Lipophilic, Acid-Stable, Adenosine Deaminase-Activated Anti-HIV Prodrugs for Central Nervous System Delivery. 2. 6-Halo and 6-Alkoxy Prodrugs of 2′-β-Fluoro-2′,3′-dideoxyinosine

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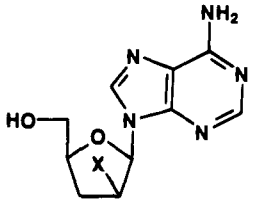
A series of 6-halo-(F-, Cl-, Br-, I-) and 6-alkoxy-(OMe-, OEt-) 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl) purines (F-ddN) have been synthesized and characterized with the objective of finding compounds which might be superior to existing drugs for the treatment of HIV in the central nervous system. These compounds, which contain lipophilic 6-substituents, were chosen as acid-stable prodrugs for the anti-HIV-active F-ddN, 9-(2,3−dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl) hypoxanthine (F-ddI), because of their potential to increase blood−brain-barrier penetration relative to F-ddI. All the new compounds were more lipophilic than the currently approved anti-AIDS drugs. Partition coefficient increases of 30- and 110-fold were achieved, relative to didanosine (ddI), for the 6-chloro- and 6-ethoxy analogues. 2′-Fluoro substitution abolished the pH 1, acid-catalyzed cleavage of the nucleoside glycosylic bond. However, pH 1, acid-catalyzed hydrolysis of the 6-fluoro substituent to produce F-ddI was observed to occur at a rate ($t_{1/2}$ 0.54 h) which was ca. 40−170 times faster than that of the other prodrugs. The utility of the F-ddNs as prodrugs for F-ddI depends upon their ability to act as substrates for adenosine deaminase. The relative rates of adenosine deaminase-catalyzed prodrug hydrolysis to F-ddI varied by a factor of >25 000 with the 6-fluoro- and 6-ethoxy analogues reacting the fastest and slowest, respectively. All of the prodrugs possessed anti-HIV activity in the phytohemagglutinin-stimulated peripheral blood mononuclear cell test system and a qualitative correlation exists between prodrug anti-HIV activity and adenosine deaminase hydrolysis rates.

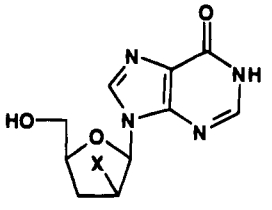
Introduction

The complications resulting from HIV infection of the central nervous system (CNS)[1] are major problems of AIDS, especially in advanced disease.[2−5] The observed neurological dysfunction, which includes a progressive, AIDS-related dementia, appears to be directly related to the presence of virus in the CNS, since HIV has many characteristics of the neurotropic lentiviruses.[6] In addition to the above symptomatic problems, the CNS can be a sanctuary for the virus since the brain's natural defense mechanism, the blood−brain barrier (BBB), effectively excludes some drugs which might provide an effective treatment. Of the currently approved anti-AIDS drugs, zidovudine (AZT) appears to have the best BBB or choroid plexus penetration properties achieving CSF/plasma ratios of 20−60%.[7,8] However, since the other clinically approved anti-HIV drugs have significantly lower ratios,[8] and all the approved drugs produce significant toxicities as well as resistant viruses, there is a continuing need for more effective drugs for the treatment of HIV in the CNS.

While there is evidence that there may be a component of facilitated transport for some of the dideoxynucleosides (ddNs) in BBB penetration,[9,10] passive diffusion is a general, lipophilicity-related mechanism[11] which has been shown to be the way ddA (**1a**) and F-ddA (**1b**) penetrate murine leukemic, human erythrocytic, and MOLT-4 cells.[12] The objective of this study was, therefore, the preparation of anti-HIV-active molecules with increased BBB penetration. We chose to investigate lipophilic, 2′-F-dideoxypurine nucleoside prodrugs of F-ddI (**2b**), taking advantage of (a) the well-known ability of adenosine deaminase (ADA) to hydrolyze the 6-substituent of many 6-substituted purine nucleosides[13] and (b) the unusual chemical and enzymatic stability that the 2′-fluorine atom confers on these compounds both before and after enzymatic conversion to F-ddI (**2b**, Scheme 1), a compound whose *in vitro* activity is similar to that of ddI (**2a**).[14,15]



1a, X = H (ddA)
1b, X = F (FddA)



2a, X = H (ddI)
2b, X = F (FddI)

Hansch and others have shown that CNS penetration and the resulting biological activity often are related to the lipophilic properties of a molecule, expressed as its octanol−aqueous partition coefficient (*P*).[11,16] However, facilitating CNS entry, while necessary, is not sufficient of itself to produce a useful drug, since once the target site is reached, the molecule must have activity against HIV or be converted to an active material.[10,15,17,18] Since ddA (**2a**) and F-ddA (**2b**) as well as numerous other

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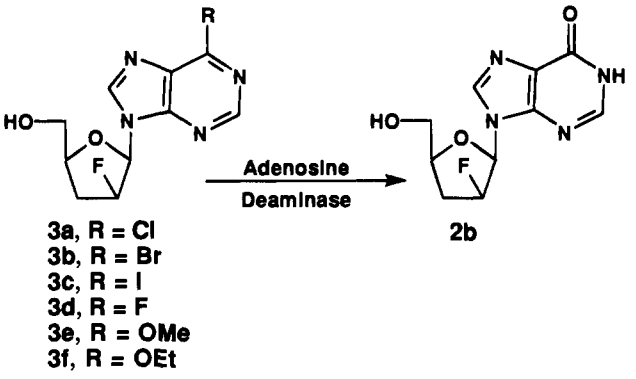
Scheme 1



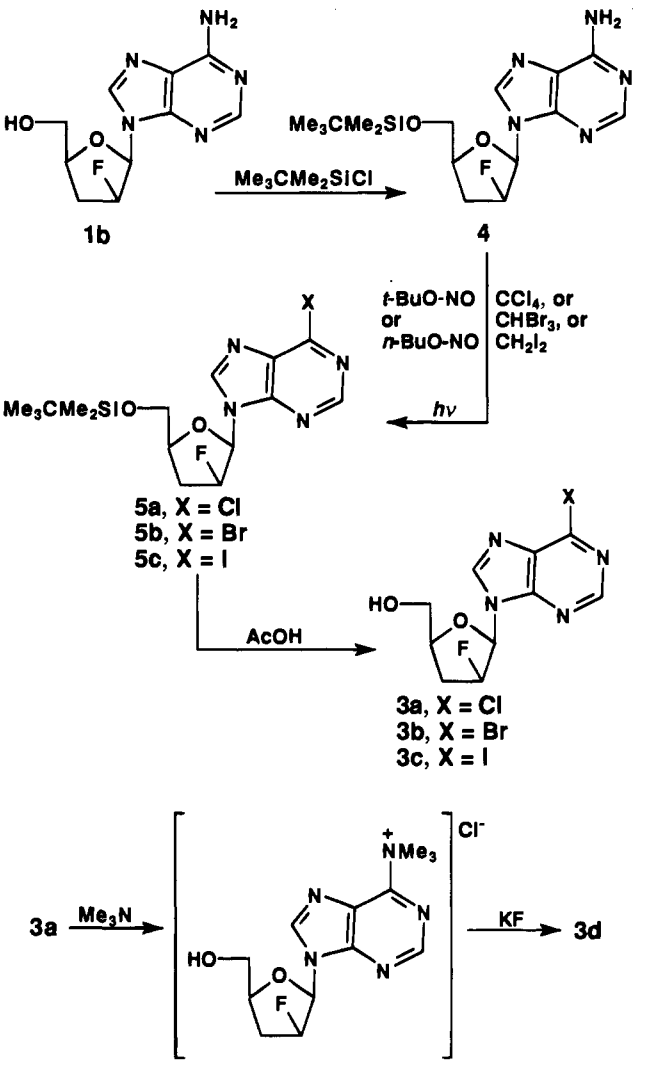
6-substituted purine nucleosides are converted to their inosine counterparts by the enzyme adenosine deaminase (ADA),[13,14b] our approach to optimizing BBB penetration began with the design and synthesis of a series of 6-substituted 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)purine analogues with log *P* values >0.0. This approach requires that a significant fraction of the lipophilic prodrug be transported intact into the CNS where it undergoes ADA-catalyzed conversion to the more hydrophilic, anti-HIV-active inosine analogue, F-ddI (Scheme 1).[15] Moreover, prodrug activation in the CNS appears feasible since not only is ADA present in the CNS,[18] but its level has been shown to increase as a consequence of certain infectious cerebral diseases,[19] including AIDS.[20] The challenge in this prodrug approach is the discovery of a molecule with the optimum combination of several generally unrelated, but structure-dependent properties: anti-HIV activity, lipophilicity, ADA hydrolysis rate, as well as systemic metabolism and elimination. The present study addresses some of the initial *in vitro* aspects of this challenge.

Chemistry

Synthesis. 2′,3′-Dideoxypurine nucleosides (e.g., **1a**, **2a**) are known to be very unstable under acidic conditions resulting in cleavage of the glycosylic bond.[14,21] Our earlier work has shown that the incorporation of a fluorine atom to the sugar 2′-position of a purine ddN (**1b**, **2b**) stabilizes these compounds toward acid decomposition as well as enzymatic catabolism.[14,15] Acid stability is an important factor in achieving a convenient oral drug formulation for this type of anti-HIV drug. While the increase in acid stability is the same whether the stereochemistry of the 2′-fluorine atom is "up" (*threo*) or "down" (*erythro*), the same can not be said for the anti-HIV activity since only the *threo*-2′-F-ddNs have significant activity.[14,22] For this reason, all the target compounds in this study (**3a**–**f**) contain 2′-fluoro substitution in the "up" configuration.

Two synthetic routes were employed to prepare compounds **3a**–**f**. Scheme 2 shows the route to the 6-halo-2′-fluoro analogues. Starting with F-ddA (**1b**),[14] silylation gave the 5′-*tert*-butyldimethylsilyl derivative, **4**. The general procedure of Nair,[23a] i.e., diazotization followed by photoinduced radical halogenation, provided the desired 6-halo-ddNs, which were deprotected under acidic conditions to produce the target molecules, **5a**–**c**. The radical halogenation procedure was not appropriate, however, for the synthesis of the 6-fluoro compound, **3d**. This material was synthesized from the

Scheme 2

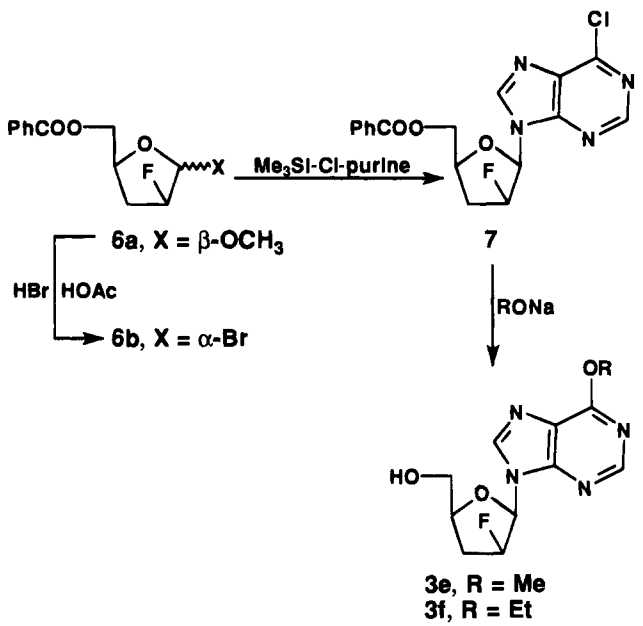


6-chloro compound (**3a**) using the general procedure of Robins[23b] by way of the unisolated 6-trimethylammonio compound, followed by a displacement reaction with fluoride ion (Scheme 2).

The 6-alkoxy analogues (**3e**, **3f**) were prepared from the fluoro sugar, **6a**, as shown in Scheme 3. We have previously described **6a** as a versatile intermediate for the synthesis of 2′-F-ddNs.[24] HBr-activation of **6a** to produce **6b**, and a subsequent reaction with silylated 6-chloropurine gave the 5′-benzoyl-protected 6-chloro intermediate, **7**. Reaction of **7** with the appropriate sodium alcoholate produced the target 6-methoxy (**3e**) and ethoxy (**3f**) analogues after removal of the 5′-blocking group. Compound **7** was very susceptible to nucleophilic displacement reactions. Attempted preparation of **3a** by deprotection of **7** with methanolic ammonia was unsuccessful, producing a mixture of the 6-methoxy compound, **3e** and F-ddA (**1b**).

Lipophilicity. Using a convenient, microscale method developed in this laboratory,[25] octanol/pH 7.0 partition coefficients (*P*) were determined for the target nucleosides (Table 1). An ca. 6-fold range was observed between the *P* values of the most hydrophilic (6-F, **2a**) and most hydrophobic (6-OEt, **3f**) of the new compounds. All of the compounds synthesized proved to be equal to or more lipophilic than AZT. The 6-chloro (**3a**) and

Scheme 3



6-ethoxy (**3f**) prodrugs were 36 and 111 times more lipophilic, respectively, than ddI[25] (Table 1).

Acid Stability. Because stability in stomach acid plays such a critical role in the development of a convenient oral dosage form for any purine dideoxynucleoside,[14,21] this property was evaluated for **3a–f** in pH 1.0 buffer solution at 37 °C. F-ddA (**1b**) and F-ddI (**2b**) previously were shown to be stable under these conditions, while ddA (**1a**) and ddI (**2a**) decomposed rapidly ($t_{1/2}$ ca. 30 s) by cleavage of the glycosilic bond.[14] Figure 1 shows that most of the new F-ddNs are quite stable ($t_{1/2}$ > 20 h) under these conditions, slowly producing F-ddI. F-ddI (**2b**), the anti-HIV-active product which is slowly produced from the 6-halo compounds (**3a–c**) through acid-catalyzed decomposition, results from the hydrolysis of the group in the 6-position. This is in contrast to the typical acid-catalyzed glycosilic cleavage of the nonfluorinated dideoxynucleosides,[21] which results in cleavage at the 9-position, producing only inactive products (aglycon and dideoxy sugar). The single 6-halo exception to slow hydrolysis is the 6-fluoro analogue (**3d**) with a $t_{1/2}$ of 0.54 h at pH 1.0. At pH 7.4, however, this reaction is 1100 times slower (data not shown). At pH 1.0, the 6-alkoxy analogues (**3e,f**) produced only very minor amounts of acidic decomposition products which were not identified.

Biology

Enzymatic Hydrolysis by Adenosine Deaminase. Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4, ADA) is a ubiquitous catabolic enzyme present

Table 1. Octanol–Water Partition Coefficients and Chromatographic Properties of Some Purine 2′-β-Fluoro-2′,3′-dideoxynucleosides

compd no.	6-R	measured[a] log *P*	HPLC mobile phase[b]	isocratic[c] *k*′	λ_{max}[d] (nm)
2b	OH (F-ddI)	−1.210 ± 0.017	A	0.5	247
3a	Cl	0.315 ± 0.016	C	3.7	264
3b	Br	0.443 ± 0.009	C	4.4	265
3c	I	0.627 ± 0.007	C	7.2	275
3d	F	0.056 ± 0.009	B	3.2	245
3e	OCH_3	0.245 ± 0.009	B	3.1	249
3f	OC_2H_5	0.803 ± 0.008	D	9.6	249
AZT		0.052 ± 0.009	C	ND[e]	266
2a	OH (ddI)	−1.242 ± 0.028	A	ND	249

[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 to determine log *P*: (A) 10%, (B) 15%, (C) 20%, or (D) 24% CH_3CN in pH 7.0, 0.01M phosphate buffer. [c] Average capacity factor ($k' = (t_r - t_0)/t_0$) for isocratic elution with 15% CH_3CN in pH 7.0, 0.010 M phosphate buffer. [d] Wavelengths determined on-the-fly in HPLC mobile phase. [e] Not determined.

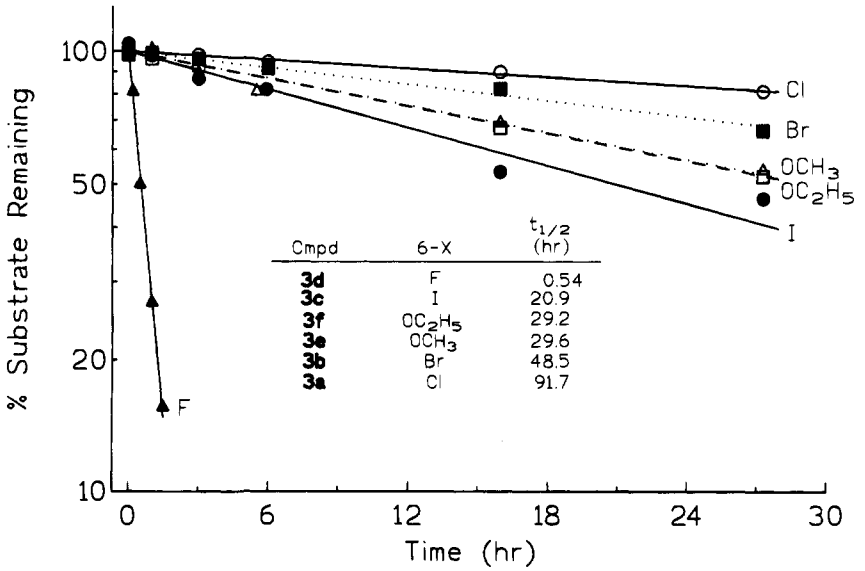


Figure 1. Acid hydrolysis kinetics of 6-substituted 2′-fluoro-2′,3′-dideoxynucleosides at pH 1 and 37 °C producing F-ddI (**2b**). The initial substrate concentration was 50 μM for all compounds. See the Experimental Section for conditions and kinetic calculations. Symbols: **3a** (○), **3b** (■), **3c** (●), **3d** (▲), **3e** (□), **3f** (△). Compound **2b** (F-ddI) is stable under these conditions.[14]

Table 2. Relative Adenosine Deaminase Hydrolysis Rates[a]

compd no.	6-R	disappearance of substrate	appearance of F-ddI
1b	NH_2 (F-ddA)	100	100
3d	F	202	233
3a	Cl	2.0	1.6
3b	Br	1.7	1.5
3c	I	0.28	0.21
3e	OCH_3	0.63	0.64
3f	OC_2H_5	0.008	–[b]
1a	NH_2 (ddA)	2080	2025

[a] Standard Conditions: 50 μM substrate; pH 7.4, 0.01 M phosphate buffer; 37 °C. Disappearance rate constant (per unit ADA) normalized to F-ddA. [b] Conversion to F-ddI detectable, but not sufficient to calculate rate of appearance.

Table 3. Anti-HIV Activity in the PHA/PBL System

		% protection[a] at various concentrations (μM)					
compd no.	6-R	5	10	20	40	80	IC_{50} (μM)
3a	Cl	40	70	92	97	98	5.9
3b	Br	31	71	90	97	99	6.5
3c	I	27	45	81	96	96	10.4
3d	F	58	83	97	99	99	< 5
3e	OCH_3	18	35	49	75	92	20.0
3f	OC_2H_5	32	39	58	79	93	14.1
2b	OH (F-ddI)	54	61	89	97	98	< 5
2a	OH (ddI)	97	96	98	98	98	< 5

[a] Activity (% protection) expressed as the percent reduction in HIV p24 Gag protein expression relative to HIV-infected, untreated controls.

in many animal and human tissues.[26] In addition to converting adenosine to inosine, this enzyme catalyzes the hydrolysis of numerous 6-substituted purine nucleosides to inosine and guanosine analogues.[13,23b] It is possible that any 6-substituted FddN which interacts with ADA, no matter how slowly, is a potential prodrug form of F-ddI. This is because the rate of human whole-body ADA hydrolysis is unknown, and therefore it is not possible to predict the hydrolysis rate for a 6-substituted analogue in humans. A compound found to be slowly hydrolyzed *in vitro* (e.g., in CD4+ T-cells or by the isolated enzyme) might be ideal in a clinical situation where vast amounts of ADA are present. We are addressing a preclinical/clinical-ADA correlation in a separate study.

In studies using commercially available, purified enzyme, the relative rates for ADA-catalyzed prodrug hydrolysis (Scheme 1) varied by more than a factor of 25 000 (Table 2). In the ADA study, analogous to the findings in the acid-catalyzed hydrolysis experiments (see above), the 6-fluoro compound (**3d**) was by far the most reactive compound, producing F-ddI almost 170 times faster than the 6-chloro analogue (**3a**), and at a rate double that of the 6-amino compound, F-ddA (**1b**).

Anti-HIV Activity. The HIV-infected, phytohemagglutinin-stimulated peripheral blood mononuclear cell (PHA-PBM) test system employed in this study uses a decrease in HIV-1 p24 Gag protein production relative to an untreated control as the measure of HIV-exposed cell protection and compound activity.[27,28] Compounds **3a–f** produced varying degrees of activity as shown in Table 3. These data are representative and are from an experiment where all compounds shown were tested in direct comparison. No significant toxicity to uninfected PBM cells was noted at the drug concentrations used. Good dose responses were observed for activities (% protection), resulting in anti-HIV potencies (IC_{50} values) in the 5–20 μM range. A correlation is observed between % protection at 5 μM concentration (Table 3) and the relative rates for ADA hydrolysis (Table 2) among the 6-halo compounds (**3a–d**). The alkoxy analogues give similar results, but the ethoxy compound (**3f**) is somewhat more active than would have been predicted from the ADA data.

Earlier work with the 6-methylamino analogue as well as preliminary work with **3a** in the ATH8 cell line indicated that 2′-deoxycoformycin (dCF) had the ability to abolish anti-HIV activity, through its potent ADA inhibitory effect which prevented the inactive prodrugs from being metabolized to F-ddI.[15] This dCF effect was also noted in a series of dideoxynucleosides incorporating unfluorinated sugars.[28] In an attempt to further explore this effect, similar experiments were performed using a variety of dCF concentrations with PHA-PBM cells. This study, however, was unsuccessful because dCF alone exerted greater toxicity effects on PHA-PBM cells than on ATH8 cells, making combination dCF/drug data uninterpretable.

Discussion

In addition to stabilizing purine dideoxynucleosides under acidic conditions, 2′-F substitution also alters the rates of several important enzymatic reactions. The ADA-catalyzed deamination of F-ddA (**1b**) is 10–20 times slower than that of ddA (**1a**).[12c,14b,15] While ddI (**2a**), the deamination product of ddA, is readily catabolized by the enzyme purine nucleoside phosphorylase (PNP) to hypoxanthine and dideoxyribose, the corresponding fluoroinosine analogue, F-ddI (**2b**), is not a substrate for PNP.[12c] These properties make F-ddNs attractive as clinical candidates, and F-ddA has been selected for clinical trial by the National Cancer Institute. Anti-HIV studies with ADA-dependent prodrugs pose certain testing problems. An intrinsically inactive F-ddI prodrug, subjected to the very small amounts of ADA present in some cell culture test systems,[12c,15] may not be converted to an active inosine (e.g., F-ddI) or guanosine[14c] analogue rapidly enough to demonstrate activity (note the F-ddN rate differences in Table 2). This could lead to discarding a potentially useful prodrug for humans where the ADA levels are high.[29] On the other hand, a compound with a very rapid *in vitro* deamination rate, while appearing to be very active, would be of little value as a prodrug in humans since it would be converted immediately (like ddA)[29] to a hydrophilic inosine analogue before prodrug transport benefits might be used to advantage for CNS penetration. *In vivo* studies are currently planned to assess how all these factors affect the CNS penetration properties of **1b**, **2a**, **3a**, and **3e** in the rat.

Since *in vitro* drug test systems are only relevant in so far as they relate to the clinical situation, only when we can correlate the effect of human ADA levels with isolated enzyme test data will it be possible to determine with certainty whether an ADA-activated prodrug might have clinical utility. Since F-ddA (**1b**) can be considered as a F-ddI prodrug, we hope to use the human deamination rates obtained from an anticipated clinical pharmacokinetic study of F-ddA to devise an *in vitro* method for estimating the whole-body ADA effects on ADA-activated prodrugs.

Experimental Section

Silica gel column chromatography was performed on silica gel 60 (E. Merck, 230–400 mesh) and analytical TLC was performed on Analtech Uniplates silica gel GF with the solvents indicated. Detection of compounds by TLC was accomplished either by UV light or by 10% methanol–sulfuric acid spray, followed by heating on a hot plate. Proton NMR spectra were recorded in the solvents indicated at 250 MHz on a Bruker AC-250 instrument. Chemical shifts are expressed as δ values with reference to Me_4Si. 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 1500. Glycerol or 3-nitrobenzyl alcohol[30] were used as sample matrices, 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 8.4–8.9 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 matrix background was automatically subtracted. Accurate mass measurement of the protonated molecular ion (MH^+) peak was carried out in a separate experiment at a dynamic resolution of 2500 using a voltage scan over a limited mass range and signal acquisition in a multichannel analysis mode. Software-based peak matching was then employed using selected glycerol peaks within the mass range as internal references.

Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

HPLC Analysis of Dideoxynucleosides. Solutions and diluted enzymatic reaction mixtures of dideoxynucleosides were analyzed on a liquid chromatography system consisting of a Spectra-Physics Analytical P2000 binary gradient pump, an AS3000 automatic sample injector also from Spectra-Physics, and a Waters Model 994 programmable photodiode array UV detector. Dideoxynucleosides were separated on a 4.6 × 250 mm 5-μm Altex/Beckman Ultrasphere C18 column that was protected by a 4.6 × 30 mm Brownlee Spheri-5 RP-18 precolumn. This column system was eluted isocratically at 1.0 mL/min with a mobile phase of 15% CH_3CN in pH 7.0, 0.01 M phosphate buffer so that all dideoxynucleosides had retention times in the range of 9–22 min. Enzymatic and hydrolysis reaction products were identified by coincidence of retention time with standards and by comparison of full-scan (210–360 nm) on-the-fly UV spectra obtained with the diode array detector. Peak areas were measured by a Spectra-Physics 4400 Chromjet integrator which was interfaced to a Thermo Separations Products WOW multichannel chromatography data system running on a Zeos 486/DX2 66MHz personal computer. For kinetic studies, these data were plotted as a function of time and curve-fit to either a first order exponential decay ($y = Ae^{-kt}$) or exponential association ($y = A(1 - e^{-kt})$) using GraphPad (Version 4.0, ISI Software, Philadelphia, PA), a PC-based curve-fitting program.

Measurement of Octanol–Water Partition Coefficients. 1-Octanol/pH 7.0 buffer partition coefficients (P) were determined for individual compounds using a microscale shake-flask method employing HPLC analysis of both the buffer and 1-octanol phases.[25] This determination was conducted in triplicate.

Acid Stability of Sugar-Fluorinated Dideoxynucleosides. Fifty microliters of a 1.0 mM aqueous solution of a dideoxynucleoside was added to 0.95 mL of pH 1.0 HCl/KCl buffer in a 1.5-mL polypropylene microcentrifuge tube (Eppendorf, Hamburg, Germany) that had been prewarmed to 37 °C. A 50-μL aliquot was immediately removed for analysis and the tube incubated at 37 °C in an Eppendorf Model 5320 thermostated heater. This initial sample aliquot was diluted with 0.45 mL of pH 7.4, 0.1 M phosphate buffer. Subsequent 50-μL samples were taken at predetermined times and treated in the same manner as the first. The concentration of dideoxynucleoside in each sample was determined by HPLC analysis of a 50-μL aliquot as described above.

Enzymatic Hydrolysis by Adenosine Deaminase. Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4) from calf intestinal mucosa was prepared as previously described[15] to give a stock solution of 1 mg/mL (250–280 units/mL) in pH 7.4, 0.01 M phosphate buffer. The adenosine deaminase inhibitor, 2′-deoxycoformycin (dCF) was obtained from the Pharmaceutical Resources Branch, NCI, and used as a 1.7 mM solution in distilled water. Relative rates of hydrolysis of selected nucleosides by ADA and characterization of products were carried out as previously described.[15] Briefly, 0.05–1.0 unit of ADA was added to 1.0 mL of a 50 μM solution of a given compound in pH 7.4, 0.01 M phosphate buffer and then incubated at 37 °C. Fifty-microliter aliquots were withdrawn at timed intervals and hydrolysis was quenched by mixing with 0.450 mL distilled water and 2 μL of 1.7 mM dCF. The diluted sample was heated at 95 °C for 1 min to insure enzyme deactivation, and the decrease in substrate concentration and the formation of product was followed over time by HPLC analysis. One unit is defined as the amount of ADA that hydrolyzes 1.0 μmol of adenosine per min at 25 °C.

Determination of Anti-HIV Activity. We have described the determination of anti-HIV activity using the PHA-PBM system previously.[27,28] Briefly, PBMs, which had been prepared from a HIV sero-negative donor and stimulated with PHA 3 days prior to drug addition, were incubated with various concentrations of the test compounds for 2 h and then exposed to a 250 tissue culture infectious dose ($TCID_{50}$) of HIV-$1_{ERS104pre}$. These cells were cultured in complete medium [15% fetal calf serum (FCS)] supplemented with rIL-2 at 37 °C. On culture day 8, the supernatant was harvested and p24 Gag protein was determined by RIA. These experiments were performed in quadruplicate.

6-Amino-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (4). A suspension of **1b** (2.00 g, 7.89 mmol) in dry DMF (30 mL) was treated with a premixed DMF solution (20 mL) of *tert*-butyldimethylsilyl chloride (1 mM) and imidazole (2.5 mM). The resulting solution was stirred at room temperature under nitrogen for 40 min and then slowly quenched with water (100 mL). The mixture was extracted with EtOAc (3 × 100 mL), and the combined organic extract was washed with brine (2 × 100 mL), dried (Na_2SO_4), and finally reduced to dryness under vacuum. The crude product was purified by flash column chromatography using silica gel and CH_2Cl_2:MeOH (10:0 to 9:1) to give **4** as an amorphous solid which was used directly in the following steps without further purification: ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $Si(CH_3)_2$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 2.40 (m, 1 H, H-3′$_a$), 2.55 (m, 1 H, H-3′$_b$), 3.85 (d, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.25 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 5.55 (br s, 2 H, NH_2), 6.30 (dd, $J_{1',F}$ = 18, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1′), 8.15 (d, $J_{8,F}$ = 2.6 Hz, 1 H, H-8), 8.35 (s, 1 H, H-2).

6-Chloro-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (5a). This method is an adaptation of the method of Nair.[23a] A suspension of **4** (1.90 g, 5.17 mmol) in freshly distilled *tert*-butyl nitrite (24 mL, 201.7 mmol) was heated to 80 °C and treated with freshly distilled CCl_4 (250 mL) while being maintained under a nitrogen atmosphere. Stirring and heating (80 °C) continued under direct incandescent illumination (200 W bulb) for 3 h. The reaction mixture was then cooled, concentrated under reduced pressure, and purified by flash column chromatography using silica gel and hexane:EtOAc (10:0 to 7:3) to give 0.790 g (39.5%) of **5a** which was used in the next step without additional purification: ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $Si(CH_3)_2$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 2.45 (m, 1 H, H-3′$_a$), 2.60 (m, 1 H, H-3′$_b$), 3.85 (m, 2 H, H-5′$_{a,b}$), 4.30 (m, 1 H, H-4′), 5.35 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.40 (dd, $J_{1',F}$ = 18, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1′), 8.50 (d, $J_{8,F}$ = 2.4 Hz, 1 H, H-8), 8.72 (s, 1 H, H-2).

6-Bromo-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (5b). Starting with 0.50 g (1.36 mmol) of **4** in the presence of freshly distilled *n*-butyl nitrite (8 mL, 68.2 mmol) and bromoform (20 mL), the above procedure was repeated to give **5b** (0.328 g, 56%), which was chromatographed in the same manner as **5a** and used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $Si(CH_3)_2$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 2.45 (m, 1 H, H-3′$_a$), 2.60 (m, 1 H, H-3′$_b$), 3.85 (m, 2 H, H-5′$_{a,b}$),

4.30 (m, 1 H, H-4′), 5.35 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.40 (dd, $J_{1',F}$ = 17, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 8.45 (d, $J_{8,F}$ = 2.3 Hz, 1 H, H-8), 8.70 (s, 1 H, H-2).

6-Iodo-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (5c). Starting with 0.50 g (1.36 mmol) of **4** in the presence of freshly distilled *n*-butyl nitrite (6 mL, 51.2 mmol) and diiodomethane (8 mL), the above procedure was repeated except that the reaction was performed over a 24 h period. The reaction mixture was concentrated, washed with water (2 × 50 mL), dried (Na_2SO_4), and chromatographed in the same manner as **5a** to give **5c** (0.345 g, 53%). This compound was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $Si(CH_3)_2$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 2.45 (m, 1 H, H-3′$_a$), 2.55 (m, 1 H, H-3′$_b$), 3.85 (d, 2 H, H-5′$_{a,b}$), 4.30 (m, 1 H, H-4′), 5.30 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.37 (dd, $J_{1',F}$ = 17, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1′), 8.42 (d, $J_{8,F}$ = 2.4 Hz, 1 H, H-8), 8.60 (s, 1 H, H-2).

General Procedure for the Removal of the 5-*O*-*tert*-Butyldimethylsilyl Group. A solution of each 5′-*O*-protected nucleoside (ca. 3 mmol) in THF (20 mL) was treated with 80% aqueous acetic acid (100 mL). The resulting solution was stirred at room temperature for 16–24 h, concentrated under reduced pressure, coevaporated three times with CH_2Cl_2, and dried under high vacuum to remove traces of acetic acid. The crude products were purified by flash column chromatography using silica gel and CH_2Cl_2:MeOH (10:0 to 9:1) as eluant to give pure products that were recrystallized from CH_2Cl_2:ether mixtures to give the following analytical samples:

6-Chloro-9-[2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (3a): 79% yield; mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 1 H, OH), 2.55 (m, 2 H, H-3′$_{a,b}$), 3.85 (dd, *J* = 12, 4.8 Hz, 1 H, H-5′$_a$), 4.00 (dd, *J* = 12, 2 Hz, 1 H, H-5′$_b$), 4.40 (m, 1 H, H-4′), 5.38 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.40 (dd, $J_{1',F}$ = 16.5, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 8.50 (d, $J_{8,F}$ = 2.1 Hz, 1 H, H-8), 8.75 (s, 1 H, H-2); FAB MS *m*/*z* (relative intensity) 273 (MH^+, 100), 155 (b + 2H, 55). Anal. ($C_{10}H_{10}ClFN_4O_2$) C, H, N.

6-Bromo-9-[2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (3b): 83% yield; mp 142–143 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (br s, 1 H, OH), 2.55 (m, 2 H, H-3′$_{a,b}$), 3.80 (dd, *J* = 12, 4.8 Hz, 1 H, H-5′$_a$), 3.95 (dd, *J* = 12, 2 Hz, 1 H, H-5′$_b$), 4.40 (m, 1 H, H-4′), 5.38 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.40 (dd, $J_{1',F}$ = 17, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 8.45 (d, $J_{8,F}$ = 1.3 Hz, 1 H, H-8), 8.70 (s, 1 H, H-2); FAB MS *m*/*z* (relative intensity) 319 (^{81}Br MH^+, 96), 317 (^{79}Br MH^+, 100), 201 (^{81}Brb + 2H, 64), 199 (^{79}Brb + 2H, 64). Anal. ($C_{10}H_{10}BrFN_4O_2$) C, H, N.

6-Iodo-9-[2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (3c): 82% yield; mp 159–161 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 2 H, OH), 2.60 (m, 2 H, H-3′$_{a,b}$), 3.80 (dd, *J* = 12, 4.8 Hz, 1 H, H-5′$_a$), 3.95 (dd, *J* = 12, 2 Hz, 1 H, H-5′$_b$), 4.40 (m, 1 H, H-4′), 5.38 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.40 (dd, $J_{1',F}$ = 17, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 8.41 (br s, 1 H, H-8), 8.63 (s, 1 H, H-2); FAB MS *m*/*z* (relative intensity) 365 (MH^+, 100), 247 (b + 2H, 65). Anal. ($C_{10}H_{10}FIN_4O_2 \cdot 0.75H_2O$) C, H, N.

6-Fluoro-9-[2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (3d). A solution of **3a** (0.10 g, 0.36 mmol) in 1,2-dimethoxyethane (10 mL) was treated with liquid trimethylamine (5 mL, condensed at −30 °C). The mixture was stirred at room temperature for 30 min under a nitrogen atmosphere, and the precipitated solid was filtered rapidly and washed with anhydrous ether (15 mL) (Note: due to the very unstable and hygroscopic nature of this salt, all these operations were conducted in a glovebag under nitrogen). The salt was then dried at high vacuum for 15 min and used immediately. A suspension of anhydrous KF (0.20 g, 3.4 mmol) in anhydrous DMF (4 mL) was heated at 80 °C for 30 min and then cooled to 40 °C. The trimethylammonium salt was quickly added to the KF suspension, and the reaction mixture was stirred and heated overnight at 40–50 °C. The reaction mixture was cooled and filtered. The filtrate was concentrated under vacuum and purified by preparative TLC (silica gel 1500 μm, EtOAc) to give **3d** (R_f = 0.42, 0.034 g, 37%) as the major product. This material decomposed when in contact with silica gel to give **2b**, thus making it difficult to secure an analytical sample: ^{1}H NMR (D_2O) δ 2.25 (m, 1 H, H-3′$_a$), 2.60 (m, 1 H, H-3′$_b$), 3.70 (m, 2 H, H-5′$_{a,b}$), 4.35 (m, 1 H, H-4′), 5.38 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.40 (dd, $J_{1',F}$ = 18, $J_{1',2'}$ = 3.2 Hz, 1 H, H-1′), 8.52 (s, 1 H, H-2), 8.61 (d, $J_{8,F}$ = 2 Hz, 1 H, H-8); FAB MS *m*/*z* (relative intensity) 257 (MH^+, 58), 139 (b + 2H, 100). The analytical purity of this compound was assessed to be 98% by HPLC. A second band was extracted from the analytical TLC plate R_f 0.24 (0.021 g, 20%) which proved to be 6-(dimethylamino)-9-[2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine. This compound was fully characterized, and it will be reported elsewhere in conjuction with a paper on 6-amino-substituted analogues. This method of fluorination was identical to that used by Robins and Basom.[23b]

6-Chloro-9-(5-*O*-benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (7). This compound was synthesized as reported previously.[24]

6-*O*-Methyl-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (3e). A suspension of **7** (0.560 g, 1.48 mmol) in MeOH (80 mL) was treated with sodium metal (0.70 g, 30.4 mmol), and the mixture was stirred at room temperature for 30 min. After the pH of the resulting solution was adjusted to ca. 7 with 1 M HCl, the solution was concentrated under vacuum. The crude product was purified twice by flash column chromatography using silica gel and CH_2Cl_2:MeOH (10:0 to 9:1) as eluant to give pure **3e** (0.347 g, 87%) as a foam: ^{1}H NMR ($CDCl_3$) δ 2.50 (m, 1 H, H-3′$_a$), 2.62 (m, 1 H, H-3′$_b$), 3.30 (br s, 1 H, OH), 3.80 (dd, *J* = 12, 4.5 Hz, 1 H, H-5′$_a$), 4.00 (dd, *J* = 12, 2 Hz, 1 H, H-5′$_b$), 4.20 (s, 3 H, OCH_3), 4.40 (m, 1 H, H-4′), 5.35 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.35 (dd, $J_{1',F}$ = 16, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1′), 8.40 (d, $J_{8,F}$ = 1.8 Hz, 1 H, H-8), 8.55 (s, 1 H, H-2); FAB MS *m*/*z* (relative intensity) 269 (MH^+, 100), 151 (b + 2H, 75). Anal. ($C_{11}H_{13}FN_4O_3 \cdot 0.5H_2O$) C, H, N.

6-*O*-Ethyl-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (3f). An identical approach starting with **7** in ethanol (0.30 g, 0.79 mmol) produced **3f** (0.157 g, 70%) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.50 (t, 3 H, CH_2CH_3), 2.50 (m, 1 H, H-3′$_a$), 2.60 (m, 1 H, H-3′$_b$), 3.40 (br s, 1 H, OH), 3.80 (dd, *J* = 12, 4.8 Hz, 1 H, H-5′$_a$), 4.00 (dd, *J* = 12, 2 Hz, 1 H, H-5′$_b$), 4.40 (m, 1 H, H-4′), 4.65 (q, 2 H, CH_2CH_3), 5.35 (dm, $J_{2',F}$ = 54 Hz, 1 H, H-2′), 6.35 (dd, $J_{1',F}$ = 17, $J_{1',2'}$ = 3.6 Hz, 1 H, H-1′), 8.15 (d, $J_{8,F}$ = 1.9 Hz, 1 H, H-8), 8.50 (s, 1 H, H-2); FAB MS *m*/*z* (relative intensity) 283 (MH^+, 100), 165 (b + 2H, 68). Anal. ($C_{12}H_{15}FN_4O_3 \cdot 0.25H_2O$) C, H, N.

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(1) Abbreviations used: ADA, adenosine deaminase; BBB, blood–brain barrier; CNS, central nervous system; dCF, 2′-deoxycoformycin; ddA, 2′,3′-dideoxyadenosine; ddI, 2′,3′-dideoxyinosine; F-ddA, 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)adenine; F-ddI, 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)hypoxanthine; F-ddN, 2′-β-fluoro-2′,3′-dideoxynucleosides; HIV, human immunodeficiency virus type 1; *P*, octanol/pH 7.0 buffer partition coefficient; PHA-PBM, phytohemagglutinin-stimulated peripheral blood mononuclear cells; PNP, purine nucleoside phosphorylase.

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