Synthesis and Anticancer Activity of Various 3'-Deoxy Pyrimidine Nucleoside Analogues and Crystal Structure of 1-(3-Deoxy-β-D-*threo*-pentofuranosyl)cytosine[1]

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Various 3'-deoxy pyrimidine nucleoside analogues have been synthesized for evaluation as potential anticancer and antiviral agents. Among these compounds, 1-(3-deoxy-β-D-*threo*-pentofuranosyl)cytosine (**10**, 3'-deoxy-ara-C) and 3'-deoxycytidine (**22**) had significant anticancer activity against CCRF-CEM, L1210, P388, and S-180 cancer cell lines in vitro, producing ED_{50} values of 2, 10, 5, and 34 μM, respectively, for 3'-deoxy-ara-C (**10**); and 25, 5, 2.5, and 15 μM, respectively, for 3'-deoxycytidine (**22**). Thus, 3'-deoxy-ara-C (**10**) was 12.5 times more active against CCRF-CEM cells than 3'-deoxycytidine (**22**). The 2'-*O*-acetyl, 5'-*O*-acetyl, and 2',5'-di-*O*-acetyl derivatives of 3'-deoxy-ara-C (**10**), compounds **34**, **31**, and **30**, demonstrated anticancer activity in the same range as 3'-deoxy-ara-C (**10**) against CCRF-CEM, L1210, P388, and S-180 cells. The 5'-*O*-acetyl derivative (**31**) had significantly greater activity against CCRF-CEM with an ED_{50} value of 0.4, but this compound also showed similar activity, as did 3'-deoxy-ara-C, against L1210, P388, and S-180 with ED_{50} values of 3, 3, and 13 μM, respectively. 3'-Deoxy-ara-C was also evaluated in vitro against HSV-2, HCMV, and GPCMV viruses and was found to be not very active with respective IC_{50} values of 110, 220, and 1000 μM. The single-crystal structure of 3'-deoxy-ara-C (**10**) was determined by X-ray crystallography. There are two molecules of the nucleoside and one molecule of water in the asymmetric unit. The sugar moieties of the two nucleoside molecules adopt different conformations. In molecule A, the ring pucker is C3'-endo with $P = 18.7°$ and $\tau_m = 37.3°$, while the CH_2OH side chain is gauche$^+$. In molecule B, the ring pucker is C2'-endo with $P = 156.8°$ and $\tau_m = 37.8°$ and the side chain is trans.

Several natural and synthetic cytosine nucleoside analogues have potent anticancer or antiviral activity.[2] For example, 1-β-D-arabinofuranosylcytosine (ara-C) is a potent anticancer agent[3] and 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) is a potent antiviral agent.[4] However, many of the biologically active compounds have problems, such as rapid deamination to an inactive uracil nucleoside analogue, or too low a therapeutic index for human use. Hence, many structural modifications have been made in an attempt to circumvent these problems.[5-14]

We have evaluated some of the biological properties of 1-(3-deoxy-β-D-*threo*-pentofuranosyl)cytosine (**10**, 3'-deoxy-ara-C), a compound first reported by Kreis et al.[15] to be resistant to enzymatic deamination by cytidine deaminase from mouse kidney, but the synthetic methodology and the physical and spectroscopic properties of this compound were not described. Recently, Webb et al.[16] reported the synthesis of 3'-deoxy-ara-C (**10**).

3'-Deoxy-ara-C (**10**) also has been synthesized in our laboratory by a different methodology and evaluated against several neoplastic cell lines as well as viruses in culture. Our preliminary findings indicate that 3'-deoxy-ara-C (**10**) has significant anticancer activity against CCRF-CEM, L1210, and P388 neoplastic cell lines.

On the basis of these findings, various 3'-deoxy pyrimidine nucleoside analogues have been synthesized as potential anticancer and/or antiviral agents.

The present report describes the syntheses of these compounds and the evaluation of their anticancer activity against various neoplastic cell lines in vitro, and the X-ray crystallographic analysis of 3'-deoxy-ara-C (**10**). To our best knowledge, this is the first crystal structure determination of a 3'-deoxyarabinofuranosyl nucleoside analogue.

Chemistry

1-(3-Deoxy-β-D-*threo*-pentofuranosyl)cytosine (**10**, 3'-

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(1) Inquiries regarding X-ray crystallography should be addressed to G. I. Birnbaum.
(2) Townsend, L. B.; Cheng, C. C. In *Antineoplastic and Immunosuppressive Agents*; Springer-Verlag: New York, 1974; Vol. 1, p 113.
(3) Cohen, S. S. *Prog. Nucleic Acid Res. Mol. Biol.* **1966**, *5*, 1.
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(12) Beisler, J. A.; Abbasi, M. M.; Driscoll, J. S. *Biochem. Pharmacol.* **1977**, *26*, 2469.
(13) Bobek, M.; Kavai, I.; Sharma, R. A.; Grill, S.; Dutschman, G.; Cheng, Y. C. *J. Med. Chem.* **1987**, *30*, 2154.
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(15) Kreis, W.; Watanabe, K. A.; Fox, J. J. *Helv. Chim. Acta* **1978**, *61*, 1011.
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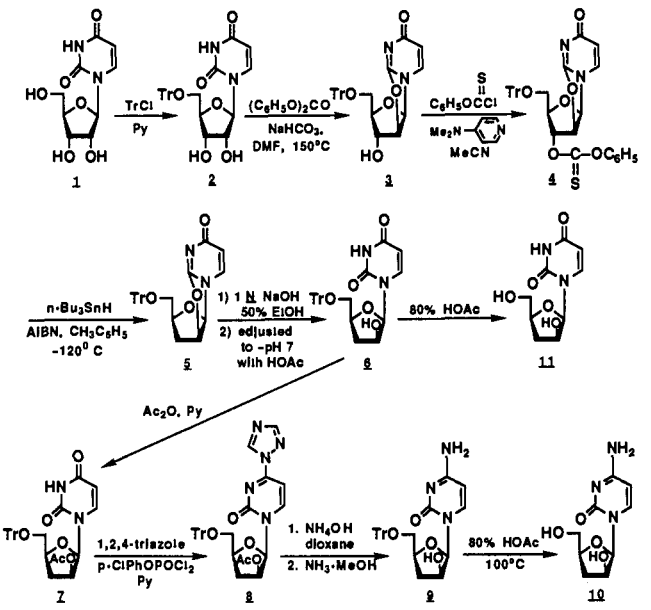
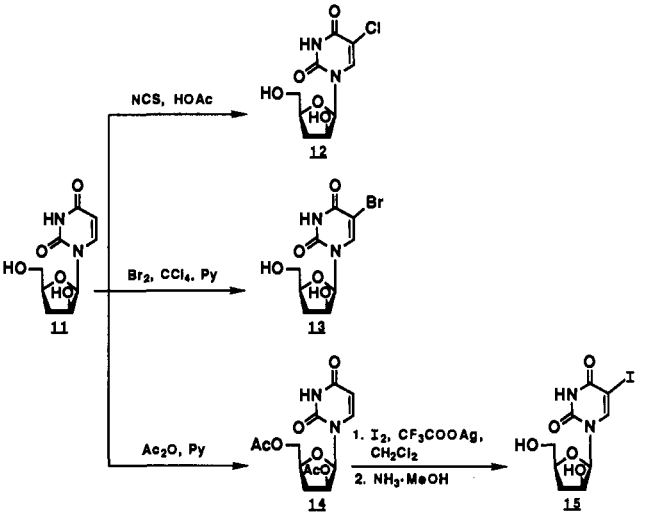
Scheme I



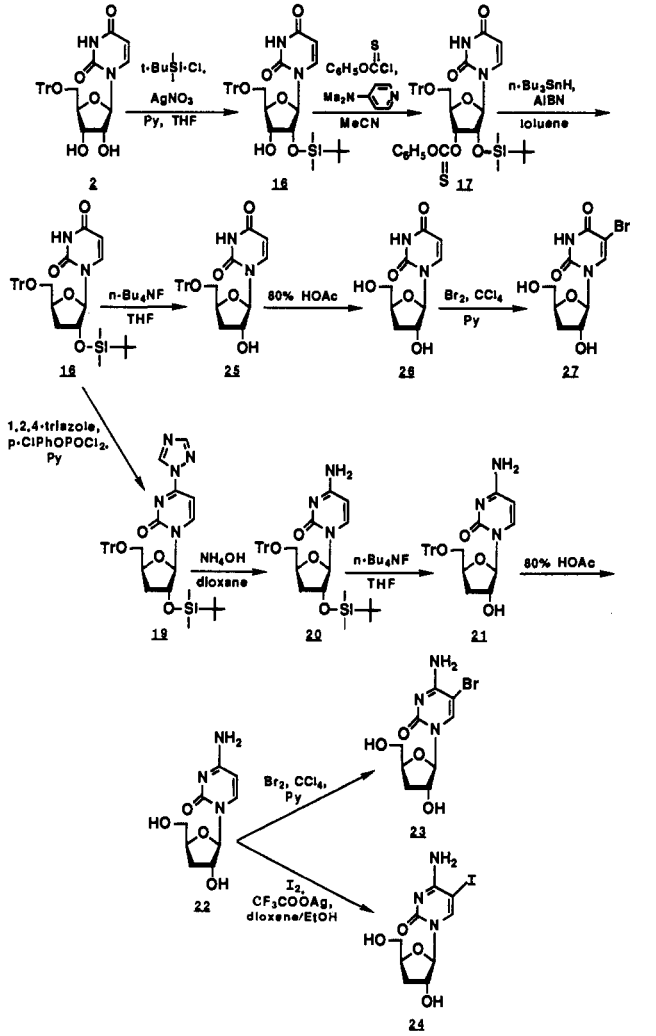
deoxy-ara-C) was synthesized as follows: Tritylation[17] of uridine (1) with trityl chloride in dry pyridine gave the 5′-*O*-trityl derivative **2**. Conversion[18] of **2** to the corresponding 2,2′-anhydro analogue **3** was accomplished by the reaction of compound **2** with diphenyl carbonate and sodium bicarbonate in DMF at 150 °C. Treatment[19] of **3** with phenyl chlorothionocarbonate and 4-(dimethylamino)pyridine in MeCN under nitrogen at room temperature gave the 3′-*O*-phenoxythiocarbonyl derivative **4**. Reduction[19] of **4** with tri-*n*-butyltin hydride and 2,2′-azobis(2-methylpropionitrile) (AIBN) in toluene at reflux temperature produced the 3′-deoxy nucleoside analogue **5**. Treatment of **5** with 1 N NaOH, followed by neutralization with HOAc to pH 7, afforded the arabinoside **6**. Acetylation of **6** with acetic anhydride in pyridine gave the acetate **7**. Treatment[20] of **7** with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature produced the 4-triazolylpyrimidinone derivative **8**. Reaction of **8** with aqueous ammonia in dioxane, followed by methanolic ammonia, gave the cytidine derivative **9**. Detritylation of **9** with 80% HOAc at 100 °C afforded the desired product **10**. The synthetic sequence is described in Scheme I. Recently, Webb et al.[16] reported the synthesis of 3′-deoxy-ara-C (**10**) which involved a key step of reductive cleavage of 2′,3′-anhydrolyxofuranosyl nucleoside. This method, however, runs a risk of producing a certain amount of 2′-deoxy-β-D-xylosyl derivative, which might be difficult to separate. The physical state and melting point of **10** were not described in their report. Our synthetic route unambiguously gave **10** which were fine white crystals with a melting point of 172–173 °C and gave satisfactory elemental microanalyses. Detritylation of **6** with 80% HOAc yielded 1-(3-deoxy-β-D-*threo*-pentofuranosyl)uracil (**11**).

5-Halogen-substituted 3′-deoxyarauridine analogues (**12**, **13**, and **15**) were prepared as described in Scheme II. After direct chlorination[21] of compound **11** with *N*-

Scheme II



Scheme III



chlorosuccinimide (NCS) in glacial acetic acid at reflux temperature for 0.5 h, treatment of the resulting crude product, which was contaminated with a small amount of acetate side products, with methanolic ammonia yielded the 5-chloro analogue **12**. Bromination[22] of 11 in pyridine

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(18) Hampton, A.; Nichol, A. W. *Biochemistry* **1966**, *5*, 2076.
(19) Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.
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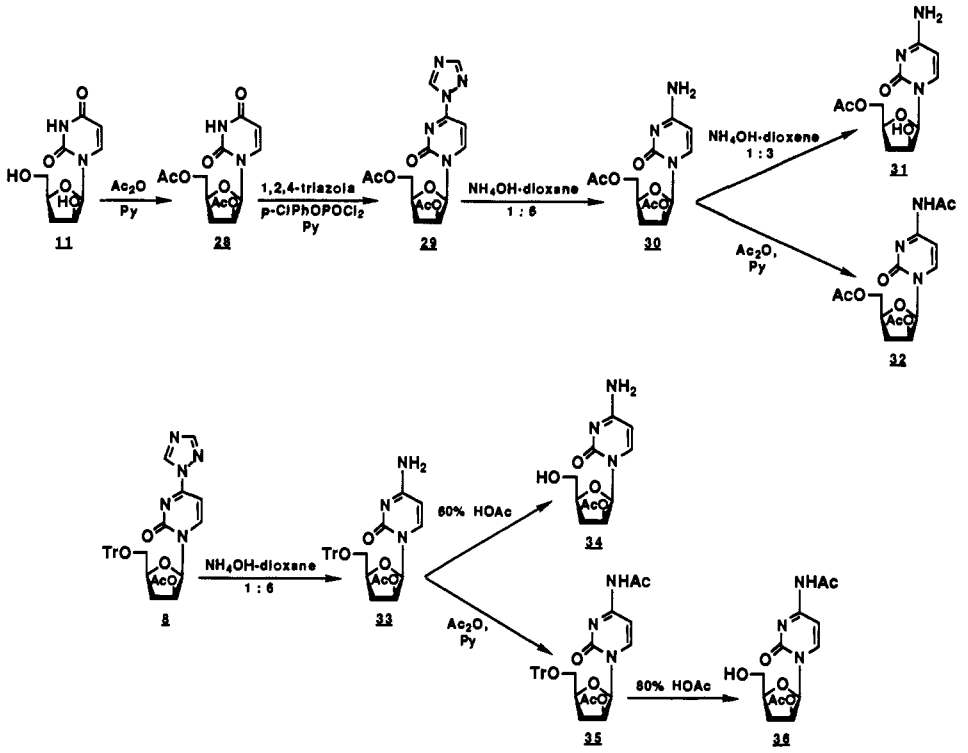
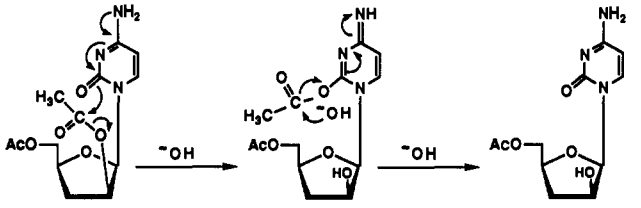
Scheme IV



with bromine in carbon tetrachloride gave the corresponding 5-bromo arabinoside **13**. Iodination[23] of the diacetate **14** with iodine and silver trifluoroacetate in dry methylene chloride, followed by treatment of the resultant 5-iodo derivative with methanolic ammonia produced the 5-iodo analogue **15**.

3'-Deoxycytidine was synthesized as described in Scheme III. Protection of the 2'-OH group of compound **2** with *tert*-butyldimethylsilyl chloride/silver nitrate/pyridine in tetrahydrofuran[24] gave **16**, which was then treated with phenyl chlorothionocarbonate and 4-(dimethylamino)pyridine in MeCN under nitrogen at room temperature to yield **17**. Reduction of **17** with tri-*n*-butyltin hydride and 2,2'-azobis(2-methylpropionitrile) (AIBN) in toluene afforded the 3'-deoxy analogue **18**. Treatment of **18** with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature gave **19**. Reaction of **19** with aqueous ammonia in dioxane yielded the cytidine derivative **20**. Desilylation of **20** with tetra-*n*-butylammonium fluoride in THF produced **21**, which was then heated with 80% HOAc at 100 °C to afford 3'-deoxycytidine **22**.[25] Similarly, compound **18** was converted to **25** and subsequently to **26**.[25] Bromination of **22** and **26** in pyridine with bromine in carbon tetrachloride gave the corresponding 5-bromo analogues, **23** and **27**, respectively. Iodination of **22** with iodine and silver trifluoroacetate in dry methylene chloride produced the 5-iodo analogue **24**.

Scheme V



Various acetate derivatives of 3'-deoxy-ara-C (**10**) were also synthesized as shown in Scheme IV. Acetylation of compound **11** with acetic anhydride in pyridine gave the diacetate derivative **28**, which was then converted to the 4-triazolylpyrimidinone derivative **29**. Treatment of **29** with aqueous ammonia in dioxane (1:6, v/v) for 2 h afforded the 3'-deoxy-ara-C diacetate **30**. Compound **30** was stirred with a mixture of NH_4OH/dioxane (1:3, v/v) at room temperature for 2 h. The 2'-*O*-acetyl group was selectively hydrolyzed to yield 1-(5-*O*-acetyl-3-deoxy-1-β-D-*threo*-pentofuranosyl)cytosine (**31**) (Scheme IV). The structure of compound **31** was assigned by NMR spectrum. There were two *O*-acetyl methyl singlets at δ 1.80 and 2.05 ppm in compound **30**. The 2'-*O*-acetyl group was assigned to the δ 1.80 signal. This was based on the anisotropy effect resulting from the 5,6 double bond of the base. The 2'-*O*-acetyl group of compound **30** was in the cone of positive shielding from the 5,6 double bond. Therefore, it would have a lower chemical shift value than that of the 5'-*O*-acetyl group methyl signal, similar to those results in the uracil analogues series.[26] The 2'-*O*-acetyl methyl singlet of **30** at δ 1.80 ppm was absent in **31**. Furthermore, while comparing the chemical shifts of the 2'-hydrogens of compounds **30** and **31**, it was found that there was a

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(24) Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Tetrahedron Lett.* **1981**, *22*, 4775.
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(26) Robins, M. J.; Maccoss, M. In *Chemistry and Biology of Nucleosides and Nucleotides*; Academic Press, Inc: New York, NY, 1978; p 311.
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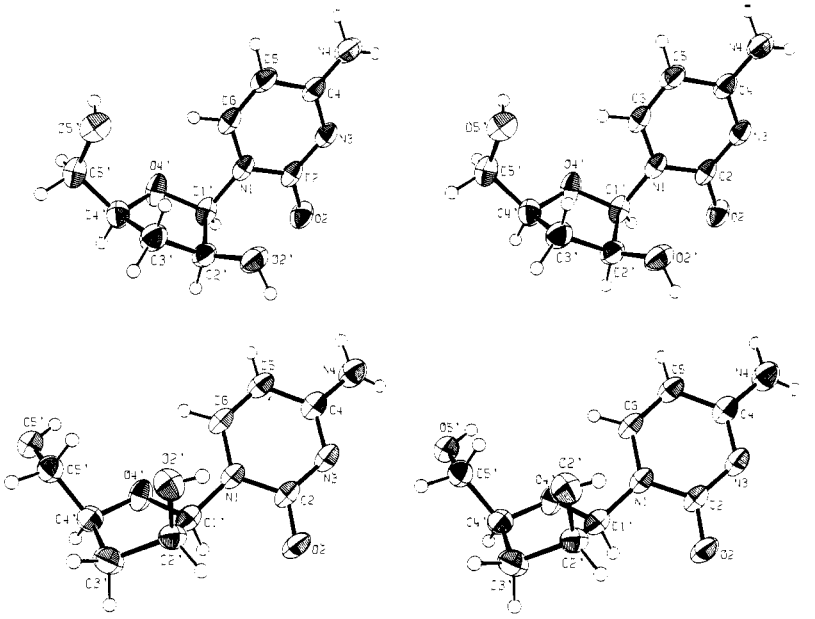

Figure 1. Stereoscopic views of molecule A (top) and molecule B (bottom); the thermal ellipsoids correspond to 50% probability.

significant upfield shift of 1.30 ppm of the 2′-hydrogen in compound **31**, indicating that the 2′-*O*-acetyl group was removed. The rationale, that the 2′-*O*-acetyl group is more sensitive to alkaline hydrolysis than the less hindered 5′-*O*-acetyl group (**30** to **31**), may be attributed to an intramolecular catalysis as shown in Scheme V.

Acetylation of **30** with acetic anhydride in pyridine yielded the triacetate analogue **32**. For the synthesis of 5′-open nucleoside **34**, the 5′-*O*-trityl-blocked intermediate **8** was used as the starting material. Treatment of **8** with NH_4OH/dioxane (1:6, v/v), followed by selective cleavage of 5′-*O*-trityl group with 80% acetic acid, gave **34**. The NMR signals of 2′-acetoxy methyl singlet and 2′-H were in agreement with those in compound **30**. Acetylation of compound **33** produced **35**, which was then treated with 80% acetic acid at 100 °C to afford N^4- and 2′-O-acetylated derivative (**36**).

X-ray Crystal Structure Analysis of 3′-Deoxy-ara-C (10). In order to determine the conformation of 3′-deoxy-ara-C (**10**), we carried out an X-ray structure analysis. The material crystallizes with two molecules of the nucleoside and one molecule of water in the asymmetric unit. Views of the two molecules are shown in Figure 1. The sugar moieties of the nucleoside adopt different conformations. In molecule A, the ring pucker is C3′-endo[28] with $P = 18.7°$ and $\tau_m = 37.3°$ while the CH_2OH side chain is gauche$^+$. In molecule B, the ring pucker is C2′-endo with $P = 156.8°$ and $\tau_m = 37.8°$, and the side chain is trans. These two ring puckers are the most common ones in most nucleosides. On the basis of the gauche effect on the C1′–O4′ and C2′–O2′ bonds, one would expect the C2′-endo pucker to be somewhat more stable, but the energy barrier between the two conformations is quite low.[29] The intramolecular distances, different for each ring pucker, may affect the substrate properties of 3′-deoxy-ara-C (**10**) for phosphorylation of 5′-OH. A correlation between the sugar ring conformation and activity of nucleoside analogues against human immunodeficiency virus was recently discussed by Van Roey et al.[30]

Both observed side-chain rotamers are favored by the gauche effect and the gauche$^+$ rotamer is often further stabilized by an intramolecular C6–H···O5′ hydrogen bond.[29] However, this is not possible in arabinonucleosides with a C2′-endo ring pucker unless O2′ and O5′ are hydrogen-bonded, hence, the trans rotamer in molecule B.

It is remarkable that the glycosyl torsion angles χ (C2–N1–C1′–O4′) are very similar in both molecules (−151.9° in molecule A and −155.3° in molecule B) despite the different sugar ring puckers. This structure determination therefore serves to confirm our earlier tentative conclusion[29] that these two conformational parameters (χ and P) are not correlated, although such a correlation had been previously suggested.[31] It is also noteworthy that the lengths of the glycosyl bond (N1–C1′) are significantly different in the two molecules: 1.491 (3) Å in molecule A and 1.462 (3) Å in molecule B; it is generally assumed that this bond length varies with the glycosyl torsion angle.[30] This difference in a bond length is the largest one between the two molecules. Most other corresponding bond lengths (their esd's range from 0.002 to 0.003 Å) are the same within experimental error and have normal values.

There is an intricate network of nine distinct intermolecular N–H···O, O–H···O, and O–H···N hydrogen bonds connecting the nucleoside and water molecules in the crystal. In particular, the water molecules connect the two independent nucleoside molecules by donating a proton to O2 in each of them. Furthermore, there is partial stacking of the cytosine bases of molecules A and B, thus further stabilizing the crystal structure.

Biological Evaluation

The synthesized compounds were evaluated for their anticancer activity in vitro against CCRF-CEM, L1210, P388, and S-180 cancer cell lines. 3′-Deoxy-ara-C (**10**) and 3′-deoxycytidine (**22**) demonstrated comparable anticancer activity against L1210, P388, and S-180, with ED_{50} values of 10, 5, and 34 μM, respectively, for 3′-deoxy-ara-C (**10**);

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Table I. Anticancer Activity of 3'-Deoxy Pyrimidine Nucleoside Analogues in Vitro

	ED_{50}[a] values in μM against cell lines			
compd	CCRF-CEM	L1210	P388	S180
10	2	4	5	34
11	>100	>100	>100	>100
12	>100	>100	>100	>100
13	>100	>100	>100	>100
15	>100	>100	>100	>100
22	25	5	2.5	15
23	>100	>100	>100	>100
24	>100	>100	>100	>100
26	>100	50	25	>100
27	>100	>100	>100	>100
30	1	20	10	30
31	0.4	3	3	13
32	23	>100	>100	>100
34	3	5	2	20
36	>100	>100	>100	>100
ara-C	0.005	0.05	0.02	0.2

[a] The ED_{50} values were estimated from dose–response curves compiled from at least two independent experiments and represent the drug concentration required to inhibit replication of the respective CCRF-CEM, P388, L1210, and S-180 cell lines by 50%.

and 5, 2.5, and 15 μM, respectively, for 3'-deoxycytidine (**22**). However, 3'-deoxy-ara-C (**10**) was found to be 12.5 times more active than 3'-deoxycytidine (**22**) against CCRF-CEM cancer cells, with respective ED_{50} values of 2 and 25 μM. The 2'-*O*-acetyl, 5'-*O*-acetyl, and 2',5'-di-*O*-acetyl derivatives of 3'-deoxy-ara-C (**10**), compounds **34**, **31**, and **30**, respectively, were as active as the parent compounds with the exception that the 5'-*O*-acetyl derivative of 3'-deoxy-ara-C, compound **31**, was 5 times more active than 3'-deoxy-ara-C (**10**) against CCRF-CEM cancer cells with an ED_{50} value of 0.4 μM. Conversely, the N^4-acetylated derivatives of 3'-deoxy-ara-C (**10**), compounds **32** and **36**, respectively, and other 3'-deoxy nucleoside analogues had no significant anticancer activity against these cell lines at concentrations as high as 100 μM. The loss of anticancer activity of the prodrugs, compounds **32** and **36**, may be due to the incomplete enzymatic hydrolysis of the N^4-acetyl group in the cell to the parent compound, 3'-deoxy-ara-C (**10**). Although 3'-deoxy-ara-C (**10**) was much less active than ara-C, 3'-deoxy-ara-C (**10**) was resistant to enzymatic deamination by cytidine deaminase from mouse kidney, whereas ara-C was rapidly deaminated to the inactive ara-U.[15] The results of the anticancer activity of these compounds are summarized in Table I.

3'-Deoxy-ara-C (**10**) was also evaluated against herpes simplex virus type 2 (HSV-2) on Vero cells, human cytomegalovirus (HCMV) on MRC-5 cells, and guinea pig cytomegalovirus (GPCMV) on guinea pig embryo cells in vitro and was found not to be very active, producing IC_{50} values of 110, 220, and >1000 μM, respectively. The IC_{50} values were expressed as the concentration (μM) that inhibits viral replication by 50%.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker WM-500 spectrometer with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. The mass spectra (at 70 eV) were provided by the Yale University Chemical Instrumentation Center. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value.

5'-*O*-Trityluridine (2). Uridine (1, 20.0 g, 0.082 mol) and chlorotriphenylmethane (trityl chloride, 25.2 g, 0.09 mol) were dissolved in dry pyridine (200 mL). The reaction mixture was stirred overnight at room temperature, and was then heated at ~100 °C for 4 h. The solution was evaporated in vacuo to dryness. The residue was dissolved in methylene chloride (400 mL) and washed with water (5 × 200 mL). The solvent was removed under reduced pressure, and the resulting residue was coevaporated twice with ethanol to afford a solid which was then recrystallized twice from ethanol to yield 31.5 g (79%): mp 195–196 °C (lit.[17] mp 200 °C); NMR (Me_2SO-d_6) δ 3.35 (d, 2 H, 5'-H), 3.82–4.16 (m, 3 H, 2'-, 3'-, and 4'-H), 5.05 (br s, 1 H, 3'-OH, D_2O exchangeable), 5.20 (d, 1 H, 5-H), 5.30 (br s, 1 H, 2'-OH, D_2O exchangeable), 5.72 (d, 1 H, 1'-H), 7.25 (m, 15 H, phenyl), 7.60 (d, 1 H, 6-H).

2,2'-Anhydro-1-(5-*O*-trityl-β-D-*threo*-pentofuranosyl)-uracil (3). A mixture of 5-*O*-trityluridine (**2**, 4.86 g, 10.0 mmol), diphenyl carbonate (3.43 g, 16.0 mmol), and sodium bicarbonate (100 mg) in 15 mL of dry DMF was heated to 150 °C with stirring until evolution of CO_2 ceased (~60 min). The reaction mixture was cooled and then poured into 150 mL of ether to give a gum which was solidified when triturated with ether. The solid was filtered, washed with ether, and recrystallized from methanol to give 3.88 g (83%) of product: mp 215–217 °C; TLC, R_f 0.23 ($CHCl_3$/MeOH, 10:1, v/v); NMR (Me_2SO-d_6) δ 2.62–3.05 (m, 2 H, 5'-H), 3.32 (m, 1 H, 3'-OH, D_2O exchangeable), 4.00–4.30 (m, 2 H, 2'- and 4'-H), 5.15 (dd, 1 H, 3'-H), 5.78 (d, 1 H, 5-H), 6.24 (d, 1 H, 1'-H), 7.05–7.35 (m, 15 H, phenyl), 7.82 (d, 1 H, 6-H).

2,2'-Anhydro-1-[3-*O*-(phenoxythiocarbonyl)-5-*O*-trityl-β-D-*threo*-pentofuranosyl]uracil (4). To a stirred suspension of 2,2'-anhydro-1-(5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (**3**, 470 mg, 1.0 mmol) and 4-(dimethylamino)pyridine (367 mg, 3.0 mmol) in dry acetonitrile (60 mL), phenyl chlorothionocarbonate (0.21 mL, 1.5 mmol) was added dropwise under nitrogen at room temperature. After the reaction mixture was stirred at room temperature for 22 h, the solvent was evaporated to dryness in vacuo to give a residue which was partitioned between ethyl acetate and water (50 mL/50 mL). The water phase was extracted with ethyl acetate (2 × 20 mL). The combined ethyl acetate solution was washed with water, dried ($MgSO_4$), and evaporated to dryness under diminished pressure to yield a foam (800 mg) which was then chromatographed on a silica gel (100 g) column (EtOAc/MeOH, 20:1, v/v) to afford 310 mg (51%) of white foam product: UV (MeOH) shoulder at 251 nm (ε 9859) and λ_{max} 236 nm (ε 16 080); UV (0.01 N NaOH) shoulder at 251 nm (ε 9507) and λ_{max} 235 nm (ε 16 100); UV (0.01 N HCl) shoulder at 252 nm (ε 11 972) and λ_{max} 236 nm (ε 16 670); MS *m/e* 469 (M^+ – $CSOC_6H_5$), 243 [$(C_6H_5)_3C^+$]; NMR (Me_2SO-d_6) δ 2.80–3.20 (m, 2 H, 5'-H), 4.00 (s, 1 H, 2'-H), 4.50 (m, 1 H, 4'-H), 5.65 (dd, 1 H, 3'-H), 5.80 (d, 1 H, *J* = 7.5 Hz, 5-H), 6.35 (d, 1 H, *J* = 5.8 Hz, 1'-H), 7.10–7.40 (m, 20 H, phenyl), 7.85 (d, 1 H, *J* = 7.4 Hz, 6-H). Anal. ($C_{35}H_{28}N_2O_6S$·CH_3OH) C, H, N.

2,2'-Anhydro-1-(3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (5). To a stirred suspension of 2,2'-anhydro-1-[3-*O*-(phenoxythiocarbonyl)-5-*O*-trityl-β-D-*threo*-pentofuranosyl]uracil (**4**, 650 mg, 1.07 mmol) and 2,2'-azobis(2-methylpropionitrile) (AIBN, 180 mg, 1.1 mmol) in dry toluene, tri-*n*-butyltin hydride (*n*-Bu_3SnH, 0.58 mL, 2.1 mmol) was added dropwise under nitrogen at room temperature. The mixture was stirred at 110–120 °C for 6 h, after which the solvent was evaporated to dryness in vacuo to yield a residue. This residue was then chromatographed on a silica gel (100 g) column (EtOAc/MeOH, 12.5:1, v/v) to afford 430 mg (88%) of white solid which was recrystallized from acetone: mp 196–198 °C; UV (MeOH) shoulder at 248 nm (ε 6763) and λ_{max} 232 nm (ε 11 350); UV (0.01 N NaOH) shoulder at 250 nm (ε 7990) and λ_{max} 232 nm (ε 12 350); UV (0.01 N HCl) shoulder at 251 nm (ε 7870) and λ_{max} 231 nm (ε 12 470); NMR (Me_2SO-d_6) δ 2.45–2.51 (m, 2 H, 3'-H), 2.77 (m, 1 H, 5'-H_a), 2.87 (m, 1 H, 5'-H_b), 4.52 (m, 1 H, 4'-H), 5.52 (m, 1 H, 2'-H), 5.84 (d, 1 H, *J* = 7.5 Hz, 5-H), 6.20 (d, 1 H, *J* = 5.5 Hz, 1'-H), 7.20–7.33 (m, 15 H, trityl), 7.92 (d, 1 H, *J* = 7.4 Hz, 6-H); MS *m/e* 453 (M^+), 243 [$(C_6H_5)_3C^+$]. Anal. ($C_{28}H_{24}N_2O_4$) C, H, N.

1-(3-Deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (6). A mixture of 2,2'-anhydro-1-(3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (**5**, 227 mg, 0.5 mmol), 1 N NaOH (1.4 mL), and 50% ethanol (20 mL) was stirred at room temperature for 2 h. The solution was neutralized with HOAc/EtOH (1:1, v/v) to ~pH 7. The resulting white solid was collected by filtration,

washed with water, dried, and recrystallized from ethanol to give 200 mg (85%) of product: mp 183–185 °C; UV (MeOH) λ_{max} 262 nm (ϵ 8370), λ_{min} 240 nm; UV (0.01 N NaOH) λ_{max} 263 nm (ϵ 7450), λ_{min} 247 nm; UV (0.01 N HCl) λ_{max} 264 nm (ϵ 8123), λ_{min} 249 nm; NMR (Me_2SO-d_6) δ 1.64–1.85 (m, 1 H, 3′-H_a), 2.15–2.35 (m, 1 H, 3′-H_b), 3.22 (m, 2 H, 5′-H), 4.05–4.36 (m, 2 H, 2′- and 4′-H), 5.30 (d, 1 H, 2′-OH, D_2O exchangeable), 5.30 (d, 1 H, J = 8.0 Hz, 5-H), 5.88 (d, 1 H, J = 5.0 Hz, 1′-H), 7.19–7.40 (m, 15 H, trityl), 7.48 (d, 1 H, J = 8.0 Hz, 6-H); MS m/e 471 (M^+ + 1), 243 [$(C_6H_5)_3C^+$]. Anal. ($C_{28}H_{26}N_2O_5$) C, H, N.

1-(2-*O*-Acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (7). Acetic anhydride (2 mL) was added dropwise to a stirred solution of 1-(3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (**6**, 374 mg, 0.8 mmol) in dry pyridine (20 mL) at 0 °C (ice/water bath). The reaction mixture was stirred at 4 °C for 18 h. Methanol was then added to the solution, and the solvents were removed in vacuo to give a residue. This residue was dissolved in ethyl acetate (50 mL), washed with water (2 × 10 mL), and dried ($MgSO_4$). The ethyl acetate solution was evaporated under reduced pressure to dryness. The resulting white foam was crystallized from MeOH/hexane to yield 312 mg (78%) of product: mp 107–109 °C; UV (MeOH) λ_{max} 261 nm (ϵ 9940), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 260 nm (ϵ 8530), λ_{min} 248 nm; UV (0.01 N HCl) λ_{max} 265 nm (ϵ 16 390), λ_{min} 254 nm; NMR (Me_2SO-d_6) δ 1.89 (s, 3 H, CH_3CO), 1.95–2.55 (m, 2 H, 3′-H), 3.38 (m, 2 H, 5′-H), 4.10–4.32 (m, 1 H, 4′-H), 5.37 (d, 1 H, 2′-H), 5.41 (d, 1 H, J = 7.9 Hz, 5-H), 5.10 (d, 1 H, J = 5.0 Hz, 1′-H), 7.12–7.41 (m, 15 H, trityl), 7.60 (d, 1 H, J = 7.9 Hz, 6-H); MS m/e 513 (M^+ + 1), 243 [$(C_6H_5)_3C^+$]. Anal. ($C_{30}H_{28}N_2O_6$) C, H, N.

1-(2-*O*-Acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-4-(1,2,4-triazolyl)pyrimidinone (8). To a stirred solution of 1-(2-*O*-acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (**7**, 1.95 g, 3.80 mmol) in dry pyridine (100 mL), 4-chlorophenyl phosphorodichloridate (1.24 mL, 7.60 mmol) was added dropwise at 0 °C (ice/water bath). After 2 min, 1,2,4-triazole (1.05 g, 15.2 mmol) was added to the above solution, and the reaction mixture was stirred at room temperature for another 48 h. The solvent was removed in vacuo to give a residue which was then chromatographed on a silica gel (50 g) column (EtOAc, R_f 0.77) to afford 1.56 g (75%) of product: mp 75–78 °C; NMR ($CDCl_3$) δ 1.72 (s, 3 H, CH_3CO), 1.95–2.45 (m, 2 H, 3′-H), 3.35 (d, 2 H, 5′-H), 4.15–4.40 (m, 1 H, 4′-H), 5.40–5.60 (m, 1 H, 2′-H), 6.15 (d, 1 H, 1′-H), 6.70 (d, 1 H, 5-H), 7.10–7.35 (m, 15 H, trityl), 8.02 (s, 1 H, triazolyl 3-H), 8.25 (d, 1 H, 6-H), 9.20 (s, 1 H, triazolyl 5-H).

This compound was pure enough and used for the next preparation immediately without further purification.

1-(3-Deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)cytosine (9). 1-(2-*O*-Acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-4-(1,2,4-triazolyl)pyrimidinone (**8**) obtained as described above was dissolved in 80 mL of NH_4OH/dioxane (1:3, v/v), and the reaction mixture was stirred for 3 h at room temperature in a Wheaton pressure bottle. The solution was evaporated to dryness in vacuo to give 1-(2-*O*-acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)cytosine which was then dissolved in 130 mL of saturated methanolic ammonia. The solution was stirred in a Wheaton pressure bottle overnight at room temperature. The solvent was evaporated to dryness under reduced pressure to yield a residue which was absorbed in 10 g of silica gel and then chromatographed on a silica gel (90 g) column (EtOAc/MeOH, 12.5:1, v/v) to afford 1.43 g (80%) of foam. The analytical sample was obtained by crystallization of the foam from C_6H_6/Et_2O: UV (MeOH) λ_{max} 273 nm (ϵ 7430), λ_{min} 253 nm; UV (0.01 N NaOH) λ_{max} 271 nm (ϵ 7427), λ_{min} 253 nm; UV (0.01 N HCl) λ_{max} 283 nm (ϵ 10 870), λ_{min} 249 nm; NMR (Me_2SO-d_6) δ 1.67 (m, 1 H, 3′-H_a), 2.27 (m, 1 H, 3′-H_b), 3.10 (m, 1 H, 5′-H_a), 3.23 (m, 1 H, 5′-H_b), 4.14 (m, 1 H, 2′-H), 4.24 (m, 1 H, 4′-H), 5.16 (d, 1 H, 5′-OH, D_2O exchangeable), 5.57 (d, 1 H, J = 7.4 Hz, 5-H), 5.94 (d, 1 H, J = 4.5 Hz, 1′-H), 6.90–7.10 (br d, 2 H, 4-NH_2, D_2O exchangeable), 7.25–7.40 (m, 15 H, trityl), 7.52 (d, 1 H, J = 7.4 Hz, 6-H); MS m/e 470 (M^+ + 1), 243 [$(C_6H_5)_3C^+$]. Anal. ($C_{28}H_{27}N_3O_4$) C, H, N.

1-(3-Deoxy-β-D-*threo*-pentofuranosyl)cytosine (10). 1-(3-Deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)cytosine (**9**, 330 mg, 0.7 mmol) was dissolved in 10 mL of 80% acetic acid solution and stirred at ~100 °C for 25 min. The solvent was removed in vacuo to yield a residue which was then chromatographed on a silica gel (10 g) column (EtOAc/MeOH, 4:1, v/v) to afford 110 mg (69%) of white crystals: mp 172–174 °C; UV (MeOH) λ_{max} 274 nm (ϵ 7200), λ_{min} 254 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ϵ 7850), λ_{min} 250 nm; UV (0.01 N HCl) λ_{max} 282 nm (ϵ 11 600), λ_{min} 242 nm; NMR (Me_2SO-d_6) δ 1.72 (m, 1 H, 3′-H_a), 2.24 (m, 1 H, 3′-H_b), 3.34–3.54 (m, 2 H, 5′-H), 3.98 (m, 1 H, 4′-H), 4.23 (m, 1 H, 2′-H), 5.08 (br s, 1 H, 5′-OH, D_2O exchangeable), 5.18 (br s, 1 H, 2′-OH, D_2O exchangeable), 5.66 (d, 1 H, J = 7.4 Hz, 5-H), 5.86 (d, 1 H, J = 4.3 Hz, 1′-H), 6.82–7.20 (br d, 2 H, 4-NH_2, D_2O exchangeable), 7.65 (d, 1 H, J = 7.4 Hz, 6-H), MS m/e 228 (M^+ + 1), 112 (cytosine + 1). Anal. ($C_9H_{13}N_3O_4$) C, H, N.

1-(3-Deoxy-β-D-*threo*-pentofuranosyl)uracil (11). Compound **6** (1.30 g, 2.76 mmol) was heated in 15 mL of 80% acetic acid at 110–120 °C with stirring for 20 min. The solution was evaporated under reduced pressure to give a residue which was washed with ether and dissolved in water (20 mL). The water solution was extracted with methylene chloride (2 × 10 mL), and the water layer was evaporated under reduced pressure to dryness. The residue was recrystallized from acetone to afford 0.55 g (87%) of product as white crystals: mp 130–132 °C; NMR (DMSO-d_6) δ 1.76 (m, 1 H, 3′-H_a), 2.21 (m, 1 H, 3′-H_b), 3.50–3.60 (m, 2 H, 5′-H), 4.00 (m, 1 H, 4′-H), 4.34 (m, 1 H, 2′-H), 5.55 (d, 1 H, J = 8.1 Hz, 5-H), 5.88 (d, 1 H, J = 4.7 Hz, 1′-H), 7.75 (d, 1 H, J = 8.1 Hz, 6-H). Anal. ($C_9H_{12}N_2O_5$) C, H, N.

1-(3-Deoxy-β-D-*threo*-pentofuranosyl)-5-chlorouracil (12). A mixture of compound **11** (250 mg, 1.1 mmol) and *N*-chlorosuccinimide (200 mg, 1.5 mmol) in 10 mL of glacial acetic acid was heated at reflux temperature with stirring for 30 min and then evaporated in vacuo to dryness. The residue was coevaporated with small portions (3 × 10 mL) of methanol and then dissolved in 20 mL of saturated NH_3/MeOH solution and stirred at room temperature in a Wheaton pressure bottle. The solvent was evaporated to dryness under reduced pressure to yield a glass, which was absorbed in 2 g of silica gel and then chromatographed on a silica gel (100 g) column (EtOAc/MeOH, 20:1, v/v) to afford 185 mg (64%) of white crystals: mp 132–134 °C; UV (MeOH) λ_{max} 280 nm (ϵ 11 991), λ_{min} 243 nm; UV (0.01 N NaOH) λ_{max} 280 nm (ϵ 11 534), λ_{min} 252 nm; UV (0.01 N HCl) λ_{max} 280 nm (ϵ 13 019), λ_{min} 244 nm; NMR (DMSO-d_6) δ 1.77 (m, 1 H, 3′-H_a), 2.21 (m, 1 H, 3′-H_b), 3.54 (m, 1 H, 5′-H_a), 3.64 (m, 1 H, 5′-H_b), 4.01 (m, 1 H, 2′-H), 4.35 (m, 1 H, 4′-H), 5.16 (t, 1 H, 5′-OH, D_2O exchangeable), 5.38 (d, 1 H, 2′-OH, D_2O exchangeable), 5.86 (d, 1 H, J = 4.9 Hz, 1′-H), 8.16 (s, 1 H, 6-H), 11.75 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{11}N_2O_5Cl$) C, H, N.

1-(3-Deoxy-β-D-*threo*-pentofuranosyl)-5-bromouracil (13). To a stirred solution of compound **11** (300 mg, 1.31 mmol) in anhydrous pyridine (20 mL) was added a solution of bromine (0.12 mL, 2 mmol) in carbon tetrachloride. The reaction mixture was stirred at room temperature for 2 h and then evaporated in vacuo to dryness. The residue was coevaporated with small portions (3 × 10 mL) of ethanol and purified on a silica gel (100 g) column (EtOAc/MeOH, 20:1, v/v) to afford 200 mg (50%) of white crystals: mp 182–184 °C; UV (MeOH) λ_{max} 277 nm (ϵ 9930), λ_{min} 240 nm; UV (0.01 N NaOH) λ_{max} 281 nm (ϵ 9005), λ_{min} 246 nm; UV (0.01 N HCl) λ_{max} 282 nm (ϵ 12 585), λ_{min} 246 nm; NMR (DMSO-d_6) δ 1.76 (m, 1 H, 3′-H_a), 2.20 (m, 1 H, 3′-H_b), 3.53 (m, 1 H, 5′-H_a), 3.63 (m, 1 H, 5′-H_b), 4.02 (m, 1 H, 2′-H), 4.35 (m, 1 H, 4′-H), 5.17 (t, 1 H, 5′-OH, D_2O exchangeable), 5.39 (d, 1 H, 2′-OH, D_2O exchangeable), 5.85 (d, 1 H, J = 4.9 Hz, 1′-H), 8.24 (s, 1 H, 6-H). Anal. ($C_9H_{11}N_2O_5Br$) C, H, N.

1-(2,5-Di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)uracil (14). Acetic anhydride (2 mL) was added dropwise to a stirred solution of compound **11** (500 mg, 2.19 mmol) in dry pyridine (20 mL) at 0 °C (ice/water bath). The reaction mixture was stirred at room temperature overnight and evaporated in vacuo to dryness. The residue was coevaporated with toluene (2 × 10 mL) to give 600 mg (88%) of white foam, which was used immediately without further purification for the next preparation: NMR ($CDCl_3$) δ 2.05 and 2.15 (two s, each 3 H, CH_3CO), 2.10 (m, 1 H, 3′-H_a), 2.62 (m, 1 H, 3′-H_b), 4.35 (m, 3 H, 4′- and 5′-H), 5.50 (m, 1 H, 2′-H), 5.85 (d, 1 H, 5-H), 6.15 (d, 1 H, 1′-H), 7.60 (d, 1 H, 6-H), 9.72 (br s, 1 H, NH, D_2O exchangeable).

1-(3-Deoxy-β-D-*threo*-pentofuranosyl)-5-iodouracil (15). To a stirred mixture of compound **14** (600 mg, 1.92 mmol) and silver trifluoroacetate (440 mg, 2.00 mmol) in dry methylene chloride (20 mL) was added dropwise a solution of iodine (665

mg, 2.62 mmol) in dry methylene chloride (10 mL) at 0 °C (ice/water bath). The mixture was stirred at room temperature for 14 h and TLC showed that the reaction was complete. The suspension was filtered and washed with methylene chloride (10 mL). The combined filtrate was washed successively with saturated sodium bicarbonate solution, water, sodium thiosulfate solution, and water, dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel (120 g) column, with EtOAc as eluant to afford 640 mg (76%) of 1-(2,5-di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)-5-iodouracil as a white foam: NMR ($CDCl_3$) δ 1.75–2.65 (m, 2 H, 3'-H), 2.05 (s, 3 H, CH_3CO), 4.35 (m, 3 H, 4'-H and 5'-H), 5.50 (m, 1 H, 2'-H). The diacetate (150 mg, 0.34 mmol) described above was stirred with 30 mL of saturated NH_3/MeOH solution at room temperature in a Wheaton pressure bottle overnight. The solvent was removed under reduced pressure to dryness. The residue was absorbed in 0.5 g of silica gel and chromatographed on a silica gel (100 g) column (EtOAc/MeOH, 10:1, v/v) to afford 70 mg (58%) of white crystals: mp 187–188 °C; UV (MeOH) λ_{max} 288 nm (ε 7908), λ_{min} 247 nm; UV (0.01 N NaOH) λ_{max} 285 nm (ε 7790), λ_{min} 252 nm; UV (0.01 N HCl) λ_{max} 291 nm (ε 9974), λ_{min} 250 nm; NMR (DMSO-d_6) δ 1.73 (m, 1 H, 3'-H_a), 2.18 (m, 1 H, 3'-H_b), 3.52 (m, 1 H, 5'-H_a), 3.63 (m, 1 H, 5'-H_b), 4.01 (m, 1 H, 2'-H), 4.33 (m, 1 H, 4'-H), 5.16 (t, 1 H, 5'-OH, D_2O exchangeable), 5.38 (m, 1 H, 2'-OH, D_2O exchangeable), 5.83 (d, 1 H, J = 5.0 Hz, 1'-H), 8.24 (s, 1 H, 6-H), 11.60 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{11}N_2O_5$) C, H, N.

2'-*O*-(*tert*-Butyldimethylsilyl)-5'-*O*-trityluridine (16). To a mixture of 5'-*O*-trityluridine (**2**, 1.48 g, 3.04 mmol), pyridine (1.47 mL, 18.24 mmol), and silver nitrate (0.62 g, 3.65 mmol) in anhydrous THF (20 mL) was added *tert*-butyldimethylsilyl chloride (0.50 g, 3.34 mmol) at room temperature. The reaction mixture was stirred at room temperature and monitored by TLC (CH_2Cl_2/MeOH, 8:0.4, v/v). After three days, the reaction was complete. The mixture was filtered, and the filtrate was evaporated in vacuo to dryness. The residue was dissolved in methylene chloride (30 mL) and washed with 5% sodium bicarbonate solution (2 × 10 mL), and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by silica gel (100 g) column chromatography (CH_2Cl_2/MeOH, 8:0.3, R_f 0.68) to yield 1.05 g (57%) of the title compound: UV (MeOH) λ_{max} 265 nm (ε 8635), λ_{min} 227 nm; UV (0.01 N NaOH) λ_{max} 264 nm (ε 20 169), λ_{min} 250 nm; UV (0.01 N HCl) λ_{max} 268 nm (ε 12 066), λ_{min} 252 nm; NMR (DMSO-d_6) δ 0.05–0.07 (2 s, 6 H, two CH_3), 0.84 (s, 9 H, *tert*-butyl), 3.22–3.55 (m, 2 H, 5'-H), 3.98 (m, 1 H, 4'-H), 4.05–4.12 (m, 1 H, 2'-H), 4.21–4.24 (m, 1 H, 3'-H), 5.13 (d, 1 H, 3'-OH, D_2O exchangeable), 5.30 (d, 1 H, J = 8.0 Hz, 5-H), 5.74 (d, 1 H, J = 4.1 Hz, 1'-H), 7.24–7.38 (m, 15 H, ArH), 7.73 (d, 1 H, J = 8.0 Hz, 6-H), 11.38 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{34}H_{40}N_2O_6Si$) C, H, N.

2'-*O*-(*tert*-Butyldimethylsilyl)-3'-*O*-(phenoxythiocarbonyl)-5'-*O*-trityluridine (17). To a stirred solution of compound **16** (13.0 g, 21.6 mmol) and 4-(dimethylamino)pyridine (7.92 g, 64.8 mmol) in dry acetonitrile (450 mL) was added dropwise phenyl chlorothiocarbonate (4.49 mL, 32.4 mmol) under nitrogen at room temperature. The reaction mixture was stirred for 65 h at room temperature and then evaporated to dryness in vacuo. The resultant residue was dissolved in CH_2Cl_2 (250 mL), washed with water (30, 20, and 10 mL each), dried ($MgSO_4$), and evaporated to dryness in vacuo to yield 27.7 g of crude product which was purified by silica gel chromatography (CH_2Cl_2/MeOH, 8:0.2, v/v, R_f 0.64) to afford 13.2 g (83%) of product: UV (MeOH) λ_{max} 259 nm (ε 12 428), λ_{min} 237 nm; UV (0.01 N NaOH) λ_{max} shoulder at 256 nm (ε 19 075) and λ_{max} 228 nm (ε 27 312); UV (0.01 N HCl) λ_{max} shoulder at 258 nm (ε 15 618) and λ_{max} 227 nm (ε 21 909); MS m/e 736 (M^+), 583 ($M^+ - CSOC_6H_5$); NMR (DMSO-d_6) δ 0.01–0.06 (2 s, 6 H, two CH_3), 0.85 (s, 9 H, *tert*-butyl), 3.35 (m, 1 H, 5'-H_a), 3.42 (m, 1 H, 5'-H_b), 4.36 (m, 1 H, 4'-H), 4.74 (t, 1 H, 2'-H), 5.60 (d, 1 H, J = 8.1 Hz, 5-H), 5.82 (m, 1 H, 3'-H), 5.86 (d, 1 H, J = 6.3 Hz, 1'-H), 7.09–7.60 (m, 20 H, ArH), 7.71 (d, 1 H, J = 8.1 Hz, 6-H), 11.53 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{41}H_{44}N_2O_7SSi$) C, H, N.

2'-*O*-(*tert*-Butyldimethylsilyl)-3'-deoxy-5'-*O*-trityluridine (18). To a stirred suspension of compound **17** (0.92 g, 1.25 mmol) and 2,2'-azobis(2-methylpropionitrile) (AIBN, 0.21 g, 1.3 mmol) in dry toluene (90 mL) was added dropwise tri-*n*-butyltin hydride (0.67 mL, 2.4 mmol) under nitrogen at room temperature. The reaction mixture was heated at 110–120 °C for 3 h and TLC showed that the reaction was complete. The solvent was evaporated to dryness in vacuo and the crude product was purified by silica gel (80 g) chromatography (CH_2Cl_2/MeOH, 8:2, v/v, R_f 0.68) to yield 0.41 g (56%) of a colorless foam: UV (MeOH) λ_{max} 266 nm (ε 11 347), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 265 nm (ε 10 264), λ_{min} 247 nm; UV (0.01 N HCl) λ_{max} 266 nm (ε 11 209), λ_{min} 254 nm; NMR (DMSO-d_6) δ 0.06–0.07 (2 s, 6 H, two CH_3), 0.84 (s, 9 H, *tert*-butyl), 1.78–1.86 (m, 1 H, 3'-H_a), 2.08–2.16 (m, 1 H, 3'-H_b), 3.30–3.41 (m, 2 H, 5'-H), 4.40 (m, 1 H, 4'-H), 4.45 (m, 1 H, 2'-H), 5.19 (d, 1 H, 5-H), 5.62 (d, 1 H, 1'-H), 7.28–7.54 (m, 15 H, ArH), 7.78 (d, 1 H, 6-H), 11.36 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{34}H_{40}N_2O_5Si$) C, H, N.

5'-*O*-Trityl-3'-deoxycytidine (21). To a stirred solution of compound (**18**) (14.0 g, 23.94 mmol) in dry pyridine (370 mL) was added dropwise 4-chlorophenyl phosphorodichloridate (8.3 mL, 51.2 mmol), followed by the addition of 1,2,4-triazole (12.38 g, 179.2 mmol) in an ice/water bath. The reaction mixture was stirred at room temperature for 48 h, and the solvent was evaporated to dryness in vacuo to give a dark purple syrup (**19**). This syrup was dissolved in 1050 mL of NH_4OH/dioxane (1:2, v/v) and stirred for 3 h at room temperature. The solution was evaporated to dryness under diminished pressure. The resulting residue was then dissolved in methylene chloride (500 mL), washed with water, and dried ($MgSO_4$). The methylene chloride solution was evaporated to dryness under reduced pressure to give a glass (**20**) which was dissolved in anhydrous THF (500 mL) and stirred with 144 mL of 1 M tetra-*n*-butylammonium fluoride under nitrogen for 1 h at room temperature. The solvent was removed in vacuo to dryness and the crude product was chromatographed on a silica gel column (EtOAc/MeOH, 6:1, v/v, R_f 0.31) to yield 6.8 g of product: UV (MeOH) λ_{max} 273 nm (ε 7296), λ_{min} 251 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ε 6780), λ_{min} 253 nm; UV (0.01 N HCl) λ_{max} 283 nm (ε 8847), λ_{min} 249 nm; NMR (DMSO-d_6) δ 1.75 (m, 1 H, 3'-H_a), 2.05 (m, 1 H, 3'-H_b), 3.22–3.32 (m, 2 H, 5'-H), 4.15 (m, 1 H, 2'-H), 4.40 (m, 1 H, 4'-H), 5.45 (d, 1 H, J = 7.4 Hz, 5-H), 5.53 (d, 1 H, 2'-OH, D_2O exchangeable), 5.68 (s, 1 H, 1'-H), 7.10–7.20 (d, 2 H, 4-NH, D_2O exchangeable), 7.26–7.39 (m, 15 H, ArH), 7.76 (d, 1 H, J = 7.4 Hz, 6-H), MS m/e 470 ($M^+ + 1$). Anal. ($C_{28}H_{27}N_3O_4 \cdot CH_3OH$) C, H, N.

3'-Deoxycytidine (22). Compound **21** (0.75 g, 1.60 mmol) was dissolved in 80 mL of 80% acetic acid solution and stirred at 110–130 °C for 30 min. The solvent was removed in vacuo to yield a residue which was extracted with ether (2 × 25 mL). The remaining solid cake was dissolved in a minimal amount of MeOH and chromatographed on a silica gel (80 g) column (EtOAc/MeOH, 4:1.5, v/v, R_f 0.17). The product was recrystallized from EtOH and weighed 170 mg. An additional 70 mg was recovered from the mother liquor to afford a total yield of 240 mg (67%): mp 230–232 °C (lit[25] 224–230 °C); UV (MeOH) λ_{max} (ε 9075), λ_{min} 252 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ε 9711), λ_{min} 249 nm; UV (0.01 N HCl) λ_{max} 282 (nm (ε 14 245), λ_{min} 242 nm; NMR (DMSO-d_6) δ 1.68 (m, 1 H, 3'-H_a), 1.85 (m, 1 H, 3'-H_b), 3.52 (m, 1 H, 5'-H_a), 3.72 (m, 1 H, 5'-H_b), 4.08 (d, 1 H, 2'-H), 4.25 (m, 1 H, 4'-H), 5.01 (t, 1 H, 5'-OH, D_2O exchangeable), 5.45 (d, 1 H, 2'-OH, D_2O exchangeable), 5.63 (s, 1 H, 1'-H), 5.64 (d, 1 H, J = 7.4 Hz, 5-H), 6.99 (br s, 1 H, 4-NH_a, D_2O exchangeable), 7.08 (br s, 1 H, 4-NH_b, D_2O exchangeable), 7.91 (d, 1 H, J = 7.4 Hz, 6-H). Anal. ($C_9H_{13}N_3O_4$) C, H, N.

3'-Deoxy-5-bromocytidine (23). To a stirred solution of 3'-deoxycytidine (**22**, 150 mg, 0.66 mmol) in pyridine (40 mL) was added dropwise a solution of bromine (180 mg, 1.12 mmol) in carbon tetrachloride (1 mL) at room temperature. The reaction mixture was stirred for 30 min and TLC showed that the reaction was complete. The solvent was removed in vacuo to yield a residue which was dissolved in water (20 mL), washed with methylene chloride (2 × 15 mL). The water layer was evaporated to dryness in vacuo and chromatographed on a silica gel (80 g) column (EtOAc/MeOH, v/v, 4:1, R_f 0.5) to afford 160 mg (80%) of product: mp 205–207 °C; UV (MeOH) λ_{max} 290 nm (ε 7200), λ_{min} 264 nm; UV (0.01 N NaOH) λ_{max} 288 nm (ε 7446), λ_{min} 262 nm; UV (0.01 N HCl) λ_{max} 300 nm (ε 9894), λ_{min} 256 nm; NMR (DMSO-d_6) δ 1.64 (m, 1 H, 3'-H_a), 1.94 (m, 1 H, 3'-H_b), 3.52 (m, 1 H, 5'-H_a), 3.81 (m, 1 H, 5'-H_b), 4.13 (m, 1 H, 2'-H), 4.31 (m, 1 H, 4'-H), 5.24 (t, 1 H, 5'-OH, D_2O exchangeable), 5.53 (d, 1 H,

2′-OH, D_2O exchangeable), 5.58 (s, 1 H, 1′-H), 6.90 (br s, 1 H, 4-NH_a, D_2O exchangeable), 7.73 (br s, 1 H, 4-NH_b, D_2O exchangeable), 8.52 (s, 1 H, 6-H); MS *m/e* 305 ($M^+ - 1$). Anal. ($C_9H_{12}BrN_3O_4$) C, H, N.

3′-Deoxy-5-iodocytidine (24). To a stirred suspension of 3-deoxycytidine (**22**, 150 mg, 0.55 mmol) and silver trifluoroacetate (290 mg, 1.32 mmol) in 2 mL of EtOH and 30 mL of dry dioxane was added dropwise a solution of iodine (0.5 g, 1.98 mmol) in 15 mL of dry dioxane at 0 °C (ice bath). Upon completion of the addition, the reaction mixture was then stirred for an additional 5 h at room temperature and filtered, and the filtrate was evaporated to dryness in vacuo. The residue was chromatographed on silica gel (85 g) column (EtOAc/MeOH, 4:1, v/v, R_f 0.48) to give 0.1 g (43%) of product: mp 214–215 °C; UV (MeOH) λ_{max} 295 (ϵ 6010), λ_{min} 266 nm; UV (0.01 N NaOH) λ_{max} 295 nm (ϵ 6728), λ_{min} 264 nm; UV (0.01 N HCl) λ_{max} 310 nm (ϵ 9591), λ_{min} 262 nm: NMR (DMSO-d_6) δ 1.60 (m, 1 H, 3′-H_a), 1.92 (m, 1 H, 3′-H_b), 3.51 (m, 1 H, 5′-H_a), 3.78 (m, 1 H, 5′-H_b), 4.09 (d, 1 H, 2′-H), 4.30 (m, 1 H, 4′-H), 5.21 (s, 1 H, OH, D_2O exchangeable), 5.51 (s, 1 H, OH, D_2O, exchangeable), 5.58 (s, 1 H, 1′-H), 6.51 (br s, 1 H, 4-NH_a, D_2O exchangeable), 7.70 (br s, 1 H, 4′-NH_b, D_2O exchangeable), 8.53 (s, 1 H, 6-H). Anal. ($C_9H_{12}N_3O_4I$) C, H, N.

3′-Deoxyuridine (26). A solution of compound **18** (3.13 g, 5.35 mmol) in THF (100 mL) was stirred with 22 mL of 1 M tetra-*n*-butylammonium fluoride in THF at room temperature for 1.5 h. The reaction mixture was evaporated in vacuo to give a residue which was dissolved in methylene chloride (50 mL), washed with water (2 × 20 mL), and dried ($MgSO_4$). The solvent was removed under reduced pressure to afford 5′-*O*-trityl-3′-deoxyuridine (**25**) which was used for the next step without further purification. Ditritylation was carried out by heating compound **25** with 250 mL of 80% acetic acid at 110–120 °C for 30 min. The solvent was then evaporated to dryness in vacuo, and the resulting residue was chromatographed on a silica gel (80 g) column (CH_2Cl_2/MeOH, 10:1, v/v, R_f 0.41) to yield 0.68 g (56%) of the desired compound: mp 174–176 °C (lit.[25a] mp 175.5–177.5 °C; lit[25b] mp 178 °C; lit[25c] mp 180–181 °C) UV (MeOH) λ_{max} 263 nm (ϵ 10958), λ_{min} 232 nm; UV (0.01 N NaOH) λ_{max} 263 nm (ϵ 9724), λ_{min} 238 nm; UV (0.01 N HCl) λ_{max} 265 nm (ϵ 11007), λ_{min} 233 nm; NMR (DMSO-d_6) δ 1.74 (m, 1 H, 3′-H_a), 1.94 (m, 1 H, 3′-H_b), 3.51 (m, 1 H, 5′-H_a), 3.71 (m, 1 H, 5′-H_b), 4.21 (m, 1 H, 2′-H), 4.26 (m, 1 H, 4′-H), 5.07 (t, 1 H, 5′-OH, D_2O exchangeable), 5.52 (s, 1 H, 2′-OH, D_2O exchangeable), 5.53 (d, 1 H, J = 8.0 Hz, 5-H_a), 5.62 (d, 1 H, J = 1.2 Hz, 1′-H), 7.95 (d, 1 H, J = 8.0 Hz, 6-H), 11.27 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{12}N_2O_5$) C, H, N.

3′-Deoxy-5-bromouridine (27). To a stirred solution of 3′-deoxyuridine (**26**, 150 mg, 0.66 mmol) in pyridine (40 mL) was added dropwise a solution of bromine (179 mg, 1.12 mmol) in carbon tetrachloride (1 mL) at room temperature. The reaction mixture was stirred for 1 h and TLC showed that the reaction was complete. The solvent was removed in vacuo to give a residue which was dissolved in water (20 mL) and washed with methylene chloride (3 × 15 mL). The water layer was evaporated to dryness in vacuo, and the resultant crude product was purified by silica gel (85 g) column chromatography (EtOAc/MeOH, 8:1, R_f 0.65) to give 100 mg (50%) of product as amorphous solid: UV (MeOH) λ_{max} 281 nm (ϵ 8589), λ_{min} 243 nm; UV (0.01 N NaOH) λ_{max} 278 nm (ϵ 6619), λ_{min} 252 nm; UV (0.01 N HCl) λ_{max} 282 nm (ϵ 8786), λ_{min} 244 nm; MS *m/e* 307 (M^+); NMR (DMSO-d_6) δ 1.70 (m, 1 H, 3′-H_a), 1.98 (m, 1 H, 3′-H_b), 3.53 (m, 1 H, 5′-H_a), 3.75 (m, 1 H, 5′-H_b), 4.28 (m, 1 H, 4′-H), 4.32 (m, 1 H, 2′-H), 5.25 (t, 1 H, 5′-OH, D_2O exchangeable), 5.53 (d, 1 H, 2′-OH, D_2O exchangeable), 5.59 (s, 1 H, 1′-H), 8.61 (s, 1 H, 6-H), 11.72 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{11}N_2O_5Br$) C, H, N.

1-(2,5-Di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)-uracil (28). Acetic anhydride (2 mL) was added dropwise to a stirred solution of compound **11** (0.58 g, 2.54 mmol) in dry pyridine (20 mL) at 0 °C (ice/water bath). The reaction mixture was stirred overnight and evaporated to dryness under reduced pressure. The residue was coevaporated with EtOH (10 mL), toluene (10 mL), and methylene chloride (10 mL), subsequently, to afford 0.7 g of crude product as a white foam which was used immediately for the next preparation without further purification: NMR ($CDCl_3$) δ 2.05 and 2.15 (two s, each 3 H, 2′,5′-di-*O*-acetyl), 2.10 (m, 1 H, 3′-H_a), 2.62 (m, 1 H, 3′-H_b), 4.35 (m, 3 H, 4′- and 5′-H), 5.50 (m, 1 H, 2′-H), 5.85 (d, 1 H, 5-H), 6.15 (d, 1 H, 1′-H), 7.60 (d, 1 H, 6-H), 9.72 (br s, 1 H, 3-NH, D_2O exchangeable).

1-(2,5-Di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)-4-(1,2,4-triazolyl)pyrimidinone (29). To a stirred solution of compound **28** (0.65 g, 2.18 mmol) in dry pyridine (15 mL) was added dropwise 4-chlorophenyl phosphorodichloridate (2.45 g, 10.0 mmol) at 0–5 °C (ice/water bath), followed by the addition of 1,2,4-triazole (2.0 g, 29.0 mmol). The reaction mixture was stirred at room temperature for 3 days and then concentrated to dryness under reduced pressure. The residue was dissolved in methylene chloride (60 mL), washed with water (3 × 20 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to give a glass which was chromatographed on a silica gel (100 g) column (EtOAc, R_f 0.29) to afford 0.62 g (81%) of product: mp 88–90 °C; UV (MeOH) λ_{max} 254 nm (ϵ 10397), λ_{min} 281 nm; UV (0.01 N NaOH) λ_{max} 252 nm (ϵ 9390), λ_{min} 280 nm; UV (0.01 N HCl) λ_{max} 252 nm (ϵ 10006), λ_{min} 280 nm; NMR ($CDCl_3$) δ 1.80 (s, 3 H, 2′-*O*-acetyl), 2.05 (s, 3 H, 5′-*O*-acetyl), 2.07 (m, 1 H, 3′-H_a), 2.53 (m, 1 H, 3′-H_b), 4.10–4.50 (m, 3 H, 4′- and 5′-H), 5.60 (m, 1 H, 2′-H), 6.12 (d, 1 H, J = 4.8 Hz, 1′-H), 7.00 (d, 1 H, J = 8.1 Hz, 5-H), 8.05 (s, 1 H, triazolyl 3-H), 8.15 (d, 1 H, J = 8.1 Hz, 6-H), 9.20 (s, 1 H, triazolyl 5-H). Anal. ($C_{15}H_{17}N_5O_6 \cdot 0.25H_2O$) C, H, N.

1-(2,5-Di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)cytosine (30). Compound **29** was dissolved in 20 mL of NH_4OH/dioxane (1:6, v/v) and the reaction mixture was stirred at room temperature in a Wheaton pressure bottle. The reaction was monitored by TLC and was stopped just when the starting material disappeared (~2 h). The solution was evaporated to dryness in vacuo, and the residue was chromatographed on a silica gel (100 g) column, with EtOAc/MeOH (4:1, v/v) as eluting solvent. The desired fractions with a R_f of 0.24 were collected and concentrated to afford 0.22 g (71%) of product as white crystals: mp 177–179 °C; UV (MeOH) λ_{max} 272 nm (ϵ 6226), λ_{min} 253 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ϵ 6962), λ_{min} 253 nm; UV (0.01 N HCl) λ_{max} 281 nm (ϵ 8546), λ_{min} 241 nm; NMR ($CDCl_3$) δ 1.85 (m, 1 H, 3′-H_a), 1.80 (s, 3 H, 2′-*O*-acetyl), 2.05 (s, 3 H, 5′-*O*-acetyl), 2.53 (m, 1 H, 3′-H_b), 4.05–4.40 (m, 3 H, 4′- and 5′-H), 5.45 (m, 1 H, 2′-H), 5.85 (d, 1 H, J = 8.0 Hz, 5-H), 6.15 (d, 1 H, J = 4.8 Hz, 1′-H), 6.70 (br s, 2 H, 4-NH_2, D_2O exchangeable), 7.55 (d, 1 H, J = 8.0 Hz, 6-H). Anal. ($C_{13}H_{17}N_3O_6$) C, H, N.

1-(5-*O*-Acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)cytosine (31). Compound **30** (156 mg, 0.5 mmol) was dissolved in 10 mL of NH_4OH/dioxane (1:3, v/v) and stirred at room temperature. The reaction was monitored by TLC and was stopped when the starting material disappeared (~3 h). The solvent was removed under reduced pressure to yield a glass which was chromatographed on a silica gel (100 g) column, with EtOAc/MeOH (3:1, v/v) as eluant. The desired fractions with a R_f of 0.20 were collected and concentrated to afford 100 mg (75%) of product as white crystals: mp 190–192 °C; UV (MeOH) λ_{max} 274 nm (ϵ 14610), λ_{min} 252 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ϵ 15452), λ_{min} 250 nm; UV (0.01 N HCl) λ_{max} 283 nm (ϵ 11987), λ_{min} 243 nm; NMR (DMSO-d_6) δ 1.70 (m, 1 H, 3′-H_a), 2.03 (s, 3 H, 5′-acetyl), 2.19 (m, 1 H, 3′-H_b), 4.00–4.35 (m, 4 H, 2′-H, 4′-H, and 5′-H), 5.20 (d, 1 H, 2′-OH, D_2O exchangeable), 5.65 (d, 1 H, J = 7.8 Hz, 5-H), 5.85 (d, 1 H, J = 4.8 Hz, 1′-H), 6.98 (br s, 2 H, NH_2, D_2O exchangeable), 7.48 (d, 1 H, J = 7.8 Hz, 6-H). Anal. ($C_{11}H_{15}N_3O_5 \cdot 0.25H_2O$) C, H, N.

1-(2,5-Di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)-*N*⁴-acetylcytosine (32). Acetic anhydride (1 mL) was added dropwise to a stirred solution of compound **30** (110 mg, 0.35 mmol) in dry pyridine (10 mL) at room temperature. The reaction mixture was stirred overnight and then evaporated to dryness in vacuo. The residue was chromatographed on a silica gel (80 g) column (EtOAc/MeOH, 10:1, v/v, R_f 0.58) to afford 50 mg (42%) of product as white crystals: mp 154–156 °C; UV (MeOH) λ_{max} 352 nm (ϵ 12076), λ_{min} 275 nm; UV (0.01 N NaOH) λ_{max} 253 nm (ϵ 12278), λ_{min} 275 nm; UV (0.01 N HCl) λ_{max} 251 nm (ϵ 12346), λ_{min} 274 nm; NMR ($CDCl_3$) δ 1.90 (m, 1 H, 3′-H_a), 1.95 (s, 3 H, *N*⁴-acetyl), 2.10 (m, 1 H, 3′-H_b), 2.15 (s, 3-H, 2′-*O*-acetyl), 2.30 (s, 1 H, 5′-*O*-acetyl), 4.30 (m, 2 H, 5′-H), 5.60 (m, 1 H, 2′-H), 6.20 (d, 1 H, J = 5.0 Hz, 1′-H), 7.50 (d, 1 H, J = 8.1 Hz, 5-H), 8.00 (d, 1 H, J = 8.1 Hz, 6-H). Anal. ($C_{15}H_{19}N_3O_7 \cdot 0.75H_2O$) C, H, N.

1-(2-*O*-Acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)cytosine (34). Compound **8** (1.0 g, 1.8 mmol) was dissolved in 20 mL of NH_4OH/dioxane (1:3, v/v), and the reaction mixture was stirred

Table II. Crystal Data

formula	$C_9H_{12}N_3O_4 \cdot 0.5H_2O$
formula weight	235.22
crystal size, mm	0.60 × 0.60 × 0.75
space group	$P2_12_12_1$
formula units/unit cell	8
cell dimensions	
a, Å	11.139 (1)
b, Å	11.363 (1)
c, Å	16.963 (1)
V, Å^3	2147.0
range, deg	$0 < 2\theta < 152$
calcd density, g cm^{-3}	1.462
linear absorption coefficient, cm^{-1}	9.1

at room temperature and monitored by TLC. The reaction was stopped just when the starting material disappeared (~3 h). The solvent was removed in vacuo to give a residue which was chromatographed on a silica gel (100 g) column (EtOAc/MeOH, 4:1, v/v, R_f 0.41) to give 1-(2-*O*-acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)cytosine (**33**) as a foam: yield, 0.69 g (79%); NMR ($CDCl_3$) δ 1.80 (s, 3 H, 2'-*O*-acetyl), 2.05 (m, 1 H, 3'-H_a), 2.35 (m, 1 H, 3'-H_b), 3.35 (m, 2 H, 5'-H), 4.25 (m, 1 H, 4'-H), 5.45 (m, 1 H, 2'-H), 5.60 (d, 1 H, 5-H), 6.15 (d, 1 H, 1'-H), 7.10–7.55 (m, 15 H, ArH), 7.65 (d, 1 H, 6-H), 8.15 (s, 2 H, 4-NH_2, D_2O exchangeable). The above described compound **33** (0.69 g, 1.35 mmol) was stirred with 80% acetic acid at 100–110 °C for 20 min. The solvent was removed in vacuo to give a residue which was purified by silica gel (80 g) column chromatography (EtOAc/MeOH, 2:1, v/v, R_f 0.35) to afford 210 mg (72%) of target product as white crystals: mp 98–100 °C; UV (MeOH) λ_{max} 270 nm (ε 7392), λ_{min} 249 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ε 8371), λ_{min} 251 nm; UV (0.01 N HCl) λ_{max} 281 nm (ε 11553), λ_{min} 243 nm; NMR (DMSO-d_6) δ 1.80 (s, 3 H, 2'-*O*-acetyl), 1.90 (m, 1 H, 3'-H_a), 2.41 (m, 1 H, 3'-H_b), 3.57 (m, 2-H, 5'-H), 4.20 (m, 1 H, 4'-H), 5.00 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.32 (m, 1 H, 2'-H), 5.65 (d, 1 H, J = 8.0 Hz, 5-H), 6.00 (d, 1 H, J = 4.9 Hz, 1'-H), 7.05 (s, 2 H, NH_2, D_2O exchangeable), 7.65 (d, 1 H, J = 8.0 Hz, 6-H). Anal. ($C_{11}H_{15}N_3O_5 \cdot 0.25H_2O$) C, H, N.

1-(2-*O*-Acetyl-3-deoxy-β-D-*threo*-pentofuranosyl)-N^4-acetylcytosine (36). Acetic anhydride (2 mL) was added dropwise to a stirred solution of compound **33** (0.55 g, 1.08 mmol) in dry pyridine (15 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure to give a residue which was coevaporated with methylene chloride (20 mL) and then chromatographed on a silica gel (100 g) column (EtOAc/MeOH, 10:1, v/v, R_f 0.72) to give 1-(2-*O*-acetyl-3-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-N^4-acetylcytosine (**35**, 0.42 g, 70%): NMR ($CDCl_3$) δ 1.80 (s, 3 H, N^4-acetyl), 1.85–2.10 (m, 1 H, 3'-H_a), 2.21 (s, 3 H, 2'-*O*-acetyl), 2.30–2.50 (m, 1 H, 3'-H_b), 3.35 (d, 2 H, 5'-H), 4.41 (m, 1 H, 4'-H), 5.55 (m, 1 H, 2'-H), 6.20 (d, 1 H, 1'-H), 7.15–7.60 (m, 16 H, 5-H and ArH), 8.00 (d, 1 H, 6-H). Compound **35** (0.4 g, 0.72 mmol) was stirred in 10 mL of 80% acetic acid at 100–110 °C for 20 min. After cooling, the solvent was removed in vacuo to give a residue which was coevaporated with methylene chloride (10 mL) and then chromatographed on a silica gel (80 g) column (CH_2Cl_2/MeOH, 10:1, v/v, R_f 0.43) to afford 150 mg (67%) of product as white crystals: mp 217–218 °C; UV (MeOH) λ_{max} 250 nm (ε 14631), λ_{min} 274 nm; UV (0.01 N NaOH) λ_{max} 250 nm (ε 14631), λ_{min} 274 nm; UV (0.01 N HCl) λ_{max} 249 nm (ε 14319), λ_{min} 273 nm; NMR (DMSO-d_6) δ 1.80 (s, 3 H, N^4-acetyl), 2.05 (m, 1 H, 3'-H_a), 2.15 (s, 3 H, 2'-*O*-acetyl), 2.40 (m, 1 H, 3'-H_b), 3.75 (d, 2-H, 5'-H), 4.25 (m, 1 H, 4'-H), 5.15 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.75 (m, 1 H, 2'-H), 6.20 (d, 1 H, J = 5.0 Hz, 1'-H), 7.35 (d, 1 H, J = 8.1 Hz, 5-H), 8.42 (d, 1 H, J = 8.1 Hz, 6-H). Anal. ($C_{13}H_{17}N_3O_6$) C, H, N.

Single-Crystal X-ray Analysis of 3'-Deoxy-ara-C (10). Colorless crystals were obtained from ethanol. The cell dimensions were determined from angular settings of 25 high-order ($90° < 2\theta < 147°$) reflections. Intensities were measured with Ni-filtered Cu Kα radiation on an Enraf-Nonius CAD-4 diffractometer, using $\omega/2\theta$ scans with variable ranges and speeds. Both hkl and $\bar{h}kl$ reflections were measured. After averaging, there were 2535 unique reflections of which 74 with $I < 3\sigma(I)$ were considered unobserved. The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary. Crystal data are given in Table II.

The structure was determined with MULTAN78.[32] Atomic coordinates and anisotropic temperature parameters of non-hydrogen atoms were refined by full-matrix least-squares. All hydrogen atoms were located on difference Fourier maps and their coordinates and isotropic B's were also refined. The atomic scattering factors were taken from the *International Tables for X-ray Crystallography*.[33] The refinement converged to a conventional residual index R = 0.032 for 2460 observed reflections (the 220 reflection suffered from secondary extinction and was given zero weight) and R = 0.033 for all reflections. A final difference Fourier map showed no significant features.

All calculations were performed with the NRCVAX system of programs.[34] Figure 1 was drawn with the ORTEP program of Johnson.[35]

Anticancer Assays. Anticancer activity was assessed by growth inhibition studies with use of murine L1210 leukemia, P388 leukemia, Sarcoma 180, and human CCRF-CEM lymphoblastic leukemia cells as described below.

Murine L1210, P388, and S-180 cells were maintained as suspension cultures in Fischer's Medium and CCRF-CEM cells were maintained as a suspension culture in Roswell Park Memorial Institute Medium, both media supplemented with 10% horse serum and all cells maintained at 37 °C in a humidified atmosphere of 5% CO_2-95% air. Under these conditions, the generation time for L1210, P388, S-180, and CCRF-CEM cells is approximately 12, 12, 18, and 20 h, respectively. Each compound, at various concentrations, was added to L1210, P388, S-180, and CCRF-CEM cells (2 × 10^4 cells/mL) in their exponential phase of growth. The cell number of the drug-free cultures (control), as well as that of the cultures supplemented with the tested compounds, were determined after 24, 48, and 72 h of growth.

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Supplementary Material Available: Tables III–VII of the final atomic parameters, anisotropic thermal parameters, bond lengths and angles, torsion angles, and details of hydrogen bonds of compound **10** (5 pages). Ordering information is given on any current masthead page.

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