Highly Water-Soluble Lipophilic Prodrugs of the Anti-HIV Nucleoside Analogue 2',3'-Dideoxycytidine and Its 3'-Fluoro Derivative

Stephen G. Kerr and Thomas I. Kalman*

Department of Medicinal Chemistry, State University of New York at Buffalo, Buffalo, New York 14260.
Received January 3, 1992

The synthesis of a novel series of lipophilic prodrug derivatives of the anti-HIV drugs 2',3'-dideoxycytidine (ddC, 1) and 3'-fluoro-ddC (2), involving N^4-substitution with (*N,N*-dialkylamino)methylene side chains, is described. The increase in the partition coefficients for the prodrug series, compared to those of the parent drugs 1 and 2, ranged from 1.5- to 122-fold and from 1.6- to 175-fold, respectively. At pH 7.4, 37 °C, the hydrolytic $t_{1/2}$ values ranged from 2 to 52 h, the diisopropyl derivatives (**3d** and **4d**) being most stable in the series. **3d** and **4d** were ≥4-fold and 1.7-fold more soluble in water than 1 and 2, respectively. The in vitro antiretroviral activities of **3d**, **4a**, and **4d** were evaluated; the results indicate efficient prodrug-to-drug conversion under the assay conditions. The results of this investigation demonstrate that it is indeed feasible to chemically modify certain nucleoside analogues with inferior solubility properties to simultaneously achieve significantly enhanced lipid and water solubility.

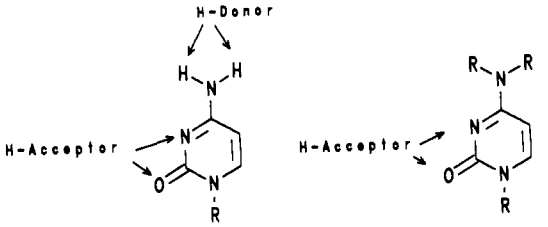
Introduction

Invasion of the central nervous system (CNS) by the human immunodeficiency virus (HIV), the causative agent of AIDS, leads to serious neurological disorders and may be a factor in the development of persistent HIV infections.[1-6] Many investigations have focused on the development of agents which can more readily penetrate the CNS by crossing the blood–brain barrier. These studies involved esterification of the 5'-hydroxyl group of the parent anti-HIV nucleoside,[7-10] e.g., zidovudine (AZT),[7-9] 2',3'-didehydro-3'-deoxythymidine (d4T), and 3'-azido-2',3'-dideoxyuridine,[10] or modification of the nucleoside base with lipophilic functional groups[11,12] or the phosphate groups of nucleotides.[13,14] These investigations aimed at increasing lipid solubility, since the correlation between lipophilicity, membrane permeability, and CNS penetration has long been established.[15-17]

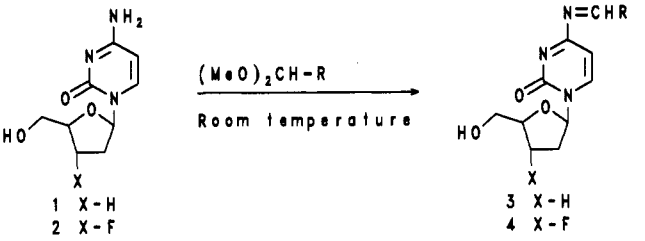
Experimental evidence suggests that selective transport of nucleosides into the CNS exists.[18,19] Accordingly, thymidine and uridine analogues are preferred compared to cytidine analogues by the uptake system.[18] AZT has a relatively high partition coefficient and readily enters the cerebrospinal fluid[18,20] possibly by both passive diffusion and active transport. In contrast, ddC, a potent anti-HIV agent currently undergoing clinical trials,[21-25] does not penetrate the CNS as well as AZT,[18,21,26-28] perhaps due to its lower lipophilicity and/or inferior substrate activity in the carrier-mediated uptake system.

The candidate drugs chosen for prodrug modification were ddC (1) and 3'-fluoro-ddC (2).[29,30] It is important to emphasize that the enhancement of lipophilicity to facilitate brain uptake of an anti-HIV drug should not compromise water solubility. The primary target cells of HIV are most abundant in the circulation, and drug availability for cellular uptake (and tissue distribution) is a function of the unbound drug concentration in the plasma. Many investigations,[7-14] including those utilizing the dihydropyridine–pyridinium redox chemical delivery system,[31] were aiming at selective accumulation in the CNS. In most instances lipophilicity was increased at the expense of water solubility. In our prodrug design we wanted to satisfy several requirements. We intended to achieve the following: large increase in lipophilicity without sacrificing water solubility; spontaneous hydrolysis of the prodrug to the drug with sufficient acid stability to permit oral administration; and systematic structural variability in the lipophilic moiety to permit the selection

Chart I



Scheme I



Compound	R
3a 4a	$N(CH_3)_2$
3b 4b	$N(C_2H_5)_2$
3c 4c	$N(n\text{-}C_3H_7)_2$
3d 4d	$N(i\text{-}C_3H_7)_2$
3e 4e	$N(C_5H_{10})$
3f 4f	$N(C_4H_8O)$
3g 4g	$N(C_4H_8)$

of structures with optimal physicochemical properties. Placing a variable lipophilic substituent on the exocyclic

* Author to whom correspondence should be sent at Department of Medicinal Chemistry, State University of New York at Buffalo, 457 Cooke Hall, Buffalo, NY 14260.

(1) Price, R. W.; Brew, B.; Sidtis, J.; Rosenblum, M.; Scheck, A.; Cleary, P. The Brain in AIDS: Central Nervous System HIV Infection and AIDS Dementia Complex. *Science* **1988**, *239*, 586–592.
(2) Fauci, A. S. The Human Immunodeficiency Virus: Infectivity and Mechanisms of Pathogenesis. *Science* **1988**, *239*, 617–622.
(3) Yarchoan, R.; Broder, S. Development of Antiretroviral Therapy for the Acquired Immunodeficiency Syndrome and Related Disorders. *N. Eng. J. Med.* **1987**, *316*, 557–564.
(4) Mitsuya, H.; Broder, S. Strategies for Antiviral Therapy in AIDS. *Nature* **1987**, *325*, 773–778.
(5) Lane, H. C.; Fauci, A. S. *In AIDS. Modern Concepts and Therapeutic Challenges*; Broder, S. Ed.; M. Dekker: New York, 1987; pp 185–203.

0022-2623/92/1835-1996$03.00/0

Table I. Partition Coefficients and $t_{1/2}$ Values of the N^4-[(Dialkylamino)methylene] Derivatives of ddC (1) and 3′-Fluoro-ddC (2)

compd	P^a	half-life ($t_{1/2}$),[b] h	compd	P^a	half-life ($t_{1/2}$),[b] h
3a	0.112	3.8	**4a**	0.229	4.66
3b	0.873	8.67	**4b**	1.688	10.17
3c	8.87	13.14	**4c**	22.38	15.58
3d	5.3	47.51	**4d**	13.8	52.08
3e	1.14	6.4	**4e**	2.28	6.3
3f	0.108	3.92	**4f**	0.211	3.74
3g	0.341	2.13	**4g**	0.688	2.57
1 (ddC)	0.073		**2** (3′-F-ddC)	0.128	

[a] Partition coefficient in 1-octanol/PBS (pH 7.4) at 25 °C. [b] $t_{1/2}$ = the time required for 50% hydrolysis in PBS (pH 7.4) at 37 °C.

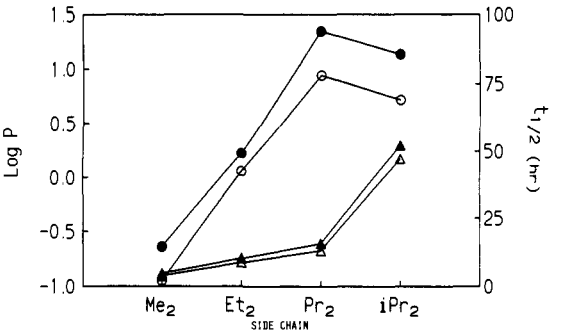


Figure 1. Circles, log P; triangles, $t_{1/2}$. Open symbols, ddC derivatives (3); filled symbols, 3′-fluoro-ddC derivatives (4). Side chains: $R_2NCH{=}N$.

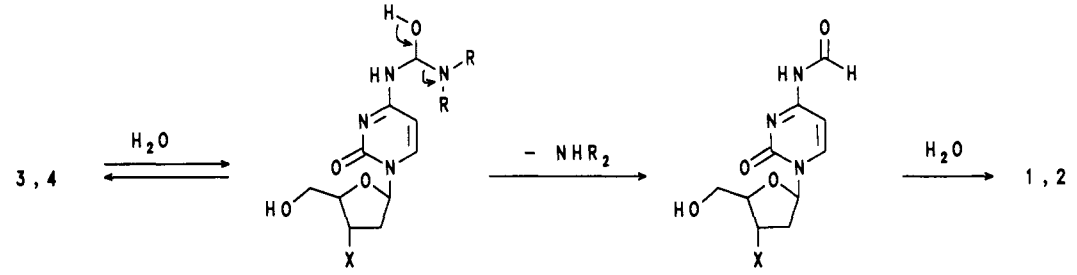
Table II. Ratios of the Partition Coefficients between the Side-Chain Analogues of 3′-Fluoro-ddC (2) and ddC (1)

compound	ratio	log ratio (π_F)
3a, 4a	2.04	0.31
3b, 4b	1.93	0.29
3c, 4c	2.52	0.40
3d, 4d	2.6	0.41
3e, 4e	2.01	0.30
3f, 4f	1.95	0.29
3g, 4g	2.02	0.31
1, 2	1.75	0.24
		av 0.32 ± 0.05

amino group of the nucleoside was expected to incrementally increase lipophilicity and, simultaneously, enhance water solubility due to the loss of the NH_2 group as a donor of two intermolecular H-bonds (Chart I) which otherwise

(6) Gartner, S.; Markovits, P.; Markovits, D. M.; Kaplan, M. H.; Gallo, R. C.; Popovik, M. The Role of Mononuclear Phagocytes in HTLV-III/LAV Infection. *Science* **1986**, *233*, 215–219.
(7) Aggarwal, S. K.; Gogu, S. R.; Rangan, S. R. S.; Agrawal, K. C. Synthesis and Biological Evaluation of Prodrugs of Zidovudine. *J. Med. Chem.* **1990**, *33*, 1505–1510.
(8) Torrence, P. F.; Kinjo, J.; Lesiak, K.; Balzarini, J.; De Clercq, E. AIDS Dementia: Synthesis and Properties of a Derivative of 3′-azido-3′-deoxythymidine (AZT) that may become 'Locked' into the Central Nervous System. *FEBS Lett.* **1988**, *234*, 135–140.
(9) Kawaguchi, T.; Ishikawa, K.; Seki, T.; Kazuhiko, J. Ester Prodrugs of Zidovudine. *J. Pharm. Sci.* **1990**, *79*, 531–533.
(10) (a) Palomino, E.; Kessel, D.; Horwitz, J. P. A Dihydropyridine Carrier System for Sustained Delivery of 2′,3′-Dideoxynucleoside to the Brain. *J. Med. Chem.* **1989**, *32*, 622–625. (b) Chu, C. K.; Bhadti, V. S.; Doshi, K. J.; Etse, E. T.; Gallo, J. M.; Boudinot, F. D.; Schinazi, R. F. 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. *J. Med. Chem.* **1990**, *33*, 2188–2192.
(11) Palomino, E.; Meltsner, B. R.; Kessel, D.; Horwitz, J. P. Synthesis and in Vitro Evaluation of Some Modified 4-Thiopyrimidine Nucleosides for Prevention or Reversal of AIDS Associated Neurological Disorders. *J. Med. Chem.* **1990**, *33*, 258–263.
(12) Barchi, J. J.; Marquez, V. E.; Driscoll, J. S.; Ford, H.; Mitsuya, H.; Shirasaka, T.; Aoki, S.; Kelley, J. A. Potential Anti-AIDS Drugs. Lipophilic, Adenosine Deaminase-Activated Prodrugs. *J. Med. Chem.* **1991**, *34*, 1647–1655.
(13) Henin, Y.; Gouyette, C.; Schwartz, O.; Debouzy, J.; Neumann, J.; Dinh, T. Lipophilic Glycosyl Phosphotriester Derivatives of AZT: Synthesis, NMR Transmembrane Transport Study, and Antiviral Activity. *J. Med. Chem.* **1991**, *34*, 1830–1837.
(14) Curley, D.; McGuigan, C.; Devine, K. G.; O'Connor, T. J.; Jeffries, D. J.; Kinchington, D. Synthesis and Anti-HIV Evaluation of Some Phosphoramidate Derivatives of AZT: Studies on the Effect of Chain Elongation on Biological Activity. *Antiviral Res.* **1990**, *14*, 345–356.
(15) Hansch, C.; Steward, A. R.; Anderson, S. M.; Bentley, D. The Parabolic Dependance of Drug Action upon Lipophilic Character as Revealed by a Study of Hypnotics. *J. Med. Chem.* **1968**, *11*, 1–11.
(16) Rall, D. P.; Zubrod, C. G. Mechanism of Drug Absorption and Excretion. Passage of Drugs in and out of the Central Nervous System. *Annu. Rev. Pharmacol.* **1962**, *2*, 109–128.
(17) Oldendorf, W. H. Lipid Solubility and Drug Penetration of the Blood Brain Barrier. *Proc. Soc. Exp. Biol. Med.* **1974**, *147*, 813–816.
(18) Collins, J. M.; Klecker, R. W.; Kelley, J. A.; Roth, J. S.; McCully, C. L.; Balis, F. M.; Poplack, D. G. Pyrimidine Dideoxyribonucleosides: Selectivity of Penetration into Cerebrospinal Fluid. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 466–470.
(19) Cornford, E. M.; Oldendorf, W. H. Independent Blood Brain Barrier Transport Systems for Nucleic Acid Precursors. *Biochim. Biophys. Acta* **1975**, *394*, 211–219.
(20) Klecker, R. W.; Collins, J. M.; Yarchoan, R.; Thomas, R.; Jenkins, J. F.; Broder, S.; Myers, C. E. Plasma and Cerebrospinal Fluid Pharmacokinetics of 3′-Azido-3′-deoxythymidine: A Novel Pyrimidine Analog with Potential Application for the Treatment of Patients with AIDS and Related Diseases. *Clin. Pharmacol. Ther.* **1987**, *41*, 407–412.
(21) Klecker, R. W.; Collins, J. M.; Yarchoan, R.; Thomas, R.; McAtee, N.; Broder, S.; Myers, C. E. Pharmacokinetics of 2′,3′-Dideoxycytidine in Patients with AIDS and Related Disorders. *J. Clin. Pharmacol.* **1988**, *28*, 837–842.
(22) Kelley, J. A.; Litterst, C. L.; Roth, J. S.; Vistica, D. T.; Poplack, D. G.; Cooney, D. A.; Nadkarni, M.; Balis, F. M.; Broder, S.; Johns, D. G. The Disposition and Metabolism of 2′,3′-Dideoxycytidine, an in Vitro Inhibitor of HTLV-III Infectivity, in Mice and Monkeys. *Drug. Metab. Dispos.* **1987**, *15*, 595–601.
(23) Tomaszewski, J. E.; Grieshaber, C. K.; Balzarini, J.; Johns, D. G.; Smith, A. C.; Liao, J. T.; Collins, W. T. Toxicologic and Pharmacokinetic Evaluation of 2′,3′-Dideoxycytidine (ddCyd, NSC-606170), a Potential Drug to Treat AIDS. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 440.
(24) Broder, S. Pharmacodynamics of 2′,3′-Dideoxycytidine: An Inhibitor of Human Immunodeficiency Virus. *Am. J. Med.* **1990**, *88* (Suppl. 5B), 2S–7S.
(25) Yarchoan, R.; Thomas, R. V.; Allain, J. P.; McAtee, N.; Dubinsky, R.; Mitsuya, H.; Lawley, T. J.; Safai, B.; Meyers, C. E.; Perno, C. F.; Klecker, R. W.; Wills, R. J.; Fischl, M. A.; McNeely, M.; Pluda, J. A.; Leuther, M.; Collins, J. M.; Broder, S. Phase I Studies of 2′,3′-Dideoxycytidine in Severe Human Immunodeficiency Virus Infection as a Single Agent and Alternating with Zidovudine (AZT). *Lancet* **1988**, *i*, 76–81.
(26) Mitsuya, H.; Broder, S. Inhibition of the in Vitro Infectivity and Cytopathic Effect of Human T-Lymphotropic Virus Type III/Lymphoadenopathy-Associated Virus (HTLV-III/LAV) by 2′,3′-Dideoxynucleosides. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911–1915.
(27) Dahlberg, J. E.; Mitsuya, H.; Blam, S. B.; Broder, S.; Aaronson, S. A. Broad Spectrum Antiretroviral Activity of 2′,3′-Dideoxynucleosides. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2469–2473.
(28) Broder, S. Dideoxycytidine (ddC): A potent Antiretroviral Agent for Human Immunodeficiency Virus Infection. *Am. J. Med.* **1990**, *88* (Suppl. 5B), 1S.

Scheme II



stabilize the solid state and tend to decrease water solubility. We rationalized that N^4-[(dialkylamino)methylene] substituents would be able to fulfill these rather stringent requirements. Previous studies of the (dimethylamino)-methylene derivatives of exocyclic amino groups of nucleosides, including that of the arabinosyladenine derivative, have been reported,[32-34] and the initial results of our investigations on **4a**, the (dimethylamino)methylene derivative of **2**, were recently published.[35-37]

Chemistry

The synthesis of the prodrug series **3** and **4** proceeded smoothly by condensing the parent drugs **1** and **2**, respectively, with an excess of the appropriate formamide dimethyl acetal as outlined in Scheme I. In this way, N^4-substituted (*N,N*-dimethyl-, (*N,N*-diethyl-, (*N,N*-di-*n*-propyl-, and (*N,N*-diisopropylamino)methylene derivatives, as well as piperidino-, morpholino- and pyrrolidinomethylene derivatives, of ddC (**3a–g**) and 3′-F-ddC (**4a–g**), were prepared.

Lipophilicity

Partition coefficients (*P*) were determined for all compounds using mixtures of octanol and phosphate-buffered saline solution, pH 7.4, at room temperature. The results are shown in Table I. *P* values for the ddC derivatives ranged from 0.11 for the least lipophilic morpholino compound (**3f**) to 8.95 for the most lipophilic di-*n*-propyl derivative (**3c**). Thus, an increase in lipophilicity of 1.48–122 times that of the parent **1** ($P = 0.073$) was achieved. In the 3′-F-ddC series, the same trend was observed, with slightly, but consistently, higher *P* values: 1.65–175 times that of the parent **2** ($P = 0.128$). The diisopropyl derivatives (**3d** and **4d**) were also highly lipophilic, with *P* values of 73–108 times those of the parent compounds, respectively. An expected linear correlation exists between the carbon number of the dialkylamino side chain and log *P* values (Figure 1) for the homologous series dimethyl to di-*n*-propyl (**3a–c**, **4a–c**). The relationship between the log *P* values of the di-*n*-propyl (**3c**, **4c**) and diisopropyl derivatives (**3d**, **4d**) in either series is in agreement with the estimated 0.2 unit lower log *P* value for the branched-chain, compared to the corresponding straight-chain derivative.[38] The observation that the 3′-F-ddC derivatives (**4a–g**) have higher *P* values than their counterparts in the ddC series (**3a–g**) suggests that the 3′-fluoro substituent enhances the lipophilicity of the nucleoside. The logarithm of the ratios of the *P* values between the two series for each side-chain derivative (**a–g**) represents the contribution of the fluoro group to the lipophilicity of the molecule analogous to the lipophilic substituent constant, π. The results are summarized in Table II; the average π_F value of 0.32 ± 0.05 obtained is significantly larger than the predicted value of −0.17 for aliphatics.[38,39] This indicates that nucleosides fluorinated at the 3′-position of the sugar moiety associate less with the aqueous phase than expected. The same conclusion can be reached when the fluoro substituent effect is calculated from the lipophilicity data of nucleoside analogues published recently by other laboratories.[40,41] In view of the differential effects of the 3′-F and 3′-H atoms on the puckering of the 2′,3′-dideoxyribose ring,[37,42] the relatively high average π_F value may arise from a combination of atomic substitution and molecular conformational effects, the latter apparently predominating.

Hydrolytic Stability

The prodrugs decompose to the corresponding unmodified drugs by spontaneous hydrolysis, which involves a

(29) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. 3′-Substituted 2′,3′-Dideoxynucleosides Analogues as Potential Anti-HIV (HTLV-III/LAV) Agents. *J. Med. Chem.* **1987**, *30*, 1270–1278.

(30) Kowollik, G.; Etzold, G.; von Janta-Lipinski, M.; Gaertner, K.; Langen, P. Ein Neur Zugang zu 1-(2,3-Didesoxy-3-fluor-β-D-ribofuranoyl)-pyrimidinen. *J. Prakt. Chem.* **1973**, *315*, 895–900.

(31) Bodor, N.; Farag, H. H.; Brewster, M. E. Site-Specific, Sustained Release of Drugs to the Brain. *Science* **1981**, *214*, 1370–1372.

(32) Zemlička, J.; Holý, A. Preparation of N-Dimethylaminomethylene Derivatives—A New Method of a Selective Substitution of Nucleoside Amino Groups. *Collect. Czech. Chem. Commun.* **1967**, *32*, 3159–3168.

(33) Holý, A.; Zemlička, J. A Study on Hydrolysis of N-Dimethylaminomethylene Cytidine, -Adenosine, -Guanosine, and Related 2′-Deoxy Compounds. *Collect. Czech. Chem. Commun.* **1969**, *34*, 2449–2458.

(34) Hanessian, S. The N^6-(Dimethylamino)methylene Derivative of 9-β-D-Arabinosyladenine as an Antiviral Agent. *J. Med. Chem.* **1973**, *16*, 290–292.

(35) Kalman, T. I. Design of an Anti-HIV Prodrug. N^4-Dimethylaminomethylene-2′,3′-Dideoxy-3′-Fluorocytidine (DDFC). *Ann. N.Y. Acad. Sci.* **1990**, *616*, 538–539.

(36) Kalman, T. I.; Reddy, A. R. V. Chemistry and Biological Activity of the Anti-HIV Prodrug N^4-Dimethylaminomethylene-2′,3′-Dideoxy-3′-Fluorocytidine (DDFC). *Ann. N.Y. Acad. Sci.* **1990**, *616*, 540–541.

(37) Kalman, T. I.; Cody, V.; Reddy, A. R. V. Chemistry and Biological Activity of a Prototype Anti-HIV Dideoxynucleoside Prodrug NSC-D614989. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 409.

(38) Leo, A.; Hansch, C.; Elkins, D. Partition Coefficients and their Uses. *Chem. Rev.* **1971**, *71*, 525–616.

(39) Craig, P. N. Interdependence Between Physical Parameters and Selection of Substituent Groups for Correlation Studies. *J. Med. Chem.* **1971**, *14*, 680–684.

(40) Cheung, A. P.; Kenney, D. Partition Coefficients and Capacity Factors of Some Nucleoside Analogues. *J. Chromatogr.* **1990**, *506*, 119–131.

(41) Balzarini, J.; Cools, M.; De Clercq, E. Estimation of the Lipophilicity of Anti-HIV Nucleoside Analogues by Determination of the Partition Coefficient and Retention Time on a Lichrospher 60 RP-8 HPLC Column. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 413–422.

(42) Cody, V.; Kalman, T. I. Conformational Analysis of Two Anti-HIV Nucleoside Analogues 2′,3′-Dideoxy-3′-Fluorocytidine and its N^4-Dimethylaminomethylene Prodrug Derivative. *Nucleosides Nucleotides* **1992**, *11*, 731–738.

Table III. Stability ($t_{1/2}$), Solubility, and pK_a Values of the N^4-[(Diisopropylamino)methylene] Side-Chain Derivatives (3d, 4d) of ddC (1) and 3'-F-ddC (2), Respectively

	stability ($t_{1/2}$),[a] h						
	serum				1 N		
compound	0%	10%	20%	90%	HCl	solubility[b]	pK_a[c]
3d	47.5	47.3	51.2	65.6	3.9	>300	5.28
4d	52.1	52.4	56.5	86.7	5.8	~25	5.13
1 (ddC)						~78	4.42
2 (3'-F-ddC)						~15	4.22

[a] $t_{1/2}$ is the time required for 50% hydrolysis at 37 °C. [b] Solubility (mg/mL) in double-distilled water at room temperature. [c] Determined spectrophotometrically at room temperature.

common N^4-formyl intermediate[36] (Scheme II) and follows pseudo-first-order kinetics. Since N^4-[(dimethylamino)-methylene] substitution of cytosine nucleosides causes a pronounced (>45-nm) bathochromic shift in their UV spectra,[32,33] their hydrolytic decomposition[33] as well as that of our prodrug series is easily determined spectrophotometrically. Table I lists the half-lives ($t_{1/2}$ values) of the target compounds in phosphate-buffered saline solution, pH 7.4, at 37 °C.

The substitution by fluorine at the 3'-position of the sugar did not influence the rate of hydrolysis. In contrast, the structure of the dialkylamino moiety of the side chain had a marked effect. In both series the pyrrolidine analogues (**3g**, **4g**) were the least stable, with half-lives of 2–3 h, whereas the diisopropyl derivatives (**3d**, **4d**) were the most stable with half-lives >47 h. The $t_{1/2}$ values of the diisopropyl analogues (**3d**, **4d**) were more than 3 times larger than those of the di-*n*-propyl analogues (**3c**, **4c**), the second most stable derivatives. The heterocyclic derivatives (**3e–g**, **4e–g**) were more susceptible to hydrolysis than the corresponding open-chain derivatives (**3a–d**, **4a–d**) having the same (or similar) number of carbon atoms; i.e., the 5-membered pyrrolidino (**3g**, **4g**) and 6-membered morpholino (**3f**, **4f**) and piperidino (**3e**, **4e**) derivatives had significantly lower $t_{1/2}$ values than the corresponding diethylamino analogue (**3b**, **4b**). The observed differences in stability must be attributed to structural variations in the side chain, leading to steric and electronic effects. Thus, bulky groups may shield the amidine double bond from attack by a water molecule, whereas electron-donating groups may enhance resonance stabilization of the conjugated side chain. According to the X-ray structure of **4a**,[42] the N^4-side chain is coplanar with the pyrimidine ring, suggesting that a resonance-stabilized conjugated system does indeed exist. Such a conjugated system should be present in all of the prodrug derivatives, as evidenced by their identical λ_{max} values (314–317 nm). In the case of the diisopropyl derivatives **3d** and **4d** the large $t_{1/2}$ values are likely due to both steric hindrance and resonance effect. A linear correlation exists between carbon number of the dialkylamino side chains and $t_{1/2}$ values (Figure 1) for the homologous series dimethyl to di-*n*-propyl (**3a–c**, **4a–c**). The relationship between the half-lives within the heterocyclic series (**3e–g**, **4e–g**) is more complex. The pyrrolidino (**3g**, **4g**) and morpholino (**3f**, **4f**) derivatives are more unstable than the piperidino (**3e**, **4e**) analogue. The stability of the latter lies in between that of the dimethyl and diethyl derivatives (**3a,b**, **4a,b**).

The half-lives of the most stable diisopropyl analogues (**3d**, **4d**) were also determined in human serum and 1 N HCl solutions at 37 °C. The results are shown in Table III. No evidence for serum-catalyzed hydrolysis could be obtained. To the contrary, increase in serum concentration resulted in slightly increased $t_{1/2}$ values, suggesting that binding to serum proteins may afford protection from hydrolysis. In acidic solution the $t_{1/2}$ values decreased by an order of magnitude, but remained sufficiently large to consider oral administration of these prodrugs feasible. The $t_{1/2}$ values of the less stable dimethylamino derivatives **3a** and **4a** were decreased in acid to the same extent (data not shown).

Table IV. Antiviral Activities

	Rousher MuLV[a]		HIV[b]	
compound	ED_{50}[c]	IC_{50}[d]	ED_{50}[c]	IC_{50}[d]
3d	20.6	>1000	0.065	>200
4a	214	>1000	0.23	>200
4d	518	>1000	0.675	>200
1 (ddC)	2.2	>1000	ND[e]	
2 (3'-F-ddC)	158	>1000	0.16	>3200

[a] Rousher murine leukemia virus in vitro UV-XC plaque reduction assay using SC-1 cells in culture. [b] Reduction of HIV cytopathic effect in CEM-IW human lymphocytes in culture. [c] Concentration (μM) required to produce 50% antiviral effect. [d] Concentration (μM) required to produce 50% cytotoxicity. [e] Not determined under the same conditions.

Water Solubility

The estimated solubilities of the most stable diisopropyl derivatives (**3d**, **4d**) in double-distilled water at room temperature are listed in Table III. These prodrugs showed greater solubilities than their respective parent compounds. The diisopropyl derivative **3d** was found to be extremely soluble (>300 mg/mL). The side-chain modification resulted in >3-fold increase in water solubility. In the case of **4d**, the solubility was 1.7 times higher than that of the parent nucleoside analogue **2**. Since the N^4-substitution resulted only in a small increase in the pK_a values (0.9 unit) to 5.2–5.3 (Table III), protonation of the prodrugs could not be responsible for the increase in water solubility. This increase in water solubility is not unreasonable on the basis of the rationale discussed above. Albert[43] has previously shown that hydrophilic substituents, such as hydroxy or amino groups, on π-deficient heterocycles (including nucleosides) have an "insolubilizing effect" which tends to dramatically decrease the water solubility of these compounds. This is due to the preference of these compounds to form intermolecular H-bonds with each other rather than with water. One way to increase the water solubility would be to remove the insolubilizing groups. Jones et al.[44] made certain quinazoline antifolates more water soluble by removing the 2-amino group from the heterocycle. In the present work the hydrogen atoms of the amino group were removed instead, since the amino group of the parent drug is required for biological activity. The results demonstrate that substituting the exocyclic amino groups of poorly soluble nucleosides with *appropriate* side chains can significantly increase lipophilicity with concomitant elevation of water solubility.

Biological Data

The antiviral activities of **3d**, **4a**, and **4d** in two in vitro retroviral assay systems are summarized in Table IV. In the Rousher murine leukemia virus plaque reduction assay, which is relatively insensitive to ddC (1) and its derivatives, the diisopropylamino derivatives (**3d** and **4d**) were 3.3- and

(43) Albert, A. *Heterocyclic Chemistry*; The Athlone Press: University of London, 1968; Chapter 4, pp 71–74.
(44) Jones, T. R.; Thornton, T. J.; Flinn, A.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. Quinazoline Antifolates Inhibiting Thymidylate Synthase: 2-Desamino Derivatives with Enhanced Solubility and Potency. *J. Med. Chem.* **1989**, *32*, 847–852.

9.4-fold less potent than their respective parent drugs, indicating that efficient prodrug-to-drug conversion does occur. In the more sensitive anti-HIV assay system, the diisopropylamino derivative of 3′-fluoro-ddC (**4d**) was 4.2-fold less potent than its parent (**2**) and only 10-fold less active than the corresponding ddC derivative (**3d**). In both systems, the shorter lived dimethylamino derivative of 3′-fluoro-ddC (**4a**) was more active than the corresponding diisopropyl analogue (**4d**). None of the compounds showed any detectable cytotoxicity at the highest concentrations tested. Naturally, for a more meaningful prodrug/drug comparison of biological activities an appropriate in vivo model of AIDS should be employed. The planning of such studies has recently been initiated.

Conclusions

The results of this investigation demonstrate that poorly lipid-soluble antiviral nucleosides bearing an exocyclic amino group[45] can be subjected to proper chemical modification to yield prodrug series of increasing lipophilicity with retention or increase in water solubility. It is reasonable to suggest that other classes of compounds may similarly be susceptible to beneficial alteration of their solubility characteristics by analogous manipulation of their structure. In this study the (diisopropylamino)-methylene derivatives (**3d** and **4d**) have emerged superior to other members of the two prodrug series. Their optimal combination of desirable physicochemical properties, enhanced lipophilicity, high stability, and increased water solubility, suggests favorable pharmacokinetic characteristics, warranting their in vivo evaluation.

Experimental Section

Melting points were determined in open-end capillary tubes on a Mel-Temp apparatus and are uncorrected. UV spectra were recorded on Cary 118C or Hitachi U-2000 spectrophotometers. UV data of all new compounds were taken in distilled water and are reported as λ_{max} values with ϵ (molar extinction coefficient) given in parentheses. Proton NMR were recorded in DMSO-d_6 on Varian EM390 or Varian Gemini 300 spectrometers, and chemical shifts δ are reported in ppm relative to tetramethylsilane. TLC analyses were performed on Uniplate GHLF silica gel (Analtech). All reactions were carried out on a flame-dried apparatus under argon. Solvents and reagents were purchased from Aldrich. Human serum was obtained from Sigma. ddC was provided by NIH. Potassium phosphate buffered saline (PBS) solution, pH 7.4, was prepared by adjusting 0.08 M K_2HPO_4 in 0.9% NaCl to pH 7.4 with 0.08 M KH_2PO_4 in 0.9% NaCl.

Partition Coefficient Measurements. 1-Octanol/aqueous phase partition coefficients were determined at room temperature using the shake-flask procedure. An aliquot (12.5 μL) of a 10^{-2} M solution of the compound in PBS was diluted to 2.5 mL with the aqueous phase (PBS), previously saturated with octanol. An equal volume of octanol, previously saturated with the aqueous phase, was added to give a total volume of 5 mL. The mixture was shaken vigorously for 1 h, and the contents were allowed to stand for 15 min. The two phases were separated and centrifuged at 1000g for 5 min. The UV absorbance for both phases were measured at 317 or 271 nm for the side-chain derivative or parent drug, respectively. The partition coefficients were calculated from the ratio of the absorbance between the octanol and aqueous phases.

Half-Life ($t_{1/2}$) Determinations. Solutions of 5×10^{-5} M of each prodrug derivative in PBS (and also in 1 N HCl for **3d** and **4d**) were incubated at 37 °C. At regular time intervals the absorbances were recorded at 317 nm. A plot of the natural logarithm of the absorbance vs time gave a straight line with negative slope equal to the pseudo-first-order hydrolysis rate constant, from which $t_{1/2}$ values were calculated. For the determination of $t_{1/2}$ values in human serum, an aliquot of 100 μL of a 10^{-2} M stock solution of the diisopropylamino derivatives (**3d** and **4d**) was mixed with 100 μL of serum (for 10% serum solution) or 200 μL of serum (for 20% serum solution) and diluted to 1000 μL with buffer, or the 100-μL aliquot was mixed with 900 μL of serum (for 90% serum solution). These solutions were incubated at 37 °C, and at regular time intervals 50-μL aliquots were withdrawn and diluted to 1000 μL with the buffer and absorbance was measured at 317 nm. The half-life values were calculated as indicated above.

pK_a Measurements. pK_a's of the compounds **1**, **2**, **3d**, and **4d** were determined by UV spectroscopy following the method of Albert and Serjeant.[46]

Biological Testing. Antiretroviral activities were determined at the National Institutes of Health in two different in vitro systems. Data obtained with the Rousher leukemia virus grown on SC-1 cells were provided by the National Institute of Allergy and Infectious Diseases. The National Cancer Institute provided the testing data obtained in their anti-HIV screening system. Cytotoxicity data refer to 50% growth inhibitory activities determined by tetrazolium dye assay.

General Procedure for the Preparation of the (Dialkylamino)methylene Derivatives of ddC (1) and 3′-Fluoro-ddC (2). To the dried nucleoside, ddC (**1**) or 3′-fluoro-ddC (**2**)[29] (0.3–1.3 mmol), in dry DMF (10 mL/mmol of nucleoside) was added excess of the appropriate dialkylformamide dimethyl acetal[47] (3–20 equiv), and the contents were allowed to stir at room temperature in the absence of moisture for several hours. After TLC analysis indicated the consumption of all starting material, the contents were evaporated to dryness under reduced pressure in a rotary evaporator, and the residue was allowed to crystallize from an ethanol/ether mixture and dried in vacuo.

***N*⁴-[(Dimethylamino)methylene]-2′,3′-dideoxycytidine (3a).** A total of 211 mg (1 mmol) of **1** was reacted with dimethylformamide dimethyl acetal (1.19 g, 10 mmol) to give **3a** (248 mg, 93%): mp 160–161 °C; UV 314 nm (31 100); ^{1}H NMR δ 1.8 (m, 3 H, 3′-CH_2, 2′-CH_2), 2.25 (m, 1 H, 2′-CH_2), 2.98 (s, 3 H, CH_3), 3.1 (s, 3 H, CH_3), 3.6 (m, 2 H, 5′-CH_2), 4.0 (m, 1 H, 4′-CH), 5.0 (t, 1 H, OH), 5.9 (m, 2 H, 5-CH, 1′-CH), 8.1 (d, 1 H, 6-CH), 8.55 (s, 1 H, N=CH). Anal. ($C_{12}H_{18}N_4O_3$) C, H, N.

***N*⁴-[(Diethylamino)methylene]-2′,3′-dideoxycytidine (3b).** A total of 75 mg (0.35 mmol) of **1** was reacted with diethylformamide dimethyl acetal (0.9 g, 6.12 mmol) to yield **3b** (92 mg, 88%): mp 147 °C; UV 316 nm (31 500); ^{1}H NMR δ 1.12 (t, 3 H, CH_2CH_3), 1.17 (t, 3 H, CH_2CH_3), 1.85 (m, 3 H, 3′-CH_2, 2′-CH_2), 2.3 (m, 1 H, 2′-CH_2), 3.5 (q, 4 H, CH_2CH_3), 3.5 (m, 1 H, 5′-CH_2), 3.7 (m, 1 H, 5′-CH_2), 4.05 (m, 1 H, 4′-CH), 5.05 (t, 1 H, OH), 5.92 (m, 2 H, 5-CH, 1′-CH), 8.14 (d, 1 H, 6-CH), 8.6 (s, 1 H, N=CH). Anal. ($C_{14}H_{22}N_4O_3$) C, H, N.

***N*⁴-[(Dipropylamino)methylene]-2′,3′-dideoxycytidine (3c).** A total of 92 mg (0.44 mmol) of **1** was reacted with dipropylformamide dimethyl acetal (0.9 g, 5.14 mmol) to yield, after crystallization from a mixture of ethyl acetate and hexane, **3c** (121 mg, 86%): mp 82 °C; UV 316 nm (39 000); ^{1}H NMR δ 0.84 (t, 3 H, CH_2CH_3), 0.86 (t, 3 H, CH_2CH_3), 1.56 (q, 4 H, CH_2CH_3), 1.8 (m, 3 H, 3′-CH_2, 2′-CH_2), 2.3 (m, 1 H, 2′-CH_2), 3.4 (m, 4 H, $CH_2CH_2CH_3$), 3.55 (m, 1 H, 5′-CH_2), 3.7 (m, 1 H, 5′-CH_2), 4.05 (m, 1 H, 4′-CH), 5.05 (t, 1 H, OH), 5.92 (m, 2 H, 5-CH, 1′-CH), 8.14 (d, 1 H, 6-CH), 8.61 (s, 1 H, N=CH). Anal. ($C_{16}H_{26}N_4O_3$) C, H, N.

***N*⁴-[(Diisopropylamino)methylene]-2′,3′-dideoxycytidine (3d).** A total of 153 mg (0.72 mmol) of **1** was reacted with diisopropylformamide dimethyl acetal (1.26 g, 7.2 mmol) to yield **3d** (185 mg, 79%): mp 147–149 °C; UV 317 nm (39 200); ^{1}H NMR δ 1.22 (d, 6 H, CH_3), 1.28 (d, 6 H, CH_3), 1.8 (m, 3 H, 3′-CH_2, 2′-CH_2), 2.3 (m, 1 H, 2′-CH_2), 3.56 (m, 1 H, 5′-CH_2), 3.71 (m, 1 H, 5′-CH_2), 3.8 (heptet, 1 H, J = 6.66 Hz, $CH(CH_3)_2$), 4.05 (m,

(45) Some N^6- and N^2-substituted adenine and guanine nucleoside analogues were similarly prepared and showed significantly greater stability (higher $t_{1/2}$ values) than the corresponding cytosine nucleoside analogues.

(46) Albert, A.; Serjeant, E. P. *Ionization Constants of Acids and Bases*; John Wiley & Sons: New York, 1962; Chapter 4, pp 69–91.

(47) Bredereck, H.; Simchen, G.; Rebsdat, S.; Kantlehner, W.; Horn, P.; Wahl, R.; Hoffmann, H.; Grieshaber, P. Darstellung und Eigenschaften der Amidacetale und Aminalester. *Chem. Ber.* **1968**, *101*, 41–50.

1 H, 4'-CH), 4.71 (heptet, 1 H, J = 6.71 Hz, CH(CH_3)$_2$), 5.05 (t, 1 H, OH), 5.93 (m, 2 H, 5-CH, 1'-CH), 8.15 (d, 1 H, 6-CH), 8.71 (s, 1 H, N=CH). Anal. ($C_{16}H_{26}N_4O_3$) C, H, N.

N^4-(Piperidinomethylene)-2',3'-dideoxycytidine (3e). A total of 75 mg (0.35 mmol) of **1** was reacted with *N*-(dimethoxymethyl)piperidine (0.9 g, 5.6 mmol) to yield, after crystallization from a mixture of ethanol, ethyl acetate, and ether, **3e** (65 mg, 60%): mp 146–148 °C; UV 316 nm (35 000); ^{1}H NMR δ 1.6 (m, 6 H, CH_2), 1.85 (m, 3 H, 3'-CH_2, 2'-CH_2), 2.3 (m, 1 H, 2'-CH_2), 3.5–3.8 (m, 6 H, 5'-CH_2, NCH_2), 4.05 (m, 1 H, 4'-CH), 5.07 (t, 1 H, OH), 5.9 (m, 2 H, 5-CH, 1'-CH), 8.14 (d, 1 H, 6-CH), 8.61 (s, 1 H, N=CH). Anal. ($C_{15}H_{22}N_4O_3$) C, H, N.

N^4-(Morpholinomethylene)-2',3'-dideoxycytidine (3f). A total of 99 mg (0.47 mmol) of **1** was reacted with *N*-(dimethoxymethyl)morpholine (0.9 g, 5.6 mmol) to give **3f** (72 mg, 50%): mp 170–171 °C; UV 315 nm (33 500); ^{1}H NMR δ 1.8 (m, 3 H, 3'-CH_2, 2'-CH_2), 2.2 (m, 1 H, 2'-CH_2), 3.5–3.8 (m, 10 H, 5'-CH_2, NCH_2CH_2O), 4.05 (m, 1 H, 4'-CH) 5.92 (m, 2 H, 5-CH, 1'-CH), 8.2 (d, 1 H, 6-CH), 8.66 (s, 1 H, N=CH). Anal. ($C_{14}H_{20}N_4O_4$) C, H, N.

N^4-(Pyrrolidinomethylene)-2',3'-dideoxycytidine (3g). A total of 96 mg (0.45 mmol) of **1** was reacted with *N*-(dimethoxymethyl)pyrrolidine (0.9 g, 6.2 mmol) to yield **3g** (93 mg, 70%): mp 80 °C; UV 317 nm (37 000); ^{1}H NMR δ 1.7–1.9 (m, 7 H, 3'-CH_2, 2'-CH_2, CH_2), 2.2 (m, 1 H, 2'-CH_2), 3.45 (t, 2 H, J = 6.6 Hz, NCH_2), 3.55 (m, 1 H, 5'-CH_2), 3.64 (t, 2 H, J = 6.0 Hz, NCH_2), 3.7 (m, 1 H, 5'-CH_2), 4.05 (m, 1 H, 4'-CH), 5.07 (t, 1 H, OH), 5.9 (m, 2 H, 5-CH, 1'-CH), 8.14 (d, 1 H, 6-CH), 8.77 (s, 1 H, N=CH). Anal. ($C_{14}H_{20}N_4O_3 \cdot {}^1/_4H_2O$) C, H, N.

N^4-[(Dimethylamino)methylene]-3'-fluoro-2',3'-dideoxycytidine (4a). A total of 300 mg (1.3 mmol) of **2** was reacted with dimethylformamide dimethyl acetal (0.45 g, 3.78 mmol) to give **4a** (279 mg, 75%): mp 203 °C; UV 317 nm (34 900); ^{1}H NMR δ 2.0–2.3 (m, 2 H, 2'-CH_2), 3.03 (s, 3 H, CH_3), 3.17 (s, 3 H, CH_3), 3.55–3.63 (m, 2 H, 5'-CH_2), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27.4 Hz, 4'-CH), 5.2 (t, 1 H, OH), 5.3 (d, 1 H, $J_{3'\text{-}F}$ = 53.4 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.2 (dd, 1 H, 1'-CH), 7.97 (d, 1 H, 6-CH), 8.63 (s, 1 H, N=CH). Anal. ($C_{12}H_{17}N_4O_3F$) C, H, N.

N^4-[(Diethylamino)methylene]-3'-fluoro-2',3'-dideoxycytidine (4b). A total of 60 mg (0.26 mmol) of **2** was reacted with diethylformamide dimethyl acetal (0.9 g, 6.12 mmol) to yield **4b** (50 mg, 61%): mp 148 °C; UV 316 nm (33 000); ^{1}H NMR δ 1.12 (t, 3 H, CH_2CH_3), 1.18 (t, 3 H, CH_2CH_3), 2.0–2.28 (m, 2 H, 2'-CH_2), 3.5 (q, 4 H, C$H_2$$CH_3$), 3.6 (m, 2 H, 5'-$CH_2$), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27.4 Hz, 4'-CH), 5.2 (t, 1 H, OH), 5.3 (d, 1 H, $J_{3'\text{-}F}$ = 53 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.25 (dd, 1 H, 1'-CH), 7.97 (d, 1 H, 6-CH), 8.62 (s, 1 H, N=CH). Anal. ($C_{14}H_{21}N_4O_3F$) C, H, N.

N^4-[(Dipropylamino)methylene]-3'-fluoro-2',3'-dideoxycytidine (4c). A total of 84 mg (0.36 mmol) of **2** was reacted with dipropylformamide dimethyl acetal (0.9 g, 5.14 mmol) to yield **4c** (104 mg, 83%): mp 118 °C; UV 317 nm (39 000); ^{1}H NMR δ 0.85 (t, 3 H, CH_2CH_3), 0.86 (t, 3 H, CH_2CH_3), 1.6 (m, 4 H, CH_2CH_3), 2.0–2.2 (m, 2 H, 2'-CH_2), 3.5 (m, 4 H, $CH_2CH_2CH_3$), 3.6 (m, 2 H, 5'-CH), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27.3 Hz, 4'-CH), 5.2 (t, 1 H, OH), 5.3 (d, 1 H, $J_{3'\text{-}F}$ = 53.8 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.2 (dd, 1 H, 1'-CH), 7.98 (d, 1 H, 6-CH), 8.65 (s, 1 H, N=CH). Anal. ($C_{16}H_{25}N_4O_3F$) C, H, N.

N^4-[(Diisopropylamino)methylene]-3'-fluoro-2',3'-dideoxycytidine (4d). A total of 202 mg (0.88 mmol) of **2** was reacted with diisopropylformamide dimethyl acetal (0.9 g, 5.14 mmol) to yield, after evaporation, silica gel column chromatography with 1–2% MeOH in CH_2CH_2, and crystallization from a mixture of ethyl acetate and hexane, **4d** (235 mg, 78%): mp 119–121 °C: UV 318 nm (39 600); ^{1}H NMR δ 1.23 (d, 6 H, CH_3), 1.27 (d, 6 H, CH_3), 2.0–2.3 (m, 2 H, 2'-CH_2), 3.6 (m, 2 H, 5'-CH_2), 3.83 (heptet, 1 H, J = 6.7 Hz, CH(CH_3)$_2$), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27 Hz, 4'-CH), 4.7 (heptet, 1 H, J = 6.85 Hz, CH(CH_3)$_2$), 5.2 (t, 1 H, OH), 5.4 (d, 1 H, $J_{3'\text{-}F}$ = 53.9 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.2 (dd, 1 H, 1'-CH), 7.97 (d, 1 H, 6-CH), 8.72 (s, 1 H, N=CH). Anal. ($C_{16}H_{25}N_4O_3F$) C, H, N.

N^4-(Piperidinomethylene)-3'-fluoro-2',3'-dideoxycytidine (4e). A total of 74.6 mg (0.32 mmol) of **2** was reacted with *N*-(dimethoxymethyl)piperidine (0.9 g, 5.6 mmol) to yield **4e** (95 mg, 90%): mp 198 °C; UV 316.6 nm (36 200); ^{1}H NMR δ 1.6 (m, 6 H, CH_2), 2.05–2.3 (m, 2 H, 2'-CH_2), 3.5–3.75 (m, 6 H, 5'-CH_2, NCH_2), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27.3 Hz, 4'-CH), 5.2 (t, 1 H, OH), 5.3 (d, 1 H, $J_{3'\text{-}F}$ = 53.8 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.2 (dd, 1 H, 1'-CH), 7.96 (d, 1 H, 6-CH), 8.6 (s, 1 H, N=CH). Anal. ($C_{15}H_{21}N_4O_3F$) C, H, N.

N^4-(Morpholinomethylene)-3'-fluoro-2',3'-dideoxycytidine (4f). A total of 94 mg (0.41 mmol) of **2** was reacted with *N*-(dimethoxymethyl)morpholine (0.9 g, 5.6 mmol) to yield **4f** (103 mg, 77%): mp 205 °C; UV 316 nm (33 400); ^{1}H NMR δ 2.0–2.2 (m, 2 H, 2'-CH_2), 3.6–3.75 (m, 10 H, 5'-CH_2, NCH_2CH_2O), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27.3 Hz, 4'-CH), 5.2 (t, 1 H, OH), 5.3 (d, 1 H, $J_{3'\text{-}F}$ = 53 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.2 (dd, 1 H, 1'-CH), 8.0 (d, 1 H, 6-CH), 8.7 (s, 1 H, N=CH). Anal. ($C_{14}H_{19}N_4O_4F$) C, H, N.

N^4-(Pyrrolidinomethylene)-3'-fluoro-2',3'-dideoxycytidine (4g). A total of 100 mg (0.44 mmol) of **2** was reacted with *N*-(dimethoxymethyl)pyrrolidine (0.9 g, 6.2 mmol) to yield **4g** (106 mg, 78%): mp 191–193 °C; UV 317 nm (35 800); ^{1}H NMR δ 1.8 (m, 4 H, CH_2), 2.0–2.3 (m, 2 H, 2'-CH_2), 3.46 (t, 2 H, J = 6.1 Hz, NCH_2), 3.6 (m, 2 H, 5'-CH_2), 3.67 (t, 2 H, J = 6.1 Hz, NCH_2), 4.2 (d, 1 H, $J_{4'\text{-}F}$ = 27 Hz, 4'-CH), 5.2 (t, 1 H, OH), 5.3 (d, 1 H, $J_{3'\text{-}F}$ = 53 Hz, 3'-CH), 6.0 (d, 1 H, 5-CH), 6.24 (dd, 1 H, 1'-CH), 7.96 (d, 1 H, 6-CH), 8.8 (s, 1 H, N=CH). Anal. ($C_{14}H_{19}N_4O_3F$) C, H, N.

Acknowledgment. This investigation was supported in part by NIH Grant R01 AI27251.