodichloridate (1.757 g, 7.16 mmol), and TEA (1.449 g, 14.32 mmol, 1995 μL) in DCM (30 mL), to yield 1.621 g (72.5%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.36, 9.07.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 7.35-7.15 (4H, m, OPh), 4.48-4.36 (1H, bs, NH), 4.22-4.04 (1H, m, CH$_2$CH$_3$), 3.76-3.74 (3H, 2s, CH$_2$O), 1.49-1.46 (3H, m, CH$_2$H$_2$).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 21.0 (CH$_3$CH), 50.8, 51.1 (CH$_2$CH$_3$), 53.4 (CH$_3$O), 121.9, 122.1, 122.3, 122.4 ('o', OPh), 130.6, 130.4, 130.2 ('m', OPh), 132.0 ('p', OPh), 148.6 ('ipso', OPh), 173.5 (COOCH$_3$).

Synthesis of 4-chlorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate.

C$_{11}$H$_{14}$Cl$_2$NO$_4$P, MW=326.11.

This is synthesised according to Standard procedure 4, using L-alanine ethyl ester hydrochloride (1.000 g, 6.50 mmol), 4-chlorophenylphosphorodichloridate (1.595 g, 6.50 mmol), and TEA (1.315 g, 13.00 mmol, 1810 μL) in DCM (20 mL), to yield 1.794 mg (yield 84.7%) of product.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.54, 9.25.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 7.44-7.21 (4H, m, OPh), 4.59 (1H, bs, NH), 4.33-4.13 (3H, m, OCH$_2$CH$_3$ + CH$_2$CH$_3$), 1.57-1.56 (3H, m, CH$_3$CH), 1.43-1.21 (3H, m, OCH$_2$CH$_3$).
Synthesis of 4-nitrophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{16}H_{16}Cl_2NO_4P$, MW=388.18.

This is synthesised according to *Standard procedure 4*, using L-alanine benzyl ester hydrochloride (1.000 g, 4.63 mmol), 4-chlorophenylphosphodichloride (1.136 g, 4.63 mmol), and TEA (937.0 mg, 9.26 mmol) in DCM (40 mL), to yield 1534 mg (yield 86.5%) of crude product used without further purification.

$^{13}P$-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.43, 9.16.

$^{1}H$-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.42-7.08 (9H, m, OP$\phi$ CH$_2$Ph), 5.19 (2H, s, CH$_2$Ph), 4.61-4.54 (1H, bs, NH), 4.26-4.10 (1H, m, CHCH$_3$), 1.42-1.38 (3H, m, CH$_3$CH).

$^{13}C$-NMR (CDCl$_3$; 75 MHz): $\delta$ 20.9, 21.0 (CH$_3$CH), 51.0, 51.2 (CHCH$_3$), 68.1, 68.2 (OCH$_2$Ph), 122.3, 122.4 ('o', OP$\phi$), 128.8, 129.1, 130.4 ('o', 'm', 'p', CH$_2$Ph+OP$\phi$), 131.9 ('ipso', CH$_2$Ph), 135.3 ('p', OP$\phi$), 148.5 ('ipso', OP$\phi$), 172.7, 172.8 (COOCH$_2$Ph).

Synthesis of phenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

$C_{11}H_{15}ClNO_4P$, MW=291.67.
This is synthesised according to **Standard procedure 4**, using 2-aminoisobutyrate methyl ester hydrochloride (583.5 mg, 3.75 mmol), phenyl dichlorophosphate (791.1 mg, 3.75, 560 µL), and TEA (758.9 mg, 7.5 mmol, 1045 µL) in DCM (20 mL), to yield 1.041 g (95.2%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 6.99 (s).

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 7.41-7.17 (5H, m, OPh), 4.98 (1H, bs, NH), 3.80 (3H, s, OCH$_3$), 1.71-1.69 (6H, 2s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 27.3, 27.2, 27.0 ([CH$_3$]$_2$C), 53.6 (OCH$_3$), 58.8 ([CH$_3$]$_2$), 120.0, 121.1 ('o' OPh), 126.2 ('p', OPh), 130.3 ('m', OPh) 145.7 ('p', OPh), 150.2, 150.3 ('ipso', OPh), 175.6, 175.7 (COOCH$_3$).

**Synthesis of phenyl-(ethyl-2-amino-2-methylpropanoate)-phosphonochloridate.**

C$_{12}$H$_{17}$CINO$_4$P, MW=305.69.

This is synthesised according to **Standard procedure 4**, using 2-aminoisobutyrate ethyl ester hydrochloride (628.6 mg, 3.75 mmol), phenyl dichlorophosphate (791.1 mg, 3.75, 560 µL), and TEA (758.9 mg, 7.5 mmol, 1045 µL) in DCM (20 mL), to yield 1.018 g (88.8%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 7.02 (s)

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 7.23-7.37 (5H, m, OPh), 4.98 (1H, bs, NH), 4.24 (2H, q, $^3$J=7.1 Hz, OCH$_3$CH$_3$), 1.70, 1.68 (6H, 2s, [CH$_3$]$_2$C), 1.30 (3H, t, $^3$J=7.1 Hz, OCH$_2$CH$_3$).
13C-NMR (CDCl3; 75 MHz): δ 14.5 (CH3CH2O), 27.3, 26.9 ([CH3]2C), 58.7 ([CH3]2), 62.7 (OCH2CH3), 121.1, 121.0 (’o’, OPh), 127.6 (’p’, OPh), 130.7 (’m’, OPh), 150.4 (’ipso’, OPh), 175.2, 175.1 (COOCH2CH3).

C17H17ClNO4P, MW= 367.76.

\[ \text{\includegraphics[width=0.5\textwidth]{structure.png}} \]

This is synthesised according to Standard procedure 4, using 2-aminoisobutyrate benzyl ester hydrochloride (861.4 mg, 3.75 mmol), phenyl dichlorophosphate (791.1 mg, 3.75, 560 µL), and TEA (758.9 mg, 7.5 mmol, 1045 µL) in DCM (30 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 6:4) affording 580 mg (42.2%) of oil.

31P-NMR (CDCl3, 121 MHz): δ 6.79 (s)

1H-NMR (CDCl3; 300 MHz): δ 7.45-7.27 (10H, m, OPh+CH2Ph), 5.28 (2H, s, CH2Ph), 4.81, 4.78 (1H, 2bs, NH), 1.78, 1.75 (6H, 2s, [CH3]2C).

13C-NMR (CDCl3; 75 MHz): δ 27.3, 26.9 ([CH3]C), 53.9 ([CH3]2), 60.9 (CH2Ph), 121.0, 126.3, 128.6, 129.0, 129.1, 130.3, 135.5 (OPh, CH2Ph), 135.5 (’ipso’, CH2Ph), 150.3, 150.2 (’ipso’, OPh), 175.0, 175.2 (COOCH2Ph).

20 Synthesis of 4-nitrophenoxy-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

25
This is synthesised according to *Standard procedure 4*, using 2-aminoisobutyrate methyl ester hydrochloride (290.0 mg, 1.89 mmol), 4-nitrophenylphosphodichloride (483.3 mg, 1.89 mmol), and TEA (382.5 mg, 3.78 mmol, 526.9 µL) in DCM (15 mL), to yield 486 mg (yield 76.4%) of crude product used without further purification.

³¹P-NMR (CDCl₃, 121 MHz): δ 6.61 (s)

¹H-NMR (CDCl₃; 300 MHz): δ 8.25 (2H, d, J=9.0 Hz, OPh), 7.43 (2H, d, J=9.0 Hz, OPh), 4.91-4.87 (1H, 2bs, NH), 3.79 (3H, s, OCH₃), 1.69-1.66 (6H, 2s, [CH₃]₂C).

¹³C-NMR (CDCl₃; 75 MHz): δ 27.0, 27.1, 27.3 ([CH₃]₂C), 53.8 (OCH₃), 59.2 (CH₃), 121.7, 121.8 (o' OPh), 126.2 (m', OPh), 145.7 (p', OPh), 154.8, 154.7 (ipso', OPh), 175.4, 175.6 (COOCH₃).

**Synthesis of 4-nitrophenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate.**

C₁₂H₁₆ClN₂O₆P, MW=350.69.

This is synthesised according to *Standard procedure 4*, using 2-aminoisobutyrate ethyl ester hydrochloride (270.0 mg, 1.61 mmol), 4-nitrophenylphosphodichloride (412.3 mg, 1.61 mmol), and TEA (325.8 mg, 3.22 mmol, 448.8 µL) in DCM (15 mL), to yield 500 mg (yield 88.5%) of crude product used without further purification.

³¹P-NMR (CDCl₃, 121 MHz): δ 6.64 (s)
1H-NMR (CDCl3; 300 MHz): δ 8.35 (2H, d, 3J=9.0 Hz, OPh), 7.53 (2H, d, 3J=9.0 Hz, OPh), 4.99-4.96 (1H, 2bs, NH), 4.34 (2H, q, 3J=7.1 Hz, OCH2CH3), 1.79-1.76 (6H, 2s, [CH3]2C), 1.40 (3H, t, 3J=7.1 Hz, OCH2CH3).
13C-NMR (CDCl3; 75 MHz): δ 14.5 (OCH2CH3), 27.0, 27.3 ([CH3]2C), 59.1, 59.2 (OCH2CH3), 121.7, 121.8 (‘o’ OPh), 126.2 (‘m’, OPh), 145.7 (‘p’, OPh), 154.7, 154.8 (‘ipso’, OPh), 175.4, 175.6 (COOCH2CH3).

Synthesis of 4-nitrophenyl-(benzyl-2-amino-2-methylpropanoate)-phosphorochloridate.

C17H16ClN3O6P, MW=412.76.

This is synthesised according to Standard procedure 4, using 2-aminoisobutyrate benzyl ester hydrochloride (578 mg, 2.52 mmol), 4-nitrophenolphosphodicloride (645 mg, 2.52 mmol), and TEA (510 mg, 5.04 mmol, 702.5 μL) in DCM (20 mL), to yield 936 mg (yield 90.0%) of crude product used without further purification.

31P-NMR (CDCl3, 121 MHz): δ 6.56 (s)
1H-NMR (CDCl3; 300 MHz): δ 8.29 (2H, d, 3J=9.0 Hz, OPh), 7.47 (2H, d , 3J=9.0 Hz, OPh), 7.40-7.37 (5H, m, CH2Ph), 5.27 (2H, s, CH2Ph), 5.04-5.01 (1H, 2bs, NH), 1.77-1.74 (6H, 2s, [CH3]2C).
13C-NMR (CDCl3; 75 MHz): δ 27.0, 27.3, ([CH3]2C), 59.2 ([CH3]2C), 68.5 (OCH2Ph), 121.6, 121.7, 126.2, 128.6, 129.1, (‘o’, ‘m’, ‘p’, CH2Ph+ OPh), 135.7 (‘ipso’, CH2Ph), 145.7 (‘p’, OPh), 154.7, 154.8 (‘ipso’, OPh), 175.8, 175.9 (COOCH2Ph).

Synthesis of 4-chlorophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

C11H14Cl2NO4P, MW=326.11
This is synthesised according to **Standard procedure 4**, using 2-aminoisobutyrate methyl ester hydrochloride (280.0 mg, 1.82 mmol), 4-chlorophenylphosphodichloride (447.4 mg, 1.82 mmol), and TEA (368.3 mg, 3.64 mmol, 507.3 μL) in DCM (20 mL), to yield 554 mg (yield 91.1%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 7.05 (s)

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.38 (2H, d, $^3$J=9.0 Hz, OPh), 7.28-7.24 (2H, 2d, $^3$J=9.0 Hz, OPh), 4.87-4.83 (1H, 2bs, NH), 3.84 (3H, s, OCH$_3$), 1.73-1.71 (6H, 2s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 27.0, 27.3, ([CH$_3$]$_2$C), 53.7 (OCH$_3$), 58.9 ([CH$_3$]$_2$), 122.5 ('o', OPh), 129.7 ('m', OPh), 131.8 ('p', OPh) 148.7, 148.9 ('ipso', OPh), 175.5, 175.7 (COOCH$_3$).

**Synthesis of 4-chlorophenyl-(ethyl-2-amino-2-methylpropanoate)-phosphorochloridate.**

C$_{12}$H$_{16}$Cl$_2$NO$_4$P, MW=340.14.

This is synthesised according to **Standard procedure 4**, using 2-aminoisobutyrate ethyl ester hydrochloride (293.4 mg, 1.75 mmol), 4-chlorophenylphosphodichloride (430.0 mg,
1.75 mmol), and TEA (354.2 mg, 3.50 mmol, 488.0 μL) in DCM (15 mL), to yield 571.7 mg (yield 96.1%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 7.09 (s)

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.38 (2H, d, $^3$J=9.1 Hz, OPh), 7.26 (2H, d, $^3$J=9.1 Hz, OPh), 4.88-4.84 (1H, 2bs, NH), 4.29 (2H, q, $^3$J=7.1 Hz, OCH$_2$CH$_3$), 1.74-1.70 (6H, 2s, CH$_3$), 1.35 (3H, t, $^3$J=7.1 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 145.0 (OCH$_2$CH$_3$), 27.0, 27.3 ([CH$_3$]$_2$C), 58.9 ([C]$_2$CH$_3$), 62.8 (OCH$_2$CH$_3$), 122.5 ('o', OPh), 130.4 ('m', OPh), 131.8 ('p', OPh), 148.7, 148.8 ('ipso', OPh), 175.1, 175.3 ('COOCH$_2$CH$_3$).

Synthesis of 4-chlorophenyl-(benzyl-2-amino-2-methylpropanoate)-
phosphorochloridate.

C$_{17}$H$_{16}$Cl$_2$NO$_4$P, MW=402.21.

This is synthesised according to Standard procedure 4, using 2-aminoisobutyrate benzyl ester hydrochloride (402.0 mg, 1.75 mmol), 4-chlorophenylphosphodichloride (430 mg, 1.75 mmol), and TEA (354.2 mg, 3.50 mmol, 488.0 μL) in DCM (15 mL), to yield 657.9 mg (yield 93.5%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 7.00 (s)

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.39-7.12 (9H, m, CH$_2$Ph+ OPh), 5.18 (2H, s, CH$_2$Ph), 4.75-4.72 (1H, 2bs, NH), 1.68-1.65 (6H, 2s, [CH$_3$]$_2$C).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 27.0, 27.3, ([CH$_3$]$_2$C), 59.0 ([C]$_2$CH$_3$), 68.4 (OCH$_2$Ph), 122.5, 128.6, 129.1, 130.7 ('o', 'm', 'p', CH$_2$Ph+ OPh), 131.8 ('p', CH$_2$Ph), 135.4 ('p', OPh), 148.6, 148.7 ('ipso', OPh), 174.9, 175.1 ('COOCH$_2$Ph').

C\textsubscript{18}H\textsubscript{18}ClF\textsubscript{3}NO\textsubscript{4}P, MW=435.76.

This is synthesised according to Standard procedure 4, using 2-aminoisobutyrate benzyl ester hydrochloride (341.0 mg, 1.49 mmol), 4-(trifluoromethyl)-phenyl-phosphodichloridate (414.3 mg, 1.49 mmol), and TEA (300.5 mg, 2.97 mmol, 413.9 µL) in DCM (15 mL), to yield 623.9 mg (96.4%) of crude product used without further purification.

\textsuperscript{31}P-NMR (CDCl\textsubscript{3}, 121 MHz): \( \delta \) 6.74 (s)

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}; 300 MHz): \( \delta \) 7.66 (2H, d, \( J=8.8 \) Hz, O\textsubscript{Ph}), 7.42-7.30 (7H, m, O\textsubscript{Ph}+CH\textsubscript{2}Ph), 5.25 (2H, s, CH\textsubscript{2}Ph), 4.95-4.91 (1H, 2bs, NH), 1.75-1.72 (6H, 2s, [CH\textsubscript{3}]\textsubscript{2}C).

\textsuperscript{13}C-NMR (CDCl\textsubscript{3}; 75 MHz): \( \delta \) 26.9, 27.0, 27.3 ([CH\textsubscript{3}]\textsubscript{2}C), 59.1 (C[CH\textsubscript{3}]\textsubscript{2}), 68.4 (CH\textsubscript{2}Ph), 121.1, 121.4, 127.7, 128.4, 128.5, 128.6, 128.9 (‘o’, ‘m’, ‘p’, O\textsubscript{Ph}+CH\textsubscript{2}Ph), 124.2 (CF\textsubscript{3}, \( J=265 \) Hz), 135.4 (‘ipso’, CH\textsubscript{2}Ph), 152.6, 152.7 (‘ipso’, O\textsubscript{Ph}), 174.9, 175.0 (COOCH\textsubscript{2}Ph).

Synthesis of Phenyl-(methoxy-\( \alpha,\alpha \)-cycloleucinyl)-phosphorochloridate.

C\textsubscript{13}H\textsubscript{13}CINO\textsubscript{4}P, MW=317.70.
This is synthesised according to *Standard procedure 4*, using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.885 g, 5.01 mmol), phenyl dichlorophosphate (1.12 g, 0.749 ml, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.266 g (81%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 7.90.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.4-7.2 (5H, m, OPh), 4.3 (1H, bs, NH), 3.75 (3H, 2s, OCH$_3$), 2.15 (4H, m, 4H cyclopentane), 1.9-1.7 (4H, m, 4H cyclopentane).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 24.4 (2CH$_2$ cyclopent), 38.8, 38.7, 38.6 (2CH$_2$ cyclopent), 53.3, 53.2 (CH$_3$O), 66.6 (Cg cyclopentane), 121.1, 121.0 (‘o’ OPh), 126.3 (‘p’, OPh), 130.3, 130.2 (‘m’, OPh), 150.2 (‘ipso’, OPh), 174.8 (COOCH$_3$).

**Synthesis of Phenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.**

C$_{14}$H$_{19}$ClNO$_4$P, MW=331.73.

This is synthesised according to *Standard procedure 4*, using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (955 mg, 5.01 mmol), phenyl dichlorophosphate (1.12 g, 5.01 mmol, 749 µL), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL). The crude was purified by flash chromatography (ethyl acetate/petroleum ether 7:3) affording 1.457 g (89%) of oil.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 8.04, 7.97.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.4-7.1 (5H, m, OPh), 4.7 (1H, bs, NH), 4.2 (2H, 2q, $^3$J=7.1 Hz, OCH$_2$CH$_3$), 2.15 (4H, m, 4H cyclopentane), 1.9-1.7 (4H, m, 4H cyclopentane), 1.30 (3H, t, $^3$J=7.1 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 14.5 (CH$_3$CH$_2$), 24.5 (2CH$_2$ cyclopent), 38.8, 38.7, 38.6, 38.5 (2CH$_2$ cyclopent), 62.0 (CH$_3$CH$_2$), 68.3 (Cg cyclopentane), 120.9 (‘o’ OPh), 126.3 (‘p’, OPh), 130.3 (‘m’, OPh), 150.3-150.2 (‘ipso’, OPh), 174.9-174.8 (COOCH$_2$CH$_3$).
Synthesis of Phenyl-(benzoxy-\(\alpha,\alpha\)-cycloleucinyl)-phosphorochloridate.

\(\text{C}_{19}\text{H}_{21}\text{ClNO}_4\text{P}, \text{MW}=393.80.\)

\[
\begin{array}{c}
\text{Ph} \quad \text{O} \quad \text{P} \quad \text{Cl} \\
\text{BnO} \quad \text{NH} \quad \text{O} \\
\end{array}
\]

This is synthesised according to *Standard procedure 4*, using benzyl-1-amo\(\text{n}\)-1-cyclopentanoate hydrochloride salt (0.984 g, 3.84 mmol), phenyl-dichlorophosphate (0.577 ml, 3.84 mmol), and TEA (1.08 mL, 7.69 mmol) in DCM (30 mL), to yield 1.485 g (98%) of crude product used without further purification.

\(^{31}\text{P}-\text{NMR (CDCl}_3, 121 \text{ MHz): } \delta 7.85.\)

\(^{1}\text{H}-\text{NMR (CDCl}_3; 300 \text{ MHz): } \delta 7.3-7.0 (10\text{H, m, OPh}_\text{Ph}+\text{CH}_2\text{Ph}), 5.2 (2\text{H, s, CH}_2\text{Ph}), 4.95-4.65 (1\text{H, bs, N[H]}), 2.25-2.1 (4\text{H, m, 4H cyclopentane}), 1.9-1.7 (4\text{H, m, 4H cyclopentane}).\)

\(^{13}\text{C}-\text{NMR (CDCl}_3; 75 \text{ MHz): } \delta 24.4, 24.3 (2\text{CH}_2 \text{cyclopent}), 38.8, 38.7, 38.5 (2\text{CH}_2 \text{cyclopent}), 67.3 (\text{Cg cyclopentane}), 68.0 (\text{CH}_2\text{Ph}), 121.0 ('o' \text{ OPh}), 126.4 (‘p’, OPh), 130.1, 129.0, 128.8 (‘m’OPh, \text{CH}_2\text{Ph}), 135.4 (‘ipso’, \text{CH}_2\text{Ph}), 150.1 (‘ipso’, OPh), 173.4 (\text{COOCH}_2\text{Ph}).\)

Synthesis of p-fluorophenyl-(methoxy-\(\alpha,\alpha\)-cycloleucinyl)-phosphorochloridate.

\(\text{C}_{13}\text{H}_{16}\text{ClNO}_4\text{P, MW}=335.70.\)

\[
\begin{array}{c}
\text{F} \quad \text{O} \quad \text{P} \quad \text{Cl} \\
\text{MeO} \quad \text{NH} \quad \text{O} \\
\end{array}
\]

This is synthesised according to *Standard procedure 4*, using methyl-1-amo\(\text{n}\)-1-cyclopentanoate hydrochloride salt (0.885 g, 5.01 mmol), para-
fluorophenyl dichlorophosphite (1.21 g, 5.01 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.65 g (99%) of crude product used without further purification.

$^3$P-NMR (CDCl$_3$, 121 MHz): δ 8.61.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.3-7.2 (2H, m, OPh), 7.1-7.0 (2H, m, OPh), 4.7 (1H, bs, NH), 3.78 (3H, 2s, OCH$_3$), 2.25-2.15 (4H, m, 4H cyclopentane), 2.0-1.8 (4H, m, 4H cyclopentane).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 24.4 (2CH$_2$ cyclopentan), 38.7, 38.6, 38.5 (2CH$_2$ cyclopent), 53.3 (CH$_3$O), 66.3-66.2 (C$_9$ cyclopentane), 117.1-116.8 ('o' OPh), 122.6-122.5 ('m', OPh), 146.1-145.9 ('ipso', OPh), 159.0 ('p', OPh), 175.3-175.2 (COOCH$_3$).

Synthesis of p-fluorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

C$_{14}$H$_{18}$ClFNO$_4$P, MW=349.72.

This is synthesised according to Standard procedure 4, using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (955 mg, 5.01 mmol), para-fluorophenyl dichlorophosphite (1.21g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in DCM (40 mL), to yield 1.64 g (94%) of crude product used without further purification.

$^3$P-NMR (CDCl$_3$, 121 MHz): δ 8.70.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.3-7.2 (2H, m, OPh), 7.1-7.0 (2H, m, OPh), 4.8 (1H, bs, NH), 4.2 (2H, 2q, $^3$J=7.1 Hz, OCH$_3$CH$_3$), 2.25-2.1 (4H, m, 4H cyclopentane), 2.0-1.8 (4H, m, 4H cyclopentane), 1.4 (3H, t, $^3$J=7.1 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 14.4 (CH$_3$CH$_2$), 24.4 (2CH$_2$ cyclopent), 38.8, 38.7, 38.6, 38.5 (2CH$_2$ cyclopent), 62.3 (CH$_3$CH$_2$), 68.3 (C$_9$ cyclopentane), 117.4, 117.0 ('o' OPh), 122.7, 122.6 ('m', OPh), 146.1, 146.0 ('ipso', OPh), 159.0 ('p', OPh), 174.9 (COOCH$_3$CH$_3$).
Synthesis of p-fluorophenyl-(benzoyl-α,α-cycloleucinyl)-phosphorochloridate.
C_{19}H_{20}ClFNO_{4}P, MW= 411.79.

This is synthesised according to Standard procedure 4, using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (1.281 g, 5.01 mmol), para-fluorophenyl-dichlorophosphatate (1.21 g, 5.01 mmol), and TEA (1.4 mL, 10 mmol) in DCM (40 mL), to yield 1.85 g (90%) of crude product used without further purification.

^{31}P-NMR (CDCl3, 121 MHz): δ 7.85.

^{1}H-NMR (CDCl3; 300 MHz): δ 7.65-7.4 (5H, m, CH_{2}Ph), 7.3-7.2 (2H, m, OPh), 7.1-7.0 (2H, m , OPh), 5.2 (2H, s, CH_{2}Ph), 4.6 (1H, bs, N\textit{H}), 2.2-2.1 (4H, m, 4H cyclopentane), 2.0-1.8 (4H, m, 4H cyclopentane).

^{13}C-NMR (CDCl3; 75 MHz): δ 24.5 (2CH_{2} cyclopentane), 38.9, 38.8, 38.6, 38.5 (2CH_{2} cyclopentane), 68.1 (C_{s}cyclopentane), 68.4 (CH_{2}Ph), 117.0, 116.8 ('o' OPh), 122.6, 122.5 ('m' OPh) 129.1, 129.0, 128.8, 128.7 (CH_{2}Ph), 135.7 ('ipso', CH_{2}Ph), 146.1, 145.9 ('ipso', OPh), 159.0 ('p', OPh), 174.6 (COOCH_{2}Ph).

Synthesis of p-nitrophenyl-(methoxy-α,α-cycloleucinyl)-phosphorochloridate.
C_{13}H_{16}ClN_{2}O_{6}P, MW=362.70.

This is synthesised according to Standard procedure 4, using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.885 g, 5.01 mmol), para-
nitrophenyldichlorophosphate (1.632 g, 5.01 mmol), and TEA (1.4 mL, 10 mmol) in DCM
(40 mL), to yield 1.601 g (90%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 8.02.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 8.2 (2H, 2d, $^3J$=8 Hz, OPh), 7.32 (2H, 2d, $^3J$=8 Hz OPh),
4.9 (1H, bs, NH), 3.71 (3H, s, OCH$_3$), 2.25-2.00 (4H, m, 4H cyclopentane), 1.95-1.7 (4H,
m, 4H cyclopentane).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 24.3 (2CH$_2$ cyclopent), 38.7, 38.6 (2CH$_2$ cyclopent), 53.3
(CH$_3$O), 68.6 (CH$_3$ cyclopentane), 121.8, 121.7 ('o' OPh), 126.0 ('m', OPh), 145.6 ('ipso',
OPh), 154.8, 154.7 ('p', OPh), 175.1-175.0 (COOCH$_3$).

Synthesis of p-nitrophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

C$_{14}$H$_{16}$ClN$_2$O$_6$P, MW=376.73.

![Structural diagram]

This is synthesised according to Standard procedure 4, using ethyl-1-amino-1-
cyclopentanate hydrochloride salt (955 mg, 5.01 mmol), para-
nitrophenyldichlorophosphate (1.362 g, 5.01 mmol), and TEA (1.4 mL, 10.02 mmol) in
DCM (40 mL), to yield 1.669 g (90%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 7.95.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 8.1 (2H, 2d, $^3J$=8 Hz, OPh), 7.28 (2H, 2d, $^3J$=8 Hz OPh),
4.8 (1H, bs, NH), 4.2 (2H, 2q, $^3J$=7.1 Hz, OCH$_2$CH$_3$), 2.2-2.0 (4H, m, 4H cyclopentane),
1.95-1.7 (4H, m, 4H cyclopentane), 1.27 (3H, t, $^3J$=7.1 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 14.4 (CH$_3$CH$_2$), 24.4 (2CH$_2$ cyclopent), 38.8, 38.7 (2CH$_2$
cyclopent), 62.4(CH$_3$CH$_2$), 68.5 (CH$_3$ cyclopentane), 121.8, 121.1 ('o' OPh), 126.1, 125.9
('m', OPh), 145.6 ('ipso', OPh), 154.8 ('p', OPh), 174.9 (COOCH$_2$CH$_3$).
Synthesis of p-nitrophenyl-(benzoxyl-α,α-cycloleuciny1)-phosphorochloridate.

C_{19}H_{26}ClIN_{2}O_{8}P, MW= 438.80.

This is synthesised according to Standard procedure 4, using benzyl-1-amino-1-cyclopentanoate hydrochloride salt (0.835 g, 3.25 mmol), para-nitrophenyl-dichlorophosphate (0.85 g, 3.25 mmol), and TEA (0.91 mL, 6.7 mmol) in DCM (30 mL), to yield 1.215 g (85%) of crude product used without further purification.

^{31}P-NMR (CDCl_{3}, 121 MHz): δ 7.99, 7.90.

^{1}H-NMR (CDCl_{3}; 300 MHz): δ 8.1 (2H, 2d, J=8 Hz, OPh), 7.4-7.2 (7H, m, OPh+CH_{2}Ph), 5.18 (2H, s, CH_{2}Ph), 5.0 (1H, bs, NH), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

^{13}C-NMR (CDCl_{3}; 75 MHz): δ 24.4 (2CH_{2} cyclopent), 38.8, 38.7, 38.6, 38.5 (2CH_{2} cyclopent), 68.0 (CH_{2}Ph), 68.6 (C_{6} cyclopentane), 121.8, 121.7 ('o' OPh), 126.1, 125.9 ('m' OPh) 129.1, 129.0, 128.8, 128.6 (CH_{2}Ph), 135.7 ('ipso', CH_{2}Ph), 145.6 ('ipso', OPh), 154.8, 154.7 ('p', OPh), 174.5, 174.4 (COOCH_{2}Ph).

Synthesis of p-chlorophenyl-(methoxy-α,α-cycloleuciny1)-phosphorochloridate.

C_{13}H_{16}Cl_{2}NO_{4}P, MW=352.15.

This is synthesised according to Standard procedure 4, using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.443 g, 2.5 mmol), para-
chlorophenyldichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 ml, 5 mmol) in DCM (20 mL), to yield 0.852 g (98%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 9.55, 9.5.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 7.35-7.15 (4H, m, OPh), 4.95 (1H, bs, NH), 3.78 (3H, s, OCH$_3$), 2.2-2.00 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 24.3 (2CH$_2$ cyclopent), 38.7 (2CH$_2$ cyclopent), 53.3 (CH$_3$O), 68.6 (C$_{4}$cyclopentane), 122.0 (’o’ OPh), 130.1 (’m’, OPh), 133.2 (’p’, OPh), 149.9 (’ips’, OPh), 175.1-175.0 (COOCH$_3$).

10 Synthesis of p-chlorophenyl-(ethoxy-α,α-cycloleuciny1)-phosphorochloridate.

C$_{14}$H$_{18}$Cl$_2$NO$_4$P, MW=366.18.

This is synthesised according to Standard procedure 4, using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (0.477 g, 2.5 mmol), para-chlorophenyldichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 0.880 g (97%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 9.85, 9.70.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 7.35-7.15 (4H, m, OPh), 4.9 (1H, bs, NH), 4.22 (2H, 2q, $^3$J=7.1 Hz, OCH$_2$CH$_3$), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane), 1.27 (3H, t, $^3$J=7 Hz, OCH$_2$CH$_3$).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 14.4 (CH$_3$CH$_2$), 24.4 (2CH$_2$ cyclopent), 38.8, 38.7 (2CH$_2$ cyclopent), 62.5, 62.4 CH$_3$CH$_2$, 68.1 (C$_{4}$cyclopentane), 122.2, 122.1 (’o’ OPh), 130.1 (’m’, OPh), 133.2 (’p’, OPh), 149.8 (’ips’, OPh), 174.8 (COOCH$_2$CH$_3$).

25 Synthesis of p-chlorophenyl-(benzoxy-α,α-cycloleuciny1)-phosphorochloridate.

C$_{19}$H$_{20}$Cl$_2$NO$_4$P, MW= 428.25.
This is synthesised according to Standard procedure 4, using benzyl-1- amino-1-cyclopentanoate hydrochloride salt (0.640 g, 2.5 mmol), para-chlorophenyl-dichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 1.041 g (97%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.39, 8.95.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.4-7.15 (9H, m, OPh + CH$_2$Ph), 5.20 (2H, s, CH$_2$Ph), 5.0 (1H, bs, NH), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 24.4 (2CH$_2$ cyclopentan), 38.8, 38.7, 38.6 (2CH$_2$ cyclopent), 68.1, 68.0 (CH$_2$Ph), 68.2 (C$_4$ cyclopentan), 121.9, 121.8 (‘o’ OPh), 130.5, 130.4, 129.3, 129.2 (‘m’ OPh, CH$_2$Ph), 133.2 (‘p’, OPh), 135.7 (‘ipso’, CH$_2$Ph), 149.9 (‘ipso’, OPh), 174.3, 174.2 (COOCH$_3$Ph).

Synthesis of p-trifluorophenyl-(methoxy-α,α-cycloeucinyl)-phosphorochloridate.

C$_{14}$H$_{16}$ClF$_3$NO$_4$P, MW=385.70.

This is synthesised according to Standard procedure 4, using methyl-1-amino-1-cyclopentanoate hydrochloride salt (0.443 g, 2.5 mmol), para-trifluorophenyl dichlorophosphate (0.700 g, 2.5 mmol), and TEA (0.7 ml, 5 mmol) in DCM (20 mL), to yield 0.931 g (97%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 8.80, 8.62.
1H-NMR (CDCl3; 300 MHz): δ 7.65 (2H, 2d, 3J=8 Hz, OPH), 7.35 (2H, 2d, 3J=8 Hz OPPh), 5.02 (1H, bs, NH), 3.78 (3H, s, OCH3), 2.25-2.05 (4H, m, 4H cyclopentane), 1.95-1.7 (4H, m, 4H cyclopentane).

13C-NMR (CDCl3; 75 MHz): δ 22.8 (2CH2 cyclopent), 37.5, 37.2 (2CH2 cyclopent), 51.5 (CH3O), 68.4 (Cq cyclopentane), 120.0 (‘o’, OPPh), 124.8 (d, J=270Hz, CF3), 126.6 (‘m’, OPPh), 129.5 (‘p’,q, J=32Hz, OPPh), 152.8 (‘ipso’, OPPh), 175.2 (COOCH3).

Synthesis of p-trifluorophenyl-(ethoxy-α,α-cycloleucinyl)-phosphorochloridate.

C15H15ClF3NO4P, MW=399.73.

This is synthesised according to Standard procedure 4, using ethyl-1-amino-1-cyclopentanoate hydrochloride salt (0.477 g, 2.5 mmol), para-trifluorophenylidichlorophosphate (0.700 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 0.950 g (89%) of crude product used without further purification.

31P-NMR (CDCl3, 121 MHz): δ 8.49.

1H-NMR (CDCl3; 300 MHz): δ 7.45 (2H, m, OPPh), 7.2 (2H, m, OPPh), 5.12 (1H, bs, NH), 4.05 (2H, m, OCH2CH3), 2.15-2.0 (4H, m, 4H cyclopentane), 1.9-1.65 (4H, m, 4H cyclopentane), 1.2 (3H, 2t, 3J=7 Hz, OCH2CH3).

13C-NMR (CDCl3; 75 MHz): δ 14.3 (CH3CH2), 24.2, 24.1 (2CH2 cyclopent), 38.6, 38.5, 38.4 (2CH2 cyclopent), 62.0 CH2CH2), 68.4 (Cq cyclopentane), 121.5 (‘o’, OPPh), 125.0 (d, J=270Hz, CF3), 127.5 (‘m’, OPPh), 129.9 (‘p’,q, J=32Hz, OPPh), 152.8, 152.7 (‘ipso’, OPPh), 174.9, 174.6 (COOCH2CH3).

Synthesis of p-trifluorophenyl-(benzoxy-α,α-cycloleucinyl)-phosphorochloridate.

C20H26ClF3NO4P, MW= 461.80.
This is synthesised according to \textit{Standard procedure 4}, using benzyl-\text{-}L-amino-\text{-}1-cyclopentanoate hydrochloride salt (0.700 g, 2.73 mmol), para-trifluorophenyl-dichlorophosphate (0.75 g, 2.73 mmol), and TEA (0.75 mL, 5.47 mmol) in DCM (25 mL), to yield 1.089 g (86\%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.39, 8.95.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.50 (2H, m, OPh), 7.4-7.15 (7H, m, OPh + CH$_2$Ph), 5.20 (2H, s, CH$_2$Ph), 4.95 (1H, bs, NH), 2.2-2.0 (4H, m, 4H cyclopentane), 1.95-1.75 (4H, m, 4H cyclopentane).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 24.3 (2CH$_2$ cyclopentan), 38.8, 38.7, 38.6 (2CH$_2$ cyclopent), 68.1, 68.0 (CH$_2$Ph), 68.2 (Cg cyclopentane), 121.4, 121.3 (‘o’, OPh), 125.1 (d, J=270Hz, CF$_3$), 126.6 (‘m’, OPh) 129.2, 128.8, 127.8 (Bn), 129.8 (‘p’,q , J=32Hz, OPh), 135.7 (‘ipso’, CH$_2$Ph), 153.5 (‘ipso’, OPh), 174.5, 174.4 (COOCH$_2$Ph).

\textbf{Synthesis of Phenyl-(methoxy-\text{-}L-phenylalaninyl)-phosphorochloridate.}

C$_{16}$H$_{17}$ClNO$_4$P, MW=353.74.

This is synthesised according to \textit{Standard procedure 4}, using L-phenylalanine methyl ester hydrochloride (1.08 g, 5 mmol), phenyldichlorophosphate (1.12 g, 0.75 ml, 5 mmol), and TEA (1.4ml, 10 mmol) in DCM (40 mL), to yield 1.626 g (92\%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.1, 8.95.
1H-NMR (CDCl3; 300 MHz): δ 7.3-7.1 (10H, m, CH2Ph+ OPh), 5.00 (1H, bs, NH), 4.35 (1H, m, CHphenylala), 3.79 (3H, 2s, CH3O), 3.00 (2H, m, CH2Ph)

13C-NMR (CDCl3; 75 MHz): δ 36.3 (CH2phenylalanine), 53.0 (CH3O), 56.6, 56.5 (CHphenylala), 121.0 (‘O’ OPh), 126.4 (‘p’, OPh), 130.2 (‘m’, OPh), 150.2 (‘ipso’, OPh), 174.1 (COOCH3).

Synthesis of Phenyl-(methoxy-L-leucinyl)-phosphorochloridate

C13H15ClNO4P, MW=319.72.

This is synthesised according to Standard procedure 4, using L-leucine methyl ester hydrochloride (0.91 g, 5 mmol), phenyldichlorophosphate (1.12 g, 0.75 ml, 5 mmol), and TEA (1.4 ml, 10 mmol) in DCM (40 mL), to yield 1.58 g (99%) of crude product used without further purification.

31P-NMR (CDCl3, 121 MHz): δ 9.45. 9.35.

1H-NMR (CDCl3; 300 MHz): δ 7.4-7.2 (5H, m, OPh), 4.90 (1H, bs, NH), 3.95 (1H, m, CHCH2CH(CH3)2), 3.78 (3H, s, OCH3), 1.8 (1H, m, CHCH2CH(CH3)2), 1.8-1.5 (2H, m, CHCH2CH(CH3)2), 1.0-0.9 (6H, m, CHCH2CH(CH3)2).

13C-NMR (CDCl3; 75 MHz): δ 23.2, 23.1, 22.4, 22.3 (2C, CHCH2CH(CH3)2), 24.9, 24.8 (CHCH2CH(CH3)2), 43.6 (CHCH2CH(CH3)2), 53.2 (CH3O), 53.7, 53.6 (CHCH2CH(CH3)2), 120.9 (‘O’ OPh), 126.4 (‘p’, OPh), 130.2 (‘m’, OPh), 150.1 (‘ipso’, OPh), 173.6 (COOCH3).

Synthesis of Phenyl-(benzoxy-L-leucinyl)-phosphorochloridate.

C19H23ClNO4P, MW = 395.82.
This is synthesised according to **Standard procedure 4**, using L-leucine benzyl ester hydrochloride (1.29 g, 5.0 mmol), phenyl-dichlorophosphate (1.12 g, 0.75 ml, 5.0 mmol), and TEA (1.4 mL, 10.0 mmol) in DCM (40 mL), to yield 1.88 g (95%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 9.93, 9.57.

$^1$H-NMR (CDCl$_3$, 300 MHz): δ 7.5-7.2 (10H, m, OPh+CH$_2$Ph), 5.2 (2H, 2s, CH$_2$Ph), 4.95 (1H, bs, NH), 4.2-4.1 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.95-1.80 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.7 (2H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.0-0.9 (6H, m, CHCH$_2$CH(CH$_3$)$_2$).

$^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 23.2, 23.1, 22.4, 22.3 (2C, CHCH$_2$CH(CH$_3$)$_2$), 24.9 (CHCH$_2$CH(CH$_3$)$_2$), 43.5 (CHCH$_2$CH(CH$_3$)$_2$), 53.8, 53.3 (CHCH$_2$CH(CH$_3$)$_2$), 67.8, 67.7 (CH$_2$Ph), 120.7 (‘o’ OPh), 126.4 (‘p’, OPh), 130.2, 129.1, 128.8, 128.7 (‘m’ OPh, CH$_2$Ph), 135.8 (‘ipso’, CH$_2$Ph), 150.2 (‘ipso’, OPh), 174.1 (COOCH$_2$Ph).

**Synthesis of p-nitrophenyl-(benzoxyl-L-leucinyl)-phosphorochloridate.**

C$_{19}$H$_{22}$ClN$_{2}$O$_6$P, MW = 440.81.

This is synthesised according to **Standard procedure 4**, using L-leucine benzyl ester hydrochloride (1.08 g, 5.01 mmol), para-nitrophenyl-dichloro phosphate (1.362 g, 5.01 mmol), and TEA (1.4 mL, 1.4 mmol) in DCM (40 mL), to yield 2.08g (95%) of crude product used without further purification.

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 9.87, 9.38.
102

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 8.25-8.10 (2H, m, OPh), 7.35-7.25 (7H, m, OPh + CH$_2$Ph), 5.15 (2H, 2s, CH$_2$Ph), 4.95 (1H, bs, NH), 4.15 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.95 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.7 (2H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.0-0.9 (6H, m, CHCH$_2$CH(CH$_3$)$_2$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 23.2, 23.1, 22.1, 22.0 (2C, CHCH$_2$CH(CH$_3$)$_2$), 24.8 (CHCH$_2$CH(CH$_3$)$_2$), 43.4, 43.3 (CHCH$_2$CH(CH$_3$)$_2$), 54.2, 53.9 (CHCH$_2$CH(CH$_3$)$_2$), 68.0, 67.9 (CH$_2$Ph), 121.6 (‘o’ OPh), 126.2, 126.1 (‘m’ OPh), 129.2, 129.0 (CH$_2$Ph), 135.4, 135.3 (‘ipso’, CH$_2$Ph), 145.8, 145.7 (‘ipso’, OPh), 154.7, 154.5 (‘p’, OPh), 173.0, 172.8 (COOCH$_2$Ph).

10

Synthesis of p-chlorophenyl-(benzoxyl-L-leucinyl)-phosphorochloridate.

C$_{19}$H$_{22}$Cl$_2$NO$_4$P, MW= 430.26.

This is synthesised according to Standard procedure 4, using L-leucine benzyl ester hydrochloride (0.644 g, 2.5 mmol), para-chlorophenyl-dichlorophosphate (0.613 g, 2.5 mmol), and TEA (0.7 mL, 5 mmol) in DCM (20 mL), to yield 0.968 g (90%) of crude product used without further purification.

20

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 9.71, 9.55.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.4-7.0 (9H, m, OPh + CH$_2$Ph), 5.15 (2H, s, CH$_2$Ph), 4.5 (1H, d, $^3$J=7Hz, NH), 4.0 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.9-1.8 (1H, m, CHCH$_2$CH(CH$_3$)$_2$), 1.7 (2H, m, CHCH$_2$CH(CH$_3$)$_2$), 0.85 (6H, m, CHCH$_2$CH(CH$_3$)$_2$).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 23.4, 23.3, 22.5, 22.4 (2C, CHCH$_2$CH(CH$_3$)$_2$), 25.0 (CHCH$_2$CH(CH$_3$)$_2$), 43.8, 43.7 (CHCH$_2$CH(CH$_3$)$_2$), 54.0, 53.8 (CHCH$_2$CH(CH$_3$)$_2$), 68.2 (CH$_2$Ph), 122.5 (‘o’ OPh), 130.5, 130.4, 129.3, 129.2 (‘m’ OPh, CH$_2$Ph), 133.2 (‘p’, OPh), 135.7 (‘ipso’, CH$_2$Ph), 149.9, 149.8 (‘ipso’, OPh), 173.4, 173.2 (COOCH$_2$Ph).
Synthesis of 4-chlorophenyl-(methyl-2-amino-2-methylpropanoate)-phosphorochloridate.

C₁₁H₁₄Cl₂NO₄P, MW=326.11.

This is synthesised according to *Standard procedure 4*, using 2-aminoisobutyrate methyl ester hydrochloride (280.0mg, 1.82 mmol), 4-chlorophenylphosphorochloridate (447.4 mg, 1.82 mmol), and TEA (368.3 mg, 3.64 mmol, 507.3 µL) in DCM (20 mL), to yield 554 mg (yield 91.1%) of crude product used without further purification.

³¹P-NMR (CDCl₃, 121 MHz): δ 7.05 (s)

¹H-NMR (CDCl₃; 300 MHz): δ 7.38 (2H, d, ³J=9.0 Hz, OPh), 7.29-7.24 (2H, 2d, ³J=9.0 Hz, OPh), 4.87-4.83 (1H, 2bs, NH), 3.84 (3H, s, OCH₃), 1.73-1.71 (6H, 2s, [CH₃]₂C).

¹³C-NMR (CDCl₃; 75 MHz): δ 27.0, 27.3, ([CH₃]₂C), 53.7 (OCH₃), 58.9 ([CH₃]₂C), 122.5 ('o', OPh), 129.7 ('m', OPh), 131.8 ('p', OPh) 148.7, 148.9 ('ipso', OPh), 75.5, 175.7 (COOCH₃).

Synthesis of 4-chlorophenyl-phosphorochloridate.

C₆H₄Cl₂O₂P, MW=245.43.

This was synthesised according to *Standard procedure 3*, using phosphorus-oxychloride (1533 mg, 10.00 mmol, 932 µL), 4-chlorophenol (1.285 g, 10.00 mmol) and TEA (1.011 g, 10.00 mmol, 1394 µL) in ethylether (100 mL) to give an oil (1.897 g, 77.3 % yield).
$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 5.18.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.45 (2H, d, $^3$$J$=9.0 Hz, OPh), 7.30 (2H, d, $^3$$J$=9.0 Hz, OPh).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 122.5 ('o', OPh), 130.6 ('m', OPh), 133.2 ('p', OPh), 148.5 ('ipso', OPh).

Synthesis of 4-(trifluoromethyl)-phenyl-phosphodichloridate.

C$_7$H$_4$ClF$_3$O$_3$P, MW=278.98.

![Chemical Structure]

This was synthesised according to Standard procedure 3, using phosphorus-oxychloride (1.570 mg, 10.24 mmol, 954.5 µL), 4-trifluoromethylphenol (1660 g, 10.24 mmol) and TEA (1.036 g, 10.24 mmol, 1427 µL) in ethylether (100 mL) to give an oil (2.521 g, 88.2% yield).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): δ 4.75.

$^1$H-NMR (CDCl$_3$; 300 MHz): δ 7.77 (2H, d, $^3$$J$=8.4 Hz, OPh), 7.49 (2H, d, $^3$$J$=8.4 Hz, OPh).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): δ 121.6 ('o', OPh), 123.6 (CF$_3$, $J$=271 Hz, OPh), 128.2 ('m', OPh), 129.7 ('p', $J$=33 Hz), 152.7 ('ipso', OPh).

Synthesis of 4-fluorophenyl-phosphodichloridate.

C$_6$H$_4$ClFO$_2$P, MW=228.97.

![Chemical Structure]
This was synthesised according to *Standard procedure 3*, using phosphorus-oxychloride (1.395 mL, 15.00 mmol), 4-chlorophenol (1.68 g, 15.00 mmol) and TEA (2.1 mL, 15.00 mmol) in ethylether (140 mL) to give an oil (3.96 g, 96 % yield).

$^{31}$P-NMR (CDCl$_3$, 121 MHz): $\delta$ 5.52.

$^1$H-NMR (CDCl$_3$; 300 MHz): $\delta$ 7.15 (2H, d, $^3J$=8.0 Hz, OPh), 7.05 (2H, d, $^3J$=8.0 Hz, OPh).

$^{13}$C-NMR (CDCl$_3$; 75 MHz): $\delta$ 116.8 (‘o’, OPh), 122.1 (‘m’, OPh), 146.7 (‘p’, OPh), 158.7 (‘ips’, OPh).

Experimental data are given in Table I illustrating the activity of compounds embodying the present invention, and of some comparative compounds, with respect to human breast cancer cell line MDA MB231, human colon cancer cell line HT115 and human prostrate cancer cell line PC-3. The compounds include those whose preparations are described above and compounds made by preparative methods corresponding to the methods described above.

The experimental procedures used human colon cancer cell line (HT115), human prostate cancer cell line (PC-3), human breast cancer cell line (MDA MB 231) and normal human umbilical vein endothelial cell (HUVEC). Compounds were diluted over a range of concentrations and added to cells over 1 to 3 days. The cytotoxicity was determined using a MTT assay at the end of each experiment.

In the Table:

ArO refers to Ar as defined above with respect to formula I;

J refers to the moiety of the present compounds represented by, respectively, ROCOCR’R’”NH-, as defined above with respect to formula I, or, with respect to Examples 51, 52 and 53, HOCOCR’R”NH-, as defined above with respect to formula II; and
B refers to the base moiety of the present compounds as defined above with respect to formula I or formula II.

BVU stands for 2-bromovinyl uridine.

5

5-(C=CC[O]O)MeU stands for methyl propenoate-2'-deoxyuridine.

GemCyt stands for Gemcitabine.

10 Examples A, 1, 67 and G are comparative Examples.

Example A is 5-(2-Bromovinyl)-2'-deoxyuridine.

Example 1 is Example 1 above corresponding to compound (7) above.

15 Example 67 is propenate-2'-deoxyuridine.

Example G is gemcitabine.

20 Examples 51, 52 and 53 are compounds embodying formula II above.

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<td>86</td>
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<td>68.8</td>
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Gemcitabine (Example G in the Table) and compound CPF31 (Example 31 in the Table: gemcitabine-[(phenyl-(benzoxy-L-alanyl))-phosphate] were compared in a mouse model with xenografts of human cancer (colon HT115 and prostrate PC3).

Mice were dosed daily at a range of concentrations (0.01-10μM) and tumour volume assessed versus control.

Kaplan-Meier statistics were computed regarding incident-free survival.

In the attached drawings:

Figure 1 shows for the mouse xenograft the tumour volume for prostate data at day 13 using Gemzar™ (gemcitabine available ex. Lilly);

Figure 2 shows for the mouse xenograft the tumour volume for prostate data at day 13 using CPF31;

Figure 3 shows the incident free survival functions v. day for each of CPF31 and gemcitabine; and
Figure 4 shows for the mouse xenograft the tumour volume for colon data at day 24 using, respectively, Gemzar and compound CPF31.

Referring to the drawings, CPF31 can be seen to be significantly less toxic than gemcitabine.

CPF31 was significantly effective at reducing prostate and colon tumour volume relative to control at daily dosing of 5 and 10 μM (3 and 6 μg/ml). Gemcitabine was not effective at the highest non-toxic concentration.

Gemzar is seen from Figure 1 to be toxic above 1μM. In contrast, CPF31 is seen from Figure 2 to have substantially lower toxicity.

Figure 3 shows that CPF31 has significantly lower side effects on a comparable basis: 3 animals show serious toxicity (10% body mass loss) in GMZ and in CPF31 on day 10, collectively 4 in GMZ and 1 in CPF31 on day 11 and 5 in GMZ and 1 in CPF on day 13. Using Chi square analysis by combining 5 and 10μM groups, the significance is p=0.193, 0.078 and 0.0289 on day 10, 11 and 13. It is clear that by day 13, CPF31 displayed significantly less side effects, and the anti-cancer effects continue to exceed that of Gemzar.

Figure 3 shows the Kaplan-Meier survival curve, incidence free survival: based on the loss according to weight loss. A Cox proportion analysis shows that CPF31 is far less toxic than GMZ based on the weight-loss calculated loss (p=0.043).

CPF31 was found to be active at 5μM in vitro, whereas Gemzar was found to be active at 600μM, with respect to the same colon cell line. Figure 4 shows the results of testing both in vivo at 5μM. The greater activity of CPF31 in reducing tumour volume is shown in Figure 4.
CLAIMS.

1. A chemical compound having formula I:

\[
\begin{array}{c}
\text{R} - \text{O} - \text{C} - \begin{array}{c}
\text{R'}
\end{array} \begin{array}{c}
\text{N} \end{array} - \begin{array}{c}
\text{P} \end{array} - \begin{array}{c}
\text{O}
\end{array} - \begin{array}{c}
\text{Ar}
\end{array} \\
\text{OH}
\end{array}
\]

wherein:

R is selected from the group comprising alkyl, aryl and alkylaryl;
R' and R" are independently selected from the group comprising H, alkyl and alkylaryl, or R' and R" together form an alkylene chain so as to provide, together with the C atom to which they are attached, a cyclic system;
Q is selected from the group comprising –O- and –CH₂–;
X and Y are independently selected from the group comprising H, F, Cl, Br, I, OH and methyl (–CH₃);
Ar is a monocyclic aromatic ring moiety or a fused bicyclic aromatic ring moiety, either of which said ring moieties is carbocyclic or heterocyclic and is optionally substituted;
Z is selected from the group comprising H, alkyl and halogen; and
n is 0 or 1,

wherein when n is 0, Z' is -NH₂ and a double bond exists between position 3 and position 4, and
when n is 1, Z' is –O;

or a pharmaceutically acceptable derivative or metabolite of a compound of formula I;

with the proviso that, except where R is 2-Bu (–CH₂–CH(CH₃)₂) and one of R' and R" is H and one of R' and R" is methyl (–CH₃), when n is 1 and X and Y are both H, then Ar is not unsubstituted phenyl (–C₆H₅).
2. A compound according to claim 1 wherein R is selected from the group comprising a C_{1-16} primary or secondary alkyl group, a C_{5-7} carbocyclic aryl group or a C_{1-6}alkylC_{5-11} aryl group.

5 3. A compound according to claim 2 wherein R is selected from the group comprising methyl (-CH₃), ethyl (-C₂H₅) and benzyl (-CH₂C₆H₅).

4. A compound according to claim 3 wherein R is benzyl.

10 5. A compound according to any one of the preceding claims wherein Ar is an optionally substituted C₆ monocyclic aromatic ring moiety, i.e. is optionally substituted phenyl.

6. A compound according to claim 5 wherein Ar is selected from the group comprising -C₆H₅, pCF₃C₆H₄-, pFC₆H₄-, pNO₂C₆H₄-, pClC₆H₄- and oClC₆H₄-.

7. A chemical compound having formula II:

wherein n, Q, R, R', R'', X, Y, Z and Z' have the meanings described in claim 1, and additionally R can be H, with provisos that:

when n is 1, X and Y are both H, R is methyl (-CH₃), one of R' and R'' is H and one of R' and R'' is methyl (-CH₃), then Z is not -CH=CHBr;

when n is 1, X and Y are both H, R is methyl (-CH₃), one of R' and R'' is H and one of R' and R'' is phenylethyl, phenylmethyl, indol-3-ylmethyl or indol-3-yylethyl, then Z is not F;

and

when n is 0, X is not H.
8. A compound according to any one of the preceding claims wherein \( R' \) and \( R'' \) are, independently, selected from the group comprising H, \( \text{C}_{1-6} \) primary, secondary and tertiary alkyl, \( \text{C}_{1-3} \) alkyl\( \text{C}_{5-7} \) aryl, or, when together they form an alkyene chain, they provide, together with the C atom to which they are attached, a \( \text{C}_{3-8} \) carbocyclic aliphatic ring.

9. A compound according to claim 8 wherein \( R' \) and \( R'' \) are, independently, selected from the group comprising H, methyl, benzyl and \(-\text{CH}_2\text{CH(CH}_3)_2\), or, \( R' \) and \( R'' \) together with the C atom to which they are attached, provide a \( \text{C}_{5-6} \) ring.

10. A compound according to claim 9 wherein \( R' \) and \( R'' \) are each methyl.

11. A compound according to claim 9 wherein one of \( R' \) and \( R'' \) is H and one of \( R' \) and \( R'' \) is methyl.

12. A compound according to claim 9 wherein the carbocyclic ring is a pentyl ring.

13. A compound according to any one of the preceding claims wherein \( R' \) and \( R'' \) correspond to the side chains of a naturally occurring amino acid.

14. A compound according to any one of the preceding claims wherein \( Z \) is selected from the group comprising H, \( \text{C}_{1-6} \) alkyl, substituted \( \text{C}_{1-6} \) alkyl, \( \text{C}_{1-6} \) alkenyl, substituted \( \text{C}_{1-6} \) alkenyl, \( \text{C}_{1-6} \) alkynyl, and halogen.

15. A compound according to any one of the preceding claims wherein \( Q \) is O.

16. A compound according to any one of the preceding claims wherein when \( n = 1 \), each of \( X \) and \( Y \) is H.

17. A compound according to any one of claims 1 to 15 wherein when \( n = 0 \), each of \( X \) and \( Y \) is F.
18. A compound according to any one of claims 1 to 15 wherein when n is 0, X is OH and Y is H.

19. A compound according to any one of claims 1 to 15 wherein when n is 0, X is H and Y is OH.

20. A compound selected from the group comprising:

(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[phenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 3)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[phenyl-(benzoyxy-L-alaninyl)]-phosphate (CPF 2)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-fluorophenyl-(methoxy-L-alaninyl)]-phosphate (CPF 5)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-fluorophenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 6)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-fluorophenyl-(benzoyxy-L-alaninyl)]-phosphate (CPF 7)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-nitrophenyl-(methoxy-L-alaninyl)]-phosphate (CPF 10)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-nitrophenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 9)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-nitrophenyl-(benzoyxy-L-alaninyl)]-phosphate (CPF 8)
(E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-[para-(trifluoromethyl)-phenyl-(methoxy-L-alaninyl)]-phosphate (CPF 15)
(E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-[para-(trifluoromethyl)-phenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 25)
(E)-5-(2-Bromovinyl)-2′-deoxyuridine-5′-[para-trifluorophenyl-(benzoyxy-L-alaninyl)]-phosphate (CPF 4)
(E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-[4-chlorophenyl-(methoxy-L-alaninyl)]-phosphate (CPF 13)
(E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-[4-chlorophenyl-(ethoxy-L-alaninyl)]-phosphate (CPF 11)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxyl-L-alaninyl)]-phosphate (CPF 12)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-α,α-dimethylglycinyl)]-phosphate (CPF 26)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(ethoxy-α,α-dimethylglycinyl)]-phosphate (CPF 27)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxyl-α,α-dimethylglycinyl)]-phosphate (CPF 14)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(methoxy-α,α-dimethylglycinyl)]-phosphate (CPF 45)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(ethoxy-α,α-dimethylglycinyl)]-phosphate (CPF 46)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(benzoxyl-α,α-dimethylglycinyl)]-phosphate (CPF 47)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(methoxy-α,α-dimethylglycinyl)]-phosphate (CPF 42)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(ethoxy-α,α-dimethylglycinyl)]-phosphate (CPF 43)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxyl-α,α-dimethylglycinyl)]-phosphate (CPF 44)
(E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[para-(trifluoromethyl)-phenyl-(benzoxyl-α,α-dimethylglycinyl)]-phosphate (CPF 48)
(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-α,α-cyclocleucinyl)]-phosphate (CPF 16)
(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(ethoxy-α,α-cyclocleucinyl)]-phosphate (CPF 17)
(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxyl-α,α-cyclocleucinyl)]-phosphate (CPF 18)
(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-nitrophenyl-(methoxy-α,α-cyclocleucinyl)]-phosphate (CPF 19)
(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-nitrophenyl-(ethoxy-α,α-cyclocleucinyl)]-phosphate (CPF 20)
(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-nitrophenyl-(benzoxo-α,α-cycloleucinyl)]-phosphate (CPF 21)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 22)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(ethoxy-α,α-cycloleucinyl)]-phosphate (CPF 23)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-fluorophenyl-(benzoxo-α,α-cycloleucinyl)]-phosphate (CPF 24)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-chlorophenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 25)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-chlorophenyl-(ethoxy-α,α-cycloleucinyl)]-phosphate (CPF 26)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-chlorophenyl-(benzoxo-α,α-cycloleucinyl)]-phosphate (CPF 27)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-L-phenylalaninyl)]-phosphate (CPF 28)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-trifluorophenyl-(methoxy-α,α-cycloleucinyl)]-phosphate (CPF 29)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[para-trifluorophenyl-(benzoxo-α,α-cycloleucinyl)]-phosphate (CPF 30)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-L-phenylalaninyl)]-phosphate (CPF 31)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-L-leucinyl)]-phosphate (CPF 32)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxo-L-leucinyl)]-phosphate (CPF 33)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxo-L-leucinyl)]-phosphate (CPF 34)

(E)-5-(2-Bromovinyl)-2'-deoxyuridine-5'-[phenyl-(2-butyl-L-alaninyl)]-phosphate (CPF 35)
Gemcitabine-[phenyl-(benzoxyl-L-alaninyl)]-phosphate (CPF 31)

Gemcitabine-[para-chlorophenyl-(benzoxyl-L-alaninyl)]-phosphate (CPF 40) and

Gemcitabine-[para-chlorophenyl-(benzoxyl-α,α-dimethylglycicylinyl)]-phosphate (CPF 41).

21. A compound according to any one of claims 1 to 6, claim 20, or to any one of claims 8 to 19 as dependent on any one of claims 1 to 6, for use in a method of treatment, preferably in the prophylaxis or treatment of cancer, with the proviso that when n is 1, X and Y are both H, one of R' and R'' is H and one of R' and R'' is methyl (CH₃), R is 2-Bu (-CH₂-CH-(CH₃)₂) or R is benzyl (-CH₂C₆H₅), then Ar can be unsubstituted phenyl (-C₆H₅).

22. Use of a compound according to any one of claims 1 to 6, claim 20, or to any one of claims 8 to 19 as dependent on any one of claims 1 to 6, in the manufacture of a medicament for the prophylaxis or treatment of cancer, with the proviso set out in claim 21.

23. A method of prophylaxis or treatment of cancer comprising administration to a patient in need of such treatment an effective dose of a compound according to any one of claims 1 to 6, claim 20, or to any one of claims 8 to 19 as dependent on any one of claims 1 to 6, with the proviso set out in claim 21.

24. A pharmaceutical composition comprising a compound according to any one of claims 1 to 6, claim 20, or to any one of claims 8 to 19 as dependent on any one of claims 1 to 6, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

25. A method of preparing a pharmaceutical composition comprising the step of combining a compound according to any one of claims 1 to 6, claim 20 or any one of claims 8 to 19 as dependent on any one of claims 1 to 6, with a pharmaceutically acceptable excipient, carrier or diluent.
26. A process for the preparation of a compound of formula I according to claim 1, the process comprising reacting of a compound of formula (III):

\[(\text{III})\]

with a compound of formula (IV)

\[(\text{IV})\]

wherein Ar, n, Q, R, R', R'', X, Y, Z' and Z'' have the meanings described in claim 1.
**FIG. 2**

* p=0.096 vs control;  ** p=0.094 vs control
FIG. 4

Colon data @ 24 days

* significant vs control;
** significant vs GMZ