policies of the Department of Health and Human Services nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. These investigations were also supported in part by the AIDS Basic Research Programme of the European Community and by grants from the Belgian Foods voor Geneeskundig Wetenschappelijk Onderzoek. R.S. is a Senior Research Assistant from the National Fund for Scientific Research (Belgium). The excellent technical assistance of Anita Camps, Frieda De Meyer, and Anita Van Lierde is gratefully acknowledged.

**Registry No.** 2, 140438-62-6; 3, 132065-10-2; 4, 140438-63-7; 6, 91469-55-5; 7, 132065-12-4; 8, 132065-13-5; 9, 132152-82-0; 10, 132065-14-6; 11, 140438-64-8; 12, 140438-65-9; 13, 140438-66-0; 14, 140438-67-1; 15, 132065-16-8; 16, 132065-15-7; 4-chlorobenzenediazonium chloride, 2028-74-2; 5-amino-4,6-dichloropyrimidine, 5413-85-4; 2-amino-4,6-dichloropyrimidine, 56-05-3; diethoxymethyl acetate, 14036-06-7.

**Supplementary Material Available:** Single-crystal X-ray structural analysis data for compound 3 including tables of atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement coefficients, and torsional angles (12 pages); observed and calculated structure factors (4 pages). Ordering information is given on any current masthead page.

# Chemistry and Anti-HIV Properties of 2′-Fluoro-2′,3′-dideoxyarabinofuranosylpyrimidines

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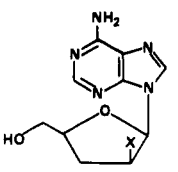
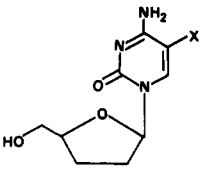
The synthesis, chemistry, biochemistry, and anti-HIV activity of a series of 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)pyrimidines have been studied in an attempt to find useful anti-AIDS drugs. Synthesis is carried out via a 2,3-dideoxyribose intermediate which facilitates the preparation of analogues by removing the sugar 3′-hydroxyl group prior to, rather than after, condensation with a uracil or cytosine aglycon. The 2′-F-dd-uridine analogues **7a–d** (with H, F, Cl, and $CH_3$ substitution in the 5-position) as well as the 4-deoxy compound (**12b**) are nonprotective to ATH8 or CEM cells infected with HIV-1. In the corresponding cytidine series, the 5-chloro analogue (**11**) is inactive. However, 2′-fluoro-2′,3′-dideoxyarabinosylcytosine, **10a**, and its 5-fluoro analogue, **10b**, are both active. While neither compound is as potent as ddC or 5-F-ddC (**2b**), **10b** gives complete protection against the cytopathic effects of HIV in both host cell lines. 2′-Fluoro substitution confers increased chemical and enzymatic stability on dideoxynucleosides. Even though dideoxy pyrimidine nucleosides are inherently more stable than the corresponding purine analogues toward acid-catalyzed cleavage of the glycosidic bond, 2′-fluoro substitution (**10a**) still increases stabilization relative to ddC (**2b**). No detectable deamination by partially purified cytidine deaminase is observed with the 2′-fluoro compounds **10a**, **10b**, or **11** under conditions which rapidly deaminate cytidine. A small amount of 2′-F-dd-*ara*-U (**7a**) is formed from **10a** in monkey plasma after >24 h of exposure. The octanol–water partition coefficients for the dideoxynucleosides in this study indicate their hydrophilic character, with log *P* values varying from −0.28 to −1.18.

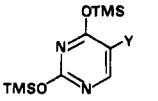
The anti-HIV properties of 2′,3′-dideoxynucleosides (ddN) such as **1a** and **2a** are now well established[1,2] and considerable effort continues to be expended on the determination of the effects of substituents on activity and potency in this series.[3] Fluorine has always has been a favored substituent in nucleoside antiviral investigations because of its size similarity and electronegativity difference relative to hydrogen.[4] Fluoro-substituted ddNs as potential anti-HIV agents have been the subject of more than 30 reports since 1987.[4,5] Our interest in this area started with the discovery that 5-F-ddC, **2b**, was as active and potent as ddC itself[6] and has continued with compounds in the 2′-F-dd-purine[7-9] series (e.g. **1b**) which possess unusual chemical[7] and biochemical[10] properties relative to their nonfluorinated parents. Several recent reports regarding anti-HIV studies with 2′-fluoro pyrimidine derivatives[11] have prompted us to report our own studies in this area.



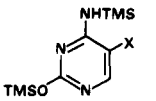

**1a**, X = H
**1b**, X = F




**2a**, X = H
**2b**, X = F




**5a**, Y = H
**b**, Y = F
**c**, Y = Cl
**d**, Y = $CH_3$




**8a**, X = H
**b**, X = F

## Chemistry

A major benefit of 2′-fluoro substitution in the dideoxy purine series[7,8] is an abrogation of the extreme acid-instability of 2′,3′-dideoxy purine nucleosides[12] since acid stability is a desirable property for the production of oral

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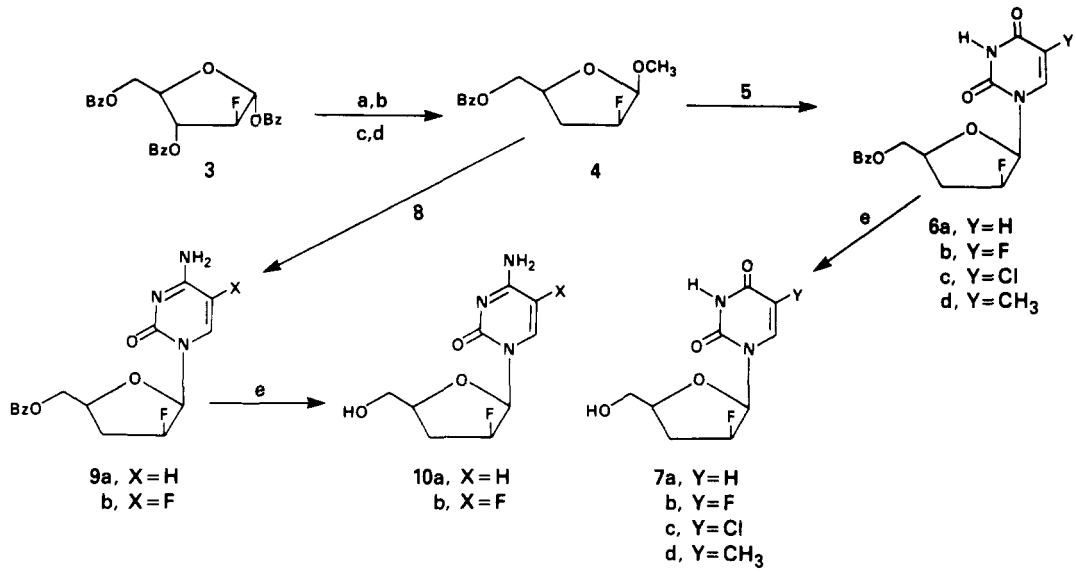
**Scheme I**[a]




[a] (a) (1) HBr/AcOH, $CH_2Cl_2$, room temperature; (2) $K_2CO_3$/MeOH/THF, room temperature (64%); (b) BzCl/Py, −30 °C (78%); (c) (1) NaH/$CS_2$/DMF, 0 °C; (2) MeI, 0 °C (99%); (d) $Bu_3SnH$/AIBN, toluene, reflux (86%); (e) $NH_3$/MeOH, room temperature.

drug formulations. In contrast to the dideoxy purine nucleosides, acid stability is not a significant problem in the conventional 2′,3′-dideoxy pyrimidine nucleoside series. Therefore, the major rationale for fluoro modification of

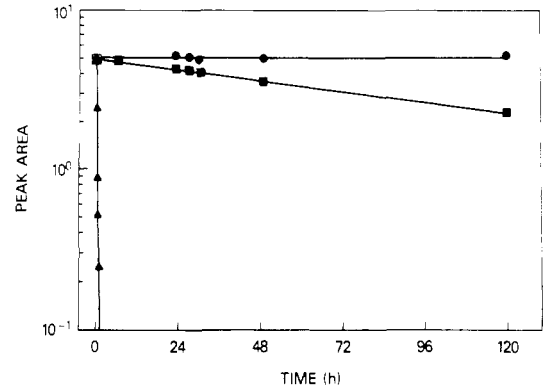



**Figure 1.** Concentration vs time curves for 2′-F-dd-ara-C (**10a**, ●), ddC (**2a**, ■), and d4C (**13**, ▲) in 0.1 N HCl (pH 1.1) at 37 °C.


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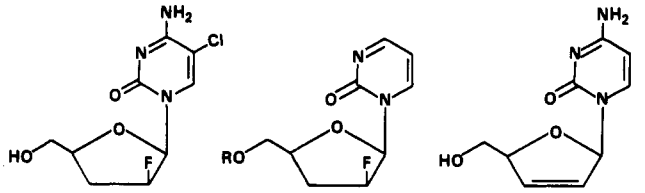
Table I. Octanol-Water Partition Coefficients and Chromatographic Properties of Some 2',3'-Dideoxy Pyrimidine Nucleosides and Related Compounds

| compound | partition coefficients: log $P^a$ | partition coefficients: HPLC mobile phase[b] | chromatographic properties: $k^c$ | chromatographic properties: $\lambda_{max}$ (nm) |
|---|---|---|---|---|
| 5-Cl-2'-F-dd-*ara*-U (**7c**) | −0.28 ± 0.01 | E | 14.24 | 275 |
| 5-Me-2'-F-dd-*ara*-U (**7d**) | −0.44 ± 0.01 | E | 15.38 | 266 |
| 5-Cl-2'-F-dd-*ara*-C (**11**) | −0.46 ± 0.02 | F | 13.07[d] | 285 |
| 5,2'-$F_2$-dd-*ara*-U (**7b**) | −0.72 ± 0.01 | C | 6.21 | 268 |
| 2'-F-dd-*ara*-U (**7a**) | −0.87 ● 0.00 | C | 5.37 | 260 |
| 5,2'-$F_2$-dd-*ara*-C (**10b**) | −0.92 ± 0.02 | F | 5.48[d] | 280 |
| ddU | −1.00 ± 0.01 | C | 3.92 | 264 |
| 5-F-ddC (**2b**) | −1.09 ± 0.01 | D | 3.27[d] | 280 |
| 2'-F-dd-*ara*-C (**10a**) | −1.18 ± 0.02 | D | 3.18[d] | 270 |
| ddC (**2a**) | −1.33 ± 0.01 | B | 2.37[d] | 271 |
| ddC-ene (**13**) | −1.57 ± 0.03 | B | 2.23[e] | 270 |
| uridine[f] | −1.94 ± 0.01 | A | 1.09[e] | 263 |
| cytidine | −2.29 ± 0.04 | A | 0.55[e] | 267 |

[a] Mean ± standard deviation of three independent determinations. [b] The following mobile phases were used at 1.0 mL/min with a 4.6 × 250 mm 5-μm Ultrasphere ODS column: (A) 2%, (B) 4%, (C) 6%, (D) 7%, or (E) 10% $CH_3CN$ in pH 7.0, 0.01 M phosphate buffer and (F) gradient elution. All dideoxynucleosides had a retention time of 4–9 min with mobile phases A–E. [c] $k = (v_r - v_0)/v_0$ or $(t_r - t_0)/t_0$; a mobile phase of 4% $CH_3CN$ in pH 7.0, 0.01 M phosphate buffer was used. [d] 4.6 × 250 mm 5-μm Ultrasphere ODS column only; $v_0$ = 1.90 mL. [e] 4.6 × 250 mm 5-μm Ultrasphere ODS column with guard column; $v_0$ = 2.31 mL. [f] Internal standard for acid stability studies.

dideoxy pyrimidine nucleosides is related to possible increases in therapeutic index and potency, as well as the potential for reduced or altered toxicity relative to an active, nonfluorinated parent compound.

The compounds described here were synthesized by a route utilizing the fluorodideoxy sugar, **4**, as a common intermediate (Scheme I). Compound **4** facilitates the preparation of 2'-F-ddNs by eliminating the necessity for performing the Barton reduction each time a new analogue is prepared. A recent report[11e] describes a different synthetic approach to the preparation of a similar intermediate. In our studies, **4** is prepared from **3**[13] in four steps[14,15] via a methyl xanthate intermediate. Reaction of the silylated pyrimidines **5** and **8** with the 1-α-bromo derivative generated in situ from **4**[15] gives mainly the protected nucleosides, **6** and **9**, which are debenzoylated with methanolic ammonia to yield the target compounds **7a–d** and **10a,b**. Because of the difficulty in obtaining 5-chlorocytosine, **11** was prepared via the corresponding uridine **7c**, by utilizing the 4-triazole derivative. Compound **12b** is a member of the zebularine family. Zebularine (4-deaminocytidine) and its analogues have significant biological properties.[16] **12b** was prepared by the route described in Scheme I in order to determine whether biological activity could be extended into the 2',3'-dideoxy series.



**11** **12a, R = Bz** **b, R = H** **13**

The partition coefficients determined for this series of nucleosides covered a range of 2.0 log units, a 100-fold variation in lipophilicity. However, the log *P* values were all negative (Table I), indicating hydrophilic character. Individually, all the cytosine dideoxynucleosides were chromatographically well-behaved with symmetrical peaks on reverse-phase HPLC in a buffered mobile phase containing a low percentage of organic modifier (Table I). However, **2b** and **10a** were not separable by HPLC, and when coinjected, coeluted as one unresolved peak. For these two dideoxycytidine analogues, the addition of a fluorine atom gave similar, minor increases in hydrophobicity, independent of whether the substitution was in the sugar or the base.

## Acid Stability

The cleavage of the glycosidic bond in 2',3'-dideoxycytidine (ddC, **2a**) and 2'-F-dd-*ara*-C (**10a**) was studied at pH 1 and 37 °C over a 120-h period. ddC is hydrolyzed slowly (Figure 1) with the concomitant formation of cytosine. As is the case in the dideoxy purine nucleoside series,[8,9] 2'-fluorine substitution increased acid stability, and **10a** was completely stable during this time period under the above described conditions with no decrease in **10a** concentration or formation of cytosine observed over a 5-day period. As a general rule, the 2',3'-dideoxy pyrimidine nucleosides are not nearly as acid-sensitive as the corresponding dideoxy purine nucleosides. This is illustrated by the relative rates of decomposition (pH 1, 37 °C) of ddC, **2a** ($t_{1/2}$ = 109 h, Figure 1), and 2',3'-dideoxyadenosine, **1a** ($t_{1/2}$ = 35 s).[7] However, sugar modification can change dideoxycytidine acid stability in a dramatic fashion. For example, with the addition of a 2',3'-double bond to ddC to produce d4C (**13**), the hydrolysis rate is increased over 400-fold (Figure 1).

## Cytidine Deaminase Stability

**Partially Purified Enzyme.** Using our standard conditions for evaluating aminonucleoside stability to cytidine deaminase (CDA), cytidine, the natural substrate, has a $t_{1/2}$ = 2.5 min for conversion to uridine by this enzyme. In contrast, cytosine ddNs are very resistant to the action of cytidine deaminase (CDA). After 23 h, ddC (**2a**) and 5-F-ddC (**2b**) show only a slight concentration decrease (ca. 5%), while no deamination is observed with the 2'-F-dd-*ara*-C analogues **10a**, **10b**, and **11** (data not shown). These data are consistent with earlier studies on ddC[17a–c]

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**Table II.** Direct Comparison of the Effect of ddC and Fluorinated Analogues **10a**, **10b**, and 11 on HIV-Induced Cytopathic Effects in ATH8 Cells[a]

| compound | concentration ($\mu$M) | % protection | % cytotoxicity |
|---|---|---|---|
| **2a** (ddC) | 0.2,[b] 0.5, 1.0, 5.0 | 93, 100, 100, 88 | 6, 0, 9, 1 |
| **2b** (5-F-ddC) | 0.2, 0.5, 1.0, 5.0 | 71, 96, 100, 99 | 0, 0, 1, 4 |
| **10a** (2′-F-dd-*ara*-C) | 0.2, 0.5, 1.0, 5.0 | 3, 25, 56, 53 | 0, 8, 4, 1 |
| **10b** (2′,5-$F_2$-dd-*ara*-C) | 0.2, 0.5, 1.0, 5.0 | 0, 8, 7, 73 | 0, 0, 8, 0 |

[a] Compounds evaluated in the same experiment. See Experimental Section for details. [b] Concentration giving 93% protection from HIV-1-induced cytopathic effects with 6% cytotoxicity.

**Table III.** Effect of Pyrimidine Nucleosides on HIV-Induced Cytopathogenesis in CEM Cells

| compd | $n$[a] | $EC_{50}$, $\mu$M[b] | $IC_{50}$, $\mu$M[c] | TI[d] |
|---|---|---|---|---|
| **2a** | 3 | 0.3 | 120 | 380 |
| **2b** | 4 | 0.1 | >40 | >400 |
| **10a** | 8 | 1.1 | 43 | 39 |
| **10b** | 4 | 57.7 | >580 | >10 |

[a] Number of averaged experiments. [b] Concentration required to inhibit HIV cytopathic effect by 50%. [c] Concentration inhibitory to 50% cell growth. [d] Therapeutic index ($IC_{50}/EC_{50}$).

as well as 3′-deoxycytidine analogues[17d] and shed no light on the observation[11c] that tetrahydrouridine, a CDA inhibitor, enhances the anti-HIV potency of **2a** and **10a**.

**Monkey Plasma.** In an earlier in vivo study in rhesus monkeys, a species with high CDA levels,[17c] 8–9% formation of ddU is observed 24 h after iv administration of ddC. For this reason, the in vitro stabilities of 20 $\mu$M ddC (**2a**) and **10a** were compared in rhesus monkey plasma. A very slow and parallel decrease in the concentration of these two dideoxynucleosides is observed (14% loss over 28 h) which correlates well with the earlier in vivo study of **2a** stability in the monkey.[17c] Although a small amount of ddU could be detected in the ddC incubation mixture after 22 h in vitro, the corresponding deamination product **7a** was not observed for **10a**.

## Anti-HIV Activity

Our laboratory[7] and others have established the relationship between the stereochemistry of 2′-fluoro dideoxy purine nucleoside substitution and anti-HIV activity. De Clercq and co-workers also established this relationship for the 3′-position, showing that the anti-HIV-effective stereochemistry for fluorine was "down" in the 3′-position, in contrast to "up" in the 2′-position.[5a] We were particularly interested to determine whether a similar relationship would be observed in the 2′-fluorodideoxy pyrimidine nucleoside series because of the excellent anti-HIV activity found for the 3′-fluoro-2′,3′-dideoxyuridines,[18–20] especially

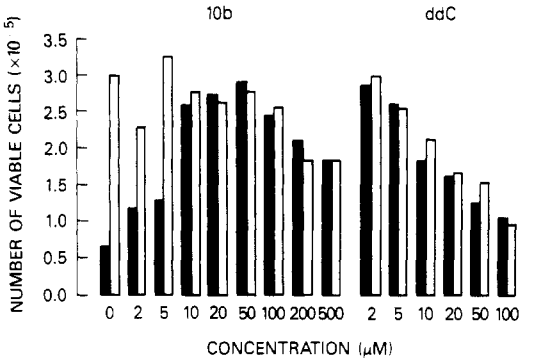


**Figure 2.** Inhibition of the cytopathic effect of HIV-1 by 2′,5-di-F-dd-ara-C (**10b**) and ddC (**2a**) in ATH8 cells. Open bars indicate cellular toxicity in uninfected, treated cells at the indicated concentrations. Closed bars indicate compound efficacy in HIV-infected cells.

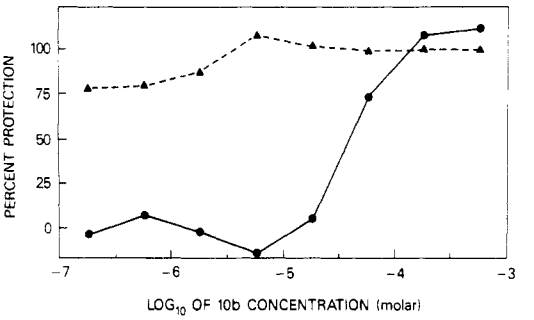


**Figure 3.** Protection of CEM cells from the cytopathic effect of HIV-1 by 2′-5-di-F-dd-ara-C (**10b**, ●). Uninfected, untreated cell growth (▲).

when the aglycon contained 5-chloro substitution. Compounds **2b**, **7a–d**, **10a,b**, **11**, and **12b** were evaluated in vitro as inhibitors of the cytopathic effects of HIV-1 in ATH8 and CEM cells. However, none of the 2′-fluoro-2′,3′-dideoxy-ara-U analogues (**7a–d**) synthesized in this study were protective against HIV in either host cell line.

In the 2′-fluoro-dd-*ara*-C series, the parent molecule, **10a**, has been shown to have anti-HIV activity in several host cell systems.[11a–d] Our findings, using the ATH8 and CEM systems (Tables II and III) are consistent with those data. It is clear from comparing **2b** and **10a** with ddC (**2a**) that fluorination at C2′ has a significantly greater effect on potency and activity than fluorination of the aglycon at C5.[6] In contrast to the activity observed with 3′-F-5-Cl-2′,3′-ddC,[21] the corresponding 2′-F-5-Cl-dd-*ara*-C analogue, **11**, was devoid of activity.

The 2′,5′-difluoro analogue (**10b**) was reported to be inactive against HIV in C8166 cells at 100 $\mu$M concentration.[11b] It was noted in that study that the lack of activity was surprising in view of the reported anti-HIV

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properties of the individual monofluorinated compounds, **10a**[11b] and **2b**.[6] We conducted a direct comparison of the anti-HIV activity of ddC (**2a**), 5-F-ddC (**2b**), 2'-F-dd-ara-C (**10a**), and 2',5-diF-dd-ara-C (**10b**) in the ATH8 system (Table II). As we previously reported,[6] **2a** and **2b** are completely protective in the 0.5–1.0 μM concentration range without significant host cell toxicity. The 2'-fluoro analogue, **10a**, is active, but less so and is not as potent. At 1.0 μM, a concentration giving 100% protection with ddC and **10a**, the difluoro analogue **10b** is both inactive and nontoxic to ATH8 cells (Table II). At 5 μM, however, definite activity is observed. This suggested additional experiments at higher concentrations. Figure 2 shows the results of a typical dose–response experiment. Complete protection without significant toxicity to ATH8 cells was achieved at concentrations between 10 and 100 μM. Complete protection with some toxicity was observed even at 500 μM, the highest concentration evaluated. The difference in activity noted between these results and those of the previous study[11b] is not unusual when different host cell lines are used for anti-HIV evaluation, and is consistent with the well-established fact that different host cell lines can yield antiviral drug potencies differing by over 1000-fold.[22] A similar spectrum of results is obtained utilizing the HIV-infected CEM cell line. Table III shows similar high anti-HIV potencies for **2a** and **2b**, with a somewhat lower potency observed after the addition of a fluorine atom to the 2'-position (**10a**). The difluoro compound proved to be ca. 500 times less potent than the compounds without sugar fluorination, but proved to be completely protective if used at a high enough concentration (Figure 3).

## Experimental Section

5-Chloro-*ara*-C (HPLC internal standard for monkey plasma CDA stability studies), ddU, ddC (**2a**), and d4C (**13**) were obtained from the Drug Synthesis and Chemistry Branch, DTP, DCT, NCI. Compounds **2b**,[6] **3**,[13] **4**,[15] **9a**,[15] and **10a**[16] were prepared as previously described. The parent compounds for **5a–d** and **8a,b** were obtained commercially, as was uridine (HPLC internal standard for acid-stability studies). Rhesus monkey plasma was provided by Dr. David Poplack, Pediatric Oncology Branch, COP, DCT, NCI. Melting points were taken on a Mel-Temp II apparatus and were uncorrected. UV spectra were recorded on a Beckman Model 34 spectrophotometer or on-the-fly during HPLC analysis on a Perkin-Elmer LC-235 diode-array spectrophotometer. Proton NMR spectra were run on a Varian XL-200 spectrometer. Chemical shifts are given in ppm relative to TMS and are referenced against the solvent in which the samples were run. Analytical and preparative TLC analyses were performed on Uniplate GHLF silica gel (Analtech, 250 and 1000 μm, respectively). Moisture-sensitive reactions were run under argon in flasks previously dried at 110 °C. Ether and THF were distilled from sodium/benzophenone ketyl. Silylation reagents were premixed in 1-mL vials (Alltech).

Positive ion fast atom bombardment (FAB) mass spectra were obtained on a VG 7070E mass spectrometer operated at an accelerating voltage of 6 kV and a resolution of 1500. Glycerol and 3-nitrobenzyl alcohol (NBA) were used as sample matrices, and ionization was effected by a beam of xenon atoms derived by charge-exchange neutralization of a 1.0–1.2 mA beam of xenon ions accelerated through 8.4–8.9 kV. Spectra were acquired under the control of a VG 11/250 $J^+$ data system at a scan speed of 10 s/decade, and the matrix background was automatically subtracted.

**General Coupling Procedure of Pyrimidine Bases with 5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-α-D-*threo*-pentofuranosyl Bromide.** This intermediate bromo sugar was generated in situ in every instance from methyl 5-*O*-benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranoside (**4**).[15] A solution of **4** (0.5–1.5 mmol) in $CH_2Cl_2$ (10–15 mL) was treated with an HBr solution in AcOH [33% (4.1 M), 6–8 equiv] and stirred at room temperature for 1 h. The reaction mixture was then concentrated under reduced pressure at room temperature and immediately subjected to high vacuum to remove traces of AcOH. The residue was dissolved in $CH_2Cl_2$ (50 mL), washed with water (3 × 10 mL) and saturated aqueous $NaHCO_3$ (3 × 10 mL), dried ($Na_2SO_4$), and concentrated in vacuo to give ca. a 95% yield of crude bromo sugar. Separately, the pyrimidines (3.5 equiv) were persilylated by stirring a suspension of the base in dry $CH_3CN$ (10 mL) with excess of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 3 mL) at room temperature for 30 min, or until a clear solution was obtained. The solvent and excess reagent were removed under high vacuum, and the residual oil was protected from ambient moisture. The oily residue was immediately dissolved in dry 1,2-dichloroethane (20 mL) and the resulting solution was added to a freshly made solution of bromosugar in dry 1,2-dichloroethane (20 mL). The reaction mixture was then heated at reflux (48 h for uracil analogues and 4 h for cytosine analogues) under a nitrogen atmosphere, cooled, treated with MeOH (10 mL), and filtered through a Celite pad. The filtrate was reduced to dryness at reduced pressure and the residue purified by preparative silica gel TLC (Analtech 2000 μm) with the solvent mixture indicated below to give the desired products, as either amorphous foams or crystalline solids.

**1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (6a).** This compound was obtained as a foam in 49% yield after preparative TLC (5% MeOH in $CH_2Cl_2$) and used directly in the subsequent step: $^1H$ NMR ($CDCl_3$) δ 2.20–2.80 (m, 2 H, H-3'$_{a,b}$), 4.58 (m, 3 H, H-4', H-5'$_{a,b}$), 5.28 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2'), 5.70 (dd, $J_{5,6}$ = 8 Hz, $J_{5,NH}$ = 2 Hz, 1 H, H-5; the $J$ = 2 Hz disappears after $D_2O$ exchange), 6.05 (dd, $J_{1',F}$ = 20 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1'), 7.35–8.10 (m, 6 H, H-6, Ph), 8.18 (br s, 1 H, NH, disappears after $D_2O$ exchange).

**1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-fluorouracil (6b).** This compound was obtained as a solid in 76% yield after preparative TLC (5% MeOH in $CH_2Cl_2$): mp 159–162 °C (MeOH–ether); $^1H$ NMR (MeOH-$d_4$) δ 2.05–2.80 (m, 2 H, H-3'$_{a,b}$), 4.46 (m, 3 H, H-4', H-5'$_{a,b}$), 5.20 (dm, $J_{2',2F}$ = 53 Hz, 1 H, H-2'), 5.95 (ddd, $J_{1',2F}$ = 19 Hz, $J_{1',2'}$ = 3 Hz, $J_{1',5F}$ = 1.6 Hz, 1 H, H-1'), 7.30–8.00 (m, 6 H, H-6, Ph). Anal. ($C_{16}H_{14}F_2N_2O_5$) C, H, N.

**1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-chlorouracil (6c).** This compound was obtained as a solid in 56% yield (64% after recovery of starting material) after preparative TLC (5% MeOH in $CH_2Cl_2$): mp 180–182 °C (MeOH–ether); $^1H$ NMR ($CDCl_3$) δ 2.25–2.85 (m, 2 H, H-3'$_{a,b}$), 4.60 (m, 3 H, H-4', H-5'$_{a,b}$), 5.28 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2'), 6.20 (dd, $J_{1',F}$ = 19 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1'), 7.40–8.15 (m, 6 H, H-6, Ph), 8.48 (br s, 1 H, NH, disappears after $D_2O$ exchange). Anal. ($C_{16}H_{14}ClFN_2O_5 \cdot 0.2H_2O$) C, H, N.

**1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)thymine (6d).** This compound was obtained as a foam in 50% yield after preparative TLC (5% MeOH in $CH_2Cl_2$) and used directly in the subsequent step: $^1H$ NMR ($CDCl_3$) δ 1.85 (s, 3 H, $CH_3$), 2.25–2.85 (m, 2 H, H-3'$_{a,b}$), 4.58 (m, 3 H, H-4', H-5'$_{a,b}$), 5.25 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2'), 6.06 (dd, $J_{1',F}$ = 20 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1'), 7.40–8.15 (m, 6 H, H-6, Ph), 8.22 (br s, 1 H, NH, disappears after $D_2O$ exchange).

**1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-fluorocytosine (9b).** This compound was obtained as a solid in 66% yield after preparative TLC (10% MeOH in $CH_2Cl_2$): mp 163–166 °C (EtOAc–ether) [lit.[11b] mp 165–167 °C]; $^1H$ NMR (MeOH-$d_4$) δ 2.10–2.80 (m, 2 H, H-3'$_{a,b}$), 4.50 (m, 3 H, H-4', H-5'$_{a,b}$), 5.20 (dm, $J_{2',2F}$ = 53 Hz, 1 H, H-2'), 5.94 (ddd, $J_{1',2F}$ = 19 Hz, $J_{1',2'}$ = 2.7 Hz, $J_{1',5F}$ = 1.7 Hz, H-1'), 7.30–8.00 (m, 6 H, H-6, Ph). Anal. ($C_{16}H_{15}F_2N_3O_4 \cdot 0.5H_2O$) C, H, N. Traces of another isomer, presumably the α-anomer ($R_f$ = 0.40), also were detected but not isolated.

**General Deblocking Procedure.** A solution of the protected nucleoside (0.1–0.4 mmol) in saturated methanolic ammonia (20 mL) was stirred in a pressure bottle at room temperature for 16–24 h. The solvent was removed under vacuum, and the residue was

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purified by preparative silica gel TLC (Analtech, 2000 μm) to give the desired final compounds.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (7a).** This compound was obtained from **6a** as a solid in 58% yield after preparative TLC (10% MeOH in $CH_2Cl_2$): mp 157–159 °C (MeOH–ether) [lit.[11b] mp 159–160 °C]; $^1H$ NMR ($D_2O$) δ 2.00 (m, 1 H, H-3′$_a$), 2.40 (m, 1 H, H-3′$_b$), 3.60 (m, 2 H, H-5′$_{a,b}$), 4.20 (m, 1 H, H-4′), 5.20 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2′), 5.70 (d, $J_{5,6}$ = 8 Hz, 1 H, H-5), 5.95 (dd, $J_{1',F}$ = 19 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1′), 7.70 (dd, $J_{5,6}$ = 8 Hz, $J_{6,F}$ = 2 Hz, 1 H, H-6); MS (FAB, glycerol) *m/z* (rel inten) 231 ($MH^+$, 67), 113 (b + 2 H, 100). Anal. ($C_9H_{11}FN_2O_4$) C, H, N.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-fluorouracil (7b).** This compound was obtained from **6b** as a solid in 61% yield after preparative TLC (10% MeOH in $CH_2Cl_2$): mp 156–158 °C (MeOH–ether); $^1H$ NMR ($D_2O$) δ 2.00 (m, 1 H, H-3′$_a$), 2.45 (m, 1 H, H-3′$_b$), 3.62 (m, 2 H, H-5′$_{a,b}$), 4.20 (m, 1 H, H-4′), 5.20 (dm, $J_{2',2'F}$ = 53 Hz, 1 H, H-2′), 5.94 (dm, $J_{1',2'F}$ = 18 Hz, 1 H, H-1′), 7.86 (dd, $J_{6,5F}$ = 7 Hz, $J_{6,2'F}$ = 2 Hz, 1 H, H-6); MS (FAB, glycerol) *m/z* (rel inten) 249 ($MH^+$, 100), 131 (b + 2 H, 51). Anal. ($C_9H_{10}F_2N_2O_4 \cdot 0.25H_2O$) C, H, N.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-chlorouracil (7c).** This compound was obtained from **6c** as a solid in 65% yield after preparative TLC (10% MeOH in $Cl_2Cl_2$): mp 167–168 °C (MeOH–ether); $^1H$ NMR ($D_2O$) δ 2.00 (m, 1 H, H-3′$_a$), 2.45 (m, 1 H, H-3′$_b$), 3.62 (m, 2 H, H-5′$_{a,b}$), 4.19 (m, 1 H, H-4′), 5.20 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2′), 5.94 (dd, $J_{1',F}$ = 18 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1′), 7.97 (d, $J_{6,F}$ = 2 Hz, 1 H, H-6); MS (FAB, NBA) *m/z* (rel inten) 265 ($MH^+$, 100), 147 (b + 2 H, 64). Anal. ($C_9H_{10}FClN_2O_4 \cdot 0.5H_2O$) C, H, N.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)thymine (7d).** This compound was obtained from **6d** as a solid in 67% yield after preparative TLC (10% MeOH in $CH_2Cl_2$): mp 163–165 °C (MeOH–ether) [lit.[11d] mp 162–164 °C]; $^1H$ NMR ($D_2O$) δ 1.78 (s, 3 H, C*H*$_3$), 2.00 (m, 1 H, H-3′$_a$), 2.45 (m, 1 H, H-3′$_b$), 3.65 (m, 2 H, H-5′$_{a,b}$), 4.20 (m, 1 H, H-4′), 5.20 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2′), 5.96 (dd, $J_{1',F}$ = 19 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1′), 7.56 (d, $J_{6,F}$ = 1 Hz, 1 H, H-6); MS (FAB, glycerol) *m/z* (rel inten) 245 ($MH^+$, 100), 127 (b + 2 H, 62). Anal. ($C_{10}H_{13}FN_2O_4$) C, H, N.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-fluorocytosine (10b).** This compound was obtained from **9b** as a tan solid in 98% yield after preparative TLC (25% MeOH in $CH_2Cl_2$): mp 135–138 °C (MeOH–ether) [lit.[11b] mp 195–196 °C]; $^1H$ NMR ($D_2O$) δ 2.00 (m, 1 H, H-3′$_a$), 2.50 (m, 1 H, H-3′$_b$), 3.65 (m, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.25 (dm, $J_{2',2'F}$ = 53 Hz, 1 H, H-2′), 5.92 (dm, $J_{1',2'F}$ = 18 Hz, 1 H, H-1′), 7.83 (dd, $J_{6,5F}$ = 6.5 Hz, $J_{6,2'F}$ = 1.5 Hz, 1 H, H-6) (a small triplet and quartet at δ 1.05 and 3.42, respectively, corresponded to traces of ethyl ether used for recrystallization; this solvent remains tenaciously attached to the material despite prolonged heating under vacuum. This phenomenon might explain the melting point discrepancy); MS (FAB, glycerol) *m/z* (rel inten) 248 ($MH^+$, 100), 130 (b + 2 H, 52). Anal. ($C_9H_{11}F_2N_3O_3 \cdot 0.8H_2O$) C, H, N.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-5-chlorocytosine (11).** A solution of **6c** (0.180 g, 0.488 mmol) in dry pyridine (10 mL) was treated with a premixed solution of $POCl_3$ (92 μL, 0.98 mmol) and 1,2,4-triazole (0.270 g, 3.91 mmol) in 15 mL of dry pyridine. The reaction mixture was stirred at room temperature for 3 h. Volatiles were removed in vacuo, and the residue was dissolved in $CH_2Cl_2$, washed with water (2 × 50 mL), and dried ($Na_2SO_4$). The solvent was evaporated to give crude triazole derivative as a brown foam. This material was transferred to a pressure bottle and stirred for 16 h in the presence of saturated methanolic ammonia (40 mL). After removal of the volatiles under reduced pressure, the residue was dissolved in a 3:1 mixture of $CH_2Cl_2$–MeOH, filtered through a short pad of Celite to remove inorganic salts, and again reduced to dryness. Purification of the residue by preparative TLC (Analtech 2000 μm; 15% MeOH in $CH_2Cl_2$) gave 0.082 g (67%) of **11** as a white foam ($R_f$ = 0.32). Crystallization from MeOH–acetone–ether produced a white crystalline solid, mp 180–182 °C; $^1H$ NMR ($D_2O$) δ 2.00 (m, 1 H, H-3′$_a$), 2.40 (m, 1 H, H-3′$_b$), 3.62 (m, 2 H, H-5′$_{a,b}$), 4.20 (m, 1 H, H-4′), 5.20 (dm, $J_{2',F}$ = 53 Hz, 1 H, H-2′), 5.90 (dd, $J_{1',F}$ = 18 Hz, $J_{1',2'}$ = 3 Hz, 1 H, H-1′), 7.92 (d, $J_{6,F}$ = 1.5 Hz, 1 H, H-6); MS (FAB, NBA) *m/z* (rel inten) 264 ($MH^+$, 100), 146 (b + 2 H, 53). Anal. ($C_9H_{11}ClFN_3O_3 \cdot 0.5H_2O$) C, H, N. A second band ($R_f$ = 0.49) provided an amorphous solid in 10% yield which was confirmed to be identical to **7c**.

**1-(5-*O*-Benzoyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)pyrimidin-2(1*H*)-one (12a).** Compound **4** (105 mg, 0.39 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and treated with 0.6 mL of a 32% solution of HBr in acetic acid at room temperature for 12 h. Argon was bubbled through the solution for 10 min, and the solvent was concentrated. The residue was taken up in water and extracted with $CH_2Cl_2$ (3×). The combined extracts were washed with water, dried ($Na_2SO_4$), and concentrated. 1,2-Dihydro-2-oxopyrimidine (100 mg, 1.04 mmol) was suspended in $CH_3CN$ (5 mL) and a 1-mL ampoule of BSTFA was added by syringe. After stirring for 30 min, the solvent was removed in vacuo, and the residue was dissolved in 1,2-dichloroethane (5 mL). The crude bromosugar was dissolved in 5 mL of $ClCH_2CH_2Cl$ and added to the silylated base via canula (argon pressure). This mixture was refluxed for 12 h, and the solvent was concentrated. The crude reaction mixture was dissolved in $CH_2Cl_2$, and the particulates were filtered through Celite. After evaporation, the residue was purified by silica chromatography ($CH_2Cl_2$–2% MeOH/$CH_2Cl_2$) affording 60 mg of product (46%). An analytically pure sample was obtained by HPLC on Partisil 10 (50-cm column, 5% MeOH–$CH_2Cl_2$, eluant): $^1H$ NMR ($CDCl_3$) δ 8.59 (dd, *J* = 3.0, 3.9 Hz, H4), 8.00–8.12 (m, H6 and benzoyl), 7.39–7.61 (m, benzoyl), 6.35 (dd, *J* = 4.0, 6.8 Hz, H5), 6.08 (dd, *J* = 2.6 and 19.4 Hz, H1′), 5.49 (dm, $^2J_{H,F}$ = 53.1 Hz, H2′), 4.66 (m, H4′), 4.54 (center of AB multiplet of an ABX system, H5′, 5″), 2.62 (m, H3′), 2.37 (m, 3″). Anal. ($C_{16}H_{15}FN_2O_4 \cdot 0.5H_2O$) C, H, N.

**1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)pyrimidin-2(1*H*)-one (12b).** Compound **12a** (50 mg, 0.157 mmol) was treated with ammonia-saturated methanol in a pressure bottle at 0 °C for 5 h. Argon was bubbled through the solution to evaporate the methanol. The residue was taken up in water and washed with $CH_2Cl_2$ (3×). The layers were separated, and the aqueous portion was lyophilized to an oil. Purification by HPLC on C18 using 7% $CH_3CN/H_2O$ as eluant afforded pure material (51% yield): $^1H$ NMR ($D_2O$) δ 8.50 (dd, *J* = 2.7, 4.4 Hz, H4), 8.30 (m, H6), 6.62 (dd, *J* = 4.4, 6.7 Hz, H5), 6.00 (dd, *J* = 3.2, 17.7 Hz, H1′), 5.36 (dm, $^2J_{H,F}$ = 53.5 Hz, H2′), 4.32 (m, H4′), 3.53–3.71 (m, H5′, H5″), MS (FAB) *m/z* 215 ($MH^+$). Anal. ($C_9H_{11}FN_2O_3$) C, H, N.

**Acid Stability of Cytosine Dideoxynucleosides.** Dideoxynucleosides were weighed (0.14–0.22 mg) and transferred directly to 20-mL glass scintillation vials, and an appropriate amount (0.12–0.28 mg) of uridine was added to each vial as an internal standard. The nucleoside and internal standard were dissolved in 10 mL of 0.1 N HCl (pH 1.1) at 37 °C. A 0.5-mL aliquot was immediately removed for analysis, and the remainder incubated at 37 °C in a Dubnoff metabolic shaking incubator. The initial sample was added to 0.5 mL of chilled 0.1 N NaOH and vortexed to neutralize the HCl and to terminate any hydrolysis. Subsequent 0.5-mL samples were taken at predetermined times and treated in the same manner as the first sample. The concentration of cytosine and remaining nucleoside in each sample was determined by HPLC analysis of a 50-μL aliquot (see below).

**Compound Stability to CDA. Isolated Enzyme.** Partially purified mouse kidney cytidine deaminase (CDA) was prepared from 4.7 g of kidneys from female CD2F-1 mice according to published procedures.[23,24] The resultant pH 7.0, 0.01 M phosphate buffer solution of partially purified enzyme had an activity of 0.10 units/mL (a unit of CDA activity is the amount of enzyme that deaminates cytidine to uridine at a rate of 1 μmol/min at 25 °C and pH 7.0) or 0.012 μmol/min per mg of protein. Cytidine and the dideoxycytidine analogues **2a**, **2b**, **10a**, **10b**, and **11** were evaluated as substrates for mouse kidney cytidine deaminase using

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0.80 mL of pH 7.0, 0.01 M phosphate buffer and 0.20 mL of partially purified CDA. These were mixed in a 1.5-mL Eppendorf microfuge tube and equilibrated at 37 °C in a Thermistat Model 5320 block heater. The equilibrated solution was spiked with the amount of concentrated buffer–substrate solution required to give a 50 μM final concentration. After vortexing to insure homogeneity, 50-μL aliquots of the CDA incubation mixture were removed as a function of time (0, 4, 15, 19, and 23 h for the dideoxynucleosides). These aliquots were diluted with 0.40 mL of pH 7.0 phosphate buffer, and the CDA was inhibited with 50 μL of aqueous 1 mM tetrahydrouridine. The extent of substrate conversion and product formation was evaluated by isocratic HPLC analysis (see below) of a 100-μL aliquot of the diluted reaction mixture.

**Monkey Plasma.** Two 5-mL aliquots of rhesus monkey plasma were warmed to 37 °C in a Dubnoff metabolic shaking incubator. To one aliquot was added 50 μL of ddC (**2a**) in DMSO (0.49 μg/μL) and to the other, 100 μL of **10a** in DMSO (0.27 μg/μL). After mixing, a 0.50-mL aliquot was taken from each sample and diluted 1:1 with distilled water. Twenty microliters of 1-β-D-arabinofuranosyl-5-chlorocytosine in DMSO (96.6 μg/mL) was added to each sample as an internal standard. The diluted plasma was then ultrafiltered in an Amicon Centrifree micropartition unit to remove protein and centrifuged at 1000*g* in a high-speed centrifuge. Subsequent 0.5-mL aliquots were removed from each of the two incubating plasma samples at 2, 4, 6, 22, and 28 h and treated in the same manner. The concentration of dideoxynucleoside remaining was determined by HPLC analysis (see below) of a 50-μL aliquot of the ultrafiltrate.

**Chromatography of Pyrimidine Dideoxynucleosides.** A 4.6 × 250 mm 5-μm Ultrasphere-ODS column protected by a 4.6 × 30 Brownlee Spheri-5 RP-18 precolumn was eluted with 4% $CH_3CN$ in 0.01 M, pH 6.8 phosphate buffer at a flow rate of 1.0 mL/min. For partition coefficient determination (see below) of individual compounds, the same analytical column without a guard column and the same mobile phase were used. For determination of the partition coefficients of the components of dideoxynucleoside mixtures, a 12-min linear gradient of 2–14% $CH_3CN$ in 0.01 M, pH 6.8 phosphate buffer was employed. A 50-μL sample was injected and the dideoxynucleosides were detected at either 270 or 273 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. Peak areas were measured with a Spectra-Physics SP4400 Chromjet computing integrator. For kinetic studies, these data were plotted as a function of time and, where possible, fitted to either first-order decomposition ($y = Ae^{-kt}$) or to first-order formation [$y = A(1 - e^{-kt})$] using GraphPad, a PC-based curve-fitting program. Data points were weighted by the function $1/x^2$ for this nonlinear regression analysis.

**Determination of Octanol–Water Partition Coefficients.** 1-Octanol–water partition coefficients (*P*) were determined for individual compounds or for mixture components by a microscale shake-flask method employing HPLC analysis of both the buffer and 1-octanol phases.[25] A 20-μL aliquot of a 0.5 mg/mL DMSO or pH 7.0 buffer solution of the dideoxynucleoside was dissolved in 1.0 mL of octanol which had been saturated with pH 7.0, 0.01 M potassium phosphate buffer. This solution was mixed thoroughly with 1.0 mL of buffer saturated with 1-octanol in a 2-mL Mixxor apparatus at 24–26 °C. After standing for 15 min, the phases were separated, centrifuged at 600*g* for 5 min, and the relative concentration of the nucleoside in each phase determined by HPLC analysis of a 50-μL aliquot. The partition coefficient was calculated by dividing the absolute area of the appropriate integrated peak from the octanol phase by that of the area from the buffer phase.

**HIV Cytopathic Effect Inhibition Assays. In ATH8 Cells.** The HIV cytopathic effect inhibition assay was performed as previously described.[9] In summary, 200 000 target $CD4^+$ ATH8 cells were exposed to cell-free HIV-$1/III_B$ at a dose of 1000 $TCID_{50}$ (50% tissue culture infectious dose) for 1 h, resuspended in 2 mL of fresh culture media containing interleukin 2, and cultured at 37 °C with or without test compounds in 5% $CO_2$-containing humidified air. On day 8 in culture, the viable cells were counted by using the dye-exclusion method. Compounds found to be active were evaluated 2–4 times in separate experiments. Data reported are from representative tests. The percent protection against the virus was determined by the following formula: 100 × [[(the number of viable cells exposed to HIV-1 and cultured in the presence of the compound) − (the number of viable cells exposed to HIV-1 and cultured in the absence of compound)]/[(the number of viable cells cultured alone) − (the number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]]. The percent toxicity of a compound on the target cells was determined by the following formula: 100 × [1 − (the number of total viable cells cultured in the presence of the compound)/(the number of total viable cells cultured alone)]. Calculated percentages equal to or less than zero are expressed as 0%.

**In CEM Cells.** This procedure was conducted by the anti-HIV drug testing program of the National Cancer Institute and has been described previously.[26] Briefly, the candidate drug is dissolved in DMSO, diluted with culture medium, and added to HIV-infected T4+ CEM lymphocytes uzing AZT as a positive control. Uninfected cells treated with drug and infected cells with and without drug are used as controls. After incubation at 37 °C in a 5% $CO_2$ atmosphere for 6 days, the XTT assay is utilized to determine activity.

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