Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2685748 A1 2008/11/14

(21) 2 685 748

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2008/05/14
(87) Date publication PCT/PCT Publication Date: 2008/11/14
(85) Entrée phase nationale/National Entry: 2009/11/12
(86) N° demande PCT/PCT Application No.: US 2008/006109
(30) Priorité/Priority: 2007/05/14 (US60/930,154)

(51) Cl.Int./Int.Cl. *C07H 19/20* (2006.01), *A61K 31/7076* (2006.01), *A61P 31/14* (2006.01), *A61P 31/18* (2006.01), *A61P 31/20* (2006.01), *C07H 19/173* (2006.01)

(71) Demandeurs/Applicants:
RFS PHARMA, LLC., US;
EMORY UNIVERSITY, US;
UNIVERSITY OF PITTSBURGH, US

(72) Inventeurs/Inventors:
SCHINAZI, RAYMOND F., US;
MELLORS, JOHN W., US;
SLUIS-CREMER, NICOLAS PAUL, US;
AMBLARD, FRANK, US; ...

(54) Titre : NUCLEOSIDES AZIDOPURIQUES POUR LE TRAITEMENT D'INFECTIONS VIRALES
(54) Title: AZIDO PURINE NUCLEOSIDES FOR TREATMENT OF VIRAL INFECTIONS

Genotypes of xxLAI viruses

Virus (xxLAI)	Mutations in RT
HIV-1K65R	K65R
HIV-1K70E	K70E
HIV-1L74V	L74V
HIV-1M184V	M184V
HIV-1AZT2	D67N, K70R, T215F, K219Q
HIV-1AZT3	M41L, L210W, T215Y
HIV-1AZT7	M41L, D67N, K70R, T215F, K219Q
HIV-1AZT9	M41L, D67N, K70R, L210W, T215Y, K219Q
HIV-1Q151M	M41L, A62V, V75I, F77L, F116Y, Q151M
HIV-169Insertion	M41L, SS insert between 69 and 70, L210W, T215Y

FIG.1

(57) Abrégé/Abstract:
The present invention is directed to compounds, compositions and methods for treating or preventing viral infec-tions, in particular, HIV, HBV, and HCV, in human patients or other animal hosts. The compounds are 3'-azido-2',3'-dideoxy purine nucleosides or phosphonates, and pharmaceutically acceptable, salts, prodrugs, and other derivatives thereof. In particular, the com-pounds show potent antiviral activity against HIV-1 resistance mutants including HIV-1_{K65R}, HTV-1_{K70E}, HIV-1_{L74V}, HIV-1_{M184V}, HIV-1_{Q151M} and inhibitory activity against HIV-1 RT harboring TAMS or insertion mutations including HIV-1_{AZT3}, HIV-1_{AZT7}, HIV-1_{AZT9}, HIV-1_{Q151M}, or HIV-$1_{69insertion}$. In one embodiment, the compounds are 3'-azido-ddA, 3'-azido-ddG, or combinations thereof, administered with one or more additional antiviral agents that select for TAM mutations and/or the M 184V mutation, along with a pharmaceutically acceptable carrier.

CA 2685748 A1 2008/11/14

(21) 2 685 748

(13) A1

(72) **Inventeurs(suite)/Inventors(continued)**: COATS, STEVEN J., US; SHI, JUNXING, US; WHITAKER, RICHARD ANTHONY, US

(74) **Agent**: RICHES, MCKENZIE & HERBERT LLP

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 November 2008 (27.11.2008)

PCT

(10) International Publication Number
WO 2008/143846 A1

(51) International Patent Classification:
C07H 19/16 (2006.01) *C07D 473/34* (2006.01)

(21) International Application Number:
PCT/US2008/006109

(22) International Filing Date: 14 May 2008 (14.05.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/930,154 14 May 2007 (14.05.2007) US

(71) Applicants *(for all designated States except US)*: **RFS PHARMA, LLC** [US/US]; 1860 Montreal Road, Tucker, GA 30084 (US). **EMORY UNIVERSITY** [US/US]; 1599 Clifton Road, NE, 4th Floor, Mailstop 1599-001-1 AZ, Atlanta, GA 30322 (US). **UNIVERSITY OF PITTSBURGH** [US/US]; 200 Gardner Steel Conference Center, Thackeray And O'Hara Streets, Pittsburgh, PA 15260 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SCHINAZI, Raymond F.** [US/US]; 1860 Montreal Road, Tucker, GA 30084 (US). **MELLORS, John W.** [US/US]; 201 Beech Street, Pittsburgh, PA 15218 (US). **SLUIS-CREMER, Nicolas Paul** [SA/US]; 2002 Lenox Oval, Pittsburgh, PA 15237 (US). **AMBLARD, Frank** [FR/US]; 1505 Summit Pointe Way, NE, Atlanta, GA 30329 (US). **COAST, Steven J.** [US/US]; 764 Alberta Drive, McDonough, GA 30252 (US). **SHI, Junxing** [US/US]; 3086 Canter Way, Duluth, GA 30097 (US). **WHITAKER, Richard Anthony** [US/US]; 3661 Willowwind Court, Loganville, GA 30052 (US).

(74) Agent: **BRADIN, David**; Intellectual Property/Technology Law, P.O. Box 14329, Research Triangle Park, NC 27709 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: AZIDO PURINE NUCLEOSIDES FOR TREATMENT OF VIRAL INFECTIONS

Genotypes of xxLAI viruses

Virus (xxLAI)	Mutations in RT
HIV-1K65R	K65R
HIV-1K70E	K70E
HIV-1L74V	L74V
HIV-1M184V	M184V
HIV-1AZT2	D67N, K70R, T215F, K219Q
HIV-1AZT3	M41L, L210W, T215Y
HIV-1AZT7	M41L, D67N, K70R, T215F, K219Q
HIV-1AZT9	M41L, D67N, K70R, L210W, T215Y, K219Q
HIV-1Q151M	M41L, A62V, V75I, F77L, F116Y, Q151M
HIV-169Insertion	M41L, SS insert between 69 and 70, L210W, T215Y

FIG.1

(57) Abstract: The present invention is directed to compounds, compositions and methods for treating or preventing viral infections, in particular, HIV, HBV, and HCV, in human patients or other animal hosts. The compounds are 3'-azido-2',3'-dideoxy purine nucleosides or phosphonates, and pharmaceutically acceptable, salts, prodrugs, and other derivatives thereof. In particular, the compounds show potent antiviral activity against HIV-1 resistance mutants including HIV-1_{K65R}, HTV-1_{K70E}, HIV-1_{L74V}, HIV-1_{M184V}, HIV-1_{Q151M} and inhibitory activity against HIV-1 RT harboring TAMS or insertion mutations including HIV-1_{AZT3}, HIV-1_{AZT7}, HIV-1_{AZT9}, HIV- 1_{Q151M}, or HIV-$1_{69insertion}$. In one embodiment, the compounds are 3'-azido-ddA, 3'-azido-ddG, or combinations thereof, administered with one or more additional antiviral agents that select for TAM mutations and/or the M 184V mutation, along with a pharmaceutically acceptable carrier.

WO 2008/143846 A1

WO 2008/143846 A1

GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

Azido purine nucleosides for treatment of viral infections

Cross-Reference to Related Applications

This application claims the benefit under 35 USC 119 of U.S. Provisional Patent Application No. 60/930,154 filed May 14, 2007. The disclosure of said U.S. Provisional Patent Application No. 60/930,154 is hereby incorporated herein by reference, in its respective entirety, for all purposes.

Field of the Invention

The present invention is directed to compounds, methods and compositions for treating or preventing viral infections using nucleoside analogues. More specifically, the invention describes 3'-azido 3'-deoxy purine and modified purine nucleoside analogues, pharmaceutically acceptable salts, prodrugs, or other derivatives thereof, and the use thereof in the treatment of a viral infection, and in particular a human immunodeficiency virus (HIV-1 and HIV-2) or hepatitis B virus (HBV) infection.

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), or hepatitis C virus (HCV). The challenge in developing antiviral therapies is to inhibit viral replication without injuring the host cell. 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, TTP, 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 nonnucleoside RT inhibitors (NNRTI).

NRTI are analogs of 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. Some nucleosides in their triphosphate form also inhibit the viral enzyme reverse transcriptase.

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, we provide a brief overview of the mutations and molecular mechanisms of HIV-1 resistance to NRTI. Two kinetically distinct molecular mechanisms of HIV-1 resistance to NRTI have been proposed (see Sluis-Cremer N, Arion D, Parniak MA. Molecular mechanisms of HIV-1 resistance to nucleoside reverse transcriptase inhibitors (NRTIs). *Cell Mol. Life Sci.* **2000**; *57*, 1408-22). One mechanism involves selective decreases in NRTI-TP *versus* normal dNTP incorporation during viral DNA synthesis. This resistance mechanism has been termed discrimination. The second mechanism involves selective

removal of the chain-terminating NRTI-monophosphate (NRTI-MP) from the prematurely terminated DNA chain (see Arion D, Kaushik N, McCormick S, Borkow G, Parniak MA. Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry.* **1998**, *37*, 15908-17; Meyer PR, Matsuura SE, Mian AM, So AG, Scott WA. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. *Mol. Cell.* **1999**, *4*, 35-43). This mechanism has been termed excision.

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

a) K65R: The K65R mutation in HIV-1 RT decreases susceptibility to all FDA approved NRTI, with the exception of AZT (see Parikh UM, Koontz DL, Chu CK, Schinazi RF, Mellors JW. *In vitro* activity of structurally diverse nucleoside analogs against human immunodeficiency virus type 1 with the K65R mutation in reverse transcriptase. *Antimicrob. Agents Chemother.* **2005**, *49*, 1139-44). This mutation also markedly decreases susceptibility to essentially all NRTI currently in development. Residue K65 resides in the β3-β4 loop in the "fingers" subdomain of the 66kDa subunit of HIV-1 RT, and in the crystal structure of the ternary HIV-1 RT-template/primer (T/P)-dNTP complex, the ε-amino group of K65 interacts with the γ-phosphate of the bound dNTP substrate (see Huang H, Chopra R, Verdine GL, Harrison SC. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science.* **1998**, *282*, 1669-75). Pre-steady-state kinetic analyses have demonstrated that K65R confers resistance to ddATP (active metabolite of ddI), 3TCTP, carbovir-TP (CBVTP, active metabolite of ABC) and tenofovir-diphosphate (tenofovir-DP) by selectively reducing kpol without affecting K_d (see Selmi B, Boretto J,

Sarfati SR, Guerreiro C, Canard B. Mechanism-based suppression of dideoxynucleotide resistance by K65R human immunodeficiency virus reverse transcriptase using an alpha-boranophosphate nucleoside analogue. *J. Biol. Chem.* **2001**, *276*, 48466-72; Deval J, White KL, Miller MD, Parkin NT, Courcambeck J, Halfon P, Selmi B, Boretto J, Canard B. Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations. *J. Biol. Chem.* **2004**, *279*, 509-16). However, for ddCTP and DXGTP (the active metabolite of DAPD) the resistance mechanism involves both reduction in kpol and increase in K_d (see Selmi B, Boretto J, Sarfati SR, Guerreiro C, Canard B. Mechanism-based suppression of dideoxynucleotide resistance by K65R human immunodeficiency virus reverse transcriptase using an alpha-boranophosphate nucleoside analogue. *J. Biol. Chem.* **2001**, *276*, 48466-72; Furman PA, Jeffrey J, Kiefer LL, Feng JY, Anderson KS, Borroto-Esoda K, Hill E, Copeland WC, Chu CK, Sommadossi JP, Liberman I, Schinazi RF, Painter GR. Mechanism of action of 1-beta-D-2,6-diaminopurine dioxolane, a prodrug of the human immunodeficiency virus type 1 inhibitor 1-beta-D-dioxolane guanosine. *Antimicrob. Agents Chemother.* **2001**, *45*, 158-65). Structural studies suggest that the K65R mutation in HIV-1 RT distorts optimal positioning of the NRTI-TP in the active site which results in decreased catalytic efficiency of incorporation (see Selmi B, Boretto J, Sarfati SR, Guerreiro C, Canard B. Mechanism-based suppression of dideoxynucleotide resistance by K65R human immunodeficiency virus reverse transcriptase using an alpha-boranophosphate nucleoside analogue. *J. Biol. Chem.* **2001**, *276*, 48466-72; Sluis-Cremer N, Arion D, Kaushik N, Lim H, Parniak MA. Mutational analysis of Lys65 of HIV-1 reverse transcriptase. *Biochem. J.* **2000**, *348*, 77-82).

b) K70E: The K70E mutation was initially selected *in vitro* with adefovir (see Cherrington JM, Mulato AS, Fuller MD, Chen MS. Novel mutation (K70E) in human immunodeficiency virus type 1 reverse transcriptase confers decreased susceptibility to 9-[2-(phosphonomethoxy)ethyl]adenine *in vitro*. *Antimicrob. Agents Chemother.* **1996**, *40*, 2212-6), but was also recently observed in selection experiments using D-d4FC (Reverset) (see Hammond JL, Parikh UM, Koontz DL, Schlueter-Wirtz S, Chu CK, Bazmi HZ, Schinazi RF, Mellors JW. *In vitro* selection and analysis of human immunodeficiency virus type 1 resistant to derivatives of beta-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine. *Antimicrob. Agents Chemother.* **2005**, *49*, 3930-2). Interestingly, the K70E mutation has become more prevalent in clinical samples since the introduction of tenofovir, and it was recently reported in 10% of

antiretroviral-naïve subjects receiving the triple NRTI combination of tenofovir, ABC and 3TC (see Ross L, Gerondelis P, Liao Q, Wine B, Lim M, Shaefer M, Rodriguez A, Limoli K, Huang W, Parkin NT, Gallant J, Lanier R. Selection of the HIV-1 reverse transcriptase mutation K70E in antiretroviral-naïve subjects treated with tenofovir/abacavir/lamivudine therapy. *Antiviral Ther.* **2005**; *10*, S102). We have demonstrated that K70E confers resistance to tenofovir-DP, CBVTP, and 3TCTP through a discrimination mechanism involving reduction in kpol with little effect on K_d (see Sluis-Cremer N, Argoti Tores P, Grzybowski J, Parikh U, Mellors, JW. Molecular Mechanism of Tenofovir, Abacavir and Lamivudine Resistance by the K70E Mutation in HIV-1 Reverse Transcriptase, 13th Conference on Retroviruses and Opportunistic Infections, February 5-9 **2006**, Denver, CO, Abstract 152).

c) L74V*:* The L74V mutation was originally identified as causing ddI resistance (see Winters MA, Shafer RW, Jellinger RA, Mamtora G, Gingeras T, Merigan TC. Human immunodeficiency virus type 1 reverse transcriptase genotype and drug susceptibility changes in infected individuals receiving dideoxyinosine monotherapy for 1 to 2 years. *Antimicrob. Agents Chemother.* **1997**, *41*, 757-62), but has also been associated with ABC, ddC, and DXG/DAPD (Amdoxovir) resistance (see Hammond JL, Parikh UM, Koontz DL, Schlueter-Wirtz S, Chu CK, Bazmi HZ, Schinazi RF, Mellors JW. *In vitro* selection and analysis of human immunodeficiency virus type 1 resistant to derivatives of beta-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine. *Antimicrob. Agents Chemother.* **2005**, *49*, 3930-2; Winters MA, Shafer RW, Jellinger RA, Mamtora G, Gingeras T, Merigan TC. Human immunodeficiency virus type 1 reverse transcriptase genotype and drug susceptibility changes in infected individuals receiving dideoxyinosine monotherapy for 1 to 2 years. *Antimicrob. Agents Chemother.* **1997**, *41*, 757-62; Miller V, Ait-Khaled M, Stone C, Griffin P, Mesogiti D, Cutrell A, Harrigan R, Staszewski S, Katlama C, Pearce G, Tisdale M. HIV-1 reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. *AIDS.* **2000**, *14*, 163-71; Bazmi HZ, Hammond JL, Cavalcanti SC, Chu CK, Schinazi RF, Mellors JW. *In vitro* selection of mutations in the human immunodeficiency virus type 1 reverse transcriptase that decrease susceptibility to (-)-beta-D-dioxolane-guanosine and suppress resistance to 3'-azido-3'-deoxythymidine. *Antimicrob. Agents Chemother.* **2000**, *44*, 1783-8). Pre-steady-state kinetic experiments have demonstrated that the L74V mutation confers resistance to ddATP by decreasing kpol without impacting on K_d. Molecular modeling suggests that the L74V mutation leads to the

loss of a stabilizing interaction between the nucleotide base of the incoming nucleotide and the side-chain of Leu-74. This can induce a rotation of the base (7° for ddATP compared with dATP), which indirectly affects the positioning of the phosphates (see Deval J, Navarro JM, Selmi B, Courcambeck J, Boretto J, Halfon P, Garrido-Urbani S, Sire J, Canard B. A loss of viral replicative capacity correlates with altered DNA polymerization kinetics by the human immunodeficiency virus reverse transcriptase bearing the K65R and L74V dideoxynucleoside resistance substitutions. *J. Biol. Chem.* **2004**, *279*, 25489-96).

d) Q151M complex: The Q151M complex consists of a cluster of mutations in HIV-1 RT that includes the Q151M mutation plus four additional mutations: A62V, V75I, F77L and F116Y. The Q151M mutation generally occurs first before the acquisition of the other mutations (see Ueno T, Shirasaka T, Mitsuya H. Enzymatic characterization of human immunodeficiency virus type 1 reverse transcriptase resistant to multiple 2',3'-dideoxynucleoside 5'-triphosphates. *J. Biol. Chem.* **1995**, *270*, 23605-11; Matsumi S, Kosalaraksa P, Tsang H, Kavlick MF, Harada S, Mitsuya H. Pathways for the emergence of multi-dideoxynucleoside-resistant HIV-1 variants. *AIDS*. **2003**, *17*, 1127-37). Although rare (~1% prevalence among resistance databases), the Q151M complex is most often selected by regimens containing d4T and ddI (see Balotta C, Violin M, Monno L, Bagnarelli P, Riva C, Facchi G, Berlusconi A, Lippi M, Rusconi S, Clementi M, Galli M, Angarano G, Moroni M. Prevalence of multiple dideoxynucleoside analogue resistance (MddNR) in a multicenter cohort of HIV-1-infected Italian patients with virologic failure. *J. Acquir. Immune Defic. Syndr.* **2000**, *24*, 232-40). The mechanism of resistance mediated by Q151M and the Q151M complex involves a selective reduction in the catalytic rate constant (kpol) for incorporation of NRTI-TP (see Deval J, Selmi B, Boretto J, Egloff MP, Guerreiro C, Sarfati S, Canard B. The molecular mechanism of multidrug resistance by the Q151M human immunodeficiency virus type 1 reverse transcriptase and its suppression using alpha-boranophosphate nucleotide analogues. *J. Biol. Chem.* **2002**, *277*, 42097-104).

e) M184I/V: The M184I/V mutation in HIV-1 RT causes high-level (>100-fold) resistance to 3TC and FTC resistance (see Schinazi RF, Lloyd RM Jr, Nguyen MH, Cannon DL, McMillan A, Ilksoy N, Chu CK, Liotta DC, Bazmi HZ, Mellors JW. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. *Antimicrob. Agents Chemother.* **1993**, *37*, 875-81; Faraj A, Agrofoglio LA, Wakefield JK, McPherson S, Morrow CD, Gosselin G, Mathe C, Imbach JL, Schinazi RF, Sommadossi JP. Inhibition of

human immunodeficiency virus type 1 reverse transcriptase by the 5'-triphosphate beta enantiomers of cytidine analogs. *Antimicrob. Agents Chemother.* **1994**, *38*, 2300-5). However, this mutation also confers resistance to ABC, ddC, ddI, (-)dOTC, and L-d4FC (see Hammond JL, Parikh UM, Koontz DL, Schlueter-Wirtz S, Chu CK, Bazmi HZ, Schinazi RF, Mellors JW. *In vitro* selection and analysis of human immunodeficiency virus type 1 resistant to derivatives of beta-2',3'- didehydro-2',3'-dideoxy-5-fluorocytidine. *Antimicrob. Agents Chemother.* **2005**, *49*, 3930-2; Winters MA, Shafer RW, Jellinger RA, Mamtora G, Gingeras T, Merigan TC. Human immunodeficiency virus type 1 reverse transcriptase genotype and drug susceptibility changes in infected individuals receiving dideoxyinosine monotherapy for 1 to 2 years. *Antimicrob. Agents Chemother.* **1997**, *41*, 757-62; Miller V, Ait-Khaled M, Stone C, Griffin P, Mesogiti D, Cutrell A, Harrigan R, Staszewski S, Katlama C, Pearce G, Tisdale M. HIV-1 reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. *AIDS.* **2000**, *14*, 163-71). Pre-steady-state kinetic analyses have demonstrated that M184V exerts a profound effect on the K_d for 3TCTP, without impacting on kpol (see Deval J, White KL, Miller MD, Parkin NT, Courcambeck J, Halfon P, Selmi B, Boretto J, Canard B. Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations. *J. Biol. Chem.* **2004**, *279*, 509-16; Feng JY, Anderson KS. Mechanistic studies examining the efficiency and fidelity of DNA synthesis by the 3TC-resistant mutant (184V) of HIV-1 reverse transcriptase. *Biochemistry.* **1999**, *38*, 9440-8) M184 forms part of the highly conserved YMDD motif, and crystal structures of 3TC-resistant M184I RT, obtained in the presence or absence of a nucleic acid substrate, suggests that steric hindrance between the oxathiolane ring of 3TCTP and the side chain of the β-branched amino acids (Val or Ile) at position 184 reduces inhibitor binding thus increasing K_d (see Gao HQ, Boyer PL, Sarafianos SG, Arnold E, Hughes SH. The role of steric hindrance in 3TC resistance of human immunodeficiency virus type-1 reverse transcriptase. *J. Mol. Biol.* **2000**, *300*, 403-18).

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 human immunodeficiency virus type 1 reverse transcriptase: 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 0.8-4mM) 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. Each of these is described below.

a) TAMS: AZT resistance correlates with multiple mutations in RT, including M41L, D67N, K70R, L210W, T215F/Y and K219E/Q (Kerr SG, Anderson KS. Pre-steady-state kinetic characterization of wild type and 3'-azido-3'-deoxythymidine (AZT) resistant human immunodeficiency virus type 1 reverse transcriptase: implication of RNA directed DNA polymerization in the mechanism of AZT resistance. *Biochemistry*. **1997**, *36*, 14064-70; Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science*. **1989**, *246*, 1155-8; Kellam P, Boucher CA, Larder BA. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1934-8; Harrigan PR, Kinghorn I, Bloor S, Kemp SD, Najera I, Kohli A, Larder BA. Significance of amino acid variation at human immunodeficiency virus type 1 reverse transcriptase residue 210 for zidovudine susceptibility. *J. Virol.* **1996**, *70*, 5930-4). Because each of these mutations has also been selected with failure of D4T therapy, (see Hooker DJ, Tachedjian G, Solomon AE, Gurusinghe AD, Land S, Birch C, Anderson JL, Roy BM, Arnold E, Deacon NJ An *in vivo* mutation from leucine to tryptophan at position 210 in human immunodeficiency virus type 1 reverse transcriptase contributes to high-level resistance to 3'-azido-3'-deoxythymidine. *J. Virol.* **1996**, *70*, 8010-8) they have been termed thymidine-analog mutations or TAMS. In general, three or more TAMS are required to show high-level resistance to AZT and cross-resistance to other NRTI. Available biochemical data indicate that AZTMP and d4TMP are the best substrates for the excision reaction carried out by HIV-1 RT containing TAMS (see Meyer PR, Matsuura SE, Schinazi RF, So AG, Scott WA. Differential removal of thymidine nucleotide analogues from blocked DNA chains by

human immunodeficiency virus reverse transcriptase in the presence of physiological concentrations of 2'-deoxynucleoside triphosphates. *Antimicrob. Agents Chemother.* **2000**, *44*, 3465-72; Naeger LK, Margot NA, Miller MD. ATP-dependent removal of nucleoside reverse transcriptase inhibitors by human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **2002**, *46*, 2179-84). However, ddAMP can also be excised, albeit less efficiently than AZTMP. In contrast, cytidine analogs and CBVTP are reported to be poor substrates of the excision reaction (see Naeger LK, Margot NA, Miller MD. ATP-dependent removal of nucleoside reverse transcriptase inhibitors by human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **2002**; *46*, 2179-84). For HIV-1 RT to effectively excise AZT from the 3'-end of a primer, the chain-terminating AZT-MP must reside in the nucleotide-binding site (N-site) of the RT active site (Naeger LK, Margot NA, Miller MD. ATP-dependent removal of nucleoside reverse transcriptase inhibitors by human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **2002**, *46*, 2179-84). Under physiological conditions, the binding of the next-correct dNTP can drive the terminating nucleotide into the primer-binding site (P-site) resulting in the formation of a dead-end complex. Formation of this complex prevents the excision reaction from occurring. Several studies have shown that the excision of AZT-MP by HIV-1 RT containing TAMS is much less sensitive (>50-fold) to inhibition by the next-correct dNTP than other NRTI analogs that lack a 3'-azido group (see Meyer PR, Matsuura SE, Schinazi RF, So AG, Scott WA. Differential removal of thymidine nucleotide analogues from blocked DNA chains by human immunodeficiency virus reverse transcriptase in the presence of physiological concentrations of 2'-deoxynucleoside triphosphates. *Antimicrob. Agents Chemother.* **2000**, *44*, 3465-72; Boyer PL, Sarafianos SG, Arnold E, Hughes SH. Selective excision of AZTMP by drug-resistant human immunodeficiency virus reverse transcriptase. *J. Virol.* **2001**, *75*, 4832-42). These data help to explain why TAMS confer higher resistance to AZT than to d4T. While it has been suggested that the 3'-azido group of the AZT-MP-terminated primer is the primary structural determinant for the excision phenotype (see Boyer PL, Sarafianos SG, Arnold E, Hughes SH. Selective excision of AZTMP by drug-resistant human immunodeficiency virus reverse transcriptase. *J. Virol.* **2001**, *75*, 4832-42; Sarafianos SG, Clark AD Jr, Das K, Tuske S, Birktoft JJ, Ilankumaran P, Ramesha AR, Sayer JM, Jerina DM, Boyer PL, Hughes SH, Arnold E. Structures of HIV-1 reverse transcriptase with pre- and post-translocation AZTMP-terminated DNA. *EMBO J.* **2002**, *21*, 6614-24), we demonstrated that 3'-azido-2',3'-ddA and

3'-azido-2',3'-ddG retain their potency against AZT-resistant virus (see 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). This indicates that the nucleoside base has an important influence on the efficiency of excision resulting from TAMS.

b) T69S Insertions: HIV-1 RT containing dipeptide insertions (typically Ser-Ser, Ser-Gly or Ser-Ala) between codons 69 and 70, together with the amino acid substitutions T69S, T215Y and other TAMS have been identified in heavily NRTI-experienced patients, albeit at low prevalence (0.5-2.7%) (see Winters MA, Merigan TC. Insertions in the human immunodeficiency virus type 1 protease and reverse transcriptase genes: clinical impact and molecular mechanisms. *Antimicrob. Agents Chemother.* **2005**, *49*, 2575-82). In phenotypic assays, viral isolates containing insertion mutations in RT demonstrate high-level resistance to AZT, and moderate levels of resistance to other NRTI, such as d4T, ddC, ddI, ABC and tenofovir. In combination with TAMS (in particular T215Y), the dipeptide insertions in HIV-1 RT confer enhanced ATP-dependent phosphorolytic activity that facilitates removal of terminating AZTMP, d4TMP, ddAMP or tenofovir, even when relatively high levels of dNTPs are present in the reaction (see Meyer PR, Lennerstrand J, Matsuura SE, Larder BA, Scott WA. Effects of dipeptide insertions between codons 69 and 70 of human immunodeficiency virus type 1 reverse transcriptase on primer unblocking, deoxynucleoside triphosphate inhibition, and DNA chain elongation. *J. Virol.* **2003**, *77*, 3871-7; Boyer PL, Sarafianos SG, Arnold E, Hughes SH. Nucleoside analog resistance caused by insertions in the fingers of human immunodeficiency virus type 1 reverse transcriptase involves ATP-mediated excision. *J. Virol.* **2002**, *76*, 9143-51; Mas A, Parera M, Briones C, Soriano V, Martinez MA, Domingo E, Menendez-Arias L. Role of a dipeptide insertion between codons 69 and 70 of HIV-1 reverse transcriptase in the mechanism of AZT resistance. *EMBO J.* **2000**, *19*, 5752-61).

Based on the structure-activity results described above, certain 3'-azido purine nucleosides (APN) emerged as a lead class of nucleoside analogs that demonstrate good activity against both HIV-1_{AZT2} and HIV-1_{K65R}. To further characterize the activity of these nucleosides, 3'-azido-ddA and 3'-azido-2',3'-ddG were evaluated against a panel of mutant viruses. This panel included recombinant viruses with K65R (HIV-1_{K65R}), L74V (HIV-1_{L74V}),

M184V (HIV-1_{M184V}), different combinations of TAMS (e.g. M41L/L210W/T215Y (HIV-1_{AZT3}), M41L/D67N/K70R/T215F/K219Q (HIV-1_{AZT7}), or M41L/D67N/K70R/L210W/T215Y/K219Q (HIV-1_{AZT9}), and multi-NRTI resistance complexes (e.g. A62V/V75I/F77L/F116Y/Q151M (HIV-1_{Q151M}) or M41L/69SS/ L210W/T215Y (HIV-$1_{69insertion}$)). The results show that both 3'-azido-ddA and 3'-azido-ddG are active against viruses with the K65R, L74V or M184V mutation. Both compounds, in comparison with AZT, were also remarkably active against all TAM-containing viruses. For example, HIV-1AZT7 was > 500-fold resistance to AZT, however less than 3.5-fold resistance was noted for this virus for 3'-azido-ddA and 3'-azido-ddG. Both 3'-azido-ddA and 3'-azido-ddG, however, were less active against HIV-1_{Q151M} and 3'-azido-ddG also lost activity against HIV-$1_{69insertion}$.

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. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer.

In industrialized countries, the high-risk group for HBV infection includes those in contact with HBV carriers or their blood samples. The epidemiology of HBV is very similar

to that of HIV/AIDS, which is a reason why HBV infection is common among patients infected with HIV or suffering from AIDS. However, HBV is more contagious than HIV.

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

A major problem in treatment of HIV and HBV is the selection for drug resistance. After taking antiviral drugs for a short period, viral mutations are selected, which render the drug a much less potent inhibitor of viral production. Even current combination therapy cannot avoid drug resistance.

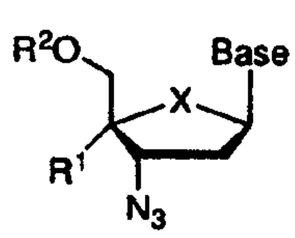
In light of the fact that acquired immune deficiency syndrome, AIDS-related complex, and hepatitis B virus have reached epidemic levels worldwide, and have tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat these diseases, with agents that have low toxicity to the host.

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

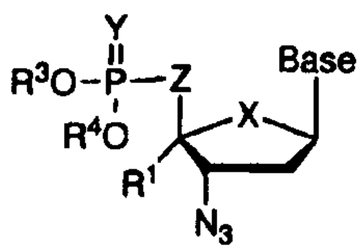
Summary of the Invention

The present invention provides compounds, methods and compositions for treating or preventing an HIV-1, HIV-2, HBV, or flaviviridae infection, such as an HCV 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 an HIV-1, HIV-2, HBV or HCV. 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 infected with HIV-1, HIV-2, HBV, or HCV are also disclosed. The formulations can further include at least one further therapeutic agent. In addition, the present invention includes processes for preparing such compounds.

The compounds described herein include β-D and β-L-3'-azido-2',3'-dideoxy purine nucleosides and phosphonates. In one embodiment, the active compound is of formula (**I**)–(**IV**):



(I)



(II)

or a pharmaceutically acceptable salt or prodrug thereof, wherein

i) X is O, CH_2, S, SO_2, NH, P=O(OH), C=CH_2, C=CHF, or C=CF_2;

ii) Y is O or S;

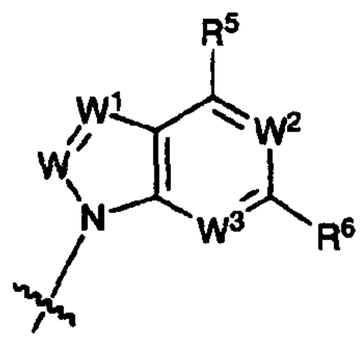
iii) Z is -CH_2, -CH_2CH_2, -CH_2O, -CH_2S, or -CH_2NH (all where a carbon atom is connected to a phosphorus atom);

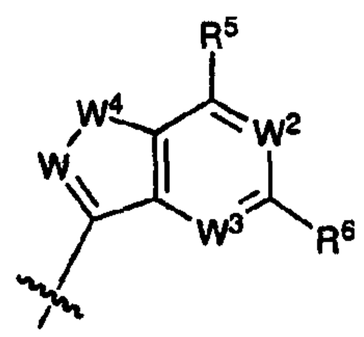
iv) R^1 is hydrogen, alkyl, haloalkyl (including but not limited to CH_2F, CF_3), halo, azido, cyano, nitro, amino, alkylamino, dialkylamino, alkenyl, alkynyl, haloalkenyl (including but not limited to Br-vinyl), alkoxy, alkenoxy, alkylthio, acyloxy, alkyloxyacyl, alkylcarbonyl, acylthio, or acylamino;

v) R^2 is H, phosphate (including but not limited to monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug), phosphothioate, carbonyl substituted with an alkyl (including but not limited to C_1-C_6), alkenyl (including but not limited to C_2-C_6), alkynyl (including but not limited to C_2-C_6), aryl (including but not limited to C_6-C_{10}), or other pharmaceutically acceptable leaving group, which, when administered *in vivo*, is capable of providing a compound wherein R^2 is H or phosphate, sulfonate ester (including but not limited to alkyl or arylalkyl sulfonyl, for example, methanesulfonyl), benzyl (wherein the phenyl group is optionally substituted with one or more substituents as described in the definition of aryl given above), a lipid (including but not limited to a phospholipid), an amino acid, a peptide, or cholesterol;

vi) R^3 and R^4 are, independently, hydrogen, phosphate, diphosphate, or a group that is preferentially removed in a hepatocyte to yield the corresponding H group. The term "preferentially removed in a hepatocyte" as used herein means at least part of the group is removed in a hepatocyte at a rate higher than the rate of removal of the same group in a non-hepatocytic cell (e.g., fibroblast or lymphocyte). It is therefore contemplated that the removable group includes all pharmaceutically acceptable groups that can be removed by a reductase, esterase, cytochrome P450 or any other specific liver enzyme. Alternative contemplated groups can also include groups that are not necessarily preferentially removed in a hepatocyte, but effect at least some accumulation and/or specific delivery to a hepatocyte (e.g., esters with selected amino acids, including valine, leucine, isoleucine, or polyarginine or polyaspartate); and

vii) Base is purine or modified purine of the general formula of **(III)**-**(IV)**:





(III) **(IV)**

wherein:

each W, W^1, W^2 and W^3 is independently N, CCF_3, $CC(O)NH_2$, CC(O)NHR', $CC(O)N(R')_2$, CC(O)OH, CC(O)OR' or CR^5;

W^4 is independently O, S, NH or NR';

each R^5 and R^6 is chosen independently from H, halogen (F, Cl, Br, I), CN, N_3, NO_2, OH, NH_2, SH, OR', NHR', $N(R')_2$, SR', OCOR', NHCOR', N(COR')COR', SCOR', OCOOR', NHCOR', CH_2OH, CH_2CN, CH_2N_3, COOH, COOR', $CONH_2$, CONHR, $CON(R')_2$, CH_2COOH, CH_2COOR', CH_2CONH_2, CH_2CONHR', $CH_2CON(R')_2$, alkyl (including but not limited to C_1-C_6), alkenyl (including but not limited to C_2-C_6), and alkynyl (including but not limited to C_2-C_6), cycloalkyl (including but not limited to C_3-C_8), aryl

wherein for formula (I) where base is formula (III), R^5 cannot be Cl, Br, I, C_{1-6} alkyloxy, C_{3-6} cycloalkyloxy, aryloxy, arylalkoxy, amino which is substituted by one or two substituents independently selected from C_{1-6} alkyl and C_{3-6} cycloalkyl, or 4 to 6 membered heterocyclic ring containing at least one nitrogen atom which ring is bonded to the purine base via a/the nitrogen atom, if R^1 and R^2 are H, W is CH, W^1, W^2 and W^3 are N, and R^6 is NH_2 or NHR^7 where R^7 is acyl;

wherein for formula (I) where base is formula (III), R^6 cannot be NH_2 when R^5 is OH and R^6 cannot be H when R^5 is NH_2, if R^1 and R^2 are H, W is CH, W^1, W^2 and W^3 are N; and

each R' is independently a lower alkyl (C_1-C_6 alkyl), lower alkenyl, lower alkynyl, lower cycloalkyl (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 aspect, W is CH and W^1-W^3 are N. In another aspect, R^5 adjacent to W^2 is a halo group, hydroxyl group, an alkoxy group, or an amine group, where the amine group is optionally substituted with an alkyl group, hydroxyalkyl group, aminoalkyl group, cycloalkyl group, alkenyl group, or alkynyl group. In yet another aspect, R^6 is H or NH_2.

In another aspect, the compounds are 3'-azido-ddA and/or 3'-azido-ddG, in combination with drugs that select for TAM mutations and/or drugs that select for the M184V mutation. The compounds described herein can be in the form of the isolated β-L- or β-D- configuration, or a mixture thereof, including but not limited to a racemic mixture.

In addition, the compounds described herein are inhibitors of HBV and/or HCV. Therefore, these compounds can also be used to treat patients that are co-infected with both HIV-1 or HIV-2 and HBV and/or HCV.

Brief Description of the Figures

Figure 1 is a graphic representation of the genotypes of xxLAI viruses.

Figures 2A-2B are graphic representations of the anti-HIV activity of 3'-azido-2',3'-ddA and 3'-azido-2',3'-ddG against a panel of drug-resistant HIV-1.

Figures 4A-4B are graphic representations of deamination by adenosine deaminase.

Figure 5 is a chart showing the development of a virus resistant to 3'-azido-2',3'-dideoxy guanosine (3'-azido-ddG, also referred to herein as compound 56) over time (weeks).

Figure 6 is a summary of treatment with 3'-azido-ddG and the mutations selected over time.

Detailed Description

The 3'-azido-2',3'-dideoxy purine nucleosides described herein show improved inhibitory activity against HIV, HBV, and flaviviridae viruses, including those with mutated RT enzymes. 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, HBV, and/or flaviviridae viruses, such as HCV. The methods involve administering an effective amount of one or more of the 3'-azido-2',3'-dideoxy purine nucleosides 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 term "independently" is used herein to indicate that the variable, which is independently applied, varies independently from application to application. Thus, in a compound such as R"XYR", wherein R" is "independently carbon or nitrogen," both R" can be carbon, both R" can be nitrogen, or one R" can be carbon and the other R" nitrogen.

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

As used herein, the term "substantially free of" or "substantially in the absence of" refers to a nucleoside composition that includes at least 85 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 nucleoside. In a preferred embodiment, the compounds described herein are substantially free of enantiomers.

Similarly, the term "isolated" refers to a nucleoside 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 nucleoside, the remainder comprising other chemical species or enantiomers.

The term "alkyl," as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbons, including both substituted and unsubstituted alkyl groups. The alkyl group can be optionally substituted with any moiety that does not otherwise interfere with the reaction or that provides an improvement in the process, including but not limited to but limited to halo, haloalkyl, hydroxyl, carboxyl, acyl, aryl, acyloxy, amino, amido, carboxyl derivatives, alkylamino, dialkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, thiol, imine, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrozine, carbamate, phosphonic acid, phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference. Specifically included are CF_3 and CH_2CF_3

In the text, whenever the term C(alkyl range) is used, the term independently includes each member of that class as if specifically and separately set out. The term "alkyl" includes C_{1-22} alkyl moieties, and the term "lower alkyl" includes C_{1-6} 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. 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. The alkynyl group can be optionally substituted with any moiety that does not adversely affect the reaction process, including but not limited to those described above for alkyl moeities. Non-limiting examples

of suitable alkynyl groups include ethynyl, propynyl, hydroxypropynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 4-methoxypentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, and hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals.

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

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

The term "aryl", alone or in combination, means a carbocyclic aromatic system containing one, two or three rings wherein such rings can be attached together in a pendent manner or can be fused. Non-limiting examples of aryl include phenyl, biphenyl, or naphthyl, or other aromatic groups that remain after the removal of a hydrogen from an aromatic ring. The term aryl includes both substituted and unsubstituted moieties. The aryl group can be optionally substituted with any moiety that does not adversely affect the process, including but not limited to but not limited to those described above for alkyl moieties. Non-limiting examples of substituted aryl include heteroarylamino, N-aryl-N-alkylamino, N-heteroarylamino-N-alkylamino, heteroaralkoxy, arylamino, aralkylamino, arylthio, monoarylamidosulfonyl, arylsulfonamido, diarylamidosulfonyl, monoaryl amidosulfonyl, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, hydroxyaralkyl, hydoxyheteroaralkyl, haloalkoxyalkyl, aryl, aralkyl, aryloxy, aralkoxy, aryloxyalkyl, saturated heterocyclyl, partially saturated heterocyclyl, heteroaryl, heteroaryloxy, heteroaryloxyalkyl, arylalkyl, heteroarylalkyl, arylalkenyl, and heteroarylalkenyl, carboaralkoxy.

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

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

The term "acyl" refers to a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl or lower alkyl, alkoxyalkyl

including but not limited to methoxymethyl, aralkyl including but not limited to benzyl, aryloxyalkyl such as phenoxymethyl, aryl including but not limited to phenyl optionally substituted with halogen (F, Cl, Br, I), alkyl (including but not limited to C_1, C_2, C_3, and C_4) or alkoxy (including but not limited to C_1, C_2, C_3, and C_4), sulfonate esters such as alkyl or aralkyl sulphonyl including but not limited to methanesulfonyl, the mono, di or triphosphate ester, 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" refers to a nonaromatic cyclic group wherein there is at least one heteroatom, such as oxygen, sulfur, nitrogen, or phosphorus in the ring.

Nonlimiting examples of heteroaryl and heterocyclic groups include furyl, furanyl, pyridyl, pyrimidyl, thienyl, isothiazolyl, imidazolyl, tetrazolyl, pyrazinyl, benzofuranyl, benzothiophenyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, isoindolyl, benzimidazolyl, purinyl, carbazolyl, oxazolyl, thiazolyl, isothiazolyl, 1,2,4-thiadiazolyl, isooxazolyl, pyrrolyl, quinazolinyl, cinnolinyl, phthalazinyl, xanthinyl; hypoxanthinyl, thiophene, furan, pyrrole, isopyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, pyrimidine or pyridazine, and pteridinyl, aziridines, thiazole, isothiazole, 1,2,3-oxadiazole, thiazine, pyridine, pyrazine, piperazine, pyrrolidine, oxaziranes, phenazine, phenothiazine, morpholinyl, pyrazolyl, pyridazinyl, pyrazinyl, quinoxalinyl, xanthinyl, hypoxanthinyl, pteridinyl, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, pyrazolopyrimidinyl, adenine, N^6-alkylpurines, N^6-benzylpurine, N^6-halopurine, N^6-vinypurine, N^6-acetylenic purine, N^6-acyl purine,N^6-hydroxyalkyl purine, N^6-thioalkyl purine, thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrmidine, uracil, N^5-alkylpyrimidines, N^5-benzylpyrimidines, N^5-halopyrimidines, N^5-vinylpyrimidine, N^5-acetylenic pyrimidine, N^5-acyl pyrimidine, N^5-hydroxyalkyl purine, and N^6-thioalkyl purine, and isoxazolyl. The heteroaromatic group can be optionally substituted as described above for aryl. The heterocyclic or heteroaromatic group can be optionally substituted with one or more substituent selected from halogen, haloalkyl, alkyl, alkoxy, hydroxy, carboxyl derivatives, amido, amino, alkylamino, dialkylamino. The heteroaromatic can be partially or totally hydrogenated as desired. As a nonlimiting example, dihydropyridine can be used in place of pyridine. Functional oxygen and nitrogen groups on the heterocyclic or heteroaryl group can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, and *t*-butyldiphenylsilyl, trityl or substituted trityl, alkyl groups, acyl groups such as acetyl and propionyl, methanesulfonyl, and p-toluenelsulfonyl. The heterocyclic or heteroaromatic group can be substituted with any moiety that does not adversely affect the reaction, including but not limited to but not limited to those described above for aryl.

The term "host," as used herein, refers to a unicellular or multicellular organism in which the virus can replicate, including but not limited to cell lines and animals, and, preferably, humans. Alternatively, the host can be carrying a part of the viral genome, whose replication or function can be altered by the compounds of the present invention. The term

host specifically refers to infected cells, cells transfected with all or part of the viral genome and animals, in particular, primates (including but not limited to chimpanzees) and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly contemplated by the present invention (such as for use in treating chimpanzees).

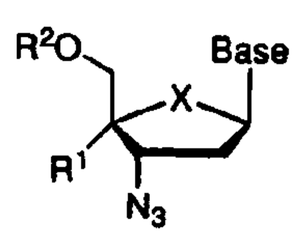
The term "pharmaceutically acceptable salt or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of a nucleoside compound which, upon administration to a patient, provides the nucleoside 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 text of which is incorporated by reference. As an alternative to the use of valine itself, a functional equivalent of the amino acid can be used (*e.g.*, an acid halide such as the acid chloride, or an

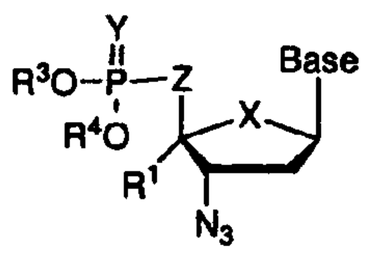
acid anhydride). In such a case, to avoid undesirable side-reactions, it may be advantageous to use an amino-protected derivative.

II. Active Compound

In one embodiment of the invention, the active compound is of formula (I)–(IV):



(I)



(II)

or a pharmaceutically acceptable salt or prodrug thereof, wherein

i) X is O, CH_2, S, SO_2, NH P=O(OH), C=CH_2, C=CHF, or C=CF_2;

ii) Y is O or S;

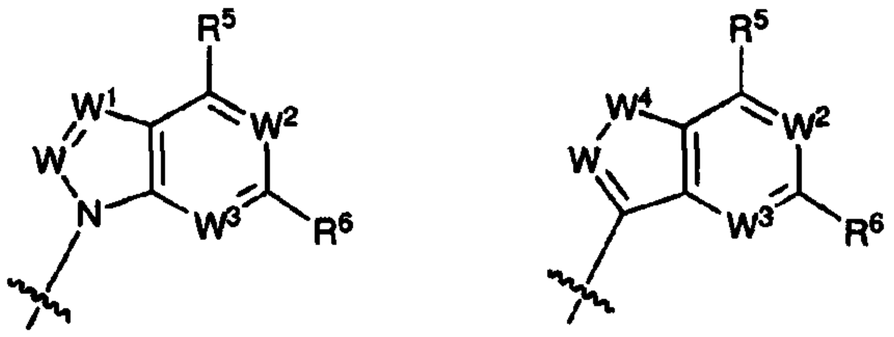
iii) Z is CH_2, CH_2CH_2, CH_2O, CH_2S, or CH_2NH (all where a carbon atom is connected to a phosphorus atom);

iv) R^1 is hydrogen, alkyl, haloalkyl (including but not limited to CH_2F and CF_3), halo, azido, cyano, nitro, amino, alkylamino, dialkylamino, alkenyl, alkynyl, haloalkenyl (including but not limited to Br-vinyl), alkoxy, alkenoxy, alkylthio, acyloxy, alkyloxyacyl, alkylcarbonyl, acylthio, or acylamino;

v) R^2 is H, phosphate (including but not limited to monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug), phosphothioate, carbonyl substituted with an alkyl (including but not limited to C_1-C_6), alkenyl (including but not limited to C_2-C_6), alkynyl (including but not limited to C_2-C_6), aryl (including but not limited to C_6-C_{10}), or other pharmaceutically acceptable leaving group, which, when administered *in vivo*, is capable of providing a compound wherein R^2 is H or phosphate, sulfonate ester (including but not limited to alkyl or arylalkyl sulfonyl, for example, methanesulfonyl), benzyl (wherein the phenyl group is optionally substituted with one or more substituents as described in the definition of aryl given above), a lipid (including but not limited to a phospholipid), an amino acid, a peptide, or cholesterol;

vi) R^3 and R^4 are, independently, hydrogen, phosphate, diphosphate, or a group that is preferentially removed in a hepatocyte to yield the corresponding H group. The term "preferentially removed in a hepatocyte" as used herein means at least part of the group is removed in a hepatocyte at a rate higher than the rate of removal of the same group in a non-hepatocytic cell (e.g., fibroblast or lymphocyte). It is therefore contemplated that the removable group includes all pharmaceutically acceptable groups that can be removed by a reductase, esterase, cytochrome P450 or any other specific liver enzyme. Alternative contemplated groups may also include groups that are not necessarily preferentially removed in a hepatocyte, but effect at least some accumulation and/or specific delivery to a hepatocyte (e.g., esters with selected amino acids, including valine, leucine, isoleucine, or polyarginine or polyaspartate); and

vii) Base is purine or modified purine of the general formula of **(III)**-**(IV)**:



(III) **(IV)**

wherein:

each W, W^1, W^2 and W^3 is independently N, CCF_3, $CC(O)NH_2$, CC(O)NHR', $CC(O)N(R')_2$, CC(O)OH, CC(O)OR' or CR^5;

W^4 is independently O, S, NH or NR';

each R^5 and R^6 is chosen independently from H, halogen (F, Cl, Br, I), CN, N_3, NO_2, OH, NH_2, SH, OR', NHR', $N(R')_2$, SR', OCOR', NHCOR', N(COR')COR', SCOR', OCOOR', NHCOR', CH_2OH, CH_2CN, CH_2N_3, COOH, COOR', $CONH_2$, CONHR, $CON(R')_2$, CH_2COOH, CH_2COOR', CH_2CONH_2, CH_2CONHR', $CH_2CON(R')_2$, alkyl (including but not limited to C_1-C_6), alkenyl (including but not limited to C_2-C_6), and alkynyl (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}), acyl (including but not limited to C_2-C_6), arylalkyl, and alkylaryl;

wherein for formula (I) where base is formula (III), R^5 cannot be Cl, Br, I, C_{1-6} alkyloxy, C_{3-6} cycloalkyloxy, aryloxy, arylalkoxy, amino which is substituted by one or two substituents independently selected from C_{1-6} alkyl and C_{3-6} cycloalkyl, or 4 to 6 membered heterocyclic ring containing at least one nitrogen atom which ring is bonded to the purine base via a/the nitrogen atom, if R^1 and R^2 are H, W is CH, W^1, W^2 and W^3 are N, and R^6 is NH_2 or NHR^7 where R^7 is acyl;

wherein for formula (I) where base is formula (III), R^6 cannot be NH_2 when R^5 is OH and R^6 cannot be H when R^5 is NH_2, if R^1 and R^2 are H, W is CH, W^1, W^2 and W^3 are N; and

each R' is independently a lower alkyl (C_1-C_6 alkyl), lower alkenyl, lower alkynyl, lower cycloalkyl (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 aspect, W is CH and W^1-W^3 are N. In another aspect, R^5 adjacent to W^2 is a halo group, hydroxyl group, an alkoxy group, or an amine group, where the amine group is optionally substituted with an alkyl group, hydroxyalkyl group, aminoalkyl group, cycloalkyl group, alkenyl group, or alkynyl group. In yet another aspect, R^6 is H or NH_2.

In another embodiment, the compound is 3'-azido-ddA or 3'-azido-ddG, either alone or together, each or both in combination with one or more antiviral compounds that select for TAM mutations and/or the M184V mutation.

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.

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.

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.

Any of the nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including but not limited to sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones & N. Bischofberger, *Antiviral Research*, **1995**, *27*, 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

The active nucleoside can also be provided as a 5'-phosphoether lipid or a 5'-ether lipid, as disclosed in the following references, which are incorporated by reference: 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 the 5'-OH position of the nucleoside 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 nucleosides of the present invention, or lipophilic preparations, include WO

89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910, WO 94/26273, WO 96/15132, EP 0 350 287, EP 93917054.4, and WO 91/19721.

V. 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
Intron A (interferon alfa-2b)	interferon	Schering-Plough
Pegasys **(Peginterferon alfa-2a)**	interferon	Roche
Epivir-HBV	nucleoside	GlaxoSmithKline

Drug Name	Drug Class	Company
(lamivudine; 3TC)	analogue	
Hepsera (Adefovir Dipivoxil)"	nucleotide analogue	Gilead Sciences
Emtriva® (emtricitabine; FTC)	nucleoside analogue	Gilead Sciences
Entecavir	nucleoside analogue	Bristol-Myers Squibb
Clevudine (CLV, L-FMAU)	nucleoside analogue	Pharmasset
ACH 126, 443 (L-Fd4C)	nucleoside analogue	Achillion Pharmaceuticals
AM 365	nucleoside analogue	Amrad
Amdoxovir (AMDX, DAPD)	nucleoside analogue	RFS Pharma LLC
LdT (telbivudine)	nucleoside analogue	Idenix
CS-1220	nucleoside analogue	Emory University
Theradigm	Immune stimulant	Epimmune
Zadaxin (thymosin)	Immune stimulant	SciClone
EHT 899	viral protein	Enzo Biochem
Dexelvuecitabine/Reverset/D-D4FC	nucleoside analogue	Pharmasset
APD	nucleoside analogue	RFS Pharma
HBV DNA vaccine	Immune stimulant	PowderJect (UK)
MCC 478	nucleoside analogue	Eli Lilly
valLdC (valtorcitabine)	nucleoside analogue	Idenix

Drug Name	Drug Class	Company
ICN 2001	nucleoside analogue	ICN
Racivir	nucleoside analogue	Pharmasset
Robustaflavone	nucleoside analogue	Advanced Life Sciences
LM-019c		Emory University
Penciclovir	nucleoside analogue	
Famciclovir		
D XG	nucleoside analogue	
ara-AMP prodrugs		
HBV/MF59		
HDP-P-acyclovir	nucleoside analogue	
Hammerhead ribozymes		
Glycosidase Inhibitors		
Pegylated Interferon		
Human Monoclonal Antibodies		

HIV Therapies: Protease Inhibitors (PIs)

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Invirase®	saquinavir (Hard Gel Cap)	SQV (HGC)	Ro-31-8959	Hoffmann-La Roche
Fortovase®	saquinavir (Soft Gel Cap)	SQV (SGC)		Hoffmann-La Roche
Norvir®	ritonavir	RTV	ABT-538	Abbott Laboratories
Crixivan®	indinavir	IDV	MK-639	Merck & Co.
Viracept®	nelfinavir	NFV	AG-1343	Pfizer
Agenerase®	amprenavir	APV	141W94 or VX-478	GlaxoSmithKline
Kaletra®	lopinavir + ritonavir	LPV	ABT-378/r	Abbott Laboratories
Lexiva®	fosamprenavir		GW-433908 or VX-175	GlaxoSmithKline
Aptivus®	tripanavir	TPV	PNU-140690	Boehringer Ingelheim
Reyataz®	atazanavir		BMS-232632	Bristol-Myers Squibb
	brecanavir		GW640385	GlaxoSmithKline
Prezista™	darunavir		TMC114	Tibotec

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

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

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

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

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

HIV Therapies: Other Classes of Drugs

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

Cellular Inhibitors

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

Entry Inhibitors (including Fusion Inhibitors)

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
Fuzeon™	enfuvirtide		T-20	Trimeris
			T-1249	Trimeris
			AMD-3100	AnorMED, Inc.
	CD4-IgG2		PRO-542	Progenics Pharmaceuticals
			BMS-488043	Bristol-Myers Squibb
	aplaviroc		GSK-873,140	GlaxoSmithKline
	Peptide T			Advanced

Brand Name	Generic Name	Abbreviation	Experimental Code	Pharmaceutical Company
				Immuni T, Inc.
			TNX-355	Tanox, Inc.
	maraviroc		UK-427,857	Pfizer
CXCR4 Inhibitor				
	AMD070		AMD11070	AnorMED, Inc.
CCR5 antagonist				
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

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, anti-HCV or anti-herpetic agent or 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.

VI. Pharmaceutical Compositions

Hosts, including but not limited to humans, infected with a human immunodeficiency virus, a hepatitis B or C virus, 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.

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

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

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

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

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

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

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

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

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

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

Liposomal suspensions (including but not limited to liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in US Pat. No. 4,522,811 (incorporated by reference). For example, liposome formulations can be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl

choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

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

AIBN	2,2'-azobisisobutyronitrile
BuLi	n-butyllithium
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
h	hour/hours
M	molar
MeCN	acetonitrile
MeOH	methanol
min	minute
NaOMe	sodium methoxide
Py	pyridine
rt or RT	room temperature
TBAF	tetra-*N*-butylammonium fluoride
TBAT	tetrabutylammonium triphenyldifluorosilicate
TBDMSCl	*tert*-butyl dimethyl silyl chloride
THF	tetrahydrofuran

TMSBr	trimethylsilyl bromide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TsCl	*p*-methylbenzene sulfonyl chloride

VII. General Schemes for Preparing Active Compounds

Methods for the facile preparation of 3'-azido-2',3'-dideoxy purine nucleosides and phosphonates are also provided. The 3'-azido-2',3'-dideoxy purine nucleosides and phosphonates 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.

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, the synthesis of 3'-azido-2',3'-dideoxy purine nucleosides **I** from 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines.

Scheme 2 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines from ribo-sugar or ribo-nucleosides.

Scheme 3 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines from xylo-sugar.

Scheme 4 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines from deoxyribo-sugar.

Scheme 5 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of carbocyclic purine nucleosides.

Scheme 6 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 3'-azido-2',3'-dideoxy purine nucleosides by manipulation at 2 or 6-position of 3'-azido-2',3'-dideoxy purine nucleosides.

Scheme 7 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 3'-azido-2',3'-dideoxy purine nucleoside phosphonates.

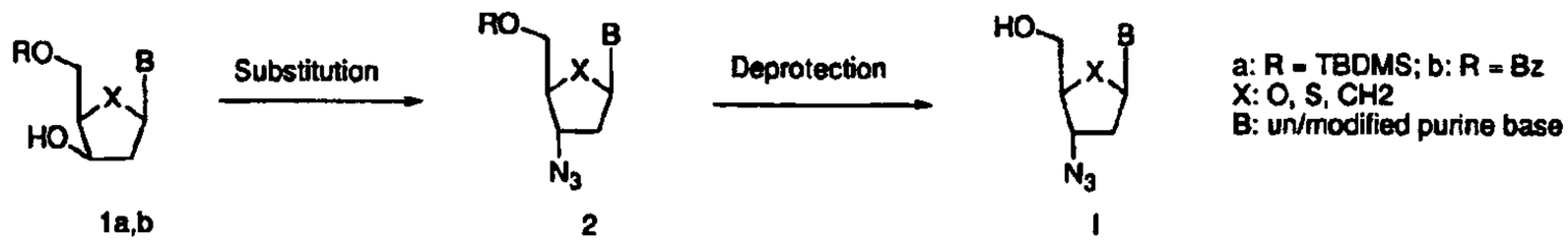
Scheme 8 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of carbocyclic 3'-azido-2',3'-dideoxy purine nucleoside phosphonates.

Scheme 9 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 3'-azido-2',3'-dideoxy purine nucleoside phosphonates.

Scheme 10 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 3'-azido-2',3'-dideoxyguanosine.

Scheme 11 is a non-limiting example of the synthesis of active compounds of the present invention, and in particular, the synthesis of 3'-azido-2',3'-dideoxyguanosine analogs **(62-65)**.

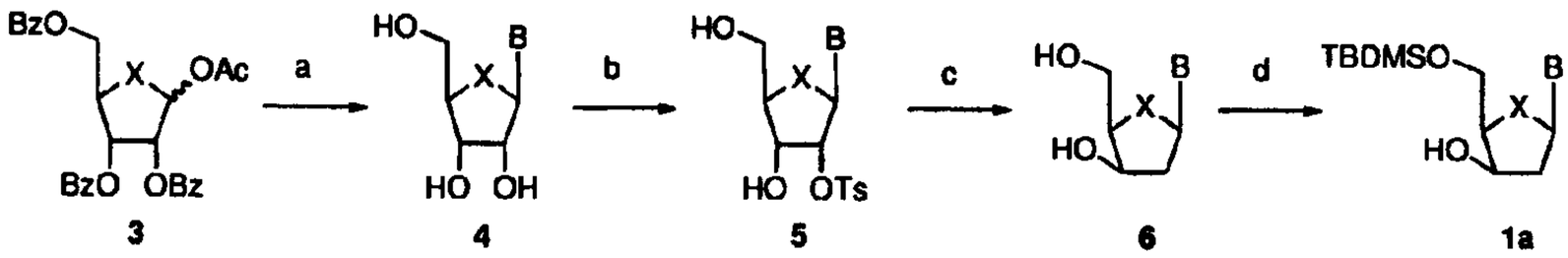
In one embodiment, the method includes azido substitution of a 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purine **I**, either directly under Mitsunobu conditions (see Marchand et al., *Nucleosides Nucleotides & Nucleic Acids*, **2000**, *19*, 205-17), or *via* a sulfonate ester intermediate, with a lithium azide, sodium azide, or ammonium azide, followed by deprotection, as depicted in Scheme 1. The sulfonate ester can be methanesulfonate, tosylate, triflate, or other suitable leaving group, and deprotection conditions can be varied depending upon the 5'-*O*-protection. The protection groups at 5'-position can be ester (such as Bz, Ac), ether (such as trityl or MOM), silyl (such as TBDMS or TBDPS) or other protecting groups. In general, methanolic ammonia is used for removing ester protection, and acidic conditions such as HOAc or HCl, can be used for removing trityl protection. For deprotecting a silyl group, either TBAF or NH_4F can be used.



1a,b 2 I

Scheme 1. Synthesis of 3'-azido-2',3'-dideoxy purine nucleosides **I** from 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines.

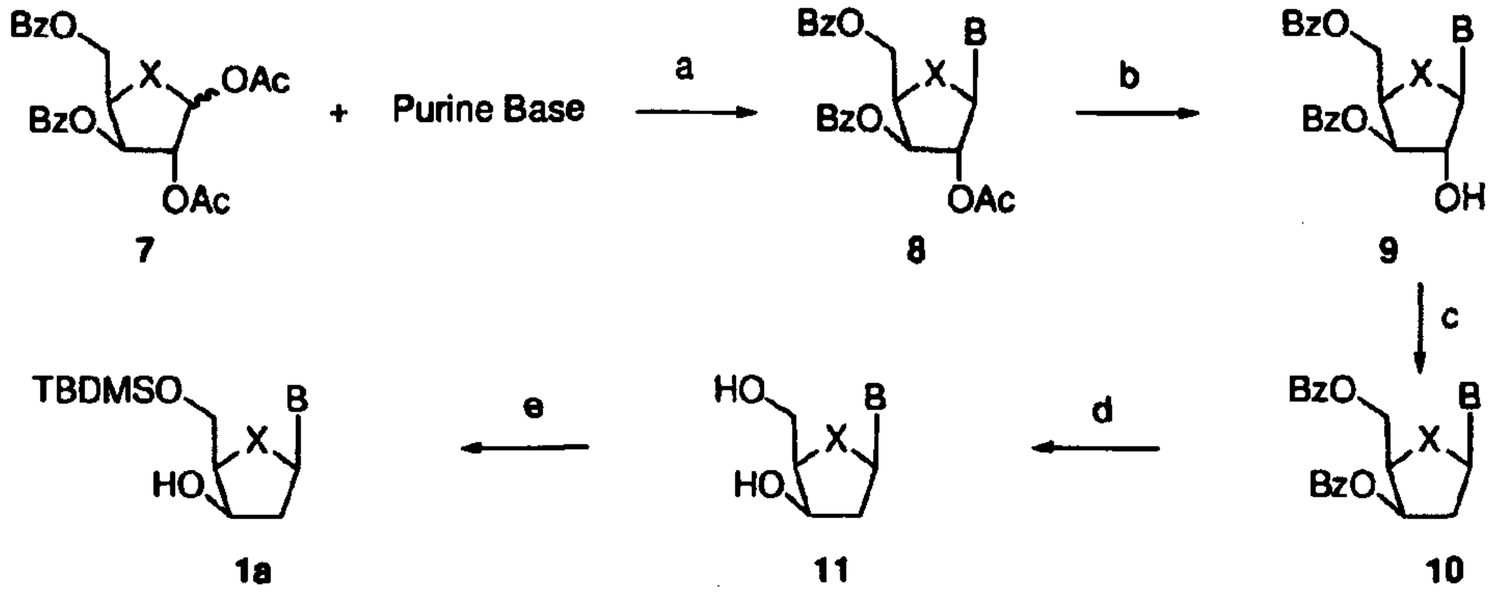
Compounds **1** can be prepared by various approaches. The first approach shown in Scheme 2 is based on Robins' procedure which transforms 2'-O-tosyl nucleosides **5** to 2'-deoxy-3'-up nucleosides **6** by deoxygenation and concomitant inversion of 3'-hydroxyl in a one-pot manner (see Hansske et al., *J. Am. Chem. Soc.* **1983**, *105*, 6736). The tosylates **5** can be prepared from purine nucleosides **4** by Wagner-Moffatt procedure (see Wagner et al., *J. Org. Chem.* **1974**, *39*, 24), whereas the purine nucleosides **4** can be either prepared from condensation of ribo-sugar **3** (X = O, S) with purine (or modified purine) base, or obtained from commercially available sources. After protection of 5'-hydroxyl group, the 3'-hydroxyl-up nucleosides **1a** are obtained.



3 4 5 6 1a

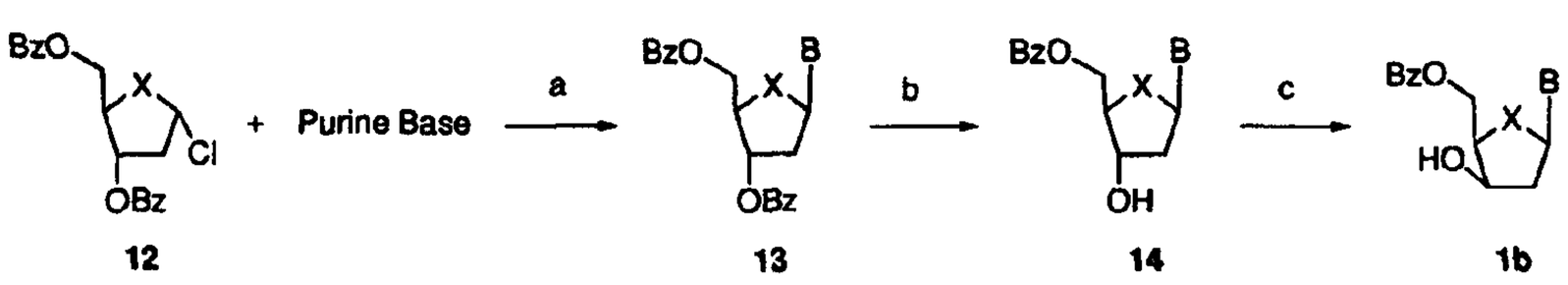
Scheme 2. Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines **1a** from ribo-sugar or ribo-nucleosides. X = O, S, CH_2; B = protected or unprotected purine base; Reagents and conditions: (a) when X = O or S: i) TMSOTf, toluene, protected or silylated purine; ii) NH_3, MeOH; (b) i) Bu_2SnO, MeOH; ii) TsCl, Et_3N; (c) $LiEt_3BH$, THF, DMSO; (d) TBDMSCl, imidazole, DMF.

The second approach utilizes condensation of xylo-sugar **7** with silylated or protected purine or modified purine base. The resulting xylo-nucleosides **8** can be selectively deacylated and deoxygenated to give compounds **10**. After deprotection and silylation, compounds **10** can be converted to **1a** (Scheme 3).



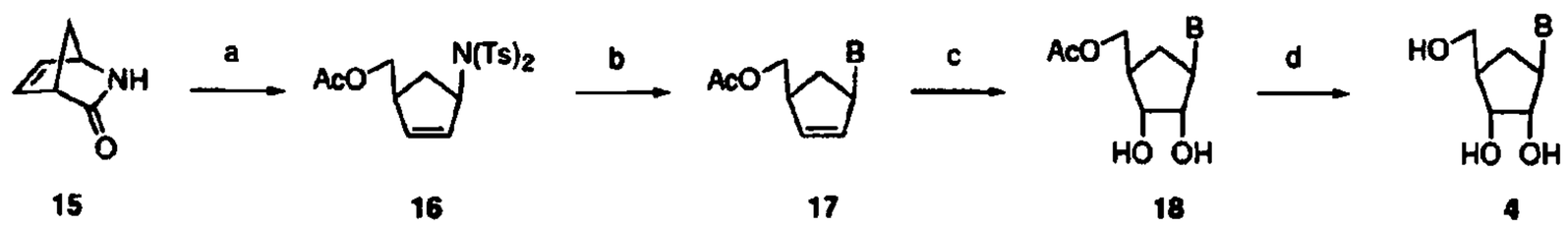
Scheme 3. Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines **1a** from xylo-sugar. X = O or S; B = protected or unprotected purine base; Reagents and conditions: (a) TMSOTf, toluene; (b) $H_2NNH_2.H_2O$, AcOH, Py; (c) i) PhOCSCl, DMAP, MeCN; ii) $(Me_3Si)_3SiH$, AIBN, dioxane; (d) NH_3, MeOH; (e) TBDMSCl, imidazole, DMF.

A third approach for preparing compounds **1** involves the condensation of a 2-deoxy-sugar **12** with silylated or protected purine base or modified purine base. The obtained benzoylated 2'-deoxy purine nucleosides **13** can be converted to 3'-unprotected compounds **14** by deprotection and selective benzoylation. Inversion of the 3'-hydroxyl group using Herdewijn's procedure transforms **14** to **1b** (Scheme 4).



Scheme 4. Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)purines **1b** from deoxyribo-sugar. X = O or S; B = protected or unprotected purine base; Reagents and conditions: (a) MeCN; (b) i) NH_3, MeOH; ii) BzCl, Py; (c) i) $(CF_3SO_2)_2O$, Py, CH_2Cl_2, H_2O; ii) MeOH, $NaHCO_3$.

For synthesizing carbocyclic nucleosides **4**, the Jung's method can be employed. This method involves the conversion of Vince lactam **15** to a pentenylamino sulfonate **16** followed by a Trost addition (see Jung et al., *J. Org. Chem.* **1994**, *59*, 4719-20). The resulting unsaturated carbocyclic nucleosides **17** can be oxidized to **18**, and the latter compounds can be deprotected to the carbocyclic nucleosides **4** (Scheme 5). From compounds **4**, following the procedures described in Schemes 1 and 2, the carbocyclic analogs **I** can be prepared.

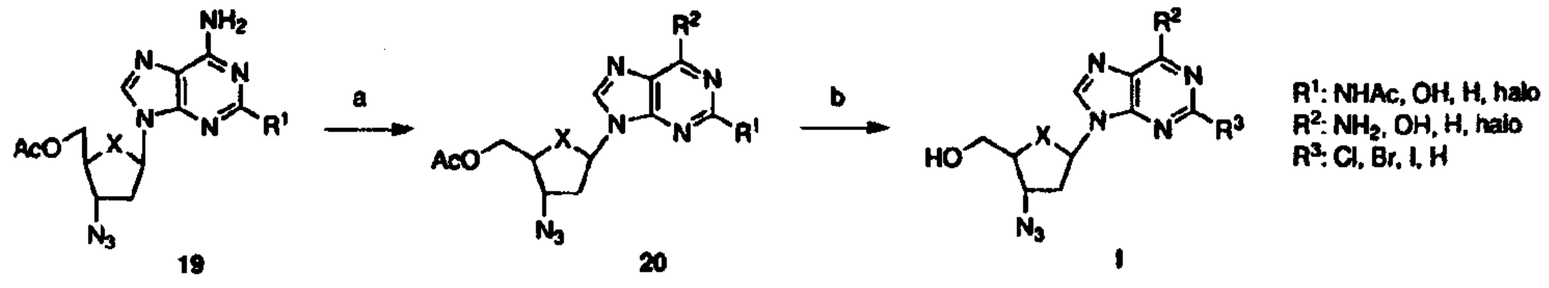


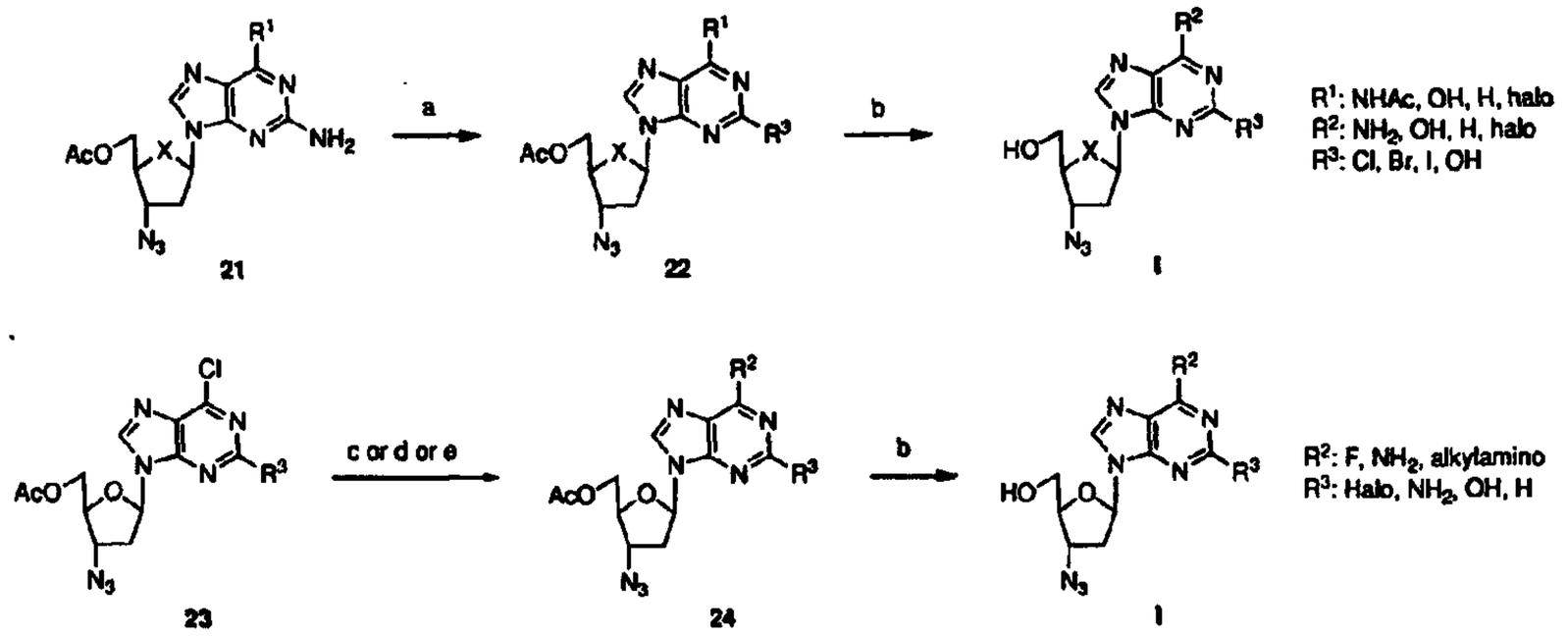
15 16 17 18 4

Scheme 5. Synthesis of carbocyclic purine nucleosides **4**. B = protected or unprotected purine base; Reagents and conditions: (a) ref. Jung et al., J. Org. Chem. 1994, 59, 4719-4720; (b) i) $Pd(OAc)_2$, $P(OiPr)_3$, THF, n-BuLi; ii) NaH, DMSO, purine base; (c) OsO_4; (d) NH_3, MeOH.

For synthesizing 4'-substituted 3'-azido purine nucleosides, multiple methodologies can be used, before or after purine base coupling. For example, the 4'-5'-unsaturated sugar can be used to introduce a variety of substituents in the 4' position through the epoxide (see Haraguchi et al., *J. Org. Chem.* **2006**, *71*, 4433-38) or iodine/nucleophile combination (see Connolly et al., 2005, WO2005/000864 A1). In another example, the 4'-*C*-hydroxymethyl can be prepared from formaldehyde or its equivalent and converted into multiple substituents at the 4'-position (see Kohgo, et al., *Nucleosides & Nucleotides* **2004**, *23*, 671-90; Siddiqui, et al., *J. Med. Chem.* **2004**, *47*, 5041-8).

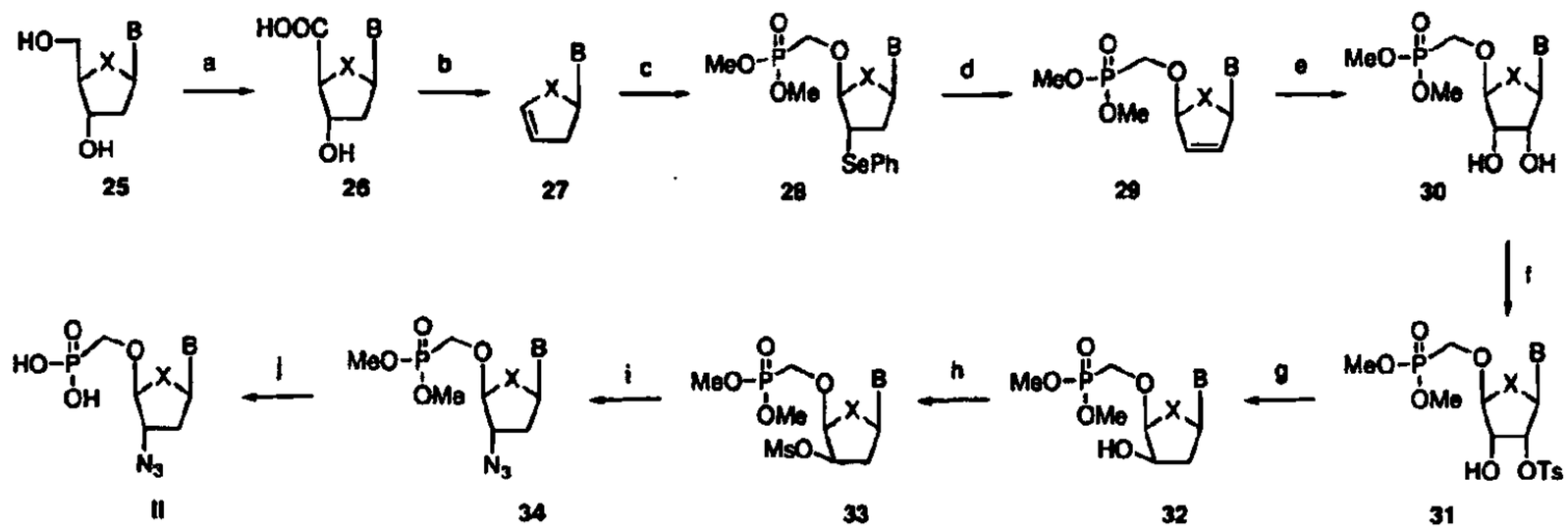
For synthesizing 2- and/or 6-modified 3'-azido purine nucleosides, the methodology of manipulation of functionality can be employed (Scheme 6). For example, the Robins' diazotization method can be used to synthesize 2- or 6-substituted purine nucleosides, in which the amino group is converted to halogen or hydrogen through a diazo intermediate. 6-Fluoro substituted nucleosides can be synthesized from 6-chloro compounds **23** via a trimethylammonium salt intermediate (see ref. Gurvich et al., *Nucleosides & Nucleotides* **1999**, *18*, 2327-33; Kim et al., *J. Med. Chem.* **1999**, *42*, 324-8). From 6-chloro compounds **23**, other 6-alkylamino substituted nucleosides can also be prepared. These preparations are depicted in Scheme 6. Other functionality transformation can be also made by other reactions known to those skilled in the art without departing from the spirit and scope of the present invention.





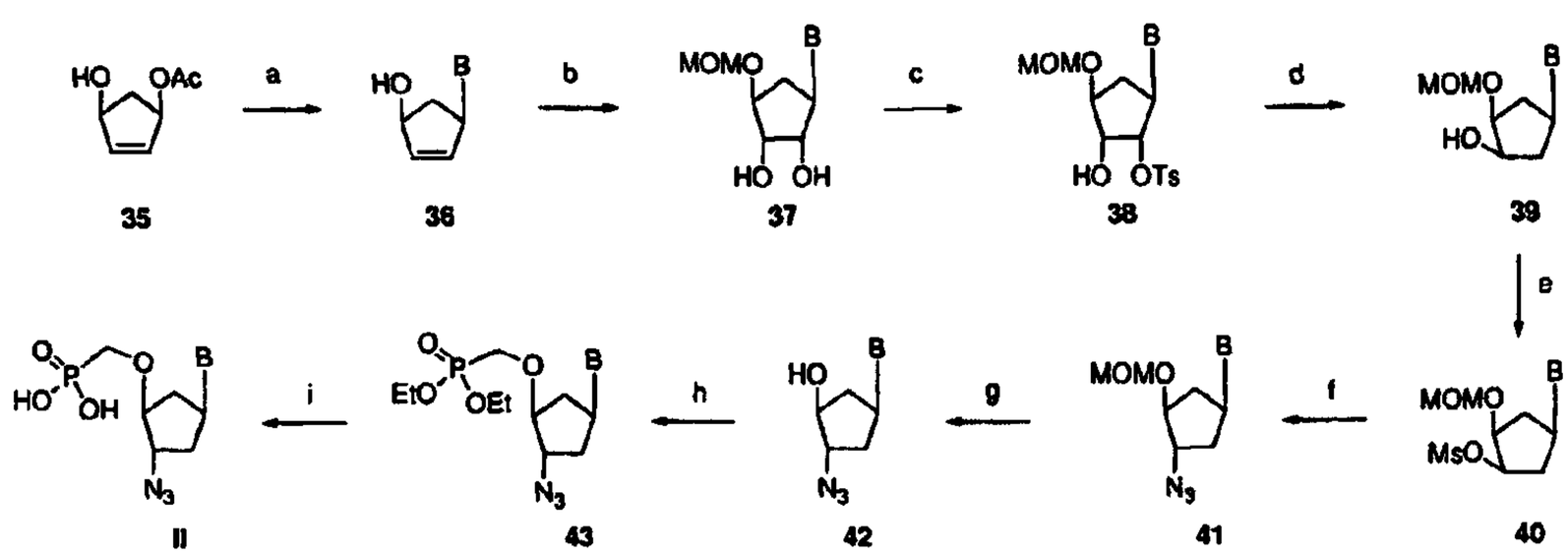
Scheme 6. Synthesis of 3'-azido-2',3'-dideoxy purine nucleosides by manipulation at 2 or 6-position of 3'-azido-2',3'-dideoxy purine nucleosides. X = O, S, CH_2; Reagents and conditions: (a) ref. Francom et al., *J. Org. Chem.* **2002**, *67*, 6788-96; Francom et al., J. Org. Chem. 2003, 68, 666-669; (b) NH_3, MeOH, 0 °C; (c) i) NMe_3, dimethoxyethane; ii) TBAT, DMF; (d) i) NMe_3, DMF, THF; ii) KF, DMF; (e) NH_3 or alkylamine.

3'-Azido-2',3'-dideoxy purine nucleoside phosphonates **II** (R^3 and R^4 = H, X = O, S) can be synthesized by adopting Kim's method (see Kim et al., *J. Org. Chem.* **1991**, *56*, 2642). The key intermediates furanoid glycals **27** can be prepared from 2'-deoxy nucleosides **25** utilizing Horwitz method (see Zemlicka et al., *J. Am. Chem. Soc.* **1972**, *94*, 3213-8). From the glycals **27**, the (dimethylphosphono)methoxy functionality can be introduced either through phenylselenyl chloride addition followed by substitution with dimethyl (hydroxymethyl)phosphonate in the presence of silver perchlorate, or directly with the aid of N-(phenylseleno)phthalimide or iodine bromide. Elimination of phenylselenyl or iodo groups results in the formation of the double bond products **29**, which give rise to ribonucleosides **30** upon oxidation. The ribonucleosides **30** can be converted to mesylates **33** by adopting Robins' procedure (see Hansske et al., *J. Am. Chem. Soc.* **1983**, *105*, 6736) followed by mesylation, a similar synthesis as described in Scheme 2. Substitution with azide followed by deprotection converts **33** to 3'-azido-2',3'-dideoxy purine nucleoside phosphonates **II**, as depicted in Scheme 7.



Scheme 7. Synthesis of 3'-azido-2',3'-dideoxy purine nucleoside phosphonates **II**. X = O or S; B = protected or unprotected purine base; Reagents and conditions: (a) Pt, pH 9 or CrO_3, Py or $KMnO_4$; (b) DMF, dimethylformamide dineopentyl acetal, 80-90 °C; (c) i) PhSeCl, -70 °C; ii) $AgClO_4$, $(MeO)_2P(=O)CH_2OH$; or N-(phenylseleno)phthalimide, $(MeO)_2P(=O)CH_2OH$; (d) $NaIO_4$; (e) OsO_4; (f) i) Bu_2SnO, MeOH; ii) TsCl, Et_3N; (g) $LiEt_3BH$, THF, DMSO; (h) MsCl, Py; (i) LiN_3, DMF; (j) TMSBr.

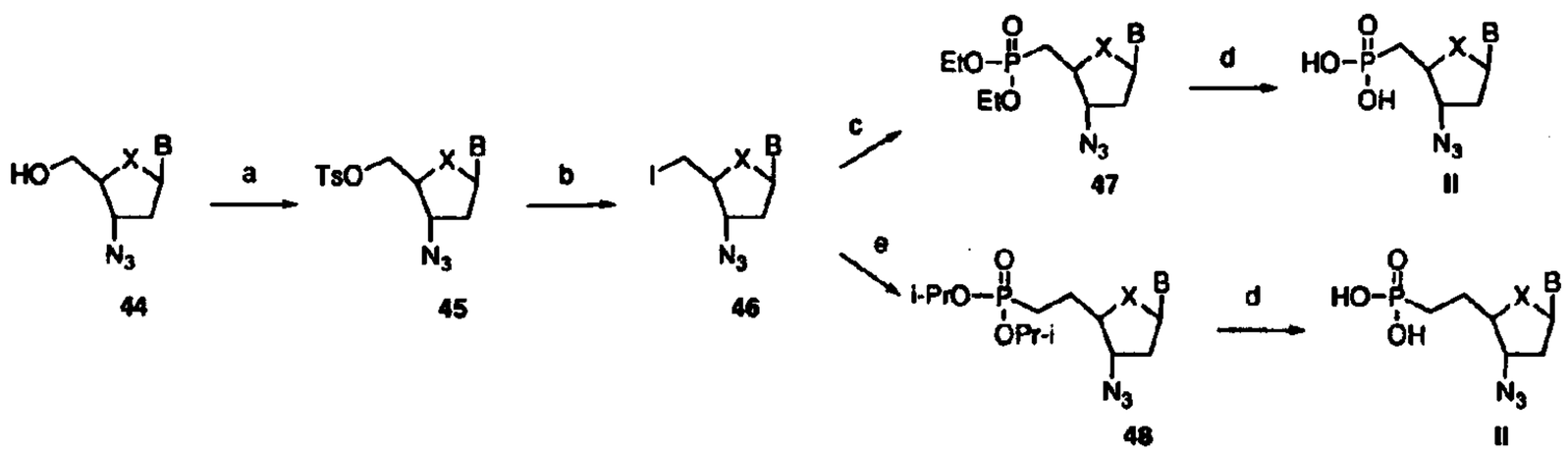
Because of the stability of 4'-hydroxy carbocyclic nucleosides, the carbocyclic nucleosides **36** can be prepared directly from cyclopentenol ester **35** via Trost reaction. Protection and oxidation of **36** gives rise to carbocyclic nucleosides **37**, which can be converted to mesylates **40** in a similar fashion as described in Scheme 2. Substitution of mesylates **40** with azide followed by deprotection gives rise to 3'-azido compounds **42**, which can be condensed with $(EtO)(OH)P(=O)CH_2OTs$ to result in the phosphonate esters **43**. Through a deprotection reaction, the carbocyclic 3'-azidonucleoside phosphonates **II** (R^3 and R^4 = H, X = CH_2) can be obtained (Scheme 8).



Scheme 8. Synthesis of carbocyclic 3'-azido-2',3'-dideoxy purine nucleoside phosphonates **II**. B = protected or unprotected purine base; Reagents and conditions: (a) $Pd(PPh_3)_4$, PPh_3, NaH, Base; (b) i) $CH_2(OMe)_2$,CH_2Cl_2, TfOH; ii) OsO_4; (c) i) Bu_2SnO, MeOH; ii) TsCl, Et_3N; (d)$LiEt_3BH$, THF, DMSO; (e) MsCl, Py; (f) LiN_3, DMF; (g) CF_3COOH, CH_2Cl_2; (h) (EtO)(HO)P(=O)CH_2OTs, NaH; (i) TMSBr.

5'-Deoxynucleoside phosphonates **II** (Z = CH_2) can be synthesized from 5'-iodo compounds **46**, which are prepared from nucleosides **44** via tosylation and iodination. Substitution of the iodo compounds **46** with triethyl phosphate, followed by deprotection, 3'-azido purine nucleoside phosphonates **II** are obtained (Scheme 9). This method has been used widely for synthesizing 5'-deoxynucleoside phosphonates (see Holy, et al., Tetrahedron Lett. 1967, 881-884).

The 5'-methylene phosphonates **II** (Z = CH_2CH_2) can also be synthesized from 5'-iodo compounds **46** by condensation with diisopropyl lithiomethane phosphonate, followed by deprotection, a method used by Wolff-Kugel and Halazy (see Wolff-Kungel, Halazy, *Tetrahedron Lett.* **1991**, *32*, 6341-4). These procedures are depicted in Scheme 9.



Scheme 9. Synthesis of 3'-azido-2',3'-dideoxy purine nucleoside phosphonates **II**. X = O, S, CH_2; B = protected or unprotected purine base; Reagents and conditions: (a) TsCl, Py; (b) NaI, EtCOMe; (c) $(EtO)_3P$; (d) TMSBr; (e) $LiCH_2P(=O)(iPrO)_2$.

Modified purines of the general formula (**IV**) can be prepared by multiple methods, including but not limited to: 1) *C*-heteroarylation of a sugar with a heteroaryl bromomagnesium salt (see Cornia, M. et al., *J. Org. Chem.* **1991**, *40*, 19-34); 2) Knoevenagel type condesation between a indole-2-thione (or purine-8-thione) with a ribofuranose derivative (see Chen, JJ et al., *Nucleosides Nucleotides & Nucleic Acids*, **2005**, *24*, 1417-37); or Friedel-Crafts type glycosylation of benzothiophene with 1-O-Me-deoxyribose promoted by $SnCl_4$/AgOTfa (see Hainke, S. et al., *Org. Biomol. Chem.* **2005**, *23*, 2233-8); and 4) a general method for aryl *C*-glycosylation involves the coupling of organocadmium compounds or Normantcuprates with protected ribofuranosyl chlorides (see Ren, RXF, et al., *J. Am. Chem. Soc.* **1996**, *118*, 7671-78).

In addition to the above described methods, other approaches, such as transglycosylation (see Robins et al., *J. Med. Chem.* **1989**, *32*, 1763-8; Freeman et al., *Bioorg. Med. Chem.* **1995**, *3*, 447-58), 3'-azido sugar-base condensation, (see Fleet et al., *Tetrahedron* **1988**, *44*, 625-36), and those described in a recent review article (see Pathak, *Chem. Rev.* **2002**, *102*, 1623-67), can be used as well to synthesize 3'-azido purine nucleosides and phosphonates.

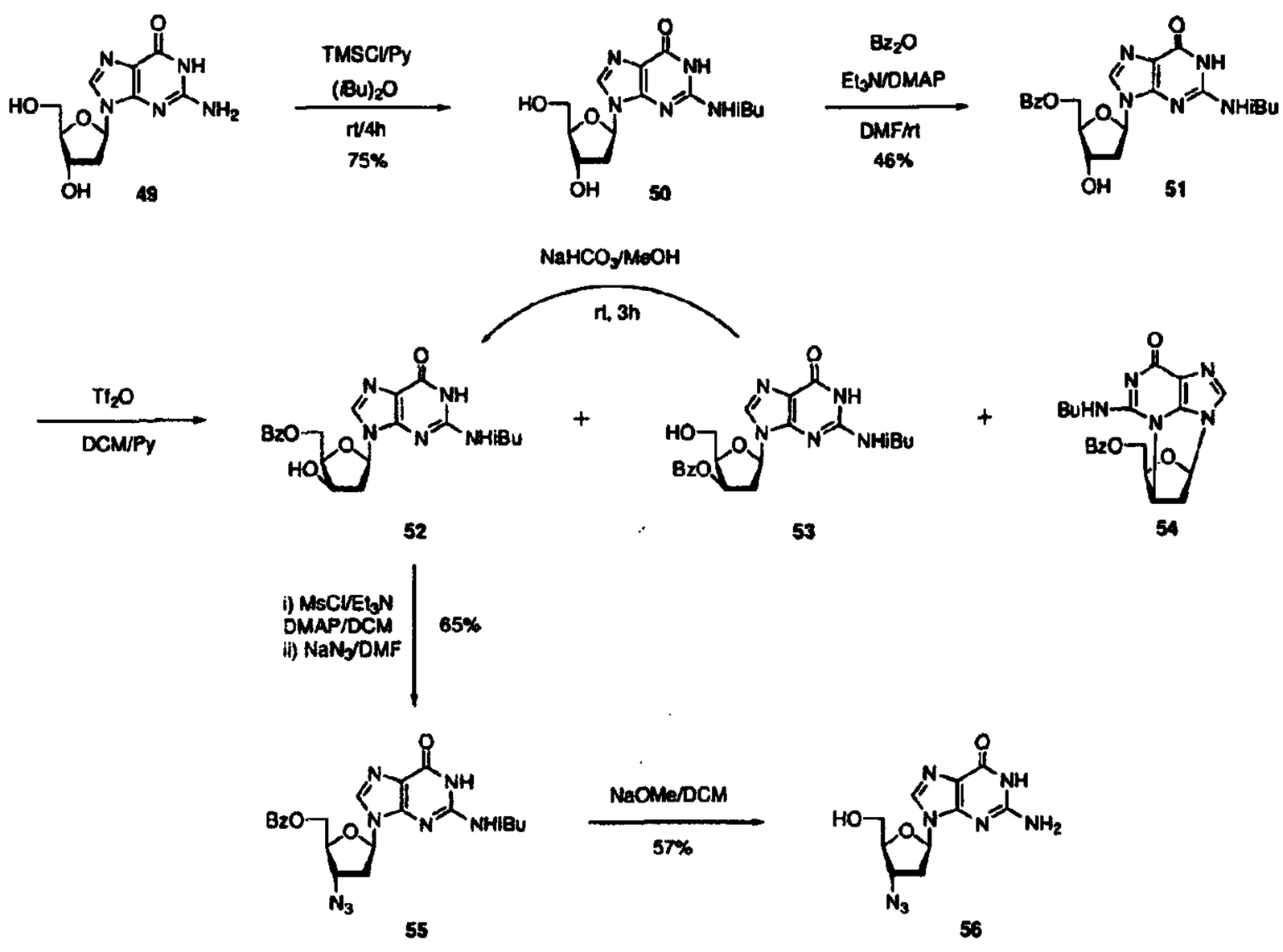
The present invention is further illustrated in the following examples. Schemes 10-11 and Examples 1-13 show preparative methods for synthesizing 3'-azido-purines, and Examples 14-26 show a biological evaluation of the 3'-azido purine nucleoside analogues. It will be understood by one of ordinary skill in the art that these examples are in no way

limiting and that variations of detail can be made without departing from the spirit and scope of the present invention.

Specific Examples

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

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



Scheme 10. Synthesis of 3'-azido-2',3'-dideoxyguanosine (**56**).

Example 1

N^2-Isobutyryl-2'-deoxyguanosine (50)

2'-Deoxyguanosine (**49**) (5 g, 18.72 mmol) was coevaporated with pyridine (100 mL) three times and suspended in dry pyridine (100 mL). Trimethylchlorosilane (11.88 mL, 93.63 mmol) was added, and the resulting solution was stirred at room temperature for 2 h. Isobutyric anhydride (15.54 mL, 93.65 mmol) was added, and the mixture was stirred at room temperature for 4 h under argon atmosphere. The reaction was cooled in an ice bath, and water (30 mL) was added. After 15 min, 29% aqueous ammonia (30 mL) was added, and the reaction was stirred for 15 min. The solution was then evaporated to near dryness, and the residue was dissolved in water (300 mL). The aqueous layer was washed with dichloromethane (150 mL) and crystallization occurred quickly in water. The compound was filtrated then dried overnight under vacuum to afford the title compound **50** (4.75 g, 75%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.01-1.10 (m, 6H, 2xCH_3), 2.20-2.26 (m, 1H, H-2'), 2.46-2.57 (m, 1H, H-), 2.71-2.76 (m, 1H, H-), 3.43-3.55 (m, 2H, H-5', H-5''), 3.77-3.81 (m, 1H, H-4'), 4.31-4.35 (m, 1H, H-), 4.93 (br s, OH), 5.29 (br s, OH), 6.17 (t, 1H, J = 6.0 Hz, H-1'), 8.20 (s, 1H, H-8), 10.97 (br s, 2x NH).

Example 2

5'-O-Benzoyl-N^2-isobutyryl-2'-deoxyguanosine (51)

To a solution of N^2-isobutyryl-2'-deoxyguanosine (**50**) (1 g, 2.96 mmol) in anhydrous DMF (44 mL) were added Et_3N (1.5 mL) and 4-dimethylaminopyridine (15 mg, 0.12 mmol). A solution of benzoic anhydride (740 mg, 3.27 mmol) in anhydrous DMF (10 mL) was added dropwise to this solution over a period of 2 h with stirring. The reaction was stirred overnight at room temperature. The solvent was evaporated and the mixture was purified by column chromatography on silica gel eluting with CH_2Cl_2-MeOH (9:1) to give the title compound **51** (0.6 g, 46%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.03-1.09 (m, 6H, 2xCH_3), 2.32-2.39 (m, 1H, H-2'), 2.47-2.73 (m, 2H, H-2'', isobutyryl CH), 4.08-4.12 (m, 1H, H-), 4.35-4.40 (m, 1H, H-5'), 4.44-4.48 (m, 1H, H-5''), 4.51-4.55 (m, 1H, H-), 5.52 (br s, 1H, 5'-OH),

6.22 (t, 1H, *J* = 6.4 Hz, H-1'), 7.47-7.51 (m, 2H benzoyl), 7.60-7.64 (m, 1H benzoyl), 7.86-7.91 (m, 2H benzoyl), 8.15 (s, 1H, H-8), 11.61 (br s, NH), 12.04 (br s, NH).

Example 3

N^2-*Isobutyryl-9-(5-O-benzoyl-2-deoxy-β-D-threo-pentofuranosyl)-guanine (52)*

To a suspension of **51** (5 g, 11.33 mmol) in anhydrous dichloromethane (200 mL) and anhydrous pyridine (30 mL) was added dropwise trifluoromethanesulfonic anhydride (5.8 mL, 33.99 mmol) at 0°C. After removal of the cooling bath, the reaction was stirred at room temperature for 30 min until the reaction mixture cleared up. Then water (20 mL) was added and the reaction was further stirred for 3h at room temperature. The organic layer was separated and evaporated. The residual oil was then purified by column chromatography on silica gel eluting with CH_2Cl_2-MeOH (95:5) yielding the title compound **52** (0.5 g, 10%), together with N^2-isobutyryl-9-(3-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)-guanine (**53**) (1.93 g, 39%) and N^2-isobutyryl-9-(5-*O*-benzoyl-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-($N^3 \rightarrow 3'$)-cycloguanine (**54**) (0.89 g, 18%).

Data for **52**: ^{1}H NMR (DMSO-d_6) β 1.06-1.08 (m, 6H, 2xCH_3), 2.27-2.31 (m, 1H, H-2'), 2.67-2.77 (m, 2H, H-2'', isobutyryl CH), 4.26-4.42 (m, 1H), 4.44-4.47 (m, 2H), 4.54-4.59 (m, 1H), 5.65 (d, 1H, *J* = 4.0 Hz, 3'-OH), 6.15 (d, 1H, *J* = 6.4 Hz, H-1'), 7.46-7.51 (m, 2H benzoyl), 7.59-7.62 (m, 1H benzoyl), 7.90-7.92 (m, 2H benzoyl), 8.20 (s, 1H, H-8), 11.68 (br s, NH), 12.04 (br s, NH).

Data for **53**: ^{1}H NMR (DMSO-d_6) δ 1.05-1.08 (m, 6H, 2xCH_3), 2.68-2.76 (m, 2H, H-2', isobutyryl CH), 2.91-2.99 (m, 1H, H-2''), 3.68-3.76 (m, 2H, H-5', H-5''), 4.25-4.29 (m, 1H, H-4'), 4.93 (t, 1H, *J* = 5.6 Hz, 5'-OH), 5.63-5.65 (m, 1H, H-3'), 5.18-5.23 (m, 1H, H-1'), 7.45-7.49 (m, 2H benzoyl), 7.61-7.65 (m, 1H benzoyl), 7.79-7.82 (m, 2H benzoyl), 8.11 (s, 1H, H-8), 11.68 (br s, NH), 11.99 (br s, NH).

Data for **54**: ^{1}H NMR (DMSO-d_6) δ 0.94–0.96 (m, 3H, CH_3), 1.00-1.02 (m, 3H, CH_3), 2.26-2.34 (m, 1H), 2.55-2.58 (m, 1H), 2.73-2.78 (m, 1H), 4.20 (dd, 1H, *J* = 4.5 Hz, *J* = 9.0 Hz, H-5'), 4.41 (dd, 1H, *J* = 4.5 Hz, *J* = 9.0 Hz, H-5''), 4.73-4.78 (m, 1H, H-4'), 5.61-5.64 (m, 1H, H3'), 6.44 (d, 1H, *J* = 3.0 Hz, H-1'), 7.40-7.44 (m, 2H benzoyl), 7.58-7.62 (m, 1H benzoyl), 7.69-7.72 (m, 2H benzoyl), 8.00 (s, 1H, H-8), 12.69 (br s, NH).

Example 4

Partial isomerisation of 53 to 52

A solution of **53** (3.05 g, 6.91 mmol) and $NaHCO_3$ (488 mg, 5.8 mmol) in MeOH (30 mL) was stirred at room temperature for 3 h. After evaporation of solvent, the residue was purified by chromatography on silica gel eluting with CH_2Cl_2-MeOH (95:5) to give **52** (1.3 g, 43%) and **53** (1.7 g, 56%).

Example 5

N^2-*Isobutyryl-9-(3-Azido-5-O-benzoyl-2,3-dideoxy-β-D-threo-pentofuranosyl)-guanine (55)*

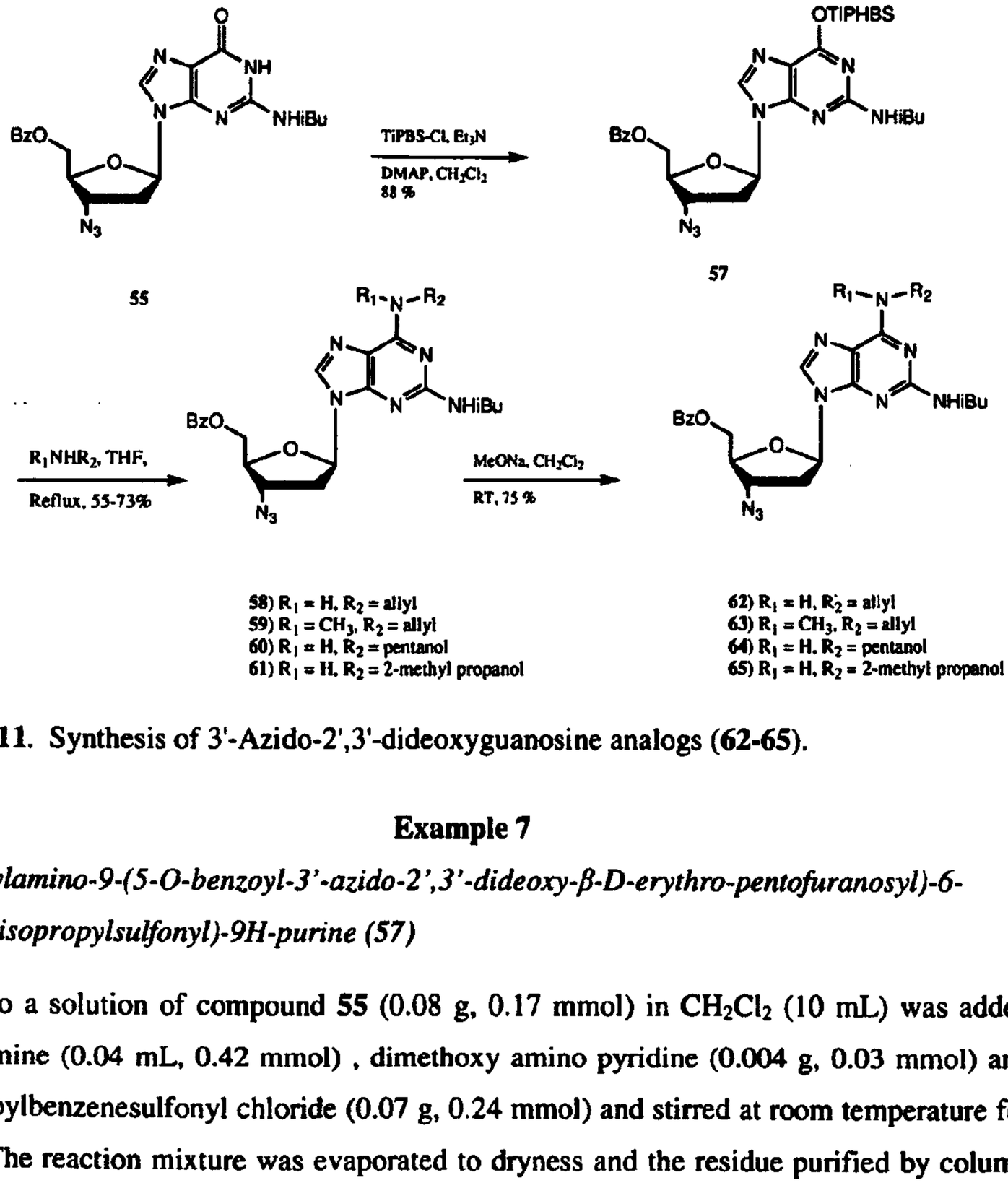
To a mixture of **52** (290 mg 0.65 mmol) in dichloromethane (30 mL) were added 4-dimethylaminopyridine (12 mg, 0.065 mmol) and Et_3N (0.45 mL), followed by methanesulfonylchloride dropwise (0.121 mL, 1.30 mmol) at 0 °C. The resulting mixture was stirred at 0°C for 40 min under argon then hydrolyzed with water (20 mL). The organic layer was separated and evaporated. The residual oil was diluted in anhydrous DMF (20 mL). To the solution was added sodium azide (410 mg, 6.5 mmol) and the mixture was heated at 120 °C for 2 h under argon. The reaction was cooled to room temperature, diluted with AcOEt and washed with water. The organic layer was evaporated and the residue was then purified by column chromatography on silica gel column eluting with CH_2Cl_2-MeOH (9:1) to give **55** (200 mg, 65%) as a white solid. IR 2104 cm^{-1} (N_3); ^1H NMR (DMSO-d_6) δ 1.08–1.12 (m, 6H, 2xCH_3), 2.50-2.79 (m, 2H, H-2', isobutyryl CH), 2.91-3.01 (m, 1H, H-2'), 4.18-4.23 (m, 1H, H-4'), 4.42-4.56 (m, 2H, H-5', H-5''), 4.83-4.89 (m, 1H, H-3'), 6.21 (t, 1H, J = 5.4 Hz, H-1'), 7.45-7.50 (m, 2H benzoyl), 7.62-7.66 (m, 1H benzoyl), 7.85-7.88 (m, 2H benzoyl), 8.20 (s, 1H, H-8), 11.53 (br s, NH), 11.91 (br s, NH).

Example 6

3'-Azido-2',3'-dideoxyguanosine (56) (also referred to as 3'-azido-ddG)

To a solution of **55** (1.4 g, 3.00 mmol) in CH_2Cl_2 (180 mL) was added NaOMe (0.5 M solution in MeOH, 12 mL). The reaction solution was stirred at 45 °C for 4 h and then evaporated to dryness. The residue was purified by column chromatography on silica gel column eluting with AcOEt/MeOH/H_2O (75:20:5) to give the title compound **56** (500 mg,

57%) as a white solid. IR 2104 cm^{-1} (N_3); ^1H NMR (DMSO-d_6) δ 2.35-2.50 (m, 1H, H-2'), 2.71-2.78 (m, 1H, H-2''), 3.51-3.57 (m, 2H, H-5', H-5''), 3.83-3.86 (m, 1H, H-4'), 4.51-4.58 (m, 1H, H-3'), 5.08-5.14 (m, 1H, 5'-OH), 6.05 (t, 1H, J = 6.3 Hz, H-1'), 6.53 (br s, 2H, NH_2), 7.91 (s, 1H, H-8), 10.68 (br s, 1H, NH).



58) R_1 = H, R_2 = allyl
59) R_1 = CH_3, R_2 = allyl
60) R_1 = H, R_2 = pentanol
61) R_1 = H, R_2 = 2-methyl propanol

62) R_1 = H, R_2 = allyl
63) R_1 = CH_3, R_2 = allyl
64) R_1 = H, R_2 = pentanol
65) R_1 = H, R_2 = 2-methyl propanol

Scheme 11. Synthesis of 3'-Azido-2',3'-dideoxyguanosine analogs (**62-65**).

Example 7

2-Isobutylamino-9-(5-O-benzoyl-3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-(2,4,6-triisopropylsulfonyl)-9H-purine (57)

To a solution of compound **55** (0.08 g, 0.17 mmol) in CH_2Cl_2 (10 mL) was added triethylamine (0.04 mL, 0.42 mmol) , dimethoxy amino pyridine (0.004 g, 0.03 mmol) and trisiopropylbenzenesulfonyl chloride (0.07 g, 0.24 mmol) and stirred at room temperature for 6-10 h. The reaction mixture was evaporated to dryness and the residue purified by column chromatography EtOAc: Hexane (3:2) to afford **57** (0.08 g, 88%) as a pale yellow solid. ^1H NMR (DMSO-d_6): δ 0.90-0.96 (m, 6H, 2 x CH_3),1.06-1.18 (m, 18H, isopropyl), 2.56-2.59 (m, 1H, H-2'a), 2.69-2.74 (m, 1H, H-2'b), 2.90-2.95 (m, 1H), 3.01-3.06 (m, 1H, CH-isopropyl), 4.01-4.11 (m, 3H, H-4',CH-isopropyl), 4.40-4.50 (m, 2H, H-5'b, H-5'a), 5.62-5.5(m, 1H, H-3'), 6.29-6.30 (m, 1H, H-1'), 7.33-7.39 (m, 4H, Ar), 7.53-7.57 (m, 1H, Ar),

7.73-7.74 (m, 2H, Ar), 8.49 (s, 1H, H-8). LCMS Calcd for $C_{36}H_{44}N_8O_7S$ 732.3, Observed (M+1) 733.4.

Example 8

2-Isobutylamino-9-(5-O-benzoyl-3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-allylamino-9H-purine (58)

To a solution of compound **57** (0.07 g, 0.09 mmol) in THF (10 mL) was added allylamine (0.03 g, 0.47 mmol) and refluxed at 55° C for 15 h. The reaction mixture was evaporated to dryness and the residue purified by column chromatography CH_2Cl_2: MeOH (9:1) to afford **58** (0.04 g, 83 %) as a syrup. ^{1}H NMR ($CDCl_3$): δ 1.18-1.20 (s, 6H, 2 x CH_3), 2.43-2.50 (m, 1H, H-2'a), 3.01-3.03 (m, 1H, H-2'b), 4.09-4.18 (m, 2H, H-5'a), 4.25-4.28(m, 1H, H-5'b), 4.44-4.53 (m, 2H, H-4',CH_2 allyl), 5.10-5.25 (m, 3H, H-3', allyl), 5.90-5.96 (m, 1H, CH allyl), 6.10-6.13 (m, 1H, H-1'), 7.32-7.35 (m, 2H, Ar), 7.46-7.48 (m, 1H, Ar), 7.55 (s, 1H, H-8), 7.89-7.91 (m, 2H, Ar). LCMS Calcd for $C_{24}H_{27}N_9O_4$ 505.2, Observed (M+1) 506.3.

Example 9

2-Amino-9-(3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-allylamino-9H-purine (62)

To a solution of Compound **58** (0.04 g, 0.07 mmol) in CH_2Cl_2 (10 mL) was added NaOMe (0.03 mL) of 0.5 M solution in MeOH. The reaction mixture was stirred at room temperature for 24 h, evaporated to dryness and purified by column chromatography on silica gel CH_2Cl_2: MeOH (9:1) to afford **62** (0.019 g, 73 %) as a white solid.^{1}H NMR ($CDCl_3$): δ 2.26-2.31 (dd, 1H, J = 5.6 Hz, 13.6 Hz, H-2'a), 3.09-3.12 (m, 1H, H-2'b), 3.69-3.73 (d, 1H, J = 12.8 Hz, H-5'a), 3.97-4.01 (d, 1H, J = 12.8 Hz, H-5'b), 4.19 (m, 3H, H-4', CH_2 allyl), 4.53-4.55 (d, 1H, J = 6.0 Hz, H-3'), 4.83 (brs, 2H, NH_2), 5.14-5.16 (d, 1H, J = 8.0 Hz, allyl), 5.23-5.27 (d, 1H, J = 16.0 Hz, allyl), 5.88-5.92 (m, 2H, CH allyl, NH), 6.04-6.08 (m, 1H, H-1'), 7.46 (s, 1H, H-8). LCMS Calcd for $C_{13}H_{19}N_9O_2$ 331.1, Observed (M+1) 332.1.

Example 10

2-Isobutylamino-9-(5-O-benzoyl-3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-N-methylallylamino-9H-purine (59)

To a solution of compound **57** (0.07 g, 0.09 mmol) in THF (10 mL) was added *N*-methylallylamine (0.04 mL, 0.63 mmol) and refluxed at 55 °C for 15 h. The reaction mixture was evaporated to dryness and the residue purified by column chromatography CH_2Cl_2: MeOH (9:1) to afford **59** (0.035 g, 73 %) as a syrup. ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 6H, 2 x CH_3), 2.47-2.54 (m, 1H, H-2'a), 3.10-3.17 (m, 1H, H-2'b), 4.19-4.24 (m, 1H, H-5'a), 4.49-4.54 (m, 2H, H-5'b, H-4'), 4.68-4.72 (m, 2H, CH_2 allyl), 5.13-5.18 (m, 3H, H-3', CH_2allyl), 5.89-5.95(m, 1H, CHallyl), 6.12-6.15 (m, 1H, H-1'), 7.35-7.37 (m, 2H, Ar), 7.48-7.50 (m, 1H, Ar), 7.66 (s, 1H, H-8), 7.91-7.93 (m, 2H, Ar). LCMS Calcd for $C_{25}H_{29}N_9O_4$ 519.2, Observed (M+1) 520.3.

Example 11

2-Amino-9-(3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-N-methylallylamino-9H-purine (63)

To a solution of Compound **59** (0.03 g, 0.05 mmol) in CH_2Cl_2 (10 mL) was added NaOMe (0.015 mL) of 0.5 M solution in MeOH. The reaction mixture was stirred at room temperature for 24 h, evaporated to dryness and purified by column chromatography on silica gel CH_2Cl_2:MeOH (9:1) to afford **63** (0.015 g, 75 %) as a white solid. ^{1}H NMR ($CDCl_3$): δ 2.26-2.31 (m, 1H, H-2'a), 2.45 (s, 3H, CH_3), 3.10-3.14 (m, 1H, H-2'b), 3.71-3.80 (m, 1H, H-5'a), 3.97-4.01 (d, 1H, J = 13 Hz, H-5'b), 4.18 (m, 1H, H-4'), 4.65 (m, 1H, H-3'), 4.85(brs, 2H, NH_2), 5.14-5.16 (m, 2H, CH_2allyl), 5.88-5.92 (m, 1H, CH-allyl), 6.04-6.08 (m, 1H, H-1'), 7.62 (s, 1H, H-8). LCMS Calcd for $C_{14}H_{19}N_9O_2$ 345.3, Observed (M+1) 346.2.

Example 12

2-Isobutylamino-9-(5-O-benzoyl-3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-aminopentanol-9H-purine (60)

To a solution of compound **57** (0.08 g, 0.1 mmol) in THF (10 mL) was added aminopentanol (0.05 g, 0.54 mmol) and refluxed at 55° C for 15 h. The reaction mixture was evaporated to dryness and the residue purified by column chromatography CH_2Cl_2:MeOH (9:1) to afford **60** (0.03 g, 56 %) as a syrup. ^{1}H NMR ($CDCl_3$): δ 1.19-1.21 (s, 6H, 2 x CH_3),

1.43-1.492 (m, 2H, alkyl), 1.56-1.67 (m, 8H, alkyl), 2.49-2.56 (m, 2H, H-2'a, $CH(CH_3)_2$), 3.13-3.19 (m, 1H, H-2'b), 3.45-3.62 (m, 4H, H-5'a, H-5'b, CH_2OH), 4.20-4.25 (m, 1H, H-4'), 4.50-4.55 (m, 1H, H-3'), 6.12-6.15 (m, 1H, H-1'), 6.20 (s, 1H, NH), 7.36-7.38 (m, 2H, Ar), 7.49-7.53 (m, 1H, Ar), 7.68 (s, 1H, H-8), 7.91-7.94 (m, 2H, Ar). LCMS Calcd for C26H33N9O5 551.2, Observed (M+1) 552.3.

2-Amino-9-(3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-aminopentanol-9H-purine (64)

To a solution of Compound **57** (0.04 g, 0.07 mmol) in CH_2Cl_2 (10 mL) was added NaOMe (0.03 mL) of 0.5 M solution in MeOH. The reaction mixture was stirred at room temperature for 24 h, evaporated to dryness and purified by column chromatography on silica gel CH_2Cl_2:MeOH (9:1) to afford **64** (0.019 g, 73 %) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 6H, $2XCH_3$), 2.29-2.33 (dd, 1H, J = 4.4 Hz, 12.4 Hz, H-2'a), 3.03-3.08 (m, 1H, H-2'b), 3.62-3.72 (m, 4H, H-5'a, CH_2OH), 3.96-3.99 (d, 1H, J = 13.2 Hz, H-5'b), 4.18 (s, 1H, H-4'), 4.53-4.54 (d, 1H, J = 6.4 Hz, H-3'), 4.87 (brs, 2H, NH_2), 6.03-6.07 (m, 1H, H-1'), 6.27 (brs, 1H, NH), 7.46 (s, 1H, H-8). LCMS Calcd for $C_{15}H_{25}N_9O_3$ 377.4; Observed (M+1) 378.2.

Example 13

2-Amino-9-(3'-azido-2',3'-dideoxy-β-D-erythro-pentofuranosyl)-6-N-2-methyl-2-amino-propanol-9H-purine (65)

To a solution of compound **57** (0.03 g, 0.04 mmol) in THF (10 mL) was added 2-methyl-2-aminopropanol (0.01 mL, 0.13 mmol) and refluxed at 55 °C for 4 h. The reaction mixture was evaporated to dryness and used for the next reaction without purification. To the residue in CH_2Cl_2 (10 mL) was added NaOMe (0.02 mL) of 0.5 M solution in MeOH. And stirred at room temperature for 24 h, evaporated to dryness and purified by column chromatography on silica gel CH_2Cl_2:MeOH (9:1) to afford **65** (0.015 g, 75 %) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.25 (m, 8H, alkyl), 2.25-2.30 (dd, 1H, J = 5.2 Hz, 14.8 Hz, H-2'a), 3.07-3.14 (m, 1H, H-2'b), 3.49 (brs, 2H, 2 X OH), 3.59-3.62 (m, 2H,CH_2OH), 3.68-3.72 (d, 1H, J = 13.2 Hz, H-5'a), 3.96-3.99 (d, 1H, J = 12.8 Hz, H-5'b), 4.17 (m, 1H, H-4'), 4.52-4.53 (d, 1H, J = 5.6 Hz, H-3'), 4.93 (brs, 2H, NH_2), 6.02-6.06 (m, 1H, H-1'6.27 (brs, 1H, NH), 7.45 (s, 1H, H-8). LCMS Calcd for $C_{14}H_{21}N_9O_3$ 363.3, Observed (M+1) 364.2.

Example 14

Anti-HIV (in PBM cells) Assay

Anti-HIV-1 activity of the compounds was determined in human peripheral blood mononuclear (PBM) cells as described previously (see Schinazi R.F., McMillan A., Cannon D., Mathis R., Lloyd R.M. Jr., Peck A., Sommadossi J.-P., St. Clair M., Wilson J., Furman P.A., Painter G., Choi W.-B., Liotta D.C. Antimicrob. Agents Chemother. **1992**, 36, 2423; Schinazi R.F., Sommadossi J.-P., Saalmann V., Cannon D., Xie M.-Y., Hart G., Smith G., Hahn E. Antimicrob. Agents Chemother. **1990**, 34, 1061). Stock solutions (20-40 mM) of the compounds were prepared in sterile DMSO and then diluted to the desired concentration in growth medium. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantified on day 6 after infection by a reverse transcriptase assay using $(rA)_n \cdot (dT)_{12-18}$ as template-primer. The DMSO present in the diluted solution (< 0.1%) had no effect on the virus yield. AZT was included as positive control. The antiviral EC_{50} and EC_{90} were obtained from the concentration-response curve using the median effective method described previously (see Chou T.-C. & Talalay P. *Adv. Enzyme Regul.* **1984**, *22*, 27-55; Belen'kii M.S. & Schinazi R.F. *Antiviral Res.* **1994**, *25*, 1-11).

Example 15

Assess Incorporation of Novel APN-TPs by HIV-1 RT

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

ii) Pre-steady-state Kinetic Analyses: A [γ32P]-ATP 5'-end labeled 20 nucleotide DNA primer (5'-TCGGGCGCCACTGCTAGAGA-3') annealed to a 57 nucleotide DNA template (5'-CTCAGACCCTTTTAGTCAGAATGGAAANTCTCTAGCAGTGGCGCCCG AACAGGGACA-3') was used in all experiments. The DNA templates contained either a T or C at position 30 (N), which allowed evaluation of the kinetics of single nucleotide incorporation using the same 20 nucleotide primer. Rapid quench experiments were carried out using a Kintek RQF-3 instrument (Kintek Corporation, Clarence, PA). In all experiments, 300 nM RT and 60nM DNA template/primer (T/P) were pre-incubated in reaction buffer (50mM Tris-HCl pH 7.5, 50 mM KCl) prior to mixing with an equivalent volume of nucleotide in the same reaction buffer containing 20mM $MgCl_2$. Reactions were terminated at times ranging from 10 ms to 30 min by quenching with 0.5M EDTA, pH 8.0. The quenched samples were mixed with an equal volume of gel loading buffer (98% deionized formamide, 10 mM EDTA and 1mg/mL each of bromophenol blue and xylene cyanol), denatured at 85°C for 5min, and the products were separated from the substrates on a 7M urea-16%

polyacrylamide gel. Product formation was analyzed using a Bio-Rad GS525 Molecular Imager (Bio-Rad Laboratories, Inc., Hercules, CA).

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

Example 16

Assess Anti-HIV Activity and Cellular Toxicity of Novel APNs

i) Viruses: Stock virus was prepared using the xxHIV-1LAI clone75 by electroporating (Gene Pulser; Bio-Rad) 5 to 10 μg of plasmid DNA into 1.3 x 10^7 MT-2 cells. At 7 days post-transfection, cell-free supernatant was harvested and stored at –80°C. The genotype of stock viruses was confirmed by extraction of RNA from virions, treatment of the extract with DNase I, amplification of the full-length coding region (amino acids 1 to 560) of RT by RT-PCR, purification of the PCR product, and sequence determination of the PCR product using a Big Dye terminator kit (v. 3.1) on an ABI 3100 automated DNA sequencer (Applied Biosystems, Foster City, Calif.). The 50% tissue culture infective dose ($TCID_{50}$) for the virus stock was determined for MT-2 cells, P4/R5 cells or PBM cells by three-fold endpoint dilution assays (six wells per dilution) and calculated using the Reed and Muench equation (see Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 1938; 27:493-497).

ii) Single-Replication-Cycle Drug Susceptibility Assay: In a 96-well plate, two- or three-fold serial dilutions of an inhibitor were added to P4/R5 cells in triplicate. Cells were infected with the amount of virus that yielded a relative light unit value of 100 in the no-drug, virus-infected control wells. At 48 h post-infection, a cell lysis buffer and luminescent substrate (Gal-Screen; Tropix/Applied Biosystems) was added to each well, and relative light

unit values were determined using a luminometer (ThermoLabSystems, Waltham, Mass.). Inhibition of virus replication was calculated as the concentration of compound required to inhibit virus replication by 50% (EC_{50}).

iii) Multiple-Replication-Cycle Drug Susceptibility Assay: In a 96-well plate, three-fold serial dilutions of an inhibitor were added to MT-2 cells in triplicate. The cells were 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 was determined using a commercial enzyme-linked immunosorbent assay (DuPont, NEN Products, Wilmington, Del.). EC_{50} values were calculated as described above.

iv) Drug Susceptibility Assays in PBM Cells: PBM cells were isolated by Ficoll–Hypaque discontinuous gradient centrifugation from healthy seronegative donors, as described previously (see Schinazi RF, Cannon DL, Arnold BH, Martino-Saltzman D. Combinations of isoprinosine and 3'-azido-3'-deoxythymidine in lymphocytes infected with human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 1988; 32:1784-1787; Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie MY, Hart GC, Smith GA. Hahn E.F. Activities of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 1990; 34:1061-1067). Cells were stimulated with phytohemagglutinin A (PHA, Difco, Sparks, MD) for 2–3 days prior to use. Infections were done in bulk for 1 h, either with 100 TCID50/1 x 10^7 cells for a flask (T25) assay or with 200 TCID50/6 x 10^7 cells/well for the 24-well plate assay. Cells were added to a plate or a flask containing a 10-fold serial dilution of the test compound. At 5 days post-infection, culture supernatants were harvested and treated with 0.5% Triton X-100. The p24 antigen concentration in the supernatants was determined as described above. EC_{50} and fold-resistance values were calculated as described above.

v) Cellular Toxicity Assays: All APNs were 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 were seeded at 5 x 10^3 to 5 x 10^4 cells/well in 96-well cell culture plates containing 10-fold serial dilutions of the test drug. The cultures were incubated for 2–4 days, after which 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye solution (Promega, Madison, WI) were added to each well and incubated overnight. The reaction was stopped with stop solubilization solution

(Promega, Madison, WI) and plates were read at a wavelength of 570 nm. The median 50% cytotoxic concentration (CC_{50}) was determined from the concentration–response curve using the median effect method.

Example 17

Assess Activity of APNs against Drug-Resistant HIV

Analogs identified above as having improved activity compared with the parent analog, and less cellular toxicity, were further evaluated for activity against a panel of drug resistant viruses. This allowed elucidation of cross-resistance profiles of the novel analogs and comparison to resistance determined for 3'-azido-ddA and 3'-azido-ddG. The drug resistant viruses used in this study included 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-$1_{69Insertion}$. The genotypes of these viruses are described above and provided in Figure 1. All of these mutant viruses were generated in our HIV-1xxLAI clone.

Example 18

Assess Activity of APNs against Drug-Resistant HIV

i) Viruses and Drug Susceptibility Assays: Virus stocks were prepared as described above. Drug susceptibility assays were performed using the single- and multiple-replication-cycle assays also described above. Inhibition of virus replication was calculated as the concentration of compound required to inhibit virus replication by 50% (EC_{50}). Fold resistance values were 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 were log10 transformed and compared using a two-sample Student's *t* test with Sigma Stat software (Jandel Scientific). *P* values less than 0.05 were considered to be statistically significant.

To further characterize the activity of these nucleosides, 3'-azido-ddA and 3'-azido-2',3'-ddG were evaluated against a panel of mutant viruses. This panel included recombinant viruses with K65R, L74V (HIV-1_{L74V}), M184V (HIV-1_{M184V}), different combinations of TAMS (e.g. M41L/L210W/T215Y (HIV-1_{AZT3}), M41L/D67N/K70R/T215F/K219Q (HIV-1_{AZT7}) or M41L/D67N/K70R/L210W/T215Y/K219Q (HIV-1_{AZT9}), and multi-NRTI

resistance complexes (e.g. A62V/V75I/F77L/ F116Y/Q151M (HIV-1_{Q151M}) or M41L/69SS/ L210W/T215Y (HIV-$1_{69insertion}$)). The results, presented in Figure 13, show that both 3'-azido-ddA and 3'-azido-ddG are active against viruses with the K65R, L74V or M184V mutation. Both compounds, in comparison with AZT, were also remarkably active against all TAM-containing viruses. For example, HIV-1AZT7 was > 500-fold resistance to AZT, however less than 3.5-fold resistance was noted for this virus for 3'-azido-ddA and 3'-azido-ddG. Both 3'-azido-ddA and 3'-azido-ddG, however, were less active against HIV-1_{Q151M} and 3'-azido-ddG also lost activity against HIV-$1_{69insertion}$.

Example 19

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

i) Enzymes: The following mutant HIV-1 RT enzymes were used in this study: K65R RT, K70E RT, L74V RT, M184V RT, AZT2 RT, AZT3 RT, Q151M RT and 69Insert RT. The genotypes of AZT2, AZT3, Q151M and 69Insert RT are identical to those described in Figure 12. *E. coli* protein expression vectors for each of these mutant RTs were developed, and protein expression and purification were performed as described previously. Protein concentration and active site concentration was determined as described above.

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

iii) Excision Assays: The ATP-mediated phosphorolytic excision of the novel analogs from chain-terminated template/primer was carried out using WT RT, AZT2 RT, AZT3 RT and 69Insert RT. The 20 nucleotide DNA primer described above was 5'-end labeled with [γ32P]-ATP and then annealed to the appropriate 57 nucleotide DNA template. The 3'-end of the primer was chain-terminated by incubation with WT RT and 100μM of the appropriate modified nucleotide analog for 30 min at 37°C. The ^{32}P-labeled, chain-terminated 21 nucleotide primer was further purified by extraction of the appropriate band after 7M urea-16% acrylamide denaturing gel electrophoresis. The purified chain-terminated primer was then re-annealed to the appropriate DNA template for use in phosphorolysis experiments. The phosphorolytic removal of APN-MP was 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 was initiated by the addition of 3.0 mM ATP and 10 mM $MgCl_2$. Inorganic pyrophosphatase (0.01 U) was present throughout the reaction. After defined incubation periods, aliquots were 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 were separated by denaturing gel electrophoresis, and the disappearance of substrate coincident with formation of product was analyzed using a Bio-Rad GS525 Molecular Imager. Data were fit to the following single exponential equation to determine the apparent rate (kATP) of ATP-mediated excision: [product] = A[exp(-kATPt)], where A represents the amplitude for product formation. Dead-end complex formation was 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 20

Mitochondrial Toxicity Assays in HepG2 Cells:

i) Effect of APNs on Cell Growth and Lactic Acid Production: The effect of the APNs on the growth of HepG2 cells was determined by incubating cells in the presence of 0 μM, 0.1 μM, 1 μM, 10 μM and 100 μM drug. Cells (5 x 10^4 per well) were 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 was determined using a hemocytometer. To measure the effects of the nucleoside analogs on lactic acid production, HepG2 cells from a stock culture were diluted and plated in 12-well culture plates at 2.5 x 10^4 cells per well. Various concentrations (0 μM, 0.1 μM, 1 μM, 10 μM and 100 μM) of nucleoside analog were added, and the cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere for 4 days. At day 4 the number of cells in each well were determined and the culture medium collected. The culture medium was 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 APN analogs would indicate a drug-induced cytotoxic effect.

ii) Effect on APNs on Mitochondrial DNA Synthesis: a realtime 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 analogues. Antimicrob. Agents Chemother. 2002; 46: 3854-60). This assay was 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 were seeded at 5,000 cells/well in collagen-coated 96-well plates. APN analogs were added to the medium to obtain final concentrations of 0 μM, 0.1μM, 10 μM and 100 μM. On culture day 7, cellular nucleic acids were prepared by using commercially available columns (RNeasy 96 kit; Qiagen). These kits co-purify RNA and DNA, and hence, total nucleic acids were eluted from the columns. The mitochondrial cytochrome *c* oxidase subunit II (COXII) gene and the ß-actin or rRNA gene were amplified from 5 μl of the eluted nucleic acids using a multiplex Q-PCR protocol with suitable primers and probes for both target and reference amplifications. For COXII the following sense, probe and antisense primers are used, respectively: 5'-TGCCCGCCATCATCCTA-3', 5'-tetrachloro-6-carboxyfluorescein-TCCTCATCGCCCTCCCATCCC-TAMRA-3' and 5'-CGTCTGTTATGTAAAGGATGCGT-3'. For exon 3 of the ß-actin gene (GenBank accession number E01094) the sense, probe, and antsense 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 were obtained for all genes, the comparative *CT* method was used to investigate potential inhibition of mitochondrial DNA synthesis. The comparative *CT* method uses arithmetic formulas in which the amount of target (COXII gene) is normalized to the amount of an endogenous reference (the ß-actin or rRNA gene) and is relative to a calibrator (a control with no drug at day 7). The arithmetic formula for this approach is given by 2-ΔΔCT, where ΔΔ*CT* is (*CT* for average target test sample - *CT* for target control) - (*CT* for average reference test -*CT* for reference control) (see Johnson MR, K Wang, JB Smith, MJ Heslin,

RB Diasio. Quantitation of dihydropyrimidine dehydrogenase expression by real-time reverse transcription polymerase chain reaction. Anal. Biochem. 2000; 278:175-184). A decrease in mitochondrial DNA content in cells grown in the presence of drug would indicate 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 analogues on mitochondrial functions in HepG2 cells. Biochem. Pharmacol. 1996; 52:1577-1584; Lewis W, Levine ES, Griniuviene B, Tankersley KO, Colacino JM, Sommadossi JP, Watanabe KA, Perrino FW. Fialuridine and its metabolites inhibit DNA polymerase gamma at sites of multiple adjacent analog incorporation, decrease mtDNA abundance, and cause mitochondrial structural defects in cultured hepatoblasts. Proc Natl Acad Sci U S A. 1996; 93: 3592-7; Pan-Zhou XR, L Cui, XJ Zhou, JP Sommadossi, VM Darley-Usmar. Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells. *Antimicrob. Agents Chemother.* **2000**, *44*, 496-503). For example, electron micrographs of HepG2 cells incubated with 10 μM fialuridine (FIAU; 1,2'-deoxy-2'-fluoro-1-D-arabinofuranosly-5-iodo-uracil) showed the presence of enlarged mitochondria with morphological changes consistent with mitochondrial dysfunction. To determine if APNs promoted morphological changes in mitochondria, HepG2 cells (2.5 x 10^4 cells/mL) were seeded into tissue cultures dishes (35 by 10 mm) in the presence of 0 μM, 0.1 μM, 1 μM, 10 μM and 100 μM APN analog. At day 8, the cells were fixed, dehydrated, and embedded in Eponas described previously. Thin sections were prepared, stained with uranyl acetate and lead citrate, and then examined using transmission electron microscopy.

Example 21

Mitochondrial Toxicity Assays in Neuro2A Cells

To estimate the potential of APNs nucleoside analogs to cause neuronal toxicity, mouse Neuro2A cells (American Type Culture Collection 131) were 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}) were 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 were carried out as described above. In all experiments, ddC and AZT were used as control nucleoside analogs.

Example 22

Effect of 3'-Azido-2',3'-dideoxypurine 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 were 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 was 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 was carried out to determine the catalytic efficiency of incorporation (k/K) for DNA polymerase γ for APN-TP and natural dNTP substrates. This allowed determination of the relative ability of this enzyme to incorporate modified analogs and predict toxicity. Pre-steady-state kinetic analyses of incorporation of APN nucleotide analogs by DNA polymerase γ were 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 (250nM) and small (1.25mM) subunits of polymerase γ and 60nM DNA template/primer in 50mM Tris-HCl, 100mM NaCl, pH 7.8, was added to a solution containing $MgCl_2$ (2.5mM) and various concentrations of nucleotide analogs. Reactions were quenched and analyzed as described previously. Data were fit to the same equations as described above.

iii) Assay for *Human Polymerase γ 3' 5' Exonuclease Activity:* The human polymerase γ exonuclease activity was studied by measuring the rate of formation of the cleavage products in the absence of dNTP. The reaction was initiated by adding $MgCl_2$ (2.5mM) to a pre-incubated mixture of polymerase γ large subunit (40nM), small subunit (270nM), and 1,500nM chain-terminated template/primer in 50mM Tris-HCl, 100mM NaCl, pH 7.8, and quenched with 0.3M EDTA at the designated time points. All reaction mixtures were 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 were fitted by linear regression with Sigma Plot (Jandel Scientific). The slope of the line was 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 23

Assay for Bone Marrow Cytotoxicity

Primary human bone marrow mononuclear cells were obtained commercially from Cambrex Bioscience (Walkersville, MD). CFU-GM assays were 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 used 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 was used as a positive control. Cells were incubated in the presence of the compound for 14-18 days at 37°C with 5% CO_2, and colonies of greater than 50 cells are counted using an inverted microscope to determine IC_{50}. The 50% inhibitory concentration (IC_{50}) was obtained by least-squares linear regression analysis of the logarithm of drug concentration versus BFU-E survival fractions. Statistical analysis was performed with Student's *t* test for independent non-paired samples.

Example 24

Anti-HBV assay

The anti-HBV activity of the compounds was 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 were compared with that of the untreated controls. Control cultures with tetracycline [Tet (+)] were also maintained to determine the basal levels of HBV expression. 3TC was included as positive control.

Example 25

Cytotoxicity assay

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

Example 26

Adenosine Deaminase Assay

To determine the propensity for deamination of the APN nucleosides by adenosine deaminase, compounds were incubated with the commercially available purified enzyme, and the reaction was followed spectrophotmetrically. Reaction conditions were 50 mM potassium phosphate, pH 7.4, with 50 μM APN nucleoside in 0.5 mL at 25°C. Reaction time was 7 minutes with 0.002 units of enzyme and 120 minutes with 0.2 units of enzyme. (The unit definition of adenosine deaminase is one unit will deaminate 1.0 μmol of adenosine to inosine per minute at pH 7.5 at 25°C.) Deoxyadenosine was the positive control which was 59% deaminated under the given conditions in 7 minutes with 0.002 units of enzyme. Deoxyguanosine was the negative control. Optical density was measured at 265 nm or 285 nm. The difference in optical density between the beginning and the end of the experiment was divided by the extinction coefficient then multiplied by the volume of the reaction to determine the number of mols of substrate transformed into product. Mols of product were divided by mols of substrate equivalent to a 100% complete reaction then multiplied by 100 to obtain percent deamination. The limit of detection was 0.001 optical density units.

Example 27

Selection of Resistant Viruses to Compound 56

Peripheral blood mononuclear (PBM) cells[1] were seeded at 1 x 10^7 cells in a total of 5 mL of RPMI-1640 (Mediatech Inc., Herndon, VA) containing 100 mL heat inactivate 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 were treated with compound **56** at 0.1 µM for one hour prior to inoculation with HIV-1_{LAI}[2] at 100 x $TCID_{50}$. The treated PBM cell group and a control nontreated PBM cell group were allowed to infect for one hour. An additional 5 mL RTU medium was added to each flask and cells were incubated for 6 days at 37 °C.

On day 6, 1 mL of supernatant from each flask was removed and spun at 9,740 g at 4°C for 2 hr. This viral pellet was then resuspended in virus solubilization buffer for RT analysis. Total RNA was isolated from culture supernatants using the commercial QIAmp

---

[1] PBM cells were separated by ficoll-hypaque (Histopaque 1077: Sigma) density gradient centrifugation from Buffy coats obtained from the American Red Cross (Atlanta, GA). Buffy coats were derived from healthy seronegative donors. Cells were activated with 3 ug/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 ug/mL streptomycin, 1.6 mM L-glutamine (Mediatech Inc., Herndon, VA), for 2-3 days prior to use.

[2] HIV-1/LAI was obtained from the Center for Disease Control and Prevention was 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, was selected to begin the infected pool.

applied drug pressure on weeks where the virus appeared to be resistant.

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

V75I was selected as early as week 21 in the compound **56** treated viral pool. At approximately week 41, F77L and H221Y were also observed in the treated viral pool.

Example 28

Synthesis of Nucleoside analog triphosphates

Nucleoside analog triphosphates were synthesized from the corresponding 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 will be purified 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.

Summary of Conclusions

Figure 1 is a graphic representation of the genotypes of xxLAI viruses. All of the listed mutant viruses were generated in an HIV-1xxLAI clone.

Figures 2A-2B are graphic representations of the anti-HIV activity of 3'-azido-2',3'-ddA and 3'-azido-2',3'-ddG against a panel of drug-resistant HIV-1. The data show that both 3'-azido-ddA and 3'-azido-ddG are active against viruses with the K65R, L74V or M184V mutation. Both compounds, in comparison with AZT, were also active against all TAM-containing viruses.

nucleosides prepared to date have shown antiviral activity.

Figures 4A-4B are graphic representations of deamination by adenosine deaminase. Compounds that are substrates of adenosine deaminase *in vitro* can be converted to the 6-oxo nucleoside *in vivo*. For example, deoxyadenosine is converted to deoxyinosine *in vitro* and would be predicted to undergo conversion to deoxyinosine in vivo.

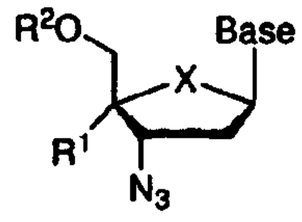
Figure 5 is a graphic representation of the development of compound **56** resistant virus as of week 47. V75I was selected as early as week 21 in the compound **56** treated viral pool. At approximately week 41, F77L and H221Y were also observed in the treated viral pool.

Figure 6 is a graphic representation summarizing compound **56** treatment of PBM cells inoculated with HIV-1_{LAI} and the resulting selected mutations. The compound **56** treated pool first resulted in V75V/I as early as week 21 and after increased selective pressure from compound **56**, F77L and H221Y were observed.

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

Claims:

1. A compound of Formula (I):



I

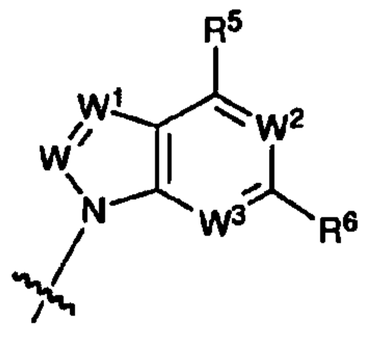
or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is O, CH_2, S, SO_2, NH, P=O(OH), C=CH_2, C=CHF, or C=CF_2;

R^1 is hydrogen, alkyl, haloalkyl (including CH_2F, CF_3), halo, azido, cyano, nitro, amino, alkylamino, dialkylamino, alkenyl, alkynyl, haloalkenyl (including Br-vinyl), alkoxy, alkenoxy, alkylthio, acyloxy, alkyloxyacyl, alkylcarbonyl, acylthio, or acylamino;

R^2 is H, phosphate (including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug), phosphothioate, carbonyl substituted with an alkyl (including C_1-C_6), alkenyl (including C_2-C_6), alkynyl (including C_2-C_6), aryl (including C_6-C_{10}), or other pharmaceutically acceptable leaving group, which, when administered *in vivo*, is capable of providing a compound wherein R^2 is H or phosphate, sulfonate ester (including alkyl or arylalkyl sulfonyl), benzyl (wherein the phenyl group is optionally substituted with one or more substituents as described in the definition of aryl given above), a lipid (including a phospholipid), an amino acid, a peptide, or cholesterol,

wherein the Base is purine or modified purine of the general formula (III):



III

wherein:

each W, W^1, W^2 and W^3 is independently N, CH, CF, CCl, CBr, CI, CCN, CCH_3, CCF_3, $CC(O)NH_2$, CC(O)NHR', $CC(O)N(R')_2$, CC(O)OH, CC(O)OR' or CR^5;

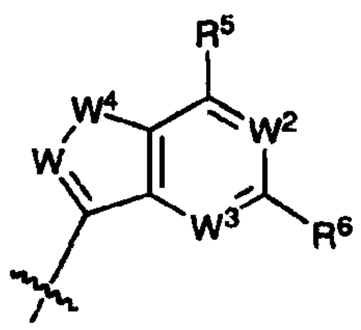
each R^5 and R^6 is chosen independently from H, halogen, CN, N_3, NO_2, OH, NH_2, SH, OR', NHR', $N(R')_2$, SR', OCOR', NHCOR', N(COR')COR', SCOR', OCOOR', NHCOR', CH_2OH, CH_2CN, CH_2N_3, COOH, COOR', $CONH_2$, CONHR, $CON(R')_2$, CH_2COOH, CH_2COOR', CH_2CONH_2, CH_2CONHR', $CH_2CON(R')_2$, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, C_{3-8} cycloalkyl, aryl, heteroaryl, acyl, arylalkyl, and alkylaryl;

with the proviso that if R^1 and R^2 are H, W is CH, W^1, W^2 and W^3 are N, and R^6 is NH_2 or NHR^7 where R^7 is acyl then R^5 cannot be Cl, Br, I, C_{1-6} alkyloxy, C_{3-6} cycloalkyloxy, aryloxy, arylalkoxy, amino which is substituted by one or two substituents independently selected from C_{1-6} alkyl and C_{3-6} cycloalkyl, or 4 to 6 membered heterocyclic ring containing at least one nitrogen atom which ring is bonded to the purine base via the nitrogen atom; and

with the proviso wherein for formula (I) where base is formula (III), R^6 cannot be NH_2 when R^5 is OH and R^6 cannot be H when R^5 is NH_2, if R^1 and R^2 are H, W is CH, W^1, W^2 and W^3 are N; and

each R' is independently a lower alkyl (C_1-C_6 alkyl), lower alkenyl, lower alkynyl, lower cycloalkyl (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, or

Base is a purine or modified purine of the general formula (IV):



IV

wherein:

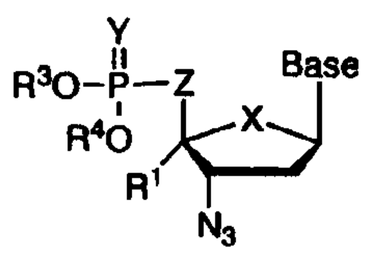
each W, W^2 and W^3 is independently N, CCF_3, $CC(O)NH_2$, CC(O)NHR', $CC(O)N(R')_2$, CC(O)OH, CC(O)OR' or CR^5;

W^4 is independently O, S, NH or NR';

each R^5 and R^6 is chosen independently from H, halogen, CN, N_3, NO_2, OH, NH_2, SH, OR', NHR', $N(R')_2$, SR', OCOR', NHCOR', N(COR')COR', SCOR', OCOOR', NHCOR', CH_2OH, CH_2CN, CH_2N_3, COOH, COOR', $CONH_2$, CONHR, $CON(R')_2$, CH_2COOH, CH_2COOR', CH_2CONH_2, CH_2CONHR', $CH_2CON(R')_2$, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-8} cycloalkyl, aryl, heteroaryl, acyl, arylalkyl, and alkylaryl; and

each R' is independently a C_{1-6} alkyl, C_{3-6} cycloalkyl, aryl, alkylaryl, or arylalkyl.

2. A compound of Formula (II):



II

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is O, CH_2, S, SO_2, NH, P=O(OH), $C=CH_2$, C=CHF, or $C=CF_2$;

Y is O or S;

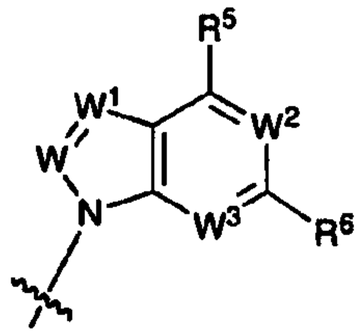
Z is CH_2, CH_2CH_2, CH_2O, CH_2S, or CH_2NH (wherein a carbon atom is connected to a phosphorus atom);

R^1 is hydrogen, alkyl, haloalkyl, halo, azido, cyano, nitro, amino, alkylamino, dialkylamino, alkenyl, alkynyl, haloalkenyl, alkoxy, alkenoxy, alkylthio, acyloxy, alkyloxyacyl, alkylcarbonyl, acylthio, or acylamino;

R^3 and R^4 are, independently, hydrogen, phosphate, diphosphate, or a group that is preferentially removed in a hepatocyte to yield the corresponding H group, wherein the term "preferentially removed in a hepatocyte" means that at least part of the group is removed in a hepatocyte at a rate higher than the rate of removal of the same group in a

non-hepatocytic cell , or the removable group is a pharmaceutically acceptable group that can be removed by a reductase, esterase, cytochrome P450 or other enzyme;

wherein the Base is purine or modified purine of the general formula (III):



III

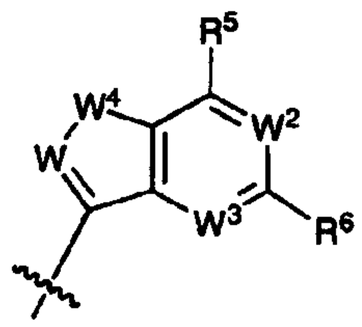
wherein:

each W, W^1, W^2 and W^3 is independently N, CH, CF, CCl, CBr, CI, CCN, CCH_3, CCF_3, $CC(O)NH_2$, CC(O)NHR', $CC(O)N(R')_2$, CC(O)OH, CC(O)OR' or CR^5;

each R^5 and R^6 is chosen independently from H, halogen (F, Cl, Br, I), CN, N_3, NO_2, OH, NH_2, SH, OR', NHR', $N(R')_2$, SR', OCOR', NHCOR', N(COR')COR', SCOR', OCOOR', NHCOR', CH_2OH, CH_2CN, CH_2N_3, COOH, COOR', $CONH_2$, CONHR, $CON(R')_2$, CH_2COOH, CH_2COOR', CH_2CONH_2, CH_2CONHR', $CH_2CON(R')_2$, $C_{1\text{-}6}$ alkyl, $C_{2\text{-}6}$ alkenyl, and $C_{2\text{-}6}$ alkynyl, $C_{3\text{-}8}$ cycloalkyl, aryl, heteroaryl, acyl, arylalkyl, and alkylaryl;

each R' is independently a $C_{1\text{-}6}$ alkyl, $C_{3\text{-}6}$ cycloalkyl, aryl, alkylaryl, or arylalkyl, or

wherein the Base is purine or modified purine of the general formula (IV):



IV

wherein:

each W, W^2 and W^3 is independently N, CH, CF, CCl, CBr, CI, CCN, CCH_3, CCF_3, $CC(O)NH_2$, CC(O)NHR', $CC(O)N(R')_2$, CC(O)OH, CC(O)OR' or CR^5;

W^4 is independently O, S, NH or NR';

each R^5 and R^6 is chosen independently from H, halogen, CN, N_3, NO_2, OH, NH_2, SH, OR', NHR', $N(R')_2$, SR', OCOR', NHCOR', N(COR')COR', SCOR', OCOOR', NHCOR', CH_2OH, CH_2CN, CH_2N_3, COOH, COOR', $CONH_2$, CONHR, $CON(R')_2$, CH_2COOH, CH_2COOR', CH_2CONH_2, CH_2CONHR', $CH_2CON(R')_2$, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-8} cycloalkyl, aryl, heteroaryl, acyl, arylalkyl, and alkylaryl;

each R' is independently a C_{1-6} alkyl, C_{3-6} cycloalkyl, aryl, alkylaryl, or arylalkyl.

3. The compounds of either of claims 1-2, wherein the compounds are in the β-L- or β-D configuration, or a racemic mixture thereof.

4. 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 3 to a patient in need of treatment thereof.

5. 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 3 to a patient in need of prophylaxis thereof.

6. 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 3 to a patient in need of treatment thereof.

7. The method of Claim 4, 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.

8. 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 3 in a pharmaceutically acceptable carrier in combination with another anti-HIV agent.

9. The method of Claim 8, 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.

10. 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 3, in a pharmaceutically acceptable carrier, in combination with another anti-HIV agent, to a patient in need or prophylaxis thereof.

11. A method for treating a host infected with HBV, comprising administering an effective amount of a compound of any of Claims 1 to 3 to a patient in need of treatment thereof.

12. A method for preventing an HBV infection, comprising administering a phrophylactically effective amount of a compound of any of Claims 1 to 3 to a patient in need of prophylaxis thereof.

13. 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 3 to a patient in need of treatment thereof.

14. A method for treating a host infected with HBV that includes administering an effective amount of a compound of any of Claims 1 to 3 in a pharmaceutically acceptable carrier in combination with another anti-HBV agent.

15. A method for preventing an HBV infection, comprising administering a prophylactically effective amount of a compound of any of Claims 1 to 3 in a pharmaceutically acceptable carrier, in combination with another anti-HBV agent, to a patient in need of prophylaxis thereof.

16. A method for treating a host infected with HCV, comprising administering an effective amount of a compound of any of Claims 1 to 3 to a patient in need of treatment thereof.

17. A method for preventing an HCV infection, comprising administering a phrophylactically effective amount of a compound of any of Claims 1 to 3 to a patient in need of prophylaxis thereof.

18. A method for treating a host infected with HCV that includes administering an effective amount of a compound of any of Claims 1 to 3 in a pharmaceutically acceptable carrier in combination with another anti-HCV agent.

19. A method of treating an HIV-1 of HIV-2 infection, comprising administering an effective treatment amount of 3'-azido-ddA, 3'-azido-ddG, or combinations thereof, in combination with one or more additional antiviral agents, where the one or more additional antiviral agents select for TAM mutations and/or for the M184V mutation.

20. A pharmaceutical composition, comprising 3'-azido-ddA, 3'-azido-ddG, or combinations thereof, and one or more additional antiviral agents that select for TAM mutations and/or the M184V mutation, along with a pharmaceutically acceptable carrier.

Genotypes of xxLAI viruses

Virus (xxLAI)	Mutations in RT
HIV-1K65R	K65R
HIV-1K70E	K70E
HIV-1L74V	L74V
HIV-1M184V	M184V
HIV-1AZT2	D67N, K70R, T215F, K219Q
HIV-1AZT3	M41L, L210W, T215Y
HIV-1AZT7	M41L, D67N, K70R, T215F, K219Q
HIV-1AZT9	M41L, D67N, K70R, L210W, T215Y, K219Q
HIV-1Q151M	M41L, A62V, V75I, F77L, F116Y, Q151M
HIV-169Insertion	M41L, SS insert between 69 and 70, L210W, T215Y

FIG.1

Anti-HIV activity of 3'-azido-2',3'-ddA and 3'-azido-2',3'-ddG against a p of drug-resistant HIV-1.

	AZT		3'-azido-ddA		3'-azido-ddG	
	EC_{50} (μM)	Fold[g]	EC_{50} (μM)	Fold[g]	EC_{50} (μM)	Fold[g]
WT	0.19 ± 0.11	-	10.7 ± 4.9	-	2.1 ± 0.9	-
K65R	0.21 ± 0.15	1.1	9.8 ± 8.4	0.9	5.0 ± 1.3	2.3
L74V	0.21	1.1	13.7 ± 5.7	1.2	2.9 ± 0.9	1.4
M184V	0.18 ± 0.16	1	8.9 ± 2.3	0.8	1.7 ± 0.1	0.8
[a]AZT2	10.4 ± 8.9	54.4	24.2 ± 3.7	2.2	5.2 ± 2.3	2.5
[b]AZT3	11.9 ± 11.6	62.7	19.4 ± 8.1	1.8	3.7 ± 1.4	1.8
[c]AZT7	96.7 ± 29.3	507.4	31.6 ± 3.7	2.9	7.6 ± 2.2	3.5
[d]AZT9	58.6 ± 9.2	307.4	37.5 ± 6.2	3.5	7.9 ± 4.9	3.7
[e]Q151M	213.7	1124	70.1 ± 10.8	6.5	72.9 ± 29.5	34.7
[f]69 insertion	204.6	1076	29.9 ± 5.9	2.8	26.4 ± 8.8	12.5

FIG.2A

EC50 values were determined by measuring inhibiti on of luminescence in P4/R5 cells using a single cycl e repli cation assay.
a: M41L/L210W/T215Y
b: D67N/K70R/T215F/K219Q
c: M41L/D67N/K70R/T 215F/K219Q
d: M41L/D67N/K70R/L 210W/T215Y/K 219Q
e: A62V/V 75I/F77L/ F116Y/Q151M
f: M41L/SS insert betwe en 69 and 70/L210W/T215Y
g: Fold resistance values were determined by divi ding the EC50 for mutant HIV-1 by the EC 50 for WT HIV-1.

FIG.2B

SUBSTITUTE SHEET (RULE 26)

Deamination of Nucleosides by Adenosine Deaminase

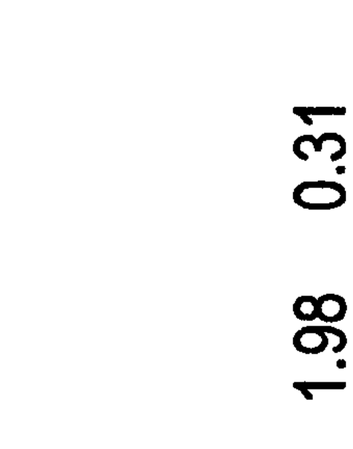
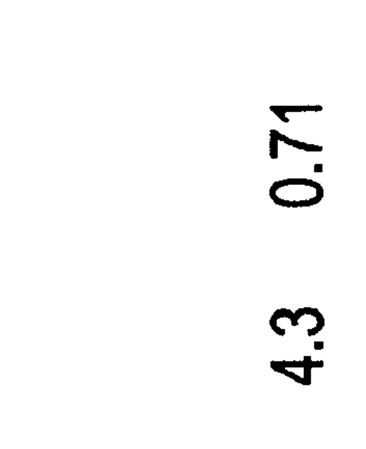
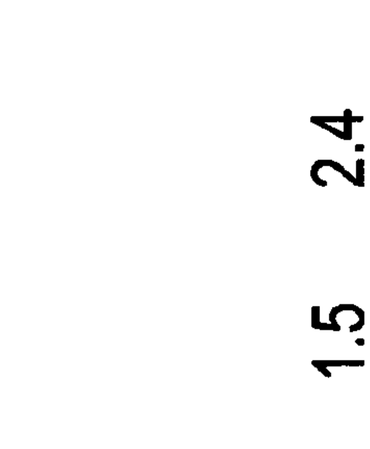
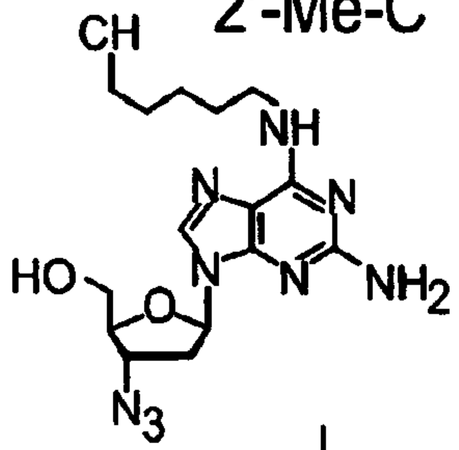
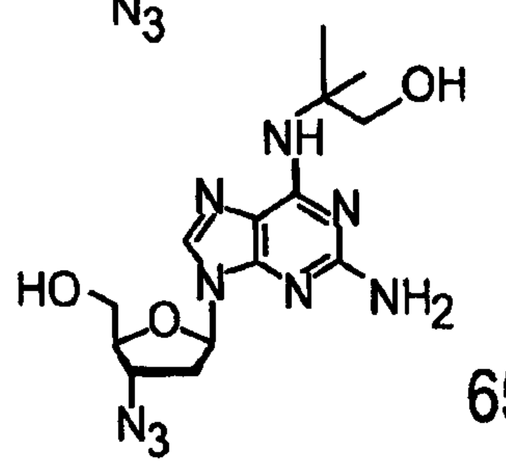
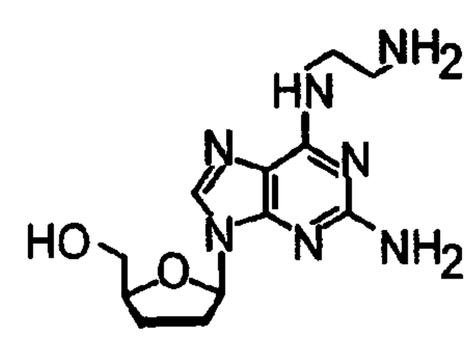
Structure	Compound	HIV-1/LAI (PBM)[a] (Ec50 µM)	HIV-1/LAI (PBM)[a] (Ec90 µM)	HIV-2/pROD10 (PBM) (Ec50 µM)	HIV-2/pROD10 (PBM) (Ec90 µM)	HBV Activity (Ec50 µM)	HBV Activity (Ec90 µM)	HSV-1 Activity[c] (Ec50 µM)	HSV-1 Activity[c] (Ec90 µM)	HCV Activity[d] (Ec50 µM)	HCV Activity[d] (Ec90 µM)	Toxicity (Ic50 µM)[b] PBM	Toxicity (Ic50 µM)[b] CEM	Toxicity (Ic50 µM)[b] Vero
	3TC	0.067	0.32	0.075	0.42	n/a	<10	n/a	n/a	n/a	n/a	>	>100	>100
	AZT	0.0050	0.020	0.0076	0.064	n/a	n/a	0.16	0.42	n/a	n/a	>	14.3	50.6
	ACV	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	>100
	2'-Me-C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1.3	5.4	29.4	24.5	>100
	75	0.18	1.98	0.31	2.0	>10	>10	>100	>100	>10	>10	>100	>100	>100
	76	0.50	4.3	0.71	7.4	>10	>10	>100	>100	>10	>10	>100	>100	>100
	77	0.40	1.5	2.4	6.1	>10	>10	>100	>100	>10	>10	>100	27.0	32.0
	78													
	56													
	62													
	63													

FIG.3A

SUBSTITUTE SHEET (RULE 26)

Deamination of Nucleosides by Adenosine Deaminase

Structure	Compound	HIV-1/LAI (PBM)[a] (Ec_{50} µM)	HIV-1/LAI (PBM)[a] (Ec_{90} µM)	HIV-2/pROD10 (PBM) (Ec_{50} µM)	HIV-2/pROD10 (PBM) (Ec_{90} µM)	HBV Activity (Ec_{50} µM)	HBV Activity (Ec_{90} µM)	HSV-1 Activity[c] (Ec_{50} µM)	HSV-1 Activity[c] (Ec_{90} µM)	HCV Activity[d] (Ec_{50} µM)	HCV Activity[d] (Ec_{90} µM)	Toxicity (Ic_{50} µM)[b] PBM	Toxicity (Ic_{50} µM)[b] CEM	Toxicity (Ic_{50} µM)[b] Vero
	3TC 75	0.067	0.32	0.075	0.42	n/a	<10	n/a	n/a	n/a	n/a	>	>100	>100
	AZT 76	0.0050	0.020	0.0076	0.064	n/a	n/a	n/a	n/a	n/a	n/a	>	14.3	50.6
	ACV 77	n/a	n/a	n/a	n/a	n/a	n/a	0.16	0.42	n/a	n/a	n/a	n/a	>100
	2'-Me-C 78	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1.3	5.4	29.4	24.5	>100
	64	86.5	>10	>100	>100	>10	>10	>100	>100	>10	>10	>100	>100	>100
	65	52.5	>10	>100	>100	>10	>10	>100	>100	>10	>10	>100	>100	>100
														

Deamination of Nucleosides by Adenosine Deaminase

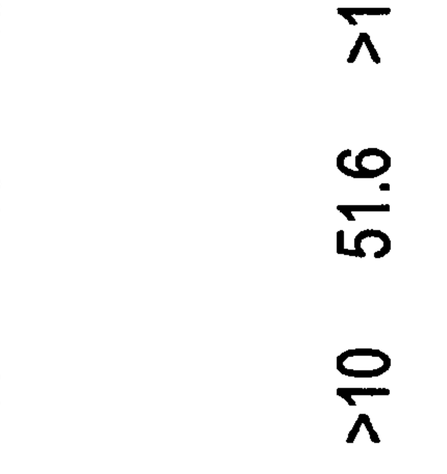
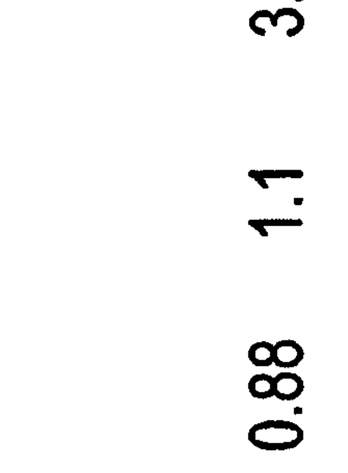
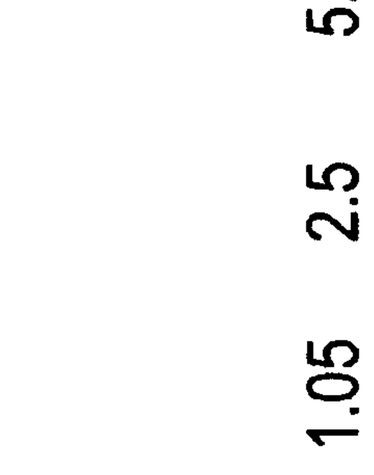
Structure	Compound	HIV-1/LAI (PBM)[a] (Ec50 µM)/(Ec90 µM)	HIV-2/pROD10 (PBM) (Ec50 µM)/(Ec90 µM)	HBV Activity (Ec50 µM)/(Ec90 µM)	HSV-1 Activity[c] (Ec50 µM)/(Ec90 µM)	HCV Activity[d] (Ec50 µM)/(Ec90 µM)	Toxicity (IC50 µM)[b] PBM	Toxicity (IC50 µM)[b] CEM	Toxicity (IC50 µM)[b] Vero
	3TC	0.067 0.32	0.075 0.42	n/a <10	n/a n/a	n/a n/a	> >100	>100	>100
	AZT	0.0050 0.020	0.0076 0.064	n/a n/a	n/a n/a	n/a n/a	> 14.3	50.6	
	ACV	n/a n/a	n/a n/a	n/a n/a	0.16 0.42	n/a n/a	n/a	n/a	>100
	2'-Me-C	n/a n/a	n/a n/a	n/a n/a	n/a n/a	1.3 5.4	29.4	24.5	>100
	67	59.9 >10	51.6 >100	>10 >10	>100 >100	>10 >10	>100	>100	>100
	68	0.19 0.88	1.1 3.8	>10 >10	>100 >100	>10 >10	>100	>100	>100
	69	0.21 1.05	2.5 5.9	>10 >10	>100 >100	>10 >10	>100	>100	>100

FIG.3C

CA 02685748 2009-11-12

SUBSTITUTE SHEET (RULE 26)

Deamination of Nucleosides by Adenosine Deaminase

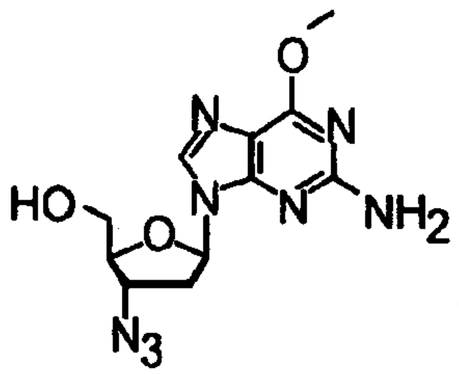
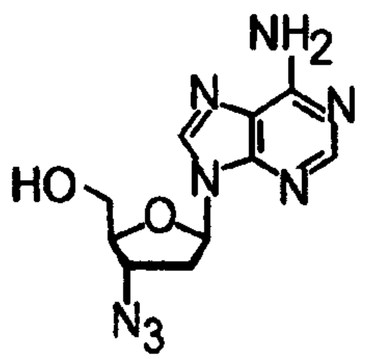
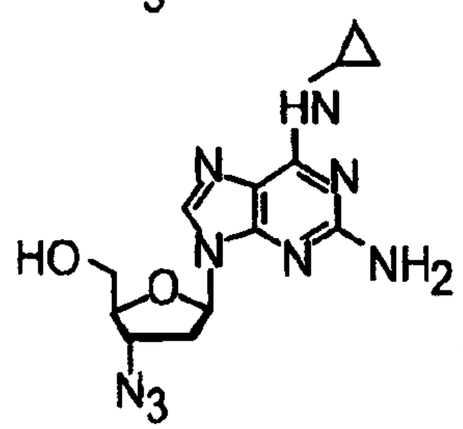
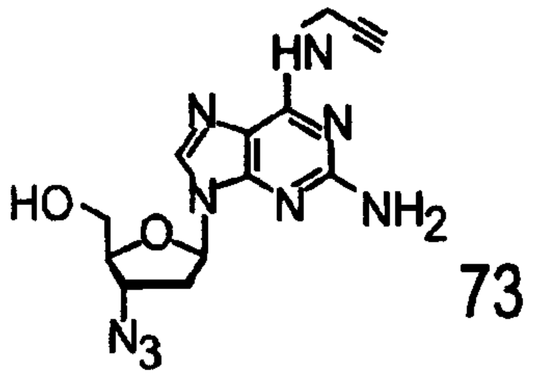
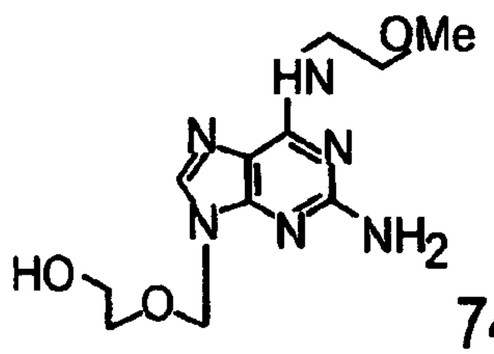
Structure	Compound	HIV-1/LAI (PBM)[a] (Ec_{50} µM)	HIV-1/LAI (PBM)[a] (Ec_{90} µM)	HIV-2/pROD10 (PBM) (Ec_{50} µM)	HIV-2/pROD10 (PBM) (Ec_{90} µM)	HBV Activity (Ec_{50} µM)	HBV Activity (Ec_{90} µM)	HSV-1 Activity[c] (Ec_{50} µM)	HSV-1 Activity[c] (Ec_{90} µM)	HCV Activity[d] (Ec_{50} µM)	HCV Activity[d] (Ec_{90} µM)	Toxicity (Ic_{50} µM)[b] PBM	Toxicity (Ic_{50} µM)[b] CEM	Toxicity (Ic_{50} µM)[b] Vero
3TC	75	0.067	0.32	0.075	0.42	n/a	<10	n/a	n/a	n/a	n/a	>	>100	>100
AZT	76	0.0050	0.020	0.0076	0.064	n/a	n/a	n/a	n/a	n/a	n/a	>	14.3	50.6
ACV	77	n/a	n/a	n/a	n/a	n/a	n/a	0.16	0.42	n/a	n/a	n/a	n/a	>100
2'-Me-C	78	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1.3	5.4	29.4	24.5	>100
	70	0.57	1.4	0.71	6.6	>10	>10	>100	>100	>10	>10	32.1	>100	>100
	71	0.36	2.7	41.9	94.9	>10	>10	>100	>100	>10	>10	>74.3	86.2	>100
	72	1.5	8.8	3.1	15.8	>10	>10	>100	>100	>10	>10	>100	>100	>100

FIG.3D

SUBSTITUTE SHEET (RULE 26)

Deamination of Nucleosides by Adenosine Deaminase

Structure	Compound	HIV-1/LAI (PBM)[a] (Ec_{50} µM)	HIV-1/LAI (PBM)[a] (Ec_{90} µM)	HIV-2/pROD10 (PBM) (Ec_{50} µM)	HIV-2/pROD10 (PBM) (Ec_{90} µM)	HBV Activity (Ec_{50} µM)	HBV Activity (Ec_{90} µM)	HSV-1 Activity[c] (Ec_{50} µM)	HSV-1 Activity[c] (Ec_{90} µM)	HCV Activity[d] (Ec_{50} µM)	HCV Activity[d] (Ec_{90} µM)	Toxicity (Ic_{50} µM)[b] PBM	Toxicity (Ic_{50} µM)[b] CEM	Toxicity (Ic_{50} µM)[b] Vero
3TC	75	0.067	0.32	0.075	0.42	n/a	<10	n/a	n/a	n/a	n/a	>	>100	>100
AZT	76	0.0050	0.020	0.0076	0.064	n/a	n/a	n/a	n/a	n/a	n/a	>	14.3	50.6
ACV	77	n/a	n/a	n/a	n/a	n/a	n/a	0.16	0.42	n/a	n/a	n/a	n/a	>100
2'-Me-C	78	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1.3	5.4	29.4	24.5	>100
	73													
	74	2.1	16.2	n/a	n/a	n/a	n/a	n/a	n/a	>10	>10	>100	>100	>100

[a]HIV drug susceptibility assay was done as previously described in Schinazi, R.F., Sommadossi, J.P., Saalmann, V., Cannon, D.L., Xie, M.-W., Hart, G.C., Smith, G.A., and Hahn, E.F. "Activity of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1." *Antimicrob. Agents Chemother*. **1990**, *34*,1061-7.
[b]Cytotoxicity assays in PBM, CEM and Vero cells were done as previously described in Stuyver, L.J., Lostia, S., Adams, M., Mathew, J., Pai, B.S., Grier, J., Tharnish, P., Choi, Y., Chong, Y., Choo, H., Chu, C.K., Otto, M.J., Schinazi, R.F. "Antiviral activities and cellular toxicities of modified 2',3'-dideoxy-2',3'-didehydrocytidine analogues" *Antimicrob. Agents Chemother*. **2002**, *46*, 3854-60.

SUBSTITUTE SHEET (RULE 26)

FIG.3F

[c]HSV-1 drug susceptibility assay was 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.

[d]**HCV Replican Assay**[1]: Huh 7 Clone B cells containing HCV Replicon RNA were seeded in a 96-well plate at 5000 cells/well, and the compounds were tested at 10 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 (ΔCtHCV). 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 ΔCt rRNA values. **77** (2'-Me-*C)* was used as the control. To determine **EC_{90} and IC_{50}** values[2], **Ct: values** were first converted into fraction of starting material[3] and then were used to calculate the % **inhibition**.

References:

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

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

3. Applied Biosystems Handbook

FIG.3G

Deamination of Nucleosides by Adenosine Deaminase.

Structure	Compound Number	Extinction Coefficient at pH 7.4	Deamination in 7 min (0.002 units Adenosine Deaminase)	Deamination in 120 min (0.2 units Adenosine Deaminase)
2'-deoxyadenosine		ε_{265}=14.3 mM^{-1} cm^{-1}	59.3%	105.5%
2'-deoxyguanosine		ε_{265}=9.6 mM^{-1} cm^{-1}	below level of detection	below level of detection

FIG.4A

Deamination of Nucleosides by Adenosine Deaminase.

Compound Number	Extinction Coefficient at pH 7.4	Deamination in 7 min (0.002 units Adenosine Deaminase)	Deamination in 120 min (0.2 units Adenosine Deaminase)
69	ε_{285}=19.6 mM^{-1} cm^{-1}	below level of detection	below level of detection
72	ε_{285}=9.6 mM^{-1} cm^{-1}	59.3%	105.5%
62	ε_{285}=10.9 mM^{-1} cm^{-1}	below level of detection	7.6%

Structure

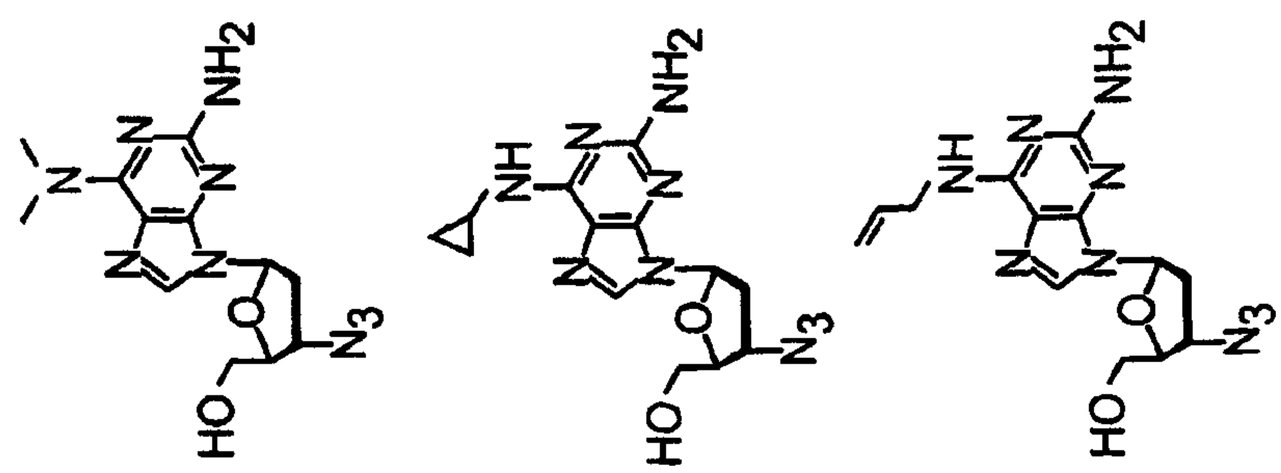


FIG.4B

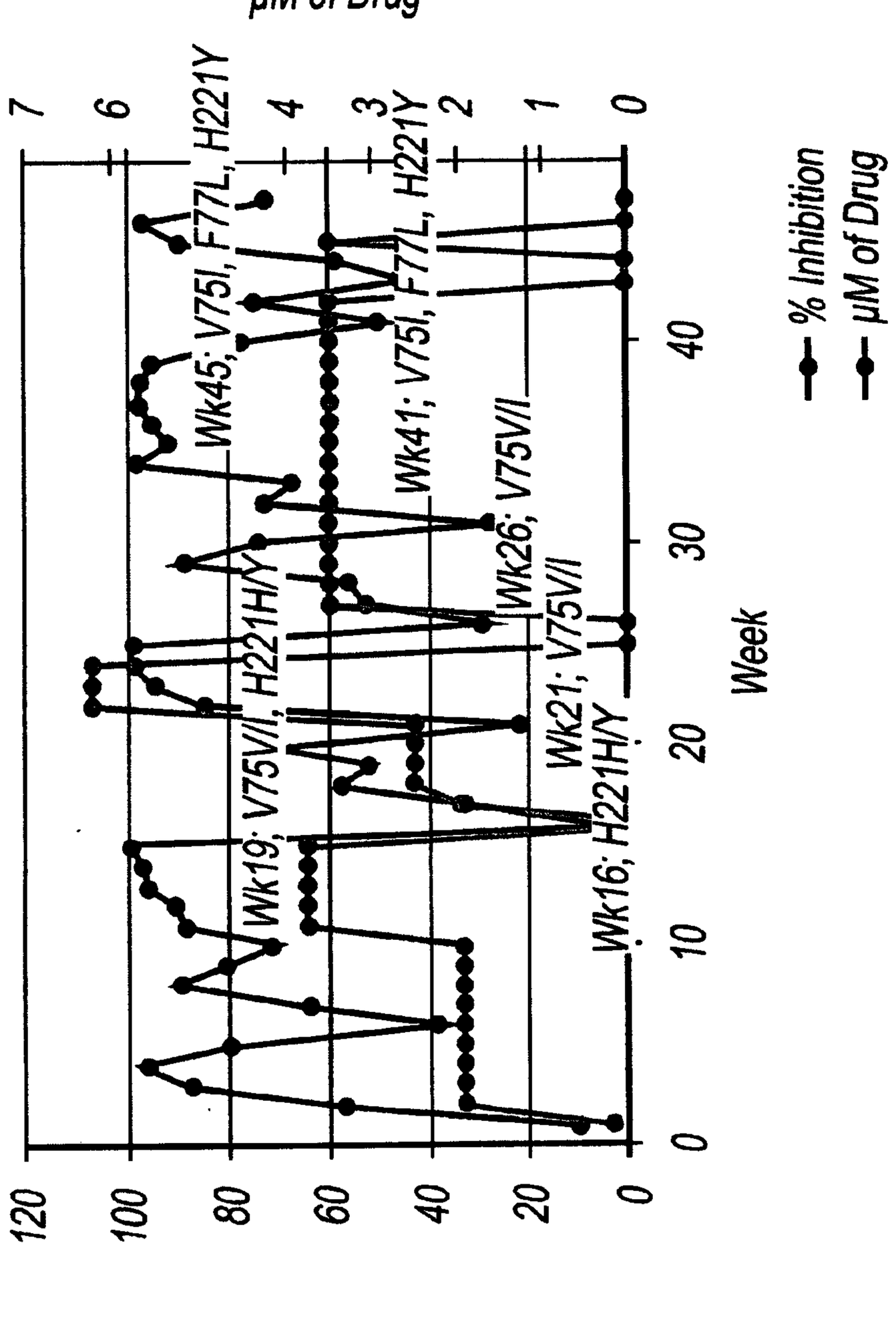

μM of Drug
% Inhibition
μM of Drug
Week
Wk45; V75I, F77L, H221Y
Wk41; V75I, F77L, H221Y
Wk26; V75V/I
Wk21; V75V/I
Wk19; V75V/I, H221H/Y
Wk16; H221H/Y

FIG.5

Summary of Compound 56 Treatment and Mutations Selected in human PBM cells

Week	Compound 56 Treatment (μM)	Mutations
16	0.0	
19	2.5	H221H/Y
21	2.5	V75V/I, H221H/Y
26	0.0	V75V/I
41	3.5	V75V/I
45	3.5	V75I, F77L, H221Y
		V75I, F77L, H221Y

FIG.6

Genotypes of xxLAI viruses

Virus (xxLAI)	Mutations in RT
HIV-1K65R	K65R
HIV-1K70E	K70E
HIV-1L74V	L74V
HIV-1M184V	M184V
HIV-1AZT2	D67N, K70R, T215F, K219Q
HIV-1AZT3	M41L, L210W, T215Y
HIV-1AZT7	M41L, D67N, K70R, T215F, K219Q
HIV-1AZT9	M41L, D67N, K70R, L210W, T215Y, K219Q
HIV-1Q151M	M41L, A62V, V75I, F77L, F116Y, Q151M
HIV-169Insertion	M41L, SS insert between 69 and 70, L210W, T215Y

FIG.1