and the mixture was stirred at room temperature for 18 h.

Water (0.5 mL) was added and the mixture stirred for an additional 30 min to hydrolyze the excess sulfonyl chloride. The pyridine and water were then removed by evaporation under reduced pressure, final traces being removed by azeotroping with toluene (2 × 5 mL). The residue was partitioned between dichloromethane (20 mL) and water (20 mL), the layers were separated, and the organic phase was washed with saturated aqueous copper(II) sulfate solution, dried over magnesium sulfate, and evaporated. Purification of the resulting material by flash chromatography eluting with ethyl acetate–hexane (1:1, v/v) gave **43**: 812 mg (69%); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.75 (3 H, d, J = 2 Hz, 5-CH_3), 3.52 (2 H, m, 5′-CH_2), 4.20 (1 H, m, 4-CH), 5.30 (1 H, m, 2′-CH), 5.44 (1 H, m, 3′-CH), 6.30 (1 H, m, 1′-CH), 7.24–7.50 (16 H, m, ArH and 6-CH), 9.96 (1 H, s, NH).

3′-Deoxy-2′,3′-didehydro-2′-fluoro-5′-*O*-tritylthymidine (44). A solution of sodium hydroxide (150 mg, 3.8 mmol) in water (1.5 mL) was added to a solution of **43** (712 mg, 1.27 mmol) in ethanol (50 mL), and the mixture was heated under reflux for 2.5 h. After the mixture cooled to room temperature, the solvent was removed under reduced pressure and the residue taken up in water (30 mL). The product was extracted with dichloromethane (3 × 30 mL), and the extracts were combined, dried over magnesium sulfate, and evaporated. Purification by flash chromatography eluting with hexane–ethyl acetate (2:1, v/v) gave **44**: 312 mg (55%); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.32 (3 H, s, 5-CH_3), 3.40 (2 H, m, 5′-CH_2), 4.95 (1 H, br s, 4′-CH), 5.72 (1 H, s, 3′-CH), 6.98 (1 H, br s, 1′-CH), 7.28–7.50 (16 H, m, ArH and 6-CH), 9.15 (1 H, s, NH).

3′-Deoxy-2′,3′-didehydro-2′-fluorothymidine (45). A solution of hydrogen chloride in chloroform (0.31 M, 2.4 mL, 0.75 mmol) was added to an ice-cooled solution of **44** (312 mg, 0.7 mmol) in chloroform (20 mL). After 30 min the solvent was evaporated under reduced pressure and the residue purified by flash chromatography eluting with ethyl acetate to give the product **45**, 130 mg (78%). A sample was recrystallized from ethyl acetate to give white crystals; mp 152–154 °C; MS m/e (+FAB) 243 $(M + H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.91 (3 H, s, 5-CH_3), 3.75–3.82 (1 H, m, 5′-CH_2), 3.91–3.99 (1 H, m, 5′-CH_2), 4.93 (1 H, m, 4′-CH), 5.72 (1 H, s, 3′-CH), 6.88 (1 H, m, 1′-CH), 7.58 (1 H, s, 6-CH), 8.30 (1 H, br s, NH). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

Acknowledgment. We thank Dr. W. A. Thomas and his staff in our Physical Chemistry Department for elemental analysis, HPLC separations, mass spectra, NMR spectra, and optical rotations.

Synthesis and Anti-HIV-1 Activity of 2′-"Up"-Fluoro Analogues of Active Anti-AIDS Nucleosides 3′-Azido-3′-deoxythymidine (AZT) and 2′,3′-Dideoxycytidine (DDC)[1,†]

Kyoichi A. Watanabe,*[,‡] Kazuho Harada,[‡] Joanna Zeidler,[‡] Jasenka Matulic-Adamic,[‡] Kiyobumi Takahashi,[‡] Wu-Yun Ren,[‡] Ling-Chin Cheng,[‡] Jack J. Fox,[‡] Ting-Chao Chou,[§] Qing-Yu Zhu,[§] Bruce Polsky,[∥] Jonathan W. M. Gold,[∥] and Donald Armstrong[∥]

Sloan-Kettering Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021. *Received March 21, 1989*

1-(3-Azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)thymine (**6**, F-AZT) and 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)cytosine (**12**, F-DDC) were synthesized from the potent antiherpes virus nucleosides 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (**1**, FMAU) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) in the hope that introduction of a 2′-"up"-fluoro substituent might potentiate the anti-HIV activity of AZT and DDC. FMAU (**1**) was converted in three steps into 2,3′-anhydro-1-(2-fluoro-2-deoxy-5-*O*-trityl-β-D-lyxofuranosyl)thymine (**4**), which when treated with NaN_3 followed by detritylation afforded **6**. F-DDC was prepared by two methods. Tritylation of FIAC followed by treatment of the product with thiocarbonyldiimidazole afforded the 5′-*O*-trityl-3′-*O*-(imidazolyl)thiocarbonyl nucleoside **9**. Upon radical reduction of **9** with Bu_3SnH and AIBN, 5′-*O*-trityl-DDC **10** was obtained. Compound **10** was detritylated to give **12**, which (when obtained by this procedure) resisted crystallization, but the diacetate **12′** was obtained in crystalline form. Alternatively, FAC (**14**) was converted into N^4,$O^{5'}$-dibenzoyl derivative **15**, which was treated with thiocarbonyldiimidazole. Reduction of **16** with Bu_3SnH/AIBN followed by debenzoylation afforded **12**, which was obtained in crystalline form. F-AZT did not exhibit any significant activity against the human immunodeficiency virus (HIV) in vitro. F-DDC, however, showed activity against HIV-1, but the therapeutic index is much inferior to that of AZT.

Our previous studies with uracil and cytosine nucleosides bearing 2′-fluoro substituents in the "up" (arabino) configuration provided a host of potent agents against many DNA viruses.[2-5] Most notable among these are 2′-fluoro-5-iodo-ara-C (FIAC, Figure 1) and 2′-fluoro-5-methyl-ara-U (FMAU), both of which are effective in vitro and in vivo against Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2), and Varicella zoster virus (VZV). Both compounds inhibited human cytomegalovirus (HCMV)[5-7] in vitro, as well as Epstein-Barr virus (EBV).[8]

† Parts of this work were presented at the 192nd National Meeting of the American Chemical Society, April, 1987, Denver, CO, and the 2nd Annual Meeting of the AIDS National Cooperative Drug Discovery and Development Group, November, 1988, Oakland, CA.

‡ Laboratory of Organic Chemistry.

§ Laboratory of Biochemical Pharmacology.

∥ Infectious Disease Service and Special Microbiology Laboratory, Department of Medicine.

(1) Nucleosides 154. For Nucleosides 153, see: Sochacka, E.; Nawrot, B.; Pankiewicz, K.; Watanabe, K. A. *J. Med. Chem.*, in press.
(2) Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21.
(3) For a review, see: Fox, J. J.; Lopez, C.; Watanabe, K. A. In *Medicinal Chemistry Advances*; De Las Heras, F. G., Vega, S., Eds.; Pergamon Press: New York, 1981; pp 27–40.
(4) Lopez, C.; Watanabe, K. A.; Fox, J. J. *Antimicrob. Agents Chemother.* **1980**, *17*, 803.
(5) Colacino, J. M.; Lopez, C. *Antimicrob. Agents Chemother.* **1985**, *28*, 252.

0022-2623/90/1833-2145$02.50/0

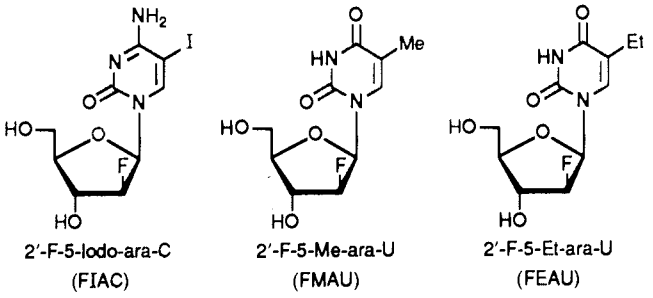


Figure 1.

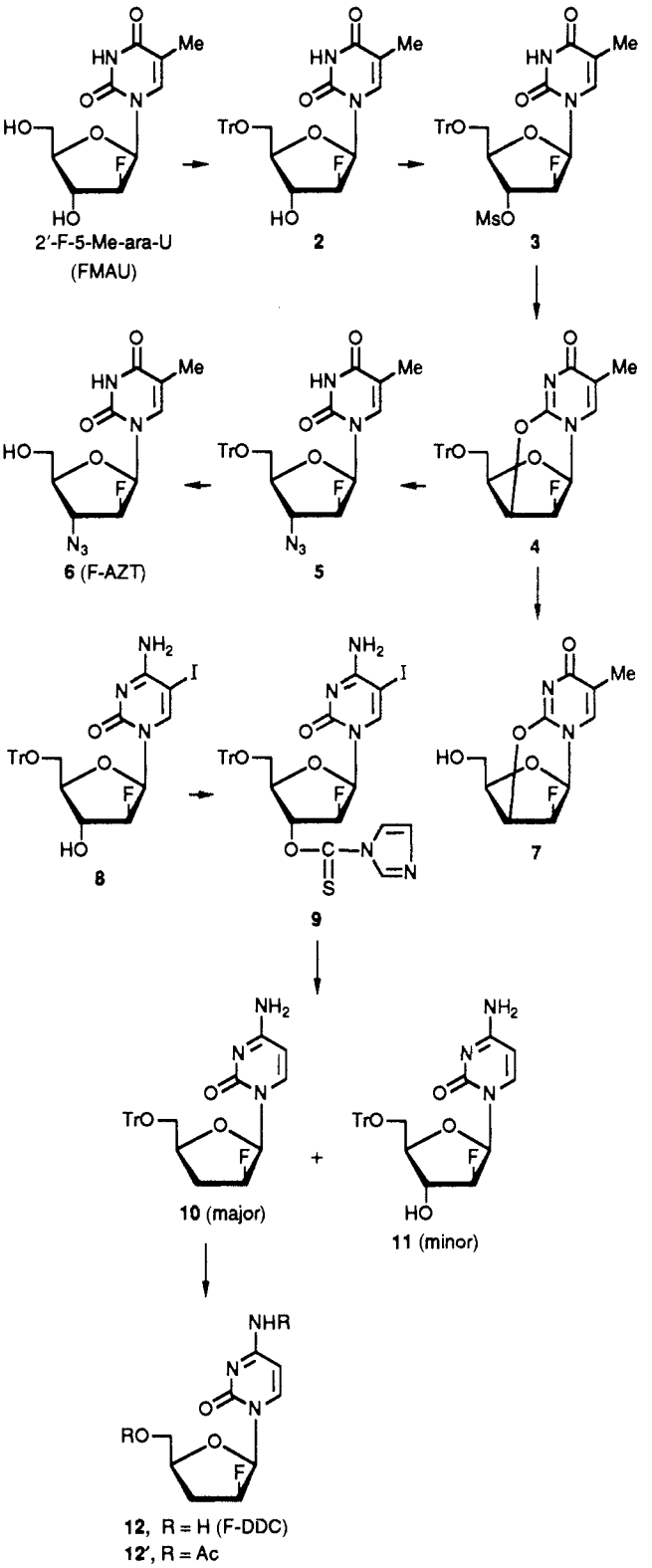
Rather comprehensive structure–activity relationship studies in our laboratory showed[2,3,9,10] that the 2′-fluoro substituent in the "up" (arabino) configuration confers far better antiviral activity than does the 2′-OH or the 2′-H. Fluorine at C-2′ was also shown to be a better choice than other halogeno substituents at this locus.[11]

Mitsuya et al. reported that 3′-azido-3′-deoxythymidine (AZT)[12] and 2′,3′-dideoxycytidine (DDC)[13,14] have shown very potent in vitro activity against HIV using the ATH8 cell line. In this report, we describe the synthesis of 1-(3-azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)thymine (**6**, F-AZT) and 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)cytosine (**12**, F-DDC), the AZT and DDC analogues containing the 2′-fluoro substituent in the "up" (arabino) configuration, in the hope that such nucleosides may exert even more potent anti-HIV activity.

F-AZT was synthesized in four steps from FMAU (**1**, Scheme I) which was tritylated to **2** and then mesylated to crystalline product **3**. Treatment of **3** with NaN_3 in aqueous DMF afforded two major products: the 2,3′-anhydro nucleoside **4** and the desired 3′-azido nucleoside **5**. It is interesting to note that formation of the "up"-azido isomer of **5** was not detected in the above reaction, although 5′-*O*-trityl-3′-*O*-mesylthymidine afforded almost equal amounts of the *erythro* (3′-"down") and *threo* (3′-"up") azido products upon treatment with NaN_3 in DMF, and the *threo* azido was further coverted into 6,3′-imino-bridged nucleoside.[15] Detritylation of **5** with aqueous HOAc gave F-AZT (**6**). The structure of **4** was firmly established by its conversion into **5** by treatment with NaN_3 in DMF.

For the synthesis of F-DDC (**12**), FIAC[2] was tritylated to **8**, which was treated with thiocarbonyldiimidazole to give the 3′-*O*-imidazolylthiocarbonate **9**. Treatment of **9** with tributyltin hydride in THF in the presence of 2,2′-azobis(2-methylpropiononitrile) (AIBN)[16] afforded 1-(2,3-dideoxy-2-fluoro-5-*O*-trityl-β-D-*threo*-pento-

Scheme I



(6) Schinazi, R. F.; Peters, J.; Sokol, M. K.; Nahmias, A. J. *Antimicrob. Agents Chemother.* **1983**, *24*, 95.
(7) Mar, E.-C.; Patel, P. C.; Cheng, Y.-C.; Fox, J. J.; Watanabe, K. A.; Huang, E.-S. *J. Gen. Virol.* **1984**, *65*, 47.
(8) Lin, J.-C.; Smith, M.-C.; Cheng, Y.-C.; Pagano, J. S. *Science* **1983**, *221*, 579.
(9) Watanabe, K. A.; Su, T.-L.; Reichman, U.; Greenberg, N.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1984**, *27*, 91.
(10) Su, T.-L.; Watanabe, K. A.; Schinazi, R. F.; Fox, J. J. *J. Med. Chem.* **1986**, *29*, 151.
(11) Watanabe, K. A.; Su, T.-L.; Klein, R. S.; Chu, C. K.; Matsuda, A.; Chun, M. W.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1983**, *26*, 152.
(12) Mitsuya, H.; Weinhold, K.; Furman, P. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096.
(13) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911.
(14) Mitsuya, H.; Jarrett, R. F.; Matsukura, M.; Veronese, F. D. M.; DeVico, A. L.; Sarngadharan, M. G.; Johns, D. G.; Reitz, M. S.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2033.
(15) Matsuda, A.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1980**, *45*, 3274.
(16) (a) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574. (b) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *47*, 485. (c) Matsuda, A.; Chu, C. K.; Reichman, U.; Pankiewicz, K.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1981**, *46*, 3603. (d) Mansuri, M. M.; Sarrett, J. E.; Wos, J. A.; Tortolani, D. R.; Brodfuehrer, P. R.; Howell, H. G.; Martin, J. C. *J. Org. Chem.* **1989**, *54*, 4780.

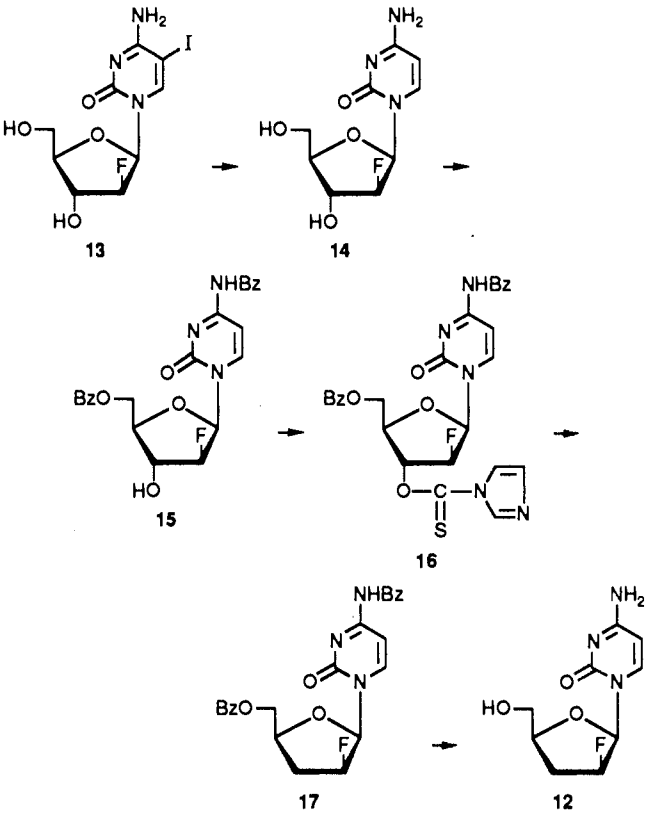
Table I. Anti-HIV-1 Effects and Cytotoxicity of F-AZT and F-DDC in MT4 Cells

	anti-HIV-1 effects: EC_{50}, μM		cytotoxicity:[a] IC_{50}, μM		selectivity ratio: IC_{50}/EC_{50}			
compd	P_{24} ELISA A	RT assay B	MT4 C	HL60[c] D	C/A	D/A	C/B	D/B
F-AZT	168	103	212	440	1.26	2.62	2.06	4.27
AZT	0.006	0.0045	53	96	8833	16000	11778	21333
F-DDC	2.66	2.54	872	707	328	266	343	278
F-DDC + THU[b]	0.73	0.71						
DDC	0.236	0.24	313	100	1326	424	1304	417
DDC + THU	0.075	0.063	339	190	4520	2533	5381	3016

[a] Inhibition of cell growth as determined by the trypan blue exclusion assay using a hemocytometer. [b] THU, 3,4,5,6-tetrahydrouridine (10 μM), a cytosine nucleoside deaminase inhibitor. [c] Human promyelocytic leukemic cells (HL-60) are not the target cells for HIV-1. This experiment was performed for cytotoxicity only.

furanosyl)cytosine (**10**) contaminated with a small amount of 5'-*O*-trityl-FAC (**11**). After purification by silica gel chromatography, **10** was de-*O*-tritylated with trifluoroacetic acid (TFA) in 1-butanol to F-DDC (**12**). Although showing a single spot on TLC and giving a ^1H NMR spectrum consistent with the structure of F-DDC, this compound resisted crystallization, and nitrogen analysis was about 2% off, as reported in the Experimental Section.[17] After acetylation, however, crystalline diacetate **12'** was obtained, which was analyzed correctly. Alternatively, FAC (**14**, Scheme II), obtained by deiodination of FIAC, was converted into the $N^4,O^{5'}$-dibenzoyl derivative **15** by the procedure of Ishido et al.[18] Thiocarbonylation of **15** to **16**, followed by radical reduction and debenzoylation, afforded crystalline F-DDC[19] (**12**). For preparation of the pure product in larger scale, the latter method proved to be far superior to the former.

Scheme II



Biological Studies

Initial Screening with Immunofluorescence Assay (IFA). These nucleoside analogues were screened preliminarily by indirect IFA[20] against the HTLV-III_B strain of HIV-1, using H9 cells as the target with 10^3 tissue culture infectious $doses_{50}$ ($TCID_{50}$) of virus. The HIV antigens were detected. Though initially *noncrystalline* F-DDC (**12**)[17] exhibited very little activity, in later studies using highly purified, crystalline **12** significant anti-HIV activity was detected. Very slight toxicity was observed at 100 μM of F-DDC. In H9 cells F-AZT did not exhibit any significant activity against HIV-1 at drug concentrations of 1.0, 10, and 100 μM after 8 days of treatment, whereas F-DDC inhibited 44, 89, and 100% of HIV replication.

Enzyme-Linked Immunosorbent Assay (ELISA) and Reverse Transcriptase (RT) Assay. The anti-HIV-1 EC_{50} values were measured in MT4 cells at five to six different concentrations of the test compounds, by using the P_{24} antigen ELISA and RT assays.[12-14] The IC_{50} values for cytotoxicity were tested in MT4 and HL60 cells by the XTT-microculture tetrazolium assay and trypan blue exclusion assay. It should be noted, however, that F-DDC exhibited significant activity against HIV-1 replication in MT4 cells (Table I, ED_{50} 2.66 μM in the P_{24} ELISA assay and 2.54 μM in the RT assay). It showed low cytotoxicity, as shown by the median-effect concentration for growth inhibition of MT4 cells (IC_{50} 1.48 mM in the XTT assay and 0.87 mM in the trypan blue exclusion assay) and of HL60 cells (IC_{50} 1.87 mM in the XTT assay and 0.71 mM in the trypan blue exclusion assay). F-AZT did not show significant anti-HIV-1 activity (ED_{50} of 168 μM for the P_{24} ELISA assay and 103 μM in the RT assay; IC_{50} was 212 μM in MT4 cells and 440 μM in HL60 cells in the trypan blue exclusion assay).

In the presence of pyrimidine nucleoside deaminase inhibitor, THU (10 μM), the anti-HIV potency of F-DDC and of DDC increased 3–4-fold. F-AZT and F-DDC exhibited lower cytotoxicity than AZT, as measured by the trypan blue exclusion method (Table I).

The use of THU, the known inhibitor of pyrimidine nucleoside deaminase, would have been expected to have little influence on the antiviral potency of F-DDC, since DDC has been reported previously[28] not to be a substrate for the deaminase. In the present studies, however, THU consistently produced strong enhancement of the anti-HIV-1 effect of DDC and F-DDC in both P_{24}-ELISA and RT assays, but decreased the cytotoxic effect in both MT4

(17) In a preliminary IFA experiment, this noncrystalline compound did not exhibit activity against HIV-1 in H9 cells.
(18) Nishino, S.; Yamamoto, H.; Nagata, Y.; Ishido, Y. *Nucleosides Nucleotides* **1986**, *5*, 159.
(19) Recently, F-DDC was reported as its HCl salt. Van Aerschot, A.; Herdewijn, P.; Balzarini, J.; Pauwels, R.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 1743.
(20) (a) Mitsuya, H.; Popovic, M.; Yarchoan, R.; Matsushita, S.; Gallo, R. C.; Broder, S. *Science* **1984**, *226*, 172. (b) Popovic, M.; Sarngadharn, M. G.; Read, E.; Gallo, R. C. *Science* **1984**, *224*, 497.

and HL60 cells (Table I). The mechanism for these selective and favorable effects is unclear. It is known[29] that one nucleoside may inhibit the transport of other nucleosides. Whether THU affects the intracellular nucleotide pool and whether THU enhances anti-HIV effects of other nucleoside analogues remain to be studied.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). Elemental analysis were performed by M.H.W. Laboratories, Phoenix, AZ. All the new compounds were analyzed correctly (±0.4%) unless otherwise specified. ^{1}H NMR spectra were recorded on a JEOL PFT-100 or a JEOL FX90 spectrometer with Me_4Si as the internal standard. Mass spectra were recorded at the Mass Spectrometric Biotechnology Resource, Rockefeller University, with use of N_2O and H_2O for the negative chemical ionization (OH^-/NCI) technique.

1-(2-Deoxy-2-fluoro-5-*O*-trityl-β-D-arabinofuranosyl)thymine (2). A mixture of FMAU (2.0 g, 7.69 mmol) and TrCl (2.8 g, 10.0 mmol) in dry pyridine (35 mL) was stirred overnight at room temperature and then heated at 100 °C for 90 min. The mixture was poured onto ice (500 g). The precipitates were washed several times with water, air-dried, and crystallized from C_6H_6–petroleum ether to give **2** (3.26 g, 85%): mp 195–200 °C; ^{1}H NMR ($CDCl_3$) δ 1.73 (3 H, s, 5-Me), 2.98 (1 H, d, 3′-OH), 3.43 (2 H, d, H-5′,5″, spacing 4.12 Hz), 4.03 (1 H, m, H-4′), 4.29 (1 H, dm, H-3′, $J_{3',F}$ = 23.5 Hz), 5.05 (1 H, dm, H-2′, $J_{2',F}$ = 55.7 Hz), 6.25 (1 H, dd, H-1′, $J_{1',2'}$ = 3.3, $J_{1',F}$ = 19.2 Hz), 7.23–7.60 (16 H, m, H-6, Tr), 9.11 (1 H, s, NH). Anal. ($C_{29}H_{25}FN_2O_5$), C, H, N.

1-(2-Deoxy-2-fluoro-3-*O*-mesyl-5-*O*-trityl-β-D-arabinofuranosyl)thymine (3). A mixture of **2** (1.50 g, 3.0 mmol) and MsCl (0.7 mL, 9.0 mmol) in pyridine (20 mL) was stirred at 0 °C for 2 h. The mixture was poured onto an ice–water mixture (100 mL), and the precipitates, collected by decantation, were dissolved in $CHCl_3$ (100 mL), dried (Na_2SO_4), and concentrated in vacuo, and the residue chromatographed on a silica gel column (hexane–EtOAc, 10:1) to give **3** (1.64 g, 97%): mp 164–166 °C (recrystallized from EtOH–petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.73 (3 H, s, 5-Me), 3.04 (3 H, s, Ms), 3.50 (2 H, m, H-5′,5″), 4.16 (1 H, m, H-4′), 5.28 (1 H, dm, H-2′, $J_{2',F}$ = 50.0 Hz), 5.37 (1 H, dm, H-3′, $J_{3',F}$ = 23.6 Hz), 6.22 (1 H, dd, H-1′, $J_{1',2'}$ = 3.4, $J_{1',F}$ = 19.5 Hz), 7.23–7.52 (16 H, m, H-6, Tr), 7.52 (1 H, s, NH). Anal. ($C_{30}H_{29}FN_2O_7S$), C, H, N.

2,3′-Anhydro-1-(2-deoxy-2-fluoro-5-*O*-trityl-β-D-lyxofuranosyl)thymine (4). To a refluxing solution of **3** (3.58 g, 6.2 mmol) in 90% aqueous EtOH (100 mL) was added dropwise 1 N NaOH (5.9 mL). After 2.5 h, the mixture was concentrated in vacuo. The residue was triturated with H_2O (100 mL), and the precipitates were crystallized from Me_2CO to give **4** (1.87 g, 63%): mp 133–135 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.94 (3 H, s, 5-Me), 3.38 (2 H, m, H-5′,5″), 4.30 (1 H, m, H-4′), 5.20 (2 H, dm, H-1′,3′, $J_{1',F} = J_{3',F}$ = 27 Hz), 5.46 (1 H, dt, H-2′, $J_{1',2'} = J_{2',3'}$ = 4.1, $J_{2',F}$ = 49.95 Hz), 6.92 (1 H, d, H-6, $J_{1',6}$ = 1.1 Hz), 7.16–7.46 (15 H, m, Tr). Anal. ($C_{29}H_{25}FN_2O_4$) C, H, N.

1-(3-Azido-2,3-dideoxy-2-fluoro-5-*O*-trityl-β-D-arabinofuranosyl)thymine (5). A. Treatment of 4 with LiN_3. A mixture of **4** (1.67 g, 3.5 mmol) and LiN_3 (0.68 g, 13.8 mmol) in dry DMF (30 mL) was heated at 120–130 °C for 18 h under N_2 atmosphere. The mixture was concentrated in vacuo, and the residue was partitioned between H_2O and $CHCl_3$ (100 mL each). The organic layer was separated, dried (Na_2SO_4), and concentrated, and the residue was chromatographed on a silica gel column (CH_2Cl_2–MeOH, 100:1 v/v) to give **5** (1.50 g, 83%) as a colorless foam. An aliquot was crystallized from $CHCl_3$–hexane (568 mg, 43.1%): mp 74–76 °C; ^{1}H NMR ($CDCl_3$) δ 1.76 (3 H, s, 5-Me), 3.45 (2 H, m, H-5′,5″), 3.88 (1 H, m, H-4′), 4.32 (1 H, ddd, H-3′, $J_{2',3'}$ = 2.5, $J_{3',4'}$ = 5.5, $J_{3',F}$ = 22 Hz), 5.10 (1 H, ddd, H-2′, $J_{1',2'}$ = 4.1, $J_{2',3'}$ = 2.5, $J_{2',F}$ = 52 Hz), 6.15 (1 H, dd, H-1′, $J_{1',2'}$ = 4.1, $J_{1',F}$ = 17.3 Hz), 7.26–7.52 (16 H, m, H-6, Tr), 8.55 (1 H, br s, NH). Anal. ($C_{29}H_{26}FN_5O_4$) C, H, N.

B. Direct Treatment of 3 with NaN_3. A mixture of **3** (1.45 g, 2.5 mmol) and NaN_3 (488 mg, 7.5 mmol) in DMF (10 mL) was heated at reflux for 2 h. The solvent was removed in vacuo, and the residue was partitioned between H_2O (10 mL) and $CHCl_3$ (20 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated, and the residue was chromatographed on a silica gel column ($CHCl_3$–MeOH, 30:1 v/v) to give crude **5** as a colorless foam (799 mg).

A second component (608 mg) was eluted from the column ($CHCl_3$–MeOH, 10:1 v/v). Crystallization from $CHCl_3$–hexane afforded **2,3′-anhydro-1-(2-deoxy-2-fluoro-5-*O*-trityl-β-D-lyxofuranosyl)thymine (4)** (500 mg, 40.1%), mp 133–135 °C dec. The ^{1}H NMR and IR spectra of this product were identical with those of **4** reported above.

1-(3-Azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)thymine (6). A solution of **5** (300 mg, 0.6 mmol) in 80% aqueous HOAc (10 mL) was heated at 90 °C for 1 h. The solvent was removed in vacuo, the residue was triturated well with H_2O and then filtered. The filtrate was concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$ then $CHCl_3$–EtOH, 10:1 v/v) to give a pale yellow oil which was crystallized from Me_2CO–petroleum ether: 135 mg (83%), mp 45–50 °C; IR (KBr) 2130 cm^{-1} (N_3); ^{1}H NMR (Me_2SO-d_6) δ 1.78 (3 H, s, 5-Me), 3.77 (2 H, m, H-5′,5″), 3.85 (1 H, m, H-4′), 4.52 (1 H, ddd, H-3′, $J_{2',3'}$ = 5.2, $J_{3',4'}$ = 7.1, $J_{3',F}$ = 22.5 Hz), 5.37 (1 H, dt, H-2′, $J_{1',2'} = J_{2',3'}$ = 5.2, $J_{2',F}$ = 53.3 Hz), 5.34 (1 H, t, 5′-OH), 6.15 (1 H, dd, H-1′, $J_{1',2'}$ = 5.2, $J_{1',F}$ = 11 Hz), 7.61 (1 H, s, H-6). Anal. ($C_{10}H_{12}FN_5O_4$) C, H, N.

2,3′-Anhydro-1-(2-deoxy-2-fluoro-β-D-lyxofuranosyl)thymine (7). In a manner similar to that outlined above, **4** was de-O-tritylated to give the title compound, mp 214–215.5 °C. Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

1-(2-Deoxy-2-fluoro-5-*O*-trityl-β-D-arabinofuranosyl)-5-iodocytosine (8). A mixture of FIAC[2] (2.5 g, 6.74 mmol) and TrCl (2.25 g, 8.1 mmol) in anhydrous pyridine (30 mL) was stirred for 2 days at room temperature and then heated at 90 °C for 1 h. The mixture was concentrated in vacuo, and the residue was triturated with hot $CHCl_3$ (3 × 100 mL) and H_2O (2 × 100 mL) and dried in vacuo to give **8** (3.84 g, 93%). Recrystallization from THF–Et_2O gave an analytical sample: mp 243 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.25 (2 H, m, H-5′,5″), 4.01 (1 H, m, H-4′), 4.28 (1 H, m, H-3′), 5.63 (1 H, br s, 3′-OH), 5.97 (1 H, s, NH), 6.09 (1 H, dd, H-1′, $J_{1',2'}$ = 3.57, $J_{1',F}$ = 19.49 Hz), 7.37 (15 H, m, Tr), 7.83 (1 H, s, H-6), 8.06 (1 H, s, NH). Anal. ($C_{28}H_{25}FIN_3O_4$) C, H, N.

1-[2-Deoxy-2-fluoro-3-*O*-[(imidazol-1-yl)thiocarbonyl]-5-*O*-trityl-β-D-arabinofuranosyl]-5-iodocytosine (9). A mixture of **8** (1.84 g, 3.0 mmol) and 1,1′-thiocarbonyldiimidazole (1.34 g, 2.5 equiv) in anhydrous DMF (15 mL) was stirred for 2 days at room temperature. The mixture was partitioned between EtOAc (250 mL) and H_2O (100 mL). The organic layer was separated, washed (H_2O, 2 × 50 mL), dried (Na_2SO_4), and concentrated to about 75 mL. After 12 h at room temperature, **9** crystallized and was collected by filtration: 1.54 g (71%); mp 220–221 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.45 (2 H, m, H-5′,5″), 4.51 (1 H, m, H-4′), 5.67 (1 H, dd, H-2′, $J_{1',2'}$ = 3.48, $J_{2',F}$ = 58.24 Hz), 6.06 (1 H, dd, H-3′, $J_{2',3'}$ = 0, $J_{3',4'}$ = 4.48, $J_{3',F}$ = 28.62 Hz), 6.36 (1 H, dd, H-1′, $J_{1',2'}$ = 3.48, $J_{1',F}$ = 20.66 Hz), 6.88 (1 H, br s, NH), 7.09, 7.86, 8.56 (1 H each, imidazole), 7.40 (15 H, m, Tr), 7.99 (2 H, m, H-6, NH). Anal. ($C_{32}H_{27}FIN_5O_4S$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-5-*O*-trityl-β-D-arabinofuranosyl)cytosine (10). Compound **9** (250 mg, 0.35 mmol) was dissolved in anhydrous THF (12 mL) under reflux. To the solution was added dropwise a solution of *n*-Bu_3SnH (0.5 mL, 1.8 mmol) and AIBN (32 mg) in THF (6 mL). The mixture was refluxed for an additional hour and then concentrated in vacuo. The residue was triturated several times with *n*-hexane and then placed on a silica gel column that was washed with CH_2Cl_2–EtOH (97:3 v/v). Compound **10** (120 mg, 74%) was obtained as a colorless foam: ^{1}H NMR (Me_2SO-d_6) δ 1.78–2.89 (2 H, m, H-3′,3″), 3.17 (2 H, d, H-5′,5″), 4.34 (1 H, m, H-4′), 5.20 (1 H, dm, H-2′, $J_{2',F}$ = 53.76 Hz), 5.65 (1 H, d, H-5, $J_{5,6}$ = 7.41 Hz), 6.00 (1 H, dd, H-1′, $J_{1',2'}$ = 3.29, $J_{1',F}$ = 18.93 Hz), 7.20–7.32 (18 H, m, Tr, H-6, NH_2). Anal. ($C_{28}H_{26}FN_3O_3$) C, H, N.

The column was washed further with CH_2Cl_2–EtOH (94:6 v/v). **5′-*O*-Trityl-FAC (11)** (29 mg, 17%) was eluted from the column: MS (OH^-/NCI) *m/z* 486 ($M - H^-$); ^{1}H NMR (Me_2SO-d_6) δ 3.25 (2 H, m, H-5′,5″), 3.97 (1 H, m, H-4′), 4.22 (1 H, m, H-3′), 4.93 (1 H, dm, H-2′, $J_{2',F}$ = 53.78 Hz), 5.66 (2 H, m, H-5, 3′-OH, $J_{5,6}$ = 7.41 Hz), 6.16 (1 H, dd, H-1′, $J_{1',2'}$ = 4.48, $J_{1',F}$ = 17.92 Hz),

7.02–7.65 (18 H, m, H-6, Tr, NH_2). Anal. ($C_{28}H_{26}FN_3O_4$) C, H, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (FAC, 14). FIAC (**13**, 3.0 g, 8.1 mmol) was dissolved in EtOH (150 mL), and the solution was adjusted to pH 10 by addition of concentrated NH_4OH and then reduced over Pd–C (10%, 1.5 g) in an H_2 atmosphere for 2 h with initial pressure of 20 psi. The catalyst was removed by filtration, and the filtrate was passed through an Amberlite IRA-400 (OH^-) column. The methanolic eluate was concentrated in vacuo, and the residue was crystallized from (EtOH–H_2O) to give **14** (1.6 g, 81%), mp 136–138 °C. The ^1H NMR spectrum was identical with that of an authentic sample.[21]

1-(5-*O*-Benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-*N*⁴-benzoylcytosine (15). To a stirred suspension of **14** (3.12 g, 12.7 mmol) in dry pyridine (240 mL) was added dropwise a solution of BzCl (3.30 mL, 28.43 mmol) in dry pyridine (240 mL), at room temperature, over a period of 2 h. After stirring for an additional 30 min, the reaction was quenched with MeOH. The mixture was concentrated in vacuo to a syrup which was chromatographed on a silica gel column (CH_2Cl_2–MeOH 60:1 and 40:1 v/v) to afford **15** (4.15 g, 72%): mp 191–192 °C (from EtOH); ^1H NMR (Me_2SO-d_6) δ 4.20–4.71 (4 H, m, H3′,4′,5′,5″), 5.19 (1 H, dm, H2′, $J_{2',F}$ = 52.02 Hz), 6.26 (1 H, dd, H1′, $J_{1',2'}$ = 3.56, $J_{1',F}$ = 18.66 Hz), 7.37 (1 H, d, H5, $J_{5,6}$ = 7.41 Hz), 7.40–8.12 (10 H, m, Bz), 8.21 (1 H, d, H-6, $J_{5,6}$ = 7.41 Hz), 11.50 (1 H, br s, NH). Anal. ($C_{23}H_{20}FN_3O_6$) C, H, N.

1-[5-*O*-Benzoyl-2-deoxy-2-fluoro-3-*O*-[(imidazol-1-yl)-thiocarbonyl]-β-D-arabinofuranosyl]-*N*⁴-benzoylcytosine (16). A mixture of **15** (2.10 g, 4.63 mmol) and 1,1-thiocarbonyldiimidazole (2.05 g, 11.5 mmol) in anhydrous DMF (20 mL) was stirred overnight at room temperature and then partitioned between EtOAc (250 mL) and H_2O (100 mL). The organic layer was separated, washed (H_2O, 2 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo to ca. 75 mL (bath temperature 35 °C), and the solution was diluted with *n*-hexane. Compound **16** precipitated and was collected by filtration and recrystallized from EtOAc to give 2.04 g (78%): mp 169–170 °C; ^1H NMR ($CDCl_3$) δ 4.64–4.90 (3 H, m, H-4′,5′,5″), 5.77 (1 H, dd, H-2′, $J_{1',2'}$ = 2.74, $J_{2',F}$ = 52.14 Hz), 6.14 (1 H, dd, H-3′, $J_{3',4'}$ = 1.92, $J_{3',F}$ = 19.21 Hz), 6.52 (1 H, dd, H-1′, $J_{1',2'}$ = 2.74, $J_{1',F}$ = 20.72 Hz), 7.03, 8.12 (1 H each, imidazole), 7.30–8.17 (11 H, m, 2Bz and imidazole), 9.53 (1 H, br s, NH). Anal. ($C_{27}H_{22}FN_5O_6S$) C, H, N.

1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-*N*⁴-benzoylcytosine (17). Compound **16** (1.0 g, 1.77 mmol) was suspended in dry toluene (37 mL), and the suspension was purged with Ar for 15 min and then heated to 110–120 °C. A solution of *n*-Bu_3SnH (4.38 mL, 16.29 mmol) and AIBN (60 mg) in dry toluene (23 mL) was purged with Ar for 10 min, and then the solution of **16** was added dropwise during a 15-min period. The mixture was heated under reflux for 1 h. After removal of the solvent in vacuo, the residue was chromatographed on a silica gel column using $CHCl_3$ and $CHCl_3$–MeOH (100:1 v/v). Compound **16** eluted and was crystallized from EtOH: 636 mg (87%); mp 170–171 °C; ^1H NMR ($CDCl_3$) δ 2.24–2.90 (2 H, m, H-3′,3″), 4.45–4.83 (3 H, m, H-4′,5′,5″), 5.45 (1 H, dm, H-2′, $J_{2',F}$ = 53.23 Hz), 6.14 (1 H, dd, H-1′, $J_{1',2'}$ = 2.47, $J_{1',F}$ = 19.49 Hz), 7.27–8.19 (10 H, m, 2Bz), 8.15 (1 H, br s, NH). Anal. ($C_{23}H_{20}FN_3O_5$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)cytosine (12, F-DDC). Method A. From 10. Compound **10** (151 mg, 0.32 mmol) was dissolved in a mixture of *n*-BuOH and TFA (3:1 v/v). The solution, after being kept at room temperature overnight, was diluted with *n*-BuOH (20 mL) and then concentrated in vacuo. The residue was triturated with EtOH (2 × 30 mL) and Et_2O (3 × 50 mL) and then dissolved in H_2O (10 mL). The aqueous solution was filtered and the filtrate concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$–MeOH, 4:1) to obtain **12** (68 mg, 93%) as a colorless foam (hygroscopic): MS (OH^-/NCI) *m/z* 228 ($M - H^-$); ^1H NMR (Me_2SO-d_6) δ 1.74–2.52 (2 H, m, H-3′,3″), 3.50 (2 H, m, H-5′,5″), 4.21 (1 H, m, H-4′), 4.83 (1 H, t, 5′-OH), 5.20 (1 H, dm, H-2′, $J_{2',F}$ = 54.61 Hz), 5.71 (1 H, d, H-5, $J_{5,6}$ = 7.41 Hz), 5.92 (1 H, dd, H-1′, $J_{1',2'}$ = 2.29, $J_{1',F}$ = 16.66 Hz), 7.15 (2 H, br s, NH_2), 7.65 (1 H, dd, H-6, $J_{5,6}$ = 7.41, $J_{6,F}$ = 1.38 Hz). Anal. ($C_9H_{12}FN_3O_3 \cdot 3H_2O$) Calcd: C, 38.16; H, 6.36; N, 14.84. Found: C, 38.04; H, 6.00; N, 12.51. On acetylation of **12** (80 mg) with Ac_2O/pyridine, crystalline diacetate **12′** was formed (86 mg, 79%): mp 223–224 °C (from MeOH); ^1H NMR (Me_2SO-d_6) δ 1.76–3.06 (8 H, m, OAc, NAc, H-3′,3″), 4.17–4.69 (3 H, H-4′,5′,5″), 5.36 (1 H, dm, H-2′, $J_{2',F}$ = 54.06 Hz), 6.04 (1 H, dd, H-1′, $J_{1',2'}$ = 3.3, $J_{1',F}$ = 18.80 Hz), 7.25 (1 H, d, H-5, $J_{5,6}$ = 7.41 Hz), 8.02 (1 H, dd, H-6, $J_{5,6}$ = 7.41, $J_{6,F}$ = 1.1 Hz), 10.92 (1 H, br s, NH exchangeable). Anal. ($C_{13}H_{16}FN_3O_5$) C, H, N.

Method B. From 17. Compound **17** (640 mg, 1.46 mmol) was dissolved in 0.3 N NaOMe in MeOH (18 mL), and the solution was stirred for 3 h at room temperature. The mixture was diluted with MeOH (30 mL) and then was neutralized with Amberlite IRC-50 (H^+) (10 mL). The resin was removed by filtration, the filtrate concentrated in vacuo, and the residue dissolved in H_2O (30 mL) and extracted with $CHCl_3$ (2 × 15 mL) and Et_2O (2 × 15 mL). The aqueous layer was concentrated in vacuo, and the residue was crystallized from EtOH to give 255 mg (82%) of **12** as colorless microcrystals, mp 203–206 °C. The ^1H NMR spectrum of this sample was identical with that of **12** prepared by method A. Anal. ($C_9H_{12}FN_3O_3$) C, H, N.

Anti-HIV-I Assay. Preliminary Screening Using H9 Cells. Experiments were performed in 24-well tissue culture plates, using 5×10^5 cells in a total volume of 1 mL. The cells were preincubated with nucleoside for 60 min before incubation with 10^3 $TCID_{50}$ of virus. The cultures were refed every 3–4 days with fresh medium containing the appropriate concentrations of each drug. On day 8, cells were harvested, and HIV antigens were detected by IFA using human serum containing high titers of anti-HIV antibodies. Cells were assessed for drug toxicity by trypan blue dye exclusion. Results are expressed as percent inhibition of infection in cultures containing experimental drugs, compared with control, untreated cultures. Zidovudine was routinely used as a positive control for inhibition.

Anti-HIV-I Assay Using MT4 Cells. MT4 cells were infected with HIV-I at 200 $TCID_{50}$ viruses per 10^6 cells. After an adsorption period of 1 h at 37 °C, 5% CO_2, unadsorbed virus was removed by washing once with fresh medium and centrifuging at 1000 rpm for 10 min. The cells were then suspended in fresh medium and made a cell suspension of 10^6 cells/3 mL. The cell suspension was distributed into 12-well plates (3 mL/well) and various concentrations of the test compounds were added immediately. After 5 days of incubation at 37 °C, 5% CO_2, HIV-1 P24 core antigen and RT activity in the supernatants of the cell cultures were determined. Untreated and uninfected cell control, and untreated but infected cell control were included in each experiment. Cell-free supernatant fluids of the cell cultures were assayed by HIV-1 P24 core antigen ELISA[22] (Du Pont–NEN Research Products, Boston, MA) and RT assay[23] after 5 days of incubation. For activity measurements, the procedure described in the Du Pont HIV-1 P24 core antigen ELISA kit was directly employed. AZT was routinely used as a positive control for inhibition of HIV-1 replication.

Cytotoxicity Assay. The cytotoxicity of the agents was determined in duplicate in 96-well microplates by XTT-microculture tetrazolium assay[24] and trypan blue exclusion assay. 2′,3′-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT) was prepared at 1 mg/mL in prewarmed (37 °C) medium without serum. Phenazine methosulfate (PMS) was prepared at 5 mM (1.53 mg/mL) in PBS. Fresh XTT and PMS were mixed together to make an 0.075 mM PMS–XTT solution (25 μL of the stock PMS was added per 5 mL of 1 mg/mL XTT). Fifty microliters of this mixture was added to each well of the cell culture after 4-day exposure to the agents. After incubation at 37 °C for 6 h, the 96-well plates were

(21) Reichman, U.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1975**, *42*, 233.

(22) Hartshorn, K. L.; Vogt, M. W.; Chou, T.-C.; Blumberg, R. S.; Byington, R.; Schooley, R. T.; Hirsch, M. S. *Antimicrob. Agents Chemother.* **1987**, *31*, 168.

(23) Sprira, T. J.; Bozeman, L. H.; Holman, R. C.; Warfield, D. T.; Phillips, S. K.; Feorino, P. M. *J. Clin. Microbiol.* **1987**, *25*, 97.

(24) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Noftiger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.

mixed, and absorbance at 450 and 630 nm was measured with a microplate reader (EL340, Bio-TEK Instruments, Winooski, VT).

Experimental Data Analysis. The dose–effect relationships of at least five different concentrations of each compound (plus no-drug control) were analyzed by the median-effect plot[25,26] using computer software[27] for automated analysis. The analysis provided anti-HIV-1 EC_{50} (median-effect concentrations), IC_{50} (median-inhibitory concentrations), and other dose–effect-related parameters.

Acknowledgment. This investigation was supported in part by funds from the National Cancer Institute and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USDHHS (Grants Nos. CA-08748 and AI-26056). The authors are indebted to Penny A. Baron for her excellent assistance in carrying out the anti-HIV assay in H9 cells. We thank Dr. Stephen Cohen of the Mass Spectrometry Biotechnology Resource, Rockefeller University, for recording the mass spectra.

(25) Chou, T.-C.; Talalay, P. *Adv. Enz. Regul.* **1984**, *22*, 27.
(26) Chou, T.-C.; Talalay, P. In *New Avenues in Developmental Cancer Chemotherapy*, Bristol-Myers Symposium Series; Harrap, K. R., Connors, T. A., Eds.; Academic Press: New York, 1987; pp 37–64.
(27) Chou, J.; Chou, T.-C. *Dose-effect Analysis with Microcomputers: Quantitation of ED_{50}, LD_{50}, Synergism, Antagonism, Low-dose Risk, Receptor Ligand Binding and Enzyme Kinetics*; IBM-PC Series; Elsevier-Biosoft, Elsevier Science Publ.: Cambridge, UK, 1987.
(28) Cooney, D. A.; Donald, M.; Mitsuya, H.; McMahon, J. B.; Nadkarni, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1986**, *35*, 2065.
(29) Kesseler, D.; Shurin, S. *Biochim. Biophys. Acta* **1968**, *163*, 179.

Synthesis and Anti-HIV Activity of Several 2′-Fluoro-Containing Pyrimidine Nucleosides

Roman Z. Sterzycki,* Ismail Ghazzouli, Vera Brankovan,† John C. Martin, and Muzammil M. Mansuri*

Department of Antiinfective Chemistry, Bristol-Myers Squibb Co., 5 Research Parkway, Wallingford, Connecticut 06492-7660, and Oncogen, Seattle, Washington 98121. *Received December 27, 1989*

Several 2′-fluoroarabino-2′,3′-dideoxy- and 2′-fluoro-2′,3′-unsaturated 2′,3′-dideoxy pyrimidine nucleoside analogues are reported. The saturated analogues 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)thymine (2′-*threo*-FddT, **33**), 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (2′-*threo*-FddU, **22**) were readily prepared from the corresponding 2′-deoxy-2′-fluoroarabinosyl nucleoside analogue by radical deoxygenation of the 3′-OH. The unsaturated compounds 1-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)thymine (2′-Fd4T, **40**) and 1-[5-*O*-(monomethoxytrityl)-2-fluoro-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl]uracil (**39**) were synthesized by an elimination reaction of the *O*-2,3′-anhydro-2′-fluoro-*lyxo* derivatives under basic conditions. The cytidine analogues **28** and **41** were prepared by amination of the corresponding uridine derivatives; compounds **28** and **41** were deprotected to give 1-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)cytidine (2′-*threo*-FddC, **29**) and 1-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)cytosine (2′-Fd4C, **42**), respectively. All of these novel compounds were evaluated in vitro against human immunodeficiency virus (HIV) (LAV isolate). 2′-*threo*-FddC (**29**) was the most active of the newly synthesized substances against HIV with an ID_{50} of 0.8 μg/mL; ddC had an ID_{50} of 0.007 μg/mL. Because of its potency in the initial tests, **29** was further evaluated in both T cells and macrophage/monocyte cell lines, with several different isolates of HIV. Although 2′-*threo*-FddC (**29**) exhibited good antiviral activity in these systems, it was less active than AZT in these assays. At 1 μM the inhibition of CFU-GM by **29** was found to be 35–40%; this is slightly higher than seen with AZT.

Introduction

An extensive effort has been conducted during the last few years to discover chemotherapeutic agents to counteract acquired immune deficiency syndrome (AIDS), which results from infection with human immunodeficiency virus (HIV).[1] 3′-Azido-3′-deoxythymidine (AZT, **1**) is currently the only drug available for the treatment of HIV infection.[2] There are, however, significant side effects with AZT; the most severe of these side effects are associated with bone marrow toxicity.[3,4] Long-term treatment of patients with AZT can also result in the emergence of low-sensitivity viral strains.[5] There is, therefore, a need for other effective but less toxic chemotherapeutic alternatives to AZT.

Several different classes of nucleoside analogues have already been identified as anti-HIV agents (Figure 1). These compounds fall into three main structural types: (1) the 3′-substituted purine or pyrimidine nucleosides such as AZT (**1**), 3′-azido-2′,3′-dideoxyuridine (AZU, **2**),[6] 3′-azido-2′,3′-dideoxyguanosine (AZG, **3**),[7] and 3′-fluoro-3′-deoxythymidine (3′-FddT, **4**),[8] (2) saturated dideoxy purine or pyrimidine nucleosides such as 2′,3′-dideoxyadenosine (ddA, **5**), or 2′,3′-dideoxyinosine (ddI, 6), or 2′,3′-dideoxycytidine (ddC, 7);[9] and (3) 2′,3′-unsaturated pyrimidine nucleosides as exemplified by 1-(2,3-dideoxy-β-D-

† Oncogen.

(1) (a) Barre-Sinoussi, F.; Chermann, J. C.; Rey, R.; Nugeyre, M. T.; Chamaret, S.; Gruest, C.; Dauguet, C.; Axler-Blin, C.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science (Washington, D.C.)* **1983**, *220*, 868. (b) Broder, S.; Gallo, R. C. *N. Engl. J. Med.* **1984**, *311*, 1292. (c) Broder, S.; Gallo, R. C. *Annu. Rev. Immunol.* **1985**, *3*, 321.
(2) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooley, R. T.; Jackson, G. G.; Durack, D. T.; King, D. *N. Engl. J. Med.* **1987**, *317*, 185. (b) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *82*, 7096.
(3) Sommadossi, J. P.; Carlisle, R. *Antimicrob. Agents Chemother.* **1987**, *31*, 452.
(4) (a) Yarchoan, R.; Klecker, R. W.; Weinhold, K. J.; Markham, P. D.; Lyerly, H. K.; Durack, D. T.; Gelmann, E.; Lehrman, S. N.; Blum, R. M.; Barry, D. W.; Shearer, G. M.; Fischl, M. A.; Mitsuya, H.; Gallo, R. C.; Collins, J. M.; Bolognesi, D. P.; Myers, C. E.; Broder, S. *Lancet* **1986**, *i*, 575. (b) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Lehrman, S. N. *N. Engl. J. Med.* **1987**, *317*, 192.
(5) Larder, B. A.; Darby, G.; Richman, D. D. *Science* **1989**, *243*, 1731.

0022-2623/90/1833-2150$02.50/0