Notes

Synthesis and HIV Inhibition Activity of 2′,3′-Dideoxy-3′-*C*-hydroxymethyl Nucleosides

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A series if 2′,3′-dideoxy-3′-*C*-hydroxymethyl purine nucleosides were prepared based on the photochemical ring expansion of a chiral cyclobutanone precursor, (2*S*)-*trans*-2,3-bis[(benzoyloxy)methyl]cyclobutanone, in the presence of a 6-substituted purine. Both α- and β-anomers are produced in this transformation. Deprotection was effected by reaction of the photoadducts with saturated methanolic ammonia. Nine purine nucleosides were tested for their inhibitory effect of HIV IIIB virus on H9 cells. The 6-hexyloxy and adenine derivatives **4e**,**c**, respectively, appeared to be most effective at inhibiting viral reproduction with **4c** comparable in activity to ddI and AZT.

Introduction

Structurally modified nucleosides have become recognized as potential antiviral[2] and anticancer[3] agents. Specifically, certain 2′,3′-dideoxy ribonucleosides have been shown to protect ATH8 and MT4 cells against the cytopathology of the human immunodeficiency virus (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS).[4] These nucleosides include 6-amino-, 6-halo-, and 6-alkoxypurine-substituted derivatives. We have recently reported on a new method for the synthesis of 2′,3′-dideoxynucleosides[7] and 2′,3′-dideoxy-3′-*C*-hydroxymethyl nucleosides[5,6] based on the photochemical ring expansion of chiral cyclobutanones. Our method generates both anomers of these chiral nucleosides. In view of the activity reported for 6-alkoxypurine 2′,3′-dideoxy nucleosides as HIV inhibitors[4] (specifically the 6-(hexyloxy)purine derivatives) and the activity of some 2′,3′-dideoxy-3′-*C*-(hydroxymethyl)ribosidic nucleosides,[8] we were interested in extending our novel synthesis to include some other 6-substituted purine 2′,3′-dideoxy-3′-*C*-hydroxymethyl nucleosides and to test their HIV inhibitory effect. These latter compounds are close structural analogues to the natural 2′-deoxy nucleosides and the naturally occurring oxetanocin along with their derivatives which have been shown to be effective as anti-HIV-1[9] and anti-herpes virus agents.[10] Two other known nucleoside analogues, azidothymidine (zidovudine, AZT) and dideoxyinosine (didanosine, ddI), have been found to inhibit viral replication and to improve survival and reduce infection rate in patients with AIDS.[11,12] In this study we report on the synthesis of the α- and β-anomers of 6-chloro-, 6-amino-, 6-methoxy-, 6-hexyloxy-, and the unsubstituted purin-9-yl-2′,3′-dideoxy-3′-*C*-(hydroxymethyl)ribosides from the photochemical ring expansion of chiral ketone **1**, as well as the HIV-1 inhibition studies of these. Two of these compounds showed inhibitory effect on HIV-1 replication. Their effect was compared with that of AZT and ddI.

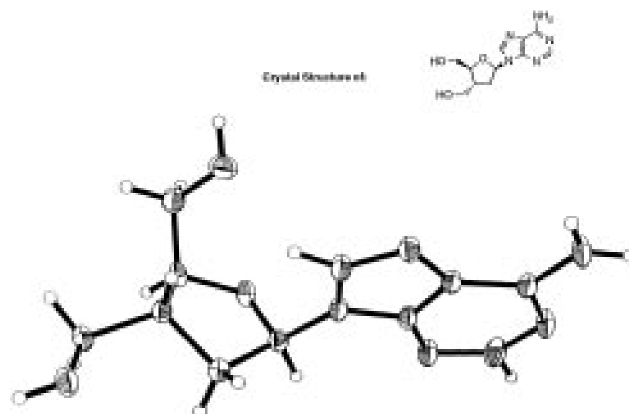

Figure 1. Crystal structure of nucleoside **4c**.

Synthesis

The chiral cyclobutanone **1** was prepared according to a modified procedure[6] as described by Ahmad.[13] The optical purity of this ketone was in excess of 98%.[6] Irradiation of ketone **1** in the presence of alcohols or reagents incorporating acidic N-H functions gives rise to ring expansion products and cycloelimination as the principal pathways. For example, irradiation of this ketone in the presence of 6-chloropurine resulted in the formation of an anomeric mixture of the protected nucleoside **2b** and the cycloelimination product **3** in 43% and 10% yields, respectively. Photoadducts **2a**,**b** were reported in our recent study of the photochemical ring expansion of ketone **1** in the presence of purine and 6-chloropurine, respectively.[6] Irradiation of ketone **1** in acetonitrile in the presence of 6-methoxypurine or 6-(hexyloxy)purine gave the corresponding photoadducts **2d**,**e** in 41% and 30% yields, respectively, along with some cycloelimination product **3** (15%).

The photoadduct **2b** could be converted to the adenine derivatives **4c** and **5c** with methanolic ammonia at 100

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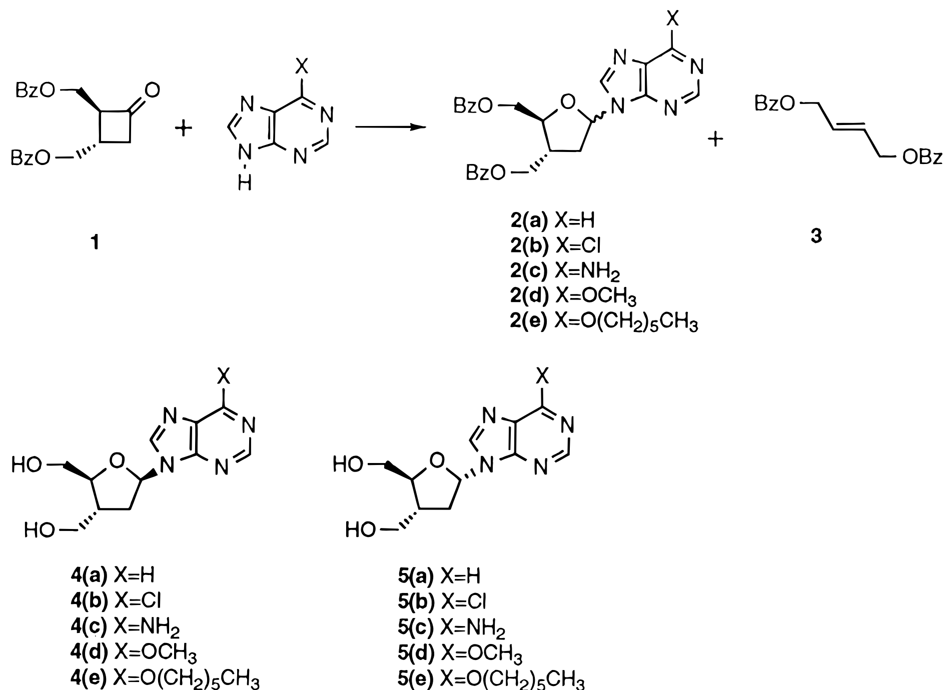
Scheme 1



°C in a sealed tube. Separation of **4c** and **5c** could be achieved by preparative HPLC. A crystal structure of **4c** was determined (see Figure 1) and confirmed the assignment of the regiochemistry for the oxacarbene insertion to the N(9)-H position of the purine ring in the original photoadduct **2b**. The crystal structure also confirms the absolute structure assignment of **4c** as the β-anomer. Under the conditions for debenzoylation employed (100 °C in sealed tube), ammonia acts as an efficient nucleophile to displace the chloro group. However, when debenzoylation was effected in methanolic ammonia under ambient conditions, no adenine nucleoside **4c** or **5c** was produced. Instead anomeric mixtures of the chloro nucleosides **4b** and **5b** and methoxy nucleosides **4d** and **5d** were formed in 19% and 41% yields, respectively. Under the latter conditions, methanol acts as a nucleophile to displace the chloride substituent.

Debenzoylation of the photoadducts **2a**,**d**,**e** gave an anomeric mixture of the corresponding deprotected nucleosides **4** and **5** in good yields. The anomeric mixture could be separated by preparative thin-layer chromatography. Assignment of the α- and β-anomeric configurations was based on characteristic ^{1}H NMR features in the 1-D and 2-D COSY and NOESY spectra. It has been shown that ring protons *syn* to the base are more deshielded than those which are *anti*.[14-16] The anomer which has a downfield shift for H-4′ was assigned to the α-anomer. In each of the α-anomers **5**, the H-4′ signal was observed at 4.1 ppm, whereas that for the β-anomers **4** appeared at 4.3 ppm. This anisotropic effect of the base was also apparent in the chemical shifts of both H-2′ and -2″ protons where the signals for the α-anomers were well resolved whereas the peaks for the β-anomers were overlapped. The former showed three separate multiplets for H-2′, H-2″, and H-3′, whereas the latter showed only two multiplets. Further confirmation was obtained from 2-D NOESY spectroscopy of the two anomeric series **4** and **5**. The β-anomers **4** exhibited a NOE correlation for the signals associated with H-1′ and H-4′, whereas no such correlation could be observed for the α-anomers **5**.

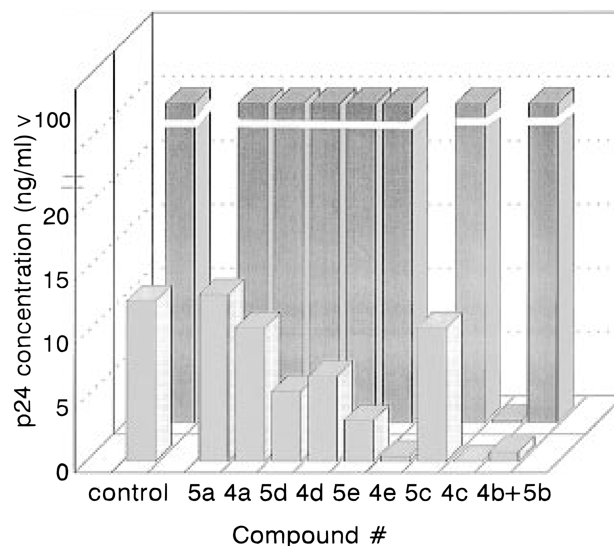


Figure 2. H9 cell infection by HIV-1 IIIB in the presence of 50 μM adenosine analogues: (light gray) day 7 and (dark gray) day 11.

Inhibition Studies

The effect of the nine purine nucleosides on HIV-1 replication was examined in H9 cells. All nine compounds showed no evidence of cytotoxicity to H9 cells while in culture over 14 days at the concentrations tested (1−100 μM; data not shown). Of the nine derivatives tested, only compounds **4c**,**e** significantly inhibited acute infection of H9 cells by the laboratory strain HIV IIIB (Figure 2). Although compounds **4b** and **5b** (anomeric mixture) inhibited virus infection at day 7, the effect was lost by day 11 in the culture (Figure 2). The antiviral effect of compounds **4c**,**e** was dose dependent with the higher concentration (50 μM) being more effective at inhibiting virus at day 14 (Figure 3).

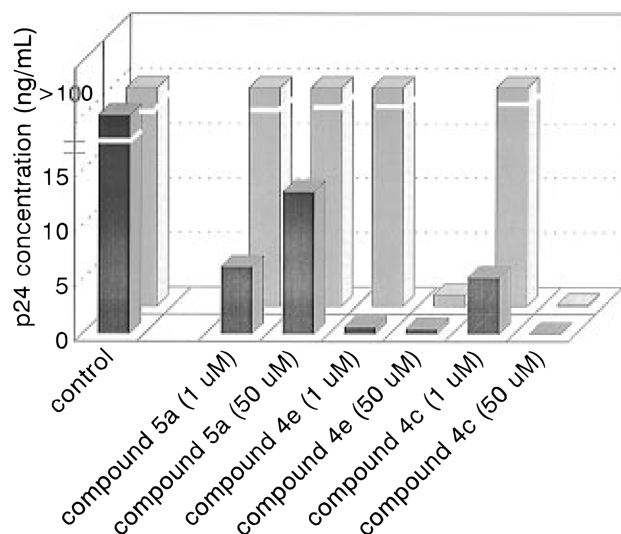


Figure 3. Results of H9 cell infection by HIV-1 IIIB in the presence of compounds **5a** and **4e**,**c**: (black) day 7 and (gray) day 14.

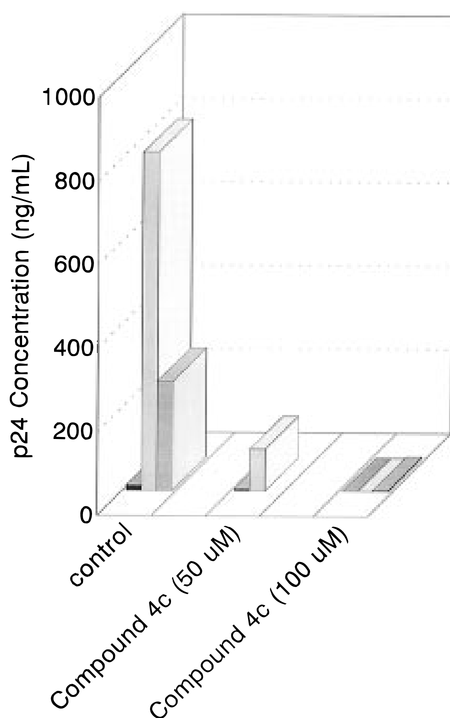


Figure 4. Summary of results of H9 cell infections by HIV-1 IIIB in the presence of compound **4c**: (black) day 7, (light gray) day 11, and (dark gray) day 14.

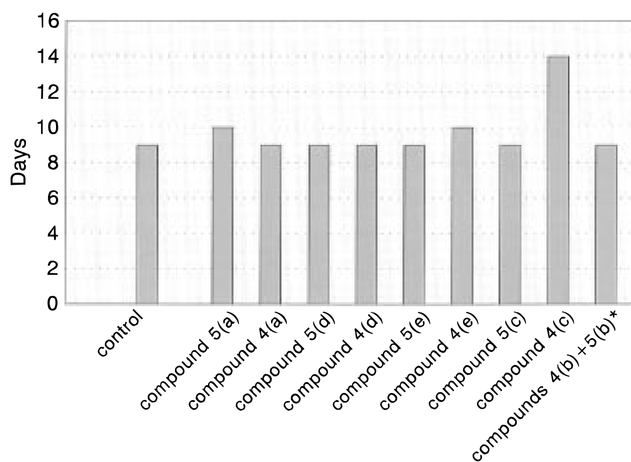


Figure 5. Day of infection syncytia occurred at 50 μM nucleoside (*tested as an anomeric mixture).

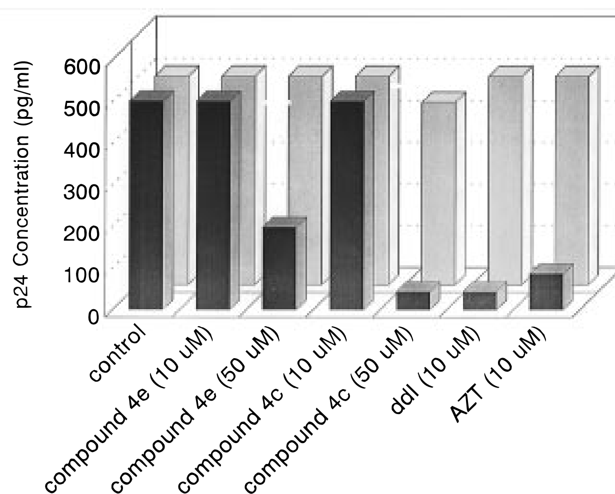


Figure 6. Results of H9 cell infection by HIV-1 IIIB in the presence of compounds **4c**,**e**, ddI, and AZT: (black) day 9 and (gray) day 15.

Compound **4c** appeared to be the most effective at inhibiting virus production with almost complete suppression at 100 μM (Figure 4) as well as being the only compound to significantly delay the onset of syncytial giant cells in culture (Figure 5). Nucleosides **4e**,**c** were then compared to the standard anti-HIV agents AZT and ddI for inhibition of HIV-1 replication (Figure 6). As shown in Figure 6, the inhibitory effect of compound **4c** is very similar to that observed for ddI and AZT at 10 μM.

In summary, adenosine analogue **4c** is not toxic to H9 cells at the concentration up to 100 μM and significantly inhibits HIV-1 replication in an acute infection system. Further studies of this compound would include examining its antiviral effect in human peripheral blood lymphocytes following infection with clinical isolates of HIV-1 as well as the antiviral effects against AZT and ddI resistant isolates of HIV-1.

Experimental Section

General. Melting points (mp) were determined on a Reichert melting point apparatus and are uncorrected. Ultraviolet (UV) absorption spectra were recorded on an HP8452a diode array spectrophotometer. Infrared (IR) spectra were obtained on a Perkin Elmer 1310 spectrometer as thin films or KBr pellets. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX 400 (400 MHz) spectrometer using samples in $CDCl_3$ solution with TMS internal standard or D_2O with TSP as internal standard. Mass spectra (MS) were recorded at 70 eV on a Kratos profile mass spectrometer. High-resolution mass spectra (HRMS) were obtained at the McMaster Regional Centre for Mass Spectrometry using a VG ZAB-E instrument. Optical rotations were determined using a Perkin Elmer 24 polarimeter at a wavelength of 589 mm with a 1.0 dm cell containing a total volume of 1 mL. Photolysis were performed using a Hanovia 450 W medium pressure mercury arc lamp in a water-cooled quartz immersion well. Pyrex test tubes containing the samples were strapped around this well, and the assembly was immersed in an ice−water bath. The samples were degassed for 30 min prior to irradiation. All solvents used were dried and distilled. Microanalyses were carried out by Guelph Chemical Laboratories Ltd. The X-ray crystal structure determination was performed on a Siemens R3m/v diffractometer using a total of 1706 reflections. Preparative HPLC was performed using a Waters RCM 25 × 10 cartridge at the University of Toronto. Ketone

1 was prepared by a modified procedure[6] as described in the literature.[13] The β-alkoxypurines were prepared by known methods[4] from purin-6-yltrimethylammonium chloride (Aldrich Chemical Co.). Anomeric mixtures of protected nucleosides **2a**,**b** were prepared according to a procedure reported by us recently.[6]

1-*N*-(6-Methoxypurine-9-yl)-5-*O*-benzoyl-3-*C*-[(benzoyloxy)methyl]-2,3-dideoxy-α- and -*β*-D-*erythro*-furanoside (2d). A solution consisting of ketone **1** (136 mg, 0.4 mmol) and 6-methoxypurine (180 mg, 1.2 mmol) in 150 mL of acetonitrile was irradiated for 36 h. Evaporation of the solvent followed by preparative thin-layer chromatography (CH_2Cl_2:CH_3OH, 95:5) gave 14 mg (12%) of **3**[6] and 81 mg (41%) of the title compound as a pale yellow solid: IR 1715, 1652 cm^{-1}; ^{1}H NMR δ 8.50, 8.48 (2 s, 1H), 8.15 (s, 1H), 8.07–8.01 and 7.65–7.53 and 7.50–7.38 (m, 10H), 6.40–6.37 (m, 1H), 4.84–4.40 (m, 5H), 4.18 and 4.17 (2 s, 3H), 3.08–2.88 (m, 2H), 2.66–2.56 (m, 1H).

1-*N*-[6-(Hexyloxy)purin-9-yl]-5-*O*-benzoyl-3-*C*-[(benzoyloxy)methyl]-2,3-dideoxy-α- and -*β*-*erythro*-furanoside (2e). A solution consisting of ketone **1** (68 mg, 0.2 mmol) and 6-(hexyloxy)purine (132 mg, 0.6 mmol) in 160 mL of acetonitrile was irradiated for 36 h. Evaporation of the solvent followed by preparative TLC (CH_2Cl_2:ethyl acetate, 95:5) gave 9 mg (15%) of **3** and 34 mg (30%) of the title compound. Further separation by preparative TLC (CH_2Cl_2:hexane, 3:2) afforded 14 mg (13%) of the β-anomer and 14 mg of the α-anomer.

α-**Anomer:** IR 1714, 1645 cm^{-1}; ^{1}H NMR δ 8.94 (s, 1H), 8.15 (s, 1H), 8.07–7.95 and 7.62–7.40 (m, 10H), 6.41–6.39 (m, 1H), 4.75–4.49 (m, 5H), 4.48–4.44 (t, 2H), 3.26–3.22 (m, 1H), 3.05–2.98 (m, 1H), 2.62–2.56 (m, 1H), 1.93–1.87 (m, 2H), 1.55–1.50 and 1.38–1.35 (m, 6H), 0.92 (t, 3H); MS (FAB) *m/z* 559 (M + 1).

***β*-Anomer:** IR 1715, 1652 cm^{-1}; ^{1}H NMR δ 8.47 (s, 1H), 8.08 (s, 1H), 8.07–8.03 and 7.61–7.44 (m, 10H), 6.39–6.37 (m, 1H), 4.86–4.56 (m, 5H), 4.54–4.50 (t, 2H), 3.01–2.81 (m, 3H), 1.93–1.87 (m, 2H), 1.53–1.35 (m, 6H), 0.93–0.90 (t, 3H); MS (FAB) *m/z* 559 (M + 1)

9-[2′,3′-Dideoxy-3′-*C*-(hydroxymethyl)-α- and -*β*-D-*erythro*-pentofuranosyl)adenine (5 and 4). The identical procedure was used as described by Sammuelsson.[8] A solution consisting of 220 mg (0.46 mmol) of **2b**[6] was treated with 15 mL of saturated methanolic ammonia (precooled in liquid N_2) at 100 °C in a sealed thick wall tube for 24 h. The solvent was removed, and the residue was dissolved in water and washed with CH_2Cl_2 (3 × 5 mL). The aqueous layer was concentrated to a small volume, and the residue was purified by reverse phase column chromatography (H_2O:CH_3OH, 9:1) followed by preparative HPLC (H_2O:CH_3OH:CH_3CN, 58:37:5). The α-anomer **5c** was eluted first followed by the β-anomer **4c**. Yields: 44 mg (23%) of **5c** and 61 mg (33%) of **4c**.

α-**Anomer 5c:** mp 166–168 °C (no lit. mp reported[8]); $[\alpha]^{22}_D$ +41.5° (*c* 0.135, H_2O); UV (H_2O) λ_{max} 260 nm (ϵ = 12 050); ^{1}H NMR (D_2O, TSP) δ 8.33 (s, 1H), 8.19 (s, 1H), 6.35 (m, 1H), 4.31 (m, 1H), 3.89–3.84 (d × d, *J* = 2.7, 13.0 Hz, 1H), 3.75–3.71 (d × d and overlapping d, *J* = 5.5, 13.0, 5.9 Hz, 3H), 2.88–2.77 (m, 1H), 2.68–2.54 (m, 1H), 2.50–2.40 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 158.2, 155.2, 151.0, 142.3, 121.5, 85.0, 83.9, 65.3, 64.8, 43.9, 37.5; MS (FAB) *m/z* 266 (M + 1).

***β*-Anomer 4c:** mp 192.5–194 °C (lit.[17] mp 192–193 °C); $[\alpha]^{22}_D$ −23° (*c* 0.135, H_2O); UV (H_2O) λ_{max} 260 nm (ϵ = 10 650); ^{1}H NMR (D_2O) δ 8.32 (s, 1H), 8.14 (s, 1H), 6.32 (m, 1H), 4.13 (m, 1H), 3.90–3.87 (d × d, *J* = 2.5, 12.6 Hz, 1H), 3.77 (d, *J* = 5.3 Hz, 2H), 3.76–3.71 (d × d, *J* = 4.7, 12.6 Hz, 1H), 2.72–2.61 (m, 2H), 2.58–2.45 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 158.1, 155.2, 150.9, 142.6, 121.4, 85.3, 85.0, 65.4, 64.9, 43.4, 37.6; MS (FAB) *m/z* 266 (M + 1).

Debenzoylation of Protected Nucleosides 2a–e General Procedure. Approximately 0.15 mmol of protected nucleoside **2** was treated with 10 mL of saturated methanolic ammonia for 24 h at room temperature. The solvent was evaporated and the residue dissolved in water and washed with CH_2Cl_2 (3 × 3 mL). After evaporation of water *in vacuo* below 40 °C, the residue was purified by preparative TLC (eluting solvent and yields specified below).

9-[2′,3′-Dideoxy-3′-*C*-(hydroxymethyl)-α- and -*β*-D-*erythro*-pentofuranosyl]purine (5a and 4a). Eluting solvent for preparative TLC was ethyl acetate:methanol (8:2). Total yield: 88% total (1:1) each of **4a** and **5a**.

α-**Anomer 5a:** mp 161–163 °C; $[\alpha]^{22}_D$ + 17° (*c* 0.5, H_2O); UV (H_2O) λ_{max} 264 nm (ϵ = 8209); ^{1}H NMR (D_2O) δ 9.08 (s, 1H), 8.89 (s, 1H), 8.66 (s, 1H), 6.48 (m, 1H), 4.34–4.30 (m, 1H), 3.84–3.80 (d × d, *J* = 2.5, 13.0 Hz, 1H), 3.72–3.62 (d × d plus overlapping d, *J* = 5.7, 5.9, 13.0 Hz, 3H), 2.84–2.74 (m, 1H), 2.58–2.52 (m, 1H), 2.52–2.47 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 157.2, 154.7, 150.8, 142.3, 123.5, 87.9, 86.2, 65.3, 64.5, 44.4, 37.4; MS (FAB) *m/z* 251 (M + 1).

***β*-Anomer 4a:** mp 167–169 °C; $[\alpha]^{22}_D$ +3.2° (*c* 0.25, CH_3OH); UV (H_2O) λ_{max} 264 nm (ϵ = 7896); ^{1}H NMR (D_2O) δ 9.08 (s, 1H), 8.89 (s, 1H), 8.68 (s, 1H), 6.46 (m, 1H), 4.11–4.08 (m, 1H), 3.82–3.78 (d × d, *J* = 2.8, 12.7 Hz, 1H), 3.71 (d, *J* = 5.7 Hz, 2H), 3.66–3.62 (d × d, *J* = 5.0, 12.7 Hz, 1H), 2.77–2.66 (m, 2H), 2.53–2.46 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 157.8, 154.8, 150.9, 141.6, 122.4, 87.7, 87.4, 65.0, 43.6, 37.6; MS (FAB) *m/z* 252 (M + 2).

6-Chloro-9-[2′,3′-dideoxy-3′-*C*-(hydroxymethyl)-α- and -*β*-D-*erythro*-pentofuranosyl]purine (5b and 4b). Eluting solvent for preparative TLC was ethyl acetate:methanol (85:15). Total yield: 19% (~1:1 mixture of anomers) in addition to 41% of the 6-methoxy nucleosides **4d** and **5d**.

α-**Anomer:** mp 199–201 °C; $[\alpha]^{22}_D$ +35.2° (*c* 0.165, CH_3OH); UV (H_2O) λ_{max} 260 nm (ϵ = 14 074); ^{1}H NMR (D_2O) δ 8.27 (s, 1H), 8.13 (s, 1H), 6.28 (m, 1H), 4.26–4.20 (m, 1H), 3.81–3.77 (d × d, *J* = 2.7, 12.6 Hz, 1H), 3.68–3.62 (d × d plus overlapping d, *J* = 5.7, 5.9, 12.6 Hz, 3H), 2.78–2.70 (m, 1H), 2.55–2.48 (m, 1H), 2.42–2.35 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 158.4, 155.5, 151.2, 142.7, 121.7, 87.5, 85.9, 65.4, 64.6, 44.3, 37.4; MS (FAB) *m/z* 285 (M + 1).

***β*-Anomer:** mp 198–200 °C; $[\alpha]^{22}_D$ −12.7° (*c* 0.275, CH_3OH); UV (H_2O) λ_{max} 260 nm (ϵ = 13 468); ^{1}H NMR (D_2O) δ 8.25 (s, 1H), 8.10 (s, 1H), 6.26 (m, 1H), 4.07–4.03 (m, 1H), 3.81–3.76 (d × d, *J* = 2.7, 12.7 Hz, 1H), 3.69–3.68 (d, *J* = 5.6 Hz, 2H), 3.63–3.59 (d × d, *J* = 5.0, 12.7 Hz, 1H), 2.64–2.57 (m, 2H), 2.46–2.40 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 158.3, 155.3, 151.2, 142.8, 121.9, 87.4, 87.1, 65.5, 64.9, 37.6; MS (FAB) *m/z* 284 (M), 286 (M + 2), 155 (6-chloropurine +1).

6-Methoxy-9-[2′,3′-dideoxy-3′-*C*-(hydroxymethyl)-α- and -*β*-D-*erythro*-pentofuranosyl]purine (5d and 4d). Eluting solvent for preparative TLC was ethyl acetate:methanol (95:5). Total yield 72% (1:1 mixture of anomers).

α-**Anomer:** mp 121–123.5 °C; $[\alpha]^{22}_D$ +3.5° (*c* 0.73, CH_3OH); UV (H_2O) λ_{max} 252 nm (ϵ = 12 380); ^{1}H NMR (D_2O) δ 8.37 (s, 1H), 8.34 (s, 1H), 6.33 (m, 1H), 4.25–4.18 (m, 1H), 4.05 (s, 3H), 3.76–3.72 (d × d, *J* = 2.7, 12.6 Hz, 1H), 3.63–3.58 (d × d plus overlapping d, *J* = 5.7, 5.9, 12.6 Hz, 3H), 2.77–2.68 (m, 1H), 2.52–2.43 (m, 1H), 2.42–2.33 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 160.1, 155.0, 153.4, 144.5, 124.0, 87.9, 86.1, 65.3, 64.6, 57.9, 44.3, 37.4; MS (FAB) *m/z* 281.

***β*-Anomer:** mp 138–140 °C; $[\alpha]^{22}_D$ −6.7° (*c* 0.75, CH_3OH); UV (H_2O) λ_{max} 252 nm (ϵ = 9771); ^{1}H NMR (D_2O) δ 8.36 (s, 1H), 8.30 (s, 1H), 6.30 (m, 1H), 4.04 (s, 3H), 4.00–3.97 (m, 1H), 3.76–3.70 (d × d, *J* = 2.7 Hz, 1H), 3.63–3.61 (d, *J* = 5.6 Hz, 2H), 3.56–3.50 (d × d, *J* = 5.0, 12.5 Hz, 1H), 2.64–2.50 (m, 2H), 2.43–2.34 (m, 1H); ^{13}C NMR (D_2O, TSP) δ 160.1, 154.9, 153.2, 144.7, 123.9, 87.7, 87.2, 65.4, 64.9, 57.8, 43.3, 37.5; MS (FAB) *m/z* 281 (M + 1), 151 (6-methoxypurine + 1).

6-(Hexyloxy)-9-[2′,3′-dideoxy-3′-*C*-(hydroxymethyl)-α- and -*β*-D-*erythro*-pentofuranosyl]purine (5e and 4e). Eluting solvent for preparative TLC was ethyl acetate:methanol (95:5). Total yield: 84% (1:1 mixture of anomers).

α-**Anomer:** mp 98–100 °C; $[\alpha]^{22}_D$ +32.5° (*c* 0.6, CH_3OH); UV (H_2O) λ_{max} 252 nm (ϵ = 12 718); ^{1}H NMR (D_2O) δ 8.42 (s, 1H), 8.40 (s, 1H), 6.38 (m, 1H), 4.53–4.50 (t, 2H), 4.30–4.25 (m, 1H), 3.82–3.78 (d × d, *J* = 2.5, 12.6 Hz, 1H), 3.68–3.63 (d × d plus overlapping d, *J* = 5.3, 5.8, 12.6 Hz, 3H), 2.82–2.75 (m, 1H), 2.59–2.51 (m, 1H), 2.48–2.41 (m, 1H), 1.82–1.78 (m, 2H), 1.45–1.41 (m, 2H), 1.32–1.24 (m, 4H), 0.82–0.78 (t, 3H); ^{13}C NMR (D_2O, TSP) δ 158.2, 155.2, 151.0, 142.3, 121.5, 87.8, 86.3, 71.1, 65.3, 64.7, 44.2, 37.5, 33.7, 33.6, 31.0, 27.5, 16.0; MS (FAB) *m/z* 352 (M + 2), 222.

***β*-Anomer:** mp 102−105 °C; $[\alpha]^{22}_D$ −6.0° (*c* 0.4, CH_3OH); UV (H_2O) λ_{max} 252 nm (ϵ = 9832); ^1H NMR (D_2O) δ 8.42 (s, 1H), 8.40 (s, 1H), 6.37 (m, 1H), 4.53−4.50 (t, 2H), 4.09−4.04 (m, 1H), 3.82−3.77 (d × d, J = 2.7, 12.7 Hz, 1H), 3.71−3.69 (d, J = 5.6 Hz, 1H), 3.64−3.60 (d × d, J = 5.0, 12.7 Hz, 1H), 2.71−2.61 (m, 2H), 2.50−2.42 (m, 1H), 1.85−1.79 (m, 2H), 1.45−1.39 (m, 2H), 1.30−1.22 (m, 4H), 0.81−0.78 (t, 3H); ^{13}C NMR (D_2O, TSP) δ 160.5, 154.7, 153.9, 144.2, 124.3, 87.7, 87.2, 71.3, 65.4, 64.9, 43.4, 37.6, 33.6, 33.5, 30.9, 27.6, 16.1; MS (FAB) m/z 351 (M + 1), 221 (6-(hexyloxy)purine + 1).

Inhibition Studies. The CD4 + T-lymphoblastoid H9 cell line (NCI, Bethesda, MD) was grown in RPMI 1640 medium, supplemented with 20% heat-inactivated fetal bovine serum (Sigma Chemicals, St. Louis, MO), penicillin (250 μg/mL), streptomycin (250 μg/mL), L-glutamine (2 mM), and Hepes buffer (10 mM). Cells (2 × 10^6/2 mL) were cultured in 12-well tissue culture dishes (Costar, Cambridge, MA). The adenosine analogues were dissolved in PBS. Cells were cultured for up to 14 days in the presence of various analogues tested at 1, 50, and 100 μM and examined for the presence of cytotoxicity by trypan blue exclusion (0.4%; Gibco, Grand Island, NY). Acute infections were performed by adding 500 $TCID_{50}$ HIV IIIB/10^6 cells to cultures in the presence or absence of nucleoside, with no subsequent wash step. Cells were examined for the presence of syncytial giant cells every 2 days. Medium was changed at least twice per week. Supernatants were collected on days 7, 11, and 14 and examined for p24 antigen using the Abbot ELISA kit according to the manufacturer's guidelines.

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