NUCLEOSIDE CYCLIC PHOSPHORAMIDATES FOR THE TREATMENT OF RNA-DEPENDENT RNA VIRAL INFECTION

Abstract: The present invention provides nucleoside cyclic phosphoramidates of structural formula (I) which are precursors to inhibitors of RNA-dependent RNA viral polymerase. These compounds are precursors to inhibitors of RNA-dependent RNA viral replication and are useful for the treatment of RNA-dependent RNA viral infection. They are particularly useful as precursors to inhibitors of hepatitis C virus (HCV) NS5B polymerase, as precursors to inhibitors of HCV replication, and/or for the treatment of hepatitis C infection. The invention also describes pharmaceutical compositions containing such nucleoside cyclic phosphoramidates alone or in combination with other agents active against RNA-dependent RNA viral infection, in particular HCV infection. Also disclosed are methods of inhibiting RNA-dependent RNA polymerase, inhibiting RNA viral replication, and/or treating RNA-dependent RNA viral infection with the nucleoside cyclic phosphoramidates of the present invention. (I)
TITLE OF THE INVENTION

NUCLEOSIDE CYCLIC PHOSPHORAMIDATES FOR THE TREATMENT OF RNA-DEPENDENT RNA VIRAL INFECTION

This application claims the benefit of U.S. Provisional Application No. 60/876,034, filed December 20, 2006, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention is concerned with nucleoside cyclic phosphoramidates, their synthesis, and their use as precursors to inhibitors of RNA-dependent RNA viral polymerase. The compounds of the present invention are precursors to inhibitors of RNA-dependent RNA viral replication and are therefore useful for the treatment of RNA-dependent RNA viral infection. They are particularly useful as precursors to inhibitors of hepatitis C virus (HCV) NS5B polymerase, as precursors to inhibitors of HCV replication, and for the treatment of hepatitis C infection.

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 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 off-spring. 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. 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 state of the art in the treatment of HCV infection has been reviewed, and reference is made to the following publications: B. Dymock, et al., "Novel approaches to the treatment of hepatitis C virus infection," Antiviral Chemistry & Chemotherapy, 11: 79-96 (2000); H. Rosen, et al., "Hepatitis C virus: current understanding and prospects for future therapies," Molecular Medicine Today, 5: 393-399 (1999); D. Moradpour, et

Different approaches to HCV therapy have been taken, which include the inhibition of viral serine proteinase (NS3 protease), helicase, and RNA-dependent RNA polymerase (NS5B), and the development of a vaccine.

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. NS5B polymerase is therefore considered to be an essential component in the HCV replication complex [see K. Ishi, et al., "Expression of Hepatitis C Virus NS5B Protein: Characterization of Its RNA Polymerase Activity and RNA Binding," Hepatology, 29: 1227-1235 (1999) and V. Lohmann, et al., "Biochemical and Kinetic Analyses 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.


It has now been found that nucleoside cyclic phosphoramidates of the present invention are precursors to potent inhibitors of RNA-dependent RNA viral replication and in
particular HCV replication. The phosphoramidates are converted *in vivo* into their nucleoside 5'-phosphate (nucleotide) derivatives which are converted into the corresponding nucleoside 5'-triphosphate derivatives which are inhibitors of RNA-dependent RNA viral polymerase and in particular HCV NS5B polymerase. The instant nucleoside phosphoramidates are useful to treat RNA-dependent RNA viral infection and in particular HCV infection.

It is therefore an object of the present invention to provide nucleoside cyclic phosphoramidates which are useful as precursors to inhibitors of RNA-dependent RNA viral polymerase and in particular as precursors to inhibitors of HCV NS5B polymerase.

It is another object of the present invention to provide nucleoside cyclic phosphoramidates which are useful as precursors to inhibitors of the replication of an RNA-dependent RNA virus and in particular as precursors to inhibitors of the replication of hepatitis C virus.

It is another object of the present invention to provide nucleoside cyclic phosphoramidates which are useful in the treatment of RNA-dependent RNA viral infection and in particular in the treatment of HCV infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside cyclic phosphoramidates of the present invention in association with a pharmaceutically acceptable carrier.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside cyclic phosphoramidates of the present invention for use as precursors to inhibitors of RNA-dependent RNA viral polymerase and in particular as precursors to inhibitors of HCV NS5B polymerase.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside cyclic phosphoramidates of the present invention for use as precursors to inhibitors of RNA-dependent RNA viral replication and in particular as precursors to inhibitors of HCV replication.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside cyclic phosphoramidates of the present invention for use in the treatment of RNA-dependent RNA viral infection and in particular in the treatment of HCV infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside cyclic phosphoramidates of the present invention in combination with other agents active against an RNA-dependent RNA virus and in particular against HCV.

It is another object of the present invention to provide methods for the inhibition of RNA-dependent RNA viral polymerase and in particular for the inhibition of HCV NS5B polymerase.
It is another object of the present invention to provide methods for the inhibition of RNA-dependent RNA viral replication and in particular for the inhibition of HCV replication.

It is another object of the present invention to provide methods for the treatment of RNA-dependent RNA viral infection and in particular for the treatment of HCV infection.

It is another object of the present invention to provide methods for the treatment of RNA-dependent RNA viral infection in combination with other agents active against RNA-dependent RNA virus and in particular for the treatment of HCV infection in combination with other agents active against HCV.

It is another object of the present invention to provide nucleoside cyclic phosphorimidates and their pharmaceutical compositions for use as a medicament for the inhibition of RNA-dependent RNA viral replication and/or the treatment of RNA-dependent RNA viral infection and in particular for the inhibition of HCV replication and/or the treatment of HCV infection.

It is another object of the present invention to provide for the use of the nucleoside cyclic phosphorimidates of the present invention and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of RNA-dependent RNA viral replication and/or the treatment of RNA-dependent RNA viral infection and in particular for the inhibition of HCV replication and/or the treatment of HCV infection.

These and other objects will become readily apparent from the detailed description which follows.

SUMMARY OF THE INVENTION

The present invention relates to compounds of structural formula I of the indicated stereochemical configuration:

\[
\begin{align*}
  &\begin{array}{c}
    \text{O} \\
    \text{O} \\
    \text{HN} \\
    \text{O} \\
    \text{OR}^6
  \end{array} \\
  &\begin{array}{c}
    \text{B} \\
    \text{R}^1 \\
    \text{R}^2 \\
    \text{R}^4 \\
    \text{R}^5
  \end{array}
\end{align*}
\]

(1),

and pharmaceutically acceptable salts thereof; wherein

\[
\begin{align*}
  &\begin{array}{c}
    \text{OR}^{10} \\
    \text{NHR}^9
  \end{array} \\
  &\begin{array}{c}
    \text{N} \\
    \text{O}
  \end{array}
\end{align*}
\]

B is \(\text{or }\), wherein the asterisk denotes the point of attachment to the rest of the compound;
n is 0, 1, or 2;
R1 is hydrogen, methyl, or fluoromethyl;
R2 is fluoro or OR3;
R3 is selected from the group consisting of hydrogen, methyl, C1-16 alky carbonyl, C2-18 alkenylcarbonyl, Ci-IO alkyloxycarbonyl, C3-6 cycloalkylcarbonyl, and an amino acyl residue of structural formula:

\[
\begin{align*}
&\text{R}^7 \\
&\text{O} \\
&\text{N} \\
&\text{R}^6
\end{align*}
\]

R4 is hydrogen, C1.5 alkyl, phenyl, or benzyl; wherein alkyl is optionally substituted with one substituent selected from the group consisting of fluorine, hydroxy, methoxy, amino, carboxy, carbamoyl, guanidino, mercapto, methylthio, 1H-imidazolyl, and 1H-indol-3-yl; and wherein phenyl and benzyl are optionally substituted with one to two substituents independently selected from the group consisting of halogen, hydroxy, and methoxy;

R5 is hydrogen or methyl;
or R4 and R5 together with the carbon atom to which they attached form a 3- to 6-membered aliphatic spirocyclic ring system;

R6 is hydrogen, Ci-16 alkyl, C2-20 alkenyl, (CH2)nC3-6 cycloalkyl, phenyl, benzyl, or adamantyl; wherein alkyl, alkenyl, cycloalkyl, and adamantyl are optionally substituted with one to three substituents independently selected from halogen, hydroxy, carboxy, C1-8 alkoxy; and wherein phenyl and benzyl are optionally substituted with one to three substituents independently selected from halogen, hydroxy, cyano, C1.4 alkoxy, trifluoromethyl, and trifluoromethoxy;

R7 is hydrogen, C1.5 alkyl, or phenyl C0-2 alkyl;

R8 is hydrogen, C1.4 alkyl, C1-4 acyl, benzoyl, C1.4 alkyloxycarbonyl, phenyl C0-2 alkyloxycarbonyl, C1.4 alkyloxycarbonyl, C1.4 alkylaminocarbonyl, phenyl C0-2 alkylaminocarbonyl, C1.4 alkylsulfonyl, or phenyl C0-2 alkylsulfonfonyl;

R9 is hydrogen, C0-8 alkylcarbonyl, C1-8 alkyloxycarbonyl, or [(di-C0-8 alkylamino)-C0-8 alkoxy]carbonyl; and

RIO is hydrogen, C0-8 alkyl, or C1-8 alkylcarbonyl.

The compounds of formula I are useful as precursors to inhibitors of RNA-dependent RNA viral polymerase and in particular of HCV NS5B polymerase. They are also precursors to inhibitors of RNA-dependent RNA viral replication and in particular of HCV replication and are useful for the treatment of RNA-dependent RNA viral infection and in particular for the treatment of HCV infection.
Without limitation as to their mechanism of action, the cyclic phosphoramidates of the present invention act as precursors of the corresponding nucleoside 5'-monophosphates. Endogenous kinase enzymes convert the 5'-monophosphates into their 5'-triphosphate derivatives which are the inhibitors of the RNA-dependent RNA viral polymerase. Thus, the cyclic phosphoramidates may provide for more efficient target cell penetration than the nucleoside itself, may be less susceptible to metabolic degradation, and may have the ability to target a specific tissue, such as the liver, resulting in a wider therapeutic index allowing for lowering the overall dose of the antiviral agent.

Also encompassed within the present invention are pharmaceutical compositions containing the compounds alone or in combination with other agents active against RNA-dependent RNA virus and in particular against HCV as well as methods for the inhibition of RNA-dependent RNA viral replication and for the treatment of RNA-dependent RNA viral infection.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to compounds of structural formula I as set forth in the Summary of the Invention above. The compounds of formula I are useful as precursors to inhibitors of RNA-dependent RNA viral polymerase. They are also precursors to inhibitors of RNA-dependent RNA viral replication and are useful for the treatment of RNA-dependent RNA viral infection.

A first embodiment of the present invention is a compound of Formula I-A, or a pharmaceutically acceptable salt thereof:

![Formula I-A](image)

(I-A);

and all variables are as originally defined (i.e., as defined in the Summary of the Invention).

A second embodiment of the present invention is a compound of Formula I-Bl, or a pharmaceutically acceptable salt thereof:
A third embodiment of the present invention is a compound of Formula I-B2, or a pharmaceutically acceptable salt thereof:

and all variables are as originally defined.

A fourth embodiment of the present invention is a compound of Formula I or Formula I-A or Formula I-B1 or Formula I-B2, or a pharmaceutically acceptable salt thereof, wherein:

R6 is hydrogen, C_{i6} alkyl, C2-20 alkenyl, (CH2)nC3-6 cycloalkyl, phenyl, benzyl, or adamantyl; wherein alkyl, alkenyl, cycloalkyl, and adamantyl are optionally substituted with one to three substituents independently selected from halogen, hydroxy, carboxy, C_{i4} alkoxy; and wherein phenyl and ben2yl are optionally substituted with one to three substituents independently selected from halogen, hydroxy, cyano, C1-4 alkoxy, trifluoromethyl, and trifluoromethoxy;

R9 is hydrogen, C_{i8} alkylcarbonyl, or C_{i8} alkyloxy carbonyl;

and all other variables are as originally defined.

In a fifth embodiment of the compounds of the present invention, R1 is methyl or fluoromethyl; R2 is hydroxy; and all other variables are as originally defined or as defined in any one of the preceding embodiments, hi a class of this embodiment, R1 is methyl.
In a sixth embodiment of the compounds of the present invention, R1 is methyl or fluoromethyl, and R.2 is fluoro; and all other variables are as originally defined or as defined in any one of the preceding embodiments. In a class of this embodiment, R1 is methyl.

In a seventh embodiment of the compounds of the present invention, Rβ is hydrogen and R4 is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, isopropyl, isobutyl, 2-methyl-1-propyl, hydroxymethyl, fluoromethyl, mercaptomethyl, carboxymethyl, carbamoylmethyl, 1-hydroxyethyl, 2-carboxyethyl, 2-carbamoylethyl, 2-methylthioethyl, 4-amino-l-butyl, 3-amino-l-propyl, 3-guanidino-1-propyl, 1H-imidazol-4-ylmethyl, phenyl, benzyl, 4-hydroxybenzyl, and 1H-indol-3-ylmethyl; and all other variables are as originally defined or as defined in any one of the preceding embodiments. In a class of this embodiment, R4 is methyl or benzyl. In a subclass of this class, R4 is methyl.

In an eighth embodiment of the compounds of the present invention, R6 is C1-8 alkyl, cyclohexyl, cyclopentyl, cyclohexymethyl, 2-cyclohexylethyl, or 3-cyclohexyl-n-propyl, each of which is optionally substituted with one to three substituents independently selected from fluorine, hydroxy, and C1-6 alkoxy; and all other variables are as originally defined or as defined in any one of the preceding embodiments. In a class of this embodiment, R6 is ethyl, butyl, or heptyl.

In a ninth embodiment of the compounds of the present invention, R6 is C1-8 alkyl, cyclohexyl, or cyclopentyl, each of which is optionally substituted with one to three substituents independently selected from fluorine, hydroxy, and C1-4 alkoxy; and all other variables are as originally defined or as defined in any one of the preceding embodiments. In a class of this embodiment, R6 is ethyl, butyl, or heptyl.

In a tenth embodiment of the compounds of the present invention, R4 is methyl, R5 is hydrogen, and R6 is ethyl, butyl, or heptyl; and all other variables are as originally defined or as defined in any one of the preceding embodiments.

In an eleventh embodiment of the compounds of the present invention, R9 is hydrogen; and all other variables are as originally defined or as defined in any one of the preceding embodiments.

A twelfth embodiment of the present invention is a compound of Formula II:
or a pharmaceutically acceptable salt thereof, wherein:
R4 is Ci-4 alkyl;
R6 is C1-8 alkyl, C1-8 alkyl substituted with C1-6 alkoxy, cyclohexyl, cyclopentyl,
cyclohexylmethyl, 2-cyclohexylethyl, or 3-cyclohexyl-n-propyl; and
R9 is H, C1-8 alkylcarbonyl, or [(di-Ci-4 alkylamino)-Ci_4 alkoxy]carbonyl.

A thirteenth embodiment of the present invention is a compound of Formula
m-A:

or a pharmaceutically acceptable salt thereof, wherein R4, R5, and Rβ are as originally defined or
as defined in any one of the preceding embodiments.

A fourteenth embodiment of the present invention is a compound of Formula
m-B:
or a pharmaceutically acceptable salt thereof, wherein R.4, R5, and R.6 are as originally defined or as defined in any one of the preceding embodiments.

A fifteenth embodiment of the present invention is a compound of Formula I which is selected from the group consisting of the compounds set forth in Examples 1 to 23 and pharmaceutically acceptable salts thereof. In a sub-embodiment, the compounds are selected from the group consisting of the compounds set forth in Examples 1 to 14 and pharmaceutically acceptable salts thereof.

Illustrative but nonlimiting examples of compounds of the present invention of structural formula I which are useful as precursors to inhibitors of RNA-dependent RNA viral polymerase are the following:

and pharmaceutically acceptable salts thereof.

In one embodiment of the present invention, the nucleoside cyclic phosphoramideates of the present invention are useful as precursors to inhibitors of positive-sense single-stranded RNA-dependent RNA viral polymerase, inhibitors of positive-sense single-stranded RNA-dependent RNA viral replication, and/or for the treatment of positive-sense single-stranded RNA-dependent RNA viral infection. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA virus is a *Flaviviridae* virus or a *Picornaviridae* virus. In a subclass of this class, the *Picornaviridae* virus is a rhinovirus, a
poliovirus, or a hepatitis A virus. In a second subclass of this class, the Flaviviridae virus is
selected from the group consisting of hepatitis C virus, yellow fever virus, dengue virus, West Nile virus, Japanese encephalitis virus, Banzi virus, and bovine viral diarrhea virus (BVDV). In a subclass of this subclass, the Flaviviridae virus is hepatitis C virus.

Another aspect of the present invention is concerned with a method for inhibiting RNA-dependent RNA viral polymerase, a method for inhibiting RNA-dependent RNA viral replication, and/or a method for treating RNA-dependent RNA viral infection in a mammal in need thereof comprising administering to the mammal a therapeutically effective amount of a compound of structural formula I.

In one embodiment of this aspect of the present invention, the RNA-dependent RNA viral polymerase is a positive-sense single-stranded RNA-dependent RNA viral polymerase, in a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA viral polymerase is a Flaviviridae viral polymerase or a Picornaviridae viral polymerase, in a subclass of this class, the Picornaviridae viral polymerase is rhinovirus polymerase, poliovirus polymerase, or hepatitis A virus polymerase, in a second subclass of this class, the Flaviviridae viral polymerase is selected from the group consisting of hepatitis C virus polymerase, yellow fever virus polymerase, dengue virus polymerase, West Nile virus polymerase, Japanese encephalitis virus polymerase, Banzi virus polymerase, and bovine viral diarrhea virus (BVDV) polymerase. in a subclass of this subclass, the Flaviviridae viral polymerase is hepatitis C virus polymerase.

In a second embodiment of this aspect of the present invention, the RNA-dependent RNA viral replication is a positive-sense single-stranded RNA-dependent RNA viral replication. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA viral replication is Flaviviridae viral replication or Picornaviridae viral replication, in a subclass of this class, the Picornaviridae viral replication is rhinovirus replication, poliovirus replication, or hepatitis A virus replication. In a second subclass of this class, the Flaviviridae viral replication is selected from the group consisting of hepatitis C virus replication, yellow fever virus replication, dengue virus replication, West Nile virus replication, Japanese encephalitis virus replication, Banzi virus replication, and bovine viral diarrhea virus replication.

In a subclass of this subclass, the Flaviviridae viral replication is hepatitis C virus replication.

In a third embodiment of this aspect of the present invention, the RNA-dependent RNA viral infection is a positive-sense single-stranded RNA-dependent viral infection. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA viral infection is Flaviviridae viral infection or Picornaviridae viral infection. In a subclass of this class, the Picornaviridae viral infection is rhinovirus infection, poliovirus infection, or hepatitis A virus infection. In a second subclass of this class, the Flaviviridae viral infection is selected from the group consisting of hepatitis C virus infection, yellow fever virus infection, dengue virus
infection, West Nile virus infection, Japanese encephalitis virus infection, Banzi virus infection, and bovine viral diarrhea virus infection, hi a subclass of this subclass, the Flaviviridae viral infection is hepatitis C virus infection.

Throughout the instant application, the following terms have the indicated meanings:

The alkyl groups specified above are intended to include those alkyl groups of the designated length in either a straight or branched configuration. Exemplary of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary-butyl, pentyl, isopentyl, hexyl, isohexyl, and the like.

The term "naphthyl" encompasses both 1-naphthyl (α-naphthyl) and 2-naphthyl (β-naphthyl).

The term "adamantyl" encompasses both 1-adamantyl and 2-adamantyl.

By the term "optionally substituted benzyl" is meant -CH2-phenyl wherein the phenyl moiety is optionally substituted.

The term "alkenyl" shall mean straight or branched chain alkenes of two to twenty total carbon atoms, or any number within this range (e.g., ethenyl, propenyl, butenyl, pentenyl, oleyl, etc.).

The term "cycloalkyl" shall mean cyclic rings of alkanes of three to eight total carbon atoms, or any number within this range (e.g., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl).

The term "alkoxy" refers to straight or branched chain alkoxides of the number of carbon atoms specified (e.g., C1-4 alkoxy), or any number within this range [e.g., methoxy (MeO-), ethoxy, isoproxy, etc.].

The term "alkylthio" refers to straight or branched chain alkylsulfides of the number of carbon atoms specified (e.g., C1-4 alkylthio), or any number within this range [e.g., methylthio (MeS-), ethylthio, isopropylthio, etc.].

The term "alkylamino" refers to straight or branched alkylamines of the number of carbon atoms specified (e.g., C1-4 alkylamino), or any number within this range [e.g., methylamino, ethylamino, isopropylamino, t-butylamino, etc.].

The term "alkylsulfonyl" refers to straight or branched chain alkylsulfones of the number of carbon atoms specified (e.g., C1-6 alkylsulfonyl), or any number within this range [e.g., methylsulfonyl (MeSO2-), ethylsulfonyl, isopropylsulfonyl, etc.].

The term "alkyloxycarbonyl" refers to straight or branched chain esters of a carboxylic acid or carbamic acid group present in a compound of the present invention having the number of carbon atoms specified (e.g., C1-8 alkyloxycarbonyl), or any number within this range [e.g., methyloxycarbonyl (MeOOC-), ethyloxycarbonyl, or butyloxycarbonyl].
The term "alkylcarbonyl" refers to straight or branched chain alkyl acyl group of the specified number of carbon atoms (e.g., C1-8 alkylcarbonyl), or any number within this range [e.g., methyloxycarbonyl (MeOCO-), ethyloxycarbonyl, or butyloxycarbonyl]. The term "halogen" is intended to include the halogen atoms fluorine, chlorine, bromine and iodine.

An asterisk (*) at the end of a bond denotes the point of attachment to the rest of the compound.

The term "phosphoryl" refers to -P(0)(0H)2-

The term "diphosphoryl" refers to the radical having the structure:

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*P-O-P-O
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The term "triphosphoryl" refers to the radical having the structure:

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*P-O-P-O-P-O
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When R7 in the amino acyl residue embodiment of R3 is a substituent other than hydrogen in the formula

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R7
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the amino acyl residue contains an asymmetric center and is intended to include the individual R- and S-stereoisomers as well as iJS-diastereoisomeric mixtures. In one embodiment, the stereochemistry at the stereogenic carbon corresponds to that of an 5-amino acid, that is, the naturally occurring alpha-amino acid stereochemistry, as depicted in the formula:

```
R7
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The term "substituted" shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed or claimed, the
substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or plurally.

The term "5'-triphosphate" refers to a triphosphoric acid ester derivative of the 5'-hydroxyl group of a nucleoside compound of the present invention having the following general structural formula IV:

![Structural formula IV](image)

wherein B, R1, and R2 are as defined above. In aspects of this definition, the term refers to either or both of the derivatives of formula FV-A and FV-B:

![Derivatives FV-A and FV-B](image)

The term "composition", as in "pharmaceutical composition," is intended to encompass a product comprising the active ingredient(s) and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically acceptable carrier.

The terms "administration of" and "administering a" compound should be understood to mean providing a compound of the invention or a prodrug of a compound of the invention to the individual in need.

Another aspect of the present invention is concerned with a method of inhibiting HCV NS5B polymerase, inhibiting HCV replication, or treating HCV infection with a compound of the present invention in combination with one or more agents useful for treating HCV infection. Such agents active against HCV include, but are not limited to, ribavirin, levovirin, viramidine, nitazoxanide, thymosin alpha-1, interferon-β, interferon-α, pegylated interferon-α (peginterferon-α), a combination of interferon-α and ribavirin, a combination of peginterferon-α
and ribavirin, a combination of interferon-α and levovirin, and a combination of peginterferon-α and levovirin. Interferon-α includes, but is not limited to, recombinant interferon-α2a (such as Roferon interferon available from Hoffmann-LaRoche, Nutley, NJ), pegylated interferon-α2a (Pegasys™), interferon-α2b (such as Intron-A interferon available from Schering Corp., Kenilworth, NJ), pegylated interferon-α2b (Peghitron™), a recombinant consensus interferon (such as interferon alfacon-1), and a purified interferon-α product. Amgen's recombinant consensus interferon has the brand name Infergen®. Levovirin is the L-enantiomer of ribavirin which has shown immunomodulatory activity similar to ribavirin. Viramidine represents an analog of ribavirin disclosed in WO 01/60379 (assigned to ICN Pharmaceuticals). In accordance with this method of the present invention, the individual components of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment, and the term "administering" is to be interpreted accordingly. It will be understood that the scope of combinations of the compounds of this invention with other agents useful for treating HCV infection includes in principle any combination with any pharmaceutical composition for treating HCV infection. When a compound of the present invention or a pharmaceutically acceptable salt thereof is used in combination with a second therapeutic agent active against HCV, the dose of each compound may be either the same as or different from the dose when the compound is used alone.

For the treatment of HCV infection, the compounds of the present invention may also be administered in combination with an agent that is an inhibitor of HCV NS3 serine protease. HCV NS3 serine protease is an essential viral enzyme and has been described to be an excellent target for inhibition of HCV replication. Both substrate and non-substrate based inhibitors of HCV NS3 protease inhibitors are disclosed in WO 98/22496, WO 98/46630, WO 99/07733, WO 99/07734, WO 99/38888, WO 99/50230, WO 99/64442, WO 00/09543, WO 00/59929, GB-2337262, WO 02/18369, WO 02/08244, WO 02/48116, WO 02/48172, WO 05/037214, and U.S. Patent No. 6,323,180. HCV NS3 protease as a target for the development of inhibitors of HCV replication and for the treatment of HCV infection is discussed in B.W. Dymock, "Emerging therapies for hepatitis C virus infection," Emerging Drugs, 6: 13-42 (2001). Specific HCV NS3 protease inhibitors combinable with the compounds of the present invention include BILN2061, VX-950, SCH6, SCH7, and SCH-503034.

Ribavirin, levovirin, and viramidine may exert their anti-HCV effects by modulating intracellular pools of guanine nucleotides via inhibition of the intracellular enzyme inosine monophosphate dehydrogenase (IMPDH). IMPDH is the rate-limiting enzyme on the biosynthetic route in de novo guanine nucleotide biosynthesis. Ribavirin is readily phosphorylated intracellularly and the monophosphate derivative is an inhibitor of IMPDH. Thus, inhibition of IMPDH represents another useful target for the discovery of inhibitors of
HCV replication. Therefore, the compounds of the present invention may also be administered in combination with an inhibitor of IMPDH, such as VX-497, which is disclosed in WO 97/41211 and WO 01/00622 (assigned to Vertex); another IMPDH inhibitor, such as that disclosed in WO 00/25780 (assigned to Bristol-Myers Squibb); or mycophenolate mofetil [see A.C. Allison and E.M. Eugui, Agents Action, 44 (Suppl.): 165 (1993)].

For the treatment of HCV infection, the compounds of the present invention may also be administered in combination with the antiviral agent amantadine (1-aminoadamantane) [for a comprehensive description of this agent, see J. Kirschbaum, Anal. Profiles Drug Subs. 12: 1-36 (1983)].

The compounds of the present invention may also be combined for the treatment of HCV infection with antiviral 2'-C-branched ribonucleosides disclosed in R. E. Harry-O'kuru, et al., J. Org. Chem., 62: 1754-1759 (1997); M. S. Wolfe, et al., Tetrahedron Lett., 36: 761-7614 (1995); U.S. Patent No. 3,480,613 (Nov. 25, 1969); US Patent No. 6,777,395 (Aug. 17, 2004); US Patent No. 6,914,054 (July 5, 2005); International Publication Numbers WO 01/90121 (29 November 2001); WO 01/92282 (6 December 2001); WO 02/32920 (25 April 2002); WO 02/057287 (25 July 2002); WO 02/057425 (25 July 2002); WO 04/002422 (8 Jan. 2004); WO 04/002999 (8 January 2004); WO 04/003000 (8 January 2004); WO 04/002422 (8 January 2004); US Patent Application Publications 2005/0107312; US 2005/0090463; US 2004/0147464; and US 2004/0063658; the contents of each of which are incorporated by reference in their entirety. Such 2'-C-branched ribonucleosides include, but are not limited to, 2'-C-methylcytidine, 2'-fluoro-2'-C-methylcytidine 2'-C-methyluridine, 2'-C-methyladenosine, 2'-C-methylguanosine, and 9-(2-C-methyl-β-D-ribofuranosyl)-2,6-diaminopurine; the corresponding amino acid esters of the furanose C-2', C-3', and C-5' hydroxyls (such as 3'-O-(L-valyl)-2'-C-methylcytidine dihydrochloride, also referred to as valopicitabine dihydrochloride or NM-283 and 3'-O-(L-valyl)-2'-fluoro-2'-C-methylcytidine), and the corresponding optionally substituted cyclic 1,3-propanediol esters of their 5'-phosphate derivatives.

The compounds of the present invention may also be combined for the treatment of HCV infection with other nucleosides having anti-HCV properties, such as those disclosed in US Patent No. 6,864,244 (Mar. 8, 2005); WO 02/51425 (4 July 2002), assigned to Mitsubishi Pharma Corp.; WO 01/79246, WO 02/32920, and WO 02/48165 (20 June 2002), assigned to Pharmasset, Ltd.; WO 01/68663 (20 September 2001), assigned to ICN Pharmaceuticals; WO 99/43691 (2 Sept. 1999); WO 02/18404 (7 March 2002), assigned to Hoffmann-LaRoche; U.S. 2002/0019363 (14 Feb. 2002); WO 02/100415 (19 Dec. 2002); WO 03/026589 (3 Apr. 2003); WO 03/026675 (3 Apr. 2003); WO 03/093290 (13 Nov. 2003); US 2003/0236216 (25 Dec. 2003); US 2004/0006007 (8 Jan. 2004); WO 04/011478 (5 Feb. 2004); WO 04/013300 (12 Feb. 2004); US 2004/0063658 (1 Apr. 2004); and WO 04/028481 (8 Apr. 2004).
In one embodiment, nucleoside HCV NS5B polymerase inhibitors that may be combined with the nucleoside derivatives of the present invention are selected from the following compounds: 4'-azido-cytidine; 4-amino-7-(2-C-methyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-i]pyrimidine; 4-amino-7-(2-C-hydroxymethyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-i]pyrimidine; 4-amino-7-(2-C-fluoromethyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-i]pyrimidine; 4-amino-5-fluoro-7-(2-C-methyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-i]pyrimidine; 2-amino-7-(2-C-methyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-i]pyrimidine; and pharmaceutically acceptable salts and prodrugs thereof.

The compounds of the present invention may also be combined for the treatment of HCV infection with non-nucleoside inhibitors of HCV polymerase such as those disclosed in WO 01/77091 (18 Oct. 2001), assigned to Tularik, Inc.; WO 01/47883 (5 July 2001), assigned to Japan Tobacco, Inc.; WO 02/04425 (17 January 2002), assigned to Boehringer Ingelheim; WO 02/06246 (24 Jan. 2002), assigned to Istituto di Ricerche di Biologia Molecolare P. Angeletti S.P.A.; WO 02/20497 (3 March 2002); WO 2005/016927 (in particular JTK003), assigned to Japan Tobacco, Inc.; the contents of each of which are incorporated herein by reference in their entirety; and HCV-796 (Viropharma Inc.).

In one embodiment, non-nucleoside HCV NS5B polymerase inhibitors that may be combined with the nucleoside derivatives of the present invention are selected from the following compounds: 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-7-oxo-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1-carboxylic acid; 14-cyclohexyl-6-(2-morpholin-4-ylethyl)-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-3-methoxy-5,6,7,8-tetrahydroindolo[2,1-a]benzodiazocine-1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-3-methoxy-6-methyl-5,6,7,8-tetrahydroindolo[2,1-a]benzodiazocine-1-carboxylic acid; methyl {[(14-cyclohexyl-3-methoxy-6-methyl-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocin-1-yl)carbonyl]amino} sulfonyl)acetate; ([(M-cyclohexyl-S-methoxy-6-methyl-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocin-1-yl)carbonyl] amino) sulfonyl)acetic acid; 14-cyclohexyl-N-{[(dimethylamino)sulfonyl]-3-methoxy-6-methyl-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocin-1-yl}carbonyl] amino) sulfonyl)acetic acid; 3-chloro-14-cyclohexyl-6-[2-(dimethylamino)ethyl]7-oxo-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-11-carboxylic acid; TV-11-carboxy-14-cyclohexyl-7,8-dihydro-6H-indolo[1.2-e][1.5]benzoxazocin-7-yl)-IV (N-dimethylamino)bis(trifluoroacetate); 14-cyclohexyl-7,8-dihydro-6H-indolo[1.2-e][1.5]benzoxazocine-1-carboxylic acid; 14-cyclohexyl-6-methyl-7-oxo-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-3-methoxy-7-oxo-5,6,7,8-tetrahydroindolo[2,1-a]
a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-[3-(dimethylamino)propyl]-7-oxo-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-7-oxo-6-(2-piperidin-1-ylethyl)-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-(morpholin-4-ylethyl)-7-oxo-5,6,7,8-tetrahydroindolo[2, 1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-[2-(diethylamino)ethyl]-7-oxo-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-[(dimethylamino)sulfonyl]-7-oxo-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-N-[4-cyclopentyl-6-[(dimethylamino)ethyl]-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-N-[6-allyl-14-cyclohexyl-3-methoxy-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 13-cyclohexyl-5-(1-carboxylic acid; 14-cyclohexyl-6-[2-(dimethylamino)ethyl]-5,6,7,8-tetrahydroindolo[2,1-a][2,5]benzodiazocine-1 1-carboxylic acid; 13-cyclohexyl-5-methyl-4,5,6,7-tetrahydrofuro[3′,2′:6,7][1,4]diazocino[1,8-a]indole-10-carboxylic acid; 15-cyclohexyl-6-[2-(dimethylamino)ethyl]-7-oxo-6,7,8,9-tetrahydro-5H-indolo[2, 1-a][2,6]benzodiazonine-12-carboxylic acid; 15-cyclohexyl-8-oxo-6,7,8,9-tetrahydro-5H-indolo[2,1-a][2,5]benzodiazonine-12-carboxylic acid; 13-cyclohexyl-6-oxo-6J-dihydro-SH-indolo[1,2-d][1,4]benzodiazepine-10-carboxylic acid; and pharmaceutically acceptable salts thereof.

By "pharmaceutically acceptable" is meant that the carrier, diluent, or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Also included within the present invention are pharmaceutical compositions comprising the nucleoside cyclic phosphoramidates of the present invention in association with a pharmaceutically acceptable carrier. Another example of the invention is a pharmaceutical composition made by combining any of the compounds described above and a pharmaceutically acceptable carrier. Another illustration of the invention is a process for making a pharmaceutical composition comprising combining any of the compounds described above and a pharmaceutically acceptable carrier.

Also included within the present invention are pharmaceutical compositions useful for inhibiting RNA-dependent RNA viral polymerase in particular HCV NS5B polymerase comprising an effective amount of a compound of the present invention and a pharmaceutically acceptable carrier. Pharmaceutical compositions useful for treating RNA-dependent RNA viral infection in particular HCV infection are also encompassed by the present
invention as well as a method of inhibiting RNA-dependent RNA viral polymerase in particular HCV NS5B polymerase and a method of treating RNA-dependent viral replication and in particular HCV replication. Additionally, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of a compound of the present invention in combination with a therapeutically effective amount of another agent active against RNA-dependent RNA virus and in particular against HCV. Agents active against HCV include, but are not limited to, ribavirin, levovirin, viramidine, thymosin alpha-1, an inhibitor of HCV NS3 serine protease, interferon-α, pegylated interferon-α (peginterferon-α), a combination of interferon-α and ribavirin, a combination of peginterferon-α and ribavirin, a combination of interferon-α and levovirin, and a combination of peginterferon-α and levovirin. Interferon-α includes, but is not limited to, recombinant interferon-α2a (such as Roferon interferon available from Hoffmann-LaRoche, Nutley, NJ), interferon-α2b (such as Intron-A interferon available from Schering Corp., Kenilworth, NJ), a consensus interferon, and a purified interferon-α product. For a discussion of ribavirin and its activity against HCV, see J.O. Saunders and S.A. Raybuck, "Inosine Monophosphate Dehydrogenase: Consideration of Structure, Kinetics, and Therapeutic Potential," *Ann. Rep. Med. Chem.*, 35: 201-210 (2000).

Another aspect of the present invention provides for the use of the nucleoside cyclic phosphoramidates and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of RNA-dependent RNA viral replication, in particular HCV replication, and/or the treatment of RNA-dependent RNA viral infection, in particular HCV infection. Yet a further aspect of the present invention provides for the nucleoside cyclic phosphoramidates and their pharmaceutical compositions for use as a medicament for the inhibition of RNA-dependent RNA viral replication, in particular HCV replication, and/or for the treatment of RNA-dependent RNA viral infection, in particular HCV infection.

The pharmaceutical compositions of the present invention comprise a compound of structural formula I as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

In practical use, the compounds of structural formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms
depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally as, for example, liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Compounds of structural formula I may also be administered parenterally.

Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and
storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyl (e.g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of structural formula I are administered orally.

For oral administration to humans, the dosage range is 0.01 to 1000 mg/kg body weight in divided doses. In one embodiment the dosage range is 0.1 to 100 mg/kg body weight in divided doses. In another embodiment the dosage range is 0.5 to 20 mg/kg body weight in divided doses. For oral administration, the compositions are preferably provided in the form of tablets or capsules containing 1.0 to 1000 milligrams of the active ingredient, particularly, 1.5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art. This dosage regimen may be adjusted to provide the optimal therapeutic response.

The compounds of the present invention contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereoisomeric mixtures and individual diastereoisomers. When R₅ is hydrogen and R₄ in the amino acyl residue attached to the phosphorus atom in structural formula I is a substituent other than hydrogen in the formula

\[
\begin{align*}
\text{R}^4 & \\
\text{N} & \\
\text{CO}_2 & \\
\text{R}^6 &
\end{align*}
\]

the amino acid residue contains an asymmetric center and is intended to include the individual R- and S-stereoisomers as well as RS-stereoisomeric mixtures. In one embodiment, the stereochemistry at the stereogenic carbon corresponds to that of an 5-amino acid, that is, the naturally occurring alpha-amino acid stereochemistry, as depicted in the formula:
The tetrasubstituted phosphorus in compounds of structural formula I constitutes another asymmetric center, and the compounds of the present invention are intended to encompass both stereochemical configurations at the phosphorus atom.

The present invention is meant to comprehend nucleoside cyclic phosphorimidates having the β-D stereochemical configuration for the five-membered furanose ring as depicted in the structural formula below, that is, nucleoside cyclic phosphorimidates in which the substituents at C-1 and C-4 of the five-membered furanose ring have the β-stereochemical configuration ("up" orientation as denoted by a bold line).

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

Some of the compounds described herein may exist as tautomers such as keto-enol tautomers. The individual tautomers as well as mixtures thereof are encompassed with compounds of structural formula I. Example of keto-enol tautomers which are intended to be encompassed within the compounds of the present invention are illustrated below:

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Compounds of structural formula I may be separated into their individual diastereoisomers by, for example, fractional crystallization from a suitable solvent, for example methanol or ethyl acetate or a mixture thereof, or via chiral chromatography using an optically active stationary phase.

Alternatively, any stereoisomer of a compound of the structural formula I may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

The compounds of the present invention may be administered in the form of a pharmaceutically acceptable salt. The term "pharmacaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts of basic compounds encompassed within the term "pharmacaceutically acceptable salt" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts of basic compounds of the present invention include, but are not limited to, the following: acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide and valerate. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof include, but are not limited to, salts derived from inorganic bases including aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, mangamous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium,
magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, cyclic amines, and basic ion-exchange resins, such as arginine, betaine, caffeine, choline, N,N-dibenzylethlenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylhnoφ holine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, moφ holine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

Also, in the case of a carboxylic acid (-COOH) or hydroxyl group being present in the compounds of the present invention, pharmaceutically acceptable prodrug esters of carboxylic acid derivatives, such as methyl, ethyl, or pivaloyloxymethyl esters or prodrug acyl derivatives of the ribose C-2', C-3', and C-5' hydroxyls, such as O-acetyl, O-pivaloyl, O-benzoyl and O-aminoacetyl, can be employed. Included are those esters and acyl groups known in the art for modifying the bioavailability, tissue distribution, solubility, and hydrolysis characteristics for use as sustained-release or prodrug formulations. The contemplated derivatives are readily convertible in vivo into the required compound. Thus, in the methods of treatment of the present invention, the terms "administering" and "administration" is meant to encompass the treatment of the viral infections described with a compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound in vivo after administration to the mammal, including a human patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs," ed. H. Bundgaard, Elsevier, 1985, which is incorporated by reference herein in its entirety.

Preparation of the Nucleoside Cyclic Phosphoramidates of the Invention:

2'-C-Methylcytidine was prepared as described in the literature by C. Pierra et al., *Nucleosides, Nucleotides and Nucleic Acids*, 24: 767 (2005) or J.A. Piccirilli et al., *J. Org. Chem.*, 64: 747 (1999). 2'-Deoxy-2'-fluoro-2'-C-methylcytidine is prepared as described in *J Med. Chem.*, 48: 5504-5508 (2005). The aryl phosphorochloridates for the phosphorylation reactions were prepared according to the methods described in U.S. Patent No. 6,455,13, the contents of which are incorporated by reference herein in their entirety. The phosphorylation reactions to generate the aryl phosphoramidates of the present invention were carried out following the methods described in U.S. Patent No. 6,455,513 and C. McGuigan, et al., *J. Med. Chem.*, 36: 1048 (1993). For example, 4-chlorophenol was reacted with phosphorus oxychloride which was followed by coupling with different amino acid salts to give 4-chlorophenoxy phosphorochloridates which were then coupled with the nucleoside in the presence of a suitable base, such as /-butylmagnesium chloride (see M. Uchiyama et al. *J Org. Chem.*, 58: 373 (1993).
and Scheme 1). The resulting intermediates were dissolved in a suitable solvent, such as DMSO, and treated with potassium tert-butoxide to effect the cyclization by displacement of $p$-chlorophenol.

**General Procedures:**

All solvents were obtained from commercial sources and were used without further purification. Reactions were carried out under an atmosphere of nitrogen in oven dried (110 °C) glassware. Organic extracts were dried over sodium sulfate (Na$_2$SO$_4$), and were concentrated (after filtration of the drying agent) on rotary evaporators operating under reduced pressure. Flash chromatography was carried out on silica gel following published procedures (W.C. Still *et al.*, J. Org. Chem., 43: 2923 (1978)) or on commercial flash chromatography systems (Biotage corporation and Jones Flashmaster II) utilising pre-packed columns.

Reagents were usually obtained directly from commercial suppliers (and used as supplied) or are readily accessible using routine synthetic steps that are either reported in the scientific literature or are known to those skilled in the art.

1H and 31p NMR spectra were recorded on Bruker AM series spectrometers operating at (reported) frequencies between 300 and 600 MHz. Chemical shifts (δ) for signals corresponding to non-exchangeable protons (and exchangeable protons where visible) are recorded in parts per million (ppm) relative to tetramethylsilane and are measured using the residual solvent peak as reference. Signals are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad, and combinations thereof); coupling constant(s) in hertz (Hz); number of protons. Mass spectral (MS) data were obtained on a Perkin Elmer API 100, or Waters MicroMass ZQ, operating in negative (ES- or positive (ES+) ionization mode and results are reported as the ratio of mass over charge (m/z) for the parent ion only. Preparative scale HPLC separations were carried out on a Waters 2525 pump, equipped with a 2487 dual absorbance detector, on a TSP Spectra system P4000 equipped with a UV1000 absorption module or on an automated, mass-triggered Waters Micromass system incorporating a 2525 pump module, a Micromass ZMD detector and a 2525 collection module. Compounds were eluted with linear gradients of water and MeCN both containing 0.1% trifluoroacetic acid or formic acid using flow rates between 10 and 40 mL/min. Symmetry C18 columns (7 μM, 19 x 300 mm) were used as stationary phase.

The following abbreviations are used in the examples, the schemes and the tables:

aq.: aqueous; Ar: aryl; atm: atmosphere; CCl$_4$: carbon tetrachloride; DCM: dichloromethane; DMF: N,N-dimethylformamide; DMSO: dimethylsulfoxide; eq.: equivalent(s); ES = electrospray (MS); Et$_3$N: triethylamine; EtOAc: ethyl acetate; Et$_2$O: diethyl ether; h: hour(s); Me: methyl; MeCN: acetonitrile; MeOH: methanol; min: minutes; MS: mass spectrum; NMR:
nuclear magnetic resonance; N,N-DMA: \( \Lambda,\alpha \)-dimethylacetamide; PE: petroleum ether; Py: pyridine; quant.: quantitative; RP-HPLC: reversed phase high-performance liquid chromatography; RT: room temperature; sec: second(s); TFA: trifluoroacetic acid; and THF: tetrahydrofuran.

The Examples below provide illustrations of the conditions used for the preparation of the compounds of the present invention. These Examples are not intended to be limitations on the scope of the instant 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 can be used to prepare these and other compounds of the present invention. All temperatures are degrees Celsius unless otherwise noted.

![Chemical Structures]

**EXAMPLE 1**

**Step 1:** «-Butyl 7\(V\)-[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate

To 4-chlorophenyl dichlorodiphosphate in DCM (0.16 M) was added (2S)-1-butoxy-1-oxopropan-2-aminium chloride (1.0 eq), after cooling to -78 \( ^\circ\)C, Et\(_3\)N (2.0 eq.) was
added neat and the reaction was left to warm to room temperature overnight. All volatiles were removed and the resulting white solid washed with Et₂O, filtered and evaporated in vacuo to obtain a colourless oil as a 1:1 mixture of diastereomers. 31p NMR (400 MHz, CDCl₃): 9.5 and 9.3 ppm.

Step 2: H-Butyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1(2 H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]-methoxy]-{(4-chlorophenoxy)phosphoryl]-amino}propanoate

2'-C-Methylcytidine (evaporated twice from toluene) was diluted with THF (0.097 M). The resulting slurry was cooled to -78 °C, then tert-butylmagnesium chloride (as 1.0M solution in THF, 2.0 eq.) was added. The mixture was immediately warmed to 0 °C, stirred for thirty min and again cooled to -78 °C, then tert-butyl iV-[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate (as 1.0 M solution in THF, 2.0 eq.) was added dropwise. The reaction was allowed to reach room temperature overnight, and quenched by the addition of water. The aqueous phase was extracted three times with EtOAc, the combined organic phases were washed with brine and dried over Na₂SO₄. The crude was purified by column chromatography on silica gel (DCM/MeOH gradient from 90/10 to 80/20), the resulting off white solid was redissolved in DMSO and purified by RP-HPLC (stationary phase: column Symmetry C18, 7 µm, 19 x 300 mm. Mobile phase: acetonitrile/H₂O buffered with 0.1% TFA). Fractions containing the pure compounds were combined and freeze-dried to afford the title compounds as TFA-salts as a 1.07:1* mixture (First Eluting: Second Eluting).

1H NMR (300 MHz, OMSO-d₆) 8 8.05 and 8.04* (d, J = 7.74 Hz and J* = 7.96 Hz, IH), 7.46-7.38 (m, 2H), 7.34-7.25 (m, 2H), 6.10* and 6.08 (d, J* = 7.96 and J = 7.74 Hz, IH), 6.02* and 6.01 (s, IH), 4.67-4.53 (m, IH), 4.53-4.37 (m, IH), 4.25-4.08 (m, 3H), 4.07-3.93 (m, IH), 3.84* and 3.81 (d, J = 4.64 Hz and J* = 4.64 Hz, IH), 1.71-1.56 (m, 2H), 1.48-1.35 (m, 5H), 1.22 (s, 3H), 0.97 (t, J = 7.19 Hz, 3H); 31p NMR: (300 MHz CD₃OD) 8: 5.16, 5.09* ppm; MS (ES+) m/z 575 (M+ H)+
Step 3: n-Butyl (2S)-2-{[(4aR, 6R, 7R, 7aR)-6-(4-amino-2-aminopyridin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,2,3]dioxaphosphinin-2-yl]amino}propanoate

n-Butyl (2S)-2-{[(2R, 3R, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]methoxy} (4-chlorophenoxy) phosphoryl]amino]propanoate TFA-salt (as a 1:1 mixture of diastereomers) was diluted with anhydrous DMSO (0.018 M), then potassium tert-butoxide (3.0 eq.) was added and the reaction stirred at room temperature for 15 min. The reaction was quenched by the addition of 1N HCl.

The crude was purified by RP-HPLC (stationary phase: column Symmetry C18, 7 µm, 19 x 300 mm. Mobile phase: acetonitrile/H₂O buffered with 0.1% TFA). Fractions containing the pure compounds were combined and freeze dried to afford the title compounds as a white powder as TFA-salts:

First Eluting:

1H NMR (300 MHz, OMSO-d₆) δ 8.80 (br s, IH), 8.28 (br s, IH), 7.71 (d, J = 7.74 Hz, IH), 6.12-5.94 (m, 3H), 5.87 (t, J = 11.39 Hz, IH), 4.67-4.45 (m, 2H), 4.43-4.19 (m, IH), 4.18-4.03 (m, 3H), 3.95-3.81 (m, IH), 1.65-1.52 (m, 2H), 1.43-1.34 (m, 2H), 1.32 (d, J = 7.08 Hz, 3H), 1.09 (s, 3H), 0.91 (t, J = 7.30 Hz, 3H), 31p NMR: (300 MHz OMSO-d₆) δ: 4.58 ppm; MS (ES+) m/z 447 (M+ H)⁺

Second Eluting:

1H NMR (300 MHz, DMSO-J) δ 8.93 (br s, IH), 8.29 (br s, IH), 7.92 (d, J = 7.74 Hz, IH), 6.13-5.94 (m, 4H), 4.69-4.55 (m, IH), 4.53-4.37 (m, IH), 4.19-4.11 (m, 2H), 4.08 (t, J = 6.52 Hz, 2H), 3.88-3.73 (m, IH), 1.64-1.52 (m, 2H), 1.44-1.32 (m, 2H), 1.29 (d, J = 7.08 Hz, 3H), 1.13 (s, 3H), 0.91 (t, J = 7.19 Hz, 3H), 31p NMR: (300 MHz DMSO-J) δ: 6.63 ppm; MS (ES+) m/z 447 (M+ H)⁺

EXAMPLE 2

Step 1: Ethyl N-[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate
Following the procedure described for Step 1 of Example 1, treatment of a DCM (0.16 M) solution of para-chlorophenyl dichlorodiphosphate with L-alanine ethyl ester hydrochloride (1.0 eq.) and Et$_3$N (2.0 eq.) provided the title compound as a colourless oil as a 1:1 mixture of diastereomers.

- $^3$I$_p$ NMR (300 MHz, CDCl$_3$): $\delta$ 9.43 and 9.11 ppm.

Step 2: Ethyl (2S)-2-{{[(2R, 3R, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]methoxy}(4-chlorophenoxy) phosphoryl]amino}propanoate

Following the procedure described for Step 2 of Example 1, 2'-C-methylcytidine (evaporated twice from toluene) in THF (0.097 M) was cooled to -78 °C, then tert-butylmagnesium chloride (as 1.0M solution in THF, 2.2 eq.) was added, followed by the addition of ethyl N-[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate (as a 1.0M solution in THF, 2.0 eq.). The crude was purified by column chromatography on silica gel (DCM/MeOH gradient from 90/10 to 80/20), the resulting off white solid was redissolved in DMSO and purified by RP-HPLC (stationary phase: column Symmetry C18, 7 μm, 19 x 300 mm. Mobile phase: acetonitrile/H$_2$O buffered with 0.1% TFA). Fractions containing the pure compounds were combined and freeze-dried to afford the title compounds as a white powder as TFA-salt as a 1:1.1* mixture (First Eluting: Second Eluting).

$^1$H NMR (300 MHz, $OMSO-d_6$): $\delta$ 8.03 and 8.02* (d, $J = 7.74$ Hz and $J^* = 7.96$ Hz, IH), 7.47-7.36 (m, 2H), 7.35-7.20 (m, 2H), 6.12-6.04 (m, IH), 6.02 and 6.01* (s, IH), 4.67-4.53 (m, IH), 4.53-4.37 (m, IH), 4.25-4.11 (m, 3H), 4.06-3.93 (m, IH), 3.83* and 3.80 (d, $J^* = 4.20$ Hz and $J = 4.22$ Hz, IH), 1.44-1.35 (m, 3H), 1.32-1.24 (m, 3H), 1.21 (m, 3H); $^3$I$_p$ NMR: (300 MHz, CD$_3$OD) $\delta$: 5.20, 5.10* ppm; MS (ES+) m/z 547 (M+H)+
Step 3: Ethyl (2S)-2-{{(4a/?, 6R, 7a/?)-6-(4-amino-2-aminopyridin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-\(H\)-furo[3,2-d][1,2,3] dioxaphosphinin-2-yl] amino}propanoate

Following the procedure described for Step 3 of Example 1, ethyl (2S)-2-{{[(2R, 3R, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]methoxy} (4-chlorophenoxy) phosphoryl} amino}propanoate TFA-salt (as a 1:1 mixture of diastereomers; first eluting: second eluting) was diluted with anhydrous DMSO (0.018 M), then potassium tert-butoxide (3.0 eq.) was added and the reaction stirred at room temperature for thirty min. The reaction was quenched by the addition of IN HCl. The crude was purified by RP-HPLC (stationary phase: column Symmetry C18, 7 µm, 19 x 300 mm. Mobile phase: acetonitrile/H\(_2\)O buffered with 0.1% TFA). Fractions containing the pure compounds were combined and freeze dried to afford the title compounds as a white powder as TFA-salts:

First Eluting:
1H NMR (300 MHz, CD\(_3\)OD): \(\delta\) 7.90 (d, J = 7.74 Hz, 1H), 6.19-6.08 (m, 2H), 4.70-4.51 (m, 2H), 4.40-4.29 (m, 2H), 4.23 (q, J = 7.16 Hz, 2H), 4.19-4.09 (m, 2H), 4.03-3.89 (m, 1H), 1.44 (d, J = 7.07 Hz, 3H), 1.32 (t, J = 6.97 Hz, 3H), 1.30 (s, 3H), 31p NMR: (300 MHz, CD\(_3\)OD): \(\delta\) 8.35 ppm; MS (ES+) m/z 419 (M+ H)+

Second Eluting:
1H NMR (300 MHz, CD\(_3\)OD): \(\delta\) 7.89 (d, J = 7.83 Hz, 1H), 6.19-6.09 (m, 2H), 4.72-4.56 (m, 2H), 4.40-4.29 (m, 2H), 4.23 (q, J = 7.15 Hz, 2H), 4.19-4.12 (m, 2H), 4.03-3.92 (m, 2H), 1.44 (d, J = 7.07 Hz, 3H), 1.32 (t, J = 7.07 Hz, 3H), 1.30 (s, 3H), 31p NMR: (300 MHz, CD\(_3\)OD): \(\delta\) 7.24 ppm; MS (ES+) m/z 419 (M+ H)+

EXAMPLE 3

Step 1: H-Heptyl \(N\)-[chloro(4-chlorophenoxy)phosphoryl]-L-alaninate
Following the procedure described for Step 1 of Example 1, treatment of a DCM (0.098 M) solution of para-chlorophenyl dichlorodiphosphate with L-alanine n-heptyl ester hydrochloride(1.0 eq.) and Et$_3$N (2.0 eq.) provided the title compound as a colourless oil as a 1:1 mixture of diastereomers.

31p NMR (300 MHz, CD$_3$OD): $\delta$ 9.5 and 9.2

Step 2: n-Heptyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1 (2 H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]methoxy]-(4-chlorophenoxy) phosphoryl]-amino]propanoate

Following the procedure described for Step 2 of Example 1, 2'-C-methylcytidine (evaporated twice from toluene) in THF (0.097 M) was cooled to -78 °C, then tert-butylmagnesium chloride (as 1.0M solution in THF, 2.2 eq.) was added, followed by the addition of $\text{N}^\text{-heptyl}$-[chloro-(4-chlorophenoxy)]phosphoryl]-L-alaninate (as a 1.0M solution in THF, 2.0 eq.). The crude was purified by column chromatography on silica gel (DCM/MeOH gradient from 90/10 to 80/20), the resulting off white solid was redissolved in DMSO and purified by RPHPLC (stationary phase: column Symmetry C18, 7 μm, 19 x 300 mm. Mobile phase: acetonitrile/H$_2$O buffered with 0.1% TFA). Fractions containing the pure compounds were combined and freeze dried to afford the title compounds as a white powder as TFA-salt as a 1.1*:1 mixture (First Eluting:Second Eluting).

1H NMR (400 MHz, CD$_3$OD) $\delta$ 7.99 and 7.96* (d, J = 8.09 Hz and J* = 8.08 Hz, IH), 7.40-7.37 (m, 2H), 7.29-7.23 (m, 2H), 6.06 and 6.04* (d, J = 8.09 Hz and J* = 8.08 Hz, IH), 5.99* and 5.98 (s, IH), 4.61-4.51 (m, IH), 4.48-4.37 (m, IH), 4.18-4.05 (m, 3H), 4.01-3.93 (m, IH), 3.79* and 3.78 (d, J* = 9.1 Hz and J = 9.09 Hz, IH), 1.65-1.59 (m, 2H), 1.39-1.30 (m, 1IH), 1.18 and 1.17* (s, 3H), 0.92-0.89 (t, J = 6.57 Hz, 3H), NH$_2$, NH, 2 x OH not visible, 31p NMR: (400 MHz CD$_3$OD): $\delta$ 4.03* and 3.98 ; MS (ES+) m/z 618 (M+ H)+

Step 3: n-Heptyl (2S)-2-{{[4ai?}, 67?; IR, 7ai?]6-(4-amino-2-aminopyridin-1(2 H)-yl)-l-hydroxy-7-methyl-2-oxidotetrahydro-4/-furo[3,2-d][1,2,3] dioxaphosphinin-2-yl] amino]propanoate
Following the procedure described for Step 3 of Example 1, «-heptyl (2S)-2-[([(2R, 3R, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1(2 H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl]methoxy} (4-chlorophenoxy) phosphoryl]amino)propanoate TFA-salt (as a 1:1 mixture of diastereomers) was diluted with anhydrous DMSO (0.018 M), then potassium tert-butoxide (2.0 eq.) was added and the reaction stirred at room temperature for thirty min. The reaction was quenched by the addition of IN HCl. The crude was purified by RP-HPLC (stationary phase: column Symmetry C18, 7 µm, 19 x 300 mm. Mobile phase: acetonitrile/H₂O buffered with 0.1% TFA). Fractions containing the pure compounds were combined and freeze dried to afford the title compounds as a white powder as TFA-salts:

First Eluting:
1H NMR (400 MHz, CD₃OD): δ 7.80 (d, J = 7.58 Hz, IH), 6.13-6.12 (bs, 2H), 4.67-4.63 (m, IH), 4.52-4.46 (m, 2H), 4.22-4.14 (m, 3H), 3.98-3.91 (m, IH), 1.72-1.65 (qt, J = 6.82 Hz, 2H), 1.45-1.43 (d, J = 7.07 Hz, 3H), 1.39-1.33 (m, 8H), 1.26 (bs, 3H), 0.92 (t, J = 6.52 Hz, 3H), NH₂, NH, 2 x OH not visible, 31p NMR: (400 MHz, CD₃OD): δ:5.25; MS (ES+) m/z 489 (M+ H)+

Second Eluting:
1H NMR (400 MHz, CD₃OD) δ 7.85 (d, J = 7.83 Hz, IH), 6.12-6.10 (m, 2H), 4.63-4.60 (m, 2H), 4.34-4.28 (m, IH), 4.17-4.12 (m, 3H), 3.99-3.91 (m, IH), 1.68 (qt, J = 6.82 Hz, 2H), 1.41 (d, J = 7.07 Hz, 3H), 1.38-1.31 (m, 8H), 1.27 (bs, 3H), 0.92 (t, J = 6.82 Hz, 3H), NH₂, NH, 2 x OH not visible, 31p NMR: (400 MHz CD₃OD) δ 7.16, MS (ES+) m/z 489 (M+ H)+

EXAMPLES 4-19

Examples 4-19 set forth in Table 1 were following the methods detailed for Examples 1-3.
TABLE 1

<table>
<thead>
<tr>
<th>Ex.</th>
<th>R&lt;sup&gt;4&lt;/sup&gt;</th>
<th>R&lt;sup&gt;6&lt;/sup&gt;</th>
<th>Example name</th>
<th>MS (ES&lt;sup&gt;+&lt;/sup&gt;) m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Me</td>
<td>tert-butyl</td>
<td>tert-butyl N-[(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2] dioxaphosphin-2-yl]-L-alaninate</td>
<td>447</td>
</tr>
<tr>
<td>5</td>
<td>Me</td>
<td>n-butyl</td>
<td>n-butyl N-[(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2] dioxaphosphin-2-yl]-L-alaninate</td>
<td>447</td>
</tr>
<tr>
<td>6</td>
<td>Et</td>
<td>Et</td>
<td>ethyl (2S)-2-{[(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2] dioxaphosphin-2-yl]amino} butanoate</td>
<td>433</td>
</tr>
<tr>
<td>7</td>
<td>iBu</td>
<td>Et</td>
<td>ethyl N-[(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2] dioxaphosphin-2-yl]-L-leucinate</td>
<td>461</td>
</tr>
<tr>
<td>8</td>
<td>nPr</td>
<td>Et</td>
<td>ethyl N-[(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2] dioxaphosphin-2-yl]-L-norvalinate</td>
<td>447</td>
</tr>
<tr>
<td></td>
<td>Me</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>----</td>
<td>--------------------------</td>
<td>-----------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Me</td>
<td>cyclopentyl</td>
<td>cyclopentyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Me</td>
<td>Et</td>
<td>ethyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Me</td>
<td>n-heptyl</td>
<td>n-heptyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Me</td>
<td>cyclohexyl</td>
<td>cyclohexyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Me</td>
<td>isopentyl</td>
<td>isopentyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Me</td>
<td>3-methoxy-propyl</td>
<td>3-methoxypropyl N[(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Me</td>
<td>2-Et-butyl</td>
<td>2-ethylbutyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Me</td>
<td>2-nPr-pentyl</td>
<td>2-propylpentyl N-(4aR,6R,7R,7aR)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate</td>
<td></td>
</tr>
</tbody>
</table>
EXAMPLE 20

Ethyl (2S)-2-((4ai?,6i?,7i?,7ai?)-7-hydroxy-7-methyl-2-oxido-6-[2-oxo-4-[(2-
propylpentanoyl)amino]pyrimidin-l(2H)-yl]tetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-
yl]amino)butanoate, prepared according the procedure described for Example 6, was diluted with a solution THF:DMF (5:2, 0.03 M). Et3N (4.0 eq.) and DMAP (0.2 eq.) were added then the mixture was cooled to 0°C and 2-propylpentanoyl chloride (2.5 eq.) was added drop-wise. The mixture was immediately warmed to room temperature and stirred for 2 hours. The reaction was quenched by the addition of IN HCl (aq) to bring the pH to 6, and then the solvent was removed in vacuum. The crude was purified by RP-HPLC (stationary phase: column Symmetry C18, 7 µm, 19 x 300 mm. Mobile phase: acetonitrile/H2O buffered with 0.1% TFA) to afford the title compound as white powder (36%).
1H NMR (300 MHz, DMSO-\textit{d}_6) \delta 10.95 (s, IH), 8.12 (d, J = 7.53 Hz, IH), 7.28 (d, J = 7.44 Hz, IH), 6.06 (s, IH), 5.95 (dd, J = 13.15 Hz, J = 10.14 Hz, IH), 4.68-4.58 (m, IH), 4.52-4.37 (m, IH), 4.17-4.12 (m, 2H), 4.10 (q, J = 7.05 Hz, 2H), 3.72-3.59 (m, IH), 2.66-2.56 (m, IH), 1.70-1.46 (m, 4H), 1.39-1.21 (m, 6H), 1.19 (t, J = 7.05 Hz, 3H), 1.04 (s, 3H), 0.89-0.8 (m, 6H); 31p NMR: (300 MHz, \textit{OMSO}-\textit{d}_6) \delta: 5.78 ppm; MS (ES+) m/z 559 (M+H)+

**EXAMPLES 21-23**

Examples 21-23 as set forth in Table 2 were prepared following the method described in Example 20.

**TABLE 2**

<table>
<thead>
<tr>
<th>Ex.</th>
<th>R^4</th>
<th>R^6</th>
<th>R^9</th>
<th>Example name</th>
<th>MS (ES+) m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Me</td>
<td>Et</td>
<td>2-propylpentanoyl</td>
<td>ethyl N-{(4aR,6R,7R,7aR)-7-hydroxy-7-methyl-2-oxido-6-[2-oxo-4-[(2-propylpentanoyl)amino]pyrimidin-1(2H)-yl]tetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl}-L-alaninate</td>
<td>545</td>
</tr>
<tr>
<td>22</td>
<td>Me</td>
<td>\textit{n}-heptyl</td>
<td>2-propylpentanoyl</td>
<td>\textit{n}-heptyl N-{(4aR,6R,7R,7aR)-7-hydroxy-7-methyl-6-{4-[(1-methylene-2-propylpentyl)amino]-2-oxopyrimidin-1(2H)-yl}-2-oxidotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-2-yl}-L-alaninate</td>
<td>615</td>
</tr>
</tbody>
</table>
BIOLOGICAL ASSAYS:

The assay employed to measure the inhibition of HCV replication is described below.

A. Assay for Inhibition of HCV RNA Replication:

The compounds of the present invention are evaluated for their ability to affect the replication of Hepatitis C Virus RNA in cultured hepatoma (HuH-7) cells containing a subgenomic HCV Replicon. The details of the assay are described below. This Replicon assay is a modification of that described in V. Lohmann, F. Korner, J-O. Koch, U. Herian, L. Theilmann, and R. Bartenschlager, "Replication of a Sub-genomic Hepatitis C Virus RNAs in a Hepatoma Cell Line," Science 285:110 (1999).

Protocol:

The assay is an in situ Ribonuclease protection, Scintillation Proximity based-plate assay (SPA). 10,000 - 40,000 cells are plated in 100-200 µL of media containing 0.8mg/mL G418 in 96-well cytostar plates (Amersham). Compounds are added to cells at various concentrations up to 100 µM in 1% DMSO at time 0 to 18 h and then cultured for 24-96 h. Cells are fixed (20 min, 10% formalin), permeabilized (20 min, 0.25% Triton X-100/PBS) and hybridized (overnight, 50°C) with a single-stranded 33P RNA probe complementary to the (+) strand NS5B (or other genes) contained in the RNA viral genome. Cells are washed, treated with RNAse, washed, heated to 65°C and counted in a Top-Count. Inhibition of replication is read as a decrease in counts per minute (cpm).

Human HuH-7 hepatoma cells, which are selected to contain a subgenomic replicon, carry a cytoplasmic RNA consisting of an HCV 5′ non-translated region (NTR), a neomycin selectable marker, an EMCV IRES (internal ribosome entry site), and HCV non-structural proteins NS3 through NS5B, followed by the 3′ NTR.

Representative compounds tested in the replication assay exhibit EC50’s less than 100 micromolar. For example, the title compounds of Examples 1-23 were tested in the replicon assay and were found to have EC50 values as set forth in Table 3 below.
TABLE 3

<table>
<thead>
<tr>
<th>Example No.</th>
<th>Replicon Assay EC₅₀ (µM)</th>
<th>Example No.</th>
<th>Replicon Assay EC₅₀ (µM)</th>
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<td>1</td>
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<td>&gt;10</td>
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</tr>
<tr>
<td>12</td>
<td>&gt;20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Assay for Intracellular Metabolism:

Part One

The compounds of the present invention can also be evaluated for their ability to enter a human hepatoma cell line and be converted intracellularly into the corresponding nucleoside 5'-mono-, di-, and triphosphates.

Two cell lines, HuH-7 and HBIIOA, are used for intracellular metabolism studies of the compounds of the present invention. HuH-7 is a human hepatoma cell line, and HBIIOA denotes a clonal line derived from HuH-7 cells that harbors the HCV bicistronic replicon. HuH-7 cells are plated in complete Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and HBIIOA cells in the same containing G418 (0.8 mg/mL) at 1.5 x 10⁶ cells/60-mm dish such that cells were 80% confluent at the time of compound addition. Tritiated compound is incubated at 2 µM in the cell medium for 3 or 23 h. Cells are collected, washed with phosphate-buffered saline, and counted. The cells are then extracted in 70% methanol, 20 mM EDTA, 20 mM EGTA, and centrifuged. The lysate is dried, and radiolabeled nucleotides are analyzed using an ion-pair reverse phase (C-18) HPLC on a Waters Millenium system connected to an in-line β-RAM scintillation detector (IN/US Systems). The HPLC mobile phases consists of (a) 10 mM potassium phosphate with 2 mM tetrabutylammonium hydroxide and (b) 50% methanol containing 10 mM potassium phosphate with 2 mM tetrabutyl-ammonium hydroxide. Peak identification is made by comparison of retention times to standards. Activity is expressed as picomoles of nucleotide detected in 10⁶ HuH-7 or HBIIOA cells.
Part Two

The compounds of the present invention were evaluated for their ability to penetrate cells (human hepatoma cell line, hepatocytes) and undergo intracellular conversion to the triphosphate. The method utilized a variety of cell lines and compounds. Following the incubation of compounds with cells, samples are extracted and quantified by HPLC.

Cells are prepared according to the following protocols:

Cells in suspension: for cryopreserved cells the protocol by In Vitro Technologies (Edison, NJ, USA) for cryopreserved cell handling was followed.

For fresh cells preparation the protocol published in Xenobiotica 2005, 35 (1035-54); Giuliano C et al. was followed.

Cells were resuspended to an appropriate cell density (generally 10^6 cells/mL; single donor or pool of 10 donors) in Hepatocyte Basal medium (Clonetics, CC-3199) and 0.2 mL/well were transferred to sterile 96 well round bottom assay plate (Costar 3788).

Compounds were added in DMSO at 1:1000 dilution, mixed by gentle swirling and incubated at 37 °C under carbogen in a Dubnoff Metabolic Shaking Incubator. Aliquots of the cell suspension were removed at different times, centrifuged at 4 °C for 20 seconds. For adherent cell lines the cells were plated out approximately 1 day in advance in 6-well tissue-culture treated plates in appropriate media and incubated at 37°C/5% CO2- 24 hours after plating, cells were treated with compounds diluted at 1:1000 and incubated for an appropriate period of time at 37°C/5% CO2- In all cases the incubation media was removed by aspiration and then the cells were extracted with cold 70% MeOH, 20 mM EDTA and 20 mM EGTA and centrifuged. The lysate was dried under nitrogen, purified by solid-phase extraction, and stored at -20 °C until analysis.

The dried lysate was analyzed using ZIC-HILIC SeQuant column (100 x 2.1 mm, 5 µm) on an Agilent 1100 HPLC connected to an API 4000 mass-spectrometer equipped with an electrospray interface (ESI). The mass spectrometer was operated in negative ion electrospray mode. The HPLC mobile phases consisted of: Eluent A: Water with 0.1% formic acid. B: Acetonitrile with 0.1% formic acid. Peak identification was made by comparison of retention times to standards. Activity was expressed as area under the concentration curve (AUC, µMxh).

Representative compounds were incubated with human hepatocytes for 2 hours and shown to form high levels of nucleoside triphosphate (Table 4).

<table>
<thead>
<tr>
<th>Example No.</th>
<th>Human Hepat. AUC (µM x h)</th>
<th>Example No.</th>
<th>Human Hepat. AUC (µM x h)</th>
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</tr>
<tr>
<td>5</td>
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<td>23</td>
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</tbody>
</table>

Table 4.
The nucleoside cyclic phosphoramidates of the present invention can also be evaluated for cellular toxicity and anti-viral specificity in the counterscreens described below.

C. COUNTERSCREENS:

The ability of the nucleoside cyclic phosphoramidates of the present invention to inhibit human DNA polymerases is measured in the following assays.

a. Inhibition of Human DNA Polymerases alpha and beta:

<table>
<thead>
<tr>
<th>6</th>
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<th>16</th>
<th>55</th>
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<td>12</td>
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<td>47</td>
</tr>
</tbody>
</table>

The nucleoside cyclic phosphoramidates of the present invention can also be evaluated for cellular toxicity and anti-viral specificity in the counterscreens described below.

C. COUNTERSCREENS:

The ability of the nucleoside cyclic phosphoramidates of the present invention to inhibit human DNA polymerases is measured in the following assays.

a. Inhibition of Human DNA Polymerases alpha and beta:

- **Reaction Conditions:**
  - 50 µL reaction volume
- **Reaction buffer components:**
  - 20 mM Tris-HCl, pH 7.5
  - 200 µg/mL bovine serum albumin
  - 100 mM KCl
  - 2 mM β-mercaptoethanol
  - 10 mM MgCl₂
  - 1.6 µM dA, dG, dC, dTTP
  - α-³²P-dATP

- **Enzyme and template:**
  - 0.05 mg/mL gapped fish sperm DNA template
  - 0.01 U/µL DNA polymerase α or β

- **Preparation of gapped fish sperm DNA template:**
  - Add 5 µL 1M MgCl₂ to 500 µL activated fish sperm DNA (USB 70076);
  - Warm to 37°C and add 30 µL of 65 U/µL of exonuclease III (GibcoBRL 18013-OH);
  - Incubate 5 min at 37°C;
  - Terminate reaction by heating to 65°C for 10 min;
  - Load 50-100 µL aliquots onto Bio-spin 6 chromatography columns (Bio-Rad 732-6002) equilibrated with 20 mM Tris-HCl, pH 7.5;
  - Elute by centrifugation at 1,000Xg for 4 min;
Pool eluate and measure absorbance at 260 nm to determine concentration.

The DNA template is diluted into an appropriate volume of 20 mM Tris-HCl, pH 7.5 and the enzyme is diluted into an appropriate volume of 20 mM Tris-HCl, containing 2 mM β-mercaptoethanol, and 100 mM KCl. Template and enzyme are pipetted into microcentrifuge tubes or a 96 well plate. Blank reactions excluding enzyme and control reactions excluding test compound are also prepared using enzyme dilution buffer and test compound solvent, respectively. The reaction is initiated with reaction buffer with components as listed above. The reaction is incubated for 1 hour at 37°C. The reaction is quenched by the addition of 20 μL 0.5M EDTA. 50 μL of the quenched reaction is spotted onto Whatman DE81 filter disks and air dried. The filter disks are repeatedly washed with 150 mL 0.3M ammonium formate, pH 8 until 1 mL of wash is < 100 cpm. The disks are washed twice with 150 mL absolute ethanol and once with 150 mL anhydrous ether, dried and counted in 5 mL scintillation fluid.

The percentage of inhibition is calculated according to the following equation: % inhibition = [(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

b. Inhibition of Human DNA Polymerase gamma:

The potential for inhibition of human DNA polymerase gamma can be measured in reactions that include 0.5 ng/μL enzyme; 10 μM dATP, dGTP, dCTP, and TTP; 2 μCi/reaction [α-33P]-dATP, and 0.4 μg/μL activated fish sperm DNA (purchased from US Biochemical) in a buffer containing 20 mM Tris pH8, 2 mM β-mercaptoethanol, 50 mM KCl, 10 mM MgCl2, and 0.1 μg/μL BSA. Reactions are allowed to proceed for 1 h at 37°C and are quenched by addition of 0.5 M EDTA to a final concentration of 142 mM. Product formation is quantified by anion exchange filter binding and scintillation counting. Compounds are tested at up to 50 μM.

The percentage of inhibition is calculated according to the following equation: % inhibition = [(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

The ability of the nucleoside cyclic phosphoramidates of the present invention to inhibit HIV infectivity and HTV spread can be measured in the following assays:

c. HIV Infectivity Assay

Assays can be performed with a variant of HeLa Magi cells expressing both CXCR4 and CCR5 selected for low background β-galactosidase (β-gal) expression. Cells are infected for 48 h, and β-gal production from the integrated HTV-I LTR promoter is quantified with a chemiluminescent substrate (Galactolight Plus, Tropix, Bedford, MA). Inhibitors are
titrated (in duplicate) in twofold serial dilutions starting at 100 µM; percent inhibition at each concentration is calculated in relation to the control infection.

d. Inhibition of HTV Spread

The ability of the compounds of the present invention to inhibit the spread of the human immunodeficiency virus (HIV) can be measured by the method described in U.S. Patent No. 5,413,999 (May 9, 1995), and J.P. Vacca, et al., Proc. Natl. Acad. Sci., 91: 4096-4100 (1994), which are incorporated by reference herein in their entirety.

The nucleoside cyclic phosphoramidates of the present invention are also screened for cytotoxicity against cultured hepatoma (HuH-7) cells containing a subgenomic HCV Replicon in an MTS cell-based assay as described in the assay below. The HuH-7 cell line is described in H. Nakabayashi, et al., Cancer Res., 42: 3858 (1982).

e. Cytotoxicity assay:

Cell cultures can be prepared in appropriate media at concentrations of approximately 1.5 x 10^5 cells/mL for suspension cultures in 3 day incubations and 5.0 x 10^4 cells/mL for adherent cultures in 3 day incubations. 99 µL of cell culture are transferred to wells of a 96-well tissue culture treated plate, and 1 µL of 100-times final concentration of the test compound in DMSO is added. The plates are incubated at 37°C and 5% CO₂ for a specified period of time. After the incubation period, 20 µL of CellTiter 96 Aqueous One Solution Cell Proliferation Assay reagent (MTS) (Promega) is added to each well and the plates are incubated at 37°C and 5% CO₂ for an additional period of time up to 3 h. The plates are agitated to mix well and absorbance at 490 nm is read using a plate reader. A standard curve of suspension culture cells is prepared with known cell numbers just prior to the addition of MTS reagent. Metabolically active cells reduce MTS to formazan. Formazan absorbs at 490 nm. The absorbance at 490 nm in the presence of compound is compared to absorbance in cells without any compound added.


The following assays can be employed to measure the activity of the compounds of the present invention against other RNA-dependent RNA viruses:
a. Determination of In Vitro Antiviral Activity of Compounds Against Rhinovirus (Cytopathic Effect Inhibition Assay):


**Viruses:**

Rhinovirus type 2 (RV-2), strain HGP, is used with KB cells and media (0.1% NaHCO₃, no antibiotics) as stated in the Sidwell and Huffman reference. The virus, obtained from the ATCC, is from a throat swab of an adult male with a mild acute febrile upper respiratory illness. Rhinovirus type 9 (RV-9), strain 211, and rhinovirus type 14 (RV-14), strain Tow, are also obtained from the American Type Culture Collection (ATCC) in Rockville, MD. RV-9 is from human throat washings and RV-14 is from a throat swab of a young adult with upper respiratory illness. Both of these viruses are used with HeLa Ohio-1 cells (Dr. Fred Hayden, Univ. of VA) which are human cervical epitheloid carcinoma cells. MEM (Eagle's minimum essential medium) with 5% Fetal Bovine serum (FBS) and 0.1% NaHCO₃ is used as the growth medium. Antiviral test medium for all three virus types was MEM with 5% FBS, 0.1% NaHCO₃, 50 µg gentamicin/mL, and 10 mM MgCl₂.

2000 µg/mL is the highest concentration used to assay the compounds of the present invention. Virus was added to the assay plate approximately 5 min after the test compound. Proper controls are also run. Assay plates are incubated with humidified air and 5% CO₂ at 37°C. Cytotoxicity is monitored in the control cells microscopically for morphologic changes. Regression analysis of the virus CPE data and the toxicity control data gives the ED50 (50% effective dose) and CC50 (50% cytotoxic concentration). The selectivity index (SI) is calculated by the formula: SI = CC50 ÷ ED50.

b. Determination of In Vitro Antiviral Activity of Compounds Against Dengue, Banzi, and Yellow Fever (CPE Inhibition Assay)

Assay details are provided in the Sidwell and Huffman reference above.

**Viruses:**

Dengue virus type 2, New Guinea strain, is obtained from the Center for Disease Control. Two lines of African green monkey kidney cells are used to culture the virus (Vero) and to perform antiviral testing (MA-104). Both Yellow fever virus, 17D strain, prepared from infected mouse brain, and Banzi virus, H 336 strain, isolated from the serum of a febrile boy in South Africa, are obtained from ATCC. Vero cells are used with both of these viruses and for assay.
Cells and Media:
MA-104 cells (BioWhittaker, Inc., Walkersville, MD) and Vero cells (ATCC) are used in
Medium 199 with 5% FBS and 0.1% NaHCO₃ and without antibiotics.
Assay medium for dengue, yellow fever, and Banzi viruses is MEM, 2% FBS, 0.18% NaHCO₃
and 50 µg gentamicin/mL.

Antiviral testing of the compounds of the present invention can be performed according to the
Sidwell and Huffman reference and similar to the above rhinovirus antiviral testing. Adequate
cytopathic effect (CPE) readings are achieved after 5-6 days for each of these viruses.

c. Determination of In Vitro Antiviral Activity of Compounds Against West Nile Virus (CPE
Inhibition Assay)
Assay details are provided in the Sidwell and Huffman reference cited above. West Nile virus,
New York isolate derived from crow brain, is obtained from the Center for Disease Control.
Vero cells are grown and used as described above. Test medium is MEM, 1% FBS, 0.1%
NaHCO₃ and 50 µg gentamicin/mL.

Antiviral testing of the compounds of the present invention can be performed following the
methods of Sidwell and Huffman which are similar to those used to assay for rhinovirus activity.
Adequate cytopathic effect (CPE) readings are achieved after 5-6 days.

d. Determination of In Vitro Antiviral Activity of Compounds Against rhino, yellow fever.
dengue, Banzi, and West Nile Viruses (Neutral Red Uptake Assay)
After performing the CPE inhibition assays above, an additional cytopathic
detection method can be used which is described in "Microtiter Assay for Interferon:
Microspectrophotometric Quantitation of Cytopathic Effect," Appl. Environ. Microbiol. 31: 35-
38 (1976). A Model EL309 microplate reader (Bio-Tek Instruments Inc.) is used to read the
assay plate. ED₅₀'s and CD₅₀'s are calculated as above.

EXAMPLE OF A PHARMACEUTICAL FORMULATION
As a specific embodiment of an oral composition of a compound of the present
invention, 50 mg of the compound of Example 1 can be formulated with sufficient finely divided
lactose to provide a total amount of 580 to 590 mg to fill a size 0 hard gelatin capsule.

While the invention has been described and illustrated in reference to specific
embodiments thereof, those skilled in the art will appreciate that various changes, modifications,
and substitutions can be made therein without departing from the spirit and scope of the
invention. For example, effective dosages other than the preferred doses as set forth hereinabove may be applicable as a consequence of variations in the responsiveness of the human being treated for severity of the HCV infection. Likewise, the pharmacologic response observed may vary according to and depending upon the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended therefore that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.
WHAT IS CLAIMED IS:

1. A compound of the structural formula I:

   \[ \text{(I),} \]

   or a pharmaceutically acceptable salt thereof; wherein

   - B is
   - n is 0, 1, or 2;
   - R1 is hydrogen, methyl, or fluoromethyl;
   - R2 is fluoro or OR3;
   - R3 is selected from the group consisting of hydrogen, methyl, Ci-16 alkylcarbonyl, C2-18 alkenylcarbonyl, Ci-io alkoxy carbonyl, C3-6 cycloalkylcarbonyl, C3-6 cycloalkyloxycarbonyl, and an amino acyl residue of structural formula:

   \[ \text{;} \]

   - R4 is hydrogen, C1.5 alkyl, phenyl, or benzyl; wherein alkyl is optionally substituted with one substituent selected from the group consisting of fluorine, hydroxy, methoxy, amino, carboxy, carbamoyl, guanidino, mercapto, methylthio, 1H-imidazolyl, and 1H-indol-3-yl; and wherein phenyl and benzyl are optionally substituted with one to two substituents independently selected from the group consisting of halogen, hydroxy, and methoxy;

   - R5 is hydrogen or methyl;
   - or R4 and R5 together with the carbon atom to which they attached form a 3- to 6-membered aliphatic spirocyclic ring system;

   - R6 is hydrogen, Ci_i6 alkyl, C2-20 alkenyl, (CH2)nC3-6 cycloalkyl, phenyl, benzyl, or adamantyl; wherein alkyl, alkenyl, cycloalkyl, and adamantyl are optionally substituted
with one to three substituents independently selected from halogen, hydroxy, carboxy, C1-8 alkoxy; and wherein phenyl and benzyl are optionally substituted with one to three substituents independently selected from halogen, hydroxy, cyano, C1-4 alkoxy, trifluoromethyl, and trifluoromethoxy;

R7 is hydrogen, C1.5 alkyl, or phenyl C0-2 alkyl;
R8 is hydrogen, C1.4 alkyl, C1.4 acyl, benzoyl, C1.4 alkylxycarbonyl, phenyl C0-2 alkylxycarbonyl, C1.4 alkylaminocarbonyl, phenyl C0-2 alkylaminocarbonyl, C1.4 alkylsulfonyl, or phenyl C0-2 alkylsulfonyl;
R9 is hydrogen, C1-8 alkyloxycarbonyl, C1-8 alkylxycarbonyl, or [(di-C1-8 alkylamino)-C1-8 alkoxy]carbonyl; and
R10 is hydrogen, C1-8 alkyl, or C1-8 alkyloxycarbonyl.

2. The compound of Claim 1, or a pharmaceutically acceptable salt thereof, wherein the compound is a compound of Formula I-A:

![Formula I-A](image)

3. The compound of Claim 1, or a pharmaceutically acceptable salt thereof, wherein the compound is a compound of Formula I-B1:

![Formula I-B1](image)
4. The compound of Claim 2, or a pharmaceutically acceptable salt thereof, wherein
                      R⁶ is hydrogen, C\1 6 alkyl, C2-20 alkenyl, (CH2)nC3-6 cycloalkyl, phenyl, benzyl, or
                      adamantyl; wherein alkyl, alkenyl, cycloalkyl, and adamantyl are optionally substituted
                      with one to three substituents independently selected from halogen, hydroxy, carboxy,
                      C1-4 alkoxy; and wherein phenyl and benzyl are optionally substituted with one to three
                      substituents independently selected from halogen, hydroxy, cyano, C1.4 alkoxy,
                      trifluoromethyl, and trifluoromethoxy; and
                      R.9 is hydrogen, Cl-8 alkylcarbonyl, or C1-8 alkoxy carbonyl.

5. The compound of any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R1 is methyl or fluoromethyl, and R2 is hydroxy.

6. The compound of Claim 5, or a pharmaceutically acceptable salt thereof, wherein R1 is methyl.

7. The compound of any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R1 is methyl or fluoromethyl, and R2 is fluoro.

8. The compound of any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R5 is hydrogen and R4 is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, isopropyl, isobutyl, 2-methyl-1-propyl, hydroxymethyl, fluoromethyl, mercaptomethyl, carboxymethyl, carbamoylmethyl, 1-hydroxyethyl, 2-carboxyethyl, 2-carbamoyl ethyl, 2-methylthio ethyl, 4-amino-1-butyl, 3-amino-1-propyl, 3-guanidino-1-propyl, 1/-imidazol-4- ylmethyl, phenyl, benzyl, 4-hydroxybenzyl, and 1H-indol-3-ylmethyl.

9. The compound of any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R6 is Cl-8 alkyl, cyclohexyl, cyclopentyl, cyclohexylmethyl, 2-cyclohexylethyl, or 3-cyclohexyl-n-propyl, each of which is optionally substituted with one to three substituents independently selected from fluorine, hydroxy, and Ci_6 alkoxy.

10. The compound of Claim 9, or a pharmaceutically acceptable salt thereof, wherein R6 is ethyl, butyl, or heptyl.

11. The compound of any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R4 is methyl, R5 is hydrogen, and R6 is ethyl, butyl, or heptyl.
12. The compound of Claim 1, which is a compound of Formula II:

![Formula II](image)

or a pharmaceutically acceptable salt thereof, wherein:

- $R_4$ is $Ci_4$ alkyl;
- $R_6$ is $C_1-8$ alkyl, $Ci_8$ alkyl substituted with $C\beta$ alkoxy, cyclohexyl, cyclopentyl, cyclohexylmethyl, 2-cyclohexylethyl, or 3-cyclohexyl-n-propyl; and
- $R_9$ is $H$, $C_1-8$ alkylcarbonyl, or [(di-$Ci_4$ alkoxy)-$Ci_4$ alkoxy]carbonyl.

13. The compound of Claim 1, which is a compound of Formula III-A:

![Formula III-A](image)

or a pharmaceutically acceptable salt thereof.

14. The compound of Claim 13, which is a compound of Formula III-B:
or a pharmaceutically acceptable salt thereof.

15. The compound of claim 1, which is selected from the group consisting of:
   n-butyl (2S)-2-[(4aR, 6R, 7R, 7aR)-6-(4-amino-2-aminopyridin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,2,3]dioxaphosphinin-2-yl]amino)propanoate;
   ethyl (2S)-2-[(4ai?, 6R, IR, 7ai?)]-6-(4-amino-2-aminopyridin-l(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,2,3] dioxaphosphinin-2-yl]amino)propanoate;
   ß-heptyl (2S)-2-[(4ai?, 6R, IR, 7ai?)]-6-(4-amino-2-aminopyridin-l(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,2,3] dioxaphosphinin-2-yl]amino)propanoate;
   tert-butyl N-[(4ai?, 6i?, 7i?, 7ai?)]-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,3,2] dioxaphosphinin-2-yl]-L-alaninate;
   ethyl [4ai?, 6i?, 7i?, 7ai?]]-6-(4-amino-2-oxopyrimidin-l(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,3,2] dioxaphosphinin-2-yl]-L-leucinate;
   ethyl N-[(4ai?, 6i?, 7i?, 7ai?)]-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,3,2] dioxaphosphinin-2-yl]-L-norvalinate;
   cyclopentyl N-[(4ai?, 6i?, 7i?, 7ai?)]-6-(4-amino-2-oxopyrpirnidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,3,2] dioxaphosphinin-2-yl]-L-alaninate;
   ethyl [4ai?, 6i?, 7i?, 7ai?]]-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4-H-furo[3,2-d][1,3,2] dioxaphosphinin-2-yl]-L-alaninate;
cyclohexyl N-[(4a\text{R},6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-i][1,3,2]dioxaphosphinin-2-yl]-L-alaninate;

isopentyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-i][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

3-methoxypropyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

2-ethylbutyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-i][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

2-propylpentyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

2-(hexyloxy)ethyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

cycloheptyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-t][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

3-cyclohexylpropyl N-[(4a\text{R},5\text{R},7\text{i}?,7\text{ai}?)-6-(4-amino-2-oxopyrimidin-1(2H)-yl)-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-i][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

ethyl (25)-2-{{(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-7-hydroxy-7-methyl-2-oxido-6-[2-oxo-4-[(2-propylpentanoyl)amino]pyrimidin-1(2H)-yl]tetrahydro-4 \text{H}fiuro[3,2-d][1,3,2]-dioxaphosphinin-2-yl} [amino]butanoate;

ethyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-7-hydroxy-7-methyl-2-oxido-6-[2-oxo-4-[(2-propylpentanoyl)amino]pyrimidin-1(2H)-yl]tetrahydro-4 \text{H}furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

{-heptyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-7-hydroxy-7-methyl-6-[4-[(1-methylene-2-propylpentyl)amino]-2-oxopyrimidin-1(2H)-yl]2-oxidotetrahydro-4 \text{H}furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl]-L-alaninate;

{-heptyl N-[(4a\text{ai}?,6\text{i}?,7\text{i}?,7\text{ai}?)-6-[4-{{(3-(dimethylamino)propoxy[carboxyl]amino)-2-oxopyrimidin-1(2H)-yl}-7-hydroxy-7-methyl-2-oxidotetrahydro-4 \text{H}furo[3,2-d][1,3,2]-dioxaphosphinin-2-yl}}]-L-alaninate;

and pharmaceutically acceptable salts thereof.

16. A pharmaceutical composition comprising a compound of any one of Claims 1 to 15, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

17. Use of a compound of any one of Claims 1 to 15, or a pharmaceutically acceptable salt thereof, for the treatment of hepatitis C virus infection in a mammal.
18. Use of a compound of any one of Claims 1 to 15, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of hepatitis C virus infection in a mammal.
INTERNATIONAL SEARCH REPORT

PCT/US 07/25637

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/04; C07H 19/06 (2008.01)
USPC - 514/43: 514/52, 536/28.4
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)
USPC- 514/43; 514/52, 536/28 4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-514/43, 49, 52, 536/28 4, 28.5, 28 53 (text search-see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGBP, EPAB , JPAB) and Google Patent/Scholar
Search terms, cytosine, cyclic phosphoramidate, alaninate, dioxaphosphirin, nucleoside, prodrug

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 2005/0080034 A (Stand et al.) 14 April 2005 (14 04 2005) abstract, para [01 18], [0360]</td>
<td>3, 5-1 1, 13-14</td>
</tr>
</tbody>
</table>

Date of the actual completion of the international search
13 February 2008 (13 02.2008)

Date of mailing of the international search report
10 MAR 2008

Authorized officer: Lee W Young

Form PCT/ISA/210 (second sheet) (April 2007)
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 07/25637

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: 16-18
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest  ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.