Notes

In Vitro and *in Vivo* Evaluation of 6-Azido-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosylpurine and N^6-Methyl-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyladenine as Prodrugs of the Anti-HIV Nucleosides 2′-F-ara-ddA and 2′-F-ara-ddI

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In an effort to improve the pharmacokinetic properties and tissue distribution of 2′-F-ara-ddI, two lipophilic prodrugs, 6-azido-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosylpurine (FAAddP, **4**) and N^6-methyl-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyladenine (FMAddA, **5**), were synthesized and their biotransformation was investigated *in vitro* and *in vivo*, in mice. Compounds **4** and **5** were synthesized *via* the intermediate **2**. For the *in vitro* studies, FAAddP and FMAddA were incubated in mouse serum, liver homogenate, and brain homogenate. FAAddP was metabolized in liver homogenate by the reduction of the azido to the amino moiety followed by deamination, yielding 2′-F-ara-ddI. The conversion of FAAddP to 2′-F-ara-ddA was mediated by microsomal P-450 NADPH reductase system, as shown by the liver microsomal assay. FAAddP was also converted to 2′-F-ara-ddI at a slower rate in the brain than in the liver. FMAddA, however, was stable in brain homogenate and was slowly metabolized in the liver homogenate. Metabolic conversion of FMAddA *in vitro* was stimulated by the addition of adenosine deaminase. In the *in vivo* metabolism study, FAAddP underwent reduction to 2′-F-ara-ddA followed by deamination to 2′-F-ara-ddI. FMAddA did not result in increased brain delivery of 2′-F-ara-ddI *in vivo*, probably due to the slow conversion as observed in the *in vitro* studies. However, there was an increase in the half-life of 2′-F-ara-ddI produced from FMAddA. This report is the first example in the design of prodrugs using the azido group for adenine- and hypoxanthine-containing nucleosides. This interesting and novel approach can be extended to other antiviral and anticancer nucleosides.

Introduction

Didanosine (ddI) is a synthetic nucleoside structurally related to inosine with proven activity against human immunodeficiency virus (HIV).[1] It is approved for use in patients who are intolerant to zidovudine (AZT) or who have developed drug resistance to zidovudine therapy.[2] However, its various side effects,[2] chemical instability in gastric acid, and low oral bioavailability[3] limit the usefulness of ddI. Furthermore, there is evidence that ddI enters the central nervous system and the cerebrospinal fluid (CSF) less readily than AZT. The extent of ddI uptake in brain tissue and CSF relative to that in plasma was only 4.7 and 1.5%, respectively.[4−6]

In an effort to overcome the instability of ddI and 2′,3′-dideoxyadenosine (ddA) in acidic conditions, 2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosylhypoxanthine (2′-F-ara-ddI) and 2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyladenine (2′-F-ara-ddA) were synthesized.[7] Marquez *et al.*[7] reported that 2′-F-ara-ddA and 2′-F-ara-ddI were stable in acidic media and were as potent as ddI in protecting $CD4^+$ATH8 cells from the cytopathogenic effects of HIV-1. However, 2′-F-ara-ddI, as well as ddI, is relatively hydrophilic and does not readily penetrate the blood−brain barrier (BBB) in mice.[8] Recently, we synthesized a more lipophilic prodrug, 2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosylpurine (2′-F-ara-ddP), which was converted to 2′-F-ara-ddI by xanthine oxidase *in vivo*.[8] Pharmacokinetic studies indicated that 2′-F-ara-ddP increased the delivery of 2′-F-ara-ddI to the brain in mice with an increased AUC_{brain}/AUC_{serum} ratio of approximately 36% after oral and intravenous administration of 2′-F-ara-ddP.[8]

As a continuation of the development of prodrug strategies in enhancing the brain delivery of anti-HIV nucleosides by utilizing the *in vivo* biotransformation systems, we have synthesized 6-azido-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosylpurine (FAAddP, **4**) and N^6-methyl-2′,3′-dideoxy-2′-fluoro-β-D-arabinofuranosyladenine[16] (FMAddA, **5**) as prodrugs of 2′-F-ara-ddI. The

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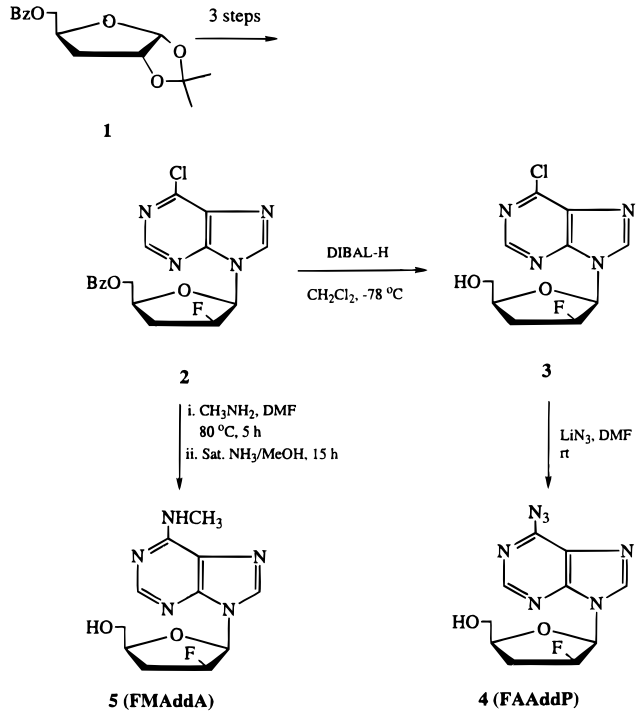
Scheme 1. Synthesis of FAAddP and FMAddP



synthesis, *in vitro* biotransformation, and *in vivo* disposition of the prodrugs are discussed in this report.

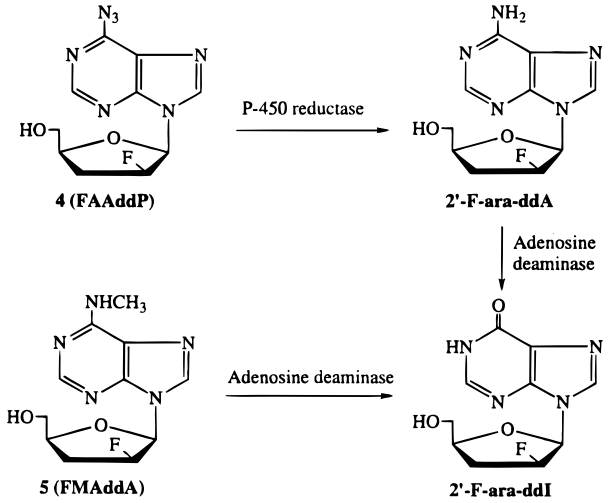
Results and Discussion

The prodrugs FAAddP (**4**) and FMAddA (**5**) were synthesized from the 6-chloropurine derivative **2** (Scheme 1). Compound **2** was synthesized from 5-*O*-benzoyl-3-deoxy-1,2-*O*-isopropylidine-α-D-ribofuranose (**1**) according to published procedures.[8] Compound **2** was debenzoylated using DIBAL-H in CH_2Cl_2 at −78 °C to obtain compound **3**. Upon treatment of compound **3** with LiN_3 in DMF at room temperature, the 6-azido derivative **4** was obtained in 73% yield. The treatment of compound **2** with methylamine in DMF at 80 °C for 5 h followed by the deprotection with saturated NH_3/MeOH for 15 h gave compound **5** quantitatively.[16]

The biotransformation of the prodrug FAAddP to 2′-F-ara-ddI probably involves a two-step metabolic process (Scheme 2). The prodrug was first metabolized to 2′-F-ara-ddA by the P-450 reductase system. A similar reduction of the azido moiety of AZT to an amino function by the cytochrome P-450 system has been recently demonstrated.[10,11] 2′-F-ara-ddA was then metabolized to 2′-F-ara-ddI by adenosine deaminase. The azido reduction assay confirmed that FAAddP was metabolized to 2′-F-ara-ddA and 2′-F-ara-ddI by the microsomal fraction of the human liver homogenate. Furthermore, *in vitro* biotransformation studies showed that direct conversion of FAAddP to 2′-F-ara-ddI by adenosine deaminase occurred at a negligible rate.

FAAddP was stable in phosphate buffer saline (PBS, pH 7.4) at 37 °C, indicating that the compound is not susceptible to chemical hydrolysis. The *in vitro* biotransformation of this prodrug in mouse serum, however, was relatively rapid with a degradation half-life of 2.41 h. Although FAAddP was metabolized in serum, no metabolites were identified. In the liver homogenate, FAAddP concentrations declined in a biphasic fashion.

Scheme 2. Biotransformation of the Prodrug FAAddP to 2′-F-ara-ddI



Over the initial 45 min, the prodrug was rapidly metabolized with a $t_{1/2}$ of 0.48 h. Subsequently, the rate of conversion was much slower ($t_{1/2}$ = 7.74 h) than the initial rate, probably due to the depletion of cofactors. The formation of 2′-F-ara-ddI paralleled the decline of the prodrug with most of the prodrug being converted to 2′-F-ara-ddI. Only low concentrations of 2′-F-ara-ddA were detected in the liver homogenate. The biotransformation of FAAddP in brain homogenate was somewhat slower than that in the liver with a half-life of 6.1 h. However, only 10% of the prodrug was converted to 2′-F-ara-ddI and 5% to 2′-F-ara-ddA over a 6 h time period. Thus, similar to the studies in serum, an unidentified pathway was responsible for the disappearance of FAAddP in mouse brain.

The metabolic conversion of FMAddA to 2′-F-ara-ddI appeared to be a one-step process, facilitated by adenosine deaminase. The prodrug was stable in PBS, mouse serum, and mouse brain homogenate and was slowly metabolized to 2′-F-ara-ddI in the liver homogenate ($t_{1/2}$ = 9.1 h). However, upon addition of adenosine deaminase to PBS ($t_{1/2}$ = 0.46 h) and brain homogenate ($t_{1/2}$ = 3.7 h), virtually all of the prodrug was converted to 2′-F-ara-ddI.

The pharmacokinetics of the active nucleoside, 2′-F-ara-ddI, were investigated in mice. Concentrations of 2′-F-ara-ddI in serum and brain after intravenous administration of 20 mg/kg of the compound are illustrated in Figure 1. Serum concentrations of 2′-F-ara-ddI declined rapidly with a half-life of 0.41 h (Table 1). Brain concentrations of the nucleoside peaked at approximately 20 min, remained relatively constant for 30 min, and subsequently declined in parallel with serum concentrations. Relative brain exposure (r_e) of the 2′-fluoronucleoside was 16.5%. Total clearance of 2′-F-ara-ddI was 2.18 (L/h)/kg and was moderate relative to the hepatic blood flow (5 (L/h)/kg) and renal blood flow (3.6 (L/h)/kg) in mice.[12] Steady-state volume of distribution was 0.78 L/kg, hence indicating that the compound was distributed intracellularly to a moderate extent.

Concentrations of FAAddP, 2′-F-ara-ddA, and 2′-F-ara-ddI in the serum and the brain after intravenous administration of 55 mg/kg of FAAddP are shown in Figure 2A,B. Serum concentrations of the prodrug

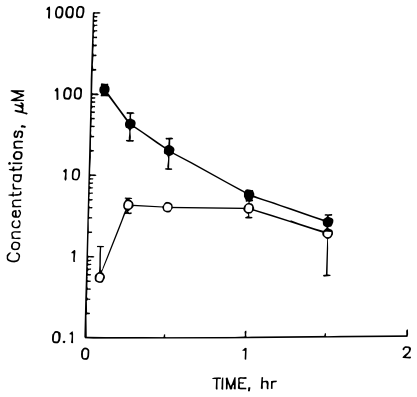


Figure 1. Mean ± SD serum (●) and brain (○) concentrations of 2′-F-ara-ddI after intravenous administration of 20 mg/kg 2′-F-ara-ddI to mice.

declined rapidly with a half-life of 0.22 h (Table 1). Total clearance and steady-state volume of distribution of the prodrug were 1.12 (L/h)/kg and 0.58 L/kg, respectively. Thus, clearance of FAAddP was 2-fold slower than that of 2′-F-ara-ddI, and the distribution was slightly less extensive. In serum samples, low levels of 2′-F-ara-ddA and higher concentrations of 2′-F-ara-ddI were observed. The elimination half-life of 2′-F-ara-ddI after FAAddP administration was longer than that after the administration of 2′-F-ara-ddI. The elimination half-life of 2′-F-ara-ddA was 2.9 h. The higher concentration of 2′-F-ara-ddI compared to 2′-F-ara-ddA is in agreement with the results of the *in vitro* studies. Approximately 30% of the intravenously administered dose of the FAAddP was converted to 2′-F-ara-ddI.

FAAddP distributed rapidly into the brain with peak brain levels observed at the first sampling time (Figure 2B). The relative brain exposure of the prodrug was 6.3%, while exposures of 2′-F-ara-ddA and 2′-F-ara-ddI were 55.8% and 19.7%, respectively. Thus, brain exposure to FAAddP was relatively low, and the relative brain exposure to 2′-F-ara-ddI after intravenous administration of FAAddP was similar to that after administration of 2′-F-ara-ddI. Although the relative brain exposure for 2′-F-ara-ddA was relatively high, brain concentrations were low.

To compare the disposition of 2′-F-ara-ddI after the administration of FAAddP to that after the administration of 2′-F-ara-ddI, AUC (area under curve) values were normalized for dose. As shown in Table 1, the dose-normalized AUC values for 2′-F-ara-ddI in the serum and the brain after intravenous administration of 55 mg/kg of FAAddP were 3−4-fold lower than those after the administration of 20 mg/kg of 2′-F-ara-ddI.

Concentrations of FAAddP, 2′-F-ara-ddA, and 2′-F-ara-ddI in the serum after oral administration of 55 mg/kg FAAddP are depicted in Figure 2C. Absorption of FAAddP was rapid with peak serum concentrations of the compounds achieved 0.5 h after dosing. Oral bioavailability of FAAddP was 19%, indicating incomplete absorption, owing in part to its poor solubility. Brain concentrations of FAAddP, 2′-F-ara-ddA, and 2′-F-ara-ddI were below the limit of quantitation because of the low oral bioavailability of FAAddP. Similar to intravenous study of FAAddP, higher concentrations of 2′-F-ara-ddI when compared to 2′-F-ara-ddA were observed, suggesting that the metabolism of FAAddP to 2′-F-ara-ddA is the rate-limiting step in the formation of 2′-F-ara-ddI.

As with FAAddP, a higher dose of FMAddA had to be administered to measure the levels of 2′-F-ara-ddI. Concentrations of FMAddA and 2′-F-ara-ddI in serum and brain after intravenous administration of 112 mg/kg of FMAddA are depicted in Figure 3. Serum concentrations of FMAddA declined rapidly with a half-life of 0.46 h (Table 1). Total clearance (1.93 (L/h)/kg) and steady-state volume of distribution of FMAddA (0.79 L/kg) were similar to those of 2′-F-ara-ddI. Only 5.6% of the administered prodrug was converted to 2′-F-ara-ddI due the low levels of ADA in mice. The relative brain exposure of the prodrug was 7.5%; however, inconsistent with *in vitro* studies, no 2′-F-ara-ddI was detected in brain samples even at the relatively high dose administered.

In summary, FAAddP underwent reduction to 2′-F-ara-ddA followed by deamination to the active compound 2′-F-ara-ddI. FMAddA did not result in increased brain delivery of the prodrug and was slowly converted to 2′-F-ara-ddI to prove to be effective. In this study we have demonstrated a new approach in the design of azido prodrugs, by utilizing the P-450 NADPH reductase system. This has a great potential in increasing the lipophilicity of the drugs and, thus, achieving the effective concentrations in the brain even though it was unsuccessful in the present study. The application of this biotransformation system utilizing azido moiety to design prodrugs for other potentially active antiviral and anticancer agents with the possibility of improving the pharmacokinetics is in progress.

Experimental Section

Melting points were determined on a Mel-Temp II laboratory device and are uncorrected. The ^{1}H NMR spectra were recorded on a JEOL FX 90 Q FT spectrometer, with tetramethylsilane as the internal standard; chemical shifts are reported in parts per million (δ), and the signals are quoted as s (singlet), d (doublet), t (triplet), m (multiplet), dm (double of multiplet), or brt (broad triplet). UV spectra were recorded on a Beckman DU-7 spectrophotometer. TLC were performed on Uniplates (silica gel) purchased from Analtech Co. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

6-Chloro-9-(5-*O*-benzoyl-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)purine (2). Compound **2** was prepared from compound **1** according to previously published procedures:[8] UV (MeOH) λ_{max} 263.5 nm (lit.[8] UV (MeOH) λ_{max} 263.5 nm).

6-Chloro-9-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)-purine (3). A solution of compound **2** (1.34 g, 3.56 mmol) in CH_2Cl_2 (40 mL) was cooled to −78 °C under nitrogen, and DIBAL-H (10.5 mL, 1 M solution in CH_2Cl_2) was added slowly. The reaction mixture was stirred at −78 °C for 45 min and quenched by the slow addition of MeOH. The reaction mixture was warmed to room temperature, and the solvent was evaporated *in vacuo*. The residue was dissolved in hot MeOH and filtered through a pad of Celite. Upon concentration of the filtrate, the residue was purified on a silica gel column (5% MeOH in $CHCl_3$) to obtain pure compound **3** (0.74 g, 75%): mp 157−158 °C; UV (MeOH) λ_{max} 263.5 nm (lit.[9] UV (MeOH) λ_{max} 260 nmm); $[\alpha]^{25}_D$ +52.3 (*c* 0.5, MeOH) (lit.[9] $[\alpha]^{25}_D$ +55.7 (*c* 1.4, MeOH)).

6-Azido-9-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)-purine (4). A solution of **3** (1.0 g, 3.67 mmol) in DMF (25 mL) and LiN_3 (0.90 g, 18.3 mmol) was stirred at room temperature for 24 h. The DMF was evaporated under high vacuum to yield a white solid which was boiled in MeOH and filtered three times to yield pure **4** (0.75 g, 73.5%) as a white

Table 1. Pharmacokinetics Parameters for 2′-F-ara-ddI, FAAddP, 2′-F-ara-ddA, and FMAddA following Administration of 20 mg/kg 2′-F-ara-ddI, 55 mg/kg FAAddP, or 112 mg/kg FMAddA to Mice

compd measd	dose (mg/kg)	route of administration	substrate	$AUC_{0-\tau}$ (μM·h)	AUC (μM·h)	$t_{1/2}$ (h)	AUC/dose ((μM·h/μmol)/kg)
			2′-F-ara-ddI Administered				
2′-F-ara-ddI	20	iv	serum	36.2	38.0	0.41	0.48
			brain	5.6	6.3	0.47	0.08
			FAAddP Administered				
FAAddP	55	iv	serum	199.3	199.8	0.22	1.01
			brain	12.0	12.6	0.22	0.06
2′-F-ara-ddA			serum	3.0	8.6	2.92	0.04
			brain	3.9	4.8	0.91	0.02
2′-F-ara-ddI			serum	26.5	26.9	0.68	0.13
			brain	4.8	5.3	0.96	0.03
			FAAddP Administered				
FAAddP	55	oral	serum	34.2	37.5	1.53	0.19
			brain	ND[a]	ND	ND	ND
2′-F-ara-ddA			serum	0.8	1.0	1.74	0.005
			brain	ND	ND	ND	ND
2′-F-ara-ddI			serum	6.7	9.2	2.92	0.046
			brain	ND	ND	ND	ND
			FMAddA Administered				
FMAddA	112	iv	serum	296.8	298.8	0.46	0.68
			brain	39.0	39.5	0.98	0.09
2′-F-ara-ddI			serum	10.1	11.2	0.52	0.025
			brain	ND	ND	ND	ND

[a] ND, not detected.

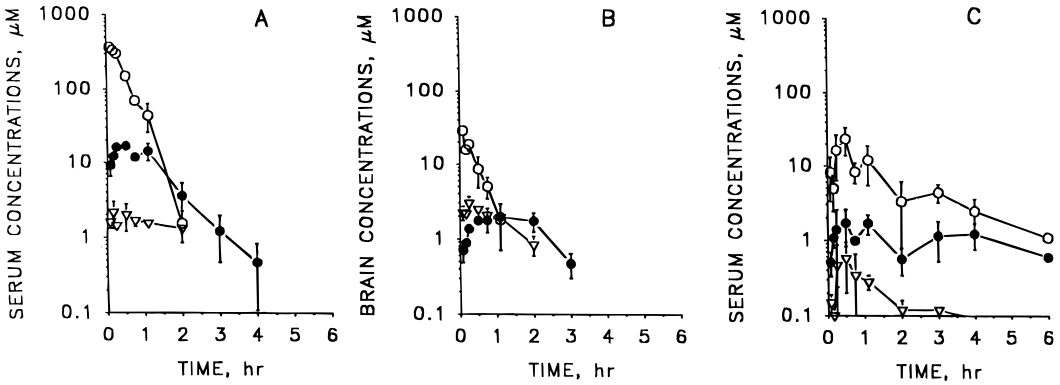


Figure 2. Mean ± SD concentrations of FAAddP (○), 2′-F-ara-ddA (▽), and 2′-F-ara-ddI (●) in serum (A) and brain (B) after intravenous administration and in serum (C) after oral administration of 55 mg/kg FAAddP to mice.

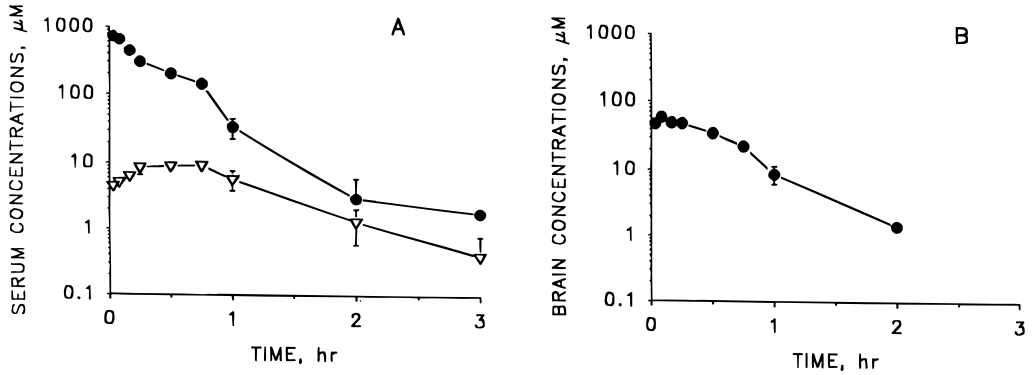


Figure 3. Mean ± SD serum (A) and brain (B) concentrations of FMAddA (●) and 2′-F-ara-ddI (▽) after intravenous administration of 55 mg/kg FMAddA to mice.

solid: mp 209 °C dec; UV (H_2O) λ_{max} 287.5 (ϵ 7471, pH 7), 287.5 (ϵ 7327, pH 2), 234 nm (ϵ 9478, pH 11); ^{1}H NMR (DMSO-d_6) δ 2.03−2.95 (m, 2H), 3.67 (brt, 1H), 5.09 (t, 1H, D_2O exchangeable), 5.57 (m, 1H), 6.61 (dd, 1H), 8.84 (d, 1H), 10.15 (s, 1H). Anal. ($C_{10}H_{10}FN_7O_2$) C, H, N.

***N*^{6}-Methyl-9-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)adenine[9,16] (5).** A solution of **2** (1.60 g, 4.25 mmol) in DMF (50 mL) and methylamine (3 mL) was sealed in a steel bomb and heated at 80 °C for 5 h. After cooling, the solvent was evaporated, NH_3 in MeOH (150 mL) was added, and the mixture was stirred overnight. Evaporation of the solvent yielded crude product, which was purified by silica gel column chromatography to yield pure **5**[9,16] (1.13 g, quantitative yield) as a hygroscopic foam: UV (MeOH) λ_{max} 264 nm (lit.[9,16] UV (MeOH) λ_{max} 265 nm); $[\alpha]^{25}_D$ +56.1 (*c* 0.58, MeOH) (lit.[9] $[\alpha]^{25}_D$ +56.57 (*c* 1.9, MeOH)).

***In Vitro* Stability in Serum, Brain, and Liver Homogenate.** Liver and brain homogenate were prepared in a 1:1 (g:mL) ratio with isotonic 0.05 M phosphate buffer, pH 7.4. FAAddP (70 μM) or FMAddA (50 μM) was added to the mouse

serum, the brain homogenate, or the liver homogenate, and the mixture was incubated in a shaker water bath at 37 °C. Aliquots of 100 μL were removed at time zero and at selected times for up to 6 h. Concentrations of the compounds were determined by HPLC.

Azido Reduction Assay. The analysis of azido-reducing activity was described previously.[11]

Deamination of 2′-F-ara-ddA and FMAddA by Adenosine Deaminase. Samples (1.5 mL) of 2′-F-ara-ddA (80 μM) or FMAddA (50 μM) were prepared in 0.05 M isotonic phosphate buffer, pH 7.4, and placed into a shaking water bath at 37 °C. The reaction was initiated by the addition of 15 μL of adenosine deaminase (type VII from calf intestinal mucosa, Sigma Chemical Co., St. Louis, MO). The final activity in the incubation media was 0.05 unit/mL for 2′-F-ara-ddA and 1.0 unit/mL for FMAddA. At specified time intervals, aliquots of 100 μL were withdrawn for the determination of 2′-F-ara-ddA and 2′-F-ara-ddI or FMAddA and 2′-F-ara-ddI concentrations.

Animal Studies. Animal studies were approved by the University of Georgia Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Female NIH-Swiss mice (Harland Sprague−Dawley, Indianapolis, IN) weighing 24−28 g were housed in 12 h light/12 h dark, constant-temperature (22 °C) environment and had free access to standard laboratory chow and water. Animals were acclimatized to this environment for 1 week prior to the experiments.

2′-F-ara-ddI, dissolved in physiological saline (15 mg/mL), was administered intravenously *via* tail vein injection at a dose of 20 mg/kg (79 μmol/kg). FAAddP (55 mg/kg; 197 μmol/kg) was administered intravenously as a solution in DMSO (15 mg/mL) or orally by a gavage as a suspension in physiological saline. FMAddA, dissolved in saline (15 mg/mL), was administered intravenously at a dose of 112 mg/kg (437 μmol/kg). At selected time intervals, mice (three animals per each time point) were anesthetized with diethyl ether and sacrificed by exsanguination *via* left ventricular heart puncture. Serum was harvested from blood collected. The brain was excised, rinsed with normal saline, blotted dry, and weighed. Serum and brain samples were frozen at −20 °C until analysis.

Analytical Methodology. Concentrations of FAAddP, FMAddA, 2′-F-ara-ddA, and 2′-F-ara-ddI in PBS, serum, brain, and liver homogenate were determined by high-performance liquid chromatography (HPLC). The brain or liver tissue were homogenized in a 1:1 (g:mL) ratio with ice-cold isotonic 0.05 M phosphate buffer, pH 7.4. Buffer, serum or tissue homogenate (100 μL) was mixed with 10 μL of internal standard (25 μg/mL of 3′-azido-2′,3′-dideoxyuridine, AZddU). Acetonitrile (600 μL) containing 0.1% acetic acid was added while the mixture was vortexed to precipitate proteins. The tubes were centrifuged at 3000 rpm for 5 min, and the supernatant was transferred to a clean tube. Supernatant was evaporated to dryness under a stream of nitrogen gas at room temperature. The residual film was reconstituted in 110 μL of mobile phase, and 50 μL was injected onto the HPLC.

Chromatographic separations were performed using a Hypersil ODS column 150 × 4.6 mm, 5 μm particle size (Alltech Associates, Deerfield, IL), preceded by a guard column packed with 30−40 μm pellicular Perisorb RP-18. Mobile phase flow rate was 2 mL/min. For the analysis of FAAddP and 2′-F-ara-ddA, the mobile phase consisted of 7% (v/v) acetonitrile in 80 mM sodium acetate, pH 5.0. The retention times for 2′-F-ara-ddI, FAAddP, and AZddU were 4.5, 7.9, and 5.1 min, respectively. The mobile phase for the analysis of 2′-F-ara-ddI consisted of 4.2% (v/v) acetonitrile in 40 mM sodium acetate, pH 4.1, yielding retention times of 3.78 and 7.6 min, for 2′-F-ara-ddI and AZddU, respectively. For the analysis of FMAddA in serum and liver homogenate, a mobile phase of 7.5% acetonitrile in 40 mM sodium acetate, pH 6.0, was used. The retention time for FMAddA was 8.8 min and that for AZddU was 4.9 min. For FMAddA analysis in brain homogenate, the mobile phase consisted of 7.5% acetonitrile in 10 mM K_2HPO_2 (pH 7.2), yielding retention times for FMAddA and AZddU of 7.8 and 4.3 min, respectively. Eluants were monitored at a UV wavelength of 260 nm.

Nucleoside standards ranging from 0.01 to 100 μg/mL, prepared in blank PBS, serum, brain homogenate, and liver homogenate were treated the same as unknown samples. Samples with nucleoside concentrations greater than 100 μg/mL were diluted with the appropriate blank matrix. The limit of quantitation (signal-to-noise ratio of 3:1) for the 2′-fluoronucleosides in all biological media was 0.1 μg/mL. Extraction recovery was greater than 80% for all compounds. The intra- and interassay relative standard deviations (RSDs) for each compound were less than 10% in all media.

Data Analysis *in Vitro* Studies. Linear regression of the natural logarithm of nucleoside analogue concentrations as a function of time were used to determine first-order degradation rate constants (k) and associated half-lives ($t_{1/2} = 0.693/k$) in PBS, serum, liver homogenate, and brain homogenate.

Data Analysis *in Vivo* Studies. Nucleoside concentration as a function of time data were analyzed by a noncompartmental technique. The area under the serum or brain nucleoside mean ($n = 3$) concentration versus time curve and the first moment (AUMC) were determined by Lagrange polynomial interpolation and integration from time zero to the last sample time ($AUC_{0-\tau}$) with extrapolation to time infinity using the least-squares terminal slope (λ_z).[14] The last three to five time points were used to obtain λ_z. Half-life was calculated from $0.693/\lambda_z$. For intravenously administered compounds, total clearance (CL_T) was calculated from dose/AUC and steady-state volume of distribution (V_{ss}) from dose × AUMC/AUC^2. The fraction of the prodrug converted to parent compound (f_c) was calculated from $AUC_{p\leftarrow pd} \times CL_T/dose_{pd}$, where $AUC_{p\leftarrow pd}$ is the AUC of the parent compound after administration of the prodrug ($dose_{pd}$) and CL_T is the clearance of the parent compound.[15] Relative brain exposure (r_e) was calculated from AUC_{brain}/AUC_{serum}.

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