Synthesis and Anti-Hepatitis B Virus Activity of 9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)purine Nucleosides

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Received April 7, 1997[⊗]

Since the discovery of 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-FMAU) as a potent anti-HBV and anti-EBV agent, we have studied the structure−activity relationships of 2′-deoxy-2′-fluoro-β-L-arabinofuranosylpyrimidine nucleosides as anti-HBV agents. Therefore it is rational to extend this study to the purine nucleosides. Thus, 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-L-arabinofuranosyl bromide (**1**), which was prepared from L-xylose *via* a multistep procedure, was coupled with several purines by the sodium salt method. From this general synthesis, 10 purine nucleosides containing the 2-deoxy-2-fluoro-β-L-arabinofuranosyl moiety have been obtained. The anti-HBV activity and toxicity of the synthesized nucleosides were evaluated in HepG2 2.2.15 cells. Among them, the adenine (**10**) and hypoxanthine (**15**) derivatives exhibit good *in vitro* anti-HBV activity (EC_{50} = 1.5 and 8 μM, respectively) without significant toxicity up to 200 μM.

Introduction

As part of our continuing efforts to synthesize nucleosides as antiviral agents, we have recently reported 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-FMAU) as a potent antiviral agent against hepatitis B virus (HBV) as well as Epstein-Barr virus (EBV).[1−3] Compared to the corresponding D-enantiomer, L-FMAU exhibits more potent anti-HBV activity *in vitro* without any significant cytotoxicity in a variety of cell lines such as CEM, 2.2.15, H1, and bone marrow progenitor cells. Additionally, L-FMAU does not interfere with the mitochondrial function, which has been the major concern for some of the antiviral nucleosides such as FIAU and DDC.[4−6] *In vitro* studies with primary duck hepatocytes indicated that L-FMAU also exhibits potent antiviral activity against duck hepatitis B virus (DHBV; EC_{50} = 0.1 μM). Oral administration of L-FMAU (40 mg/kg/day) for 5 days to Peking ducks congenitally infected with chronic DHBV markedly reduced the viremia level without any abnormalities.[7] Furthermore, *in vivo* efficacy studies in woodchucks chronically infected with woodchuck hepatitis virus indicated that L-FMAU effectively suppresses the virus during the drug treatment at 10 mg/kg/day, and no significant viral rebound was noted after discontinuation of the drug up to 24 weeks.[8] L-FMAU is currently undergoing preclinical toxicology studies.

Recently, we have also reported the structure−activity relationships of 2′-fluoro-β-L-arabinofuranosyl pyrimidines as anti-HBV agents,[9] from which we found that L-FMAU exhibits the most potent anti-HBV activity among these pyrimidine nucleosides, whereas two other derivatives, namely, 2′-deoxy-2′-fluoro-β-L-arabinofuranosylcytosine (L-FAC) and its 5-iodocytosine derivative (L-FIAC), show good *in vitro* anti-HBV activity. These encouraging results prompted us to extend our studies to the purine nucleosides.

Previously, several purine nucleosides containing the 2-deoxy-2-fluoro-β-D-arabinofuranosyl moiety have been synthesized as potential antiviral and antileukemic agents.[10−12] One of the analogues, 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (2′-F-ara-G), was found to be selectively toxic to human T-cell leukemia.[11,12] More recently, a purine analogue, 2,4-diamino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine, has been reported to exhibit good *in vitro* anti-HBV activity.[13] It is well known that incorporation of a fluorine atom at the 2′-position of purine nucleosides can increase the stability of these compounds toward chemical as well as metabolic degradation.[14,15] Recent studies with antiviral L-nucleosides suggest that different affinities toward anabolic and catabolic enzymes exist between the biologically active L-nucleosides and their D-counterparts, which could account for the enhanced antiviral potency of the L-isomers.[16,17] On the basis of these observations, it is interesting to see whether the incorporation of the 2-deoxy-2-fluoro-β-L-arabinofuranosyl moiety into purine nucleosides may influence the biological activity. We wish to report herein the synthesis and anti-HBV activity of 9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)purine nucleosides.

Results and Discussion

Chemistry. The synthesis of the purine nucleosides reported in this paper was accomplished by the coupling of glycosyl bromide **1** with the sodium salts[18] of the corresponding purines **2**−**4** followed by derivatization. The sugar intermediate **1** was prepared from L-xylose, *via* a multistep procedure as we reported earlier,[9] with the adaption of the new fluorination method reported by Chou *et al.*[19] Thus, the fully blocked 6-chloropurine derivative **5** was obtained in 65% isolated yield by the condensation of **1** with the sodium salt of **2**. Ammonolysis by an appropriate amine with concomittant or subsequent deacylation in saturated NH_3/CH_3OH gave the 6-substituted amino analogues **10** and **12**−**14** in good yields (66−82%). The 6-unsubstituted purine

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⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1997.

S0022-2623(97)00233-1 CCC: $14.00

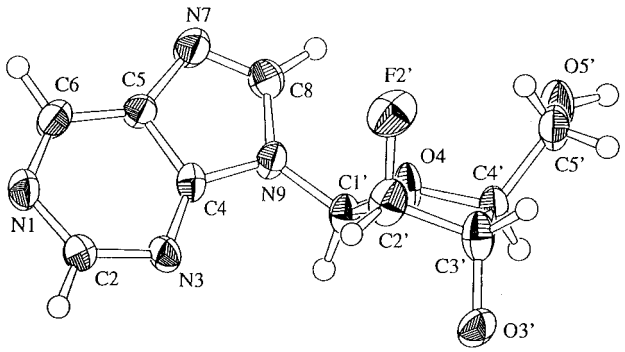


Figure 1. ORTEP drawing of 9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-9*H*-purine (**11**).

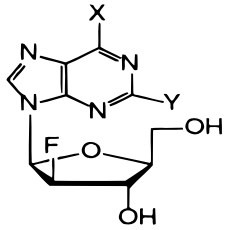
nucleoside **11** was obtained in 86% yield by dehalogenation of **5** with 10% Pd−C in EtOAc in the presence of Et_3N followed by deacylation with saturated NH_3/CH_3OH. Treatment of **5** with mercaptoethanol in the presence of $NaOCH_3$ in refluxing methanol gave the hypoxanthine derivative **15** in 64% yield. Our attempt to synthesize the hypoxanthine derivative **15** by the deamination of **10** with adenosine deaminase was unsuccessful. This suggests that **10** is not as good a substrate of this enzyme as the corresponding D-enantiomer, which can be degraded at a comparable rate as adenosine.[15] The 6-mercaptopurine derivative **16** was obtained in 65% yield by the treatment of **5** with thiourea in refluxing ethanol followed by deacylation with saturated NH_3/CH_3OH.

Coupling of the sodium salt of 2,6-dichloropurine in acetonitrile with **1** gave the fully blocked nucleoside **17** in 29% yield, from which the 2-chloroadenine derivative **21** was obtained in 81% yield by direct ammonolysis in saturated NH_3/CH_3OH at 90 °C. Treatment of **17** with LiN_3 in refluxing EtOH followed by hydrogenation on 5% Pd−C and subsequent deacylation gave the 2,6-diaminopurine analogue **22** in 40% yield. Although the coupling reaction of the sodium salt of 2-amino-6-chloropurine with **1** in DMF gave a complex mixture, the desired product **20** was isolated in 9% yield and was converted to the guanine derivative **23** in 70% yield by reaction with mercaptoethanol in refluxing methanol in the presence of $NaOCH_3$.

All the nucleosides obtained were characterized by microanalysis and spectroscopic methods, and the physical data are consistent with those of the known D-isomers. In addition to the presence of a doublet for H-8 in the ^1H NMR spectra, which results from the coupling with the 2′-arabino fluorine atom ($J_{F-H8} = 1.6-3.0$ Hz), the β-configuration was also confirmed by the C1′−F coupling constant ($J_{C1'-F} = 15-18$ Hz) in the ^{13}C NMR spectra of the intermediates **5**, **17**, and **21**.[20] Furthermore, the configuration of compound **11** has been unambiguously determined by single-crystal X-ray crystallography,[21] and the ORTEP diagram is shown in Figure 1.

Anti-HBV Activities. Anti-HBV activity and cytotoxicity of the synthesized nucleosides were evaluated in 2.2.15 cells as described before,[1] and the results are summarized in Table 1. Initially, we synthesized three compounds, namely, the adenine **10**, hypoxanthine **15**, and guanine **23** derivatives. We found **10** and **15** exhibit significant *in vitro* anti-HBV activity ($EC_{50} = 1.5$ and 8 μM) without significant cytotoxicity up to 200 μM, while **23** does not show any anti-HBV activity up to 200 μM.

Table 1. Anti-HBV Activity and Toxicity of 9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)purine Nucleosides



				toxicities, IC_{50} (μM)	
no.	X	Y	HBV (2.2.15) EC_{50} (μM)	2.2.15	CEM
11	H	H	>10	>200	ND[a]
10	NH_2	H	1.5	>200	90
12	NHMe	H	>10	>200	ND
13	cyclopropylamino	H	>10	>200	ND
14	NMe_2	H	>10	>200	ND
15	OH	H	8	>200	>100
16	SH	H	>10	>200	ND
21	NH_2	Cl	>10	>200	ND
22	NH_2	NH_2	>10	180	ND
23	OH	NH_2	>10	>200	>100

[a] ND: not determined.

Further exploration of the structure modification revealed that the free 6-NH_2 group is preferred for the anti-HBV activity, since either attachment of a small alkyl group (methyl, cyclopropyl, dimethyl) to or substitution of an NH_2 group with a hydrogen atom results in the loss of anti-HBV activity. Similar findings have been observed in the study of L-nucleosides with other sugar moieties.[22] Although the hypoxanthine derivative **15** exhibits moderate antiviral activity, the 6-mercapto analogue **16** does not show any significant activity up to 10 μM. Substitutions with either a chlorine atom (**21**) or an NH_2 group (**22**) at the 2-position of compound **10** also decrease the anti-HBV activity.

In summary, we have synthesized a number of purine nucleosides containing the 2-deoxy-2-fluoro-β-L-arabinofuranosyl moiety. From the structure−activity relationships studies, we have found that the adenine and hypoxanthine derivatives (**10** and **15**) exhibit moderately potent *in vitro* anti-HBV activity without significant cytotoxicity.

Experimental Section

Melting points were determined on a Mel-temp II apparatus and are uncorrected. NMR spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR with Me_4Si as internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC were performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220−440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. UV spectra were obtained on a Beckman DU 650 spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, or Galbraith Laboratories, Inc., Knoxville, TN.

9-(3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-β-L-arabinofuranosyl)-9*H*-6-chloropurine (5). A mixture of 6-chloropurine (**2**; 0.19 g, 1.2 mmol) and NaH (60 mg, 1.5 mmol, 60% in oil) in anhydrous CH_3CN (5 mL) was stirred under Ar at room

temperature for 30 min, to which **1** (0.42 g, 1.0 mmol) in CH_3CN (10 mL) was added. The resulting mixture was stirred at room temperature for 4 h, filtered, and washed with CH_2Cl_2. The combined filtrate was evaporated to dryness to give a mixture that was separated on a silica gel column (100:1 $CHCl_3$:CH_3OH). The major product was collected and recrystallized from EtOH to give **5** as a white solid (270 mg, 65.6%): mp 101−105 °C; UV (EtOH) λ_{max} 235.0, 264.5 nm; $[\alpha]^{25}_D$ +29.84 (*c* 0.22, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.78 (s, 1H, H-2), 8.42 (d, 1H, H-8, J = 2.9 Hz), 8.12−7.44 (m, 10H, benzoyl), 6.71 (dd, 1H, H-1′, $J_{1',2'}$ = 2.6 Hz, $J_{1',F}$ = 21.9 Hz), 5.80 (dd, 1H, H-3′, $J_{3',F}$ = 21.9 Hz), 5.40 (dd, 1H, H-2′, $J_{2',F}$ = 50.0 Hz), 4.84 (m, 2H, H-5′a,b), 4.64 (m, 1H, H-4′); ^{13}C NMR ($CDCl_3$) 152.64, 144.90 (CO), 134.71, 134.59, 133.92, 130.39, 130.24, 130.16, 129.91, 129.21, 129.00 (Ar), 92.93 (d, J = 192.96 Hz, C-2′), 84.29 (d, J = 17.01 Hz, C-1′), 81.95 (C-4′), 76.89 (C-3′), 63.52 (C-5′). Anal. ($C_{24}H_{18}ClFN_4O_5 \cdot C_2H_5OH$) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)adenine (10). A solution of **5** (0.15 g, 0.30 mmol) in saturated NH_3/CH_3OH (20 mL) was sealed in a stainless steel bomb and heated at 90 °C for 16 h. The solvent was evaporated, and the residue was purified by preparative TLC (7:1 $CHCl_3$:CH_3OH) and recrystallized from MeOH to give **10** as white crystals (60 mg, 74%): mp 231−233 °C; UV (H_2O) λ_{max} 256.0 (ϵ 18 171) (pH 2), 258.5 (ϵ 17 679) (pH 7), 258.0 nm (ϵ 18 674) (pH 11); $[\alpha]^{25}_D$ −44.55 (*c* 0.11, H_2O); ^{1}H NMR (DMSO-d_6) δ 8.23 (d, 1H, H-8, J = 1.9 Hz), 8.15 (s, 1H, H-2), 7.34 (bs, 2H, NH_2, D_2O exchangeable), 6.40 (dd, 1H, H-1′, $J_{1',2'}$ = 4.6 Hz, $J_{1',F}$ = 14.4 Hz), 5.94 (d, 1H, 3′-OH, D_2O exchangeable), 5.18 (dt, 1H, H-2′, $J_{2',F}$ = 52.7 Hz), 5.12 (t, 1H, 5′-OH, D_2O exchangeable), 4.44 (dt, 1H, H-3′, $J_{3',F}$ = 19.8 Hz), 3.83 (m, 1H, H-4′), 3.64 (m, 2H, H-5′a,b); FABMS m/z 270 $(M + 1)^+$. Anal. ($C_{10}H_{12}FN_5O_3$) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)-9*H*-purine (11). A mixture of **5** (0.25 g, 0.50 mmol), 10% Pd−C (150 mg) in EtOAc (30 mL), and Et_3N (5 mL) was subjected to hydrogenolysis at 40 psi for 5 h. After filtration through a Celite pad and washing with EtOAc, the combined filtrate was evaporated to dryness to give **6** as a white solid: UV (MeOH) λ_{max} 230.5, 262.0 nm. It was then stirred in saturated NH_3/CH_3OH at room temperature for 16 h. The solvent was evaporated, and the residue was purified by silica gel column chromatography (10:1 $CHCl_3$:CH_3OH). Recrystallization from MeOH gave **11** as white crystals (0.11 g, 86%): mp 174−176 °C; UV (H_2O) λ_{max} 261.5 (ϵ 6673) (pH 2), 261.5 (ϵ 6343) (pH 7), 262.0 nm (ϵ 6330) (pH 11); $[\alpha]^{25}_D$ −61.49 (*c* 0.10, H_2O); ^{1}H NMR (DMSO-d_6) δ 9.24 (s, 1H, H-6), 9.01 (s, 1H, H-2), 8.78 (d, 1H, H-8, J = 1.8 Hz), 6.60 (dd, 1H, H-1′, $J_{1',2'}$ = 4.7 Hz, $J_{1',F}$ = 13.1 Hz), 6.05 (d, 1H, 3′-OH, D_2O exchangeable), 5.33 (dt, 1H, H-2′, $J_{2',F}$ = 52.6 Hz), 5.16 (t, 1H, 5′-OH, D_2O exchangeable), 4.50 (dm, 1H, H-3′, $J_{3',F}$ = 19.0 Hz), 3.91 (m, 1H, H-4′), 3.67 (m, 2H, H-5′a,b); FABMS m/z 255 $(M + 1)^+$. Anal. ($C_{10}H_{11}FN_4O_3$) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)-N^6-methyl-9*H*-purine (12). A solution of **5** (0.16 g, 0.32 mmol) in MeOH (10 mL) and methylamine (10 mL, 40% in H_2O) in a sealed stainless steel bomb was heated at 85 °C for 12 h. Removal of solvent and purification by silica gel column chromatography (10:1 $CHCl_3$:CH_3OH) gave **12** as a pale white solid (75 mg, 82%): mp 91−94 °C; UV (H_2O) λ_{max} 261.5 (ϵ 12 601) (pH 2), 265.0 (ϵ 10 985) (pH 7), 265.0 nm (ϵ 11 647) (pH 11); $[\alpha]^{25}_D$ −37.43 (*c* 0.17, H_2O); ^{1}H NMR (DMSO-d_6) δ 8.25 (bs, 2H, H-8, H-2), 7.94 (bs, 1H, NH, D_2O exchangeable), 6.42 (dd, 1H, H-1′, $J_{1',2'}$ = 4.6 Hz, $J_{1',F}$ = 14.2 Hz), 5.99 (d, 1H, 3′-OH, D_2O exchangeable), 5.20 (dt, 1H, H-2′, $J_{2',F}$ = 52.7 Hz), 5.15 (t, 1H, 5′-OH, D_2O exchangeable), 4.46 (dt, 1H, H-3′, $J_{3',F}$ = 19.0 Hz), 3.86 (m, 1H, H-4′), 3.67 (m, 2H, H-5′ab), 2.96 (s, 3H, NHC**H**$_3$); FABMS m/z 284 $(M + 1)^+$. Anal. ($C_{11}H_{14}FN_5O_3$) C, H, N.

***N*^{6}-Cyclopropyl-9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-9*H*-purine (13).** A solution of **5** (0.11 g, 0.22 mmol) in THF (20 mL) and cyclopropylamine (1 mL) was heated in a sealed steel bomb at 90 °C for 4 h and then evaporated to dryness to give **8** as a syrup: UV (MeOH) λ_{max} 230.0, 268.5 nm. The crude product thus obtained was treated with saturated NH_3/CH_3OH at room temperature for 16 h. Removal of solvent followed by purification on preparative TLC (9:1 $CHCl_3$:CH_3OH) and crystallization from MeOH gave **13** as white needles (45 mg, 66%): mp 131−133 °C; UV (H_2O) λ_{max} 265.0 (ϵ 21 609) (pH 2), 268.5 (ϵ 19 202) (pH 7), 268.5 nm (ϵ 20 138) (pH 11); $[\alpha]^{25}_D$ −42.47 (*c* 0.11, H_2O); ^{1}H NMR (DMSO-d_6) δ 8.24 (bs, 2H, H-8, H-2), 8.02 (bs, 1H, NH, D_2O exchangeable), 6.40 (dd, 1H, H-1′, $J_{1',2'}$ = 4.6 Hz, $J_{1',F}$ = 14.1 Hz), 5.96 (d, 1H, 3′-OH, D_2O exchangeable), 5.18 (dt, 1H, H-2′, $J_{2',F}$ = 52.5 Hz), 5.12 (t, 1H, 5′-OH, D_2O exchangeable), 4.42 (dt, 1H, H-3′, $J_{3',F}$ = 18.8 Hz), 3.85 (m, 1H, H-4′), 3.64 (m, 2H, H-5′ab), 3.15 (m, 1H, C**H**$(CH_2)_2$), 0.72 (d, 4H, CH(C**H**$_2)_2$); FABMS m/z 310 $(M + 1)^+$. Anal. ($C_{13}H_{16}FN_5O_3 \cdot H_2O$) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)-N^6,N^6-dimethyl-9*H*-purine (14). A mixture of **5** (0.2 g, 0.4 mmol) and *N*,*N*-dimethylamine (0.3 mL, 40% in H_2O) in 1,4-dioxane (20 mL) was stirred at room temperature for 1 h and then evaporated to dryness to give **7** as a syrup: UV (MeOH) λ_{max} 273.0 nm. The crude **7** was treated with saturated NH_3/CH_3OH at room temperature for 16 h; removal of solvent and purification by silica gel column chromatography (10:1 $CHCl_3$:CH_3OH) gave **14** as a foam (80 mg, 67%); an analytical sample was obtained by crystallization from 2-propanol to give **14** as a white solid: mp 152−154 °C; UV (H_2O) λ_{max} 267.0 (ϵ 15 912) (pH 2), 274.0 (ϵ 15 121) (pH 7), 273.5 nm (ϵ 16 547) (pH 11); $[\alpha]^{25}_D$ −47.88 (*c* 0.11, H_2O); ^{1}H NMR (DMSO-d_6) δ 8.25 (d, 1H, H-8, J = 1.8 Hz), 8.22 (s, 1H, H-2), 6.42 (dd, 1H, H-1′, $J_{1',2'}$ = 4.6 Hz, $J_{1',F}$ = 14.2 Hz), 5.96 (d, 1H, 3′-OH, D_2O exchangeable), 5.18 (dt, 1H, H-2′, $J_{2',F}$ = 52.7 Hz), 5.12 (t, 1H, 5′-OH, D_2O exchangeable), 4.43 (dt, 1H, H-3′, $J_{3',F}$ = 18.9 Hz), 3.84 (m, 1H, H-4′), 3.64 (m, 2H, H-5′ab), 3.16 (s, 6H, NH(C**H**$_3)_2$); FABMS m/z 298 $(M + 1)^+$. Anal. ($C_{12}H_{16}FN_5O_3 \cdot 0.8H_2O$) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)hypoxanthine (15). To a solution of **5** (0.2 g, 0.4 mmol) in methanol (20 mL) was added $NaOCH_3$ (91 mg, 1.6 mmol) followed by 2-mercaptoethanol (0.11 mL, 1.6 mmol). The mixture was refluxed under Ar for 4 h. The solvent was evaporated, and the residue was dissolved in 50% MeOH/H_2O, neutralized with Dowex 50w × 8 (H^+) resin, and then filtered and washed with 50% MeOH/H_2O. Removal of solvent gave a syrup that was purified on preparative TLC (4:1 CH_2Cl_2:CH_3OH) and then loaded on a short silica gel column. Elution with EtOAc gave **15** as a white solid (70 mg, 64%): mp >118 °C dec; UV (H_2O) λ_{max} 248.0 (ϵ 8097) (pH 2), 248.5 (ϵ 6068) (pH 7), 252.5 nm (ϵ 8680) (pH 11); $[\alpha]^{25}_D$ −45.13 (*c* 0.12, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.20 (d, 1H, H-8, J = 1.9 Hz), 8.08 (s, 1H, H-2), 6.35 (dd, 1H, H-1′, $J_{1',2'}$ = 4.7 Hz, $J_{1',F}$ = 13.3 Hz), 5.90 (d, 1H, 3′-OH, D_2O exchangeable), 5.21 (dt, 1H, H-2′, $J_{2',F}$ = 52.7 Hz), 5.10 (t, 1H, 5′-OH, D_2O exchangeable), 4.42 (dt, 1H, H-3′, $J_{3',F}$ = 18.9 Hz), 3.84 (m, 1H, H-4′), 3.63 (dm, 2H, H-5′a,b); FABMS m/z 271 $(M + 1)^+$. Anal. ($C_{10}H_{11}FN_4O_4 \cdot$EtOAc) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)-9*H*-purine-6-thiol (16). A mixture of **5** (0.4 g, 0.8 mmol) and thiourea (76 mg, 1.0 mmol) in EtOH (25 mL) was stirred at reflux for 2 h and then cooled in an ice−water bath. The white precipitate formed was filtered and washed with EtOH (5 mL) to give **9** (0.33 g, 82%): UV (MeOH) λ_{max} 323.0, 227.5 nm (shoulder). Compound **9** was treated with saturated NH_3/CH_3OH at room temperature for 15 h and then evaporated to dryness. The residue was purified by silica gel column chromatography (10:1 $CHCl_3$:CH_3OH) to give **16** as a white solid (0.14 g, 81%): mp 224−226 °C; UV (H_2O) λ_{max} 321.0 (ϵ 16 697) (pH 2), 315.5 (ϵ 8796) (pH 7), 309.5 nm (ϵ 16 246) (pH 11); $[\alpha]^{25}_D$ −37.39 (*c* 0.10, H_2O); ^{1}H NMR (DMSO-d_6) δ 13.86 (s, 1H, NH, D_2O exchangeable), 8.42 (d, 1H, H-8, J = 1.6 Hz), 8.22 (s, 1H, H-2), 6.38 (dd, 1H, H-1′, $J_{1',2'}$ = 4.8 Hz, $J_{1',F}$ = 12.8 Hz), 5.98 (d, 1H, 3′-OH, D_2O exchangeable), 5.22 (dt, 1H, H-2′, $J_{2',F}$ = 52.4 Hz), 5.14 (t, 1H, 5′-OH, D_2O exchangeable), 4.42 (dt, 1H, H-3′, $J_{3',F}$ = 18.6 Hz), 3.86 (m, 1H, H-4′), 3.66 (dm, 2H, H-5′a,b); FABMS m/z 287 $(M + 1)^+$. Anal. ($C_{10}H_{11}FN_4O_3S$) C, H, N.

9-(3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-β-L-arabinofuranosyl)-2,6-dichloro-9*H*-purine (17). A mixture of 2,6-dichloropurine (**3**; 0.75 g, 4.0 mmol) and NaH (95%, 0.15 g, 6.0 mmol) in CH_3CN (20 mL) was stirred under Ar at room temperature for 30 min. To this was added **1** (0.84 g, 0.20 mmol), and the mixture was stirred at room temperature for

3 h and then washed with CH_2Cl_2. The combined filtrate was evaporated to dryness to give a mixture that was purified by silica gel column chromatography (4:1 hexanes:EtOAc). The major product was collected to give **17** as a white foam (0.31 g, 29%); an analytical pure sample was obtained by crystallization from MeOH: mp 153−154 °C; UV (MeOH) λ_{max} 273.5 nm; $[\alpha]^{25}_D$ +26.80 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.40 (d, 1H, H-8, J = 3.0 Hz), 8.10−7.44 (m, 10H, benzoyl), 6.65 (dd, 1H, H-1′, $J_{1',2'}$ = 2.7 Hz, $J_{1',F}$ = 21.8 Hz), 5.78 (dd, 1H, H-3′, $J_{3',F}$ = 16.9 Hz), 5.42 (dd, 1H, H-2′, $J_{2',F}$ = 49.8 Hz), 4.84 (m, 2H, H-5′a,b), 4.64 (m, 1H, H-4′); ^{13}C NMR ($CDCl_3$) 166.15, 165.14 (CO), 153.25, 152.44, 152.12, 145.18, 145.12, 134.32, 133.53, 130.57, 129.99, 129.72, 129.55, 129.21, 128.80, 128.68, 128.60, 127.97 (Ar), 92.57 (d, J = 193.00 Hz, C-2′), 83.91 (d, J = 17.0 Hz, C-1′), 81.73 (C-4′), 76.46 (C-3′), 63.08 (C-5′). Anal. ($C_{24}H_{17}Cl_2FN_4O_5$) C, H, N.

2-Chloro-9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)adenine (21). Compound **17** (130 mg, 0.245 mmol) was treated with saturated NH_3/CH_3OH (20 mL) in a sealed bomb at 90 °C for 7 h. Removal of solvent followed by purification on a silica gel column (10:1−7:1 $CHCl_3$:CH_3OH) gave **21** as a foam (60 mg, 81%); an analytical sample was obtained by crystallization from 2-propanol to give **21** as a white solid: mp 226−228 °C; UV (H_2O) λ_{max} 263.0 (ϵ 14 858) (pH 2), 263.0 (ϵ 13 438) (pH 7), 263.0 nm (ϵ 13 073) (pH 11); $[\alpha]^{25}_D$ −31.86 (*c* 0.10, H_2O); ^{1}H NMR (DMSO-d_6) δ 8.27 (d, 1H, H-8, J = 1.9 Hz), 7.89 (bs, 2H, NH_2, D_2O exchangeable), 6.31 (dd, 1H, H-1′, $J_{1',2'}$ = 4.7 Hz, $J_{1',F}$ = 13.7 Hz), 5.95 (d, 1H, 3′-OH, D_2O exchangeable), 5.22 (dt, 1H, H-2′, $J_{2',F}$ = 52.5 Hz), 5.08 (t, 1H, 5′-OH, D_2O exchangeable), 4.41 (dt, 1H, H-3′, $J_{3',F}$ = 18.7 Hz), 3.83 (m, 1H, H-4′), 3.65 (dm, 2H, H-5′a,b); FABMS m/z 304 $(M + 1)^+$. Anal. ($C_{10}H_{11}ClFN_5O_3$·0.15IPr-OH) C, H, N.

2,6-Diamino-9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-9*H*-purine (22). A mixture of **17** (130 mg, 0.245 mmol) and LiN_3 (48 mg, 1.0 mmol) in 95% EtOH was stirred at reflux for 40 min and then evaporated to dryness. The residue was extracted with $CHCl_3$, washed with water, dried ($MgSO_4$). Removal of solvent gave **18** as a syrup: UV (MeOH) λ_{max} 295.5 nm. It was redissolved in EtOH (20 mL) and stirred with 10% Pd−C (20 mg) under H_2 at 1 atm for 2 h and then filtered and washed with EtOAc. The combined filtrate was evaporated to dryness and purified by silica gel column chromatography (10:1 $CHCl_3$:CH_3OH) to give **19** as a syrup (0.11 g, 91%): UV (MeOH) λ_{max} 255.0, 279.0 nm. **19** was treated with saturated NH_3/CH_3OH (15 mL) at room temperature for 16 h and then evaporated to dryness. The residue was triturated with acetone, and the solid was recrystallized from MeOH to give **22** as a white solid (28 mg, 40% total yield from **17**): mp 220−223 °C; UV (H_2O) λ_{max} 251.5 (ϵ 9680), 289.5 (ϵ 8196) (pH 2), 255.5 (ϵ 6751), 278.5 (ϵ 7302) (pH 7), 255.0 (ϵ 7941), 278.0 nm (ϵ 8279) (pH 11); $[\alpha]^{25}_D$ −44.24 (*c* 0.12, H_2O); ^{1}H NMR (DMSO-d_6) δ 7.79 (d, 1H, H-8, J = 2.0 Hz), 6.80 (bs, 2H, NH_2, D_2O exchangeable), 6.17 (dd, 1H, H-1′, $J_{1',2'}$ = 4.2 Hz, $J_{1',F}$ = 16.0 Hz), 5.90 (bs, 3H, 3′-OH, NH_2, D_2O exchangeable), 5.08 (t, 1H, 5′-OH, D_2O exchangeable), 5.07 (dt, 1H, H-2′, $J_{2',F}$ = 52.4 Hz), 4.37 (dt, 1H, H-3′, $J_{3',F}$ = 18.2 Hz), 3.80 (m, 1H, H-4′), 3.61 (m, 2H, H-5′a,b); FABMS m/z 285 $(M + 1)^+$. Anal. ($C_{10}H_{13}FN_6O_3$) C, H, N.

2-Amino-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-L-arabinofuranosyl)-6-chloro-9*H*-purine (20). A mixture of 2-amino-6-chloropurine (**4**; 0.44 g, 2.6 mmol) and NaH (80 mg, 3.0 mmol, 95% in mineral oil) in anhydrous DMF (10 mL) was stirred under Ar at room temperature for 15 min. To this was added **1** (0.87 g, 2.0 mmol) in DMF (20 mL), and the mixture was stirred at room temperature for 30 h. It was evaporated to dryness, and the residue was purified on a silica gel column (100:1 $CHCl_3$:CH_3OH) to give a mixture of mainly two products (*N*-9 and *N*-7). Separation on preparative TLC (3:2 hexane: EtOAc) followed by recrystallization in MeOH gave the desired product **20** (fast moving band) as a white solid (95 mg, 9.3%): mp 101−103 °C; UV (MeOH) λ_{max} 232.5, 307.5 nm; $[\alpha]^{25}_D$ +42.46 (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.11, 7.43 (m, 10H, benzoyl), 8.04 (d, 1H, H-8, J = 3.0 Hz), 6.45 (dd, 1H, H-1′, $J_{1',2'}$ = 2.7 Hz, $J_{1',F}$ = 22.2 Hz), 5.77 (dd, 1H, H-3′, $J_{3',F}$ = 16.6 Hz), 5.32 (dd, 1H, H-2′, $J_{2',F}$ = 50.1 Hz), 5.13 (bs, 2H, NH_2, D_2O exchangeable), 4.81 (m, 2H, H-5′a,b), 4.58 (m, 1H, H-4′); ^{13}C NMR ($CDCl_3$) 165.20, 162.13 (CO), 148.15, 141.70, 134.62, 133.86, 130.37, 130.06, 129.18, 128.97, 113.74 (Ar), 92.95 (d, J = 193.39 Hz, C-2′), 83.78 (d, J = 17.20, C-1′), 81.42 (C-4′), 76.84 (C-4′), 63.62 (C-5′). Anal. ($C_{24}H_{19}ClFN_5O_5$·1.5H_2O) C, H, N.

9-(2-Deoxy-2-fluoro-β-L-arabinofuranosyl)guanine (23). A suspension of **20** (80.0 mg, 0.156 mmol), 2-mercaptoethanol (45 μL, 0.63 mmol), and $NaOCH_3$ (36 mg, 0.63 mmol) in MeOH (10 mL) was stirred at reflux under Ar for 6 h. The solvent was evaporated, and the residue was dissolved in 50% MeOH/H_2O, neutralized with Dowex 50w × 8 (H^+) resin, filtered, and washed with 50% MeOH/H_2O. The filtrate was evaporated to dryness and then triturated with acetone. The residue was further triturated with ether to leave a white solid which was recrystallized from water to give a white solid (31 mg, 70%): mp 249−251 °C; UV (H_2O) λ_{max} 254.0 (ϵ 11 786) (pH 2), 254.0 (ϵ 10 786) (pH 7), 254.5 nm (ϵ 11 214) (pH 11); $[\alpha]^{25}_D$ −24.96 (*c* 0.10, H_2O); ^{1}H NMR (DMSO-d_6) δ 7.80 (d, 1H, H-8, J = 2.1 Hz), 6.14 (dd, 1H, H-1′, $J_{1',2'}$ = 4.2 Hz, $J_{1',F}$ = 16.0 Hz), 5.92 (d, 1H, 3′-OH, D_2O exchangeable), 5.12 (dt, 1H, H-2′, $J_{2',F}$ = 52.4 Hz), 5.12 (t, 1H, 5′-OH, D_2O exchangeable), 4.36 (dt, 1H, H-3′, $J_{3',F}$ = 17.9 Hz), 3.82 (m, 1H, H-4′), 3.62 (dm, 2H, H-5′a,b); FABMS m/z 286 $(M + 1)^+$. Anal. ($C_{10}H_{12}FN_5O_4$) C, H, N.

Acknowledgment. This research was supported by Grant AI 33655 from the National Institutes of Health. We thank Dr. Michael G. Bartlett for performing the mass spectroscopy.

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JM970233+