The spectrophotometric titrations were carried out by adding 50-μL portions (total of 26) of drug solution to 2.5 mL of approximately 1×10^{-4} M DNA solution and after 5 min measuring the absorbance at the wavelength used for the drug. The amount of free and bound drug in solution after each addition was calculated by the method of Muller and Crothers.[16] Data that was in the range of 65-100% bound, except for bisantrene, was used to analyze the drug binding by the McGhee-von Hippel equation. Each experiment was run at least three times and the data is a composite of three runs. For most compounds, there was little variation between runs; however, solubility problems were encountered with bisantrene. They were overcome by dissolving the bisantrene in 0.01 M sodium phosphate and 0.001 M EDTA at pH 7, without the NaCl. Addition of small volumes of this solution to the DNA solution made little difference in the total ionic strength (~0.11 M), but as larger volumes were added it fell, reaching a minimum of ~0.07 M when a total of 1.3 mL of bisantrene solution was added.

Melt Transition Temperatures. The buffer for these experiments was 0.01 M Na_3PO_4, 0.001 M EDTA, at pH 7.0. Into both the sample and reference cuvette were placed 3 mL of 5×10^{-5} M calf thymus DNA solution and the appropriate amount of drug solution to provide a ratio of five base pairs per drug molecule. The sample cuvette was heated from 25 to 110 °C at 1 °C per minute, while the absorbance at 260 nm was monitored.

Calculations. Curve fitting of the McGhee-von Hippel equation to spectrophotometric titration data was accomplished by use of the program FUNFIT[17] and the smoothing cubic spline function was obtained from IMSL routines,[9] both on a VAX computer.

pK_a Determinations. Solutions were prepared by dissolving 0.2 mmol of each compound as free base or dihydrochloride in sufficient 0.0392 N HCl to give 1 mequiv of acid beyond that required to protonate all amino groups. These solutions were stirred and titrated with 0.0242 N NaOH while the pH was measured on a Sargent-Welch Model IP pH meter. Data were graphed and pK_a values were determined from points on the curve where 0.5 equiv of base per each functional group had been added. Insolubility at higher pH values prevented accurate determinations of pK_a values for **3** and **7**; however, they clearly were dications at pH 7.0. Bisantrene (**2**) was so insoluble that it was titrated in very dilute solution. One milliequivalent of it was dissolved in 10 mL of 0.00392 N HCl and titrated with 0.00242 N NaOH. At the point where there was somewhat more monocation than dication present, bisantrene precipitated and could not be titrated further. Results of these titrations are given in Table IV.

Acknowledgment. This investigation was supported in part by Grant CA 37798 awarded by the National Cancer Institute, DHHS.

Supplementary Material Available: Plots of the McGhee-von Hippel equation for **4**, **5**, **7**, **3**, and **2** (Figures 7-11) and melt transition temperature curves for **6-8** and **9-11** (Figures 12 and 13) (7 pages). Ordering information is given on any current masthead page.

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Synthesis and Structure-Activity Relationships of 6-Substituted 2′,3′-Dideoxypurine Nucleosides as Potential Anti-Human Immunodeficiency Virus Agents

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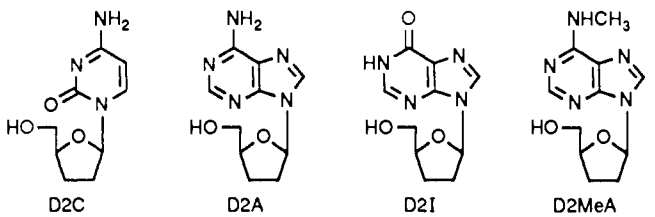
In order to study the structure-activity relationships of 2′,3′-dideoxypurine nucleosides as potential anti-HIV agents, various 6-substituted purine analogues have been synthesized and examined in virus-infected and uninfected human peripheral blood mononuclear cells. N^6-methyl-2′,3′-dideoxyadenosine (D2MeA, **7a**) was initially synthesized from adenosine via 2′,3′-*O*-bisxanthate **3**. As extension of this reaction to other N^6-substituted compounds failed, a total synthetic method utilizing 2′,3′-dideoxyribose derivative **9** was used for the synthesis of other purine nucleosides. An acid-stable derivative of N^6-methyl-2′,3′-dideoxyadenosine, 2′-fluoroarabinofuranosyl analogue **32** (D2MeFA), has been synthesized from the appropriate carbohydrate **24** by condensation with N^6-methyladenine **23**. Among these compounds, N^6-methyl derivative (D2MeA) **7a** proved to be one of the most potent antiviral agents. The order of potency for the 6-substituted compounds was NHMe > NH_2 > Cl ≈ $N(Me)_2$ > SMe > OH ≈ NHEt > SH > NHBn ≈ H. The results suggest that a bulk tolerance effect at the 6-position of the 2′,3′-dideoxypurine nucleoside may dictate the antiviral activity of these compounds. Acid-stable analogue **32** (D2MeFA) was found to be 20-fold less potent than the parent compound. Both D2MeA and D2MeFA were resistant to calf intestine adenosine deaminase. The presence of a fluorine atom in the carbohydrate moiety greatly increased stability to acid, making D2MeFA a potential orally active antiviral agent that could be useful for the treatment of retroviral infections in humans.

Certain dideoxyncleosides exhibit potent antiviral activities against human immunodeficiency viruses (HIV) in vitro. 2′,3′-dideoxycytidine (D2C),[1] 2′,3′-dideoxyadenosine (D2A),[1] and 2′,3′-dideoxyinosine (D2I)[2] (Chart I) are currently undergoing clinical trials in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. The exact mechanism by which these nucleosides suppress the replication of HIV is not fully understood. It is reported that 2′,3′-dideoxynucleosides as their triphosphates inhibit the HIV reverse transcriptase and can cause chain termination of DNA.[1,3-6]

Chart I



† University of Georgia.
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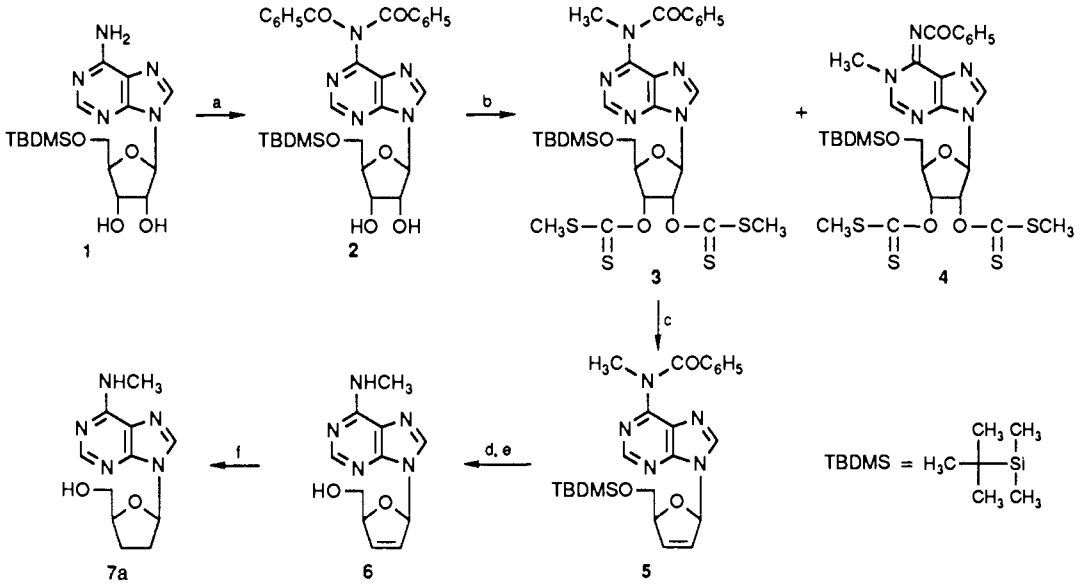
Scheme I[a]



[a] Reagents: (a) TMSCl, C_6H_5COCl, pyridine; (b) CS_2, DMSO, NaOH, CH_3I; (c) Bu_3SnH, AIBN, benzene; (d) TBAF, THF; (e) NH_3/MeOH; (f) H_2, Pd/C, EtOH–H_2O.

D2A is a potent anti-HIV nucleoside in vitro, which undergoes rapid deamination by adenosine deaminase to D2I.[1,7-10] Interestingly, D2I is partially phosphorylated to D2I monophosphate, which is converted to D2A monophosphate, and subsequently to D2A triphosphate. Thus, it has been proposed that the antiviral activity of D2I may be attributed to D2A triphosphate.[8,9] Therefore, D2I can be considered to be a prodrug of D2A monophosphate.

Recently, we have reported the structure–activity relationships of 2′,3′-dideoxy- and 2′,3′-didehydro-2′,3′-dideoxypyrimidine and purine nucleosides along with some pyrimidine C-nucleosides as anti-HIV agents.[10] From these studies, we discovered that N^6-methyl-2′,3′-dideoxyadenosine (D2MeA) was the most potent compound among purine nucleosides. Thus, it was of interest to extend the structure–activity relationships to other purine nucleosides related to D2MeA.

Results and Discussion

Recently, we have reported a general synthesis of 2′,3′-dideoxy- and 2′,3′-didehydro-2′,3′-dideoxynucleosides from the corresponding ribonucleosides.[11] In this method bisxanthates were reduced by tributyltin hydride to the 2′,3′-unsaturated nucleosides, which on catalytic hydrogenation yielded 2′,3′-dideoxynucleosides. Since the method has been found to be general, it was applied to the synthesis of D2MeA from adenosine (Scheme I). Thus, in order to prevent the formation of thiocarbamate, 5′-protected adenosine was reacted with chlorotrimethylsilane, followed by benzoyl chloride in pyridine. N^6,N^6-Dibenzoyladenosine derivative **2** obtained was treated with CS_2 and 5 N aqueous NaOH solution in DMSO, followed by an excess of CH_3I. Two major products obtained in the above reaction were separated by column chromatography. The less polar compound (TLC, benzene–EtOAc 2:1, R_f = 0.74) was found to be bisxanthate **3**, formed by the debenzoylation of one of the N^6-benzoyl groups and subsequent N^6-methylation. The compound with lower R_f (0.34) was identified as N^6-(benzoylimino)-N_1-methyl derivative **4** on the basis of ^1H NMR spectral characteristics and NOE experiments. Irradiation of the N_1–CH_3 signal in **4** resulted in the enhancement of the H-2 proton signal (41%) and a similar observation was made when the H-2 signal was irradiated (enhancement of N_1–CH_3 by 21%). The irradiation of the N^6–CH_3 signal of **3** did not show any effect on the intensity of the H-2 proton signal. Bisxanthates **3** and **4** were found to be unstable and **3** was converted into 2′,3′-unsaturated nucleoside **5** by treatment with tributyltin hydride. Compound **5** was desilylated to the free nucleoside **6** by treatment with tetra-*n*-butylammonium fluoride (TBAF), followed by the debenzoylation with a methanolic ammonia solution. The desired nucleoside D2MeA **7a** previously prepared from 2′-deoxyadenosine[12] was obtained by catalytic hydrogenation of **6**. Extension of the above alkylation reaction (**2** to **5**) with other alkyl iodides failed to give N^6-alkylated products such as **5**. Furthermore, a large-scale reaction did not give

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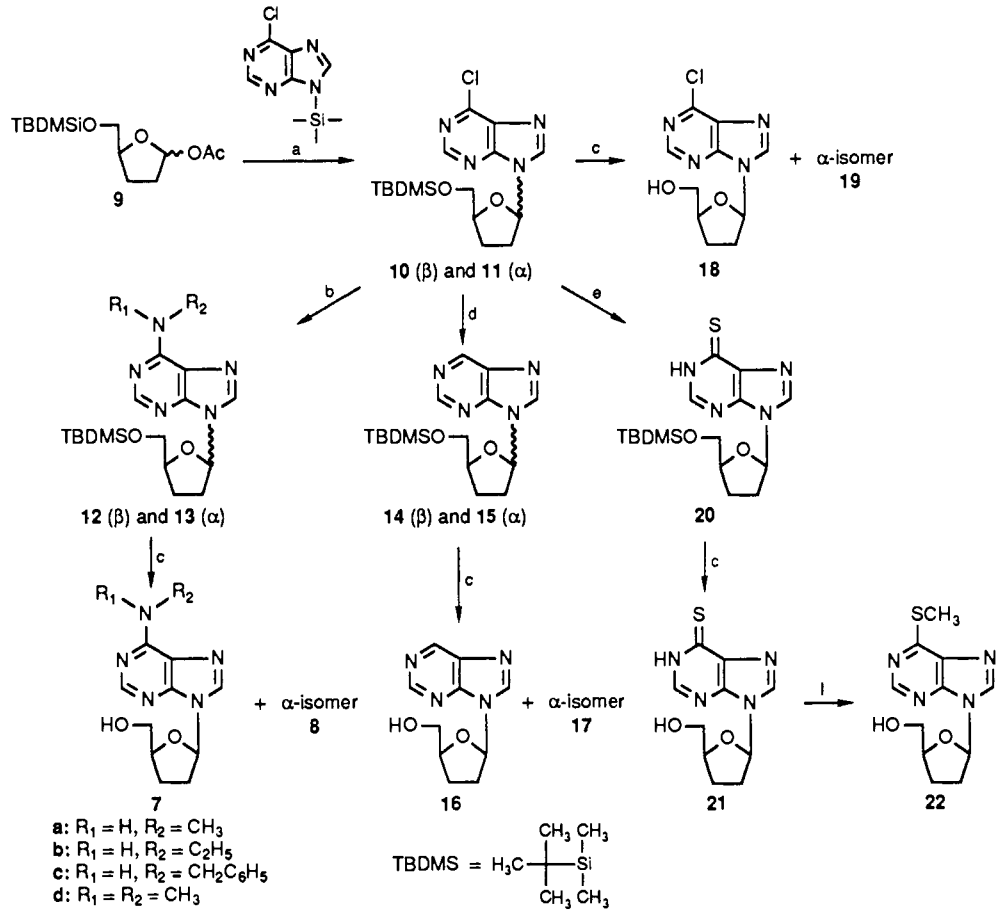
Scheme II[a]



[a] Reagents: (a) TMSOTf, $ClCH_2CH_2Cl$; (b) RNH_2, MeOH; (c) TBAF, THF; (d) Pd/C, H_2, NH_4OH, MeOH; (e) NaSH, MeOH; (f) NaOMe, CH_3I, MeOH.

a reproducible yield of the final product **7a**.

In order to circumvent this problem as well as to synthesize new 2',3'-dideoxynucleosides a total synthetic method was used (Scheme II). Our approach was to use 2,3-dideoxyribose derivative **9**[13] as a key intermediate, which can then be condensed with an appropriate heterocyclic moiety. Thus, the 5(*S*)-[[(*tert*-butyldimethylsilyl)oxy]methyly-5(*H*)-furan-2-one[14,15] was catalytically reduced to the saturated lactone and converted to the lactol by treatment with DIBAL, which was acetylated to obtain the desired carbohydrate intermediate **9** in good yield.

Coupling of **9** with trimethylsilylated 6-chloropurine in the presence of trimethylsilyl triflate in 1,2-dichloroethane[16] yielded an α,β-mixture (**11** and **10**, 1:1). The anomeric mixture could be separated by a column chromatography over silica gel using hexanes–EtOAc (6:1) as the eluent. However, the chromatographic separation was more readily effected after the removal of the *tert*-butyldimethylsilyl group to obtain **19** and **18** in good yields. Attempted condensation of **9** with trimethylsilylated 6-chloropurine in the presence of $EtAlCl_2$[13] gave **10** and **11** (1:1) along with an α,β-mixture of N_7-substituted 6-chloropurine derivatives. Nucleophilic displacement reaction of a mixture of **10** and **11** with aliphatic amines and benzylamine was carried out to give the desired N^6-alkyl- and N^6-benzyl-2',3'-dideoxyadenosine derivatives as shown in Scheme II. Thus, the reaction of a mixture of 6-chloropurine derivatives **10** and **11** with excess amine at 110 °C in a steel bomb for 16 h yielded the corresponding N^6-substituted adenosine derivatives **12** and **13**. Since the α,β-mixture of these compounds was inseparable, they were deprotected with 1 M TBAF/THF to yield the desired N^6-substituted dideoxyadenosines as a mixture of **7a–d** and **8a–c**. The chromatographic separation of the α- and β-isomers was accomplished by using a silica gel column. The assignment of anomeric configuration was made by ^1H NMR spectra: the anomeric protons of the α-anomers (**8a–c**) were observed further downfield than those of the corresponding β-anomers. Furthermore, the H-4' proton of the α-isomers appears downfield from that observed for the β-anomers, and the H-5' protons of the α-anomers appear upfield from those observed for the β-anomers[13] (Table II). The less polar product (R_f = 0.43, $CHCl_3$–MeOH 10:1) obtained from reaction of methylamine with **10** and **11** and subsequent deprotection was assigned as N^6-methyl-2',3'-dideoxyadenosine on the basis of ^1H NMR and was found to be identical with **7a**, obtained by the hydrogenation of **6** as shown in Scheme I. This provided additional support for the assignment of the anomeric configuration made for the 2',3'-dideoxyadenosines.

9-(2,3-Dideoxyribofuranosyl)-6-chloropurines **10** and **11** were also utilized for the synthesis of other purine nucleosides such as **16**, **21**, and **22**. Thus, catalytic hydrogenation of a mixture of **10** and **11** in methanol containing aqueous ammonia, in the presence of 10% palladium on

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Table I. Physical Constants of 6-Substituted 2′,3′-Dideoxypurine Nucleosides

no.	mp °C (solv)[a]	$[\alpha]_D$, deg	formula	anal.	TLC R_f (solv)[b]
2			$C_{30}H_{35}N_5O_6Si$	C,H,N	0.29 (D)
4			$C_{28}H_{37}N_5O_5S_4Si\cdot0.25C_6H_6$	C,H,N	
5	148–150 (d)		$C_{24}H_{31}N_5O_3Si$	C,H,N	0.34 (E)
6	139–145 (ei)		$C_{11}H_{13}N_5O_2$	C,H,N	
10	syrup		$C_{16}H_{25}ClN_4O_2Si$	C,H,N	0.34 (C)
11	104–105 (f)		$C_{16}H_{25}ClN_4O_2Si$	C,H,N	0.38 (C)
7a	128–129	−11.5 (*c* 1.0, MeOH)	$C_{11}H_{15}N_5O_2\cdot0.75H_2O$	C,H,N	0.43 (A)
8a	foam	+47.07 (*c* 1.058, Me_2SO)	$C_{11}H_{15}N_5O_2\cdot0.75H_2O$	C,H,N	0.32 (A)
7b	foam		$C_{12}H_{17}N_5O_2\cdot0.50H_2O$	C,H,N	0.36 (A)
8b	98–99 (gh)	+34.33 (*c* 1.068, MeOH)	$C_{12}H_{17}N_5O_2$	C,H,N	0.27 (A)
7c	foam	−5.63 (*c* 0.764, MeOH)	$C_{17}H_{19}N_5O_2\cdot0.25H_2O$	C,H,N	0.66 (A)
8c	foam	+26.2 (*c* 1.087, MeOH)	$C_{17}H_{19}N_5O_2\cdot0.25H_2O$	C,H,N	0.52 (A)
7d	156–158 (gh)	−13.76 (*c* 1.01, MeOH)	$C_{12}H_{17}N_5O_2$	C,H,N	
16	149–151 (gh)	−3.55 (*c* 1.04, MeOH)	$C_{10}H_{12}N_4O_2$	C,H,N	0.37 (A)
17	116–118 (gh)	+27.19 (*c* 1.07, MeOH)	$C_{10}H_{12}N_4O_2$	C,H,N	0.29 (A)
18	97–99 (gh)	+8.35 (*c* 1.174, MeOH)	$C_{10}H_{11}ClN_4O_2\cdot0.1MeOH$	C,H,N,Cl	0.47 (A)
19	71–73 (gi)	+20.73 (*c* 1.1438 $CHCl_3$)	$C_{10}H_{11}ClN_4O_2\cdot0.025H_2O$	C,H,N	0.41 (A)
20	>200 (je)		$C_{16}H_{26}N_4O_2SSi$	C,H,N,S	
21	188–190 (kl)	−27.02 (*c* 1.047, Me_2SO)	$C_{10}H_{12}N_4O_2S$	C,H,N	
22	105–107 (g)	−6.97 (*c* 1.05, MeOH)	$C_{11}H_{14}N_4O_2S$	C,H,N,S	0.25 (B)
26	foam	+39.7 (*c* 1.00, MeOH)	$C_{11}H_{14}N_5FO_3$	C,H,N,F	
27	234–235 (e)	+62.0 (*c* 0.10, MeOH)	$C_{11}H_{14}N_5FO_3$	C,H,N,F	
28	foam		$C_{17}H_{28}N_5FO_3Si\cdot0.25H_2O$	C,H,N,F	
30	83–85 (i)		$C_{19}H_{30}N_5FO_4SSi$	C,H,N,F,S	
31	105–110		$C_{17}H_{28}N_5FO_2Si$	C,H,N,F	
32	hygroscopic solid	+36.57 (*c* 0.7, MeOH)	$C_{11}H_{14}N_5FO_2\cdot0.5H_2O$	C,H,N,F	

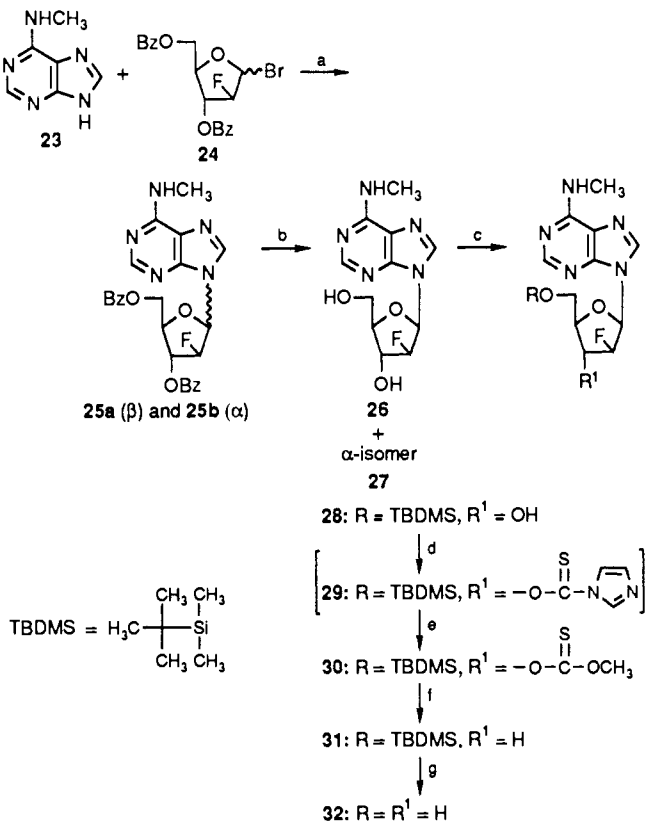
[a] Solvents: d, acetone; e, MeOH; f, petroleum ether (35–60 °C); g, hexanes; h, EtOAc, i, EtOEt; j, C_6H_6; k, EtOH; l, H_2O. [b] Solvents: A, $CHCl_3$–MeOH (10:1); B, $CHCl_3$–MeOH (100:3); C, hexanes–EtOAc (2:1); D, $CHCl_3$–MeOH (50:1); E, C_6H_6–EtOAc (2:1).

charcoal,[17] yielded a mixture of dechlorinated products **14** and **15**. This mixture was deprotected with 1 M TBAF and chromatographically separated to yield (2,3-dideoxyribofuranosyl)purines **16** (β) and **17** (α) as colorless, crystalline compounds.

Reaction of 6-chloropurine derivative **10** with thiourea in refluxing ethanol[18] or with sodium thiosulfate in an ethanol-water mixture at refluxing temperature[19] resulted in deglycosylation. However, the desired product **20** could be obtained in good yields by the reaction of **10** with anhydrous methanolic sodium hydrogen sulfide in the presence of hydrogen sulfide.[20,21] Desilylation of **20** yield 6-mercaptopurine derivative **21**, which could be S-methylated with methyl iodide in methanol containing sodium methoxide to give 6-methylthiopurine derivative **22**.

Preliminary studies have indicated that N^6-methyl-2′,3′-dideoxyadenosine **7a** is as unstable as D2A in acidic media.[22] Introduction of fluorine at the 2′-position (arabinofuranosyl configuration) of D2A is reported to increase the stability of the glycosyl bond under acidic condition while the anti-HIV activity is maintained.[23,24] In view of the potent anti-HIV activity exhibited by N^6-methyl-(2,3-dideoxyribofuranosyl)adenine **7a**[10] and the potent anti-herpes simplex virus activity[25–27] reported for pyrimidine nucleosides containing the 2′-fluoro-β-D-arabinofuranosyl moiety, the synthesis of a more acid-stable

Scheme III[a]



[a] Reagents: (a) NaH, DMF; (b) $MeOH/NH_3$; (c) TBDMSCl, imidazole, DMF; (d) thiocarbonyldiimidazole, DMF; (e) MeOH; (f) Et_3B, Bu_3SnH, benzene; (g) TBAF, THF.

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analogue of D2MeA such as **32** was highly desirable as a potential orally active anti-HIV agent.

The condensation of **24**[28] with the sodium salt of N^6-methyladenine, prepared in situ by the reaction of **23** with NaH in DMF yielded an inseparable anomeric mixture of **25a** and **25b** (β:α, 2:1) in 62% yield (Scheme III). Debenzoylation of the mixture using a saturated solution of methanolic ammonia and flash column chromatography of the crude product yielded α-anomer **27** as a white, crystalline solid (33%) and β-anomer **26** as a foam (65%). Protection of 5'-OH group of **26** with a *tert*-butyldimethylsilyl moiety and treatment of the protected nucleoside **28** with excess N,N'-thiocarbonyldiimidazole in DMF at 80 °C for 10 h yielded imidazole intermediate **29**, which on treatment with methanol at 60 °C gave the crystalline methyl thionocarbonate **30**. Deoxygenation of **30** was accomplished by the treatment with tributyltin hydride and triethylborane in anhydrous benzene at room temperature[29] to obtain **31** in excellent yield. Desilylation with TBAF gave the desired N^6-methyl-9-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)adenine **32**.

Antiviral Results

The antiviral activity and cytotoxicity of D2MeA and related compounds in human peripheral blood mononuclear (PBM) cells are shown in Table III. Among the purine analogues synthesized and evaluated for anti-HIV-1 activity, N^6-methyl-2',3'-dideoxyadenosine (D2MeA, **7a**) was one of the most potent purine nucleoside tested, with a median effective concentration (EC_{50} ± SD) of 0.26 ± 0.12 μM (mean determination using cells from five different donors). D2MeA was slightly more potent than D2A (EC_{50} = 0.64 ± 0.38 μM); the greater variability in the antiviral activity of D2A may be related to the different levels of adenosine deaminase and other enzymes involved in its antiviral activity in various donor cells. Among the other 6-modified 2',3'-dideoxy analogues evaluated, the N^6-ethyl, N^6,N^6-dimethyl, 6-chloro, and 6-mercaptomethyl analogues (**7b**, **7d**, **18**, and **22**) had significant antiviral activity. The order of antiviral activity for the N-substituted adenosine analogues was D2MeA > D2A > D2Me2A > D2EtA and the *N*-benzyl derivative D2BnA was inactive. This suggests that there is some bulk tolerance effect at the 6-position. The increased antiviral activity of D2MeA may be due to the enzymatic stability against adenosine deaminase, which metabolizes D2A to the less potent D2I.

The lack of activity of D2P derivative **16** may be attributed to the fact that it may not bind to the nucleoside kinases, which is required for any biological activity. It is interesting to note that the 2'-fluoro analogue of D2MeA (**32**) was about 20-fold less potent than D2MeA (EC_{50} = 0.26 vs 4.3 μM) although Marquez et al.[23] have observed that the introduction of 2'-fluoro (arabinofuranosyl) into D2A maintains the anti-HIV potency. With the exception of D2BnA **7c**, which was toxic at 10 μM, none of the compounds evaluted exhibited toxicity to PBM cells when evaluated up to 100 μM. The detailed kinetic analysis for the deamination studies will be reported elsewhere.

In contrast to the low stability of D2MeA and D2A, D2MeFA was found to be completely resistant to acid (pH 2.0) for more than 2 weeks.[22] This compound was nontoxic to Vero and PBM cells when tested up to 200 μM. Although D2MeFA is less potent than D2MeA, because of its greater chemical and biological stability, D2MeFA deserves further evaluation as a potential antiviral agent for the treatment of HIV infections.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX 90Q Fourier transform spectrometer or a Bruker AM 250 NMR spectrometer for the 90- and 250-MHz ^{1}H NMR spectra, respectively, with Me_4Si as internal standard: chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). UV spectra were obtained on a Bausch and Lomb Spectronic 2000 spectrometer or Beckman DU-7 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA or Galbraith Laboratories, Inc., Knoxville, TN.

N^6,N^6-Dibenzoyl-5'-*O*-(*tert*-butyldimethylsilyl)adenosine (2). To a suspension of 5'-(*O*-*tert*-butyldimethylsilyl)adenosine (1, 11.41 g, 30 mmol) (dried twice by the coevaporation with 100 mL of pyridine) in dry pyridine (150 mL) was added chlorotrimethylsilane (20 mL, 156 mmol). After stirring of the mixture for 30 min, benzoyl chloride (17.4 mL, 150 mmol) was added and stirring was continued for 2.5 h. The mixture was cooled in an ice bath and water (50 mL) was added. After 5 min, aqueous $NaHCO_3$ solution (100 mL) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was evaporated to near dryness, and the residue obtained was dissolved in $CHCl_3$, dried (Na_2SO_4), and concentrated to a syrup. Column chromatography over silica gel using $CHCl_3$–MeOH (50:1 → 50:1.5) yielded 14.72 g (83.5%) of **2**. Anal. ($C_{30}H_{35}N_5O_6Si$) C, H, N.

N^6-Benzoyl-N^6-methyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-bis-*O*-[(methylthio)thiocarbonyl]adenosine (3) and N^6-(Benzoylimino)-N_1-methyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-bis-*O*-[(methylthio)thiocarbonyl]adenosine (4). To a solution of **2** (5.53 g, 9.7 mmol) in DMSO (12 mL) containing CS_2 (9.8 mL, 163 mmol) was added 5 N NaOH solution (9.8 mL) in two portions. The mixture was stirred at room temperature for 20 min. Methyl iodide (19.7 mL, 316 mmol) was added dropwise and the stirring was continued for 35 min. TLC (benzene–EtOAc, 2:1) of the reaction mixture indicated the presence of two major products (R_f = 0.74 and 0.34). The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (100 mL × 4). The combined organic layer was washed with water, dried (Na_2SO_4), and evaporated to yield 6.5 g of a glassy material. Separation by column chromatography over silica gel using C_6H_6–EtOAc (4:1 → 2:1) as the eluent and evaporation of the appropriate fractions yielded **3** (2.16 g, 33%): UV (MeOH) λ_{max} 280; 280 (pH 11); 281 nm (pH 2). As compound **3** was found to be unstable, it was used in the next reaction without further characterization.

Further elution of the column using C_6H_6–EtOAc (2:1) yielded the more polar product (2.73 g, 42%), which was identified as N_1-methyl-N^6-(benzylamino) derivative **4**: UV (MeOH) λ_{max} 280; 279 (pH 11); 282 nm (pH 2). Anal. ($C_{28}H_{37}N_5O_5S_4Si$·0.25C_6H_6) C, H, N.

N^6-Benzoyl-N^6-methyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-didehydro-2',3'-dideoxyadenosine (5). To a boiling solution of **3** (2.1 g, 3.1 mmol) in dry benzene (30 mL) was added a solution of Bu_3SnH (5.4 mL, 20 mmol) and AIBN (0.5 g) in benzene (10 mL) during 10 min. The reaction mixture was refluxed for 20 min and benzene was evaporated under reduced pressure. The residue was purified by chromatography over silica gel using benzene–EtOAc as the eluent. Recrystallization from acetone yielded **5** (0.76 g, 53%) as colorless crystals. Anal. ($C_{24}H_{31}N_5O_3Si$) C, H, N.

N^6-Methyl-2',3'-didehydro-2',3'-dideoxyadenosine (6). To

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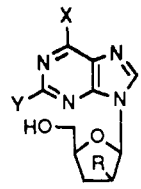
Table II. ^{1}H NMR Signals Observed for 6-Substituted-2,3-dideoxypurine Nucleosides

no.	δ[c] (multiplicity; J, Hz) H-1′	H-2′	H-3′	H-4′	H-5′	other signals
2	6.04 (d, $J_{1',2'}$ = 5.10)	4.23 (m)	4.68 (m)	3.60–4.13 (m)		8.69 (s, H-8), 8.71 (s, H-2), 0.01 (s, $SiMe_2$), 0.83 (s, *t*-Bu), 5.28 (d, 2′-(3′)-OH), 6.04 (d, 3′-(2′)-OH), 7.28–7.96 (m, C_6H_5)[a]
3	6.37–6.87 (m, H-1′, H-2′, and H-3′)			4.53 (m)	3.92 (b, d, $J_{5',4'}$ = 2.7)	8.56 (s, H-8), 8.59 (s, H-2), 0.03 (s, $SiMe_2$), 0.83 (s, *t*-Bu), 2.54 and 2.63 (2 s, S-CH_3), 3.67 (s, N-CH_3), 7.03–7.49 (m, C_6H_5)[a]
4	6.25–6.75 (m, H-1′, H-2′, and H-3′)			4.55 (m)	3.90 (m)	8.07 (s, H-8), 8.46 (s, H-2), 0.03 (s, $SiMe_2$), 0.84 (s, *t*-Bu), 2.50 and 2.55 (2 s, S-CH_3), 3.66 (s, N-CH_3), 7.20–7.55 (m, aromatic), 7.85–8.00 (m, aromatic)[a]
5	7.12 (b s)	6.28 (d, $J_{2',3'}$ = 4.8)	6.60 (d, $J_{3',2'}$ = 4.8)	5.00 (m)	3.84 (d, $J_{5',4'}$ = 3.8)	8.51 (s, H-8), 8.66 (s, H-2), 0.00 (s, $SiMe_2$), 0.87 (s, *t*-Bu), 3.75 (s, N-CH_3), 7.17-7.56 (m, C_6H_5)[a]
6	6.96 (m)	6.12 (ddd, J = 1.5, 2.1, 6.1)	6.47 (ddd, J = 1.6, 1.6, 6.2)	4.88 (m)	3.60 (b s)	8.15 (s, H-8), 8.22 (s, H-2), 3.00 (d, J = 3.80, N-CH_3), 5.02 (b s, OH), 7.68 (bd, NH)[a]
7a	6.23 (apparent t, $J_{1',2'}$ = 5.05, 5.27)	2.42 (m)	2.05 (m)	4.13 (m)	3.60 (m)	8.21 (s, H-8), 8.33 (s, H-2), 2.99 (d, J = 4.40, N-CH_3), 5.03 (t, OH), 7.67 (b m, NH)[a]
8a	6.29 (t, $J_{1',2'}$ = 5.28)	2.43 (m)	1.98 (m)	4.38 (m)	3.45 (m)	8.22 (s, H-8), 8.25 (s, H-2), 2.99 (d, J = 4.78, N-CH_3), 4.75 (t, OH), 7.65 (b m, NH)[a]
7b	6.22 (dd, $J_{1',2'}$ = 5.05, 5.27)	2.40 (m)	2.10 (m)	4.13 (m)	3.55 (m)	8.19 (s, H-8), 8.33 (s, H-2), 1.17 (t, J = 7.25, CH_3), 5.00 (b m, OH), 7.70 (b t, NH)[b]
8b	6.34 (apparent t, $J_{1',2'}$ = 5.05, 5.49)	2.68 (m)	2.05 (m)	4.55 (7)	3.55 (m)	7.86 (s, H-8), 8.34 (s, H-2), 1.31 (t, J = 7.25, CH_3), 5.85 (b m, NH)[b]
7c	6.23 (apparent t, $J_{1',2'}$ = 5.05, 5.49)	2.38 (m)	2.05 (m)	4.13 (m)	3.55 (m)	8.19 (s, H-8), 8.37 (s, H-2), 4.75 (b d, CH_2), 5.01 (t, OH), 7.29 (m, C_6H_5)[a]
8c	6.30 (t, $J_{1',2'}$ = 5.28)	1.60–2.65 (m)		4.38 (m)	3.45 (apparent t, J = 5.27, 4.83)	8.21 (s, H-8), 8.28 (s, H-2), 4.76 (t, CH_2 and OH), 7.30 (m, C_6H_5)[a]
7d	6.23 (apparent t, $J_{1',2'}$ = 4.61, 5.28)	2.40 (m)	2.05 (m)	4.10 (m)	3.60 (m)	8.20 (s, H-8), 8.36 (s, H-2), 3.30 (s, CH_3), 5.00 (t, OH)[a]
10	6.40 (dd, $J_{1',2'}$ = 3.52, 5.28)	2.50 (m)	2.13 (m)	4.30 (m)	3.77 (dd, J = 3.3, 11.43) 4.05 (dd, J = 2.85, 11.2)	8.64 (s, H-8), 8.72 (s, H-2), 0.11 (s, $SiMe_2$), 0.92 (s, *t*-Bu)[b]
11	6.39 (dd, $J_{1',2'}$ = 3.96, 5.50)	2.53 (m)	2.13 (m)	4.53 (m)	3.70 (m)	8.24 (s, H-8), 8.74 (s, H-2), 0.10 (s, $SiMe_2$), 0.92 (s, *t*-Bu)[b]
12d	6.33 (apparent t, $J_{1',2'}$ = 4.17, 5.49)	2.44 (m)	2.08 (m)	4.23 (m)	3.83 (m)	8.09 (s, H-8), 8.32 (s, H-2), 0.09 (s, $SiMe_2$), 0.91 s, *t*-Bu), 3.53 (s, CH_3)[b]
16	6.28 (apparent t, $J_{1',2'}$ = 5.56, 5.86)	2.00–3.00 (m)		4.35 (m)	3.83 (m)	8.30 (s, H-8), 8.97 (s, H-2), 9.15 (s, H-6)[b]
17	6.43 (apparent t, $J_{1',2'}$ = 4.98, 5.27)	2.68 (m)	2.15 (m)	4.60 (m)	3.75 (m)	8.23 (s, H-8), 8.98 (s, H-2), 9.15 (s, H-6)[b]
18	6.31 (apparent t, $J_{1',2'}$ = 5.06, 5.71)	1.95–2.80 (m)		4.38 (m)	3.88 (m)	8.50 (s, H-8), 8.74 (s, H-2)[b]
19	6.42 (apparent t, $J_{1',2'}$ = 4.83, 5.27)	1.80–2.85 (m)		4.63 (m)	3.75 (m)	8.24 (s, H-8), 8.75 (s, H-2)[b]
20	6.28 (dd, $J_{1',2'}$ = 3.05, 6.55)	2.44 (m)	2.12 (m)	4.26 (m)	3.77 (dd, J = 3.43, 11.31) 3.97 (dd, J = 3.31, 11.30)	8.03 (d, J = 2.21, H-8), 8.38 (s, H-2), 0.09 (s, $SiMe_2$), 0.91 (s, *t*-Bu), 9.44 (b s, NH)[a,b]
21	6.22 (dd, $J_{1',2'}$ = 3.81, 5.27)	2.40 (m)	2.03 (m)	4.10 (m)	3.53 (m)	8.18 (s, H-8), 8.51 (s, H-2), 4.93 (t, OH)[a]
22	6.18 (apparent t, $J_{1',2'}$ = 5.90, 6.15)	2.00–3.00 (m)		4.33 (m)	3.83 (m)	8.10 (s, H-8), 8.70 (s, H-2), 2.72 (s, S-CH_3), 4.81 (dd, OH)[b]
26	6.42 (dd, $J_{1',F}$ = 14.53, $J_{1',2'}$ = 4.62)	5.20 (dt, $J_{2',F}$ = 52.51, $J_{2',3'}$ = 3.84)	4.42 (dm, $J_{3',F}$ = 19.12, $J_{3',4'}$ = 4.61)	3.83 (m)	3.72 (m)	8.22 (d, J = 2.20, H-8), 8.23 (s, H-2), 3.17 (d, J = 5.82, N-CH_3), 5.10 (t, 5′-OH), 5.94 (d, 3′-OH), 7.73 (b d, NH)[a]
27	6.25 (dd, $J_{1',F}$ = 16.26, $J_{1',2'}$ = 3.07)	5.72 (dt, $J_{2',F}$ = 52.07, $J_{2',3'}$ = 3.63)	4.28–4.52 (m)		3.58 (m)	8.26 (s, H-8), 8.30 (s, H-2), 3.02 (d, J = 5.05, N-CH_3), 4.96 (t, 5′-OH), 6.08 (d, 3′-OH), 7.81 (b, d, NH)[a]
28	6.43 (dd, $J_{1',F}$ = 13.23, $J_{1',2'}$ = 4.84)	5.25 (dt, $J_{2',F}$ = 52.96, $J_{2',3'}$ = 4.68)	4.46 (dm, $J_{3',F}$ = 19.44)		3.87 (m)	8.15 (d, J = 1.76, H-8), 8.25 (s, H-2), 0.07 (s, $SiMe_2$), 0.89 (s, *t*-Bu), 3.00 (d, J = 3.96, N-CH_3), 5.99 (d, 3′-OH), 7.77 (b d, NH)[a]
30	6.48 (dd, $J_{1',F}$ = 21.98, $J_{1',2'}$ = 2.86)	5.23 (dd, $J_{2',F}$ = 49.88)	5.93 (dd, $J_{3',F}$ = 15.06, $J_{3',4'}$ = 2.41)	4.24 (m)	3.98 (d, $J_{5',4'}$ = 4.59)	8.04 (d, J = 3.08, H-8), 8.39 (s, H-2), 0.12 (s, $SiMe_2$), 0.93 (s, *t*-Bu), 3.22 (d, J = 5.05, N-CH_3), 4.11 (s, O-CH_3), 5.81 (b d, NH)[b]
31	6.30 (dd, $J_{1',F}$ = 18.46, $J_{1',2'}$ = 3.30)	5.27 (dq, $J_{2',F}$ = 54.27, $J_{2',3'}$ = 3.52)	2.48 (dq, $J_{3',F}$ = 31.86, $J_{3',4'}$ = 2.86)	4.27 (m)	3.84 (d, $J_{5',4'}$ = 4.83)	8.07 (d, J = 2.63, H-8), 8.39 (s, H-2), 0.12 (s, $SiMe_2$), 0.93 (s, *t*-Bu), 3.22 (d, J = 5.05, N-CH_3), 5.80 (b d, NH)[b]
32	6.26 (dd, $J_{1',F}$ = 17.57, $J_{1',2'}$ = 3.73)	5.34 (dq, $J_{2',F}$ = 53.80, $J_{2',3'}$ = 4.17)	2.54 (dm, $J_{3',F}$ = 29.00, $J_{3',4'}$ = 2.86)	4.37 (m)	3.88 (t, $J_{5',4'}$ = 3.52)	7.97 (d, J = 2.19, H-8), 8.38 (s, H-2), 3.19 (d J = 5.06, N-CH_3), 4.37 (bm, OH), 6.10 (b d, NH)[b]

[a] Me_2SO. [b] $CDCl_3$. [c] Part per million downfield from TMS.

a solution of **5** (0.71 g, 1.5 mmol) in dry THF (10 mL) was added 1 M Bu_4NF/THF (2.5 mL) and the mixture was stirred for 25 min. The solvent was evaporated and the residue was purified by filtration through a silica gel column using $CHCl_3$–MeOH (20:1) as the eluent. The resulting compound (0.49 g) was then treated with a saturated methanolic ammonia solution (15 mL) for 25

Table III. Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentrations of Various Purine Nucleosides in PBM Cells



no.	compd	X	Y	R	ED_{50}, μM	IC_{50}, μM
	D2A	NH_2	H	H	0.62	>100
	D2I	OH	H	H	5.5	>100
	D2G	OH	NH_2	H	0.88	>100
7a	D2MeA	$NHCH_3$	H	H	0.26	>100
7b	D2EtA	NHC_2H_5	H	H	5.2	>100
7c	D2BnA	$NHCH_2C_6H_5$	H	H	>100	~10.0
7d	D2Me2A	$N(CH_3)_2$	H	H	2.3	>100
16	D2P	H	H	H	>100	>100
18	D2ClP	Cl	H	H	2.1	>100
21	D2SHP	SH	H	H	53.9	>100
22	D2SMeP	SCH_3	H	H	3.6	>100
32	D2MeFA	$NHCH_3$	H	F	4.3	>100

h at room temperature. Evaporation of the solvent followed by chromatographic purification (silica gel, $CHCl_3$–MeOH, 10:1) yielded a solid. Recrystallization from MeOH–ether yielded **6** (0.28 g, 73% from **5**): UV (MeOH) λ_{max} 264 (ε 16 590); 265 (ε 15 860) (pH 11); 267 nm (ε 14 000) (pH 2). Anal. ($C_{11}H_{13}N_5O_2$) C, H, N.

N^6-Methyl-2',3'-dideoxyadenosine (7a)[12] from 6. A suspension of **6** (92 mg, 0.37 mmol) and 10% Pd/C (37 mg) in EtOH–H_2O (4:1, 10 mL) was hydrogenated at 55 psi at room temperature for 5 h. The catalyst was filtered off, and the filtrate was evaporated to a small volume, ether was added, and the mixture was cooled in a refrigerator to yield **7a** as colorless powder (55 mg, 59%). Anal. ($C_{11}H_{15}N_5O_2 \cdot 0.75H_2O$) C, H, N.

9-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-β-D-ribofuranosyl]-6-chloropurine (10) and α-Anomer 11. A mixture of 6-chloropurine (5.10 g, 33 mmol), hexamethyldisilazane (100 mL), and chlorotrimethylsilane (9 mL, 71 mmol) was refluxed for 2 h. The clear solution obtained was concentrated in vacuo and the residue was coevaporated with toluene (25 mL × 2) to obtain a pale yellow solid, which was used for the next reaction without purification. To the solid prepared above was added a solution of sugar acetate **9** (8.22 g, 30 mmol) in anhydrous $ClCH_2CH_2Cl$ (150 mL). Trimethylsilyl triflate (2.5 mL, 13 mmol) was added dropwise and the reaction mixture was stirred for 30 min. The resulting solution was poured into an ice-cold mixture of CH_2Cl_2 and saturated $NaHCO_3$ solution (2:1, 450 mL), stirred for 15 min, and filtered through a Celite pad. The organic layer was washed with saturated $NaHCO_3$ solution (225 mL) and brine (225 mL) and dried (Na_2SO_4). The solvents were removed by distillation under reduced pressure to yield a mixture of α- and β-anomers **11** and **10** (1:1) as a colorless solid (10 g, 82%). Analytical samples of **10** and **11** were obtained by column chromatography over silica gel using EtOAc–hexanes (1:6) as the eluent. **11** (α-anomer): UV (MeOH) λ_{max} 265 nm. Anal. ($C_{16}H_{25}ClN_4O_2Si$) C, H, N. **10** (β-anomer): UV (MeOH) λ_{max} 265 nm. Anal. ($C_{16}H_{25}ClN_4O_2Si$) C, H, N.

β- and α-N^6-Methyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-dideoxyadenosines (12a and 13a). A solution of **10** and **11** (0.74 g, 2 mmol) and methylamine (40 wt % solution in H_2O, 10 mL) in MeOH (50 mL) was heated at 110 °C in a steel bomb for 16 h. After cooling, the solvents were removed by distillation under vacuum. The residue syrup was dissolved in CH_2Cl_2 (100 mL), washed with water and brine, and dried. Evaporation of the solvent yielded a syrup, which was purified by column chromatography (silica gel 230–400 mesh) using CH_2Cl_2–MeOH (10:0.2) as the eluent. A mixture of **12a** and **13a** was obtained as a syrup (0.45 g, 61%) after the evaporation of the appropriate fractions: UV (MeOH) λ_{max} 266 nm. Anal. ($C_{17}H_{29}N_5O_2Si \cdot 0.5H_2O$) C, H, N.

β- and α-N^6-Ethyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-dideoxyadenosines (12b and 13b). A solution of **10** and **11** (0.37 g, 1 mmol) and ethylamine (70 wt % solution in H_2O, 2 mL) in MeOH (20 mL) was treated according to the procedure described for **12a** and **13a**. Chromatographic purification of the crude product (silica gel) using $CHCl_3$–MeOH (40:1) as the eluent yielded a mixture of **12b** and **13b** (0.25 g, 67%) as a colorless syrup: UV (MeOH) λ_{max} 267 nm. Anal. ($C_{18}H_{31}N_5O_2Si \cdot 0.25\ H_2O$) C, H, N.

β- and α-N^6-Benzyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-dideoxyadenosine (12c and 13c). A solution of **10** and **11** (0.74 g, 2 mmol) and benzylamine (0.214 g, 2 mmol) in MeOH (30 mL) was treated according to the procedure described for **12a** and **13a** to obtain a mixture of α- and β-anomers **13c** and **12c** (0.49 g, 55%) as a colorless syrup. An analytical sample was obtained by preparative TLC on a silica gel plate using CH_2Cl_2–MeOH (50:1) as the solvent system: UV (MeOH) λ_{max} 271 nm. Anal. ($C_{23}H_{33}N_5O_2Si$) C, H, N.

β- and α-N^6,N^6-Dimethyl-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-dideoxyadenosine (12d and 13d). A solution of **10** and **11** (0.37 g, 1 mmol) and dimethylamine (40 wt % solution in H_2O, 5 mL) in MeOH (30 mL) was treated according to the procedure described for **12a** and **13a**. Purification of crude product by preparative TLC on a silica gel plate using CH_2Cl_2–MeOH (50:1) as the solvent system yielded an analytical sample of a mixture of α- and β-anomers **13d** and **12d** as a colorless syrup (0.26 g, 68%): UV (MeOH) λ_{max} 275 nm. Anal. ($C_{18}H_{31}N_5O_2Si$) C, H, N.

β-Anomer **12d** (R_f = 0.21, $CHCl_3$–MeOH, 50:1) could be separated by a column chromatography over silica gel (230–600 mesh) using CH_2Cl_2–MeOH (10:1) as the eluent. Compound **12d** was used in the next reaction without further purification.

β- and α-9-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxyribofuranosyl]purine (14 and 15). A solution of **10** and **11** (0.37 g, 1 mmol) in MeOH (40 mL) containing concentrated NH_4OH (0.63 mL, 8.25 mmol) and 10% Pd/C (50 mg) was hydrogenated at 15 psi at room temperature for 1.5 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The residue was then stirred with boiling hexanes (50 mL) for 10 min and filtered. Evaporation of the solvent yielded a mixture of α- and β-anomers **15** and **14** as a colorless syrup (0.30 g, 90%): UV (MeOH) λ_{max} 263 nm. Anal. ($C_{16}H_{26}N_4O_2Si \cdot 0.2C_6H_{14}$) C, H, N.

9-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-β-D-ribofuranosyl]-6-mercaptopurine (20). A stream of H_2S was bubbled through a refluxing solution of **10** (0.34 g, 0.9 mmol) in anhydrous MeOH (40 mL) for 30 min. NaSH in anhydrous MeOH (1 N, 2.8 mL) was added dropwise while the solution was heated and the introduction of H_2S was continued for 1 h. The yellowish solution was cooled to room temperature and the pH was adjusted to 6–7 with 1 N methanolic acetic acid. Solvents were removed by distillation under vacuum. On trituration with water the residue yielded a white precipitate, which was filtered, washed with water, and dried. Crystallization from C_6H_6–MeOH yielded **20** as a colorless solid (0.30 g, 89%): UV (MeOH) λ_{max} 325; 317 nm (pH 11). Anal. ($C_{16}H_{26}N_4O_2SSi$) C, H, N, S.

β- and α-9-(2,3-Dideoxyribofuranosyl)-6-chloropurine (18 and 19). A mixture of α- and β-anomers **11** and **10** (0.74 g, 2 mmol) in dry THF (30 mL) was deprotected with 1 M Bu_4NF/THF (2.0 mL, 2 mmol). After evaporation of the solvent, the residue was chromatographed over silica gel (230–400 mesh) using $CHCl_3$–MeOH (58:1) as the eluent to give pure α-anomer **19** (0.22 g, 43%) and β-anomer **18** (0.19 g, 37%) as well as a mixture of α- and β-anomers (0.06 g, 12%). The α- and β-anomers obtained as syrups were individually triturated with ether and cooled overnight in a refrigerator. The crystals obtained were filtered and dried. **18**: UV (MeOH) λ_{max} 265 (ε 8910); 264 (ε 8720) (pH 11); 264 nm (ε 8540) (pH 2). Anal. ($C_{10}H_{11}ClN_4O_2 \cdot 0.1MeOH$) C, H, N, Cl. **19**: UV (MeOH) λ_{max} 265 (ε 9250); 265 (ε 9145) (pH 11); 264 nm (ε 9190) (pH 2). Anal. ($C_{10}H_{11}ClN_4O_2 \cdot 0.025H_2O$) C, H, N.

N^6-Methyl-2',3'-dideoxyadenosine (7a)[12] and α-Anomer 8a. To an ice-cold solution of **12a** and **13a** (0.73 g, 2 mmol) in anhydrous THF (30 mL) was added 1 M Bu_4NF/THF (2.0 mL, 2 mmol) dropwise. The reaction mixture was stirred at about 5–10 °C for 1 h. The solvent was removed by distillation under reduced pressure, and the anomers were separated by column chromatography (silica gel 230–400 mesh) using $CHCl_3$–MeOH (10:0.3) as the eluent. α-Anomer **8a** (0.165 g, 33%) was obtained as a foam

and β-anomer **7a** (0.16 g, 32%) was obtained as a colorless powder after the evaporation of the appropriate fractions. **8a**: UV (MeOH) λ_{max} 266 (ε 13600); 267 (ε 13900) (pH 11); 264 nm (ε 13600) (pH 2). Anal. ($C_{11}H_{15}N_5O_2 \cdot 0.75H_2O$) C, H, N. **7a**: UV nMeOH) λ_{max} 267 (ε 15300); 267 (ε 14800) (pH 11); 265 nm (ε 14180) (pH 2).

***N*-Ethyl-2',3'-dideoxyadenosine (7b) and α-Anomer 8b.** A mixture of α- and β-anomers **13b** and **12b** (0.77 g, 2 mmol) was deprotected with 1 M Bu_4NF/THF (2 mL, 2 mmol) as described for **7a** and **8a**. Chromatography of the crude product using $CHCl_3$–MeOH (100:2.5) as the eluent yielded α-anomer **8b** (0.195 g, 36%) as colorless needles and β-anomer **7b** (0.162 g, 30%) as a foam (hygroscopic). **8b**: UV (MeOH) λ_{max} 267 (ε 18970); 268 (ε 19750) (pH 11); 266 nm (ε 18280) (pH 2). Anal. ($C_{12}H_{17}N_5O_2$) C, H, N. **7b**: UV (MeOH) λ_{max} 267; 268 (pH 11); 264 nm (pH 2). Anal. ($C_{12}H_{17}N_5O_2 \cdot 0.50H_2O$) C, H, N.

***N*^{6}-Benzyl-2',3'-dideoxyadenosine (7c) and α-Anomer 8c.** A mixture of α- and β-anomers **13c** and **12c** (0.44 g, 1 mmol) was deprotected with 1 M Bu_4NF/THF (1 mL, 1 mmol) according to the procedure described for **7a** and **8a**. Chromatography (silica gel 230–400 mesh) of the crude product using $CHCl_3$–MeOH (50:1) as the eluent yielded β-anomer **7c** (0.16 g, 49%) and α-anomer **8c** (0.14 g, 43%) as a foam. **7c**: UV (MeOH) λ_{max} 270 (ε 21040); 270 nm (ε 21360) (pH 11); 270 nm (ε 19650) (pH 2). Anal. ($C_{17}H_{19}N_5O_2 \cdot 0.25H_2O$) C, H, N. **8c**: UV (MeOH) λ_{max} 270 (ε 16000); 270 (ε 17050) (pH 11); 271 nm (ε 15000) (pH 2). Anal. ($C_{17}H_{19}N_5O_2 \cdot 0.25H_2O$) C, H, N.

***N*^{6},*N*^{6}-Dimethyl-2',3'-dideoxyadenosine (7d).** Compound **12d** (0.76 g, 2 mmol) was desilylated with 1 M Bu_4NF/THF (2 mL, 2 mmol). Chromatography (silica gel 230–400 mesh) of the crude product using CH_2Cl_2–MeOH (5:1) yielded **7d** as a colorless solid (0.25 g, 48%): UV (MeOH) λ_{max} 275 (ε 20140); 276 (ε 20300) (pH 11); 276 nm (ε 17030) (pH 2). Anal. ($C_{12}H_{17}N_5O_2$) C, H, N.

9-(2,3-Dideoxy-β-D-ribofuranosyl)purine (16) and α-Anomer 17. A mixture of α- and β-anomers **15** and **14** (0.67 g, 2 mmol) in dry THF (25 mL) was desilylated with 1M Bu_4NF/THF (2 mL, 2 mmol). Chromatographic separation (silica gel 230–400 mesh, $CHCl_3$–MeOH, 50:1) yielded α-anomer **17** (0.164 g, 37%) as colorless crystals and β-anomer **16** (0.120 g, 27%) as needles. **17**: UV (MeOH) λ_{max} 263 (ε 8285); 264 (ε 8050) (pH 11); 261 nm (ε 6800) (pH 2). Anal. ($C_{10}H_{12}N_4O_2$) C, H, N. **16**: UV (MeOH) λ_{max} 264 (ε 6860); 264 (ε 7090) (pH 11); 261 (nm (ε 5400) (pH 2). Anal. ($C_{10}H_{12}N_4O_2$) C, H, N.

9-(2,3-Dideoxy-β-D-ribofuranosyl)-6-mercaptopurine (21). A suspension of **20** (0.18 g, 0.5 mmol) in THF (30 mL) was deprotected with an excess of 1 M Bu_4NF/THF (2 mL, 2 mmol). The reaction mixture was stirred at room temperature for 24 h. After the removal of solvent, the purification of the residue by column chromatography (silica gel 230–400 mesh) using $CHCl_3$–MeOH (10:1) as the eluent yielded **21** as a colorless solid (0.08 g, 65%): UV λ_{max} (MeOH) 324 (ε 24720); 311 (ε 23780), 235 (sh) (pH 12); 325 (ε 20350), 226 nm (ε 6580) (pH 1). Anal. ($C_{10}H_{12}N_4O_2S$) C, H, N.

9-(2,3-Dideoxy-β-D-ribofuranosyl)-6-(methylthio)purine (22). A solution of **21** (0.20 g, 0.8 mmol) in MeOH (20 mL) containing NaOMe (0.8 mmol, prepared by dissolving 0.0184 g of Na in MeOH) was stirred with MeI (0.2 mL) for 30 min at room temperature. After the removal of the solvent, the residue was purified by column chromatography (silica gel 230–400 mesh) using $CHCl_3$–MeOH (10:0.3) as the eluent. Evaporation of the solvents yielded a colorless syrup, which on trituration with hexanes gave a solid. Recrystallization from hexanes–EtOAc afforded **22** as colorless needles (0.124 g, 59%): UV (MeOH) λ_{max} 283 (ε 19655), 290 (sh); 292 (ε 19440) (pH 11); 295 nm (ε 15620) (pH 1). Anal. ($C_{11}H_{14}N_4O_2S$) C, H, N, S.

***N*^{6}-Methyl-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (25b) and α-Anomer 25a.** To a suspension of *N*^{6}-methyladenine **23** (1.16 g, 7.8 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.31 g, 7.8 mmol). After the evolution of hydrogen had ceased, a solution of 3,5-di-*O*-benzoyl-1-bromo-2-deoxy-2-fluoro-α-D-arabinofuranose (**24**,[28] 3 g, 7.1 mmol) in DMF (5 mL) was added dropwise; the reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was diluted with H_2O (100 mL), neutralized with acetic acid, and extracted with EtOAc (100 mL × 3). The combined organic layer was dried, the solvent was evaporated, and the mixture was purified by column chromatography ($CHCl_3$–MeOH, 50:1), giving an inseparable anomeric mixture of **25a** and **25b** (2.16 g, 62%).

***N*^{6}-Methyl-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (26) and α-Anomer 27.** A mixture of **25a** and **25b** (1.47 g, 3 mmol) was debenzoylated by stirring with saturated MeOH/NH_3 (30 mL) at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography ($CHCl_3$–MeOH, 20:1). Evaporation of the appropriate fractions and trituration of the residue with ether yielded **27** (0.28 g, 33%): UV (MeOH) λ_{max} 266 (ε 17195); 266 (ε 17072) (pH 11); 262 nm (ε 17490) (pH 2). Anal. ($C_{11}H_{14}N_5FO_3$) C, H, N, F. **26** (0.55 g, 65%): UV (MeOH) λ_{max} 266 (ε 12808); 266 (ε 13080) (pH 11); 262 nm (ε 13870) (pH 2). Anal. ($C_{11}H_{14}N_5FO_3$) C, H, N, F.

***N*^{6}-Methyl-9-[5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]adenine (28).** To a solution of **26** (1.13 g, 4 mmol) in DMF (20 mL) was added imidazole (0.82 g, 12 mmol) and *tert*-butyldimethylsilyl chloride (1.2 g, 8 mmol). The reaction mixture was heated with stirring at 40 °C for 48 h. The solvent was evaporated, and the residue was purified by column chromatography ($CHCl_3$–MeOH, 15:1), giving **28** as a foam (1.19 g, 75%): UV (MeOH) λ_{max} 265 nm. Anal. ($C_{17}H_{28}N_5FO_3Si \cdot 0.25H_2O$) C, H, N, F.

***N*^{6}-Methyl-9-[5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-fluoro-3-*O*-(methoxythiocarbonyl)-β-D-arabinofuranosyl]adenine (30).** To a solution of **28** (0.8 g, 2 mmol) in DMF (20 mL) was added *N*,*N*'-thiocarbonyldiimidazole (0.72 g, 4 mmol), and the reaction mixture was heated with stirring at 80 °C for 14 h. The solvent was removed in vacuo and the residue was dissolved in anhydrous MeOH and heated with stirring at 60 °C for 2 h. The solvent was evaporated, and the residue was purified by column chromatography ($CHCl_3$–MeOH, 25:1). Evaporation of the solvents and trituration of the residue with ether gave **30** (0.62 g, 65%): UV (MeOH) λ_{max} 264 nm. Anal. $C_{19}H_{30}N_5FO_4SSi$) C, H, N, F, S.

***N*^{6}-Methyl-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl]adenine (31).** To a solution of **30** (0.42 g, 0.9 mmol) and Et_3B (1 M solution in hexane, 1.06 mL, 1.06 mmol) in anhydrous benzene (5 mL) was added dropwise *n*-Bu_3SnH (0.36 mL, 1.34 mmol at 20 °C under an argon atmosphere, and the reaction mixture was stirred at 20 °C for 1 h. The solvent was evaporated, and the residue was partitioned between hexane (150 mL) and acetonitrile (200 mL). The acetonitrile layer was washed three times with hexane (150 mL × 3) to remove Et_3B and *n*-Bu_3SnH. Acetonitrile was evaporated and the residue was purified by column chromatography ($CHCl_3$–MeOH, 10:1) to give **31** (0.29 g, 85%), which crystallized on drying under vacuum: UV (MeOH) λ_{max} 265 nm. Anal. ($C_{17}H_{28}N_5FO_2Si$) C, H, N, F.

***N*^{6}-Methyl-9-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)adenine (32).** To a solution of **31** (0.23 g, 0.6 mmol) in THF (10 mL) was added 1 M Bu_4NF/THF (0.87 mL, 0.87 mmol), and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated, and the residue was purified by column chromatography ($CHCl_3$–MeOH, 10:1) to give **32** (0.15 g, 94%) as a hygroscopic foam: UV (MeOH) λ_{max} 265 (*e* 11750); 265 (ε 13510) (pH 11); 261 nm (ε 15530) (pH 2). Anal. ($C_{11}H_{14}N_5FO_2 \cdot 0.5H_2O$) C, H, N, F.

Antiviral Evaluation Procedures. The procedures for the antiviral assays in human peripheral blood mononuclear (PBM) cells have been described previously.[30] Briefly, uninfected phytohemagglutinin-stimulated human PBM cells were infected with HIV-1 (strain LAV-1) (about 63000 disintegrations/min of reverse transcriptase (RT) activity per 107 cells/10 mL of medium). The drugs were then added to duplicate or triplicate cultures. Uninfected and untreated PBM cells were grown in parallel at equivalent cell concentraions as controls. The cultures were maintained in a humidified 5% CO_2–95% air incubator at 37 °C for 6 days after infection, at which point all cultures were sampled for supernatant RT activity. Previous studies had indicated that maximum RT levels were obtained at that time.[10,30-33]

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The supernatant was clarified, and the virus particles were then pelleted at 40000 rpm for 30 min by using a rotor (70.1 Ti; Beckman Instruments, Inc., Fullerton, Calif.) and suspended in virus-disrupting buffer. The RT assay was performed by a modification of the method of Spira et al.[34] in 96-well microdilution plates using (rA)n·(dT)$_{12-18}$ as template-primer. The RT results were expressed in dpm/milliliter of originally clarified supernatant. The drugs were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells and also in African green Monkey kidney (Vero) cells that were obtained from the American Type Culture Collection, Rockville, MD. The Vero cells were maintained in minimum essential medium supplemented with 2% heat-inactivated fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL), and the toxicity assay was performed as described previously.[34] The PBM and Vero cells were cultured with and without drug for 6 and 4 days, respectively, at which time they were counted for cell proliferation and viability by using the trypan blue exclusion method.[30,34] Only the effects on cell growth are reported since these correlated well with cell viability. The median effective concentration (EC_{50}) and inhibitory concentration (IC_{50}) values were derived from the computer-generated median-effect plot of the dose-effect data as described previously.[35]

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Synthesis and Bioactivity of a New Class of Rigid Glutamate Analogues. Modulators of the *N*-Methyl-D-aspartate Receptor

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A variety of derivatives of azetidine-2,4-dicarboxylic acid were synthesized and examined for their ability to stimulate $^{45}Ca^{2+}$ uptake in cultures of cerebellar granule cells. Of the compounds tested, the *cis*-azetidine-2,4-dicarboxylic acid (**10f**) was found to be the most potent agent in potentiating glutamate, aspartate, or *N*-methyl-D-aspartate (NMDA) stimulated $^{45}Ca^{2+}$ uptake at the NMDA receptor. The mechanism of action of **10f** was further investigated in [^{3}H]MK-801 binding assays and [^{3}H]GABA release from cultured embryonic rat forebrain neurons. All of the results from the functional studies of azetidine **10f** are consistent with a selectivity of action at the NMDA receptor. Moreover, azetidine **10f** appears to exhibit a dual type of action, behaving as a glutamate-like agonist at higher concentrations and as a positive modulator at concentrations below 50 μM.

Currently four L-glutamate receptor subtypes have been identified on the basis of the ligand structural features essential for receptor binding, as well as, in part, the coupling of these recognition sites to specific signal transduction systems.[1] The *N*-methyl-D-aspartate (NMDA) receptors are activated by glutamate, aspartate, and NMDA, and are competitively antagonized by D-2-amino-5-phosphonovaleric acid (D-APV) or noncompetitively by phencyclidine (PCP) and MK-801. NMDA receptors are coupled to a Na^+/Ca^{2+} permeable ion channel which exhibits a voltage dependent Mg^{2+} blockade. The NMDA receptor operated ion channel is noncompetitively blocked by Zn^{2+}, which appears to act at a site distinct from that for Mg^{2+}.[2] Quisqualate receptors are activated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and are antagonized by agents such as γ-glutamyl aminomethanesulfonate (GAMS) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).[3] One subtype of quisqualate receptor is linked to a fast response mediated by Na^+ channels and a second type to a slower response involving the activation of a phospholipase C with the production of inositol 1,4,5-trisphosphate. Kainate receptors are also antagonized by CNQX and their response mediated by Na^+ ion channels, much like that of one of the quisqualate receptor subtypes.[4]

Of these four glutamate receptor subtypes, the NMDA receptor in particular has captured the attention of many neurobiologists, for it has been shown to play a key role

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