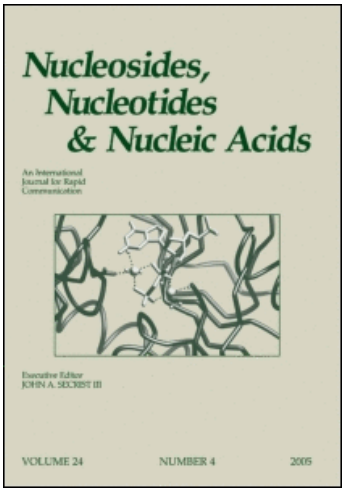

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SYNTHESIS AND BIOLOGICAL EVALUATION OF ENDOCYCLIC 2′,3′-DIDEHYDRO-2′,3′-DIDEOXYMETHANOCARBA ADENOSINE

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ABSTRACT

This is the first report describing the synthesis and conformation of methanocarba nucleosides incorporating an *endo* (β-face) cyclopropyl at the 2′,3′ position of 2′,3′-didehydro-2′,3′-dideoxy carbocyclic nucleosides. These nucleoside isosteres have been shown to exist in a unique extreme eastern conformation. This prediction was confirmed by x-ray crystallography and high resolution NMR spectroscopy. As expected, the methanocarba adenosine compound was neither a substrate nor an inhibitor of adenosine deaminase. However, some of the compounds synthesized demonstrated moderate antiviral activity against HSV-1. The methanocarba adenosine and its triphosphate form were evaluated as inhibitors of HIV-1 reverse transcriptase.

INTRODUCTION

The concept of enzymatically stable nucleoside analogues led to the synthesis of carbocyclic nucleosides. However, as a result of altered stereoelectronic effects, carbocyclic nucleosides are generally less biologically

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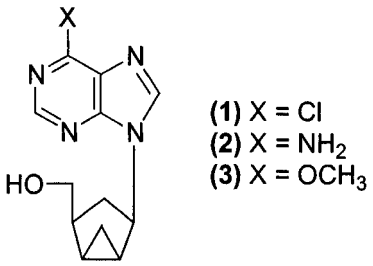


Figure 1. Methanocarba adenosine isosteres. Note that although only one enantiomer is depicted in each structure, all compounds and intermediates are racemic mixtures.

effective than their nucleoside counterparts.[1,2] In solution, the sugar moieties of nucleosides exist in a rapid dynamic equilibrium between extreme conformations as defined in the pseudorotational cycle.[3,4] In the solid state however, only one of these conformations is present. Therefore, it is difficult to correlate which conformation of a nucleoside is responsible for its biological activity.

It has been shown that substituting a bicyclo[3.1.0]hexane moiety for the nucleoside glycone locks the ring pucker of the nucleoside analogue so that the conformation is the same in both the solution and solid states.[5] The rigid methanocarba nucleoside may then result in a stable nucleoside-receptor interaction with the target enzyme. Synthesizing carbocyclic nucleoside analogues incorporating rigid bicyclo[3.1.0]hexanes allows structure activity relationships to be evaluated correlating the defined conformations with specific enzyme activity.

Based on the exceptional antiviral activity of the 2′,3′-didehydro-2′,3′-dideoxy carbocyclic nucleosides previously synthesized in our laboratory, we prepared bicyclo[3.1.0]hexane nucleosides incorporating an *endo* cyclopropyl at the 2′,3′-didehydro-2′,3′-dideoxy position.[6] Because both cyclopropyl and vinyl groups demonstrate similar unsaturated properties, the *exo* 2′,3′-methanocarba nucleosides have recently been synthesized.[7,8] X-ray crystallography and high resolution NMR spectroscopy confirmed that the *endo* methanocarba adenosine **2** compound reported here (Fig. 1) exists in a unique extreme eastern (C3′-*endo*, when C3′ supplants the oxygen of the furanose) conformation. The methanocarba nucleosides were synthesized and evaluated for activity against HSV-1 and HIV-1. The triphosphate nucleotide analogue of compound **2** was also synthesized and tested as an inhibitor of HIV-1 reverse transcriptase.

RESULTS

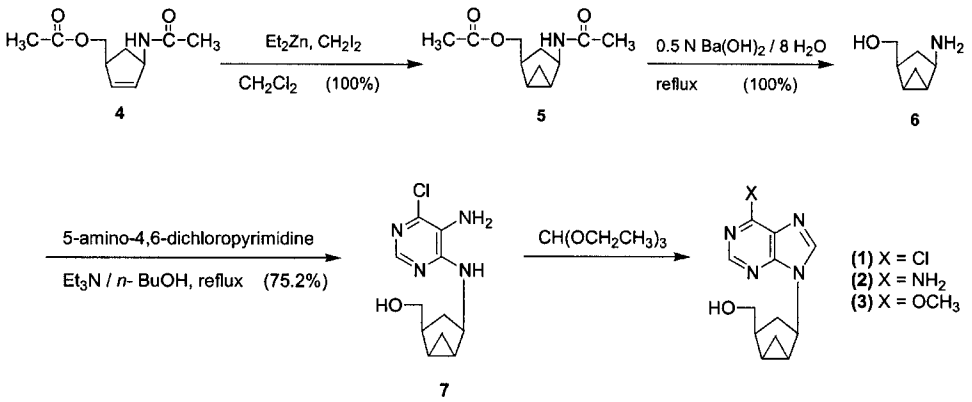
Chemical Synthesis

The basic strategy of synthesizing the methanocarba purine nucleoside analogues involved two steps. The first step was the synthesis of a functio-

nalized amino bicyclo[3.1.0]hexane moiety. The heterocylic purine was then constructed from the amino bicyclohexane. The versatile lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one, was utilized as the starting material because the rigid bicyclo[2.2.1]heptene allowed for control of the stereochemistry of the incoming substituent reactions.[9–11] This unsaturated lactam offered a *cis* orientation for the hydroxymethyl and the heterocyclic functions. The *endo* bicyclic template, **6**, was constructed from the racemic lactam in four steps. The lactam underwent acidic hydrolysis as previously described in the literature to yield the *cis*-1,3-cyclopentene system which was subsequently esterified and acetylated to give the blocked amino cyclopentene.[10] The methyl ester was then reduced, and the corresponding alcohol was acetylated to give compound **4**.[10–13] Modified Simmons-Smith reaction conditions were employed for the diastereoselective delivery of the incoming methylene function to generate the acetylated *endo* bicyclo[3.1.0]hexane, **5**, in a quantitative yield as shown in Sch. 1.[14–16] The formation of the *endo* isomer was consistent with the anticipated amide-directed cyclopropanation.[16] The amide and ester moieties of compound **5** were removed by barium hydroxide hydrolysis to the bicyclic amino alcohol, **6**.

The *endo* bicyclo[3.1.0]hexane template, **6**, and 5-amino-4,6-dichloropyrimidine were condensed in refluxing *n*-butanol in the presence of triethylamine to yield the pyrimidinylamino compound, **7**. Cyclization of compound **7** was subsequently completed with triethyl orthoformate and acid to give the corresponding purine. Any resulting formate ester was removed by mild acid hydrolysis.[17] The halide of the 6-chloropurine, **1**, was displaced with ammonia to generate methanocarba adenosine, **2**. When compound **1** was heated in methanol, displacement of the chlorine from the heterocyclic ring gave the methoxy derivative, **3**.

The triphosphate derivative was synthesized from the methanocarba adenosine isostere using a "one pot" procedure.[18] The methanocarba



Scheme 1. Synthesis of compounds **1**, **2**, and **3** using the *endo*-bicyclo [3.1.0]hexane.

adenosine isostere, **2**, was first treated with phosphorous oxychloride to generate the phosphoric dichloride intermediate. Bis(tributylammonium) pyrophosphate was then added directly to the mixture and the triphosphate derivative was isolated after the neutral mixture was eluted by a linear gradient of aqueous triethylamine bicarbonate through an anion exchange column. Bis(tributylammonium) pyrophosphate was generated by passing an aqueous solution of tetrasodium pyrophosphate decahydrate through a cation exchange column directly into a solution of tributylamine in ethanol.[19]

DISCUSSION

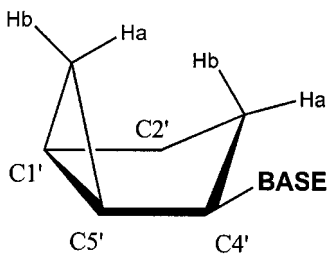
The regiostereochemical assignments for the conformations of the adenosine isostere, **2**, were confirmed by high-resolution ^{1}H NMR spectra analysis, including COSY and NOSEY spectra. The x-ray crystal structure of the adenosine isostere, **2**, was also evaluated.

Two-Dimensional ^{1}H NMR Spectra Analysis

The vicinal couplings between different protons were identified as cross signals in the HOMO-COSY 2D spectra. The results of the COSY spectra are summarized in Table 1. NOSEY spectral analysis provided definitive

Table 1. Cross Signals Observed in HOMO-COSY 2 D Spectra of the Methanocarba Adenosine Isostere

	H-4′	CH_2	H-2′	H-3′a	H-5′	H-1′	H-3′b	H-6′a	H-6′b
H-4′				X	X		X		
CH_2OH			X						
H-2′		X		X		X	X		
H-3′a	X		X				X		
H-5′	X					X		X	X
H-1′			X		X			X	X
H-3′b	X		X	X					
H-6′a					X	X			X
H-6′b					X	X		X	



Compound **2**

evidence for the regiochemistry assigned for the methanocarba andenosine compound synthesized (Fig. 2). It is important to note that there are cross signals between correlating H-1′, H-2′, H-4′, and H-5′ confirming that these four protons are all below the plane of the six-membered ring. This confirms that the cyclopropyl was added to form the *endo* bicyclic hexane. The NOESY spectra also confirms that a 6′-Ha and 3′-Hb interaction is occurring. There are also interactions between 6′-Hb and 1′-H and between 3′-Ha and 4′-H.

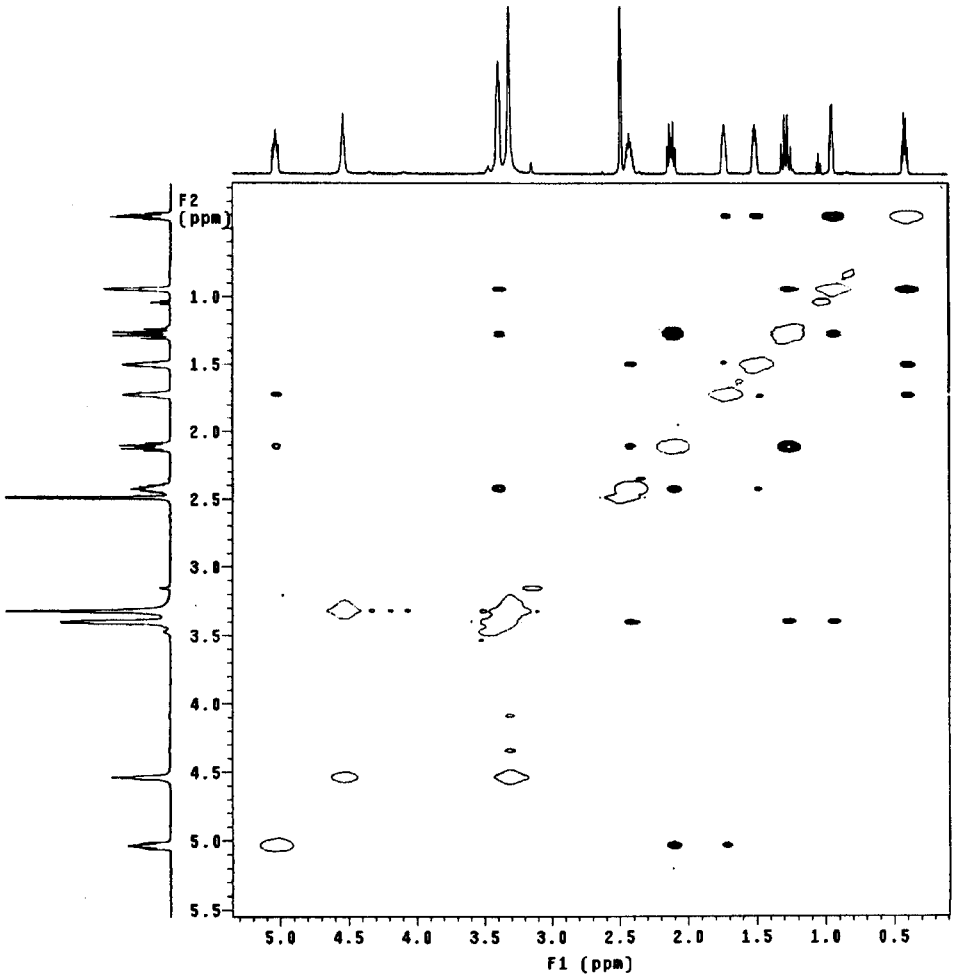


Figure 2. NOSEY spectra of compound **2**.

X-Ray Crystallographic Analysis of Methanocarba Adenosine

The adenine rings in the crystal structure are stacked with an extensive intermolecular H-bonding network among the neighboring methanocarba adenosine molecules. It is suggested that intermolecular interactions in the crystal do not have a major impact on the extent of puckering in the sugar ring (Ψ_{max}). But, the interactions can tune the mode of puckering (P), the glycosidic conformation (χ), and the C2′-CH_2OH conformation (γ) to maximally optimize hydrogen bonding and stacking interactions (Fig. 3).

The pseudorotational value, P, was obtained from calculations based on the crystal structure data obtained. If the pseudorotational concept is applied to the bicyclo[3.1.0]hexane ring, the phase angle, P, is 92.5°. This defines the pucker as C3′-*endo* if the C3′ is taken to be analogous to the furanose O4′. The pseudosugar is unique in that it is confined to the eastern conformation. This is important because in natural nucleosides containing riboses and deoxyriboses, P falls in the ranges of 0°–36° and 144°–180°. The existence of these narrow ranges implies the existence of the true minimum energy regions. The potential barriers near $P = 90°$ and 270° arise when the molecule pseudorotates from one energy minimum to the other. It is speculated that the pseudorotational pathway along the $P = 90°$ point is the favored mode of north-south interconverion.[20] Therefore, this compound in its uniqueness is an extremely useful tool for studying the conformational preferences of adenosine metabolizing enzymes.

BIOLOGICAL EVALUATION

The *endo* methanocarba adenosine isostere, **2**, was not recognized by the adenosine deaminase enzyme. This result is in support of a previous study

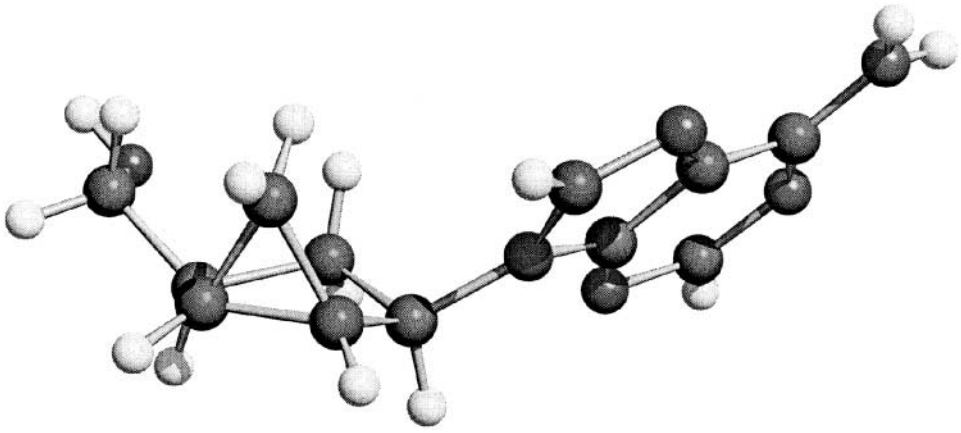


Figure 3. The molecular structure of compound **2** as determined by X-ray crystallography.

demonstrating the conformational preference of adenosine deaminase[21]. Compounds were tested against a DNA virus, Herpes simplex virus, type 1 (strain F), in African green monkey kidney cells (VERO cell line). Both compounds **2** and **3** were shown to inhibit HSV-1 with an IC_{50} of 40 μM. Compound **2** showed no cytotoxicity and compound **3** showed only slight cytotoxicity at 400 μM. The 6-chloropurine compound, **1**, was the most active with an $IC_{50} = 4.0$ μM. As expected, compound **1** was also the most cytoxic as cycotoxicity was observed at 40 μM. Compound **2** was evaluated for antiviral activity against HIV-1 but was found to be inactive (the IC_{50} value was greater than 100 μM, AZT demonstrated an IC_{50} value of 0.003 μM). From this study, it can be postulated that the inactivity of this compound could be a result of poor anabolism to the mono-, di-, or triphosphate level or of the poor substrate properties of the 5′-triphosphate toward the viral encoded reverse transcriptase. Therefore, the methanocarba nucleoside triphosphate of **2** was tested for HIV-1 reverse transcriptase activity. Interestingly, the *endo* methanocarba nucleotide inhibited reverse transcriptase with an IC_{50} of 15 μM. From this, it can be concluded that the parent nucleoside form is probably not anabolized intracellularly to the triphosphate form.

EXPERIMENTAL SECTION

Crystal Structure

The adenosine nucleoside analogue, **2**, was recrystallized by evaporation from methanol/water (10:1). All calculations were preformed with SGI INDY R4400-SC and/or Pentium computers using the SHELXTL V5.10 suite of programs. A clear colorless $0.14 \times 0.11 \times 0.06\,mm^3$ triangular crystal was used for data collection on an automated Bruker SMART diffractometer platform equipped with a graphite monochromator. The frame time was 25 seconds and the detector distance was 4.95 cm. Lattice parameters were determined from 20 reflections within θ range 1.47° to 25.13°. The data collection range of *hkl* was $-6 \le h \le 3$, $-9 \le k \le 9$, $-16 \le 1 \le 16$ with 98.7% completeness to $\theta = 25.13°$. The data collection was conducted using MoKα radiation. A randomly oriented region of reciprocal space was surveyed to the extent of 1.3 hemispheres and to a resolution of 0.84 Å. Three major sections of frames were collected with 0.30° steps in ω at 3 different φ settings and a detector position of −28° in 2θ. The intensity data were corrected for absorption and decay.[22] Final cell constants were calculated from the *xyz* centroids of 567 strong reflections from the actual data collection after integration.

Biological Experimental

Adenosine Deaminase Assay

Adenosine deaminase (150-200 units/mg protein), originally isolated from calf intestinal mucosa, was obtained from Sigma Chemical Company, St. Louis, Missouri. Adenosine deaminase solution was prepared by dilution into potassium phosphate buffer (0.05M, pH 7.4) to the concentration of approximately 100 units/mL. The enzymatic reaction was preformed in 1 mL potassium phosphate buffer containing 40 μM compound and 50 milliunits of adenosine deaminase solution. The assay was monitored by following the decrease in absorbance at 265 nm (37°C) for 15 min. If there was no change in absorbance within 15 min, an additional enzyme (50 milliunits) was added and the reaction was continued overnight. A decrease in UV absorbance indicated that the test compound was deaminated and thus a substrate for adenosine deaminase. The inhibitory effects of the test compounds against adenosine deaminase were evaluated by comparing the rate of deamination of adenosine with or without the presence of test compound. If deamination rate was delayed by the coexistance of the test compound, the compound was considered an inhibitor of adenosine deaminase.

Antiviral Assays

HSV-1

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 lines) grown in minimal essential media supplemented with 5% fetal calf serum in COSTAR 96-well microtiter plates were infected with an inoculum of HSV-1, strain F. Final concentrations of 100 μg/mL followed by ten fold serial dilutions of test compounds were added to the wells (100 μg/mL to 0.1 μg/mL). Virus controls contained no test compounds. Other wells remained free of virus to determine the drug toxicity effects against the VERO cells. Acyclovir was used as the positive control. The 96-well plates were incubated for 3 days at 37°C in a humidified atmosphere with 5% CO_2 until the maximum CPE were observed in the virus control cultures. The cell monolayers were microscopically examined for virus induced CPE and for drug cytotoxicity. The IC_{50} value is the concentration of the test compound the decreased the total number of plaques 50% to that present in the control wells with no test compound.

HIV-1

The HIV inhibitory assay of the compounds were evaluated by microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood

mononuclear cells (PBMCs), which quantify the ability of a compound to inhibit HIV-induced cell killing or HIV replication.[23] AZT was used as the positive control. Quantification was performed with the tetrazolium dye 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2M-tetrazolium-5-carboxanilide (XTT), which is metabolized to a colored formazan product by the viable cells.[24]

HIV-1 Teverse Transcriptase

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 $MgCl_2$, 4mM β-mercaptoethanol, 3% glycerol, 1 mg/mL bovine serum albumin, 3.33 μg/mL of primed 16S rRNA from E. 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 previously.[25] 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.[26] Assays were done in duplicate. An initial range finding experiment was first conducted. The data from this experiment was then used to narrow the concentration range for each compound, which was then tested in smaller increments. The experiments were repeated in duplicate and these two experiments were used to calculate the average IC_{50} values.

Chemical Experimental

General Synthesis

All chemicals and solvents were reagent grade unless otherwise specified. The chemicals and solvents used were obtained from Aldrich Chemical Company, Milwaukee, WI. All intermediates and final compounds were racemic mixtures. Analytical thin layer chromatography (TLC) was performed on 0.25 mm layers of Merck silica gel 60 F_{254} on glass-backed plates. The plates were visualized by viewing under UV light and by exposure to iodine vapor unless otherwise indicated. Column chromatography was performed on Merck silica gel 60 Å, 230–400 mesh. Chromatography on silica involved dissolving the crude material in a suitable solvent, treatment of

the solution with silica gel, followed by evaporation of the solvent and then drying of the silica *in vacuo*. The dried compounds containing silica were then loaded onto a slurry packed column of silica gel and eluted with the solvent system listed. Then, the fractions containing pure product (as indicated by TLC) were combined and concentrated under reduced pressure. Evaporation under reduced pressure refers to the use of a rotary evaporator using aspirator pressure. Evaporation *in vacuo* was carried out with a rotary evaporator under 0.2 mm Hg of pressure at a temperature below 30°C. The melting points were determined in open capillary tubes heated in a Mel-Temp II melting point apparatus and are uncorrected. Routine mass spectra, elemental analysis, ^{1}H, and ^{31}P NMR spectra were recorded using standard methods. Positive-ion fast-atom bombardment mass spectra (FABMS) were obtained on a VG 7070E-HF spectrometer. MBNA was used as the sample matrix and ionization was effected by a beam of xenon atoms. The peaks listed are those from the molecular ion, designated M^+, and fragments that can be assigned as plus or minus relative to the molecular ion. The elemental analyses were preformed by M-H-W Laboratories in Phoenix, AZ. The ^{1}H and ^{31}P NMR spectra were obtained on Varian Unity VAC-200 or VAC-300 with an automatic sample changer or on a Varian Inova, VI-500 in the solvent listed and are given in parts per million (δ). The peak multiplicities are abbreviated as follows: broad, br; singlet, s; doublet, d; triplet, t; and multiplet, m.

(±)-*cis*-endo-Acetic acid 4-acetylamino-bicyclo[3.1.0]hex-2-yl methyl ester (5). Compound **4** (2.04 g, 10.4 mmol) was dissolved in anhydrous methylene chloride (50 mL) and cooled to 0 °C under N_2 (g) as diethylzinc (100 mL, 1 M in hexanes) was carefully added. The clear yellow solution was stirred for 30 min. Diiodomethane (8.91 mL, 111 mmol) was diluted with methylene chloride (40 mL) and then added dropwise to the above cold diethylzinc/**4** solution. The yellow and white mixture continued stirring as the reaction mixture warmed to room temperature overnight (19 h). Saturated sodium chloride (40 mL) and 3N hydrochloric acid (80 mL) were added to the reaction over 15 min. Methylene chloride (100 mL) and saturated sodium sulfite (40 mL) were then added to the clear yellow mixture. The aqueous layer was removed and washed with chloroform (100 mL). The chloroform layer and the methylene chloride layer were combined and washed with saturated sodium bicarbonate (200 mL). The orange organic layer was removed and dried over anhydrous magnesium sulfate and concentrated to a yellow oil, **5** (2.20 g, 10.4 mmol, 100%): R_f value 0.778 (isopropanol : ammonium hydroxide : water − 7:1:2); 1H NMR (200 MHz, dimethyl-d_6 sulfoxide): δ 7.74–7.78 (1H, d, N*H*), 4.23–4.33 (1H, m, *H*-4′), 3.81–4.00 (2H, m, OCH_2), 2.32–2.43 (1H, m, *H*-2′), 1.99 (3H, s, CH_3CO_2), 1.76 (3H, s, NCOCH_3), 1.64–1.79 (1H, m, *H*-3′a), 1.34–1.43 (1H, m, *H*-5′),

1.20–1.31 (1H, m, *H*-1′), 0.560–0.730 (1H, m, *H*-3′b), 0.459–0.522 (1H, m, *H*-6′a), 0.158–0.263 (1H, m, *H*-6′b). Anal. calcd. for $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.39; H, 8.24; N, 6.61.

(±)-*cis*-endo-(4-Amino-bicyclo[3.1.0]hex-2-yl)methanol (6). Compound **5** (2.16 g, 10.2 mmol) was refluxed with aqueous barium hydroxide (0.5N, 250 mL) overnight (17.5 h), cooled to 0°C and carefully neutralized with carbon dioxide (g). The white precipitate was removed by filtration and the clear filtrate was concentrated to dryness. The dry solid was triturated with water (2 × 50 mL) and the water mixture was filtered through Celite 545. The yellow filtrate was passed through an anion (-OH) exchange column (Amberlite IRA-400, 15-50 wet mesh, activated from the chloride form by washing with 1N sodium hydroxide). The clear solution was concentrated *in vacuo* (37 °C, 0.2 mm Hg) to a white solid. Absolute ethanol (150mL) was added, and the white precipitate was removed by filtration. The clear filtrate was concentrated to an oil, **6** (1.31 g, 10.3 mmol, 100%): mp (dec) 93–95°C; R_f value 0.416 (isopropanol : ammonium hydroxide : water, 7:1:2); ^{1}H NMR (200 MHz, dimethyl-d_6 sulfoxide): δ 6.41 (1H, b, N*H*-a, D_2O exchangeable), 4.33 (1H, b, O*H*, D_2O exchangeable), 4.03 (1H, b, N*H*-b, D_2O exchangeable), 3.30–3.43 (1H, m, *H*-4′), 3.23–3.26 (2H, m, CH_2OH), 2.03–2.13 (1H, m, *H*-2′), 1.51–1.65 (1H, m, *H*-3′a), 1.31–1.14 (2H, m, *H*-1′ and *H*-5′), 0.468–0.300 (2H, m, *H*-3′b and *H*-6′a), 0.016–0.086 (1H, m, *H*-6′b). MS (FAB) 127.19, $(M+H)^+$.

(±)-*cis*-endo-{4-[(5-Amino-6-chloro-pyrimidin-4-yl)amino]-bicyclo[3.1.0]-hex-2-yl}methanol (7). Compound **6** (400 mg, 3.14 mmol) was combined with 5-amino-4,6-dichloropyrimidine (781 mg, 4.40 mmol) in 1-butanol (50 mL). Triethylamine (3.12 mL) was added and the mixture was refluxed for 45 h. The brown mixture was concentrated *in vacuo* to an orange residue and purified by flash column chromatography (hexanes/ethyl acetate – 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6) to a fluffy white solid, **7** (601 mg, 2.36 mmol, 75.2%): mp 176–178 °C; R_f value 0.241 (ethyl acetate); ^{1}H NMR (200 MHz, dimethyl-d_6 sulfoxide): δ 7.71 (1H, s, *H*-2), 6.58–6.65 (1H, d, N*H*, D_2O exchangeable), 5.09 (2H, s, NH_2, D_2O exchangeable), 4.59–4.62 (1H, m, *H*-4′), 4.44–4.50 (1H, t, O*H*, D_2O exchangeable), 3.30–3.36 (2H, m, CH_2OH), 2.20–2.32 (1H, m, *H*-2′), 1.95–1.81 (1H, m, *H*-3′a), 1.36–1.62 (1H, m, *H*-5′), 1.27–1.34 (1H, m, *H*-1′), 0.633–0.801 (1H, m, *H*-3′b), 0.483–0.545 (1H, m, *H*-6′a), 0.152–0.256 (1H, m, *H*-6′b). Anal. calcd. for $C_{11}H_{15}N_4OCl$: C, 51.87; H, 5.94; N, 22.0. Found: C, 52.10; H, 5.97; N, 22.21.

(±)-*cis*-endo-[4-(6-Chloro-9*H*-purin-9-yl)bicyclo[3.1.0]hex-2-yl]-methanol (1). Compound **7** (519 mg, 2.04 mmol) was mixed with triethyl ortho-formate (13 mL) and hydrochloric acid (12N, 0.50 mL). The clear yellow

solution was then stirred overnight (14 h). The reaction mixture was concentrated and hydrochloric acid (0.5 N, 12 mL) was added. After the yellow solution was stirred at room temperature for 1 h, it was neutralized to pH 7.2 with aqueous sodium hydroxide (1N, 27 mL) and concentrated to a thick white mixture. The mixture was purified by flash column chromatography (5% methanol/methylene chloride) to a dark brown residue (646 mg) and recrystallized (ethyl acetate) to yield **1** as a white solid (378 mg, 1.43 mmol, 70.1%): mp 129–130°C; R_f value 0.459 (10% methanol/methylene chloride); ^{1}H NMR (200 MHz, dimethyl-d_6 sulfoxide): δ 8.82 (1H, s, *H*-2), 8.77 (1H, s, *H*-8), 5.15–5.20 (1H, m, *H*-4′), 4.55–4.60 (1H, b, O*H*, D_2O exchangeable), 3.70–3.46 (2H, d, CH_2OH), 2.38–2.48 (1H, m, *H*-2′), 2.17–2.28 (1H, m, *H*-3′a), 1.77–1.81 (1H, m, *H*-5′), 1.52–1.58 (1H, m, *H*-1′) 1.31–1.43 (1H, m, *H*-3′b), 0.936–0.984 (1H, m, *H*-6′a), 0.461–0.431 (1H, m, *H*-6′b). Anal. calcd. for $C_{12}H_{13}N_4OCl$: C, 54.44; H, 4.95; N, 21.17; Cl, 13.39. Found: C, 54.16; H, 5.06; N, 21.49; Cl, 13.61.

(±)-*cis*-endo-[4-(6-Amino-9*H*-purin-9-yl)bicyclo[3.1.0]hex-2-yl]-methanol (2). Compound **1** (707 mg, 2.67 mmol) was placed in a bomb. Excess liquid ammonia was added. The bomb was sealed and heated at 75°C for 42 h. The bomb was then cooled, and the ammonia was evaporated. The remaining white solid was purified by flash column chromatography (methanol/methylene chloride, 0%–15%) to yield **2** as a fine white powder (611 mg, 2.49 mmol, 93.2%): mp 123–124 °C; R_f value 0.478 (20% methanol/methylene chloride); ^{1}H NMR (500 MHz, dimethyl-d_6 sulfoxide): δ8.25 (1H, s, *H*-2), 8.12 (1H, s, *H*-8), 7.18 (2H, s, NH_2-C6, D_2O exchangeable), 5.01–5.06 (1H, m, *H*-4′), 4.54 (1H, t, O*H*, D_2O exchangeable), 3.39–3.40 (2H, m, CH_2OH), 2.40–2.45 (1H, m, *H*-2′), 2.09–2.14 (1H, m, *H*-3′a), 1.71–1.75 (1H, m, *H*-5′), 1.48–1.52 (1H, m, *H*-1′), 1.24–1.31 (1H, m, *H*-3′b), 0.934–0.960 (1H, m, *H*-6′a), 0.391–0.433 (1H, m, *H*-6′b). Anal. calcd. for $C_{12}H_{15}N_5O$: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.63; H, 6.17; N, 28.62.

(±)-*cis*-endo-[4-(6-Methoxy-9*H*-purin-9-yl)bicyclo[3.1.0]hex-2-yl]-methanol (3). Compound **1** (537 mg, 2.11 mmol) was mixed with triethyl orthoformate (10 mL) and hydrochloric acid (12N, 0.50 mL) and stirred overnight (14 h). When dilute hydrochloric acid (0.5N, 12 mL) was added to the white reaction mixture, it turned to a clear yellow solution. After the solution stirred at room temperature for 1 h, sodium hydroxide (1N, 32 mL) was added until the pH of the solution was slightly basic. Methanol (10 mL) was added, and the reaction mixture was concentrated *in vacuo* to a residue. The residue was purified by flash column chromatography (10% methanol/chloroform) and recrystallized (ethyl acetate) to yield **3** (357 mg, 1.46 mmol, 69.3%): mp 163–164 °C; R_f value 0.404 (10% methanol/methylene chloride);

^{1}H NMR (300 MHz, dimethyl-d_6 sulfoxide): δ 8.50 (1H, s, *H*-2), 8.49 (1H, s, *H*-8), 5.09–5.17 (1H, m, *H*-4′), 4.53–4.56 (1H, t, O*H*, D_2O exchangeable), 3.99–4.06 (3H, s, CH_3), 3.38–3.49 (2H, m, CH_2OH), 2.38–2.40 (1H, m, *H*-2′), 2.11–2.21 (1H, m, *H*-3′a), 1.72–1.80 (1H, m, *H*-5′), 1.48–1.56 (1H, m, *H*-1′), 1.15–1.41 (1H, m, *H*-3′b), 0.928–0.971 (1H, m, *H*-6′a), 0.380–0.452 (1H, m, *H*-6′b). Anal. calcd. for $C_{13}H_{16}N_4O_2$: C, 59.99; H, 6.20; N, 21.52. Found: C, 60.06; H, 6.34; N, 21.95.

(±)-*cis*-endo-P-{[4-(6-Amino-9*H*-purin-9-yl)bicyclo[3.1.0]hex-2-yl] methyl} ester triphosphoric acid. Compound **2** (100 mg, 0.407 mmol) was suspended in dry trimethylphosphate (4 mL) and cooled to 0 °C. Phosphorus oxychloride (0.100 mL, 1.05 mmol, 166 mg) was then added and the cool mixture continued stirring at 0 °C for two hours. The reaction mixture was then stirred at room temperature for 2 h and recooled 0 °C. Bis (tributylammonium) pyrophosphate (1.11 g, 2.04 mmol), and tributylamine (3.11 mg, 4.12 mL) in dimethylformamide (6 mL) was added to the above reaction mixture. After the mixture stirred 0°C for 15 min, it was poured into ice water (200 mL). This neutral solution was stored at 4 °C overnight, concentrated *in vacuo* and lypholized to an oil. Ether (2 × 125 mL) was added to the oil to extract some of the remaining trimethyl phosphate. The residue was concentrated and dissolved in water (20 mL) and applied to a 4 × 40 cm column containing DEAE Sephadex A-25 (25 g), HCO_3^- form. The elution was performed by a linear gradient of water (1 L) to 0.75 M triethylamine bicarbonate (1.5 L). The fractions containing the triphosphate compound were concentrated and lyophilized. The solid was evaporated several times with ethanol (4 × 100 mL) to remove the triethylammonium bicarbonate, and then lyophilized to yield the triphosphate of compound 2 as a clear, colorless fluffy solid (227 mg crude, UV: 52.6% with A = 0.44054, $\varepsilon = 15.4 \times 10^3$, 119 mg, 0.246 mmol, 60.4% actual); ^{1}H NMR (300 MHz, dimethyl-d_6 sulfoxide): δ 8.29 (1H, s, *H*-2), 8.13 (1H, s, *H*-8), 7.17 (2H, s, NH_2-C6, D_2O exchangeable), 5.06 (1H, m, *H*-4′), 3.39 (2H, m, CH_2OH), 2.49 (1H, m, *H*-2′), 2.08 (1H, m, *H*-3′a), 1.75 (1H, m, *H*-5′), 1.50 (1H, m, H-1′), 1.35–1.43 (1H, m, H-3′b), 0.846-0.905 (1H, m, H-6′a), 0.414–0.433 (1H, m, *H*-6′b). ^{31}P NMR (300 MHz, 1.6 mL Hepes Buffer, pH7 containing 0.5 mL deuterium oxide and 0.05 mL EDTA, 150mg/mL): δ −21.65 (P_β), −10.24 (P_α), −6.57 (P_γ). MS (FAB) 486.0, $(M+H)^+$; 484.2, $(M-H)^-$.

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