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

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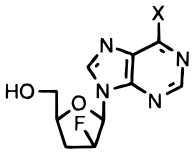
A series of 6-substituted amino analogs of 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl) purines (F-ddN) has been synthesized and characterized with the objective of finding compounds which might be superior to existing drugs for the treatment of HIV in the central nervous system. These compounds are intended to be more lipophilic than the currently approved anti-HIV drugs for better blood−brain barrier penetration. Subsequent adenosine deaminase (ADA)-catalyzed hydrolysis of these prodrugs in the brain is expected to produce the anti-HIV agent, 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)hypoxanthine (F-ddI). The new compounds, synthesized from the corresponding 6-chloro analog, include F-ddN which contain methylamino, ethylamino, dimethylamino, hydroxylamino, methoxyamino, benzyloxyamino, hydrazino, and nitro substituents in the 6-position. The 6-nitro analog was isolated as an unexpected product during the preparation of the 6-chloro derivative. Among the analogs with anti-HIV activity, the ethylamino and dimethylamino compounds are ca. 100 times more lipophilic than ddI or F-ddI. As expected, 2′-fluoro substitution protects the compounds from acid-catalyzed glycosylic cleavage. Only the hydroxylamino and nitro analogs underwent any nonenzymatic hydrolysis at pH 1.0 or 7.4. This reaction, however, results in hydrolysis of the group in the 6-position rather than glycosylic bond cleavage. ADA catalyzes the hydrolysis of the 6-substituents at rates which vary from slightly slower ($NO_2$, 1.7×) to much slower (NHEt, 5000×) than F-ddA. The 6-dimethylamino analog is the only compound which possesses anti-HIV activity ($ED_{50}$ 18 μM) without ADA hydrolysis. With the exception of the two inactive alkoxyamino compounds, the other prodrugs exhibited cellular protection in the HIV-1/PHA-PBM system with $IC_{50}$ potencies of 7−40 μM.

## Introduction

HIV infection of the central nervous system (CNS)[1] and the attendant AIDS-related dementia remains a major problem for people with AIDS.[2] Although significant progress has been made in the discovery of new therapeutic approaches to the systemic treatment of HIV infection,[3] a continuing effort is needed for the specific problem of CNS HIV infection.[4a] Among the enzymes used in the enzyme-activated prodrug approach to this problem are xanthine oxidase[4b] and adenosine deaminase (ADA).[5] The detailed rationale for our ADA approach has been described previously.[5] In general, this involves the design of molecules which might penetrate the blood−brain barrier (BBB) better than the drugs currently available. Specifically, it utilizes fluorinated dideoxypurine nucleoside (F-ddN) analogs with increased lipophilicity for better BBB diffusion, in anticipation that these compounds will be enzymatically activated by adenosine deaminase (ADA) in the CNS to 2′-β-fluoro-2′,3′-dideoxyinosine (F-ddI, **1**).[5,6] F-ddI is an anti-HIV-active relative of F-ddA (**2**),[6,7a] a compound scheduled for clinical trial in AIDS patients in the near future.[8] F-ddI possesses several favorable qualities as an anti-HIV agent such as acid stability for ease of oral formulation[6] and a lack of catabolic inactivation by purine nucleoside phosphorylase (PNP).[9]




**1**, X = OH (F-ddI)
**2**, X = $NH_2$ (F-ddA)

## Chemistry

**Synthesis.** Since it is the 2′-fluoro group which confers acid stability on the normally acid-unstable 2′,3′-dideoxypurine nucleosides,[5−8,10] this functionality is included in all the prodrugs synthesized. Fluorine in the 2′-β-configuration (*threo*) is utilized, since the initially prepared 2′-α-fluoro (*erythro*) compounds, while acid-stable,[7a] are inactive as anti-HIV agents.[5,7] Although the prodrugs themselves could possess anti-HIV activity, earlier compounds evaluated in this series indicated that conversion to **1** was necessary before activity was observed.[5b]

The amino analogs **4**−**6** are prepared from the 5′-benzoyl-protected 6-chloro compound, **3a**, by straightforward nucleophilic displacement reactions with various amine derivatives (Scheme 1). We prepared **4** by a different procedure earlier.[5b] The syntheses of **7**−**10** proceed from the deprotected compound, **3b**, an analog

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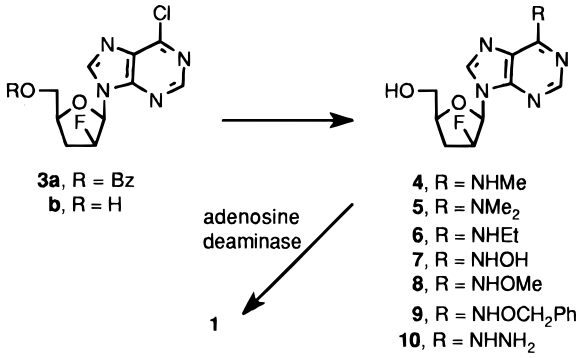
**Scheme 1**




we described previously in this series.[5a] Since the ultimate fate of these prodrugs involves ADA-catalyzed hydrolysis of the 6-substituent (Scheme 1), and because it is known that larger groups are more resistant to this reaction,[11] the 6-substituents were chosen in an attempt to keep the variations simple in order to see how minor structural changes, e.g. **4** vs **5** and **5** vs **6**, influence the ADA-catalyzed reaction. Hydroxylamino functionality (**7**) was included since this group has been shown to be a good substrate for ADA in the adenosine series.[11,12] In addition, if hydrolyzed at a rate which produced concentrations of F-ddI adequate for anti-HIV activity, alkoxyamino substitution could provide an entry to progressively more lipophilic nucleosides through longer alkyl groups. The methoxyamino (**8**) and benzyloxyamino (**9**) analogs were synthesized in an attempt to exploit this approach. Although the 6-hydrazino riboside is reported to be unstable in aqueous solution,[13a] the dideoxy analog, **10**, was prepared and evaluated because the riboside was shown in earlier studies to be a substrate for ADA.[13b] In addition, *N*-alkylhydrazino compounds would provide an entry to longer chain, more lipophilic analogs if the parent compound proved active. The 6-dimethylamino analog, **5**, was prepared in good yield as described above. This compound also had been observed earlier as a minor side product during the preparation of the 6-fluoro prodrug from **3b** *via* a 6-trimethylammonium intermediate.[5a]

The 6-chloro analogs, **3a** and **3b**, are critical intermediates in the preparation of the prodrugs reported here (Scheme 1). If the deblocked chloro compound (**3b**) is required, **3a** cannot be used as an intermediate since removal of the 5′-benzoyl protecting group with methanolic ammonia produces a mixture of F-ddA (**1b**) and the corresponding 6-methoxy compound.[5a] However, changing 5′-protection to *tert*-butyldimethylsilyl (**11**) prior to photochlorination provides an intermediate (**12**) (Scheme 2) which can be deblocked with fluoride ion[5a] to give **3b** as the major product. Upon repeating this reaction, we were able to isolate a minor product (10%), the blocked 6-nitro analog (**13**). This nitro group proved to be quite reactive. Attempted 5′-deblocking of **13** with fluoride ion produced the previously described[5a] 6-fluoro analog, **14**. Alternatively, removal of the 5′-TBDMS group with aqueous acetic acid provides the deblocked nitro compound, **15** (Scheme 2). While this material proved somewhat difficult to characterize since it is hydrolytically unstable and partially converted to F-ddI (**1**) during the deprotection procedure, the use of appropriate chromatographic conditions allowed the isolation of **15**. In addition to the expected NMR and mass spectral data, **15** was further characterized by reduction to the known 6-amino compound, F-ddA (**2**).

**Scheme 2**

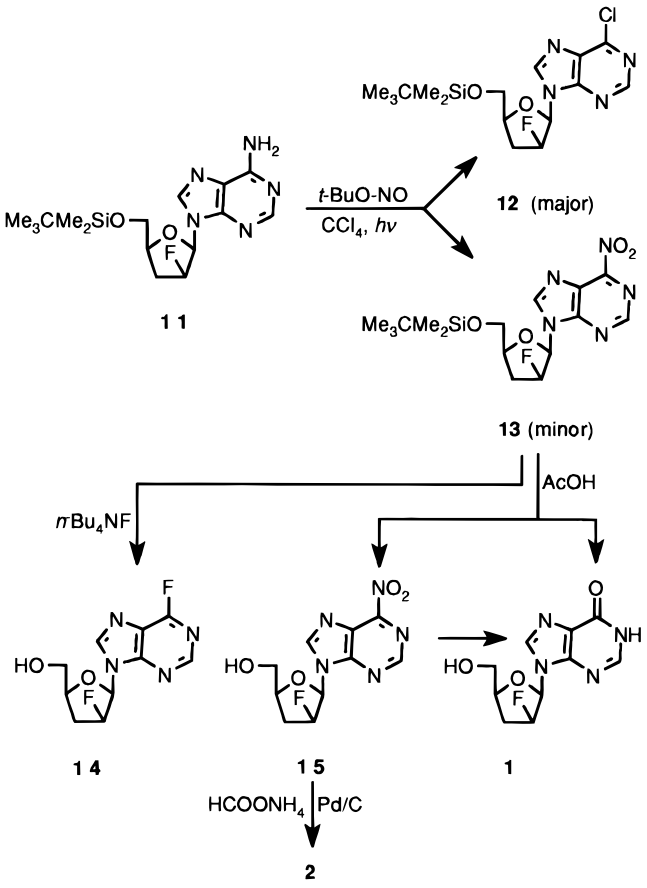
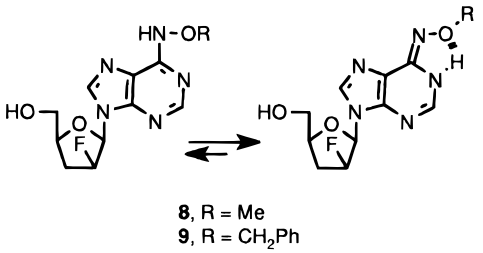



**Chart 1**




The proton NMR spectra of the *O*-alkylhydroxylamino derivatives **8** and **9** in DMSO-$d_6$ indicates an equilibrium between the alkoxyamino and alkoxyimino tautomers (Chart 1). The alkoxyimino tautomer appears to be favored, possibly because of a stabilizing intramolecular hydrogen bond formed between the imino proton and the oxygen atom. This is evidenced by the observed splitting of the H-2 signal ($J_{2,NH}$ ca. 3.6 Hz) in both **8** and **9**, indicating the presence of the imino tautomer. After deuterium exchange, the minor (alkoxyamino) tautomers are no longer detectable.

**Lipophilicity.** Octanol/buffer (pH 7.4) partition coefficients, *P*, were obtained for the prodrugs prepared in this study. They were determined by a convenient microscale shake-flask method utilizing HPLC/UV quantitation.[14] By convention, they are reported as log *P* values in Table 1. The (benzyloxy)amino compound, **9**, is over 170 times more lipophilic than the hydroxylamino prodrug, **7**, the most hydrophilic of the new compounds. Compounds **5** and **6**, the most lipophilic of the compounds with anti-HIV activity, are 100 times more lipophilic than either ddI or F-ddI. Compound **9**, while 290 times more lipophilic than ddI, is inactive with an $IC_{50} > 80$ $\mu$M under our experimental condi-

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

| compd | 6-substituent | measured[a] log *P* | HPLC mobile phase[b] | isocratic[c] *k*′ | $\lambda_{max}$[d] (nm) |
|---|---|---|---|---|---|
| **1** | OH (F-ddI)[e] | −1.210 ± 0.017 | A | 0.5 | 247 |
| **2** | $NH_2$ (F-ddA) | −0.183 ± 0.004 | B | 0.8 | 259 |
| **4** | $NHCH_3$ | 0.273 ± 0.008 | C | 1.7 | 265 |
| **5** | $N(CH_3)_2$ | 0.797 ± 0.007 | D | 6.1 | 275 |
| **6** | $NHC_2H_5$ | 0.779 ± 0.010 | D | 3.8 | 266 |
| **7** | NHOH | −0.983 ± 0.019 | F | −[f] | 265 |
| **8** | $NHOCH_3$ | −0.414 ± 0.007 | A | 1.1 | 267 |
| **9** | $NHOC_7H_7$ | 1.255 ± 0.010 | E | 27.1 | 268 |
| **15** | $NO_2$ | −0.395 ± 0.006 | C | 2.6 | 305 |

[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) 12%, (C) 15%, (D) 20%, or (E) 25% $CH_3CN$ in pH 7.0, 0.01 M phosphate buffer; (F) 12.5% $CH_3CN$ in pH 3.0, 0.005 M heptanesulfonic acid. [c] Average capacity factor ($k' = (t_r - t_0)/t_0$) for isocratic elution with 15% $CH_3CN$ (mobile phase C) in pH 7.0, 0.010 M phosphate buffer. [d] Wavelengths determined on-the-fly in HPLC mobile phase. [e] Reference log *P* values for F-ddI, ddI (−1.242) and AZT (0.052) are from ref 5a. [f] Could not be chromatographed using mobile phase C.

tions. The experimental partition coefficient values were internally consistent, giving good agreement with calculated values using the standard π value of +0.5 for methyl and methylene groups (**2**, **4**, **5**, **6** and **7**, **8**).[15] We were not able to discover chromatographic conditions suitable for determining the *P* value for the hydrazino compound, **10**.

**Stability at pH 1.0 and 7.4.** Normally, ddN are quite unstable to the acidic conditions encountered in the stomach,[16] producing inactive products through glycosylic cleavage, and making oral formulations difficult. Because 2′-fluoro substitution had previously conferred acid stability on ddN,[5−8] stability at pH 1.0 was evaluated for several of the new compounds (**4**, **6**, **7**). No decomposition of any type was observed by HPLC analysis for **4** or **6** over a 24 h period. The hydroxylamino analog, **7**, however, decomposes at pH 1.0 with a half-life of 103 h to produce unidentified products which did not include either **1** or **2**, compounds which are stable under these conditions.[5−7]

Several compounds (**5**, **6**, **7**, **15**) were evaluated at the pH of the biological test system (7.4) in an attempt to determine whether there were any nonenzymatic, hydrolytic effects in addition to those observed in the presence of ADA (see below). While **5** and **6**, as well as **2**, were unaffected over a 24 h period, **7** and **15** showed slow changes at pH 7.4. The hydroxylamino analog **7** produced both **1** and **2** with $t_{1/2} = 105$ h (Figure 1). The mechanism for the production of **2** remains to be studied. The nitro compound (**15**) is hydrolyzed to F-ddI (**1**) with a half-life of 27.4 h. This observation is consistent with the difficulty encountered in isolating **15**.

## Biology

**Enzymatic Hydrolysis by Adenosine Deaminase.** The prodrugs in this study have been designed with the possibility of ADA-catalyzed hydrolysis to the anti-HIV agent, F-ddI, **1** (Scheme 1). It is well-known that, in addition to the amino group, adenosine deaminase (ADA) catalyzes the hydrolysis of other 6-position functionality in purine nucleosides.[11,12,13b,17] The compounds in this study were subjected to ADA-catalyzed

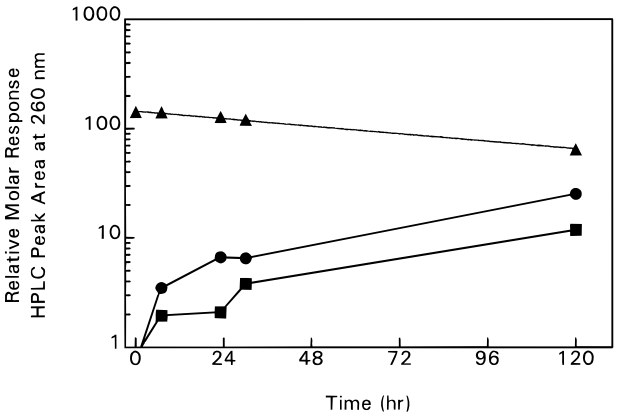



**Figure 1.** Hydrolysis kinetics of the 6-NHOH analog (**7**) in pH 7.4 phosphate buffered saline at 37 °C and an initial 50 μM concentration. The curve for the hydrolysis of **7** (▲) is the best nonlinear regression fit ($r^2 = 0.993$) for monoexponential decay. The appearance curves for F-ddI, **1** (●), and F-ddA, **2** (■), are plotted point-to-point. The peak area for F-ddI has been adjusted to reflect the fact that the HPLC-UV detector molar response at 260 nm is 0.48× that at the $\lambda_{max}$ of 247 nm.

**Table 2.** Relative Adenosine Deaminase Hydrolysis Rates[a]

| compd | 6-substituent | disappearance of substrate[b] | appearance of F-ddI |
|---|---|---|---|
| **2** (F-ddA) | $NH_2$ | 100 | 100 |
| **3b**[c] | Cl | 2.0 | 1.6 |
| **4** | $NHCH_3$ | 0.9 | 0.74 |
| **5** | $N(CH_3)_2$ | NR[d] | NR |
| **6** | $NHC_2H_5$ | 0.02 | −[e] |
| **7** | NHOH | 9.0 | 8.2 |
| **8** | $NHOCH_3$ | 0.02 | −[e] |
| **9** | $NHOC_7H_7$ | NR | NR |
| **10** | $NHNH_2$ | ND[f] | 3.2 |
| **14**[c] | F | 202 | 233 |
| **15** | $NO_2$ | 58 | 58 |

[a] Standard conditions: 50 μM substrate; pH 7.4, 0.01 M phosphate buffer; 37 °C. [b] Disappearance rate per unit ADA normalized to that of F-ddA. [c] Reference 5a. [d] No reaction; decrease in substrate or conversion to F-ddI not detectable after 24 h using 1 unit/mL ADA. [e] Conversion to F-ddI detectable, but not sufficient to calculate rate of appearance. [f] Not determined because of inability to measure parent compound by HPLC.

hydrolysis with commercially available, purified enzyme (1.0 unit/mL) at prodrug concentrations (50 μM) which produced *in vitro* anti-HIV activity for previous members of this series.[5a] Table 2 shows the rates of this reaction relative to that of the 6-amino compound, F-ddA (**2**). Relative rates at 1.0 unit/mL ADA are given for compound comparison because the amount of ADA in the anti-HIV test system used (PHA/PBM cells and media) is not known. The analytical HPLC method measures not only the disappearance of prodrug, but also the resulting production of F-ddI, allowing hydrolysis quantitation in two ways. The time course of this reaction is shown in Figure 2 for the 6-hydroxylamino analog **7**. ADA-catalyzed hydrolysis was not detected for either the 6-dimethylamino (**5**) or the (benzyloxy)-amino (**9**) analog. The addition of a methyl group (**4**) to the 6-amino function of F-ddA (**2**) reduced its hydrolysis rate about 100-fold. Ethyl group addition (**6**) gave a significantly greater reduction (5000×), producing a rate similar to that found for the methoxyamino compound (**8**). The hydroxyamino (**7**) hydrolysis rate is only about 10 times slower than that for the amino compound, consistent with data reported for the adenosine analog.[11,12] The fastest ADA-catalyzed hydroly-

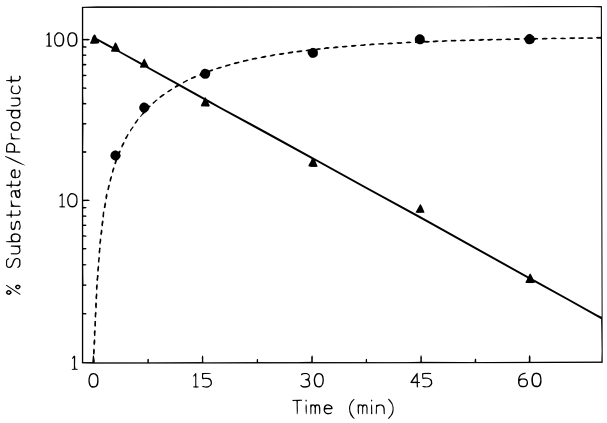



**Figure 2.** Hydrolysis kinetics of 50 $\mu$M 6-NHOH analog (**7**) by 1.0 unit/mL of adenosine deaminase at pH 7.4 and 37 °C. A unit of adenosine deaminase is the amount of enzyme which hydrolyzes a standard substrate (e.g. adenosine) at a rate of 1.0 $\mu$mol/min at 25 °C and pH 7.5. The curve depicting the enzymatic hydrolysis of **7** (▲) is the best nonlinear regression fit ($r^2 = 0.998$) for monoexponential decay. The appearance curve for the resulting F-ddI, **1** (●), product represents the best nonlinear regression fit ($r^2 = 0.996$) to the equation for monoexponential association ($y = A(1 - e^{-bt})$. See Experimental Section for hydrolysis conditions and kinetic calculations.

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

| compd | 6-substituent | $IC_{50}$ ($\mu$M) | compd | 6-substituent | $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|---|
| **1** (F-ddI) | OH | 6.3 | **8** | NHOMe | >80 |
| **2** (F-ddA) | $NH_2$ | 3.3 | **9** | $NHOCH_2Ph$ | >80 |
| **3b** | Cl | 5.9[a] | **10** | $NHNH_2$ | 36.3 |
| **4** | NHMe | 13.1 | **14** | F | <5[a] |
| **5** | $NMe_2$ | 18.0 | **15** | $NO_2$ | 9.6 |
| **6** | NHEt | 11.1 | **ddI** | | 0.3 |
| **7** | NHOH | 6.8 | | | |

[a] Reference 5a.

sis rate among the new compounds belongs to the nitro analog (**15**) which is only fractionally slower than F-ddA.

**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 relative to an untreated control as the measure of HIV-exposed cell protection and compound activity.[18,19] Compounds **1**−**10**, **14**, and **15**, along with ddI as a positive control, were evaluated in this system (Table 3). These data are representative of duplicate evaluations and are from an experiment where all the new compounds were tested in direct comparison. No significant toxicity to uninfected PBM cells was noted at the 10−80 $\mu$M drug concentrations used, and good dose response data were obtained for the active compounds (i.e. $IC_{50}$ < 80 $\mu$M).

While no quantitative correlation exists between $IC_{50}$ values (Table 3) and ADA hydrolysis rates (Table 2), a trend can be discerned. Compounds with the faster F-ddI conversion rates are generally among the more potent prodrugs (**2**, **15**, **7**, **3b**,[5a] and **14**.[5a] F-ddA (**2**) is seen to be about twice as potent as F-ddI (**1**), its hydrolysis product. While this would be abnormal for the other compounds in this study whose anti-HIV activity depends on the metabolic generation of F-ddI, it is the usual situation for F-ddA.[6] F-ddA is directly metabolized to F-ddATP by deoxycytidine kinase[9] in addition to being catabolized to F-ddI and utilizing the 5′-nucleotidase pathway for conversion to F-ddATP (the anti-HIV-active metabolite for all the compounds in this series). Among the other analogs, the hydroxylamino compound, **7**, decomposes nonenzymatically in pH 7.4 buffer to give F-ddA as well as F-ddI with a $t_{1/2} = 105$ h (Figure 1). This might explain the somewhat higher than expected potency of this compound (based only on the ADA rate) in our 7-day anti-HIV assay. A somewhat similar effect is operational in the case of the 6-$NO_2$ analog, **15**, which produces F-ddI at relatively rapid rates by both pH 7.4 solvolysis ($t_{1/2} = 27.4$ h) and ADA-catalyzed hydrolysis (Table 2). Although the inactive compounds in this study (alkoxyamino analogs **8** and **9**) are at the low end of the hydrolysis rates as might be expected, the alkylamino compounds, **4** and **6**, also have slow ADA rates (Table 2), but are protective (Table 3). The dimethylamino analog **5** is an anomaly in this data set. No ADA-catalyzed hydrolysis is observed with this compound, yet an 18 $\mu$M anti-HIV $IC_{50}$ value is obtained. This compares well with a 17.3 $\mu$M value found in a separate PHA-PBM experiment, as well as a 149 $\mu$M $IC_{50}$ value in the standard NCI CEM/HIV-1 screening system[20] (data not shown). Earlier work has shown that the activity of 6-substituted dideoxypurine prodrugs is dependent upon conversion to their corresponding inosine analogs.[5b,19] It is possible that this might not be the case with **5** which may have intrinsic activity of its own.

## Discussion

A useful prodrug in this series must be optimized for a number of unrelated, but structurally dependent properties (lipophilicity, ADA hydrolysis rate, acid-stability, systemic metabolism and elimination). One critical event for prodrug activation is the necessity for an adequate concentration of ADA in the CNS. Recent reports indicate that this is probable, and that the level of this enzyme may increase in both the CSF and serum during HIV infection.[21,22] In addition, a significant amount of work supporting the CNS prodrug concept has been reported for the nonfluorinated, 6-halo-2′,3′-dideoxypurine nucleosides, both *in vitro*[19] and in animal models.[23−27] While these compounds and their active metabolite, ddI, are both acid-sensitive and catabolized at rates higher than those for the 2′-fluoro derivatives, a similarly enhanced CNS penetration should also be realized with the fluorinated prodrugs.

With the addition of the compounds described previously,[5] a varied set of 14 6-substituted-2′-fluoro-2′,3′-dideoxypurine derivatives is available to test the hypothesis that an acid-stable, lipophilic, ADA-activated prodrug might provide a useful CNS-directed anti-HIV drug. The groups introduced into the 6-position include various substituted-amino, halogen, and alkoxy groups. Active prodrugs have been prepared with ADA hydrolysis rates that vary by a factor of >25000 (e.g. F vs OEt). The partition coefficients for the complete series span a 180-fold range (NHOH to OBn), with several of the active prodrugs 100 times more lipophilic than ddI. At this point it is not possible to determine which compound in the series might have the optimum combination of properties for CNS treatment. One major variable bearing on this question is the rate of human whole-body ADA drug hydrolysis, which will affect prodrug ability to reach the brain. This question should be answered in a pharmacokinetic phase I study scheduled as part of an upcoming clinical trial with F-ddA (**2**). Meanwhile, a comparison of the CNS penetration

properties of the 6-chloro prodrug **3b**, ddA, ddI, F-ddA, and F-ddI is underway in rats.

## Experimental Section

Column chromatography was performed on silica gel 50 (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 $\delta$ 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[28] 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 measurements of protonated molecular ion ($MH^+$) peaks were carried out in separate experiments 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.** Except as noted, solutions and diluted enzymatic reaction mixtures of dideoxynucleosides were analyzed on a reversed phase, high-performance liquid chromatography (RP-HPLC) system that has been previously described.[5a] This system contained a Thermo Separations Products Model AS3000 automatic, refrigerated, sample injector that was maintained at 5 °C. Dideoxynucleoside retention time was adjusted to be in the range 5−22 min by changing the percentage of mobile phase organic modifier (Table 1). Analysis of the 6-NHOH analog, **7**, however, required the use of ion pairing for separation on the standard 4.6 × 250 mm 5-$\mu$m Beckman Ultrasphere C18 column that was used for all dideoxynucleoside separations. This reversed phase, ion pairing (RP-IP) HPLC was conducted on the above column without using a precolumn and by employing a mobile phase consisting of 12.5% $CH_3CN$ in aqueous 0.005 M heptanesulfonic acid ion pair cocktail (Regis Chemical Co.) that had been adjusted to pH 3.0 with 1.0 N sodium hydroxide. Before mixing and use, all mobile phase components were filtered and then degassed by sonication and/or sparging with ultra-high-purity helium (Matheson Gas Products). Where possible, 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 66 MHz 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 Prism (Version 1.0, GraphPad Software), a PC-based curve-fitting program.

**Measurement of Octanol−Water Partition Coefficients.** 1-Octanol/pH 7.4 (0.01 M potassium phosphate) 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[14] (Table 1). Determinations were conducted in triplicate. Because of chromatographic constraints, the $P$ for the 6-NHOH compound **7** was determined by difference after measurement of compound concentration in the aqueous phase both before and after partitioning with 1-octanol. Satisfactory chromatographic conditions were not found for the determination of the $P$ value for the hydrazino compound, **10**.

**Hydrolytic Stability (pH 7.4) of Sugar-Fluorinated Dideoxynucleosides.** The hydrolytic stability of compounds **4**−**7** and **15** was evaluated at 37 °C in pH 7.4, 0.1 M phosphate-buffered saline solution (PBS, Biofluids). For each compound, 1.0 mL PBS was added to a 1.5 mL polypropylene microcentrifuge tube (Eppendorf) and prewarmed to 37 °C in an Eppendorf Model 5436 Thermomixer before being spiked to a concentration of approximately 50 $\mu$M. After mixing, a 50 $\mu$L aliquot was immediately removed for analysis and the remaining solution incubated at 37 °C. This initial sample aliquot was mixed with 50 $\mu$L of the appropriate chilled (4 °C) mobile phase, and a 20 $\mu$L aliquot was analyzed by HPLC. Subsequent 50 $\mu$L samples were taken at predetermined times and treated in the same manner as the first.

**Acid Stability (pH 1.0) of Sugar-Fluorinated Dideoxynucleosides.** The pH 1.0 acid stability of compounds **4**−**7** and **15** was evaluated at 37 °C as previously described.[5a] The only modification made was for compound **7** where the 50 $\mu$L sample aliquots were diluted with 0.45 mL of chilled (4 °C) pH 3.0, 0.005 M heptanesulfonic acid ion pair cocktail prior to HPLC analysis instead of pH 7.0 phosphate buffer. The concentration of dideoxynucleoside in each sample was determined by HPLC analysis of a 50 $\mu$L 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[5b] 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 2.6 mM solution in distilled water. Relative ADA hydrolysis rates for selected nucleosides and characterization of products were carried out as previously described.[5]

**6-Chloro-9-(5-*O*-benzoyl-2,3-dideoxy-2-fluoro-*β*-D-*threo*-pentofuranosyl)-9*H*-purine (3a) and 6-Chloro-9-(2,3-dideoxy-2-fluoro-*β*-D-*threo*-pentofuranosyl)-9*H*-purine (3b).** These compounds were synthesized as described in refs 10a and 5b, respectively.

**6-(Methylamino)-9-(2,3-dideoxy-2-fluoro-*β*-D-*threo*-pentofuranosyl)-9*H*-purine (4).** This compound has previously been synthesized by a different route.[5b] A suspension of **3a** (1.44 g, 3.82 mmol) in EtOH (8 mL) was treated with a 40% solution of aqueous methylamine (22 mL) and stirred at 40 °C for 30 min under a nitrogen atmosphere. The solution was cooled and concentrated under reduced pressure to give an oily residue. In order to ensure complete neutralization of the product due to the volatile nature of methylamine, the residue was dissolved in MeOH and treated with a few drops of 10% NaOH. The solution was again reduced to dryness, and the crude product was purified by flash column chromatography using silica gel and a 0−10% gradient of MeOH in $CH_2Cl_2$ as eluant. Collection of the proper fractions produced 0.878 g (86%) of **4** as a foam: $^1$H NMR ($D_2O$) $\delta$ 2.20 (m, 1 H, H-3′$_a$), 2.60 (m, 1 H, H-3′$_b$), 2.85 (br s, 3 H, NHC$H_3$), 3.68 (m, 2 H, H-5′$_{a,b}$), 4.30 (m, 1 H, H-4′), 5.35 (dm, $J_{2',F}$ = 53.6 Hz, 1 H, H-2′), 6.08 (dd, $J_{1',F}$ = 18.6, $J_{1',2'}$ = 3.2 Hz, 1 H, H-1′), 7.84 (s, 1 H, H-2), 8.05 (d, $J_{8,F}$ = 2.4 Hz, 1 H, H-8); FAB MS $m/z$ (relative intensity) 268 ($MH^+$, 100), 150 ($bH_2^+$, 70). Anal. ($C_{11}H_{14}FN_5O_2 \cdot 0.5H_2O$) C, H, N.

**6-(Dimethylamino)-9-(2,3-dideoxy-2-fluoro-*β*-D-*threo*-pentofuranosyl)-9*H*-purine (5).** A suspension of **3a** (0.215 g, 0.57 mmol) in EtOH (5 mL) was treated with a 40% solution of aqueous dimethylamine (10 mL) and heated at reflux for 4 h. The solution was cooled and concentrated under reduced pressure. The residue was purified by preparative TLC (Analtech; silica gel, 2000 $\mu$m) using $CH_2Cl_2$:MeOH (9:1) as eluant to give 0.125 g (78%) of **5** as a solid: mp 141−143 °C ($CH_2Cl_2$:ether); $^1$H NMR ($D_2O$) $\delta$ 2.15 (m, 1 H, H-3′$_a$), 2.55 (m, 1 H, H-3′$_b$), 3.10 (br s, 6 H, N($CH_3$)$_2$), 3.65 (m, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.25 (dm, $J_{2',F}$ = 53.5 Hz, 1 H, H-2′), 6.05 (dd, $J_{1',F}$ = 18.6, $J_{1',2'}$ = 3.0 Hz, 1 H, H-1′), 7.80 (s, 1 H, H-2), 8.00 (d, $J_{8,F}$ = 2.3 Hz, 1 H, H-8); FAB MS $m/z$ (relative intensity) 282 ($MH^+$, 100), 164 (b + 2 H, 34). Anal. ($C_{12}H_{16}FN_5O_2$) C, H, N.

**6-(Ethylamino)-9-(2,3-dideoxy-2-fluoro-*β*-D-*threo*-pentofuranosyl)-9*H*-purine (6).** A suspension of **3a** (0.260 g, 0.69 mmol) in excess aqueous ethylamine (70%, 30 mL) was heated at reflux for 20 h. The solution was cooled and concentrated under reduced pressure, and the residue was purified by flash

column chromatography, in the same manner as for compound **4**, to give 0.180 g (93%) of **6** as a foam: $^1$H NMR ($D_2O$) $\delta$ 1.10 (t, 3 H, $CH_2CH_3$), 2.15 (m, 1 H, H-3′$_a$), 2.55 (m, 1 H, H-3′$_b$), 3.30 (br q, 2 H, $CH_2CH_3$), 3.65 (m, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.22 (dm, $J_{2',F}$ = 53.5 Hz, 1 H, H-2′), 6.03 (dd, $J_{1',F}$ = 18.6, $J_{1',2'}$ = 3.2 Hz, 1 H, H-1′), 7.85 (s, 1 H, H-2), 8.03 (d, $J_{8,F}$ = 2.4 Hz, 1 H, H-8); FAB MS *m/z* (relative intensity) 282 ($MH^+$, 100), 164 (b + 2 H, 40). Anal. ($C_{12}H_{16}FN_5O_2 \cdot 0.75H_2O$) C, H, N.

**6-(Hydroxyamino)-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine, Hydrochloride (7).** A solution of **3b** (0.095 g, 0.35 mmol) in dry THF (5 mL) was treated with excess of *O*-(trimethylsilyl)hydroxylamine (2.5 mL) and heated at reflux for 20 h. The solution was cooled, quenched with MeOH (5 mL), and concentrated under reduced pressure. The crude product was purified by flash column chromatography using silica gel and a 0−20% gradient of MeOH in $CH_2Cl_2$ as eluant to give 0.067 g (63%) of **7** as a foam. An analytical sample was obtained after purification by reversed phase C-18 chromatography using a 0−10% gradient of MeOH in water as eluant. Lyophilization of the product-containing fractions afforded pure **7** as an amorphous white solid: $^1$H NMR ($D_2O$) $\delta$ 2.18 (m, 1 H, H-3′$_a$), 2.54 (m, 1 H, H-3′$_b$), 3.68 (m, 2 H, H-5′$_{a,b}$), 4.30 (m, 1 H, H-4′), 5.30 (dm, $J_{2',F}$ = 53.5 Hz, 1 H, H-2′), 6.15 (dd, $J_{1',F}$ = 18.4, $J_{1',2'}$ = 3.0 Hz, 1 H, H-1′), 7.88 (s, 1 H, H-2), 8.09 (d, $J_{8,F}$ = 1.8 Hz, 1 H, H-8); FAB MS *m/z* (relative intensity) 270 ($MH^+$, 100), 152 (b + 2 H, 33). Anal. ($C_{10}H_{12}FN_5O_3 \cdot HCl$) C, H, N.

**6-(Methoxyamino)-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (8).** A solution of **3b** (0.150 g, 0.55 mmol) in dry THF (15 mL) was treated with excess of *O*-methylhydroxylamine (8.50 g). *O*-Methylhydroxylamine was obtained as a clear liquid by distillation (bp 48−50 °C) of a KOH-neutralized aqueous solution of *O*-methylhydroxylamine hydrochloride. The reaction mixture was heated at reflux for 20 h, cooled, and concentrated under reduced pressure. The crude product was purified by preparative TLC (Analtech; silica gel, 2000 μm) using $CH_2Cl_2$:MeOH (9:1) as eluant to give 0.145 g (93%) of **8** as a solid: mp 200−202 °C dec (EtOH:ether); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 2.20 (m, 1 H, H-3′$_a$), 2.55 (m, 1 H, H-3′$_b$), 3.60 (m, 2 H, H-5′$_{a,b}$), 3.65 and 3.66 (singlets, 3 H, $CH_3O$), 4.15 (m, 1 H, H-4′), 5.01 (m, 1 H, OH), 5.40 (dm, $J_{2',F}$ = 54.3 Hz, 0.8 H, H-2′), 5.45 (dm, $J_{2',F}$ = 54.3 Hz, 0.2 H, H-2′), 6.15 (dd, $J_{1',F}$ = 15.3, $J_{1',2'}$ = 3.8 Hz, 0.8 H, H-1′), 6.38 (dd, $J_{1',F}$ = 15.3, $J_{1',2'}$ = 3.8 Hz, 0.2 H, H-1′), 7.56 (d, $J_{2,NH}$ = 3.6 Hz, 0.8 H, H-2), 8.00 (d, $J_{8,F}$ = 1.9 Hz, 0.8 H, H-8), 8.30 (br s, 0.2 H, H-2), 8.36 (br s, 0.2 H, H-8), 11.00 (br s, 0.2 H, NH), 11.30 (br s, 0.8 H, NH); $^1$H NMR ($Me_2SO$-$d_6$ + $D_2O$) $\delta$ 2.20 (m, 1 H, H-3′$_a$), 2.55 (m, 1 H, H-3′$_b$), 3.60 (m, 2 H, H-5′$_{a,b}$), 4.15 (m, 1 H, H-4′), 5.40 (dm, $J_{2',F}$ = 54.3 Hz, 1 H, H-2′), 6.15 (dd, $J_{1',F}$ = 15.3, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1′), 7.75 (br s, 1 H, H-2), 8.10 (br s, 1 H, H-8); FAB MS *m/z* (relative intensity) 284 ($MH^+$, 100), 166 (b + 2 H, 27). Anal. ($C_{11}H_{14}FN_5O_3 \cdot 0.5H_2O$) C, H, N.

**6-[(Benzyloxy)amino]-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (9).** A solution of **3b** (0.10 g, 0.36 mmol) in dry THF (10 mL) was treated with excess of *O*-benzylhydroxylamine[29] (4 g) and triethylamine (50 μL). The reaction mixture was heated at reflux for 36 h, cooled, and concentrated under reduced pressure. The crude product was purified by preparative TLC (Analtech; silica gel, 1500 μm) using $CH_2Cl_2$:MeOH (9:1) as eluant to give 0.040 g (30%) of **9** as a foam [some **3b** (0.028 g, 28%) was also isolated]: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 2.20 (m, 1 H, H-3′$_a$), 2.50 (m, 1 H, H-3′$_b$), 3.60 (m, 2 H, H-5′$_{a,b}$), 4.15 (m, 1 H, H-4′), 5.01 (m, 3 H, $PhCH_2O$, OH), 5.35 (dm, $J_{2',F}$ = 54.3 Hz, 0.8 H, H-2′), 5.45 (dm, $J_{2',F}$ = 54.3 Hz, 0.2 H, H-2′), 6.15 (dd, $J_{1',F}$ = 15.2, $J_{1',2'}$ = 3.9 Hz, 0.8 H, H-1′), 6.38 (dd, $J_{1',F}$ = 15.2, $J_{1',2'}$ = 3.9 Hz, 0.2 H, H-1′), 7.20−7.50 (m, 5 H, Ph), 7.60 (d, $J_{2,NH}$ = 3.7 Hz, 0.8 H, H-2), 8.00 (d, $J_{8,F}$ = 2.1 Hz, 0.8 H, H-8), 8.36 (br s, 0.2 H, H-2), 8.40 (br s, 0.2 H, H-8), 11.05 (br s, 0.2 H, NH), 11.30 (d, $J_{2,NH}$ = 3.7 Hz, 0.8 H, NH); $^1$H NMR ($Me_2SO$-$d_6$ + $D_2O$) $\delta$ 2.20 (m, 1 H, H-3′$_a$), 2.50 (m, 1 H, H-3′$_b$), 3.60 (m, 2 H, H-5′$_{a,b}$), 4.15 (m, 1 H, H-4′), 5.00 (s, 2 H, $PhCH_2$), 5.35 (dm, $J_{2',F}$ = 54.3 Hz, 1 H, H-2′), 6.15 (dd, $J_{1',F}$ = 15.2, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1′), 7.20−7.50 (m, 5 H, Ph), 7.70 (br s, 1 H, H-2), 8.05 (br s, 1 H, H-8); FAB MS *m/z* (relative intensity) 360 ($MH^+$, 100), 242 (b + 2 H, 24), 91 ($PhCH_2^+$, 44). Anal. ($C_{17}H_{18}FN_5O_3 \cdot 1.5H_2O$) C, H, N.

**6-Hydrazino-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine, Hydrochloride (10).** Compound **3b** (0.128 g, 0.47 mmol) was treated with anhydrous hydrazine (2 mL), and the resulting solution was stirred at room temperature for 30 min. After the addition of 2-propanol (5 mL), the reaction mixture was reduced to dryness under vacuum. The residue was purified by flash column chromatography on silica gel using a 0−20% gradient of MeOH in $CH_2Cl_2$ as eluant to give 0.113 g (79%) of **10** as a hygroscopic hydrochloride salt: $^1$H NMR (MeOH-$d_4$) $\delta$ 2.40 (m, 1 H, H-3′$_a$), 2.60 (m, 1 H, H-3′$_b$), 3.75 (m, 2H, H-5′$_{a,b}$), 4.30 (m, 1 H, H-4′), 5.37 (dm, $J_{2',F}$ = 54.2 Hz, 1 H, H-2′), 6.35 (dd, $J_{1',F}$ = 16.4, $J_{1',2'}$ = 3.6 Hz, 1 H, H-1′), 8.32 (s, 1 H, H-2), 8.36 (d, $J_{8,F}$ = 2.2 Hz, 1 H, H-8); FAB MS *m/z* (relative intensity) 269 ($MH^+$, 100), 151 (b + 2 H, 62). Anal. ($C_{10}H_{13}FN_6O_2 \cdot HCl \cdot 1.75H_2O$) C, H, N.

**6-Nitro-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (13).** This compound was obtained as a minor byproduct during the diazotization/radical chlorination reaction of 6-amino-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (**11**) with *tert*-butyl nitrite and $CCl_4$.[5a] A suspension of **11** (1.90 g, 5.17 mmol) in freshly distilled *tert*-butyl nitrite (24 mL, 210.7 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 mixure 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 0.79 g (39%) of 6-chloro-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (**12**).[5a] This was followed by **13** (0.20 g, 10%), which was used in the next step without additional purification: $^1$H NMR ($CDCl_3$) $\delta$ 0.15 (s, 6 H, $Me_2Si$), 0.95 (s, 9 H, $Me_3C$), 2.45 (m, 1 H, H-3′a), 2.60 (m, 1 H, H-3′b), 3.90 (m, 2 H, H-5′a,b), 4.35 (m, 1 H, H-4′), 5.40 (dm, $J_{2',F}$ = 54.0 Hz, 1 H, H-2′), 6.50 (dd, $J_{1',F}$ = 15.5, $J_{1',2'}$ = 3.6 Hz, 1 H, H-1′), 8.75 (d, $J_{8,F}$ = 2.2 Hz, 1 H, H-8), 9.00 (s, 1 H, H-2).

**Deprotection of 6-Nitro-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (13) To Give 14 or 15. A. With Tetra-*n*-butylammonium Fluoride.** A solution of **13** (0.010 g, 0.025 mmol) in THF (4 mL) was treated with a 1 M solution of tetra-*n*-butylammonium fluoride in THF (TBAF, 0.2 mL) and stirred at room temperature for 1 h. The solution was directly applied to a preparative TLC (Analtech; silica gel, 1500 μm) which was developed with EtOAc as eluant to give 0.0032 g (50%) of 6-fluoro-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (**14**) as a foam.[5a]

**B. With AcOH.** A solution of **13** (0.10 g, 0.251 mmol) in THF (4 mL) was treated with a 4:1:1 mixture of AcOH:THF:water (6 mL) and stirred at room temperature for 16 h. The solution was reduced to dryness under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc as eluant to give 0.0382 g (54%) of 6-nitro-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (**15**) as a solid, mp 148−150 °C. This sample was >96% pure by HPLC analysis with <4% F-ddI identified as the impurity: $^1$H NMR ($CDCl_3$) $\delta$ 2.55 (m, 1 H, H-3′a,b), 3.95 (m, 2 H, H-5′a,b), 4.45 (m, 1 H, H-4′), 5.45 (dm, $J_{2',F}$ = 54.0 Hz, 1 H, H-2′), 6.52 (dd, $J_{1',F}$ = 15.2, $J_{1',2'}$ = 3.7 Hz, 1 H, H-1′), 8.85 (d, $J_{8,F}$ = 1.6 Hz, 1 H, H-8), 9.05 (s, 1 H, H-2); FAB MS *m/z* (relative intensity) 284 ($MH^+$, 100), 166 (b + 2 H, 62); accurate mass positive ion FAB MS *m/z* 284.1092 ($MH^+$, calcd 284.0795). Compound **15** could not be purified completely because of facile hydrolytic conversion to F-ddI. Changing the flash chromatographic eluant from EtOAc to 10% MeOH in $CH_2Cl_2$ also permited the isolation of 0.012 g (19%) of F-ddI which was identified by comparison with an authentic sample.[6]

**Reduction of 6-Nitro-9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-9*H*-purine (15) to the 6-Amino Analog 2.** A suspension of **15** (0.022 g, 0.07 mmol) in EtOH (5 mL) was treated with ammonium formate (0.020 g, 0.31 mmol) and 10% Pd/C (0.020 g), and the mixture was stirred at room

temperature for 2 h. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (Analtech; silica gel, 1500 $\mu$m) using $CH_2Cl_2$:MeOH (9:1) as eluant to give 0.012 g (60%) of **2**: mp 225−227 °C (lit.[6] mp 227 °C) as the major product, and 0.0033 g (17%) of **1** as the minor product, mp 199−201 °C (lit.[6] mp 204 °C).

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(1) Abbreviations: ADA, adenosine deaminase; AIDS, acquired immunodeficiency syndrome; BBB, blood−brain barrier; CNS, central nervous system; dCF, 2′-deoxycoformycin; ddA, 2′,3′-dideoxyadenosine; ddI, 2′,3′-dideoxyinosine; ddN, 2′,3′-dideoxynucleosides; F-ddA, 9-(2,3-dideoxy-2-fluoro-$\beta$-D-*threo*-pentofuranosyl)adenine; F-ddI, 9-(2,3-dideoxy-2-fluoro-$\beta$-D-*threo*-pentofuranosyl)hypoxanthine; F-ddN, 2′-$\beta$-fluoro-2′,3′-dideoxynucleosides; HIV, human immunodeficiency virus type 1; $IC_{50}$, compound concentration inhibiting HIV cytopathogenic effects by 50%; NCI, National Cancer Institute; *P*, octanol/pH 7.4 buffer partition coefficient; PHA-PBM, phytohemagglutinin-stimulated peripheral blood mononuclear cells; PNP, purine nucleoside phosphorylase.

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