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Synthesis and Anti-HIV Activity of Isonucleosides

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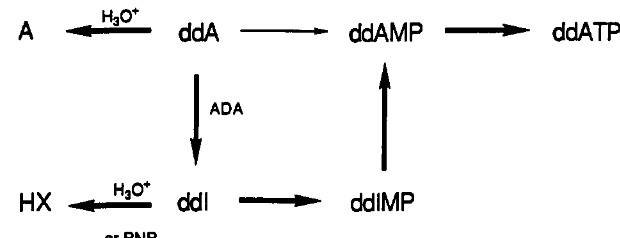
A series of isomeric 2′,3′-dideoxynucleosides which contains a modified carbohydrate moiety has been prepared. This class of compounds was designed to mimic the activity of known anti-HIV dideoxynucleosides, while imparting enhanced chemical and enzymatic stability. Isonucleosides containing the standard heterocyclic bases (A, C, G, T) were synthesized via nucleophilic addition of the base to an isomeric sugar unit. Modified derivatives were generated by manipulation of the intact isonucleoside. Two of the compounds prepared, iso-ddA (1) and iso-ddG (**6**), exhibit significant and selective anti-HIV activity, as well as beneficial hydrolytic stability.

Introduction

There is considerable interest in the use of inhibitors of human immunodeficiency virus (HIV) reverse transcriptase for the treatment of acquired immune deficiency syndrome (AIDS). 3′-Azido-3′-deoxythymidine (AZT), the first approved drug for the treatment of this disease, acts through this mechanism, and has been found to be clinically efficacious in the treatment of AIDS and AIDS-related complex (ARC), as well as in the management of asymptomatic HIV infection.[1,2] A number of other 2′,3′-dideoxynucleosides that have exhibited anti-HIV activity in cell culture are currently being evaluated in the clinic.[3,4] However, nucleosides that have shown activity in infected patients also have demonstrated toxicities and/or other side effects.[3,5] Clearly, there is a need for better therapeutic agents for the treatment of AIDS and the less severe stages of HIV infection.

2′,3′-Dideoxyadenosine (ddA) is an effective inhibitor of HIV replication in cell culture. Its action is the consequence of the intracellular formation of the corresponding 5′-triphosphate (ddATP) which inhibits the viral reverse transcriptase.[6,7] Analysis of the metabolism of ddA has revealed that the primary pathway in the formation of the triphosphate is not direct, but involves the formation of 2′,3′-dideoxyinosine (ddI) and its corresponding 5′-monophosphate (ddIMP), followed by formation of ddA-5′-monophosphate (ddAMP), and finally ddATP (Scheme I).[8] Moreover, ddA is highly susceptible to acid hydrolysis to adenine (A), as well as to catabolism by adenosine deaminase (ADA) to ddI.[7,9] Consequently, ddI has been chosen in preference to ddA for clinical study in the treatment of AIDS. Nonetheless, ddI is itself acid unstable

Scheme I



Scheme II

Iso-sugar
(2, R' = Ts)
Iso-nucleosides
Iso-nucleoside Derivatives

requiring co-administration of an antacid to patients when given by the oral route, and is susceptible to catabolic

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2',3'-Dideoxyadenosine

Iso-dideoxyadenosine 1

Figure 1.

elimination via hypoxanthine (HX) by the action of purine nucleoside phosphorylase (PNP).[4,9,10]

In light of the need for more stable, effective antiviral agents, we undertook the synthesis of a new class of 2',3'-dideoxynucleosides exemplified by 1[11] (Figure 1). Transposition of the 3' carbon and ring oxygen of the 2',3'-dideoxyribose unit was designed to provide enhanced chemical and enzymatic stability to the "isomeric" dideoxyadenosine via elimination of the labile glycosidic bond. Others have also attempted to obtain more stable analogs of ddA by the preparation of 2-chloro-, 2-bromo-, and 2-fluoro-2',3'-dideoxyadenosine and 2-chloro-2',3'-didehydro-2',3'-dideoxyadenosine.[12] In each case, antiviral activity was observed in cell culture only at inhibitor concentrations near the cytotoxic dose. By contrast, N^6-methyl-2',3'-dideoxyadenosine is a selective inhibitor of HIV replication in cell culture, and is reported to be resistant to enzymatic degradation by adenosine deaminase.[13] The susceptibility of this compound to hydrolysis via either acid or the action of purine nucleoside phosphorylase, however, was not reported. Herein we describe the synthesis and anti-HIV activity of the isomeric 2',3'-dideoxynucleosides, a novel series of hydrolytically stable anti-HIV agents.

Chemistry

A strategy for the synthesis of this class of compounds is depicted in Scheme II. It was envisioned that an appropriate carbohydrate could be utilized to generate a

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novel isosugar unit. Incorporation of each standard purine and pyrimidine base (A, C, G, T) as a late step in the synthetic sequence could efficiently provide the first generation of isomeric dideoxynucleosides. A number of related compounds containing modifications of the heterocyclic base would be available through manipulation of these intact "nucleosides".

The required isomeric sugar moiety (2, R′ = Ts) was prepared from an *erythro*-pentofuranose as described previously.[11] While well-documented preparations of carbocyclic nucleosides via the amino-sugar are available,[14] we chose instead to prepare the isonucleosides via direct displacement reactions of the nucleo-base onto the isosugar.[15] This strategy enabled us to assemble a number of diverse isonucleosides in a short period of time.

Table I illustrates the isonucleosides prepared. Synthesis of the "standard" isonucleosides took place via direct displacement reactions of the isosugar with the appropriate heterocyclic base. Iso-ddA (1) was generated in 35% yield by the reaction of adenine, K_2CO_3, and 18-crown-6 with isosugar 2.[11] The pyrimidine analogs, iso-ddC (3) and iso-ddT (4), were prepared analogously, but in much lower yield. In these cases, O-alkylation and bis-1,3-alkylation, respectively, were tentatively identified as side reactions. A similar displacement reaction using 2-amino-6-chloropurine as the nucleophile afforded isonucleoside 5; subsequent acid hydrolysis then afforded iso-ddG (6) in 33% overall yield.[16] To complete this primary series, iso-ddA was treated with $NaNO_2$ in HOAc to generate iso-ddI (7).[17]

Structure elucidation of these products was based on spectroscopic methods. The UV absorbances of the isonucleosides were measured, and compared to those of known substituted purines and pyrimidines.[18] In all cases, the absorbance maxima of each isonucleoside could be correlated with the appropriately alkylated nucleo-base (i.e. N-9 substitution in the purine series, and N-1 substitution in the pyrimidine series).

Additional purine isonucleosides were prepared by using 6-chloropurines 8 and 5 as intermediates. Nucleophilic displacement of 2 with 6-chloropurine afforded 8 in 30% yield. Alternatively, this compound could be synthesized

Table I

isonucleoside base	compd no.	starting material	reaction conditions (yield)
	1	2	adenine, K_2CO_3, 18-crown-6 (35%)
	3	2	cytosine, K_2CO_3, 18-crown-6 (15%)
	4	2	thymine, K_2CO_3, 18-crown-6 (11%)
	5	2	2-amino-6-chloropurine, K_2CO_3, 18-crown-6 (30%)
	6	5	HCl, H_2O (41%)
	7	1	$NaNO_2$, HOAc (73%)
	8	2	6-chloropurine, K_2CO_3, 18-crown-6 (25%)
	9	8	$MeNH_2$ (89%)
	10	8	H_2, Pd/C (95%)
	11	8	KF, Me_3N (58%)
	12	8	thiourea, propanol (86%)
	13	12	CH_3I, NH_4OH (85%)
	14	5	NH_3 (59%)
	15	5	H_2, Pd/C (78%)
	16	5	KF, Me_3N (46%)
	17	5	thiourea, propanol (48%)

in a five-step sequence via the primary amine, as described elsewhere.[11] The N^6-methyl isonucleoside 9 was prepared by reaction of 8 with methylamine. Catalytic hydrogenation of 8 using Pd/C afforded the purine analog 10.[19]

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Table II. In Vitro Anti-HIV Activity of Isonucleosides

compd	ED_{50} (μM)[a]	CD_{50} (μM)[b]
1 (iso-ddA)	5–15	>100
3 (iso-ddC)	>100	>100
4 (iso-ddT)	>100	>100
5	>100	>100
6 (iso-ddG)	10–50	>500
7 (iso-ddI)	>100	>100
8	>100	>100
9	>100	>100
10	>100	>100
11	>100	>100
12	>100	>100
13	>100	>100
14	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100
ddA[c]	5–10	>200
ddG[c]	5–10	>200

[a] Effective dose of compound which provides 50% protection of ATH-8 cells against the cytopathic effect of HIV. [b] Cytotoxic dose of compound which reduced by 50% the viability of uninfected ATH-8 cells. [c] Data from ref 6a.

Synthesis of the 6-fluoroisopurine (11) was carried out by treatment of 8 with trimethylamine and KF.[20] The 6-mercaptopurine derivative 12 could be generated by reaction of 8 with thiourea.[21] Conversion of 12 to the S-methylated analog (13) took place using iodomethane.[22]

Analogous reactions were carried out using the 2-amino-6-chloropurine compound (5) as starting material to generate isodideoxyguanosine derivatives. Through these reactions, the 2,6-diamino (14), 2-amino (15), 2-amino-6-fluoro (16), and 2-amino-6-mercapto (17) purine derivatives were synthesized.

Results and Discussion

All of the isonucleosides described were tested for anti-HIV activity in ATH-8 cells according to a protocol previously described.[6a] As can be seen in Table II, iso-ddA (1) and iso-ddG (6) exhibited a substantial level of anti-HIV activity. Against HIV-1 (HTLV-III_B strain) IC_{50}s of 5–15 and 10–50 μM, respectively, were measured. These determinations were made in several ($\geq$3) experiments, with a standard such as AZT always included; a range is reported as the nature of the assay makes a more precise determination tenuous, at best. In the case of iso-ddA, this activity is comparable to that described for ddA in certain experiments.[6a] Iso-ddG however, is less potent than its counterpart ddG. Neither of these compounds exhibited cytotoxicity at the highest concentrations tested (up to 200 μM). Of the remaining isonucleosides, including iso-ddI, iso-ddC, and N^6-methylisodideoxyadenosine, no significant antiviral activity nor overt cytotoxicity was evident at the highest concentration tested (100 μM). This pattern of activity is in contrast to the 2′,3′-dideoxynucleosides in which case a number of purines as well as pyrimidines exhibit anti-HIV activity. The lack of antiviral activity of iso-ddI, coupled with the resistance of iso-ddA to adenosine deaminase,[9] suggests that the mechanism of activation of iso-ddA may be less circuitous than that observed for ddA/ddI. If the conversion of iso-ddA to its triphosphate (iso-ddATP) is direct, as suggested by these results, iso-ddA may offer certain advantages over the ddA/ddI system.

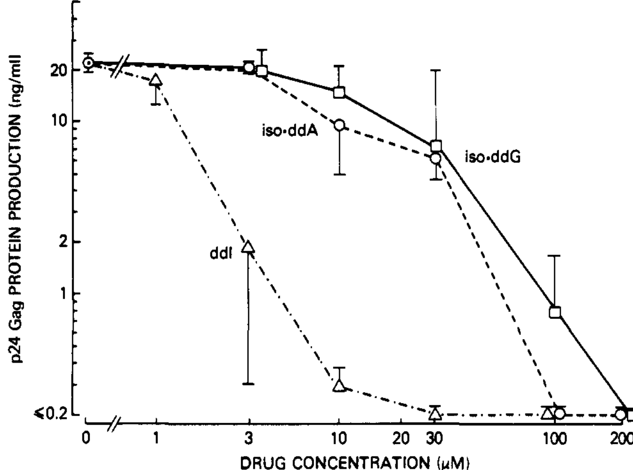

Figure 2. Anti-HIV activity of iso-ddA, iso-ddG, and ddI in AZT-resistant strains. p24 gag protein production (ng/mL) at various concentrations of the drugs tested. Assay of 5 × 10^5 PHA-stimulated human PBM infected at 20 $TCID_{50}$ infectious dose of HIV-1 isolated from an HIV-1 infected patient incubated at various drug concentrations; supernatant assayed on day 7. IC_{50} for drugs tested: iso-ddA = 8.2 μM; iso-ddG = 17.5 μM; ddI = 1.28 μM.

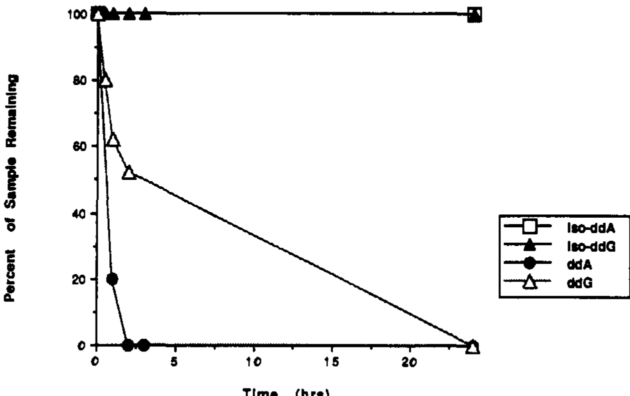

Figure 3. Stability of isonucleosides and 2′,3′-dideoxynucleosides at pH 3.

Iso-ddA and iso-ddG were further tested against AZT-resistant strains of HIV-1 isolated from an HIV-1 infected patient. In phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM), both compounds showed antiviral activity; however both were less potent than ddI under the conditions of the assay using inhibition of p24 gag production as endpoint (see Figure 2). The concentrations of iso-ddA and iso-ddG required to inhibit HIV replication by 50% (IC_{50}) were 8.5 and 17.5 μM, respectively. An IC_{50} of 1.28 μM was measured in this assay for ddI. The activities of the isonucleosides in this system reflect those observed in the assays performed using laboratory stains of HIV-1 in ATH-8 cells.

As the isonucleosides were designed to possess enhanced acid stability, we carried out studies comparing the half-

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lives of iso-ddA and iso-ddG with those of ddA and ddG. Under conditions which mimic the gastric environment (pH 3 and 37 °C)[23] both 2′,3′-dideoxynucleosides were rapidly hydrolyzed to their purine and carbohydrate components. ddA exhibited a half-life of less than 1 h, while ddG's half-life was measured at approximately 2 h. In sharp contrast, iso-ddA and iso-ddG showed no evidence of any decomposition under the same conditions for the duration of the 24-h experiment (Figure 3).

Recent literature has suggested a correlation between X-ray-determined nucleoside structure and degree of anti-HIV activity.[24] Application of this "model" to the conformation of iso-ddA as determined by X-ray analysis[11] reveals a further distinction of the isonucleosides. The anti glycosyl orientation and the gauche,gauche orientation of the hydroxymethyl substituent found in the major rotamer of iso-ddA are typical of nucleoside conformations.[25] However, while most anti-HIV nucleosides have been observed to adopt a furanose structure centered at a C3′-exo conformation,[24] the carbohydrate pucker observed in iso-ddA is best described as C2′-exo.[26] Further evidence that a C3′-exo furanose conformation may not be predictive of activity comes from the work of Fiandor and Tam, who describe an AZT isostere which was shown to adopt the "active" C3′-exo conformation, but was ineffective as an anti-HIV agent.[27] We propose that a specific carbohydrate conformation may not be a requirement for, nor predictive of, anti-HIV activity. The low-energy barrier between C3′-exo and C2′-exo furanose structures should allow either access to the "active" conformation.

The isonucleosides, in particular iso-ddA and iso-ddG, represent a novel class of anti-HIV agents. Their activity profile, acid stability, resistance to enzymatic degradation, and conformation distinguish these molecules from other known antiviral nucleosides, and may offer certain advantages in antiviral therapy. It is anticipated that additional studies will further delineate the characteristics of this novel structural class. These results will be reported in due course.

Experimental Section

Physical Chemistry. Infrared spectra (IR) were recorded on a Digilab FTS 15-E spectrometer. Mass spectra (MS) were obtained on either a VG7070-HF or a VGZAB mass spectrometer. Nuclear magnetic resonance spectra (NMR) were obtained on either a Varian XL-200-FT or a Varian XL-400 instrument. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane, with coupling constants (J) in hertz (Hz).

Chromatography. Silica gel 60 (230–400 mesh) from Merck was used for flash column chromatography. Preparative HPLC was carried out on a LDC preparative instrument using an ES Industries 10 μ chromegabond MC18 (50 cm × 23 mm) column; UV detection at appropriate wavelength and a CH_3CN/H_2O gradient elution system were used. Analytical TLC was performed using Merck precoated silica gel 60 F_{254} plates. Analytical HPLC was performed on a Waters instrument, using a Hamilton PRP-1 (4.6 mm × 250 mm) column, UV detection at 260 nm, and a mobile phase consisting of 10% CH_3CN/H_2O.

(2*R*-*trans*)-Tetrahydro-4-[[(4-methylphenyl)sulfonyl]oxy]-2-furanmethanol (2). A solution of 3-deoxy-1,2-*O*-(1-methylethylidene)-α-D-*erythro*-pentofuranose 4-methylbenzenesulfonate (40 g, 0.12 mol) in 1% (v/v) acetic acid/methanol (800 mL) was heated at 70 °C for 3 days. After cooling to room temperature, the pH of the solution was adjusted to 6 with addition of a sodium methoxide solution. The mixture was then evaporated to dryness in vacuo to afford a foamy solid. To this substance were added 160 mL of dry pyridine and tosyl chloride (50 g), and the mixture was stirred at 0 °C for 15 h. After being poured into 400 mL of ice/water, the mixture was extracted with 2 × 400 mL of CH_2Cl_2 and washed successively with 1 N HCl, saturated $NaHCO_3$ solution, and water. The organic solution was then dried and evaporated under vacuum to give 35.7 g of (3*S*-*trans*)-tetrahydro-5-(dimethoxymethyl)-3-furanol 4-methylbenzenesulfonate as a mobile liquid (93% yield): ^1H NMR (200 MHz, $CDCl_3$), δ 2.10 (m, 2 H, 4-H1, H2), 2.46 (s, 3 H, Ar-CH_3), 3.41 (s, 6 H, $(OCH_3)_2$), 3.92 (m, 2 H, 2-H1, H2), 4.20 (m, 2 H, 5-H, $CH(OMe)_2$), 5.13 (m, 1 H, 3-H), 7.35 (d, 2 H, J = 8 Hz, Ar-H3, H5), 7.80 (d, 2 H, J = 8 Hz, Ar-H2, H6).

A mixture of (3*S*-*trans*)-tetrahydro-5-(dimethoxymethyl)-3-furanol 4-methylbenzenesulfonate (10.0 g, 31.6 mmol), 1% CF_3CO_2H (50 mL), and dioxane (80 mL) in a 250-mL round-bottom flask fitted with a reflux condenser was warmed to 80 °C for 5 h. After cooling to room temperature, the solution was adjusted to pH 7 using 1 N NaOH, treated with $NaBH_4$ (1.2 g, 31.7 mmol), and allowed to stir at room temperature for an additional 30 min. The volatile solvents were partially evaporated, and the residue was extracted with EtOAc (100 mL × 3). The organic fraction was dried over $MgSO_4$, filtered, evaporated, and purified by flash column chromatography (60% EtOAc/40% hexane) to afford 3.6 g of recovered starting material and 2.7 g (49% yield based on recovered starting material) of 2 as a colorless oil which solidified upon refrigeration: ^1H NMR (200 MHz, $CDCl_3$) δ 1.98–2.14 (m, 3 H, 3-H1, H2, OH), 2.52 (s, 3 H, CH_3), 3.52, 3.82 (AB of ABX, 2 H, J_{AB} = 12 Hz, J_{AX} = 6 Hz, J_{BX} = 3 Hz, CH_2OH), 3.93, 4.04 (AB of ABX, 2 H, J_{AB} = 11 Hz, J_{AX} = 1 Hz, J_{BX} = 4 Hz, 5-H1, H2), 4.25 (m, 1 H, 2-H), 5.10 (m, 1 H, 4-H), 7.40 (d, 2 H, J = 9 Hz, Ar-H3, H5), 7.84 (d, 2 H, J = 9 Hz, Ar-H2, H6); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 21.68 (CH_3), 34.05 (C3-CH_2), 63.45 (CH_2OH), 72.95 (C5-CH_2), 78.44 (C1-CH), 81.86 (C4-CH), 127.78, 130.01 (Ar-CH), 145.08 (Ar-C-S); IR ($CHCl_3$) 3630, 3465, 3020, 1015 cm^{-1}; MS 241 (M^+ − CH_2OH).

(2*R*-*cis*)-4-(6-Amino-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (1, Iso-ddA). To a flame-dried argon-blanketed round-bottom flask equipped with a magnetic stir bar and condenser were added adenine (50 mg, 0.37 mmol), K_2CO_3 (100 mg, 0.73 mmol), 18-crown-6 (97 mg, 0.37 mmol), and DMF (10 mL). This mixture was allowed to stir at room temperature for 30 min under a stream of argon. A solution of (2*R*-*trans*)-tetrahydro-4-[[(4-methylphenyl)sulfonyl]oxy]-2-furanmethanol (2) (100 mg, 0.37 mmol) in 2 mL of DMF was then added dropwise to the stirring mixture; the resultant mixture was warmed to 80 °C and stirred at that temperature overnight. After removal of the solvent in vacuo, the solid residue was purified by flash column chromatography using a 10% MeOH/90% CH_2Cl_2 solution as eluant. The isolated material was further purified by reverse-phase HPLC using a gradient elution system of CH_3CN/H_2O to yield 30 mg (35%) of 1 as a white solid. An analytical sample was recrystallized from methanol: mp 183–185 °C; $[\alpha]_D$ +47.91° (c 0.67, MeOH); UV λ_{max} (methanol) 260 nm (ϵ 13 990); ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.09 (ddd, 1 H, J = 13, 8, 5 Hz, 3′-H1), 2.55 (m, 1 H, 3′-H2), 3.53, 3.61 (2 × m, 2 × 1 H, CH_2OH), 4.01 (m, 3 H, 5′-H1, H2, 2′-H), 4.93 (dd, 1 H, J = 5, 5 Hz, OH), 5.16 (m, 1 H, 4′-H), 7.21 (br s, 2 H, NH_2), 8.14, 8.25 (2 × s, 2 × 1 H, H-2, H-8); IR (KBr) 3450–3200, 3115, 1645, 1605 cm^{-1}; MS 235 (M^+). Anal. ($C_{10}H_{13}N_5O_2$) C, H, N.

(2*R*-*cis*)-4-Amino-1-[tetrahydro-5-(hydroxymethyl)-3-furanyl]-2(1*H*)-pyrimidinone (3, Iso-ddC). To a flame-dried argon-blanketed round-bottom flask equipped with a magnetic stir bar and condenser were added cytosine (41 mg, 0.37 mmol), K_2CO_3 (101 mg, 0.73 mmol), 18-crown-6 (97 mg, 0.37 mmol), and DMF (5 mL). This mixture was allowed to stir at room tem-

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perature for 30 min under a stream of argon. A solution of (2*R-trans*)-tetrahydro-4-[[(4-methylphenyl)sulfonyl]oxy]-2-furanmethanol (2) (100 mg, 0.37 mmol) in 2 mL of DMF was then added dropwise to the stirring mixture; the resultant mixture was warmed to 80 °C and stirred at that temperature overnight. After removal of the solvent in vacuo, the solid residue was purified by flash column chromatography using a 10% MeOH/90% CH_2Cl_2 solution as eluant. An analytically pure sample could be obtained using reverse-phase HPLC with a gradient elution system of CH_3CN/H_2O to yield 12 mg (15%) of 3 as a very hygroscopic white solid: $[\alpha]_D$ = +19.33° (*c* = 0.36, MeOH); UV λ_{max} (MeOH) 274 nm; UV λ_{max} (pH 1) 213, 283 nm; UV λ_{max} (pH 13) 230, 272 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 1.67 (ddd, 1 H, *J* = 13, 8, 5 Hz, 4'-H1), 2.36 (ddd, 1 H, *J* = 13, 8, 8 Hz, 4'-H2), 3.47, 3.58 (AB of ABX, 2 H, J_{AB} = 12 Hz, J_{AX} = 4 Hz, J_{BX} = 3 Hz, CH_2OH) 3.77 (d, 2 H, *J* = 5 Hz, 2'-H1, H2), 3.85 (m, 1 H, 5'-H), 4.85 (br, 1 H, OH), 5.14 (m, 1 H, 3'-H), 5.68 (d, 1 H, *J* = 7 Hz, 5-H), 6.97 (br, 2 H, NH_2), 7.67 (d, 1 H, *J* = 7 Hz, 6-H); IR (KBr) 3340, 3200, 1650, 1605 cm^{-1}; MS 211 (M^+). Anal. ($C_9H_{13}N_3O_3 \cdot {}^1/_3H_2O$) C, H, N. O-Alkylated side product: R_f (20% MeOH/80% CH_2Cl_2) 0.80; ^1H NMR (400 MHz, Me_2SO-d_6) δ 1.72 (ddd, 1 H, *J* = 13, 7, 3 Hz, 4'-H1), 2.31 (ddd, 1 H, *J* = 13, 7, 7 Hz, 4'-H2), 3.41 (ddd, 1 H, *J* = 11, 6, 6 Hz, CH of CH_2OH), 3.47 (ddd, 1 H, *J* = 11, 6, 5 Hz, CH of CH_2OH), 3.82 (m, 3 H, 2'-H1, H2, 5'-H), 4.72 (t, 1 H, *J* = 6 Hz, OH), 5.30 (m, 1 H, 3'-H), 6.06, 7.83 (d, 2 H, *J* = 6 Hz, H-5, H-6), 6.84 (br, 2 H, NH_2).

(2*R-cis*)-1-[Tetrahydro-5-(hydroxymethyl)-3-furanyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (4, Iso-ddT). To a flame-dried argon-blanketed round-bottom flask equipped with a magnetic stir bar and condenser were added thymine (92 mg, 0.73 mmol), K_2CO_3 (202 mg, 1.46 mmol), 18-crown-6 (194 mg, 0.73 mmol), and DMF (15 mL). This mixture was allowed to stir at room temperature for 30 min under a stream of argon. A solution of (2*R-trans*)-tetrahydro-4-[[(4-methylphenyl)sulfonyl]oxy]-2-furanmethanol (2) (200 mg, 0.73 mmol) in 2 mL of DMF was then added dropwise to the stirring mixture; the resultant mixture was warmed to 80 °C and stirred at that temperature overnight. After removal of the solvent in vacuo, the solid residue was purified by flash column chromatography using a 10% MeOH/90% CH_2Cl_2 solution as eluant. The isolated material was further purified by reverse-phase HPLC using a gradient elution system of CH_3CN/H_2O to yield 18 mg (11%) of 4 as a hygroscopic white solid: mp 85–86 °C; $[\alpha]_D$ = −11.41° (*c* = 0.26, MeOH); UV λ_{max} (pH 1) 206, 272 nm; UV λ_{max} (pH 7) 272 nm; UV λ_{max} (pH 13) 269 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 1.77 (s, 3 H, CH_3), 2.38 (m, 1 H, 4'-H1), 2.51 (m, 1 H, 4'-H2), 3.50, 3.65 (2 × m, 2 × 1 H, CH_2OH), 3.75, 3.87 (AB of ABX, 2 H, J_{AB} = 10 Hz, J_{AX} = 6.5 Hz, J_{BX} = 2.0 Hz, 2'-H1, H2), 3.84 (m, 1 H, 5'-H), 4.97 (dd, 1 H, *J* = 5.5, 5.5 Hz, OH), 5.11 (m, 1 H, 3'-H), 7.63 (s, 1 H, H-6), 11.34 (br, 1 H, NH); IR (KBr) 3400, 1940, 1690, 1120–1035 cm^{-1}; MS 226 (M^+). Anal. ($C_{10}H_{14}N_2O_4 \cdot {}^1/_3H_2O$) C, H, N. Bis-alkylated side product: R_f (10% MeOH/90% CH_2Cl_2) 0.35; ^1H NMR (200 MHz, CD_3OD) δ 1.90 (m, 1 H, 4'-H), 1.98 (s, 3 H, CH_3), 2.16 (m, 1 H, 4'-H), 2.34 (m, 1 H, 4'-H), 2.53 (m, 1 H, 4'-H), 3.62–4.26 (m, 10 H), 5.32 (br, 1 H, 3'-H), 5.74 (m, 1 H, 3'-H), 7.24 (s, 1 H, H-6).

(2*R-cis*)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (5). To a flame-dried argon-blanketed round-bottom flask equipped with a magnetic stir bar and condenser were added 2-amino-6-chloropurine (63 mg, 0.37 mmol), K_2CO_3 (100 mg, 0.73 mmol), 18-crown-6 (97 mg, 0.37 mmol), and DMF (10 mL). This mixture was allowed to stir at room temperature for 60 min under a stream of argon. A solution of (2*R-trans*)-tetrahydro-4-[[(4-methylphenyl)sulfonyl]oxy]-2-furanmethanol (2) (100 mg, 0.37 mmol) in 2 mL of DMF was then added dropwise to the stirring mixture; the resultant mixture was warmed to 80 °C and stirred at that temperature overnight. After removal of the solvent in vacuo, the solid residue was purified by flash column chromatography using a 5% MeOH/95% CH_2Cl_2 solution as eluant. The isolated material was further purified by reverse-phase HPLC using a gradient elution system of CH_3CN/H_2O to yield 30 mg (30%) of 5 as a white solid. Recrystallization from EtOH afforded analytically pure material: mp 208–210 °C; $[\alpha]_D$ = +7.74° (*c* = 0.155, MeOH); UV λ_{max} (EtOH) 221, 240, 303 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.04 (ddd, 1 H, *J* = 13, 8, 4.5 Hz, 3'-H1), 2.50 (m, 1 H, 3'-H2), 3.52, 3.62 (2 × m, 2 × 1 H, CH_2OH), 3.91, 4.04 (AB of ABX, 2 H, J_{AB} = 10 Hz, J_{AX} = 6 Hz, J_{BX} = 3 Hz, 5'-H1, H2), 3.98 (m, 1 H, 2'-H), 4.94 (dd, 1 H, *J* = 5, 5 Hz, OH), 5.03 (m, 1 H, 4'-H), 6.92 (br s, 2 H, NH_2), 8.26 (s, 1 H, H-8); IR (KBr) 3430–3205, 1638, 1615, 1560 cm^{-1}; MS 269 (M^+). Anal. ($C_{10}H_{12}N_5O_2Cl$) C, H, N.

(3*R-cis*)-2-Amino-1,9-dihydro-9-[tetrahydro-5-(hydroxymethyl)-3-furanyl]-6*H*-purin-6-one (6, Iso-ddG). A solution of (2*R-cis*)-4-(2-amino-6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (5) (170 mg, 0.63 mmol) in 1 N HCl (25 mL) was heated to reflux temperature for 3 h. The reaction mixture was neutralized with 40% KOH solution and then evaporated to afford a white solid. Purification via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system afforded 65 mg (41%) of 6 as a white solid. Analytically pure material was obtained by recrystallization from H_2O: mp 305–309 °C; $[\alpha]_D$ = −19.09° (*c* = 0.11, H_2O); UV λ_{max} (pH 0) 205, 253, 278 nm; UV λ_{max} (pH 6) 252, 270 nm; UV λ_{max} (pH 13) 255, 268 nm; ^1H NMR (200 MHz, CD_3OD) δ 2.04 (ddd, 1 H, *J* = 15, 8, 5 Hz, 4'-H1), 2.54 (ddd, 1 H, *J* = 15, 8, 8 Hz, 4'-H2), 3.57, 3.74 (AB of ABX, 2 H, J_{AB} = 12 Hz, J_{AX} = 5 Hz, J_{BX} = 3 Hz, CH_2OH), 3.90–4.08 (m, 3 H, 2'-H1, H2; 5'-H), 5.03 (m, 1 H, 3'-H), 7.86 (s, 1 H, H-8); IR (KBr) 3400, 1690 cm^{-1}; MS 237 (M + H). Anal. ($C_{10}H_{13}N_5O_3 \cdot {}^3/_4H_2O$) C, H, N.

(3*R-cis*)-1,9-Dihydro-9-[tetrahydro-5-(hydroxymethyl)-3-furanyl]-6*H*-purin-6-one (7, Iso-ddI). A solution of (2*R-cis*)-4-(6-amino-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (1) (61 mg, 0.26 mmol) in glacial acetic acid (5 mL) was treated with $NaNO_2$ (90 mg, 1.29 mmol). This mixture was allowed to stir at room temperature overnight, at which time TLC showed the presence of starting material. Additional $NaNO_2$ (90 mg, 1.29 mmol) was added to the reaction mixture, which continued to stir for 24 h further. Evaporation of the solvent, followed by flash column chromatography (10% MeOH/90% CH_2Cl_2) afforded 45 mg (73%) of 7 as a white solid. The product was dissolved in 10 mL of MeOH and stirred with IR45 resin for 3 h. Filtration, evaporation, and recrystallization from EtOH afforded 7 free of HOAc residue: mp 220 °C; $[\alpha]_D$ = +36.04° (*c* = 0.23, MeOH); UV λ_{max} (MeOH) 248 nm; UV λ_{max} (pH 1) 248 nm; UV λ_{max} (pH 13) 253 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.05 (ddd, 1 H, *J* = 13, 8, 5 Hz, 4'-H1), 2.54 (m, 1 H, 4'-H2), 3.53, 3.62 (br AB, 2 H, J_{AB} = 11 Hz, CH_2OH), 3.93–4.02 (m, 3 H, 2'-H1, H2, 5'-H), 4.94 (br s, 1 H, OH), 5.16 (m, 1 H, 3'-H), 8.05, 8.20 (2 × s, 2 × 1 H, H-2, H-8), 12.28 (br, 1 H); IR (KBr) 3400, 3150, 1698, 1682, 1591 cm^{-1}; MS 236 (M^+). Anal. ($C_{10}H_{12}N_4O_3$) C, H, N.

(2*R-cis*)-4-(6-Chloro-9*H*-purin-9-yl)tetrahydrofuran-2-methanol (8). To a flame-dried argon-blanketed round-bottom flask equipped with a magnetic stir bar and condenser were added 6-chloropurine (435 mg, 2.8 mmol), K_2CO_3 (870 mg, 6.3 mmol), 18-crown-6 (100 mg, 0.38 mmol), and DMF (40 mL). This mixture was allowed to stir at room temperature for 30 min under a stream of argon. A solution of 2 (870 mg, 3.2 mmol) in DMF (10 mL) was then added dropwise to the stirring mixture. The resultant mixture was warmed to 80 °C and stirred at that temperature overnight. After filtration, the solvent was removed in vacuo and the resultant product purified by flash column chromatography using a 5% MeOH/95% CH_2Cl_2 solution as eluant. This material was further purified by reverse-phase HPLC using a CH_3CN/H_2O gradient elution system to yield 178 mg (25%) of 8 as a white solid: mp 143–144 °C; $[\alpha]_D$ = +26.80° (*c* = 0.05, MeOH); UV λ_{max} (MeOH) 264 nm; UV λ_{max} (pH 1) 265 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.15 (ddd, 1 H, *J* = 13, 8, 4 Hz, 3'-H1), 2.64 (ddd, 1 H, *J* = 13, 8, 6 Hz, 3'-H2), 3.54, 3.66 (AB of ABMX, 2 H, J_{AB} = 12 Hz, J_{AM} = 5 Hz, J_{AX} = 5 Hz, J_{BM} = 5 Hz, J_{BX} = 4 Hz, CH_2OH), 3.98, 4.15 (AB of ABX, 2 H, J_{AB} = 10 Hz, J_{AX} = 6 Hz, J_{BX} = 2 Hz, 5'-H1, H2), 4.03 (m, 1 H, 2'-H), 4.97 (dd, 1 H, *J* = 5, 5 Hz, OH), 5.34 (m, 1 H, 4'-H), 8.80, 8.82 (2 × s, 2 × 1 H, H-2, H-8); IR (KBr) 3450, 1591, 1565, 1555 cm^{-1}; MS 254 (M^+). Anal. ($C_{10}H_{11}N_4O_2Cl$) C, H, N.

Alternative Preparation of 8. A solution of (2*R-trans*)-tetrahydro-4-[[(4-methylphenyl)sulfonyl]oxy]-2-furanmethanol (2) (150 mg, 0.55 mmol) in DMF (3 mL) was cooled to −5 °C and treated with NaN_3 (90 mg, 1.38 mmol). The solution was allowed to warm to room temperature and then heated at 70 °C for 5 h. After removal of DMF, the residue was partitioned between EtOAc (50 mL) and H_2O (10 mL). The organic layer was removed and then washed with saturated $NaHCO_3$ solution (10 mL) and saturated NaCl solution (10 mL). The aqueous layers were combined

and back-washed with EtOAc (50 mL × 3). All organic fractions were pooled, dried over $MgSO_4$, filtered, and evaporated to provide 71 mg of the corresponding azide as a colorless oil. This material was combined with MeOH (5 mL) and 10% Pd/C (15 mg, cat.) in a flame-dried 25-mL round-bottom flask. The flask was blanketed with H_2 and allowed to stir at room temperature for 3 h. The catalyst was removed via filtration, and the solvent evaporated to provide 54 mg (84%) of (2*R-cis*)-4-aminotetrahydro-2-furanmethanol as a colorless oil which required no further purification: ^1H NMR (400 MHz, $CDCl_3$) δ 1.73 (ddd, 1 H, J = 13, 4, 2 Hz, 3-H1), 2.26 (ddd, 1 H, J = 13, 9, 6 Hz, 3-H2), 2.76 (br s, 3 H, NH_2, OH), 3.53, 3.82 (AB of ABX, 2 H, J_{AB} = 12 Hz, J_{AX} = 3 Hz, J_{BX} = 2 Hz, CH_2OH), 3.71–3.78 (m, 3 H, 5-H1, H2, 4-H), 4.27 (m, 1 H, 2-H); IR ($CHCl_3$) 3635, 1015 cm^{-1}; MS 99 ($M^+ - H_2O$).

To a 50-mL round-bottom flask fitted with a reflux condenser was added (2*R-cis*)-4-aminotetrahydro-2-furanmethanol (184 mg, 1.57 mmol), 5-amino-4,6-dichloropyrimidine (770 mg, 4.70 mmol), triethylamine (0.44 mL, 3.16 mmol), and *tert*-butyl alcohol (16 mL). The reaction mixture was heated to reflux temperature under an atmosphere of argon for 72 h, at which time the volatile materials were removed by rotary evaporation. The residue was dissolved in 100 mL of EtOAc, washed with saturated $NaHCO_3$ solution (50 mL) and saturated NaCl solution (50 mL), and then dried over $MgSO_4$. After filtration and evaporation, the product was purified by flash column chromatography (50% EtOAc/hexane) to yield 220 mg (58%) of (2*R-cis*)-4-[(5-amino-6-chloro-4-pyrimidinyl)amino]tetrahydrofuran-2-methanol]: UV λ_{max} (MeOH) 206, 265, 295 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 1.90 (br dd, 1 H, J = 14, 4 Hz, 3′-H1), 2.48 (ddd, 1 H, J = 14, 10, 7 Hz, 3′-H2), 2.89 (br s, 1 H, OH), 3.50 (br s, 2 H, NH_2), 3.62, 3.94 (br AB, 2 H, J = 12 Hz, CH_2OH), 3.84, 3.93 (AB of ABX, 2 H, J_{AB} = 9.5 Hz, J_{AX} = 4 Hz, J_{BX} = 1 Hz, 5′-H1, H2), 4.21 (m, 1 H, 2′-H), 4.72 (m, 1 H, 4′-H), 6.24 (br d, 1 H, J = 7 Hz, NH), 8.03 (s, 1 H, Ar-H); IR ($CHCl_3$) 3625, 3350, 1618, 1582 cm^{-1}; MS 244 (M^+).

A solution of (2*R-cis*)-4-[(5-amino-6-chloro-4-pyrimidinyl)-amino]tetrahydrofuran-2-methanol (220 mg, 0.90 mmol) and diethoxymethyl acetate (5 mL) in a 10-mL round-bottom flask fitted with a reflux condenser was heated to 100 °C for 120 h. The volatile materials were removed via rotary evaporation, and the residue was recombined with toluene (5 mL) and *p*-toluenesulfonic acid (5 mg, cat.). After stirring at room temperature for 1 h and removal of toluene, the contents of the flask were purified by flash column chromatography (10% MeOH/90% CH_2Cl_2) to afford 109 mg (48%) of **8** as a white solid.

(2*R-cis*)-Tetrahydro-4-[6-(methylamino)-9*H*-purin-9-yl]-2-furanmethanol (9). A solution of 40 mg (0.157 mmole) of (2*R-cis*)-4-(6-chloro-9*H*-purin-9-yl)tetrahydrofuran-2-methanol (**8**) in 10 mL of 40% methylamine in water was sealed in a steel bomb and heated in an oil bath at 60 °C for 5 days. The mixture was cooled, evaporated, and purified by flash column chromatography using 10% MeOH/90% CH_2Cl_2 solution as eluant, which gave 35 mg (89%) of the desired product (**9**). An analytically pure sample could be obtained via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system: mp 273–275 °C; $[\alpha]_D$ = 0° (c = 0.1, MeOH); UV λ_{max} (EtOH) 208, 265 nm; ^1H NMR (200 MHz, CD_3OD) δ 2.08 (ddd, 1 H, J = 3, 8, 13 Hz, 3′-H1), 2.58 (ddd, 1 H, J = 8, 8, 13 Hz, 3′-H2), 3.05 (br s, 3 H, NCH_3), 3.57, 3.75 (AB of ABX, 2 H, J_{AB} = 13 Hz, J_{AX} = 5 Hz, J_{BX} = 4 Hz, CH_2OH), 3.94–4.10 (m, 3 H, 5′-H1, H2, 2′-H), 5.17 (m, 1 H, 4′-H), 8.16, 8.23 (2 × s, 2 × 1 H, H-2, H-8); IR (KBr) 3400, 3200, 1678 cm^{-1}; MS 249 (M^+). Anal. ($C_{11}H_{15}N_5O_2 \cdot H_2O$) C, H, N.

(2*R-cis*)-Tetrahydro-4-(9*H*-purin-9-yl)-2-furanmethanol (10). A mixture of (2*R-cis*)-4-(6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (**8**) (40 mg, 0.16 mmol), sodium acetate (20 mg, 0.5 mmol), and Pd/C (5 mg, cat.) in absolute ethanol (10 mL) was hydrogenated at 45 psi for 16 h in a Parr shaker. The mixture was then filtered, evaporated, and purified by flash column chromatography (5% MeOH/95% CH_2Cl_2) to yield 33 mg (95%) of **10** as a colorless oil. An analytically pure sample could be obtained via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system as eluant: mp 82–83 °C; $[\alpha]_D$ = +17.52° (c = 0.31, MeOH); UV λ_{max} 263 nm; ^1H NMR (200 MHz, CD_3OD) δ 2.15 (ddd, 1 H, J = 3.5, 8, 13.5 Hz, 3′-H1), 2.64 (ddd, 1 H, J = 8, 8, 13.5 Hz, 3′-H2), 3.58, 3.79 (AB of ABX, 2 H, J_{AB} = 12 Hz, J_{AX} = 4 Hz, J_{BX} = 3.5 Hz, CH_2OH), 4.02, 4.16 (AB of ABX, 2 H, J_{AB} = 10 Hz, J_{AX} = 6 Hz, J_{BX} = 2.5 Hz, 5′-H1, H2), 4.10 (m, 1 H, 2′-H), 5.40 (m, 1 H, 4′-H), 8.72, 8.86, 8.99 (3 × s, 3 × 1 H, H-2, H-6, H-8); IR (KBr) 3305, 1597, 1582 cm^{-1}; MS 221 (m/z). Anal. ($C_{10}H_{12}N_4O_2 \cdot {}^1/_4H_2O$) C, H, N.

(2*R-cis*)-4-(6-Fluoro-9*H*-purin-9-yl)tetrahydrofuran-2-methanol (11). Under argon, to a stirred solution of 114 mg (0.448 mmol) of (2*R-cis*)-4-(6-chloro-9*H*-purin-9-yl)tetrahydrofuran-2-methanol (**8**) in 4 mL of dry dimethylformamide was added 18 μL (0.2 mmol) of trimethylamine (distilled over calcium hydride) followed by 383 mg (6.6 mmol) of potassium fluoride (dried at 100 °C under high vacuum overnight). The resulting suspension was stirred under argon at room temperature overnight, and then the solids were removed via filtration. The filtrate was evaporated to dryness, and the residue purified by flash column chromatography using a 7% MeOH/93% CH_2Cl_2 solution as eluant to yield 61.8 mg (58%) of the desired product. An analytically pure sample could be obtained via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system: mp 114–115 °C; $[\alpha]_D$ = +18.26 (c = 1.0, MeOH); UV λ_{max} (EtOH) 248 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.15 (ddd, 1 H, J = 13, 8, 5 Hz, 3′-H1), 2.64 (ddd, 1 H, J = 13, 8, 8 Hz, 3′-H2), 3.55, 3.66 (2 × m, 2 × 1 H, CH_2OH), 3.97–4.06 (m, 2 H, 5′-H1, 2′-H), 4.14 (dd, 1 H, J = 10, 2.5 Hz, 5′-H2), 4.97 (app t, 1 H, J = 5 Hz, OH), 5.37 (m, 1 H, 4′-H), 8.70, 8.80 (2 × s, 2 × 1 H, H-2, H-8); ^{19}F NMR (376 MHz, $CDCl_3$) δ −72.7; IR (KBr) 3625, 3340, 1622, 1572 cm^{-1}; MS 239 (M + H). Anal. ($C_{10}H_{11}N_4O_2F \cdot {}^1/_4H_2O$) C, H, N.

(3*R-cis*)-1,9-Dihydro-9-[tetrahydro-5-(hydroxymethyl)-3-furanyl]-6*H*-purine-6-thione (12). A stirred solution of 150 mg (0.59 mmol) of (2*R-cis*)-4-(6-chloro-9*H*-purin-9-yl)tetrahydrofuran-2-methanol (**8**) and 54 mg (0.71 mmol) of thiourea in 10 mL of propyl alcohol was heated to reflux temperature for 3 h. The mixture was cooled, and the solids which precipitated during heating were collected, washed with propyl alcohol, and dried overnight at room temperature under high vacuum to give 128.5 mg (86%) of **12** as a white solid: mp 294–296 °C dec; $[\alpha]_D$ = +57.33° (c = 0.30, MeOH); UV λ_{max} (H_2O) 213, 321 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.06 (ddd, J = 13, 8, 5 Hz, 1 H, 4′-H1), 2.59 (ddd, J = 13, 8, 8 Hz, 1 H, 4′-H2), 3.52, 3.63 (br AB, 2 H, J = 10.5 Hz, CH_2OH), 3.94, 4.07 (AB of ABX, 2 H, J_{AB} = 10 Hz, J_{AX} = 6 Hz, J_{BX} = 2 Hz, 2′-H1, H2), 3.99 (m, 1 H, 5′-H), 4.96 (br s, 1 H, OH), 5.18 (br d, 1 H, J = 5 Hz, 3′-H), 8.21, 8.41 (2 × s, 2 × 1 H, H-2, H-8), 13.72 (br, 1 H, NH); IR (KBr) 3415, 1598 cm^{-1}; MS 252 (M^+). Anal. ($C_{10}H_{12}N_4O_2S$) C, H, N.

(2*R-cis*)-Tetrahydro-4-[6-(methylthio)-9*H*-purin-9-yl]-2-furanmethanol (13). To a stirred suspension of 50 mg (0.198 mmol) of (3*R-cis*)-1,9-dihydro-9-[tetrahydro-5-(hydroxymethyl)-3-furanyl]-6*H*-purine-6-thione (**12**) in 2 mL of water at room temperature was added 1 drop of ammonium hydroxide which afforded a clear solution. Methyl iodide (16.5 μL, 0.265 mmol) was added, and the solution was stirred at room temperature for 2 h and then lyophilized overnight. The residue was dissolved in 1 mL of water and stirred with Ag 2- ×8 OH^- resin for 30 min. After removal of the resin by filtration and washing with water, the resulting solution was lyophilized overnight to give 45 mg (85%) of **13** as a white, very hygroscopic solid. An analytically pure sample could be obtained via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system: UV λ_{max} (MeOH) 216, 230 (infl), 282, 289 nm; ^1H NMR (400 MHz, CD_3OD) δ 2.20 (m, 1 H, 3′-H1), 2.70 (m, 4 H, 3′-H2, SCH_3), 3.66, 3.85 (AB of ABX, J_{AB} = 12 Hz, J_{AX} = 4 Hz, J_{BX} = 3 Hz, 2 H, CH_2OH), 4.08, 4.20 (AB of ABX, J_{AB} = 10 Hz, J_{AX} = 6 Hz, J_{BX} = 1 Hz, 2 H, 5′-H1, H2), 4.13 (m, 1 H, 2′-H), 5.38 (m, 1 H, 4′-H), 8.59, 8.68 (2 × s, 2 × 1 H, H-2, H-8); IR (KBr) 3380, 1572 cm^{-1}; MS 266 (M^+). Anal. ($C_{11}H_{14}N_4O_2S \cdot {}^1/_4H_2O$) C, H, N.

(2*R-cis*)-4-(2,6-Diamino-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (14). To (2*R-cis*)-4-(2-amino-6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (**5**) (40 mg, 0.15 mmol) in a stainless steel bomb was condensed 40 mL of liquid NH_3. The bomb was sealed and heated to 60 °C for 60 h. The contents of the bomb were diluted with methanol, evaporated, and then purified using flash column chromatography (20% MeOH/80% CH_2Cl_2) to afford 22 mg (59%) of **14** as a white solid. An analytically pure sample could be obtained via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system: mp 156–158 °C; $[\alpha]_D$ = +11.81° (c = 0.11, MeOH); UV λ_{max} (MeOH) 213, 254, 280 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.01 (ddd, 1 H, J = 12, 9,

5 Hz, 3'-H1), 2.54 (m, 1 H, 3'-H2), 3.53, 3.58 (br AB, 2 H, J = 12 Hz, CH_2OH), 3.94 (m, 3 H, 5'-H1, H2, 2'-H), 4.90 (m, 1 H, OH), 4.95 (m, 1 H, 4'-H), 5.75 (br s, 2 H, NH_2), 6.64 (br s, 2 H, NH_2), 7.83 (s, 1 H, H-8); IR (KBr) 3440–3200, 1630, 1595 cm^{-1}; MS 250 (M^+). Anal. ($C_{10}H_{14}N_6O_2\cdot{}^1/_3H_2O$) C, H, N.

(2*R*-*cis*)-4-(2-Amino-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (15). A mixture of (2*R*-*cis*)-4-(2-amino-6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (5) (63 mg, 0.23 mmol), sodium acetate (20 mg, 0.5 mmol), and Pd/C (5 mg, cat.) in absolute ethanol (10 mL) was hydrogenated at 35 psi for 24 h in a Parr shaker. The mixture was then filtered, evaporated, and purified by flash column chromatography (15% MeOH/85% CH_2Cl_2) to yield 42 mg (78%) of 15 as a white solid. An analytically pure sample could be obtained via reverse-phase HPLC using a CH_3CN/H_2O gradient elution system: mp 67–69 °C; $[\alpha]_D$ = −4.30° (c = 0.16, MeOH); UV λ_{max} (MeOH) 221, 245, 309 nm; ^1H NMR (200 MHz, CD_3OD) δ 2.16 (ddd, 1 H, J = 13.5, 8, 5 Hz, 3'-H1), 2.64 (ddd, 1 H, J = 13.5, 8, 8 Hz, 3'-H2), 3.63 (br s, 1 H, OH), 3.65, 3.83 (AB of ABX, 2 H, J_{AB} = 12.5 Hz, J_{AX} = 5 Hz, J_{BX} = 3 Hz, CH_2OH), 4.00–4.19 (m, 3 H, 5'-H1, H2, 2'-H), 5.22 (m, 1 H, 4'-H), 8.31, 8.54 (2 × s, 2 × 1 H, H-2, H-8); IR (KBr) 3400–3205, 2925, 2870, 1612, 1578 cm^{-1}; MS 235 (M^+). Anal. ($C_{10}H_{13}N_5O_2\cdot{}^1/_2H_2O$) C, H, N.

(2*R*-*cis*)-4-(2-Amino-6-fluoro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (16). Under argon, to a stirred solution of 32.7 mg (0.121 mmol) of (2*R*-*cis*)-4-(2-amino-6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (5) in 2 mL of dry dimethylformamide was added 4.9 μL (0.054 mmol) of trimethylamine (distilled over calcium hydride) followed by 98.7 mg (1.7 mmol) of potassium fluoride (dried at 100 °C under high vacuum overnight). The resulting suspension was heated under argon at 40 °C for 7 days. After removal of solids the filtrate was evaporated to dryness and the residue purified by flash column chromatography using an 8% MeOH/92% CH_2Cl_2 solution as eluant to provide 14 mg (46%) of the desired product as a white solid: mp 188–189 °C; $[\alpha]_D$ = 0° (c = 0.24, MeOH); UV λ_{max} (EtOH) 216, 243, 250 (sh), 288 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 2.04 (ddd, J = 13, 8, 5 Hz, 1 H, 3'-H1), 2.50 (m, obscured by solvent, 3'-H2), 3.52, 3.61 (AB of ABMX, J_{AB} = 11 Hz, J_{AM} = 5 Hz, J_{AX} = 5.5 Hz, J_{BM} = 5 Hz, J_{BX} = 5.5 Hz, 2 H, CH_2OH), 3.91, 4.02 (AB of ABX, J_{AB} = 9.5 Hz, J_{AX} = 6 Hz, J_{BX} = 3 Hz, 2 H, 5'-H1, H2), 3.97 (m, 1 H, 2'-H), 4.94 (app t, J = 5.5 Hz, 1 H, OH), 5.05 (m, 1 H, 4'-H), 6.92 (br s, 2 H, NH_2), 8.23 (s, 1 H, H-8); ^{19}F NMR (376 MHz, Me_2SO-d_6) δ −72.5; IR (KBr) 3445–3210, 1642 cm^{-1}; MS 253 (M^+). Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N.

(3*R*-*cis*)-2-Amino-1,9-dihydro-9-[tetrahydro-5-(hydroxymethyl)-3-furanyl]-6*H*-purine-6-thione (17). A solution of 100 mg (0.37 mmol) of (2*R*-*cis*)-4-(2-amino-6-chloro-9*H*-purin-9-yl)tetrahydro-2-furanmethanol (5) and 34 mg (0.445 mmol) of thiourea in 10 mL of propyl alcohol was heated to reflux for 6 h and then kept at room temperature overnight. Solids which precipitated were collected to give 60 mg of the crude product. Further purification by recrystallization from methanol–water afforded 48 mg (48%) of 17 as a white solid: mp 295–298 °C dec; $[\alpha]_D$ = +31.6° (c = 0.26, MeOH); UV λ_{max} (MeOH) 207, 260, 344 nm; ^1H NMR (400 MHz, Me_2SO-d_6) δ 1.98 (m, 1 H, 4'-H1), 2.54 (m, 1 H, 4'-H2), 3.52, 3.60 (2 × m, 2 × 1 H, CH_2OH), 3.85–4.02 (m, 3 H, 2'-H1, H2, 5'-H), 4.94 (m, 2 H, OH, 3'-H), 6.68 (br s, 2 H, NH_2), 8.02 (s, 1 H, H-8), 11.88 (br s, 1 H, NH); IR (KBr) 3410–3080, 1628, 1602, 1555 cm^{-1}; MS 267 (M^+). Anal. ($C_{10}H_{13}N_5O_2S\cdot{}^3/_4H_2O$) C, H, N.

Determination of HIV-1 Gag Protein Production in PHA-Stimulated Human PBM. Unfractionated peripheral blood mononuclear cells (PBM) were obtained by Ficoll-Hypaque gradient centrifugation of heparinized venous blood obtained from a normal volunteer. PBM were then stimulated with phytohemagglutinin (5 μg/mL) and incubated at 37 °C in complete media (RPMI 1640, 15% undialyzed, heat-inactivated fetal bovine serum, 15 units/mL recombinant Interleukin-2 (Advanced Biotechnologies Inc., Columbia, MD), 4 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin) for 24 h before cells were washed with RPMI at 4 °C and resuspended in complete media and plated at 5 × 10^5 cells per well in 2 mL of media. A twenty 50% tissue culture infectious dose ($TCID_{50}$) of HIV-$1_{(ERS\ 205\ pre)}$ isolated from an HIV-1 infected patient, and known to be AZT resistant in vitro, was used to infect the PHA-PBM and the appropriate amount of drug tested was added to each well. Cells were incubated at 37 °C for 7 days with removal of half of the culture media volume on day 4 and reconstitution with fresh complete media and appropriate quantity of drug. p24 gag protein production was measured from culture supernatant collected on day 7 by radioimmunoassay (DuPont).

Stability Studies of ddA and Iso-ddA. Each compound (1 mg) was dissolved in pH 3 buffer solution (1 mL) and incubated at 37 °C. Stability studies were conducted using HPLC analyses to measure the disappearance of starting material.

Stability Studies of ddG and Iso-ddG. Each compound (2 mg) was dissolved in 0.7 mL of a 0.1 N CD_3CO_2D solution, incubated at 37 °C, and monitored by 200-MHz NMR at specific time intervals. The disappearance of the resonance due to the C-8 proton of the guanine ring of ddG (7.58 ppm) and iso-ddG (7.69 ppm) with the concomitant appearance of a new resonance at 7.62 ppm due to the C-8 proton of the hydrolysis product guanine (G) was used to monitor the stability.

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