Synthesis and Anti-HIV Evaluation of 2′,3′-Dideoxyribo-5-chloropyrimidine Analogues: Reduced Toxicity of 5-Chlorinated 2′,3′-Dideoxynucleosides

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In view of the selective anti-HIV activity of 2′,3′-dideoxy-3′-fluoro-5-chlorouridine (11), a series of eight 2′,3′-dideoxy-5-chloropyrimidines were synthesized and evaluated for their inhibitory activity against human immunodeficiency virus type 1 (HIV-1) replication in MT-4 cells. A marked improvement in selectivity was noted for the 5-chlorouracil derivatives of 2,3-dideoxyribofuranose, 3-azido-2,3-dideoxyribofuranose, and 3-fluoro-2,3-dideoxyribofuranose, mainly due to decreased toxicity of the compounds for the host cells. While chlorination of 2′,3′-dideoxycytidine removed the anti-HIV activity, introduction of a chlorine at the C-5 position of 3′-fluoro-, 3′-azido- or 2′,3′-didehydro-2′,3′-dideoxycytidine led to reduced cytotoxicity with only slightly reduced anti-HIV activity. X-ray analysis showed compound 11 to have two molecules in the asymmetric unit with χ = −168.8 (3)° and −131.3 (3)° and P = 179 (1)° and 163 (1)°, respectively; thus revealing no close resemblance to 3′-azido-3′-deoxythymidine (AZT).

Various stages in the replicative cycle of human immunodeficiency virus (HIV) could be envisaged as points of attack for antiviral agents, e.g. virus adsorption to the cell membrane, uncoating, reverse transcription of viral RNA, integration of the viral genome into cellular DNA, transcription to viral mRNA, translation of viral mRNA to precursor proteins, processing of viral proteins to glycoproteins, and budding of the virus particles. Virtually all nucleoside analogues that have been reported to inhibit HIV replication are assumed to interact at the reverse transcriptase level.

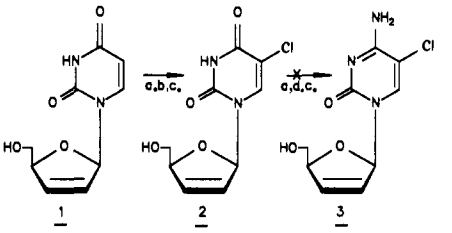
We have recently demonstrated that 3′-fluoro-2′,3′-dideoxy-5-chlorouridine (FddClUrd, 11) is a potent and selective inhibitor of HIV-1 and HIV-2 replication.[2-4] Introduction of a chlorine atom at the C-5 position of uracil resulted in a markedly reduced toxicity of 11 compared to that of 2′,3′-dideoxy-3′-fluorouridine (FddUrd) and its thymine counterpart (FddThd).[3,4] We therefore envisaged the synthesis of several 5-chloro-2′,3′-dideoxynucleosides in both the uridine and cytidine series, namely the 5-chlorouracil and 5-chlorocytosine derivatives of 2,3-dideoxyribofuranose, 2,3-didehydro-2,3-dideoxyribofuranose, 3-azido-2,3-dideoxyribofuranose, and 3-fluoro-2,3-dideoxyribofuranose.

Following prior conversion to their corresponding 5′-triphosphate analogues, these nucleosides inhibit transcription of the viral RNA to DNA either by competitive inhibition or by chain termination following their incorporation at the 3′-end of the DNA chain.

Chemistry

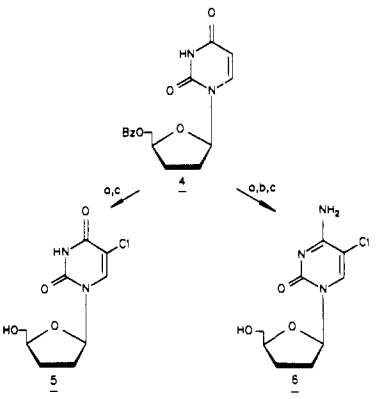
All chlorinations of the C-5 position of the uracil ring were carried out with *N*-chlorosuccinimide as described for the synthesis of FddClUrd (11).[2] After acylation with acetic anhydride in pyridine, 2′,3′-didehydro-2′,3′-dideoxyuridine (d4Urd)[5] was treated with 1.3 equiv of *N*-chlorosuccinimide for 30 min at 100 °C. Flash chromatographic purification, followed by treatment with methanol saturated with ammonia yielded 44% (overall) of 2′,3′-didehydro-2′,3′-dideoxy-5-chlorouridine (d4ClUrd, 2 Scheme I). Likewise, 5′-*O*-benzoyl-2′,3′-dideoxyuridine was treated with *N*-chlorosuccinimide and subsequently debenzoylated to afford 75% of 2′,3′-dideoxy-5-chlorouridine (ddClUrd, 5). 3′-Azido-2′,3′-dideoxyuridine (AzddUrd)[6] was acetylated, chlorinated with *N*-chlorosuccinimide, and deacetylated to give 3′-azido-2′,3′-dideoxy-5-chlorouridine (AzddClUrd, 8) in 45% overall yield.

Scheme I[a]



[a] (a) C_5H_5N, $(CH_3CO)_2O$; (b) NCS, C_5H_5N, 30 min at 100 °C; (c) NH_3, CH_3OH; (d) $(CF_3SO_2)_2O$, C_5H_5N.

Scheme II[a]



[a] (a) NCS, C_5H_5N, 45 min at 90 °C; (b) $(CF_3SO_2)_2O$, C_5H_5N; (c) NH_3, CH_3OH.

At first the 2′,3′-dideoxycytidine analogues were synthesized from their 2′,3′-dideoxyuridine counterparts by treatment with trifluoromethanesulfonic anhydride, followed by addition of ammonia in methanol.[7] However,

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(1) To whom correspondence should be addressed concerning X-ray analysis.
(2) Van Aerschot, A.; Herdewijn, P.; Balzarini, J.; Pauwels, R.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 1743.
(3) Balzarini, J.; Van Aerschot, A.; Pauwels, R.; Baba, M.; Schols, D.; Herdewijn, P.; De Clercq, E. *Mol. Pharmacol.* **1989**, *35*, 571.
(4) Balzarini, J.; Van Aerschot, A.; Herdewijn, P.; De Clercq, E. *Biochem. Pharmacol.* **1989**, *38*, 869.
(5) Horwitz, J. P.; Chua, J.; Da Rooge, M. A.; Noel, M.; Klundt, I. L. *J. Org. Chem.* **1966**, *31*, 205.
(6) Lin, T.-S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 544.

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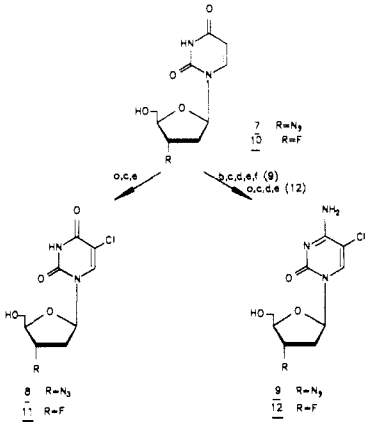
Scheme III[a]

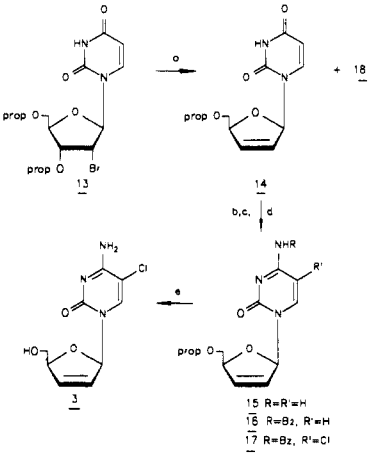


[a] (a) C_5H_5N, $(CH_3CO)_2O$; (b) MeOTrCl, C_5H_5N; (c) NCS, C_5H_5N, 30 min at 100 °C; (d) $(CF_3SO_2)_2O$, C_5H_5N; (e) NH_3, CH_3OH; (f) 80% CH_3COOH, 30 min at 60 °C.

reaction with phosphorus oxychloride and triazole, followed by ammonia, proved to be a superior method. 2′,3′-Dideoxy-5-chlorocytidine (ddClCyd, **6**) was synthesized by starting from **4** (Scheme II). After chlorination, the 5′-O-benzoylated derivative of **5** was isolated by column purification and treated with 2 equiv of trifluoromethanesulfonic anhydride in 1,2-dichloroethane–pyridine (4:1) for 4 h at room temperature. The mixture was subsequently poured into methanol saturated with ammonia and stirred overnight at ambient temperature. Purification on silica gel gave **6** in 17% overall yield. During the synthesis of **6**, side products were also noticed, but no attempts were made to isolate and characterize them. Because of the low yield of this conversion, the reaction was studied in more detail for the 3′-fluoro analogue. After treatment of 5′-*O*-acetyl-2′,3′-dideoxy-3′-fluoro-5-chlorouridine[2] with trifluoromethanesulfonic anhydride for 3 h at ambient temperature, TLC analysis showed complete reaction of the starting product to base-line material. The reaction mixture was poured into methanol saturated with ammonia and stirred overnight, after which TLC analysis indicated the presence of two major nucleosidic products. Apparently, treatment with ammonia not only displaced the (trifluoromethyl)sulfonyl moiety by attack of ammonia at C-4 (yielding cytosine derivatives), but in addition it also hydrolyzed the intermediate, giving rise again to the starting product. This could be explained by direct nucleophilic attack on sulfur with recovery of the uracil base (however, partial sulfonylation at N^3 cannot be excluded). After chromatographic purification, compound **11** (FddClUrd) was obtained in 43% yield, whereas only 22% of 2′,3′-dideoxy-3′-fluoro-5-chlorocytidine (FddClCyd, **12**) was isolated (Scheme III).

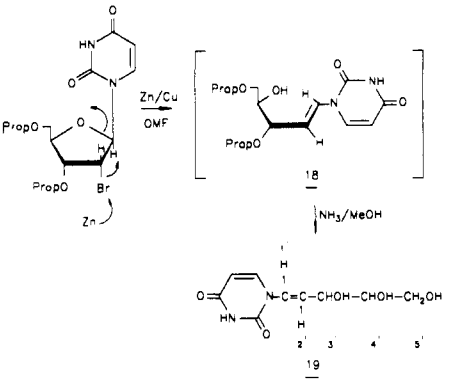
Chlorination of AzddUrd,[6] protected with a 5′-*O*-monomethoxytrityl group, with 1.5 equiv of *N*-chlorosuccinimide proceeded in 91% yield to give 5′-*O*-(monomethoxytrityl)-3′-azido-2′,3′-dideoxy-5-chlorouridine. Conversion to the 2′,3′-dideoxycytidine analogue was done with trifluoromethanesulfonic anhydride as for the synthesis of **6**. Due to the lipophilic and base-stable trityl group, extraction with ethyl acetate and purification of the intermediate was possible. This revealed the presence of 40% of the starting product although it had reacted completely with the anhydride according to TLC analysis. In addition, 50% of 5′-*O*-(monomethoxytrityl)-3′-azido-2′,3′-dideoxy-5-chlorocytidine was isolated as a foam. Detritylation with 80% acetic acid gave 34% of the target compound **9** (AzddClCyd) (15% overall from **7**).

Scheme IV[a]



[a] (a) Zn/Cu, HOAc; (b) o-$ClC_6H_4OP(O)Cl_2$, triazole, C_5H_5N followed by NH_4OH; (c) Bz_2O, C_5H_5N; (d) NCS, C_5H_5N, 30 min at 100 °C; (e) NH_3, CH_3OH.

Scheme V



Analogous conversion of **2** to **3** was not possible, using monomethoxytrityl as protecting group. 5′-O-protected **3** could be isolated in low yield, but the compound did not survive the detritylation conditions. An alternative strategy was aimed at synthesizing first 2′,3′-dideoxy-2′,3′-didehydrocytidine (d4Cyd) and chlorinating it afterward. Treatment of a 2′,3′-*trans*-halo acetate or a 2′,3′-*cis*-halo acetate with zinc/acetic acid appeared to be a convenient way to prepare olefinic nucleosides as shown by Classon et al.[8] Robins reported a modification of this method for synthesizing d4Ado by means of a zinc–copper couple in DMF.[9] This strategy was followed to prepare d4Urd on a larger scale.[10] The readily available 2′-bromo-3′,5′-di-*O*-propionyl-2′-deoxyuridine[11] was treated with approximately 5 equiv of a zinc–copper couple and 2 equiv of acetic acid in anhydrous DMF to yield 61% of **14**. Besides uracil (yield not determined), resulting from cleavage of the glycosidic bond, a minor side product (**18**)

(7) Herdewijn, P.; Van Aerschot, A. *Nucleosides Nucleotides* **1989**, *8*, 933.

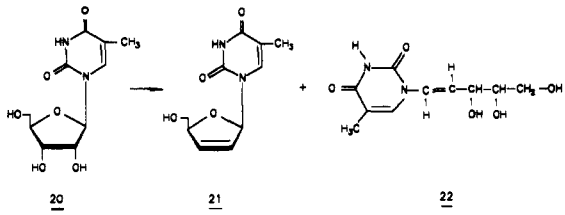
(8) Classon, B.; Garegg, P. J.; Samuelsson, B. *Acta Chem. Scand.* **1982**, *B36*, 251.

(9) Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.* **1984**, *25*, 367.

(10) During the course of this work, this same strategy has been used to prepare d4Urd and d4Thd by Mansuri et al. Mansuri, M. M.; Starrett, J. E. Jr.; Wos, J. A.; Tortolani, D. R.; Brodfuehrer, P. R.; Howell, H. G.; Martin, J. C. *J. Org. Chem.* **1989**, *54*, 4780.

(11) Marumoto, R.; Honjo, M. *Chem. Pharm. Bull.* **1974**, *22*, 128.

Scheme VI



20 21 22

was also isolated. According to NMR analysis it consisted of a mixture of two products containing two propionyl groups. On UV analysis it displayed a bathochrome shift. After deacylation, **19** was isolated in 7% yield from **13** (Scheme V). Its structure can be explained by trans elimination with the 4'-hydroxylate as the leaving moiety, instead of the *cis*-propionate at the 3'-position. UV analysis showed a bathochrome shift of **19**, as compared to that of d4Urd, to 281 nm, and MS gave a molecular ion at 228. NMR analysis showed a double bond with a large coupling constant of 14.4 Hz, indicating a trans orientation and four D_2O exchangeable protons (three hydroxyl groups and the amide proton). Selective decoupling experiments confirmed the proposed structure **19**.

The same reaction procedure with 5-methyluridine (**20**) afforded 32% of d4Thd (**21**) and 4.6% of (*E*)-1-(3(*S*),4(*R*),5-trihydroxypent-1-en-1-yl)thymine (**22**) (Scheme VI). This product also showed a bathochrome shift to 286 nm and a large coupling constant of 14.4 Hz in the ^1H NMR spectrum, indicating a trans configuration.

Propionylated d4Urd derivative **14** was converted to its d4Cyd counterpart **15** in a one-pot reaction following the method of Sung (Scheme IV).[12] Treatment with 2-chlorophenyl dichlorophosphate and 1,2,4-triazole for 15 h at room temperature, followed by addition of concentrated ammonia for 1 h and subsequent evaporation, yielded, after purification on silica gel, 81% of compound **15**. Prior tests with 3',5'-di-*O*-benzoyl-2'-deoxycytidine and 3',5',*N*-tribenzoyl-2'-deoxycytidine indicated that the latter gave better results on chlorination. Therefore, protection of the base moiety of **15** was done with benzoic anhydride in pyridine, affording 93% of compound **16**. Chlorination of this base-protected material was possible in modest yield: treatment with 1.5 equiv of *N*-chlorosuccinimide for 30 min at 100 °C afforded 38% of chlorinated nucleoside **17**, along with 21% of recovered starting product **16**. Prolonging the reaction time or increasing the amount of chlorinating agent only caused more decomposition. Deacylation with methanol saturated with ammonia finally yielded 2',3'-didehydro-2',3'-dideoxy-5-chlorocytidine (d4ClCyd, **3**) in 62% yield. Since a larger scale preparation was needed for the cytidine analogues **9** and **12**, an alternative strategy was developed for conversion of the uridine analogues **8** and **11** to **9** and **12**. As the method of Sung[12] works well for conversion of d4Urd to d4Cyd, a modification of this procedure was used (method B), avoiding the use of the expensive 2-chlorophenyl dichlorophosphate. Without isolating the intermediates, the 5'-O-protected derivatives of **8** and **11** were treated with 2 equiv of phosphorus oxychloride and 8 equiv of 1,2,4-triazole for 1 h at room temperature, after which an excess of concentrated ammonia was added for 5 min. After deprotection, cytidine analogues **9** and **12** were isolated in 61% and 66% overall yield (protection, conversion, deprotection), respectively. This method thus clearly proved superior to method A, which needs trifluoromethanesulfonic anhydride. The latter reagent seems to be valuable only in the purine series, as demonstrated with the synthesis of 9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-2,6-diaminopurine,[13] but not in the pyrimidine series.

Table I. Comparative Potency and Selectivity of 2',3'-Dideoxynucleoside Analogues as Inhibitors of HIV-1 Replication in MT-4 Cells

compound	ED_{50},[a] μM	CD_{50},[b] μM	SI[c]
d4ClUrd (2)	>20	44	<2.2
d4Urd[d]	>20	39	<2
d4ClCyd (3)	22	185	8.4
d4Cyd[e]	0.13	7.9	61
ddClUrd (5)	81	>500	>6
ddUrd[f]	210	>625	>3
ddClCyd (6)	>500	>500	
ddCyd[f]	0.3	40	120
AzddClUrd (8)	0.72	213	296
AzddUrd[d]	0.43	39	90
AzddClCyd (9)	9	877	97
AzddCyd[f]	7.6	160	21
FddClUrd (11)[d,g,h]	0.38	535	1408
FddUrd[d]	0.06	1.1	18
FddClCyd (12)	26	>1000	>38
FddCyd[f]	16	26	1.6
19	>500	>500	
22	475	>500	>1.0
d4Thd	0.05	19	380
AzddThd (AZT)[g]	0.003	4.81	1603

[a] Effective dose of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [b] Cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50%. [c] Selectivity index or ratio of CD_{50} to ED_{50}. [d] Data taken from ref 4. [e] Data taken from ref 29. [f] Data taken from ref 30. [g] Data taken from ref 3. [h] Data taken from ref 2.

Antiviral Activity

The anti-HIV-1 activity and cytotoxicity of the test compounds were evaluated in MT-4 cells (Table I). Substitution of a chlorine at C-5 of d4Cyd (**3**) resulted in a 100-fold decrease of antiviral activity, while the cytotoxicity was decreased only 25-fold. Both d4Urd and its 5-chloro-substituted derivative (**2**) were inactive at subtoxic concentrations. In contrast, introduction of a chlorine atom at C-5 of the uracil moiety of ddUrd (**5**), AzddUrd (**8**), and FddUrd (**11**) resulted in an improvement of their anti-HIV selectivity. This improvement in selectivity was mainly due to decreased toxicity, rather than increased antiviral activity of the test compounds. Indeed, compound **8** was about 5-fold and compound **11** was 500-fold less cytotoxic than their parent compounds AzddUrd and FddUrd. Consequently, compound **11** was about as selective an anti-HIV agent as AZT.

Introduction of a chlorine atom at C-5 of the cytosine moiety of ddCyd (compound **6**) removed the antiviral (and cytotoxic) activity. In contrast, compounds **9** and **12**, the 5-chloro-substituted derivatives of AzddCyd and FddCyd, exhibited similar antiviral activity as their parent compounds, but proved markedly less cytotoxic (Table I). Thus, akin to **11** (FddClUrd), compounds **9** (AzddClCyd) and **12** (FddClCyd) may be considered as selective anti-HIV agents.

Our preliminary findings indicate that the ddCyd derivatives **9** and **12** have poor substrate affinity for cytidine deaminase. Moreover, under the experimental conditions that lead to complete deamination of cytidine to uridine, there was no spectrophotometric evidence of deamination of either **9** or **12**. Addition of 1000 μM 2'-deoxycytidine annihilated the antiviral effects of **9** and **12**. In contrast,

(12) Sung, W. L. *J. Chem. Soc., Chem. Commun.* **1981**, 1089.

(13) Herdewijn, P.; Van Aerschot, A. *Tetrahedron Lett.* **1989**, *30*, 855.

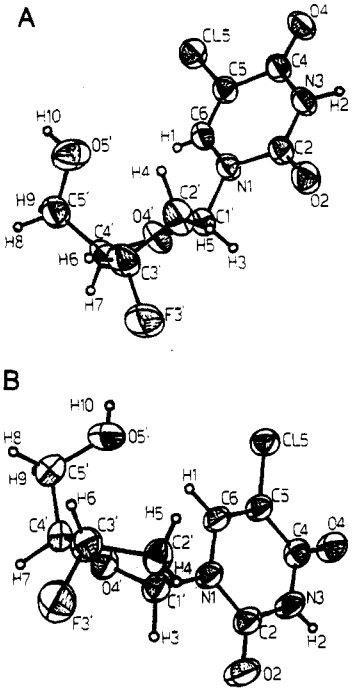


Figure 1. An ORTEP view[16] showing the numbering scheme of the molecules A and B.

addition of 250 μM thymidine did not affect the anti-HIV activity of **9** and **12** although it reversed the antiviral activity of FddClUrd and AzddClUrd (data not shown). These observations suggest that the cytidine derivatives do not need to be deaminated to the corresponding uridine derivatives to exert their antiviral effects. In this respect, compounds **9** and **12** should not simply be considered as prodrugs of FddClUrd and AzddClUrd, but anti-HIV agents in their own right.

The acyclic nucleoside derivatives of uracil (**19**) and thymine (**22**) had no marked anti-HIV activity.

X-ray Crystallographic Studies

The structure of compound **11**, the most potent anti-HIV agent among the 5-chlorinated nucleosides, was analyzed by X-ray crystallography and its conformation was compared to that of AZT.[14]

2′,3′-Dideoxy-3′-fluoro-5-chlorouridine: $C_9H_{10}N_2O_4ClF$, M_r = 264.64, monoclinic, $P2_1$, a = 5.8093 (3) Å, b = 18.4108 (9) Å, c = 10.2558 (5) Å, β = 95.693 (5)°, V = 1091.49 (9) Å^3, Z = 4, D_x = 1.610 Mg m^{-3}, Ni-filtered Cu Kα radiation, λ = 1.54178 Å, μ = 3.3717 mm^{-1}, $F(000)$ = 544, T = 298 K. Final R = 0.035 for 1766 unique observed reflections. The asymmetric unit contains two molecules (A and B). For molecule A: the N-glycosidic torsion angle χ has a value of −168.8 (3)° in the anti range; the sugar pucker is ${}^2_3T$ with P = 179 (1)° and ψ_m = 32 (1)° and the C4′–C5′ conformation is +*sc* with γ = 55.8 (4)°. For molecule B: the N-glycosidic torsion angle χ has a value of −131.3 (3)° in the anti range; the sugar pucker is ${}^2E$ with P = 163 (1)° and ψ_m = 32 (1)° and the C4′–C5′ conformation is +*sc* with γ = 51.1 (4)°. The conformational parameters are in accordance with the IUPAC–IUB Joint Commission on Biochemical Nomenclature guidelines.[15] The molecules in the crystal structure are linked by an intensive network of hydrogen bonds, including three of the less common (C–)H···O types.

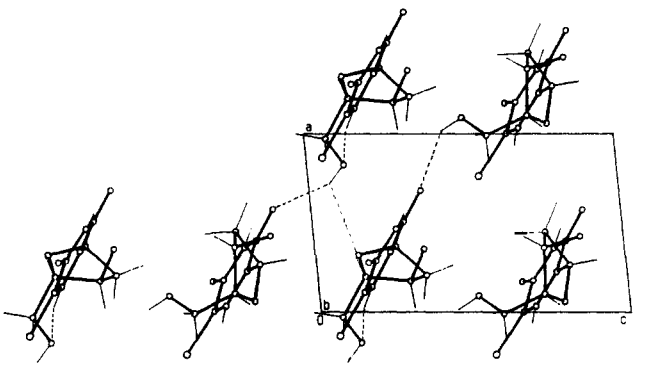


Figure 2. A PLUTO plot[27] showing the intramolecular hydrogen bonds and the most important intermolecular hydrogen bonds in the crystal structure as indicated by dashed lines.

An ORTEP view[16] of molecules A and B with the atomic numbering scheme is shown in Figure 1, parts A and B. Figure 2 shows the intramolecular hydrogen bonds and the classical intermolecular hydrogen bonds. The network of classical intermolecular hydrogen bonds contains one bifurcated or "three-centered" system.[17] This system involves O5′B, so that it donates its O5′-H to both O2A(x − 1, y, z − 1) and O4′B(x − 1, y, z). O5′A donates its O5′-H to O2B(x − 1, y, z) only. Molecule B has also one intramolecular hydrogen bond: C6B–H1B···O5′B. This nonclassical bond can reasonably be described as a hydrogen bond by satisfying the description established by Taylor and Kennard,[18] i.e. this hydrogen bond favors the +*sc* conformer with anti-oriented base moieties over the other two possible staggered forms (−*sc* and *ap*). Apart from the described hydrogen bonds there are three other (C–)H···O contacts present, which have also been interpreted as hydrogen bonds by Taylor and Kennard.[18] There is no close conformational similarity between compound **11** and AZT. This latter compound has two separate molecules in its asymmetric unit with χ = −125.9 (5)° and −172.0 (5)° and a sugar ring pucker P = 171 (1)° and 213 (1)°, respectively.[14]

Experimental Section

All methods and reagents were as previously described.[2] A 10% stock solution of trifluoromethanesulfonic anhydride was made in 1,2-dichloroethane and kept at −20 °C for 3 weeks at most. Electron-impact mass spectra were obtained by direct insertion on a AEI MS-12 mass spectrometer with 130–150 °C source temperature, 8 kV accelerating voltage, 100 μA trap current, and 70 eV ionization energy (relative intensity between brackets; M, molecular ion; B, base fragment; S, sugar fragment).

2′,3′-Didehydro-2′,3′-dideoxy-5-chlorouridine (2). An amount of 375 mg (1.78 mmol) of 2′,3′-didehydro-2′,3′-dideoxyuridine[5] was treated with 10 mL of pyridine–acetic anhydride (8:2) for 2 h at room temperature when TLC indicated complete acylation. The mixture was concentrated, dissolved in 50 mL of EtOAc and washed twice with a saturated aqueous $NaHCO_3$ solution. The organic layer was dried, evaporated, and coevaporated with pyridine. The oily residue was dissolved in 10 mL of anhydrous pyridine and 300 mg (2.25 mmol) of *N*-chlorosuccinimide was added. The mixture was heated for 30 min at 100 °C and poured into 100 mL of a 5% aqueous $NaHCO_3$ solution containing 2% sodium thiosulfate. The solution was extracted twice with EtOAc and the organic layer was dried and evaporated.

(14) Dyer, I.; Low, N.; Tollin, P.; Wilson, H. R.; Howie, R. A. *Acta Crystallogr.* **1988**, *C44*, 767.
(15) IUPAC-IUB Joint Commission on Biochemical Nomenclature. *Pure Appl. Chem.* **1983**, *55*, 1273.
(16) Johnson, C. K. *ORTEP. A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations.* Report ORNL-5138 (Third Revision); Oak Ridge National Laboratory, TN 1976.
(17) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(18) Taylor, R.; Kennard, O. *J. Am. Chem. Soc.* **1982**, *104*, 5063.

Flash purification of the dark brown mass on 40 g of silica gel ($CHCl_3$–MeOH 97:3) yielded a yellow foam which was treated for 6 h at room temperature with 100 mL of MeOH saturated with ammonia. Evaporation and purification ($CHCl_3$–MeOH 97:3 to 94:6) yielded 191 mg (0.78 mmol, 44% overall) of the title compound as a foam. The analytical sample (95 mg) was recrystallized twice from MeOH–Et_2O: mp 140–142 °C dec; UV (MeOH) λ_{max} 276 nm (ϵ = 9750); MS m/z 244 (1, M), 146 (42, B + H), 99 (100, S); ^1H NMR (DMSO-d_6) δ 3.65 (m, H-5', H-5''), 4.83 (m, H-4'), 5.1 (br, 5'-OH), 5.93 (dt, H-2', $J_{2',3'}$ = 6 Hz), 6.41 (dt, H-3', $J_{2',3'}$ = 6 Hz), 6.80 (m, H-1'), 8.25 (s, H-6), 11.75 (br, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 61.8 (C-5'), 87.7 (C-4'), 89.6 (C-1'), 107.0 (C-5), 125.8 (C-2'), 135.5 (C-3'), 138.6 (C-6), 149.9 (C-2), 159.1 (C-4) ppm. Anal. ($C_9H_9ClN_2O_4$) C, H, N.

2',3'-Dideoxy-5-chlorouridine (5). A portion of 420 mg (1.14 mmol) of 5'-*O*-benzoyl-2',3'-dideoxyuridine (**4**) and 400 mg (3 mmol) of *N*-chlorosuccinimide was dissolved in 20 mL of anhydrous pyridine and the mixture was heated for 45 min at 90 °C. Most of the pyridine was removed in vacuo and the dark brown residue was dissolved in 100 mL of $CHCl_3$ and washed twice with a saturated solution of $NaHCO_3$ containing 2% sodium thiosulfate. The organic layer was dried and evaporated and the residue was purified on silica gel ($CHCl_3$–MeOH 98:2), yielding 400 mg of a brown oil, which was treated with ammonia in methanol for 15 h at ambient temperature. Chromatographic purification ($CHCl_3$–MeOH 95:5) yielded 210 mg (0.85 mmol, 75%) of the title compound: mp 154–155 °C dec; UV (MeOH) λ_{max} 278 nm (ϵ = 9500); MS m/z 246 (11, B), 147 (17, B + 2 H), 146 (7, B + H), 101 (100, S); ^1H NMR (DMSO-d_6) δ 1.75–2.15 (m, H-3', H-3''), 2.20–2.50 (m, H-2', H-2''), 3.67 (m, H-5', H-5''), 4.07 (m, H-4'), 5.21 (t, 5'-OH), 5.92 (dd, H-1'), 8.48 (s, H-6), 11.80 (br, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 24.0 (C-3'), 32.3 (C-2'), 61.3 (C-5'), 82.1 (C-4'), 86.0 (C-1'), 106.4 (C-5), 138.0 (C-6), 149.5 (C-2), 159.1 (C-4) ppm. Anal. ($C_9H_{11}ClN_2O_4 \cdot 0.3H_2O$) C, H, N.

2',3'-Dideoxy-5-chlorocytidine (6). A portion of 560 mg (1.77 mmol) of 5'-*O*-benzoyl-2',3'-dideoxyuridine (**4**) was treated with *N*-chlorosuccinimide as for the preparation of **5**, yielding 540 mg (1.54 mmol) of benzoylated **5** as a brown oil. The oil was coevaporated twice with anhydrous pyridine, dissolved in 20 mL of dichloroethane–pyridine (4:1) and cooled in an ice–salt bath. To the resulting solution, 5 mL of a 10% solution of trifluoromethanesulfonic anhydride was added dropwise over 5 min. After 4 h at room temperature, the mixture was poured in 100 mL of methanol saturated with ammonia, and stirred for 15 h. Chromatographic purification ($CHCl_3$–MeOH 94:6) yielded 72 mg (0.29 mmol, 17%) of the title compound: mp 172 °C; UV (MeOH) λ_{max} 289 nm (ϵ = 6875); MS m/z 245 (17, M), 146 (84, B + 2 H), 145 (100, B + H), 110 (77, B + H – Cl), 101 (52, S); ^1H NMR (DMSO-d_6) δ 1.70–2.40 (m, H-2', H-2'', H-3', H-3''), 3.68 (m, H-5', H-5''), 4.05 (m, H-4'), 5.17 (t, 5'-OH), 5.87 (dd, H-1'), 7.16 (br) and 7.72 (br) (NH_2), 8.39 (s, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 23.9 (C-3'), 32.8 (C-2'), 61.3 (C-5'), 82.0 (C-4'), 86.3 (C-1'), 98.2 (C-5), 139.4 (C-6), 153.6 (C-2), 161.4 (C-4) ppm. Anal. ($C_9H_9ClN_3O_3$) C, H, N.

3'-Azido-2',3'-dideoxy-5-chlorouridine (8). A portion of 250 mg (0.99 mmol) of 3'-azido-2',3'-dideoxyuridine[6] (**7**) was coevaporated with anhydrous pyridine and dissolved in 25 mL of pyridine–acetic anhydride (4:1). The mixture was stirred for 2 h at room temperature, evaporated, and coevaporated twice with toluene to remove excess anhydride. The residue was dissolved in 10 mL of anhydrous pyridine and 270 mg (2 mmol) of *N*-chlorosuccinimide was added. The mixture was heated for 30 min at 100 °C, when TLC ($CHCl_3$–MeOH 95:5) indicated complete reaction. Most of the pyridine was removed in vacuo and the residue was dissolved in 25 mL of $CHCl_3$ and washed twice with a 5% aqueous $NaHCO_3$ solution containing 2% sodium thiosulfate. The organic layer was evaporated and the remaining oil was treated overnight at 4 °C with 50 mL of methanol saturated with ammonia. Chromatographic purification ($CHCl_3$–MeOH 97:3) yielded the title compound **8**, which crystallized from EtOAc to yield 128 mg (0.44 mmol, 45%): mp 170 °C; IR (KBr) 2100 cm^{-1} (N_3); UV (MeOH) λ_{max} 277 nm (ϵ = 9450); MS m/z 287 (8, M), 147 (6, B + 2 H), 146 (13, B + H), 142 (100, S); ^1H NMR (DMSO-d_6) δ 2.36 (m, H-2', H-2'', partially hidden by DMSO), 3.65 (m, H-5', H-5''), 3.86 (m, H-3'), 4.44 (q, H-4'), 5.35 (br, 5'-OH), 6.03 (t, H-1', J = 6 Hz), 8.30 (s, H-6), 11.8 (br, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 37.6 (C-2'), 60.2 (C-3'), 61.1 (C-5'), 85.2 and 85.4 (C-1', C-4'), 108.1 (C-5), 138.5 (C-6), 150.2 (C-2), 160.0 (C-4) ppm. Anal. ($C_9H_{10}ClN_5O_4$) C, H, N.

3'-Azido-2',3'-dideoxy-5-chlorocytidine (9). Method A. (a) A 2.6-g portion (4.95 mmol) 5'-*O*-(monomethoxytrityl)-3'-azido-2',3'-dideoxyuridine[19] was reacted with 1.0 g (7.5 mmol) of *N*-chlorosuccinimide in 100 mL of pyridine essentially as described for the synthesis of **8**. Workup and chromatographic purification yielded 2.52 g (4.5 mmol, 91%) of a light brown foam. UV (MeOH) λ_{max} 276 nm; ^1H NMR ($CDCl_3$) δ 2.45 (m, H-2', H-2''), 3.44 (t, J = 2.6 Hz, H-5', H-5''), 3.79 (s, CH_3O), 3.99 (m, H-3'), 4.29 (m, H-4'), 6.15 (t, J = 6.2 Hz, H-1'), 7.95 (s, H-6), 9.02 (br, NH) ppm.

(b) The 5-chlorinated nucleoside of the previous preparation (2.52 g, 4.5 mmol) was coevaporated with anhydrous pyridine and dissolved in 60 mL of dichloroethane–pyridine (5:1). To the cooled solution (0 °C) was added dropwise over 20 min 20 mL of a 10% solution of trifluoromethanesulfonic anhydride in 1,2-dichloroethane. After 3 h at room temperature TLC ($CHCl_3$–MeOH 95:5) indicated complete conversion of the starting product to base-line material, and the mixture was poured into 200 mL of methanol saturated with ammonia. The solution was stirred overnight at room temperature after which TLC revealed the presence of the starting material and of a new product in approximately equal amounts. After concentration, the residue was dissolved in EtOAc and washed with water and with brine. The organic layer was dried, evaporated, and purified, yielding 1.00 g (1.78 mmol, 40%) of recovered 5'-O-monomethoxytritylated **8** and 1.26 g (2.25 mmol, 50%) of 5'-*O*-(monomethoxytrityl)-3'-azido-2',3'-dideoxy-5-chlorocytidine as a foam [UV (MeOH) λ_{max} 288 nm]. This foam was treated with 100 mL of 80% acetic acid for 30 min at 60 °C. After adsorption on silica gel, the mixture was purified ($CHCl_3$ to $CHCl_3$–MeOH 94:6), yielding 380 mg (1.32 mmol) of a light yellow foam, which crystallized from MeOH–diethyl ether: yield 221 mg (0.77 mmol, 34%); mp 173–175 °C dec; UV (MeOH) λ_{max} 289 nm (ϵ = 7300), λ_{min} 264 nm; MS m/z 286 (5, M), 146 (46, B + 2 H), 145 (100, B + H), 142 (S, 9), 110 (27, B + H – Cl); ^1H NMR (DMSO-d_6) δ 2.34 (m, H-2', H-2''), 3.67 (m, H-5', H-5''), 3.86 (m, H-3'), 4.37 (q, J = 5.5 Hz, H-4'), 5.3 (br, 5'-OH), 6.03 (t, J = 6 Hz, H-1'), 7.20 (br) and 7.83 (br) (NH_2), 8.20 (s, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 37.5 (C-2'), 59.5 (C-3'), 60.4 (C-5'), 84.5 and 85.2 (C-1', C-4'), 99.2 (C-5), 139.2 (C-6), 153.6 (C-2), 161.5 (C-4) ppm. Anal. ($C_9H_{11}ClN_6O_3$) C, H, N.

Method B. A 2.0-g amount (4.03 mmol) 5'-*O*-trityl-3'-azido-2',3'-dideoxyuridine[6] was treated with 1.06 g (8 mmol) of *N*-chlorosuccinimide in 75 mL of anhydrous pyridine for 30 min at 95 °C. Workup as usual gave 1.98 g (3.73 mmol) of a brown foam (UV λ_{max} 277 nm). This chlorinated material was coevaporated with anhydrous pyridine and a premixed solution of 0.75 mL (8 mmol) of phosphorus oxychloride and 2.2 g (32 mmol) of 1,2,4-triazole in 70 mL of anhydrous pyridine was added. The mixture was stirred for 1 h at room temperature, after which 5 mL of concentrated ammonia was added at once. The mixture heated up considerably and was quickly cooled in an ice bath. After 5 min at room temperature the volatiles were removed in vacuo and the residue was suspended in 50 mL of $CHCl_3$–MeOH (1:1) and filtered over a small layer of Celite to remove most of the inorganic salts. After evaporation of the filtrate, the residue was purified partially on silica gel, yielding 2.2 g of an unpure brown foam, which was used as such for the detritylation reaction. The foam was dissolved in 50 mL of $CHCl_3$–MeOH (3:2), and 760 mg (4 mmol) of *p*-toluenesulfonic acid monohydrate was added. After 2 h at room temperature, TLC indicated that almost no reaction had taken place, and 1.5 g more of acid was added. After an additional 2 h the mixture was neutralized with ammonia; the products were adsorbed on some silica gel and purified by column chromatography on silica gel (50 g, elution $CHCl_3$–MeOH 93:7). Crystallization from MeOH–Et_2O afforded 706 mg (2.46 mmol) of **9** (61% overall yield).

2',3'-Dideoxy-3'-fluoro-5-chlorocytidine (12). Method A. After coevaporation with anhydrous pyridine 760 mg (2.47 mmol) of 5'-*O*-acetyl-2',3'-dideoxy-3'-fluoro-5-chlorouridine[2] was dissolved in 24 mL of dichloroethane–pyridine (5:1) and cooled in an ice–salt

(19) Reference 7 describes the synthesis of the 5'-*O*-trityl derivative.

bath. A 10% solution of trifluoromethanesulfonic anhydride in dichloroethane (10 mL) was added dropwise over 10 min, and the mixture was stirred for 3 h at ambient temperature. TLC ($CHCl_3$–MeOH 95:5) indicated complete reaction of the starting product to base-line material, and the contents were poured in 100 mL of methanol saturated with ammonia and stirred for 15 h. After this time TLC ($CHCl_3$–MeOH 9:1) revealed two major nucleoside products of which the faster moving one comigrated with FddClUrd (11). Flash chromatographic purification ($CHCl_3$–MeOH 97:3 to 9:1) yielded 286 mg (1.08 mmol, 43%) of recovered 11 and 600 mg of an unpure brown foam. After extensive purification, 148 mg (0.56 mmol, 22%) of the title compound was isolated as a white foam, which crystallized from MeOH–acetone: mp 184–185 °C; UV (MeOH) λ_{max} 288 nm (ϵ = 6700), λ_{min} 265 nm; MS m/z 263 (15, M), 146 (34, B + 2 H), 145 (100, B + H), 119 (13, S), 110 (30, B + H – Cl); ^{1}H NMR (DMSO-d_6) δ 1.90–2.60 (m, H-2′, H-2″, partially hidden by DMSO), 3.63 (d, J = 3.5 Hz, H-5′, H-5″), 4.20 (dt, J = 3.5 Hz, $J_{4',F}$ = 27.3 Hz, H-4′), 5.2 (br, 5′-OH), 5.29 (dd, J = 4 Hz, $J_{3',F}$ = 54 Hz, H-3′), 6.17 (dd, J = 5.7 and 8.5 Hz, H-1′), 7.3 (br) and 7.9 (br) (NH_2), 8.11 (s, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 38.3 (J = 20 Hz, C-2′), 60.9 (J = 11 Hz, C-5′), 85.2 (J = 23.2 Hz, C-4′), 85.5 (C-1′), 95.0 (J = 173.3 Hz, C-3′), 99.4 (C-5), 139.1 (C-6), 153.5 (C-2), 161.4 (C-4) ppm. Anal. ($C_9H_{11}ClFN_3O_3$) C, H, N.

Method B. A portion of 1.06 g (4 mmol) of 11 was treated with 3 g (13 mmol) of benzoic anhydride in 60 mL of anhydrous pyridine in the presence of 10 mg of 4-(dimethylamino)pyridine for 2 h at room temperature. After workup the 5′-O-benzoylated material was coevaporated with anhydrous pyridine and a premixed solution of 0.75 mL (8 mmol) of phosphorus oxychloride and 2.2 g (32 mmol) of 1,2,4-triazole in 70 mL of pyridine was added. The mixture was stirred for 1 h, cooled in an ice bath, and 5 mL of concentrated ammonia was added. After 5 min the mixture was evaporated and the residue was suspended in 50 mL of $CHCl_3$–MeOH (1:1) and filtered over a small path of Celite. Flash chromatography yielded 2.3 g of an unpure foam, which was deacylated with methanol saturated with ammonia. Purification on silica gel (50 g, elution $CHCl_3$–MeOH 93:7) and crystallization from MeOH–Et_2O gave 701 mg (2.65 mmol) of 12 (66% overall yield).

5′-*O*-Propionyl-2′,3′-didehydro-2′,3′-dideoxyuridine (14) and (*E*)-1-(3(*S*),4(*R*),5-Trihydroxypent-1-en-1-yl)uracil (19). A 9.5-g portion (22.65 mmol) of 2′-bromo-3′,5′-di-*O*-propionyl-2′-deoxyuridine[11] (13) was dissolved in 150 mL of anhydrous DMF. To the stirred solution was added 2 mL of acetic acid and 8 g of zinc–copper couple,[20] which resulted in a temperature raise to about 40 °C. After 30 min the mixture was filtered over a small path of Celite and the filtrate was neutralized with some ammonia. Evaporation in vacuo yielded an oil which was dissolved in $CHCl_3$ and washed twice with a saturated aqueous $NaHCO_3$ solution. Chromatographic purification ($CHCl_3$ to $CHCl_3$–MeOH 96:4) yielded 3.66 g (13.8 mmol, 61%) of the title compound 14 with UV, MS, and NMR analysis in accordance with the analysis of 2′,3′-didehydro-2′,3′-dideoxyuridine.[5]

Further elution afforded 900 mg (2.64 mmol, 12%) of a di-O-propionylated product, 18: UV (MeOH) λ_{max} 281 nm, λ_{min} 247 nm; MS m/z 340 (trace, M), 266 (3, M – CH_3CH_2COOH), 224 (7, M – $CHOHCH_2OCOCH_2CH_3$ + H), 223 (10, M – $CHOHCH_2OCOCH_2CH_3$), 168 (10, 224 – CH_3CHCO), 167 (20, 223 – CH_3CHCO), 112 (7, B + H), 81 (65, M – B – 2 CH_3CH_2COOH), 57 (100, CH_3CH_2CO). ^{1}H and ^{13}C NMR showed the product to be a mixture of two di-O-propionylated compounds, resulting from partial transesterification under the described reaction conditions. The product was therefore fully characterized after treatment with methanol saturated with ammonia for 15 h at room temperature. Purification yielded 380 mg (1.66 mmol, 7% from 13) of the title product 19, which crystallized from methanol–diethyl ether: mp 142–143 °C; UV (MeOH) λ_{max} 281 nm (ϵ 11.960), 270 nm (sh); MS m/z 228 (trace, M), 210 (3, M – H_2O), 180 (1, 210 – CH_2O), 179 (2, 210 – CH_2OH), 168 (21, M – $CHOHCH_2OH$ + H), 167 (100, M – $CHOHCH_2OH$), 124 (19, 167 – HNCO), 113 (7, B + 2 H), 112 (18, B + H); ^{1}H NMR (DMSO-d_6) δ 3.41 (m, H-5′, H-5″), 3.45 (m, H-4′), 4.05 (q, J = 5.5 Hz, H-3′), 4.44 (t, 5′-OH), 4.65 (d, 3′-OH), 5.07 (d, 4′-OH), 5.72 (d, J = 8 Hz, H-5), 5.92 (dd, J = 14.4 and 6.1 Hz, H-2′), 6.96 (dd, J = 14.4 and 0.8 Hz, H-1′), 7.91 (d, J = 8 Hz, H-6), 11.45 (br, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 63.1 (C-5′), 70.5 (C-3′), 75.0 (C-4′), 102.5 (C-5), 119.5 (C-2′), 124.1 (C-1′), 140.9 (C-6), 149.5 (C-2), 163.1 (C-4) ppm. Anal. ($C_9H_{12}N_2O_5$) C, H, N.

5′-*O*-Propionyl-2′,3′-didehydro-2′,3′-dideoxycytidine (15). After coevaporating twice with anhydrous pyridine, 1.46 g (5.46 mmol) of 5′-*O*-propionyl-2′,3′-didehydro-2′,3′-dideoxyuridine (14) and 2.4 g (35 mmol) of triazole were dissolved in 50 mL of anhydrous pyridine, and 2.5 mL (15 mmol) of 2-chlorophenyl dichlorophosphate was added. The red-brown mixture was left overnight at room temperature, after which 8 mL of concentrated ammonia (33%) was added. After stirring for 1 h, TLC ($CHCl_3$–MeOH 8:2) revealed one major nucleoside and the mixture was concentrated, adsorbed on 20 g of silica gel, and put on top of a small silica gel column (20 g). Stepwise elution [(1) $CHCl_3$, (2) $CHCl_3$–MeOH 95:5, (3) $CHCl_3$–MeOH 85:15] gave an unpure oil which was rechromatographed ($CHCl_3$–MeOH 92:8), affording 1.18 g (4.42 mmol, 81%) of the title product still containing small impurities: UV (MeOH) λ_{max} 271 and 237 nm, λ_{min} 255 nm; MS m/z 265 (M); ^{1}H NMR ($CDCl_3$) δ 1.11 (3 H, t, J = 7.5 Hz, CH_3), 2.33 (2 H, q, J = 7.5 Hz, CH_2CH_3), 4.27 (2 H, m, H-5′, H-5″), 5.03 (1 H, m, H-4′), 5.90 (1 H, d, J = 7.5 Hz, H-5), 5.97 (1 H, m, H-2′), 6.18 (1 H, m, H-3′), 7.02 (1 H, m, H-1′), 7.48 (1 H, d, J = 7.5 Hz, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 8.8 (CH_3), 27.1 (CH_2CH_3), 64.6 (C-5′), 84.1 (C-4′), 90.9 (C-1′), 95.3 (C-5), 127.9 (C-2′), 131.9 (C-3′), 140.4 (C-6), 156.3 (C-2), 165.7 (C-4), 173.8 (C=O) ppm.

***N*-Benzoyl-5′-*O*-propionyl-2′,3′-didehydro-2′,3′-dideoxycytidine (16).** Cytidine analogue 15 obtained in the previous preparation (1.18 g, 4.42 mmol) was coevaporated twice with anhydrous pyridine and dissolved in 40 mL of pyridine. A 2.3-g amount (10 mmol) of benzoic anhydride was added and the mixture was stirred for 2 h at room temperature, when another 2.3 g was added. After 2 h more, TLC ($CHCl_3$–MeOH 95:5) indicated complete reaction and the mixture was concentrated. The residue was dissolved in $CHCl_3$ and washed twice with a saturated $NaHCO_3$ solution. Chromatographic purification of the organic layer afforded 1.52 g (4.10 mmol, 93%) of the title compound as a white foam: UV (MeOH) λ_{max} 261 and 304 nm, λ_{min} 287 nm; ^{1}H NMR ($CDCl_3$) δ 1.14 (3 H, t, J = 7.5 Hz, CH_3), 2.34 (2 H, q, J = 7.5 Hz, CH_2CH_3), 4.23 (1 H, dd, J = 3 Hz and 12.5 Hz, H-5′), 4.53 (1 H, dd, J = 4 Hz and 12.5 Hz, H-5″), 5.16 (1 H, m, H-4′), 6.17 (2 H, m, H-2′, H-3′), 6.98 (1 H, m, H-1′), 7.35–7.63 (4 H, m, aromatic H, H-5), 7.83–8.17 (3 H, m, aromatic H, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 8.8 (CH_3), 27.2 (CH_2CH_3), 64.1 (C-5′), 85.2 (C-4′), 92.0 (C-1′), 96.7 (C-5), 127.6 (2 C_o), 127.9 (C-2′), 128.7 (2 C_m), 131.7 (C-3′), 132.9 (C_p and C_x), 144.1 (C-6), 154.7 (C-2), 162.4 (C-4), 167.0 (NHC(O)R), 173.6 (COOR) ppm.

***N*-Benzoyl-5′-*O*-propionyl-2′,3′-didehydro-2′,3′-dideoxy-5-chlorocytidine (17).** An amount of 1.2 g (3.25 mmol) of 16 was coevaporated with pyridine and dissolved in 40 mL of anhydrous pyridine. After addition of 665 mg (5 mmol) of *N*-chlorosuccinimide, the mixture was heated for 30 min at 100 °C and cooled. TLC ($CHCl_3$–MeOH 95:5) revealed a product with higher mobility and a series of side products, along with some starting material. After concentration, the black residue was dissolved in $CHCl_3$ and washed twice with a $NaHCO_3$ solution. After two chromatographic purifications, 250 mg (0.67 mmol, 21%) of the starting product 16 was recovered and 500 mg (1.23 mmol, 38%) of the title product 17 was isolated as a white foam: UV (MeOH) λ_{max} 263 and 331 nm, λ_{min} 290 nm; ^{1}H NMR ($CDCl_3$) δ 1.17 (3 H, t, J = 7.5 Hz, CH_3), 2.40 (2 H, q, J = 7.5 Hz, CH_2CH_3), 4.23 (1 H, dd, J = 3 Hz and 13 Hz, H-5′), 4.56 (1 H, dd, J = 4 Hz and 13 Hz, H-5″), 5.10 (1 H, m, H-4′), 5.95 (1 H, dm, H-2′), 6.32 (1 H, dm, H-3′), 6.97 (1 H, m, H-1′), 7.38–7.57 (3 H, m, aromatic H), 7.90 (1 H, s, H-6), 8.25–8.39 (2 H, m, aromatic H) ppm; ^{13}C NMR ($CDCl_3$) δ 8.9 (CH_3), 27.3 (CH_2CH_3), 63.8 (C-5′), 85.0 (C-4′), 90.6 (C-1′), 109.0 (C-5), 126.8 (C-2′), 128.1 (2 C_o), 130.0 (2 C_m), 132.9 (C_p), 133.6 (C-3′), 136.1 (C_x), 137.7 (C-6), 147.3 (C-2), 155.5 (C-4), 173.8 (COOR) ppm.

2′,3′-Didehydro-2′,3′-dideoxy-5-chlorocytidine (3). 5-Chlorinated cytidine analogue 17 obtained in the previous preparation was reacted with 50 mL of methanol saturated with ammonia for 8 h at room temperature. After evaporation, the

(20) In *Reagents for Organic Synthesis*; Fieser, M., Fieser, L. F., Eds.; Interscience: New York, 1975; Vol. 5, p 758.

residue was purified by flash chromatography ($CHCl_3$–MeOH 94:6), affording 188 mg (0.77 mmol, 62%) of the title product **3**, which crystallized from MeOH–diethyl ether: mp 144–145 °C; UV (MeOH) λ_{max} 288 nm (ϵ = 6760), λ_{min} 267 nm; MS m/z 243 (2, M), 145 (97, B + H), 110 (100, B + H – Cl), 98 (48, S – H); ^{1}H NMR (DMSO-d_6) δ 3.63 (d, J = 2.6 Hz after exchange with D_2O, H-5', H-5''), 4.81 (m, H-4'), 5.07 (t, J = 5 Hz, 5'-OH), 5.91 (dm, $J_{2',3'}$ = 5.7 Hz, H-2'), 6.34 (dm, $J_{2',3'}$ = 5.7 Hz, H-3'), 6.84 (m, H-1'), 7.21 (br) and 7.84 (br) (NH_2), 8.14 (s, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ 61.9 (C-5'), 87.4 (C-4'), 90.2 (C-1'), 99.0 (C-5), 126.7 (C-2'), 134.4 (C-3'), 140.1 (C-6), 153.9 (C-2), 161.5 (C-4) ppm. Anal. ($C_9H_{10}ClN_3O_3$) C, H, N.

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (d4Thd, 21) and (*E*)-1-(3(*S*),4(*R*),5-Trihydroxypent-1-en-1-yl)thymine (22). 5-Methyluridine (**20**) (9.3 g, 36 mmol) was reacted with 17 mL (230 mmol) of acetyl bromide in acetonitrile as described for the synthesis of **13**.[11] After workup and flash purification to remove most of the brown tar, the impure product (12.1 g) was treated with 9.1 g (140 mmol) of a Zn/Cu couple[20] and 3.25 mL (57 mmol) of glacial acetic acid in 250 mL of anhydrous DMF. After stirring for 30 min at room temperature, the suspension was filtered over a small path of Celite and concentrated in vacuo. The oil was dissolved in 200 mL of $CHCl_3$ and washed twice with a saturated aqueous $NaHCO_3$ solution. Evaporation of the organic layer and chromatographic purification gave 5'-O-acylated d4Thd and a di-O-acylated side product. Treatment of the respective compounds with ammonia in methanol afforded, after purification on silica gel, 2.6 g (11.6 mmol, 32% from **20**) of d4Thd[6] (**21**) and 400 mg (1.66 mmol, 4.6% from **20**) of the title product **22**, which was recrystallized from methanol. **22**: mp 150–151 °C; UV (MeOH) λ_{max} 286 nm (ϵ = 10060), λ_{max} 231 nm (ϵ = 9300); MS m/z 242 (trace, M), 224 (4, M – H_2O), 194 (3, 224 – CH_2O), 193 (3, 224 – CH_2OH), 182 (23, M – CHOHCH_2OH + H), 181 (100, M – CHOHCH_2OH), 138 (13, 181 – HNCO), 127 (9, B + 2 H), 126 (35, B + H); ^{1}H NMR (DMSO-d_6) δ 3.43 (m, H-4', H-5', H-5''), 4.03 (m, H-3'), 4.40 (t, 5'-OH), 4.56 (d, 3'-OH), 4.98 (d, 4'-OH), 5.90 (dd, J = 14.4 and 6.1 Hz, H-2'), 6.99 (d, J = 14.4 Hz, H-1'), 7.83 (s, H-6), 10.30 (br, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 12.4 (CH_3), 63.3 (C-5'), 71.0 (C-3'), 75.1 (C-4'), 111.1 (C-5). 119.0 (C-2'), 125.0 (C-1'), 137.1 (C-6), 150.0 (C-2), 164.5 (C-4) ppm. Anal. ($C_{10}H_{14}N_2O_5$) C, H, N.

Single-Crystal X-ray Analysis. Yellowish prismatic crystal obtained at room temperature from an ethanol–water solution: dimensions ~0.35 × 0.2 × 0.2 mm; Hilger & Watts computer-controlled four-circle diffractometer, Ni-filtered Cu Kα radiation, $\omega/2\theta$ scan technique ($2\theta_{max}$ = 130°, $0 \le h \le 7$, $-22 \le k \le 22$, $-12 \le l \le 12$); cell dimensions by least-squares refinement of the setting angles of 24 reflections with $37 < 2\theta < 51°$, space group $P2_1$ from systematic absences, $0k0$ for k odd. Four standard reflections (040, 002, 230, 07–2) monitored after every 50 reflections showed no significant decrease in intensity per hour: 4009 reflections measured, 1875 unique reflections, 1766 unique observed reflections [$I > 3\sigma(I)$], Lorentz–polarization corrections, absorption corrections by the method of North et al.[21] based on two reflections (40–1, 100) with values between 0.9924 and 0.7423, scattering factors from Cromer et al.[22] and Stewart et al.[23] (for H). R_{int} = 0.037. The structure was solved by MULTAN 82.[24] The E-map calculated from the solution with the best figure of merit revealed 18 of the 34 non-hydrogen atoms in the asymmetric unit. The remaining atoms were obtained from a subsequent Fourier synthesis. A difference synthesis revealed the position of two hydrogen atoms (attached to atoms 05'A and 05'B). All other hydrogen atoms were included at calculated positions (C–H and N–H distances 0.95 Å). The hydrogen atoms were refined with fixed isotropic temperature factors 1.3 times that of the parent atom. All other atoms were refined anisotropically on F by full-matrix least-squares calculations. The refinement converged at R = 0.035, wR = 0.045, s = 0.523. $w = (C_0 + C_1|F_o| + C_2|F_o|^2 + C_3|F_o|^3)^{-1}$, where C_0 = 100, C_1 = 1, C_2 = 0.001, C_3 = 0.000005. A total of 367 refined parameters gave max shift/esd = 0.03; the minimum and maximum electron density were –0.417 and 0.211 e Å^{-3}. The number of reflections per refined variable was 1766/367 = 4.8. All calculations were performed on a PDP-11/73 microcomputer using SDP/PDP[25] and PARST.[26]

Antiviral Assay Procedures. HIV (strain HTLV-III$_B$) was prepared from the culture supernatant of persistently HTLV-III$_B$-infected MT-4 cells. The antiviral activity assays[28] were based on the protection of HIV-infected MT-4 cells against HIV-induced cytopathogenicity. They were run in parallel with the cytotoxicity assays aimed at establishing the toxicity of the compounds for uninfected MT-4 cells.

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Supplementary Material Available: All further information concerning the X-ray analysis (atomic coordinates and equivalent isotropic thermal parameters, bond lengths, bond angles, torsion angles, and geometry of the hydrogen bonds) is available as supplementary material (5 pages). Ordering information is given on any current masthead page.

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