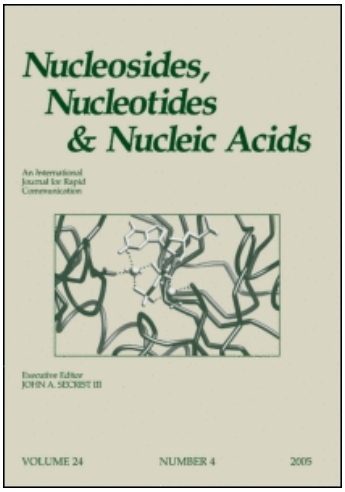

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 12 February 2002

To cite this Article Gudmundsson, Kristjan S. , Daluge, Susan M. , Condreay, Lynn D. and Johnson, Lance C.(2002) 'SYNTHESIS OF NOVEL 8-SUBSTITUTED CARBOCYCLIC ANALOGS OF 2',3'-DIDEOXYADENOSINE WITH ACTIVITY AGAINST HEPATITIS B VIRUS', Nucleosides, Nucleotides and Nucleic Acids, 21: 11, 891 — 901
To link to this Article: DOI: 10.1081/NCN-120016613
URL: http://dx.doi.org/10.1081/NCN-120016613

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 21, Nos. 11 & 12, pp. 891–901, 2002

SYNTHESIS OF NOVEL 8-SUBSTITUTED CARBOCYCLIC ANALOGS OF 2′,3′-DIDEOXYADENOSINE WITH ACTIVITY AGAINST HEPATITIS B VIRUS

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ABSTRACT

Synthesis and antiviral activity of several new 8-substituted carbocyclic analogs of D-2′,3′-dideoxyadenosine are described. The new 8-substituted analogs were synthesized via lithiation of carbocyclic 2′,3′-dideoxyadenosine followed by quenching with electrophiles. This methodology allows for a divergent synthesis of a variety of 8-substituted analogs from carbocyclic 2′,3′-dideoxyadenosine in high yields. 8-Methyl and 8-halogenated carbocyclic 2′,3′-dideoxyadenosine analogs showed 6–25 fold more activity against hepatitis B virus than the unsubstituted carbocyclic D-2′,3′-dideoxyadenosine.

INTRODUCTION

2′,3′-Dideoxyribofuranosides have received considerable attention as this class of nucleosides includes several compounds with useful clinical anti-HIV activity (e.g., ddI and ddC).[1] The 2′3′-dideoxyribofuranosides suffer from lack of stability of the glycosidic bond and 2′3′-dideoxyadenosine (ddA)

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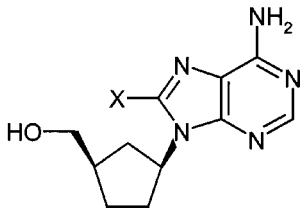
DOI: 10.1081/NCN-120016613 1525-7770 (Print); 1532-2335 (Online)



1a-g

a, X=CH_3; b, X=Cl; c, X=Br; d, X=I; e, X=$NHCH_3$; f, X=$CON(CH_3)_2$; g, X=phenyl.

Figure 1.

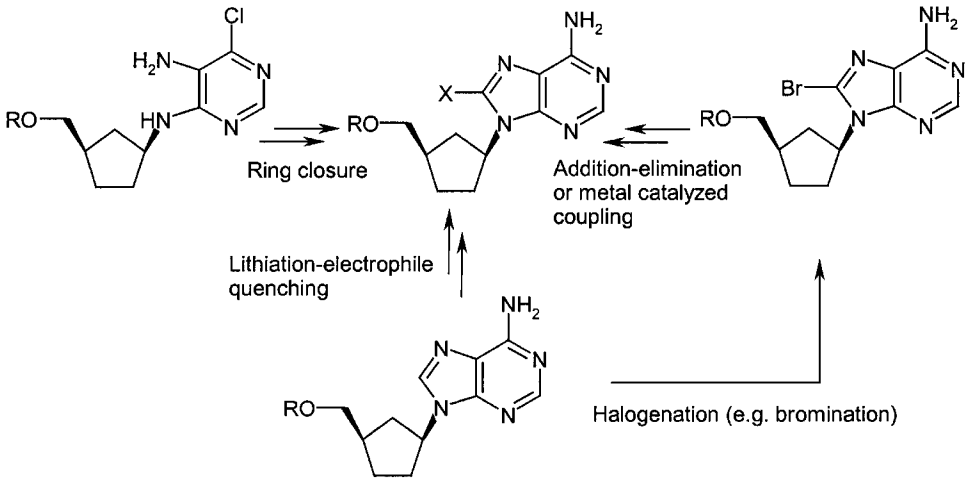
also suffers from rapid deamination by adenosine deaminase. Several 2′,3′-dideoxyribofuranoside analogs substituted at the C-8 position of adenine have been synthesized[2] and evaluated for hydrolytic[3] and enzymatic stability,[4] but these analogs have not been reported to have significant antiviral activity.[5] The carbocyclic analogs of D- and L-ddA[6] and numerous other carbocyclic-2′,3′-dideoxynucleoside analogs[7] have been described and reported to have antiviral activity, including activity against hepatitis B virus.[6,7] Since carbocyclic and ribofuranoside analogs often have very different antiviral activity, we became interested in the synthesis of 8-substituted carbocyclic D-ddA analogs. These have, to the best of our knowledge, not been reported.

Herein we describe a facile route for the synthesis of several novel 8-substituted carbocyclic dideoxyadenosine analogs (**1a-g**) and their antiviral activity against hepatitis B virus (HBV).

RESULTS AND DISCUSSION

We envisioned several potential routes toward the desired 8-substituted carbocyclic dideoxyadenosine analogs as outlined in Sch. 1. Synthesis of 8-substituted derivatives by condensation of orthoformates and diaminopyrimidines to form the purine ring system is well precedented for carbocyclic nucleosides,[6,7] but this route is only viable for a limited number of 8-substituted analogs.

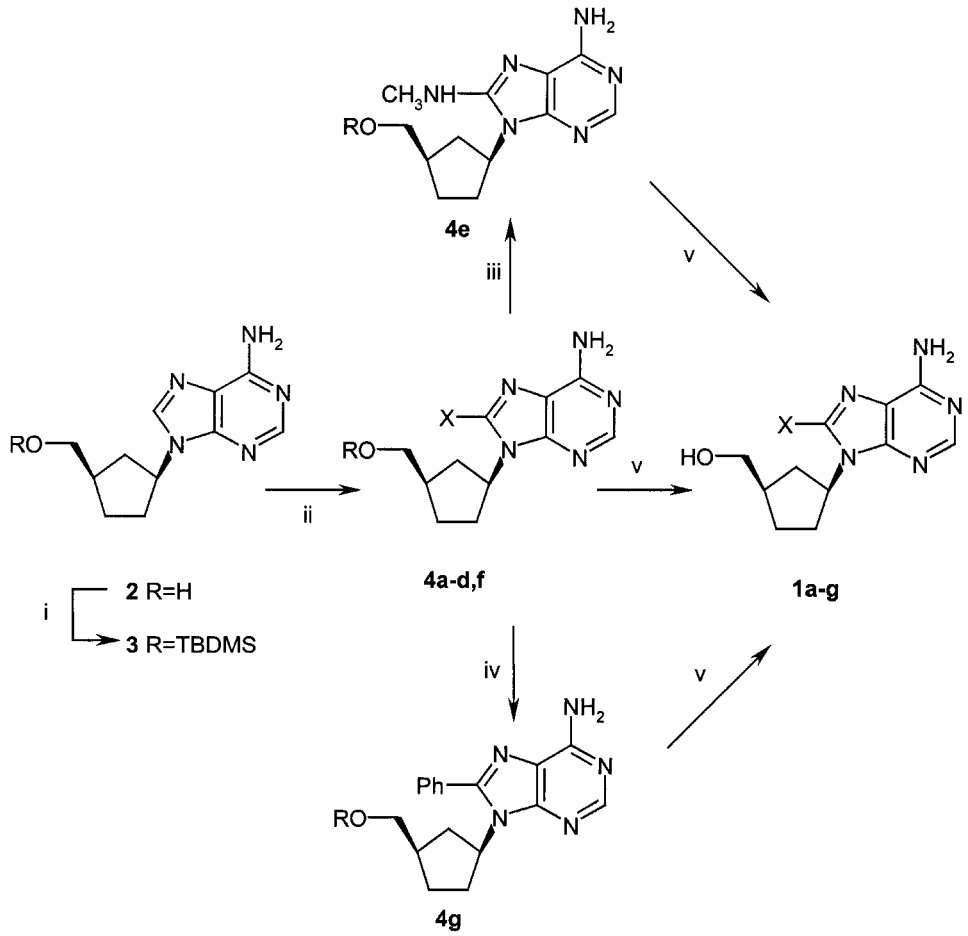
As we desired a highly divergent route that could be used to add a variety of functionality at the C-8 position we initially focused our attention on C-8 halogenation of carbocyclic D-ddA. The 8-halogen could subsequently be replaced by addition-elimination or metal catalyzed coupling reactions to give the desired 8-substituted compounds. Carbocyclic D-ddA (**2**) was synthesized in a similar manner as described in the literature.[6] Silylation of **2** using TBDMSCl and imidazole gave the silyl protected carbocyclic dideoxyadenosine (**3**). Bromination of the C-8 position of **3** using N-bromosuccinimide in dichloromethane was successful, but gave a



Scheme 1.

relatively low and poorly reproducible yield (5-35%) of **4c**. Attempts to improve the yield by use of other solvents (such as N,N-dimethylformamide, dioxane, acetonitrile) failed. Similarly, attempts to brominate the unprotected carbocyclic dideoxyadenosine (**2**) using N-bromosuccinimide in various solvents gave, again, low and poorly reproducible yield of the desired **1c** (5–15%).

We then decided to study lithiation of **3** followed by quenching of the lithiated adenosine derivative with electrophiles using conditions similar to those previously described for lithiation-electrophile quenching of 2′,3′-dideoxyribofuranosyl derivatives.[2] We found that lithiation of **3** using excess lithium diisopropylamide (LDA, 5 equiv) in tetrahydrofuran at −78°C followed by quenching with an electrophile (e.g., Br_2) gave consistently a good yield (e.g., 81% of the 8-bromo derivative, **4c**) of the desired products. For optimal yields, 5–6 equivalents of LDA were required, fewer equivalents gave lower yield of the desired product and resulted in recovery of more unreacted starting material. Other bases (such as lithium tetramethylpiperidine, lithium bis(trimethylsilyl)amide) did not improve the yield. These LDA lithiation-electrophile quenching conditions were used for a variety of electrophiles to give good yields of **4a–d** and **4f**. The 8-methylamine derivative **4e** was obtained by an addition-elimination reaction, whereby treatment of the 8-iododerivative **4d** with methylamine at 130°C gave **4e**. The 8-phenyl derivative **4g** was synthesized by palladium catalyzed coupling of **4d** and phenylboronic acid. Desilylation, using tetrabutylammonium fluoride, gave the desired compounds **1a–g** in excellent overall yields.



Scheme 2. a, X = CH_3; b, X = Cl; c, X = Br; d, X = I; e, X = $NHCH_3$; f, X = $CON(CH_3)_2$; g, X = phenyl. (i) TBDMSCl (1.3 equiv), imidazole, DMF at RT; (ii) LDA (5 eq) at $-78°C$ in THF, then add electrophile (2–3 equiv); (iii) Treatment of **4d** (X = I) with CH_3NH_2 at 130°C; (iv) Treatment of **4d** (X = I) with $PhB(OH)_2$ (1.4 equiv), $Pd(PPh_3)_4$ (0.3 equiv), Na_2CO_3 (aq, 2 equiv) in DME at 80°C; (v) TBAF (2 equiv), THF, RT.

Several of the 8-substituted carbocyclic D-ddA analogs showed interesting activity against HBV as outlined in Table 1.

Thus the 8-methyl analog (**1a**) and the 8-halogenated analogs (**1b–1d**) showed 6–25 fold better activity against HBV than the unsubstituted carbocyclic D-ddA (**2**). The 8-methylamine derivative (**1e**) showed similar activity to **2**, while the 8-dimethylcarboxamide (**1f**) and the 8-phenyl analog (**1g**) showed no HBV activity, possibly due to the steric bulkiness of these substituents. For all the 8-substituted carbocyclic analogs that showed activity against HBV, the IC_{50} for activity was clearly delineated from cytotoxicity. None of the carbocyclic D-ddA derivatives (**1a–1g**) had activity against HIV-1 in MT-4

Table 1. Anti-HBV Activity[a] and Cytotoxicity[a] of Carbocyclic ddA (**2**) and 8-Substituted Analogs in HepG2-2.2.15 Cells

Compound Number	HBV Activity[b] IC_{50} (μM)	HepG2 Toxicity[b] CC_{50} (μM)	Selectivity Index (CC_{50}/IC_{50})
2	33	>200	>6
1a	6	>200	>33
1b	12	>200	>16
1c	2	48	24
1d	1.3	9	7
1e	33	>200	>6
1f	47	>200	>4
1g	>200	>200	–

[a]Assay conditions are described in the Experimental section.
[b]Antiviral 50% inhibitory concentration (IC_{50}) and 50% cytotoxic concentration (CC_{50}). Results are averages from duplicate experiments.

lymphocytes that was separate from cytotoxicity.[8] Of particular interest was the anti-HBV activity and selectivity of the 8-methyl (**1a**), 8-chloro (**1b**) and 8-bromo (**1c**) substituted carbocyclic D-ddA derivatives. Additional analogs related to these are being synthesized and studied.

EXPERIMENTAL

Chemistry

Nuclear magnetic resonance (NMR) spectra were obtained at 300 MHz on Varian Unity Plus NMR spectrophotometer. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Elemental analysis were performed by Atlantic Microlab Inc. Flash column chromatography was performed using Merck Silica gel 60 (230–400 mesh), and the stated solvent system under pressure. Mass spectra were obtained on Micromass Platform mass spectrometers from Micromass Ltd. Altrincham, UK, using Electrospray Ionization.

9-[(1*S*,3*R*)-3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)cyclopentyl]-9*H*-purin-6-amine (3). To a solution of carbocyclic D-ddA ([(1*R*,3*S*)-3-(6-amino-9*H*-purin-9-yl)cyclopentyl]methanol (**2**), 1.0 g, 4.3 mmol) in N,N-dimethylformamide (10 mL) was added imidazole (0.44 g, 6.4 mmol) and t-butylchlorodimethylsilane (1.4 mL, 5.6 mmol). The resulting mixture was stirred at room temperature for 12 h. Dichloromethane was added to the reaction mixture and the organic phase washed with water and brine. The organic phase was dried over magnesium sulfate. Filtration and concentration

followed by purification by flash chromatography (5% methanol in chloroform) gave **3** (1.4 g, 94%) as a white solid. ^{1}H-NMR ($CDCl_3$): δ 8.28 (s, 1H), 7.88 (s, 1H), 5.70 (broad s, 2H), 4.84 (m, 1H), 3.60 (m, 2H), 1.6–2.4 (m, 7H), 0.84 (s, 9H), 0.0 (s, 6H); MS m/z 348 (M + H). Anal. Calcd for $C_{17}H_{29}N_5O_2Si$: C 58.75, H 8.41, N 20.15. Found, C 58.73, H 8.34, N 20.21.

[(1*R*,3*S*)-3-(6-Amino-8-methyl-9*H*-purin-9-yl)cyclopentyl]methanol (1a). Diisopropylamine (2.0 mL, 14.4 mmol) was added to a solution of n-butyllithium (9 mL of 1.6 M solution in hexane, 14.4 mmol) in tetrahydrofuran (20 mL) at − 78°C. The resulting mixture was stirred for 5 min, then a solution of compound **3** (1.0 g, 2.9 mmol) in dry tetrahydrofuran (10 mL) was added. The dark reaction mixture was stirred for 30 min, then iodomethane (0.5 mL, 7.3 mmol) was added. Reaction was stirred at − 78°C for additional 3 h, then quenched by addition of excess acetic acid. Ethyl acetate and water are added to the reaction mixture and the phases were separated. The ethyl acetate phase was washed with saturated aqueous sodium bicarbonate, water and brine and dried over magnesium sulfate. Filtration and concentration followed by purification by flash chromatography (5% methanol in chloroform) gave 9-[(1*S*,3*R*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)cyclopentyl]-8-methyl-9*H*-purin-6-amine (**4a**, 0.50 g, 48%) as a solid. This solid (0.5 g, 1.4 mmol) was dissolved in tetrahydrofuran (10 mL) and to this solution was added tetrabutylammonium fluoride (2.0 mL of 1 M solution in tetrahydrofuran, 2.0 mmol). The resulting reaction was stirred at room temperature overnight, then concentrated *in vacuo*. Purification by flash chromatography (5% methanol in chloroform) gave **1a** (230 mg, 67%) as a solid. ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 5.58 (broad s, 2H), 4.83 (m, 1H), 4.45 (m, 1H), 3.85 (m, 2H), 2.63 (s, 3H), 2.5 (m, 3H), 2.05 (m, 2H), 1.9 (m, 2H); MS m/z 248 (M + H). Anal. Calcd for $C_{12}H_{17}N_5Ox1/3\ H_2O$: C 56.91, H 7.03, N 27.65. Found, C 56.94, H 6.94, N 27.70.

[(1*R*,3*S*)-3-(6-Amino-8-chloro-9*H*-purin-9-yl)cyclopentyl]methanol (1b). Diisopropylamine (0.73 g, 1.0 mL, 7.2 mmol) was added to a solution of n-butyllithium (4.5 mL of 1.6 M solution in hexane, 7.2 mmol) in tetrahydrofuran (10 mL) at − 78°C. The resulting mixture was stirred for 5 min then a solution of compound **3** (0.5 g, 1.4 mmol) in dry tetrahydrofuran (10 mL) was added. The dark reaction mixture was stirred for 30 min, then hexachloroethane (1.0 g, 4.2 mmol) was added. Reaction was stirred at −78°C for additional 4 h, then quenched by addition of excess acetic acid. Ethyl acetate and water are added to the reaction mixture and the phases were separated. The organic phase was washed with water and brine and dried over magnesium sulfate. Filtration and concentration followed by purification by flash chromatography (10% methanol in chloroform) gave **4b** (0.47 g, 86%) as a solid. This solid (0.47 g, 1.2 mmol) was dissolved in

tetrahydrofuran (10 mL) and to this solution was added tetrabutylammonium fluoride (3.0 mL of 1 M solution in tetrahydrofuran, 3.0 mmol). The resulting reaction mixture was stirred at room temperature overnight, then concentrated *in vacuo*. Purification by flash chromatography (10% methanol in chloroform), followed by recrystallization from acetonitrile, gave **1b** (0.26 g, 79%) as a solid. ^{1}H NMR ($CDCl_3$): δ 8.34 (s, 1H), 5.66 (broad s, 2H), 5.03 (m, 1H), 3.82 (m, 3H), 1.6–2.6 (m, 7H); MS m/z 268 (M + H). Anal. Calcd for $C_{11}H_{14}N_5OCl$: C 49.35, H 5.27, N 26.16. Found, C 49.37, H 5.28, N 26.21.

[(1*R*,3*S*)-3-(6-Amino-8-bromo-9*H*-purin-9-yl)cyclopentyl]methanol (1c). Treatment of **3** (0.5 g, 1.4 mmol) with lithium diisopropylamide (7.2 mmol, made *in situ* from n-butyllithium and diisopropylamine), followed by addition of bromine (0.57 g, 0.19 mL, 3.6 mmol), gave 498 mg (81%, 98% yield based on recovered starting material) of **4c** as a yellow solid. This solid (0.50 g, 1.2 mmol) was dissolved in tetrahydrofuran (10 mL) and to the solution was added tetrabutylammonium fluoride (3.0 mL of 1 M solution, 3.0 mmol). The resulting solution was stirred at room temperature overnight, then concentrated *in vacuo*. Purification by flash chromatography (10% methanol in chloroform) gave **1c** (0.34 g, 91%, 74% yield for the two step synthesis from **3**) as a solid. ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H), 6.03 (broad s, 2H), 4.88 (m, 1H), 3.76 (m, 2H), 1.7–2.5 (m, 7H); MS m/z 313 (M + H). Anal. Calcd for $C_{11}H_{14}N_5OBr$ x1/3CH_3OH: C 42.16, H 4.79, N 21.69. Found, C 42.13, H 4.69, N 21.48.

Alternatively, treatment of **3** (0.5 g, 1.44 mmol) with N-bromosuccinimide (0.76 g, 4.3 mmol) in dichloromethane (10 mL) at room temperature for 24 h also gave, after deprotection, 72 mg (16% yield for the two step synthesis from **3**) of **1c** as a solid.

9-[(1*S*,3*R*)-3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)cyclopentyl]-8-iodo-9*H*-purin-6-amine (4d). Treatment of **3** (1.0 g, 2.9 mmol) with lithium diisopropylamide (15 mmol, made *in situ* from n-butyllithium and diisopropylamine) at −78°C in a similar fashion as described above, followed by addition of iodine (1.9 g, 7.5 mmol) gave, after purification by flash chromatography (10% methanol in chloroform), 1.0 g (73%) yield of **4d** as a solid (100% yield based on recovered starting material). ^{1}H NMR ($CDCl_3$): δ 8.16 (s, 1H), 5.60 (broad s, 2H), 4.75 (m, 1H), 3.63 (m, 2H), 1.7–2.5 (m, 7H), 0.84 (s, 9H), 0.0 (s, 6H); MS m/z 474 (M + H). Anal. Calcd for $C_{17}H_{28}N_5OSiI$: C 43.14, H 5.96, N 14.79. Found, C 43.52, H 5.97, N 14.72.

[(1*R*,3*S*)-3-(6-Amino-8-iodo-9*H*-purin-9-yl)cyclopentyl]methanol (1d). Compound **4d** (0.3 g, 0.63 mmol) was dissolved in tetrahydrofuran (10 mL) and to this solution was added tetrabutylammonium fluoride (1.0 mL of 1 M solution in tetrahydrofuran, 1.0 mmol). The resulting reaction

was stirred at room temperature overnight, then concentrated *in vacuo*. Purification by flash chromatography (15% methanol in chloroform), followed by recrystallization from acetonitrile, gave 0.16 g (71%) of **1d** as a solid. ^{1}H NMR (d_6-DMSO): δ 8.07 (s, 1H), 7.33 (broad s, 2H), 4.80 (m, 1H), 4.62 (t, 1H), 3.47 (m, 2H), 1.6–2.2 (m, 7H); MS m/z 360 (M + H). Anal. Calcd for $C_{11}H_{14}N_5OI$: C 36.79, H 3.93, N 19.50. Found, C 36.98, H 3.93, N 19.24.

{(1*R*,3*S*)-3-[6-amino-8-(methylamino)-9*H*-purin-9-yl]cyclopentyl}methanol (1e). Compound **4d** (0.41 g, 0.87 mmol) was treated with methylamine in methanol (10 mL of 2 M solution in methanol) at 130°C in a steel vessel for 12 h. The reaction was cooled to room temperature and concentrated *in vacuo*. Purification by flash chromatography (10% methanol in chloroform) gave 0.33 g (quantitative yield) of **4e** as a solid. Compound **4e** (0.33 g, 0.87 mmol) was dissolved in tetrahydrofuran (10 mL) and to this solution was added tetrabutylammonium fluoride (2.0 mL of 1 M solution in tetrahydrofuran, 2.0 mmol). The resulting reaction was stirred at room temperature overnight, then concentrated *in vacuo*. Purification by flash chromatography (20% methanol in ethyl acetate), followed by concentration from dichloromethane, gave 0.16 g (70%) of **1e** as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 1H), 5.41 (m, 1H), 5.34 (broad s, 2H), 4.75 (m, 1H), 4.6 (broad s, 1H), 3.75 (d, 2H), 2.99 (d, 3H), 1.7–2.4 (m, 7H); MS m/z 263 (M + H). Anal. Calcd for $C_{12}H_{18}N_6Ox0.3\ CH_2Cl_2$: C 51.33, H 6.51, N 29.20. Found, C 51.49, H 6.69, N 29.50.

6-Amino-9-[(1*S*,3*R*)-3-(hydroxymethyl)cyclopentyl]-*N*,*N*-dimethyl-9*H*-purine-8-carboxamide (1f). Treatment of **3** (0.5 g, 1.4 mmol) with lithium diisopropylamide (7.5 mmol, made *in situ* from n-butyllithium and diisopropylamine) at −78°C in a similar fashion as described above, followed by addition of dimethylcarbamyl chloride (0.3 mL, 3.0 mmol) gave, after purification by flash chromatography (10% methanol in chloroform), 0.26 g (43%) yield of **4f** as a solid. This solid (0.26 g, 0.62 mmol) was dissolved in tetrahydrofuran (10 mL) and to this solution was added tetrabutylammonium fluoride (1.2 mL of 1 M solution in tetrahydrofuran, 1.2 mmol). The resulting reaction was stirred at room temperature overnight, then concentrated in vacuo. Purification by flash chromatography (20% methanol in dichloromethane) gave 0.15 g (79%) of **1f** as a white foam. ^{1}H NMR ($CDCl_3$): δ 8.37 (s, 1H), 5.95 (broad s, 2H), 5.05 (m, 1H), 4.1 (m, 1H), 3.80 (m, 2H), 3.21 (s, 6H), 1.7–2.4 (7H); MS m/z 305 (M + H). Anal. Calcd for $C_{14}H_{20}N_6O_2x0.45\ CH_2Cl_2$: C 50.66, H 6.15, N 24.53. Found, C 50.56, H 6.52, N 24.54.

[(1*R*,3*S*)-3-(6-Amino-8-phenyl-9*H*-purin-9-yl)cyclopentyl]methanol (1g). Compound **4d** (0.7 g, 1.5 mmol) was dissolved in dimethoxyethane

(10 mL). To this solution was added phenylboronic acid (0.26 g, 2.1 mmol), tetrakis(triphenylphosphine)palladium(0) (0.35 g, 0.4 mmol) and aqueous sodium carbonate (0.37 g, 2.98 mmol in 2 mL of water). The resulting reaction was heated at 80°C for 12 h. This mixture was cooled to room temperature, then water and ethyl acetate were added. The phases were separated and the organic phase washed with water, brine and dried over magnesium sulfate. The organic phase was filtered and concentrated to dryness *in vacuo*. Purification by flash chromatography (10% methanol in chloroform) gave 0.45 g (71%) of **4g** as a solid. Compound **4g** (0.45 g, 1.1 mmol) was dissolved in tetrahydrofuran (10 mL) and to this solution was added tetrabutylammonium fluoride (2.0 mL of 1 M solution in tetrahydrofuran, 2.0 mmol). The resulting reaction was stirred at room temperature overnight, then concentrated *in vacuo*. Purification by flash chromatography (10% methanol in dichloromethane) gave 0.24 g (71%) of **1g** as a solid. ^{1}H NMR (d_6-DMSO): δ 8.18 (s, 1H), 7.70 (m, 2H), 7.60 (m, 3H), 7.36 (broad s, 2H), 4.60 (m, 1H), 4.62 (t, 1H), 3.48 (m, 2H), 1.6–2.6 (m, 7H); MS m/z 310 (M + H). Anal. Calcd for $C_{17}H_{19}N_5Ox0.1\ CH_2Cl_2$: C 64.61, H 6.09, N 22.03. Found, C 64.56, H 6.08, N 21.92.

Antiviral (HBV) Assay

Antiviral potency and growth inhibition potential of compounds was determined using the assay developed by Jansen et al.[9] Briefly, HepG2-2.2.15 cells constitutively producing HBV[10] were seeded into 96 well microtiter plates at a density of 5×10^3 per well and growth medium containing drug was replaced every other day for 9 days. Supernatants were then collected and analyzed for HBV content. Samples were tested in conjunction with both positive (.448 fg/μL plasmid DNA) and negative (RPMI medium supplemented with 2 mM L-glutamine and 10% fetal calf serum) controls. Data was normalized to non-drug treated cells, and expressed as a percent of control for analysis.

Evaluation of toxicity (i.e., growth inhibition) was made by fixing monolayers with 70% ethanol, and staining with bisbenzimide H33342 for 1 h at 37°C. Fluorescence values of drug treated cells were compared to non-drug treated cells and expressed as a percentage of control. HBV detection (and hence efficacy determination) was performed by "capturing" virus from supernatants on anti-HBsAg coated plates, washing, denaturing to release HBV DNA, performing PCR with biotinylated primers, streptavidin capture of biotinylated PCR products with concomitant probe hybridization, addition of substrate, and reading optical densities of the colorimetric reaction. Dilutions of a standardized HBV-containing supernatant were included on every plate, and HBV DNA concentrations of test wells were calculated from this HBV standard curve.

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Received July 31, 2002
Accepted September 4, 2002