addition of the tested analogue. The reciprocal of membrane resistance in chloride-containing solution was assumed to be total membrane conductance (G_m) and the same parameter measured in chloride-free solution was considered largely potassium conductance (G_K). The mean chloride conductance G_{Cl} of each experimental group of fibers was estimated as the mean G_m minus the mean G_K. Sodium and other small conductances were neglected. These determinations were made in several fibers of different preparations at three or more concentrations of each examined analogue in order to construct the dose-response curves for G_{Cl}. Moreover, for each compound the resultant conductances vs. concentration curves were fit to a single site binding equation with a nonlinear least-squares method from which the $IC_{50} \pm SD$ (concentration required for half-maximal G_{Cl} block ± standard deviation) was determined. The excitability characteristics of the sampled fibers were determined intracellularly[15] at a different concentration of each compound by observing the intracellular membrane potential response recorded from one microelectrode to a square-wave constant-current delivered by a second microelectrode inserted within 100 μm from the voltage electrode. In each fiber the membrane potential was set by a steady holding current to −80 mV, before passing the depolarizing pulses.

For in vivo studies some of the tested compounds, thoroughly mixed in a bolus, were administrated in a single dose to different groups of rats. Electromyographic recordings were performed at different times, allowing sufficient time for the substance to distribute and for the effect to develop fully. Data are expressed as means ± SEM. Significance of differences between group means was calculated by the Student's t test. The estimates for SEM of G_{Cl} were obtained from the variances of G_m and G_K, assuming no covariance, by standard methods.[17] Standard deviations for the IC_{50} values were calculated from the variance-covariance matrix obtained during nonlinear fitting procedures.

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3′-Substituted 2′,3′-Dideoxynucleoside Analogues as Potential Anti-HIV (HTLV-III/LAV) Agents†

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A series of 2′,3′-unsaturated and 3′-substituted 2′,3′-dideoxynucleoside analogues of purines and pyrimidines have been synthesized and evaluated for their inhibitory activity against human immunodeficiency virus (HIV). The 2′,3′-unsaturated analogues of 2′,3′-dideoxycytidine (ddeCyd) and 2′,3′-dideoxythymidine (ddeThd), 3′-azido-2′,3′-dideoxythymidine (AzddThd), 3′-fluoro-2′,3′-dideoxythymidine, 2′,3′-dideoxycytidine (ddCyd), and 2′,3′-dideoxyadenosine (ddAdo) emerged as the most potent inhibitors of HIV-induced cytopathogenicity in the human T lymphocyte cell lines ATH8 and MT4. In ATH8 cells ddCyd, ddeCyd, and ddAdo had the highest therapeutic index whereas in MT4 cells AzddThd, ddThd, ddCyd, and ddAdo were the most selective. Derivatives from ddThd in which the substituent group was linked to the 3′-carbon atom via a thio, sulfonyl, or oxygen bridge were far less inhibitory to HIV than was AzddThd.

Acquired immunodeficiency syndrome (AIDS) is an immunosuppressive disease characterized by an immune impairment associated with life-threatening opportunistic infections and a high susceptibility to unusual forms of certain neoplasms (i.e., Kaposi's sarcoma).[1-3] Human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), recently designated as human immunodeficiency virus (HIV),[4] has been recognized as the etiologic agent of AIDS.[5,6] Several compounds with different chemical structures (i.e., Suramin,[7-9] Evans Blue,[9] aurintricarboxylic acid,[10] HPA-23,[11,12] phosphonoformic acid,[9,13] ribavirin,[14] interferon-α,[15] AL-721,[16] and 2′,3′-dideoxyribonucleosides[17-20]) have been reported as having significant inhibitory effect against HIV in vitro. Of these compounds, 2′,3′-dideoxyribonucleosides have thus far proven to be the most potent antiretroviral agents in vitro.[17-20] Indeed, Mitsuya and co-workers reported that 3′-azido-2′,3′-dideoxythymidine (AzddThd) protected human T cells (clone ATH8) against the cytopathogenic effect of HIV at 1–5 μM,[17] and 2′,3′-dideoxycytidine (ddCyd) completely suppressed HIV-induced cytopathogenicity in vitro at 0.5 μM.[18] The 2′,3′-unsaturated analogue of ddCyd, ddeCyd, proved equally effective as ddCyd in protecting ATH8 cells against HIV,[19] while ddeThd, the 2′,3′-unsaturated analogue of 2′,3′-dideoxythymidine (ddThd), was

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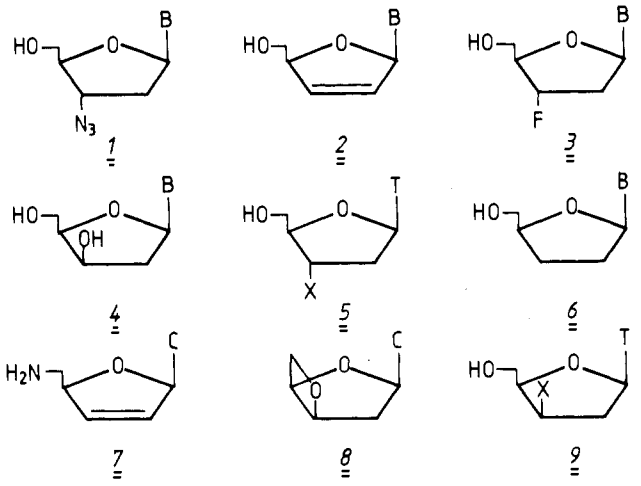
Scheme I



a, thymin-1-yl (T)
b, cytosin-1-yl (C)
c, adenin-9-yl (A)
d, uracil-1-yl (U)
e, guanin-9-yl (G)
f, 5-methylcytosin-1-yl
g, 5-ethyluracil-1-yl
h, N^4,5-dimethylcytosin-1-yl
i, 5-fluorouracil-1-yl
j, 5-iodouracil-1-yl
k, N_3
l, Cl
m, Br
n, I
o, OMe
p, OEt
q, OCH_2COONa
r, OSO_2CH_3
s, SEt
t, SCH_2CH_2OH
u, SCN

also found to be a potent and selective inhibitor of HIV.[20] Recently, Lin et al. reported on the antiviral activity of various 3′-azido, 3′-amino, 2′,3′-unsaturated, and 2′,3′-dideoxy analogues of pyrimidine deoxyribonucleosides against retroviruses.[21] From their studies, AzddThd, 5-bromo- and 5-iodo-3′-azido-2′,3′-dideoxyuridine, ddeThd, ddeCyd, and ddCyd emerged as the most active agents against Moloney murine leukemia virus.

The present study describes the synthesis of a series of nucleoside analogues and their anti-HIV activity, with two different target cell lines (ATH8 and MT4) for infection. From this study, several 2′,3′-dideoxythymidine and 2′,3′-dideoxycytidine derivatives emerged as potent and selective inhibitors of HIV-induced cytopathogenicity.

Chemistry

3′-*O*-Methylthymidine (**5o**) and 3′-[(2-hydroxyethyl)-thio]-3′-deoxythymidine (**5t**) were synthesized by using the same modus operandi as described for the synthesis of **5p**[22] and **5s**,[23] respectively (Scheme I).

Although 9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine (**1c**) was previously synthesized by Imazawa[24] via a transglycosylation procedure, an alternative procedure was used here. The starting material for the synthesis of **1c**, 9-[2-deoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine, was synthesized by monomethoxytritylation of **4c**.[25] However, difficulties were obtained during the workup procedure when the synthesis of **4c** from 2′-*O*-tosyladenosine[25] was carried out in gram amounts. Therefore, we protected the 5′-hydroxyl function of 2′-*O*-tosyladenosine[26] by monomethoxytritylation (75%) before the product was subjected to the rearrangement conditions[25] (78%). Mesylation of 5′-protected **4c** at the 3′-position, followed by reaction with sodium azide in dimethylformamide and deprotection with *p*-toluenesulfonic acid, afforded the 3′-azido compound **1c** in satisfactory yield.

1-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)thymine (**9k**) was obtained by 5′-deprotection with acetic acid of 1-(3-azido-2,3-dideoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine.[27] However, careful examination of the TLC (EtOAc) revealed 3′-azido-3′-deoxythymidine (**1a**) as a minor impurity. Both compounds can be separated by column chromatography with EtOAc as eluent.

3′-Thiocyanato-3′-deoxythymidine (**5u**) was synthesized by nucleophilic displacement of the mesyloxy group of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] with potassium thiocyanate, followed by deprotection with acetic acid. 3′-Deoxy-2′-thymidinene (**2a**) was isolated as the major compound in the reaction of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] with tetrabutylammonium fluoride in tetrahydrofuran, followed by detritylation. Reaction of 5′-*O*-trityl-3′-azido-3′-deoxythymidine[28] with 1-methylimidazole in the presence of phosphoryl chloride,[29] followed by a workup procedure with ammonia or methylamine and detritylation, afforded the 4-substituted 3′-azido-3′-deoxythymidine analogues **1f** and **1h**, respectively. The synthesis of **1f** has been previously described;[30] in our procedure it was isolated as the HCl salt.

5-Ethyl-3′-azido-2′,3′-dideoxyuridine (**1g**) was synthesized from 5-ethyl-2′-deoxyuridine[31] according to a classical reaction sequence.[28,37] After protecting of the 5′-hydroxy

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Table I. ^{1}H NMR[a] and ^{13}C NMR[b] Spectral Data of 3′-Fluoro-2′,3′-dideoxynucleosides

	H-1′[c]	$J_{1',2'}$, $J_{1',2''}$	H-2′, H-2″	H-3′	$J_{3',F}$	H-4′[d]	$J_{4',F}$	H-5′, H-5″
3a	6.22	7.9, 6.8	1.94–2.64	5.44	53.6	4.16	27.7	3.64
3g	6.21	7.9, 6.7	2.03–2.68	5.30	53.6	4.16	27.7	3.64
3b	6.25	8.6, 5.9	1.75–2.54	5.27	54.3	4.15	27.0	3.58
3c	6.39	9.0, 5.7	2.50–3.30	5.44	53.4	4.25	26.8	3.62
	C-1′	C-2′	$J_{C2',F}$	C-3′	$J_{C3',F}$	C-4′	$J_{C4',F}$	C-5′	$J_{C5',F}$
3a	83.9	37.0	19.5	94.8	173.3	84.9	22.0	60.9	11.0
3g	83.9	36.9	19.5	94.8	173.3	84.8	22.0	60.8	11.0
3b	85.1	37.7	20.75	94.9	173.3	84.7	22.0	60.9	11.0
3c	84.2	36.9	20.8	95.1	173.3	85.6	22.6	61.2	11.0

[a] All ^{1}H NMR spectra were taken in Me_2SO-d_6 with Me_4Si as internal standard; chemical shifts in δ values (ppm); coupling constants in hertz. [b] All ^{13}C NMR spectra were taken in Me_2SO-d_6, which was used as internal standard (39.6 ppm); coupling constants in hertz. [c] H-1′ appears as a double doublet. [d] H-4′ appears as a doublet of broad triplets.

group by tritylation, the 3′-hydroxyl group of 5-ethyl-2′-deoxyuridine was mesylated with mesyl chloride in pyridine. Reversal of the configuration at the 3′-carbon atom was performed by refluxing in the presence of sodium hydroxide. This reaction proceeded via a 2,3′-anhydro intermediate. Mesylation of the 3′-hydroxyl group and nucleophilic displacement with azide, followed by detritylation, afforded 5-ethyl-3′-azido-2′,3′-dideoxyuridine in a total yield of 71% (starting from 5-ethyl-2′-deoxyuridine). 5-Ethyl-2′,3′-dideoxyuridine (**6g**) was synthesized from 5′-*O*-benzoyl-5-ethyl-2′-deoxyuridine according to a reaction sequence described by Prisbe.[32]

As mentioned before, when a solution of 1-(2,3-dideoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine in tetrahydrofuran was treated with tetrabutylammonium fluoride (TBAF) at room temperature, 5′-*O*-trityl-3′-deoxy-2′-thymidinene was formed as the major compound, with 5′-*O*-trityl-3′-fluoro-3′-deoxythymidine as the minor compound. After detritylation with 80% acetic acid, only 5% of 3′-fluoro-3′-deoxythymidine was isolated. The synthesis of 3′-fluoro-3′-deoxythymidine (**3a**) has been previously reported by Langen et al.[33,34] They described the opening of the 2,3′-anhydro bond of 2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine with HF–AlF_3 (28% yield) and the reaction of 3′-*O*-mesylthymidine with KHF_2[34] (14% yield). The same authors also synthesized 3′-deoxy-3′-fluorothymidine from 2,3′-anhydro-1-(2-deoxy-5-*O*-mesyl-β-D-*threo*-pentofuranosyl)thymine in a two-step procedure (total yield 28%).[33]

However, we wanted to investigate a more general procedure for the synthesis of 3′-fluoro-2′,3′-dideoxynucleosides, which would also be applicable to purine nucleosides. Therefore, the fluorinating agent (diethylamino)sulfurtrifluoride (DAST)[35] was reacted with four different 2′-deoxynucleosides. In all cases, the desired compounds were obtained in good to moderate yield. The reaction of 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[36,37] with DAST in benzene–tetrahydrofuran gave 5′-*O*-trityl-3′-fluoro-3′-deoxythymidine in very good yield. Some destruction took place during the detritylation procedure, and the final yield of 3′-fluoro-3′-deoxythymidine (**3a**) was 62%. Only 3% of 3′-deoxy-2′-thymidinene could be isolated. The ratio of the amounts of **3a** and **2a**, obtained in the reaction with DAST, was opposite to the ratio obtained in the reaction with TBAF. The same reaction sequence (DAST in benzene, 80% HOAc) applied on 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-5-ethyluracil yielded 65% of 3′-fluoro-2′,3′-dideoxy-5-ethyluridine **3g**.

The reaction of 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)cytosine with DAST was less straightforward. 5′-*O*-Trityl-3′-fluoro-2′,3′-dideoxycytidine was obtained only in 36% yield. When the heterocyclic base was protected at N^4 with a benzoyl group, no significant amount of the 3′-fluoro compound could be isolated. 2′,3′-Dideoxy-3′-fluorocytidine **3b** has been previously synthesized from 2′,3′-dideoxy-3′-fluorouridine via a multistep sequence in low yield.[33]

The purine nucleoside 9-[2,3-dideoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine was treated with DAST in CH_2Cl_2. From this reaction mixture was isolated the 3′-fluoro compound **3c** in 52% yield. Although some detritylation was observed, this reaction showed the usefulness of DAST in the presence of acid-labile groups (glycosidic bound and monomethoxytrityl ether). The observed detritylation may be partly responsible for the lower yield compared to that in the reaction with the uridine analogues (protected with a trityl group in place of a monomethoxytrityl group). Deprotection of the 5-hydroxyl group with 2% *p*-toluenesulfonic acid in dichloromethane–methanol gave 3′-fluoro-2′,3′-dideoxyadenosine **3c** in 62% yield.

The ^{1}H NMR and ^{13}C NMR spectral data of the fluoro compounds are given in Table I. The almost identical chemical shifts and coupling constants obtained for the different compounds suggest that they possess a similar configuration.

Previously published methods were used to synthesize the following sugar-modified nucleosides: 3′-azido-3′-deoxythymidine[28] (**1a**), 3′-chloro-3′-deoxythymidine[38] (**5l**), 3′-bromo-3′-deoxythymidine[39,40] (**5m**), 3′-iodo-3′-deoxythymidine[41,42] (**5n**), 3′-(ethylthio)-3′-deoxythymidine[23] (**5s**), 3′-*O*-ethylthymidine[22] (**5p**), 3′-*O*-(carboxymethyl)thymidine[43,44] (**5q**), 3′-*O*-mesylthymidine[39] (**5r**), 1-(2-deoxy-3-*O*-mesyl-β-D-*threo*-pentofuranosyl)thymine[28] (**9r**), 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine[25] (**4c**), 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine[36] (**4a**), 1-(2-deoxy-β-D-*threo*-pentofuranosyl)cytosine[45] (**4b**), 2′,3′-dideoxy-2′-cytidinene[46] (**2b**), 2′,3′-dideoxy-2′-uridinene[47] (**2d**),

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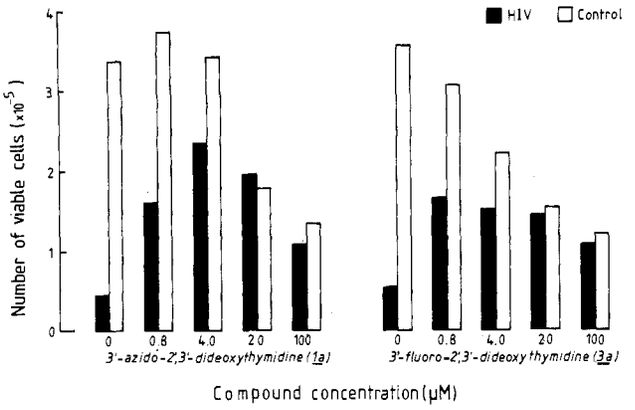

Figure 1. Inhibition of the cytopathogenicity of HIV for ATH8 cells by AzddThd and 3′-fluoro-ddThd. Viability of the cells was measured after an incubation period of 7 days (mock-infected cells incubated in the presence of different concentrations of the test compounds, □; HIV-infected cells incubated in the presence of different concentrations of the test compounds, ■).

2′,3′-dideoxy-2′-adenosinene[48] (**2c**), 2′,3′-dideoxyuridine[47] (**6d**), 1-(2-deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)cytosine[46] (**8**), 5′-amino-2′,3′,5′-trideoxy-2′-cytidinene[49,50] (**7**), and 2′,3′-dideoxythymidine[47] (**6a**).

Antiviral Activity

The compounds evaluated for their anti-HIV effect can mainly be divided into five different structural classes: (i) 3′-substituted 2′,3′-dideoxythymidine analogues; (ii) 2′,3′-dideoxycytidine analogues, modified in the sugar and/or base moiety; (iii) 2′-deoxyxylo pyrimidine and 2′-deoxyxylo purine analogues; (iv) 3′-substituted and unsubstituted 5-ethyl-2′,3′-dideoxyuridine analogues; and (v) 3′-substituted 2′,3′-dideoxyadenosine analogues. Included in each class of compounds were the parental unmodified 2′,3′-dideoxyribonucleosides and the corresponding 2′,3′-didehydro-2′,3′-dideoxyribonucleosides. Mitsuya and Broder, using an immortalized T cell line (ATH8) that is highly sensitive to the infectivity of HIV, found that various 2′,3′-dideoxynucleosides (i.e., ddCyd, ddThd, ddAdo, ddIno, ddGuo), akin to the previously reported 3′-azido-ddThd (AzddThd),[17] inhibited both the cytopathogenicity and replication of HIV at doses that were not toxic to the host cells.[18] Among the 3′-substituted ddThd analogues reported in this paper, only 3′-fluoro (**3a**) and 3′-azido-ddThd (**1a**) were highly effective in protecting ATH8 cells against the cytopathic effect of HIV (Table II, Figure 1). However, **3a** was more cytotoxic to the cells than AzddThd, and its in vitro therapeutic index, expressed as the ratio of compound concentration required to reduce the cell viability of normal uninfected ATH8 cells by 50% (ID_{50}) to the compound concentration required to achieve 50% protection of ATH8 cells against HIV (ED_{50}), was somewhat lower than that of AzddThd (10 and 19, respectively). None of the other 3′-halogeno derivatives of ddThd [i.e., 3′-chloro-, 3′-bromo-, 3′-iodo-ddThd (compounds **5l**, **5m**, **5n**)] had a significant, if any, inhibitory effect against HIV (ED_{50} > 100 μM). Also, substituents linked to the 3′-carbon of the sugar moiety of ddThd via a thio (i.e., **5s**, **5t**, **5u**), sulfonyl (**5r**), or oxygen (i.e., **5o**, **5p**, **5q**) bridge did not afford products capable of protecting ATH8 cells against HIV at concentrations lower than 100 μM. In contrast, the 2′,3′-unsaturated derivative of ddThd, ddeThd (**2a**), proved markedly effective in inhibiting HIV-induced cytopathogenicity in ATH8 cells. Its ED_{50} was comparable to that of AzddThd (4.1 μM and 2.4 μM, respectively), but it was less toxic for ATH8 cells than AzddThd (ID_{50} = 110 μM and 45 μM, respectively), which makes ddeThd a valuable candidate for further examination as a potential anti-HIV drug in vivo.

Within the class of the ddCyd analogues, the 2′,3′-unsaturated derivative of ddCyd, ddeCyd (**2b**), proved, like ddCyd itself, very effective as an inhibitor of HIV in vitro. Its effective antiviral dose was 0.30 μM, and its cytotoxic dose was 30 μM. More detailed investigations revealed that both compounds had comparable antiviral, antimetabolic, and cytostatic properties. 3′-Azido-5-methyl-ddCyd (**1f**) was totally devoid of anti-HIV activity when evaluated in the ATH8 cell system. However, deamination of **1f** at the nucleoside level by Cyd/dCyd deaminase or at the nucleotide level by dCMP deaminase should convert this compound directly to the antiviral-active drug AzddThd or its 5′-monophosphate, AzddTMP. The fact that **1f** was not antivirally active suggests that the compound is neither phosphorylated by dCyd kinase nor deaminated by Cyd/dCyd (or dCMP) deaminase. This is consistent with the observation that ddCyd is a poor substrate for human kidney Cyd/dCyd deaminase.[51] As

Table II. Comparative Potency and Selectivity of 2′,3′-Dideoxyribonucleoside Analogues as Inhibitors of HIV Replication in ATH8 Cells

compd	ED_{50},[a] μM	ID_{50},[b] μM	therapeutic index (ratio ID_{50}/ED_{50})
1a	2.4	45	19
1c	4.8	27	5.6
1f	>100	>100	
1g	>100	>100	
1h	>100	>100	
2a	4.1	110	27
2b	0.30	30	100
2c	40	52	1.3
2d	>100	107	<1.1
3a	1.4	15	10
3b	8	>250	>31
3g	>500	>500	
4a	>100	>100	
4b	>100	>100	
4c	>100	>100	
5l	>500	>500	
5m	>500	180	<0.4
5n	>500	>500	
5o	>100	88	<0.9
5p	>100	>100	
5q	>100	>100	
5r	>100	>100	
5s	>100	100	<1
5t	>100	>100	
5u	>100	>100	
6a	100	>2000	>20
6d	>500	>500	
7	260	>500	>1.9
8	400	>500	>1.2
9r	>100	>100	
ddCyd	0.20	35	175
ddAdo	2.7	>500	>148

[a] Effective dose of compound, achieving a 50% protection of ATH8 cells against the cytopathic effect of HIV. [b] Inhibitory dose of compound, required to reduce the viability of normal uninfected ATH8 cells by 50%.

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(49) Adachi, T.; Arai, Y.; Inoue, I.; Saneyoshi, M. *Carbohydr. Res.* **1980**, *78*, 67.
(50) Adachi, T.; Yamada, Y.; Inoue, I.; Saneyoshi, M. *Synthesis* **1977**, 45.
(51) Kelly, J. A.; Litterst, C. L.; Roth, J. S.; Vistica, D. T.; Poplack, D. G.; Cooney, D. A.; Nadkarni, M.; Balis, F. M.; Broder, S.; Johns, D. G. *Drug Metab. Dispos.*, in press.

a consequence, **1f** cannot be considered as a prodrug of AzddThd. Also, Mitsuya and Broder reported that 3′-azido-ddCyd was much less effective as an antiretroviral agent than ddCyd in ATH8 cells.[52] Hence, the total lack of antiretroviral activity of **1f** and **1h** is not surprising.

The fact that none of the 2′-deoxyxylo purine and 2′-deoxyxylo pyrimidine derivatives showed any protective effect against the HIV-infected ATH8 cells suggests that the 3′-hydroxyl in the "up" position is not compatible with the compound being recognized as a substrate for the enzymes involved in the metabolism (i.e., nucleoside and nucleotide kinases) or as an inhibitor for the enzyme (reverse transcriptase) involved in the final action of the compounds. The same is true for the 3′-epimer of AzddThd (**9k**), which is totally devoid of anti-HIV activity.

Of the ddUrd analogues tested [i.e., 3′-azido-5-ethyl-ddUrd (**1g**), 3′-fluoro-5-ethyl-ddUrd (**3g**), 2′,3′-didehydro-ddUrd (**2d**), ddUrd (**6**), and 3′-azido-5-methyl-ddUrd (**1a**)], none, except for **1a**, showed any appreciable antiretroviral activity (ED_{50} > 100 or even > 500 μM) (Table II). These data were confirmed with HIV-infected MT4 cells (Table III). For 3′-azido-5-ethyl-ddUrd (**1g**), our observations are in marked contrast with the data recently presented by R. Schinazi and co-workers at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy (New Orleans, LA). (See also *Chem. Eng. News* **1986**, *64*(49), 7–14.) They reported that **1g** was equally active against HIV as AzddThd, but markedly less toxic than AzddThd when measured in a variety of normal cells (Vero, H9, peripheral mononuclear cells, fibroblasts). Compounds **1g**, **3g**, and **6g** were not the only 5-substituted 2′,3′-dideoxyuridine analogues that were found inactive against HIV; other examples of inactive compounds include **1i** and **1j** (Mitsuya and Broder, unpublished data).

Among the ddAdo analogues tested, 3′-azido-ddAdo (**1c**) had similar antiretroviral activity as ddAdo (ED_{50} = 3–5 μM) but was considerably more cytostatic. In contrast, the 2′,3′-unsaturated derivative of ddAdo, ddeAdo (**2c**), was much less active.

All compounds mentioned above were further evaluated in the human T4 cell line MT4.[53] These cells form macroscopically visible clusters upon cultivation at 37 °C. When clusters of uninfected or HIV-infected cells treated in the presence of an antivirally protective dose of the test compound are converted into a single cell suspension (by pipetting), they recluster within 4 h. Unprotected HIV-infected cells do not recluster. We compared the differential potencies of the test compounds against HIV-infected MT4 cells with those obtained in the ATH8 cell system. We found that within each class of compounds the order of antiretroviral potency was almost identical irrespective of whether the test system used was ATH8 or MT4 (Table III). However, depending upon the structural class of compounds evaluated, the concentrations at which the compounds showed a protective effect against HIV-infected MT4 cells varied considerably from those required to protect ATH8 cells against the cytopathic effect of HIV. The most striking differences were noted within the class of the 3′-substituted ddThd analogues. For example, AzddThd, ddeThd, and ddThd afforded 50% protection against HIV-infected MT4 cells at concentrations of 0.008 μM, 0.05 μM, and 1.25 μM, respectively, that is, 100–300-fold lower than the concentration required to protect HIV-infected ATH8 cells. The extremely high sensitivity of MT4 cells for the ddThd analogues was further confirmed by a cytopathogenicity assay in HIV-infected MT4 cells (data not shown). The biochemical basis for the differential activity of these compounds in both cell lines is now under investigation.

In conclusion, several analogues of ddThd and ddCyd (i.e., **2a**, **2b**, **3a**, **3b**) have been found to be potent inhibitors of HIV-induced cytopathogenicity in vitro. It seems imperative to pursue them for more extensive pharmacological studies in the scope of developing an appropriate chemotherapy for retrovirus infections (i.e., AIDS).

Experimental Section

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 257 spectrophotometer on samples in potassium bromide disks at 1.5%. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. Mass spectra were determined with an AEI MS-12 apparatus. The ^1H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, br = broad signal, q = quadruplet, m = multiplet) unless stated otherwise. Precoated Merck silica gel F254 plates were used for TLC, and the spots were examined with UV light and sulfuric acid–anisaldehyde spray. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Anhydrous solvents were obtained as follows: tetrahydrofuran was obtained by distillation after reflux overnight with lithium aluminum hydride; pyridine was refluxed overnight in *p*-toluenesulfonyl chloride, distilled, refluxed overnight in potassium hydroxide, and distilled again; dichloromethane was stored for 1 week in anhydrous calcium chloride, filtered, and distilled; ethanol was dried by distillation after it had been refluxed overnight with magnesium–iodine; water was removed from *N*,*N*-dimethylformamide by distillation with benzene followed by distillation in vacuo; acetonitrile was first refluxed on phosphorus pentoxide and distilled; benzene was dried by distillation after it had been refluxed in the presence of sodium.

9-[2-Deoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine. A. A mixture of 100 mg (0.4 mmol) of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine[25] and 149 mg (0.48 mmol) of 4-anisylchlorodiphenylmethane in 5 mL of anhydrous pyridine was stirred overnight. Methanol was added, and the reaction mixture was evaporated, diluted with $CHCl_3$ (20 mL), washed with H_2O (2 × 20 mL), dried, evaporated, and coevaporated with toluene. The title compound was purified by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 95:5] and precipitated from Et_2O (yield 72%): UV (MeOH) λ_{max} 259 nm (ε 15 300); ^1H NMR ($CDCl_3$) δ 2.28–3.00 (m, 2 H, H-2′ and H-2″), 3.56 (m, 2 H, H-5′ and H-5″), 3.73 (s, 3 H, CH_3), 4.02 (m, 1 H, H-4′), 4.34 (m, 1 H, H-3′), 6.06 (dd, 1 H, *J* = 8.8 Hz and 2.6 Hz, H-1′), 6.51 (br s, 2 H, NH_2), 6.73 (d, 2 H, *o*-anisyl), 7.08–7.50 (m, 12 H, trityl), 7.87 and 8.14 (s, s, 1 H, 1 H, H-2 and H-8); ^{13}C NMR ($CDCl_3$) 40.6 (C-2′), 54.9 (CH_3), 62.5 (C-5′), 70.65 (C-3′), 84.0 and 84.4 (C-1′ and C-4′), 120.4 (C-5), 140.4 (C-8), 147.9 (C-4), 151.9 (C-2), 155.8 (C-6) ppm (values for the monomethoxytrityl group are not mentioned).

B. A mixture of 4.21 g (10 mmol) of 2′-*O*-tosyladenosine[26] and 4.01 g (13 mmol) of 4-anisylchlorodiphenylmethane in 100 mL of anhydrous pyridine was stirred overnight at room temperature. The reaction mixture was evaporated, diluted with $CHCl_3$ (250 mL), washed with water (2 × 250 mL), dried, evaporated, and coevaporated with toluene. Purification by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 98:2] afforded 5.2 g (75% yield) of 2′-*O*-tosyl-5′-*O*-(monomethoxytrityl)adenosine, which was precipitated from $CHCl_3$–Et_2O. Further elution of the column with $CHCl_3$–MeOH, 90:10, gave 500 mg (12%) of the starting material after crystallization from H_2O. UV (MeOH) λ_{max} 260 nm (ε 12 800); ^1H NMR ($CDCl_3$) δ 2.36 (s, 3 H, CH_3-phenyl), 3.48 (m, 2 H, H-5′ and H-5″), 3.74 (s, 3 H, CH_3O), 4.32 (m, 1 H, H-4′), 4.76 (m, 1 H, H-3′), 5.12 (br s, 1 H, 3′-OH), 5.80 (dd, 1 H, H-2′), 6.14 (d, 1 H, *J* = 6.4 Hz, H-1′), 6.26 (br s, 2 H, NH_2), 6.85 (2 d, 4 H, o-anisyl and *m*-tosyl), 7.05–7.58 (m, 14 H, other aromatic protons), 7.83 and 8.04 (s, s, 1 H, 1 H, H-2 and H-8); ^{13}C NMR

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Table III. Toxicity and Inhibition of HIV Replication in MT4 Cells by Various 2′,3′-Dideoxyribonucleoside Analogues

compd	assay	concentration, μM				
		125	5	0.2	0.008	0.00032
1a	Tox[a]	++	++	–	–	–
	Prot[b]	ND[c]	ND	+++	++	+
1c	Tox	++	+	–	ND	ND
	Prot	ND	++	–	–	ND
1f	Tox	+	–	–	ND	ND
	Prot	+++	++	+	ND	ND
1g	Tox	++	–	–	ND	ND
	Prot	ND	+	–	–	–
1h	Tox	–	–	–	ND	ND
	Prot	+++	+	–	ND	ND
2a	Tox	++	++	+	ND	ND
	Prot	ND	ND	+++	+	–
2b	Tox	++	–	–	ND	ND
	Prot	ND	+++	++	–	ND
2c	Tox	++	–	–	–	–
	Prot	–	–	–	ND	ND
2d	Tox	++	–	–	ND	ND
	Prot	ND	–	–	–	ND
3a	Tox	++	++	++	+	–
	Prot	ND	ND	ND	+	+
3b	Tox	++	++	+	–	–
	Prot	ND	ND	++	–	–
3c	Tox	–	–	–	ND	ND
	Prot	+++	+	–	ND	ND
3g	Tox	–	–	–	ND	ND
	Prot	+	–	–	ND	ND
4a	Tox	–	–	–	ND	ND
	Prot	++	–	–	ND	ND
4b	Tox	++	+	–	ND	ND
	Prot	ND	–	–	–	ND
4c	Tox	+	–	–	ND	ND
	Prot	–	–	–	ND	ND
5l	Tox	++	–	–	ND	ND
	Prot	ND	+	–	–	ND
5m	Tox	++	–	–	ND	ND
	Prot	ND	+	–	–	ND
5n	Tox	++	–	–	ND	ND
	Prot	ND	–	–	–	ND
5o	Tox	++	+	–	ND	ND
	Prot	ND	+	–	–	ND
5p	Tox	++	–	–	ND	ND
	Prot	ND	+	–	–	ND
5q	Tox	–	–	–	ND	ND
	Prot	–	–	–	ND	ND
5r	Tox	–	–	–	ND	ND
	Prot	+++	+	–	ND	ND
5s	Tox	++	–	–	ND	ND
	Prot	ND	+	–	–	ND
5t	Tox	+	–	–	ND	ND
	Prot	+	–	–	ND	ND
5u	Tox	++	–	–	ND	ND
	Prot	ND	+	–	–	ND
6a	Tox	–	–	–	ND	ND
	Prot	ND	+++	+	–	ND
6d	Tox	–	–	–	ND	ND
	Prot	+++	–	–	ND	ND
6g	Tox	–	–	–	–	ND
	Prot	–	–	–	–	ND
7	Tox	+	–	–	ND	ND
	Prot	++	–	–	ND	ND
8	Tox	–	–	–	ND	ND
	Prot	+	–	–	ND	ND
9k	Tox	–	–	–	ND	ND
	Prot	–	–	–	ND	ND
9r	Tox	+	–	–	ND	ND
	Prot	+++	–	–	ND	ND
ddCyd	Tox	++	–	–	ND	ND
	Prot	ND	+++	++	–	ND
ddAdo	Tox	–	–	–	ND	ND
	Prot	+++	++	–	ND	ND

[a] Toxicity of the compound determined by evaluation of cluster morphology and "reclustering" properties in mock-infected MT4 cells (++, >90% toxicity; +, 10–40% toxicity; –, <10% toxicity). [b] Protection of MT4 cells against HIV replication determined by cluster morphology and "reclustering" properties (+++, >90% protection; ++, 40–60% protection; +, 10–40% protection; –, <10% protection). [c] Not determined.

($CDCl_3$) 21.3 (CH_3-phenyl), 55.0 (CH_3O), 63.1 (C-5′), 70.6 (C-3′), 79.8 (C-2′), 84.4 and 85.0 (C-4′ and C-1′), 119.6 (C-5), 139.5 (C-8), 149.1 (C-4), 152.6 (C-2), 155.2 (C-6) ppm (values for the trityl carbon atoms are not mentioned).

To a solution of 4.16 g (6 mmol) of 2′-*O*-tosyl-5′-*O*-(monomethoxytrityl)adenosine in 60 mL of anhydrous THF, cooled in an ice bath, was added 60 mL of 1 M solution of lithiumn triethylborohydride in THF. After the reaction mixture was stirred overnight at room temperature, 10 mL of H_2O was added and the reaction mixture was evaporated. The residue was diluted with CH_2Cl_2 (200 mL), washed with H_2O (2 × 200 mL), dried, and evaporated, leaving an oil, which was purified by column chromatography ($CHCl_3$–MeOH, 97:3). The title compound was precipitated from $CHCl_3$–Et_2O (yield 2.45 g, 4.7 mmol, 78%).

9-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-adenine (1c). Methanesulfonyl chloride (0.17 mL) was added to a solution of 366 mg (0.7 mmol) of 9-[2-deoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine in 5 mL of anhydrous pyridine at 0 °C. The mixture was kept overnight in the refrigerator. After addition of 1 mL of H_2O, the reaction mixture was warmed up to room temperature, evaporated, diluted with $CHCl_3$ (20 mL), washed with H_2O (2 × 20 mL), dried, and evaporated. Column chromatographic purification [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 97:3] gave 405 mg (0.67 mmol, 96%) of 9-(2-deoxy-3-*O*-mesyl-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl)adenine as a foam. The mesylation of the 3′-OH group was verified by NMR spectroscopy. ^1H NMR ($CDCl_3$) showed the appearance of a singlet at δ 2.71 (CH_3SO_2), a downfield shift for H-3′ (δ 5.40), and a broad singlet at δ 6.54 (NH_2) as the only exchangeable protons. The ^{13}C NMR spectrum showed a strong downfield shift for C-3′ (78.3 ppm) and the appearance of the methylsulfonyl group at 38.3 ppm. Sodium azide (100 mg) was added to a solution of 360 mg (0.6 mmol) of 9-[2-deoxy-3-*O*-mesyl-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine in 5 mL of anhydrous DMF, and the reaction mixture was heated at 100 °C for 2 h. After cooling to room temperature and evaporation of the solvent, the mixture was taken up in $CHCl_3$ (20 mL) and washed with H_2O (1 × 20 mL). The organic layer was dried and evaporated. TLC ($CHCl_3$–MeOH, 95:5, R_f 0.44) revealed only one compound. This product was dissolved in 10 mL of CH_2Cl_2–MeOH (4:1) containing 2% *p*-toluenesulfonic acid and stirred for 15 min. After 0.25 mL of Et_3N was added, the mixture was evaporated and applied onto a silica gel column, which was eluted with $CHCl_3$–MeOH (95:5). This yielded 100 mg (0.36 mmol, 60%) of **1c** after crystallization from EtOH. IR, UV, ^1H NMR, and melting point were in agreement with those reported by Imazawa et al.:[24] mp 189–191 °C; IR (KBr) 2110 cm^{-1} (N_3); UV (MeOH) λ_{max} 259 nm (ε 15 600); ^1H NMR (Me_2SO-d_6) δ 2.32–2.66 (m, H-2′), 2.78–3.16 (m, H-2″), 3.60 (m, H-5′ and H-5″), 3.95 (q, H-4′), 4.61 (m, H-3′), 5.34 (t, *J* = 5.7 Hz, OH), 6.32 (t, *J* = 6.6 Hz, H-1′), 7.32 (br s, NH_2), 8.15, 8.34 (2 s, H-2 and H-8).

3′-*O*-Methylthymidine (5o). 3′-*O*-Methylthymidine was synthesized by using exactly the same modus operandi as described by Hampton et al.[22] for the synthesis of 3′-*O*-ethylthymidine **5p**: mp 133–135 °C ($MeOH$–Et_2O); MS, *m*/*e* 256 (M^+); UV (H_2O) λ_{max} 266 nm (ε 9730), (0.2 N NaOH) λ_{max} 265 nm (ε 7570); ^1H NMR (Me_2SO-d_6) δ 1.80 (s, 3 H, CH_3), 2.17 (m, 2 H, H-2′ and H-2″), 3.28 (s, 3 H, CH_3O), 3.56 (m, 2 H, H-5′ and H-5″), 3.90 (m, 2 H, H-3′ and H-4′), 5.07 (t, 1 H, 5′-OH), 6.07 (dd, *J* = 7.9 Hz and 6.2 Hz, 1 H, H-1′), 7.68 (d, 1 H, H-6), 11.25 (br s, 1 H, NH). Anal. ($C_{11}H_{16}N_2O_5$) C, H, N.

3′-[(2-Hydroxyethyl)thio]-3′-deoxythymidine (5t). The same modus operandi as followed for the synthesis of the 3′-(ethylthio) derivative **5s**[23] was used for the synthesis of **5t**, except that 1-(2′-deoxy-3′-*O*-mesyl-5′-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] was used as starting material, ethanethiol was substituted by 2-mercaptoethanol, and the title compound (**5t**) was crystallized from Et_2O (50% yield): mp 147 °C; MS, *m*/*e* 302 (M^+); UV (MeOH) λ_{max} 266 nm (ε 10 100); ^1H NMR (Me_2SO-d_6–D_2O) δ 1.78 (d, 3 H, *J* = 1.3 Hz, CH_3), 2.10–2.56 (m, 2 H, H-2′ and H-2″), 2.67 (t, 2 H, *J* = 6.7 Hz, CH_2S), 3.30–3.84 (m, 6 H, H-3′, H-4′, H-5′, H-5″, and CH_2O), 6.04 (dd, 1 H, *J* = 6.6 Hz and 4.8 Hz, H-1′), 7.76 (d, 1 H, H-6). Anal. ($C_{12}H_{18}N_2O_5S$) C, H, N.

3′-Thiocyanato-3′-deoxythymidine (5u). A mixture of 996 mg (1.77 mmol) of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] and 777 mg (8 mmol) of potassium thiocyanate in 20 mL of anhydrous DMF was heated at 100 °C for 10 h. The reaction mixture was evaporated, diluted with $CHCl_3$ (50 mL), washed with H_2O (2 × 50 mL), dried, and evaporated. The resulting oil was purified by column chromatography ($CHCl_3$–MeOH, 99:1), and the 5′-*O*-trityl derivative was detritylated with 20 mL of 80% acetic acid. After heating for 20 min at 100 °C, the solvent was evaporated and 3′-thiocyanato-3′-deoxythymidine (**5u**) was purified by column chromatography ($CHCl_3$–MeOH, 97:3) and crystallized from MeOH (105 mg, 0.37 mmol, 21%): mp 114–116 °C; UV (H_2O) λ_{max} 266 nm (ε 9600), (0.1 N NaOH) λ_{max} 266 nm (ε 8200); MS, *m*/*e* 283 (M^+), 252 (M^+ – CH_2OH), 158 (M^+ – $C_5H_5N_2O_2$), 126 ($C_5H_5N_2O_2$ + H^+), 99 (158 – HSCN); IR (KBr) 2150 cm^{-1} (SCN); ^1H NMR (pyridine-d_5) δ 1.83 (s, 3 H, *J* = 0.88 Hz, CH_3), 2.82 (m, 2 H, H-2′ and H-2″), 3.94–4.60 (m, 4 H, H-3′, H-4′, H-5′, and H-5″), 6.63 (dd, 1 H, *J* = 6.6 Hz and 4.8 Hz, H-1′), 7.97 (d, 1 H, H-6), 13.1 (br s, 1 H, NH). Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N.

3′-Azido-5-methyl-2′,3′-dideoxycytidine (1f) and 1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(methylamino)-5-methyl-2(1*H*)-pyrimidinone (1h). A solution of 1.02 g (2 mmol) of 3′-azido-5′-*O*-trityl-3′-deoxythymidine[28] in 20 mL of a mixture of CH_3CN–pyridine (1:1) was added to a suspension containing 0.7 mL of phosphoryl chloride and 2 mL of *N*-methylimidazole in 15 mL of CH_3CN at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was divided into two equal parts. To one part was added 10 mL of ammonia (33%). The other part was diluted with 10 mL of methylamine (50% in H_2O). After being stirred for 2 h at room temperature, both solutions were evaporated, diluted with $CHCl_3$ (100 mL), washed with H_2O (2 × 100 mL), dried, and evaporated. The reaction products were purified by column chromatography ($CHCl_3$–MeOH, 98:2 and 99:1 for parts 1 and 2, respectively). Both compounds were fully characterized after detritylation with 80% acetic acid (100 °C, 20 min) and a second column chromatography ($CHCl_3$–MeOH, 95:5, and $CHCl_3$–MeOH, 97:3, respectively). **a. 3′-Azido-5-methyl-2′,3′-dideoxycytidine:** 52% yield; UV (H_2O) λ_{max} 275 nm (ε 9700); IR (KBr) 2090 cm^{-1} (N_3); MS, *m*/*e* 266 M^+. Further identification was performed on the hydrochloride salt, which was obtained by treatment of a methanolic solution of the nucleoside with 1 N HCl in MeOH followed by addition of Et_2O and crystallization: mp 176 °C dec; ^1H NMR (D_2O) δ 2.05 (d, 3 H, *J* = 0.7 Hz, CH_3), 2.55 (m, 2 H, H-2′ and H-2″), 3.90 (m, 2 H, H-5′ and H-5″), 4.06 (m, 1 H, H-4′), 4.34 (m, 1 H, H-3′), 6.16 (t, 1 H, *J* = 6.15 Hz, H-1′), 7.95 (d, 1 H, H-6). Anal. ($C_{10}H_{15}N_6O_3Cl$) C, H, N. **b. 1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(methylamino)-5-methyl-2(1*H*)-pyrimidinone** (53% yield) was precipitated from MeOH–Et_2O: UV (MeOH) λ_{max} 272 nm (ε 9900); IR (KBr) 2100 cm^{-1} (N_3); MS, *m*/*e* 280 (M^+); ^1H NMR (Me_2SO-d_6) δ 1.87 (d, 3 H, CH_3), 2.26 (t, 2 H, H-2′ and H-2″), 2.81 (d, 3 H, *J* = 4.6 Hz, CH_3NH), 3.62 (m, 2 H, H-5′ and H-5″), 3.83 (m, 1 H, H-4′), 4.35 (m, 1 H, $J_{3',4'}$ = 4.4 Hz, H-3′), 5.19 (br s, 1 H, OH), 6.11 (t, 1 H, *J* = 6.6 Hz, H-1′), 7.20 (q, 1 H, NH), 7.56 (d, 1 H, H-6). Anal. ($C_{11}H_{16}N_6O_3$) C, H, N.

1-(3-*O*-Mesyl-5-*O*-trityl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-ethyluracil. A mixture of 1 g (3.9 mmol) of 5-ethyl-2′-deoxyuridine[30] and 1.39 g (5 mmol) of trityl chloride in 50 mL of anhydrous pyridine was heated at 50 °C for 4 h and kept overnight at room temperature. The reaction mixture was cooled to 0 °C, mesyl chloride (1 mL) was added, and the reaction mixture was stored in the refrigerator overnight. After addition of H_2O (1 mL), the solvent was evaporated, and the resulting oil was diluted with EtOAc (100 mL), washed with H_2O (2 × 100 mL), dried, and evaporated. TLC analysis revealed mainly one compound ($CHCl_3$–MeOH, 97:3, R_f 0.48), which was purified by flash chromatography on silica ($CHCl_3$–MeOH, 99:1) for ^1H NMR characterization (2.05 g, 3.56 mmol, 91% yield): ^1H NMR ($CDCl_3$) δ 1.86 (t, 3 H, *J* = 7.47 Hz, CH_3CH_2), 1.96 (dq, 2 H, CH_2CH_3), 2.54 (m, 2 H, H-2′ and H-2″), 3.02 (s, 3 H, CH_3SO_2), 3.48 (m, 2 H, H-5′ and H-5″), 4.31 (m, 1 H, H-4′), 5.38 (m, 1 H, H-3′), 6.40 (dd, 1 H, *J* = 5.9 Hz and 8.2 Hz, H-1′), 7.35 (m, 16 H, trityl and H-6).

1-(5-*O*-Trityl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-ethyluracil. The foam obtained in the previous reaction was dissolved in EtOH (50 mL); 20 mL of 1 N NaOH and 50 mL of H_2O were added, and the reaction mixture was refluxed. The

course of the reaction was followed by UV spectroscopy. During the reaction, the UV maximum changed first to a shoulder at 252 nm and then returned to 266 nm, which indicated the formation of a 2,3′-anhydro compound as an intermediate. After 5 h, the reaction mixture was cooled, concentrated to 60 mL, cooled in an ice bath, and acidified with 0.5 N HCl to pH 4. The precipitate was collected, washed thoroughly with H_2O, and dried in vacuo: 1.7 g (3.4 mmol, 96% yield); R_f ($CHCl_3$–MeOH, 95:5) 0.58; UV (MeOH) λ_{max} 266 nm (ϵ 10 100); ^1H NMR ($CDCl_3$) δ 1.91 (t, 3 H, J = 7.47 Hz, CH_2CH_3), 1.98–2.60 (m, 4 H, CH_2CH_3, H-2′ and H-2″), 3.32–3.76 (m, 3 H, H-5′, H-5″, and OH), 4.09 (m, 1 H, H-4′), 4.40 (m, 1 H, H-3′), 6.17 (br d, 1 H, H-1′), 7.22 (m, 17 H, trityl and H-6), 10.07 (s, NH). Anal. ($C_{30}H_{30}N_2O_5$) C, H, N.

3′-Azido-5-ethyl-2′,3′-dideoxyuridine (1g). Mesyl chloride (1 mL) was added to a cooled (ice bath) solution of 1.7 g (3.4 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-ethyluracil in 20 mL of anhydrous pyridine, and the reaction mixture was kept at 4 °C overnight and for an additional 5 h at room temperature. After addition of H_2O (1 mL), the reaction mixture was evaporated, diluted with $CHCl_3$ (50 mL), washed with H_2O (2 × 50 mL), dried, and evaporated, leaving an oil (one spot on TLC, $CHCl_3$–MeOH, 95:5, R_f 0.65), which was dissolved in DMF (10 mL) and heated for 2 h at 100 °C in the presence of 1 g of sodium azide. After cooling to room temperature, the reaction mixture was poured into 250 mL of ice water; the precipitate was collected, washed with H_2O (2 × 100 mL), dissolved in 50 mL of 80% acetic acid, and heated for 15 min at 100 °C. Evaporation, coevaporation with toluene, and chromatographic purification [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 98:2] yielded 787 mg (2.8 mmol, 82%) of **1g**: mp (diisopropyl ether) 116–116.5 °C; MS, m/e 281 (M^+); UV (MeOH) λ_{max} 265 nm (ϵ 9680); IR (KBr) 2090 cm^{-1} (N_3^-); ^1H NMR (Me_2SO-d_6) δ 1.04 (t, 3 H, J = 7.47 Hz, CH_3), 2.22 (q, CH_2CH_3), 2.20–2.59 (m, H-2′ and H-2″), 3.61 (m, 2 H, H-5′ and H-5″), 3.83 (m, 1 H, H-4′), 4.41 (m, 1 H, H-3′), 5.21 (br t, 1 H, OH), 6.10 (t, 1 H, J = 6.4 Hz, H-1′), 7.65 (s, 1 H, H-6), 11.25 (br s, 1 H, NH). Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

5′-*O*-Benzoyl-5-ethyl-2′-deoxyuridine. A solution of 0.48 mL (4 mmol) of benzoyl chloride in pyridine (25 mL) was added dropwise to a stirred solution of 1 g (3.9 nmol) of 5-ethyl-2′-deoxyuridine[31] in pyridine (25 mL) at 0 °C over a period of 1 h. MeOH was added, and, after being stirred for 10 min, the reaction mixture was evaporated and applied to a silica gel column eluting with $CHCl_3$–MeOH (95:5). The title compound was crystallized from MeOH–Et_2O: 1.1 g (3.05 mmol, 83%); mp 178–179 °C; UV (MeOH) λ_{max} 266 nm (ϵ 11 000); ^1H NMR ($CDCl_3$–CD_3OD, 2:1) δ 0.91 (t, 3 H, J = 7.47 Hz, CH_3), 2.12 (q, 2 H, CH_2CH_3), 2.28 (m, 2 H, H-2′ and H-2″, partly hidden by CH_2CH_3), 4.20–4.76 (m, 4 H, H-3′, H-4′, H-5′, and H-5″), 6.29 (t, 1 H, 6.8 Hz, H-1′), 7.12–7.64 (m, 4 H, H-6 and phenyl), 7.90–8.10 (m, 2 H, phenyl).

5-Ethyl-2′,3′-dideoxyuridine (6g). The modus operandi was exactly the same as used by Prisbe in ref 32 for the synthesis of 2′,3′-dideoxythymidine, except that the 3′-*O*-(methoxythio)-carbonyl derivative has not been crystallized and that tributyltin hydride was used instead of a mixture of bis(tributyltin) oxide and polymethylhydrosiloxane. The benzoyl group was removed with MeOH saturated with NH_3: total yield 77%; mp 117–118 °C; UV (MeOH) λ_{max} 267 nm (ϵ 10 000); ^1H NMR ($CDCl_3$) δ 1.10 (t, 3 H, J = 7.47 Hz, CH_3), 1.90–2.55 (m, 6 H, CH_2CH_3, H-2′, H-2″, H-3′ and H-3″), 3.82 (m, 2 H, H-5′ and H-5″), 4.12 (m, 1 H, H-4′), 6.08 (dd, 1 H, J = 4.0 Hz and 6.6 Hz, H-1′), 7.48 (s, 1 H, H-6), 9.16 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 12.7 (CH_3), 20.1 (CCH_3), 25.2 (C-3′), 32.2 (C-2′), 63.4 (C-5′), 81.4 (C-4′), 86.4 (C-1′), 116.3 (C-5), 135.6 (C-6), 150.5 (C-2), 163.7 (C-4). Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

1-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)thymine (9k). A solution of 1-(3-azido-2,3-dideoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[27] (1.02 g, 2 mmol) in 80% acetic acid was heated for 15 min at 100 °C. The reaction mixture was evaporated, coevaporated with toluene, and isolated after two chromatographic purifications [(1) $CHCl_3$–MeOH, 95:5; (2) EtOAc], yielding the azidothymidine epimer **9k** as a foam (300 mg, 56%). We did not succeed in crystallizing this compound: MS, m/e 267 (M^+); IR (KBr) 2100 cm^{-1} (N_3); UV (MeOH) λ_{max} 267 nm (ϵ 9970); ^1H NMR (Me_2SO-d_6) δ 1.81 (s, 3 H, CH_3), 2.10 (m, 1 H, H-2′), 2.76 (m, 1 H, H-2″), 3.68 (m, 2 H, H-5′ and H-5″), 3.96 (m, 1 H, H-4′), 4.44 (m, 1 H, H-3′), 4.97 (br t, 1 H, OH), 6.01 (dd, 1 H, J = 3.5 Hz and 7.5 Hz, H-1′), 7.48 (s, 1 H, H-6), 11.25 (br s, 1 H, NH); Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

1-(5-*O*-Trityl-2-deoxy-β-D-*threo*-pentofuranosyl)cytosine. A solution of 5.2 g (8 mmol) of 3′-*O*-mesyl-5′-*O*-trityl-*N*-benzoyl-2′-deoxycytidine in a mixture of EtOH (300 mL), H_2O (100 mL), and 1 N NaOH (8 mL) was stirred overnight. After addition of another 50 mL of 1 N NaOH, the solution was heated at 100 °C for 3 h, cooled, and neutralized with 1 N HCl. After evaporation of ethanol, the precipitate was collected, washed twice with H_2O, and dried in vacuo (3.65 g, 94%): UV (MeOH) λ_{max} 270 nm (ϵ 9400); ^1H NMR (Me_2SO-d_6) δ 1.81 (m, 1 H, H-2′), 2.53 (m, 1 H, H-2″), 3.28 (m, 2 H, H-5′ and H-5″), 4.14 (m, 2 H, H-3′ and H-4′), 5.13 (br d, 1 H, 3′-OH), 5.64 (d, 1 H, J = 7.47 Hz, H-5), 6.06 (br d, 1 H, J = 6.6 Hz, H-1′), 7.08 (br s, NH_2), 7.38 (m, 15 H, trityl), 7.65 (d, 1 H, H-6).

1-(2′-Deoxy-β-D-*threo*-pentofuranosyl)cytosine[45] (4b). This product was synthesized according to the method described by Ohtsuka et al.[45] However, the monomethoxytrityl instead of the trityl group was used as blocking group for the 5′-hydroxyl group. Detritylation was performed with 80% acetic acid at 100 °C for 15 min. The reaction mixture was chromatographed ($CHCl_3$–MeOH, 80:20), and the title compound was isolated as the hydrochloride (a solution of the nucleoside in H_2O was acidified with 0.5 N HCl to pH 2, the water was evaporated, and the residue was crystallized from MeOH): UV (H_2O) λ_{max} 272 nm (ϵ 9750) (for comparison with literature data,[14] the UV spectrum was taken before the nucleoside was converted to the hydrochloride salt); mp (decomposition started at 170 °C and was fast at 179 °C); R_f ($CHCl_3$–MeOH–25% NH_3, 7:3:0.5) 0.48; ^1H NMR (Me_2SO-d_6) δ 2.03 (m, 1 H, H-2′), 2.38–2.72 (m, 1 H, H-2″), 3.74 (m, 2 H, H-5′ and H-5″), 3.90 (m, 1 H, H-4′), 4.43 (m, 1 H, H-3′), 5.96 (dd, 1 H, J = 6.6 Hz, and 1.2 Hz, H-1′), 6.17 (d, 1 H, J = 7.9 Hz, H-5), 8.13 (d, 1 H, H-6), 8.70 (br s, 1 H, NH), 9.73 (br s, 1 H, NH); ^{13}C NMR (Me_2SO-d_6) 40.8 (C-2′), 59.1 (C-5′), 68.2 (C-3′), 85.9 (C-1′), 86.4 (C-4′), 93.0 (C-5), 145.3 (C-6), 147.0 (C=O), 159.6 (C=O) ppm.

3′-Deoxy-2′-thymidinene[47] (2a). A solution of 500 mg (0.89 mmol) of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] in 10 mL of THF, containing 1 M TBAF, was stored at room temperature for 32 h. TLC of the reaction mixture revealed one major compound and two minor compounds (one of these being the starting material). After evaporation of the solvent, the reaction mixture was divided between $CHCl_3$ (50 mL) and H_2O (50 mL). The organic layer was dried and evaporated. Column chromatographic purification ($CHCl_3$–MeOH, 98:2) gave only the major compound, 5′-*O*-trityl-3′-deoxy-2′-thymidinene,[48] in a pure form: ^1H NMR ($CDCl_3$) δ 1.28 (d, 3 H, CH_3), 3.39 (m, 2 H, H-5′ and H-5″), 4.96 (m, 1 H, H-4′), 5.88 (ddd, 1 H, J = 6.0 Hz, 2.2 Hz, and 1.25 Hz, H-2′), 6.36 (ddd, 1 H, J = 6.0 Hz and 2 × 1.75 Hz, H-3′), 7.05 (m, 1 H, H-1′), 7.27 (m, 16 H, trityl and H-6).

In a second preparation, the crude reaction mixture was stirred for 15 min in 80% acetic acid at 100 °C, evaporated, and coevaporated with toluene. TLC of the reaction mixture showed extensive destruction of the nucleosides. However, the two compounds that showed an UV absorption *and* a positive anisaldehyde–sulfuric acid test were isolated by column chromatography ($CHCl_3$–MeOH, 98:2) and identified as 3′-deoxy-2′-thymidinene (**2a**, 85 mg) and 3′-fluoro-3′-deoxythymidine (22 mg). **2a**: mp 164–165 °C (lit.[16] mp 165–166 °C); UV (H_2O) λ_{max} 265 nm (lit.[16] λ_{max} 266 nm); ^1H NMR (Me_2SO-d_6) δ 1.75 (s, 3 H, CH_3), 3.60 (m, 2 H, H-5′ and H-5″), 4.76 (m, 1 H, H-4′), 4.98 (t, 1 H, 5′-OH), 5.92 (m, 1 H, H-2′), 6.39 (m, 1 H, H-3′), 6.81 (m, 1 H, H-1′), 7.62 (s, 1 H, H-6), 11.26 (br s, 1 H, NH).

3′-Fluoro-3′-deoxythymidine (3a). To a solution of 485 mg (1 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine[36,37] in 20 mL of anhydrous benzene containing 1 mL of THF (solubility) was added 0.5 mL of DAST. The reaction mixture was stirred for 2 h, poured into a 5% solution of sodium bicarbonate (20 mL), and extracted with EtOAc (2 × 20 mL). TLC of the reaction mixture revealed mainly one compound. The organic layer was dried, evaporated, and treated for 15 min at 100 °C with 80% acetic acid. After evaporation, the reaction mixture was purified by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 99:1], yielding 7 mg (3%) of 3′-deoxy-2′-thymidinene (**2a**) and 152 mg (62%) of 3′-fluoro-3′-deoxythymidine (**3a**).

Melting point (176–177 °C) and spectroscopic data (UV, ^{1}H NMR) were identical with those described in the literature:[33,34] ^{1}H NMR (Me_2SO-d_6) δ 1.82 (s, 3 H, CH_3), 5.20 (br t, 1 H, 5′-OH), 7.71 (d, 1 H, J = 1.1 Hz, H-6), 11.29 (br s, 1 H, NH); ^{13}C NMR (Me_2SO-d_6) 12.2 (CH_3), 109.8 (C-5), 135.8 (C-6), 150.5 (C-2), 163.7 (C-4) ppm. (Values for the sugar protons and carbon atoms are presented in Table I.)

1-(3-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-ethyluracil (3g). DAST (1 mL) was added to a solution of 996 mg (2 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-ethyluracil in 40 mL of anhydrous benzene. The reaction mixture was kept for 2 h at room temperature, washed with 20 mL of 5% sodium bicarbonate solution (2×), dried, and evaporated. This reaction mixture showed mainly one compound on TLC. Heating for 15 min at 100 °C in 80% acetic acid and evaporation of the solvent yielded an oil, which was applied onto a silica column and eluted with $CHCl_3$–MeOH (99.5:0.5) followed by $CHCl_3$–MeOH (98:2). **3g** was crystallized from MeOH–Et_2O (335 mg, 65%): mp 175–176 °C; UV (MeOH) λ_{max} 266 nm (ϵ 9680); MS, m/e 258 (M^+); R_f ($CHCl_3$–MeOH, 90:10) 0.40; ^{1}H NMR (Me_2SO-d_6) δ 1.02 (t, 3 H, J = 7.47 Hz, CH_2CH_3), 2.22 (br q, CH_2CH_3), 5.18 (br t, 1 H, 5′-OH), 7.66 (d, 1 H, H-6), 11.27 (br s, 1 H, NH) (other values are presented in Table I); ^{13}C NMR (Me_2SO-d_6) 12.7 (CH_3), 19.5 (CH_2), 115.4 (C-5), 135.0 (C-6), 150.2 (C-2), 163.1 (C-4) ppm (other values are presented in Table I). Anal. ($C_{11}H_{15}O_4N_2F$) C, H, N.

1-(3-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)cytosine (3b). DAST (0.5 mL) was added to a suspension of 485 mg (1 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)cytosine in a mixture of anhydrous CH_2Cl_2–THF (5:5, 10 mL). After the addition of DAST, the suspension cleared up immediately. The reaction mixture was stirred for 2 h at room temperature. After addition of $CHCl_3$ (10 mL), the mixture was poured into 20 mL of 5% $NaHCO_3$ solution. The organic layer was separated, and the water layer was extracted again with $CHCl_3$ (20 mL). The combined organic layer was dried, evaporated, and purified by column chromatography. The product with R_f 0.32 ($CHCl_3$–MeOH, 90:10) was isolated (177 mg, 36%) and treated with 80% acetic acid for 15 min at 100 °C. Evaporation of the reaction mixture followed by column chromatography [(1) $CHCl_3$–MeOH, 95:5; (2) $CHCl_3$–MeOH, 90:10] and crystallization from MeOH afforded 49 mg (59% for the detritylation reaction) of **3b**: mp 225 °C dec; UV (H_2O) λ_{max} 271 nm (ϵ 9200), (0.01 N HCl) λ_{max} 279 nm (ϵ 14050); MS, m/e 229 (M^+). Values not mentioned in Table I were as follows: ^{1}H NMR (Me_2SO-d_6) δ 5.12 (br t, 1 H, 5′-OH), 5.74 (d, 1 H, J = 7.47 Hz, H-5), 7.16 (br s, 2 H, NH_2), 7.76 (d, 1 H, J = 7.47 Hz, H-6); ^{13}C NMR (Me_2SO-d_6) 94.2 (C-5), 140.7 (C-6), 156.2 (C-2), 165.5 (C-4) ppm. Anal. ($C_9H_{12}O_3N_3F$) C, H, N.

9-(3-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-adenine (3c). A mixture of 786 mg (1.5 mmol) of 9-[5-*O*-(monomethoxytrityl)-2-deoxy-β-D-*threo*-pentofuranosyl]adenine and 0.75 mL of DAST in 30 mL of anhydrous CH_2Cl_2 was kept for 1 h at room temperature and poured into 30 mL of 5% $NaHCO_3$ solution. The organic layer was dried, evaporated, and purified by column chromatography. 9-[5-*O*-(Monomethoxytrityl)-3-fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]adenine was eluted with $CHCl_3$–MeOH (98.5:1.5) (411 mg, 0.78 mmol, 52%): ^{1}H NMR ($CDCl_3$) δ 2.61–3.27 (m, 2 H, H-2′ and H-2″), 3.41 (d, 2 H, J = 4.4 Hz, H-5′ and H-5″), 3.78 (s, 3 H, OCH_3), 4.45 (dt, 1 H, $J_{4',F}$ = 26 Hz, H-4′), 5.36 (dm, 1 H, $J_{3',F}$ = 54.5 Hz, H-3′), 5.93 (br s, 2 H, NH_2), 6.44 (dd, 1 H, J = 6.15 Hz and 8.35 Hz, H-1′), 6.79 (d, 2 H, J = 9.2 Hz, *o*-phenyl), 7.25 (m, trityl), 7.93 and 8.23 (2 s, 2 × 1 H, H-8 and H-2). Detritylation was performed by treating the monomethoxytrityl compound (370 mg, 0.7 mmol) with 2% *p*-toluenesulfonic acid in CH_2Cl_2–MeOH (4:1) (20 mL) for 15 min at room temperature. After addition of 1.05 mL of 2 N NaOH, the reaction mixture was evaporated and purified by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 97:3]. The 3′-fluoro compound **3c** was crystallized from MeOH (110 mg, 0.43 mmol, 62%): mp 188.5–190.0 °C; UV (MeOH) λ_{max} 259 nm (ϵ 15950); R_f ($CHCl_3$–MeOH, 90:10) 0.45. Values not mentioned in Table I were as follows: ^{1}H NMR (Me_2SO-d_6) δ 5.49 (t, 1 H, 5′-OH), 7.30 (s, 2 H, NH_2), 8.14 and 8.33 (2 s, 2 × 1 H, H-8 and H-2); ^{13}C NMR (Me_2SO-d_6) 139.7 (C-8), 152.4 (C-2), 156.2 (C-6) ppm. Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N.

Antiviral Test Procedures. HIV infection was carried out with the HTLV-III_B strain. The virus was prepared from the culture supernatant of a persistently HTLV-III_B infected H9 cell line. The antiviral assays, based on an inhibition of HIV-induced cytopathogenicity in human T4 lymphocyte (ATH8) cells, were carried out by following previously established procedures.[17,18]

The antiviral assays using MT4 as the target cell line were based on the MT4 cell cluster characteristics and "reclustering" properties. Briefly, MT4 cells were infected with HIV (300 $CCID_{50}$/well) and seeded at a density of 2.5 × 10^5 cells/mL in the presence of varying concentrations of test compound. Five days after incubation at 37 °C, the cell cluster morphology of the MT4 cells was evaluated microscopically. Clusters of HIV-infected MT4 cells that had been protected by the compounds could be readily distinguished from HIV-infected MT4 cell clusters that had not been protected. These cell aggregates were then converted into a single cell suspension by pipetting and incubated at 37 °C for an additional 4 h, followed by microscopic assessment of the "reclustering" properties of the treated cell cultures. Mock-infected MT4 cells were evaluated under the same conditions. Reclustering of the mock-infected cells and cells treated with an antivirally protective dose of test compound occurred within a 4-h period. Nonprotected cells no longer reclustered. A more detailed description of the assay will be published elsewhere.

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