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(54) **D-AMINO ACID COMPOUNDS FOR LIVER DISEASE**

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(57) **ABSTRACT**

Provided herein are compounds, compositions and methods for the treatment of liver disease and conditions, including HCV infections. In certain embodiments, compounds and compositions of nucleoside derivatives are disclosed, which can be administered either alone or in combination with other anti-viral agents.

## D-AMINO ACID COMPOUNDS FOR LIVER DISEASE

### FIELD

[0001] Provided herein are compounds, methods and pharmaceutical compositions for use in treatment of liver diseases and conditions, including viral infections such as hepatitis C virus infections in hosts in need thereof. In certain embodiments, D-amino acids linked to therapeutic nucleoside analogs are provided which display remarkable efficacy and bioavailability for the treatment of, for example, HCV infection in a human.

### BACKGROUND

[0002] The hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide. (Boyer, N. et al., *J. Hepatol.* 32:98-112, 2000). HCV causes a slow growing viral infection and is the major cause of cirrhosis and hepatocellular carcinoma (Di Besceglie, A. M. and Bacon, B. R., *Scientific American*, October: 80-85, 1999; Boyer, N. et al., *J. Hepatol.* 32:98-112, 2000). It is estimated there are about 130-170 million people with chronic hepatitis C virus infection, and there are about 350,000 deaths from hepatitis C-related liver diseases each year (Hepatitis C Fact Sheet, *World Health Organization Fact Sheet No. 164*, June 2011). Cirrhosis caused by chronic hepatitis C infection accounts for 8,000-12,000 deaths per year in the United States, and HCV infection is the leading indication for liver transplantation.

[0003] HCV infection becomes chronic in about 75% of cases, with many patients initially being asymptomatic. The first symptoms of HCV infection are often those of chronic liver disease. About 20 to 30% of patients with chronic hepatitis due to HCV develop cirrhosis, although this may take decades. Development of cirrhosis due to HCV also increases the risk of hepatocellular cancer (The Merck Manual Online, *Chronic Hepatitis*, available at [www.merckmanuals.com/professional/hepatic\\_and\\_biliary\\_disorders/hepatitis/chronic\\_hepatitis.html](http://www.merckmanuals.com/professional/hepatic_and_biliary_disorders/hepatitis/chronic_hepatitis.html), last revision February 2007).

[0004] In light of the fact that HCV infection has reached epidemic levels worldwide, and has tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat hepatitis C that have low toxicity to the host. Further, given the rising threat of other flaviviridae infections, there remains a strong need to provide new effective pharmaceutical agents that have low toxicity to the host. Therefore, there is a continuing need for effective treatments of flavivirus infections and HCV infections.

### SUMMARY

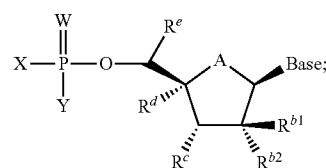
[0005] Provided herein are compounds useful for treatment of liver diseases and conditions, for example, for the treatment of flavivirus infections such as HCV infections.

[0006] The compounds comprise D-amino acids linked to therapeutic moieties. In certain embodiments the D-amino acid compounds display high tissue levels of active species, remarkable efficacy, or bioavailability, or all, for the treatment of, for example, liver disease and conditions in a human in need thereof. Some of the compounds are based, in part, on the discovery that the active component of certain therapeutic moieties linked to D-amino acids can accumulate favorably in liver cells when the compounds are administered to subjects.

[0007] In certain embodiments, the compounds provided herein are useful in the prevention and treatment of Flaviviridae infections and other related conditions such as anti-Flaviviridae antibody positive and Flaviviridae-positive conditions, chronic liver inflammation caused by HCV, cirrhosis, fibrosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-Flaviviridae antibody or Flaviviridae-antigen positive or who have been exposed to a Flaviviridae. In particular embodiments, the Flaviviridae is hepatitis C. In certain embodiments, the compounds are used to treat any virus that replicates through an RNA-dependent RNA polymerase.

[0008] A method for the treatment of a Flaviviridae infection in a host, including a human, is also provided that includes administering an effective amount of a compound provided herein, administered either alone or in combination or alternation with another anti-Flaviviridae agent, optionally in a pharmaceutically acceptable carrier.

[0009] In certain embodiments, provided herein are compounds according to formula (2001):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof, wherein: Base is a nucleobase; A is S or O; W is S or O; X is a D-amino acid residue, or an ester thereof; Y is hydrogen, —OR<sup>1</sup>, —SR<sup>1</sup>, or —NR<sup>1</sup>R<sup>2</sup>; R<sup>b1</sup> is alkyl, cycloalkyl, —H, azido, cyano, or halogen; R<sup>b2</sup> is —OH, —Cl, —F, —H, azido, cyano, amino, or alkoxyl, or, in the alternative, R<sup>b1</sup> and R<sup>b2</sup>, along with the carbon atom to which they are attached, join to form a three-membered carbocyclic or heterocyclic ring; R<sup>c</sup> is —H or —OH, or, in the alternative, Y and R<sup>c</sup> join to form a six-membered heterocyclic ring wherein Y and R<sup>c</sup> together represent a single divalent —O—; R<sup>d</sup> is —H, —F, azido, or allenyl; or, in the alternative, R<sup>b2</sup> and R<sup>d</sup> join to form alkylene or substituted alkylene; R<sup>e</sup> is —H or alkyl; each R<sup>1</sup> is independently alkyl, cycloalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, substituted alkyl or hydantoinylalkyl; and each R<sup>2</sup> is independently hydrogen or alkyl.

[0010] In one aspect, the compounds provided herein are provided or administered in combination with a second therapeutic agent, such as one useful for the treatment or prevention of HCV infections. Exemplary second therapeutic agents are provided in detail elsewhere herein.

[0011] In another aspect, provided herein are pharmaceutical compositions, single unit dosage forms, and kits suitable for use in treating or preventing disorders such as HCV infections which comprise a therapeutically or prophylactically effective amount of a compound provided herein and a therapeutically or prophylactically effective amount of a second therapeutic agent such as one useful for the treatment or prevention of HCV infections.

[0012] In certain embodiments, a method of treatment of a liver disease or disorder is provided comprising administer-

ing to an individual in need thereof a treatment effective amount of a compound provided herein.

**[0013]** Flaviviridae which can be treated are, e.g., discussed generally in *Fields Virology*, Fifth Ed., Editors: Knipe, D. M., and Howley, P. M., Lippincott Williams & Wilkins Publishers, Philadelphia, Pa., Chapters 33-35, 2006. In a particular embodiment of the invention, the Flaviviridae is HCV. In an alternate embodiment, the Flaviviridae is a flavivirus or pestivirus. In certain embodiments, the Flaviviridae can be from any class of Flaviviridae. In certain embodiments, the Flaviviridae is a mammalian tick-borne virus. In certain embodiments, the Flaviviridae is a seabird tick-borne virus. In certain embodiments, the Flaviviridae is a mosquito-borne virus. In certain embodiments, the Flaviviridae is an Aroa virus. In certain embodiments, the Flaviviridae is a Dengue virus. In certain embodiments, the Flaviviridae is a Japanese encephalitis virus. In certain embodiments, the Flaviviridae is a Kokobera virus. In certain embodiments, the Flaviviridae is a Ntaya virus. In certain embodiments, the Flaviviridae is a Spondweni virus. In certain embodiments, the Flaviviridae is a Yellow fever virus. In certain embodiments, the Flaviviridae is a Entebbe virus. In certain embodiments, the Flaviviridae is a Modoc virus. In certain embodiments, the Flaviviridae is a Rio Bravo virus.

**[0014]** Specific flaviviruses include, without limitation: Absetarov, Aedes, Alfuy, Alkhurma, Apoi, Aroa, Bagaza, Banzi, Bukalasa bat, Bouboui, Bussuquara, Cacipacore, Calbertado, Carey Island, Cell fusing agent, Cowbone Ridge, *Culex, Dakar bat, Dengue 1, Dengue 2, Dengue 3, Dengue 4, Edge Hill, Entebbe bat, Gadgets Gully, Hanzalova, Hypr, Ilheus, Israel turkey meningoencephalitis, Japanese encephalitis, Jugra, Jutiapa, Kadam, Kamiti River, Karshi, Kedougou, Kokobera, Koutango, Kumlinge, Kunjin, Kyasanur Forest disease, Langat, Louping ill, Meaban, Modoc, Montana myotis leukoencephalitis, Murray valley encephalitis, Nakiwogo, Naranjal, Negishi, Ntaya, Omsk hemorrhagic fever, Phnom-Penh bat, Powassan, Quang Binh, Rio Bravo, Rocio, Royal Farm, Russian spring-summer encephalitis, Saboya, St. Louis encephalitis, Sal Vieja, San Perlita, Saumarez Reef, Sepik, Sokuluk, Spondweni, Stratford, Tembusu, Tick-borne encephalitis, Turkish sheep encephalitis, Tyuleny, Uganda S, Usutu, Wesselsbron, West Nile, Yaounde, Yellow fever, Yokose, and Zika.*

**[0015]** Pestiviruses which can be treated are discussed generally in *Fields Virology*, Fifth Ed., Editors: Knipe, D. M., and Howley, P. M., Lippincott Williams & Wilkins Publishers, Philadelphia, Pa., Chapters 33-35, 2006. Specific pestiviruses include, without limitation: bovine viral diarrhea virus ("BVDV"), classical swine fever virus ("CSFV," also called hog cholera virus), and border disease virus ("BDV").

#### DESCRIPTION OF EXEMPLARY EMBODIMENTS

**[0016]** Provided herein are compounds, compositions and methods useful for treating liver disorders such as HCV infection in a subject. Further provided are dosage forms useful for such methods.

#### Definitions

**[0017]** When referring to the compounds provided herein, the following terms have the following meanings unless indicated otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is

commonly understood by one of ordinary skill in the art. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

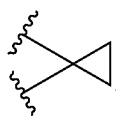
**[0018]** The term "alkyl," as used herein, unless otherwise specified, refers to a saturated straight or branched hydrocarbon. In certain embodiments, the alkyl group is a primary, secondary, or tertiary hydrocarbon. In certain embodiments, the alkyl group includes one to ten carbon atoms, i.e., C<sub>1</sub> to C<sub>10</sub> alkyl. In certain embodiments, the alkyl group is selected from the group consisting of methyl, CF<sub>3</sub>, CCl<sub>3</sub>, CFCl<sub>2</sub>, CF<sub>2</sub>Cl, ethyl, CH<sub>2</sub>CF<sub>3</sub>, CF<sub>2</sub>CF<sub>3</sub>, propyl, isopropyl, butyl, isobutyl, secbutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The term includes both substituted and unsubstituted alkyl groups, including halogenated alkyl groups. In certain embodiments, the alkyl group is a fluorinated alkyl group. Non-limiting examples of moieties with which the alkyl group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., *Protective Groups in Organic Synthesis*, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

**[0019]** The term "lower alkyl," as used herein, and unless otherwise specified, refers to a saturated straight or branched hydrocarbon having one to six carbon atoms, i.e., C<sub>1</sub> to C<sub>6</sub> alkyl. In certain embodiments, the lower alkyl group is a primary, secondary, or tertiary hydrocarbon. The term includes both substituted and unsubstituted moieties.

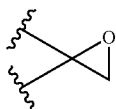
**[0020]** The term "upper alkyl," as used herein, and unless otherwise specified, refers to a saturated straight or branched hydrocarbon having seven to thirty carbon atoms, i.e., C<sub>7</sub> to C<sub>30</sub> alkyl. In certain embodiments, the upper alkyl group is a primary, secondary, or tertiary hydrocarbon. The term includes both substituted and unsubstituted moieties.

**[0021]** The term "cycloalkyl," as used herein, unless otherwise specified, refers to a saturated cyclic hydrocarbon. In certain embodiments, the cycloalkyl group may be a saturated, and/or bridged, and/or non-bridged, and/or a fused bicyclic group. In certain embodiments, the cycloalkyl group includes three to ten carbon atoms, i.e., C<sub>3</sub> to C<sub>10</sub> cycloalkyl. In some embodiments, the cycloalkyl has from 3 to 15 (C<sub>3-15</sub>), from 3 to 10 (C<sub>3-10</sub>), or from 3 to 7 (C<sub>3-7</sub>) carbon atoms. In certain embodiments, the cycloalkyl group is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexylmethyl, cycloheptyl, bicyclo[2.1.1]hexyl, bicyclo[2.2.1]heptyl, decalinyl or adamantyl. The term includes both substituted and unsubstituted cycloalkyl groups, including halogenated cycloalkyl groups. In certain embodiments, the cycloalkyl group is a fluorinated cycloalkyl group. Non-limiting examples of moieties with which the cycloalkyl group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary.

**[0022]** "Cyclopropylene," as used herein, refers to a divalent cyclopropane group. In certain embodiments, a cyclopropylene group is of formula



[0023] “Oxiranylene,” as used herein, refers to a divalent oxirane group. In certain embodiments, a oxiranylene group is of formula



[0024] “Alkylene” refers to divalent saturated aliphatic hydrocarbon groups particularly having from one to eleven carbon atoms which can be straight-chained or branched. In certain embodiments, the alkylene group contains 1 to 10 carbon atoms. The term includes both substituted and unsubstituted moieties. This term is exemplified by groups such as methylene ( $-\text{CH}_2-$ ), ethylene ( $-\text{CH}_2\text{CH}_2-$ ), the propylene isomers (e.g.,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  and  $-\text{CH}(\text{CH}_3)\text{CH}_2-$ ) and the like. The term includes groups having more than one double bond, such as allenes comprising an allenylene ( $>\text{C}=\text{C}=\text{C}<$ ) or allenyl ( $>\text{C}=\text{C}=\text{CH}_2$ ) group. The term includes halogenated alkylene groups. In certain embodiments, the alkylene group is a fluorinated alkylene group. Non-limiting examples of moieties with which the alkylene group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, alkylaryl, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, and phosphonate, either unprotected, or protected as necessary.

[0025] “Alkenyl” refers to monovalent olefinically unsaturated hydrocarbon groups, in certain embodiment, having up to about 11 carbon atoms, from 2 to 8 carbon atoms, or from 2 to 6 carbon atoms, which can be straight-chained or branched and having at least 1 to 2 sites of olefinic unsaturation. The term includes both substituted and unsubstituted moieties. Exemplary alkenyl groups include ethenyl (i.e., vinyl, or  $-\text{CH}=\text{CH}_2$ ), n-propenyl ( $-\text{CH}_2\text{CH}=\text{CH}_2$ ), isopropenyl ( $-\text{C}(\text{CH}_3)=\text{CH}_2$ ), and the like. The term includes halogenated alkenyl groups. In certain embodiments, the alkenyl group is a fluorinated alkenyl group. Non-limiting examples of moieties with which the alkenyl group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary.

[0026] The term “cycloalkenyl,” as used herein, unless otherwise specified, refers to an unsaturated cyclic hydrocarbon. In certain embodiments, cycloalkenyl refers to mono- or multicyclic ring systems that include at least one double bond. In certain embodiments, the cycloalkenyl group may be a bridged, non-bridged, and/or a fused bicyclic group. In certain embodiments, the cycloalkyl group includes three to ten carbon atoms, i.e.,  $\text{C}_3$  to  $\text{C}_{10}$  cycloalkyl. In some embodiments, the cycloalkenyl has from 3 to 7 ( $\text{C}_{3-10}$ ), or from 4 to

7 ( $\text{C}_{3-7}$ ) carbon atoms. The term includes both substituted and unsubstituted cycloalkenyl groups, including halogenated cycloalkenyl groups. In certain embodiments, the cycloalkenyl group is a fluorinated cycloalkenyl group. Non-limiting examples of moieties with which the cycloalkenyl group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary.

[0027] “Alkenylene” refers to divalent olefinically unsaturated hydrocarbon groups, in certain embodiments, having up to about 11 carbon atoms or from 2 to 6 carbon atoms which can be straight-chained or branched and having at least 1 or from 1 to 2 sites of olefinic unsaturation. This term is exemplified by groups such as ethenylene ( $-\text{CH}=\text{CH}-$ ), the propenylene isomers (e.g.,  $-\text{CH}=\text{CHCH}_2-$  and  $-\text{C}(\text{CH}_3)=\text{CH}-$  and  $-\text{CH}=\text{C}(\text{CH}_3)-$ ) and the like. The term includes both substituted and unsubstituted alkenylene groups, including halogenated alkenylene groups. In certain embodiments, the alkenylene group is a fluorinated alkenylene group. Non-limiting examples of moieties with which the alkenylene group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary.

[0028] “Alkynyl” refers to acetylenically unsaturated hydrocarbon groups, in certain embodiments, having up to about 11 carbon atoms or from 2 to 6 carbon atoms which can be straight-chained or branched and having at least 1 or from 1 to 2 sites of alkynyl unsaturation. Non-limiting examples of alkynyl groups include acetylenic, ethynyl ( $-\text{C}\equiv\text{CH}$ ), propargyl ( $-\text{CH}_2\text{C}\equiv\text{CH}$ ), and the like. The term includes both substituted and unsubstituted alkynyl groups, including halogenated alkynyl groups. In certain embodiments, the alkynyl group is a fluorinated alkynyl group. Non-limiting examples of moieties with which the alkynyl group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, carbonyl, sulfanyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary.

[0029] The term “aryl,” as used herein, and unless otherwise specified, refers to phenyl, biphenyl or naphthyl. The term includes both substituted and unsubstituted moieties. An aryl group can be substituted with any described moiety, including, but not limited to, one or more moieties selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), alkyl, haloalkyl, hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., *Protective Groups in Organic Synthesis*, John Wiley and Sons, Second Edition, 1991.

[0030] “Alkoxy” refers to the group  $-\text{OR}'$  where  $\text{R}'$  is alkyl or cycloalkyl. Alkoxy groups include, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxo, n-hexoxo, 1,2-dimethylbutoxy, and the like.

[0031] “Alkoxy carbonyl” refers to a radical  $-\text{C}(\text{O})-$  alkoxy where alkoxy is as defined herein.

**[0032]** The term “heterocyclylalkyl” refers to a radical -alkyl-heterocyclyl, where alkyl and heterocyclyl are as defined herein.

**[0033]** The term “alkylcarbonylthioalkyl” refers to a radical -alkyl-S—C(O)-alkyl, where alkyl is as defined herein.

**[0034]** The term “alkoxycarbonylalkyl” refers to a radical -alkyl-C(O)-alkoxy, where alkyl and alkoxy are as defined herein.

**[0035]** The term “arylalkoxycarbonylalkyl” refers to a radical -alkyl-C(O)-alkoxy-aryl, where alkyl, alkoxy and aryl are as defined herein.

**[0036]** The term “alkylcarbonylalkoxy(arylalkyl)” refers to a radical -alkoxy(-alkyl-aryl)-C(O)-alkyl, where alkyl, alkoxy and aryl are as defined herein.

**[0037]** The term “(alkoxycarbonyl)(alkoxycarbonylamino)alkyl” refers to a radical -alkyl(-carbonyl-alkoxy)(-amino-carbonyl-alkoxy), where alkyl, carbonyl, alkoxy, and amino are as defined herein.

**[0038]** The term “cycloalkylcarbonylalkoxy” refers to a radical -alkoxy-C(O)-cycloalkyl, where alkoxy and cycloalkyl are as defined herein.

**[0039]** The term “alkoxycarbonylaminoalkylcarbonylthioalkyl” refers to a radical -alkyl-S—C(O)—NH-alkyl-C(O)-alkoxy or -alkyl-S—C(O)-alkyl-NH—C(O)-alkoxy, where alkyl and alkoxy are as defined herein.

**[0040]** The term “hydroxylalkylcarbonylthioalkyl” refers to a radical -alkyl-S—C(O)-alkyl-OH, where alkyl is as defined herein.

**[0041]** The term “aminoalkylcarbonylalkoxycarbonylthioalkyl” refers to a radical -alkyl-S—C(O)-alkoxy-C(O)—NH-alkyl or -alkyl-S—C(O)-alkoxy-C(O)-alkyl-NH<sub>2</sub>, where alkyl and alkoxy are as defined herein.

**[0042]** The term “alkoxycarbonylaminoalkyl” refers to a radical -alkyl-NH—C(O)-alkoxy or —NH-alkyl-C(O)-alkoxy, where alkyl and alkoxy are as defined herein.

**[0043]** The term “hydroxylalkyl” refers to a radical -alkyl-OH, where alkyl is as defined herein.

**[0044]** The term “aminoalkylcarbonylalkoxy” refers to a radical -alkoxy-C(O)-alkyl-NH<sub>2</sub> or -alkoxy-C(O)—NH-alkyl, where alkyl and alkoxy are as defined herein.

**[0045]** “Amino” refers to the radical —NH<sub>2</sub> or —NH—R, where each R is independently alkyl, aryl, or cycloalkyl.

**[0046]** “Amino alcohol” refers to the radical —NHLOH, wherein L is alkylene.

**[0047]** “Carboxyl” or “carboxy” refers to the radical —C(O)OH.

**[0048]** The term “alkylamino” or “arylamino” refers to an amino group that has one or two alkyl or aryl substituents, respectively. In certain embodiments, the alkyl substituent is upper alkyl. In certain embodiments, the alkyl substituent is lower alkyl. In another embodiment, the alkyl, upper alkyl, or lower alkyl is unsubstituted.

**[0049]** “Halogen” or “halo” refers to chloro, bromo, fluoro or iodo.

**[0050]** “Monoalkylamino” refers to the group alkyl-NR'—, wherein R' is selected from hydrogen and alkyl or cycloalkyl.

**[0051]** “Thioalkoxy” refers to the group —SR' where R' is alkyl or cycloalkyl.

**[0052]** The term “heterocyclyl” or “heterocyclic” refers to a monovalent monocyclic non-aromatic ring system and/or multicyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, or N; and the remaining ring atoms are carbon atoms. In certain

embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. Heterocyclyl groups are bonded to the rest of the molecule through the non-aromatic ring. In certain embodiments, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include a fused or bridged ring system, and in which the nitrogen or sulfur atoms may be optionally oxidized, the nitrogen atoms may be optionally quaternized, and some rings may be partially or fully saturated, or aromatic. The heterocyclyl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of such heterocyclic radicals include, but are not limited to, azepinyl, benzodioxanyl, benzodioxolyl, benzofuranonyl, benzopyranonyl, benzopyranyl, benzotetrahydrofuranlyl, benzotetrahydrothienyl, benzothiopyranlyl, benzoxazinyl,  $\beta$ -carbonyl, chromanyl, chromonyl, cinnolinyl, coumarinyl, decahydroisoquinolinyl, dihydrobenzothiazinyl, dihydrobenzoxazinyl, dihydrofuryl, dihydroisindolyl, dihydropyranlyl, dihydropyrazolyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, imidazolidinyl, imidazolyl, indolinyl, isobenzotetrahydrofuranlyl, isobenzotetrahydrothienyl, isochromanyl, isocoumarinyl, isoindolinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisindolyl, oxazolidinonyl, oxazolidinyl, oxiranyl, piperazinyl, piperidinyl, 4-piperidinyl, pyrazolidinyl, pyrazolinyl, pyrrolidinyl, pyrrolinyl, quinclidinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydropyranlyl, tetrahydrothienyl, thiamorpholinyl, thiazolidinyl, tetrahydroquinolinyl, and 1,3,5-trithianyl. In certain embodiments, heterocyclic may also be optionally substituted as described herein.

**[0053]** The term “heteroaryl” refers to refers to a monovalent monocyclic aromatic group and/or multicyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S and N in the ring. Heteroaryl groups are bonded to the rest of the molecule through the aromatic ring. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, furanyl, imidazolyl, isothiazolyl, isoxazolyl, oxadiazolyl, oxadiazolyl, oxazolyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, tetrazolyl, triazinyl and triazolyl. Examples of bicyclic heteroaryl groups include, but are not limited to, benzofuranlyl, benzimidazolyl, benzoisoxazolyl, benzopyranlyl, benzothiadiazolyl, benzothiazolyl, benzothienyl, benzotriazolyl, benzoxazolyl, furopyridyl, imidazopyridinyl, imidazothiazolyl, indoliziny, indolyl, indazolyl, isobenzofuranlyl, isobenzothienyl, isoindolyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxazolopyridinyl, phthalazinyl, pteridinyl, purinyl, pyridopyridyl, pyrrolopyridyl, quinolinyl, quinoxalinyl, quinazolinyl, thiadiazolopyrimidyl, and thienopyridyl. Examples of tricyclic heteroaryl groups include, but are not limited to, acridinyl, benzindolyl, carbazolyl, dibenzofuranlyl, perimidinyl, phenanthrolinyl, phenanthridinyl, phenarsazinyl, phenazinyl,

phenothiazinyl, phenoxazinyl and xanthenyl. In certain embodiments, heteroaryl may also be optionally substituted as described herein.

**[0054]** The term “alkylaryl” refers to an aryl group with an alkyl substituent. The term “aralkyl” or “arylalkyl” refers to an alkyl group with an aryl substituent.

**[0055]** The term “alkylheterocyclyl” refers to a heterocyclyl group with an alkyl substituent. The term heterocyclylalkyl refers to an alkyl group with a heterocyclyl substituent.

**[0056]** The term “alkylheteroaryl” refers to a heteroaryl group with an alkyl substituent. The term heteroarylalkyl refers to an alkyl group with a heteroaryl substituent.

**[0057]** The term “protecting group” as used herein and unless otherwise defined refers to a group that is added to an oxygen, nitrogen or phosphorus atom to prevent its further reaction or for other purposes. A wide variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis.

**[0058]** “Pharmaceutically acceptable salt” refers to any salt of a compound provided herein which retains its biological properties and which is not toxic or otherwise undesirable for pharmaceutical use. Such salts may be derived from a variety of organic and inorganic counter-ions well known in the art. Such salts include, but are not limited to: (1) acid addition salts formed with organic or inorganic acids such as hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, sulfamic, acetic, trifluoroacetic, trichloroacetic, propionic, hexanoic, cyclopentylpropionic, glycolic, glutaric, pyruvic, lactic, malonic, succinic, sorbic, ascorbic, malic, maleic, fumaric, tartaric, citric, benzoic, 3-(4-hydroxybenzoyl)benzoic, picric, cinnamic, mandelic, phthalic, lauric, methanesulfonic, ethanesulfonic, 1,2-ethane-disulfonic, 2-hydroxyethanesulfonic, benzenesulfonic, 4-chlorobenzenesulfonic, 2-naphthalenesulfonic, 4-toluenesulfonic, camphoric, camphorsulfonic, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 3-phenylpropionic, trimethylacetic, tert-butylacetic, lauryl sulfuric, gluconic, benzoic, glutamic, hydroxynaphthoic, salicylic, stearic, cyclohexylsulfamic, quinic, muconic acid and the like acids; or (2) base addition salts formed when an acidic proton present in the parent compound either (a) is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion or an aluminum ion, or alkali metal or alkaline earth metal hydroxides, such as sodium, potassium, calcium, magnesium, aluminum, lithium, zinc, and barium hydroxide, ammonia or (b) coordinates with an organic base, such as aliphatic, alicyclic, or aromatic organic amines, such as ammonia, methylamine, dimethylamine, diethylamine, picoline, ethanolamine, diethanolamine, triethanolamine, ethylenediamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylene-diamine, chlorprocaine, diethanolamine, procaine, N-benzylphenethylamine, N-methylglucamine piperazine, tris(hydroxymethyl)-aminomethane, tetramethylammonium hydroxide, and the like.

**[0059]** Pharmaceutically acceptable salts further include, by way of example only and without limitation, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium and the like, and when the compound contains a basic functionality, salts of non-toxic organic or inorganic acids, such as hydrohalides, e.g. hydrochloride and hydrobromide, sulfate, phosphate, sulfamate, nitrate, acetate, trifluoroacetate, trichloroacetate, propionate, hexanoate, cyclopentylpropionate, glycolate, glutarate, pyruvate, lactate, malonate, succinate, sorbate, ascorbate, malate, maleate, fumarate, tartarate, citrate, benzoate, 3-(4-hydroxybenzoyl)

benzoate, picrate, cinnamate, mandelate, phthalate, laurate, methanesulfonate (mesylate), ethanesulfonate, 1,2-ethanedisulfonate, 2-hydroxyethanesulfonate, benzenesulfonate (besylate), 4-chlorobenzenesulfonate, 2-naphthalenesulfonate, 4-toluenesulfonate, camphorate, camphorsulfonate, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylate, glucoheptonate, 3-phenylpropionate, trimethylacetate, tert-butylacetate, lauryl sulfate, gluconate, benzoate, glutamate, hydroxynaphthoate, salicylate, stearate, cyclohexylsulfamate, quinate, muconate and the like.

**[0060]** As used herein, the term “nucleobase” refers to the base portion of a nucleoside or nucleotide. In certain embodiments, a nucleobase is a purine or pyrimidine base, as defined herein.

**[0061]** The term “purine” or “pyrimidine” base refers to, but is not limited to, adenine, N<sup>6</sup>-alkylpurines, N<sup>6</sup>-acylpurines (wherein acyl is C(O)(alkyl, aryl, alkylaryl, or arylalkyl), N<sup>6</sup>-benzylpurine, N<sup>6</sup>-halopurine, N<sup>6</sup>-vinylpurine, N<sup>6</sup>-acetylenic purine, N<sup>6</sup>-acyl purine, N<sup>6</sup>-hydroxyalkyl purine, N<sup>6</sup>-alkylaminopurine, N<sup>6</sup>-thioalkyl purine, N<sup>2</sup>-alkylpurines, N<sup>2</sup>-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azapyrimidine, including 6-azacytosine, 2- and/or 4-mercaptopyrimidine, uracil, 5-halouracil, including 5-fluorouracil, C<sup>5</sup>-alkylpyrimidines, C<sup>5</sup>-benzylpyrimidines, C<sup>5</sup>-halopyrimidines, C<sup>5</sup>-vinylpyrimidine, C<sup>5</sup>-acetylenic pyrimidine, C<sup>5</sup>-acyl pyrimidine, C<sup>5</sup>-hydroxyalkyl purine, C<sup>5</sup>-amidopyrimidine, C<sup>5</sup>-cyanopyrimidine, C<sup>5</sup>-iodopyrimidine, C<sup>6</sup>-iodo-pyrimidine, C<sup>5</sup>-Br-vinyl pyrimidine, C<sup>6</sup>-Br-vinyl pyrimidine, C<sup>5</sup>-nitropyrimidine, C<sup>5</sup>-amino-pyrimidine, N<sup>2</sup>-alkylpurines, N<sup>2</sup>-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracil, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, and pyrazolopyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 7-deazaguanine, 7-deazaadenine, 2,6-diaminopurine, and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyl dimethylsilyl, and t-butyl diphenylsilyl, trityl, alkyl groups, and acyl groups such as acetyl and propionyl, methanesulfonyl, and p-toluenesulfonyl.

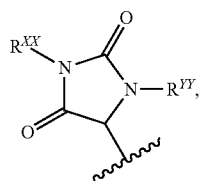
**[0062]** The term “acyl” or “O-linked ester” refers to a group of the formula C(O)R', wherein R' is alkyl or cycloalkyl (including lower alkyl), carboxylate residue of amino acid, aryl including phenyl, alkaryl, arylalkyl including benzyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxymethyl; or substituted alkyl (including lower alkyl), aryl including phenyl optionally substituted with chloro, bromo, fluoro, iodo, C<sub>1</sub> to C<sub>4</sub> alkyl or C<sub>1</sub> to C<sub>4</sub> alkoxy, sulfonate esters such as alkyl or arylalkyl sulfonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxy-trityl, substituted benzyl, alkaryl, arylalkyl including benzyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxymethyl. Aryl groups in the esters optimally comprise a phenyl group. In particular, acyl groups include acetyl, trifluoroacetyl, methylacetyl, cyclopropylacetyl, propionyl, butyryl, hexanoyl, heptanoyl, octanoyl, neo-heptanoyl, phenylacetyl, 2-acetoxy-2-phenylacetyl, diphenylacetyl, α-methoxy-α-trifluoromethyl-phenylacetyl, bromoacetyl, 2-nitro-benzenecacetyl, 4-chloro-benzenecacetyl, 2-chloro-2,2-diphenylacetyl, 2-chloro-2-phenylacetyl, trimethylacetyl, chlorodifluoroacetyl, perfluoroacetyl, fluoroacetyl, bromodifluoroacetyl, methoxyacetyl, 2-thiophenecacetyl, chlorosulfonylacetyl, 3-methoxyphenyl-

lacetyl, phenoxyacetyl, tert-butylacetyl, trichloroacetyl, monochloro-acetyl, dichloroacetyl, 7H-dodecafluoro-heptanoyl, perfluoro-heptanoyl, 7H-dodeca-fluoroheptanoyl, 7-chlorododecafluoro-heptanoyl, 7-chlorododecafluoro-heptanoyl, 7H-dodecafluoroheptanoyl, 7H-dodeca-fluoro-heptanoyl, nonafluoro-3,6-dioxa-heptanoyl, nonafluoro-3,6-dioxaheptanoyl, perfluoroheptanoyl, methoxybenzoyl, methyl 3-amino-5-phenylthiophene-2-carboxyl, 3,6-dichloro-2-methoxy-benzoyl, 4-(1,1,2,2-tetrafluoroethoxy)-benzoyl, 2-bromo-propionyl, omega-aminocapryl, decanoyl, n-pentadecanoyl, stearyl, 3-cyclopentyl-propionyl, 1-benzene-carboxyl, O-acetylmandelyl, pivaloyl acetyl, 1-adamantane-carboxyl, cyclohexane-carboxyl, 2,6-pyridinedicarboxyl, cyclopropane-carboxyl, cyclobutane-carboxyl, perfluorocyclohexyl carboxyl, 4-methylbenzoyl, chloromethyl isoxazoly carbonyl, perfluorocyclohexyl carboxyl, crotonyl, 1-methyl-1H-indazole-3-carbonyl, 2-propenyl, isovaleryl, 1-pyrrolidinedicarbonyl, 4-phenylbenzoyl.

**[0063]** The term “amino acid” refers to naturally occurring and synthetic  $\alpha$ ,  $\beta$   $\gamma$  or  $\delta$  amino acids, and includes but is not limited to, amino acids found in proteins, i.e. glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine. In certain embodiments, the amino acid is in the L-configuration. Alternatively, the amino acid can be a derivative of alanyl, valinyl, leucinyl, isoleuccinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, histidinyl,  $\beta$ -alanyl,  $\beta$ -valinyl,  $\beta$ -leucinyl,  $\beta$ -isoleuccinyl,  $\beta$ -prolinyl,  $\beta$ -phenylalaninyl,  $\beta$ -tryptophanyl,  $\beta$ -methioninyl,  $\beta$ -glycyl,  $\beta$ -serinyl,  $\beta$ -threoninyl,  $\beta$ -cysteinyl,  $\beta$ -tyrosinyl,  $\beta$ -asparaginyl,  $\beta$ -glutaminyl,  $\beta$ -aspartoyl,  $\beta$ -glutaroyl,  $\beta$ -lysinyl,  $\beta$ -argininyl or  $\beta$ -histidinyl.

**[0064]** The term “amino acid derivative” refers to a group derivable from a naturally or non-naturally occurring amino acid, as described and exemplified herein. Amino acid derivatives are apparent to those of skill in the art and include, but are not limited to, ester, amino alcohol, amino aldehyde, amino lactone, and N-methyl derivatives of naturally and non-naturally occurring amino acids. In an embodiment, an amino acid derivative is provided as a substituent of a compound described herein, wherein the substituent is  $\text{—NH—G}$  ( $\text{S}_C\text{—C(O)—Q}$  or  $\text{—OC(O)G(S}_C\text{)—Q}$ , wherein Q is  $\text{—SR}$ ,  $\text{—NRR}$  or alkoxyl, R is hydrogen or alkyl,  $\text{S}_C$  is a side chain of a naturally occurring or non-naturally occurring amino acid and G is  $\text{C}_1\text{—C}_2$  alkyl. In certain embodiments, G is  $\text{C}_1$  alkyl and  $\text{S}_C$  is selected from the group consisting of hydrogen, alkyl, heteroalkyl, arylalkyl and heteroarylalkyl.

**[0065]** As used herein, the term “hydantoinyl” refers to the group



where  $\text{R}^{\text{XX}}$  and  $\text{R}^{\text{YY}}$  are each independently hydrogen or lower alkyl.

**[0066]** As used herein, the term “hydantoinylalkyl” refers to the group -alkyl-hydantoinyl, where alkyl and hydantoinyl are as described herein.

**[0067]** The term “substantially free of” or “substantially in the absence of” with respect to a nucleoside composition refers to a nucleoside composition that includes at least 85 or 90% by weight, in certain embodiments 95%, 98%, 99% or 100% by weight, of the designated enantiomer of that nucleoside. In certain embodiments, in the methods and compounds provided herein, the compounds are substantially free of enantiomers.

**[0068]** Similarly, the term “isolated” with respect to a nucleoside composition refers to a nucleoside composition that includes at least 85, 90%, 95%, 98%, 99% to 100% by weight, of the nucleoside, the remainder comprising other chemical species or enantiomers.

**[0069]** “Solvate” refers to a compound provided herein or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of solvent bound by non-covalent intermolecular forces. Where the solvent is water, the solvate is a hydrate.

**[0070]** “Isotopic composition” refers to the amount of each isotope present for a given atom, and “natural isotopic composition” refers to the naturally occurring isotopic composition or abundance for a given atom. Atoms containing their natural isotopic composition may also be referred to herein as “non-enriched” atoms. Unless otherwise designated, the atoms of the compounds recited herein are meant to represent any stable isotope of that atom. For example, unless otherwise stated, when a position is designated specifically as “H” or “hydrogen,” the position is understood to have hydrogen at its natural isotopic composition.

**[0071]** “Isotopic enrichment” refers to the percentage of incorporation of an amount of a specific isotope at a given atom in a molecule in the place of that atom’s natural isotopic abundance. For example, deuterium enrichment of 1% at a given position means that 1% of the molecules in a given sample contain deuterium at the specified position. Because the naturally occurring distribution of deuterium is about 0.0156%, deuterium enrichment at any position in a compound synthesized using non-enriched starting materials is about 0.0156%. The isotopic enrichment of the compounds provided herein can be determined using conventional analytical methods known to one of ordinary skill in the art, including mass spectrometry and nuclear magnetic resonance spectroscopy.

**[0072]** “Isotopically enriched” refers to an atom having an isotopic composition other than the natural isotopic composition of that atom. “Isotopically enriched” may also refer to a compound containing at least one atom having an isotopic composition other than the natural isotopic composition of that atom.

**[0073]** As used herein, “alkyl,” “cycloalkyl,” “alkenyl,” “cycloalkenyl,” “alkynyl,” “aryl,” “alkoxy,” “alkoxycarbonyl,” “amino,” “carboxyl,” “alkylamino,” “arylamino,” “thioalkoxy,” “heterocyclyl,” “heteroaryl,” “alkylheterocyclyl,” “alkylheteroaryl,” “acyl,” “aralkyl,” “alkaryl,” “purine,” “pyrimidine,” “carboxyl” and “amino acid” groups optionally comprise deuterium at one or more positions where hydrogen atoms are present, and wherein the deuterium composition of the atom or atoms is other than the natural isotopic composition.

**[0074]** Also as used herein, “alkyl,” “cycloalkyl,” “alkenyl,” “cycloalkenyl,” “alkynyl,” “aryl,” “alkoxy,” “alkoxy-

carbonyl,” “carboxyl,” “alkylamino,” “arylamino,” “thioalkoxy,” “heterocyclyl,” “heteroaryl,” “alkylheterocyclyl,” “alkylheteroaryl,” “acyl,” “aralkyl,” “alkaryl,” “purine,” “pyrimidine,” “carboxyl” and “amino acid” groups optionally comprise carbon-13 at an amount other than the natural isotopic composition.

**[0075]** As used herein, EC<sub>50</sub> refers to a dosage, concentration or amount of a particular test compound that elicits a dose-dependent response at 50% of maximal expression of a particular response that is induced, provoked or potentiated by the particular test compound.

**[0076]** As used herein, the IC<sub>50</sub> refers to an amount, concentration or dosage of a particular test compound that achieves a 50% inhibition of a maximal response in an assay that measures such response.

**[0077]** The term “host,” as used herein, refers to any unicellular or multicellular organism in which the virus can replicate, including cell lines and animals, and in certain embodiments, a human. Alternatively, the host can be carrying a part of the Flaviviridae viral genome, whose replication or function can be altered by the compounds of the present invention. The term host specifically includes infected cells, cells transfected with all or part of the Flaviviridae genome and animals, in particular, primates (including chimpanzees) and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention (such as chimpanzees).

**[0078]** As used herein, the terms “subject” and “patient” are used interchangeably herein. The terms “subject” and “subjects” refer to an animal, such as a mammal including a non-primate (e.g., a cow, pig, horse, cat, dog, rat, and mouse) and a primate (e.g., a monkey such as a cynomolgous monkey, a chimpanzee and a human), and for example, a human. In certain embodiments, the subject is refractory or non-responsive to current treatments for hepatitis C infection. In another embodiment, the subject is a farm animal (e.g., a horse, a cow, a pig, etc.) or a pet (e.g., a dog or a cat). In certain embodiments, the subject is a human.

**[0079]** As used herein, the terms “therapeutic agent” and “therapeutic agents” refer to any agent(s) which can be used in the treatment or prevention of a disorder or one or more symptoms thereof. In certain embodiments, the term “therapeutic agent” includes a compound provided herein. In certain embodiments, a therapeutic agent is an agent which is known to be useful for, or has been or is currently being used for the treatment or prevention of a disorder or one or more symptoms thereof.

**[0080]** “Therapeutically effective amount” refers to an amount of a compound or composition that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. A “therapeutically effective amount” can vary depending on, inter alia, the compound, the disease and its severity, and the age, weight, etc., of the subject to be treated.

**[0081]** “Treating” or “treatment” of any disease or disorder refers, in certain embodiments, to ameliorating a disease or disorder that exists in a subject. In another embodiment, “treating” or “treatment” includes ameliorating at least one physical parameter, which may be indiscernible by the subject. In yet another embodiment, “treating” or “treatment” includes modulating the disease or disorder, either physically (e.g., stabilization of a discernible symptom) or physiologically (e.g., stabilization of a physical parameter) or both. In

yet another embodiment, “treating” or “treatment” includes delaying the onset of the disease or disorder.

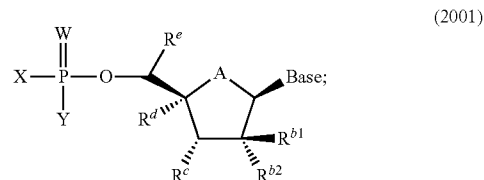
**[0082]** As used herein, the terms “prophylactic agent” and “prophylactic agents” as used refer to any agent(s) which can be used in the prevention of a disorder or one or more symptoms thereof. In certain embodiments, the term “prophylactic agent” includes a compound provided herein. In certain other embodiments, the term “prophylactic agent” does not refer to a compound provided herein. For example, a prophylactic agent is an agent which is known to be useful for, or has been or is currently being used to prevent or impede the onset, development, progression and/or severity of a disorder.

**[0083]** As used herein, the phrase “prophylactically effective amount” refers to the amount of a therapy (e.g., prophylactic agent) which is sufficient to result in the prevention or reduction of the development, recurrence or onset of one or more symptoms associated with a disorder, or to enhance or improve the prophylactic effect(s) of another therapy (e.g., another prophylactic agent).

**[0084]** Compounds

**[0085]** Provided herein are D-amino acid compounds useful for the treatment of liver diseases and conditions, for example, Flaviviridae infections such as HCV infection. The D-amino acid compounds can be formed as described herein and used for the treatment of, for example, Flaviviridae infections such as HCV infection.

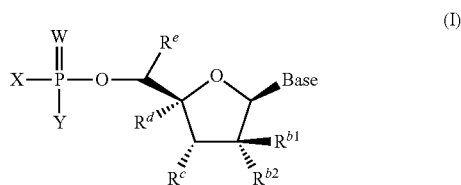
**[0086]** In certain embodiments, provided herein are compounds according to formula (2001):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof, wherein: Base is a nucleobase; A is S or O; W is S or O; X is a D-amino acid residue, or an ester thereof; Y is hydrogen, —OR<sup>1</sup>, —SR<sup>1</sup>, or —NR<sup>1</sup>R<sup>2</sup>; R<sup>b1</sup> is alkyl, cycloalkyl, —H, azido, cyano, or halogen; R<sup>b2</sup> is —OH, —Cl, —F, —H, azido, cyano, amino, or alkoxy, or, in the alternative, R<sup>b1</sup> and R<sup>b2</sup>, along with the carbon atom to which they are attached, join to form a three-membered carbocyclic or heterocyclic ring; R<sup>c</sup> is —H or —OH, or, in the alternative, Y and R<sup>c</sup> join to form a six-membered heterocyclic ring wherein Y and R<sup>c</sup> together represent a single divalent —O—; R<sup>d</sup> is —H, —F, azido, or allenyl; or, in the alternative, R<sup>b2</sup> and R<sup>d</sup> join to form alkylene or substituted alkylene; R<sup>e</sup> is —H or alkyl; each R<sup>1</sup> is independently alkyl, cycloalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, substituted alkyl or hydantoinylalkyl; and each R<sup>2</sup> is independently hydrogen or alkyl. In certain embodiments of Formula (2001), R<sup>b1</sup> and R<sup>b2</sup>, along with the carbon atom to which they are attached, join to form a three-membered carbocyclic or heterocyclic ring. In certain embodiments, R<sup>b1</sup> and R<sup>b2</sup>, along with the carbon atom to which they are attached, join to form cyclopropylene or oxiranylene.

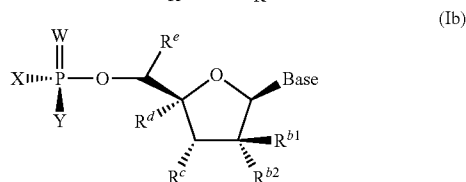
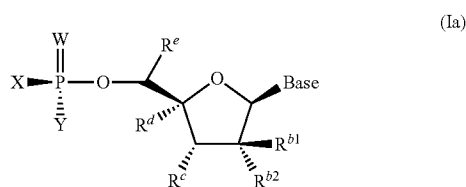


[0087] In certain embodiments, provided herein are compounds according to Formula (I):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

[0088] In certain embodiments, provided herein are compounds according to Formula (Ia) or (Ib):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof. In certain embodiments,  $R_P$  compounds are provided. In certain embodiments,  $S_P$  compounds are provided.

[0089] In Formula (I), (Ia) or (Ib), Base is any nucleobase known to those of skill in the art. Base can be a naturally occurring nucleobase, or it can be a non-natural nucleobase known to those of skill in the art. In certain embodiments, Base is a purine or pyrimidine nucleobase. In particular embodiments, Base is guanosine, uracil, cytosine, adenine or a derivative thereof. Exemplary nucleobases are described herein.

[0090] In Formula (I), (Ia) or (Ib), W is S or O. In certain embodiments, W is S. In certain embodiments, W is O.

[0091] In Formula (I), (Ia) or (Ib), X is a D-amino acid residue, or an ester thereof. X can be any D-amino acid residue known to those of skill in the art. X can be the D-enantiomer of a naturally occurring amino acid residue, or X can be the D-enantiomer of a non-natural amino acid residue. In particular embodiments, X is D-alanine, D-phenylalanine, D-valine or D-leucine. In preferred embodiments, X is D-alanine. The ester can be any ester known to those of skill in the art. In particular embodiments, the ester is an alkyl ester. In certain embodiments, the ester is selected from the group consisting of ethyl ester, propyl ester, n-propyl ester, isopropyl ester, butyl ester, t-butyl ester, n-butyl ester, and cyclopentyl ester.

[0092] In Formula (I), (Ia) or (Ib), Y is hydrogen,  $-OR^1$ ,  $-SR^1$ , or  $-NR^1R^2$ . Each  $R^1$  is independently alkyl, cycloalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, substituted alkyl or hydantoinylalkyl. In certain embodiments,

each  $R^1$  is independently alkyl, cycloalkyl, heterocyclylalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkylcarbonylthioalkyl, alkoxyalkyl, arylalkoxyalkyl, alkoxyalkyl, alkoxyalkoxy(arylalkyl), (alkoxyalkoxy)(alkoxyalkoxy)alkyl, cycloalkylcarbonylalkoxy, alkoxyalkylcarbonylalkoxy, alkoxyalkylcarbonylthioalkyl, hydroxylalkyl, aminoalkyl, aminoalkylcarbonylalkoxy, aminoalkylcarbonylthioalkyl, or hydantoinylalkyl. Each  $R^2$  is independently hydrogen or alkyl. In particular embodiments,  $R^2$  is H.

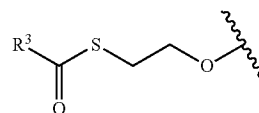
[0093] In Formula (I), (Ia) or (Ib),  $R^c$  is  $-H$  or  $-OH$ . In the alternative, in certain embodiments, Y and  $R^c$  join to form a six-membered heterocyclic ring wherein Y and  $R^c$  together represent a single divalent  $-O-$ . In these embodiments, the compounds comprise a cyclic phosphate group linking the 3' and 5' carbons of the nucleoside sugar.

[0094] In Formula (I), (Ia) or (Ib),  $R^{b1}$  is  $-CH_3$ ,  $-H$ , azido, cyano, or halogen. In certain embodiments,  $R^{b1}$  is  $-CH_3$ . Also in Formula (I), (Ia) or (Ib),  $R^{b2}$  is  $-OH$ ,  $-Cl$ ,  $-F$ ,  $-H$ , azido, cyano, amino, or alkoxy. In certain embodiments,  $R^{b2}$  is  $-OH$ . In certain embodiments,  $R^{b2}$  is  $-Cl$ . In certain embodiments,  $R^{b2}$  is  $-F$ . In certain embodiments of Formula (I), (Ia) or (Ib),  $R^{b1}$  and  $R^{b2}$ , along with the carbon atom to which they are attached, join to form a three-membered carbocyclic or heterocyclic ring. In certain embodiments,  $R^{b1}$  and  $R^{b2}$ , along with the carbon atom to which they are attached, join to form cyclopropylene or oxiranylene.

[0095] In Formula (I), (Ia) or (Ib),  $R^d$  is  $-H$ ,  $-F$ , azido, or allenyl. In certain embodiments,  $R^d$  is  $-H$ . In the alternative, in certain embodiments,  $R^{b2}$  and  $R^d$  join to form alkylene or substituted alkylene. In particular embodiments,  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ . In particular embodiments, the  $-CH_2-$  is linked to the 4' carbon of the sugar, and the  $-O-$  is linked to the 2' carbon of the sugar.

[0096] In Formula (I), (Ia) or (Ib),  $R^e$  is  $-H$  or alkyl. In particular embodiments,  $R^e$  is  $-H$ .

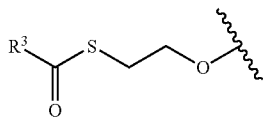
[0097] In certain embodiments according to Formula (I), (Ia) or (Ib),  $R^e$  is  $-H$ ;  $R^d$  is  $-H$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ ;  $R^c$  is  $-H$  or  $-OH$ ; and  $R^2$  is H. In particular embodiments,  $R^e$  is  $-H$ ;  $R^d$  is  $-H$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is H; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In particular embodiments,  $R^e$  is  $-H$ ;  $R^d$  is  $-H$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is H; Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



wherein  $R^3$  is alkyl, alkoxyalkyl, aminoalkyl, hydroxylalkyl, or aminoalkylcarbonylalkoxy.

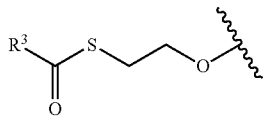
[0098] In certain embodiments according to Formula (I), (Ia) or (Ib),  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ; and  $R^2$  is H. In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is H; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is H; Base is selected from guanosine, uracil, cytosine, adenine or a deriva-

tive thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



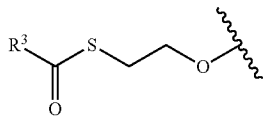
wherein  $R^3$  is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxyl.

**[0099]** In certain embodiments according to Formula (I), (Ia) or (Ib),  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2CH_2-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ; and  $R^2$  is  $H$ . In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2CH_2-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is  $H$ ; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2CH_2-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^e$  is  $-H$  or  $-OH$ ;  $R^2$  is  $H$ ; Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



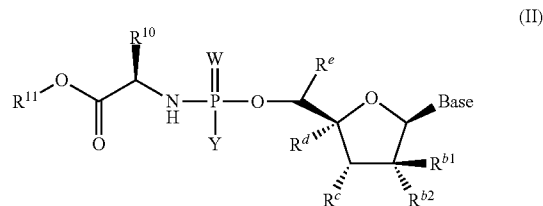
wherein  $R^3$  is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxyl.

**[0100]** In certain embodiments according to Formula (I), (Ia) or (Ib),  $R^e$  is  $-H$ ;  $R^d$  is  $-H$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ ;  $R^2$  is  $H$ ; and Y and  $R^e$  together represent a single divalent  $-O-$ . In particular embodiments,  $R^e$  is  $-H$ ;  $R^d$  is  $-H$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ ;  $R^2$  is  $H$ ; Y and  $R^c$  together represent a single divalent  $-O-$ ; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In particular embodiments,  $R^e$  is  $-H$ ;  $R^d$  is  $-H$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ ;  $R^2$  is  $H$ ; Y and  $R^c$  together represent a single divalent  $-O-$ ; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



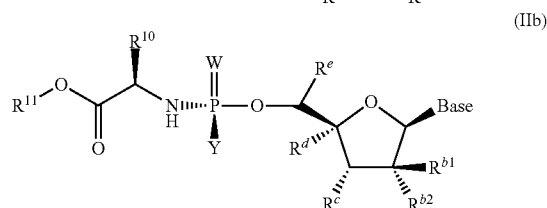
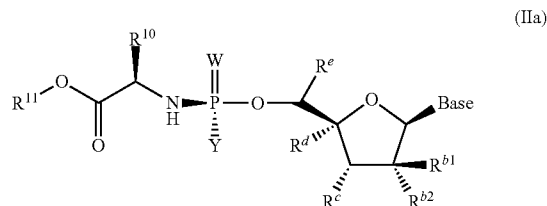
wherein  $R^3$  is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxyl.

**[0101]** In certain embodiments, provided herein are compounds according to Formula (II):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

**[0102]** In certain embodiments, provided herein are compounds according to Formula (IIa) or (IIb):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof. In certain embodiments,  $R_p$  compounds are provided. In certain embodiments,  $S_p$  compounds are provided.

**[0103]** In Formulas (II), (IIa) and (IIb), the symbols  $R^{b1}$ ,  $R^{b2}$ ,  $R^c$ ,  $R^d$ ,  $R^e$ , W, Y and Base have the meanings provided above.

**[0104]** In Formulas (II), (IIa) and (IIb), each  $R^{10}$  is independently alkyl, arylalkyl, heteroarylalkyl or a side chain of a naturally occurring amino acid, other than hydrogen. In particular embodiments,  $R^{10}$  is methyl, isopropyl, t-butyl or benzyl.

**[0105]** In Formulas (II), (IIa) and (IIb), each  $R^{11}$  is independently alkyl, cycloalkyl or  $-H$ . In particular embodiments, each  $R^{11}$  is ethyl, propyl, isopropyl, n-propyl, butyl, n-butyl, t-butyl or cyclopentyl.

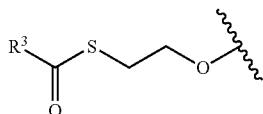
**[0106]** In certain embodiments of Formulas (II), (IIa) and (IIb),  $R^c$  is  $-H$  or  $-OH$ . In the alternative, in certain embodiments, Y and  $R^c$  join to form a six-membered heterocyclic ring wherein Y and  $R^c$  together represent a single divalent  $-O-$ . In these embodiments, the compounds comprise a cyclic phosphate group linking the 3' and 5' carbons of the nucleoside sugar.

**[0107]** In certain embodiments of Formulas (II), (IIa) and (IIb),  $R^{b1}$  is  $-CH_3$ . Also in certain embodiments,  $R^{b2}$  is  $-OH$ ,  $-Cl$  or  $-F$ . In certain embodiments,  $R^{b2}$  is  $-OH$ . In certain embodiments,  $R^{b2}$  is  $-Cl$ . In certain embodiments,  $R^{b2}$  is  $-F$ .

**[0108]** In certain embodiments of Formulas (II), (IIa) and (IIb),  $R^d$  is —H. In the alternative, in certain embodiments,  $R^{b2}$  and  $R^d$  join to form alkylene or substituted alkylene. In particular embodiments,  $R^{b2}$  and  $R^d$  form —CH<sub>2</sub>—O—. In particular embodiments, the —CH<sub>2</sub>— is linked to the 4' carbon of the sugar, and the —O— is linked to the 2' carbon of the sugar.

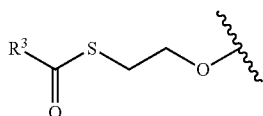
**[0109]** In certain embodiments of Formulas (II), (IIa) and (IIb), R<sup>e</sup> is —H or alkyl. In particular embodiments, R<sup>e</sup> is —H.

**[0110]** In certain embodiments according to Formula (II), (IIa) or (IIb), R<sup>e</sup> is —H; R<sup>d</sup> is —H; R<sup>b1</sup> is —CH<sub>3</sub>; R<sup>b2</sup> is —OH, —Cl or —F; R<sup>c</sup> is —H or —OH; and R<sup>2</sup> is H. In particular embodiments, R<sup>e</sup> is —H; R<sup>d</sup> is —H; R<sup>b1</sup> is —CH<sub>3</sub>; R<sup>b2</sup> is —OH, —Cl or —F; R<sup>c</sup> is —H or —OH; R<sup>2</sup> is H; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In particular embodiments, R<sup>e</sup> is —H; R<sup>d</sup> is —H; R<sup>b1</sup> is —CH<sub>3</sub>; R<sup>b2</sup> is —OH, —Cl or —F; R<sup>c</sup> is —H or —OH; R<sup>2</sup> is H; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



wherein R<sup>3</sup> is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxy. In particular embodiments according to this paragraph, R<sup>10</sup> is methyl, isopropyl, t-butyl or benzyl; and R<sup>11</sup> is ethyl, propyl, isopropyl, n-propyl, butyl, n-butyl, t-butyl or cyclopentyl.

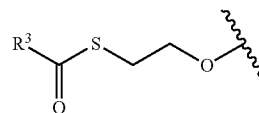
[0111] In certain embodiments according to Formula (II), (IIa) or (IIb),  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ; and  $R^2$  is  $H$ . In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is  $H$ ; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2-O-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is  $H$ ; Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



wherein R<sup>3</sup> is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxy. In particular embodiments according to this paragraph, R<sup>10</sup> is methyl, isopropyl, t-butyl or benzyl; and R<sup>11</sup> is ethyl, propyl, isopropyl, n-propyl, butyl, n-butyl, t-butyl or cyclopentyl.

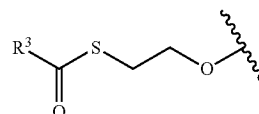
**[0112]** In certain embodiments according to Formula (II), (IIa) or (IIb),  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2CH_2-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ; and  $R^2$  is  $H$ . In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2CH_2-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is  $H$ ; and Base is selected from

guanosine, uracil, cytosine, adenine or a derivative thereof. In certain embodiments,  $R^e$  is  $-H$ ;  $R^{b2}$  and  $R^d$  form  $-CH_2CH_2-$ ;  $R^{b1}$  is  $-CH_3$ ;  $R^c$  is  $-H$  or  $-OH$ ;  $R^2$  is  $H$ ; Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



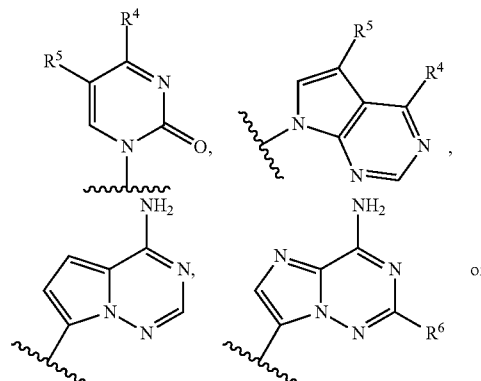
wherein R<sup>3</sup> is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxy. In particular embodiments according to this paragraph, R<sup>10</sup> is methyl, isopropyl, t-butyl or benzyl; and R<sup>11</sup> is ethyl, propyl, isopropyl, n-propyl, butyl, n-butyl, t-butyl or cyclopentyl.

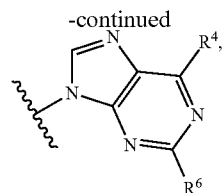
**[0113]** In certain embodiments according to Formula (II), (IIa) or (IIb),  $R^e$  is —H;  $R^d$  is —H;  $R^{b1}$  is —CH<sub>3</sub>;  $R^{b2}$  is —OH, —Cl or —F;  $R^2$  is H; and Y and  $R^e$  together represent a single divalent —O—. In particular embodiments,  $R^e$  is —H;  $R^d$  is —H;  $R^{b1}$  is —CH<sub>3</sub>;  $R^{b2}$  is —OH, —Cl or —F;  $R^2$  is H; Y and  $R^e$  together represent a single divalent —O—; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof. In particular embodiments,  $R^e$  is —H;  $R^d$  is —H;  $R^{b1}$  is —CH<sub>3</sub>;  $R^{b2}$  is —OH, —Cl or —F;  $R^2$  is H; Y and  $R^e$  together represent a single divalent —O—; and Base is selected from guanosine, uracil, cytosine, adenine or a derivative thereof; and X is D-alanine, or an ester thereof. In certain embodiments according to this paragraph, Y is alkyl, aryl, arylalkyl, cycloalkyl or



wherein R<sup>3</sup> is alkyl, alkoxycarbonylaminoalkyl, hydroxyalkyl, or aminoalkylcarbonylalkoxyl. In particular embodiments according to this paragraph, R<sup>10</sup> is methyl, isopropyl, t-butyl or benzyl; and R<sup>11</sup> is ethyl, propyl, isopropyl, n-propyl, butyl, n-butyl, t-butyl or cyclopentyl.

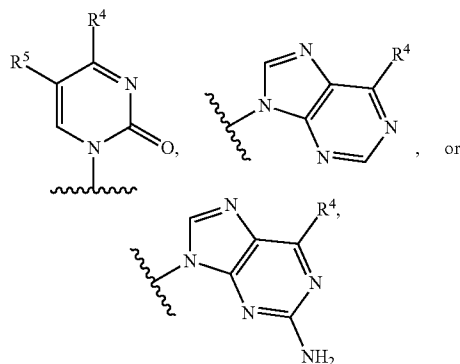
[0114] In certain embodiments, a compound of any of Formulas (I), (Ia), (Ib), (II), (IIa), or (Ith) is provided wherein: each Base is independently





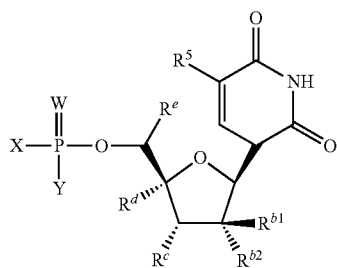
or a tautomer thereof; each  $R^4$  is independently hydrogen, hydroxyl, hydroxylamine, alkylamino, halogen, sulfanyl, amino or alkoxy; each  $R^5$  is independently hydrogen, halogen or methyl; and each  $R^6$  is independently hydrogen, amino, or halo.

**[0115]** In certain embodiments, a compound of any of Formulas (I), (Ia), (Ib), (II), (IIa) or (IIIb) is provided wherein: each Base is independently

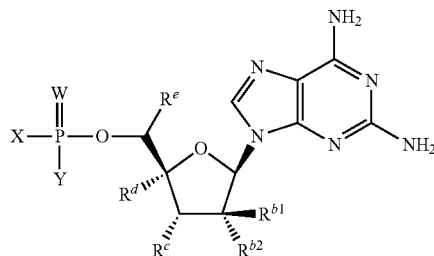
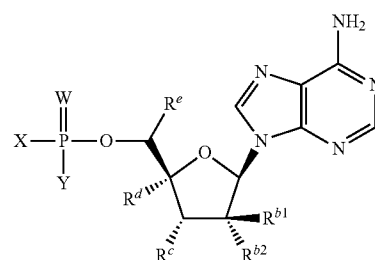
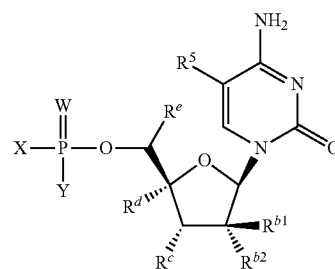
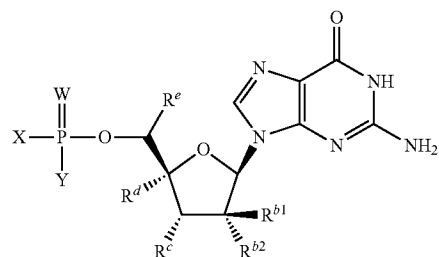
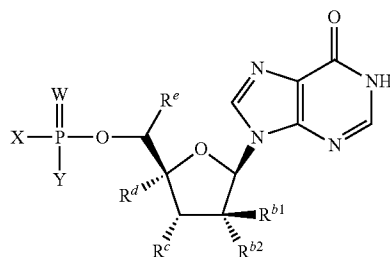


or a tautomer thereof; each  $R^4$  is independently hydrogen, hydroxyl, hydroxylamine, halogen, sulfanyl, amino or alkoxy; and each  $R^5$  is independently hydrogen, halogen or methyl. In an embodiment, each  $R^4$  is alkylamino. In an embodiment, each  $R^4$  is alkylamino having from seven to thirty carbon atoms. In an embodiment, each  $R^4$  is alkylamino having from fifteen to thirty carbon atoms. In an embodiment, each  $R^4$  is alkylamino having from twenty to thirty carbon atoms. In an embodiment, each  $R^4$  is alkylamino having from seven to fifteen carbon atoms. In an embodiment, each  $R^4$  is alkylamino having from seven to twenty carbon atoms. In an embodiment, each  $R^4$  is alkylamino having from ten to twenty carbon atoms.

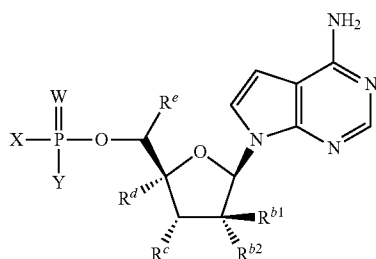
**[0116]** In certain embodiments, a compound of any of the following Formulas is provided:



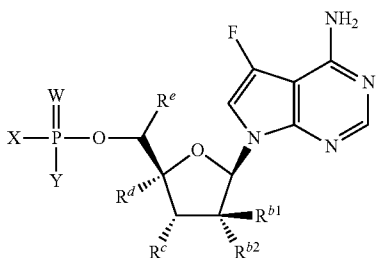
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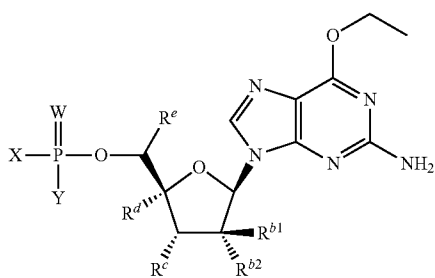
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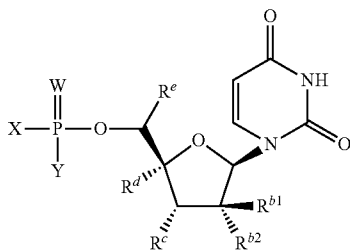
(1002)

or pharmaceutically acceptable salts, solvates, stereoisomeric forms or polymorphic forms thereof, wherein:  $R^{b1}$ ,  $R^{b2}$ ,  $R^c$ ,  $R^d$ ,  $R^e$ , W, X, A and Y are as defined in the context of Formula (I); and each  $R^5$  is independently hydrogen, halogen or methyl.

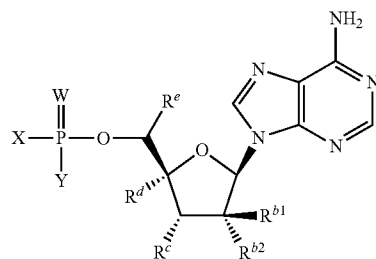
[0117] In certain embodiments, provided herein are compounds according to any of the following Formulas:



(XVII)

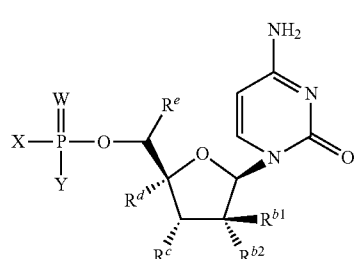


(XVIII)



(XIX)

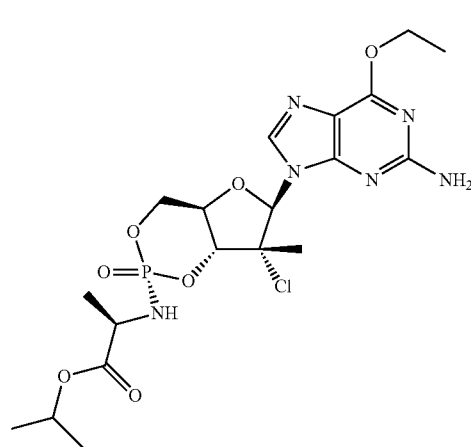
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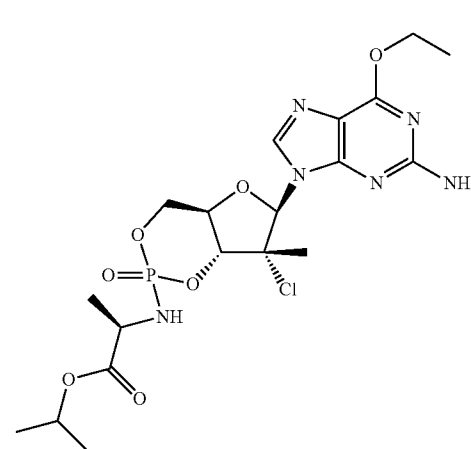
(XX)

or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof, wherein  $R^{b1}$ ,  $R^{b2}$ ,  $R^c$ ,  $R^d$ ,  $R^e$ , W, X, and Y are as defined in the context of Formula (I). In certain embodiments, a compound of Formula (XVII) is provided. In certain embodiments, a compound of Formula (XVIII) is provided. In certain embodiments, a compound of Formula (XIX) is provided. In certain embodiments, a compound of Formula (XX) is provided.

[0118] In certain embodiments, provided herein are compounds according to any of the following formulas:

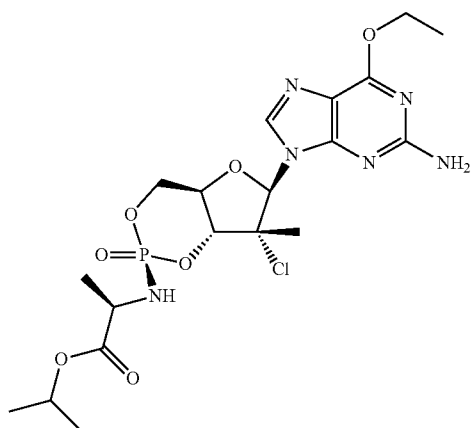


(9ib)



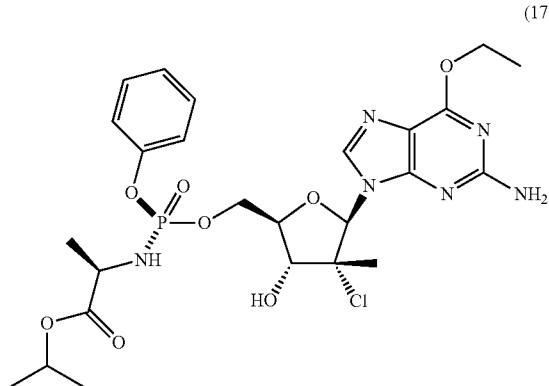
(9i)

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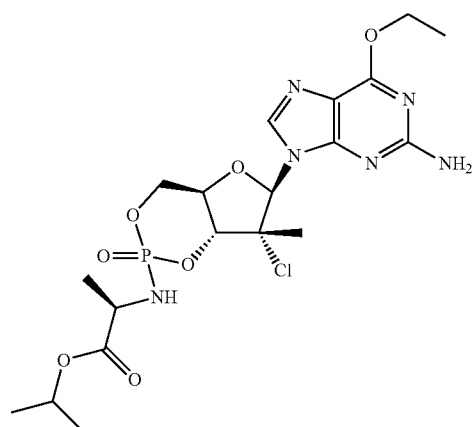


(9ia)

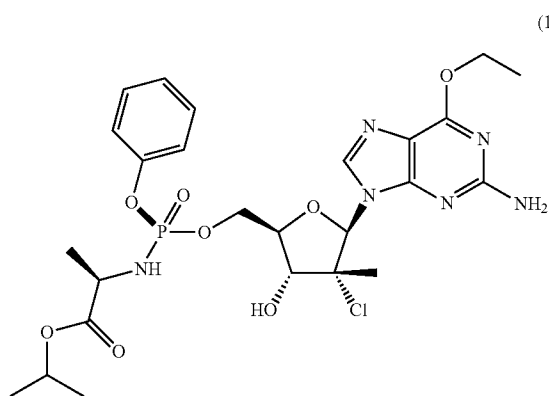
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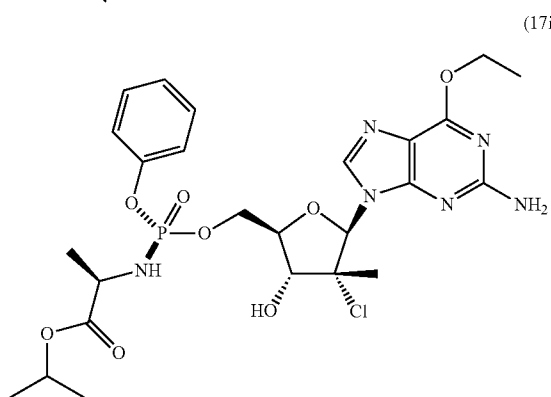
(17ia)



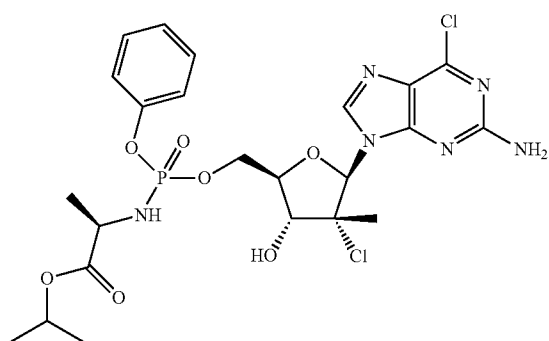
(10)



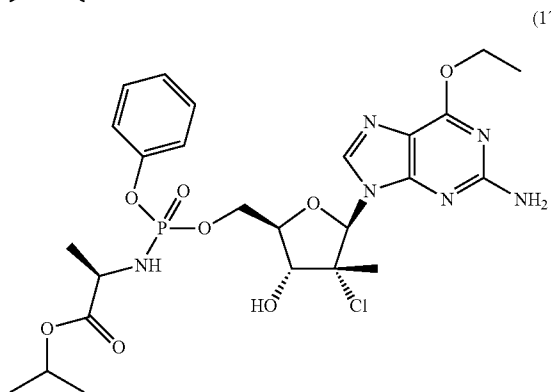
(18)



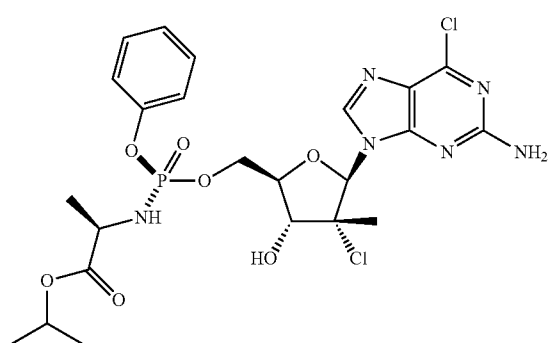
(17ib)



(19i)



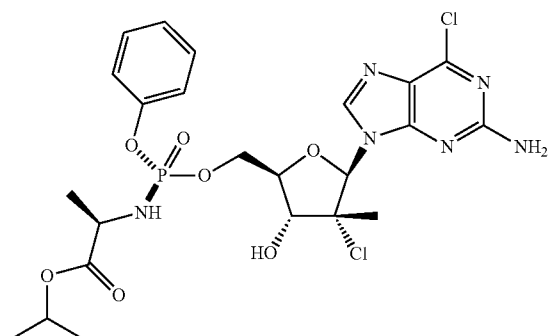
(17i)



(19ia)

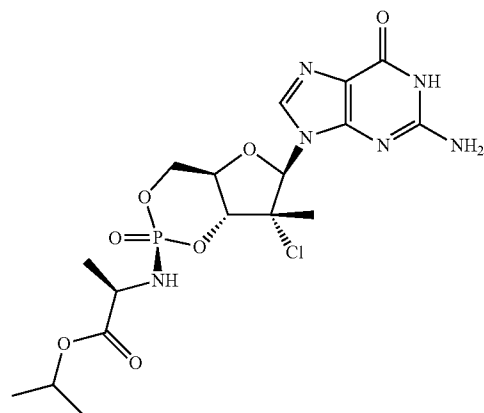
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(19ib)

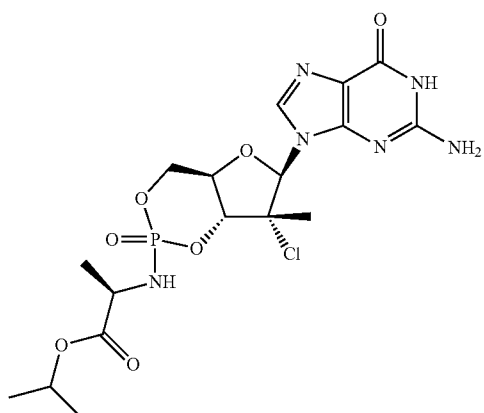


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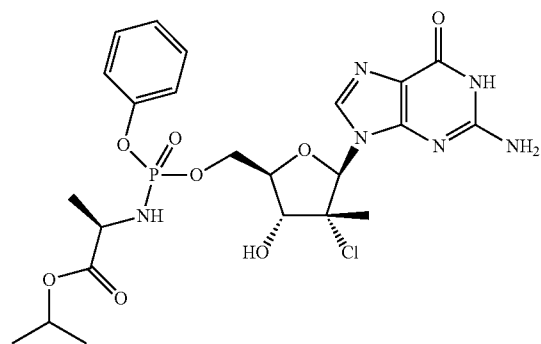
(20ai)



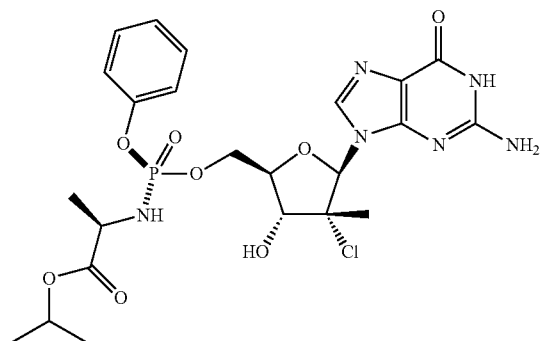
(20i)



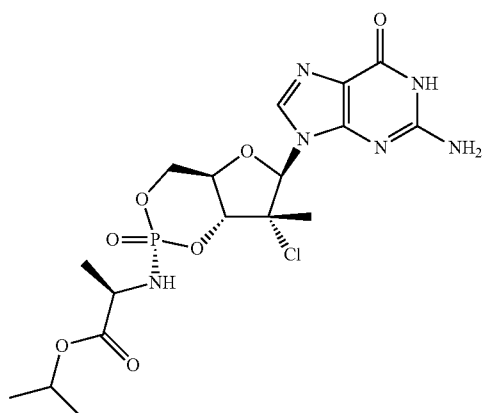
(21i)



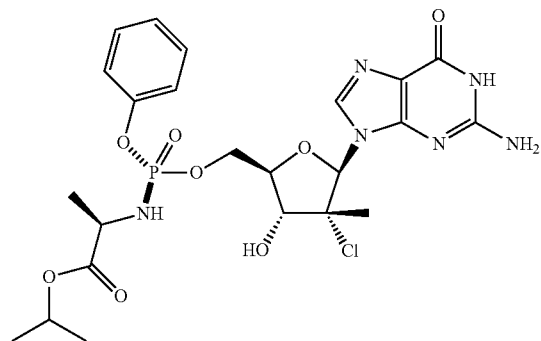
(21ai)



(20bi)

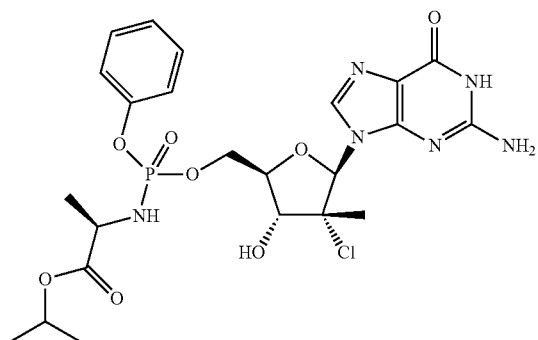


(21bi)



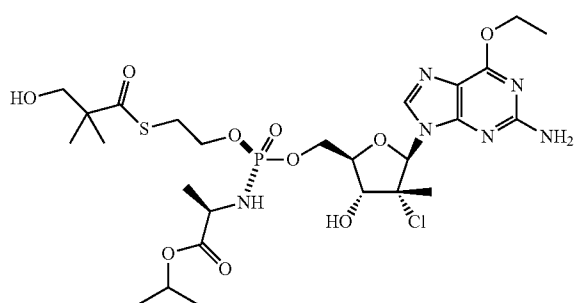
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(27i)

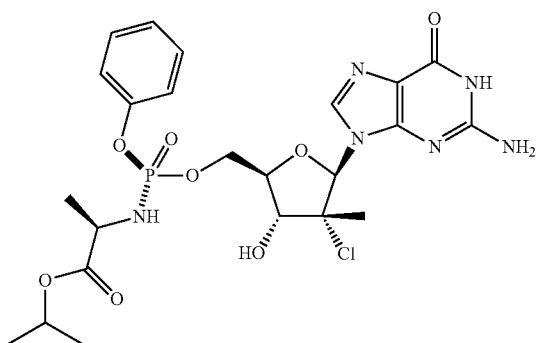


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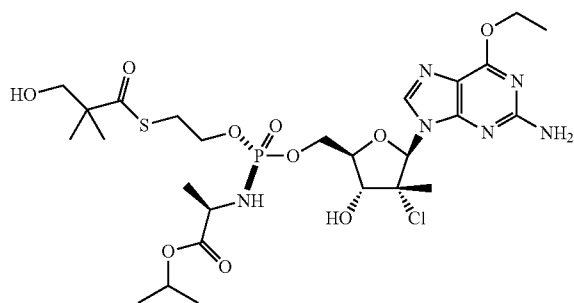
(33ia)



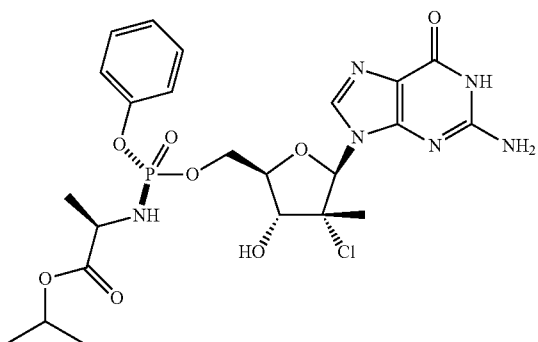
(27ai)



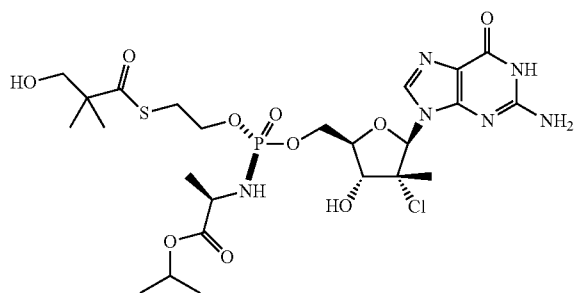
(33ib)



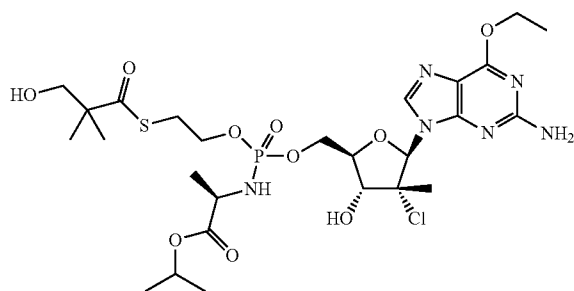
(27bi)



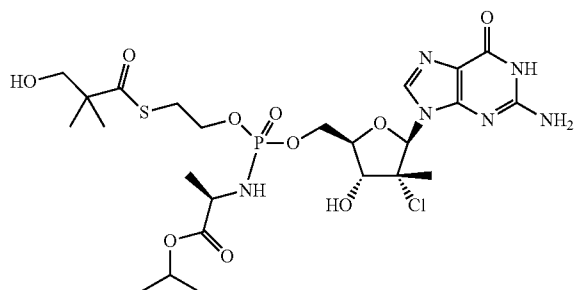
(35bi)



(33i)



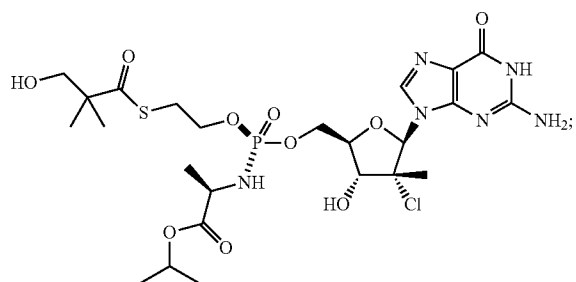
(35i)





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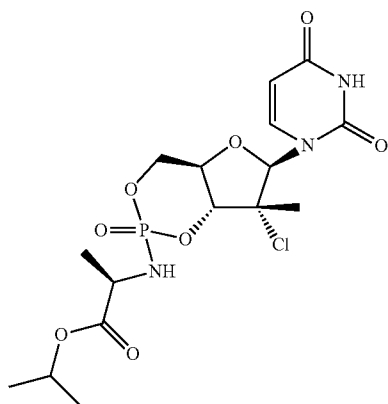
(35ai)



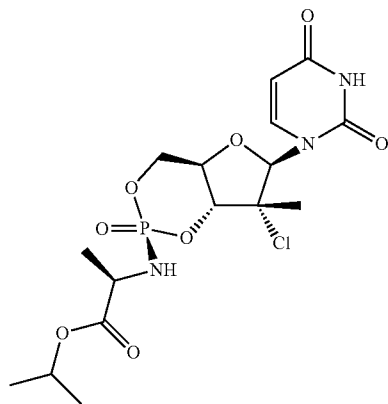
or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

[0119] In certain embodiments, provided herein are compounds according to any of the following formulas:

(38i)

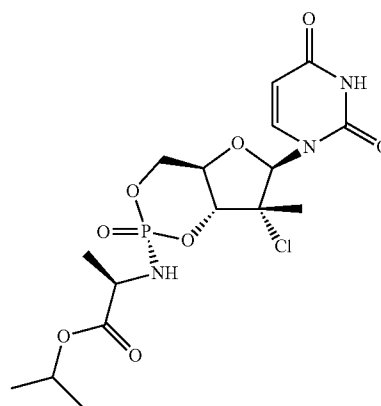


(38ia)

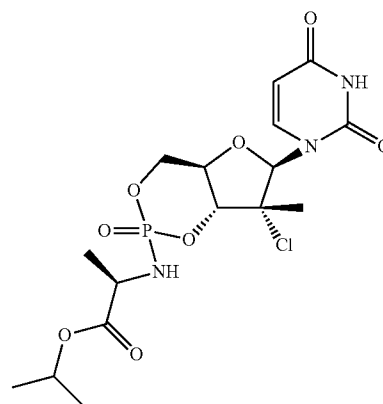


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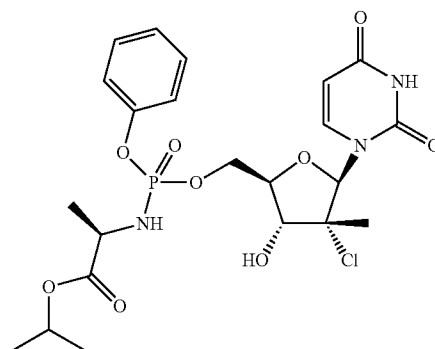
(38ib)



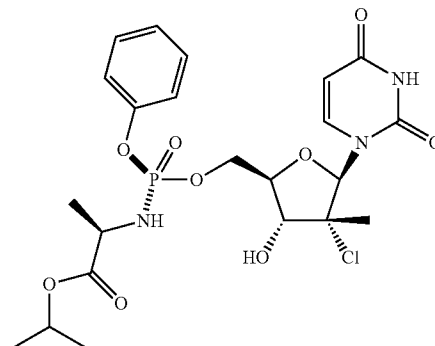
(39)



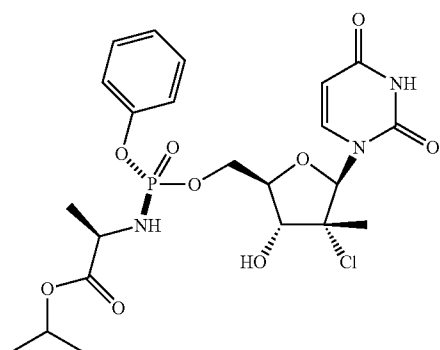
(40i)



(40ia)

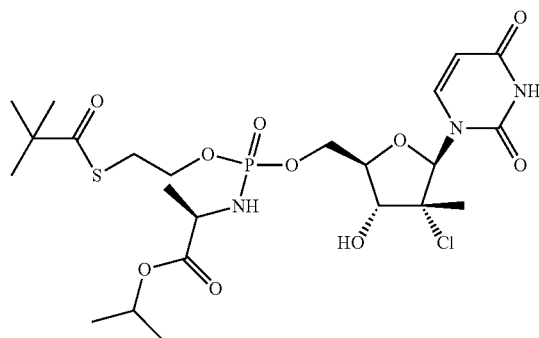


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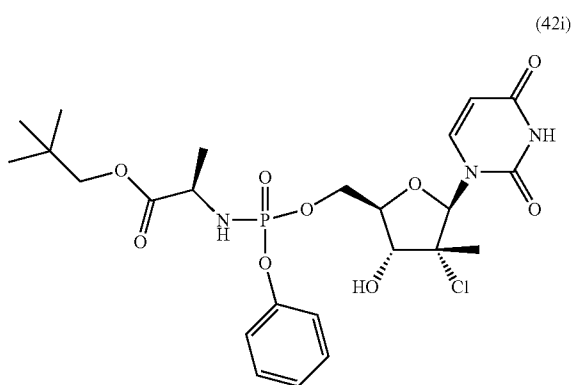


(40ib)

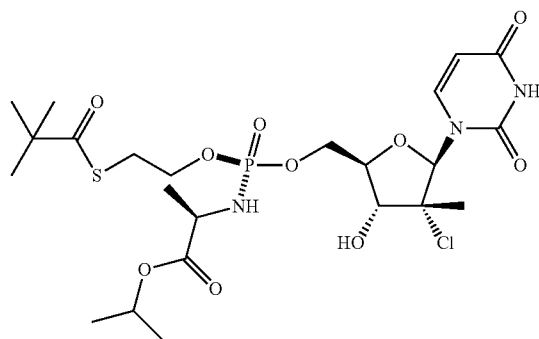
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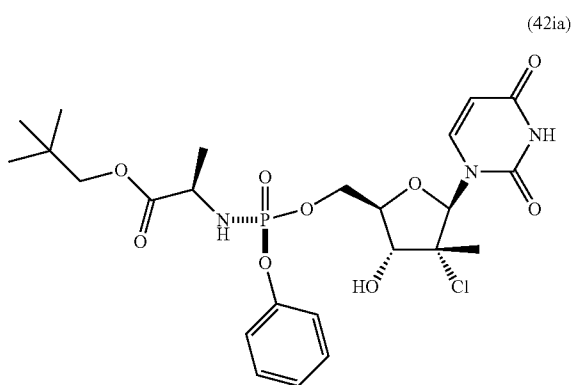
(43i)



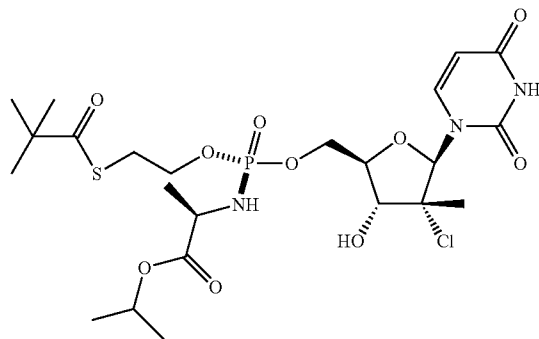
(42i)



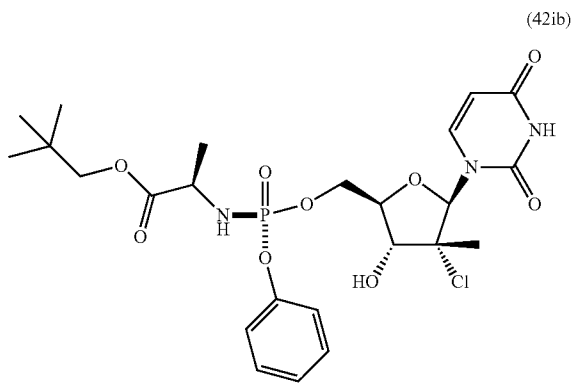
(43ia)



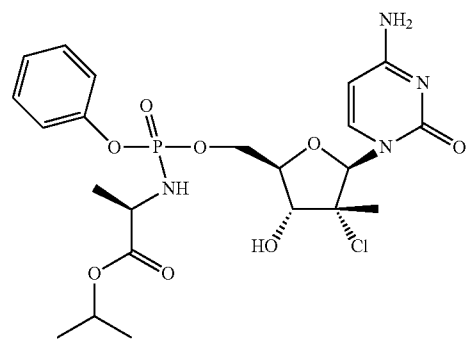
(42ia)



(43ib)

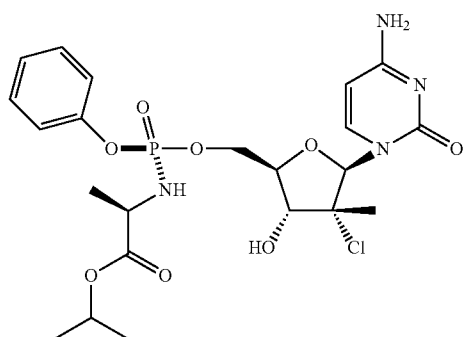


(42ib)



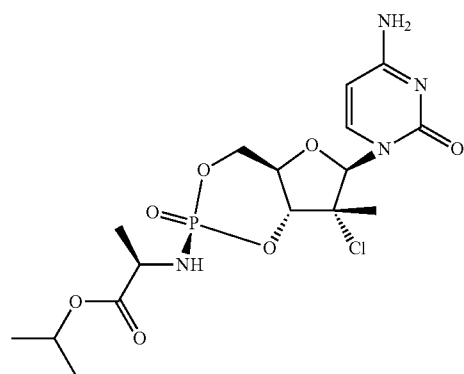
(56i)

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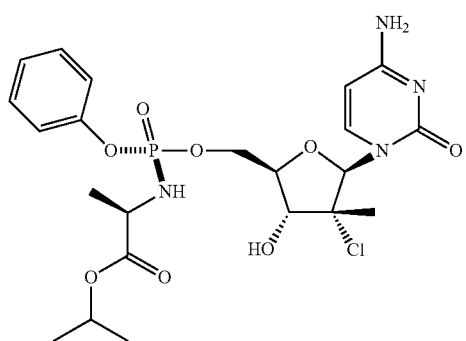


(56ia)

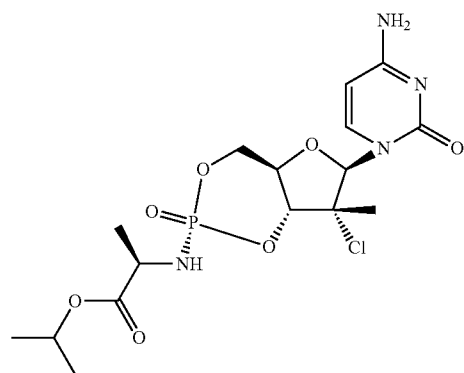
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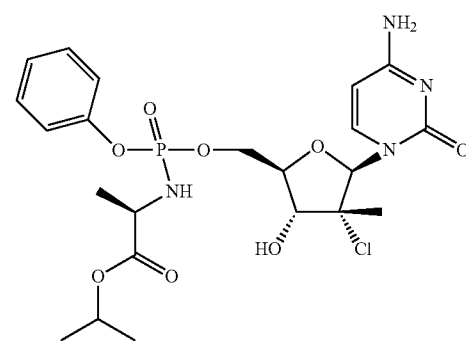
(58ia)



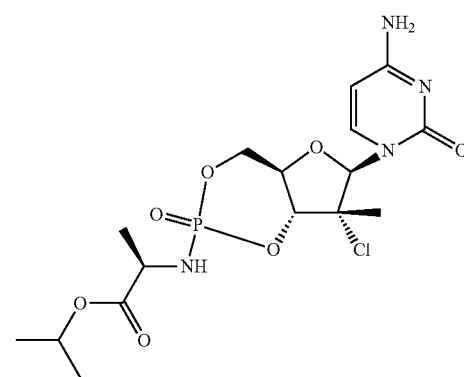
(56ib)



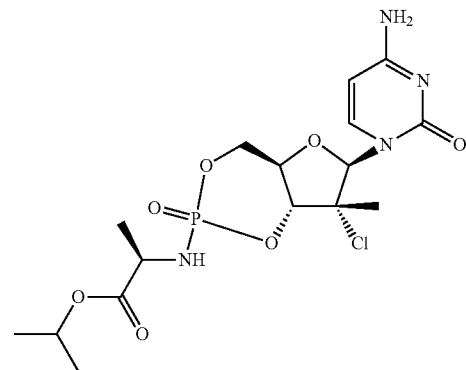
(58ib)



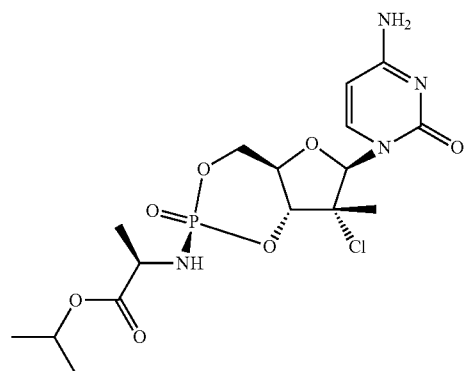
(57)



(63i)

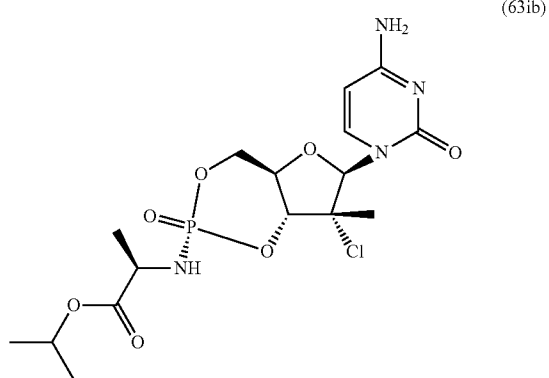


(58i)

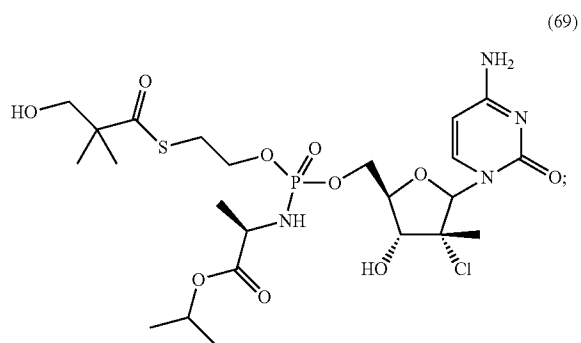
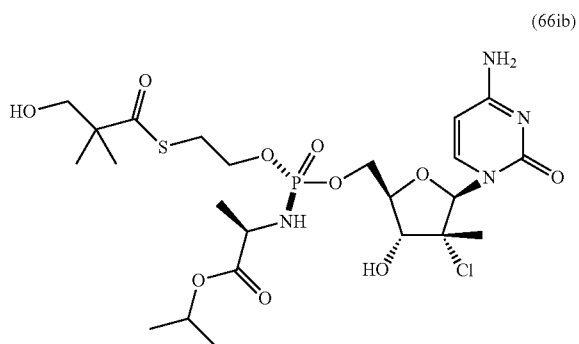
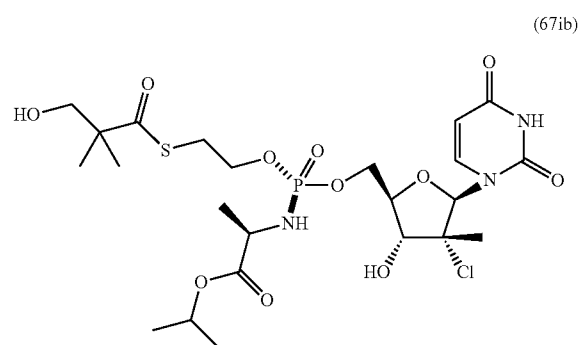
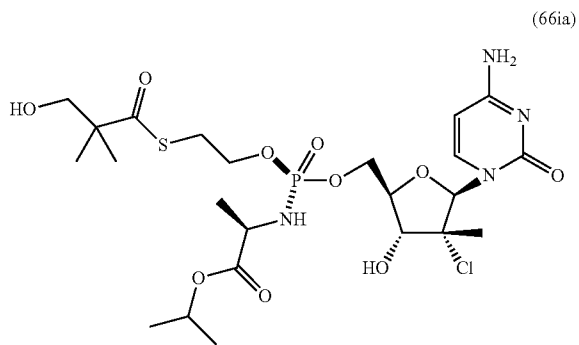
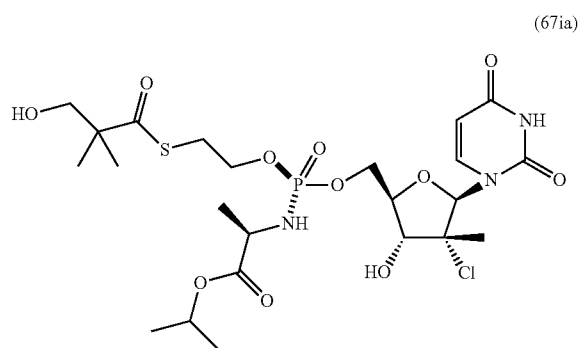
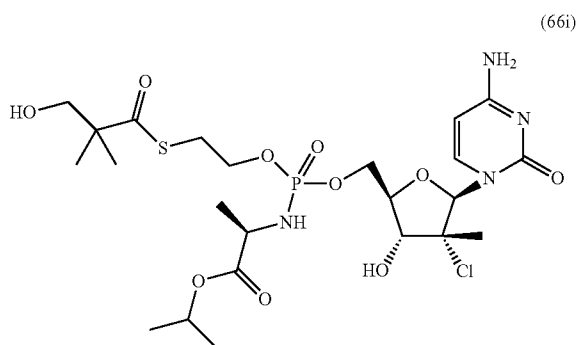
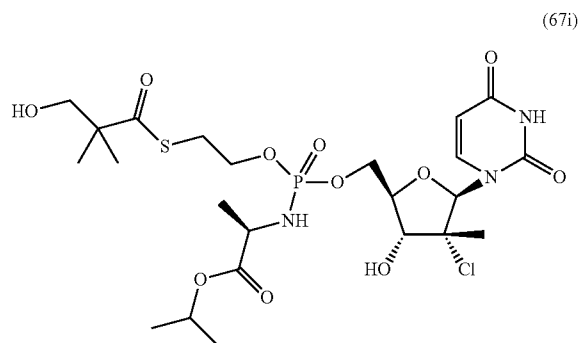


(63ia)

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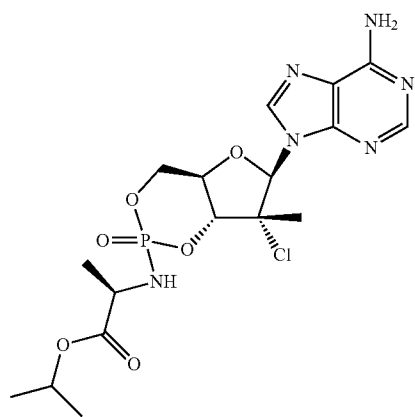


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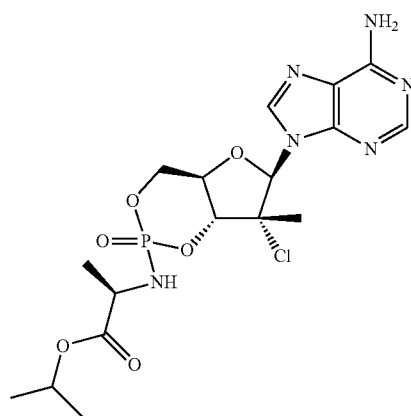


or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

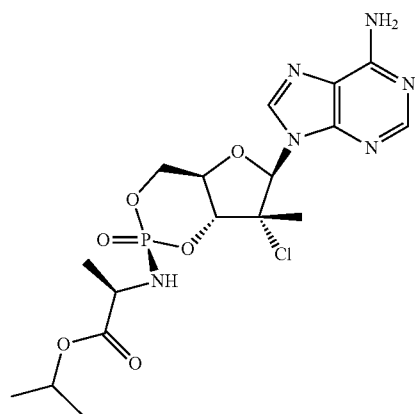
[0120] In certain embodiments, provided herein are compounds according to any of the following formulas:



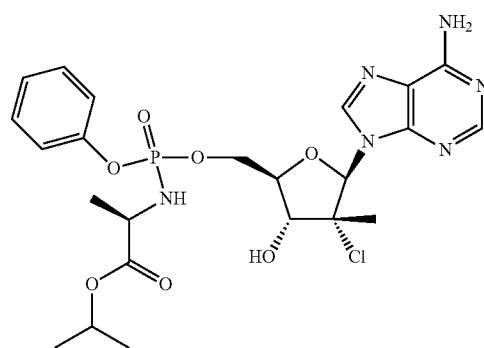
(77i)



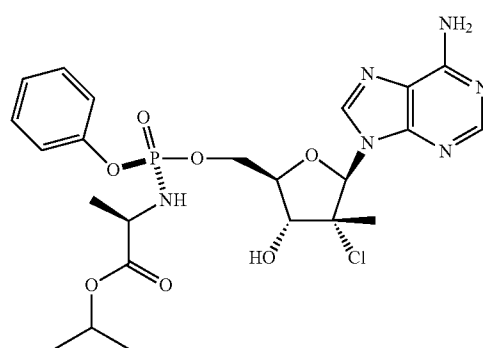
(78)



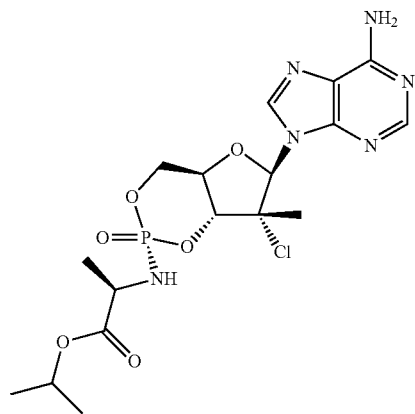
(77ia)



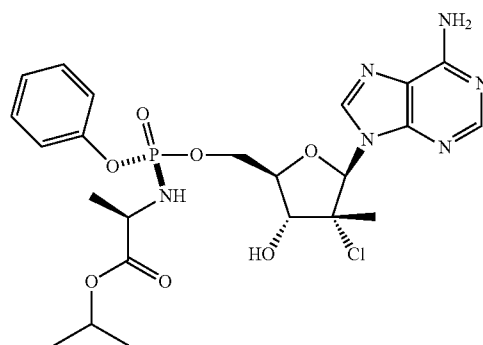
(79i)



(79ia)

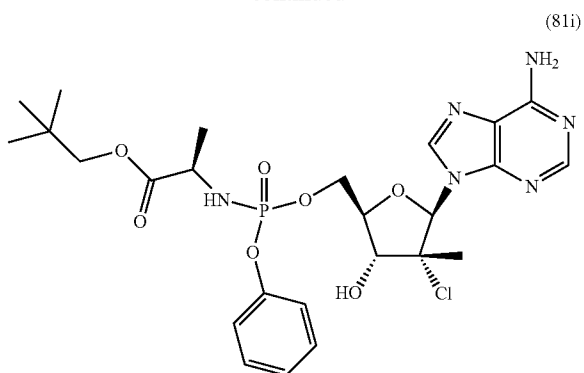


(77ib)

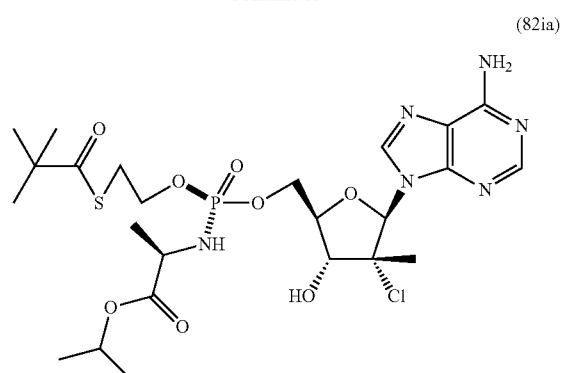


(79ib)

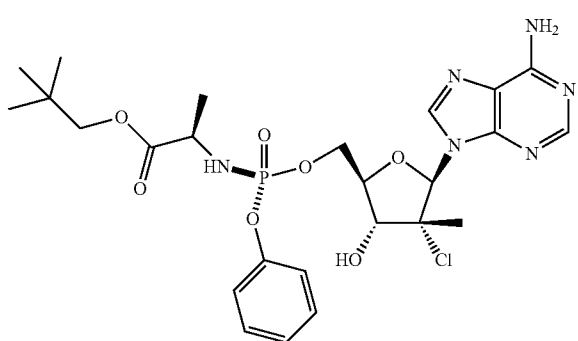
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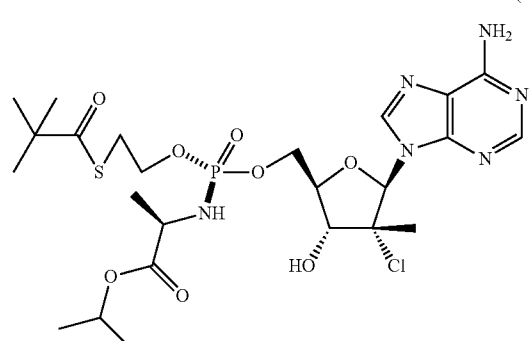
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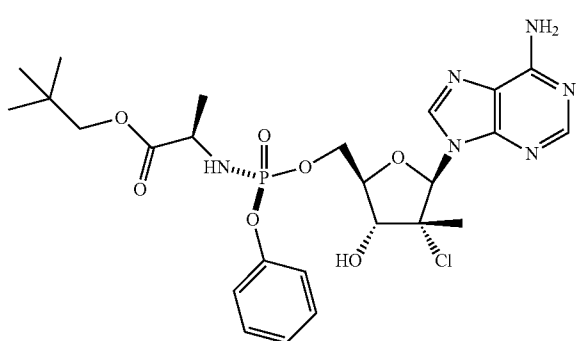
(81ia)



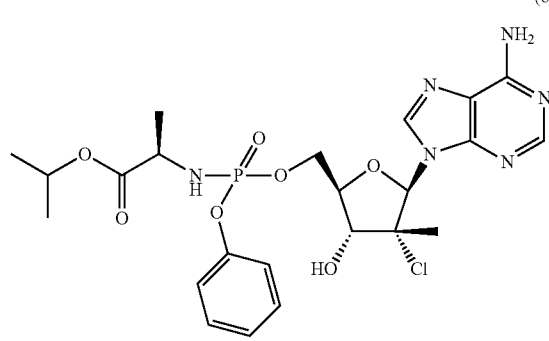
(82ib)



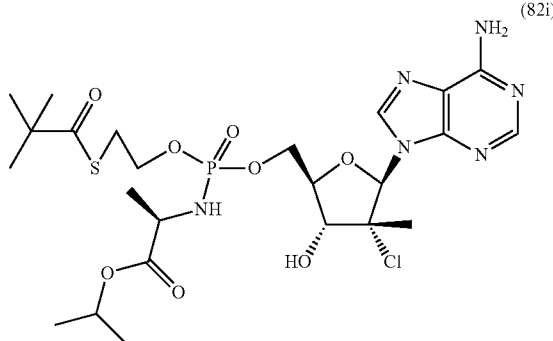
(81ib)



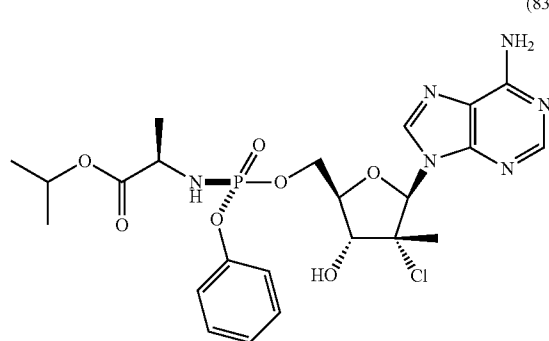
(83i)



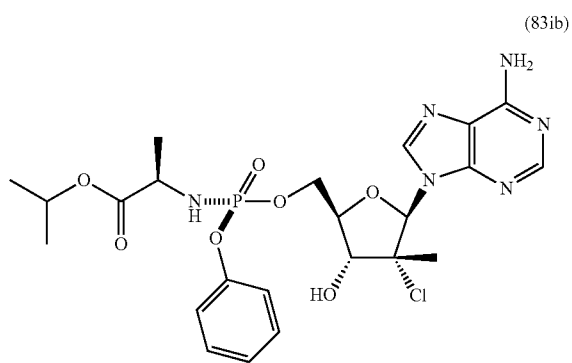
(82i)



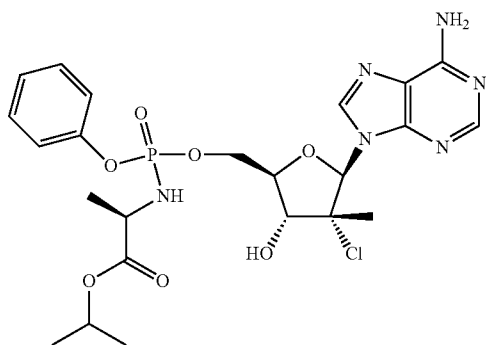
(83ia)



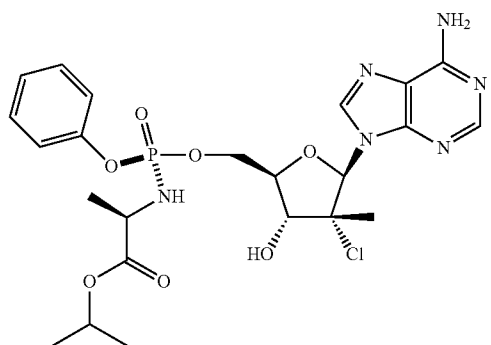
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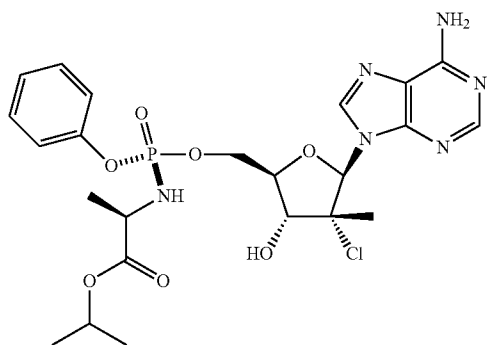
(92i)



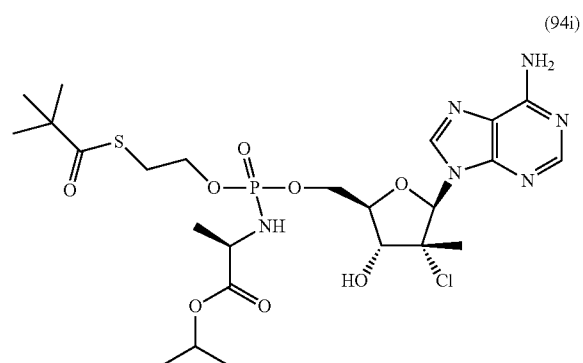
(92ia)



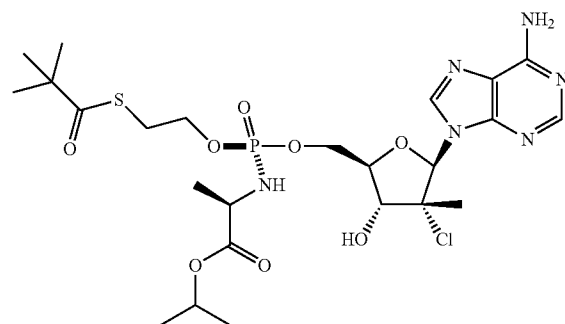
(92ib)



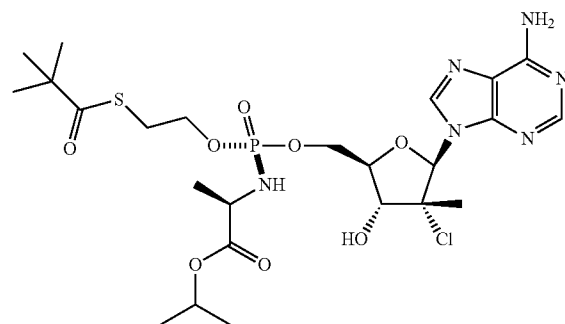
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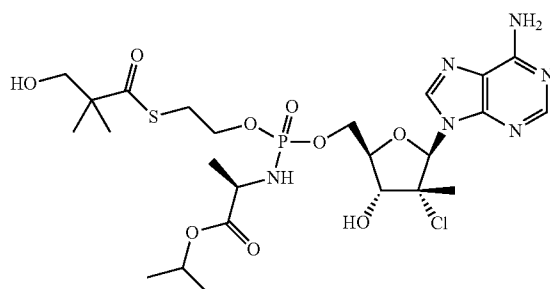
(94ia)



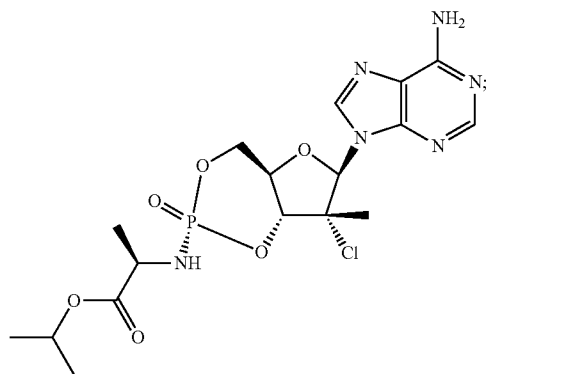
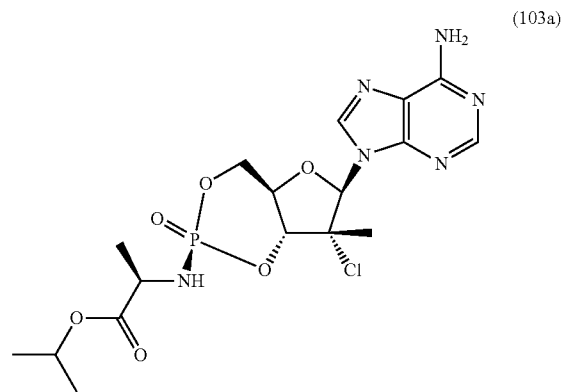
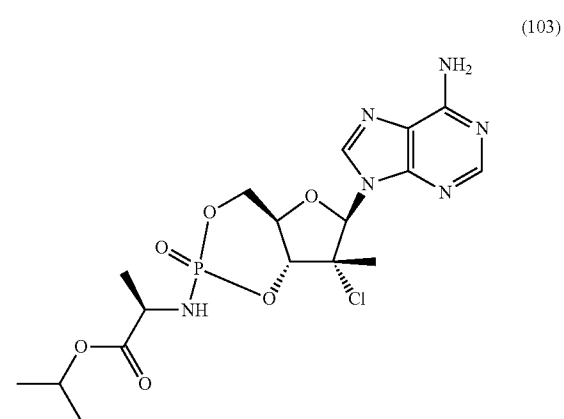
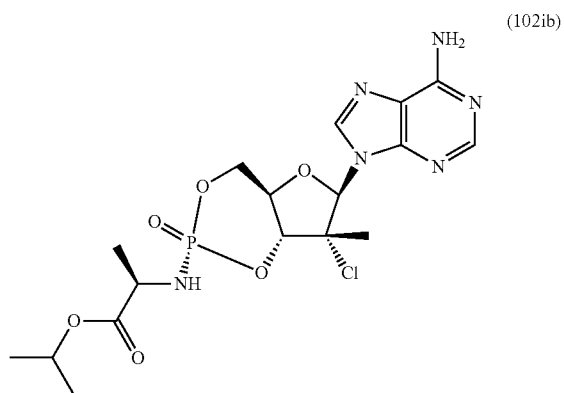
(94ib)



(96i)



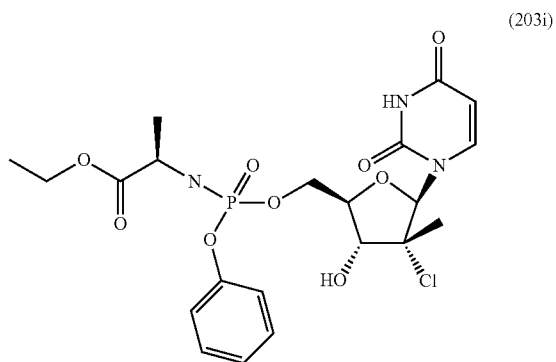
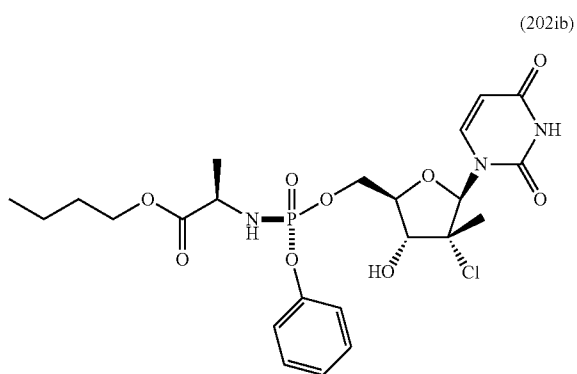
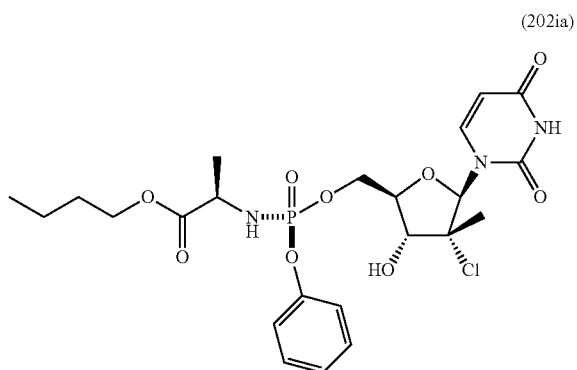
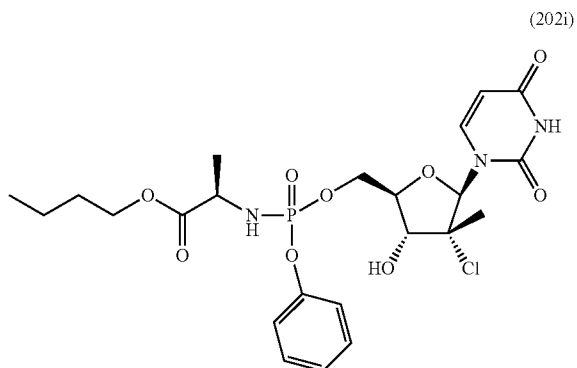
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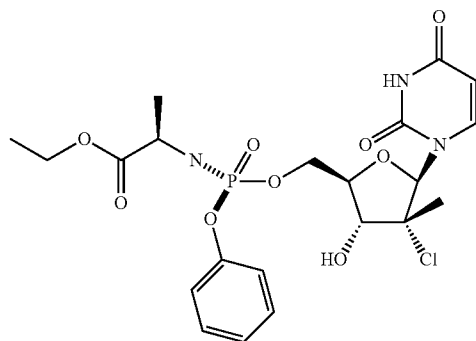
or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

**[0121]** In certain embodiments, provided herein are compounds according to any of the following formulas:

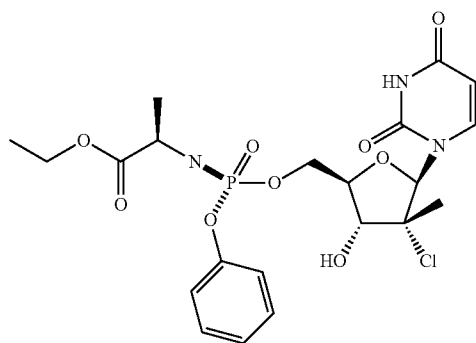


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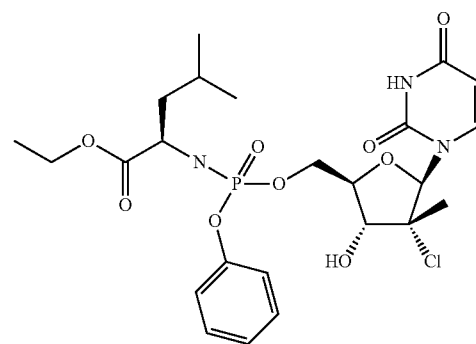
(203ia)



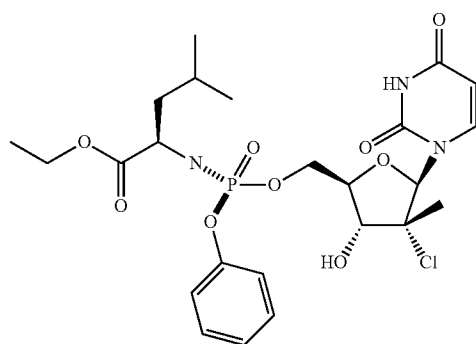
(203ib)



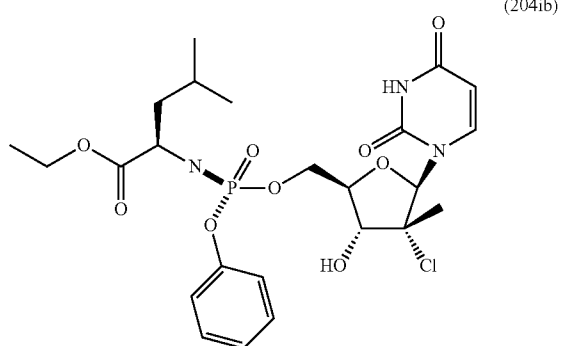
(204i)



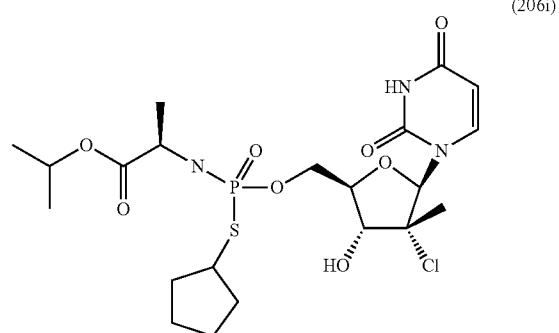
(204ia)



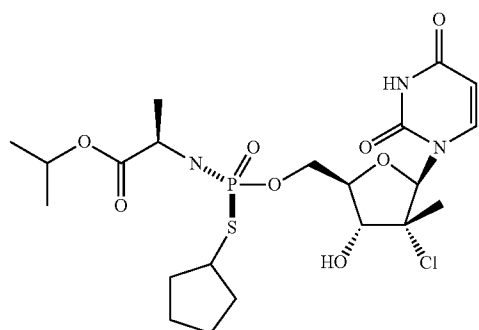
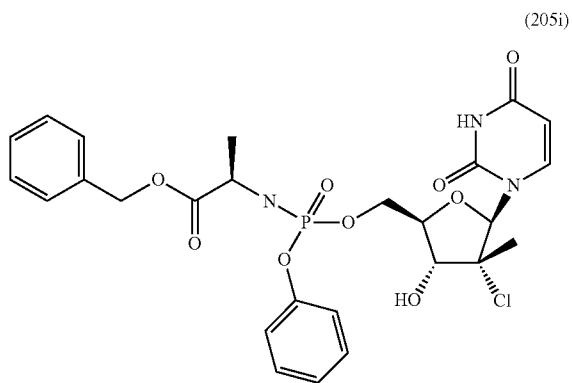
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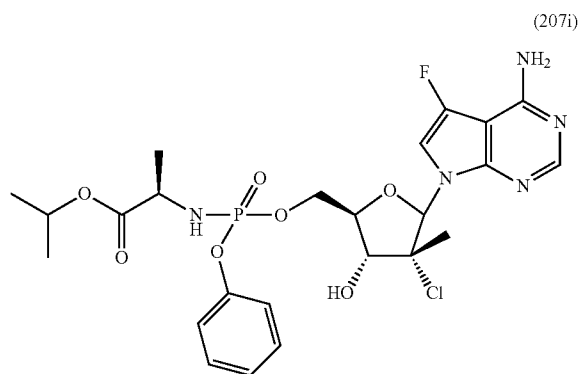
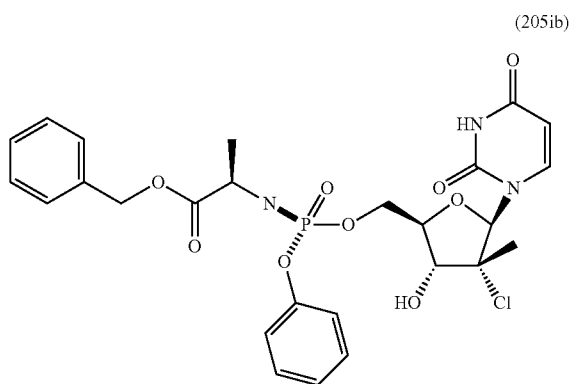
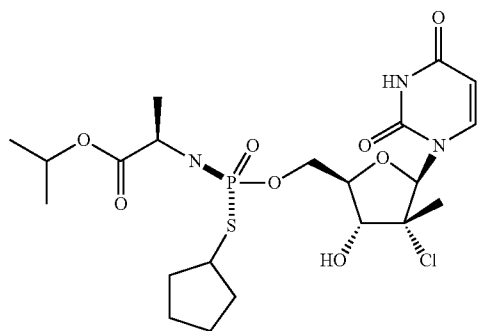
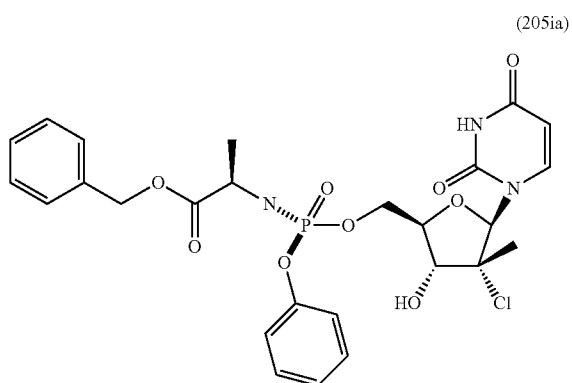
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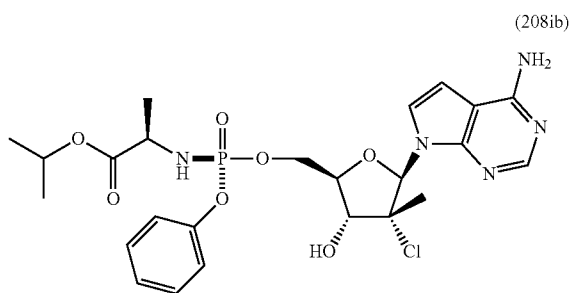
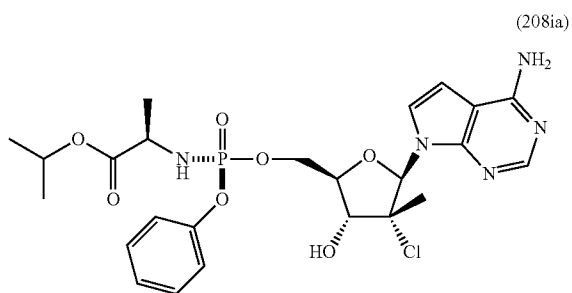
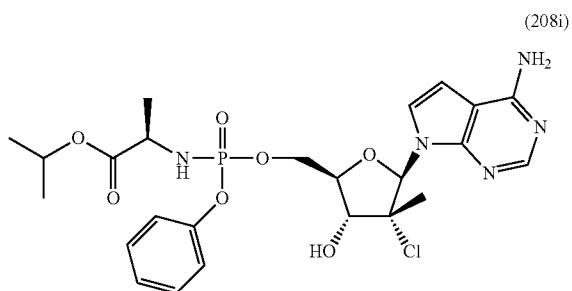
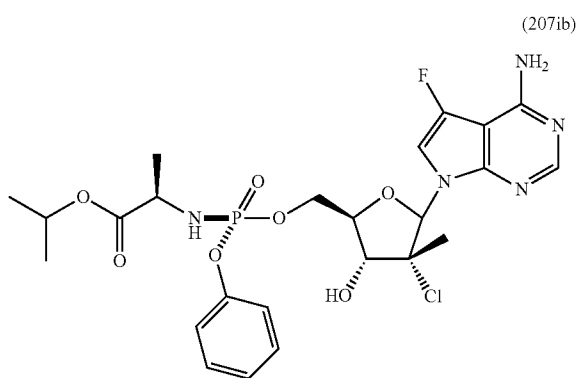
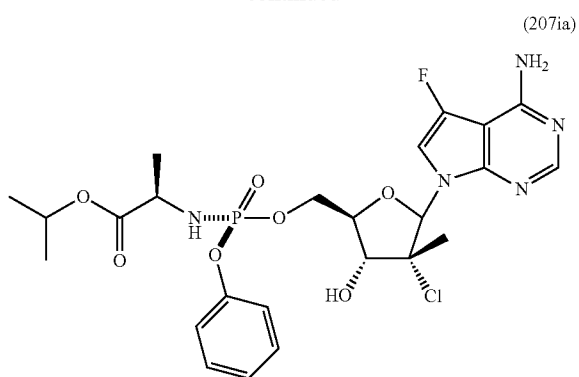
(206ia)



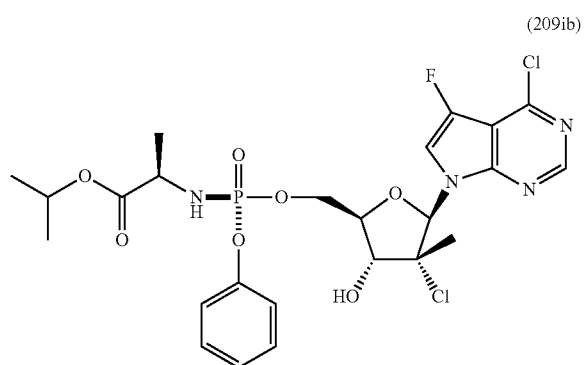
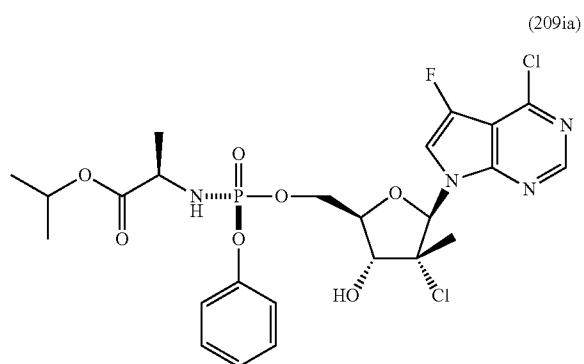
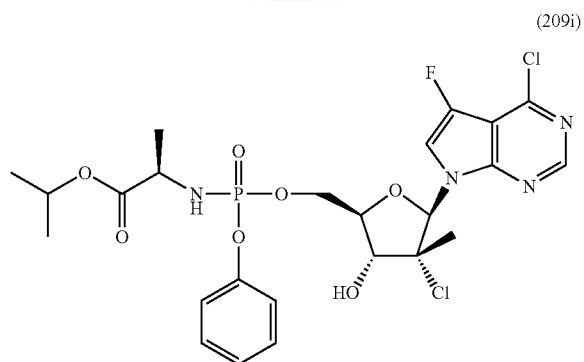
(206ib)



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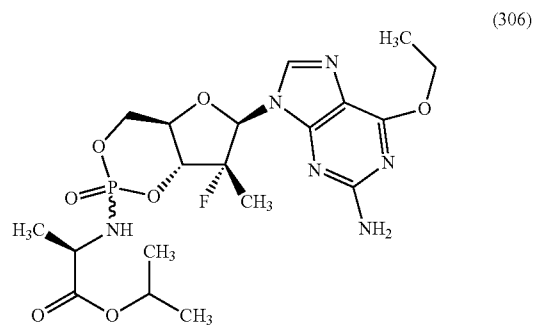


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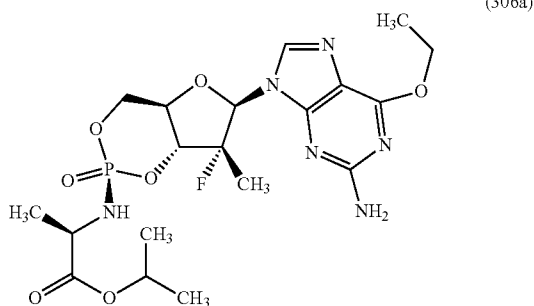


or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

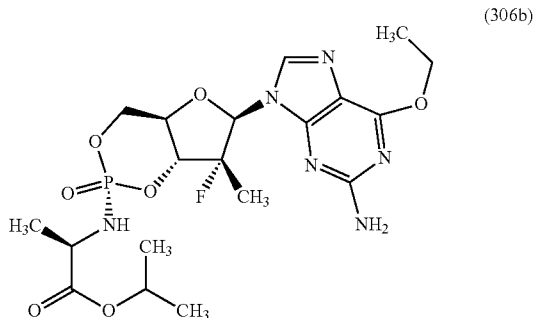
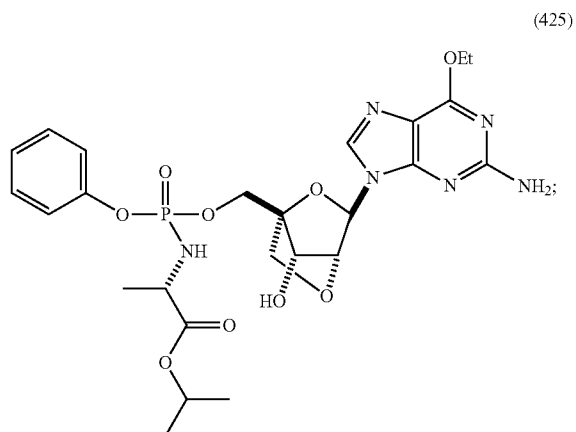
**[0122]** In certain embodiments, provided herein are compounds according to any of the following formulas:



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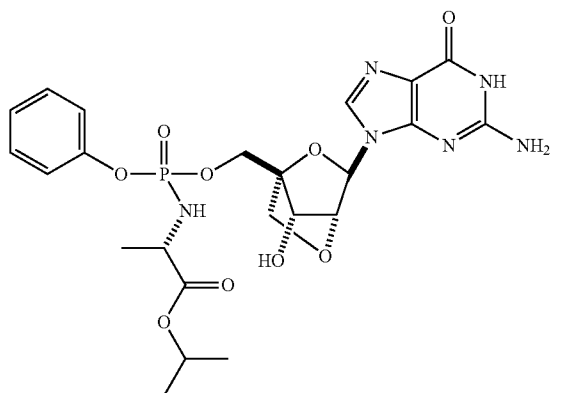


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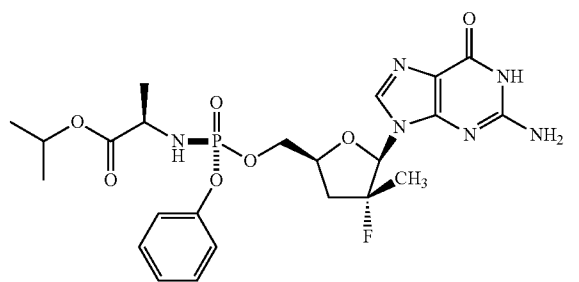
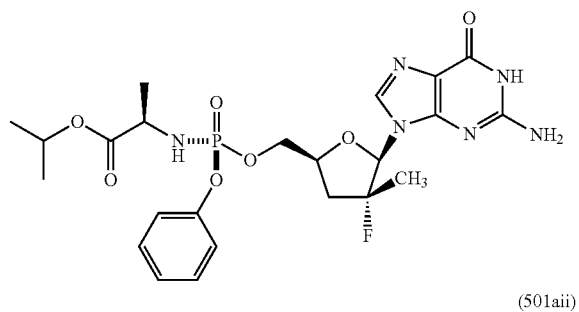
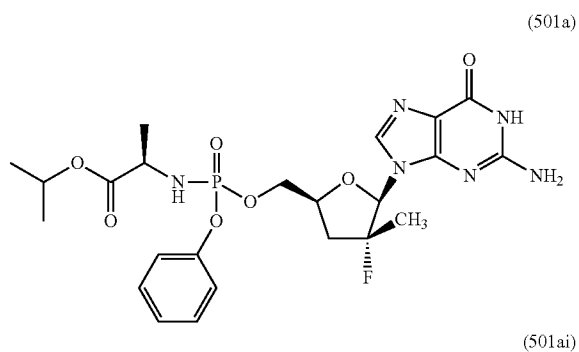
or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

[0123] In certain embodiments, a compound of formula according to formula 401 or 425 is provided:



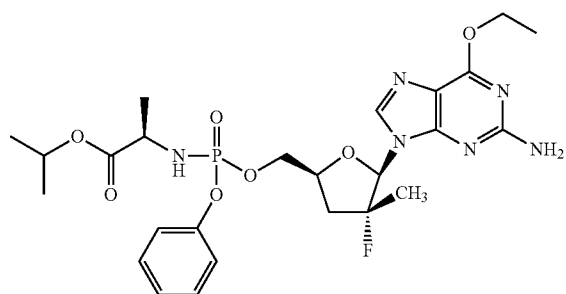
or a pharmaceutically acceptable salt, solvate, phosphate, prodrug, stereoisomeric form, tautomeric form or polymorphic form thereof.

[0124] In certain embodiments, provided herein are compounds according to any of the following formulas:



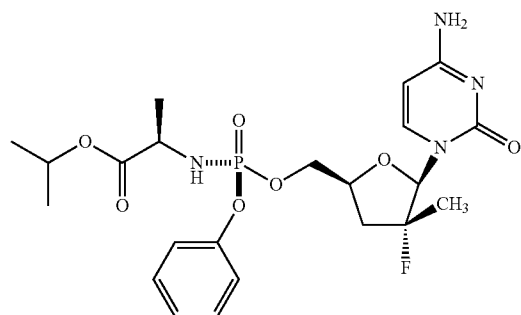
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(502a)



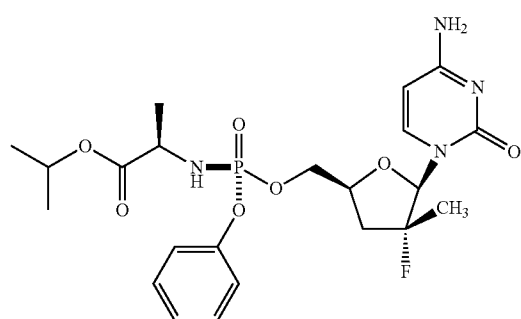
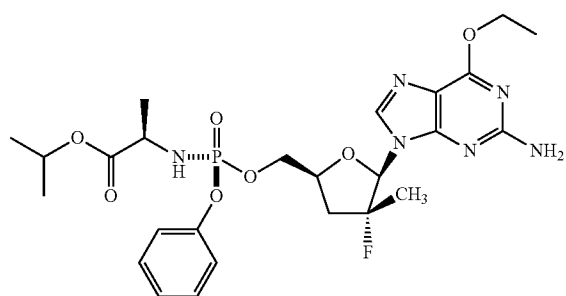
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(503ai)

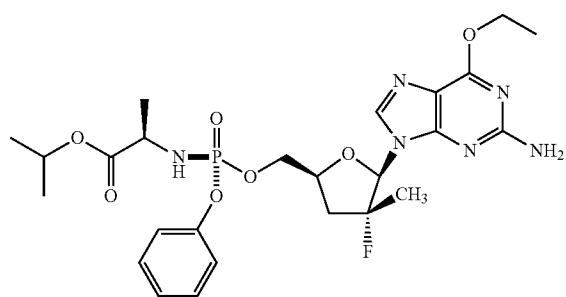


(503aii)

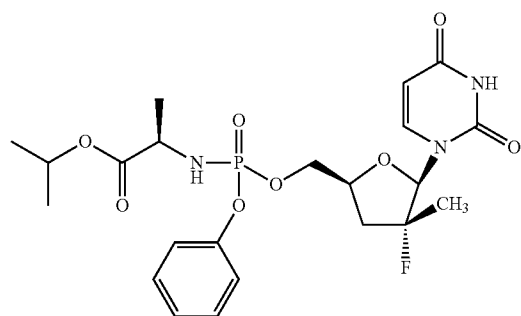
(502ai)



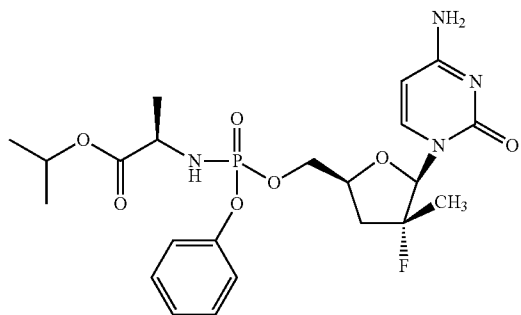
(502aii)



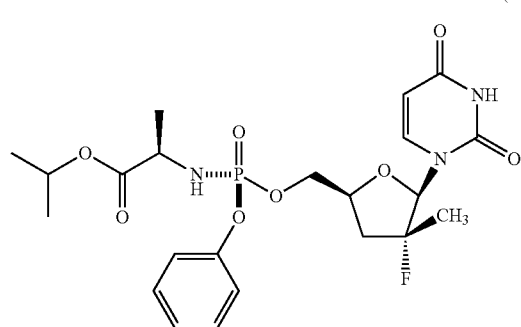
(504a)



(503a)

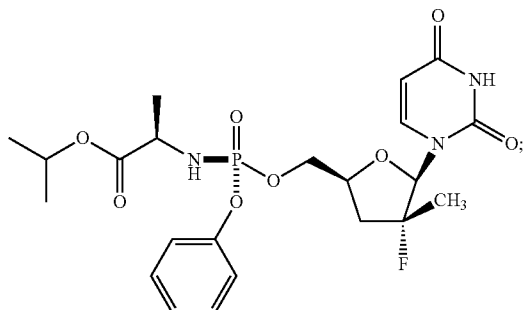


(504ai)



-continued

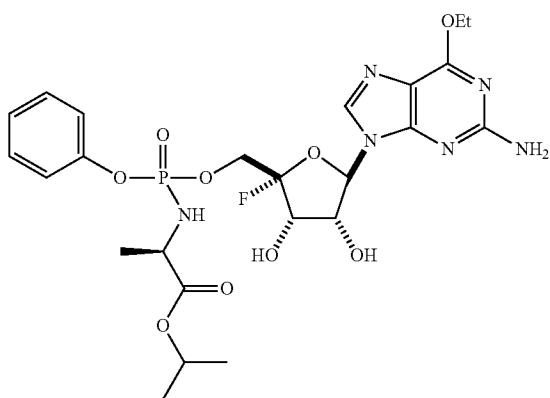
(504aii)



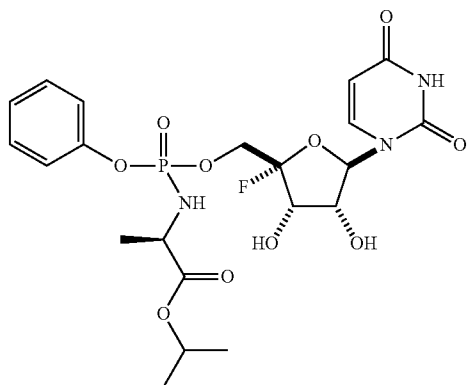
or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

**[0125]** In certain embodiments, provided herein are compounds according to any of the following formulas:

(601a)

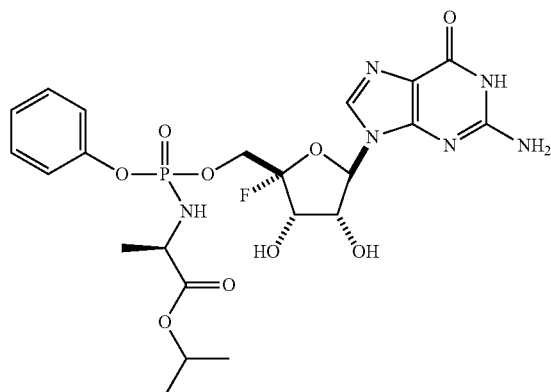


(602a)



-continued

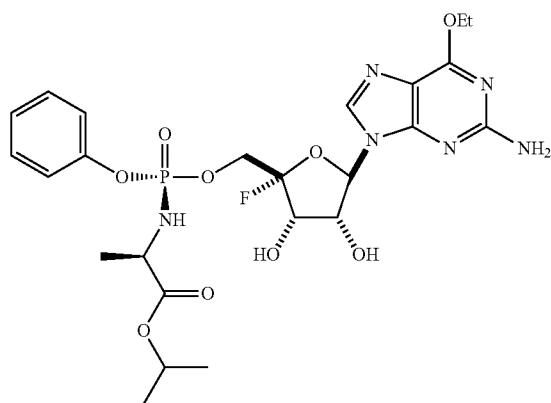
(603a)



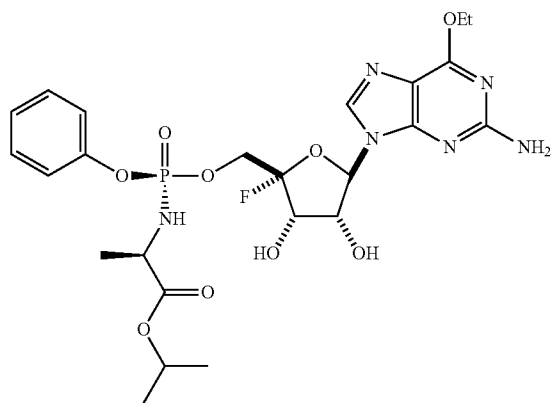
or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

**[0126]** In certain embodiments, provided herein are compounds according to any of the following formulas:

(601ai)

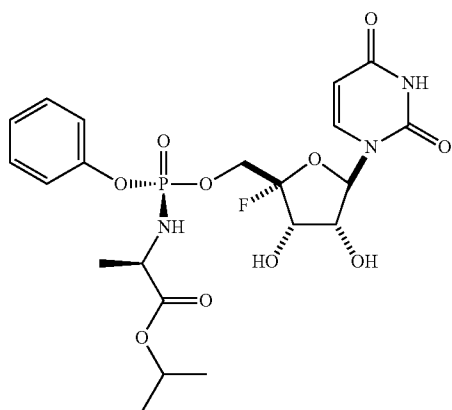


(601aii)



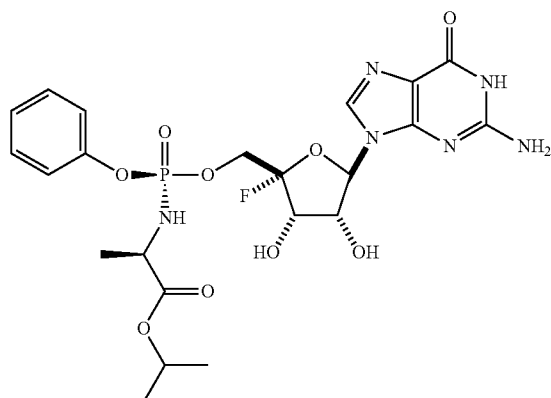
-continued

(602ai)



-continued

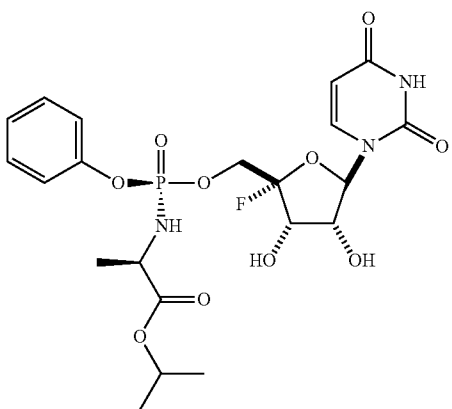
(603aii)



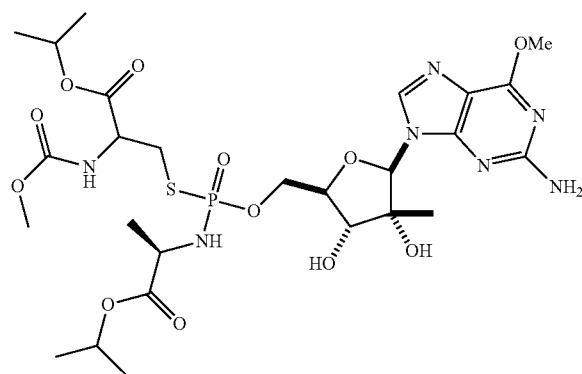
or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

**[0127]** In certain embodiments provided herein are compounds according to any of the following formulas:

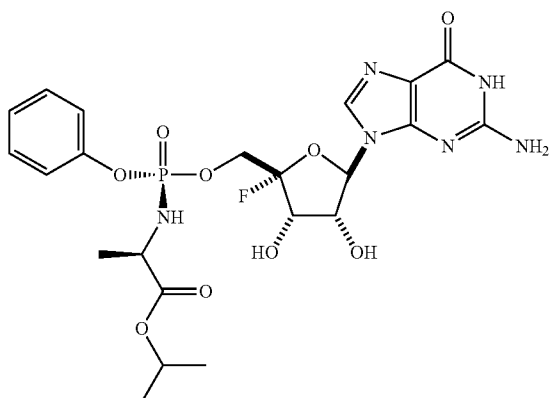
(602aii)



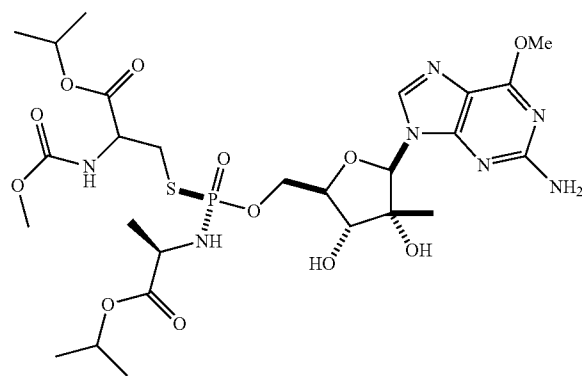
(802a)



(603ai)

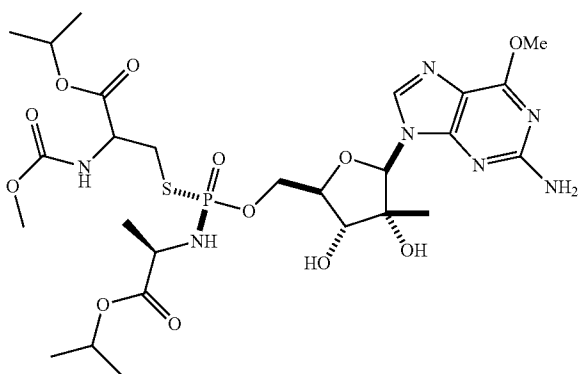


(802ai)

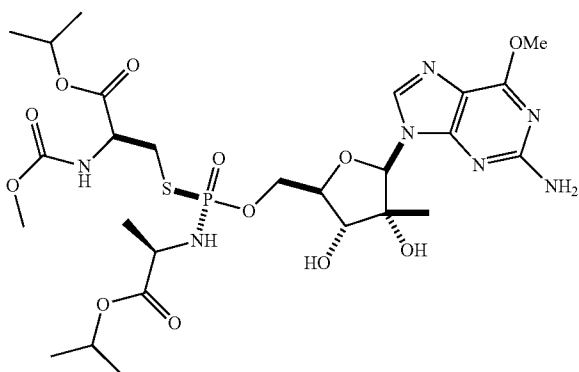


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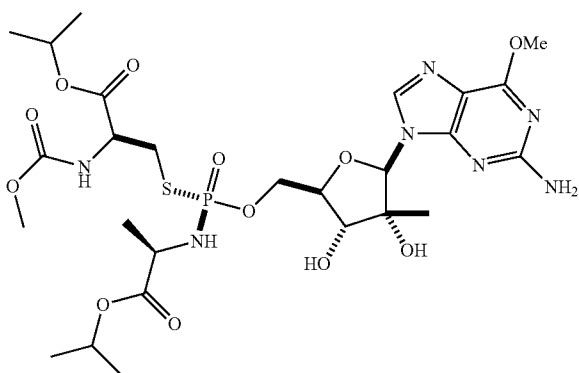
(802aii)



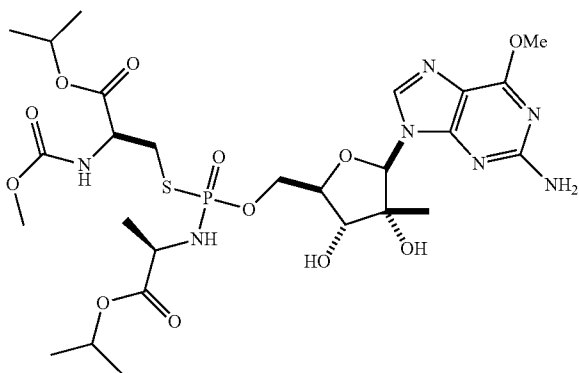
(802axi)



(802axii)

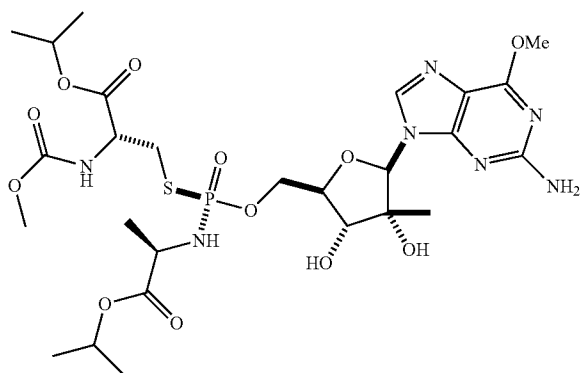


(802ax)

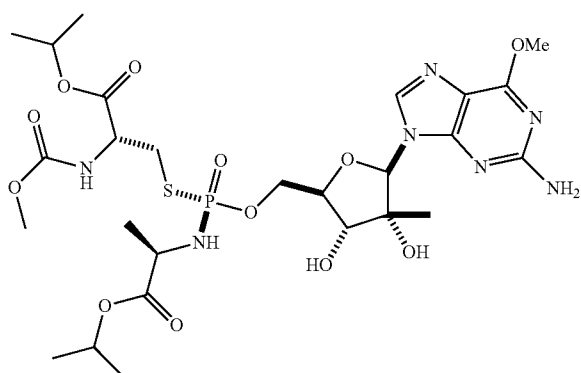


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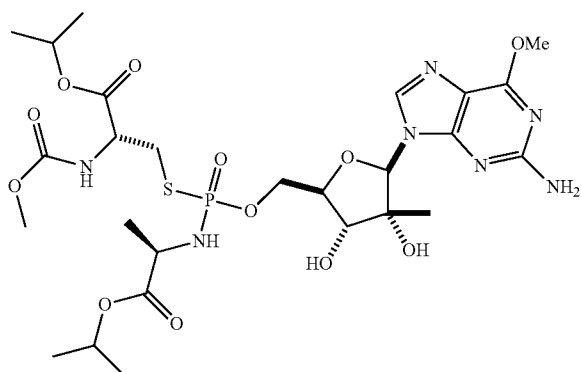
(802ayi)



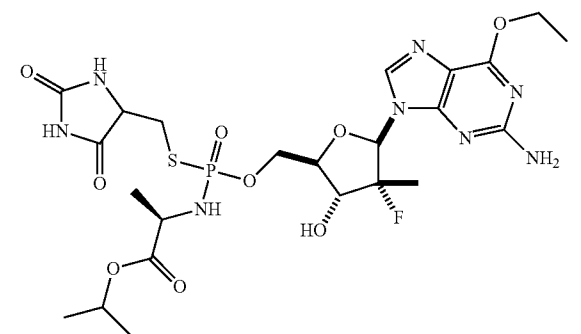
(802ayii)



(802ay)



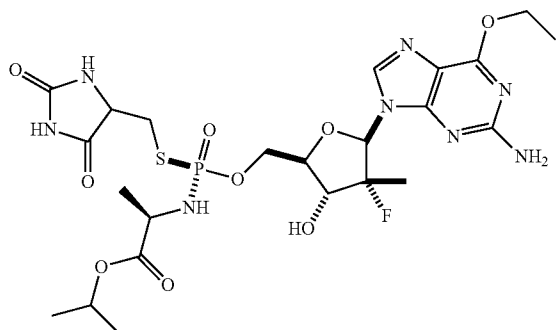
(803a)





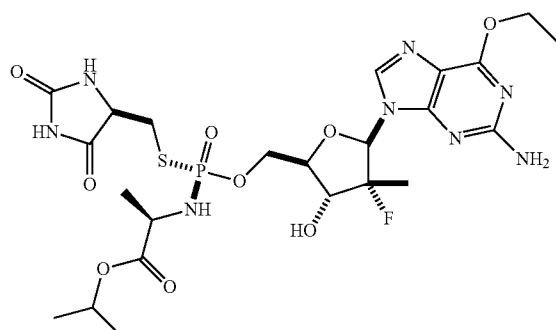
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(803ai)

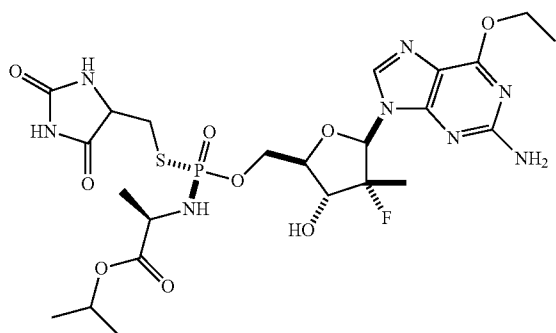


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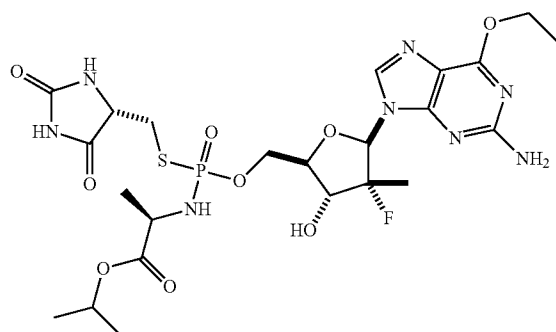
(803axii)



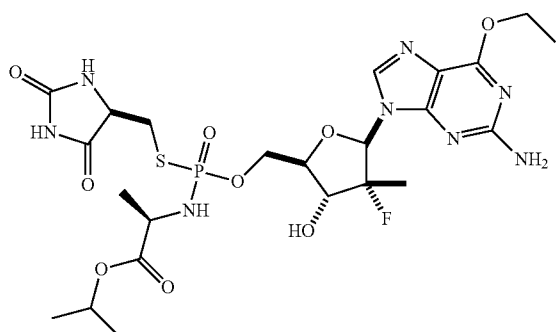
(803aii)



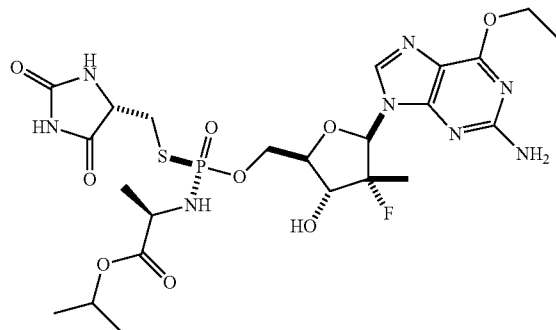
(803ay)



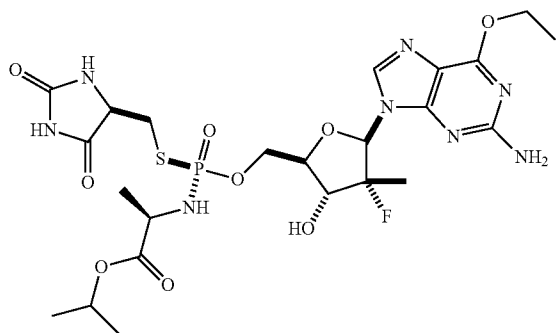
(803ax)



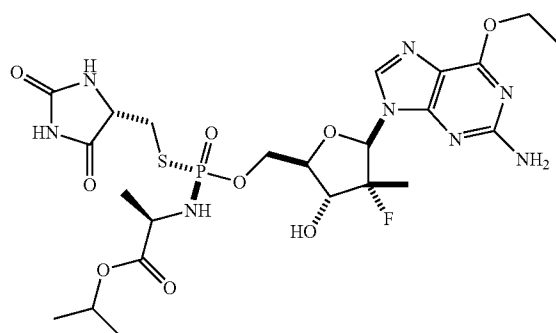
(803ayi)



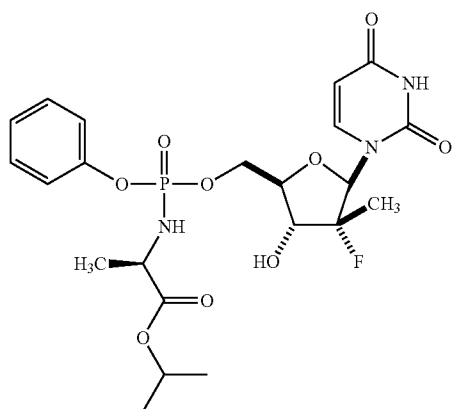
(803axi)



(803ayii)

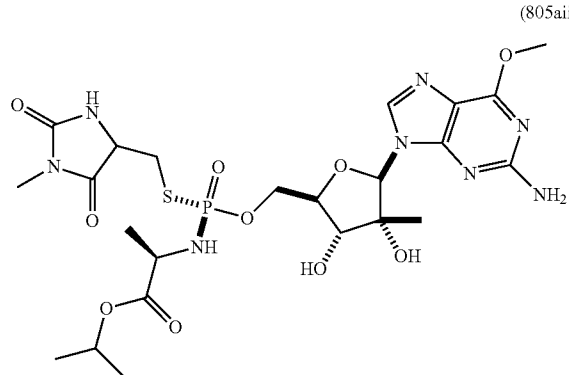


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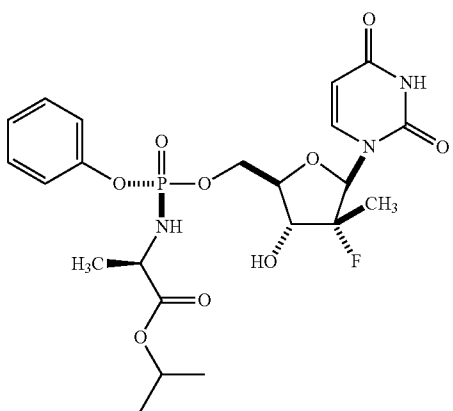


(804a)

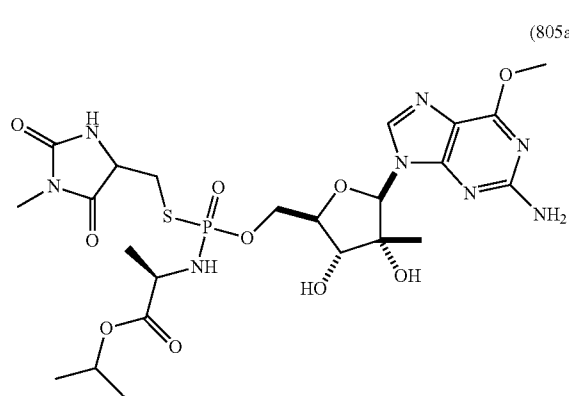
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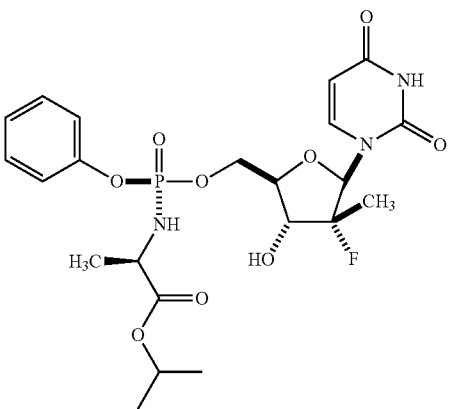
(805aii)



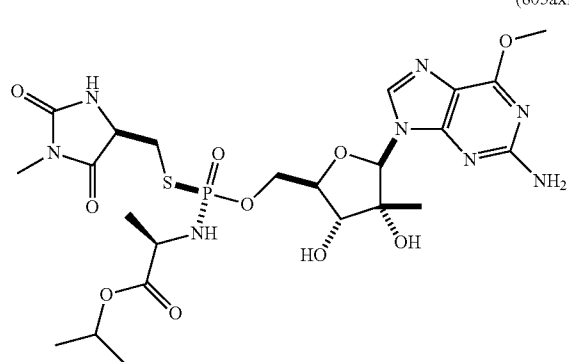
(804ai)



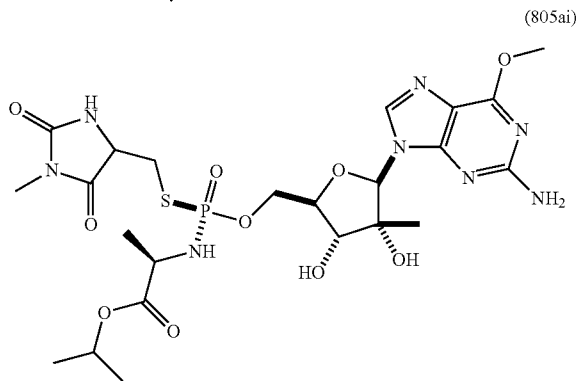
(805a)



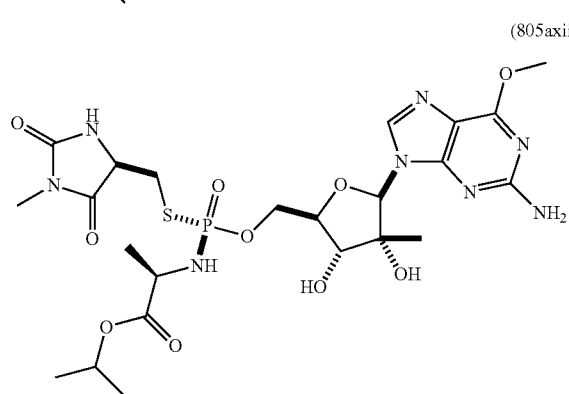
(804aii)



(805axi)

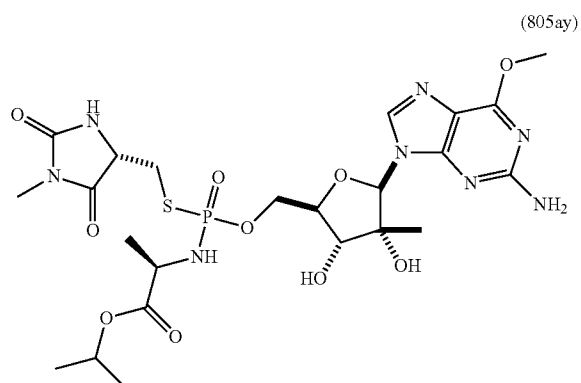
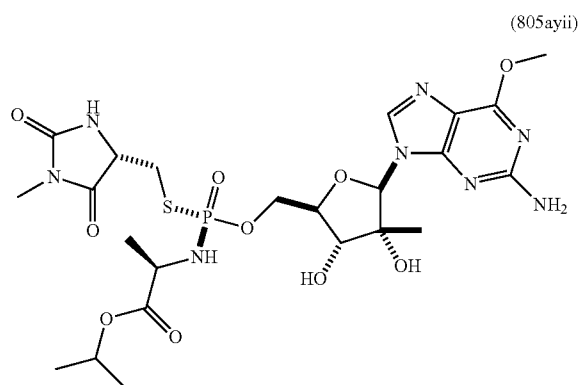
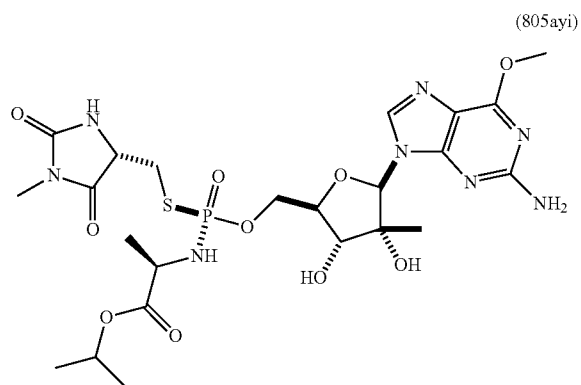
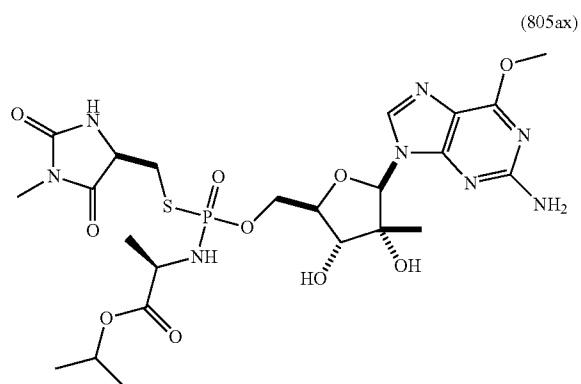


(805ai)

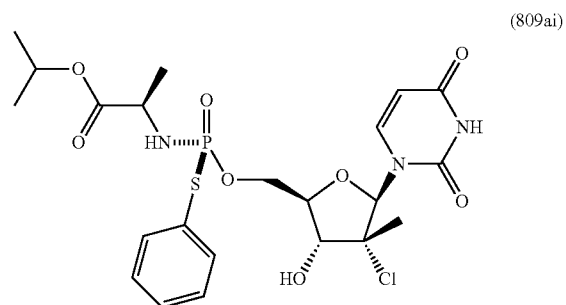
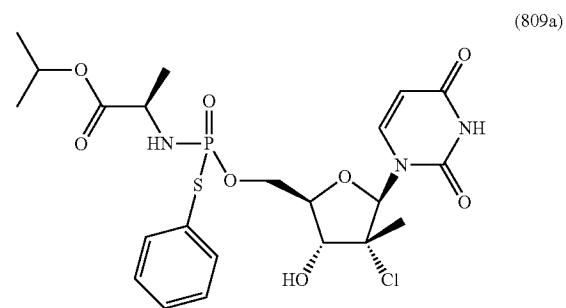
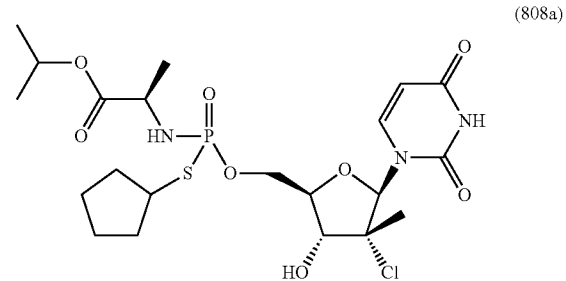
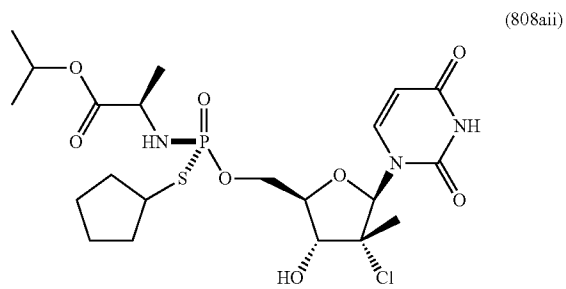
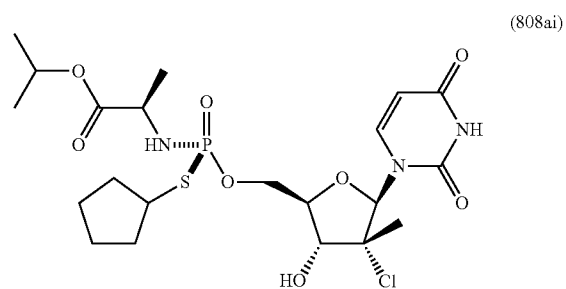


(805axii)

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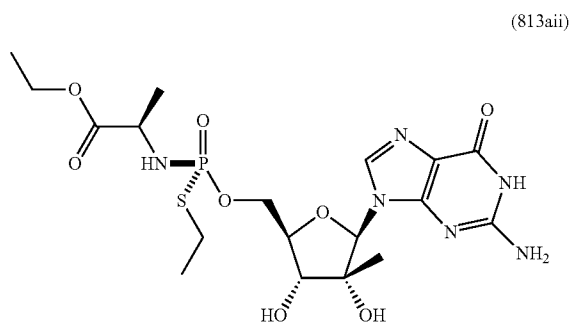


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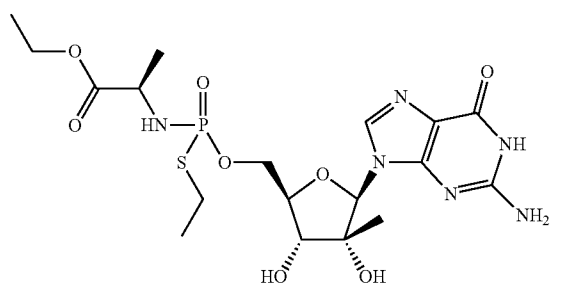




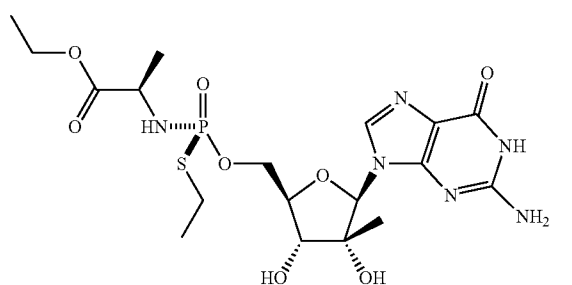
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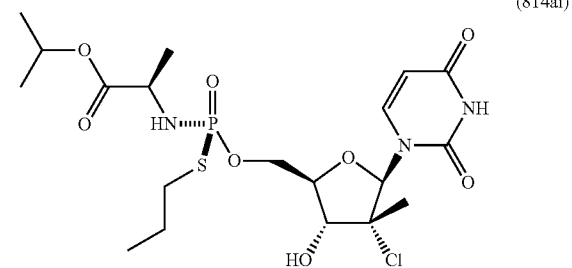
(813a)



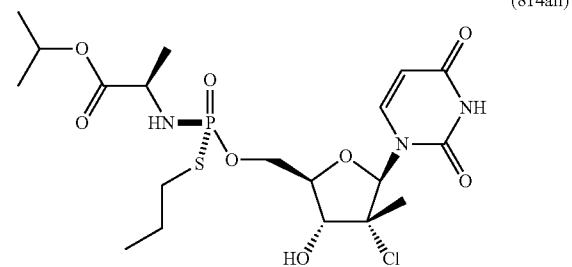
(813ai)



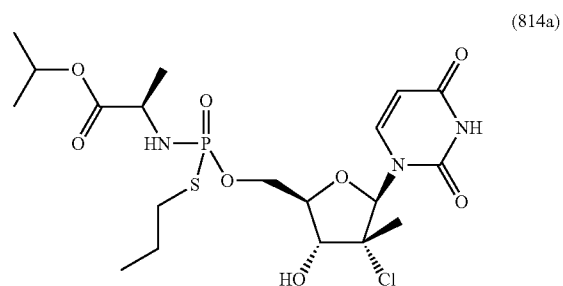
(814ai)



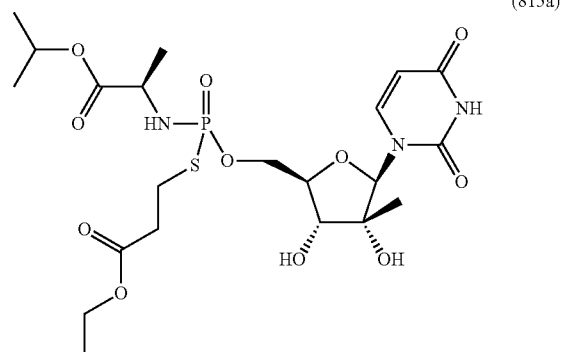
(814aii)



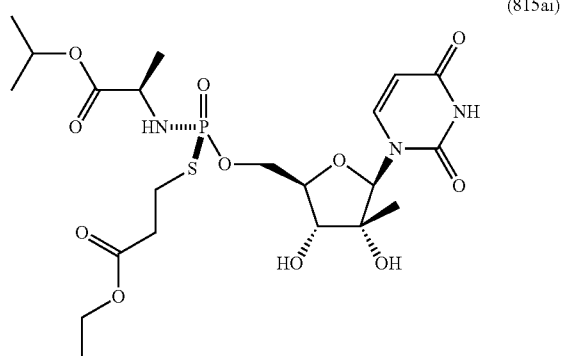
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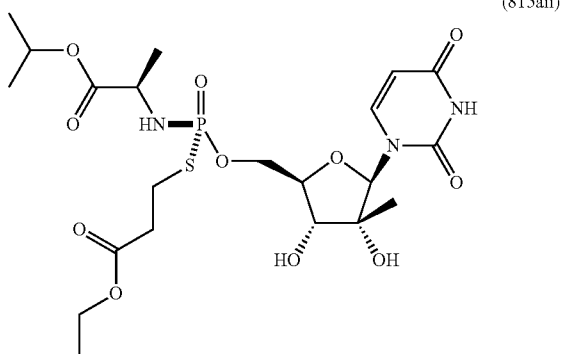
(815a)



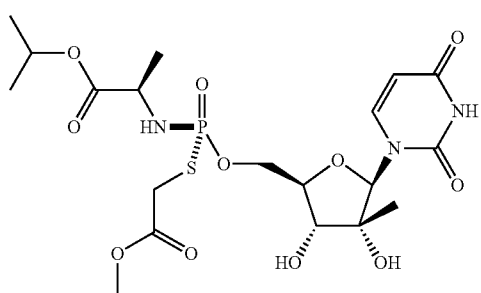
(815ai)



(815aii)

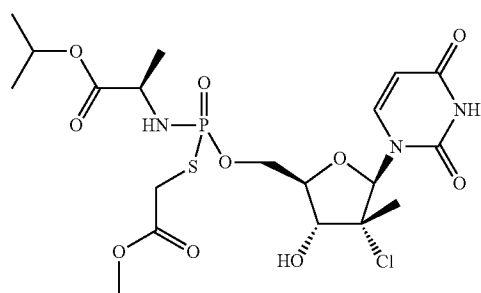


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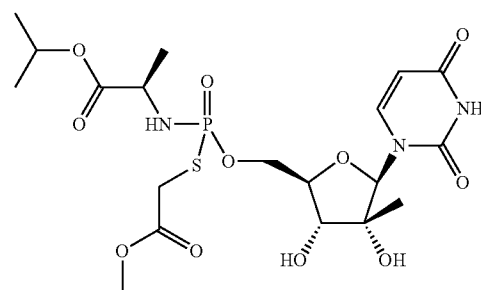


(816aii)

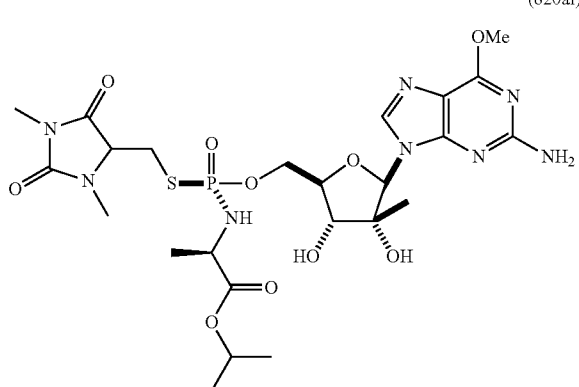
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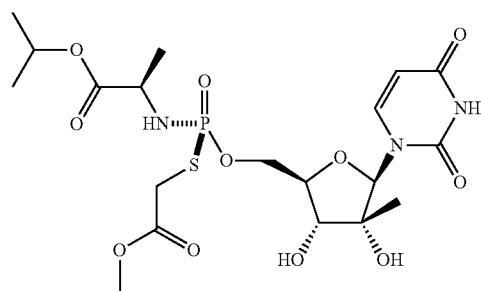
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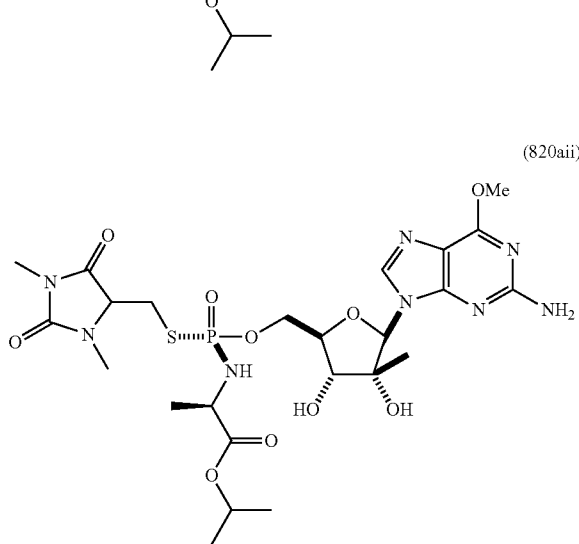
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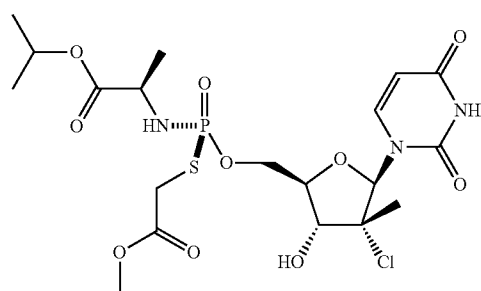
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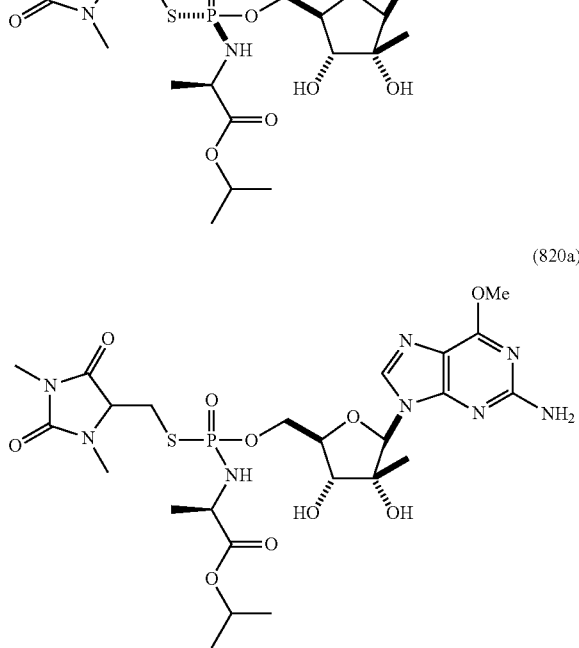
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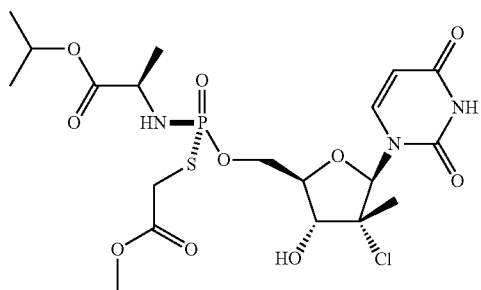
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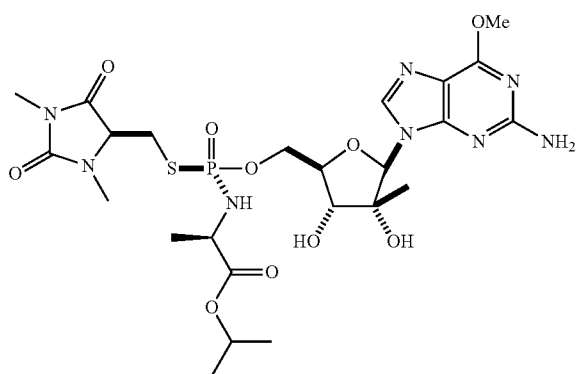
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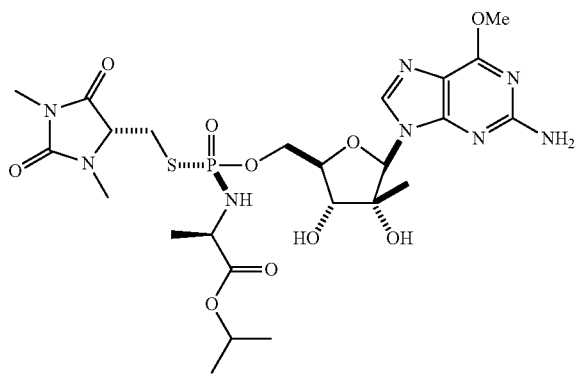
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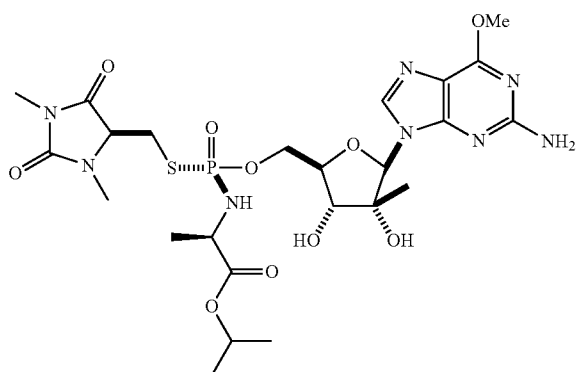


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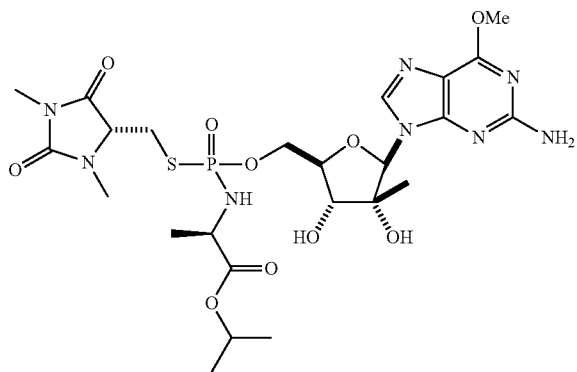
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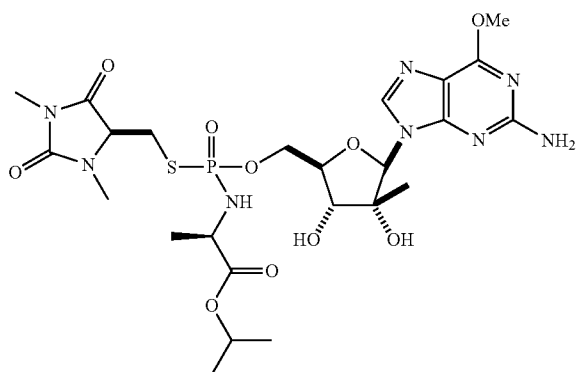
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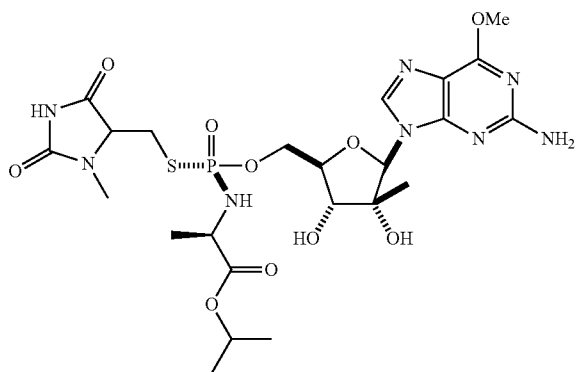
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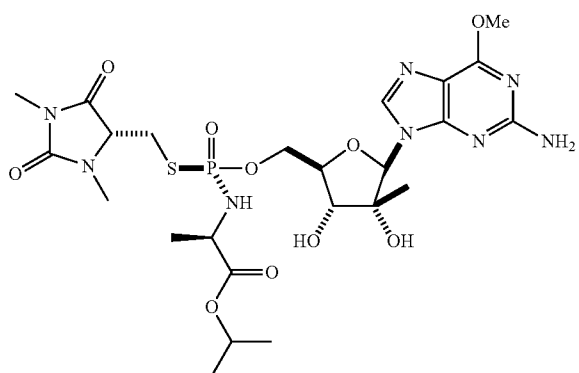
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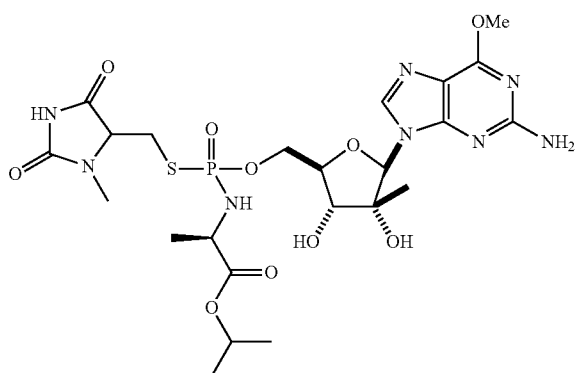
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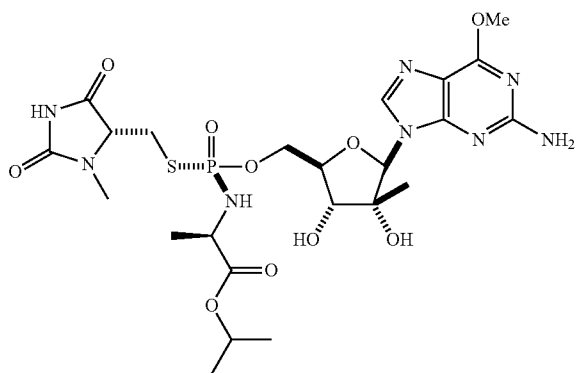


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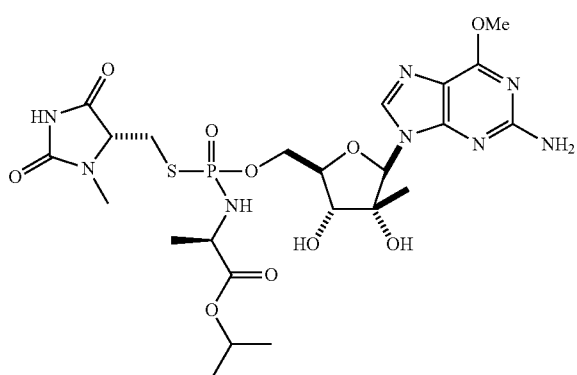


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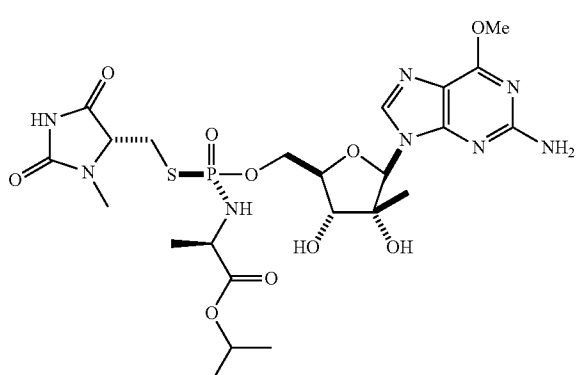
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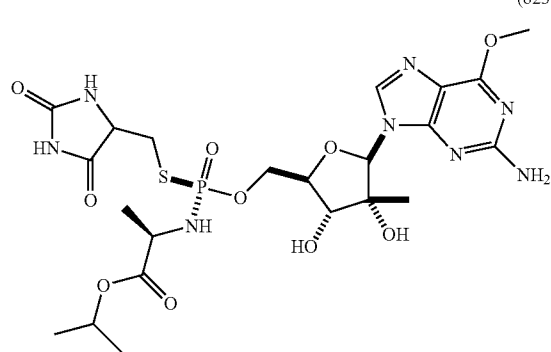
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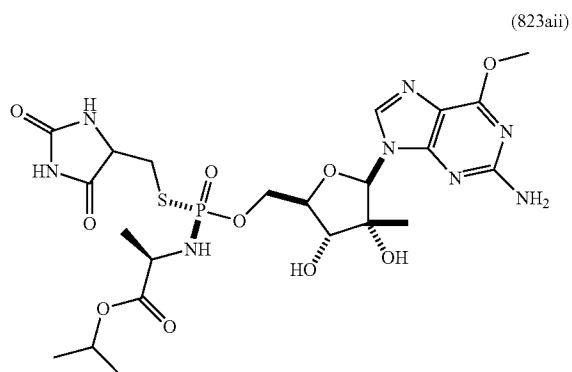


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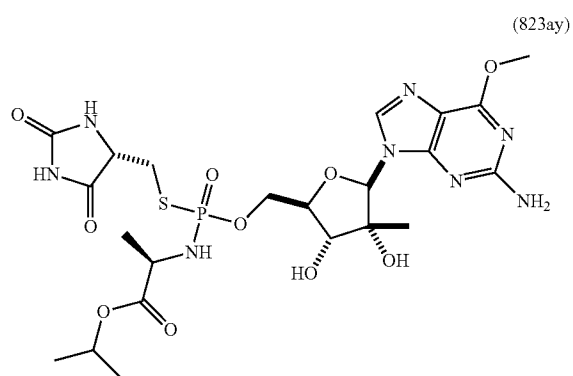
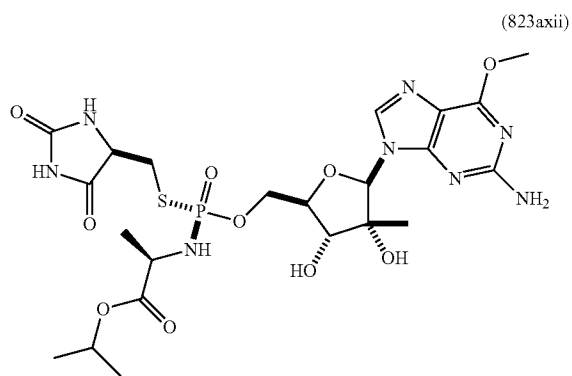
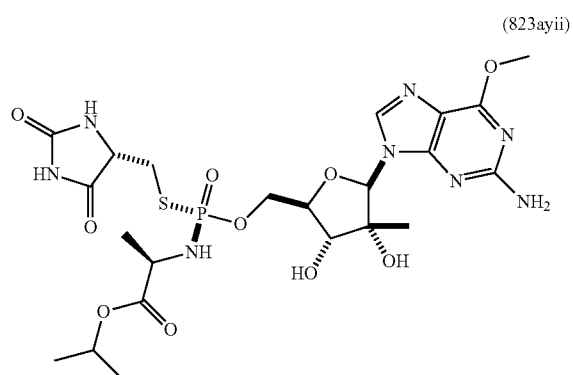
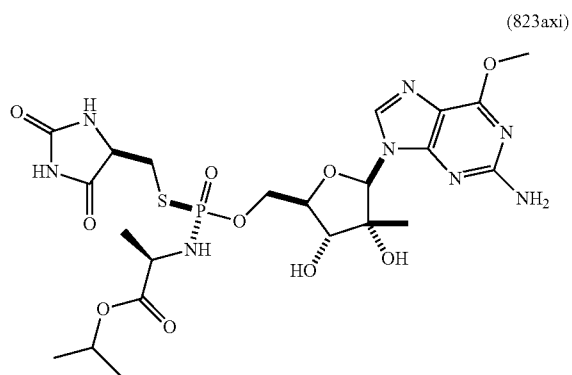
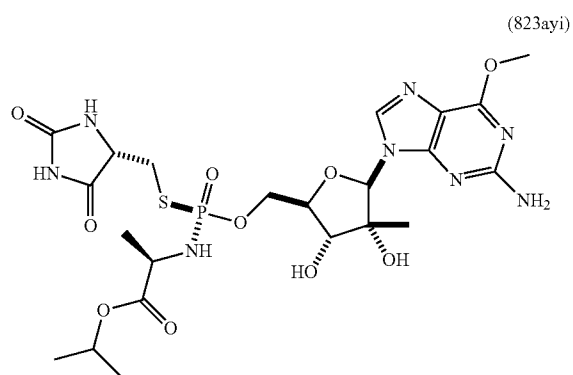
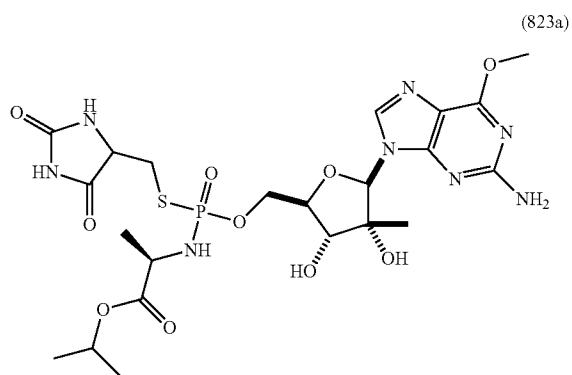
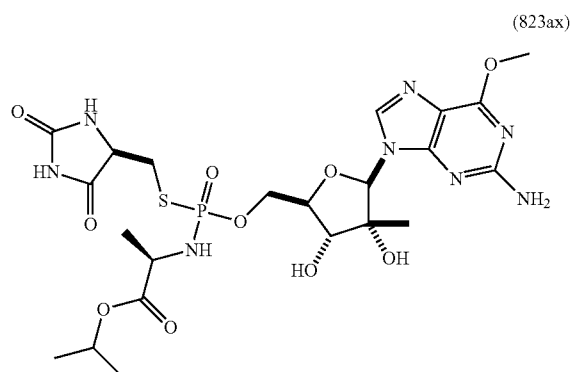


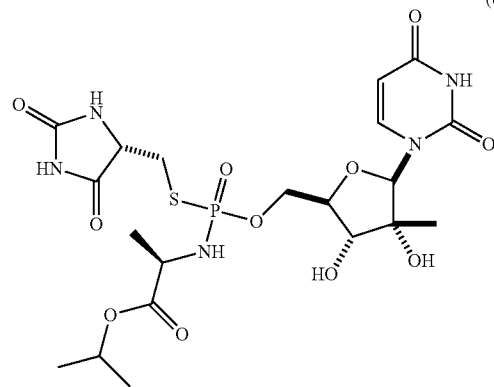
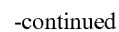


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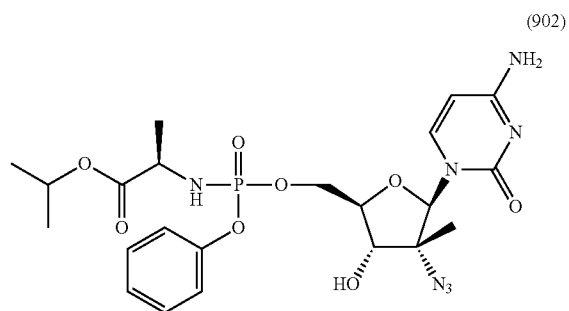


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(902a)

Chemical structure of compound 902a, showing a pyrimidine ring substituted with an amino group (NH<sub>2</sub>) and a carbonyl group (C=O). The pyrimidine is linked to a ribose sugar via a phosphate group. The ribose has a hydroxyl group (OH) and an azide group (N<sub>3</sub>). The phosphate group connects the ribose to a chiral center (C\*) which is also bonded to a benzene ring and an amide group. The amide group is further linked to an isopropoxy group (OCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>).

(901)

CC(C)OC(=O)[C@H](C)NP(=O)(Oc1ccccc1)O[C@H]2C[C@@H](O)[C@H](C#N)[C@H]2Oc3cc(N)c(=O)nc3=O

(902b)

(901a)

Chemical structure of compound (901a) is shown, which is a pyridine derivative. The structure includes a pyridine ring substituted with an amino group (NH<sub>2</sub>) and a carbonyl group (C=O). The pyridine ring is connected to a five-membered ring containing a hydroxyl group (OH) and a cyano group (CN). This five-membered ring is linked via a phosphate group to a chiral center (marked with a wedge bond) which is part of a carbamate group. The carbamate group is further connected to an isopropoxy group (O-CH(CH<sub>3</sub>)<sub>2</sub>). A benzene ring is also attached to the phosphate group.

(903)

Chemical structure of a nucleotide derivative, showing a phosphate group linked to a ribose sugar, which is further linked to a pyrimidine base (labeled with NH<sub>2</sub> and O). The structure includes a chiral center marked with a wedge bond and a hydrogen atom marked with a dash bond.

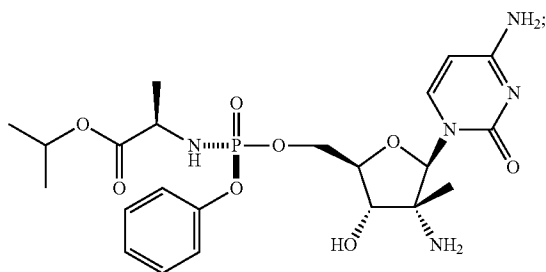
(901b)

(903a)

CC(C)OC(=O)[C@H](C)NCP(=O)(Oc1ccccc1)OP[C@@H]2C[C@H](O)[C@H](N)[C@H]2N

-continued

(903b)



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof.

[0129] In an embodiment, provided herein are compounds comprising a D-amino acid, or ester thereof, linked to a drug. In certain embodiments, the drug is a drug for treating a liver disease or condition. In certain embodiments, the liver disease or condition is hepatitis, fatty liver disease, cirrhosis, liver cancer, biliary cirrhosis, sclerosing cholangitis, Budd-Chiari syndrome, hemochromatosis, Wilson's disease, Gilbert's syndrome, biliary atresia, alpha-1 antitrypsin deficiency, alagille syndrome, or progressive familial intrahepatic cholestasis. In certain embodiments, the drug is a drug for treating hepatitis C. In certain embodiments, the drug is an interferon, a nucleotide analogue, a polymerase inhibitor, an NS3 protease inhibitor, an NS5A inhibitor, an entry inhibitor, a non-nucleoside polymerase inhibitor, a cyclosporine immune inhibitor, an NS4A antagonist, an NS4B-RNA binding inhibitor, a locked nucleic acid mRNA inhibitor, or a cyclophilin inhibitor.

[0130] In some embodiments, provided herein are:

[0131] (a) compounds as described herein and pharmaceutically acceptable salts and compositions thereof;

[0132] (b) compounds as described herein and pharmaceutically acceptable salts and compositions thereof for use in the treatment and/or prophylaxis of a liver disorder including Flaviviridae infection, especially in individuals diagnosed as having a Flaviviridae infection or being at risk of becoming infected by hepatitis C;

[0133] (c) processes for the preparation of compounds as described herein as described in more detail elsewhere herein;

[0134] (d) pharmaceutical formulations comprising a compound as described herein, or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent;

[0135] (e) pharmaceutical formulations comprising a compound as described herein or a pharmaceutically acceptable salt thereof together with one or more other effective anti-HCV agents, optionally in a pharmaceutically acceptable carrier or diluent;

[0136] (f) a method for the treatment and/or prophylaxis of a host infected with Flaviviridae that includes the administration of an effective amount of a compound as described herein its pharmaceutically acceptable salt or composition; or

[0137] (g) a method for the treatment and/or prophylaxis of a host infected with Flaviviridae that includes the administration of an effective amount of a compounds as described herein, its pharmaceutically acceptable salt or

composition in combination and/or alternation with one or more effective anti-HCV agent.

#### Optically Active Compounds

[0138] It is appreciated that compounds provided herein have several chiral centers and may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that any racemic, optically-active, diastereomeric, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound provided herein, which possess the useful properties described herein is within the scope of the invention. It being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

[0139] In particular, since the 1' and 4' carbons of a nucleoside are chiral, their non-hydrogen substituents (the base and the CHOR groups, respectively) can be either cis (on the same side) or trans (on opposite sides) with respect to the sugar ring system. The four optical isomers therefore are represented by the following configurations (when orienting the sugar moiety in a horizontal plane such that the oxygen atom is in the back): cis (with both groups "up", which corresponds to the configuration of naturally occurring B-D nucleosides), cis (with both groups "down", which is a non-naturally occurring B-L configuration), trans (with the C2' substituent "up" and the C4' substituent "down"), and trans (with the C2' substituent "down" and the C4' substituent "up"). The "D-nucleosides" are cis nucleosides in a natural configuration and the "L-nucleosides" are cis nucleosides in the non-naturally occurring configuration.

[0140] Likewise, most amino acids are chiral (designated as L or D, wherein the L enantiomer is the naturally occurring configuration) and can exist as separate enantiomers.

[0141] Examples of methods to obtain optically active materials are known in the art, and include at least the following.

[0142] i) physical separation of crystals—a technique whereby macroscopic crystals of the individual enantiomers are manually separated. This technique can be used if crystals of the separate enantiomers exist, i.e., the material is a conglomerate, and the crystals are visually distinct;

[0143] ii) simultaneous crystallization—a technique whereby the individual enantiomers are separately crystallized from a solution of the racemate, possible only if the latter is a conglomerate in the solid state;

[0144] iii) enzymatic resolutions—a technique whereby partial or complete separation of a racemate by virtue of differing rates of reaction for the enantiomers with an enzyme;

[0145] iv) enzymatic asymmetric synthesis—a synthetic technique whereby at least one step of the synthesis uses an enzymatic reaction to obtain an enantiomerically pure or enriched synthetic precursor of the desired enantiomer;

[0146] v) chemical asymmetric synthesis—a synthetic technique whereby the desired enantiomer is synthesized from an achiral precursor under conditions that produce asymmetry (i.e., chirality) in the product, which may be achieved using chiral catalysts or chiral auxiliaries;

- [0147] vi) diastereomer separations—a technique whereby a racemic compound is reacted with an enantiomerically pure reagent (the chiral auxiliary) that converts the individual enantiomers to diastereomers. The resulting diastereomers are then separated by chromatography or crystallization by virtue of their now more distinct structural differences and the chiral auxiliary later removed to obtain the desired enantiomer;
- [0148] vii) first- and second-order asymmetric transformations—a technique whereby diastereomers from the racemate equilibrate to yield a preponderance in solution of the diastereomer from the desired enantiomer or where preferential crystallization of the diastereomer from the desired enantiomer perturbs the equilibrium such that eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the diastereomer;
- [0149] viii) kinetic resolutions—this technique refers to the achievement of partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the enantiomers with a chiral, non-racemic reagent or catalyst under kinetic conditions;
- [0150] ix) enantiospecific synthesis from non-racemic precursors—a synthetic technique whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;
- [0151] x) chiral liquid chromatography—a technique whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase. The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;
- [0152] xi) chiral gas chromatography—a technique whereby the racemate is volatilized and enantiomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral adsorbent phase;
- [0153] xii) extraction with chiral solvents—a technique whereby the enantiomers are separated by virtue of preferential dissolution of one enantiomer into a particular chiral solvent;
- [0154] xiii) transport across chiral membranes—a technique whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane which allows only one enantiomer of the racemate to pass through.
- [0155] In some embodiments, compositions of 2'-chloro nucleoside analog compounds that are substantially free of a designated enantiomer of that compound. In certain embodiments, in the methods and compounds of this invention, the compounds are substantially free of enantiomers. In some embodiments, the composition includes that includes a compound that is at least 85, 90%, 95%, 98%, 99% to 100% by weight, of the compound, the remainder comprising other chemical species or enantiomers.
- [0156] Isotopically Enriched Compounds
- [0157] Also provided herein are isotopically enriched compounds, including but not limited to isotopically enriched 2'-chloro nucleoside analog compounds.
- [0158] Isotopic enrichment (for example, deuteration) of pharmaceuticals to improve pharmacokinetics ("PK"), pharmacodynamics ("PD"), and toxicity profiles, has been demonstrated previously with some classes of drugs. See, for example, Lijinsky et. al., *Food Cosmet. Toxicol.*, 20: 393 (1982); Lijinsky et. al., *J. Nat. Cancer Inst.*, 69: 1127 (1982); Mangold et. al., *Mutation Res.* 308: 33 (1994); Gordon et. al., *Drug Metab. Dispos.*, 15: 589 (1987); Zello et. al., *Metabolism*, 43: 487 (1994); Gately et. al., *J. Nucl. Med.*, 27: 388 (1986); Wade D, *Chem. Biol. Interact.* 117: 191 (1999).
- [0159] Isotopic enrichment of a drug can be used, for example, to (1) reduce or eliminate unwanted metabolites, (2) increase the half-life of the parent drug, (3) decrease the number of doses needed to achieve a desired effect, (4) decrease the amount of a dose necessary to achieve a desired effect, (5) increase the formation of active metabolites, if any are formed, and/or (6) decrease the production of deleterious metabolites in specific tissues and/or create a more effective drug and/or a safer drug for combination therapy, whether the combination therapy is intentional or not.
- [0160] Replacement of an atom for one of its isotopes often will result in a change in the reaction rate of a chemical reaction. This phenomenon is known as the Kinetic Isotope Effect ("KIE"). For example, if a C—H bond is broken during a rate-determining step in a chemical reaction (i.e. the step with the highest transition state energy), substitution of a deuterium for that hydrogen will cause a decrease in the reaction rate and the process will slow down. This phenomenon is known as the Deuterium Kinetic Isotope Effect ("DKIE"). (See, e.g., Foster et al., *Adv. Drug Res.*, vol. 14, pp. 1-36 (1985); Kushner et al., *Can. J. Physiol. Pharmacol.*, vol. 77, pp. 79-88 (1999)).
- [0161] The magnitude of the DKIE can be expressed as the ratio between the rates of a given reaction in which a C—H bond is broken, and the same reaction where deuterium is substituted for hydrogen. The DKIE can range from about 1 (no isotope effect) to very large numbers, such as 50 or more, meaning that the reaction can be fifty, or more, times slower when deuterium is substituted for hydrogen. High DKIE values may be due in part to a phenomenon known as tunneling, which is a consequence of the uncertainty principle. Tunneling is ascribed to the small mass of a hydrogen atom, and occurs because transition states involving a proton can sometimes form in the absence of the required activation energy. Because deuterium has more mass than hydrogen, it statistically has a much lower probability of undergoing this phenomenon.
- [0162] Tritium ("T") is a radioactive isotope of hydrogen, used in research, fusion reactors, neutron generators and radiopharmaceuticals. Tritium is a hydrogen atom that has 2 neutrons in the nucleus and has an atomic weight close to 3. It occurs naturally in the environment in very low concentrations, most commonly found as T<sub>2</sub>O. Tritium decays slowly (half-life=12.3 years) and emits a low energy beta particle that cannot penetrate the outer layer of human skin. Internal exposure is the main hazard associated with this isotope, yet it must be ingested in large amounts to pose a significant health risk. As compared with deuterium, a lesser amount of tritium must be consumed before it reaches a hazardous level. Substitution of tritium ("T") for hydrogen results in yet a

stronger bond than deuterium and gives numerically larger isotope effects. Similarly, substitution of isotopes for other elements, including, but not limited to,  $^{13}\text{C}$  or  $^{14}\text{C}$  for carbon,  $^{33}\text{S}$ ,  $^{34}\text{S}$ , or  $^{36}\text{S}$  for sulfur,  $^{15}\text{N}$  for nitrogen, and  $^{17}\text{O}$  or  $^{18}\text{O}$  for oxygen, may lead to a similar kinetic isotope effect.

**[0163]** For example, the DKIE was used to decrease the hepatotoxicity of halothane by presumably limiting the production of reactive species such as trifluoroacetyl chloride. However, this method may not be applicable to all drug classes. For example, deuterium incorporation can lead to metabolic switching. The concept of metabolic switching asserts that xenogens, when sequestered by Phase I enzymes, may bind transiently and re-bind in a variety of conformations prior to the chemical reaction (e.g., oxidation). This hypothesis is supported by the relatively vast size of binding pockets in many Phase I enzymes and the promiscuous nature of many metabolic reactions. Metabolic switching can potentially lead to different proportions of known metabolites as well as altogether new metabolites. This new metabolic profile may impart more or less toxicity.

**[0164]** The animal body expresses a variety of enzymes for the purpose of eliminating foreign substances, such as therapeutic agents, from its circulation system. Examples of such enzymes include the cytochrome P450 enzymes ("CYPs"), esterases, proteases, reductases, dehydrogenases, and monoamine oxidases, to react with and convert these foreign substances to more polar intermediates or metabolites for renal excretion. Some of the most common metabolic reactions of pharmaceutical compounds involve the oxidation of a carbon-hydrogen (C—H) bond to either a carbon-oxygen (C=O) or carbon-carbon (C=C) pi-bond. The resultant metabolites may be stable or unstable under physiological conditions, and can have substantially different pharmacokinetic, pharmacodynamic, and acute and long-term toxicity profiles relative to the parent compounds. For many drugs, such oxidations are rapid. These drugs therefore often require the administration of multiple or high daily doses.

**[0165]** Therefore, isotopic enrichment at certain positions of a compound provided herein will produce a detectable KIE that will affect the pharmacokinetic, pharmacologic, and/or toxicological profiles of a compound provided herein in comparison with a similar compound having a natural isotopic composition.

#### **[0166] Preparation of Compounds**

**[0167]** The compounds provided herein can be prepared, isolated or obtained by any method apparent to those of skill in the art. Compounds provided herein can be prepared according to the Exemplary Preparation Schemes in the Examples provided below. Reaction conditions, steps and reactants not provided in the Exemplary Preparation Schemes would be apparent to, and known by, those skilled in the art.

#### **[0168] Pharmaceutical Compositions and Methods of Administration**

**[0169]** Compounds provided herein can be formulated into pharmaceutical compositions using methods available in the art and those disclosed herein. Any of the compounds disclosed herein can be provided in the appropriate pharmaceutical composition and be administered by a suitable route of administration.

**[0170]** The methods provided herein encompass administering pharmaceutical compositions containing at least one compound as described herein, if appropriate in the salt form, either used alone or in the form of a combination with one or

more compatible and pharmaceutically acceptable carriers, such as diluents or adjuvants, or with another anti-HCV agent.

**[0171]** In certain embodiments, the second agent can be formulated or packaged with the compound provided herein. Of course, the second agent will only be formulated with the compound provided herein when, according to the judgment of those of skill in the art, such co-formulation should not interfere with the activity of either agent or the method of administration. In certain embodiments, the compound provided herein and the second agent are formulated separately. They can be packaged together, or packaged separately, for the convenience of the practitioner of skill in the art.

**[0172]** In clinical practice the active agents provided herein may be administered by any conventional route, in particular orally, parenterally, rectally or by inhalation (e.g. in the form of aerosols). In certain embodiments, the compound provided herein is administered orally.

**[0173]** Use may be made, as solid compositions for oral administration, of tablets, pills, hard gelatin capsules, powders or granules. In these compositions, the active product is mixed with one or more inert diluents or adjuvants, such as sucrose, lactose or starch.

**[0174]** These compositions can comprise substances other than diluents, for example a lubricant, such as magnesium stearate, or a coating intended for controlled release.

**[0175]** Use may be made, as liquid compositions for oral administration, of solutions which are pharmaceutically acceptable, suspensions, emulsions, syrups and elixirs containing inert diluents, such as water or liquid paraffin. These compositions can also comprise substances other than diluents, for example wetting, sweetening or flavoring products.

**[0176]** The compositions for parenteral administration can be emulsions or sterile solutions. Use may be made, as solvent or vehicle, of propylene glycol, a polyethylene glycol, vegetable oils, in particular olive oil, or injectable organic esters, for example ethyl oleate. These compositions can also contain adjuvants, in particular wetting, isotonicizing, emulsifying, dispersing and stabilizing agents. Sterilization can be carried out in several ways, for example using a bacteriological filter, by radiation or by heating. They can also be prepared in the form of sterile solid compositions which can be dissolved at the time of use in sterile water or any other injectable sterile medium.

**[0177]** The compositions for rectal administration are suppositories or rectal capsules which contain, in addition to the active principle, excipients such as cocoa butter, semi-synthetic glycerides or polyethylene glycols.

**[0178]** The compositions can also be aerosols. For use in the form of liquid aerosols, the compositions can be stable sterile solutions or solid compositions dissolved at the time of use in apyrogenic sterile water, in saline or any other pharmaceutically acceptable vehicle. For use in the form of dry aerosols intended to be directly inhaled, the active principle is finely divided and combined with a water-soluble solid diluent or vehicle, for example dextran, mannitol or lactose.

**[0179]** In certain embodiments, a composition provided herein is a pharmaceutical composition or a single unit dosage form. Pharmaceutical compositions and single unit dosage forms provided herein comprise a prophylactically or therapeutically effective amount of one or more prophylactic or therapeutic agents (e.g., a compound provided herein, or other prophylactic or therapeutic agent), and a typically one or more pharmaceutically acceptable carriers or excipients. In

a specific embodiment and in this context, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” includes a diluent, adjuvant (e.g., Freund’s adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water can be used as a carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E. W. Martin.

**[0180]** Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well-known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a subject and the specific active ingredients in the dosage form. The composition or single unit dosage form, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

**[0181]** Lactose free compositions provided herein can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmacopia (USP)SP(XXI)/NF (XVI). In general, lactose free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Exemplary lactose free dosage forms comprise an active ingredient, microcrystalline cellulose, pre gelatinized starch, and magnesium stearate.

**[0182]** Further encompassed herein are anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long term storage in order to determine characteristics such as shelf life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, New York, 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

**[0183]** Anhydrous pharmaceutical compositions and dosage forms provided herein can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine can

be anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

**[0184]** An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions can be packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

**[0185]** Further provided are pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

**[0186]** The pharmaceutical compositions and single unit dosage forms can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such compositions and dosage forms will contain a prophylactically or therapeutically effective amount of a prophylactic or therapeutic agent, in certain embodiments, in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration. In a certain embodiment, the pharmaceutical compositions or single unit dosage forms are sterile and in suitable form for administration to a subject, for example, an animal subject, such as a mammalian subject, for example, a human subject.

**[0187]** A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, intramuscular, subcutaneous, oral, buccal, sublingual, inhalation, intranasal, transdermal, topical, transmucosal, intra-tumoral, intra-synovial and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal or topical administration to human beings. In an embodiment, a pharmaceutical composition is formulated in accordance with routine procedures for subcutaneous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

**[0188]** Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a subject, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil in water emulsions, or a water in oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a subject; and sterile solids (e.g.,

crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a subject.

**[0189]** The composition, shape, and type of dosage forms provided herein will typically vary depending on their use. For example, a dosage form used in the initial treatment of viral infection may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the maintenance treatment of the same infection. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed herein will vary from one another will be readily apparent to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton Pa. (2000).

**[0190]** Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[0191]** Typical dosage forms comprise a compound provided herein, or a pharmaceutically acceptable salt, solvate or hydrate thereof lie within the range of from about 0.1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning or as divided doses throughout the day taken with food. Particular dosage forms can have about 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0, 100, 200, 250, 500 or 1000 mg of the active compound.

**[0192]** Oral Dosage Forms

**[0193]** Pharmaceutical compositions that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton Pa. (2000).

**[0194]** In certain embodiments, the oral dosage forms are solid and prepared under anhydrous conditions with anhydrous ingredients, as described in detail herein. However, the scope of the compositions provided herein extends beyond anhydrous, solid oral dosage forms. As such, further forms are described herein.

**[0195]** Typical oral dosage forms are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

**[0196]** Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or non-aqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

**[0197]** For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

**[0198]** Examples of excipients that can be used in oral dosage forms include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

**[0199]** Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

**[0200]** Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL PH 101, AVICEL PH 103 AVICEL RC 581, AVICEL PH 105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC 581. Suitable anhydrous or low moisture excipients or additives include AVICEL PH 103™ and Starch 1500 LM.

**[0201]** Disintegrants are used in the compositions to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

**[0202]** Disintegrants that can be used in pharmaceutical compositions and dosage forms include, but are not limited



to, agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, pre gelatinized starch, other starches, clays, other algin, other celluloses, gums, and mixtures thereof.

**[0203]** Lubricants that can be used in pharmaceutical compositions and dosage forms include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB O SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

**[0204]** Delayed Release Dosage Forms

**[0205]** Active ingredients such as the compounds provided herein can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,639,480; 5,733,566; 5,739,108; 5,891,474; 5,922,356; 5,972,891; 5,980,945; 5,993,855; 6,045,830; 6,087,324; 6,113,943; 6,197,350; 6,248,363; 6,264,970; 6,267,981; 6,376,461; 6,419,961; 6,589,548; 6,613,358; and 6,699,500; each of which is incorporated herein by reference in its entirety. Such dosage forms can be used to provide slow or controlled release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients provided herein. Thus encompassed herein are single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled release.

**[0206]** All controlled release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled release formulations include extended activity of the drug, reduced dosage frequency, and increased subject compliance. In addition, controlled release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

**[0207]** Most controlled release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradu-

ally and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

**[0208]** In certain embodiments, the drug may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In certain embodiments, a pump may be used (see, Sefton, *CRC Crit. Ref Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in a subject at an appropriate site determined by a practitioner of skill, i.e., thus requiring only a fraction of the systemic dose (see, e.g., Goodson, *Medical Applications of Controlled Release*, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)). The active ingredient can be dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethyleneterephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer, that is insoluble in body fluids. The active ingredient then diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active ingredient in such parenteral compositions is highly dependent on the specific nature thereof, as well as the needs of the subject.

**[0209]** Parenteral Dosage Forms

**[0210]** In certain embodiments, provided are parenteral dosage forms. Parenteral dosage forms can be administered to subjects by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses subjects' natural defenses against contaminants, parenteral dosage forms are typically, sterile or capable of being sterilized prior to administration to a subject. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

**[0211]** Suitable vehicles that can be used to provide parenteral dosage forms are well known to those skilled in the

art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

**[0212]** Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms.

**[0213]** Transdermal, Topical & Mucosal Dosage Forms

**[0214]** Also provided are transdermal, topical, and mucosal dosage forms. Transdermal, topical, and mucosal dosage forms include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences, 16<sup>th</sup>, 18<sup>th</sup> and 20<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980, 1990 & 2000); and Introduction to Pharmaceutical Dosage Forms, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

**[0215]** Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed herein are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane 1,3 diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are nontoxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., Remington's Pharmaceutical Sciences, 16<sup>th</sup>, 18<sup>th</sup> and 20<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980, 1990 & 2000).

**[0216]** Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients provided. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

**[0217]** The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as

stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery enhancing or penetration enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

**[0218]** Dosage and Unit Dosage Forms

**[0219]** In human therapeutics, the doctor will determine the posology which he considers most appropriate according to a preventive or curative treatment and according to the age, weight, stage of the infection and other factors specific to the subject to be treated. In certain embodiments, doses are from about 1 to about 1000 mg per day for an adult, or from about 5 to about 250 mg per day or from about 10 to 50 mg per day for an adult. In certain embodiments, doses are from about 5 to about 400 mg per day or 25 to 200 mg per day per adult. In certain embodiments, dose rates of from about 50 to about 500 mg per day are also contemplated.

**[0220]** In further aspects, provided are methods of treating or preventing an HCV infection in a subject by administering, to a subject in need thereof, an effective amount of a compound provided herein, or a pharmaceutically acceptable salt thereof. The amount of the compound or composition which will be effective in the prevention or treatment of a disorder or one or more symptoms thereof will vary with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The frequency and dosage will also vary according to factors specific for each subject depending on the specific therapy (e.g., therapeutic or prophylactic agents) administered, the severity of the disorder, disease, or condition, the route of administration, as well as age, body, weight, response, and the past medical history of the subject. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

**[0221]** In certain embodiments, exemplary doses of a composition include milligram or microgram amounts of the active compound per kilogram of subject or sample weight (e.g., about 10 micrograms per kilogram to about 50 milligrams per kilogram, about 100 micrograms per kilogram to about 25 milligrams per kilogram, or about 100 microgram per kilogram to about 10 milligrams per kilogram). For compositions provided herein, in certain embodiments, the dosage administered to a subject is 0.140 mg/kg to 3 mg/kg of the subject's body weight, based on weight of the active compound. In certain embodiments, the dosage administered to a subject is between 0.20 mg/kg and 2.00 mg/kg, or between 0.30 mg/kg and 1.50 mg/kg of the subject's body weight.

**[0222]** In certain embodiments, the recommended daily dose range of a composition provided herein for the conditions described herein lie within the range of from about 0.1 mg to about 1000 mg per day, given as a single once-a-day dose or as divided doses throughout a day. In certain embodiments, the daily dose is administered twice daily in equally divided doses. In certain embodiments, a daily dose range should be from about 10 mg to about 200 mg per day, in other embodiments, between about 10 mg and about 150 mg per day, in further embodiments, between about 25 and about 100 mg per day. It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Fur-

thermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with subject response.

**[0223]** Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to prevent, manage, treat or ameliorate such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with the composition provided herein are also encompassed by the herein described dosage amounts and dose frequency schedules. Further, when a subject is administered multiple dosages of a composition provided herein, not all of the dosages need be the same. For example, the dosage administered to the subject may be increased to improve the prophylactic or therapeutic effect of the composition or it may be decreased to reduce one or more side effects that a particular subject is experiencing.

**[0224]** In certain embodiment, the dosage of the composition provided herein, based on weight of the active compound, administered to prevent, treat, manage, or ameliorate a disorder, or one or more symptoms thereof in a subject is 0.1 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 10 mg/kg, or 15 mg/kg or more of a subject's body weight. In another embodiment, the dosage of the composition or a composition provided herein administered to prevent, treat, manage, or ameliorate a disorder, or one or more symptoms thereof in a subject is a unit dose of 0.1 mg to 200 mg, 0.1 mg to 100 mg, 0.1 mg to 50 mg, 0.1 mg to 25 mg, 0.1 mg to 20 mg, 0.1 mg to 15 mg, 0.1 mg to 10 mg, 0.1 mg to 7.5 mg, 0.1 mg to 5 mg, 0.1 to 2.5 mg, 0.25 mg to 20 mg, 0.25 to 15 mg, 0.25 to 12 mg, 0.25 to 10 mg, 0.25 mg to 7.5 mg, 0.25 mg to 5 mg, 0.5 mg to 2.5 mg, 1 mg to 20 mg, 1 mg to 15 mg, 1 mg to 12 mg, 1 mg to 10 mg, 1 mg to 7.5 mg, 1 mg to 5 mg, or 1 mg to 2.5 mg.

**[0225]** In certain embodiments, treatment or prevention can be initiated with one or more loading doses of a compound or composition provided herein followed by one or more maintenance doses. In such embodiments, the loading dose can be, for instance, about 60 to about 400 mg per day, or about 100 to about 200 mg per day for one day to five weeks. The loading dose can be followed by one or more maintenance doses. In certain embodiments, each maintenance dose is, independently, about from about 10 mg to about 200 mg per day, between about 25 mg and about 150 mg per day, or between about 25 and about 80 mg per day. Maintenance doses can be administered daily and can be administered as single doses, or as divided doses.

**[0226]** In certain embodiments, a dose of a compound or composition provided herein can be administered to achieve a steady-state concentration of the active ingredient in blood or serum of the subject. The steady-state concentration can be determined by measurement according to techniques available to those of skill or can be based on the physical characteristics of the subject such as height, weight and age. In certain embodiments, a sufficient amount of a compound or composition provided herein is administered to achieve a steady-state concentration in blood or serum of the subject of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL. In some embodiments, loading doses can be administered to achieve steady-state blood or serum concentrations of about 1200 to about 8000 ng/mL, or about 2000 to about 4000 ng/mL for one to five days. In certain embodiments, maintenance doses can be administered to achieve a steady-state concentration in

blood or serum of the subject of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL.

**[0227]** In certain embodiments, administration of the same composition may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months. In other embodiments, administration of the same prophylactic or therapeutic agent may be repeated and the administration may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

**[0228]** In certain aspects, provided herein are unit dosages comprising a compound, or a pharmaceutically acceptable salt thereof, in a form suitable for administration. Such forms are described in detail herein. In certain embodiments, the unit dosage comprises 1 to 1000 mg, 5 to 250 mg or 10 to 50 mg active ingredient. In particular embodiments, the unit dosages comprise about 1, 5, 10, 25, 50, 100, 125, 250, 500 or 1000 mg active ingredient. Such unit dosages can be prepared according to techniques familiar to those of skill in the art.

**[0229]** The dosages of the second agents are to be used in the combination therapies provided herein. In certain embodiments, dosages lower than those which have been or are currently being used to prevent or treat HCV infection are used in the combination therapies provided herein. The recommended dosages of second agents can be obtained from the knowledge of those of skill. For those second agents that are approved for clinical use, recommended dosages are described in, for example, Hardman et al., eds., 1996, Goodman & Gilman's The Pharmacological Basis Of Basis Of Therapeutics 9<sup>th</sup> Ed, McGraw-Hill, New York; Physician's Desk Reference (PDR) 57<sup>th</sup> Ed., 2003, Medical Economics Co., Inc., Montvale, N.J., which are incorporated herein by reference in its entirety.

**[0230]** In various embodiments, the therapies (e.g., a compound provided herein and the second agent) are administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours apart. In various embodiments, the therapies are administered no more than 24 hours apart or no more than 48 hours apart. In certain embodiments, two or more therapies are administered within the same patient visit. In other embodiments, the compound provided herein and the second agent are administered concurrently.

**[0231]** In other embodiments, the compound provided herein and the second agent are administered at about 2 to 4 days apart, at about 4 to 6 days apart, at about 1 week part, at about 1 to 2 weeks apart, or more than 2 weeks apart.

**[0232]** In certain embodiments, administration of the same agent may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

In other embodiments, administration of the same agent may be repeated and the administration may be separated by at least at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

[0233] In certain embodiments, a compound provided herein and a second agent are administered to a patient, for example, a mammal, such as a human, in a sequence and within a time interval such that the compound provided herein can act together with the other agent to provide an increased benefit than if they were administered otherwise. For example, the second active agent can be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. In certain embodiments, the compound provided herein and the second active agent exert their effect at times which overlap. Each second active agent can be administered separately, in any appropriate form and by any suitable route. In other embodiments, the compound provided herein is administered before, concurrently or after administration of the second active agent.

[0234] In certain embodiments, the compound provided herein and the second agent are cyclically administered to a patient. Cycling therapy involves the administration of a first agent (e.g., a first prophylactic or therapeutic agents) for a period of time, followed by the administration of a second agent and/or third agent (e.g., a second and/or third prophylactic or therapeutic agents) for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improve the efficacy of the treatment.

[0235] In certain embodiments, the compound provided herein and the second active agent are administered in a cycle of less than about 3 weeks, about once every two weeks, about once every 10 days or about once every week. One cycle can comprise the administration of a compound provided herein and the second agent by infusion over about 90 minutes every cycle, about 1 hour every cycle, about 45 minutes every cycle. Each cycle can comprise at least 1 week of rest, at least 2 weeks of rest, at least 3 weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.

[0236] In other embodiments, courses of treatment are administered concurrently to a patient, i.e., individual doses of the second agent are administered separately yet within a time interval such that the compound provided herein can work together with the second active agent. For example, one component can be administered once per week in combination with the other components that can be administered once every two weeks or once every three weeks. In other words, the dosing regimens are carried out concurrently even if the therapeutics are not administered simultaneously or during the same day.

[0237] The second agent can act additively or synergistically with the compound provided herein. In certain embodiments, the compound provided herein is administered concurrently with one or more second agents in the same pharmaceutical composition. In another embodiment, a compound provided herein is administered concurrently with one or more second agents in separate pharmaceutical compositions. In still another embodiment, a compound provided

herein is administered prior to or subsequent to administration of a second agent. Also contemplated are administration of a compound provided herein and a second agent by the same or different routes of administration, e.g., oral and parenteral. In certain embodiments, when the compound provided herein is administered concurrently with a second agent that potentially produces adverse side effects including, but not limited to, toxicity, the second active agent can advantageously be administered at a dose that falls below the threshold that the adverse side effect is elicited.

#### [0238] Kits

[0239] Also provided are kits for use in methods of treatment of a liver disorder such as HCV infections. The kits can include a compound or composition provided herein, a second agent or composition, and instructions providing information to a health care provider regarding usage for treating the disorder. Instructions may be provided in printed form or in the form of an electronic medium such as a floppy disc, CD, or DVD, or in the form of a website address where such instructions may be obtained. A unit dose of a compound or composition provided herein, or a second agent or composition, can include a dosage such that when administered to a subject, a therapeutically or prophylactically effective plasma level of the compound or composition can be maintained in the subject for at least 1 days. In some embodiments, a compound or composition can be included as a sterile aqueous pharmaceutical composition or dry powder (e.g., lyophilized) composition.

[0240] In some embodiments, suitable packaging is provided. As used herein, "packaging" includes a solid matrix or material customarily used in a system and capable of holding within fixed limits a compound provided herein and/or a second agent suitable for administration to a subject. Such materials include glass and plastic (e.g., polyethylene, polypropylene, and polycarbonate) bottles, vials, paper, plastic, and plastic-foil laminated envelopes and the like. If e-beam sterilization techniques are employed, the packaging should have sufficiently low density to permit sterilization of the contents.

#### [0241] Methods of Use

[0242] In certain embodiments, provided herein are methods for the treatment and/or prophylaxis of a host infected with Flaviviridae that includes the administration of an effective amount of a compounds provided herein, or a pharmaceutically acceptable salt thereof. In certain embodiments, provided herein are methods for treating an HCV infection in a subject. In certain embodiments, the methods encompass the step of administering to the subject in need thereof an amount of a compound effective for the treatment or prevention of an HCV infection in combination with a second agent effective for the treatment or prevention of the infection. The compound can be any compound as described herein, and the second agent can be any second agent described in the art or herein. In certain embodiments, the compound is in the form of a pharmaceutical composition or dosage form, as described elsewhere herein.

[0243] Flaviviridae that can be treated are discussed generally in *Fields Virology*, Editors: Fields, B. N., Knipe, D. M., and Howley, P. M., Lippincott-Raven Publishers, Philadelphia, Pa., Chapter 31, 1996. In a particular embodiment of the invention, the Flaviviridae is HCV. In an alternate embodiment of the invention, the Flaviviridae is a flavivirus or pestivirus. Specific flaviviruses include, without limitation: Absettarov, Alfuy, Apoi, Aroa, Bagaza, Banzi, Bouboui, Bus-

suquara, Cacipacore, Carey Island, Dakar bat, Dengue 1, Dengue 2, Dengue 3, Dengue 4, Edge Hill, Entebbe bat, Gadgets Gully, Hanzalova, Hypr, Ilheus, Israel turkey meningoencephalitis, Japanese encephalitis, Jugra, Jutiapa, Kadam, Karshi, Kedougou, Kokobera, Koutango, Kumlinge, Kunjin, Kyasanur Forest disease, Langat, Louping ill, Meaban, Modoc, Montana myotis leukoencephalitis, Murray valley encephalitis, Naranjal, Negishi, Ntaya, Omsk hemorrhagic fever, Phnom-Penh bat, Powassan, Rio Bravo, Rocio, Royal Farm, Russian spring-summer encephalitis, Saboya, St. Louis encephalitis, Sal Vieja, San Perlita, Saumarez Reef, Sepik, Sokuluk, Spondweni, Stratford, Tembusu, Tyulenyi, Uganda S, Usutu, Wesselsbron, West Nile, Yaounde, Yellow fever, and Zika.

**[0244]** Pestiviruses that can be treated are discussed generally in *Fields Virology*, Editors: Fields, B. N., Knipe, D. M., and Howley, P. M., Lippincott-Raven Publishers, Philadelphia, Pa., Chapter 33, 1996. Specific pestiviruses include, without limitation: bovine viral diarrhea virus ("BVDV"), classical swine fever virus ("CSFV," also called hog cholera virus), and border disease virus ("BDV").

**[0245]** In certain embodiments, the subject can be any subject infected with, or at risk for infection with, HCV. Infection or risk for infection can be determined according to any technique deemed suitable by the practitioner of skill in the art. In certain embodiments, subjects are humans infected with HCV.

**[0246]** In certain embodiments, the subject has never received therapy or prophylaxis for an HCV infection. In further embodiments, the subject has previously received therapy or prophylaxis for an HCV infection. For instance, in certain embodiments, the subject has not responded to an HCV therapy. For example, under current interferon therapy, up to 50% or more HCV subjects do not respond to therapy. In certain embodiments, the subject can be a subject that received therapy but continued to suffer from viral infection or one or more symptoms thereof. In certain embodiments, the subject can be a subject that received therapy but failed to achieve a sustained virologic response. In certain embodiments, the subject has received therapy for an HCV infection but has failed to show, for example, a  $2 \log_{10}$  decline in HCV RNA levels after 12 weeks of therapy. It is believed that subjects who have not shown more than  $2 \log_{10}$  reduction in serum HCV RNA after 12 weeks of therapy have a 97-100% chance of not responding.

**[0247]** In certain embodiments, the subject is a subject that discontinued an HCV therapy because of one or more adverse events associated with the therapy. In certain embodiments, the subject is a subject where current therapy is not indicated. For instance, certain therapies for HCV are associated with neuropsychiatric events. Interferon (IFN)-alpha plus ribavirin is associated with a high rate of depression. Depressive symptoms have been linked to a worse outcome in a number of medical disorders. Life-threatening or fatal neuropsychiatric events, including suicide, suicidal and homicidal ideation, depression, relapse of drug addiction/overdose, and aggressive behavior have occurred in subjects with and without a previous psychiatric disorder during HCV therapy. Interferon-induced depression is a limitation for the treatment of chronic hepatitis C, especially for subjects with psychiatric disorders. Psychiatric side effects are common with interferon therapy and responsible for about 10% to 20% of discontinuations of current therapy for HCV infection.

**[0248]** Accordingly, provided are methods of treating or preventing an HCV infection in subjects where the risk of neuropsychiatric events, such as depression, contraindicates treatment with current HCV therapy. In certain embodiments, provided are methods of treating or preventing HCV infection in subjects where a neuropsychiatric event, such as depression, or risk of such indicates discontinuation of treatment with current HCV therapy. Further provided are methods of treating or preventing HCV infection in subjects where a neuropsychiatric event, such as depression, or risk of such indicates dose reduction of current HCV therapy.

**[0249]** Current therapy is also contraindicated in subjects that are hypersensitive to interferon or ribavirin, or both, or any other component of a pharmaceutical product for administration of interferon or ribavirin. Current therapy is not indicated in subjects with hemoglobinopathies (e.g., thalassemia major, sickle-cell anemia) and other subjects at risk from the hematologic side effects of current therapy. Common hematologic side effects include bone marrow suppression, neutropenia and thrombocytopenia. Furthermore, ribavirin is toxic to red blood cells and is associated with hemolysis. Accordingly, in certain embodiments, provided are methods of treating or preventing HCV infection in subjects hypersensitive to interferon or ribavirin, or both, subjects with a hemoglobinopathy, for instance thalassemia major subjects and sickle-cell anemia subjects, and other subjects at risk from the hematologic side effects of current therapy.

**[0250]** In certain embodiments, the subject has received an HCV therapy and discontinued that therapy prior to administration of a method provided herein. In further embodiments, the subject has received therapy and continues to receive that therapy along with administration of a method provided herein. The methods can be co-administered with other therapy for HCV and/or HCV according to the judgment of one of skill in the art. In certain embodiments, the methods or compositions provided herein can be co-administered with a reduced dose of the other therapy for HCV and/or HCV.

**[0251]** In certain embodiments, provided are methods of treating a subject that is refractory to treatment with interferon. For instance, in some embodiments, the subject can be a subject that has failed to respond to treatment with one or more agents selected from the group consisting of interferon, interferon  $\alpha$ , pegylated interferon  $\alpha$ , interferon plus ribavirin, interferon  $\alpha$  plus ribavirin and pegylated interferon  $\alpha$  plus ribavirin. In some embodiments, the subject can be a subject that has responded poorly to treatment with one or more agents selected from the group consisting of interferon, interferon  $\alpha$ , pegylated interferon  $\alpha$ , interferon plus ribavirin, interferon  $\alpha$  plus ribavirin and pegylated interferon  $\alpha$  plus ribavirin. A pro-drug form of ribavirin, such as taribavirin, may also be used.

**[0252]** In certain embodiments, the subject has, or is at risk for, co-infection of HCV with HIV. For instance, in the United States, 30% of HIV subjects are co-infected with HCV and evidence indicates that people infected with HIV have a much more rapid course of their hepatitis C infection. Maier and Wu, 2002, *World J Gastroenterol* 8:577-57. The methods provided herein can be used to treat or prevent HCV infection in such subjects. It is believed that elimination of HCV in these subjects will lower mortality due to end-stage liver disease. Indeed, the risk of progressive liver disease is higher in subjects with severe AIDS-defining immunodeficiency than in those without. See, e.g., Lesens et al., 1999, *J Infect*

*Dis* 179:1254-1258. In certain embodiments, compounds provided herein have been shown to suppress HIV in HIV subjects. Thus, in certain embodiments, provided are methods of treating or preventing HIV infection and HCV infection in subjects in need thereof.

**[0253]** In certain embodiments, the compounds or compositions are administered to a subject following liver transplant. Hepatitis C is a leading cause of liver transplantation in the U.S., and many subjects that undergo liver transplantation remain HCV positive following transplantation. In certain embodiments, provided are methods of treating such recurrent HCV subjects with a compound or composition provided herein. In certain embodiments, provided are methods of treating a subject before, during or following liver transplant to prevent recurrent HCV infection.

**[0254]** Assay Methods

**[0255]** Compounds can be assayed for HCV activity according to any assay known to those of skill in the art.

**[0256]** Further, compounds can be assayed for accumulation in liver cells of a subject according to any assay known to those of skill in the art. In certain embodiments, a compound can be administered to the subject, and a liver cell of the subject can be assayed for the compound or a derivative thereof, e.g. a nucleoside, nucleoside phosphate or nucleoside triphosphate derivative thereof.

**[0257]** In certain embodiments, a 2'-chloro nucleoside analog compound is administered to cells, such as liver cells, in vivo or in vitro, and the nucleoside triphosphate levels delivered intracellularly are measured, to indicate delivery of the compound and triphosphorylation in the cell. The levels of intracellular nucleoside triphosphate can be measured using analytical techniques known in the art. Methods of detecting ddATP are described herein below by way of example, but other nucleoside triphosphates can be readily detected using the appropriate controls, calibration samples and assay techniques.

**[0258]** In certain embodiments, ddATP concentrations are measured in a sample by comparison to calibration standards made from control samples. The ddATP concentrations in a sample can be measured using an analytical method such as HPLC LC MS. In certain embodiments, a test sample is compared to a calibration curve created with known concentrations of ddATP to thereby obtain the concentration of that sample.

**[0259]** In certain embodiments, the samples are manipulated to remove impurities such as salts ( $\text{Na}^+$ ,  $\text{K}^+$ , etc.) before analysis. In certain embodiments, the lower limit of quantitation is about  $\sim 0.2$  pmol/mL for hepatocyte cellular extracts particularly where reduced salt is present.

**[0260]** In certain embodiments, the method allows successfully measuring triphosphate nucleotides formed at levels of 1-10,000 pmol per million cells in e.g. cultured hepatocytes and HepG2 cells.

**[0261]** Second Therapeutic Agents

**[0262]** In certain embodiments, the compounds and compositions provided herein are useful in methods of treatment of a liver disorder, that comprise further administration of a second agent effective for the treatment of the disorder, such as HCV infection in a subject in need thereof. The second agent can be any agent known to those of skill in the art to be effective for the treatment of the disorder, including those currently approved by the FDA.

**[0263]** In certain embodiments, a compound provided herein is administered in combination with one second agent.

In further embodiments, a second agent is administered in combination with two second agents. In still further embodiments, a second agent is administered in combination with two or more second agents.

**[0264]** As used herein, the term "in combination" includes the use of more than one therapy (e.g., one or more prophylactic and/or therapeutic agents). The use of the term "in combination" does not restrict the order in which therapies (e.g., prophylactic and/or therapeutic agents) are administered to a subject with a disorder. A first therapy (e.g., a prophylactic or therapeutic agent such as a compound provided herein) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (e.g., a prophylactic or therapeutic agent) to a subject with a disorder.

**[0265]** As used herein, the term "synergistic" includes a combination of a compound provided herein and another therapy (e.g., a prophylactic or therapeutic agent) which has been or is currently being used to prevent, manage or treat a disorder, which is more effective than the additive effects of the therapies. A synergistic effect of a combination of therapies (e.g., a combination of prophylactic or therapeutic agents) permits the use of lower dosages of one or more of the therapies and/or less frequent administration of said therapies to a subject with a disorder. The ability to utilize lower dosages of a therapy (e.g., a prophylactic or therapeutic agent) and/or to administer said therapy less frequently reduces the toxicity associated with the administration of said therapy to a subject without reducing the efficacy of said therapy in the prevention or treatment of a disorder. In addition, a synergistic effect can result in improved efficacy of agents in the prevention or treatment of a disorder. Finally, a synergistic effect of a combination of therapies (e.g., a combination of prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of either therapy alone.

**[0266]** The active compounds provided herein can be administered in combination or alternation with another therapeutic agent, in particular an anti-HCV agent. In combination therapy, effective dosages of two or more agents are administered together, whereas in alternation or sequential-step therapy, an effective dosage of each agent is administered serially or sequentially. The dosages given will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. In certain embodiments, an anti-HCV (or anti-pestivirus or anti-flavivirus) compound that exhibits an  $\text{EC}_{50}$  of 10-15  $\mu\text{M}$ . In certain embodiments, less than 1-5  $\mu\text{M}$ , is desirable.

**[0267]** It has been recognized that drug-resistant variants of flaviviruses, pestiviruses or HCV can emerge after prolonged

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

**[0268]** Any of the viral treatments described in the Background of the Invention can be used in combination or alternation with the compounds described in this specification. Non-limiting examples of second agents include:

**[0269]** HCV Protease inhibitors: Examples include Medivir HCV Protease Inhibitor (HCV-PI or TMC435) (Medivir/Tibotec); MK-7009 (Merck), RG7227 (ITMN-191) (Roche/Pharmasset/InterMune), boceprevir (SCH 503034) (Schering), SCH 446211 (Schering), naltaprevir SCH900518 (Schering/Merck), ABT-450 (Abbott/Enanta), ACH-1625 (Achillion), BI 201335 (Boehringer Ingelheim), PHX1766 (Phenomix), VX-500 (Vertex) and telaprevir (VX-950) (Vertex). Further examples of protease inhibitors include substrate-based NS3 protease inhibitors (Attwood et al., *Antiviral peptide derivatives*, PCT WO 98/22496, 1998; Attwood et al., *Antiviral Chemistry and Chemotherapy* 1999, 10, 259-273; Attwood et al., Preparation and use of amino acid derivatives as anti-viral agents, German Patent Pub. DE 19914474; Tung et al., Inhibitors of serine proteases, particularly hepatitis C virus NS3 protease, PCT WO 98/17679), including alphaketoamides and hydrazinoureas, and inhibitors that terminate in an electrophile such as a boronic acid or phosphonate (Llinas-Brunet et al., Hepatitis C inhibitor peptide analogues, PCT WO 99/07734); Non-substrate-based NS3 protease inhibitors such as 2,4,6-trihydroxy-3-nitro-benzamide derivatives (Sudo K. et al., *Biochemical and Biophysical Research Communications*, 1997, 238, 643-647; Sudo K. et al., *Antiviral Chemistry and Chemotherapy*, 1998, 9, 186), including RD3-4082 and RD3-4078, the former substituted on the amide with a 14 carbon chain and the latter processing a para-phenoxyphenyl group; and Sch 68631, a phenanthrenequinone, an HCV protease inhibitor (Chu M. et al., *Tetrahedron Letters* 37:7229-7232, 1996).

**[0270]** SCH 351633, isolated from the fungus *Penicillium griseofulvum*, was identified as a protease inhibitor (Chu M. et al., *Bioorganic and Medicinal Chemistry Letters* 9:1949-1952). Eglin c, isolated from leech, is a potent inhibitor of several serine proteases such as *S. griseus* proteases A and B, a-chymotrypsin, chymase and subtilisin. Qasim M. A. et al., *Biochemistry* 36:1598-1607, 1997.

**[0271]** U.S. patents disclosing protease inhibitors for the treatment of HCV include, for example, U.S. Pat. No. 6,004, 933 to Spruce et al., which discloses a class of cysteine protease inhibitors for inhibiting HCV endopeptidase 2; U.S. Pat. No. 5,990,276 to Zhang et al., which discloses synthetic inhibitors of hepatitis C virus NS3 protease; U.S. Pat. No. 5,538,865 to Reyes et al.; WO 02/008251 to Corvas International, Inc., and U.S. Pat. No. 7,169,760, US2005/176648, WO 02/08187 and WO 02/008256 to Schering Corporation. HCV inhibitor tripeptides are disclosed in U.S. Pat. Nos. 6,534,523, 6,410,531, and 6,420,380 to Boehringer Ingelheim and WO 02/060926 to Bristol Myers Squibb. Diaryl

peptides as NS3 serine protease inhibitors of HCV are disclosed in WO 02/48172 and U.S. Pat. No. 6,911,428 to Schering Corporation. Imidazoleidinones as NS3 serine protease inhibitors of HCV are disclosed in WO 02/08198 and U.S. Pat. No. 6,838,475 to Schering Corporation and WO 02/48157 and U.S. Pat. No. 6,727,366 to Bristol Myers Squibb. WO 98/17679 and U.S. Pat. No. 6,265,380 to Vertex Pharmaceuticals and WO 02/48116 and U.S. Pat. No. 6,653, 295 to Bristol Myers Squibb also disclose HCV protease inhibitors. Further examples of HCV serine protease inhibitors are provided in U.S. Pat. No. 6,872,805 (Bristol-Myers Squibb); WO 2006000085 (Boehringer Ingelheim); U.S. Pat. No. 7,208,600 (Vertex); US 2006/0046956 (Schering-Plough); WO 2007/001406 (Chiron); US 2005/0153877; WO 2006/119061 (Merck); WO 00/09543 (Boehringer Ingelheim), U.S. Pat. No. 6,323,180 (Boehringer Ingelheim) WO 03/064456 (Boehringer Ingelheim), U.S. Pat. No. 6,642,204 (Boehringer Ingelheim), WO 03/064416 (Boehringer Ingelheim), U.S. Pat. No. 7,091,184 (Boehringer Ingelheim), WO 03/053349 (Bristol-Myers Squibb), U.S. Pat. No. 6,867,185, WO 03/099316 (Bristol-Myers Squibb), U.S. Pat. No. 6,869, 964, WO 03/099274 (Bristol-Myers Squibb), U.S. Pat. No. 6,995,174, WO 2004/032827 (Bristol-Myers Squibb), U.S. Pat. No. 7,041,698, WO 2004/043339 and U.S. Pat. No. 6,878,722 (Bristol-Myers Squibb).

**[0272]** Thiazolidine derivatives which show relevant inhibition in a reverse-phase HPLC assay with an NS3/4A fusion protein and NS5A/5B substrate (Sudo K. et al., *Antiviral Research*, 1996, 32, 9-18), especially compound RD-1-6250, possessing a fused cinnamoyl moiety substituted with a long alkyl chain, RD4 6205 and RD4 6193;

**[0273]** Thiazolidines and benzanilides identified in Kakiuchi N. et al., J. EBS Letters 421, 217-220; Takeshita N. et al., *Analytical Biochemistry*, 1997, 247, 242-246;

**[0274]** A phenanthrenequinone possessing activity against protease in a SDS-PAGE and autoradiography assay isolated from the fermentation culture broth of *Streptomyces* sp., SCH 68631 (Chu M. et al., *Tetrahedron Letters*, 1996, 37, 7229-7232), and SCH 351633, isolated from the fungus *Penicillium griseofulvum*, which demonstrates activity in a scintillation proximity assay (Chu M. et al., *Bioorganic and Medicinal Chemistry Letters* 9, 1949-1952);

**[0275]** Helicase inhibitors (Diana G. D. et al., Compounds, compositions and methods for treatment of hepatitis C, U.S. Pat. No. 5,633,358; Diana G. D. et al., Piperidine derivatives, pharmaceutical compositions thereof and their use in the treatment of hepatitis C, PCT WO 97/36554);

**[0276]** HCV polymerase inhibitors, including nucleoside and non-nucleoside polymerase inhibitors, such as ribavirin, viramidine, clemizole, filibuvir (PF-00868554), HCV POL, NM 283 (valopicitabine), MK-0608, 7-Fluoro-MK-0608, MK-3281, IDX-375, ABT-072, ABT-333, ANA598, BI 207127, GS 9190, PSI-6130, R1626, PSI-6206, PSI-938, PSI-7851, PSI-7977, RG1479, RG7128, HCV-796 VCH-759 or VCH-916.

**[0277]** Gliotoxin (Ferrari R. et al., *Journal of Virology*, 1999, 73, 1649-1654), and the natural product cerulenin (Lohmann V. et al., *Virology*, 1998, 249, 108-118);

**[0278]** Interfering RNA (iRNA) based antivirals, including short interfering RNA (siRNA) based antivirals, such as Sirna-034 and others described in International Patent Publication Nos. WO/03/070750 and WO 2005/012525, and US Patent Publication No. US 2004/0209831.



**[0279]** Antisense phosphorothioate oligodeoxynucleotides (S-ODN) complementary to sequence stretches in the 5' non-coding region (NCR) of the virus (Alt M. et al., *Hepatology*, 1995, 22, 707-717), or nucleotides 326-348 comprising the 3' end of the NCR and nucleotides 371-388 located in the core coding region of the HCV RNA (Alt M. et al., *Archives of Virology*, 1997, 142, 589-599; Galderisi U. et al., *Journal of Cellular Physiology*, 1999, 181, 251-257);

**[0280]** Inhibitors of IRES-dependent translation (Ikeda N et al., Agent for the prevention and treatment of hepatitis C, Japanese Patent Pub. JP-08268890; Kai Y. et al., Prevention and treatment of viral diseases, Japanese Patent Pub. JP-10101591);

**[0281]** HCV NS5A inhibitors, such as BMS-790052 (daclatasvir, Bristol-Myers Squibb), PPI-461 (Presidio Pharmaceuticals), PPI-1301 (Presidio Pharmaceuticals), IDX-719 (Idenix Pharmaceuticals), AZD7295 (Arrow Therapeutics, AstraZeneca), EDP-239 (Enanta), ACH-2928 (Achillion), ACH-3102 (Achillion), ABT-267 (Abbott), or GS-5885 (Gilead);

**[0282]** HCV entry inhibitors, such as celgosivir (MK-3253) (MIGENIX Inc.), SP-30 (Samaritan Pharmaceuticals), ITX4520 (iTherX), ITX5061 (iTherX), PRO-206 (Progenics Pharmaceuticals) and other entry inhibitors by Progenics Pharmaceuticals, e.g., as disclosed in U.S. Patent Publication No. 2006/0198855.

**[0283]** Ribozymes, such as nuclease-resistant ribozymes (Maccjak, D. J. et al., *Hepatology* 1999, 30, abstract 995) and those disclosed in U.S. Pat. No. 6,043,077 to Barber et al., and U.S. Pat. Nos. 5,869,253 and 5,610,054 to Draper et al.; and

**[0284]** Nucleoside analogs have also been developed for the treatment of Flaviviridae infections.

**[0285]** In certain embodiments, the compounds provided herein can be administered in combination with any of the compounds described by Idenix Pharmaceuticals in International Publication Nos. WO 01/90121, WO 01/92282, WO 2004/003000, 2004/002422 and WO 2004/002999.

**[0286]** Other patent applications disclosing the use of certain nucleoside analogs that can be used as second agents to treat hepatitis C virus include: PCT/CA00/01316 (WO 01/32153; filed Nov. 3, 2000) and PCT/CA01/00197 (WO 01/60315; filed Feb. 19, 2001) filed by BioChem Pharma, Inc. (now Shire Biochem, Inc.); PCT/US02/01531 (WO 02/057425; filed Jan. 18, 2002); PCT/US02/03086 (WO 02/057287; filed Jan. 18, 2002); U.S. Pat. No. 7,202,224; U.S. Pat. No. 7,125,855; U.S. Pat. No. 7,105,499 and U.S. Pat. No. 6,777,395 by Merck & Co., Inc.; PCT/EP01/09633 (WO 02/18404; published Aug. 21, 2001); US 2006/0040890; 2005/0038240; 2004/0121980; U.S. Pat. No. 6,846,810; U.S. Pat. No. 6,784,166 and U.S. Pat. No. 6,660,721 by Roche; PCT Publication Nos. WO 01/79246 (filed Apr. 13, 2001), WO 02/32920 (filed Oct. 18, 2001) and WO 02/48165; US 2005/0009737; US 2005/0009737; U.S. Pat. No. 7,094,770 and U.S. Pat. No. 6,927,291 by Pharmasset, Ltd.

**[0287]** Further compounds that can be used as second agents to treat hepatitis C virus are disclosed in PCT Publication No. WO 99/43691 to Emory University, entitled "2'-Fluoronucleosides". The use of certain 2'-fluoronucleosides to treat HCV is disclosed.

**[0288]** Other compounds that can be used as second agents include 1-amino-alkylcyclohexanes (U.S. Pat. No. 6,034,134 to Gold et al.), alkyl lipids (U.S. Pat. No. 5,922,757 to Chojkier et al.), vitamin E and other antioxidants (U.S. Pat. No. 5,922,757 to Chojkier et al.), squalene, amantadine, bile

acids (U.S. Pat. No. 5,846,964 to Ozeki et al.), N-(phosphonoacetyl)-L-aspartic acid, (U.S. Pat. No. 5,830,905 to Diana et al.), benzenedicarboxamides (U.S. Pat. No. 5,633,388 to Diana et al.), polyadenylic acid derivatives (U.S. Pat. No. 5,496,546 to Wang et al.), 2',3'-dideoxyinosine (U.S. Pat. No. 5,026,687 to Yarchoan et al.), benzimidazoles (U.S. Pat. No. 5,891,874 to Colacino et al.), plant extracts (U.S. Pat. No. 5,837,257 to Tsai et al., U.S. Pat. No. 5,725,859 to Omer et al., and U.S. Pat. No. 6,056,961), and piperidines (U.S. Pat. No. 5,830,905 to Diana et al.).

**[0289]** In certain embodiments, a compound of a formula provided herein, or a composition comprising a compound of a formula provided herein, is administered in combination or alternation with a second anti-viral agent selected from the group consisting of an interferon, a nucleotide analogue, a polymerase inhibitor, an NS3 protease inhibitor, an NS5A inhibitor, an entry inhibitor, a non-nucleoside polymerase inhibitor, a cyclosporine immune inhibitor, an NS4A antagonist, an NS4B-RNA binding inhibitor, a locked nucleic acid mRNA inhibitor, a cyclophilin inhibitor, and combinations thereof.

**[0290]** Exemplary Second Therapeutic Agents for Treatment of HCV

**[0291]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus interferon, such as Intron A® (interferon alfa-2b) and; Roferon A® (Recombinant interferon alfa-2a), Infergen® (consensus interferon; interferon alfacon-1), PEG-Intron® (pegylated interferon alfa-2b), and Pegasys® (pegylated interferon alfa-2a). In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with ribavirin and in combination or alternation with an anti-hepatitis C virus interferon. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with ribavirin, in combination or alternation with an anti-hepatitis C virus interferon, and in combination or alternation with an anti-hepatitis C virus protease inhibitor. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus interferon and without ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus interferon, in combination or alternation with an anti-hepatitis C virus protease inhibitor, and without ribavirin.

**[0292]** In certain embodiments, the anti-hepatitis C virus interferon is infergen, IL-29 (PEG-Interferon lambda), R7025 (Maxy-alpha), Belerofon, Oral Interferon alpha, BLX-883 (Locteron), omega interferon, multiferon, medusa interferon, Albuferon or REBIF®.

**[0293]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus polymerase inhibitor, such as ribavirin, viramidine, HCV POL, NM 283 (valopicitabine), MK-0608, 7-Fluoro-MK-0608, PSI-6130, R1626, PSI-6206, PSI-938, R1479, HCV-796, VX-950 (Telaprevir, Vertex), GS 9190 NN (Gilead), GS 9256 (Gilead), PSI-7792 (BMS), BI 207127 (BI), R7128 (Roche), or PSI-7977 (Pharmasset), PSI-938 (Pharmasset), VX-222 (Vertex), ALS-2200 (Vertex), ALS-2158 (Vertex), MK-0608 (Merck), TMC649128 (Medivir), PF-868554 (Pfizer), PF-4878691



(Pfizer), ANA598 (Roche), VCH-759 (Vertex), IDX184 (Idenix), IDX375 (Idenix), A-837093 (Abbott), GS 9190 (Gilead), GSK625433 (GlaxoSmithKline), ABT-072 (Abbott), ABT-333 (Abbott), INX-189 (Inhibitex), or EDP-239 (Enanta).

**[0294]** In certain embodiments, the one or more compounds provided herein can be administered in combination with ribavirin and an anti-hepatitis C virus interferon, such as Intron A® (interferon alfa-2b) and Pegasys® (Peginterferon alfa-2a); Roferon A® (Recombinant interferon alfa-2a), Infergen® (consensus interferon; interferon alfacon-1), PEG-Intron® (pegylated interferon alfa-2b), Zalbin (albinterferon alfa-2b), omega interferon, pegylated interferon lambda, and Pegasys® (pegylated interferon alfa-2a).

**[0295]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus protease inhibitor such as ITMN-191, SCH 503034 (bocepravir), VX950 (telaprevir), VX985, VX500, VX813, PHX1766, BMS-650032, GS 9256, BI 201335, IDX320, R7227, MK-7009 (vaniprevir), TMC435, BMS-791325, ACH-1625, ACH-2684, ABT-450, or AVL-181.

**[0296]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an HCV NS5A inhibitor, such as BMS-790052 (daclatasvir, Bristol-Myers Squibb), PPI-461 (Presidio Pharmaceuticals), PPI-1301 (Presidio Pharmaceuticals), IDX-719 (Idenix Pharmaceuticals), AZD7295 (Arrow Therapeutics, AstraZeneca), EDP-239 (Enanta), ACH-2928 (Achillion), ACH-3102 (Achillion), ABT-267 (Abbott), or GS-5885 (Gilead).

**[0297]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus vaccine, such as TG4040, PeviPRO™, CGI-5005, HCV/MF59, GV1001, IC<sub>41</sub>, GNI-103, GenPhar HCV vaccine, C-Vaxin, CSL123, Hepavaxx C, ChronVac-C® or INNO0101 (E1).

**[0298]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus monoclonal antibody, such as MBL-HCV1, AB68 or XTL-6865 (formerly HepX-C); or an anti-hepatitis C virus polyclonal antibody, such as cicavir.

**[0299]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an anti-hepatitis C virus immunomodulator, such as Zadaxin® (thymalfasin), SCV-07, NOV-205 or Oglufanide.

**[0300]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with cyclophilin inhibitor, such as Enanta cyclophilin binder, SCY-635, or Debio-025.

**[0301]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with Nexavar, doxorubicin, PI-88, amantadine, JBK-122, VGX-410C, MX-3253 (Ceglosivir), Suvus (BIVN-401 or virostat), PF-03491390 (formerly IDN-6556), G126270, UT-231B, DEBIO-025, EMZ702, ACH-0137171, MitoQ, ANA975, AVI-4065, Bavtuxinab (Tarvacin), Alinia (nitrazoxanide) or PYN17.

**[0302]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with telaprevir, bocepravir, interferon alfacon-1, inter-

feron alfa-2b, pegylated interferon alpha 2a, pegylated interferon alpha 2b, ribavirin, or combinations thereof.

**[0303]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with a protease inhibitor. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with telaprevir. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with bocepravir.

**[0304]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with a protease inhibitor and in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with telaprevir and in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with bocepravir and in combination or alternation with ribavirin.

**[0305]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with a protease inhibitor and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with telaprevir and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with bocepravir and not in combination or alternation with ribavirin.

**[0306]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an interferon. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with interferon alfacon-1. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with interferon alfa-2b. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with pegylated interferon alpha 2a. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with pegylated interferon alpha 2b.

**[0307]** In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with an interferon and in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with interferon alfacon-1 and in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with interferon alfa-2b and in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with pegylated interferon alpha 2a and in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with pegylated interferon alpha 2b and in combination or alternation with ribavirin.

**[0308]** In certain embodiments, one or more compounds can be administered in combination or alternation with one or more of the second agents provided herein and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered

in combination or alternation with an interferon and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with interferon alfacon-1 and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with interferon alfa-2b and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with pegylated interferon alpha 2a and not in combination or alternation with ribavirin. In certain embodiments, one or more compounds provided herein can be administered in combination or alternation with pegylated interferon alpha 2b and not in combination or alternation with ribavirin.

#### EXAMPLES

**[0309]** As used herein, the symbols and conventions used in these processes, schemes and examples, regardless of whether a particular abbreviation is specifically defined, are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry. Specifically, but without limitation, the following abbreviations may be

used in the examples and throughout the specification: g (grams); mg (milligrams); mL (milliliters);  $\mu$ L (microliters); mM (millimolar);  $\mu$ M (micromolar); Hz (Hertz); MHz (megahertz); mmol (millimoles); hr or hrs (hours); min (minutes); MS (mass spectrometry); ESI (electrospray ionization); TLC (thin layer chromatography); HPLC (high pressure liquid chromatography); THF (tetrahydrofuran);  $\text{CDCl}_3$  (deuterated chloroform); AcOH (acetic acid); DCM (dichloromethane); DMSO (dimethylsulfoxide);  $\text{DMSO-d}_6$  (deuterated dimethylsulfoxide); EtOAc (ethyl acetate); MeOH (methanol); and BOC (t-butyloxycarbonyl).

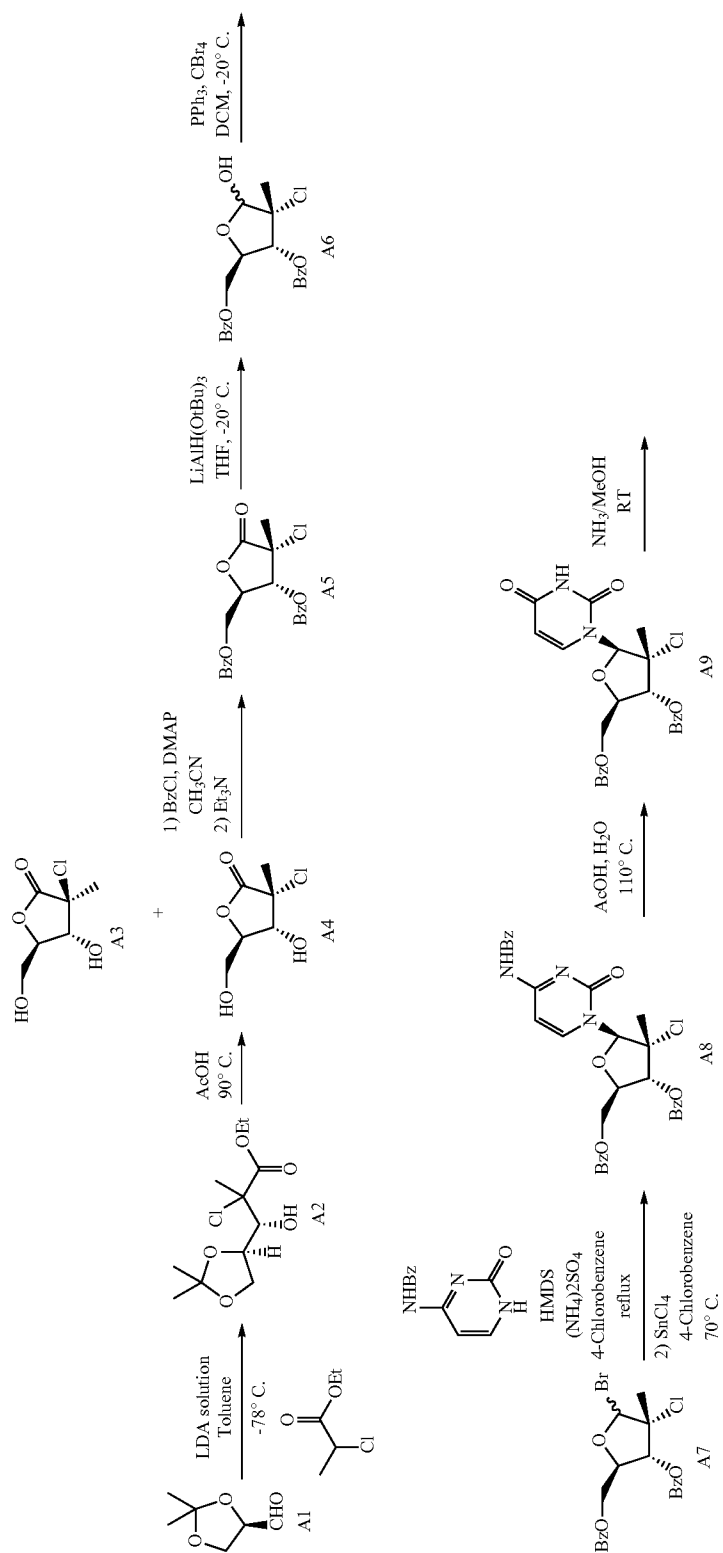
**[0310]** For all of the following examples, standard work-up and purification methods known to those skilled in the art can be utilized. Unless otherwise indicated, all temperatures are expressed in  $^{\circ}\text{C}$ . (degrees Centigrade). All reactions are conducted at room temperature unless otherwise noted. Synthetic methodologies illustrated herein are intended to exemplify the applicable chemistry through the use of specific examples and are not indicative of the scope of the disclosure.

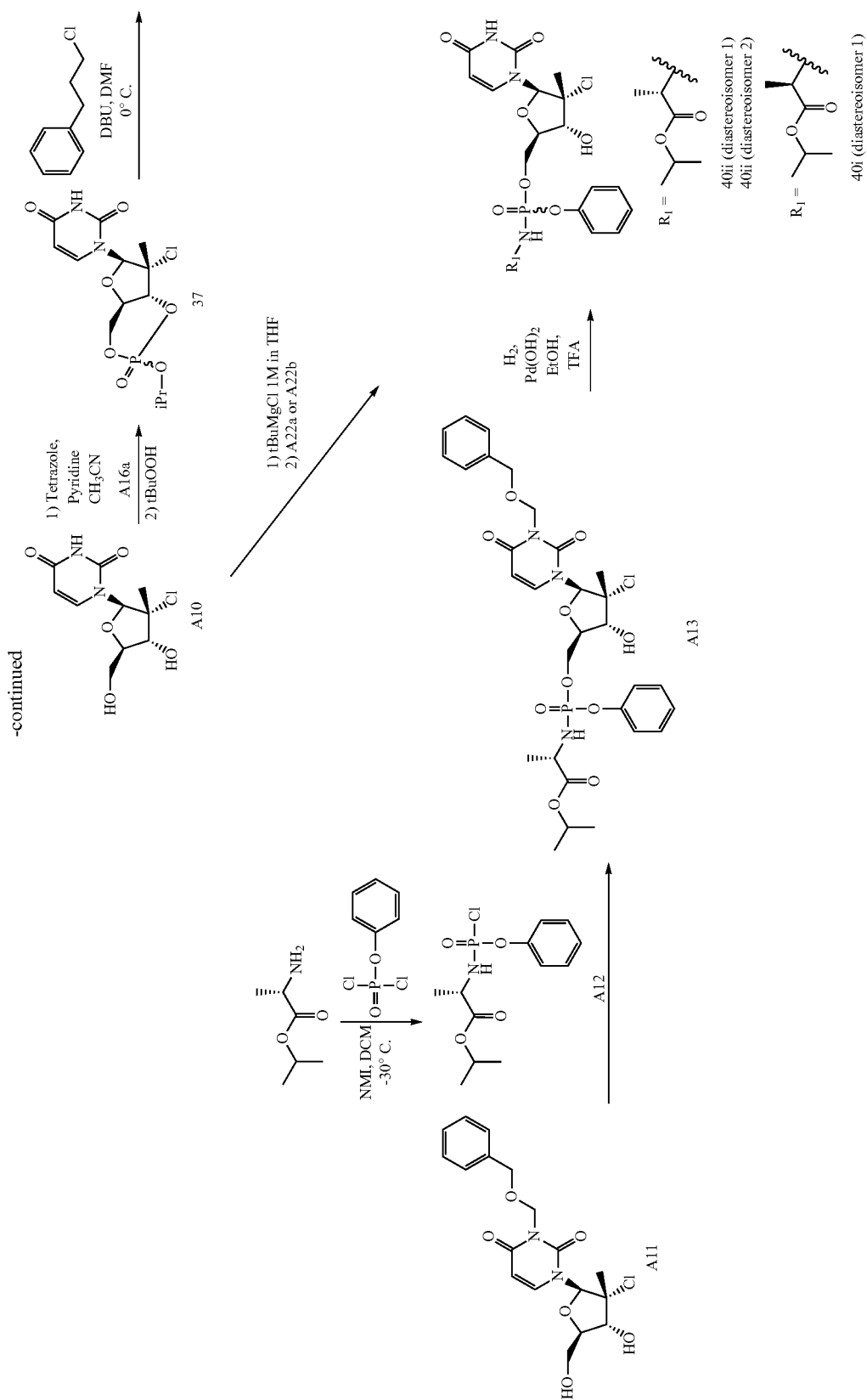
#### Example 1

##### Preparation of 2'-Chloro Nucleoside Analogs

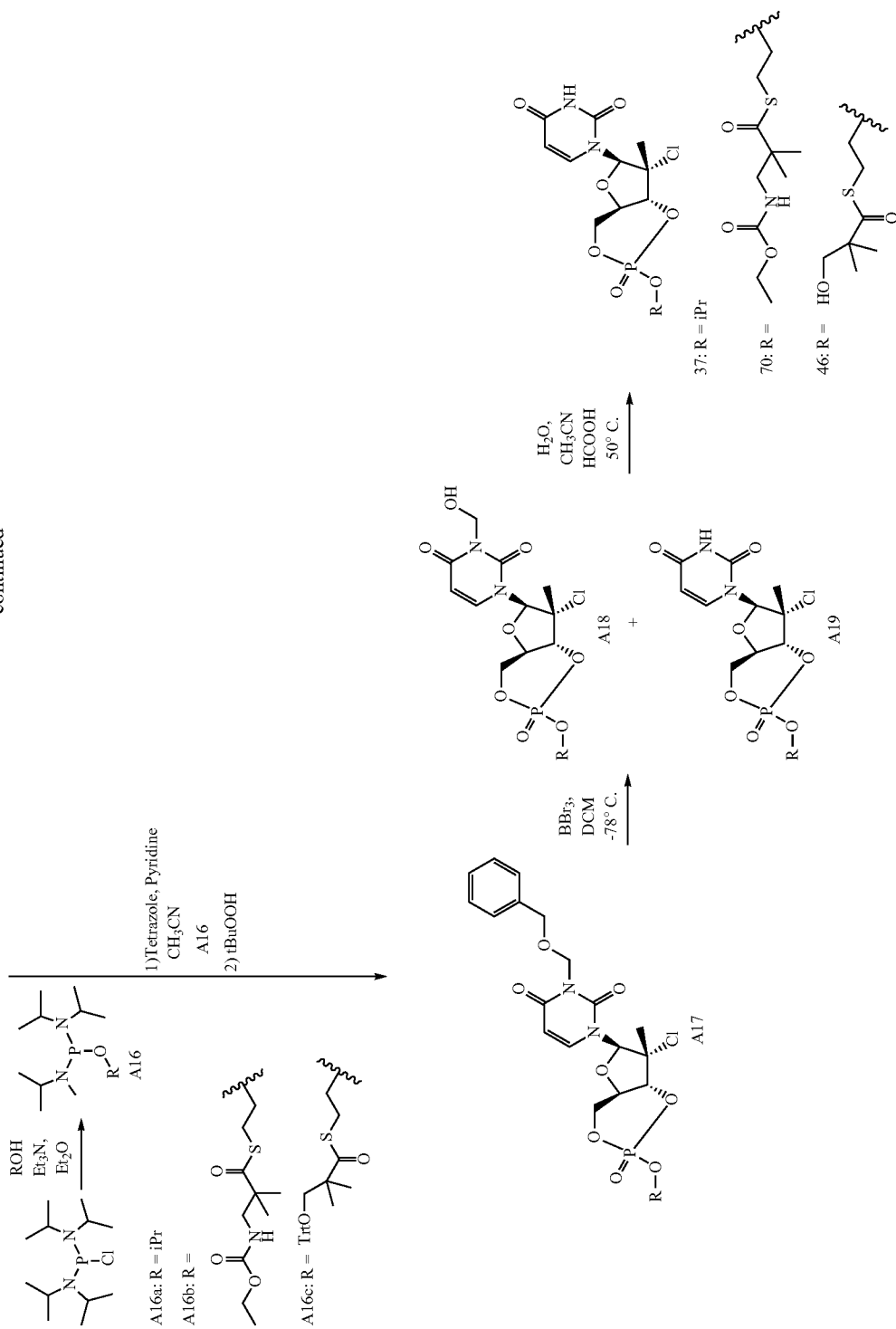
**[0311]**

Scheme 1



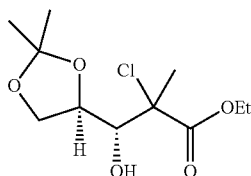


-continued-



Ethyl(3R)-2-chloro-3-[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]-3-hydroxy-2-methylpropanoate (A2)

[0312]



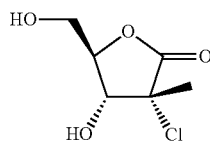
[0313] A 5 L flange flask was fitted with a thermometer, nitrogen inlet, pressure equalizing dropping funnel, bubbler, and a suba-seal. Methyl lithium solution (1.06 L, 1.6 M in diethylether, 1.7 equiv.) was added, and the solution was cooled to about  $-25^{\circ}\text{C}$ . Diisopropyl amine (238 ml, 1.7 equiv.) was added using the dropping funnel over about 40 minutes. The reaction was left stirring, allowing to warm to ambient temperature overnight.  $\text{CO}_2(\text{g})/\text{acetone}$  cooling was applied to the LDA solution, cooling to about  $-70^{\circ}\text{C}$ .

[0314] R-Glyceraldehyde dimethylacetal solution (50% in DCM) was evaporated down to  $\sim 100$  mbar at a bath temp of  $35^{\circ}\text{C}$ ., to remove the DCM, then azeotroped with anhydrous hexane (200 ml), under the same Büchi conditions.  $^1\text{H}$  NMR was used to confirm that all but a trace of DCM remained.

[0315] The fresh aldehyde (130 g, 1 mol) and ethyl 2-chloropropionate (191 ml, 1.5 equiv.) were placed in a 1 L round bottom flask, which was filled with toluene (800 ml). This solution was cooled in a  $\text{CO}_2(\text{g})/\text{acetone}$  bath, and added via cannula to the LDA solution over about 50 minutes, keeping the internal temperature of the reaction mixture cooler than  $-60^{\circ}\text{C}$ . The mixture was stirred with cooling (internal temp. slowly fell to  $\sim -72^{\circ}\text{C}$ .) for 90 min, then warmed to room temperature over 30 minutes using a water bath. This solution was added to a sodium dihydrogen phosphate solution equivalent to 360 g of  $\text{NaH}_2\text{PO}_4$  in 1.5 L of ice/water, over about 10 minutes, with ice-bath cooling. The mixture was stirred for 20 minutes, then transferred to a sep. funnel, and partitioned. The aqueous layer was further extracted with EtOAc (2x1 L), and the combined organic extracts were dried over sodium sulfate. The volatiles were removed in vacuo (down to 20 mbar). The resultant oil was hydrolyzed crude.

(3R,4R,5R)-3-chloro-4-hydroxy-5-(hydroxymethyl)-3-methyloxolan-2-one (A4)

[0316]

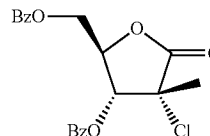


[0317] The crude oil A2 was taken up in acetic acid (1.5 L, 66% in water) and heated to  $90^{\circ}\text{C}$ . over one hour, then at held at that temperature for one hour. Once the mixture had cooled to room temperature, the volatiles were removed in vacuo, and azeotroped with toluene (500 ml). The resultant oil was combined with some mixed material from an earlier synthesis

and columned in two portions (each  $\sim 1.25$  L of silica,  $38 \rightarrow 75\%$  EtOAc in DCM). The lower of the two main spots is the desired material; fractions containing this material as the major component were combined and the solvent removed in vacuo to give 82 g of orange solid whose  $^1\text{H}$  NMR showed the material to be of about 57% purity (of the remainder 29% was the indicated epimer). This material was recrystallized from toluene/butanone (600 ml/ $\sim 185$  ml), the butanone being the 'good' solvent. The resultant solid was filtered washing with toluene and hexane, and dried in vacuo to give product of about 92% purity (30 g).

(2R,3R,4R)-2-[(benzoyloxy)methyl]-4-chloro-4-methyl-5-oxoxolan-3-yl benzoate (A5)

[0318]

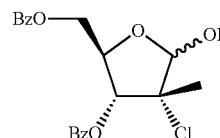


[0319] A 2 L 3-neck round bottom flask was fitted with an overhead stirrer, thermometer and pressure equalizing dropping funnel ( $\rightarrow \text{N}_2$ ). The intermediate A4 (160 mmol) in acetonitrile (1 L) was added, followed by 4-dimethylaminopyridine (3.2 mmol) and benzoyl chloride (352 mmol). Finally triethylamine (384 mmol) was added over 10 minutes using the dropping funnel. The addition of the triethylamine is accompanied by a mild exotherm, which obviated the addition of a cold water bath to keep the internal temperature below  $25^{\circ}\text{C}$ . The reaction was stirred at ambient temperature for 2.5 hours. The reaction mixture was transferred to a sep. funnel with EtOAc (2 L) and half saturated brine (2 L), and partitioned. The aqueous layer was re-extracted with EtOAc (1 L). The combined organic layers were washed with 50% sodium bicarbonate/25% brine (1.5 L) and dried over sodium sulfate, to give 62 g of solid. This was recrystallized from 1.8 L of 1:1 toluene/trimethylpentane ( $95^{\circ}\text{C}$ .), to give 52.4 g of product.

[0320]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  (ppm) 1.91 (s, 3H), 4.57 (dd,  $J=5.12$  Hz and  $J=12.57$  Hz, 1H), 4.77 (dd,  $J=3.29$  Hz and  $J=12.68$  Hz, 1H), 4.92-4.96 (m, 1H), 5.60 (d,  $J=8.36$  Hz, 1H), 7.38-7.66 (m, 6H), 7.97-7.99 (m, 2H), 8.08-8.10 (m, 2H); MS (ESI)  $m/z=411.1$  ( $\text{MNa}^+$ ).

3,5-Di-O-benzoyl-2-C-chloro-2-C-methyl-D-ribofuranose (A6)

[0321]



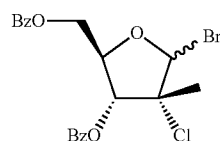
[0322] To a solution of A5 (14.48 mmol) in anhydrous tetrahydrofuran (70 ml) was added under inert atmosphere at  $-35^{\circ}\text{C}$ .,  $\text{LiAlH}(\text{OtBu})_3$  (1M in tetrahydrofuran, 21.7 mmol) over a 30 min period. The reaction mixture was stirred for 1 hour at  $-20^{\circ}\text{C}$ . and quenched by addition of a saturated  $\text{NH}_4\text{Cl}$  solution, keeping the temperature below  $0^{\circ}\text{C}$ . Ethyl

acetate was added and the white suspension was filtered through a pad of celite and washed with ethyl acetate. The filtrate was extracted with ethyl acetate twice. The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (eluent: petroleum ether/ethyl acetate 0 to 20%). The product was dried in vacuum (50° C.) overnight to afford expected intermediate as a colorless oil in 96% yield (mixture  $\alpha/\beta$ : 45/55).

[0323]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  (ppm) 1.74 (s, 1.75H<sub>B</sub>), 1.76 (s, 1.25H<sub>C</sub>), 4.42-4.69 (m, 3H), 5.30 (d, J=12.8 Hz, 0.55H<sub>B</sub>), 5.43-5.47 (m, 0.45H<sub>C</sub>), 5.60 (d, J=7.0 Hz, 0.55H<sub>B</sub>), 5.78 (d, J=7.0 Hz, 0.45H<sub>C</sub>), 7.35-7.41 (m, 2H), 7.45-7.56 (m, 3H), 7.59-7.65 (m, 1H), 7.96-8.04 (m, 2H), 8.06-8.14 (m, 2H); MS (ESI)  $m/z$ =413 (MNa<sup>+</sup>).

3,5-Di-O-benzoyl-2-C-chloro-2-C-methyl-D-ara-  
binofuranosyl bromide (A7)

[0324]

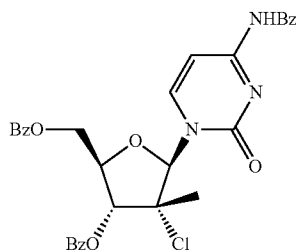


[0325] To a solution of A6 (12.80 mmol) in anhydrous dichloromethane (80 ml) was added under inert atmosphere at -20° C., triphenylphosphine (18.0 mmol). The reaction mixture was stirred for 15 minutes at -20° C. and CBr<sub>4</sub> (19.20 mmol) was added. The reaction mixture was then stirred for 1 hour at -20° C. The crude was partially concentrated under reduced pressure (bath temperature below 30° C.) and directly purified by chromatography on silica gel (eluent: petroleum ether/ethyl acetate 0 to 30%) to afford a mixture of 0 sugar A7a (1.67 g) and a sugar A7b (2.15 g) as a colorless gum in 66% global yield.

[0326]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\beta$  sugar  $\delta$  (ppm) 1.93 (s, 3H), 4.60-4.88 (m, 3H), 6.08 (d, J=7.9 Hz, 1H), 6.62 (s, 1H), 7.31-7.38 (m, 2H), 7.41-7.55 (m, 3H), 7.59-7.65 (m, 1H), 8.00-8.05 (m, 2H), 8.06-8.12 (m, 2H);  $\alpha$  sugar  $\delta$  (ppm) 1.88 (s, 3H), 4.66-4.89 (m, 3H), 5.37 (d, J=4.88 Hz, 1H), 6.44 (s, 1H), 7.41-7.55 (m, 4H), 7.54-7.65 (m, 2H), 8.00-8.05 (m, 2H), 8.14-8.20 (m, 2H); MS (ESI)  $m/z$ =476/478 (MNa<sup>+</sup>).

3',5'-Di-O-benzoyl-2'-C-chloro-2'-C-methyl-4-ben-  
zoyl-cytidine (A8)

[0327]

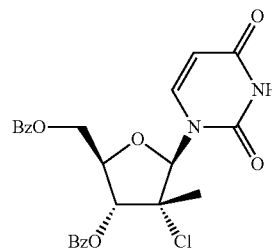


[0328] To a suspension of N-benzoyl cytosine (9.48 mmol), and a catalytic amount of ammonium sulfate in 4-chlorobenzene (24 ml) was added HMDS (28.44 mmol). The reaction mixture was heated during 2 hours at 140° C. The solvent was removed under inert atmosphere and the residue was taken in 4-chlorobenzene (15 ml). Then, A7b (4.74 mmol) in chlorobenzene (10 ml) was added dropwise to the reaction mixture followed by SnCl<sub>4</sub> (14.22 mmol) dropwise. The reaction mixture was stirred at 70° C. overnight, cooled to room temperature and diluted with dichloromethane and a saturated NaHCO<sub>3</sub> solution. The white suspension was filtered through a pad of celite and washed with dichloromethane. The filtrate was extracted with dichloromethane twice. The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under reduced pressure to afford expected intermediate as a white solid in 89% yield.

[0329]  $^1\text{H}$  NMR (DMSO, 400 MHz):  $\delta$  (ppm) 1.58 (s, 3H), 4.68-4.81 (m, 3H), 5.68 (brs, 1H), 6.55 (brs, 1H), 7.36 (d, J=7.84 Hz, 1H), 7.39-7.76 (m, 9H), 7.88-8.07 (m, 6H), 8.30 (d, J=7.84 Hz, 1H); MS (ESI)  $m/z$ =588 (MH<sup>+</sup>).

3',5'-Di-O-benzoyl-2'-C-chloro-2'-C-methyluridine  
(A9)

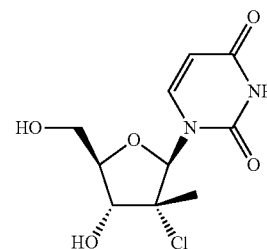
[0330]



[0331] A suspension of A8 (4.19 mmol) in an acetic acid/water mixture (67 ml/17 ml, v/v), was heated at 110° C. for 3 hours. The reaction mixture was evaporated to dryness and co-evaporated with toluene (three times) to afford expected intermediate in quantitative yield as an oil which was directly used for the next step; MS (ESI)  $m/z$ =485 (MH<sup>+</sup>).

2'-C-Chloro-2'-C-methyluridine (301)

[0332]

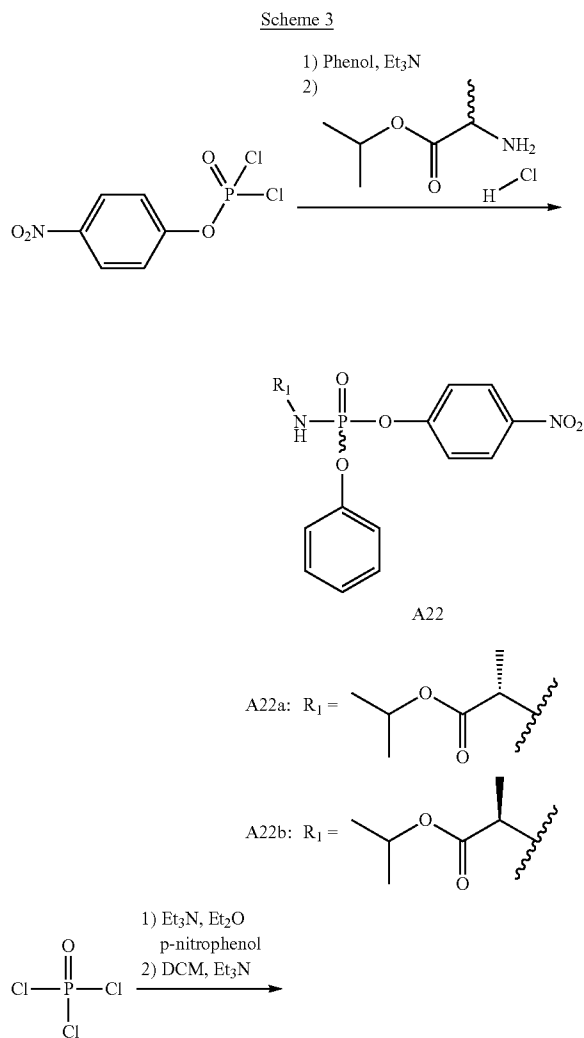


**[0333]** Intermediate A9 (4.19 mmol) in 7 N methanolic ammonia (80 ml) was stirred at room temperature for 24 hours. The mixture was evaporated to dryness, diluted with water and transferred into a separatory funnel. The aqueous layer was extracted with dichloromethane and water was removed under reduced pressure. The residue was purified by flash RP18 gel chromatography (eluent: water/acetonitrile 0 to 40%) to afford pure expected compound as a white foam in 79% yield.

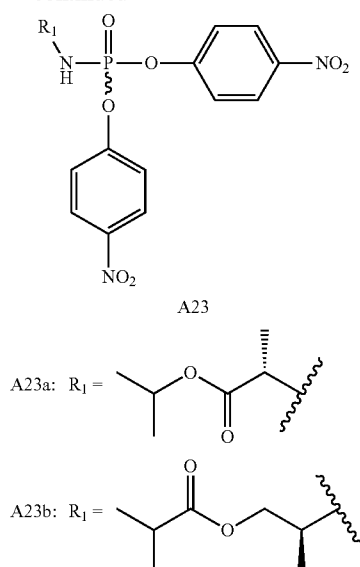
**[0334]**  $^1\text{H}$  NMR (DMSO, 400 MHz):  $\delta$  (ppm) 1.44 (s, 3H), 3.60-3.68 (m, 1H), 3.80-3.94 (m, 3H), 5.39 (t,  $J=4.45$  Hz, 1H), 5.63 (d,  $J=8.26$  Hz, 1H), 5.93 (d,  $J=5.72$  Hz, 1H), 6.21 (s, 1H), 8.16 (d,  $J=8.90$  Hz, 1H), 11.44 (m, 1H); MS (ESI)  $m/z=277$  ( $\text{MH}^+$ ).

#### General Method D

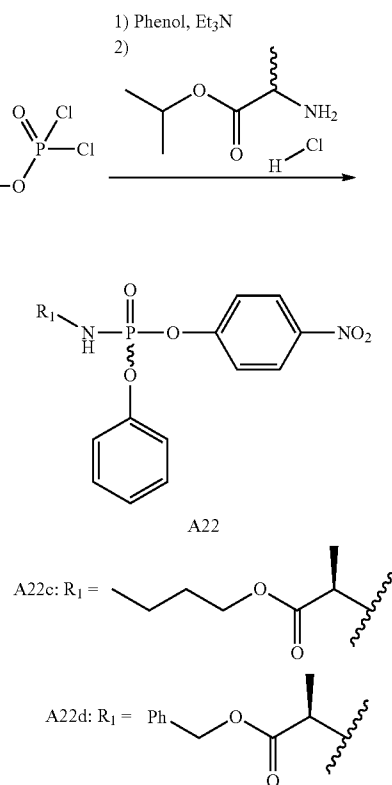
**[0335]** The following procedure was used to obtain intermediates A22a, A22b, A22c and A22d.



-continued



#### Scheme 3A



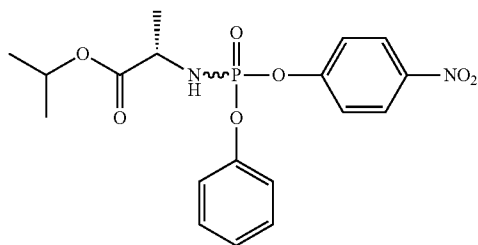
**[0336]** To a stirred solution of 4-nitrophenyl dichlorophosphate (Aldrich) (14.91 mmol) in DCM (30 mL) was added a solution of phenol (Aldrich) (14.91 mmol) and TEA (16.40 mmol) in DCM (30 mL) at  $-78^\circ\text{C}$ . over a period of 20



minutes. The reaction mixture was stirred at  $-78^{\circ}\text{C}$ . during 30 minutes and then, transferred into another round-bottom flask containing L- or D-alanine isopropyl ethyl ester hydrochloride (14.91 mmol) in DCM (30 mL) at  $0^{\circ}\text{C}$ . To the mixture was added TEA (31.31 mmol) over a period of 15 minutes. The reaction mixture was stirred at  $0^{\circ}\text{C}$ . during 1 hour and then, the solvent was evaporated. The residue was triturated with ethyl acetate (45 mL) and the white solid was filtered-off. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (eluent: petroleum ether-petroleum ether/ethyl acetate 20%) to give the expected intermediate.

Isopropyl(2S)-2-[[[4-nitrophenoxy)-phenoxy-phosphoryl]amino]propanoate (A22a)

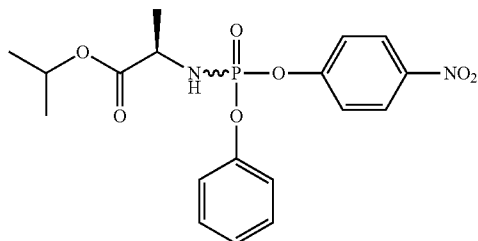
[0337]



[0338] 60% yield;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm) 1.15 (d,  $J=6.26$  Hz, 3H), 1.16 (d,  $J=6.26$  Hz, 3H), 1.33 (m, 3H), 3.83 (dd,  $J=9.7$  and  $11.76$  Hz, 1H), 3.97-4.08 (m, 1H), 4.94 (heptuplet,  $J=6.26$  Hz, 1H), 7.11-7.19 (m, 3H), 7.27-7.35 (m, 4H), 8.16 (dd,  $J=1.72$  and  $9.07$  Hz, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm)-3.21 (s, 0.45P), -3.18 (s, 0.55P); MS (ESI)  $m/z=409.14$  ( $\text{MH}^+$ ).

Isopropyl(2R)-2-[[[4-nitrophenoxy)-phenoxy-phosphoryl]amino]propanoate (A22b)

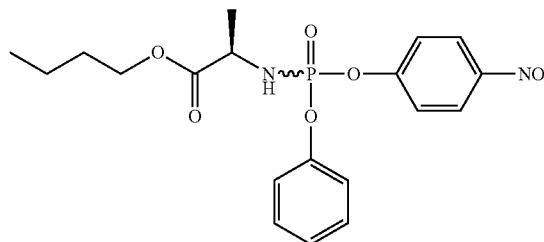
[0339]



[0340] 80% yield;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm) 1.22 (d,  $J=6.28$  Hz, 3H), 1.23 (d,  $J=6.28$  Hz, 3H), 1.40 (m, 3H), 3.91-3.96 (m, 1H), 4.05-4.13 (m, 1H), 5.01 (heptuplet,  $J=6.30$  Hz, 1H), 7.19-7.25 (m, 3H), 7.33-7.41 (m, 4H), 8.22 (dd,  $J=1.74$  Hz and  $8.95$  Hz, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm)-3.21 (s, 0.45P), -3.18 (s, 0.55P); MS (ESI)  $m/z=409.14$  ( $\text{MH}^+$ ).

Butyl (2R)-2-[[[4-nitrophenoxy)-phenoxy-phosphoryl]amino]propanoate (A22c)

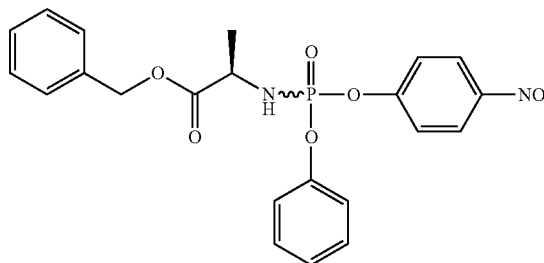
[0341]



[0342] 72% yield; yellow oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm) 0.92 (t,  $J=7.35$  Hz, 3H), 1.30-1.39 (m, 2H), 1.40-1.43 (m, 3H), 1.56-1.63 (m, 2H), 3.84-3.89 (m, 1H), 4.08-4.18 (m, 3H), 7.18-7.26 (m, 3H), 7.33-7.41 (m, 4H), 8.23 (dd,  $J=1.77$  Hz and  $9.01$  Hz, 2H); MS (ESI)  $m/z=423$  ( $\text{MH}^+$ ).

Benzyl (2R)-2-[[[4-nitrophenoxy)-phenoxy-phosphoryl]amino]propanoate (A22d)

[0343]



[0344] 89% yield; yellow oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm) 1.41-1.44 (m, 3H), 3.82-3.88 (m, 1H), 4.13-4.25 (m, 1H), 5.14-5.15 (m, 2H), 7.18-7.24 (m, 3H), 7.28-7.38 (m, 9H), 8.16-8.21 (m, 2H); MS (ESI)  $m/z=457$  ( $\text{MH}^+$ ).

#### General Method F

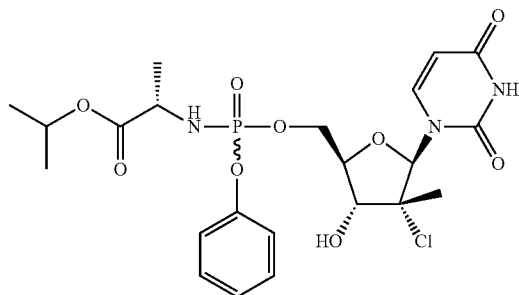
[0345] The following procedure was used to obtain compounds 40i and 40ii.

[0346] To a solution of compound 301 (15 mmol) in THF (5 mL/mmol) was added tert-butylmagnesium chloride (1M in THF) (31 mmol) over a period of 10 minutes. Appropriate intermediate A22 (18 mmol) in THF (20 mL) was added and the reaction mixture was stirred at room temperature during 3 days. The reaction mixture was quenched with saturated aqueous solution of ammonium chloride. The residue was suspended in ethyl acetate and washed with water. The organic layer was washed with aqueous sodium bicarbonate and brine, dried over  $\text{MgSO}_4$ , filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (eluent: DCM-DCM/MeOH 2%) to separate the diastereoisomers.

Compound 40ii

Diastereoisomer 2

[0347]

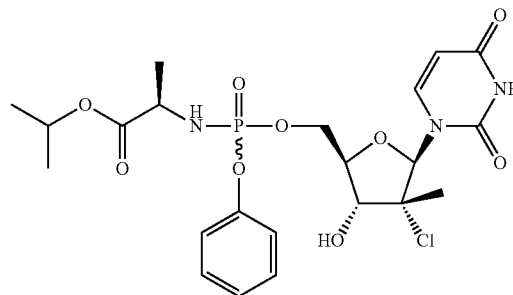


[0348] White solid; 13% yield;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  (ppm) 1.24-1.26 (m, 6H), 1.36 (d,  $J=7.04$  Hz, 3H), 1.59 (s, 3H), 3.69-3.77 (m, 1H), 3.91-3.99 (m, 2H), 4.17-4.19 (m, 1H), 4.43-4.59 (m, 2H), 5.01-5.06 (m, 1H), 5.68 (d,  $J=8.20$  Hz, 1H), 6.42 (s, 1H), 7.21-7.39 (m, 5H), 7.60 (d,  $J=8.20$  Hz, 1H), 8.14 (s, 1H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm) 3.47 (s, 1P); MS (ESI)  $m/z=546.2$  ( $\text{MH}^+$ ).

Compound 40i

Diastereoisomer 1

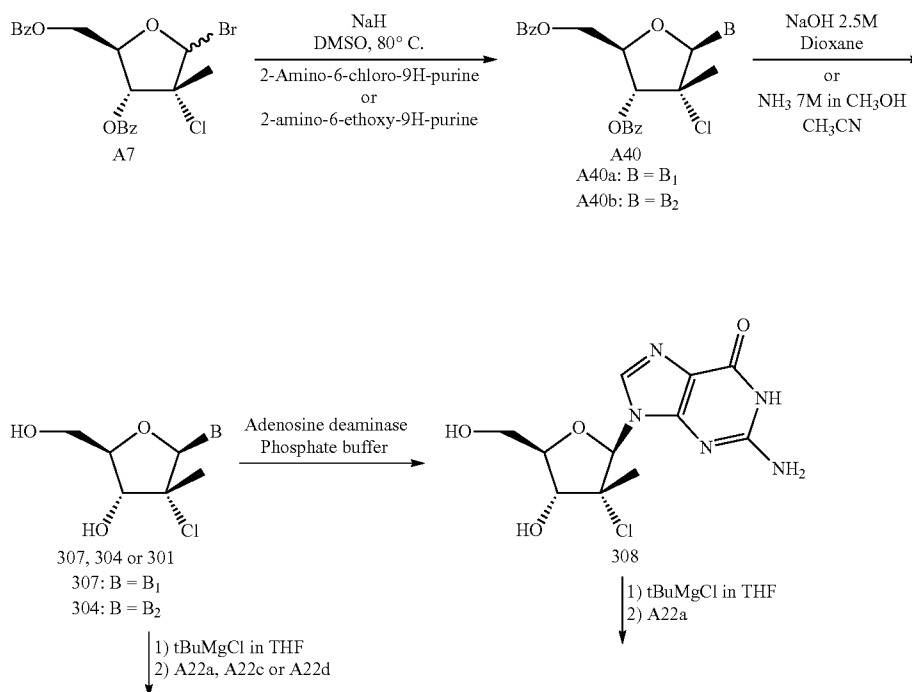
[0349]



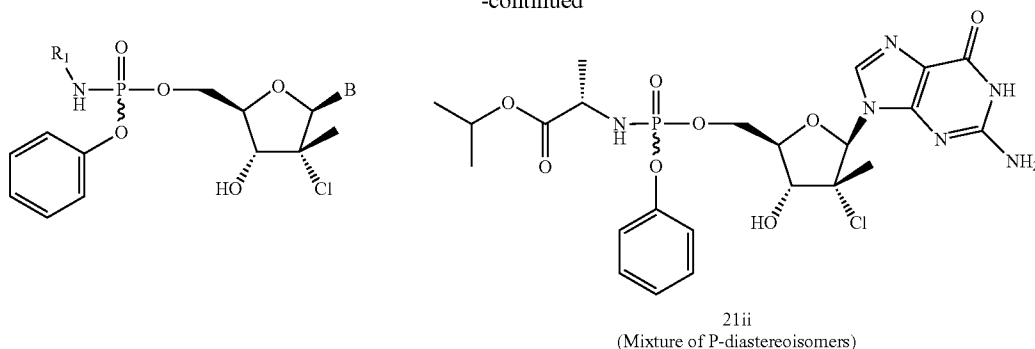
[0350] In this case, after chromatography on silica gel, the mixture of diastereoisomers was purified by preparative HPLC.

[0351] White solid; 3% yield;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.25 (d,  $J=6.25$  Hz, 6H), 1.38 (d,  $J=7.04$  Hz, 3H), 1.51 (s, 3H), 3.66-3.74 (m, 2H), 3.82-3.96 (m, 2H), 4.15 (dd,  $J=1.62$  and 9.24 Hz, 1H), 4.39-4.53 (m, 2H), 5.03 (heptuplet,  $J=6.26$  Hz, 1H), 5.56 (dd,  $J=2.29$  and 8.18 Hz, 1H), 6.39 (s, 1H), 7.19-7.26 (m, 3H), 7.34-7.43 (m, 3H), 8.06 (s, 1H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm) 3.35 (s, 1P); MS (ESI)  $m/z=546.20$  ( $\text{MH}^+$ ).

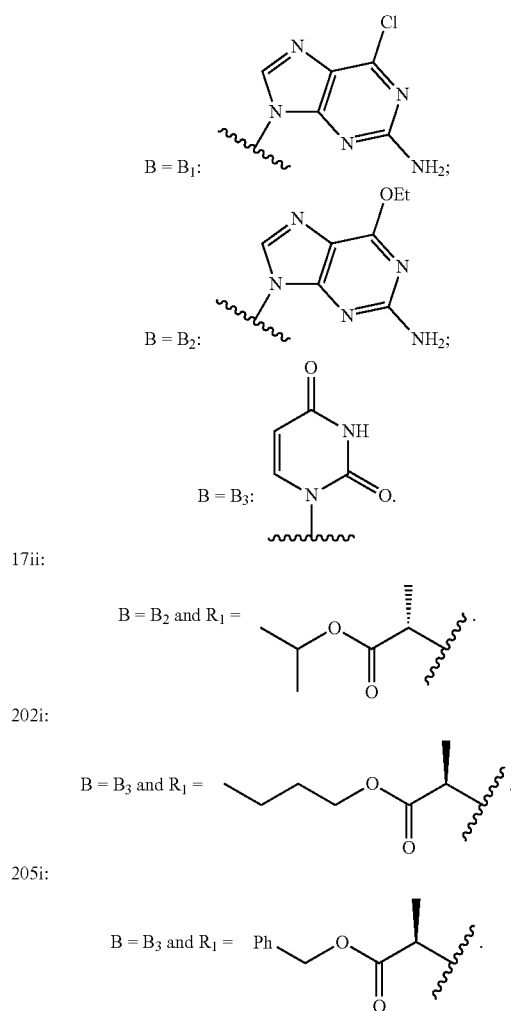
Scheme 8



-continued



[0352] The following abbreviations are used in Scheme 8:



#### General Method K

[0353] The following procedure was used to obtain compounds 202i and 205i.

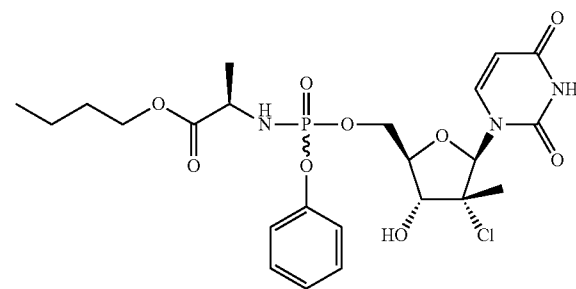
[0354] To a solution of compound 301 (0.72 mmol) in anhydrous THF (7 mL/mmole) under nitrogen at room tem-

perature was added tert-butyldimethylsilyl chloride (1M in THF) (1.52 mmol) followed by compound A22c or A22d (0.795 mmol) solubilized in THF (4 mL/mmole). DMSO (4 mL/mmole) was added and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane and washed with H<sub>2</sub>O. The organic phase was dried, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (eluent: DCM/MeOH 0 to 2%) followed by purification by preparative HPLC to give the expected pure diastereoisomers.

#### Compound 202i

Mixture of Diastereoisomers

[0355]



#### 202i P-Diastereoisomer 1

[0356] 15% yield; white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ (ppm) 0.93 (t, J=7.37 Hz, 3H), 1.32-1.40 (m, 2H), 1.40 (d, J=7.04 Hz, 3H), 1.51 (s, 3H), 1.56-1.65 (m, 2H), 3.63 (d, J=7.70 Hz, 1H), 3.70-3.75 (m, 1H), 3.82-3.86 (m, 1H), 3.92-4.02 (m, 1H), 4.08-4.19 (m, 3H), 4.39-4.52 (m, 2H), 5.56 (d, J=8.20 Hz, 1H), 6.39 (s, 1H), 7.19-7.26 (m, 3H), 7.34-7.38 (m, 2H), 7.41 (d, J=8.21 Hz, 1H), 8.10 (s, 1H); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 161.98 MHz): δ (ppm) 4.27 (s, 1P); MS (ESI, EI<sup>+</sup>) m/z=560 (MH<sup>+</sup>).

#### 202i P-Diastereoisomer 2

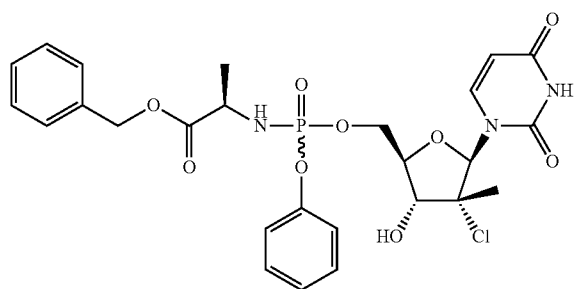
[0357] 18% yield; white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ (ppm) 0.92 (t, J=7.35 Hz, 3H), 1.30-1.38 (m, 2H), 1.37 (d, J=7.13 Hz, 3H), 1.57-1.62 (m, 2H), 1.61 (s, 3H),

3.45-3.53 (m, 2H), 4.00-4.20 (m, 5H), 4.46-4.59 (m, 2H), 5.63 (d, J=8.26 Hz, 1H), 6.44 (s, 1H), 7.19-7.22 (m, 3H), 7.34-7.38 (m, 2H), 7.66 (d, J=8.18 Hz, 1H), 8.04 (s, 1H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm) 3.84 (s, 1P); MS (ESI,  $\text{EI}^+$ )  $m/z=560$  ( $\text{MH}^+$ )

## Compound 205i

Mixture of Diastereoisomers

[0358]



## 205i P-Diastereoisomer 1

**[0359]** 12% yield; white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  (ppm) 1.40 (d, J=7.08 Hz, 3H), 1.49 (s, 3H), 3.55 (d, J=7.83 Hz, 1H), 3.65-3.70 (m, 1H), 3.77-3.81 (m, 1H), 3.97-4.04 (m, 1H), 4.07-4.10 (m, 1H), 4.28-4.45 (m, 2H), 5.16 (s, 2H), 5.54 (d, J=8.22 Hz, 1H), 6.36 (s, 1H), 7.18-7.23 (m, 3H), 7.31-7.38 (m, 8H), 7.99 (s, 1H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm) 4.07 (s, 1P); MS (ESI,  $\text{EI}^+$ )  $m/z=594$  ( $\text{MH}^+$ ).

## 205i P-Diastereoisomer 2

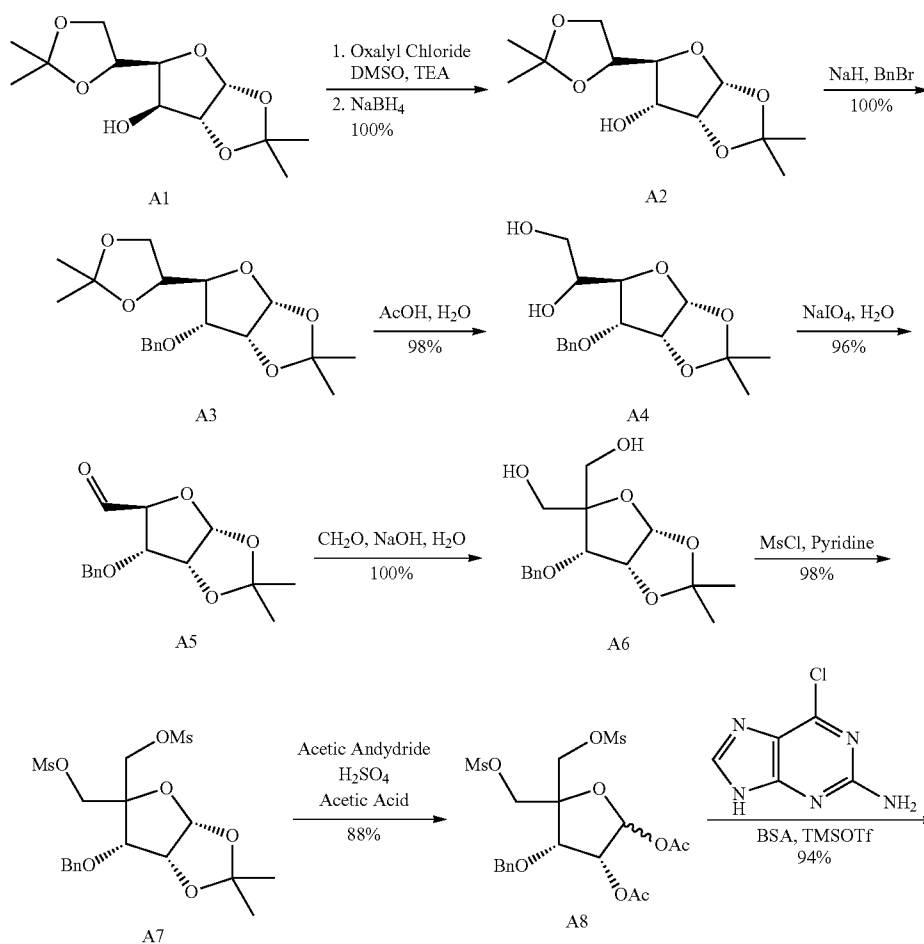
**[0360]** 19% yield; white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  (ppm) 1.38 (d, J=7.11 Hz, 3H), 1.59 (s, 3H), 3.41 (d, J=7.94 Hz, 1H), 3.51-3.56 (m, 1H), 3.98-4.02 (m, 1H), 4.09-4.19 (m, 2H), 4.43-4.57 (m, 2H), 5.13 (s, 2H), 5.61 (d, J=8.27 Hz, 1H), 6.43 (s, 1H), 7.18-7.22 (m, 3H), 7.28-7.39 (m, 7H), 7.63 (d, J=8.16 Hz, 1H), 8.16 (s, 1H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 161.98 MHz):  $\delta$  (ppm) 3.69 (s, 1P); MS (ESI,  $\text{EI}^+$ )  $m/z=594$  ( $\text{MH}^+$ ).

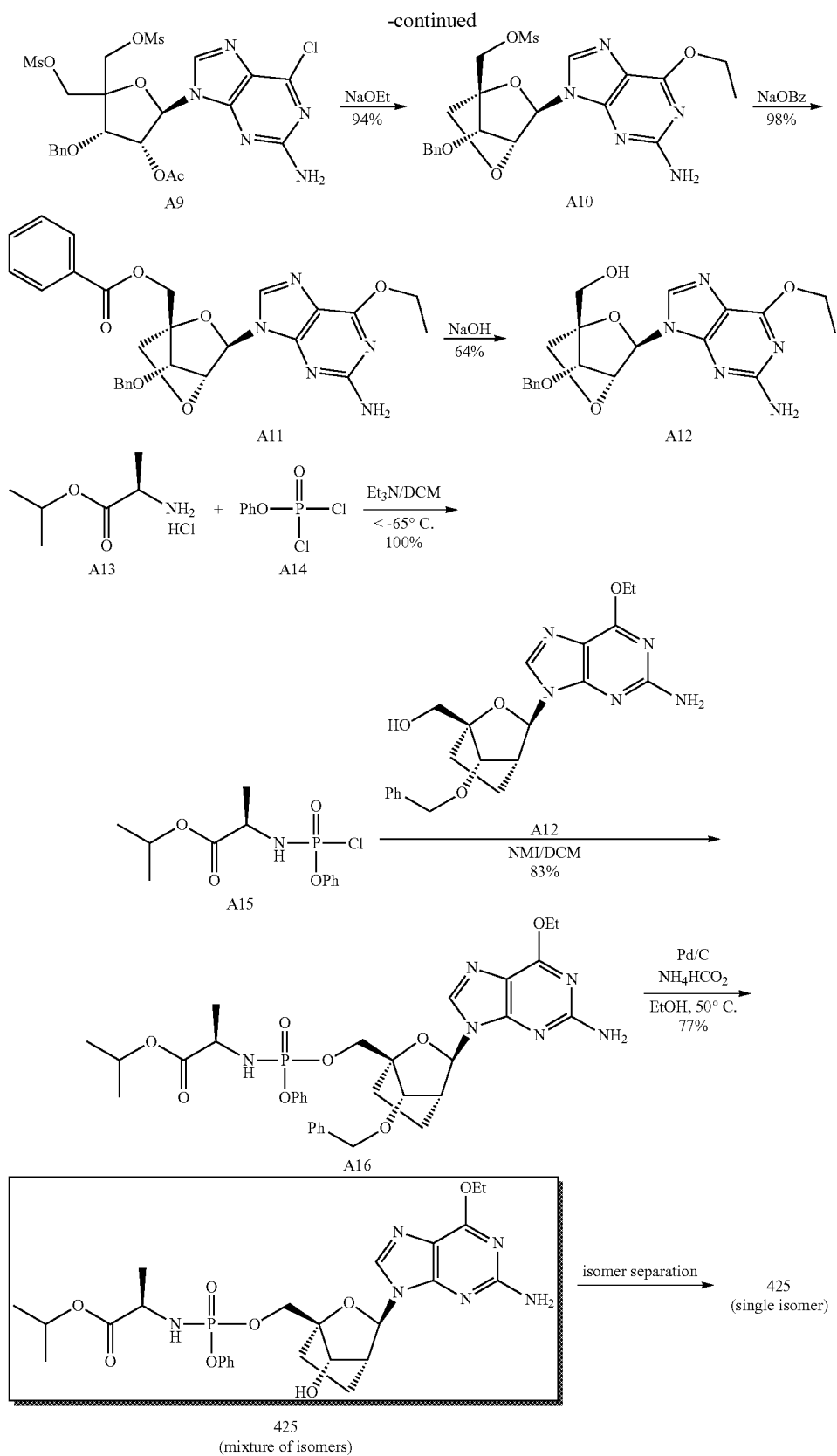
## Example 1B

## Preparation of Bridged Nucleosides

[0361]

Scheme 1

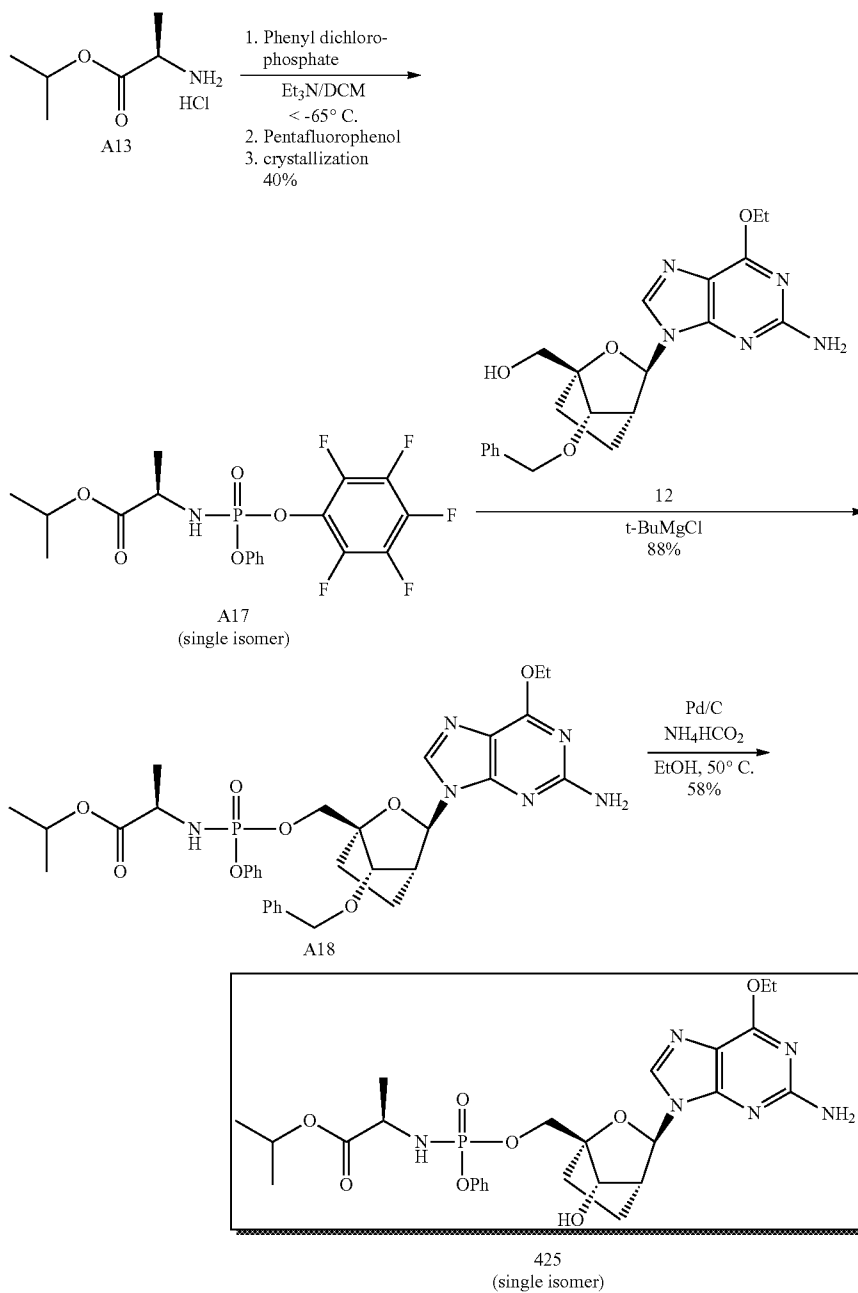




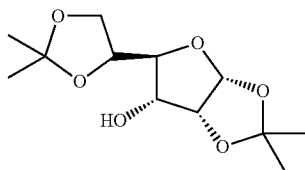
[0362] Isomer separation was carried out using a preparative SFC system equipped with an AS-H chiral column and

using methanol/CO<sub>2</sub> as the mobile phase. Synthesis of single diastereomers was performed as provided in Scheme 2.

Scheme 2



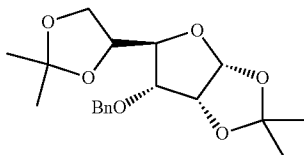
Preparation of compound A2:



A2

**[0363]** DMSO (163.7 mL, 2.31 mol) was added drop-wise to a solution of oxalyl chloride (97.5 mL, 1.15 mol) in DCM (1.5 L) at  $-78^{\circ}\text{C}$ . After 15 min at this temperature a solution of A1 (200 g, 0.77 mol) in DCM (500 mL) was added drop-wise. After additional 15 min at  $-78^{\circ}\text{C}$ , triethylamine (536 mL, 3.84 mol, 5 eq) was added drop-wise. The reaction mixture was allowed to warm to  $-20^{\circ}\text{C}$ , then ethanol (1 L) and water (0.5 L) were added followed by portion-wise addition of  $\text{NaBH}_4$  (30.2 g, 0.8 mol, 1.04 eq). The reaction mixture was allowed to warm to room temperature and stirred for 18 hrs. The reaction mixture was poured into 1M HCl aqueous solution and extracted with DCM. The organic layers were washed with water, brine, dried ( $\text{MgSO}_4$ ) and evaporated to give A2 (200 g, 100%) as an off-white solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.80 (d, 1H), 4.60 (dd, 1H), 4.30 (dt, 1H), 4.10-3.99 (m, 3H), 3.80 (dd, 1H), 2.57 (d, 1H), 1.56 (s, 3H), 1.45 (s, 3H), 1.39 (s, 3H), 1.38 (s, 3H).

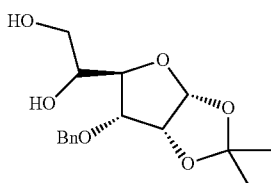
## Preparation of compound A3

**[0364]**

A3

**[0365]** NaH (60% in mineral oil, 14.4 g, 0.36 mol) was suspended in acetonitrile (600 mL) and cooled to  $0^{\circ}\text{C}$ . A solution of A2 (78.0 g, 0.3 mol) in acetonitrile (600 mL) was added drop-wise followed by a solution of benzyl bromide (42.8 mL, 0.36 mol) in acetonitrile (100 mL). The reaction mixture was stirred for 4 h before careful addition of methanol (100 mL). The reaction mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc. The organic layers were combined and dried ( $\text{MgSO}_4$ ) and evaporated to give A3 (115 g, 100%) as a white solid.  $^1\text{H}$  NMR  $\delta$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.41-7.28 (m, 5H), 5.74 (d, 1H), 4.77 (d, 1H), 4.59 (d, 1H), 4.57 (d, 1H), 4.37 (ddd, 1H), 4.13 (dd, 1H), 4.04-3.92 (m, 2H), 3.88 (dd, 1H), 1.58 (s, 3H), 1.36 (s, 3H), 1.35 (s, 3H).

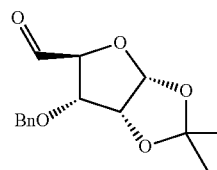
## Preparation of compound A4

**[0366]**

A4

**[0367]** Acetic acid in water (80%, 1 L) was added to A3 (100 g, 0.29 mol) and the mixture stirred at r.t. for 42 h. The reaction mixture was poured into a solution of NaOH solution (540 g in 3 L water) with vigorous stirring then extracted with EtOAc ( $\times 3$ ). The organic layers were combined and dried ( $\text{MgSO}_4$ ) then evaporated to give A4 (86.7 g, 98%) as yellow oil.  $^1\text{H}$  NMR  $\delta$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.47-7.28 (m, 5H), 5.77 (d, 1H), 4.78 (d, 1H), 4.65-4.50 (m, 2H), 4.16-4.10 (m, 1H), 4.03-3.97 (m, 1H), 3.92 (dd, 1H), 3.75-3.61 (m, 2H), 2.50-2.38 (m, 2H), 1.59 (s, 3H), 1.36 (s, 3H).

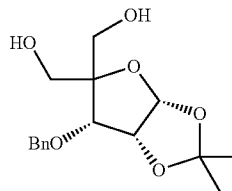
## Preparation of compound A5

**[0368]**

A5

**[0369]** A solution of A4 (23.66 g, 76.2 mmol) in water (250 mL) was slowly added to a solution of sodium periodate (19.08 g, 89.2 mmol) in water (125 mL) at  $0^{\circ}\text{C}$ . After 30 min., ethylene glycol (2.5 mL) was added and the reaction mixture extracted with EtOAc. The organic layers were combined, dried ( $\text{MgSO}_4$ ) and evaporated to give A5 (20.44 g, 96%) as yellow oil.  $^1\text{H}$  NMR  $\delta$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  9.61 (d, 1H), 7.42-7.24 (m, 5H), 5.81 (d, 1H), 4.75 (d, 1H), 4.63 (d, 1H), 4.60 (t, 1H), 4.49 (dd, 1H), 3.85 (dd, 1H), 1.60 (s, 3H), 1.36 (s, 3H).

## Preparation of compound A6

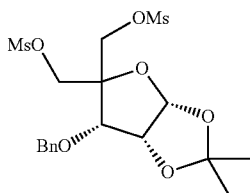
**[0370]**

A6

**[0371]** Aqueous 37% formaldehyde (40 mL) followed by 1N NaOH (200 mL) were added to a solution of A5 (20.44 g, 73.45 mmol) in water (150 mL) and dioxane (50 mL) at  $0^{\circ}\text{C}$ . The reaction mixture was stirred at r.t. for 7 days and then partitioned between EtOAc and brine. The organic layers were combined, dried ( $\text{MgSO}_4$ ) and evaporated to give A6 (22.79 g, 100%) as a pale yellow oil.  $^1\text{H}$  NMR  $\delta$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.41-7.28 (m, 5H), 5.76 (d, 1H), 4.80 (d, 1H), 4.62 (dd, 1H), 4.52 (d, 1H), 4.21 (d, 1H), 3.90 (dd, 2H), 3.78 (dd, 1H), 3.55 (dd, 1H), 2.37 (t, 1H), 1.89 (dd, 1H), 1.63 (s, 3H), 1.33 (s, 3H).

## Preparation of compound A7

[0372]

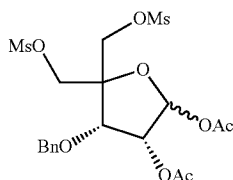


A7

[0373] A solution of A6 (84.98 g, 273.83 mmol) in pyridine (360 mL) was cooled to 0° C. and MsCl (63.89 mL, 825.47 mmol) was added portion wise. After addition, the reaction slurry was stirred at room temperature for 3 hrs before cooled back to 14° C. 55 mL water was added drop-wise over 23 min and temperature rose up to 53° C. Then pyridine was mostly removed using rotary evaporator (bath temperature <40° C.). The mixture was then partitioned into EtOAc 550 mL and water 500 mL. Organic layer was further washed with brine and the first aqueous layer was also back extracted with EtOAc (200 mL). Organic layers were combined and dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated and chased with acetonitrile (100 mL×2) and gave yellow solid A7 (136.61 g, 91.83%). <sup>1</sup>H NMR δ (400 MHz, CDCl<sub>3</sub>) δ 7.42-7.28 (m, 5H), 5.80 (d, 1H), 4.90 (d, 1H), 4.80 (d, 1H), 4.67 (m, 1H), 4.60 (d, 1H), 4.33 (2, 1H), 4.20 (d, 1H), 4.16 (d, 1H), 3.10 (s, 3H), 3.00 (s, 3H), 1.70 (s, 3H), 1.36 (s, 3H) LCMS [M] 485.2.

## Preparation of compound A8

[0374]

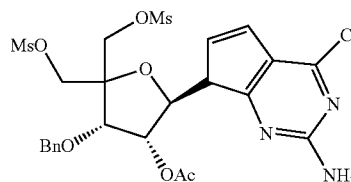


A8

[0375] Compound A7 (136.61 g, 268.9 mmol) was slowly dissolved into acetic acid (1.25 L) and the solution was cooled to 7° C. before acetic anhydride (190 mL, 2010 mmol, 7.5 eq) and concentrated H<sub>2</sub>SO<sub>4</sub> (1.72 mL, 33 mmol, 0.12 eq) were added. The solution was stirred for another 10 min. before warmed up to room temperature. The solution was stirred at room temperature for 18 hrs and then cooled to 9° C. 120 mL of water was added over 2 min and the mixture was stirred at room temperature for 1 hr, before it was partitioned between water 964 mL and DCM 1140 mL. Organic layer was isolated and evaporated in order to remove acetic acid and gave yellow oil. The oil was re-dissolved into DCM (820 mL) and washed twice with saturated NaHCO<sub>3</sub> solution. The solution was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to give yellow oil A8 (127.3 g, 94.7%). <sup>1</sup>H NMR δ (400 MHz, CDCl<sub>3</sub>) δ 7.41-7.29 (m, 5H), 6.20 (s, 1H), 5.40 (d, 1H), 4.64 (d, 1H), 4.52 (m, 2H), 4.44 (m, 1H), 4.39 (d, 1H), 4.31 (d, 1H), 4.21 (m, 1H). LCMS [M+CH<sub>3</sub>COO-] 569.0.

## Preparation of compound A9

[0376]

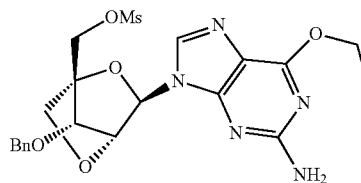


A9

[0377] At room temperature, into the mixture of A8 (113.04 g, 221.4 mmol) and chloro-purine (41.31 g, 243.6 mmol) in 1,2-dichloroethane (1.36 L), was added N,O-Bis(trimethylsilyl)acetamide (108.4 mL, 443.3 mmol). The slurry was then heated to 81° C. for 40 min. and cooled back to 29° C. TMSOTf (81.34 mL, 444.9 mmol) was added all at once and temperature rose to 36° C. The mixture was then heated to 81° C. again for 2 hrs before it was cooled down to room temperature. 1,2-dichloroethane was then removed using rotary evaporator and remaining mixture was partitioned into DCM (1.15 L) and saturated NaHCO<sub>3</sub> solution (0.64 L). Solid crashed and slurry was filtered and solid rinsed with 65 mL of DCM. Filtrate and rinse were combined and the organic layer was washed again with sat NaHCO<sub>3</sub> solution, 5% brine solution and dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated to give a foamy solid A9 (136.79 g, 94.7%). <sup>1</sup>H NMR δ (400 MHz, CDCl<sub>3</sub>) δ 7.69 (s, 1H), 7.34-7.27 (m, 5H), 5.92 (d, 1H), 5.57 (t, 1H), 5.32 (b, 2H), 5.13 (d, 1H), 4.73 (d, 1H), 4.60 (m, 3H), 4.33 (t, 2H), 2.97 (s, 3H), 2.94 (s, 3H), 2.04 (s, 3H) LCMS [M] 620.15

## Preparation of compound A10

[0378]



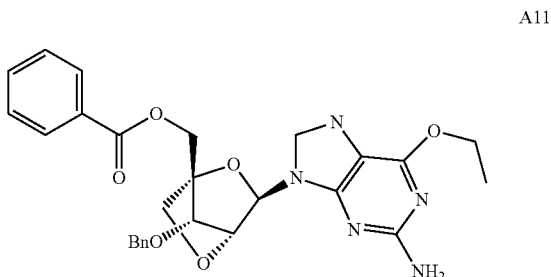
A10

[0379] Compound A9 (136.79 g, 207.4 mmol) was mixed with 1.3 L THF and 1.3 L EtOH. The solution was cooled to 0° C., before NaOEt (95%, 81.71 g, 1140.6 mmol) was added in portions. The mixture was stirred and warmed up to room temperature over 18 hrs. The mixture was then cooled to 0° C. before HC12N (650 mL) was added in portions. Organic solvents were removed and remaining crude oil was partitioned into 1.0 L EtOAc and 150 mL water. Aqueous layer was back extracted with EtOAc (200 mL) and all organic layers were combined and washed with 5% brine solution (400 mL×4). Organic layer was isolated and dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated and gave brown powder A10 (100.0 g, 93.9%). <sup>1</sup>H NMR δ (400 MHz, CDCl<sub>3</sub>) δ 7.63 (s, 1H), 7.37-7.30 (m, 5H), 5.93 (d, 1H), 4.93 (b, 2H), 4.76 (s, 1H), 4.73-4.54 (m, 6H), 4.40 (s, 1H), 4.20 (d, 1H), 4.01 (d, 1H), 3.04 (s, 3H), 1.49 (t, 3H). LCMS [M+H] 492.19.



## Preparation of compound A11

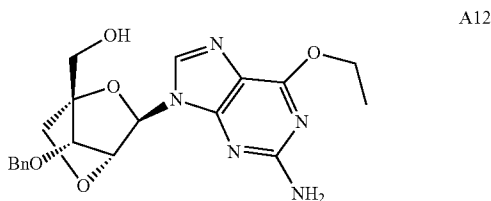
[0380]



[0381] Compound A10 (113.5 g, 219.7 mmol) was dissolved into DMSO (114 mL). Then NaOBz powder (99.41 g, 689.9 mmol) was added. The slurry was heated to 97° C. for 2.5 hrs before it was cooled down and mixed with water (1 L), and then with EtOAc (1 L). Aqueous layer was further washed with EtOAc (0.7 L) and all EtOAc layers were combined and washed with saturated NaHCO<sub>3</sub> solution (0.72 L), and with 5% brine solution (0.75 L×2). EtOAc layer was dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated and gave powder A11 (118.6 g, 93.6%). <sup>1</sup>H NMR δ (400 MHz, CDCl<sub>3</sub>) δ 7.93 (t, 2H), 7.87 (s, 1H), 7.69 (m, 1H), 7.53 (m, 2H), 7.34-7.28 (m, 5H), 6.54 (b, 2H), 5.92 (s, 1H), 4.82 (s, 1H), 4.76 (d, 2H), 4.71 (d, 2H), 4.59 (s, 1H), 4.46 (m, 2H), 4.12 (m, 1H), 4.05 (m, 1H), 1.36 (t, 3H). LCMS [M+H] 518.27.

## Preparation of compound A12

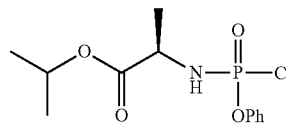
[0382]



[0383] Compound A11 (118.6 g, 214.5 mmol) was dissolved into THF (1.1 L). Then into the solution was added NaOH aq (NaOH 30.89 g, 772.2 mmol, 3.6 eq. with water 0.5 L) at room temperature. The mixture was stirred over 16 hrs and then heated to 35° C. for 6.5 hrs. The reaction mixture was cooled to 1° C. and HCl (1N 550 mL) was added. Organic layer and aqueous layer were separated. The aqueous layer was back extracted with EtOAc (0.5 L) and both organic layers were combined and washed with saturated NaHCO<sub>3</sub> (450 mL) and then with 5% brine (450 mL×2). Brine washes were combined and washed with EtOAc (200 mL). All organic layers were combined and dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated and gave crude solid (103 g). The crude solid was purified on column (1.5 kg Gold combiflash column, with solvents DCM and EtOAc), and gave pure solid compound A12 (56.49 g, 96%). <sup>1</sup>H NMR δ (400 MHz, CDCl<sub>3</sub>) δ 7.93 (s, 1H), 7.34-7.27 (m, 5H), 6.53 (br, 2H), 5.84 (s, 1H), 5.15 (t, 1H), 4.65 (d, 3H), 4.46 (q, 2H), 4.29 (s, 1H), 3.95 (d, 1H), 3.81 (m, 3H), 3.18 (d, 1H), 1.36 (t, 3H). LCMS [M+H] 414.20.

## Preparation of compound A15

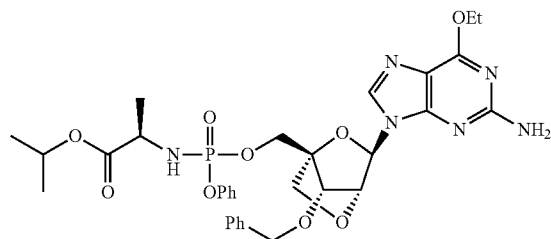
[0384]



[0385] To a stirred solution of D-alanine isopropyl ester HCl A13 (14.2 g, 84.66 mmol) and phenyl dichlorophosphate 14 (12.6 mL, 84.66 mmol) in DCM (142 mL) at -70° C. was added a solution of triethylamine (24.7 mL) in DCM (142 mL) over 50 min. The mixture was stirred at this temperature for additional 1.5 hrs. The mixture was filtered through a sintered glass funnel and the filtrate was concentrated under reduced pressure. The residue was triturated with TBME (120 mL), filtered off and rinsed with TBME (2×120 mL). The combined filtrate was concentrated under reduced pressure to give A15 (25.9 g, 100%), which was used for the following coupling reaction without further purification.

## Preparation of compound A16

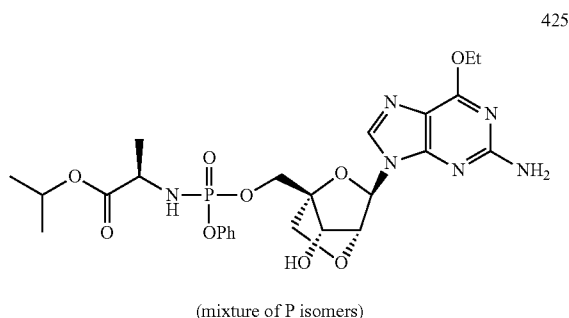
[0386]



[0387] To a stirred solution of nucleoside A12 (10 g, 24.19 mmol) and N-methylimidazole (15.4 mL, 193.52 mmol) in DCM (200 mL) at 5° C., was added a solution of compound A15 (25.9 g, 84.66 mmol) in DCM (45 mL) over 1 hr. The mixture was allowed to warm to rt overnight and then concentrated under reduced pressure to give yellow oil. This oil was diluted with EtOAc (200 mL) and water (200 mL). The organic layer was separated, washed with 5% aqueous ammonium chloride solution (2×200 mL) and 5% brine solution (200 mL), dried (sodium sulphate), filtered and concentrated under reduced pressure to give crude product (29.9 g). The crude compound was chromatographed using EtOAc/dichloromethane 3:2 gradient to give product A16 (13.8 g, 83%) as off-white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 7.92 (s, 1H), 7.17-7.34 (m, 10H), 6.54 (br s, 2H), 6.07 (q, 1H), 5.88 (d, 1H), 4.84 (m, 1H), 4.76 (d, 1H), 4.68 (d, 1H), 4.47 (m, 5H), 4.03 (m, 1H), 3.84 (m, 2H), 1.37 (t, 3H), 1.19 (m, 4H), 1.13 (m, 6H); <sup>31</sup>P NMR 3.70, 3.48; HPLC (test20) 5.42 min; LCMS 16.35 min (M<sup>+</sup>+H) 683.33.

## Preparation of 425 isomer mixture

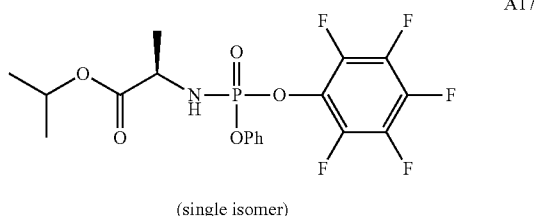
[0388]



[0389] To a stirred mixture of Pd/C (5.4 g) in EtOH (140 mL) at 22° C. was added a solution of nucleoside A16 (13.8 g, 20.21 mmol) in EtOH (560 mL), and the reaction mixture was heated to 50° C. for 45 min. The crude mixture was filtered through a Celite pad and rinsed with MeOH (4×250 mL). The combined filtrate was concentrated under reduced pressure to give 12.4 g of crude product. The crude compound was chromatographed using 0-5% MeOH/dichloromethane gradient to give 425 (mixture of isomers, 9.3 g, 77% yield) as an off-white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 7.96 (s, 1H), 7.17-7.37 (m, 5H), 6.54 (br s, 2H), 6.06 (q, 1H), 5.90 (d, 1H), 5.81 (d, 1H), 4.86 (m, 1H), 4.32-4.47 (m, 6H), 3.97 (d, 1H), 3.79 (m, 2H), 1.37 (t, 3H), 1.34 (m, 3H), 1.16 (m, 6H); <sup>31</sup>P NMR 3.83, 3.63; HPLC (test20) 4.34 min; LCMS 11.72 min (M++H) 593.27.

## Preparation of the compound A17

[0390]

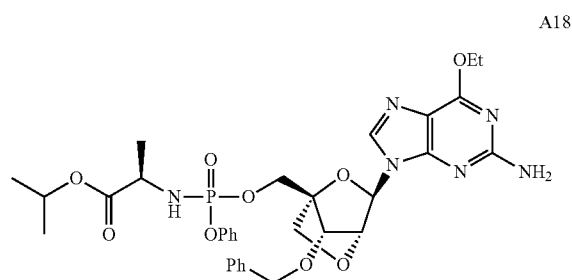


[0391] To a stirred solution of D-alanine isopropyl ester hydrochloride A13 (20 g, 119.3 mmol) and phenyl dichlorophosphate (25.3 g, 17.9 mL, 118.8 mmol) in anhydrous dichloromethane (150 mL) was added a solution of triethylamine (25.4 g, 35 mL, 251.3 mmol) in anhydrous dichloromethane (150 mL) at -70° C. over 45 min dropwise. The reaction mixture was stirred at this temperature for additional 30 min and then allowed to warm to 0° C. over 2 h and stirred for 1 h. To this mixture was added a solution of 2,3,4,5,6-pentafluorophenol (22 g, 119.5 mmol) and triethylamine (1.3 g, 17 mL, 122 mmol) in anhydrous dichloromethane (75 mL) over 40 min. The crude mixture was stirred at 0° C. for 2 h and then stored at 5° C. over night. The white solid (triethylamine hydrochloride) was filtered off and washed with dichloromethane (1×25 mL). The filtrate was concentrated under reduced pressure, the residue was triturated with TBME (300 mL) and the triethylamine hydrochloride salt was removed by

filtration. The cake was washed with dichloromethane (2×25 mL) and the combined filtrate was concentrated under reduced pressure to give the crude solid containing even mixture of diastereomers. The mixture was triturated with 20% EtOAc in hexanes (200 mL) to give 29.5 g of compound A17 as a white solid. This was further purified using a mixture of IPA (240 mL) and water (290 mL) to give the desired compound A17 (21.5 g, 40%). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz) δ -1.56; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.40-7.36 (m, 2H), 7.29-7.21 (m, 3H), 5.10-5.01 (1H), 4.21-4.02 (m, 2H), 1.47 (d, J=7.2 Hz, 3H), 1.29-1.24 (m, 6H).

## Preparation of compound A18

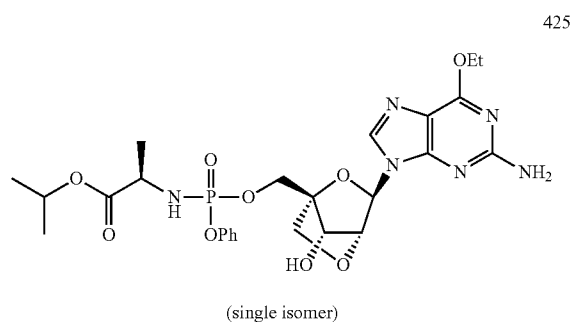
[0392]



[0393] To the stirred solution of compound A17 (1.5 g, 3.63 mmol) in dry THF (35 mL) was added a 1.0 M solution of tert-butylmagnesium chloride in THF (4.5 mL, 5.4 mmol) over 7 min at -9° C. The reaction mixture was stirred at that temperature for 10 min and a solution of compound A2 (2 g, 4.4 mmol) in THF (10 mL) was added over 10 min at -9° C. The crude reaction mixture was stirred at that for additional 40 min, warmed to rt over a period of 1 h, and then quenched with 2 N HCl (20 mL). Toluene (100 mL) was added and the layers separated, aqueous layer re-extracted with toluene (50 mL). The combined toluene layer was washed with brine (1×50 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the crude product (4.2 g). The crude compound was chromatographed using 0-5% MeOH/dichloromethane gradient to give product A18 (2.2 g, yield ~88%). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz) δ 2.43; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.68 (s, 1H), 7.29-7.22 (m, 9H), 7.16-7.12 (m, 1H), 5.90 (s, 1H), 5.02-4.98 (m, 2H), 4.64-4.55 (m, 2H), 4.46-4.45 (m, 3H), 4.13-4.11 (m, 2H), 4.02-3.92 (m, 2H), 3.83-3.80 (m, 1H), 1.48 (t, J=7.2 Hz, 3H), 1.39-1.37 (m, 4H), 1.27-1.19 (m, 6H); LCMS: 683.33 (MH+).

## Preparation of 425

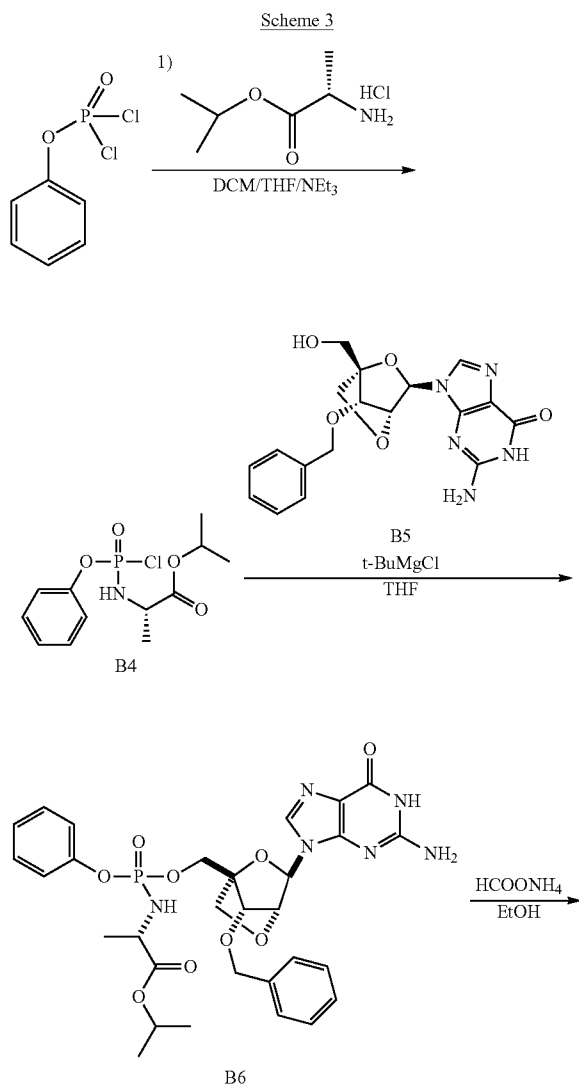
[0394]



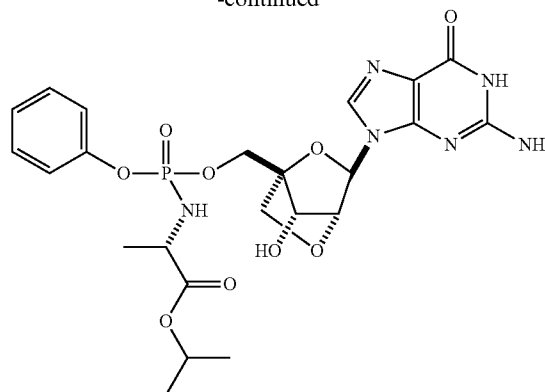
**[0395]** To a stirred solution of compound A18 (2.0 g, 2.93 mmol) in ethanol (40 mL) was added Pd/C (10%, 1.1 g). The crude mixture was heated at 50° C. and ammonium formate (0.96 g, 15.24 mmol) was added. The reaction mixture was heated for additional 1.5 hrs and filtered over a pad of celite. The celite bed washed with MeOH (30 mL) and the filtrate was concentrated to give 3 g of crude product. The crude product was chromatographed using 0-5% MeOH/dichloromethane gradient to give 25 (1.0 g, yield 58%). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz) δ 3.61; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ 7.93 (s, 1H), 7.38-7.34 (m, 2H), 7.23-7.15 (m, 3H), 6.15 (bs, 2H), 6.09-6.04 (m, 1H), 5.95 (d, J=4 Hz, 1H), 5.80 (s, 1H), 4.89-4.86 (m, 1H), 4.51-4.29 (m, 6H), 3.99 (d, J=8 Hz, 1H), 3.82-3.73 (m, 2H), 1.35 (t, J=7.2 Hz, 3H), 1.23 (d, J=7.2 Hz, 3H), 1.16-1.14 (m, 6H); LCMS: 593.23 (MH<sup>+</sup>).

## Preparation of 401

**[0396]**



-continued



401

(2S)-isopropyl 2-((((1R,3R,4R,7S)-3-(2-amino-6-oxo-1H-purin-9(6H)-yl)-7-(benzyloxy)-2,5-dioxabicyclo[2.2.1]heptan-1-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (B6)

**[0397]** To a solution of phenyl dichlorophosphate (7394, 4.7 mmol) in THF (10 mL) at -55.6° C. under Argon, was added L-alanine isopropyl ester (827 mg, 4.93 mmol, 1.05 eq.) dissolved in 8.3 mL of DCM over 5 min. (-44.3° C.), Triethylamine (1.38 mL, 9.87 mmol, 2.1 eq.) was added over 3 min (-48.6° C. → -40° C.). The reaction was kept <-30° C. and reaction followed by LCMS, <sup>1</sup>H and <sup>31</sup>P NMR that indicated reaction completion after 25 min. to give compound B4.

**[0398]** To a suspension of B5 (1 g, 2.35 mmol, 0.5 eq.) in THF/DCM (10/5 mL) at -40° C. under Argon, was added t-BuMgCl (5.17 mL, 5.17 mmol, 1.1 eq.) over 4 min. (-32.1° C.). The reaction mixture was kept stirring at 0° C. for 45 min (B5 completely solubilized). To this solution cooled at -50° C. was added the previous chlorophosphoramidate solution (compound 4) over 7 min (-36.8° C.) and 5 mL of THF was used to rinse the remaining phosphoramidate compound. The reaction was kept stirring at 0° C. for 30 min., the reaction was followed by LCMS.

**[0399]** To the reaction mixture was added 25 mL 5% brine and 25 mL ethyl acetate. The organic was separated then washed with 20 mL 5% brine. The organic layer was then dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to give 2.47 g of a yellow oil. The crude product was purified by Combiflash (80 g gold column, DCM 100% → DCM/MeOH 90/10). Compound B6 was isolated as a white solid (818.8 mg, 51%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.90 (d, J=4 Hz, 1H), 7.36-7.30 (m, 7H), 7.19-7.15 (m, 3H), 6.54 (br s, 2H), 6.08 (m, 1H), 5.88 (d, J=8 Hz, 1H), 5.01 (m, 1H), 4.77 (d, J=12 Hz, 1H), 4.66 (m, 2H), 4.50-4.38 (m, 5H), 4.02 (dd, J=8 Hz, 20 Hz, 1H), 3.86-3.74 (m, 2H), 1.39 (t, J=8 Hz, 3H), 1.23 (m, 9H). <sup>31</sup>P NMR (400 MHz, DMSO-d<sub>6</sub>) δ 3.72, 3.65. (M+H<sup>+</sup>) 709.

(2S)-isopropyl 2-((((1R,3R,4R,7S)-3-(2-amino-6-oxo-1H-purin-9(6H)-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1] heptan-1-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (401)

**[0400]** To a solution of B6 (350 mg, 0.484 mmol) in ethanol (17.5 mL) was added Pd/C (128.7 mg, 0.121 mmol, 0.25 eq.). The mixture was heated at 50° C. and ammonium formate (152.6 mg, 2.42 mmol, Seq.) was added. The reaction mixture was kept stirring at 50° C. for 30 min. After cooling down to room temperature, the mixture was filtered through a celite pad and solid rinsed with 3×10 mL of methanol. The filtrate was concentrated and dissolved in 10 mL DCM, washed with 10 mL ¼ saturated. NaHCO<sub>3</sub> solution, 10 mL water. The second organic solution was extracted with 10 mL DCM. The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to give 311 mg of a white solid. This crude product was purified using Combiflash (4 g gold column DCM 100%→DCM/MeOH 90/10). Compound 7 was isolated as a white solid (237 mg, 79%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.95 (s, 1H), 7.37-7.35 (m, 2H), 7.22-7.18 (m, 3H), 6.54 (br s, 2H), 6.04 (m, 1H), 5.90-5.76 (m, 2H), 4.87 (sept, J=4 Hz, 1H), 4.49-4.4 (m, 5H), 4.30 (t, J=4 Hz, 1H), 4.01 (dd, J=8 Hz, 24 Hz, 1H), 3.85 (m, 2H), 1.38 (t, J=8 Hz, 3H), 1.25 (m, 3H), 1.16 (m, 6H). <sup>31</sup>P NMR (400 MHz, DMSO-d<sub>6</sub>) δ 3.85, 3.73. (M+H<sup>+</sup>).

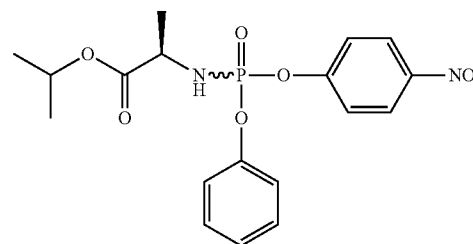
#### Example 1C

#### Compound 502a

**[0401]**

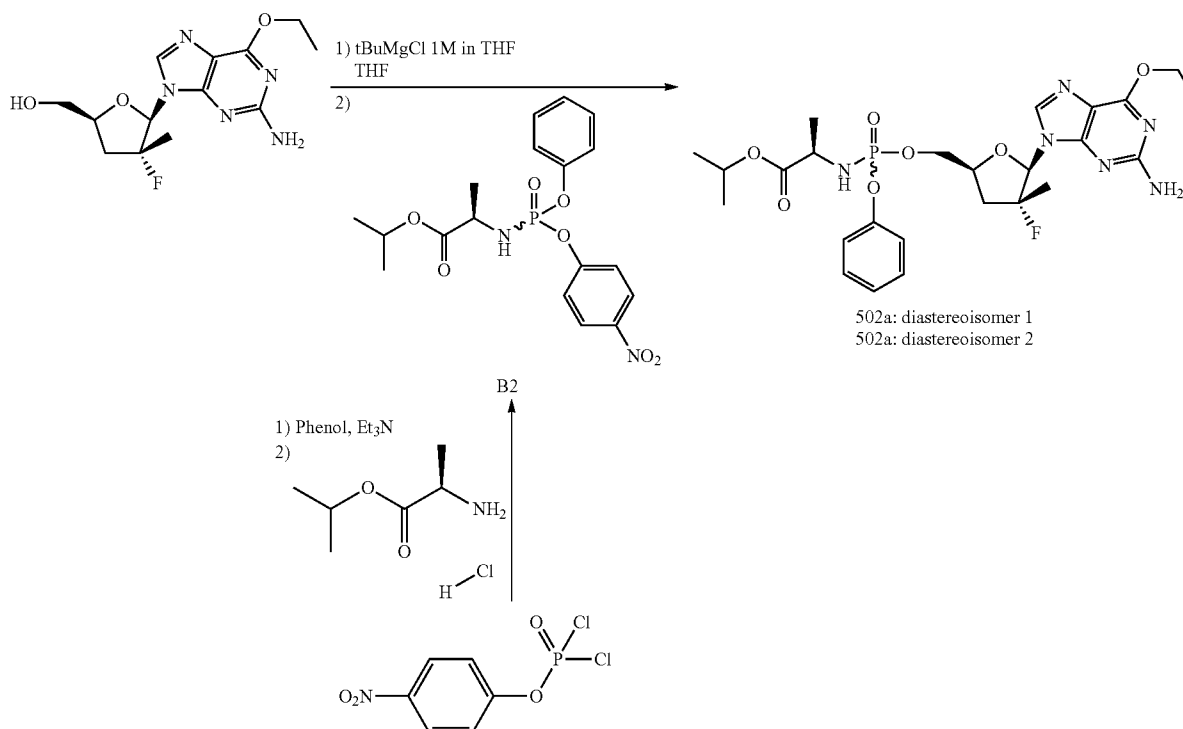
#### Intermediate B2

**[0402]**



**[0403]** To a stirred solution of 4-nitrophenyl dichlorophosphate (Aldrich) (35.97 mmol) in DCM (2 mL/mmol) was added a solution of phenol (Aldrich) (35.97 mmol) and TEA (39.57 mmol) in DCM (2 mL/mmol) at -78° C. over a period of 20 minutes. The reaction mixture was stirred at -78° C. during 30 minutes and then, transferred into another round-bottom flask containing D-alanine isopropyl ester hydrochloride (35.97 mmol) in DCM (2 mL/mmol) at 0° C. To the mixture was added TEA (31.31 mmol) over a period of 15 minutes. The reaction mixture was stirred at 0° C. during 1 hour and then, the solvent was evaporated. The residue was triturated with ethyl acetate (45 mL) and the white solid was filtered-off. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (eluent: petroleum ether-petroleum ether/ethyl acetate 20%) to give the expected compound in 80% yield; <sup>1</sup>H NMR

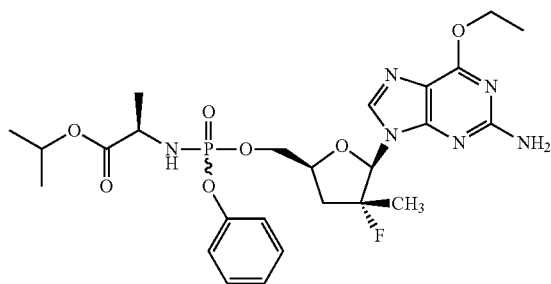
Scheme 2



(CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm) 1.22 (d, J=6.28 Hz, 3H), 1.23 (d, J=6.28 Hz, 3H), 1.40 (m, 3H), 3.91-3.96 (m, 1H), 4.05-4.13 (m, 1H), 5.01 (heptuplet, J=6.30 Hz, 1H), 7.19-7.25 (m, 3H), 7.33-7.41 (m, 4H), 8.22 (dd, J=1.74 and 8.95 Hz, 2H); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 161.98 MHz):  $\delta$  (ppm)-3.21 (s, 0.45P), -3.18 (s, 0.55P); MS (ESI) m/z=409.14 (MH<sup>+</sup>).

Compound 502a

[0404]



[0405] To a solution of 3'-deoxy nucleoside (0.803 mmol) in anhydrous THF (4 mL) at room temperature under nitrogen was added dropwise tert-butylmagnesium chloride (1M in THF) (1.69 mmol) followed by DMSO (0.6 mL). The heterogeneous reaction mixture was stirred during 30 minutes at room temperature. Compound B2 (0.964 mmol) in THF (2.4 mL) was added dropwise and the reaction mixture was abandoned at room temperature all the weekend. The reaction mixture was quenched with saturated aqueous solution of NH<sub>4</sub>Cl and diluted with ethyl acetate. The mixture was extracted with ethyl acetate and the organic layer was washed with H<sub>2</sub>O and NaHCO<sub>3</sub>. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (eluent: CH<sub>2</sub>Cl<sub>2</sub>—CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH) and by preparative HPLC to give two pure diastereoisomers.

Compound 502a, Diastereoisomer 1

[0406] white solid; 14% yield; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  (ppm) 1.09 (d, J=6.24 Hz, 3H), 1.12 (d, J=6.24 Hz, 3H), 1.13 (d, J=21.79 Hz, 3H), 1.19 (d, J=7.11 Hz, 3H), 1.35 (t, J=7.11 Hz, 3H), 2.28-2.37 (m, 1H), 3.70-3.80 (m, 1H), 4.23-4.29 (m, 1H), 4.35-4.40 (m, 1H), 4.45 (q, J=7.11 Hz, 2H), 4.47-4.51 (m, 1H), 4.83 (heptuplet, J=6.24 Hz, 1H), 6.04-6.09 (m, 1H), 6.06 (d, J=18.25 Hz, 1H), 6.56 (s, 2H), 7.14-7.17 (m, 1H), 7.20-7.22 (m, 2H), 7.32-7.36 (m, 2H), 7.94 (s, 1H); <sup>31</sup>P NMR (DMSO-d<sub>6</sub>, 161.98 MHz)  $\delta$  (ppm) 3.6 (s, 1P); MS (ESI) m/z=581.12 (MH<sup>+</sup>).

Compound 502a, Diastereoisomer 2

[0407] white solid; 6% yield; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  (ppm) 1.10 (d, J=6.21 Hz, 3H), 1.11 (d, J=6.21 Hz, 3H), 1.13 (d, J=6.95 Hz, 3H), 1.16 (d, J=21.98 Hz, 3H), 1.35 (t, J=7.10 Hz, 3H), 2.28-2.37 (m, 1H), 3.70-3.80 (m, 1H), 4.26-4.32 (m, 1H), 4.38-4.43 (m, 1H), 4.44 (q, J=7.12 Hz, 2H), 4.47-4.54 (m, 1H), 4.81 (heptuplet, J=6.23 Hz, 1H), 5.98 (dd, J=9.96 Hz and 12.72 Hz, 1H), 6.09 (d, J=18.27 Hz, 1H), 6.55 (s, 2H), 7.14-7.18 (m, 3H), 7.33-7.37 (m, 2H), 7.99 (s, 1H); <sup>31</sup>P NMR (DMSO-d<sub>6</sub>, 161.98 MHz)  $\delta$  (ppm) 3.97 (s, 1P); MS (ESI) m/z=581.08 (MH<sup>+</sup>).

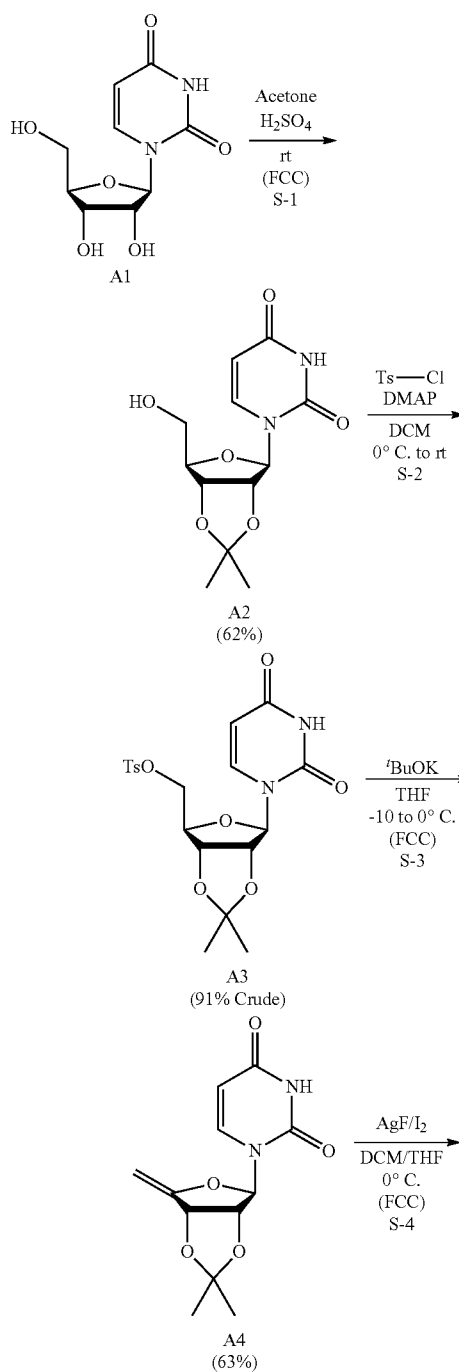
Example 1D

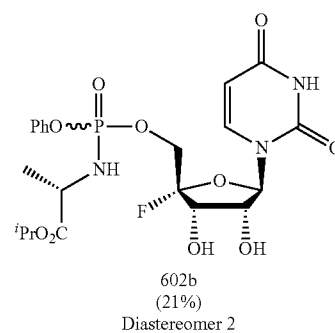
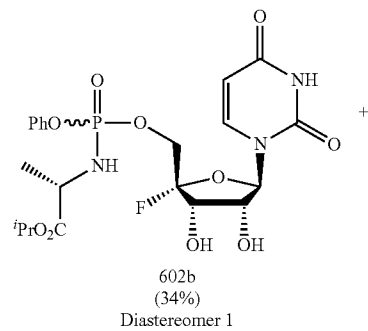
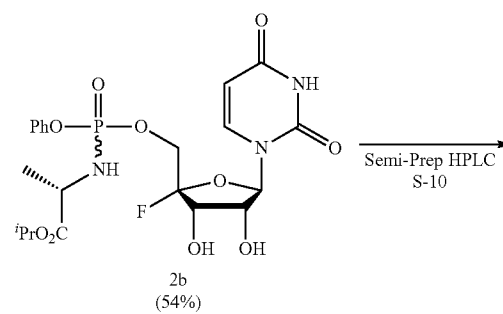
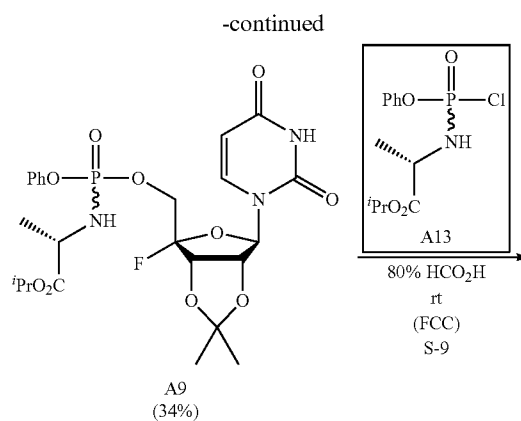
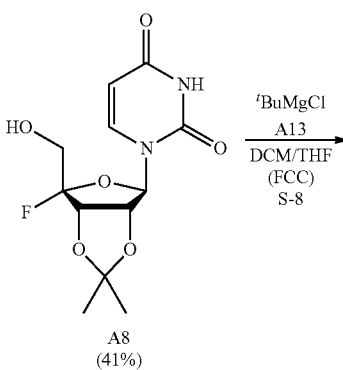
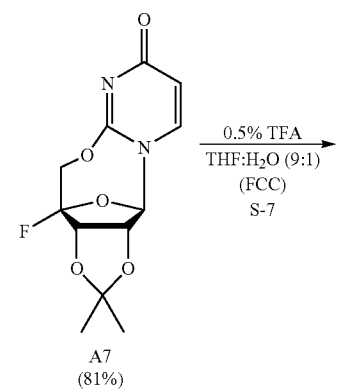
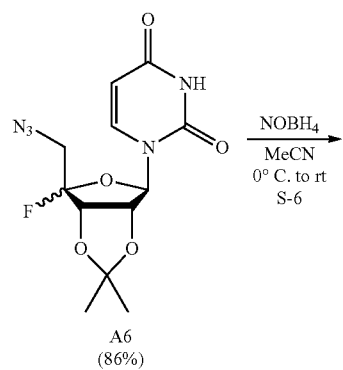
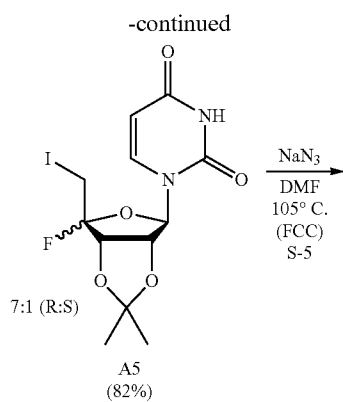
Preparation of 4'-fluoro nucleosides

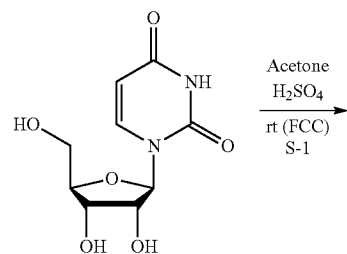
Compound 602b

Two Diastereomers

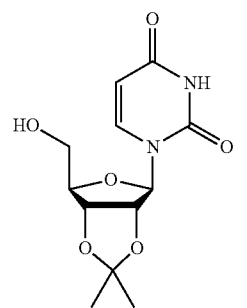
[0408]





**[0409] Step 1:**

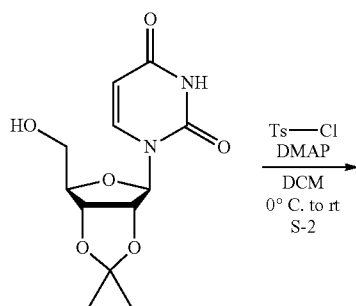
A1  
Chemical Formula:  $C_9H_{12}N_2O_6$   
Molecular Weight: 244.20



A2  
Chemical Formula:  $C_{12}H_{16}N_2O_6$   
Molecular Weight: 284.27

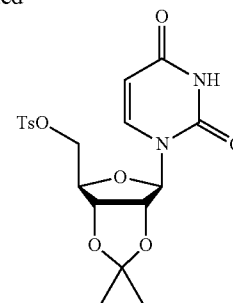
**[0410]** Uridine (10 grams, 40.1 mmol) was dissolved in acetone (100 mL) containing sulfuric acid (conc., 1.0 mL). After stirring at room temperature overnight, the mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (Silica Gel, 100% DCM to 4% MeOH/DCM) to afford 11.0 grams of the acetonide A2 (98%).

**[0411]** HPLC (Method A, 254 nm) split peak at 2.3 and 2.49, 99.6A %;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.34 (s, 3H), 1.56 (s, 3H), 2.50-2.60 (br-s, 1H), 3.79-3.90 (m, 2H), 4.27 (m, 1H), 4.95 (m, 1H), 5.02 (m, 1H), 5.53 (d, 1H), 5.71 (dd, 1H), 7.33 (d, 1H), 8.03 (br s, 1H).

**[0412] Step 2:**

A2  
Chemical Formula:  $C_{12}H_{16}N_2O_6$   
Molecular Weight: 284.27

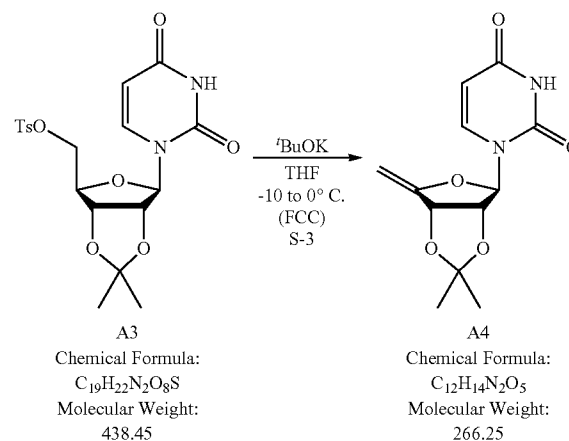
-continued



A3  
Chemical Formula:  $C_{12}H_{22}N_2O_8S$   
Molecular Weight: 438.45

**[0413]** The acetonide A2 (11.0 g, 38.7 mmol) was suspended in dichloromethane (110 mL). Dimethylaminopyridine (DMAP, 11.8 g, 96.8 mmol, 2.5 eq) was added and the mixture stirred at room temperature until the acetonide had fully dissolved. The mixture was cooled to ca. 0° C. (ice-bath) and tosyl chloride (8.85 g, 46.4 mmol, 1.2 eq) was added in 5 portions. After the addition was complete, the ice bath was removed and the mixture stirred for 1 hour. HPLC analysis showed the reaction to be complete. The mixture was transferred to a separatory funnel and was washed with aqueous HCl (1N, 2×100 mL), aqueous sodium bicarbonate (saturated, 100 mL), and brine (100 mL). The organic solution was dried over magnesium sulfate and was concentrated under reduced pressure affording the crude tosylate. (15.47 g, 91%). The crude product A3 (purity; ca 86% by NMR) was used without purification for Step-3.

**[0414]** HPLC (Method A, 254 nm), 4.86 min; LCMS (m/e 327.05,  $M^+$ -Uracil);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.29 (s, 3H), 1.50 (s, 3H), 2.40 (s, 3H), 4.22 (m, 2H), 4.30 (m, 1H), 4.76 (dd, 1H), 4.90 (dd, 1H), 5.61 (d, 1H), 5.67 (d, 1H), 7.21 (d, 1H), 7.29 (d, 2H), 7.72 (d, 2H), 9.39 (br s, 1H).

**[0415] Step 3:**

A3  
Chemical Formula:  $C_{19}H_{22}N_2O_8S$   
Molecular Weight: 438.45

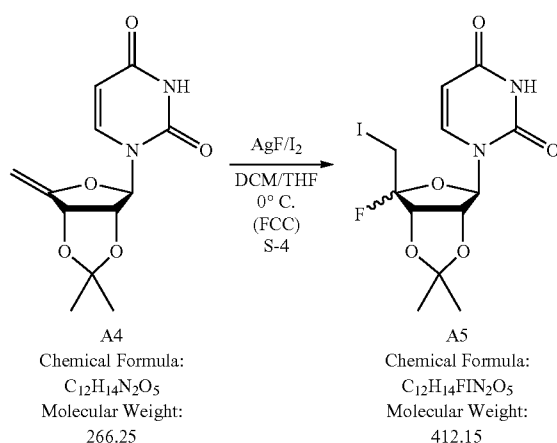
A4  
Chemical Formula:  $C_{12}H_{14}N_2O_5$   
Molecular Weight: 266.25

**[0416]** The crude tosylate A3 (39.5 g, 78.7 mmol) was dissolved in THF (100 mL) and was cooled to -10° C. Potassium t-butoxide (26.5 g, 236 mmol, 3 eq) was added forming a solid mass. An additional 250 mL of THF was added to ensure adequate stirring. The mixture was stirred for 30 min-

utes and HPLC analysis showed that the reaction was complete. Silica gel (60 g) was added and the mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (Silica Gel, 100% DCM to 4% MeOH/DCM) to afford 13.2 g (62%) of the enol ether A4.

**[0417]** HPLC (Method A, 272 nm), 3.24 min, 98% A; LCMS ( $M^+ + 1$  m/e 267.09);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.41 (s, 3H); 1.53 (s, 3H); 4.42 (m, 1H); 4.60 (m, 1H); 5.05 (m, 1H); 5.33 (m, 1H); 5.67 (s, 1H); 5.75 (dd, 1H); 7.20 (d, 1H); 9.60 (br s, 1H).

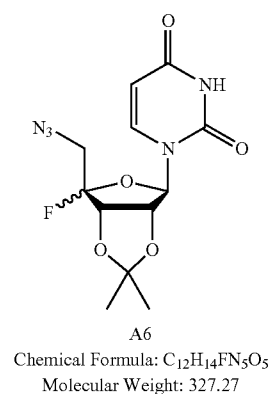
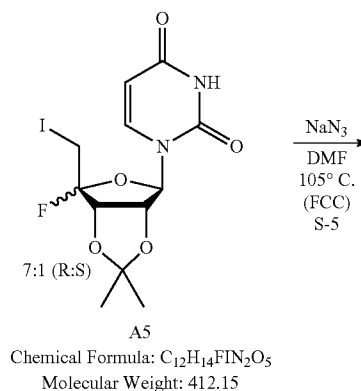
**[0418]** Step 4:



**[0419]** The nucleosidic enol-ether A4 (7.34 g, 27.6 mmol, 1 eq) and finely crushed silver fluoride (17.5 g, 138 mmol, 5 eq) were added to a flask containing dichloromethane (520 mL, DCM was needed to ensure adequate stirring of the heterogeneous mixture.) The suspension was stirred rapidly and cooled to  $0^\circ\text{C}$ . In a separate flask, iodine (14.0 g, 55.2 mmol, 2 eq) was dissolved in THF (40 mL). (The limited solubility of iodine in DCM resulted in incomplete reaction when DCM was used for preparing the iodine solution.) The iodine solution was transferred to a slow-addition funnel and was added to the reaction mixture over 70 minutes. This addition rate provided a 7:1 ratio of the desired isomer (R) to undesired isomer (S). The mixture was stirred for 10 min at which point HPLC analysis showed the reaction to be complete. The reaction mixture was quenched by the addition of an aqueous solution of  $\text{Na}_2\text{S}_2\text{O}_3$  and  $\text{NaHCO}_3$  (5 wt % each, 300 mL total volume). The mixture was filtered through Celite™ and the filter pad washed with DCM. The biphasic mixture was transferred to a separatory funnel and the phases were separated. The organic phase was dried with magnesium sulfate and the mixture concentrated under reduced pressure affording ca. 11 g of crude product. The crude product was purified by flash column chromatography (Silica Gel, 0 to 60% EtOAc/heptane) to provide AS as a beige colored solid. The crude solid was dissolved in DCM (20 mL) which was then added to heptane (200 mL) giving AS as a white-colored solid. (A5, 10.4 g, 82%).

**[0420]** HPLC (Method A, 254 nm); AS (4.18 and 4.38 min) 97% A, 7:1 R:S;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.16 (br s, 1H); 7.20 (d, 1H); 5.77 (d, 1H); 5.65 (s, 1H); 5.16 (m, 1H); 5.10 (m, 1H); 3.53 (m, 1H); 3.48 (m, 1H); 1.59 (s, 3H); 1.38 (s, 3H);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ )  $\delta$  -101.91 (1F, A5-R, Major), -94.16 (0.165 F, Minor, A5-S).

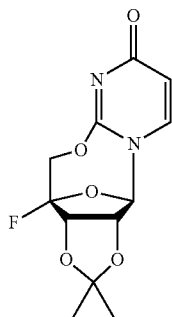
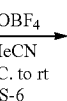
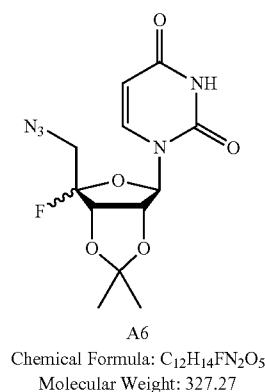
**[0421]** Step 5:



**[0422]** The iodofluorinated nucleoside AS (2.4 g, 5.8 mmol, 1 eq) was dissolved in DMF (24 mL). Sodium azide (1.9 g, 29 mmol, 5 eq) was added and the mixture stirred and heated at  $100^\circ\text{C}$  overnight. HPLC analysis indicated that the reaction was incomplete. Additional sodium azide (378 mg, 5.8 mmol, 1 eq) was added and the reaction continued for another 105 minutes. HPLC analysis showed that the reaction was nearly complete. The mixture was allowed to cool to room temperature and ethyl acetate (75 mL) and water (50 mL) were added. The mixture was then transferred to a separatory funnel and the phases were split. The aqueous phase was extracted with ethyl acetate (25 mL). The combined organic layers were washed with water (4x50 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Silica Gel, 0 to 60% EtOAc/heptane) to provide 1.63 g of the desired azide A6 (86%).

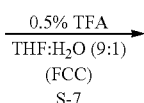
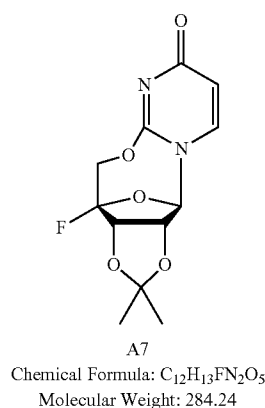
**[0423]** HPLC (Method A, 254 nm); A6, 3.96 min, 4.09 min;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.90 (br s, 1H); 7.18 (d, 1H); 5.77 (dd, 1H); 5.68 (s, 1H); 5.10 (m, 2H); 3.57 (d, 1H); 3.54 (s, 1H); 1.60 (s, 3H); 1.38 (s, 3H);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ )  $\delta$  -109.70 (1F, A6-R, Major), -102.10 (0.280 F, A6-S, Minor).



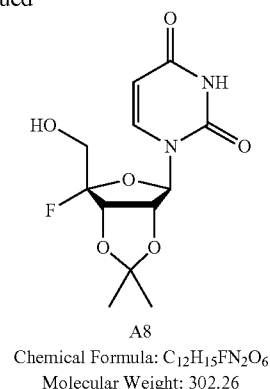
**[0424]** Step 6:

**[0425]** The azido nucleoside A6 (0.988 g, 3.2 mmol, 1 eq) was dissolved in acetonitrile (10 mL). The mixture was cooled to 0° C. (ice-bath) and nitrosyl tetrafluoroborate (1.06 g, 9.06 mmol, 3 eq) was added in a single portion. The mixture was stirred for 30 minutes at 0° C. The ice-bath was removed and the mixture stirred for 1 hour at room temperature. HPLC analysis showed the reaction to be complete. The reaction was quenched by the addition of 50% brine/50%  $Na_2HPO_4$  (20 mL). The mixture was transferred to a separatory funnel and was extracted with dichloromethane (3×20 mL). The combined organic extracts were dried with magnesium sulfate and concentrated under reduced pressure affording 0.699 g (81%) of crude A7. The crude material was used in Step 7 without further purification.

**[0426]** HPLC (Method A, 254 nm); A7, 2.77 min; LCMS ( $M^+ + 1$ ,  $m/e = 285$ ).

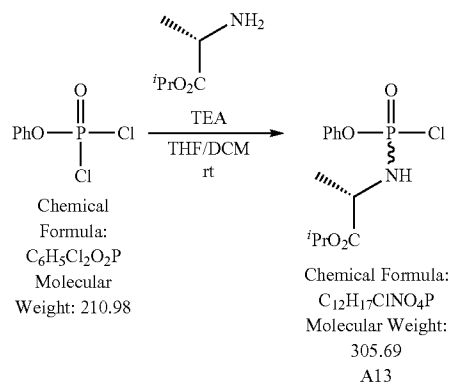
**[0427]** Step 7:

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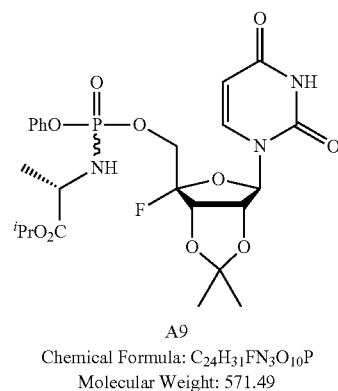
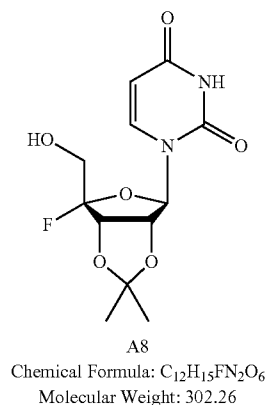


**[0428]** The nucleoside A7 (699 mg, 2.5 mmol, 1 eq) was dissolved in THF (6.3 mL) and water (0.7 mL). TFA (35  $\mu$ L) was added and the mixture stirred for 1 hour at room temperature. HPLC analysis showed that the reaction was complete. The mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (Silica Gel, 100% DCM to 4% MeOH/DCM) to provide 308 mg (41%) of the hydroxymethyl nucleoside A8.

**[0429]** HPLC (Method A, 254 nm); A8, 2.74 min; LCMS ( $M^- - 1$ ,  $m/e = 301$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.38 (s, 3H), 1.59 (s, 3H), 2.41 (br s, 1H), 3.82 (d, 2H), 5.10 (d, 1H), 5.24 (m, 1H), 5.72 (s, 1H), 5.77 (d, 1H), 7.23 (d, 1H), 9.06 (br s, 1H);  $^{19}F$  NMR (376 MHz,  $CDCl_3$ )  $\delta$  -115.65.

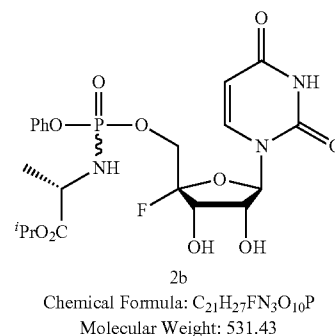
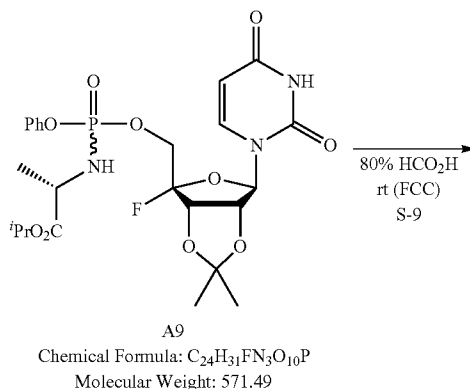
**[0430]** Step 8a:

**[0431]** Phenyl dichlorophosphate (495  $\mu$ L, 3.31 mmol, 1 eq) was dissolved in THF. The mixture was cooled to -66° C. In a separate flask, a solution of isopropyl alanine (583 mg, 3.48 mmol, 1.05 eq) in DCM (6 mL) was prepared. This solution was added to the solution of the dichlorophosphate over 5 minutes. Triethylamine (966  $\mu$ L, 6.95 mmol, 2.1 eq) was then added over 3 minutes maintaining the temperature at -66° C. The mixture was stirred for 25 minutes and this solution was used for Step 8 without further purification.

**[0432]** Step 8:

**[0433]** The nucleoside A8 (500 mg, 1.65 mmol, 0.5 eq) was dissolved in THF (5 mL) forming a clean solution. The mixture was stirred and cooled to  $-43^{\circ}C$ . *t*-Butyl magnesium chloride (1M in THF, 3.64 mL, 3.64 mmol, 1.1 eq) was added drop-wise over 5 minutes. The mixture was cooled to  $50^{\circ}C$ . and the solution of the chlorophosphamidate A13 (3.31 mmol, 1 eq) was added drop-wise via a syringe over 7 minutes. (The solution became brown-colored and cloudy.) The mixture was stirred for 30 minutes and analyzed by HPLC. The mixture was warmed to  $0^{\circ}C$ . and stirred for 30 minutes. LCMS analysis indicated the reaction to be complete. Brine (5%, 10 mL) was added, the mixture was transferred to a reparatory funnel and was extracted with ethyl acetate (3x15 mL). The organic extracts were dried over magnesium sulfate and were concentrated under reduced pressure. The crude product was purified by flash column chromatography (Silica Gel, 100% DCM to 4% MeOH/DCM) to afford 324 mg (34%) of the mixture of the phosphoramidate diastereomers A9.

**[0434]** HPLC (Method A, 254 nm); A9, 4.87 min, 4.95 min 1.8:1 ratio of diastereomers; LCMS ( $M^{-1}$ ,  $m/e=570$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.18 (m, 6H), 1.31 (m, 3H), 1.35 (m, 3H), 1.55 (s, 3H), 3.98 (m, 2H), 4.28 (m, 2H), 4.98 (m, 2H), 5.20 (m, 1H), 5.69 (m, 1H), 5.78 (s, 1H), 7.20, 7.28 (m, 6H), 9.22, 9.41 (2 s, 1H);  $^{19}F$  NMR (376 MHz,  $CDCl_3$ )  $\delta$  -113.99 (m, 1F), -113.53 (m, 0.6F);  $^{31}P$  NMR (162 MHz,  $CDCl_3$ ), 2.33, 2.32 (2 s, 1P).

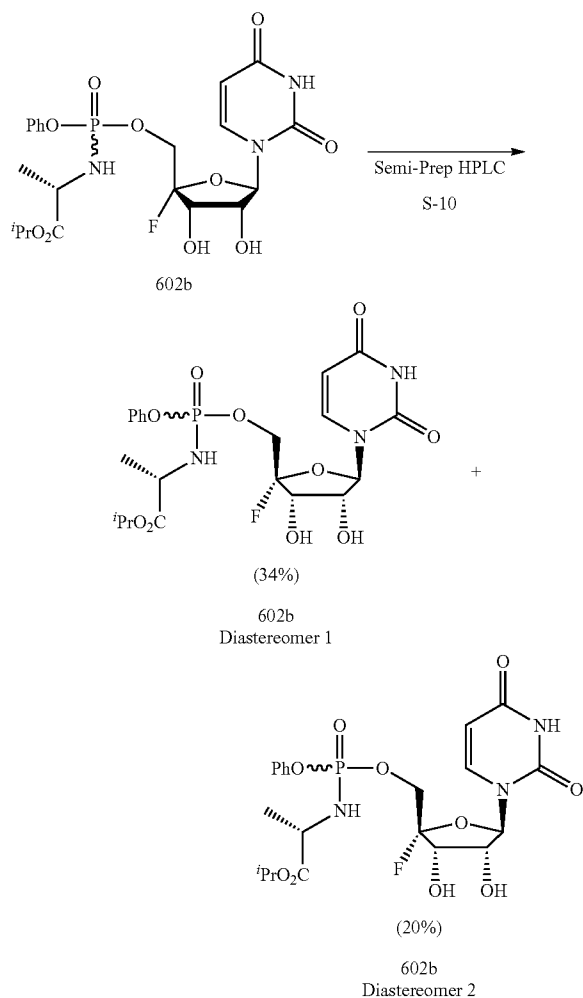
**[0435]** Step 9:

**[0436]** The nucleoside A9 (548 mg, 0.959 mmol, 1 eq) was dissolved in formic acid (80%, 35 mL). The mixture was stirred a room temperature for 3 hour and 45 minutes. HPLC analysis showed the reaction to be complete. The reaction mixture was transferred to a reparatory funnel, was diluted with brine (35 mL) and was extracted with ethyl acetate (3x40 mL). The combined organic extracts were dried over magnesium sulfate and were concentrated under reduced pressure. The crude product was purified by flash column chromatography (Silica Gel, 100% DCM to 10% MeOH/DCM) to afford 296 mg (58%) of the mixture of the phosphoramidate diastereomers 602b.

**[0437]** HPLC (Method A, 254 nm); 2b, 3.80 min; LCMS ( $m/e=532$  ( $M^{+1}$ ), 512( $M^{+}-F$ );  $^1H$  NMR (400 MHz,  $CD_3OD$ )  $\delta$  1.18 (m, 6H), 1.28 (m, 3H), 3.27 (s, 1H), 3.85 (m, 1H), 4.28 (m, 3H), 4.47 (dd, 1H), 4.93 (m, 1H), 5.60 (d, 0.3H), 5.65 (d, 0.67H), 5.96 (m, 1H), 7.18 (m, 3H), 7.32 (m, 2H), 7.51 (d, 1H);  $^{19}F$  NMR (376 MHz,  $CD_3OD$ )  $\delta$  -123.73 (m, 2.2 F), -123.96 (m, 1F);  $^{31}P$  NMR (162 MHz,  $CD_3OD$ ), 3.43 (m, 2.2P), 3.59 (m, 1P).

Step 10: Semi-Preparative HPLC Separation of the Diastereomers of 602b

[0438]



[0439] The mixture of diastereomers 602b was separated using a Phenomenex Luna C18 (2) and PrepMethod A. Approximately 290 mg of 602b was dissolved in 2 mL of methanol/heptanes (80:20) to give a 145 mg/mL solution. Four 500  $\mu$ L injections were made. The fractions from the separations were analyzed by analytical HPLC (Method B). The suitable fractions were combined and concentrated providing 50 mg (34%) of 602b diastereomer 1 (13.99 min, 97.6 A %, >99.9% de) and 30 mg (20%) of 602b diastereomer 2 (19.50 min, 96.8 A %, 94.2% de).

[0440] PrepMethod A:

[0441] Gilson prep HPLC system with GX-281 liquid handler and 322 pump. Phenomenex Luna C18(2) column, 150 $\times$  21.20 mm, 5  $\mu$ m. Mobile phase 40/60 MeOH/water. Flow=22 ml/min.

[0442] HPLC Method B:

[0443] Luna C18 (2), 5  $\mu$ m, 3.0 $\times$ 150 mm. Mobile Phase: 45% Methanol:Water (Isocratic). Flow=0.6 mL min<sup>-1</sup>, 25 min runtime. DAD detector monitored at 214 and 260 nm.

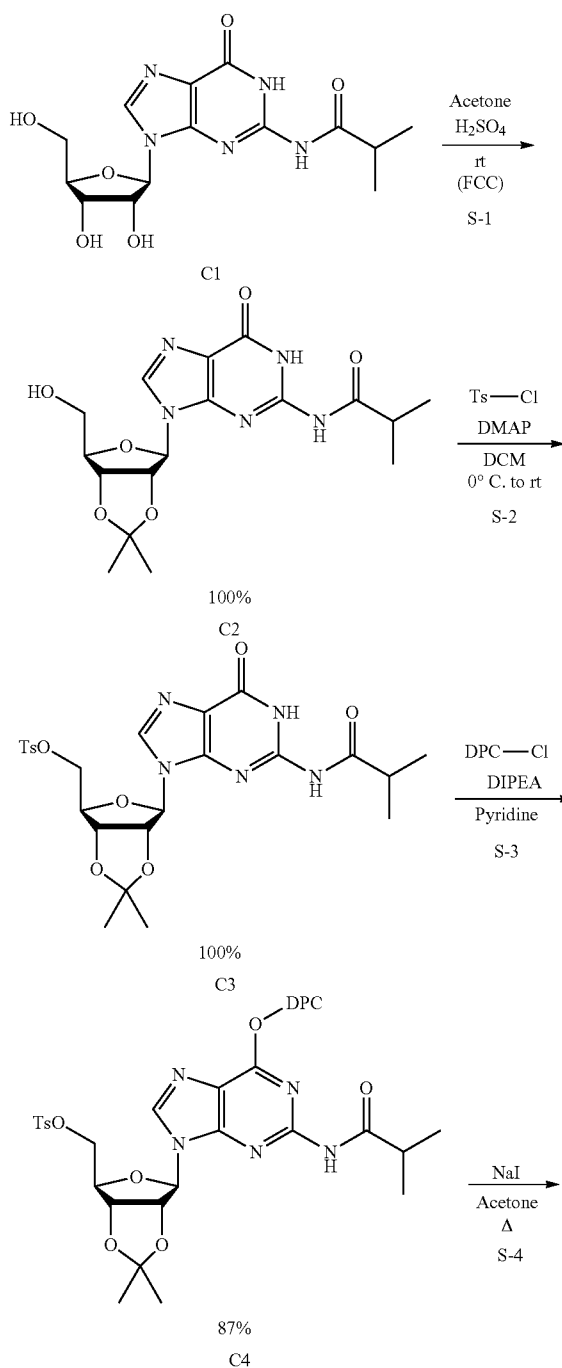
[0444] HPLC Method A:

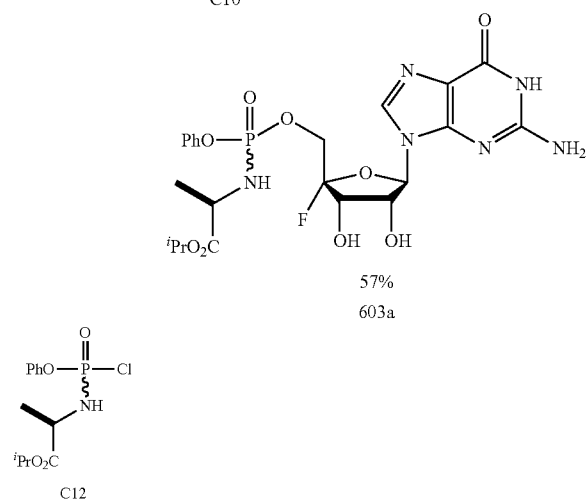
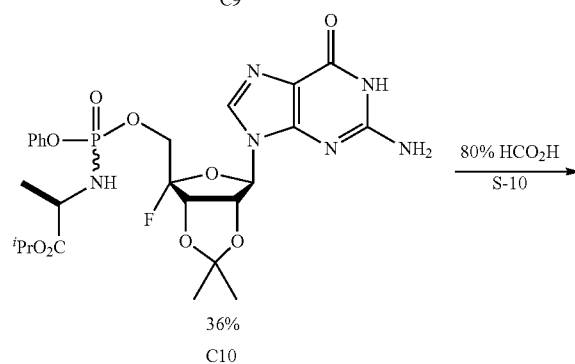
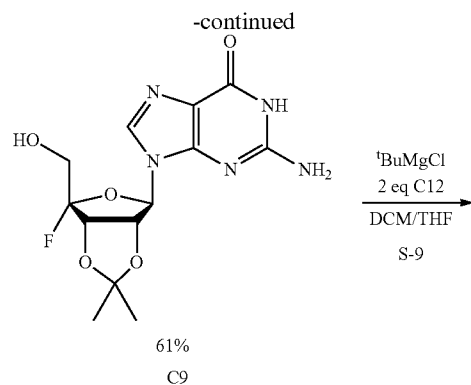
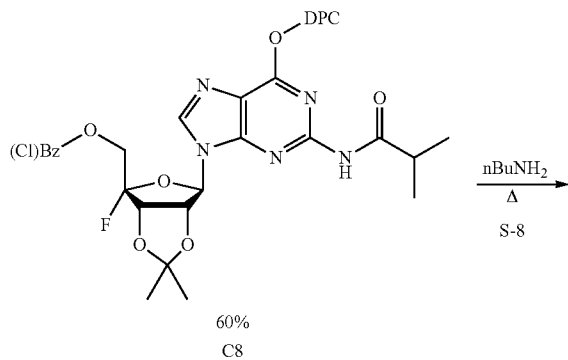
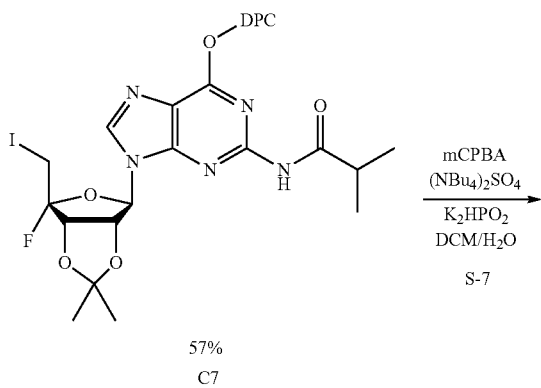
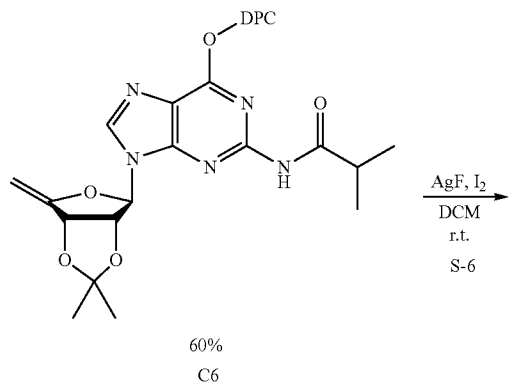
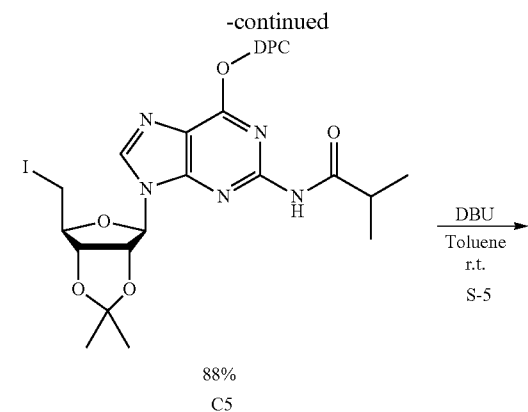
[0445] Agilent Technologies 1100 Series HPLC with diode array detector. Mobile Phase: ACN/NH<sub>4</sub>OAc pH 4.4 buffer (5% to 80% over 10 min); Flow=1.4 ml min<sup>-1</sup>. DAD detector monitored at 254 and 272 nm.

Compound 603a

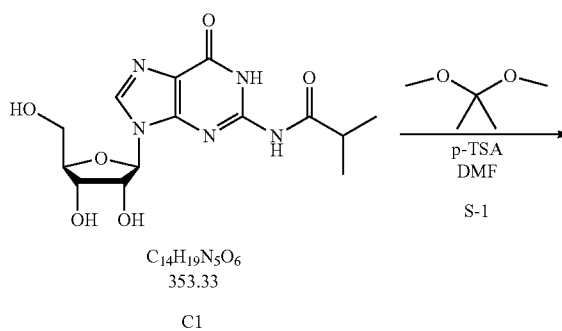
Single Diastereomer

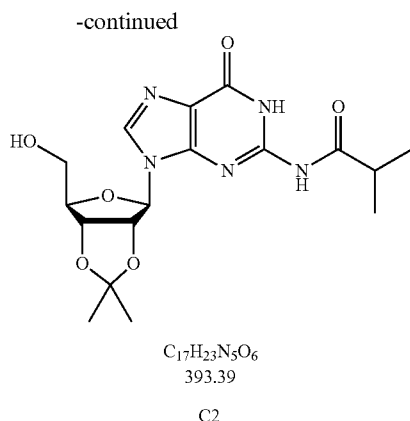
[0446]





[0447] Step 1:

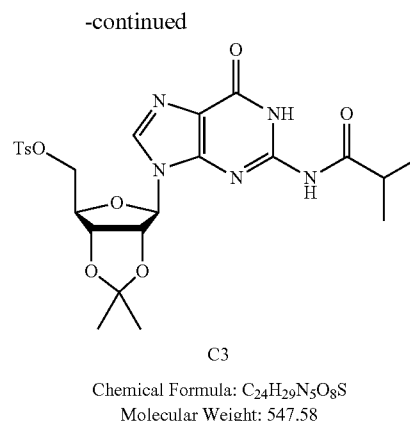
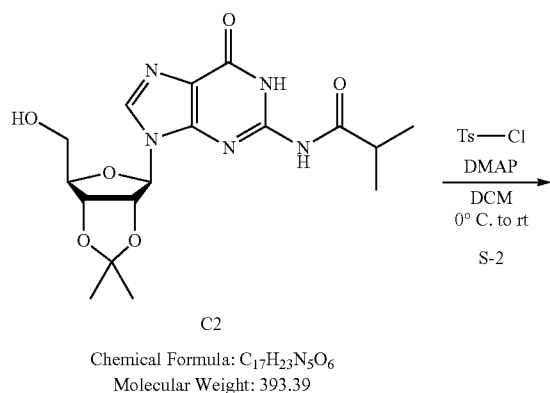




**[0448]** The nucleoside C1 (10 g, 28.3 mmol) was dissolved in a 1:1 mixture of dimethoxypropane (50 mL, 408 mmol, 14.4 eq) and dimethylformamide (DMF, 50 mL). p-Toluenesulfonic acid monohydrate (p-TSA, 2.05 g, 10.77 mmol, 0.380 eq) was added and the mixture was stirred at room temperature for 48 hours. Initially, 0.1 eq of p-TSA was added; after 24 hours, the reaction was only 50% complete. Additional aliquots of p-TSA (0.28 eq total) were needed to drive the reaction to completion. The reaction mixture was concentrated on a rotary evaporator and the residue was dissolved in dichloromethane (DCM, 300 mL). The mixture was transferred to a separatory funnel and was washed with saturated sodium bicarbonate solution (300 mL). The aqueous phase was back-extracted with 2×100 mL of DCM and the combined organic phases were dried over magnesium sulfate and were concentrated under reduced pressure affording the crude product C2 (1.2 g, 108%). (<sup>1</sup>H NMR analysis showed that the crude product contained DMF).

**[0449]** HPLC (Method A, 254 nm), RT 3.4 min; LCMS (M<sup>-</sup>-1 m/e=392) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.11 (br s, 1H), 9.20 (br s, 1H), 7.66 (d, 2H), 7.58 (s, 1H), 7.27 (d, 2H), 5.79 (d, 1H), 5.22 (dd, 1H), 5.12 (dd, 1H), 4.49 (dd, 1H), 4.33 (m, 1H), 4.31 (m, 1H), 3.84 (m, 1H), 3.70 (m, 1H), 2.65 (sept, 1H), 2.37 (br s, 1H), 1.51 (s, 3H), 1.28 (s, 3H), 1.18 (a-t, 6H).

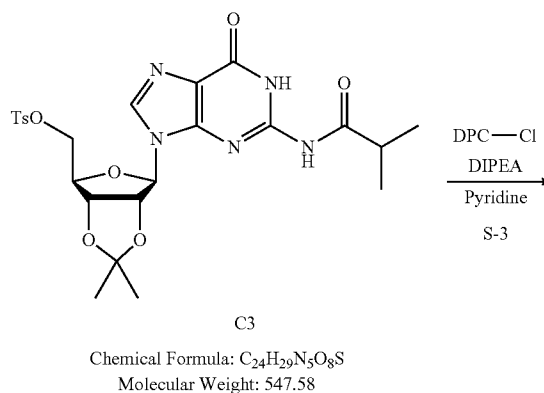
**[0450]** Step 2:



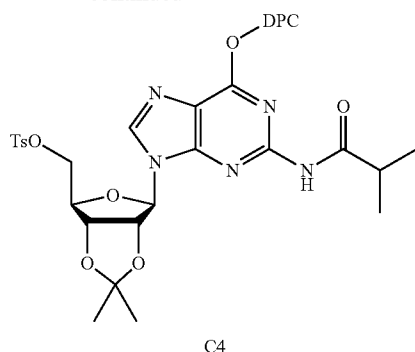
**[0451]** The crude nucleoside C2 (12.1 g, 28.3 mmol) was dissolved in dichloromethane (DCM, 125 mL) under argon. Dimethylaminopyridine (DMAP, 8.6 g, 70.8 mmol, 2.5 eq) was added and the mixture was cooled in an ice-bath. Tosyl chloride (TsCl, 7.0 g, 36.8 mmol, 1.3 eq) was added and the mixture was stirred at 0° C. for 30 minutes. The ice-bath was removed and the mixture was allowed to stir at room temperature for an additional 30 minutes. HPLC analysis showed that the reaction was complete. The mixture was transferred to a separatory funnel and was diluted with DCM (125 mL). The DCM solution was washed with 1M HCl (2×100 mL), saturated bicarbonate solution (100 mL), and brine (100 mL). The mixture was dried over magnesium sulfate and was concentrated under reduced pressure affording 15.73 g of the desired product C3 (101%, contains DMF).

**[0452]** HPLC (Method A, 254 nm), RT 4.78 min; LCMS (M<sup>+</sup>+1, m/e=548); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.11 (br s, 1H) 9.20 (br s, 1H), 7.66 (d, 2H), 7.58 (s, 1H), 7.27 (d, 2H), 5.79 (d, 1H), 5.22 (dd, 1H), 5.12 (dd, 1H), 4.49 (dd, 1H), 4.33 (m, 1H), 4.31 (m, 1H), 3.84 (m, 1H), 3.70 (m, 1H), 2.65 (sept, 1H), 2.37 (br s, 1H), 1.51 (s, 3H), 1.28 (s, 3H), 1.18 (d, 3H), 1.14 (d, 3H).

**[0453]** Step 3:



-continued

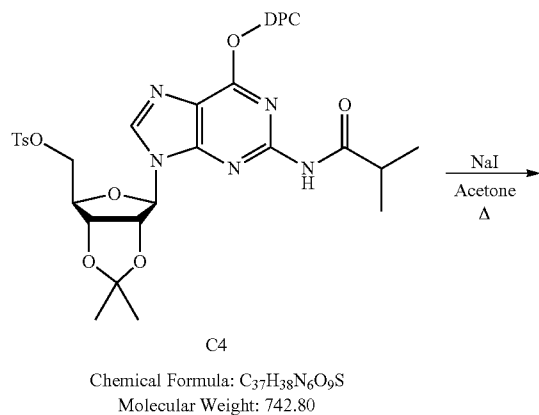


Chemical Formula:  $C_{37}H_{38}N_6O_9S$   
Molecular Weight: 742.80

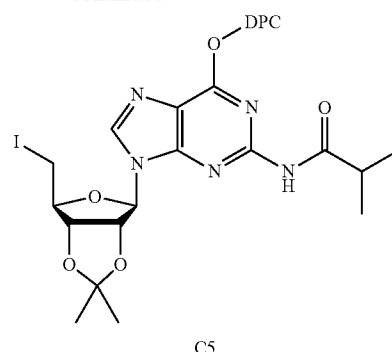
**[0454]** The nucleoside C3 (8.0 g, 14.6 mmol) was dissolved in pyridine (80 mL) under an argon atmosphere. Diisopropylethylamine (DIPEA, 5.08 mL, 29.2 mmol, 2 eq) was added followed by diphenylcarbamoyl chloride (DPC-Cl, 3.72 g, 1.1 eq). The mixture was stirred at room temperature under an argon atmosphere for 1 hour. HPLC analysis indicated the reaction to be complete. The mixture was quenched by the addition of water (15 mL) and was concentrated under reduced pressure. The residue was transferred to a separatory funnel with DCM (150 mL). The DCM solution was washed with aqueous HCl (1M, 100 mL), dried over magnesium sulfate, and was concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 0→50% EtOAc/heptanes) to provide 9.5 g of C4 (87%).

**[0455]** HPLC (Method A, 254 nm), RT 6.53 min; LCMS ( $M^+ + 1$ ,  $m/e = 743$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.99 (br s, 1H), 7.81 (s, 1H), 7.35 (m, 12H), 6.92 (d, 2H), 5.91 (d, 1H), 5.42 (dd, 1H), 5.14 (dd, 1H), 4.40 (m, 1H), 4.29 (m, 2H), 2.61 (sept, 1H), 2.10 (s, 3H), 1.50 (s, 3H), 1.29 (s, 3H), 1.18 (2d, 6H).

**[0456]** Step 4:



-continued

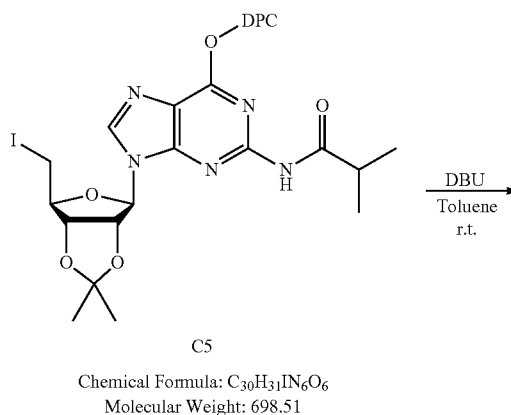


Chemical Formula:  $C_{30}H_{31}IN_6O_6$   
Molecular Weight: 698.51

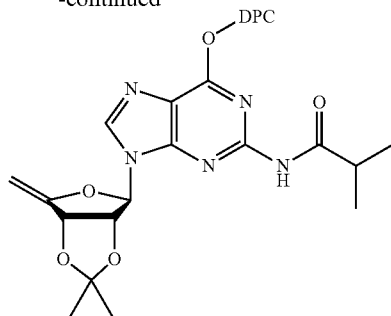
**[0457]** The nucleoside C4 (9.5 g, 12.8 mmol) was dissolved in acetone (100 mL) under an argon atmosphere. Sodium iodide (13.4 g, 89.6 mmol, 7 eq) was added and the mixture was refluxed overnight. LCMS analysis indicated that the reaction was complete. The mixture was allowed to cool and was concentrated under reduced pressure. The mixture was transferred to a separatory funnel with DCM (100 mL) and was washed with a mixture of 5% sodium bicarbonate and 5% sodium thiosulfate (75 mL total). The organic phase was dried over magnesium sulfate and concentrated under reduced pressure affording 9 grams of a dark-colored foam. The crude material was purified by flash column chromatography (silica gel, 0→50% EtOAc/heptanes) to provide 7.82 g of C5 (88%).

**[0458]** HPLC (Method A, 254 nm), RT 6.39 min; LCMS ( $M^+ + 1$ ,  $m/e = 699$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.96 (s, 1H), 7.95 (br s, 1H), 7.30 (m, 10H), 6.00 (d, 1H), 5.40 (m, 2H), 4.40 (m, 1H), 3.45 (m, 1H), 3.20 (dd, 1H), 2.67 (m, 1H), 1.54 (s, 3H), 1.34 (s, 3H), 1.19 (m, 6H).

**[0459]** Step 5:



-continued



C6

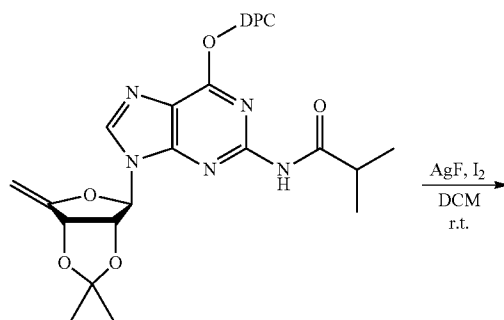
Chemical Formula:  $C_{30}H_{30}N_6O_6$   
Molecular Weight: 570.60

**[0460]** The nucleoside C5 (7.82 g, 11.2 mmol) was dissolved in toluene. 1,8-Diazabicyclo[5.4.0] undec-7-ene

(DBU, 5.0 mL, 33.6 mmol, 3 eq) was added dropwise over 3 minutes. The mixture was stirred at room temperature for ca 64 hours. HPLC analysis indicated the reaction to be complete. The reaction mixture was diluted with DCM (50 mL) and saturated sodium bicarbonate solution (50 mL). This mixture was transferred to a separatory funnel along with additional portions of DCM (100 mL) and saturated sodium bicarbonate solution (50 mL). The layers were separated and the organic phase dried over magnesium sulfate and was concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 0→4% MeOH/DCM) affording 3.83 g of the desired product C6 (60%).

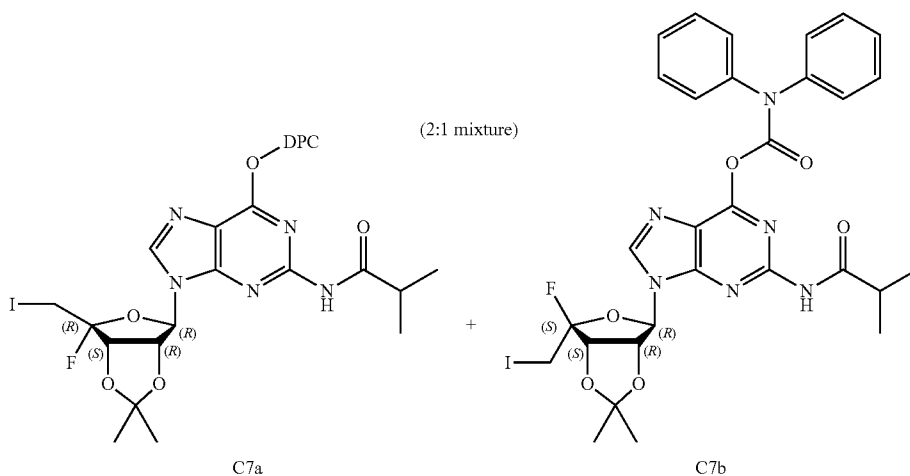
**[0461]** HPLC (Method A, 254 nm), RT 6.04 min; LCMS ( $M^+ + 1$ ,  $m/e = 571$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.88 (s, 1H), 7.87 (br s, 1H), 7.27 (m, 10H), 6.11 (s, 1H), 5.88 (d, 1H), 5.27 (d, 1H), 4.48 (m, 1H), 4.39 (m, 1H), 2.75 (m, 1H), 1.50 (s, 3H), 1.38 (s, 3H), 1.19 (m, 6H).

**[0462]** Step 6:



C6

Chemical Formula:  $C_{30}H_{30}N_6O_6$   
Molecular Weight: 570.60



C7a

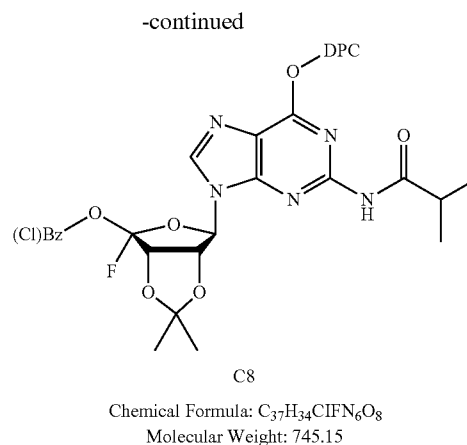
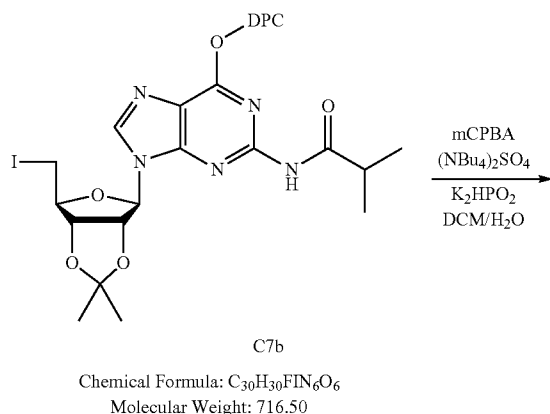
C7b

Chemical Formula:  $C_{30}H_{30}FIN_6O_6$   
Molecular Weight: 716.50

**[0463]** The nucleoside C6 (1.1 g, 1.9 mmol) was dissolved in DCM (10 mL). Freshly crushed silver fluoride (1.22 g, 9.6 mmol, 5 eq) was added. In a separate flask, iodine (627 mg, 2.5 mmol, 1.3 eq) was dissolved in DCM (10 mL). The iodine solution was added drop-wise to the solution of the nucleoside over 30 minutes. After stirring for 5 minutes, HPLC analysis indicated that the reaction was incomplete. An additional 5 eq of crushed silver fluoride (1.22 g, 9.6 mmol) was added followed by the portion-wise addition of solid iodine (0.5 eq, 125 mg) over 5 minutes. After stirring at room temperature for 5 minutes, HPLC analysis showed that the reaction was complete. The mixture was quenched by the addition of 20 mL of a mixture of 5% sodium bicarbonate and 5% sodium thiosulfate. The mixture was filtered through Celite™ and was transferred to a reparatory funnel. (Some finely divided solids were not removed by the Celite™ filtration and were present in the organic phase.) The organic solution was dried over magnesium sulfate and was concentrated under reduced pressure providing the crude product (1.45 g). The crude product was a 2:1 mixture of C7a and C7b. The crude product was purified by flash column chromatography (silica gel, 0→50% EtOAc) affording 396 mg of the desired diastereomer C7a (29%). In another reaction, the desired diastereomer, C7a, was obtained in 57% yield after chromatography accompanied by a 21% isolated yield of the “S diastereomer”.

**[0464]** HPLC (Method A, 254 nm), RT 6.47 min; LCMS ( $M^+ + 1$ ,  $m/e = 717$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 8.01 (br s, 1H), 7.89 (s, 1H), 7.34 (m, 10H), 6.27 (s, 1H), 6.10 (dd, 1H), 5.10 (d, 1H), 3.70 (m, 1H), 3.66 (s, 1H), 2.61 (sept, 1H), 1.58 (s, 3H), 1.32 (s, 3H), 1.20 (m, 6H);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ );  $\delta$  -101.14 (m, 1F).

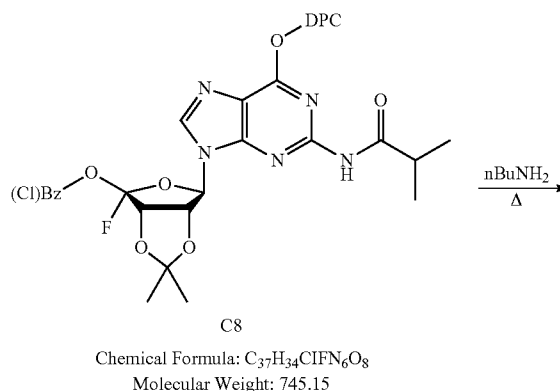
**[0465]** Step 7:



**[0466]** The nucleoside C5 (837 mg, 1.17 mmol) was dissolved in DCM (17 mL). In a separate flask, a solution of potassium hydrogen phosphate (306 mg, 1.76 mmol, 1.5 eq) in water (1 mL) was prepared. This solution along with bis (tetrabutylammonium)sulfate (50% in water, 2.34 mL, 1.17 mmol, 131) were added to the solution of the nucleoside. m-Chloroperbenzoic acid (mCPBA, 1.21 g, 7.02 mmol, 6 eq) was added and the mixture was stirred rapidly at room temperature overnight. HPLC analysis indicated the reaction to be complete. The mixture was transferred to a reparatory funnel and was washed with a mixture of 5% sodium bicarbonate and 5% sodium thiosulfate (20 mL total volume). The layers were separated and the organic phase was dried over magnesium sulfate and was concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 0→50% EtOAc/heptane) providing the desired product C8 (525 mg, 60%).

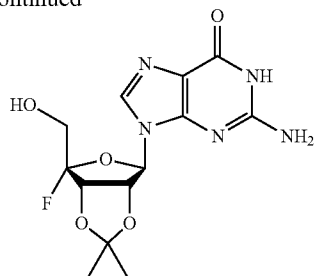
**[0467]** HPLC (Method A, 254 nm), RT 6.93 min; LCMS ( $M^+ + 1$ ,  $m/e = 745$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.15 (br s, 1H), 7.93 (m, 1H), 7.91 (s, 1H), 7.82 (m, 1H), 7.20-7.55 (m, 12H), 6.25 (s, 1H), 6.11 (dd, 1H), 5.11 (d, 1H), 4.66 (dd, 1H), 4.49 (a-t, 1H), 2.49 (m, 1H), 1.59 (s, 3H), 1.35 (s, 3H), 1.07 (m, 6H);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ );  $\delta$  -110.89 (m, 1F).

**[0468]** Step 8:





-continued



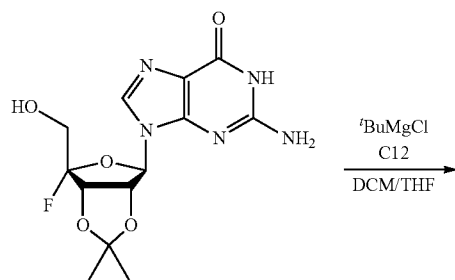
C9

Chemical Formula:  $C_{13}H_{16}FN_5O_5$   
Molecular Weight: 341.30

**[0469]** The nucleoside C8 (1.92 g, 2.58 mmol) was dissolved in *n*-butyl amine (19 mL) forming a green-colored solution. The mixture was stirred and heated to 80° C. for 30 minutes. (The color of the solution had turned red). HPLC analysis indicated the reaction to be complete. The mixture was concentrated under reduced pressure. DCM (20 mL) was added to the red oil forming a thick precipitate. The precipitate was removed by filtration and was washed with copious amounts of cold DCM providing a white-colored solid. This solid was dried in a vacuum oven overnight affording 538 mg of the desired product C9 (61%)

**[0470]** HPLC (Method A, 254 nm), RT 2.66 min; LCMS ( $M^- - 1$ ,  $m/e = 340$ );  $^1H$  NMR (400 MHz, MeOD)  $\delta$  7.87 (s, 1H), 6.32 (s, 1H), 5.44 (dd, 1H), 5.17 (dd, 1H), 3.75 (s, 1H), 3.73 (s, 1H), 1.58 (s, 3H), 1.38 (s, 3H);  $^{19}F$  NMR (376 MHz,  $CDCl_3$ );  $\delta$  -116.90 (m, 1F).

**[0471]** Step 9:



C9

Chemical Formula:  $C_{13}H_{16}FN_5O_5$   
Molecular Weight: 341.30

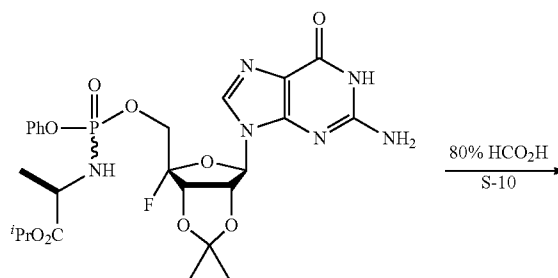
**[0472]** Preparation of (2R)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate, C12. Phenyl dichlorophosphate (437  $\mu$ L, 2.93 mmol) was dissolved in THF (4 mL) and was cooled to -66° C. with dry-ice/acetone. In a separate flask, D-Ala isopropyl ester (516 mg, 3.08 mmol, 1.05 eq) was dissolved in DCM (5 mL). This solution was added to the solution of the dichlorophosphate drop wise over 5 minutes. Triethylamine (855  $\mu$ L, 6.15 mmol, 2.1 eq) was added drop wise over 5 minutes and the mixture was stirred for 30 minutes at -66° C. The formation of the chlorophosphoramidate reagent C12 was shown to be complete by  $^1H$  NMR,  $^{31}P$  NMR and LCMS.

**[0473]** LCMS ( $M^- - Cl + OH - 1$ ,  $m/e = 286$ );  $^{31}P$  NMR (162 MHz,  $CDCl_3$ ),  $\delta$  8.08 (1P), 7.72 (1P).

**[0474]** The nucleoside C9 (500 mg, 1.46 mmol, 0.5 eq) was suspended in THF (5 mL) and was cooled to -66° C. *t*-Butyl magnesium chloride (1 M in THF, 3.22 mL, 3.22 mmol, 1.1 eq) was slowly added over 5 minutes. The mixture was stirred for 5 minutes followed by the addition of the chlorophosphate C12 (prepared above) over 8 minutes. The dry-ice bath was replaced with an ice-bath and the reaction mixture was stirred at 0° C. for 30 minutes. HPLC analysis indicated the reaction to be complete. The mixture was quenched by the addition of 20% sodium chloride (NaCl, 25 mL) and was extracted with DCM (2 $\times$ 10 mL). The organic solution was washed with brine (25 mL), dried over  $MgSO_4$  and was concentrated. The crude product was purified by flash column chromatography (0 $\rightarrow$ 10% MeOH/DCM) to provide 317 mg of C10 (36%) as a single diastereomer. Later column cuts provided an additional 365 mg (41%) of product which was 85% pure.

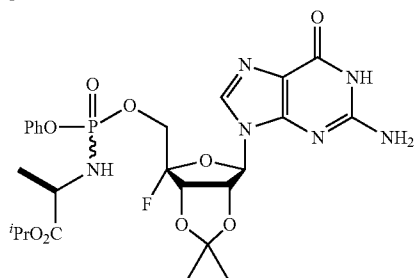
**[0475]** HPLC (Method A, 254 nm), RT 6.26 min; LCMS ( $M^+ + 1$ ,  $m/e = 611$ ).

**[0476]** Step 10:



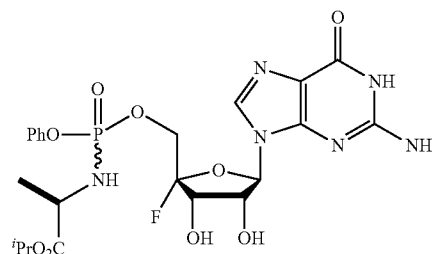
C10

Chemical Formula:  $C_{25}H_{32}FN_6O_9P$   
Molecular Weight: 610.5285



C10

Chemical Formula:  $C_{25}H_{32}FN_6O_9P$   
Molecular Weight: 610.5285



603a

Chemical Formula:  $C_{22}H_{28}FN_6O_9P$   
Molecular Weight: 570.4647

[0477] The nucleoside C10 (315 mg, 0.52 mmol) was dissolved in 80% formic acid (15 mL) and was allowed to stir at room temperature for 15 hours. HPLC analysis showed the reaction to be complete. The mixture was concentrated under reduced pressure and the crude material was purified by flash column-chromatography (silica gel, 0→10% MeOH/DCM) to afford 168 mg of 3a (57%) as a single diastereomer.

[0478] HPLC (Method A, 254 nm), RT 3.52 min; LCMS ( $M^+ + 1$ ,  $m/e = 571$ );  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.70 (br s, 1H), 7.84 (s, 1H), 7.33 (m, 2H), 7.16 (m, 3H), 6.56 (br s, 2H), 6.03 (m, 2H), 5.92 (br s, 1H), 5.35 (br s, 1H), 4.83 (m, 1H), 4.65 (dd, 1H), 4.44 (m, 1H), 4.19 (m, 2H), 3.71 (m, 1H), 1.21 (m, 3H), 1.14 (m, 6H);  $^{19}\text{F}$  NMR ( $\text{DMSO}-d_6$ , 376 MHz); 6-120.7 (m, 1F);  $^{31}\text{P}$  NMR (162 MHz,  $\text{DMSO}-d_6$ )  $\delta$  3.53 (1P).

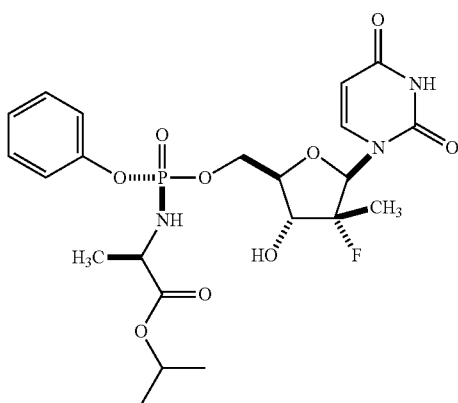
[0479] HPLC Method A:

[0480] Agilent Technologies 1100 Series HPLC with diode array detector. Mobile Phase: ACN/ $\text{NH}_4\text{OAc}$  pH 4.4 buffer (5% to 80% over 10 min). Flow =  $1.4 \text{ mL min}^{-1}$ . DAD detector monitored at 254 and 272 nm.

#### Example 1E

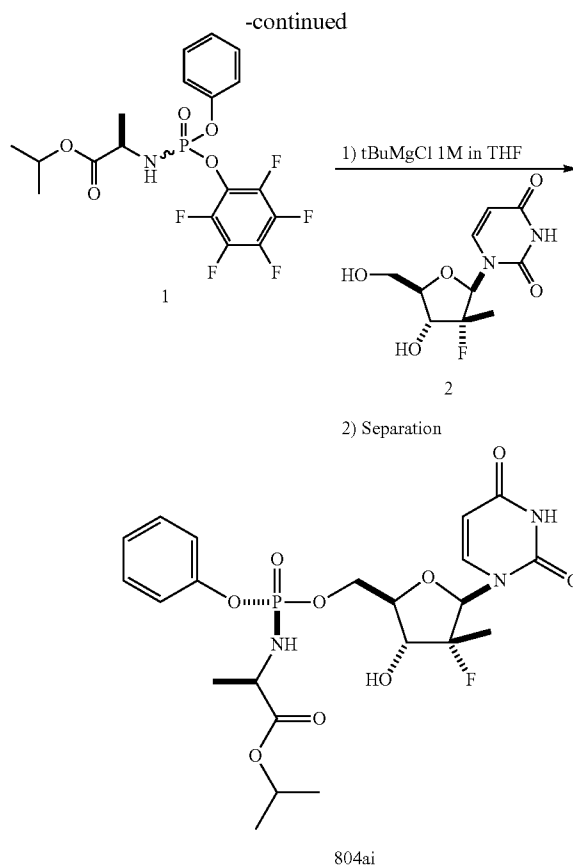
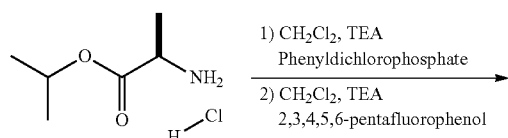
Preparation of Diastereomerically Pure D-Alanine, N-((R<sub>p</sub>,2'R)-2'-deoxy-2'-fluoro-2'-methyl-P-phenyl-5'-uridylyl)-, 1-methylethyl ester Compound (804ai)

[0481]



(804ai)

Scheme 1



Compound 1

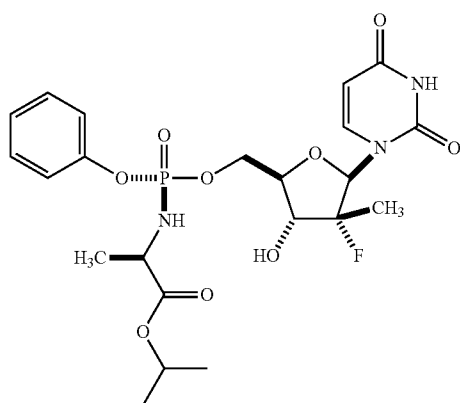
[0482] To a stirred solution of D-Alanine isopropyl ester hydrochloride (47.7 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (1.05 mL/mmol) was added TEA (98.30 mmol) at  $-70^\circ\text{C}$ . over 15 minutes dropwise. To this mixture was added a solution of phenyl dichlorophosphate (47.7 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (1.05 mL/mmol) over 1 hour. The reaction mixture was stirred at this temperature for additional 30 minutes and then allowed to warm to  $0^\circ\text{C}$ . over 2 hours. To this mixture was added a solution of pentafluorophenol (47.7 mmol) and TEA (52 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL). The reaction mixture was stirred at  $0^\circ\text{C}$ . during 1 hour. The triethylamine salt was filtered washed with  $\text{CH}_2\text{Cl}_2$ . The filtrate was concentrated under reduced pressure, the residue was triturated with TBME (150 mL). The heterogeneous mixture was filtered and the solid was rinsed with TBME. The filtrate was concentrated and the residue was triturated with a mixture of hexane/ethyl acetate 20% (100 mL). The suspension was

filtered and the solid was rinsed with a mixture of hexane/ethyl acetate 20% and dried to give the expected compound 1 in 11% yield as a single isomer.

[0483]  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 1.23-1.26 (m, 6H), 1.46 (d,  $J=7.02$  Hz, 3H), 3.94 (dd,  $J=9.47$  Hz and 12. Hz, 1H), 4.09-4.19 (m, 1H), 4.99-5.09 (m, 1H), 7.19-7.27 (m, 3H), 7.34-7.38 (m, 2H).

D-Alanine, N-(( $R_P$ ,2'R)-2'-deoxy-2'-fluoro-2'-methyl-P-phenyl-5'-uridylyl)-, 1-methylethyl ester (804ai)

[0484]



(804ai)

[0485] Compound 2 was prepared according to published procedures. To a solution of compound 2 (4.23 mmol) in THF (3.92 mL/mmol) at  $-5^\circ\text{C}$ . under nitrogen was added drop-

wise tert-butyilmagnesium chloride (1M in THF) (8.92 mmol). The heterogeneous reaction mixture was stirred during 30 minutes at  $-5^\circ\text{C}$ . and 30 minutes at room temperature. The reaction mixture was cooled down to  $-5^\circ\text{C}$ . under nitrogen and compound 1 (5.07 mmol) in THF (18 mL) was added dropwise. The reaction mixture was stirred at  $-5^\circ\text{C}$ . to  $0^\circ\text{C}$ . overnight. The reaction mixture was quenched with aqueous solution of HCl 1N (20 mL) at  $-5^\circ\text{C}$ . and extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with  $\text{H}_2\text{O}$ ,  $\text{Na}_2\text{CO}_3$  aq 5%,  $\text{H}_2\text{O}$  and brine. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (eluent: 100%  $\text{CH}_2\text{Cl}_2$  to  $\text{CH}_2\text{Cl}_2$ : $\text{CH}_3\text{OH}$  95:5) to give the desired pure isomer as a white powder in 77% yield.

[0486] The crystal structure of pure isomer was obtained. The crystal structure showed the pure isomer corresponds to the  $R_P$  isomer of Formula 804ai.

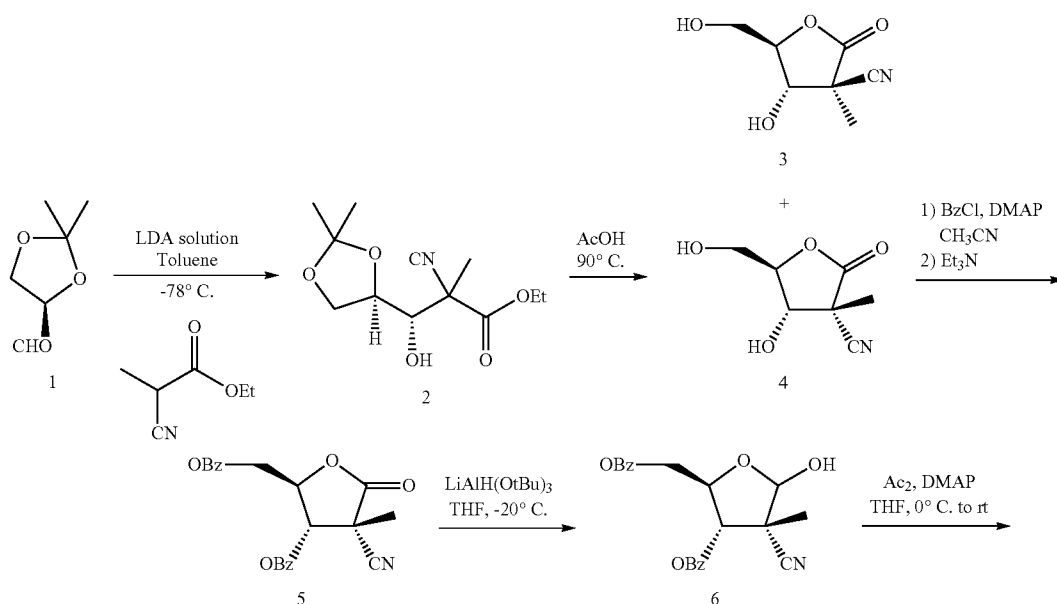
[0487]  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 1.25 (d,  $J=6.26$  Hz, 6H), 1.33 (d,  $J=22.32$  Hz, 3H), 1.38 (d,  $J=6.97$  Hz, 3H), 3.61-3.63 (m, 1H), 3.72-3.98 (m, 3H), 4.06-4.10 (m, 1H), 4.39-4.51 (m, 2H), 5.03 (sept,  $J=6.22$  Hz, 1H), 5.58 (dd,  $J=2.29$  Hz and 8.19 Hz, 1H), 6.16 (d,  $J=19.05$  Hz, 1H), 7.19-7.26 (m, 4H), 7.34-7.38 (m, 2H), 8.43 (brs, 1H);  $^{31}\text{P}$  NMR (161.98 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 4.29 (s, 1P). LCMS (ESI+)  $m/z$  530.2  $[\text{M}+\text{H}]^+$  100%. LCMS (ESI-)  $m/z$  528.2  $[\text{M}-\text{H}]^-$  100%.

#### Example 1F

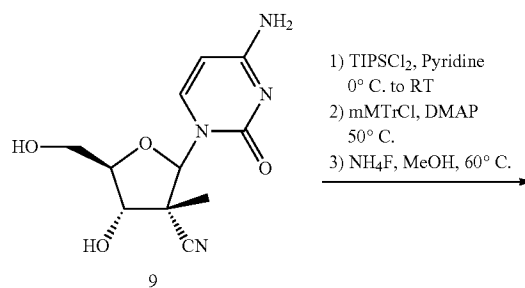
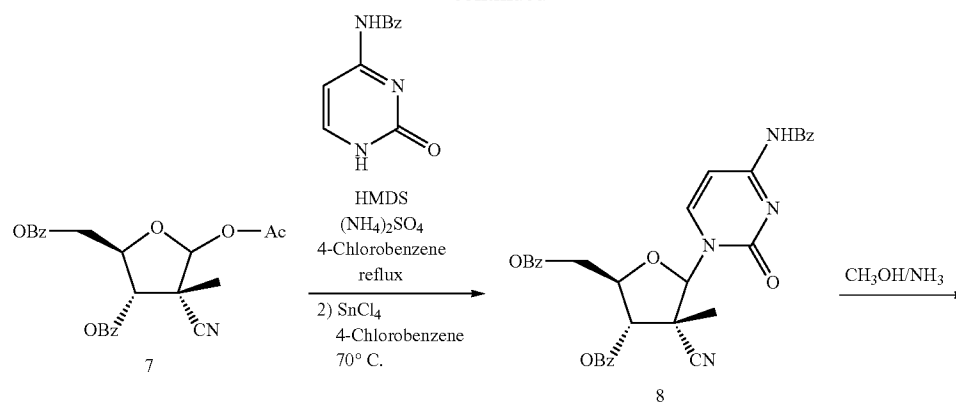
#### Preparation of 2'-cyano, azido and amino nucleosides

[0488]

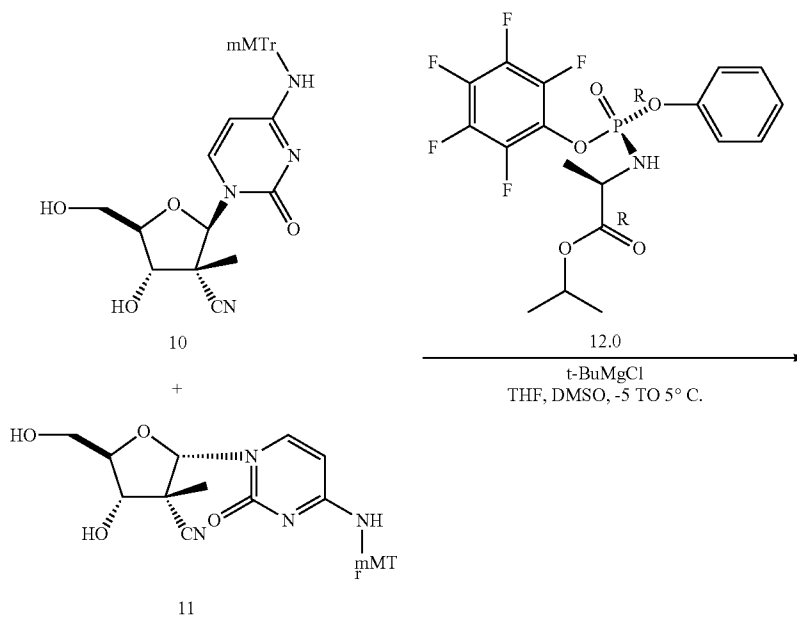
Scheme 1

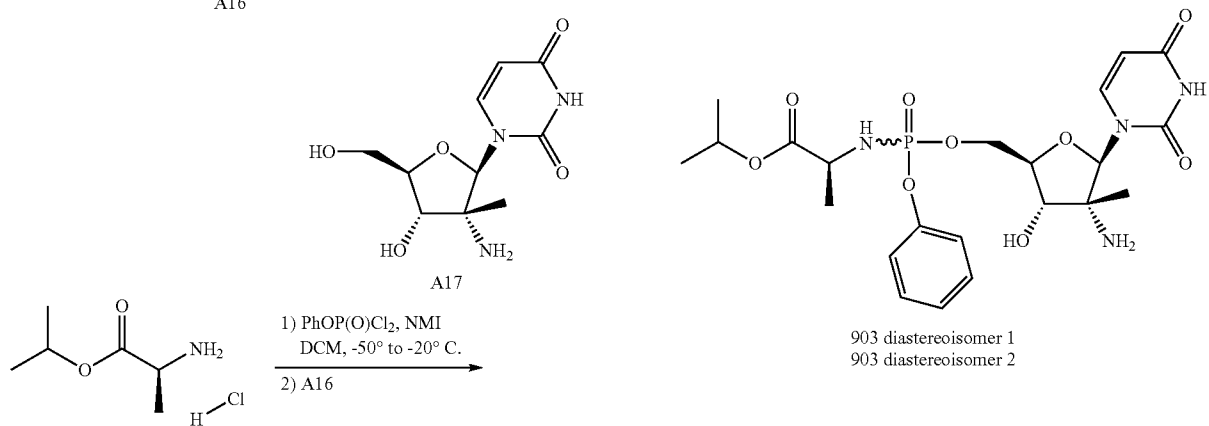
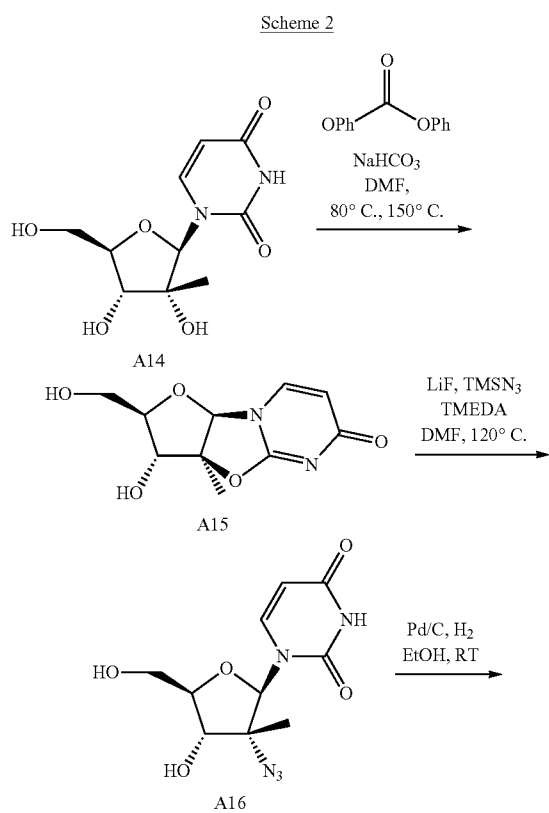
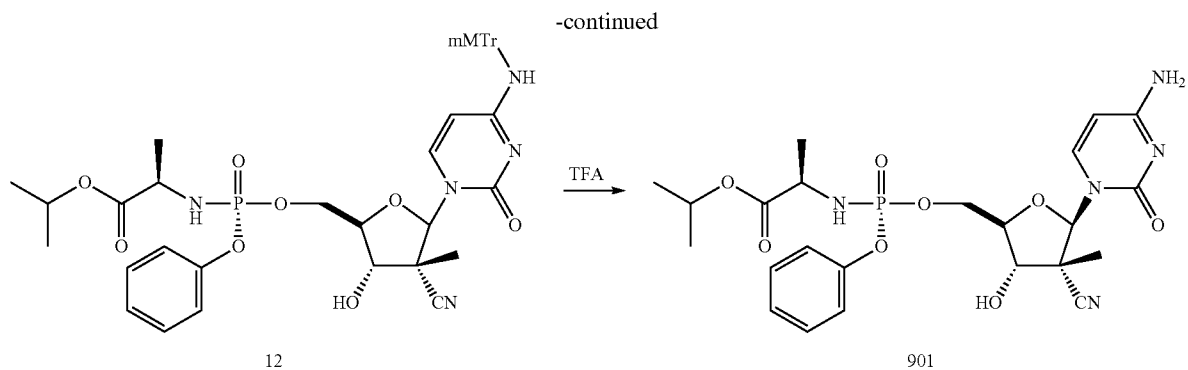


-continued



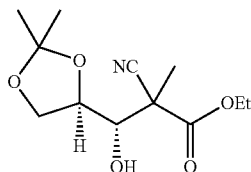
9a:  $\alpha$ -anomer  
9b:  $\beta$ -anomer





Ethyl 2-cyano-3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylbutanoate (A2)

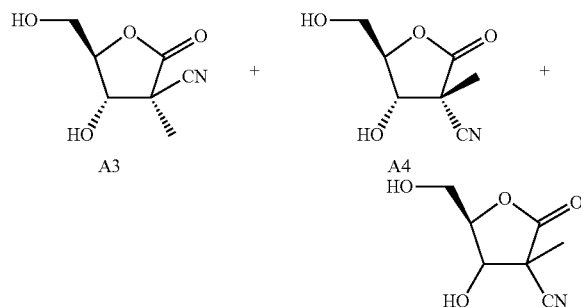
[0489]



[0490] A 5 L flange flask was fitted with a thermometer, nitrogen inlet, pressure equalizing dropping funnel, bubbler, and a subseal. Methyl lithium solution (956 mL, 1.6M in diethylether, 1.7 equiv.) was added, and the solution was cooled to about  $-25^{\circ}\text{C}$ . Diisopropyl amine (214 mL, 1.7 equiv.) was added using the dropping funnel over about 40 minutes. The reaction was left stirring, allowing to warm to ambient temperature overnight.  $\text{CO}_2(\text{s})$ /acetone cooling was applied to the LDA solution, cooling to about  $-70^{\circ}\text{C}$ . R-Glyceraldehyde dimethylacetal solution (50% in DCM) was evaporated down to  $\sim 100$  mbar at a bath temp of  $35^{\circ}\text{C}$ , to remove the DCM, then azeotroped with anhydrous hexane ( $2 \times 100$  mL), under vacuum. The fresh aldehyde (120 g, 0.9 mol) and ethyl 2-cyanopropionate (170 mL, 1.5 equiv.) were placed in a 1 L round bottom flask, which was filled with toluene (800 mL). This solution was cooled in a  $\text{CO}_2(\text{s})$ /acetone bath, and added via cannula to the LDA solution over about 50 minutes, keeping the internal temperature of the reaction mixture cooler than  $-55^{\circ}\text{C}$ . The mixture was stirred with cooling (internal temp. slowly fell to  $\sim -72^{\circ}\text{C}$ ) for 90 min, then warmed to room temperature over 30 minutes using a water bath. This solution was added to a sodium dihydrogen phosphate solution 300 g of  $\text{NaH}_2\text{PO}_4$  in 1.5 L of ice/water, over about 10 minutes, with ice-bath cooling. The mixture was stirred for 20 minutes, then filtered and transferred to a reparatory funnel, and partitioned. The solid was further washed with EtOAc ( $2 \times 1$  L) and the washings were used to extract the aqueous. The combined organic extracts were dried over sodium sulfate. The volatiles were removed in vacuo. The resultant oil was hydrolyzed crude.

3-Cyano-4-hydroxy-5-(hydroxymethyl)-3-methyloxolan-2-one

[0491]

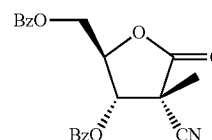


[0492] The crude oil was taken up in acetic acid (1.5 L, 66% in water) and heated to  $90^{\circ}\text{C}$ . over one hour, then at held at

that temperature for one hour. Once the mixture had cooled to room temperature, the volatiles were removed in vacuo, and azeotroped with toluene ( $2 \times 500$  mL). The resultant oil was combined with some mixed material from an earlier synthesis and columned in two portions (each  $\sim 1.25$  L of silica,  $0 \rightarrow 12.5\% \rightarrow 25\% \rightarrow 50\%$  EtOAc in DCM). The lower of the two main spots is the desired material; fractions containing this material as the major component were combined and the solvent removed in vacuo to give 85.4 g of a brown oil as a mixture of 3 diastereomers (15:8:2).

((2R,3 S,4R)-3-(benzoyloxy)-4-cyano-4-methyl-5-oxotetrahydrofuran-2-yl)methyl benzoate (A5)

[0493]

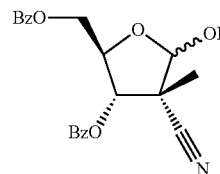


[0494] A 2 L 3-neck round bottom flask was fitted with an overhead stirrer, thermometer and pressure equalizing dropping funnel under nitrogen. 3-Cyano-4-hydroxy-5-(hydroxymethyl)-3-methyloxolan-2-one (85.4 g, 0.50 mol) in acetonitrile (1.5 L) was added, followed by 4-dimethylaminopyridine (700 mg) and benzoyl chloride (128 mL, 2.2 equiv.). Finally triethylamine (167 mL, 2.4 equiv.) was added over 10 minutes using the dropping funnel. The addition of the triethylamine is accompanied by a mild exotherm, which obviated the addition of a cold water bath to keep the internal temperature below  $25^{\circ}\text{C}$ . The reaction was stirred at ambient temperature for 2.5 hours. The reaction mixture was transferred to a separating funnel with EtOAc (2.5 L) and half saturated brine (2.5 L), and partitioned. The aqueous layer was re-extracted with EtOAc (1.5 L). The combined organic layers were washed with 50% Sodium bicarbonate/25% Brine (1.5 L) and dried over sodium sulphate. The resultant brown solid was twice recrystallized from hexane/chloroform, to give  $\sim 15$  g of product of the desired purity. The mother liquors from the recrystallizations were further recrystallized from chloroform/hexanes several times to give a further 15 g of product.

[0495]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm) 8.11 (dm,  $J=8.3$  Hz, 2H), 7.98 (dm,  $J=8.4$  Hz, 2H), 7.66 (tm,  $J=7.5$  Hz, 1H), 7.59 (tm,  $J=7.5$  Hz, 1H), 7.49 (tm,  $J=7.6$  Hz, 2H), 7.43 (tm,  $J=7.6$  Hz, 2H), 5.54 (d,  $J=6.5$  Hz, 1H), 4.97-5.02 (m, 1H), 4.77 (dd,  $J=12.7$ , 3.5 Hz, 1H), 4.66 (dd,  $J=12.7$ , 4.7 Hz, 1H), 1.88 (s, 3H).

3,5-Di-O-benzoyl-2-C-cyano-2-C-methyl-D-ribofuranose (A6)

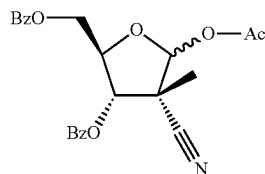
[0496]



[0497] To a solution of A5 (81.08 mmol) in anhydrous tetrahydrofuran (650 mL) was added under inert atmosphere at  $-35^{\circ}\text{C}$ .,  $\text{LiAlH}(\text{OtBu})_3$  (1.0 M in tetrahydrofuran, 21.7 mmol) over a 20 minutes period. The reaction mixture was stirred for 1 hour at  $-20^{\circ}\text{C}$ . and quenched by addition of a saturated  $\text{NH}_4\text{Cl}$  solution, keeping the temperature below  $0^{\circ}\text{C}$ . Ethyl acetate was added and the white suspension was filtered through a pad of celite and washed with ethyl acetate. The filtrate was extracted with ethyl acetate twice. The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The expected intermediate was used without further purification for the next step. MS (ESI)  $m/z=404$  ( $\text{MNa}^+$ ).

1-O-Acetyl-3,5-di-O-benzoyl-2-C-cyano-2-C-methyl-D-arabinofuranose (A7)

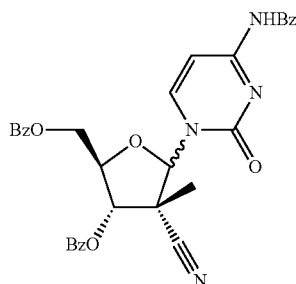
[0498]



[0499] To a solution of A6 (81.0 mmol) in anhydrous tetrahydrofuran (420 mL) was added dropwise under inert atmosphere (nitrogen) at  $0^{\circ}\text{C}$ ., acetic anhydride (405.0 mmol) followed by 4-dimethylaminopyridine (8.1 mmol). The reaction mixture was allowed to warm-up to room temperature and was stirred for 1 hour. The crude was partially concentrated under reduced pressure, partitioned with dichloromethane and a saturated  $\text{NaHCO}_3$  solution, then transferred into a separatory funnel. The organic layer was extracted, dried, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel [eluent: petroleum ether/ethyl acetate: 0 to 100%] to afford a  $\alpha,\beta$  sugar mixture A7 in 96% overall yield (2 steps). MS (ESI)  $m/z=869.2$  ( $2\text{MNa}^+$ ).

3',5'-Di-O-benzoyl-2'-C-cyano-2'-C-methyl-4-benzoyl- $\alpha,\beta$ -cytidine (A8)

[0500]

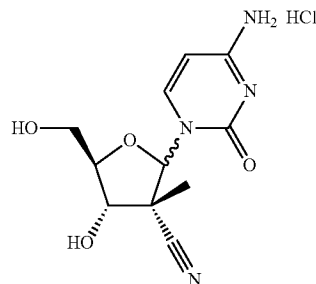


[0501] To a suspension of N-benzoyl cytosine (23.62 mmol), and a catalytic amount of ammonium sulfate in 4-chlorobenzene (60 mL) was added HMDS (70.85 mmol). The reaction mixture was heated at  $140^{\circ}\text{C}$ . overnight. The

solvent was removed under inert atmosphere and the residue was taken in 4-chlorobenzene (20 mL). Then, 7 (11.81 mmol) in chlorobenzene (40 mL) was added dropwise to the reaction mixture followed by  $\text{SnCl}_4$  (23.62 mmol) dropwise. The reaction mixture was stirred at  $70^{\circ}\text{C}$ . overnight, cooled to room temperature and diluted with dichloromethane and a saturated  $\text{NaHCO}_3$  solution. The white suspension was filtered through a pad of celite and washed with dichloromethane. The filtrate was extracted with dichloromethane twice. The combined organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated under reduced pressure to afford expected nucleoside as an  $\alpha,\beta$  mixture. Crude material was used without further purification for the next step. MS (ESI)  $m/z=598.2$  ( $\text{MH}^+$ ).

2'-C-Cyano-2'-C-methyl- $\alpha,\beta$ -cytidine, hydrochloride form (A9)

[0502]



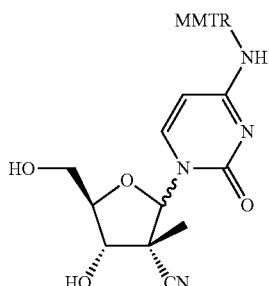
[0503] A suspension of A8 (11.8 mmol) in 7N methanolic ammonia (150 mL) was stirred at room temperature for 3 days in a stainless steel pressure reactor. The mixture was evaporated to dryness, diluted with water and transferred into a separatory funnel. The aqueous layer was extracted with dichloromethane and water was removed under reduced pressure. Crude residue was diluted with ethanol (50 mL) and 10 mL of 1.25 N HCl in dioxan were added. Concentration of the reaction mixture under reduced pressure followed by 3 co-evaporations with absolute ethanol afforded a precipitate which was filtrated-off and washed with absolute ethanol to give pure expected compound as a white solid in 41% overall yield (2 steps) (57/43  $\alpha,\beta$  mixture).

[0504]  $^1\text{H}$  NMR (DMSO, 400 MHz)  $\delta$  (ppm) 1.15 (s, 3H $\beta$ ), 1.51 (s, 3H $\alpha$ ), 3.45-3.95 (m, 3H $\alpha,\beta$ ), 4.00-4.10 (m, 1H $\alpha,\beta$ ), 4.98 (brs, 1H $\alpha$ ), 5.29 (brs, 1H $\beta$ ), 5.80 (d,  $J=7.40$  Hz, 1H $\beta$ ), 5.89 (d,  $J=7.40$  Hz, 1H $\alpha$ ), 5.95 (s, 1H $\alpha$ ), 6.22 (s, 1H $\beta$ ), 6.42 (brd, 1H $\alpha,\beta$ ), 7.53 (brs, 1H $\alpha,\beta$ ), 7.76 (d,  $J=7.40$  Hz, 1H $\alpha$ ), 7.89 (brs, 1H $\alpha,\beta$ ), 7.96 (d,  $J=7.40$  Hz, 1H $\beta$ ); MS (ESI)  $m/z=267$  ( $\text{MH}^+$ )

[0505] Compound A9b: The white solid A9 was triturated with a mixture of methanol/triethylamine/water; and filtered to afford an off-white solid A9a as  $\alpha$ -anomer, and a filtrate. The filtrate was concentrated under reduced pressure and purified by flash chromatography on silica gel [eluent: DCM/methanol:80/20, with 1% of Et<sub>3</sub>N] to afford the expected  $\beta$ -anomer A9b. Off-white solid,  $^1\text{H}$  NMR (DMSO, 400 MHz)  $\delta$  (ppm) 1.13 (s, 3H), 3.60-3.65 (m, 1H), 3.77-3.90 (m, 3H), 5.26 (brt, 1H), 5.73 (d,  $J=7.42$  Hz, 1H), 6.24 (s, 1H), 6.38 (brd, 1H), 7.29 (brd, 2H), 7.88 (d,  $J=7.42$  Hz, 1H); MS (ESI)  $m/z=267$  ( $\text{MH}^+$ ).

## Compound A10

[0506]

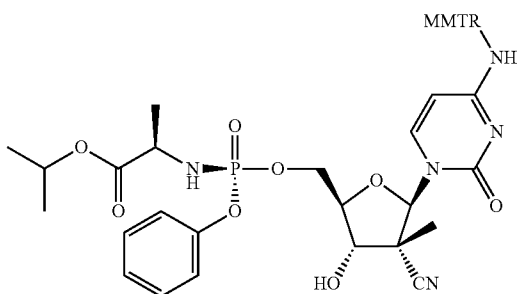


[0507] To a solution of compound A9 (2.31 mmol) in dry pyridine (16 mL) and DMF (was added dropwise TIPSCl<sub>2</sub> (2.54 mmol) under nitrogen atmosphere. The reaction was stirred for 5 hours at room temperature. Then, DMAP (2.31 mmol) and mMTTrCl (2.77 mmol) were added at room temperature and the reaction mixture was stirred at 55° C. overnight. The reaction mixture was slowly added to a saturated solution of NaHCO<sub>3</sub>. The aqueous layer was extracted with DCM and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude was diluted in MeOH (16 mL) and NH<sub>4</sub>F (11.55 mmol) was added. The reaction mixture was stirred at 60° C. during 1.5 hour and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel [eluent: DCM to DCM/MeOH 95/5] to afford a mixture of expected  $\beta$  nucleoside 10 (270 mg, beige foam, 22% overall yield) and a nucleoside A11 (416 mg).

[0508] Compound A10:  $\beta$  (ppm) 0.97 (s, 3H), 3.51-3.87 (m, 4H), 3.71 (s, 3H), 5.23 (brt, 1H), 6.06 (s, 1H), 6.26 (d, J=7.50 Hz, 1H), 6.35 (brd, J=4.50 Hz, 1H), 5.80 (d, J=7.40 Hz, 1H $\beta$ ), 6.80-7.40 (m, 14H), 7.80 (d, J=7.50 Hz, 1H), 8.51 (brs, 1H); MS (ESI)  $m/z$ =537.2 (MH<sup>+</sup>).

## Compound A12

[0509]

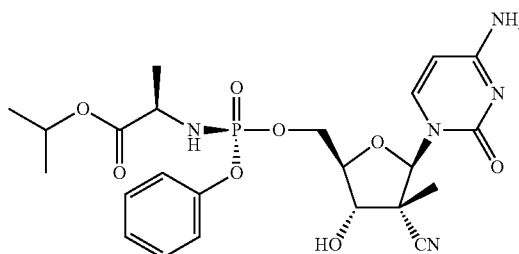


[0510] To as solution of compound A10 (0.39 mmol) in anhydrous THF (2 mL) under nitrogen at -5° C. was added dropwise tert-butylmagnesium chloride (1.0M in THF) (0.82 mmol). The white suspension was stirred at this temperature for 15 minutes and then warmed to ambient temperature and stirred for an additional 20 minutes. The reaction mixture was cooled down to 0° C. and compound A12.0 (0.47 mmol) solubilized in THF (2 mL) was added dropwise. DMSO (0.4

mL) was added and the mixture was stirred at 7° C. overnight. The reaction mixture was diluted with dichloromethane and washed with H<sub>2</sub>O. The organic phase was dried, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel [eluent: DCM/MeOH 0 to 4%] to give the expected compound in 68% yield. MS (ESI)  $m/z$ =806.2 (MH).

## Compound 901

[0511]



[0512] To a solution of compound A12 (0.27 mmol) in DCM (10 mL) was added dropwise TFA (2.67 mmol) under nitrogen. The reaction mixture was stirred at room temperature overnight. The reaction mixture was purified directly by flash chromatography on silica gel and by preparative HPLC to give the expected compound as a white powder.

[0513] <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  (ppm) 1.15 (d, J=3.06 Hz, 3H), 1.17 (d, J=3.06 Hz, 3H), 1.25 (brd, 6H), 3.65 (brd, J=13.0 Hz, 1H), 3.77-3.91 (m, 2H), 4.00 (brd, J=7.22 Hz, 1H), 4.70 (t, J=8.30 Hz, 1H), 4.88 (heptuplet, J=6.30 Hz, 1H), 5.28 (brs, 1H), 5.76 (d, J=7.52 Hz, 1H), 6.28 (s, 1H), 6.34 (q, J=10.30 Hz, J=10.36 Hz), 7.17-7.27 (m, 3H), 7.31-7.42 (m, 4H), 7.83 (d, J=7.57 Hz, 1H); <sup>31</sup>P NMR (DMSO, 161.98 MHz):  $\delta$  (ppm) 3.48 (s, 1P); MS (ESI)  $m/z$ =536.2 (MH<sup>+</sup>).

## Example 2

## HCV Replicon Assay

[0514] Huh-7-derived cell line (Zluc) that harbors an HCV genotype 1b replicon and a luciferase reporter gene was grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM GlutaMAX, 1% MEM nonessential amino acids, 100 IU/mL penicillin, 100  $\mu$ g/mL streptomycin, and 0.5 mg/mL Geneticin® (G418). For dose response testing the cells were seeded in 96-well plates at 7.5 $\times$ 10<sup>3</sup> cells per well in a volume of 50  $\mu$ L, and incubated at 37° C./5% CO<sub>2</sub>. Drug solutions were made up freshly in Huh-7 media as 2 $\times$  stocks. Ten additional 5-fold dilutions were prepared from these stocks in DMEM without G418. At least three hours after Zluc cells were seeded, drug treatment was initiated by adding 50  $\mu$ L of drug dilutions to the plates in duplicate. Final concentrations of drug ranged from 100  $\mu$ M to 0.0000512  $\mu$ M. Cells were then incubated at 37° C./5% CO<sub>2</sub>. Alternatively, compounds were tested at two concentrations (1  $\mu$ M and 10  $\mu$ M). In all cases, Huh-7 (which do not harbors the HCV replicon) served as negative control. After 72 hours of incubation, the inhibition of HCV replication was measured by quantification of photons emitted after mono-oxygenation of 5'-fluoroluciferin to oxyfluoroluciferin by firefly luciferase. For this, media was removed from the plates via gentle tapping. Fifty microliters



of ONE-glo luciferase assay reagent was added to each well. The plates were shaken gently for 3 min at room temperature and luminescence was measured on a Victor<sup>3</sup> V 1420 multi-label counter (Perkin Elmer) with a 1 second read time using a 700 nm cut-off filter. The EC<sub>50</sub> values were calculated from dose response curves from the resulting best-fit equations determined by Microsoft Excel and XLfit 4.1 software. When screening at two fixed concentrations, the results were expressed as % inhibition at 1  $\mu$ M and 10  $\mu$ M.

[0515] For cytotoxicity evaluation, Zluc cells were treated with compound as described herein, and cell viability was monitored using the CellTiter-Blue Cell Viability Assay (Promega) by adding 20  $\mu$ L of the assay solution to each well. The plates were then incubated at 37° C./5% CO<sub>2</sub> for at least 3 hours. Fluorescence was detected in plates using excitation and emission wavelengths of 560 and 590 nm, respectively, in a Victor<sup>3</sup> V 1420 multilabel counter (Perkin Elmer) and CC<sub>50</sub> values were determined using Microsoft Excel and XLfit 4.1 software.

[0516] Compounds presented in Table 2 below were assayed according to the replicon assay described herein.

TABLE 2

HCV Replicon Activity					
HCV Replicon			HCV Replicon		
Compound	EC <sub>50</sub>	CC <sub>50</sub>	Compound	EC <sub>50</sub>	CC <sub>50</sub>
Reference	EC <sub>50</sub>	CC <sub>50</sub>	Reference	EC <sub>50</sub>	CC <sub>50</sub>
Compound 40ii	++++	+	Compound 40ii	+++	+
Diastereomer 1			Diastereomer 2		
Compound 40i	+	+	Compound 202i	+++	+
Diastereomer 1			Diastereomer 2		
Compound 202i	++	+	Compound 205i	+++	+
Diastereomer 1			Diastereomer 2		
Compound 205i	++	+	Compound 603a	+++	++
Diastereomer 1			Single Diastereomer		
Compound 401	++	++	Compound 401	+++	++
Diastereomer 1			Diastereomer 2		
Compound 425	+	++	Compound 425	+	++
Diastereomer 1			Diastereomer 2		
Compound 502a	++	++	Compound 502a	+	++
Diastereomer 1			Diastereomer 2		
Compound 602b	+	++	Compound 602b	++	++
Diastereomer 1			Diastereomer 2		

EC<sub>50</sub> is provided as follows:

++++  $\leq$  250 nM, 250 nM < +++  $\leq$  1  $\mu$ M, 1  $\mu$ M < ++  $\leq$  10  $\mu$ M, and + > 10  $\mu$ M

CC<sub>50</sub> is provided as follows:

++  $\leq$  50  $\mu$ M, + > 50  $\mu$ M

### Example 3

#### Metabolism Assays

[0517] Assay for the Release of Active Metabolite in Huh-7 Cells.

[0518] Huh-7 cells were plated in 1 mL culture medium (DMEM, containing glucose, L-glutamine and sodium pyruvate, 10% FBS, 100 IU/mL penicillin, 100  $\mu$ g/mL streptomycin, 2 mM GlutaMAX, 1% MEM non-essential amino acids) at the concentration 0.8, 0.4 and 0.2 million cells per well on 6 well plates for 24, 48 and 72 hr treatment, respectively. Plated cells were incubated overnight at 37° C. in an incubator.

[0519] The following morning test compound was diluted to 20  $\mu$ M from a stock solution in DMSO in fresh culture medium pre-warmed to 37° C. and 1 mL of the solution/well

was added to cells. A final medium volume per well was 2.0 mL, test compound concentration in well was 10  $\mu$ M and final DMSO concentration was 0.1%.

[0520] After 24, 48 or 72 hr, the medium was carefully removed and cell monolayers were washed twice with 2 mL ice-cold PBS per well. Following the last wash, all PBS was carefully removed and 1.0 mL of extraction solution (ice-cold 70% methanol) added. The plate was tightly covered with Parafilm, plastic plate cover and Parafilm again and an intracellular content was extracted at -20° C. for 24 hr.

[0521] After 24 hr the extracts were transferred into polypropylene microfuge tubes and dry on a refrigerated centrifuge concentrator. Dry residues were reconstituted in 250  $\mu$ L of HPLC-grade water and centrifuged at 16,000 $\times$ g for 10 min. Aliquots (100  $\mu$ L each) of the supernatants were transferred into a 96 well plate and internal standard (4 ng/mL final concentration) was added as the internal standard (IS) for LC-MS/MS analysis.

[0522] Abbreviations:

[0523] FHH=fresh human hepatocytes; Ms=Mouse; MSH=fresh mouse hepatocyte.

[0524] Assay for the Release of Active Metabolite in Primary Hepatocytes:

[0525] Plates of fresh human and mouse hepatocytes were obtained on ice. The medium was removed and replaced with hepatocyte culture medium (William's E supplemented with penicillin-streptomycin, 1% L-glutamine, 1% insulin-transferrin-selenium and 0.1  $\mu$ M Dexamethasone (Invitrogen) or with Invitro GRO HI medium complemented with Torpedo antibiotics (Celsis)). Cells were left overnight in an incubator at 37° C. to acclimatize to culture and the medium.

[0526] Hepatocyte incubations were conducted at a final volume of 0.5 mL hepatocyte culture medium/well (0.8 million cells/well for human and 0.5 million cells/well for mouse; 12 well plate no overlay, collagen coat). Culture medium from overnight incubation of cells was removed and replaced with fresh medium, pre-warmed to 37° C., containing 10  $\mu$ M of test compound from a stock solution in DMSO (final DMSO concentration was 0.1%). At each specific time point, incubation medium was removed and cell monolayers were carefully washed two times with ice-cold PBS. Following the last wash, all PBS was carefully removed and 1.0 mL of extraction solution (ice-cold 70% methanol/30% water) added. Cells were scraped off and suspended in the extraction solution, transferred to 2 mL polypropylene microfuge tubes and intracellular contents extracted overnight at -20° C.

[0527] After the overnight treatment the cellular extracts were prepared by centrifugation at 16,000 $\times$ g for 10 min to remove cellular debris. The remaining sample was then dried using a refrigerated centrifuge concentrator. Dry extracts were reconstituted in 1000  $\mu$ L of HPLC-grade water and centrifuged at 16,000 $\times$ g for 10 min. Aliquots (100  $\mu$ L each) of the supernatant were transferred into a 96 well plate and internal standard (4 ng/mL final concentration) was added as the internal standard (IS) for LC-MS/MS analysis.

[0528] The incubation time points were 6, 24 and 48 hours for human hepatocytes and 1, 4, 8, 12 and 24 hours for mouse hepatocytes. Results are provided in Table 4 below.

TABLE 4

Formation of Active Metabolite in Huh-7 cells and Hepatocytes				
Cells	Compound 40ii Diastereomer 2	Compound 40ii Diastereomer 1	Compound 40i Diastereomer 1	Compound 40i Diastereomer 2
Huh-7 TP $C_{max}$ (pmol/mill cells)	294	123	ND	ND
Huh-7 TP (24 hr)	240	25	ND	ND
Huh-7 TP (48 hr)	294	96	ND	ND
Huh-7 TP (72 hr)	144	123	ND	ND
Huh-7 TP AUC (pmol · hr/mill cells)	14544	4380	ND	ND
FHH TP AUC (pmol · hr/mill cells)	4934	ND	ND	ND
FHH TP $C_{max}$ (pmol/mill cells)	197	ND	ND	ND
FHH TP (6 hr)	197	ND	ND	ND
FHH TP (24 hr)	89	ND	ND	ND
FHH TP (48 hr)	59	ND	ND	ND
MsH AUC (pmol · hr/mill cells)	1794	ND	4052	1073
MsH $C_{max}$ (pmol/mill cells)	87		198	89

<sup>a</sup>ND = not determined<sup>b</sup>BLD = below limit of detection

## Example 4

Pharmacokinetics of Plasma Nucleoside and Liver  
Triphosphate Following a Single Oral Dose in CD-1  
Mice**[0529]** Abbreviations:**[0530]** Ms=Mouse; TP=triphosphate.

**[0531]** A single oral dose of Compound 1 at 10 mg/kg in PEG 200 (dose volume 5 mL/kg) was administered to nine CD-1 male mice. Five untreated animals were used for the collection of control plasma and liver. Terminal plasma and liver samples were collected from three animals per time point at 4, 12 and 24 hours post dose. Liver specimens were collected from all animals immediately after the incision. Freezing forceps stored in liquid nitrogen were used to freeze the liver before excision.

**[0532]** Plasma samples were analyzed for nucleoside by LC-MS/MS. The internal standard (IS) was either 2'-MeG-D3 or tiapride. For protein precipitation and extraction, each plasma sample (50  $\mu$ L) was treated with 500  $\mu$ L of 0.2% formic acid in acetonitrile and 20  $\mu$ L of the internal standard working solution. After vortexing and centrifugation, 5004 of the sample extracts were transferred to a new plate, dried under N<sub>2</sub> at ~28° C. and reconstituted with 75  $\mu$ L of 0.2% FA

in water. The extracts were chromatographed on an Aquasil C18 column using a gradient system of 0.2% formic acid in water and acetonitrile. The analytes were detected and quantified by tandem mass spectrometry in positive ion mode on an MDS Sciex API5000 equipped with a Turbo Ion-spray® interface. The calibration range was 0.500 (LLOQ) to 200 ng/mL in mouse plasma.

**[0533]** Liver samples were analyzed for the active species nucleoside triphosphate by LC-MS/MS. The triphosphate levels were assayed by homogenizing (on ice) a known weight of mouse liver with 4 $\times$  volume of 0.95 M trichloroacetic acid (TCA). Internal standard solution was added to the homogenate followed by neutralization with 20% ammonium hydroxide solution and addition of 500  $\mu$ L 1% formic acid. The tissue samples were extracted by weak anion exchange solid phase extraction (SPE). Post extraction, the eluates were evaporated under nitrogen, followed by reconstitution before injection onto the LC-MS/MS system. The samples were chromatographed on a Luna NH<sub>2</sub> column using a gradient system of ammonium acetate (1 mM to 20 mM and pH 8.0 to pH 10.0) in water and acetonitrile (70:30). The analyte was detected and quantified by tandem mass spectrometry in positive ion mode on an API4000 equipped with a Turbo Ion-spray® interface.

**[0534]** Results are provided in Table 5 below.

TABLE 4

Mouse plasma and liver pharmacokinetic parameters				
Compound	Ms Plasma nucleoside $C_{max}$ (pmol/mL at 1 $\mu$ mol/kg)	Ms Plasma nucleoside AUC (pmol · hr/mL at 1 $\mu$ mol/kg)	Ms Liver TP $C_{max}$ (pmol/g at 1 $\mu$ mol/kg)	Ms Liver TP AUC (pmol · hr/g at 1 $\mu$ mol/kg)
Compound 40ii Diastereomer 1	86	970	71	840

TABLE 4-continued

Mouse plasma and liver pharmacokinetic parameters				
Compound	Ms Plasma nucleoside $C_{max}$ (pmol/mL at 1 $\mu$ mol/kg)	Ms Plasma nucleoside AUC (pmol $\cdot$ hr/mL at 1 $\mu$ mol/kg)	Ms Liver TP $C_{max}$ (pmol/g at 1 $\mu$ mol/kg)	Ms Liver TP AUC (pmol $\cdot$ hr/g at 1 $\mu$ mol/kg)
Compound 40ii	99	1300	34	560
Diastereomer 2				
Compound 40i	94	1400	520	6200
Diastereomer 1				
Compound 40i	64	1000	430	4400
Diastereomer 2				
Compound 202i	—	1700	—	8400
Diastereomer 1				
Compound 202i	—	1700	—	7200
Diastereomer 2				
Compound 202ii	—	—	160	1200
Diastereomer 1				
Compound 202ii	—	—	84	850
Diastereomer 2				
Compound 205i	—	1400	—	5600
Diastereomer 1				
Compound 205i	—	1700	—	6900
Diastereomer 2				

ND = Not determined;

BLQ = below limit of quantitation.

## Example 4A

## Pharmacokinetics of Plasma Nucleoside and Liver Triphosphate Following a Single Oral Dose in CD-1 Mice

**[0535]** Abbreviations:**[0536]** Ms=Mouse; TP=triphosphate.

**[0537]** A single oral dose of test compound at 2 mg/kg and 10 mg/kg in PEG 200 (dose volume 1 mL/kg and 5 mL/kg) was administered to nine CD-1 male mice. Five untreated animals were used for the collection of control plasma and liver. Terminal plasma and liver samples were collected from three animals per time point at 4, 12 and 24 hours post dose. Liver specimens were collected from all animals immediately after the incision. Freezing forceps stored in liquid nitrogen were used to freeze the liver before excision.

**[0538]** Plasma samples were analyzed for nucleoside by LC-MS/MS. The internal standard (IS) was either 2'-MeG-D3 or tiapride. For protein precipitation and extraction, each plasma sample (50  $\mu$ L) was treated with 500  $\mu$ L of 0.2% formic acid in acetonitrile and 20  $\mu$ L of the internal standard working solution. After vortexing and centrifugation, 500  $\mu$ L of the sample extracts were transferred to a new plate, dried under  $N_2$  at  $\sim 28^\circ C$ . and reconstituted with 75  $\mu$ L of 0.2% FA in water. The extracts were chromatographed on an Aquasil C18 column using a gradient system of 0.2% formic acid in water and acetonitrile. The analytes were detected and quantified by tandem mass spectrometry in positive ion mode on an MDS Sciex API5000 equipped with a Turbo IonSpray® interface. The calibration range was 0.500 (LLOQ) to 200 ng/mL in mouse plasma.

**[0539]** Liver samples were analyzed for the active species nucleoside triphosphate by LC-MS/MS. The triphosphate levels were assayed by homogenizing (on ice) a known

weight of mouse liver with 4 $\times$  volume of 0.95 M trichloroacetic acid (TCA). Internal standard solution was added to the homogenate followed by neutralization with 20% ammonium hydroxide solution and addition of 500  $\mu$ L 1% formic acid. The tissue samples were extracted by weak anion exchange solid phase extraction (SPE). Post extraction, the eluates were evaporated under nitrogen, followed by reconstitution before injection onto the LC-MS/MS system. The samples were chromatographed on a Luna NH<sub>2</sub> column using a gradient system of ammonium acetate (1 mM to 20 mM and pH 8.0 to pH 10.0) in water and acetonitrile (70:30). The analyte was detected and quantified by tandem mass spectrometry in positive ion mode on an API4000 equipped with a Turbo IonSpray® interface.

**[0540]** Results are provided in Table 3 below.

TABLE 4A

Mouse plasma and liver pharmacokinetic parameters		
Cells	Compound 425 Diastereomer 1	Compound 425 Diastereomer 2
Ms Plasma nucleoside AUC (pmol $\cdot$ hr/mL after 2 mg/kg dose)	46	26
Ms Liver TP AUC (pmol $\cdot$ hr/g at after 2 mg/kg dose)	3900	1900
Ms Plasma nucleoside AUC (pmol $\cdot$ hr/mL after 10 mg/kg dose)	130	75
Ms Liver TP AUC (pmol $\cdot$ hr/g at after 10 mg/kg dose)	7500	2500

## Example 4B

## Pharmacokinetics of Plasma Nucleoside and Liver Triphosphate Following a Single Oral Dose in CD-1 Mice

[0541] Abbreviations:

[0542] Ms=Mouse; TP=triphosphate;

[0543] A single oral dose of test compound at 10 mg/kg and/or 2 mg/kg in PEG 200 (dose volume 5 mL/kg) was administered to nine CD-1 male mice. Five untreated animals were used for the collection of control plasma and liver. Terminal plasma and liver samples were collected from three animals per time point at 4, 12 and 24 hours post dose. Liver specimens were collected from all animals immediately after the incision. Freezing forceps stored in liquid nitrogen were used to freeze the liver before excision. Only liver samples were analyzed for triphosphate levels.

[0544] Liver samples were analyzed for the active species nucleoside triphosphate by LC-MS/MS. The triphosphate levels were assayed by homogenizing (on ice) a known weight of mouse liver with 4x volume of 0.95 M trichloroacetic acid (TCA) in water. Internal standard solution was added to the homogenate and mixed. Sample homogenates were centrifuged at 16.1 krpm for 5 minutes. Supernatants were transferred to 96 well plates and injected onto the LC-MS/MS system. The samples were chromatographed on a Luna NH<sub>2</sub> column using a gradient system of ammonium acetate (1 mM to 20 mM and pH 8.0 to pH 10.0) in water and acetonitrile (70:30). The analyte was detected and quantified by tandem mass spectrometry using the analyte specific MRM transition on an API4000 equipped with a Turbo Ion-spray® interface.

[0545] Results are provided in Table 3 below.

TABLE 4B

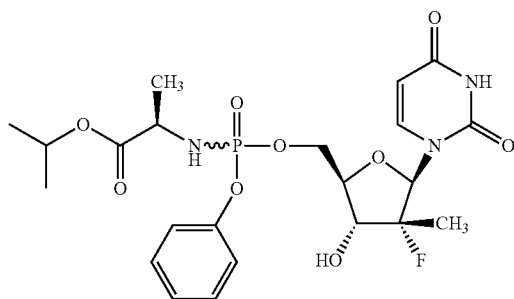
Mouse liver pharmacokinetic parameters					
Cells	Compound 602b Diastereomer 1	Compound 602b Diastereomer 2	Compound 603a Single Diastereomer	Compound 603b Diastereomer 1	Compound 603b Diastereomer 2
Ms Liver TP AUC (pmol · hr/g at 1 µmol/kg) following a single 10 mg/kg dose	5500	3600	120	110	—
Ms Liver TP AUC (pmol · hr/g at 1 µmol/kg) following a single 2 mg/kg dose	4100	3200	—	—	—

## Example 4C

## Plasma and Liver Pharmacokinetics Following a Single Oral Dose in CD-1 Mice

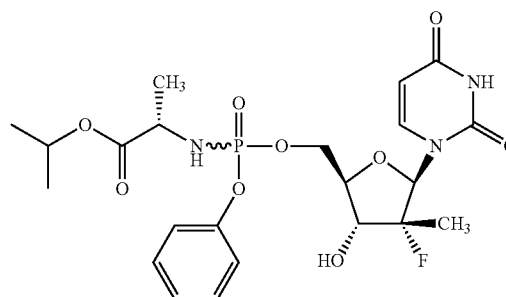
[0546]

(804a)



-continued

(804b)



[0547] Abbreviations:

[0548] Ms=Mouse; 2'-Me-2'-F-U=2'-methyl-2'-fluorouridine; 2'-Me-2'-F-U TP=2'-methyl-2'-fluorouridine triphosphate; 2'-F-2'-Me-G=2'-fluoro-2'-methyl-guanosine.

[0549] A single oral dose of test compound at 10 mg/kg for 804a or 25 mg/kg for 804b in PEG 200 (dose volume 5 mL/kg) was administered to nine CD-1 male mice. Five untreated animals were used for the collection of control plasma and liver. Terminal plasma and liver samples were collected from three animals per time point at 4, 12 and 24 hours post dose. Liver specimens were collected from all animals immediately after the incision. Freezing forceps stored in liquid nitrogen were used to freeze the liver before excision.

[0550] Plasma samples were analyzed for 2'-methyl-2'-fluorouridine (2'-Me-2'-F-U) by LC-MS/MS. The internal standard (IS) was D<sub>3</sub>-2'-F-2'-Me-G. For protein precipitation and extraction, each plasma sample (50 µL) was treated with 500 µL of 0.2% formic acid in acetonitrile and 20 µL of the internal standard working solution. After vortexing and centrifugation, 500 µL of the sample extracts were transferred to a new plate, dried under N<sub>2</sub> at ~28° C. and reconstituted with 75 µL of 0.2% FA in water. The extracts were chromatographed on an Aquasil C18 column using a gradient system of 0.2% formic acid in water and acetonitrile. The analytes were detected and quantified by tandem mass spectrometry in positive ion mode on an MDS Sciex API5000 equipped with a Turbo Ion-spray® interface. The calibration range was 0.500 (LLOQ) to 200 ng/mL in mouse plasma. The corresponding range for molar units is 1.92 to 769 pmol/mL.

[0551] Liver samples were analyzed for the active species 2'-methyl-2'-fluorouridine triphosphate (2'-Me-2'-F-U TP) by LC-MS/MS. 2'-Me-2'-F-U TP levels were assayed by

homogenizing (on ice) a known weight of mouse liver with 4× volume of 0.95 M trichloroacetic acid (TCA). Internal standard solution was added to the homogenate followed by neutralization with 20% ammonium hydroxide solution and addition of 500  $\mu$ L 1% formic acid. The tissue samples were extracted by weak anion exchange solid phase extraction (SPE). Post extraction, the eluates were evaporated under nitrogen, followed by reconstitution before injection onto the LC-MS/MS system. The samples were chromatographed on a Luna NH<sub>2</sub> column using a gradient system of ammonium acetate (1 mM to 20 mM and pH 8.0 to pH 10.0) in water and acetonitrile (70:30). The analyte was detected and quantified by tandem mass spectrometry in positive ion mode on an API4000 equipped with a Turbo Ionspray® interface. The calibration range was 10 to 10000 pmol/mL in mouse liver homogenate (50 to 50000 pmol/g of mouse liver).

[0552] Results are provided in Table 5 below.

[0557] Liver samples were analyzed for the relevant nucleoside triphosphate by LC-MS/MS. The triphosphate levels were assayed by homogenizing (on ice) a known weight of liver with 4× volume of 0.95 M trichloroacetic acid (TCA). Appropriate internal standard solution was added to the homogenate followed by neutralization with 20% ammonium hydroxide solution and addition of 500  $\mu$ L 1% formic acid. The tissue samples were extracted by weak anion exchange solid phase extraction (SPE). Post extraction, the eluates were evaporated under nitrogen, followed by reconstitution before injection onto the LC-MS/MS system. The samples were chromatographed on a Luna NH<sub>2</sub> column using a gradient system of ammonium acetate (1 mM to 20 mM and pH 8.0 to pH 10.0) in water and acetonitrile (70:30). The analyte was detected and quantified by tandem mass spec-

TABLE 4C

Mouse plasma and liver pharmacokinetic parameters				
Cells	Compound (804a)		Compound (804b)	
	Ia (R <sub>p</sub> isomer)	Ib (S <sub>p</sub> isomer)	Diastereomer 1 (S <sub>p</sub> isomer)	Diastereomer 2 (R <sub>p</sub> isomer)
Ms Plasma 2'-Me-2'-F-U	480	260	320	320
AUC (pmol · hr/mL at 1 $\mu$ mol/kg)				
Ms Liver 2'-Me-2'-F-U TP	3200	430	250	310
AUC (pmol · hr/g at 1 $\mu$ mol/kg)				

## Example 5

Pharmacokinetics of Liver Triphosphate and Plasma Prodrug and Nucleoside Following a Single Oral Dose in Cynomolgus Monkeys

[0553] Abbreviations:

[0554] Mo=Monkey; TP=triphosphate; AUC=area under the concentration curve.

[0555] For each compound, a single oral dose at 10 mg/kg in PEG 200 (dose volume 3 mL/kg) was administered to cynomolgus monkeys. Untreated animals were used for the collection of control liver. Plasma samples were collected at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours for compound 37, diastereomer 2. Terminal liver samples were collected from three animals per time point at 6, 12 and 24 hours post dose for compound 37, diastereomer 2 and at 6 hours post dose for compound 44, diastereomer 2. Liver specimens were collected from all animals immediately after the incision. Freezing forceps stored in liquid nitrogen were used to freeze the liver before excision.

[0556] Plasma samples were analyzed for the prodrug and nucleoside by LC-MS/MS. For protein precipitation and extraction, each plasma sample (50  $\mu$ L) was treated with 500  $\mu$ L of 0.2% formic acid in acetonitrile and 20  $\mu$ L of an appropriate internal standard working solution. After vortexing and centrifugation, 5004 of the sample extracts were transferred to a new plate, dried under N<sub>2</sub> at ~28° C. and reconstituted with 75  $\mu$ L of 0.2% FA in water. The extracts were chromatographed on an Aquasil C18 column using a gradient system of 0.2% formic acid in water and acetonitrile. The analytes were detected and quantified by tandem mass spectrometry in positive ion mode on an MDS Sciex API4000 equipped with a Turbo Ionspray® interface.

trometry in positive ion mode on an API4000 equipped with a Turbo Ionspray® interface. Results are provided in Table 5 below.

TABLE 5

Pharmacokinetics of the prodrug and nucleoside in plasma and triphosphate in liver of Cynomolgus monkeys			
Compound	Compound 37 Diastereomer 2	Compound 40ii Diastereomer 2	Compound 40i Diastereomer 1
Dose (mg/kg)	10	10	10
Dose-normalized parameters <sup>a</sup>			
Plasma prodrug			
C <sub>max</sub> (pmol/mL)	840	ND <sup>b</sup>	
T <sub>max</sub> (hr)	4	ND	
AUC <sub>0-24</sub> (pmol · hr/mL)	4000	ND	
Plasma nucleoside			
C <sub>max</sub> (pmol/mL)	51	ND	
T <sub>max</sub> (hr)	4	ND	
AUC <sub>0-24</sub> (pmol · hr/mL)	650	ND	
Nucleoside triphosphate in Liver			
C <sub>6</sub> (pmol/g)	1500	120	270
C <sub>max</sub> (pmol/g)	1700	ND	
T <sub>max</sub> (hr)	12	ND	
AUC <sub>0-24</sub> (pmol · hr/g)	29000	ND	

<sup>a</sup>The C<sub>max</sub>, C<sub>6</sub> and AUC<sub>0-24</sub> data are normalized to 1  $\mu$ mol/kg dose

<sup>b</sup>ND = not determined

## Example 6

## Hydrolysis of D-Alanine Prodrugs by Cathepsin a (CatA) and/or Carboxylesterase 1 (CES1)

## Introduction

**[0558]** The HCV NS5B RNA-dependent RNA polymerase is essential for the viral life cycle and thus, is a target for antiviral therapy. The active site of NS5B is well conserved among the six genotypes of HCV and therefore, nucleos(t)ide analogs can act pan-genotypically. Furthermore, nucleotide inhibitors are typically not cross-resistant to other classes of direct acting antivirals and can have a higher barrier to resistance compared to non-nucleoside, protease and non-structural protein 5A (NS5A) inhibitors of HCV, making this class of HCV antivirals useful in a of combination HCV antiviral therapy.

**[0559]** Nucleoside analogs are typically competitive inhibitors of endogenous nucleosides and may act through chain termination upon incorporation into the nascent HCV RNA chain during replication (Eldrup, et al. 2004, Structure-Activity Relationship of Purine Ribonucleosides for Inhibition of Hepatitis C Virus RNA-Dependent RNA Polymerase. *J. Med. Chem.* 47: 2283-2295). However, upon cell entry a nucleoside analog must first be phosphorylated to the active triphosphate species (Gardelli, et al 2009, Phosphoramidate prodrugs of 2'-C-methylcytidine for therapy of hepatitis C virus infection. *J. Med. Chem.* 52:5394-5407; Stein and Moore, 2001, Phosphorylation of nucleoside analog antiretrovirals: a review for clinicians. *Pharmacotherapy* 21:11-34; Tomassini, et al 2005, Inhibitory effect of 2'-substituted nucleosides on hepatitis C virus replication correlates with metabolic properties in replicon cells. *Antimicrob. Agents Chemother.* 49:2050-2058; Murakami, et al 2007, Mechanism of activation of  $\beta$ -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine and inhibition of hepatitis C virus NS5B RNA polymerase. *Antimicrob. Agents Chemother.* 51:503-509). A barrier to first generation nucleoside inhibitors was the often inefficient conversion of the nucleoside to a nucleotide monophosphate (NMP) by cellular kinases (Gardelli, et al 2009, Phosphoramidate prodrugs of 2'-C-methylcytidine for therapy of hepatitis C virus infection. *J. Med. Chem.* 52:5394-5407; Stein and Moore, 2001, Phosphorylation of nucleoside analog antiretrovirals: a review for clinicians. *Pharmacotherapy* 21:11-34; Murakami, et al 2007, Mechanism of activation of  $\beta$ -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine and inhibition of hepatitis C virus NS5B RNA polymerase. *Antimicrob. Agents Chemother.* 51:503-509).

**[0560]** Second generation nucleoside analogs have been designed as liver-targeted nucleotide prodrugs, which bypass the rate-limiting NMP conversion to active species by delivering the nucleoside as a monophosphate prodrug. As GS-7977, Z4 and Z2 are pyrimidine nucleotide prodrugs that act by inhibition of the HCV NS5B RNA-dependent RNA polymerase through a 2' modified UTP metabolite.

**[0561]** The intracellular metabolism (anabolism) of nucleotide analogs is critical to their antiviral activity. A first step in the metabolism of nucleotide prodrugs is the removal of the prodrug moiety by cellular enzymes followed by the activation of the nucleoside monophosphate analog by host cell kinases for the sequential phosphorylation of the parent nucleos(t)ide analog to the 5'-triphosphate form, the biologi-

cally active metabolite. Removal of the prodrug moiety often involves sequential or independent work of different cellular enzymes.

**[0562]** In vivo Z4 and Z2 appear to be effectively liver-targeted with a high liver:plasma ratio of drug metabolites. Both prodrugs are readily converted to the triphosphate (TP) metabolite in the liver of mice and monkey producing more TP than GS-7977. The TP derivatives of Z4 and Z2 selectively inhibit wild-type HCV NS5B enzyme in vitro with submicromolar  $IC_{50}$  values. When tested in a genotype 1b HCV replicon-bearing human hepatoma cell line (Huh-7), however, Z4 and Z2 were largely inactive and failed to inhibit replicon reproduction ( $EC_{50} > 50 \mu M$ ). The in vitro antiviral inactivity of Z4 and Z2 is thought to reflect an inability of Huh-7 replicon cells to metabolize the prodrug moiety.

**[0563]** The first step of GS-7977 activation includes hydrolysis of the carboxyl ester by cathepsin A (CatA) and/or carboxylesterase 1 (CES1) (Saboulard et al, 2009, Characterization of the Activation Pathway of Phosphoramidate Triester Prodrugs of Stavudine and Zidovudine. *Molecular Pharmacology*. 56:693-704; Murakami et al, 2010, Mechanism of Activation of PSI-7851 and Its Diastereoisomer PSI-7977, *JBC*, 285(45):34337-34347; Sofia et al, 2010, Discovery of PSI-35366, a novel purine nucleotide prodrug for the treatment of hepatitis C virus. *J. Med. Chem.* 53:7202-7218). Since CES1 is reported to be underexpressed in Huh-7 replicon cells, CatA appears to be the major enzyme that hydrolyzes GS-7977 in these cells (Murakami et al, 2010, Mechanism of Activation of PSI-7851 and Its Diastereoisomer PSI-7977, *JBC*, 285(45):34337-34347).

## Methods

**[0564]** In this example the hydrolysis of the two D-alanine prodrugs Z2 (2'-C1,2'-MeUMP, diastereoisomer  $R_p$ ) and Z4 (2'-F, 2'-MeUTP, diastereoisomer  $R_p$ ) using CatA and CES1 was compared with activation of the L-alanine prodrugs Y1 (2'-MeUTP,  $S_p$  stereoisomer), GS-7977 (X1, diastereoisomer  $S_p$ ) and PSI-7976 (X2, diastereoisomer  $R_p$ ).

**[0565]** CatA, cathepsin L (CatL) and CES1 were purchased from R & D Systems (Minneapolis, Minn.). Prior to the enzymatic hydrolysis reactions, CatA was activated according to the manufacturer's instruction. Briefly, CatA (0.05  $\mu g/\mu L$ ) was incubated with CatL (0.005  $\mu g/\mu L$ ) for 30 min at 37° C. in 25 mM MES pH 6.0 containing 5 mM DTT. The reaction was stopped by addition of the CatL specific inhibitor E64 (10  $\mu M$ ).

**[0566]** The CatA assay was performed at 37° C. The reaction mixture contained 25 mM MES buffer pH 6.0, 100 mM NaCl, 4 mM DTT and 100  $\mu M$  of the compound. The reaction was started by addition of the activated CatA enzyme to a final concentration of 0.005  $\mu g/\mu L$ . One hundred- $\mu L$  aliquots were taken after 0.5 min, 3 hrs and 18 hrs of incubation. Reactions were stopped by mixing the sample with an equal volume of ice-cold methanol, and were loaded on a HPLC for analysis.

**[0567]** CES1 assay was performed at 37° C. in the reaction mixture containing 50 mM Tris/HCl buffer pH 7.5 and 100  $\mu M$  of the compound. Reaction was started by addition of the CES1 to the final concentration 0.01  $\mu g/mL$ . 100  $\mu L$  aliquots were taken after 0.5 min, 3 hrs and 21 hrs of the incubation and the reaction was stopped by mixing with 100  $\mu L$  of the ice-cold methanol prior to HPLC analysis.

**[0568]** Samples were analyzed by HPLC using 5  $\mu C$ -18, 4.6x250 mm Phenomenex® Columbus column (Phenom-

enex USA, CA). The mobile phase consisted of buffer A (25 mM potassium phosphate with 5 mM tetrabutylammonium dihydrogen phosphate pH 6.3) and buffer B (100% methanol). HPLC gradient conditions are shown in Table 6.

TABLE 6

Time (min)	% A	% B	Flow (mL/min)
0	100	0	1
15	70	30	1
30	50	50	1
65	50	50	1
70	95	5	1

## Results

[0569] As shown in Table 7, both CatA and CES1 hydrolyzed GS-7977 and its diastereoisomer PSI-7076. However, CatA cleaved GS-7977 ( $S_P$  configuration) 10 times more efficiently than its  $R_P$  diastereoisomer, while CES 1 preferentially hydrolyzed the  $R_P$  diastereoisomer PSI-7976. These results are in good agreement with the literature (Murakami, et al 2010, Mechanism of Activation of PSI-7851 and Its Diastereoisomer PSI-7977, JBC, 285(45):34337-34347).

TABLE 7

Compound	Reference number	$S_P/R_P$	EC <sub>50</sub> , (μM)	Huh-7 pmol*hr/ 10 <sup>6</sup> cellsAUC <sub>0-72</sub>	Liver TP pmol*hr/g	CatA	CES1
GS-7977	X1	$S_P$	0.25	63555	250	100% @ 18 h	12%/3 h; 15%/21 h
L-Ala-2'F,2'MeUTP							
PSI-7976	X2	$R_P$	2.08	6527	310	10% @ 18 h	56%/3 h; 94%/21 h
L-Ala-2'F,2'MeUTP							
L-Ala-2'MeUTP	Y1	$S_P$	0.17	63740	420	100 @ 3 h	Not tested
D-Ala-2'Cl,2'MeUTP	Z1	$S_P$	7		4400	0%	4.5% @ 21 h
	Z2	$R_P$	5.9; 14; 47	436.9	6200	0%	23% @ 3 h; 49% @ 21 h
D-Ala-2'F,2'MeUTP	Z3	$S_P$	17		430	0%	0%
	Z4	$R_P$	>50	720.4	3200	0%	10% @ 3 h; 26% @ 21 h

[0570] In contrast, CatA was unable to hydrolyze any of the D-Ala-prodrugs tested. However, both Z2 and Z4 were processed by CES 1.

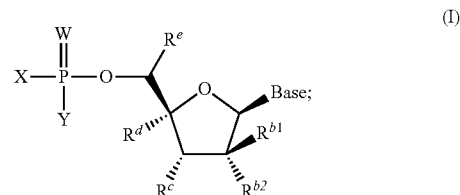
[0571] Since Huh-7 replicon-bearing cells have been found to express little or no CES1, CatA is the major enzyme that hydrolyzes GS-7977 in these cells (Murakami et al, 2010, Mechanism of Activation of PSI-7851 and Its Diastereoisomer PSI-7977, JBC, 285(45):34337-34347). The inability of CatA to activate the D-Ala-prodrugs Z2 and Z4 may explain the inactivity of these compounds in Huh-7 replicon-bearing cells, since the lack of in vitro activity is believed to reflect low production of the active TP moiety in Huh-7 replicon cells.

[0572] In vivo, high expression of CES1 in the liver coupled with high catalytic efficiency and possible involvement of other liver enzyme appears to result in efficient conversion of Z2 and Z4 to their corresponding triphosphate metabolites.

[0573] All publications, patents, and patent applications cited in this specification are herein incorporated by reference as if each individual publication, patent, or patent application

were specifically and individually indicated to be incorporated by reference. While the claimed subject matter has been described in terms of various embodiments, the skilled artisan will appreciate that various modifications, substitutions, omissions, and changes may be made without departing from the spirit thereof. Accordingly, it is intended that the scope of the claimed subject matter is limited solely by the scope of the following claims, including equivalents thereof.

1. A compound according to formula (I):



or a pharmaceutically acceptable salt, solvate, stereoisomeric form, tautomeric form or polymorphic form thereof, wherein:

Base is a nucleobase;

W is S or O;

X is a D-amino acid residue, or an ester thereof;

Y is hydrogen, —OR<sup>1</sup>, —SW, or —NR<sup>1</sup>R<sup>2</sup>;

R<sup>b1</sup> is —CH<sub>3</sub>, —H, azido, cyano, or halogen;

R<sup>b2</sup> is —OH, —Cl, —F, —H, azido, cyano, amino, or alkoxy;

R<sup>c</sup> is —H or —OH, or, in the alternative, Y and R<sup>c</sup> join to form a six-membered heterocyclic ring wherein Y and R<sup>c</sup> together represent a single divalent —O—;

R<sup>d</sup> is —H, —F, azido, or allenyl; or, in the alternative, R<sup>b2</sup> and R<sup>d</sup> join to form alkylene or substituted alkylene;

R<sup>e</sup> is —H or alkyl;

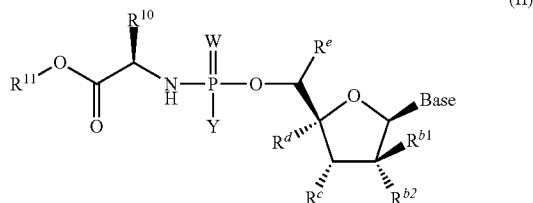
each R<sup>1</sup> is independently alkyl, cycloalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, substituted alkyl or hydantoinylalkyl; and

each R<sup>2</sup> is independently hydrogen or alkyl.

2. The compound of claim 1 wherein each R<sup>1</sup> is independently alkyl, cycloalkyl, heterocyclylalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkylcarbonylthioalkyl, alkoxy-carbonylalkyl, arylalkoxy-carbonylalkyl, alkylcarbonyla-

lkoxy(arylalkyl), (alkoxycarbonyl)(alkoxycarbonylamino) alkyl, cycloalkylcarbonylalkoxy, alkoxycarbonylaminoalkylcarbonylthioalkyl, hydroxylalkylcarbonylthioalkyl, aminoalkylcarbonylalkoxycarbonylthioalkyl, or hydantoinylalkyl.

3. The compound of claim 1 according to formula (II):



wherein each Y is independently  $-\text{OR}^1$ ,  $-\text{SR}^1$ , or  $-\text{NR}^1\text{R}^2$ ;

each  $\text{R}^{10}$  is independently alkyl, arylalkyl, heteroarylalkyl, cycloalkyl or a side chain of a naturally occurring amino acid other than hydrogen; and

each  $\text{R}^{11}$  is independently alkyl or  $-\text{H}$ .

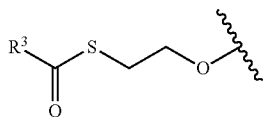
4. The compound of claim 1 wherein  $\text{R}^e$  and  $\text{R}^d$  are H.

5-10. (canceled)

11. The compound of claim 1 wherein W is O, and  $\text{R}^{b2}$  is Cl, F or OH.

12. (canceled)

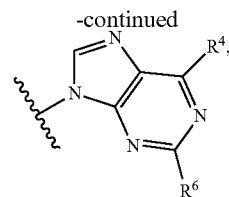
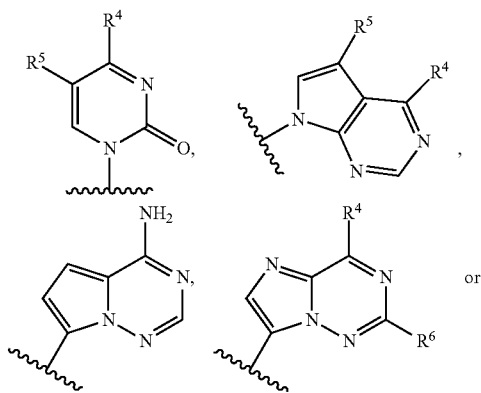
13. The compound of claim 1 wherein Y is



and  $\text{R}^3$  is alkyl, alkoxycarbonylaminoalkyl, hydroxylalkyl, or aminoalkylcarbonylalkoxyl.

14. The compound claim 1 wherein:

each Base is independently



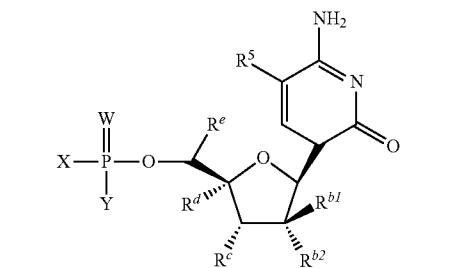
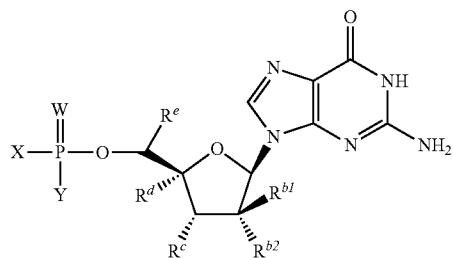
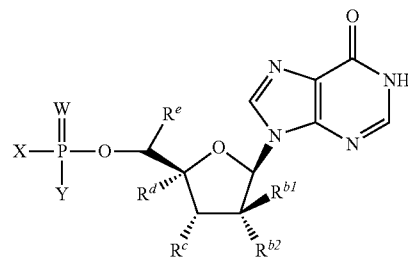
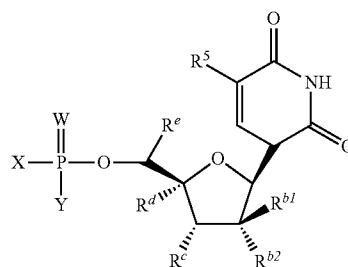
or a tautomer thereof;

each  $\text{R}^4$  is independently hydrogen, hydroxyl, hydroxylamine, alkylamino, halogen, sulfanyl, amino or alkoxyl;

each  $\text{R}^5$  is independently hydrogen, halogen or methyl; and each  $\text{R}^6$  is independently hydrogen, amino, or halo.

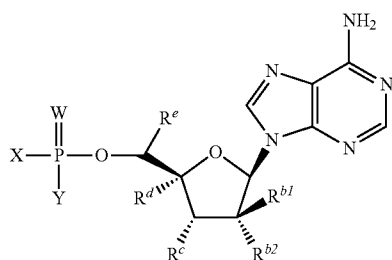
15. The compound of claim 14 wherein  $\text{R}^4$  is alkylamino.

16. The compound of claim 1 according to any of the following formulas:



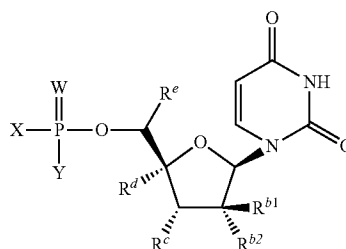


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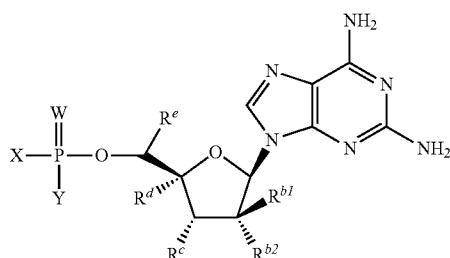


(XII)

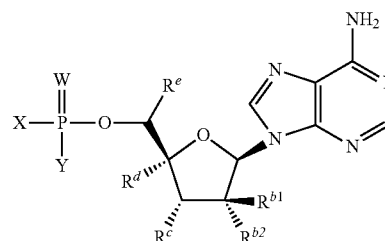
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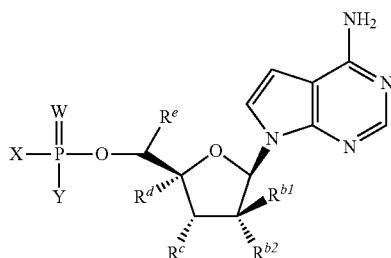
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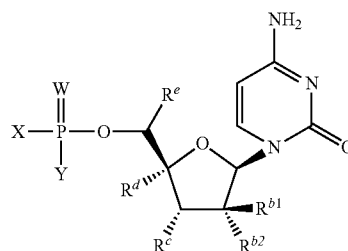
(XIII)



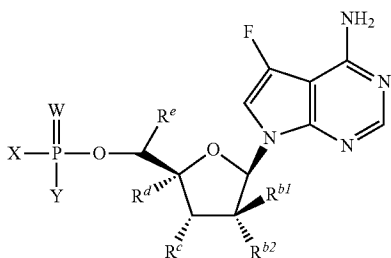
(XIX)



(1001)

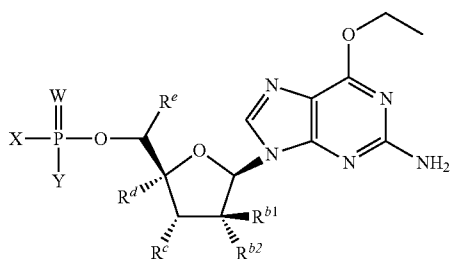


(XX)



(1002)

17. The compound of claim 1 according to any of the following formulas:



(XVII)

18. The  $R_P$  compound of claim 1.

19. The  $S_P$  compound of claim 1.

20. A pharmaceutical composition comprising the compound of claim 1 and a pharmaceutically acceptable excipient, carrier or diluent.

21. The pharmaceutical composition of claim 20, wherein the composition is an oral formulation.

22. A method for the treatment of a host infected with a hepatitis C virus, comprising the administration of an effective treatment amount of a compound of claim 1.

23. The method of claim 22, wherein the host is a human.

24. The method of claim 22, wherein the administration directs a substantial amount of the compound, or pharmaceutically acceptable salt or stereoisomer thereof, to a liver of the host.

25. The method of claim 22, wherein the compound or composition is administered in combination or alternation with a second anti-viral agent selected from the group consisting of an interferon, a nucleotide analogue, a polymerase inhibitor, an NS3 protease inhibitor, an NS5A inhibitor, an entry inhibitor, a non-nucleoside polymerase inhibitor, a cyclosporine immune inhibitor, an NS4A antagonist, an NS4B-RNA binding inhibitor, a locked nucleic acid mRNA inhibitor, a cyclophilin inhibitor, and combinations thereof.

26. The method of claim 25, wherein the second anti-viral agent is selected from the group consisting of telaprevir, boceprevir, interferon alfacon-1, interferon alfa-2b, pegylated interferon alpha 2a, pegylated interferon alpha 2b, ribavirin, and combinations thereof.

27. The method of claim 25, wherein the second anti-viral agent is selected from the group consisting of telaprevir, boceprevir, interferon alfacon-1, interferon alfa-2b, pegy-

lated interferon alpha 2a, pegylated interferon alpha 2b, and combinations thereof, and further wherein the administration is not in combination or alternation with ribavirin.

**28.** A method of treating a liver disease or condition comprising administering to a host in need thereof a compound comprising a D-amino acid linked to a therapeutic moiety.

\* \* \* \* \*