Potential Anti-AIDS Drugs. 2′,3′-Dideoxycytidine Analogues

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5-Substituted 2′,3′-dideoxycytidine analogues have been synthesized and evaluated in vitro for their capabilities to protect $T4^+$ lymphocytes from the cytopathic effects of the HTLV-III/LAV (HIV) virus, the causative agent of acquired immunodeficiency syndrome (AIDS). These analogues were designed to be more lipophilic than 2′,3′-dideoxycytidine (ddC) in order to enhance central nervous system penetration. Earlier reports had shown that ddC is a potent protective agent. When ddC is substituted at the 5-position with either methyl or bromo substituents, activity is completely abolished. However, when the substitution is fluoro (5-F-ddC), both activity and potency are retained. 2′,3′-Dideoxy-5-azacytidine is also protective but more toxic than ddC or 5-F-ddC. In a different approach, an attempt was made to utilize ddCMP, ddTMP, and ddAMP as preformed nucleotides in order to circumvent the generally low level of phosphorylation achieved with dideoxynucleosides which function as relatively poor substrates for the cellular kinases. Only ddAMP is as active as its nucleoside precursor. Because ddAMP is not more active than ddA at low concentrations, it is possible that the active agent is ddA which is generated from ddAMP prior to cell entry.

Acquired immunodeficiency syndrome (AIDS) is a fatal disease which has reached epidemic proportions among certain high-risk groups. It is caused by a human pathogenic retrovirus known variously as human T-lymphotropic virus type III (HTLV-III), lymphadenopathy-associated virus (LAV), and human immunodeficiency virus (HIV). This virus is cytopathic for helper/inducer T-cells.[1-4]

Although there is no effective therapy for AIDS, a number of compounds are currently under study as HIV inhibitors, both in clinical trials and in model systems. Among the potential anti-AIDS drugs being studied are suramin and its analogs,[5,6] ribavirin,[6,7] foscarnet,[8] and HPA-29.[9] Biological response modifiers such as α and γ interferon, interleukin-2, and monoclonal antibodies are also being evaluated.[9] Comparative data for a number of these materials have been reviewed recently.[10] Retroviral DNA polymerase (reverse transcriptase) plays a unique and essential role in the life cycle of HIV and this enzyme can be a target for antiviral therapy.

Historically, nucleosides have been among the best drugs for treating DNA and RNA viral infections. Several known active agents of this class [e.g., 3′-azido-3′-deoxythymidine (AZT), ribavirin] have been evaluated against HIV in vitro[6,11] and AZT, a compound with activity in the test system used in the present study, has recently completed a placebo-controlled, randomized clinical trial.[12] Other compounds very effective in protecting cells against the cytopathic effects of HIV in vitro are 2′,3′-dideoxy (dd) analogues of physiologically important nucleosides.[13] These compounds, as their 5′-triphosphates, are known to terminate growing DNA chains because they lack the 3′-hydroxyl group required for further polymerization. This termination process forms the basis for the Sanger DNA sequencing method.[14] While different cell types appear to vary significantly in their abilities to phosphorylate 2′,3′-dideoxynucleosides to the mono-, di-, and triphosphate levels,[15,16] the corresponding end-product triphosphates are known to strongly inhibit reverse transcriptase, as well as β and γ DNA polymerase.[15,17] However, DNA polymerase α, the key synthetic enzyme for DNA replication during cell growth and an enzyme with repair functions as well, is much less affected.[15]

While the 2′,3′-dideoxy and analogues of adenosine, guanosine, inosine, thymidine, and cytidine are all very effective in protecting $T4^+$ lymphocytes against the cytopathic effects of HIV in vitro, 2′,3′-dideoxycytidine (1, ddC) appears to be the most potent member of the group on the basis of molarity. At the noncytotoxic concentrations of 0.5 and 5.0 μM, ddC provided essentially 100% protection to $T4^+$ lymphocytes exposed to what would be an otherwise cytopathic dose of HIV virus. These data led us to undertake a study of 5-substituted 2′,3′-dideoxycytidine analogues as inhibitors of HIV infection. An analysis of such inhibitors might be relevant not only to HIV but also to a broad spectrum of pathogenic retroviruses which have medical and veterinary implications, since the viral DNA polymerase is highly conserved among retroviruses.

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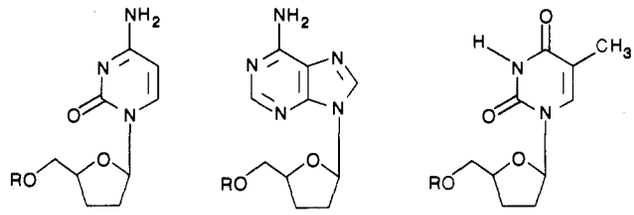
(1) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. *Science (Washington, D.C.)* **1984**, *224*, 497-500.
(2) Gallo, R. C.; Sarngadharan, M. G.; Popovic, M.; Shaw, G. M.; Hahn, B.; Wong-Staal, F.; Robert-Guroff, M.; Salahuddian, Z.; Markham, P. D. *Prog. Allergy* **1986**, *37*, 1-45.
(3) Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Charmaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzinoux, C.; Rozenbaum, W.; Montagnier, L. *Science (Washington, D.C.)* **1983**, *220*, 868-871.
(4) Montagnier, L. *Prog. Allergy* **1986**, *37*, 46-64.
(5) Mitsuya, H.; Popovic, M.; Yarchoan, R.; Matsushita, S.; Gallo, R. C.; Broder, S. *Science (Washington, D.C.)* **1984**, *226*, 172-174.
(6) Balzarini, J.; Mitsuya, H.; DeClercq, E.; Broder, S. *Int. J. Cancer* **1986**, *37*, 451-457.
(7) McCormick, J. B.; Getchell, J. P.; Mitchell, S.; Hicks, D. R.; Heath, J. International Conference on AIDS 1985, Atlanta, April 14-17, 1985; p 72.
(8) Sarin, P. S.; Taguchi, Y.; Sun, D.; Thornton, A.; Gallo, R. C.; Oberg, B. *Biochem. Pharmacol.* **1985**, *34*, 4075, 4079.
(9) Lane, H. C.; Fauchi, A. S. *Ann. Intern. Med.* **1985**, *103*, 714-718.
(10) Oberg, B. *J. Antimicrob. Chemo.* **1986**, *17*, 549-551.
(11) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096-7100.
(12) Yarchoan, R.; Klecker, R. W.; Weinhold, K. J.; Markham, P. D.; Lyerly, H. K.; Durack, D. T.; Gelmann, E.; Lehrman, S. N.; Blum, R. M.; Barry, D. W.; et al. *Lancet* **1986**, *1*, 575-580.
(13) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911-1915.
(14) Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463-5467.
(15) Waqar, M. A.; Evans, M. J.; Manley, K. F.; Hughes, R. G.; Huberman, J. A. *J. Cell Physiol.* **1984**, *121*, 402-408.
(16) Cooney, D. A.; Dalal, M.; Mitsuya, H.; McMahon, J. B.; Nadkarni, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1986**, *35*, 2065-2068.
(17) Furmanski, P.; Bourguignon, G. J.; Bolles, C. S.; Corombos, J. D.; Das, M. R. *Cancer Lett.* **1980**, *8*, 307-315.



1: R = H
24: R = $PO_3(NH_4)_2$
2: R = H
25: R = PO_3Na_2
3: R = H
26: R = PO_3Na_2

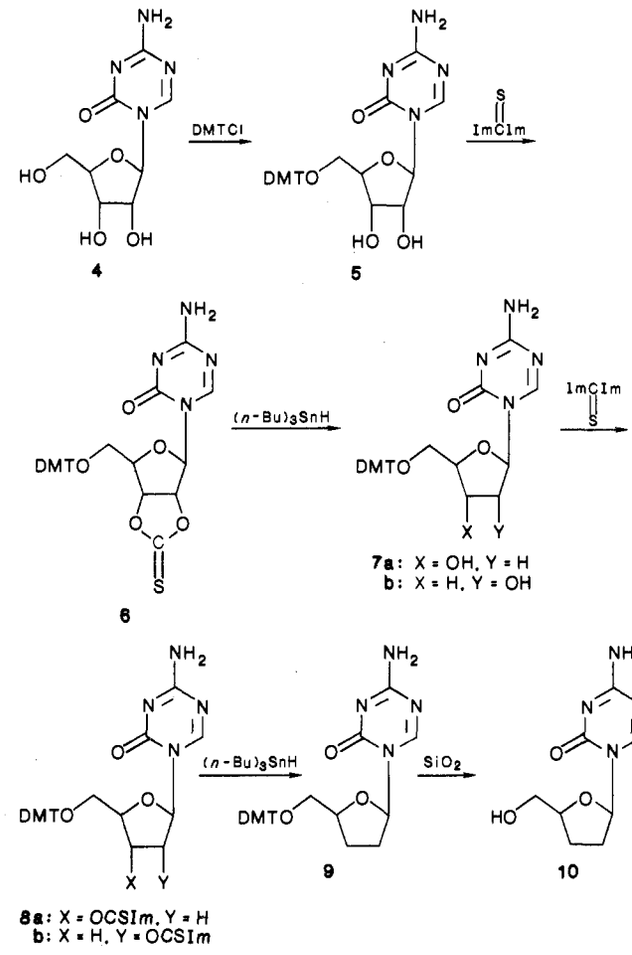
Results and Discussion

Numerous 5-substituted 2′-deoxypyrimidine nucleosides have been prepared in attempts to discover chemotherapeutic agents for DNA viral infections. Although most of the agents evaluated have been analogues of thymidine (5-substituted 2′-deoxyuridines), a small number of 2′-deoxycytidine derivatives with 5-alkyl or 5-halo substitution also have been studied and found to be effective in vitro against the herpes viruses HSV-1 and HSV-2.[18] This encouraged us to prepare a small series of 5-substituted cytidine analogues containing the dideoxyribose moiety in order to study the effect of this substitution on anti-HIV activity and on central nervous system (CNS) penetration, which is low for ddC.

We felt that it might be possible to determine a structure–activity trend for 5-substitution with just a few additional compounds since the parent compound, 1 (proton substitution at position 5), is known to have excellent in vitro activity against HIV.[13] It was hoped that the comparison of the effects of 5-methyl (**15**) vs. 5-bromo (**17**) and hydrogen (**1**) vs. 5-fluoro (**22**) and 5-aza (**10**), as well as the small atoms (**1**, **10**, **22**) vs. the larger groups (**15**, **17**), might indicate the relative importance of electronic and steric effects within a group of compounds which were more lipophilic than the parent molecule.

The 2′,3′-dideoxy-5-azacytidine analogue **10** was prepared first in order to determine whether a reactive heterocyclic base[19,20] might result in enzyme inactivation as well as oligonucleotide termination. The synthesis of 2′,3′-dideoxy-5-azacytidine (**10**) was approached with caution because of the known instability of 5-azacytidine analogues toward nucleophilic attack[20,21] and the sensitivity of dideoxynucleosides to acidic conditions.[22,23] After the 5′-hydroxyl of **4** was blocked with the dimethoxytrityl group[24] to give **5** (Scheme I), a reductive procedure[25,26] was followed to remove one of the secondary hydroxyl groups. This involved the preparation of the cyclic thiocarbonate **6** with thiocarbonyldiimidazole followed by reduction with tri-*n*-butyltin hydride to give a mixture of 2′- and 3′-deoxy derivatives (**7a**, **7b**). Repetition of this procedure removed the second hydroxyl group and gave **9** via a mixture of monothiourethanes (**8a**, **8b**). The dimethoxytrityl group

Scheme I[a]

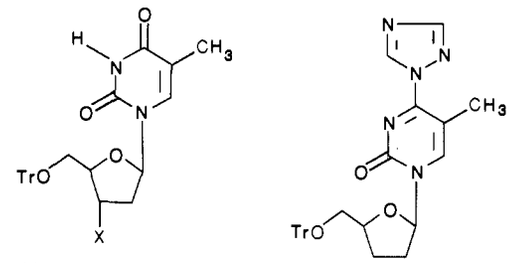


4
5
6
7a: X = OH, Y = H
b: X = H, Y = OH
8a: X = OCSIm, Y = H
b: X = H, Y = OCSIm
9
10

[a] DMT = dimethoxytrityl, Im = imidazolyl.

was removed by mild hydrolysis on silica gel[27] to produce the target compound, **10**.

The 5-methyl analogue **15** was prepared from thymidine by employing the reductive sequence described above. 5′-Tritylthymidine (**11**) was converted to the imidazoylthiocarbonyl derivative **12**, which was reduced to the dideoxy analogue **13**. The conversion of **13** to produce 5-methyl-2′,3′-dideoxycytidine (**15**) was accomplished by ammonolysis of the intermediate 4-triazole derivative **14**[28] and deprotection.



11: X = OH
12: X = OCS(imidazolyl)
13: X = H
Tr = trityl
14

The 5-bromo analogue **17** was easily prepared from 2′,3′-dideoxycytidine (**1**) by bromination with *N*-bromosuccinimide by using the general method of Srivastava and Nagpal.[29] The synthesis of the final analogue in this series,

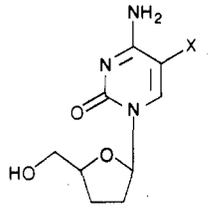
(18) DeClercq, E. In *Approaches to Antiviral Agents*; Harnden, M. R., Ed.; VCH: Deerfield Beach, FL, 1985; pp 57–99.
(19) Wu, J. C.; Santi, D. V. In *Biochemistry and Biology of DNA Methylation*; Cantoni, G. L., Razin, A., Eds.; Alan R. Liss: New York, 1985; pp 119–129.
(20) Beisler, J. *J. Med. Chem.* **1978**, *21*, 204–208.
(21) Beisler, J.; Abbasi, M. M.; Driscoll, J. S. *J. Med. Chem.* **1979**, *22*, 1230–1234.
(22) York, J. L. *J. Org. Chem.* **1981**, *46*, 2171–2173.
(23) Garrett, E. R.; Seydel, J. K.; Sharpen, A. J. *J. Org. Chem.* **1966**, *31*, 2219–2227.
(24) Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 430–440.
(25) Barton, D. H. R.; McConbie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574–1585.
(26) Barton, D. H. R.; McConbie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1718–1723.
(27) Lehrfeld, J. *J. Org. Chem.* **1967**, *32*, 2544–2546.
(28) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171–1176.

Table I. Protective Effect of 2′,3′-Dideoxynucleosides against HTLV-III/LAV (HIV) Pathogenesis

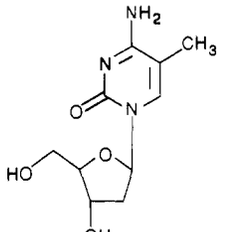
compound	concn, μM	protective effect,[a] %	cytotoxicity,[b] %
ddC (1)	0.05, 0.5, 5, 50	18, 97, 100, 77	0, 0, 0, 30
ddA (2)	1, 5, 10, 50, 100, 200	42, 98, 100, 100, 100, 100	0, 6, 0, 0, 0, 0
ddT (3)	10, 50, 100, 200, 500, 1000, 2000	3, 9, 19, 77, 68, 46, 43	8, 8, 13, 6, 30, 40, 53
dd-5-AC (10)	1, 10, 50	16, 58, 38	13, 19, 62
5-CH_3-ddC (15)	2, 10, 100	2, 7, 3	23, 27, 44
5-Br-ddC (17)	1, 10, 30, 200	6, 8, 5, 0	14, 14, 2, 0
5-F-ddC (22)	0.05, 0.5, 5, 50	5, 100, 100, 88	5, 0, 0, 9
ddCMP (24)	0.5, 2, 10	13, 9, 74	0, 7, 0
ddAMP (25)	1, 5, 10, 50, 100, 200	25, 91, 100, 83, 100, 100	2, 10, 6, 4, 0, 5
ddTMP (26)	10, 50, 200	7, 18, 15	9, 9, 10

[a] The percentage of protective effect of a nucleoside was determined by the following formula: 100 × [(number of viable cells exposed to HTLV-III and cultured in the presence of the nucleoside) − (number of viable cells exposed to HTLV-III cultured in the absence of the nucleoside)] ÷ [(number of viable cells cultured alone) − (number of viable cells exposed to HTLV-III in the absence of the nucleoside)]. [b] The percentage of cytotoxicity was determined by the following formula: 100 × [1 − (number of viable cells cultured in the presence of the nucleoside) ÷ (number of viable cells cultured alone)].

5-fluoro-2′,3′-dideoxycytidine (**22**), started with the preparation of the 5′-*O*-trimethylacetyl (pivaloyl) analogue (**18**) of 5-fluoro-2′-deoxyuridine. By use of the Prisbe and Martin modification[30] of the above described deoxygenation sequence, **19** was reduced to produce the dideoxy analogue **20**, which was converted to the 4-thio derivative **21** with Lawesson's reagent.[31] Treatment of **21** with ammonia gave **22**.



15: X = CH_3
17: X = Br
22: X = F



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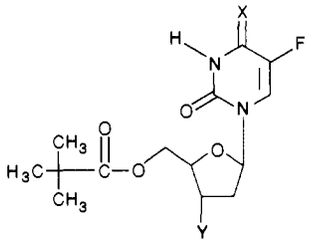

18: X = O, Y = OH
19: X = O, Y = $OCSOCH_3$
20: X = O, Y = H
21: X = S, Y = H

The 5-aza analogue **10** has significant anti-HIV activity at a 10 μM concentration but is clearly more cytotoxic than ddC (Table I). This is consistent with the finding that the ribofuranosyl and 2′-deoxyribofuranosyl analogues of 5-azacytosine are both very cytotoxic antitumor agents.[32,33]

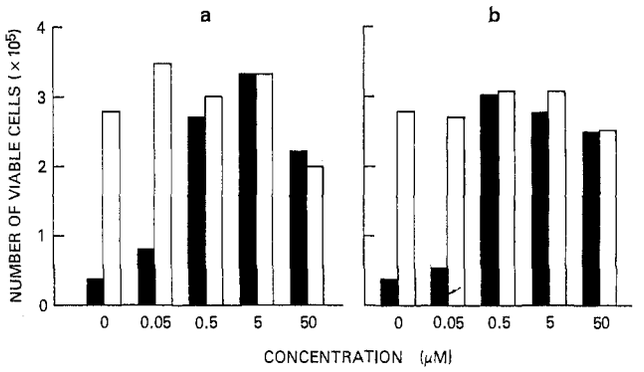

Figure 1. Inhibition of cytopathic effect of HIV by (a) ddC (1) and (b) 5-F-ddC (**22**) against ATH8 cells. ATH8 cells (2×10^5) were preexposed to polybrene, exposed to HTLV-III_B (2000 virus particles per cell) in culture tubes (solid columns) in the presence or absence of various concentrations of the two 2′,3′-dideoxynucleosides. Control cells (open columns) were similarly treated, but were not exposed to the virus. On day 5, total viable cells were counted.

5-Methyl-2′,3′-dideoxycytidine (**15**) is devoid of protective effects but begins to show cytotoxicity at 100 μM. By comparison, the nucleoside with only partial reduction of the sugar, 5-methyl-2′-deoxycytidine (**16**), is neither protective nor cytotoxic (data not shown). The 5-bromo-2′,3′-dideoxy analogue **17** is also ineffective. 2′,3′-Dideoxy-5-fluorocytidine (**22**), however, is as potent and protective to HIV-infected cells as the lead compound, **1** (Figure 1). A biochemical study is planned to determine if the observed activities can be correlated with deoxycytidine kinase and thymidine kinase substrate specificity. A CNS penetration study in rhesus monkeys is currently underway and will be reported separately.

As mentioned above, 2′,3′-dideoxynucleosides must be converted to their 5′-triphosphates to be active against HIV reverse transcriptase, and the rate at which these nucleotides are formed appears to vary with cell type.[16] The first step in this metabolic process, the formation of the monophosphate, is critical since a large nucleoside K_m value for the appropriate deoxynucleoside kinase can result in poor reverse transcriptase inhibitory activity. Additionally, reduced kinase activity can be a cause for the development of resistance as is the case with nucleoside antitumor agents.[34] While it is generally thought that nucleotides do not readily penetrate cells because of their ionic character and relatively low lipophilicity, the nucleoside monophosphates as active drugs would be superior to unphosphorylated compounds for obvious reasons. In 1975, Plunkett and Cohen reported that 2′,3′-dideoxyadenosine 5′-phosphate (**25**) appeared to enter mouse fibroblasts intact with cytotoxic consequences.[35] This prompted us to compare the activities of the 5′-monophosphates of the dideoxy analogues of cytidine (**24**), adenosine (**25**), and thymidine (**26**) in our HIV assay.

Thymidine monophosphate (**26**) is the best candidate to test this approach because of the unusually high doses of **3** required for the protection of HIV-infected cells relative to the other dideoxy nucleosides.[13] The high doses of **3** required for protection probably relate to the very slow

(29) Srivastava, P. C.; Nagpal, K. L. *Experientia* **1970**, *26*, 220.
(30) Prisbe, E. J.; Martin, J. C. *Synth. Commun.* **1985**, *15*, 401–409.
(31) Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 223–228.
(32) Driscoll, J. S.; Johns, D. G.; Plowman, J. *Invest. New Drugs* **1985**, *3*, 331–334.
(33) Chabot, G. G.; Momparler, R. L. *Cancer Treat. Rep.* **1984**, *68*, 1483–1487.
(34) Chabner, B. A.; Myers, C. E. In *Cancer: Principles and Practice of Oncology*, 2nd ed.; DeVita, V. T., Hellman, S., Rosenberg, S. A., Eds.; Lippincott: Philadelphia, 1985; p 300.
(35) Plunkett, W.; Cohen, S. S. *Cancer Res.* **1975**, *35*, 1547–1554.

rate of phosphorylation for this compound.[15] If preformed metabolite, i.e., **26**, were able to penetrate the cell membrane, triphosphate formation might be facilitated with a lowered effective dose resulting. This did not occur (Table I), and **26** was essentially inactive and noncytotoxic at 200 μM, a concentration which afforded considerable protection with the nucleoside **3**. This was also the case with ddCMP (**24**), although significant protection was beginning to be observed at doses approximately 10 times higher than were required for activity with ddC (**1**). The results with ddAMP (**25**) were somewhat different in that the nucleotide appeared to be just as active as the nucleoside **2** over a wide concentration range. However, **25** was not more potent at low concentrations, leading to the suspicion that conversion of **25** to **2** might be occurring through the action of a phosphatase. Nevertheless, it is possible that **25** might offer some practical advantage over the relatively insoluble **2** in a clinical formulation.

Experimental Section

Biological Procedures. HIV cytopathic effect assay was performed with ATH8 cells as previously described.[13] Briefly, 2×10^5 ATH8 cells were exposed to HTLV-III$_B$ virus (2000 virus particles/cell) for 45 min after treatment with polybrene, resuspended in 2 mL of culture medium containing interleukin 2 in the presence or absence of various concentrations of compounds, and incubated in culture tubes at 37 °C in 5% CO_2/95% air humidifed atmosphere. Control cells were treated similarly but were not exposed to the virus. At various time points on days 5–7 of culture, the total viable cells were counted in a hemocytometer by the trypan blue dye exclusion method.

Chemical Procedures. Commercially available synthetic reagents were purchased from the Aldrich Chemical Co. 5-Azacytidine, 2′-deoxy-5-fluorouridine, and 2′,3′-dideoxycytidine were obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, NCI. 5-Methyl-2′-deoxycytidine (**16**) was purchased from Sigma Chemical Co. The dideoxynucleosides **1–3** and their monophosphates **24–26** were obtained from Pharmacia P-L Biochemicals. Preparative TLC plates were Taper Plates from the Analtech Corp. Thomas-Hoover melting points were uncorrected. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN. ^{1}H NMR data were obtained on a Varian XL-200 instrument. Positive-ion fast-atom-bombardment (FAB) mass spectra were acquired with a VG Analytical 7070E mass spectrometer operated under the control of a VG 2035 data system.

5′-*O*-(4,4′-Dimethoxytrityl)-5-azacytidine (5). To a solution of 5-azacytidine (2.20 g, 9 mmol) in dry pyridine (45 mL) was added 4,4′-dimethoxytrityl chloride (3.15 g, 9.3 mmol), and the mixture was stirred overnight. The reaction mixture was poured into ice–water and extracted with chloroform. The combined organic extracts were dried ($MgSO_4$) and chromatographed through a silica gel column (ethyl acetate–10% methanol/ethyl acetate) to give 2.89 g (59%) of a white product: NMR ($CDCl_3$ + D_2O) δ 3.35 (m, 2 H), 3.70 (s, 6 H), 4.26 (m, 3 H), 5.80 (d, 1 H), 6.80 (m, 4 H), 7.31 (m, 9 H), 8.45 (s, 1 H). Anal. ($C_{29}H_{30}N_4O_7 \cdot 0.5H_2O$) C, H. N.

5′-*O*-(4,4′-Dimethoxytrityl)-5-azacytidine 2′,3′-*O*-(Cyclic thiocarbonate) (6). To a solution of compound **5** (0.55 g, 1 mmol) in anhydrous acetonitrile (25 mL) was added 1,1′-thiocarbonyldiimidazole (0.45 g, 2.27 mmol), and the mixture was stirred under nitrogen overnight. The solvent was evaporated and the residue was chromatographed through a silica gel column (ethyl acetate) to give 0.53 g (90%) of a white foam: NMR (D_2O) δ 3.49 (m, 2 H), 3.80 (s, 6 H), 4.42 (m, 2 H), 4.63 (m, 1 H), 5.78 (d, 1 H), 6.80 (m, 4 H), 7.31 (m, 9 H), 7.98 (s, 1 H). Anal. ($C_{30}H_{28}N_4O_7S$) C, H, N, S (ΔC = −0.54).

Mixture of 5′-*O*-(4,4′-Dimethoxytrityl) Derivatives of 2′-Deoxy- and 3′-Deoxy-5-azacytidine (7a, 7b). To a solution of compound **6** (0.53 g, 0.90 mmol) in anhydrous toluene (25 mL) was added α,α'-azoisobutyronitrile (AIBN) (0.02 g, 0.12 mmol) and tri-*n*-butyltin hydride (1.7 mL, 6.52 mmol). The mixture was heated to 110–120 °C for 2 h, the solvent was evaporated, and the residue was purified on preparative TLC (5% methanol/ethyl acetate) to give 0.19 g (41%) of mixture of **7a** and **7b**, which was used without further purification.

2′,3′-Dideoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-azacytidine (9). To a solution of the mixture of **7a** and 7b (0.38 g, 0.72 mmol) in anhydrous acetonitrile (10 mL) was added, 1,1′-thiocarbonyldiimidazole (0.3 g, 1.87 mmol), and the mixture was stirred under nitrogen for 24 h. Solvent was evaporated and the residue was chromatographed through a silica gel column (ethyl acetate → 10% methanol/ethyl acetate) to give 0.32 g (71%) of a mixture of **8a** and **8b**. This mixture was dissolved in anhydrous toluene (20 mL), AIBN (0.02 g, 0.12 mmol) and tri-*n*-butyltin hydride (1.9 mL, 7.28 mmol) were added, and the mixture was heated to 110–120 °C for 2 h. The solvent was evaporated and the residue was purified by preparative TLC (10% methanol/ethyl acetate) to give 0.12 g (47%) of a white foam: NMR ($CDCl_3$ + D_2O) δ 2.20 (m, 4 H), 3.37 (m, 2 H), 3.80 (s, 6 H), 4.23 (m, 1 H), 5.97 (d, 1 H, J = 5 Hz), 6.80 (m, 4 H), 7.31 (m, 9 H), 8.5 (s, 1 H). Anal. ($C_{29}H_{30}N_4O_5 \cdot 0.75H_2O$) C, H, N.

2′,3′-Dideoxy-5-azacytidine (10). Compound **9** (0.08 g, 0.16 mmol) was dissolved in chloroform (2 mL) and poured onto a silica gel column (3 g, Whatman Partisil 40). The column was washed with benzene (40 mL) and left at ambient temperature for 24 h. The column was eluted with ethyl acetate followed by 10% methanol/ethyl acetate to give 0.02 g of crude product along with 0.04 g of unchanged starting material. The crude product was dissolved in water and filtered through a Millex-GS filter, and the filtrate was chromatographed through a C_{18} reverse-phase column (10% methanol/water) to give 0.005 g (30%) of white, lyophilized product: mp 247 °C dec; NMR (D_2O) δ 2.06 (m, 4 H), 3.72 (m, 2 H), 4.18 (m, 1 H), 5.89 (d, 2 H, J = 5 Hz), 8.51 (s, 1 H); FAB mass spectrum, m/z (relative intensity) 113 (b + 2 H, 100), 213 (MH^+, 88); MH^+ calcd 213.0988, found 213.0991 ± 0.0020 (n = 8). Anal. ($C_8H_{12}N_4O_3 \cdot 0.25H_2O$) C, H, N ($\Delta$N = −0.50).

5′-*O*-Tritylthymidine (11). This compound was prepared from thymidine and triphenylmethyl chloride in dry pyridine by the procedure of Horwitz and Urbanski.[36] Recrystallization from acetone/benzene gave a white solid, mp 129–131 °C (lit.[36] mp 128–130 °C).

3′-*O*-(1-Imidazolylthiocarbonyl)-5′-*O*-tritylthymidine (12). To a solution of 11 (0.49 g, 1 mmol) in dry acetonitrile (15 mL) was added 0.42 g (2.1 mmol) of 1,1′-thiocarbonyldiimidazole, and the solution was heated to 90 °C for 3 h under nitrogen. The reaction mixture was cooled, the solvent evaporated, and the residue chromatographed on a preparative TLC plate (ethyl acetate) to give 0.43 g (72%) of a white foam: NMR ($CDCl_3$) δ 1.45 (s, 3 H), 2.71 (m, 3 H), 3.57 (m, 2 H), 4.34 (m, 1 H), 6.14 (m, 1 H), 6.45 (br s, 1 H), 7.29 (m, 15 H), 7.61 (s, 1 H), 7.65 (s, 1 H), 8.09 (s, 1 H), 8.66 (s, 1 H).

3′-Deoxy-5′-*O*-tritylthymidine (13). To a solution of compound **12** (0.35 g, 0.58 mmol) in dry toluene (20 mL) was added AIBN (0.05 g, 0.29 mmol) and tri-*n*-butyltin hydride (3.1 mL, 11.5 mmol), and the mixture was heated to 120 °C for 1 h. The reaction mixture was cooled, the solvent was evaporated, and the residue was chromatographed on a preparative TLC plate (1:1 ethyl acetate/hexane) to give 0.15 g (55%) of a white foam: NMR ($CDCl_3$ + D_2O) δ 2.06 (s, 3 H), 2.14 (m, 3 H), 2.45 (m, 1 H), 3.42 (m, 2 H), 4.12 (m, 1 H), 6.14 (m, 1 H), 7.20 (m, 15 H), 7.62 (s, 1 H).

2′,3′-Dideoxy-5′-*O*-(tritylribosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2-one (14). Triethylamine (8.9 mL, 64 mmol) was added to a stirred, 0 °C mixture of 1,2,4-triazole (4.61 g, 66.92 mmol), phosphoryl chloride (1.34 mL, 14.89 mmol), and acetonitrile (20 mL). To the resulting mixture was added a solution of compound **13** (1.16 g, 2.47 mmol) in acetonitrile (10 mL), and the reaction mixture was stirred under ambient conditions for 1.5 h. After the solvent was evaporated, the residue was dissolved in chloroform. This solution was washed with saturated sodium bicarbonate solution and dried ($MgSO_4$). The solvent was evaporated and the residue was chromatographed on a preparative TLC plate (ethyl acetate) to give 0.69 g (54%) of a white product: NMR ($CDCl_3$) δ 2.0 (s, 3 H), 2.1 (m, 3 H), 2.4 (m, 1 H), 3.4 (m, 2 H), 4.2 (m, 1 H), 6.1 (m, 1 H), 7.2 (m, 16 H),

(36) Horwitz, J. P.; Urbanski, J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3300–3302.

8.0 (s, 1 H), 8.2 (s, 1 H). Anal. ($C_{31}H_{29}N_5O_3 \cdot H_2O$) C, H, N.

2′,3′-Dideoxy-5-methylcytidine (15). To a solution of compound 14 (0.52 g, 1 mmol) in dioxane (10 mL) was added 2 mL of concentrated ammonium hydroxide, and the solution was stirred for 5 h. After the solvents were evaporated, the residue was again dissolved in dioxane (10 mL) and the solution was stirred with 5 mL of AG 50W-X8 resin overnight. The resin mixture was placed in a column and washed with 50% aqueous methanol followed by 2 N NH_4OH. The ammonium hydroxide eluent was lyophilized to give a crude product, which was further purified through a C_{18} reverse-phase column (15% CH_3OH/water) to give 0.11 g (48%) of a lyophilized white solid: NMR (D_2O) δ 1.8 (m, 1 H), 2.0 (s, 3 H), 2.1 (m, 2 H), 2.45 (m, 1 H), 3.8 (m, 2 H), 4.25 (m, 1 H), 6.14 (m, 1 H), 7.78 (s, 1 H); FAB mass spectrum, m/z (relative intensity) 101 (sugar, 14), 126 (b + 2 H, 100), 226 (MH^+, 19). Anal. ($C_{10}H_{15}N_3O_3 \cdot 0.75H_2O$) C, H, N.

2′,3′-Dideoxy-5-bromocytidine (17). To a solution of 2′,3′-dideoxycytidine (0.127 g, 0.6 mmol) in dry DMF (1.5 mL) was added *N*-bromosuccinimide (0.117 g, 0.65 mmol), and the mixture was stirred under nitrogen overnight. Solvent was removed in vacuo and the residue was chromatographed on a preparative TLC plate (ethyl acetate/methanol/triethylamine, 90:10:1) to give 0.097 g (57%) of a white solid: mp 188–190 °C dec after recrystallization from acetone/hexane; NMR (D_2O) δ 1.94 (m, 2 H), 2.02 (m, 1 H), 2.45 (m, 1 H), 3.74 (dd, 1 H), 4.01 (dd, 1 H), 4.23 (m, 1 H), 6.01 (dd, 1 H), 8.70 (s, 1 H); FAB mass spectrum, m/z (relative intensity) 112 (b + 3 H − Br, 99), 190 ([^{79}Br]b + 2 H, 68), 192 ([^{81}Br]b + 2 H, 70), 212 (M + 2 H − Br, 100), 290 ([^{79}Br]MH^+, 35), 292 ([^{81}Br]MH^+, 35). Anal. ($C_9H_{12}N_3BrO_3$) C, H, N, Br.

1-[2′-Deoxy-5′-*O*-(trimethylacetyl)-β-D-ribofuranosyl]-5-fluorouracil (18). To a solution of 2′-deoxy-5-fluorouridine (1.23 g, 5 mmol) in anhydrous pyridine (10 mL) was added trimethylacetyl (pivaloyl) chloride (0.7 mL, 5.6 mmol), and the mixture was stirred overnight. Pyridine and the excess trimethylacetyl chloride were removed in vacuo, and the residue was dissolved in cold water. After extraction with chloroform, the combined organic extracts were dried ($MgSO_4$), the solvent was removed in vacuo, and the residue was chromatographed through a silica gel column (Kieselgel 60, 1:1 ethyl acetate/hexane) to give 1.16 g (70%) of product. Recrystallization from ethyl acetate/hexane gave white needles: mp 135.5–136.5 °C; NMR ($CDCl_3$) δ 1.23 (s, 9 H), 2.04 (m, 1 H), 2.57 (m, 2 H), 4.27 (m, 1 H), 4.42 (m, 2 H), 6.25 (m, 1 H), 7.62 (d, J = 6 Hz, 1 H), 9.7 (br s, 1 H). Anal. ($C_{14}H_{19}FN_2O_6$) C, H, N, F.

2′-Deoxy-3′-(methoxythiocarbonyl)-5′-*O*-(trimethylacetyl)-5-fluorouridine (19). To a solution of compound 18 (0.66 g, 2 mmol) in anhydrous acetonitrile (30 mL) was added 1,1′-thiocarbonyldiimidazole (0.80 g, 4 mmol), and the mixture was heated at 90 °C under nitrogen overnight. Solvent was evaporated, anhydrous methanol (10 mL) was added, and the resulting mixture heated to 60–65 °C for 2 h. Solvent was evaporated and the residue was chromatographed through a silica gel column (Kieselgel 60, ethyl acetate/hexane, 7:3) to give 0.54 g (67%) of a white product: mp 128–128.5 °C after recrystallization from acetone/hexane; NMR ($CDCl_3$) δ 1.24 (s, 9 H), 1.56 (m, 1 H), 2.05 (m, 1 H), 2.63 (m, 1 H), 4.08 (s, 3 H), 4.35 (m, 1 H), 4.52 (m, 2 H), 6.28 (m, 1 H), 7.61 (d, J = 6 Hz, 1 H), 8.61 (br s, 1 H). Anal. ($C_{16}H_{21}N_2O_7FS$) C, H, N, S, F.

2′,3′-Dideoxy-5′-*O*-(trimethylacetyl)-5-fluorouridine (20). To a solution of compound 19 (0.40 g, 1 mmol) in dry toluene (20 mL) were added AIBN (0.08 g, 0.5 mmol) and tri-*n*-butyltin hydride (5.4 mL, 20 mmol). The mixture was heated at 120–125 °C for 0.5 h. The solvent was removed and the residue purified on a preparative TLC plate (1:1 ethyl acetate/hexane) to give 0.21 g (68%) of a white foam: NMR ($CDCl_3$) δ 1.16 (s, 9 H), 1.78 (m, 1 H), 1.98 (m, 2 H), 2.42 (m, 1 H), 4.26 (m, 3 H), 5.96 (m, 1 H), 7.64 (d, J = 6 Hz, 1 H), 9.87 (br s, 1 H). Anal. ($C_{14}H_{19}N_2O_5F \cdot 0.5H_2O$) C, H, N, F.

2′,3′-Dideoxy-5′-*O*-(trimethylacetyl)-4-thioxo-5-fluorouridine (21). To a solution of compound 20 (0.41 g, 1.30 mmol) in anhydrous benzene (30 mL) was added 1.15 g (2.8 mmol) of Lawesson's reagent,[31] and the mixture was heated to 110 °C for 2 h. The reaction mixture was cooled and filtered through a bed of Celite. Solvent was evaporated and the residue was chromatographed through a silica gel column (Kieselgel 60, 1:1 ethyl acetate/hexane) to give 0.27 g (62%) of a greenish foam, which was used without further purification.

2′,3′-Dideoxy-5-fluorocytidine (22). To a solution of compound 21 (0.5 g, 1.5 mmol) in chloroform (5 mL) was added 75 mL of saturated methanolic ammonia, and the mixture was heated in a stainless steel bomb at 138 °C (ca. 250 psi) for 24 h. Upon cooling, the solvent was evaporated and the residue was treated with fresh methanol (50 mL) and filtered to eliminate the insoluble pivaloylamide and inorganic impurities. The clear solution was reduced to dryness and the off-white residue was dissolved in water (7 mL) and purified through a C_{18} reverse-phase column (7.5 in. × 1.5 in. diameter) using 10% MeOH/H_2O as eluant and a flow rate of 3 mL/min. Fractions (15 mL) were monitored by UV (254 nm), and collection of product began after 2 h. Collection of pure product lasted 1 h, and the combined fractions were lyophilized to give 210 mg (60%) of a white product: mp 100 °C soften, 155–160 °C; NMR (D_2O) δ 1.78 (m, 1 H), 2.06 (m, 2 H), 2.44 (m, 1 H), 3.82 (m, 2 H), 4.22 (m, 1 H), 6.02 (m, 1 H), 8.06 (d, J = 6 Hz, 1 H); FAB mass spectrum, m/z (relative intensity) 130 (b + 2 H, 100), 230 (MH^+, 76). Anal. ($C_9H_{12}N_3O_3F \cdot 0.75H_2O$) C, H, N.

Acknowledgment. We thank Dr. James A. Kelley for the mass spectral determinations including the exact mass measurement for compound **10**.

Registry No. 1, 7481-89-2; **2**, 4097-22-7; **3**, 3416-05-5; **4**, 320-67-2; **5**, 107036-45-3; **6**, 107036-46-4; **7a**, 107036-47-5; **7b**, 107036-48-6; **8a**, 107036-49-7; **8b**, 107036-50-0; **9**, 107036-51-1; **10**, 107036-52-2; 11, 7791-71-1; **12**, 107036-53-3; **13**, 107036-54-4; **14**, 107036-55-5; **15**, 107036-56-6; **17**, 107036-57-7; **18**, 107036-58-8; **19**, 107036-59-9; **20**, 107036-60-2; **21**, 107036-61-3; **22**, 107036-62-4; **24**, 107133-41-5; **25**, 107132-15-0; **26**, 107132-16-1; thymidine, 50-89-5; 1,2,4-triazole, 288-88-0; 2′-deoxy-5-fluorouridine, 50-91-9.