Fluorinated Sugar Analogues of Potential Anti-HIV-1 Nucleosides[1]

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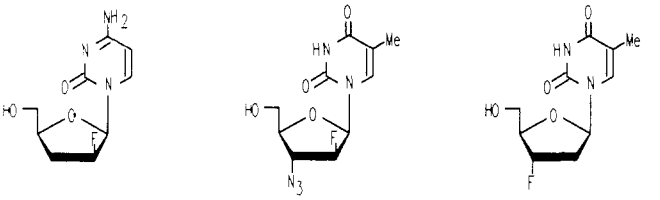
In order to obtain agents with therapeutic indices superior to those of AZT, FLT, or D4T, several analogues of anti-HIV-1 nucleosides were synthesized. These include 2′,3′-dideoxy-2′,3′-difluoro-5-methyluridine (**13**), its arabino analogue **19**, arabino-5-methylcytosine analogue **21**, 3′-deoxy-2′,3′-didehydro-2′-fluorothymidine (**25**), 3′-azido-2′,3′-dideoxy-2′-fluoro-5-methyluridine (**29**), 2′-azido-3′-fluoro-2′,3′-dideoxy-5-methyluridine (**31**), and 2′,3′-dideoxy-2′-fluoro-5-methyluridine (**37**). These new nucleosides were screened for their activity against HIV and feline TLV in vitro. None of the compounds showed significant activity. It is interesting to note that such a small modification in the sugar moiety of active anti-HIV nucleosides (i.e., displacement of hydrogen by fluorine) almost completely inactivate the agents.

Introduction

A recent report from our laboratories[2] as well as others[3,4] show that 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)cytosine (F-DDC, Chart I) exhibited activity against HIV-1. On the other hand, 1-(3-azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)thymine (F-AZT, Chart I) was inactive against HIV-1 in both H9 and MT4 cells.[2,4] These results prompted us to further study fluorine-containing analogues of anti-HIV-1 nucleosides to evaluate the influence of the 2′-fluorine substituent on antiviral activity. In this report, we describe the synthesis and preliminary anti-HIV screening results of several 2′- and 3′-fluorinated nucleosides.

We were interested in 2′,3′-dideoxy-2′,3′-difluoro-*ribo* and -*arabino* nucleosides, and 2′,3′-didehydro-2′,3′-dideoxy-2′-fluoro nucleosides, since they are close analogues of the active 2′,3′-dideoxy nucleosides and 2′,3′-olefinic nucleosides.[5] We were also interested in 3′-azido-2′,3′-dideoxy-2′-fluoro-5-methyluridine and its 2′-azido-3′-fluoro isomer. During the course of this investigation, we synthesized 3′-deoxy-3′-fluorothymidine (FLT) in model experiments and observed an intriguing mesyl migration, which is also reported herein. Recently FLT was found to be very active against HIV-1.[6-8] FLT was originally synthesized by Etzold et al.[9] by opening the 2,3′-anhydro linkage of 2,3′-anhydrothymidine (**1**, Scheme I). Later, Herdewijn et al.[10] reported the synthesis of FLT by treatment of 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine with DAST. We utilized the readily accessible 2,3′-anhydro-1-(5-*O*-mesyl-β-D-*threo*-pentofuranosyl)thymine[11] (**2**) as the starting material, which was treated with HF/AlF_3 according to the method of Etzold et al.[9] to yield 3′-deoxy-3′-fluoro-5′-*O*-mesylthymidine (**3**) in 28% yield. Occasionally, we observed the formation of 3′,5′-di-*O*-mesylthymidine[12] (**5**) as the major product (up to 40% yield). The reproducibility of this reaction, however, was rather poor. Apparently, the mesylate ion produced by degradation attacked **2**. Actually, addition of methanesulfonic acid (MsOH) to the reaction produced **5** consistently. It was found that treatment of **2** with MsOH in dry dioxane also gave **5** as the major product. These results are very intriguing, since mesylate has long been considered to be nonnucleophilic. It should be noted that Langen et al.[13] also made a similar observation when they treated **2** with HF/DMF. They obtained **3** as the major product together with **5** (7%) and 2,5′-anhydro-3′-*O*-mesylthymidine (3%). Treatment of **3** with KOAc in Ac_2O afforded 5′-*O*-acetyl derivative **4**. Saponification of

Chart I



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[‡] Infectious Disease Service and Special Microbiology Laboratory, Department of Medicine.
[§] Laboratory of Biochemical Pharmacology.

(1) Nucleosides. 158. The chemistry part of this work together with some preliminary biological data were presented at the 2nd Annual Meeting of the AIDS National Cooperative Drug Discovery and Development Group, November 1988, Oakland, CA. During the preparation of this manuscript for publication, 2′,3′-dideoxy-2′,3′-difluorouridine and -cytidine were reported (Van Aerschot, A.; Balzarini, J.; Pauwels, R.; Kerremans, L.; De Clercq, E.; Herdewijn, P. *Nucleosides Nucleotides* **1989**, *8*, 1121.), as well as 1-(2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)uracil, -cytosine, and 1-(2,3-didehydro-2,3-dideoxy-2-fluoro-β-D-*glycero*-pentoenofuranosyl)thymine (Martin, J. A.; Bushnell, D. J.; Dunkan, I. B.; Dunsdon, S. J.; Hall, M. J.; Machin, P. J.; Merrett, J. H.; Parkes, K. E. B.; Roberts, N. A.; Thomans, G. J.; Galpin, S. A.; Kinchington, D. *J. Med. Chem.* **1990**, *33*, 2137.).
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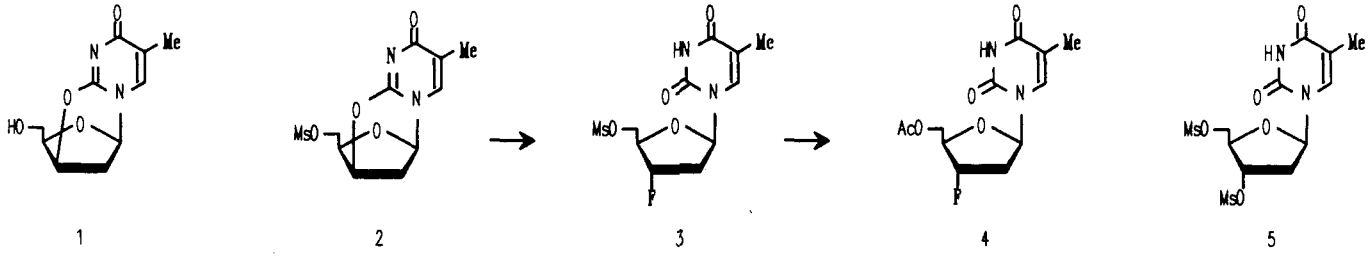
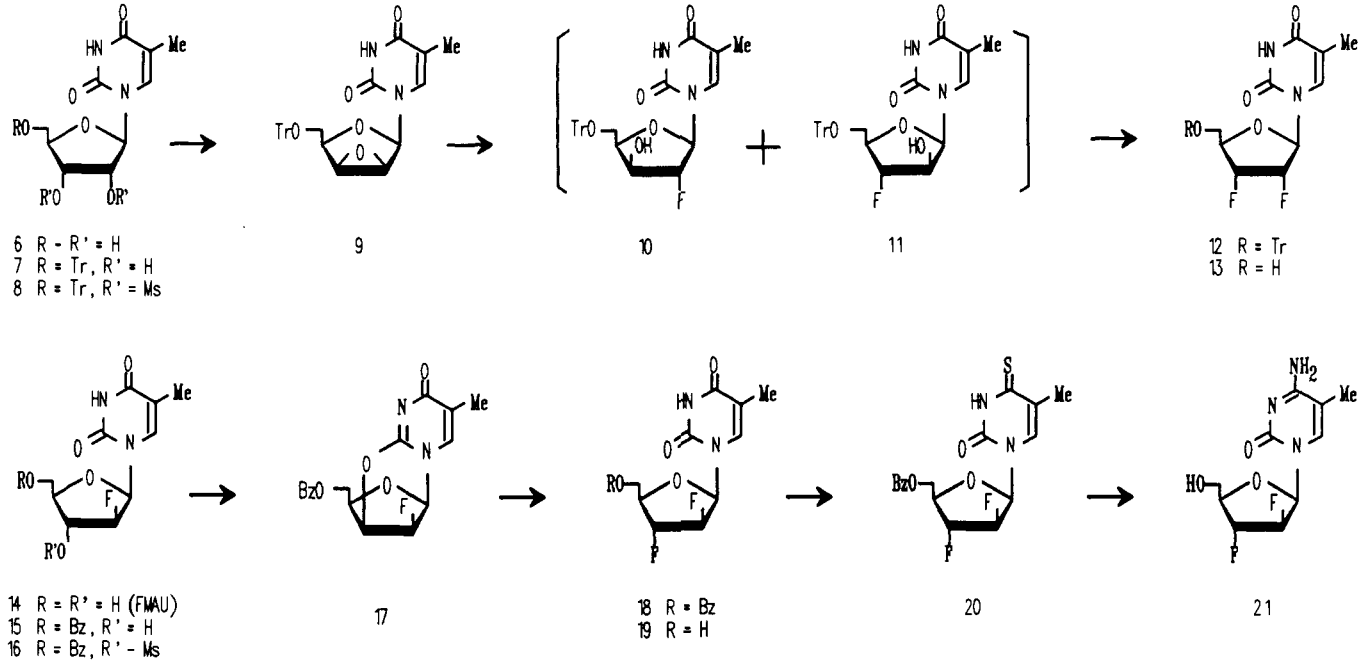
Scheme I



Scheme II



4 with methanolic ammonia gave FLT.

For the synthesis of 2′,3′-difluoro nucleosides, ribofuranosylthymine (**6**, Scheme II) served as the starting material, which was prepared by two different routes: the mercuric cyanide–nitromethane condensation procedure[14] and hydroxymethylation of uridine followed by reduction.[15] After tritylation and mesylation, the known dimesylate **8** was obtained, which was converted into the 2′,3′-*lyxo*-epoxide **9** with base. Treatment of epoxide **9** with KHF_2 in 2-ethoxyethanol at 140 °C afforded a mixture of the 2′-fluoro-*xylo* and 3′-fluoro-*arabino* nucleoside (**10** and **11**, respectively), which, without separation, was treated with DAST to afford the protected nucleoside **12**. De-O-tritylation of **12** gave crystalline 2′,3′-dideoxy-2′,3′-difluoro-5-methyluridine (**13**, 2′-F-*ribo*-FLT).

For the synthesis of 1-(2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)thymine (**19**, 2′-F-*arabino*-FLT), FMAU (**14**)[16] was selectively benzoylated to give 5′-*O*-benzoyl-FMAU (**15**), which was mesylated to **16**. Treatment of **16** with DBU[17] to anhydro nucleoside **17**, followed by HF/AlF_3 treatment[9] afforded 2′,3′-difluoro-*arabino* nucleoside **18**, which was de-O-benzoylated to give **19**. Compound **18** was thiated with P_2S_5 in dioxane,[18] and then ammonolysis of product **20** afforded 2′,3′-difluoro-5-methylarabinocytidine (**21**) in crystalline form.

As an analogue of 2′,3′-didehydro-3′-deoxythymidine (D4T), we synthesized 1-(2,3-didehydro-2,3-dideoxy-2-fluoro-β-D-*glycero*-pentofuranosyl)thymine[5] (**25**, Scheme III), starting also from FMAU (**14**), which was converted in two steps into 3′-*O*-mesyl-5′-*O*-trityl-FMAU (**22**). Conversion of **22** into 2,3′-anhydro intermediate **23** followed by butoxide treatment according to the method of Horwitz et al.[19] afforded 2′,3′-olefin **24**, which was de-O-tritylated to the desired nucleoside **25**.

When the *lyxo*-epoxide **9** was treated with NH_4N_3/EtOH[20] or NaN_3 in the presence of HOAc,[21] the major product was 3′-azido-*arabino* nucleoside **26**. However, by treatment of **9** with LiN_3/EtOH, during which the reaction condition became very basic, regioselectivity of the epoxide opening was lost,[22] and both **26** and 2′-azido-*xylo* isomer

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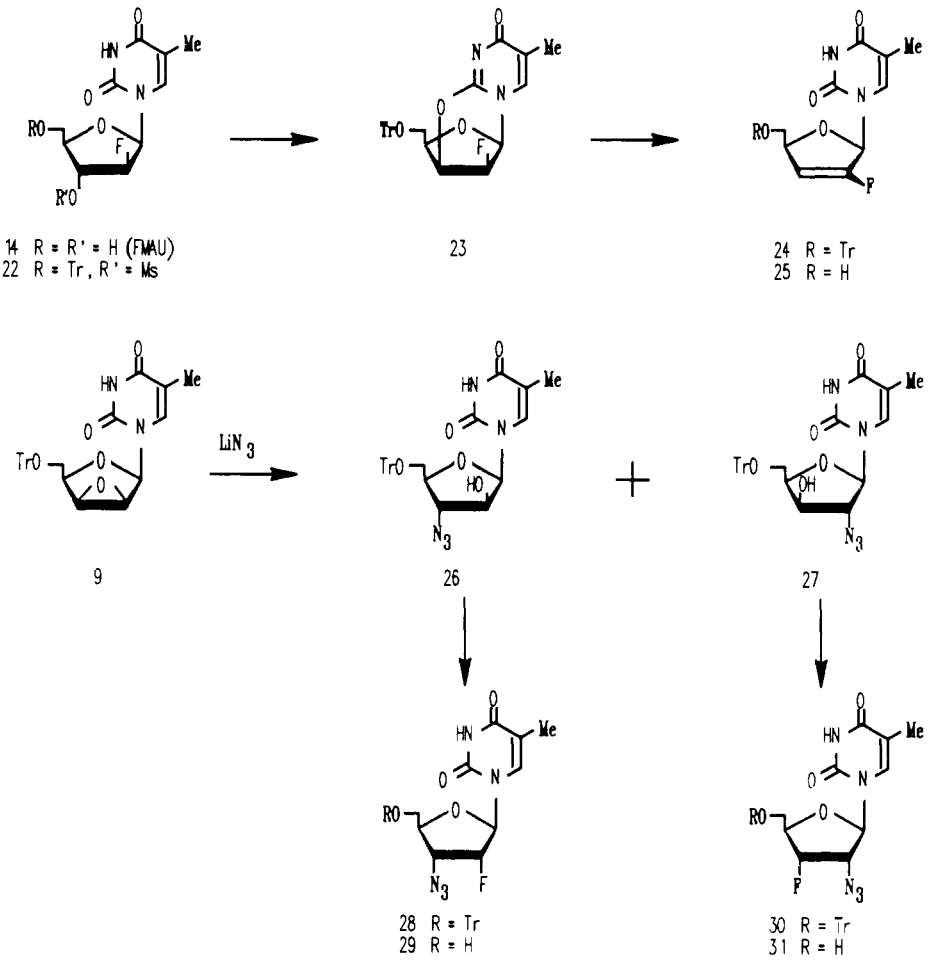
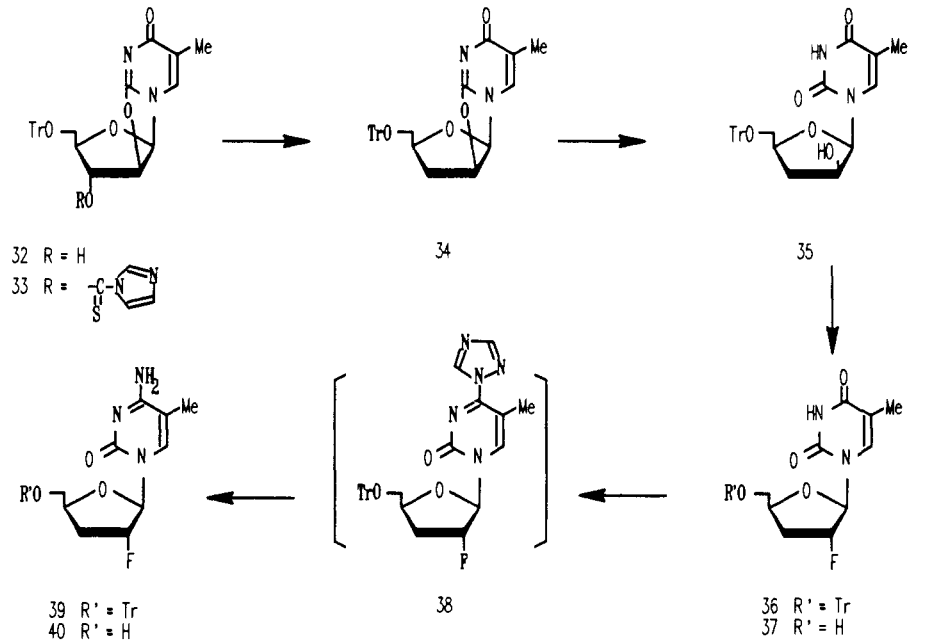
Scheme III



Scheme IV



27 were obtained in about a 3:2 ratio. After separation of these isomers, each was converted into the corresponding *ribo* fluoride **28** and **30**, respectively, by treatment with DAST. Subsequent de-O-tritylation afforded the fluorinated *ribo* analogue of AZT (**29**) and its isomer **31**.

We also synthesized 2′-fluoro-2′,3′-dideoxy-5-methyluridine (**37**, Scheme IV) as an isomer of FLT, and 5-methylcytosine derivative **40**. 2,2′-Anhydro nucleoside (**32**)[23] was converted into 3′-*O*-[1-imidazolyl(thiocarbonyl)]

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derivative **33**, which was reduced under Barton's conditions[24-26] to give 3′-deoxy-2,2′-anhydro nucleoside **34** in high yield. Mild alkaline treatment of **34** afforded *threo* (2′-β-OH) nucleoside **35**, which was further converted into the 2′-fluoro-*erythro* derivative **36** by treatment with DAST. Detritylation of **36** in 80% aqueous acetic acid gave the targeted nucleoside **37**. The corresponding cytosine analogue **40** was prepared from the 5′-*O*-trityl derivative **36** via triazolyl intermediates **38** by the known procedure.[27]

Biological Studies

These nucleoside analogues were screened preliminarily by indirect IFA[28] against the HTLV-III$_B$ strain of HIV-1, with H9 cells as the target with 10^3 tissue culture 50% infectious doses ($TCID_{50}$) of virus. HIV antigens were detected. None of the compounds described in this report showed significant anti-HIV activity.

These nucleoside analogues were also screened in vitro for inhibition of the replication of an animal oncoretrovirus, the amphotropic murine leukemia virus (MuLV), by an inhibition of infectious foci assay. The NS292 strain of MuLV was used to infect susceptible mink cells (CCL64) and the outcome of infection or inhibition of infection was determined by detection of MuLV antigens in the mink cells by using an indirect immunofluorescent assay.[29]

For cytotoxicity determination, human promyelocytic leukemic cells (HL-60) were used. Inhibition of cell growth was measured by XTT-microculture tetrazolium assay and trypan blue exclusion assay, using five or six different concentrations of each fluorinated nucleoside. The median inhibitory concentrations (IC_{50}s) for compound **21** were 234 and 123 μM, respectively, for the above two assays. All other compounds tested showed IC_{50} greater than 1 mM, indicating negligible cytotoxicity.

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). TLC was performed on Analtech Uniplates with short-wavelength UV light for visualization. Elementary analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^{1}H NMR spectra were recorded on a JEOL FX90Q spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), and dd (double doublet). Values given for coupling constants are first order.

3′-Deoxy-3′-fluoro-5′-*O*-mesylthymidine (3). A mixture of **2**[11] (1.0 g, 3.3 mmol), 0.2% (v/v) HF/dioxane (90 mL) and $AlF_3 \cdot 3H_2O$ (0.3 g, 2.2 mmol) was placed in a sealed steel vessel and heated at 170 °C for 90 min. After cooling to room temperature, the mixture was treated with H_2O (3 mL) and $CaCO_3$ (0.5 g, 5 mmol). The solid inorganic salts were removed by filtration. The filtrate was dried (Na_2SO_4) and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 3:1, v/v) to give **3** (300 mg, 28%) after recrystallization from MeOH: mp 161–163 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.79 (3 H, s, Me), 2.31 (1 H, m, H2′, $J_{2',F}$ = 14.31 Hz), 2.60 (1 H, m, H2″, $J_{2'',F}$ = 7.6 Hz), 3.24 (3 H, s, Ms), 4.43 (2 H, m, H5′,5″), 4.43 (1 H, dm, H4′, $J_{4',F}$ = 22.3, $J_{3',4'}$ = 4.0 Hz), 5.36 (1 H, dm, H3′, $J_{3',F}$ = 53.6, $J_{3',4'}$ = 4.0 Hz), 6.24 (1 H, dd, H1′, $J_{1',2'}$ = 7.68, $J_{1',2''}$ = 7.13 Hz), 7.50 (1 H, d, H6, $J_{Me,6}$ = 1.3 Hz), 11.4 (1 H, s, NH, exchangeable). Anal. Calcd ($C_{11}H_{15}FN_2O_6S$) C, H, F, N, S.

Treatment of 2 with MsOH in Dioxane. A mixture of **2** (0.5 g, 1.65 mmol) and MsOH (164 mg, 1.65 mmol) in dry dioxane (15 mL) was heated under reflux for 1.5 h. The mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ (50 mL). The $CHCl_3$ solution was washed with H_2O (3 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo, and the residue was chromatographed on a silica gel column (2 × 30 cm) using $CHCl_3$/MeOH (50:1, v/v) as the eluent to give 341 mg (52%) of 3′,5′-di-*O*-mesylthymidine (**5**): mp 157–158 °C darkening, 168 °C dec (lit.[13] mp 168–169 °C); the ^{1}H NMR spectrum of this sample was identical with that reported for 3′,5′-di-*O*-mesylthymidine.[30]

5′-*O*-Acetyl-3′-deoxy-3′-fluorothymidine (4). A mixture of **3** (200 mg, 0.62 mmol), KOAc (200 mg), and Ac_2O (12 mL) was heated at 135 °C for 3 h, and then concentrated in vacuo. Traces of Ac_2O were removed by coevaporations with toluene, and the residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, v/v) to give, after recrystallization from MeOH, **4** (100 mg, 57%): mp 97–99 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.78 (3 H, s, Me), 2.02 (3 H, s, Ac), 2.54 (1 H, dm, H2′, $J_{2',F}$ = 14.3 Hz), 2.37 (1 H, dm, H2″, $J_{2'',F}$ = 7.7 Hz), 4.22 (2 H, m, H5′,5″), 4.38 (1 H, dm, H4′, $J_{4',F}$ = 27.2 Hz), 5.32 (1 H, dm, H3′, $J_{3',F}$ = 53.6, $J_{3',4'}$ = 4.2 Hz), 6.21 (1 H, dd, H1′, $J_{1',2'}$ = 7.4, $J_{1',2''}$ = 6.5 Hz), 7.48 (1 H, d, H6, $J_{Me,6}$ = 1.3 Hz), 11.4 (1 H, s, NH, exchangeable). Anal. Calcd ($C_{12}H_{15}FN_2O_5$) C, H, F, N.

1-(2,3-Di-*O*-mesyl-5-*O*-trityl-β-D-ribofuranosyl)thymine (8). A solution of **6** (9.6 g, 37 mmol) in pyridine (95 mL) was treated with TrCl (11.34 g, 40 mmol). The mixture was kept overnight at 0 °C, and then heated at 70 °C for 3 h. The mixture was cooled to 0 °C, and MsCl (3.5 g, 31 mmol) was added. After 1 h at 0 °C, the mixture was left at room temperature overnight and then poured onto an ice/water mixture (2 L). The solid precipitates were collected and dissolved in $CHCl_3$ (50 mL), and the solution was washed (H_2O), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 49:1, v/v) to give 13.4 g (71%) of **8**: mp 107–110 °C (from Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 1.46 (3 H, s, Me), 3.27 and 3.37 (6 H, ds, 2 Ms), 3.40 (2 H, m, H5′,5″), 4.07 (1 H, m, H4′), 5.53 (2 H, m, H2′,3′), 6.01 (1 H, d, H1′, $J_{1',2'}$ = 4.1 Hz), 7.26–7.52 (16 H, m, Tr and H6), 11.54 (1 H, s, NH, exchangeable). Anal. Calcd ($C_{27}H_{36}N_2O_{10}S \cdot {}^1/_2H_2O$) C, H, N, S.

1-(2,3-Anhydro-5-*O*-trityl-β-D-lyxofuranosyl)thymine (9). To a solution of **8** (15 g, 23.3 mmol) in EtOH (100 mL) was added 1 *N* NaOH (15 mL) with stirring. The mixture was heated at 60 °C for 3 h and then cooled to room temperature. After neutralization with 80% HOAc, the mixture was concentrated in vacuo to about 30 mL and then poured onto ice/water (5 mL). The precipitates were collected and recrystallized from EtOH to give **9** (10.5 g, 95%): mp 130–134 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.65 (3 H, s, Me), 3.30 (2 H, m, H5′,5″), 4.06 (2 H, very narrow m, H3′,4′), 4.26 (1 H, s, H2′), 6.12 (1 H, s, H1′), 7.33 (16 H, m, Tr and H6), 8.72 (1 H, br s, NH). Anal. Calcd ($C_{25}H_{29}N_2O_5$) C, H, N.

1-(2,3-Dideoxy-2,3-difluoro-5-*O*-trityl-β-D-ribofuranosyl)thymine (12). A mixture of **9** (1.0 g, 2.1 mmol), KHF_2 (0.39 g, 5 mmol), $EtOCH_2CH_2OH$ (10 mL), and H_2O (2 mL) was heated at 145 °C for 15 h, and then the solvents were removed in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 49:1, v/v) to give a mixture of 1-(3-deoxy-3-fluoro-5-*O*-trityl-β-D-arabinofuranosyl)thymine (**10**) and 1-(2-deoxy-2-fluoro-5-*O*-trityl-β-D-xylofuranosyl)thymine (**11**) (0.75 g), which was dissolved in benzene (30 mL) and treated with DAST (0.97 g, 6 mmol) at −5 °C. After 2 h at room temperature, the mixture was poured onto ice/water (20 mL). The organic layer was separated, washed (10% $NaHCO_3$ 2 × 15 mL, and H_2O 2 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, v/v) to give 0.67 g (42%) of **12**; mp 145–148 °C (crystallized from MeOH); ^{1}H NMR (Me_2SO-d_6) δ 1.54 (3 H, s, Me), 3.24 (2 H, m, H5′,5″), 4.28 (1 H, dm, H4′, $J_{4',F3'}$ = 20.0 Hz), 5.49 (1 H, dm, H3′, $J_{2',3'}$ = 4.9, $J_{3',F2'}$ = 8.5, $J_{3',F3'}$ = 50.8 Hz), 5.56 (1 H, dd, H2′, $J_{2',F2'}$ = 50.3, $J_{2',F3'}$ = 8.5, $J_{1',2'}$ = 3.6, $J_{2',3'}$ = 4.9 Hz),

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6.04 (1 H, dd, H1′, $J_{1',2'}$ = 3.6, $J_{1',2'}$ = 15.0 Hz), 7.34 (16 H, m, Tr and H6), 11.52 (1 H, s, NH). Anal. Calcd ($C_{28}H_{26}F_2N_2O_4$) C, H, F, N.

1-(2,3-Dideoxy-2,3-difluoro-β-D-ribofuranosyl)thymine (13). A solution of **12** (0.6 g, 2 mmol) in 80% HOAc (15 mL) was heated at 100 °C for 15 min and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 9:1, v/v) to give 0.16 g (47%) of **13**: mp 204–206 °C (crystallized from EtOH); UV (0.01 N HCl) λ_{max} 264.0 nm (ε 10 900), λ_{min} 233.8 (ε 2900), (0.01 N NaOH) λ_{max} 264.4 nm (ε 7100), 227.2 (ε 8800), λ_{min} 245.2 (ε 5100); ^{1}H NMR (Me_2SO-d_6) δ 1.84 (3 H, s, Me), 3.72 (2 H, m, H5′,5″), 4.32 (1 H, dm, H4′, $J_{4',F3'}$ = 23.8 Hz), 5.32 (1 H, ddq, H3′, $J_{3',F3'}$ = 53.3, $J_{3',F2'}$ = 6.8, $J_{2',3'}$ = 4.9, $J_{3',4'}$ = 3.0 Hz), 5.43 (1 H, ddt, H2′, $J_{1',2'}$ = 4.9, $J_{2',3'}$ = 4.8, $J_{2',F2'}$ = 51.5, $J_{2',F3'}$ = 15.1 Hz), 5.45 (1 H, t, 5′-OH, exchangeable), 6.12 (1 H, dd, H1′, $J_{1',2'}$ = 5.4, $J_{1',F2'}$ = 15.2 Hz), 7.76 (1 H, d, H6, $J_{Me,6}$ = 1.38 Hz), 11.53 (1 H, s, NH, exchangeable). Anal. Calcd ($C_{10}H_{12}F_2N_2O_4$) C, H, F, N.

1-(5-O-Benzoyl-2-deoxy-2-fluoro-3-O-mesyl-β-D-arabinofuranosyl)thymine (16). A solution of FMAU (2.6 g, 10 mmol) in pyridine (80 mL) was treated with BzCl (1.4 g, 10 mmol) overnight at −10 °C. MsCl (1.14 g, 10 mmol) was then added, and the mixture was stirred overnight at room temperature. The reaction was quenched by addition of ice/water (200 mL) with vigorous stirring. The precipitates were collected by filtration, washed (H_2O), air-dried, dissolved in a small amount of $CHCl_3$, and chromatographed on a silica gel column ($CHCl_3$/MeOH, 9:1, v/v) to give, after crystallization from $CHCl_3$/Et_2O, 2.8 g (65%) of **16**: mp 136–137 °C. ^{1}H NMR (Me_2SO-d_6) δ 1.65 (3 H, s, Me), 3.74 (3 H, s, Ms), 4.51 (1 H, m, H4′), 4.65 (2 H, m, H5′,5″), 5.56 (1 H, ddd, H2′, $J_{2',F}$ = 51.75, $J_{1',2'}$ = $J_{2',3'}$ = 4.5 Hz), 5.54 (1 H, ddd, H3′, $J_{3',F}$ = 18.86, $J_{3',4'}$ = 4.0, $J_{2',3'}$ = 4.5 Hz), 6.29 (1 H, dd, H1′, $J_{1',F}$ = 16.43, $J_{1',2'}$ = 4.5 Hz), 7.39 (1 H, s, H6), 7.46–8.08 (5 H, m, Bz), 11.53 (1 H, s, NH, exchangeable). Anal. Calcd ($C_{18}H_{19}FN_2O_7S$) C, H, F, N.

2,3′-Anhydro-1-(5-O-benzoyl-2-deoxy-2-fluoro-D-lyxofuranosyl)thymine (17). A mixture of **16** (1.11 g, 2.5 mmol) and DBU (0.46 g, 3 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 3 days and then filtered. The filtrate was concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 19:1, v/v) to give 0.70 g (80%) of **17**: mp 245–248 °C (from MeOH); ^{1}H NMR (Me_2SO-d_6) δ 1.73 (3 H, s, Me), 4.43 (2 H, m, H5′,5″), 4.78 (1 H, m, H4′), 5.48 (1 H, dd, H3′, $J_{3',4'}$ = 2.5, $J_{3',F}$ = 7.8 Hz), 5.96 (1 H, dt, H2′, $J_{1',2'}$ = $J_{2',3'}$ = 4.0, $J_{2',F}$ = 49.4 Hz), 5.97 (1 H, dd, H1′, $J_{1',2'}$ = 4.0, $J_{1',F}$ = 6.4 Hz), 7.30 (1 H, d, H6, $J_{Me,6}$ = 1.8 Hz), 7.66 (5 H, m, Bz). Anal. Calcd ($C_{17}H_{15}FN_2O_5$) C, N, F, N.

1-(5-O-Benzoyl-2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)thymine (18). A mixture of **17** (174 mg, 0.5 mmol), AlF_3 (50 mg, 0.6 mmol), and HF (0.03 mL) in dioxane (50 mL) was heated at 170 °C for 1 h in a sealed steel vessel. After cooling to room temperature, the mixture was treated with H_2O (3 mL) and $CaCO_3$ (0.5 g). The solid inorganic materials were removed by filtration. The filtrate was dried (Na_2SO_4) and concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$) to give 55 mg (42%) of **18** (a foam): ^{1}H NMR (Me_2SO-d_6) δ 1.61 (3 H, s, Me), 4.56 (1 H, dm, H4′, $J_{3',4'}$ = 4.6, $J_{4',F3'}$ = 21.1 Hz), 4.67 (2 H, m, H5′,5″), 5.54 (1 H, dq, H3′, $J_{2',3'}$ = 3.0, $J_{3',4'}$ = 4.6, $J_{3',F3'}$ = 51.0, $J_{3',F2'}$ = 8.5 Hz), 5.69 (1 H, dq, H2′, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 3.0, $J_{2',F2'}$ = 51.0, $J_{2',F3'}$ = 14.1 Hz), 6.30 (1 H, dd, H1′, $J_{1',2'}$ = 4.4, $J_{1',F2'}$ = 17.8 Hz), 7.34 (1 H, s, H6), 7.43–8.10 (5 H, m, Bz), 11.53 (1 H, br s, NH). Anal. Calcd for $C_{16}H_{16}F_2N_2O_5 \cdot {}^1/_2H_2O$: C, 54.40; H, 4.57; F, 7.46; N, 10.12. Found: C, 54.38; H, 4.03; F, 7.79; N, 10.04. The value for hydrogen was off by 0.54%, but this intermediate was used directly in the next step.

1-(2,3-Dideoxy-2,3-difluoro-β-D-arabinofuranosyl)thymine (19). A solution of **18** (65 mg, 0.18 mmol) in NH_3/MeOH (25 mL) was stirred overnight at room temperature, and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 9:1, v/v) to give 39 mg (83%) of **19**, mp 131–134 °C (from EtOH); UV (0.01 N HCl) λ_{max} 264.8 nm (ε 10 100), λ_{min} 233.6 (ε 2900), (0.01 N NaOH) λ_{max} 265.6 (ε 7300), 227.6 (ε 8500), λ_{min} 244.4 (ε 4500); ^{1}H NMR (Me_2SO-d_6) δ 1.78 (3 H, s, Me), 3.69 (2 H, m, H5′,5″), 4.13 (1 H, dm, H4′, $J_{4',F3'}$ = 22.6, $J_{3',4'}$ = 2.8 Hz), 5.28 (1 H, t, 5′-OH, exchangeable), 5.35 (1 H, ddt, H3′, $J_{2',3'}$ = 4.6, $J_{3',4'}$ = 4.7, $J_{3',F3'}$ = 54.6, $J_{3',F2'}$ = 12.3 Hz), 5.52 (1 H, ddd, H2′, $J_{1',2'}$ = 3.0, $J_{2',F2'}$ = 56.1, $J_{2',F3'}$ = 12.3 Hz), 6.19 (1 H, dd, H1′, $J_{1',2'}$ = 4.4, $J_{1',F2'}$ = 17.6 Hz), 7.53 (1 H, d, H6, $J_{Me,6}$ = 1.9 Hz), 11.48 (1 H, s, NH). Anal. Calcd ($C_{10}H_{12}F_2N_2O_4$) C, H, F, N.

1-(5-O-Benzoyl-2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)-4-thiothymidine (20). To a stirred suspension of **18** (0.985 g, 2.7 mmol) in dry dioxane (50 mL) was added P_2S_5 (500 mg, 2.25 mmol). The mixture was heated under reflux for 1 h. A second charge of P_2S_5 (0.5 g) was added, and the heating and stirring continued for another hour. The mixture was concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$) to give 50 mg (30%) of **20**: mp 110–114 °C (from EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.86 (3 H, s, Me), 4.60 (1 H, dm, H4′, $J_{4',F3'}$ = 14.5 Hz), 4.71 (2 H, m, H5′,5″), 5.90 (1 H, dq, H3′, $J_{2',3'}$ = 3.9, $J_{3',F3'}$ = 50.8, $J_{3',F2'}$ = 15.1 Hz), 5.90 (1 H, dq, H2′, $J_{1',2'}$ = 4.0, $J_{2',3'}$ = 3.9, $J_{2',F2'}$ = 50.8, $J_{2',F3'}$ = 13.72 Hz), 6.25 (1 H, dd, H1′, $J_{1',2'}$ = 4.0, $J_{1',F2'}$ = 16.4 Hz), 7.35 (1 H, s, H6), 7.46–8.25 (5 H, m, Bz). Anal. Calcd ($C_{16}H_{16}F_2N_2O_4S$) C, H, F, N, S.

1-(2,3-Dideoxy-2,3-difluoro-β-D-arabinofuranosyl)-5-methylcytosine (21). A solution of **20** (229 mg, 0.62 mmol) in NH_3/MeOH (10 mL) was heated in a sealed steel vessel at 100 °C for 3 days, and then the mixture was concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, v/v) to give 117 mg (75%) of **21**: mp 198–201 °C (from EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.84 (3 H, s, Me), 3.66 (2 H, m, H5′,5″), 4.13 (1 H, dm, H4′, $J_{3',4'}$ = 3.2, $J_{4',F3'}$ = 19.2 Hz), 5.21 (1 H, t, 5′-OH, exchangeable), 5.27 (1 H, ddq, H3′, $J_{2',3'}$ = 2.0, $J_{3',4'}$ = 3.2, $J_{3',F2'}$ = 15.9, $J_{3',F3'}$ = 50.5 Hz), 5.43 (1 H, ddq, H2′, $J_{1',2'}$ = 4.1, $J_{2',F2'}$ = 46.5, $J_{2',F3'}$ = 15.9 Hz), 6.18 (1 H, dd, H1′, $J_{1',2'}$ = 4.10, $J_{1',F2'}$ = 18.9 Hz), 7.18 (2 H, m, NH_2, exchangeable), 7.42 (1 H, s, H6); UV (0.01 N HCl) λ_{max} 283.2 nm (ε 14 600), 210.4 (13 800), λ_{min} 241.6 (ε 4120), (0.01 N NaOH) λ_{max} 274.4 nm (ε 10 080), 237.2 (8600), λ_{min} 254.0 (ε 7100). Anal. Calcd ($C_{10}H_{13}F_2N_3O_3$) C, H, F, N.

1-(2-Deoxy-2-fluoro-3-O-mesyl-5-O-trityl-β-D-arabinofuranosyl)thymine (22). A mixture of FMAU (14; 4.8 g, 18 mmol) and TrCl (5.67 g, 20.3 mmol) in pyridine (100 mL) was heated at 50 °C for 2 h and then cooled to 0 °C. MsCl (1.5 mL, 19.5 mmol) was added, and the mixture was kept at 4 °C overnight. The reaction was quenched by addition of H_2O (3 mL) and concentrated in vacuo. The residue was dissolved in AcOEt (150 mL), washed (H_2O, 3 × 100 mL), dried (Na_2SO_4), and concentrated to dryness, and the residue chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, v/v) to give 7.0 g (68%) of **22**: mp 167–170 °C (from MeOH); ^{1}H NMR (Me_2SO-d_6) δ 1.62 (3 H, s, Me), 3.13 (3 H, s, Ms), 3.41 (2 H, m, H5′,5″), 4.25 (1 H, m, H4′), 5.43 (1 H, dm, H3′, $J_{3',F}$ = 19.3 Hz), 5.60 (1 H, ddd, H2′, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 4.6, $J_{2',F}$ = 51.8 Hz), 6.26 (1 H, dd, H1′, $J_{1',2'}$ = 4.4, $J_{1',F}$ = 14.4 Hz), 7.26–7.37 (16 H, m, Tr and H6), 11.54 (1 H, s, NH). Anal. Calcd ($C_{28}H_{29}FN_2O_7S \cdot {}^1/_2H_2O$) C, H, F, N, S.

2,3′-Anhydro-1-(2-deoxy-2-fluoro-5-O-trityl-β-D-lyxofuranosyl)thymine (23). A solution of **22** (3.0 g, 5.39 mmol) and DBU (1.5 mL) in CH_2Cl_2 (25 mL) was heated under reflux for 8 h and then concentrated to dryness. The residue was chromatographed on a silica gel column ($CHCl_3$) to give 2.18 g (85%) of **23**: mp 253–255 °C (from MeOH); ^{1}H NMR (Me_2SO-d_6) δ 1.80 (3 H, s, Me), 3.13 (2 H, m, H5′,5″), 4.61 (1 H, m, H4′), 5.40 (1 H, dm, H3′, $J_{2',3'}$ = 3.8, $J_{3',4'}$ = 7.6, $J_{3',F}$ = 4.5 Hz), 5.89 (1 H, ddd, H2′, $J_{1',2'}$ = 3.3, $J_{2',3'}$ = 3.8, $J_{2',F}$ = 49.7 Hz), 5.96 (1 H, br dd, H1′, $J_{1',2'}$ = 3.3, $J_{1',F}$ = 5.2 Hz), 7.30 (15 H, m, Tr), 7.66 (1 H, s, H6). Anal. Calcd ($C_{28}H_{24}FN_2O_4$) C, H, N.

1-(3-Deoxy-2,3-didehydro-2-fluoro-5-O-trityl-β-D-*glycero*-2-enopentofuranosyl)thymine (24). A suspension of **23** (646 mg, 1.37 mmol) and *t*-BuOK (270 mg, 2.4 mmol) in DMSO (10 mL) was stirred at room temperature for 2 h and then filtered. The filtrate was concentrated in vacuo, and the residue chromatographed on a silica gel column ($CHCl_3$/MeOH, 49:1, v/v) to give 600 mg (92%) of **24**: mp 176–180 °C (from EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.27 (3 H, s, Me), 3.21 (2 H, m, H5′,5″), 4.98 (1 H, m, H4′), 6.17 (1 H, t, H1′, $J_{1',2'}$ = $J_{1',F}$ = 1.5 Hz), 6.81 (1 H, m, H3′), 7.32 (16 H, m, Tr and H6), 11.52 (1 H, s, NH). Anal. Calcd ($C_{28}H_{25}FN_2O_4$) C, H, N.

1-(3-Deoxy-2,3-didehydro-2-fluoro-β-D-*glycero*-2-enopentofuranosyl)thymine (25). A solution of **24** (0.6 g, 1.27 mmol) in 80% HOAc (10 mL) was heated under reflux for 20 min

and then concentrated in vacuo. The residue was chromatographed on a column of silica gel ($CHCl_3$/MeOH, 9:1, v/v) to give 100 mg (31%) of **25**: mp 154–159 °C (from EtOH/H_2O); ^1H NMR (Me_2SO-d_6) δ 1.76 (3 H, s, Me), 3.61 (2 H, m, H5′,5″), 4.79 (1 H, m, H4′), 5.15 (1 H, t, 5′-OH), 5.99 (1 H, m, H1′), 6.76 (1 H, m, H3′), 7.88 (1 H, d, H6), 11.43 (1 H, s, NH). Anal. Calcd ($C_{10}H_{11}FN_2O_4$) C, H, N.

Treatment of Epoxide 9 with LiN_3: Synthesis of 1-(3-Azido-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)thymine (26) and 1-(2-Azido-2-deoxy-5-*O*-trityl-β-D-xylofuranosyl)thymine (27). A mixture of **9** (2.0 g, 9.0 mmol) and LiN_3 (0.49 g, 10 mmol) in EtOH (50 mL) was heated under reflux for 2 h and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, v/v). Compound **27** (830 mg, 20%) eluted from the column first followed by **26** (1.3 g, 30%).

Compound **26**: mp 128–133 °C (from MeOH/$CHCl_3$); IR (KBr) ν 2100 cm^{-1} (N_3); ^1H NMR (Me_2SO-d_6) δ 1.60 (3 H, s, Me), 3.35 (2 H, m, H5′,5″), 3.54 (1 H, m, H4′), 3.85 (1 H, m, H3′), 4.35 (1 H, m, H2′), 6.08 (1 H, d, H1′, $J_{1',2'}$ = 6.0 Hz), 6.11 (1 H, d, 2′-OH), 7.37 (16 H, m, Tr and H6), 11.34 (1 H, s, NH). Anal. Calcd ($C_{25}H_{27}N_5O_5$) C, H, N.

Compound **27**: mp 125–130 °C (from MeOH/$CHCl_3$); IR (KBr) ν 2100 cm^{-1} (N_3); ^1H NMR (Me_2SO-d_6) δ 1.61 (3 H, s, Me), 3.17 (2 H, m, H5′,5″), 4.17 (2 H, m, H3′,4′), 4.23 (1 H, m, H2′), 5.15 (1 H, d, 3′-OH), 5.82 (1 H, d, H1′, $J_{1',2'}$ = 2.7 Hz), 7.35 (16 H, m, Tr and H6), 11.42 (1 H, s, NH). Anal. Calcd ($C_{25}H_{27}N_5O_5 \cdot {}^1/_4H_2O$) C, H, N.

2′-Azido-2′,3′-dideoxy-3′-fluoro-5′-*O*-trityl-5-methyluridine (30). To a solution of **27** (800 mg, 1.6 mmol) in benzene (30 mL) was added DAST (970 mg, 6 mmol) dropwise at −5 °C. The mixture was stirred for 2 h at room temperature and then poured onto ice/water (50 mL). The organic layer was separated, washed successively with 10% $NaHCO_3$ (2 × 15 mL) and H_2O (2 × 15 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, v/v) to give 380 mg (47%) of **30**: mp 110–115 °C (from MeOH); ^1H NMR (Me_2SO-d_6) δ 1.47 (3 H, s, Me), 3.24 (2 H, m, H5′,5″), 4.29 (1 H, dm, H4′, $J_{4',F}$ = 23.3, $J_{3',4'}$ = 2.5 Hz), 4.45 (1 H, dm, H2′, $J_{2',F}$ = 15.3, $J_{2',3'}$ = 4.3 Hz), 4.49 (1 H, dq, H3′, $J_{3',F}$ = 53.8, $J_{2',3'}$ = 4.5 Hz), 5.98 (1 H, d, H1′, $J_{1',2'}$ = 7.7 Hz), 7.35 (16 H, m, H6 and Tr), 11.42 (1 H, s, NH). Anal. Calcd ($C_{29}H_{26}FN_5O_4$) C, H, F, N.

In a similar manner, **26** (950 mg, 1.9 mmol) was converted into **1-(3-azido-2,3-dideoxy-2-fluoro-β-D-ribofuranosyl)thymine (28)** (460 mg, 65%): mp 109–112 °C (from MeOH); ^1H NMR (Me_2SO-d_6) δ 1.54 (3 H, s, Me), 3.34 (2 H, m, H5′,5″), 4.12 (1 H, dm, H4′, $J_{3',4'}$ = 9.9 Hz), 4.60 (1 H, ddd, H3′, $J_{3',F}$ = 24.0, $J_{2',3'}$ = 4.9, $J_{3',4'}$ = 9.9 Hz), 5.50 (1 H, ddd, H2′, $J_{2',F}$ = 52.8, $J_{2',3'}$ = 4.9, $J_{1',2'}$ = 2.0 Hz), 5.92 (1 H, dd, H1′, $J_{1',2'}$ = 2.1, $J_{1',F}$ = 20.2 Hz), 7.35 (16 H, m, H6 and Tr), 11.51 (1 H, s, NH). Anal. Calcd ($C_{29}H_{26}FN_5O_4$) C, H, F, N.

2′-Azido-2′,3′-dideoxy-3′-fluoro-5-methyluridine (31). A solution of **30** (0.38 g, 0.7 mmol) in 80% aqueous AcOH (10 mL) was heated under reflux for 15 min and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$/MeOH, 95:5, v/v) to give 0.12 g (60%) of **31**: mp 130–134 °C; IR (KBr) ν 2100 cm^{-1} (N_3); UV (0.1 N HCl) λ_{max} 265.2 nm (ε 9800), 203.4 (ε 10 000), λ_{min} 234.0 (ε 4630), (0.1 N NaOH) λ_{max} 266.0 nm (ε 7200), 227.6 (ε 7300), λ_{min} 244.8 (ε 4630); ^1H NMR (Me_2SO-d_6) δ 1.85 (3 H, s, Me), 3.70 (2 H, m, H5′,5″), 4.35 (1 H, dm, H4′, $J_{4',F}$ = 28.0 Hz), 4.51 (1 H, dm, H2′, $J_{2',F}$ = 26.9 Hz), 5.42 (1 H, dd, H3′, $J_{3',2'}$ = 4.5, $J_{3',4'}$ = 2.0, $J_{3',F}$ = 54.1 Hz), 5.47 (1 H, br, 5′-OH), 6.10 (1 H, d, H1′, $J_{1',2'}$ = 8.2 Hz), 7.74 (1 H, d, H6), 11.20 (1 H, br, NH). Anal. Calcd ($C_{10}H_{12}FN_5O_4$) C, H, N.

In a similar manner, 0.46 g (64%) of **3′-azido-2′,3′-dideoxy-2′-fluoro-5-methyluridine (29)** was prepared from 0.95 g (1.9 mmol) of **28**: IR (KBr) ν 2160 cm^{-1} (N_3); UV (0.1 N HCl) λ_{max} 264.8 nm (ε 11 200), 203.2 (ε 11 650), λ_{min} 232.4 (ε 4020), (0.1 N NaOH) λ_{max} 265.2 nm (ε 10 500), 226.8 (ε 10 600), λ_{min} 243.2 (ε 8200); ^1H NMR (Me_2SO-d_6) δ 1.76 (3 H, s, Me), 3.82 (2 H, m, H5′,5″), 3.98 (1 H, m, H4′), 4.33 (1 H, ddd, H3′, $J_{2',3'}$ = 4.4, $J_{3',4'}$ = 9.6, $J_{3',F}$ = 22.8 Hz), 5.43 (1 H, ddd, H2′, $J_{1',2'}$ = 2.0, $J_{2',3'}$ = 3.8, $J_{2',F}$ = 52.4 Hz), 5.48 (1 H, t, 5′-OH), 5.91 (1 H, dd, H1′, $J_{1',2'}$ = 2.0, $J_{1',F}$ = 19.0 Hz), 11.31 (1 H, s, NH). Anal. Calcd ($C_{10}H_{12}FN_5O_4$) C, H, N, F.

2,2′-Anhydro-1-(3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine (34). A mixture of **32**[31] (1.35 g, 2.80 mmol) and 1,1′-(thiocarbonyl)diimidazole (669 mg, 3.75 mmol) in DMF (20 mL) was stirred at room temperature for 4 h. The reaction was quenched by addition of H_2O (100 mL), and the precipitates collected were dissolved in $CHCl_3$ (50 mL). The solution was washed (H_2O, 15 mL), dried (Na_2SO_4), and concentrated to give crude **33** (1.49 g, 90%).

To a suspension of crude **33** (1.35 g, 2.80 mmol) in a 1:1 (v/v) toluene–MeCN (80 mL) was added *n*-Bu_3SnH (2.25 mL, 8.37 mmol) and a catalytic amount of AIBN, and the mixture was heated under reflux for 5 h. After cooling, the mixture was concentrated in vacuo, and the residue chromatographed over a silica gel column (CH_2Cl_2/MeOH, 10:1, v/v) to give **34** (450 mg, 35%): mp 220–221 °C (EtOH); ^1H NMR (Me_2SO-d_6) δ 1.78 (3 H, s, Me), 2.02–2.62 (2 H, m, H3′,3″), 2.70–2.90 (2 H, m, H5′,5″), 4.40–4.70 (1 H, m, H4′), 5.48 (1 H, t, H2′, $J_{1',2'}$ = $J_{2',3'}$ = 5.4 Hz), 6.20 (1 H, d, H1′, $J_{1',2'}$ = 5.4 Hz), 7.20–7.45 (15 H, m, Tr), 7.82 (1 H, s, H6). Anal. Calcd ($C_{29}H_{26}N_2O_4$) C, H, N.

1-(3-Deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine (35). To a solution of **34** (436 mg, 0.94 mmol) in MeCN (20 mL) was added 1 N NaOH (2.55 mL). After stirring at room temperature for 1.5 h, the mixture was neutralized with CO_2 and then concentrated in vacuo. The residue was extracted with CH_2Cl_2 (2 × 50 mL), and the combined extracts were dried (Na_2SO_4) and concentrated, and the residue was crystallized from MeOH/H_2O to give **35** (452 mg, 99%): mp 113–115 °C (EtOH/H_2O); ^1H NMR (Me_2SO-d_6) δ 1.59 (3 H, s, Me), 2.10–2.40 (2 H, m, H3′,3″), 3.10–3.30 (2 H, m, H5′,5″), 4.25–4.40 (2 H, m, H2′,4′), 5.92 (1 H, d, $J_{1',2'}$ = 4.9 Hz), 7.20–7.45 (16 H, m, H6 and Tr), 11.27 (1 H, s, NH). Anal. Calcd ($C_{29}H_{28}N_2O_5$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-5-*O*-trityl-β-D-*erythro*-pentofuranosyl)thymine (36). To a solution of **35** (439 mg, 0.9 mmol) in CH_2Cl_2 (10 mL) was added DAST (0.43 mL) at −60 °C, and then the mixture was allowed to warm to room temperature. After 2 h at room temperature, the reaction was quenched by addition of 10% aqueous $NaHCO_3$ (30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue chromatographed on a silica gel column (CH_2Cl_2/MeOH, 15:1, v/v) to give 300 mg (67%) of **36**: mp 107–110 °C (CH_2Cl_2/petroleum ether); ^1H NMR (Me_2SO-d_6) δ 1.45 (3 H, s, Me), 2.00–2.60 (2 H, m, H3′,3″), 3.29–3.32 (2 H, m, H5′,5″), 4.30–4.55 (1 H, m, H4′), 5.41 (1 H, dd, H2′, $J_{2',F}$ = 54.1 Hz), 5.89 (1 H, d, H1′, $J_{1',F}$ = 20.0 Hz), 7.25–7.58 (16 H, m, H6 and Tr), 11.41 (1 H, s, NH). Anal. Calcd ($C_{29}H_{27}FN_2O_4$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-β-D-*erythro*-pentofuranosyl)thymine (37, 2′,3′-Dideoxy-2′-fluoro-5-methyluridine). A solution of **36** (67 mg, 0.14 mmol) in 80% aqueous HOAc (4 mL) was heated at 80 °C for 30 min and then concentrated in vacuo. The residue was chromatographed on a silica gel column (CH_2Cl_2/MeOH, 3:2, v/v) to give **37** as colorless needles from EtOAc (22 mg, 65%): UV (H_2O) λ_{max} 267.0 nm (ε 8760), λ_{min} 234.5 (ε 2230), (0.01 N HCl) λ_{max} 267.0 nm (ε 8600), λ_{min} 234.5 (ε 2110), (0.01 N NaOH) λ_{max} 267.0 nm (ε 6720), λ_{min} 244.0 (ε 4080); ^1H NMR (Me_2SO-d_6) δ 1.75 (3 H, s, Me), 1.80–2.50 (2 H, m, H3′,3″), 3.40–3.90 (2 H, m, H5′,5″), 4.15–4.40 (1 H, m, H4′), 5.23 (1 H, t, 5′-OH), 5.29 (1 H, dd, H2′, $J_{2',F}$ = 54.3 Hz), 5.88 (1 H, d, H1′, $J_{1',F}$ = 18.4 Hz), 7.84 (1 H, s, H6), 11.32 (1 H, s, NH). Anal. Calcd ($C_{10}H_{11}FN_2O_4$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-5-*O*-trityl-β-D-*erythro*-pentofuranosyl)-5-methylcytosine (39). To a solution of **36** (700 mg, 1.44 mmol) in pyridine (15 mL) was added, at 4 °C, first 4-chlorophenyl phosphorodichloridate (0.23 mL, 1.4 mmol) and then 1,2,4-triazole (195 mg, 2.8 mmol). The mixture was stirred at room temperature for 3 days and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (75 mL), and the solution was washed with H_2O (2 × 25 mL) and aqueous $NaHCO_3$ (25 mL), dried ($MgSO_4$), and then concentrated to dryness to give crude **38** as a foam.

The crude **38**, without purification, was dissolved in 1:3 (v/v) NH_4OH/dioxane (20 mL). After being stirred for 5 h at room

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temperature, the mixture was concentrated in vacuo. The residue was chromatographed on a silica gel column (CH_2Cl_2/MeOH, 15:1, v/v) to afford **39** (301 mg, 43%): mp 129–131 °C (CH_2Cl_2/petroleum ether); UV (H_2O/MeOH) λ_{max} 278.0 nm (ϵ 8000), λ_{min} 254.0 (ϵ 5970), (0.01 N HCl) λ_{max} 289.0 nm (ϵ 10570), λ_{min} 249.0 (ϵ 2450), (0.01 N NaOH) λ_{max} 278.0 (ϵ 7650), λ_{min} 255.5 (ϵ 5660); ^{1}H NMR ($CDCl_3$) δ 1.41 (3 H, s, Me), 1.97–2.70 (2 H, m, H3′,3″), 3.34 (1 H, dd, H5′, $J_{5',5''}$ = 10.8, $J_{4',5'}$ = 3.57 Hz), 3.65 (1 H, dd, H5″, $J_{5',5''}$ = 10.8, $J_{4',5''}$ = 2.2 Hz), 4.58 (1 H, m, H4′), 5.06 (1 H, dd, H2′, $J_{2',3'}$ = 3.0, $J_{2',F}$ = 50.6 Hz), 6.01 (1 H, d, H1′, $J_{1',F}$ = 16.7 Hz), 7.32 (15 H, m, Tr), 7.74 (1 H, s, H6). Anal. Calcd ($C_{29}H_{28}FN_3O_3$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-β-D-*erythro*-pentofuranosyl)-5-methylcytosine (40, 2′,3′-Dideoxy-2′-fluoro-5-methylcytidine). A solution of **39** (200 mg, 0.42 mmol) in 80% aqueous HOAc (4 mL) was heated at 80 °C for 30 min and then concentrated in vacuo. Traces of HOAc were removed by several coevaporations with toluene. The residue was chromatographed on a silica gel column (CH_2Cl_2/MeOH, 3:2, v/v) to give **40** (91 mg, 91%): mp 179–180 °C (EtOH/Et_2O), UV (H_2O) λ_{max} 276.5 nm (ϵ 7450), λ_{min} 253.5 (ϵ 4440), (0.01 N HCl) λ_{max} 286.0 nm (10340), λ_{min} 244.0 (ϵ 1190), (0.01 N NaOH) λ_{max} 276.5 nm (ϵ 7280), λ_{min} 254.0 (ϵ 4410); ^{1}H NMR (Me_2SO-d_6) δ 1.82 (3 H, s, Me), 1.91–2.36 (2 H, m, H3′,3″), 3.70 (2 H, m, H5′,5″), 4.30 (1 H, m, H4′), 5.13 (1 H, dd, H2′, $J_{2',3'}$ = 3.0, $J_{2',F}$ = 52.3 Hz), 5.21 (1 H, bs, OH), 5.86 (1 H, d, H1′, $J_{1',F}$ = 18.4 Hz), 6.83 (1 H, bs, NH), 7.28 (1 H, bs, NH), 7.78 (1 H, s, H6). Anal. Calcd ($C_{10}H_{14}FN_3O_3 \cdot {}^1/_3H_2O$) C, H, N.

Anti-HIV-1 Assay. Preliminary Screening Using H9 Cells. H9 cells were preincubated with 10^3 TCI_{50} of HIV, washed, resuspended in conditioned medium, and added to 96-well plates containing 1-fold serial dilutions (in duplicate) of nucleoside. The cultures were refed every 3–4 days with fresh medium containing the appropriate concentrations of each drug. On day 7, cells were harvested onto slides and HIV antigens were detected by IFA using human serum containing high titers of polyclonal 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.

Activity against MuLV. Susceptible mink CCL64 cells were seeded on 12-well multispot tissue culture slides at 7×10^5/mL in 0.05 mL and incubated at 37 °C overnight, then treated with the nucleoside for 1 h before infection with MuLV NS292 (infectious titer of 8×10^4/mL). After 1 h, the tissue culture medium containing MuLV was removed and replaced with medium containing various concentrations of the nucleosides being tested. The slides were incubated at 37 °C in moist chambers for 3 days. On day 5, the slides were washed in PBS for 5 min, dipped in D_2O, and fixed in absolute MeOH for 5 min at room temperature.

Infectious MuLV foci were quantitated in the culture by IFA.[29] Fifty microliters of a 1:60 dilution of a high-titered rabbit anti MuLV gs serum was placed on each well of the slides, and the slides were incubated in a moist chamber at 37 °C for 1 h. After incubation, the slides were washed successively with PBS (2 × 5 min) and H_2O (5 min) and air-dried. Fifty microliters of a 1:80 dilution of goat anti-rabbit IgG FITC conjugate was added to each well and the slides were incubated at 37 °C for 1 h in a humidified chamber. The slides were then washed with PBS (2 × 5 min) and H_2O followed by counterstaining with 0.025% Evans blue for 7 min. After counterstaining, the slides were washed (PBS and H_2O) and dried, one drop of 50% glycerin in PBS was placed on each well, and a cover slip was applied. Slides were read under a Zeiss epiilluminated UV microscope. The number of MuLV antigen positive foci in treated wells was compared with that in the untreated control wells of each slide, and the percent MuLV reduction was calculated as follows:

$$100 - \frac{\text{no. foci in treated well}}{\text{no. foci in untreated well}} \times 100 = \% \text{ reduction of MuLV replication}$$

3′-Azido-3′-deoxythymidine was routinely used as a positive control for inhibition.

Cytotoxicity Assay. The cytotoxicity of the agents was determined in duplicate in 96-well microplates by XTT-microculture tetrazolium assay,[32] and trypan blue exclusion assay. 1′,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 (26 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 mixed, and absorbance at 450 nm and 630 nm was measured with a microplate reader (EL340, Bio-TEK Instruments, Winooski, VT).

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