(30 mg, 68%) was obtained by filtration, followed by washing with ether thoroughly, and dried. Mp: 121-126 °C. R_f (B): 0.46. HPLC (3): 14.8, 15.3 min. ^{1}H NMR δ ($CDCl_3$): 7.55-7.10 (10 H, m, 2 C_6H_5), 4.98 (1 H, m, Dpa αCH), 4.53 (1 H, m, Arg CH), 4.32 (1 H, m, Dpa βCH), 4.20 (1 H, m, Pro CH), 3.38-1.10 (26 H, m, 13 CH_2). MS m/z (FAB): 618 (M^{*+}).

D-**Dpa-Pro-Argψ(COCH$_2$)Gly-pip (1a)** (30 mg, 75%). Mp: 146-151 °C. R_f (B): 0.41. HPLC (3): 14.73, 16.54 min. ^{1}H NMR δ ($CDCl_3$): 7.55-7.10 (10 H, m, 2 C_6H_5), 7.10-6.10 (4 H, m, guanidino H), 4.98 (1 H, m, Dpa αCH), 4.53 (1 H, m, Arg CH), 4.32 (1 H, m, Dpa βCH), 4.20 (1 H, m, Pro CH), 3.38-1.15 (26 H, m, 13 CH_2). MS m/z (FAB): 619 (M + H), 641 (M + Na). Anal. ($C_{34}H_{47}N_7O_4$·2TFA·4H_2O): C, H, N.

L-**Dpa-Pro-Argψ(COCH$_2$)Gly-pip (1b)** (30 mg, 43%). Mp: 136-140 °C. R_f (B): 0.41. ^{1}H NMR δ ($CDCl_3$): 7.43-7.10 (10 H, m, 2 C_6H_5), 4.99 (1 H, m, Dpa αCH), 4.56 (1 H, m, Arg CH), 4.35 (1 H, m, Dpa βCH), 4.20 (1 H, m, Pro CH), 3.40-1.15 (26 H, m, 13 CH_2). MS m/z (FAB): 619 (M + H). Anal. ($C_{34}H_{47}N_7O_4$·2CF_3CO_2H·3.5H_2O): C, H, N.

D,L-α**Nal-Pro-Argψ(COCH$_2$)Gly-pip (1d)** (72 mg, 82%). Mp: 123-127 °C. HPLC (3): 13.33, 13.75 min. ^{1}H NMR δ ($CDCl_3$): 8.15-7.25 (7 H, m, $C_{10}H_7$), 5.26 (1 H, m, Nal CH), 4.64 (1 H, m, Arg αCH), 4.23 (1 H, m, Pro αCH), 3.46 (2 H, m, Nal CH_2), 3.42-1.10 (26 H, m, 13 CH_2). MS m/z (FAB): 592 (M^{*+}). Anal. ($C_{32}H_{45}N_7O_4$·2TFA·3H_2O): C, H, N.

D,L-β**Nal-Pro-Argψ(COCH$_2$)Gly-pip (1e)** (35 mg, 30%). MS m/z (FAB): 592 (M^{*+}). Anal. ($C_{32}H_{45}N_7O_4$·2TFA·3.5H_2O): C, H, N.

D-β**Nal-Pro-Argψ(COCH$_2$)Gly-pip (1f)** (28 mg, 68%). HPLC (3): 11.6, 12.4 min. ^{1}H NMR δ ($CDCl_3$): 7.70-7.21 (7 H, m, $C_{10}H_7$), 4.78 (1 H, m, Nal CH), 4.46 (1 H, m, Arg CH), 4.18 (1 H, m, Pro CH), 3.47 (2 H, m, Nal CH_2), 3.40-1.10 (26 H, m, 13 CH_2). MS m/z (FAB): 593 (M + H). Anal. ($C_{32}H_{45}N_7O_4$·2TFA·4H_2O): C, H, N.

D,L-**Fgl-Pro-Argψ(COCH$_2$)Gly-pip (1g)** (59 mg, 81%). R_f (B): 0.46. ^{1}H NMR δ ($CDCl_3$): 7.77-7.15 (8 H, m, $C_{13}H_8$), 5.04 (1 H, m, Fgl αCH), 4.76 (1 H, m, Arg CH), 4.64 (1 H, m, Fgl βCH), 4.53 (1 H, m, Pro CH), 3.45-1.05 (26 H, m, 13 CH_2). MS m/z (FAB): 6.17 (M^{*+}). Anal. ($C_{34}H_{45}N_7O_4$·2TFA·3.5H_2O): C, H. N.

Enzymatic Assay.[15] Kinetic studies were made in 0.1 M sodium phosphate buffer containing 0.2 M NaCl, 0.5% polyethylene glycol 6000, and 0.02% sodium azide at pH 7.5. The assays were performed in the presence or absence of inhibitor at a concentration sufficient to give approximately 50% inhibition at a single concentration of substrate S-2238 (10 μM). The assay was carried out with a range of substrate concentrations at and around the K_m for substrate/thrombin (20-5 μM). The final concentration of human α-thrombin[16] used was 0.19 nM, and the assays were carried out at 37 °C. The inhibition constant (K_i) was calculated from a Lineweaver-Burk plot.

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3′,3′-Difluoro-3′-deoxythymidine: Comparison of Anti-HIV Activity to 3′-Fluoro-3′-deoxythymidine

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3′,3′-Difluoro-3′-deoxythymidine (**3**) has been synthesized in four steps from thymidine, and characterized by ^{1}H NMR and NOE experiments. The J_{HF} coupling constants support a conformation in solution that is predominantly 2′-endo (*S*). Although conformationally and sterically nucleoside **3** may resemble other thymidine analogs which are active against HIV-1, **3** is virtually inactive.

One of the most active nucleoside analogs against HIV-1 is 3′-fluoro-3′-deoxythymidine (**1**).[3,4] This nucleoside is a more potent inhibitor of HIV-1 replication in vitro than 3′-azidothymidine (**2**) (Table I). We previously reported that the analog with an additional 3′-fluoro substituent, 3′,3′-difluoro-3′-deoxythymidine (**3**), is virtually inactive against HIV-1.[5] On the basis of calculations using MINDO/3, a semiempirical, all-valence-electron molecular orbital method, we postulated that the 3′-fluoro, located on the same face of the furanose as the thymine, strongly influenced the conformation about the glycosidic bond and led to a substantial preference for the N (3′-endo, 2′-exo) conformation of the deoxyribose. On the basis of NMR experiments reported here, that prediction is not born out.

The synthesis of 3′,3′-difluoro-3′-deoxythymidine is outlined in Figure 1. Although the synthesis was previously reported, the experimental details, the structure determination, and the data on antiviral activity have not been published previously.[6,7] The first two steps, tritylation of thymidine and the oxidation of 5′-*O*-trityl-thymidine to yield 3′-ketothymidine (**5**) have been previously described in detail.[8] Fluorination was accom-

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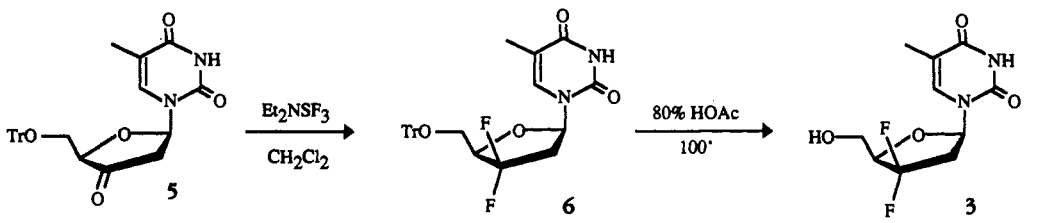
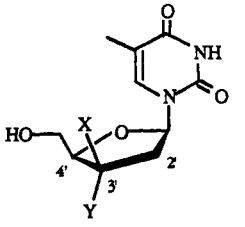


Figure 1.

Table I. Anti-HIV Activity and HIV Reverse Transcriptase Inhibition by Dideoxythymidine Analogs



1: X = H, Y = F; 3′-fluoro-3′-deoxythymidine
2: X = H, Y = N_3; 3′-azido-3′-deoxythymidine (AZT)
3: X, Y = F; 3′,3′-difluoro-3′-deoxythymidine
4: X, Y = H; 3′-deoxythymidine (ddT)

compd	IC_{50}[a] of 5′-triphosphate for HIV reverse transcriptase, μM	ED_{50}[b] HIV-induced cytopathicity, μM	CD_{50},[c] μM
1	<0.05	0.001	0.197
2	0.04	0.004	20
3	8.8 ± 2.4	38.6 ± 2.8	48.4 ± 0.6
4	<0.05	6	>625

[a] IC_{50} is defined as the concentration of drug that inhibited 50% of the enzyme activity of the non-drug-containing assay. Values determined by Y.-C. Cheng et al.[18] [b] ED_{50} is defined as the dose required to affect a 50% reduction in the cytopathic effect of HIV for MT4 cells. [c] CD_{50} is defined as the cytotoxic dose required to reduce the number of viable cells in the untreated MT4 cell cultures by 50%.

plished using (diethylamido)sulfur trifluoride (DAST) in methylene chloride, but the yield was very poor and at least seven additional side products were obtained. The desired product, 5′-*O*-trityl-3′,3′-difluoro-3′-deoxythymidine (**6**) was purified by preparative thin-layer chromatography. The trityl protecting group was removed by heating compound **6** in glacial acetic acid at 100 °C.

An NOE experiment allowed us to unequivocally assign the H-2′ (δ 2.67 ppm) and H-2″ (δ 2.85 ppm) protons of nucleoside **3**. The lower field signal at 2.85 ppm (H-2″) was enhanced by 5.0% on irradiation of the anomeric proton (H-1′). The higher field signal at 2.67 ppm (H-2′) was not enhanced. Irradiation of the thymine C-6 proton at 7.65 ppm led to a 7.4% enhancement of the resonance at 2.67 ppm, further confirming that this signal was due to H-2′. There was a significantly smaller NOE effect between the H-6 proton and the H-1′ proton (2.2%). With this information in hand, we were able to assign the coupling constants shown in Table II.

The J_{HF} couplings for **3** support the predominance of the 2′-endo conformer in solution. The magnitude of $J_{2'F3''}$ is greater than the magnitude of $J_{2'F3'}$ (18.7 versus 9.0 Hz). This is only possible if the torsion angle H2′–C2′–C3′–F3″ is between −120 and −180° and H2″–C2′–C3′–F3′ is between 60 and 120°. If the 3′-endo conformation were more highly populated, then the relative positions of the two fluorines with respect to the two C2 protons would be switched, and $J_{2'F3'}$ would be greater than $J_{2'F3''}$.

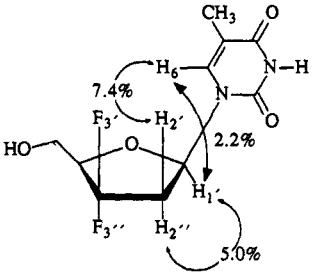
One can estimate a value for the dihedral angle C1′–C2′–C3′–C4′ (ν_2) in nucleoside **3** by assuming that it is close

Table II. 3′,3′-Difluoro-3′-deoxythymidine NMR Data

Coupling Constants

$J_{1'2'}$	$J_{1'2''}$	$J_{2'F3'}$	$J_{2'F3''}$	$J_{2''F3'}$	$J_{2''F3''}$
8.1	7.05	14.6	18.7	9.0	14.6

Nuclear Overhauser Effects



in magnitude to the dihedral angle H2′–C2′–C3′–F3′ (ϕ), which can be calculated from the coupling constant $J_{2'F3'}$ = 14.6 Hz by the equation $J_{HF} = 31 \cos^2 \phi$.[9] The calculated value of ϕ is 49.4°. This is higher than expected since τ_m for nucleosides is normally between 36 and 42°.[10] However, two factors have been neglected in this calculation, which could give lower values for ϕ. First, the observed coupling constant is a result of averaging all conformations. The actual coupling constant for molecules in the *S* conformation may be higher, which would yield a lower calculated value for ϕ. The relationship $J_{HF} = 31 \cos^2 \phi$ is based on compounds containing a single fluorine substituent and no additional electronegative groups. For example, the axial–axial coupling between H and F in glycopyranosyl fluorides falls in the range 23.4–25.4 Hz.[11] If we were to assume the maximum coupling constant to be 26 Hz, then $J_{HF} = 26 \cos^2 \phi$, which for J = 14.6 Hz yields ϕ = 43°. This is in the range expected for ν_2 (or τ_m).

In comparison, Joecks et al. determined the sugar conformation of 3′-fluoro-3′-deoxythymidine (**1**) in solution from NMR data and estimated P = 160° and ϕ_m = 48° with the relative populations of the N and *S* conformers 0.12:0.88.[12]

In summary, on the basis of the NMR data, 3′,3-difluoro-3′-deoxythymidine appears to assume predominantly an *S* conformation in solution, and in that respect is similar to other thymidine analogs active against HIV.[13–16] If nucleoside **3** is primarily in the *S* conformation,

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there must still be other conformational differences that position the C5'–O5' group in an unfavorable position for nucleoside kinase and/or reverse transcriptase (as the 5'-triphosphate) binding. The optimal torsional angles γ, χ, and P found for nucleosides are generally similar in the phosphorylated molecules;[17] hence, structural features found in the modified nucleosides described here may well carry over to the 5'-triphosphate. From the HIV reverse transcriptase inhibition study of Cheng et al.,[18] it would appear that the lack of activity of nucleoside **3** at least partially results from the less effective binding of its 5'-triphosphate to this enzyme. The inhibitory concentration is at least 175 times greater than that for the 5'-triphosphates of either 3'-deoxythymidine (**4**) (ddT) or 3'-fluoro-3'-deoxythymidine (**1**).

The observation that ddT (in its 5'-triphosphate form) is as an effective inhibitor of reverse transcriptase as AZT (5'-triphosphate), but is less active as an anti-HIV agent than AZT, may be ascribed to ddT being a poorer substrate for thymidine kinase, which implies that a polar substituent may be necessary in the down position at C3' for favorable recognition by this enzyme. Alternatively, it has been suggested that the C3'-exo conformation (P near 198°) may be required for effective binding and transformation of nucleoside analogs to their 5'-phosphates by nucleoside kinases,[4] and this conformation may not be as readily accessible for ddT and 3',3'-difluoro-3'-deoxythymidine.

There are a number of possible reasons why the previous MINDO/3 calculations predicted that **3** would exist predominantly in an N conformation.[5] It has been reported that MINDO/3 gives erroneous results for *gem*-difluoro compounds because F–F repulsion is underestimated.[19] Furthermore, MINDO/3 does not deal adequately with hydrogen bonding and, consequently, results which implicate a dipole–dipole attraction between H-6 and the 3'-fluoro must be equally suspect.[20] It would clearly be of interest to determine the crystal structure of **3**, and compare the results with more refined ab initio methods.

Aside from conformational considerations, nucleoside **3** differs substantially from thymidine, AZT, and 3'-fluoro-3'-deoxythymidine in one important respect, the direction in space of the dipole due to the two C–F bonds at C-3'. Rather than pointing downward (relative to the base), this dipole would lie much closer to the plane of the deoxyribose. This could certainly contribute to the inability of **3** to serve as a substrate for thymidine kinase and for its 5'-triphosphate to be a poor substrate for reverse transcriptase.

Experimental Section

General. All glassware was washed in a base bath, rinsed with distilled water, and dried at 110 °C for 8 h prior to use. Dichloromethane and pyridine were dried and distilled prior to use. Diethyl ether, chloroform, methanol, ethyl acetate, and toluene were reagent grade and used without further purification. Thymidine was purchased from U.S. Biochemical Corp. Molecular sieve powder (3 Å, <10-μm particles Sigma no. M1885) was heated at 325 °C in a box furnace for at least 3 h and was allowed to cool in a desiccator prior to use. Triphenylmethyl chloride and (diethylamido)sulfur trifluoride (DAST) were purchased from Aldrich Chemical Co. and used without further purification. Nitrogen gas was purchased as prepurified and used without further purification. 5'-*O*-Tritylthymidine was prepared by the method of Munson[21] and recrystallized from hot toluene. The preparation of 3'-keto-5'-*O*-tritylthymidine has been reported previously.[22] Pyridinium dichromate was prepared by the method of Corey and Schmidt[23] and dried in a vacuum oven.

Prep-TLC was performed using Whatman PK5F 1000-μm glass-backed silica plates and using chloroform/methanol [95/5] as the solvent. ^{1}H, ^{13}C, and ^{19}F NMR spectra were obtained on a Varian VXR-500 instrument at 499.9, 125.7, and 383 MHz, respectively. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ or DMSO-d_6 and referenced to TMS; ^{19}F NMR spectra were run in $CDCl_3$ and referenced to $CFCl_3$. Melting points were determined using a Buchi 510 apparatus and are uncorrected. FAB mass spectra were recorded by the Mass Spectroscopy Laboratory, Department of Medicinal Chemistry and Pharmacognosy, Purdue University. HPLC data was acquired using a Beckman Model 338 system interfaced to an IBM PS/2 Model 70 computer equipped with Beckman System Gold chromatography software. Samples were eluted from a 4-mm diameter C-18 ultrasphere column using 75/25 acetonitrile/water (Fisher HPLC grade) at 1.5 mL/min and detected at 254 nm.

Preparation of 5'-*O*-Trityl-3'-deoxy-3',3'-difluorothymidine (6). 5'-*O*-Trityl-3'-ketothymidine (**5**) (0.8315 g) was dissolved in 30 mL of methylene chloride and placed into a 50-mL 2-necked round-bottom flask. The flask was purged with nitrogen, and DAST (0.6 mL) was added dropwise to the solution. Nitrogen was flushed through the solution for 10 min before addition of the DAST, and a blanket of nitrogen was maintained throughout the reaction time. The solution was allowed to stir at room temperature for 10 h, after which time the mixture was poured into a stirred saturated aqueous solution of sodium bicarbonate. The two-phased mixture was transferred to a separatory funnel and the organic layer washed two more times with aqueous bicarbonate. The organic layer was dried over sodium sulfate and the solvent removed under reduced pressure to yield a yellowish foamy residue. Prep-TLC (chloroform/methanol [95/5]) showed six spots, with the material of R_f value 0.57 corresponding to the product (72.6 mg, 12%): ^{1}H NMR ($CDCl_3$) δ 7.65 (s, H6), 7.45–7.2 (m, Ar), 6.36 (t, H1', J = 7.50 Hz), 4.15 (ddd, H4', J = 4.88 Hz), 3.49–3.40 (m, H5' and H5''), 2.84 (m, H2'), 2.55 (m, H2'), 1.42 ppm

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(s, Me); ^{13}C NMR ($CDCl_3$) δ 163.22 (C4), 150.05 (C2), 142.67 (Ar), 134.42 (C6), 128.49 (Ar), 127.82 (Ar), 127.42 (Ar), 112.01 (C5), 87.74 (Ph_3C), 80.91 (C1'), 80.68 (t, C4'), 60.92 (C5'), 40.21 (t, J_{CF} = 23.35 Hz, C2'), 11.87 ppm (CH_3); ^{19}F NMR δ -98.16 (dq), -109.89 ppm (ddt); HRMS (FAB) calculated for (M + H) 505.1939, found 505.1926.

Preparation of 3',3'-Difluoro-3'-deoxythymidine (3). Deprotection of 5'-*O*-trityl-3',3'-difluoro-3'-deoxythymidine was accomplished by heating the compound in 80% acetic acid at 100 °C for 15 min and after prep-TLC (silica gel; chloroform/methanol [95/5] as solvent) and elution through a P-2 Biogel column 18.6 mg (66%) of 3',3'-difluoro-3'-deoxythymidine was isolated: ^1H NMR (DMSO-d_6) 7.65 (s, H6), 6.23 (dd, H1', $J_{1'2'}$ = 7.05 Hz, $J_{1'2'}$ = 8.1 Hz), 5.26 (t, 5'OH, J = 5.3 Hz), 4.11 (m, H4'), 3.68 (m, H5' and H5''), 2.85 (dddd, H2', $J_{1'2''}$ = 7.05 Hz, $J_{2'2''}$ = 14.7 Hz, $J_{2'F3'}$ = 9.0 Hz, $J_{2'',F3''}$ = 14.6 Hz), 2.66 (dddd, H2', $J_{1'2'}$ = 8.1 Hz, $J_{2'F3'}$ = 14.6 Hz, $J_{2',F3''}$ = 18.7 Hz), 1.80 ppm (s, Me); ^{13}C NMR (DMSO-d_6) 163.30 (C4), 150.09 (C2), 135.15 (C6), 127.0 (t, J = 250 Hz, C3'), 109.97 (C5), 80.80 (dd, J = 24.7 and 28.8 Hz, C4'), 80.14 (dd, J = 5.8 and 7.5 Hz, C1'), 58.33 (C5'), 12.15 (CH_3); the C2' resonance was obscured by DMSO; HRMS (FAB) calculated for (M + H) 263.0843, found 263.0842.

Biological Evaluation. Anti-HIV activity was determined by monitoring inhibition of cytopathic effect of HIV for MT-4 cells.[24,25]

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Synthesis and Antiviral Properties of (±)-5'-Noraristeromycin and Related Purine Carbocyclic Nucleosides. A New Lead for Anti-Human Cytomegalovirus Agent Design

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(±)-5'-Noraristeromycin (**3**) has been prepared in three steps beginning with the 2,3-*O*-isopropylidene derivative of (±)-(1α,2β,3β,4α)-4-amino-1,2,3-cyclopentanetriol (**7**). Also prepared from the same starting material were the related hypoxanthine (**4**), guanine (**5**), and 2,6-diaminopurine (**6**) analogues. Compounds **3**-**6** were evaluated for antiviral activity against a large number of viruses with marked activity being observed for **3** towards vaccinia virus, human cytomegalovirus, vesicular stomatitis virus, parainfluenza (type 3) virus, measles virus, respiratory syncytial virus, reovirus (type 1), and the arenaviruses Junin and Tacaribe. None of the compounds showed cytotoxicity to the host cell monolayers used in the antiviral studies. Both **3** and **6** have been found to be inhibitors of *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase), which likely accounts for their antiviral activity. Inhibition of AdoHcy hydrolase represents a new approach to human cytomegalovirus drug design that should be pursued. Also, the activity of **3** should be further scrutinized for the treatment of pox-, rhabdo-, paramyxo-, reo-, and arenavirus infections.

Carbocyclic nucleosides have become increasingly relevant to the design of biologically meaningful agents.[1] Aristeromycin (**1**)[2] is the carbocyclic nucleoside analogue of adenosine that displays antiviral properties as a result of its inhibition of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase.[3] The clinical potential of **1** is, however, limited

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