Synthesis and Anti-human Immunodeficiency Virus (HIV-1) Activity of 3′-Deoxy-3′-(triazol-1-yl)thymidines and 2′,3′-Dideoxy-3′-(triazol-1-yl)uridines, and Inhibition of Reverse Transcriptase by Their 5′-Triphosphates[1)]

Kosaku HIROTA,*[,a] Hiroshi HOSONO,[a] Yukio KITADE,[a] Yoshifumi MAKI,[a] Chung K. CHU,[b] Raymond F. SCHINAZI,[c] Hideo NAKANE,[d] and Katsuhiko ONO[d]

Department of Medicinal Chemistry, Gifu Pharmaceutical University,[a] Mitahora-higashi, Gifu 502, Japan, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The University of Georgia,[b] Athens, Georgia 30602, U.S.A., Department of Pediatrics, Emory University School of Medicine and Veterans Affairs Medical Center,[c] Atlanta, Georgia 30033, U.S.A., and Laboratory of Viral Oncology, Aichi Cancer Center Research Institute,[d] Chikusa-ku, Nagoya 464, Japan. *Received March 29, 1990*

3′-Deoxy-3′-(1,2,3-triazol-1-yl)thymidines (5a, 6a, 8a, 11a, and 12a) and 2′,3′-dideoxy-3′-(1,2,3-triazol-1-yl)uridines (5b, 6b, 8b, 11b, and 12b) were synthesized as cyclic analogues of 3′-azido-3′-deoxythymidine (AZT) and 3′-azido-2′,3′-dideoxyuridine (CS-87) by the cyclization of 5′-trityl derivatives (1a, b) of AZT and CS-87 using α-ketophosphorus ylides and with acetylenic compounds followed by deprotection of the 5′-trityl group. It was hypothesized that the triazole nitrogen atoms could mimic and distorted azido group. However, no significant activity against human immunodeficiency virus type 1 (HIV-1) was observed with any of these compounds. 5′-Triphosphates (17a and 18a, b), prepared from 5a and 6a, b, were inactive against HIV-1 and Rauscher murine leukemia virus (RLV) reverse transcriptases.

Keywords 3′-deoxythymidine; 2′,3′-dideoxyuridine; triazole; 5′-triphosphate; reverse transcriptase; anti-human immunodeficiency virus activity; inhibition; Wittig reagent; cycloaddition; dimethyl acetylenedicarboxylate

Since 3′-azido-3′-deoxythymidine (AZT, Zidovudine) was found to have excellent activity against human immunodeficiency virus type 1 (HIV-1) which gives rise to the acquired immunodeficiency syndrome (AIDS),[2)] a number of sugar-modified nucleosides have been synthesized and their anti-HIV-1 activity has been evaluated.[3)] Among them, several 3′-substituted 3′-deoxythymidines, possessing various substituents instead of the 3′-azido group of AZT, have been investigated as one of the targets for the development of more selective and potent agents against HIV-1.[3-5)]

Although AZT is known to exert its activity as a triphosphate by inhibiting the HIV-1 reverse transcriptase,[6)] the crucial role of the 3′-azido group for enzyme inhibition is still obscure. Recently, the crystal structure of AZT was determined by X-ray diffraction[7,8)] and on the basis of their results Camerman *et al.* have presented[7)] an intriguing hypothesis on the role of the 3′-azido group for the inhibition of the reverse transcriptase by AZT: the azido group may bind tightly to the poly- and/or mono-nucleotide binding site of the enzyme. Thus, it was of interest to synthesize 3′-(triazol-1-yl)-substituted pyrimidine nucleosides as 3′-azido-cyclized analogues of AZT and 3′-azido-2′,3′-dideoxyuridine (CS-87),[5)] which is currently undergoing clinical trials.[9)] The 5′-triphosphates of the 3′-(triazol-1-yl)nucleosides were also synthesized to evaluate their inhibition potentials for the reverse transcriptase.

Azido compounds are known to cyclize to triazole derivatives by reacting with acetylenic compounds and Wittig reagents such as α-keto phosphorus ylides.[10)] Our initial approach for the formation of the triazole ring at the 3′-position of pyrimidine nucleosides was to utilize Wittig

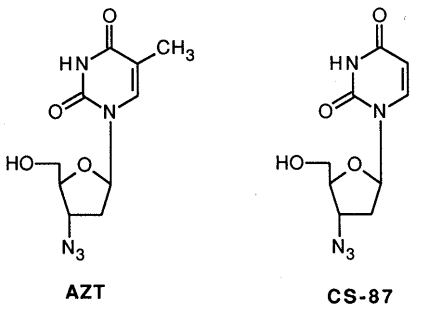


Chart 1

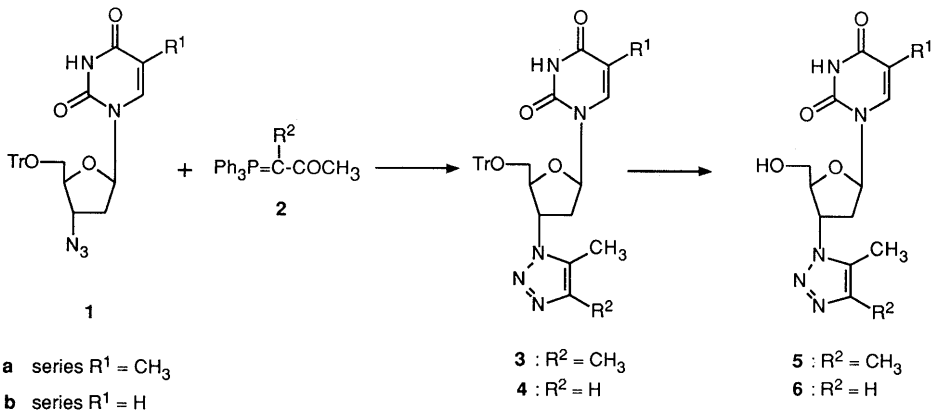


Chart 2

reagents.[11] Starting compounds, 5′-trityl derivatives **1a,b** of AZT and CS-87, were prepared according to the published procedure.[12,13] Treatment of **1a,b** with 2-oxo-3-methylpropylidentriphenylphosphorane (**2**: R^2=Me) in dry toluene under reflux resulted in the formation of the 3′-(4,5-dimethyl-1,2,3-triazol-1-yl)pyrimidine nucleosides **3a,b** in 48% and 57% yields, respectively. In a similar manner, the reaction of **1a,b** with 2-oxopropylidentriphenylphosphorane (**2**: R^2=H) afforded the corresponding 5-methyl-1,2,3-triazoles **4a,b** in 49% and 63% yields, respectively. A positional isomer, 4-methyl-1,2,3-triazole derivative, was not detected in the reaction mixture. The exclusive formation of 5-methyltriazoles in the reaction of azido compounds with the Wittig reagent (**2**: R^2=H) has been well demonstrated.[14,15] Deprotection of the 5′-trityl group in **3a,b** and **4a,b** was smoothly performed upon treatment with 80% acetic acid at 100 °C to give the 3′-(triazol-1-yl)thymidines **5a,6a** and 3′-(triazol-1-yl)uridines **5b,6b** in high yields.

1,3-Dipolar cycloaddition of acetylenic compounds to **1a,b** was examined as a second route for the formation of the triazole ring. The reaction of **1a,b** with dimethyl acetylenedicarboxylate (DMAD) in refluxing toluene led to the formation of the corresponding triazoles **7a,b** in 96% and 90% yields, respectively,[16] which were deprotected to give the desired triazoles **8a,b**.

On the other hand, the cycloaddition of **1a** to methyl propiolate (MP) gave a mixture of two positional isomers **9a** and **10a** (63% and 27% yields), which were detritylated to give 3′-(triazol-1-yl)thymidines **11a** and **12a**, respectively.

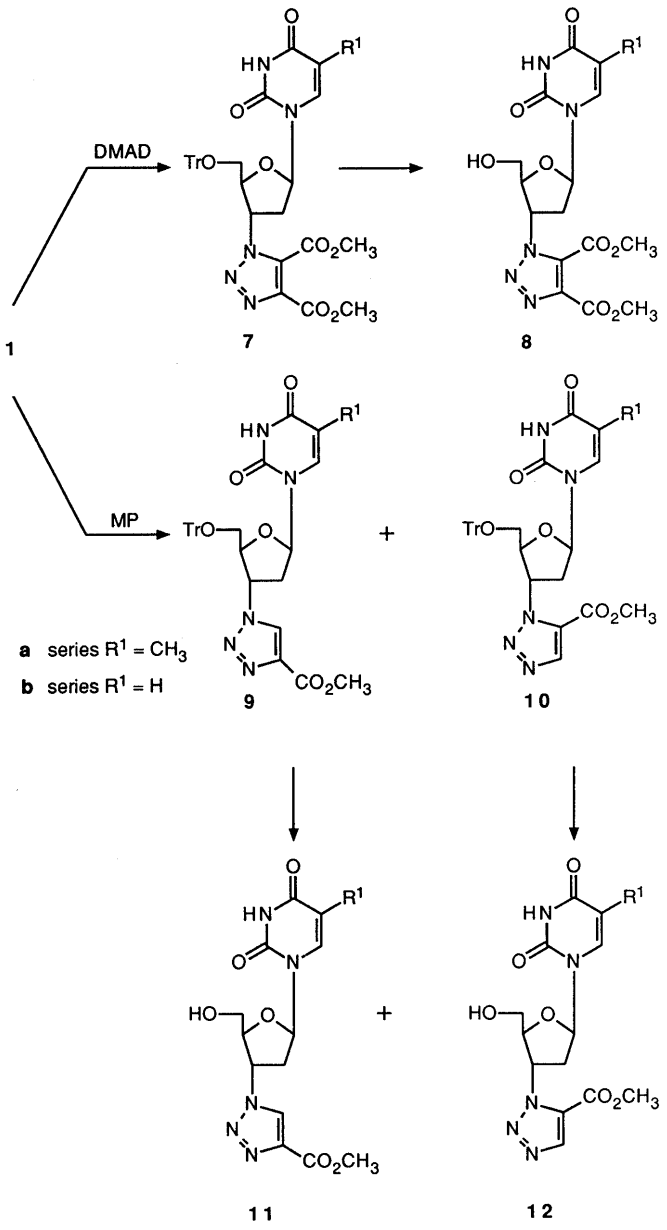
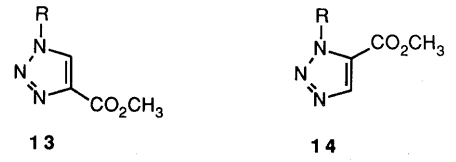


Chart 3



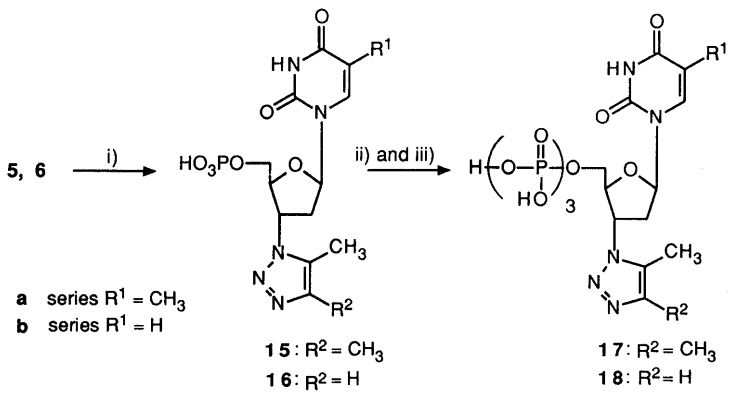
R=2,3,5-tri-*O*-benzoyl-1-β-D-ribofuranosyl

Chart 4

TABLE I. Chemical Shift of Triazole Ring Proton (δ Values)[a]

1,4-Disubstituted 1,2,3-triazole No.	5-H	1,5-Disubstituted 1,2,3-triazole No.	4-H
11a	9.10	**12a**	8.46
11b	9.08	**12b**	8.46
13	9.11[b]	**14**	8.41[b]

a) Measured in DMSO-d_6. *b*) Cited from reference 17.



i) $POCl_3$ in $(CH_3O)_3PO$ ii) carbonyldiimidazole iii) $H_3P_2O_7^-$ n-$Bu_3\overset{+}{N}H$

Chart 5

Alonso *et al.* have reported[17] a method for unequivocal assignment of the positional isomers of 1-glycosyl-1,2,3-triazole-4(and 5)-carboxylates (*e.g.* **13** and **14** in Chart 4) obtained from the reaction of glycosylazides with methyl propiolate by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy: in the chemical shift of the triazole ring protons, the 5-H of 4-carboxylates appears at a lower field than the 4-H of 5′-carboxylates. Thus, the structures of **11a** and **12a** were deduced by comparison of the chemical shifts of the triazole ring protons with those of known 1,4- and 1,5-disubstituted triazoles **13** and **14**, respectively (see, Table I). Analogous treatment of the azidouridine **1b** with methyl propiolate and subsequent detritylation of the resulting cyclic adducts **9b** and **10b** afforded 3′-(triazol-1-yl)uridines **11b** and **12b**, respectively.

In order to examine the inhibition against the reverse transcriptase, the 5′-triphosphates of the 3′-(triazol-1-yl)nucleosides **5a** and **6a, b** were prepared as shown in Chart 5. Phosphorylation of **5a** and **6a, b** by phosphorus oxychloride in trimethyl phosphate gave the corresponding 5′-monophosphates **15a** and **16a, b**, which were allowed to react with *N,N′*-carbonyldiimidazole and were subsequently treated with tributylammonium pyrophosphate to give the desired 5′-triphosphates **17a** and **18a, b**.

The anti-HIV-1 activity of compounds **5a, b** and **6a, b** were evaluated using HIV-1 infected human peripheral blood mononuclear (PBM) cells. No appreciable antiviral activity ($EC_{50} > 100\ \mu M$, AZT = 0.006 μM) was observed with these compounds. When the triphosphates **17a** and **18a, b** were tested for inhibitory activity of HIV-1 and Rauscher murine leukemia virus (RLV) reverse transcriptases, they did not show appreciable inhibitory activity ($IC_{50} > 50\ \mu M$, AZT-5′-triphosphate = 0.15 μM) against the enzyme. These results suggest that even if 3′-(triazol-1-yl)nucleosides are triphosphorylated by cellular kinases, they may not be able to inhibit the reverse transcriptase.

In conclusion, the three nitrogen atoms of the 3′-triazole ring are not biologically equivalent to the azido group of AZT and CS-87. The resulting inactivity may be attributed to both bulkiness and dissimilar electronic nature of the triazole ring.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analyses were carried out at the microanalytical laboratory of our university. Infrared (IR) spectra were taken on a Hitachi 215 instrument from KBr pellets. ^{1}H-NMR were recorded on a JEOL JNX-270 spectrometer, using tetramethylsilane in $CDCl_3$ or sodium 2,2-dimethyl-2-silapentane-5-sulfonate in $(CD_3)_2SO$ as internal references. Chemical shifts are quoted in parts per million (s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet, br = broad, dd = double doublet, dt = double triplet). Mass spectra (MS) were measured at 70 eV with a JEOL JMS-D300 spectrometer. Column chromatography was carried out on silica gel (Wako gel C-300). High-performance liquid chromatography (HPLC) was performed on a Shimadzu SPD-6A apparatus. The column employed was Wakosil 5C18 (Wako).

3′-Deoxy-3′-(4,5-dimethyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-tritylthymidine (3a) A mixture of 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (**1a**)[12] (0.51 g, 1 mmol) and 2-oxo-3-methylpropylidentriphenylphosphorane[18] (0.365 g, 1.1 mmol) in dry toluene (5 ml) was refluxed for 24 h under argon. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (3 : 2). The appropriate fractions were collected and the solvent was removed under reduced pressure to give **3a** (0.27 g, 48%) as a foam. MS *m/z*: 563 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.67 (3H, d, J = 1.3 Hz, CH_3), 2.01 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.67 (1H, m, H-2′), 3.10 (1H, m, H-2′), 3.22 (1H, dd, J = 11.0, 3.0 Hz, H-5′), 3.65 (1H, dd, J = 11.0, 3.0 Hz, H-5′), 4.54 (1H, m, H-4′), 5.06 (1H, m, H-3′), 6.50 (1H, t, J = 6.0 Hz, H-1′), 7.28–7.40 (15H, m, Tr), 7.67 (1H, d, J = 1.3 Hz, H-6), 8.02 (1H, br s, NH).

2′,3′-Dideoxy-3′-(4,5-dimethyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-trityluridine (3b) A mixture of 3′-azido-2′,3′-dideoxy-5′-*O*-trityluridine (**1b**)[13] (0.248 g, 0.5 mmol) and 2-oxo-3-methylpropylidentriphenylphosphorane[18] (0.183 g, 0.55 mmol) in dry toluene (5 ml) was refluxed for 24 h under argon. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (1 : 1). The appropriate fractions were collected and the solvent was removed under reduced pressure to give **3b** (0.157 g, 57%), which was used in the next step without further purification. The structure of this compound was fully characterized after detritylation.

3′-Deoxy-3′-(5-methyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-tritylthymidine (4a) A mixture of **1a**[12] (0.51 g, 1 mmol) and 2-oxo-3-propylidentriphenylphosphorane[18] (0.350 g, 1.1 mmol) in dry toluene (5 ml) was refluxed for 15 h under argon. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (7 : 3). The appropriate fractions were collected and the solvent was removed under reduced pressure to give **4a** (0.27 g, 49%) as a foam. MS *m/z*: 549 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.63 (3H, s, CH_3), 2.11 (3H, s, CH_3), 2.72 (1H, m, H-2′), 3.12 (1H, m, H-2′), 3.21 (1H, dd, J = 11.0, 6.0 Hz, H-5′), 3.68 (1H, dd, J = 11.0, 6.0 Hz, H-5′), 4.53 (1H, m, H-4′), 5.12 (1H, m, H-3′), 6.53 (1H, t, J = 6.0 Hz, H-1′), 7.29—7.40 (16H, m, Tr and triazole), 7.68 (1H, s, H-6), 7.95 (1H, br s, NH).

2′,3′-Dideoxy-3′-(5-methyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-trityluridine (4b) A mixture of **1b**[13] (0.248 g, 0.5 mmol) and 2-oxo-3-methylpropylidentriphenylphosphorane[18] (0.175 g, 0.55 mmol) in dry toluene (5 ml) was refluxed for 15 h under argon. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (3 : 2). The appropriate fractions were collected and the solvent was removed under reduced pressure to give **4b** (0.168 g, 63%), which was used in the next step without further purification. The structure of this compound was fully characterized after detritylation.

3′-Deoxy-3′-(4,5-dimethyl-1*H*-1,2,3-triazol-1-yl)thymidine (5a) A mixture of **3a** (0.113 g, 0.2 mmol) in 80% acetic acid (5 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from ethyl acetate to give **5a** (0.046 g, 72%) as a foam. *Anal.* Calcd for $C_{14}H_{19}N_5O_4$: C, 52.33; H, 5.96; N, 21.80. Found: C, 52.03; H, 5.99; N, 21.38. High-resolution MS: 321.1470 (M^+, $C_{14}H_{19}N_5O_4$ requires 321.1437). ^{1}H-NMR (DMSO-d_6) δ: 1.90 (3H, s, CH_3), 2.26 (3H, s, CH_3), 2.33 (3H, s, CH_3), 2.65—2.84 (2H, m, H-2′), 3.68 (1H, dt, J = 4.7, 12.0 Hz, H-5′), 3.78 (1H, dt, J = 4.7, 12.0 Hz, H-5′), 4.28 (1H, m, H-4′), 5.16 (1H, m, H-3′), 5.39 (1H, t, OH), 6.56 (1H, t, J = 6.8 Hz, H-1′), 7.89 (1H, d, J = 0.9 Hz, H-6), 11.43 (1H, br s, NH).

2′,3′-Dideoxy-3′-(4,5-dimethyl-1*H*-1,2,3-triazol-1-yl)uridine (5b) A mixture of **3b** (0.110 g, 0.2 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was crystallized from ethyl acetate and recrystallized from ethanol to give **5b** (0.045 g, 74%), mp 254—255 °C. *Anal.* Calcd for $C_{13}H_{17}N_5O_4 \cdot 1/4C_2H_5OH$: C, 50.86; H, 5.85; N, 21.97. Found: C, 50.83; H, 5.76; N, 22.15. MS *m/z*: 307 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.26 (3H, s, CH_3), 2.32 (3H, s, CH_3), 2.65 (1H, m, H-2′), 2.81 (1H, m, H-2′), 3.72 (2H, m, H-5′), 4.30 (1H, d, J = 3.4 Hz, H-4′), 5.16 (1H, m, H-3′), 5.39 (1H, br s, OH), 5.78 (1H, d, J = 8.1 Hz, H-5), 6.54 (1H, t, J = 6.4 Hz, H-1′), 8.04 (1H, d, J = 8.1 Hz, H-6), 11.45 (1H, br, NH).

3′-Deoxy-3′-(5-methyl-1*H*-1,2,3-triazol-1-yl)thymidine (6a) A mixture of **4a** (0.110 mg, 0.2 mmol) in 80% acetic acid (5 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from ethyl acetate to give **6a** (0.054 g, 89%), mp 225—226 °C. *Anal.* Calcd for $C_{13}H_{17}N_5O_4 \cdot 1/8CH_3COOC_2H_5$: C, 50.94; H, 5.76; N, 22.00. Found: C, 50.80; H, 5.58; N, 21.38. High-resolution MS: 307.1262 (M^+, $C_{13}H_{17}N_5O_4$ requires 307.1280). ^{1}H-NMR (DMSO-d_6) δ: 1.90 (3H, s, CH_3), 2.41 (3H, s, CH_3), 2.65—2.83 (2H, m, H-2′), 3.69 (1H, m, H-5′), 3.79 (1H, m, H-5′), 4.28 (1H, m, H-4′), 5.21 (1H, m, H-3′), 5.40 (1H, t, J = 5.1 Hz, OH), 6.59 (1H, t, J = 6.8 Hz, H-1′), 7.63 (1H, s, triazole), 7.90 (1H, d, J = 1.0 Hz, H-6), 11.44 (1H, br s, NH).

2′,3′-Dideoxy-3′-(5-methyl-1*H*-1,2,3-triazol-1-yl)uridine (6b) A mixture

of **4b** (0.107 g, 0.2 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was crystallized from ethyl acetate and recrystallized from ethanol to give **6b** (0.048 g, 82%), mp 242—243 °C. *Anal.* Calcd for $C_{12}H_{15}N_5O_4 \cdot 1/3\,H_2O$: C, 48.16; H, 5.28; N, 23.40. Found: C, 48.29; H, 5.16; N, 23.09. MS *m/z*: 293 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.41 (3H, s, CH_3), 2.70 (1H, m, H-2′), 2.81 (1H, m, H-2′), 3.73 (2H, m, H-5′), 4.30 (1H, m, H-4′), 5.22 (1H, m, H-3′), 5.43 (1H, br, OH), 5.78 (1H, d, J=8.1 Hz, H-5), 6.57 (1H, t, J=6.4 Hz, H-1′), 7.63 (1H, s, triazole), 8.05 (1H, d, J=8.1 Hz, H-6), 11.47 (1H, br, NH).

3′-Deoxy-3′-(4,5-dimethoxycarbonyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-trityl-thymidine (7a) A mixture of **1a**[12] (0.204 g, 0.4 mmol) and DMAD (0.071 g, 0.5 mmol) in dry carbon tetrachloride (5 ml) was refluxed for 24 h. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (7 : 3). The appropriate fractions were collected and the solvent was removed under reduced pressure to give **7a** (0.251 g, 96%) as a foam. ^{1}H-NMR ($CDCl_3$) δ: 1.53 (3H, s, CH_3), 2.80 (1H, m, H-2′), 3.01 (1H, m, H-2′), 3.44 (1H, dd, J=10.7, 3.0 Hz, H-5′), 3.57 (1H, dd, J=10.7, 3.0 Hz, H-5′), 3.86 (3H, s, CH_3), 3.98 (3H, s, CH_3), 4.57 (1H, m, H-4′), 5.90 (1H, dt, J=9.0, 4.3 Hz, H-3′), 6.63 (1H, t, J=6.4 Hz, H-1′), 7.26—7.55 (15H, m, Tr), 7.62 (1H, s, H-6), 8.55 (1H, s, NH).

2′,3′-Dideoxy-3′-(4,5-dimethoxycarbonyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-trityluridine (7b) A mixture of **1b**[13] (0.248 g, 0.5 mmol) and DMAD (0.071 g, 0.5 mmol) in dry toluene (5 ml) was refluxed for 10 h. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (7 : 3). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **7b** (0.287 g, 90%), which was used in the next step without further purification. The structure of this compound was fully identified after detritylation.

3′-Deoxy-3′-(4,5-dimethoxycarbonyl-1*H*-1,2,3-triazol-1-yl)thymidine (8a) A mixture of **7a** (0.241 g, 0.37 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from water to give **8a** (0.125 g, 83%), mp 104—105 °C. *Anal.* Calcd for $C_{16}H_{19}N_5O_8 \cdot H_2O$: C, 44.96; H, 4.95; N, 16.39. Found: C, 45.03; H, 4.83; N, 16.45. MS *m/z*: 365 ($M^+ - CO_2$). ^{1}H-NMR (DMSO-d_6) δ: 1.89 (3H, s, CH_3), 2.72 (1H, m, H-2′), 2.86 (1H, m, H-2′), 3.79 (1H, br s, H-5′), 3.98 (3H, s, CH_3), 4.02 (3H, s, CH_3), 4.40 (1H, m, H-4′), 5.44 (1H, t, J=4.7 Hz, OH), 5.69 (1H, m, H-3′), 6.61 (1H, dd, J=9.0, 5.0 Hz, H-1′), 7.95 (1H, s, H-6), 11.46 (1H, br s, NH).

2′,3′-Dideoxy-3′-(4,5-dimethoxycarbonyl-1*H*-1,2,3-triazol-1-yl)uridine (8b) A mixture of **7b** (0.191 g, 0.3 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from water to give **8b** (0.088 g, 83%), mp 104—105 °C. *Anal.* Calcd for $C_{15}H_{17}N_5O_8 \cdot H_2O$: C, 43.59; H, 4.63; N, 16.94. Found: C, 43.83; H, 4.36; N, 17.18. MS *m/z*: 409 (M^+). ^{1}H-NMR (DMSO-d_6), δ: 2.68 (1H, m, H-2′), 2.90 (1H, m, H-2′), 3.78 (1H, m, H-5′), 3.97 (3H, s, CH_3), 4.01 (3H, s, CH_3), 4.42 (1H, m, H-4′), 5.45 (1H, t, J=4.7 Hz, OH), 5.69 (1H, m, H-3′), 5.80 (1H, dd, J=8.1, 1.7 Hz), 6.60 (1H, dd, J=7.7, 6.4 Hz, H-1′), 8.10 (1H, d, J=8.1 Hz, H-6), 11.48 (1H, br s, NH).

Reaction of 1a with Methyl Propiolate A mixture of **1a**[12] (0.306 g, 0.6 mmol) and methyl propiolate (0.151 g, 1.8 mmol) in dry toluene (4 ml) was refluxed for 24 h under argon. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (7 : 3). The early-eluting fractions gave 3′-deoxy-3′-(5-methoxycarbonyl-1,2,3-triazol-1-yl)-5′-*O*-tritylthymidine (**10a**) (0.096 g, 27%), mp 106—107 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.51 (3H, d, J=1.0 Hz, CH_3), 2.78 (1H, m, H-2′), 3.01 (1H, m, H-2′), 3.50 (1H, m, H-5′), 3.88 (3H, s, CH_3), 4.57 (1H, m, H-4′), 6.30 (1H, m, H-3′), 6.67 (1H, t, J=6.4 Hz, H-1′), 7.28—7.43 (15H, m, Tr), 7.67 (1H, d, J=1.0 Hz, H-6), 8.15 (1H, s, triazole), 8.67 (1H, s, NH). The later-eluting fractions gave 3′-deoxy-3′-(4-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-tritylthymidine (**9a**) (0.225 g, 63%), mp 121—122 °C. MS *m/z*: 593 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.66 (3H, s, CH_3), 2.81 (1H, m, H-2′), 3.11 (1H, m, H-2′), 3.35 (1H, dd, J=10.7, 2.9 Hz, H-5′), 3.68 (3H, dd, J=10.7, 2.9 Hz, H-5′), 3.95 (3H, s, CH_3), 4.40 (1H, m, H-4′), 5.38 (1H, m, H-3′), 6.40 (1H, t, J=6.4 Hz, H-1′), 7.28—7.41 (15H, m, Tr), 7.56 (1H, d, J=1.0 Hz, H-6), 8.01 (1H, s, triazole), 8.93 (1H, s, NH).

Reaction of 1b with Methyl Propiolate A mixture of **1b**[13] (0.496 g, 1 mmol) and methyl propiolate (0.252 g, 3 mmol) in dry toluene (5 ml) was refluxed for 24 h. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with benzene–ethyl acetate (7 : 3). The early-eluting fractions gave 2′,3′-dideoxy-3′-(5-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-trityluridine (**10b**) (0.150 g, 26%), which was used in the next step without further purification. The later-eluting fractions gave 2′,3′-dideoxy-3′-(4-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)-5′-*O*-trityluridine (**9b**) (0.360 g, 62%), which was used in the next step without further purification. The structures of **9b** and **10b** were fully characterized after detritylation.

3′-Deoxy-3′-(4-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)thymidine (11a) A mixture of **9a** (0.065 g, 0.11 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from ethanol to give **11a** (0.028 g, 74%), mp 244—245 °C. *Anal.* Calcd for $C_{14}H_{17}N_5O_6 \cdot 1/5H_2O$: C, 47.38; H, 4.94; N, 19.73. Found: C, 47.60; H, 4.81; N, 19.52. MS *m/z*: 351 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 1.90 (3H, s, CH_3), 2.70—2.97 (2H, m, H-2′), 3.75 (2H, m, H-5′), 3.94 (3H, s, CH_3), 4.33 (1H, m, H-4′), 5.36 (1H, t, J=5.1 Hz, OH), 5.53 (1H, m, H-3′), 6.51 (1H, t, J=6.4 Hz, H-1′), 7.90 (1H, d, J=0.9 Hz, H-6), 9.10 (1H, s, triazole), 11.46 (1H, br s, NH).

2′,3′-Dideoxy-3′-(5-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)uridine (11b) A mixture of **9b** (0.232 g, 0.4 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from ethyl acetate to give **11b** (0.118 g, 87%), mp 257—259 °C. *Anal.* Calcd for $C_{13}H_{15}N_5O_6 \cdot 1/4H_2O$: C, 45.69; H, 4.57; N, 20.49. Found: C, 45.80; H, 4.40; N, 20.37. MS *m/z*: 320 ($M^+ - OH$). ^{1}H-NMR (DMSO-d_6) δ: 2.76 (1H, m, H-2′), 2.89 (1H, m, H-2′), 3.75 (2H, m, H-5′), 3.93 (3H, s, CH_3), 4.36 (1H, m, H-4′), 5.37 (1H, t, J=4.7 Hz, OH), 5.52 (1H, m, H-3′), 5.78 (1H, d, J=8.0 Hz, H-5), 6.49 (1H, t, J=6.4 Hz, H-1′), 8.06 (1H, d, J=8.0 Hz, H-6), 9.08 (1H, s, triazole), 11.48 (1H, br s, NH).

3′-Deoxy-3′-(5-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)thymidine (12a) A mixture of **10a** (0.060 g, 0.1 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from ethyl acetate to give **12a** (0.025 g, 71%), mp 227—228 °C. *Anal.* Calcd for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.88; N, 19.94. Found: C, 47.56; H, 4.76; N, 19.83. MS *m/z*: 351 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 1.90 (3H, s, CH_3), 2.67—2.84 (2H, m, H-2′), 3.82 (2H, m, H-5′), 3.97 (3H, s, CH_3), 4.41 (1H, m, H-4′), 5.47 (1H, t, J=4.7 Hz, OH), 5.99 (1H, m, H-3′), 6.63 (1H, dd, J=8.1, 6.4 Hz, H-1′), 8.01 (1H, s, H-6), 8.46 (1H, s, triazole), 11.45 (1H, br s, NH).

2′,3′-Dideoxy-3′-(5-methoxycarbonyl-1*H*-1,2,3-triazol-1-yl)uridine (12b) A mixture of **10b** (0.116 g, 0.2 mmol) in 80% acetic acid (2 ml) was heated at 100 °C for 30 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was recrystallized from ethyl acetate to give **12b** (0.050 g, 75%), mp 207—208 °C. *Anal.* Calcd for $C_{13}H_{15}N_5O_6 \cdot 1/4H_2O$: C, 45.69; H, 4.57; N, 20.49. Found: C, 45.74; H, 4.42; N, 20.42. MS *m/z*: 306 ($M^+ - CH_2OH$). ^{1}H-NMR (DMSO-d_6) δ: 2.69 (1H, m, H-2′), 2.87 (1H, m, H-2′), 3.81 (2H, m, H-5′), 3.97 (3H, s, CH_3), 4.43 (1H, d, J=3.0 Hz, H-4′), 5.48 (1H, t, J=4.7 Hz, OH), 5.80 (1H, d, J=8.1 Hz, H-5), 5.98 (1H, m, H-3′), 6.61 (1H, dd, J=8.1, 6.0 Hz, H-1′), 8.15 (1H, d, J=8.1 Hz, H-6), 8.46 (1H, s, triazole), 11.47 (1H, s, NH).

Preparation of Nucleoside 5′-Monophosphates (15a, 16a, and 16b)[19] General Procedure: A mixture of nucleoside (**5a**, **6a**, and **6b**) (0.1 mmol) in trimethyl phosphate (1 ml) was allowed to stand for 5 min. Phosphorous oxychloride (0.120 ml, 0.4 mmol) was added dropwise to the solution and the mixture was stored in the refrigerator overnight. The mixture was poured into ice-cold water (30 ml) and extracted with ether (30 ml × 3). The water-layer was neutralized with ammonium hydroxide. The solution was diluted with water to 300 ml and applied to a diethylaminoethylcellulose Sephadex A-25 column (16 × 20 cm) preequilibrated with water. Elution was with a linear gradient of 0 (600 ml) to 0.2 M (600 ml) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were

collected and concentrated *in vacuo*, and water was added to and evaporated from the resulting residue to remove residual triethylammonium bicarbonate. The desired products [**15a** (29%), **16a** (24%), and **16b** (68%)] were isolated as the triethylammonium salt. Their structures were confirmed by enzymatic degradation using alkaline phosphatase.

Preparation of Nucleoside 5′-Triphosphates (17a, 18a, and 18b)[20] General Procedure: A mixture of **15a**, **16a**, or **16b** and 1,1′-carbonyldiimidazole (10 eq) was dissolved in dry *N*,*N*-dimethylformamide (DMF). The reaction mixture was stirred for 2 h and methanol (0.01 ml) was added to the mixture. After stirring for 30 min, a solution of tri-*n*-butylammonium pyrophosphate (0.4 M, 1—2 ml) was added and the mixture was stirred overnight. The solution was diluted with water to 300 ml and applied to a DEAE-Sephadex A-25 column (1.6 × 20 cm) preequilibrated with water. Elution was with a linear gradient of 0 (600 ml) to 0.5 M (600 ml) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were collected and concentrated *in vacuo*, and water was added and the solvent was evaporated to remove the residual triethylammonium bicarbonate. The desired products [**17a** (32%), **18a** (90%), and **18b** (30%)] were isolated as the triethylammonium salts. Their structures were confirmed by enzymatic degradation using alkaline phosphatase.

Enzymatic Degradation 5′-Dephosphorylation of 5′-monophosphates (**15a**, **16a**, and **16b**) and 5′-Triphosphates (**17a**, **18a**, and **18b**) was effected *via* alkaline phosphatase exposure using 0.4 OD_{258} of the substrate and 0.06 unit of the enzyme in Tris–acetate (0.2 M, pH 8.8), $MgCl_2$ (0.001 M), and a total volume of 100 μl. Incubation was at 37 °C for 2 h. The digested products were confirmed by comparison with the corresponding nucleosides (**5a**, **6a**, and **6b**), respectively, using HPLC.

Antiviral Assay in Human PBM Cells Three-day-old phyohemagglutinin-stimulated PBM cells (10^6 cell/ml) from hepatitis B and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100—50% tissue culture infections dose 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 later the supernatant was clarified and the virus pelleted. The reverse transcriptase activity associated with 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.*[21] and Spira *et al.*,[22] except that fungizone was not included in the medium. 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 dpm, respectively.

Assay for Reverse Transcriptase Reverse transcriptase activity was measured with $(rA)_n(dT)_{12-18}$ as the template·primer under the optimized reaction conditions specified for each of the RLV- and HIV-reverse transcriptases.[23] The reaction mixture contained the following components: 50 mM Tris–HCl, pH 8.0; 5 μg/ml $(rA)_n(dT)_{12-18}$ (1 : 1); 10 μM [^{3}H]dTTP (400 cpm/pmol); 5 mM dithiothreitol; 50 mM KCl; 15% (v/v) glycerol; 0.2 mM $MnCl_2$ for RLV-reverse transcriptase and 5 mM $MgCl_2$ for HIV-reverse transcriptase.

References and Notes

1) This paper is part 67 of a series entitled "Pyrimidines." For Part 66 see, K. Hirota, H. Sajiki, Y. Kitade, and Y. Maki, *Tetrahedron*, **46**, 3431 (1990).
2) H. Mitsuya , K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. Nusinoff-Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Border, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 7096 (1985).
3) E. De Clercq, A. Van Aerschot, P. Herdewijn, M. Baba, R. Pauwels, and J. Balzarini, *Nucleosides & Nucleotides*, **8**, 659 (1989).
4) T. V. Kutateladze, A. M. Kritzyn, V. L. Florentjev, V. N. Kavsan, Z. G. Chidgeavadze, and R. S. Beabealashvilli, *FEBS Lett.*, **207**, 205 (1986); N. Dyatkina, S. Minassian, M. Kukhanova, A. Krayevsky, M. von Janta-Lipinsky, Z. G. Chidgeavadze, and R. S. Beabealashvilli, *ibid.*, **219**, 151 (1987); P. Herdewijn, J. Balzarini, E. De Clercq, R. Pauwels, M. Baba, S. Broder, and H. Vanderhaeghe, *J. Med. Chem.*, **30**, 1270 (1987); P. Herdewijn, J. Balzarini, M. Baba, R. Pauwels, A. Van Aerschot, G. Janssen, and E. De Clercq, *ibid.*, **31**, 2040 (1988); M. Okabe, R.-C. Sun, S. Y.-K. Tam, L. J. Todaro, and D. L. Coffen, *J. Org. Chem.*, **53**, 4780 (1988); G. W. J. Fleet, J. C. Son, and A. E. Derome, *Tetrahedron*, **44**, 625 (1988); A. Calvo-Mateo, M. J. Camarasa, A. Diaz-Ortiz, F. G. De las Heras, and A. Alemany, *ibid.*, **44**, 4895 (1988); A. Calvo-Mateo, M.-J. Camarasa, A. Diaz-Ortiz, and F. G. De las Heras, *Tetrahedron Lett.*, **29**, 941 (1988); K. E. B. Parkes and K. Taylor, *ibid.*, **29**, 2995 (1988); S. L. Schreiber and N. Ikemoto, *ibid.*, **29**, 3211 (1988); M. Maillard, A. Faraj, F. Frappier, J.-C. Florent, D. S. Grierson, and C. Monneret, *ibid.*, **30**, 1955 (1989); M. J. Camarasa, A. Diaz-Ortiz, A. Calvo-Mateo, F. G. De las Heras, J. Balzarini, and E. De Clercq, *J. Med. Chem.*, **32**, 1732 (1989); C. K. Chu, B. Doboszewski, W. Schmidt, and G. V. Ullas, *J. Org. Chem.*, **54**, 2767 (1989); R. Rosowsky, R. M. Ruprecht, and V. C. Solan, *Nucleosides & Nucleotides*, **8**, 491 (1989); M. J. Camarasa, A. Diaz-Ortiz, A. Calvo-Mateo, F. G. De las Heras, J. Balzarini, and E. De Clercq, *ibid.*, **8**, 837 (1989); J. Fiandor, D. M. Huryn, B. Sluboski, L. J. Tadaro, and S. Y.-K. Tam, *ibid.*, **8**, 1107 (1989); R. Sterzycki, M. Mansuri, V. Brankovan, R. Buroker, I. Ghazzouli, M. Hitchcock, J.-P. Sommadossi, and J. C. Martin, *ibid.*, **8**, 1115 (1989); A. Van Aerschot, J. Balzarini, R. Pauwels, P. Wigerinck, E. De Clercq, and P. Herdewijn, *ibid.*, **8**, 1125 (1989); J. Hiebl, E. Zbiral, J. Balzarini, and E. De Clercq, *J. Med. Chem.*, **33**, 845 (1990).
5) C. K. Chu, R. F. Schinazi, M. K. Ahn, G. V. Ullas, and Z. P. Gu, *J. Med. Chem.*, **32**, 612 (1989).
6) F. A. Furman, J. A. Fyfe, M. H. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. Nusinoff-Lehrman, D.-P. Bolognesi, S. Broder, H. Mitsuya, and D. W. Barry, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8333 (1986).
7) A. Camerman, D. Mastropaolo, and N. Camerman, *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 8239 (1987).
8) G. I. Birnbaum, J. Giziewicz, E. J. Gabe, T. Lin, and W. H. Prusoff, *Can. J. Chem.*, **65**, 2135 (1987); P. V. Roey, J. M. Salerno, W. L. Duax, C. K. Chu, M. K. Ahn, and R. F. Schinazi, *J. Am. Chem. Soc.*, **110**, 2277 (1988); R. Parthasarathy and H. Kim, *Biochem. Biophys. Res.Commun.*, **152**, 351 (1988).
9) J. L. Marx, *Science*, **244**, 287 (1989).
10) H. Wamhoff, "Comprehensive Heterocyclic Chemistry," Vol. 5, ed. by K. T. Potts, Pergamon Press, Oxford, 1984, pp. 669—732; K. T. Finley, "The Chemistry of Heterocyclic Compounds; Triazoles: 1, 2, 3," ed. by J. A. Montgomery, John Wiley & Sons, Inc., New York, 1980.
11) G. R. Harvey, *J. Org. Chem.*, **31**, 1587 (1966); W. Schorkhuber and E. Zbiral, *Justus Liebig. Ann. Chem.*, **1980**, 1455.
12) R. P. Glinski, M. S. Khan, R. L. Kalamas, and M. B. Sporn, *J. Org. Chem.*, **38**, 4299 (1973).
13) T.-S. Lin and W. R. Mancini, *J. Med. Chem.*, **26**, 544 (1983).
14) P. Ykmann, G. Labbe, and G. Smets, *Tetrahedron*, **27**, 845 (1971).
15) E. Zbiral, *Synthesis*, **1974**, 775.
16) Since completing this work, synthesis of 3′-(1*H*-1,2,3-triazol-1-yl)-3′-deoxythymidines by 1,3-dipolar cycloaddition of AZT to various alkynes have been reported independently: D. Habich, W. Barth, and M. Rosner, *Heterocycles*, **29**, 2083 (1989).
17) G. Alonso, M. T. Garcia-Lopez, G. Garcia-Munoz, R. Madroñero, and M. Rico, *J. Heterocycl. Chem.*, **7**, 1269 (1970).
18) F. Ramirez and S. Dershowitz, *J. Org. Chem.*, **22**, 41 (1957).
19) M. Yoshikawa and T. Takanishi, *Bull. Chem. Soc. Jpn.*, **42**, 3505 (1969).
20) H. Sawai, T. Shibata, and M. Ohno, *Tetrahedron Lett.*, **47**, 4573 (1979).
21) J. S. McDougal, S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaramen, and L. S. Martin, *J. Immun. Meth.*, **76**, 171 (1985).
22) T. J. Spira, L. H. Bozeman, R. C. Halman, D. I. Warfield, S. K. Phillips, and P. M. Feorino, *J. Clin. Microbiol.*, **25**, 97 (1987).
23) K. Ono, H. Nakane, F. Barre-Sinoussi, and J.-C. Chermann, *Eur. J. Biochem.*, **176**, 305 (1988).