Synthesis and Antiviral Activity of Various 3′-Azido Analogues of Pyrimidine Deoxyribonucleosides against Human Immunodeficiency Virus (HIV-1, HTLV-III/LAV)

Tai-Shun Lin,*[1a] Ji-Yu Guo,[1b] Raymond F. Schinazi,[1c] Chung K. Chu,[1d] Jia-Ning Xiang,[1a] and Willian H. Prusoff[1a]

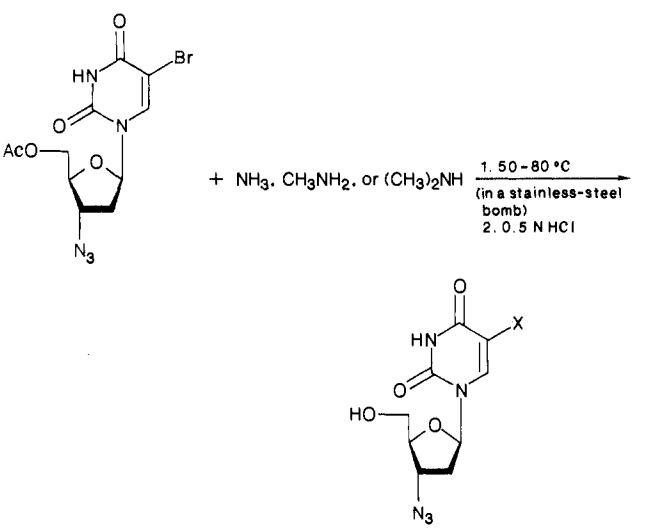
Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510, Veterans Administration Medical Center (Atlanta) and Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30303, and College of Pharmacy, University of Georgia, Athens, Georgia 30602.
Received July 6, 1987

Various 3′-azido analogues of pyrimidine deoxyribonucleosides have been synthesized and tested against human immunodeficiency virus (HIV-1, HTLV-III/LAV) in human peripheral blood mononuclear cells. Among these compounds, the 3′-azido analogues of thymidine (**2**), 3-(3-oxo-1-propenyl)thymidine (**21**), 2′-deoxyuridine (**1**), 2′-deoxy-5-bromouridine (**5**), 2′-deoxy-5-fluorocytidine (**19**), 2′-deoxy-5-iodouridine (**6**), 2′-deoxycytidine (**18**), 2′-deoxy-5-fluorouridine (**4**), 2′-deoxy-5-thiocyanatouridine (**16**), 2′-deoxy-5-methylcytidine (**20**), 2′-deoxy-5-aminouridine (**7**), and 2′-deoxy-5-hydroxyuridine (**10**) were found to have signficant antiviral activity, with EC_{50} values of 0.002, 0.01, 0.2, 1.0, 1.0, 1.1, 1.2, 4.8, 5.1, 5.1, 6.2, and 10 μM, respectively. The structure–activity relationships are discussed.

3′-Azido-3′-deoxythymidine (AZT) was found by Mitsuya et al.[2] to be a potent inhibitor of the replication of the human immunodeficiency virus (HIV), which is accepted to be responsible for the clinical syndrome termed AIDS. Furman et al.[3] investigated the metabolism of AZT and found it to be sequentially phosphorylated to the 5′-mono-, -di-, and -triphosphate analogues. As the triphosphate analogue, AZT inhibits the utilization of dTTP by reverse transcriptase and may be incorporated in the terminal position of DNA, thereby preventing elongation.[3] In either event, the synthesis of HIV-1 DNA is prevented and hence inhibition of HIV-1 replication. Other sites of inhibition may also be involved; however, this has not been established. When AZT was combined with recombinant alpha A interferon, a synergistic inhibition of HIV-1 in cell culture was found by Hartshorn et al.;[4] however, when AZT was combined with ribavirin, Vogt et al.[5] found it was antagonistic.

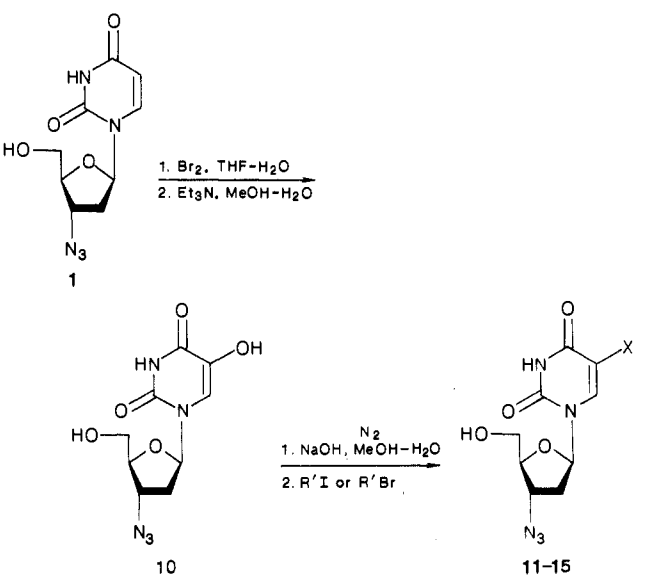
AZT in a preliminary 6-week clinical trial was reported by Yarchoan et al.[6] to be well absorbed from the gi tract, to cross the blood brain barrier, and to produce positive clinical improvement. Although AZT is not considered a

Scheme I



7–9: X = NH_2·HCl, $NHCH_3$·HCl, $N(CH_3)_2$·HCl

Scheme II



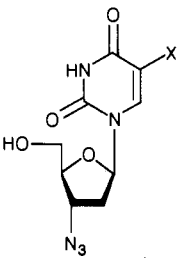
R′ = CH_3, CH_2CH_3, $CH_2C{\equiv}CH$, $CH_2CH{=}CH_2$, CH_2CN; X = OCH_3, OCH_2CH_3, $OCH_2C{\equiv}CH$, $OCH_2CH{=}CH_2$, OCH_2CN

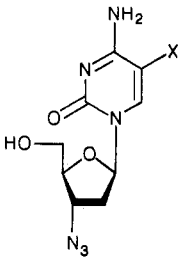
(1) (a) Yale University. (b) Visiting Scientist at Yale from the Institute of Materia Medica, Chinese Academy of Medical Science, Beijing, The People's Republic of China. (c) Veterans Administration Medical Center (Atlanta) and Department of Pediatrics, Emory University School of Medicine. (d) College of Pharmacy, University of Georgia.

(2) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Border, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096.
(3) Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Nusinoff-Lehrman, S.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333.
(4) Hartshorn, K. L.; Vogt, M. W.; Chou, T.-C.; Blumberg, R. S.; Byington, R.; Schooley, R. T.; Hirsch, M. S. *Antimicrob. Agents Chemother.* **1987**, *31*, 168.
(5) Vogt, M. W.; Hartshorn, K. L.; Furman, P. A.; Chow, T.-C.; Fyfe, J. A.; Coleman, L. A.; Crumpacker, C.; Schooley, R. T.; Hirsch, M. S. *Science (Washington, D.C.)* **1987**, *235*, 1376.
(6) Yarchoan, R.; Weinhold, K. J.; Lyerly, H. K.; Gelmann, E.; Blum, R. M.; Shearer, G. M.; Mitsuya, H.; Collins, J. M.; Myers, C. E.; Klecker, R. W.; Markham, P. D.; Durack, D. T.; Nusinoff-Lehrman, S.; Barry, D. W.; Fischl, M. A.; Gallo, R. C.; Bolognesi, D. P.; Broder, S. *Lancet* **1986**, *1*, 575.
(7) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911.
(8) Lin, T.-S.; Schinazi, R. F.; Chen, M. S.; Kinney-Thomas, E.; Prusoff, W. H. *Biochem. Pharmacol.* **1987**, *36*, 311.
(9) Balzarini, J.; Pauwels, R.; Herdewijn, P.; DeClercq, E.; Cooney, D. A.; Kang, G.-J.; Dalal, M.; Johns, D. G.; Broder, S. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 735.

0022-2623/88/1831-0336$01.50/0

Chart I. Structure Formulas of Various 3′-Azido Analogues of Pyrimidine Deoxyribonucleosides

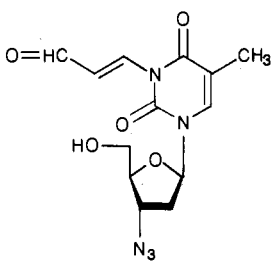




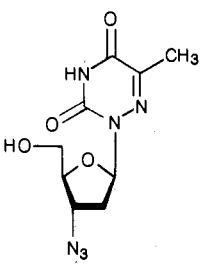
1: X = H
2: X = CH_3
3: X = CF_3
4: X = F
5: X = Br
6: X = I
7: X = NH_2·HCl
8: X = $NHCH_3$·HCl
9: X = $N(CH_3)_2$·HCl

10: X = OH
11: X = OCH_3
12: X = OCH_2CH_3
13: X = $OCH_2C{\equiv}CH$
14: X = $OCH_2CH{=}CH_2$
15: X = OCH_2CN
16: X = SCN
17: X = SCH_3

18: X = H
19: X = F
20: X = CH_3



21



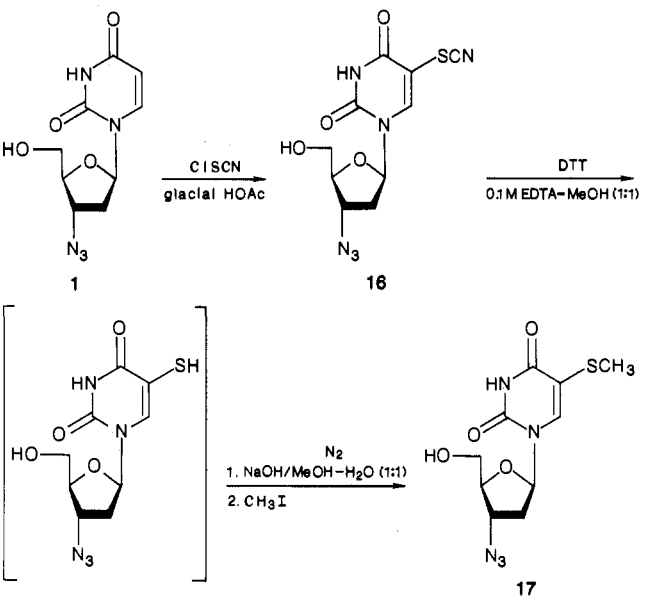
26

cure, its ability to prolong life of AIDS patients, in spite of bone marrow suppression observed in some patients, has encouraged the evaluation of other nucleosides.[7-13] AZT, as well as several other nucleoside analogues that are inhibitory to HIV-1 in vitro, was first synthesized by Horwitz et al.[14,15]

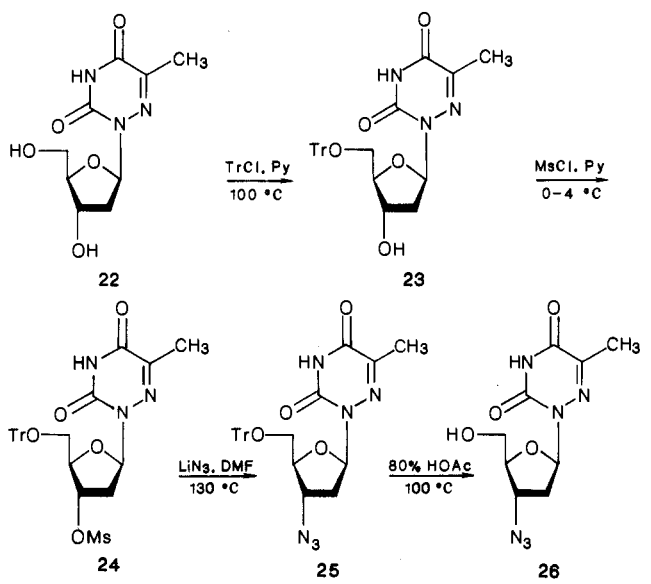
Several reviews have appeared recently that evaluate the various compounds for their activity against HIV-1, as well as a discussion of the AIDS problem in general.[16-19] The present paper describes the synthesis of a variety of 3′-azido nucleosides and their antiviral activity against HIV-1 in cell culture and provides a discussion of their structure–activity relationship.

Chemistry. Various 3′-azido analogues of pyrimidine 2′-deoxyribofuranosyl nucleosides (Chart I) have been synthesized and tested as potential anti-HIV agents. Compounds **1**,**3–6**, and **18–20** were synthesized by the methodology previously described.[10,20,21] Compound **2** was prepared by the methodology of Horwitz et al.[15] with minor modification.[22] Treatment of 3′-azido-5′-*O*-acetyl-2′,3′-dideoxy-5-bromouridine[10] with ammonia or other appropriate amines at 50–80 °C in a stainless-steel container gave the respective 5-amino or alkylamino derivatives **7–9** (Scheme I). Bromination[23] of 3′-azido-2′,3′-dideoxyuridine (**1**)[20] with Br_2–H_2O at room temperature, followed by treatment with MeOH–H_2O in the presence of triethylamine, produced 3′-azido-2′,3′-dideoxy-5-hydroxyuridine (**10**). Alkylation of compound **10** with the appropriate alkyl halide (iodide or bromide) in the presence of NaOH in MeOH–H_2O under N_2 afforded the corresponding 5-alkoxy analogues **11–15** (Scheme II).[24-26] 3′-Azido-2′,3′-dideoxy-5-thiocyanatouridine (**16**) was synthesized by reacting compound **1** with chlorothiocyanogen (ClSCN), which was prepared from chlorine and dry KSCN in glacial acetic

Scheme III



Scheme IV



(10) Lin, T.-S.; Chen, M. S.; McLaren, C.; Gao, Y. S.; Ghazzouli, I.; Prusoff, W. H. *J. Med. Chem.* **1987**, *30*, 440.
(11) Baba, M.; Pauwels, R.; Herdewijn, P.; DeClercq, E.; Desmyter, J.; Vandeputte, M. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 128.
(12) Hartmann, H.; Hunsmann, G.; Eckstein, F. *Lancet* **1987**, *1*, 40.
(13) Dahlberg, J. E.; Mitsuya, H.; Blam, S. B.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2469.
(14) Horwitz, J. P.; Chua, J.; Noel, M.; Donnatti, J. T. *J. Org. Chem.* **1967**, *32*, 817.
(15) Horwitz, J. P.; Chua, J.; Noel, J. M. *J. Org. Chem.* **1964**, *29*, 2076.
(16) Robins, R. K. *Chem. Eng. News* **1986**, *64*, 28.
(17) DeClercq, E. *J. Med. Chem.* **1986**, *29*, 1561.
(18) Oberg, B. J. *Antimicrob. Chemother.* **1986**, *17*, 549.
(19) Sandstrom, E. *Drugs* **1986**, *31*, 462.
(20) Lin, T.-S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 544.
(21) Lin, T.-S.; Gao, Y. S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 1691.
(22) Lin, T.-S.; Prusoff, W. H.; *J. Med. Chem.* **1978**, *21*, 109.
(23) Podrebarac, E. G., Cheng, C. C. *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, 1968; Vol. 1, pp 412–413.
(24) Torrence, P. F.; Spencer, J. W.; Bobst, A. M.; Descamps, J.; DeClercq, E. *J. Med. Chem.* **1978**, *21*, 228.
(25) Otter, B. A.; Taube, A.; Fox, J. J. *J. Org. Chem.* **1971**, *36*, 1251.
(26) Huang, G. F.; Okada, M.; DeClercq, E.; Torrence, P. F. *J. Med. Chem.* **1981**, *24*, 390.

Table I. Antiviral Activity of Various 3′-Azido Analogues of Pyrimidine Deoxyribonucleosides on the Replication of Human Immunodeficiency Virus (HIV-1, HTLV-III/LAV) in Human Peripheral Blood Mononuclear Cells

compd	EC_{50}(HIV-1), μM	compd	EC_{50}(HIV-1), μM
1	0.2[32]	12	54
2	0.002 (0.23)[10]	13	38
3	>100	14	>100
4	4.8	15	16
5	1.0 (2.3)[10]	16	5.1
6	1.1	17	>100
7	6.2	18	1.2
8	>100	19	1.0
9	>100	20	5.1
10	~10	21	0.01
11	70	26	>100

acid.[27] Treatment of compound **16** with dithiothreitol (DTT) in 0.1 M EDTA–MeOH (1:1), followed by methyl iodide in the presence of NaOH in MeOH–H_2O under N_2, yielded 3′-azido-2′,3′-dideoxy-5-(methylthio)uridine (**17**; Scheme III).[28,29]

Treatment[30] of 3′-azido-3′-deoxythymidine (**2**) with triethylamine in DMF at room temperature for 1 h followed by propiolaldehyde at −78 °C for 2 h and then at room temperature overnight yielded the 3-oxo-1-propenyl derivative **21**.

Tritylation of 6-azathymidine (**22**)[31] with trityl chloride in pyridine at 100 °C gave the 5′-*O*-trityl-protected nucleoside **23**, which was methanesulfonated with methanesulfonyl chloride in pyridine at 0–4 °C. The resultant sulfonate **24** was then treated with lithium azide in DMF at 130 °C to afford the 5′-*O*-trityl-3′-azido derivative **25**. Detritylation of compound **25** with 80% acetic acid at 100 °C produced 3′-azido-3′-deoxy-6-azathymidine **26** (Scheme IV).

Antiviral Activity. These compounds were tested against HIV-1, and the antiviral activity was expressed by the concentration (μM) that inhibits 50% of viral replication.

Among the 3′-azido analogues of pyrimidine deoxyribonucleosides, 3′-azido-3′-deoxythymidine (**2**, AZT) was the most active against HIV-1 in vitro with an EC_{50} value of 0.002 μM. Conversely, 3′-azido-3′-deoxy-6-azathymidine (**26**) was practically inactive (EC_{50} > 100 μM). The 3′-azido derivatives of 3-(3-oxo-1-propenyl)thymidine (**21**), 2′-deoxyuridine (**1**), 2′-deoxy-5-bromouridine (**5**), 2′-deoxy-5-fluorocytidine (**19**), 2′-deoxy-5-iodouridine (**6**), 2′-deoxycytidine (**18**), 2′-deoxy-5-fluorouridine (**4**), 2′-deoxy-5-thiocyanatouridine (**16**), 2′-deoxy-5-methylcytidine (**20**), 2′-deoxy-5-aminouridine (**7**), and 2′-deoxy-5-hydroxyuridine (**10**) also demonstrated significant antiviral activity with EC_{50} values of 0.01, 0.2, 1.0, 1.0, 1.1, 1.2, 4.8, 5.1, 5.1, 6.2, and 10 μM, respectively. However, the 3′-azido derivatives of 2′-deoxy-5-[(cyanomethyl)oxy]uridine (**15**), 2′-deoxy-5-(2-propynyloxy)uridine (**13**), 2′-deoxy-5-ethoxyuridine (**12**), and 2′-deoxy-5-methoxyuridine (**11**) only showed moderate antiviral activity with EC_{50} values of 16, 38, 54, and 70 μM, respectively. The other 3′-azido derivatives in this series, compounds **3**, **8**, **9**, **14**, and **17**, were found to be practically inactive (EC_{50} > 100 μM). These compounds were not toxic to the host human PBM cell at >100 μM except compound **21**, which was toxic at ~10 μM. The results are summarized in Table I.

Structure–Activity Relationships. It appears that the substituents in the 5-position of the 2′-deoxynucleoside analogues affect the antiviral activity. These effects may be related to their substrate activity for thymidine kinase or cytidine kinase (for compounds **18–20**), which is required for activation, 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 hydrogen at carbon 5 of the uracil base in compound **1** with a methyl group produces the most active compound (**2**, AZT). Conversely, replacement of the hydrogen with a trifluoromethyl group (**3**) results in the loss of the antiviral activity, whereas substitution of the hydrogen at carbon 5 in **1** with a fluoro, bromo, iodo, amino, hydroxyl, and thiocyanato group (compounds **4–7**, **10**, and **16**, respectively) retains a significant amount of antiviral activity. Substitution of the hydrogen in 5-amino and 5-hydroxyl group in compounds **7** and **10** with an alkyl group (methyl, ethyl, etc.) markedly reduces the antiviral activity. Substitution of the N^3-hydrogen in the pyrimidine base in compound **2** with an 3-oxo-1-propenyl moiety produces the second most active compound (**21**) in this group. However, substitution of carbon 6 in compound **2** with a nitrogen (**26**) results in the loss of antiviral activity. In the 2′-deoxycytidine series, replacement of the hydrogen at carbon 5 in compound **18** with a fluoro group (**19**) yields a compound with equal antiviral activity. Substitution of the hydrogen with a methyl group (**20**), however, results in the reduction of antiviral activity. There seems to be no clear relationship between the antiviral activity and either the electron-withdrawing or the electron-donating capacity of the substituents in the 5-position of the nucleoside analogues.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded 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. The mass spectra (at 70 eV) were provided by Yale University Chemical Instrumentation Center. TLC was performed on EM precoated silical 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-aminouridine Hydrochloride (7). Liquid ammonia (70 mL) was added to 3′-azido-5′-*O*-acetyl-2′,3′-dideoxy-5-bromouridine[10] (2.5 g, 6.70 mmol) in a stainless-steel container. The container was sealed and kept at ~50 °C for 24 h. The excess ammonia was allowed to evaporate by blowing over dry N_2. The brown residue was dissolved in 50 mL of 0.1 N hydrochloric acid, and the solution was passed through a column (2 × 20 cm) packed with Dowex 50-X8 (H^+) ion-exchange resin. The column was first eluted with H_2O to remove any unreacted starting material and other byproducts and then eluted with 0.5 N hydrochloric acid. Fractions containing the product, as determined by TLC (CH_2Cl_2–MeOH, 7:3, R_f 0.75) were combined and evaporated in vacuo at <40 °C to a volume of ~15 mL. Addition of methanol (40 mL) to the solution caused immediate formation of colorless crystals of **7**, which were collected by filtration: yield, 0.60 g (30%); mp 215–218 °C dec; IR (KBr) 4.76 μm (azido); UV (EtOH) λ_{max} 297 nm, λ_{min} 257 nm; NMR

(27) Torrence, P. F.; Waters, J. A.; Witkop, B. *Synthetic Procedures in Nucleic Acid Chemistry*, Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, 1968; Vol. 1, pp 367–370.
(28) Nagamachi, T.; Fourrey, J. L.; Torrence, P. F.; Waters, J. A.; Witkop, B. *J. Med. Chem.* **1974**, *17*, 403.
(29) Dinan, F. J.; Bardos, T. J. *J. Med. Chem.* **1980**, *23*, 569.
(30) Johnson, F.; Pillai, K. M. R.; Grollman, A. P.; Tseng, L.; Takeshita, M. *J. Med. Chem.* **1984**, *27*, 954.
(31) Shiau, G. T.; Prusoff, W. H. *Carbohydr. Res.* **1978**, *62*, 175.
(32) Schinazi, R. F.; Chu, C. K.; Ahn, M.-K.; Sommadossi, J.-P.; McClure, H. *J. Cellular Biochem.* **1987**, *11D* (suppl), 74.

(Me_2SO-d_6) δ 2.34–2.36 (m, 2 H, 2′-H), 3.63–3.66 (m, 2 H, 5′-H), 3.93 (q, 1 H, 4′-H), 4.39 (q, 1 H, 3′-H), 4.2–5.0 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.02 (t, 1 H, 1′-H), 8.31 (s, 1 H, 6′-H), 8.80–10.4 (br s, 3 H, 5-NH_3^+, D_2O exchangeable), 12.0 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 269 (M^+ – HCl). Anal. ($C_9H_{12}N_6O_4HCl$) C, H, N.

Compounds **8** and **9** were synthetized by the same methodology as described for the preparation of compound **7** except that the appropriate amines were used, and the reaction temperature was maintained at ~80 °C.

3′-Azido-2′,3′-dideoxy-5-(methylamino)uridine hydrochloride (8): mp 184–187 °C dec; IR (KBr) 4.77 μm (azido); UV (0.01 N HCl) λ_{max} 266 nm (ε 11 300), λ_{min} 232 nm, shoulder at 304 nm; UV (0.01 N NaOH) λ_{max} 293 nm ε 8400), λ_{min} 263 nm; NMR (Me_2SO-d_6) δ 2.30–2.35 (m, 1 H, 2′-H_a), 2.38–2.44 (m, 1 H, 2′-H_b), 2.67 (s, 3 H, 5-*N*-CH_3), 3.60–3.63 (m, 1 H, 5′-H_a), 3.66–3.69 (m, 1 H, 5′-H_b), 3.89 (m, 1 H, 4′-H), 4.40 (q, 1 H, 3′-H), 4.80–6.30 (br s, 3 H, 5′-OH and 5-NH_2^+, D_2O exchangeable), 6.06 (t, 1 H, 1′-H), 7.86 (s, 1 H, 6-H), 11.8 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 283 (M^+ – HCl). Anal. ($C_{10}H_{14}N_6O_4HCl$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-(dimethylamino)uridine hydrochloride (9): mp 205–208 °C dec; IR (film) 4.75 μm (azido); UV (EtOH) λ_{max} 299 nm, λ_{min} 264 nm; NMR (Me_2SO-d_6) δ 2.34–2.42 (m, 1 H, 2′-H_a), 2.43–2.46 (m, 1 H, 2′-H_b), 2.55 [s, 6 H, 5-*N*-$(CH_3)_2$], 3.61–3.65 (m, 1 H, 5′-H_a), 3.69–3.72 (m, 1 H, 5′-H_b), 3.90 (m, 1 H, 4′-H), 4.42 (q, 1 H, 3′-H), 4.60–6.00 (br s, 2 H, 5′-OH and 5-NH^+, D_2O exchangeable), 6.05 (t, 1 H, 1′-H), 8.22 (s, 1 H, 6-H), 11.9 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 297 (M^+ – HCl). Anal. ($C_{11}H_{16}N_6O_4HCl$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-hydroxyuridine (10). Bromine was added dropwise to a stirred solution of 3′-azido-2′,3′-dideoxyuridine[20] (1, 3.0 g, 11.9 mmol) in 60 mL of H_2O and 40 mL of THF at room temperature until a light-yellow color persisted (ca. 0.8 mL of bromine). Air was then bubbled through the solution until it became almost colorless. The solution was cooled in an ice–water bath, and 90 mL of triethylamine was added in several portions so that the temperature of the solution did not exceed 25 °C. The resulting solution was kept overnight and evaporated under diminished pressure (water pump) below 40 °C to dryness. The residue was chromatographed twice on a silica gel column (CH_2Cl_2–MeOH, 5:1, R_f 0.69) to afford 0.65 g (20%) of the desired product: mp 193 °C dec; IR (film) 4.80 μm (azido); UV (EtOH) λ_{max} 282 nm, λ_{min} 248 nm; NMR (Me_2SO-d_6) δ 2.21–2.26 (m, 1 H, 2′-H_a) 2.31–2.37 (m, 1 H, 2′-H_b), 3.61 (m, 2 H, 5′-H), 3.82 (m, 1 H, 4′-H), 4.38 (m, 1 H, 3′-H), 5.20 (s, 1 H, 5-OH, D_2O exchangeable), 6.10 (t, 1 H, 1′-H), 7.33 (s, 1 H, 6-H), 8.70 (s, 1 H, 5′-OH, D_2O exchangeable), 11.5 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 270 (M^+ + 1). Anal. ($C_9H_{11}N_5O_5$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-methoxyuridine (11). To a stirred solution of 3′-azido-2′,3′-dideoxyuridine (1; 2.0 g, 7.9 mmol) in a mixture of 25 mL of THF and 40 mL of H_2O at room temperature was added dropwise bromine until a light-yellow color persisted. Air was then bubbled through the solution until it became almost colorless. Triethylamine (30 mL) and methanol (10 mL) were added to the clear solution (cooled in an ice–water bath). The resultant solution was kept at room temperature for 4 h and then evaporated in vacuo below 40 °C to dryness. The residue (compound **10**) was used for the next step without further purification.

The above residue was dissolved in a mixture of MeOH (25 mL) and H_2O (17 mL) to which 8 mL of 1 N NaOH was added. Methyl iodide (5.7 g, 40 mmol, 2.5 mL) was added to the solution. The reaction mixture was allowed to proceed for 7 days at room temperature, and the progress was monitored by TLC (EtOAc–hexane, 5:1, R_f 0.78). The solvents were removed in vacuo (~40 °C). The residue was chromatographed on a silica gel column (EtOAc–hexane, 2:1) to afford 0.17 g (8% based on 1) of product: mp 116–118 °C; IR (film) 4.83 μm (azido); UV (EtOH) λ_{max} 280 nm, λ_{min} 242 nm; NMR (Me_2SO-d_6) δ 2.33–2.37 (m, 1 H, 2′-H_a), 2.44–2.48 (m, 1 H, 2′-H_b), 3.20 (s, 3 H, 5-OCH_3), 3.61 (m, 1 H, 5′-H_a), 3.69 (m, 1 H, 5′-H_b), 3.86 (m, 1 H, 4′-H), 4.37 (q, 1 H, 3′-H), 5.39 (t, 1 H, 5′-OH, D_2O exchangeable), 6.04 (t, 1 H, 1′-H), 8.45 (s, 1 H, 6-H); MS, *m/e* 283 (M^+), 268 (M^+ – CH_3). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

Compounds **12–15** were synthesized by the same methodology as described for the synthesis of compound **11**, except that the appropriate alkyl halides (bromide or iodide were employed, and in some cases, the reaction time was shorter.

3′-Azido-2′,3′-dideoxy-5-ethoxyuridine (12): mp 118–120 °C; IR (film) 4.82 μm (azido); UV (EtOH) λ_{max} 281 nm, λ_{min} 242 nm; NMR (Me_2SO-d_6) δ 1.24 (t, 3 H, CH_3) 2.25–2.28 (m, 1 H, 2′-H_a), 2.39–2.42 (m, 1 H, 2′-H_b), 3.60–3.62 (m, 1 H, 5′-H_a), 3.65–3.68 (m, 1 H, 5′-H_b), 3.83 (m, 1 H, 4′-H), 3.80 (q, 2 H, OCH_2), 4.41 (m, 1 H, 3′-H), 5.31 (t, 1 H, 5′-OH, D_2O exchangeable), 6.11 (t, 1 H, 1′-H), 7.53 (s, 1 H, 6-H), 11.5 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 297 (M^+), 298 (M^+ + 1). Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-(2-propynyloxy)uridine (13). The reaction time for the synthesis of this compound was 36 h: mp 208–211 °C dec; IR (film) 4.80 μm; UV (EtOH) λ_{max} 278 nm, λ_{min} 238 nm; NMR (Me_2SO-d_6) δ 2.28–2.31 (m, 1 H, 2′-H_a), 2.36–2.39 (m, 1 H, 2′-H_b), 2.66 (m, 1 H, CH), 3.84 (m, 1 H, 4′-H), 4.41 (m, 1 H, 3′-H), 4.60 (s, 2 H, OCH_2), 5.27 (t, 1 H, 5′-OH, D_2O exchangeable), 6.11 (t, 1 H, 1′-H), 7.86 (s, 1 H, 6-H), 11.6 (s, 1 H, 3-NH); MS, *m/e* 308 (M^+ + 1). Anal. ($C_{12}H_{13}N_5O_5$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-(propenyloxy)uridine (14). The reaction time was 24 h for the synthesis of this compound: mp 164 °C dec; IR (film) 4.80 μm (azido); UV (EtOH) λ_{max} 280 nm, λ_{min} 243 nm; NMR ($CDCl_3$) δ 2.40–2.50 (m, 2 H, 2′-H), 3.82 (m, 1 H, 4′-H), 3.95–4.05 (m, 2 H, 5′-H), 4.36–4.40 (q, 1 H, 3′-H), 4.43 (m, 2 H, OCH_2), 4.44 (m, 1 H, 5′-OH, D_2O exchangeable), 5.27–5.38 (m, 2 H, C=CH_2), 5.92–6.00 (m, 1 H, CCH=C), 6.15 (t, 1 H, 1′-H), 7.40 (s, 1 H, 6-H), 8.20–9.80 (br s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 309 (M^+), 310 (M^+ + 1). Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-[(cyanomethyl)oxy]uridine (15). The reaction time was 48 h for the synthesis of this compound: mp 161–163 °C dec; IR (film) 4.77 μm (azido); UV (EtOH) λ_{max} 273 nm, λ_{min} 237 nm; NMR (Me_2SO-d_6) δ 2.28–2.33 (m, 1 H, 2′-H_a), 2.38–2.42 (m, 1 H, 2′-H_b), 3.56–3.68 (m, 2 H, 5′-H), 3.86 (m, 1 H, 4′-H), 4.41 (q, 1 H, 3′-H), 4.92 (s, 2 H, OCH_2CN), 5.30 (t, 1 H, 5′-OH, D_2O exchangeable), 6.06 (t, 1 H, 1′-H), 7.90 (s, 1 H, 6-H), 11.7 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 307 (M^+ – 1), 308 (M^+). Anal. ($C_{11}H_{12}N_6O_5$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-thiocyanatouridine (16). 3′-Azido-2′,3′-dideoxyuridine (1; 0.9 g, 3.6 mmol) was dried for 2 h at 100 °C in vacuo and then added to a solution of ClSCN, which was prepared from chlorine (3.6 g, 50 mmol) and dry KSCN (5.4 g, 55 mmol) in 200 mL of glacial acetic acid. The reaction was conducted under anhydrous conditions and stirred at room temperature. The progress of the reaction was monitored by TLC. As soon as TLC showed the complete disappearance of the starting material (compound 1; EtOAc–hexane, 4:1, R_f 0.24), the reaction was terminated by adding cyclohexane (10 mL) and stirring for 20 min. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo at <40 °C. The residue was extracted with ether and the insoluble material was dissolved in MeOH and chromatographed on a silica gel column (EtOAc–hexane, 4:1, R_f 0.61) to give 0.18 g (16%) of the product: mp 144–146 °C dec; IR (film) 4.76 μm (azido); UV (EtOH) λ_{max} 275 nm, λ_{min} 238 nm; NMR (Me_2SO-d_2) δ 2.34–2.38 (m, 1 H, 2′-H_a) 2.47–2.49 (m, 1 H, 2′-H_b), 3.60 (m, 1 H, 5′-H_a), 3.70 (m, 1 H, 5′-H_b), 3.86 (q, 1 H, 4′-H), 4.36 (q, 1 H, 3′-H), 5.41 (t, 1 H, 5′-OH, D_2O exchangeable), 5.99 (t, 1 H, 1′-H), 8.65 (s, 1 H, 6-H), 12.0 (s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 308 (M^+ – 2), 309 (M^+ – 1), 310 (M^+). Anal. ($C_{10}H_{10}N_6O_4S$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-(methylthio)uridine (17). 3′-Azido-2′,3′-dideoxy-5-thiocyanatouridine (16; 90 mg, 0.20 mmol) was dissolved in 10 mL of 0.1 M EDTA (pH 7.8) and 10 mL of MeOH, and then dithiothreitol (DTT; 100 mg, 0.65 mmol) was added to the solution. When the reaction was completed as monitored by the appearance of the 332-nm band in the UV spectrum, the resulting colorless solution was evaporated to dryness in vacuo at <40 °C. The residue was suspended in MeOH with stirring and then filtered. The filtrate was concentrated to ~3 mL and filtered again. Under an atmosphere of N_2, the filtrate was diluted with MeOH to 10 mL, and then 5 mL of H_2O and 4 mL of 0.1 N NaOH were added followed by 0.1 mL of CH_3I (0.23 g, 1.6 mmol). The reaction mixture was stirred at room temperature overnight under an atmosphere of N_2. Evaporation of the solvents in vacuo afforded a white solid, which was chromatographed on a silica gel column (EtOAc–hexane, 3:1, R_f 0.43) and then rechromatographed on a preparative TLC plate (2 mm) twice to give 18 mg (20% based on **16**) of product: mp 115–118 °C; IR

(film) 4.80 μm (azido); UV (EtOH) λ_{max} 291 nm, λ_{min} 261 nm; NMR (Me_2SO-d_6) δ 2.18 (s, 3 H, 5-SCH_3), 2.18–2.23 (m, 1 H, 2′-H_a), 2.32–2.37 (m, 1 H, 2′-H_b), 3.60 (m, 2 H, 5′-H), 3.81 (m, 1 H, 4′-H), 4.39 (q, 1 H, 3′-H), 5.29 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.13 (t, 1 H, 1′-H), 7.53 (s, 1 H, 6-H), 11.0–12.0 (br s, 3-NH, D_2O exchangeable); MS, *m/e* 299 (M^+), 300 ($M^+ + 1$). Anal. ($C_{10}H_{13}N_5O_4S$) C, H, N.

3-(3-Oxo-1-propenyl)-3′-azido-3′-deoxythymidine (21). 3′-Azido-3′-deoxythymidine (**2**; 1.0 g, 3.7 mmol) was dissolved in anhydrous DMF (3 mL) containing triethylamine (0.52 g, 3.7 mmol), and the mixture was stirred for 1 h at room temperature. The solution was cooled to −78 °C (acetone–dry ice bath), and propiolaldehyde (6.3 g, ~100 mmol) was added in one portion. The reaction mixture was stirred at −78 °C for 2 h and then allowed to come up to room temperature slowly overnight. Excess reagents and DMF were removed in vacuo, and the residue was chromatographed on a silica gel column (EtOAc–hexane, 2:1). The product was obtained as almost colorless needles. (0.15 g, 13%): mp 107–108 °C; TLC R_f 0.6 (EtOA–hexane, 2:1); IR (film) 4.85 μm (azido); UV (EtOH) λ_{max} 267 nm, λ_{min} 235 nm; NMR (Me_2SO-d_6) δ 2.34–2.40 (m, 1 H, 2′-H_a), 2.44–2.49 (m, 1 H, 2′-H_b), 3.61–3.64 (m, 1 H, 5′-H_a), 3.68–3.72 (m, 1 H, 5′-H_b), 3.88 (m, 1 H, 4′-H), 4.40 (q, 1 H, 3′-H), 5.31 (t, 1 H, 5′-OH, D_2O exchangeable), 6.11 (t, 1 H, 1′-H), 7.07 (q, 1 H, =CH), 7.91 (s, 1 H, 6-H), 8.16 (d, 1 H, NCH=), 9.61 (d, 1 H, CHO); MS, *m/e* 322 ($M^+ + 1$). Anal. ($C_{13}H_{15}N_5O_5$) C, H, N.

6-Azathymidine (22): mp 150–153 °C (lit.[30] mp 153–154 °C); NMR (Me_2SO-d_6) δ 2.03 (m, 1 H, 2′-H_a), 2.10 (s, 3 H, 5-CH_3), 2.40 (m, 1 H, 2′-H_b), 3.34 (dd, 1 H, 5′-H_a), 3.46 (dd, 1 H, 5′-H_b), 3.68 (dd, 1 H, 4′-H), 4.27 (dd, 1 H, 3′-H), 4.60 (br s, 1 H, 5′-OH, D_2O exchangeable, 5.13 (br s, 1 H, 3′-OH, D_2O exchangeable), 6.31 (dd, 1 H, 1′-H) 11.9 (br s, 1 H, 3-NH, D_2O exchangeable).

5′-*O*-Trityl-6-azathymidine (23). A solution of 6-azathymidine (**22**; 0.6 g, 2.5 mmol) and triphenylmethyl chloride (0.7 g, 0.25 mmol) in 70 mL of pyridine was heated with stirring at ~100 °C for 1 h. The reaction mixture was evaporated in vacuo to give a syrup, which was triturated with H_2O. The resulting solid was collected by filtration and recrystallized from CH_2Cl_2 to yield 0.6 g (40%) of product: mp 105–108 °C, NMR (Me_2SO-d_6) δ 2.00 (s, 3 H, 5-CH_3), 2.08 (m, 1 H, 2′-H_a), 2.36 (m, 1 H, 2′-H_b), 3.03 (m, 2 H, 5′-H), 3.94 (m, 1 H, 4′-H), 4.25 (m, 1 H, 3′-H), 5.11 (d, 1 H, 3′-OH, D_2O exchangeable), 6.16 (dd, 1 H, 1-H), 7.10–7.20 (m, 15 H, phenyl), 12.1 (br s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 485 (M^+). Anal. ($C_{28}H_{27}N_3O_5$) C, H, N.

3′-Azido-3′-deoxy-5′-*O*-trityl-6-azathymidine (25). Methanesulfonyl chloride (1.47 g, 12.8 mmol) was added to a solution of compound **23** (0.13 g, 0.30 mmol) in 15 mL of pyridine. The reaction mixture was stirred at 4 °C for 20 h, and then the solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (25 mL), and the solution was washed with $NaHCO_3$ solution and H_2O and dried over anhydrous Na_2SO_4. The solvent was removed under diminished pressure to give a syrup (compound **24**), which was used in the following reaction without further purification.

A solution of compound **24** (0.41 g, 0.72 mmol) and lithium azide (0.30 g, 7.2 mmol) in 20 mL of DMF was heated with stirring at 130 °C (oil bath) for 2 h. The reaction mixture was then poured into ice–water and extracted several times with CH_2Cl_2. The combined CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The resulting syrup was chromatographed on a preparative silica gel plate (2 mm) (EtOAc–CH_2Cl_2, 1:2) to afford 0.13 g (33%) of product: mp 83–85 °C; IR (film) 4.75 μm (azido); NMR (Me_2SO-d_6) δ 1.86 (s, 3 H, 5-CH_3), 2.30 (m, 1 H, 2′-H_a), 2.55 (m, 1 H, 2′-H_b), 3.16 (m, 2 H, 5′-H), 3.98 (m, 1 H, 4′-H), 4.40 (m, 1 H, H′-3), 6.16 (dd, 1 H, 1′-H), 7.10–7.20 (m, 15 H, phenyl), 12.1 (br s, 1 H, 3-NH, D_2O exchangeable); MS, *m/e* 510 (M^+). Anal. ($C_{28}H_{26}N_6O_4$) C, H, N.

3′-Azido-3′-deoxy-6-azathymidine (26). Compound **25** (0.22 g, 0.40 mmol) in 20 mL of 80% acetic acid was heated with stirring for 20 min. The solution was evaporated to dryness, and the residue was chromatographed on a preparative silica gel plate (2 mm) (EtOAc–CH_2Cl_2, 1:2). The product was isolated as a glass and weighed 0.1 g (86%): IR (film) 4.75 μm (azido); UV (EtOH) λ_{max} 264 nm, λ_{min} 226 nm; NMR ($CDCl_3$) δ 2.18 (s, 3 H, 5-CH_3), 2.31 (m, 1 H, 2′-H_a), 2.68 (m, 1 H, 2′-H_b), 3.65 (m, 1 H, 5′-H_a), 3.84 (m, 1 H, 5′-H_b), 3.96 (m, 1 H, 4′-H), 4.37 (m, 1 H, 3′-H), 5.10 (br s, 1 H, 5′-OH, D_2O exchangeable), 6.42 (dd, 1 H, 1′-H). MS, *m/e* 269 ($M^+ + 1$), 270 ($M^+ + 2$).

Antiviral Test Procedures. Three-day-old mitogen-stimulated human peripheral blood mononuclear cells (10^6 cells/mL) were infected with HIV-1 (strain LAV) at a concentration of about 100 $TCID_{50}$ per mL and cultured in the presence and absence of various concentrations of compounds. The drugs were added about 45 min after infection. Five days after infection, the supernatant was clarified, and the virus was pelleted. The reverse transcriptase activity in the disrupted virus was determined. The methods used for culturing the PBM cells, harvesting the virus, and determining the reverse transcriptase activity were those described by McDougal et al.[33] and Spira et al.[34] The virus-infected control had about 2×10^5 dpm per mL of reverse transcriptase activity. The blank and uninfected cell control values were about 300 and 1000 dmp, respectively. Drugs with potent activity were retested with different PBM cells.

The effects of the drugs on the growth of uninfected human PBM cells were also established. Mitogen-stimulated PBM cells (3.8×10^5 cells/mL) were cultured in the presence and absence of drugs under the same conditions as those used for the antiviral assays described above. The cells were counted daily for 5 days by using the trypan blue exclusion method. The EC_{50} was determined by the median effect method.[35]

Acknowledgment. This research was supported by U.S. Public Health Service Grants CA-05262, CA-45410, CA-28852, and CA-44094 from the National Cancer Institute and by a Merit Award from the Veterans Administration. The technical assistance of D. Mozdziesz is gratefully acknowledged.

Registry No. 1, 84472-85-5; **2**, 30516-87-1; **3**, 87190-84-9; **4**, 87190-74-7; **5**, 105784-82-5; **6**, 85236-92-6; **7**, 111495-87-5; **8**, 111495-88-6; **9**, 111495-89-7; **10**, 111495-90-0; **11**, 111495-91-1; **12**, 111495-92-2; **13**, 111495-93-3; **14**, 111495-94-4; **15**, 111495-95-5; **16**, 111495-96-6; **17**, 111495-97-7; **18**, 84472-89-9; **19**, 87190-80-5; **20**, 87190-79-2; **21**, 111495-98-8; **22**, 13410-30-5; **23**, 111524-66-4; **24**, 111495-99-9; **25**, 111496-00-5; **26**, 111496-01-6; HC≡CCHO, 624-67-9; 3′-azido-5′-*O*-acetyl-2′,3′-dideoxy-5-bromouridine, 105784-85-8.

(33) McDougal, J. S.; Cort, S. P.; Kennedy, M. S.; Cabridilla, C. D.; Feorino, P. M.; Francis, D. P.; Hicks, D.; Kalyanaramen, V. S.; Martin, L. S. *J. Immunol. Methods* **1985**, *76*, 171.
(34) Spira, T. J.; Bozeman, L. H.; Holman, R. C.; Warfield, D. I.; Phillips, S. K.; Feorino, P. M. *J. Clin. Microbiol.* **1987**, *25*, 97.
(35) Schinazi, R. F.; Chou, T. C.; Scott, R. J.; Yao, X.; Nahmias, A. J. *Antimicrob. Agents Chemother.* **1986**, *30*, 491.