be found in the recent work of Mauldin et al.[29]

Without additional information the mechanism by which the Pt(II) dimers and cyclotrimers exert their antitumor effects is unknown. However, these compounds would be expected to undergo substitution reactions, most likely via an S_N2 process,[22] initially producing acyclic compounds which could react with DNA and other cellular components. The fact that two or three platinum(II) centers are involved suggests that the compounds are potentially more disruptive on a molecular basis to biological processes than are mononuclear platinum species. Studies in progress are attempting to more clearly define the substitution chemistry of the Pt(II) dimers and trimers in solution.

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Brain Targeting of Anti-HIV Nucleosides: Synthesis and in Vitro and in Vivo Studies of Dihydropyridine Derivatives of 3′-Azido-2′,3′-dideoxyuridine and 3′-Azido-3′-deoxythymidine

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A significant number of patients with AIDS and AIDS-related complex develop neurological complications. Therefore, it is critical that anti-HIV agents penetrate the blood–brain barrier and suppress viral replication in the brain. In an effort to increase the brain delivery of anti-HIV nucleosides, in vitro and in vivo pharmacokinetics of dihydropyridine derivatives of 3′-azido-2′,3′-dideoxyuridine (AzddU, AZDU, or CS-87) and 3′-azido-3′-deoxythymidine (AZT, Zidovudine) have been studied. In vitro studies of the prodrugs (AzddU-DHP and AZT-DHP) in human serum, mouse serum, and mouse brain homogenate indicated that the rates of serum conversion from prodrugs to parent drugs are species dependent: mouse brain homogenate > mouse serum > human serum. Half-lives in human serum, mouse serum, and mouse brain homogenate are 4.33, 0.56, 0.17 h, respectively, for AzddU and 7.70, 1.40, and 0.18 h, respectively, for AZT. In vivo studies of AzddU-DHP and AZT-DHP showed that the prodrugs have areas under the serum concentration–time curves (AUC) similar to those of the parent drugs. The AUC in serum for AzddU following prodrug administration is 25.79 μg h/mL, which is similar to the value of 25.83 μg h/mL when AzddU was administered. Analogously, the serum AUCs for AZT when AZT-DHP and AZT were administered are 25.38 and 26.64 μg h/mL, respectively. However, the brain AUCs for both AzddU and AZT derived from prodrugs, being 11.43 and 11.28 μg h/mL, respectively, are greater than the brain AUCs for AzddU (2.09 μg h/mL) and AZT (1.21 μg h/mL) when the parent drugs were administered. Thus, the relative brain exposure (r_e) for AzddU (5.47) and AZT (9.32) indicate a significant increase in exposure to the anti-HIV nucleosides following prodrug administrations. The results of extended half-lives of the synthesized prodrugs in human serum along with the higher r_e values in vivo warrant studies in larger animals to determine the potential usefulness of the prodrugs in humans.

A significant number of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex develop neurological complications.[1-8] Human immunodeficiency virus (HIV) can replicate in the brain and the infected brain serves as a reservoir for the virus.[5] It is well documented that HIV has been isolated from the cerebrospinal fluid (CSF) of AIDS patients[9,10] as well as from brains of postmortem AIDS patients. Although the mechanism of HIV-induced central nervous system (CNS) dysfunction is still unclear, it has been proposed that the virus is carried into the brain by infected macrophages/monocytes.[5,6,11] The neurological disorder associated with the HIV infection may be the result of interference of endogenous neurotropic substances by gp120 of HIV.[12] Regardless of the mechanism by which HIV causes the CNS disorders, it is critical that anti-HIV agents penetrate the blood–brain barrier and suppress viral replication in the brain. However, most available chemotherapeutic agents either do not cross the blood–brain barrier or cross to only a small extent. Despite this general rule, there is evidence that 3′-azido-3′-deoxythymidine (AZT) can penetrate into cerebrospinal fluid (CSF),[13] although it has not been demonstrated that AZT is actually able to cross the blood–brain barrier in humans. Patients receiving AZT

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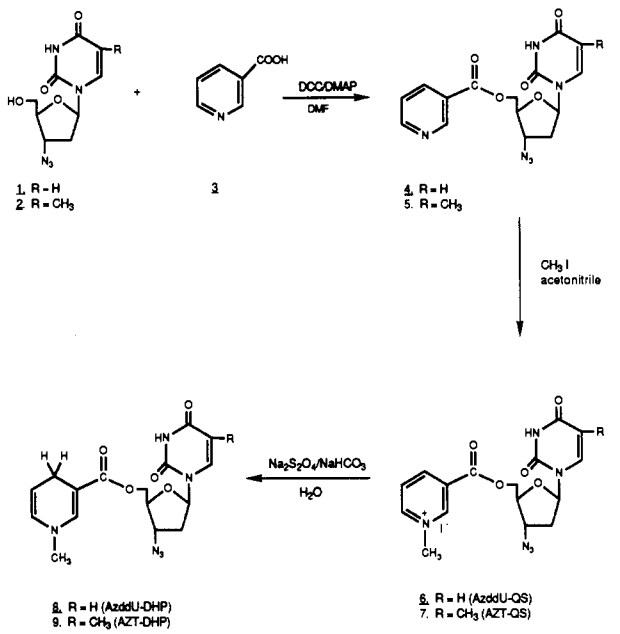
showed a general improvement of neurological functions including attention, memory, motor function, and general cognitive ability.[14] It has been reported that 60% of the serum concentration of AZT can be accounted for in CSF in humans.[13] However, it is unknown whether the concentration in CSF is sufficient to effectively suppress viral replication in the CNS.

As a part of our drug discovery program for AIDS, we have identified several promising anti-HIV nucleosides such as 3′-azido-2′,3′-dideoxyuridine (AzddU, AZDU, or CS-87),[15,16] 3′-azido-2′,3′-dideoxy-5-methylcytidine (CS-92),[15,17] and 2′,3′-dideoxy-N^6-methyladenosine (CS-176).[18,19] Among these compounds, the forerunner, AzddU, is currently undergoing phase I clinical trials in patients with AIDS and AIDS-related complex. AzddU appears to be less potent than AZT against HIV in a peripheral blood mononuclear (PBM) cell screening system as well as in MT-4 cell lines, although the differences in potency in CEM and CD4+HeLa cells are not as great as that in PBM cells.[20] The lower activity in PBM cells appears to be related to a lower affinity of AzddU for the enzyme responsible for its initial phosphorylation.[16] An advantage over AZT is its significantly lower toxicity on bone marrow cells.[21] Although AzddU has been found to cross the blood–brain barrier in mice (vide infra)[22] and is transported into CSF in monkeys,[23] drug concentration in the human CNS has not yet been determined. Furthermore, even if it does penetrate the CNS, as suggested by the animal studies, the extent of transport as well as the duration of the drug in the CNS in human may not be predictable or sufficient enough to effectively suppress viral replication. Therefore, it was of interest to develop an antiviral prodrug which can more readily penetrate the blood–brain barrier than the parent nucleoside and maintain an effective brain concentration of the drug.

Various strategies for increasing drug delivery to the brain have been considered. However, the prodrug approach, in which the increased lipophilic nature of the prodrug may facilitate passive diffusion across the blood–brain barrier with subsequent conversion to the parent compound by simple chemical hydrolysis as well as by brain enzymes, seems to be advantageous. Bodor et al.[24]

Scheme I



reported a novel approach utilizing a dihydropyridine–pyridinium salt type of redox system, in which a biologically active compound linked to a lipophilic dihydropyridine carrier can readily penetrate the blood–brain barrier. Oxidation of the carrier moiety to the hydrophilic pyridinium salt (quaternized salt) in the brain slows its elimination due to a "lock-in" mechanism of polar compounds in the brain, while elimination from the general circulation of the hydrophilic pyridinium salt is accelerated. Subsequent cleavage of the quaternary carrier drug results in sustained delivery of the drug in the brain with reduction of general toxicity due to the rapid elimination of the polar quaternary salt. This method has been explored as a general strategy of targeted drug delivery to the brain.

Recently, Torrence et al.[25] reported the synthesis of a dihydropyridine derivative of AZT which was found to be active in a MT-4 cell screening system and could be easily oxidized to a quaternary salt in rat brain cytosol in vitro. Gogu et al.[26] studied the same prodrug in murine bone marrow cells and found it to be significantly less toxic than the parent compound AZT. Little et al.[27] also studied the dihydropyridine derivative of AZT in rats and dog and found significantly higher concentrations in brain and CSF, respectively. Palomino et al.[28] have also reported the synthesis and the pharmacokinetics of a dihydropyridine derivative of 3′-deoxy-2′,3′-didehydrothymidine (D4T).[29-31] Studies in mice demonstrated that the dihydropyridine derivative crosses the blood–brain barrier and is oxidized

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Table I. In Vitro Studies of AzddU-DHP and AZT-DHP at 37 °C

compound	degradation rate constant (k,h^{-1})				half-life, h			
	human serum	mouse serum	mouse brain hom	phosphate buffer (pH 7.4)	human serum	mouse serum	mouse brain hom	phosphate buffer (pH 7.4)
AzddU-DHP	0.16	1.24	4.15	0.33	4.33	0.56	0.17	2.13
AzddU-QS (from AzddU-DHP)	0.14	1.36	1.07	0.80	4.95	0.51	0.65	0.87
AZT-DHP	0.09	0.49	3.89	0.21	7.70	1.40	0.18	3.23
AZT-QS (from AZT-DHP)	0.02	0.64	0.41	0.55	34.65	1.08	1.69	1.25

by cerebral tissue to the quaternary salt, resulting in the sustained release of D4T.

As a part of our continuing efforts to develop anti-HIV nucleoside prodrugs targeted toward the brain, we report the synthesis and in vitro and in vivo studies in mice of dihydropyridine derivatives of AzddU and AZT.

Results and Discussion

The syntheses of the dihydropyridine derivatives of AzddU and AZT were similar to those reported for the syntheses of the dihydropyridine prodrug of AZT[25-27] and D4T.[28] In these earlier reports,[25,28] nicotinyl chloride was used for the esterification of 5′-OH group. However, we have found that nicotinyl chloride gives N^3-nicotinyl derivatives as a byproduct. Therefore, a nicotinic acid and N,N'-dicyclohexylcarbodiimide coupling method was used in order to avoid the N^3-nicotinyl derivatives (Scheme I). Quaternization of **4** to **6** was accomplished in good yields by refluxing **4** with methyl iodide in acetonitrile. However, the highly hygroscopic nature of quaternary salts **6** made it difficult to obtain the correct elemental analyses. Reduction of the quaternary products **6** with sodium dithionite in degassed water under argon gave a good yield of dihydropyridine derivatives **8**. However, some difficulties have been experienced in obtaining an analytical sample of AzddU dihydropyridine derivative **8** due to a minor conversion to the quaternized compounds by air oxidation, which underwent further hydrolysis to the parent nucleosides. The AZT prodrug **9** was found to be more stable than that of AzddU, **8**. Throughout the in vivo and in vitro experiments, fresh solutions were necessary for the biological evaluations.

Table I gives the degradation rate constants and associated half-lives for dihydropyridine derivatives **8** and **9** and their quaternary salts in biological media as well as phosphate buffer (pH 7.4). Interestingly, both the AzddU and AZT dihydropyridine derivatives show the greatest stability in human serum, followed by phosphate buffer (pH 7.4), mouse serum, and brain homogenate. The extended half-lives of dihydropyridine derivatives (greater than 4 h) in human serum would allow sufficient time to cross the blood–brain barrier in appreciable concentrations. However, it is expected that in the case of oral administration, first-pass metabolism may play a role in determining the stability and half-life of the prodrugs. The degradation rates for the AzddU derivatives are higher than the corresponding reactions for the AZT derivatives. These results are consistent with the stability observed during the chemical synthesis of the prodrugs **8** and **9**. The DHP prodrugs **8** and **9** are converted to parent nucleosides **1** and **2**, respectively, by either oxidation to the QS **6** or **7** followed by hydrolysis or by direct hydrolysis. AzddU quarternary salt (AzddU-QS) and AZT quaternary salt (AZT-QS) have longer half-lives than their DHP derivatives in human serum and mouse brain homogenate, whereas in mouse serum the half-lives are similar. Examination of the data suggest that the degradation pathways of both AzddU-DHP and AZT-DHP are similar in each biological media in vitro. On the basis of concentration–time profiles, prodrug to parent drug conversions seem to be favored through the quaternary salt intermediates in brain homogenates. In human and mouse serum, prodrug concentrations were sustained and quaternary salt concentrations were relatively low, possibly indicating that parent drugs are predominantly formed by direct hydrolysis of the prodrugs. These prolonged half-lives of AzddU-DHP and AZT-DHP in human serum seem to indicate that the prodrugs may be potentially useful in delivering anti-HIV nucleoside to the brain.

Table II. In Vivo Studies of AzddU-DHP and AZT-DHP in Mice in Comparison to Those of AzddU and AZT

compound	AUC,[a] μg h/mL		r_e[b]	$t_{1/2}$, h	EC_{50},[c] μM
	serum	brain[d]			
AzddU-DHP	4.43				1.0
AzddU-QS (from AzddU-DHP)	1.21	3.91			
AzddU (from AzddU-DHP)	25.79	11.43	5.47	4.34	
AzddU (administered itself)	25.83	2.09		0.84	0.2
AZT-DHP	1.27				1.6×10^{-3}
AZT-QS (from AZT-DHP)	0.60	1.29			
AZT (from AZT-DHP)	25.38	11.28	9.32	15.8	
AZT (administered itself)	26.64	1.21		0.54	2×10^{-3}

[a] AUC: areas under the serum and brain concentration–time curves. [b] r_e: relative brain exposure $(AUC)_{pd \to p}/(AUC)_p$; r_e values greater than 1 indicate favorable brain delivery of the parent nucleosides following prodrug administration. [c] In vitro anti-HIV results in peripheral blood mononuclear cells. [d] It was assumed that 1 g of brain is equivalent to 1 mL.

The area under the curve (AUC) for the serum and brain after administration of DHP derivatives and parent nucleosides in mice are shown in Table II. The AUCs in serum and brain for the DHP derivatives and QS species are small relative to the AUCs for the parent compounds derived from DHP administration. DHP concentrations were detected at only 1 or 2 sample times in brain, and thus, the AUC could not be determined. These data suggest that DHP derivatives disappear rapidly to the quarternary salts in the mouse serum and brain as suggested by the in vitro data. Brain concentrations of the quaternary salt species could be measured for up to 2 h for AzddU-QS and up to 6 h for AZT-QS. In both cases, the concentrations were small as indicated by the AUCs. The maximum brain AzddU-QS and AZT-QS concentrations occured at 5 min, the first measured time point, and were 3.88 and 1.84 μg/mL, respectively. The AUC in serum for AzddU following prodrug administration is 25.79 μg h/mL and very similar to the value of 25.83 μg h/mL obtained for AzddU when it was administered. Analogously, the serum AUCs for AZT when prodrug and parent compounds were administered were 25.38 and 26.64 μg h/mL, respectively. However, the brain AUCs for both AzddU and AZT derived from prodrug, being 11.43 and 11.28 μg h/mL, respectively, are greater than the brain AUCs for AzddU (2.09 μg h/mL) and AZT (1.21 μg h/mL) when the parent drugs were administered.

Table II also gives the relative brain exposure (r_e) and apparent brain half-lives of AzddU at AZT. Figures 1 and 2 illustrate brain AzddU and AZT concentrations, respectively, that were measured following both prodrug (AzddU-DHP and AZT-DHP) and parent compound

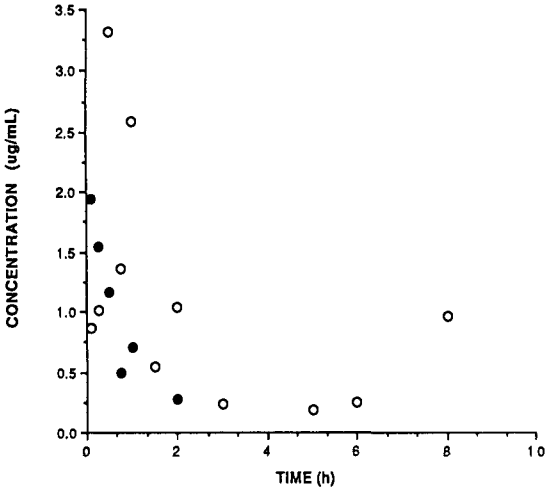

Figure 1. Mouse brain concentrations of AZddU following administration of AZddU itself (●) and as AZddU-DHP (○).

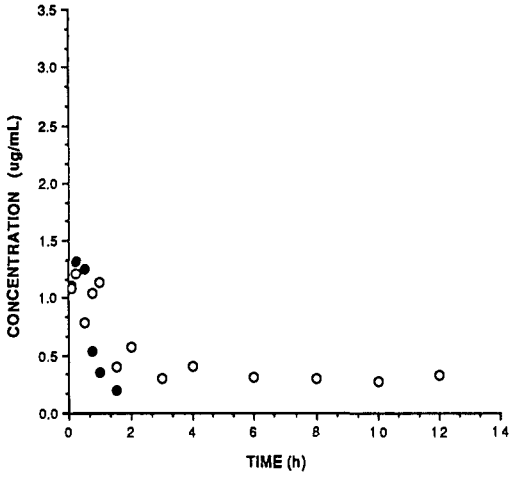

Figure 2. Mouse brain concentrations of AZT following administration of AZT itself (●) and as AZT-DHP (○).

(AzddU and AZT) administrations. The r_e value of 5.47 for AzddU and 9.32 for AZT indicate a significant increase in exposure to these compounds following prodrug administrations. The greater octanol/water partition coefficients of the dihydropyridine derivatives (21.62 and 53.09 for AzddU-DHP and AZT-DHP, respectively) relative to the parent compounds (0.45 and 1.10 for AzddU and AZT, respectively) are consistent with the notion that the lipophilic dihydropyridine derivatives cross the blood–brain barrier more readily than the parent compounds.

Comparison of the apparent brain half-lives for AzddU (4.34 vs 0.84 h) and AZT (15.8 vs 0.54 h) following prodrug administration indicates an increased retention of each compound in the brain following prodrug administration compared to the values obtained for parent drug administrations. The apparent qualifier refers to the possibility that the terminal concentration phase may be a function of the formation rate of AzddU or AZT from either their dihydropyridine or their quaternary salt precursors. In any case, the r_e and half-life values clearly demonstrate that AzddU and AZT have a longer residence time in the brain following prodrug administration than is found following parent drug administration. The nucleoside prodrugs were compared to the parent compound for their ability to inhibit HIV replication in human lymphocytes. Although the AZT prodrug had similar potency to AZT, the AzddU prodrug was 5-fold less potent than AzddU in PBM cells (Table II), which is not consistent with the in vitro data in human serum. However, the results suggest that the prodrugs were readily converted to the parent compounds in vitro.

Optimizing brain delivery of active anti-HIV compounds is an important criteria for successful treatment of AIDS. It appears that the chemical delivery system designed by Bodor et al.[24] does work to a reasonable extent in mice. Certainly similar brain uptake studies are warranted in larger animals, and consideration of means to administer dihydropyridine derivatives clinically should be pursued.

Experimental Section

Synthesis. ^{1}H NMR spectra were recorded on a JEOL FX 90Q Fourier transform spectrometer using 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 Bausch and Lomb Spectronic 2000 spectrometer or Beckman DU-7 spectrophotometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Elemental analysis were performed by Atlantic Microlab Inc., Norcross, GA.

3′-Azido-2′,3′-dideoxy-5′-*O*-(3-pyridylcarbonyl)uridine (4). A mixture of 3′-azido-2′,3′-dideoxyuridine (10.0 g, 39.5 mmol), nicotinic acid (7.8 g, 63.4 mmol), 1,3-dicyclohexylcarbodiimide (13.6 g, 66.0 mmol), and 4-(dimethylamino)pyridine (0.85 g) in 75 mL of DMF was stirred for 12 h. The precipitated dicyclohexylurea was filtered off and the filter cake was washed with DMF. The filtrate was evaporated, and the residue was extracted with $CHCl_3$, washed with water, dried (Na_2SO_4), and evaporated. Chromatography of the residue over a silica gel column using 1% $CHCl_3$ in EtOAc gave 10.5 g (74%) of the product: mp 125–127 °C; UV (MeOH) λ_{max} 214, 261 nm; IR (KBr) 2100 (N_3), 1730 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.93 (br s, 1 H, NH, exchangeable), 9.26 (d, 1 H, J = 1.46 Hz, pyridine 2-H), 8.84 (dd, 1 H, J = 1.46 Hz, 4.84 Hz, pyridine 6-H), 8.31 (dt, 1 H, J = 1.75 Hz, 6.15 Hz, pyridine 4-H), 7.50–7.27 (m, 2 H, pyridine 5-H and 6-H), 6.05 (t, 1 H, J = 6.15 Hz, 1′-H), 5.66 (d, 1 H, J = 8.2 Hz, 5-H), 4.64 (d, 2 H, J = 3.81 Hz, 5′-H), 4.49–4.14 (m, 2 H, 3′- and 4′-H), 2.55 (t, 2 H, J = 6.1 Hz, 2′-H). Anal. ($C_{15}H_{14}N_6O_5$) C, H, N.

3′-Azido-2′,3′-dideoxy-5′-*O*-[(1-methyl-3-pyridinio)carbonyl]uridine (6). To a solution of **3** (4.0 g, 11.17 mmol) in 50 mL of dry acetonitrile was added methyl iodide (6.8 g, 48.0 mmol) and the mixture was heated at 50–60 °C for 12 h. The solvent was evaporated under reduced pressure and the residue was washed with ether and dichloromethane and dried to yield a hygroscopic solid (4.5 g, 80%): ^{1}H NMR (DMSO-d_6) δ 11.3 (br s, 1 H, NH, exchangeable), 9.53 (s, 1 H, pyridine 2-H), 9.22 (br d, 1 H, J = 5.5 Hz, pyridine 6-H), 8.99 (br d, 1 H, J = 7.9 Hz, 4-H), 8.29 (t, 1 H, J = 7.0 Hz, pyridine 5-H), 7.69 (d, 1 H, J = 7.9 Hz, 6-H), 6.19 (t, 1 H, 7.0 Hz, 1′-H), 5.59 (d, 1 H, J = 7.9 Hz, 5-H), 4.69–4.60 (m, 3 H, 3′- and 5′-H), 4.44 (s, 3 H, NCH_3), 4.28–4.11 (m, 1 H, 4′-H), 2.70–2.10 (m, 2 H, 2′-H). Anal. ($C_{16}H_{17}N_6O_5I\cdot1.5H_2O$) C, H, N, I.

3′-Azido-2′,3′-dideoxy-5′-*O*-[(1,4-dihydro-1-methyl-3-pyridyl)carbonyl]uridine (8). To a stirred ice-cold solution of **4** (2.0 g, 3.8 mmol) in 50 mL of degassed water was added sodium bicarbonate (2.7 g, 32.1 mmol) followed by sodium dithionite (90%, 3.1 g, 16 mmol). The mixture was stirred under argon for 1 h. The solid which separated was extracted with degassed CH_2Cl_2, and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to give 1.0 g (70%) of **5** as a yellow foamy solid: UV (MeOH) λ_{max} 209 (ϵ 19 500), 260 (ϵ 11 360), 360 nm (ϵ 6340); IR KBr) 2100 (N_3), 1700 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.30 (br s, 1 H, NH, exchangeable), 7.53 (d, 1 H, J = 8.13 Hz, 6-H), 7.01 (d, 1 H, J = 1.32 Hz, pyridine 2-H), 6.12 (t, 1 H, J = 6 Hz, 1′-H), 5.77–5.57 (m, 2 H, pyridine 6-H and 5-H), 4.79 (dt, 1 H, J = 3.7 Hz, 7.8 Hz, pyridine 5-H), 4.39 (d, 2 H, J = 2.86 Hz, 5′-H), 4.25–4.10 (m, 2 H, 3′- and 4′-H), 3.06 (m, 2 H, pyridine 4-H), 2.95 (s, 3 H, NCH_3), 2.53–2.25 (m, 2 H, 2′-H). Anal. ($C_{16}H_{18}N_6O_5$) C, H, N.

3′-Azido-3′-deoxy-5′-*O*-[(1,4-dihydro-1-methyl-3-pyridyl)carbonyl]thymidine (9). The synthetic method for **9** was similar to that of AzddU and was reported previously.[25,26,30]

Analysis of AzddU-DHP and AzddU-QS or AZT-DHP and AZT-QS. To a 100-μL aliquot of serum or brain homogenate

containing the internal standard (O-acetophenitidine) was added 300 μL of cold acetonitrile with vigorous mixing to precipitate proteins. Mixing was continued for 30 s, and then 100 mg of sodium chloride was added to each tube, and the tubes were briefly mixed and centrifuged at 10000 rpm for 5 min. For serum, 200 μL of supernatant obtained from centrifugation was evaporated to dryness under nitrogen at 25 °C whereas for brain homogenates all of the supernatant was evaporated to dryness. The residual film was reconstituted in 100 μL of mobile phase and a 75-90-μL aliquot was injected onto the HPLC system.

Chromatographic separations were achieved on a Hypersil ODS analytical column (5 μ, 4.6 × 150 mm) preceded by a guard column filled with 30-40 μm pellicular RP-18 perisorb material. The mobile phase consisted of 30% (v/v) acetonitrile in 40 mM aqueous sodium acetate containing 4 mM sodium lauryl sulfate. The mobile phase flow rate was 2 mL/min and the detector wavelength was set at 260 nm. AzddU and AZT were quantitated in serum and brain homogenates by a previously published method.[22] Serum and brain homogenate samples prepared for prodrug and quaternary salt were processed immediately for HPLC analyses to avoid degradation. All analytical procedures had intra- and interday coefficients of variations less than 15%.

In Vitro Stability Determination in Serum, Brain Homogenate, and Phosphate Buffer (pH 7.4). AzddU-DHP or AZT-DHP (as a 5 or 1 mg/mL standard in DMSO) were added to give an initial concentration of either 50 or 10 μg/mL, respectively, in 3 mL of phosphate buffer (pH 7.4) and freshly collected human serum, mouse serum, or brain homogenate (1:1 g/mL in a physiological phosphate buffer) maintained at 37 °C on a shaking water bath. One-hundred microliter aliquots were withdrawn at time zero and at 0.083, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 10 h after adding the prodrugs. The aliquots were analyzed for the dihydropyridine, quaternary salt, and parent drug moieties as described above.

In Vivo Studies. Female NIH-Swiss mice weighing 25-30 g were administered intravenously with AzddU (50 mg/kg), AZT (50 mg/kg), AzddU-DHP (73.9 mg/kg, equivalent to 50 mg/kg AzddU), or AZT-DHP (72.7 mg/kg, equivalent to 50 mg/kg AZT) dissolved in DMSO (50 mg/mL) through a tail vein over 30 s. Animals were momentarily restrained during dosing and then placed in individual cages and allowed food and water ad libitum. Three mice each were killed at 5, 15, 30, 45, and 60 min and 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h following dosing. Animals were killed by exsanguination via 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. A brain homogenate was prepared in a 1:1 (g/mL) ratio with ice-cold, pH 7.4, isotonic phosphate buffer. Serum and brain samples were processed immediately for the analysis of the prodrug and salt by an ion-pair HPLC method. Parent drug was analyzed separately by an HPLC method.[22]

Data Analysis. In Vitro Studies. The terminal phase concentrations for AzddU-DHP AzddU-QS, AZT-DHP, and AZT-QS were used to determine degradation rate constant (k) and the associated half-life in each media. The degradation rate constant (K) was equal to the slope of the equation obtained from linear regression of the natural log of the concentration versus time values in the terminal phase. The terminal phase concentrations for the quaternary salt species could represent the formation of the salt from the prodrug or degradation of the salt to the parent compound. In the current analysis, it is assumed that the degradation rate of the salt is slower than the formation rate from the dihydropyridine derivatives.

In Vivo Studies. The measured mouse serum and brain concentrations were used to calculate the area under the serum or brain concentration-time curve (AUC) from time zero to infinity. A Lagrange polynomial integration method[32] was used to obtain the AUC to the last observed time point, t_n. The AUC from t_n to infinity was estimated by C_N/k, where C_N equals the observed concentration at t_n and k equals the terminal disposition rate constant. The terminal disposition rate constant (k) was equal to the slope of the equation obtained from linear regression of the natural log of the concentration and time values in the terminal phase.

The relative brain exposure[33] to AzddU and AZT was calculated as $r_e = (AUC)_{pd\rightarrow p}/(AUC)_p$, where $(AUC)_{pd\rightarrow p}$ equals the area under the AzddU or AZT brain concentration-time curve following administration of the prodrug (PD), and $(AUC)_P$ is the same area obtained following administration of the parent (P) compounds AzddU or AZT. r_e values greater than 1 indicate favorable brain delivery of the parent compounds following prodrug administration.

An apparent elimination half-life for AzddU and AZT in brain following prodrug and parent drug administration was calculated as $0.693/k$, where k was determined as described above.

Antiviral Assays. The antiviral activity of the DHP analogues of AZT and AzddU were determined In PBM cells infected with HIV-1 (strain LAV) as described previously.[15] For comparison, AZT and AzddU were included.

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Organic Phosphorus Compounds. 5. (4-Benzothiazol-2-ylbenzyl)amidophosphonate as Potent Calcium Antagonistic Vasodilators[1]

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Structural modifications of the calcium antagonist fostedil (KB-944) and their coronary vasodilator activity are described. Amidophosphonates **4a-m**, lactam amidophosphonates **7a-l**, and diamide dilactam **10** were prepared, and their coronary vasodilator activity was assessed in dogs. Many compounds exhibited coronary vasodilator activity superior to that of fostedil. Among them, the 2-oxopyrrolidine derivative **7a** was the most effective compound. Its action as a coronary vasodilator was 3 and 2 times more potent than that of fostedil and diltiazem hydrochloride, respectively.

Compounds of general structure 1 have been shown to exhibit coronary vasodilator activity[2,3] by binding to the protein of the voltage-sensitive calcium channel present on the membrane of smooth muscles.[4-6] Among these

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