upon recrystallization from MeOH/$CHCl_3$: mp 255–258 °C dec; IR (KBr) 2114 (N_3), 1752 (lactone), 1662 (pyridone), 1614 (aromatic), 1440, 1392, 1995, 1260, 1250, 1230, 1100, 1048, 785, 760, 722, and 170 cm^{-1}; ^1H NMR ($CHCl_3$) δ 1.03 (t, 3, J = 7 Hz, H-18), 2.20 (m, 2, H-19), 5.30 (s, 2, H-5), 5.49 (AB q, 2, J = 17 Hz, $\Delta\nu$ = 90 Hz, H-17), 7.52 (s, 1, H-14), 7.68 (t, 1, H = 8 Hz, H-11), 7.84 (t, 1, 8 Hz, H-10), 7.94 (d, 1, 8 Hz, H-12), 8.24 (d, 1, 8 Hz, H-9), 8.41 (s, 1, H-7). Anal. ($C_{20}H_{15}N_5O_3 \cdot 0.25H_2O$) C, H, N.

20-Amino-20-deoxy-(20*RS*)-camptothecin Hydrochloride (2e). A solution of azide **2d** (100 mg, 0268 mmol) in absolute EtOH (15 mL) containing concentrated HCl (0.6 mL) and 10% Pd/C (75 mg) was subjected to 1 atm of H_2 at room temperature for 20 h. The catalyst was removed by Celite filtration and the solvent was evaporated to afford **2e** as a pale orange-yellow solid (96 mg, 93%), and recrystallization from MeOH/$CHCl_3$ gave the pure material as a beige solid: mp 285–287 °C dec; IR (KBr) 3650–3120, 3120–2300 (C-H, amine HCl), 1750 (lactone), 1660 (pyridone), 1595 (aromatic), 1498, 1470, 1403, 1245, 1172, 1132, 1045, 785, 765, and 718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, 3, J = 7.5 Hz, H-18), 1.91 (m, 2, H-19), 1.96 (b s, 3, NH_3), 5.29 (s, 2, H-5), 5.49 (AB q, 2, J = 17 Hz, $\Delta\nu$ = 92 Hz, H-17), 7.65 (t, 1, J = 7 Hz, H-11), 7.78 (s, 1, H-14), 7.83 (t, 1, J = 7 Hz, H-10), 7.92 (d, 1, J = 7 Hz, H-12), 8.22 (d, 1, J = 7 Hz, H-9), 8.38 (s, 1, H-7). Anal. ($C_{20}H_{17}N_3O_3 \cdot 1.4HCl \cdot 2.2H_2O$) C, H, Cl, N.

(20*S*)-Camptothecin-21-lactam (2f). 17-Acetoxy-21-isopropyl amide derivative **19**[14] of 20(*S*)-camptothecin (267 mg, 0.595 mmol) was suspended in CH_2Cl_2 (2 mL) in a 23-mL capacity Teflon-lined bomb (Parr 4745) containing a magnetic stirbar and the mixture was chilled to -78 °C. Ammonia (8 mL) was condensed in the bomb, the bomb was sealed, and the stirred mixture was left at ambient temperature for 18 h. After recooling, the bomb was opened, and the contents were evaporated to provide a yellow solid consisting primarily of lactam **2f**. The sample was chromatographed as a dispersion on Celite through SiO_2 (18 g, 50 mL of $CHCl_3$, 300 mL each of 2% MeOH/$CHCl_3$ and 3% MeOH/$CHCl_3$) to provide pure **2f** as a pale yellow solid (155 mg, 75%) which was recrystallized from MeOH/$CHCl_3$: mp 310–315 °C with prior darkening above 250 °C; IR (KBr) 3400, 3240 (NH, OH), 2975, 2937, 2880, 1672 (lactam), 1655 (pyridone), 1600, 1583, 1496, 1460, 1440, 1400, 1354, 1228, 1176, 1132, 1083, 1000, 918, 838, 789, 773, 764, and 726; ^1H NMR (DMSO-d_6) δ 0.81 (t, 3, J = 7 Hz, H-18), 1.76 (m, 2, H-19), 4.26 (AB q, 2, J = 17.5 Hz, $\Delta\nu$ = 50 Hz, with one proton further coupled, J = 4 Hz, with lactam proton), 5.28 (s, 2, H-5), 5.66 (s, 1, 20-OH), 7.35 (s, 1, H-14), 7.70 (t, 1, J = 7 Hz, H-11), 7.86 (t, 1, J = 7 Hz, H-10), 8.15 (m, 2, H-9 and H-12), 8.34 (d, 1, J = 4 Hz, NH), 8.68 (s, 1, H-7). Anal. ($C_{20}H_{17}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Acknowledgment. This investigation was supported by U. S. Public Health Service Research Grant R01-CA38996 from the National Cancer Institute. L-1210 assays were conducted by Southern Research Institute; 9KB and 9PS assays were done by the Department of Microbiology, School of Medicine, University of Miami.

Registry No. **1a**, 7689-03-4; **1b**, 31456-25-4; **2c**, 124717-74-4; **2d**, 124717-75-5; **2e**, 124717-76-6; **2f**, 118514-60-6; **3**, 124717-72-2; **4**, 124717-73-3; **7**, 36276-24-1; **8**, 124717-60-8; **9**, 124717-61-9; **10**, 124717-62-0; **11**, 124717-64-2; **12**, 124717-65-3; **13**, 124717-66-4; **14**, 124717-67-5; **15**, 124717-68-6; **16**, 124717-69-7; **17**, 124717-70-0; **18**, 124717-71-1; **19**, 69181-16-4; $HO_2CCH_2CO_2H$, 141-82-2; *o*-$NH_2C_6H_4CHO \cdot HCl$, 51723-15-0; ethyl 2-[2-carboxy-4-[1-(2,5-dioxolyl)]phenyl]ethanoate, 124717-63-1; topoisomerase I, 80449-01-0.

Acid-Stable 2′-Fluoro Purine Dideoxynucleosides as Active Agents against HIV

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2′,3′-Dideoxy purine nucleosides have anti-HIV activity in vitro and the inosine analogue is being clinically evaluated. The instability of these compounds toward acidic conditions complicates oral administration. The effect of the addition of a fluorine atom to the 2′-position was investigated by preparing the fluorine-containing 2′-*erythro* and 2′-*threo* isomers of ddA and the *threo* isomer of ddI. All fluorine-containing compounds were indefinitely stable to acidic conditions which completely decomposed ddI (**1**) and ddA (**2**) in minutes. While the fluorine-containing *erythro* isomer, **5**, was inactive, the *threo* isomers, 2′-F-dd-ara-A (**3**) and 2′-F-dd-ara-I (**4**), were just as potent and active in protecting CD4+ ATH8 cells from the cytopathogenic effects of HIV-1 as the parent drugs. Exposure to pH 1 at 37 °C prior to testing destroyed the activity of ddA and ddI but left the anti-HIV properties of **3** and **4** unchanged. The fluorinated analogues also protected cells exposed to HIV-2 and inhibited *gag* gene product expression but not as effectively as the parent compounds. The fluorine-containing analogues appear to be somewhat more toxic in vitro to the antigen- and mitogen-driven proliferation of immunocompetent cells than their corresponding parent compounds.

Fluorine substitution has been extensively investigated in drug research and biochemistry as a means of enhancing biological activity and increasing chemical or metabolic stability.[1] Important factors in the substitution of fluorine for hydrogen are (1) the comparable size of the two atoms, (2) the powerful electron withdrawing properties of fluorine relative to hydrogen, and (3) the increased stability of the carbon–fluorine bond relative to the carbon–hydrogen bond. In terms of size, fluorine has a small van der Waals radius (1.35 Å) which closely resembles that of hydrogen (1.20 Å).[2] Therefore, replacement of a hydrogen by fluorine in a bioactive molecule is expected to cause minimal steric perturbations with respect to the molecule's mode of binding to receptors or enzymes. In contrast, since fluorine is the most electronegative of the elements,[2] its powerful electron withdrawing properties can profoundly

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[‡] Clinical Oncology Program.
[§] Laboratory of Biochemical Pharmacology.

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(2) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960; p 93.

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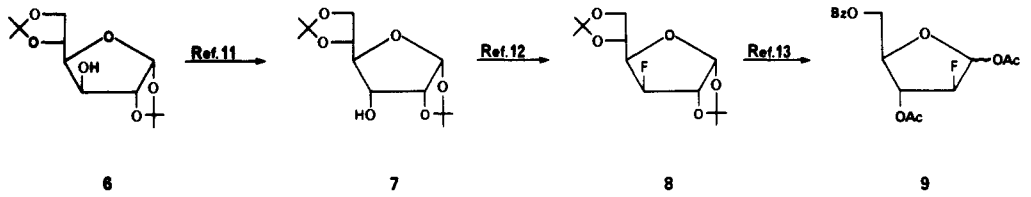
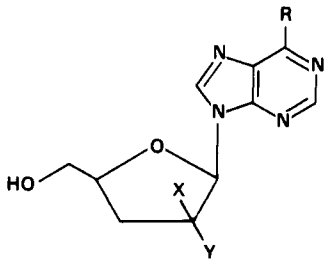
Scheme I



6 7 8 9

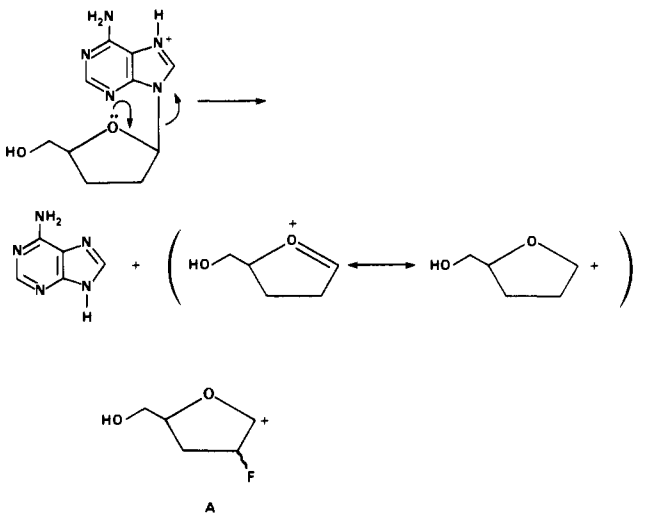
affect chemical reactivity.[3] These characteristics of fluorine attracted our attention as a possible means of overcoming the chemical and enzymatic instability characteristic of purine dideoxynucleosides.[4]

Among the anti-HIV active purine dideoxynucleosides,[5a] 2',3'-dideoxyinosine (ddI, 1) is a compound currently under clinical investigation.[5b] This compound, as well as its metabolic precursor, 2',3'-dideoxyadenosine (ddA, 2), shows potent activity and better selectivity in vitro against HIV than AZT in ATH8 cells.[5a,c] Although all dideoxynucleosides are hydrolytically less stable than their ribosyl forms, this difference reaches staggering proportions in the case of ddA which undergoes acid-catalyzed cleavage of the glycosylic linkage at a rate 40 000 times faster than adenosine.[4] At pH 1 and 37 °C, the half-life of ddA is only 35 s,[6] and this instability clearly complicates any intended use of this drug by oral administration. Since oral drug administration is a very attractive and suitable strategy for treating a large patient population for long periods of time, modification of ddA/ddI which renders these agents stable to the acidic conditions present in the human stomach is highly desirable.



1, X=Y=H; R=OH (ddI)
2, X=Y=H; R=NH_2 (ddA)
3, X=F; Y=H; R=NH_2 (2'-F-dd-ara-A)
4, X=F; Y=H; R=OH (2'-F-dd-ara-I)
5, X=H; Y=F; R=NH_2

Acid-catalyzed hydrolysis of purine nucleosides is thought to proceed by an A1 mechanism in which the protonated nucleoside dissociates in the rate-controlling step to a glycosyl carbonium ion and free purine (Figure 1).[4] It was felt that introduction of an electronegative fluorine atom at position C-2' adjacent to the reaction center should destabilize the resulting oxocarbonium ion and decrease the rate of hydrolysis (structure A, Figure 1).



A

Figure 1. Proposed mechanism for the acid-catalyzed decomposition of dideoxypurine nucleosides.[4]

The synthesis of the *threo* (3) and *erythro* (5) isomers, as communicated earlier,[6] confirmed this hypothesis by demonstrating that both compounds were completely inert to acid hydrolysis under the conditions that rapidly hydrolyzed dideoxyadenosine. Although minimal steric changes were expected to occur after fluorine substitution in either isomer, the influence of this substitution on the conformation of the sugar ring (i.e., C-3' endo vs C-2' endo) for the individual isomers could have important biological consequences that were impossible to predict beforehand.[7] Evaluation of 3 and 5 in the ATH8/HIV system indicated that only the *threo* isomer (3, 2'-F-dd-ara-A) was an effective anti-HIV agent. The protective effect of 3 was virtually indistinguishable from its parent compound, ddA.[6]

Another important consequence of fluorine substitution with its resulting increased chemical stability of the glycosylic bond is the possibility of avoiding enzymatic cleavage of ddI (1) by purine nucleoside phosphorylase.[8] ddI is readily formed from ddA via adenosine deaminase.[9] This is also true for the 2'-fluorinated analogue. While this deamination has little effect on the resulting anti-HIV activity since both ddA and ddI (and the corresponding fluorine-containing compounds) are equally effective against the HIV virus,[10] the cleavage of ddI by purine nucleoside phosphorylase could cause a reduction of useful levels of ddI in the cytoplasm. The fluorinated inosine analogue, 4 (2'-F-dd-ara-I), corresponding to the active

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(9) Cooney, D. A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1987**, *36*, 1765.
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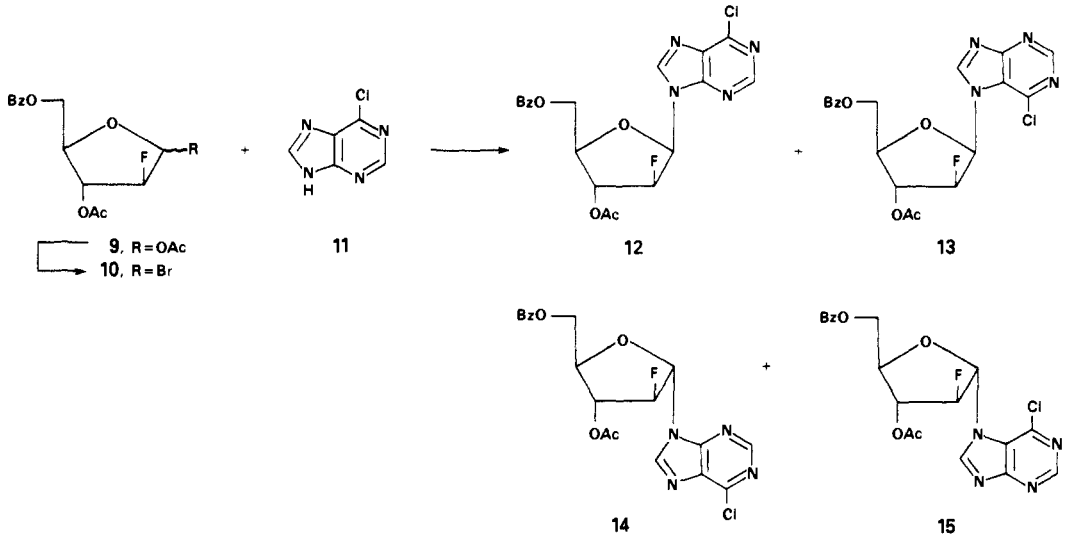
Scheme II



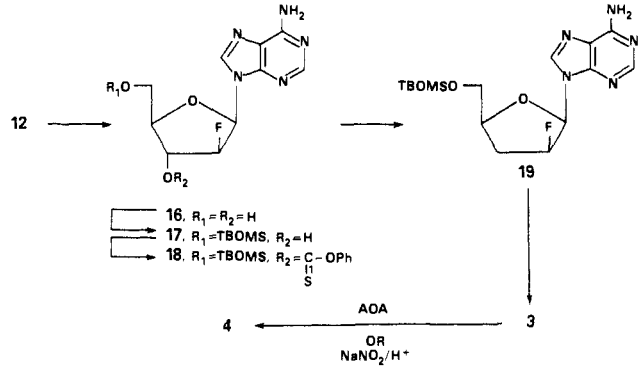
threo isomer **3**, was synthesized chemically in an attempt to overcome both the chemical and enzymatic cleavage problems observed with ddI.

In this paper we describe the detailed chemical syntheses of the *threo* and *erythro* 2′-fluoro-2′,3′-dideoxyadenosine isomers (**3** and **5**) and the *threo* isomer of 2′-fluoro-2′,3′-dideoxyinosine (**4**). Anti-HIV-1 activity is described in the ATH8 system both before and after exposure to acid conditions similar to those of the human stomach, and the results are directly compared with the nonfluorinated parent drugs, **1** and **2**. Anti-HIV-2 activity and the inhibition of *gag* protein expression are also shown. The substrate properties of the adenosine analogues with the catabolic enzyme adenosine deaminase is examined, along with the effects of **1**–**4** on the antigen- and mitogen-induced proliferation of immunocompetent T- and B-cells.

Chemistry

Commercially available diacetone D-glucose (**6**) was readily transformed in two steps into the corresponding 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**7**, Scheme I).[11] Subsequently, a one-step conversion of **7** to 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**8**) was then accomplished in 70% yield with the use of (diethylamido)sulfur trifluoride (DAST), according to the method of Tewson and Welch (Scheme I).[12] From this point onward, the five-step sequence of Reichman and Watanabe was employed to convert **8** to the key intermediate 1,3-di-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose (**9**, mixture of anomers, Scheme I).[13] After reaction of **9** with HBr in acetic acid, the glycosylation procedure developed by Montogomery et al. for the synthesis of 2,6-dichloro-9-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine was attempted.[14] The in situ generated β-bromo sugar **10** was reacted with 6-chloropurine (**11**) in refluxing dichloromethane over molecular sieves (4A) for 15 h to give an expected mixture of four isomers (Scheme II). Unfortunately, the yield of the desired 9β-isomer (**12**) from this procedure was very low (8%). From the same mixture, the other isomers—7β (**13**), 9α (**14**), and 7α (**15**)—were also isolated and characterized. In this series, the 2′-fluorine in the "up" configuration possesses a characteristic long-range coupling with the H-8 proton of the purine that is only observable in the β-isomers (**12** and **13**).[15] Discrimination between the 9β- and 7β-isomers was easily accomplished by examining their UV spectra at the 6-aminopurine stage and by comparison with the reported spectral and physical data for authentic **16** (vide infra).[16,17] A significantly better yield of **12** (25%) was later realized by employing phase-transfer catalytic conditions with benzyltriethylammonium chloride. Conversion of **12** to the known product **16** by ammonolysis with concentrated methanolic ammonia under pressure produced **16** in 90% yield (Scheme III). All of the spectral properties of **16** matched those reported previously for this compound.[16] Selective protection of the 5′-hydroxyl function of **16** with *tert*-butyldimethylsilyl chloride, followed by the well-known two-stage deoxygenation sequence of Robins and Wilson,[18] gave compound **19**. Removal of the protective group with tetra-*n*-butylammonium fluoride gave the desired *threo* isomer **3**, which was fully characterized (see the Experimental Section).

Scheme III



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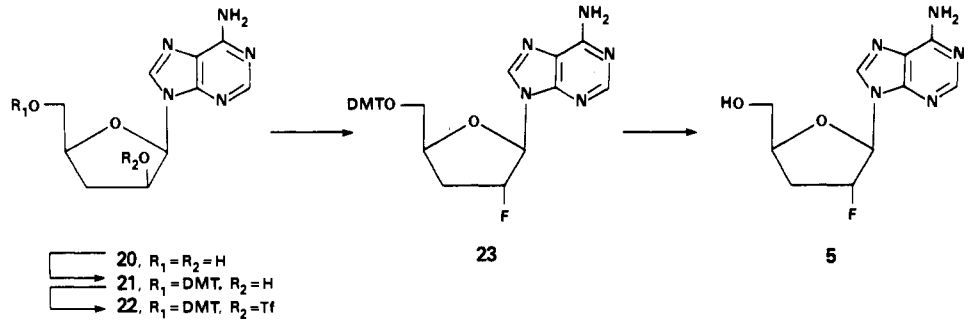
Scheme IV



For the synthesis of the *erythro* isomer **5**, we made use of compound **20**.[19,20] As shown in Scheme IV, protection of **20** with dimethoxytrityl chloride, followed by activation of the 2'-hydroxy group via formation of the corresponding triflate derivative, allowed the expected S_N2 displacement by fluoride to give **23**. Removal of the dimethoxytrityl group proceeded quantitatively to give the desired *erythro* isomer **5**. When a similar approach to this was attempted with cordycepin (3'-deoxyadenosine) as the substrate, only elimination products were obtained. Recently, however, this type of displacement has been realized by Herdewijn et al., albeit in very low yield.[21] Finally, as indicated in Scheme III, the corresponding inosine analogue **4** was prepared from **3** by either enzymatic (adenosine deaminase, ADA) or chemical ($NaNO_2$/HOAc) deamination. The ^1H NMR spectrum of 2'-F-dd-ara-I (**4**) shows the characteristic doublet at δ 8.23 for the H-8 proton of the purine, plus all of the other highly diagnostic proton resonances affected in varying degrees by the presence of the fluorine atom.

Chemical and Biological Properties

As reported earlier,[6] a major reason for synthesizing the 2'-fluoro analogue, **3**, was the hypothesis that this substitution might stabilize the compound toward acidic conditions which are known to rapidly decompose ddA.[4] An acid-stable compound would facilitate an oral drug formulation since ddA (**2**) had a half-life of less than 1 min at pH 1 and 37 °C.[6] The 2'-fluorinated analogue, **3**, however, was shown to be stable indefinitely under these conditions.[6]

Since adenosine deaminase (ADA) is such an ubiquitous enzyme, it was necessary to determine whether ADA converted **3** to the corresponding inosine analogue **4**. This reaction is known to take place with the parent compound, ddA.[9] Figure 2 shows that while **3** is a substrate for ADA, it is deaminated about 15 times slower than **2**. This has been confirmed and further quantitated by Hao et al. who also determined that 2'-F-dd-ara-I (**4**), unlike ddI (**1**), is not a substrate for the catabolic enzyme purine nucleoside phosphorylase (PNP).[22]

Since **4** would, therefore, be expected to be formed from **3** in vivo, the acid stability of **4** was determined (Figure 3) under conditions which roughly approximate the human

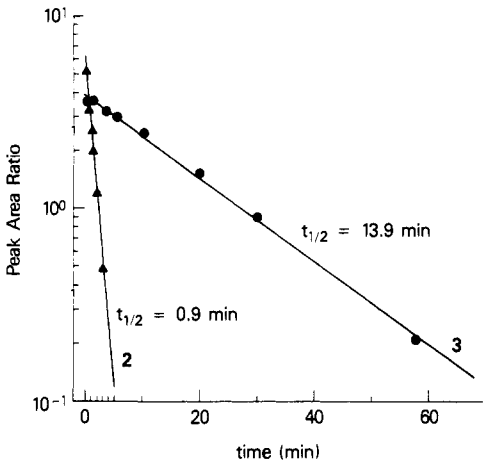


Figure 2. Concentration (peak area ratio to internal standard) vs time curve for 2',3'-dideoxyadenosine (▲) and 2'-F-dd-ara-A (●) after incubation with 0.3 unit of adenosine deaminase at pH 7.5 and 25 °C. The initial concentration of **2** was 55 μg/mL, while that of **3** was 40 μg/mL.

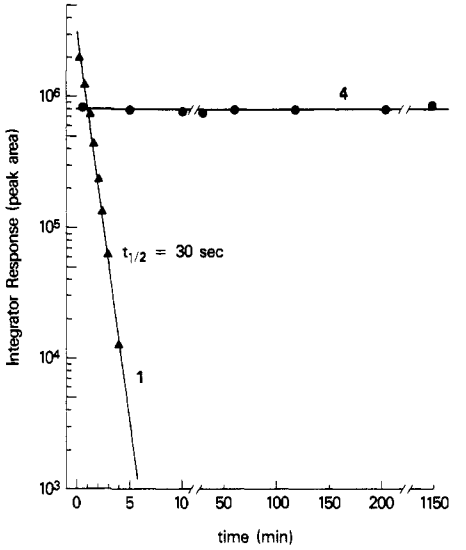


Figure 3. Concentration vs time profile for 2',3'-dideoxyinosine (▲) and 2'-F-dd-ara-I (●) at pH 1 and 37 °C. The time scale for the first 10 min is greatly expanded. The initial concentration of **1** was 9.2 μg/mL, while that of **4** was 34.5 μg/mL.

stomach environment (pH 1 and 37 °C). While ddI (**1**) had $t_{1/2}$ of 30 s under these conditions, 2'-F-dd-ara-I was completely stable. These results are similar to those previously reported[6] for the adenosine analogues, ddA (**2**) and 2'-F-dd-ara-A (**3**).

Figure 4 shows that the fluoro compounds **4** and **3** possess activity and potency against HIV which are similar to that of their parent compounds, ddI and ddA (**1**, **2**). However, as expected, quite different results are obtained

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Table I. Percent *gag* Protein Expression in H9 Cells

compound	evaluation day	concentration, μM					
		0	5	20	50	100	200
1 (ddI)	7	76	73	9	0	0	0
	10	73	53	30	0	0	0
	12	83	74	48	3	0	0
2 (ddA)	7	79	48	1	0	0	0
	10	70	48	0	0	0	0
	12	84	86	8	2	0	0
3 (2′-F-dd-ara-A)	7	79	58	61	18	10	0
	10	71	73	37	20	34	0
	12	79	66	53	19	50	0
4 (2′-F-dd-ara-I)	7	76	54	58	23	34	7
	10	71	82	52	24	12	10
	12	74	61	61	57	53	60

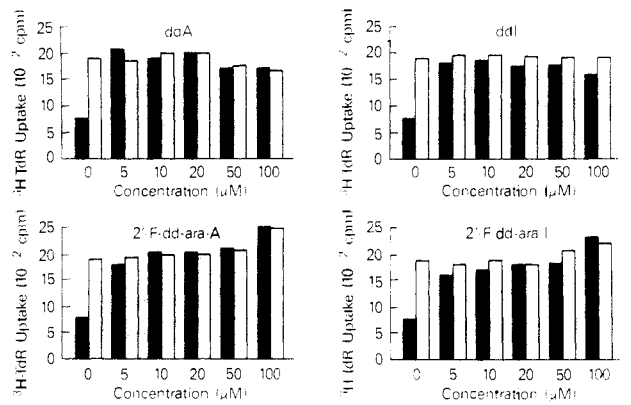

Figure 4. Protection of ATH8 cells from the cytopathogenic effects of HIV-1. Open bars are proportional to the number of viable cells on days 5–7 in the presence of the indicated concentration of drug (toxicity control). The solid bars measure the same parameter when HIV-1 was incubated with the cells in the presence of drug.

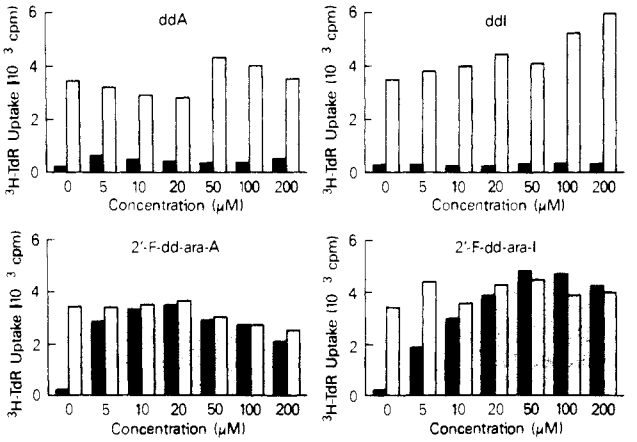

Figure 5. Anti-HIV-1 activity (ATH8) after 30 min HCl treatment (pH 1.0, 37 °C) and lyophilization prior to testing.

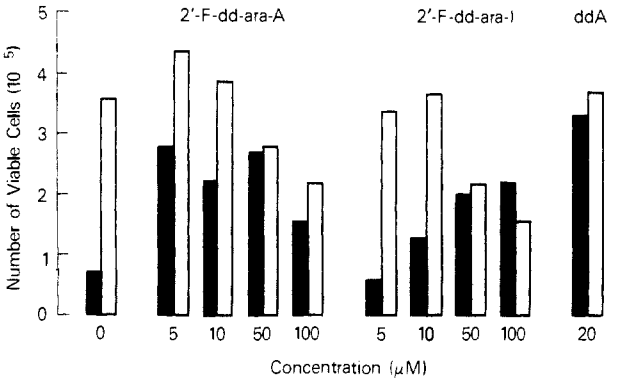

Figure 6. Protection of ATH8 cells against the cytopathogenic effect of HIV-2. ATH8 cells (2×10^5) were exposed to HIV-2 (100 virus particles per cell) and cultured in the presence or absence of drug (closed columns). Control cells were similarly treated but not exposed to the virus (open columns). On day 7 in culture, the total viable cells were counted by dye exclusion methods.

if all four compounds are exposed for 30 min to acidic conditions (pH 1, 37 °C) prior to anti-HIV testing. As seen in Figure 5, ddA and ddI were cleaved by acid treatment and have lost their activity while the 2′-fluoro analogues, 3 and 4, are unchanged and retain their activity and potency.

A practical effect of the acid stability of 2′-F-dd-ara-A was shown when this compound was found to have increased oral bioavailability relative to ddA in beagle dogs.[23]

Compounds 3 and 4 also protected ATH8 cells from the cytopathogenic effect of HIV-2 (Figure 6). In this experiment, the protective effect of the fluorine-containing nucleosides approached that of the antiretroviral dideoxynucleoside, ddA. However, when the fluorine-containing nucleosides were tested in the p24 *gag* protein expression assay using H9 cells, these compounds were less effective than the parent compounds in suppressing the *gag* protein expression (Table I). In this experiment, 20 μM ddI (1) also failed to completely block the p24 *gag* protein expression in H9 cells, which is somewhat different from our previous data.[5a] This difference may depend on the virus inoculum used, different cell types, and/or the sensitivity difference. A dideoxynucleoside depends critically on cellular kinases for the metabolic steps required to produce a 5′-triphosphate, the active form of the drug. It is, therefore, not surprising that a compound's activity will vary with the cell type used to some extent, since the occurrence and relative amounts of these kinases vary from one cell type to another. In any event, it seems that the fluorine-containing nucleosides are less potent than their parent compounds in these H9 cell assays.

An assessment of the cytotoxicity effects of compounds 1–4 on the antigen-driven proliferation of immune T-cells and the mitogen-driven proliferation of blood mononuclear cells was undertaken (Figure 7). Although the effects are generally similar, adenosine analogues are more toxic than inosine analogues, and the fluorinated compounds appear to be more toxic than their parent compounds.

Experimental Section

Chemistry. All chemical reagents were commercially available. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian XL-200 instrument. Proton chemical shifts

(23) Stoltz, M. L.; El-hawari, M.; Litle, L.; Smith, A. C.; Tomaszewski, J. E.; Grieshaber, C. K. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 535.

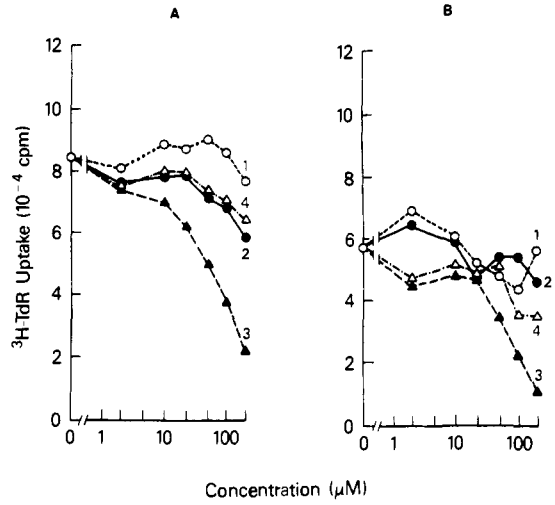

Figure 7. Effect of compounds 1–4 on the in vitro immune reactivity of normal lymphocytes. A. Normal clonal helper/inducer tetanus toxoid specific TM-11 cells (10^5) stimulated with tetanus toxoid plus autologous peripheral blood mononuclear cells (PBM) which had been irradiated. B. PBM (10^5) stimulated with pokeweed mitogen.

are expressed as δ values with reference to Me_4Si. UV spectra were recorded in a Beckman Model 34 spectrophotometer. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 241 polarimeter. Positive-ion fast atom bombardment (FAB) mass spectra were obtained on a VG 7070E mass spectrometer. Samples were dissolved in a glycerol matrix, and ionization was effected by a beam of xenon atoms derived by neutralizing xenon ions accelerated through 8.6 kV. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN. Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) from calf intestinal mucosa (Sigma Chemical Co.), Type VIII, 400 units/mL in 3.2 M $(NH_4)_2SO_4$ was diluted 1:10 with distilled water before use. 2',3'-Dideoxyadenosine and 2',3'-dideoxyinosine were obtained from the Drug Synthesis and Chemistry Branch, NCI.

Enzymatic Deamination of ddA. ddA (110 μg) was dissolved in 2.0 mL of pH 7.5 buffer at room temperature. To this was added 7.5 μL of diluted adenosine deaminase solution (0.3 unit), which was mixed by vortexing. A 200-μL aliquot of the enzyme–ddA solution was removed for analysis and diluted with 0.8 mL of cold distilled water. After addition of 20 μL of N^6-methyl-2'-deoxyadenosine (96.7 μg/mL in dimethyl sulfoxide) as an internal standard, 0.50 mL of the diluted sample was ultrafiltered to remove enzyme in a Centrifree Micropartition unit by centrifugation at 2000*g* (0 °C). The remainder of the sample was maintained on ice for 35 min to check reaction quenching at 0 °C, after which time it too was ultrafiltered. The enzyme–ddA solution was placed in a Dubnoff metabolic shaking incubator at 25 °C, and 100-μL aliquots were analyzed by HPLC as a function of time (see below).

Enzymatic Deamination of 3. Compound **3** (80 μg, 0.32 μmol) was reacted with adenosine deaminase at 25 °C in the manner described above. When **3** no longer could be detected by HPLC analysis after 3.5 h, the remaining mixture was ultrafiltered to give a solution of **4** in pH 7.5 buffer. This solution was used directly to determine the acid stability of **4**.

Acid Stability of Dideoxynucleosides. ddI (92 μg) was added to pH 1 buffer prewarmed to 37 °C. The solution was shaken at 37 °C, and a 0.5-mL aliquot was removed immediately, with subsequent aliquots at timed intervals for HPLC analysis. The aliquots were neutralized with 0.2 N NaOH (0.7 mL) to terminate hydrolysis and then chilled on ice until HPLC analysis. For determination of the acid stability of **4**, 0.95 mL of an ultrafiltered solution of enzymatically produced compound (see above) was warmed to 37 °C, the pH was adjusted to 1.0, and 0.10-mL aliquots were withdrawn at timed intervals. These aliquots were neutralized with 0.13 mL of 0.1 N NaOH and diluted with 0.30 mL of distilled water before addition of N^6-methyl-2'-deoxyadenosine internal standard and HPLC analysis.

Acid-Treated Samples for Anti-HIV Testing. Duplicate samples of approximately 1.0 mg of each dideoxynucleoside (1–4) were dissolved in 2.00 mL of 0.1 N HCl by sonication for 5 min. Each dideoxynucleoside solution was then incubated for 30 min at 37 °C. Individual solutions were then neutralized to pH 6.5–7.5 with 2.04 mL of 0.1 N NaOH. One set of samples was lyophilized and then tested for its ability to confer protection against HIV in ATH8 cells (see below). The other set of samples was diluted with distilled water and analyzed by HPLC to determine the concentration of dideoxynucleoside remaining. Lyophilization recovery of **3** was determined by a separate triplicate experiment to be 95 ± 3%.

HPLC Analysis of Dideoxy Purine Nucleosides. Relative concentrations of dideoxynucleosides were measured by the HPLC analysis of 100-μL aliquots of either ultrafiltered or neutralized samples. A 4.6 × 250 mm 5-μm Ultrasphere-ODS column (Altex/Beckman), protected by a Waters guard column packed with 37–50-μm Vydac 201SC, was eluted with 8.5% CH_3CN in 0.01 M pH 6.8 phosphate buffer at a flow rate of 1.0 mL/min. Dideoxynucleosides and decomposition products were detected at 260 nm with a Gilson 116 variable-wavelength detector. Peak identity was determined from coincidence of retention times with standards and by comparison of on-the-fly UV spectra obtained with a Perkin-Elmer LC-235 diode array detector. In this system the compounds of interest had the following capacity factors (k') and maximum UV absorbances: ddI, k' = 1.89, 249 nm; ddA, k' = 3.79, 260 nm; **4**, k' = 1.92, 247 nm; **3**, k' = 3.99, 259 nm; N^6-methyl-2'-deoxyadenosine, k' = 4.44, 265 nm. Peak areas and peak heights were measured simultaneously on a Spectra-Physics SP4200 computing integrator. For kinetic studies, these data were plotted as a function of time and, where possible, fitted to first order decomposition kinetics using MLAB, a nonlinear least-squares curve-fitting program.

6-Chloro-9-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine (12). Method A. Compound **9** (0.23 g, 0.68 mmol) was dissolved in anhydrous methylene chloride (4 mL) and treated with a solution (0.5 mL) of 30% HBr in acetic acid. The reaction was monitored by TLC (silica gel, ethyl acetate/hexane, 1:1). After 20 min, an additional 2.5 mL of the HBr/AcOH solution was required to drive the reaction to completion (1 h after the second addition). Traces of acetic acid were removed by several additions of toluene followed by evaporation. The bromo sugar **10**, obtained as a yellowish syrup, was dissolved in anhydrous methylene chloride (10 mL), and to this solution, oven-dried 4A molecular sieves (ca. 6 mL) and 6-chloropurine (**11**) (0.108 g, 0.70 mmol) were added. The resulting mixture was refluxed for 15 h, cooled, filtered, and evaporated to dryness. The residue was chromatographed on preparative TLC plates (Analtech 2000 μ) developed with ethyl acetate/hexane (3:2). As expected, four isomers were observed by analytical TLC with R_f values of 0.40, 0.31, 0.19, and 0.13, [silica gel, ethyl acetate/hexane (1:1)]. The major band (R_f 0.31), which corresponded to the desired 9β-isomer, afforded 0.024 g (8.4%) of pure **12** as a white foam; ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1 H, H-2), 8.36 (d, $J_{8,F}$ = 2.9 Hz, 1 H, H-8), 8.05–8.09 (m, 2 H, aromatic phenyl protons), 7.45–7.58 (m, 3 H, aromatic phenyl protons), 6.59 (dd, $J_{1',F}$ = 22.0 Hz, $J_{1',2'}$ = 2.7 Hz, 1 H, H-1'), 5.53 (dd, $J_{3',F}$ = 16.2 Hz, $J_{3',4'}$ = 2.5 Hz, 1 H, H-3'), 5.21 (dd, $J_{2',F}$ = 50.0 Hz, $J_{1',2'}$ = 2.7 Hz, 1 H, H-2'), 4.73 (m, 2 H, H-5'$_{a,b}$), 4.45 (m, 1 H, H-4'), 2.21 (s, 3 H, CH_3CO). Anal. ($C_{19}H_{16}ClFN_4O_5 \cdot 0.25EtOAc$) C, H, N.

The 7β-isomer (**13**, R_f 0.19) had an almost identical ^{1}H NMR spectrum except for the following resonances: δ 8.92 (s, 1 H, H-2), 8.63 (d, $J_{8,F}$ = 2.9 Hz, 1 H, H-8), and 6.90 (dd, $J_{1',F}$ = 22.0 Hz, $J_{1',2'}$ = 2.7 Hz, 1 H, H-1'). Anal. ($C_{19}H_{16}ClFN_4O_5$) C, H, N.

The other isomers were characterized spectrally by ^{1}H NMR and the following assignments were made. 9α-Isomer (**14**, R_f 0.40): ^{1}H NMR δ 8.80 (s, 1 H, H-2), 8.29 (s, 1 H, H-8), 6.50 (d, $J_{1',F}$ = 14 Hz, 1 H, H-1'). 7α-Isomer (**15**, R_f 0.13): ^{1}H NMR δ 8.91 (s, 1 H, H-2), 8.42 (s, 1 H, H-8), 6.92 (d, $J_{1',F}$ = 14 Hz, 1 H, H-1').

Method B. Compound **9** (0.55 g, 1.62 mmol) was dissolved in anhydrous methylene chloride (5 mL) and treated with a 30% HBr solution in acetic acid (2 mL). After 1 h, excess HBr was expelled out by bubbled air and the resulting solution was reduced to dryness under vacuum. The residue was dissolved in toluene and evaporated to dryness. This procedure was repeated twice to remove excess acetic acid. The bromo sugar residue (**10**) was

then dissolved in methylene chloride (6 mL). In a separate flask, a suspension of 6-chloropurine (11) (0.275 g, 1.76 mmol) in methylene chloride (6 mL) containing benzyltriethylammonium chloride (0.080 g) was treated with 25% aqueous NaOH (3.6 mL). The resulting mixture was shaken vigorously for 5 min before the methylene chloride solution containing the bromo sugar was added. After the addition of **10**, the reaction mixture was shaken for 1 h at room temperature. Methylene chloride (25 mL) was then added to facilitate the separation of the two layers and the milky aqueous layer was extracted four times with methylene chloride (25 mL). The organic extract was dried (Na_2SO_4), and the solvent was removed under vacuum. The crude oil obtained was added to a silica gel column (Merck Kiesegel 60, 70–230 mesh) and eluted with hexane/ethyl acetate (1:1) to provide 0.172 g (25%) of the major component which corresponded to the desired 9β-isomer (**12**), identical with the material obtained by using method A.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (16). The protected chloropurine **12** (0.108 g) was heated in a sealed steel bomb (external temperature 95–100 °C) with saturated methanolic ammonia (120 mL) for 3.5 days. During evaporation of the solvent, 0.040 g of the product crystallized. Concentration of the mother liquor and purification by silica gel chromatography (Merck Kiesegel 60, 70–230 mesh) using 10% methanol in methylene chloride as eluant, afforded an extra 0.020 g of the desired product (combined yield 90%). An analytical sample of **16** was obtained by recrystallization from methanol: mp 230–231 °C (lit.[16] mp 232–234 °C); $[\alpha]^{24}_D$ = +22.7° (*c* 0.03, water) (lit.[16] $[\alpha]^{24}_D$ = +22.6° (*c* 0.7, water); UV $(H_2O)_{max}$ 260 nm (ε 14 970).

6-Amino-9-[5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-9*H*-purine (17). A solution of compound **16** (0.032 g, 0.12 mmol) in anhydrous DMF (1 mL) was treated with 132 μL of a reagent mixture consisting of *tert*-butyldimethylsilyl chloride (1 mM) and imidazole (2.5 mM) in DMF and the mixture was stirred at room temperature. Additional amounts of this reagent (40 and 15 μL) were added after 30 min and 1 h, respectively, until TLC analysis (silica gel, chloroform/methanol, 9:1) indicated that reaction was complete. After the addition of 10 drops of methanol to quench the reaction, the mixture was reduced to dryness after the addition of a small amount of silica gel. The sample, adsorbed on silica gel, was loaded on top of a short silica gel column (Merck Kiesegel 60, 70–230 mesh) packed in chloroform. The desired sample was eluted with a step gradient from 2 to 10% methanol in chloroform and was obtained as a white solid (0.040 mg, 85%): mp 185 °C; ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1 H, H-2), 8.10 (d, $J_{8,F}$ = 2.6 Hz, H-8), 6.53 (dd, $J_{1',F}$ = 18 Hz, $J_{1',2'}$ = 3.4 Hz, 1 H, H-1'), 5.72 (br s, 2 H, NH_2), 5.11 (dm, $J_{2',F}$ = 52.5 Hz, 1 H, H-2'), 4.68 (dm, $J_{3',F}$ = 17.5 Hz, 1 H, H-3'), 4.04 (m, 1 H, H-4'), 3.89 (m, 2 H, H-5'$_{a,b}$), 0.91 (s, 9 H, $(CH_3)_3CSi$), 0.10 (s, 6 H, $(CH_3)_2Si$). This compound was used directly for the next step.

6-Amino-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]-9*H*-purine (19). Compound **17** (28.7 mg, 0.075 mmol) was treated with 0.5 mL (0.083 mmol) of a stock solution of (dimethylamido)pyridine [DMAP, 0.092 g (0.753 mmol) in 2 mL of dry acetonitrile]. Anhydrous DMF (0.2 mL) was added to achieve complete solution of the starting material. The reaction mixture was then treated with 0.5 mL (0.083 mmol) of a stock solution of phenyl chlorothiocarbonate [46 μL (0.332 mmol) in 2 mL of anhydrous acetonitrile] and the resulting mixture was allowed to react at room temperature for 1 h when additional amounts of the DMAP solution (0.2 mL) and phenyl chlorothionocarbonate solution (0.2 mL) were required to drive the reaction to completion. After an additional 30 min, TLC analysis (silica gel, ethyl acetate) indicated that the reaction was complete. A small volume of methanol was added to quench the reaction and the mixture was concentrated under vacuum, the product dissolved in methylene chloride and purified by preparative TLC (Analtech 2000 μ, ethyl acetate/hexane, 3:2) to give 0.029 g of the intermediate **18** as a white solid. Compound **18** (0.019 g, 0.037 mmol) was immediately treated with 1 mL (0.015 mmol) of a stock solution of 2,2'-azobis(2-methylpropionitrile) [AIBN, 0.012 g (0.073 mmol) in 5 mL of anhydrous toluene] and with tributyltin hydride (20.5 μL, 0.074 mmol) and the resulting mixture heated to 85–95 °C. After 30 min, additional amounts of tributyltin hydride (20 μL) and the AIBN stock solution (0.5 mL) were added. The reaction was judged to be complete after an additional 30 min reflux. After cooling, the entire reaction mixture (ca. 1.5 mL) was loaded on a preparative TLC plate (Analtech 2000 μ) and developed with methylene chloride/methanol (19:1). Compound **19** (0.010 g, 71%) was isolated from a single major band as a pure solid: mp 204 °C; ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1 H, H-2), 8.15 (d, $J_{8,F}$ = 2.6 Hz, H-8), 6.31 (dd, $J_{1',F}$ = 18 Hz, $J_{1',2'}$ = 3.2 Hz, H-1'), 5.58 (br s, 2 H, NH_2), 5.28 (dm, $J_{2',F}$ = 54.0 Hz, 1 H, H-2'), 4.28 (m, 1 H, H-4'), 3.85 (m, 2 H, H-5'$_{a,b}$), 2.36–2.62 (m, 2 H, H-3'$_{a,b}$), 0.90 (s, 9 H, $(CH_3)_3CSi$), 0.10 (s, 6 H, $(CH_3)_2Si$). This material was used immediately in the final step of the reaction sequence.

9-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)adenine (3). Tetrabutylammonium fluoride (33 μL of a 1 M solution in THF) was added to a solution of **19** (0.010 g) in anhydrous THF (0.5 mL). After 1 min of stirring at room temperature, a precipitate was formed and 10 min later the reaction mixture was loaded onto a preparative TLC plate (Analtech 2000 μ) which was developed with 5% methanol in methylene chloride. From the isolated band, 0.0053 g (78%) of **3** was isolated. This material was dissolved in water and lyophilized to give a fluffy white powder which was recrystallized from ethanol: mp 227 °C; ^{1}H NMR (D_2O) δ 8.23 (d, $J_{8,F}$ = 2.6 Hz, 1 H, H-8), 8.05 (s, 1 H, H-2), 6.25 (dd, $J_{1',F}$ = 18.7 Hz, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1'), 5.35 (dm, $J_{2',F}$ = 53.7 Hz, 1 H, H-2'), 4.37 (m, 1 H, H-4'), 3.75 (m, 2 H, H-5'$_{a,b}$), 2.10–2.82 (m, 2 H, H-3'$_{a,b}$); $[\alpha]^{24}_D$ +57.8° (*c* 0.083, H_2O); accurate mass positive ion FAB MS *m/z* 254.1031 (MH^+, calcd 254.1052). Anal. ($C_{10}H_{12}FN_5O_2$) C, H, F, N.

9-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)hypoxanthine (4). Method A. Compound **3** (0.013 g, 0.051 mmol) dissolved in 5 mL of water was treated with 40 μL of a suspension containing adenosine deaminase from calf intestinal mucosa (EC 3.5.4.4, Sigma Chemical Co., 402.8 units/mL) and the mixture was stirred at room temperature overnight. The reaction mixture was chromatographed on a C-18 reversed phase column (Baker C-18 bonded silica gel) and eluted first with water and then with a water/methanol solution (10:1). The UV-active fractions were combined and lyophilized to give **4** as a white fluffy solid (0.010 g, 77%). Recrystallization first from acetone/methanol and later from ethanol, produced a white crystalline solid: mp 204 °C; ^{1}H NMR (D_2O) δ 8.23 (d, $J_{8,F}$ = 2.6 Hz, H-8), 8.14 (s, 1 H, H-2), 6.32 (dd, $J_{1',F}$ = 18.0 Hz, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1'), 5.40 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2'), 4.36 (m, 1 H, H-4'), 3.75 (m, 2 H, H-5'$_{a,b}$), 2.12–2.82 (m, 2 H, H-3'$_{a,b}$); $[\alpha]^{24}_D$ +58.9° (*c* 0.087, H_2O); accurate mass positive ion FAB MS *m/z* 255.0893 (MH^+, calcd 255.0893). Anal. ($C_{10}H_{11}FN_4O_3$) C, H, F, N.

Method B. Compound **3** (0.040 g, 0.158 mmol) in water (5 mL) was treated with sodium nitrite (0.30 g, 4.35 mmol) in glacial acetic acid (1.5 mL). The reaction mixture was tightly capped in a reaction vessel and stirred at room temperature for 20 h. Following concentration of the mixture under reduced pressure, water was added and the solution reduced to dryness twice to remove traces of acetic acid. The residue was then chromatographed as described above, and the appropriate fractions were combined and lyophilized to give 0.037 g (95%) of **4** which was identical with that obtained by using method A.

9-(3-Deoxy-β-D-*threo*-pentofuranosyl)adenine (20). This compound was synthesized by Dr. Terrence C. Owen (University of South Florida) by the method of Hanskee and Robins[20] under contract N01 CM 437639 to the DS&CB, DTP, NCI.

6-Amino-9-[5-*O*-(4,4'-dimethoxytrityl)-3-deoxy-β-D-*threo*-pentofuranosyl]-9*H*-purine (21). A mixture of **20** (0.116 g, 0.47 mmol), dimethoxytrityl chloride (0.19 g, 0.56 mmol), DMAP (5 mg), and triethylamine (105 μL) in anhydrous pyridine (5 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated to dryness and dissolved in methylene chloride. The insoluble material was removed by filtration and the filtrate was flash chromatographed on silica gel (Merck Kiesegel 60, 230–400 mesh) with a solution of methanol in methylene chloride (step gradient from 1% to 10%). The appropriate fractions were pooled and the desired product, **21** (0.94 g, 37%), was obtained as a white solid, mp 110–112 °C. This material was used immediately for the next step.

9-(2,3-Dideoxy-2-fluoro-β-D-*erythro*-pentofuranosyl)adenine (5). The DMT-protected nucleoside **21** (0.225 g, 0.40 mmol) was dissolved in anhydrous THF (5 mL) and treated with

80% NaH (0.015 g, 0.51 mmol). The resulting mixture was then cooled to 0 °C for 1 h. After cooling it further with a mixture of dry ice and 2-propanol, trifluoromethanesulfonyl chloride (54 μL, 0.51 mmol) was added and the mixture stirred for 2 h. The reaction mixture then was reduced in volume and applied directly to a preparative TLC plate (Analtech, 2000 μ) to give a major band that corresponded to the triflate **22** (0.205 g, 74%). Part of this material (0.113 g, 0.16 mmol) was dissolved in dry THF (2 mL) and treated with tetrabutylammonium fluoride (175 μL, 1 M solution in THF) at room temperature for 30 min. Preparative silica gel TLC chromatography (Analtech, 2000 μ) developed with ethyl acetate afforded 0.072 g (78%) of the desired protected compound, **23**. This material was dissolved in 2 mL of methylene chloride and treated with trichloroacetic acid (2 mL, 3% solution in methylene chloride) at room temperature. After 5 min, the solution was loaded on a silica gel preparative TLC plate (Analtech, 2000 μ) which was developed with a methylene chloride/methanol solution (9:1). After its isolation from the plate, compound **5** was obtained as a white solid (0.03 g, 94%). It was recrystallized from methanol to give small flakes: mp 235 °C; ^{1}H NMR (D_2O) δ 8.48 (s, 1 H, H-8), 8.23 (s, 1 H, H-2), 6.31 (d, $J_{1',F}$ = 17.2 Hz, 1 H, H-1'), 5.54 (dd, $J_{2',F}$ = 52.2 Hz, $J_{2',3'a}$ = 3.4 Hz, 1 H, H-2'), 4.54 (m, 1 H, H-4'), 3.97 (dd, $J_{5'a,5'b}$ = 12.4 Hz, $J_{5'a,4'}$ = 2.6 Hz, 1 H, H-5'$_a$), 3.70 (dd, $J_{5'b,5'a}$ = 12.4 Hz, $J_{5'b,4'}$ = 3.6 Hz, 1 H, H-5'$_b$), 2.27–2.70 (m, 2 H, H-3'$_{a,b}$); accurate mass positive ion FAB MS *m/z* 254.1059 (MH^+, calcd 254.1052). Anal. ($C_{10}H_{12}FN_5O_2$) C, H, N.

Antiviral Activity against HIV in Vitro. Experiments on the protection of CD4+ ATH8 cells from the cytopathogenic effects of HIV-1 were carried out as previously described[24] with the exception that incorporation of [^{3}H]thymidine rather than the trypan blue exclusion method was used to quantitate protection of these susceptible cells. The target ATH8 cells were pulsed with [^{3}H]thymidine for 24 h and harvested onto glass filters and the radioactivity counted. This method is similar to that described earlier.[5a] The inhibition of the infectivity and cytopathic effect of HIV-2 was determined by exposing ATH8 cells (2×10^5) to an exceedingly potent HIV-2/LAV preparation (100 virus particles/cell). Cells were cultured in the presence of drug and/or virus. On day 7, the total viable cells were counted by the trypan dye exclusion method. In inhibition of *gag* protein expression experiments, H9 cells (10^5) were exposed to HIV-1 (500 virus particles per cell) and cultured in the absence and presence of various concentrations of **1–4**. On days 7, 10, and 12 in culture, the percentage of the target H9 cells expressing p24 *gag* protein of HIV-1 was determined by indirect immunofluorescence assay using a murine monoclonal antibody (M26). Toxicity to antigen- and mitogen-driven proliferation of immunocompetent cells was conducted as previously described.[5a] The data are the average of triplicate determinations.

Supplementary Material Available: The NMR spectrum of 2'-F-dd-ara-I (**4**) (1 page). Ordering information is given on any current masthead.

(24) Kim, C.-H.; Marquez, V. E.; Broder, S.; Mitsuya, H.; Driscoll, J. S. *J. Med. Chem.* **1987**, *30*, 862.

Synthesis and Biological Activity of Novel Quaternary Ammonium Derivatives of Alkylglycerols as Potent Inhibitors of Protein Kinase C

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Alkylglycerols such as *rac*-1-*O*-octadecyl-2-*O*-methylglycerophosphochocholine (Et-18-OMe) have shown an inhibitory effect on the metastasis and growth of various cancer cell lines. Alkyl phospholipids have been shown to accumulate at the surface in several cell lines, the selectivity of which is still not clearly understood. A consequence of this action may lead to the inhibition of cell membrane related protein kinase C (PKC). The goal of this research was to develop ether lipid inhibitors of PKC to augment antineoplastic activity. This led to the synthesis and in vitro testing of a series of novel quaternary ammonium derivatives of alkylglycerols. The biological testing of these analogues on PKC stimulated with *rac*-1-*O*-oleoyl-2-*O*-acetylglycerol showed several analogues with inhibition comparable to that of Et-18-OMe.

Since the discovery of protein kinase C (PKC),[1] the function of this ubiquitous kinase as a regulatory enzyme has been extensively investigated. Central to our interests is the interaction between PKC and naturally-occurring phospholipids and diacylglycerols.[1,2] The hydrolysis of phosphatidylinositol by phospholipase C yields diacylglycerol which acts as a stimulus for PKC.[1-3] Activation of PKC results in the translocation of the enzyme to the cell membrane, where phosphatidylserine and diacylglycerol, in the presence of calcium, are able to fully activate the enzyme.[2] Contrary to the action of diacylglycerols, endogenous alkylacylglycerols derived by phosphocholine hydrolysis[4-6] are inhibitors of PKC. In addition, synthetic dialkyl linked glycerols inhibit activation of PKC by diacylglycerols.[6]

Synthetic alkyl analogues of phosphatidylcholine such as *rac*-1-*O*-octadecyl-2-*O*-methylglycerophosphocholine (Et-18-OMe, Chart I) have also shown an inhibitory effect on this kinase.[7,8] Alkyl phospholipids are also known to

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0022-2623/90/1833-0985$02.50/0 © 1990 American Chemical Society