9-(*trans*-4-Hydroxy-2-buten-1-yl)guanine (2b). A solution of compound **6b** (1.29 g, 5 mmol) in 0.1 M HCl (30 mL) was refluxed for 5 h. The mixture was evaporated, and the residue was coevaporated with water (25 mL) and finally dissolved in the same solvent (30 mL). This solution was stirred with Dowex 1 (acetate, 10 g, wet weight) for 1 h at room temperature. The resin was filtered off, it was repeatedly washed with water (total of 1 L) and the filtrate was evaporated. The residue was chromatographed on a silica gel column (65 g) using solvent S_2 as an eluent. The appropriate UV-absorbing fractions were collected and evaporated to give a white solid, which was crystallized from an acetone-methanol-water (7:2:1) mixture (15 mL), giving 0.77 g (70%) of **2b**, mp 231-233 °C, homogeneous on TLC (S_4). Two more recrystallizations afforded 0.60 g (54%): mp 269 °C; UV (0.01 M Na_2HPO_4, pH 7) max 252 nm (ϵ 13 800), 272 sh (δ 10 200); NMR (CD_3SOCD_3) δ 10.65 (s, 1, purine ring NH), 7.63 (s, 1, purine ring H), 6.50 (s, 2, NH_2), 5.74 and 5.59 (2 td, 2, *trans*-CH=CH, J = 15 Hz), 4.75 (poorly resolved t, 1, OH), 4.54 and 3.89 (d and an apparent s, 4, CH_2); MS, m/e (ion, relative abundance) 221 (M, 1.6), 202 (1.4), 190 (M - CH_2OH, 4.8), peaks of m/e 151, 109, 69, 55, and 44 were found in the mass spectrum of guanine.[28] Anal. (C, H, N - hemihydrate).

Deamination of 9-(*trans*-4-Hydroxy-2-buten-1-yl)adenine (2a) with Adenosine Deaminase. Compound **2a** (0.5 mg, 2.4 μmol) and adenosine deaminase from calf intestine (type II, Sigma Chemical Co., St. Louis, MO, 0.4 units) were incubated in 0.05 M Na_2HPO_4 (pH 7.5, 0.4 mL) at room temperature with magnetic stirring. The final concentration of **2a** was 5.5×10^{-3} M. Aliquots were removed, and they were examined by TLC (S_2) and, after appropriate dilution, by UV spectroscopy. The reaction was complete after 19 h (UV max 250 nm). Adenine, which is a very weak substrate,[29] was completely resistant to deamination under the conditions of assay. By contrast, adenosine was quantitatively deaminated at 4.3×10^{-5} M with the same amount of enzyme in 4 min as shown by UV spectrophotometric assay.[30]

Acknowledgment. This investigation was supported by NIH Grant CA 32779 from the National Cancer Institute, Bethesda, MD, and by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. Thanks are due to the Central Instrumentation Facility, Department of Chemistry, Wayne State University, for measurements of ^1H NMR (Dr. O. Mols) and mass spectra (Dr. R. J. Hood). We also thank Dr. D. Kessel, Department of Pharmacology and Internal Medicine, Wayne State University School of Medicine, for assays in murine leukemia L 1210 cell culture and to Dr. H. E. Renis, Cancer Research, The Upjohn Company, Kalamazoo, MI, for antiviral screening. We also thank a referee for providing us with ref 18 and 19.

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(31) **Note added in proof**: Compounds **2a** and **2b** were inactive against vaccinia virus up to 320 μg/mL and HTLV-III (AIDS) virus (Canonico, P. G.; Broder, S., personal communications).

Synthesis and Antiviral Activity of Various 3'-Azido, 3'-Amino, 2',3'-Unsaturated, and 2',3'-Dideoxy Analogues of Pyrimidine Deoxyribonucleosides against Retroviruses[1]

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Various 3'-azido, 3'-amino, 2',3'-unsaturated, 2',3'-dideoxy, and 5-substituted analogues of pyrimidine deoxyribonucleosides have been prepared and tested against Moloney-murine leukemia virus (M-MULV), a mammalian T-lymphotropic retrovirus in vitro. Among these compounds, the 3'-azido analogues of thymidine, 2'-deoxy-5-bromouridine, and 2'-deoxy-5-iodouridine, the 2',3'-unsaturated analogue of thymidine and 2'-deoxycytidine, and 2',3'-dideoxycytidine were found to be most active, with ED_{50} values of 0.02, 1.5, 3.0, 2.5, 3.7, and 4.0 μM, respectively. These active compounds were nontoxic to the host SC-1 cells up to 100 μM concentration. The 3'-azido analogues of thymidine and 2'-deoxy-5-bromouridine were also tested in vitro against HTLV-III/LAV/AAV ("AIDS" virus) and found to be significantly active, with ED_{50} values of 0.23 and 2.3 μM, respectively. The structure-activity relationships are discussed.

There is a need for compounds that may be effective in the therapy of acquired immune deficiency syndrome (AIDS). It has been estimated that one million or more individuals in the United States have been exposed to the HTLV-III/LAV virus, the putative causative agent of AIDS, and as of May 1986 over 20 000 cases of AIDS have been reported. Of those diagnosed as having AIDS 3 or more years ago, 85% are now dead.

Some compounds have been identified as having an inhibitory effect against retroviruses, particularly the HTLV-III/LAV virus, and hence of potential use in the therapy of AIDS. These include HPA-23,[3,4] interferon,[5] ribavirin,[6] phosphonoformate,[7,8] ansamycin,[9] suramin,[10-12]

(1) This research has been presented in part; see: Gao, Y. S.; Lin, T. S.; Prusoff, W. H. In *Abstracts of Papers*, 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 1982; American Chemical Society: Washington, DC, 1982; CARB 55.
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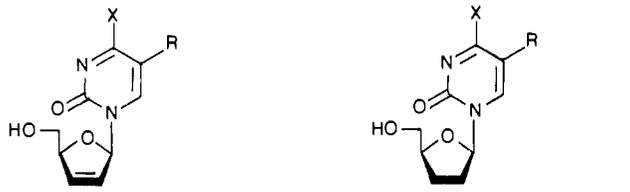


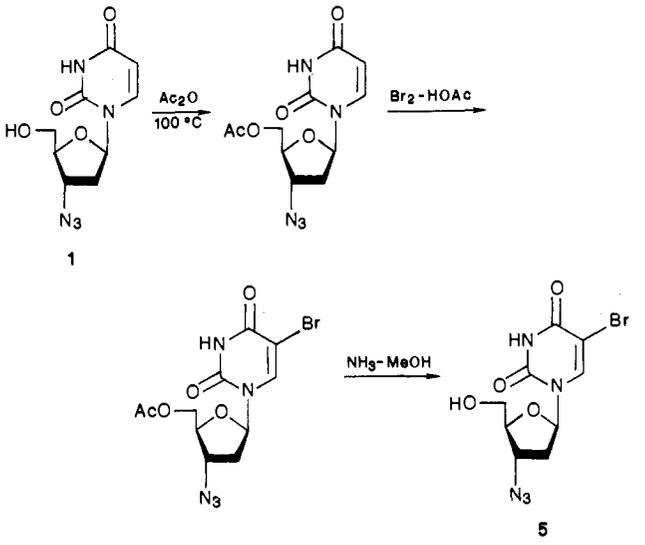
Figure 1. Structure formulae of various 3′-azido, 3′-amino, 5′-azido, 5′-amino, 3′,5′-diazido, 3′,5′-diamino, 2′,3′-unsaturated, and 2′,3′-dideoxy analogues of pyrimidine deoxyribonucleosides.

AL-721,[13] 3′-azido-3′-deoxythymidine,[14-18] and more recently several 2′,3′-dideoxynucleosides[19] of which 2′,3′-dideoxycytidine was most potent.

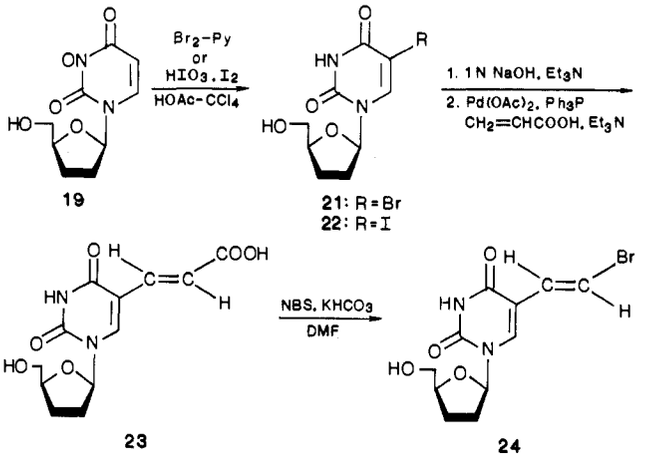
The present paper emanates from the finding that 3′-azido-3′-deoxythymidine has activity against the HTLV-III/LAV virus,[18] as previously reported for the Friend virus, which is another retrovirus,[14,15] and is in clinical trial.[20] Ostertag et al.[15] suggested that this compound could possibly be used to inhibit viral DNA replication by interfering with DNA chain elongation, without significantly affecting cell viability. Horwitz et al.[21] were the first to synthesize 3′-azido-3′-deoxythymidine, and later as part of a program concerned with the synthesis of 3′-amino nucleosides, Lin and Prusoff[22] synthesized 3′-azido-3′-deoxythymidine by a modification of the procedure of Horwitz et al.[21]

The present paper describes the synthesis of a number of nucleoside analogues and their antiviral activity against a retrovirus, Moloney-murine leukemia virus, which was used as a primary screen, as well as the antiviral activity

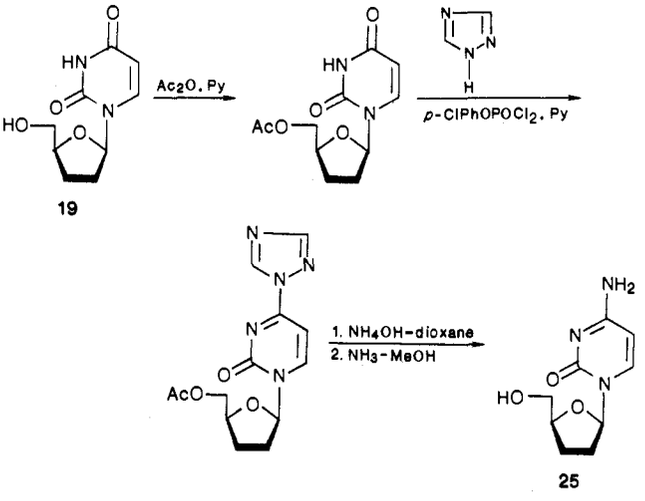
Scheme I



Scheme II



Scheme III



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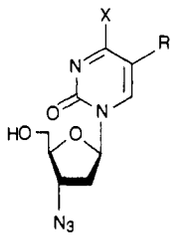
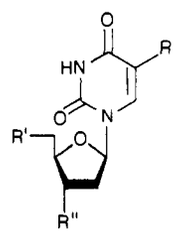
of the most potent compounds derived from the primary screen, against the HTLV-III/LAV/AAV virus.

Chemistry

Various 3′-azido, 3′-amino, 2′,3′-unsaturated, 2′,3′-dideoxy, and 5-substituted pyrimidine 2′-deoxyribofuranosyl nucleoside analogues (Figure 1) have been synthesized and tested as potential antiretrovirus agents. The 3′-azido derivatives **1**, **3**, **4**, **6**, **7–9**, 5′-azido derivative **10**, 3′,5′-diazido derivative **11**, 3′-amino derivative **13**, 5′-amino derivative **14**, and 3′,5′-diamino derivative **15** were synthesized by the methodology previously described.[22-24] Compounds **16**, **17**, **19**, and **20** were prepared by the procedures of Horwitz et al.[25,26] Compounds **2** and **12** were fabricated by the methodology of Horwitz et al.[21] with minor modification.[22] 3′-Azido-2′,3′-dideoxy-5-bromouridine (**5**) was synthesized by treatment of 3′-azido-2′,3′-dideoxyuridine (**1**)[23] with acetic anhydride at 100 °C, followed by bromination[27] of the acetate with bromine and subsequent removal of the 5-*O*-acetyl protecting group in methanolic ammonia (Scheme I). Bromination[28] or iodination[29] of 2′,3′-dideoxyuridine (**19**)[26] with bromine in pyridine or iodine and iodic acid in glacial acetic acid gave the 5-bromo and 5-iodo analogues **21** and **22**, respectively. Reaction of 5-iodo derivative **22** with palladium acetate, acrylic acid, and triphenylphosphine in the presence of triethylamine and 1 N NaOH solution in dioxane[30] afforded the corresponding (*E*)-5-(2-carboxyvinyl)-2′,3′-dideoxyuridine (**23**), which was subsequently treated with *N*-bromosuccinimide and $KHCO_3$ in DMF[31] to yield (*E*)-5-(2-bromovinyl)-2′,3′-dideoxyuridine (**24**) (Scheme II). 2′,3′-Unsaturated cytidine analogue **18** and 2′,3′-dideoxycytidine (**25**) were first synthesized by Horwitz et al.;[26] however, a different route was adopted for the preparation of **18** and **25** from the 2′,3′-unsaturated uridine derivative **16** and 2′,3′-dideoxyuridine **19**, respectively. Acetylation of compounds **16** and **19** with acetic anhydride in pyridine gave the corresponding acetates, which were then treated with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature[32] to yield the 4-triazolylpyrimidinone derivatives. Subsequent treatment of the 4-triazolylpyrimidinone derivatives with aqueous ammonia in dioxane (1:3) for several hours and then methanolic ammonia overnight at room temperature[32] yielded compounds **18** and **25**, respectively (Scheme III).



1: R = H, X = OH
2: R = CH_3, X = OH
3: R = CF_3, X = OH
4: R = F, X = OH
5: R = Br, X = OH
6: R = I, X = OH
7: R = H, X = NH_2
8: R = F, X = NH_2
9: R = CH_3, X = NH_2



10: R = CH_3, R′ = N_3, R″ = OH
11: R = CH_3, R′ = R″ = N_3
12: R = CH_3, R′ = OH, R″ = NH_2
13: R = CF_3, R′ = OH, R″ = NH_2
14: R = CH_3, R′ = NH_2, R″ = OH
16: R = CH_3, R′ = R″ = NH_2

Table I. Antiviral Activity of Various 3′-Azido, 3′-Amino, 2′,3′-Unsaturated, and 2′,3′-Dideoxy Analogues of Pyrimidine Deoxyribonucleosides on the Replication of Moloney Murine Leukemia Virus (M-MULV) in Vitro

compd	ED_{50},[a] μM (M-MULV)	compd	ED_{50},[a] μM (M-MULV)
1	52	13	>100
2	0.02	14	>100
3	>100	15	>100
4	>100	16	>100
5	1.5	17	2.5
6	3.0	18	3.7
7	58	19	>100
8	>100	20	>100
9	>100	21	>100
10	>100	22	>100
11	17	24	>100
12	42	25	4.0

[a] The ED_{50} values were estimated from dose–response curves and represent the drug concentration required to inhibit 50% of the syncytial forming units of the virus.

Antiviral Activity

These compounds were tested against Moloney-murine leukemia virus (M-MULV) a mammalian T-lymphotropic retrovirus. The antiviral activity was expressed by the effective dose (μM) that inhibits 50% of the syncytial forming units.

Among the 3′-azido and 3′-amino derivatives of 2′-deoxyuridine, 3′-azido-3′-deoxythymidine (**2**) was the most active against M-MULV in vitro with an ED_{50} value of 0.02 μM. The 3′-azido analogues of 5-bromo- and 5-iodo-2′-deoxyuridine, compounds **5** and **6**, also showed significant antiviral activity with ED_{50} values of 1.5 and 3.0 μM, respectively. The 3′-azido derivative of 2′-deoxyuridine **1** and the 3′,5′-diazido and 3′-amino derivatives of thymidine, **11** and **12**, demonstrated moderate antiviral activity, giving ED_{50} values of 52, 17, and 42 μM, respectively. Conversely, the 3′-azido analogue of 2′-deoxycytidine **7** only showed moderate inhibition against M-MULV with an ED_{50} value of 58 μM. The other 3′-azido and 3′-amino derivatives in this group were found to be practically inactive. Among the 2′,3′-unsaturated and 2′,3′-dideoxy derivatives of pyrimidine deoxyribonucleosides, the 2′,3′-unsaturated analogue of thymidine **17** and 2′-deoxycytidine **18** and the 2′,3′-dideoxy analogue of 2′-deoxycytidine **25** produced significant antiviral activity with ED_{50} values of 2.5, 3.7, and 4.0 μM, respectively. The results are summarized in Table I. Whereas Wagar et al.,[33] reported that 2′,3′-dideoxythymidine (**20**) caused 50% inhibition of M-MULV replication in 3T3 cells at a concentration of 10 μM, we found this compound to be practically inactive at a concentration of 100 μM. This inactivity may be due to poor transport or metabolism of the compound by the host cells used, or equally as plausible to the strain of M-MULV used being less susceptible to inhibition because of differences

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in affinity of the formed 2',3'-dideoxythymidine triphosphate for the reverse transcriptases encoded by the two strains of M-MULV.

The two most active 3'-azido analogues of thymidine and 5-bromo-2'-deoxyuridine, compounds **2** and **5**, were selected for evaluation in vitro against HTLV-III/LAV/AAV, the "AIDS" virus, and were found to be significantly active, with ED_{50} values of 0.23 and 2.3 μM, respectively. Compound **2** was about 10 times more activie than compound **5** under our experimental conditions.

Structure-Activity Relationships

There appears to be a relationship between the antiviral activity and the electron-withdrawing capacity of the substituents in the 5-position of the nucleoside analogues as evidenced by the decrease in the antiviral activity when the methyl moiety is replaced ($CF_3 > I > Br > CH_3$). However, when the substituent on carbon-5 is F or H, there is also a marked loss of antiviral activity. These differences may be related to their substrate activity for thymidine kinase, which is required for activation, or to differences in metabolic conversion to the di- or triphosphate, or to the relative affinity of the nucleoside analogue triphosphate for the reverse transcriptase.

Replacement of the uracil moiety of the 3'-azido nucleoside analogue (**1**) with a cytosine (**7**) did not affect its antiviral activity; however, when the substituent on carbon-5 of the pyrimidine moiety was either F or CH_3 (compounds **8** and **9**), the antiviral activity was markedly reduced. This could be explained if these 3'-azido-2',3'-dideoxycytidine analogues were required to be deaminated by deoxycytidine deaminase for which the 5-methyl or 5-F analogues were not substrates. However, other possibilities include differences in metabolic conversion to the di- and triphosphates, as well as relative affinities of the triphosphate analogues for the reverse transcriptase.

The azido group in the 3'-position of the deoxyribose moiety of the thymidine (**2**) is critical since transfer to the 5'-position (**10**) results in a very marked decrease in activity. However, retention of the 3'-azido group with addition of an azido to the 5'-position (**11**) increased the antiviral activity relative to **10**, but decreased its activity relative to **2**. Thus a primary hydroxyl in the 5'-position is beneficial. Although one presumes the 5'-hydroxyl is required for substrate activity for thymidine kinase, this can not be a prerequisite for the antiviral activity of the 3',5'-diazido analogue **11**, unless the 5'-azido moiety were hydrolytically cleaved, either chemically or enzymically, to a hydroxyl, which is most unlikely.

Reduction of the 3'-azido moiety of **2** to an amino group (**12**) markedly decreased activity; however, moderate antiviral activity was retained. Whether the activity of **12** is related to a bonafide antiviral activity of this compound or to the cytotoxic properties[22] of **12** is not clear.

Replacement of the 3'-azido moiety of **2** with H (**20**) resulted in loss of activity; however, subsequent removal of a hydrogen atom from both the 2'- and 3'-carbon produced **17**; thus the azido moiety per se is not an absolute requirement for the antiviral activity of the thymidine analogues. However, replacement of the methyl group of **17** with a hydrogen (**16**) resulted in loss of activity.

The 3'-azido moiety conferred moderate antiviral activity to 2'-deoxycytidine (**7**), but this moiety is not an absolute requirement since its replacement with a hydrogen (**25**) resulted in a very marked increase in activity. Subsequent removal of a hydrogen from the 2'- and 3'-carbon of **25** to produce **18** did not affect the increase in antiviral activity from that of **7**. Deamination of **25** produced **19** with a concomitant loss of antiviral activity, which could not be recovered by insertion of a variety of substituents on the carbon-5 of the uracil moiety (**20–24**).

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded at 60 MHz on a Varian T-60 spectrometer or at 500 MHz on a Brucker WM-500 spectrometer with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. IR spectra were taken on the Perkin-Elmer 21 spectrophotometer. 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 obtained for those elements were within ±0.4% of the theoretical values.

3'-Azido-2',3'-dideoxy-5-bromouridine (5). A suspension of 3'-azido-2',3'-dideoxyuridine[23] (1.0 g, 3.95 mmol) in 25 mL of acetic anhydride was heated until dissolution occurred. A solution of bromine (0.7 g, 4.35 mmol) in 3 mL of glacial acetic acid was added to the above solution with cooling, to maintain a temperature at 25 °C. After being kept overnight in the cold (4 °C), the solution was evaporated to dryness under reduced pressure to give 3'-azido-5'-*O*-acetyl-2',3'-dideoxy-5-bromouridine as a thick syrup, which was dissolved immediately in 50 mL of absolute methanol containing 7 g of anhydrous ammonia. This solution was then kept at 4 °C for 48 h. The solvent was removed in vacuo at ~30 °C, and the residue was chromatographed on a silica gel column (CH_2Cl_2–EtOAc, 1:1). The fractions containing the desired product (R_f 0.51) were pooled together, and the solvent was evaporated in vacuo to afford 0.55 g (42%) of white plate-like crystals: mp 150–151 °C; IR (KBr) 4.70 (azido) μm; UV (EtOH) λ_{max} 278 nm (ϵ 14 300); UV (EtOH) λ_{min} 240 nm; UV (0.1 N HCl) λ_{max} 278 nm (ϵ 9400); UV (0.1 N HCl) λ_{min} 240 nm; UV (0.1 N NaOH) λ_{max} 274 nm (ϵ 6800); UV (0.1 N NaOH) λ_{min} 248 nm; NMR (Me_2SO-d_6) δ 2.28–2.33 (m, 1 H, 2'-H_a), 2.42–2.46 (m, 1 H, 2'-H_b), 3.59 (d, 1 H, 5'-H_a), 3.68 (d, 1 H, 5'-H_b), 3.83 (m, 1 H, 4'-H), 4.37 (m, 1 H, 3'-H), 5.35 (br s, 1 H, 5'-OH, D_2O exchangeable), 6.01 (t, 1 H, 1'-H), 8.37 (s, 1 H, 6-H), 11.81 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{10}BrN_5O_4$) C, H, Br, N.

2',3'-Dideoxyuridin-2'-ene (16): mp 155–156 °C (lit.[25] mp 153–154 °C); NMR (Me_2SO-d_6) δ 3.57 (d, 2 H, 5'-H), 4.76 (m, 1 H, 4'-H), 5.58 (d, 1 H, 5-H), 5.90 (m, 1 H, 3'-H, vinyl), 6.39 (m, 1 H, 2'-H, vinyl), 6.79 (m, 1 H, 1'-H), 7.73 (d, 1 H, 6-H).

2',3'-Dideoxycytidin-2'-ene (18). This compound was prepared from compound **16** by the methodology described for the synthesis of compound **24**: yield, 40% (based on compound **16**); mp 162–163 °C (lit.[26] mp 168–169 °C); UV (0.1 N HCl) λ_{max} 275 nm (ϵ 11 340), λ_{min} 237 nm; UV (0.1 N NaOH) λ_{max} 267 nm (ϵ 7010), λ_{min} 247 nm; NMR (Me_2SO-d_6) δ 3.56 (m, 2 H, 5'-H), 4.75 (m, 1 H, 4'-H), 4.95 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.68 (d, 1 H, 5-H), 5.88 (m, 1 H, 3'-H, vinyl), 6.33 (m, 1 H, 2'-H, vinyl), 6.89 (m, 1 H, 1'-H), 7.12–7.19 (br d, 1 H, 4-NH_2, D_2O exchangeable), 7.68 (d, 1 H, 6-H).

2',3'-Dideoxyuridine (19): mp 116–117 °C (lit.[25] mp 117.5–118.5 °C); NMR (Me_2SO-d_6) δ 1.78–2.31 (m, 4 H, 2'- and 3'-H), 3.49–3.70 (m, 2 H, 5'-H), 3.98–4.06 (m, 1 H, 4'-H), 5.58 (d, 1 H, 5-H), 5.94 (t, 1 H, 1'-H), 7.94 (d, 1 H, 6-H).

2',3'-Dideoxy-5-bromouridine (21). A solution of bromine (0.53 g, 3.3 mmol) in CCl_4 (1.8 mL) was added to a solution of compound **19** (0.62 g, 2.9 mmol) in 14 mL of pyridine. The reaction mixture was stirred at room temperature for 2 h. The solvents were evaporated to dryness in vacuo. The residue was coevaporated with MeOH several times and crystallized from H_2O–MeOH (4:1, v/v) to yield 0.42 (49%) of colorless needles: mp 172–173 °C (lit.[28] mp 178–179 °C); UV (H_2O) λ_{max} 282 nm; NMR (Me_2SO-d_6) δ 1.52–2.40 (br m, 4 H, 2'- and 3'-H), 3.46–3.80 (m, 2 H, 5'-H), 3.80–4.28 (m, 2 H, 4'-H and 5'-OH, D_2O exchangeable), 5.90 (m, 1 H, 1'-H), 8.50 (s, 1 H, 6-H), 11.6 (br s, 1 H, 3-NH, D_2O exchangeable).

2',3'-Dideoxy-5-iodouridine (22). A mixture of compound **19** (1.0 g, 4.7 mmol), iodic acid (0.27 g, 1.50 mmol), and iodine (0.45 g, 1.70 mmol) in a mixture of glacial HOAc (4 mL), CCl_4 (1 mL), and H_2O (1 mL) was stirred at 45 °C for 2 h. The solvents were removed in vacuo at ~40 °C. The resulting residue was coevaporated five times with MeOH and chromatographed on

a silica gel column ($CHCl_3$–EtOH, 4:1) to give 0.4 g (31%) of product: mp 150 °C dec; UV (EtOH) λ_{max} 285 nm (ϵ 8640), λ_{min} 248 nm; UV (0.01 N HCl) λ_{max} 290 nm (ϵ 8480), λ_{min} 250 nm; UV (0.01 N NaOH) λ_{max} 276 nm (ϵ 6000), λ_{min} 250 nm; NMR (Me_2SO-d_6) δ 1.79–1.93 (m, 2 H, 3′-H), 1.93–2.06 (m, 1 H, 2′-H_a or H_b), 2.18–2.32 (m, 1 H, 2′-H_a or H_b), 3.48–3.54 (m, 1 H, 5′-H_a or H_b), 3.70–3.77 (m, 1 H, 5′-H_a or H_b), 4.05 (m, 1 H, 4′-H), 5.20 (t, 1 H, 5′-OH, D_2O exchangeable), 5.87 (q, 1 H, 1′-H), 8.56 (s, 1 H, 6-H), 11.6 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{11}IN_2O_3$) C, H, N.

(*E*)-5-(2-Carboxyvinyl)-2′,3′-dideoxyuridine (23). A solution of the 5-iodo derivative **22** (1.0 g, 2.9 mmol) in 3 mL of 1 N NaOH and 0.5 mL of triethylamine (3.5 mmol) was added dropwise to a stirred solution of palladium acetate (0.034 g, 0.2 mmol), triphenylphosphine (0.078 g, 0.3 mmol), acrylic acid (0.41 mL, 6 mmol), and triethylamine (0.5 mL, 3.5 mmol) in 4 mL of dioxane at ~95 °C (oil bath). After the addition, the reaction mixture was heated at the same temperature for an additional 3 h, cooled to room temperature, and filtered to remove most of the palladium metal. A 20% aqueous KOH solution was added to the filtrate. The excess solvent and triethylamine was removed under reduced pressure, and the remaining solution was left overnight at room temperature in order to hydrolyze the 2-carboxyethyl ester of the 2-carboxyvinyl derivative, which has been formed to some extent as a byproduct during the reaction by Michael addition on the double bond of the acrylic acid. The solution was filtered again and then carefully acidified with concentrated hydrochloric acid to pH 2 while cooling in an ice bath. The resultant solid was collected by filtration and washed twice with H_2O and then with chilled acetone until the washings were colorless. After drying, the product weighed 0.41 g (49%): mp 260 °C dec; UV (EtOH) λ_{max} 304 nm, λ_{min} 278 nm; NMR (Me_2SO-d_6) δ 1.60–245 (br m, 4 H, 2′- and 3′-H), 3.34–3.92 (m, 2 H, 5′-H), 4.10 (m, 1 H, 4′-H), 5.32 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.08 (m, 1 H, 1′-H), 6.72 (d, 1 H, vinyl H_A), 7.31 (d, 1 H, vinyl H_B), 8.62 (s, 1 H, 6-H), 11.6 (br s, 1 H, COOH, D_2O exchangeable). Anal. ($C_{12}H_{14}N_2O_6$) C, H, N.

(*E*)-5-(2-Bromovinyl)-2′,3′-dideoxyuridine (24). A solution of *N*-bromosuccinimide (0.26 g, 1.5 mmol) in 1.5 mL of DMF was added slowly, during a period of half an hour, to a stirred solution of compound **23** (0.34 g, 1.5 mmol) in 3 mL of DMF containing 0.34 g of solid $KHCO_3$. The reaction mixture was further stirred at room temperature for 2 h and then filtered to remove the insoluble material. The filtrate was evaporated in vacuo at a bath temperature of >45 °C. The oily residue was taken up in H_2O and evaporated again. This operation was repeated three times. The gummy residue was dissolved in water and the pH was adjusted to 6.5. The solution was concentrated and kept at 4 °C overnight. The resultant crystals were collected by filtration, washed with H_2O, and dried, to yield 0.25 g (53%) of product. The analytical sample was obtained by silica gel column chromatography ($CHCl_3$–EtOH, 10:1): mp 160–162 °C dec; UV (EtOH) λ_{max} 295 nm, λ_{min} 254 nm; NMR (Me_2SO-d_6) δ 1.81–1.89 (br m, 2 H, 3′-H), 1.97–2.03 (m, 1 H, 2′-H_a), 2.24–2.31 (m, 1 H, 2′-H_b), 3.52–3.57 (m, 1 H, 5′-H_a), 3.58–3.77 (m, 1 H, 5′-H_b), 4.04 (m, 1 H, 4′-H), 5.02 (br s, 1 H, 5′-OH, D_2O exchangeable), 5.93 (m, 1 H, 1′-H), 6.81 (d, 1 H, vinyl H_A), 7.21 (d, 1 H, vinyl H_B), 8.24 (s, 1 H, 6-H), 11.2 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_{13}BrN_2O_4$) C, H, Br, N.

2′,3′-Dideoxycytidine (25). Acetic anhydride (0.96 g, 9.45 mmol) was added slowly to a stirred solution of compound **19** (0.40 g, 1.89 mmol) in 10 mL of pyridine at 0 °C (ice bath). The resultant solution was allowed to stand overnight at 4 °C. The solvent and the excess acetic anhydride was removed in vacuo. The remaining residue was dissolved in 50 mL of $CHCl_3$ and washed in a separatory funnel with 50-mL portions of H_2O (three times), saturated $NaHCO_3$ (two times), and H_2O again (two times). The $CHCl_3$ solution was clarified with Norit, dried with anhydrous $MgSO_4$, and filtered. The filtrate was then concentrated to a residue, which was used immediately without further purification for the next preparation.

The acetate was dissolved in 10 mL of pyridine. While the mixture was stirred in a cold-water bath, 4-chlorophenyl phosphorodichloridate (0.70 g 2.84 mmol) was added dropwise, followed by the addition of 1,2,4-triazole (0.39 g, 5.68 mmol). The mixture was stirred at room temperature for 3 days and then concentrated under reduced pressure (~30 °C). The resulting residue was dissolved in 25 mL of CH_2Cl_2 and washed with 25 mL of H_2O (two times) and 50% $NaHCO_3$ solution (25 mL). The CH_2Cl_2 solution was clarified with Norit, dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness in vacuo to yield a glassy residue (4-triazolylpyrimidinone derivative), which was dissolved in 20 mL of NH_4OH–dioxane (1:3). The mixture was stirred for 5 h at room temperature in a Wheaton pressure bottle. This solution was then concentrated and the remaining residue was stirred overnight in the pressure bottle at room temperature in 20 mL of saturated methanolic ammonia. The solution was then reduced to a small volume in vacuo and chromatographed on a silica gel column ($CHCl_3$–MeOH, 3:1, R_f 0.4) to afford 0.18 g (45% based on **19**) of product: mp 209–210 °C (lit.[26] mp 215–217 °C); UV (0.1 N HCl) λ_{max} 280 nm (ϵ 17720), λ_{min} 238 nm; UV (0.1 N NaOH) λ_{max} 270 nm (ϵ 8410), λ_{min} 247 nm; NMR (Me_2SO-d_6) δ 1.72–1.88 (m, 3 H, 2′-H_a and 3′-H), 2.21–2.29 (m, 1 H, 2′-H_b), 3.54 (m, 1 H, 5′-H_a), 3.66 (m, 1 H, 5′-H_b), 4.01 (m, 1 H, 4′-H), 4.98 (t, 1 H, 5′-OH, D_2O exchangeable), 5.68 (d, 1 H, 5-H), 5.92 (q, 1 H, 1′-H), 7.04 (br d, 2 H, 4-NH_2, D_2O exchangeable), 7.89 (d, 1 H, 6-H).

Antiviral Test Procedures. SC-1 and XC cells were obtained from American Tissue Culture Collection (Rockville, MD) and grown in Eagle's Medium (EMEM) supplemented with 10% fetal calf serum. Moloney-murine leukemia virus, a mammalian T-lymphotropic retrovirus, was propagated in 3T3 mouse cells.

The viral inhibition was determined by using the XC assay as described by Rowe et al.,[34] with slight modification. Briefly, monolayer cultures of SC-1 cells were infected with approximately 100 PFU of M-MULV. After 1 h of incubation at 37 °C, inoculum was removed, and test compounds were added at various concentrations in culture medium. After 5 days of incubation the compounds were removed and the SC-1 cells were killed by irradiation with UV light. XC cells were added on top of this UV-irradiated M-MULV-infected SC-1 monolayer and syncytium were scored 4 days later.

The antiviral activity was expressed by the effective dose (μM) that inhibits 50% of the syncytial forming units.

Measurement of anti-HTLV-III/LAV/AAV activity was carried out in cultures of HUT 78 cells infected with the virus and maintained at 37 °C. Every 3 days one-third of the culture media was replenished with fresh media containing the appropriate concentrations of drug. The reverse transcriptase activities of the media at day 27 were analyzed by using poly(C)·oligo(dG) as template primer in the presence of 10 mM $MgCl_2$ and [^{3}H]dGTP.

Acknowledgment. This research was supported by USPHS Grants CA-28852 and CA-05262. We also acknowledge the support of Northeast NMR Facility at Yale University for the high-resolution NMR spectra, made possible by a grant from the Chemical Division of the NSF (CHE-7916210). Assays for anti-HTLV-III activity were performed by Dr. B. Poiesz, Regional Oncology Center, SUNY Health Science Center, Syracuse, NY.