

from 0.68 to 4.04 ng/AI per mL/h.

Test compounds dissolved in DMSO were added to the incubation mixture. At the concentration employed, DMSO inhibits the generation of angiotensin I by <10%. All values are reported as percent of the vehicle (DMSO) control response. The amount of angiotensin I measured was corrected for endogenous angiotensin I in the plasma.

The IC_{50} values were obtained by plotting three or more inhibitor concentrations on semilog paper and estimating the concentration producing 50% inhibition.

Chymotrypsin Stability Studies. Stock solutions of the renin inhibitors in methanol (1 mg/mL) were prepared. A 20- μ L aliquots of this solution was then added to 3 mL of 0.03 M sodium phosphate buffer/0.1 M NaCl, pH 6.9, containing 10 μ g/mL bovine chymotrypsin (Sigma C-4129) and the mixture was incubated at 37 °C. At 0, 15, 45, 90, and 180 min, 0.4 mL was removed

and diluted with acetonitrile. A blank in which the buffer/chymotrypsin solution was heated in boiling H_2O for 30 min to inactivate the enzyme prior to addition of the renin inhibitor was also run for each inhibitor. A 100- μ L aliquot of the incubation mixture was analyzed by injection onto an Alltech (C-8 5 μ m Econosil 250 mm \times 4.6 mm) column equilibrated with 65% acetonitrile/35% 0.1% TEA, pH 3.2. A Waters Lambda-Max LC Spectrophotometer at 214 nm was used for detection and the Spectra Physics SP4270 integrator was used for quantitation. The results are expressed as percent parent remaining (chymotrypsin treatment - blank) following incubation for 3 h.

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Synthesis, Antiretrovirus Effects, and Phosphorylation Kinetics of 3'-Isocyano-3'-deoxythymidine and 3'-Isocyano-2',3'-dideoxyuridine

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The silylated AzddThd 5 and AzddUrd 6 prepared from 2,3'-anhydronucleoside derivatives 3 and 4 were transformed to formamides 7 and 8 by using the sequence $RN_3 \rightarrow RN=P(C_6H_5) \rightarrow RNHCHO$. Formamides 7 and 8 were dehydrated to the protected 3'-isocyano derivatives 9 and 10; deblocking gave 11 and 12. Neither 3'-isocyano-3'-deoxythymidine (11) nor 3'-isocyano-2',3'-dideoxyuridine (12) showed anti-HIV activity at noncytotoxic concentrations. ddThd derivative 11 was considerably more toxic to MT-4 cells than ddUrd derivative 12; it also had a much greater affinity (K_i) for MT-4 cell dThd kinase than ddUrd derivative 12. Both compounds appear to be linear mixed-type inhibitors of MT-4 cell dThd kinase.

Since the discovery of 3'-azido-3'-deoxythymidine (AZT) as an antiretroviral agent,¹ a number of structurally related nucleoside analogues have been synthesized and evaluated for their antiretroviral properties (for a review see refs 2 and 3). Recently, analogous compounds containing the electronically comparable cyano,^{4b,5-7} ethynyl,⁸ thiocyno,^{4a,b} and isothiocyno group^{4b} instead of the azido group have been synthesized.

To our knowledge, the 3'-isocyano-substituted derivatives of 3'-deoxythymidine and 2',3'-dideoxyuridine have not been reported yet.²² The most important difference between the chemical properties of the azido and the isocyano groups is the electrophilicity of the first and the nucleophilicity of the second.

We have recently described direct transformation of the azido group to the formamido group,⁹ thus avoiding the disadvantages of the sequence $RN_3 \rightarrow RNH_2 \rightarrow RNHCHO$. With respect to the easily practicable transformation of the azido to the isocyano group,⁹ we applied this functionality interchange to the protected 3'-azido-3'-deoxythymidine (AzddThd, AZT) and 3'-azido-2',3'-dideoxyuridine (AzddUrd).

The individual steps are summarized in Scheme I. In the first step, 5'-O-tert-butylidimethylsilylthymidine (1) and the corresponding 2'-deoxyuridine derivative 2 were treated with triphenylphosphine-diethyl azodicarboxylate¹⁰ to give cyclonucleoside derivatives 3 and 4, respectively. By a nucleophilic opening reaction with sodium azide in DMF- H_2O (9:1, v/v),¹¹ these compounds were transformed to the protected AzddThd derivative 5 and AzddUrd derivative 6. Then the P-N ylides obtained

by Staudinger reaction were treated with acetic formic anhydride¹² to give the intermediates 5a and 6a. Enols 5b and 6b, which were in equilibrium with imino acetates 5a and 6a, were transformed to the formamides 7 and 8. The yields were about 90%. Dehydration to isocyano derivatives 9 and 10 was achieved according to the procedure of Ugi.¹³ Finally, the 5'-O-tert-butylidimethylsilyl group was removed by tetrabutylammonium fluoride.¹⁴

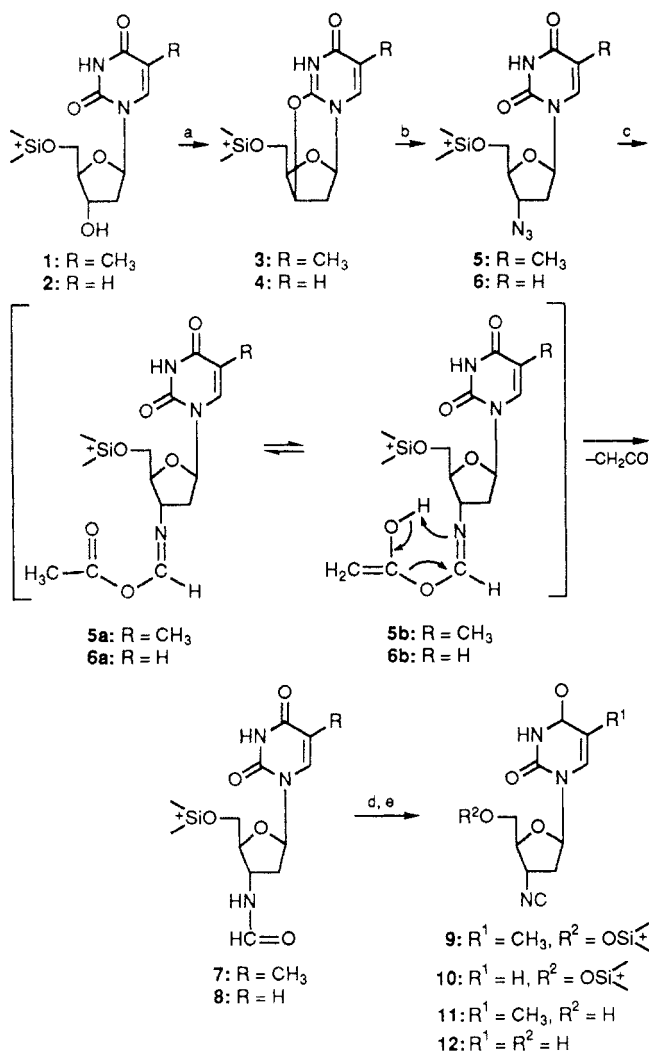
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Table I. Inhibitory Effects of 3'-Isocyano-3'-deoxythymidine and 3'-Isocyano-2',3'-dideoxyuridine on HIV-1 and HIV-2 Replication in MT-4 Cells

compound	cytopathogenicity: ED ₅₀ , ^a μ M		CD ₅₀ , ^b μ M	selectivity index ^c
	HIV-1-induced	HIV-2-induced		
3'-isocyano-ddThd	>0.8	>0.8	0.88 \pm 0.1	<1.1
3'-isocyano-ddUrd	>500	>500	\geq 500	\leq 1
AzddThd (AZT)	0.004 \pm 0.001	0.004 \pm 0.001	6.3 \pm 2.5	1575

^a 50% effective dose or dose required to inhibit HIV-induced cytopathogenicity in MT-4 cells (average values \pm standard deviation).^b 50% cytotoxic dose or dose required to reduce the viability of MT-4 cells by 50% (average values \pm standard deviation). ^c Selectivity index or CD₅₀/ED₅₀ ratio.**Scheme I^a**^a (a) TPP, DEAD, toluene; (b) NaN₃, DMF-H₂O = 9:1; (c) TPP, HCO₂COCH₃, toluene; (d) POCl₃, [(CH₃)₃CH]₂NH, CH₂Cl₂; (e) TBAF, THF.**Antiviral Activity of 3'-Isocyano-ddThd (11) and 3'-Isocyano-ddUrd (12) and Kinetics of Their Interaction with the Phosphorylation of [methyl-³H]dThd**

The anti-HIV-1 and -HIV-2 activity and cytotoxicity data of 3'-isocyano-ddThd 11 and 3'-isocyano-ddUrd 12 are shown in Table I. None of the compounds proved effective as inhibitors of HIV replication in MT-4 cells at subtoxic concentrations. 3'-Isocyano-ddThd (11) was considerably more toxic to MT-4 cells than 3'-isocyano-ddUrd (12) (CD₅₀ = 0.88 and >500 μ M, respectively). In contrast, AzddThd (AZT) was effective against HIV-1 and HIV-2 at 0.004 μ M and cytotoxic at 6.3 μ M (Table I). Consequently, the selectivity index of AzddThd was 1575.

Unlike AzddThd, 3'-isocyano-ddThd (11) and 3'-isocyano-ddUrd (12) were not inhibitory to simian AIDS-related virus (SRV) induced giant-cell formation in Raji cells (data not shown). Also in contrast with AzddThd, both 3'-isocyano-ddThd (11) and -ddUrd (12) were equally cytostatic to thymidine kinase deficient (TK⁻) Raji and normal Raji/0 cells (data not shown). Thus, dThd kinase seems to play an important role in the antiviral and cytostatic action of AzddThd, but not 3'-isocyano-ddThd (11) or 3'-isocyano-ddUrd (12).

3'-Isocyano-ddThd (11) and 3'-isocyano-ddUrd (12) were evaluated for their interaction with [methyl-³H]dThd phosphorylation by partially purified MT-4 cell dThd kinase. 3'-Isocyano-ddThd (11) proved to be a more potent inhibitor of [methyl-³H]dThd phosphorylation than 3'-isocyano-ddUrd (12) (K_i = 90 and 2000 μ M, respectively) (Figure 1). In fact, both compounds showed kinetics of interaction with MT-4 cell dThd kinase that were clearly different from that of AzddThd. In contrast with AzddThd, which is a very potent competitive inhibitor of dThd phosphorylation by dThd kinase,¹⁹ 3'-isocyano-substituted ddThd (11) and ddUrd (12) analogues proved to be linear mixed-type inhibitors of dThd phosphorylation by MT-4 cell extracts. This is evident from the Lineweaver-Burk plots where the lines intersect left from the y axis (Figure 1) and the replots of the Lineweaver-Burk plots (Dixon slope versus [Inhibitor]) (Figure 1, intersect).

The fact that 3'-isocyano-ddThd (11) has a much greater inhibitory effect on the phosphorylation of dThd by MT-4 cell extracts than 3'-isocyano-ddUrd (12) most likely accounts for the greater toxicity of the former to MT-4 cells (Table I). The lack of antiretrovirus activity of both compounds may be ascribed to inappropriate phosphorylation of 3'-isocyano-ddUrd (12) and 3'-isocyano-ddThd (11) to the corresponding 5'-triphosphates and/or poor affinity of these 5'-triphosphate derivatives to the HIV-specified reverse transcriptase. The latter issue could be directly addressed by evaluation of the interaction of the 5'-triphosphates with the reverse transcriptase.

Experimental Section

Melting points were determined on a Kofler apparatus and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker WM 250 spectrometer operating at 250 MHz for ¹H and 62.9 MHz for ¹³C. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. If not indicated otherwise, CDCl₃ was used as solvent. Precoated Merck silica gel F 254 plates were used for TLC, and the spots were examined with UV light and by spraying with a solution of 2% Ce(NO₃)₄ in 2 N H₂SO₄ followed by heating at 200 °C. Flash chromatography¹⁶ was performed with 230–400 mesh silica gel from E. Merck. Abbreviations used are EA (ethylacetate) and PE (petroleum ether).

Infrared spectra were recorded with a Perkin-Elmer 377 spectrophotometer. Mass spectra were recorded on a Varian CH-7 apparatus (70 eV). The source of the anhydrous solvents was as follows: tetrahydrofuran was obtained by distillation after reflux

with potassium-benzophenone; toluene was dried by distillation after it had been refluxed in the presence of sodium; DMF was refluxed on CaH₂ and distilled; dichloromethane was refluxed on phosphorus pentoxide and distilled.

5'-O-(tert-Butyldimethylsilyl)thymidine (1). This compound was prepared from thymidine as described for 2: yield 86%; mp 192–194 °C (lit.¹⁶ mp 193–194 °C); TLC *R_f* (EA) 0.51; ¹H NMR δ 0.08 (s, 6, Si(CH₃)₂), 0.90 (s, 9, SiC(CH₃)₃), 1.89 (s, 3, 5-CH₃), 2.06 (ddd, 1, *J*_{2'a,2'b} = 13.7 Hz, *J*_{2'a,1'} = 4.5 Hz, *J*_{2'a,3'} = 3.9 Hz, 2'-Ha), 2.40 (dd, 1, *J*_{2'b,1'} = 8.8 Hz, 2'-Hb), 3.46 (br s, 1, 3'-OH, D₂O exchangeable), 3.80 (d, 1, *J*_{5'a,5'b} = 11.8 Hz, 5'-Ha), 3.90 (d, 1, 5'-Hb), 4.06 (br s, 1, 4'-H), 4.43 (d, 1, 3'-H), 6.39 (dd, 1, 1'-H), 7.53 (s, 1, 6-H), 9.81 (br s, 1, 3-H, D₂O exchangeable). Anal. (C₁₆H₂₈N₂O₅Si) C, H, N.

5'-O-(tert-Butyldimethylsilyl)-2'-deoxyuridine (2). A solution of 1.51 g (10.0 mmol) of TBDMSiCl in 5 mL of dry DMF was added slowly to a stirred solution of 2.07 g (9.1 mmol) of 2'-deoxyuridine and 1.36 g (20.0 mmol) of imidazole in 20 mL of dry DMF at -10 °C. The mixture was stirred for 1 h in the cold, and then at room temperature overnight. DMF was removed in vacuo. The remaining residue was partitioned between H₂O and CH₂Cl₂. The organic phase was dried over Na₂SO₄, concentrated, and purified by flash chromatography (PE-EA = 1:1) to yield 89% of a colorless hygroscopic solid; TLC *R_f* (EA) 0.65. This material was used directly in the next step. Surprisingly, there is no report on the synthesis and the NMR data of this compound in the literature although GC retention data¹⁷ and characteristic MS fragmentation pathways¹⁸ are reported. 2: ¹H NMR δ 0.11 (s, 6, Si(CH₃)₂), 0.91 (s, 9, SiC(CH₃)₃), 2.14 (ddd, 1, *J*_{2'a,2'b} = 14.0 Hz, *J*_{2'a,1'} = 7.0 Hz, *J*_{2'a,3'} = 4.0 Hz, 2'-Ha), 2.45 (ddd, 1, *J*_{2'b,1'} = 7.0 Hz, *J*_{2'b,3'} = 6.0 Hz, 2'-Hb), 3.08 (br s, 1, 3'-OH, D₂O exchangeable), 3.88 (ABX system, 2, *J*_{5'a,5'b} = 12.0 Hz, *J*_{5'a,4'} = *J*_{5'b,4'} = 2.0 Hz, 5'-Ha, 5'-Hb), 4.07 (m, 1, 4'-H), 4.46 (m, 1, 3'-H), 5.70 (d, 1, *J*_{5,6} = 8.0 Hz, 5-H), 6.38 (t, 1, 1'-H), 7.93 (d, 1, 6-H), 9.37 (br s, 1, 3-H, D₂O exchangeable). Anal. (C₁₅H₂₆N₂O₅Si) C, H, N.

2,3'-Anhydro-5'-O-(tert-butyldimethylsilyl)thymidine (3). A suspension of 6.02 g (16.9 mmol) of 1 and 50 mL of toluene containing 5.09 g (19.4 mmol) of triphenylphosphane (TPP) was heated to 80 °C on an oil bath, and then THF (10 mL) was added to get a clear solution. Subsequently, 3.06 mL (19.4 mmol) of diethyl azodicarboxylate (DEAD) was added. After 30 min, TLC (EA) indicated complete reaction. The solution was allowed to cool down and was stored at 4 °C overnight. On the next day, 4.47 g (78%) of crystalline 3 was obtained. The filtrate was evaporated and purified by flash chromatography; excess TPP and TPPO were eluted with EA, and 0.73 g (13%) of 3 was eluted with acetone: total yield 91%; recrystallization from EA gave colorless crystals; mp 175–176 °C; TLC (EA) *R_f* 0.05; ¹H NMR δ 0.06 and 0.08 (s, 3, Si(CH₃)₂), 0.89 (s, 9, SiC(CH₃)₃), 1.96 (s, 3, 5-CH₃), 2.46 (ddd, 1, *J*_{2'a,2'b} = 13.0 Hz, *J*_{2'a,1'} = 3.6 Hz, *J*_{2'a,3'} = 2.9 Hz, 2'-Ha), 2.73 (d, 1, 2'-Hb), 3.79 (ABX system, 2, *J*_{5'a,5'b} = 10.1 Hz, *J*_{5'a,4'} = *J*_{5'b,4'} = 7.2 Hz, 5'-Ha, 5'-Hb), 4.44 (m, 1, 4'-H), 5.19 (br s, 1, 3'-H), 5.52 (d, 1, 1'-H), 6.98 (s, 1, 6-H); MS (250 °C) *m/e* 323 (1), 281 (20) (M⁺ - C(CH₃)₃). Anal. (C₁₆H₂₆N₂O₄Si) C, H, N.

2,3'-Anhydro-5'-O-(tert-butyldimethylsilyl)-2'-deoxyuridine (4). A solution of 2.00 g (5.84 mmol) of 2, 1.84 (7.00 mmol) of TPP, and 20 mL of toluene was heated to 80 °C on an oil bath. DEAD (1.104 mL, 7.00 mmol) was added. After 30 min, TLC (EA) indicated complete reaction. After removal of the solvent, the residue was recrystallized from EA, yielding 88% of a colorless material. An analytical sample was prepared by recrystallization from EA twice. It was impossible to obtain a sharp melting point. At 150 °C we observed a change in crystal structure, and decomposition took place between 180 and 190 °C. 4: ¹H NMR δ 0.06 (s, 6, Si(CH₃)₂), 0.88 (s, 9, SiC(CH₃)₃), 2.49 (ddd, 1, *J*_{2'a,2'b} = 3.3 Hz, *J*_{2'a,3'} = 1.8 Hz, 2'-Ha), 2.76 (d, 1, *J*_{2'a,2'b} = 13.4 Hz, 2'-Hb), 3.79 (ABX system, 2, *J*_{5'a,5'b} = 11.1 Hz, *J*_{5'a,4'} = *J*_{5'b,4'} = 6.7 Hz, 5'-Ha, 5'-Hb), 4.29 (m, 1, 4'-H), 5.22 (br s, 1, 3'-H),

5.70 (d, 1, 1'-H), 5.91 (d, 1, *J*_{5,6} = 7.3 Hz, 5-H), 7.25 (d, 1, 6-H); MS (200 °C) *m/e* 309 (1), 267 (21) (M⁺ - CH(CH₃)₃). Anal. (C₁₅H₂₄N₂O₄Si) C, H, N.

3'-Azido-5'-O-(tert-butyldimethylsilyl)-3'-deoxythymidine (5). Compound 3 (507 mg, 1.5 mmol) was allowed to react with 293 mg (4.5 mmol) of sodium azide in 20 mL of DMF-H₂O mixture¹¹ (9:1, v/v) heated under reflux for 11 h to give compound 5. After removal of the solvent, H₂O (10 mL) and CH₂Cl₂ (50 mL) were added. The organic layer was dried over Na₂SO₄ and concentrated and the residue was chromatographed with PE-EA = 2:1 as the eluting system: yield 58%; mp 83–84 °C; TLC *R_f* (ether-acetone = 9:1) 0.81; ¹H NMR δ 0.15 (s, 6, Si(CH₃)₂), 0.96 (s, 9, SiC(CH₃)₃), 1.95 (s, 3, 5-CH₃), 2.24 (ddd, 1, *J*_{2'a,2'b} = 14.8 Hz, *J*_{2'a,1'} = 7.4 Hz, *J*_{2'a,3'} = 7.4 Hz, 2'-Ha), 2.46 (ddd, 1, *J*_{2'b,1'} = 7.4 Hz, *J*_{2'b,3'} = 3.7 Hz, 2'-Hb), 3.80 (dd, 1, *J*_{5'a,4'} = 1.9 Hz, *J*_{5'a,5'b} = 9.3 Hz, 5'-Ha), 3.93–4.00 (m, 2, *J*_{5,4'} = 1.9 Hz, 4'-H, 5'-Hb), 4.26 (m, 1, 3'-H), 6.24 (t, 1, 1'-H), 7.47 (s, 1, 6-H), 8.81 (br s, 1, 3-H); ¹³C NMR δ -5.56, -5.45, 12.43, 18.26, 25.82, 37.86, 60.37, 62.79, 84.35, 84.49, 110.97, 134.93, 150.38, 163.96; MS (130 °C) *m/e* 324 (7) (M⁺ - C(CH₃)₃); IR (KBr) 2100 cm⁻¹ (N₃). Anal. (C₁₆H₂₇N₅O₄Si) C, H, N.

3'-Azido-5'-O-(tert-butyldimethylsilyl)-2',3'-dideoxyuridine (6). Compound 6 was prepared in the same manner as 5, with 4 as starting material: yield 55%; mp 88–89 °C; TLC *R_f* (ether-acetone = 9:1) 0.80; ¹H NMR δ 0.11 (s, 6, Si(CH₃)₂), 0.91 (s, 9, SiC(CH₃)₃), 2.29 (ddd, 1, *J*_{2'a,2'b} = 13.8 Hz, *J*_{2'a,1'} = 6.0 Hz, *J*_{2'a,3'} = 8.0 Hz, 2'-Ha), 2.46 (ddd, 1, *J*_{2'b,1'} = 6.0 Hz, *J*_{2'b,3'} = 6.0 Hz, 2'-Hb), 3.78 (dd, 1, *J*_{5'a,5'b} = 12.0 Hz, *J*_{5'a,4'} = 2.0 Hz, 5'-Ha), 3.98–4.04 (m, 2, 4'-H, 5'-Hb), 4.21 (m, 1, *J*_{3,4'} = 6.0 Hz, 3'-H), 5.68 (dd, 1, *J*_{5,6} = 8.0 Hz, *J* = 1.8 Hz, 5-H), 6.21 (t, 1, 1'-H), 7.88 (d, 1, 6-H), 9.06 (br s, 1, 3-H); ¹³C NMR δ -5.78, -5.66, 18.15, 25.68, 38.31, 59.31, 62.23, 84.61, 84.80, 102.13, 139.61, 150.32, 163.62; MS (90 °C) *m/e* 310 (6) (M⁺ - C(CH₃)₃), 267 (53); IR (CH₂Cl₂) 2115 cm⁻¹ (N₃). Anal. (C₁₅H₂₅N₅O₄Si) C, H, N.

5'-O-(tert-Butyldimethylsilyl)-3'-deoxy-3'-formamidothymidine (7). To 3.0 g (7.87 mmol) of 5 and 20 mL of toluene was added 2.47 g (9.44 mmol) of TPP and 1.04 g (11.8 mmol) of acetic formic anhydride,¹² at 0 °C. Then the solution was heated for 4 h at 60 °C. The product was allowed to crystallize overnight at 4 °C from the reaction mixture. The yield of crystalline 7 was 2.281 g (76%). The filtrate was evaporated and after flash chromatography (ether-acetone = 9:1 till all triphenylphosphane oxide (TPPO) was eluted, then acetone alone) 0.443 g (15%) of 7 was obtained: total yield 91%; mp 170–172 °C; TLC *R_f* (ether-acetone = 9:1) 0.08; ¹H NMR δ 0.11 (s, 6, Si(CH₃)₂), 0.90 (s, 9, SiC(CH₃)₃), 1.90 (s, 3, 5-CH₃), 2.14 (ddd, 1, *J*_{2'a,2'b} = 13.6 Hz, *J*_{2'a,1'} = 9.3 Hz, *J*_{2'a,3'} = 7.2 Hz, 2'-Ha), 2.29 (dd, 1, *J*_{2'b,1'} = 5.8 Hz, 2'-Hb), 3.89 (br s, 2, 5'-Ha, 5'-Hb), 4.05 (br s, 1, 4'-H), 4.53 (m, 1, 3'-H), 6.34 (dd, 1, 1'-H), 7.62 (s, 1, 6-H), 8.35 (d, 1, *J*_{NH,3'} = 8.0 Hz, NHCHO), 8.67 (s, 1, CHO), 9.82 (br s, 1, 3-H); ¹³C NMR (CD₃OD) δ -5.27, -5.19, 12.70, 19.28, 26.47, 38.93, 49.39, 64.16, 85.93, 86.43, 111.52, 137.29, 152.21, 163.54, 166.19; MS (200 °C) *m/e* 368 (1) (M⁺ - CH₃), 326 (31) (M⁺ - C(CH₃)₃). Anal. (C₁₇H₂₉N₃O₅Si) C, H, N.

5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-3'-formamidouridine (8). To 1.50 g (4.01 mmol) of 6 dissolved in 10 mL of toluene were added 1.28 g (4.90 mmol) of TPP and 0.540 g of acetic formic anhydride¹² at 0 °C. Then the solution was heated for 4 h at 60 °C. After cooling (ice bath), crystalline 8 settled down in the reaction bottle. The yield of crystalline 8 was 0.75 g (50%). The filtrate was evaporated, and after flash chromatography 0.38 g (25%) of 8 was obtained: total yield 75%; mp 178–180 °C; TLC *R_f* (ether-acetone = 9:1) 0.08; ¹H NMR δ 0.11 (s, 6, Si(CH₃)₂), 0.87 (s, 9, SiC(CH₃)₃), 2.22 (ddd, 1, *J*_{2'a,2'b} = 13.0 Hz, *J*_{2'a,1'} = 7.5 Hz, *J*_{2'a,3'} = 7.5 Hz, 2'-Ha), 2.37 (ddd, 1, *J*_{2'b,1'} = 5.5 Hz, *J*_{2'b,3'} = 2.3 Hz, 2'-Hb), 3.90 (br s, 2, 5'-Ha, 5'-Hb), 4.10 (d, 1, *J*_{3,4'} = 2.0 Hz), 4.54 (m, 1, 3'-H), 5.75 (d, 1, *J*_{5,6} = 8.0 Hz, 5-H), 6.35 (dd, 1, 1'-H), 7.83 (br s, 1, NHCHO), 8.00 (d, 1, 6-H), 8.27 (s, 1, NHCHO), 10.46 (br s, 1, 3-H); ¹³C NMR δ -5.56, 18.29, 25.85, 38.12, 49.91, 64.05, 85.31, 87.09, 103.02, 140.02, 151.36, 161.90, 163.66; MS (210 °C) *m/e* 354 (2), 312 (95) (M⁺ - C(CH₃)₃). Anal. (C₁₆H₂₇N₃O₅Si) C, H, N.

5'-O-(tert-Butyldimethylsilyl)-3'-deoxy-3'-isocyanothymidine (9). To a solution of 0.716 g (1.87 mmol) of 7, dichloromethane (20 mL), and 0.707 mL (5.04 mmol) of diisopropylamine was added 0.192 mL (2.06 mmol) of phosphoryl

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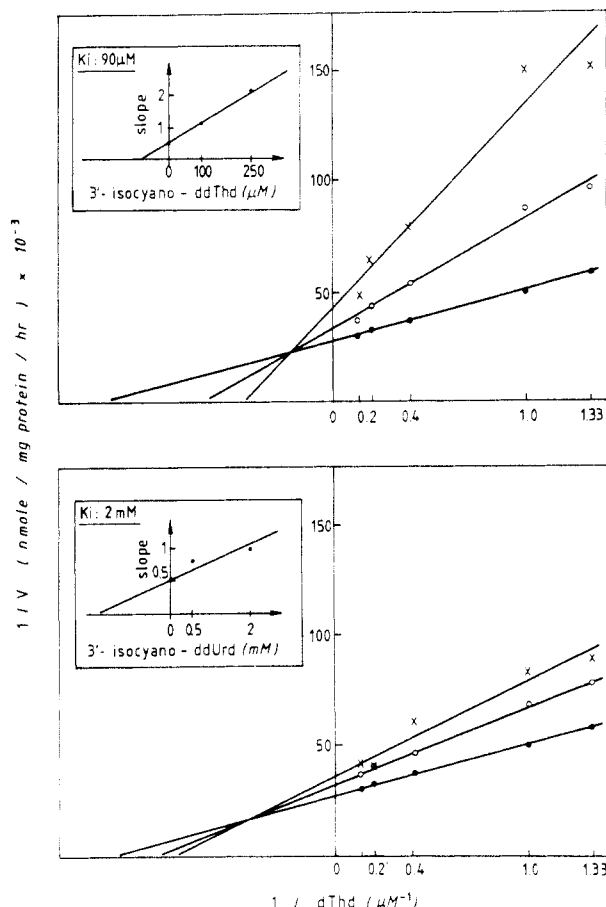


Figure 1. Lineweaver-Burk ($1/V$ versus $1/[S]$) and Dixon (slope/ $[I]$ versus $[I]$) plots for MT-4 cytosol thymidine kinase activity (with [*methyl-³H]dThd as the substrate) in the presence of 3'-isocyno-ddThd and 3'-isocyno-ddUrd. Concentrations of 3'-isocyno-ddThd were 0 μ M (\bullet), 100 μ M (\circ) and 250 μ M (\times); concentrations of 3'-isocyno-ddUrd were 0 μ M (\bullet), 500 μ M (\circ) and 2000 μ M (\times).*

chloride¹³ in 3 mL of CH_2Cl_2 dropwise under stirring at 0 °C. Stirring was continued for 1 h at 0 °C and overnight at room temperature. Then the reaction mixture was poured into a cooled solution of Na_2CO_3 (0.40 g) in 10 mL of H_2O . After stirring for 1 h more, CH_2Cl_2 was added and the organic layer was washed once with water, dried with Na_2SO_4 , and evaporated. The residue in ether-acetone = 9:1 (v/v) was applied to a flash column containing 25 g of silica gel. The column was eluted first with ether-acetone = 9:1 and 124 mg of crude **9** was obtained. More crude product (183 mg) could be eluted by treating the column several times with acetone. The combined fractions were purified by flash chromatography (25 g of silica gel, PE-EA = 1:1): yield of pure **9** 239 mg (35%); mp 149–151 °C; TLC R_f (ether-acetone = 9:1) 0.73; ^1H NMR δ 0.08 (s, 6, $\text{Si}(\text{CH}_3)_2$), 0.89 (s, 9, $\text{Si}(\text{CH}_3)_3$), 1.87 (s, 3, 5- CH_3), 2.31 (ddd, 1, $J_{2'a,2'b} = 13.3$ Hz, $J_{2'a,1'} = 6.1$ Hz, $J_{2'a,3'} = 6.1$ Hz, 2'-Ha), 2.63 (ddd, 1, $J_{2'b,1'} = 6.1$ Hz, $J_{2'b,3'} = 4.8$ Hz, 2'-Hb), 3.85 (ABX system, 2, $J_{5'a,5'b} = 13.3$ Hz, $J_{5'a,4'} = 1.9$ Hz, 5'-Ha, 5'-Hb), 4.14–4.27 (m, 2, 3'-H, 4'-H), 6.26 (t, 1, 1'-H), 7.29 (s, 1, 6-H), 8.90 (br s, 1, 3-H); ^{13}C NMR δ -5.58, -5.46, 12.40, 18.24, 25.79, 39.03, 51.13, 61.81, 84.41, 84.94, 111.27, 134.70, 150.21, 159.59, 163.78; MS (160 °C) m/e 308 (7) ($\text{M}^+ - \text{C}(\text{CH}_3)_3$), 281 (5); IR (CH_2Cl_2) 2140 cm^{-1} ($\text{N}=\text{C}$). Anal. ($\text{C}_{17}\text{H}_{27}\text{N}_3\text{O}_4\text{Si}$) C, H, N.

5'-O-(*tert*-Butyldimethylsilyl)-2',3'-dideoxy-3'-isocyno-uridine (10). Compound **10** was prepared in the same manner as **9**, with **8** as starting material: yield 47%; mp 106–108 °C; TLC R_f (ether-acetone = 9:1 (v/v)) 0.78; ^1H NMR δ 0.12 (s, 6, $\text{Si}(\text{CH}_3)_2$), 0.92 (s, 9, $\text{Si}(\text{CH}_3)_3$), 2.41 (ddd, 1, $J_{2'a,2'b} = 12.7$ Hz, $J_{2'a,1'} = 5.4$ Hz, $J_{2'a,3'} = 5.4$ Hz, 2'-Ha), 2.69 (ddd, 1, $J_{2'b,1'} = 5.4$ Hz, $J_{2'b,3'} = 5.4$ Hz, 2'-Hb), 3.92 (ABX system, 2, $J_{5'a,5'b} = 10.9$ Hz, $J_{5'a,4'} = 1.5$ Hz, 5'-Ha, 5'-Hb), 4.18–4.30 (m, 2, 3'-H, 4'-H), 5.68 (d, 1, $J_{5,6} = 7.3$ Hz, 5-H), 6.27 (t, 1, 1'-H), 7.76 (d, 1, 6-H), 9.07 (br s, 1, 3-H); ^{13}C NMR δ -5.64, -5.52, 18.28, 25.80, 39.67, 61.49, 84.84,

85.36, 102.50, 139.33, 150.03, 160.07, 163.04; MS (150 °C) m/e (%) 294 (8) ($\text{M}^+ - \text{C}(\text{CH}_3)_3$), 267 (5); IR (CH_2Cl_2) 2150 cm^{-1} ($\text{N}=\text{C}$). Anal. ($\text{C}_{16}\text{H}_{25}\text{N}_3\text{O}_4\text{Si}$) C, H, N.

3'-Isocyno-3'-deoxythymidine (11). To a solution of 531 mg (1.45 mmol) of **9** in dry THF (10 mL) was added 3.19 mL of a 0.5 M solution of TBAF in dry THF. After 30 min, TLC (CHCl_3 - CH_3OH = 5:1) indicated complete reaction. THF was removed in vacuo and the residue was chromatographed with CHCl_3 - MeOH as the solvent system: yield 332 mg (91%); mp 150–152 °C; TLC R_f (CHCl_3 - MeOH = 5:1) 0.58; ^1H NMR (acetone- d_6) δ 1.79 (d, 3, $J_{\text{CH}_3,6} = 1.0$ Hz, 5- CH_3), 2.67 (t, 2, $J_{2,1'} = J_{2,3'} = 6.5$ Hz, 2'-Ha, 2'-Hb), 3.87 (ABX system, 2, $J_{5'a,5'b} = 12.5$ Hz, $J_{5'a,4'} = 2.0$ Hz, $J_{5'b,4'} = 3.0$ Hz, 5'-Ha, 5'-Hb), 4.21 (m, 1, 4'-H), 4.56 (m, 2, $J_{3',4'} = 6.5$ Hz, 3'-H, 5'-OH), 6.31 (t, 1, 1'-H), 7.69 (d, 1, 6-H), 10.06 (br s, 1, 3-H); IR (KBr) 2147, 2138 cm^{-1} ($\text{N}=\text{C}$). Anal. ($\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_4$) C, H, N.

3'-Isocyno-2',3'-dideoxyuridine (12). To a solution of 509 mg (1.45 mmol) of **10** in dry THF (10 mL) was added 2.9 mL of a 0.5 M solution of TBAF in dry THF. After 20 min deblocking was finished. THF was removed in vacuo and the residue was chromatographed with MeOH - CHCl_3 = 1:30 as the solvent system: yield 88%; mp 179–182 °C; TLC R_f (CHCl_3 - MeOH = 5:1) 0.50; ^1H NMR (acetone- d_6) δ 2.68 (t, 2, $J_{2,1'} = J_{2,3'} = 6.5$ Hz, 2'-Ha, 2'-Hb), 3.87 (ABX system, 2, $J_{5'a,5'b} = 12.5$ Hz, $J_{5'a,4'} = 2.0$ Hz, $J_{5'b,4'} = 4.0$ Hz, 5'-Ha, 5'-Hb), 4.23 (m, 1, 4'-H), 4.54 (m, 2, $J_{3',4'} = 6.5$ Hz, 3'-H, 5'-OH), 5.60 (d, 1, $J_{5,6} = 8.0$ Hz), 6.29 (t, 1, 1'-H), 7.86 (d, 1, 6-H), 10.06 (br s, 1, 3-H); IR (KBr) 2146 cm^{-1} ($\text{N}=\text{C}$). Anal. ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_4$) C, H, N.

Antiviral Test Procedure. The HTLV-III_B strain of HIV-1 and the LAV-2 strain of HIV-2 were used throughout all experiments. The viruses were prepared from the culture supernatant of HTLV-III_B-infected MT-4 and LAV-2-infected CEM cells. SRV was prepared from the supernatants of SRV-infected Raji/0 cells. The antiviral assays were based on the protection of HIV-infected MT-4 cells against virus-induced cytopathogenicity^{19,20} or SRV-infected Raji cells against virus-induced giant-cell formation. They were run in parallel with the cytotoxicity assays aimed at establishing the toxicity of the compounds for uninfected MT-4 cells.

Enzyme Assay. Thymidine (dThd) kinase was prepared from exponentially growing MT-4 cells. The enzyme fractions precipitated between 30% and 70% $(\text{NH}_4)_2\text{SO}_4$ were used in our experiments and [*methyl-³H]dThd served as the radiolabeled substrate. The methods for preparing the enzyme extract and determining the enzymatic activity have been described previously.^{19,21}*

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