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Enhanced Brain Delivery of an Anti-HIV Nucleoside 2′-F-ara-ddI by Xanthine Oxidase Mediated Biotransformation

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In order to enhance the brain delivery of 2′-F-ara-ddI, 2′-F-ara-ddP **6** was synthesized and its *in vitro* and *in vivo* bioconversion reaction studied. For the study, a new efficient synthetic method for 2′-F-ara-ddP **6** was developed from 5-benzoyl-1,2-*O*-isopropylidene-3-deoxyribose **1**. For *in vitro* study 2′-F-ara-ddP was incubated in pH 2, mouse liver homogenate, and mouse serum at 37 °C. No degradation was observed in pH 2 and serum, while in liver homogenate 2′-F-ara-ddP was almost completely converted to 2′-F-ara-ddI within 20 min ($t_{1/2}$ = 3.54 min). In order to determine the role of xanthine oxidase in the conversion of 2′-F-ara-ddP to 2′-F-ara-ddI, *in vitro* studies were conducted in phosphate buffer (pH 7.4) in the presence or absence of allopurinol, in which the half-lives of 2′-F-ara-ddP were 7.4 and 3.4 h, respectively, indicating the conversions were catalyzed by the xanthine oxidase. A similar experiment with aldehyde oxidase isolated from the human liver did not affect the biotransformation. The biotransformation was also detected in the brain homogenate, although the rate of conversion was low and incomplete. In order to assess the bioconversion *in vivo*, pharmacokinetic studies of 2′-F-ara-ddP and 2′-F-ara-ddI were conducted in mice. The maximum serum concentrations of 2′-F-ara-ddI administered itself and as 2′-F-ara-ddP reached 48.1 ± 10.00 and 89.3 ± 26.0 μM and were observed in 1 and 0.25 h, respectively. The data indicate that 2′-F-ara-ddI is absorbed at a slower rate than that of 2′-F-ara-ddP. The bioavailability of the prodrug after oral administration was 60.7%. The concentration of 2′-F-ara-ddI following oral administration of 2′-ara-ddI was close to the detection limits while 2′-F-ara-ddI was detected at significantly higher concentrations in the brain after oral administration of 2′-F-ara-ddP. From this study, we have administered the enhanced brain delivery of anti-HIV nucleoside utilizing an *in vivo* biotransformation system.

A significant number of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex develop neurological complications due to the infection caused by human immunodeficiency virus (HIV) in the central nervous system (CNS).[1-10] The progressive HIV infection in the CNS is described as an "AIDS dementia complex" characterized by abnormalities in cognition, motor performance, behavior, and other brain defects. It has been observed that the progression of the HIV infection in children, including the dimentia, is faster than in adults in that the incubation period from initial HIV infection to the onset of progressive encephalopathy varied from 2 months to 5 years.[11] It is well documented that HIV has been isolated from the cerebrospinal fluid (CSF) of AIDS patients as well as from brains of postmortem AIDS patients.[12,13] Although its pathogenesis is not well understood, the HIV brain infection is confined mainly to macrophages, microglia, and multinucleated cells that are formed by virus-induced cell fusion.[14] It has been proposed that HIV is carried into the brain by infected monocytes/macrophages.[5-7,15] Regarding the CNS dysfunction by the HIV infection, Brenneman *et al.*[16] suggested that the neuronal deficits associated with HIV may not be entirely a result of infectivity but that gp 120 shed from HIV could directly produce the neuropathology as a result of its interference with endogenous neurotropic substances.

Regardless of the mechanism by which HIV causes the CNS dysfunction, it is critical that anti-HIV agents penetrate the blood–brain barrier (BBB) and suppress HIV replication in order to alleviate the CNS dysfunction. However, most available chemotherapeutic agents either do not cross the BBB or cross only to a small extent. Despite this general rule, there is evidence that 3′-azido-3′-deoxythymidine (AZT) can penetrate cerebrospinal fluid (CSF).[17] More importantly, it has been reported that AZT can partially reverse the HIV-induced neurological complications[18,19] and the brain metabolic abnormalities.[20] Since the dementia caused by HIV can be reversible in certain individuals, questions have been raised whether the dementia is a metabolic problem.[21] Although AZT seems to penetrate the BBB and partially reverse the dementia complex, the dosage currently used to treat AIDS may not be adequate to suppress the viral replication and prevent the infection in the CNS.[22] Furthermore, two other AIDS chemotherapeutic agents, ddI and ddC, do not seem to penetrate the BBB to any significant extent.[23] Therefore, it is of interest to develop nontoxic anti-HIV prodrugs that can more readily penetrate the BBB than the parent nucleosides and maintain effective brain concentration of parent nucleosides.

Various strategies for increasing drug delivery to the brain have been reported.[24] However, the prodrug approach seems to be the most suitable for anti-HIV nucleosides. For this approach to be successful, the modified nucleosides as lipophilic prodrugs must diffuse through the BBB with subsequent conversion to the parent compounds by simple chemical and/or enzymatic cleavage.

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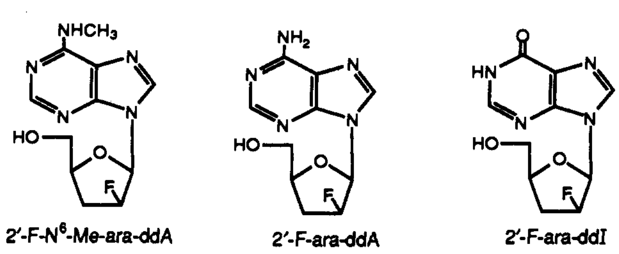


Figure 1.

Bodor *et al.*[25] developed an approach utilizing a dihydropyridine (DHP)–pyridinium salt redox system in which a biologically active compound linked to a lipophilic DHP carrier can readily penetrate the BBB.

Recently, this concept has been explored for several anti-HIV nucleosides. For example, AZT (3′-azido-3′-deoxythymidine), d4T (2′,3′-didehydro-2′,3′-dideoxythymidine), and AZdU (3′-azido-2′,3′-dideoxyuridine) have been modified to dihydropyridine derivatives and *in vitro* as well as *in vivo* studies conducted.[26–35]

In vivo studies indicated that significantly greater amounts of AZT, AZdU, d4T, and ddC could be delivered to the brain following prodrug administration than the parent drugs.[27,30,32–35] However, the major limitation of the approach was found to be the facile oxidation of the DHP-modified prodrugs by oxygen in air as well as in solution, which made even iv formulation difficult. Due to the instability, an oral formulation has not been studied with the DHP-modified prodrugs. Other types of AZT prodrugs have also been synthesized and *in vitro* as well as *in vivo* characteristics studied that include AZT derivatives of glycosyl phosphotriester,[34] esters of fatty acids,[36,37] and retinoic acid.[31] By utilizing these prodrugs, the increased brain delivery and/or the increased half-life of AZT has been achieved to a certain extent.

Prodrugs of other anti-HIV nucleosides have been synthesized which include 2′,3′-dideoxy-6-halopurines[38–41] and 2′-fluoro-2′,3′-dideoxy-6-halopurines.[42–44] These lipophilic compounds of purine derivatives have been synthesized to improve brain delivery.

In this paper we report a biochemical approach to enhance the brain delivery of a ddI derivative that utilizes the brain drug metabolizing system. Although ddI has been approved by the FDA as an anti-HIV drug for the treatment of patients with AIDS and AIDS related complex, who cannot tolerate or develop resistance against AZT, its lack of stability in acidic media by oral administration coupled with side effects such as pancreatitis and peripheral neuropathy limit its usefulness as an orally active anti-HIV drug.[23] Our laboratories and others have been interested in developing anti-HIV nucleosides overcoming these disadvantages of ddI.[42–44] As a part of these efforts, we have reported the synthesis of 2′,3′-dideoxy-N^6-methyladenosine and found it to be the most potent compound among purine nucleosides in peripheral blood mononuclear (PBM) cells.[44] However, like ddA it was found to be unstable in acidic medium, resulting in the glycosyl bond cleavage. Thus, in order to increase the acid stability of 2′,3′-dideoxy-N^6-methyladenosine, the 2′-fluoro derivative was synthesized.[44] However, in PBM cells the anti-HIV activity of the 2′,3′-dideoxy-2′-fluoro-N^6-methylarabinofuranosyladenosine (N^6-Me-2′-F-ara-ddA) was not as potent as that of 2′,3′-dideoxy-N^6-methyladenosine[44] (Figure 1). Marquez *et al.*[42] have also synthesized 2′-fluoropurine nucleosides to overcome the acid instability of anti-HIV purine nucleosides. They have reported that 2′-F-ara-ddA and 2′-F-ara-ddI were as potent as the parent compounds (ddA and ddI) in protecting CD4 + ATH8 cells from the cytopathogenic effects of HIV-1 and were stable in acidic conditions. Since ddI does not significantly cross the BBB,[23] our strategy was to design a prodrug which is more lipophilic and could be converted to ddI after cell penetration by utilizing the *in vivo* biotransformation system such as xanthine oxidase. It should be noted that the xanthine oxidase has been known to be present in the brain.[45] The first compound, which we prepared to test the concept, was 2′,3′-dideoxyribofuranosylpurine (ddP),[44] which lacked a substitution at C6-position and was expected to be converted to ddI by the xanthine oxidase *in vivo* (Figure 2). Previously, the xanthine oxidase system has been exploited to increase the bioavailability and solubility of acyclovir.[47,48] However, ddP was found to be as unstable in acidic conditions (pH 2) ($t_{1/2}$ = 0.82 min) as ddI or ddA. As a consequence, acid-stable 2′-F-ara-ddP (Figure 2) was prepared as shown in Scheme 1 to study the *in vivo* pharmacokinetics and biotransformation to 2′-F-ara-ddI in mice. The 2′-F-ara-ddP was previously prepared by Barchi *et al.*[43] from the

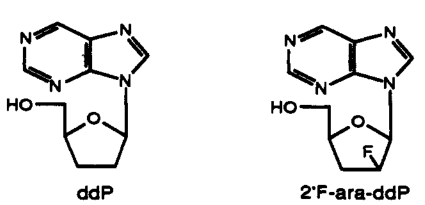
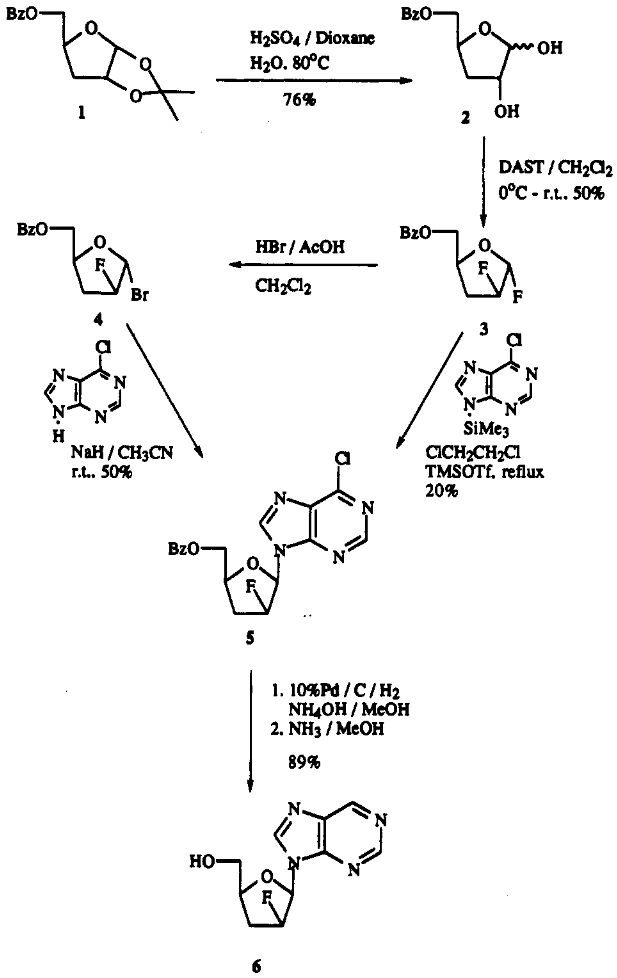
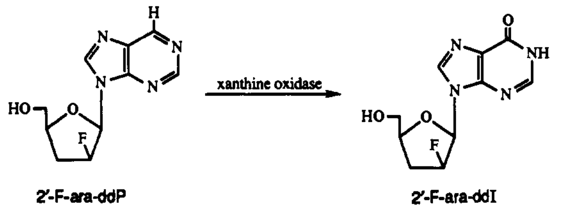


Figure 2.

Scheme 1



Scheme 2



deoxygenation of 3′-OH and found to be inactive in the ATH8 cells system. It should be mentioned that 2′-F-ara-ddI is undergoing clinical trials, as an anti-HIV agent.

Results and Discussion

The desired compound, 2′-F-ara-ddP **6** was synthesized from **1**, which could be readily prepared from 1,2:5,6-diacetone-glucose (Scheme 1). The 1,2-isopropylidene group of **1** was removed by sulfuric acid in dioxane at 80 °C to obtain the 1,2-dihydroxy derivative **2**, which was treated with (diethylamino)sulfur trifluoride (DAST) to give the 1,2-difluoro derivative **3**. The difluoro sugar **3** was directly condensed with the silylated 6-chloropurine using TMSOTf as a catalyst to obtain the 5′-benzoyl-protected nucleoside **5**. Although this procedure gave the desired condensed product **5**, it not only required refluxing temperature but also produced a low yield (20%). Thus, the 1,2-difluoride **3** was converted to the bromo derivative **4**, which was condensed with the 6-chloropurine using the sodium salt method in CH_3CN to obtain an improved yield (50%). The final product **6** was prepared by dehalogenation of the 6-chloro group of **5** by treating with 10% Pd–C/H_2 followed by debenzoylation with methanolic ammonia. Although the compound **6** was previously synthesized by deoxygenation of the 3′-hydroxy group at the nucleoside stage,[43] the procedure reported in Scheme 1 is not only being readily adapted for a multigram synthesis, but also the method can be used for the synthesis of other similar 2′-fluoro-2′,3′-dideoxy nucleosides.

In order to determine the chemical and biological stabilities, 2′-F-ara-ddP was incubated in pH 2, mouse liver homogenate, and mouse serum at 37 °C for 24 h. No degradation was observed in pH 2 and in the serum. However, in mouse liver homogenate 2′-F-ara-ddP was almost completely converted to 2′-F-ara-ddI within 20 min ($t^{1/2}$ = 3.54 min), which suggested that extensive enzymatic oxidation, possibly by xanthine oxidase, occurred (Scheme 2). In order to determine the role of xanthine oxidase in the conversion of 2′-F-ara-ddP to 2′-F-ara-ddI, *in vitro* stability studies of 2′-F-ara-ddP were conducted in phosphate buffer (pH 7.4) in the presence or absence of the xanthine oxidase inhibitor, allopurinol (Figure 3). The half-lives of 2′-F-ara-ddP in the absence and presence of the allopurinol was 7.4 and 3.4 h, respectively, indicating that the 2′-F-ara-ddP to 2′-F-ara-ddI conversion in the liver can be catalyzed by the xanthine oxidase biotransformation system.

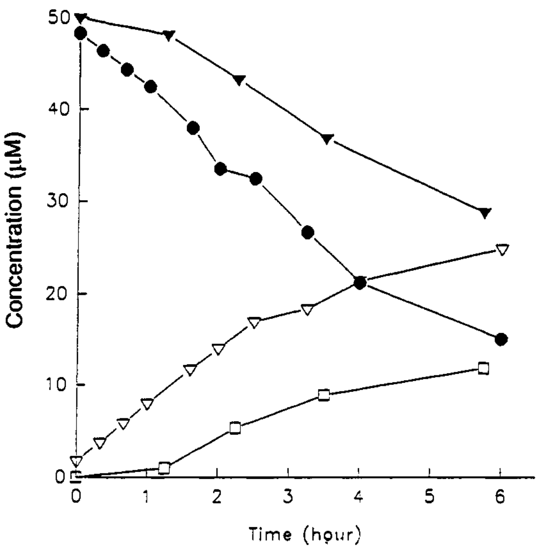


Figure 3. Kinetics of 2′-F-ara-ddP disappearance (●, ▲) and 2′-F-ara-ddI formation (▽, □) in absence or presence of 50 μM allopurinol, respectively, in physiological phosphate buffer, pH 7.4, at 37 °C catalyzed by xanthine oxidase (0.2 unit/mL). (Each data points the mean + SD.)

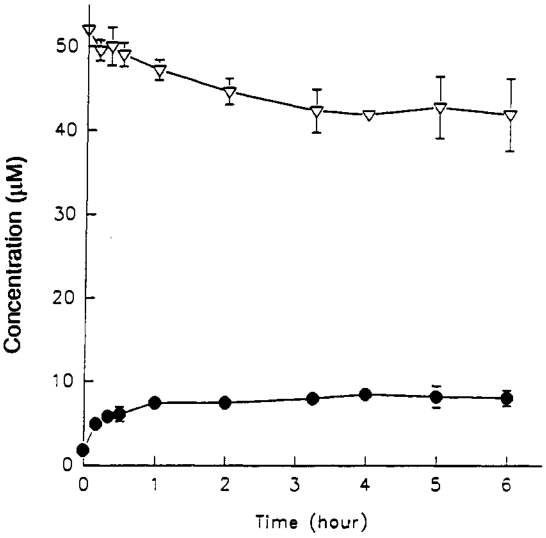


Figure 4. Conversion of 2′-F-ara-ddP (▽) to 2′-F-ara-ddI (●) in brain homogenate at 37 °C.

Since 2′-F-ara-ddP is more hydrophobic than 2′-F-ara-ddI, we expected that 2′-F-ara-ddP can penetrate the BBB more readily and be converted to the active anti-HIV nucleoside, 2′-F-ara-ddI, provided that xanthine oxidase exists in the brain and can oxidize the 2′-F-ara-ddP.[45] In order to test this hypothesis, studies of 2′-F-ara-ddP in mouse brain homogenate were conducted which demonstrated the biotransformation of 2′-F-ara-ddP to 2′-F-ara-ddI and the rate of 2′-F-ara-ddI formation corresponded to the rate of 2′-F-ara-ddP disappearance as shown in Figure 4. However, only a maximum of 15.6% of 2′-F-ara-ddP was converted to 2′-F-ara-ddI, and the reaction was completed within 1 h. Longer incubation did not lead to a higher percentage of the conversion. The low percentage of 2′-F-ara-ddP oxidation in brain homogenate may be due to a number of reasons, such as (a) nonspecific binding of 2′-F-ara-ddP, (b) inactivation of the enzyme during incubation, (c) inhibition of the enzyme by products of the reaction. Regardless of reasons for the low conversion to the ddI derivatives, it is interesting to know that 2′-F-ara-ddP is biotransformed to its inosine derivative. In order to determine the role of the xanthine oxidase in the metabolism of 2′-F-ara-ddP in brain homogenate, various concentrations of allopurinol were added to the brain homogenate along with the 2′-F-ara-ddP. However, the biotransformation of 2′-F-ara-ddP was not inhibited by allopurinol at 50 or 500 μM. The ineffectiveness of allopurinol in inhibiting the bioconversion is not clear; however, it might be due to nonspecific binding of allopurinol to brain homogenate, thus pre-

Table 1. Pharmacokinetic Parameters of 2′-F-ara-ddP and 2′-F-ara-ddI in Mice after Oral Administration at Doses 100 (420 μM) and 105.1 (414 μM) mg/kg, respectively

	Serum			brain			
compound	AUC (μmol L)/h	$t_{1/2}$, h	F, %	AUC (μmol L)/h	$t_{1/2}$, h	Cl,[b] L/kg	V_{SS},[c] L/kg
2′F-ara-ddP (oral)	153	0.79	60.7	32.3	0.65	2.9	2.32
2′F-ara-ddP (iv)	252	0.4		120	0.42	1.76	1.12
2′F-ara-ddI (oral)	154	4.28		ND[a]	ND	2.88	14.5
2′F-ara-ddI (from 2′-F-ara-ddP oral)	134	1.73		47.9	0.93		
2′F-ara-ddI (from 2′-F-ara-ddP iv)	132	0.39		48	0.64		

[a] ND = nondetectable. [b] Cl values for oral administrations are apparent clearances (i.e. Cl/F). [c] V_{SS} values for oral administrations are apparent volumes of distribution (i.e. V_{SS}/F).

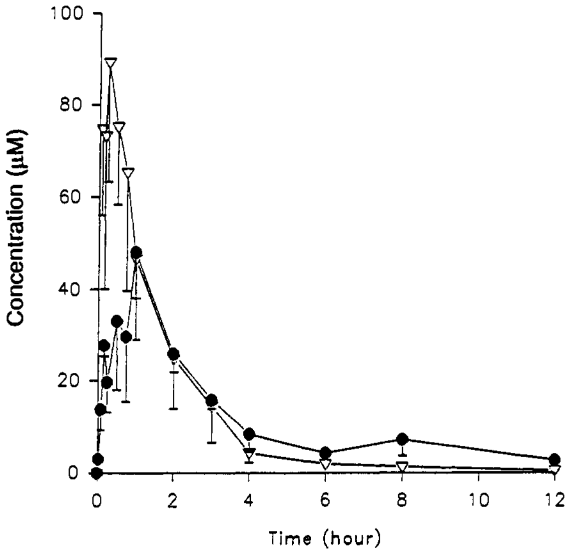


Figure 5. 2′-F-ara-ddI serum concentrations following 100 mg/kg of 2′-F-ara-ddP (▽) or 105.1 mg/kg of 2′-F-ara-ddI (●) oral administration in mice. (The data are expressed as mean + SD.)

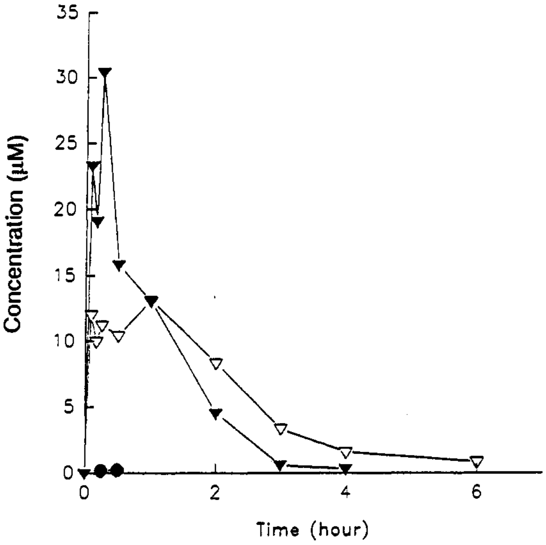


Figure 6. 2′-F-ara-ddI (▽) and 2′-F-ara-ddP (▼) concentrations in mouse brain after oral administration of 2′-F-ara-ddP at dose 100 mg/mL. (●) Brain concentration of 2′-F-ara-ddI administered itself at equimolar dose.

venting its inhibition of xanthine oxidase. Aldehyde oxidase (specific activity: 3.4 nM/mg of protein per min), isolated from human liver, was also explored for its ability to catalyze the oxidation of the 2′-F-ara-ddP.[50] This enzyme catalyzes the oxidation of a variety of aldehydes as well as a number of non-aldehyde heterocyclic compounds including 5-substituted pyrimidinone.[50] However, the aldehyde oxidase failed to convert 2′-F-ara-ddP to 2′-F-ara-ddI under the conditions described.

The results of *in vitro* experiments in buffer, liver, and brain homogenate encouraged us to conduct comparative pharmacokinetic studies of 2′-F-ara-ddP and 2′-F-ara-ddI in mice (Table 1). The serum concentration–time profiles of 2′-F-ara-ddI after single oral administration of 2′-F-ara-ddI and 2′-F-ara-ddP at equimolar doses are shown in Figure 5. The maximum serum concentrations of 2′-F-ara-ddI administered itself and as 2′-F-ara-ddP reached 48.1 ± 10.0 and 89.3 ± 26.0 μM (mean ± SD) and were observed in 1 and 0.25 h, respectively. These data suggest that 2′-F-ara-ddI is absorbed at a slower rate compared to the more lipophilic 2′-F-ara-ddP. To evaluate the bioavailabilities of 2′-F-ara-ddP and 2′-F-ara-ddI after the prodrug oral administration, iv studies of 2′-F-ara-ddP were conducted. Interestingly, the bioavailability of the prodrug after oral administration was 60.7%, whereas the AUCs for 2′-F-ara-ddI following iv and oral prodrug administration were found to be very similar in serum (132 and 134 (μmol h)/L, respectively), as well as in the brain (48 and 47.9 (μmol h)/L, respectively). The terminal half-life of 2′-F-ara-ddI after iv administration of 2′-F-ara-ddP was the same as that of the prodrug (0.39 and 0.4 h, respectively), indicative of formation-rate limited elimination. The difference in AUCs of 2′-F-ara-ddP after iv and oral administration is either due to first-pass metabolism of the prodrug or incomplete absorption, although the former is more likely.

Figure 6 shows concentrations of 2′-F-ara-ddI and 2′-F-ara-ddP in the brain following oral administrations of the compounds. The concentrations of 2′-F-ara-ddI following oral administration of 2′-F-ara-ddI was close to the detection limits and could be measured at only two time points. This indicates very low permeability of the blood–brain barrier for 2′-F-ara-ddI. On the contrary, 2′-F-ara-ddP was detected at significantly higher concentrations in the brain, suggesting the enhanced BBB permeability of 2′-F-ara-ddP. Figure 6 also shows that the 2′-F-ara-ddI brain concentration–time profile after prodrug administration. The mean maximum brain concentration of 2′-F-ara-ddI following prodrug administration was approximately half of that of 2′-F-ara-ddP (13.3 vs 30.5 μM); however, 2′-F-ara-ddI was detected for a longer time than 2′-F-ara-ddP. Comparison of the AUCs for 2′-F-ara-ddI (47.9 (μmol h)/L) and 2′-F-ara-ddP (32.3 (μmol h)/L) as well as the apparent brain half-lives (0.65 and 0.93 h, respectively) also indicate an increased retention of 2′-F-ara-ddI in the brain tissue following prodrug administration. These results suggest a slower efflux of 2′-F-ara-ddI from the brain compared to the conversion rate of 2′-F-ara-ddP to 2′-F-ara-ddI in the brain.

In summary, in an effort to increase the brain delivery of anti-HIV nucleosides, a prodrug, 2′-F-ara-ddP, was synthesized, and its *in vitro* and *in vivo* biotransformation to 2′-F-ara-ddI, the active anti-HIV agent, was studied. From this study, we have demonstrated that xanthine oxidase was responsible for the metabolism of the prodrug based on *in vitro* studies in serum. However, the enzymatic basis of this reaction in the brain or *in vivo* has not been elucidated. The 2′-F-ara-ddP prodrug does warrant further investigation based on its stability following oral administration and ability to increase 2′-F-ara-ddI brain concentrations. Further biological and biochemical studies of 2′-F-ara-ddP, including *in vivo* pharmacokinetic studies in monkeys, are planned and will be reported elsewhere.

Experimental Section

Chemical Synthesis. Melting points were determined on a Mel-temp II laboratory device and uncorrected. The ^{1}H NMR spectra were recorded on a JEOL FX 90 Q or Bruker 300 fourier transform spectrometer for 90- or 300-MHz ^{1}H NMR spectra, respectively, with Me_4Si as internal standard; chemical shifts are reported in parts per million (δ), and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). UV spectra were obtained on a Beckman DU-7 spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, or Galbraith Laboratories, Inc., Knoxville, TN.

5-*O*-Benzoyl-3-deoxy-D-ribofuranose (2). A solution of **1** (10.0 g, 35.97 mmol) in 75% aqueous dioxane (200 mL) and concentrated H_2SO_4 (1.50 mL) was stirred at 70–80 °C for 4 h. The reaction mixture was cooled in an ice bath, neutralized with solid $NaHCO_3$, filtered, and evaporated to give a crude product. The crude product was purified by silica gel column chromatography ($CHCl_3$–MeOH, 20:1) to yield **2** as a pale yellow oil, which was crystallized from CH_2Cl_2–hexane to give **2** (6.5 g, 76%) as white crystals: mp 71–72 °C; ^{1}H NMR (DMSO-d_6) δ 1.82–1.97 (m, 2H, H-3), 3.98 (d, $J_{2,3}$ = 3.5 Hz, 1H, H-2), 4.23 (dd, $J_{5a,5b}$ = 11.4 and $J_{5a,4}$ = 6.9 Hz, 1H, H_a-5), 4.37 (dd, $J_{5b,5a}$ = 11.4 and $J_{5b,4}$ = 2.96 Hz, 1H, H_b-5), 4.46 (m, 1H, H-4), 5.05 (br s, 2H, H-1 and OH, becomes sharp singlet after D_2O exchange), 6.22 (br s, 1H, OH), 7.52–8.02 (m, 5H, Ar). Anal. ($C_{12}H_{14}O_5$) C, H.

5-*O*-Benzoyl-2,3-dideoxy-1,2-difluoro-*α*-D-arabinofuranose (3). To a solution of **2** (10.0 g, 42.04 mmol) in dry CH_2Cl_2 under nitrogen atmosphere at −78 °C was added slowly with stirring DAST (22.2 mL, 168.06 mmol). The reaction mixture was stirred at −78 °C for an additional 15 min, and the temperature was raised to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C, aqueous $NaHCO_3$ solution was added, and the mixture was stirred for 30 min. The organic layer was separated, washed with water, dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness to give an oil, which was purified by silica gel column chromatography (hexane–EtOAc, 9:1) to yield pure **3** (5.06 g, 49.8%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.14 (qd, $J_{3a,F}$ = 28.7, $J_{3a,3b}$ = 15.0 and $J_{3a,4}$ = 2.9, $J_{3a,2}$ = 2.9 Hz, 1H, H_a-3), 2.55 (m, 1H, H_b-3), 4.43 (dd, $J_{5a,5b}$ = 12.0 and $J_{5a,4}$ = 6.2 Hz, 1H, H_a-5), 4.49 (dd $J_{5b,5a}$ = 11.9 and $J_{5b,4}$ = 4.0 Hz, 1H, H_b-5), 4.79 (m, 1H, H-4), 5.17 (dm, $J_{2,F}$ = 51.7 Hz, 1H, H-2), 5.95 (dd, $J_{1,F}$ = 59.9 and $J_{1,2}$ = 5.0, 1H, H-1), 7.42–8.09 (m, 5H, Ar); $[\alpha]^{25}_D$ +44.77 (*c* 0.6, MeOH). Anal. ($C_{12}H_{14}O_5$) C, H, F.

5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-*α*-D-arabinofuranosyl Bromide (4). To a solution of **3** (5.0 g, 20.66 mmol) in CH_2Cl_2 (50 mL) was added 30% HBr in AcOH (20 mL, 103.3 mmol, 45% w/v), and the reaction mixture was stirred at room temperature for 2 h. TLC (hexane–EtOAc, 6:1) indicated a very polar compound and a slightly nonpolar compound compared to the starting material. (After workup all the nonpolar compound was converted to the polar compound). The reaction mixture was concentrated in vacuo and dried under high vacuum to remove AcOH. The residue was dissolved in CH_2Cl_2 (100 mL), washed with water and saturated $NaHCO_3$ solution, dried (Na_2SO_4), filtered, and concentrated to give **9** which was used in the condensation step without further purification.

6-Chloro-9-(5-*O*-benzoyl-2,3-dideoxy-2-fluoro-*β*-D-arabinofuranosyl)purine (5). A mixture of 6-chloropurine (6.38 g, 41.32 mmol, 2 equiv) and NaH (1.98 g, 49.58 mmol, 2.2 equiv, 60% suspension in oil) was stirred in anhydrous acetonitrile (50 mL) under a nitrogen atmosphere at room temperature for 30 min. Freshly prepared bromo sugar **4** (1 equiv) in acetonitrile (50 mL) was added to the reaction mixture, and stirring was continued for 3 h. The reaction mixture was diluted with $CHCl_3$ (100 mL) and filtered through a pad of Celite, and the Celite pad was washed with $CHCl_3$. The combined filtrates were concentrated, redissolved in EtOAc, and washed with water and saturated NaCl solution. After drying (Na_2SO_4), it was filtered and concentrated to give crude **5**, which was purified on silica gel column chromatographty (hexane–EtOAc, 2.5:1) to give pure **5** (3.80 g, 48.8%) as a white solid. SMall amounts of the 9α isomer[42] (0.38 g, 4.8%) were also isolated: mp 129 °C (lit.[42] mp 129–130 °C); UV (MeOH) λ_{max} 263.5 nm; $[\alpha]^{25}_D$ +73.8 (*c* 0.5, MeOH).

9-(2,3-Dideoxy-2-fluoro-*β*-D-arabinofuranosyl)purine (6). To a solution of **5** (1.40 g, 3.72 mmol) in MeOH–THF (150:5 mL) were added NH_4OH (5 mL) and 10% Pd–C (0.20 g), and the mixture was stirred at room temperature under 50 psi hydrogen for 3 h. Then the reaction mixture was filtered through Celite, the solvent was evaporated, NH_3/MeOH (100 mL, saturated at 0 °C) was added, and the mixture was stirred overnight. After the solvent was evaporated, the residue obtained was purified by silica gel column chromatography ($CHCl_3$–MeOH, 20:1) to yield chromotographically pure **6**, which was crystallized from acetone–ether to give **6** (0.79 g, 89%) as white crystals: mp 113–114 °C (lit.[43] mp 93–95 °C); ^{1}H NMR (DMSO-d_6) δ 9.23 (s, 1H, H-6 or H-2), 8.99 (s, 1H, H-2 or H-6), 8.80 (s, 1H, H-8), 6.53 (dd, J = 3.8 and 18.4 Hz, 1′-H), 5.54 (dm, $J_{2'H,F}$ = 54.4 Hz, 1 H, 2′-H), 5.11 (t, J = 5.6 Hz, 1H, D_2O exchangeable, 5′-H), 4.24 (m, 1H, 4′-H), 3.64 (m, 2 H, 5′-H and 5″-H), 2.51–2.75 (m, 1H, 3′-H), 2.17–2.45 (m, 1H, 3″-H); ^{13}C NMR (DMSO-d_6) δ 152.3, 150.6, 148.2, 145.4, 133.3, 92.8, 89.8, 83.6, 83.4, 78.2, 62.7, 32.2, 32; MS (EI) *m/z* (relative intensity) 309 (MH^+, 65), 308 (M^+, 48), 208 (100); UV (MeOH) λ_{max} 262 (ϵ 7336), 243 (sh, ϵ 4331), 208.5 nm (ϵ 8405); λ_{min} 220 (ϵ 1632); IR (KBr) 3376, 1582, 1495, 1408, 1211, 1126 cm^{-1}; $[\alpha]^{20}_D$ +63.0 (*c* 0.51, MeOH) [lit.[43] $[\alpha]^{20}_D$ +38.1 (*c* 1.8, MeOH)]. Anal. ($C_{10}H_{11}FN_4O_2$) C, H, N, F.

***In Vitro* Stability Studies in Serum, Brain, and Liver Homogenate.** Samples of mouse serum, liver, or brain homogenate (1.5 mL each) prepared in a 1:1 (g:mL) ratio with isotonic phosphate buffer, pH 7.4, which contain 2′-F-ara-ddP at the concentration of 50 μM, were incubated at 37 °C under mixing. Aliquots of 50 mL were removed at time 0 and for up to 8 h after adding 2′-F-ara-ddP to measure 2′-F-ara-ddP and 2′-F-ara-ddI concentrations.

Enzymatic Oxidation of 2′-F-ara-ddP. Samples (1.5-mL) of 2′-F-ara-ddP (50 μM) were prepared in 0.05 M isotonic phosphate buffer (pH 7.4) in the absence or presence of the xanthine oxidase inhibitor allopurinol (50 μM) and placed into a shaking water bath (37 °C). Reaction was initiated by addition of 15 μL of xanthine oxidase (from buttermilk; purchased from Sigma Chemical Co.) solution (final activity in incubation media was 0.2 unit/mL: 1 unit is the amount of enzyme which converts xanthine to uric acid at the rate of 1.0 μmol/min at 25 °C and pH 7.5). At specified time intervals, aliquots of 50 μL were withdrawn to analyze 2′-F-ara-ddP and 2′-F-ara-ddI concentration.

Animal Studies. Female NIH-Swiss mice (Harland Farms) weighing 24–28 g were housed in a 12 h light/12 h dark constant-temperature (20 °C) environment and had free access to standard laboratory chow and water. 2′-F-ara-ddP and 2′-F-ara-ddI solutions (15 mg/mL) were prepared in physiological saline and administered intragastrically (2′-F-ara-ddP and 2′-F-ara-ddI) or intravenously (2′-F-ara-ddP) through a tail vein over 30 s at a dose of 100 or 106.8 mg/kg (equimolar to 2′-F-ara-ddP dose). Animals were placed in individual cages and allowed food and water *ad libitum*. Mice (three animals per each time point) were killed by exsanguination *via* a left ventricle heart puncture after anesthetization with diethyl ether. Serum was harvested from blood collected from the heart. The brain was excised, rinsed with normal saline, blotted dry, and weighed. The brain and serum samples were stored at −20 °C until analyzed.

Analysis of 2′-F-ara-ddP and 2′-F-ara-ddI. The brain or liver tissue was thawed and homogenized in a 1:1 (g:mL) ratio with ice-cold isotonic phosphate buffer (pH 7.4). A buffer, serum, or tissue homogenate aliquot of 50 μL was mixed with 10 μL of internal standard solution (3′-azido-2′,3′-dideoxyuridine, 25 μg/mL). Acetonitrile (600 μL) containing 0.1% acetic acid was added while vortexing to precipitate proteins. The tubes were centrifuged at 3000*g* for 5 min. Supernatant obtained from centrifugation was evaporated to dryness under a nitrogen stream. The residual film was reconstituted in 60 μL of mobile phase, and an aliquot of 50 μL was injected into HPLC.

HPLC Conditions. Chromatographic separations were performed on a Hypersil ODS column (Alltech Associates, 150 × 4.5 mm, 5-μm particle size) preceded by a guard column packed with 30–40-μm pellicular Perisorb RP-18. The mobile phase consisted of 4.3% (v/v) acetonitrile, 40 mM sodium acetate, and 10 mM K_2HPO_4, pH 7.2. The mobile-phase flow rate was 2 mL/min, and the detection wavelength was 260 nm. Under the chromatographic conditions described, 2′-F-ara-ddI, 2′-F-ara-ddP, and internal standard retention times were 5.85, 12.66, and 12.21 min, respectively. The sensitivity limit for 2′-F-ara-ddI and 2′-F-ara-ddP (signal-to-noise ratio of 3:1) was 50 and 100 ng/mL, respectively, in each biological media. The intra- and interassay coefficient of variation for each compound was less than 5% in buffer and less than 8% in serum and tissue homogenates.

Data Analysis. Pharmacokinetic parameters for 2′-F-ara-ddP and 2′-F-ara-ddI were calculated by noncompartmental techniques. The area under the plasma concentration–time curve (AUC) was determined using a Lagrange polynomial integration method.[51] Bioavailability (F) was determined as AUC_{or}/AUC_{iv} where AUC_{iv} and AUC_{or} are the areas under the serum concentration–time curve at oral and intravenous administration, respectively. The elimination half-life ($t_{1/2}$) was found as: $t_{1/2} = 0.693/K$, where K is the terminal elimination rate constant equal to the slope obtained from the equation of linear regression for the natural log of the concentration versus time values in the terminal phase. The rate constants of conversion of the prodrug to the parent drug in tissue homogenate were assumed to be first-order. Rate equations were fit by nonlinear regression methods.[52]

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