Potential Anti-AIDS Drugs. Lipophilic, Adenosine Deaminase-Activated Prodrugs

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Selected acid-stable (2′-fluoro-2′,3′-dideoxyarabinofuranosyl)adenine nucleosides containing methyl groups and other lipophilic functions at various positions in the adenine ring were prepared and evaluated as anti-HIV agents. The N^6-methyl (**1f**), N^6-benzoyl (**1g**), and 6-chloro (**1i**) analogues had modest activity, giving 30–50% protection to ATH8 cells infected with HIV. 2-Methyl (**1d**), 8-methyl (**1h**), and 2,N^6-dimethyl (**1e**) substitution, as well as N^1-oxide (**21**) formation, abolished the activity of the parent compound (**1a**). Several of these compounds, originally designed as agents for treating HIV in the central nervous system, were further investigated as substrates for adenosine deaminase (ADA). Kinetic experiments showed that ADA catalyzed the formation of the anti-HIV active inosine compound **1b** from the N^6-methyl analogue **1f** in a quantitative manner. The anti-HIV activity of **1f** and **1i** was abolished when the ADA inhibitor, 2′-deoxycoformycin, was added to the test mixture. In contrast, the activity of **1f** was significantly enhanced when ADA was added to the test system. These data indicate that **1f** and **1i** are prodrug forms of **1b** in systems containing ADA.

Introduction

Central nervous system (CNS) complications in AIDS patients are often deadly and varied.[1-4] Because the CNS can be a sanctuary for HIV as well as a site for significant physiologic damage, the need for effective anti-HIV drugs which can be delivered to the CNS has been recognized.[5-7] In an attempt to enhance blood–brain barrier (BBB) penetration, dideoxyadenosine (ddA) analogues with increased lipophilicity were synthesized and evaluated, in vitro, in the ATH8/HIV system. During this investigation, it was discovered that in addition to the normal kinase activation of a 2′,3′-dideoxynucleoside (ddN) to its active 5′-triphosphate metabolite, catabolism by adenosine deaminase was also a requirement for activity for some of the newly synthesized compounds.

The blood–brain barrier protects the brain from potentially harmful materials in the systemic circulation. Unfortunately, this phenomenon can also exclude useful drugs.[8] Lipophilic, nonionic, low molecular weight materials generally appear to have the best passive diffusion properties for CNS penetration.[9] It also has been reported that an active transport mechanism may play a role in BBB penetration of some nucleosides[10,11] although the structural requirements necessary to make use of this possibility are not well-defined. Hansch and co-workers have pioneered the use of octanol/water partition coefficients (log P) to correlate compound structure with CNS penetration.[12] We felt that this approach might provide an appropriate hypothesis for maximizing the CNS anti-AIDS activity for the 2′,3′-dideoxynucleoside series. A preliminary account of this work has been presented.[13]

The log P of AZT (0.05, Table I) indicates that this compound is really neither lipophilic nor hydrophilic but partitions into octanol and pH 7.0 buffer almost equally. AZT, however, is one of the more lipophilic compounds investigated clinically, and it enters the CNS better than ddC.[10,14] The failure of ddC to achieve significant CNS levels might be related to the more hydrophilic nature (log P = −1.33) of this compound relative to AZT. For this reason we decided to prepare ddN more lipophilic than AZT for anti-HIV evaluation.

The design of lipophilic compounds is not difficult given the general principles and lipophilic substituent constants generated by Hansch and others in earlier QSAR studies.[15] The more uncertain part of the problem is retention of anti-HIV activity after making the appropriate structural modifications, since the activity of dideoxynucleosides is critically dependent on a series of enzymatic events, any one of which might be adversely affected by a structural

Table I. Octanol/Water Partition Coefficients and Chromatographic Properties of 2′,3′-Dideoxynucleosides

compd	log P[a]	HPLC mobile phase[b]	λ_{max},[c] nm
21	−1.38 ± 0.06	B	231, 260
ddC	−1.33 ± 0.01	A	271
ddI	−1.24 ± 0.03	C	249
1b	−1.21 ± 0.02	C	247
1c	−0.40 ± 0.01	D	261
ddA	−0.29 ± 0.01	D	260
1a	−0.18 ± 0.01	D	259
AZT	0.05 ± 0.01	F	266
1h	0.10 ± 0.01	D	260
1d	0.12 ± 0.02	D	260
1f	0.27 ± 0.01	E	265
1i	0.32 ± 0.02	F	264
1e	0.64 ± 0.01	F	268
1g	0.73 ± 0.02	F	280

[a] Mean ± standard deviation of three independent determinations. [b] The following mobile phases were used at 1.0 mL/min with a 4.6 × 250 mm 5-μm Ultrasphere ODS column: A, 4%; B, 7%; C, 10%; D, 12%; E, 15%, or F, 20% CH_3CN in pH 7.0, 0.01 M phosphate buffer. All dideoxynucleosides had a retention time of 4–9 min under the above conditions. [c] Wavelength determined on-the-fly in HPLC mobile phase.

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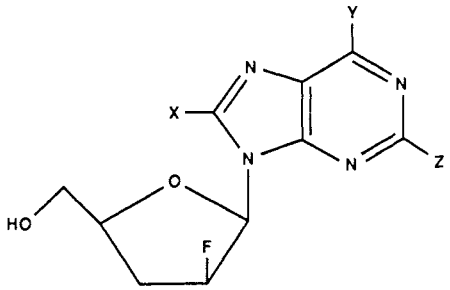
change. For this reason, we set out to make the most minor structural changes possible—the addition of a methyl group (which should increase a log P value by ca. 0.5)[15] at various purine positions. We chose to modify purines rather than pyrimidines because purines are the more lipophilic class (see Table I). Among the dideoxypurines, we chose to modify ddA rather than ddI, for the same reason, and always included a 2′-fluoro substituent for its acid stabilizing properties.[16]

After the synthesis and evaluation of the target compounds, it became clear that the above structural modifications, at best, produced compounds with modest activity relative to the unmethylated parent (**1a**). Since



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a) X=H; Y=NH_2; Z=H
b) X=H; Y=OH; Z=H
c) X=H; Y=H; Z=H
d) X=H; Y=NH_2; Z=CH_3
e) X=H; Y=$NHCH_3$; Z=CH_3
f) X=H; Y=$NHCH_3$; Z=H
g) X=H; Y=NHCOPh; Z=H
h) X=CH_3; Y=NH_2; Z=H
i) X=H; Y=Cl; Z=H

these new analogues did not meet our criteria of activity for further CNS developmental work, the investigation was refocused to determine what role adenosine deaminase (ADA) might play in activation or deactivation. It is known that ADA can utilize a number of riboside and 2′-deoxyriboside-containing purine nucleosides as alternate substrates.[17,18] Inosine analogue **1b**, which would be metabolically generated from our 6-substituted analogues, has established anti-HIV activity in vitro.[16] If this conversion occurred, the new compounds might have anti-HIV prodrug possibilities.

Results

Chemistry. The compounds of interest were prepared via a common carbohydrate precursor, 1,3,5-tri-*O*-benzoyl-2′-fluoro-α-D-ribofuranose (**2a**).[19] Anomeric bromination with 30% HBr/acetic acid followed by condensation of the resulting sugar **2b** with the appropriate pertrimethylsilylated base afforded protected nucleosides **3** and **8** as intermediates in the synthesis of target compounds **1c–e** (Scheme I). Fusion of **2b** with purine gave mainly **3a** along with minor percentages of β-N-7 and α-N-9 nucleosides **3b** and **3c**. The analogous coupling of 2-methyladenine gave **8a** as the major product along with 25% of the α-N-9 compound **8b** and very minor amounts of N-7 and N^6 alkylated bases.

Ammonolysis of the acyl groups with ammonia-saturated methanol in a pressure bottle at 4 °C afforded 2′-fluoro 2′-deoxynucleosides **4a** and **9a** in high yield.

The stereochemistries of the major products were assigned by NMR coupling constants based on precedent[20] and the regiochemistries (N-9 vs N-7) by UV[21] and one dimensional NOE difference (1D NOE) spectra. In general, $^3J_{1',2'}$ in β-nucleosides with a 2′-*ara* substituent is in the range of 3–4 Hz whereas the α-nucleosides show extremely small or no coupling between the 1′- and 2′-protons. Also characteristic of β-*D*-2′-*ara*-fluoro nucleosides of the purine series is the small but finite coupling (1–3 Hz) of the 2′-fluorine atom to H8 of the base[22] ($^5J_{8,F}$ = 2.5 Hz in these compounds). Distinguishing the N-7 and N-9 regioisomers of the 2-methyladenine derivatives **9a** and **9b** was based on the significant difference in the UV maxima of alkyl-substituted adenines.[21] This correlation was used previously to distinguish the N-7 and N-9 isomers of compound **1a**.[16] Since the methyl group at the 2-position has little effect on the position of the UV maxima, compound **9a**, whose UV maximum was centered at 260 nm, was assigned the N-9 structure. In the case where unsubstituted purine is the base (compounds **4a** and **4b**), the assignment of regiochemistry based on a similar argument is ambiguous (maxima of **4a** and **4b** are 261 and 264 nm, respectively). The ^{1}H NMR spectrum of **4a** was virtually identical with that of **4b** except for a subtle difference in the chemical shifts of the H1′ protons. When H1′ or **4b** was irradiated in a 1D NOE experiment, positive enhancements were observed at H6 and H8 in addition to the expected enhancement at H2′. When a similar experiment was performed on compound **4a**, a positive NOE was observed on only one base proton (H8). These data suggest the regiochemistries of **4a** and **4b** to be N-9 and N-7, respectively. This qualitative assessment was confirmed by conversion of the known 5′-protected adenosine derivative **16** to the corresponding nebularine analogue **7** by reductive deamination[23] (Scheme II) and spectroscopic comparison to **7** prepared from **4a** (Scheme I).

Selective 5′-protection of compounds **4a** and **9a** yielded alcohols **5** and **10**, while monobenzoylation[24] of **10** gave **13**, all in high yield (Scheme I). Reductive deoxygenation of the 3′-hydroxyl groups of compounds **5** and **10** to produce **7** and **12** proceeded without incident through methyl xanthate **6** and methoxy(thiocarbonyl) derivative[25] **11**, respectively. Acid-catalyzed deprotection of **7** and **12** gave the corresponding targets **1c** and **1d**. Preparation of the xanthate of **13** produced N^6-methylated product **14**, the precursor of nucleoside **1e**. Radical deoxygenation of **14** and sequential deblocking of the resulting product **15**, via

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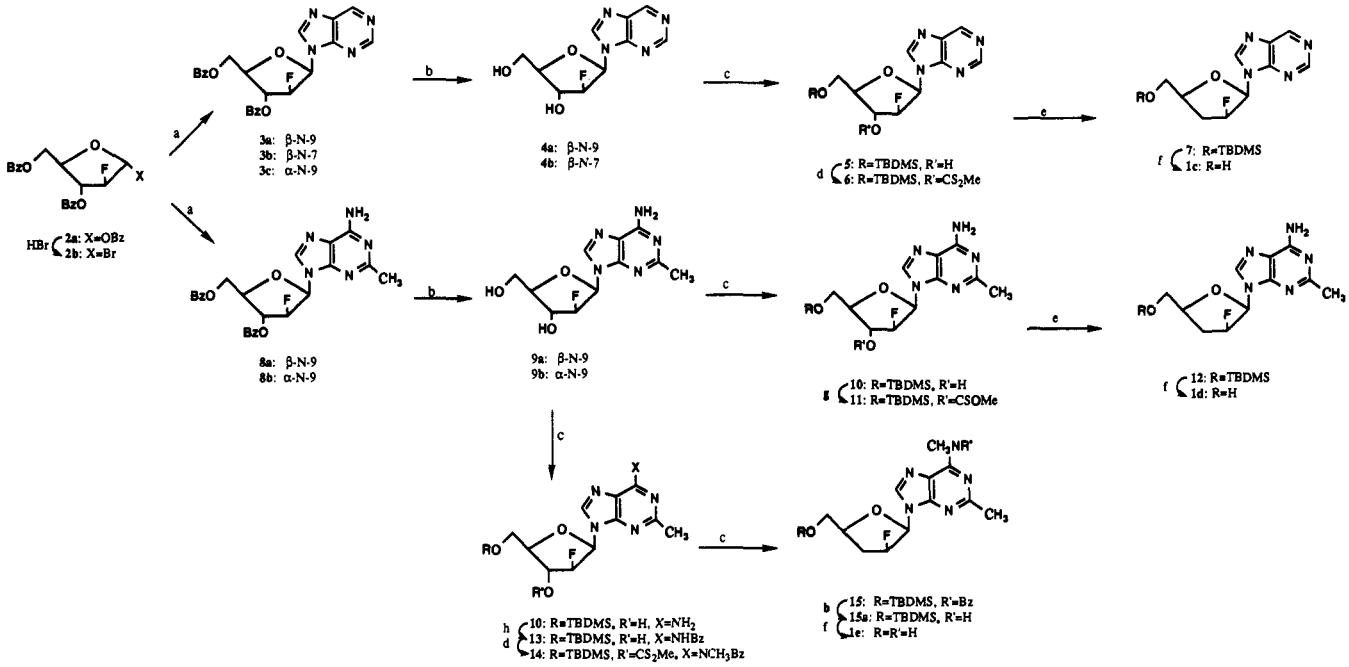
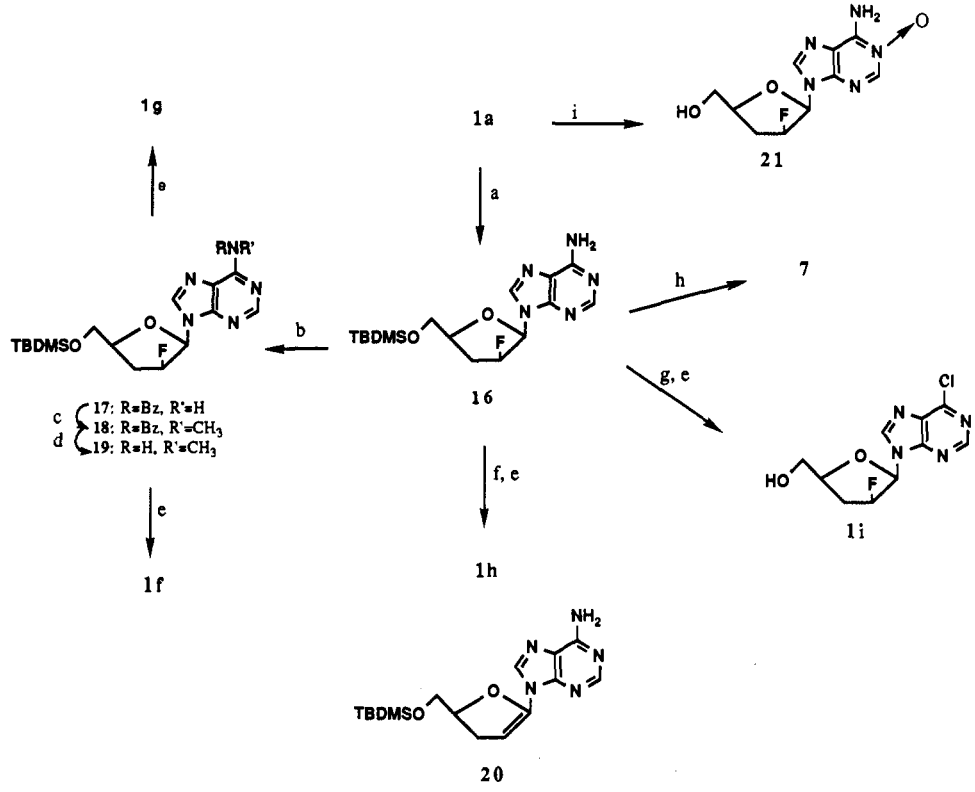
Scheme I[a]



[a] (a) TMS-base, neat, 100 °C; (b) NH_3/MeOH; (c) TBDMS-Cl, imidazole, DMF; (d) CS_2, NaH, MeI, DMF; (e) Bu_3SnH, AIBN, toluene, 90 °C; (f) 80% AcOH, 90 °C; (g) 1,1'-thiocarbonyldiimidazole, DMF then MeOH, reflux; (h) BzCl, Pyr, then NaOH.

Scheme II[a]



[a] (a) TBDMS-Cl, imidazole, DMF; (b) BzCl, Pyr, then NaOH; (c) NaH, MeI, DMF; (d) NH_3/MeOH; (e) 80% AcOH; (f) LDA, MeI, −78 °C; (g) *t*-BuONO, CCl_4, 85 °C, 200-W bulb; (h) *t*-BuONO, THF, 85 °C, 200-W bulb; (i) H_2O_2, AcOH.

15a, afforded the desired, 2,N^6-dimethyladenine analogue **1e**.

Compounds **1f–h** were prepared from the known 5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-dideoxy nucleoside **16**[16] (Scheme II). Dibenzoylation of **16** followed by NaOH cleavage[24] to the monobenzoyl derivative and desilylation afforded N^6-benzoyl nucleoside **1g**. Compound **1f** was prepared from **17** via methylation and sequential deblocking of the N- and O-protecting groups as for **1e**. Direct lithiation[26] of the 8-position of **16**, followed by quenching with methyl iodide and deprotection, gave the 8-substituted derivative **1h** in low to moderate yield. A significant amount of olefin **20** was formed from base-

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Scheme III

catalyzed elimination of hydrofluoric acid. Loss of the absorption for H1′ and the appearance of an olefinic multiplet at 5.58 ppm, which couples strongly to the H3′ methylene in the ^{1}H NMR spectrum, provided evidence for structure **20**.

6-Chloro derivative **1i** was prepared via compound **16** by the method of Nair[23] (Scheme II). Direct replacement of the 6-amino group with chlorine proceeds in modest but acceptable yield (ca. 50%). Compound **1a** was oxidized to its 1-oxide **21** by the action of hydrogen peroxide in acetic acid.

Partition Coefficients. Molecules with a 100-fold range of lipophilicities were designed within the 2′-fluoro purine ddN series (Table I). Octanol/pH 7.0 buffer partition coefficients were determined by using a newly developed microscale method. N^6-Benzoyl compound **1g** was the most lipophilic compound produced in this series with an octanol/pH 7.0 buffer partition coefficient (P) of 5.4 (log P 0.73). The 1-oxide of ddA (**21**) was the most hydrophilic compound synthesized with a P of 0.04 (log P = −1.38). Lipophilicities of these two compounds are 5 times greater and 27 times less than AZT, respectively. The other compounds synthesized had intermediate P values. The addition of a methyl group normally increases the log P value of a compound by about 0.5.[27] In the dideoxynucleoside series, however, the lipophilicity constant (π value) for a methyl group proved to be somewhat less, 0.3–0.4 (Table I).

Anti-HIV Activity in Vitro. Initially, the various monomethyl analogues (**1d,f,h**) were prepared in an attempt to produce a modest increase in lipophilicity without adversely affecting the anti-HIV activity of the parent compound, **1a**. N^6-Methyl analogue **1f**[13] was the only monomethyl compound with any activity, and that activity was reduced relative to that of **1a** (Table II). Activity with the corresponding nonfluorinated compound had been reported earlier by Chu and co-workers,[28] and the activity of **1f** against HIV in peripheral blood mononuclear cells was recently reported by the same group.[29] Compound **1e** is the 2-methyl analogue of **1f**. As with **1d**, the methyl group in the 2-position abolished activity. Substitution of N^6 with a benzoyl group (**1g**) resulted in the preservation of modest activity. Generation of the 1-oxide (**21**) of the parent compound, **1a**, or removal of the 6-amino group to produce nebularine analogue **1c** abolished activity. 6-Chloro analogue **1i**, however, provided ca. 50% protection to HIV-exposed ATH8 cells. This finding, as well as the activity found for **1f**, made us suspect that the 6-substituted compounds were perhaps being converted to an active metabolite. A reasonable explanation was that ADA catalyzed the hydrolysis of these compounds to the known

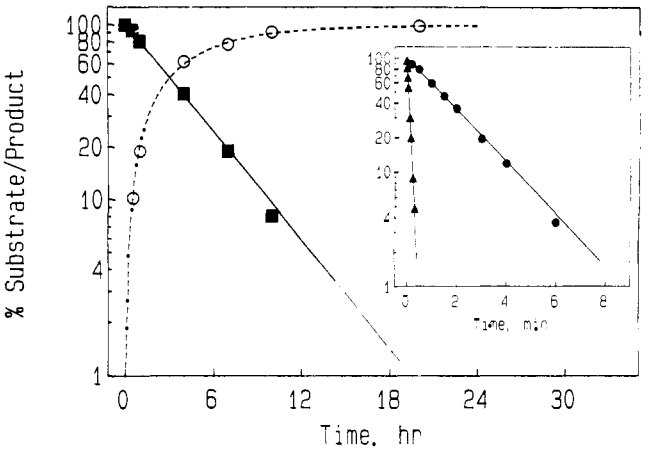

Figure 1. Hydrolysis kinetics and 2′-F-dd-*ara*-I formation during the incubation of 50 μM N^6-CH_3-2′-F-dd-*ara*-A (**1f**) with 0.7 units/mL of adenosine deaminase at 37 °C. A unit of adenosine deaminase is the amount of enzyme which hydrolyzes a standard substrate (e.g. adenosine) at the rate of 1.0 μmol/min at 25 °C and pH 7.5. See the Experimental Section for hydrolysis conditions. Symbols and measured $t_{1/2}$: N^6-CH_3-2′-F-dd-*ara*-A (■), 3.0 h; 2′-F-dd-*ara*-I (○), 3.0 h [appearance $t_{1/2}$]. Insert: Adenosine deaminase hydrolysis kinetics for 50 μM ddA and 2′-F-dd-*ara*-A. The hydrolysis curve for ddA has been extrapolated from kinetics obtained with 0.07 unit/mL of adenosine deaminase under identical conditions. Symbols and measured $t_{1/2}$: ddA (▲), 5 s; 2′-F-dd-*ara*-A (●), 1.33 min.

active inosine analogue **1b** (Scheme III). The ATH8 cells and the incubation medium (which contains 15% fetal calf serum) contain ADA, which can act on the ddN during the 7 day in vitro anti-HIV test. For this reason, we decided to quantitate how rapidly N^6-methyl compound **1f** was converted to **1b** by ADA.

Adenosine Deaminase. Kinetic data already available indicated that the deamination of N^6-methyladenosine riboside analogues was slow.[17] Reaction of N^6-methyl analogue **1f** with ADA as the isolated enzyme (0.7 units/mL) at 37 °C showed that a hydrolysis reaction occurred at a rate substantial enough to be easily quantified (Figure 1, $t_{1/2}$ = 3.0 h), but which was 135 times slower than that of **1a** (Figure 1, inset). It was also established that the corresponding inosine analogue **1b** was the product formed, and that the rate of formation of **1b** corresponded to the rate of disappearance of **1f** (Figure 1). Dideoxyadenosine and its 2′-fluoro analogue **1a** were deaminated at much faster rates ($t_{1/2}$ = 5 and 80 s, respectively) under these conditions (Figure 1, inset). As expected,[30] no deamination was observed under the same conditions with N-oxide **21**. Similarly, N^6-benzoyl compound **1g** was unaffected by ADA.

Because of the ubiquitous nature of ADA in vivo, it occurred to us that the ADA-catalyzed hydrolysis of 6-substituted dideoxypurine nucleoside analogues might possibly be used to advantage in anti-AIDS therapy in general, and in CNS therapy, in particular. The ADA reaction might be of general utility if the proper hydrolysis rate could be achieved for a compound which was converted into an active material, e.g. **1b**. In addition, if a compound could be designed which was catabolized slowly enough by ADA in the peripheral circulation to allow transport into the CNS, but fast enough by ADA in the CNS to provide therapeutic concentrations of an active, more hydrophilic inosine analogue, then a drug-delivery

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Table II. Effect of 2′-Deoxycoformycin on the Anti-HIV Activity of 2′,3′-Dideoxyadenosine Analogues in ATH-8 Cells[a]

compd	dCF (5μM)	concn, μM	% protection	% toxicity
1a	−	0,20,50	0,70,84	0,0,0
	+	0,20,50	0,68,48	11,17,54
1f	−	0,5,10,20,50,100	0,13,24,32,37,27	0,0,0,0,6,44
	+	0,5,10,20,50,100	0,1,2,0,0,0	11,3,7,0,46,70
1g	−	0,5,20,50,100	0,8,30,29,21	0,2,0,0,8
1i	−	0,10,20,50,100	0,48,57,53,35	0,5,26,46,52
	+	0,10,20,50,100	3,15,11,14,17	14,49,58,56,72
ddA	−	10	100	0
	+	10	86	22
ddI	−	20	100	0

[a] ATH8 cells were exposed to HIV-1/III_B for 1 h and cultured in the presence of various concentrations of each compound. On day 8, the total viable cells were counted. Orders of numbers in the column for concentrations correspond to the orders of numbers in other columns.

Table III. Adenosine Deaminase/Dideoxynucleoside Kinetic Parameters[a]

substrate	K_M, M	v_{max}, μmol/min per unit	measured rel rate[b]
ddA	1.4×10^{-5}	1.32	100
2′-F-dd-*ara*-A (1a)	3.3×10^{-4}	2.3×10^{-1}	6.0
6-Cl-2′-F-dd-*ara*-P (1i)	7.5×10^{-3}	2.6×10^{-2}	0.06
N^6-CH_3-2′-F-dd-*ara*-A (1f)	1.1×10^{-4}	5.5×10^{-4}	0.04

[a] pH 7.4 and 37 °C. [b] Measured at 50 μM substrate concentration.

system could be available which provided enhanced CNS prodrug entry with reduced therapeutic drug exit. This would be a variation on the CNS "locked-in" effect of very polar molecules, which is a part of the dihydropyridine CNS prodrug system developed by Bodor and co-workers[31] and is important since it is generally thought that compounds which enter the CNS easily also exit easily.[32] The reported values for ADA in the CNS are somewhat variable, but it appears clear that certain CNS diseases, especially meningeal tuberculosis, greatly increase ADA activity relative to normal controls.[33,34] Whether AIDS causes a similar effect on ADA CNS levels is, however, presently, unknown.

Since it is reported that ADA hydrolyzes a number of groups other than the amino function in the 6-position of purine nucleosides,[17,18] 6-chloro derivative 1i also appeared to be an attractive target, since the π value for an aromatic chlorine is +0.71. This should result in a compound (1i) with a predicted log P value of 0.31 based on that of 1c. Table I shows that this is the case. Another reason for the interest in 1i is the recently demonstrated anti-HIV activity of the non-fluoro sugar analogues of the 2′,3′-dideoxy-6-halopurines in multiple CD4+ cell systems, including the ATH8 system.[35] Because of the reproducible, albeit unspectacular, activity found for 1i and 1f (Table II), we decided to examine the role of ADA by determining the enzyme kinetics for the hydrolysis of several active 2′-fluoro analogues (1a,f,i, Scheme III) relative to the nonfluorinated dideoxynucleoside ddA.

As seen in Table III, there are significant variations in both the binding affinities (K_M) and the maximum reaction velocities (v_{max}) among the 2′-fluoro analogues and between ddA. None of the 2′-fluoro dideoxynucleosides bound as tightly or reacted as rapidly with ADA as the parent compound, ddA. Therefore, large differences thus exist in the measured relative rates of ADA hydrolysis determined at 50 μM substrate for these compounds as compared to ddA. Both the 6-chloro (1i) and 6-methylamino (1f) analogues are hydrolyzed much more slowly (ca. 1700 and 2500 times slower, respectively) than ddA. While 1f was bound to ADA about 70 times tighter than 1i, its v_{max} was 50 times slower, resulting in the measured relative rates being fairly similar. Compound 1a was hydrolyzed 17 times slower than ddA, which compares with a value of ca. 10 times slower determined previously under slightly different conditions.[36] Relative rates were measured with 50 μM substrate (Table III), since this concentration produced an anti-HIV protective effect with the compounds shown in Table II. Preliminary experiments indicate that the ADA level in media alone (no ATH8 cells) is more than 1000 times lower than the 0.7 unit/mL concentration used in our isolated enzyme experiments. ADA is also present in a number of cell lines,[37] including ATH8 cells.[36] Additional studies are underway to quantitate the effects of media and cellular ADA on ddN analogues and will be reported at a future time.

In order to further establish the importance of ADA in the anti-HIV experiments, the effect of the powerful ADA inhibitor 2′-deoxycoformycin (dCF) was evaluated. dCF affected the compounds in this study in different ways when added to the in vitro anti-HIV test system (Table II). dCF alone (5 μM) did not produce significant toxicity or protection of ATH8 cells against the effects of HIV-1. Similarly, there were no important changes in the protection afforded by ddA and its 2′-fluoro analogue 1a in the presence of dCF. Toxicity, however, appeared to be potentiated in each instance. The ddA/dCF anti-HIV results are consistent with reported data.[37] The effects observed were quite different when dCF was used in combination with the N^6-methyl (1f) and 6-chloro (1i) compounds. In these cases, protection was either abolished or greatly decreased relative to experiments conducted in the absence of dCF (Table II). This is qualitatively consistent with the isolated enzyme data shown in Figure 1 and Table III.

As a consequence of the above results, studies were conducted to evaluate the effect of augmenting the HIV/ATH8 test system with additional ADA. In these experiments (data not shown), the combination of 10 μM 1f (N^6-methyl analogue) and ADA (0.7 unit/mL) gave 90% protection, whereas 1f, alone, at the same concentration, gave only 13% protection. Compound 1a with supplementary ADA under the same conditions did not change its activity. ADA by itself neither influenced the viability nor protected infected ATH8 cells. These data suggest that 1f and 1i are prodrug forms of 2′-F-dd-*ara*-I (1b), which require hydrolysis by ADA as a necessary step in their activation.

Experimental Section

Melting points were taken on a Mel-Temp II apparatus and were uncorrected. UV spectra were recorded on a Beckman Model

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34 spectrophotometer or on-the-fly during HPLC analysis on a Perkin-Elmer LC-235 diode array spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter at the sodium D line. Infrared spectra were recorded on a Perkin-Elmer 727B spectrophotometer. Proton and ^{13}C NMR spectra were run on a Varian XL-200 spectrometer at 200 and 50 MHz, respectively. Chemical shifts are given in ppm relative to TMS and are referenced against the solvent in which the samples were run. Analytical and preparative TLC analyses were performed on Uniplate GHLF silica gel (Analtech, 250 and 1000 μm, respectively). Column chromatography was accomplished with Kieselgel 60 (mesh size 230–400). Reverse-phase purification was performed either on C_{18} disposable extractions columns (J. T. Baker, 6 mL) or at medium pressure on bonded phase C_{18} silica gel. Moisture-sensitive reactions were run under argon in flasks previously dried at 110 °C. Ether and THF were distilled from sodium/benzophenone ketyl. All other solvents came from Sure Seal bottles purchased from Aldrich. Silylation reagents were premixed in 1-mL vials and purchased from Alltech.

Positive ion fast atom bombardment mass spectra were obtained on a VG 7070E mass spectrometer operated at an accelerating voltage of 6 kV and a resolution of 2000. Glycerol was used as the sample matrix and ionization was effected by a beam of xenon atoms derived by charge-exchange neutralization of a 1.0–1.2 mA beam of xenon ions accelerated through 8.0–9.2 kV. Spectra were acquired under the control of a VG 11/250 J^+ data system at a scan speed of 10 s/decade, and the background due to the glycerol matrix was automatically subtracted. Accurate mass measurements of the protonated molecular ions (MH^+) for compounds **1d** and **1i** were obtained by a limited mass range voltage scan at a resolution of 3000 with repetitive data accumulation and averaging under computer control. The MH^+ peak was then mass measured with the appropriate data system software using selected glycerol peaks within the mass range as internal references.

A 50-μL aliquot of a 10 mg/mL suspension of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) from calf intestinal mucosa was centrifuged at 600*g* for 3 min. The residue remaining after removal of the $(NH_4)_2SO_4$ supernatant was dissolved in 0.5 mL of pH 7.4 tris(hydroxymethyl)aminomethane (Tris) buffer to give an enzyme solution of 1 mg/mL (274 units/mL). 2′-Deoxycoformycin (dCF), 9-(2′,3′-dideoxy-2′-fluoroarabinosyl)adenine (2′-F-dd-*ara*-A, **1a**), and 9-(2′,3′-dideoxy-2′-fluoroarabinosyl)hypoxanthine (2′-F-dd-*ara*-I, 1b) were obtained from Dr. Karl Flora, Pharmaceutical Resources Branch, NCI. A pH 7.4 buffer was prepared by adjusting 0.01 M Tris to pH 7.4 with 0.5 N HCl. The 1.0 N HCl, HPLC-grade acetonitrile, monobasic potassium phosphate, sodium hydroxide, and ultrapure Tris were all commercially available and were used without further purification.

Enzymatic Deamination of Adenosine Analogues. Solutions (1 mM) of dideoxynucleoside substrates (ddA, **1a**,**f**,**g**,**i**, and **21**) were prepared in 0.01 M pH 7.4 Tris buffer, while 1 mM and 2 μM solutions of the adenosine deaminase inhibitor 2′-deoxycoformycin were prepared in distilled water. A 50-μL aliquot of substrate solution was diluted with 0.95 mL of 0.01 M Tris buffer in a 1.5-mL Eppendorf tube and equilibrated at 37 °C in a Dubnoff metabolic shaking incubator. Reaction was initiated by addition of 2.5 μL of adenosine deaminase solution (0.7 unit). At specified time intervals, a 50-μL aliquot of the reaction mixture was withdrawn and quenched by mixing with 0.45 mL of cold 2 μM 2′-deoxycoformycin. This diluted sample was ultrafiltered to remove enzyme in a Centrifree Micropartition unit by centrifugation at 600*g* at 4 °C in a high-speed refrigerated centrifuge. The decrease in substrate concentration was monitored by HPLC analysis of the resultant ultrafiltrate (see below). The ability of 2′-deoxycoformycin to inhibit deamination under the above conditions was evaluated by adding 2 μL of 1.0 mM inhibitor to diluted substrate solutions at the time of equilibration. Reaction, sampling, and analysis were carried out as above. In additional experiments, four to six concentrations of each substrate (ddA, **1a**,**f**,**i**) were reacted with ADA (0.01, 0.03, or 0.4 units, depending on substrate) at pH 7.4 and 37 °C. Initial hydrolysis rates for each substrate concentration were measured through linear least squares curve fitting of the concentration vs time data for reaction of the first 10% of substrate. K_M and v_{max} values for each substrate were then determined from a graphical Lineweaver–Burke plot of these initial rates.

HPLC Analysis of Dideoxypurine Nucleosides. Concentrations of dideoxynucleosides were measured by the HPLC analysis of 100-μL aliquots of ultrafiltered samples. A 4.6 × 250 mm 5-μm Ultrasphere-ODS column, protected by a Waters guard column packed with 37–50 μm Vydac 201SC, was eluted with 10–20% CH_3CN in 0.01 M pH 7.0 phosphate buffer at a flow rate of 1.0 mL/min. Dideoxynucleosides and deamination products were detected at the appropriate wavelength of maximum absorption with a Gilson 116 variable-wavelength detector. Peak identity was determined from coincidence of retention times with standards and by comparison of on-the-fly UV spectra obtained with a Perkin-Elmer LC-235 diode-array detector. Peak areas and peak heights were measured simultaneously on a Spectra-Physics SP4200 computing integrator. For kinetic studies, these data were plotted as a function of time and, where possible, fitted to a first-order decomposition using Graph-Pad, a commercial nonlinear least squares curve fitting program.

Measurement of Octanol/Water Partition Coefficients. *n*-Octanol/water partition coefficients (*P*) were determined by a microscale shake-flask procedure,[38] which was a modification of an earlier method.[39] A 20-μL aliquot of a 0.5 mg/mL DMSO solution of the dideoxynucleoside was dissolved in 1.0 mL of octanol-saturated, pH 7.0, 0.01 M potassium phosphate buffer. This was mixed thoroughly with 1.0 mL of buffer-saturated *n*-octanol in a 2-mL Mixxor apparatus at 24–26 °C and then allowed to stand for 15 min. The phases were separated and centrifuged at 600*g* for 5 min, and the relative concentration of sample in each phase was determined by HPLC analysis of a 50-μL aliquot. The partition coefficient was calculated by dividing the absolute area of the appropriate integrated peak from the octanol phase by that of the buffer phase.

HIV Cytopathic Effect Inhibition Assay. The HIV cytopathic effect inhibition assay was performed as previously described.[7] Briefly, 200 000 target $CD4^+$ ATH8 cells were exposed to cell-free HIV-$1/III_B$ at a dose of 43 (or 1087 for compound **1g**) $TCID_{50}$ (50% tissue culture infectious dose) for 1 h, resuspended in 2 mL of fresh culture media containing interleukin 2, and cultured at 37 °C with or without test compounds in 5% CO_2-containing humidified air. On day 8 in culture, the viable cells were counted by using the dye-exclusion method. All compounds, including inactives, were evaluated a minimum of two times in separate experiments. Data reported in Table II are from representative tests. The percent protection against the virus was determined by the following formula: 100 × [[(the number of viable cells exposed to HIV-1 and cultured in the presence of the compound) − (the number of viable cells exposed to HIV-1 and cultured in the absence of compound)]/[(the number of viable cells cultured alone) − (the number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]]. The percent toxicity of a compound on the target cells was determined by the following formula: 100 × [1 − (the number of total viable cells cultured in the presence of the compound)/(the number of total viable cells cultured alone)]. Calculated percentages equal to or less than zero are expressed as 0%.

9-(3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-9*H*-purine (3a). Purine (250 mg, 2.08 mmol) was suspended in dry acetonitrile (7 mL, argon atmosphere) and treated with BSTFA (1-mL vial, Pierce) at room temperature. After 30 min the homogeneous solution was evaporated to dryness in vacuo. The resulting yellow oil was placed under an argon atmosphere and compound **2b** (807 mg, 1.90 mmol), dissolved in dry CH_2Cl_2 (10 mL), was added to the (trimethylsilyl)purine via syringe. After removal of the solvent on the rotoevaporator (40 °C) the neat mixture was heated to 100 °C and rotated under vacuum for 45 min. After cooling to room temperature, the resulting syrup was dissolved in CH_2Cl_2, filtered, and concentrated. Purification on silica gel (1% MeOH/CH_2Cl_2–3% MeOH/CH_2Cl_2)

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afforded 564.2 mg (63.9%) of a 3:1 mixture of isomers **3a** and **3b** as a foam along with a minor amount (<5%) of **3c**. ^{1}H NMR of the major component **3a** ($CDCl_3$): δ 9.19 (s, H2 or H6), 9.01 (s, H2 or H6), 8.40 (d, J = 2.8 Hz, H8), 8.0–8.2 (m, aromatic), 7.3–7.7 (m, aromatic), 6.75 (dd, J = 2.7 and 12.4 Hz, H1′), 5.80 (dd, J = 2.7 and 16.3 Hz, H3′), 5.38 (dd, J = 2.7 and 49.8 Hz, H2′), 4.82 (d, H5′, H5″), 4.62 (q, H4′). The mixture was carried through the next step.

9-(2′-Deoxy-2′-fluoro-β-D-arabinofuranosyl)-9*H*-purine (4a). A 3:1 mixture of **3a** and **3b** (563 mg, 1.21 mmol) was placed in a pressure bottle and treated with a solution of ammonia-saturated methanol (10 mL). The mixture was kept at 10 °C for 14 h. Argon was bubbled through the solution for 5 min and the methanol was removed. The resulting gum was taken up in water and washed with CH_2Cl_2 (3×). The aqueous layer was freeze-dried and purified first by silica gel flash chromatography (FC) (5% MeOH/CH_2Cl_2 eluant) and subsequently by reverse-phase chromatography (C_{18}, linear gradient of H_2O–10% MeOH/H_2O) affording 285 mg of the diol as a powder (92%) in the same isomer ratio (3:1). ^{1}H NMR (DMSO-d_6) for the major component **4a**: δ 9.22 (s, H2 or H6), 8.98 (s, H2 or H6), 8.75 (d, J = 1.8 Hz, H8), 6.58 (dd, J = 4.9 and 13.0 Hz, H1′), 5.30 (dt, J = 4.4 Hz and 52.6 Hz, H2′), 4.49 (dt, J = 4.4 Hz and 19.0 Hz, H3′), 3.89 (q, J = 4.9 Hz, H4′), 3.68 (m, H5′, H5″). This was also carried through to the next step. Anal. ($C_{10}H_{11}N_4O_3F$·0.2H_2O) C, H, N, F.

9-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl]-9*H*-purine (5). A mixture of **4a** and **4b** (285 mg, 1.12 mmol) was dissolved in dry dimethylformamide (DMF, 4 mL), treated with 2 mL of a premixed solution of TBDMSCl/imidazole in DMF (Alltech) at room temperature, and stirred for 15 min. Water was added and the aqueous mixture was extracted with ethyl acetate (3×). The combined organic layers were washed with brine (3×), dried (Na_2SO_4), and concentrated. Silica gel FC (1:1 EtOAc/petroleum ether eluant) afforded a clean separation of the N-9 and N-7 purine isomers. The desired β-N-9 compound **5** was obtained as an oil in 62%. ^{1}H NMR ($CDCl_3$): δ 9.15 (s, H2 or H6), 8.98 (s, H2 or H6), 8.42 (d, J = 2.4 Hz, H8), 6.63 (dd, J = 4.0 and 16.7 Hz, H1′), 5.16 (ddd, J = 3.0, 3.9, and 51.8 Hz, H2′), 4.73 (dm, J = 18.1 Hz, H3′), 4.06 (q, J = 4.7 Hz, H4′), 3.91 (m, H5′, H5″), 0.91 (s, 9 H, t-Bu), 0.10 (s, 6 H, $Si(CH_3)_2$).

9-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl]-9*H*-purine (7). Method A. Compound **5** (54 mg, 0.147 mmol) and CS_2 (111 mg, 1.47 mmol) were dissolved in dry DMF and cooled to 0 °C. Sodium hydride (80% suspension, 10 mg) was added and the mixture was stirred at the same temperature for 30 min. Methyl iodide (208 mg, 1.47 mmol) was added via syringe and the solution was stirred for an additional 30 min. Water was added and the mixture was extracted with ether (3×). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Following purification on silica gel, 3′-*O*-xanthate **6** was obtained in 64% yield based on recovered starting material. This compound (30 mg, 0.066 mmol) was dissolved in toluene (3 mL); a trace of AIBN was added followed by 0.1 mL of tri-*n*-butyltin hydride. The solution was heated to 90 °C for 10 min and the solvent was evaporated. Silica FC (2% MeOH/CH_2Cl_2) afforded 15 mg (65%) of 3′-deoxy derivative **7** as a powder. Compound **6** ^{1}H NMR ($CDCl_3$): δ 9.16 (s, 1 H), 8.98 (s, 1 H), 8.42 (d, J = 3.1 Hz, H8), 6.64 (dd, J = 3.0 and 21.5 Hz, H1′), 6.25 (dd, J = 2.6 and 16.0 Hz, H3′), 5.28 (dd, J = 3.0 and 49.8 Hz, H2′), 4.31 (q, J = 4.0 Hz, H4′), 3.98 (m, H5′, H5″), 2.62 (s, 3 H), 0.91 (s, 9 H), 0.11 (s, 6 H). Compound **7** ^{1}H NMR ($CDCl_3$): δ 9.15 (s, 1 H), 8.96 (s, 1 H), 8.48 (d, J = 2.5 Hz, H8), 6.42 (dd, J = 3.5 and 16.7 Hz, H1′), 5.32 (dq, $J_{2'F}$ = 53.5 Hz, H2′), 4.30 (m, H4′), 3.83 (d, H5′, H5″), 2.59 (m, H3′), 2.44 (m, H3″), 0.92 (s, 9 H), 0.11 (s, 3 H), 0.103 (s, 3 H). Compound **7** was used as such for the next experiment.

Method B. A mixture of *tert*-butyl nitrite (1.12 g, 0.011 mmol) and dry THF (8 mL) was heated to reflux under an atmosphere of argon. The flask was illuminated with a 200-W unfrosted bulb and compound **16** (200 mg, 0.55 mmol) dissolved in 2 mL of THF was added over 15 min via syringe. After 1 h at reflux TLC analysis showed disappearance of starting material and appearance of a major less polar spot, along with several very minor compounds. The solvent was evaporated and the crude residue was purified by silica flash chromatography. The major spot was isolated and determined by NMR analysis to be identical with compound **7** prepared by method A.

9-(2′,3′-Dideoxy-2′-fluoro-β-D-arabinofuranosyl)-9*H*-purine (1c). Compound **7** (105 mg, 0.298 mmol) was dissolved in 80% acetic acid and heated to 90 °C for 35 min. The acid was removed in vacuo and the resulting syrup was eluted through a short silica column (5% MeOH/CH_2Cl_2). The nucleoside obtained was recrystallized from acetone/ether to afford needles (mp 93–95 °C) of pure **1c**. Yield of the hydrolysis was 88%. ^{1}H NMR (CD_3COCD_3): δ 9.08 (s, 1 H), 8.90 (s, 1 H), 8.66 (d, J = 2.3 Hz, H8), 6.56 (dd, J = 3.8 and 15.9 Hz, H1′), 5.52 (dm, $J_{2'F}$ = 54.4 Hz, H2′), 4.38 (m, H4′), 3.81 (m, H5′, H5″), 2.78 (dddd, H3′), 2.45 (dddd, H3″). ^{13}C NMR (CD_3COCD_3): δ 153.2, 148.9, 145.9, 92.2, 85.2, 79.3, 64.2, 33.5. FAB-MS m/z (relative intensity) 239 (MH^+, 85), 121 (bH_2^+, 100). UV (H_2O): λ_{max} 261, 205 nm. $[\alpha]^{25}_D$: +38.1° (c 1.8, MeOH). Anal. ($C_{10}H_{11}FN_4O_2$) C, H, N, F.

2-Methyl-9-(3′,5′-di-*O*-benzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)adenine (8a). 2-Methyladenine hemisulfate (250 mg, 1.26 mmol) was pertrimethylsilylated under argon at room temperature (acetonitrile, BSTFA) and the solvent was evaporated in vacuo. The resulting yellow oil was placed under an argon atmosphere and compound **2b** (500 mg, 1.18 mmol) dissolved in dry CH_2Cl_2 (8 mL) was added via syringe. The solvent was removed on the rotoevaporator (40 °C), the water bath was replaced with an oil bath (100 °C), and rotation under vacuum was continued at this temperature for 45 min. The brown syrup was dissolved in CH_2Cl_2, filtered, and concentrated. Purification on silica gel (1% MeOH/CH_2Cl_2–3% MeOH/CH_2Cl_2) afforded a mixture of isomers (71%), the desired β-N-9 nucleoside **8a** being 55% of the mixture. A pure sample of **8a** was obtained as a foam by repeated silica gel FC. ^{1}H NMR ($CDCl_3$): δ 7.3–8.2 (m, aromatics and H8), 6.59 (dd, J = 2.8 and 22.9 Hz, H1′), 5.88 (br s, NH_2), 5.76 (dd, J = 2.7 and 17.2 Hz, H3′), 5.33 (dd, J = 2.7 and 50.1 Hz, H2′), 4.79 (d, H5′, H5″), 4.55 (q, J = 4.1 Hz, H4′), 2.59 (s, 2-Me). At this point, it was expedient to carry the mixture through the next step.

2-Methyl-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-adenine (9). The mixture of **8a** and **8b** (490 mg, 1 mmol) was placed in a pressure bottle along with 10 mL of ammonia-saturated methanol. The solution was allowed to stand at 5 °C for 6 h whereupon argon was bubbled through the solution. The reaction was worked up as per compound **4**. Purification (FC, 5%–10% MeOH/CH_2Cl_2 eluant) yielded 288 mg (90%) of a 4:1 mixture of **9a** and **9b**.

A pure sample of **9a** was obtained as an oil by preparative silica gel TLC (15% MeOH/CH_2Cl_2). Compound **9a** ^{1}H NMR (D_2O): δ 8.12 (d, J = 2.1 Hz, H8), 7.22 (br s, NH_2), 6.35 (dd, J = 4.6 and 14.6 Hz, H1′), 5.16 (dt, J = 4.2 and 52.9 Hz, H2′), 4.43 (dt, J = 4.0 and 19.0 Hz, H3′), 3.81 (q, J = 4.6 Hz, H4′), 3.64 (br m, H5′, H5″), 2.38 (s, 2-CH_3). ^{13}C NMR (DMSO): δ 161.6, 155.6, 149.95, 139.0, 116.4, 95.4, 83.4, 81.2, 72.6, 60.4, 25.4. UV (MeOH): λ_{max} 260, 212 nm. $[\alpha]^{25}_D$: +31.1° (c 7, MeOH). The mixture was carried through to the next step.

2-Methyl-9-[5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl]adenine (10). The 4:1 mixture of **9a** and **9b** (63 mg, 0.22 mmol) was dissolved in dry DMF and treated with premixed silylating agent (1 mL) as described for compound **4**. After standard workup and purification on silica gel (1% MeOH/CH_2Cl_2) a clean separation of 5′-protected nucleoside derivatives was effected, the major product being the desired compound **10** (68 mg, 77%), which was obtained as an oil. ^{1}H NMR ($CDCl_3$): δ 8.03 (d, J = 2.8 Hz, H8), 6.56 (dd, J = 3.5 and 19.2 Hz, H1′), 5.73 (br s, NH_2), 5.08 (ddd, J = 2.2, 3.3, and 51.9 Hz, H2′), 4.67 (ddd, J = 2.3, 3.7, and 17.0 Hz, H3′), 4.06 (m, H4′), 3.88 (m, H5′, H5″), 2.57 (s, 2-CH_3), 0.89 (s, 6 H, t-Bu), 0.08 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$). Anal. ($C_{17}H_{28}N_5O_3F$-Si·0.5H_2O) C, H, N (C + 0.5).

2-Methyl-9-[5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-[methoxy(thiocarbonyl)]-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl]adenine (11). Compound **10** (60 mg, 0.15 mmol) was dissolved in dry DMF, the solution was heated to 80 °C, and 1,1′-thiocarbonyldiimidazole (107 mg, 0.604 mmol) was added at once. The mixture was stirred at 80 °C for 1 h and the solvent was removed in vacuo. The brown gum was dissolved in anhydrous methanol (5 mL) and this solution was refluxed for 30 min. After removal of the solvent the mixture was purified by

silica FC (80% EtOAc/petroleum ether eluant). Compound 11 was obtained as a powder in 70% yield (48 mg) for the two steps. ^{1}H NMR ($CDCl_3$): δ 8.03 (d, J = 3.3 Hz, H8), 6.47 (dd, J = 2.9 and 22.3 Hz, H1′), 5.93 (dd, J = 2.7 and 16.0 Hz, H3′), 5.63 (br s, NH_2), 5.19 (dd, J = 3.0 and 49.7 Hz, H2′), 4.23 (m, H4′), 4.10 (s, 3 H, OCH_3), 4.04–3.87 (m, H5′, H5″), 2.57 (s, 3 H, 2-CH_3), 0.91 (s, 9 H, t-Bu), 0.10 (s, 6 H, $Si(CH_3)_2$). The compound was used without further purification in the following step.

2-Methyl-9-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl]adenine (12). Compound 11 (48 mg, 0.102 mmol) was dissolved in dry toluene and AIBN (trace) was added followed by tri-n-butyltin hydride (0.1 mL). The solution was heated to 90 °C for 10 min. The solvent was evaporated and the mixture was purified by silica FC (2% $MeOH/CH_2Cl_2$). Compound 12 was obtained as a white solid in 80% yield (31.1 mg). ^{1}H NMR ($CDCl_3$): δ 8.05 (d, J = 2.9 Hz, H8), 6.28 (dd, J = 3.2 and 19.0 Hz, H1′), 5.50 (br s, NH_2), 5.23 (dm, $J_{2',F}$ = 53.6 Hz, H2′), 4.25 (m, H4′), 3.82 (m, H5′, H5″), 2.57 (s, 2-CH_3), 0.92 (s, 9 H, t-Bu), 0.10 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$). The compound was used directly in the next step.

2-Methyl-9-(2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl)adenine (1d). Compound 12 (31.1 mg, 0.082 mmol) was dissolved in 4 mL of acetic acid. To this solution was added 1 mL of water with stirring and the flask was heated to 90 °C for 1 h. The acid was removed in vacuo and the resulting gum purified first by silica FC (5% $MeOH/CH_2Cl_2$) and then by reverse-phase C_{18} chromatography eluting with a gradient of water–10% $MeOH/H_2O$. The desired nucleoside was recrystallized from acetone to afford needles (mp 237–239 °C) in 87% yield (19 mg). ^{1}H NMR (CD_3COCD_3): δ 8.08 (d, J = 2.3 Hz, H8), 6.48 (br s, NH_2), 6.32 (dd, J = 3.9 and 16.6 Hz, H1′), 5.42 (dm, $J_{2',F}$ = 54.5 Hz, H2′), 4.57 (m, H4′), 4.29 (m, H5′, H5″), 2.64 (m, H3′), 2.42 (m, H3″), 2.41 (s, 2-CH_3). ^{13}C NMR (D_2O): δ 165.2, 157.4, 151.5, 143.0, 118.4, 93.7, 87.3, 80.5, 65.8, 34.7, 26.4. FAB-MS m/z (relative intensity) 268 (MH^+, 100), 150 (bH_2^+, 53); accurate mass m/z 268.1180 (MH^+, calcd 268.1210). UV (H_2O): λ_{max} 260, 208 nm. $[\alpha]^{25}_D$: +46.1° (c 3.1, H_2O). Anal. ($C_{11}H_{14}O_2FN_5$) C, H, N.

8-Methyl-9-(2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl)adenine (1h). A solution of diisopropylamine (260 mg, 2.72 mmol) in dry THF (3 mL) was cooled to −78 °C. n-Butyllithium (1.35 mL of a 1 M solution) was added dropwise via syringe and the solution was stirred at the same temperature for 15 min. Compound 16[16] (200 mg, 0.544 mmol) dissolved in THF (3.5 mL) was next added under a positive pressure of argon and the solution was stirred for an additional 20 min. Methyl iodide (0.9 mL, 1.36 mmol) was added and after 15 min at −78 °C the reaction was quenched with acetic acid. The solution was warmed to room temperature and diluted with ether. The organic layer was washed sequentially with water, saturated $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and evaporated. After purification on silica gel, a mixture of the desired 8-methyl derivative and several minor byproducts was isolated (134 mg). This was treated with 80% acetic acid/water at 90 °C for 40 min and the acid was evaporated. Purification by preparative TLC (silica, 10% CH_3OH/CH_2Cl_2, eluant) followed by semipreparative HPLC (C_{18} silica, 15% acetonitrile/water eluant) afforded pure 1b (30 mg), which recrystallized from acetone as a white solid (mp 208–209 °C) in 22% overall yield. ^{1}H NMR (D_2O): δ 7.86 (s, H2), 6.08 (dd, J = 3.7 and 20.2 Hz, H1′), 5.21 (dm, $J_{2',F}$ = 54.5 Hz, H2′), 4.24 (m, H4′), 3.76 (dd, J = 2.5 and 12.4 Hz, H5′), 3.63 (dd, J = 4.8 and 12.4 Hz, H5″), 2.60 (m, H3′), 2.42 (s, 8-Me), 2.16 (m, H3″). ^{13}C NMR (D_2O): δ 156.6, 153.9, 153.8, 152.1, 119.3, 94.5, 89.2, 80.4, 65.5, 35.0, 17.2. FAB-MS m/z (relative intensity) 268 (MH^+, 100), 150 (bH_2^+, 35). UV (D_2O): λ_{max} 261, 209 nm. $[\alpha]^{25}_D$ = +37° (c 2.0, MeOH). Anal. ($C_{11}H_{14}FN_5O_2$) C, H, N, F.

2-Methyl-*N*^{6}-benzoyl-9-[2′-deoxy-5′-*O*-(*tert*-butyldimethylsilyl)-2′-fluoro-β-D-arabinofuranosyl]adenine (13). Benzoyl chloride (0.5 mL, 1.57 mmol) was added to a solution of compound 10 (58 mg, 0.157 mmol) in dry pyridine (3 mL) previously cooled to 0 °C. After 2 h at this temperature the reaction was quenched with saturated sodium bicarbonate solution and the resulting mixture was extracted with ethyl acetate (3×). The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated to a gum. The crude dibenzoate was dissolved in 4 mL of pyridine/methanol/water (65:30:5), cooled to 0 °C, and treated with 2 mL of 2 N NaOH solution. After 30 min at this temperature, solid NH_4Cl was added followed by water and the aqueous layer was extracted with ethyl acetate as described above. Chromatography on silica gel afforded 58 mg (78%) of monobenzoate 13 as an oil. ^{1}H NMR ($CDCl_3$): δ 8.95 (br s, NH), 8.20 (d, J = 2.7 Hz, H8), 8.04 and 7.55 (m, aromatics), 6.59 (dd, J = 3.6 and 19.0 Hz, H1′), 5.11 (ddd, $J_{2',F}$ = 51.6 Hz, H2′), 4.67 (dm, $J_{3',F}$ = 17.8 Hz, H3′), 4.03 (m, H4′), 3.87 (m, H5′, H5″), 2.75 (s, 2-CH_3), 0.90 (s, 9 H, t-Bu), 0.09 (s, 6 H, $Si(CH_3)_2$). This compound was used directly in the next step.

2,*N*^{6}-Dimethyl-*N*^{6}-benzoyl-9-[5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-xanthyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl]adenine (14). A solution of compound 13 (80 mg, 0.17 mmol) and carbon disulfide (129 mg, 1.7 mmol) in dry DMF (4 mL) was cooled to 0 °C and sodium hydride (11 mg of an 80% slurry in mineral oil) was added at once. The bright red mixture was stirred at 0 °C for 30 min. Methyl iodide (241 mg, 1.7 mmol) was added via syringe and the resulting yellow solution was stirred for an additional 20 min. Water was added and the solution was extracted with ether (3×). The combined ether layers were washed with brine (3×), dried (Na_2SO_4), and concentrated. Silica FC yielded 63.8 mg (65.4%) of the desired N-methyl-3′-xanthate as an oil along with 20% of a byproduct believed to be the N^1-methylated compound. ^{1}H NMR ($CDCl_3$): δ 8.12 (d, J = 3.1 Hz, H8), 7.1–7.5 (m, aromatics), 6.49 (dd, J = 2.9 and 22.0 Hz, H1′), 6.22 (dd, J = 2.7 and 16.5 Hz, H3′), 5.20 (dd, J = 3.1 and 49.8 Hz, H2′), 4.25 (m, H4′), 3.95 (m, H5′, H5″), 3.79 (s, NCH_3), 2.62 (s, SCH_3), 2.47 (s, 2-CH_3), 0.90 (s, t-Bu), 0.09 (s, $SiCH_3)_2$). The compound was used as such for the next step.

2,*N*^{6}-Dimethyl-*N*^{6}-benzoyl-9-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl]adenine (15). A toluene solution (3 mL) of compound 14 (63 mg, 0.109 mmol) containing AIBN (trace) and tri-n-butyltin hydride (95 mg, 0.327 mmol) was heated to 90 °C for 10 min. The solvent was evaporated and the mixture purified on silica gel (2% $MeOH/CH_2Cl_2$), affording 45 mg (86.6%) of the 3′-deoxy product 15 as an oil. ^{1}H NMR ($CDCl_3$): δ 8.15 (d, J = 2.7 Hz, H8), 7.1–7.5 (m, aromatics), 6.28 (dd, J = 3.2 and 18.4 Hz, H1′), 5.22 (dm, $J_{2',F}$ = 53.6 Hz, H2′), 4.23 (m, H4′), 3.79 (d, H5′, H5″), 3.77 (s, NCH_3), 2.3–2.6 (m, H3′, H3″), 2.44 (s, 2-CH_3), 0.89 (s, t-Bu), 0.07 (s, $Si(CH_3)_2$). This compound was used directly in the following final reaction.

2,*N*^{6}-Dimethyl-9-(2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl)adenine (1e). Compound 15 (45 mg, 0.957 mmol) was treated with a saturated solution of ammonia in methanol in a pressure bottle at room temperature. After 4 h argon was bubbled through the solution and the methanol was evaporated. The resulting oil (15a) was dissolved in THF (3 mL) and treated with tetrabutylammonium fluoride (0.1 mL of a 1.0 M solution in THF). After 5 min at 25 °C the solvent was evaporated and the oil was purified by silica preparative TLC (10% MeOH/CH_2Cl_2) and reverse-phase C_{18} chromatography (gradient of water–20% $MeOH/H_2O$). Desired nucleoside 1e was recovered as a glass in 78% (20 mg) yield over the two steps. ^{1}H NMR (CD_3COCD_3): δ 8.05 (d, J = 2.2 Hz, H8); 6.75 (br s, NH), 6.32 (dd, J = 3.9 and 16.5 Hz, H1′), 5.42 (dm, $J_{2',F}$ = 54.6 Hz, H2′), 4.63 (br s, OH), 4.29 (m, H4′), 3.75 (m, H5′, H5″), 3.11 (br s, NCH_3), 2.3–2.8 (2 dddd, H3′, H3″), 2.43 (s, 2-CH_3). ^{13}C NMR (CD_3COCD_3): δ 162.8, 156.1, 155.9, 139.6, 92.3, 85.6, 78.9, 64.2, 33.4, 29.4, 26.0. FAB-MS m/z (relative intensity) 282 (MH^+, 100), 164 (bH_2^+, 64). UV (H_2O): λ_{max} 267, 212 nm. $[\alpha]^{25}_D$: +37.8° (c 6, H_2O). Anal. ($C_{12}H_{16}N_5O_2F\cdot0.8H_2O$) C, H, N (N − 0.7).

6-Amino-9-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxy-2′-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (16). Compound 16 was prepared as previously described.[16]

6-Chloro-9-(2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyl)-9*H*-purine (1i). Compound 16 (216 mg, 0.59 mmol) was suspended in dry CCl_4 (7 mL) and 1.34 mL (11.8 mmol) of freshly distilled *tert*-butyl nitrite was added. The mixture was heated to 80 °C and illuminated with a 200-W unfrosted bulb maintained 1 in. from the reaction flask. After 12 h the reagents and solvent were evaporated in vacuo. The residue was dissolved in 10% CH_3OH/CH_2Cl_2, a small amount of Celite was added, and the solution was filtered through sodium sulfate and evaporated. Chromatography on silica gel (CH_2Cl_2 then 2% CH_3OH/CH_2Cl_2, eluant) afforded 85 mg (37%) of the 5′-protected-6-chloro de-

rivative. This was treated with 80% acetic acid at 85 °C for 30 min. The acid was evaporated and the residue was purified on a C_{18} silica extraction column (Baker, 2.5% CH_3OH/H_2O eluant) yielding 37 mg (62%) of the target nucleoside **1i**. An analytically pure sample was obtained by a second chromatography on an Altex C_{18} HPLC column employing 20% CH_3CN/H_2O. ^1H NMR (CD_3COCD_3): δ 8.78 (d, J = 2.2 Hz, H8), 8.72 (s, H2), 6.55 (dd, J = 3.4 and 15.0 Hz, H1'), 5.59 (dm $J_{2'F}$ = 54.3 Hz, H2'), 4.38 (m, H4'), 3.82 (br AB, H5', 5''), 2.72 (m, H3'), 2.46 (m, H3''). ^{13}C NMR (D_2O): δ 151.9, 151.1, 150.2, 146.4, 91.5, 78.5, 63.3, 32.3. FAB-MS m/z (relative intensity) 273 (MH^+, ^{35}Cl, 100), 239 (MH − Cl + H, 29), 155 (bH_2^+, ^{35}Cl, 70); accurate mass m/z 273.0561 (MH^+, calcd 273.0555). UV (CH_3OH): λ_{max} 260, 252 (inflection), 208 nm. $[\alpha]^{25}_D$: + 55.7° (*c* 1.4, CH_3OH). Anal. ($C_{10}H_{10}N_4O_2ClF$) C, H, N.

N^6-Benzoyl-9-[5'-*O*-(*tert*-butyldimethylsilyl)-2,3'-dideoxy-2'-fluoro-β-D-arabinofuranosyl]adenine (17). Compound **16** (200 mg, 0.545 mmol) was treated under identical conditions as in the preparation of compound **13**. After workup and purification by silica FC (2% $MeOH/CH_2Cl_2$), 206 mg (77.8%) of monobenzoate **17** was obtained as an oil along with ca. 10% of the dibenzoyl intermediate. ^1H NMR ($CDCl_3$): δ 9.11 (br s, NH), 8.79 (s, H2), 8.35 (d, J = 2.6 Hz, H8), 6.40 (dd, J = 3.4 and 17.7 Hz, H1'), 5.31 (dm, $J_{2'F}$ = 53.5 Hz, H2'), 4.32 (m, H4'), 3.85 (d, H5', H5''), 2.57 (m, H3'), 2.43 (m, H3''), 0.92 (s, *t*-Bu), 0.11 (s, $SiCH_3$), 0.10 (s, $SiCH_3$). This compound was carried to the next step.

N^6-Methyl-N^6-benzoyl-9-[5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-dideoxy-2'-fluoro-β-D-arabinofuranosyl]adenine (18). This was prepared in an identical manner to compound **14**. After workup and purification, 100 mg (47%) of desired N^6-methyl derivative **18** was recovered as an oil along with 38 mg (18%) of what was most likely the N^1-methyl derivative. ^1H NMR ($CDCl_3$): δ 8.51 (s, H2), 8.32 (d, J = 2.6 Hz, H8), 7.1–7.5 (m, aromatics), 6.31 (dd, J = 3.4 and 17.4 Hz, H1'), 5.25 (dm, $J_{2'F}$ = 53.7 Hz, H2'), 4.26 (m, H4'), 3.80 (s, NCH_3), 3.79 (m, H5', H5''), 2.53 (m, H3'), 2.39 (m, H3''), 0.90 (s, 9 H, *t*-Bu), 0.10 (s, 6 H, $Si(CH_3)_2$). This compound was used directly in the next step.

N^6-Methyl-9-(2',3'-dideoxy-2'-fluoro-β-D-arabinofuranosyl)adenine (1f). Compound **18** (100 mg, 0.206 mmol) was debenzoylated as per the other benzoates (ammonia-saturated methanol, pressure bottle, 0 °C for 4 h then room temperature for 2 h) to afford after typical workup and FC on silica N^6-methyl-5'-*O*-protected derivative **19** in >90% yield. This was dissolved in 80% glacial acetic acid and heated to 90 °C for 20 min. After evaporation of the acid and preparative TLC on silica (10% $MeOH/CH_2Cl_2$) 45 mg (82% for two steps) of desired nucleoside **1f** was obtained as a glass. Compound **19** ^1H NMR ($CDCl_3$): δ 8.38 (s, H2), 8.07 (d J = 2.7 Hz, H8), 6.29 (dd, J = 3.2 and 18.4 Hz, H1'), 6.01 (br s, NH), 5.24 (dm, $J_{2'F}$ = 53.7 Hz, H2'), 4.25 (m, H4'), 3.81 (d, H5', H5''), 3.19 (br d, J = 4.6 Hz, N-CH_3), 2.53 (m, H3'), 2.36 (m, H3''), 0.91 (s, *t*-Bu); 0.09 (s, $SiCH_3$), 0.08 (s, Si-CH_3). Compound **1f** ^1H NMR (CD_3COCD_3): δ 8.25 (br s, H2), 8.18 (d J = 2.3 Hz, H8), 7.01 (br s, NH), 6.38 (dd, J = 3.8 and 16.5 Hz, H1'), 5.42 (dm, $J_{2'F}$ = 54.4 Hz, H2'), 4.59 (br s, OH), 4.31 (m, H4'), 3.78 (br m, H5', H5''), 3.11 (br s, NCH_3), 2.30–2.83 (2 dddd, H3', H3''). ^{13}C NMR (D_2O): δ 156.8, 154.5, 148.9, 142.4, 120.0, 93.5, 87.1, 80.5, 65.9, 34.8, 29.7. FAB-MS m/z 268 (MH^+, 100), 150 (bH_2^+, 30). UV (H_2O): λ_{max} 265, 211 nm. $[\alpha]^{25}_D$: +56.8° (*c* 1.9, MeOH). Anal. ($C_{11}H_{14}O_2FN_5 \cdot 0.7H_2O$) C, H, N.

N^6-Benzoyl-9-(2',3'-dideoxy-2'-fluoro-β-D-arabinofuranosyl)adenine (1g). Compound **17** (49 mg, 0.104 mmol) was hydrolyzed with 80% acetic acid at 90 °C for 30 min. After evaporation of the acid and recrystallization from ether/CH_2Cl_2, desired nucleoside **1g** was obtained in 68% yield (23.4 mg). Mp: 187–189 °C. ^1H NMR (CD_3COCD_3): δ 9.97 (br s, NH), 8.65 (s, H2), 8.51 (d, J = 2.3 Hz, H8), 6.52 (dd, J = 3.8 and 16.1 Hz, H1'), 5.52 (dm, $J_{2'F}$ = 54.4 Hz, H2'), 4.36 (m, H4'), 4.30 (t, J = 6.0 Hz, OH), 3.80 (m, H5', H5''), 2.3–2.9 (2 dddd, H3', H3''). ^{13}C NMR (CD_3COCD_3): δ 168.9, 152.3, 151.9, 149.5, 144.5, 133.7, 133.0, 129.2, 128.4, 123.5, 91.8, 85.3, 78.7, 63.6, 32.6. FAB-MS m/z (relative intensity) 358 (MH^+, 100), 240 (bH_2^+, 61), 105 (93). UV (MeOH): λ_{max} 278, 231 (sh), 205 nm. $[\alpha]^{25}_D$: + 31.7° (*c* 1.7, MeOH). Anal. ($C_{17}H_{15}O_3FN_5 \cdot 0.2H_2O$) C, H, N, F.

9-(2',3'-Dideoxy-2'-fluoro-β-D-arabinofuranosyl)adenine 1-Oxide (21). Compound **1a** (225 mg, 0.89 mmol) was dissolved in 8 mL of acetic acid and 1.5 mL of 30% solution of hydrogen peroxide was added. The mixture was stirred at room temperature for 5 days, the flask was cooled to 0 °C and 10% palladium on carbon was added. After stirring for 30 min, the mixture was filtered through Celite and concentrated. The crude syrup was purified by flash chromatography on C_{18} silica, eluting with a gradient of water–5% $MeOH/H_2O$, affording 132 mg of product. This was recrystallized from 95% EtOH to afford 101 mg (42%) as needles. Mp: >230 °C dec. ^1H NMR (DMSO-d_6): δ 8.56 (s, H2), 8.45 (d, J = 2.0 Hz, H8), 6.30 (dd, J = 3.9 and 15.1 Hz, H1'), 5.43 (dm, $J_{2'F}$ = 54.6 Hz, H2'), 5.04 (t, J = 5.8 Hz, 5'-OH), 4.17 (m, H4'), 3.61 (br s, H5', H5''), 2.54 (m, H3'), 2.24 (m, H3''). ^{13}C NMR (DMSO-d_6): δ 148.3, 143.4, 142.5, 141.4, 117.9, 91.5, 83.5, 78.0, 62.7, 32.2. FAB-MS m/z (relative intensity) 270 (MH^+, 48), 254 ($MH\text{-}O^+$, 100), 152 (bH_2^+, 16), 136 ($bH_2\text{-}O^+$, 62). UV (H_2O): λ_{max} 232, 263 nm. $[\alpha]^{25}_D$: +12.2° (*c* 1.0, MeOH). Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N, F.

Registry No. **1a**, 110143-10-7; **1b**, 117525-25-4; **1c**, 132722-90-8; **1d**, 132722-91-9; **1e**, 132722-92-0; **1f**, 126502-17-8; **1g**, 132722-93-1; **1h**, 132722-94-2; **1h** 5'-TBDMS derivative, 132723-10-5; **1i**, 132722-95-3; **1i** 5'-TBDMS derivative, 132723-09-2; **2a**, 97614-43-2; **2b**, 97614-44-3; **3a**, 132723-01-4; **3b**, 132723-15-0; **3c**, 132723-14-9; **4a**, 109304-16-7; **4b**, 132723-16-1; **5**, 132723-02-5; **6**, 132723-03-6; **7**, 132749-36-1; **8a**, 132723-04-7; **8b**, 132723-11-6; **9a**, 132723-05-8; **9b**, 132723-12-7; **10**, 132723-06-9; **11**, 132723-07-0; **12**, 132749-37-2; **13**, 132749-38-3; **14**, 132723-08-1; **14** N^1-methyl analogue, 132723-13-8; **15**, 132722-96-4; **16**, 125542-18-9; **17**, 132722-97-5; **18**, 132722-98-6; **19**, 126502-16-7; **20**, 132722-99-7; **21**, 132723-00-3; dCF, 53910-25-1; adenosine deaminase, 9026-93-1; purine, 120-73-0; 2-methyladenine hemisulfate, 74873-18-0.