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(54) Titre : NUCLEOTIDES PYRIMIDINES ET LEURS PROMEDICAMENTS MONOPHOSPHATES POUR LE
TRAITEMENT D'INFECTIONS VIRALES ET DU CANCER

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

(57) **Abrégé/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 A¹-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.

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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.



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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 Sapovirus; 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 relevant nucleotide prodrugs and ultimately 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 (k_{pol}), 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, hepsara (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 protooncogenes) 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 oncogenes 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 an 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.

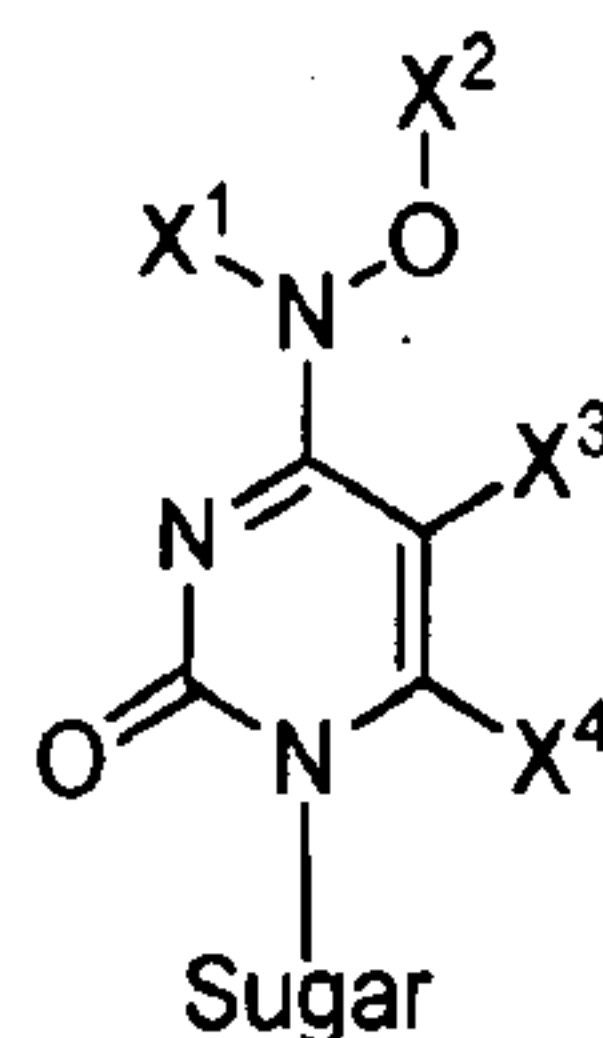
As with Hepatitis C replicons, Norovirus replicons require viral helicase, protease, and polymerase to be functional in order for replication of the replicon to occur. The replicons can be used in high throughput assays, which evaluate whether a compound to be screened for activity inhibits the ability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

REPLACEMENT SHEET



(I)

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

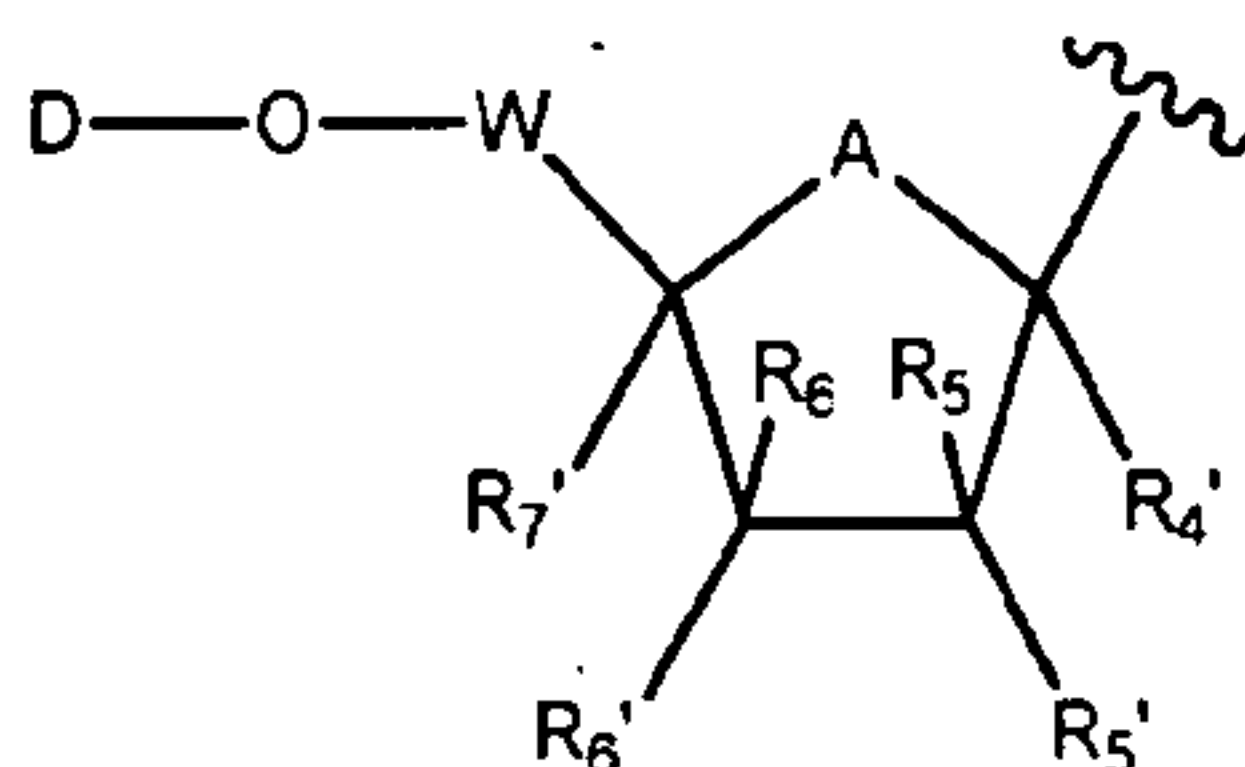
- i) X¹ is H, C₁-C₆alkyl, C₁-C₆haloalkyl, C₁-C₆alkoxy, C₂-C₆ alkenyl, C₂-C₆ alkynyl, COR¹, or COOR¹;
- ii) X² is hydrogen, CH₂-O(CO)-X⁵; CH₂-O(CO)O-X⁵ COR¹, or COOR¹

wherein each R¹ is, independently, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₆ alkyl, or C₁₋₆ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, or C₃₋₁₀ cycloalkyl

X⁵ is independently, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, alkoxy, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₆ alkyl, or C₁₋₆ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, or C₃₋₁₀ cycloalkyl

- iii) Each X³ and X⁴ is independently H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aryl, alkylaryl, halogen (F, Cl, Br, I), NH₂, OH, SH, CN, or NO₂.

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



(II)

wherein:

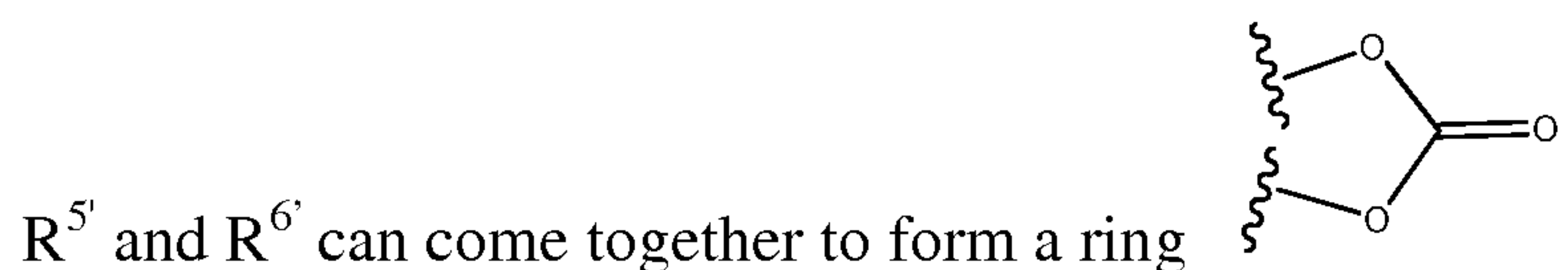
D is H, C(O)R¹, C(O)OR¹, diphosphate ester, or triphosphate ester;

R¹ is as defined above;

W is CL₂ or CL₂CL₂, wherein L independently is selected from the group consisting of H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH₂, CHF, CF₂, C=CH₂, C=CHF, or C=CF₂;

R^{4'}, R⁵, R^{5'}, R⁶, R^{6'}, and R^{7'} are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH₂, NHOH, NHNH₂, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;



In one embodiment, where Sugar is formula (II), when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H then, R⁵, R^{5'}, R⁶, R^{6'} cannot be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

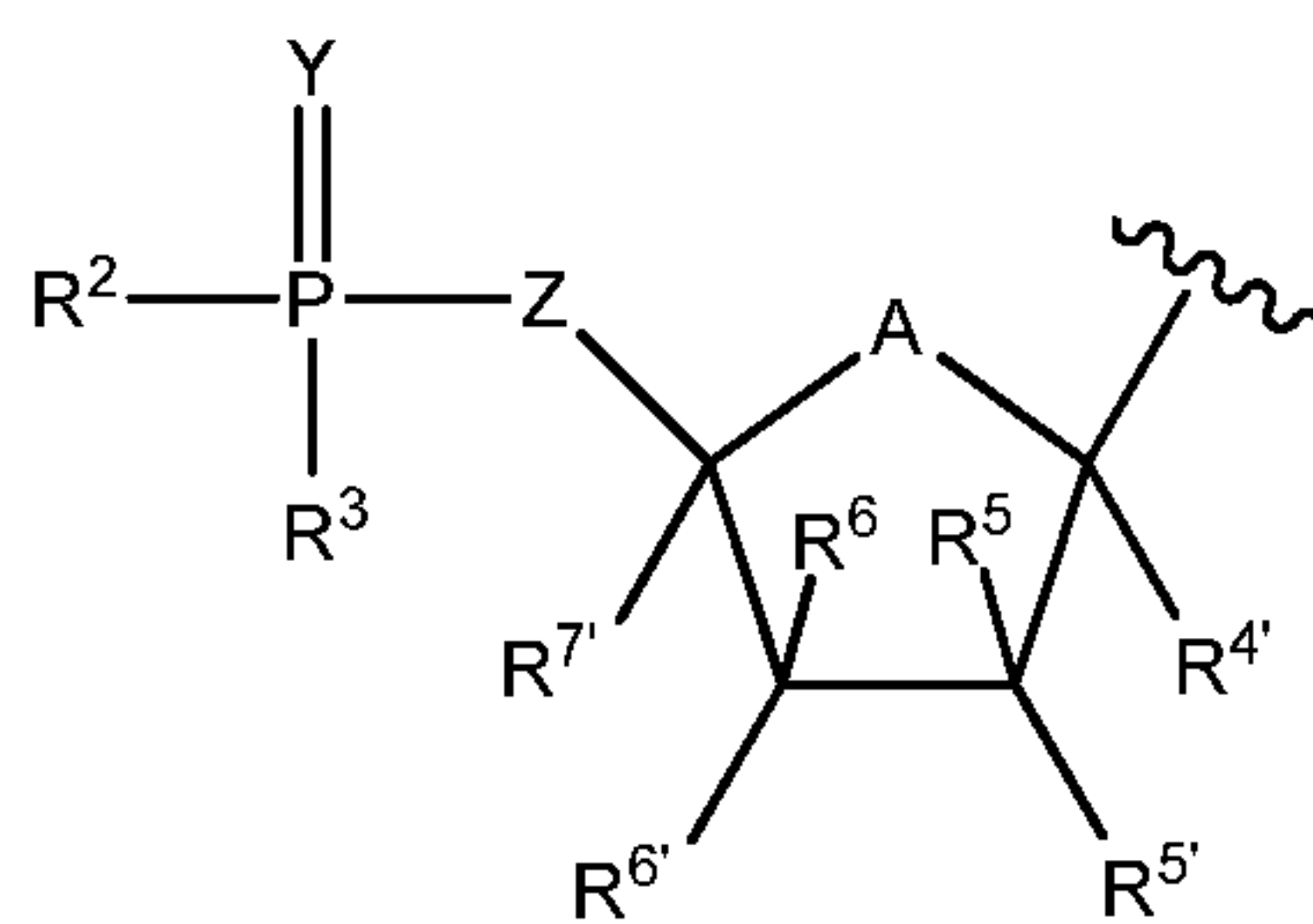
In another embodiment, R^{6'} is independently selected from the group consisting of NHOH, NHNH₂, N₃, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

In one embodiment, wherein for formula (I) where sugar is formula (II), when A is O or S, R^{7'} cannot be OH, SH, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, or NR₂.

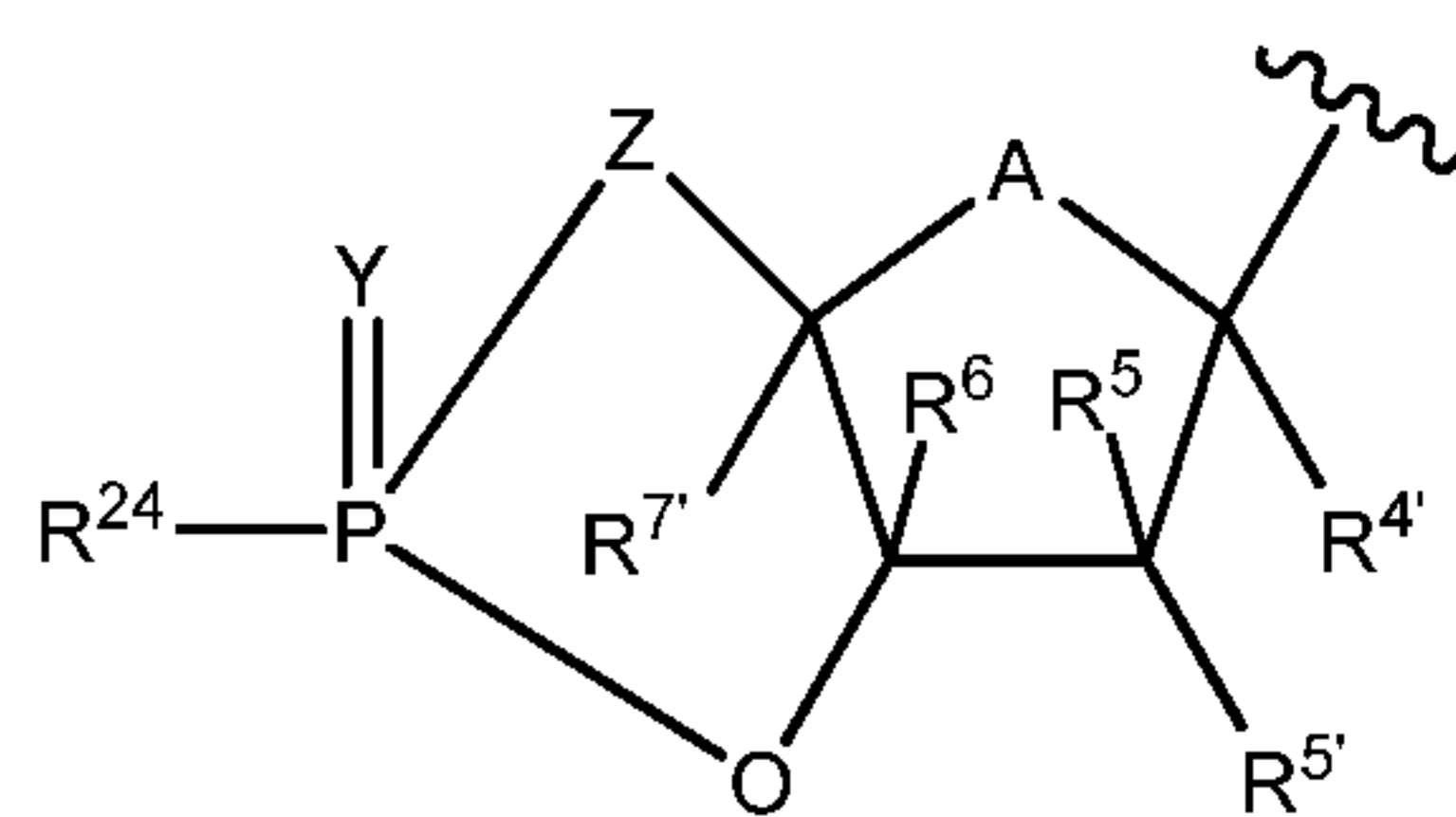
In another embodiment, R^{7'} is, independently, selected from the group consisting of H, F, Cl, Br, I, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, and R;

R is independently C₁-C₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₆ cycloalkyl, (C₃-C₆ 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:

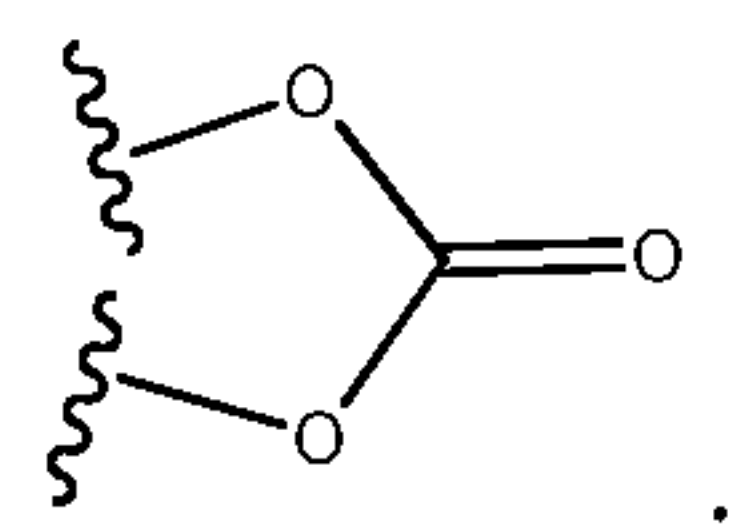
Y is O or S;

Z is selected from the group consisting of CL₂, CL₂CL₂, CL₂OCL₂, CL₂SCL₂, CL₂O, OCL₂ and CL₂NHCL₂, wherein L independently is selected from the group consisting of H, F, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH₂, CHF, CF₂, C=CH₂, C=CHF, or C=CF₂;

R^{4'}, R⁵, R^{5'}, R⁶, R^{6'}, and R^{7'} are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH₂, NHOH, NHNH₂, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

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

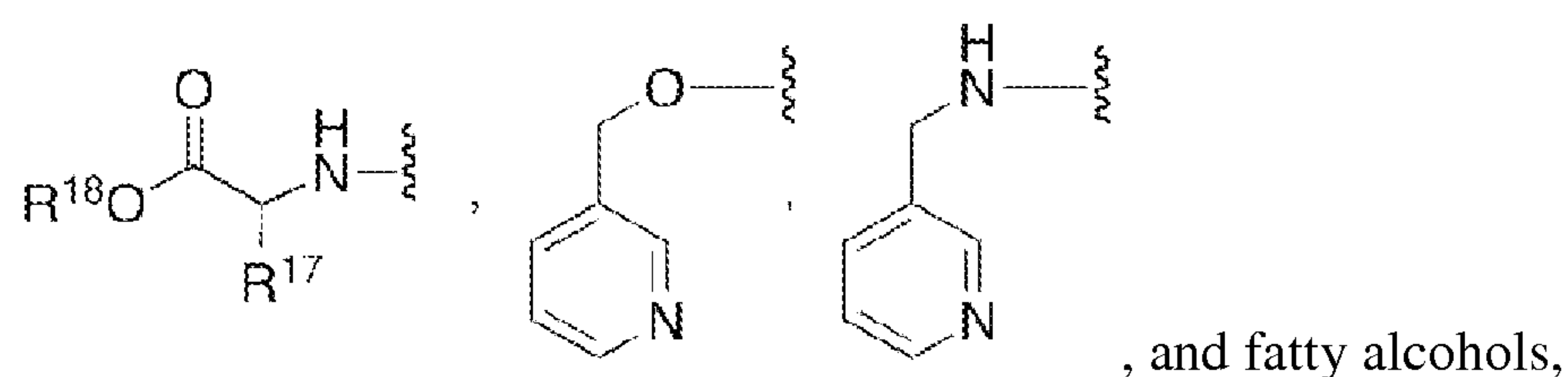


In one embodiment, where Sugar is formula (III) or (IV), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2 , NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2 .

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} ,



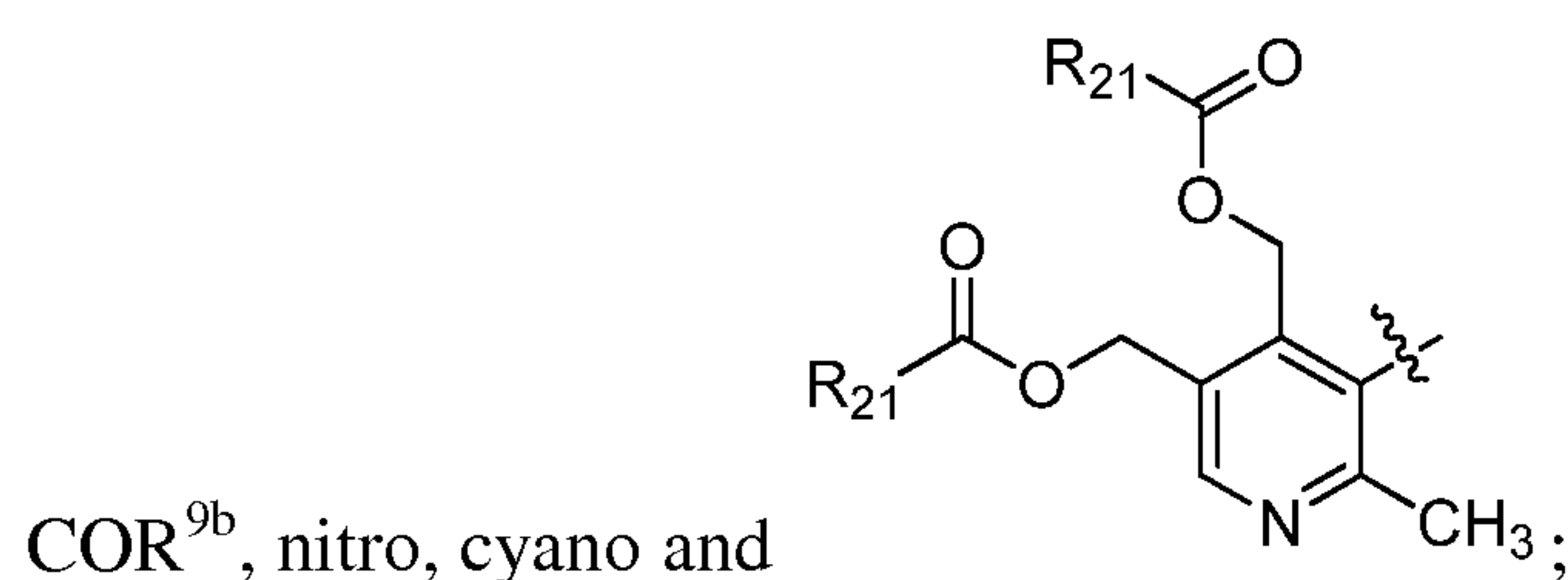
R^{15} is selected from the group consisting of H, Li, Na, K, phenyl and pyridinyl; wherein phenyl and pyridinyl are optionally substituted with zero to three substituents independently selected from the group consisting of $(CH_2)_{0-6}CO_2R^{16}$ and $(CH_2)_{0-6}CON(R^{16})_2$;

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:

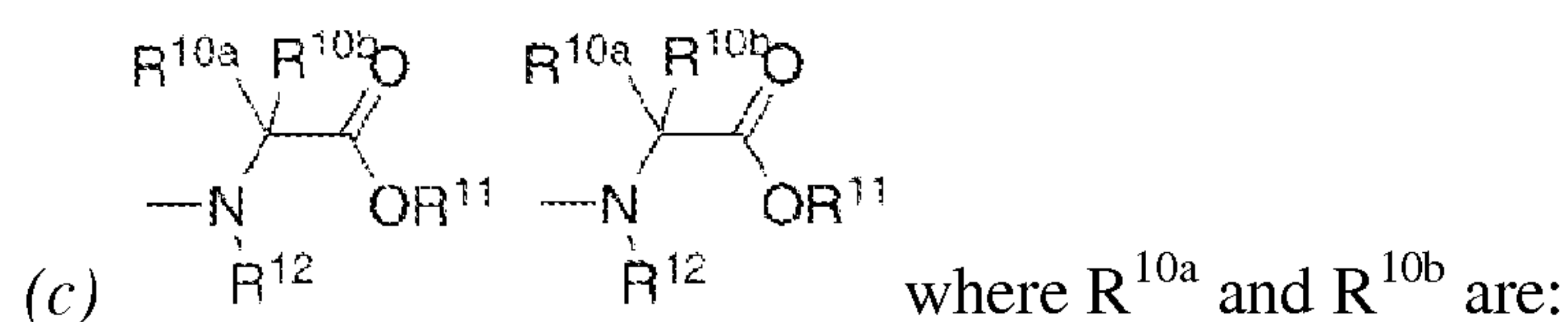
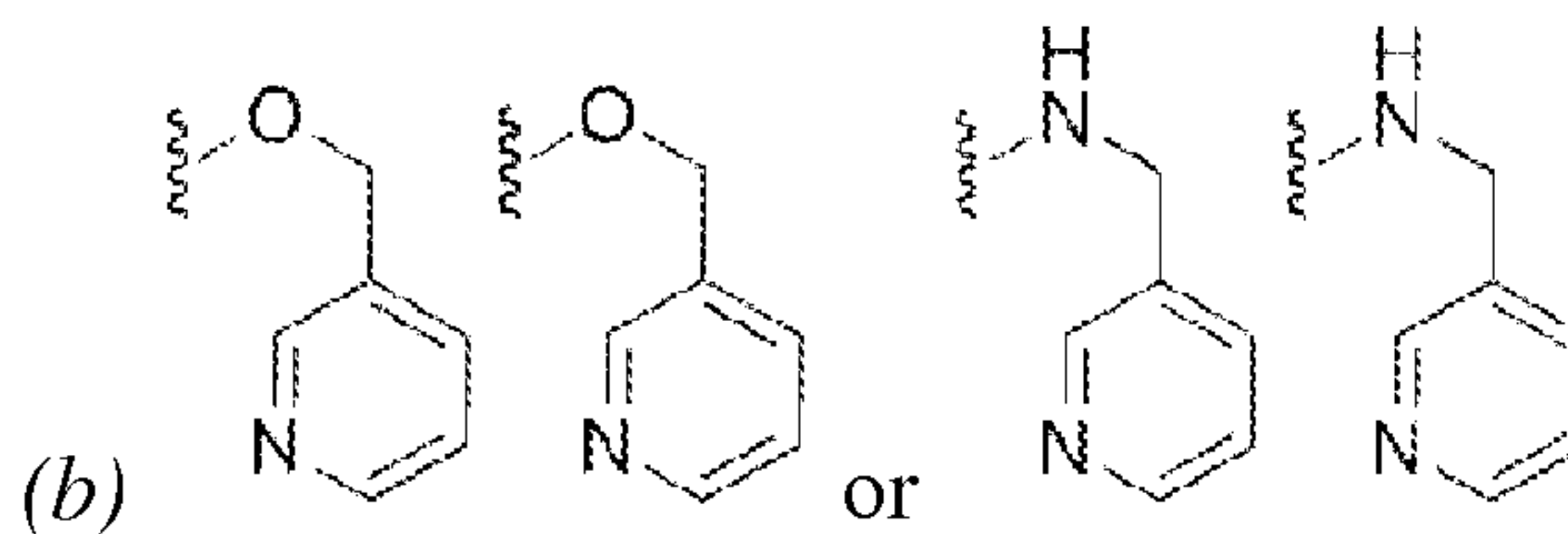
(a) OR^8 where R^8 is H, Li, Na, K, C_{1-20} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, aryl, or heteroaryl which includes, but is not limited to, phenyl or naphthyl optionally substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, 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,



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 $-N(R^{9a})_2$;



(i) independently selected from the group consisting of H, C₁₋₁₀ alkyl, -(CH₂)_rNR^{9a}₂, C₁₋₆ hydroxyalkyl, -CH₂SH, -(CH₂)₂S(O)_pMe, -(CH₂)₃NHC(=NH)NH₂, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -(CH₂)_mCOR^{9b}, aryl and aryl-C₁₋₃ alkyl, said aryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, halogen, nitro, and cyano;

(ii) R^{10a} is H and R^{10b} and R¹² together are (CH₂)₂₋₄ to form a ring that includes the adjoining N and C atoms;

(iii) R^{10a} and R^{10b} together are (CH₂)_n to form a ring;

(iv) R^{10a} and R^{10b} both are C₁₋₆ alkyl; or

(v) R^{10a} is H and R^{10b} is H, CH₃, CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or C₃₋₁₀ cycloalkyl;

p is 0 to 2;

r is 1 to 6;

n is 4 or 5;

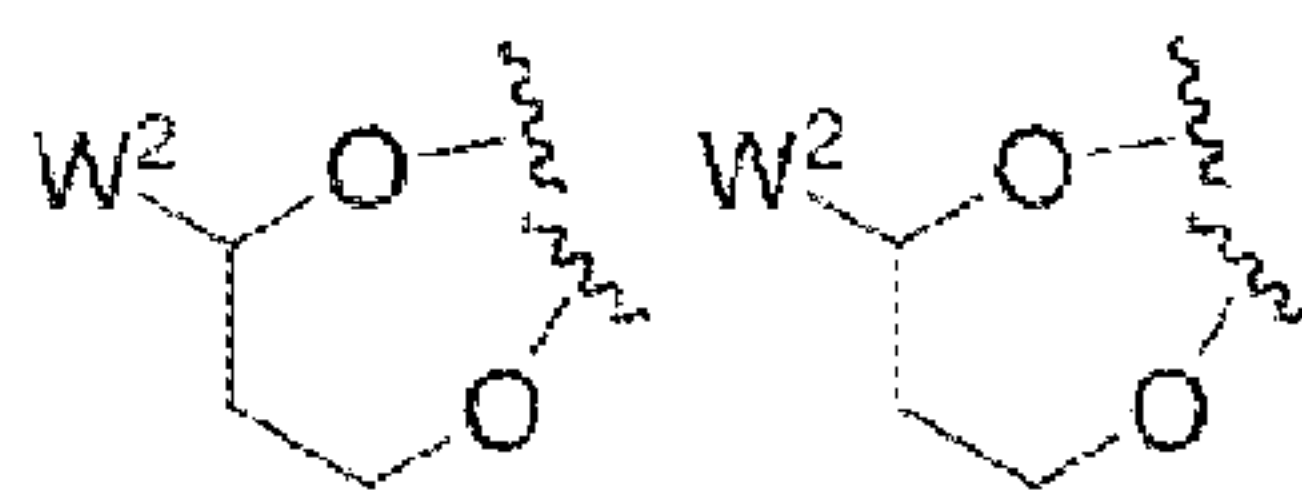
m is 0 to 3;

R¹¹ is H, C₁₋₁₀ alkyl, or C₁₋₁₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ 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:

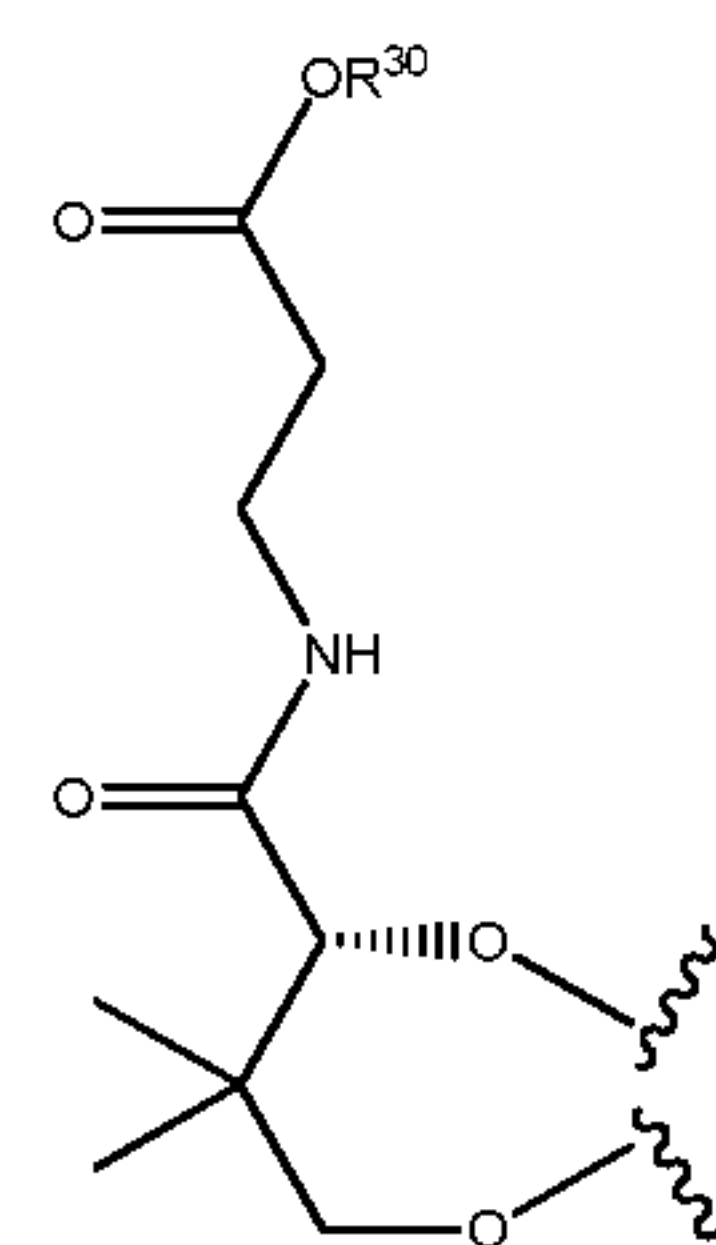
a) when there are two heteroatoms and one is O, then the other can not be O or S, and

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;

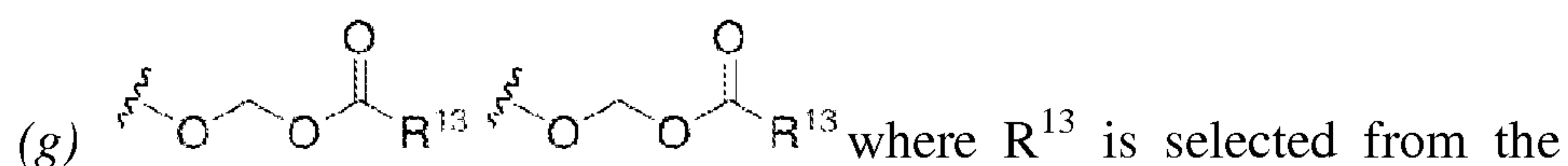
R^{9b} is $-OR^{9a}$ or $-N(R^{9a})_2$;

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



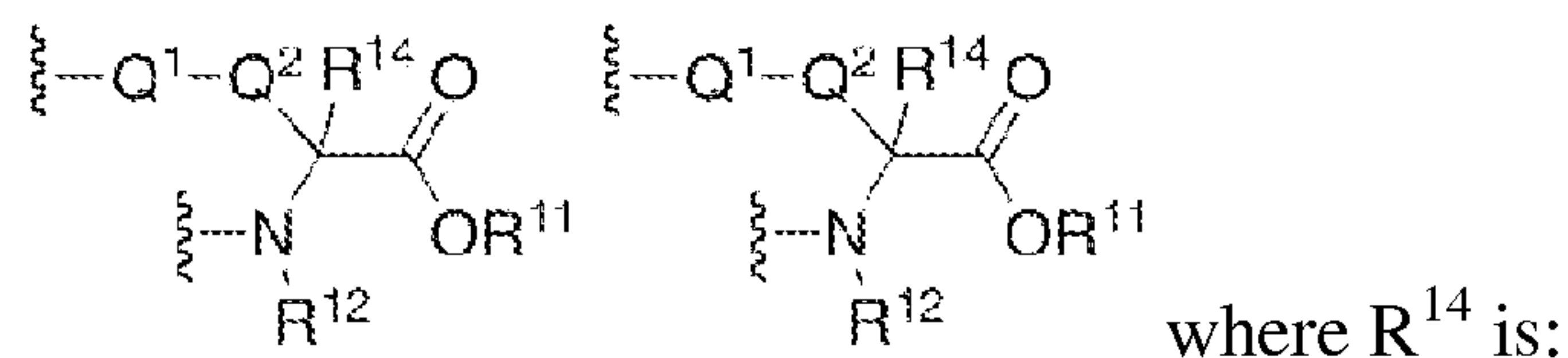
(f) R^2 and R^3 can come together to form a ring
 R^{30} is H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty

alcohol or C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;



group consisting of H, C₁₋₁₀ alkyl, C₁₋₁₀ alkyl optionally substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, and substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

(h) R² and R³ can come together to form a ring



(i) independently selected from the group consisting of H, C₁₋₁₀ alkyl, -(CH₂)_rNR₂^{9a}, C₁₋₆ hydroxyalkyl, -CH₂SH, -(CH₂)₂S(O)_pMe, -(CH₂)₃NHC(=NH)NH₂, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -(CH₂)_mCOR^{9b}, aryl, aryl-C₁₋₃ alkyl, heteroaryl and heteroaryl-C₁₋₃ alkyl, said aryl and heteroaryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, halogen, nitro, and cyano;

(ii) R¹⁴ is H, CH₃, CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or C₃₋₁₀ 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;

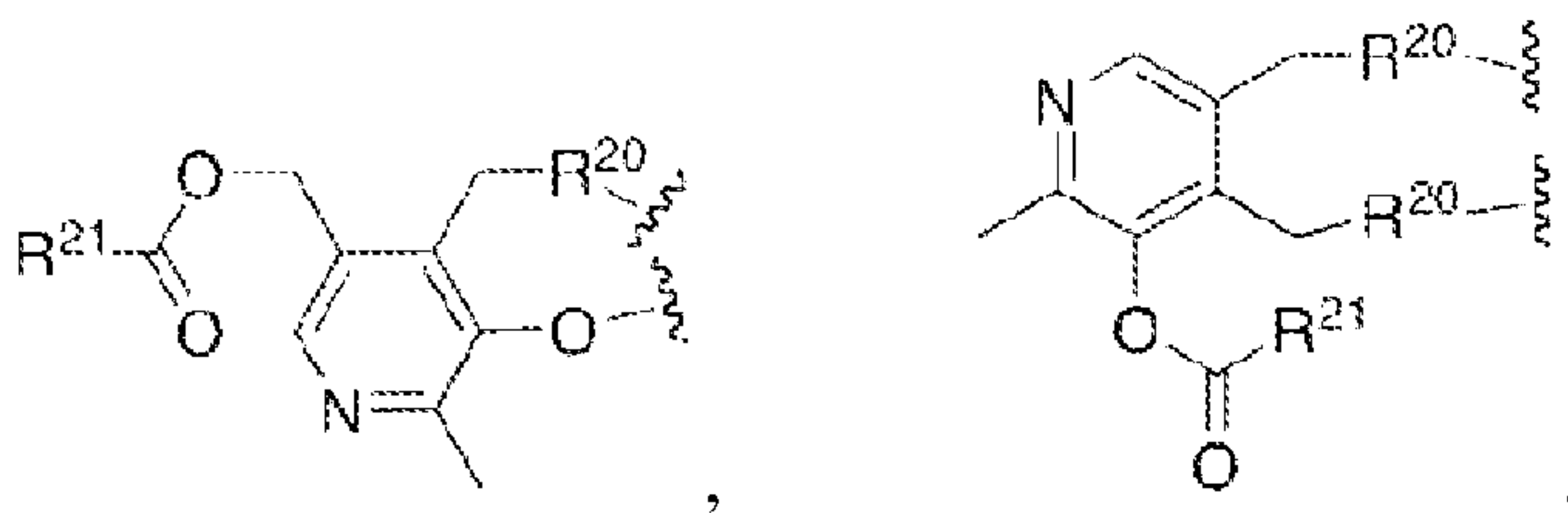
R^{11} is H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl;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

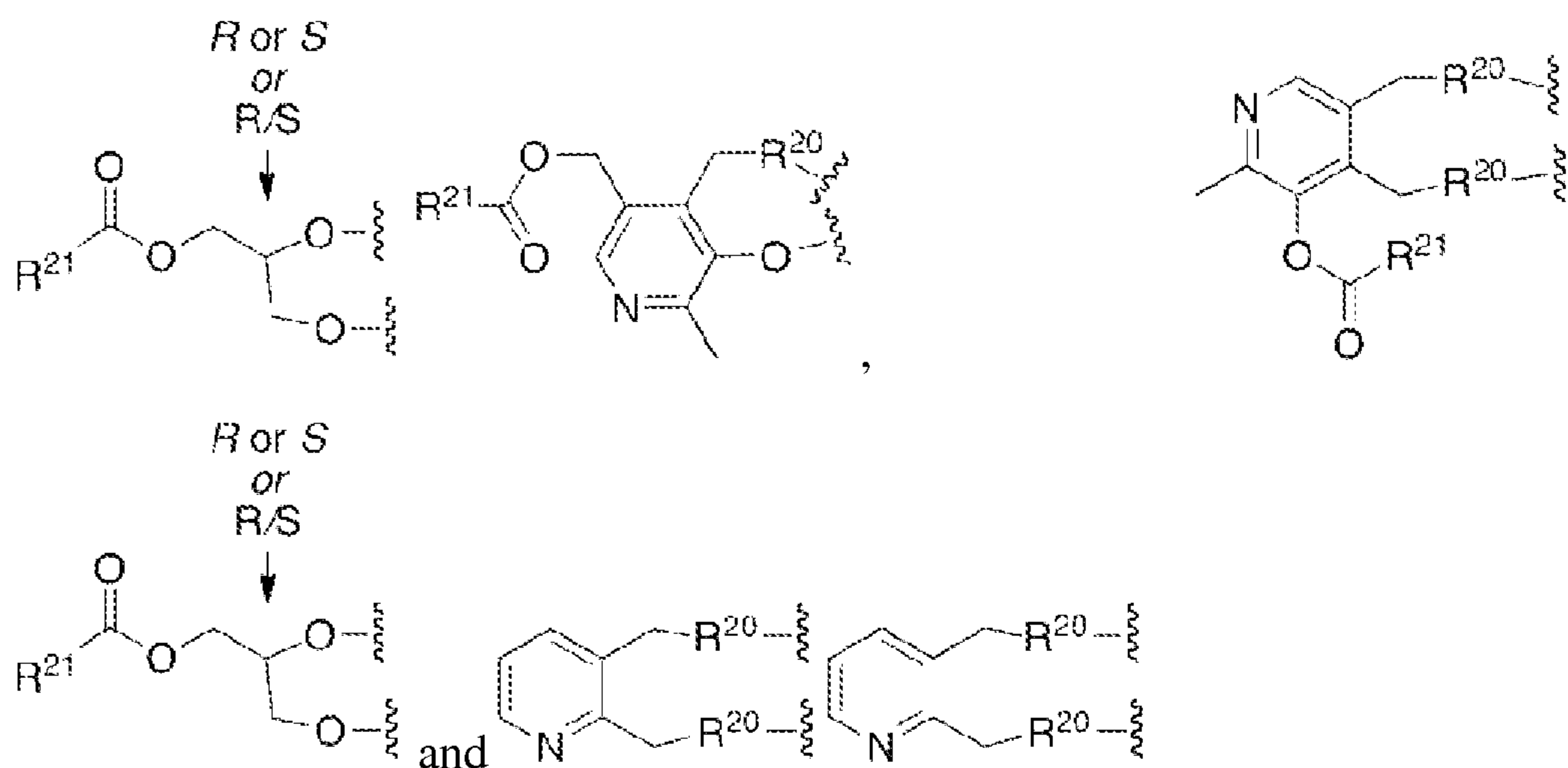
R^{12} is H or C_{1-3} alkyl, or R^{14b} and R^{12} together are $(CH_2)_{2-4}$ so as to form a ring that includes the adjoining N and C atoms;

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

consisting

of



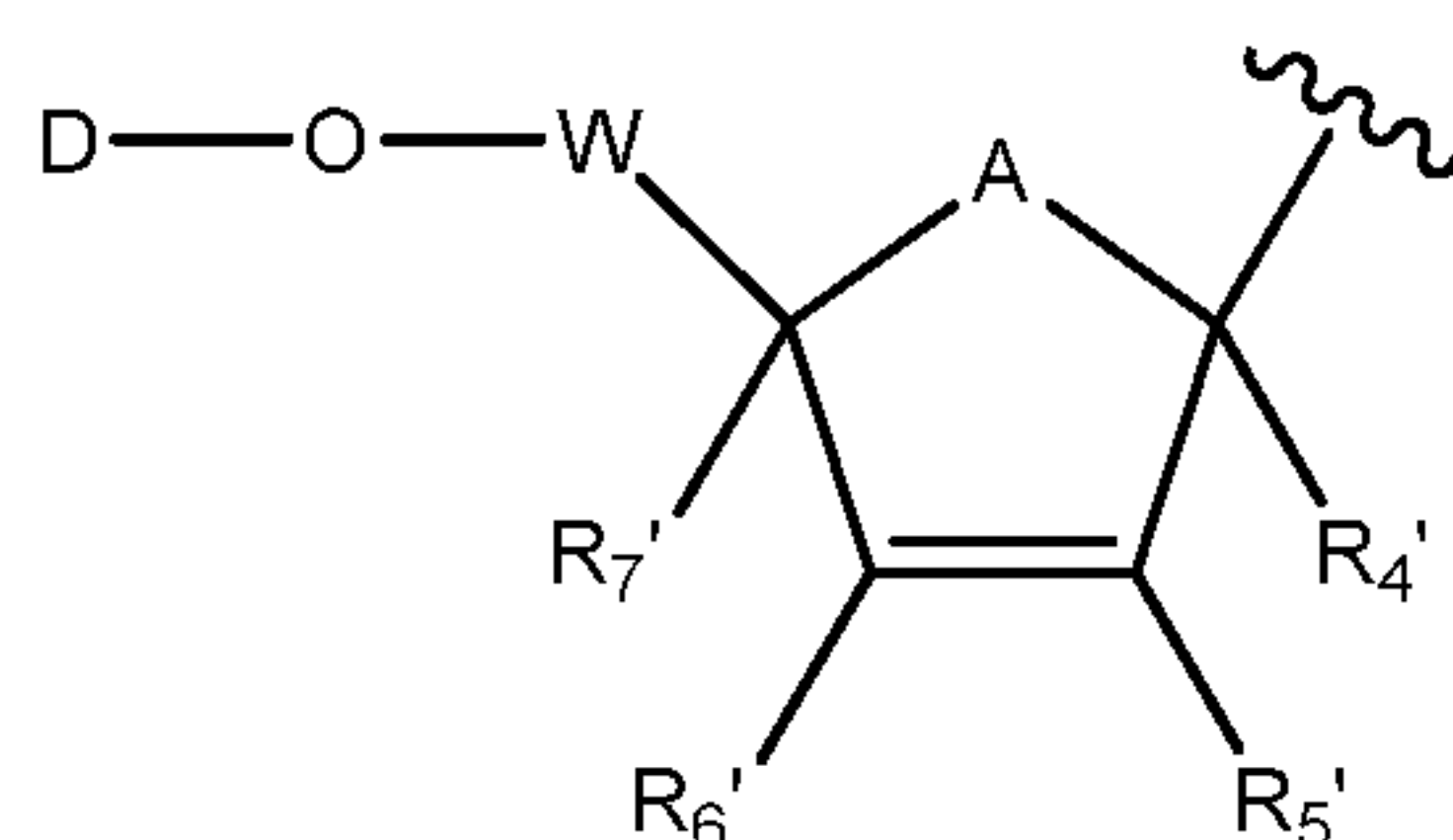


where R²⁰ is O or NH, and

R²¹ is selected from the group consisting of H, C₁₋₂₀ alkyl, C₁₋₂₀ alkenyl, the carbon chain derived from a fatty acid, and C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, and substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl, and

(j) R² is a monophosphate ester or a diphosphate ester when R³ 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)R¹, C(O)OR¹, diphosphate ester, or triphosphate ester;

R¹ is independently C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆

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alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

W is CL₂ or CL₂CL₂, wherein L independently is selected from the group consisting of H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A, R², R³, 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, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, or NR₂,

In another embodiment, R^{7'} is, independently, selected from the group consisting of H, F, Cl, Br, I, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, and R;

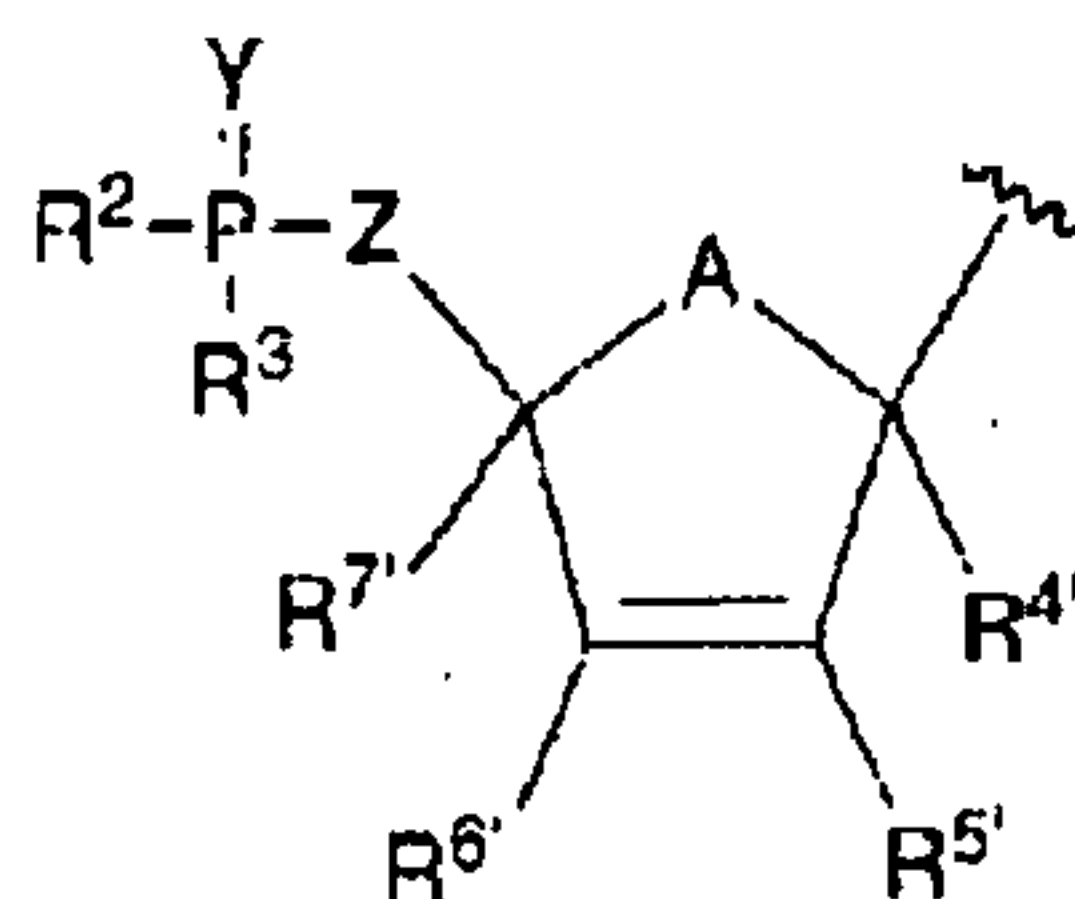
wherein R is independently C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₆ cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In one embodiment, where Sugar is of Formula (V), when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H then, R^{5'} and R^{6'} cannot be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

In another embodiment, R^{5'} and R^{6'} are independently selected from the group consisting of NHOH, NHNH₂, N₃, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

REPLACEMENT SHEET

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



(VI)

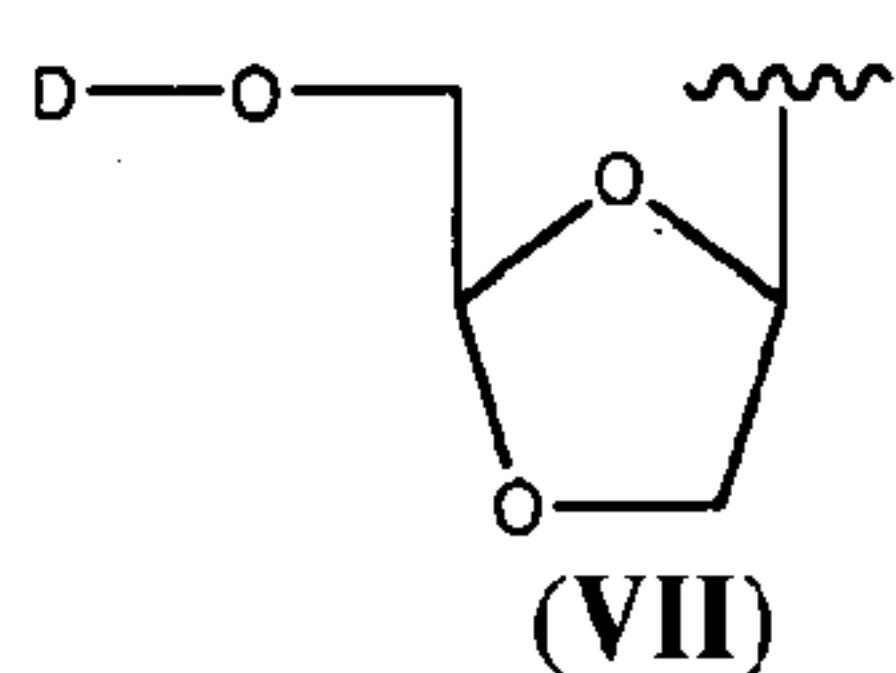
wherein:

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;

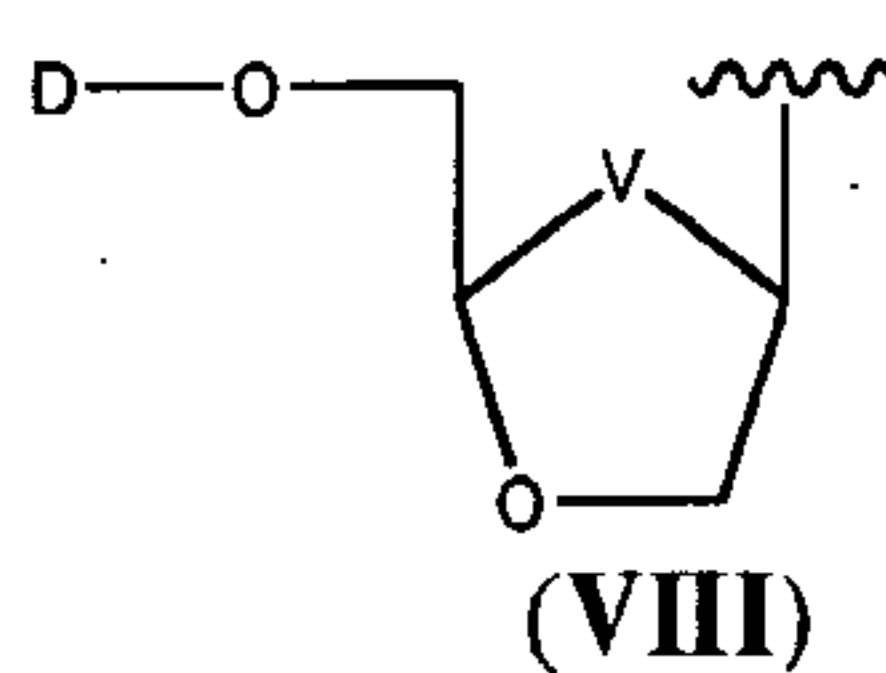
wherein for formula (I) where Sugar is of Formula (VI), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2 , NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2 ,

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.

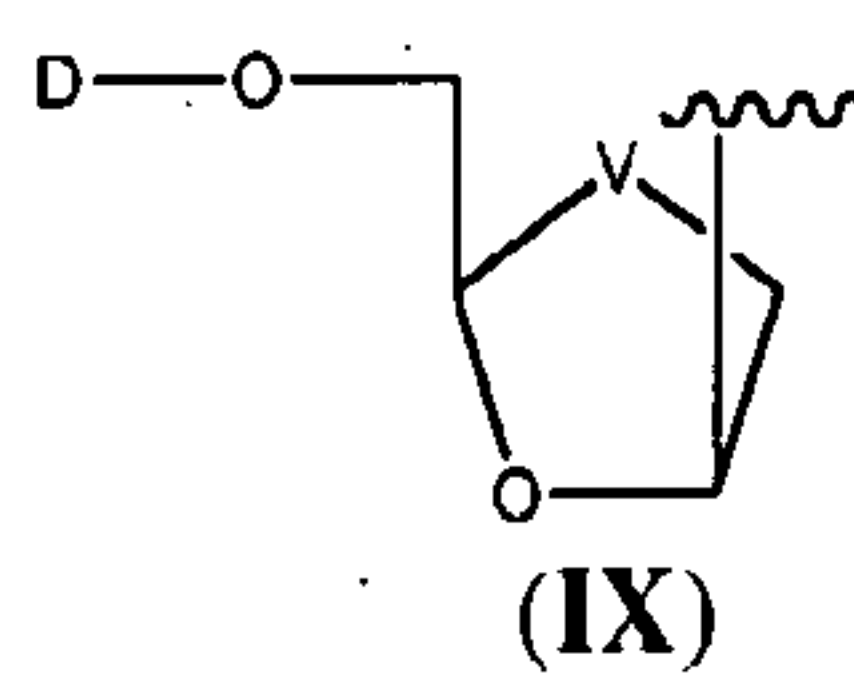
In another embodiment, Sugar is a dioxolane, an oxathiolane, or a dithiolane of the general formulas (VII), (VIII), (IX), and (X):



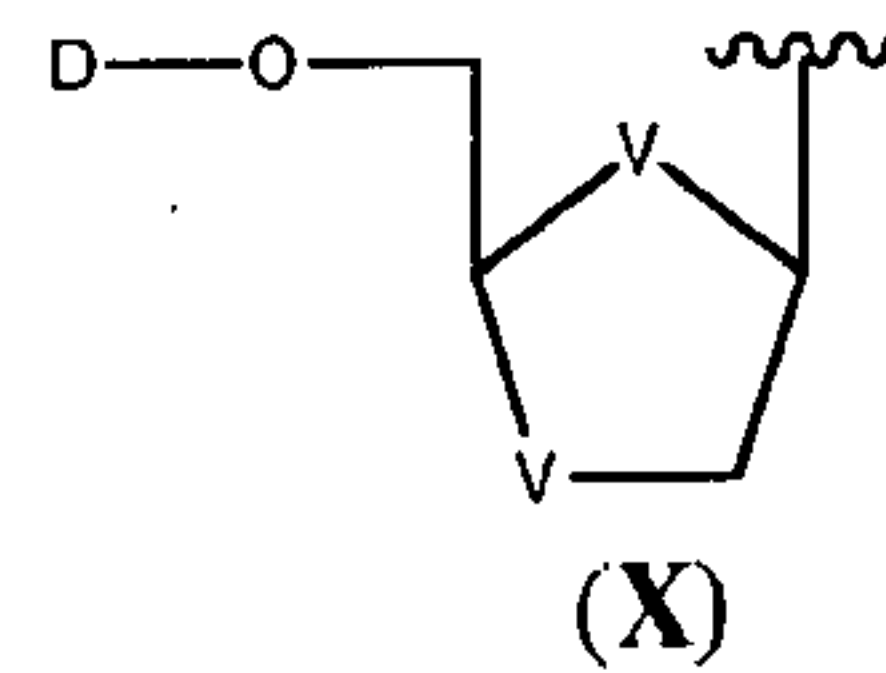
(VII)



(VIII)



(IX)



(X)

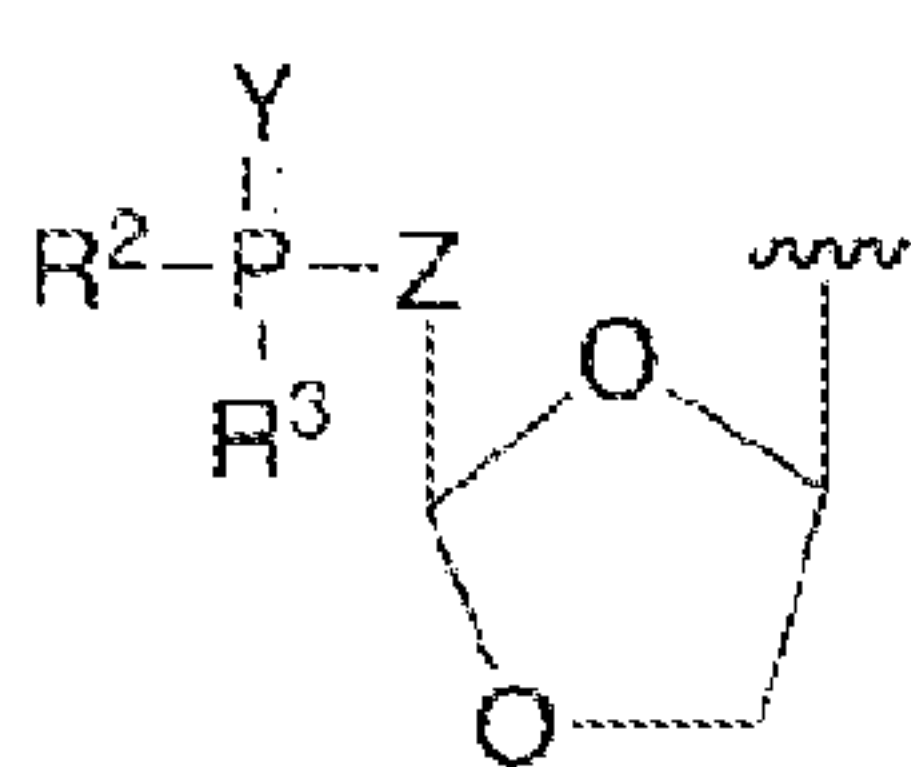
D is H, $C(O)OR^1$, diphosphate ester, or triphosphate ester;

V is, individually, S or Se;

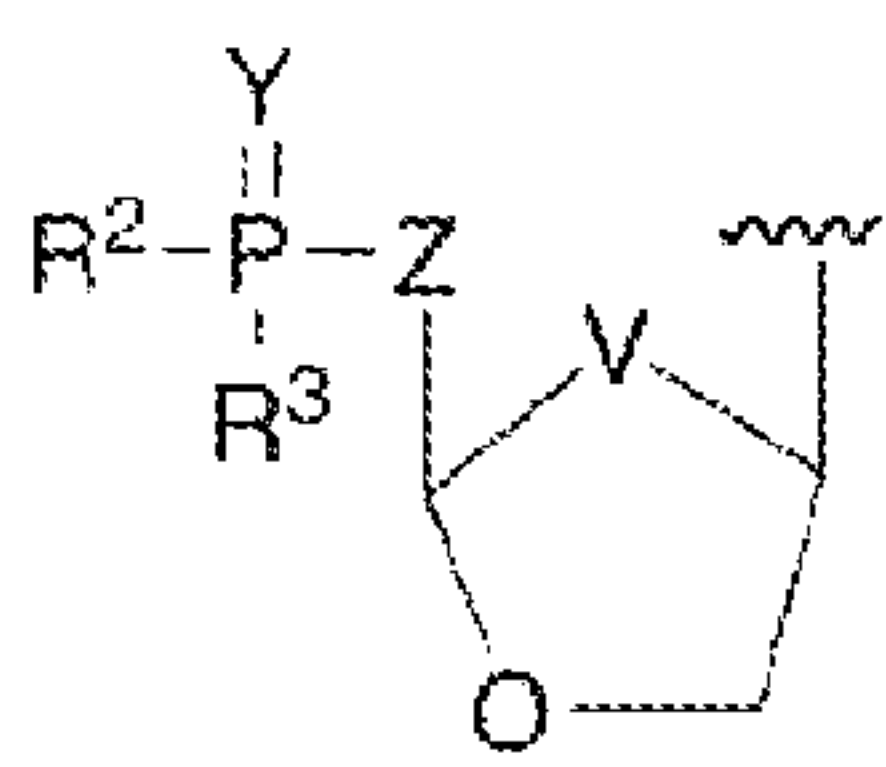
R^1 is independently C_{1-20} alkyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_1 - C_6 alkyl, C_1 - C_6 alkoxy, di(C_1 -

C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

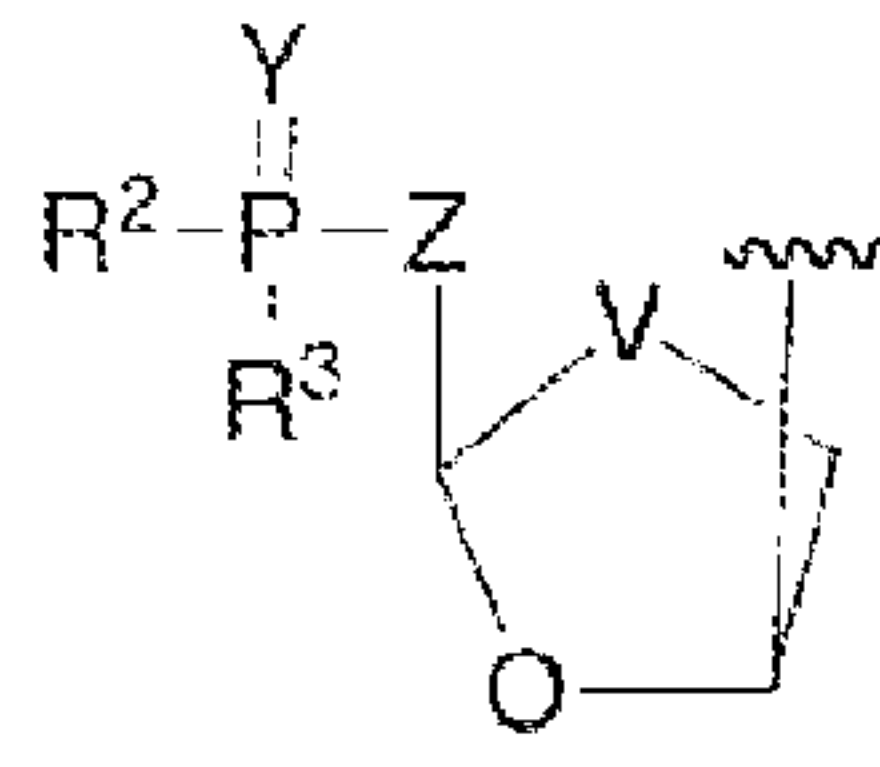
In yet another embodiment, Sugar is a dioxolane, or a oxathiolane, or dithiolane of the general Formulas (XI), (XII), (XIII), and (XIV):



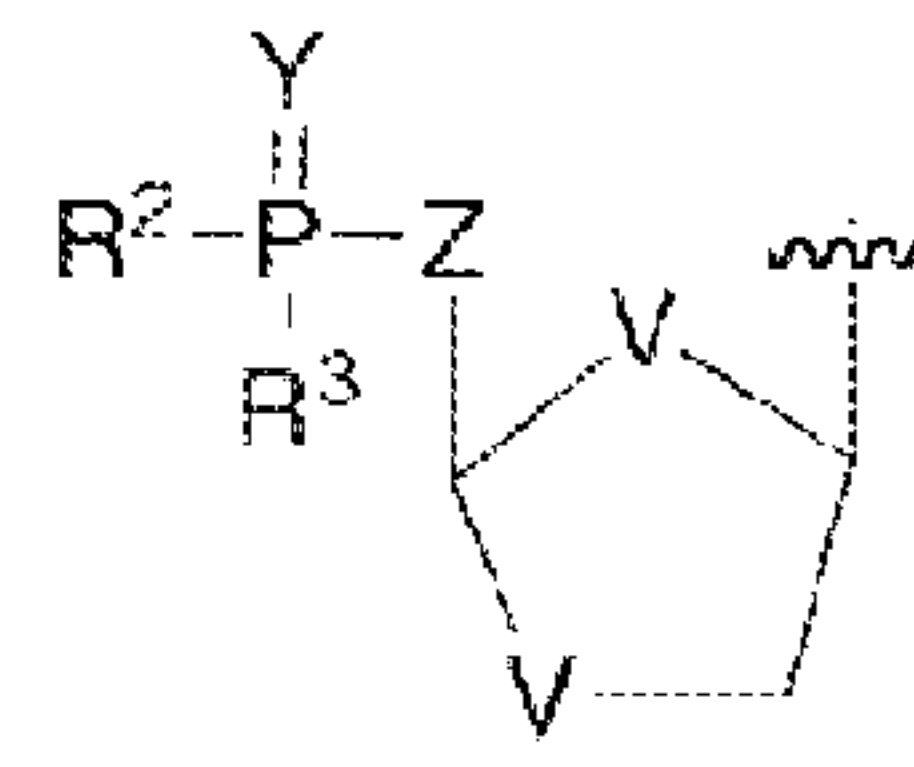
(XI)



(XII)



(XIII)



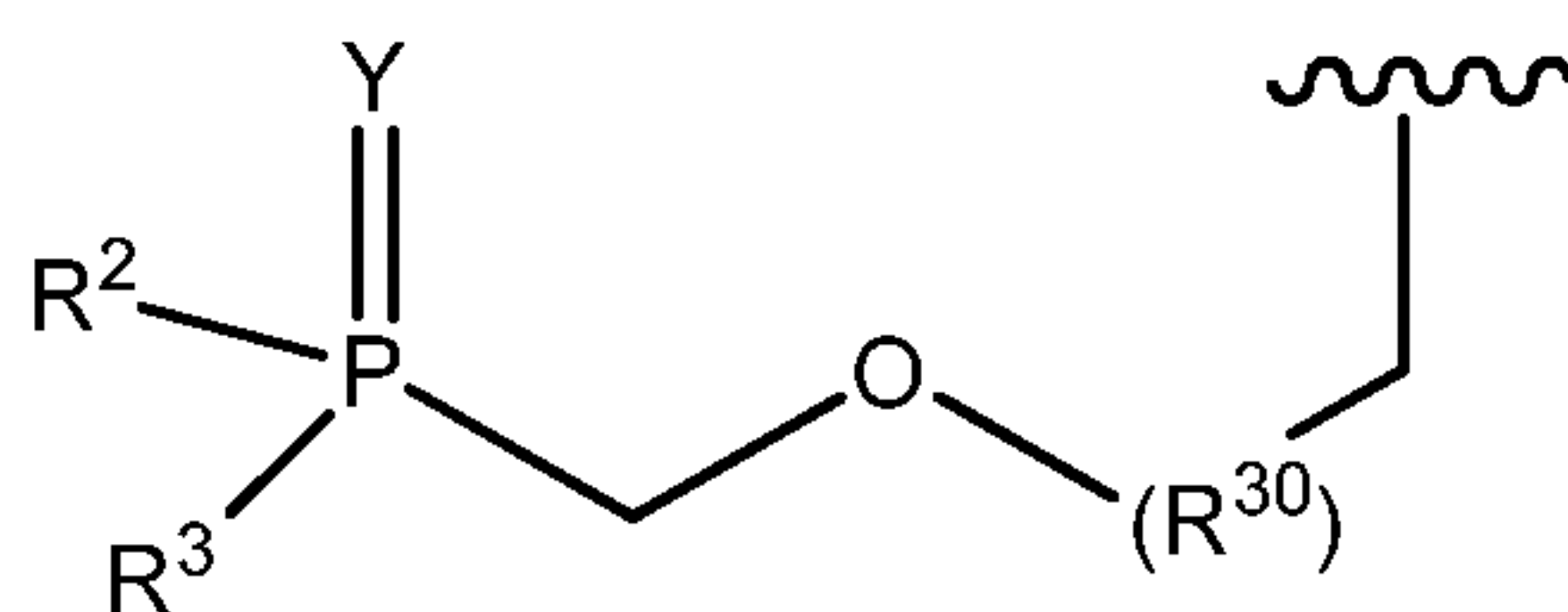
(XIV)

wherein:

V is, individually, S or Se;

R², R³, 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):



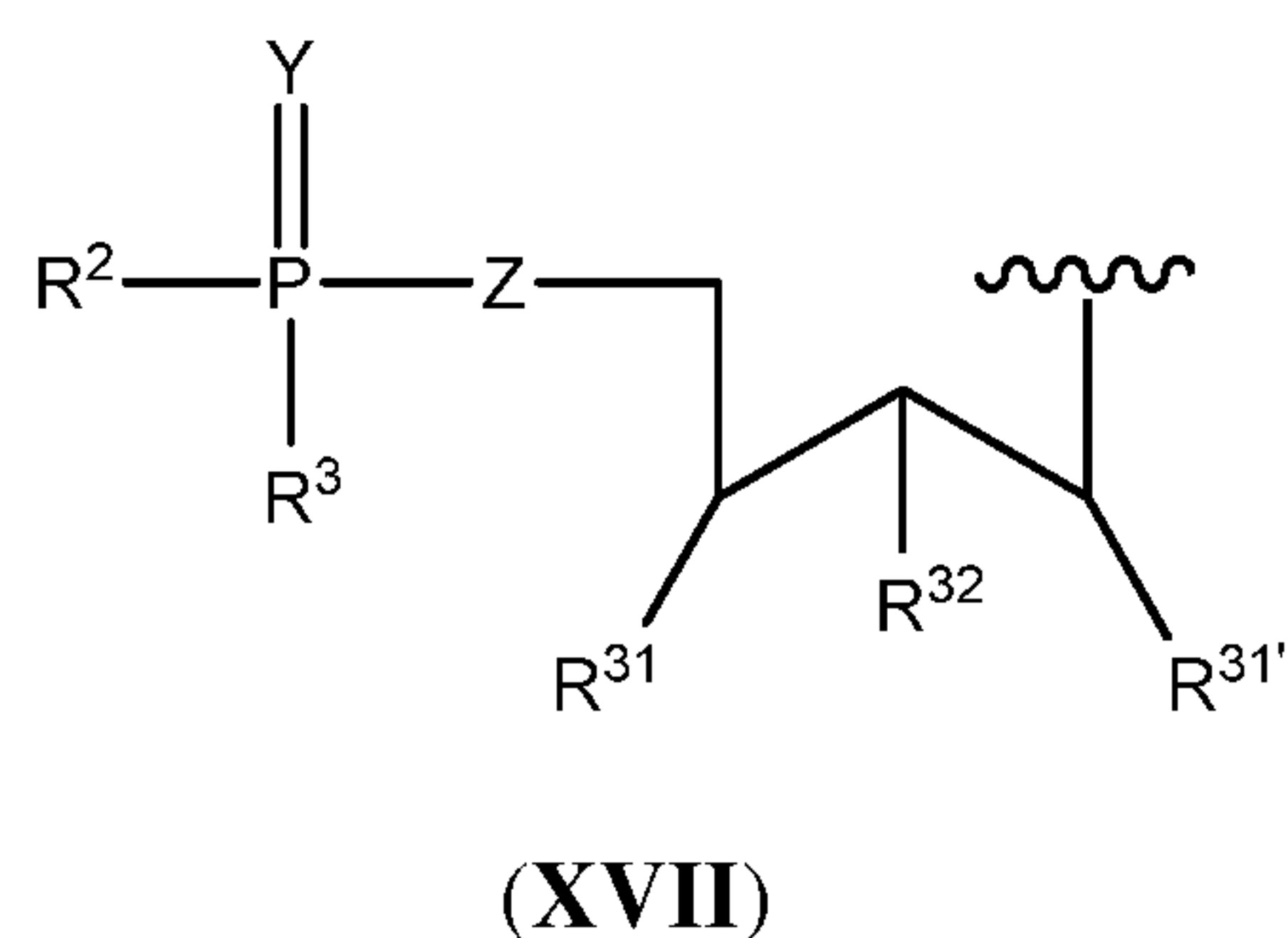
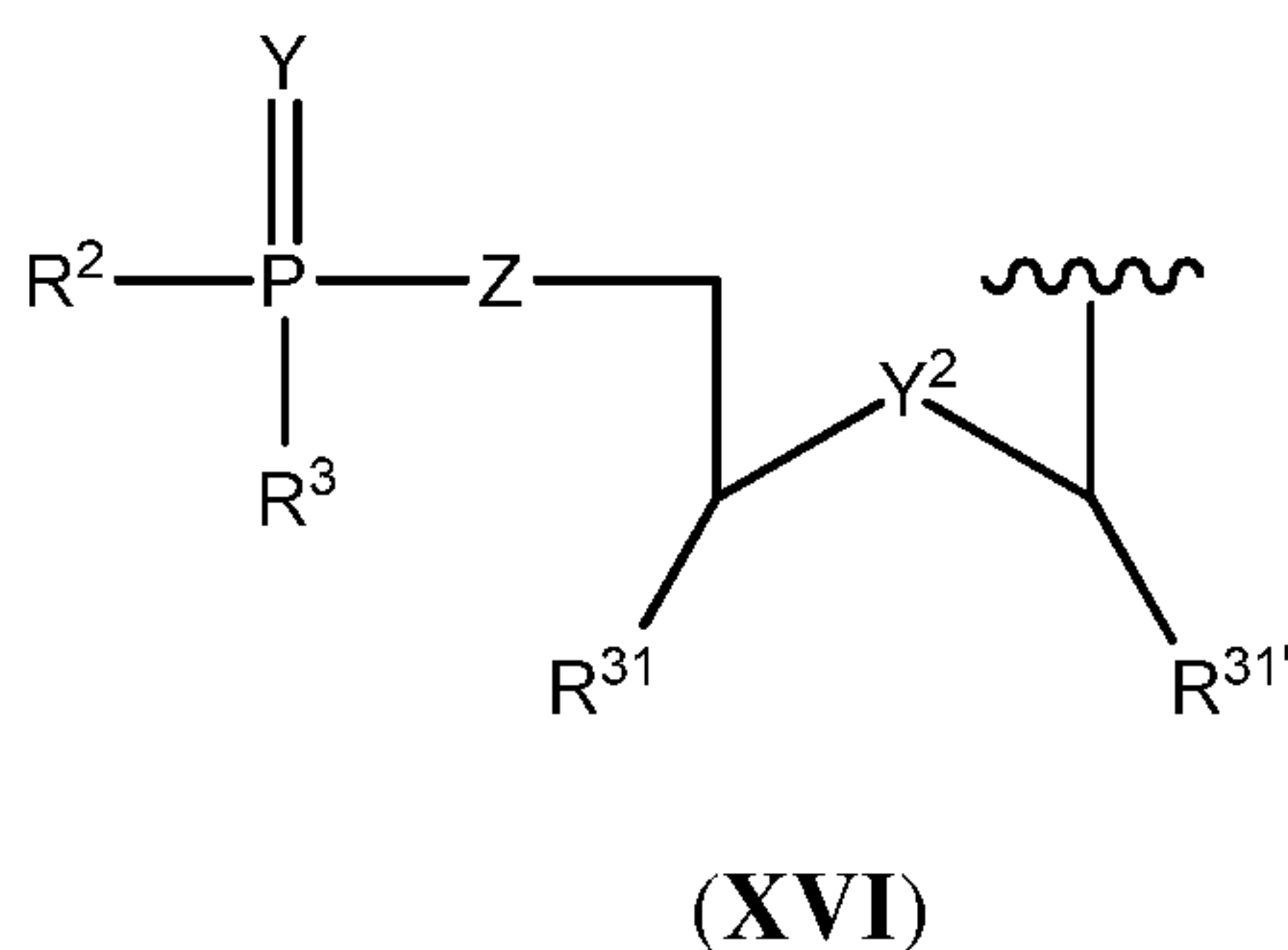
(XV)

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):



wherein:

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

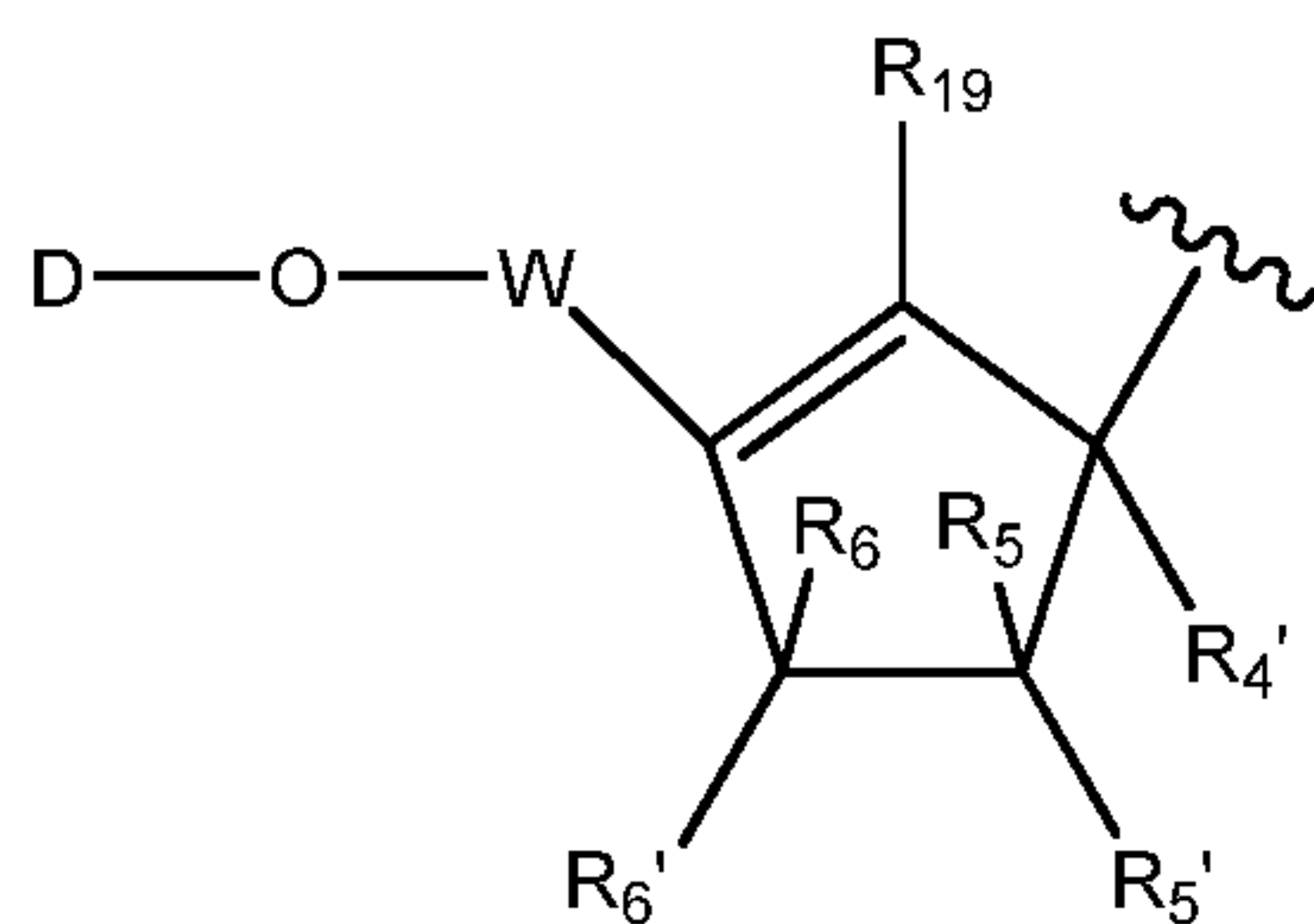
Y^2 is O, S, Se, or NR;

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'}, R⁵, R^{5'}, R⁶, and R^{6'} are as defined above;

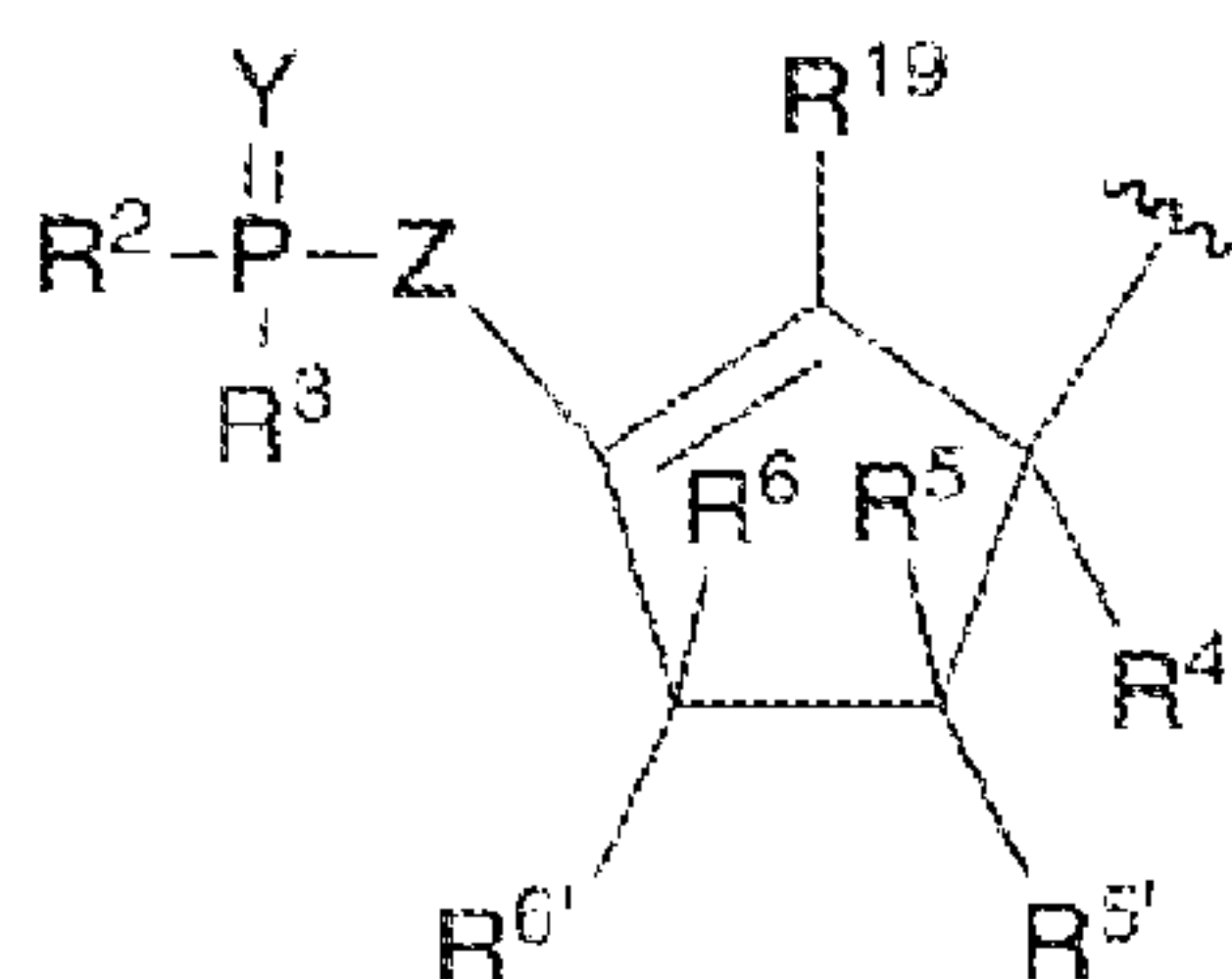
R¹⁹ is H, F, Cl, Br, I, N₃, C(O)OH, CN, C(O)NH₂, C(S)NH₂, C(O)OR, or R;

wherein R is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ 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 CH₂, R^{4'} and R¹⁹ are H, then, R⁵, R^{5'}, R⁶, R^{6'} can not be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

In another embodiment, R^{6'} can be independently selected from the group consisting of NHOH, NHNH₂, N₃, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂.

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



(XIX)

wherein:

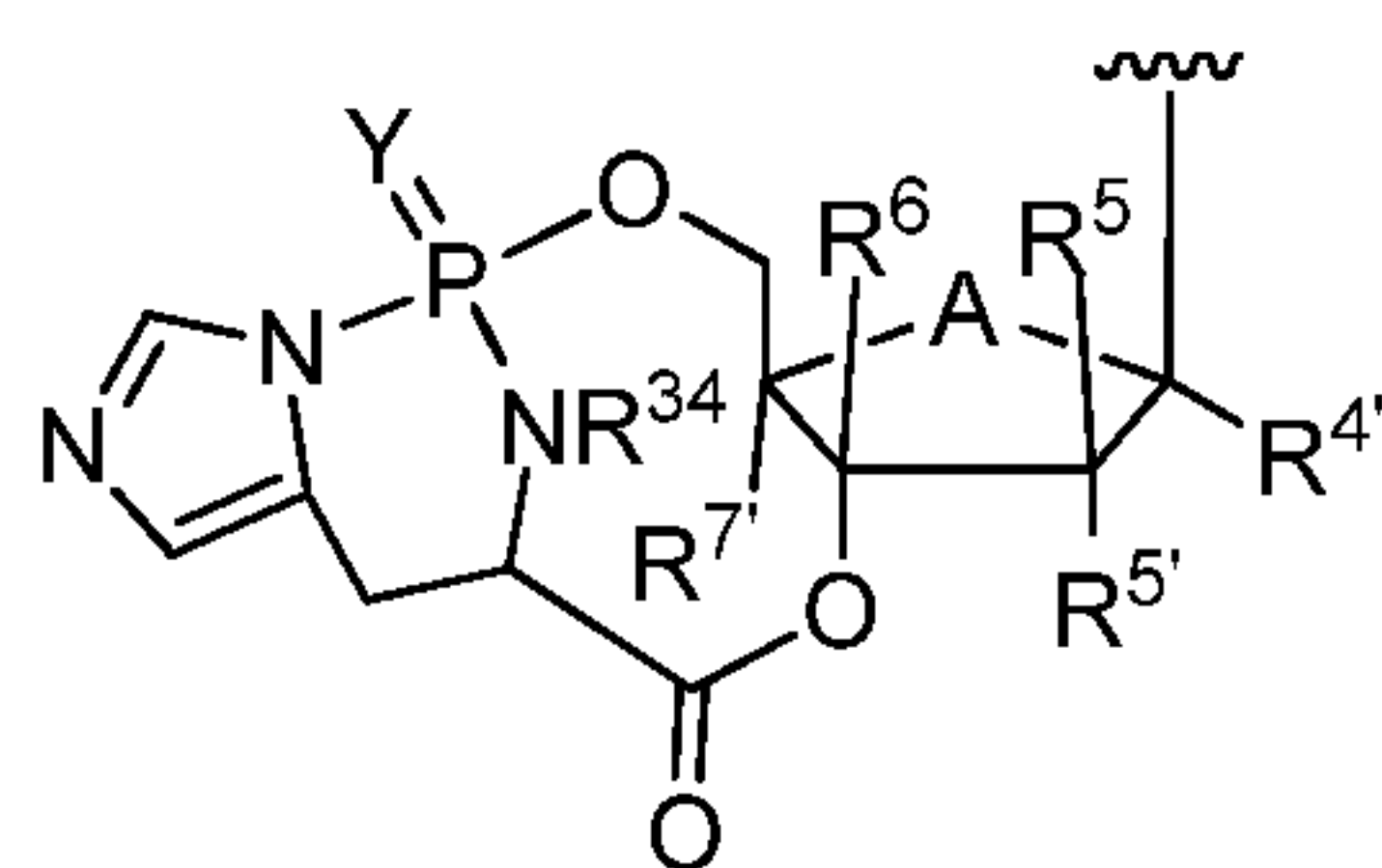
R^2 , R^3 , and Y are as defined above with respect to Formulas I, II, III and IV;

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

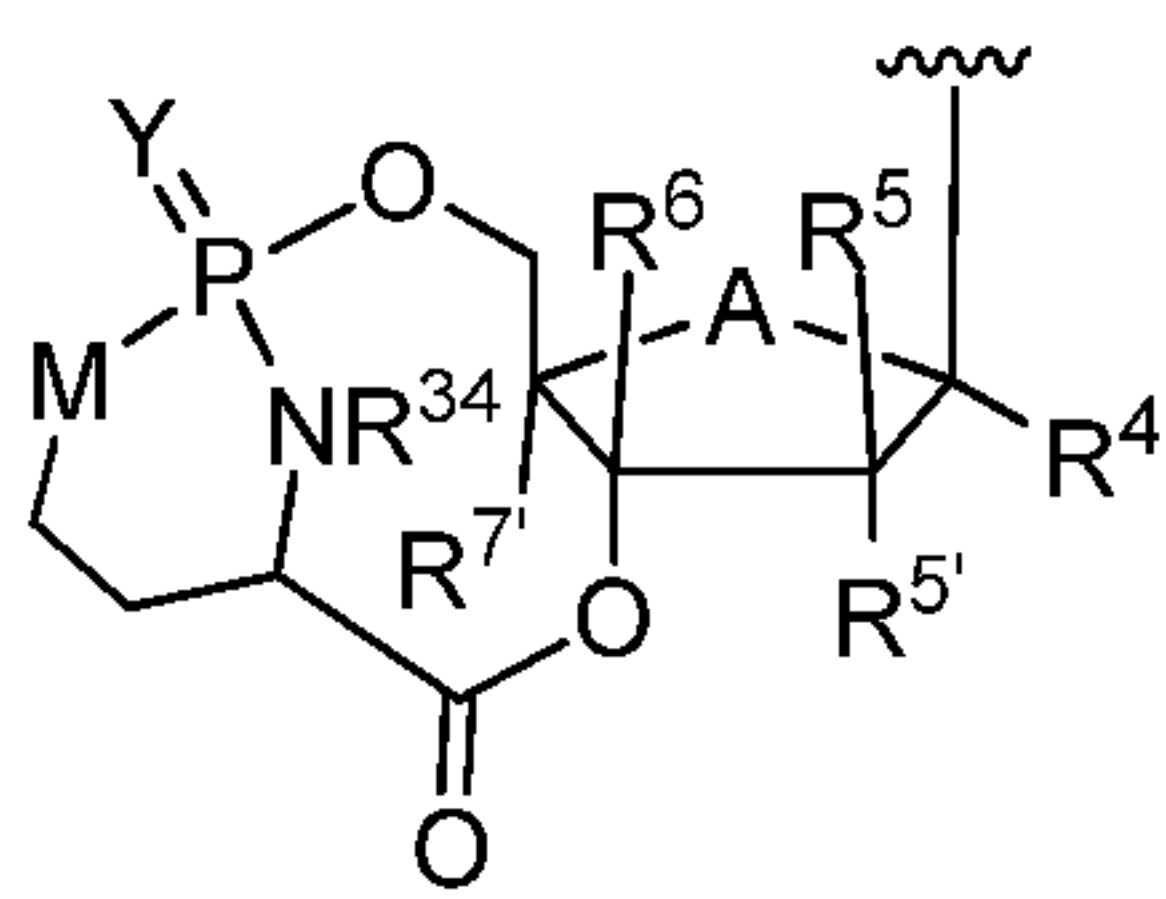
R^{19} is H, F, Cl, Br, I, N_3 , $C(O)OH$, CN, $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, or R,

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.

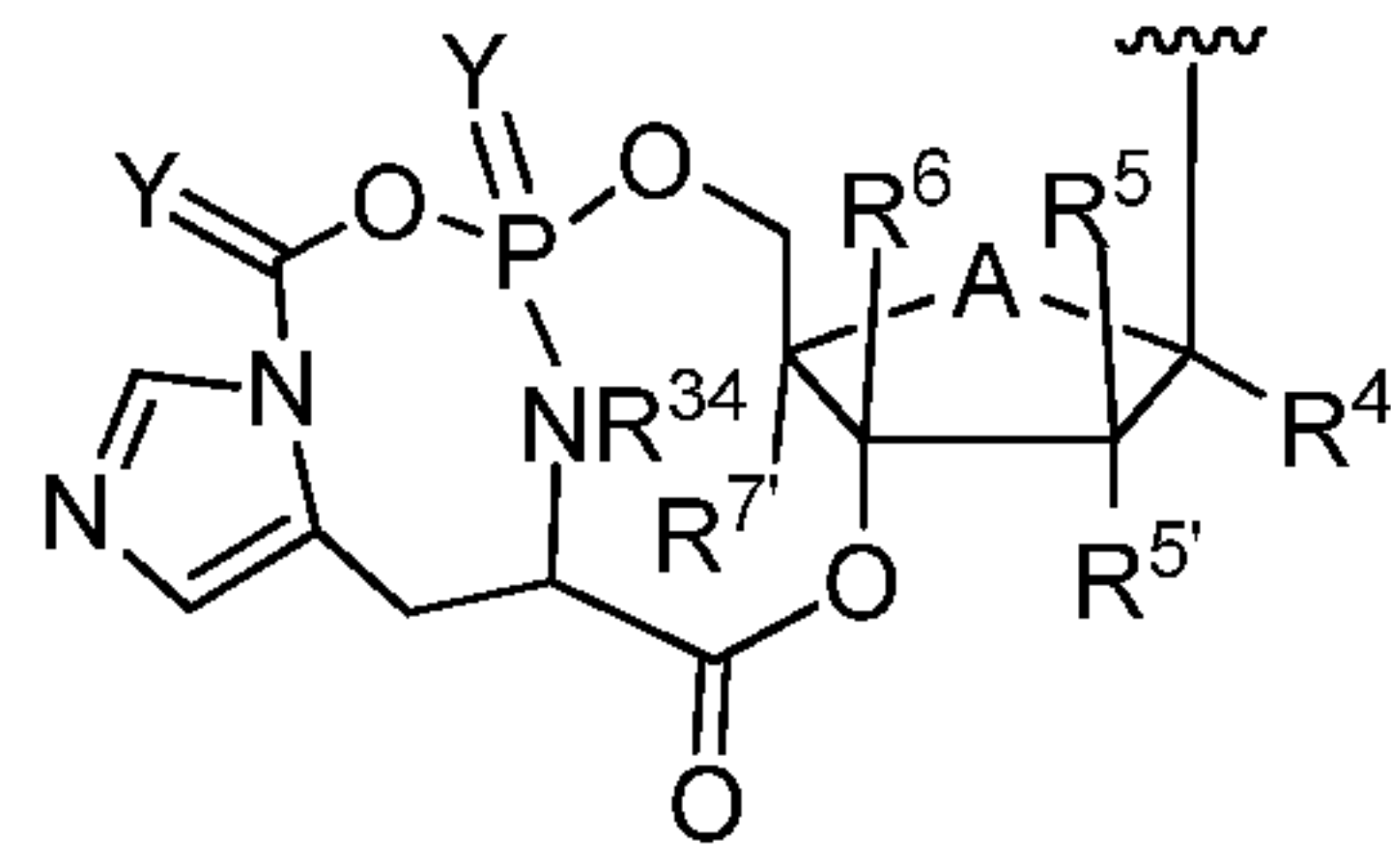
In yet another embodiment, Sugar has one of the Formulas (XX), (XXI), or (XXII):



(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;

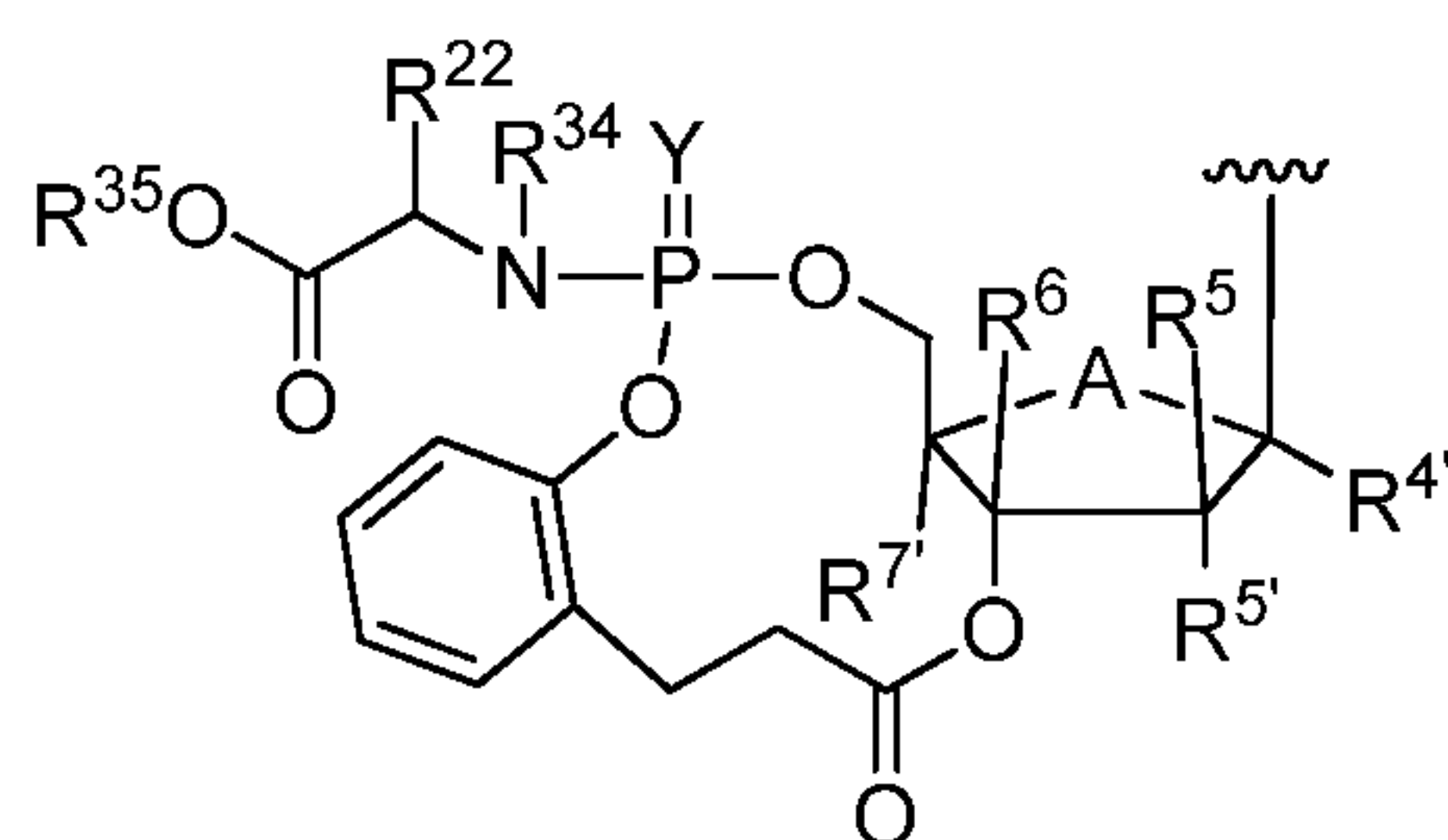
R^{34} is C_1 - C_6 alkyl;

M is O, S, or NR;

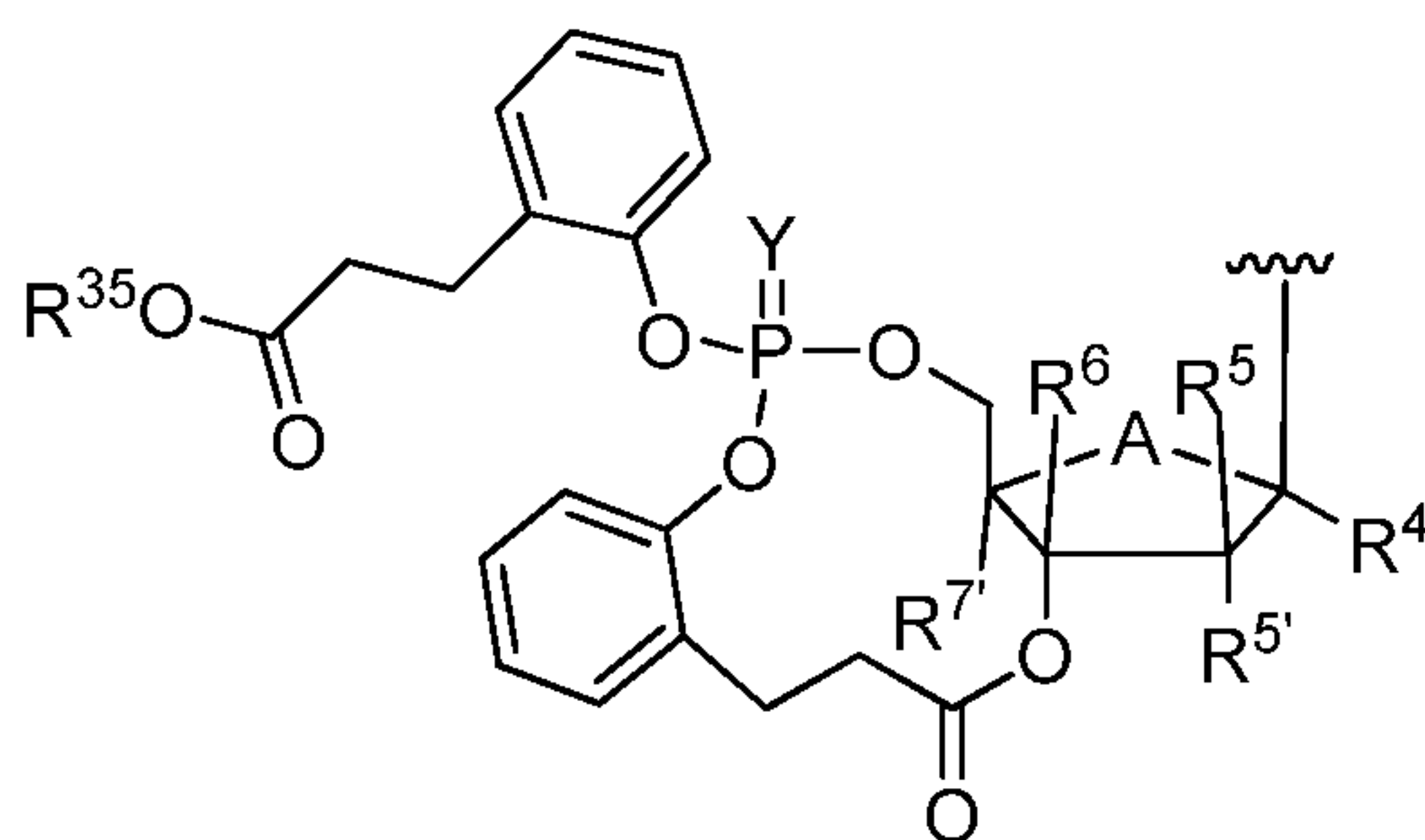
wherein R is, independently, C_1 - C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3 - C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II,

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

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



(XXIII)



(XXIV)

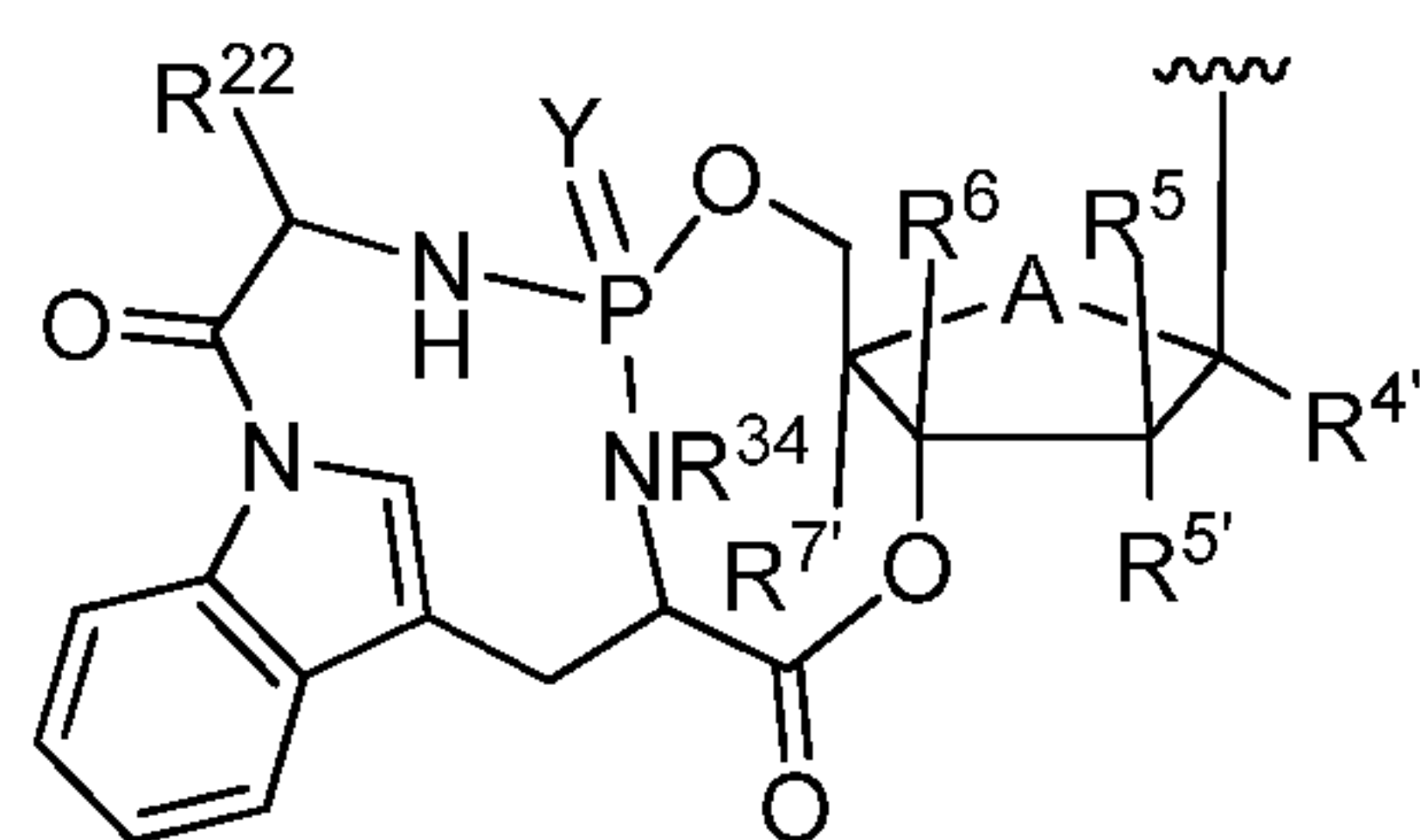
wherein:

R^{4'}, R⁵, R^{5'}, R⁶, Y, A, R^{7'}, R³⁴ are as defined above with respect to Formulas I, II, III and IV;

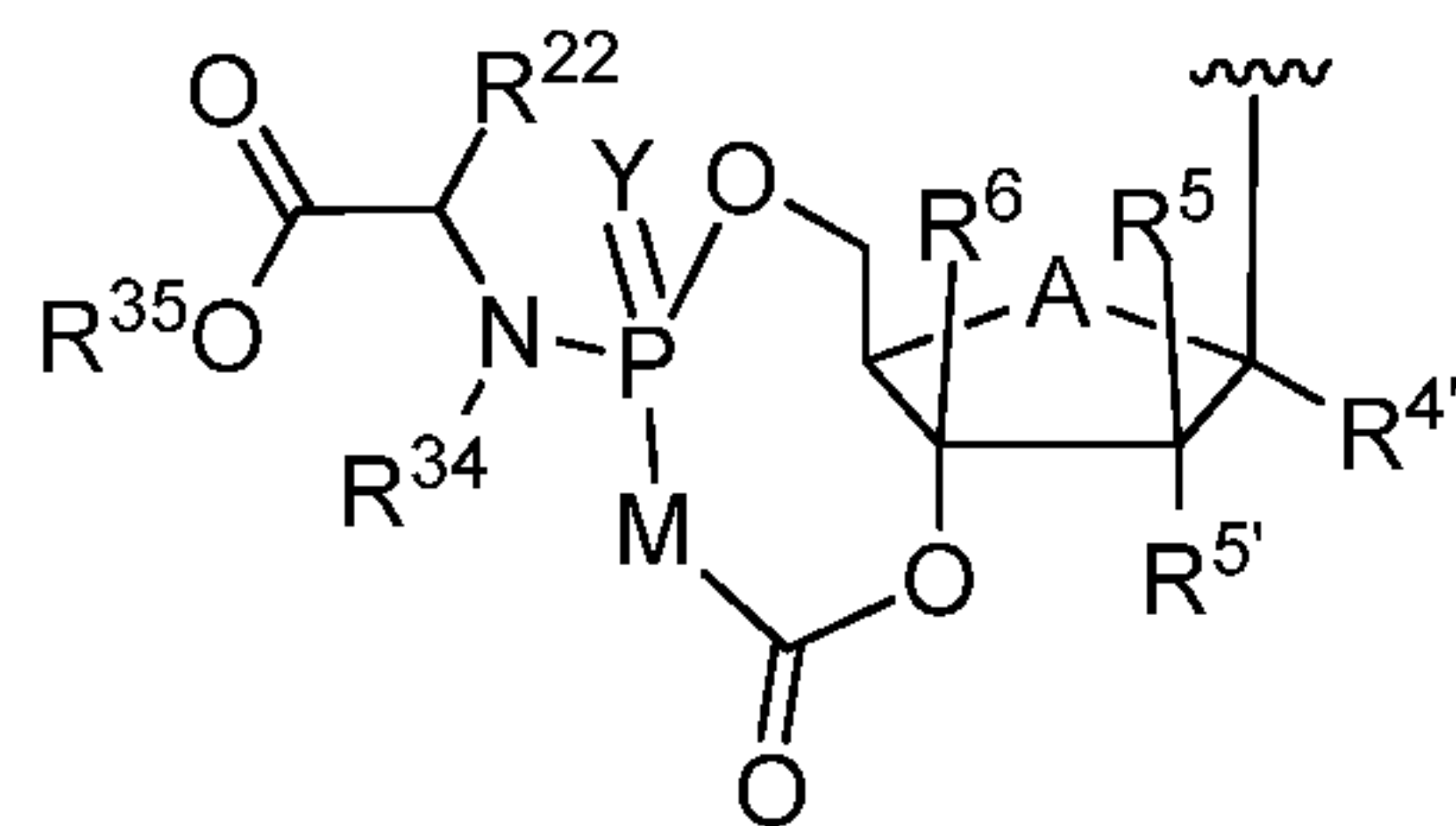
R³⁵ is H, C₁₋₁₀ alkyl, C₁₋₁₀ alkyl optionally substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl; and

R²² is H, CH₃, CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or C₃₋₆ cycloalkyl;

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



(XXV)

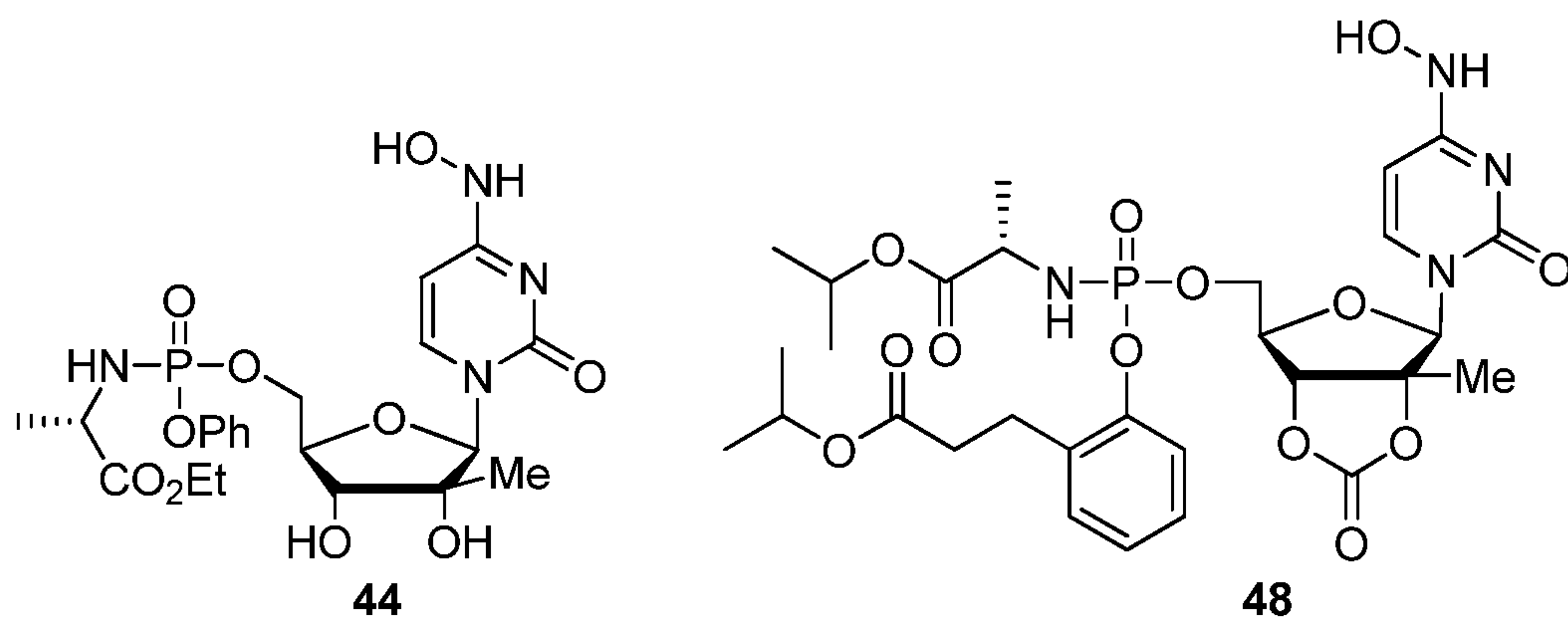
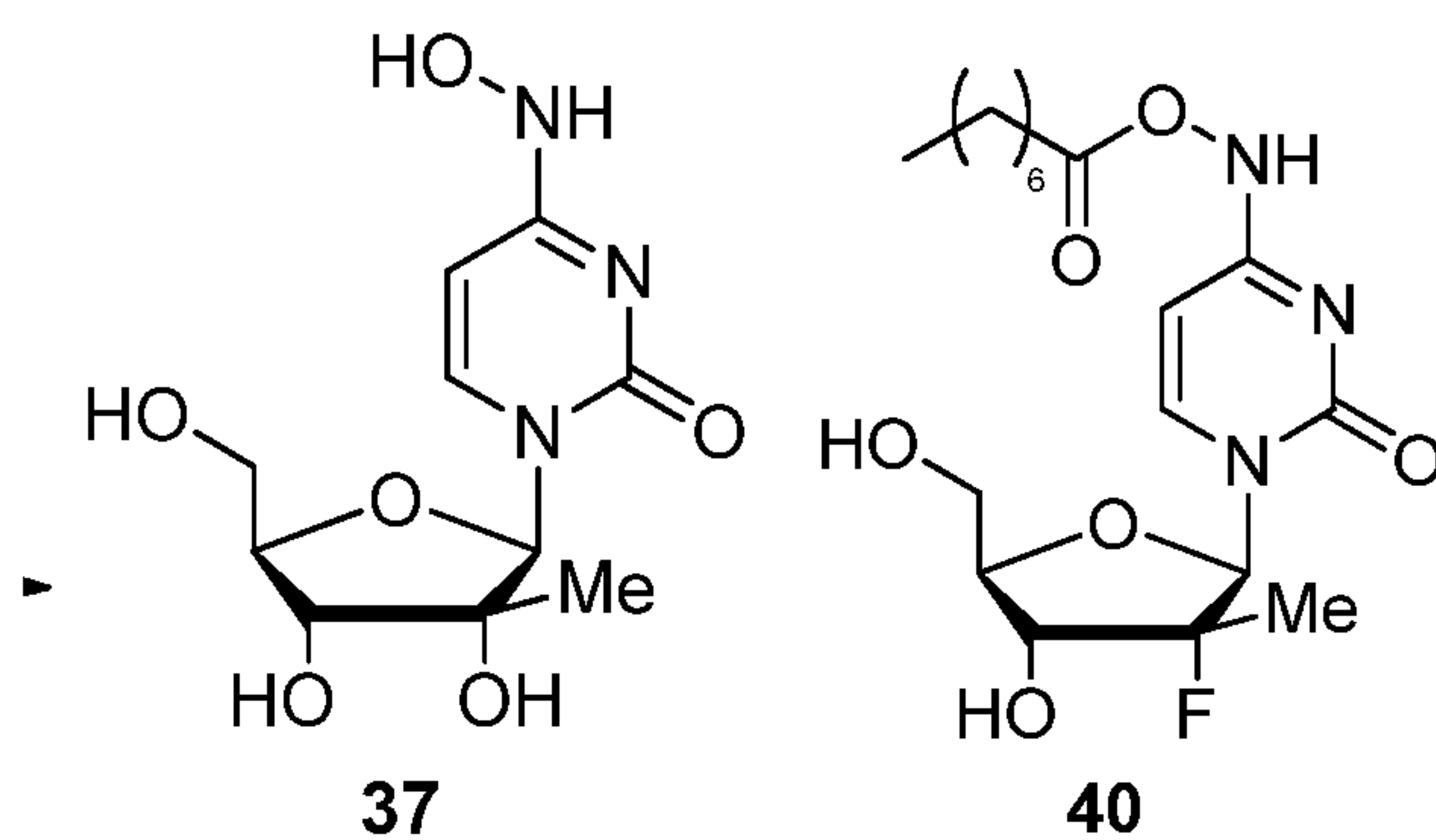


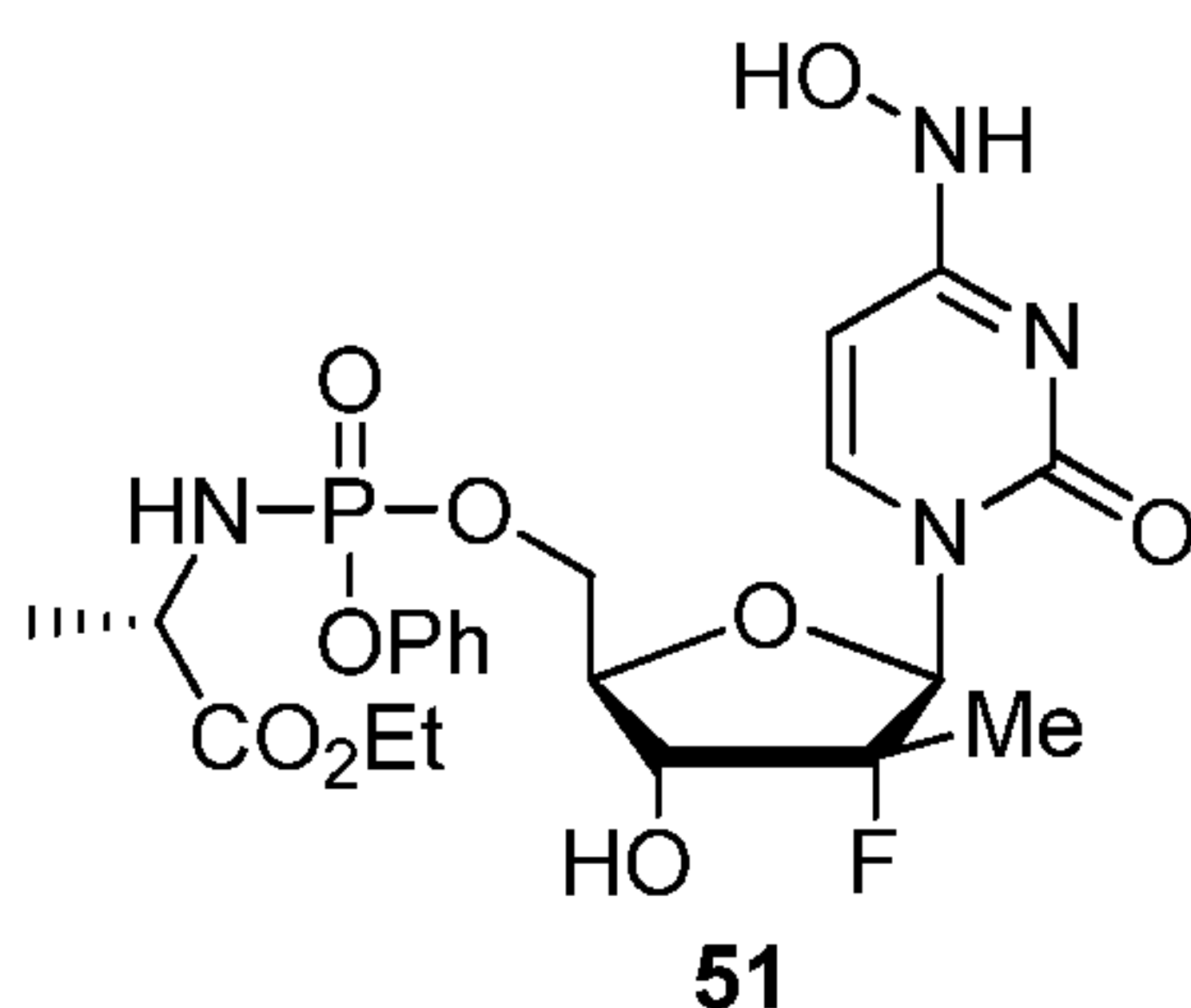
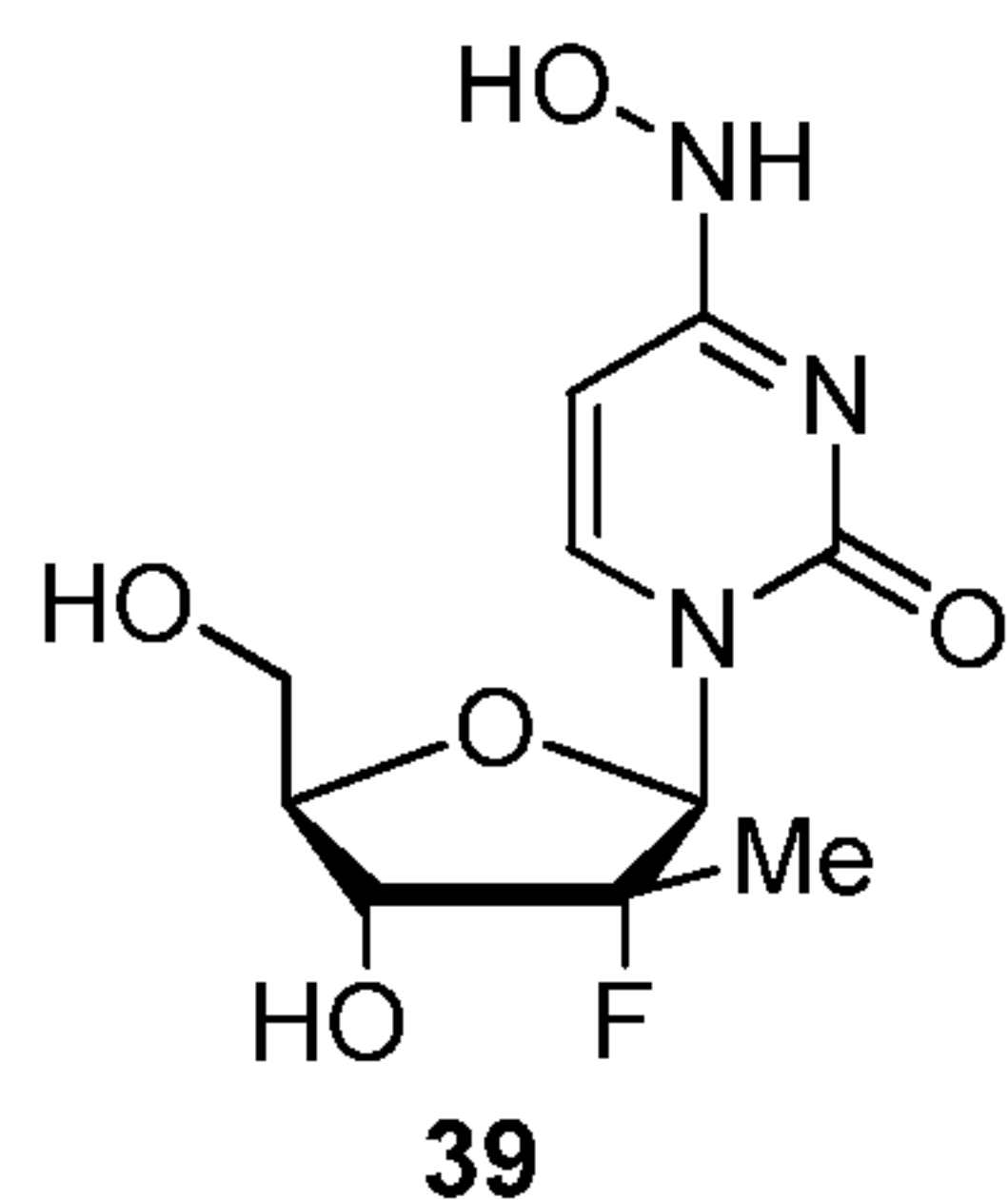
(XXVI)

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:





, 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.

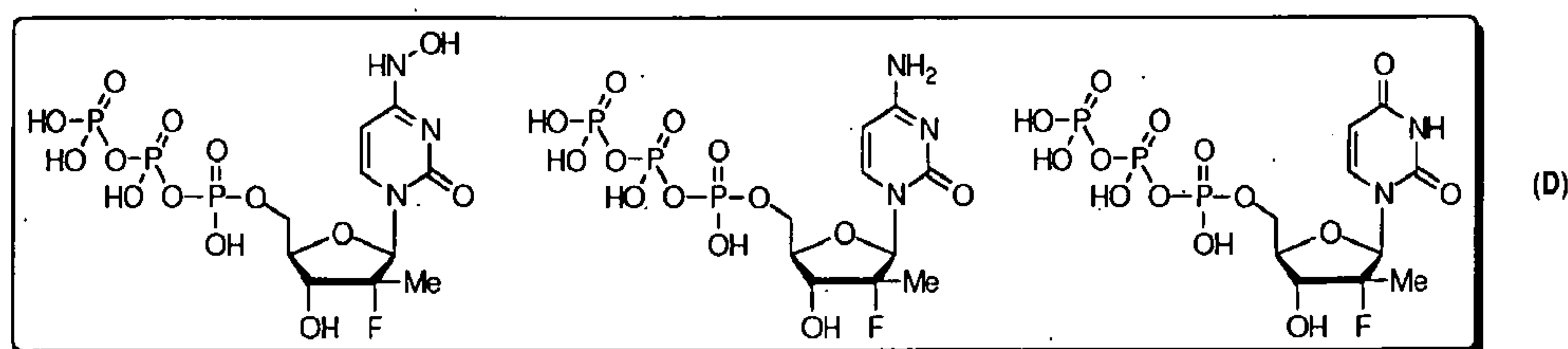
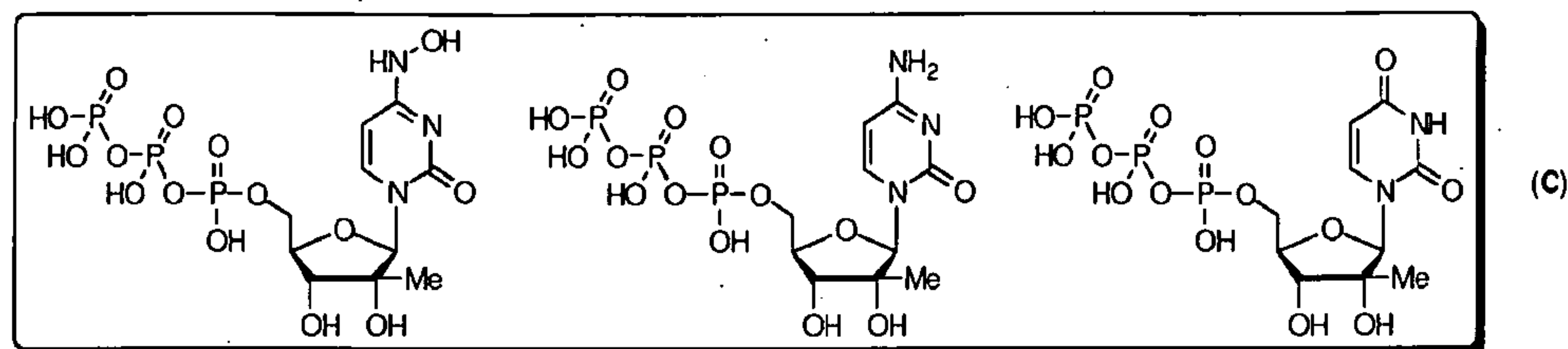
In another embodiment, the base is a pyrimidine, and one of R^5 and $R^{5'}$ is OH, Cl, or F.

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 -NH₂ moiety, and, optionally, partial conversion of the -NHOH moiety or the resulting -NH₂ 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-NH₂ 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.

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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 moiety is converted to an NH₂ 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 vice 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 R¹X¹YR², wherein R¹ is “independently carbon or nitrogen,” both R¹ can be carbon, both R¹ can be nitrogen, or one R¹ can be carbon and the other R¹ 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 CF₃ and CH₂CF₃

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 C₁₋₂₂ alkyl moieties, and the term “lower alkyl” includes C₁₋₆ 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 C₂₋₆ 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 C₂₋₆ 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 moieties. 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, hydroxyheteroaralkyl, 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 C₁, C₂, C₃, and C₄), alkoxy (including but not limited to C₁, C₂, C₃, and C₄), 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-mercaptopyrimidine, 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*-toluenesulfonyl. 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.

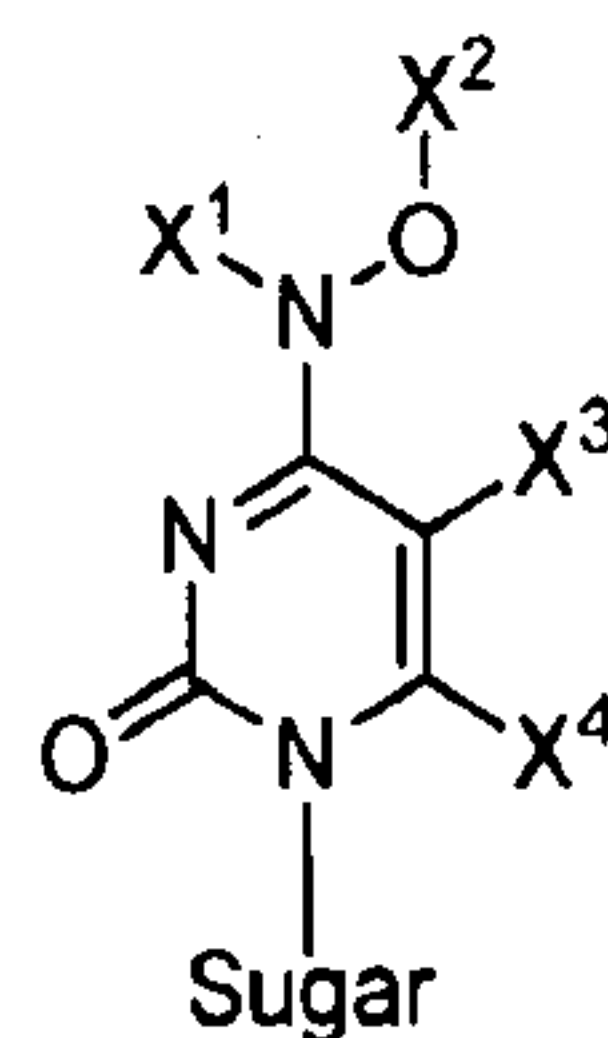
Prodrugs also include amino acid esters of the disclosed nucleosides (*see, e.g.*, European Patent Specification No. 99493, the text of which is incorporated by reference, which describes amino acid esters of acyclovir, specifically the glycine and alanine esters which show improved water-solubility 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

REPLACEMENT SHEET

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:

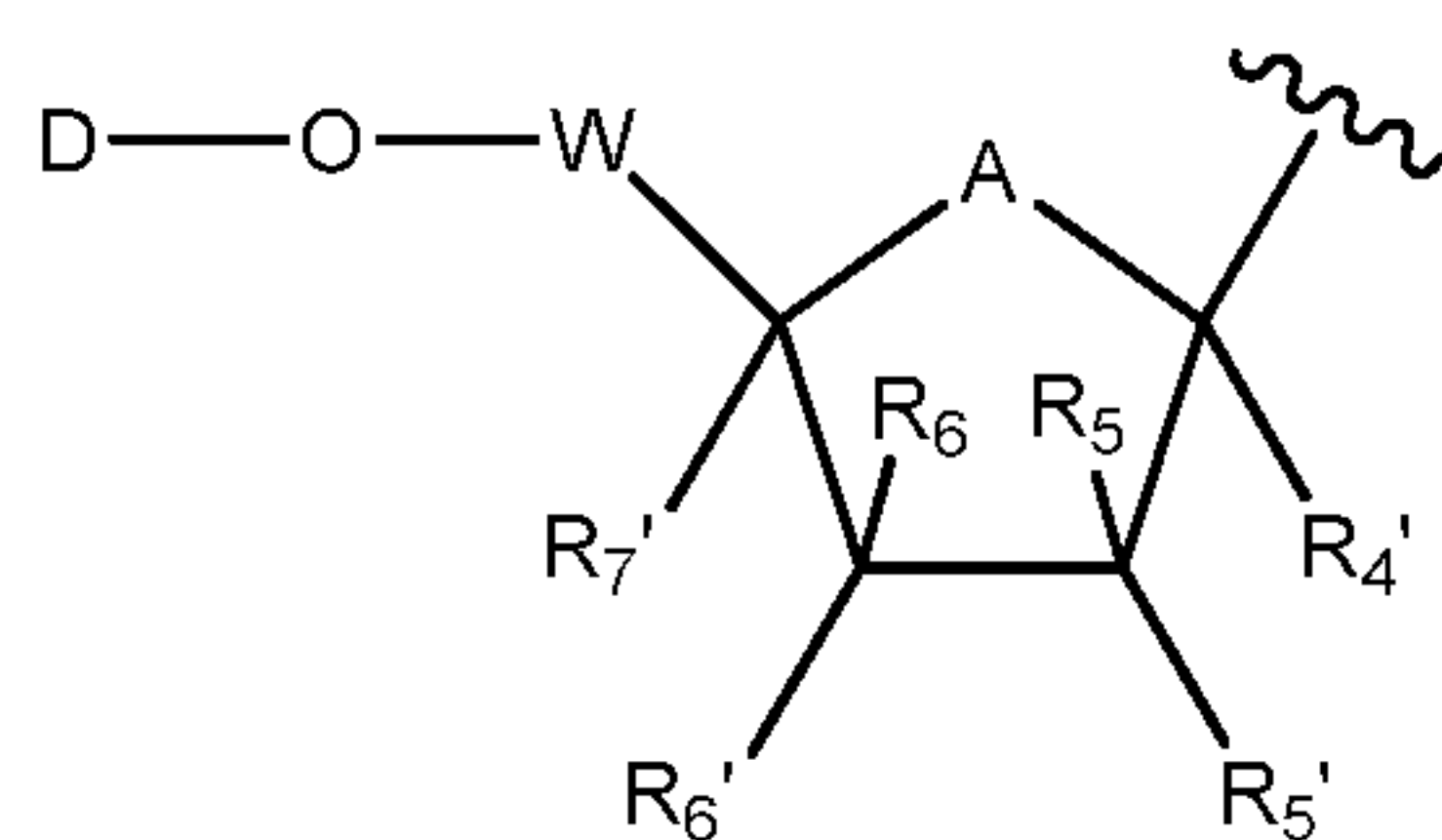
- iv) X¹ is H, C₁-C₆alkyl, C₁-C₆haloalkyl, C₁-C₆alkoxy, C₂-C₆ alkenyl, C₂-C₆ alkynyl, COR¹, or COOR¹;
- v) X² is hydrogen, CH₂-O(CO)-X⁵; CH₂-O(CO)O-X⁵, COR¹, or COOR¹.

wherein each R¹ is, independently, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₆ alkyl, or C₁₋₆ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, or C₃₋₁₀ cycloalkyl

X⁵ is independently, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, alkoxy, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₆ alkyl, or C₁₋₆ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, or C₃₋₁₀ cycloalkyl

- vi) Each X³ and X⁴ is independently H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aryl, alkylaryl, halogen (F, Cl, Br, I), NH₂, OH, SH, CN, or NO₂.

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



(II)

wherein:

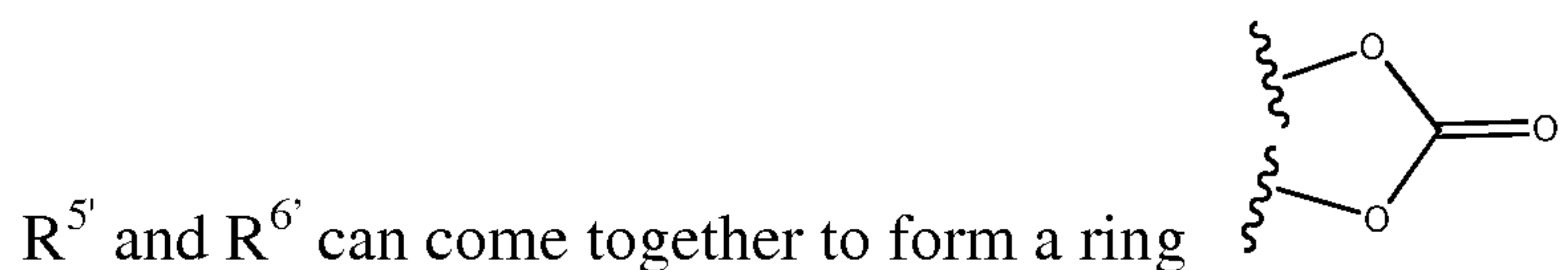
D is H, C(O)R¹, C(O)OR¹, diphosphate ester, or triphosphate ester;

R¹ is as defined above;

W is CL₂ or CL₂CL₂, wherein L independently is selected from the group consisting of H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH₂, CHF, CF₂, C=CH₂, C=CHF, or C=CF₂;

R^{4'}, R⁵, R^{5'}, R⁶, R^{6'}, and R^{7'} are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH₂, NHOH, NHNH₂, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;



In one embodiment, where Sugar is formula (II), when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H then, R⁵, R^{5'}, R⁶, R^{6'} cannot be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

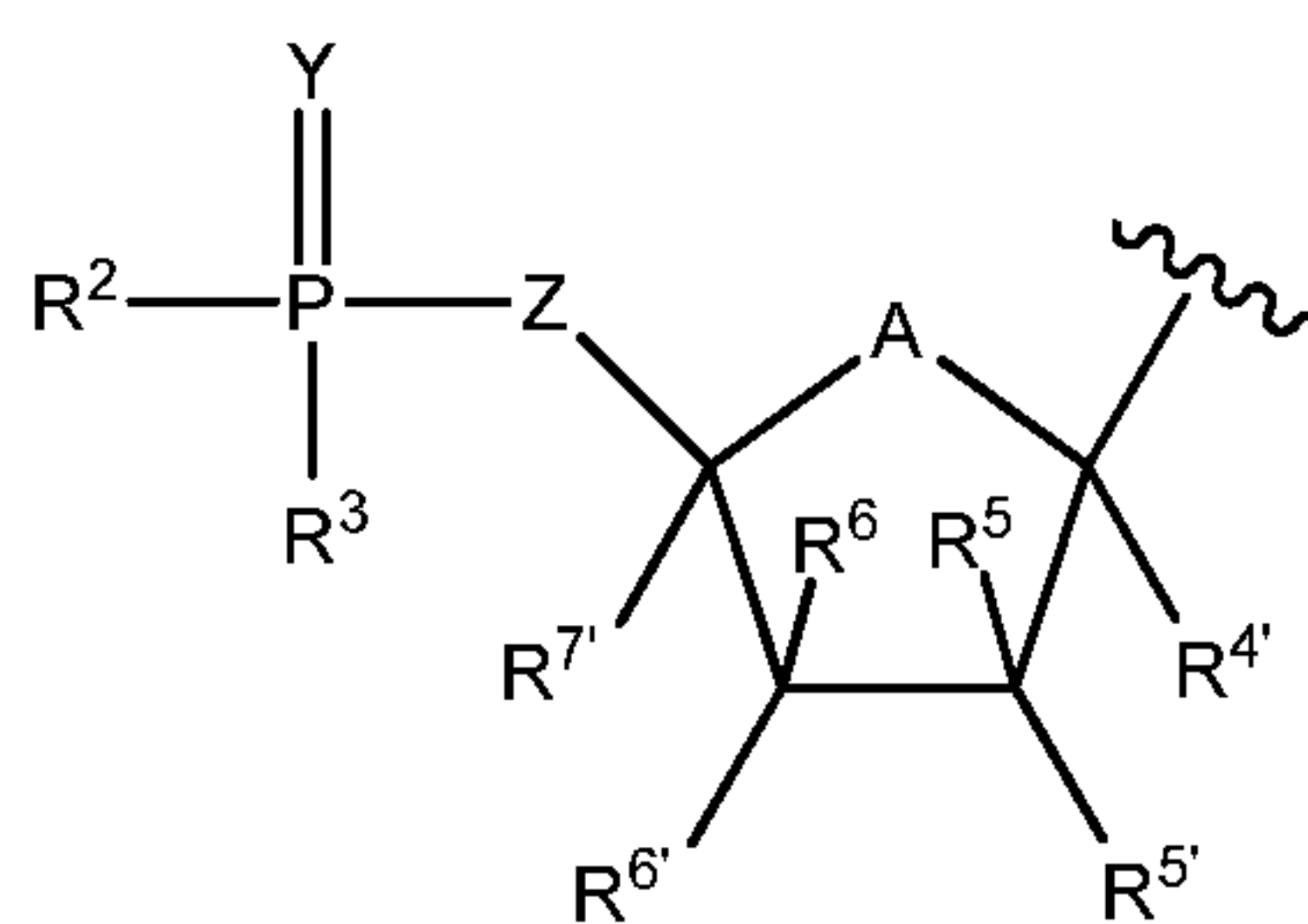
In another embodiment, R^{6'} is independently selected from the group consisting of NHOH, NHNH₂, N₃, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

In one embodiment, wherein for formula (I) where sugar is formula (II), when A is O or S, R^{7'} cannot be OH, SH, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, or NR₂.

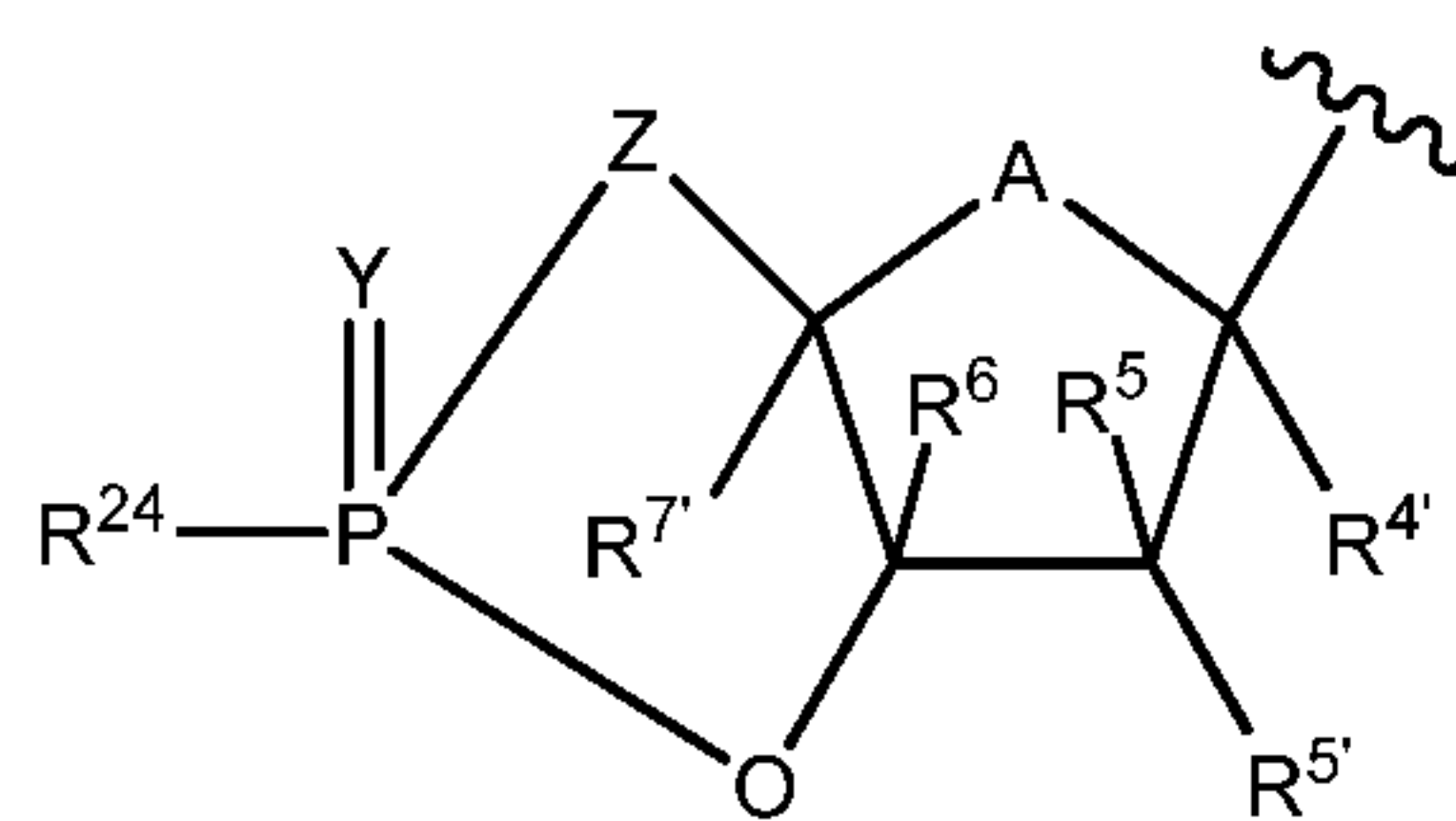
In another embodiment, $R^{7'}$ is, independently, selected from the group consisting of H, F, Cl, Br, I, N_3 , $C(O)OH$, CN, CH_2OH , $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, and R;

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:

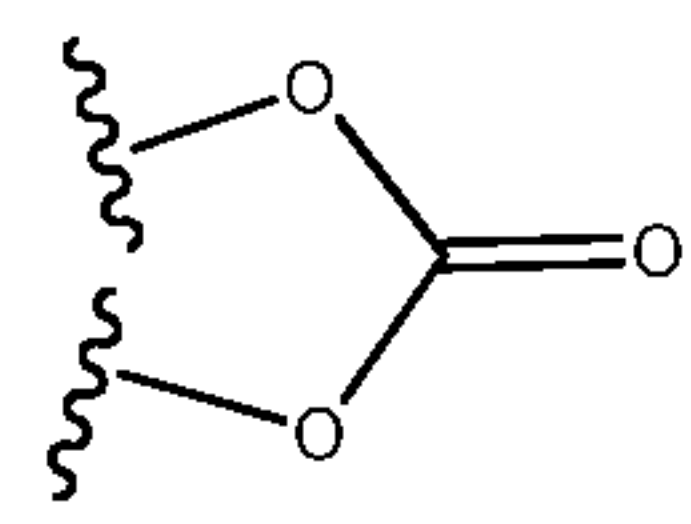
Y is O or S;

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

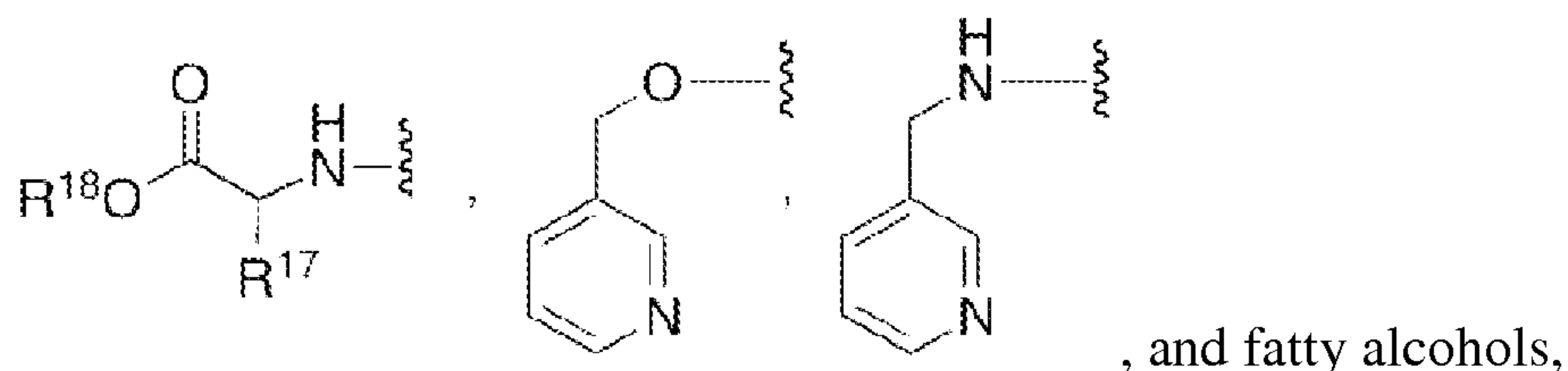


In one embodiment, where Sugar is formula (III) or (IV), when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2 , NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2 .

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} ,



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 $(CH_2)_{0-6}CON(R^{16})_2$;

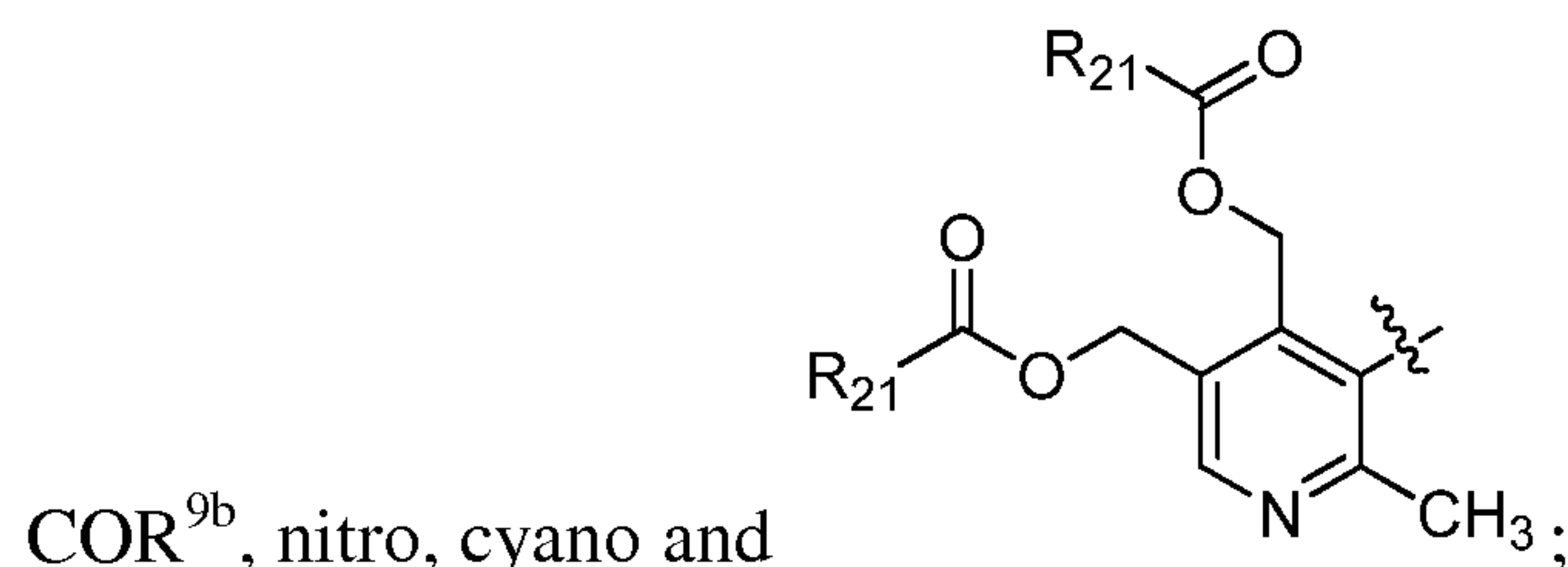
R^{17} is selected from those groups occurring in natural L-amino acids, C_{1-6} alkyl, (C_1-C_6) alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3-C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above.

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 C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or cycloalkyl.

Representative R² and R³ are independently selected from the group consisting of:

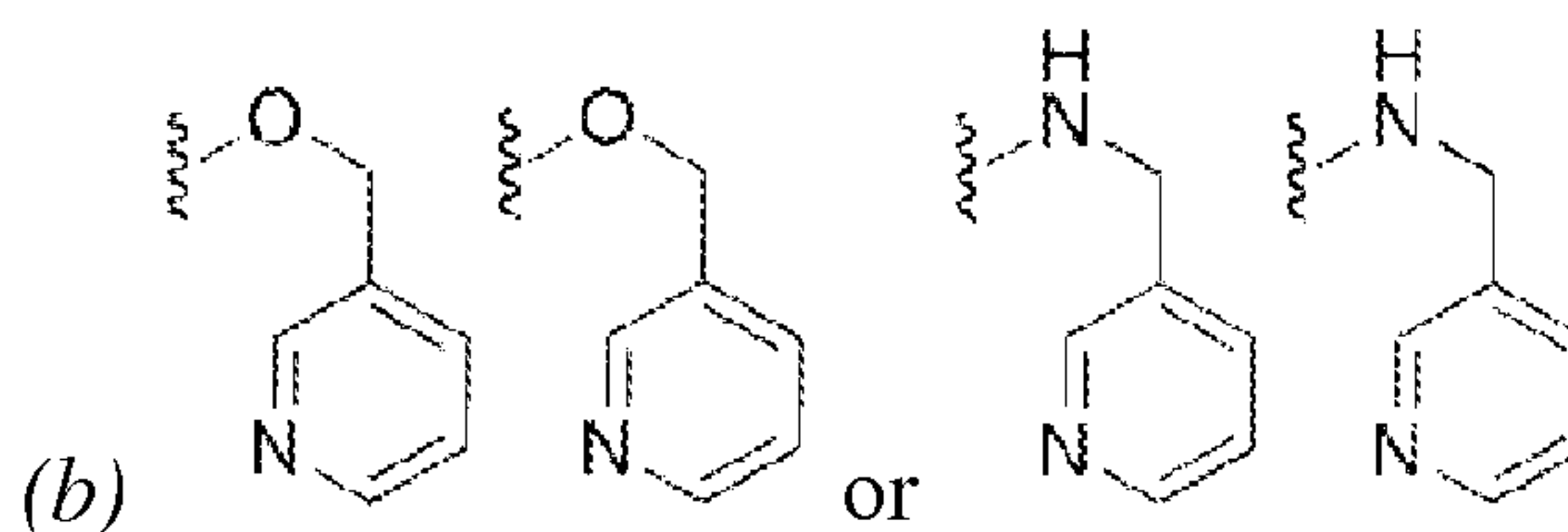
(a) OR⁸ where R⁸ is H, Li, Na, K, C₁₋₂₀ alkyl, C₃₋₆ cycloalkyl, C₁₋₆ haloalkyl, aryl, or heteroaryl which includes, but is not limited to, phenyl or naphthyl optionally substituted with one to three substituents independently selected from the group consisting of C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkoxy, (CH₂)₀₋₆CO₂R^{9a}, halogen, C₁₋₆ haloalkyl, -N(R^{9a})₂, C₁₋₆ acylamino, -NHSO₂C₁₋₆ alkyl, -SO₂N(R^{9a})₂, -SO₂C₁₋₆ alkyl,

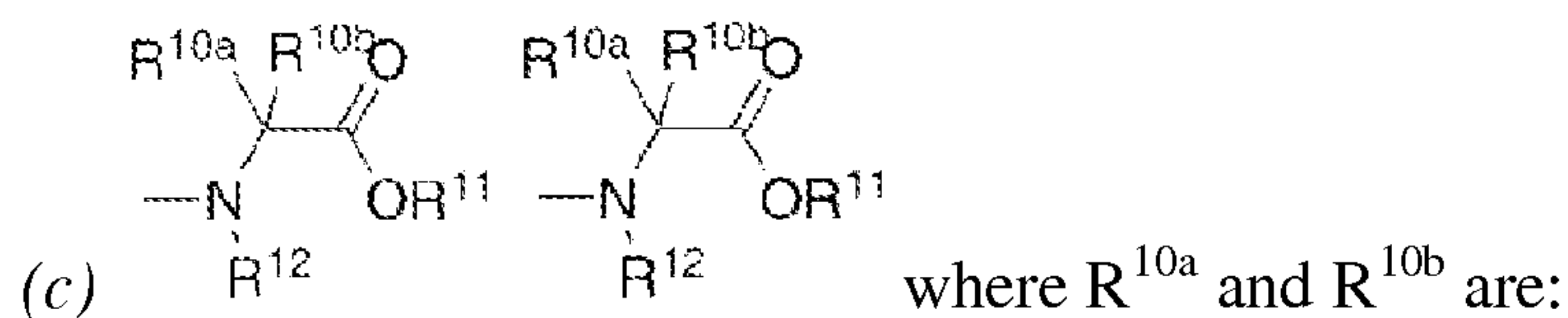


wherein R²¹ is as defined below;

R^{9a} is independently H, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

R^{9b} is -OR^{9a} or -N(R^{9a})₂;





(i) independently selected from the group consisting of H, C₁₋₁₀ alkyl, -(CH₂)_rNR^{9a}₂, C₁₋₆ hydroxyalkyl, -CH₂SH, -(CH₂)₂S(O)_pMe, -(CH₂)₃NHC(=NH)NH₂, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -(CH₂)_mCOR^{9b}, aryl and aryl-C₁₋₃ alkyl, said aryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, halogen, nitro, and cyano;

(ii) R^{10a} is H and R^{10b} and R¹² together are (CH₂)₂₋₄ to form a ring that includes the adjoining N and C atoms;

(iii) R^{10a} and R^{10b} together are (CH₂)_n to form a ring;

(iv) R^{10a} and R^{10b} both are C₁₋₆ alkyl; or

(v) R^{10a} is H and R^{10b} is H, CH₃, CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂-CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or C₃₋₁₀ cycloalkyl;

p is 0 to 2;

r is 1 to 6;

n is 4 or 5;

m is 0 to 3;

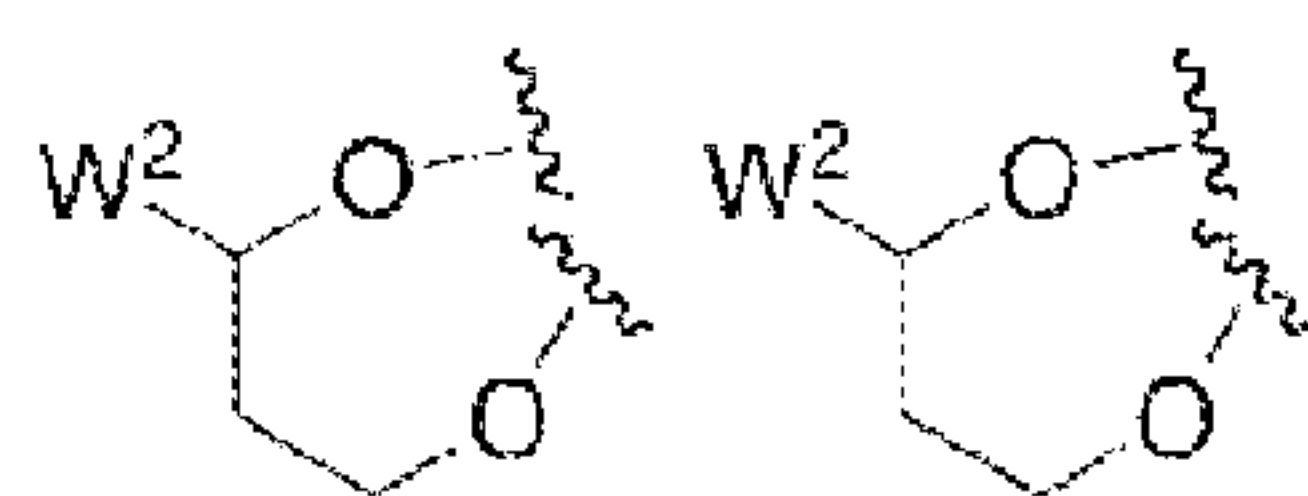
R¹¹ is H, C₁₋₁₀ alkyl, or C₁₋₁₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are

C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

R¹² is H or C₁₋₃ alkyl, or R^{10a}, or R^{10b} and R¹² together are (CH₂)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) R² and R³ can come together to form a ring



where W² 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 C₁₋₆ alkyl, CF₃, C₂₋₆ alkenyl, C₁₋₆ alkoxy, OR^{9c}, CO₂R^{9a}, COR^{9a}, halogen, C₁₋₆ haloalkyl, -N(R^{9a})₂, C₁₋₆ acylamino, CO₂N(R^{9a})₂, SR^{9a}, -NHSO₂C₁₋₆ alkyl, -SO₂N(R^{9a})₂, -SO₂C₁₋₆ 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:

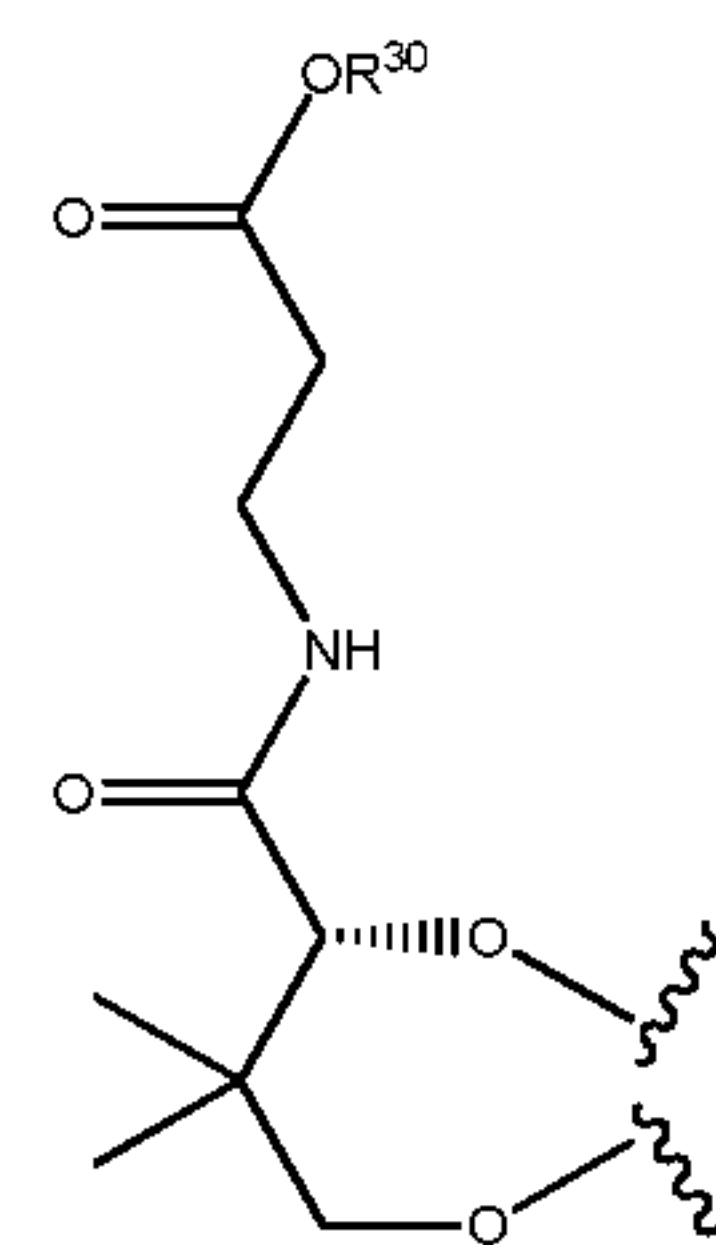
a) when there are two heteroatoms and one is O, then the other can not be O or S, and

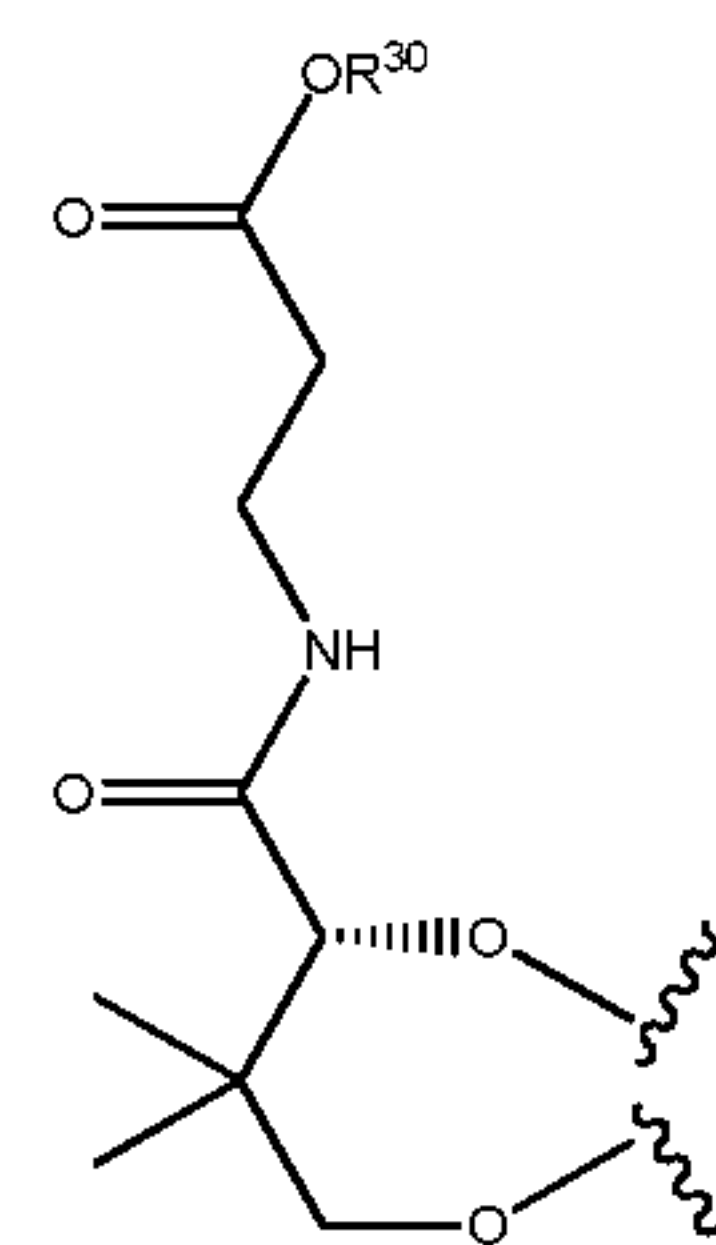
b) when there are two heteroatoms and one is S, then the other can not be O or S;

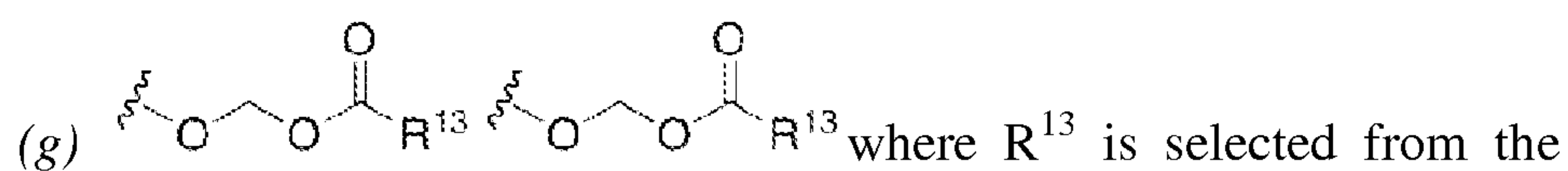
R^{9a} is independently H or C₁₋₆ alkyl;

R^{9b} is -OR^{9a} or -N(R^{9a})₂;

R^{9c} is H or C₁₋₆ acyl;

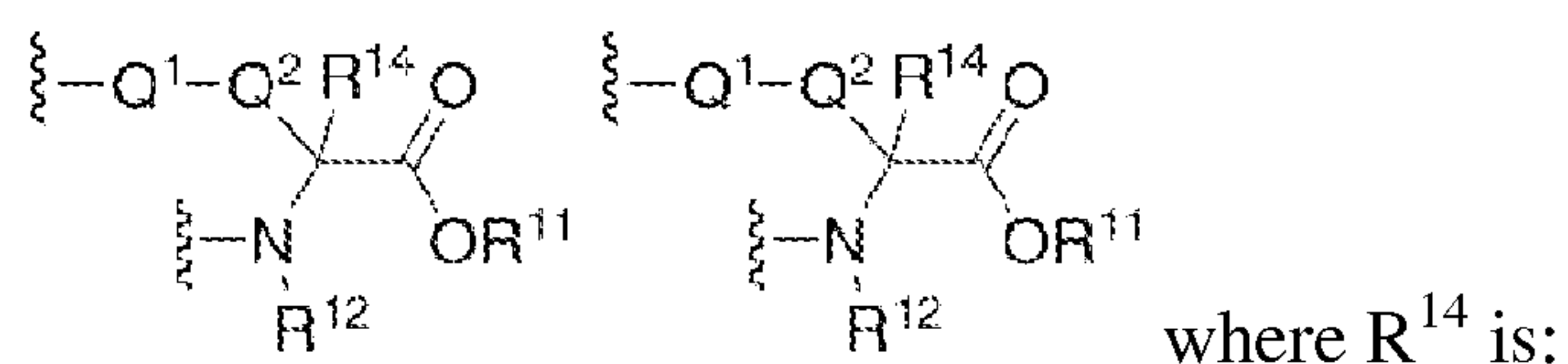


(f) R^2 and R^3 can come together to form a ring  where R^{30} is H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty alcohol or C_{1-20} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl,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



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



(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, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, halogen, nitro, and cyano;

(ii) R¹⁴ is H, CH₃, CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or C₃₋₁₀ cycloalkyl;

p is 0 to 2;

r is 1 to 6;

m is 0 to 3

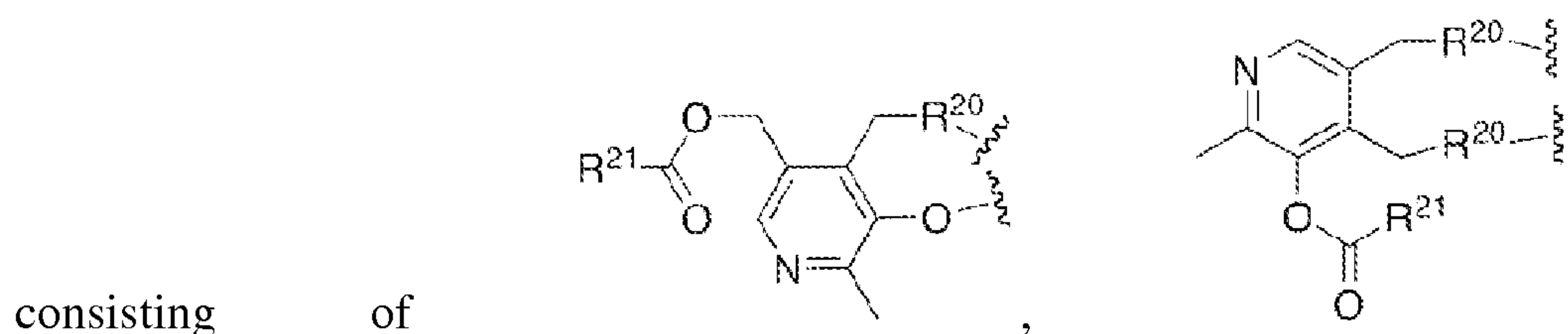
Q¹ is NR^{9a}, O, or S

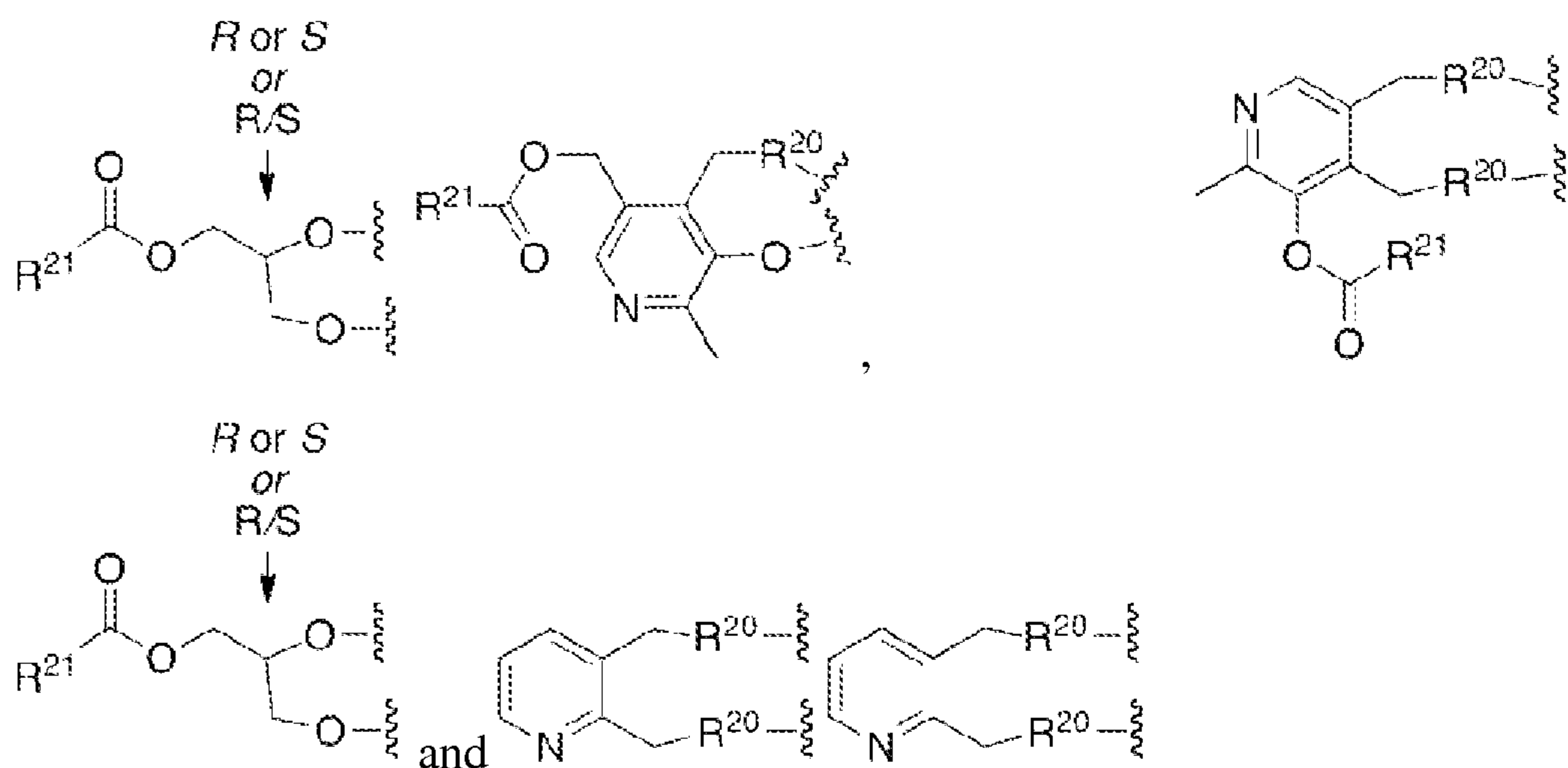
Q² is C₁₋₁₀ alkyl, C₁₋₆ hydroxyalkyl, aryl and aryl-C₁₋₃ alkyl, heteroaryl and heteroaryl-C₁₋₃ alkyl, said aryl and heteroaryl groups optionally substituted with a group selected from the group consisting of hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, fluoro, and chloro;

R¹¹ is H, C₁₋₁₀ alkyl, C₁₋₁₀ alkyl optionally substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

R¹² is H or C₁₋₃ alkyl, or R^{14b} and R¹² together are (CH₂)₂₋₄ so as to form a ring that includes the adjoining N and C atoms;

(i) R² and R³ can come together to form a ring selected from the group



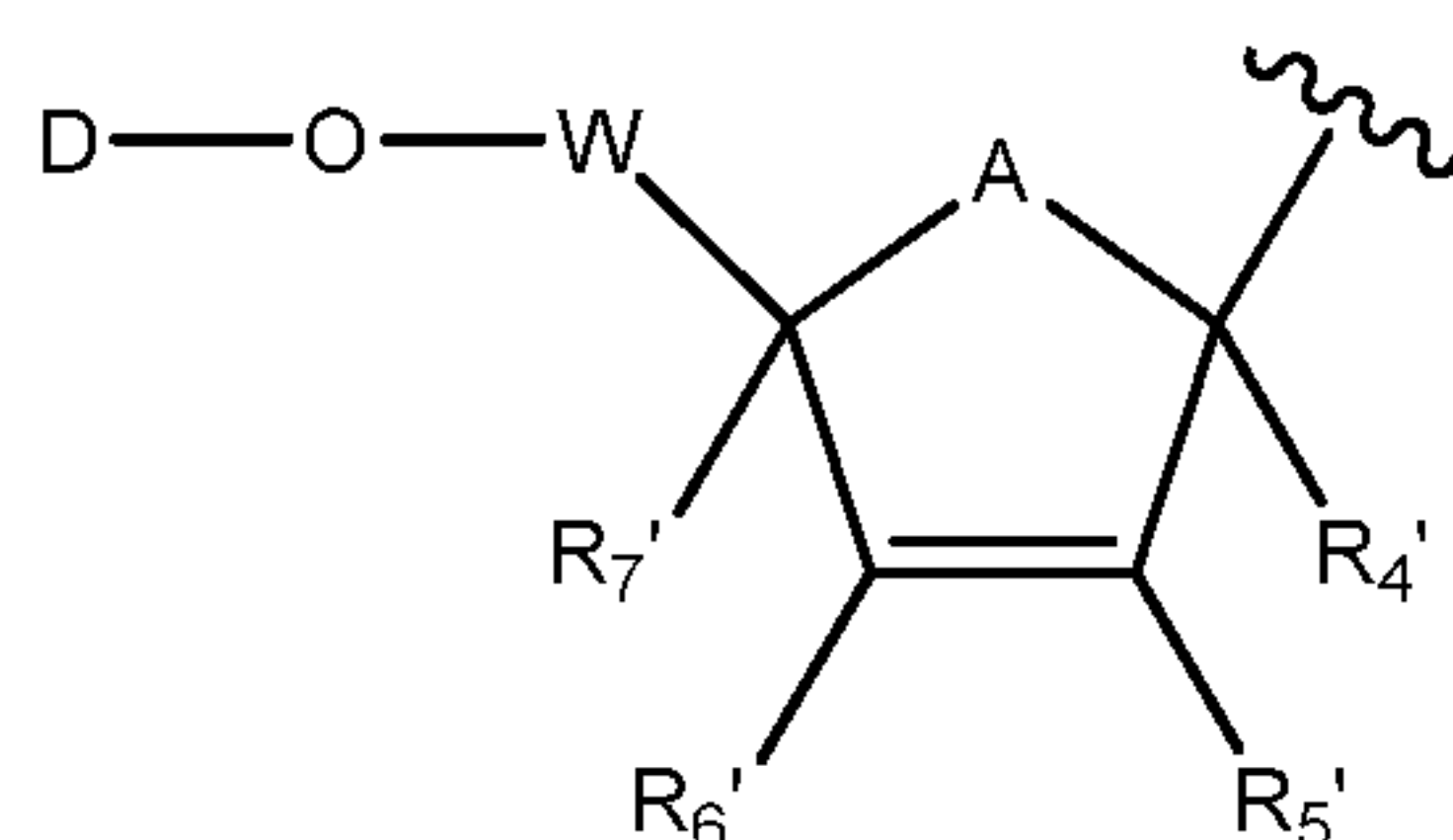


where R^{20} is O or NH, and

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, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

W is CL₂ or CL₂CL₂, wherein L independently is selected from the group consisting of H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A, R², R³, 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, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, or NR₂,

In another embodiment, R^{7'} is, independently, selected from the group consisting of H, F, Cl, Br, I, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, and R;

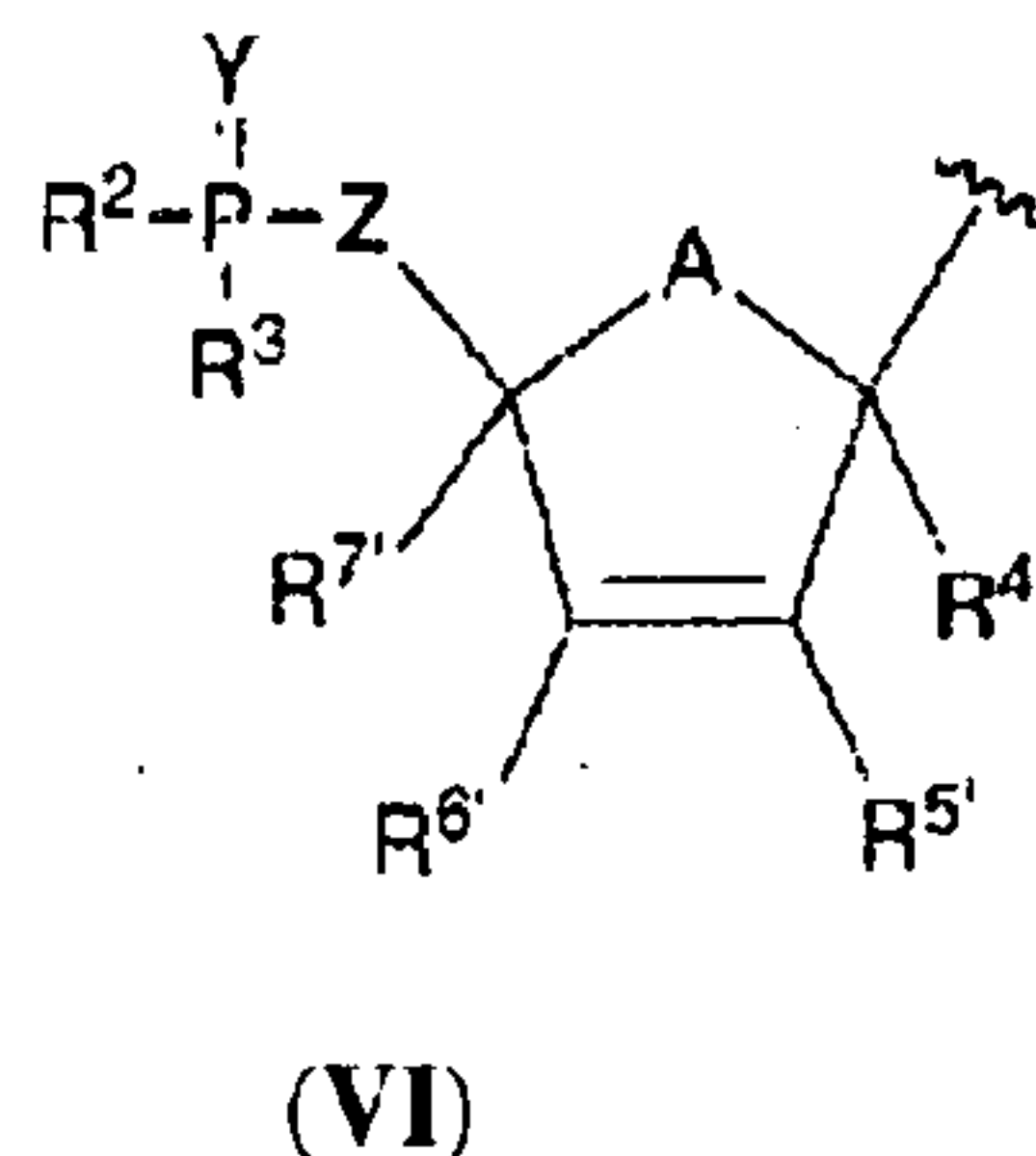
wherein R is independently C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₆ cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in connection with Formulas I, II, III and IV, for example, hydroxyalkyl, aminoalkyl, and alkoxyalkyl.

In one embodiment, where Sugar is of Formula (V), when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H then, R^{5'} and R^{6'} cannot be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

In another embodiment, R⁵ and R⁶ are independently selected from the group consisting of NHOH, NHNH₂, N₃, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

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



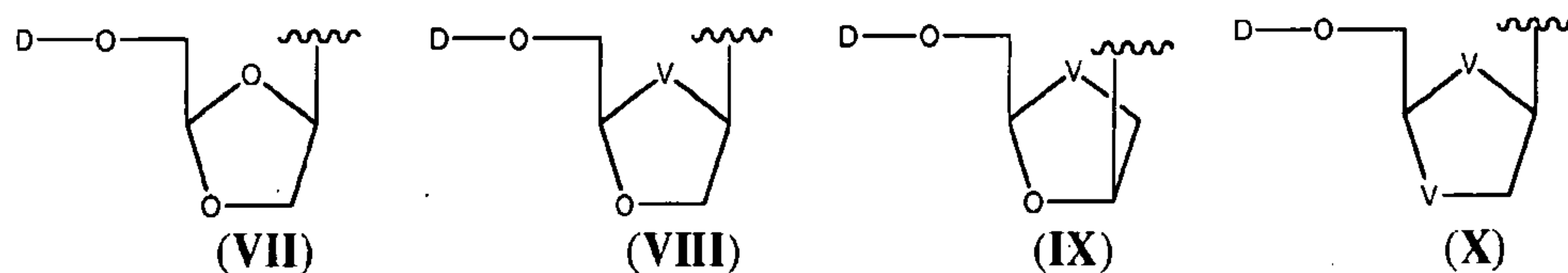
wherein:

A, R², R³, 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, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, or NR₂,

wherein R is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ 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):



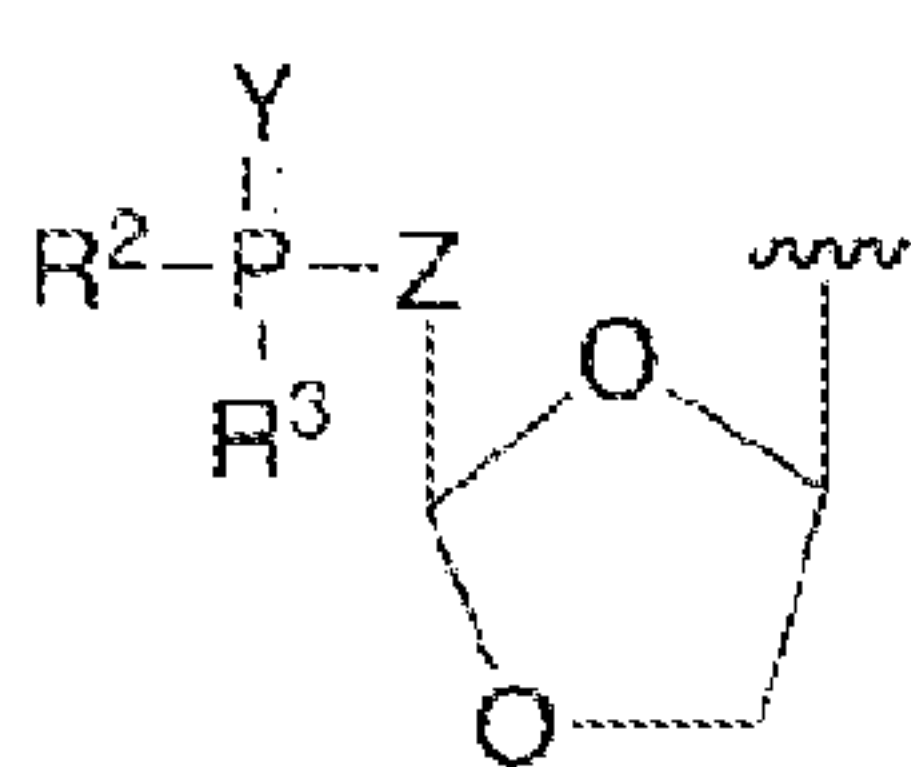
D is H, C(O)OR¹, diphosphate ester, or triphosphate ester;

V is, individually, S or Se;

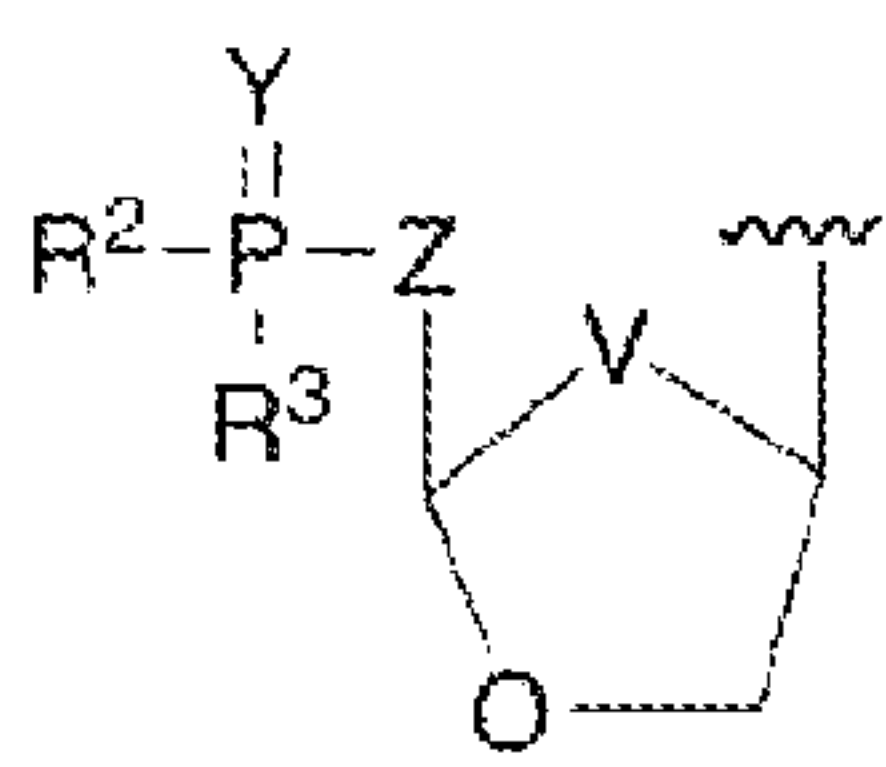
R¹ is independently C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-

C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

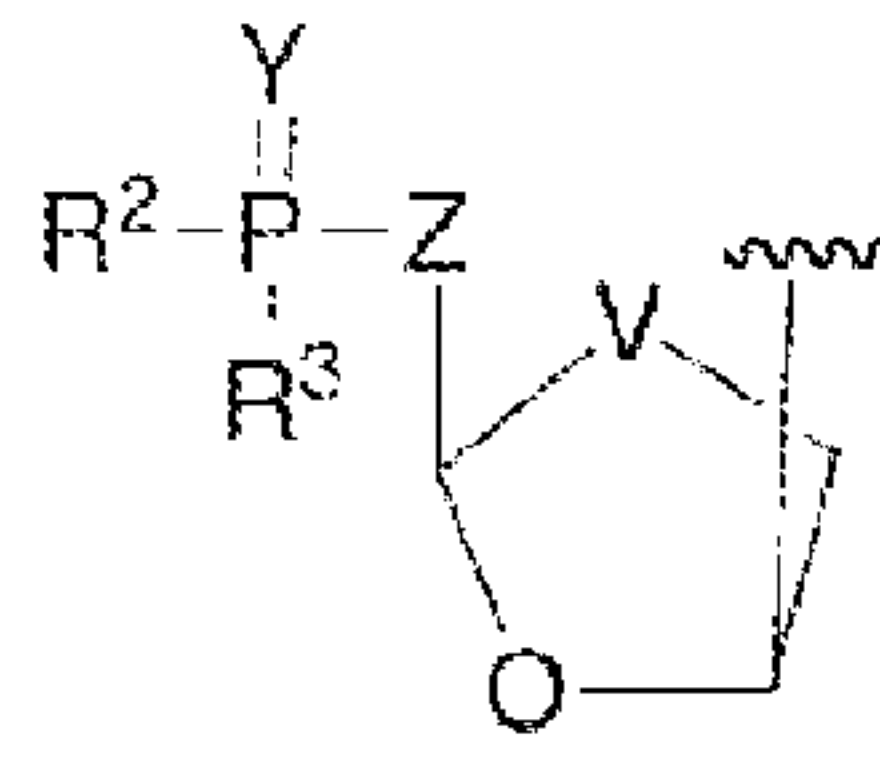
In yet another embodiment, Sugar is a dioxolane, an oxathiolane, or a dithiolane of the general Formulas (XI), (XII), (XIII), and (XIV):



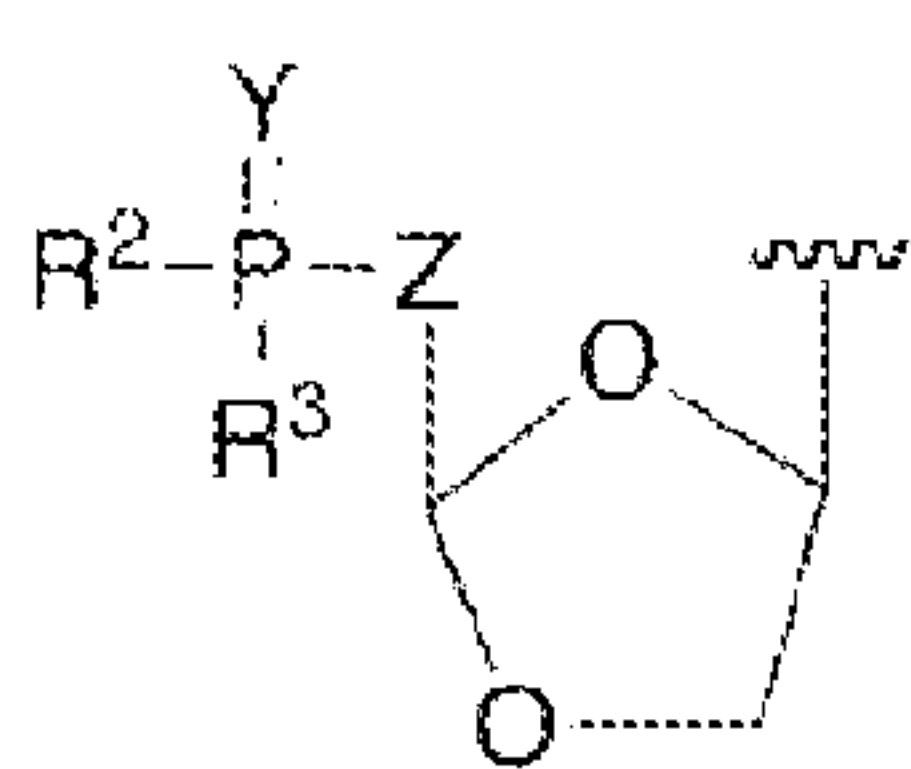
(XI)



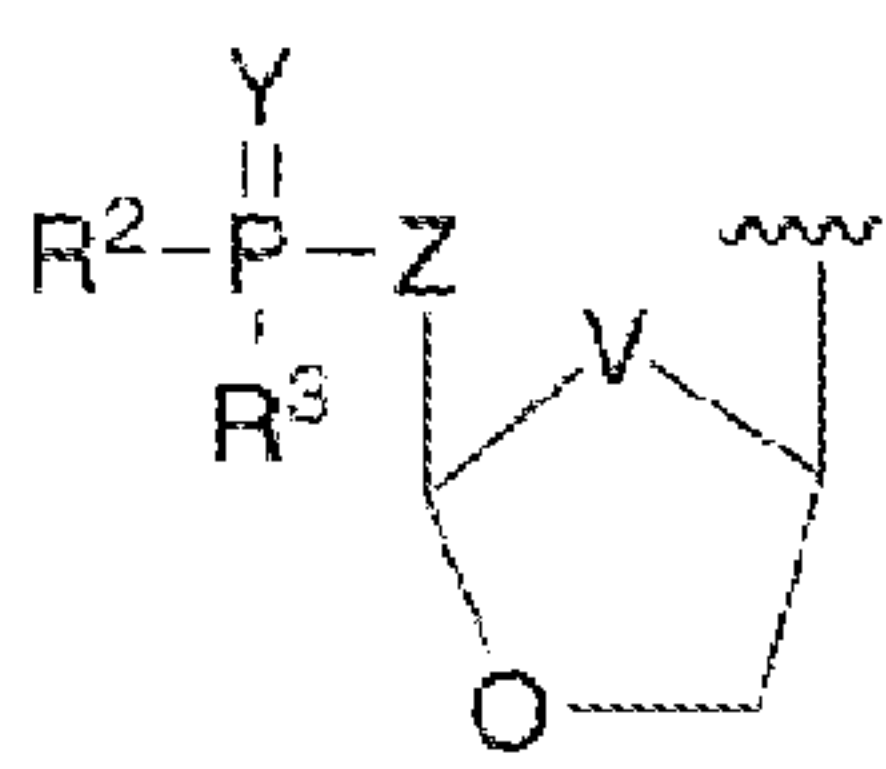
(XII)



(XIII)



(XIV)

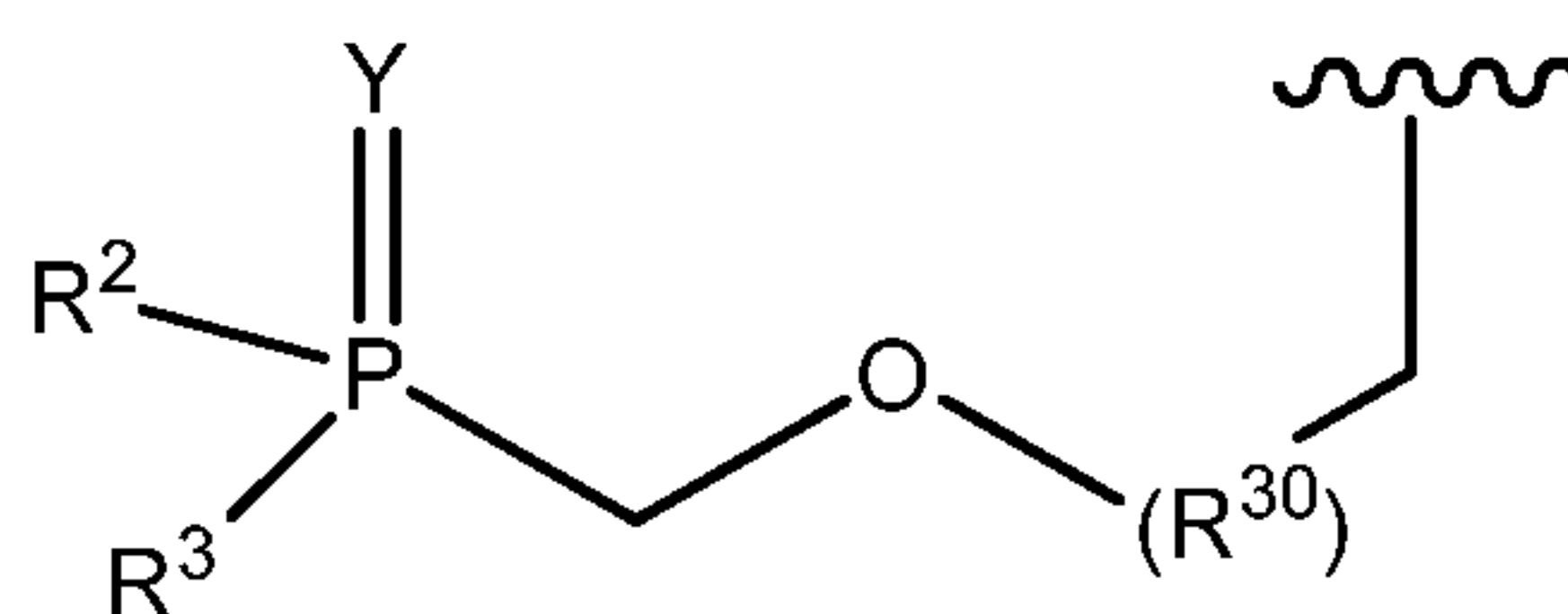


wherein:

V is, individually, S or Se;

R², R³, 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):



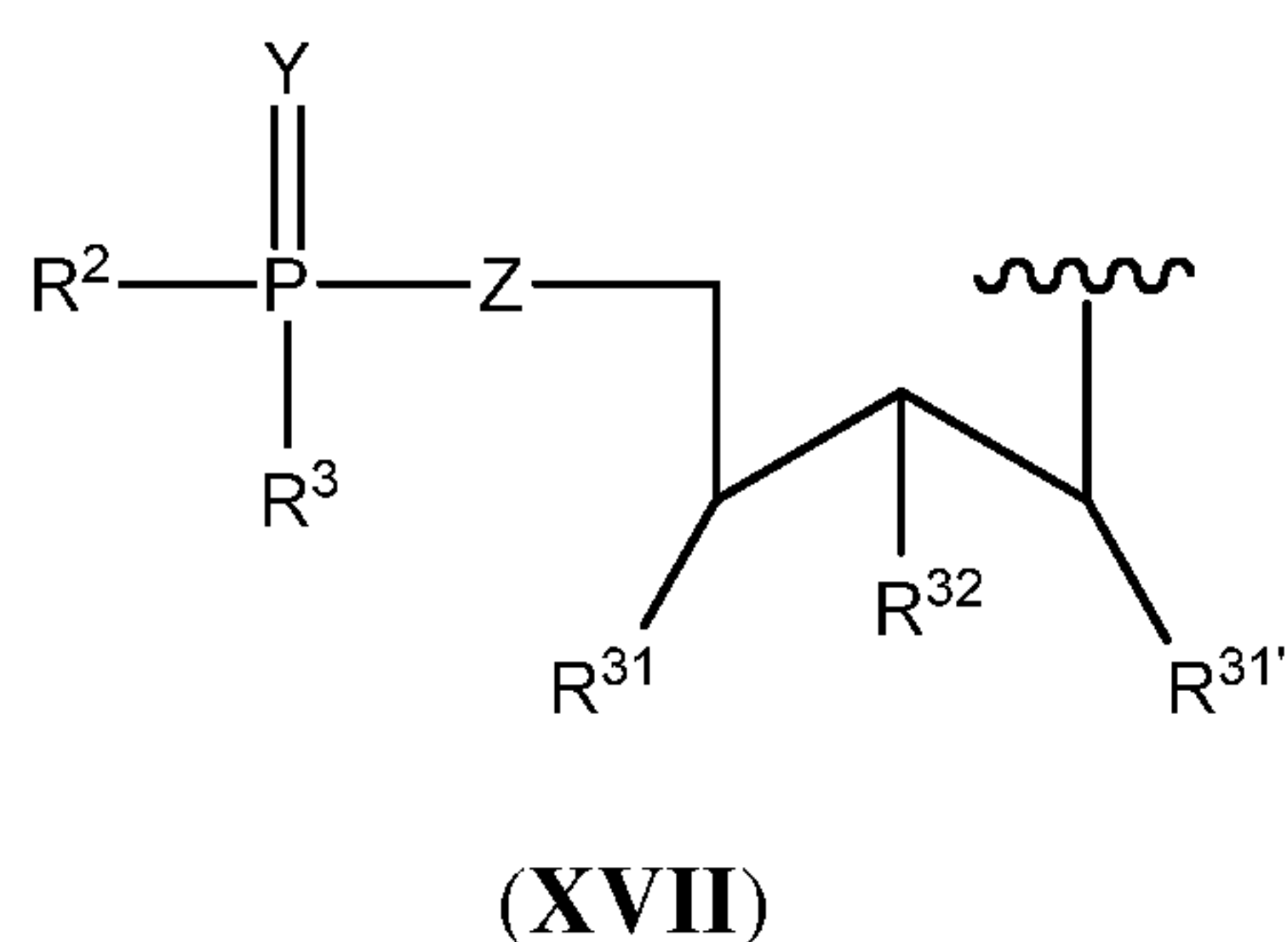
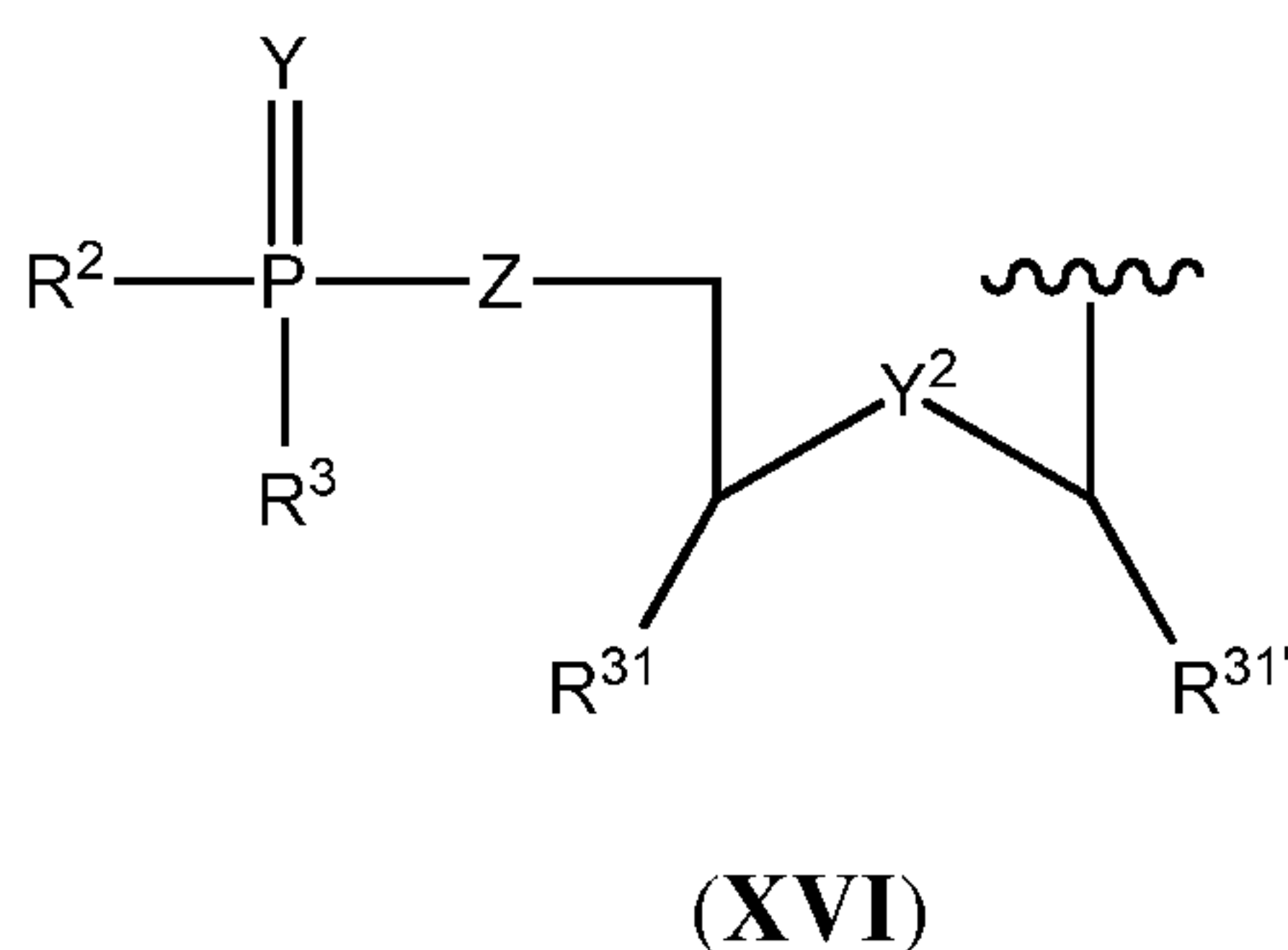
(XV)

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):



wherein:

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

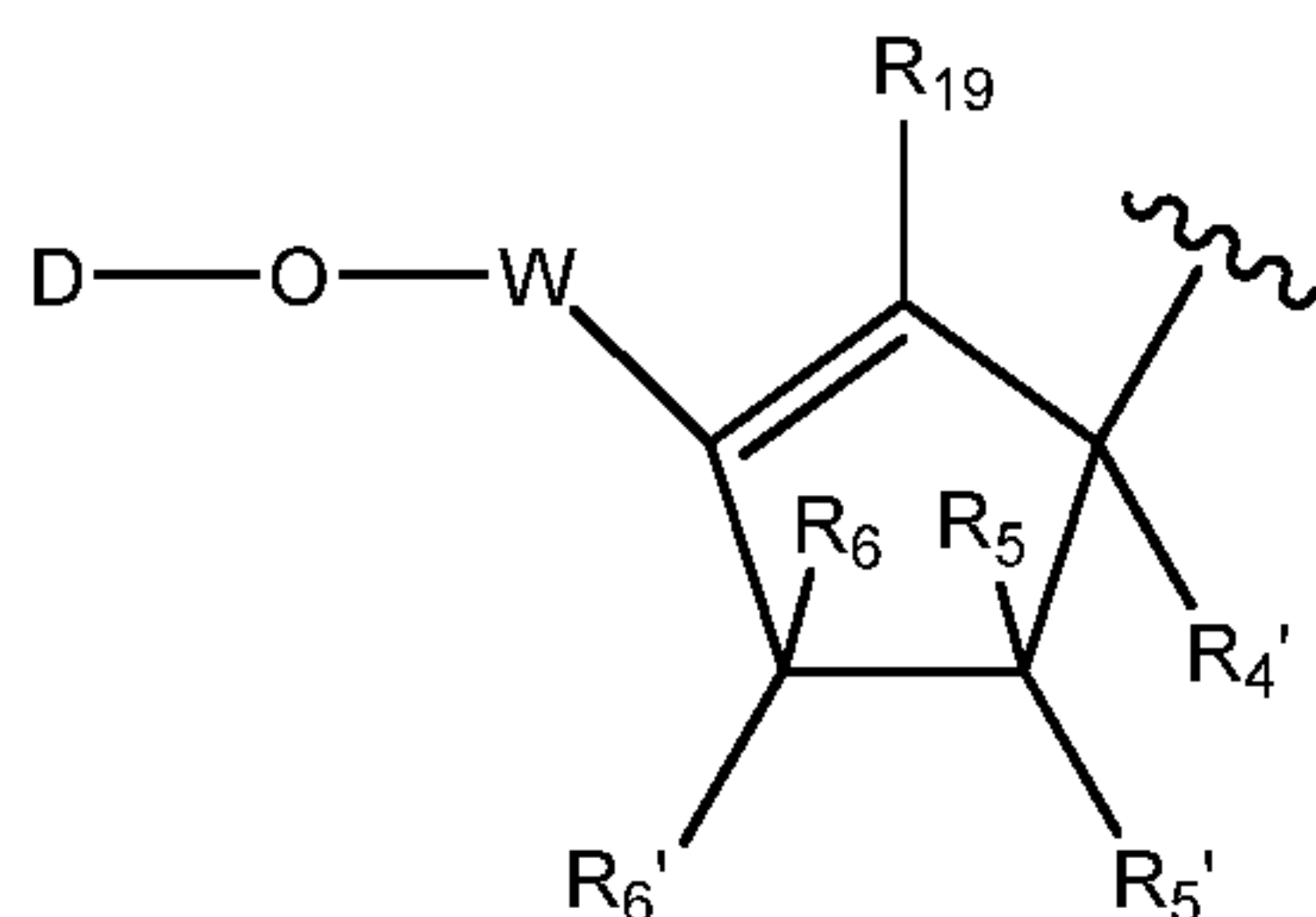
Y^2 is O, S, Se, or NR;

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;

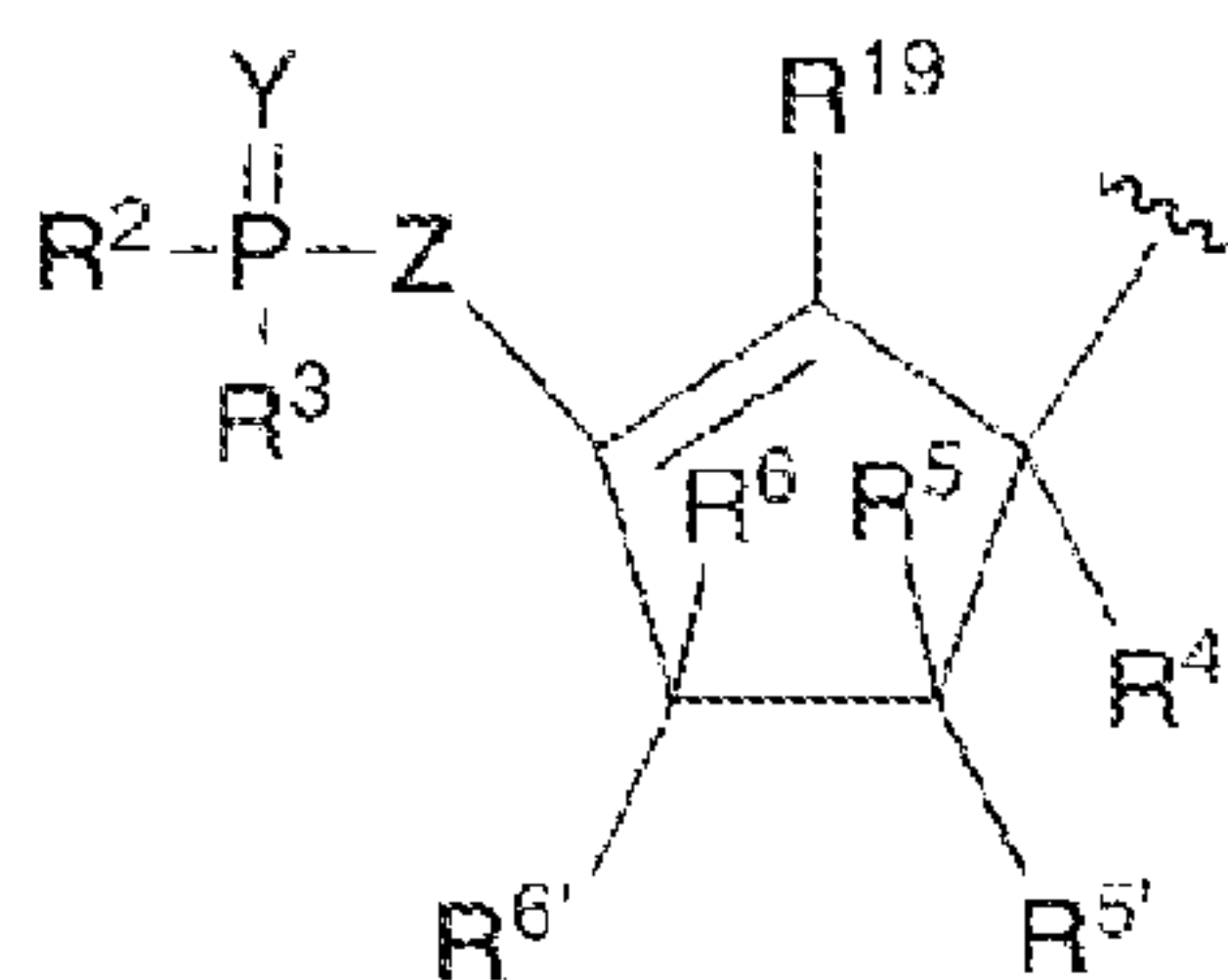
R^{19} is H, F, Cl, Br, I, N_3 , $C(O)OH$, CN, $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, or R;

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:

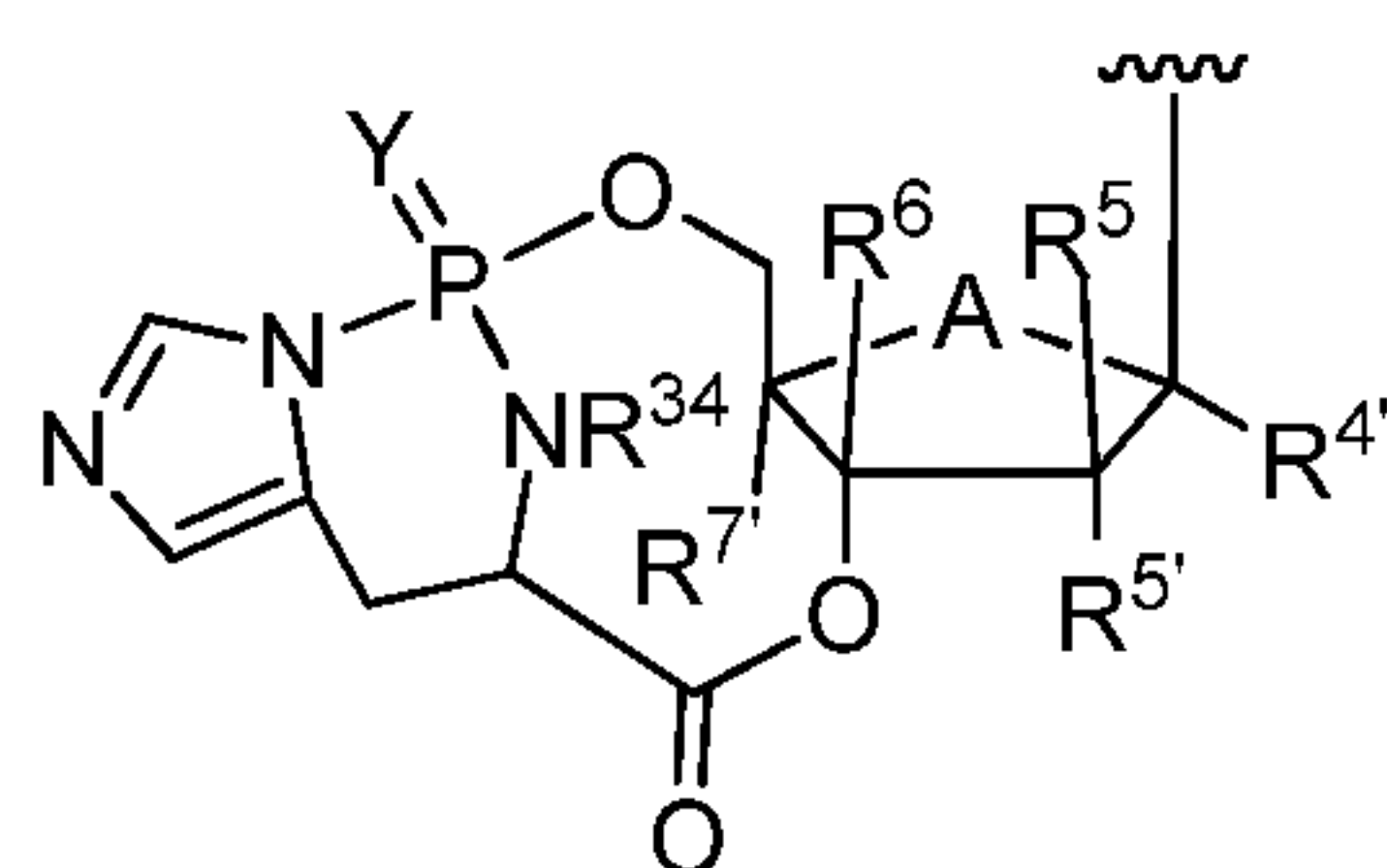
R^2 , R^3 , and Y are as defined above with respect to Formulas I, II, III and IV;

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

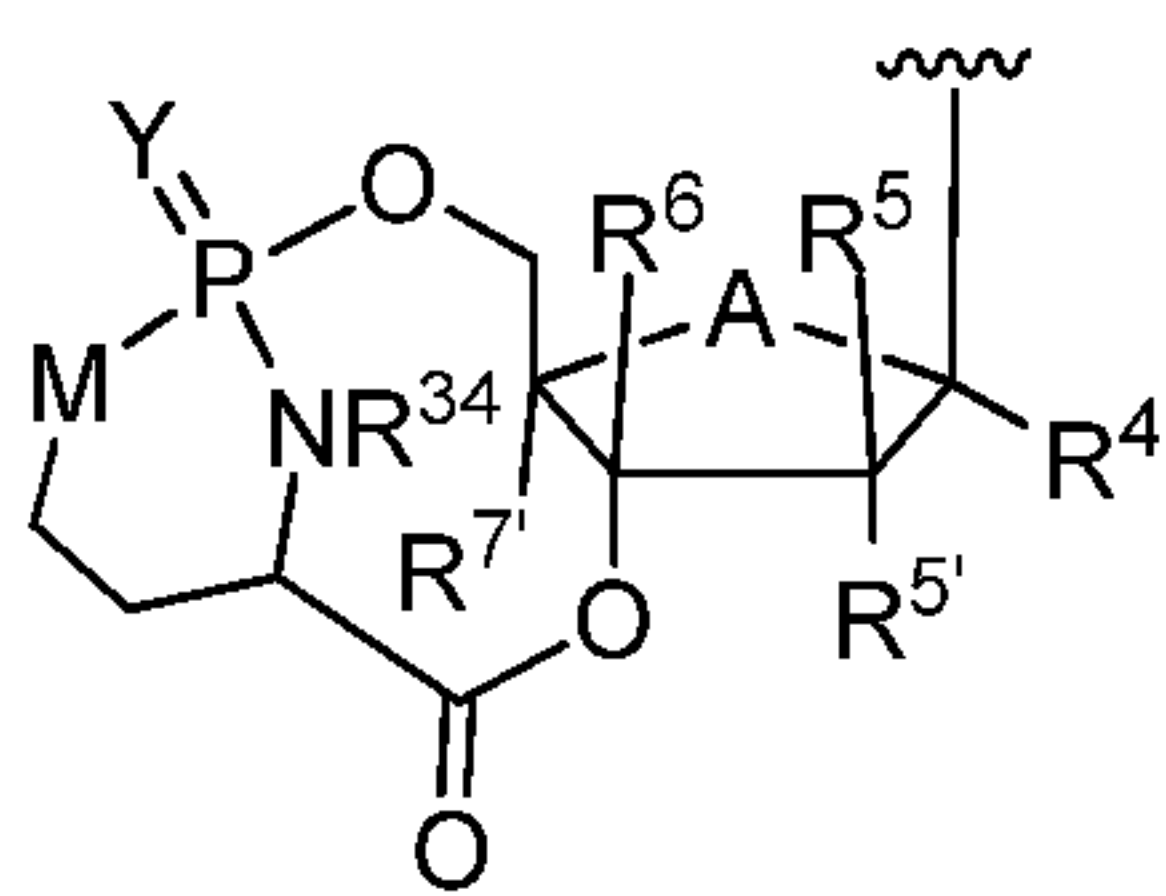
R^{19} is H, F, Cl, Br, I, N_3 , $C(O)OH$, CN, $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, or R,

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.

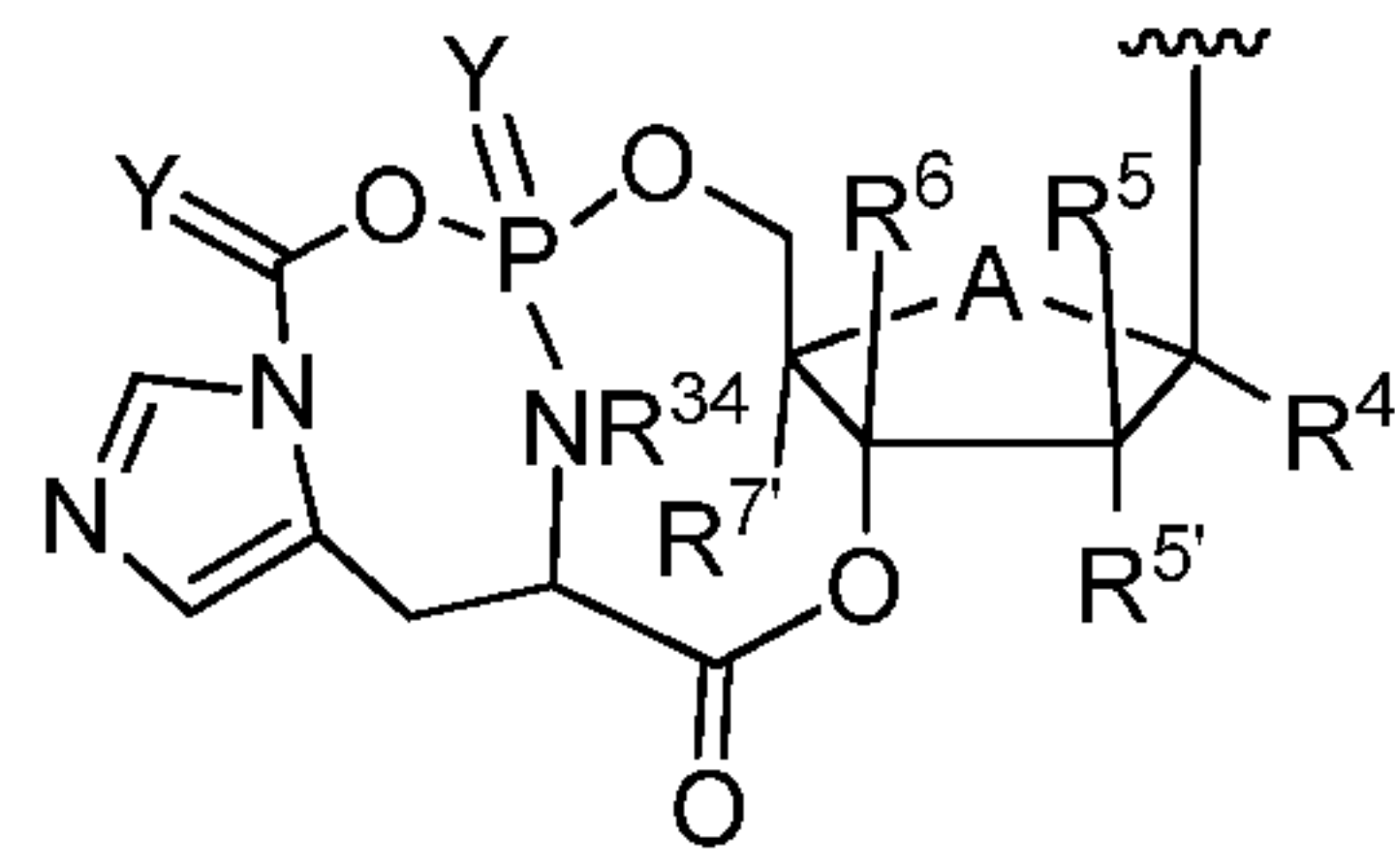
In yet another embodiment, Sugar has one of the Formulas (XX), (XXI), or (XXII):



(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;

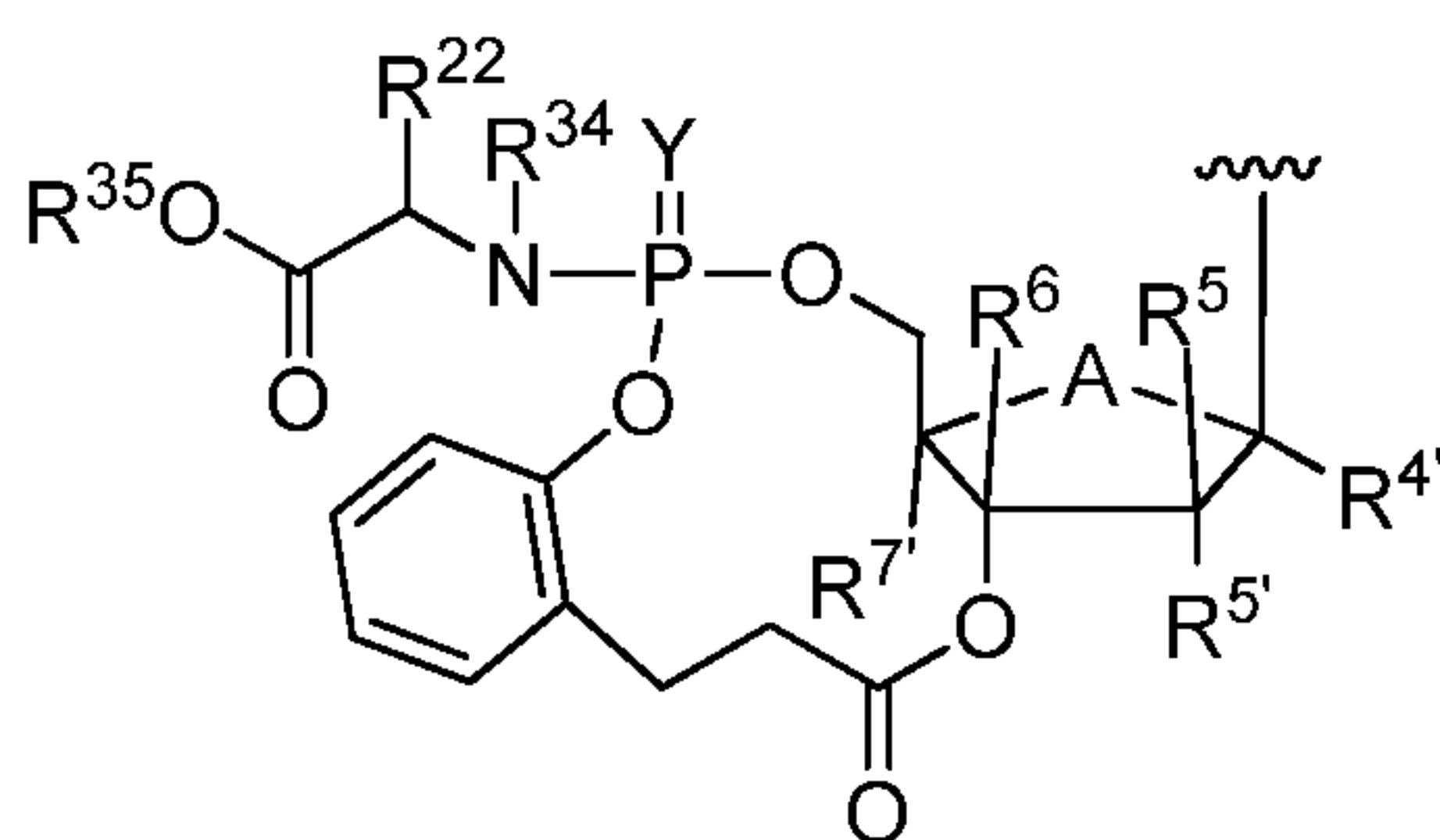
R^{34} is C_1 - C_6 alkyl;

M is O, S, or NR;

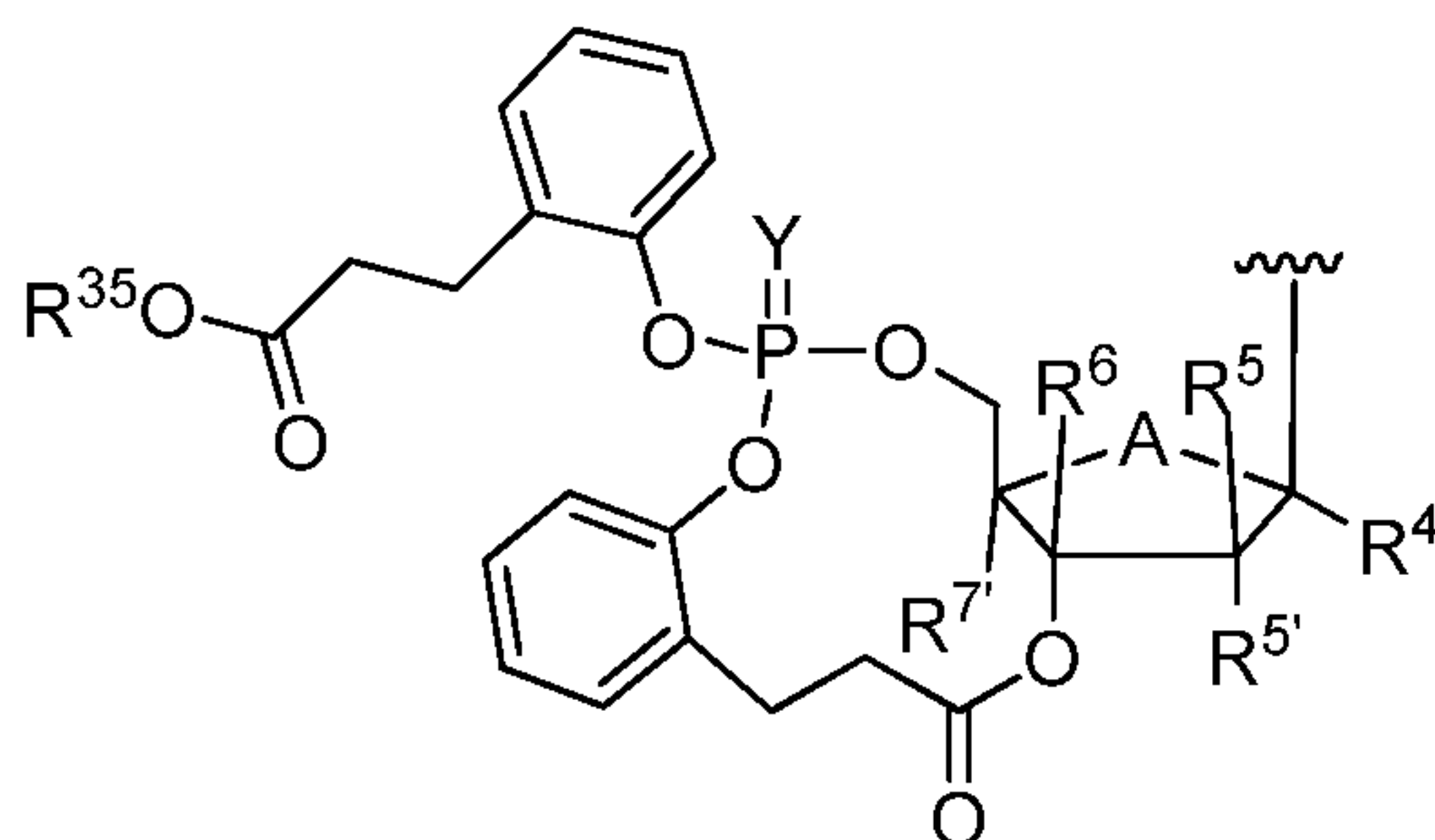
wherein R is, independently, C_1 - C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3 - C_6 cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more

substituents as defined above in 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)

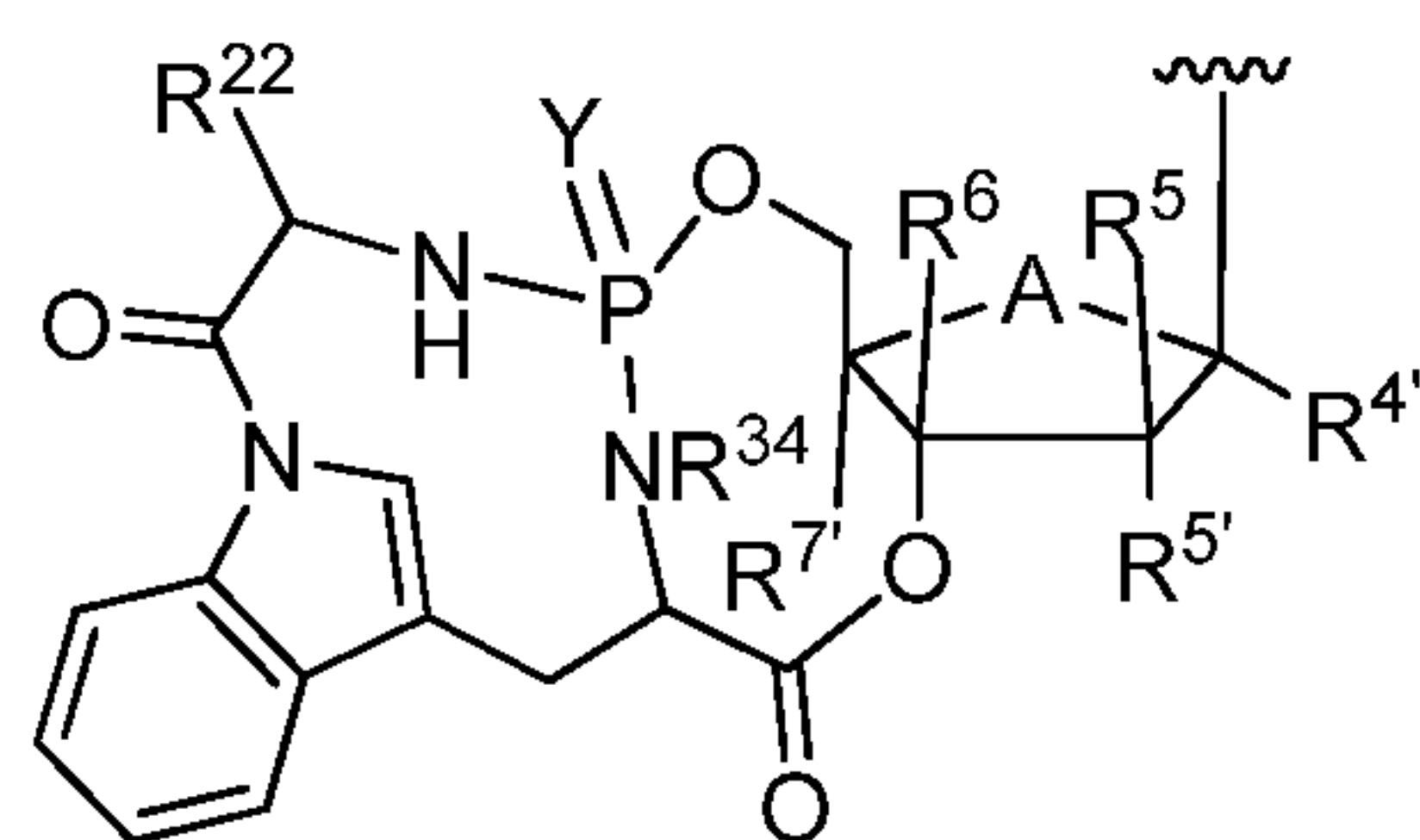
wherein:

$R^{4'}$, 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;

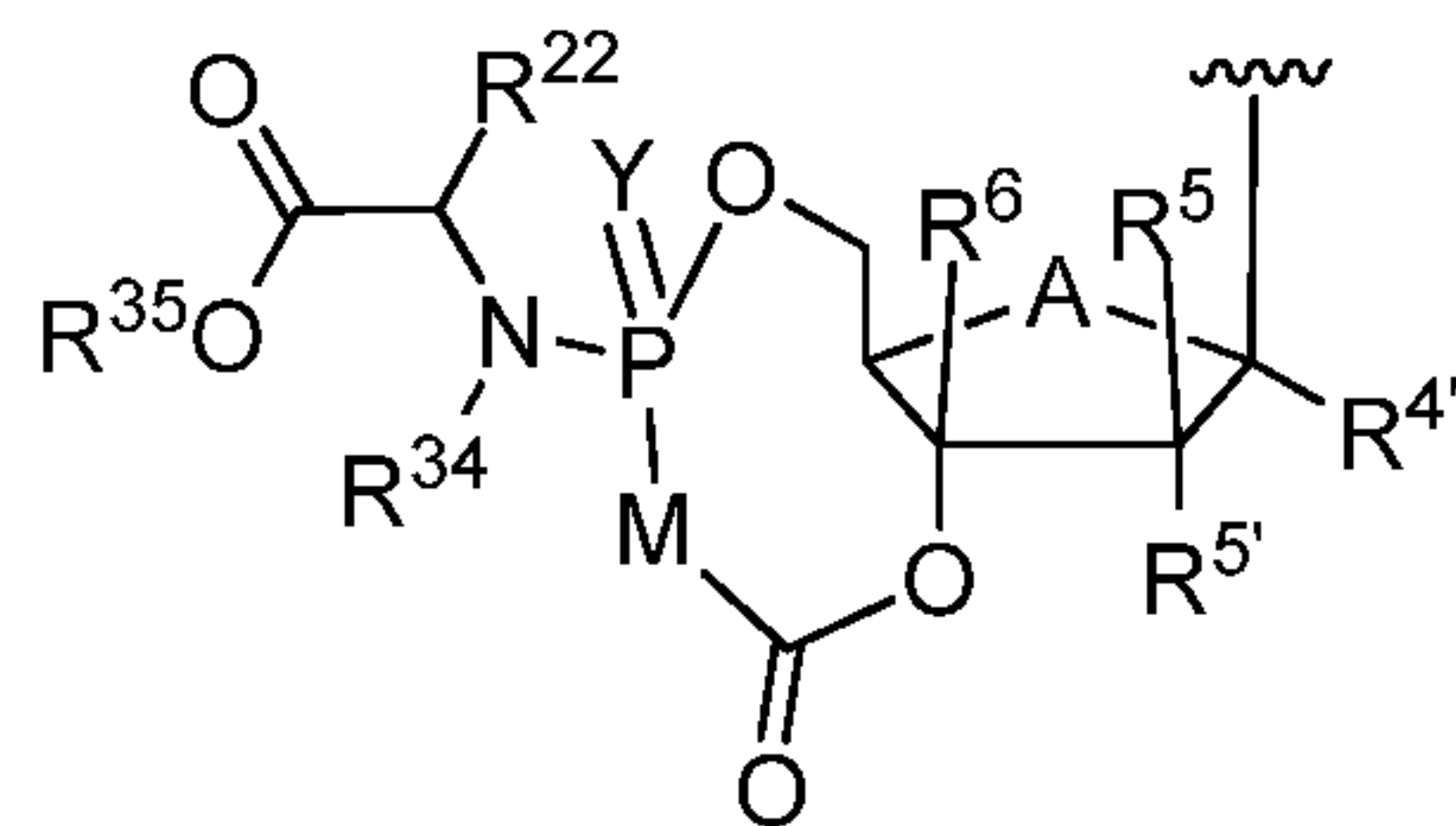
R^{35} is H, C_{1-10} alkyl, C_{1-10} alkyl optionally substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl;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

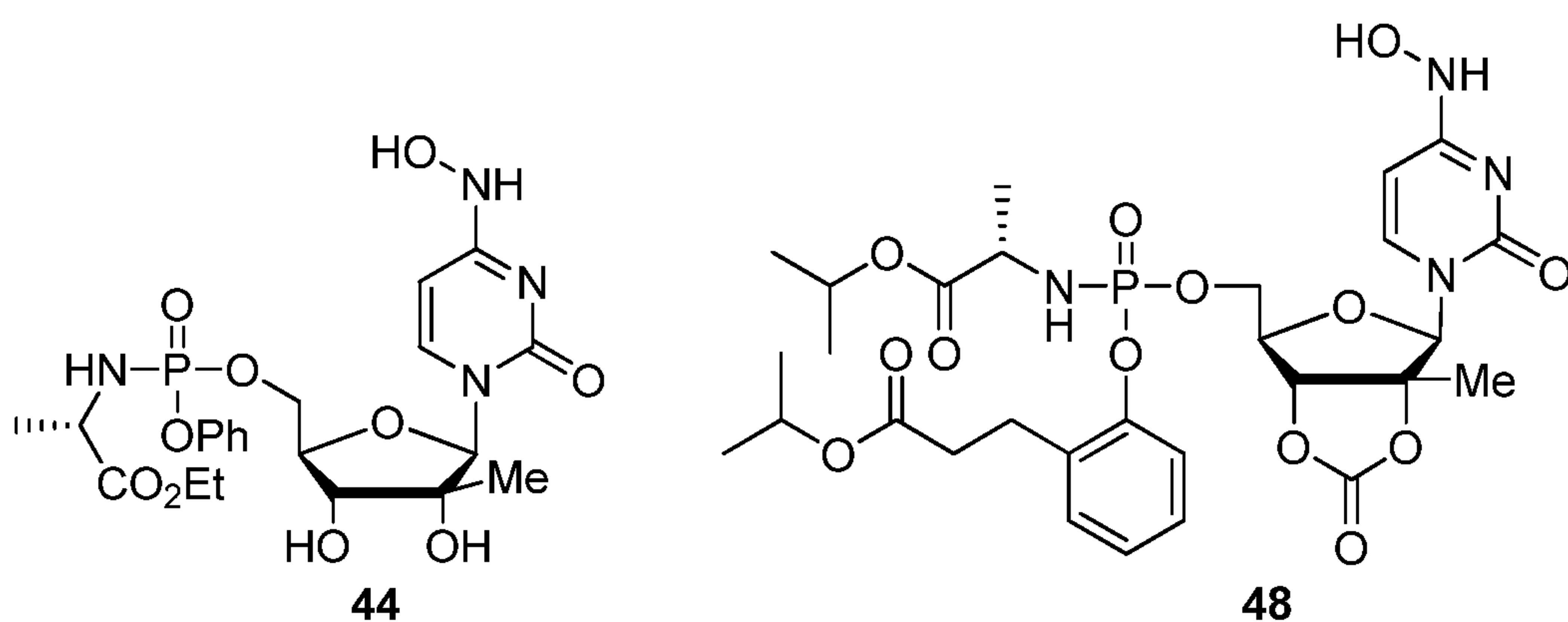
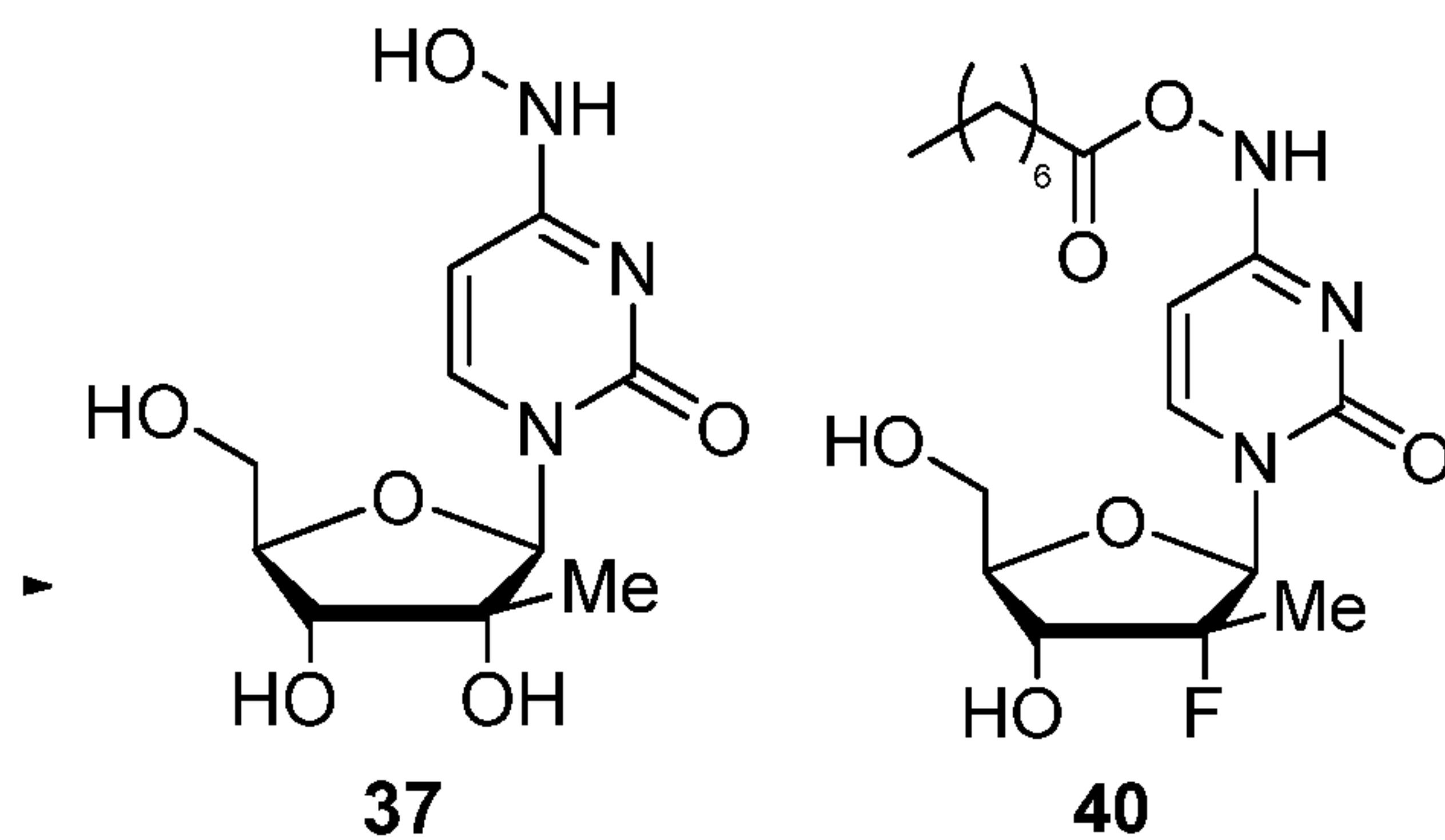


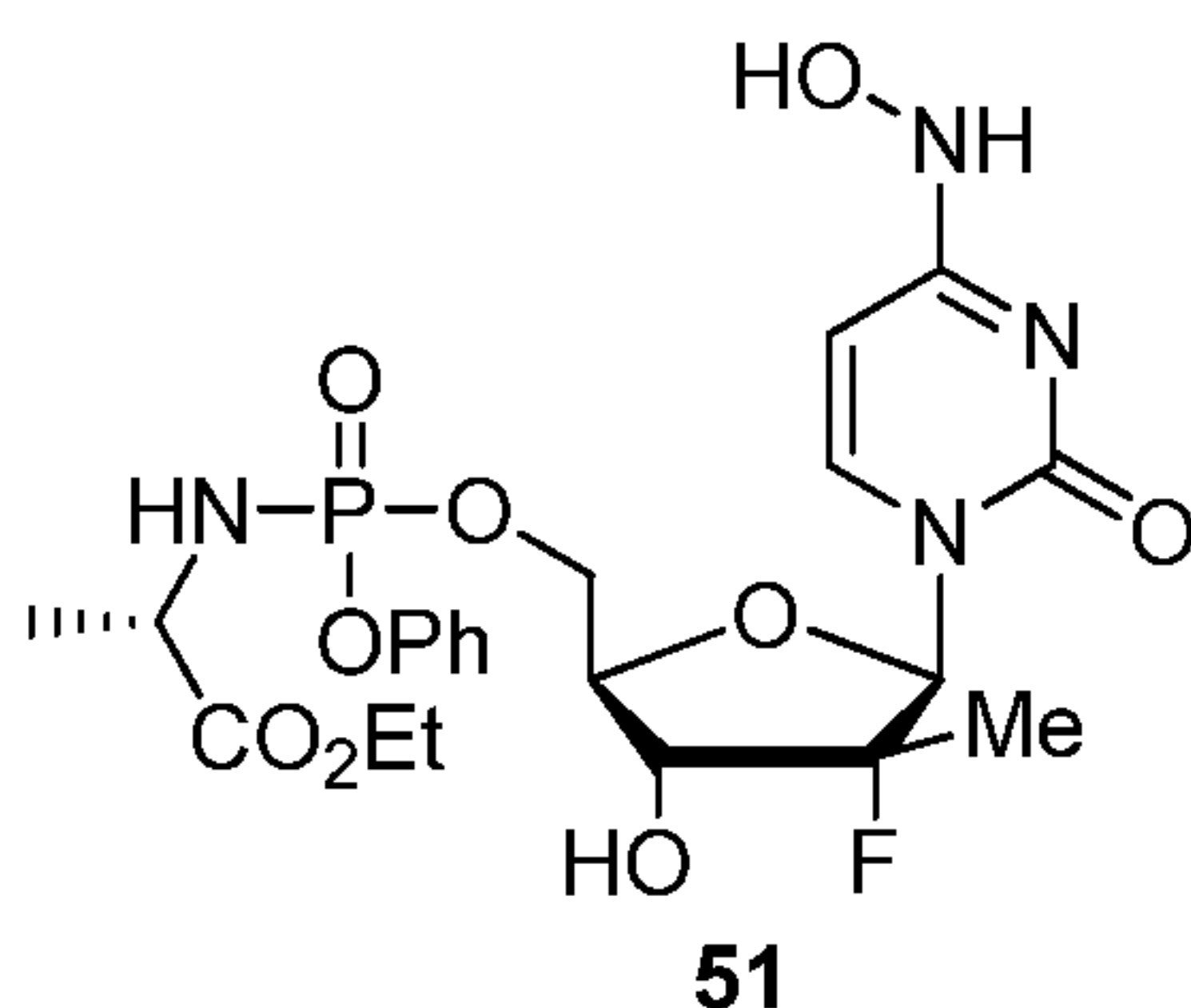
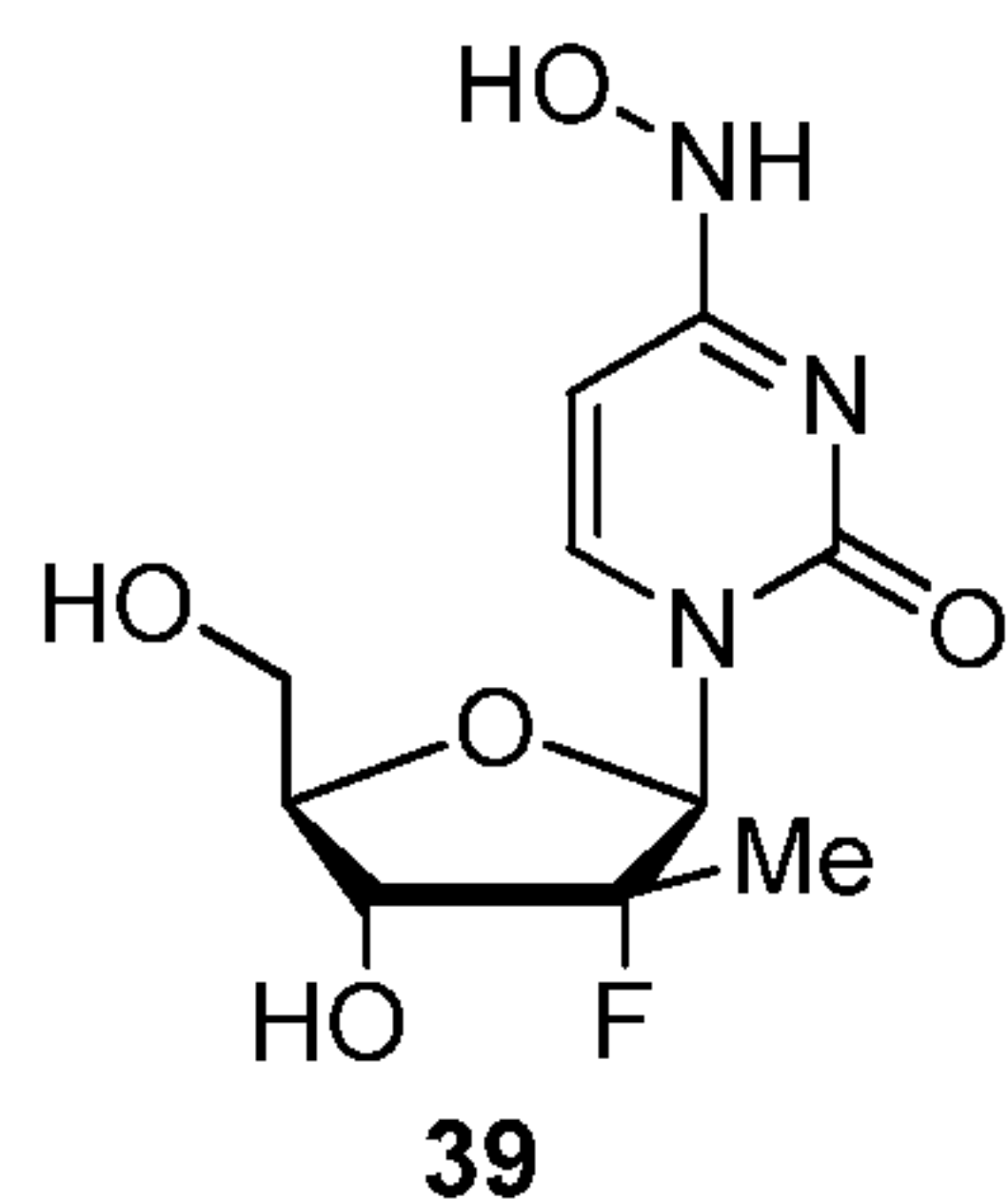
(XXVI)

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:





, 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.

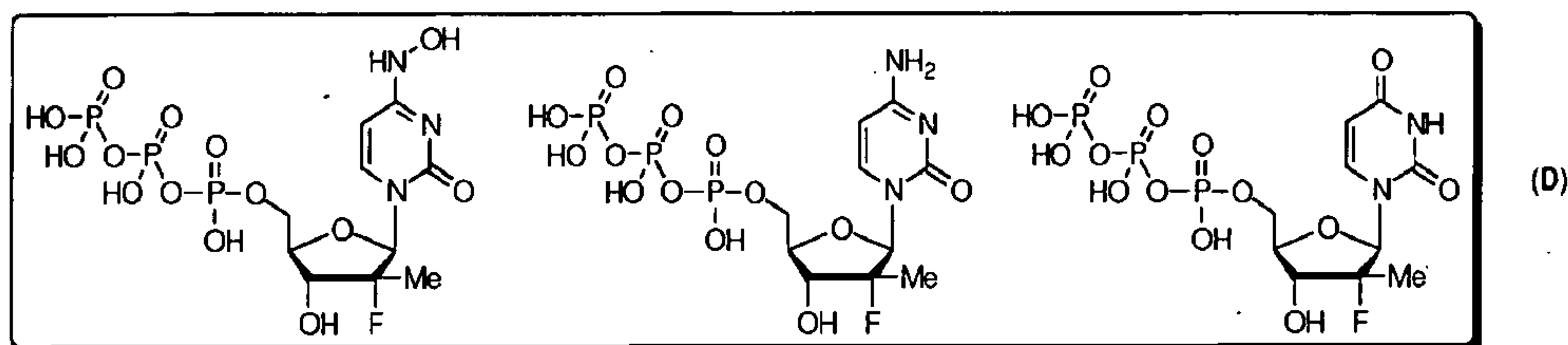
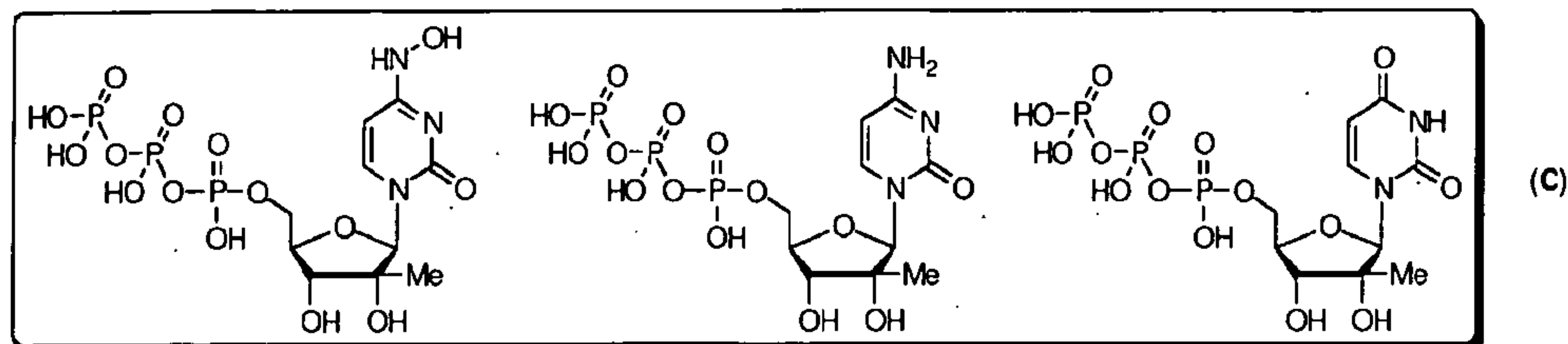
In another embodiment, the base is a pyrimidine, and one of R^5 and $R^{5'}$ is OH, Cl, or F.

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 -NH₂ moiety, and, optionally, partial conversion of the -NHOH moiety or the resulting -NH₂ 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-NH₂ 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.

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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 moiety is converted to an NH₂ 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 vice 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 analog 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 R² and/or R³ 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 lymphomas, 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-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 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 CELEBREXTM (celecoxib), 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 31, 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, HERCEPTINTM (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 (Imperial 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 Pharmaceuticals, 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 lymphocyte 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 interferon alfa-2b)	interferon	Schering-Plough
egasys peginterferon alfa-2a)	interferon	Roche
ovir-HBV lamivudine; 3TC)	nucleoside analogue	GlaxoSmithKline
epsera (Adefovir ipivoxil)"	nucleotide analogue	Gilead Sciences
ntriava® (emtricitabine; TC)	nucleoside analogue	Gilead Sciences http://www.hivandhepatitis.com/advertisement/triangle.html
ntecavir	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
IT (telbivudine)	nucleoside analogue	Idenix/Novartis

Drug Name	Drug Class	Company
S-1220	nucleoside analogue	Emory University
heradigm	Immune stimulant	Epimmune
adaxin (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
ILdC (valtorcitabine)	nucleoside analogue	Idenix
IN 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 antibodies		

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®	tipranavir	TPV	PNU-140690	Boehringer Ingelheim
Reyataz®	atazanavir		BMS-232632	Bristol-Myers Squibb
	brexnavir		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
<u>Zerit</u> ®	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
			<u>TMC-278</u>	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	Apoptosis regulation	Idun Pharma.

XTL-002	Monoclonal 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	Monoclonal Antibody	<u>XTL</u>
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, hexamethylmelamine, 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 μ M, preferably about 1.0 to 15 μ M. 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 stearyl phosphatidyl ethanolamine, stearyl 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-dimethylaminopropyl)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**.

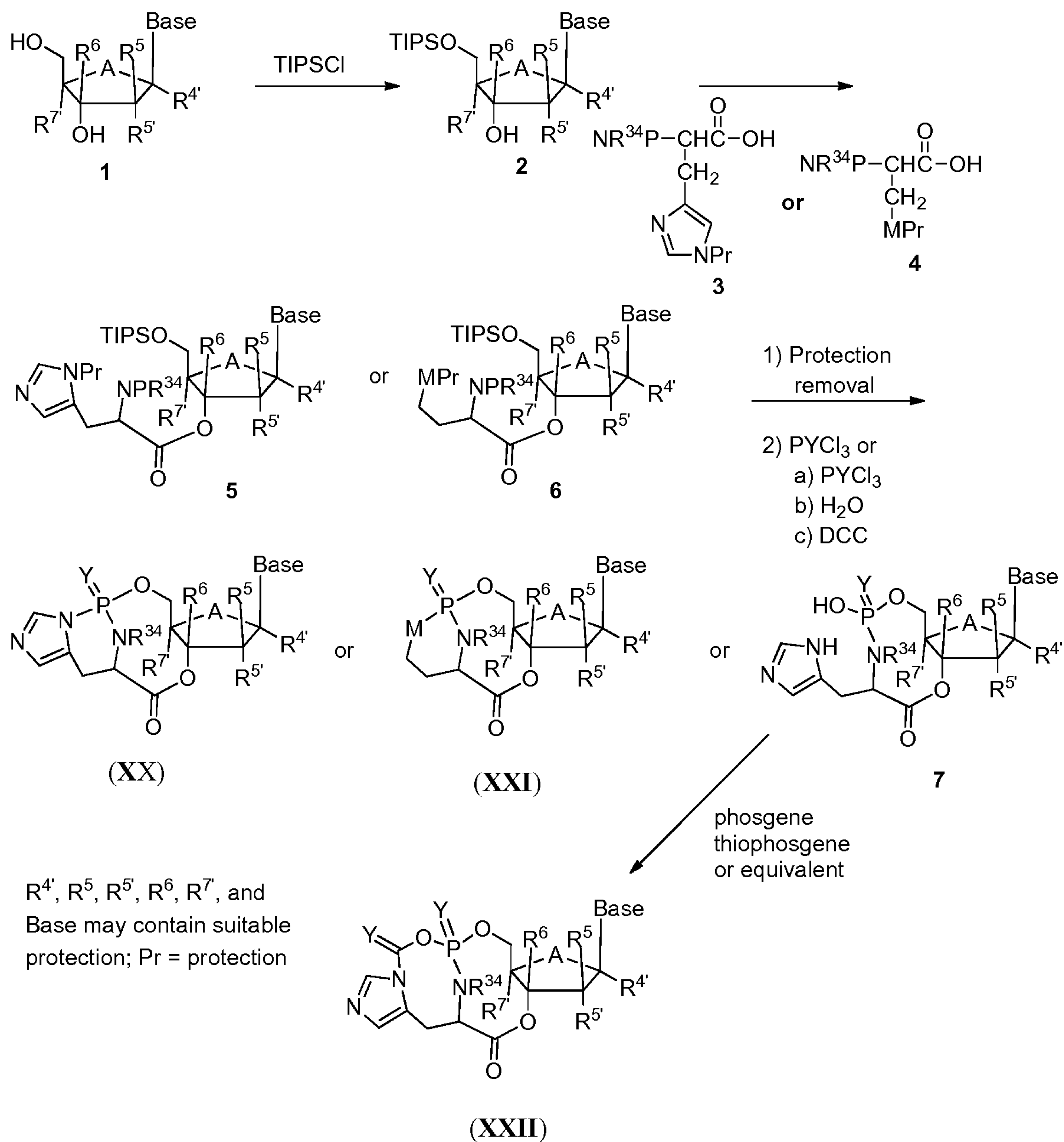
Scheme 20 is a non-limiting example of the synthesis of active compounds of the present invention, and, in particular, a synthetic approach to active compounds of the present invention, and, in particular, a synthetic approach to N^4 -hydroxycytidine 2'-C-Me nucleoside prodrugs **54** and **56**.

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 Chemotherapy: 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

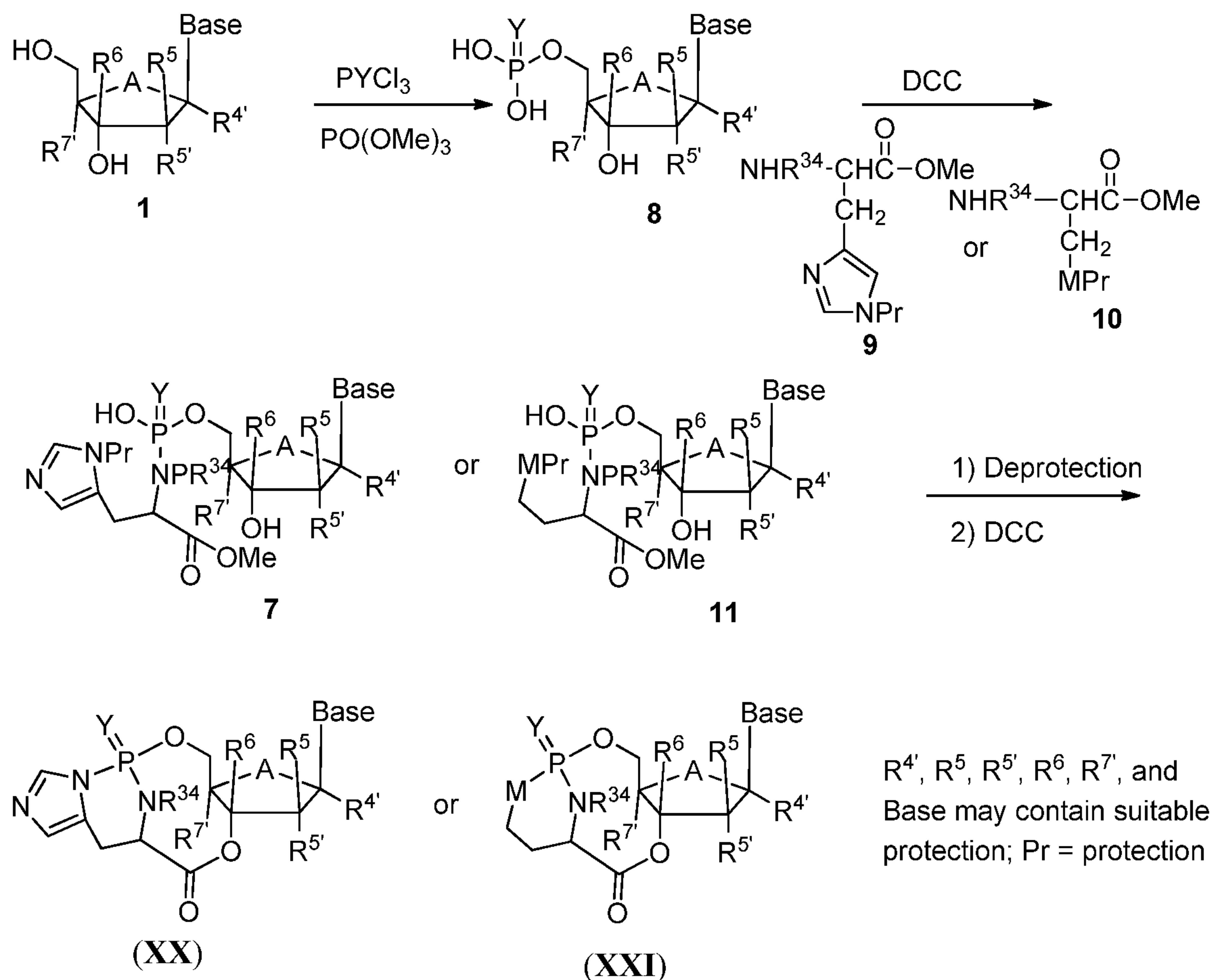
accomplished by agents such as EDC, EDC/HOBt, TBTU, or CDI to give esters **5** or **6**. After removal of protecting groups the resulting amino alcohols can be converted to the monophosphate prodrugs **XX** or **XXI** by exposure to phosphorous oxychloride or phosphorothioyl trichloride (POCl_3 or PSCl_3) 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; $\text{R}^{4'}$, R^5 , $\text{R}^{5'}$, R^6 , Y, M, R^{34} , and $\text{R}^{7'}$ are as defined in active compound section)

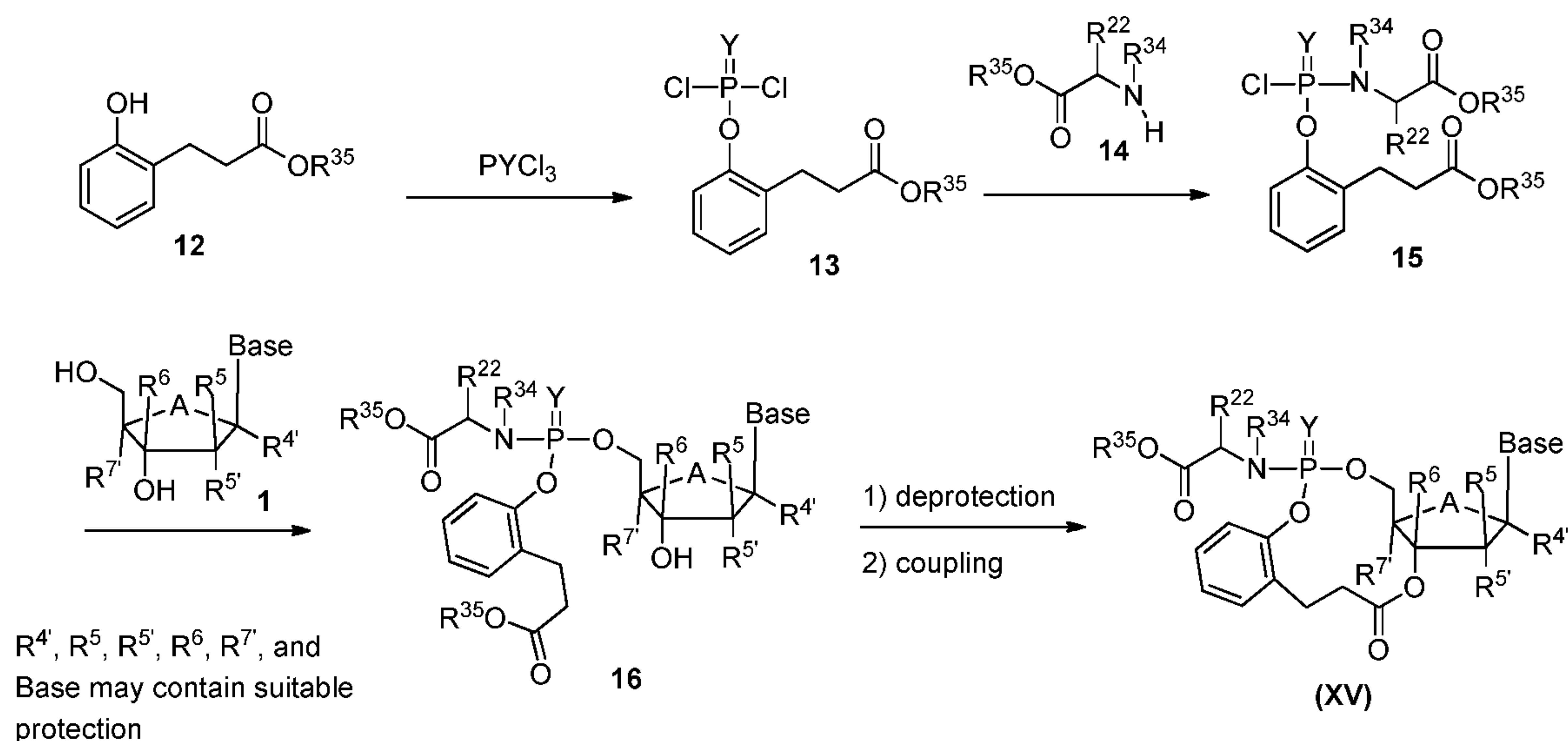
Alternatively, monophosphate prodrugs **XX**, **XXI**, **XXII** can be synthesized as outlined in Scheme 2, namely nucleoside **1** can be converted to the monophosphate, **8** directly by the action of phosphorous oxychloride or phosphorothioyl trichloride in trimethyl phosphate. Coupling to the amino 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.



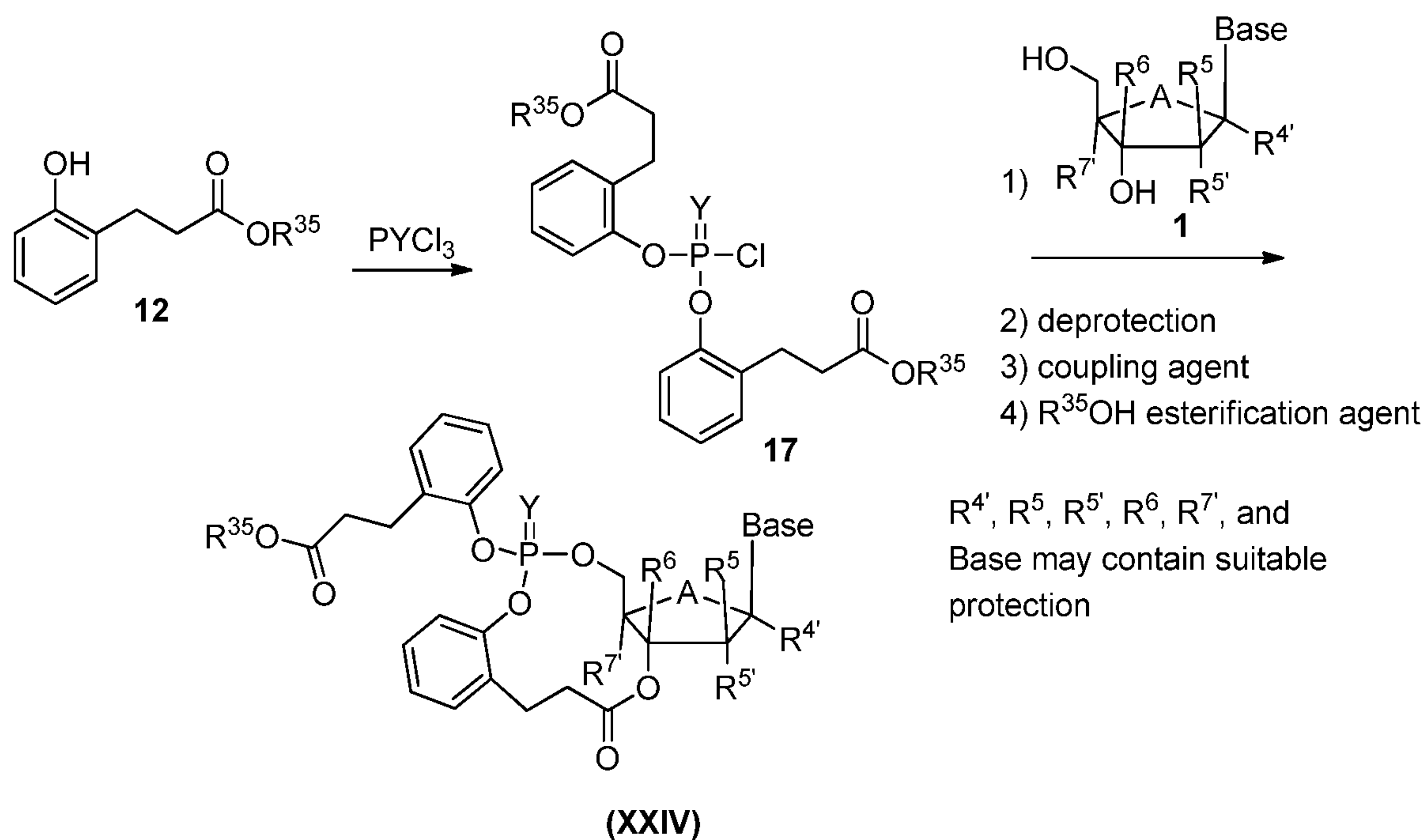
Scheme 2 An alternate synthetic approach to monophosphate prodrugs **XX**, **XXI**, **XXII**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5 , $R^{5'}$, R^6 , Y, M, R^{34} , and R^7 are as defined in active compound section)

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**.



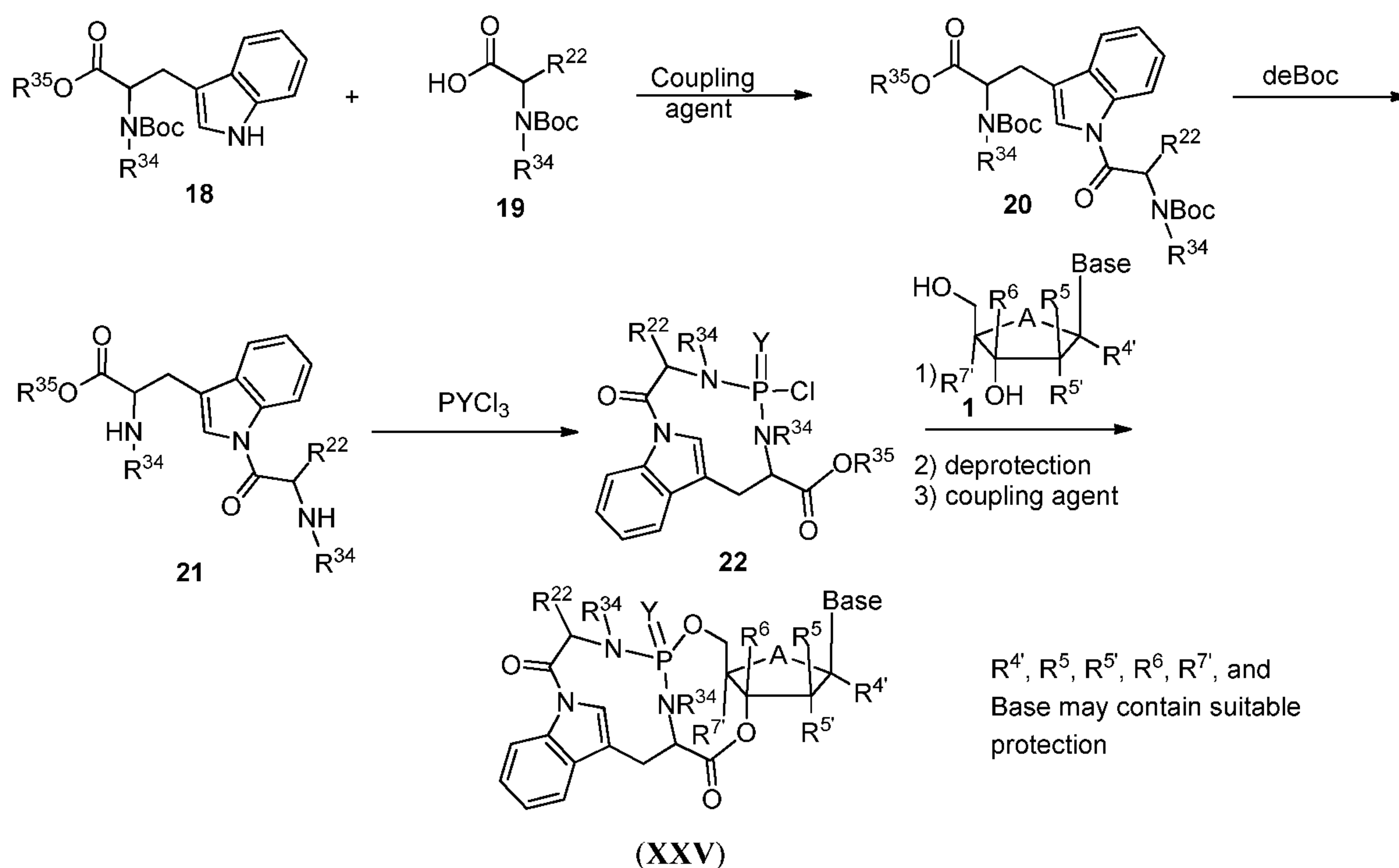
Scheme 3 A synthetic approach to monophosphate prodrug **XXIII**. (Base is a natural or unnatural nucleoside base; $\text{R}^{4'}$, R^5 , $\text{R}^{5'}$, R^6 , Y, R^{34} , R^{35} , R^{22} , and $\text{R}^{7'}$ are as defined in active compound section)

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**.



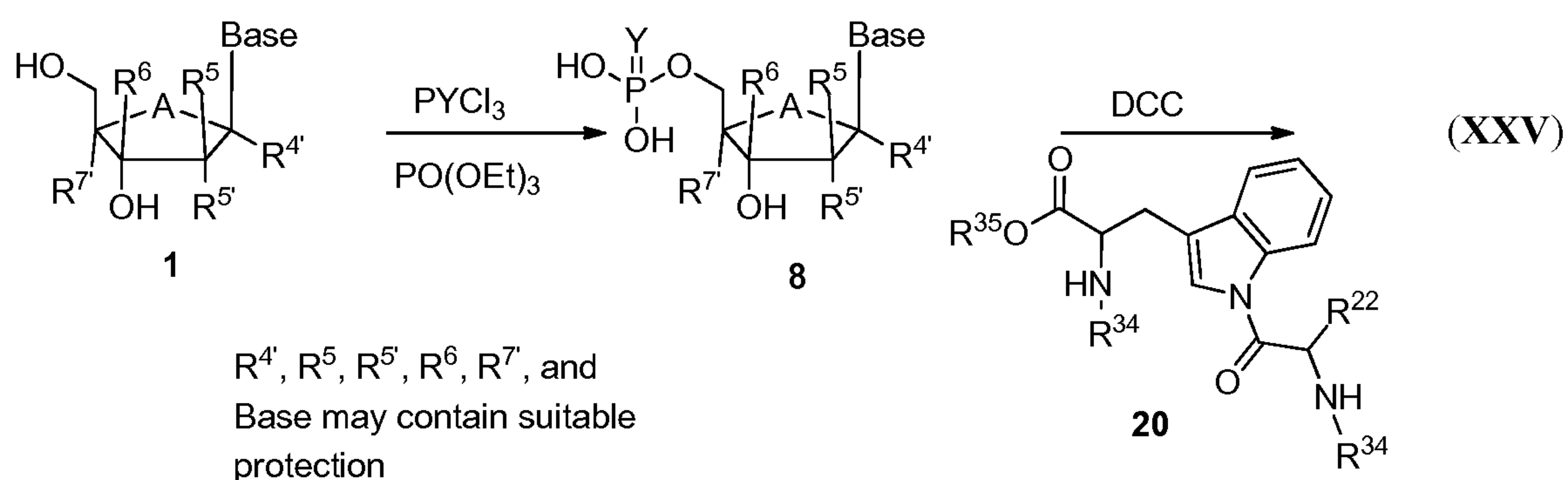
Scheme 4 A synthetic approach to monophosphate prodrug **XXIV**. (Base is a natural or unnatural nucleoside base; R^{4'}, R⁵, R^{5'}, R⁶, Y, R³⁵, and R^{7'} are as defined in active compound section)

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**.



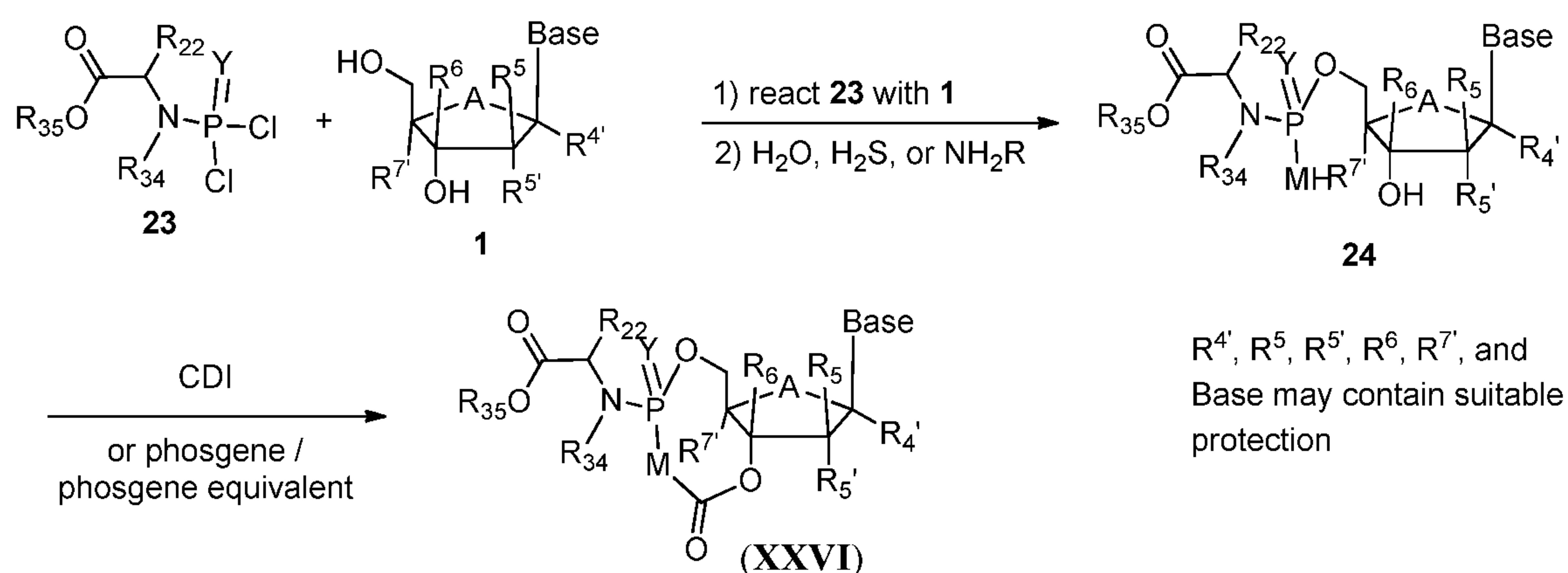
Scheme 5 A synthetic approach to monophosphate prodrug **XXV**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5 , $R^{5'}$, R^6 , Y , R^{34} , R^{35} , R^{22} , and $R^{7'}$ are as defined in active compound section)

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



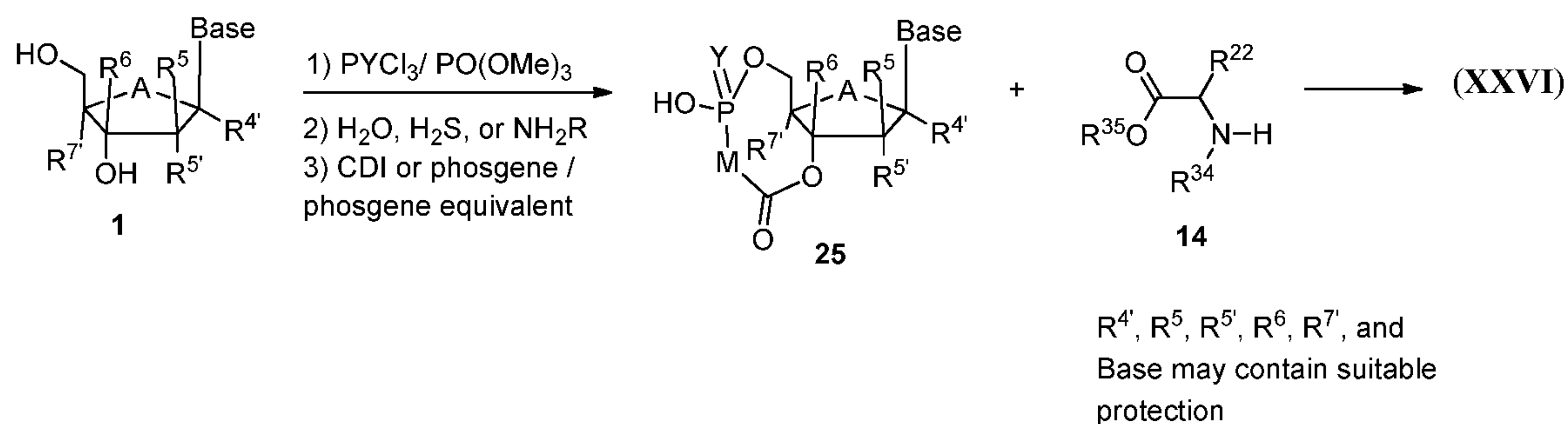
Scheme 6 An alternate synthetic approach to monophosphate prodrug **XXV**. (Base is a natural or unnatural nucleoside base; $R^{4'}$, R^5 , $R^{5'}$, R^6 , Y , R^{34} , R^{35} , R^{22} , and $R^{7'}$ are as defined in active compound section)

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**.



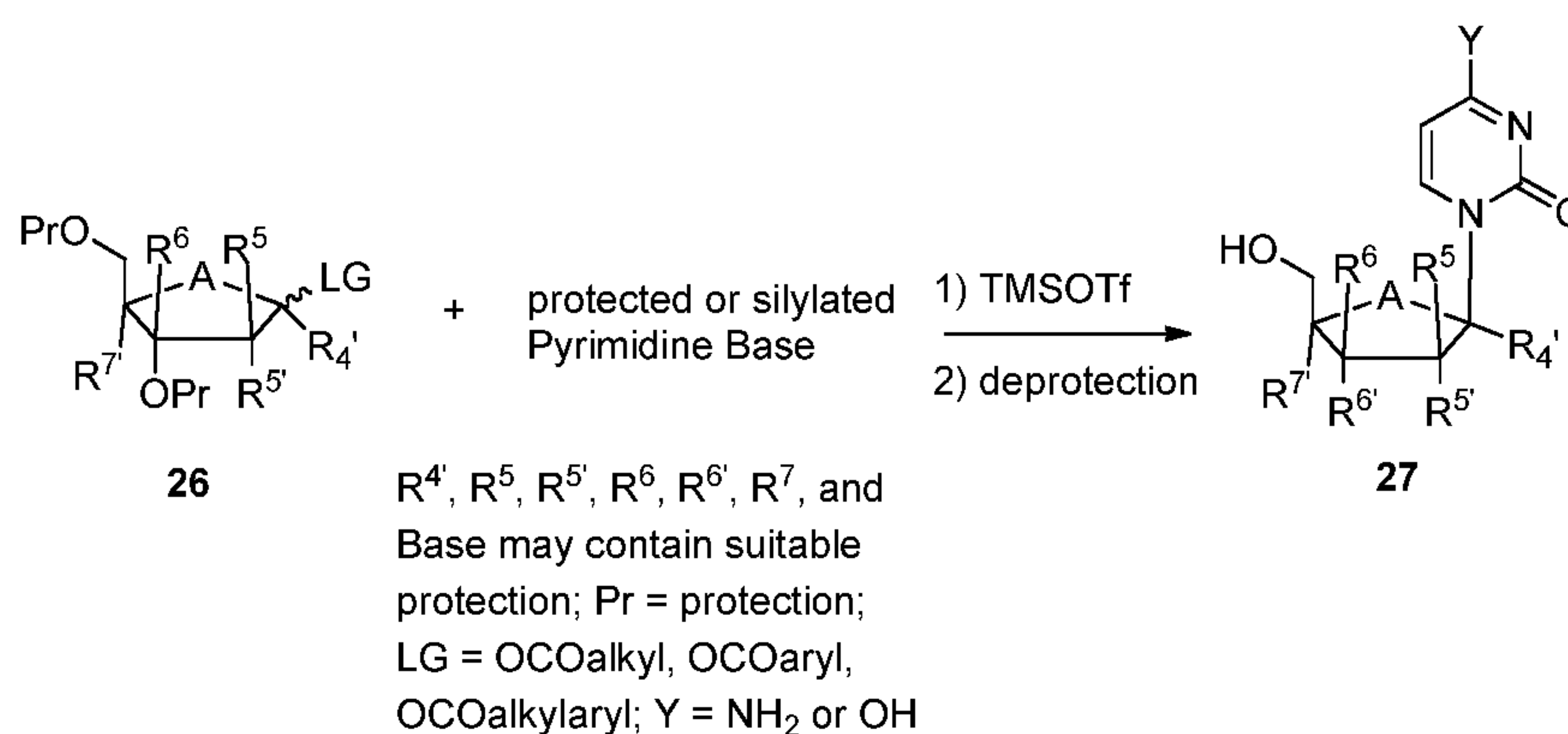
Scheme 7 A synthetic approach to monophosphate prodrug **XXVI**. (Base is a natural or unnatural nucleoside base; R^{4'}, R⁵, R^{5'}, R⁶, Y, M, R³⁴, R³⁵, R²², and R^{7'} are as defined in active compound section)

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).



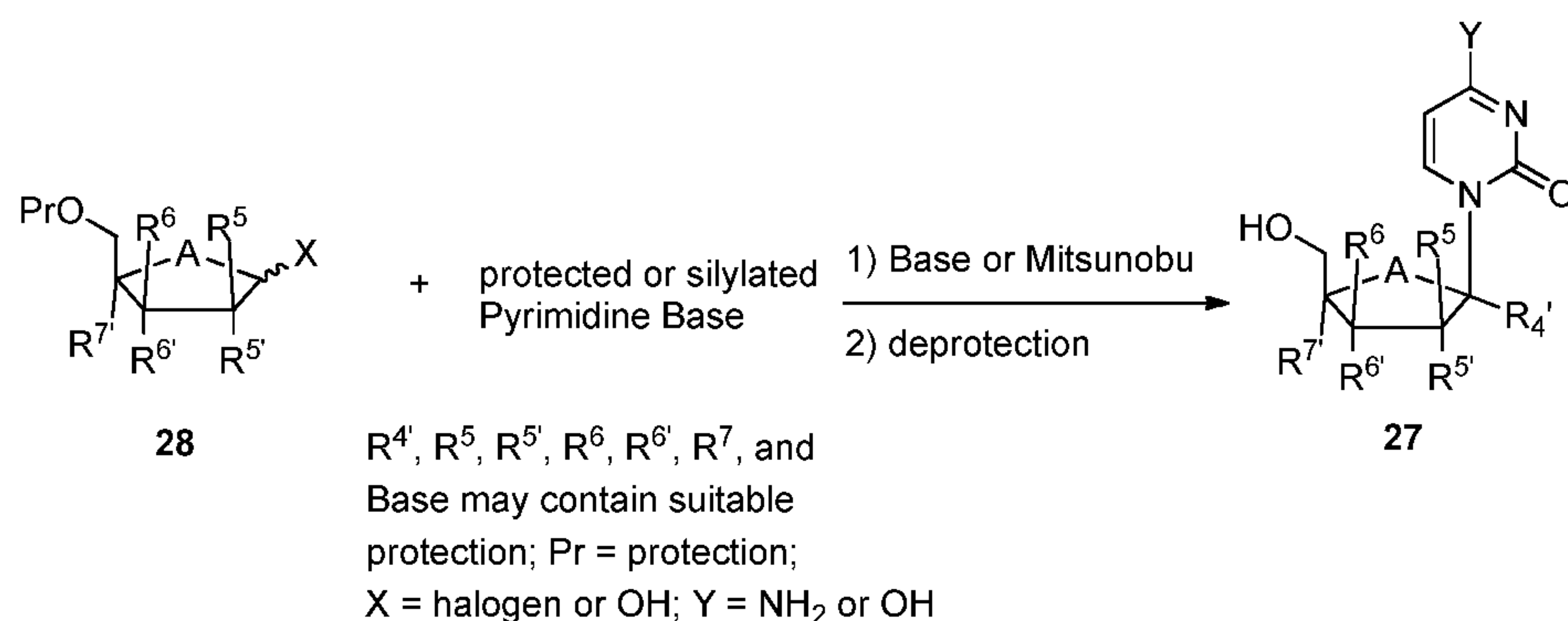
Scheme 8 An alternate synthetic approach to monophosphate prodrug **XVIII**. (Base is a natural or unnatural nucleoside base; $\text{R}^{4'}$, R^5 , $\text{R}^{5'}$, R^6 , Y , R^{34} , R^{35} , R^{22} , and $\text{R}^{7'}$ are as defined in active compound section)

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).



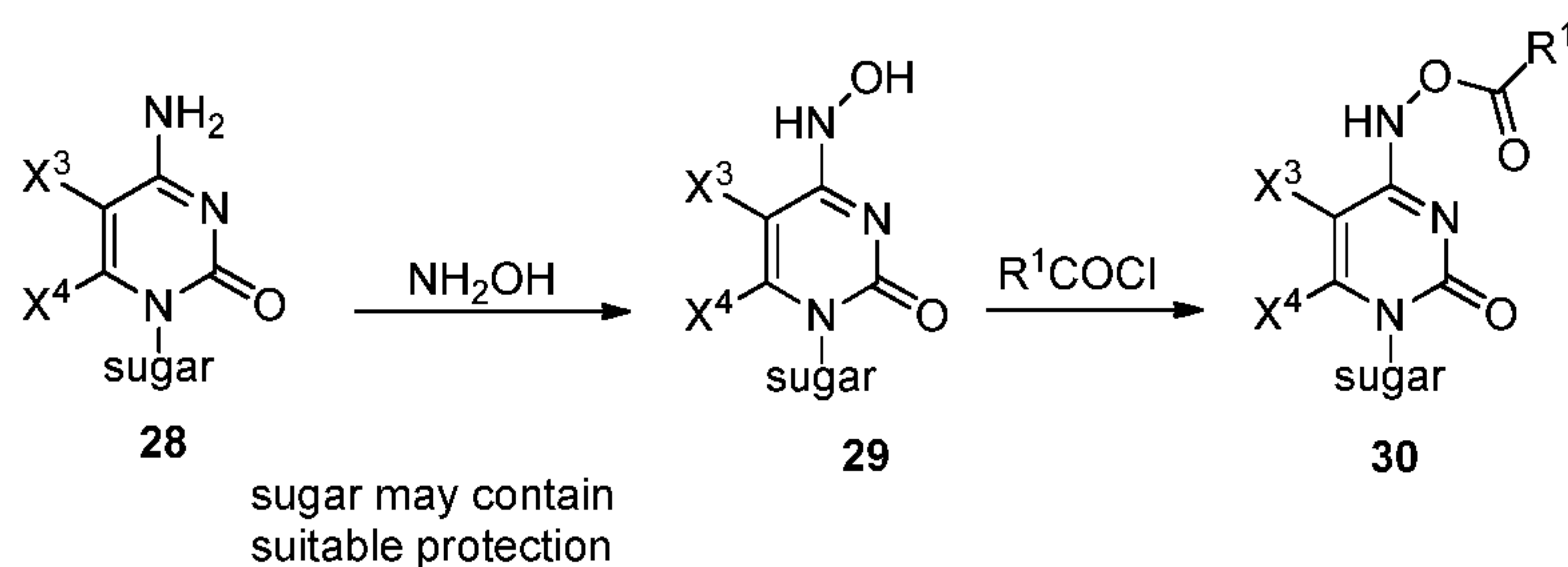
Scheme 9 A synthetic approach to nucleosides **27**. ($\text{R}^{4'}$, R^5 , $\text{R}^{5'}$, R^6 , Y , A , and $\text{R}^{7'}$ are as defined in active compound section)

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).



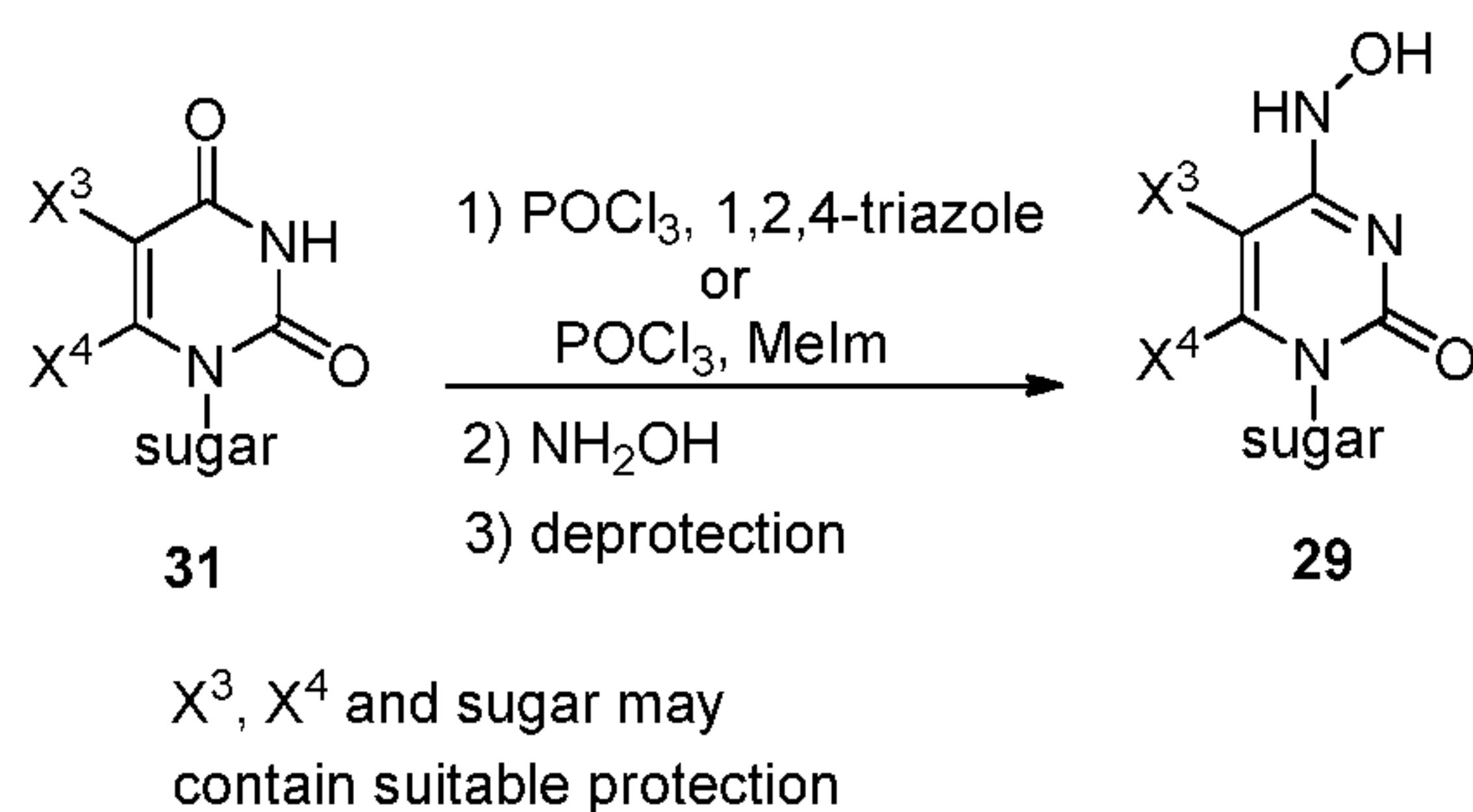
Scheme 10 An alternate synthetic approach to nucleosides **27**. ($R^{4'}$, R^5 , $R^{5'}$, R^6 , Y, $R^{6'}$ and $R^{7'}$ are as defined in active compound section)

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



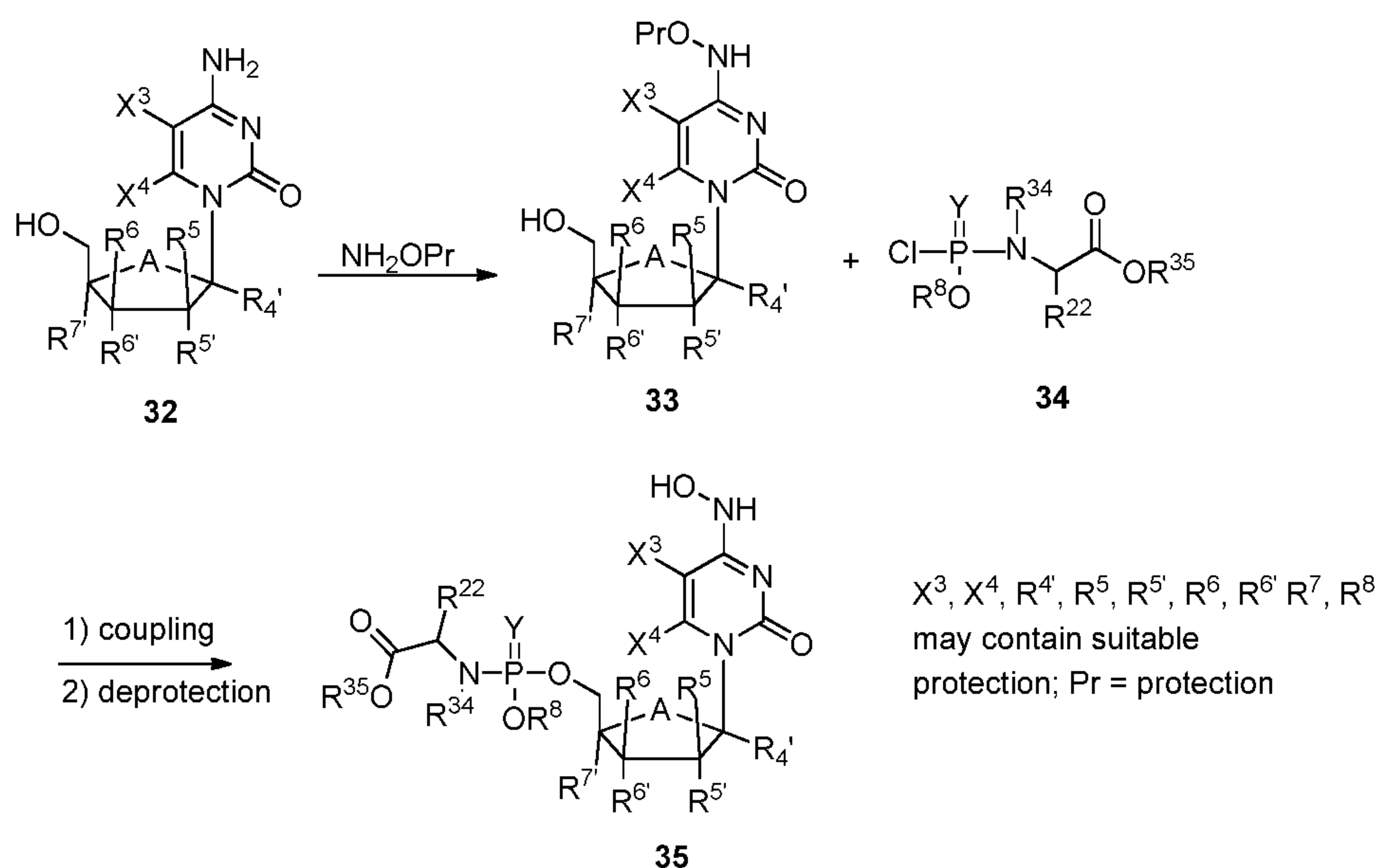
Scheme 11 Synthetic approach to nucleosides **29** and **30**. (X^3 , X^4 , R^1 and sugar are as defined in active compound section)

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 -

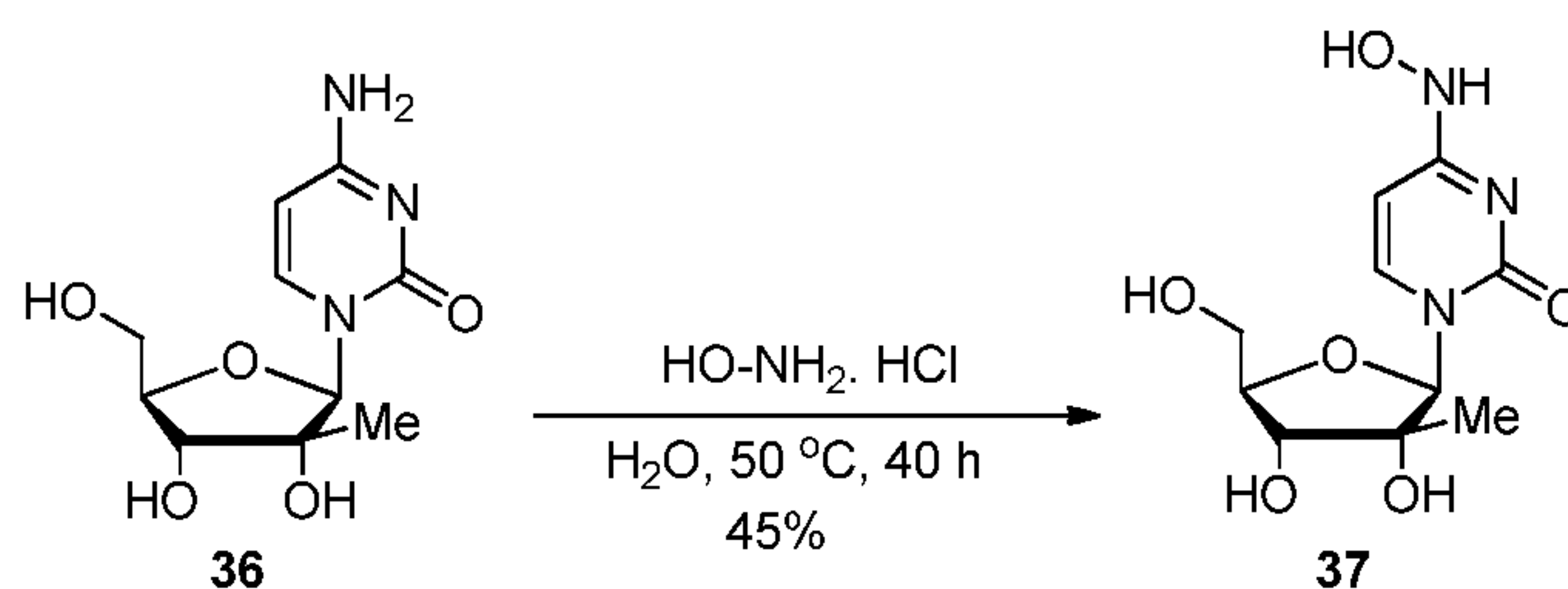
hydroxycytidine nucleosides derivatives and modified monophosphate prodrug analogs. It will be understood by one of ordinary skill in the art that these examples are in no way limiting and that variations of detail can be made without departing from the spirit and scope of the present invention.

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. ^1H 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

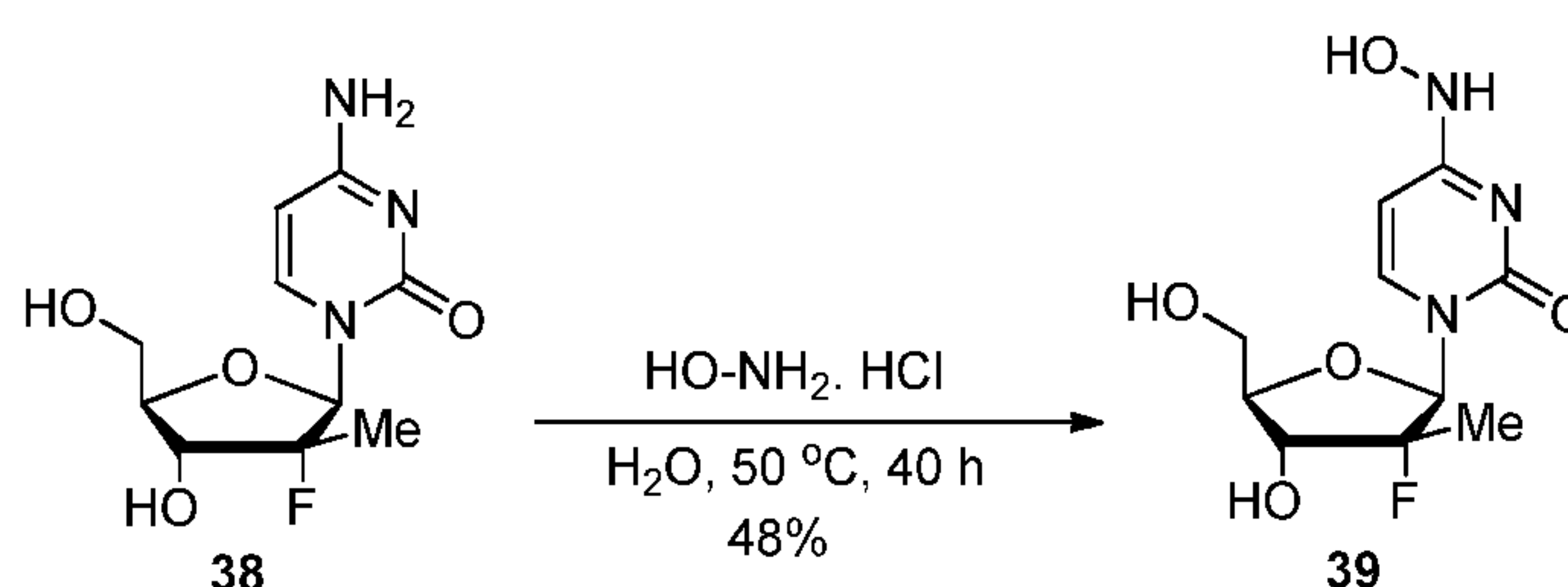


Scheme 14. Synthesis of N⁴-hydroxycytidine 2'-C-Me nucleoside **37**

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 H₂O 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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH = 95:5 to 90:10 v/v) to give **37** (0.83 g, 0.30 mmol) in 45% yield. LCMS (ESI) Calcd for C₁₀H₁₅N₃O₆ 273.2, observed (M + 1) 274.1.

Example 2

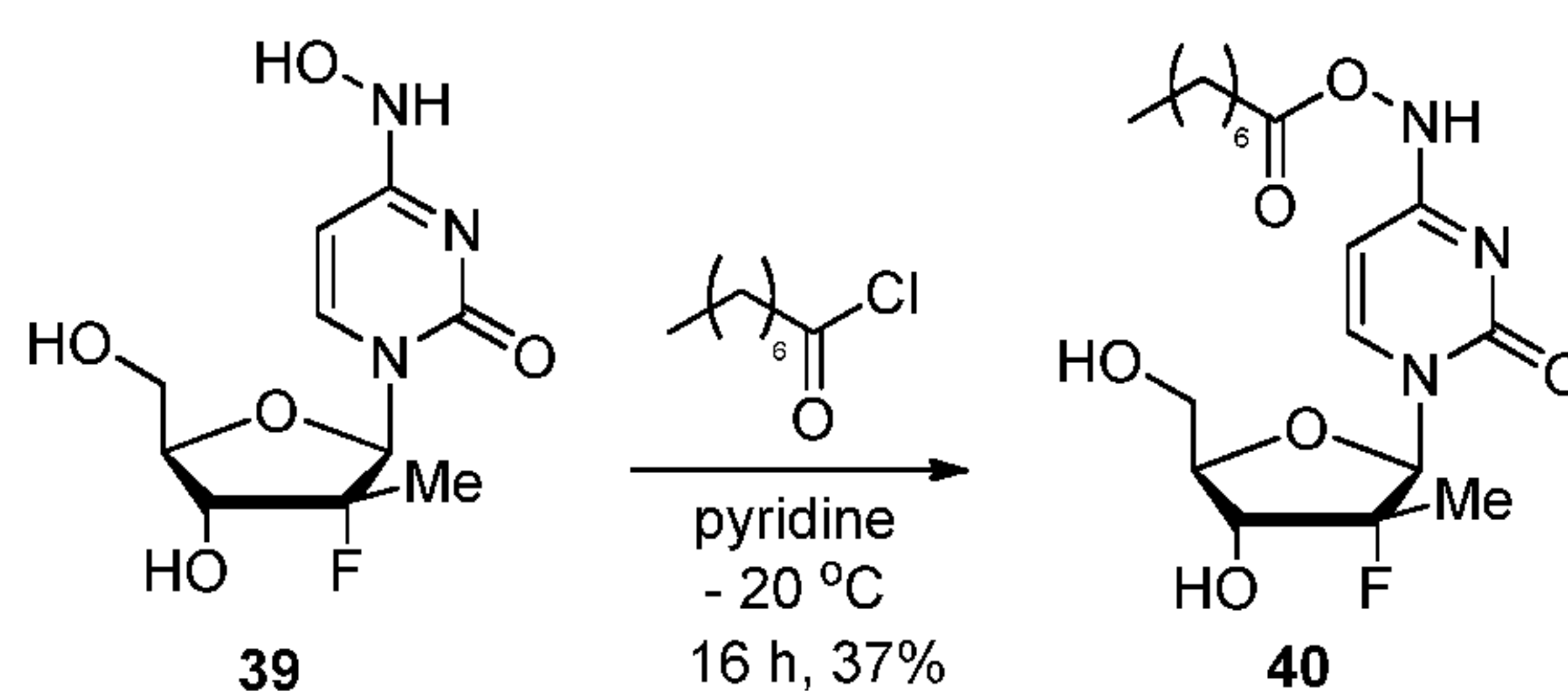


Scheme 15. Synthesis of *N*⁴-hydroxycytidine 2'-deoxy-2'-α-fluoro-2'-β-C-Me nucleoside **39**

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 H₂O 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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH = 95:5 to 90:10 v/v) to give **39** (0.51 g, 1.85 mmol) in 48% yield. LCMS (ESI) Calcd for C₁₀H₁₄FN₃O₅ 275.2, observed (M + 1) 274.3

Example 3



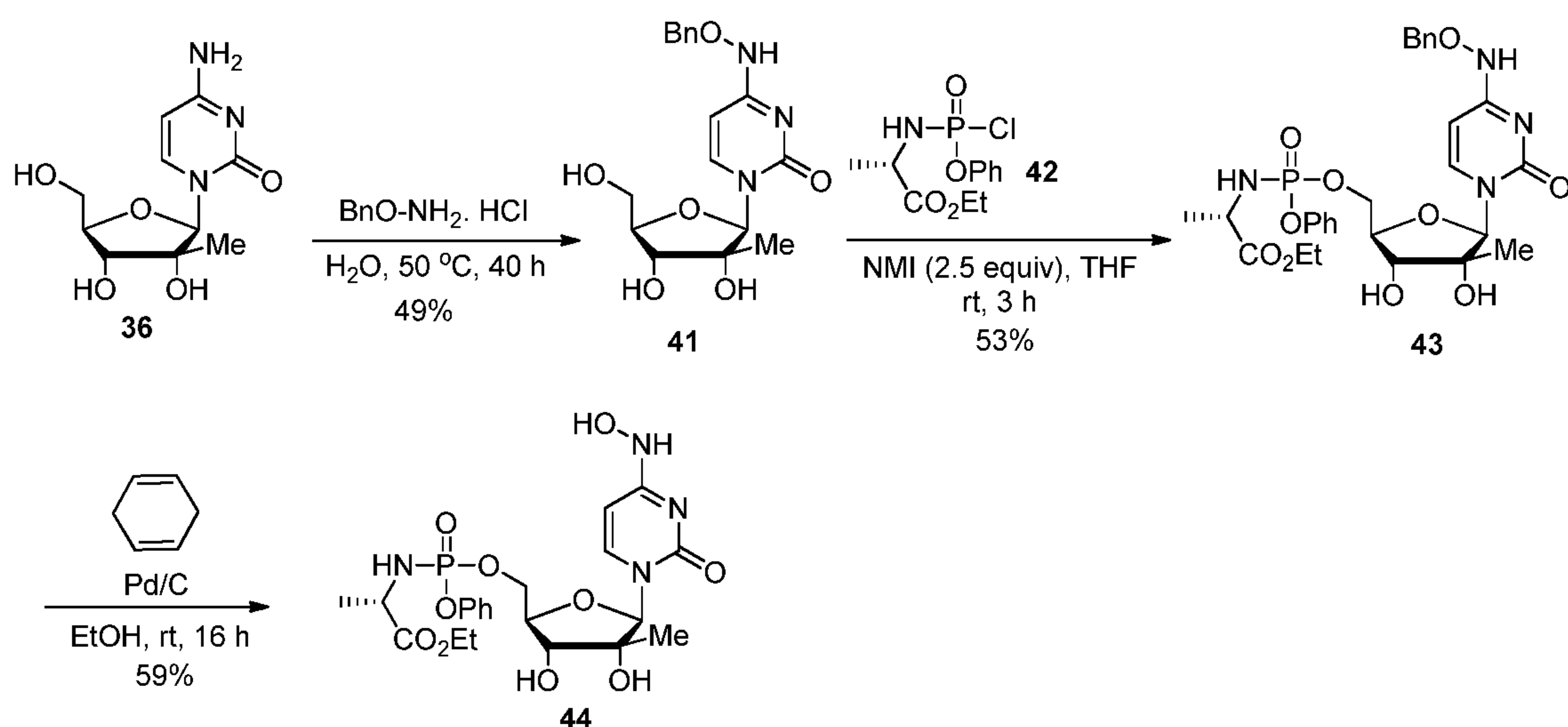
Scheme 16. Synthesis of *N*⁴-(Octanoyloxy)cytidine 2'-deoxy-2'-α-fluoro-2'-β-C-Me nucleoside **40**

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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified

by silica gel column chromatography ($\text{CH}_2\text{Cl}_2:\text{MeOH} = 95:5$ to $85:15$ v/v) to give **40** (0.04 g, 0.09 mmol) in 37% yield. LCMS (ESI) Calcd for $\text{C}_{18}\text{H}_{28}\text{FN}_3\text{O}_6$ 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 ($\text{CH}_2\text{Cl}_2:\text{MeOH} = 95:5$ to $90:10$ v/v) to give **41** (0.12 g, 0.33 mmol) in 49% yield. LCMS (ESI) Calcd for $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_6$ 363.4, observed ($M + 1$) 364.3

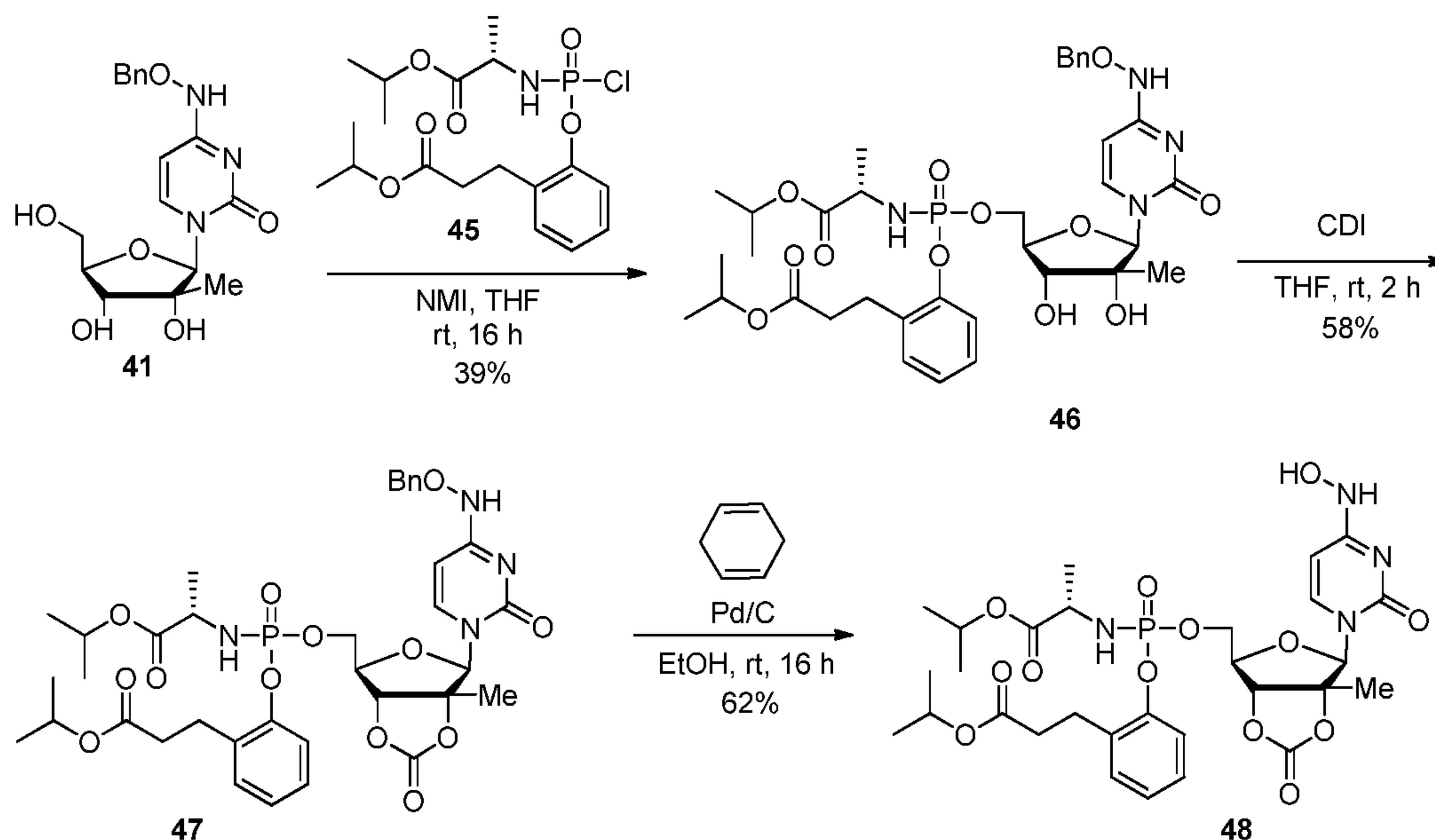
(2*S*)-ethyl 2-((((2*R*,3*R*,4*R*,5*R*)-5-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2*H*)-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate, **43**

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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH = 95:5 to 90:10 v/v) to give **43** (0.04 g, 0.06 mmol) in 53% yield. LCMS (ESI) Calcd for C₂₈H₃₅N₄O₁₀P 618.6, observed (M + 1) 619.7

(2*S*)-ethyl 2-((((2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1(2*H*)-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 (CH₂Cl₂:MeOH = 90:10) to give **44** (0.02 g, 0.04 mmol) in 59% yield. LCMS (ESI) Calcd for C₂₁H₂₉N₄O₁₀P 528.4, observed (M + 1) 528.3

Example 5



Scheme 18. Synthesis of N⁴-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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH = 95:5 to 90:10 v/v) to give **46** (0.12 g, 0.16 mmol) in 39% yield. LCMS (ESI) Calcd for C₃₅H₄₇N₄O₁₂P 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**

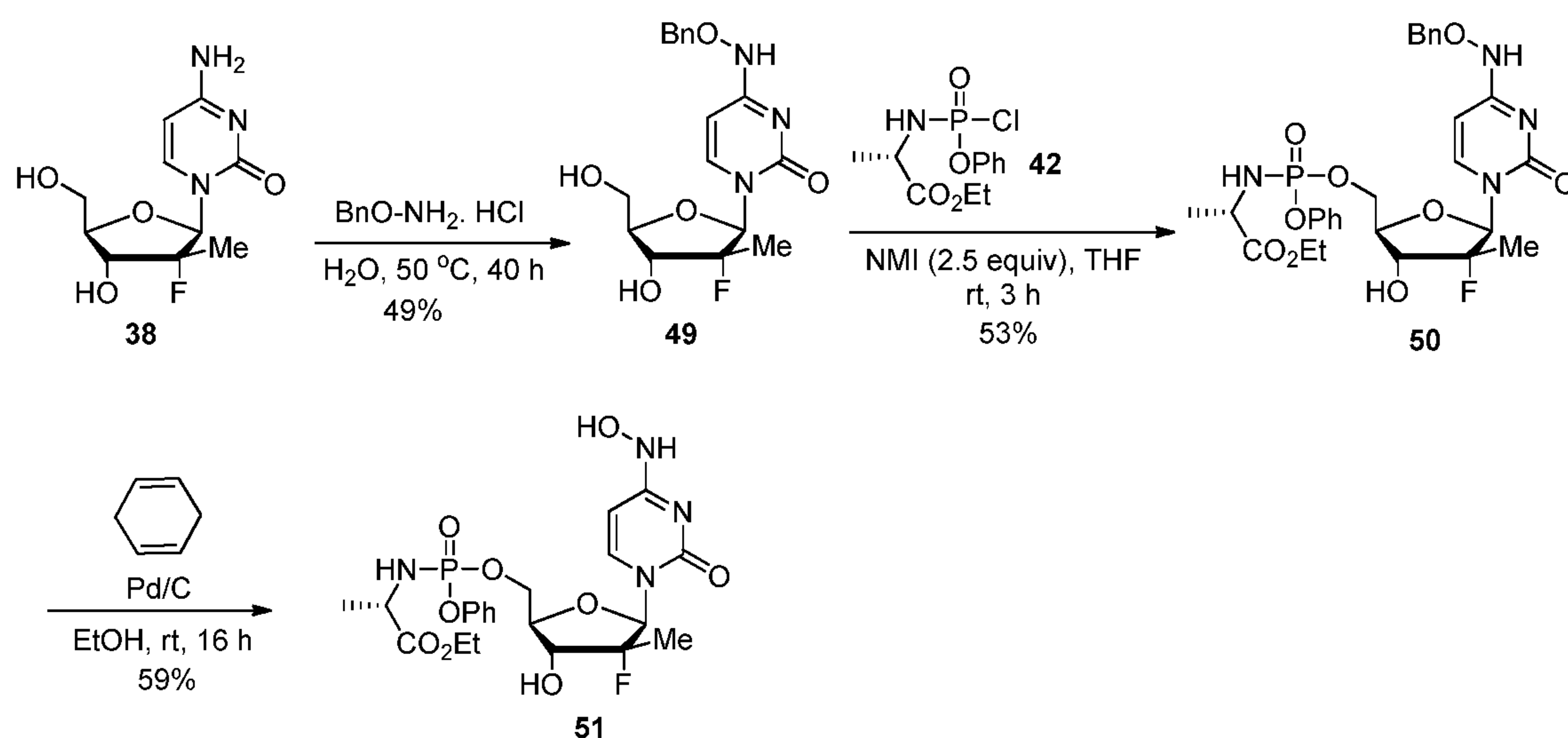
To a solution of **46** (0.04 g, 0.05 mmol) in 0.25 mL of THF was added N,N'-carbonyldiimidazole (0.02 mg, 0.12 mmol) at 0 °C. After stirring for 2 h at rt, the solution was concentrated under reduced pressure. The residue was purified by silica

gel column chromatography (Hexane:EtOAc = 5:5) to give **47** (0.02 g, 0.03 mmol) in 58% yield. LCMS (ESI) Calcd for C₃₆H₄₅N₄O₁₃P 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 (CH₂Cl₂:MeOH = 90:10) to give **48** (0.02 g, 0.04 mmol) in 62% yield. LCMS (ESI) Calcd for C₂₉H₃₉N₄O₁₃P 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 H₂O 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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH = 95:5 to 90:10 v/v) to give **49** (0.11 g, 0.30 mmol) in 39% yield. LCMS (ESI) Calcd for C₁₇H₂₀N₃O₅F 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

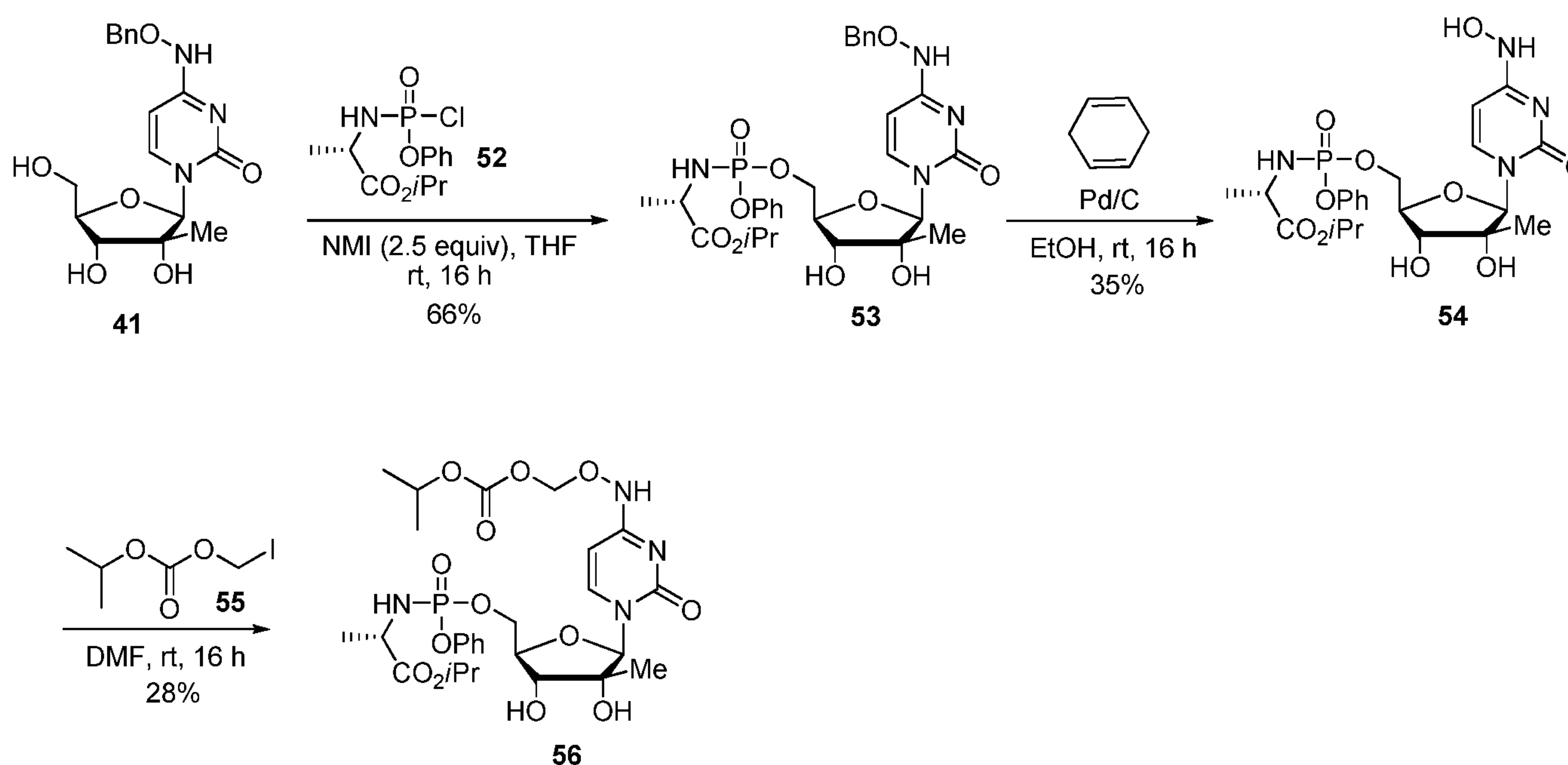
To a solution of **49** (0.15 g, 0.41 mmol) in 3 mL was added 1-methylimidazole (0.10 mL, 1.23 mmol) and 1.23 mL of a 1M solution of phenyl-(ethoxy-L-alaninyl)-phosphorochloridate **42** in THF, under argon atmosphere. After stirring for 16 h at room temperature, AcOEt (10 mL) was added and the reaction mixture was washed with water (3 x 3 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH = 95:5 to 90:10 v/v) to give **50** (0.03 g, 0.05 mmol) in 13% yield. LCMS (ESI) Calcd for C₂₈H₃₄N₄O₉PF 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 (CH₂Cl₂:MeOH = 95:5) to give **51** (0.01 g, 0.04 mmol) in 40% yield. LCMS (ESI) Calcd for C₂₁H₂₈N₄O₉PF 530.4, observed (M + 1) 531.3

Example 7



Scheme 20. Synthesis of N⁴-hydroxycytidine 2'-C-Me nucleoside prodrugs **54** and **56**

2S-isopropyl 2-((((((2*R*,3*R*,4*R*,5*R*)-5-(4-((benzyloxy)amino)-2-oxopyrimidin-1(2*H*)-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 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂: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 (CH₂Cl₂: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 Cs₂CO₃ (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, CH₂Cl₂ (5 mL) was added and the reaction mixture was washed with water (3 x 3 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂: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 CH₂Cl₂, 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 Et₃N (14 mL, 102.8 mmol) in 40 mL of CH₂Cl₂ 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 Et₂O (40 mL). The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column

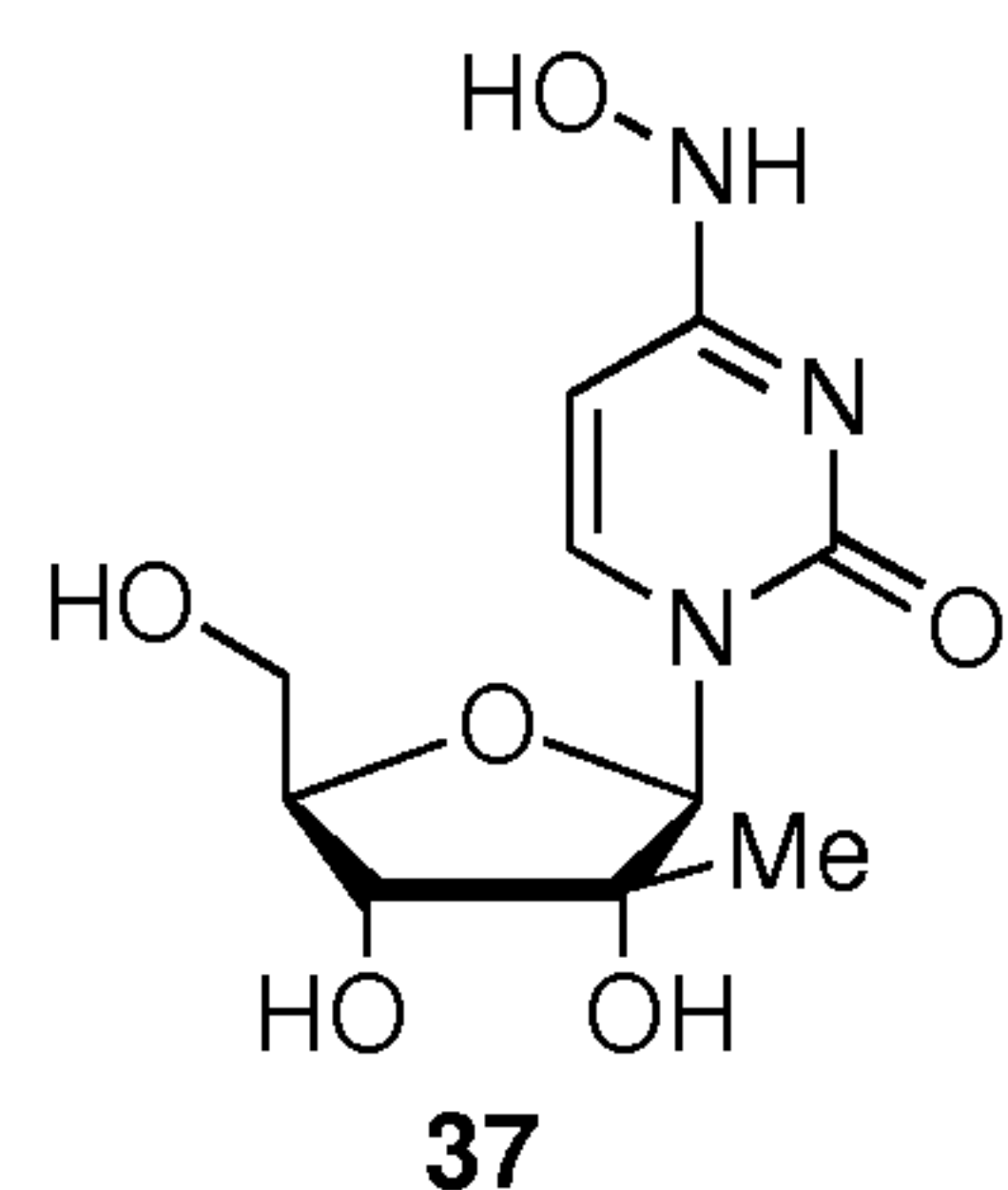
chromatography (EtOAc:hexane = 1:0 to 1:1 v/v) to give **52** (7.86 g, 26 mmol) as a colorless oil in 50% yield.

Example 8

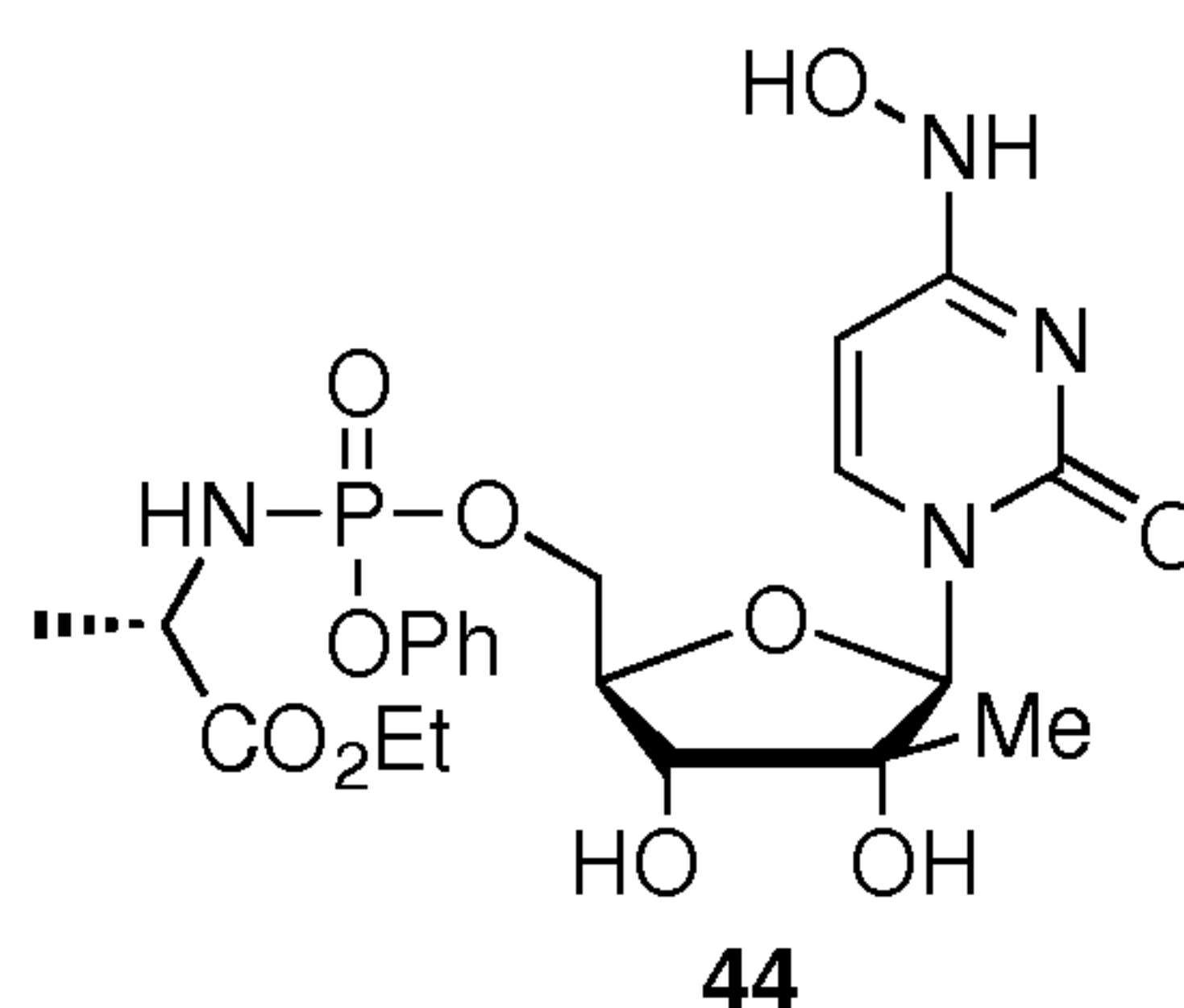
Shown below are two examples of the LC/MS qualitative analysis of nucleotides formed after 4 h incubation of 50 μ M *N*⁴-hydroxycytidine nucleosides and *N*⁴-hydroxycytidine monophosphate prodrugs in Huh-7 cells.

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 **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**



HCV EC₅₀ = > 10 μ M
 PBM IC₅₀ = >100 μ M
 CEM IC₅₀ = >100 μ M
 Vero IC₅₀ = >100 μ M
 Huh-7 IC₅₀ = >10 μ M



HCV EC₅₀ = 0.8 μ M
 PBM IC₅₀ = >100 μ M
 CEM IC₅₀ = >100 μ M
 Vero IC₅₀ = >100 μ M
 Huh-7 IC₅₀ = >10 μ M

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:

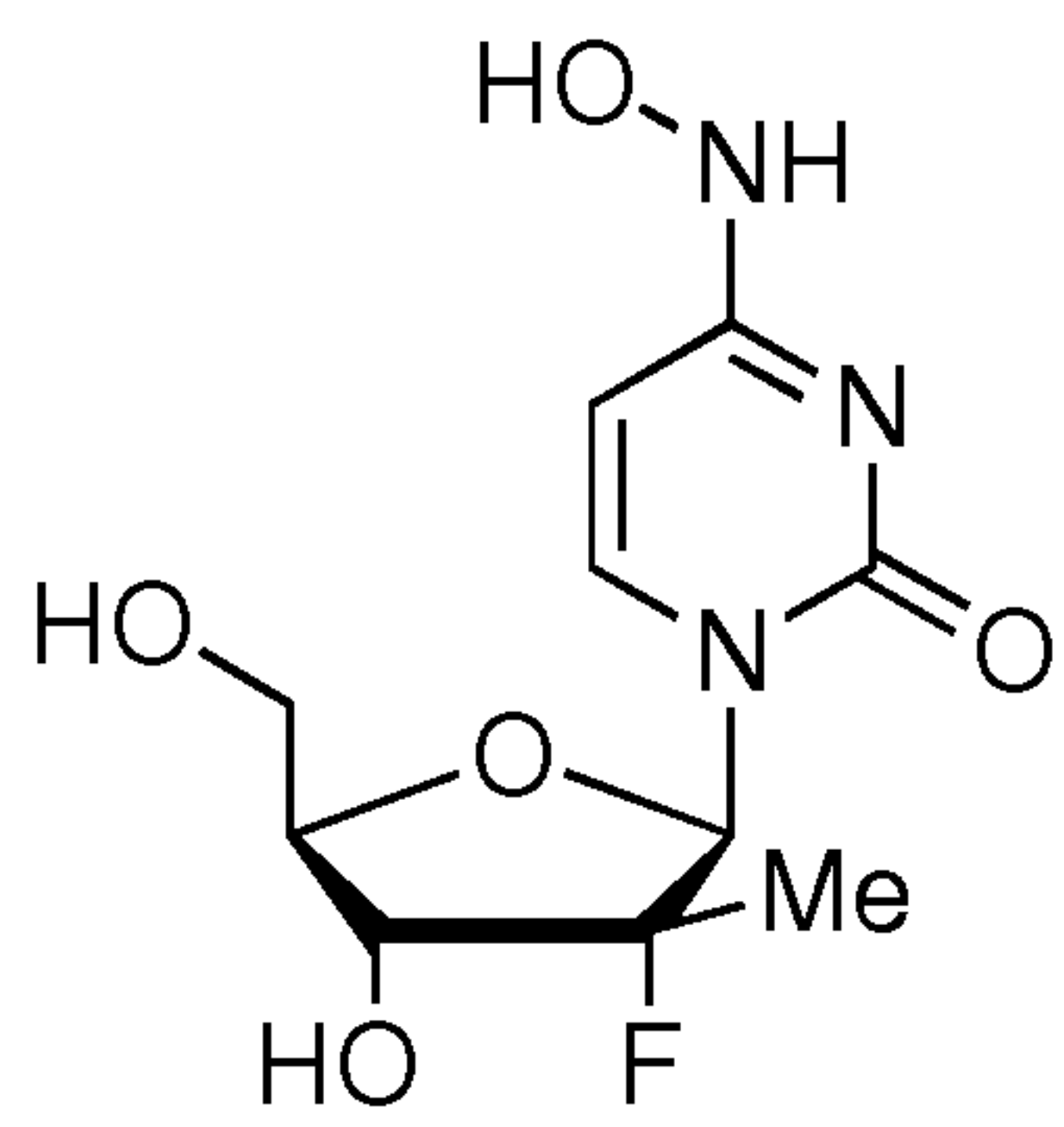
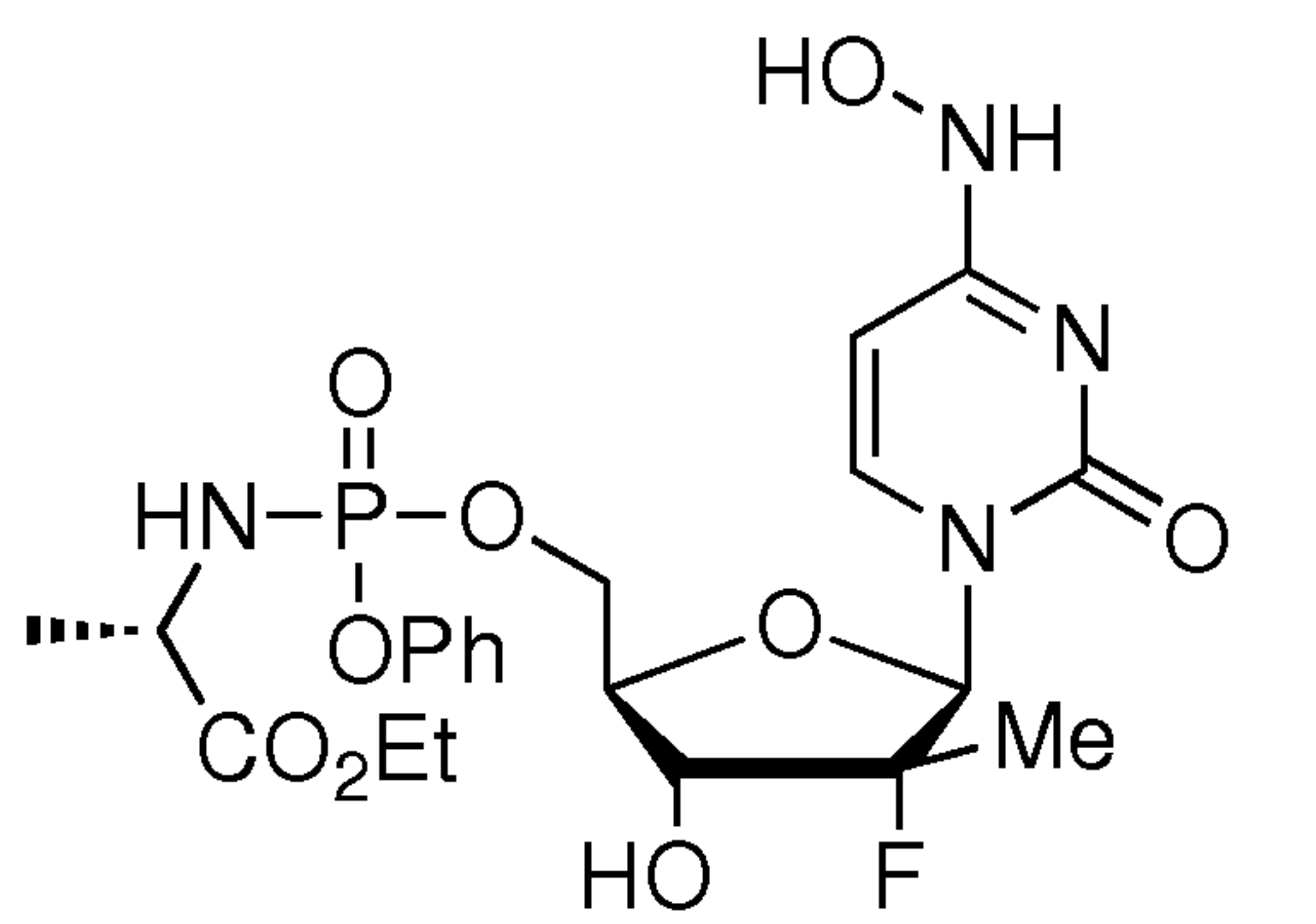
Table 2. LC/MS analysis of nucleotides formed after 4 hr incubation in Huh-7 cells of 50 μ M **37** and **44**

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	$3,075 \pm 98.5$
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	 51
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	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	39	51
Metabolites		

(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., Saalman 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•(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 EC₅₀ and EC₉₀ 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 (ϵ_{280}) 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'-

CTCAGACCCTTTTAGTCAGAATGGAAANTCTCTAGCAGTGGCGCCCCG 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 MgCl₂. 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 N⁴-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 10⁷ 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 (TCID₅₀) 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% (EC₅₀).

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.). EC₅₀ 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, Saalman 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 TCID₅₀/1 x 10⁷ cells for a flask (T25) assay or with 200 TCID₅₀/6 x 10⁷ 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. EC₅₀ 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 10³ to 5 x 10⁴ 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 (CC₅₀) can be determined from the concentration–response curve using the median effect method.

Example 12

Assess Activity N⁴-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs against Drug-Resistant HIV

Analogues identified above as having improved activity compared with the parent analogue, 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 K_d and k_{pol} 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 $[\gamma^{32}P]$ -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 100 μ M 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 (k_{ATP}) of ATP-mediated excision: $[\text{product}] = A[\exp(-k_{\text{ATP}}t)]$, 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 (5×10^4 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 2.5×10^4 cells per well. Various concentrations (0 μ M, 0.1 μ M, 1 μ M, 10 μ M and 100 μ M) of nucleoside analog can be added, and the cultures can be incubated at 37°C in a humidified 5% CO₂ 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 N⁴-hydroxycytidine nucleosides derivatives, modified monophosphate and phosphonates prodrugs analogs can be used to indicate a drug-induced cytotoxic effect.

ii) Effect on N⁴-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 μ M, 0.1 μ M, 10 μ M and 100 μ M. 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 β -actin or rRNA gene can be amplified from 5 μ l 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 β -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 β -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 $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT$ 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, Griniuvienė 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 μ M fialuridine

(FIAU; 1,2'-deoxy-2'-fluoro-1-D-arabinofuranosyl-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 (2.5×10^4 cells/mL) can be seeded into tissue culture dishes (35 by 10 mm) in the presence of 0 μ M, 0.1 μ M, 1 μ M, 10 μ M and 100 μ M 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 60 nM DNA template/primer in 50mM Tris-HCl, 100 mM NaCl, pH 7.8, can be added to a solution containing MgCl₂ (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 MgCl₂ (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% CO₂, and colonies of greater than 50 cells can be counted using an inverted microscope to determine IC₅₀. The 50% inhibitory concentration (IC₅₀) 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.

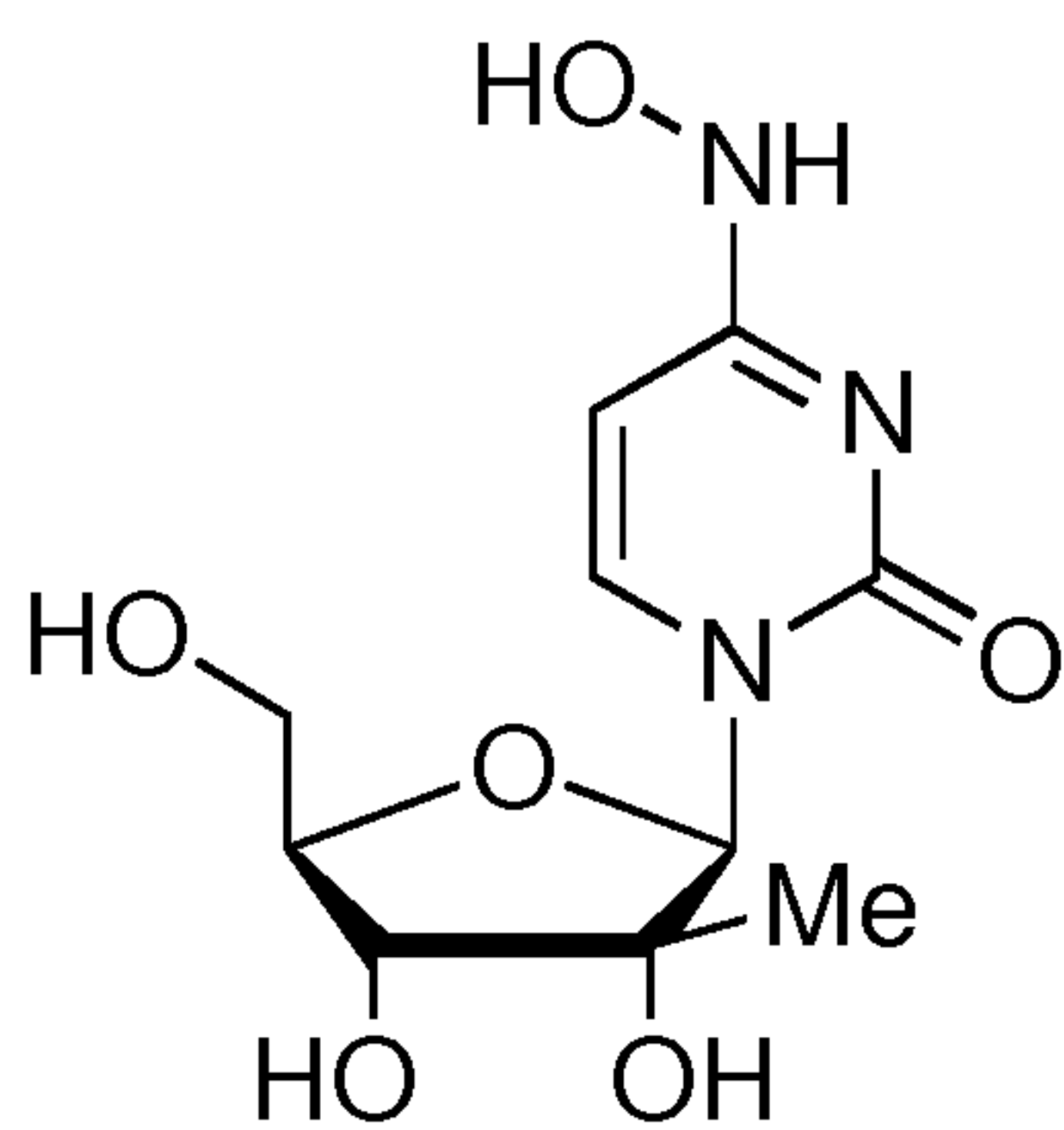
Example 20

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., Saalman 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 (IC₅₀) 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 EC₅₀, PBM IC₅₀, CEM IC₅₀, Vero IC₅₀, and Huh-7 IC₅₀ Data for Selected Compounds

**37**

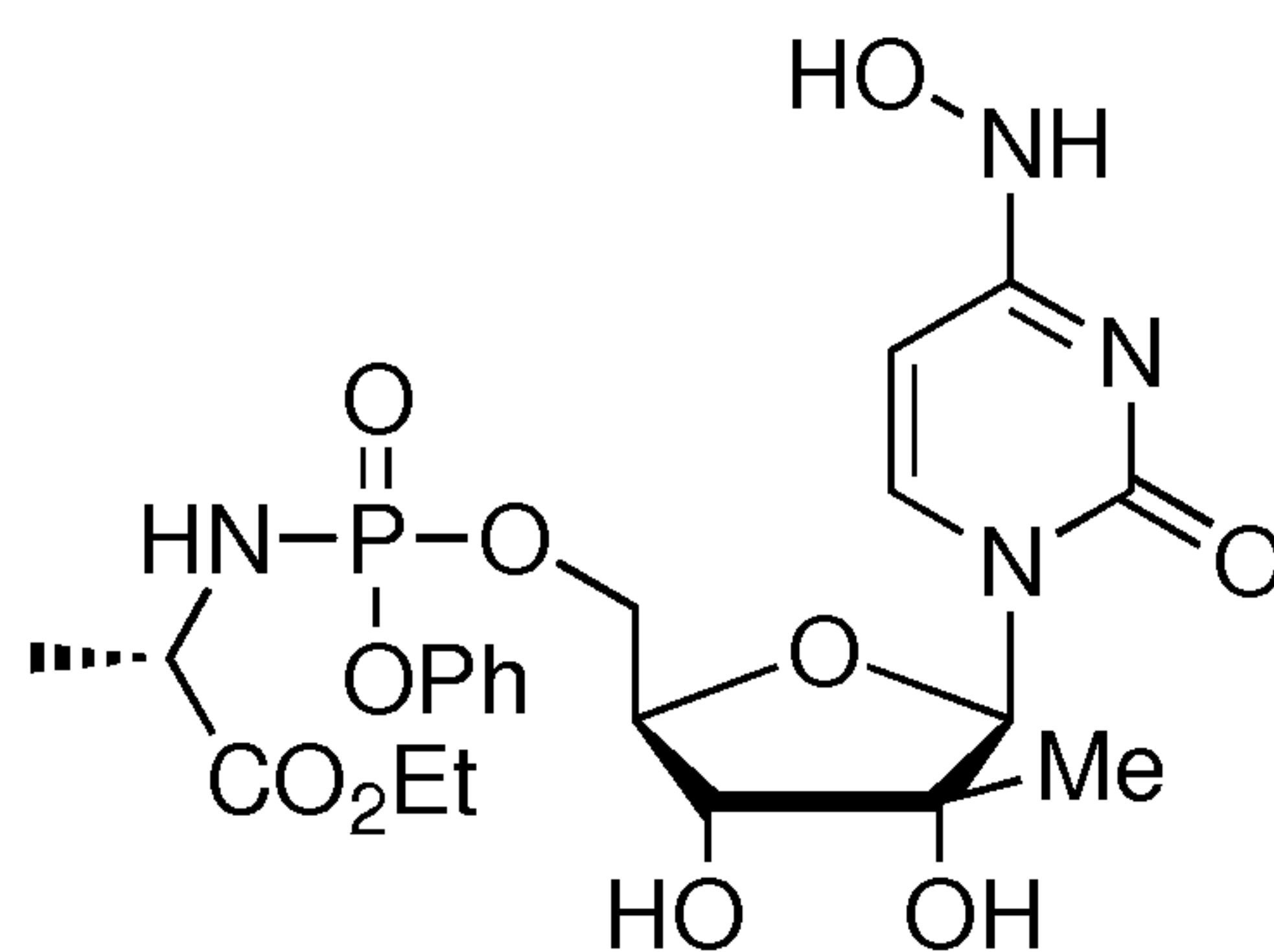
HCV EC₅₀ = > 10 μM

PBM IC₅₀ = >100 μM

CEM IC₅₀ = >100 μM

Vero IC₅₀ = >100 μM

Huh-7 IC₅₀ = >10 μM

**44**

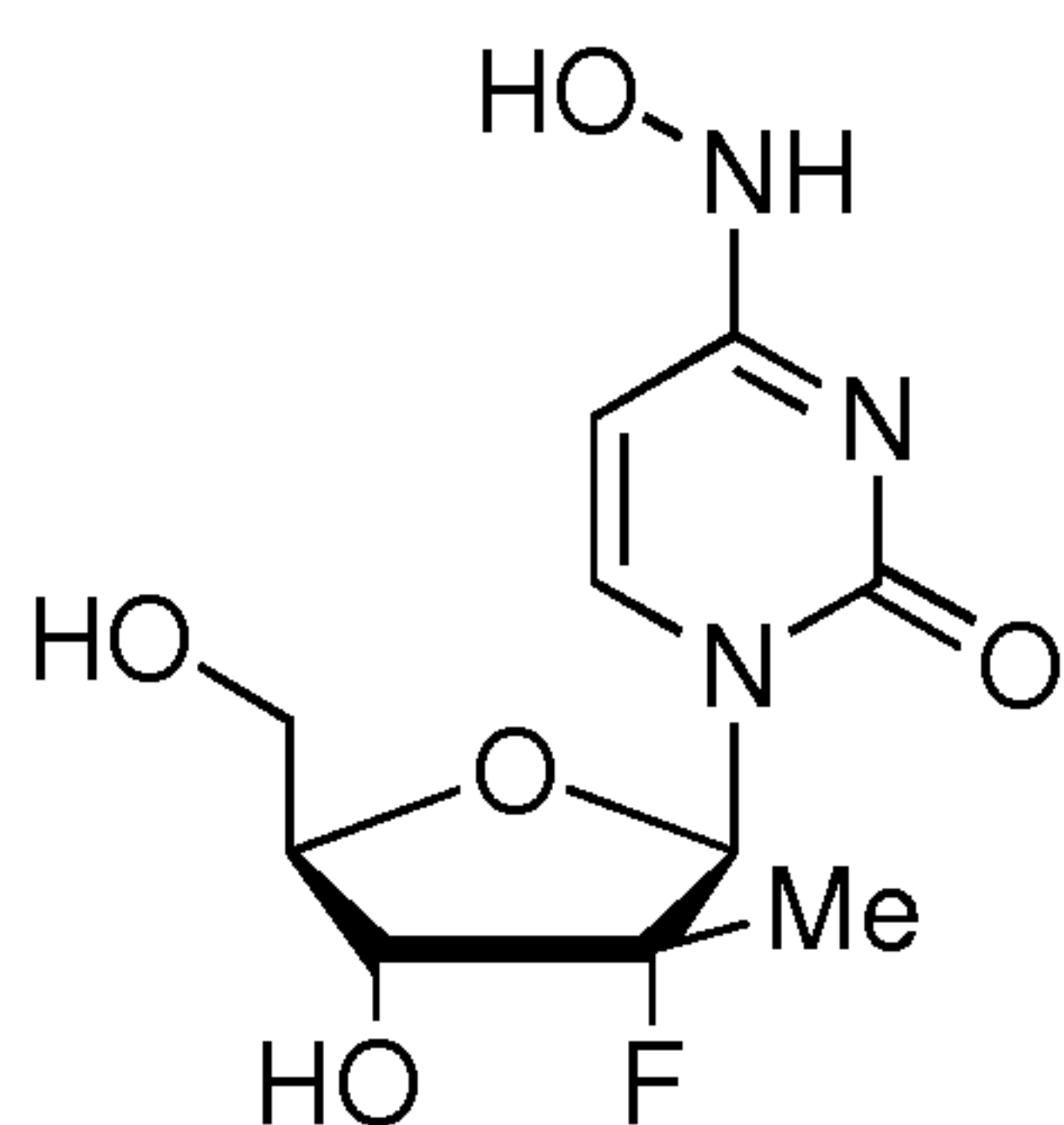
HCV EC₅₀ = 0.8 μM

PBM IC₅₀ = >100 μM

CEM IC₅₀ = >100 μM

Vero IC₅₀ = >100 μM

Huh-7 IC₅₀ = >10 μM

**39**

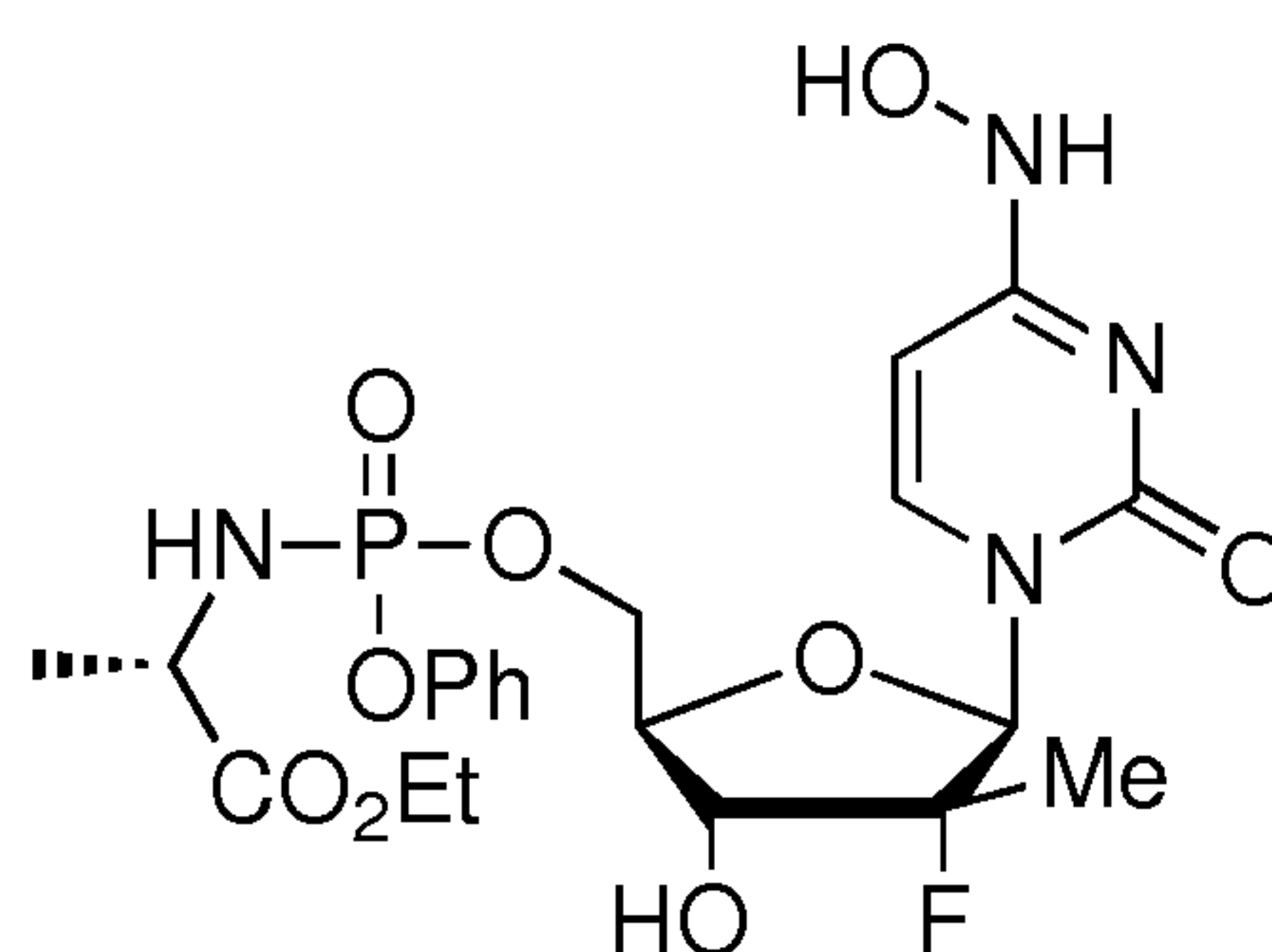
HCV EC₅₀ = > 10 μM

PBM IC₅₀ = >100 μM

CEM IC₅₀ = >100 μM

Vero IC₅₀ = >100 μM

Huh-7 IC₅₀ = >10 μM

**51**

HCV EC₅₀ = 2.6 μM

PBM IC₅₀ = >100 μM

CEM IC₅₀ = >100 μM

Vero IC₅₀ = >100 μM

Huh-7 IC₅₀ = >33 μM

Example 21

Selection of Resistant Viruses to nucleotide monophosphate prodrugs

Peripheral blood mononuclear (PBM) cells¹ can be seeded, for example, at a concentration of 1×10^7 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 TCID₅₀. 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

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 cellular 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

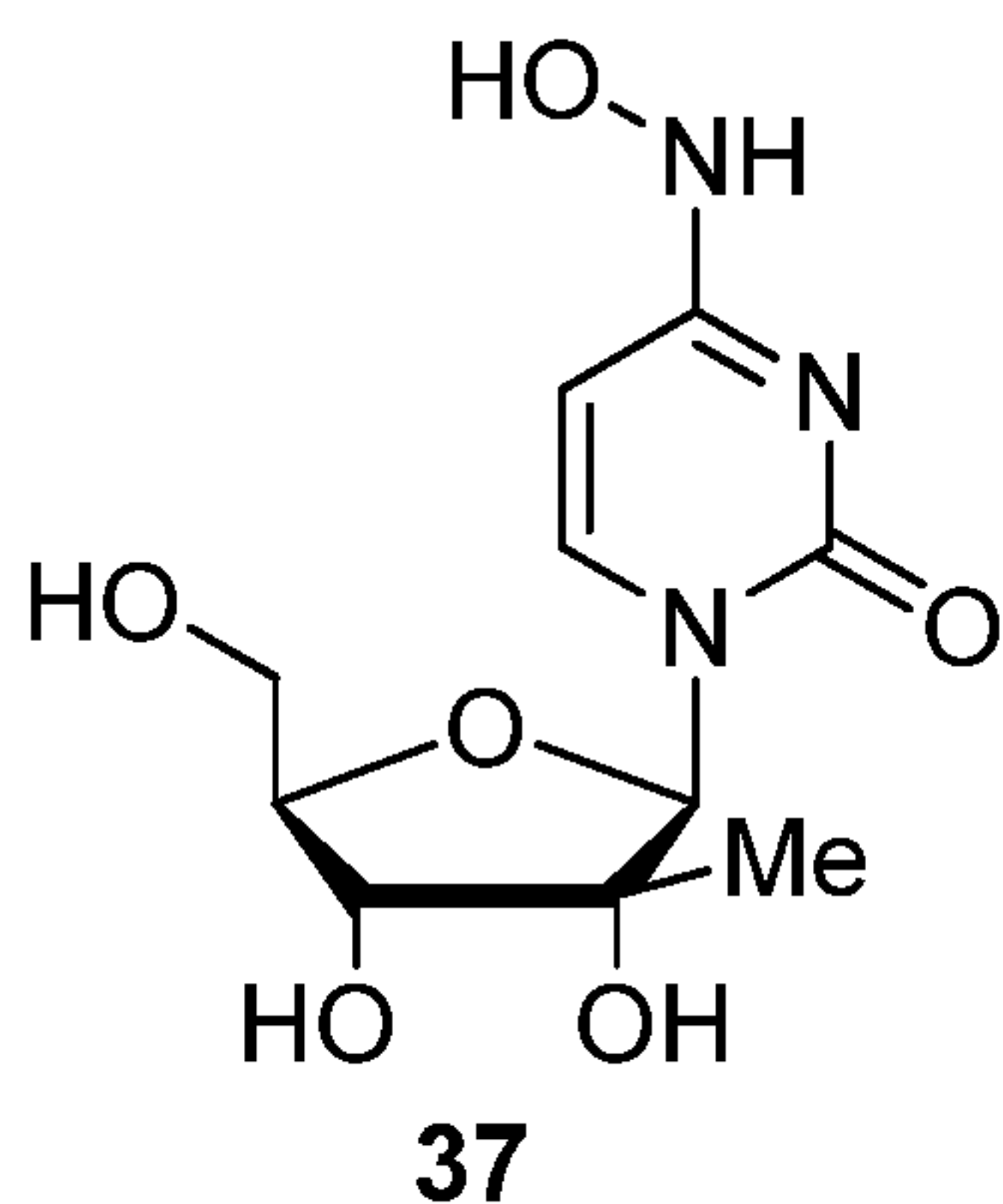
drug is added 1 h after infection. In the case where a compound blocks attachment, it will show up positive in the CPE assay, but may be negative by plaque assay. Efficacy: a minimum of six drug concentrations would be used covering a range of 100 mg/ml to 0.03 mg/ml, in 5-fold increments. From these data would be calculated the dose that inhibited viral replication by 50% (effective concentration 50; EC₅₀). Toxicity: The same drug concentrations used to determine efficacy can also be 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 stain, 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.

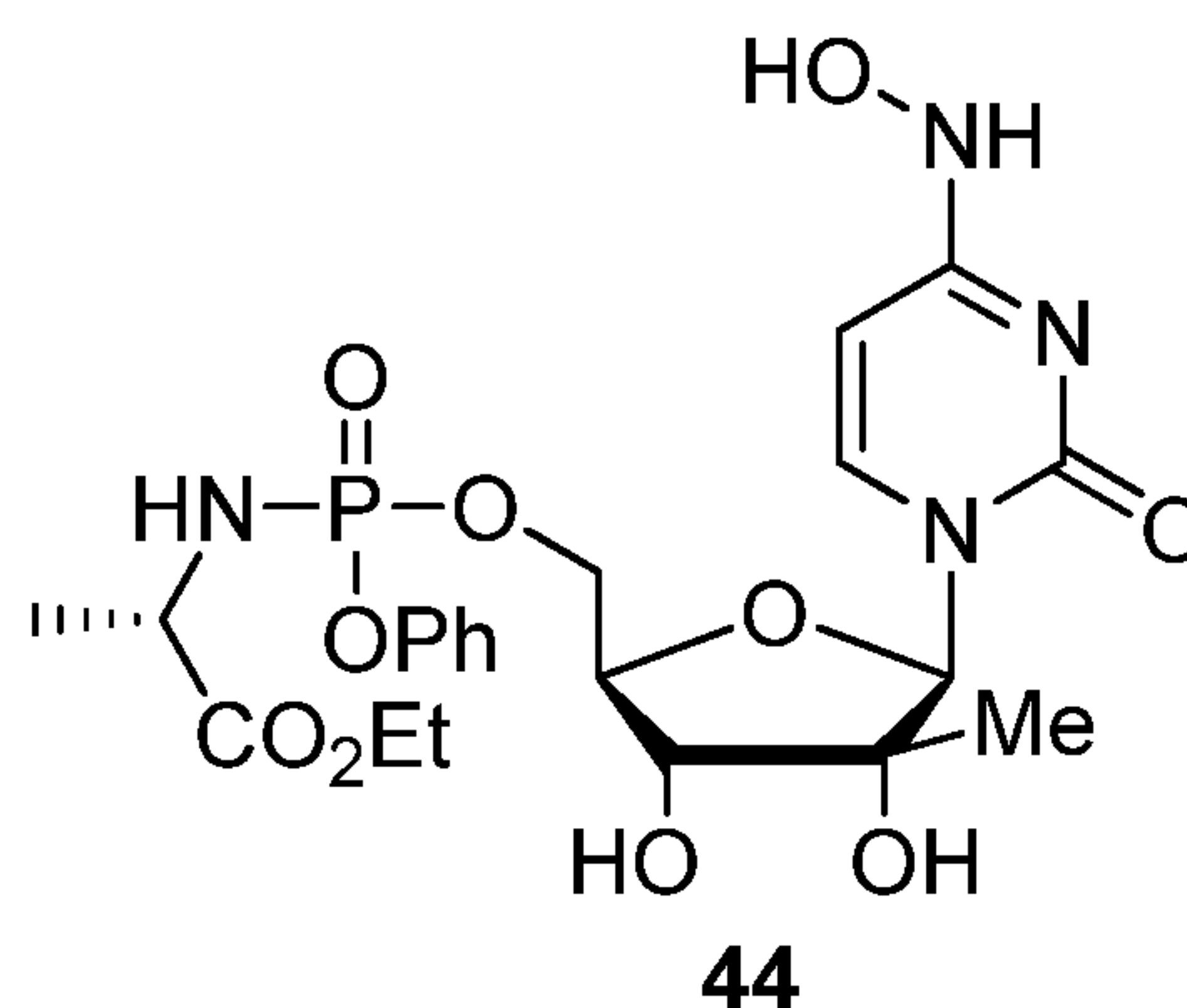
Example 24

HCV Replicon Assay¹

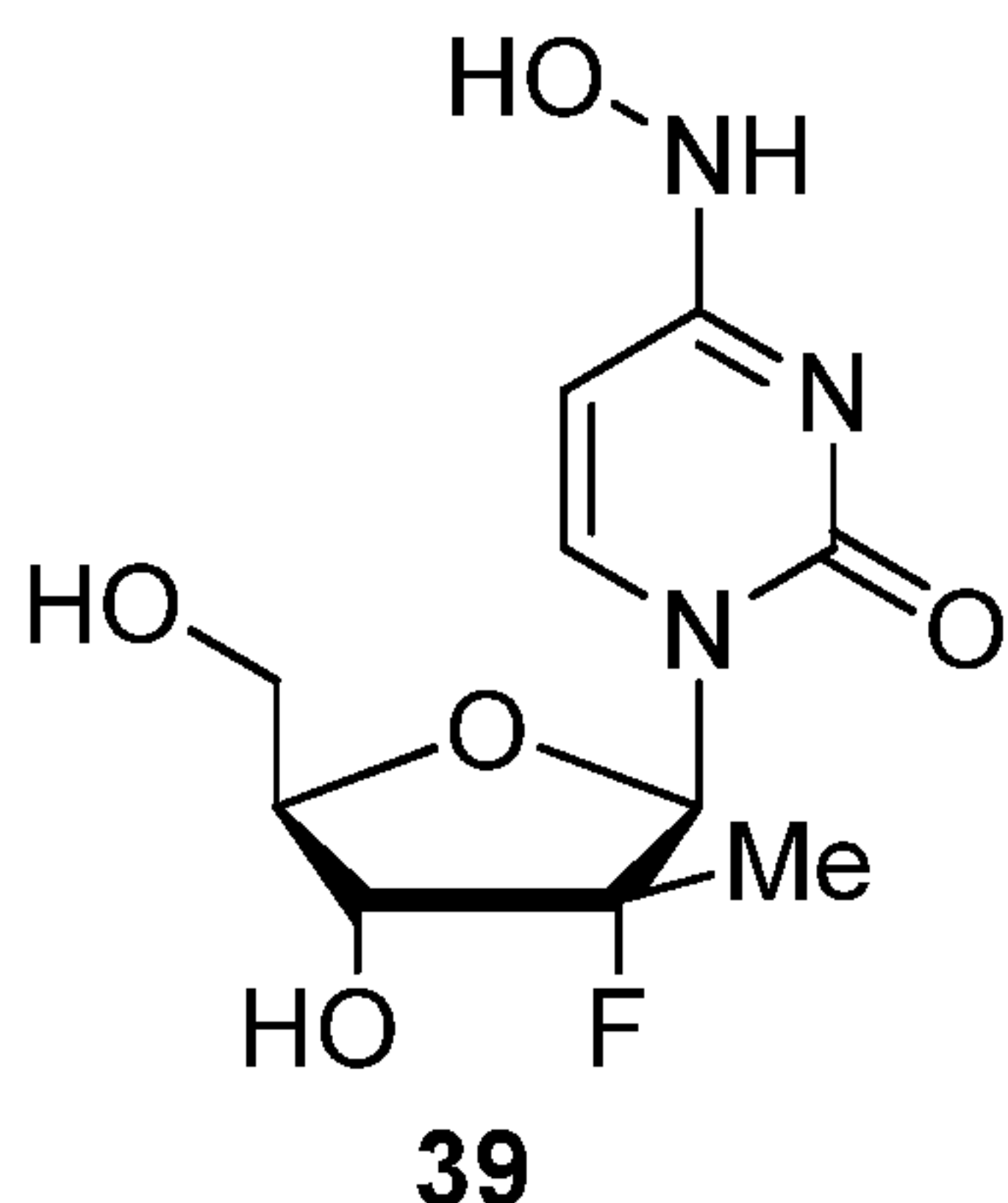
Huh 7 Clone B cells containing HCV Replicon RNA were seeded in a 96-well plate at 5000 cells/well, and the compounds tested at 10 μ M in triplicate immediately after seeding. Following five days incubation (37°C, 5% CO₂), total cellular RNA was isolated by using versaGene RNA purification kit from Gentra. 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 (Δ Ct HCV). A Δ Ct 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 Δ Ct rRNA values. (2'-Me-C) was used as the control. To determine EC₉₀ and IC₅₀ values², Δ Ct: values were first converted into fraction of starting material³ and then were used to calculate the % inhibition.



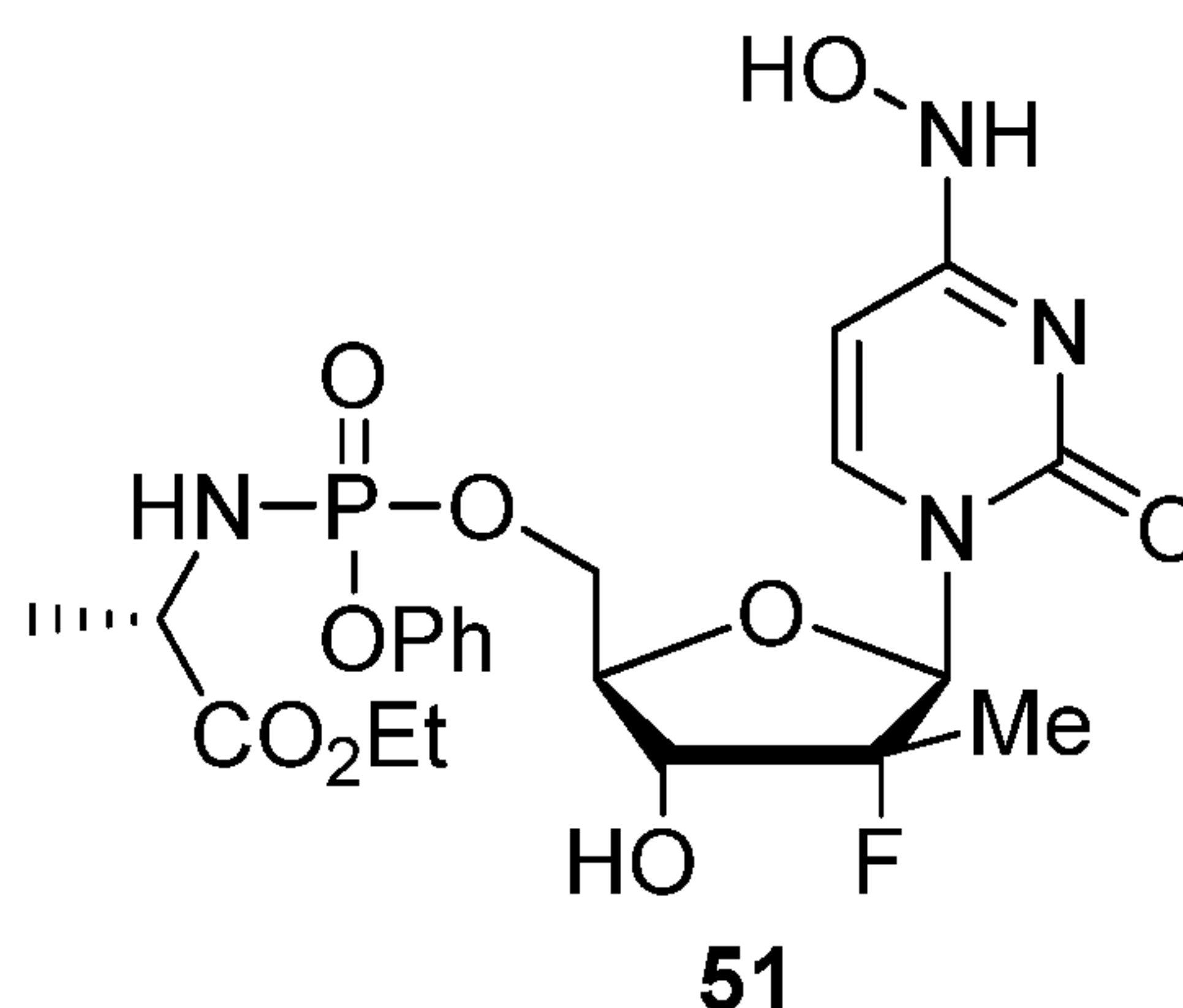
HCV EC₅₀ = > 10 μM
 PBM IC₅₀ = >100 μM
 CEM IC₅₀ = >100 μM
 Vero IC₅₀ = >100 μM
 Huh-7 IC₅₀ = >10 μM



HCV EC₅₀ = 0.8 μM
 PBM IC₅₀ = >100 μM
 CEM IC₅₀ = >100 μM
 Vero IC₅₀ = >100 μM
 Huh-7 IC₅₀ = >10 μM



HCV EC₅₀ = > 10 μM
 PBM IC₅₀ = >100 μM
 CEM IC₅₀ = >100 μM
 Vero IC₅₀ = >100 μM
 Huh-7 IC₅₀ = >10 μM



HCV EC₅₀ = 2.6 μM
 PBM IC₅₀ = >100 μM
 CEM IC₅₀ = >100 μM
 Vero IC₅₀ = >100 μM
 Huh-7 IC₅₀ = >33 μM

References:

1. Stuyver L et al., Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture. *Antimicrob. Agents Chemother.* **2003**, 47, 244-254.
2. Reed IJ & Muench H, A simple method of 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 cm² 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 2.5×10^6 cells per well in a 6-well plate and exposed to 10 μ M of [³H] labeled active compound (500 dpm/pmol) for the specified time periods.

The cells are maintained at 37°C under a 5% CO₂ atmosphere. At the selected time points, the cells are washed three times with ice-cold phosphate-buffered saline (PBS).

Intracellular active compound and its respective metabolites are extracted by incubating the cell pellet overnight at -20°C with 60% methanol. 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 μ Ci of ³H 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 (C_{max}), time when the maximum concentration is achieved (T_{max}), area under the curve (AUC), half life of the dosage concentration (T_{1/2}), clearance (CL), steady state volume and distribution (V_{ss}) 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 (EC₅₀) 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 (2×10^5 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 (5×10^5 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.

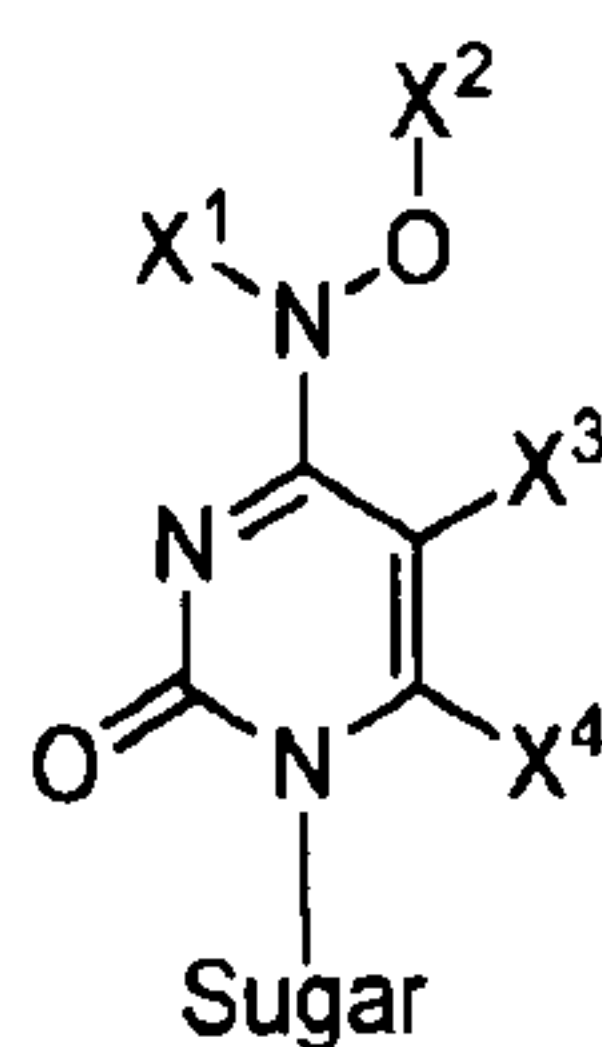
Each of the references identified in this application are incorporated herein in their entirety for all purposes.

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.

REPLACEMENT SHEET

Claims:

1. A compound of Formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X¹ is H, C₁-C₆alkyl, C₁-C₆haloalkyl, C₁-C₆alkoxy, C₂-C₆ alkenyl, C₂-C₆ alkynyl, COR¹, or COOR¹;

X² is hydrogen, CH₂-O(CO)-X⁵; CH₂-O(CO)O-X⁵, COR¹, or COOR¹,

wherein each R¹ is, independently, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₆ alkyl, or C₁₋₆ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, or C₃₋₁₀ cycloalkyl.

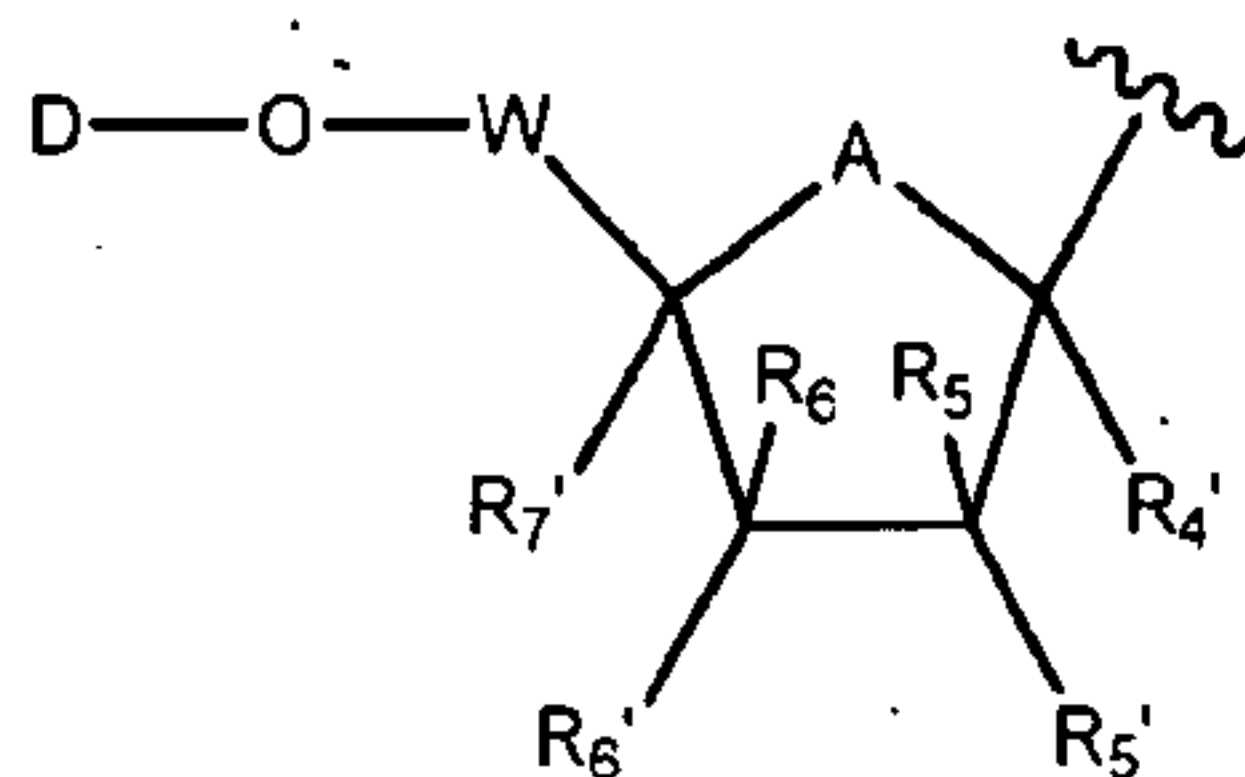
X⁵ is independently, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁-C₆ alkyl, alkoxy, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₆ alkyl, or C₁₋₆ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, or C₃₋₁₀ cycloalkyl.

each X³ and X⁴ is independently H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aryl, alkylaryl, halogen, NH₂, OH, SH, CN, or NO₂.

2. A compound of Claim 1, wherein each R² is, COR¹ or COOR¹.

REPLACEMENT SHEET

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)R¹, C(O)OR¹, diphosphate ester, or triphosphate ester;

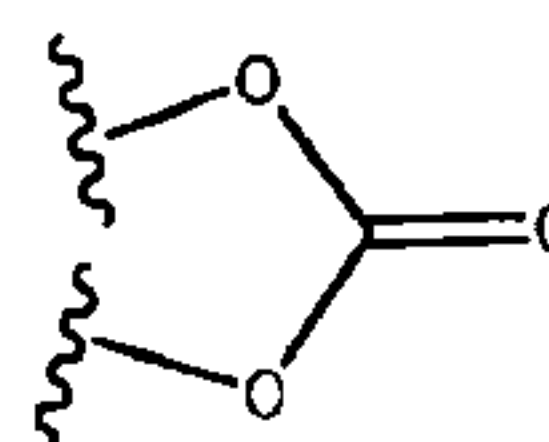
R¹ is as defined above;

W is CL₂ or CL₂CL₂, wherein L independently is selected from the group consisting of H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH₂, CHF, CF₂, C=CH₂, C=CHF, or C=CF₂;

R^{4'}, R^{5'}, R^{6'}, and R^{7'} are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH₂, NHOH, NHNH₂, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

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



wherein:

when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H then, R^{5'}, R^{6'} can not be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH,

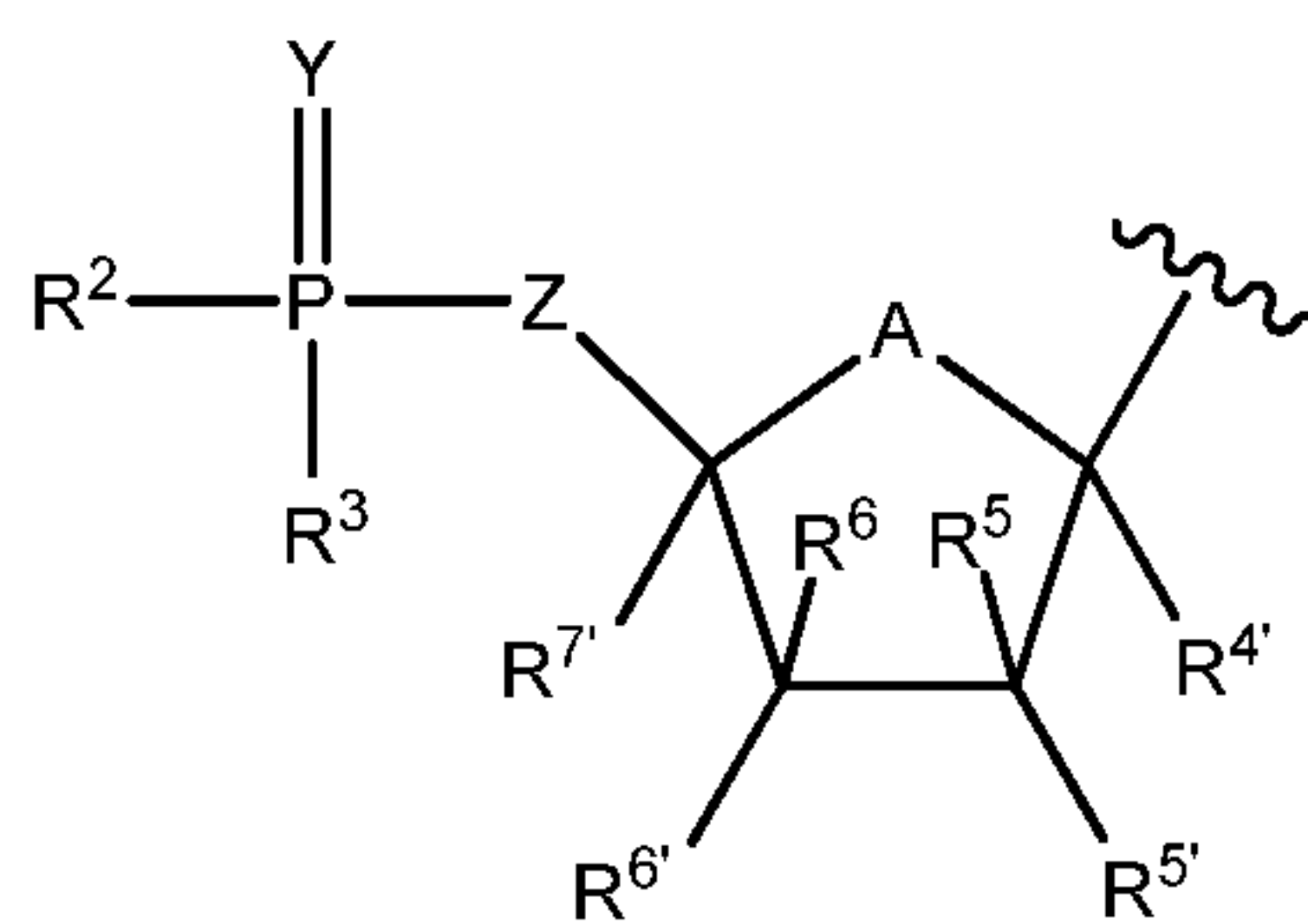
when A is O or S, $R^{7'}$ cannot be OH, SH, NH_2 , NHOH, $NHNH_2$, OR, SR, SSR, NHR, or NR_2 , and

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.

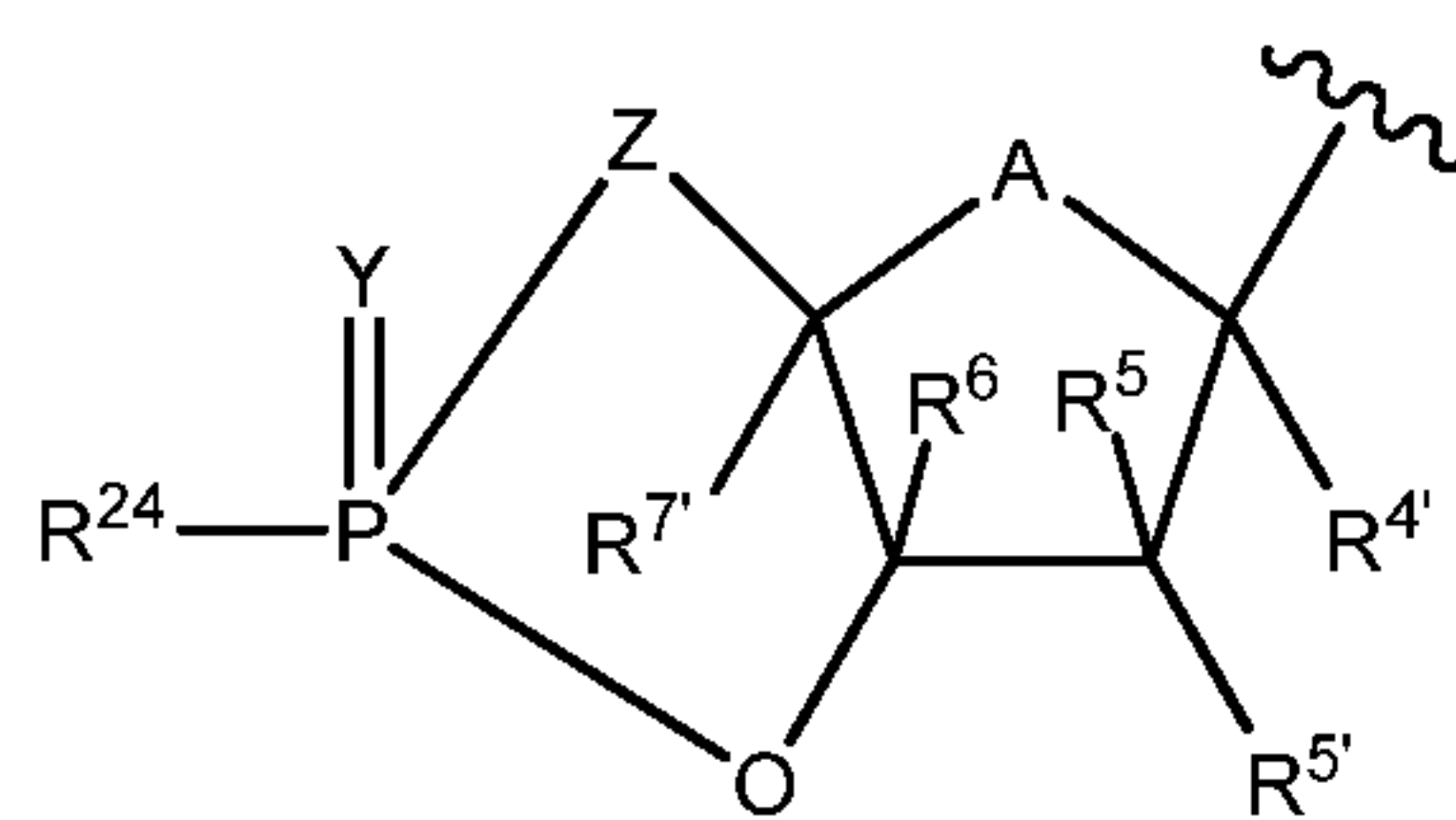
4. The compound of Claim 3, wherein R^6 is independently selected from the group consisting of NHOH, $NHNH_2$, N_3 , $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, R, OR, SR, SSR, NHR, and NR_2 , and wherein R is independently a C_1 - C_6 alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_3 - C_6 cycloalkyl) 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;

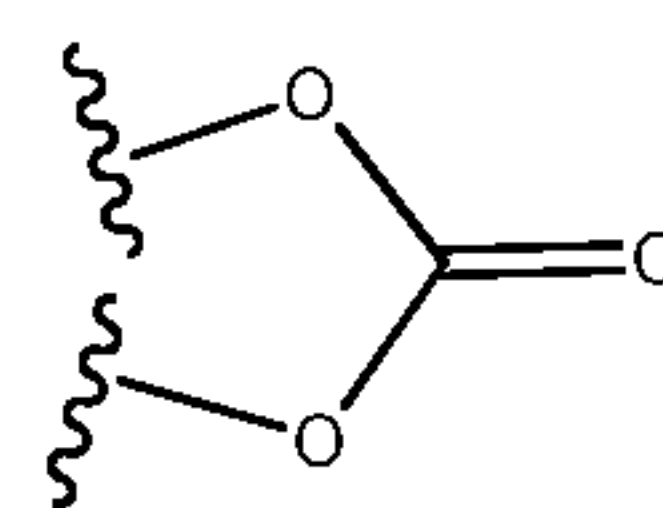
Z is selected from the group consisting of CL_2 , CL_2CL_2 , CL_2OCL_2 , CL_2SCL_2 , CL_2O , OCL_2 and CL_2NHCL_2 , wherein L independently is selected from the group consisting of H, F, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-}

₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A is O, S, CH₂, CHF, CF₂, C=CH₂, C=CHF, or C=CF₂;

R^{4'}, R⁵, R^{5'}, R⁶, R^{6'}, and R^{7'} are independently selected from the group consisting of H, F, Cl, Br, I, OH, SH, NH₂, NHOH, NHNH₂, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂;

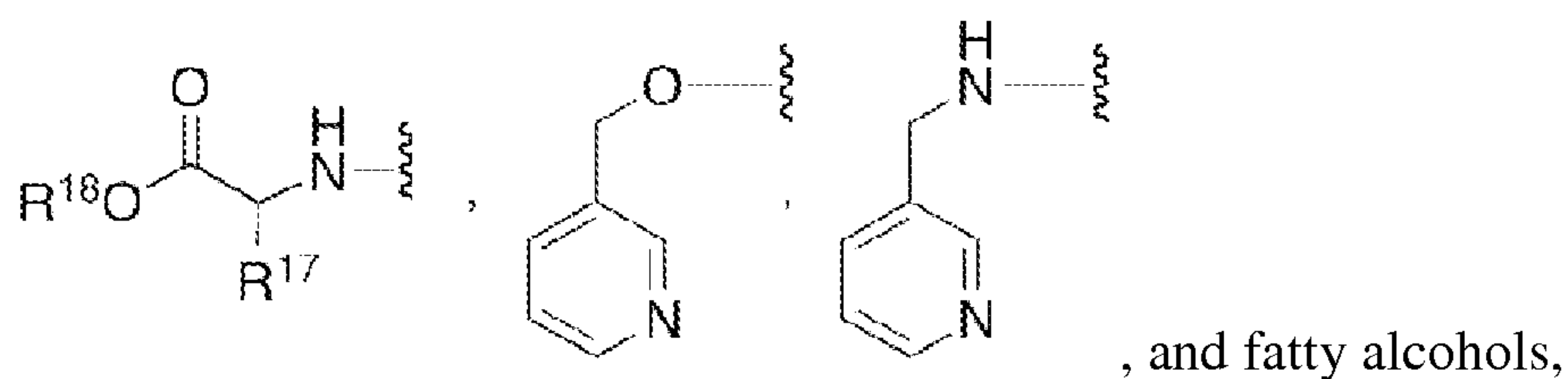
R^{5'} and R^{6'} can come together to form a ring



wherein when A is O or S, R^{7'} cannot be OH, SH, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, or NR₂, and

R is independently a C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, C₃₋₆ cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1,

R²⁴ is selected from the group consisting of OR¹⁵,



R¹⁵ 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 (CH₂)₀₋₆CO₂R¹⁶ and (CH₂)₀₋₆CON(R¹⁶)₂;

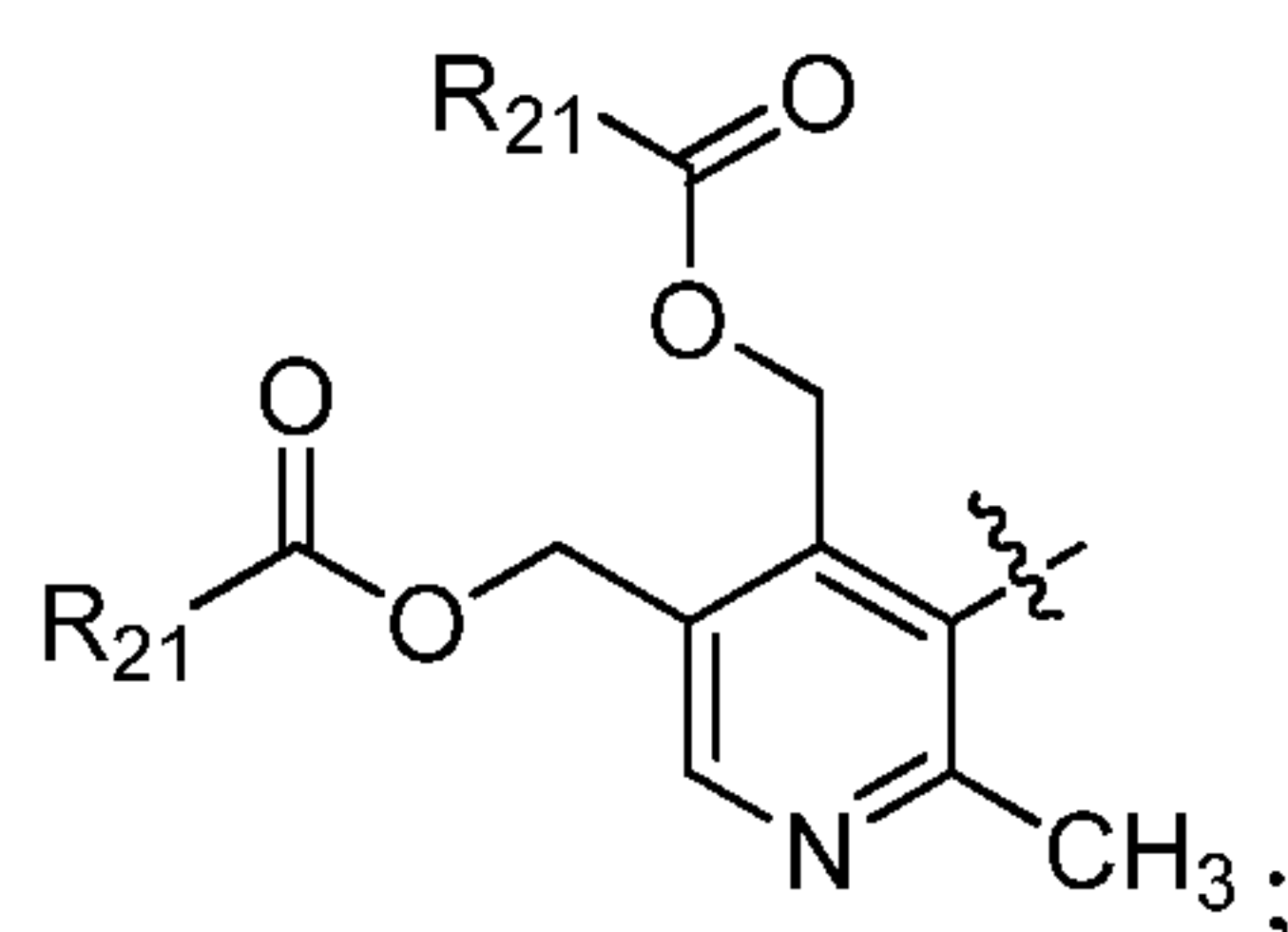
R¹⁷ is selected from those groups occurring in natural L-amino acids, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₆ cycloalkyl, aryl, alkylaryl, or arylalkyl, wherein the groups can be substituted with one or more substituents as defined above in Claim 1,

R¹⁸ is H, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-

amino, fluoro, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C_{1-C6} alkyl, C_{1-C6} alkoxy, di(C_{1-C6} alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or cycloalkyl;

R² and R³ are independently selected from the group consisting of:

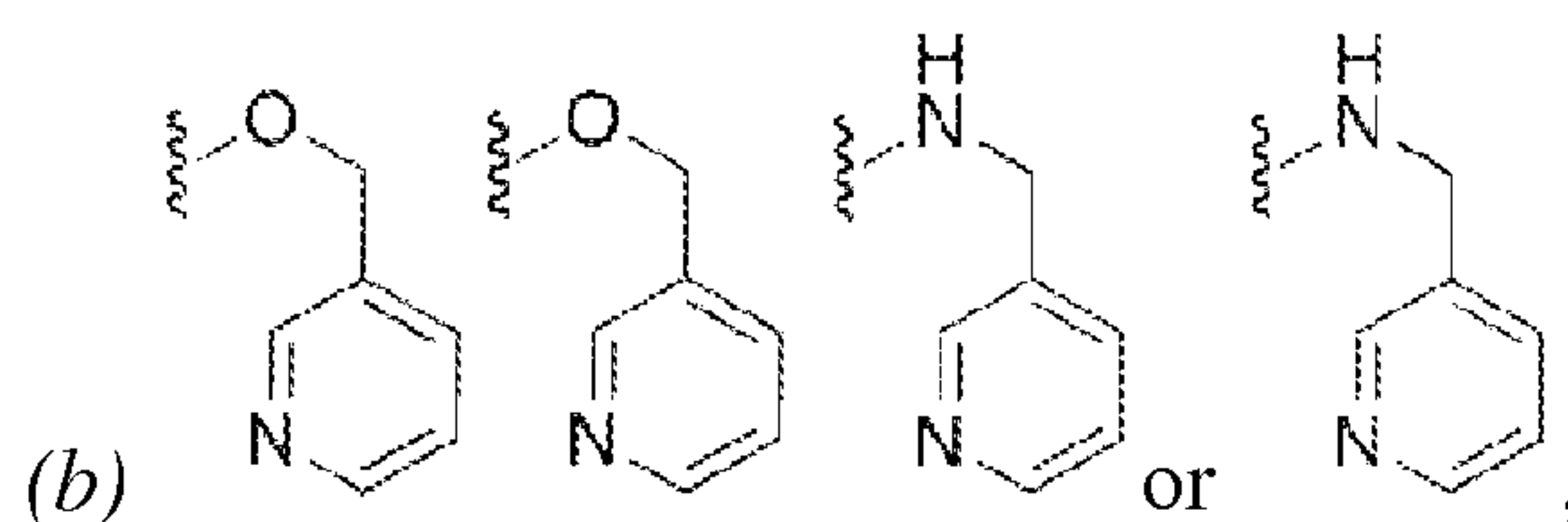
(a) OR⁸ where R⁸ is H, Li, Na, K, C₁₋₂₀ alkyl, C₃₋₆ cycloalkyl, C₁₋₆ haloalkyl, aryl, or heteroaryl, optionally substituted with one to three substituents independently selected from the group consisting of C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkoxy, (CH₂)₀₋₆CO₂R^{9a}, halogen, C₁₋₆ haloalkyl, -N(R^{9a})₂, C₁₋₆ acylamino, -NHSO₂C₁₋₆ alkyl, -SO₂N(R^{9a})₂, -SO₂C₁₋₆ alkyl, COR^{9b}, nitro, cyano and

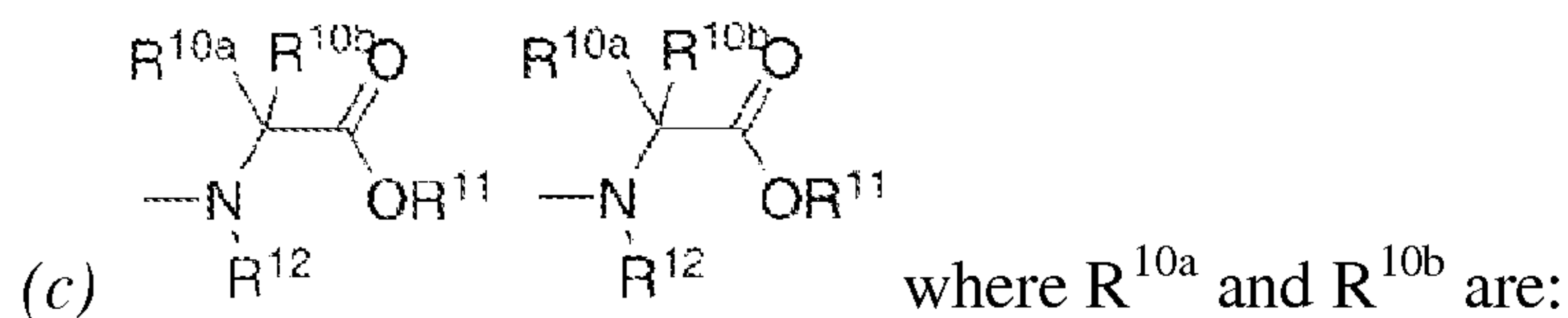


wherein R²¹ is as defined below;

R^{9a} is independently H, C₁₋₂₀ alkyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

R^{9b} is -OR^{9a} or -N(R^{9a})₂;





(i) independently selected from the group consisting of H, C₁₋₁₀ alkyl, -(CH₂)_rNR^{9a}₂, C₁₋₆ hydroxyalkyl, -CH₂SH, -(CH₂)₂S(O)_pMe, -(CH₂)₃NHC(=NH)NH₂, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -(CH₂)_mCOR^{9b}, aryl and aryl-C₁₋₃ alkyl, wherein said aryl groups are optionally substituted with a group selected from the group consisting of hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, halogen, nitro, and cyano;

(ii) R^{10a} is H and R^{10b} and R¹² together are (CH₂)₂₋₄ to form a ring that includes the adjoining N and C atoms;

(iii) R^{10a} and R^{10b} together are (CH₂)_n to form a ring;

(iv) R^{10a} and R^{10b} both are C₁₋₆ alkyl; or

(v) R^{10a} is H and R^{10b} is H, CH₃, CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or C₃₋₁₀ cycloalkyl;

p is 0 to 2;

r is 1 to 6;

n is 4 or 5;

m is 0 to 3;

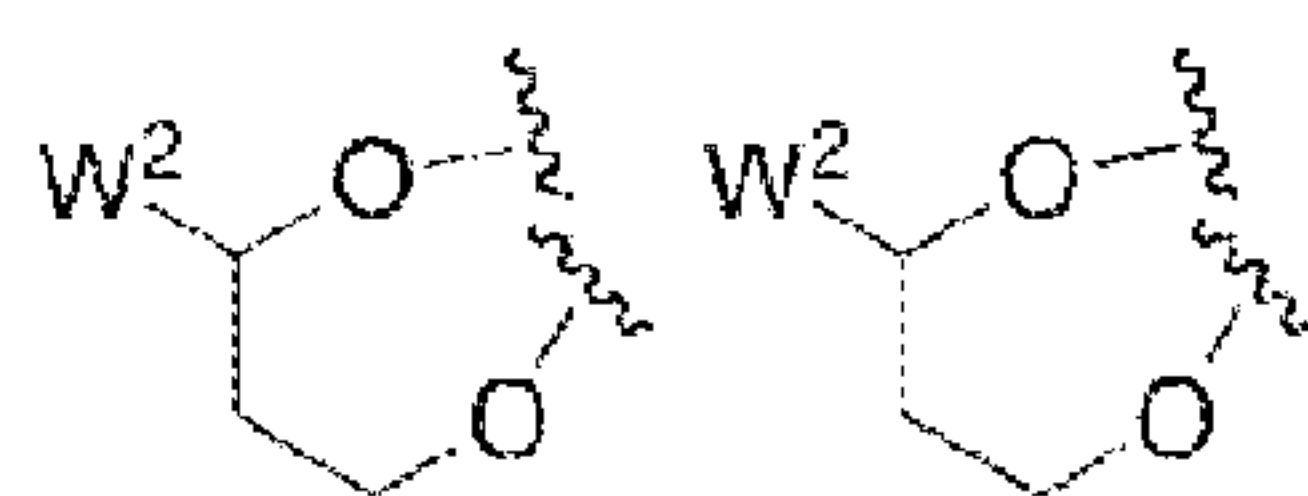
R¹¹ is H, C₁₋₁₀ alkyl, or C₁₋₁₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl;

wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

R¹² is H or C₁₋₃ alkyl, or R^{10a}, or R^{10b} and R¹² together are (CH₂)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) R² and R³ can come together to form a ring



where W² 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 C₁₋₆ alkyl, CF₃, C₂₋₆ alkenyl, C₁₋₆ alkoxy, OR^{9c}, CO₂R^{9a}, COR^{9a}, halogen, C₁₋₆ haloalkyl, -N(R^{9a})₂, C₁₋₆ acylamino, CO₂N(R^{9a})₂, SR^{9a}, -NHSO₂C₁₋₆ alkyl, -SO₂N(R^{9a})₂, -SO₂C₁₋₆ 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:

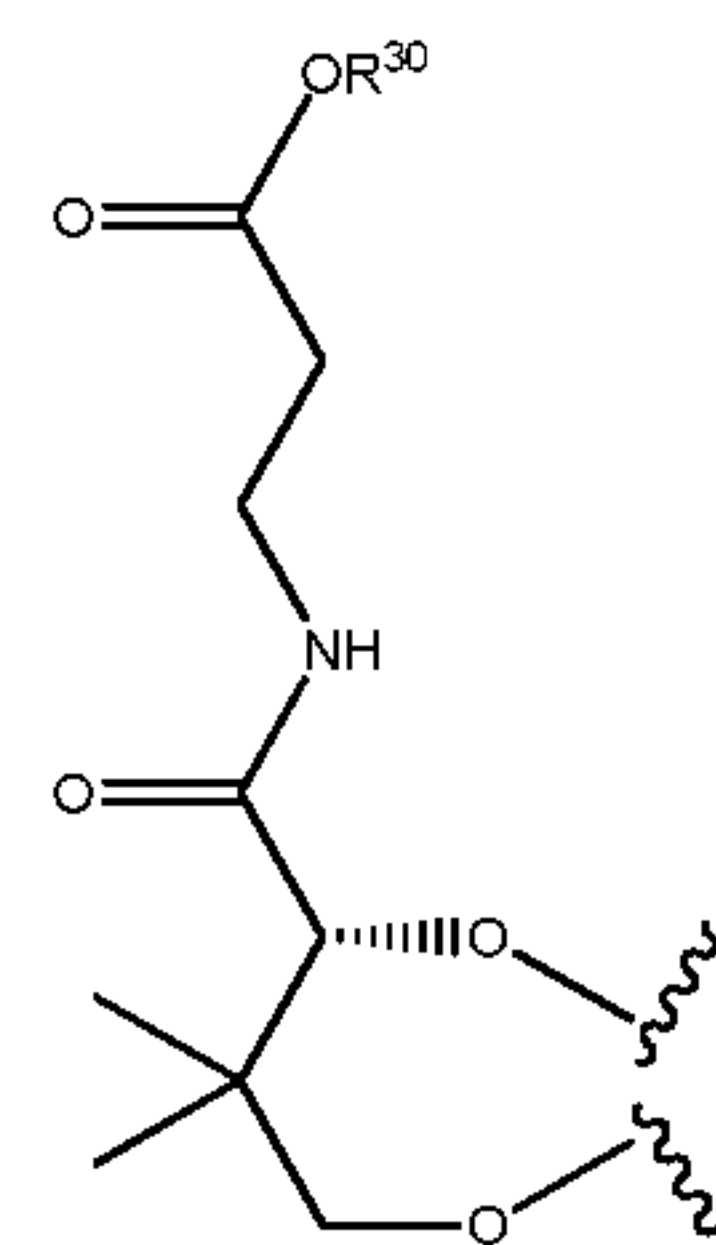
a) when there are two heteroatoms and one is O, then the other can not be O or S, and

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

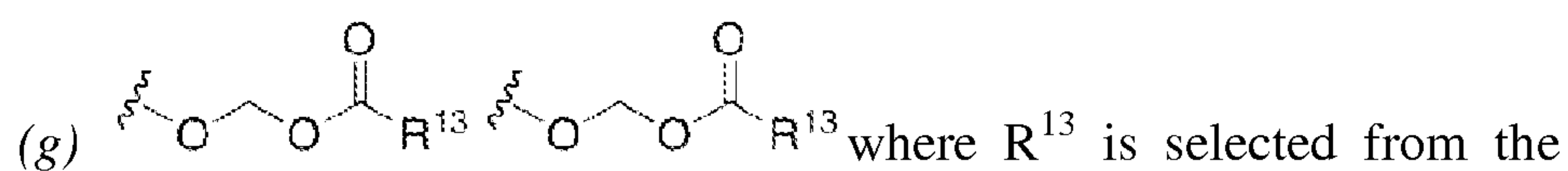
R^{9a} is independently H or C₁₋₆ alkyl;

R^{9b} is -OR^{9a} or -N(R^{9a})₂;

R^{9c} is H or C₁₋₆ acyl;

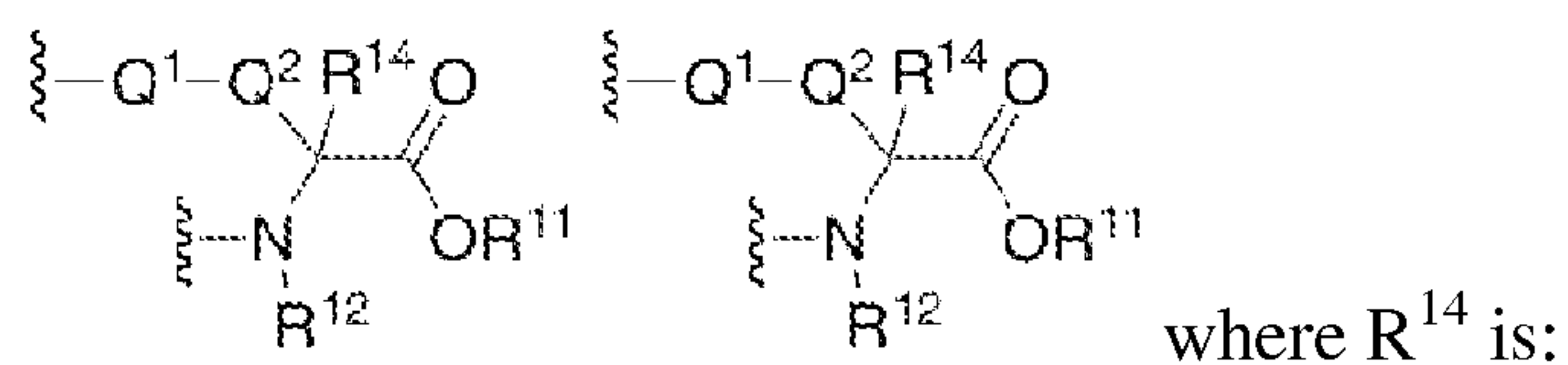


(f) R² and R³ can come together to form a ring where R³⁰ is H, C₁₋₂₀ alkyl, C₁₋₂₀ alkenyl, the carbon chain derived from a fatty alcohol or C₁₋₂₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;



group consisting of H, C₁₋₁₀ alkyl, C₁₋₁₀ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, C₃₋₁₀ cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl moiety; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁₋₆ alkyl, C₁₋₆ alkoxy, di(C₁₋₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

(h) R² and R³ can come together to form a ring



(i) independently selected from the group consisting of H, C₁₋₁₀ alkyl, - (CH₂)_rNR₂^{9a}, C₁₋₆ hydroxyalkyl, -CH₂SH, -(CH₂)₂S(O)_pMe, - (CH₂)₃NHC(=NH)NH₂, (1*H*-indol-3-yl)methyl, (1*H*-imidazol-4-yl)methyl, -(CH₂)_mCOR^{9b}, aryl, aryl-C₁₋₃ alkyl, heteroaryl, and heteroaryl-C₁₋₃ alkyl, wherein said aryl and heteroaryl groups are optionally substituted with a group selected from the group consisting of hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ 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

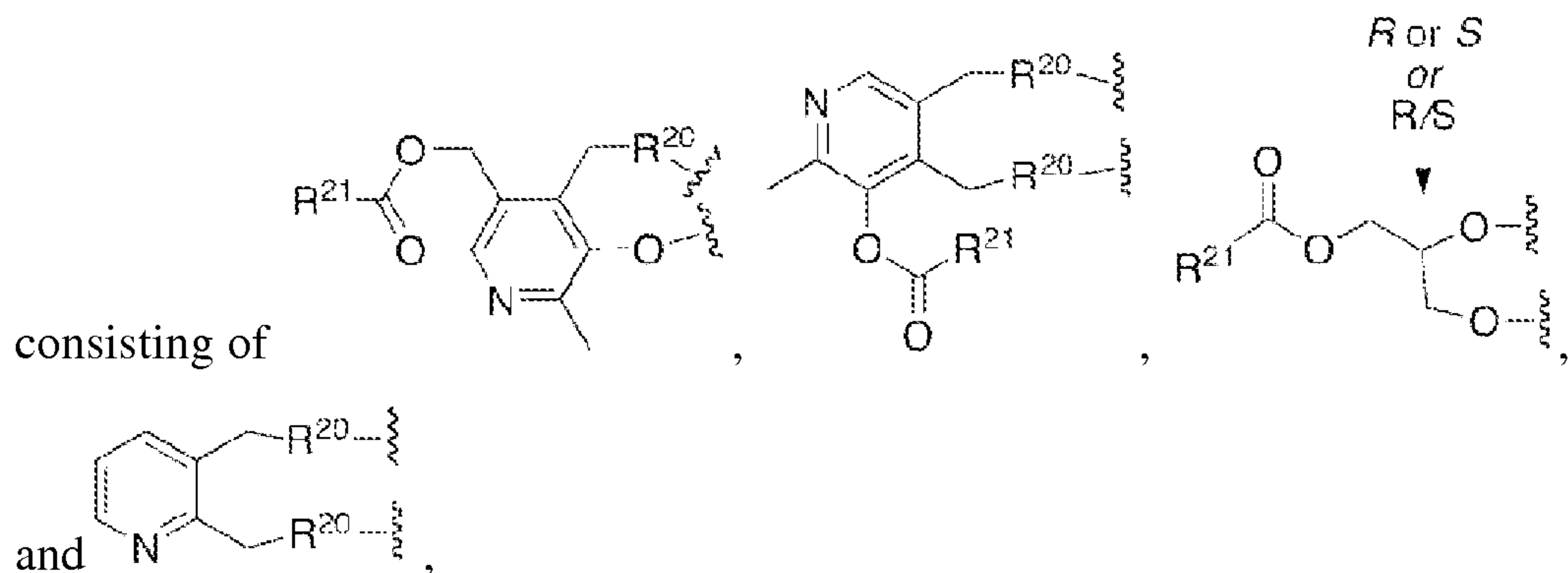
Q^1 is NR^{9a} , O, or S

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



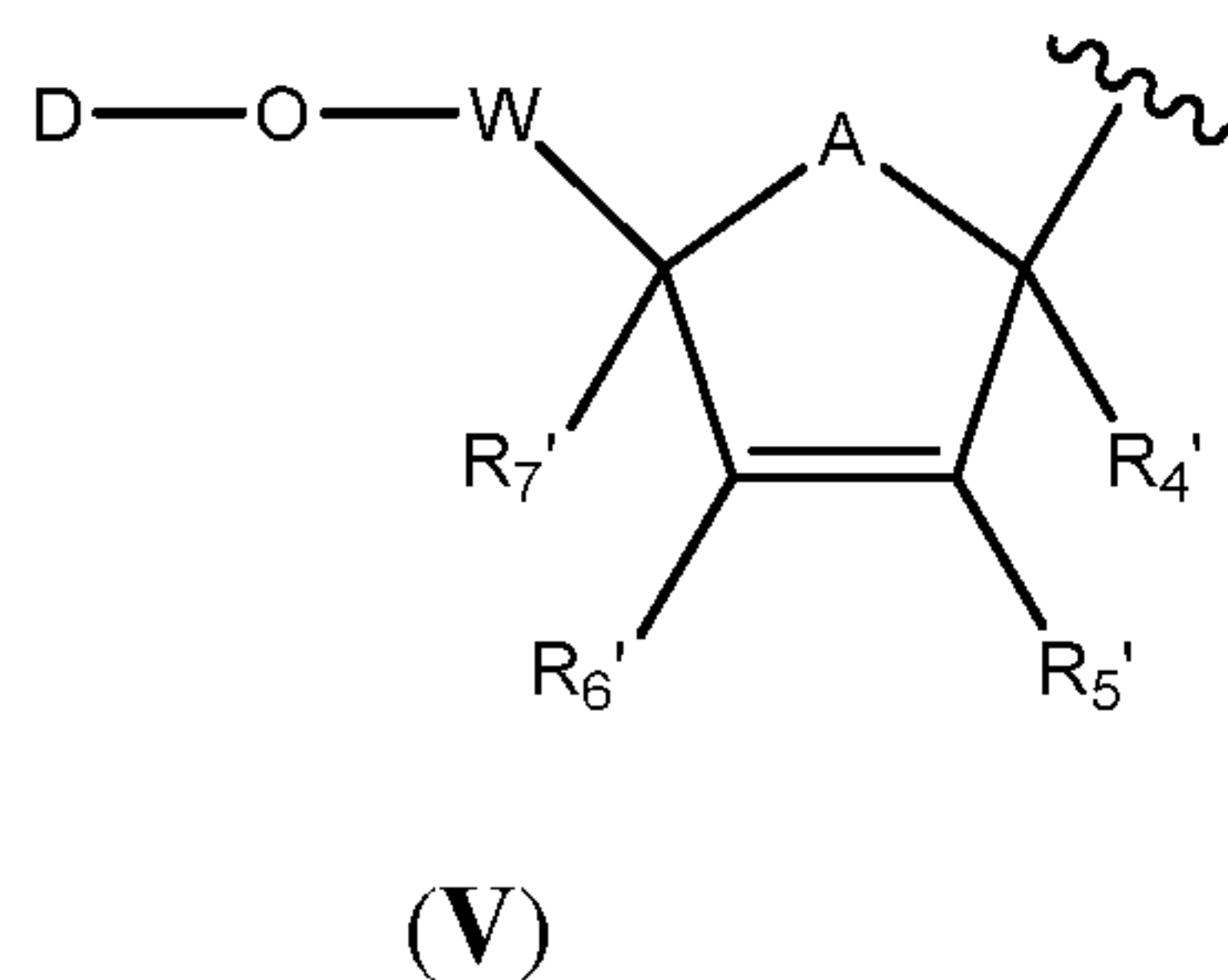
where R^{20} is O or NH and

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):



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 C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl can each optionally contain one or more heteroatoms;

A, R², R³, 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, NH₂, NHOH, NHNH₂, OR, SR, SSR, NHR, and NR₂,

wherein R is independently a C₁-C₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃-C₆ 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,

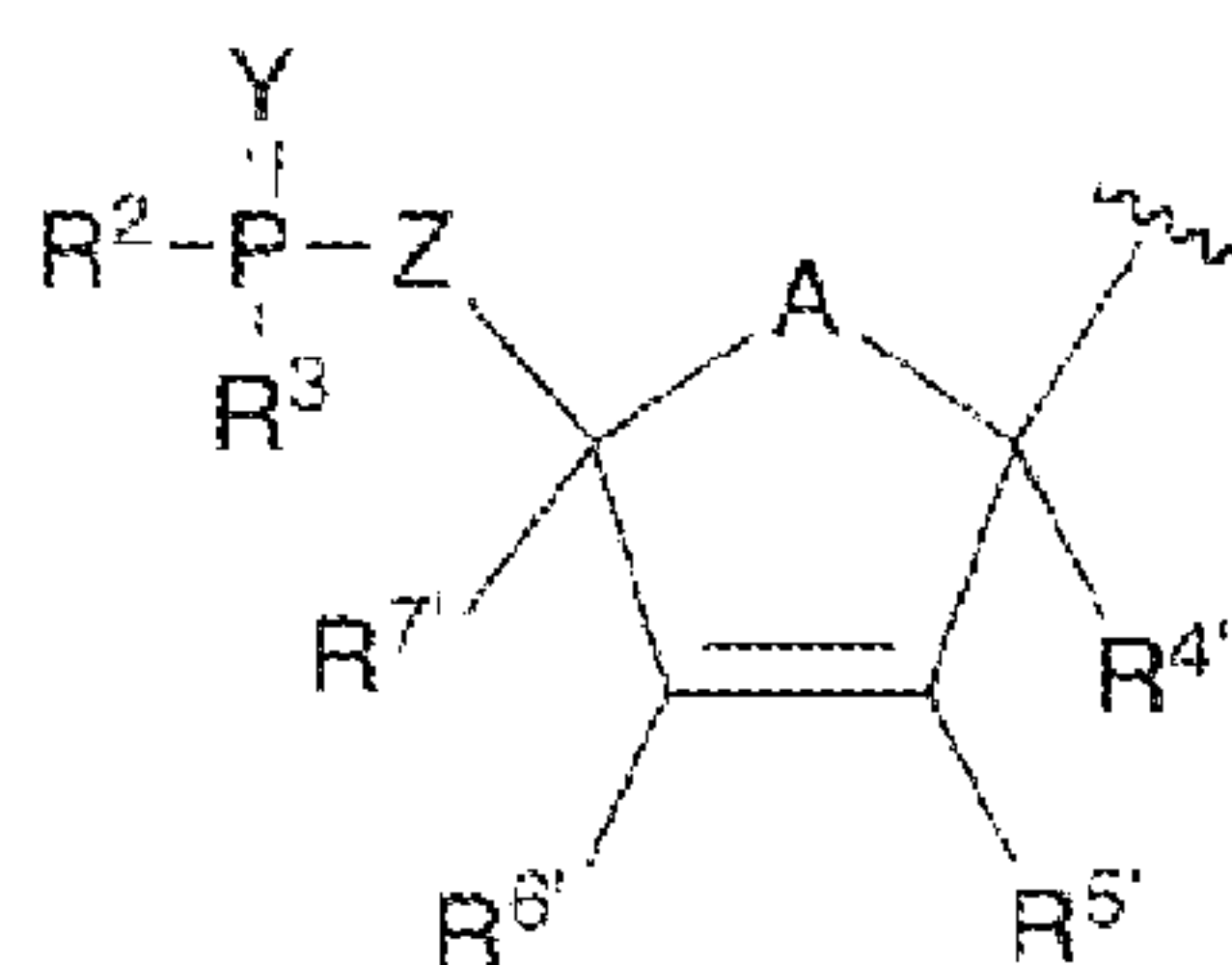
and wherein, when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H, then, R^{5'} and R^{6'} cannot be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

9. The compound of Claim 8, wherein R^{7'} is, independently, selected from the group consisting of H, F, Cl, Br, I, N₃, C(O)OH, CN, CH₂OH, C(O)NH₂, C(S)NH₂, C(O)OR, and R, wherein R is independently C₁-C₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃-C₆ 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, NHNH₂, N₃, C(O)NH₂, C(S)NH₂, C(O)OR, R, OR, SR, SSR, NHR, and NR₂.

11. The compound of Claim 8, wherein when A is O or CH₂, D is H or acyl, W is CH₂, R^{4'} and R^{7'} are H then, R^{5'} and R^{6'} cannot be H, halogen, OH, SH, OCH₃, SCH₃, NH₂, NHCH₃, CH₃, CH=CH₂, CN, CH₂NH₂, CH₂OH, or COOH.

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



(VI)

wherein:

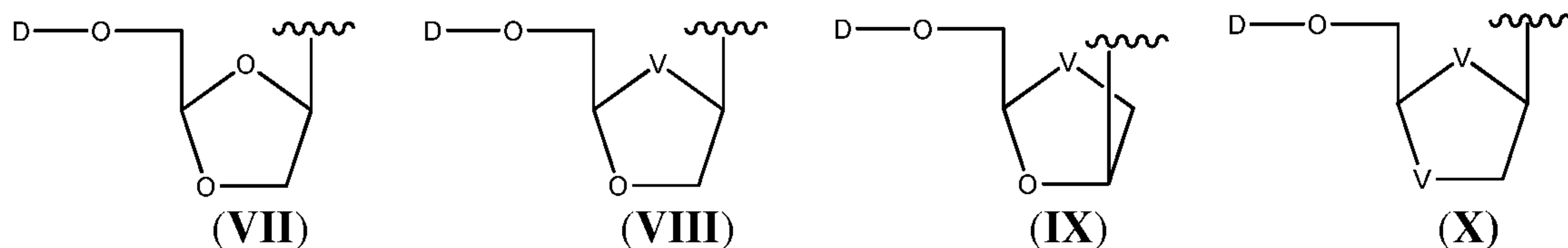
A, R^2 , R^3 , Y, Z, $R^{4'}$, $R^{5'}$, $R^{6'}$, and $R^{7'}$ are as defined above in connection with Claims 1-3;

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):



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

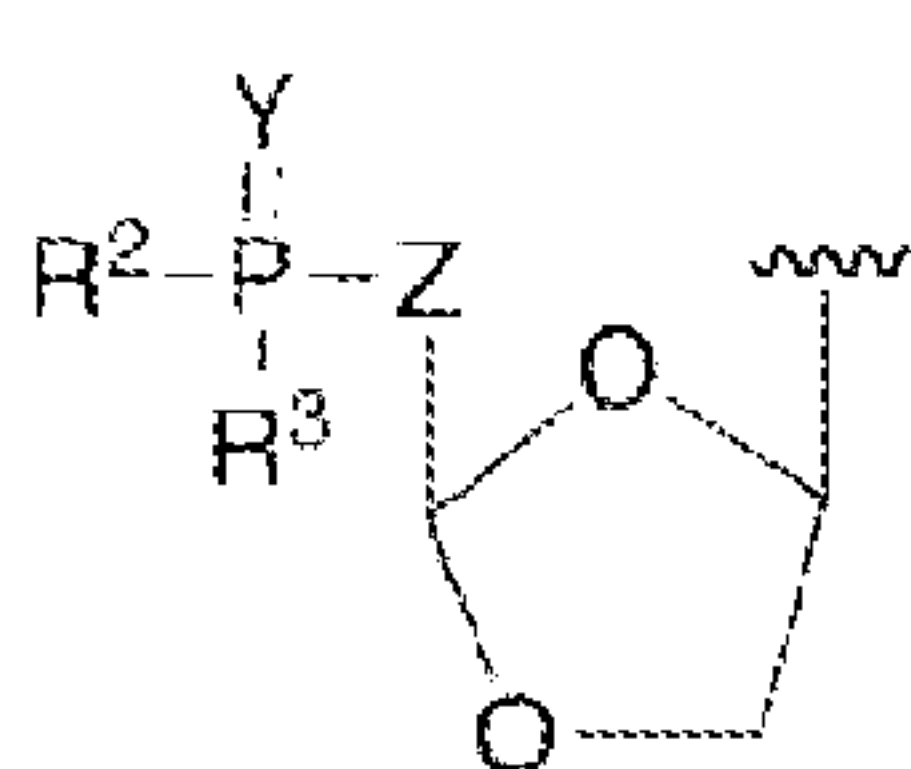
V is, individually, S or Se;

R^1 is independently C_{1-20} 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, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted

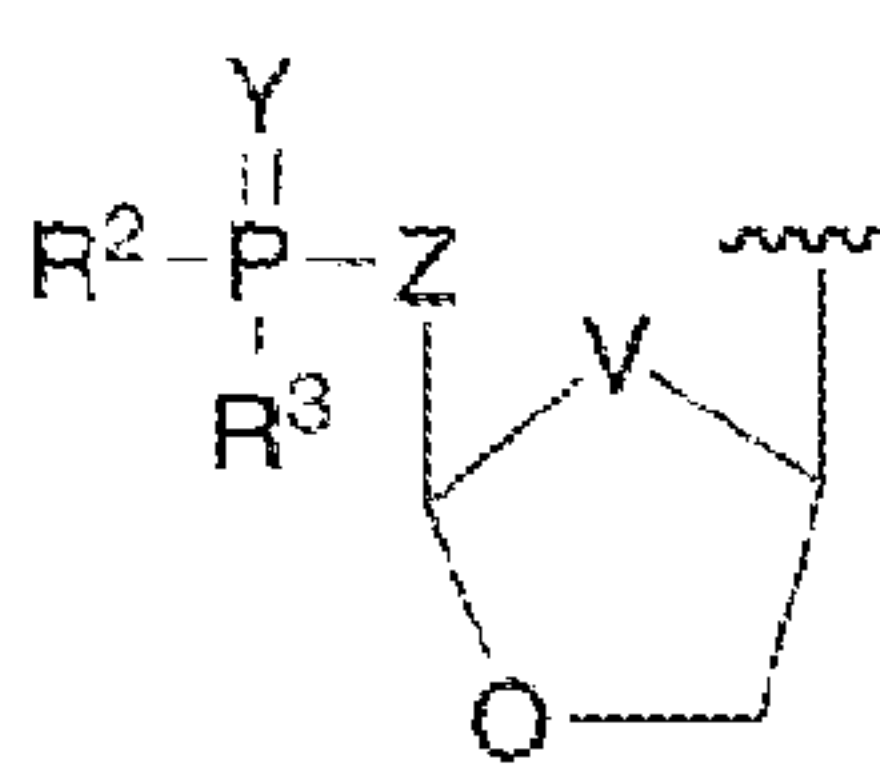
heteroaryl; wherein the substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a C₁-C₆ alkyl, C₁-C₆ alkoxy, di(C₁-C₆ alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or C₃₋₁₀ cycloalkyl alkyl;

wherein D cannot be H or acyl.

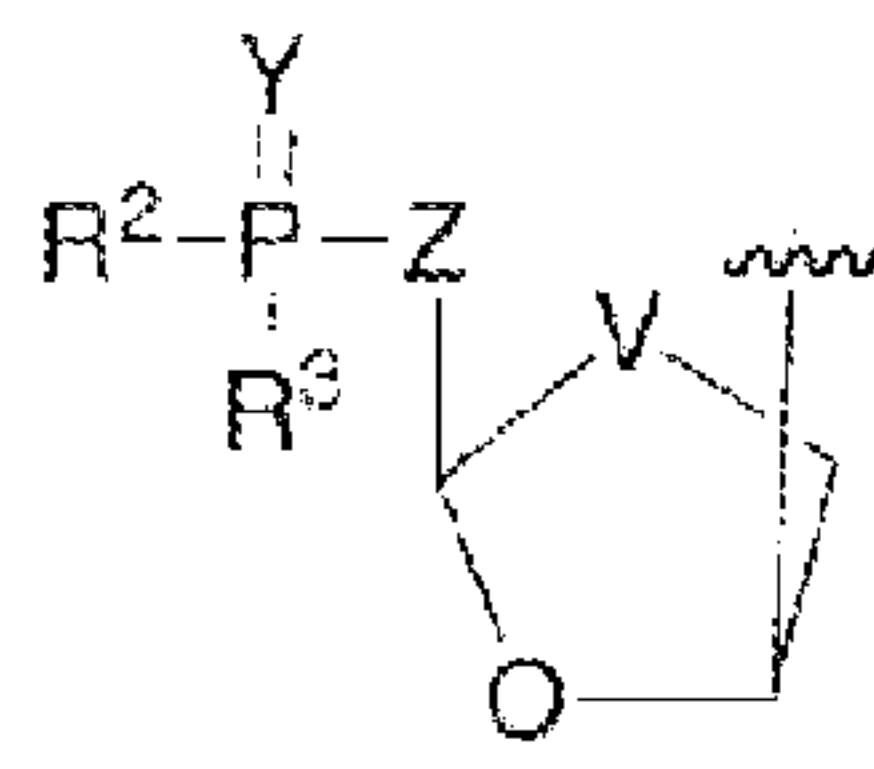
15. The compound of Claim 1, wherein Sugar is a dioxolane, oxathiolane, or dithiolane of the general formulas (XI), (XII), (XIII), and (XIV):



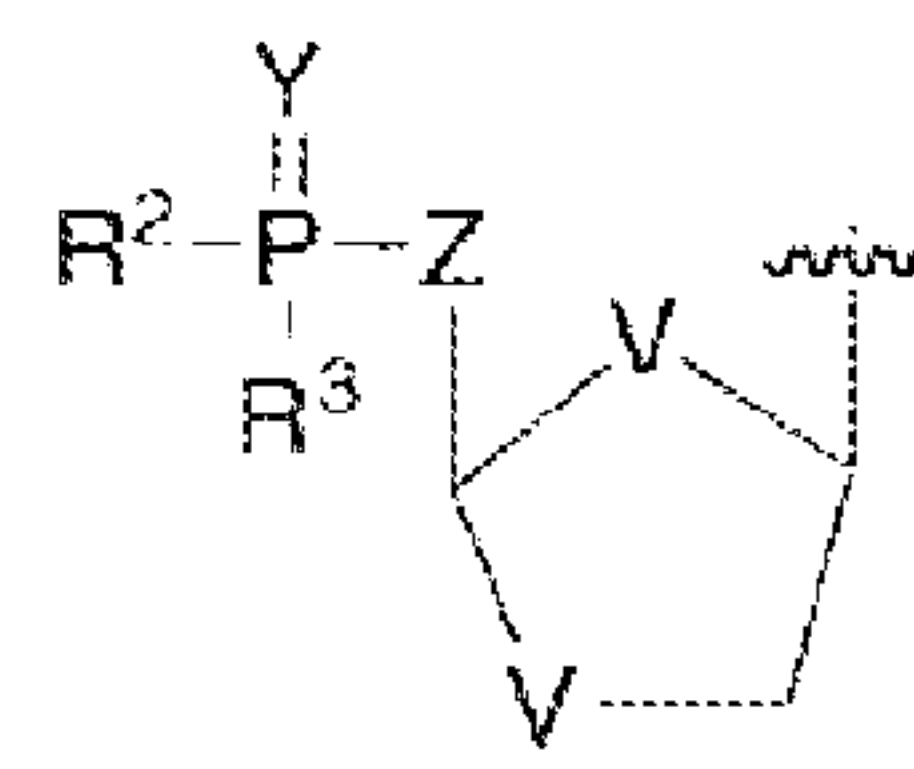
(XI)



(XII)



(XIII)



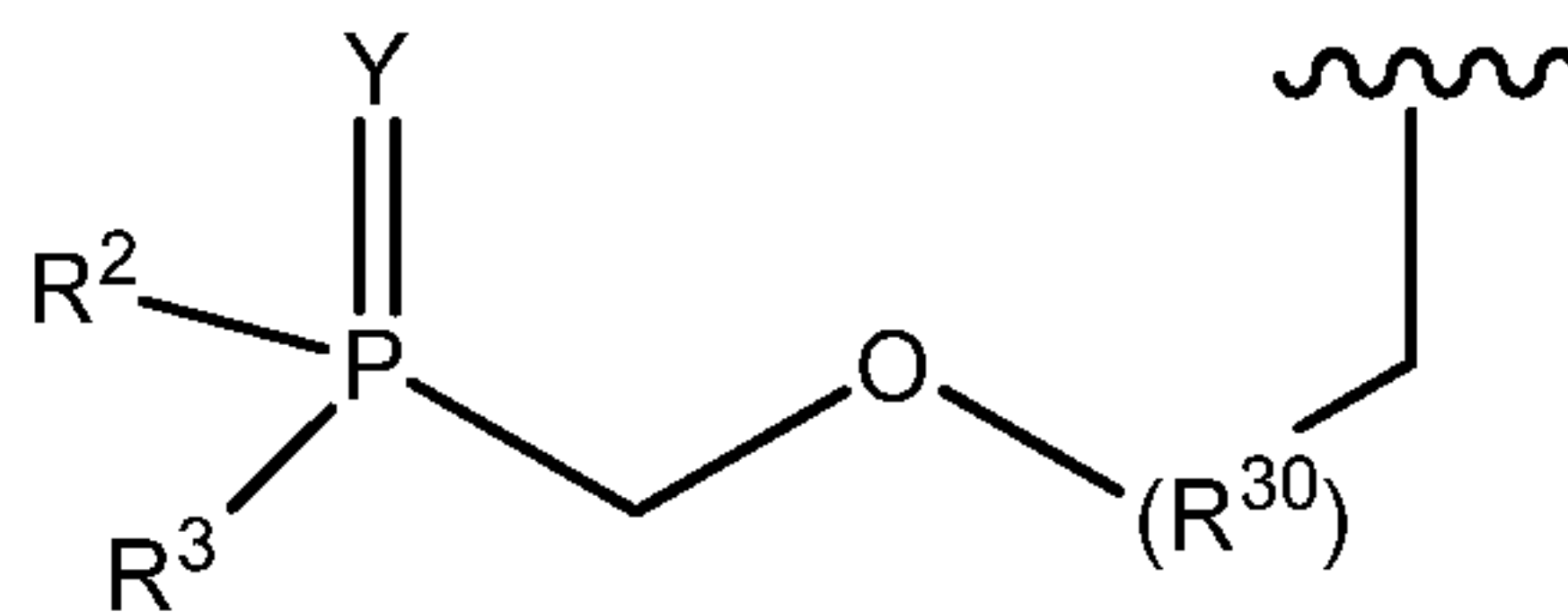
(XIV)

wherein:

V is, individually, S or Se;

R², R³, 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):



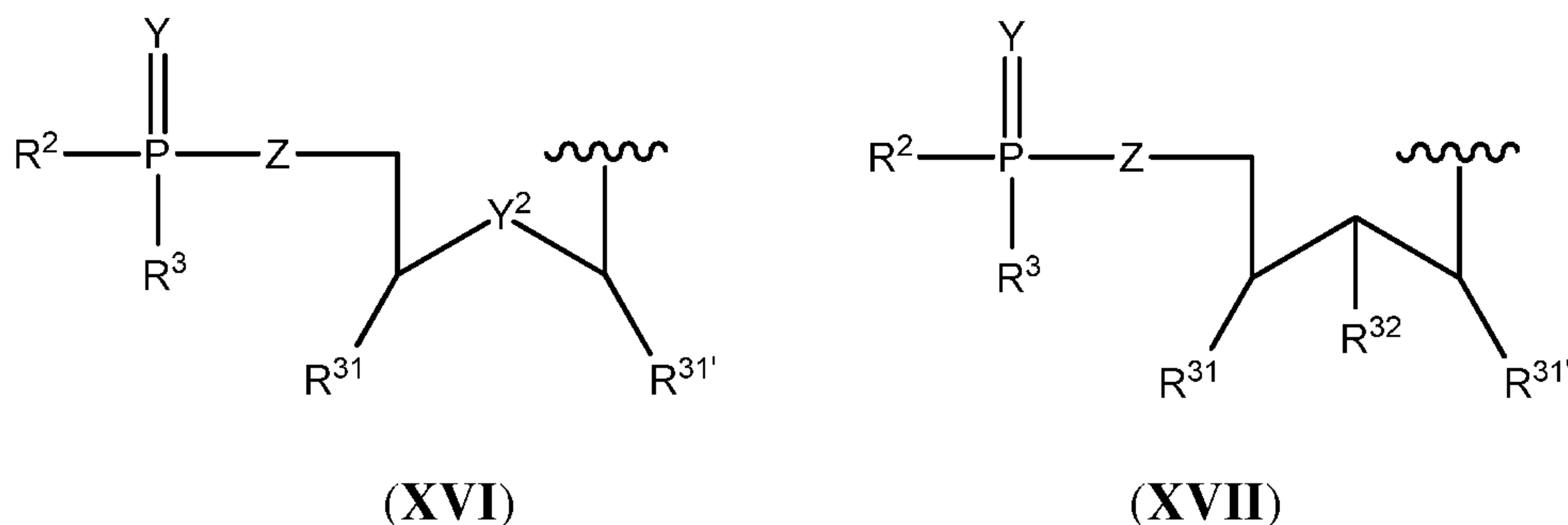
(XV)

wherein:

R², R³, 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):



wherein:

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

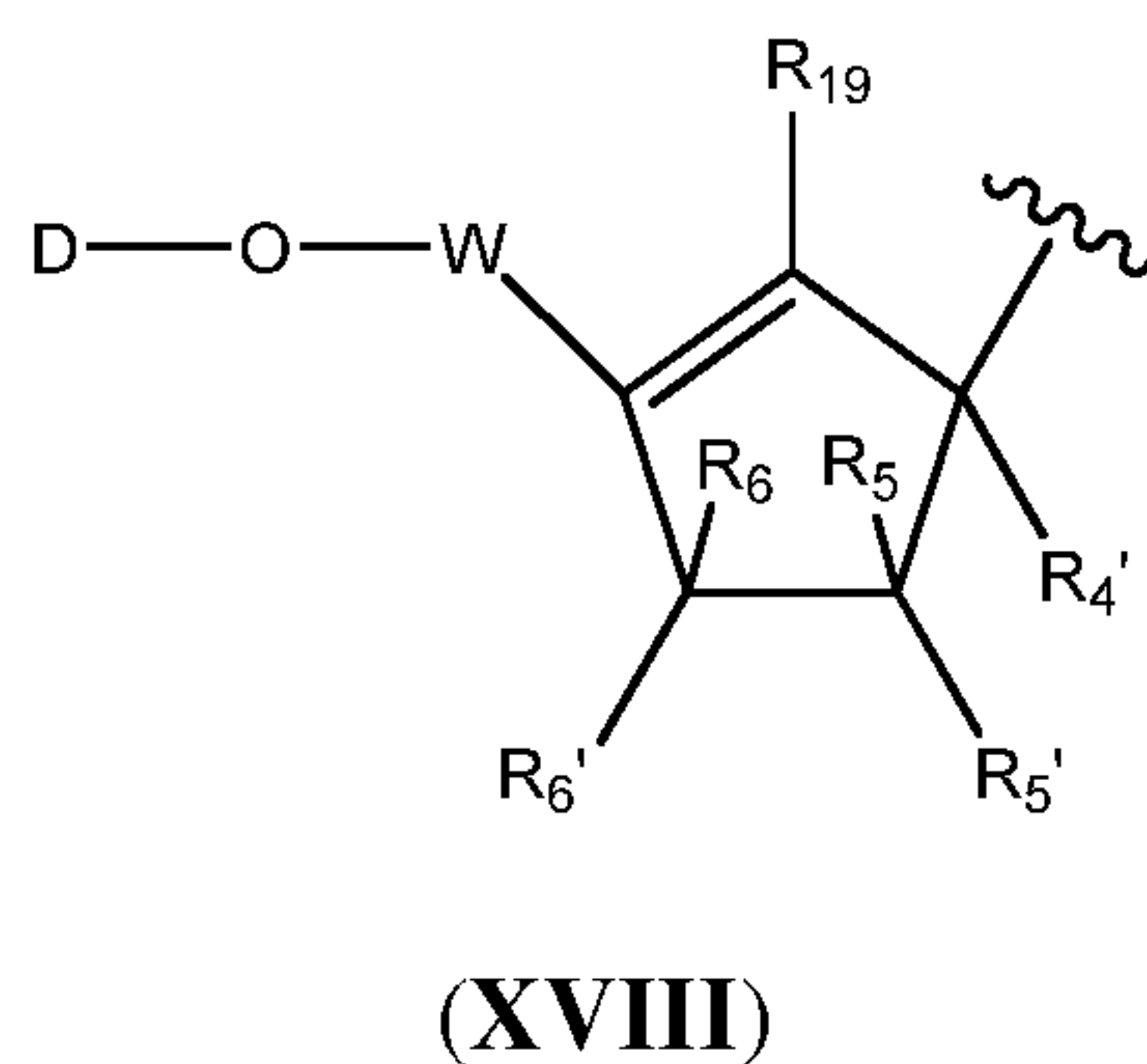
Y^2 is O, S, Se, or NR;

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)



wherein:

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

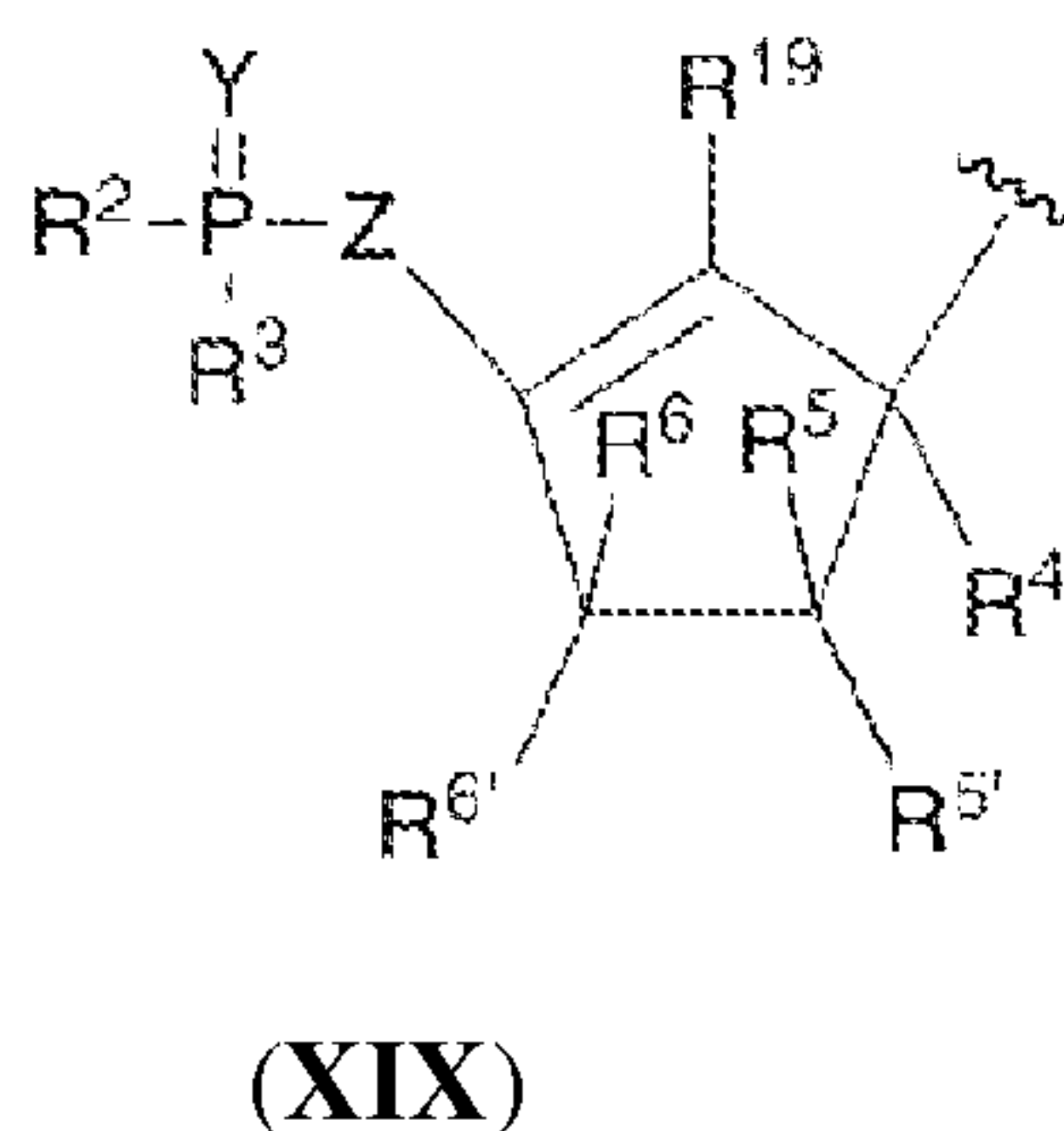
R^{19} is H, F, Cl, Br, I, N_3 , $C(O)OH$, CN, $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, or R;

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):



wherein:

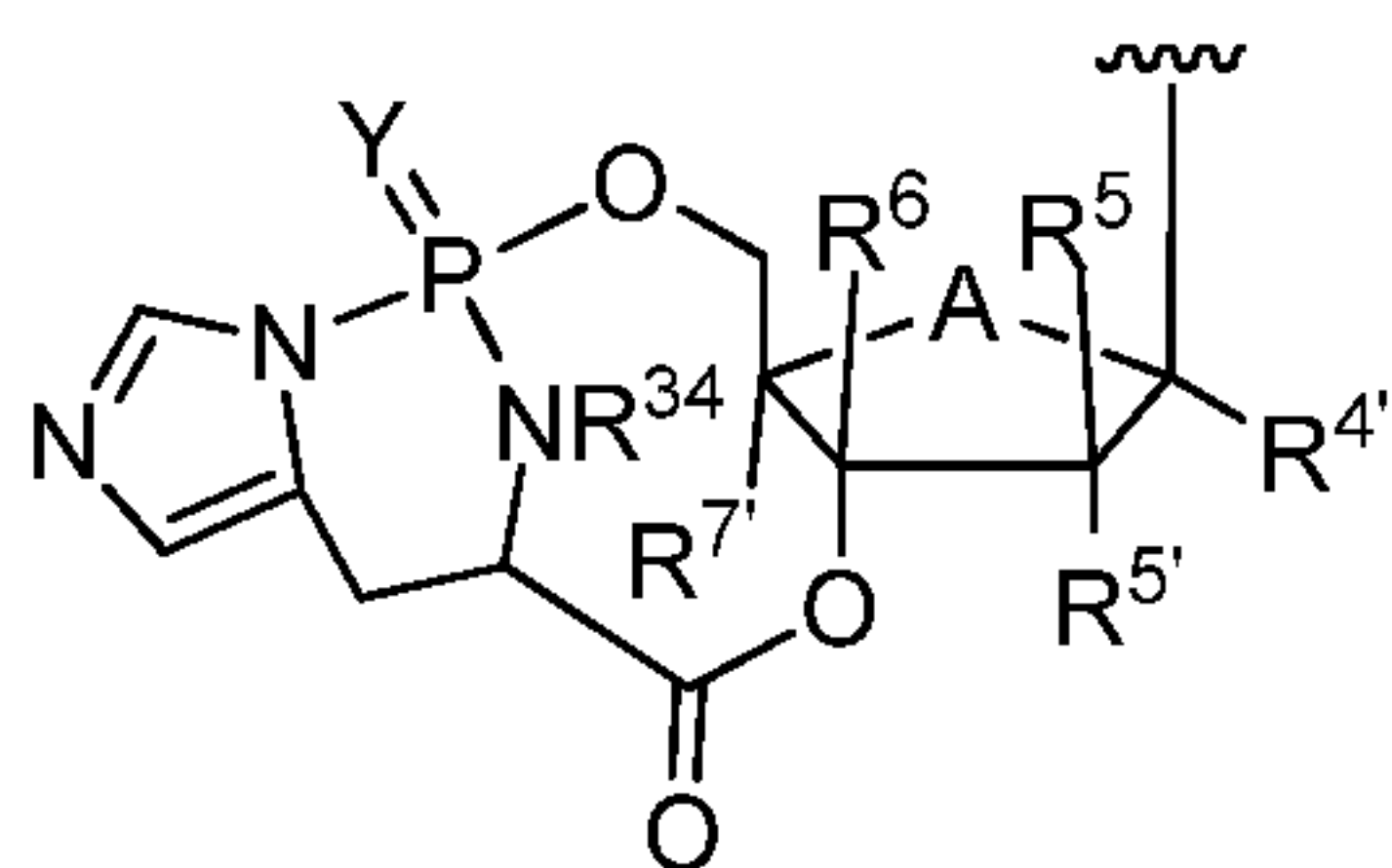
R^2 , R^3 , and Y are as defined above with respect to Claims 1-3;

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

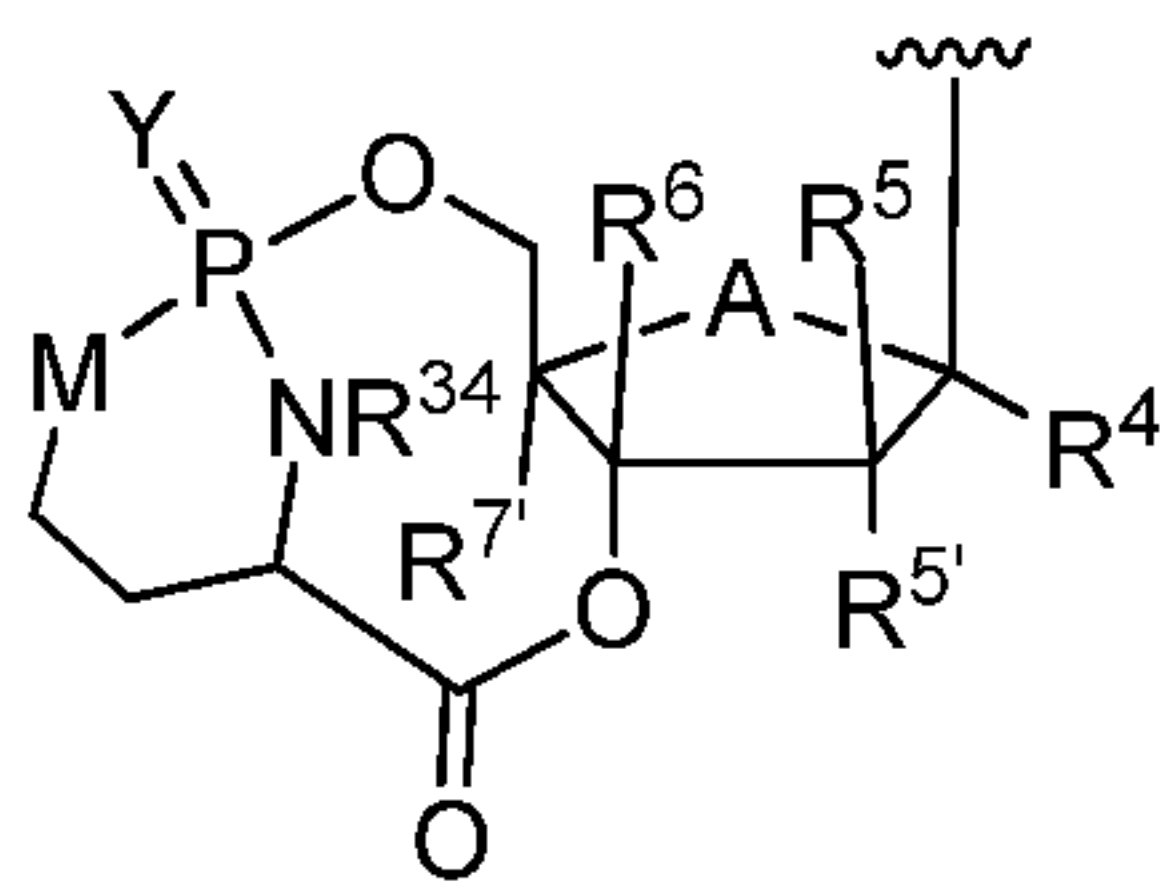
R^{19} is H, F, Cl, Br, I, N_3 , $C(O)OH$, CN, $C(O)NH_2$, $C(S)NH_2$, $C(O)OR$, or R, and

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.

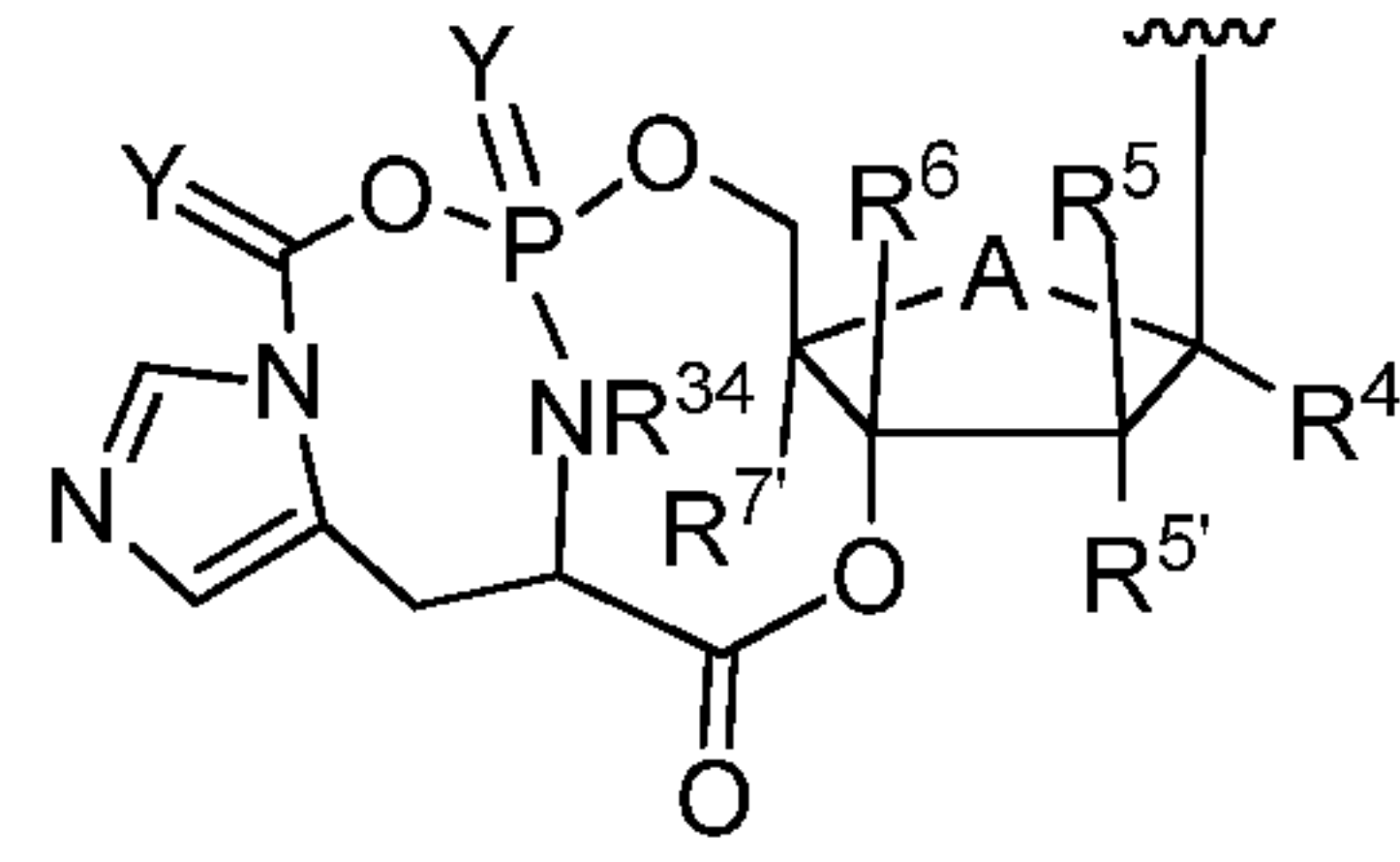
21. The compound of Claim 1, wherein Sugar has one of the Formulas (XX), (XXI), or (XXII):



(XX)



(XXI)



(XXII)

wherein:

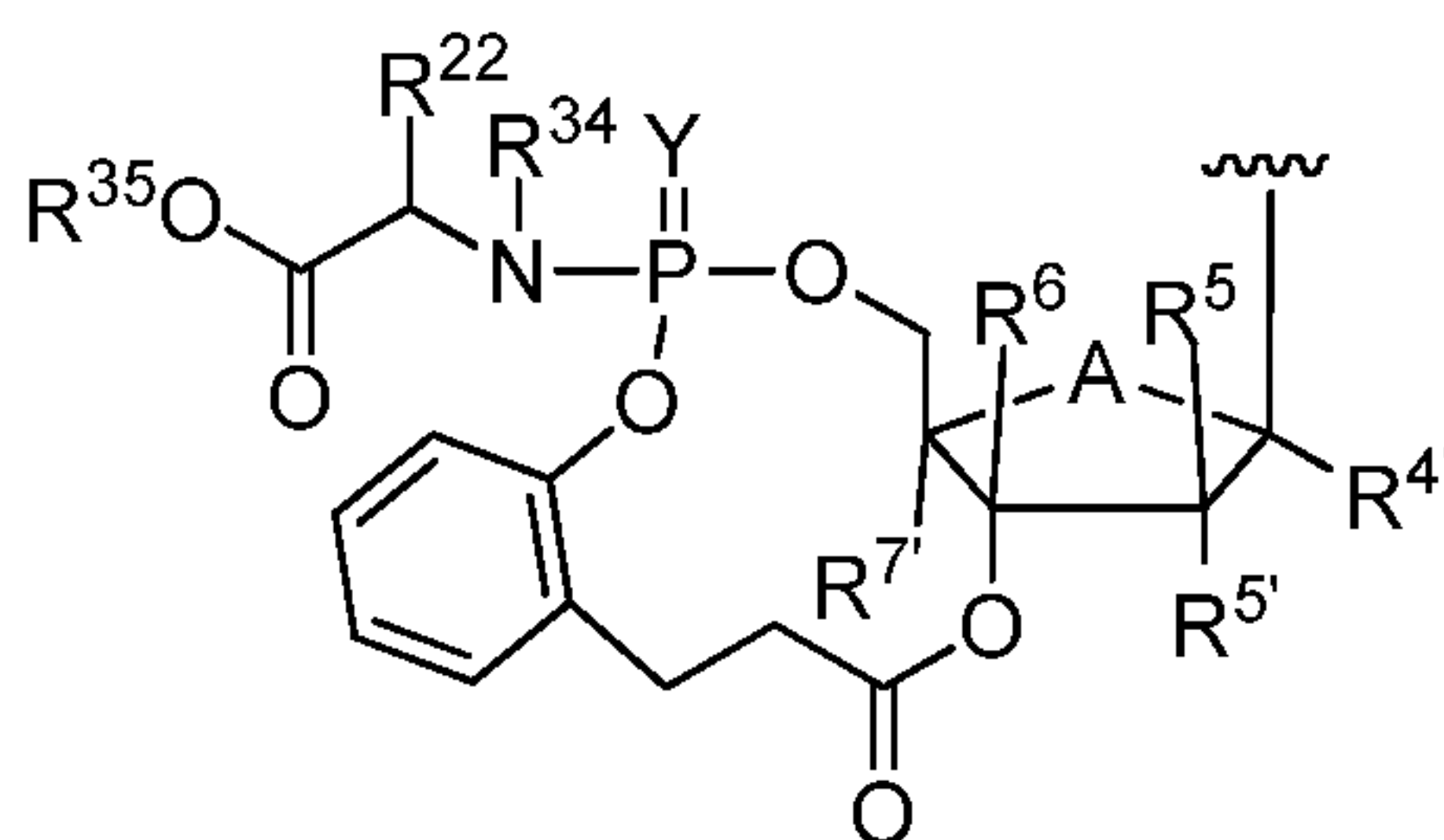
R^{4'}, R⁵, R^{5'}, R⁶, Y, A, and R^{7'} are as defined above with respect to Claims 1-3;

R³⁴ is C₁-C₆ alkyl;

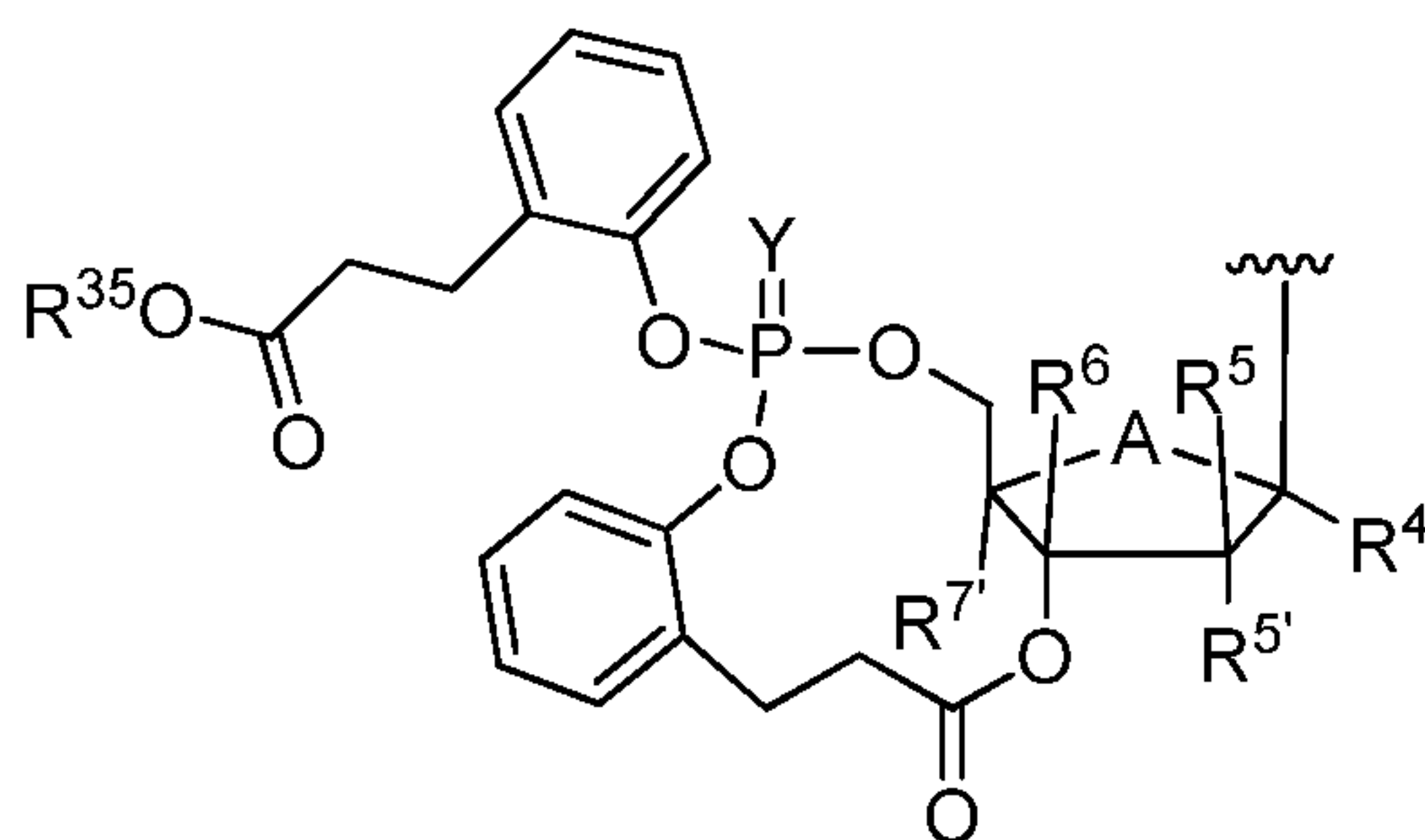
M is O, S, or NR;

wherein R is, independently, C₁-C₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃-C₆ 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)

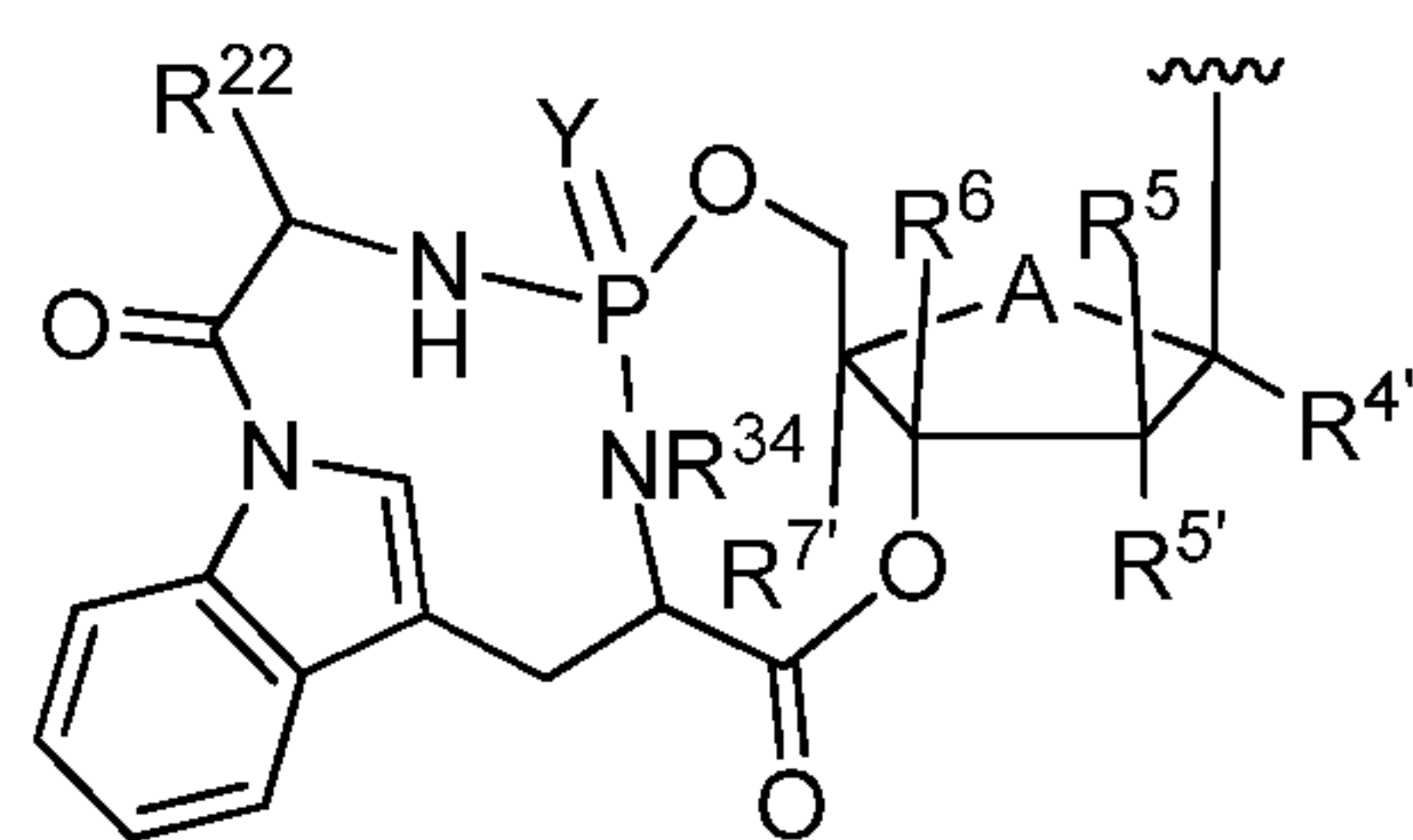
wherein:

R^{4'}, R⁵, R^{5'}, R⁶, Y, A, R^{7'}, R³⁴ are as defined above with respect to Claims 1-3;

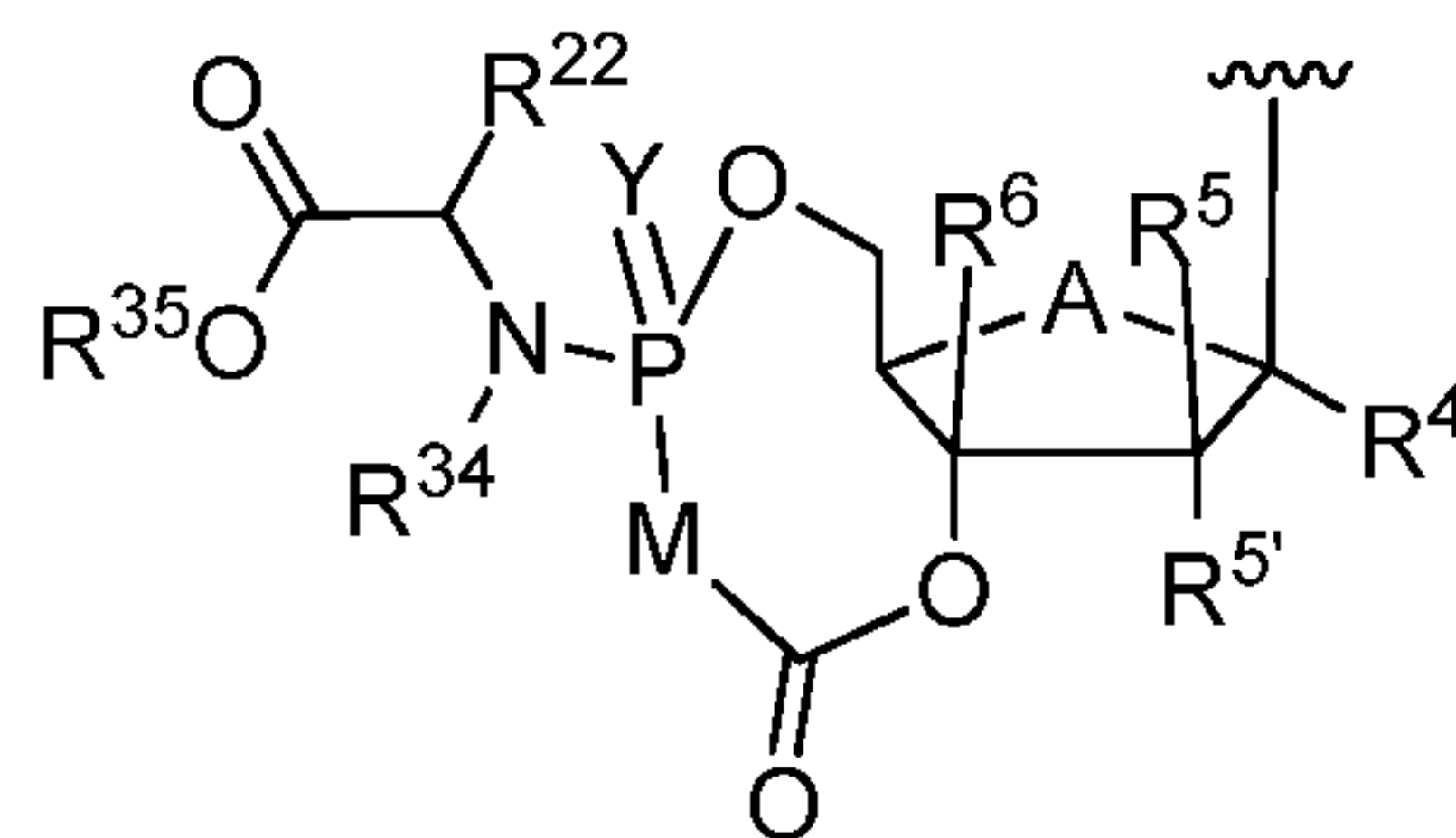
R^{35} is H, C_{1-10} alkyl, C_{1-10} alkyl substituted with a C_{1-6} alkyl, C_{1-6} alkoxy, di(C_{1-6} alkyl)-amino, fluoro, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl alkyl, cycloheteroalkyl, aryl, heteroaryl, substituted aryl, or substituted heteroaryl moiety; wherein the substituents are C_{1-5} alkyl, 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 , $CH(OH)CH_3$, $CH_2((4'-OH)-Ph)$, CH_2SH , or C_{3-6} cycloalkyl.

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



(XXV)

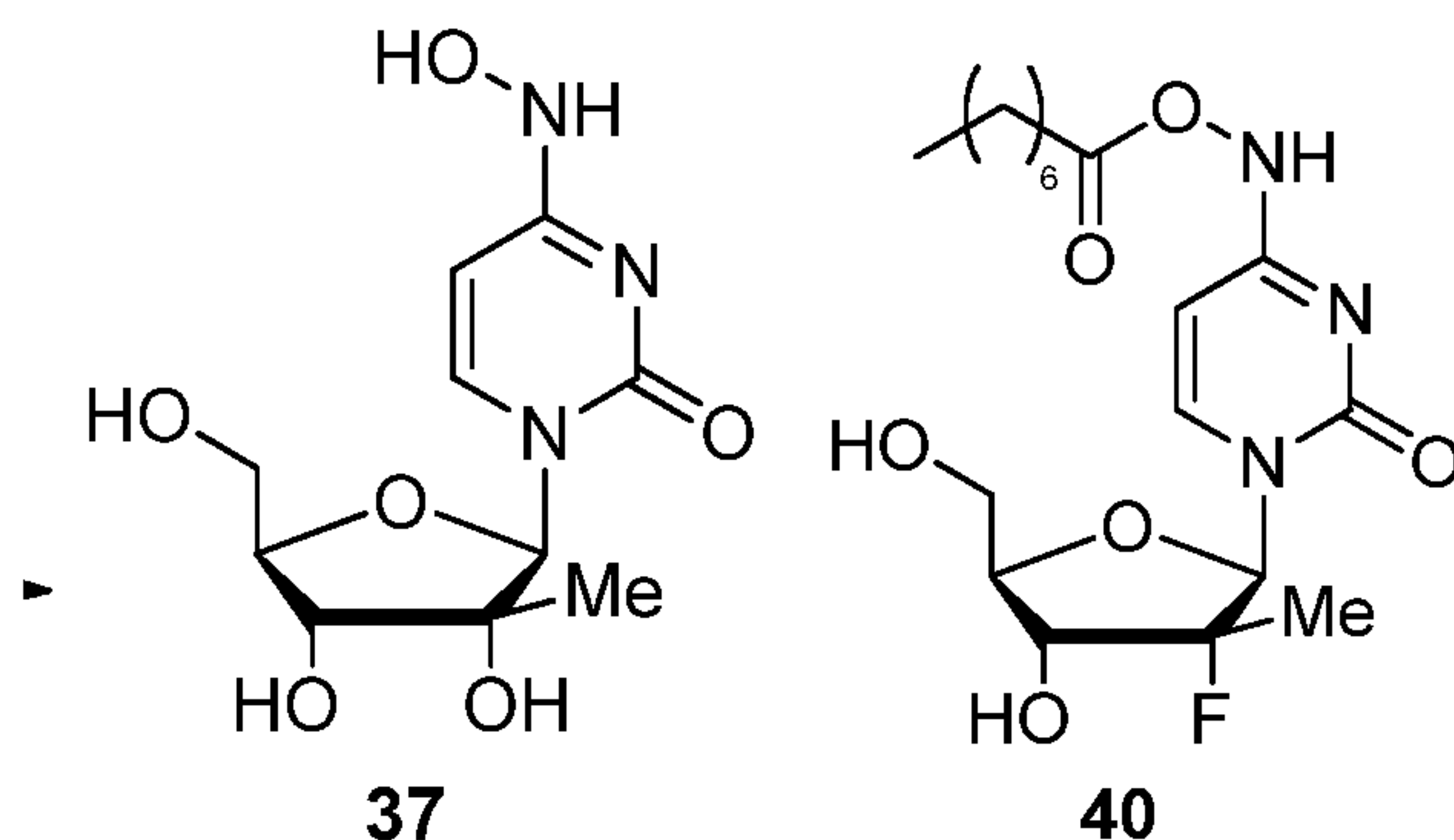


(XXVI)

wherein:

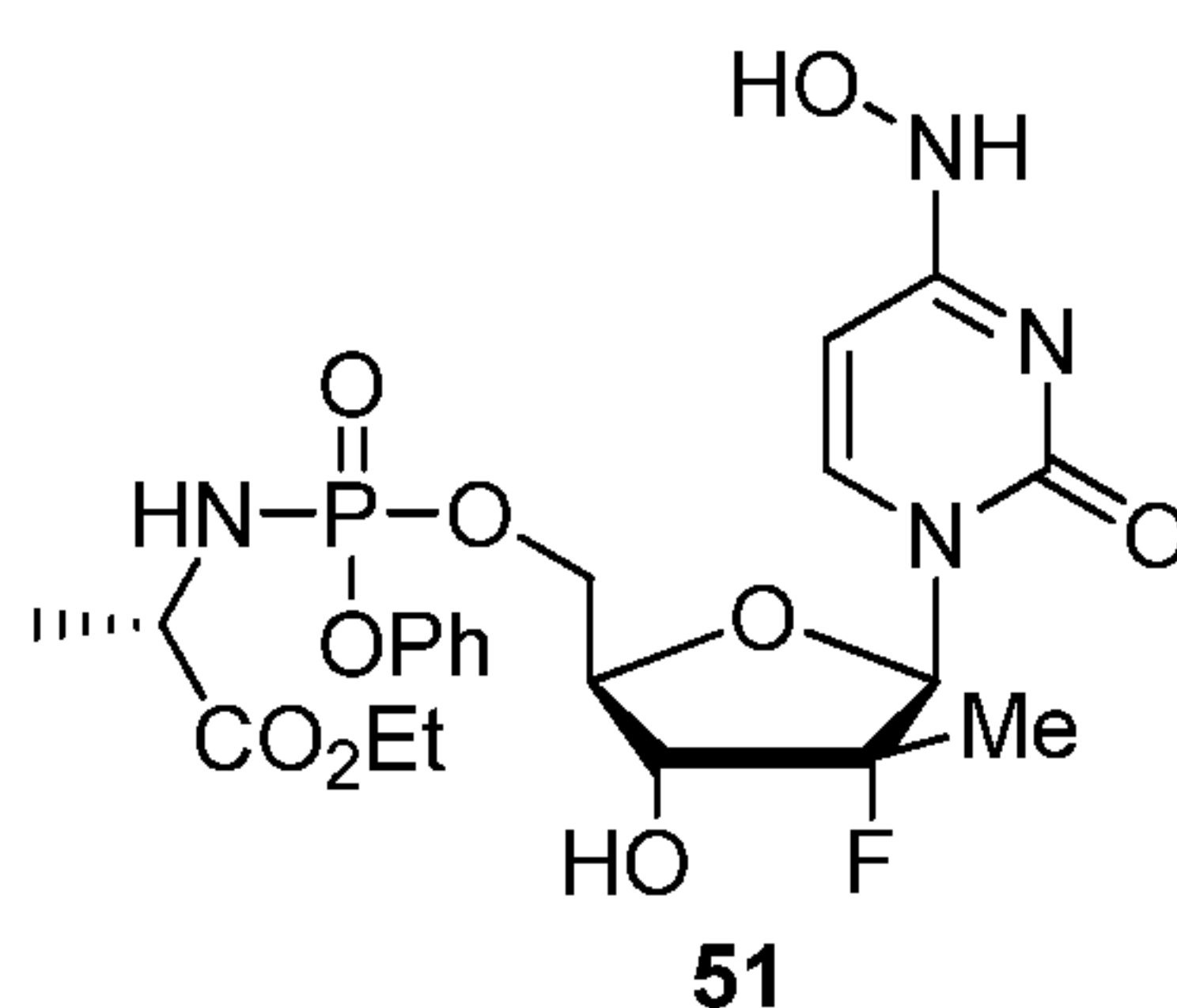
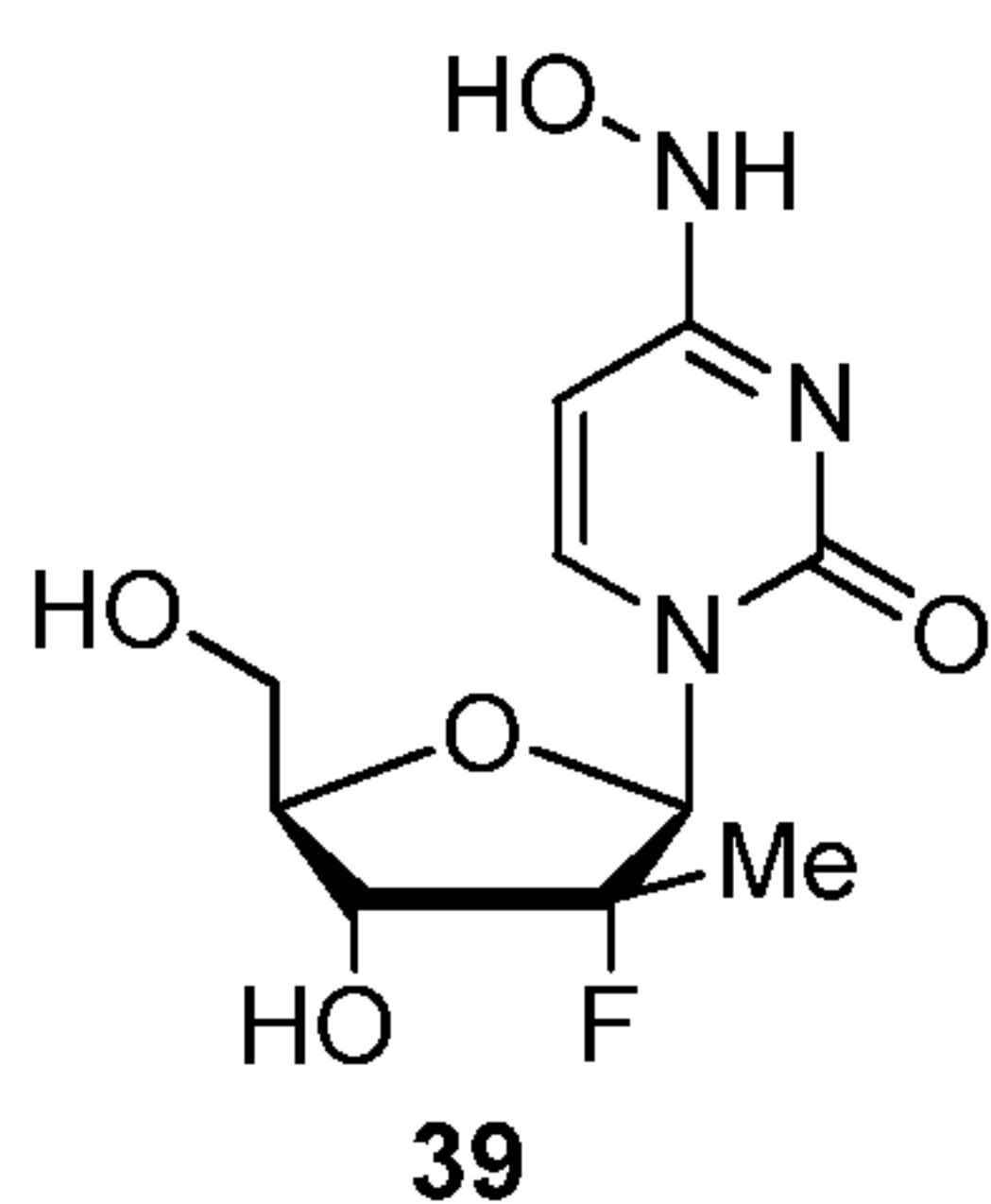
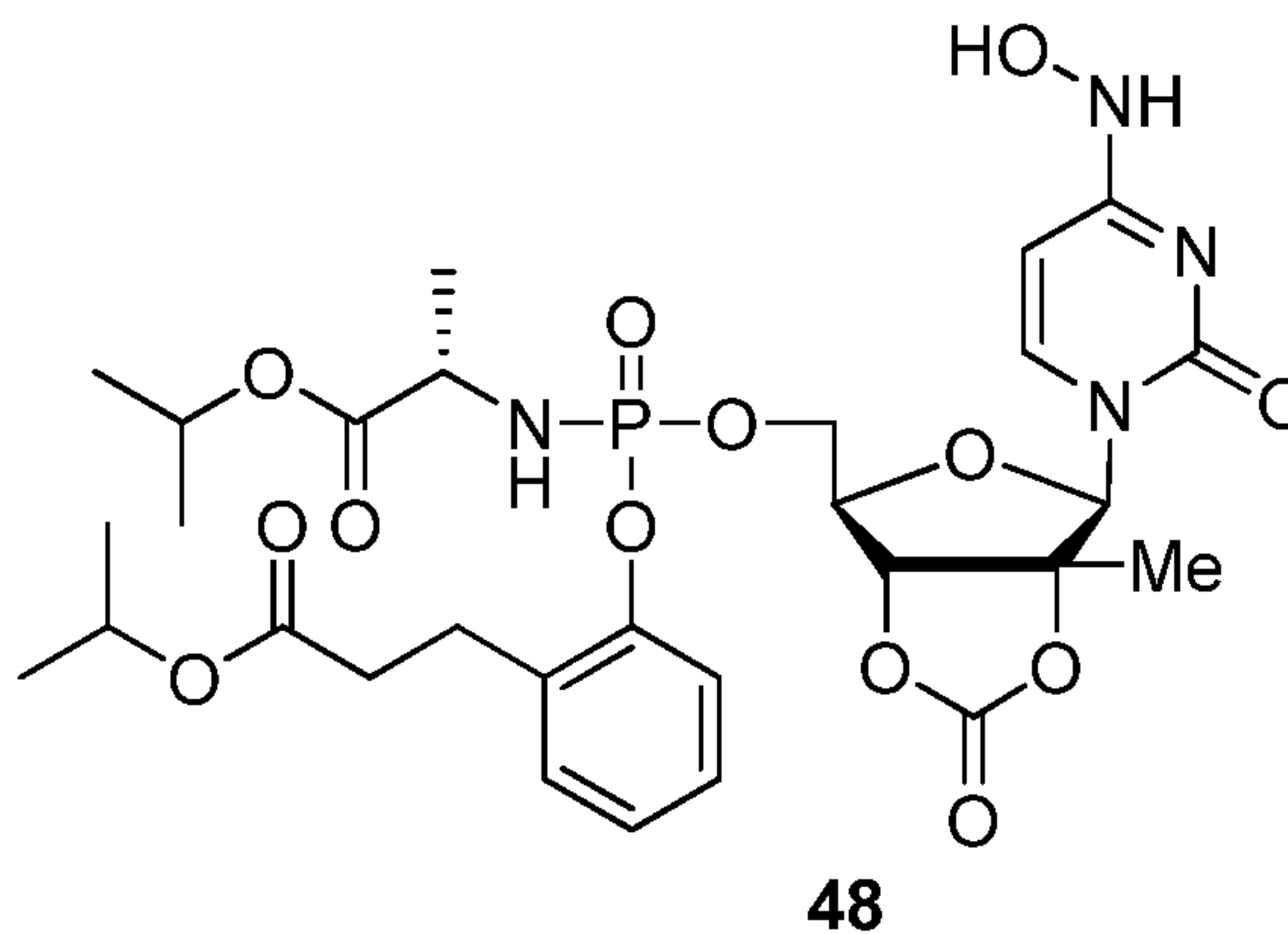
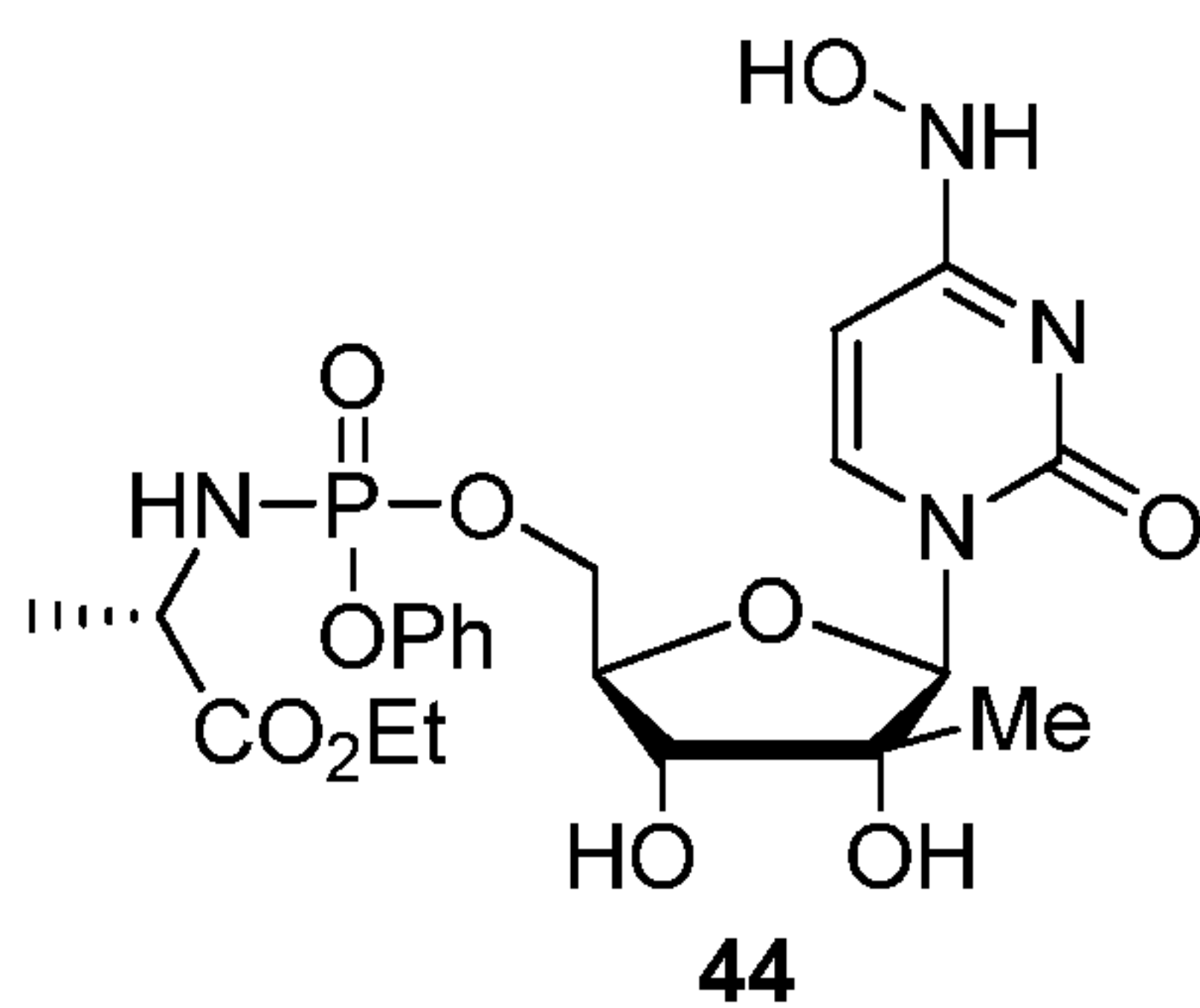
R^4 , R^5 , $R^{5'}$, R^6 , Y , M , R^7 , R^{34} , R^{35} , R^{22} are as defined above with respect to Claims 1-3.

24. A compound of one of the following formulas:



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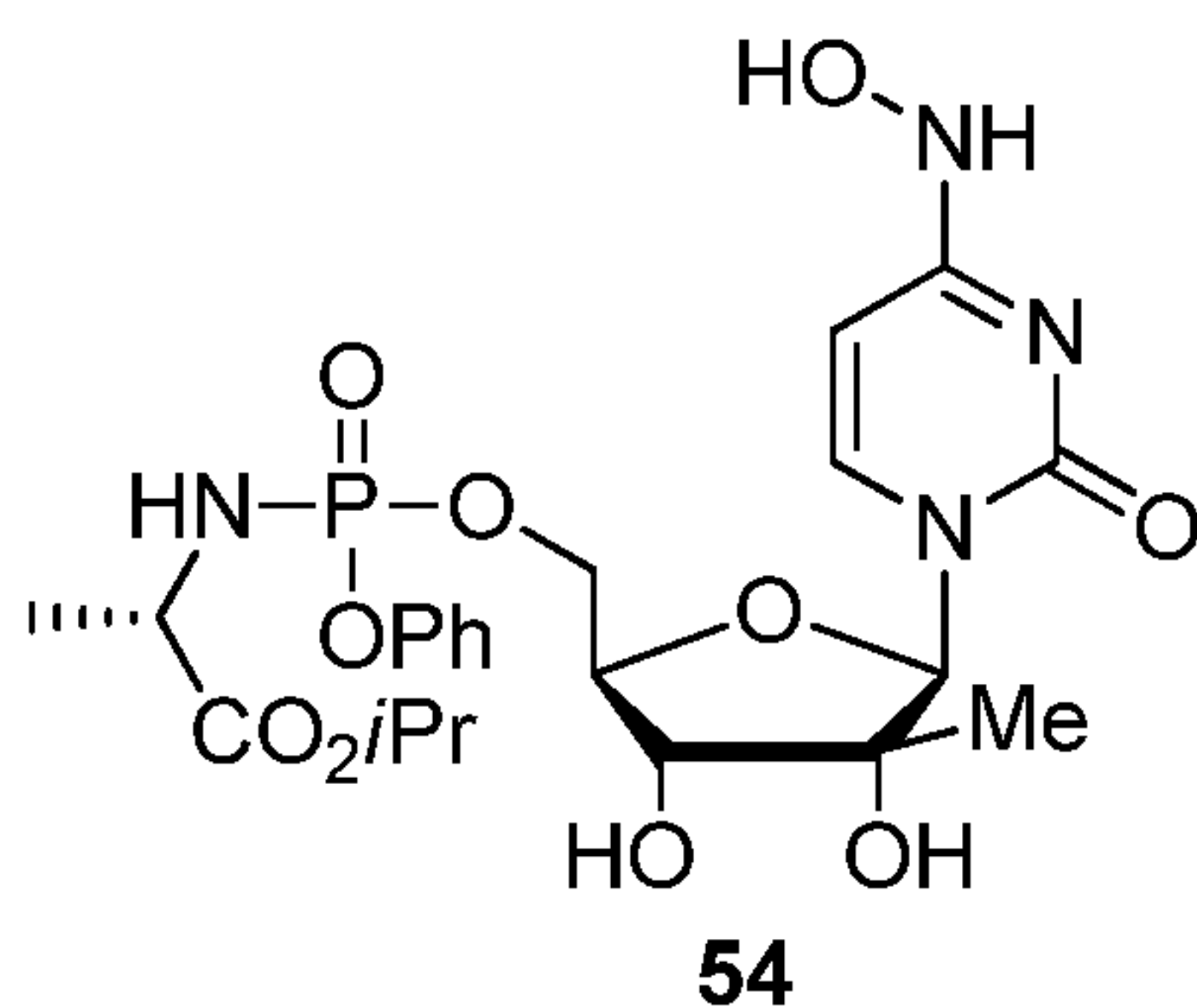
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, or pharmaceutically

acceptable salts thereof.

25. A compound of the following formula:



, 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

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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 27 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 27 to a patient in need of treatment thereof.

31. The method of Claim 28, 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 27 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 27, in a pharmaceutically acceptable carrier, in combination with another anti-HIV agent, to a patient in need of 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 27 to a patient in need of treatment thereof.

36. A method for preventing an HBV infection, comprising administering a prophylactically effective amount of a compound of any of Claims 1 to 27 to a patient in need of prophylaxis thereof.

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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 27 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 27 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 27 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 27 to a patient in need of treatment thereof.

41. A method for preventing an Norovirus or Saporovirus infection, comprising administering a prophylactically effective amount of a compound of any of Claims 1 to 27 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 27 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 27 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

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an effective amount of a compound of any of Claims 1 to 27 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 prophylactically effective amount of a compound of any of Claims 1 to 27 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 27 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 27 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 27 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 27 to a patient in need of treatment thereof.

51. 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 27 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 27 to a patient in need of treatment thereof.

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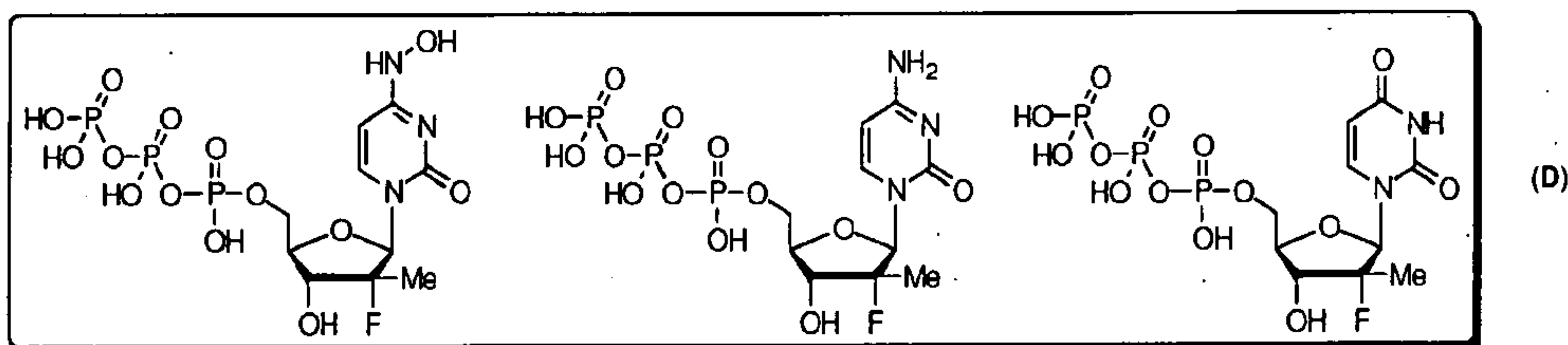
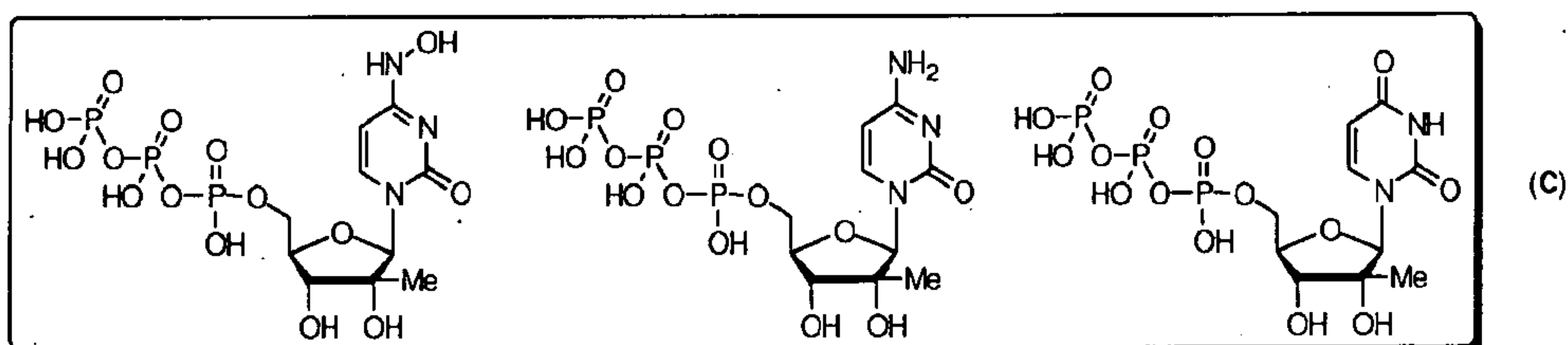
53. A method for treating a host infected with HSV-1 or HSV-2 that includes administering an effective amount of a compound of any of Claims 1 to 27 in a pharmaceutically acceptable carrier in combination with another anti-HSV-1 AND HSV-2 agent.

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 27 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 27 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 27 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-NH₂ and 4-OH pyrimidine triphosphates:



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.

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59. The use of Claim 58, 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-2 infection, 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 66, wherein the medicament further comprises another anti-cancer agent.

68. The compound of Claim 1, wherein X^2 is $\text{CH}_2\text{-O}(\text{CO})\text{O-X}^5$.

69. The use of a compound of Claim 68 in the preparation of a medicament for treating or preventing an HIV-1 or HIV-2 infection.