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Synthesis of Conformationally Restricted 2′,3′-*exo*-Methylene Carbocyclic Nucleosides Built on a Bicyclo[3.1.0]hexane Template

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Abstract—A series of 2′,3′-*exo*-methylene carbocyclic nucleosides was synthesized as potential antiviral agents. These compounds were built on a bicyclo[3.1.0]hexane template that exhibits a rigid pseudoboat conformation and is capable of maintaining an identical conformation in solid state and in solution. The structures of the synthesized compounds were elucidated by NMR and X-ray crystallography. All the compounds were tested as anti-HIV and anti-HSV agents. The chemically synthesized 5′-triphosphate analogue of 7 was evaluated directly as a reverse transcriptase inhibitor.

Introduction

Carbocyclic nucleoside analogues in which a methylene group replaces the O atom of the ribofuranose ring represent an extremely important class of potentially active therapeutic agents. For example, carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine (carbovir) has been identified as a potent and selective inhibitor of HIV-1 replication and cytopathic effects in a variety of human *t*-lymphoblastoid cell lines[1,2] and has been approved by the FDA as a drug for the treatment of AIDS. In our ongoing search for antiviral nucleosides, we have synthesized a series of 2′,3′-*exo*-methylene carbocyclic nucleosides, where the sugar moieties are conformationally restricted due to the methylene group fused between the 2′ and 3′ positions.

The sugar ring of nucleosides and nucleotides equilibrates in solution between two extreme forms of ring pucker neighboring a 2′-*exo*/3′-*endo* (Northern) conformation and the opposite 2′-*endo*/3′-*exo* (Southern) conformation resulting from gauche interactions between the furan oxygen and the 2′-and 3′-hydroxyl groups as well as the anomeric effect.[3] In the solid state, one of the two forms usually predominates and, similarly when a nucleoside or nucleotide binds to its target enzyme, only one of the two possible conformations takes part in drug–receptor interactions.[4] Loss of the furanose oxygen in carbocyclic nucleosides causes the cyclopentane ring to adopt an unusual 1′-*exo* conformation that is relatively far from the characteristic North or South conformations observed in the typical nucleosides.[5]

One of the strategies that can be used in carbocyclic nucleosides to regain the ring pucker characteristic of typical nucleosides is to construct them on a rigid bicyclic system. We have synthesized a number of purine carbocyclic nucleosides built on a rigid bicyclo [3.1.0]hexane template. Since the bicyclo [3.1.0]hexane template is known to exist rigidly in a pseudoboat shape, these compounds are able to maintain identical conformations in the solid state and in solution and thereby help define the role of sugar puckering in nucleosides by stabilizing the active receptor bound conformation. Since both a cyclopropane ring as well as a C-C double bond have unsaturated properties these nucleosides can also be expected to show anti-HIV properties similar to carbovir.[6]

Most nucleoside analogues have to be converted to the triphosphate form in order to exhibit biological activity. However, the conformation required for triphosphate production does not necessarily correlate with the conformational preference of reverse transcriptase.[7] For example, it has been proposed that the preference of AZT for the extreme south conformation is responsible for its potent anti-HIV activity.[8] However, the antipodal north conformation has been suggested for the interaction of AZT-TP with its target enzyme, HIV

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reverse transcriptase.[9] Marquez et al. have demonstrated that AZT, by its flexible nature, can adopt a south conformation required for phosphorylation by kinase enzymes and subsequently switch to a north conformation for interaction with reverse transcriptase.[7] We have synthesized the triphosphate of **7** to study its interaction with reverse transcriptase.

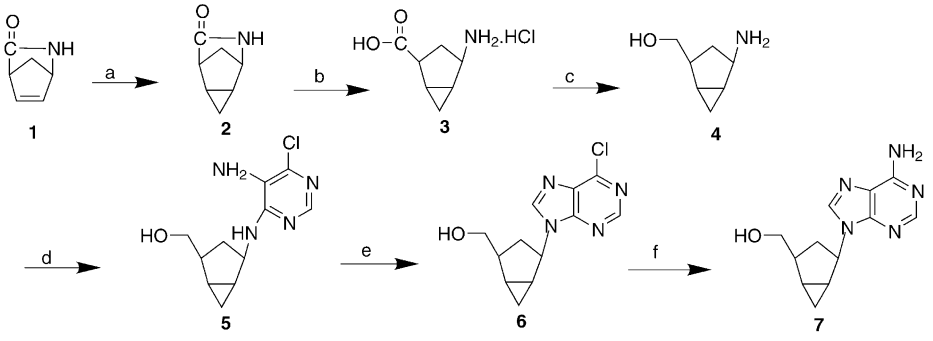
Results and Discussion

Synthesis

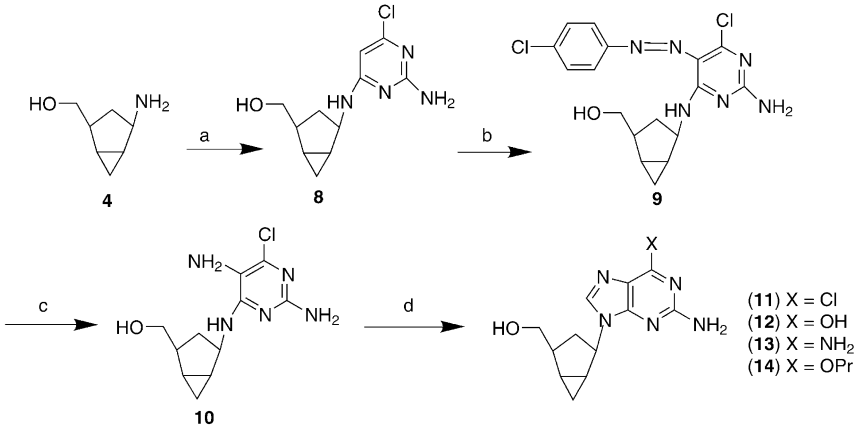
To synthesize the target compounds (±) 2′,3′-dideoxy-2′,3′-*exo*-methylene nucleosides, (±) 2-azabicyclo-[2.2.1]hept-5-en-3-one **1** (commercially available) was used as the starting material. Cyclopropanation of **1** using diazomethane and catalytic palladium acetate[10] gave the desired cyclopropyl intermediate (±)-*cis*-*exo*-2-(aza)-3-(oxo)-tricyclo[3.2.1.0]heptane, (**2**) as cyclopropanation took place from the less hindered side of the lactam (Scheme 1). Acid-catalyzed hydrolysis of **2** with 1 N HCl solution gave (±)-*cis*-*exo*-2-(amino)-4-(carboxy)-bicyclo[3.1.0]hexane hydrochloride, (**3**). Reduction of **3** with lithium aluminum hydride (LAH) in tetrahydrofuran (THF) led to the formation of the amino alcohol, (±)-*cis*-*exo*-2(amino)-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (**4**). Once the carbocyclic sugar moiety **4**, was formed, it was coupled with 5-amino-4,6-dichloropyrimidine in the presence of triethylamine and *n*-butanol to give the pyrimidylamino derivative **5**. Ring closure of **5** with triethyl orthoformate in the presence of HCl[11] gave (±)-*cis*-*exo*-2-(6-chloro-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0] hexane (**6**). Treatment of **6** with liquid ammonia under high pressure gave the adenine derivative,(±)-*cis*-*exo*-2-(6-amino-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0] hexane (**7**).

The syntheses of the 2,6-disubstituted purine analogues are shown in Scheme 2. Amine **4** was condensed with 2-amino-4,6-dichloropyrimidine to afford the pyrimidylamino derivative, **8**. This was then treated with *p*-chlorobenzenediazonium chloride by the method of Shealy and Clayton[12] to give azopyrimidine **9**. Azopyrimidine **9** was then reduced with zinc in the presence of acetic acid[2] and generated the amine **10.** Subsequent cyclization of the substituted pyrimidine with triethyl orthoformate and HCl led to the formation of (±)-*cis*-*exo*-2-(2-amino-6-chloro-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (**11**).

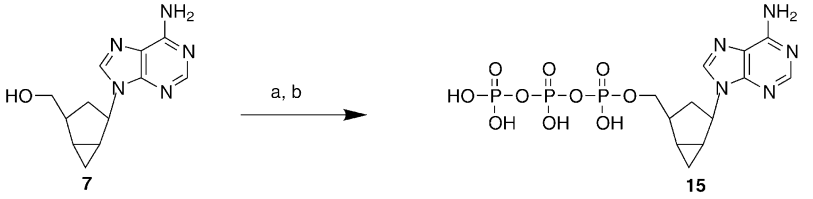
The 2-amino-6-chloropurine analogue was refluxed with 1 N HCl[13] in order to obtain the guanine analogue, **12**. The diaminopurine analogue (**13**) was obtained by displacing the 6-chloro group of **11** with liquid ammonia. Displacement of the 6-chloro group of **11** with *n*-propanol in the presence of sodium hydride gave the corresponding propyl derivative (**14**). The corresponding optically active (−) compounds (**1a**–**14a**) were also synthesized using the same procedure (see Experimental).



Scheme 1. Reagents and conditions: (a) CH_2N_2, $Pd(OAc)_2$, 24 h; (b) 1 N HCl, reflux 1.5 h; (c) LAH, THF, 24 h; (d) 5-amino-4,6-dichloropyrimidine, Et_3N, *n*-BuOH, reflux 24 h; (e) $CH(OEt)_3$, concd HCl, 24 h; (f) liq NH_3, 48 h.



Scheme 2. Reagents and conditions: (a) 2-amino-4,6-dichloropyrimidine, Et_3N, *n*-BuOH, reflux 24 h; (b) *p*-chloroaniline, 3 N HCl, $NaNO_2$, CH_3COOH, NaOAc, 24 h; (c) Zn, CH_3COOH, EtOH, reflux, 5 h; (d) $CH(OEt)_3$, concd HCl, 24 h.



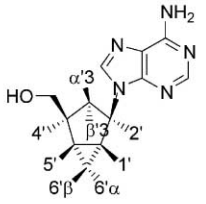
Scheme 3. Reagents and conditions: (a) $POCl_3$, $PO(OMe)_3$, 0 °C; (b) $(Bu_3NH)_2H_2P_2O_7^-$.

Synthesis of the triphosphate analogue was performed using a 'one-pot' approach by reacting the nucleoside **7** with phosphorus oxychloride and subsequently treating the monophosphate intermediate with bis(tributylammonium) pyrophosphate to give the triphosphate **15** (Scheme 3).

Stereochemical assignments of the final compounds were made on the basis of 1-D and 2-D NMR spectroscopy and X-ray crystallography. The NOESY spectra (Fig. 1) showed showed correlation between the $C_{6'}$-Hα proton (0.34–0.30 ppm) and the $C_{2'}$-H proton (4.76–4.74 ppm). Correlation was also seen between the $C_{6'}$-Hα proton (0.34–0.30 ppm) and the $C_{4'}$-H proton (1.94–1.84 ppm) indicating that the cyclopropyl was below the plane of the five-membered ring. The X-ray structure (Fig. 2) further confirmed that the cyclopropyl group was indeed below the plane of the cyclopentane ring.

Biological Results and Conclusion

Anti-HIV and anti-HSV activities of the synthesized compounds have been evaluated. Preliminary data



7

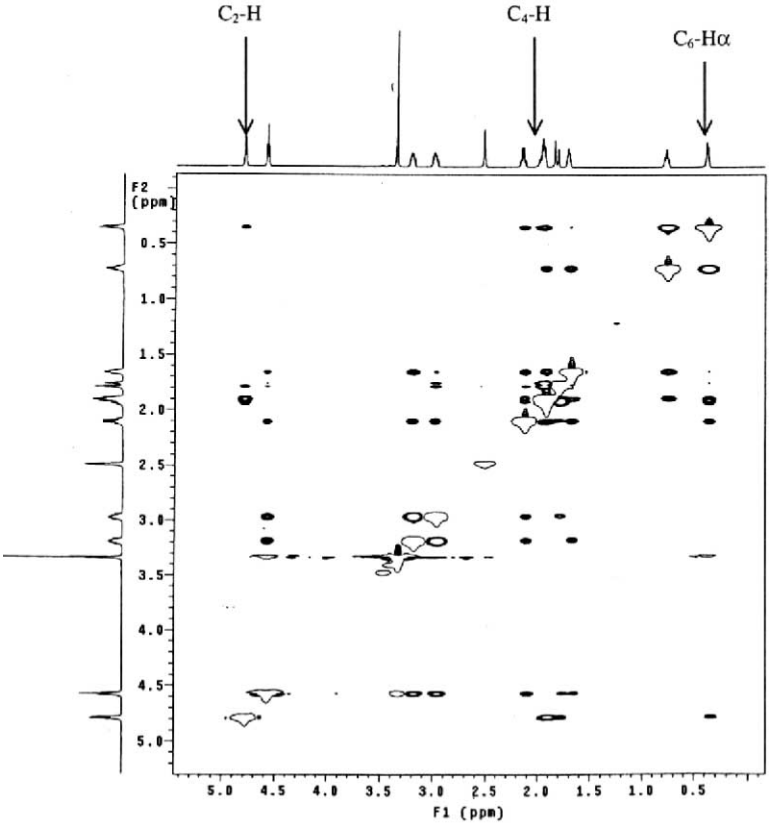


Figure 1. 2-D NOESY of compound **7**.

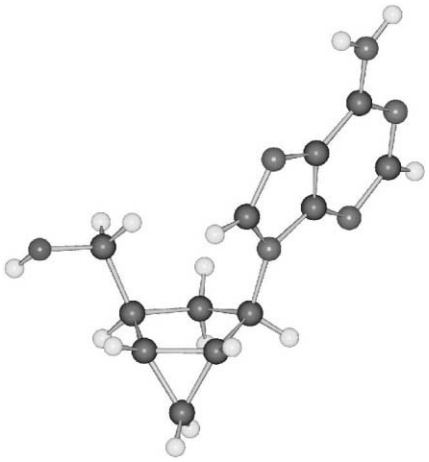


Figure 2. X-ray crystal structure of compound **7**.

showed that compounds **6** and **7** had an EC_{50} of 0.1–1 μg/mL and compounds **12**–**14** had an EC_{50} of 1–10 μg/mL as compared to Acyclovir, a potent anti-herpes compound which had an EC_{50} of 0.1 μg/mL. No toxicity was observed for any of the compounds up to 100 μg/mL. None of the compounds showed significant anti-HIV activity up to 100 μg/mL. The optically active isomers also showed similar biological activities. The triphosphate analogue **15** was evaluated against reverse transcriptase and had an IC_{50} of 36.39 μg/mL as compared to dideoxy adenosine triphosphate, which had an IC_{50} of 0.023 μg/mL.

Based on the X-ray structure of compound **7**, the angle of pseudorotation P was calculated as 91.89° which is far different from the conventional 'north' or 'south' conformation, $P = 0°$ and $P = 180°$ respectively, observed in typical nucleosides. The unusual conformation of these nucleosides may be responsible for their lack of significant activity against HIV as the compound may not be in a suitable conformation to interact tightly with reverse transcriptase, the target enzyme.

In conclusion, we have synthesized a series of 2′,3′-*exo*-methylene carbocyclic nucleosides and evaluated their structure, conformation and biological activity.

Experimental

All reactions involving moisture sensitive reagents were conducted in oven-dried glassware under nitrogen a atmosphere. Solvents were dried when necessary. All other chemicals and solvents were reagent grade unless specified otherwise and were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, USA. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian Unity 300,Varian Unity 500, GE 300 and Varian Mercury 300 spectrometers and referenced to the solvent. Chemical shifts are expressed in ppm. Peak multiplicities are abbreviated: broad, br; singlet, s; doublet, d; triplet, t; quartet, q; pentet, p; and multiplet, m. Fast-atom bombardment (FAB) mass spectra (MS) were obtained on a VG 7070E-HF instrument. Optical rotations were measured on a Rudolph polarimeter. Flash column chromatography was performed on Merck Science silica gel 60 (230–400) mesh. Thin layer chromatography (TLC) was performed on Merck Science silica gel 60 F_{254} glass plates (0.25 mm thickness). Plates were visualized by UV light, iodine vapor or anisaldehyde solution.

Diazomethane

A Diazald distillation apparatus was fitted with a 250 mL round-bottomed distillation pot and a 250 mL round-bottomed flask as a receiver. The reaction vessel was heated with a water bath to 65–70 °C, and the receiver was cooled to ca. −40 °C (controlled dry-ice/acetone conditions). Potassium hydroxide (5.1 g, 178 mmol), 2-methoxyethylether (25 mL), water (10 mL) and ether (10 mL) were taken in the distillation pot. Diazald (10 g, 47 mmol) in ether (65 mL) was added drop wise. Soon after the addition commenced, diazomethane in ether started to collect in the receiver. After the final addition, 20 mL of ether was added and allowed to distill over. This was repeated until the distillate was colourless (usually only once).

(±)-*exo*-7-Azatricyclo[3.2.1.0 < 2,4 >]octan-6-one (2). A solution of 2-azabicyclo[2.2.1]hept-5-en-3-one (500 mg, 4.58 mmol) and palladium acetate (50 mg, 0.22 mmol) in ether (10 mL) was stirred at room temperature for 15 min. To this solution CH_2N_2 in ether (80 mL) was added dropwise over a 20-min period. The reaction mixture was allowed to stir overnight at room temperature. It was then filtered over a pad of Celite and concentrated. Purification of the residue by silica gel chromatography using ethyl acetate/hexane (1:1) afforded a yellow oil. The oil was recrystallized with dichloromethane–hexane to yield 294.6 mg (52.15%) of **2** as a white solid: mp 82–83 °C; R_f 0.3 (ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.3 (br, 1H, NH), 3.79–3.78 (d, 1H, H-1), 2.7 (d, 1H, H-5), 1.59–1.35 (m, 4H, H-4, H-2, H-8, H-8), 1.29–1.25 (q, 1H, H-3), 0.82–0.75 (q, 1H, H-3). Anal. calcd for C_7H_9NO: C, 68.26; H, 7.36; N, 11.37. Found: C, 68.32; H, 7.16; N, 11.57.

(−)-*exo*-7-Azatricyclo[3.2.1.0 < 2,4 >]octan-6-one (2a). The reaction was repeated as above using (−) 2-azabicyclo [2.2.1]hept-5-en-3-one (1.00 g, 9.16 mmol), palladium acetate (1000 mg, 0.44 mmol) and CH_2N_2 in ether (160 mL). The reaction mixture was purified by silica gel chromatography (ethyl acetate/hexane, 1:1) to give a yellow oil. The oil was recrystallized with dichloromethane-hexane to yield 703.1 mg (62%) of **2a** as a white solid: mp 78–79 °C; $[\alpha]_D^{25}$ −217.793° (*c* 1, CH_2Cl_2). Anal. calcd for C_7H_9NO: C, 68.26; H, 7.36; N, 11.37. Found: C, 68.36; H, 7.13; N, 11.48.

(±)-*cis*-*exo*-2-(Amino)-4-(carboxy)-bicyclo[3.1.0]hexane hydrochloride (3). A solution of lactam **2** (500 mg, 4.06 mmol) in 1 N HCl (15 mL) was heated under reflux at 128 °C for 1.5 h. The reaction mixture was then

concentrated to dryness under reduced pressure to obtain a yellow oil. Addition of acetone to the yellow oil gave 882.3 mg (86.4%) of **3** as a white solid: mp 202–206 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.73–3.70 (d, 1H, H-2), 3.01–2.98 (d, 1H, H-4), 1.99–1.79 (m, 3H, H-3, H-3, H-1), 1.65–1.59 (q, 1H, H-5), 0.80–0.73 (q, 1H, H-6), 0.41–0.36 (q, 1H, H-6). Anal. calcd for $C_7H_{12}ClNO_2$: C, 47.33; H, 6.8; N, 7.88. Found: C, 47.51; H, 6.69; N, 7.89.

(−)-*cis-exo*-2-(Amino)-4-(carboxy)-bicyclo[3.1.0]hexane hydrochloride (3a). Compound **2a** (200 mg, 1.623 mmol) was heated under reflux with 1 N HCl as above to yield a yellow oil, which on addition of acetone afforded a white solid (244.1 mg, 84.92%): mp 202–206 °C; $[\alpha]_D^{25}$ −52.104° (*c* 1, CH_3OH). Anal. calcd for $C_7H_{12}ClNO_2$: C, 47.33; H, 6.8; N, 7.88. Found: C, 47.47; H, 6.90; N, 7.93.

(±)-*cis-exo*-2-(Amino)-4-(hydroxymethyl)-bicyclo[3.1.0]-hexane (4). To lithium aluminum hydride (500 mg, 13.0 mmol) in anhydrous tetrahydrofuran (40 mL) was added compound **3** the amino hydrochloride (577 mg, 3.2 mmol) at room temperature under N_2. The reaction mixture was stirred overnight and quenched with water (3 mL). A white solid was filtered and the filtrate was concentrated to dryness to obtain **4** as an oily residue, (300 mg, 72.5%). This oil was then used for the next step without any further purification. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.60–3.56 (dd, 1H, C*H*$_2$OH), 3.51–3.46 (dd, 1H, C*H*$_2$OH), 3.33–3.31 (d, 1H, H-2), 2.18–2.15 (m, 1H, H-4), 1.73 (m, 1H, H–3), 1.29–1.24 (m, 3H, H-3, H-5, H-1), 0.47–0.45 (m, 1H, H-6), 0.1 (q, 1H, H-6).

(−)-*cis-exo*-2-(Amino)-4-(hydroxymethyl)-bicyclo[3.1.0]-hexane (4a). The reaction was repeated as above using compound **3a** (908 mg, 5.129 mmol), lithium aluminum hydride (1.00 g, 26.0 mmol) and tetrahydrofuran (70 mL). Compound **4a** was obtained as an oil, 510.8 mg (79%).

(±)-*cis-exo*-2-[(5-Amino-6-chloro-4-pyrimidinyl)amino]-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (5). To compound **4**, the amine alcohol (419.2 mg, 3.299 mmol) were added 5-amino-4,6-dichloro-pyrimidine (811.60 mg, 4.9488 mmol), triethylamine (0.91 mL) and 1-butanol (40 mL), and the mixture was heated under reflux for 24 h. The volatile solvents were removed; the residue was adsorbed onto silica gel and it was packed into a column and eluted with chloroform followed by chloroform/methanol (30:1). The product fractions were collected and concentrated into a residue. The residue was recrystallized using ethyl acetate to yield 338.6 mg (40.3%) of **5** as a solid: mp 86–88 °C; TLC: R_f 0.41 (chloroform/methanol, 10:1). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.75 (s, 1H, H-2), 4.37–4.35 (m, 1H, H-2′), 3.57–3.54 (m, 2H, C*H*$_2$OH), 2.18–2.13 (m, 1H, H-3′), 1.81–1.74 (m, 1H, H-1′), 1.62–1.57 (m, 1H, H-4′), 1.48–1.44 (m, 2H, H-5′, H-3′), 0.60–0.55 (m, 1H, H-6′), 0.19–0.15 (q, 1H, H-6′). Anal. calcd for $C_{11}H_{15}O\ ClN_4$: C, 51.86; H, 5.93; N, 21.99. Found: C, 51.80; H, 6.01; N, 21.80.

(−)-*cis-exo*-2-[(5-Amino-6-chloro-4-pyrimidinyl)amino]-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (5a). Compound **4a** (510 mg, 4.0157 mmol) was reacted with 5-amino-4,6-dichloro-pyrimidine (987.85 mg, 6.023 mmol), triethylamine (1.12 mL) and 1-butanol (50 mL) as above. Purification of the residue by silica gel chromatography using $CHCl_3$ followed by chloroform/methanol (30:1) afforded a yellow oil, which was recrystallized using ethyl acetate–hexane to give a white solid, 591.4 mg (58%): mp 152–154 °C; $[\alpha]_D^{25}$ −8.016° (*c* 1, CH_3OH). Anal. calcd for $C_{11}H_{15}O\ ClN_4$: C, 51.86; H, 5.93; N, 21.99. Found: C, 51.77; H, 6.14; N, 21.70.

(±)-*cis-exo*-2-(6-Chloro-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (6). A mixture of compound **5** (500 mg, 1.9646 mmol), triethyl orthoformate (10.7 mL), and hydrochloric acid (12 N, 0.5 mL) was stirred overnight at room temperature. The suspension was dried in vacuo. Diluted hydrochloric acid (0.5 N, 15 mL) was added and the mixture was stirred at room temperature for 1 h. The mixture was neutralized to pH 8 with 1 N sodium hydroxide and adsorbed onto silica gel. It was packed into a column and eluted by chloroform/methanol (20:1) to yield compound **6** as a white solid, 344.8 mg (66.3%): mp 176 °C; TLC: R_f 0.26 (chloroform/methanol, 10:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.79 (s, 1H, H-8), 8.74 (s, 1H, H-2), 4.92–4.90 (d, 1H, H-2′), 4.54–4.50 (t, 1H, CH_2OH, D_2O exchangeable), 3.17–3.11 (m, 1H, C*H*$_2$OH), 2.96–2.89 (m, 1H, C*H*$_2$OH), 2.11–2.06 (m, 1H, H-3′), 1.99–1.92 (m. 2H, H-4′, H-1′), 1.84–1.79 (m, 1H, H-3′), 1.66–1.60 (m, 1H, H-5′), 0.77–0.70 (m, 1H, H-6′), 0.39–0.34 (q, 1H, H-6′). Anal. calcd for $C_{12}H_{13}O\ ClN_4$: C, 54.44; H, 4.95; N, 21.16. Found: C, 54.29; H, 4.75; N, 20.97.

(−)-*cis-exo*-2-(6-Chloro-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (6a). Compound **5a** (500 mg, 1.964 mmol) was reacted with triethyl orthoformate and hydrochloric acid as above. Purification by a silica gel column using an eluate of chloroform/methanol (30:1) gave a white solid 250 mg (50%): mp 158 °C; $[\alpha]_D^{25}$ −71.843° (*c* 1, CH_3OH). Anal. calcd for $C_{12}H_{13}O\ ClN_4$: C, 54.44; H, 4.95; N, 21.16. Found: C, 54.36; H, 5.04; N, 21.08.

(±)-*cis-exo*-2-(6-Amino-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (7). Liquid ammonia was passed into a solution of **6** (300 mg, 1.133 mmol) in methanol (5 mL) at −80 °C in a bomb. The bomb was sealed and heated at 75 °C for 48 h. Ammonia and methanol were evaporated. The residue was adsorbed onto silica gel and it was packed into a column and eluted with chloroform/methanol (10:1). The crude product was recrystallized from ethanol to yield 194.7 mg (70.06%) of **7**: mp 220 °C; TLC: R_f 0.13 (chloroform/methanol, 10:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.20 (s, 1H, H-8), 8.08 (s, 1H, H-2), 7.16 (br, 2H, NH_2, D_2O exchangeable), 4.76–4.74 (d, 1H, H-2′), 4.56–4.52 (t, 1H, CH_2OH, D_2O exchangeable), 3.30–3.11 (m, 1H, C*H*$_2$OH), 2.97–2.89 (m, 1H, C*H*$_2$OH), 2.11–2.03 (m, 1H, H-3′), 1.94–1.84 (m. 2H, H-4′, H-1′), 1.76–1.71 (m, 1H, H-3′), 1.65–1.59 (m, 1H, H-5′), 0.72–0.65 (q, 1H,

H-6′), 0.34–0.30 (q, 1H, H-6′). Anal. calcd for $C_{12}H_{15}ON_5$: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.58; H, 6.31; N, 28.24.

(−)-*cis-exo*-2-(6-Amino-9*H*-purin-9-yl)-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (7a). Compound **6a** (270 mg, 1.02 mmol) was reacted with liquid ammonia as described above. The residue was purified through a silica gel column using an eluate of chloroform/methanol (10:1) to give a white solid, 355.7 mg (76.67%): mp 238 °C; $[\alpha]_D^{25}$ −50.610° (*c* 1, CH_3OH). Anal. calcd for $C_{12}H_{15}ON_5$: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.66; H, 6.36; N, 28.40.

(±)-*cis-exo*-2-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (8). To compound **4** (428.2 mg, 3.37 mmol) was added 1-butanol (40 mL), triethylamine (2.8 mL), and 2-amino-4,6-dichloropyrimidine (829.84 mg, 5.06 mmol) and the mixture was heated under reflux for 48 h. The solvent was removed under reduced pressure and the residue was purified through a silica gel column using an eluate of chloroform followed by chloroform/methanol (20:1). The product fractions were collected and concentrated to give a white solid, which was then recrystallized using dichloromethane–hexane to yield 605.2 mg (70.6%) of **8** as a white solid: mp 179–180 °C; TLC: R_f = 0.58 (dichloromethane/methanol, 9:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.1 (br, s, 1H, NH), 6.3 (br, s, 2H, NH_2), 5.7 (s, 1H, H-1), 4.6 (m, 1H, H-2′), 4.2 (t, 1H, CH_2OH, D_2O exchangeable), 3.4 (m, 2H, CH_2OH), 2.0 (m, 1H, H-3′), 1.7 (m, 1H, H-1′), 1.5 (m, 1H, H-4′), 1.36–1.31 (m, 2H, H-3′, H-5′), 0.5 (m, 1H, H-6′), 0.01–0.00 (m, 1H, H-6′). Anal. calcd for $C_{11}H_{15}ClN_4O.0.25H_2O$: C, 51.01; H, 6.03; N, 21.63. Found: C, 50.70; H, 6.22; N, 21.64.

(−)-*cis-exo*-2-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (8a). Compound **4a** (462.3 mg, 3.6401 mmol) was reacted with 2-amino-4,6-dichloropyrimidine (895 mg, 5.46 mmol), triethylamine (3 mL) and 1-butanol (40 mL) as above. The residue was purified through a silica gel column using an eluate of chloroform followed by chloroform/methanol (35:1) to give a white solid, 511.6 mg (55.5%): mp 68 °C; $[\alpha]_D^{25}$ −67.13° (*c* 0.5, CH_3OH). Anal. calcd for $C_{11}H_{15}ClN_4O$: C, 51.86; H, 5.93; N, 21.99. Found: C, 51.73; H, 5.86; N, 22.02.

(±)-*cis-exo*-2-[(2-Amino-6-chloro-5-[(4-chlorophenyl)-azo]-4-pyrimidinyl)amino]-4-(hydroxymethyl)-bicyclo[3.1.0]-hexane (9). A cold diazonium salt solution was prepared from *p*-chloroaniline (345 mg, 2.7 mmol) in 3 N HCl (6 mL) and sodium nitrite (204 mg, 2.94 mmol) in water (1.2 mL). This solution was added to a mixture of **8** (600 mg, 2.36 mmol), acetic acid (12 mL), water (12 mL), and sodium acetate trihydrate (4.68 g). The reaction mixture was stirred overnight at room temperature. The yellow precipitate was filtered and washed with cold water until neutral, and then it was air-dried in a fume hood to yield, 638.1 mg (68.75%) of compound **9**: mp 259 °C; TLC: R_f 0.6 (dichloromethane/methanol, 9:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.36–10.34 (d, 1H, NH), 7.73–7.70 (d, 2H, aromatic H's), 7.58 (br, s, 2H), 7.54–7.51 (d, 2H, aromatic H's), 4.76–4.72 (t, 1H, CH_2OH, D_2O exchangeable), 4.52–4.46 (m, 1H, H-2′), 3.48–3.46 (m, 1H, CH_2OH), 3.45–3.41 (m, 1H, CH_2OH), 2.09–2.06 (m, 1H, H-3′), 1.7–1.6 (m, 1H, H-1′), 1.55–1.50 (m, 1H, H-4′), 1.41–1.37 m, 2H, H-3′, H-5′), 0.50–0.46 (m, 1H, H-6′), 0.12–0.10 (m, 1H, H-6′). Anal. calcd for $C_{17}H_{18}Cl_2N_6O$: C, 51.91; H, 4.61; N, 21.36. Found: C, 51.83; H, 4.76; N, 21.30.

(−)-*cis-exo*-2-[(2-Amino-6-chloro-5-[(4-chlorophenyl)-azo]-4-pyrimidinyl)amino]-4-(hydroxymethyl)-bicyclo[3.1.0]-hexane (9a). A cold diazonium salt solution prepared as described above was added to a mixture of **8a** (400 mg, 1.574 mmol), acetic acid (8 mL), water (8 mL), and sodium acetate trihydrate (3.2 g). Following the procedure above, compound was obtained as a yellow product, 402.4 mg (65%): mp 238 °C; $[\alpha]_D^{25}$ +125.06° (*c* 0.5, CH_3OCH_3). Anal. calcd for $C_{17}H_{18}Cl_2N_6O$: C, 51.91; H, 4.61; N, 21.36. Found: C, 51.76; H, 4.50; N, 21.19.

(±)-*cis-exo*-2-[(2,5-Diamino-6-chloro-4-pyrimidinyl)-amino]-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (10). To a suspension of **9** (1.227 g, 3.1221 mmol) in ethanol (92 mL) and water (41 mL) was added zinc dust (2.044 g, 31.2 mmol) and acetic acid (1 mL). The reaction mixture was heated under reflux in nitrogen for 3 h. The zinc was removed by filtration and the filtrate was concentrated to give a brown residue. The residue was then adsorbed onto silica gel and it was packed into a column and eluted with chloroform/methanol (20:1). The product fractions were concentrated to give a yellow oil. Further purification from methanol–ether yielded compound **10** as a white solid: 243.0 mg (64.7%); mp 178 °C; TLC: R_f 0.29 (chloroform/methanol, 10:1). ^{1}H NMR (CD_3OD, 300 MHz) δ 4.37–4.35 (d, 1H, H-2′), 3.59–3.56 (m, 2H, CH_2OH), 2.19–2.12 (m, 1H, H-3′), 1.84–1.74 (m, 1H, H-1′), 1.58–1.53 (m, 1H, H-4′), 1.43–1.39 (m, 2H, H-3′, H-5′), 0.58–0.51 (m, 1H, H-6′), 0.12–0.08 (m, 1H, H-6′). Anal. calcd for $C_{11}H_{16}ClN_5O$: C, 48.98; H, 5.97; N, 25.96. Found: C, 49.23; H, 6.20; N, 25.61.

(−)-*cis-exo*-2-[(2,5-Diamino-6-chloro-4-pyrimidinyl)-amino]-4-(hydroxymethyl)-bicyclo[3.1.0]hexane (10a). A mixture of **9a** (2.559 g, 6.513 mmol), zinc dust (4.259 g, 65 mmol), acetic acid (2.08 mL), water (100 mL) and ethanol (180 mL) was heated under reflux in nitrogen for 3 h and worked up as described above. The mixture was then adsorbed onto silica gel and it was packed into a column and eluted with chloroform/methanol (30:1). The product fractions were concentrated to give a yellow oil. Further purification from methanol–ether yielded **10a** as a white solid, 986.2 mg (58%); mp 186–188 °C; $[\alpha]_D^{25}$ −22.04° (*c* 0.5, CH_3OH). Anal. calcd for $C_{11}H_{16}ClN_5O{\cdot}0.5H_2O$: C, 47.40; H, 6.14; N, 25.11. Found: C, 47.20; H, 6.25; N, 25.28.

(±)-*cis-exo*-2-(2-Amino-6-chloro-9*H*-purin-9-y]-4-(hydroxy-methyl)-bicyclo[3.1.0] hexane (11). To a suspension of **10** (543 mg, 2.018 mmol) in triethyl orthoformate (11 mL) was added hydrochloric acid (12 N, 0.5 mL) and the

mixture was stirred at room temperature overnight. The suspension was dried in vacuo. Diluted hydrochloric acid (0.5 N, 15 mL) was added to the residue and the mixture was stirred at room temperature for 1 h. The reaction mixture was then neutralized to pH 8 with 1 N sodium hydroxide and concentrated to give a pale yellow residue. The residue was then adsorbed onto silica gel and it was packed into a column and eluted with chloroform/methanol (20:1) to chloroform/methanol (15:1). The product fractions were concentrated to give a white solid, 413.5 mg (73.4%). TLC: R_f 0.3 (chloroform/methanol; 10:1); mp 228–230 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.20 (s, 1H, H-8), 6.89 (br, s, 2H, NH_2, D_2O exchangeable), 4.65–4.62 (m, 1H, H-2′), 4.57–4.52 (m, 1H, CH_2OH, D_2O exchangeable), 3.29–3.14 (m, 1H, CH_2OH), 2.99–2.93 (m, 1H, CH_2OH), 2.09–2.06 (m, 1H, H-3′), 1.84–1.79 (m, 2H, H-4′, H-1′), 1.77–1.72 (m, 1H, H-3′), 1.63–1.57 (m, 1H, H-5′), 0.71–0.67 (m, 1H, H-6′), 0.33–0.28 (m, 1H, H-6′). Anal. calcd for $C_{12}H_{14}ClN_5O.0.6H_2O$: C, 49.61; H, 5.27; N, 24.09. Found: C, 49.89; H, 5.01; N, 23.75.

(−)-*cis*-*exo*-2-(2-Amino-6-chloro-9*H*-purin-9-y]-4-(hydroxymethyl)-bicyclo[3.1.0] hexane (11a). A mixture of **10a** (200 mg, 0.74 mmol), triethyl orthoformate (5.4 mL) and hydrochloric acid (12 N, 0.22 mL) was stirred at room temperature overnight. The reaction mixture was processed as described above to give a pale-yellow solid, 150 mg (75%), mp 190 °C; $[\alpha]_D^{25}$ −116.71 (*c* 0.5, CH_3OH). Anal. calcd for $C_{12}H_{14}ClN_5O$: C, 51.52; H, 5.04; N, 25.03. Found: C, 51.43; H, 4.93; N, 24.91.

(±)-*cis*-*exo*-2-(2-Amino-1,9-dihydro-6*H*-purin-6-one]-4-(hydroxymethyl)-bicyclo [3.1.0] hexane (12). A solution of **11** (100 mg, 0.3584 mmol) in 1 N HCl (10 mL) was heated under reflux for 5 h. The reaction mixture was then concentrated. The residue was then dissolved in water and the solution was neutralized to pH 7 with 6 N sodium hydroxide. A precipitate formed and the suspension was refrigerated for 1 h. It was then filtered and the solid was recrystallized using hot water to give a white solid, 52.7 mg (56.27%); mp 292–294 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.47 (s, br, 1H, OH, D_2O exchangeable), 7.75 (s, 1H, H-8), 6.40 (br, s, 2H, NH_2, D_2O exchangeable), 4.57–4.51 (m, 2H, H-2′, CH_2OH, D_2O exchangeable), 3.27–3.15 (m, 1H, CH_2OH), 2.99–2.93 (m, 1H, CH_2OH), 2.07–2.01 (m, 1H, H-3′), 1.82–1.72 (m, 2H, H-4′, H-1′), 1.65–1.62 (m, 1H, H-3′), 1.60–1.56 (m, 1H, H-5′), 0.69–0.62 (m, 1H, H-6′), 0.28–0.24 (m, 1H, H-6′). Anal. calcd for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.78; N, 26.80. Found: C, 55.20; H, 6.00; N, 26.66.

(−)-*cis*-*exo*-2-(2-Amino-1,9-dihydro-6*H*-purin-6-one]-4-(hydroxymethyl)-bicyclo [3.1.0] hexane (12a). Compound **11a** (200 mg, 0.716 mmol) was hydrolyzed with hydrochloric acid and purified as described above to yield a white solid, 18 mg (9.6%); mp 304 °C; $[\alpha]_D^{25}$ −38.5° (*c* 0.25, DMSO). Anal. calcd for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.78; N, 26.80. Found: C, 55.25; H, 5.66; N, 26.81.

(±)-*cis*-*exo*-2-(2,6-Diamino-9*H*-purin-9-y]-4-(hydroxymethyl)-bicyclo [3.1.0] hexane (13). Liquid ammonia was passed into a solution of **11** (150 mg, 0.5376 mmol) in methanol (2 mL) at −80 °C in a bomb. The bomb was sealed and heated at 75 °C for 48 h. The bomb was then opened at −80 °C and allowed to warm to room temperature. Ammonia and methanol were evaporated. The residue was adsorbed onto silica gel and it was packed into a column and eluted with chloroform/methanol (15:1) to chloroform/methanol (5:1). The product fractions were concentrated to give a crude product, which was recrystallized from ethanol to yield a white solid, 50.0 mg (36%); mp 240–242 °C; TLC: R_f 0.18 (chloroform/methanol; 15:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.77 (s, 1H, H-8), 6.61 (br, s, 2H, NH_2, D_2O exchangeable), 6.61 (br, s, 2H, NH_2, D_2O exchangeable), 4.58–4.53 (m, 2H, H-2′, CH_2OH, D_2O exchangeable), 3.19–3.14 (m, 1H, CH_2OH), 2.98–2.96 (m, 1H, CH_2OH), 2.046–2.04 (m, 1H, H-3′), 1.79–1.74 (m, 2H, H-4′, H-1′), 1.70–1.65 (m, 1H, H-3′), 1.61–1.58 (m, 1H, H-5′), 0.66–0.64 (m, 1H, H-6′), 0.27–0.26 (m, 1H, H-6′). Anal. calcd for $C_{12}H_{16}N_6O.0.4H_2O$: C, 53.87; H, 6.33; N, 31.42. Found: C, 54.02; H, 6.12; N, 31.19.

(−)-*cis*-*exo*-2-(2,6-Diamino-9*H*-purin-9-y]-4-(hydroxymethyl)-bicyclo [3.1.0] hexane (13a). Compound **11a** (150 mg, 0.537 mmol) was reacted with ammonia as described above to yield white crystals of **13a**, 75 mg (53%); mp 160 °C; MS (FAB-HR): 260 (M^+), 261 ($M^+ + 1$); $[\alpha]_D^{25}$ −106.8° (*c* 0.5, CH_3OH).

(±)-*cis*-*exo*-2-[2-Amino-6-propoxy-9*H*-purin-9-y]-4-(hydroxymethyl)-bicyclo [3.1.0] hexane (14). To compound **11** (100 mg, 0.3584 mmol) was added *n*-propanol (10 mL), sodium hydride (200 mg, 8.33 mmol) and heated under reflux for 1 h. The reaction mixture was then neutralized with acetic acid and concentrated to dryness in vacuo. The residue was then partitioned between ethyl acetate and water. The organic layer was dried over Na_2SO_4 and concentrated. The residue was adsorbed on silica gel and it was packed into a column and eluted with chloroform/methanol (50:1) to chloroform/methanol (25:1). The product fractions were concentrated to give a crude product, which was recrystallized from methanol-water to yield a white solid, 34.8 mg (31.6%); mp 132–136 °C; TLC: R_f 0.3 (chloroform/methanol; 15:1). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.92 (s, 1H, H-8), 6.36 (br, s, 2H, NH_2, D_2O exchangeable), 4.60–4.53 (m, 2H, H-2′, CH_2OH, D_2O exchangeable), 4.32–4.27 (t, 2H, OCH_2CH_2), 3.20–3.13 (m, 1H, CH_2OH), 2.98–2.92 (m, 1H, CH_2OH), 2.07–2.04 (m, 1H, H-3′), 1.81–1.76 (m, 2H, H-4′, H-1′), 1.73–1.71 (m, 2H, $CH_2CH_2CH_3$), 1.68–1.67 (m, 1H, H-3′), 1.62–1.57 (m, 1H, H-5′), 0.95–0.90 (t, 3H, CH_2CH_3), 0.68–0.64 (m, 1H, H-6′), 0.30–0.26 (m, 1H, H-6′). Anal. calcd for $C_{15}H_{21}N_5O_2.0.5H_2O$: C, 57.65; H, 7.09; N, 22.39. Found: C, 57.22; H, 7.19; N, 22.24.

(−)-*cis*-*exo*-2-[2-Amino-6-propoxy-9*H*-purin-9-y]-4-(hydroxymethyl)-bicyclo [3.1.0] hexane (14a). Compound **11a** (150 mg, 0.537 mmol) was treated with sodium hydride (300 mg, 12.49 mmol) and *n*-propanol (15 mL) as described above to give an oil, which was

recrystallized from methanol–water to yield a white solid, 60 mg (37%); mp 136–138 °C; MS (FAB-HR): 303 (M^+), 304 ($M^+ + 1$); $[\alpha]_D^{25}$ −122.32° (*c* 0.5, CH_3OH). Anal. calcd for $C_{15}H_{21}N_5O_2$. $1.25H_2O$: C, 55.28; H, 7.26; N, 21.49. Found: C, 55.43; H, 7.21; N, 21.79.

Bis(tributylammonium) pyrophosphate. A solution of tetrasodium pyrophosphate decahydrate (6.3 g, 14.1 mmol) in 100 mL of water was passed through a Dowex 50X-8 (H^+) column (25 g) directly into a solution of tributylamine (67 mL, 28.1 mmol) in ethanol (200 mL). The resulting solution was evaporated to an oil under reduced pressure. This oil was triturated with diethyl ether to give a colorless hygroscopic solid that was further dried under reduced pressure. 5.12 g (65.8%). ^{31}P NMR (D_2O, 300 MHz) δ −10.05.

(±)-*cis*-*exo*-2-(6-Amino-9*H*-purin-9-yl)-4-[(hydroxy)[hydroxy(phosphonooxy)-phosphinyl]oxy]phosphinyloxymethyl]-bicyclo[3.1.0]hexane (15). To a suspension of compound **7** (100 mg, 0.407 mmol) in dry trimethylphosphate (2.8 mL) at 0 °C was added phosphorus oxychloride (161.36 mg, 1.054 mmol), and the mixture was stirred at 0 C for 2 h. This reaction mixture was then treated with a solution of bis(tributylammonium) pyrophosphate, (1.13 g, 2.038 mmol), and tributylamine (0.32 g, 1.727 mmol) in dimethyl formamide (4.01 mL). The mixture was stirred at 0 °C for 15 min, and then poured into ice-water (100 mL), neutralized with cold aqueous triethylamine to pH 7, and lyophilized. The semisolid was then dissolved in water (20 mL), and applied to an ion-exchange column (DEAE Sephadex A-25, HCO_3^- form, 20 g). The elution was performed by a linear gradient of water (1.0 L) to 0.75 M triethylamine bicarbonate (1.5 L) and monitored at 254 nm. The fractions containing the triphosphate were combined, and concentrated under reduced pressure, and then lyophilized. The solid obtained was evaporated several times with ethanol to remove the triethylammonium bicarbonate, and then dried in vacuo to give compound **15**, 132.1 mg. TLC: R_f 0.12 (isopropanol/ammonia/water, 7:1:2). ^1H NMR (D_2O, 300 MHz) δ 8.11 (s, 1H, H-8), 7.76 (s, 1H, H-2), 4.42–4.40 (d, 1H, H-2′), 3.30–3.21 (m, 1H, CH_2OH), 3.16–3.03 (m, 1H, CH_2OH), 2.09–2.01 (m, 1H, H-3′), 1.71–1.61 (m, 1H, H-5′), 1.48–1.45 (m. 2H, H-4′, H-1′), 1.33–1.27 (m, 1H, H-3′), 0.48–0.42 (q, 1H, H-6′), 0.01–0.00 (q, 1H, H-6′); ^{31}P NMR (1.6 mL Hepes Buffer, pH 7 containing 0.5 mL D_2O and 0.05 mL EDTA, 150 mg/mL) δ-4.63 (P_γ);−9.69 (P_α), −20.55 (P_β); MS (FAB-HR): 485.2 (M^+), 486.0 ($M^+ + 1$), 484.2 ($M^+ - 1$).

Antiviral test

The in vitro antiviral assays were carried out by virus-induced cytopathogenic effects (CPE) inhibition studies. Confluent monolayers of African green monkey kidney cells (Vero cell line) grown in minimal essential media supplemented with 5% fetal calf serum in COSTAR 96-well plates were infected with an innoculum of HSV-1 (strain F). Final concentrations of 100 μg/mL followed by 10-fold serial dilutions of the test compounds were added to the wells. Some wells were used as virus controls and left free of test compound. Other wells remained free of virus to determine drug toxic effects against vero cells. Acyclovir, a potent anti-HSV agent was used as the positive control. The 96-well plates were incubated for 3 days at 37 °C in a humidified atmosphere containing 5% CO_2 until maximum CPE were observed in the virus control cultures. The cell monolayers were examined microscopically for virus-induced CPE and for drug cytotoxicity. The IC_{50} value of the inhibitor is defined as the concentration of drug that decreased the number of plaques 50% to that present in the compound free control wells.

Reverse transcriptase assay

Purified recombinant HIV-1 reverse transcriptase was obtained from the University of Alabama at Birmingham, Center for AIDS Research, Gene Expression Core Facility (supported in part by the NIH Centers for AIDS research program grant P30 AI27767). For the study of RNA transcription, HIV-1 reverse transcriptase activity was measured in 50-μL reactions containing 50 mM Tris (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, 4 mM β-mercaptoethanol, 3% glycerol, 1 mg/mL bovine serum albumin, 3.33 μg/mL of primed 16S rRNA from *Escherichia coli*, 10 μM dTTP, 10 μM dGTP, 10 μM dCTP, and 0.25 μM [^{33}P]dATP (the K_m concentration). The primer was annealed to the template at a ratio of 3 to 1 as described earlier.[14] After incubation the DNA in each sample was precipitated onto glass fiber filters using a 5% trichloroacetic acid solution containing 10 mM pyrophosphate. These filters were batch washed and counted for radioactivity.[15] Assays were done in duplicate and the IC_{50} values were reported.

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