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(54) Title: PYRIMIDINE NUCLEOTIDES AND THEIR MONOPHOSPHATE PRODRUGS FOR TREATMENT OF VIRAL INFECTIONS AND CANCER

(57) Abstract: The present invention is directed to compounds, compositions and methods for treating or preventing cancer and viral infections, in particular, HIV, HCV, Norovirus, Saporovirus, cytomegalovirus (CMV), herpes viruses (HSV-1, HSV-2), Dengue virus, Yellow fever, or HBV in human patients or other animal hosts. The compounds are certain A^-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs, and pharmaceutically acceptable, salts, prodrugs, and other derivatives thereof. In particular, the compounds show potent antiviral activity against HIV-1, HIV-2, HCV, Norovirus, Saporovirus, cytomegalovirus (CMV), herpes viruses (HSV-1, HSV-2), Dengue virus, Yellow fever, and HBV.

PYRIMIDINE NUCLEOSIDES AND THEIR MONOPHOSPHATE PRODRUGS FOR TREATMENT OF VIRAL INFECTIONS AND CANCER

Field of the Invention

The present invention is directed to compounds, methods and compositions for treating or preventing viral infections using nucleotide analogs. More specifically, the invention describes N^4-hydroxycytidine nucleosides derivatives and modified monophosphate prodrug analogs, pharmaceutically acceptable salts, or other derivatives thereof, and the use thereof in the treatment of cancer or viral infection(s), and in particular 1) human immunodeficiency virus (HIV-1 and HIV-2); 2) Flaviviridae family of viruses including hepatitis C (HCV), West Nile virus, Dengue virus, and Yellow fever; 3) Caliciviridae infection including Norovirus and Saporovirus; 4) HSV-1, HSV-2 and 5) cytomegalovirus (CMV), 6) hepatitis B virus (HBV) infection. This invention teaches how to prepare N^4-hydroxycytidine nucleoside derivatives, convert them to therapeutically relevent nucleotide prodrugs and ultimatly deliver corresponding nucleotide triphosphates to reverse transcriptases and polymerases at therapeutically-relevant concentrations.

Background of the Invention

Nucleoside analogs as a class have a well-established regulatory history, with more than 10 currently approved by the US Food and Drug Administration (US FDA) for treating human immunodeficiency virus (HIV), hepatitis B virus (HBV), herpes simplex C virus (HSV). The challenge in developing antiviral therapies is to inhibit viral replication without injuring the host cell.

Hepatitis C virus (HCV) has infected more than 180 million people worldwide. It is estimated that three to four million persons are newly infected each year, 70% of whom will develop chronic hepatitis. HCV is responsible for 50–76% of all liver cancer cases, and two thirds of all liver transplants in the developed world. Standard therapy [pegylated interferon alfa plus ribavirin (a nucleoside analog)] is only effective in 50–60% of patients and is associated with significant side-effects. The impact on standard of care by approval, in May 2011, of the two HCV protease inhibitors Incivek and Victrelis remains unclear as both drugs require response-guided

therapy regimens that can shorten the duration of IFN therapy in infected persons with an early viral response from 48 weeks to as few as 24 weeks but with a sustained virologic response (SVR) for genotype 1 HCV occurs in only about 70 to 80% when administered with IFN and RBV. (Sheridan, C. *Nature Biotech.* **2011**, *29*, 553) Therefore, there is an urgent need for new HCV drugs.

The HCV genome comprises a positive-strand RNA enclosed in a nucleocapsid and lipid envelope and consists of 9.6kb ribonucleotides, which encodes a large polypeptide of about 3000 amino acids (Dymock et al. Antiviral Chemistry & Chemotherapy 2000, 11, 79). Following maturation, this polypeptide is cut into at least 10 proteins. One of these proteins, NS5B, possesses polymerase activity and is involved in the synthesis of double-stranded RNA from the single-stranded viral RNA genome that serves as the template. The discovery of novel antiviral strategies to selectively inhibit HCV replication has long been hindered by the lack of convenient cell culture models for the propagation of HCV. This hurdle has been overcome first with the establishment of the HCV replicon system in 1999 (Bartenschlager, R., *Nat. Rev. Drug Discov.* **2002**, *1*, 911–916 and Bartenschlager, R., *J. Hepatol.* **2005**, *43*, 210–216) and, in 2005, with the development of robust HCV cell culture models (Wakita, T., et al., *Nat. Med.* **2005**, *11*, 791-6; Zhong, J., et al., *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9294-9; Lindenbach, B.D., et al., *Science* **2005**, *309*, 623-6).

HCV replication may be prevented through the manipulation of NS5B's polymerase activity via competitive inhibition of NS5B protein. Alternatively, a chain-terminator nucleoside analog also may be incorporated into the extending RNA strand. Currently, the most advanced nucleoside for the treatment of HCV is PSI-7977 (GS-7977), that is currently in phase III clinical trials as a safe and effective anti-HCV agent (Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Micolochick Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.* **2010**, 53, 7202). For reviews on nucleoside and nucleoside prodrug inhibitors of HCV NS5B see: 1) Bobeck DR, Coats SJ, Schinazi RF. Advances in nucleoside monophosphate prodrugs as anti-hepatitis C virus agents. *Antivir. Ther.* **2010**, *15*, 935-50; 2) Ray AS, Hostetler KY. Application of kinase bypass strategies to nucleoside antivirals. *Antiviral Res.* **2011**, *92,* 277-91; 3) Sofia, M. J.; Furman P. A. Symonds, W. T. Chapter 11 in Accounts in Drug Discovery: Case Studies in

Medicinal Chemistry by RSC; 4) Brown, N. A. Progress towards improving antiviral therapy for hepatitis C with hepatitis C virus polymerase inhibitors. Part I: Nucleoside analogues. Expert Opin. Invest. Drugs 2009, 709–725; 5) Beaulieu, P. L. Recent advances in the development of NS5B polymerase inhibitors for the treatment of hepatitis C virus infection. Expert Opin. Ther. Pat. 2009, 19, 145–164; 6) Koch, U.; Narjes, F. Recent Progress in the Development of Inhibitors of the Hepatitis C Virus RNA-Dependent RNA Polymerase. Curr. Top. Med. Chem. 2007, 7, 1302–1329.

Recently, several patent applications (including WO 09/086192, WO 12/040124, WO 12/040126, WO 12/040127, US 12/070415, WO 08/082601, WO 10/014134, WO 11/017389, WO 11/123586, WO 10/135569, WO 10/075549. WO 10/075554, WO 10/075517, WO 09 152095, WO 08/121634, WO 05/03147, WO WO 99/43691, WO 01/32153, WO 01160315, WO 01179246, WO 01/90121, WO 01/92282, WO 02/48165, WO 02/18404, WO 02/094289, WO 02/057287, WO 02/100415(A2), US 06/040890, WO 02/057425, EP 1674104(A1), EP 1706405(A1), US 06/199783, WO 02/32920, US 04/6784166, WO 05/000864, WO 05/021568) have described nucleoside analogs as anti-HCV agents.

In HIV, a key target for drug development is reverse transcriptase (HIV-RT), a unique viral polymerase. This enzyme is active early in the viral replication cycle and converts the virus' genetic information from RNA into DNA, a process necessary for continued viral replication. Nucleoside reverse transcriptase inhibitors (NRTI) mimic natural nucleosides. In the triphosphate form, each NRTI competes with one of the four naturally occurring 2'-deoxynucleoside-5'-triphosphate (dNTP), namely, dCTP, dTTP, dATP, or dGTP for binding and DNA chain elongation near the active site of HIV-1 RT.

Reverse transcription is an essential event in the HIV-1 replication cycle and a major target for the development of antiretroviral drugs (see Parniak MA, Sluis-Cremer N. Inhibitors of HIV-1 reverse transcriptase. *Adv. Pharmacol.* **2000**, *49*, 67-109; Painter GR, Almond MR, Mao S, Liotta DC. Biochemical and mechanistic basis for the activity of nucleoside analogue inhibitors of HIV reverse transcriptase. *Curr. Top. Med. Chem.* **2004**, *4*, 1035-44; Sharma PL, Nurpeisov V, Hernandez-Santiago B, Beltran T, Schinazi RF. Nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Curr. Top. Med. Chem.* **2004**, *4* 895-919). Two distinct groups of compounds have been identified that inhibit HIV-1 RT. These are the nucleoside or

nucleotide RT inhibitors (NRTI) and the non-nucleoside RT inhibitors (NNRTI).

NRTI are analogs of 2'-deoxyribonucleosides that lack a 3'-OH group on the ribose sugar. They were the first drugs used to treat HIV-1 infection and they remain integral components of nearly all antiretroviral regimens.

In 1985, it was reported that the synthetic nucleoside 3'-azido-3'-deoxythymidine (zidovudine, AZT), one representative NRTI, inhibited the replication of HIV. Since then, several other NRTI, including but not limited to 2',3'-dideoxyinosine (didanosine, ddI), 2',3'-dideoxycytidine (zalcitabine, ddC), 2',3'-dideoxy-2',3'-didehydrothymidine (stavudine, d4T), (-)-2',3'-dideoxy-3'-thiacytidine (lamivudine, 3TC), (-)-2',3'-dideoxy-5-fluoro-3'-thiacytidine (emtricitabine, FTC), (1*S*,4*R*)-4-[2-amino-6-(cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate (abacavir, ABC), (*R*)-9-(2-phosphonylmethoxypropyl)adenine (PMPA, tenofovir disoproxil fumarate) (TDF), and (-)-carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine (carbovir) and its prodrug abacavir, have proven effective against HIV. After phosphorylation to the 5'-triphosphate by cellular kinases, these NRTI are incorporated into a growing strand of viral DNA causing chain termination, because they lack a 3'-hydroxyl group.

In general, to exhibit antiviral activity, NRTI must be metabolically converted by host-cell kinases to their corresponding triphosphate forms (NRTI-TP). The NRTI-TP inhibit HIV-1 RT DNA synthesis by acting as chain- terminators of DNA synthesis (see Goody RS, Muller B, Restle T. Factors contributing to the inhibition of HIV reverse transcriptase by chain terminating nucleotides *in vitro* and *in vivo*. *FEBS Lett.* **1991**, *291*, 1-5). Although combination therapies that contain one or more NRTI have profoundly reduced morbidity and mortality associated with AIDS, the approved NRTI can have significant limitations. These include acute and chronic toxicity, pharmacokinetic interactions with other antiretrovirals, and the selection of drug-resistant variants of HIV-1 that exhibit cross-resistance to other NRTI.

HIV-1 drug resistance within an individual arises from the genetic variability of the virus population and selection of resistant variants with therapy (see Chen R, Quinones-Mateu ME, Mansky LM. Drug resistance, virus fitness and HIV-1 mutagenesis. *Curr. Pharm. Des.* **2004**, *10*, 4065-70). HIV-1 genetic variability is due to the inability of HIV-1 RT to proofread nucleotide sequences during replication.

This variability is increased by the high rate of HIV-1 replication, the accumulation of proviral variants during the course of HIV-1 infection, and genetic recombination when viruses of different sequence infect the same cell. As a result, innumerable genetically distinct variants (termed quasi-species) evolve within an individual in the years following initial infection. The development of drug resistance depends on the extent to which virus replication continues during drug therapy, the ease of acquisition of a particular mutation (or set of mutations), and the effect of drug resistance mutations on drug susceptibility and viral fitness. In general, NRTI therapy selects for viruses that have mutations in RT. Depending on the NRTI resistance mutation(s) selected, the mutant viruses typically exhibit decreased susceptibility to some or, in certain instances, all NRTI. From a clinical perspective, the development of drug resistant HIV-1 limits future treatment options by effectively decreasing the number of available drugs that retain potency against the resistant virus. This often requires more complicated drug regimens that involve intense dosing schedules and a greater risk of severe side effects due to drug toxicity. These factors often contribute to incomplete adherence to the drug regimen. Thus, the development of novel NRTI with excellent activity and safety profiles and limited or no cross-resistance with currently-available drugs is critical for effective therapy of HIV-1 infection.

The development of nucleoside analogs active against drug-resistant HIV-1 requires detailed understanding of the molecular mechanisms involved in resistance to this class of compounds. Accordingly, a brief overview of the mutations and molecular mechanisms of HIV-1 resistance to NRTI is provided. Two kinetically distinct molecular mechanisms of HIV-1 resistance to NRTI have been proposed (see Sluis-Cremer N, Arion D, Parniak MA. Molecular mechanisms of HIV-1 resistance to nucleoside reverse transcriptase inhibitors (NRTIs). *Cell Mol. Life Sci.* **2000**; *57*, 1408-22). One mechanism involves selective decreases in NRTI-TP *versus* normal dNTP incorporation during viral DNA synthesis. This resistance mechanism has been termed discrimination. The second mechanism involves selective removal of the chain-terminating NRTI-monophosphate (NRTI-MP) from the prematurely terminated DNA chain (see Arion D, Kaushik N, McCormick S, Borkow G, Parniak MA. Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry*. **1998**, *37*,

15908-17; Meyer PR, Matsuura SE, Mian AM, So AG, Scott WA. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. *Mol. Cell.* **1999**, *4*, 35-43). This mechanism has been termed excision.

The discrimination mechanism involves the acquisition of one or more resistance mutations in RT that improve the enzyme's ability to discriminate between the natural dNTP substrate and the NRTI-TP. In this regard, resistance is typically associated with a decreased catalytic efficiency of NRTI-TP incorporation. NRTI-TP (and dNTP) catalytic efficiency is driven by two kinetic parameters, (i) the affinity of the nucleotide for the RT polymerase active site (K_d) and (ii) the maximum rate of nucleotide incorporation (kpol), both of which can be determined using pre-steady-state kinetic analyses (see Kati WM, Johnson KA, Jerva LF, Anderson KS. Mechanism and fidelity of HIV reverse transcriptase. *J. Biol. Chem.* **1992**, *26*: 25988-97).

For the excision mechanism of NRTI resistance, the mutant HIV-1 RT does not discriminate between the natural dNTP substrate and the NRTI-TP at the nucleotide incorporation step (see Kerr SG, Anderson KS. Pre-steady-state kinetic characterization of wild type and 3'-azido-3'-deoxythymidine (AZT) resistant HIV-1 RT: implication of RNA directed DNA polymerization in the mechanism of AZT resistance. *Biochemistry*. **1997**, *36*, 14064-70). Instead, RT containing "excision" mutations shows an increased capacity to unblock NRTI-MP terminated primers in the presence of physiological concentrations of ATP (typically within the range of 0.8-4 mM) or pyrophosphate (PPi) (see Arion D, Kaushik N, McCormick S, Borkow G, Parniak MA. Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry*. **1998**, *37*, 15908-17; Meyer PR, Matsuura SE, Mian AM, So AG, Scott WA. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. *Mol. Cell.* **1999**, *4*, 35-43). NRTI resistance mutations associated with the excision mechanism include thymidine analog mutations (TAMS) and T69S insertion mutations.

Another virus that causes a serious human health problem is the hepatitis B virus (HBV). HBV is second only to tobacco as a cause of human cancer. The

mechanism by which HBV induces cancer is unknown. It is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

After a 2- to 6-month incubation period, during which the host is typically unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, resulting in abdominal pain, jaundice and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which large sections of the liver are destroyed.

Patients typically recover from the acute phase of HBV infection. In some patients, however, the virus continues replication for an extended or indefinite period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone and worldwide almost 300 million carriers. Currently (July 2012) the WHO estimates worldwide that two billion people have been infected with the hepatitis B virus and more than 240 million have chronic (long-term) liver infections. About 600,000 people die every year due to the acute or chronic consequences of hepatitis B. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer.

In industrialized countries, the high-risk group for HBV infection includes those in contact with HBV carriers or their blood samples. The epidemiology of HBV is very similar to that of HIV/AIDS, which is a reason why HBV infection is common among patients infected with HIV or suffering from AIDS. However, HBV is more contagious than HIV.

3TC (lamivudine), interferon alpha-2b, peginterferon alpha-2a, hepsera (adefovir dipivoxil), baraclude (entecavir), and Tyzeka (Telbivudine) are currently FDA-approved drugs for treating HBV infection. However, some of the drugs have severe side effects, and viral resistance develops rapidly in patients treated with these drugs.

Norovirus is one of four viral genera found in the non-enveloped positive strand RNA family *Caliciviridae*. The other three species in *Caliciviridae* are

Lagovirus, Vesivirus, and Sapovirus. Sapovirus is the only member of the genus other than Norovirus which utilizes humans as hosts. The Norovirus genome is approximately 7.56 kb with three open reading frames (ORFs). The first ORF codes for nonstructural proteins including a helicase, a protease, and a RNA directed RNA polymerase (RDRP) all of which are required for replication of the virus. The remaining two ORFs code for Capsid proteins (Jiang, X. (1993) Virology 195(1):51-61). The numerous strains of Norovirus have been classified into 5 genogroups of which I, IV, and V infect humans (Zheng, D.P., et al. (2006) Virology 346(2):312-323) and are estimated by the CDC to cause approximately 23 million gastroenteritis cases, corresponding to 40% of foodborne illness each year in the US (Mead P.S. (1999) Emerg. Infect. Dis. 5(5):607-625).

Common symptoms are vomiting, diarrhea, and intestinal cramps. Vomiting is the most common symptom in children, while diarrhea is more common in infected adults. Dehydration is a significant concern. The loss of life due to this virus is about 300 patients per year in the United States, and these deaths are usually among patients with a weak immune system (Centers for Disease Control and Prevention. "Norwalk-like viruses:" public health consequences and outbreak management. MMWR 2001;50 (No. RR-9):3). The incubation period from exposure to full infection is typically 24 to 48 hrs with approximately 30% of infected individuals showing no symptoms. Symptoms generally persist for 24 to 60 hrs (Adler, J.L. and Zickl, R., J. (1969) Infect. Dis. 119:668-673). Viral shedding may last for up to 2 weeks following the infection, however, it is not clear whether this virus is infectious.

Norovirus is transmitted primarily by the fecal-oral route through contaminated food or water, person to person contact, aerosols of vomit or stool samples. Viral titers in stool samples can reach 10^6 to 10^7 particles per mL, and particles are stable to temperatures of 0° C (32°F) to 60°C (140°F) (Duizer, E. et al., (2004) Appl. Environ. Microbiol. 70(8); 4538-4543). The virus is highly infectious, and various sources suggest infection may require inoculation of as few as 10 to 100 viral particles (Centers for Disease Control and Prevention. "Norwalk-like viruses:" public health consequences and outbreak management. MMR 2001; 50(No. RR-9):3-6). This leads to epidemics in schools, nursing homes, cruise ships, hospitals, or other locations where people congregate.

Norovirus is named for Norwalk-like viruses, a name derived from an outbreak at a school in Norwalk, Ohio in 1968. The viral particle responsible for the Norwalk illness was identified in 1972 by immune electron microscopy following passage of rectal swab filtrates through three sets of human volunteers (Kapikian, A.Z. et al. (1972) J. Virol. 10:1075-1081). In following years, the virus was called small round structured virus due to its electron microscopic image, calicivirus since it a member of the Caliciviridae family, and/or probably most commonly Norwalk-like virus after the originally isolated strain. Common names for the virus include winter vomiting virus, stomach flu, food poisoning, and viral gastroenteritis. While the outcome of infection is generally non-life threatening, the cost of loss of use of facilities and loss of productivity is great, and, consequently, a therapy for treatment of Norovirus infection in humans would be very desirable.

There is currently no approved pharmaceutical treatment for Norovirus infection (http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus-qa.htm), and this has probably at least in part been due to the lack of availability of a cell culture system. Recently, a replicon system has been developed for the original Norwalk G-I strain (Chang, K. O., et al. (2006) Virology 353:463-473). Both Norovirus replicons and Hepatitis C replicons require viral helicase, protease, and polymerase to be functional in order for replication of the replicon to occur. Most recently, an *in vitro* cell culture infectivity assay has been reported utilizing Norovirus genogroup I and II inoculums (Straub, T. M. et al. (2007) Emerg. Infect. Dis. 13(3):396-403). This assay is performed in a rotating-wall bioreactor utilizing small intestinal epithelial cells on microcarrier beads, and at least initially seems as though it would be difficult to screen a meaningful number of compounds with this system. Eventually the infectivity assay may be useful for screening entry inhibitors. Other groups, such as Ligocyte Pharmaceuticals, Inc. (http://www.ligocyte.com/) have focused on trying to develop a vaccine against Noroviruses, however, these efforts have not yet been successful and may prove difficult as has often been the case in viral systems where low replicase fidelity is an evolutionary benefit.

Proliferative disorders are one of the major life-threatening diseases and have been intensively investigated for decades. Cancer now is the second leading cause of death in the United States, and over 500,000 people die annually from this proliferative disorder. A tumor is an unregulated, disorganized proliferation of cell

growth. A tumor is malignant, or cancerous, if it has the properties of invasiveness and metastasis. Invasiveness refers to the tendency of a tumor to enter surrounding tissue, breaking through the basal laminas that define the boundaries of the tissues, thereby often entering the body's circulatory system. Metastasis refers to the tendency of a tumor to migrate to other areas of the body and establish areas of proliferation away from the site of initial appearance.

Cancer is not fully understood on the molecular level. It is known that exposure of a cell to a carcinogen such as certain viruses, certain chemicals, or radiation, leads to DNA alteration that inactivates a "suppressive" gene or activates an "oncogene." Suppressive genes are growth regulatory genes, which upon mutation, can no longer control cell growth. Oncogenes are initially normal genes (called prooncongenes) that by mutation or altered context of expression become transforming genes. The products of transforming genes cause inappropriate cell growth. More than twenty different normal cellular genes can become oncongenes by genetic alteration. Transformed cells differ from normal cells in many ways, including cell morphology, cell-to-cell interactions, membrane content, cytoskeletal structure, protein secretion, gene expression and mortality (transformed cells can grow indefinitely).

All of the various cell types of the body can be transformed into benign or malignant tumor cells. The most frequent tumor site is lung, followed by colorectal, breast, prostate, bladder, pancreas and then ovary. Other prevalent types of cancer include leukemia, central nervous system cancers, including brain cancer, melanoma, lymphoma, erythroleukemia, uterine cancer, and head and neck cancer.

Cancer is now primarily treated with one or a combination of three means of therapies: surgery, radiation and chemotherapy. Surgery involves the bulk removal of diseased tissue. While surgery is sometimes effective in removing tumors located at certain sites, for example, in the breast, colon and skin, it cannot be used in the treatment of tumors located in other areas, such as the backbone, or in the treatment of disseminated neoplastic conditions such as leukemia.

Chemotherapy involves the disruption of cell replication or cell metabolism. It is used most often in the treatment of leukemia, as well as breast, lung, and testicular cancer. There are five major classes of chemotherapeutic agents currently in use for

the treatment of cancer: natural products and their derivatives; anthacyclines; alkylating agents; antiproliferatives (also called antimetabolites); and hormonal agents. Chemotherapeutic agents are often referred to as antineoplastic agents.

Several synthetic nucleosides, such as 5-fluorouracil, have been identified that exhibit anticancer activity. 5-Fluorouracil has been used clinically in the treatment of malignant tumors, including, for example, carcinomas, sarcomas, skin cancer, cancer of the digestive organs, and breast cancer. 5-Fluorouracil, however, causes serious adverse reactions such as nausea, alopecia, diarrhea, stomatitis, leukocytic thrombocytopenia, anorexia, pigmentation and edema.

Despite the availability of a vaccine (*Crit. Rev. Clin. Lab. Sci.* **2004**, *41*, 391-427). Yellow fever virus (YFV) continues to be a serious human health concern, causing approximately 30,000 deaths each year. YFV is one of the most lethal viral infections of humans (*Expert Rev. Vaccines* **2005**, *4*, 553-574.). Of infected individuals approximately 15% will develop severe disease, with a fatality rate of 20 to 50% among those individuals. No approved therapies specific for treatment of YFV are available. Treatment is symptomatic-rest, fluids, and ibuprofen, naproxen, acetaminophen, or paracetamol may relieve symptoms of fever and aching. Aspirin should be avoided. Although the virus is endemic to Africa and South America, there is potential for outbreaks of YFV outside these areas and such imported cases have been reported (*J. Travel Med.* **2005**, *12*(Suppl. 1), S3–S11).

West Nile Virus (WNV) is from the family Flaviviridae and predominantly a mosquito-borne disease. It was first discovered in the West Nile District of Uganda in 1937. According to the reports from the Centers for Disease Control and Prevention, WNV has been found in Africa, the Middle East, Europe, Oceania, west and central Asia, and North America. Its first emergence in North America began in the New York City metropolitan area in 1999. It is a seasonal epidemic in North America that normally erupts in the summer and continues into the fall, presenting a threat to environmental health. Its natural cycle is bird-mosquito-bird and mammal. Mosquitoes, in particular the species Culex pipiens, become infected when they feed on infected birds. Infected mosquitoes then spread WNV to other birds and mammals including humans when they bite. In humans and horses, fatal Encephalitis is the most serious manifestation of WNV infection. WNV can also cause mortality in some

infected birds. There is no specific treatment for WNV infection. In cases with milder symptoms, people experience symptoms such as fever and aches that pass on their own, although even healthy people have become sick for several weeks. In more severe cases, people usually need to go to the hospital where they can receive supportive treatment.

Dengue infection is also from the family Flaviviridae and is the most important arthropod-borne infection in Singapore (*Epidemiol News Bull* **2006**, *32*,62-6). Globally, there are an estimated 50 to 100 million cases of dengue fever (DF) and several hundred thousand cases of dengue hemorrhagic fever (DHF) per year with and average fatality rate of 5%. Many patients recover from dengue infection with minimal or no residual illness. Dengue infections are usually asymptomatic, but can present with classic dengue fever, dengue haemorrhagic fever or dengue shock syndrome. Even for outpatients, the need for maintaining adequate hydration is highly important. Dengue infections can be effectively managed by intravenous fluid replacement therapy, and if diagnosed early, fatality rates can be kept below 1%. To manage the pain and fever, patients suspected of having a dengue infection should be given acetaminophen preparations. Aspirin and non-steroidal anti-inflammatory medications may aggravate the bleeding tendency associated with some dengue infection. However, some manifestations of dengue infection previously described include liver failure (*Dig Dis Sci* **2005**, *50*, 1146-7), encephalopathy (*J Trop Med Public Health* **1987**, *18*, 398-406), and Guillain-Barré syndrome (*Intern Med* **2006**, *45*, 563-4).

In light of the fact that acquired immune deficiency syndrome, AIDS-related complex, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, Yellow fever, cancer, and HBV have reached alarming levels worldwide, and have significant and in some cases tragic effects on the effected patient, there remains a strong need to provide new effective pharmaceutical agents to treat these diseases, with agents that have low toxicity to the host.

It would be advantageous to provide new antiviral or chemotherapy agents, compositions including these agents, and methods of treatment using these agents, particularly to treat drug resistant cancers or mutant viruses. The present invention provides such agents, compositions and methods.

Summary of the Invention

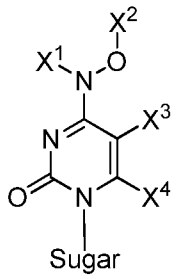
The present invention provides compounds, methods and compositions for treating or preventing cancer or an HIV-1, HIV-2, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, Yellow fever, cytomegalovirus (CMV), or HBV infection in a host. The methods involve administering a therapeutically or prophylactically-effective amount of at least one compound as described herein to treat or prevent an infection by, or an amount sufficient to reduce the biological activity of, cancer or an HIV-1, HIV-2, HCV, Norovirus, Saporovirus, HSV-1, HSV-2 Dengue virus, Yellow fever, cytomegalovirus (CMV), or HBV infection. The pharmaceutical compositions include one or more of the compounds described herein, in combination with a pharmaceutically acceptable carrier or excipient, for treating a host with cancer or infected with HIV-1, HIV-2, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, Yellow fever, cytomegalovirus (CMV), or HBV. The formulations can further include at least one further therapeutic agent. In addition, the present invention includes processes for preparing such compounds.

As with Hepatitis C replicons, Norovirus replicons require viral helicase, protease, and polymerase to be functional in order for replication of the replicon to occur. The replicons can be used in high throughput assays, which evaluate whether a compound to be screened for activity inhibits the ability of Norovirus helicase, protease, and/or polymerase to function, as evidenced by an inhibition of replication of the replicon.

The compounds described herein include β-D and β-L-N^4-hydroxycytidine nucleosides derivatives and modified monophosphate, phosphonate prodrugs. In one embodiment, the active compound is of formula (**I**):

In addition, the compounds described herein are inhibitors of HIV-1, HIV-2, HCV, Norovirus, Saporovirus, herpes viruses (HSV-1, HSV-2), Dengue virus, Yellow fever, cytomegalovirus (CMV), cancer, and/or HBV. Therefore, these compounds can also be used to treat patients that are infected or co-infected with HIV-1, HIV-2, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, Yellow fever, cancer, and/or HBV.

In one embodiment, the compound is a compound of Formula (**I**):



(**I**)

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

i) X^1 is H, C_1-C_6alkyl, C_1-C_6haloalkyl, C_1-C_6alkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, COR^1, or $COOR^1$;

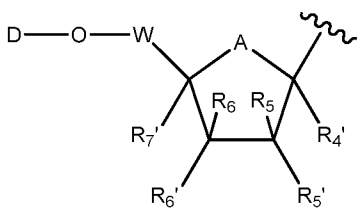
ii) X^2 is hydrogen, COR^1, or $COOR^1$

wherein each R^1 is, independently, CH_2-O(CO)-X^5; CH_2-O(CO)O-X^5 , $C_{1\text{-}20}$ alkyl, the carbon chain derived from a fatty alcohol or $C_{1\text{-}20}$ alkyl substituted with a C_1-C_6 alkyl, alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, $C_{3\text{-}10}$ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are $C_{1\text{-}6}$ alkyl, or $C_{1\text{-}6}$ alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or $C_{3\text{-}10}$ cycloalkyl

X^5 is independently, $C_{1\text{-}20}$ alkyl, the carbon chain derived from a fatty alcohol or $C_{1\text{-}20}$ alkyl substituted with a C_1-C_6 alkyl, alkoxy, $C_{3\text{-}10}$ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are $C_{1\text{-}6}$ alkyl, or $C_{1\text{-}6}$ alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or $C_{3\text{-}10}$ cycloalkyl

iii) Each X^3 and X^4 is independently H, $C_{1\text{-}6}$ alkyl, $C_{2\text{-}6}$ alkenyl, $C_{2\text{-}6}$ alkynyl, aryl, alkylaryl, halogen (F, Cl, Br, I), NH_2, OH, SH, CN, or NO_2.

In one embodiment, Sugar is ribose or a modified ribose of the general Formula (**II**):



(**II**)

wherein:

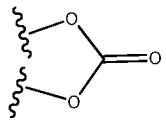
D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

R^1 is as defined above;

W is CL_2 or CL_2CL_2, wherein L independently is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH_2, CHF, CF_2, $C=CH_2$, C=CHF, or $C=CF_2$;

$R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, and $R^{7'}$ are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

$R^{5'}$ and $R^{6'}$ can come together to form a ring  .

In one embodiment, where Sugar is formula (**II**), when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H then, R^5, $R^{5'}$, R^6, $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH=CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

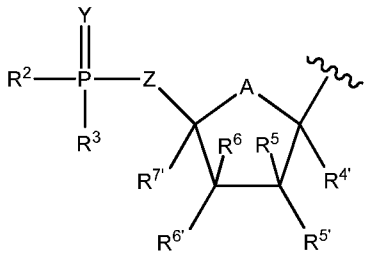
In another embodiment, $R^{6'}$ is independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

In one embodiment, wherein for formula (**I**) where sugar is formula (**II**), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2.

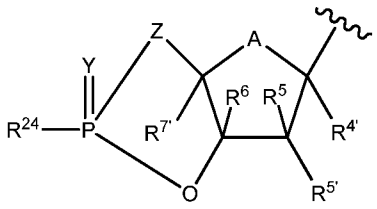
In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R;

R is independently C_1-C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_3-C_6 cycloalkyl) aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above, where representative substituents include for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In another embodiment, Sugar is ribose or modified ribose of the general formulas (**III**) or (**IV**):



(**III**)



(**IV**)

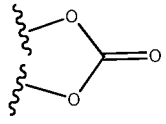
wherein:

Y is O or S;

Z is selected from the group consisting of CL_2, CL_2CL_2, CL_2OCL_2, CL_2SCL_2, CL_2O, OCL_2 and CL_2NHCL_2, wherein L independently is selected from the group consisting of H, F, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH_2, CHF, CF_2, C=CH_2, C=CHF, or C=CF_2;

$R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, and $R^{7'}$ are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

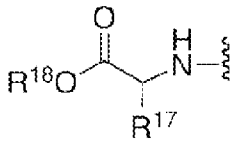
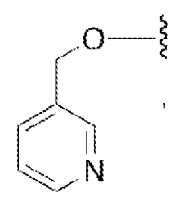
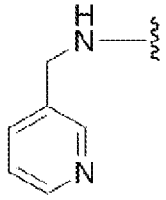
$R^{5'}$ and $R^{6'}$ can come together to form a ring 

.

In one embodiment, where Sugar is formula (**III**) or (**IV**), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2.

In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R.

R is independently a C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above.

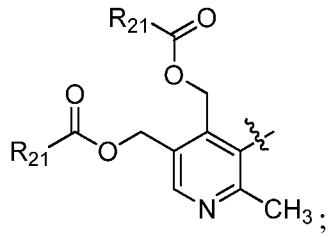
R^{24} is selected from the group consisting of OR^{15},  ,  ,  , and fatty alcohols,

R^{15} is selected from the group consisting of H, Li, Na, K, phenyl and pyridinyl; wherein phenyl and pyridinyl are optionally substituted with zero to three substituents independently selected from the group consisting of $(CH_2)_{0-6}CO_2R^{16}$ and $(CH_2)_{0-6}CON(R^{16})_2$;

R^{17} is selected from to those groups occurring in natural L-amino acids, C_{1-6} alkyl, (C_1-C_6 alkyl), C_{2-6} alkenyl, C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above.

R^{18} is H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol (such as oleyl alcohol, octacosanol, triacontanol, linoleyl alcohol, and the like) or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or cycloalkyl.

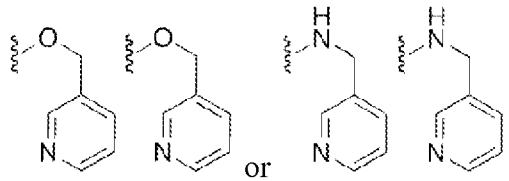
Representative R^2 and R^3 are independently selected from the group consisting of:

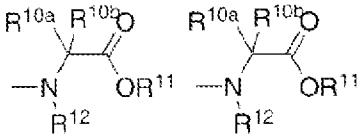
(a) OR^8 where R^8 is H, Li, Na, K, C_{1-20} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, aryl, or heteroaryl which includes, but is not limited to, phenyl or naphthyl optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, $(CH_2)_{0-6}CO_2R^{9a}$, halogen, C_{1-6} haloalkyl, $-N(R^{9a})_2$, C_{1-6} acylamino, $-NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^{9a})_2$, $-SO_2C_{1-6}$ alkyl, COR^{9b}, nitro, cyano and  ;

wherein R^{21} is as defined below;

R^{9a} is independently H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

(b)  or

(c)  where R^{10a} and R^{10b} are:

(i) independently selected from the group consisting of H, C_{1-10} alkyl, $-(CH_2)_rNR^{9a}_2$, C_{1-6} hydroxyalkyl, $-CH_2SH$, $-(CH_2)_2S(O)_pMe$, $-(CH_2)_3NHC(=NH)NH_2$, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, $-(CH_2)_mCOR^{9b}$, aryl and aryl-C_{1-3} alkyl, said aryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, halogen, nitro, and cyano;

(ii) R^{10a} is H and R^{10b} and R^{12} together are $(CH_2)_{2-4}$ to form a ring that includes the adjoining N and C atoms;

(iii) R^{10a} and R^{10b} together are $(CH_2)_n$ to form a ring;

(iv) R^{10a} and R^{10b} both are C_{1-6} alkyl; or

(v) R^{10a} is H and R^{10b} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$-$CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-10} cycloalkyl;

p is 0 to 2;

r is 1 to 6;

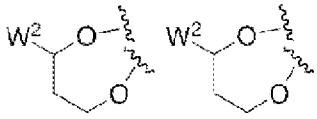
n is 4 or 5;

m is 0 to 3;

R^{11} is H, C_{1-10} alkyl, or C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{12} is H or C_{1-3} alkyl, or R^{10a}, or R^{10b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

(d) an O attached lipid (including a phospholipid), an N or O attached peptide, an O attached cholesterol, or an O attached phytosterol;

(e) R^2 and R^3 can come together to form a ring  where W^2 is selected from the group consisting of phenyl and monocyclic heteroaryl, optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, CF_3, C_{2-6} alkenyl, C_{1-6} alkoxy, OR^{9c}, CO_2R^{9a}, COR^{9a}, halogen, C_{1-6} haloalkyl, $-N(R^{9a})_2$, C_{1-6} acylamino, $CO_2N(R^{9a})_2$, SR^{9a}, $-NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^{9a})_2$, $-SO_2C_{1-6}$ alkyl, COR^{9b}, and cyano, and wherein said monocyclic heteroaryl and substituted monocyclic heteroaryl has 1-2 heteroatoms that are independently selected from the group consisting of N, O, and S, with the provisos that:

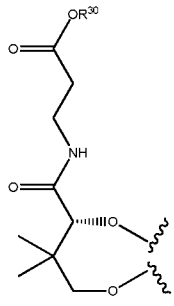
a) when there are two heteroatoms and one is O, then the other can not be O or S, and

b) when there are two heteroatoms and one is S, then the other can not be O or S;

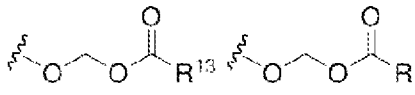
R^{9a} is independently H or C_{1-6} alkyl;

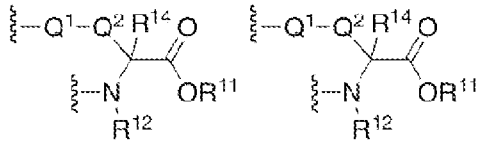
R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

R^{9c} is H or C_{1-6} acyl;

(f) R^2 and R^3 can come together to form a ring  where R^{30} is H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty

alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

(g)  where R^{13} is selected from the group consisting of H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, and substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

(h) R^2 and R^3 can come together to form a ring  where R^{14} is:

(i) independently selected from the group consisting of H, C_{1-10} alkyl, -$(CH_2)_rNR_2^{9a}$, C_{1-6} hydroxyalkyl, -CH_2SH, -$(CH_2)_2S(O)_pMe$, -$(CH_2)_3NHC(=NH)NH_2$, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -$(CH_2)_mCOR^{9b}$, aryl, aryl-C_{1-3} alkyl, heteroaryl and heteroaryl-C_{1-3} alkyl, said aryl and heteroaryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, halogen, nitro, and cyano;

(ii) R^{14} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, -$CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-10} cycloalkyl;

p is 0 to 2;

r is 1 to 6;

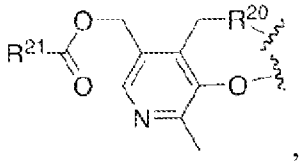
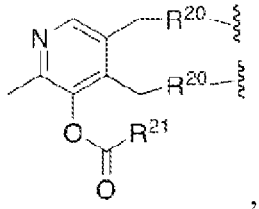
m is 0 to 3

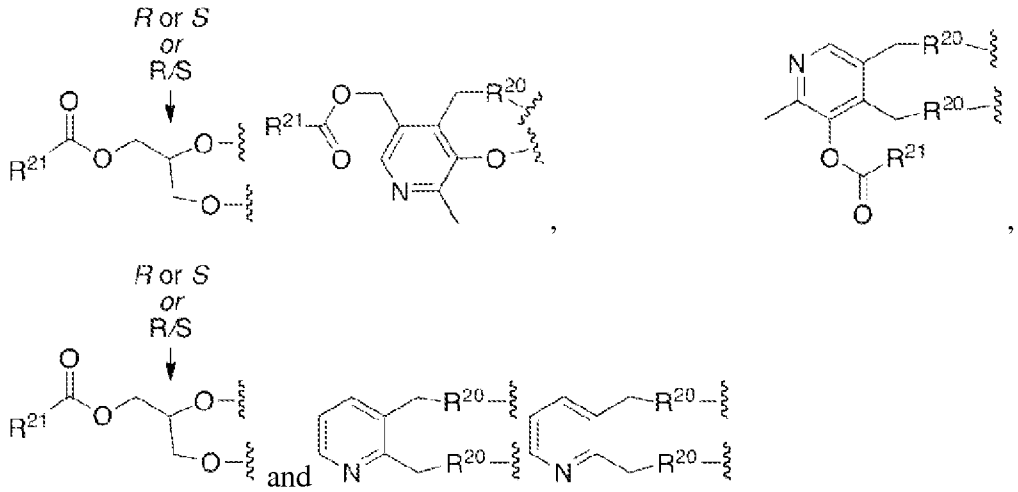
Q^1 is NR^{9a}, O, or S

Q^2 is C_{1-10} alkyl, C_{1-6} hydroxyalkyl, aryl and aryl-C_{1-3} alkyl, heteroaryl and heteroaryl-C_{1-3} alkyl, said aryl and heteroaryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, fluoro, and chloro;

R^{11} is H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{12} is H or C_{1-3} alkyl, or R^{14b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

(i) R^2 and R^3 can come together to form a ring selected from the group consisting of  ,  ,

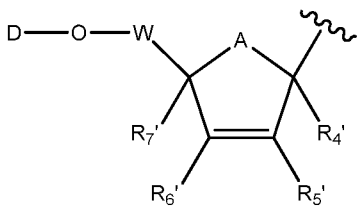


where R^{20} is O or NH, and

R^{21} is selected from the group consisting of H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty acid, and C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, and substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl, and

(j) R^2 is a monophosphate ester or a diphosphate ester when R^3 is OH, O^-K^+, O^-Li^+, or O^-Na^+.

In still another embodiment, Sugar is ribose or modified ribose of the general formula (**V**):



(**V**)

wherein:

D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

R^1 is independently C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6}

alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

W is CL_2 or CL_2CL_2, wherein L independently is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A, R^2, R^3, Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Formulas I, II, III and IV;

wherein for formula (**I**) where Sugar is formula (**V**), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2,

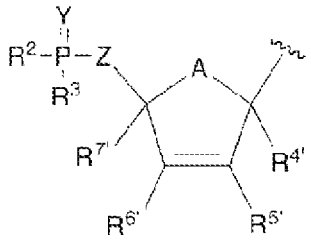
In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R;

wherein R is independently C_1-C_6 alkyl, C_{2-6} alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In one embodiment, where Sugar is of Formula (**V**), when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H then, $R^{5'}$ and $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH{=}CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

In another embodiment, R^5 and $R^{6'}$ are independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

In yet another embodiment, Sugar is a modified ribose of the general Formula (**VI**):



(**VI**)

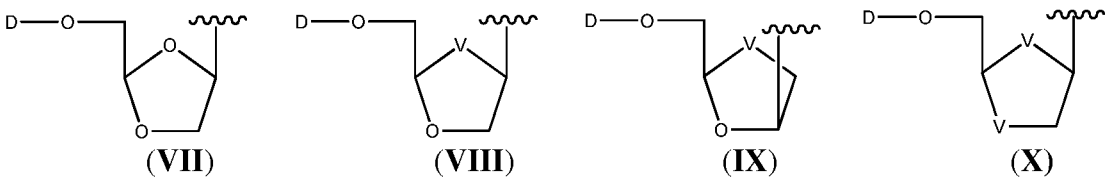
wherein:

A, R^2, R^3, Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Formulas I, II, III and IV;

wherein for formula (**I**) where Sugar is of Formula (**VI**), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2,

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In another embodiment, Sugar is a dioxolane, an oxathiolane, or a dithiolane of the general formulas (**VII**), (**VIII**), (**IX**), and (**X**):



(**VII**) (**VIII**) (**IX**) (**X**)

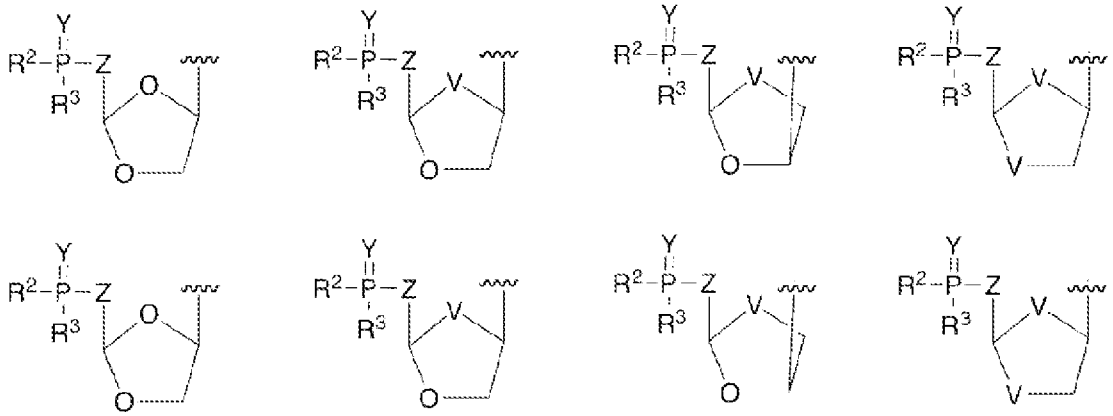
D is $C(O)OR^1$, diphosphate ester, or triphosphate ester;

V is, individually, S or Se;

R^1 is independently $C_{1\text{-}20}$ alkyl, the carbon chain derived from a fatty alcohol or $C_{1\text{-}20}$ alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-

C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

In yet another embodiment, Sugar is a dioxolane, or a oxathiolane, or dithiolane of the general Formulas (**XI**), (**XII**), (**XIII**), and (**XIV**):



(**XI**) (**XII**) (**XIII**)

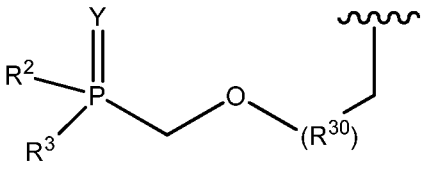
(**XIV**)

wherein:

V is, individually, S or Se;

R^2, R^3, Y, and Z are as defined above with respect to Formulas I, II, III and IV.

In still another embodiment, Sugar is a phosphonylmethoxyalkyl of the general Formula (**XV**):



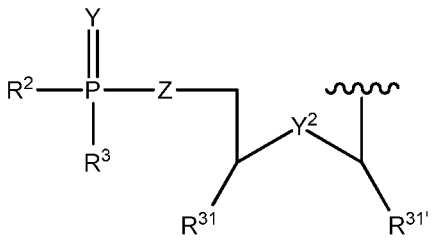
(**XV**)

wherein:

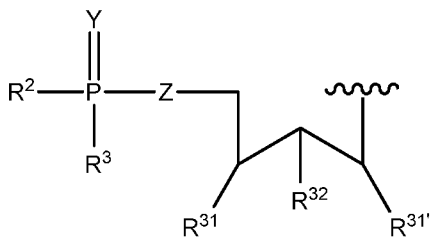
R^2, R^3, and Y are as defined above with respect to Formulas I, II, III and IV; and ;

R^{30} is selected from the group consisting of C_{1-20} alkyl, C_{2-20} alkyl (including but not limited to C_1-C_6), alkenyl (including but not limited to C_2-C_6), and C_{2-20} alkynyl, C_{3-10} (including but not limited to C_2-C_6), cycloalkyl (including but not limited to C_3-C_8), aryl (including but not limited to C_6-C_{10}), heteroaryl (including but not limited to C_6-C_{10}), arylalkyl, and alkylaryl;

In still another embodiment, Sugar is of the general formulas (**XVI**) or (**XVII**):



(**XVI**)



(**XVII**)

wherein:

R^2, R^3, Z, and Y are as defined above;

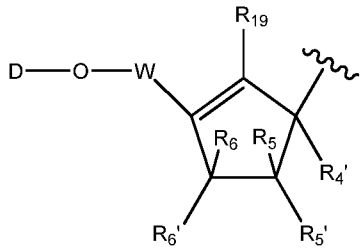
Y^2 is O, S, Se, or NR;

R is, independently, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl;

R^{31}, $R^{31'}$ and R^{32} are defined as H, CH_3, or CH_2OR^{33}; and

R^{33} is H or C_1-C_6 acyl.

In another embodiment, Sugar is a modified ribose of the general formulas (**XVIII**)



(**XVIII**)

wherein:

D, W, $R^{4'}$, R^5, $R^{5'}$, R^6, and $R^{6'}$ are as defined above;

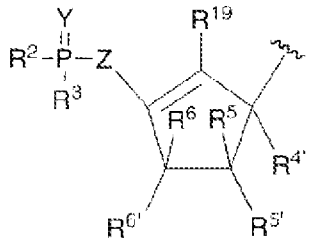
R^{19} is H, F, Cl, Br, I, N_3, C(O)OH, CN, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, or R;

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In one embodiment, where sugar is of Formula (**XVII**), when D is H or acyl, W is CH_2, $R^{4'}$ and R^{19} are H, then, R^5, $R^{5'}$, R^6, $R^{6'}$ can not be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH{=}CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

In another embodiment, $R^{6'}$ can be independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2.

In a further embodiment, Sugar is a modified ribose of Formulas (**XIX**):



(**XIX**)

wherein:

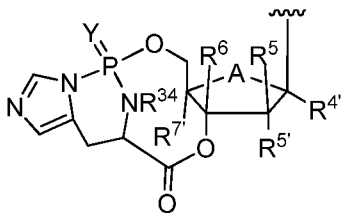
R^2, R^3, and Y are as defined above with respect to Formulas I, II, III and IV;

$R^{4'}$, R^5, $R^{5'}$, R^6, and $R^{6'}$ are as defined above;

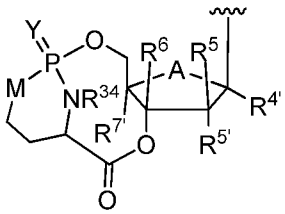
R^{19} is H, F, Cl, Br, I, N_3, C(O)OH, CN, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, or R,

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

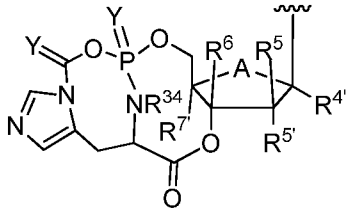
In yet another embodiment, Sugar has one of the Formulas (**XX**), (**XXI**), or (**XXII**):



(**XX**)



(**XXI**)



(**XXII**)

wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, and $R^{7'}$ are as defined above with respect to Formulas I, II, III and IV;

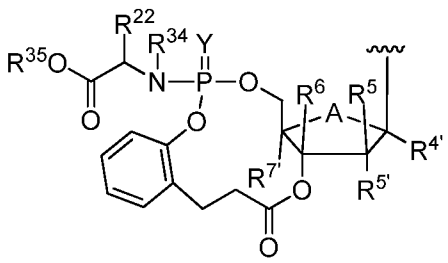
R^{34} is C_1-C_6 alkyl;

M is O, S, or NR;

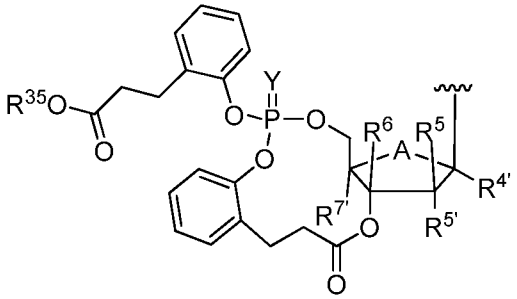
wherein R is, independently, C_1-C_6 alkyl, $C_{2\text{-}6}$ alkenyl, $C_{2\text{-}6}$ alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II,

III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl;

In another embodiment, Sugar has of one of the Formulas (**XXIII**) or (**XXIV**):



(**XXIII**)



(**XXIV**)

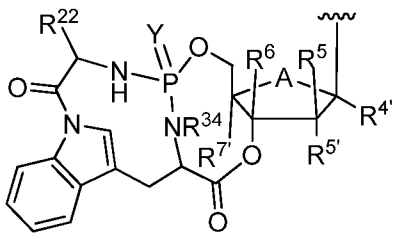
wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, $R^{7'}$, R^{34} are as defined above with respect to Formulas I, II, III and IV;

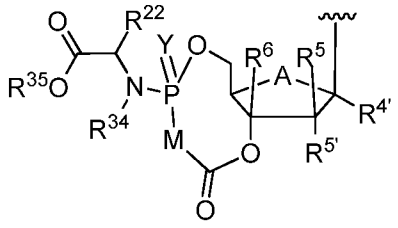
R^{35} is H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl; and

R^{22} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-6} cycloalkyl;

In still another embodiment, Sugar has one of the Formulas (**XXV**) or (**XXVI**):



(**XXV**)

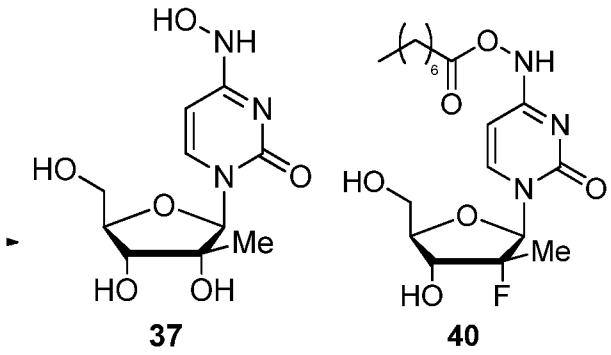


(**XXVI**)

wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, M, $R^{7'}$, R^{34}, R^{35}, R^{22} are as defined above with respect to Formulas I, II, III and IV;

In one embodiment, the compound has one of the following formulas:



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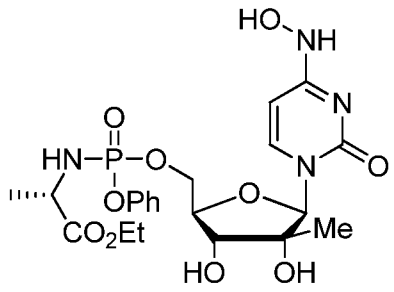

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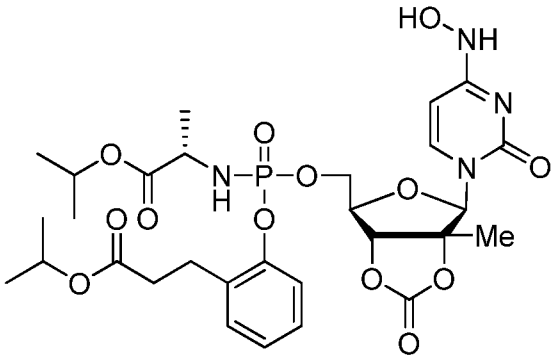

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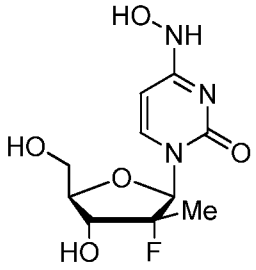

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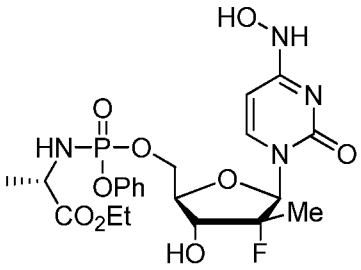

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, or pharmaceutically acceptable salts thereof.

In one embodiment, at least one of R^5 or $R^{5'}$ is F, Cl, or Me.

In another embodiment, R^5 and $R^{5'}$ are Me and F, respectively.

In another embodiment, R^5 and $R^{5'}$ are Me and Cl, respectively.

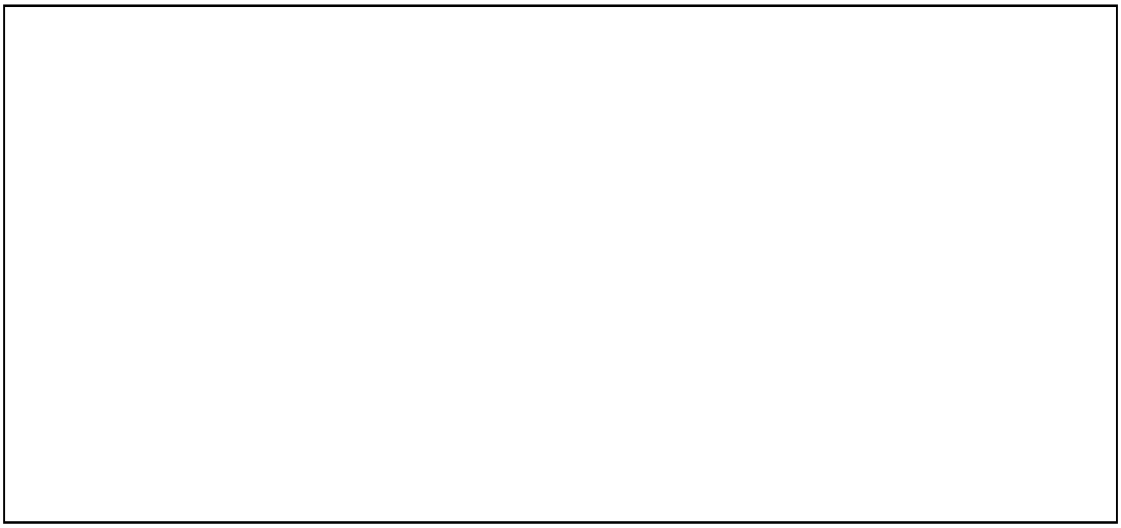
In another embodiment, L is methyl.

In another embodiment, the base is a pyrimidine, and one of R^5 and $R^{5'}$ is OH, Cl, or F.

The compounds described herein can be in the form of the β-L- or β-D-configuration, or a mixture thereof, including a racemic mixture thereof.

In those embodiments where the phosphorous portion of the compound described herein contains a chiral center, such chiral center can be in the form of the R_p- or S_p-configuration or a mixture thereof, including a racemic mixture thereof.

In one embodiment, the compounds are converted in a biological system to a mixture of pyrimidine triphosphates, due to partial conversion of the -NHOH moiety on the pyrimidine ring to an -NH_2 moiety, and, optionally, partial conversion of the -NHOH moiety or the resulting –NH_2 moiety on the pyrimidine ring to an OH moiety. An example of this type of partial conversion is shown below, where mixtures **C** or **D** of pyrimidine triphosphates include 4-NHOH, 4-NH_2 and 4-OH pyrimidine triphosphates. Such mixtures can be formed, for example, when the compound that is administered includes a prodrug on the 5'-OH moiety of the sugar. Examples of suitable prodrugs include those exemplified above.



Thus, by administering a single compound, a combination of two or three active compounds can be formed during drug metabolism, and these drugs can target a virus in different ways. For example, the analog in which the NHOH is converted, directly or indirectly, to an OH moiety behaves like a uridine analog when it is incorporated by the virus into the growing DNA or RNA strand. The analog in which the NHOH moeity is converted to an NH_2 moiety behaves like a cytosine analog when it is incorporated by the virus into the growing DNA or RNA strand. The NHOH analog can behave like either a cytosine or uridine analog when it is incorporated by the virus into the growing DNA or RNA strand. It is expected that the combination of three active triphosphates will result in different and more difficult mutation selection versus any of the single triphosphate drugs that are typically administered.

By attacking the virus in multiple ways, i.e., by presenting the virus with both U and C type analogs, the prodrug compound has a built-in mechanism for defending against viral resistance. That is, should the virus mutate to avoid taking up the U analog, it may still be susceptible to one or more of the C analogs, and vise versa, and should there be multiple C analogs, resistance to one may not confer resistance to another.

Thus, the compounds described herein can be administered as a single component, and yet provide the benefits of combination antiviral therapy. When combined with additional antiviral agents, particularly non-NNRTI antiviral agents, the combination can provide the benefits of combinations with many additional components, while providing the simplicity of including only one nucleoside prodrug.

Detailed Description

N^4-hydroxycytidine nucleosides derivatives and modified monophosphate prodrug analogs described herein show inhibitory activity against HIV, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, Yellow fever, cancer, HBV, and herpes viruses, such as HSV-1, HSV-2, and cytomegalovirus (CMV). Therefore, the compounds can be used to treat or prevent a viral infection in a host, or reduce the biological activity of the virus. The host can be a mammal, and in particular, a human, infected with HIV-1, HIV-2, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, Yellow fever, cancer, cytomegalovirus (CMV), and/or HBV. The methods involve administering an effective amount of one or more of the nucleoside or nucleotides monophosphate prodrugs described herein.

Pharmaceutical formulations including one or more compounds described herein, in combination with a pharmaceutically acceptable carrier or excipient, are also disclosed. In one embodiment, the formulations include at least one compound described herein and at least one further therapeutic agent.

The present invention will be better understood with reference to the following definitions:

I. Definitions

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

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

As used herein, the term "substantially free of" or "substantially in the absence of" refers to a nucleotide composition that includes at least 85 to 90% by weight, preferably 95% to 98 % by weight, and, even more preferably, 99% to 100% by

weight, of the designated enantiomer of that nucleotide. In a preferred embodiment, the compounds described herein are substantially free of enantiomers.

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

In some cases the phosphorus atom may be chiral herein termed "P*" or "P" which means that and that it has a designation of "R" or "S" corresponding to the accepted meanings of Cahn-Ingold-Prelog rules for such assignment. Prodrugs of Formula A may exist as a mixture of diastereomers due to the chirality at the phosphorus center. When chirality exists at the phosphorous center it may be wholly or partially Rp or Sp or any mixture thereof.

The term "alkyl," as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbons, including both substituted and unsubstituted alkyl groups. The alkyl group can be optionally substituted with any moiety that does not otherwise interfere with the reaction or that provides an improvement in the process, including but not limited to but limited to halo, haloalkyl, hydroxyl, carboxyl, acyl, aryl, acyloxy, amino, amido, carboxyl derivatives, alkylamino, dialkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, thiol, imine, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrozine, carbamate, phosphonic acid, 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. Specifically included are CF_3 and CH_2CF_3

In the text, whenever the term C(alkyl range) is used, the term independently includes each member of that class as if specifically and separately set out. The term "alkyl" includes $C_{1\text{-}22}$ alkyl moieties, and the term "lower alkyl" includes $C_{1\text{-}6}$ alkyl moieties. It is understood to those of ordinary skill in the art that the relevant alkyl radical is named by replacing the suffix "-ane" with the suffix "-yl".

The term "alkenyl" refers to an unsaturated, hydrocarbon radical, linear or branched, in so much as it contains one or more double bonds, and the term "lower alkenyl" includes C_{2-6} alkenyl moieties. The alkenyl group disclosed herein can be optionally substituted with any moiety that does not adversely affect the reaction process, including but not limited to but not limited to those described for substituents on alkyl moieties. Non-limiting examples of alkenyl groups include ethylene, methylethylene, isopropylidene, 1,2-ethane-diyl, 1,1-ethane-diyl, 1,3-propane-diyl, 1,2-propane-diyl, 1,3-butane-diyl, and 1,4-butane-diyl.

The term "alkynyl" refers to an unsaturated, acyclic hydrocarbon radical, linear or branched, in so much as it contains one or more triple bonds, and the term "lower alkynyl" includes C_{2-6} alkynyl moieties. The alkynyl group can be optionally substituted with any moiety that does not adversely affect the reaction process, including but not limited to those described above for alkyl moeities. Non-limiting examples of suitable alkynyl groups include ethynyl, propynyl, hydroxypropynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 4-methoxypentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, and hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals.

The term "alkylamino" or "arylamino" refers to an amino group that has one or two alkyl or aryl substituents, respectively.

The term "protected" as used herein and unless otherwise defined refers to a group that is added to an oxygen, nitrogen, or phosphorus atom to prevent its further reaction or for other purposes. A wide variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis, and are described, for example, in Greene et al., Protective Groups in Organic Synthesis, supra.

The term "aryl", alone or in combination, means a carbocyclic aromatic system containing one, two or three rings wherein such rings can be attached together in a pendent manner or can be fused. Non-limiting examples of aryl include phenyl, biphenyl, or naphthyl, or other aromatic groups that remain after the removal of a hydrogen from an aromatic ring. The term aryl includes both substituted and unsubstituted moieties. The aryl group can be optionally substituted with any moiety that does not adversely affect the process, including but not limited to but not limited to those described above for alkyl moieties. Non-limiting examples of substituted aryl

include heteroarylamino, N-aryl-N-alkylamino, N-heteroarylamino-N-alkylamino, heteroaralkoxy, arylamino, aralkylamino, arylthio, monoarylamidosulfonyl, arylsulfonamido, diarylamidosulfonyl, monoaryl amidosulfonyl, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, hydroxyaralkyl, hydoxyheteroaralkyl, haloalkoxyalkyl, aryl, aralkyl, aryloxy, aralkoxy, aryloxyalkyl, saturated heterocyclyl, partially saturated heterocyclyl, heteroaryl, heteroaryloxy, heteroaryloxyalkyl, arylalkyl, heteroarylalkyl, arylalkenyl, and heteroarylalkenyl, carboaralkoxy.

The terms "alkaryl" or "alkylaryl" refer to an alkyl group with an aryl substituent. The terms "aralkyl" or "arylalkyl" refer to an aryl group with an alkyl substituent.

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

The term "acyl" refers to a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl or lower alkyl, alkoxyalkyl, including but not limited to methoxymethyl, aralkyl, including but not limited to benzyl, aryloxyalkyl, such as phenoxymethyl, aryl, including but not limited to phenyl, optionally substituted with halogen (F, Cl, Br, I), alkyl (including, but not limited to C_1, C_2, C_3, and C_4), alkoxy (including but not limited to C_1, C_2, C_3, and C_4), sulfonate esters, such as alkyl or aralkyl sulphonyl, including but not limited to methanesulfonyl, mono, di or triphosphate esters, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (*e.g.*, dimethyl-t-butylsilyl) or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group. The term "lower acyl" refers to an acyl group in which the non-carbonyl moiety is lower alkyl.

The terms "alkoxy" and "alkoxyalkyl" embrace linear or branched oxy-containing radicals having alkyl moieties, such as methoxy radical. The term "alkoxyalkyl" also embraces alkyl radicals having one or more alkoxy radicals attached to the alkyl radical, that is, to form monoalkoxyalkyl and dialkoxyalkyl radicals. The "alkoxy" radicals can be further substituted with one or more halo atoms, such as fluoro, chloro or bromo, to provide "haloalkoxy" radicals. Examples of such radicals include fluoromethoxy, chloromethoxy, trifluoromethoxy, difluoromethoxy, trifluoroethoxy, fluoroethoxy, tetrafluoroethoxy, pentafluoroethoxy, and fluoropropoxy.

The term "alkylamino" denotes "monoalkylamino" and "dialkylamino" containing one or two alkyl radicals, respectively, attached to an amino radical. The terms arylamino denotes "monoarylamino" and "diarylamino" containing one or two aryl radicals, respectively, attached to an amino radical. The term "aralkylamino", embraces aralkyl radicals attached to an amino radical. The term aralkylamino denotes "monoaralkylamino" and "diaralkylamino" containing one or two aralkyl radicals, respectively, attached to an amino radical. The term aralkylamino further denotes "monoaralkyl monoalkylamino" containing one aralkyl radical and one alkyl radical attached to an amino radical.

The term "heteroatom," as used herein, refers to oxygen, sulfur, nitrogen and phosphorus.

The terms "heteroaryl" or "heteroaromatic," as used herein, refer to an aromatic that includes at least one sulfur, oxygen, nitrogen or phosphorus in the aromatic ring.

The term "heterocyclic," "heterocyclyl," and cycloheteroalkyl refer to a nonaromatic cyclic group wherein there is at least one heteroatom, such as oxygen, sulfur, nitrogen, or phosphorus in the ring.

Nonlimiting examples of heteroaryl and heterocyclic groups include furyl, furanyl, pyridyl, pyrimidyl, thienyl, isothiazolyl, imidazolyl, tetrazolyl, pyrazinyl, benzofuranyl, benzothiophenyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, isoindolyl, benzimidazolyl, purinyl, carbazolyl, oxazolyl, thiazolyl, isothiazolyl, 1,2,4-thiadiazolyl, isooxazolyl, pyrrolyl, quinazolinyl, cinnolinyl, phthalazinyl, xanthinyl, hypoxanthinyl, thiophene, furan, pyrrole, isopyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, pyrimidine or pyridazine, and pteridinyl, aziridines, thiazole, isothiazole, 1,2,3-oxadiazole, thiazine, pyridine, pyrazine, piperazine, pyrrolidine, oxaziranes, phenazine, phenothiazine, morpholinyl, pyrazolyl, pyridazinyl, pyrazinyl, quinoxalinyl, xanthinyl, hypoxanthinyl, pteridinyl, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, pyrazolopyrimidinyl, adenine, N^6-alkylpurines, N^6-benzylpurine, N^6-halopurine, N^6-vinypurine, N^6-acetylenic purine, N^6-acyl purine,N^6-hydroxyalkyl purine, N^6-thioalkyl purine, thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrmidine, uracil, N^5-

alkylpyrimidines, N^5-benzylpyrimidines, N^5-halopyrimidines, N^5-vinylpyrimidine, N^5-acetylenic pyrimidine, N^5-acyl pyrimidine, N^5-hydroxyalkyl purine, and N^6-thioalkyl purine, and isoxazolyl. The heteroaromatic group can be optionally substituted as described above for aryl. The heterocyclic or heteroaromatic group can be optionally substituted with one or more substituents selected from the group consisting of halogen, haloalkyl, alkyl, alkoxy, hydroxy, carboxyl derivatives, amido, amino, alkylamino, and dialkylamino. The heteroaromatic can be partially or totally hydrogenated as desired. As a nonlimiting example, dihydropyridine can be used in place of pyridine. Functional oxygen and nitrogen groups on the heterocyclic or heteroaryl group can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, and *t*-butyldiphenylsilyl, trityl or substituted trityl, alkyl groups, acyl groups such as acetyl and propionyl, methanesulfonyl, and p-toluenelsulfonyl. The heterocyclic or heteroaromatic group can be substituted with any moiety that does not adversely affect the reaction, including but not limited to but not limited to those described above for aryl.

The term "host," as used herein, refers to a unicellular or multicellular organism in which the virus can replicate, including but not limited to cell lines and animals, and, preferably, humans. Alternatively, the host can be carrying a part of the viral genome, whose replication or function can be altered by the compounds of the present invention. The term host specifically refers to infected cells, cells transfected with all or part of the viral genome and animals, in particular, primates (including but not limited to 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 contemplated by the present invention (such as for use in treating chimpanzees).

The term "peptide" refers to various natural or synthetic compounds containing two to one hundred amino acids linked by the carboxyl group of one amino acid to the amino group of another.

The term "pharmaceutically acceptable salt or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of a nucleotide compound which,

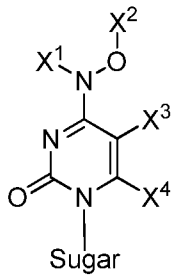
upon administration to a patient, provides the nucleotide monophosphate compound. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on functional moieties of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, or dephosphorylated to produce the active compound. The prodrug forms of the compounds of this invention can possess antiviral activity, can be metabolized to form a compound that exhibits such activity, or both.

Prodrugs also include amino acid esters of the disclosed nucleosides (*see, e.g.*, European Patent Specification No. 99493, the text of which is incorporated by reference, which describes amino acid esters of acyclovir, specifically the glycine and alanine esters which show improved water-solubility compared with acyclovir itself, and US Pat. No. 4,957,924 (Beauchamp), which discloses the valine ester of acyclovir, characterized by side-chain branching adjacent to the α-carbon atom, which showed improved bioavailability after oral administration compared with the alanine and glycine esters). A process for preparing such amino acid esters is disclosed in US Pat. No. 4,957,924 (Beauchamp), the contents of which are incorporated by reference. As an alternative to the use of valine itself, a functional equivalent of the amino acid can be used (*e.g.*, an acid halide such as the acid chloride, or an acid anhydride). In such a case, to avoid undesirable side-reactions, it may be advantageous to use an amino-protected derivative.

II. Active Compound

In one embodiment of the invention, the active compound is of Formula (**I**):

In one embodiment, the compound is a compound of Formula (**I**):



(**I**)

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

iv) X^1 is H, C_1-C_6alkyl, C_1-C_6haloalkyl, C_1-C_6alkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, COR^1, or $COOR^1$;

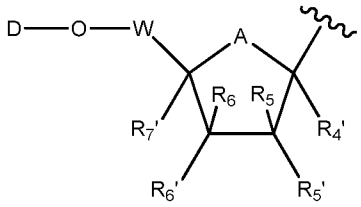
v) X^2 is hydrogen, COR^1, or $COOR^1$

wherein each R^1 is, independently, CH_2-O(CO)-X^5; CH_2-O(CO)O-X^5 ,C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-6} alkyl, or C_{1-6} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or C_{3-10} cycloalkyl

X^5 is independently, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, alkoxy, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-6} alkyl, or C_{1-6} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or C_{3-10} cycloalkyl

vi) Each X^3 and X^4 is independently H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, alkylaryl, halogen (F, Cl, Br, I), NH_2, OH, SH, CN, or NO_2.

In one embodiment, Sugar is ribose or a modified ribose of the general Formula (**II**):



(**II**)

wherein:

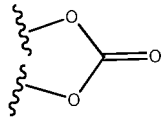
D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

R^1 is as defined above;

W is CL_2 or CL_2CL_2, wherein L independently is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH_2, CHF, CF_2, $C=CH_2$, C=CHF, or $C=CF_2$;

$R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, and $R^{7'}$ are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

$R^{5'}$ and $R^{6'}$ can come together to form a ring 

.

In one embodiment, where Sugar is formula (**II**), when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H then, R^5, $R^{5'}$, R^6, $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH=CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

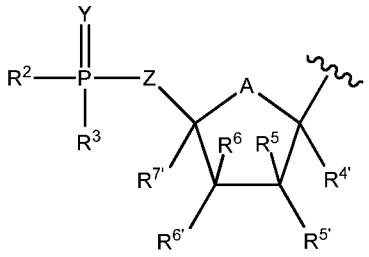
In another embodiment, $R^{6'}$ is independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

In one embodiment, wherein for formula (**I**) where sugar is formula (**II**), when A is O or S, $R^{7'}$cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2.

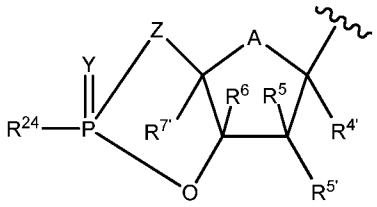
In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R;

R is independently C_1-C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_3-C_6 cycloalkyl) aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above, where representative substituents include for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In another embodiment, Sugar is ribose or modified ribose of the general formulas (**III**) or (**IV**):



(**III**)



(**IV**)

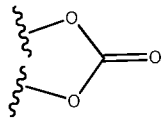
wherein:

Y is O or S;

Z is selected from the group consisting of CL_2, CL_2CL_2, CL_2OCL_2, CL_2SCL_2, CL_2O, OCL_2 and CL_2NHCL_2, wherein L independently is selected from the group consisting of H, F, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH_2, CHF, CF_2, $C=CH_2$, C=CHF, or $C=CF_2$;

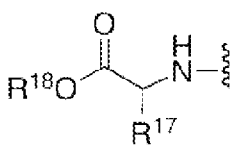
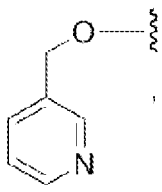
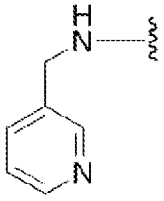
$R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, and $R^{7'}$ are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

$R^{5'}$ and $R^{6'}$ can come together to form a ring  .

In one embodiment, where Sugar is formula (**III**) or (**IV**), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2.

In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R.

R is independently a C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above.

R^{24} is selected from the group consisting of OR^{15},  ,  ,  , and fatty alcohols,

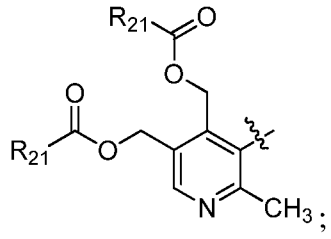
R^{15} is selected from the group consisting of H, Li, Na, K, phenyl and pyridinyl; wherein phenyl and pyridinyl are optionally substituted with zero to three substituents independently selected from the group consisting of $(CH_2)_{0-6}CO_2R^{16}$ and $(CH_2)_{0-6}CON(R^{16})_2$;

R^{17} is selected from to those groups occurring in natural L-amino acids, C_{1-6} alkyl, (C_1-C_6 alkyl), C_{2-6} alkenyl, C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above.

R^{18} is H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol (such as oleyl alcohol, octacosanol, triacontanol, linoleyl alcohol, and the like) or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl

substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or cycloalkyl.

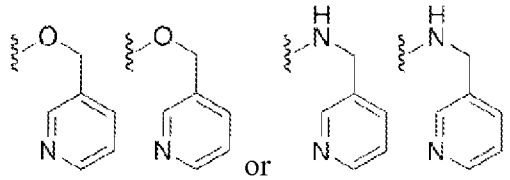
Representative R^2 and R^3 are independently selected from the group consisting of:

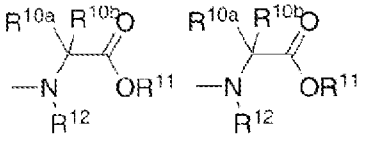
(a) OR^8 where R^8 is H, Li, Na, K, C_{1-20} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, aryl, or heteroaryl which includes, but is not limited to, phenyl or naphthyl optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, $(CH_2)_{0-6}CO_2R^{9a}$, halogen, C_{1-6} haloalkyl, $-N(R^{9a})_2$, C_{1-6} acylamino, $-NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^{9a})_2$, $-SO_2C_{1-6}$ alkyl, COR^{9b}, nitro, cyano and  ;

wherein R^{21} is as defined below;

R^{9a} is independently H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

(b)  or

(c)  where R^{10a} and R^{10b} are:

(i) independently selected from the group consisting of H, C_{1-10} alkyl, $-(CH_2)_rNR^{9a}{}_2$, C_{1-6} hydroxyalkyl, $-CH_2SH$, $-(CH_2)_2S(O)_pMe$, $-(CH_2)_3NHC(=NH)NH_2$, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, $-(CH_2)_mCOR^{9b}$, aryl and aryl-C_{1-3} alkyl, said aryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, halogen, nitro, and cyano;

(ii) R^{10a} is H and R^{10b} and R^{12} together are $(CH_2)_{2-4}$ to form a ring that includes the adjoining N and C atoms;

(iii) R^{10a} and R^{10b} together are $(CH_2)_n$ to form a ring;

(iv) R^{10a} and R^{10b} both are C_{1-6} alkyl; or

(v) R^{10a} is H and R^{10b} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$-$CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-10} cycloalkyl;

p is 0 to 2;

r is 1 to 6;

n is 4 or 5;

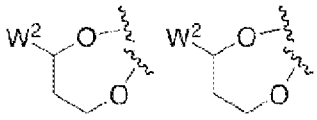
m is 0 to 3;

R^{11} is H, C_{1-10} alkyl, or C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are

C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{12} is H or C_{1-3} alkyl, or R^{10a}, or R^{10b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

(d) an O attached lipid (including a phospholipid), an N or O attached peptide, an O attached cholesterol, or an O attached phytosterol;

(e) R^2 and R^3 can come together to form a ring  where W^2 is selected from the group consisting of phenyl and monocyclic heteroaryl, optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, CF_3, C_{2-6} alkenyl, C_{1-6} alkoxy, OR^{9c}, CO_2R^{9a}, COR^{9a}, halogen, C_{1-6} haloalkyl, $-N(R^{9a})_2$, C_{1-6} acylamino, $CO_2N(R^{9a})_2$, SR^{9a}, $-NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^{9a})_2$, $-SO_2C_{1-6}$ alkyl, COR^{9b}, and cyano, and wherein said monocyclic heteroaryl and substituted monocyclic heteroaryl has 1-2 heteroatoms that are independently selected from the group consisting of N, O, and S, with the provisos that:

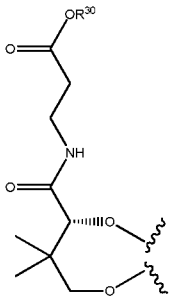
a) when there are two heteroatoms and one is O, then the other can not be O or S, and

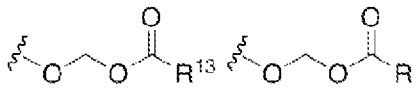
b) when there are two heteroatoms and one is S, then the other can not be O or S;

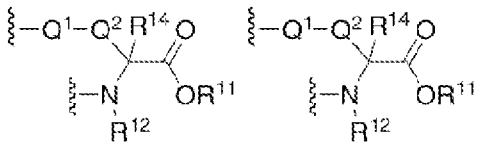
R^{9a} is independently H or C_{1-6} alkyl;

R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

R^{9c} is H or C_{1-6} acyl;

(f) R^2 and R^3 can come together to form a ring  where R^{30} is H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

(g)  where R^{13} is selected from the group consisting of H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, and substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

(h) R^2 and R^3 can come together to form a ring  where R^{14} is:

(i) independently selected from the group consisting of H, C_{1-10} alkyl, -$(CH_2)_rNR_2^{9a}$, C_{1-6} hydroxyalkyl, -CH_2SH, -$(CH_2)_2S(O)_pMe$, -$(CH_2)_3NHC(=NH)NH_2$, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -$(CH_2)_mCOR^{9b}$, aryl, aryl-C_{1-3} alkyl, heteroaryl and heteroaryl-C_{1-3} alkyl, said aryl and heteroaryl groups optionally substituted with a group selected

from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, halogen, nitro, and cyano;

(ii) R^{14} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-10} cycloalkyl;

p is 0 to 2;

r is 1 to 6;

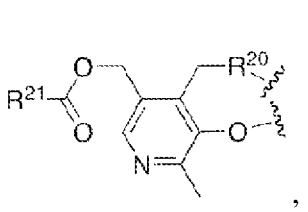
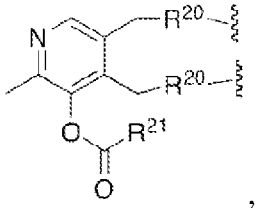
m is 0 to 3

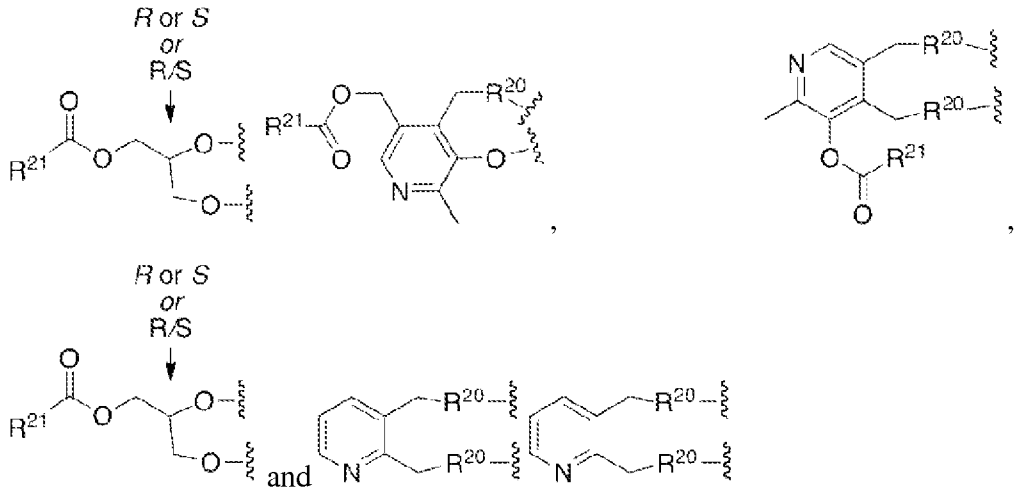
Q^1 is NR^{9a}, O, or S

Q^2 is C_{1-10} alkyl, C_{1-6} hydroxyalkyl, aryl and aryl-C_{1-3} alkyl, heteroaryl and heteroaryl-C_{1-3} alkyl, said aryl and heteroaryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, fluoro, and chloro;

R^{11} is H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{12} is H or C_{1-3} alkyl, or R^{14b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

(i) R^2 and R^3 can come together to form a ring selected from the group consisting of  ,  ,

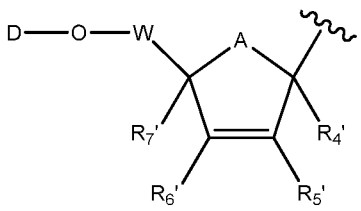


where R^{20} is O or NH, and

R^{21} is selected from the group consisting of H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty acid, and C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, and substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl, and

(j) R^2 is a monophosphate ester or a diphosphate ester when R^3 is OH, O^-K^+, O^-Li^+, or O^-Na^+.

In still another embodiment, Sugar is ribose or modified ribose of the general formula (**V**):



(**V**)

wherein:

D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

R^1 is independently C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6}

alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

W is CL_2 or CL_2CL_2, wherein L independently is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A, R^2, R^3, Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Formulas I, II, III and IV;

wherein for formula (**I**) where Sugar is formula (**V**), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2,

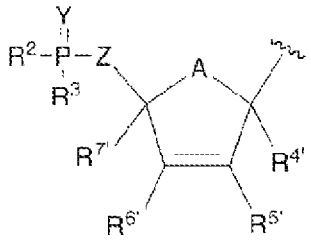
In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R;

wherein R is independently C_1-C_6 alkyl, C_{2-6} alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In one embodiment, where Sugar is of Formula (**V**), when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H then, $R^{5'}$ and $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH{=}CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

In another embodiment, R^5 and $R^{6'}$ are independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

In yet another embodiment, Sugar is a modified ribose of the general Formula (**VI**):



(**VI**)

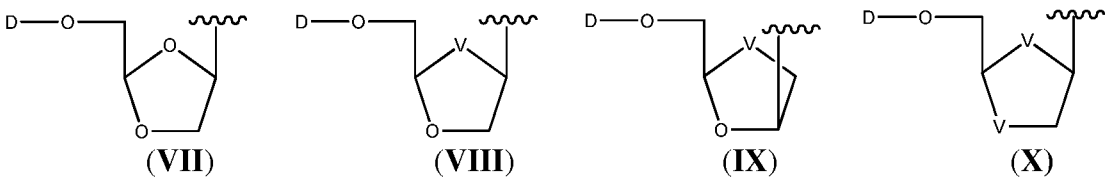
wherein:

A, R^2, R^3, Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Formulas I, II, III and IV;

wherein for formula (**I**) where Sugar is of Formula (**VI**), when A is O or S, $R^{7'}$cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2,

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In another embodiment, Sugar is a dioxolane, an oxathiolane, or a dithiolane of the general formulas (**VII**), (**VIII**), (**IX**), and (**X**):



(**VII**) (**VIII**) (**IX**) (**X**)

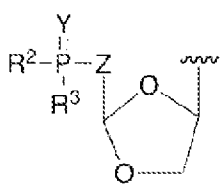
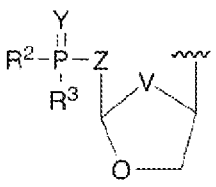
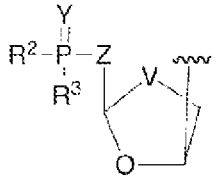
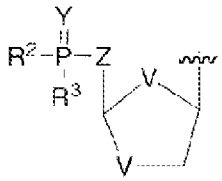
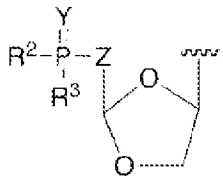
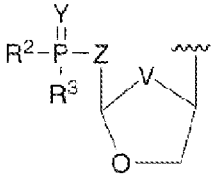
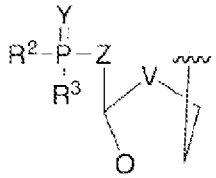
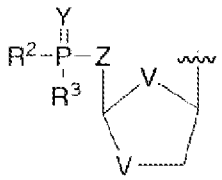
D is $C(O)OR^1$, diphosphate ester, or triphosphate ester;

V is, individually, S or Se;

R^1 is independently $C_{1\text{-}20}$ alkyl, the carbon chain derived from a fatty alcohol or $C_{1\text{-}20}$ alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-

C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

In yet another embodiment, Sugar is a dioxolane, an oxathiolane, or a dithiolane of the general Formulas (**XI**), (**XII**), (**XIII**), and (**XIV**):

(**XI**) (**XII**) (**XIII**)

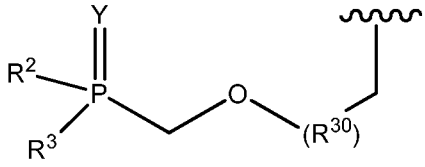
(**XIV**)

wherein:

V is, individually, S or Se;

R^2, R^3, Y, and Z are as defined above with respect to Formulas I, II, III and IV.

In still another embodiment, Sugar is a phosphonylmethoxyalkyl of the general Formula (**XV**):



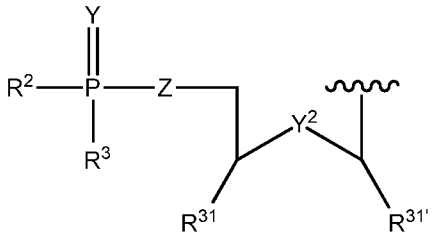
(**XV**)

wherein:

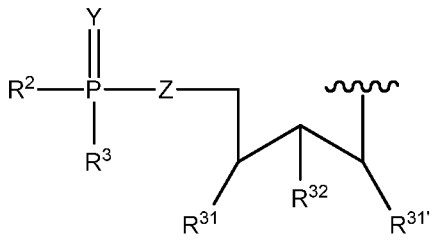
R^2, R^3, and Y are as defined above with respect to Formulas I, II, III and IV; and;

R^{30} is selected from the group consisting of C_{1-20} alkyl, C_{2-20} alkyl (including but not limited to C_1-C_6), alkenyl (including but not limited to C_2-C_6), and C_{2-20} alkynyl, C_{3-10} (including but not limited to C_2-C_6), cycloalkyl (including but not limited to C_3-C_8), aryl (including but not limited to C_6-C_{10}), heteroaryl (including but not limited to C_6-C_{10}), arylalkyl, and alkylaryl;

In still another embodiment, Sugar is of the general formulas (**XVI**) or (**XVII**):



(**XVI**)



(**XVII**)

wherein:

R^2, R^3, Z, and Y are as defined above;

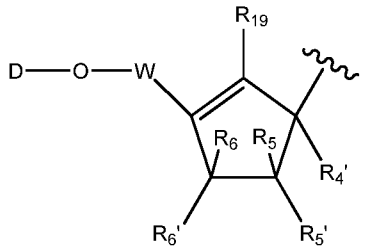
Y^2 is O, S, Se, or NR;

R is, independently, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl;

R^{31}, $R^{31'}$ and R^{32} are defined as H, CH_3, or CH_2OR^{33}; and

R^{33} is H or C_1-C_6 acyl.

In another embodiment, Sugar is a modified ribose of the general formulas (**XVIII**)



(**XVIII**)

wherein:

D, W, $R^{4'}$, R^5, $R^{5'}$, R^6, and $R^{6'}$ are as defined above;

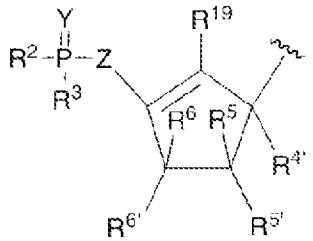
R^{19} is H, F, Cl, Br, I, N_3, C(O)OH, CN, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, or R;

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In one embodiment, where sugar is of Formula (**XVII**), when D is H or acyl, W is CH_2, $R^{4'}$ and R^{19} are H, then, R^5, $R^{5'}$, R^6, $R^{6'}$ can not be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH{=}CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

In another embodiment, $R^{6'}$ can be independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2.

In a further embodiment, Sugar is a modified ribose of Formulas (**XIX**):



(**XIX**)

wherein:

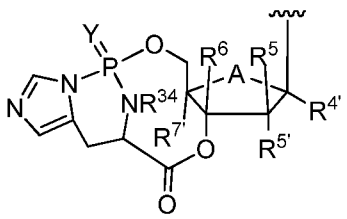
R^2, R^3, and Y are as defined above with respect to Formulas I, II, III and IV;

$R^{4'}$, R^5, $R^{5'}$, R^6, and $R^{6'}$ are as defined above;

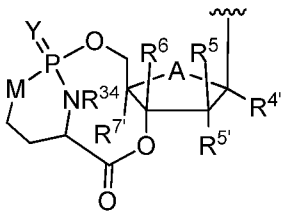
R^{19} is H, F, Cl, Br, I, N_3, C(O)OH, CN, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, or R,

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

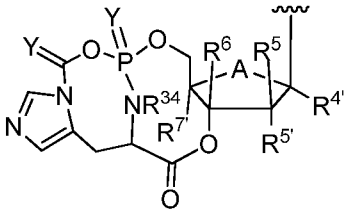
In yet another embodiment, Sugar has one of the Formulas (**XX**), (**XXI**), or (**XXII**):



(**XX**)



(**XXI**)



(**XXII**)

wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, and $R^{7'}$ are as defined above with respect to Formulas I, II, III and IV;

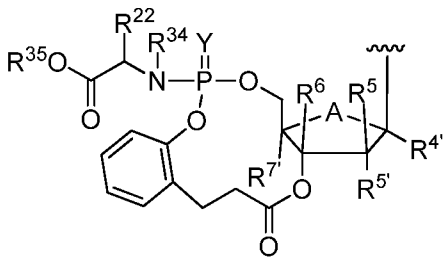
R^{34} is C_1-C_6 alkyl;

M is O, S, or NR;

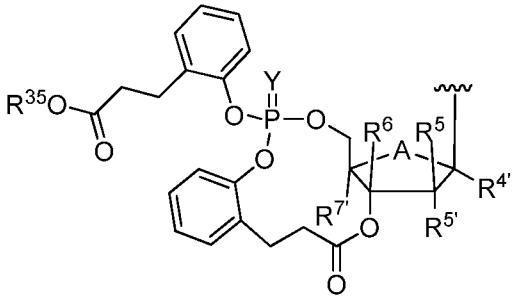
wherein R is, independently, C_1-C_6 alkyl, $C_{2\text{-}6}$ alkenyl, $C_{2\text{-}6}$ alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more

substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl;

In another embodiment, Sugar has of one of the Formulas (**XXIII**) or (**XXIV**):



(**XXIII**)



(**XXIV**)

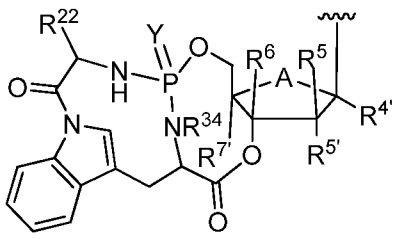
wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, $R^{7'}$, R^{34} are as defined above with respect to Formulas I, II, III and IV;

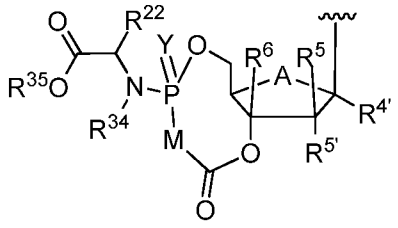
R^{35} is H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl; and

R^{22} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-6} cycloalkyl;

In still another embodiment, Sugar has one of the Formulas (**XXV**) or (**XXVI**):



(**XXV**)

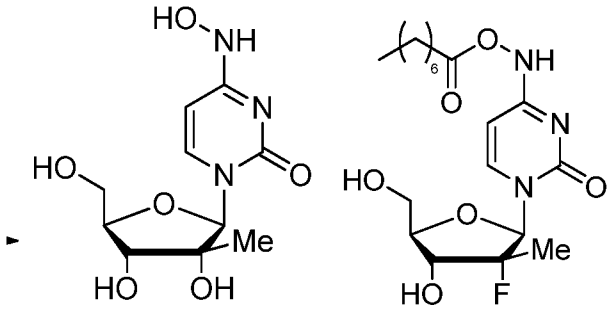


(**XXVI**)

wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, M, $R^{7'}$, R^{34}, R^{35}, R^{22} are as defined above with respect to Formulas I, II, III and IV;

In one embodiment, the compound has one of the following formulas:



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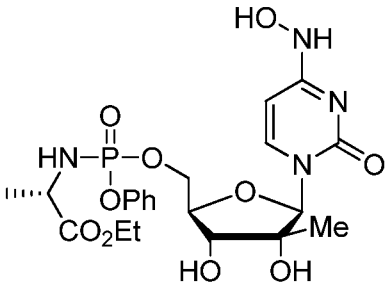

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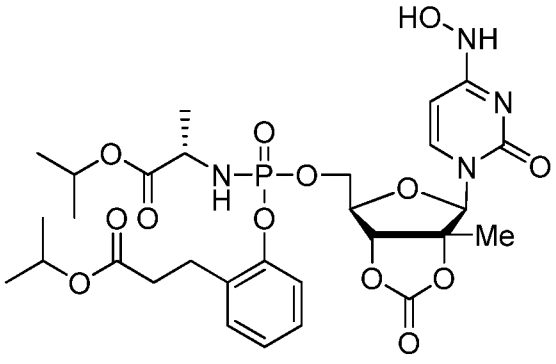

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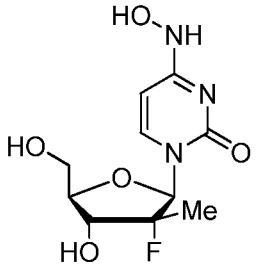

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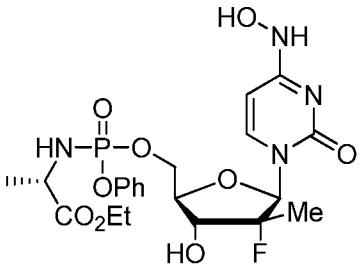

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, or pharmaceutically acceptable salts thereof.

In one embodiment, at least one of R^5 or $R^{5'}$ is F, Cl, or Me.

In another embodiment, R^5 and $R^{5'}$ are Me and F, respectively.

In another embodiment, R^5 and $R^{5'}$ are Me and Cl, respectively.

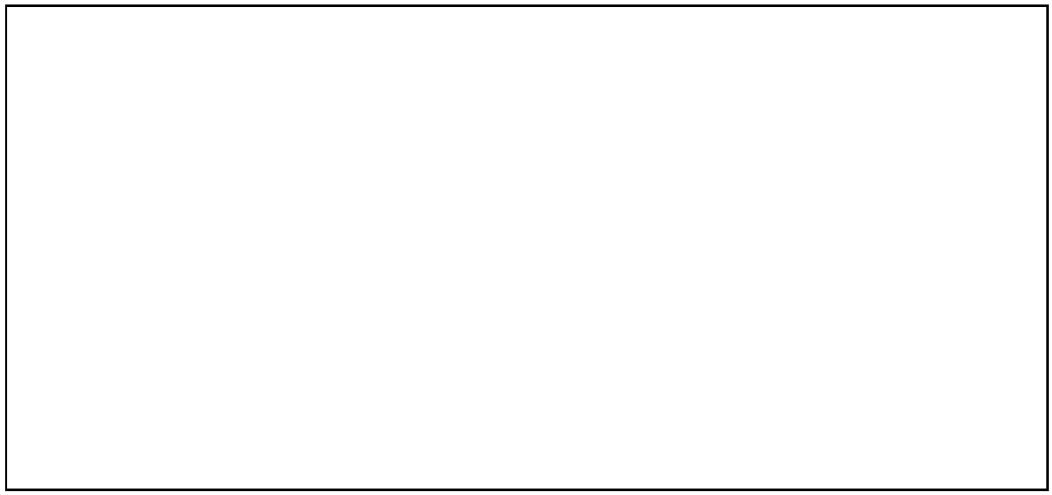
In another embodiment, L is methyl.

In another embodiment, the base is a pyrimidine, and one of R^5 and $R^{5'}$ is OH, Cl, or F.

The compounds described herein can be in the form of the β-L- or β-D-configuration, or a mixture thereof, including a racemic mixture thereof.

In those embodiments where the phosphorous portion of the compound described herein contains a chiral center, such chiral center can be in the form of the R_p- or S_p-configuration or a mixture thereof, including a racemic mixture thereof.

In one embodiment, the compounds are converted in a biological system to a mixture of pyrimidine triphosphates, due to partial conversion of the -NHOH moiety on the pyrimidine ring to an -NH_2 moiety, and, optionally, partial conversion of the -NHOH moiety or the resulting –NH_2 moiety on the pyrimidine ring to an OH moiety. An example of this type of partial conversion is shown below, where mixtures **C** or **D** of pyrimidine triphosphates include 4-NHOH, 4-NH_2 and 4-OH pyrimidine triphosphates. Such mixtures can be formed, for example, when the compound that is administered includes a prodrug on the 5'-OH moiety of the sugar. Examples of suitable prodrugs include those exemplified above.



Thus, by administering a single compound, a combination of two or three active compounds can be formed during drug metabolism, and these drugs can target a virus in different ways. For example, the analog in which the NHOH is converted, directly or indirectly, to an OH moiety behaves like a uridine analog when it is incorporated by the virus into the growing DNA or RNA strand. The analog in which the NHOH moeity is converted to an NH_2 moiety behaves like a cytosine analog when it is incorporated by the virus into the growing DNA or RNA strand. The NHOH analog can behave like either a cytosine or uridine analog when it is incorporated by the virus into the growing DNA or RNA strand. It is expected that the combination of three active triphosphates will result in different and more difficult mutation selection versus any of the single triphosphate drugs that are typically administered.

By attacking the virus in multiple ways, i.e., by presenting the virus with both U and C type analogs, the prodrug compound has a built-in mechanism for defending against viral resistance. That is, should the virus mutate to avoid taking up the U analog, it may still be susceptible to one or more of the C analogs, and vise versa, and should there be multiple C analogs, resistance to one may not confer resistance to another.

Thus, the compounds described herein can be administered as a single component, and yet provide the benefits of combination antiviral therapy. When combined with additional antiviral agents, particularly non-NNRTI antiviral agents, the combination can provide the benefits of combinations with many additional components, while providing the simplicity of including only one nucleoside prodrug.

III. Stereoisomerism and Polymorphism

The compounds described herein may have asymmetric centers and occur as racemates, racemic mixtures, individual diastereomers or enantiomers, with all isomeric forms being included in the present invention. Compounds of the present invention having a chiral center can exist in and be isolated in optically active and racemic forms. Some compounds can exhibit polymorphism. The present invention encompasses racemic, optically-active, polymorphic, or stereoisomeric forms, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein. The optically active forms can be prepared by, for example, 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 or by enzymatic resolution. One can either purify the respective nucleoside, then derivatize the nucleoside to form the compounds described herein, or purify the nucleotides themselves.

Optically active forms of the compounds can be prepared using any method known in the art, including but not limited to 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.

Examples of methods to obtain optically active materials include at least the following.

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

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

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

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

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

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

vii) first- and second-order asymmetric transformations: a technique whereby diastereomers from the 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;

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

ix) enantiospecific synthesis from non-racemic precursors: a synthetic technique whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;

x) chiral liquid chromatography: a technique whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase (including but not limited to via chiral HPLC). The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;

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

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

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

Chiral chromatography, including but not limited to simulated moving bed chromatography, is used in one embodiment. A wide variety of chiral stationary phases are commercially available.

IV. Nucleotide Salt or Prodrug Formulations

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically acceptable salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate and α-glycerophosphate. Suitable

inorganic salts can also be formed, including but not limited to, sulfate, nitrate, bicarbonate and carbonate salts.

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

The nucleotide prodrugs described herein can be administered to additionally increase the activity, bioavailability, stability or otherwise alter the properties of the nucleotide monophosphate.

A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the monophosphate or other anolog of the nucleoside will increase the stability of the nucleotide.

Examples of substituent groups that can replace one or more hydrogens on the monophosphate moiety are alkyl, aryl, steroids, carbohydrates, including but not limited to sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones & N. Bischofberger, *Antiviral Research*, **1995,** *27*, 1-17 and S.J. Hecker & M.D. Erion, *J. Med. Chem.*, **2008**, *51*, 2328-2345. Any of these can be used in combination with the disclosed nucleotides to achieve a desired effect.

The active nucleotide can also be provided as a 5'-phosphoether lipid as disclosed in the following references, which are incorporated by reference: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C. Piantadosi, "Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation," *AIDS Res. Hum. Retroviruses*, **1990**, *6*, 491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. Iyer, C.A. Wallen, S. Piantadosi, and E.J. Modest, "Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity," *J. Med. Chem.*, **1991**, *34*, 1408-14; Hosteller, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch, "Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3,-deoxythymidine," *Antimicrob. Agents Chemother.*, **1992**, *36*, 2025-29; Hostetler,

K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman, "Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides." *J. Biol. Chem.*, **1990**, *265*, 61127.

Nonlimiting examples of US patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at R^2 and/or R^3 position of the nucleotides described herein, or lipophilic preparations, include US Pat. Nos. 5,149,794 (Yatvin *et al.*); 5,194,654 (Hostetler *et al.*), 5,223,263 (Hostetler *et al.*); 5,256,641 (Yatvin *et al.*); 5,411,947 (Hostetler *et al.*); 5,463,092 (Hostetler *et al.*); 5,543,389 (Yatvin *et al.*); 5,543,390 (Yatvin *et al.*); 5,543,391 (Yatvin *et al.*); and 5,554,728 (Basava *et al.*), all of which are incorporated by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to nucleosites of the present invention, or lipophilic preparations, include WO 89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910, WO 94/26273, WO 96/15132, EP 0 350 287, EP 93917054.4, and WO 91/19721.

V. Methods of Treatment

Hosts, including but not limited to humans, infected with HIV-1, HIV-2, HBV, HCV, Norovirus, Saporovirus, HSV-1, HSV-2, Dengue virus, yellow fever, or a gene fragment thereof, can be treated by administering to the patient an effective amount of the active compound or a pharmaceutically acceptable prodrug or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The compounds can also be used to treat cancer. Patients that can be treated with the compounds described herein, and the pharmaceutically acceptable salts and prodrugs of these compounds, according to the methods of this invention include, for example, patients that have been diagnosed as having lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head and neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer or cancer of the anal region, stomach cancer, colon cancer, breast cancer, gynecologic tumors (e.g., uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma

of the cervix, carcinoma of the vagina or carcinoma of the vulva), Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system (e.g., cancer of the thyroid, parathyroid or adrenal glands), sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors of childhood, lymphocytic lymphonas, cancer of the bladder, cancer of the kidney or ureter (e.g., renal cell carcinoma, carcinoma of the renal pelvis), or neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas).

This invention also relates to a method of and to a pharmaceutical composition for inhibiting abnormal cellular proliferation in a patient which comprises an amount of a compound described herein, or a pharmaceutically acceptable salt or prodrug thereof, and an amount of one or more substances selected from anti-angiogenesis agents, signal transduction inhibitors, and antiproliferative agents.

Anti-angiogenesis agents, such as MMP-2 (matrix-metalloprotienase 2) inhibitors, MMP-9 (matrix-metalloprotienase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors, can be used in conjunction with a compound of formula 1 and pharmaceutical compositions described herein. Examples of useful COX-II inhibitors include CELEBREX™ (alecoxib), valdecoxib, and rofecoxib. Examples of useful matrix metalloproteinase inhibitors are described in WO 96/33172 (published Oct. 24, 1996), WO 96/27583 (published Mar. 7, 1996), European Patent Application No. 97304971.1 (filed Jul. 8, 1997), European Patent Application No. 99308617.2 (filed Oct. 29, 1999), WO 98/07697 (published Feb. 26, 1998), WO 98/03516 (published Jan. 29, 1998), WO 98/34918 (published Aug. 13, 1998), WO 98/34915 (published Aug. 13, 1998), WO 98/33768 (published Aug. 6, 1998), WO 98/30566 (published Jul. 16, 1998), European Patent Publication 606,046 (published Jul. 13, 1994), European Patent Publication 931,788 (published Jul. 28, 1999), WO 90/05719 (published May 331, 1990), WO 99/52910 (published Oct. 21, 1999), WO 99/52889 (published Oct. 21, 1999), WO 99/29667 (published Jun. 17, 1999), PCT International Application No. PCT/IB98/01113 (filed Jul. 21, 1998), European Patent Application No. 99302232.1 (filed Mar. 25, 1999), Great Britain patent application number 9912961.1 (filed Jun. 3, 1999), U.S. Provisional Application No. 60/148,464 (filed Aug. 12, 1999), U.S. Pat. No. 5,863,949 (issued Jan. 26, 1999), U.S. Pat. No. 5,861,510 (issued Jan. 19, 1999), and European Patent Publication 780,386 (published

Jun. 25, 1997), all of which are incorporated herein in their entireties by reference. Preferred MMP inhibitors are those that do not demonstrate arthralgia. More preferred are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (i.e. MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13).

The compounds described herein can also be used with signal transduction inhibitors, such as agents that can inhibit EGFR (epidermal growth factor receptor) responses, such as EGFR antibodies, EGF antibodies, and molecules that are EGFR inhibitors; VEGF (vascular endothelial growth factor) inhibitors, such as VEGF receptors and molecules that can inhibit VEGF; and erbB2 receptor inhibitors, such as organic molecules or antibodies that bind to the erbB2 receptor, for example, HERCEPTIN™ (Genentech, Inc. of South San Francisco, Calif., USA).

EGFR inhibitors are described in, for example in WO 95/19970 (published Jul. 27, 1995), WO 98/14451 (published Apr. 9, 1998), WO 98/02434 (published Jan. 22, 1998), and U.S. Pat. No. 5,747,498 (issued May 5, 1998), and such substances can be used in the present invention as described herein. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225 and anti-EGFR 22Mab (ImClone Systems Incorporated of New York, N.Y., USA), ABX-EGF (Abgenix/Cell Genesys), EMD-7200 (Merck KgaA), EMD-5590 (Merck KgaA), MDX-447/H-477 (Medarex Inc. of Annandale, N.J., USA and Merck KgaA), and the compounds ZD-1834, ZD-1838 and ZD-1839 (AstraZeneca), PKI-166 (Novartis), PKI-166/CGP-75166 (Novartis), PTK 787 (Novartis), CP 701 (Cephalon), leflunomide (Pharmacia/Sugen), CI-1033 (Warner Lambert Parke Davis), CI-1033/PD 183,805 (Warner Lambert Parke Davis), CL-387,785 (Wyeth-Ayerst), BBR-1611 (Boehringer Mannheim GmbH/Roche), Naamidine A (Bristol Myers Squibb), RC-3940-II (Pharmacia), BIBX-1382 (Boehringer Ingelheim), OLX-103 (Merck & Co. of Whitehouse Station, N.J., USA), VRCTC-310 (Ventech Research), EGF fusion toxin (Seragen Inc. of Hopkinton, Mass.), DAB-389 (Seragen/Lilgand), ZM-252808 (Imperical Cancer Research Fund), RG-50864 (INSERM), LFM-A12 (Parker Hughes Cancer Center), WHI-P97 (Parker Hughes Cancer Center), GW-282974 (Glaxo), KT-8391 (Kyowa Hakko) and EGFR Vaccine (York Medical/Centro de Immunologia Molecular (CIM)). These and other EGFR-inhibiting agents can be used in the present invention.

VEGF inhibitors, for example CP-547,632 (Pfizer Inc., N.Y.), AG-13736 (Agouron Pharmceuticals, Inc. a Pfizer Company), SU-5416 and SU-6668 (Sugen Inc. of South San Francisco, Calif., USA), and SH-268 (Schering) can also be combined with the compound of the present invention. VEGF inhibitors are described in, for example in WO 99/24440 (published May 20, 1999), PCT International Application PCT/IB99/00797 (filed May 3, 1999), in WO 95/21613 (published Aug. 17, 1995), WO 99/61422 (published Dec. 2, 1999), U.S. Pat. No. 5,834,504 (issued Nov. 10, 1998), WO 98/50356 (published Nov. 12, 1998), U.S. Pat. No. 5,883,113 (issued Mar. 16, 1999), U.S. Pat. No. 5,886,020 (issued Mar. 23, 1999), U.S. Pat. No. 5,792,783 (issued Aug. 11, 1998), WO 99/10349 (published Mar. 4, 1999), WO 97/32856 (published Sep. 12, 1997), WO 97/22596 (published Jun. 26, 1997), WO 98/54093 (published Dec. 3, 1998), WO 98/02438 (published Jan. 22, 1998), WO 99/16755 (published Apr. 8, 1999), and WO 98/02437 (published Jan. 22, 1998), all of which are incorporated herein in their entireties by reference. Other examples of some specific VEGF inhibitors useful in the present invention are IM862 (Cytran Inc. of Kirkland, Wash., USA); anti-VEGF monoclonal antibody of Genentech, Inc. of South San Francisco, Calif.; and angiozyme, a synthetic ribozyme from Ribozyme (Boulder, Colo.) and Chiron (Emeryville, Calif.). These and other VEGF inhibitors can be used in the present invention as described herein.

ErbB2 receptor inhibitors, such as CP-358,774 (OSI-774) (Tarceva) (OSI Pharmaceuticals, Inc.), GW-282974 (Glaxo Wellcome plc), and the monoclonal antibodies AR-209 (Aronex Pharmaceuticals Inc. of The Woodlands, Tex., USA) and 2B-1 (Chiron), can furthermore be combined with the compound of the invention, for example those indicated in WO 98/02434 (published Jan. 22, 1998), WO 99/35146 (published Jul. 15, 1999), WO 99/35132 (published Jul. 15, 1999), WO 98/02437 (published Jan. 22, 1998), WO 97/13760 (published Apr. 17, 1997), WO 95/19970 (published Jul. 27, 1995), U.S. Pat. No. 5,587,458 (issued Dec. 24, 1996), and U.S. Pat. No. 5,877,305 (issued Mar. 2, 1999), which are all hereby incorporated herein in their entireties by reference. ErbB2 receptor inhibitors useful in the present invention are also described in U.S. Provisional Application No. 60/117,341, filed Jan. 27, 1999, and in U.S. Provisional Application No. 60/117,346, filed Jan. 27, 1999, both of which are incorporated in their entireties herein by reference. The erbB2 receptor inhibitor compounds and substance described in the aforementioned PCT

applications, U.S. patents, and U.S. provisional applications, as well as other compounds and substances that inhibit the erbB2 receptor, can be used with the compounds described herein in accordance with the present invention.

The compounds can also be used with other agents useful in treating abnormal cellular proliferation or cancer, including, but not limited to, agents capable of enhancing antitumor immune responses, such as CTLA4 (cytotoxic lymphocite antigen 4) antibodies, and other agents capable of blocking CTLA4; and anti-proliferative agents such as other farnesyl protein transferase inhibitors, and the like. Specific CTLA4 antibodies that can be used in the present invention include those described in U.S. Provisional Application 60/113,647 (filed Dec. 23, 1998) which is incorporated by reference in its entirety, however other CTLA4 antibodies can be used in the present invention.

Other anti-angiogenesis agents, including, but not limited to, other COX-II inhibitors, other MMP inhibitors, other anti-VEGF antibodies or inhibitors of other effectors of vascularization can also be used.

The compounds and pharmaceutical compositions described herein can be used to treat or prevent an infection by one or more Noroviruses, as well as other viruses in the *Caliciviridae* taxonomic family.

In therapeutic use for treating Norovirus infection, the compounds and/or compositions can be administered to patients diagnosed with Norovirus infection at dosage levels suitable to achieve therapeutic benefit. By "therapeutic benefit," and grammatical equivalents, is meant the administration of the compound leads to a beneficial effect in the patient over time. For example, therapeutic benefit can be achieved when the Norovirus titer or viral load in a patient is either reduced or stops increasing.

Therapeutic benefit also can be achieved if the administration of a compound slows or halts altogether the onset of adverse symptoms that typically accompany Norovirus infections, regardless of the Norovirus titer or viral load in the patient. The compounds and/or compositions described herein may also be administered prophylactically in patients who are at risk of developing Norovirus infection, or who have been exposed to Norovirus, to prevent the development of Norovirus infection.

For example, the compounds and/or compositions thereof may be administered to patients likely to have been exposed to Norovirus.

Outbreaks of norovirus disease often occur in closed or semi-closed communities, such as long-term care facilities, hospitals, prisons, and cruise ships where once the virus has been introduced, the infection spreads very rapidly by either person-to-person transmission or through contaminated food. Many norovirus outbreaks have been traced to food that was handled by one infected person. Accordingly, it may be advantageous to provide prophylactic doses of the compounds described herein to individuals in these facilities who are likely to come into contact with Norovirus or other *Caliciviridae.*

VI. Combination or Alternation Therapy

In one embodiment, the compounds of the invention can be employed together with at least one other antiviral agent, chosen from entry inhibitors, reverse transcriptase inhibitors, protease inhibitors, and immune-based therapeutic agents.

For example, when used to treat or prevent HIV or HBV infection, the active compound or its prodrug or pharmaceutically acceptable salt can be administered in combination or alternation with another antiviral agent, such as anti-HIV, anti-HBV, or anti-HCV agent, including, but not limited to, those of the formulae above. In general, in combination therapy, effective dosages of two or more agents are administered together, whereas during alternation therapy, an effective dosage of each agent is administered serially. The dosage will depend on absorption, inactivation and excretion rates of the drug, as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

Nonlimiting examples of antiviral agents that can be used in combination with the compounds disclosed herein include those in the tables below.

Hepatitis B Therapies

Drug Name	Drug Class	Company
tron A ıterferon alfa-2b)	interferon	Schering-Plough
ɛgasys **'eginterferon alfa-2a)**	interferon	Roche
ɔivir-HBV ımivudine; 3TC)	nucleoside analogue	GlaxoSmithKline
epsera (Adefovir ipivoxil)"	nucleotide analogue	Gilead Sciences
ntriva® (emtricitabine; ΓC)	nucleoside analogue	Gilead Scienceshttp://www.hivandhepatitis.com/advertisement/triangle.html
ıtecavir	nucleoside analogue	Bristol-Myers Squibb
levudine (CLV, L- MAU)	nucleoside analogue	Pharmasset/Bukwang
CH 126, 443 (L-Fd4C)	nucleoside analogue	Achillion Pharmaceuticals
M 365	nucleoside analogue	Amrad
mdoxovir (AMDX, **APD)**	nucleoside analogue	RFS Pharma LLC
dT **(telbivudine)**	nucleoside analogue	Idenix/Novartis

Drug Name	Drug Class	Company
S-1220	nucleoside analogue	Emory University
heradigm	Immune stimulant	Epimmune
idaxin (thymosin)	Immune stimulant	SciClone
HT 899	viral protein	Enzo Biochem
exelvuecitabine/Reverset/D-4FC	nucleoside analogue	Pharmasset
PD	nucleoside analogue	RFS Pharma
BV DNA vaccine	Immune stimulant	PowderJect (UK)
CC 478	nucleoside analogue	Eli Lilly
dLdC (valtorcitabine)	nucleoside analogue	Idenix
CN 2001	nucleoside analogue	ICN
acivir	nucleoside analogue	Pharmasset/Emory University
obustaflavone	nucleoside analogue	Advanced Life Sciences

Drug Name	Drug Class	Company
M-019c		Emory University
enciclovir	nucleoside analogue	Novartis
amciclovir	nucleoside analogue	Novartis
XG	nucleoside analogue	RFS Pharma, LLC
a-AMP prodrugs		
BV/MF59		
DP-P-acyclovir	nucleoside analogue	
ammerhead ribozymes		
lycosidase Inhibitors		
egylated Interferon		
uman Monoclonal ntibodies		

HIV Therapies: Protease Inhibitors (PIs)

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Invirase®	saquinavir (Hard	SQV (HGC)	Ro-31-8959	Hoffmann-La Roche

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
	Gel Cap)			
Fortovase®	saquinavir (Soft Gel Cap)	SQV (SGC)		Hoffmann-La Roche
Norvir®	ritonavir	RTV	ABT-538	Abbott Laboratories
Crixivan®	indinavir	IDV	MK-639	Merck & Co.
Viracept®	nelfinavir	NFV	AG-1343	Pfizer
Agenerase®	amprenavir	APV	141W94 or VX-478	GlaxoSmithKline
Kaletra®	lopinavir + ritonavir	LPV	ABT-378/r	Abbott Laboratories
Lexiva®	fosamprenavir		GW-433908 or VX-175	GlaxoSmithKline
Aptivus®	tripanavir	TPV	PNU-140690	Boehringer Ingelheim
Reyataz®	atazanavir		BMS-232632	Bristol-Myers Squibb
	brecanavir		GW640385	GlaxoSmithKline
Prezista™	darunavir		TMC114	Tibotec

HIV Therapies: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company

Brand Name	**Generic Name**	**Abbreviation**	**Experimental Code**	**Pharmaceutical Company**
Retrovir®	zidovudine	AZT or ZDV		GlaxoSmithKline
Epivir®	lamivudine	3TC		GlaxoSmithKline
Combivir®	zidovudine + lamivudine	AZT + 3TC		GlaxoSmithKline
Trizivir®	abacavir + zidovudine + lamivudine	ABC + AZT + 3TC		GlaxoSmithKline
Ziagen®	abacavir	ABC	1592U89	GlaxoSmithKline
Epzicom™	abacavir + lamivudine	ABC + 3TC		GlaxoSmithKline
Hivid®	zalcitabine	ddC		Hoffmann-La Roche
Videx®	didanosine: buffered versions	ddI	BMY-40900	Bristol-Myers Squibb
Entecavir	baraclude			Bristol-Myers Squibb
Videx® EC	didanosine: delayed-release capsules	ddI		Bristol-Myers Squibb
Zerit®	stavudine	d4T	BMY-27857	Bristol-Myers Squibb

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Viread™	tenofovir disoproxil fumarate (DF)	TDF or Bis(POC) PMPA		Gilead Sciences
Emtriva®	emtricitabine	FTC		Gilead Sciences
Truvada®	Viread + Emtriva	TDF + FTC		Gilead Sciences
Atripla™		TDF + FTC + Sustiva®		Gilead/BMS/Merck
	amdoxovir	DAPD, AMDX		RFS Pharma LLC
apricitabine	AVX754		SPD 754	Avexa Ltd
	Alovudine	FLT	MIV-310	Boehringer
	Elvucitabine	L-FD4C	ACH-126443,	Achillion
	KP-1461		SN1461, SN1212	Koronis
	Racivir	RCV		Pharmasset
Dexelvucitabine	Reverset	D-D4FC	DPC 817	Pharmasset/Emory
			GS9148 and prodrugs thereof	Gilead Sciences

HIV Therapies: Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Viramune®	nevirapine	NVP	BI-RG-587	Boehringer Ingelheim
Rescriptor®	delavirdine	DLV	U-90152S/T	Pfizer
Sustiva®	efavirenz	EFV	DMP-266	Bristol-Myers Squibb
	(+)-calanolide A			Sarawak Medichem
	capravirine	CPV	AG-1549 or S-1153	Pfizer
			DPC-083	Bristol-Myers Squibb
			TMC-125	Tibotec-Virco Group
			TMC-278	Tibotec-Virco Group
			IDX12899	Idenix
			IDX12989	Idenix

HIV Therapies: Other Classes of Drugs

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Viread™	tenofovir disoproxil fumarate (DF)	TDF or Bis(POC) PMPA		Gilead Sciences

Cellular Inhibitors

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Droxia®	hydroxyurea	HU		Bristol-Myers Squibb

Entry Inhibitors (including Fusion Inhibitors)

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Fuzeon™	enfuvirtide		T-20	Trimeris
			T-1249	Trimeris
			AMD-3100	AnorMED, Inc.
	CD4-IgG2		PRO-542	Progenics Pharmaceuticals
			BMS-488043	Bristol-Myers Squibb
	aplaviroc		GSK-873,140	GlaxoSmithKline
	Peptide T			Advanced Immuni T, Inc.
			TNX-355	Tanox, Inc.
	maraviroc		UK-427,857	Pfizer
CXCR4 Inhibitor				
	AMD070		AMD11070	AnorMED, Inc.
CCR5 antagonist				

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
vicriroc		SCH-D	SCH-417690	Schering-Plough

HIV Therapies: Immune-Based Therapies

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Proleukin®	aldesleukin, or Interleukin-2	IL-2		Chiron Corporation
Remune®	HIV-1 Immunogen, or Salk vaccine		AG1661	The Immune Response Corporation
			HE2000	HollisEden Pharmaceuticals

Table of anti-Hepatitis C Compounds in Current Clinical Development

Drug Name	**Drug Category**	**Pharmaceutical Company**
PEGASYS pegylated interferon alfa–2a	Long acting interferon	Roche
INFERGEN interferon alfacon-1	Interferon, Long acting interferon	InterMune
OMNIFERON natural interferon	Interferon, Long acting interferon	Viragen
ALBUFERON	Longer acting interferon	Human Genome Sciences
REBIF interferon beta-1a	Interferon	Ares-Serono
Omega Interferon	Interferon	BioMedicine
Oral Interferon alpha	Oral Interferon	Amarillo Biosciences
Interferon gamma-1b	Anti-fibrotic	InterMune
IP-501	Anti-fibrotic	Interneuron
Merimebodib VX-497	IMPDH inhibitor (inosine monophosphate dehydrogenase)	Vertex
AMANTADINE (Symmetrel)	Broad Antiviral Agent	Endo Labs Solvay
IDN-6556	Apotosis regulation	Idun Pharma.

XTL-002	Monclonal Antibody	XTL
HCV/MF59	Vaccine	Chiron
CIVACIR	Polyclonal Antibody	NABI
	Therapeutic vaccine	Innogenetics
VIRAMIDINE	Nucleoside Analogue	ICN
ZADAXIN (thymosin alfa-1)	Immunomodulator	Sci Clone
CEPLENE histamine dihydrochloride	Immunomodulator	Maxim
VX 950 / LY 570310	Protease Inhibitor	Vertex/ Eli Lilly
ISIS 14803	Antisense	Isis Pharmaceutical/ Elan
IDN-6556	Caspase inhibitor	Idun Pharmaceuticals, Inc. http://www.idun.com
JTK 003	Polymerase Inhibitor	AKROS Pharma
Tarvacin	Anti-Phospholipid Therapy	Peregrine
HCV-796	Polymerase Inhibitor	ViroPharma /Wye
CH-6	Serine Protease	Schering
ANA971	Isatoribine	ANADYS
ANA245	Isatoribine	ANADYS

CPG 10101 (Actilon)	Immunomodulator	Coley
Rituximab (Rituxam)	Anti-CD20 Monoclonal Antibody	Genetech/IDEC
NM283 (Valopicitabine)	Polymerase Inhibitor	Idenix Pharmaceuticals
HepX™-C	Monclonal Antibody	XTL
IC41	Therapeutic Vaccine	Intercell
Medusa Interferon	Longer acting interferon	Flamel Technologies
E-1	Therapeutic Vaccine	Innogenetics
Multiferon	Long Acting Interferon	Viragen
BILN 2061	Serine Protease	Boehringer - Ingelheim
Interferon beta-1a (REBIF)	Interferon	Ares-Serono

VII. Combination Therapy for the Treatment of Proliferative Conditions

In another embodiment, the compounds, when used as an antiproliferative, can be administered in combination with another compound that increases the effectiveness of the therapy, including but not limited to an antifolate, a 5-fluoropyrimidine (including 5-fluorouracil), a cytidine analogue such as β-L-1,3-dioxolanyl cytidine or β-L-1,3-dioxolanyl 5-fluorocytidine, antimetabolites (including purine antimetabolites, cytarabine, fudarabine, floxuridine, 6-mercaptopurine, methotrexate, and 6-thioguanine), hydroxyurea, mitotic inhibitors (including CPT-11, Etoposide (VP-21), taxol, and vinca alkaloids such as vincristine and vinblastine, an alkylating agent (including but not limited to busulfan, chlorambucil, cyclophosphamide, ifofamide, mechlorethamine, melphalan, and thiotepa),

nonclassical alkylating agents, platinum containing compounds, bleomycin, an anti-tumor antibiotic, an anthracycline such as doxorubicin and dannomycin, an anthracenedione, topoisomerase II inhibitors, hormonal agents (including but not limited to corticosteroids (dexamethasone, prednisone, and methylprednisone), androgens such as fluoxymesterone and methyltestosterone, estrogens such as diethylstilbesterol, antiestrogens such as tamoxifen, LHRH analogues such as leuprolide, antiandrogens such as flutamide, aminoglutethimide, megestrol acetate, and medroxyprogesterone), asparaginase, carmustine, lomustine, hexamethyl-melamine, dacarbazine, mitotane, streptozocin, cisplatin, carboplatin, levamasole, and leucovorin. The compounds of the present invention can also be used in combination with enzyme therapy agents and immune system modulators such as an interferon, interleukin, tumor necrosis factor, macrophage colony-stimulating factor and colony stimulating factor. In one embodiment, the compounds described herein can be employed together with at least one other antiviral agent chosen from reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, entry inhibitors and polymerase inhibitors.

In addition, compounds according to the present invention can be administered in combination or alternation with one or more anti-retrovirus, anti-HBV, interferon, anti-cancer or antibacterial agents, including but not limited to other compounds of the present invention. Certain compounds described herein may be effective for enhancing the biological activity of certain agents according to the present invention by reducing the metabolism, catabolism or inactivation of other compounds, and as such, are co-administered for this intended effect.

VIII. Combination Therapy for Treating Noroviral Infections

In addition to the antiviral compounds described herein, other compounds can also be present. For example, type I interferon (IFN) is known to inhibit Norovirus replication. Certain vitamins, particularly vitamin C, are believed to be effective at treating certain viral infections. One study has shown that Vitamin A supplementation reduced the prevalence of Norovirus GII infections, increased the length of both Norovirus GI and GII shedding, and decreased the prevalence of NoV-associated diarrhea (1: J Infect Dis. 2007 Oct 1;196(7):978-85. Epub 2007 Aug 22). Lysine is known as an antiviral agent. It is also known that virus-like particles (VLPs) derived

from genogroup II (GII) Norovirus were bound to cell surface heparan sulfate proteoglycan and other negatively charged glycosaminoglycans. To treat the symptoms of infection, one can also administer an anti-emetic, an anti-diarrheal agent, and/or an analgesic.

VIII. Pharmaceutical Compositions

Hosts, including but not limited to humans, infected with a human immunodeficiency virus, a hepatitis B virus, Flaviviridae family of viruses or Caliciviridae virus or a gene fragment thereof, or cancer can be treated by administering to the patient an effective amount of the active compound or a pharmaceutically acceptable prodrug or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

A preferred dose of the compound for will be in the range of between about 0.1 and about 100 mg/kg, more generally, between about 1 and 50 mg/kg, and, preferably, between about 1 and about 20 mg/kg, of body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable salts and prodrugs can be calculated based on the weight of the parent nucleoside to be delivered. If the salt or prodrug exhibits activity in itself, the effective dosage can be estimated as above using the weight of the salt or prodrug, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. An oral dosage of 50-1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound from about 0.2 to 70 μM, preferably about 1.0 to 15 μM. This can be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

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

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

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

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

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

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including but not limited to implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid. For example, enterically coated compounds can be used to protect cleavage by stomach acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Suitable materials can also be obtained commercially.

Liposomal suspensions (including but not limited to liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in US Pat. No. 4,522,811 (incorporated by reference). For example, liposome formulations can be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on

the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

The terms used in describing the invention are commonly used and known to those skilled in the art. As used herein, the following abbreviations have the indicated meanings:

aq aqueous

CDI carbonyldiimidazole

DMF *N*,*N*-dimethylformamide

DMSO dimethylsulfoxide

EDC 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride

EtOAc ethyl acetate

h hour/hours

HOBt *N*-hydroxybenzotriazole

M molar

min minute

rt or RT room temperature

TBAT tetrabutylammonium triphenyldifluorosilicate

TBTU *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate

THF tetrahydrofuran

IX. General Schemes for Preparing Active Compounds

Methods for the facile preparation of N^4-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs are also provided. N^4-hydroxycytidine nucleosides derivatives, modified monophosphate and

phosphonates prodrugs analogs disclosed herein can be prepared as described in detail below, or by other methods known to those skilled in the art. It will be understood by one of ordinary skill in the art that these schemes are in no way limiting and that variations of detail can be made without departing from the spirit and scope of the present invention.

Generally, the nucleotides of formula **III, IV, VI, XI-XIV, XIX-XXVI** are prepared by first preparing the corresponding nucleoside, then capping the 5'-hydroxy group as a monophosphate or other analog as described herein that can be readily converted *in vivo* to an active triphosphate form of the compound.

The various reaction schemes are summarized below.

Scheme 1 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to monophosphate prodrugs **XX**, **XXI**, **XXII**.

Scheme 2 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, an alternate synthetic approach to monophosphate prodrugs **XX**, **XXI**, **XXII**.

Scheme 3 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to monophosphate prodrug **XXIII**.

Scheme 4 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to monophosphate prodrug **XXIV**.

Scheme 5 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to monophosphate prodrug **XXV**.

Scheme 6 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, an alternate synthetic approach to monophosphate prodrug **XXV**.

Scheme 7 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to monophosphate prodrug **XXVI**.

Scheme 8 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, an alternate synthetic approach to monophosphate prodrug **XXVI**.

Scheme 9 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to nucleosides **27**.

Scheme 10 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, an alternate synthetic approach to nucleosides **27**.

Scheme 11 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, a synthetic approach to nucleosides **29** and **30**.

Scheme 12 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, an alternate synthetic approach to nucleosides **30**.

Scheme 13 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to monophosphate prodrug **35**.

Scheme 14 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to N^4-hydroxycytidine 2'-*C*-Me nucleoside **37**.

Scheme 15 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to N^4-hydroxycytidine 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me nucleoside **39**.

Scheme 16 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to N^4-(Octanoyloxy)cytidine 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me nucleoside **40**.

Scheme 17 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to N^4-hydroxycytidine 2'-*C*-Me nucleoside prodrug **44**.

Scheme 18 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to N^4-hydroxycytidine 2'-*C*-Me nucleoside prodrug **48**.

Scheme 19 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to N^4-hydroxycytidine 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me nucleoside prodrug **51**.

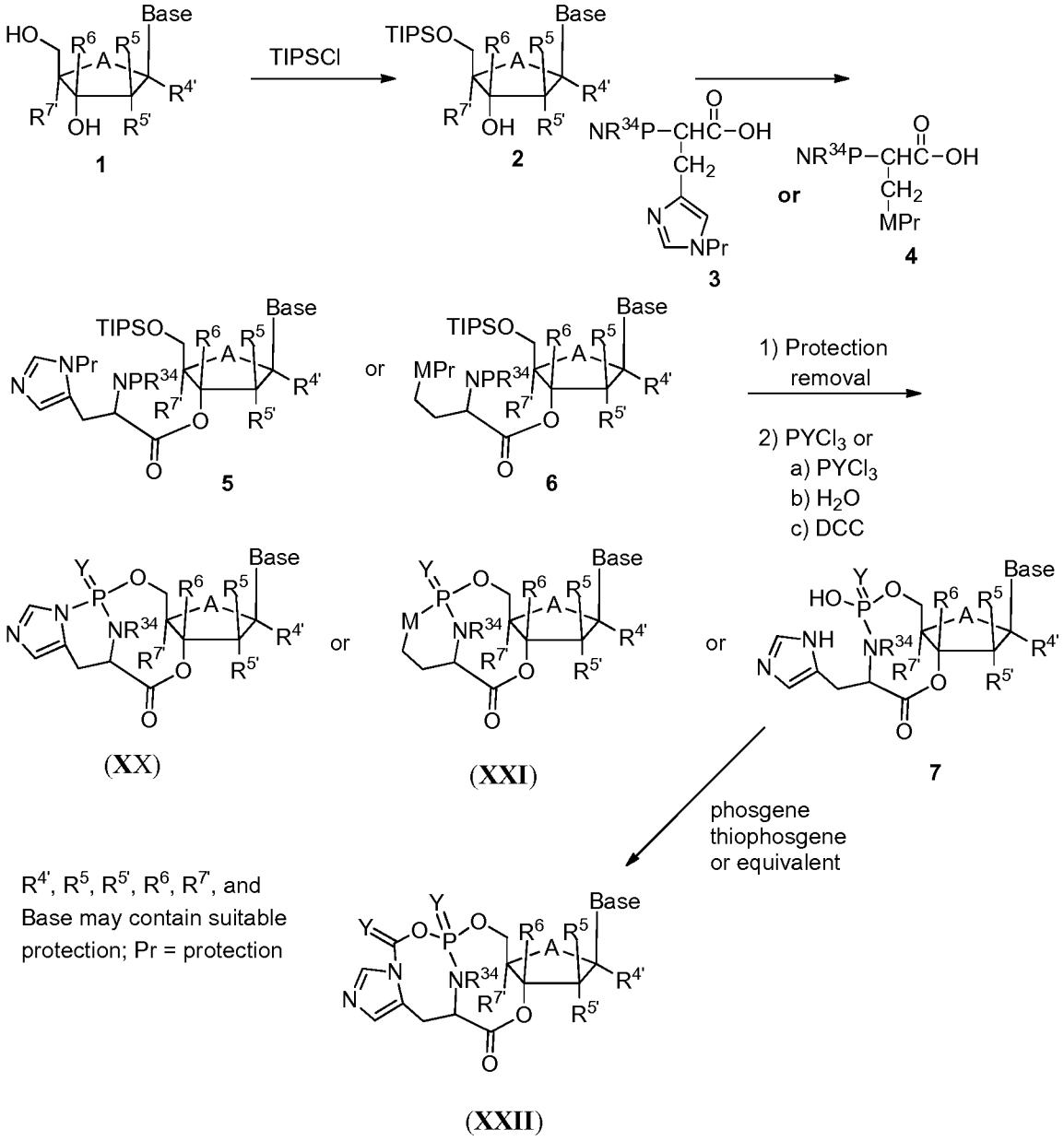
Scheme 20 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to active compounds of the present invention, and, in particular, a synthetic approach to N^4-hydroxycytidine 2'-*C*-Me nucleoside prodrugs **54** and **56**.

Scheme 21 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to monophosphate prodrug **35**.

Scheme 22 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to monophosphate prodrug **35**.

In one embodiment, nucleosides of formulas **XX**, **XXI** or **XXII** are prepared by protection of compound **1** by a group such as TIPS to provide **2** bearing a free alpha-hydroxyl group at the 3'-position of the sugar (Scheme 1). Preparation of compound **1** is accomplished by one of ordinary skill in the art, by methods outlined in: (a) Rajagopalan, P.; Boudinot, F. D; Chu, C. K.; Tennant, B. C.; Baldwin, B. H.; Antiviral Nucleosides: Chiral Synthesis and Chemotheraphy: Chu, C. K.; Eds. Elsevier: **2003**. b) Recent Advances in Nucleosides: Chemistry and Chemotherapy: Chu, C. K.; Eds. Elsevier: **2002**. c) Frontiers in Nucleosides & Nucleic Acids, **2004**, Eds. R. F. Schinazi & D. C. Liotta, IHL Press, Tucker, GA, USA, pp: 319-37 d) Handbook of Nucleoside Synthesis: Vorbruggen H. & Ruh-Pohlenz C. John Wiley & sons **2001**), and by general Schemes 9-10. Coupling of **2** with acids **3** or **4** can be

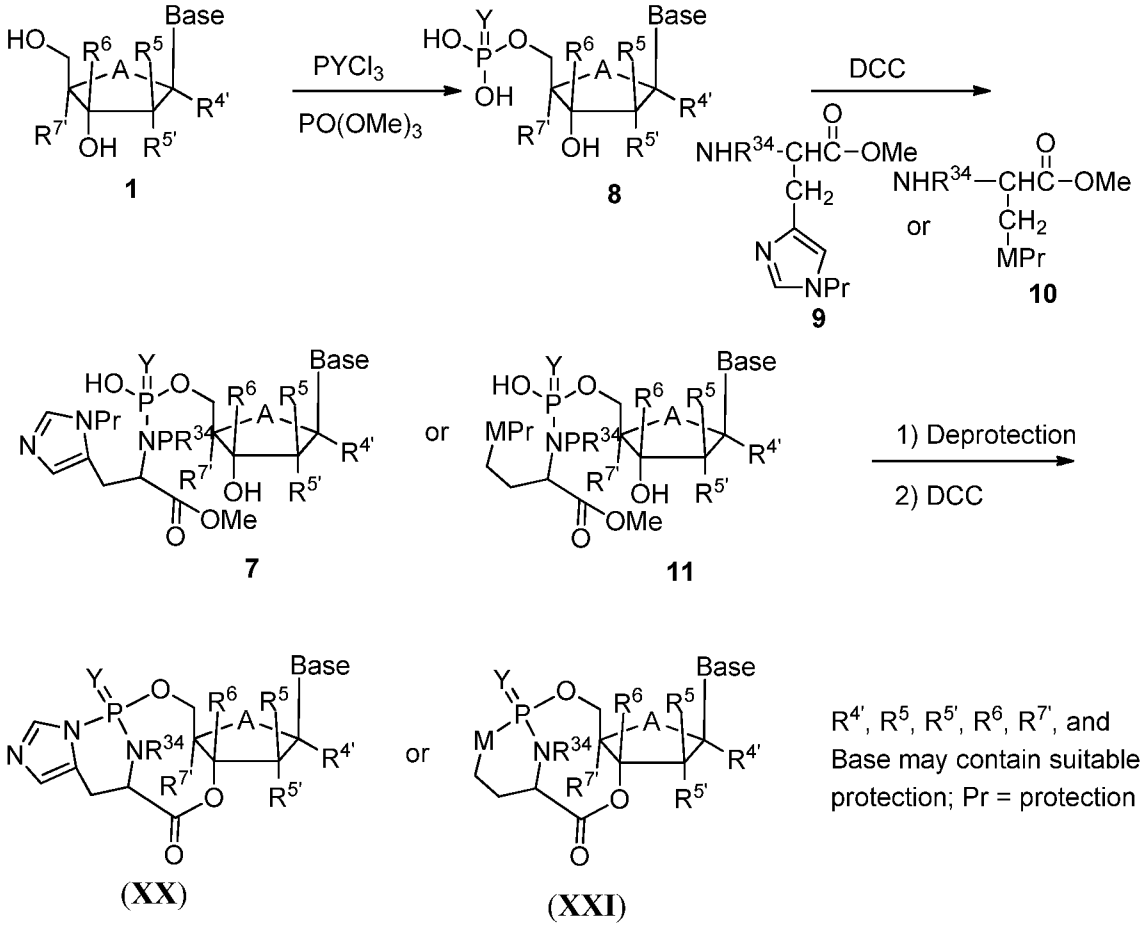
accomplished by agents such as EDC, EDC/HOBt, TBTU, or CDI to give esters **5** or **6**. After removal of protecting groups the resulting amino alcohols can be converted to the monophosphate prodrugs **XX** or **XXI** by exposure to phosphorous oxychloride or phosphorothioyl trichloride ($POCl_3$ or $PSCl_3$) or alternatively after water workup of the phosphorous oxychloride or phosphorothioyl trichloride reaction, a coupling agent such as DCC can be utilized in the formation of **XX** or **XXI**. Compound **7** can be obtained after water workup of the phosphorous oxychloride or phosphorothioyl trichloride reaction and subsequent exposure to phosgene or a phosgene equivalent such as CDI or triphosgene gives monophosphate prodrug **XXII**.



Scheme 1 A synthetic approach to monophosphate prodrugs **XX**, **XXI**, **XXII**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, M, R^{34}, and $R^{7'}$ are as defined in active compound section)

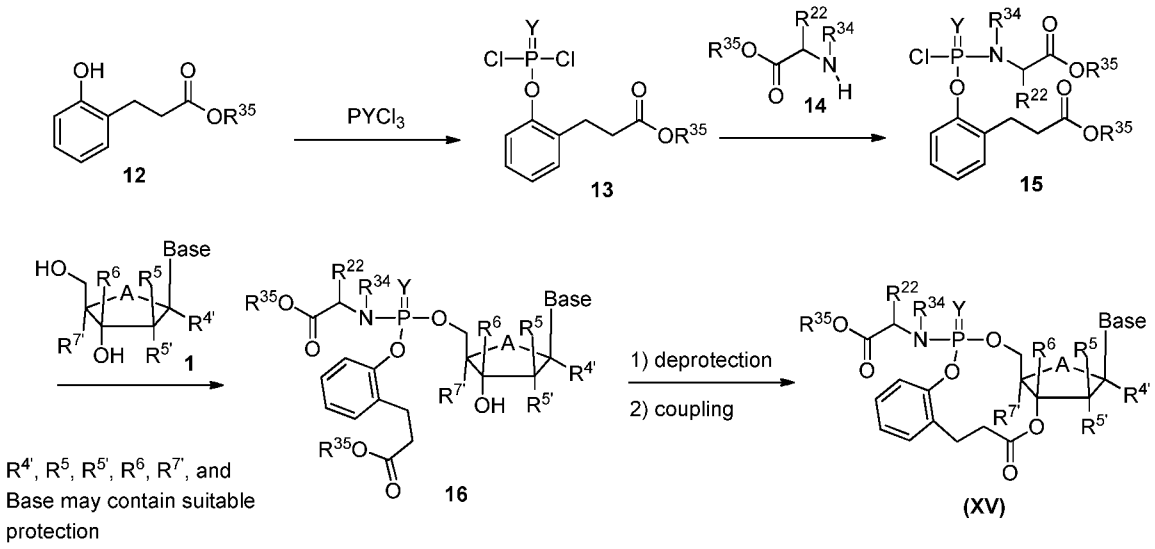
Alternatively, monophosphate prodrugs **XX**, **XXI**, **XXII** can be synthesized as outlined in Scheme 2, namely nucleoside **1** can be converted to the monophosphate, **8** directly by the action of phosphorous oxychloride or phosphorothioyl trichloride in trimethyl phosphate. Coupling to the amino esters **9** or **10** can be accomplished with standard coupling agents such as DCC to give phosphoramidates **7** and **11**. Deprotection and subsequent coupling of **7** or **11** with agents such as EDC,

EDC/HOBt, TBTU, or CDI provides monophosphate prodrugs **XX** and **XXI**. Monophosphate prodrug **XXII** can be obtained from **7** as described in Scheme 1.



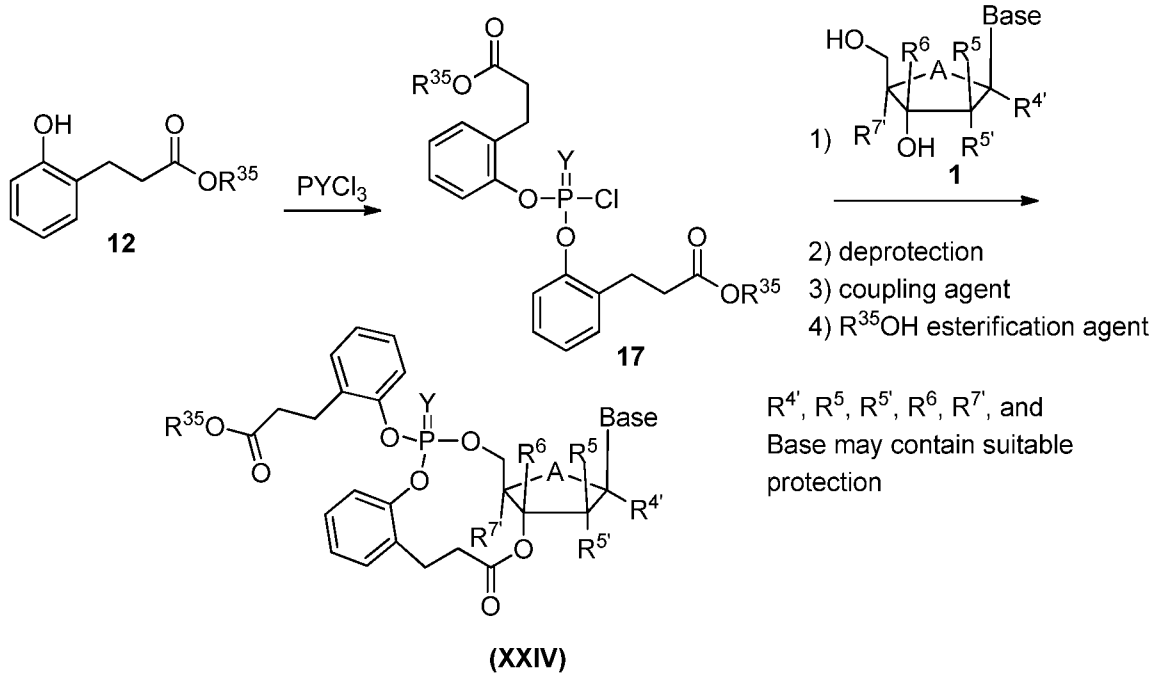
Scheme 2 An alternate synthetic approach to monophosphate prodrugs **XX**, **XXI**, **XXII**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, M, R^{34}, and $R^{7'}$ are as defined in active compound section)

Monophosphate prodrug **XXIII** can be prepared as outlined in Scheme 3 starting from phenol **12** (Scheme 3). Exposure of **12** to phosphorous oxychloride or phosphorothioyl trichloride provides **13**, which is subsequently allowed to react with an amino ester **14** to give phosphoramidate **15**. Nucleoside **1** can next be converted to monophosphate analog **16** by reaction of the 5'-hydroxyl group with the chlorophosphorylamino propanoate, **15**. Deprotection and subsequent coupling of **16** with agents such as EDC, EDC/HOBt, TBTU, or CDI provides monophosphate prodrugs **XXIII**.



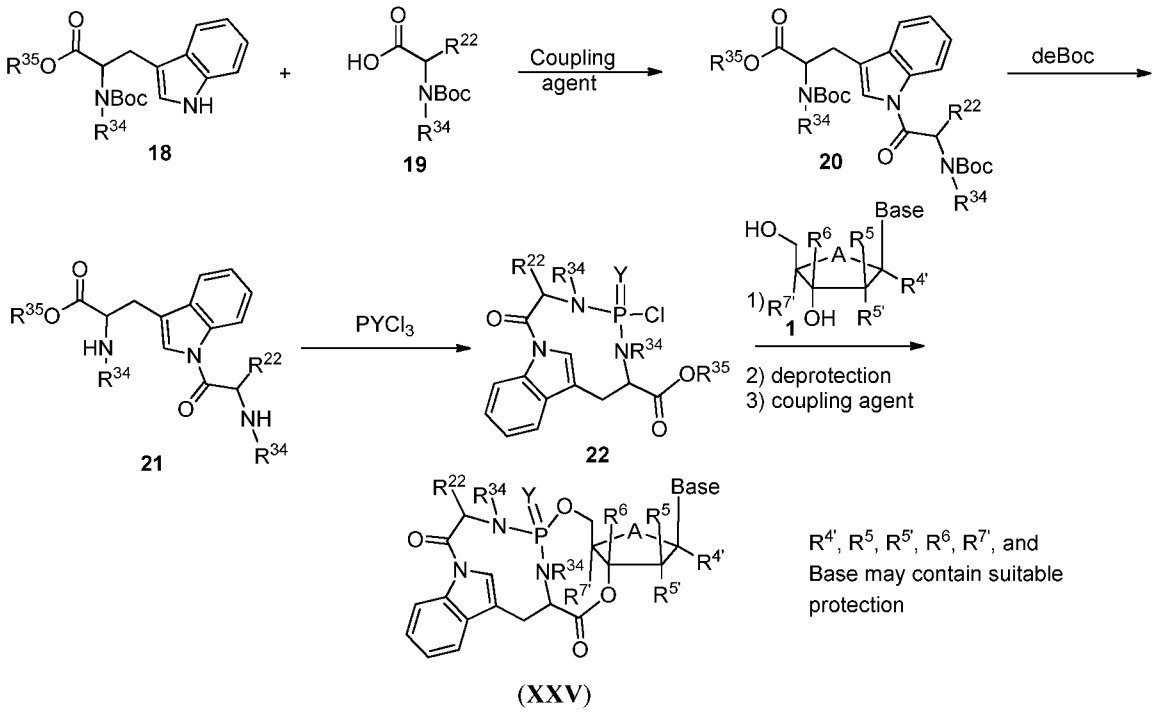
Scheme 3 A synthetic approach to monophosphate prodrug **XXIII**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, R^{34} R^{35}, R^{22}, and $R^{7'}$ are as defined in active compound section)

Monophosphate prodrug **XXIV** can be prepared by reaction of phenol **12** with phosphorous oxychloride or phosphorothioyl trichloride to provide diphenyl phosphorochloridate, **17** (Scheme 4). Nucleoside **1** can next be converted to an intermediate monophosphate analog by reaction of the 5'-hydroxyl group with the diphenyl phosphorochloridate, **17**. Deprotection and subsequent ester formation with the 3'-hydroxyl group with agents such as EDC, EDC/HOBt, TBTU, or CDI followed by reesterification with $R^{35}OH$ provides monophosphate prodrugs **XXIV**.



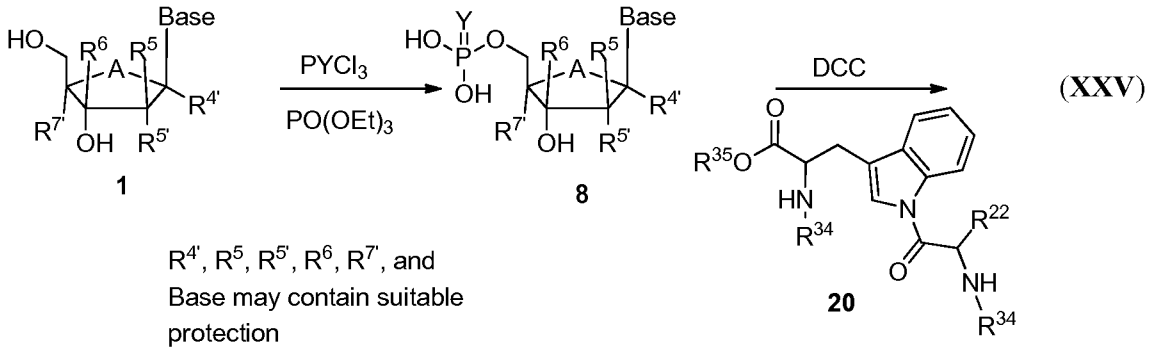
Scheme 4 A synthetic approach to monophosphate prodrug **XXIV**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, R^{35}, and $R^{7'}$ are as defined in active compound section)

Monophosphate prodrug **XXV** can be prepared by initial reaction of protected tryptophan **18** with protected amino acid **19** with coupling agents such as EDC, EDC/HOBt, TBTU, or CDI to give dipeptide **20** (Scheme 5). Removal of the amine protections gives then diamine **21** which can then be reacted with phosphorous oxychloride or phosphorothioyl trichloride to give the cyclic phosphorodiamidic chloride, **22**. Nucleoside **1** can next be converted to a monophosphate analog by reaction of the 5'-hydroxyl group with the cyclic phosphorodiamidic chloride, **22**. Deprotection and subsequent coupling of **22** with agents such as EDC, EDC/HOBt, TBTU, or CDI provides monophosphate prodrugs **XXV**.



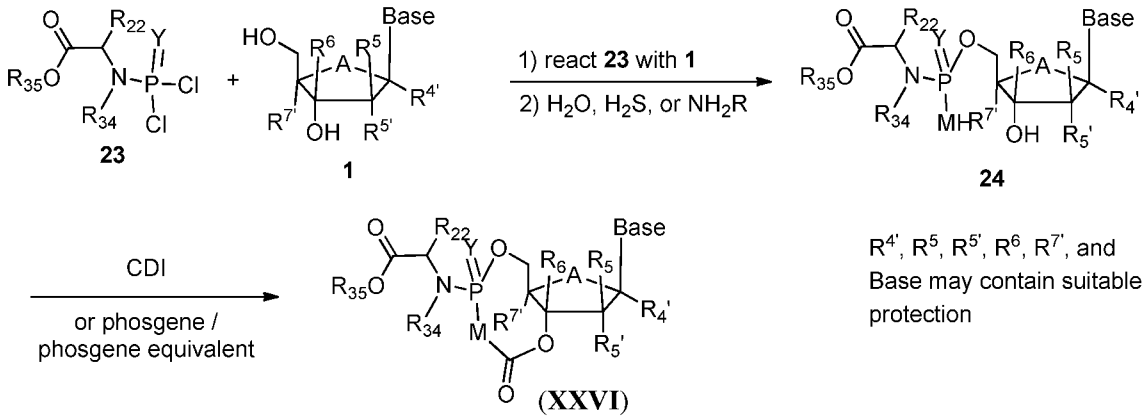
Scheme 5 A synthetic approach to monophosphate prodrug **XXV**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, R^{34}, R^{35}, R^{22}, and $R^{7'}$ are as defined in active compound section)

Alternatively, monophosphate prodrug **XXV** can be prepared from monophosphate analog **8** followed by coupling with dipeptide **20** (Scheme 6).



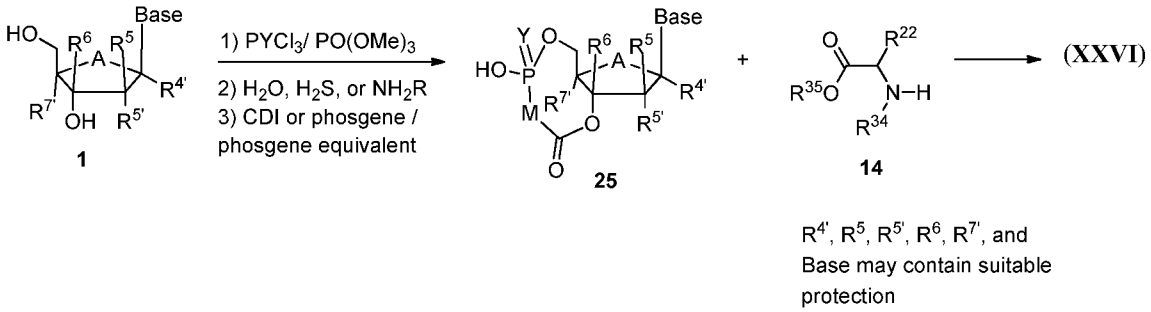
Scheme 6 An alternate synthetic approach to monophosphate prodrug **XXV**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, R^{34}, R^{35}, R^{22}, and $R^{7'}$ are as defined in active compound section)

Monophosphate prodrug **XXVI** can be prepared by initial reaction of phosphoramidic dichloride **23** with nucleoside **1** (Scheme 7). Subsequent reaction of the produced intermediate with water, hydrogen sulfide, or an amine provides monophosphate analog **24** (Scheme 7). Exposure of the bis nucleophile **24** to phosgene or a phosgene equivalent such as CDI provides monophosphate prodrugs **XXVI**.



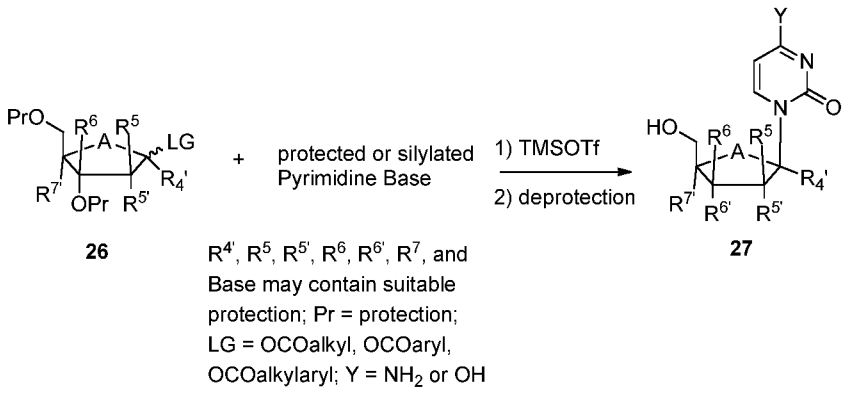
Scheme 7 A synthetic approach to monophosphate prodrug **XXVI**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, M, R^{34}, R^{35}, R^{22}, and $R^{7'}$ are as defined in active compound section)

Alternatively, monophosphate prodrug **XXVI** (where M is not NR) can be prepared by initial reaction of nucleoside **1** with phosphorous oxychloride or phosphorothioyl trichloride as shown in Scheme 8. Subsequent reaction of the produced intermediate with water or hydrogen sulfide followed by reaction with phosgene or a phosgene equivalent such as CDI provides monophosphate prodrugs **XXVI**. (Scheme 8).



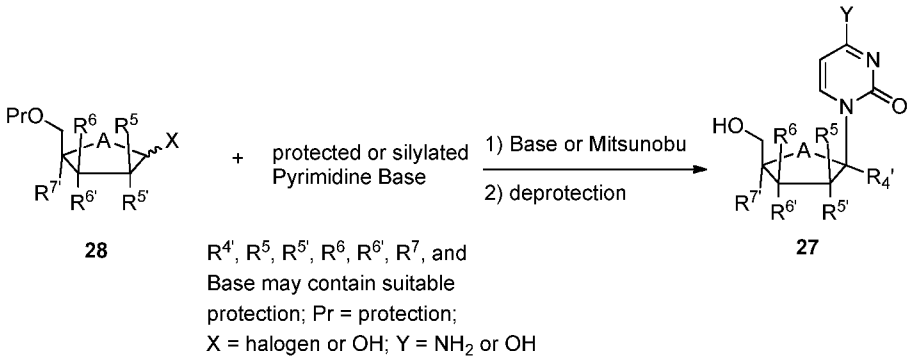
Scheme 8 An alternate synthetic approach to monophosphate prodrug **XVIII**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5, $R^{5'}$, R^6, Y, R^{34}, R^{35}, R^{22}, and $R^{7'}$ are as defined in active compound section)

Nucleoside **27** can be prepared by coupling sugar **26** with a protected or silylated pyrimidine base in the presence of Lewis acid such as TMSOTf. Deprotection of the 5'- hydroxyl gives nucleoside **27**. (Scheme 9).



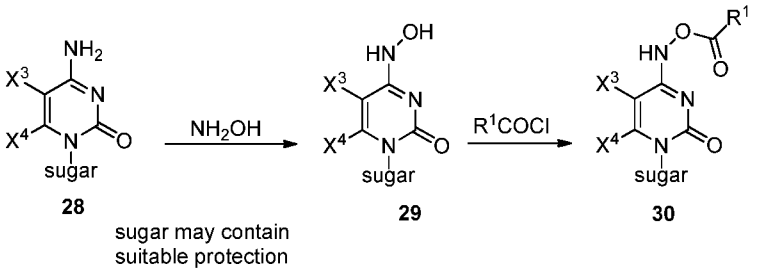
Scheme 9 A synthetic approach to nucleosides **27**. ($R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, and $R^{7'}$ are as defined in active compound section)

Alternatively, nucleoside **27** can be prepared from 1'-halo or 1'- hydroxy compound **28**. For the case of 1'-halo a protected or free pyrimidine base in the presence of a base such as triethyl amine or sodium hydride followed by deprotection would give nucleosides **27**. For the case of 1'-hydroxy a protected or free pyrimidine base in the presence of a Mitsunobu coupling agent such as diisopropyl azodicarboxylate followed by deprotection would give nucleosides **27** (Scheme 10).



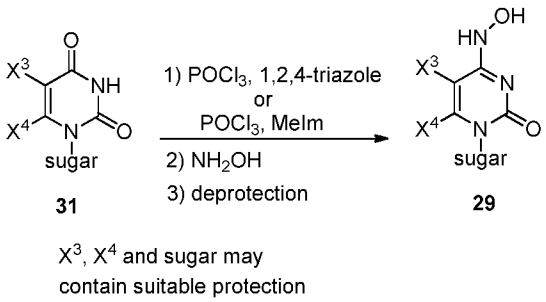
Scheme 10 An alternate synthetic approach to nucleosides **27**. ($R^{4'}$, R^5, $R^{5'}$, R^6, Y, $R^{6'}$ and $R^{7'}$ are as defined in active compound section)

N^4-hydroxycytidine nucleosides **29** can be prepared by reaction of compound **28** with hydroxylamine (Scheme 11). Subsequent reaction with various acid chlorides provides coresponding N^4-acyloxy derivatives **30**.



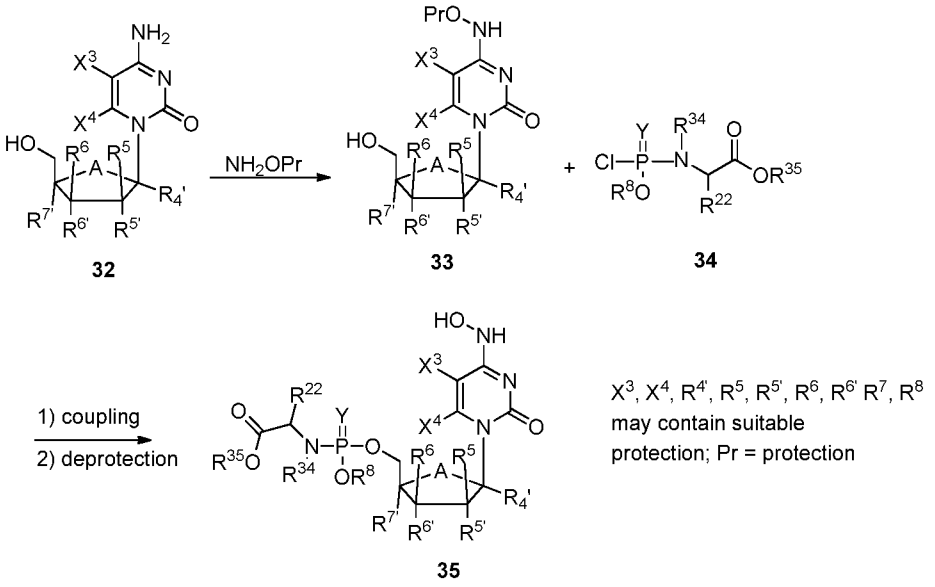
Scheme 11 Synthetic approach to nucleosides **29** and **30**. (X^3, X^4, R^1 and sugar are as defined in active compound section)

Alternatively, nucleoside **29** can be prepared by initial reaction of nucleoside **31** with phosphorous oxychloride and 1,2,4-triazole or methylimidazole as shown in scheme 12. Subsequent reaction of the produced intermediate with hydroxylamine followed by deprotection of the sugar moiety gives nucleoside **29**.



Scheme 12 An alternate synthetic approach to nucleosides **29**. (X^3, X^4, R^1 and sugar are as defined in active compound section)

Monophosphate prodrug **35** can be prepared by initial reaction of an appropriately protected hydroxylamine derivative with nucleoside **32** (Scheme 13). Subsequent reaction of **33** with phosphoramidate chloride **34** followed by necessary deprotection provides monophosphate prodrug **35**.



Scheme 13 Approach to monophosphate prodrug **35**. (X^3, X^4, Y, $R^{4'}$, R^5, $R^{5'}$, R^6, Y, R^{34}, R^{35}, R^{22}, and $R^{7'}$ are as defined in active compound section)

In some cases the phosphorus atom may be chiral herein termed "P*" or "P" which means that and that it has a designation of "R" or "S" corresponding to the accepted meanings of Cahn-Ingold-Prelog rules for such assignment. Prodrugs of Formula A may exist as a mixture of diastereomers due to the chirality at the phosphorus center. When chirality exists at the phosphorous center it may be wholly or partially Rp or Sp or any mixture thereof.

The present invention is further illustrated in the following examples. Schemes 14 - 19 and Examples 1 - 6 show preparative methods for synthesizing N^4-hydroxycytidine nucleosides derivatives and modified monophosphate prodrug analogs, and Examples 7 - 35 show methods for the biological evaluation of the N^4-

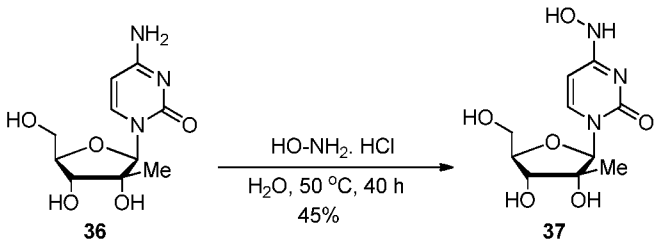
hydroxycytidine nucleosides derivatives and modified monophosphate prodrug analogs. It will be understood by one of ordinary skill in the art that these examples are in no way limiting and that variations of detail can be made without departing from the spirit and scope of the present invention.

Specific Examples

Specific compounds which are representative of this invention were prepared as per the following examples and reaction sequences; the examples and the diagrams depicting the reaction sequences are offered by way of illustration, to aid in the understanding of the invention and should not be construed to limit in any way the invention set forth in the claims which follow thereafter. The present compounds can also be used as intermediates in subsequent examples to produce additional compounds of the present invention. No attempt has necessarily been made to optimize the yields obtained in any of the reactions. One skilled in the art would know how to increase such yields through routine variations in reaction times, temperatures, solvents and/or reagents.

Anhydrous solvents were purchased from Aldrich Chemical Company, Inc. (Milwaukee). Reagents were purchased from commercial sources. Unless noted otherwise, the materials used in the examples were obtained from readily available commercial suppliers or synthesized by standard methods known to one skilled in the art of chemical synthesis. Melting points (mp) were determined on an Electrothermal digit melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken on a Varian Unity Plus 400 spectrometer at room temperature and reported in ppm downfield from internal tetramethylsilane. Deuterium exchange, decoupling experiments or 2D-COSY were performed to confirm proton assignments. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), m (multiplet). All J-values are in Hz. Mass spectra were determined on a Micromass Platform LC spectrometer using electrospray techniques. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Analytic TLC was performed on Whatman LK6F silica gel plates, and preparative TLC on Whatman PK5F silica gel plates. Column chromatography was carried out on Silica Gel or via reverse-phase high performance liquid chromatography.

Example 1

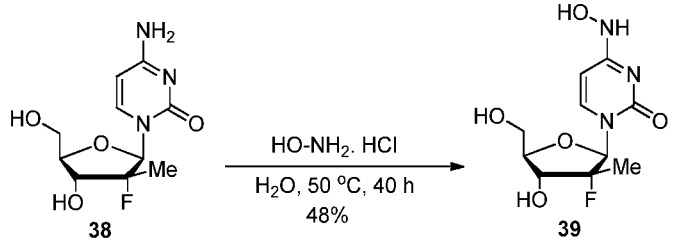


Scheme 14. Synthesis of N^4-hydroxycytidine 2'-*C*-Me nucleoside **37**

1-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-4-(hydroxyamino)pyrimidin-2(1H)-one ***37***

To a solution of **36** (0.175 g, 0.68 mmol) in 2 mL of H_2O was added hydroxylamine hydrochloride (0.24 g, 3.4 mmol). The reaction mixture was stirred at 50 °C and monitored by TLC and/or LC/MS. After 16 h, hydroxylamine hydrochloride (0.24 g, 3.4 mmol) was added and the reaction mixture was stirred 50 °C for an extra 24 h. After complete consumption of the starting material, the aqueous solution was extracted with AcOEt (3 x 5 mL). The combined organic layer were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **37** (0.83 g, 0.30 mmol) in 45% yield. LCMS (ESI) Calcd for $C_{10}H_{15}N_3O_6$ 273.2, observed (M + 1) 274.1.

Example 2

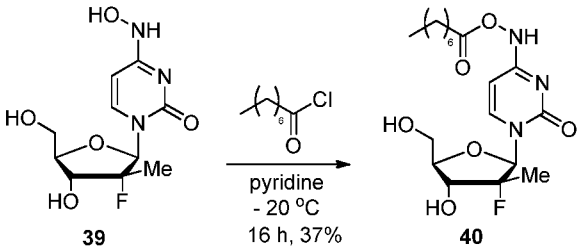


Scheme 15. Synthesis of N^4-hydroxycytidine 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me nucleoside **39**

*1-((2R,3R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-4-(hydroxyamino)pyrimidin-2(1H)-one **39***

To a solution of **38** (1 g, 3.86 mmol) in 10 mL of H_2O was added hydroxylamine hydrochloride (1.34 g, 19 mmol). The reaction mixture was stirred at 50 °C and monitored by TLC and/or LC/MS. After 16 h, hydroxylamine (1.34 g, 19 mmol) was added and the reaction mixture was stirred 50 °C for an extra 24 h. After complete consumption of the starting material, the aqueous solution was extracted with AcOEt (3 x 25 mL). The combined organic layer were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **39** (0.51 g, 1.85 mmol) in 48% yield. LCMS (ESI) Calcd for $C_{10}H_{14}FN_3O_5$ 275.2, observed (M + 1) 274.3

Example 3



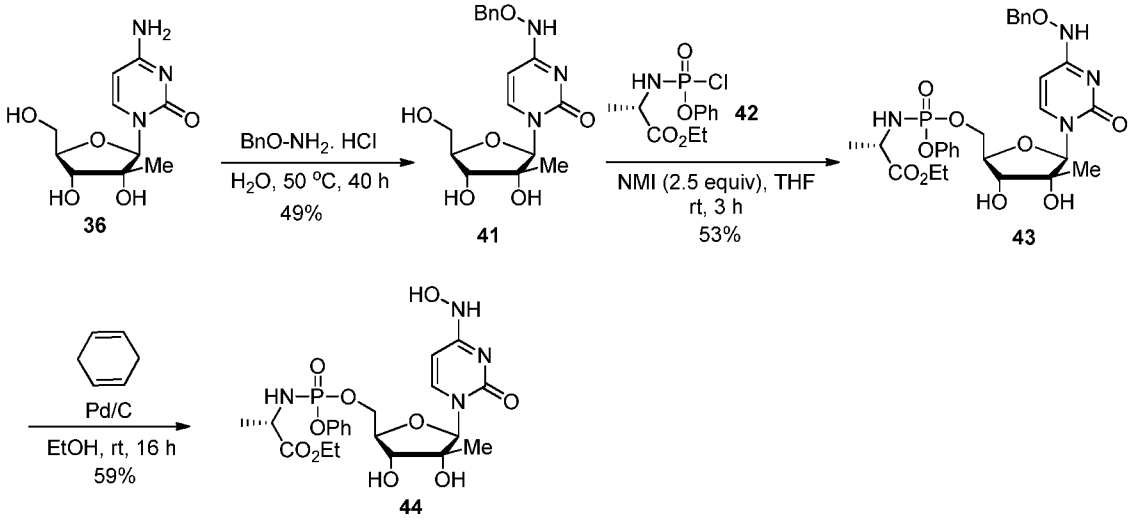
Scheme 16. Synthesis of N^4-(Octanoyloxy)cytidine 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me nucleoside **40**

*1-((2R,3R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-4-((octanoyloxy)amino)pyrimidin-2(1H)-one **40***

To a precooled (-20 °C) solution of **39** (0.06 g, 0.23 mmol) in 2 mL of anhydrous pyridine was added octanoyl chloride (44 µL, 0.26 mmol). After stirring the mixture at 4 °C for 16 h, the reaction was quenched with MeOH (2 mL) and the solution was concentrated under reduced pressure. AcOEt (10 mL) was then added and the mixture was washed with water (3 x 5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified

by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 85:15 v/v) to give **40** (0.04 g, 0.09 mmol) in 37% yield. LCMS (ESI) Calcd for $C_{18}H_{28}FN_3O_6$ 401.4, observed (M + 1) 402.3

Example 4



Scheme 17. Synthesis of N^4-hydroxycytidine 2'-*C*-Me nucleoside prodrug **44**

.4-((benzyloxy)amino)-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidin-2(1H)-one, **41**

To a solution of **36** (0.175 g, 0.68 mmol) in 2 mL of H_2O was added O-benzylhydroxylamine hydrochloride (0.70 g, 4.38 mmol). The reaction mixture was stirred at 50 °C and monitored by TLC and/or LC/MS. After 16 h, O-benzylhydroxylamine hydrochloride (0.30 g, 1.88 mmol) was added and the reaction mixture was stirred 50 °C for an extra 24 h. After complete consumption of the starting material, the aqueous solution was extracted with AcOEt (3 x 5 mL). The combined organic layer were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **41** (0.12 g, 0.33 mmol) in 49% yield. LCMS (ESI) Calcd for $C_{17}H_{21}N_3O_6$ 363.4, observed (M + 1) 364.3

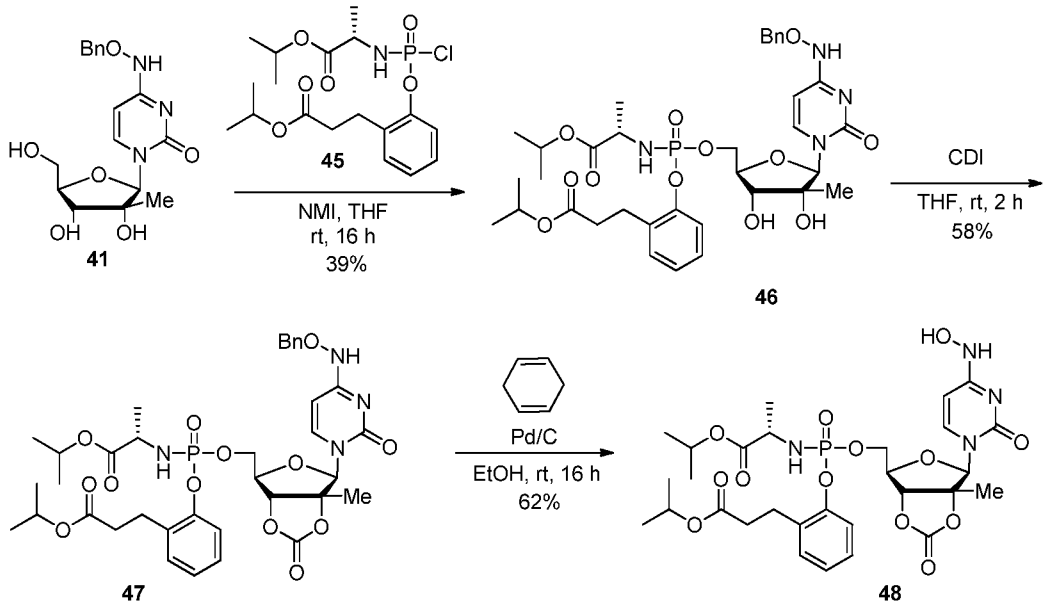
(2S)-ethyl 2-(((((2R,3R,4R,5R)-5-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate, **43**

To a solution of **41** (0.04 g, 0.12 mmol) in 2 mL was added 1-methylimidazole (0.15 mL, 0.3 mmol) and 0.3 mL of a 1M solution of phenyl-(ethoxy-L-alaninyl)-phosphorochloridate **42** in THF, under argon atmosphere. After stirring for 3 h at room temperature, AcOEt (10 mL) was added and the reaction mixture was washed with water (3 x 3 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **43** (0.04 g, 0.06 mmol) in 53% yield. LCMS (ESI) Calcd for $C_{28}H_{35}N_4O_{10}P$ 618.6, observed (M + 1) 619.7

(2S)-ethyl 2-(((((2R,3R,4R,5R)-3,4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1(2H)-yl)-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate, **44**

To a solution of **43** (0.04 g, 0.06 mmol) in 2 mL of EtOH was added 1,4-cyclohexadiene (0.1 mL) and Pd/C (0.01 g, 10% Pd on activated carbon) at rt. After stirring for 16 h at rt, the suspension was filtered on a celite pad and the collected solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 90:10) to give **44** (0.02 g, 0.04 mmol) in 59% yield. LCMS (ESI) Calcd for $C_{21}H_{29}N_4O_{10}P$ 528.4, observed (M + 1) 528.3

Example 5



Scheme 18. Synthesis of N^4-hydroxycytidine 2'-*C*-Me nucleoside prodrug **48**

isopropyl 3-(2-(((((2R,3R,4R,5R)-5-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)propanoate ***46***

To a solution of **41** (0.15 g, 0.41 mmol) in 7 mL of anhydrous THF was added 1-methylimidazole (0.07 mL, 0.83 mmol) and 0.83 mL of a 1M solution of phosphoramidate chloride **45** in THF, under argon atmosphere. After stirring for 16 h at room temperature, AcOEt (20 mL) was added and the reaction mixture was washed with water (3 x 5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **46** (0.12 g, 0.16 mmol) in 39% yield. LCMS (ESI) Calcd for $C_{35}H_{47}N_4O_{12}P$ 746.7, observed (M + 1) 747.5

Isopropyl 3-(2-(((((3aR,4R,6R,6aR)-6-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2H)-yl)-6a-methyl-2-oxotetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)(((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)propanoate ***47***

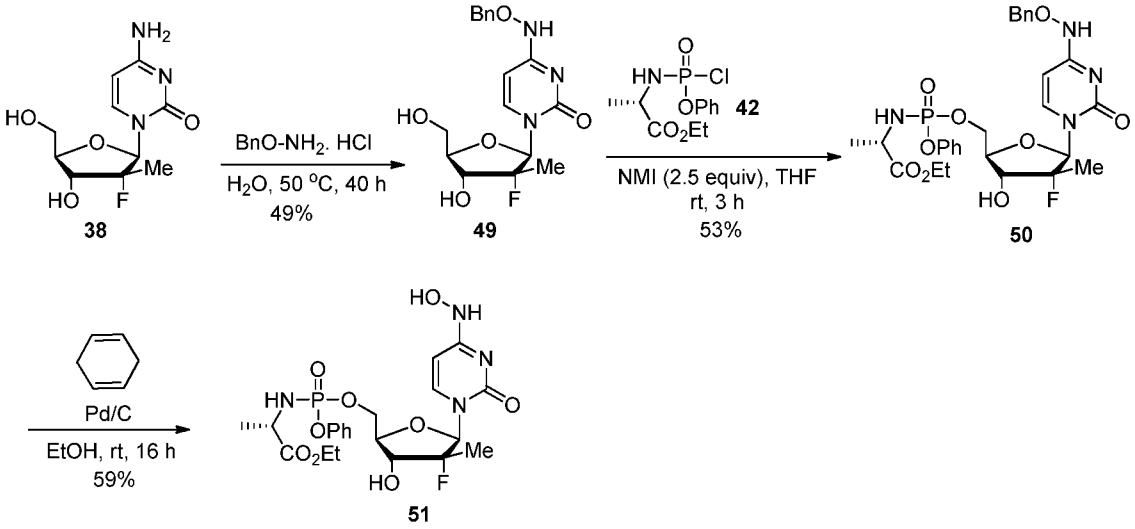
To a solution of **46** (0.04 g, 0.05 mmol) in 0.25 mL of THF was added N,N'-carbonyldiimidazole (0.02 mg, 0.12 mmol) at 0 °C. After stirring for 2 h at rt, the solution was concentrated under reduced pressure. The residue was purified by silica

gel column chromatography (Hexane:EtOAc = 5:5) to give **47** (0.02 g, 0.03 mmol) in 58% yield. LCMS (ESI) Calcd for $C_{36}H_{45}N_4O_{13}P$ 772.7, observed (M + 1) 772.5

*Isopropyl 3-(2-(((((3aR,4R,6R,6aR)-6-(4-(hydroxyamino)-2-oxopyrimidin-1(2H)-yl)-6a-methyl-2-oxotetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)((S)-1-isopropoxy-1-oxopropan-2-yl)amino)phosphoryl)oxy)phenyl)propanoate **48***

To a solution of **47** (0.02 g, 0.06 mmol) in 2 mL of EtOH was added 1,4-cyclohexadiene (0.1 mL) and Pd/C (0.01 g, 10% Pd on activated carbon) at rt. After stirring for 16 h at rt, the suspension was filtered on a celite pad and the collected solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 90:10) to give **48** (0.02 g, 0.04 mmol) in 62% yield. LCMS (ESI) Calcd for $C_{29}H_{39}N_4O_{13}P$ 682.6, observed (M + 1) 683.4

Example 6



Scheme 19. Synthesis of N^4-hydroxycytidine 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me nucleoside prodrug **51**

.

*4-((benzyloxy)amino)-1-((2R,3R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidin-2(1H)-one **49***

To a solution of **38** (0.2 g, 0.77 mmol) in 2 mL of H_2O was added O-benzylhydroxylamine hydrochloride (0.37 g, 2.31 mmol). The reaction mixture was stirred at 50 °C and monitored by TLC and/or LC/MS. After 16 h, O-benzylhydroxylamine hydrochloride (0.37 g, 2.31 mmol) was added and the reaction mixture was stirred 50 °C for an extra 24 h. After complete consumption of the starting material, the aqueous solution was extracted with AcOEt (3 x 10 mL). The combined organic layer were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **49** (0.11 g, 0.30 mmol) in 39% yield. LCMS (ESI) Calcd for $C_{17}H_{20}N_3O_5F$ 365.4, observed (M + 1) 366.3

*(2S)-ethyl 2-(((((2R,3R,4R,5R)-5-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate **50***

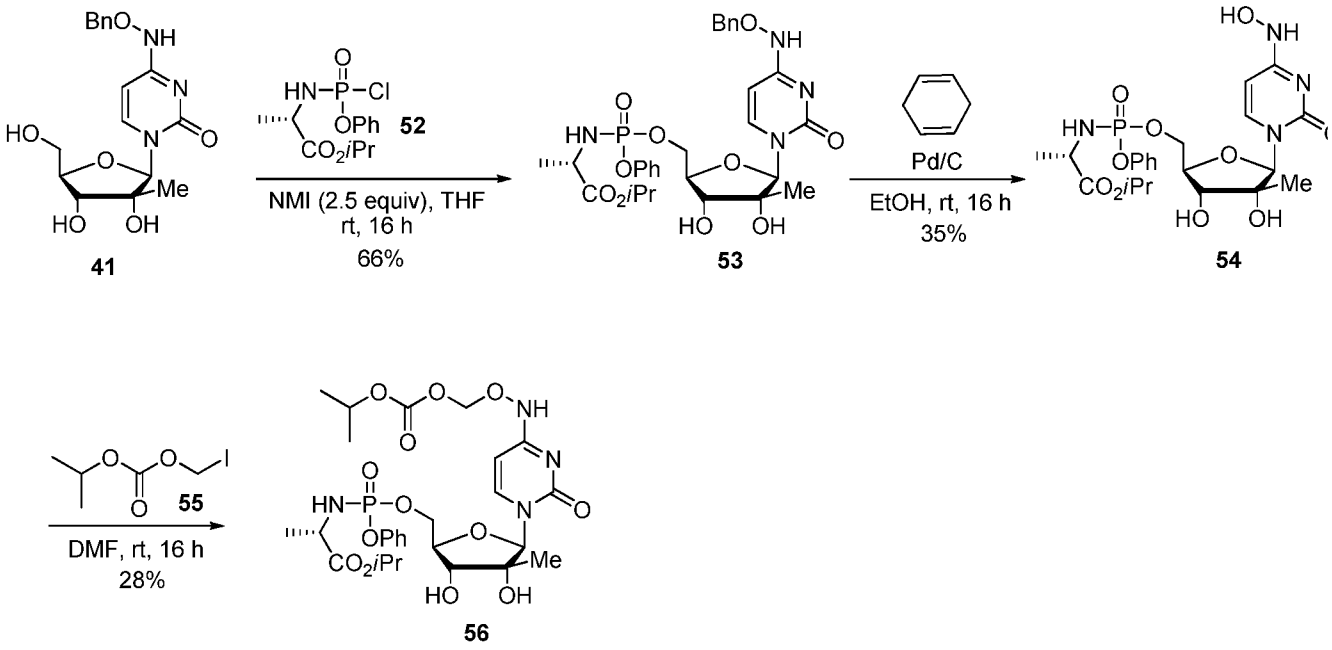
To a solution of **49** (0.15 g, 0.41 mmol) in 3 mL was added 1-methylimidazole (0.10 mL, 1.23 mmol) and 1.23 mL of a 1M solution of phenyl-(ethoxy-L-alaninyl)-phosphorochloridate **42** in THF, under argon atmosphere. After stirring for 16 h at room temperature, AcOEt (10 mL) was added and the reaction mixture was washed with water (3 x 3 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **50** (0.03 g, 0.05 mmol) in 13% yield. LCMS (ESI) Calcd for $C_{28}H_{34}N_4O_9PF$ 620.6, observed (M + 1) 621.3

*(2S)-ethyl 2-(((((2R,3R,4R,5R)-4-fluoro-3-hydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1(2H)-yl)-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate **51***

To a solution of **50** (0.03 g, 0.06 mmol) in 2 mL of EtOH was added 1,4-cyclohexadiene (0.1 mL) and Pd/C (0.01 g, 10% Pd on activated carbon) at rt. After stirring for 16 h at rt, the suspension was filtered on a celite pad and the collected solution was concentrated under reduced pressure. The residue was purified by silica

gel column chromatography (CH_2Cl_2:MeOH = 95:5) to give **51** (0.01 g, 0.04 mmol) in 40% yield. LCMS (ESI) Calcd for $C_{21}H_{28}N_4O_9PF$ 530.4, observed (M + 1) 531.3

Example 7



Scheme 20. Synthesis of N^4-hydroxycytidine 2'-*C*-Me nucleoside prodrugs **54** and **56**

2S)-isopropyl 2-(((((2R,3R,4R,5R)-5-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate, **53**

To a solution of **41** (0.13 g, 0.36 mmol) in 5 mL of THF was added 1-methylimidazole (0.07 mL, 0.9 mmol) and 0.9 mL of a 1M solution of (2S)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate **52** in THF, under argon atmosphere. After stirring for 3 h at room temperature, AcOEt (15 mL) was added and the reaction mixture was washed with water (3 x 5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 to 90:10 v/v) to give **53** (0.15 g, 0.24 mmol) in 66% yield.

(2S)-ethyl 2-(((((2R,3R,4R,5R)-3,4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1(2H)-yl)-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate, **54**

To a solution of **53** (0.06 g, 0.1 mmol) in 1.5 mL of *i*PrOH was added 1,4-cyclohexadiene (0.2 mL) and Pd/C (0.01 g, 10% Pd on activated carbon) at rt. After stirring for 16 h at rt, the suspension was filtered on a celite pad and the collected solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 90:10) to give **54** (0.02 g, 0.04 mmol) in 35% yield

(2S)-ethyl 2-(((((2R,3R,4R,5R)-3,4-dihydroxy-5-(4-((((isopropoxycarbonyl)oxy)methoxy)amino)-2-oxopyrimidin-1(2H)-yl)-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate **56**

To a solution of **54** (0.03 g, 0.055 mmol) in 0.6 mL of DMF was added Cs_2CO_3 (0.054 g, 0.165 mmol) and iodomethyl isopropyl carbonate **55** (0.027 g, 0.11 mmol). After stirring for 16 h at room temperature, CH_2Cl_2 (5 mL) was added and the reaction mixture was washed with water (3 x 3 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 95:5 v/v) to give **56** (0.01 g, 0.015 mmol) in 28% yield.

(2S)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate, **52**

To a solution of phenyl dichlorophosphate (7.88 g, 51.4 mmol) in 40 mL of CH_2Cl_2, was added L-Alanine isopropyl ester hydrochloride (8.58 g, 51.4 mmol), under argon atmosphere. The mixture was cooled down to -78 °C and a solution of Et_3N (14 mL, 102.8 mmol) in 40 mL of CH_2Cl_2 was added dropwise over 2 h. After, stirring the resulting solution at room temperature for 16 h, the white solid formed was filtered on a celite pad and washed with anhydrous Et_2O (40 mL). The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column

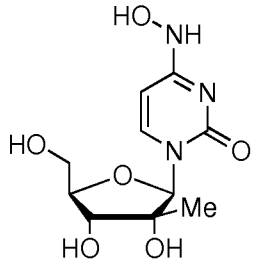
chromatography (EtOAc:hexane = 1:0 to 1:1 v/v) to give **52** (7.86 g, 26 mmol) as a colorless oil in 50% yield.

Example 8

Shown below are two examples of the LC/MS qualitative analysis of nucleotides formed after 4 h incubation of 50 μM N^4-hydroxycytidine nucleosides and N^4-hydroxycytidine monophosphate prodrugs in Huh-7 cells.

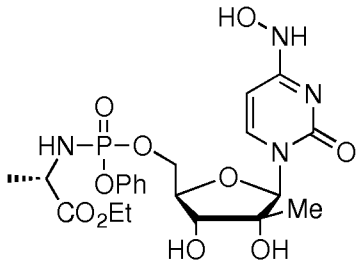
Incubation of **37** in Huh-7 cells resulted in the detection of only very low level **37**-TP (Table 1). However, incubation of monophosphate prodrug **44** in Huh-7 cells resulted in the detection of high levels of of high levels of **37**-MP, **37**-DP and **37**-TP (Table 1) along with very low levels of **36**-DP, **36**-TP and 2'-deoxy-β-*C*-Me-U-TP.

Table 1. HCV and toxicity data for MP prodrug **44** and the parent nucleoside **37**



37

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



44

HCV EC_{50} = 0.8 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM

These high levels of intracellular **37**-MP, **37**-DP and **37**-TP produced upon incubation of the MP prodrug **44** indicate that the MP prodrug bypassed the first phosphorylation step, leading to the formation of **37**-TP. The results are shown below in Table 2:

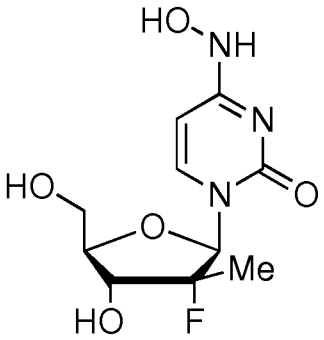
Table 2. LC/MS analysis of nucleotides formed after 4 hr incubation in Huh-7 cells of 50 μM **37** and **44**

Drugs Metabolites (pmol/10^6 cells)	**37**	**44**
2'-OH-2'-Me-U	BLOQ	BLOQ
2'-OH-2'-Me-UMP	BLOQ	BLOQ
2'-OH-2'-Me-UDP	BLOQ	BLOQ
2'-OH-2'-Me-UTP	BLOQ	4.84 ± 0.23
36	BLOQ	BLOQ
36-MP	BLOQ	BLOQ
36-DP	BLOQ	1.75 ± 0.19
36-TP	BLOQ	33.3 ± 0.15
37	BLOQ	BLOQ
37-MP	BLOQ	239.2 ± 35.2
37-DP	BLOQ	451.4 ± 31.1
37-TP	3.20 ± 1.30	3,075 ± 98.5
44	-	13.3 ± 1.7

BLOQ means below the limit of quantification

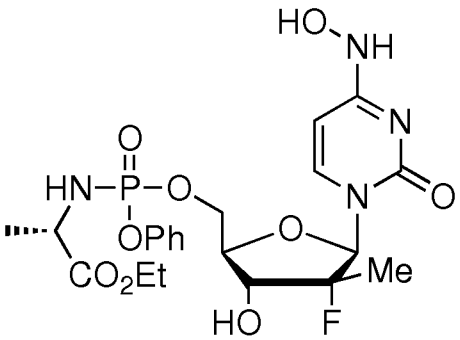
HCV and toxicity data for MP prodrug **39** and the parent nucleoside **51** is shown below in Table 3.

Table 3:



39

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



51

HCV EC_{50} = 2.6 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >33 μM

Incubation of **39** in Huh-7 cells resulted in the detection of high levels of **39** along with low levels of 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me-U-TP, **38**-DP and **38**-TP. No 39-MP, DP, -TP are detected. (Table 4)

However, incubation of monophosphate prodrug **51** in Huh-7 cells resulted in the detection of high levels of **39**, **39**-MP, **39**-DP and **39**-TP (Table 2). Low levels of **38**, **38**-MP, **38**-DP, **38**-TP and 2'-deoxy-2'-α-fluoro-2'-β-*C*-Me-U-TP were also observed.

These high levels of intracellular **39**-DP and **39**-TP produced upon incubation of the MP prodrug **51** indicate that the MP prodrug allow to bypass the first phosphorylation step leading to the formation of **39**-TP.

Table 4 LC/MS analysis of nucleotides formed

after 4 hr incubation in Huh-7 cells of 50 μM 39 and 51

Drugs Metabolites	**39**	**51**

(pmol/10^6 cells)		
2'-F-2'-Me-U	BLOQ	BLOQ
2'-F-2'-Me-UMP	BLOQ	BLOQ
2'-F-2'-Me-UDP	BLOQ	BLOQ
2'-F-2'-Me-UTP	0.68 ± 0.07	6.25 ± 0.17
38	BLOQ	5.00 ± 0.34
38-MP	BLOQ	3.24 ± 0.26
38-DP	0.42 ± 0.019	3.01 ± 0.39
38-TP	2.17 ± 0.13	20.3 ± 1.54
39	188.8 ± 15.3	144.6 ± 21.9
39-MP	BLOQ	3,452 ± 247
39-DP	BLOQ	31.6 ± 7.7
39-TP	BLOQ	364.5 ± 10.6
51	-	71.5 ± 2.3

BLOQ means below the limit of quantification

Example 9

Anti-HIV (in PBM cells) Assay

Anti-HIV-1 activity of the compounds was determined in human peripheral blood mononuclear (PBM) cells as described previously (see Schinazi R.F., McMillan A., Cannon D., Mathis R., Lloyd R.M. Jr., Peck A., Sommadossi J.-P., St. Clair M., Wilson J., Furman P.A., Painter G., Choi W.-B., Liotta D.C. Antimicrob. Agents Chemother. **1992**, 36, 2423; Schinazi R.F., Sommadossi J.-P., Saalmann V., Cannon D., Xie M.-Y., Hart G., Smith G., Hahn E. Antimicrob. Agents Chemother. **1990**, 34, 1061). Stock solutions (20-40 mM) of the compounds were prepared in sterile DMSO

and then diluted to the desired concentration in growth medium. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantified on day 6 after infection by a reverse transcriptase assay using $(rA)_n \bullet (dT)_{12-18}$ as template-primer. The DMSO present in the diluted solution (< 0.1%) had no effect on the virus yield. AZT was included as positive control. The antiviral EC_{50} and EC_{90} were obtained from the concentration-response curve using the median effective method described previously (see Chou T.-C. & Talalay P. *Adv. Enzyme Regul.* **1984**, *22*, 27-55; Belen'kii M.S. & Schinazi R.F. *Antiviral Res.* **1994**, *25*, 1-11).

Example 10

Assess Incorporation of nucleoside-TPs by HIV-1 RT

i) Protein Expression and Purification: HIV-1 RT (xxLAI background) (see Shi C, Mellors JW. A recombinant retroviral system for rapid *in vivo* analysis of human immunodeficiency virus type 1 susceptibility to reverse transcriptase inhibitors. Antimicrob Agents Chemother. 1997; 41:2781-5) was over-expressed in bacteria using the p6HRT-PROT expression vector and purified to homogeneity as described previously (see Le Grice SF, Gruninger-Leitch F. Rapid purification of homodimer and heterodimer HIV-1 reverse transcriptase by metal chelate affinity chromatography. Eur J Biochem. 1990; 187: 307-14; Le Grice SF, Cameron CE, Benkovic SJ. Purification and characterization of human immunodeficiency virus type 1 reverse transcriptase. Methods Enzymol. 1995; 262:130-44). The protein concentration of the purified enzymes was determined spectrophotometrically at 280 nm using an extinction co-efficient (ε280) of 260450M-1cm-1. Active site concentrations of RT were calculated from pre-steady-state burst experiments, as described previously (see Kati WM, Johnson KA, Jerva LF, Anderson KS. Mechanism and fidelity of HIV reverse transcriptase. J Biol. Chem. 1992; 267: 25988-97). All reactions described below were carried out using active site concentrations.

ii) Pre-steady-state Kinetic Analyses: A [γ^{32}P]-ATP 5'-end labeled 20 nucleotide DNA primer (5'-TCGGGCGCCACTGCTAGAGA-3') annealed to a 57 nucleotide DNA template (5'-

CTCAGACCCTTTTAGTCAGAATGGAAA**N**TCTCTAGCAGTGGCGCCCG AACAGGGACA-3') was used in all experiments. The DNA templates contained either a T or C at position 30 (**N**), which allowed evaluation of the kinetics of single nucleotide incorporation using the same 20 nucleotide primer. Rapid quench experiments were carried out using a Kintek RQF-3 instrument (Kintek Corporation, Clarence, PA). In all experiments, 300 nM RT and 60nM DNA template/primer (T/P) were pre-incubated in reaction buffer (50mM Tris-HCl pH 7.5, 50 mM KCl) prior to mixing with an equivalent volume of nucleotide in the same reaction buffer containing 20mM $MgCl_2$. Reactions were terminated at times ranging from 10 ms to 30 min by quenching with 0.5M EDTA, pH 8.0. The quenched samples were mixed with an equal volume of gel loading buffer (98% deionized formamide, 10 mM EDTA and 1mg/mL each of bromophenol blue and xylene cyanol), denatured at 85°C for 5min, and the products were separated from the substrates on a 7M urea-16% polyacrylamide gel. Product formation was analyzed using a Bio-Rad GS525 Molecular Imager (Bio-Rad Laboratories, Inc., Hercules, CA).

iii) Data Analysis: Data obtained from kinetic assays was fitted by nonlinear regression using Sigma Plot software (Jandel Scientific) with the appropriate equations (see Johnson KA. Rapid quench kinetic analysis of polymerases, adenosinetriphosphatases, and enzyme intermediates. Methods Enzymol. 1995; 249:38-61). The apparent burst rate constant (kobs) for each particular concentration of dNTP was determined by fitting the time courses for the formation of product to the equation: [product] = A[1-exp(-kobst)], where A represents the burst amplitude. The turnover number (kpol) and apparent dissociation constant for dNTP (K_d) was obtained by plotting the apparent catalytic rates, kobs, against dNTP concentrations and fitting the data with the following hyperbolic equation: kobs = (kpol[dNTP])/([dNTP] + K_d).

Example 11

Assess Anti-HIV Activity and Cellular Toxicity of N^4-hydroxycytidine nucleoside derivatives, modified monophosphate and phosphonate prodrug analogs

i) Viruses: Stock virus can be prepared using the xxHIV-1LAI clone75 by electroporating (Gene Pulser; Bio-Rad) 5 to 10 μg of plasmid DNA into 1.3 x 10^7 MT-2 cells. At 7 days post-transfection, cell-free supernatant can be harvested and stored at –80°C. The genotype of stock viruses can be confirmed by extraction of

RNA from virions, treatment of the extract with DNase I, amplification of the full-length coding region (amino acids 1 to 560) of RT by RT-PCR, purification of the PCR product, and sequence determination of the PCR product using a Big Dye terminator kit (v. 3.1) on an ABI 3100 automated DNA sequencer (Applied Biosystems, Foster City, Calif.). The 50% tissue culture infective dose ($TCID_{50}$) for the virus stock can be determined for MT-2 cells, P4/R5 cells or PBM cells by three-fold endpoint dilution assays (six wells per dilution) and calculated using the Reed and Muench equation (see Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 1938; 27:493-497).

ii) Single-Replication-Cycle Drug Susceptibility Assay: In a 96-well plate, two- or three-fold serial dilutions of an inhibitor were added to P4/R5 cells in triplicate. Cells were infected with the amount of virus that yielded a relative light unit value of 100 in the no-drug, virus-infected control wells. At 48 h post-infection, a cell lysis buffer and luminescent substrate (Gal-Screen; Tropix/Applied Biosystems) was added to each well, and relative light unit values were determined using a luminometer (ThermoLabSystems, Waltham, Mass.). Inhibition of virus replication was calculated as the concentration of compound required to inhibit virus replication by 50% (EC_{50}).

iii) Multiple-Replication-Cycle Drug Susceptibility Assay: In a 96-well plate, three-fold serial dilutions of an inhibitor can be added to MT-2 cells in triplicate. The cells can be infected at a multiplicity of infection of 0.01 as determined by endpoint dilution in MT-2 cells. At 7 days post-infection, culture supernatants were harvested and treated with 0.5% Triton X-100. The p24 antigen concentration in the supernatants can be determined using a commercial enzyme-linked immunosorbent assay (DuPont, NEN Products, Wilmington, Del.). EC_{50} values can be calculated as described above.

iv) Drug Susceptibility Assays in PBM Cells: PBM cells were isolated by Ficoll–Hypaque discontinuous gradient centrifugation from healthy seronegative donors, as described previously (see Schinazi RF, Cannon DL, Arnold BH, Martino-Saltzman D. Combinations of isoprinosine and 3'-azido-3'-deoxythymidine in lymphocytes infected with human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 1988; 32:1784-1787; Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie MY, Hart GC, Smith GA. Hahn E.F. Activities of 3'-azido-3'-

deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 1990; 34:1061-1067). Cells were stimulated with phytohemagglutinin A (PHA, Difco, Sparks, MD) for 2–3 days prior to use. Infections were done in bulk for 1 h, either with 100 $TCID_{50}$/1 x 10^7 cells for a flask (T25) assay or with 200 $TCID_{50}$/6 x 10^7 cells/well for the 24-well plate assay. Cells were added to a plate or a flask containing a 10-fold serial dilution of the test compound. At 5 days post-infection, culture supernatants were harvested and treated with 0.5% Triton X-100. The p24 antigen concentration in the supernatants was determined as described above. EC_{50} and fold-resistance values were calculated as described above.

v) Cellular Toxicity Assays: Nucleoside and nucleoside monophosphate prodrugs can be evaluated for their potential toxic effects on P4/R5 cells, MT-2 cells and uninfected PHA-stimulated human PBM cell. Log-phase P4/R5, MT-2, and PHA-stimulated human PBM cells can be seeded at 5 x 10^3 to 5 x 10^4 cells/well in 96-well cell culture plates containing 10-fold serial dilutions of the test drug. The cultures can be incubated for 2–4 days, after which 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye solution (Promega, Madison, WI) can be added to each well and incubated overnight. The reaction can be stopped with stop solubilization solution (Promega, Madison, WI) and plates can be read at a wavelength of 570 nm. The median 50% cytotoxic concentration (CC_{50}) can be determined from the concentration–response curve using the median effect method.

Example 12

Assess Activity N^4-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs against Drug-Resistant HIV

Analogs identified above as having improved activity compared with the parent analog, and less cellular toxicity, can be further evaluated for activity against a panel of drug resistant viruses. The drug resistant viruses used in this study can include HIV-1_{K65R}, HIV-1_{K70E}, HIV-1_{L74V}, HIV-1_{M184V}, HIV-1_{AZT2}, HIV-1_{AZT3}, HIV-1_{AZT7}, HIV-1_{AZT9}, HIV-1_{Q151M} and HIV-$1_{69Insertion}$. All of these mutant viruses can be generated in our HIV-1_{xx}LAI clone.

Example 13

Assess Activity of N^4-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs against Drug-Resistant HIV

i) Viruses and Drug Susceptibility Assays: Virus stocks can be prepared as described above. Drug susceptibility assays can be performed using the single- and multiple-replication-cycle assays also described above. Inhibition of virus replication can be calculated as the concentration of compound required to inhibit virus replication by 50% (EC_{50}). Fold resistance values can be determined by dividing the EC_{50} for mutant HIV-1 by the EC_{50} for WT HIV-1.

ii) Statistical analysis: To determine if fold-resistance values are statistically significant, EC_{50} values from at least three independent experiments can be log10 transformed and compared using a two-sample Student's *t* test with Sigma Stat software (Jandel Scientific). *P* values less than 0.05 are considered to be statistically significant.

Example 14

Assess Incorporation and Excision of Nucleotides by Mutant HIV-1 RTs

i) Enzymes: The following mutant HIV-1 RT enzymes can be used in this study: K65R RT, K70E RT, L74V RT, M184V RT, AZT2 RT, AZT3 RT, Q151M RT and 69Insert RT. *E. coli* protein expression vectors for each of these mutant RTs can be developed, and protein expression and purification can be performed as described previously. Protein concentration and active site concentration can be determined as described above.

ii) Kinetic Analyses of Nucleotide Incorporation: Pre-steady-state kinetic analyses can be used to determine the kinetic parameters Kd and kpol for each novel nucleoside-TPs for K65R, K70E RT, L74V RT, M184V RT and Q151M RT. Experimental design and data analysis can be carried out as described above.

iii) Excision Assays: The ATP-mediated phosphorolytic excision of the novel analogs from chain-terminated template/primer can be carried out using WT RT, AZT2 RT, AZT3 RT and 69Insert RT. The 20 nucleotide DNA primer described above can be 5'-end labeled with [γ^{32}P]-ATP and then annealed to the appropriate 57 nucleotide DNA template. The 3'-end of the primer can be chain-terminated by incubation with WT RT and 100μM of the appropriate modified nucleotide analog for

30 min at 37°C. The ^{32}P-labeled, chain-terminated 21 nucleotide primer can be further purified by extraction of the appropriate band after 7M urea-16% acrylamide denaturing gel electrophoresis. The purified chain-terminated primer can then be re-annealed to the appropriate DNA template for use in phosphorolysis experiments. The phosphorolytic removal of nucleoside-MP can be achieved by incubating 300 nM (active site) WT or mutant RT with 60 nM of the chain-terminated T/P complex of interest in 50 mM Tris-HCl pH 8.0, 50 mM KCl. The reaction can be initiated by the addition of 3.0 mM ATP and 10 mM $MgCl_2$. Inorganic pyrophosphatase (0.01 U) can be present throughout the reaction. After defined incubation periods, aliquots can be removed from the reaction tube and quenched with equal volumes of gel loading dye (98% deionized formamide, 10mM EDTA and 1mg/mL each of bromophenol blue and xylene cyanol). Products can be separated by denaturing gel electrophoresis, and the disappearance of substrate coincident with formation of product can be analyzed using a Bio-Rad GS525 Molecular Imager. Data can be fit to the following single exponential equation to determine the apparent rate (kATP) of ATP-mediated excision: [product] = A[exp(-kATPt)], where A represents the amplitude for product formation. Dead-end complex formation can be determined as described previously (see Meyer PR, Matsuura SE, Mian AM, So AG, Scott WA. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. Mol Cell. 1999;4:35-43; Sluis-Cremer N, Arion D, Parikh U, Koontz D, Schinazi RF, Mellors JW, Parniak MA. The 3'-azido group is not the primary determinant of 3'-azido-3'-deoxythymidine (AZT) responsible for the excision phenotype of AZT-resistant HIV-1. J Biol Chem. 2005; 280: 29047-52).

Example 15

Mitochondrial Toxicity Assays in HepG2 Cells:

i) Effect of nucleoside and nucleoside monophosphate prodrugs on Cell Growth and Lactic Acid Production: The effect on the growth of HepG2 cells can be determined by incubating cells in the presence of 0 μM, 0.1 μM, 1 μM, 10 μM and 100 μM drug. Cells (5 x 10^4 per well) can be plated into 12-well cell culture clusters in minimum essential medium with nonessential amino acids supplemented with 10% fetal bovine serum, 1% sodium pyruvate, and 1% penicillin/streptomycin and incubated for 4 days at 37°C. At the end of the incubation period, the cell number can

be determined using a hemocytometer. Also taught by Pan-Zhou X-R, Cui L, Zhou X-J, Sommadossi J-P, Darley-Usmer VM. "Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells"Antimicrob. Agents Chemother. **2000**; *44*: 496-503. To measure the effects of the nucleoside analogs on lactic acid production, HepG2 cells from a stock culture can be diluted and plated in 12-well culture plates at 2.5 x 10^4 cells per well. Various concentrations (0 μM, 0.1 μM, 1 μM, 10 μM and 100 μM) of nucleoside analog can be added, and the cultures can be incubated at 37°C in a humidified 5% CO_2 atmosphere for 4 days. At day 4, the number of cells in each well can be determined and the culture medium collected. The culture medium can be filtered, and the lactic acid content in the medium determined using a colorimetric lactic acid assay (Sigma-Aldrich). Since lactic acid product can be considered a marker for impaired mitochondrial function, elevated levels of lactic acid production detected in cells grown in the presence of N^4-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs can be used to indicate a drug-induced cytotoxic effect.

ii) Effect on *N*^{4}-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs *on Mitochondrial DNA Synthesis:* a real-time PCR assay to accurately quantify mitochondrial DNA content has been developed (see Stuyver LJ, Lostia S, Adams M, Mathew JS, Pai BS, Grier J, Tharnish PM, Choi Y, Chong Y, Choo H, Chu CK, Otto MJ, Schinazi RF. Antiviral activities and cellular toxicities of modified 2',3'-dideoxy-2',3'-didehydrocytidine analogs. Antimicrob. Agents Chemother. 2002; 46: 3854-60). This assay can be used in all studies described in this application that determine the effect of nucleoside analogs on mitochondrial DNA content. In this assay, low-passage-number HepG2 cells can be seeded at 5,000 cells/well in collagen-coated 96-well plates. Nucleoside monophosphate analogs can be added to the medium to obtain final concentrations of 0 μM, 0.1 μM, 10 μM and 100 μM. On culture day 7, cellular nucleic acids can be prepared by using commercially available columns (RNeasy 96 kit; Qiagen). These kits co-purify RNA and DNA, and hence, total nucleic acids are eluted from the columns. The mitochondrial cytochrome *c* oxidase subunit II (COXII) gene and the ß-actin or rRNA gene can be amplified from 5 μl of the eluted nucleic acids using a multiplex Q-PCR protocol with suitable primers and probes for both target and reference amplifications. For COXII the following sense, probe and antisense primers can be used, respectively: 5'-TGCCCGCCATCATCCTA-3', 5'-tetrachloro-6-

carboxyfluorescein-TCCTCATCGCCCTCCCATCCC-TAMRA-3' and 5'-CGTCTGTTATGTAAAGGATGCGT-3'. For exon 3 of the ß-actin gene (GenBank accession number E01094) the sense, probe, and antisense primers are 5'-GCGCGGCTACAGCTTCA-3', 5'-6-FAMCACCACGGCCGAGCGGGATAMRA-3' and 5'-TCTCCTTAATGTCACGCACGAT-3', respectively. The primers and probes for the rRNA gene are commercially available from Applied Biosystems. Since equal amplification efficiencies can be obtained for all genes, the comparative *CT* method can be used to investigate potential inhibition of mitochondrial DNA synthesis. The comparative *CT* method uses arithmetic formulas in which the amount of target (COXII gene) is normalized to the amount of an endogenous reference (the ß-actin or rRNA gene) and is relative to a calibrator (a control with no drug at day 7). The arithmetic formula for this approach is given by 2-ΔΔCT, where ΔΔ*CT* is (*CT* for average target test sample - *CT* for target control) - (*CT* for average reference test -*CT* for reference control) (see Johnson MR, K Wang, JB Smith, MJ Heslin, RB Diasio. Quantitation of dihydropyrimidine dehydrogenase expression by real-time reverse transcription polymerase chain reaction. Anal. Biochem. 2000; 278:175-184). A decrease in mitochondrial DNA content in cells grown in the presence of drug indicates mitochondrial toxicity.

iii) Electron Microscopic Morphologic Evaluation: NRTI induced toxicity has been shown to cause morphological changes in mitochondria (e.g., loss of cristae, matrix dissolution and swelling, and lipid droplet formation) that can be observed with ultrastructural analysis using transmission electron microscopy (see Cui L, Schinazi RF, Gosselin G, Imbach JL. Chu CK, Rando RF, Revankar GR, Sommadossi JP. Effect of enantiomeric and racemic nucleoside analogs on mitochondrial functions in HepG2 cells. Biochem. Pharmacol. **1996**, *52*, 1577-1584; Lewis W, Levine ES, Griniuviene B, Tankersley KO, Colacino JM, Sommadossi JP, Watanabe KA, Perrino FW. Fialuridine and its metabolites inhibit DNA polymerase gamma at sites of multiple adjacent analog incorporation, decrease mtDNA abundance, and cause mitochondrial structural defects in cultured hepatoblasts. Proc Natl Acad Sci U S A. 1996; 93: 3592-7; Pan-Zhou XR, L Cui, XJ Zhou, JP Sommadossi, VM Darley-Usmar. Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells. *Antimicrob. Agents Chemother.* **2000**, *44*, 496-503). For example, electron micrographs of HepG2 cells incubated with 10 μM fialuridine

(FIAU; 1,2'-deoxy-2'-fluoro-1-D-arabinofuranosly-5-iodo-uracil) can show the presence of enlarged mitochondria with morphological changes consistent with mitochondrial dysfunction. To determine if nucleoside and nucleoside monophosphate prodrugs promoted morphological changes in mitochondria, HepG2 cells (2.5 x 10^4 cells/mL) can be seeded into tissue cultures dishes (35 by 10 mm) in the presence of 0 μM, 0.1 μM, 1 μM, 10 μM and 100 μM nucleoside analog. At day 8, the cells can be fixed, dehydrated, and embedded in Eponas described previously. Thin sections can be prepared, stained with uranyl acetate and lead citrate, and then examined using transmission electron microscopy.

Example 16

Mitochondrial Toxicity Assays in Neuro2A Cells

To estimate the potential of nucleoside analogs to cause neuronal toxicity, mouse Neuro2A cells (American Type Culture Collection 131) can be used as a model system (see Ray AS, Hernandez-Santiago BI, Mathew JS, Murakami E, Bozeman C, Xie MY, Dutschman GE, Gullen E, Yang Z, Hurwitz S, Cheng YC, Chu CK, McClure H, Schinazi RF, Anderson KS. Mechanism of anti-human immunodeficiency virus activity of beta-D-6-cyclopropylamino-2',3'-didehydro-2',3'-dideoxyguanosine. *Antimicrob. Agents Chemother.* **2005**, *49*, 1994-2001). The concentrations necessary to inhibit cell growth by 50% (CC_{50}) can be measured using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide dye-based assay, as described. Perturbations in cellular lactic acid and mitochondrial DNA levels at defined concentrations of drug can be carried out as described above. In all experiments, ddC and AZT can be used as control nucleoside analogs.

Example 17

Effect of Nucleotide Analogs on the DNA Polymerase and Exonuclease Activities of Mitochondrial DNA Polymerase γ

i) *Purification of Human Polymerase γ*: The recombinant large and small subunits of polymerase γ can be purified as described previously (see Graves SW, Johnson AA, Johnson KA. Expression, purification, and initial kinetic characterization of the large subunit of the human mitochondrial DNA polymerase. *Biochemistry*. **1998**, *37*, 6050-8; Johnson AA, Tsai Y, Graves SW, Johnson KA.

Human mitochondrial DNA polymerase holoenzyme: reconstitution and characterization. Biochemistry **2000**; 39: 1702-8). The protein concentration can be determined spectrophotometrically at 280 nm, with extinction coefficients of 234,420, and 71,894 M-1 cm-1 for the large and the small subunits of polymerase γ, respectively.

ii) Kinetic Analyses of Nucleotide Incorporation: Pre-steady-state kinetic analyses can be carried out to determine the catalytic efficiency of incorporation (k/K) for DNA polymerase γ for nucleoside-TP and natural dNTP substrates. This allows determination of the relative ability of this enzyme to incorporate modified analogs and predict toxicity. Pre-steady-state kinetic analyses of incorporation of nucleotide analogs by DNA polymerase γ can be carried out essentially as described previously (see Murakami E, Ray AS, Schinazi RF, Anderson KS. Investigating the effects of stereochemistry on incorporation and removal of 5-fluorocytidine analogs by mitochondrial DNA polymerase gamma: comparison of D- and L-D4FC-TP. *Antiviral Res.* **2004**, *62*, 57-64; Feng JY, Murakami E, Zorca SM, Johnson AA, Johnson KA, Schinazi RF, Furman PA, Anderson KS. Relationship between antiviral activity and host toxicity: comparison of the incorporation efficiencies of 2',3'-dideoxy-5-fluoro-3'-thiacytidine-triphosphate analogs by human immunodeficiency virus type 1 reverse transcriptase and human mitochondrial DNA polymerase. *Antimicrob Agents Chemother.* **2004**, *48*, 1300-6). Briefly, a pre-incubated mixture of large (250 nM) and small (1.25 mM) subunits of polymerase γ and 6 0nM DNA template/primer in 50mM Tris-HCl, 100 mM NaCl, pH 7.8, can be added to a solution containing $MgCl_2$ (2.5 mM) and various concentrations of nucleotide analogs. Reactions can be quenched and analyzed as described previously. Data can be fit to the same equations as described above.

iii) Assay for *Human Polymerase γ 3' 5' Exonuclease Activity:* The human polymerase γ exonuclease activity can be studied by measuring the rate of formation of the cleavage products in the absence of dNTP. The reaction can be initiated by adding $MgCl_2$ (2.5mM) to a pre-incubated mixture of polymerase γ large subunit (40nM), small subunit (270nM), and 1,500nM chain-terminated template/primer in 50mM Tris-HCl, 100mM NaCl, pH 7.8, and quenched with 0.3M EDTA at the designated time points. All reaction mixtures can be analyzed on 20% denaturing polyacrylamide sequencing gels (8M urea), imaged on a Bio-Rad GS-525 molecular

image system, and quantified with Molecular Analyst (Bio-Rad). Products formed from the early time points were plotted as a function of time. Data can be fitted by linear regression with Sigma Plot (Jandel Scientific). The slope of the line can be divided by the active enzyme concentration in the reaction to calculate the *k*exo for exonuclease activity (see Murakami E, Ray AS, Schinazi RF, Anderson KS. Investigating the effects of stereochemistry on incorporation and removal of 5-fluorocytidine analogs by mitochondrial DNA polymerase gamma: comparison of D- and L-D4FC-TP. Antiviral Res. 2004; 62: 57-64; Feng JY, Murakami E, Zorca SM, Johnson AA, Johnson KA, Schinazi RF, Furman PA, Anderson KS. Relationship between antiviral activity and host toxicity: comparison of the incorporation efficiencies of 2',3'-dideoxy-5-fluoro-3'-thiacytidine-triphosphate analogs by human immunodeficiency virus type 1 reverse transcriptase and human mitochondrial DNA polymerase. Antimicrob Agents Chemother. 2004; 48: 1300-6).

Example 18

Assay for Bone Marrow Cytotoxicity

Primary human bone marrow mononuclear cells can be obtained commercially from Cambrex Bioscience (Walkersville, MD). CFU-GM assays can be carried out using a bilayer soft agar in the presence of 50 units/mL human recombinant granulocyte/macrophage colony-stimulating factor, while BFU-E assays use a methylcellulose matrix containing 1 unit/mL erythropoietin (see Sommadossi JP, Carlisle R. Toxicity of 3'-azido-3'-deoxythymidine and 9-(1,3-dihydroxy-2-propoxymethyl) guanine for normal human hepatopoietic progenitor cells *in vitro*. Antimicrob. Agents Chemother. 1987; 31: 452-454; Sommadossi, JP, Schinazi, RF, Chu, CK, and Xie, MY. Comparison of Cytotoxicity of the (-) and (+) enantiomer of 2',3'-dideoxy-3'-thiacytidine in normal human bone marrow progenitor cells. Biochem. Pharmacol. 1992; 44:1921-1925). Each experiment was performed in duplicate in cells from three different donors. AZT can be used as a positive control. Cells can be incubated in the presence of the compound for 14-18 days at 37°C with 5% CO_2, and colonies of greater than 50 cells can be counted using an inverted microscope to determine IC_{50}. The 50% inhibitory concentration (IC_{50}) can be obtained by least-squares linear regression analysis of the logarithm of drug concentration versus BFU-E survival fractions. Statistical analysis can be performed

with Student's *t* test for independent non-paired samples.

Example 19

Anti-HBV assay

The anti-HBV activity of the compounds can be determined by treating the AD-38 cell line carrying wild type HBV under the control of tetracycline (see Ladner S.K., Otto M.J., Barker C.S., Zaifert K., Wang G.H., Guo J.T., Seeger C. & King R.W. *Antimicrob. Agents Chemother.* **1997**, *41*, 1715-20). Removal of tetracycline from the medium [Tet (-)] results in the production of HBV. The levels of HBV in the culture supernatant fluids from cells treated with the compounds can be compared with that of the untreated controls. Control cultures with tetracycline [Tet (+)] can also be maintained to determine the basal levels of HBV expression. 3TC can be included as positive control.

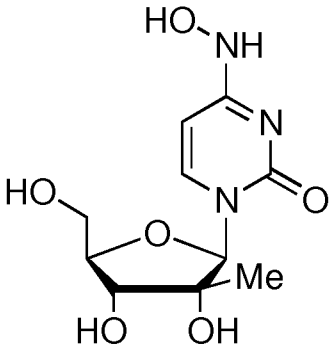
Example 20

Cytotoxicity assay

The toxicity of the compounds was assessed in Vero, human PBM, CEM (human lymphoblastoid), and can be assessed in MT-2, and HepG2 cells, as described previously (see Schinazi R.F., Sommadossi J.-P., Saalmann V., Cannon D.L., Xie M.-Y., Hart G.C., Smith G.A. & Hahn E.F. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061-67). Cycloheximide was included as positive cytotoxic control, and untreated cells exposed to solvent were included as negative controls. The cytotoxicity (IC_{50}) was obtained from the concentration-response curve using the median effective method described previously (see Chou T.-C. & Talalay P. *Adv. Enzyme Regul.* **1984**, *22*, 27-55; Belen'kii M.S. & Schinazi R.F. *Antiviral Res.* **1994**, *25*, 1-11).

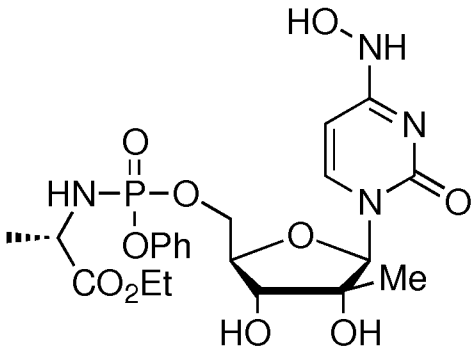
The data on Vero, human PBM, and CEM (human lymphoblastoid) cells is shown below in Table 5:

Table 5: HCV EC_{50}, PBM IC_{50}, CEM IC_{50}, Vero IC_{50}, and Huh-7 IC_{50} Data for Selected Compounds



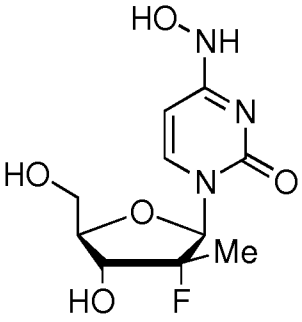
37

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



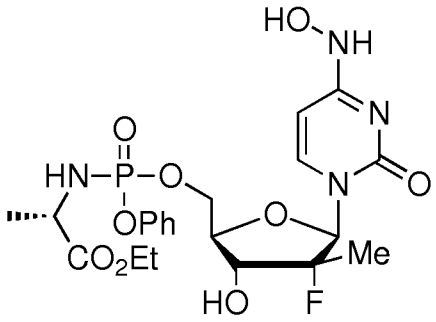
44

HCV EC_{50} = 0.8 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



39

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



51

HCV EC_{50} = 2.6 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >33 μM

Example 21

Selection of Resistant Viruses to nucleotide monophosphate prodrugs

Peripheral blood mononuclear (PBM) cells[1] can be seeded, for example, at a concentration of 1 x 10^7 cells in a total of 5 mL of RPMI-1640 (Mediatech Inc., Herndon, VA) containing 100 mL heat inactivated fetal bovine serum (Hyclone, Logan, Utah), 83.3 IU/mL penicillin, 83.3 μg/mL streptomycin (Mediatech Inc., Herndon, VA), 1.6 mM L-glutamine (Mediatech Inc., Herndon, VA), 0.0008% DEAE-Dextran (Sigma-Aldrich, St. Louis, MO), 0.047% sodium bicarbonate, and 26 IU/mL recombinant interleukin-2 (Chiron Corporation, Emeryville, CA) in two T25 flask, one control (untreated) and one treated with drug.

Naive PBM cells can be treated with *nucleotide monophosphate prodrug* at 0.1 μM for one hour prior to inoculation with HIV-1_{LAI}[2] at 100 x $TCID_{50}$. The treated PBM cell group and a control nontreated PBM cell group can be allowed to infect, for example, for one hour. An additional 5 mL RTU medium can be added to each flask and cells can be incubated, for example, for 6 days at 37 °C.

On day 6, 1 mL of supernatant from each flask can be removed and spun at 9,740 g at 4°C for 2 hr. The resulting viral pellet can then be resuspended in virus solubilization buffer for RT analysis. Total RNA can be isolated from culture

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[1] PBM cells can be separated by ficoll-hypaque (Histopaque 1077: Sigma) density gradient centrifugation from Buffy coats obtained from the American Red Cross (Atlanta, GA). Buffy coats can be derived from healthy, seronegative donors. Cells can be activated with 3 μg/mL phytohemagglutinin A (Sigma-Aldrich, St. Louis, MO) in 500 mL of RPMI-1640 (Mediatech Inc., Herndon, VA) containing 100 mL heat inactivated fetal bovine serum (Hyclone, Logan, Utah), 83.3 IU/mL penicillin, 83.3 μg/mL streptomycin, 1.6 mM L-glutamine (Mediatech Inc., Herndon, VA), for 2-3 days prior to use.

[2] HIV-1/LAI can be obtained from the Center for Disease Control and Prevention and used as the virus for the resistant pool and a multiplicity of infection (MOI) of 0.1, as determined by a limiting dilution method in PBM cells, can be selected to begin the infected pool.

supernatants using the commercial QIAmp Viral RNA mini kit (Quiagen). Sequencing can be performed in parallel between the control virus and nucleotide monophosphate prodrug treated virus to determine if there are any mutations created by the applied drug pressure on weeks where the virus appears to be resistant.

The percent inhibition of the treated viral pool relative to the untreated viral pool can be calculated and closely monitored weekly prior to treatment. The selective pressure for the viral pool can be increased from 0.1 μM to 3.5 μM (40 times the EC_{50} value) over a period of as many as 47 weeks or more.

Example 22

Synthesis of Nucleoside analog triphosphates

Nucleoside analog triphosphates were synthesized from suitably protected nucleosides, using the Ludwig and Eckstein's method. (Ludwig J, Eckstein F. "Rapid and efficient synthesis of nucleoside 5'-O-(1-thiotriphosphates), 5'-triphosphates and 2',3'-cyclophosphorothioates using 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one" *J. Org. Chem.* **1989**, *54* 631-5) The crude nucleoside analog triphosphate can be purified, for example, by FPLC using a HiLoad 26/10 Q Sepharose Fast Flow Pharmacia column and gradient of TEAB buffer (pH 7.0). The product will be characterized by UV spectroscopy, proton and phosphorus NMR, mass spectroscopy and HPLC.

The resulting triphosphates can be used as controls for the celluar pharmacology assays described above and for kinetic work with HIV-RT, HCV polymerase and other viral and human polymerases.

Example 23

Screening Assays for Activity Against HSV-1 and HSV-2

In the CPE-inhibition assay, drug can be added 1 h prior to infection so the assay system will have maximum sensitivity and detect inhibitors of early replicative steps such as adsorption or penetration as well as later events. To rule out non-specific inhibition of virus binding to cells all compounds that show reasonable activity in the CPE assay would be confirmed using a classical plaque reduction assay in which the

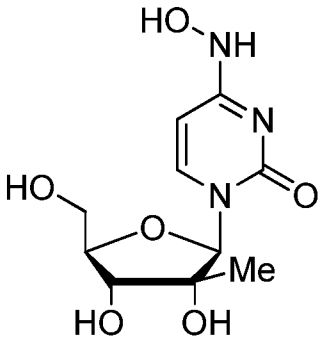
drug is added 1 h after infection. In the case where a compound blocks attachment, it will show up positive in the CPE assay, but may be negative by plaque assay. Efficacy: a minimum of six drug concentrations would be used covering a range of 100 mg/ml to 0.03 mg/ml, in 5-fold increments. From these data would be calculated the dose that inhibited viral replication by 50% (effective concentration 50; EC_{50}). Toxicity: The same drug concentrations used to determine efficacy can also used on uninfected cells in each assay to determine toxicity of each experimental compound. The drug concentration that is cytotoxic to cells as determined by their failure to take up a vital strain, neutral red.

HSV-1 drug susceptibility assay can also be done as previously described in: Schinazi, R.F., Peters, J., Williams, C.C., Chance, D., Nahmias, A.J. "Effect of combinations of acyclovir with vidarabine or its 5'-monophosphate on herpes simplex virus in cell culture and in mice." *Antimicrob. Agents Chemother.* **1982**, *22,* 499-507.

Example 24

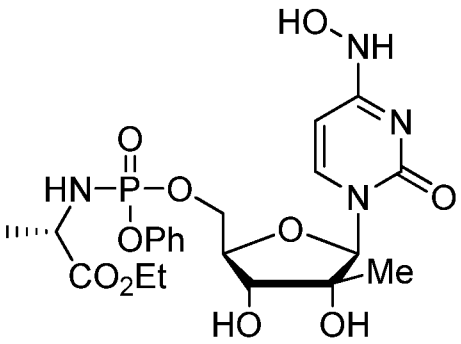
HCV Replicon Assay[1]

Huh 7 Clone B cells containing HCV Replicon RNA were seeded in a 96-well plate at 5000 cells/well, and the compounds tested at 10 μM in triplicate immediately after seeding. Following five days incubation (37°C, 5% CO_2), total cellular RNA was isolated by using versaGene RNA purification kit from Gentra. Replicon RNA and an internal control (TaqMan rRNA control reagents, Applied Biosystems) were amplified in a single step multiplex Real Time RT-PCR Assay. The antiviral effectiveness of the compounds was calculated by subtracting the threshold RT-PCR cycle of the test compound from the threshold RT-PCR cycle of the no-drug control (ΔCt HCV). A ΔCt of 3.3 equals a 1-log reduction (equal to 90% less starting material) in Replicon RNA levels. The cytotoxicity of the compounds was also calculated by using the ΔCt rRNA values. (2'-Me-*C*) was used as the control. To determine EC_{90} and IC_{5O} values[2], ΔCt: values were first converted into fraction of starting material[3] and then were used to calculate the % inhibition.



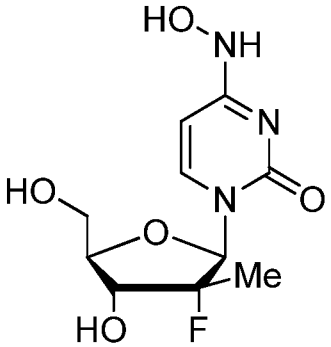
37

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



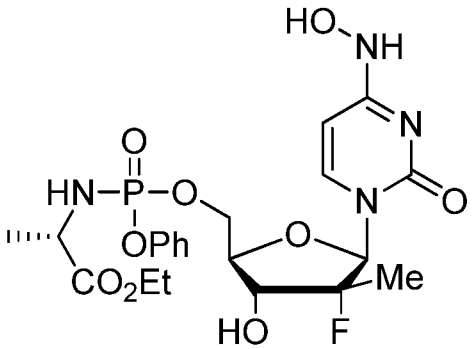
44

HCV EC_{50} = 0.8 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



39

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



51

HCV EC_{50} = 2.6 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >33 μM

References:

1. Stuyver L et al., Ribonucleoside analogue that blocks replication or bovine viral diarrhea and hepatitis C viruses in culture. *Antimicrob. Agents Chemother.* **2003,** *47*, 244-254.

2. Reed IJ & Muench H, A simple method or estimating fifty percent endpoints. *Am. J. Hyg. 27*: 497, 1938.

3. Applied Biosystems Handbook

Example 25

West Nile virus drug susceptibility can also be assayed as previously described in: Song, G.Y., Paul, V., Choo, H., Morrey, J., Sidwell, R.W., Schinazi, R.F., Chu, C.K. Enantiomeric synthesis of D- and L-cyclopentenyl nucleosides and their antiviral activity against HIV and West Nile virus. *J. Med. Chem.* **2001**, *44*, 3985-3993,

Example 26

Yellow fever drug susceptibility can also be assayed as previously described in: Julander, J.G., Furuta, Y., Shafer, K., Sidwell, R.W. Activity of T-1106 in a Hamster Model of Yellow Fever Virus Infection. *Antimicrob. Agents Chemother.* **2007**, *51*, 1962-1966.

Example 27

The human and Dengue virus polymerase assays can be performed, for example, by Replizyme Ltd. Briefly, each enzyme/compound combination can be tested in duplicate over a range of concentrations from 0.8 mM to 100 mM. The compounds can be run alongside a control (no inhibitor), a solvent dilution (for example, 0.016% to 2% DMSO) and the relevant Replizyme reference inhibitor.

A representative high throughput assay for identifying compounds with activity against Dengue is disclosed in Lim et al., A scintillation proximity assay for dengue virus NS5 2′-*O*-methyltransferase-kinetic and inhibition analyses, Antiviral Research, Volume 80, Issue 3, December 2008, Pages 360-369.

Dengue virus (DENV) NS5 possesses methyltransferase (MTase) activity at its N-terminal amino acid sequence and is responsible for formation of a type 1 cap structure, m7GpppAm2′-O in the viral genomic RNA. Optimal *in vitro* conditions for DENV2 2′-O-MTase activity can be characterized using purified recombinant protein and a short biotinylated GTP-capped RNA template. Steady-state kinetics parameters derived from initial velocities can be used to establish a robust scintillation proximity assay for compound testing. Pre-incubation studies by Lim et al., Antiviral Research, Volume 80, Issue 3, December 2008, Pages 360-369, showed that MTase–AdoMet and MTase–RNA complexes were equally catalytically competent and the enzyme

supports a random bi bi kinetic mechanism. Lim validated the assay with competitive inhibitory agents, S-adenosyl-homocysteine and two homologues, sinefungin and dehydrosinefungin. A GTP-binding pocket present at the N-terminal of DENV2 MTase was previously postulated to be the cap-binding site. This assay allows rapid and highly sensitive detection of 2′-O-MTase activity and can be readily adapted for high-throughput screening for inhibitory compounds. It is suitable for determination of enzymatic activities of a wide variety of RNA capping MTases.

This assay can be used to screen the compounds described herein for their anti-Dengue activity.

Example 28

Anti-Norovirus Activity

Compounds can exhibit anti-norovirus activity by inhibiting norovirus polymerase and/or helicase, by inhibiting other enzymes needed in the replication cycle, or by other pathways.

There is currently no approved pharmaceutical treatment for Norovirus infection, and this has probably at least in part been due to the lack of availability of a cell culture system. Recently, a replicon system has been developed for the original Norwalk G-I strain (Chang, K. O., et al. (2006) Virology 353:463-473)

Both Norovirus replicons and Hepatitis C replicons require viral helicase, protease, and polymerase to be functional in order for replication of the replicon to occur. Most recently, an *in vitro* cell culture infectivity assay has been reported utilizing Norovirus genogroup I and II inoculums (Straub, T. M. et al. (2007) Emerg. Infect. Dis. 13(3):396-403). This assay is performed in a rotating-wall bioreactor utilizing small intestinal epithelial cells on microcarrier beads. The infectivity assay can be used to screen entry inhibitors.

Example 29

Phosphorylation Assay of Nucleoside to Active Triphosphate in HepG2 cells

To determine the cellular metabolism of the compounds, HepG2 cells can be obtained from the American Type Culture Collection (Rockville, MD), and are grown in 225 cm^2 tissue culture flasks in minimal essential medium supplemented with non-essential amino acids, 1% penicillin-streptomycin. The medium can be renewed every three days, and the cells can be subcultured once a week. After detachment of the adherent monolayer with a 10 minute exposure to 30 mL of trypsin-EDTA and three consecutive washes with medium, confluent HepG2 cells can be seeded at a density of 2.5 x 10^6 cells per well in a 6-well plate and exposed to 10 μM of [^{3}H] labeled active compound (500 dpm/pmol) for the specified time periods.

The cells are maintained at 37°C under a 5% CO_2 atmosphere. At the selected time points, the cells are washed three times with ice-cold phosphate-buffered saline (PBS).

Intracellular active compound and its respective metabolites are extracted by incubating the cell pellet overnight at -20°C with 60% methanol. The extracts are then combined, dried under gentle filtered air flow and stored at -20°C until HPLC analysis.

Example 30

Bioavailability Assay in Cynomolgus Monkeys

The following procedure can be used to determine whether the compounds are bioavailable. Within 1 week prior to the study initiation, a cynomolgus monkey can be surgically implanted with a chronic venous catheter and subcutaneous venous access port (VAP) to facilitate blood collection and can undergo a physical examination including hematology and serum chemistry evaluations and the body weight recording. Each monkey (six total) receives approximately 250 μCi of ^{3}H activity with each dose of active compound at a dose level of 10 mg/kg at a dose concentration of 5 mg/mL, either via an intravenous bolus (3 monkeys, IV), or via oral gavage (3 monkeys, PO). Each dosing syringe is weighed before dosing to gravimetrically determine the quantity of formulation administered. Urine samples are collected via pan catch at the designated intervals (approximately 18-0 hours pre-dose, 0-4, 4-8 and 8-12 hours post-dosage) and processed. Blood samples are

collected as well (pre-dose, 0.25, 0.5, 1,2, 3,6, 8, 12 and 24 hours post-dosage) via the chronic venous catheter and VAP or from a peripheral vessel if the chronic venous catheter procedure should not be possible. The blood and urine samples are analyzed for the maximum concentration (Cmax), time when the maximum concentration is achieved (TmaX), area under the curve (AUC), half life of the dosage concentration (TV,), clearance (CL), steady state volume and distribution (Vss) and bioavailability (F).

Example 31

Cell Protection Assay (CPA)

The assay can be performed essentially as described by Baginski, S. G.; Pevear, D. C.; Seipel, M.; Sun, S. C. C.; Benetatos, C. A.; Chunduru, S. K.; Rice, C. M. and M. S. Collett "Mechanism of action of a pestivirus antiviral compound" PNAS USA 2000, 97 (14), 7981- 7986. MDBK cells (ATCC) are seeded onto 96-well culture plates (4,000 cells per well) 24 hours before use. After infection with BVDV (strain NADL, ATCC) at a multiplicity of infection (MOI) of 0.02 plaque forming units (PFU) per cell, serial dilutions of test compounds are added to both infected and uninfected cells in a final concentration of 0.5% DMSO in growth medium. Each dilution is tested in quadruplicate. Cell densities and virus inocula are adjusted to ensure continuous cell growth throughout the experiment and to achieve more than 90% virus-induced cell destruction in the untreated controls after four days post-infection. After four days, plates are fixed with 50% TCA and stained with sulforhodamine B. The optical density of the wells is read in a microplate reader at 550 nm.

The 50% effective concentration (EC_{50}) values are defined as the compound concentration that achieved 50% reduction of cytopathic effect of the virus.

Example 32

Plaque Reduction Assay

For a given compound, the effective concentration can be determined in duplicate 24-well plates by plaque reduction assays. Cell monolayers are infected with 100 PFU/well of virus. Then, serial dilutions of test compounds in MEM supplemented with 2% inactivated serum and 0.75% of methyl cellulose are added to the monolayers. Cultures are further incubated at 37°C for 3 days, then fixed with 50% ethanol and 0.8% Crystal Violet, washed and air-dried. Then plaques are counted to determine the concentration to obtain 90% virus suppression.

Example 33

Yield Reduction Assay

For a given compound, the concentration to obtain a 6-log reduction in viral load can be determined in duplicate 24-well plates by yield reduction assays. The assay is performed as described by Baginski, S. G.; Pevear, D. C.; Seipel, M.; Sun, S. C. C.; Benetatos, C. A.; Chunduru, S. K.; Rice, C. M. and M. S. Collett "Mechanism of action of a pestivirus antiviral compound" PNAS USA 2000,97 (14), 7981-7986, with minor modifications.

Briefly, MDBK cells are seeded onto 24-well plates (2 x 10^5 cells per well) 24 hours before infection with BVDV (NADL strain) at a multiplicity of infection (MOI) of 0.1 PFU per cell. Serial dilutions of test compounds are added to cells in a final concentration of 0.5% DMSO in growth medium. Each dilution is tested in triplicate. After three days, cell cultures (cell monolayers and supernatants) are lysed by three freeze-thaw cycles, and virus yield is quantified by plaque assay. Briefly, MDBK cells are seeded onto 6-well plates (5 x 10^5 cells per well) 24 h before use. Cells are inoculated with 0.2 mL of test lysates for 1 hour, washed and overlaid with 0.5% agarose in growth medium. After 3 days, cell monolayers are fixed with 3.5% formaldehyde and stained with 1% crystal violet (w/v in 50% ethanol) to visualize plaques. The plaques are counted to determine the concentration to obtain a 6-log reduction in viral load.

Example 34

Diagnosis of Norovirus Infection

One can diagnose a norovirus infection by detecting viral RNA in the stools of affected persons, using reverse transcription-polymerase chain reaction (RT-PCR) assays. The virus can be identified from stool specimens taken within 48 to 72 hours after onset of symptoms, although one can obtain satisfactory results using RT-PCR on samples taken as long as 7 days after the onset of symptoms. Other diagnostic methods include electron microscopy and serologic assays for a rise in titer in paired sera collected at least three weeks apart. There are also commercial enzyme-linked immunoassays available, but these tend to have relatively low sensitivity, limiting their use to diagnosis of the etiology of outbreaks. Clinical diagnosis of norovirus infection is often used, particularly when other causative agents of gastroenteritis have been ruled out.

Example 35

In Vitro Anti-Viral Activity

In vitro anti-viral activity can be evaluated in the following cell lines:

The Norwalk G-I strain (Chang, K. O., et al. (2006) Virology 353:463-473), the GII-4 strain replicon, as well other Norovirus replicons can be used in assays to determine the *in vitro* antiviral activity of the compounds described herein, or other compounds or compound libraries. In some embodiments, the replicon systems are subgenomic and therefore allow evaluation of small molecule inhibitors of non-structural proteins. This can provide the same benefits to Norovirus drug discovery that Hepatitis C replicons contributed to the discovery of therapeutics useful for treatment of that virus (Stuyver, L. J., et al. (2006) Antimicrob. Agents Chemother. 47:244-254). Both Norovirus replicons and Hepatitis C replicons require viral helicase, protease, and polymerase to be functional in order for replication of the replicon to occur. It is believed that the compounds described herein inhibit viral polymerase and/or viral helicase.

The *in vitro* cell culture infectivity assay reported using Norovirus genogroup I and II inoculums (Straub, T. M. et al. (2007) Emerg. Infect. Dis. 13(3):396-403) can also be used. This assay can be performed in a rotating-wall bioreactor utilizing small

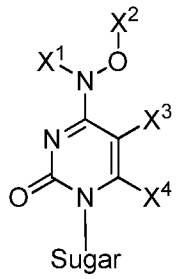
intestinal epithelial cells on microcarrier beads. The infectivity assay can be used for screening compounds for their ability to inhibit the desired virus.

Each of the references identified in this application are incorporated herein in their entirety for all purposes.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations and/or modifications as come within the scope of the following claims and their equivalents.

Claims:

1. A compound of Formula (**I**):



(**I**)

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X^1 is H, C_1-C_6alkyl, C_1-C_6haloalkyl, C_1-C_6alkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, COR^1, or $COOR^1$;

X^2 is hydrogen, COR^1, or $COOR^1$

wherein each R^1 is, independently, CH_2-O(CO)-X^5; CH_2-O(CO)O-X^5, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-6} alkyl, or C_{1-6} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or C_{3-10} cycloalkyl

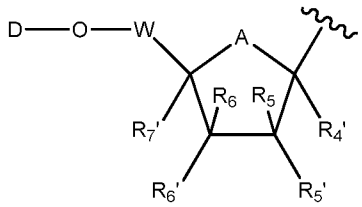
X^5 is independently, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, alkoxy, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-6} alkyl, or C_{1-6} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or C_{3-10} cycloalkyl

each X^3 and X^4 is independently H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, alkylaryl, halogen, NH_2, OH, SH, CN, or NO_2.

2. A compound of Claim 1, wherein each R^1 is, independently, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein

the substituents are C_{1-6} alkyl, or C_{1-6} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, or C_{3-10} cycloalkyl.

3. The compound of Claim 1, wherein Sugar is ribose or a modified ribose of the general Formula (**II**):



(**II**)

wherein:

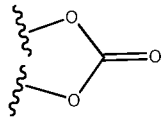
D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

R^1 is as defined above;

W is CL_2 or CL_2CL_2, wherein L independently is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH_2, CHF, CF_2, $C{=}CH_2$, C=CHF, or $C{=}CF_2$;

$R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, and $R^{7'}$ are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

$R^{5'}$ and $R^{6'}$ can come together to form a ring 

wherein:

when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H then, R^5, $R^{5'}$, R^6, $R^{6'}$ can not be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH{=}CH_2$, CN, CH_2NH_2, CH_2OH, COOH

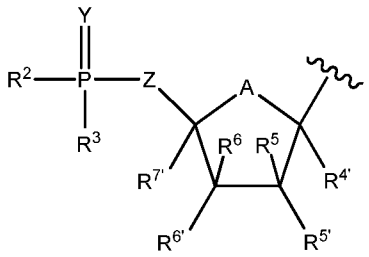
when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2, and

R is independently a C_1-C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_3-C_6 cycloalkyl) aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1.

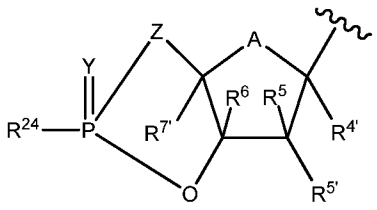
4. The compound of Claim 3, wherein $R^{6'}$ is independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2, and wherein R is independently a C_1-C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_3-C_6 cycloalkyl) aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1.

5. The compound of Claim 3, wherein $R^{7'}$ is independently selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R, and wherein R is independently a C_1-C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_3-C_6 cycloalkyl) aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1.

6. The compound of Claim 1, wherein Sugar is ribose or modified ribose of the general Formulas (**III**) or (**IV**):



(**III**)



(**IV**)

wherein:

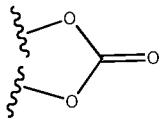
Y is O or S;

Z is selected from the group consisting of CL_2, CL_2CL_2, CL_2OCL_2, CL_2SCL_2, CL_2O, OCL_2 and CL_2NHCL_2, wherein L independently is selected from the group consisting of H, F, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-}

$_6$ alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

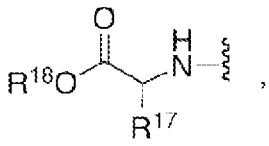
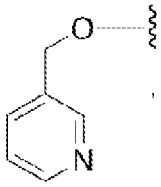
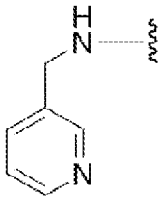
A is O, S, CH_2, CHF, CF_2, C=CH_2, C=CHF, or C=CF_2;

$R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, and $R^{7'}$ are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2;

$R^{5'}$ and $R^{6'}$ can come together to form a ring 

wherein when A is O or S, $R^{7'}$cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2, and

R is independently a C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1,

R^{24} is selected from the group consisting of OR^{15},  ,  ,  , and fatty alcohols,

R^{15} is selected from the group consisting of H, Li, Na, K, phenyl and pyridinyl; wherein phenyl and pyridinyl are optionally substituted with one to three substituents independently selected from the group consisting of $(CH_2)_{0-6}CO_2R^{16}$ and $(CH_2)_{0-6}CON(R^{16})_2$;

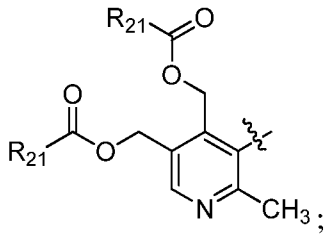
R^{17} is selected from those groups occurring in natural L-amino acids, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1,

R^{18} is H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-

amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or cycloalkyl;

R^2 and R^3 are independently selected from the group consisting of:

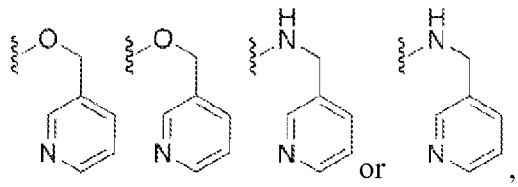
(a) OR^8 where R^8 is H, Li, Na, K, C_{1-20} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, aryl, or heteroaryl, optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, $(CH_2)_{0-6}CO_2R^{9a}$, halogen, C_{1-6} haloalkyl, $-N(R^{9a})_2$, C_{1-6} acylamino, $-NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^{9a})_2$, $-SO_2C_{1-6}$ alkyl, COR^{9b}, nitro, cyano and

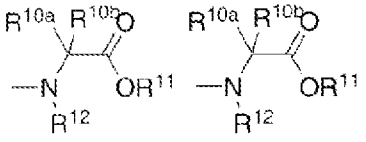


; wherein R^{21} is as defined below;

R^{9a} is independently H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

(b)  or ,

(c)  where R^{10a} and R^{10b} are:

(i) independently selected from the group consisting of H, C_{1-10} alkyl, $-(CH_2)_rNR^{9a}{}_2$, C_{1-6} hydroxyalkyl, $-CH_2SH$, $-(CH_2)_2S(O)_pMe$, $-(CH_2)_3NHC(=NH)NH_2$, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, $-(CH_2)_mCOR^{9b}$, aryl and aryl-C_{1-3} alkyl, wherein said aryl groups are optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, halogen, nitro, and cyano;

(ii) R^{10a} is H and R^{10b} and R^{12} together are $(CH_2)_{2-4}$ to form a ring that includes the adjoining N and C atoms;

(iii) R^{10a} and R^{10b} together are $(CH_2)_n$ to form a ring;

(iv) R^{10a} and R^{10b} both are C_{1-6} alkyl; or

(v) R^{10a} is H and R^{10b} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$-$CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-10} cycloalkyl;

p is 0 to 2;

r is 1 to 6;

n is 4 or 5;

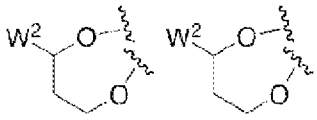
m is 0 to 3;

R^{11} is H, C_{1-10} alkyl, or C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl;

wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{12} is H or C_{1-3} alkyl, or R^{10a}, or R^{10b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

(d) an O attached lipid (including a phospholipid), an N or O attached peptide, an O attached cholesterol, or an O attached phytosterol;

(e) R^2 and R^3 can come together to form a ring  where W^2 is selected from the group consisting of phenyl or monocyclic heteroaryl, optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, CF_3, C_{2-6} alkenyl, C_{1-6} alkoxy, OR^{9c}, CO_2R^{9a}, COR^{9a}, halogen, C_{1-6} haloalkyl, $-N(R^{9a})_2$, C_{1-6} acylamino, $CO_2N(R^{9a})_2$, SR^{9a}, $-NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^{9a})_2$, $-SO_2C_{1-6}$ alkyl, COR^{9b}, and cyano, and wherein said monocyclic heteroaryl and substituted monocyclic heteroaryl has 1-2 heteroatoms that are independently selected from the group consisting of N, O, and S, with the provisos that:

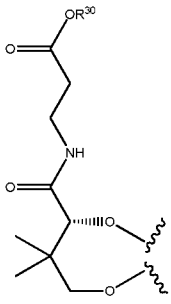
a) when there are two heteroatoms and one is O, then the other can not be O or S, and

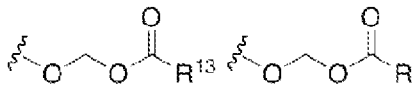
b) when there are two heteroatoms and one is S, then the other can not be O or S;

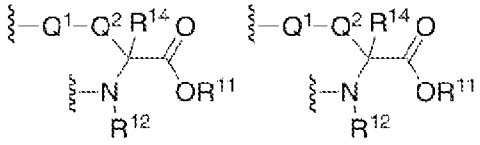
R^{9a} is independently H or C_{1-6} alkyl;

R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

R^{9c} is H or C_{1-6} acyl;

(f) R^2 and R^3 can come together to form a ring  where R^{30} is H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

(g)  where R^{13} is selected from the group consisting of H, C_{1-10} alkyl, C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl moiety; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

(h) R^2 and R^3 can come together to form a ring  where R^{14} is:

(i) independently selected from the group consisting of H, C_{1-10} alkyl, -$(CH_2)_rNR_2^{9a}$, C_{1-6} hydroxyalkyl, -CH_2SH, -$(CH_2)_2S(O)_pMe$, -$(CH_2)_3NHC(=NH)NH_2$, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -$(CH_2)_mCOR^{9b}$, aryl, aryl-C_{1-3} alkyl, heteroaryl, and heteroaryl-C_{1-3} alkyl, wherein said aryl and heteroaryl groups are optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, halogen, nitro, and cyano;

(ii) R^{14} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, CH_2((4'-OH)-Ph), CH_2SH, or C_{3-10} cycloalkyl;

p is 0 to 2;

r is 1 to 6;

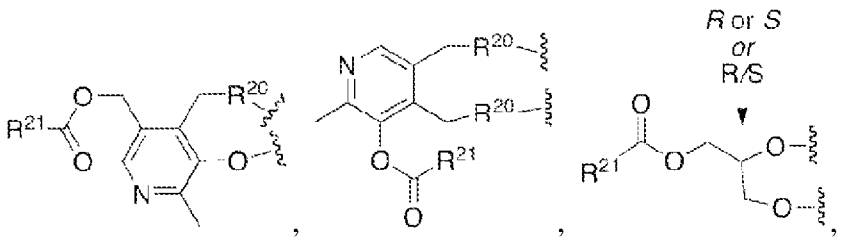
m is 0 to 3

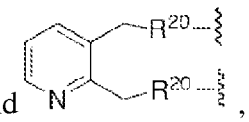
Q^1 is NR^{9a}, O, or S

Q^2 is C_{1-10} alkyl, C_{1-6} hydroxyalkyl, aryl and aryl-C_{1-3} alkyl, heteroaryl and heteroaryl-C_{1-3} alkyl, said aryl and heteroaryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C_{1-10} alkyl, C_{1-6} alkoxy, fluoro, and chloro;

R^{11} is H, C_{1-10} alkyl, C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl moiety; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

R^{12} is H or C_{1-3} alkyl, or R^{14b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

(i) R^2 and R^3 can come together to form a ring selected from the group consisting of 

and  ,

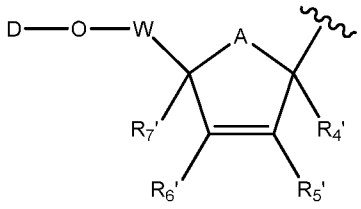
where R^{20} is O or NH and

R^{21} is selected from the group consisting of H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty acid, and C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl, and

(j) R^2 is a monophosphate ester or a diphosphate ester when R^3 is OH, O^-K^+, O^-Li^+, or O^-Na^+.

7. The compound of Claim 6, wherein $R^{7'}$ is independently selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R.

8. The compound of Claim 1, wherein Sugar is ribose or modified ribose of the general formula (**V**):



(**V**)

wherein:

D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

R^1 is independently C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

W is CL_2 or CL_2CL_2, wherein L independently is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl,

wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl can each optionally contain one or more heteroatoms;

A, R^2, R^3, Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Claims 1-3;

wherein, when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, and NR_2,

wherein R is independently a C_1-C_6 alkyl, C_{2-6} alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Claims 1-3,

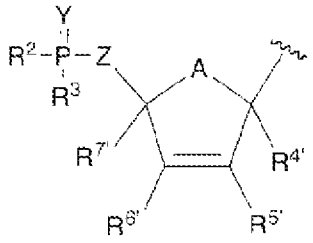
and wherein, when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H, then, $R^{5'}$ and $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH=CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

9. The compound of Claim 8, wherein $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R, wherein R is independently C_1-C_6 alkyl, C_{2-6} alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV.

10. The compound of Claim 8, wherein R^5 and $R^{6'}$ are independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2.

11. The compound of Claim 8, wherein when A is O or CH_2, D is H or acyl, W is CH_2, $R^{4'}$ and $R^{7'}$ are H then, $R^{5'}$ and $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH=CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

12. The compound of Claim 1, wherein Sugar is a modified ribose of the general Formula (**VI**):



(**VI**)

wherein:

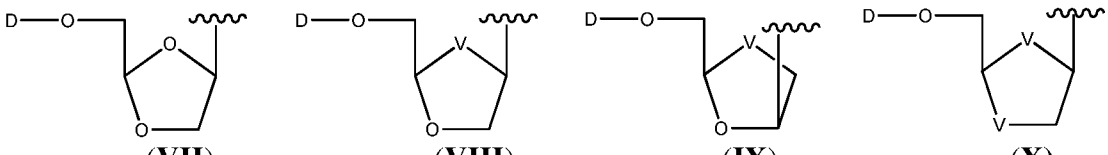
A, R^2, R^3, Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Claims 1-3;

wherein, when A is O or S, $R^{7'}$cannot be OH, SH, NH_2, NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2,

wherein R is independently a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can optionally be substituted with one or more substituents as defined above in connection with Claims 1-3.

13. The compound of Claim 12, wherein $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3, C(O)OH, CN, CH_2OH, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, and R.

14. The compound of Claim 1, wherein Sugar is a dioxolane, an oxathiolane, or a dithiolane of the general Formulas (**VII**), (**VIII**), (**IX**), and (**X**):



(**VII**) (**VIII**) (**IX**) (**X**)

D is H, $C(O)R^1$, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

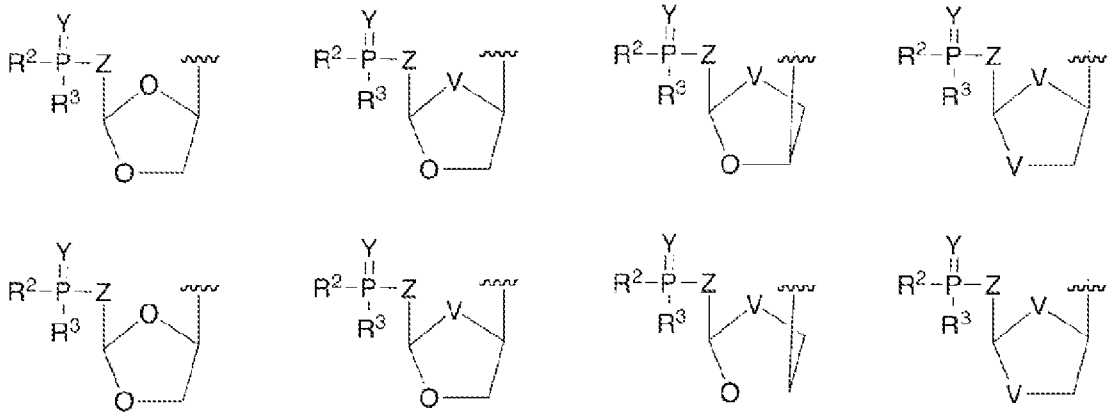
V is, individually, S or Se;

R^1 is independently $C_{1\text{-}20}$ alkyl, the carbon chain derived from a fatty alcohol or $C_{1\text{-}20}$ alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, $C_{3\text{-}10}$ cycloalkyl, $C_{3\text{-}10}$ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted

heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_1-C_6 alkyl, C_1-C_6 alkoxy, di(C_1-C_6 alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl;

wherein D cannot be H or acyl.

15. The compound of Claim 1, wherein Sugar is a dioxolane, oxathiolane, or dithiolane of the general formulas (**XI**), (**XII**), (**XIII**), and (**XIV**):



(**XI**) (**XII**) (**XIII**)

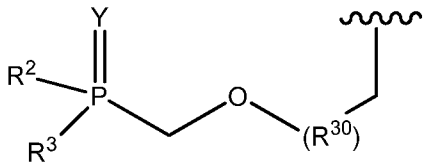
(**XIV**)

wherein:

V is, individually, S or Se;

R^2, R^3, Y, and Z are as defined above with respect to Claims 1-3.

16. The compound of Claim 1, wherein Sugar is a phosphonylmethoxyalkyl of the general Formula (**XV**):



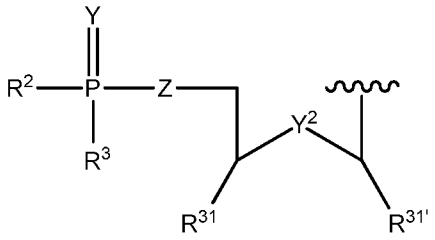
(**XV**)

wherein:

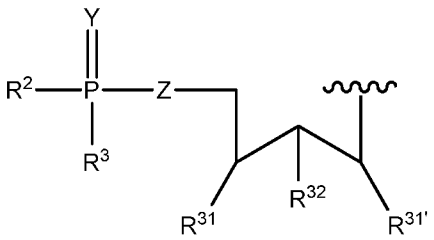
R^2, R^3, and Y are as defined above with respect to Claims 1-3; and

R^{30} is selected from the group consisting of C_{1-20} alkyl, C_{2-20} alkyl, C_{2-20} alkenyl, C_{2-20} alkynyl, C_{3-10} cycloalkyl, aryl, heteroaryl, arylalkyl, and alkylaryl.

17. The compound of Claim 1, wherein Sugar is of the general Formulas (**XVI**) or (**XVII**):



(**XVI**)



(**XVII**)

wherein:

R^2, R^3, Z, and Y are as defined above in Claims 1-3;

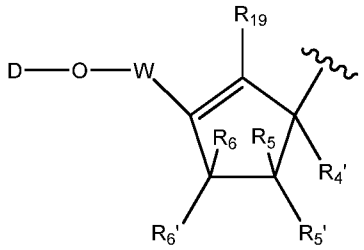
Y^2 is O, S, Se, or NR;

R is, independently, a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1,

R^{31}, $R^{31'}$ and R^{32} are H, CH_3, or CH_2OR^{33}; and

R^{33} is H or C_1-C_6 acyl.

18. The compound of Claim 1, wherein Sugar is a modified ribose of the general Formula (**XVIII**)



(**XVIII**)

wherein:

D, W, $R^{4'}$, R^5, $R^{5'}$, R^6, and $R^{6'}$ are as defined above in Claims 1-3;

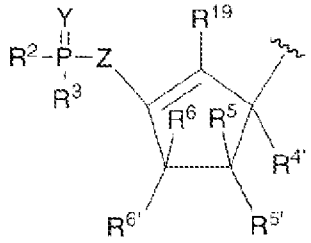
R^{19} is H, F, Cl, Br, I, N_3, C(O)OH, CN, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, or R;

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above,

wherein, when D is H or acyl, W is CH_2, $R^{4'}$ and R^{19} are H, then, R^5, $R^{5'}$, R^6, $R^{6'}$ cannot be H, halogen, OH, SH, OCH_3, SCH_3, NH_2, $NHCH_3$, CH_3, $CH{=}CH_2$, CN, CH_2NH_2, CH_2OH, or COOH.

19. The compound of Claim 18, wherein $R^{6'}$ is independently selected from the group consisting of NHOH, $NHNH_2$, N_3, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, R, OR, SR, SSR, NHR, and NR_2.

20. The compound of Claim 1, wherein Sugar is a modified ribose of the general formulas (**XIX**):



(**XIX**)

wherein:

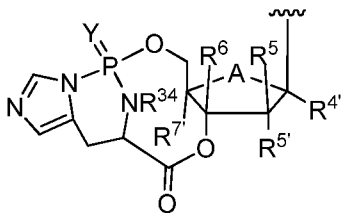
R^2, R^3, and Y are as defined above with respect to Claims 1-3;

$R^{4'}$, R^5, $R^{5'}$, R^6, and $R^{6'}$ are as defined above in Claims 1-3;

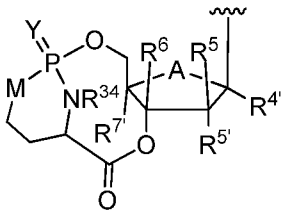
R^{19} is H, F, Cl, Br, I, N_3, C(O)OH, CN, $C(O)NH_2$, $C(S)NH_2$, C(O)OR, or R, and

wherein R is independently C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can optionally be substituted with one or more substituents as defined above in connection with Claims 1-3.

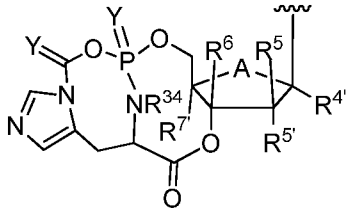
21. The compound of Claim 1, wherein Sugar has one of the Formulas (**XX**), (**XXI**), or (**XXII**):



(**XX**)



(**XXI**)



(**XXII**)

wherein:

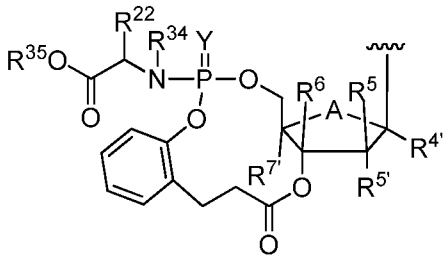
$R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, and $R^{7'}$ are as defined above with respect to Claims 1-3;

R^{34} is C_1-C_6 alkyl;

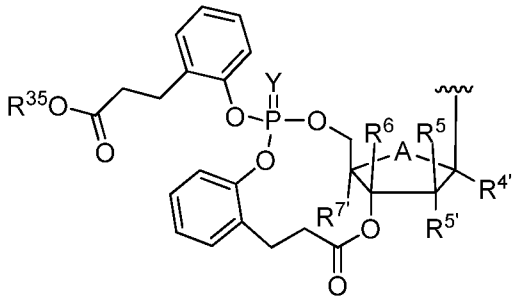
M is O, S, or NR;

wherein R is, independently, C_1-C_6 alkyl, $C_{2\text{-}6}$ alkenyl, $C_{2\text{-}6}$ alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Claims 1-3.

22. The compound of Claim 1, wherein Sugar has of one of the Formulas (**XXIII**) or (**XXIV**):



(**XXIII**)



(**XXIV**)

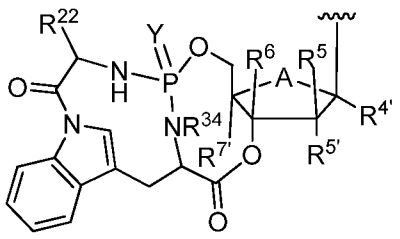
wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, A, $R^{7'}$, R^{34} are as defined above with respect to Claims 1-3;

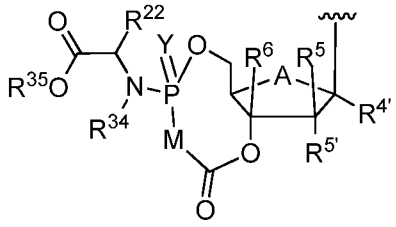
R^{35} is H, C_{1-10} alkyl, C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl moiety; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or C_{3-10} cycloalkyl alkyl; and

R^{22} is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, CH_2-indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H, $CH_2C(O)NH_2$, CH_2CH_2COOH, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $CH_2CH_2CH_2NHC(NH)NH_2$, CH_2-imidazol-4-yl, CH_2OH, $CH(OH)CH_3$, $CH_2((4'\text{-}OH)\text{-}Ph)$, CH_2SH, or C_{3-6} cycloalkyl.

23. The compound of Claim 1, wherein Sugar has one of the Formulas (**XXV**) or (**XXVI**):



(**XXV**)

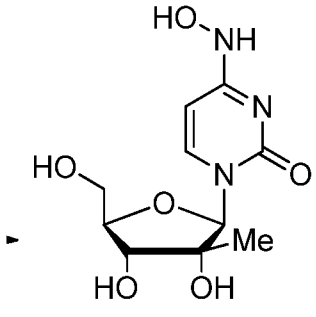


(**XXVI**)

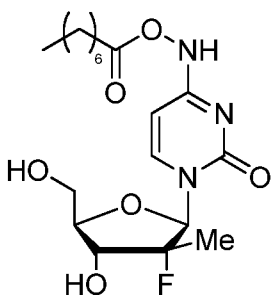
wherein:

$R^{4'}$, R^5, $R^{5'}$, R^6, Y, M, $R^{7'}$, R^{34}, R^{35}, R^{22} are as defined above with respect to Claims 1-3.

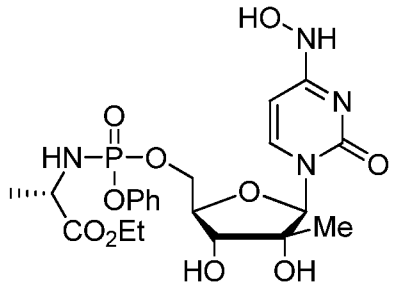
24. A compound of one of the following formulas:



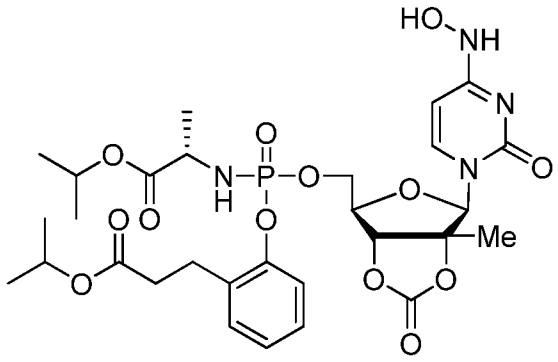
37



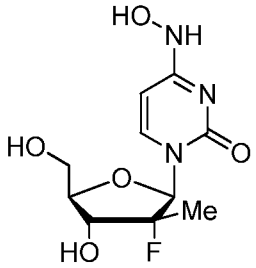
40



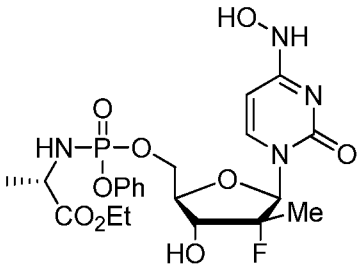
44



48



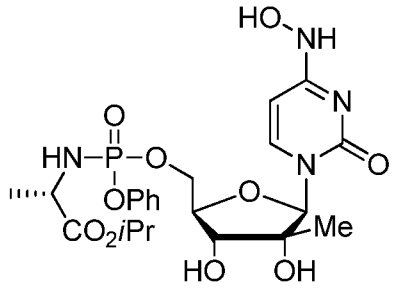
39



51

, or pharmaceutically acceptable salts thereof.

25. A compound of the following formula:



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, or pharmaceutically acceptable salts thereof.

26. The compound of any of Claims 1-25, wherein the compounds are described herein can be in the form of the β-L- or β-D-configuration, or a mixture thereof, including a racemic mixture thereof.

27. The compound of any of Claims 1-25, wherein when the phosphorous portion of the compound described herein contains a chiral center, such chiral center

can be in the form of the R_p- or S_p-configuration or a mixture thereof, including a racemic mixture thereof.

28. A method for treating a host infected with HIV-1 or HIV-2, comprising administering an effective amount of a compound of any of Claims 1 to 27 to a patient in need of treatment thereof.

29. A method for preventing an HIV-1 or HIV-2 infection, comprising administering an prophylactically-effective amount of a compound of any of Claims 1 to 25 to a patient in need of prophylaxis thereof.

30. A method for reducing the biological activity of an HIV-1 or HIV-2 infection in a host, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

31. The method of Claim 26, wherein the HIV-1 or HIV-2 infection is caused by a virus comprising a mutation selected from the group consisting of TAM mutations and the M184V mutation.

32. A method for treating a host infected with HIV-1 or HIV-2 that includes administering an effective amount of a compound of any of Claims 1 to 24 in a pharmaceutically acceptable carrier in combination with another anti-HIV agent.

33. The method of Claim 32, wherein the HIV-1 or HIV-2 infection is caused by a virus comprising a mutation selected from the group consisting of TAM mutations and the M184V mutation.

34. A method for preventing an HIV-1 or HIV-2 infection, comprising administering a prophylactically-effective amount of a compound of any of Claims 1 to 25, in a pharmaceutically acceptable carrier, in combination with another anti-HIV agent, to a patient in need or prophylaxis thereof.

35. A method for treating a host infected with HBV, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

36. A method for preventing an HBV infection, comprising administering a phrophylactically effective amount of a compound of any of Claims 1 to 25 to a patient in need of prophylaxis thereof.

37. A method for reducing the biological activity of an HBV infection in a host, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

38. A method for treating a host infected with HBV that includes administering an effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier in combination with another anti-HBV agent.

39. A method for preventing an HBV infection, comprising administering a prophylactically-effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier, in combination with another anti-HBV agent, to a patient in need of prophylaxis thereof.

40. A method for treating a host infected with Norovirus or Saporovirus, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

41. A method for preventing an Norovirus or Saporovirus infection, comprising administering a phrophylactically effective amount of a compound of any of Claims 1 to 25 to a patient in need of prophylaxis thereof.

42. A method for reducing the biological activity of an Norovirus or Saporovirus infection in a host, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

43. A method for treating a host infected with Norovirus or Saporovirus that includes administering an effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier in combination with another anti-Norovirus or anti-Saporovirus agent.

44. A method for preventing an Norovirus or Saporovirus infection, comprising administering a prophylactically-effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier, in combination with another anti-Norovirus or anti-Saporovirus agent, to a patient in need of prophylaxis thereof.

45. A method for treating a host infected with Flaviviridae family of viruses including HCV, Yellow fever, Dengue, and West Nile virus comprising administering

an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

46. A method for preventing an infection from a Flaviviridae family of viruses including HCV, Yellow fever, Dengue, and West Nile virus, comprising administering a phrophylactically effective amount of a compound of any of Claims 1 to 25 to a patient in need of prophylaxis thereof.

47. A method for reducing the biological activity of an infection with Flaviviridae family of viruses including HCV, Yellow fever, Dengue, and West Nile virus in a host, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

48. A method for treating a host infected with a with Flaviviridae family of viruses including HCV, Yellow fever, Dengue, and West Nile virus that includes administering an effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier in combination with another anti-Norovirus or anti-Saporovirus agent.

49. A method for preventing an infection from a Flaviviridae family of viruses including HCV, Yellow fever, Dengue, and West Nile virus, comprising administering a prophylactically-effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier, in combination with another anti-Norovirus or anti-Saporovirus agent, to a patient in need of prophylaxis thereof.

50. A method for treating a host infected with HSV-1 or HSV-2, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

51. A method for preventing an HSV-1 or HSV-2 infection, comprising administering a phrophylactically effective amount of a compound of any of Claims 1 to 25 to a patient in need of prophylaxis thereof.

52. A method for reducing the biological activity of an HSV-1 or HSV-2 infection in a host, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

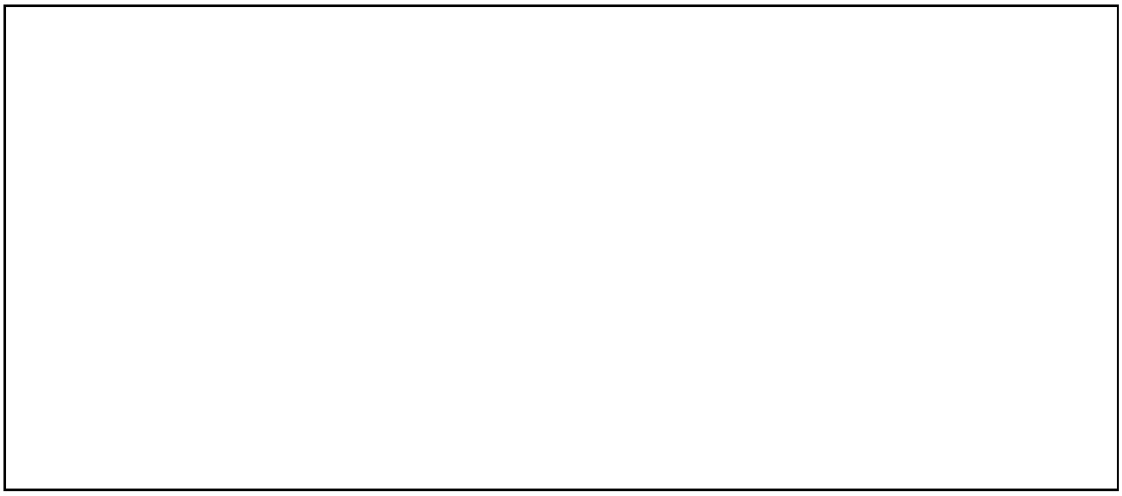
53. A method for treating a host infected with HSV-1 or HSV-2 that includes administering an effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier in combination with another anti-HSV-1 AND HSV-2 agent.

54. A method for preventing an HSV-1 or HSV-2 infection, comprising administering a prophylactically-effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier, in combination with another anti-HSV-1 or anti-HSV-2 agent, to a patient in need of prophylaxis thereof.

55. A method for treating a host with cancer, comprising administering an effective amount of a compound of any of Claims 1 to 25 to a patient in need of treatment thereof.

56. A method for treating a host with cancer that includes administering an effective amount of a compound of any of Claims 1 to 25 in a pharmaceutically acceptable carrier in combination with another anti-cancer agent.

57. The method of any of Claims 28-56, wherein the compounds are converted in a biological system to a mixture of compounds comprising mixture **C** or **D** of 4-NHOH, 4-NH_2 and 4-OH pyrimidine triphosphates:



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58. The use of a compound of any of Claims 1-27 in the preparation of a medicament for use in treating a host infected with HIV-1 or HIV-2, preventing an HIV-1 or HIV-2 infection, or reducing the biological activity of an HIV-1 or HIV-2 infection in a host.

59. The use of Claim 59, wherein the medicament further comprises another anti-HIV agent.

60. The use of a compound of any of Claims 1-27 in the preparation of a medicament for use in treating a host infected with HBV, preventing an HBV infection, or reducing the biological activity of an HBV infection in a host.

61. The use of Claim 60, wherein the medicament further comprises another anti-HBV agent.

62. The use of a compound of any of Claims 1-27 in the preparation of a medicament for use in treating a host infected with a Flaviviridae, Norovirus or Saporovirus infection, preventing a Flaviviridae, Norovirus or Saporovirus infection, or reducing the biological activity of a Flaviviridae, Norovirus or Saporovirus infection in a host.

63. The use of Claim 62, wherein the medicament further comprises another anti- Flaviviridae, anti-Norovirus or anti-Sapovirus agent.

64. The use of a compound of any of Claims 1-27 in the preparation of a medicament for use in treating a host infected with HSV-1 or HSV-2, preventing an HSV-1 or HSV-2infection, or reducing the biological activity of an HSV-1 or HSV-2 infection in a host.

65. The use of Claim 64, wherein the medicament further comprises another anti-HSV-1 or anti-HSV-2 agent.

66. The use of a compound of any of Claims 1-27 in the preparation of a medicament for use in treating cancer.

67. The use of Claim 67, wherein the medicament further comprises another anti-cancer agent.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/067309

A. CLASSIFICATION OF SUBJECT MATTER

(see extra sheet)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07H 19/06, 19/10, A61K 31/7068, A61P 31/18, 31/22, 31/14, 35/00, 31/12, C07H 19/04, A61K 31/55, 31/7076, C07H 19/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, VINITI, DWPI, EAPATIS, Espacenet, PAJ, USPTO, CIPO, DEPATISnet, Patentscope

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2005/020885 A2 (ISIS PHARMACEUTICALS, INC.) 10.03.2005, pp. 169, 209	1, 24, 26, 27 2, 16-17, 19-23, 25
X Y	US 2005/0043268 A1 (DAVID LOAKES et al.) 24.02.2005, fig. 9, claims, table 1	1, 28-30, 35-37, 40-42, 58, 60, 62 31, 33
X Y	US 2005/0026902 A1 (TIMOTHY MAZIASZ) 03.02.2005, p. 53, compound 30, abstract	1, 3-5, 28-30, 58 31, 33
X	WO 2004/037159 A2 (OBETHERAPY BIOTECHNOLOGY et al.) 06.05.2004, pp. 121, 124	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2013 (19.12.2013)	27 March 2014 (27.03.2014)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer E. Guseva Telephone No. (495)531-64-81

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/067309

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/068162 A2 (PHARMASSET LTD. et al.) 21.08.2003, example 13, abstract, pp.11-13	1, 45-49, 55-56, 62-63, 66-67
Y		43-44, 53-54, 65
X	WO 2002/032920 A2 (PHARMASSET LIMITED et al.) 25.04.2002, pp. 16-17, 26-28, 47, 52, 57, 93, 168-173, examples 39-40	1, 3-5, 18, 28-30, 35-37, 45-47, 50-52, 55-56, 58, 60, 62, 64
Y		31, 33
X	EP 0576230 A1 (ELI LILLY AND COMPANY) 29.12.1993, example 1, abstract, tables, claims	1, 3-5, 26, 50-52, 64, 55, 66
X	US 5496935 A (MAX-DELBRUCK-CENTRUM) 05.03.1996, claims, examples 4-5, 7-8, table	1, 26, 28-30, 58
Y		31, 33
X	US 2009/0105186 A1 (MAX-DELBRUECK-CENTRUM FUER MOLEKULARE MEDIZIN) 23.04.2009, paragraphs [0107] - [0167], claims	1, 6-9, 11-15, 26, 28-30 32, 34, 38-39, 55, 59, 58, 60-61, 66
Y		31, 33, 43-44, 53-54, 65
X	CN 102351931 A (HIGH & NEW TECHNOLOGY RES CT OF HENAN ACADEMY OF SICENCES et al.) 15.02.2012, pp. 48, 58, compound 5-17, abstract	1, 3-5, 55, 66
X	CN 1626543 A (RES CT FOR QUALITY EXAMINATION et al.) 15.06.2005, pp. 5, 6, abstract	1
X	FOX, Jack J. et al. Thiation of Nucleosides. II. Synthesis of 5-methyl-2'-deoxycytidine and Related Pyrimidine Nucleosides. Journal of the American Chemical Society, 1959, 81, pp.178-187, especially pp. 179, 181, 182, Experimental	1
X	SHI, Junxing et al. Synthesis and anti-viral activity of a series of D- and L-2'-deoxy-2'-fluororibonucleosides in the subgenomic HVC replicon system. Bioorganic & Medicinal Chemistry, 2005, 13(5), pp. 1641-1652, especially, pp.1641-1643, Experimental	1, 45-47, 62
X	BANKS, G. R. et al. Mutagenic Analogues of Cytosine: RNA Polymerase Template and Substrate Studies. Journal of Molecular Biology, 1971, 60(3), pp. 425-439, especially, pp. 425-427, 438	1
X	SUZUKI, Tetsuya et al. Template properties of mutagenic cytosine analogues in reverse transcription. Nucleic Acids Research, 2006, 34(22), pp. 6438-6449, especially, pp.6438-6440	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/067309

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

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

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

2. ☒ Claims Nos.: 10
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Dependent claim 10 is unclear and therefore has not been searched. Claim 10 specifies the meanings of radical R^5, however, such radical is absent in the structural formula (V) defined in independent claim 8.

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

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Classification of subject matter

International application No.
PCT/US 2013/067309

C07H 19/06 *(2006.01)*
C07H 19/10 *(2006.01)*
A61K 31/7068 *(2006.01)*
A61P 31/18 *(2006.01)*
A61P 31/22 *(2006.01)*
A61P 31/14 *(2006.01)*
A61P 35/00 *(2006.01)*
A61P 31/12 *(2006.01)*

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(71)申请人 共晶制药股份有限公司
地址 美国乔治亚州
申请人 埃默里大学
(72)发明人 弗兰克·安布拉尔
斯蒂文·J·科阿斯
雷蒙德·F·斯基那兹
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A61K 31/7068(2006.01)
A61P 31/18(2006.01)
A61P 31/22(2006.01)
A61P 31/14(2006.01)
A61P 35/00(2006.01)
A61P 31/12(2006.01)

权利要求书15页 说明书86页

(54)发明名称

用于治疗病毒感染和癌症的嘧啶核苷及其单磷酸酯前药

(57)摘要

本发明涉及用于治疗或预防在人患者或其它动物宿主中的癌症和病毒感染，特别是HIV、HCV、诺瓦克病毒、札如病毒、巨细胞病毒(CMV)、疱疹病毒(HSV-1、HSV-2)、登革热病毒、黄热病或HBV的化合物、组合物及方法。所述化合物是某些A^-羟基胞苷核苷衍生物、修饰的单磷酸盐和膦酸盐前药类似物、及其药学上可接受的盐、前药及其它衍生物。具体地说，所述化合物显示针对HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、巨细胞病毒(CMV)、疱疹病毒(HSV-1、HSV-2)、登革热病毒、黄热病及HBV的有效抗病毒活性。

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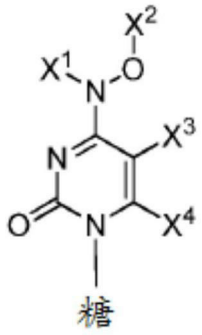
1. 一种式 (I) 化合物 :



(I)

或其药学上可接受的盐或前药,其中 :

X^1是 H、C_1-C_6烷基、C_1-C_6卤烷基、C_1-C_6烷氧基、C_2-C_6烯基、C_2-C_6炔基、COR^1或 $COOR^1$;

X^2是氢、COR^1或 $COOR^1$

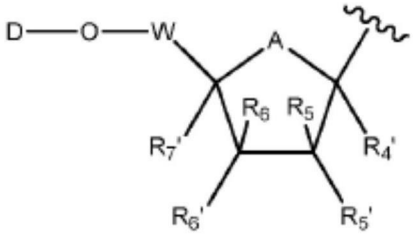
其中每个 R^1独立地是 CH_2-O(CO)-X^5、CH_2-O(CO)O-X^5、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基 :C_1-C_6烷基、烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基 ;其中所述取代基是 C_{1-6}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟或 C_{3-10}环烷基取代的 C_{1-6}烷基

X^5独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基 :C_1-C_6烷基、烷氧基、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基 ;其中所述取代基是 C_{1-6}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟或 C_{3-10}环烷基取代的 C_{1-6}烷基

X^3和 X^4各自独立地是 H、C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、芳基、烷基芳基、卤素、NH_2、OH、SH、CN 或 NO_2。

2. 如权利要求 1 所述的化合物,其中每个 R^1独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基、C_1-C_6烷基、烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基 ;其中所述取代基是 C_{1-6}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟或 C_{3-10}环烷基取代的 C_{1-6}烷基。

3. 如权利要求 1 所述的化合物,其中糖是通式 (II) 的核糖或修饰的核糖 :



(II)

其中 :

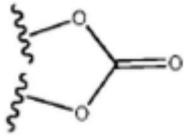
D 是 H、C(O)R^1、C(O)OR^1、二磷酸酯或三磷酸酯 ;

R^1如上所定义 ;

W 是 CL_2 或 CL_2CL_2，其中 L 独立地选自由以下组成的组 ：H、C_{1-6} 烷基、C_{2-6} 烯基及 C_{2-6} 炔基，其中 C_{1-6} 烷基、C_{2-6} 烯基及 C_{2-6} 炔基可各自任选地含有一个或多个杂原子 ；

A 是 O、S、CH_2、CHF、CF_2、C ＝ CH_2、C ＝ CHF 或 C ＝ CF_2；

$R^{4'}$、R^5、$R^{5'}$、R^6、$R^{6'}$ 及 $R^{7'}$ 独立地选自由以下组成的组 ：H、F、Cl、Br、I、OH、SH、NH_2、NHOH、$NHNH_2$、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

$R^{5'}$ 和 $R^{6'}$ 可一起形成环 

其中 ：

当 A 是 O 或 CH_2，D 是 H 或酰基，W 是 CH_2，$R^{4'}$ 和 $R^{7'}$ 是 H 时，R^5、$R^{5'}$、R^6、$R^{6'}$ 不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ＝ CH_2、CN、CH_2NH_2、CH_2OH、COOH

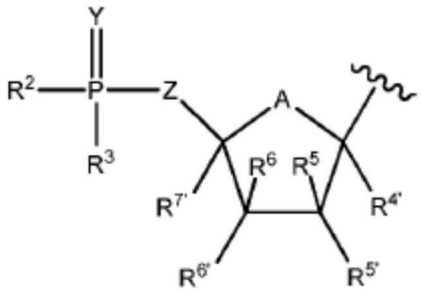
当 A 是 O 或 S 时，$R^{7'}$ 不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2，并且

R 独立地是 C_1-C_6 烷基、C_{2-6} 烯基、C_{2-6} 炔基、C_{3-6} 环烷基、(C_3-C_6 环烷基) 芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上面在权利要求 1 中所定义的取代基取代。

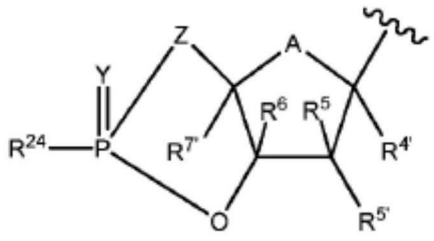
4. 如权利要求 3 所述的化合物，其中 $R^{6'}$ 独立地选自由以下组成的组 ：NHOH、$NHNH_2$、N_3、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2，并且其中 R 独立地是 C_1-C_6 烷基、C_{2-6} 烯基、C_{2-6} 炔基、C_{3-6} 环烷基、(C_3-C_6 环烷基) 芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上面在权利要求 1 中所定义的取代基取代。

5. 如权利要求 3 所述的化合物，其中 $R^{7'}$ 独立地选自由以下组成的组 ：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 及 R，并且其中 R 独立地是 C_1-C_6 烷基、C_{2-6} 烯基、C_{2-6} 炔基、C_{3-6} 环烷基、(C_3-C_6 环烷基) 芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上面在权利要求 1 中所定义的取代基取代。

6. 如权利要求 1 所述的化合物，其中糖是通式 (III) 或 (IV) 的核糖或修饰的核糖 ：



(III)



(IV)

其中 ：

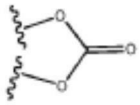
Y 是 O 或 S ；

Z 选自由以下组成的组 ：CL_2、CL_2CL_2、CL_2OCL_2、CL_2SCL_2、CL_2O、OCL_2 及 CL_2NHCL_2，其中 L 独立地选自由以下组成的组 ：H、F、C_{1-6} 烷基、C_{2-6} 烯基及 C_{2-6} 炔基，其中 C_{1-6} 烷基、C_{2-6} 烯基及 C_{2-6} 炔基可各自任选地含有一个或多个杂原子 ；

A 是 O、S、CH_2、CHF、CF_2、C ＝ CH_2、C ＝ CHF 或 C ＝ CF_2；

$R^{4'}$、R^5、$R^{5'}$、R^6、$R^{6'}$ 及 $R^{7'}$ 独立地选自由以下组成的组 ：H、F、Cl、Br、I、OH、SH、NH_2、NHOH、

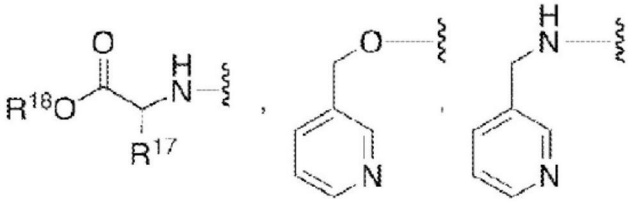
$NHNH_2$、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

$R^{5'}$ 和 $R^{6'}$ 可一起形成环 

其中当 A 是 O 或 S 时，$R^{7'}$ 不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2，并且

R 独立地是 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上面在权利要求 1 中所定义的取代基取代，

R^{24}选自由以下组成的组：OR^{15}、



及脂肪醇，

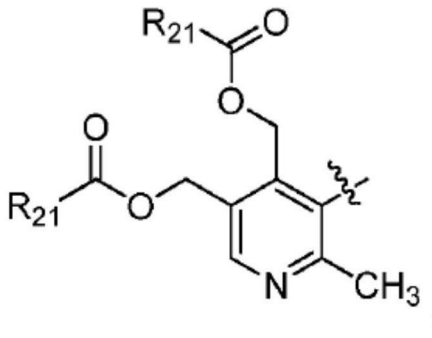
R^{15}选自由以下组成的组：H、Li、Na、K、苯基及吡啶基；其中苯基和吡啶基任选地被一个至三个独立地选自由 $(CH_2)_{0-6}CO_2R^{16}$和 $(CH_2)_{0-6}CON(R^{16})_2$组成的组的取代基取代；

R^{17}选自在天然 L- 氨基酸中存在的那些基团、C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上面在权利要求 1 中所定义的取代基取代，

R^{18}是 H、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基或环烷基取代的 C_{1-5}烷基；

R^2和 R^3独立地选自由以下组成的组：

(a)OR^8，其中 R^8是 H、Li、Na、K、C_{1-20}烷基、C_{3-6}环烷基、C_{1-6}卤烷基、芳基或杂芳基，任选地被一个至三个独立地选自由以下组成的组的取代基取代：C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、C_{1-6}烷氧基、$(CH_2)_{0-6}CO_2R^{9a}$、卤素、C_{1-6}卤烷基、$-N(R^{9a})_2$、C_{1-6}酰基氨基、$-NHSO_2C_{1-6}$烷基、$-SO_2N(R^{9a})_2$、$-SO_2C_{1-6}$烷基、COR^{9b}、硝基、氰基及

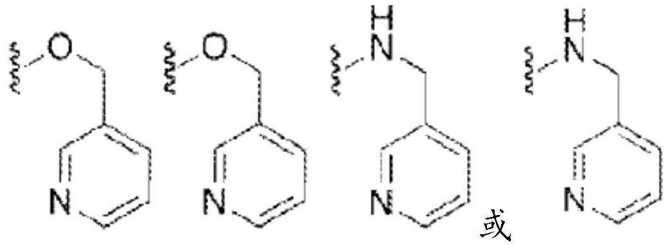


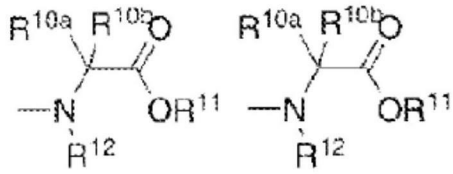
；

其中 R^{21}如下所定义；

R^{9a}独立地是 H、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二 (C_{1-6}烷基)- 氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_{1-6}烷基、C_{1-6}烷氧基、二 (C_{1-6}烷基)- 氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

R^{9b}是 $-OR^{9a}$或 $-N(R^{9a})_2$；

(b)  或 ，

(c)  其中 R^{10a}和 R^{10b}是：

(i)独立地选自由以下组成的组：H、C_{1-10}烷基、$-(CH_2)_rNR^{9a}{}_2$、C_{1-6}羟烷基、$-CH_2SH$、$-(CH_2)_2S(O)_pMe$、$-(CH_2)_3NHC(=NH)NH_2$、(1H-吲哚-3-基)甲基、(1H-咪唑-4-基)甲基、$-(CH_2)_mCOR^{9b}$、芳基及芳基-C_{1-3}烷基，其中所述芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、卤素、硝基及氰基；

(ii)R^{10a}是H且R^{10b}和R^{12}一起是$(CH_2)_{2-4}$以形成包含邻接的N和C原子的环；

(iii)R^{10a}和R^{10b}一起是$(CH_2)_n$以形成环；

(iv)R^{10a}和R^{10b}都是C_{1-6}烷基；或

(v)R^{10a}是H且R^{10b}是H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2-吲哚-3-基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$-$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2-咪唑-4-基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH或C_{3-10}环烷基；

p是0至2；

r是1至6；

n是4或5；

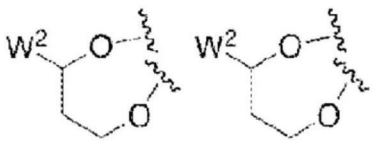
m是0至3；

R^{11}是H、C_{1-10}烷基、或被以下取代的C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基取代的C_{1-5}烷基；

R^{12}是H或C_{1-3}烷基，或R^{10a}、或R^{10b}和R^{12}一起是$(CH_2)_{2-4}$以便形成包含邻接的N和C原子的环；

(d)O连接的脂质(包括磷脂)、N或O连接的肽、O连接的胆固醇或O连接的植物甾醇；

(e)R^2和R^3可在一起形成环

 其中W^2选自由以下组成的组：苯基或单环杂芳基，其任选地被一个至三个独立地选自由以下组成的组的取代基取代：C_{1-6}烷基、CF_3、C_{2-6}烯基、C_{1-6}烷氧基、OR^{9c}、CO_2R^{9a}、COR^{9a}、卤素、C_{1-6}卤烷基、$-N(R^{9a})_2$、C_{1-6}酰基氨基、$CO_2N(R^{9a})_2$、SR^{9a}、$-NHSO_2C_{1-6}$

烷基、$-SO_2N(R^{9a})_2$、$-SO_2C_{1-6}$烷基、COR^{9b}及氰基，并且其中所述单环杂芳基和取代的单环杂芳基具有 1-2 个独立地选自由 N、O 及 S 组成的组的杂原子，前提条件是：

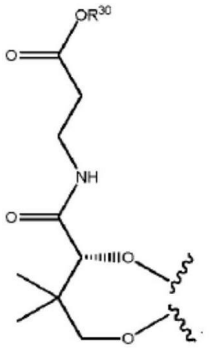
a) 当存在两个杂原子且一个是 O 时，那么另一个不能是 O 或 S，并且

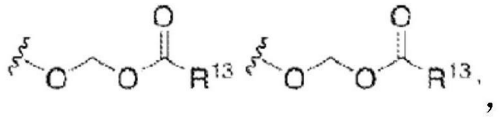
b) 当存在两个杂原子且一个是 S 时，那么另一个不能是 O 或 S；

R^{9a}独立地是 H 或 C_{1-6}烷基；

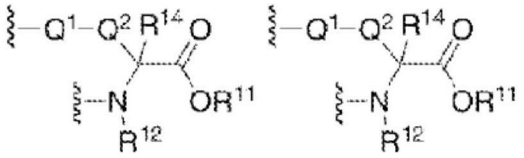
R^{9b}是 $-OR^{9a}$或 $-N(R^{9a})_2$；

R^{9c}是 H 或 C_{1-6}酰基；

(f)R^2和 R^3可在一起形成环  其中 R^{30}是 H、C_{1-20}烷基、C_{1-20}烯基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基或被 C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

(g)  其中 R^{13}选自由以下组成的组：H、C_{1-10}烷基、被以下取代的 C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基部分；其中所述取代基是 C_{1-5}烷基、或被以下取代的 C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基；

(h)R^2和 R^3可在一起形成环

 其中 R^{14}是：

(i) 独立地选自由以下组成的组：H、C_{1-10}烷基、$-(CH_2)_rNR_2^{9a}$、C_{1-6}羟烷基、$-CH_2SH$、$-(CH_2)_2S(O)_pMe$、$-(CH_2)_3NHC(=NH)NH_2$、(1H-吲哚-3-基)甲基、(1H-咪唑-4-基)甲基、$-(CH_2)_mCOR^{9b}$、芳基、芳基-C_{1-3}烷基、杂芳基及杂芳基-C_{1-3}烷基，其中所述芳基和杂芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、卤素、硝基及氰基；

(ii)R^{14}是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2-吲哚-3-基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$、$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2-咪唑-4-基、CH_2OH、$CH(OH)CH_3$、CH_2((4'-OH)-Ph)、CH_2SH 或 C_{3-10}环

烷基；

p 是 0 至 2；

r 是 1 至 6；

m 是 0 至 3

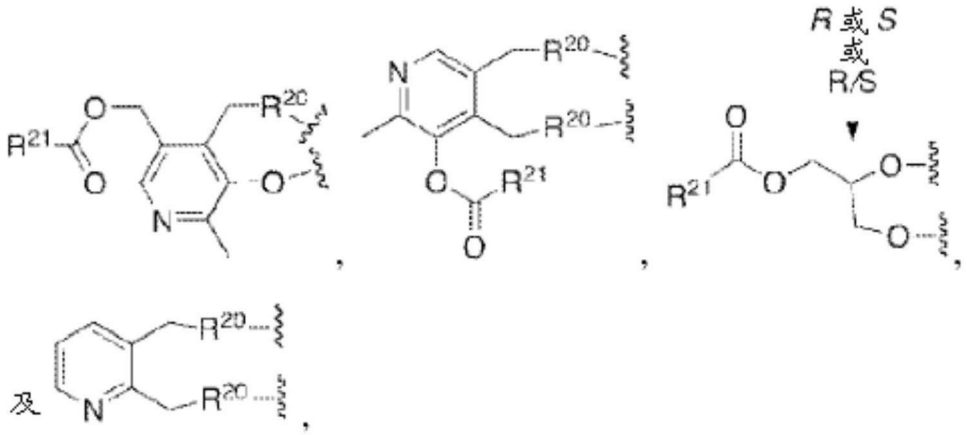
Q^1是 NR^{9a}、O 或 S

Q^2是 C_{1-10}烷基、C_{1-6}羟烷基、芳基和芳基-C_{1-3}烷基、杂芳基及杂芳基-C_{1-3}烷基，所述芳基和杂芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、氟及氯；

R^{11}是 H、C_{1-10}烷基、被以下取代的 C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基部分；其中所述取代基是 C_{1-5}烷基、或被以下取代的 C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基；

R^{12}是 H 或 C_{1-3}烷基，或 R^{14b}和 R^{12}一起是 $(CH_2)_{2-4}$以便形成包含邻接的 N 和 C 原子的环；

(i)R^2和 R^3可在一起形成选自由以下组成的环



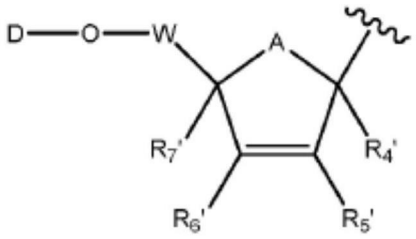
其中 R^{20}是 O 或 NH，并且

R^{21}选自由以下组成的组：H、C_{1-20}烷基、C_{1-20}烯基、来源于脂肪酸的碳链、及被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基或被 C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基，并且

(j) 当 R^3是 OH、O^-K^+、O^-Li^+或 O^-Na^+时，R^2是单磷酸酯或二磷酸酯。

7. 如权利要求 6 所述的化合物，其中 $R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、C(O)NH_2、C(S)NH_2、C(O)OR 及 R。

8. 如权利要求 1 所述的化合物，其中糖是通式 (V) 的核糖或修饰的核糖：



(V)

其中：

D 是 H、C(O)R^1、C(O)OR^1、二磷酸酯或三磷酸酯；

R^1独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）- 氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）- 氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

W 是 CL_2或 CL_2CL_2，其中 L 独立地选自由以下组成的组：H、C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基，

其中 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基可各自任选地含有一个或多个杂原子；

A、R^2、R^3、Y、Z、$R^{4'}$、$R^{5'}$、$R^{6'}$及 $R^{7'}$如上关于权利要求 1-3 所定义；

其中当 A 是 O 或 S 时，$R^{7'}$不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 及 NR_2，

其中 R 独立地是 C_1-C_6烷基、C_{2-6}烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于权利要求 1-3 所定义的取代基取代，

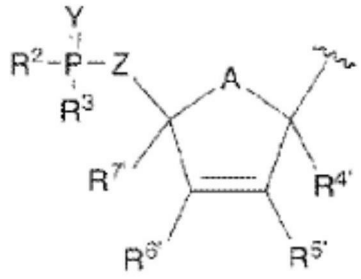
且其中，当 A 是 O 或 CH_2，D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 $R^{7'}$是 H 时，$R^{5'}$和 $R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ＝ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

9. 如权利要求 8 所述的化合物，其中 $R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、C(O)NH_2、C(S)NH_2、C(O)OR 及 R，其中 R 独立地是 C_1-C_6烷基、C_{2-6}烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基取代。

10. 如权利要求 8 所述的化合物，其中 $R^{5'}$和 $R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2。

11. 如权利要求 8 所述的化合物，其中当 A 是 O 或 CH_2，D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 $R^{7'}$是 H 时，$R^{5'}$和 $R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ＝ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

12. 如权利要求 1 所述的化合物，其中糖是通式 (VI) 的修饰的核糖：



(VI)

其中：

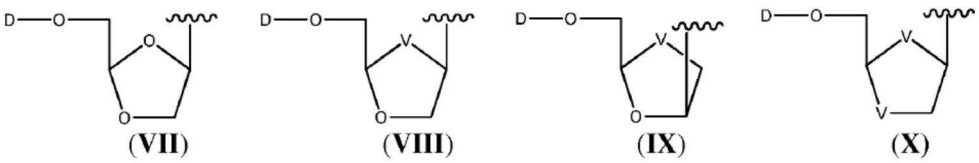
A、R^2、R^3、Y、Z、$R^{4'}$、$R^{5'}$、$R^{6'}$及 $R^{7'}$如上关于权利要求 1-3 所定义；

其中当 A 是 O 或 S 时，$R^{7'}$不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2，

其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可任选地被一个或多个如上关于权利要求 1-3 所定义的取代基取代。

13. 如权利要求 12 所述的化合物，其中 $R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 及 R。

14. 如权利要求 1 所述的化合物，其中糖是通式 (VII)、(VIII)、(IX) 及 (X) 的二氧戊环、氧硫杂环戊烷或二硫戊环：



(VII) (VIII) (IX) (X)

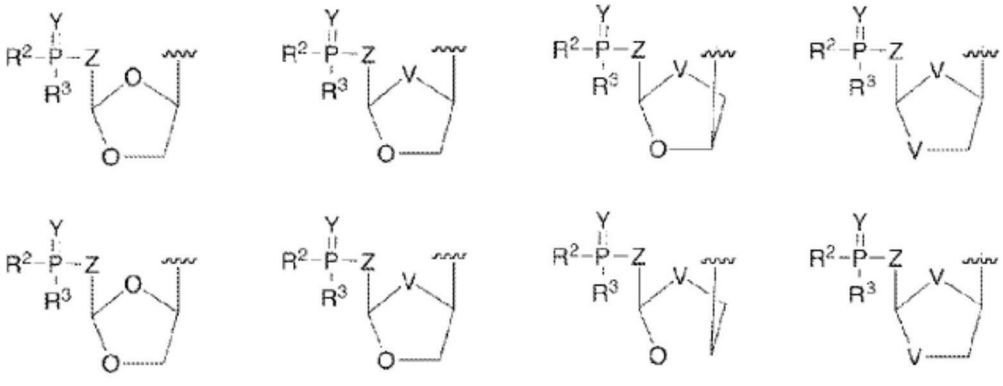
D 是 H、$C(O)R^1$、$C(O)OR^1$、二磷酸酯或三磷酸酯；

V 个别地是 S 或 Se；

R^1独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

其中 D 不能是 H 或酰基。

15. 如权利要求 1 所述的化合物，其中糖是通式 (XI)、(XII)、(XIII) 及 (XIV) 的二氧戊环、氧硫杂环戊烷或二硫戊环：



(XI) (XII) (XIII)

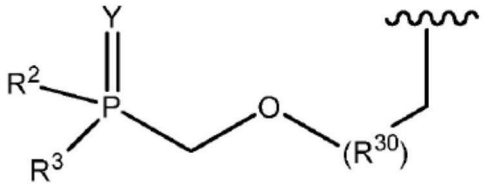
(XIV)

其中：

V 个别地是 S 或 Se；

R^2、R^3、Y 及 Z 如上关于权利要求 1-3 所定义。

16. 如权利要求 1 所述的化合物，其中糖是通式 (XV) 的膦酰基甲氧基烷基：



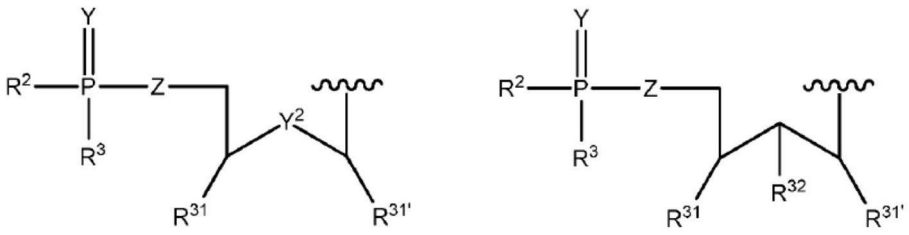
(XV)

其中：

R^2、R^3及 Y 如上关于权利要求 1-3 所定义；并且

R^{30}选自由以下组成的组：C_{1-20}烷基、C_{2-20}烷基、C_{2-20}烯基、C_{2-20}炔基、C_{3-10}环烷基、芳基、杂芳基、芳基烷基及烷基芳基。

17. 如权利要求 1 所述的化合物，其中糖具有通式 (XVI) 或 (XVII)：



(XVI) (XVII)

其中：

R^2、R^3、Z 及 Y 如上面在权利要求 1-3 中所定义；

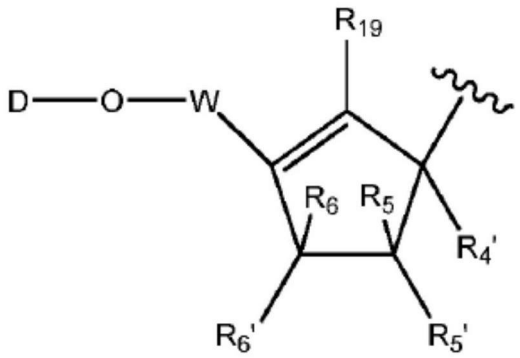
Y^2是 O、S、Se 或 NR；

R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上面在权利要求 1 中所定义的取代基取代，

R^{31}、$R^{31'}$及 R^{32}是 H、CH_3或 CH_2OR^{33}；并且

R^{33}是 H 或 C_1-C_6酰基。

18. 如权利要求 1 所述的化合物，其中糖是通式 (XVIII) 的修饰的核糖：



(XVIII)

其中：

D、W、$R^{4'}$、R^5、$R^{5'}$、R^6及 $R^{6'}$如上面在权利要求 1-3 中所定义；

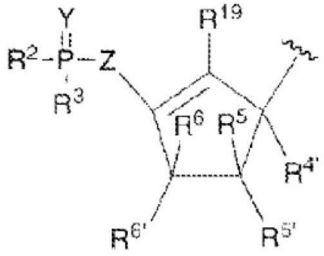
R^{19}是 H、F、Cl、Br、I、N_3、C(O)OH、CN、C(O)NH_2、C(S)NH_2、C(O)OR 或 R；

其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团被一个或多个如上所定义的取代基取代，

其中，当 D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 R^{19}是 H 时，R^5、$R^{5'}$、R^6、$R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ═ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

19. 如权利要求 18 所述的化合物，其中 $R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2。

20. 如权利要求 1 所述的化合物，其中糖是通式 (XIX) 的修饰的核糖：



(XIX)

其中：

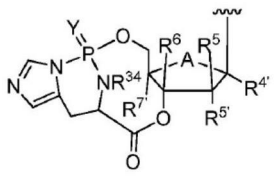
R^2、R^3及 Y 如上关于权利要求 1-3 所定义；

$R^{4'}$、R^5、$R^{5'}$、R^6及 $R^{6'}$如上面在权利要求 1-3 中所定义；

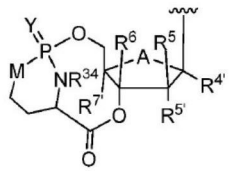
R^{19}是 H、F、Cl、Br、I、N_3、C(O)OH、CN、C(O)NH_2、C(S)NH_2、C(O)OR 或 R，并且

其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可任选地被一个或多个如上关于权利要求 1-3 所定义的取代基取代。

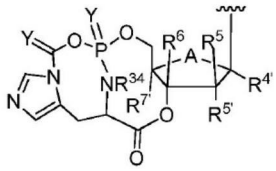
21. 如权利要求 1 所述的化合物，其中糖具有式 (XX)、(XXI) 或 (XXII) 之一：



(XX)



(XXI)



(XXII)

其中：

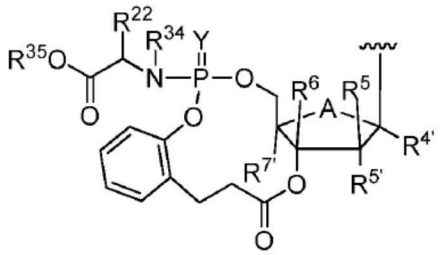
$R^{4'}$、R^5、$R^{5'}$、R^6、Y、A 及 $R^{7'}$如上关于权利要求 1-3 所定义；

R^{34}是 C_1-C_6烷基；

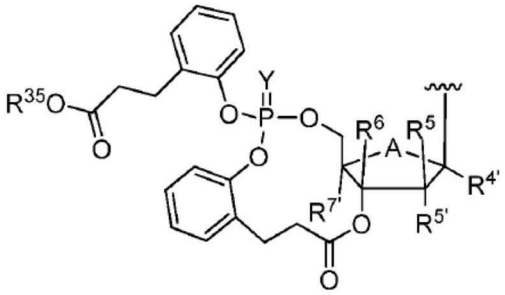
M 是 O、S 或 NR；

其中 R 独立地是 C_1-C_6烷基、C_{2-6}烯基、C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于权利要求 1-3 所定义的取代基取代。

22. 如权利要求 1 所述的化合物，其中糖具有式 (XXIII) 或 (XXIV) 之一：



(XXIII)



(XXIV)

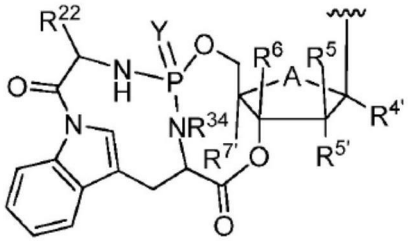
其中：

$R^{4'}$、R^5、$R^{5'}$、R^6、Y、A、$R^{7'}$、R^{34}如上关于权利要求 1-3 所定义；

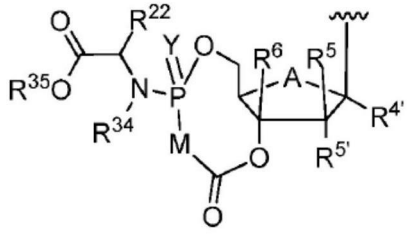
R^{35}是 H、C_{1-10}烷基、被以下取代的 C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二 (C_{1-6}烷基)- 氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基部分；其中所述取代基是 C_{1-5}烷基、或被以下取代的 C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二 (C_{1-6}烷基)- 氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基；并且

R^{22}是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2- 吲哚 -3- 基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$、$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2- 咪唑 -4- 基、CH_2OH、$CH(OH)CH_3$、CH_2((4'-OH)-Ph)、CH_2SH 或 C_{3-6}环烷基。

23. 如权利要求 1 所述的化合物，其中糖具有式 (XXV) 或 (XXVI) 之一：



(XXV)

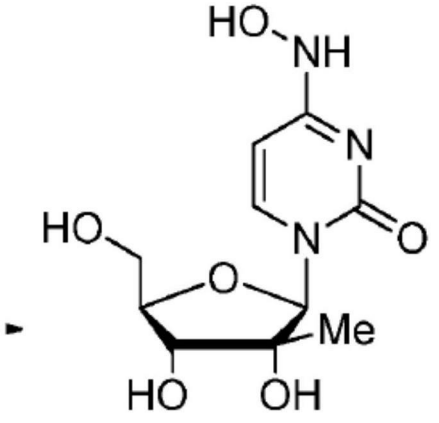


(XXVI)

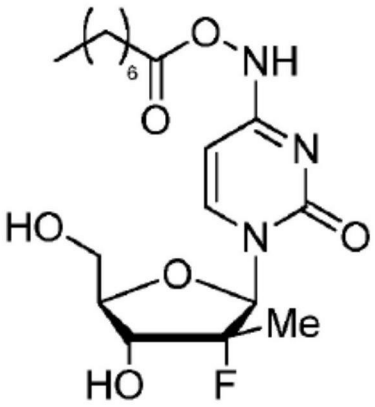
其中：

$R^{4'}$、R^5、$R^{5'}$、R^6、Y、M、$R^{7'}$、R^{34}、R^{35}、R^{22}如上关于权利要求 1-3 所定义。

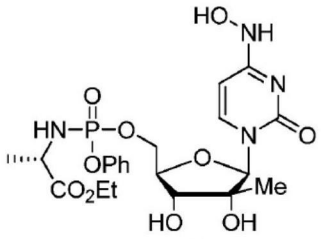
24.一种具有下式之一的化合物：



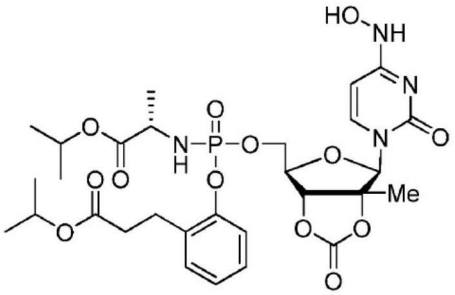
37



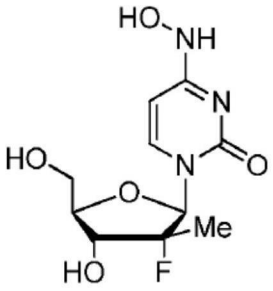
40



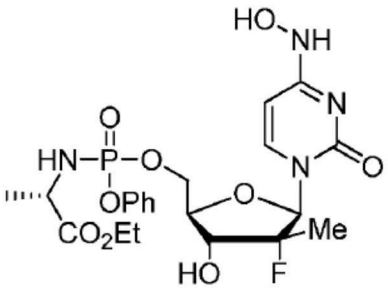
44



48



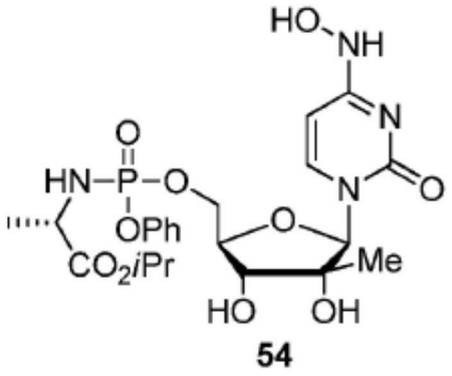
39



51

或其药学上可接受的盐。

25.一种下式的化合物：



54

或其药学上可接受的盐。

，

26. 如权利要求 1-25 中任一项所述的化合物，其中本文所述的化合物可呈以下形式：β-L- 或 β-D- 构型，或其混合物，包括其外消旋混合物。

27. 如权利要求 1-25 中任一项所述的化合物，其中当本文所述的化合物的磷部分含有手性中心时，这种手性中心可呈以下形式：R_p- 或 S_p- 构型或其混合物，包括其外消旋混合物。

28. 一种用于治疗感染了 HIV-1 或 HIV-2 的宿主的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 27 中任一项所述的化合物。

29. 一种用于预防 HIV-1 或 HIV-2 感染的方法，其包括向需要这种预防的患者施用预防有效量的如权利要求 1 至 25 中任一项所述的化合物。

30. 一种用于降低宿主中的 HIV-1 或 HIV-2 感染的生物活性的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

31. 如权利要求 26 所述的方法，其中所述 HIV-1 或 HIV-2 感染是由包含选自由 TAM 突变和 M184V 突变组成的组的突变的病毒引起。

32. 一种用于治疗感染了 HIV-1 或 HIV-2 的宿主的方法，其包括施用与另一种抗 HIV 剂组合的有效量的在药学上可接受的载体中的如权利要求 1 至 24 中任一项所述的化合物。

33. 如权利要求 32 所述的方法，其中所述 HIV-1 或 HIV-2 感染是由包含选自由 TAM 突变和 M184V 突变组成的组的突变的病毒引起。

34. 一种用于预防 HIV-1 或 HIV-2 感染的方法，其包括向需要这种预防的患者施用与另一种抗 HIV 剂组合的预防有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

35. 一种用于治疗感染了 HBV 的宿主的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

36. 一种用于预防 HBV 感染的方法，其包括向需要这种预防的患者施用预防有效量的如权利要求 1 至 25 中任一项所述的化合物。

37. 一种用于降低宿主中的 HBV 感染的生物活性的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

38. 一种用于治疗感染了 HBV 的宿主的方法，其包括施用与另一种抗 HBV 剂组合的有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

39. 一种用于预防 HBV 感染的方法，其包括向需要这种预防的患者施用与另一种抗 HBV 剂组合的预防有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化

合物。

40. 一种用于治疗感染了诺瓦克病毒或札如病毒的宿主的方法，其包含向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

41. 一种用于预防诺瓦克病毒或札如病毒感染的方法，其包括向需要这种预防的患者施用预防有效量的如权利要求 1 至 25 中任一项所述的化合物。

42. 一种用于降低宿主中的诺瓦克病毒或札如病毒感染的生物活性的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

43. 一种用于治疗感染了诺瓦克病毒或札如病毒的宿主的方法，其包括施用与另一种抗诺瓦克病毒或抗札如病毒剂组合的有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

44. 一种用于预防诺瓦克病毒或札如病毒感染的方法，其包括向需要这种预防的患者施用与另一种抗诺瓦克病毒或抗札如病毒剂组合的预防有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

45. 一种用于治疗感染了包括 HCV、黄热病、登革热及西尼罗病毒的黄病毒科病毒的宿主的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

46. 一种用于预防包括 HCV、黄热病、登革热及西尼罗病毒的黄病毒科病毒的感染的方法，其包括向需要这种预防的患者施用预防有效量的如权利要求 1 至 25 中任一项所述的化合物。

47. 一种用于降低宿主中的包括 HCV、黄热病、登革热及西尼罗病毒的黄病毒科病毒感染的生物活性的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

48. 一种用于治疗感染了包括 HCV、黄热病、登革热及西尼罗病毒的黄病毒科病毒的宿主的方法，其包括施用与另一种抗诺瓦克病毒或抗札如病毒剂组合的有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

49. 一种用于预防包括 HCV、黄热病、登革热及西尼罗病毒的黄病毒科病毒的感染的方法，其包括向需要这种预防的患者施用与另一种抗诺瓦克病毒或抗札如病毒剂组合的预防有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

50. 一种用于治疗感染了 HSV-1 或 HSV-2 的宿主的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

51. 一种用于预防 HSV-1 或 HSV-2 感染的方法，其包括向需要这种预防的患者施用预防有效量的如权利要求 1 至 25 中任一项所述的化合物。

52. 一种用于降低宿主中的 HSV-1 或 HSV-2 感染的生物活性的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

53. 一种用于治疗感染了 HSV-1 或 HSV-2 的宿主的方法，其包括施用与另一种抗 HSV-1 和 HSV-2 剂组合的有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

54. 一种用于预防 HSV-1 或 HSV-2 感染的方法，其包括向需要这种预防的患者施用与另一种抗 HSV-1 或抗 HSV-2 剂组合的预防有效量的在药学上可接受的载体中的如权利要求 1

至 25 中任一项所述的化合物。

55. 一种用于治疗具有癌症的宿主的方法，其包括向需要这种治疗的患者施用有效量的如权利要求 1 至 25 中任一项所述的化合物。

56. 一种用于治疗具有癌症的宿主的方法，其包括施用与另一种抗癌剂组合的有效量的在药学上可接受的载体中的如权利要求 1 至 25 中任一项所述的化合物。

57. 如权利要求 28-56 中任一项所述的方法，其中所述化合物在生物系统中被转化为包含 4-NHOH、4-NH_2和 4-OH 嘧啶三磷酸酯的混合物 C 或 D 的化合物的混合物：

。

58. 如权利要求 1-27 中任一项所述的化合物在制备用于治疗感染了 HIV-1 或 HIV-2 的宿主、预防 HIV-1 或 HIV-2 感染、或降低宿主中的 HIV-1 或 HIV-2 感染的生物活性的药剂中的用途。

59. 如权利要求 59 所述的用途，其中所述药剂还包含另一种抗 HIV 剂。

60. 如权利要求 1-27 中任一项所述的化合物在制备用于治疗感染了 HBV 的宿主、预防 HBV 感染、或降低宿主中的 HBV 感染的生物活性的药剂中的用途。

61. 如权利要求 60 所述的用途，其中所述药剂还包含另一种抗 HBV 剂。

62. 如权利要求 1-27 中任一项所述的化合物在制备用于治疗感染了黄病毒科、诺瓦克病毒或札如病毒感染的宿主、预防黄病毒科、诺瓦克病毒或札如病毒感染、或降低宿主中的黄病毒科、诺瓦克病毒或札如病毒感染的生物活性的药剂中的用途。

63. 如权利要求 62 所述的用途，其中所述药剂还包含另一种抗黄病毒科、抗诺瓦克病毒或抗札如病毒剂。

64. 如权利要求 1-27 中任一项所述的化合物在制备用于治疗感染了 HSV-1 或 HSV-2 的宿主、预防 HSV-1 或 HSV-2 感染、或降低宿主中的 HSV-1 或 HSV-2 感染的生物活性的药剂中的用途。

65. 如权利要求 64 所述的用途，其中所述药剂还包含另一种抗 HSV-1 或抗 HSV-2 剂。

66. 如权利要求 1-27 中任一项所述的化合物在制备用于治疗癌症的药剂中的用途。

67. 如权利要求 67 所述的用途，其中所述药剂还包含另一种抗癌剂。

用于治疗病毒感染和癌症的嘧啶核苷及其单磷酸酯前药

技术领域

[0001] 本发明涉及使用核苷酸类似物治疗或预防病毒感染的化合物、方法及组合物。更具体地说，本发明描述 N^4- 羟基胞苷核苷衍生物及修饰的单磷酸酯前药类似物、其药学上可接受的盐或其它衍生物，以及其在治疗癌症或病毒感染、且特别是以下疾病中的用途 :1) 人类免疫缺陷病毒 (HIV-1 和 HIV-2) ;2) 黄病毒科病毒，包括丙型肝炎 (HCV)、西尼罗病毒、登革热病毒和黄热病 ;3) 杯状病毒科感染，包括诺瓦克病毒和札如病毒 ;4)HSV-1、HSV-2 ; 以及 5) 巨细胞病毒 (CMV) ;6) 乙型肝炎病毒 (HBV) 感染。本发明教导如何制备 N^4- 羟基胞苷核苷衍生物，将其转化为治疗上相关的核苷酸前药并且最终将相应的核苷酸三磷酸酯在治疗上相关的浓度下递送至逆转录酶和聚合酶。

[0002] 发明背景

[0003] 作为一类的核苷类似物具有非常确定的调控史，目前有超过 10 种已得到美国食品与药物管理局 (US FDA) 的批准用于治疗人类免疫缺陷病毒 (HIV)、乙型肝炎病毒 (HBV)、单纯性疱疹 C 病毒 (HSV)。开发抗病毒疗法的挑战在于抑制病毒复制，而不会损害宿主细胞。

[0004] 丙型肝炎病毒 (HCV) 在全世界已经感染了超过一亿八千万人口。据估计每年有三至四百万人被新感染，70％的人将发展为慢性肝炎。HCV 导致 50-76％所有的肝癌病例，以及发达国家中所有肝移植的三分之二。标准疗法 [聚乙二醇化干扰素 α + 病毒唑 (核苷类似物)] 仅在 50-60％的患者中有效且与明显的副作用有关。两种 HCV 蛋白酶抑制剂 Incivek 和 Victrelis 在 2011 年 5 月得到批准的标准护理的效果仍不清楚，因为两种药物都需要反应指导的疗法方案，这会将 IFN 疗法在具有早期病毒反应的感染者中的持续时间从 48 周缩短至短至 24 周，但对于当施用 IFN 和 RBV 时在仅约 70 至 80％中存在的基因型 1 HCV 来说仍具有持续的病毒学反应 (SVR)(Sheridan,C.Nature Biotech.2011,29,553)。因此，迫切需要新的 HCV 药物。

[0005] HCV 基因组包含包裹在核衣壳和脂质包膜中的正链 RNA 并且由 9.6kb 核糖核苷酸组成，其编码约 3000 个氨基酸的大多肽 (Dymock 等人 Antiviral Chemistry&Chemotherapy 2000,11,79)。成熟之后，此多肽被切割成至少 10 个蛋白。这些蛋白质中的一个，NS5B，具有聚合酶活性并且涉及从充当模板的单链病毒 RNA 基因组合成双链 RNA。对于新型的抗病毒策略选择性地抑制 HCV 复制的发现由于缺乏用于 HCV 增殖的便利的细胞培养模型而长期受到阻碍。这个障碍首先在 1999 年通过建立 HCV 复制子系统而被克服 (Bartenschlager,R.,Nat.Rev.Drug Discov.2002,1,911-916 及 Bartenschlager,R.,J. Hepatol.2005,43,210-216)，并且在 2005 年，通过开发稳固的 HCV 细胞培养模型而得以解决 (Wakita,T., 等人 ,Nat.Med.2005,11,791-6 ;Zhong,J., 等人 ,Proc.Natl.Acad.Sci. U.S.A.2005,102,9294-9 ;Lindenbach,B.D., 等人 ,Science 2005,309,623-6)。

[0006] HCV 复制可通过经由竞争性抑制 NS5B 蛋白操纵 NS5B 的聚合酶活性来防止。或者，链终止核苷类似物也可并入延长的 RNA 链中。目前，用于治疗 HCV 的最先进的核苷是 PSI-7977(GS-7977)，其目前作为安全和有效的抗 HCV 剂处在 III 期临床试验中

(Sofia,M.J. ;Bao,D. ;Chang,W. ;Du,J. ;Nagarathnam,D. ;Rachakonda,S. ;Reddy,P. G. ;Ross,B.S. ;Wang,P. ;Zhang,H.-R ;Bansal,S. ;Espiritu,C ;Keilman,M. ;Lam,A.M. ; Micolochick Steuer,H.M. ;Niu,C ;Otto,M.J. ;Furman,P.A.J.Med.Chem.2010,53,7202)。关于 HCV NS5B 的核苷和核苷前药抑制剂的综述,参见 :1)Bobeck DR,Coats SJ,Schinazi RF.Advances in nucleoside monophosphate prodrugs as anti-hepatitis C virus agents.Antivir.Ther.2010,15,935-50 ;2)Ray AS,Hostetler KY.Application of kinase bypass strategies to nucleoside antivirals.Antiviral Res.2011,92,277-91 ;3) Sofia,M.J. ;Furman P.A.Symonds,W.T. 第 11 章,Accounts in Drug Discovery:Case Studies in Medicinal Chemistry by RSC ;4)Brown,N.A.Progress towards improving antiviral therapy for hepatitis C with hepatitis C virus polymerase inhibitors. 第 I 部 分 :Nucleoside analogues.Expert Opin.Invest.Drugs 2009,709-725 ; 5)Beaulieu,P.L.Recent advances in the development of NS5B polymerase inhibitors for the treatment of hepatitis C virus infection.Expert Opin.Ther. Pat.2009,19,145-164 ;6)Koch,U. ;Narjes,F.Recent Progress in the Development of Inhibitors of the Hepatitis C Virus RNA-Dependent RNA Polymerase.Curr.Top.Med. Chem.2007,7,1302-1329。

[0007] 近来,若干专利申请(包括 WO 09/086192、WO 12/040124、WO 12/040126、WO 12/040127、US 12/070415、WO 08/082601、WO 10/014134、WO 11/017389、WO 11/123586、WO 10/135569、WO 10/075549、WO 10/075554、WO 10/075517、WO 09152095、WO 08/121634、WO 05/03147、WO WO 99/43691、WO 01/32153、WO 01160315、WO 01179246、WO 01/90121、WO 01/92282、WO 02/48165、WO 02/18404、WO 02/094289、WO 02/057287、WO 02/100415(A2)、US 06/040890、WO 02/057425、EP 1674104(A1)、EP 1706405(A1)、US 06/199783、WO 02/32920、US 04/6784166、WO 05/000864、WO 05/021568) 已经描述了作为抗 HCV 剂的核苷类似物。

[0008] 在 HIV 中,用于药物开发的关键靶标是逆转录酶 (HIV-RT),一种独特的病毒聚合酶。此酶最初在病毒复制周期中有活性并且将病毒的遗传信息从 RNA 转化成 DNA,这是连续病毒复制所必需的过程。核苷逆转录酶抑制剂 (NRTI) 模拟天然的核苷。在三磷酸酯形式下,每个 NRTI 与四种天然存在的 2'- 脱氧核苷 -5'- 三磷酸酯 (dNTP) 中的一种竞争,即 dCTP、dTTP、dATP 或 dGTP,用于靠近 HIV-1 RT 的活性位点的结合和 DNA 链延长。

[0009] 逆转录是 HIV-1 复制周期中的必需事件并且是开发抗逆转录病毒药物的主要靶标(参见 Parniak MA,Sluis-Cremer N.Inhibitors of HIV-1 reverse transcriptase. Adv.Pharmacol.2000,49,67-109 ;Painter GR,Almond MR,Mao S,Liotta DC.Biochemical and mechanistic basis for the activity of nucleoside analogue inhibitors of HIV reverse transcriptase.Curr.Top.Med.Chem.2004,4,1035-44 ;Sharma PL,Nurpeisov V,Hernandez-Santiago B,Beltran T,Schinazi RF.Nucleoside inhibitors of human immunodeficiency virus type 1reverse transcriptase.Curr.Top.Med. Chem.2004,4895-919)。已经鉴定出抑制 HIV-1 RT 的两种不同的化合物组。它们是核苷或核苷酸 RT 抑制剂 (NRTI) 和非核苷 RT 抑制剂 (NNRTI)。

[0010] NRTI 是在核糖上缺乏 3'-OH 基团的 2'- 脱氧核糖核苷。它们是最早用于治疗

HIV-1 感染的药物并且它们保留几乎所有的抗逆转录病毒方案的整体组分。

[0011] 在 1985 年，据报道合成的核苷 3'- 叠氮基 -3'- 脱氧胸苷（齐多夫定，AZT，一种代表性的 NRTI）抑制 HIV 的复制。从那时起，包括但不限于以下的若干其它 NRTI 已被证明对 HIV 是有效的：2',3'- 双去氧肌苷（去羟肌苷，ddI）、2',3'- 二脱氧胞苷（扎西他滨，ddC）、2',3'- 双脱氧 -2',3'- 二脱氢胸苷（司他夫定，d4T）、(-)-2',3'- 双脱氧 -3'- 硫杂胞苷（拉米夫定，3TC）、(-)-2',3'- 双脱氧 -5- 氟 -3'- 硫杂胞苷（恩曲他滨，FTC）、(1S,4R)-4-[2- 氨基 -6-（环丙基 - 氨基）-9H- 嘌呤 -9- 基]-2- 环戊烯 -1- 甲醇琥珀酸酯（阿巴卡韦，ABC）、(R)-9-(2- 膦酰基甲氧丙基）腺嘌呤 (PMPA，替诺福韦地索普西富马酸盐）(TDF) 以及 (-)- 碳环 2',3'- 二脱氢 -2',3'- 双脱氧鸟嘌呤核苷（卡波韦）及其前药阿巴卡韦。在通过细胞激酶磷酸化为 5'- 三磷酸酯之后，这些 NRTI 被并入病毒 DNA 的生长链中，造成链终止，因为它们缺乏 3'- 羟基。

[0012] 一般说来，为了展现抗病毒活性，NRTI 必须通过宿主 - 细胞激酶代谢转化为其相应的三磷酸酯形式 (NRTI-TP)。NRTI-TP 通过充当 DNA 合成的链终止剂来抑制 HIV-1 RT DNA 合成（参见 Goody RS, Muller B, Restle T. Factors contributing to the inhibition of HIV reverse transcriptase by chain terminating nucleotides in vitro and in vivo. FEBS Lett. 1991, 291, 1-5）。虽然含有一种或多种 NRTI 的组合疗法已经极大地降低与 AIDS 有关的发病率和死亡率，但获批准的 NRTI 可具有显著的局限性。这些局限性包括急性和慢性毒性、与其它抗逆转录病毒的药物动力学相互作用以及对其它 NRTI 展现交叉耐药性的 HIV-1 的耐药性变体的选择。

[0013] 在个体内的 HIV-1 耐药性是由病毒群体的遗传变异性和疗法对耐药性变体的选择所造成（参见 Chen R, Quinones-Mateu ME, Mansky LM. Drug resistance, virus fitness and HIV-1mutagenesis. Curr. Pharm. Des. 2004, 10, 4065-70）。HIV-1 遗传变异性是由于 HIV-1 RT 在复制期间不能校对核苷酸序列所致。此变异性通过高速的 HIV-1 复制、前病毒变体在 HIV-1 感染过程中的积聚以及当不同序列的病毒感染同一细胞时的遗传重组而得到增强。因此，无数的遗传学上不同的变体（称为准物种）在初始感染之后的岁月里在个体内进化。耐药性的发展取决于药物疗法期间病毒复制继续的程度、获得一个具体突变（或突变组）的便利性以及耐药性突变对药物敏感性和病毒适合度的影响。一般说来，NRTI 疗法选择在 RT 中具有突变的病毒。取决于所选的 NRTI 耐药性突变，突变病毒通常展现对一些或在某些情况下所有 NRTI 的敏感性的降低。从临床观点上说，耐药性 HIV-1 的发展通过有效地减少保留针对耐药性病毒的效力的可利用药物的数量来限制未来的治疗选择。这经常需要更复杂的药物方案，其涉及密集的给药方案和由于药物毒性所致的更大的严重副作用风险。这些因素经常促成对药物方案的不完全顺从性。因此，具有优良的活性和安全概况以及与目前可利用的药物之间有限的或没有交叉耐药性的新型 NRTI 的开发是有效的 HIV-1 感染疗法的关键。

[0014] 对耐药性 HIV-1 有活性的核苷类似物的开发需要详细理解涉及对此类化合物有抗性的分子机制。因此，提供对 NRTI 有耐药性的 HIV-1 的突变和分子机制的简要概述。已经提出对 NRTI 有耐药性的 HIV-1 的两种动力学上不同的分子机制（参见 Sluis-Cremer N, Arion D, Parniak MA. Molecular mechanisms of HIV-1 resistance to nucleoside reverse transcriptase inhibitors (NRTIs). Cell Mol. Life Sci. 2000 ;57, 1408-22）。

一种机制涉及在病毒 DNA 合成期间 NRTI-TP 对比正常的 dNTP 掺入的选择性减少。这种抗性机制被称为辨别 (discrimination)。第二机制涉及从提前终止的 DNA 链上选择性除去链终止的 NRTI- 单磷酸酯 (NRTI-MP)(参见 Arion D,Kaushik N,McCormick S,Borkow G,Parniak MA.Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine(AZT):increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. Biochemistry.1998,37,15908-17 ;Meyer PR,Matsuura SE,Mian AM,So AG,Scott WA.A mechanism of AZT resistance:an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase.Mol.Cell.1999,4,35-43)。这种机制被称为切除。

[0015] 辨别机制涉及在 RT 中获得一个或多个抗性突变,这改善了酶辨别天然 dNTP 底物与 NRTI-TP 的能力。在这点上,抗性通常与 NRTI-TP 掺入的降低的催化效率有关。NRTI-TP(和 dNTP) 催化效率是由以下两种动力学参数驱动 :(i) 核苷酸对于 RT 聚合酶活性位点的亲和力 (K_d) 以及 (ii) 核苷酸掺入的最大速率 (kpol),这两者都可使用预稳态动力学分析来确定 (参见 Kati WM,Johnson KA,Jerva LF,Anderson KS.Mechanism and fidelity of HIV reverse transcriptase.J.Biol.Chem.1992,26:25988-97).

[0016] 对于 NRTI 抗性的切除机制,突变 HIV-1 RT 不能在核苷酸掺入步骤时辨别天然 dNTP 底物与 NRTI-TP(参见 Kerr SG,Anderson KS.Pre-steady-state kinetic characterization of wild type and 3'-azido-3'-deoxythymidine(AZT)resistant HIV-1 RT:implication of RNA directed DNA polymerization in the mechanism of AZT resistance.Biochemistry.1997,36,14064-70)。反之,含有“切除”突变的 RT 显示出在生理浓度的 ATP(通常在 0.8-4mM 范围内) 或焦磷酸盐 (PPi) 存在下解阻断 NRTI-MP 终止的引物的提高的能力 (参见 Arion D,Kaushik N,McCormick S,Borkow G,Parniak MA.Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine(AZT):increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. Biochemistry.1998,37,15908-17 ;Meyer PR,Matsuura SE,Mian AM,So AG,Scott WA.A mechanism of AZT resistance:an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase.Mol.Cell.1999,4,35-43)。与切除机制有关的 NRTI 抗性突变包括胸苷类似物突变 (TAMS) 和 T69S 插入突变。

[0017] 引起严重的人类健康问题的另一种病毒是乙型肝炎病毒 (HBV)。HBV 作为人类癌症病因仅在烟草之后处于第二位。HBV 诱发癌症的机制是未知的。据推测,它可直接触发肿瘤发展,或经由慢性炎症、肝硬化和与感染有关的细胞再生间接地触发肿瘤发展。

[0018] 2 至 6 个月的温育期之后 (在此期间宿主通常不知道感染),HBV 感染可造成急性肝炎和肝脏损伤,导致腹痛、黄疸和某些酶的血液水平的升高。HBV 可引起暴发型肝炎,这是一种快速进行性、经常致命的疾病形式,其中大部分肝脏被破坏。

[0019] 患者通常从 HBV 感染的急性期中痊愈。然而,在一些患者中,病毒继续复制延长的或不确定的时间,造成慢性感染。慢性感染可导致慢性持续性肝炎。感染了慢性持续性 HBV 的患者在发展中国家中最常见。截至 1991 年中期,仅在亚洲就有大约 2 亿 2 千 5 百万 HBV

的慢性携带者且全世界有将近3亿携带者。目前(2012年7月),据WHO估计全世界有二十亿人已感染了乙型肝炎病毒并且超过2亿4千万具有慢性(长期)肝脏感染。约600,000人每年死于乙型肝炎的急性或慢性后果。慢性持续性肝炎可引起疲劳、肝硬化和肝细胞癌(一种原发性肝癌)。

[0020] 在工业化国家中,HBV感染的高风险群组包括与HBV携带者或其血液样品接触的那些人。HBV的流行病学非常类似于HIV/AIDS,这是为什么HBV感染在感染了HIV或患有AIDS的患者中常见的原因。然而,HBV比HIV更具传染性。

[0021] 3TC(拉米夫定)、干扰素α-2b、聚乙二醇化干扰素α-2a、贺维力(阿德福韦酯)、贝乐克(恩替卡韦)及替泽卡(替比夫定)是目前用于治疗HBV感染的FDA批准的药物。然而,一些药物具有严重的副作用,且病毒耐药性在用这些药物治疗的患者中快速发展。

[0022] 诺瓦克病毒是在非包膜正链RNA杯状病毒科中发现的四种病毒属中的一种。杯状病毒科中的其它三个物种是兔病毒、囊病毒和札如病毒。札如病毒是除了将人作为宿主的诺瓦克病毒外的属中的唯一成员。诺瓦克病毒基因组是大约7.56kb,具有三个开放阅读框架(ORF)。第一个ORF编码包括解旋酶、蛋白酶和RNA指导的RNA聚合酶(RDRP)的非结构蛋白,这些酶全部都是复制病毒所需要的。剩余的两个ORF编码衣壳蛋白(Jiang,X.(1993)Virology 195(1):51-61)。已将众多的诺瓦克病毒株分为5个基因组,其中I、IV和V感染人(Zheng,D.P.,等人(2006)Virology 346(2):312-323)并且据CDC估计引起大约2千3百万肠胃炎病例,相当于美国每年40%的食物传染性疾病(Mead P.S.(1999)Emerg.Infect.Dis.5(5):607-625)。

[0023] 常见症状是呕吐、腹泻及肠痉挛。呕吐是儿童中最常见的症状,而腹泻在受感染的成人中更常见。脱水是一个重要问题。由于此病毒所致的丧命在美国每年有约300个患者,并且这些死亡通常发生在具有薄弱的免疫系统的患者中(Centers for Disease Control and Prevention."Norwalk-like viruses:"public health consequences and outbreak management.MMWR 2001;50(No.RR-9):3)。从对完全感染的暴露开始的温育期通常是24小时至48小时,其中有大约30%受感染的个体不显示症状。症状一般持续24至60小时(Adler,J.L.和Zickl,R.,J.(1969)Infect.Dis.119:668-673)。病毒脱落可持续感染之后长达2周,然而,并不清楚此病毒是否具有传染性。

[0024] 诺瓦克病毒主要通过粪-口途径经由污染的食物或水、人与人的接触、呕吐或粪便样品的气溶胶来传播。粪便样品中的病毒滴度可达到每毫升10^6至10^7个颗粒,并且颗粒在0℃(32℉)至60℃(140℉)的温度下是稳定的(Duizer,E.等人,(2004)Appl.Environ.Microbiol.70(8);4538-4543)。病毒是高度传染性的,并且各种来源提示感染可能需要少至10至100个病毒颗粒的接种(Centers for Disease Control and Prevention."Norwalk-like viruses:"public health consequences and outbreak management.MMR 2001;50(第RR-9期):3-6)。这导致在学校、疗养院、旅游客轮、医院或人群聚集的其它场所中的流行。

[0025] 诺瓦克样(Norwalk-like)病毒被命名为诺瓦克病毒,这是一个来源于1968年在俄亥俄州的诺瓦克的学校中的爆发的名称。造成诺瓦克疾病的病毒颗粒在1972年在三组人志愿者的直肠拭子滤液的传代之后通过免疫电子显微镜术来鉴定(Kapikian,A.Z.等人(1972)J.Virol.10:1075-1081)。在接下来的时间里,病毒因其电子显微图像而被称为小圆

结构病毒；杯状病毒，因为它是杯状病毒科的成员；和 / 或在最初分离株之后大概最常用的诺瓦克样病毒。该病毒的常见名称包括冬季呕吐病毒、胃流感、食物中毒以及病毒性胃肠炎。虽然感染的后果通常并不危急生命，但机构使用的成本损失以及生产率的损失很大，且因此非常需要一种用于治疗人的诺瓦克病毒感染的疗法。

[0026] 目前没有被批准用于诺瓦克病毒感染的药物治疗 (http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus-qa.htm)，并且这大概至少部分是由于缺乏细胞培养系统的可用性。近来，已经开发用于原始的诺瓦克 G-I 毒株的复制子系统 (Chang,K.O., 等人 (2006)Virology 353:463-473)。诺瓦克病毒复制子和丙型肝炎复制子都需要病毒解旋酶、蛋白酶和聚合酶的作用以便进行复制子的复制。最近，已经报道利用诺瓦克病毒基因组 I 和 II 接种体的体外细胞培养传染性测定 (Straub,T.M. 等人 (2007)Emerg.Infect.Dis.13(3):396-403)。此测定是在利用微载体珠上的小肠上皮细胞的旋转壁生物反应器中进行，并且至少最初似乎难以用此系统筛选有意义数量的化合物。最终，传染性测定可适用于筛选进入抑制剂。其他集团如Ligocyte Pharmaceuticals,Inc.(http://www.ligocyte.com/) 关注设法开发一种针对诺瓦克病毒的疫苗，然而，这些努力尚未成功并且可能证明经常在低复制酶保真度是一个革命性益处的病毒系统中出现的情况是困难的。

[0027] 增殖性病症是一种严重危急生命的疾病并且几十年来已进行了深入的研究。癌症现在是美国的第二主要死亡原因，且超过 500,000 人每年死于此增殖性病症。肿瘤是细胞生长不受调控的混乱的增殖。肿瘤是恶性的或癌性的，如果它具有侵袭性和转移特性的话。侵袭性是指肿瘤进入周围组织，穿透限定组织边界的基底层，进而常常进入身体的循环系统中的倾向。转移是指肿瘤迁移到身体其它区域并且确立远离初始出现部位的增殖区域的倾向。

[0028] 癌症在分子水平上还没有被完全理解。众所周知，细胞向致癌物如某些病毒、某些化学品或辐射的暴露导致 DNA 改变，这种改变使“抑制”基因失活或“致癌基因”激活。抑制基因是生长调控基因，其一旦突变，便不再能控制细胞生长。致癌基因最初是正常基因（称为前致癌基因），其通过突变或表达环境的改变而变为转化基因。转化基因的产物造成不适当的细胞生长。超过二十种不同的正常细胞基因可通过遗传变化变为致癌基因。转化的细胞在许多方面不同于正常细胞，包括细胞形态、细胞与细胞的相互作用、膜含量、细胞骨架结构、蛋白质分泌、基因表达以及死亡（转化的细胞可无限生长）。

[0029] 身体的所有不同的细胞类型均可转变成良性或恶性肿瘤细胞。最常见的肿瘤部位是肺，其次是结肠直肠、乳腺、前列腺、膀胱、胰腺以及卵巢。其它常见的癌症类型包括白血病、中枢神经系统癌症，包括脑癌、黑素瘤、淋巴瘤、红白血病、子宫癌以及头颈癌。

[0030] 癌症目前主要用以下三种方式的疗法中的一种或组合来治疗：手术、辐射及化疗。手术涉及患病组织的整体摘除。虽然手术有时在摘除位于某些部位（例如乳腺、结肠和皮肤）的肿瘤中是有效的，但其不能用于治疗位于其它区域如脊椎中的肿瘤或治疗扩散性肿瘤病状如白血病。

[0031] 化疗涉及细胞复制或细胞代谢的破坏。它最常用于治疗白血病以及乳腺癌、肺癌和睾丸癌。目前存在五种主要类别的化疗剂用于治疗癌症：天然产物及其衍生物；蒽环霉素类；烷化剂；抗增殖剂（又称为抗代谢物）；以及激素剂。化疗剂经常被称为抗肿瘤剂。

[0032] 已经鉴定展现抗癌活性的若干合成核苷如 5- 氟尿嘧啶。5- 氟尿嘧啶已在临床上

用于治疗恶性肿瘤，包括例如癌瘤、肉瘤、皮肤癌、消化器官癌以及乳腺癌。然而，5- 氟尿嘧啶引起严重的不良反应，如恶心、脱发、腹泻、口炎、白细胞血小板减少、厌食、色素沉着及浮肿。

[0033] 尽管有疫苗的可用性 (Crit.Rev.Clin.Lab.Sci.2004,41,391-427)，但黄热病病毒 (YFV) 仍然是一种严重的人类健康问题，每年造成大约 30,000 人死亡。YFV 是最致命性的人类病毒感染之一 (Expert Rev.Vaccines 2005,4,553-574.)。在受感染的个体中，大约 15％将发展成严重的疾病，在那些个体中有 20 至 50％的病死率。没有被批准的对于治疗 YFV 有特异性的疗法。治疗是症状性 - 停止 (symptomatic-rest) 流体，并且布洛芬、萘普生、醋氨酚或扑热息痛可减轻发热和疼痛症状。应当避免阿斯匹林。虽然病毒流行至非洲和南美洲，但 YFV 也可能在这些地区之外的地方爆发且已有这种输入病例的报道 (J.Travel Med.2005,12(增刊 1),S3-S11)。

[0034] 西尼罗病毒 (WNV) 来自黄病毒科且主要引起蚊传播的疾病。它首先在 1937 年于乌干达的西尼罗河地区被发现。根据来自疾病控制和预防中心的报道，已在非洲、中东、欧洲、大洋洲、西亚和中亚以及北美洲发现 WNV。其在北美洲的首次出现是在 1999 年纽约市的大都市地区。它在北美洲是季节性流行的，一般在夏季爆发且持续到秋季，呈现出对环境卫生的威胁。它的自然循环是鸟 - 蚊 - 鸟和哺乳动物。蚊子、特别是尖音库蚊物种当以受感染的鸟为食时变得受感染。受感染的蚊子然后当其叮咬时将 WNV 传播给其它鸟和包括人的哺乳动物。在人和马中，致命性脑炎是 WNV 感染最严重的表现。WNV 还可在一些受感染的鸟中引起死亡。没有用于 WNV 感染的特异性治疗。在具有轻度症状的病例中，人们经受在其自身传递的如发热和疼痛的症状，尽管甚至健康的人也会患病几周。在更严重的病例中，人们通常需要去医院，在那里他们可以接受支持治疗。

[0035] 登革热感染也来自黄病毒科并且是新加坡最重要的虫媒传播感染 (Epidemiol News Bull 2006,32,62-6)。在全球，每年估计有 5 千万至 1 亿登革热 (DF) 病例以及几十万登革热出血热 (DHF) 病例且平均病死率是 5％。许多患者从具有最少或没有残余疾病的登革热感染中痊愈。登革热感染通常是无症状的，但可表现出典型的登革热、登革出血热或登革热休克综合征。甚至对于门诊患者，对维持足够的水合的需要也是非常重要的。登革热感染可通过静脉内补液疗法得到有效的控制，并且如果及早诊断，病死率可保持低于 1％。为了控制疼痛和发热，疑似具有登革热感染的患者都应给予醋氨酚制剂。阿斯匹林和非甾族抗炎药疗法可加重与一些登革热感染有关的出血倾向。然而，先前所述的登革热感染的一些表现包括肝功能衰竭 (Dig Dis Sci 2005,50,1146-7)、脑病 (J Trop Med Public Health 1987,18,398-406)、以及格林 - 巴利综合征 (Intern Med 2006,45,563-4)。

[0036] 鉴于获得性免疫缺陷综合征、AIDS- 相关复合物、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、癌症以及 HBV 在全球已经达到警戒水平且对受影响的患者具有显著且在一些情况下悲剧性影响的事实，仍强烈需要提供治疗这些疾病的新的有效药剂，其中的药剂对宿主具有低毒性。

[0037] 有利的是，提供新的抗病毒或化疗剂、包含这些试剂的组合物以及使用这些试剂的治疗方法，特别是用于治疗耐药性癌症或突变病毒。本发明提供这种试剂、组合物及方法。

[0038] 发明概述

[0039] 本发明提供用于治疗或预防宿主中的癌症和 HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、巨细胞病毒 (CMV) 或 HBV 感染的化合物、方法及组合物。所述方法涉及施用治疗或预防有效量的至少一种如本文所述的化合物来治疗或预防癌症或 HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、巨细胞病毒 (CMV) 或 HBV 的感染或施用足以降低这些疾病的生物活性的量。药物组合物包含与药学上可接受的载体或赋形剂组合的一种或多种本文所述的化合物，所述化合物用于治疗具有癌症或感染了 HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、巨细胞病毒 (CMV) 或 HBV 的宿主。所述制剂还可包含至少一种其它治疗剂。另外，本发明包括用于制备这种化合物的方法。

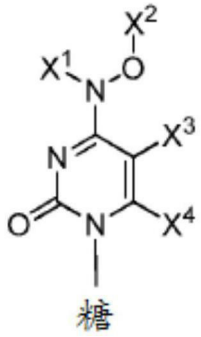
[0040] 与丙型肝炎复制子的情况一样，诺瓦克病毒复制子需要病毒解旋酶、蛋白酶和聚合酶的作用以便进行复制子的复制。复制子可用于高通量测定中，所述测定评价有待就活性被筛选的化合物是否抑制诺瓦克病毒解旋酶、蛋白酶和 / 或聚合酶发挥作用的能力，如通过抑制复制子的复制所证明。

[0041] 本文所述的化合物包括 β-D 和 β-L-N^4- 羟基胞苷核苷衍生物及修饰的单磷酸酯、膦酸酯前药。在一个实施方案中，活性化合物具有式 (I)：

[0042] 另外，本文所述的化合物是 HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、疱疹病毒 (HSV-1、HSV-2)、登革热病毒、黄热病、巨细胞病毒 (CMV) 癌症和 / 或 HBV 的抑制剂。因此，这些化合物还可用于治疗感染或合并感染有 HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、癌症和 / 或 HBV 的患者。

[0043] 在一个实施方案中，化合物是式 (I) 化合物：

[0044]



(I)

[0045] 或其药学上可接受的盐或前药，其中：

[0046] i)X^1是 H、C_1-C_6烷基、C_1-C_6卤烷基、C_1-C_6烷氧基、C_2-C_6烯基、C_2-C_6炔基、COR^1或 $COOR^1$；

[0047] ii)X^2是氢、COR^1或 $COOR^1$

[0048] 其中每个 R^1独立地是 CH_2-O(CO)-X^5、CH_2-O(CO)O-X^5、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-6}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟或 C_{3-10}环烷基取代的 C_{1-6}烷基

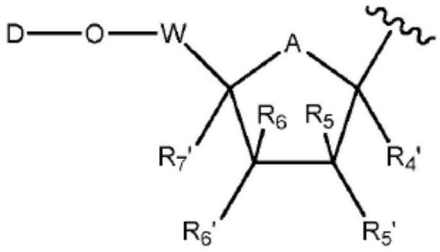
[0049] X^5独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、

烷氧基、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-6}烷基、或被C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟或C_{3-10}环烷基取代的C_{1-6}烷基

[0050] iii)X^3和X^4各自独立地是H、C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、芳基、烷基芳基、卤素(F、Cl、Br、I)、NH_2、OH、SH、CN或NO_2。

[0051] 在一个实施方案中，糖是通式(II)的核糖或修饰的核糖：

[0052]



(II)

[0053] 其中：

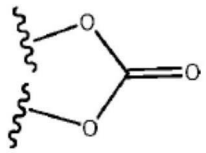
[0054] D是H、C(O)R^1、C(O)OR^1、二磷酸酯或三磷酸酯；

[0055] R^1如上所定义；

[0056] W是CL_2或CL_2CL_2，其中L独立地选自由以下组成的组：H、C_{1-6}烷基、C_{2-6}烯基及C_{2-6}炔基，其中C_{1-6}烷基、C_{2-6}烯基及C_{2-6}炔基可各自任选地含有一个或多个杂原子；

[0057] A是O、S、CH_2、CHF、CF_2、C＝CH_2、C＝CHF或C＝CF_2；

[0058] $R^{4'}$、R^5、$R^{5'}$、R^6、$R^{6'}$及$R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、OH、SH、NH_2、NHOH、$NHNH_2$、N_3、C(O)OH、CN、CH_2OH、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR及NR_2；

[0059] $R^{5'}$和$R^{6'}$可一起形成环 

。

[0060] 在一个实施方案中，其中糖是式(II)，当A是O或CH_2，D是H或酰基，W是CH_2，$R^{4'}$和$R^{7'}$是H时，R^5、$R^{5'}$、R^6、$R^{6'}$不能是H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH＝CH_2、CN、CH_2NH_2、CH_2OH或COOH。

[0061] 在另一实施方案中，$R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR及NR_2；

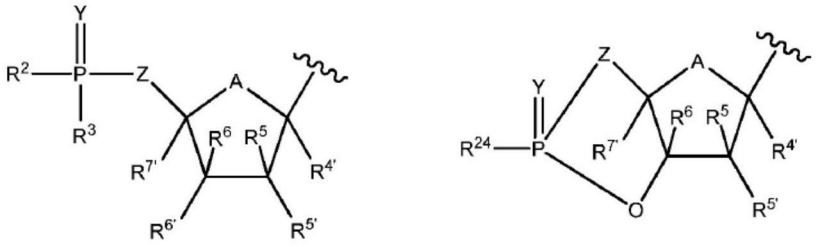
[0062] 在一个实施方案中，其中对于糖是式(II)的式(I)，当A是O或S时，$R^{7'}$不能是OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR或NR_2。

[0063] 在另一实施方案中，$R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、C(O)NH_2、C(S)NH_2、C(O)OR及R；

[0064] R独立地是C_1-C_6烷基、C_{2-6}烯基、C_{2-6}炔基、C_{3-6}环烷基、(C_3-C_6环烷基)芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基取代，其中代表性取代基包括例如羟烷基、氨基烷基和烷氧基烷基。

[0065] 在另一实施方案中，糖是通式（III）或（IV）的核糖或修饰的核糖：

[0066]



(III) (IV)

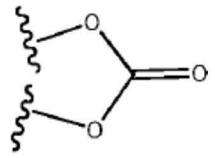
[0067] 其中：

[0068] Y 是 O 或 S；

[0069] Z 选自由以下组成的组：CL_2、CL_2CL_2、CL_2OCL_2、CL_2SCL_2、CL_2O、OCL_2及 CL_2NHCL_2，其中 L 独立地选自由以下组成的组：H、F、C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基，其中 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基可各自任选地含有一个或多个杂原子；

[0070] A 是 O、S、CH_2、CHF、CF_2、C ＝ CH_2、C ＝ CHF 或 C ＝ CF_2；

[0071] $R^{4'}$、R^5、$R^{5'}$、R^6、$R^{6'}$及 $R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、OH、SH、NH_2、NHOH、$NHNH_2$、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

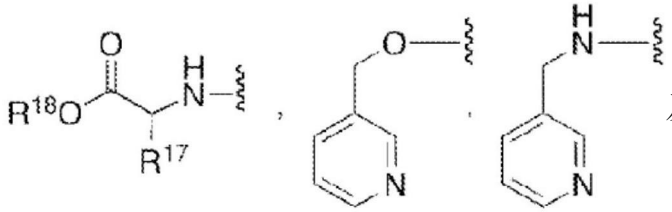
[0072] $R^{5'}$和 $R^{6'}$可一起形成环  。

[0073] 在一个实施方案中，其中糖是式（III）或（IV），当 A 是 O 或 S 时，$R^{7'}$不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2。

[0074] 在另一实施方案中，$R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 及 R。

[0075] R 独立地是 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基取代。

[0076] R^{24}选自由以下组成的组：OR^{15}、

[0077]  及脂肪醇，

[0078] R^{15}选自由以下组成的组：H、Li、Na、K、苯基及吡啶基；其中苯基和吡啶基任选地被零至三个独立地选自由 $(CH_2)_{0-6}CO_2R^{16}$和 $(CH_2)_{0-6}CON(R^{16})_2$组成的组的取代基取代；

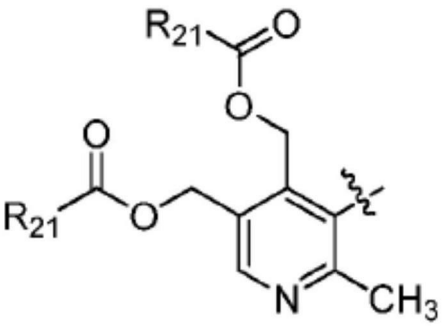
[0079] R^{17}选自在天然 L- 氨基酸中存在的那些基团、C_{1-6}烷基、（C_1-C_6烷基）、C_{2-6}烯基、C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基取代。

[0080] R^{18}是 H、C_{1-20}烷基、来源于脂肪醇（如油醇、二十八醇、三十醇、亚油醇等）的碳链或

被以下取代的 C_{1-20}烷基：C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基或环烷基取代的 C_{1-5}烷基。

[0081] 代表性 R^2和 R^3独立地选自由以下组成的组：

[0082] (a)OR^8，其中 R^8是 H、Li、Na、K、C_{1-20}烷基、C_{3-6}环烷基、C_{1-6}卤烷基、芳基或杂芳基(包括但不限于苯基或萘基)，任选地被一个至三个独立地选自由以下组成的组的取代基取代：C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、C_{1-6}烷氧基、$(CH_2)_{0-6}CO_2R^{9a}$、卤素、C_{1-6}卤烷基、$-N(R^{9a})_2$、C_{1-6}酰基氨基、$-NHSO_2C_{1-6}$烷基、$-SO_2N(R^{9a})_2$、$-SO_2C_{1-6}$烷基、COR^{9b}、硝基、氰基及



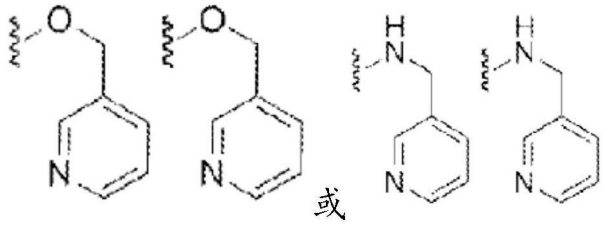
；

[0083] 其中 R^{21}如下所定义；

[0084] R^{9a}独立地是 H、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

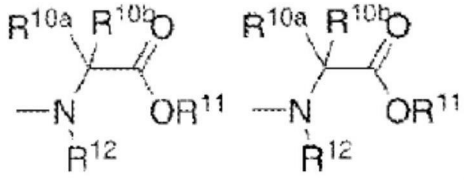
[0085] R^{9b}是 $-OR^{9a}$或 $-N(R^{9a})_2$；

[0086] (b)



或

[0087] (c)



其中 R^{10a}和 R^{10b}是：

，

[0088] (i)独立地选自由以下组成的组：H、C_{1-10}烷基、$-(CH_2)_rNR^{9a}{}_2$、C_{1-6}羟烷基、$-CH_2SH$、$-(CH_2)_2S(O)_pMe$、$-(CH_2)_3NHC(=NH)NH_2$、(1H-吲哚-3-基)甲基、(1H-咪唑-4-基)甲基、$-(CH_2)_mCOR^{9b}$、芳基及芳基-C_{1-3}烷基，所述芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、卤素、硝基及氰基；

[0089] (ii)R^{10a}是 H 且 R^{10b}和 R^{12}一起是 $(CH_2)_{2-4}$以形成包含邻接的 N 和 C 原子的环；

[0090] (iii)R^{10a}和 R^{10b}一起是 $(CH_2)_n$以形成环；

[0091] (iv)R^{10a}和 R^{10b}都是 C_{1-6}烷基；或

[0092] (v)R^{10a}是 H 且 R^{10b}是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2- 吲 哚 -3- 基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$-$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2- 咪唑 -4- 基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH 或 C_{3-10}环烷基；

[0093] p 是 0 至 2；

[0094] r 是 1 至 6；

[0095] n 是 4 或 5；

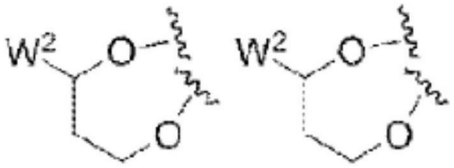
[0096] m 是 0 至 3；

[0097] R^{11}是 H、C_{1-10}烷基、或被以下取代的 C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二 (C_{1-6}烷基)- 氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_{1-6}烷基、C_{1-6}烷氧基、二 (C_{1-6}烷基)- 氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

[0098] R^{12}是 H 或 C_{1-3}烷基，或 R^{10a}、或 R^{10b}和 R^{12}一起是 $(CH_2)_{2-4}$以便形成包含邻接的 N 和 C 原子的环；

[0099] (d)O 连接的脂质（包括磷脂）、N 或 O 连接的肽、O 连接的胆固醇或 O 连接的植物甾醇；

[0100] (e)R^2和 R^3可在一起形成环

[0101]  其中 W^2选自由以下组成的组：苯基和单环杂芳基，其任选地被一个至三个独立地选自由以下组成的组的取代基取代：C_{1-6}烷基、CF_3、C_{2-6}烯基、C_{1-6}烷氧基、OR^{9c}、CO_2R^{9a}、COR^{9a}、卤素、C_{1-6}卤烷基、$-N(R^{9a})_2$、C_{1-6}酰基氨基、$CO_2N(R^{9a})_2$、SR^{9a}、$-NHSO_2C_{1-6}$烷基、$-SO_2N(R^{9a})_2$、$-SO_2C_{1-6}$烷基、COR^{9b}及氰基，并且其中所述单环杂芳基和取代的单环杂芳基具有 1-2 个独立地选自由 N、O 及 S 组成的组的杂原子，前提条件是：

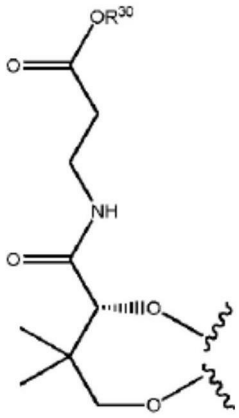
[0102] a) 当存在两个杂原子且一个是 O 时，那么另一个不能是 O 或 S，并且

[0103] b) 当存在两个杂原子且一个是 S 时，那么另一个不能是 O 或 S；

[0104] R^{9a}独立地是 H 或 C_{1-6}烷基；

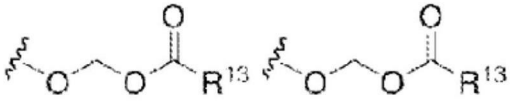
[0105] R^{9b}是 $-OR^{9a}$或 $-N(R^{9a})_2$；

[0106] R^{9c}是 H 或 C_{1-6}酰基；

[0107] (f)R^2和 R^3可在一起形成环  其中 R^{30}是 H、C_{1-20}烷基、C_{1-20}烯基、

，

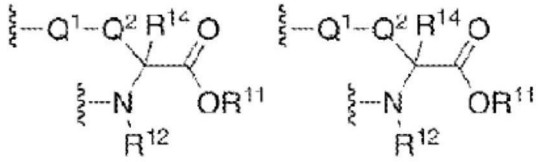
来源于脂肪醇的碳链或被以下取代的C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基或被C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基取代的C_{1-5}烷基；

[0108] (g)  其中 R^{13}选自由以下组成的组：H、C_{1-10}

，

烷基、被以下取代的C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基和取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被以下取代的C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基；

[0109] (h)R^2和 R^3可在一起形成环

[0110]  其中 R^{14}是：

，

[0111] (i)独立地选自由以下组成的组：H、C_{1-10}烷基、$-(CH_2)_rNR_2^{9a}$、C_{1-6}羟烷基、$-CH_2SH$、$-(CH_2)_2S(O)_pMe$、$-(CH_2)_3NHC(=NH)NH_2$、(1H-吲哚-3-基)甲基、(1H-咪唑-4-基)甲基、$-(CH_2)_mCOR^{9b}$、芳基、芳基-C_{1-3}烷基、杂芳基及杂芳基-C_{1-3}烷基，所述芳基和杂芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、卤素、硝基及氰基；

[0112] (ii)R^{14}是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2-吲哚-3-基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$、$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2-咪唑-4-基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH或C_{3-10}环烷基；

[0113] p是0至2；

[0114] r是1至6；

[0115] m是0至3

[0116] Q^1是NR^{9a}、O或S

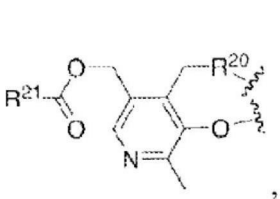
[0117] Q^2是 C_{1-10}烷基、C_{1-6}羟烷基、芳基和芳基 -C_{1-3}烷基、杂芳基及杂芳基 -C_{1-3}烷基，所述芳基和杂芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、氟及氯；

[0118] R^{11}是H、C_{1-10}烷基、被以下取代的C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被以下取代的C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基；

[0119] R^{12}是H或C_{1-3}烷基，或R^{14b}和R^{12}一起是$(CH_2)_{2-4}$以便形成包含邻接的N和C原子的环；

[0120] R^2和R^3可在一起形成选自由以下组成的组的环：

[0121]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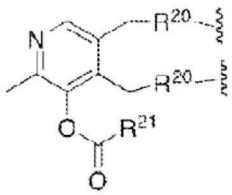
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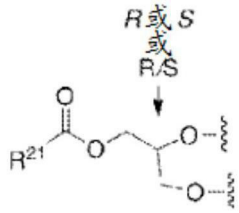
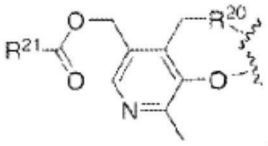
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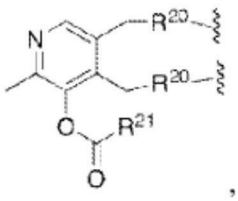
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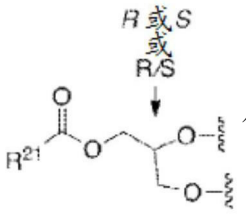
[0122]

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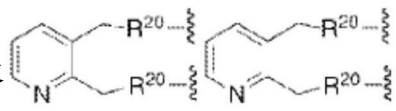


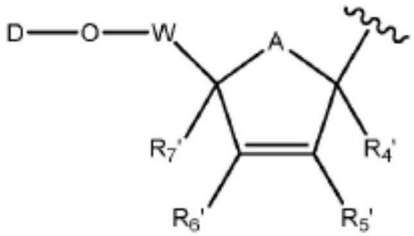
[0123] 其中R^{20}是O或NH，并且

[0124] R^{21}选自由以下组成的组：H、C_{1-20}烷基、C_{1-20}烯基、来源于脂肪酸的碳链、及被以下取代的C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基及取代的杂芳基；其中所述取代基是C_{1-5}烷基或被C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基取代的C_{1-5}烷基，并且

[0125] (j)当R^3是OH、O^-K^+、O^-Li^+或O^-Na^+时，R^2是单磷酸酯或二磷酸酯。

[0126] 在又一实施方案中，糖是通式(V)的核糖或修饰的核糖：

[0127]



(V)

[0128] 其中：

[0129] D 是 H、C(O)R^1、C(O)OR^1、二磷酸酯或三磷酸酯；

[0130] R^1独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被 C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

[0131] W 是 CL_2或 CL_2CL_2，其中 L 独立地选自由以下组成的组：H、C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基，其中 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基可各自任选地含有一个或多个杂原子；

[0132] A、R^2、R^3、Y、Z、$R^{4'}$、$R^{5'}$、$R^{6'}$及 $R^{7'}$如上关于式 I、II、III 及 IV 所定义；

[0133] 其中对于糖是式 (V) 的式 (I)，当 A 是 O 或 S 时，$R^{7'}$不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2，

[0134] 在另一实施方案中，$R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 及 R；

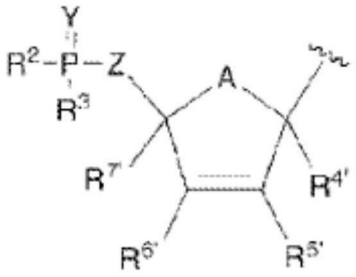
[0135] 其中 R 独立地是 C_1-C_6烷基、C_{2-6}烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基（例如羟烷基、氨基烷基及烷氧基烷基）取代。

[0136] 在一个实施方案中，其中糖具有式 (V)，当 A 是 O 或 CH_2，D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 $R^{7'}$是 H 时，$R^{5'}$和 $R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ═ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

[0137] 在另一实施方案中，R^5和 $R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、C(O)NH_2、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

[0138] 在又一实施方案中，糖是通式 (VI) 的修饰的核糖：

[0139]



(VI)

[0140] 其中：

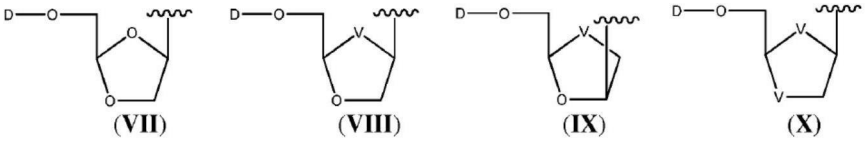
[0141] A、R^2、R^3、Y、Z、$R^{4'}$、$R^{5'}$、$R^{6'}$及$R^{7'}$如上关于式I、II、III及IV所定义；

[0142] 其中对于糖是式(VI)的式(I)，当A是O或S时，$R^{7'}$不能是OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR或NR_2，

[0143] 其中R独立地是C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式I、II、III及IV所定义的取代基（例如，羟烷基、氨基烷基及烷氧基烷基）取代。

[0144] 在另一实施方案中，糖是通式(VII)、(VIII)、(IX)及(X)的二氧戊环、氧硫杂环戊烷或二硫戊环：

[0145]



(VII) (VIII) (IX) (X)

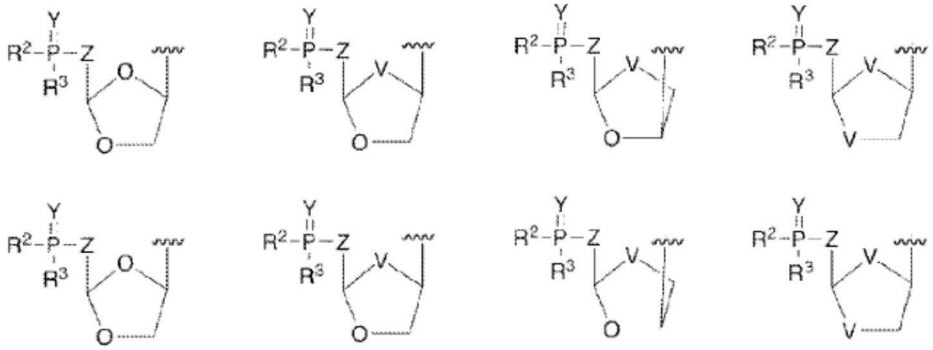
[0146] D是C(O)OR^1、二磷酸酯或三磷酸酯；

[0147] V个别地是S或Se；

[0148] R^1独立地是C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的C_{1-20}烷基：C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基取代的C_{1-5}烷基；

[0149] 在又一实施方案中，糖是通式(XI)、(XII)、(XIII)及(XIV)的二氧戊环、氧硫杂环戊烷或二硫戊环：

[0150]



(XI) (XII) (XIII)

(XIV)

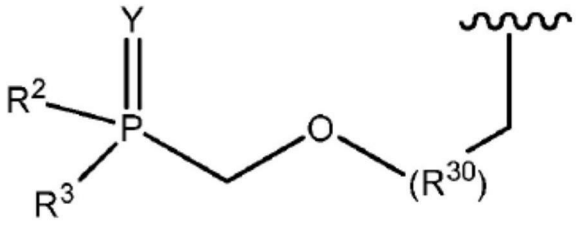
[0151] 其中：

[0152] V个别地是S或Se；

[0153] R^2、R^3、Y和Z如上关于式I、II、III和IV所定义。

[0154] 在又一实施方案中，糖是通式(XV)的磷酰基甲氧烷基：

[0155]



(XV)

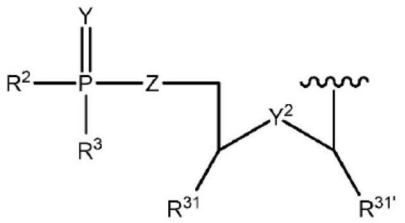
[0156] 其中：

[0157] R^2、R^3和 Y 如上关于式 I、II、III 和 IV 所定义；并且；

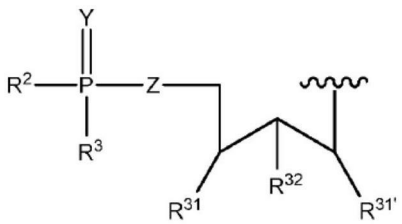
[0158] R^{30}选自由以下组成的组：C_{1-20}烷基、C_{2-20}烷基（包括但不限于 C_1-C_6）、烯基（包括但不限于 C_2-C_6）、和 C_{2-20}炔基、C_{3-10}（包括但不限于 C_2-C_6）、环烷基（包括但不限于 C_3-C_8）、芳基（包括但不限于 C_6-C_{10}）、杂芳基（包括但不限于 C_6-C_{10}）、芳基烷基以及烷基芳基；

[0159] 在又一实施方案中，糖具有通式 (XVI) 或 (XVII)：

[0160]



(XVI)



(XVII)

[0161] 其中：

[0162] R^2、R^3、Z 和 Y 如上所定义；

[0163] Y^2是 O、S、Se 或 NR；

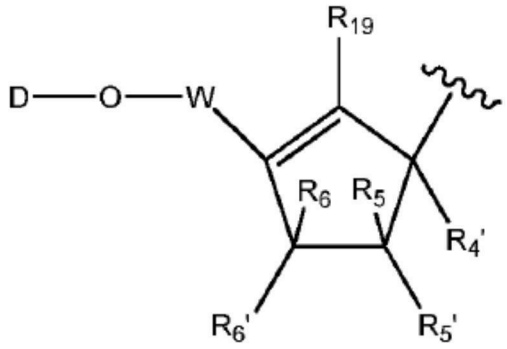
[0164] R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基（例如羟烷基、氨基烷基和烷氧基烷基）取代；

[0165] R^{31}、$R^{31'}$和 R^{32}被定义为 H、CH_3或 CH_2OR^{33}；并且

[0166] R^{33}是 H 或 C_1-C_6酰基。

[0167] 在另一实施方案中，糖是通式 (XVIII) 的修饰的核糖

[0168]



(XVIII)

[0169] 其中：

[0170] D、W、$R^{4'}$、R^5、$R^{5'}$、R^6及 $R^{6'}$如上所定义；

[0171] R^{19}是 H、F、Cl、Br、I、N_3、C(O)OH、CN、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 或 R；

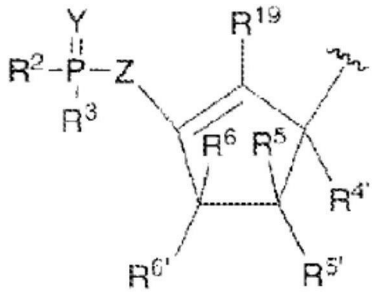
[0172] 其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基（例如，羟烷基、氨基烷基及烷氧基烷基）取代。

[0173] 在一个实施方案中，其中糖具有式 (XVII)，当 D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 R^{19}是 H 时，R^5、$R^{5'}$、R^6、$R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ＝ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

[0174] 在另一实施方案中，$R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2。

[0175] 在又一实施方案中，糖是式 (XIX) 的修饰的核糖：

[0176]



(XIX)

[0177] 其中：

[0178] R^2、R^3和 Y 如上关于式 I、II、III 和 IV 所定义；

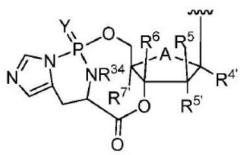
[0179] $R^{4'}$、R^5、$R^{5'}$、R^6及 $R^{6'}$如上所定义；

[0180] R^{19}是 H、F、Cl、Br、I、N_3、C(O)OH、CN、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 或 R，

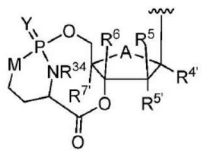
[0181] 其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基（例如，羟烷基、氨基烷基及烷氧基烷基）取代。

[0182] 在又一实施方案中，糖具有式 (XX)、(XXI) 或 (XXII) 中的一种：

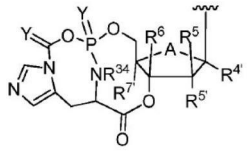
[0183]



(XX)



(XXI)



(XXII)

[0184] 其中：

[0185] $R^{4'}$、R^5、$R^{5'}$、R^6、Y、A 及 $R^{7'}$ 如上关于式 I、II、III 及 IV 所定义；

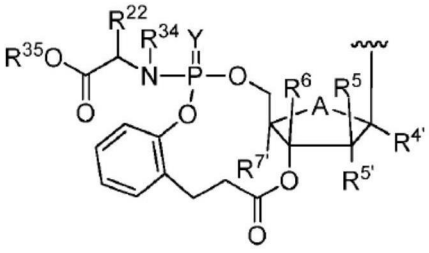
[0186] R^{34} 是 C_1-C_6 烷基；

[0187] M 是 O、S 或 NR；

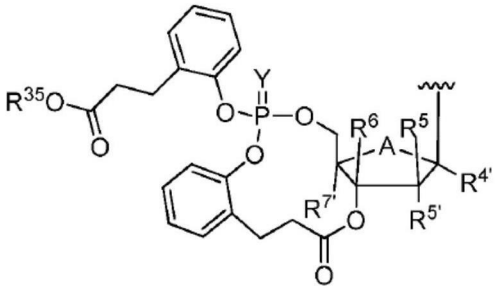
[0188] 其中 R 独立地是 C_1-C_6 烷基、C_{2-6} 烯基、C_{2-6} 炔基、C_3-C_6 环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 和 IV 所定义的取代基（例如羟烷基、氨基烷基和烷氧基烷基）取代；

[0189] 在另一实施方案中，糖具有式 (XXIII) 或 (XXIV) 中的一种：

[0190]



(XXIII)



(XXIV)

[0191] 其中：

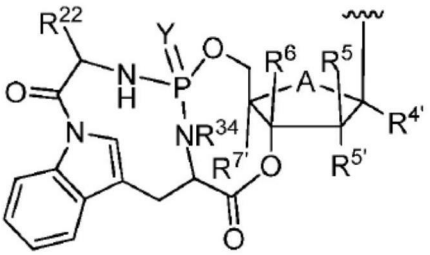
[0192] $R^{4'}$、R^5、$R^{5'}$、R^6、Y、A、$R^{7'}$、R^{34} 如上关于式 I、II、III 及 IV 所定义；

[0193] R^{35} 是 H、C_{1-10} 烷基、被以下取代的 C_{1-10} 烷基：C_{1-6} 烷基、C_{1-6} 烷氧基、二 (C_{1-6} 烷基)-氨基、氟、C_{3-10} 环烷基、C_{3-10} 环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5} 烷基、或被 C_{1-6} 烷基、C_{1-6} 烷氧基、二 (C_{1-6} 烷基)-氨基、氟、C_{3-10} 环烷基或 C_{3-10} 环烷基烷基取代的 C_{1-5} 烷基；并且

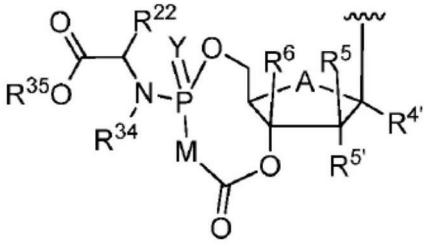
[0194] R^{22} 是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2-吲哚-3-基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$、$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2-咪唑-4-基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH 或 C_{3-6} 环烷基；

[0195] 在又一实施方案中，糖具有式 (XXV) 或 (XXVI) 中的一种：

[0196]



(XXV)



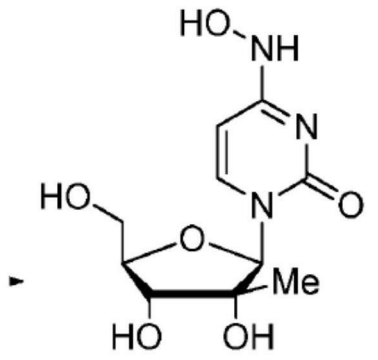
(XXVI)

[0197] 其中：

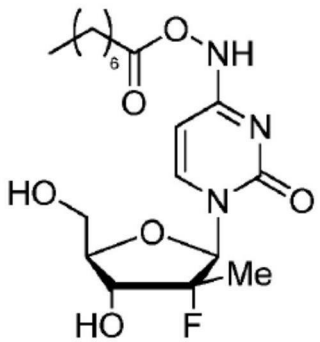
[0198] $R^{4'}$、R^5、$R^{5'}$、R^6、Y、M、$R^{7'}$、R^{34}、R^{35}、R^{22}如上关于式 I、II、III 及 IV 所定义；

[0199] 在一个实施方案中，化合物具有下式中的一种：

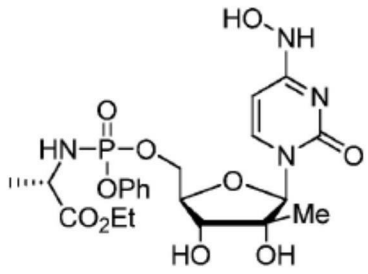
[0200]



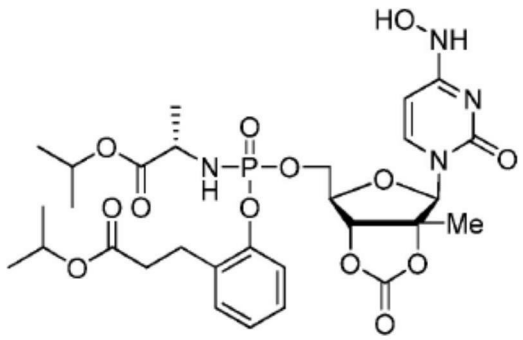
37



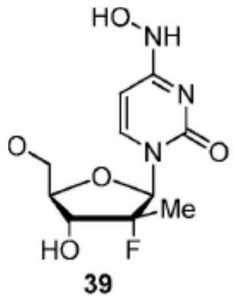
40



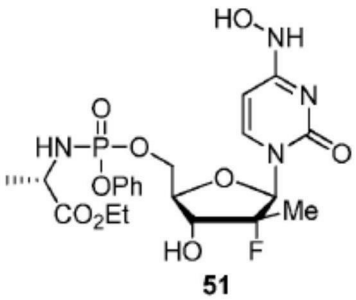
44



48



39



51

[0201] ，或其药学上可接受的盐。

[0202] 在一个实施方案中，R^5或 $R^{5'}$中的至少一个是 F、Cl 或 Me。

[0203] 在另一实施方案中，R^5和 $R^{5'}$分别是 Me 和 F。

[0204] 在另一实施方案中，R^5和 $R^{5'}$分别是 Me 和 Cl。

[0205] 在另一实施方案中，L 是甲基。

[0206] 在另一实施方案中，碱基是嘧啶，并且 R^5和 $R^{5'}$中的一个是 OH、Cl 或 F。

[0207] 本文所述的化合物可呈以下形式：β-L- 或 β-D- 构型、或其混合物，包括其外消旋混合物。

[0208] 在那些实施方案中，当本文所述的化合物的磷部分含有手性中心时，这种手性中心可呈以下形式：R_p- 或 S_p- 构型或其混合物，包括其外消旋混合物。

[0209] 在一个实施方案中，化合物在生物系统中被转化为嘧啶三磷酸酯的混合物，这是由于在嘧啶环上的 -NHOH 部分转化为 $-NH_2$部分且任选地嘧啶环上的 -NHOH 部分或所得的 $-NH_2$部分转化为 OH 部分。这类部分转化的一个实例显示如下，其中嘧啶三磷酸酯的混合物 C 或 D 包括 4-NHOH、4-NH_2及 4-OH 嘧啶三磷酸酯。这种混合物可例如当所施用的化合物包括在糖的 5'-OH 部分上的前药时形成。适合的前药的实例包括如上所例示的那些。

[0210]

[0211] 因此，通过施用单一化合物，两种或三种活性化合物的组合可在药物代谢期间形成，并且这些药物可以不同方式靶向病毒。例如，其中 NHOH 直接地或间接地转化为 OH 部分的类似物当通过病毒掺入生长中的 DNA 或 RNA 链中时的作用类似于尿苷类似物。其中 NHOH 部分转化为 NH_2部分的类似物当通过病毒掺入生长中的 DNA 或 RNA 链中时的作用类似于胞嘧啶类似物。NHOH 类似物当通过病毒掺入生长中的 DNA 或 RNA 链中时的作用可类似于胞嘧啶或尿苷类似物。预期三种活性三磷酸酯的组合对比通常施用的任何单个三磷酸酯药物将产生不同且更难的突变选择。

[0212] 通过以多种方式攻击病毒，即通过用 U 和 C 型类似物呈递病毒，前药化合物具有抵御病毒耐药性的嵌入机制 (built-in mechanism)。也就是说，如果病毒应突变以避免占用 U 类似物，则其还可对一种或多种 C 类似物敏感，且反之亦然，并且如果应存在多种 C 类似物，则对其中一种的抗性可能不会赋予对另一种的抗性。

[0213] 因此，本文所述的化合物可作为单一组分施用，且还提供组合抗病毒疗法的益处。当与另外的抗病毒剂、特别是非 NNRTI 抗病毒剂组合时，所述组合可提供与许多另外的组分的组合的益处，同时提供仅包括一种核苷前药的简单性。

[0214] 发明详述

[0215] 本文所述的N^4-羟基胞苷核苷衍生物及修饰的单磷酸酯前药类似物显示针对HIV、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、癌症、HBV和疱疹病毒如HSV-1、HSV-2、以及巨细胞病毒(CMV)的抑制活性。因此,所述化合物可用于治疗或预防宿主中的病毒感染或降低病毒的生物活性。宿主可以是感染了HIV-1、HIV-2、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病、癌症、巨细胞病毒(CMV)和/或HBV的哺乳动物且特别是人。所述方法包括施用有效量的一种或多种本文所述的核苷或核苷酸单磷酸酯前药。

[0216] 还公开了包含与药学上可接受的载体或赋形剂组合的一种或多种本文所述的化合物的药物制剂。在一个实施方案中,所述制剂包含至少一种本文所述的化合物和至少一种其它治疗剂。

[0217] 本发明将参考以下定义得到更好的理解:

[0218] I.定义

[0219] 术语"独立地"在本文中用于指示独立地应用的变量在应用与应用之间独立地变化。因此,在如R"XYR"的化合物中,其中R"“独立地是碳或氮",两个R"均可是碳,两个R"均可是氮,或一个R"可以是碳且另一个R"是氮。

[0220] 如本文所用,术语"对映异构体纯的"是指包含至少大约95%且优选地大约97%、98%、99%或100%核苷酸的单一对映异构体的核苷酸组合物。

[0221] 如本文所用,术语"基本上不含"或"基本上在不存在下"是指包含至少85至90重量%、优选地95重量%至98重量%、且甚至更优选地99重量%至100重量%的核苷酸的指定对映异构体的核苷酸组合物。在一个优选实施方案中,本文所述的化合物基本上不含对映异构体。

[0222] 类似地,术语"分离的"是指包含至少85至90重量%、优选地95重量%至98重量%、且甚至更优选地99重量%至100重量%的核苷酸且其余包含其它化学物种或对映异构体的核苷酸组合物。

[0223] 在一些情况下,磷原子可以是手性的,在本文中被称为"P*"或"P",这意味着并且是指其具有对应于对于这种指配的Cahn-Ingold-Prelog规则的所接受的含义的"R"或"S"名称。式A的前药可由于在磷中心处的手性而作为非对映异构体的混合物存在。当在磷中心处存在手性时,其可能全部或部分是Rp或Sp或其任何混合物。

[0224] 除非另作说明,如本文所用的术语"烷基"是指饱和直链、支链或环状伯烃、仲烃或叔烃,包括取代的和未被取代的烷基。烷基可任选地被不另外干扰反应或提供过程中的改善的任何部分取代,包括但不限于卤基、卤烷基、羟基、羧基、酰基、芳基、酰氧基、氨基、酰胺基、羧基衍生物、烷基氨基、二烷基氨基、芳基氨基、烷氧基、芳氧基、硝基、氰基、磺酸、硫醇、亚胺、磺酰基、硫烷基、亚硫酰基、氨磺酰基、酯、羧酸、酰胺、膦酰基、氧膦基、磷酰基、磷化氢、硫酯、硫醚、酰卤、酸酐、肟、肼、氨基甲酸酯、膦酸、膦酸酯,根据需要是未受保护或受保护的,如本领域技术人员已知,例如如在Greene, 等人,Protective Groups in Organic Synthesis,John Wiley and Sons,第二版,1991中所教导,该文献据此以引用的方式并入。具体地包括CF_3和CH_2CF_3。

[0225] 在正文中,每当使用术语C(烷基范围),该术语独立地包括那类中的每个成员,就如同具体地和单独地列出一般。术语"烷基"包括C_{1-22}烷基部分,且术语"低级烷基"包括

C_{1-6}烷基部分。本领域技术人员应理解，有关烷基是通过用后缀“-基”取代后缀“-ane”来命名。

[0226] 术语“烯基”是指直链或支链的程度以致其含有一个或多个双键的不饱和烃基，且术语“低级烯基”包括C_{2-6}烯基部分。本文所公开的烯基可任选地被不会不利地影响反应过程的任何部分取代，包括但不限于针对烷基部分上的取代基所述的那些。烯基的非限制性实例包括乙烯、甲基乙烯、异丙烯、1,2-乙烷-二基、1,1-乙烷-二基、1,3-丙烷-二基、1,2-丙烷-二基、1,3-丁烷-二基以及1,4-丁烷-二基。

[0227] 术语“炔基”是指直链或支链的程度以致其含有一个或多个三键的不饱和无环烃基，且术语“低级炔基”包括C_{2-6}炔基部分。炔基可任选地被不会不利地影响反应过程的任何部分取代，包括但不限于如上针对烷基部分所述的那些。适合的炔基的非限制性实例包括乙炔基、丙炔基、羟基丙炔基、丁炔-1-基、丁炔-2-基、戊炔-1-基、戊炔-2-基、4-甲氧基戊炔-2-基、3-甲基丁炔-1-基、己炔-1-基、己炔-2-基以及己炔-3-基、3,3-二甲基丁炔-1-基。

[0228] 术语“烷基氨基”或“芳基氨基”是指分别具有一个或两个烷基或芳基取代基的氨基。

[0229] 如本文所用且除非另外指明，术语“受保护的”是指添加到氧、氮或磷原子中以防止它的进一步反应或出于其它目的的基团。广泛多种氧和氮保护基为有机合成领域的技术人员所知且描述于例如上述Greene等人，Protective Groups in Organic Synthesis中。

[0230] 单独或组合的术语“芳基”意指含有一个、两个或三个环的碳环芳族系统，其中这种环可以悬挂方式连接在一起或可以被稠合。芳基的非限制性实例包括苯基、联苯或萘基、或在从芳族环上除去氢之后剩余的其它芳族基团。术语芳基包括取代的和未被取代的部分。芳基可任选地被不会不利地影响过程的任何部分取代，包括但不限于如上针对烷基部分所述的那些。取代的芳基的非限制性实例包括杂芳基氨基、N-芳基-N-烷基氨基、N-杂芳基氨基-N-烷基氨基、杂芳烷氧基、芳基氨基、芳烷基氨基、芳硫基、单芳基酰氨基磺酰基、芳基亚磺酰氨基、二芳基酰氨基磺酰基、单芳基酰氨基磺酰基、芳基亚硫酰基、芳基磺酰基、杂芳硫基、杂芳基亚硫酰基、杂芳基磺酰基、芳酰基、杂芳酰基、杂芳烷酰基、羟基芳烷基、羟基杂芳烷基、卤烷氧基烷基、芳基、芳烷基、芳氧基、芳烷氧基、芳氧基烷基、饱和杂环基、部分饱和杂环基、杂芳基、杂芳氧基、杂芳氧基烷基、芳基烷基、杂芳基烷基、芳基烯基以及杂芳基烯基、碳芳烷氧基。

[0231] 术语“烷芳基”或“烷基芳基”是指具有芳基取代基的烷基。术语“芳烷基”或“芳基烷基”是指具有烷基取代基的芳基。

[0232] 如本文所用的术语“卤基”包括氯、溴、碘及氟。

[0233] 术语“酰基”是指羧酸酯，其中酯基的非羰基部分选自直链、支链或环状烷基或低级烷基、烷氧基烷基，包括但不限于甲氧基甲基；芳烷基，包括但不限于苄基；芳氧基烷基如苯氧基甲基；芳基，包括但不限于苯基，任选地被卤素(F、Cl、Br、I)取代；烷基(包括但不限于C_1、C_2、C_3及C_4)；烷氧基(包括但不限于C_1、C_2、C_3及C_4)；磺酸酯，如烷基或芳烷基磺酰基，包括但不限于甲磺酰基；单磷酸酯、二磷酸酯或三磷酸酯；三苯甲基或单甲氧基三苯甲基、取代的苄基、三烷基甲硅烷基(例如二甲基-叔丁基甲硅烷基)或二苯基甲基甲硅烷基。酯中的芳基最适合包括苯基。术语“低级酰基”是指其中非羰基部分是低级烷基的酰

基。

[0234] 术语“烷氧基”和“烷氧基烷基”包含具有烷基部分的直链或支链含氧基团，如甲氧基。术语“烷氧基烷基”还包括具有一个或多个连接至烷基上以形成单烷氧基烷基和二烷氧基烷基的烷氧基的烷基。“烷氧基”可进一步被一个或多个卤原子如氟、氯或溴取代以提供“卤烷氧基”。这种基团的实例包括氟甲氧基、氯甲氧基、三氟甲氧基、二氟甲氧基、三氟乙氧基、氟乙氧基、四氟乙氧基、五氟乙氧基及氟丙氧基。

[0235] 术语“烷基氨基”表示含有分别连接至氨基上的一个或两个烷基的“单烷基氨基”和“二烷基氨基”。术语“芳基氨基”表示含有分别连接至氨基上的一个或两个芳基的“单芳基氨基”和“二芳基氨基”。术语“芳烷基氨基”包括连接至氨基上的芳烷基。术语芳烷基氨基表示含有分别连接至氨基上的一个或两个芳烷基的“单芳烷基氨基”和“二芳烷基氨基”。术语芳烷基氨基进一步表示含有连接至氨基上的一个芳烷基和一个烷基的“单芳烷基单烷基氨基”。

[0236] 如本文所用的术语“杂原子”是指氧、硫、氮及磷。

[0237] 如本文所用的术语“杂芳基”或“杂芳族”是指在芳族环中包含至少一个硫、氧、氮或磷的芳族。

[0238] 术语“杂环”、“杂环基”及“环杂烷基”是指在环中存在至少一个杂原子如氧、硫、氮或磷的非芳族环状基团。

[0239] 杂芳基和杂环基团的非限制性实例包括呋喃基 (furyl)、呋喃基 (furanyl)、吡啶基、嘧啶基、噻吩基、异噻唑基、咪唑基、四唑基、吡嗪基、苯并呋喃基、苯并噻吩基、喹啉基、异喹啉基、苯并噻吩基、异苯并呋喃基、吡唑基、吲哚基、异吲哚基、苯并咪唑基、嘌呤基、咔唑基、噁唑基、噻唑基、异噻唑基、1,2,4- 噻二唑基、异噁唑基、吡咯基、喹唑啉基、噌啉基、酞嗪基、黄嘌呤基、次黄嘌呤基、噻吩、呋喃、吡咯、异吡咯、吡唑、咪唑、1,2,3- 三唑、1,2,4- 三唑、噁唑、异噁唑、噻唑、异噻唑、嘧啶或哒嗪，及蝶啶基、氮丙啶、噻唑、异噻唑、1,2,3- 噁二唑、噻嗪、吡啶、吡嗪、哌嗪、吡咯烷、噁嗪烷 (oxazirane)、吩嗪、吩噻嗪、吗啉基、吡唑基、哒嗪基、吡嗪基、喹喔啉基、黄嘌呤基、次黄嘌呤基、蝶啶基、5- 氮杂胞苷基、5- 氮杂尿嘧啶基、三唑并吡啶基、咪唑并吡啶基、吡咯并嘧啶基、吡唑并嘧啶基、腺嘌呤、N^6- 烷基嘌呤、N^6- 苄基嘌呤、N^6- 卤代嘌呤、N^6- 乙烯嘌呤、N^6- 炔属嘌呤、N^6- 酰基嘌呤、N^6- 羟烷基嘌呤、N^6- 硫代烷基嘌呤、胸腺嘧啶、胞嘧啶、6- 氮杂嘧啶、2- 巯基嘧啶、尿嘧啶、N^5- 烷基嘧啶、N^5- 苄基嘧啶、N^5- 卤代嘧啶、N^5- 乙烯基嘧啶、N^5- 炔属嘧啶、N^5- 酰基嘧啶、N^5- 羟烷基嘌呤及 N^6- 硫代烷基嘌呤、以及异噁唑基。杂芳族基团可如上针对芳基所述任选地被取代。杂环或杂芳族基团可任选地被一个或多个选自由以下组成的组的取代基取代：卤素、卤烷基、烷基、烷氧基、羟基、羧基衍生物、酰胺基、氨基、烷基氨基及二烷基氨基。杂芳族可根据需要被部分或全部氢化。作为非限制性实例，二氢吡啶可代替吡啶来使用。在杂环或杂芳基上的官能性氧和氮基团可根据需要或希望被保护。适合的保护基是本领域技术人员所熟知的且包括三甲基甲硅烷基、二甲基己基甲硅烷基、叔丁基二甲基甲硅烷基和叔丁基二苯基甲硅烷基、三苯甲基或取代的三苯甲基、烷基、酰基如乙酰基和丙酰基、甲磺酰基以及对甲苯磺酰基。杂环或杂芳族基团可被不会不利地影响反应的任何部分取代，包括但不限于如上针对芳基所述的那些。

[0240] 如本文所用的术语“宿主”是指其中病毒可以复制的单细胞或多细胞有机体，包括

但不限于细胞系和动物且优选是人。或者，宿主可携带一部分病毒基因组，其复制或功能可由本发明化合物改变。术语宿主具体是指受感染的细胞、被病毒基因组全部或一部分转染的细胞以及动物，特别是灵长类动物（包括但不限于黑猩猩）和人。在本发明的大多数动物应用中，宿主是人患者。然而，在某些适应症中，本发明明确地考虑到了兽医应用（如用于治疗黑猩猩）。

[0241] 术语“肽”是指含有由一个氨基酸的羧基连接到另一个的氨基上的二至一百个氨基酸的各种天然或合成化合物。

[0242] 术语“药学上可接受的盐或前药”在本说明书通篇中用于描述核苷酸化合物的任何药学上可接受的形式（如酯、磷酸酯、酯的盐或相关基团），其一旦施用于患者便提供核苷酸单磷酸酯化合物。药学上可接受的盐包括由药学上可接受的无机或有机碱和酸衍生的那些盐。适合的盐包括由碱金属如钾和钠、碱土金属如钙和镁与药学领域中熟知的许多其它酸衍生的那些盐。药学上可接受的前药是指在宿主中代谢，例如水解或氧化以形成本发明化合物的化合物。前药的典型实例包括在活性化合物的官能部分上具有生物学不稳定的保护基团的化合物。前药包括可被氧化、还原、氨化、脱氨基、羟基化、脱羟基化、水解、脱水、烷基化、脱烷基化、酰化、脱酰化、磷酸化或去磷酸化以产生活性化合物的化合物。本发明化合物的前药形式可具有抗病毒活性，可被代谢形成展现这种活性的化合物，或两者。

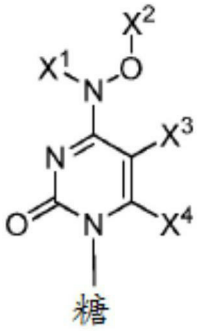
[0243] 前药还包括所公开的核苷的氨基酸酯（参见，例如欧洲专利说明书号 99493，其正文以引用的方式并入，该专利描述阿昔洛韦的氨基酸酯，具体是甘氨酸和丙氨酸酯，其显示与阿昔洛韦本身相比改善的水溶性，以及美国专利号 4,957,924(Beauchamp)，其公开了以靠近 α－碳原子的侧链支化为特征的阿昔洛韦的缬氨酸酯，其显示与丙氨酸和甘氨酸酯相比在口服施用之后改善的生物利用率）。用于制备这种氨基酸酯的方法公开于美国专利号 4,957,924(Beauchamp) 中，其内容以引用的方式并入。作为使用缬氨酸本身的一种替代方案，可使用氨基酸的功能等效物（例如，酰卤如酰氯，或酸酐）。在这种情况下，为了避免不合需要的副反应，使用氨基保护的衍生物可以是有利的。

[0244] II. 活性化合物

[0245] 在本发明的一个实施方案中，活性化合物具有式 (I)：

[0246] 在一个实施方案中，化合物是式 (I) 化合物：

[0247]



(I)

[0248] 或其药学上可接受的盐或前药，其中：

[0249] iv)X^1是 H、C_1-C_6烷基、C_1-C_6卤烷基、C_1-C_6烷氧基、C_2-C_6烯基、C_2-C_6炔基、COR^1或

$COOR^1$；

[0250] v)X^2是氢、COR^1或 $COOR^1$

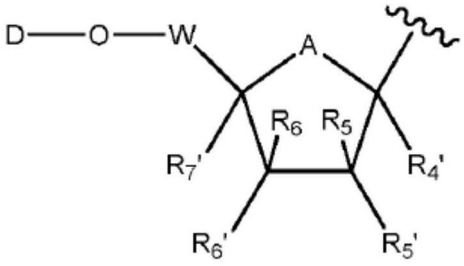
[0251] 其中每个 R^1独立地是 CH_2-O(CO)-X^5、CH_2-O(CO)O-X^5、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-6}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟或 C_{3-10}环烷基取代的 C_{1-6}烷基

[0252] X^5独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、烷氧基、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-6}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟或 C_{3-10}环烷基取代的 C_{1-6}烷基

[0253] vi)X^3和 X^4各自独立地是 H、C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、芳基、烷基芳基、卤素(F、Cl、Br、I)、NH_2、OH、SH、CN 或 NO_2。

[0254] 在一个实施方案中，糖是通式(II)的核糖或修饰的核糖：

[0255]



(II)

[0256] 其中：

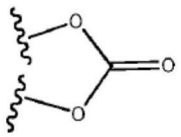
[0257] D 是 H、C(O)R^1、C(O)OR^1、二磷酸酯或三磷酸酯；

[0258] R^1如上所定义；

[0259] W 是 CL_2或 CL_2CL_2，其中 L 独立地选自由以下组成的组：H、C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基，其中 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基可各自任选地含有一个或多个杂原子；

[0260] A 是 O、S、CH_2、CHF、CF_2、C = CH_2、C = CHF 或 C = CF_2；

[0261] $R^{4'}$、R^5、$R^{5'}$、R^6、$R^{6'}$及 $R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、OH、SH、NH_2、NHOH、$NHNH_2$、N_3、C(O)OH、CN、CH_2OH、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

[0262] $R^{5'}$和 $R^{6'}$可一起形成环 

。

[0263] 在一个实施方案中，其中糖是式(II)，当 A 是 O 或 CH_2，D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 $R^{7'}$是 H 时，R^5、$R^{5'}$、R^6、$R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH = CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

[0264] 在另一实施方案中，$R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

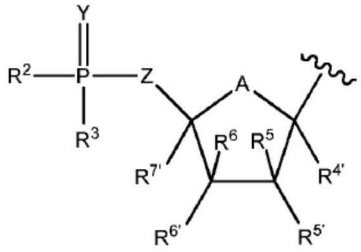
[0265] 在一个实施方案中，其中对于糖是式（II）的式（I），当 A 是 O 或 S 时，$R^{7'}$ 不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2。

[0266] 在另一实施方案中，$R^{7'}$ 独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 及 R；

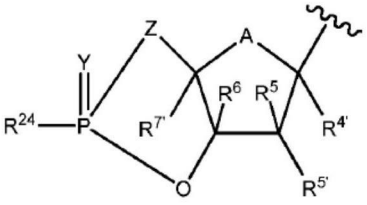
[0267] R 独立地是 C_1-C_6烷基、C_{2-6}烯基、C_{2-6}炔基、C_{3-6}环烷基、（C_3-C_6环烷基）芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基取代，其中代表性取代基包括例如羟烷基、氨基烷基和烷氧基烷基。

[0268] 在另一实施方案中，糖是通式（III）或（IV）的核糖或修饰的核糖：

[0269]



(III)



(IV)

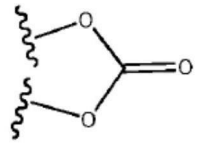
[0270] 其中：

[0271] Y 是 O 或 S；

[0272] Z 选自由以下组成的组：CL_2、CL_2CL_2、CL_2OCL_2、CL_2SCL_2、CL_2O、OCL_2及 CL_2NHCL_2，其中 L 独立地选自由以下组成的组：H、F、C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基，其中 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基可各自任选地含有一个或多个杂原子；

[0273] A 是 O、S、CH_2、CHF、CF_2、C ＝ CH_2、C ＝ CHF 或 C ＝ CF_2；

[0274] $R^{4'}$、R^5、$R^{5'}$、R^6、$R^{6'}$及 $R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、OH、SH、NH_2、NHOH、$NHNH_2$、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

[0275] $R^{5'}$和 $R^{6'}$可一起形成环 

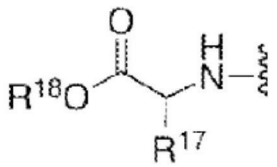
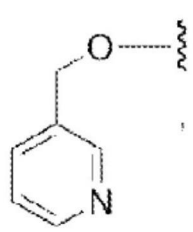
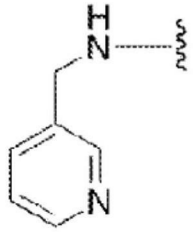
。

[0276] 在一个实施方案中，其中糖是式（III）或（IV），当 A 是 O 或 S 时，$R^{7'}$ 不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2。

[0277] 在另一实施方案中，$R^{7'}$ 独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 及 R。

[0278] R 独立地是 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基取代。

[0279] R^{24}选自由以下组成的组：OR^{15}、

[0280]    及脂肪醇，

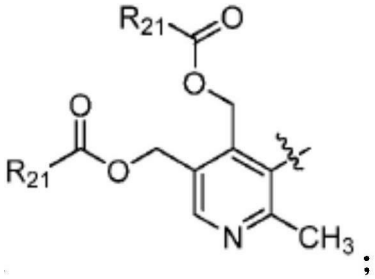
[0281] R^{15}选自由以下组成的组：H、Li、Na、K、苯基及吡啶基；其中苯基和吡啶基任选地被零至三个独立地选自由 $(CH_2)_{0-6}CO_2R^{16}$和 $(CH_2)_{0-6}CON(R^{16})_2$组成的组的取代基取代；

[0282] R^{17}选自在天然L-氨基酸中存在的那些基团、C_{1-6}烷基、(C_1-C_6烷基)、C_{2-6}烯基、C_{2-6}炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基取代。

[0283] R^{18}是H、C_{1-20}烷基、来源于脂肪醇（如油醇、二十八醇、三十醇、亚油醇等）的碳链或被以下取代的C_{1-20}烷基：C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基、环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被C_1-C_6烷基、C_1-C_6烷氧基、二(C_1-C_6烷基)-氨基、氟、C_{3-10}环烷基或环烷基取代的C_{1-5}烷基。

[0284] 代表性R^2和R^3独立地选自由以下组成的组：

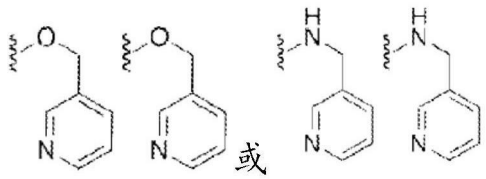
[0285] (a)OR^8，其中R^8是H、Li、Na、K、C_{1-20}烷基、C_{3-6}环烷基、C_{1-6}卤烷基、芳基或杂芳基（包括但不限于苯基或萘基），任选地被一个至三个独立地选自由以下组成的组的取代基取代：C_{1-6}烷基、C_{2-6}烯基、C_{2-6}炔基、C_{1-6}烷氧基、$(CH_2)_{0-6}CO_2R^{9a}$、卤素、C_{1-6}卤烷基、$-N(R^{9a})_2$、C_{1-6}酰基氨基、$-NHSO_2C_{1-6}$烷基、$-SO_2N(R^{9a})_2$、$-SO_2C_{1-6}$烷基、COR^{9b}、硝基、氰基及

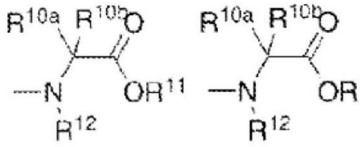
 ；

[0286] 其中R^{21}如下所定义；

[0287] R^{9a}独立地是H、C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基取代的C_{1-5}烷基；

[0288] R^{9b}是$-OR^{9a}$或$-N(R^{9a})_2$；

[0289] (b) 

[0290] (c)  其中 R^{10a} 和 R^{10b} 是：

，

[0291] (i) 独立地选自由以下组成的组：H、C_{1-10} 烷基、$-(CH_2)_rNR^{9a}{}_2$、C_{1-6} 羟烷基、$-CH_2SH$、$-(CH_2)_2S(O)_pMe$、$-(CH_2)_3NHC(=NH)NH_2$、(1H- 吲哚 -3- 基) 甲基、(1H- 咪唑 -4- 基) 甲基、$-(CH_2)_mCOR^{9b}$、芳基及芳基 $-C_{1-3}$ 烷基，所述芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10} 烷基、C_{1-6} 烷氧基、卤素、硝基及氰基；

[0292] (ii) R^{10a} 是 H 且 R^{10b} 和 R^{12} 一起是 $(CH_2)_{2-4}$ 以形成包含邻接的 N 和 C 原子的环；

[0293] (iii) R^{10a} 和 R^{10b} 一起是 $(CH_2)_n$ 以形成环；

[0294] (iv) R^{10a} 和 R^{10b} 都是 C_{1-6} 烷基；或

[0295] (v) R^{10a} 是 H 且 R^{10b} 是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2- 吲哚 -3- 基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$-$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2- 咪唑 -4- 基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH 或 C_{3-10} 环烷基；

[0296] p 是 0 至 2；

[0297] r 是 1 至 6；

[0298] n 是 4 或 5；

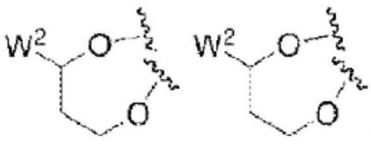
[0299] m 是 0 至 3；

[0300] R^{11} 是 H、C_{1-10} 烷基、或被以下取代的 C_{1-10} 烷基：C_{1-6} 烷基、C_{1-6} 烷氧基、二 (C_{1-6} 烷基)- 氨基、氟、C_{3-10} 环烷基、C_{3-10} 环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5} 烷基、或被 C_{1-6} 烷基、C_{1-6} 烷氧基、二 (C_{1-6} 烷基)- 氨基、氟、C_{3-10} 环烷基或 C_{3-10} 环烷基烷基取代的 C_{1-5} 烷基；

[0301] R^{12} 是 H 或 C_{1-3} 烷基，或 R^{10a}、或 R^{10b} 和 R^{12} 一起是 $(CH_2)_{2-4}$ 以便形成包含邻接的 N 和 C 原子的环；

[0302] (d) O 连接的脂质 (包括磷脂)、N 或 O 连接的肽、O 连接的胆固醇或 O 连接的植物甾醇；

[0303] (e) R^2 和 R^3 可在一起形成环

[0304]  其中 W^2 选自由以下组成的组：苯基和单环杂芳基，

其任选地被一个至三个独立地选自由以下组成的组的取代基取代：C_{1-6} 烷基、CF_3、C_{2-6} 烯基、C_{1-6} 烷氧基、OR^{9c}、CO_2R^{9a}、COR^{9a}、卤素、C_{1-6} 卤烷基、$-N(R^{9a})_2$、C_{1-6} 酰基氨基、$CO_2N(R^{9a})_2$、SR^{9a}、$-NHSO_2C_{1-6}$ 烷基、$-SO_2N(R^{9a})_2$、$-SO_2C_{1-6}$ 烷基、COR^{9b} 及氰基，并且其中所述单环杂芳基和取代的单环杂芳基具有 1-2 个独立地选自由 N、O 及 S 组成的组的杂原子，前提条件是：

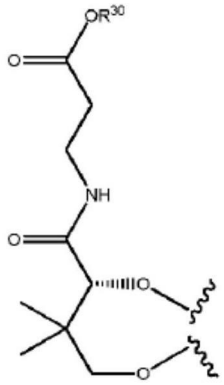
[0305] a) 当存在两个杂原子且一个是 O 时，那么另一个不能是 O 或 S，并且

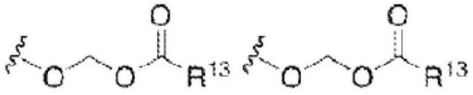
[0306] b) 当存在两个杂原子且一个是 S 时，那么另一个不能是 O 或 S；

[0307] R^{9a} 独立地是 H 或 C_{1-6} 烷基；

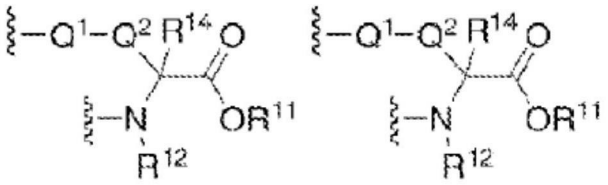
[0308] R^{9b} 是 $-OR^{9a}$ 或 $-N(R^{9a})_2$；

[0309] R^{9c}是 H 或 C_{1-6}酰基；

[0310] (f)R^2和 R^3可在一起形成环  其中 R^{30}是 H、C_{1-20}烷基、C_{1-20}烯基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基或被 C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

[0311] (g)  其中 R^{13}选自由以下组成的组：H、C_{1-10}烷基、被以下取代的 C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基和取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被以下取代的 C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基；

[0312] (h)R^2和 R^3可在一起形成环

[0313]  其中 R^{14}是：

[0314] (i)独立地选自由以下组成的组：H、C_{1-10}烷基、$-(CH_2)_rNR_2^{9a}$、C_{1-6}羟烷基、$-CH_2SH$、$-(CH_2)_2S(O)_pMe$、$-(CH_2)_3NHC(=NH)NH_2$、(1H-吲哚-3-基)甲基、(1H-咪唑-4-基)甲基、$-(CH_2)_mCOR^{9b}$、芳基、芳基-C_{1-3}烷基、杂芳基及杂芳基-C_{1-3}烷基，所述芳基和杂芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、卤素、硝基及氰基；

[0315] (ii)R^{14}是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2-吲哚-3-基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$、$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2-咪唑-4-基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH 或 C_{3-10}环烷基；

[0316] p 是 0 至 2；

[0317] r 是 1 至 6；

[0318] m 是 0 至 3

[0319] Q^1是 NR^{9a}、O 或 S

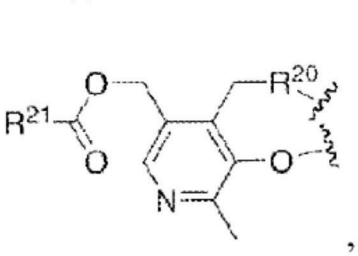
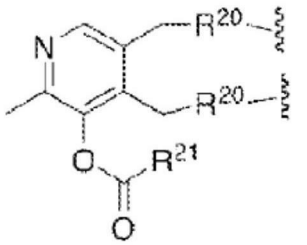
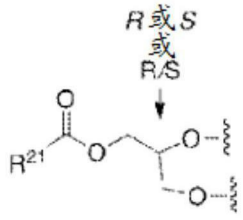
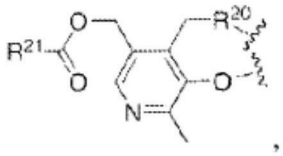
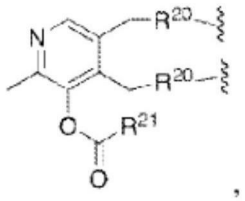
[0320] Q^2是 C_{1-10}烷基、C_{1-6}羟烷基、芳基和芳基 -C_{1-3}烷基、杂芳基及杂芳基 -C_{1-3}烷基，所述芳基和杂芳基任选地被选自由以下组成的组的基团取代：羟基、C_{1-10}烷基、C_{1-6}烷氧基、氟及氯；

[0321] R^{11}是H、C_{1-10}烷基、被以下取代的C_{1-10}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被以下取代的C_{1-5}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基；

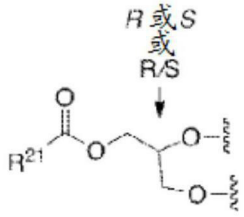
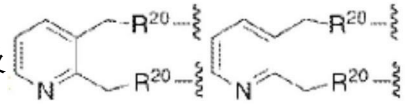
[0322] R^{12}是H或C_{1-3}烷基，或R^{14b}和R^{12}一起是$(CH_2)_{2-4}$以便形成包含邻接的N和C原子的环；

[0323] (i)R^2和R^3可在一起形成选自由以下组成的环

[0324]

[0325]


及


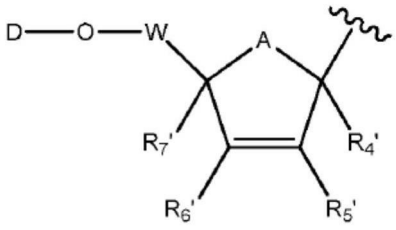
[0326] 其中R^{20}是O或NH，并且

[0327] R^{21}选自由以下组成的组：H、C_{1-20}烷基、C_{1-20}烯基、来源于脂肪酸的碳链、及被以下取代的C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基及取代的杂芳基；其中所述取代基是C_{1-5}烷基或被C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或C_{3-10}环烷基烷基取代的C_{1-5}烷基，并且

[0328] (j)当R^3是OH、O^-K^+、O^-Li^+或O^-Na^+时，R^2是单磷酸酯或二磷酸酯。

[0329] 在又一实施方案中，糖是通式(V)的核糖或修饰的核糖：

[0330]



(V)

[0331] 其中：

[0332] D 是 H、C(O)R^1、C(O)OR^1、二磷酸酯或三磷酸酯；

[0333] R^1独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_{1-6}烷基、C_{1-6}烷氧基、二（C_{1-6}烷基）-氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是C_{1-5}烷基、或被 C_{1-6}烷基、C_{1-6}烷氧基、二(C_{1-6}烷基)-氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

[0334] W 是 CL_2或 CL_2CL_2，其中 L 独立地选自由以下组成的组：H、C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基，其中 C_{1-6}烷基、C_{2-6}烯基及 C_{2-6}炔基可各自任选地含有一个或多个杂原子；

[0335] A、R^2、R^3、Y、Z、$R^{4'}$、$R^{5'}$、$R^{6'}$及 $R^{7'}$如上关于式 I、II、III 及 IV 所定义；

[0336] 其中对于糖是式 (V) 的式 (I)，当 A 是 O 或 S 时，$R^{7'}$不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2，

[0337] 在另一实施方案中，$R^{7'}$独立地选自由以下组成的组：H、F、Cl、Br、I、N_3、C(O)OH、CN、CH_2OH、C(O)NH_2、C(S)NH_2、C(O)OR 及 R；

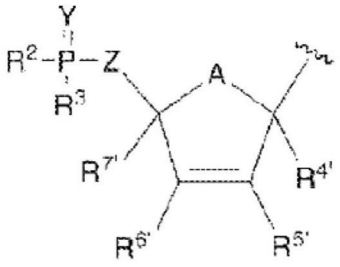
[0338] 其中 R 独立地是 C_1-C_6烷基、C_{2-6}烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基（例如羟烷基、氨基烷基及烷氧基烷基）取代。

[0339] 在一个实施方案中，其中糖具有式 (V)，当 A 是 O 或 CH_2，D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 $R^{7'}$是 H 时，$R^{5'}$和 $R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ＝ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

[0340] 在另一实施方案中，R^5和 $R^{6'}$独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、C(O)NH_2、C(S)NH_2、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2；

[0341] 在又一实施方案中，糖是通式 (VI) 的修饰的核糖：

[0342]



(VI)

[0343] 其中：

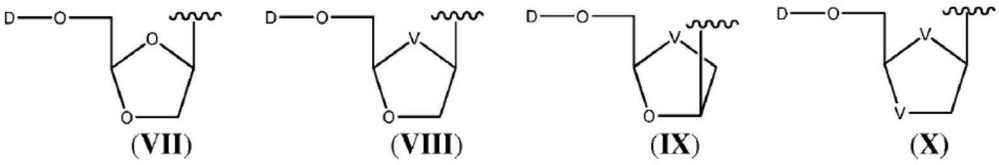
[0344] A、R^2、R^3、Y、Z、$R^{4'}$、$R^{5'}$、$R^{6'}$及 $R^{7'}$如上关于式 I、II、III 及 IV 所定义；

[0345] 其中对于糖是式 (VI) 的式 (I)，当 A 是 O 或 S 时，$R^{7'}$ 不能是 OH、SH、NH_2、NHOH、$NHNH_2$、OR、SR、SSR、NHR 或 NR_2，

[0346] 其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基（例如，羟烷基、氨基烷基及烷氧基烷基）取代。

[0347] 在另一实施方案中，糖是通式 (VII)、(VIII)、(IX) 及 (X) 的二氧戊环、氧硫杂环戊烷或二硫戊环：

[0348]



(VII) (VIII) (IX) (X)

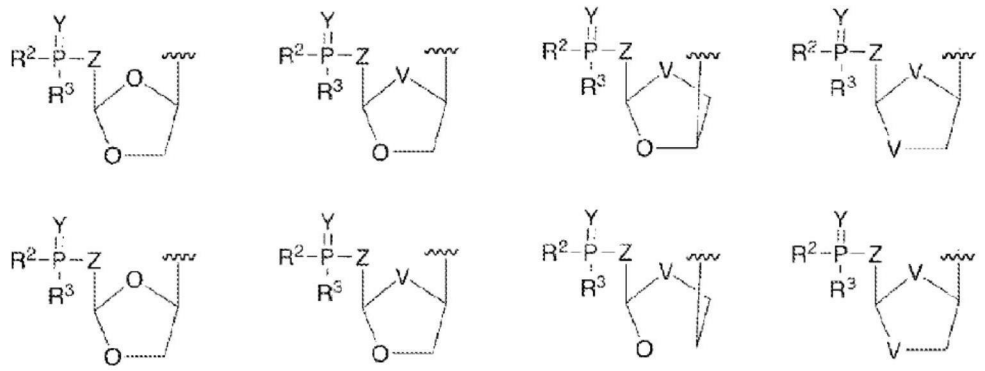
[0349] D 是 C(O)OR^1、二磷酸酯或三磷酸酯；

[0350] V 个别地是 S 或 Se；

[0351] R^1独立地是 C_{1-20}烷基、来源于脂肪醇的碳链或被以下取代的 C_{1-20}烷基：C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基、C_{3-10}环烷基烷基、环杂烷基、芳基、杂芳基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5}烷基、或被 C_1-C_6烷基、C_1-C_6烷氧基、二 (C_1-C_6烷基)- 氨基、氟、C_{3-10}环烷基或 C_{3-10}环烷基烷基取代的 C_{1-5}烷基；

[0352] 在又一实施方案中，糖是通式 (XI)、(XII)、(XIII) 及 (XIV) 的二氧戊环、氧硫杂环戊烷或二硫戊环：

[0353]



(XI) (XII) (XIII)

(XIV)

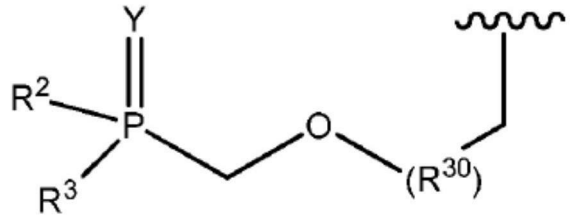
[0354] 其中：

[0355] V 个别地是 S 或 Se；

[0356] R^2、R^3、Y 和 Z 如上关于式 I、II、III 及 IV 所定义。

[0357] 在又一实施方案中，糖是通式 (XV) 的磷酰基甲氧烷基：

[0358]



(XV)

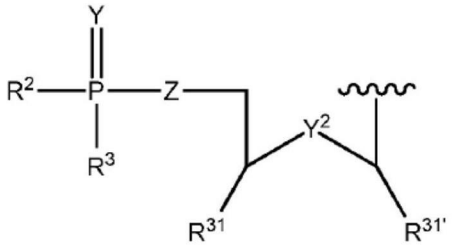
[0359] 其中：

[0360] R^2、R^3和 Y 如上关于式 I、II、III 及 IV 所定义；并且；

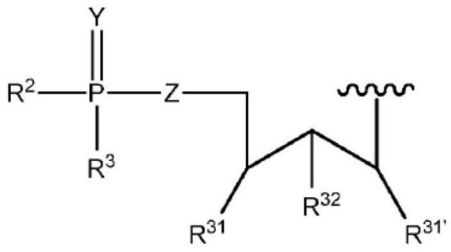
[0361] R^{30}选自由以下组成的组：C_{1-20}烷基、C_{2-20}烷基（包括但不限于 C_1-C_6）、烯基（包括但不限于 C_2-C_6）、和 C_{2-20}炔基、C_{3-10}（包括但不限于 C_2-C_6）、环烷基（包括但不限于 C_3-C_8）、芳基（包括但不限于 C_6-C_{10}）、杂芳基（包括但不限于 C_6-C_{10}）、芳基烷基以及烷基芳基；

[0362] 在又一实施方案中，糖具有通式 (XVI) 或 (XVII)：

[0363]



(XVI)



(XVII)

[0364] 其中：

[0365] R^2、R^3、Z 和 Y 如上所定义；

[0366] Y^2是 O、S、Se 或 NR；

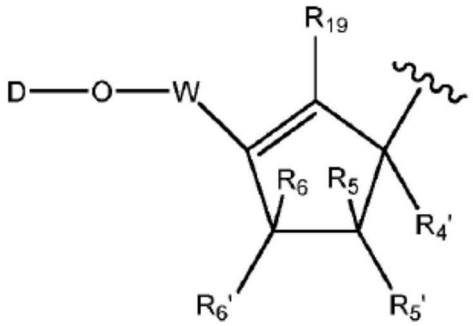
[0367] R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基（例如羟烷基、氨基烷基和烷氧基烷基）取代；

[0368] R^{31}、$R^{31'}$和 R^{32}被定义为 H、CH_3或 CH_2OR^{33}；并且

[0369] R^{33}是 H 或 C_1-C_6酰基。

[0370] 在另一实施方案中，糖是通式 (XVIII) 的修饰的核糖

[0371]



(XVIII)

[0372] 其中：

[0373] D、W、$R^{4'}$、R^5、$R^{5'}$、R^6及 $R^{6'}$如上所定义；

[0374] R^{19}是 H、F、Cl、Br、I、N_3、C(O)OH、CN、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 或 R；

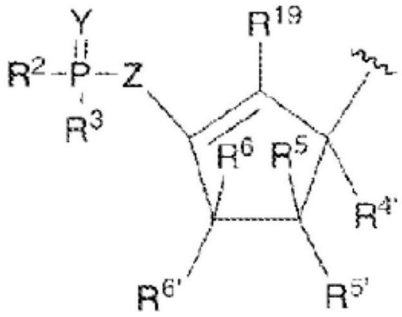
[0375] 其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上所定义的取代基（例如，羟烷基、氨基烷基及烷氧基烷基）取代。

[0376] 在一个实施方案中，其中糖具有式 (XVII)，当 D 是 H 或酰基，W 是 CH_2，$R^{4'}$和 R^{19}是 H 时，R^5、$R^{5'}$、R^6、$R^{6'}$不能是 H、卤素、OH、SH、OCH_3、SCH_3、NH_2、$NHCH_3$、CH_3、CH ＝ CH_2、CN、CH_2NH_2、CH_2OH 或 COOH。

[0377] 在另一实施方案中，$R^{6'}$可独立地选自由以下组成的组：NHOH、$NHNH_2$、N_3、$C(O)NH_2$、$C(S)NH_2$、C(O)OR、R、OR、SR、SSR、NHR 及 NR_2。

[0378] 在又一实施方案中，糖是式 (XIX) 的修饰的核糖：

[0379]



(XIX)

[0380] 其中：

[0381] R^2、R^3和 Y 如上关于式 I、II、III 及 IV 所定义；

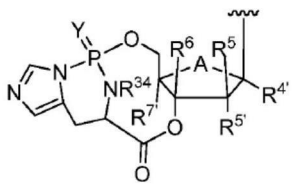
[0382] $R^{4'}$、R^5、$R^{5'}$、R^6及 $R^{6'}$如上所定义；

[0383] R^{19}是 H、F、Cl、Br、I、N_3、C(O)OH、CN、$C(O)NH_2$、$C(S)NH_2$、C(O)OR 或 R；

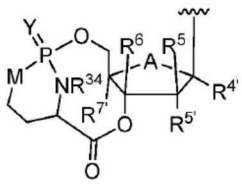
[0384] 其中 R 独立地是 C_1-C_6烷基、C_2-C_6烯基、C_2-C_6炔基、C_3-C_6环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基（例如，羟烷基、氨基烷基及烷氧基烷基）取代。

[0385] 在又一实施方案中，糖具有式 (XX)、(XXI) 或 (XXII) 中的一种：

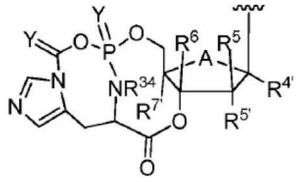
[0386]



(XX)



(XXI)



(XXII)

[0387] 其中：

[0388] $R^{4'}$、R^5、$R^{5'}$、R^6、Y、A 及 $R^{7'}$ 如上关于式 I、II、III 及 IV 所定义；

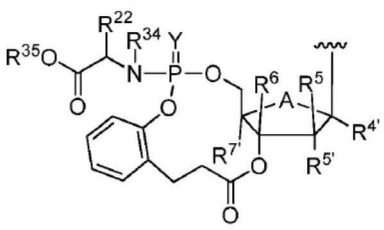
[0389] R^{34} 是 C_1-C_6 烷基；

[0390] M 是 O、S 或 NR；

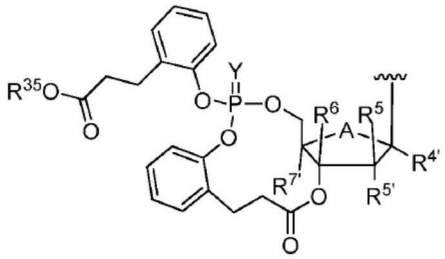
[0391] 其中 R 独立地是 C_1-C_6 烷基、C_{2-6} 烯基、C_{2-6} 炔基、C_3-C_6 环烷基、芳基、烷基芳基或芳基烷基，其中所述基团可被一个或多个如上关于式 I、II、III 及 IV 所定义的取代基（例如羟烷基、氨基烷基和烷氧基烷基）取代；

[0392] 在另一实施方案中，糖具有式 (XXIII) 或 (XXIV) 中的一种：

[0393]



(XXIII)



(XXIV)

[0394] 其中：

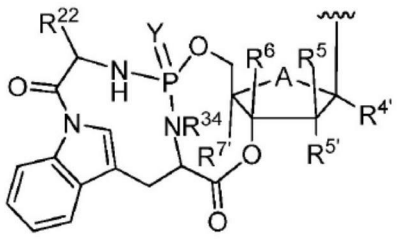
[0395] $R^{4'}$、R^5、$R^{5'}$、R^6、Y、A、$R^{7'}$、R^{34} 如上关于式 I、II、III 及 IV 所定义；

[0396] R^{35} 是 H、C_{1-10} 烷基、被以下取代的 C_{1-10} 烷基：C_{1-6} 烷基、C_{1-6} 烷氧基、二 (C_{1-6} 烷基)-氨基、氟、C_{3-10} 环烷基、C_{3-10} 环烷基烷基、环杂烷基、芳基如苯基、杂芳基如吡啶基、取代的芳基或取代的杂芳基；其中所述取代基是 C_{1-5} 烷基、或被 C_{1-6} 烷基、C_{1-6} 烷氧基、二 (C_{1-6} 烷基)-氨基、氟、C_{3-10} 环烷基或 C_{3-10} 环烷基烷基取代的 C_{1-5} 烷基；并且

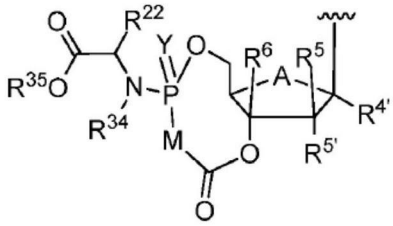
[0397] R^{22} 是 H、CH_3、CH_2CH_3、$CH(CH_3)_2$、$CH_2CH(CH_3)_2$、$CH(CH_3)CH_2CH_3$、CH_2Ph、CH_2-吲哚-3-基、$-CH_2CH_2SCH_3$、CH_2CO_2H、$CH_2C(O)NH_2$、CH_2CH_2COOH、$CH_2CH_2C(O)NH_2$、$CH_2CH_2CH_2CH_2NH_2$、$CH_2CH_2CH_2NHC(NH)NH_2$、CH_2-咪唑-4-基、CH_2OH、$CH(OH)CH_3$、$CH_2((4'-OH)-Ph)$、CH_2SH 或 C_{3-6} 环烷基；

[0398] 在又一实施方案中，糖具有式 (XXV) 或 (XXVI) 中的一种：

[0399]



(XXV)



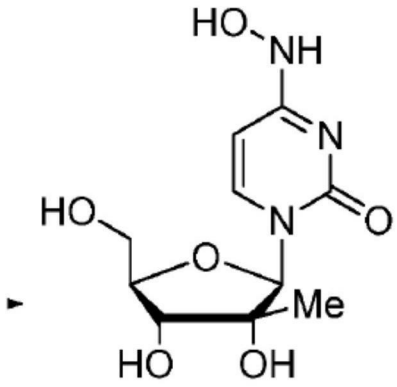
(XXVI)

[0400] 其中：

[0401] $R^{4'}$、R^5、$R^{5'}$、R^6、Y、M、$R^{7'}$、R^{34}、R^{35}、R^{22}如上关于式 I、II、III 及 IV 所定义；

[0402] 在一个实施方案中，化合物具有下式中的一种：

[0403]



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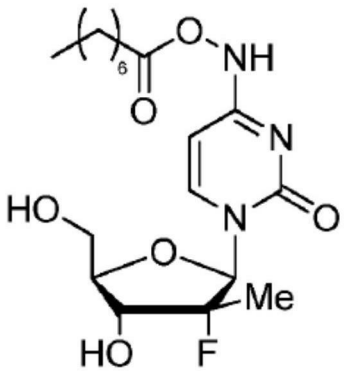

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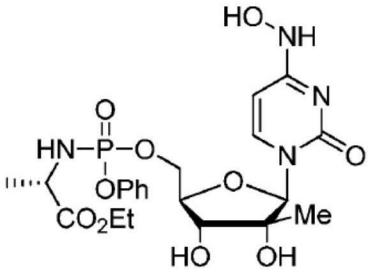
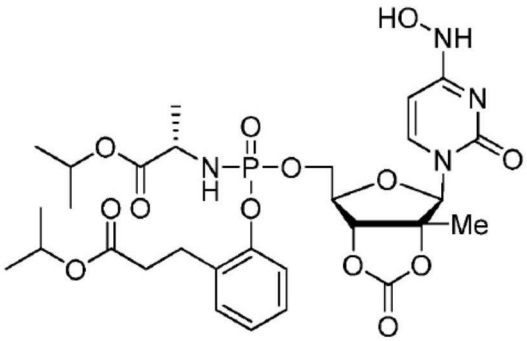
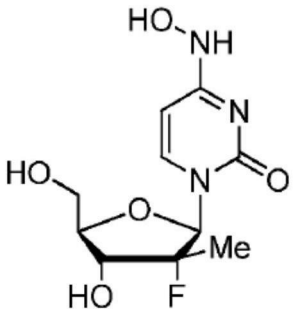


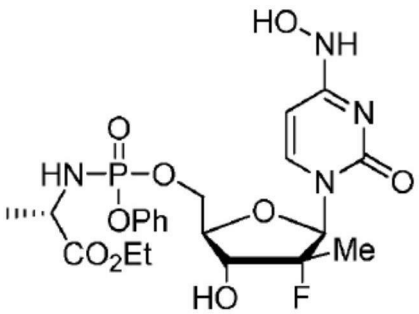
44



48



39



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[0404] 或其药学上可接受的盐。

[0405] 在一个实施方案中，R^5或 $R^{5'}$中的至少一个是 F、Cl 或 Me。

[0406] 在另一实施方案中，R^5和 $R^{5'}$分别是 Me 和 F。

[0407] 在另一实施方案中，R^5和 $R^{5'}$分别是 Me 和 Cl。

[0408] 在另一实施方案中，L 是甲基。

[0409] 在另一实施方案中，碱基是嘧啶，并且 R^5和 $R^{5'}$中的一个是 OH、Cl 或 F。

[0410] 本文所述的化合物可呈以下形式：β-L- 或 β-D- 构型、或其混合物，包括其外消旋混合物。

[0411] 在那些实施方案中，当本文所述的化合物的磷部分含有手性中心时，这种手性中心可呈以下形式：R_p- 或 S_p- 构型或其混合物，包括其外消旋混合物。

[0412] 在一个实施方案中，化合物在生物系统中被转化为嘧啶三磷酸酯的混合物，这是由于在嘧啶环上的 -NHOH 部分转化为 $-NH_2$部分且任选地嘧啶环上的 -NHOH 部分或所得的 $-NH_2$部分转化为 OH 部分。这类部分转化的一个实例显示如下，其中嘧啶三磷酸酯的混合物 C 或 D 包括 4-NHOH、$4-NH_2$及 4-OH 嘧啶三磷酸酯。这种混合物可例如当所施用的化合物包括在糖的 5'-OH 部分上的前药时形成。适合的前药的实例包括如上所例示的那些。

[0413]

[0414] 因此，通过施用单一化合物，两种或三种活性化合物的组合可在药物代谢期间形成，并且这些药物可以不同方式靶向病毒。例如，其中 NHOH 直接地或间接地转化为 OH 部分的类似物当通过病毒掺入生长中的 DNA 或 RNA 链中时的作用类似于尿苷类似物。其中 NHOH 部分转化为 NH_2部分的类似物当通过病毒掺入生长中的 DNA 或 RNA 链中时的作用类似于胞嘧啶类似物。NHOH 类似物当通过病毒掺入生长中的 DNA 或 RNA 链中时的作用可类似于胞嘧啶或尿苷类似物。预期三种活性三磷酸酯的组合对比通常施用的任何单个三磷酸酯药物将产生不同且更难的突变选择。

[0415] 通过以多种方式攻击病毒，即通过用 U 和 C 型类似物呈递病毒，前药化合物具有抵御病毒耐药性的嵌入机制。也就是说，如果病毒应突变以避免占用 U 类似物，则其还可对一种或多种 C 类似物敏感，且反之亦然，并且如果应存在多种 C 类似物，则对其中一种的抗性可能不会赋予对另一种的抗性。

[0416] 因此，本文所述的化合物可作为单一组分施用，且还提供组合抗病毒疗法的益处。当与另外的抗病毒剂、特别是非 NNRTI 抗病毒剂组合时，所述组合可提供与许多另外的组分的组合的益处，同时提供包括仅一种核苷前药的简单性。

[0417] III. 立体异构现象和多晶型现象

[0418] 本文所述的化合物可具有不对称中心并且作为外消旋体、外消旋混合物、个别非对映异构体或对映异构体存在，其中所有异构体形式都包括在本发明中。具有手性中心的本发明化合物可以光学活性和外消旋形式存在和分离。一些化合物可以展现多晶型现象。本发明包括本发明化合物的外消旋、光学活性、多晶型或立体异构形式或其混合物，其具有本文所述的有用的特性。光学活性形式可通过以下方式来制备：通过再结晶技术拆分外消旋形式，通过从光学活性起始材料来合成，通过手性合成，或通过使用手性固定相进行色谱分离或通过酶促拆分。可纯化各自的核苷，然后衍生化所述核苷以形成本文所述的化合物，或纯化核苷酸本身。

[0419] 光学活性形式的化合物可使用本领域中已知的任何方法来制备：包括但不限于通过再结晶技术拆分外消旋形式，通过从光学活性起始材料来合成，通过手性合成，或通过使用手性固定相进行色谱分离。

[0420] 获得光学活性材料的方法的实例包括至少以下。

[0421] i)晶体的物理分离：该技术手工地分离个别对映异构体的肉眼可见晶体。如果存在单独的对映异构体的晶体，即所述材料是团聚体且晶体在视觉上是不同的，便可使用此技术；

[0422] ii)同时结晶：该技术将个别的对映异构体从外消旋体溶液中单独结晶，可能只有当后者是呈固态的团聚体时才发生；

[0423] iii)酶促拆分：该技术在酶的作用下借助于针对对映异构体的不同反应速度部分或完全分离外消旋体；

[0424] iv)酶促不对称合成：在该合成技术中，合成的至少一个步骤使用酶促反应以获得所需对映异构体的对映异构纯的或富集的合成前体；

[0425] v)化学不对称合成：该合成技术在产物中产生不对称性（即手性）条件下从非手性前体合成所需对映异构体，所述合成可使用手性催化剂或手性助剂完成；

[0426] vi)非对映异构体分离：该技术通过外消旋化合物与对映异构纯的试剂（手性助剂）反应，将个别对映异构体转化为非对映异构体。所得非对映异构体然后通过色谱法或结晶借助于其现在更明显的结构差异被分离且手性助剂随后被除去以获得所需对映异构体；

[0427] vii)一级和二级不对称转化：该技术通过平衡外消旋体的非对映异构体，使得其在由所需对映异构体形成的非对映异构体溶液中占一定优势，或者是由来自所需对映异构体的非对映异构体的优先结晶作用破坏这种平衡，这样使得所有的物质最终几乎都被转化为所需对映异构体的结晶型非对映异构体。所需对映异构体然后从非对映异构体中释放；

[0428] viii)动力学拆分：该技术是指利用在动力学条件下对映异构体和手性、非外消旋试剂或催化剂反应的不同反应速率，来获得对外消旋体的部分或完全的拆分（或对部分拆分的化合物的进一步拆分）；

[0429] ix)从非外消旋前体的对映体特异性合成：在该合成技术中，从非手性起始物质获得所需对映异构体，并且在合成过程中，其立体化学完整性未受到或仅仅受到最低程度的危害；

[0430] x)手性液相色谱法：在该技术中，外消旋体的对映异构体在液体流动相中借助于它们与固定相具有不同的相互作用（包括但不限于经由手性 HPLC）而被分离。固定相可由

手性材料制成，或者流动相可含有另外的手性材料以诱导不同的相互作用；

[0431] xi)手性气相色谱法：该技术通过将外消旋体挥发，然后借助于对映异构体在气态流动相中与含有固定的非外消旋手性吸附相的柱相互作用的程度不同而将对映异构体分离出来；

[0432] xii)用手性溶剂萃取：该技术借助于一种对映异构体可以优先溶解于特定的手性溶剂中，从而实现对映异构体的分离；

[0433] xiii)透过手性膜的转运：在该技术中将外消旋体与薄膜屏障接触。屏障通常分离两种互溶液体，一种含有外消旋体，并且驱动力如浓度或压差使得优先转运透过膜屏障。由于膜的非外消旋手性性质只允许外消旋体的一种对映异构体通过，从而实现分离。

[0434] 在一个实施方案中使用包括但不限于模拟移动床色谱法的手性色谱法。广泛多种手性固定相可商购获得。

[0435] IV. 核苷酸盐或前药制剂

[0436] 在化合物具有足够碱性或酸性以形成稳定的无毒酸或碱盐的情况下，施用作为药学上可接受的盐形式的化合物可为适当的。药学上可接受的盐的实例是以形成生理学上可接受的阴离子的酸形成的有机酸加成盐，例如甲苯磺酸盐、甲磺酸盐、乙酸盐、柠檬酸盐、丙二酸盐、酒石酸盐、琥珀酸盐、安息香酸盐、抗坏血酸盐、α-酮戊二酸盐以及 α-甘油磷酸盐。还可形成适合的无机盐，包括但不限于硫酸盐、硝酸盐、碳酸氢盐及碳酸盐。

[0437] 可利用本领域公知的标准方法，例如将足够碱性的化合物，如胺与提供生理学可接受的阴离子的合适的酸进行反应来获得药学上可接受的盐。也可以制备羧酸的碱金属（例如钠、钾或锂）或碱土金属（例如钙）盐。

[0438] 可施用本文所述的核苷酸前药以另外提高核苷酸单磷酸酯的活性、生物利用率、稳定性或以其它方式改变其特性。

[0439] 许多核苷酸前药配体是已知的。一般来说，核苷的单磷酸酯或其它类似物的烷基化、酰化或其它亲脂性修饰将提高核苷酸的稳定性。

[0440] 可取代单磷酸酯部分上的一个或多个氢的取代基的实例是烷基、芳基、类固醇、碳水化合物，包括但不限于糖、1,2-二酰基甘油及醇。许多都描述于 R.Jones&N.Bischofberger,Antiviral Research,1995,27,1-17 及 S.J.Hecker&M.D.Erion,J.Med.Chem.,2008,51,2328-2345 中。这些中的任一种都可组合所公开的核苷酸使用以实现预期效果。

[0441] 如以下文献（以引用的方式并入本文）中所公开，所述活性核苷还可以 5'-磷酸醚脂形式提供:Kucera,L.S.,N.Iyer,E.Leake,A.Raben,Modest E.K.,D.L.W., 和 C.Piantadosi,"Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation,"AIDS Res.Hum.Retroviruses,1990,6,491-501;Piantadosi,C,J.Marasco C.J.,S.L.Morris-Natschke,K.L.Meyer,F.Gumus,J.R.Surles,K.S.Ishaq,L.S.Kucera,N.Iyer,C.A.Wallen,S.Piantadosi 和 E.J.Modest,"Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity,"J.Med.Chem.,1991,34,1408-14;Hosteller,K.Y.,D.D.Richman,D.A.Carson,L.M.Stuhmiller,G.M.T.van Wijk 和 H.van den Bosch,"Greatly enhanced inhibition

of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol,a lipid prodrug of 3,-deoxythymidine,"Antimicrob.Agents Chemother.,1992,36,2025-29;Hostetler,K.Y.,L.M.Stuhmiller,H.B.Lenting,H.van den Bosch 和 D.D.Richman,"Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides."J.Biol.Chem.,1990,265,61127。

[0442] 公开可(优选在本文所述的核苷酸的 R^2 和/或 R^3 位置处)共价结合到核苷中的合适的亲脂性取代基,或亲脂性制剂的美国专利的非限制性实例包括:美国专利号5,149,794(Yatvin 等人);5,194,654(Hostetler 等人);5,223,263(Hostetler 等人);5,256,641(Yatvin 等人);5,411,947(Hostetler 等人);5,463,092(Hostetler 等人);5,543,389(Yatvin 等人);5,543,390(Yatvin 等人);5,543,391(Yatvin 等人);以及5,554,728(Basava 等人),所有均以引用的方式并入。公开可连接至本发明的核苷的亲脂性取代基的外国专利申请包括:WO 89/02733、WO 90/00555、WO 91/16920、WO 91/18914、WO 93/00910、WO 94/26273、WO 96/15132、EP 0 350 287、EP 93917054.4 以及 WO 91/19721。

[0443] V. 治疗方法

[0444] 感染了 HIV-1、HIV-2、HBV、HCV、诺瓦克病毒、札如病毒、HSV-1、HSV-2、登革热病毒、黄热病或其基因片段的宿主(包括但不限于人)可通过在药学上可接受的载体或稀释剂存在下向所述患者施用有效量的活性化合物或其药学上可接受的前药或盐来治疗。活性物质可通过任何适当途径,例如口服、胃肠外、静脉内、真皮内、皮下或局部,以液体或固体形式来施用。

[0445] 所述化合物还可用于治疗癌症。可根据本发明方法用本文所述的化合物及这些化合物的药学上可接受的盐和前药治疗的患者包括例如已被诊断为具有以下疾病的患者:肺癌、骨癌、胰腺癌、皮肤癌、头颈癌、皮肤或眼内黑素瘤、子宫癌、卵巢癌、直肠癌或肛门癌、胃癌、结肠癌、乳腺癌、妇科肿瘤(例如子宫肉瘤、输卵管癌、子宫内膜癌、子宫颈癌、阴道癌或外阴癌)、何杰金氏病、食道癌、小肠癌、内分泌系统癌(例如甲状腺癌、甲状旁腺癌或肾上腺癌)、软组织肉瘤、尿道癌、阴茎癌、前列腺癌、慢性或急性白血病、儿童实体肿瘤、淋巴细胞淋巴瘤、膀胱癌、肾或输尿管癌(例如肾细胞癌、肾盂癌)、或中枢神经系统肿瘤(例如原发性 CNS 淋巴瘤、脊椎轴肿瘤、脑干神经胶质瘤或垂体腺瘤)。

[0446] 本发明还涉及用于抑制患者中的异常细胞增殖的方法和药物组合物,所述药物组合物包含一定量的本文所述的化合物或其药学上可接受的盐或前药、以及一定量的一种或多种选自抗血管生成剂、信号转导抑制剂及抗增殖剂的物质。

[0447] 抗血管生成剂,如 MMP-2(基质-金属蛋白酶 2)抑制剂、MMP-9(基质-金属蛋白酶 9)抑制剂和 COX-II(环加氧酶 II)抑制剂可以与本文所述的式 1 的化合物和药物组合物联合使用。有用的 COX-II 抑制剂的实例包括 CELEBREX™(阿来考昔)、伐地考昔及罗非考昔。有用的基质金属蛋白酶抑制剂的实例描述于 WO 96/33172(公开于 1996 年 10 月 24 日)、WO 96/27583(公开于 1996 年 3 月 7 日)、欧洲专利申请号 97304971.1(申请于 1997 年 7 月 8 日)、欧洲专利申请号 99308617.2(申请于 1999 年 10 月 29 日)、WO 98/07697(公开于 1998 年 2 月 26 日)、WO 98/03516(公开于 1998 年 1 月 29 日)、WO 98/34918(公开于 1998 年 8 月 13 日)、WO 98/34915(公开于 1998 年 8 月 13 日)、WO 98/33768(公开于 1998

年 8 月 6 日）、WO 98/30566（公开于 1998 年 7 月 16 日）、欧洲专利公布 606,046（公开于 1994 年 7 月 13 日）、欧洲专利公布 931,788（公开于 1999 年 7 月 28 日）、WO 90/05719（公开于 1990 年 5 月 331 日）、WO 99/52910（公开于 1999 年 10 月 21 日）、WO 99/52889（公开于 1999 年 10 月 21 日）、WO 99/29667（公开于 1999 年 6 月 17 日）、PCT 国际申请号 PCT/IB98/01113（申请于 1998 年 7 月 21 日）、欧洲专利申请号 99302232.1（申请于 1999 年 3 月 25 日）、英国专利申请号 9912961.1（申请于 1999 年 6 月 3 日）、美国临时申请号 60/148,464（申请于 1999 年 8 月 12 日）、美国专利号 5,863,949（授权于 1999 年 1 月 26 日）、美国专利号 5,861,510（授权于 1999 年 1 月 19 日）以及欧洲专利公布 780,386（公开于 1997 年 6 月 25 日）中，所有专利都以引用的方式整体并入本文。优选的 MMP 抑制剂是不表现关节痛的那些抑制剂。更优选的是相对于其它基质 - 金属蛋白酶（即 MMP-1、MMP-3、MMP-4、MMP-5、MMP-6、MMP-7、MMP-8、MMP-10、MMP-11、MMP-12 及 MMP-13）选择性抑制 MMP-2 和 / 或 MMP-9 的那些。

[0448] 本文所述的化合物还可与以下活性剂一起使用：信号转导抑制剂，如可抑制 EGFR（表皮生长因子受体）反应的试剂，如 EGFR 抗体、EGF 抗体和作为 EGFR 抑制剂的分子；VEGF（血管内皮生长因子）抑制剂，如 VEGF 受体和可以抑制 VEGF 的分子；和 erbB2 受体抑制剂，如与 erbB2 受体结合的有机分子或抗体，例如 HERCEPTIN™（Genentech, Inc. of South San Francisco, Calif., USA）。

[0449] EGFR 抑制剂描述于例如 WO 95/19970（公开于 1995 年 7 月 27 日）、WO 98/14451（公开于 1998 年 4 月 9 日）、WO 98/02434（公开于 1998 年 1 月 22 日）以及美国专利号 5,747,498（授权于 1998 年 5 月 5 日）中，并且这种物质可用于如本文所述的本发明中。EGFR- 抑制剂包括但不限于单克隆抗体 C225 和抗 EGFR 22Mab（ImClone Systems Incorporated of New York, N.Y., USA）、ABX-EGF（Abgenix/Cell Genesys）、EMD-7200（Merck KgaA）、EMD-5590（Merck KgaA）、MDX-447/H-477（Medarex Inc. of Annandale, N.J., USA 和 Merck KgaA）、以及化合物 ZD-1834、ZD-1838 和 ZD-1839（AstraZeneca）、PKI-166（Novartis）、PKI-166/CGP-75166（Novartis）、PTK 787（Novartis）、CP 701（Cephalon）、来氟米特（Pharmacia/Sugen）、CI-1033（Warner Lambert Parke Davis）、CI-1033/PD 183,805（Warner Lambert Parke Davis）、CL-387,785（Wyeth-Ayerst）、BBR-1611（Boehringer Mannheim GmbH/Roche）、Naamidine A（Bristol Myers Squibb）、RC-3940-II（Pharmacia）、BIBX-1382（Boehringer Ingelheim）、OLX-103（Merck&Co. of Whitehouse Station, N.J., USA）、VRCTC-310（Ventech Research）、EGF 融合毒素（Seragen Inc. of Hopkinton, Mass.）、DAB-389（Seragen/Lilgand）、ZM-252808（Imperical Cancer Research Fund）、RG-50864（INSERM）、LFM-A12（Parker Hughes Cancer Center）、WHI-P97（Parker Hughes Cancer Center）、GW-282974（Glaxo）、KT-8391（Kyowa Hakko）及 EGFR 疫苗（York Medical/Centro de Immunologia Molecular（CIM））。这些及其它 EGFR- 抑制剂可用于本发明中。

[0450] 以下 VEGF 抑制剂也可与本发明化合物组合：例如 CP-547,632（Pfizer Inc., N.Y.）、AG-13736（Agouron Pharmaceuticals, Inc.a Pfizer Company）、SU-5416 及 SU-6668（Sugen Inc. of South San Francisco, Calif., USA）和 SH-268（Schering）。VEGF 抑制剂描述于例如 WO 99/24440（公开于 1999 年 5 月 20 日）、PCT 国际申请 PCT/

IB99/00797(申请于1999年5月3日)、WO 95/21613(公开于1995年8月17日)、WO 99/61422(公开于1999年12月2日)、美国专利号5,834,504(授权于1998年11月10日)、WO 98/50356(公开于1998年11月12日)、美国专利号5,883,113(授权于1999年3月16日)、美国专利号5,886,020(授权于1999年3月23日)、美国专利号5,792,783(授权于1998年8月11日)、WO 99/10349(公开于1999年3月4日)、WO 97/32856(公开于1997年9月12日)、WO 97/22596(公开于1997年6月26日)、WO 98/54093(公开于1998年12月3日)、WO 98/02438(公开于1998年1月22日)、WO 99/16755(公开于1999年4月8日)以及WO 98/02437(公开于1998年1月22日)中,所有专利都以引用的方式整体并入本文。本发明中适用的一些具体VEGF抑制剂的其它实例是IM862(Cytran Inc.of Kirkland,Wash.,USA);Genentech,Inc.of South San Francisco,Calif.的抗VEGF单克隆抗体;以及血管酶(angiozyme),一种来自Ribozyme(Boulder,Colo.)和Chiron(Emeryville,Calif.)的合成核糖酶。这些及其它VEGF抑制剂可用于如本文所述的本发明中。

[0451] ErbB2受体抑制剂如CP-358,774(OSI-774)(它塞瓦)(OSI Pharmaceuticals,Inc.)、GW-282974(Glaxo Wellcome plc)以及单克隆抗体AR-209(Aronex Pharmaceuticals Inc.of The Woodlands,Tex.,USA)和2B-1(Chiron)可此外与本发明化合物组合,例如以下专利中所示的那些:WO 98/02434(公开于1998年1月22日)、WO 99/35146(公开于1999年7月15日)、WO 99/35132(公开于1999年7月15日)、WO 98/02437(公开于1998年1月22日)、WO 97/13760(公开于1997年4月17日)、WO 95/19970(公开于1995年7月27日)、美国专利号5,587,458(授权于1996年12月24日)以及美国专利号5,877,305(授权于1999年3月2日),所有专利都据此以引用的方式整体并入本文。适用于本发明中的ErbB2受体抑制剂还描述于1999年1月27日申请的美国临时申请号60/117,341和1999年1月27日申请的美国临时申请号60/117,346中,这两者都以引用的方式整体并入本文。erbB2受体抑制剂化合物和在上述PCT申请、美国专利和美国临时申请中描述的物质以及抑制erbB2受体的其它化合物和物质都可根据本发明与本文所述的化合物一起使用。

[0452] 化合物还可与适用于治疗异常细胞增殖或癌症的其它试剂一起使用,包括但不限于能增强抗肿瘤免疫应答的试剂,如CTLA4(细胞毒性淋巴细胞抗原4)抗体、及能阻断CTLA4的其它试剂;以及抗增殖剂如其它法呢基蛋白转移酶抑制剂及其类似物。可用于本发明中的特定的CTLA4抗体包括美国临时申请60/113,647(申请于1998年12月23日)中描述的那些,该专利以引用的方式整体并入,然而,其它CTLA4抗体也可以用于本发明。

[0453] 还可使用其它抗血管生成剂,包括但不限于其它COX-II抑制剂、其它MMP抑制剂、其它抗VEGF抗体或其它血管形成效应物的抑制剂。

[0454] 本文所述的化合物和药物组合物可用于治疗或预防一种或多种诺瓦克病毒以及杯状病毒科分类学科中的其它病毒的感染。

[0455] 在治疗诺瓦克病毒感染的治疗用途中,将化合物和/或组合物在适于实现治疗益处的剂量水平下向诊断有诺瓦克病毒感染的患者施用。“治疗益处”及语法等效物指的是化合物的施用造成患者在一段时间内的有益效果。例如,当在患者中的诺瓦克病毒滴度或病毒载量减少或停止增加时,可以实现治疗益处。

[0456] 如果化合物的施用减慢或完全停止了诺瓦克病毒感染通常伴随的不利症状的进展，那么不论患者体内的诺瓦克病毒滴度或病毒载量如何，也认为获得了治疗益处。本文所述的化合物和／或组合物还可预防性地施用于有发展诺瓦克病毒感染危险或已经暴露于诺瓦克病毒的患者，以防止诺瓦克病毒感染的发展。例如，化合物和／或其组合物可向可能已经暴露于诺瓦克病毒的患者施用。

[0457] 诺瓦克病毒疾病的爆发经常发生在封闭或半封闭的社区群体中，如长期护理机构、医院、监狱以及旅游客轮，在那些地方一旦病毒被引入，感染便会通过人与人之间的传播或经由污染的食物非常快速地传播。许多诺瓦克病毒爆发都追溯到由一个感染者触摸过的食物。因此，向很可能接触到诺瓦克病毒或其它杯状病毒科的这些机构中的个体提供预防剂量的本文所述的化合物可为有利。

[0458] VI. 组合或交替疗法

[0459] 在一个实施方案中，本发明化合物可与选自以下的至少一种其它抗病毒剂一起使用：进入抑制剂、逆转录酶抑制剂、蛋白酶抑制剂以及基于免疫的治疗剂。

[0460] 例如，当用于治疗或预防 HIV 或 HBV 感染时，活性化合物或其前药或药学上可接受的盐可与另一种抗病毒剂组合或交替施用，如抗 HIV、抗 HBV 或抗 HCV 剂，包括但不限于上式的那些物质。一般说来，在组合疗法中，有效剂量的两种或更多种试剂在一起施用，而在交替疗法期间，有效剂量的每个试剂被连续地施用。剂量将取决于药物的吸收、失活及排泄率以及本领域技术人员已知的其它因素。应注意，剂量值还将随有待缓解的病状的严重性而变化。进一步应理解的是对于任何具体的受试者、具体的给药方案和安排都应该根据个体的需要以及施用或监督组合物施用的人的专业判断并随着时间来调整。

[0461] 可与本文所公开的化合物组合使用的抗病毒剂的非限制性实例包括在下表中的那些。

[0462] 乙型肝炎疗法

[0463]

药物名称	药物类别	公司
tron A 干扰素 α-2b)	干扰素	Schering-Plough
egasys **聚乙二醇化干扰素 α-2a)**	干扰素	Roche
pivir-HBV 拉米夫定；3TC)	核苷类似物	GlaxoSmithKline
epsera (阿德福韦酯)"	核苷类似物	Gilead Sciences
mtriva® (恩曲他滨；TC)	核苷类似物	Gilead Scienceshttp://www.hivandhepatitis.com/advertisement/triangle.html：
恩替卡韦	核苷类似物	Bristol-Myers Squibb
levudine (CLV，L- MAU)	核苷类似物	Pharmasset/Bukwang
CH 126, 443 (L-Fd4C)	核苷类似物	Achillion Pharmaceuticals
M365	核苷类似物	Amrad
氨多索韦(AMDX，APD)	核苷类似物	RFS Pharma LLC
dT **(替比夫定)**	核苷类似物	Idenix/Novartis

[0464]

药物名称	药物类别	公司
S-1220	核苷类似物	Emory University
heradigm	免疫刺激剂	Epimmune
ıdaxin（胸腺素）	免疫刺激剂	SciClone
HT 899	病毒蛋白	Enzo Biochem
exelvuecitabine/Reverset/D-4FC	核苷类似物	Pharmasset
PD	核苷类似物	RFS Pharma
BV DNA 疫苗	免疫刺激剂	PowderJect (UK)
CC 478	核苷类似物	Eli Lilly
ılLdC（伐托他滨）	核苷类似物	Idenix
:N 2001	核苷类似物	ICN
acivir	核苷类似物	Pharmasset/Emory University
罗波斯塔双黄酮	核苷类似物	Advanced Life Sciences

[0465]

药物名称	药物类别	公司
M-019c		Emory University
喷昔洛韦	核苷类似物	Novartis
法昔洛韦	核苷类似物	Novartis
XG	核苷类似物	RFS Pharma, LLC
a-AMP 前药		
BV/MF59		
DP-P-阿昔洛韦	核苷类似物	
锤头状核酶		
糖苷酶抑制剂		
聚乙二醇化干扰素		

[0466]

人类单克隆抗体		

[0467] HIV 疗法 :蛋白酶抑制剂 (PI)

[0468]

商标名称	一般名称	缩写	实验代码	制药公司
Invirase®	沙奎那韦 (硬凝胶帽)	SQV(HGC)	Ro-31-8959	Hoffmann-La Roche
Fortovase®	沙奎那韦 (软凝胶帽)	SQV (SGC)		Hoffmann-La Roche
Norvir®	利托那韦	RTV	ABT-538	Abbott Laboratories
Crixivan®	茚地那韦	IDV	MK-639	Merck & Co.
Viracept®	奈非那韦	NFV	AG-1343	Pfizer
Agenerase ®	安普那韦	APV	141W94 或 VX-478	GlaxoSmithKline
Kaletra®	洛匹那韦+ 利托那韦	LPV	ABT-378/r	Abbott Laboratories
Lexiva®	福沙那韦		GW-433908 或 VX-175	GlaxoSmithKline
Aptivus®	替拉那韦	TPV	PNU-140690	Boehringer Ingelheim
Reyataz®	阿扎那韦		BMS-232632	Bristol-Myers Squibb
	贝肯那韦		GW640385	GlaxoSmithKline
Prezista™	地瑞那韦		TMC114	Tibotec

[0469] HIV 疗法 :核苷 / 核苷酸逆转录酶抑制剂 (NRTI)

[0470]

商标名称	一般名称	缩写	实验代码	制药公司
Retrovir®	齐多夫定	AZT 或 ZDV		GlaxoSmithKline
Epivir®	拉米夫定	3TC		GlaxoSmithKline
Combivir®	齐多夫定 + 拉米夫定	AZT + 3TC		GlaxoSmithKline
Trizivir®	阿巴卡韦+ 齐多夫定 + 拉米夫定	ABC + AZT + 3TC		GlaxoSmithKline
Ziagen®	阿巴卡韦	ABC	1592U89	GlaxoSmithKline
Epzicom™	阿巴卡韦+ 拉米夫定	ABC + 3TC		GlaxoSmithKline
Hivid®	扎西他滨	ddC		Hoffmann-La Roche
Videx®	去羟肌苷: 缓冲形式	ddI	BMY-40900	Bristol-Myers Squibb

[0471]

恩替卡韦	贝乐克			Bristol-Myers Squibb
Videx® EC	去羟肌苷：延迟释放胶囊	ddI		Bristol-Myers Squibb
Zerit®	司他夫定	d4T	BMY-27857	Bristol-Myers Squibb
商标名称	**一般名称**	**缩写**	**实验代码**	**制药公司**
Viread™	替诺福韦地索普西富马酸盐(DF)	TDF 或双(POC) PMPA		Gilead Sciences
Emtriva®	恩曲他滨	FTC		Gilead Sciences
Truvada®	Viread + Emtriva	TDF + FTC		Gilead Sciences
Atripla™		TDF + FTC + Sustiva®		Gilead/BMS/Merck
	氨多索韦	DAPD，AMDX		RFS Pharma LLC
阿立他滨	AVX754		SPD754	Avexa Ltd
	阿洛夫定	FLT	MIV-310	Boehringer
	艾夫他滨	L-FD4C	ACH-126443	Achillion
	KP-1461		SN1461，SN1212	Koronis
	Racivir	RCV		Pharmasset
Dexelvucitabine	Reverset	D-D4FC	DPC817	Pharmasset/Emory
			GS9148 及其前药	Gilead Sciences

[0472] HIV 疗法：非核苷逆转录酶抑制剂（NNRTI）

[0473]

商标名称	一般名称	缩写	实验代码	制药公司
Viramune®	奈韦拉平	NVP	BI-RG-587	Boehringer Ingelheim
Rescriptor®	地拉韦啶	DLV	U-90152S/T	Pfizer
Sustiva®	依法韦仑	EFV	DMP-266	Bristol-Myers Squibb
	(+)-胡桐素 A			Sarawak Medichem
	卡普韦林	CPV	AG-1549 或 S-1153	Pfizer
			DPC-083	Bristol-Myers Squibb
			TMC-125	Tibotec-Virco Group
			TMC-278	Tibotec-Virco Group
			IDX12899	Idenix
			IDX12989	Idenix

[0474] HIV 疗法 :其它类别的药物

[0475]

商标名称	一般名称	缩写	实验代码	制药公司
Viread™	替诺福韦地索普西富马酸盐 (DF)	TDF 或双(POC) PMPA		Gilead Sciences

[0476] 细胞抑制剂

[0477]

商标名称	一般名称	缩写	实验代码	制药公司
Droxia®	羟基脲	HU		Bristol-Myers Squibb

[0478] 进入抑制剂（包括融合抑制剂）

[0479]

商标名称	一般名称	缩写	实验代码	制药公司
Fuzeon™	恩夫韦地		T-20	Trimeris
			T-1249	Trimeris
			AMD-3100	AnorMED, Inc.
	CD4-IgG2		PRO-542	Progenics Pharmaceuticals
			BMS-488043	Bristol-Myers Squibb
	阿拉韦罗		GSK-873,140	GlaxoSmithKline
	肽 T			Advanced Immuni T, Inc.
			TNX-355	Tanox, Inc.
	马拉维若		UK-427,857	Pfizer
CXCR4 抑制剂				
	AMD070		AMD11070	AnorMED, Inc.
CCR5 拮抗剂				

[0480]

商标名称	一般名称	缩写	实验代码	制药公司
vicriroc		SCH-D	SCH-417690	Schering-Plough

[0481] HIV 疗法 :基于免疫的疗法

[0482]

商标名称	一般名称	缩写	实验代码	制药公司
Proleukin®	阿地白介素或 白介素-2	IL-2		Chiron Corporation
Remune®	HIV-1 免疫原或沙克疫苗		AG1661	The Immune Response Corporation :
			HE2000	HollisEden Pharmaceuticals

[0483] 在当前临床发展中的抗丙型肝炎化合物的表

[0484]

药物名称	药物类别	制药公司
派罗欣 聚乙二醇化干扰素 α-2a	长效干扰素	Roche
干复津 干扰素 α-1	干扰素，长效干扰素	InterMune
OMNIFERON 天然干扰素	干扰素，长效干扰素	Viragen
ALBUFERON	更长效干扰素	Human Genome Sciences
REBIF 干扰素 β-1a	干扰素	Ares-Serono
ω 干扰素	干扰素	BioMedicine
口服干扰素 α	口服干扰素	Amarillo Biosciences
干扰素 γ-1b	抗纤维变性	InterMune
IP-501	抗纤维变性	Intemeuron
Merimebodib VX-497	IMPDH 抑制剂(肌苷单磷酸酯脱氢酶)	Vertex
金刚烷胺 (Symmetrel)	光谱抗病毒剂	Endo Labs Solvay
IDN-6556	凋亡调控	Idun Pharma.
XTL-002	单克隆抗体	XTL
HCV/MF59	疫苗	Chiron
CIVACIR	多克隆抗体	NABI
	治疗性疫苗	Inno genetics
VIRAMIDINE	核苷类似物	ICN
ZADAXIN (胸腺素 α-1)	免疫调节剂	Sci Clone
CEPLENE 组胺二盐酸盐	免疫调节剂	Maxim
VX 950/LY 570310	蛋白酶抑制剂	Vertex/Eli Lilly
ISIS 14803	反义	Isis Pharmaceutical/ Elan
IDN-6556	半胱氨酸蛋白酶抑制剂	Idun Pharmaceuticals, Inc. http://www.idun.com
JTK 003	聚合酶抑制剂	AKROS Pharma
Tarvacin	抗磷脂疗法	Peregrine

[0485]

HCV-796	聚合酶抑制剂	ViroPharma/ AVye
CH-6	丝氨酸蛋白酶	Schering
ANA971	艾沙托立宾	ANADYS
ANA245	艾沙托立宾	ANADYS
CPG 10101 (Actilon):	免疫调节剂	Coley
利妥昔单抗(Rituxam)	抗 CD20 单克隆抗体	Genetech/IDEC
NM283 (瓦洛他滨)	聚合酶抑制剂	Idenix Pharmaceuticals
HepX™-C	单克隆抗体	XTL
IC41	治疗性疫苗	Intercell
水母干扰素	更长效干扰素	Flamel Technologies
E-1	治疗性疫苗	Innogenetics
Multiferon	长效干扰素	Viragen
BILN 2061	丝氨酸蛋白酶	Boehringer - Ingelheim
干扰素 β-1a (REBIF)	干扰素	Ares-Serono

[0486] VII. 用于治疗增殖病状的组合疗法

[0487] 在另一实施方案中，化合物当用作抗增殖剂时可与增强疗法有效性的另一种化合物组合施用，包括但不限于抗叶酸剂、5- 氟嘧啶（包括 5- 氟尿嘧啶）、胞苷类似物如 β -L-1,3- 二氧戊环基胞苷或 β -L-1,3- 二氧戊环基 5- 氟胞苷、抗代谢物（包括嘌呤抗代谢物、阿糖胞苷、氟达拉滨、5- 氟脱氧尿苷、6- 巯基嘌呤、氨甲蝶呤及 6- 硫鸟嘌呤）、羟基脲、有丝分裂抑制剂（包括 CPT-11、依托泊苷 (VP-21)、紫杉酚及长春花生物碱如长春新碱和长春花碱）、烷化剂（包括但不限于白消安、苯丁酸氮芥、环磷酰胺、异环磷酰胺、氮芥、美法仑及硫替派）、非典型烷化剂、含铂的化合物、博来霉素、抗肿瘤抗生素、蒽环霉素如阿霉素和道诺霉素、蒽二酮、拓扑异构酶 II 抑制剂、激素试剂（包括但不限于皮质甾类（地塞米松、强的松和甲基强的松）、雄激素如氟羟甲睾酮和甲基睾甾酮、雌激素如二乙基己烯雌酚、抗雌激素药如他莫昔芬、LHRH 类似物如亮丙瑞林、抗雄激素药如氟他胺、氨鲁米特、甲地孕酮和甲羟孕酮）、天冬酰胺酶、卡莫司汀、洛莫司汀、六甲基三聚氰胺、达卡巴嗪、米托坦、链脲霉素、顺铂、卡铂、左旋咪唑以及甲酰四氢叶酸。本发明化合物还可与酶治疗剂和免疫系统调节剂如干扰素、白介素、肿瘤坏死因子、巨噬细胞集落刺激因子及集落刺激因子组合使用。在一个实施方案中，本文所述的化合物可与选自以下的至少一种其它抗病毒剂一起使用 :逆转录酶抑制剂、蛋白酶抑制剂、融合抑制剂、进入抑制剂及聚合酶抑制剂。

[0488] 另外，根据本发明的化合物可与一种或多种抗逆转录病毒、抗 HBV、干扰素、抗癌或抗细菌剂，包括但不限于本发明的其它化合物组合或交替施用。本文所述的某些化合物可通过减少其它化合物的代谢、分解代谢或失活有效提高根据本发明的某些试剂的生物活性，且因此为了此预期效果而共同施用。

[0489] VIII. 用于治疗诺瓦克病毒感染的组合疗法

[0490] 除本文所述的抗病毒化合物之外，还可以存在其它化合物。例如，已知 I 型干扰素

(IFN) 抑制诺瓦克病毒复制。某些维生素 (特别是维生素 C) 被认为在治疗某些病毒感染中是有效的。一项研究显示维生素 A 补充给药减少诺瓦克病毒 GII 感染的发病，增加诺瓦克病毒 GI 和 GII 脱落的长度并且减少 NoV 相关腹泻的发病 (1:J Infect Dis.2007 年 10 月 1 日 ;196(7):978-85.Epub 2007 年 8 月 22 日)。已知赖氨酸是抗病毒剂。还已知，来源于基因组 II(GII) 诺瓦克病毒的病毒样颗粒 (VLP) 结合至细胞表面硫酸乙酰肝素蛋白聚糖及其它带负电的糖胺聚糖。为了治疗感染症状，还可以施用止吐剂、抗腹泻剂和 / 或止痛剂。

[0491] VIII. 药物组合物

[0492] 感染了人类免疫缺陷病毒、乙型肝炎病毒、黄病毒科病毒或杯状病毒科病毒或其基因片段或癌症的宿主 (包括但不限于人) 可通过在药学上可接受的载体或稀释剂存在下向所述患者施用有效量的活性化合物或其药学上可接受的前药或盐来治疗。活性物质可通过任何适当途径，例如口服、胃肠外、静脉内、真皮内、皮下或局部，以液体或固体形式来施用。

[0493] 化合物的优选剂量将在以下范围内 :在约 0.1 与约 100mg/kg 之间、更一般在约 1 与 50mg/kg 之间、且优选地在约 1 与约 20mg/kg 接受者体重 / 天之间。药学上可接受的盐和前药的有效剂量范围可基于有待递送的母体核苷的重量来计算。如果盐或前药本身展现活性，那么有效剂量可如上使用盐或前药的重量或通过本领域技术人员已知的其它方式来估算。

[0494] 化合物方便地以任何适合的单位剂型施用，包括但不限于含有 7 至 3000mg、优选地 70 至 1400mg 活性成分 / 单位剂型的剂型。50-1000mg 的口服剂量通常是适宜的。

[0495] 理想地，应施用活性成分以实现从约 0.2 至 70μM、优选地约 1.0 至 15μM 的活性化合物的峰值血浆浓度。这可例如通过静脉内注射 0.1 至 5％活性成分任选地在盐水中的溶液或施用活性成分的大丸剂来实现。

[0496] 活性化合物在药物组合物中的浓度将取决于药物的吸收、失活及排泄率以及本领域技术人员已知的其它因素。应注意剂量值还将随有待缓解的病状的严重性而变化。还应理解，对于任何具体的受试者而言，具体的剂量方案应根据个体需要和施用或监督组合物施用的人员的职业判断随时间进行调整，而且本文所列举的浓度范围只是示例性的，而不是为限制所要求保护的组合物的范围或实践。活性成分可以一次施用，或者可将其分成许多较小的剂量，而以不同的时间间隔施用。

[0497] 施用活性化合物的一个优选模式是口服。口服组合物通常将包含惰性稀释剂或可食用的载体。可以将它们包封在明胶胶囊中，或者压制成片剂。为用于口服治疗性施用目的，可以将活性化合物加入赋形剂中并以片剂、锭剂或胶囊形式使用。药学上相容的粘合剂和 / 或佐剂物质可以作为所述组合物的一部分包括在内。

[0498] 片剂、丸剂、胶囊、锭剂等可含有任何以下成分或相似性质的化合物 :粘合剂如微晶纤维素、黄蓍胶或明胶 ;赋形剂如淀粉或乳糖 ;崩解剂如藻酸、Primogel 或玉米淀粉 ;润滑剂如硬脂酸镁或 Sterotes ;助流剂如胶态二氧化硅 ;甜味剂如蔗糖或糖精 ;或者调味剂如薄荷、水杨酸甲酯或橙味调味剂。当所述单位剂型为胶囊时，除上述类型的物质外，还可含有液体载体如脂肪油。另外，单位剂型还可含有各种改变剂量单位的物理形式的其它物质，例如糖包衣物、虫胶或其它肠包衣剂。

[0499] 所述化合物可以作为酏剂、混悬液、糖浆剂、干胶片 (wafer)、咀嚼胶等的组分施

用。除活性化合物外，糖浆剂还可以含有作为甜味剂的蔗糖和某些防腐剂、染料和着色剂以及调味剂。

[0500] 还可将化合物、其药学上可接受的前药或盐与不损害所需作用的其它活性物质混合，或与补充所需作用的物质如抗生素、抗真菌剂、抗炎药或其它抗病毒药，包括但不限于其它核苷化合物混合。用于肠胃外、真皮内、皮下或局部施用的溶液或混悬液可包含下列组分：无菌稀释剂如注射用水、盐水溶液、不挥发油、聚乙二醇、甘油、丙二醇或其它合成溶剂；抗细菌剂如苄醇或对羟基苯甲酸甲酯；抗氧化剂如抗坏血酸或亚硫酸氢钠；螯合剂如乙二胺四乙酸；缓冲剂如乙酸盐、柠檬酸盐或磷酸盐，以及渗透压调节剂如氯化钠或葡萄糖。肠胃外制剂可以被包封在由玻璃或塑料制成的安瓿、一次性注射器或多剂量小瓶内。

[0501] 如果静脉内施用，那么优选载体是生理盐水或磷酸盐缓冲盐水 (PBS)。

[0502] 在一个优选实施方案中，所述活性化合物与保护所述化合物免受体内快速清除的载体共同制备，如控释制剂，包括但不限于植入物和微囊化递送系统。可使用生物可降解性、生物相容性聚合物，如乙烯乙酸乙烯酯、聚酐、聚乙醇酸、胶原、聚原酸酯及聚乳酸。例如，肠溶包衣的化合物可用来保护胃酸引起的裂解。用于制备这种制剂的方法对本领域技术人员是显而易见的。适合的材料也可以商购获得。

[0503] 脂质体悬浮液（包括但不限于靶向具有针对病毒抗原的单克隆抗体的受感染细胞的脂质体）也优选作为药学上可接受的载体。这些可根据本领域技术人员已知的方法，例如如美国专利号 4,522,811（以引用的方式并入）中所述来制备。例如，可以如下制备脂质体制剂：溶解合适的一种或多种脂质（如硬脂酰磷脂酰乙醇胺、硬脂酰磷脂酰胆碱、花生酰磷脂酰胆碱和胆固醇）于无机溶剂中，然后蒸发，在容器表面留下干脂质的薄膜。然后将活性化合物或其单磷酸酯、二磷酸酯和 / 或三磷酸酯衍生物的水溶液引入容器中。随后用手转动容器使脂类物质从容器壁上脱落并分散到脂类聚集物中，从而形成脂质体混悬液。

[0504] 描述本发明中所用的术语是常用的且为本领域技术人员所知。如本文所用，以下缩写具有指定含义：

[0505] aq 含水

[0506] CDI 羰基二咪唑

[0507] DMF N,N- 二甲基甲酰胺

[0508] DMSO 二甲亚砜

[0509] EDC 1- 乙基 -3-(3- 二甲基氨基丙基）碳二亚胺盐酸盐

[0510] EtOAc 乙酸乙酯

[0511] H 小时

[0512] HOBt N- 羟基苯并三唑

[0513] M 摩尔

[0514] min 分钟

[0515] rt 或 RT 室温

[0516] TBAT 三苯基二氟硅酸四丁基铵

[0517] TBTU O-（苯并三唑 -1- 基）-N,N,N',N'- 四甲基脲四氟硼酸盐

[0518] THF 四氢呋喃

[0519] IX. 用于制备活性化合物的一般流程

[0520] 还提供用于简易制备 N^4- 羟基胞苷核苷衍生物、修饰的单磷酸酯和膦酸酯前药类似物的方法。本文所公开的 N^4- 羟基胞苷核苷衍生物、修饰的单磷酸酯和膦酸酯前药类似物可如下所详细描述或通过本领域技术人员已知的其它方法来制备。本领域普通技术人员应当理解到这些流程决非是限制性的而且在不脱离本发明的精神和范围的情况下可对这些流程进行各种具体改变。

[0521] 通常，式 III、IV、VI、XI-XIV、XIX-XXVI 的核苷酸通过以下操作来制备：首先制备相应的核苷，然后为 5'- 羟基加帽作为本文所述的单磷酸酯或其它类似物，其可容易地在体内转化为化合物的活性三磷酸酯形式。

[0522] 各种反应流程概述如下。

[0523] 流程 1 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XX、XXI、XXII 的合成方法。

[0524] 流程 2 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XX、XXI、XXII 的替代合成方法。

[0525] 流程 3 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XXIII 的合成方法。

[0526] 流程 4 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XXIV 的合成方法。

[0527] 流程 5 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XXV 的合成方法。

[0528] 流程 6 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XXV 的替代合成方法。

[0529] 流程 7 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XXVI 的合成方法。

[0530] 流程 8 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 XXVI 的替代合成方法。

[0531] 流程 9 是合成本发明活性化合物的一个非限制性实例，且特别是核苷 27 的合成方法。

[0532] 流程 10 是合成本发明活性化合物的一个非限制性实例，且特别是核苷 27 的替代合成方法。

[0533] 流程 11 是合成本发明活性化合物的一个非限制性实例，且特别是核苷 29 和 30 的合成方法。

[0534] 流程 12 是合成本发明活性化合物的一个非限制性实例，且特别是核苷 30 的替代合成方法。

[0535] 流程 13 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 35 的合成方法。

[0536] 流程 14 是合成本发明活性化合物的一个非限制性实例，且特别是 N^4- 羟基胞苷 2'-C-Me 核苷 37 的合成方法。

[0537] 流程 15 是合成本发明活性化合物的一个非限制性实例，且特别是 N^4- 羟基胞苷 2'- 脱氧 -2'-α- 氟 -2'-β-C-Me 核苷 39 的合成方法。

[0538] 流程 16 是合成本发明活性化合物的一个非限制性实例，且特别是 N^4-（辛酰氧基）胞苷 2'- 脱氧 -2'- α - 氟 -2'- β -C-Me 核苷 40 的合成方法。

[0539] 流程 17 是合成本发明活性化合物的一个非限制性实例，且特别是 N^4- 羟基胞苷 2'-C-Me 核苷前药 44 的合成方法。

[0540] 流程 18 是合成本发明活性化合物的一个非限制性实例，且特别是 N^4- 羟基胞苷 2'-C-Me 核苷前药 48 的合成方法。

[0541] 流程 19 是合成本发明活性化合物的一个非限制性实例，且特别是 N^4- 羟基胞苷 2'- 脱氧 -2'- α - 氟 -2'- β -C-Me 核苷前药 51 的合成方法。

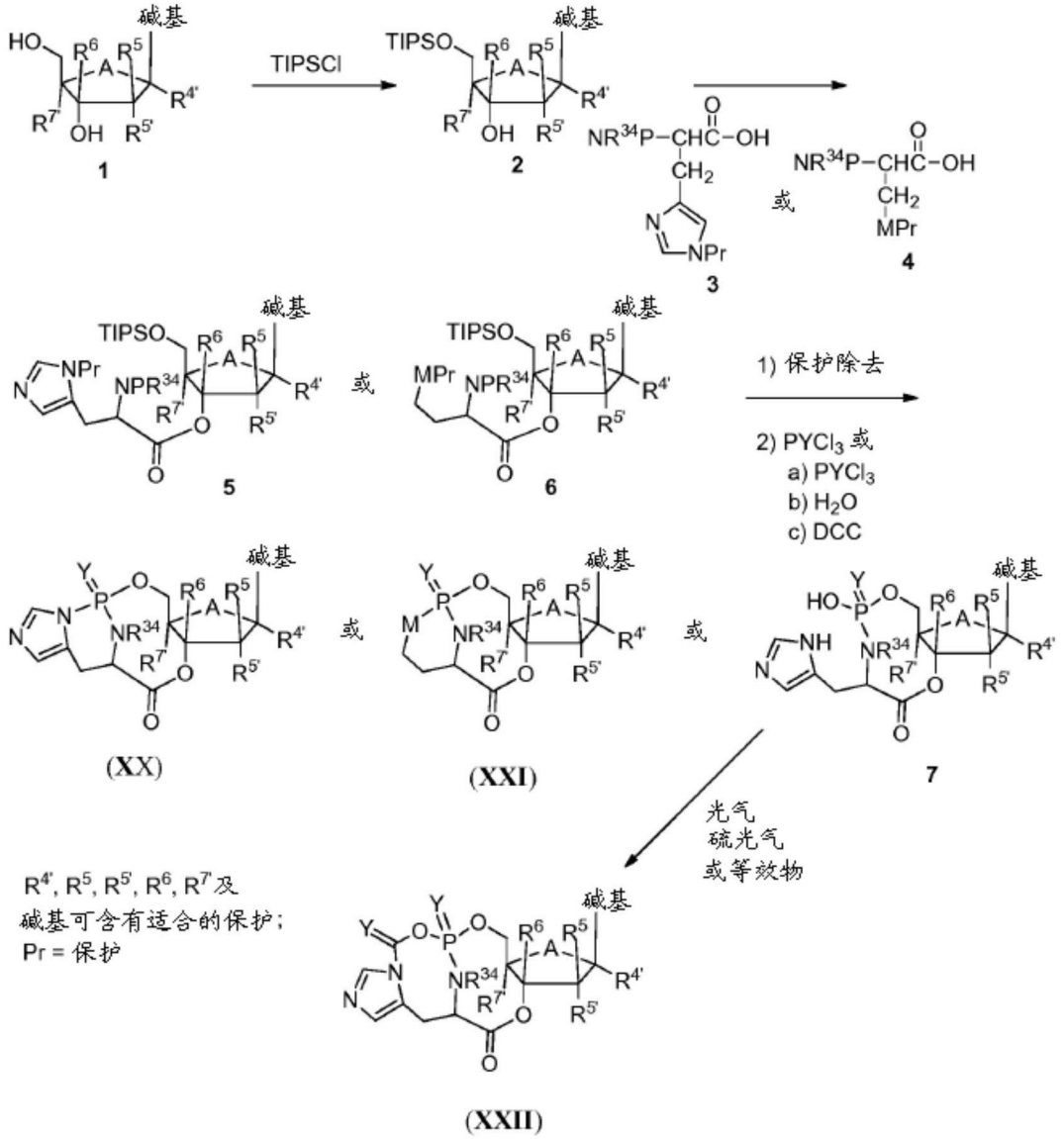
[0542] 流程 20 是合成本发明活性化合物的一个非限制性实例，且特别是本发明活性化合物的合成方法，且特别是 N^4- 羟基胞苷 2'-C-Me 核苷前药 54 和 56 的合成方法。

[0543] 流程 21 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 35 的合成方法。

[0544] 流程 22 是合成本发明活性化合物的一个非限制性实例，且特别是单磷酸酯前药 35 的合成方法。

[0545] 在一个实施方案中，式 XX、XXI 或 XXII 的核苷通过由如 TIPS 的基团保护化合物 1 以提供在糖的 3'- 位处具有游离的 α - 羟基的 2 来制备（流程 1）。化合物 1 的制备是由本领域的普通技术人员通过在以下文献中所概述的方法以及通过一般流程 9-10 来完成：(a)Rajagopalan,P. ;Boudinot,F.D ;Chu,C.K. ;Tennant,B.C ;Baldwin,B.H. ;Antiviral Nucleosides:Chiral Synthesis and Chemotheraphy:Chu,C.K. ; 编 著 Elsevier:2003 ; b)Recent Advances in Nucleosides:Chemistry and Chemotherapy:Chu,C.K. ; 编 著 Elsevier:2002 ;c)Frontiers in Nucleosides&Nucleic Acids,2004, 编 著 R.F.Schinazi&D.C.Liotta,IHL Press,Tucker,GA,USA, 第 319-37 页 ;d)Handbook of Nucleoside Synthesis:Vorbruggen H.&Ruh-Pohlenz C.John Wiley&sons 2001)。2 与酸 3 或 4 的偶合可由试剂如 EDC、EDC/HOBt、TBTU 或 CDI 来完成以得到酯 5 或 6。除去保护基之后，所得的氨基醇可通过暴露于磷酰氯或三氯硫磷 ($POCl_3$ 或 $PSCl_3$) 而转化为单磷酸酯前药 XX 或 XXI，或替代地，在磷酰氯或三氯硫磷反应的水处理之后，可在 XX 或 XXI 的形成中利用偶合剂如 DCC。化合物 7 可在磷酰氯或三氯硫磷反应的水处理之后获得且随后暴露于光气或光气等效物如 CDI 或三光气，从而得到单磷酸酯前药 XXII。

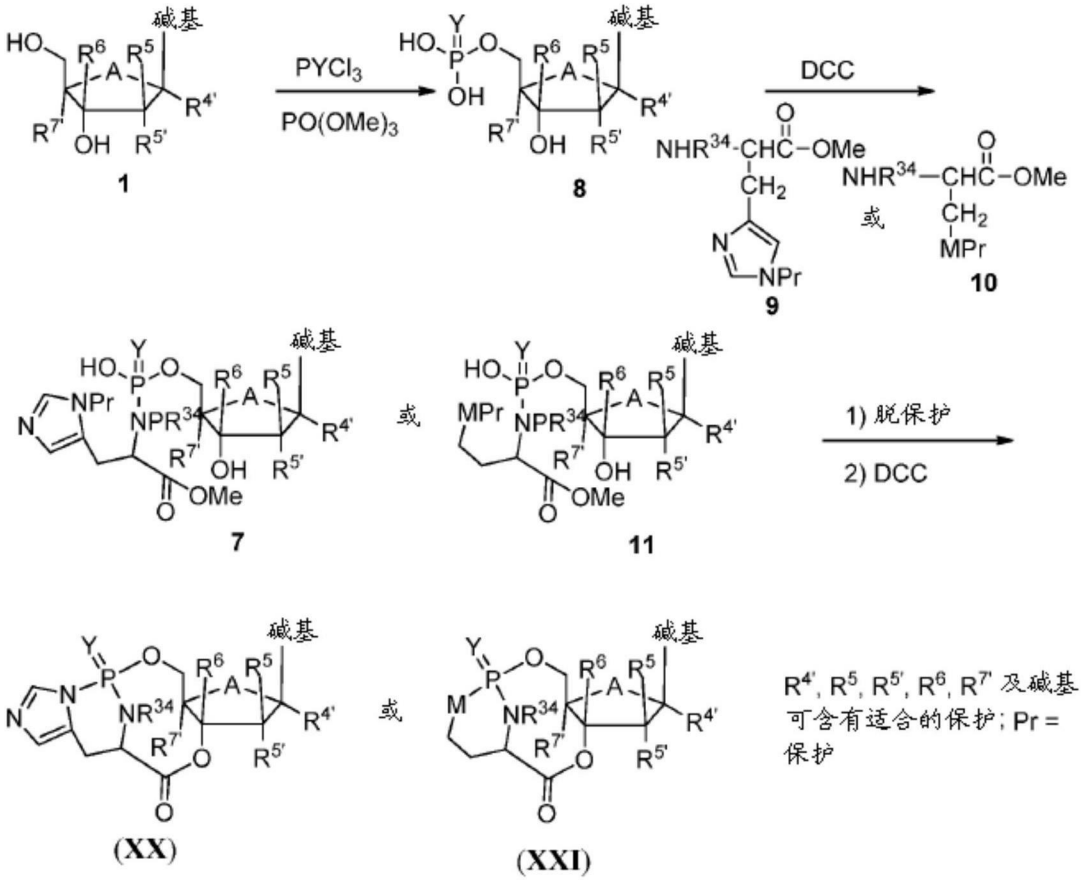
[0546]



[0547] 流程 1 单磷酸酯前药 XX、XXI、XXII 的合成方法。((碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^5、$R^{5'}$、R^6、Y、M、R^{34}及 $R^{7'}$如在活性化合物部分中所定义)

[0548] 或者,单磷酸酯前药 XX、XXI、XXII 可如流程 2 中所概述来合成,即核苷 1 可通过在磷酸三甲酯中的磷酰氯或三氯硫磷 (phosphorothioyl trichloride) 的作用直接转化为单磷酸酯 8。偶合至氨基酯 9 或 10 可用标准偶合剂如 DCC 完成以得到氨基磷酸酯 7 和 11。脱保护且随后 7 或 11 与试剂如 EDC、EDC/HOBt、TBTU 或 CDI 的偶合提供单磷酸酯前药 XX 和 XXI。单磷酸酯前药 XXII 可如流程 1 中所述从 7 处获得。

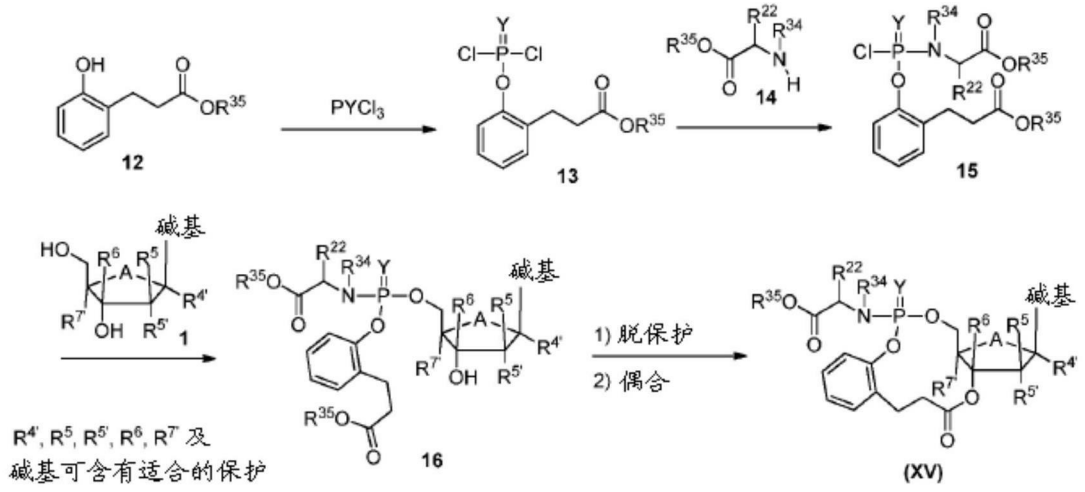
[0549]



[0550] 流程 2 单磷酸酯前药 XX、XXI、XXII 的替代合成方法。((碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^5、$R^{5'}$、R^6、Y、M、R^{34}及 $R^{7'}$如在活性化合物部分中所定义)

[0551] 单磷酸酯前药 XXIII 可如流程 3 所概述从苯酚 12 开始制备 (流程 3)。12 暴露于磷酰氯或三氯硫磷提供 13,随后允许 13 与氨基酯 14 反应以得到氨基磷酸酯 15。核苷 1 可接着通过 5'- 羟基与丙酸氯磷酰基氨酯 15 的反应转化为单磷酸酯类似物 16。脱保护且随后 16 与试剂如 EDC、EDC/HOBt、TBTU 或 CDI 的偶合提供单磷酸酯前药 XXIII。

[0552]

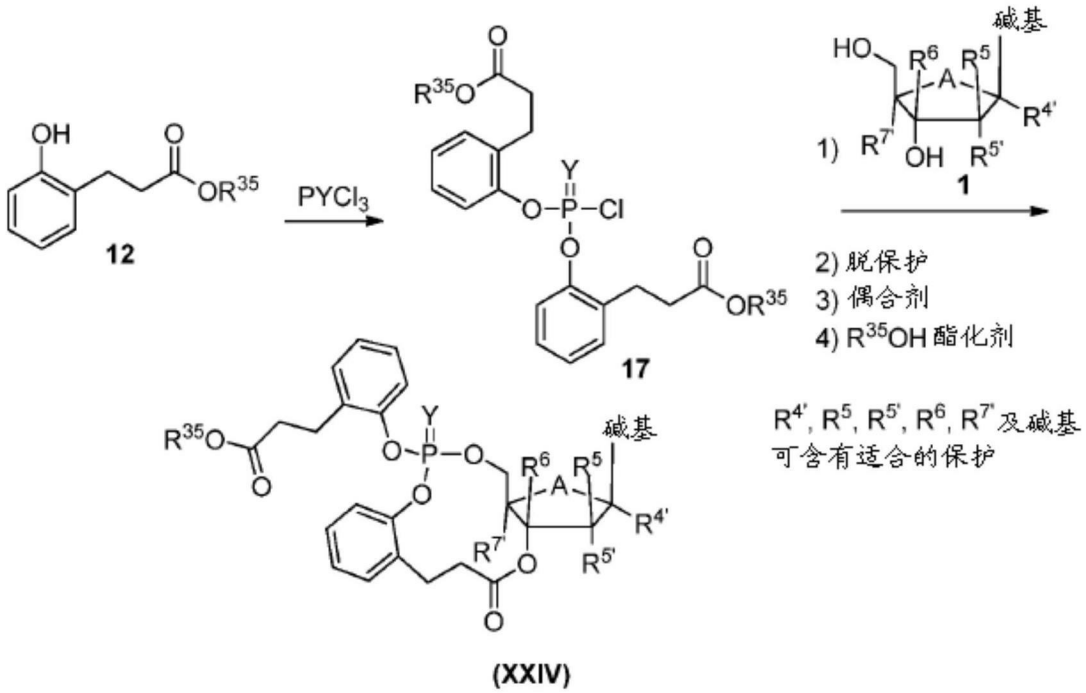


[0553] 流程 3 单磷酸酯前药 XXIII 的合成方法。(碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^5、$R^{5'}$、R^6、Y、$R^{34}R^{35}$、R^{22}及 $R^{7'}$如在活性化合物部分中所定义)

[0554] 单磷酸酯前药 XXIV 可通过苯酚 12 与磷酰氯或三氯硫磷的反应以提供氯磷酸二苯

酯 17 来制备（流程 4）。核苷 1 可接着通过 5'－羟基与氯磷酸二苯酯 17 的反应转化为中间单磷酸酯类似物。脱保护且随后 3'－羟基与试剂如 EDC、EDC/HOBt、TBTU 或 CDI 的酯形成，接着与 $R^{35}OH$ 的再酯化提供单磷酸酯前药 XXIV。

[0555]

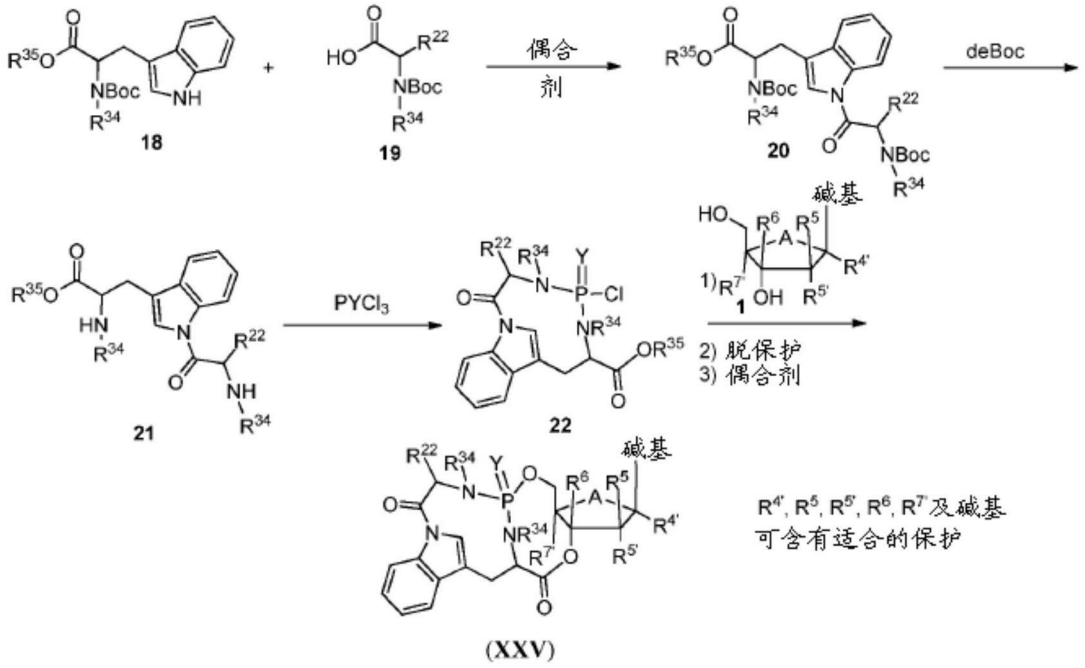


(XXIV)

[0556] 流程 4 单磷酸酯前药 XXIV 的合成方法。（碱基是天然或非天然核苷碱基；$R^{4'}$、R^5、$R^{5'}$、R^6、Y、R^{35}及 $R^{7'}$如在活性化合物部分中所定义）

[0557] 单磷酸酯前药 XXV 可通过受保护的色氨酸 18 和受保护的氨基酸 19 与偶合剂如 EDC、EDC/HOBt、TBTU 或 CDI 的初始反应以得到二肽 20 来制备（流程 5）。胺保护的除去然后得到二胺 21，其可接着与磷酰氯或三氯硫磷反应以得到环状磷酰氯二胺 22。核苷 1 可接着通过 5'－羟基与环状磷酰氯二胺 22 的反应转化为单磷酸酯类似物。脱保护且随后 22 与试剂如 EDC、EDC/HOBt、TBTU 或 CDI 的偶合提供单磷酸酯前药 XXV。

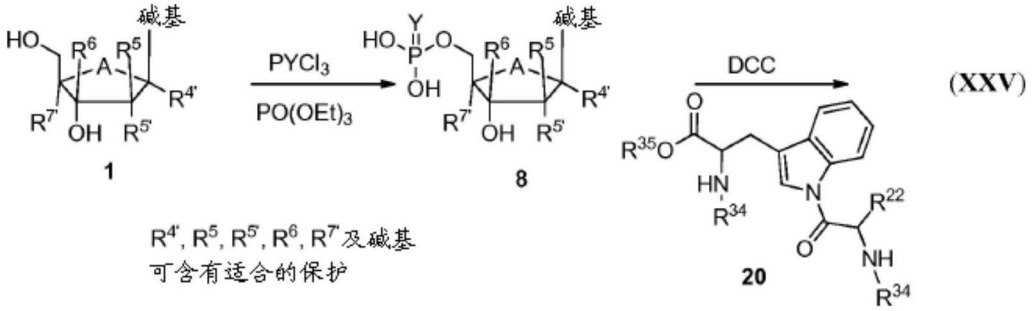
[0558]



[0559] 流程 5 单磷酸酯前药 XXV 的合成方法。(碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^{5}、$R^{5'}$、R^{6}、Y、R^{34}、R^{35}、R^{22}及 $R^{7'}$如在活性化合物部分中所定义)

[0560] 或者,单磷酸酯前药 XXV 可通过单磷酸酯类似物 8 与二肽 20 的偶合来制备(流程 6)。

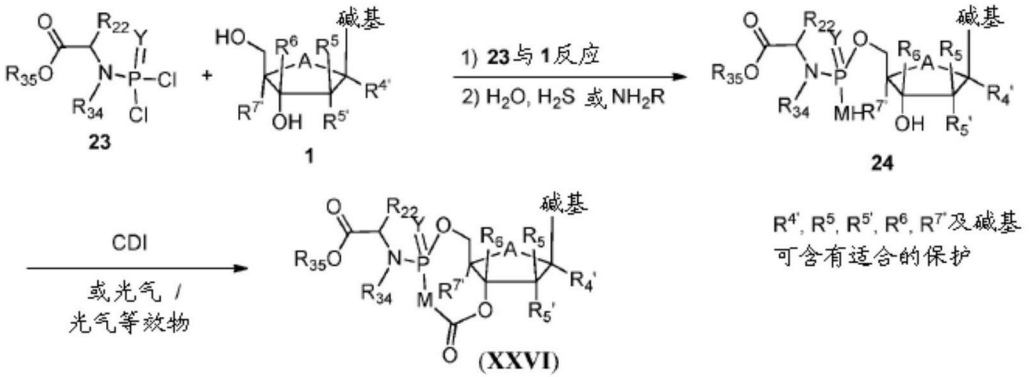
[0561]



[0562] 流程 6 单磷酸酯前药 XXV 的替代合成方法。(碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^{5}、$R^{5'}$、R^{6}、Y、R^{34}、R^{35}、R^{22}及 $R^{7'}$如在活性化合物部分中所定义)

[0563] 单磷酸酯前药 XXVI 可通过磷酰二氯 23 与核苷 1 的初始反应来制备(流程 7)。所产生的中间体与水、硫化氢或胺的随后反应提供单磷酸酯类似物 24(流程 7)。双亲核试剂 24 暴露于光气或光气等效物如 CDI 提供单磷酸酯前药 XXVI。

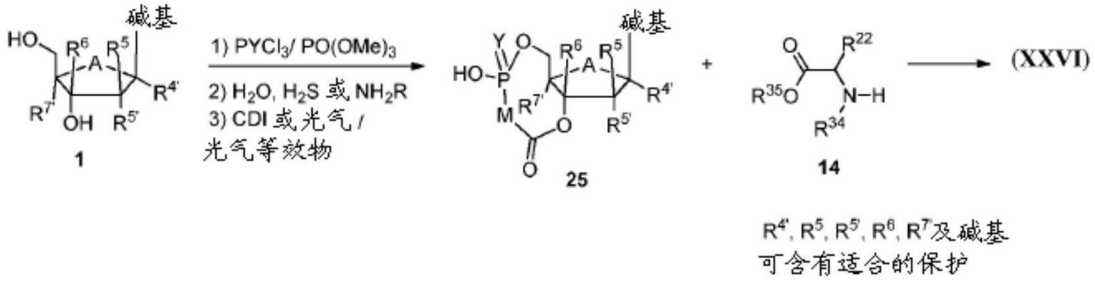
[0564]



[0565] 流程 7 单磷酸酯前药 XXVI 的合成方法。(碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^5、$R^{5'}$、R^6、Y、M、R^{34}、R^{35}、R^{22}及 $R^{7'}$如在活性化合物部分中所定义)

[0566] 或者,单磷酸酯前药 XXVI(其中 M 不是 NR) 可通过如流程 8 所示核苷 1 与磷酰氯或三氯硫磷的初始反应来制备。所产生的中间体与水或硫化氢的随后反应,接着与光气或光气等效物如 CDI 的反应提供单磷酸酯前药 XXVI。(流程 8)。

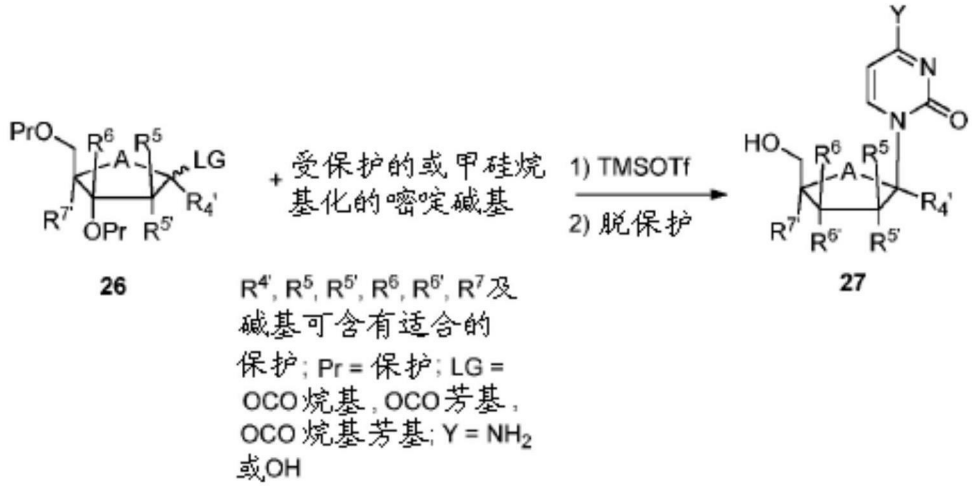
[0567]



[0568] 流程 8 单磷酸酯前药 XVIII 的替代合成方法。(碱基是天然或非天然核苷碱基 ;$R^{4'}$、R^5、$R^{5'}$、R^6、Y、R^{34}、R^{35}、R^{22}及 $R^{7'}$如在活性化合物部分中所定义)

[0569] 核苷 27 可通过在路易斯酸如 TMSOTf 存在下将糖 26 与受保护的或甲硅烷基化的嘧啶碱基偶合来制备。5'-羟基脱保护得到核苷 27。(流程 9)。

[0570]

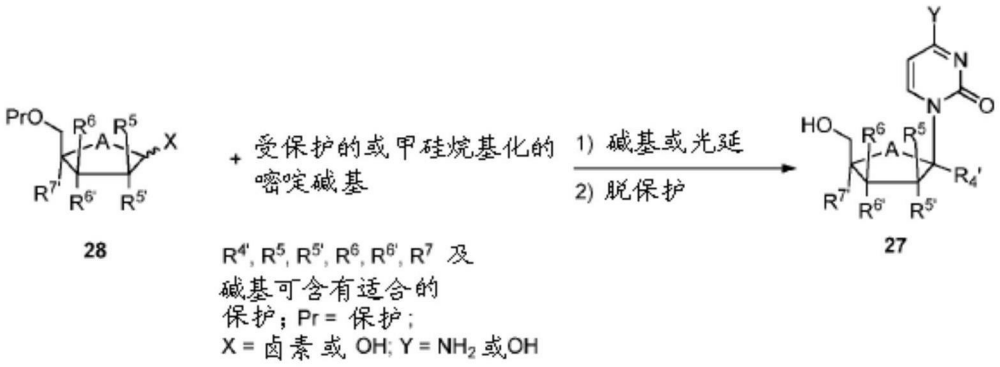


[0571] 流程 9 核苷 27 的合成方法。($R^{4'}$、R^5、$R^{5'}$、R^6、Y、A 及 $R^{7'}$如在活性化合物部分中所定义)

[0572] 或者,核苷 27 可从 1'-卤基或 1'-羟基化合物 28 来制备。对于 1'-卤基的情况,

在碱如三乙胺或氢化钠存在下保护的或游离的嘧啶碱基，接着脱保护将得到核苷 27。对于 1'- 羟基的情况，在光延偶合剂如偶氮二甲酸二异丙酯存在下保护的或游离的嘧啶碱基，接着脱保护将得到核苷 27（流程 10）。

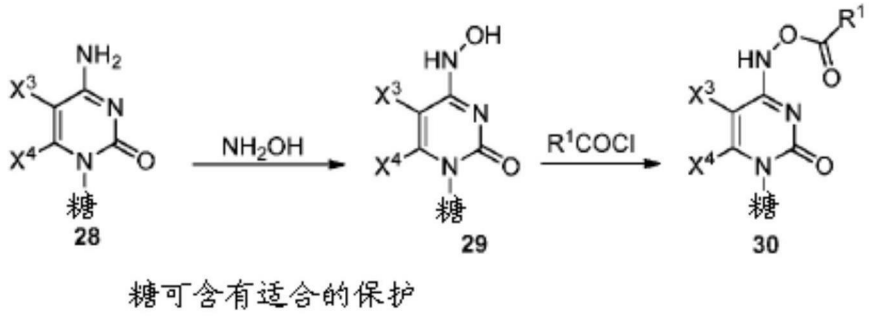
[0573]



[0574] 流程 10 核苷 27 的替代合成方法。（$R^{4'}$、R^5、$R^{5'}$、R^6、Y、$R^{6'}$及 $R^{7'}$如在活性化合物部分中所定义）

[0575] N^4- 羟基胞苷核苷 29 可通过化合物 28 与羟胺的反应来制备（流程 11）。与各种酰氯的随后反应提供相应的 N^4- 酰氧基衍生物 30。

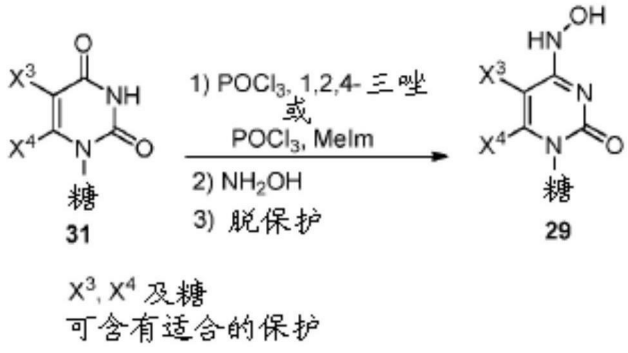
[0576]



[0577] 流程 11 核苷 29 和 30 的合成方法。（X^3、X^4、R^1及糖如在活性化合物部分中所定义）

[0578] 或者，核苷 29 可如流程 12 所示通过核苷 31 与磷酰氯和 1,2,4- 三唑或甲基咪唑的初始反应来制备。所产生的中间体与羟胺的随后反应，接着糖部分的脱保护得到核苷 29。

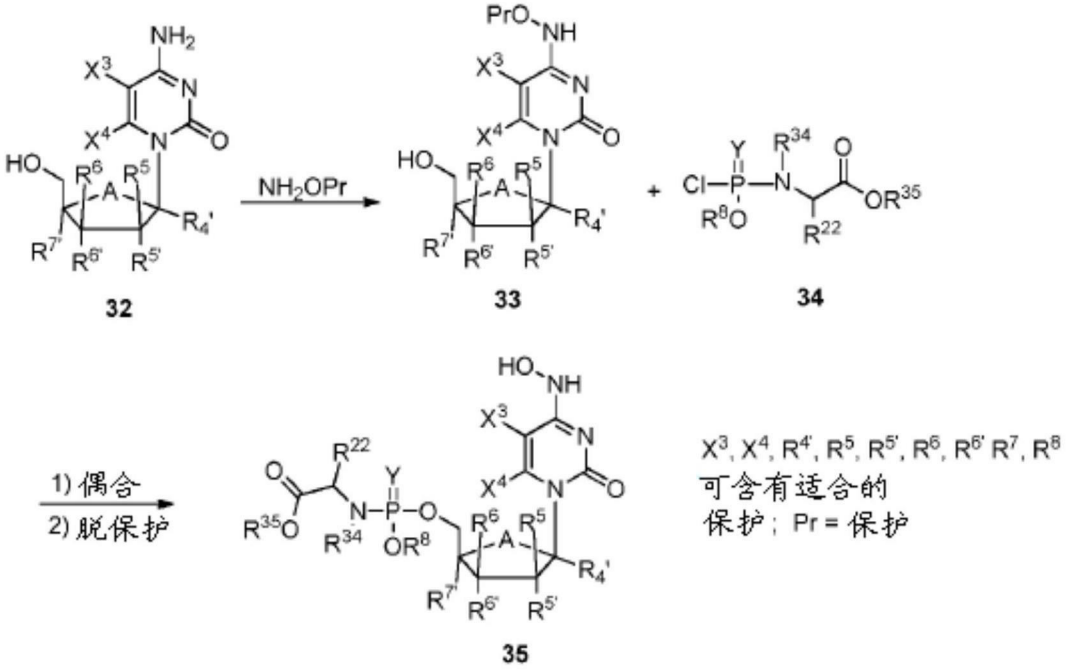
[0579]



[0580] 流程 12 核苷 29 的替代合成方法。（X^3、X^4、R^1及糖如在活性化合物部分中所定义）

[0581] 单磷酸酯前药 35 可通过适当保护的羟胺衍生物与核苷 32 的初始反应来制备（流程 13）。33 与氨基磷酸酯氯化物 34 的随后反应，接着必需的脱保护提供单磷酸酯前药 35。

[0582]



[0583] 流程 13 单磷酸酯前药 35 的方法。(X^3、X^4、Y、$R^{4'}$、R^5、$R^{5'}$、R^6、Y、R^{34}、R^{35}、R^{22}及 $R^{7'}$如在活性化合物部分中所定义)

[0584] 在一些情况下,磷原子可以是手性的,在本文中被称为“P*”或“P”,这意味着并且是指其具有对应于对于这种指配的 Cahn-Ingold-Prelog 规则的所接受的含义的“R”或“S”名称。式 A 的前药可由于在磷中心处的手性而作为非对映异构体的混合物存在。当在磷中心处存在手性时,其可能全部或部分是 Rp 或 Sp 或其任何混合物。

[0585] 本发明进一步在以下实施例中说明。流程 14-19 和实施例 1-6 显示用于合成 N^4- 羟基胞苷核苷衍生物和修饰的单磷酸酯前药类似物的制备方法,并且实施例 7-35 显示用于 N^4- 羟基胞苷核苷衍生物和修饰的单磷酸酯前药类似物的生物学评价的方法。本领域普通技术人员应当理解这些实施例决非是限制性的而且在不脱离本发明的精神和范围的情况下可以对这些实施例进行各种具体改变。

具体实施例

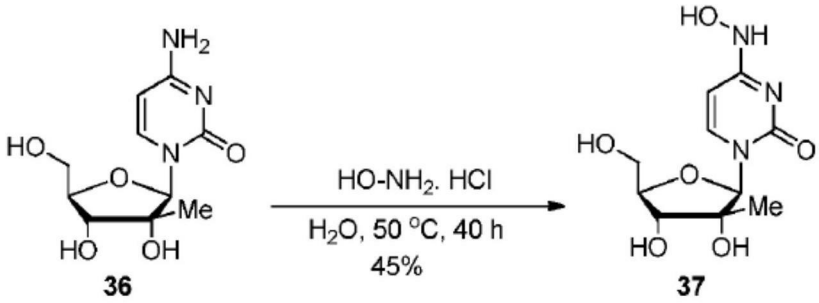
[0586] 按照以下实施例和反应顺序制备本发明代表性的具体化合物;作为说明,提供实施例和描述反应顺序的示意图,以帮助理解本发明,但不能将其解释为以任何方式限制随后的权利要求书中叙述的本发明。本发明化合物也可用作随后的实施例中的中间体以产生本发明另外的化合物。没有必要试图对任何反应中获得的产率进行优化。本领域技术人员应知道如何通过反应时间、温度、溶剂和 / 试剂的常规改变来提高所述产率。

[0587] 无水溶剂是购自 Aldrich Chemical Company, Inc. (Milwaukee)。试剂是购自商业来源。除非另外指出,否则在实施例中使用的材料得自易于获取的商业供应商,或者通过化学合成领域技术人员已知的标准方法合成。熔点 (mp) 是在电热数字熔点仪上测定,并且未校正。^1H 和 ^{13}C NMR 光谱用 Varian Unity Plus 400 光谱仪在室温下测定,且以距离内标四甲基硅烷的 ppm 低磁场报道。使用氘交换、去耦实验或 2D-COSY 以确定质子的归属。通过以下符号表示信号的多重度 :s(单峰),d(双重峰),dd(两个双重峰),t(三重峰),q(四重峰),br(宽峰),bs(宽单峰),m(多重峰)。所有 J 值都以 Hz 为单位。质谱是在 Micromass

Platform LC 分光计上使用电喷雾技术测定的。元素分析是通过 Atlantic Microlab Inc. (Norcross,GA) 进行。在 Whatman LK6F 硅胶板上进行分析 TLC,且在 Whatman PK5F 硅胶板上进行制备 TLC。柱色谱法在硅胶上或经由反相高效液相色谱法进行。

[0588] 实施例 1

[0589]



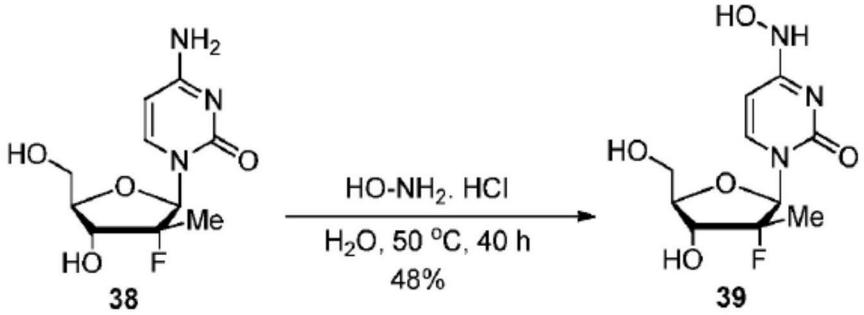
[0590] 流程 14. N^4- 羟基胞苷 2'-C-Me 核苷 37 的合成

[0591] 1-((2R,3R,4R,5R)-3,4- 二羟基 -5-(羟基甲基)-3- 甲基四氢呋喃 -2- 基)-4-(羟基氨基) 嘧啶 -2(1H)- 酮 37

[0592] 向 36(0.175g,0.68mmol) 在 2mL H_2O 中的溶液中添加羟胺盐酸盐 (0.24g,3.4mmol)。反应混合物在 50℃下搅拌且通过 TLC 和 / 或 LC/MS 监测。16h 之后,添加羟胺盐酸盐 (0.24g,3.4mmol) 且在 50℃下额外搅拌反应混合物 24h。在起始材料完全消耗之后,用 AcOEt(3×5mL) 萃取水溶液。将合并的有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5 至 90:10v/v) 纯化残余物以得到 45% 产率的 37(0.83g,0.30mmol)。LCMS(ESI) $C_{10}H_{15}N_3O_6$ 的计算值 :273.2,观测值 :(M+1)274.1。

[0593] 实施例 2

[0594]



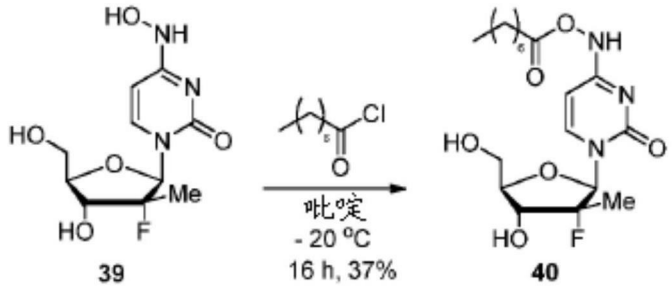
[0595] 流程 15. N^4- 羟基胞苷 2'- 脱氧 -2'-α- 氟 -2'-β-C-Me 核苷 39 的合成

[0596] 1-((2R,3R,4R,5R)-3- 氟 -4- 羟基 -5-(羟基甲基)-3- 甲基四氢呋喃 -2- 基)-4-(羟基氨基) 嘧啶 -2(1H)- 酮 39

[0597] 向 38(1g,3.86mmol) 在 10mL H_2O 中的溶液中添加羟胺盐酸盐 (1.34g,19mmol)。反应混合物在 50℃下搅拌且通过 TLC 和 / 或 LC/MS 监测。16h 之后,添加羟胺 (1.34g,19mmol) 且在 50℃下额外搅拌反应混合物 24h。在起始材料完全消耗之后,用 AcOEt(3×25mL) 萃取水溶液。将合并的有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5 至 90:10v/v) 纯化残余物以得到 48% 产率的 39(0.51g,1.85mmol)。LCMS(ESI) $C_{10}H_{14}FN_3O_5$ 的计算值 :275.2,观测值 :(M+1)274.3

[0598] 实施例 3

[0599]



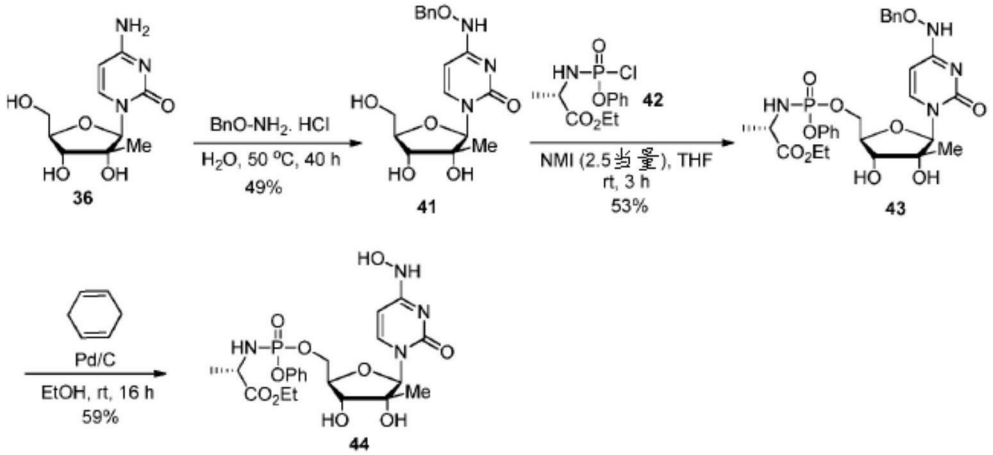
[0600] 流程 16. N^4-(辛酰氧基)胞苷 2'-脱氧-2'-α-氟-2'-β-C-Me 核苷 40 的合成

[0601] 1-((2R,3R,4R,5R)-3-氟-4-羟基-5-(羟基甲基)-3-甲基四氢呋喃-2-基)-4-((辛酰氧基)氨基)嘧啶-2(1H)-酮 40

[0602] 向 39(0.06g,0.23mmol)在 2mL 无水吡啶的预冷却(-20℃)溶液中添加辛酰氯(44μL,0.26mmol)。在 4℃下搅拌混合物 16h 之后,将反应用 MeOH(2mL)终止且在减压下浓缩溶液。然后添加 AcOEt(10mL)且将混合物用水(3×5mL)洗涤。将有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法(CH_2Cl_2:MeOH = 95:5 至 85:15v/v)纯化残余物以得到 37%产率的 40(0.04g,0.09mmol)。LCMS(ESI)$C_{18}H_{28}FN_3O_6$的计算值:401.4,观测值:(M+1)402.3

[0603] 实施例 4

[0604]



[0605] 流程 17. N^4-羟基胞苷 2'-C-Me 核苷前药 44 的合成

[0606] 4-((苄氧基)氨基)-1-((2R,3R,4R,5R)-3,4-二羟基-5-(羟基甲基)-3-甲基四氢呋喃-2-基)嘧啶-2(1H)-酮 41

[0607] 向 36(0.175g,0.68mmol)在 2mL H_2O 中的溶液中添加 O-苄基羟基胺盐酸盐(0.70g,4.38mmol)。反应混合物在 50℃下搅拌且通过 TLC 和/或 LC/MS 监测。16h 之后,O-苄基羟基胺盐酸盐(0.30g,1.88mmol)添加且在 50℃下额外搅拌反应混合物 24h。在起始材料完全消耗之后,用 AcOEt(3×5mL)萃取水溶液。将合并的有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法(CH_2Cl_2:MeOH = 95:5 至 90:10v/v)纯化残余物以得到 49%产率的 41(0.12g,0.33mmol)。LCMS(ESI)$C_{17}H_{21}N_3O_6$的计算值:363.4,观测值:(M+1)364.3

[0608] (2S)-2-(((((2R,3R,4R,5R)-5-(4-((苄氧基)氨基)-2-氧代嘧

啶 -1(2H)- 基)-3,4- 二羟基 -4- 甲基四氢呋喃 -2- 基) 甲氧基)(苯氧基) 磷酰基) 氨基) 丙酸乙酯 43

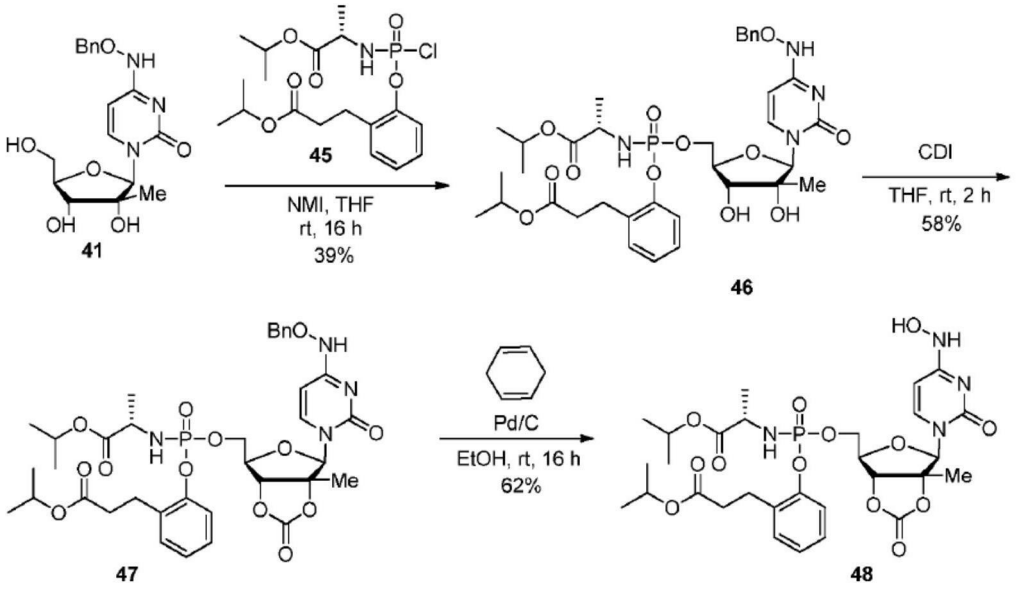
[0609] 在氩气氛围下向 41(0.04g,0.12mmol) 在 2mL 中的溶液中添加 1- 甲基咪唑 (0.15mL,0.3mmol) 和 0.3mL 苯基 -(乙氧基 -L- 丙氨酰基)- 氯磷酸酯 42 在 THF 中的 1M 溶液。在室温下搅拌 3h 之后,添加 AcOEt(10mL) 且将反应混合物用 (3×3mL) 水洗涤。将有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5 至 90:10v/v) 纯化残余物以得到 53% 产率的 43(0.04g,0.06mmol)。LCMS(ESI) $C_{28}H_{35}N_4O_{10}P$ 的计算值 :618.6,观测值 :(M+1)619.7

[0610] (2S)-2-(((((2R,3R,4R,5R)-3,4- 二羟基 -5-(4-(羟基氨基)-2- 氧代嘧啶 -1(2H)- 基)-4- 甲基四氢呋喃 -2- 基) 甲氧基)(苯氧基) 磷酰基) 氨基) 丙酸乙酯 44

[0611] 在 rt 下向 43(0.04g,0.06mmol) 在 2mL EtOH 中的溶液中添加 1,4- 环己二烯 (0.1mL) 和 Pd/C(0.01g,10% Pd/ 活性炭)。在 rt 下搅拌 16h 之后,在硅藻土垫上过滤悬浮液且将收集的溶液在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 90:10) 纯化残余物以得到 59% 产率的 44(0.02g,0.04mmol)。LCMS(ESI) $C_{21}H_{29}N_4O_{10}P$ 的计算值 :528.4,观测值 :(M+1)528.3

[0612] 实施例 5

[0613]



[0614] 流程 18. N^4- 羟基胞苷 2'-C-Me 核苷前药 48 的合成

[0615] 3-(2-(((((2R,3R,4R,5R)-5-(4-((苄氧基) 氨基)-2- 氧代嘧啶 -1(2H)- 基)-3,4- 二羟基 -4- 甲基四氢呋喃 -2- 基) 甲氧基)(((S)-1- 异丙氧基 -1- 氧代丙 -2- 基) 氨基) 磷酰基) 氧基) 苯基) 丙酸异丙酯 46

[0616] 在氩气氛围下向 41(0.15g,0.41mmol) 在 7mL 无水 THF 中的溶液中添加 1- 甲基咪唑 (0.07mL,0.83mmol) 和 0.83mL 氨基磷酸酯氯化物 45 在 THF 中的 1M 溶液。在室温下搅拌 16h 之后,添加 AcOEt(20mL) 且将反应混合物用水 (3×5mL) 洗涤。将有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5 至 90:10v/v) 纯化残余物以得到 39% 产率的 46(0.12g,0.16mmol)。LCMS(ESI) $C_{35}H_{47}N_4O_{12}P$ 的计算值 :746.7,观测值 :(M+1)747.5

[0617] 3-(2-(((((3aR,4R,6R,6aR)-6-(4-((苄氧基)氨基)-2-氧代嘧啶-1(2H)-基)-6a-甲基-2-氧代四氢呋喃并[3,4-d][1,3]间二氧杂环戊烯-4-基)甲氧基)(((S)-1-异丙氧基-1-氧代丙-2-基)氨基)磷酰基)氧基)苯基)丙酸异丙酯47

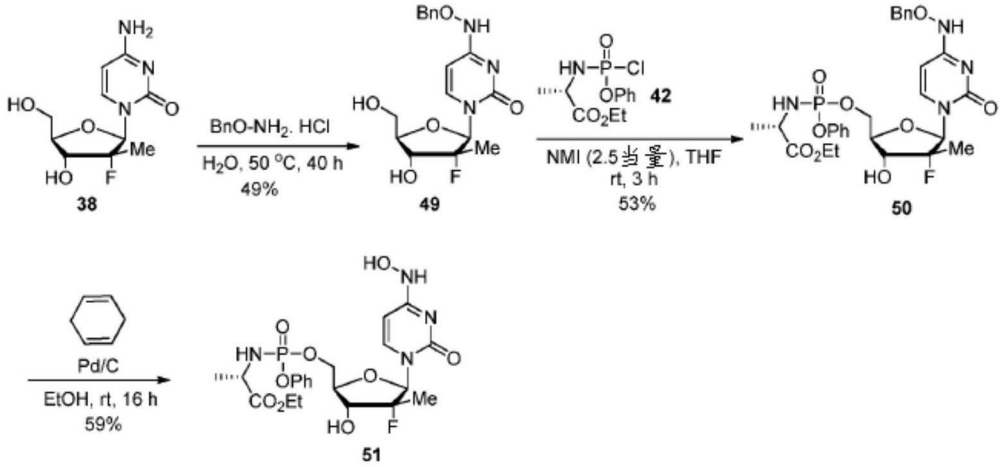
[0618] 在0℃下向46(0.04g,0.05mmol)在0.25mL THF中的溶液中添加N,N'-羰基二咪唑(0.02mg,0.12mmol)。在rt下搅拌2h之后,在减压下浓缩溶液。通过硅胶柱色谱法(己烷:EtOAc=5:5)纯化残余物以得到58%产率的47(0.02g,0.03mmol)。LCMS(ESI)$C_{36}H_{45}N_4O_{13}P$的计算值:772.7,观测值:(M+1)772.5

[0619] 3-(2-(((((3aR,4R,6R,6aR)-6-(4-(羟基氨基)-2-氧代嘧啶-1(2H)-基)-6a-甲基-2-氧代四氢呋喃并[3,4-d][1,3]间二氧杂环戊烯-4-基)甲氧基)(((S)-1-异丙氧基-1-氧代丙-2-基)氨基)磷酰基)氧基)苯基)丙酸异丙酯48

[0620] 在rt下向47(0.02g,0.06mmol)在2mL EtOH中的溶液中添加1,4-环己二烯(0.1mL)和Pd/C(0.01g,10%Pd/活性炭)。在rt下搅拌16h之后,在硅藻土垫上过滤悬浮液且将收集的溶液在减压下浓缩。通过硅胶柱色谱法(CH_2Cl_2:MeOH=90:10)纯化残余物以得到62%产率的48(0.02g,0.04mmol)。LCMS(ESI)$C_{29}H_{39}N_4O_{13}P$的计算值:682.6,观测值:(M+1)683.4

[0621] 实施例6

[0622]



[0623] 流程19. N^4-羟基胞苷2'-脱氧-2'-α-氟-2'-β-C-Me核苷前药51的合成

[0624] 4-((苄氧基)氨基)-1-((2R,3R,4R,5R)-3-氟-4-羟基-5-(羟基甲基)-3-甲基四氢呋喃-2-基)嘧啶-2(1H)-酮49

[0625] 向38(0.2g,0.77mmol)在2mL H_2O中的溶液中添加O-苄基羟基胺盐酸盐(0.37g,2.31mmol)。将反应混合物在50℃下搅拌且通过TLC和/或LC/MS监测。16h之后,添加O-苄基羟基胺盐酸盐(0.37g,2.31mmol)且在50℃下额外搅拌反应混合物24h。在起始材料完全消耗之后,用AcOEt(3×10mL)萃取水溶液。将合并的有机层经Na_2SO_4干燥,过滤且在减压下浓缩。通过硅胶柱色谱法(CH_2Cl_2:MeOH=95:5至90:10v/v)纯化残余物以得到39%产率的49(0.11g,0.30mmol)。LCMS(ESI)$C_{17}H_{20}N_3O_5F$的计算值:365.4,观测值:(M+1)366.3

[0626] (2S)-2-(((((2R,3R,4R,5R)-5-(4-((苄氧基)氨基)-2-氧代嘧啶-1(2H)-基)-4-氟-3-羟基-4-甲基四氢呋喃-2-基)甲氧基)(苯氧基)磷酰基)氨基)丙酸乙酯50

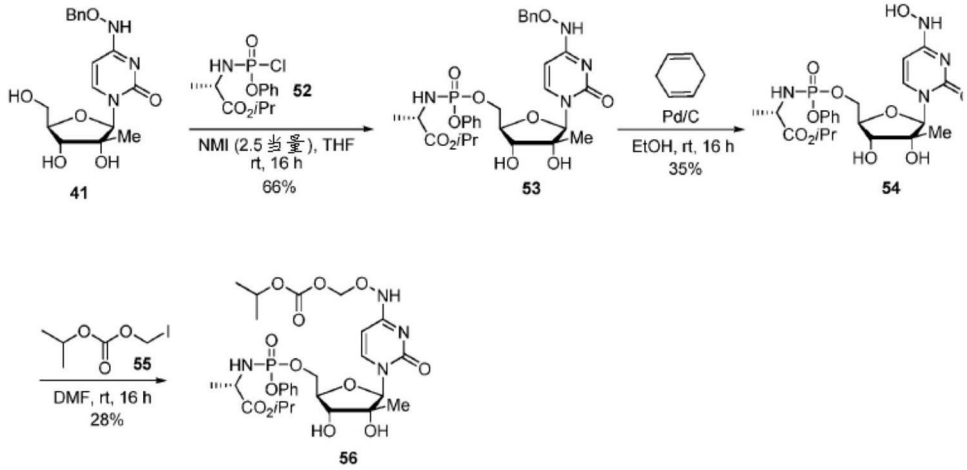
[0627] 在氩气氛围下向 49(0.15g,0.41mmol) 在 3mL 中的溶液中添加 1- 甲基咪唑 (0.10mL,1.23mmol) 和 1.23mL 苯基 -(乙氧基 -L- 丙氨酰基)- 氯磷酸酯 42 在 THF 中的 1M 溶液。在室温下搅拌 16h 之后,添加 AcOEt(10mL) 且将反应混合物用水 (3×3mL) 洗涤。将有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5 至 90:10v/v) 纯化残余物以得到 13%产率的 50(0.03g,0.05mmol)。LCMS(ESI)$C_{28}H_{34}N_4O_9PF$ 的计算值 :620.6,观测值 :(M+1)621.3

[0628] (2S)-2-(((((2R,3R,4R,5R)-4- 氟 -3- 羟基 -5-(4-(羟基氨基)-2- 氧代嘧啶 -1(2H)- 基)-4- 甲基四氢呋喃 -2- 基) 甲氧基)(苯氧基) 磷酰基) 氨基) 丙酸乙酯 51

[0629] 在 rt 下向 50(0.03g,0.06mmol) 在 2mL EtOH 中的溶液中添加 1,4- 环己二烯 (0.1mL) 和 Pd/C(0.01g,10% Pd/ 活性炭)。在 rt 下搅拌 16h 之后,在硅藻土垫上过滤悬浮液且将收集的溶液在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5) 纯化残余物以得到 40%产率的 51(0.01g,0.04mmol)。LCMS(ESI)$C_{21}H_{28}N_4O_9PF$ 的计算值 :530.4,观测值 :(M+1)531.3

[0630] 实施例 7

[0631]



[0632] 流程 20. N^4- 羟基胞苷 2'-C-Me 核苷前药 54 和 56 的合成

[0633] 2S)-2-(((((2R,3R,4R,5R)-5-(4-((苄氧基) 氨基)-2- 氧代嘧啶 -1(2H)- 基)-3,4- 二羟基 -4- 甲基四氢呋喃 -2- 基) 甲氧基)(苯氧基) 磷酰基) 氨基) 丙酸异丙酯 53

[0634] 在氩气氛围下向 41(0.13g,0.36mmol) 在 5mL THF 中的溶液中添加 1- 甲基咪唑 (0.07mL,0.9mmol) 和 0.9mL(2S)- 异丙基 2-((氯 (苯氧基) 磷酰基) 氨基) 丙酸酯 52 在 THF 中的 1M 溶液。在室温下搅拌 3h 之后,添加 AcOEt(15mL) 且将反应混合物用水 (3×5mL) 洗涤。将有机层经 Na_2SO_4 干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 95:5 至 90:10v/v) 纯化残余物以 66%产率得到 53(0.15g,0.24mmol)。

[0635] (2S)-2-(((((2R,3R,4R,5R)-3,4- 二羟基 -5-(4-(羟基氨基)-2- 氧代嘧啶 -1(2H)- 基)-4- 甲基四氢呋喃 -2- 基) 甲氧基)(苯氧基) 磷酰基) 氨基) 丙酸乙酯 54

[0636] 在 rt 下向 53(0.06g,0.1mmol) 在 1.5mL iPrOH 中的溶液中添加 1,4- 环己二烯 (0.2mL) 和 Pd/C(0.01g,10% Pd/ 活性炭)。在 rt 下搅拌 16h 之后,在硅藻土垫上过滤悬浮液且将收集的溶液在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH = 90:10) 纯化残余物

以得到 35％产率的 54(0.02g,0.04mmol)

[0637] (2S)-2-(((((2R,3R,4R,5R)-3,4- 二羟基 -5-(4-((((异丙氧基羰基）氧基）甲氧基）氨基)-2- 氧代嘧啶 -1(2H)- 基)-4- 甲基四氢呋喃 -2- 基）甲氧基)(苯氧基）磷酰基）氨基）丙酸乙酯 56

[0638] 向 54(0.03g,0.055mmol) 在 0.6mL DMF 中的溶液中添加 Cs_2CO_3(0.054g,0.165mmol) 和碘甲基异丙基碳酸酯 55(0.027g,0.11mmol)。在室温下搅拌 16h 之后,添加 CH_2Cl_2(5mL) 且将反应混合物用水 (3×3mL) 洗涤。将有机层经 Na_2SO_4干燥,过滤且在减压下浓缩。通过硅胶柱色谱法 (CH_2Cl_2:MeOH ＝ 95:5v/v) 纯化残余物以 28％产率得到 56(0.01g,0.015mmol)。

[0639] (2S)-2-((氯（苯氧基）磷酰基）氨基）丙酸异丙酯 52

[0640] 在氩气氛围下向苯基二氯磷酸酯 (7.88g,51.4mmol) 在 40mLCH_2Cl_2中的溶液中添加 L- 丙氨酸异丙酯盐酸盐 (8.58g,51.4mmol)。将混合物冷却降至 -78℃且经 2h 逐滴添加 Et_3N(14mL,102.8mmol) 在 40mL CH_2Cl_2中的溶液。在室温下搅拌所得的溶液 16h 之后,将所形成的白色固体在硅藻土垫上过滤且用无水 Et_2O(40mL) 洗涤。将有机层在减压下浓缩且通过硅胶柱色谱法纯化残余物 (EtOAc: 己烷＝ 1:0 至 1:1v/v) 以得到 50％产率的呈无色油状的 52(7.86g,26mmol)。

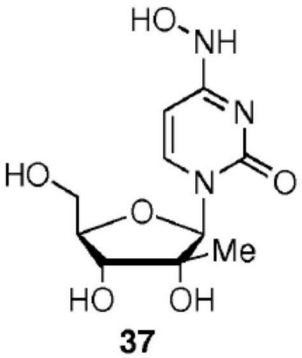
[0641] 实施例 8

[0642] 以下显示在 50μM N^4- 羟基胞苷核苷和 N^4- 羟基胞苷单磷酸酯前药在 Huh-7 细胞中温育 4h 之后形成的核苷酸的 LC/MS 定性分析的两个实施例。

[0643] 37 在 Huh-7 细胞中的温育使得仅检测到极低水平的 37-TP(表 1)。然而,单磷酸酯前药 44 在 Huh-7 细胞中的温育使得检测到高水平的 37-MP、37-DP 及 37-TP(表 1),以及极低水平的 36-DP、36-TP 及 2'- 脱氧 -β-C-Me-U-TP。

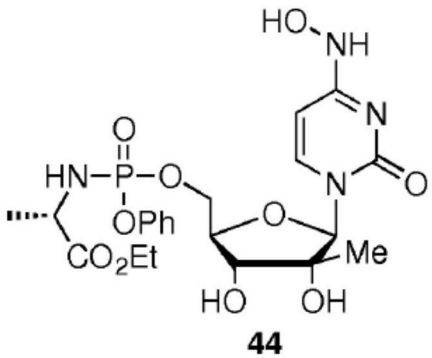
[0644] 表 1. MP 前药 44 和母体核苷 37 的 HCV 和毒性数据

[0645]



37

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



44

HCV EC_{50} = 0.8 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM

[0646] 在 MP 前药 44 的温育之后产生的这些高水平的细胞内 37-MP、37-DP 及 37-TP 指示 MP 前药绕过第一磷酸化步骤,使得形成 37-TP。结果显示于下表 2 中：

[0647] 表 2. 在 50 μ M37 和 44 在 Huh-7 细胞中温育 4 小时之后形成的核苷酸的 LC/MS 分析

[0648]

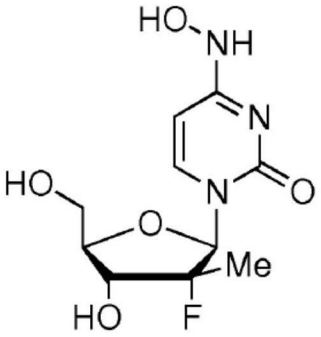
药物 代谢物 (pmol/10^6个细胞)	**37**	**44**
2'-OH-2'-Me-U	BLOQ	BLOQ
2'-OH-2'-Me-UMP	BLOQ	BLOQ
2'-OH-2'-Me-UDP	BLOQ	BLOQ
2'-OH-2'-Me-UTP	BLOQ	4.84 ± 0.23
36	BLOQ	BLOQ
36-MP	BLOQ	BLOQ
36-DP	BLOQ	1.75 ± 0.19
36-TP	BLOQ	33.3 ± 0.15
37	BLOQ	BLOQ
37-MP	BLOQ	239.2 ± 35.2
37-DP	BLOQ	451.4 ± 31.1
37-TP	3.20 ± 1.30	3,075 ± 98.5
44	-	13.3 ± 1.7

[0649] BLOQ 意指低于定量限

[0650] MP 前药 39 和母体核苷 51 的 HCV 和毒性数据显示在下表 3 中。

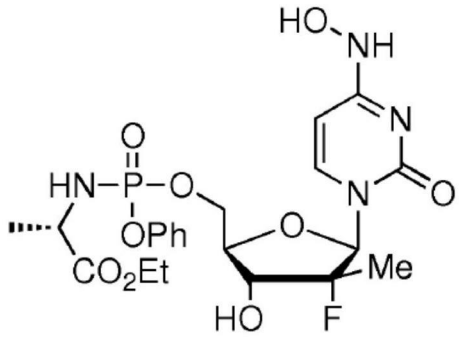
[0651] 表 3：

[0652]



39

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



51

HCV EC_{50} = 2.6 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >33 μM

[0653] 39 在 Huh-7 细胞中的温育使得检测到高水平的 39 以及低水平的 2'-脱氧-2'-α-氟-2'-β-C-Me-U-TP、38-DP 及 38-TP。未检测到 39-MP、DP、-TP。(表 4)

[0654] 然而,单磷酸酯前药 51 在 Huh-7 细胞中的温育使得检测到高水平的 39、39-MP、39-DP 及 39-TP(表 2)。还观测到低水平的 38、38-MP、38-DP、38-TP 及 2'-脱氧-2'-α-氟-2'-β-C-Me-U-TP。

[0655] 在 MP 前药 51 的温育之后产生的这些高水平的细胞内 39-DP 和 39-TP 指示 MP 前药允许绕过第一磷酸化步骤,使得形成 39-TP。

[0656] 表 4 在 50μM39 和 51 在 Huh-7 细胞中温育 4 小时之后形成的核苷酸的 LC/MS 分析

[0657]

药物 代谢物	39	51

[0658]

(pmol/10^6个细胞)		
2'-F-2'-Me-U	BLOQ	BLOQ
2'-F-2'-Me-UMP	BLOQ	BLOQ
2'-F-2'-Me-UDP	BLOQ	BLOQ
2'-F-2'-Me-UTP	0.68±0.07	6.25±0.17
38	BLOQ	5.00±0.34

38-MP	BLOQ	3.24±0.26
38-DP	0.42±0.019	3.01±0.39
38-TP	2.17±0.13	20.3±1.54
39	188.8±15.3	144.6±21.9
39-MP	BLOQ	3,452±247
39-DP	BLOQ	31.6±7.7
39-TP	BLOQ	364.5±10.6
51	-	71.5±2.3

[0659] BLOQ 意指低于定量限

[0660] 实施例 9

[0661] 抗 HIV(在 PBM 细胞中)测定

[0662] 化合物的抗 HIV-1 活性如先前所述在人周围血液单核 (PBM) 细胞中测定(参见 Schinazi R.F.,McMillan A.,Cannon D.,Mathis R.,Lloyd R.M.Jr.,Peck A.,Sommadossi J.-P.,St.Clair M.,Wilson J.,Furman P.A.,Painter G.,Choi W.-B.,Liotta D.C.Antimicrob.Agents Chemother.1992,36,2423;Schinazi R.F.,Sommadossi J.-P.,Saalmann V.,Cannon D.,Xie M.-Y.,Hart G.,Smith G.,Hahn E.Antimicrob. Agents Chemother.1990,34,1061)。化合物的储备溶液 (20-40mM) 在无菌 DMSO 中制备,然后在生长培养基中稀释至所需浓度。用原型 HIV-1LAI 在 0.01 的感染复数下感染细胞。从细胞上清液处获得的病毒在感染之后第 6 天使用 $(rA)_n \cdot (dT)$12-18 作为模板 - 引物通过逆转录酶测定来定量。存在于稀释溶液中的 DMSO(<0.1%)对病毒产量没有影响。AZT 作为阳性对照包括在内。抗病毒 EC_{50}和 EC_{90}是使用先前所述的半数有效方法从浓度 - 反应曲线中获得(参见 Chou T.-C.&Talalay P.Adv.Enzyme Regul.1984,22,27-55;Belen'kii M.S.&Schinazi R.F.Antiviral Res.1994,25,1-11)。

[0663] 实施例 10

[0664] 通过 HIV-1 RT 评定核苷 -TP 的掺入

[0665] i) 蛋白质表达和纯化:使用 p6HRT-PROT 表达载体在细菌中过表达 HIV-1 RT(xxLAI 背景)(参见 Shi C,Mellors JW.A recombinant retroviral system for rapid in vivo analysis of human immunodeficiency virus type 1 susceptibility to reverse transcriptase inhibitors.Antimicrob Agents Chemother.1997;41:2781-5) 并且如先前所述纯化至均质(参见 Le Grice SF,Gruninger-Leitch F.Rapid purification of homodimer and heterodimer HIV-1 reverse transcriptase by metal chelate affinity chromatography.Eur J Biochem.1990;187:307-14;Le Grice SF,Cameron CE,Benkovic SJ.Purification and characterization of human immunodeficiency virus type 1 reverse transcriptase.Methods Enzymol.1995;

262:130-44)。使用 260450M-1cm-1 的消光系数 (ε 280) 在 280nm 下用分光光度法测定纯化的酶的蛋白质浓度。RT 的活性位点浓度是如先前所述由预稳态破裂实验来计算 (参见 Kati WM,Johnson KA,Jerva LF,Anderson KS.Mechanism and fidelity of HIV reverse transcriptase.J Biol.Chem.1992 ;267:25988-97)。如下所述的所有反应均使用活性位点浓度进行。

[0666] ii) 预稳态动力学分析 :退火至 57 个核苷酸 DNA 模板 (5'-CTCAGACCCTTTTAGTCAGAATGGAAANTCTCTAGCAGTGGCGCCCG AACAGGGACA-3') 的 [γ ^{32}P]-ATP 5'- 末端标记的 20 个核苷酸 DNA 引物 (5'-TCGGGCGCCACTGCTAGAGA-3') 被用于所有实验中。DNA 模板含有在位置 30(N) 处的 T 或 C,这允许使用相同的 20 个核苷酸引物评价单个核苷酸掺入的动力学。快速淬火实验是使用 Kintek RQF-3 仪器 (Kintek Corporation,Clarence,PA) 进行。在所有实验中,300nM RT 和 60nM DNA 模板 / 引物 (T/P) 在反应缓冲液 (50mM Tris-HCl pH 7.5,50mM KCl) 中预温育,之后在含有 20mM $MgCl_2$的相同反应缓冲液中与相等体积的核苷酸混合。反应通过用 0.5M EDTA,pH 8.0 淬火在从 10ms 至 30min 的时间范围内终止。将淬火的样品与相等体积的凝胶加样缓冲液 (98% 去离子甲酰胺、10mM EDTA 和 1mg/mL 溴酚蓝和二甲苯苯胺 (xylene cyanol) 的每一者) 混合,在 85℃下变性 5min,并且将产物在 7M 尿素 -16% 聚丙烯酰胺凝胶上与底物分离。使用 Bio-Rad GS525 分子成像器 (Bio-Rad Laboratories,Inc.,Hercules,CA) 分析产物形成。

[0667] iii) 数据分析 :将从动力学测定中获得的数据使用 Sigma 绘图软件 (Jandel Scientific) 通过非线性回归与适当的方程拟合 (参见 Johnson KA.Rapid quench kinetic analysis of polymerases,adenosinetriphosphatases,and enzyme intermediates.Methods Enzymol.1995 ;249:38-61)。dNTP 的每个具体浓度的表观破裂速率常数 (kobs) 是通过将产物形成的时程拟合至方程来确定 :[产物] = A[1-exp(-kobst)],其中 A 代表破裂幅度。dNTP 的转换数 (kpol) 和表观电离常数 (K_d) 是通过针对 dNTP 浓度绘制表观催化速率 kobs 并将数据与以下双曲线方程拟合而获得 :kobs = (kpol[dNTP])/([dNTP]+K_d)。

[0668] 实施例 11

[0669] 评定 N^4- 羟基胞苷核苷衍生物、修饰的单磷酸酯及膦酸酯前药类似物的抗 HIV 活性和细胞毒性

[0670] i) 病毒 :病毒原种可使用 xxHIV-1LAI 克隆 75 通过将 5 至 10 μg 的质粒 DNA 电穿孔 (Gene Pulser ;Bio-Rad) 到 1.3×10^7个 MT-2 细胞中来制备。转染后 7 天,可收获不含细胞的上清液并储存在 -80℃下。病毒原种的基因型可通过以下操作来确认 :从病毒粒子提取 RNA,用 DNA 酶 I 处理提取物,通过 RT-PCR 扩增 RT 的全长编码区 (氨基酸 1 至 560),纯化 PCR 产物并且使用 Big Dye 终止剂试剂盒 (v.3.1) 在 ABI 3100 自动 DNA 测序仪 (Applied Biosystems,Foster City,Calif.) 上测定序列。可通过三倍终点稀释测定 (每份稀释液 6 个孔) 测定 MT-2 细胞、P4/R5 细胞或 PBM 细胞的病毒原种的 50% 组织培养感染剂量 ($TCID_{50}$) 并且使用 Reed 和 Muench 方程来计算 (参见 Reed LJ,Muench H.A simple method of estimating fifty per cent endpoints.Am.J.Hyg.1938 ;27:493-497)。

[0671] ii) 单个复制周期的药物敏感性测定 :在 96 孔板中,将抑制剂的两倍或三倍连续稀释液一式三份添加至 P4/R5 细胞中。将细胞用在无药物的病毒感染对照孔中产生 100 的相对光单位值的量的病毒来感染。感染后 48h,将细胞溶解缓冲液和发光

底物 (Gal-Screen ;Tropix/Applied Biosystems) 添加至每个孔中，并且使用光度计 (ThermoLabSystems,Waltham,Mass) 测定相对光单位值。病毒复制的抑制计算为抑制 50％病毒复制所需的化合物浓度 (EC_{50})。

[0672] iii) 多个复制周期的药物敏感性测定 :在 96 孔板中，可将抑制剂的三倍连续稀释液一式三份添加至 MT-2 细胞中。细胞可如在 MT-2 细胞中的终点稀释所测定在 0.01 的感染复数下被感染。感染后 7 天，将培养上清液收集并且用 0.5％ Triton X-100 处理。在上清液中的 p24 抗原浓度可使用商用的酶联免疫吸附测定 (DuPont,NEN Products,Wilmington,Del.) 来测定。EC_{50}值可如上所述来计算。

[0673] iv) 在 PBM 细胞中的药物敏感性测定 :PBM 细胞是如先前所述通过 Ficoll-Hypaque 不连续梯度离心从健康的血清阴性供体中分离 (参见 Schinazi RF,Cannon DL,Arnold BH,Martino-Saltzman D.Combinations of isoprinosine and 3'-azido-3'-deoxythymidine in lymphocytes infected with human immunodeficiency vims type 1.Antimicrob.Agents Chemother.1988 ;32:1784-1787 ; Schinazi RF,Sommadossi JP,Saalmann V,Cannon DL,Xie MY,Hart GC,Smith GA.Hahn E.F.Activities of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1.Antimicrob.Agents Chemother.1990 ;34:1061-1067)。在使用之前细胞用植物凝集素 A(PHA,Difco,Sparks,MD) 刺激 2-3 天。感染是大量进行持续 1h，对于烧瓶 (T25) 测定用 $100TCID_{50}/1\times10^{7}$个细胞或对于 24 孔板测定用 $200TCID_{50}/6\times10^{7}$个细胞。将细胞添加至含有测试化合物的 10 倍连续稀释液的板或烧瓶中。感染后 5 天，将培养上清液收集并且用 0.5％ Triton X-100 处理。上清液中的 p24 抗原浓度如上所述来测定。EC_{50}和倍数抗性值如上所述来计算。

[0674] v) 细胞毒性测定 :核苷及核苷单磷酸酯前药可就其对 P4/R5 细胞、MT-2 细胞及未感染的 PHA 刺激的人 PBM 细胞的可能毒性作用进行评价。可将对数期 P4/R5、MT-2 及 PHA 刺激的人 PBM 细胞以 5×10^{3}至 5×10^{4}个细胞 / 孔接种于含有测试化合物的 10 倍连续稀释液的 96 孔细胞培养板中。可培养培养物 2-4 天，之后可将溴化 3-(4,5- 二甲基噻唑 -2- 基)-2,5- 二苯基四唑 (MTT) 染料溶液 (Promega,Madison,WI) 添加至每个孔中并且培养过夜。将反应用终止增溶溶液 (Promega,Madison,WI) 来终止并且可在 570nm 的波长下读取板。中值 50％细胞毒性浓度 (CC_{50}) 可使用中值效果方法从浓度 - 反应曲线中测定。

[0675] 实施例 12

[0676] 评定 N^4- 羟基胞苷核苷衍生物、修饰的单磷酸酯及膦酸酯前药类似物针对耐药性 HIV 的活性

[0677] 如上所鉴定与母体类似物相比具有改善的活性和较小细胞毒性的类似物可进一步就针对一组耐药性病毒的活性进行评价。在此研究中使用的耐药性病毒可包括 $HIV\text{-}1_{K65R}$、$HIV\text{-}1_{K70E}$、$HIV\text{-}1_{L74V}$、$HIV\text{-}1_{M184V}$、$HIV\text{-}1_{AZT2}$、$HIV\text{-}1_{AZT3}$、$HIV\text{-}1_{AZT7}$、$HIV\text{-}1_{AZT9}$、$HIV\text{-}1_{Q151M}$ 及 $HIV\text{-}1_{69\,插入}$。所有这些突变病毒都可在 $HIV\text{-}1_{XX}$LAI 克隆中生成。

[0678] 实施例 13

[0679] 评定 N^4- 羟基胞苷核苷衍生物、修饰的单磷酸酯及膦酸酯前药类似物针对耐药性 HIV 的活性

[0680] i) 病毒和药物敏感性测定 :可如上所述制备病毒原液。药物敏感性测定可使用也如上所述的单个和多个复制周期测定来进行。病毒复制的抑制可计算为抑制 50％病毒复制所需的化合物浓度 (EC_{50})。抗性倍数值可通过将突变的 HIV-1 的 EC_{50}除以 WT HIV-1 的 EC_{50}来确定。

[0681] ii) 统计分析 :为了确定倍数抗性值是否在统计上是显著的,可将来自至少三个独立实验的 EC_{50}值进行 log10 转换并且使用两样品 Student's t 检验用 Sigma Stat 软件 (Jandel Scientific) 来比较。小于 0.05 的 P 值被认为在统计上是显著的。

[0682] 实施例 14

[0683] 通过突变的 HIV-1 RT 评定核苷酸的掺入和切除

[0684] i) 酶 :以下突变的HIV-1 RT酶可用于此研究中 :K65R RT、K70E RT、L74V RT、M184V RT、AZT2RT、AZT3RT、Q151M RT 及 69 插入 RT。可以开发用于这些突变型 RT 的每一个的大肠杆菌蛋白质表达载体,并且可如先前所述进行蛋白质表达和纯化。蛋白质浓度和活性位点浓度可如上所述来测定。

[0685] ii) 核苷酸掺入的动力学分析 :预稳态动力学分析可用于确定K65R、K70E RT、L74V RT、M184V RT 及 Q151M RT 的每种新型核苷 -TP 的动力学参数 Kd 和 kpol。试验设计和数据分析可如上所述进行。

[0686] iii) 切除测定 :来自链终止模板 / 引物的新型类似物的 ATP 介导的磷酸分解切除可使用 WT RT、AZT2RT、AZT3RT 及 69Insert RT 来进行。如上所述的 20 个核苷酸 DNA 引物可用 [γ ^{32}P]-ATP 在 5'- 末端标记,然后退火至适当的 57 个核苷酸 DNA 模板。引物的 3'- 末端可通过用 WT RT 和 100μM 适当修饰的核苷酸类似物在 37℃下温育 30min 来进行链终止。^{32}P 标记的链终止的 21 核苷酸引物可进一步通过在 7M 尿素 -16％丙烯酰胺变性凝胶电泳之后提取适当的条带来纯化。纯化的链终止的引物然后可与适当的 DNA 模板重退火以供在磷酸分解实验中使用。核苷 -MP 的磷酸分解去除可通过将 300nM(活性位点)WT 或突变型 RT 与 60nM 感兴趣的链终止的 T/P 复合物在 50mM Tris-HCl pH 8.0、50mM KCl 中温育来实现。反应可通过添加 3.0mM ATP 和 10mM $MgCl_2$来开始。无机焦磷酸酶 (0.01U) 可在反应自始至终都存在。在限定的温育期之后,等分试样可从反应试管中除去并且用相等体积的凝胶加样染料 (98％去离子甲酰胺、10mM EDTA 及 1mg/mL 溴酚蓝和二甲苯苯胺的每一者) 淬火。产物可通过变性凝胶电泳分离,并且可使用 Bio-Rad GS525 分子成像器分析与产物形成相一致的底物消失。数据可拟合至以下单一指数方程以测定 ATP 介导的切除的表观速率 (kATP) :[产物] = A[exp(-kATPt)],其中 A 代表产物形成的幅度。终端复合物形成可如先前所述来测定 (参见 Meyer PR,Matsuura SE,Mian AM,So AG,Scott WA.A mechanism of AZT resistance:an increase in nucleotide-dependent primer unblocking by mutant HIV-1reverse transcriptase.Mol Cell.1999 ;4:35-43 ;Sluis-Cremer N,Arion D,Parikh U,Koontz D,Schinazi RF,Mellors JW,Parniak MA.The 3'-azido group is not the primary determinant of 3'-azido-3'-deoxythymidine(AZT)responsible for the excision phenotype of AZT-resistant HIV-1.J Biol Chem.2005 ;280:29047-52)。

[0687] 实施例 15

[0688] 在 HepG2 细胞中的线粒体毒性测定 :

[0689] i) 核苷及核苷单磷酸酯前药对细胞生长和乳酸产生的影响 :对 HepG2 细胞生长的

影响可通过在0μM、0.1μM、1μM、10μM及100μM药物存在下温育细胞来确。定可将细胞(5×10^4/孔)涂于12孔细胞培养集落中的具有补充有10%胎牛血清、1%丙酮酸钠及1%青霉素/链霉素的非必需氨基酸的最小必需培养基中并且在37℃下温育4天。温育期结束时,细胞数量可使用血球计来测定。还由Pan-Zhou X-R,Cui L,Zhou X-J,Sommadossi J-P,Darley-Usmer VM."Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells"Antimicrob.Agents Chemother.2000;44:496-503所教导。为了测量核苷类似物对乳酸产生的影响,来自储备培养物的HepG2细胞可被稀释并以2.5×10^{4}个细胞/孔涂于12孔培养板中。可添加各种浓度(0μM、0.1μM、1μM、10μM及100μM)的核苷类似物,并且可将培养物在37℃下在湿润的5% CO_2气氛中温育4天。在第4天,可测定每个孔中的细胞数量并收集培养基。可将培养基过滤,并且使用比色乳酸测定(Sigma-Aldrich)来测定培养基中的乳酸含量。因为乳酸产物可被视为减弱的线粒体功能的标记物,所以在N^4-羟基胞苷核苷衍生物、修饰的单磷酸酯及膦酸酯前药类似物存在下生长的细胞中检测到的升高水平的乳酸产生可用于指示药物诱导的细胞毒性效应。

[0690] ii)N^4-羟基胞苷核苷衍生物、修饰的单磷酸酯及膦酸酯前药类似物对线粒体DNA合成的影响:已开发了精确地定量线粒体DNA含量的实时PCR测定(参见Stuyver LJ,Lostia S,Adams M,Mathew JS,Pai BS,Grier J,Tharnish PM,Choi Y,Chong Y,Choo H,Chu C K,Otto MJ,Schinazi RF.Antiviral activities and cellular toxicities of modified 2',3'-dideoxy-2',3'-didehydrocytidine analogs.Antimicrob.Agents Chemother.2002;46:3854-60)。此测定可用于本申请中所述的所有研究,所述研究确定核苷类似物对线粒体DNA含量的影响。在此测定中,低传代数的HepG2细胞可以5,000个细胞/孔接种于涂布胶原的96孔板中。核苷单磷酸酯类似物可添加至培养基中以获得0μM、0.1μM、10μM及100μM的最终浓度。在培养第7天,细胞核酸可通过使用可商购获得的柱(RNeasy 96试剂盒;Qiagen)来制备。这些试剂盒共同纯化RNA和DNA,且因此将总核酸从柱上洗脱。线粒体细胞色素C氧化酶亚基II(COXII)基因及β-肌动蛋白或rRNA基因可使用多重Q-PCR方案从5μl洗脱的核酸来扩增,其中适合的引物和探针用于靶标和参考扩增两者。对于COXII,可分别使用以下有义、探针及反义引物:5'-TGCCCGCCATCATCCTA-3'、5'-四氯-6-羧基荧光素-TCCTCATCGCCCTCCCATCCC-TAMRA-3'及5'-CGTCT GTTATGTAAAGGATGCGT-3'。对于β-肌动蛋白基因(GenBank登录号E01094)的外显子3,有义、探针及反义引物分别是5'-GCGCGGC TACAGCTTCA-3'、5'-6-FAMCACCACG GCCGAGCGGGATAMRA-3'及5'-TCTCCTTA ATGTCACGCACGAT-3'。rRNA基因的引物和探针可从Applied Biosystems商购获得。因为相等的扩增效率可对于所有基因获得,所以比较CT方法可用于研究线粒体DNA合成的可能抑制。比较CT方法使用以下算术公式,其中靶标(COXII基因)的量对内源参照物(β-肌动蛋白或rRNA基因)的量进行归一化并且相对于定标物(在第7天时没有药物的对照)。用于此方法的算术公式是由2-ΔΔCT给出,其中ΔΔCT是(平均靶标测试样品的CT-靶标对照的CT)-(平均参照测试的CT-参照对照的CT)(参见Johnson MR,K Wang,J B Smith,MJ Heslin,RB Diasio.Quantitation of dihydropyrimidine dehydrogenase expression by real-time reverse transcription polymera se chain reaction.Anal.Biochem.2000;278:175-184)。在药物存在下生长的

细胞中的线粒体 DNA 含量的下降指示线粒体毒性。

[0691] iii) 电子显微镜形态学评价：已显示 NRTI 诱发的毒性在线粒体（例如，嵴丧失、基质溶解和溶胀以及脂类小滴形成）中引起形态变化，这可使用透射电子显微术用超微结构分析来观测（参见 Cui L, Schinazi RF, Gosselin G, Imbach JL. Chu CK, Rando RF, Revankar GR, Sommadossi JP. Effect of enantiomeric and racemic nucleoside analogs on mitochondrial functions in HepG2 cells. Biochem. Pharmacol. 1996, 52, 1577-1584 ; Lewis W, Levine ES, Griniuviene B, Tankersley KO, Colacino JM, Sommadossi JP, Watanabe KA, Perrino FW. Fialuridine and its metabolites inhibit DNA polymerase gamma at sites of multiple adjacent analog incorporation, decrease mtDNA abundance, and cause mitochondrial structural defects in cultured hepatoblasts. Proc Natl Acad Sci U S A. 1996 ; 93 : 3592-7 ; Pan-Zhou XR, L Cui, XJ Zhou, JP Sommadossi, VM Darley-Usmar. Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells. Antimicrob. Agents Chemother. 2000, 44, 496-503)。例如，用 10μM 非阿尿苷 (FIAU ; 1, 2'- 脱氧 -2'- 氟 -1-D- 阿拉伯呋喃糖基 -5- 碘 - 尿嘧啶）温育的 HepG2 细胞的电子显微照片可显示存在扩大的线粒体，其具有与线粒体功能障碍一致的形态变化。为了确定核苷及核苷单磷酸酯前药是否促进线粒体中的形态变化，可将 HepG2 细胞 (2.5×10^4 个细胞 /mL) 在 0μM、0.1μM、1μM、10μM 及 100μM 核苷类似物存在下接种于组织培养盘 (35×10mm) 中。在第 8 天，可将细胞固定，脱水并且如先前所述包埋入 Eponas 中。可制备薄切片，用醋酸铀酰和柠檬酸铅染色，然后使用透射电子显微术来检验。

[0692] 实施例 16

[0693] 在 Neuro2A 细胞中的线粒体毒性测定

[0694] 为了估计核苷类似物产生神经元毒性的可能性，小鼠 Neuro2A 细胞（美国典型培养物保藏中心 131) 可用作模型系统（参见 Ray AS, H ernandez-Santiago BI, Mathew JS, Murakami E, Bozeman C, Xie MY, Dutschman GE, Gullen E, Yang Z, Hurwitz S, Cheng YC, Chu CK, McClure H, Schinazi RF, Anderson KS. Mechanism of anti-human immunodeficiency virus activity of beta-D-6-cyclopropylamino-2', 3'-didehydro-2', 3'-dideoxyguanosine. Antimicwb. Agents Chemother. 2005, 49, 1994-2001)。抑制 50% 细胞生长所必需的浓度 (CC_{50}) 可使用基于溴化 3-(4, 5- 二甲基 - 噻唑 -2- 基)-2, 5- 二苯基四唑染料的测定如所述来测量。细胞乳酸和线粒体 DNA 水平在限定的药物浓度下的扰动可如上所述进行。在所有实验中，ddC 和 AZT 可用作对照核苷类似物。

[0695] 实施例 17

[0696] 核苷酸类似物对线粒体 DNA 聚合酶 γ 的 DNA 聚合酶和核酸外切酶活性的影响

[0697] i) 人聚合酶 γ 的纯化：聚合酶 γ 的重组大和小亚基可如先前所述进行纯化（参见 Graves SW, Johnson AA, Johnson KA. Expression, purification, and initial kinetic characterization of the large subunit of the human mitochondrial DNA polymerase. Biochemistry. 1998, 37, 6050-8 ; Johnson AA, Tsai Y, Graves SW, Johnson KA. Human mitochondrial DNA polymerase holoenzyme: reconstitution and characterization. Biochemistry 2000 ; 39 : 1702-8)。蛋白质浓度可在 280nm 下对聚合酶

γ 的大和小亚基分别是 234,420 和 71,894M-1cm-1 的消光系数用分光光度法来测定。

[0698] ii) 核苷酸掺入的动力学分析 :可进行预稳态动力学分析以测定核苷 -TP 及天然 dNTP 底物的 DNA 聚合酶 γ 的掺入的催化效率 (k/K)。这允许确定此酶掺入修饰的类似物和预测毒性的相对能力。核苷酸类似物通过 DNA 聚合酶 γ 掺入的预稳态动力学分析可基本上如先前所述进行 (参见 Murakami E,Ray AS,Schinazi RF,Anderson KS.Investigating the effects of stereochemistry on incorporation and removal of 5-fluorocytidine analogs by mitochondrial DNA polymerase gamma:comparison of D-and L-D4FC-TP. Antiviral Res.2004,62,57-64 ;Feng JY,Murakami E,Zorca SM,Johnson AA,Johnson KA,Schinazi RF,Furman PA,Anderson KS.Relationship between antiviral activity and host toxicity:comparison of the incorporation efficiencies of 2',3'-dideoxy-5-fluoro-3'-thiacytidine-triphosphate analogs by human immunodeficiency virus type lreverse transcriptase and human mitochondrial DNA polymerase. Antimicrob Agents Chemother.2004,48,1300-6)。简言之,聚合酶 γ 和 60nM DNA 模板 / 引物的大 (250nM) 和小 (1.25mM) 亚基在 50mM Tris-HCl、100mM NaCl,pH 7.8 中的预温育混合物可添加至含有 $MgCl_2$(2.5mM) 及各种浓度的核苷酸类似物的溶液中。可对反应进行淬火并且如先前所述来分析。数据可如上所述拟合至同一方程。

[0699] iii) 人聚合酶 γ 3'5' 核酸外切酶活性的测定 :人聚合酶 γ 核酸外切酶活性可通过在 dNTP 不存在下测量裂解产物的形成率来研究。反应可通过添加 $MgCl_2$(2.5mM) 至聚合酶 γ 大亚基 (40nM)、小亚基 (270nM) 及 1,500nM 链终止模板 / 引物在 50mM Tris-HCl、100mM NaCl,pH 7.8 的预温育混合物中开始,并且在指定的时点用 0.3M EDTA 淬火。所有反应混合物都可在 20% 变性聚丙烯酰胺测序凝胶 (8M 尿素) 上分析,在 Bio-Rad GS-525 分子图像系统上成像,并且用 Molecular Analyst(Bio-Rad) 定量。对由早期时点形成的产物以时间函数进行绘图。数据可通过线性回归与 Sigma Plot(Jandel Scientific) 拟合。可将线的斜率除以反应中的活性酶浓度以计算核酸外切酶活性的 kexo(参见 Murakami E,Ray AS,Schinazi RF,Anderson KS.Investigating the effects of stereochemistry on incorporation and removal of 5-fluorocytidine analogs by mitochondrial DNA polymerase gamma:comparison of D-and L-D4FC-TP.Antiviral Res.2004 ;62:57-64 ;Feng JY,Murakami E,Zorca SM,Johnson AA,Johnson KA,Schinazi RF,Furman PA,Anderson KS.Relationship between antiviral activity and host toxicity:comparison of the incorporation efficiencies of 2',3'-dideoxy-5-fluoro-3'-thiacytidine-triphosphate analogs by human immunodeficiency virus type 1 reverse transcriptase and human mitochondrial DNA polymerase.Antimicrob Agents Chemother.2004 ;48:1300-6)。

[0700] 实施例 18

[0701] 骨髓细胞毒性的测定

[0702] 初级人骨髓单核细胞可从 Cambrex Bioscience(Walkersville,MD) 商购获得。CFU-GM 测定可在 50 单位 /mL 人重组粒细胞 / 巨噬细胞集落刺激因子存在下使用双层软琼脂进行,而 BFU-E 测定使用含有 1 单位 /mL 促红细胞生成素的甲基纤维素基质 (参见 Sommadossi JP,Carlisle R.Toxicity of 3'-azido-3'-deoxythymidine and 9-(1,3-dih

ydrosy-2-propoxymethyl)guanine for normal human hepatopoietic progenitor cells in vitro.Antimicrob.Agents Chemother.1987 ;31:452-454 ;Sommadossi,JP,Schinazi, RF,Chu,CK,and Xie,MY.Comparison of Cytotoxicity of the(-)and(+)enantiomer of 2',3'-dideoxy-3'-thiacytidine in normal human bone marrow progenitor cells. Biochem.Pharmacol.1992 ;44:1921-1925)。每个实验都在来自三个不同供体的细胞中一式两份进行。AZT 可用作阳性对照。细胞可在化合物存在下在 37℃和 5％ CO_2下温育 14-18 天，并且大于 50 个细胞的集落可使用倒置显微镜计数以测定 IC_{50}。50％抑制浓度 (IC_{50}) 可通过药物浓度对 BFU-E 存活分数的对数的最小二乘回归分析来获得。统计分析可用 Student's t 检验针对独立的非成对样品进行。

[0703] 实施例 19

[0704] 抗 HBV 测定

[0705] 化合物的抗 HBV 活性可通过在四环素的控制下处理携带野生型 HBV 的 AD-38 细胞系来确定（参见 Ladner S.K.,Otto M.J.,Barker C.S.,Zaifert K.,Wang G.H.,Guo J.T.,Seeger C.&King R.W.Antimicrob.Agents Chemother.1997,41,1715-20)。从培养基 [Tet(-)] 除去四环素使得产生 HBV。HBV 在来自用化合物处理的细胞的培养上清液中的水平可与未处理的对照物相比较。用四环素 [Tet(+)] 的对照培养物也可以维持以确定 HBV 表达的基础水平。3TC 作为阳性对照包括在内。

[0706] 实施例 20

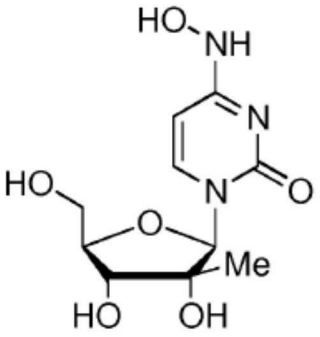
[0707] 细胞毒性测定

[0708] 化合物的毒性是在 Vero、人 PBM、CEM（人淋巴母细胞）中评定，并且可以如先前所述在 MT-2 和 HepG2 细胞中评定（参见 Schinazi R.F.,Sommadossi J.-P.,Saalmann V.,Cannon D.L.,Xie M.-Y.,Hart G.C.,Smith G.A.&Hahn E.F.Antimicrob.Agents Chemother.1990,34,1061-67)。放线菌酮作为阳性细胞毒性对照包括在内，并且暴露于溶剂的未处理的细胞作为阴性对照包括在内。细胞毒性 (IC50) 是使用先前所述的半数有效方法从浓度-反应曲线中获得（参见 Chou T.-C.&Talalay P.Adv.Enzyme Regul.1984,22,27-55 ;Belen'kii M.S.&Schinazi R.F.Antiviral Res.1994,25,1-11)。

[0709] 关于 Vero、人 PBM 及 CEM（人淋巴母细胞）细胞的数据显示于下表 5 中：

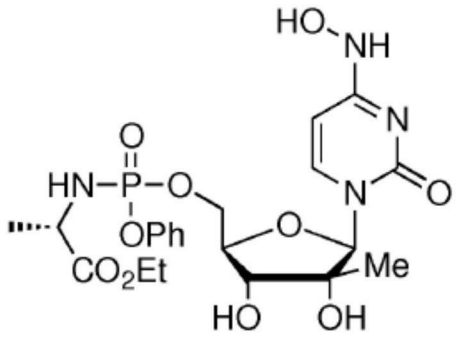
[0710] 表 5：所选化合物的 HCV EC_{50}、PBM IC_{50}、CEM IC_{50}、Vero IC_{50}及 Huh-7IC_{50}数据

[0711]



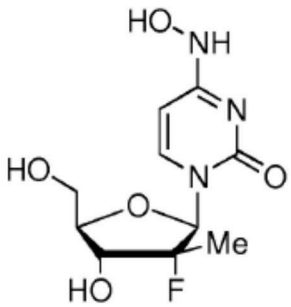
37

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



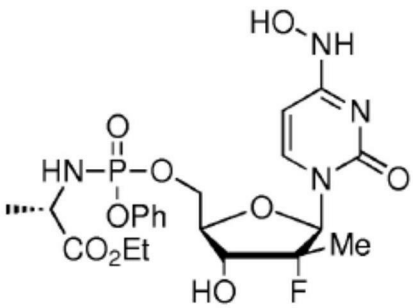
44

HCV EC_{50} = 0.8 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



39

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



51

HCV EC_{50} = 2.6 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >33 μM

[0712] 实施例 21

[0713] 抗药性病毒对核苷酸单磷酸酯前药的选择

[0714] 周围血液单核 (PBM) 细胞[1]可例如以 1×10^{7}个细胞的浓度接种于含有 100mL 热灭活的胎牛血清 (Hyclone,Logan,Utah)、83.3IU/mL 青霉素、83.3μg/mL 链霉素 (Mediatech Inc.,Herndon,VA)、1.6mM L- 谷氨酰胺 (Mediatech Inc.,Herndon,VA)、0.0008% DEAE- 右旋糖酐 (Sigma-Aldrich,St.Louis,MO)、0.047%碳酸氢钠及 26IU/mL 重组白介素 -2(Chiron Corporation,Emeryville,CA) 的总共 5mL 的 RPMI-1640(Mediatech Inc.,Herndon,VA) 中,用两个 T25 烧瓶,一个是对照 (未处理的) 且一个是用药物处理的。

[0715] 原生 PBM 细胞可在用在 $100\times TCID_{50}$下的 $HIV\text{-}1_{LAI}$[2]接种之前用在 0.1μM 下的核苷酸单磷酸酯前药处理 1 小时。处理过的 PBM 细胞群及对照未处理的 PBM 细胞群可允许感染例如 1 小时。另外的 5mLRTU 培养基可添加至每个烧瓶中并且可在 37℃下温育细胞例如

6 天。

[0716] 在第 6 天，来自每个烧瓶的 1mL 上清液可被移除并且在 4℃下以 9,740g 旋转 2 小时。所得的病毒沉淀然后可再悬浮于病毒增溶缓冲液中用于 RT 分析。总 RNA 可使用商用 QIAmp 病毒 RNA 迷你试剂盒 (Quiagen) 从培养中分离上清液。测序可在对照病毒与核苷酸单磷酸酯前药处理过的病毒之间并行进行以确定施加的药物压力是否在病毒似乎具有抗药性的数周内制造任何突变。

[0717] _____

[0718] [1]PBM 细胞可通过 ficoll-hypaque(Histopaque 1077:Sigma) 密度梯度离心从由 American Red Cross(Atlanta,GA) 处获得的血沉棕黄层中分离。血沉棕黄层可来源于健康的、血清阴性供体。细胞可在使用之前 2-3 天用在含有 100mL 热灭活胎牛血清 (Hyclone,Logan,Utah)、83.3IU/mL 青霉素、83.3μg/mL 链霉素、1.6mM L- 谷氨酰胺 (Mediatech Inc.,Herndon,VA) 的 500mL RPMI-1640(Mediatech Inc.,Herndon,VA) 中的 3μg/mL 植物凝集素 A(Sigma-Aldrich,St.Louis,MO) 来活化。

[0719] [2]HIV-1/LAI 可从 Center for Disease Control and Prevention 处获得并用作抗药性池的病毒并且如通过在 PBM 细胞中的限制稀释法所测定的 0.1 的感染复数 (MOI) 可被选出以便开始感染池。

[0720] 可以计算处理过的病毒池相对于未处理的病毒池的抑制百分比并且在处理之前每周密切监测。病毒池的选择压力可在长达 47 周或更长期间内从 0.1μM 增加到 3.5μM(40 倍的 EC_{50} 值)。

[0721] 实施例 22

[0722] 核苷类似物三磷酸酯的合成

[0723] 核苷类似物三磷酸酯是使用 Ludwig 和 Eckstein 的方法从适当保护的核苷来合成。(Ludwig J,Eckstein F."Rapid and efficient synthe sis of nucleoside 5'-O-(1-thiotriphosphates),5'-triphosphates and 2',3'-cyclophosphorothioates using 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one"J Org.Chem.1989,54631-5)。天然核苷类似物三磷酸酯可例如通过 FPLC 使用 HiLoad 26/10Q Sepharose Fast Flow Pharmacia 柱和 TEAB 缓冲液 (pH 7.0) 的梯度来纯化。产物将通过 UV 光谱学、质子和磷 NMR、质谱学及 HPLC 来表征。

[0724] 所得的三磷酸酯可用作如上所述的细胞药理学测定的对照并且用于 HIV-RT、HCV 聚合酶及其它病毒和人聚合酶的动力学研究。

[0725] 实施例 23

[0726] 针对 HSV-1 和 HSV-2 的活性的筛选测定

[0727] 在 CPE 抑制测定中，药物可在感染之前 1h 添加，这样测定系统将具有最高灵敏度并检测早期复制步骤如吸附或渗透以及稍后事件的抑制剂。为了排除结合至细胞的病毒的非特异性抑制，在 CPE 测定中显示合理活性的所有化合物将使用其中药物在感染之后 1h 添加的经典噬斑减少测定来确认。在化合物阻断连接的情况下，其在 CPE 测定中显示阳性，但可通过噬斑测定呈现阴性。功效 :最少将使用六个药物浓度，覆盖 100mg/ml 至 0.03mg/ml 的范围，以 5 倍的增量。从这些数据将计算出抑制 50％病毒复制的剂量 (有效浓度 50 ；EC_{50})。毒性 :用于测定功效的相同的药物浓度也可在测定每种实验化合物的毒性的每个测

定中针对未感染的细胞使用。如通过其不能消除极重要的毒株中性红(neutral red)所测定,药物浓度对细胞具有细胞毒性。

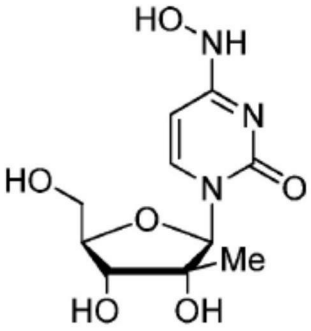
[0728] HSV-1药物敏感性测定也可如先前在以下文献中所述来进行:Schinazi,R.F.,Peters,J.,Williams,C.C.,Chance,D.,Nahmias,A.J."Effect of combinations of acyclovir with vidarabine or its 5'-monophosphate on herpes simplex virus in cell culture and in mice."Antimicrob.Agents Chemother.1982,22,499-507。

[0729] 实施例24

[0730] HCV复制子测定[1]

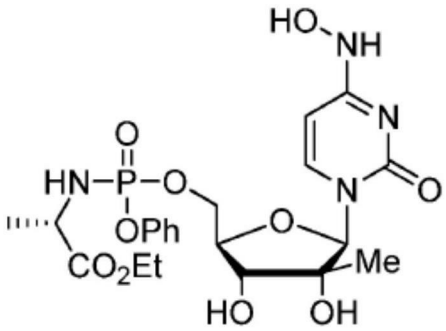
[0731] 将含有HCV复制子RNA的Huh 7Clone B细胞以5000个细胞/孔接种于96孔板中,并且在接种之后立即一式三份在10μM下测试化合物。五天温育(37℃,5% CO_2)之后,通过使用来自Gentra的versaGene RNA纯化试剂盒分离总细胞RNA。在单步多重实时RT-PCR测定中扩增复制子RNA和内部对照(TaqMan rRNA对照试剂,Applied Biosystems)。化合物的抗病毒有效性通过将无药物的对照(ΔCt HCV)的阈值RT PCR周期减去测试化合物的阈值RT-PCR周期来计算。3.3的ΔCt等于复制子RNA水平的1-log减少(等于90%较少的起始材料)。化合物的细胞毒性还通过使用ΔCt rRNA值来计算。(2'-Me-C)被用作对照。为了测定EC_{90}和IC_{50}值[2],ΔCt:值首先被转换为起始材料[3]的分率,然后用于计算抑制%。

[0732]



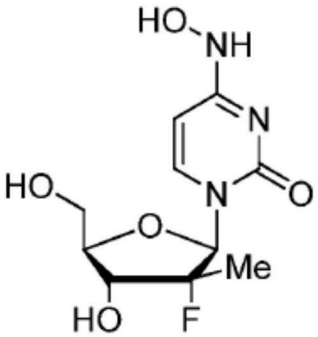
37

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



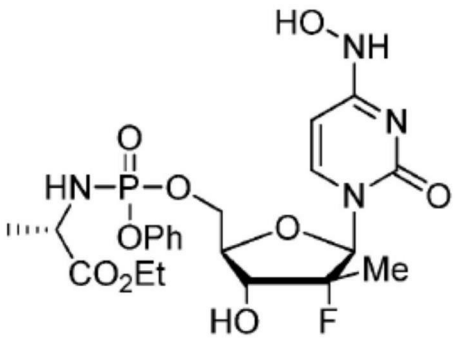
44

HCV EC_{50} = 0.8 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



39

HCV EC_{50} = > 10 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >10 μM



51

HCV EC_{50} = 2.6 μM
PBM IC_{50} = >100 μM
CEM IC_{50} = >100 μM
Vero IC_{50} = >100 μM
Huh-7 IC_{50} = >33 μM

[0733] 参考文献：

[0734] 1.Stuyver L et al.,Ribonucleoside analogue that blocks replication or bovine viral diarrhea and hepatitis C viruses in culture.Antimicrob.Agents Chemother.2003,47,244-254.

[0735] 2.Reed IJ&Muench H,A simple method or estimating fifty percent endpoints.Am.J.Hyg.27:497,1938.

[0736] 3.Applied Biosystems Handbook

[0737] 实施例 25

[0738] 西尼罗病毒药物敏感性也可以如先前在以下文献中所述来测定:Song,G.Y.,Paul,V.,Choo,H.,Morrey,J.,Sidwell,R.W.,Schinazi,R.F.,Chu,C.K.Enantiomeric synthesis of D-and L-cyclopentenyl nucleosides and their antiviral activity against HIV and West Nile virus.J.Med.Chem.2001,44,3985-3993。

[0739] 实施例 26

[0740] 黄热病药物敏感性也可如先前在以下文献中所述来测定 :Julander,J.G.,Furuta,Y.,Shafer,K.,Sidwell,R.W.Activity of T-1106 in a Hamster Model of Yellow Fever Virus Infection.Antimicob.Agents Chemother.2007,51,1962-1966。

[0741] 实施例 27

[0742] 人及登革热病毒聚合酶测定可例如通过 Replizyme Ltd 来进行。简言之,每个酶 / 化合物组合可在 0.8mM 至 100mM 浓度范围内一式两份来测试。化合物可与对照 (无抑制剂)、溶剂稀释物 (例如 0.016％至 2％ DMSO) 及有关的 Replizyme 参考抑制剂平行进行。

[0743] 用于鉴定具有针对登革热的活性的化合物的代表性高通量测定在 Lim 等人 ,A scintillation proximity assay for dengue virus NS5 2'-O-methyltransferase-kinetic and inhibition analyses,Antiviral Research, 第 80 卷 , 第 3 期 ,2008 年 12 月 , 第 360-369 页中公开。

[0744] 登革热病毒 (DENV)NS5 在其 N 末端氨基酸序列处具有甲基转移酶 (MTase) 活性并且使得在病毒基因组 RNA 中形成 1 型帽结构 m7GpppAm2'-O。对于 DENV22'-O-MTase 活性的最佳体外条件可使用纯化重组蛋白及短的生物素化 GTP- 带帽 RNA 模板来表征。衍生自初始速度的稳态动力学参数可用于确立针对化合物测试的稳固的闪烁亲近测定。通过 Lim 等人 ,Antiviral Research, 第 80 卷 , 第 3 期 ,2008 年 12 月 , 第 360-369 页的预温育研究显示 MTase-AdoMet 和 MTase-RNA 复合物具有同样的催化能力并且所述酶支持随机的 bi bi 动力学机制。Lim 验证用竞争性抑制剂 S- 腺苷 - 同型半胱氨酸及两个同系物西萘芬净和脱氢西萘芬净的测定。存在于 DENV2MTase 的 N 末端处的 GTP- 结合袋先前被假定为帽结合位点。此测定允许快速且高灵敏度的检测 2'-O-MTase 活性并且可容易地适于抑制性化合物的高通量筛选。其适用于测定广泛多种 RNA 带帽 MTase 的酶促活性。

[0745] 此测定可用于就抗登革热活性对本文所述的化合物进行筛选。

[0746] 实施例 28

[0747] 抗诺瓦克病毒活性

[0748] 化合物可通过抑制诺瓦克病毒聚合酶和 / 或解旋酶,通过抑制在复制周期中所需的其它酶或通过其它途径展现抗诺瓦克病毒活性。

[0749] 目前没有被批准用于诺瓦克病毒感染的药物治疗,并且这大概至少部分是由于缺乏细胞培养系统的可用性。近来,已经开发用于原始的诺瓦克 G-I 毒株的复制子系统 (Chang,K.O., 等人 (2006)Virology 353:463-473)。

[0750] 诺瓦克病毒复制子和丙型肝炎复制子都需要病毒解旋酶、蛋白酶和聚合酶的作用以便进行复制子的复制。最近,已经报道利用诺瓦克病毒基因组 I 和 II 接种体的体外细胞培养传染性测定 (Straub,T.M. 等人 (2007)Emerg.Infect.Dis.13(3):396-403)。此测定在利用在微载体珠上的小肠上皮细胞的旋转壁式生物反应器中进行。传染性测定可用于筛选进入抑制剂。

[0751] 实施例 29

[0752] 在 HepG2 细胞中核苷至活性三磷酸酯的磷酸化测定

[0753] 为了测定化合物的细胞代谢,HepG2 细胞可从美国典型培养物保藏中心 (Rockville,MD) 处获得,并且在 225cm^2组织培养烧瓶中在补充有非必需氨基酸、1％青霉

素 - 链霉素的最低必需培养基中生长。培养基可每三天更新一次，并且细胞可每隔一周传代培养。在以暴露于 30mL 胰蛋白酶 -EDTA 10 分钟分离贴壁单层且用培养基连续洗涤三次之后，汇合的 HepG2 细胞可以 2.5×10^6 个细胞 / 孔的密度接种于 6 孔板中并且暴露于 10 μ M [^{3}H] 标记的活性化合物 (500dpm/pmol) 达规定的时间段。

[0754] 细胞被维持在 37℃和 5％ CO_2 气氛下。在选定的时间点，将细胞用冰冷的磷酸盐缓冲盐水 (PBS) 洗涤三次。

[0755] 细胞内活性化合物及其各自的代谢物是通过在 -20℃下用 60％甲醇温育细胞沉淀过夜来提取。然后将提取物合并，在轻柔过滤的空气流下干燥并储存于 -20℃直到 HPLC 分析。

[0756] 实施例 30

[0757] 在猕猴中的生物利用率测定

[0758] 以下工序可用于确定化合物是否是生物学可利用的。在研究开始之前 1 周内，猕猴可用留置静脉导管和皮下静脉入口 (VAP) 通过手术植入以便于血液收集并且可经历体格检查，包括血液学和血清化学评价及体重记录。每个猴子 (总计六只) 经由静脉内快速浓注 (3 只猴子，IV) 或经由口服管饲法 (3 只猴子，PO) 接受大约 250 μ Ci 的 ^{3}H 活性，每个剂量的活性化合物在 10mg/kg 的剂量水平下在 5mg/mL 的剂量浓度下。在给药之前为每个给药注射器称重以通过重量分析法测定所施用制剂的量。经由在指定的间隔时间下 (给药前大约 18-0 小时、给药后 0-4、4-8 及 8-12 小时) 经由盘收集器收集尿样品并且处理。同样经由留置静脉导管和 VAP 或 (如果留置静脉导管工序不可能的话) 从外周血管收集血液样品 (给药前、给药后 0.25、0.5、1、2、3、6、8、12 及 24 小时)。分析血液和尿样品的最大浓度 (Cmax)、达到最大浓度时的时间 (TmaX)、曲线下面积 (AUC)、剂量浓度的半衰期 (TV)、清除率 (CL)、稳态容量和分布 (Vss) 以及生物利用率 (F)。

[0759] 实施例 31

[0760] 细胞保护测定 (CPA)

[0761] 所述测定可基本上如以下文献所述来进行 :Baginski,S.G. ;Pevear,D.C ;Seipel,M. ;Sun,S.C.C ;Benetatos,C.A. ;Chunduru,S.K. ;Rice,C.M. 及 M.S.Collett"Mechanism of action of a pestivirus antiviral compound"PNAS USA 2000,97(14),7981-7986。在使用之前 24 小时将 MDBK 细胞 (ATCC) 接种于 96 孔培养板上 (每孔 4,000 个细胞)。在用 BVDV(毒株 NADL，ATCC) 在每细胞 0.02 噬斑形成单位 (PFU) 的感染复数 (MOI) 下感染之后，测试化合物的连续稀释液以在生长培养基中 0.5％ DMSO 的最终浓度被添加至感染的和未感染的细胞中。每个稀释液一式四份地测试。细胞密度和病毒接种体被调节以确保整个实验中细胞的连续生长并在感染后 4 天之后实现在未处理的对照中超过 90％病毒诱导的细胞破坏。4 天之后，将板用 50％ TCA 固定并用磺酰罗丹明 B 染色。在 550nm 下在微板读取器中读取孔的光密度。

[0762] 50％有效浓度 (EC_{50}) 值被定义为化合物实现病毒的细胞病变效应减少 50％的浓度。

[0763] 实施例 32

[0764] 噬斑减少测定

[0765] 对于给定的化合物，有效浓度可通过噬斑减少测定在 24 孔板中一式两份来测定。

细胞单层用 100PFU/ 孔的病毒感染。然后，将测试化合物在补充有 2％灭活血清和 0.75％甲基纤维素的 MEM 中的连续稀释液添加至单层中。将培养物在 37℃下进一步温育 3 天，然后用 50％乙醇和 0.8％结晶紫固定，洗涤并风干。然后对噬斑进行计数以测定获得 90％病毒抑制的浓度。

[0766] 实施例 33

[0767] 产量减少测定

[0768] 对于给定的化合物，获得病毒载量的 6-log 减少的浓度可以通过产量减少测定在 24 孔板中一式两份进行测定。所述测定如以下文献所述来进行 :Baginski,S.G. ;Pevear,D.C ;Seipel,M. ;Sun,S.C.C ;Benetatos,C.A. ;Chunduru,S.K. ;Rice,C.M. 及 M.S.Collett "Mechanism of action of a pestivirus antiviral compound"PNAS USA 2000,97(14),7981-7986，其中有细微修改。

[0769] 简言之，在用 BVDV(NADL 毒株）以 0.1PFU 每细胞的感染复数 (MOI) 感染之前 24 小时将 MDBK 细胞接种于 24 孔板 (2×10^5个细胞 / 孔）上。将测试化合物的连续稀释液以在生长培养基中 0.5％ DMSO 的最终浓度添加至细胞中。每份稀释液一式三份测试。三天之后，将细胞培养物（细胞单层和上清液）通过三个冷冻 - 融化循环溶解，并且病毒产量通过噬斑测定来定量。简言之，在使用之前 24h 将 MDBK 细胞接种于 6 孔板 (5×10^5个细胞 / 孔）上。将细胞用 0.2mL 测试裂解物接种 1 小时，洗涤并且用在生长培养基中的 0.5％琼脂糖覆盖。3 天之后，将细胞单层用 3.5％甲醛固定并且用 1％结晶紫（在 50％乙醇中的 w/v）染色以目测检验噬斑。对噬斑进行计数以测定获得病毒载量的 6-log 减少的浓度。

[0770] 实施例 34

[0771] 诺瓦克病毒感染的诊断

[0772] 可以通过使用逆转录聚合酶链反应 (RT-PCR) 测定检测受影响的人的粪便中的病毒 RNA 来诊断诺瓦克病毒感染。病毒可从在症状开始之后 48 至 72 时间内采集的粪便样本中鉴定，但也可使用 RT-PCR 对在症状开始之后长达 7 天时采集的样品获得令人满意的结果。其它诊断方法包括电子显微镜和血清学测定 :至少三周时收集的双份血清中的滴度有升高。还可利用酶联免疫测定法，但它们倾向于具有相对较低的灵敏度，这限制了其对爆发的病因学诊断的使用。经常使用诺瓦克病毒感染的临床诊断，特别是当肠胃炎的其它病原体已被排除时。

[0773] 实施例 35

[0774] 体外抗病毒活性

[0775] 在下列细胞系中评估体外抗病毒活性 :

[0776] 诺瓦克 G-I 毒株 (Chang,K.O., 等人 (2006)Virology 353:463-473)、GIT4 毒株复制子以及其它诺瓦克病毒复制子可用于测定本文所述的化合物或其它化合物或化合物文库的体外抗病毒活性的测定中。在一些实施方案中，复制子系统是亚基因组，且因此允许评价非结构蛋白质的小分子抑制剂。这可向诺瓦克病毒药物发现提供相同益处，丙型肝炎复制子有助于发现适用于治疗病毒的治疗剂 (Stuyver,L.J., 等人 (2006)Antimicrob. Agents Chemother.47:244-254)。诺瓦克病毒复制子和丙型肝炎复制子都需要病毒解旋酶、蛋白酶和聚合酶的作用以便进行复制子的复制。据信本文所述的化合物抑制病毒聚合酶和 / 或病毒解旋酶。

[0777] 使用诺瓦克病毒基因组 I 和 II 接种体所报道的体外细胞培养传染性测定 (Straub,T.M. 等人 (2007)Emerg.Infect.Dis.13(3):396-403) 也可以使用。此测定可在利用在微载体珠上的小肠上皮细胞的旋转壁式生物反应器中进行。感染性测定可用于就其抑制所需病毒的能力对化合物进行筛选。

[0778] 本申请中所鉴定的每一参考文献出于所有目的以引用的方式整体并入本文中。

[0779] 虽然出于说明的目的,上述说明书通过实施例教导了本发明的原理,但应当理解本发明的实施包括落入以下权利要求及其等价物的范围内的所有常用的变化、改变和 / 或修改。

Abstract

The present invention is directed to compounds, compositions and methods for treating or preventing cancer and viral infections, in particular, HIV, HCV, Norovirus, Saporovirus, cytomegalovirus (CMV), herpes viruses (HSV-1, HSV-2), Dengue virus, Yellow fever, or HBV in human patients or other animal hosts. The compounds are certain A^-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs, and pharmaceutically acceptable, salts, prodrugs, and other derivatives thereof. In particular, the compounds show potent antiviral activity against HIV-1, HIV-2, HCV, Norovirus, Saporovirus, cytomegalovirus (CMV), herpes viruses (HSV-1, HSV-2), Dengue virus, Yellow fever, and HBV.