Synthesis of C-Nucleoside Analogues of 2′,3′-Dideoxycytidine, 3′-Azido-2′,3′-dideoxyuridine (CS-87), and 2′,3′-Dideoxy-2′,3′-didehydrocytidine

B. Doboszewski,† C. K. Chu,*,† and H. Van Halbeek‡

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy and Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602

Received December 18, 1987

C-Nucleoside analogues of 2′,3′-dideoxycytidine, 3′-azido-2′,3′-dideoxyuridine (CS-87), and 2′,3′-dideoxy-2′,3′-didehydrocytidine were synthesized from pseudouridine. The C-nucleoside analogue of 2′,3′-dideoxycytidine **4a** was prepared by stepwise deoxygenation of 1,3-dimethylpseudouridine (**11a**) followed by the ring transformation of the uracil to the isocytosine ring. Reduction of dimethylxanthate **11c** to 2′,3′-unsaturated derivative **13** followed by the ring transformation reaction afforded the C-nucleoside analogue of 2′,3′-dideoxy-2′,3′-didehydrocytidine (**5**). Finally, the C-nucleoside analogue of 3′-azido-2′,3′-dideoxyuridine (CS-87, **6**) was prepared from 2′-deoxypseudouridine (**22a**) as the intermediate, in which the 3′-OH was oxidized to a 3′-keto moiety followed by reduction to yield **25a**. Mesylation and displacement of the mesyl group of **25a** by the azide moiety followed by deblocking of the silyl protecting group yielded the desired compound **6**.

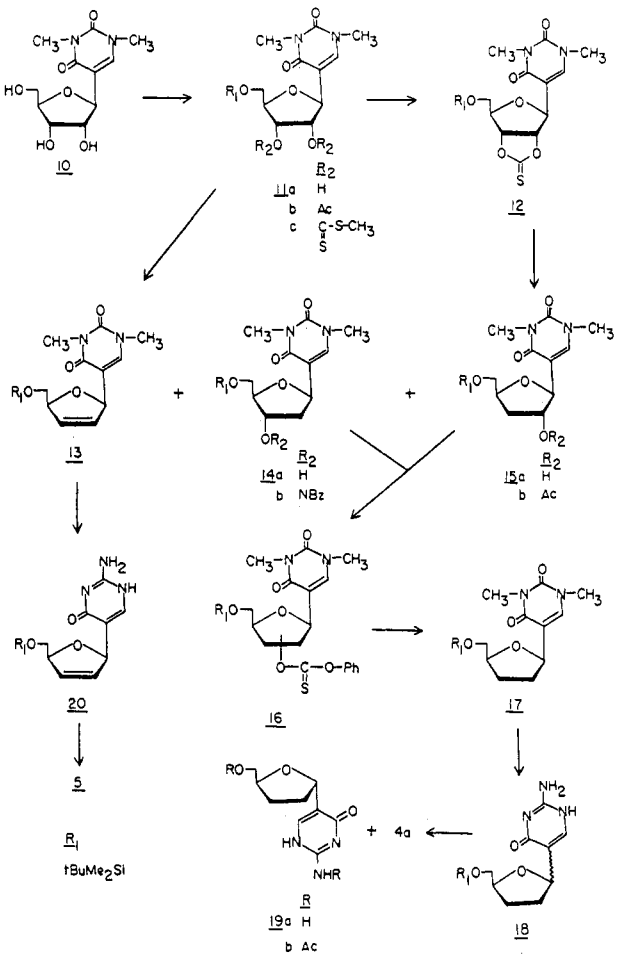
A number of nucleosides such as 3′-azido-3′-deoxythymidine (AZT),[1] 2′,3′-dideoxycytidine (**1**),[2] 2′,3′-dideoxyadenosine,[2] 2′,3′-dideoxy-2′,3′-didehydrocytidine (**2**),[3] ribavirin,[4] 3′-azido-2′,3′-dideoxy-5-ethyluridine (CS-85),[5] and 3′-azido-2′,3′-dideoxyuridine (CS-87, **3**)[6] (Figure 1) have been identified as antiviral agents for human immunodeficiency virus (HIV). At present AZT is the only one approved by the Food and Drug Administration for marketing. However, its bone marrow suppression limits the usefulness for AIDS patients and AIDS-related complex (ARC) individuals undergoing AZT therapy.[7] 2′,3′-Dideoxycytidine (**1**), which is currently undergoing clinical trials, is a potent anti-HIV agent in vitro and has been found to be less toxic than AZT.[2] 3′-Azido-2′,3′-dideoxyuridine (CS-87, **3**)[6] has also been found to be a potent antiviral agent against HIV with much less toxic effect on bone marrow cells than AZT. CS-87 is currently undergoing preclinical toxicology studies in our group.[8]

In this paper we report the syntheses of the C-nucleoside analogues **4a**, **5**, **6** of **1**, **2**, **3**, respectively, for anti-HIV screening. The rationale for this effort was that isosteric exchange of nitrogen with carbon can lead to biologically active compounds. For example, pseudoisocytidine (**7**), a C-nucleoside analogue of cytidine (**8**), and 5-azacytidine (**9**) (Figure 1), has exhibited an excellent antitumor activity[9] and has consequently undergone clinical trials.[10]

Results and Discussion

The synthetic approach to the nucleoside **4a** is outlined in Scheme I. The 5′-hydroxy group of dimethylpseudouridine (**10**)[11] was selectively protected by reacting **10** with *tert*-butylchlorodimethylsilane in the presence of imidazole[12] to give **11a** in 88% yield. Compound **11a** was characterized by ^{1}H NMR spectroscopy in its acetylated form **11b**. Anomerization, which is a general phenomenon during the synthesis of C-nucleosides,[13] was not observed during these reactions. Thiocarbonylation[14] of **11a** with 1,1′-thiocarbonyldiimidazole proceeded smoothly to give 81% of the desired product **12**. Reduction of **12** with tri-*n*-butyltin hydride[14] gave three compounds: **13**, **14a**, and **15a**, easily separable by column chromatography, in 1.4%, 45.6%, and 28.1% yields, respectively. This result was inconsistent with the previous findings,[15] where the yield of the 2′,3′-dideoxy-2′,3′-didehydro nucleoside was much higher (18%). There were no indications of anomerization during the reduction. The identification of **14a**

Scheme I



† College of Pharmacy.
‡ Complex Carbohydrate Research Center.

(1) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Broder, Nusinoff-Lehrman, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096.
(2) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911.
(3) Lin, T.-S.; Schinazi, R. F.; Chen, M. S.; Kinney-Thomas, E.; Prusoff, W. H. *Biochem. Pharmacol.* **1987**, *36*, 311.
(4) McCormick, J. B.; Getchell, J. P.; Mitchell, S. W.; Hicks, D. R. *Lancet* **1984**, ii, 1367.

0022-3263/88/1953-2777$01.50/0

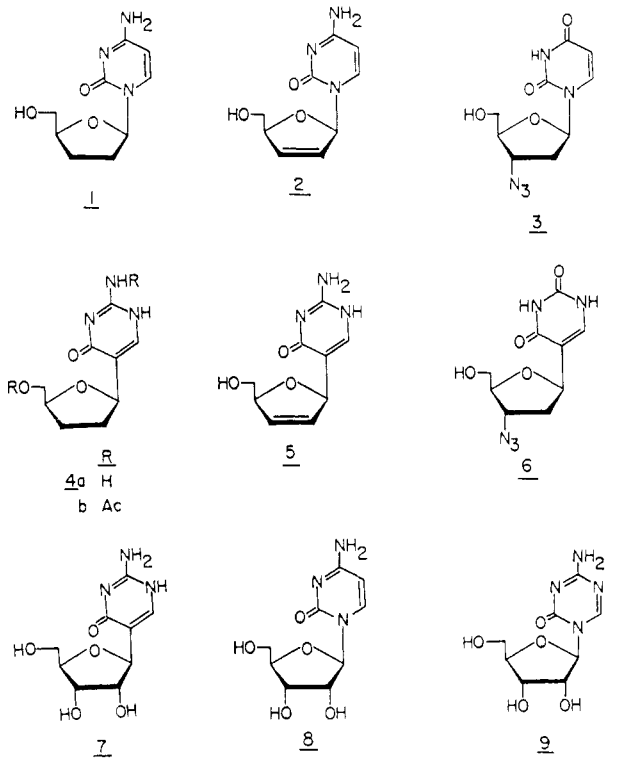


Figure 1.

and **15a** has been made on the basis of splitting patterns of anomeric protons in the ^{1}H NMR spectra. While **14a** exhibited a doublet of doublets due to two neighboring H-2′ protons, **15a** showed a broad singlet (one neighboring H-2′), as previously observed.[15]

Since the yield of the olefin **13** by the reduction of **12** was very low, alternative procedures were examined. Reduction of the diol **11a** via its dimethyl xanthate (**11c**), prepared from **11a**, carbon disulfide, and sodium hydroxide, followed by methylation with methyl iodide,[16] gave a significantly improved yield of **13** (76%). A direct method of generation of the olefin **13** was also explored, in which the diol **11a** was reacted with triphenylphosphine, iodoform, and imidazole.[17] However, this method was found to be inferior to the xanthate procedure.

Table I. 500-MHz ^{1}H NMR Characteristics[a] of Certain C-Nucleosides

	chemical shift, ppm			
proton	**4b**	**19b**	**5**	**6**
H-1′	4.755 (t)	4.802 (t)	6.068 (m)[c]	4.723 (dd)
H-2a′	2.239 (m)	2.262 (m)	6.214 (m)[c]	2.136 (m)
H-2b′	1.67 (m)[b]	1.71 (m)[b]		2.049 (m)
H-3a′	1.968 (m)	1.995 (m)	6.154 (m)[c]	4.209 (m)
H-3b′	1.65 (m)[b]	1.68 (m)[b]		
H-4′	4.112 (m)	4.319 (dq)	5.175 (m)[c]	3.756 (q)
H-5a′	4.150 (dd)	4.03 (dd)[b]	4.042 (dd)	3.48 (dd)[b]
H-5b′	4.059 (dd)	3.99 (dd)[b]	3.983 (dd)	3.45 (dd)[b]
H-6	7.729 (br s)	7.689 (br s)	8.15 (s)	7.400 (br s)
CH_3 (OAc)	2.129 (s)	2.129 (s)		
CH_3 (NAc)	2.025 (s)	2.025 (s)		

[a] Spectra were acquired at 27 °C, for DMSO-d_6 solutions of compounds **4b**, **19b**, and **6**, and for a pyridine-d_5 solution of compound **5**. Chemical shifts (δ) are downfield from tetramethylsilane; they were measured relative to DMSO-d_5 (δ 2.49) or pyridine-d_4 (δ 8.71 and 7.19), respectively, with an accuracy of ±0.002 ppm. [b] Due to higher order effects ($\Delta\nu \leq J$), chemical shifts could not be determined more accurately than ±0.01 ppm. [c] Multiplets due to long-range (four- and five-bond) couplings ($J_{1',3'}$, $J_{1',4'}$, $J_{1',6}$, $J_{2',4'}$), in addition to vicinal couplings ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'}$).

Table II. ^{1}H Coupling Constants (J) of Certain C-Nucleosides

	coupling constant			
J, Hz	**4b**	**19b**	**5**[b]	**6**[c]
1′,2a′	6.6	6.7	nd	6.1
1′,2b′	6.6	6.7		9.3
2a′,2b′	nd[d]	nd		−13.2
2a′,3a′	nd	nd	4.2[a]	3.0
2a′,3b′	nd	nd		
2b′,3a′	nd	nd		6.7
2b′,3b′	nd	nd		
3a′,3b′	nd	nd		
3a′,4′	nd	6.4	2.1[a]	4.0
3b′,4′	nd	6.4		
4′,5a′	3.4	4.0	4.0	4.0
4′,5b′	5.5	6.4	4.4	4.0
5a′,5b′	−10.7	−11.6	−11.5	nd

[a] Tentative assignment. [b] Compound **5** has only one proton attached to C-2′ (designated as H-2a′), and one proton attached to C-3′ (designated H-3a′). [c] Compound **6** has only one proton attached to C-3′ (designated as H-3a′). [d] nd J values could not be determined because of the complexity and higher order patterns of the multiplets.

In order to obtain the dideoxynucleoside (**17**), a mixture of **14a** and **15a** was converted into the 2′(3′)-phenoxythiocarbonyl derivative[18] **16**, which was reduced by tri-*n*-butyltin hydride to **17** in good yield (92%). The same compound **17** was also obtained directly from **13** in low yield (15%) via a diimide reduction[19] of the 2′,3′ carbon–carbon double bond. The dimethylpseudouridine (**17**) was then converted into 2′,3′-dideoxypseudoisocytidine (**18**) according to the known procedure.[11,20] ^{1}H NMR spectroscopy indicated that **18** was a mixture of β- and α-anomers in a 55:45 ratio. The anomers were unseparable under condition of flash chromatography.[21] The mixture **18** was then treated with tetra-*n*-butylammonium fluoride, yielding the free nucleosides **4a** and **19a**, easily separable by chromatography, in a ratio of 5:1. This β:α ratio was different from that for **18**, which indicated that the α-anomer might have been converted to the β-anomer under

(5) Schinazi, R. F.; Chu, C. K.; Feorino, P.; Sommadossi, J. P., 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, Sept 28–Oct 1, 1986.
(6) Schinazi, R. F.; Chu, C. K.; Ahn, M. K.; Sommadossi, J. P.; McClure, H. Abbott-UCLA Symposium—Human Retroviruses, Cancer and AIDS: Approaches to Prevention and Therapy, Keystone, CO, April 1–6, 1987.
(7) Richman, D. D.; Fischl, M. A.; Grieco, M. H. *N. Engl. J. Med.* **1987**, *137*, 192.
(8) National Cooperative Drug Discovery Group for AIDS, which was selected by the NIH in 1986 and is consisted of University of Georgia—Emory University—Yale University.
(9) Burchenal, J. H.; Ciovacco, K.; Kalaher, K.; O'Toole, T.; Kiefner, R.; Dowling, M. D.; Chu, C. K.; Watanabe, K. A.; Wempen, I.; Fox, J. *J. Cancer Res.* **1976**, *36*, 1520.
(10) Woodcock, T. M.; Chow, T.-C.; Tan, C. T.; Sternberg, S. S.; Philips, F. S.; Young, C. W.; Burchenal, J. H. *Cancer Res.* **1980**, *40*, 4234.
(11) Hirota, K.; Watanabe, K.; Fox, J. J. *J. Org. Chem.* **1978**, *43*, 1193.
(12) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799.
(13) Chu, C. K.; Watanabe, K. A.; Fox, J. J. *J. Heterocycl. Chem.* **1975**, *12*, 817.
(14) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *47*, 485.
(15) Matsuda, A.; Chu, C. K.; Reichman, U.; Pankiewicz, K.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1981**, *46*, 3603.
(16) (a) Barrett, A. G. M.; Barton, D. H. R.; Bielski, R. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2378. (b) Hayashi, T.; Iwaoka, T.; Takeda, N.; Ohki, E. *Chem. Pharm. Bull.* **1978**, *26*, 1786.
(17) Bessoles, M.; Abushanab, E.; Panzica, R. P. *J. Chem. Soc., Chem. Commun.* **1981**, 26.
(18) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932.
(19) Hoffman, J. M., Jr.; Schlessinger, R. H. *J. Chem. Soc., Chem. Commun.* **1971**, *12*, 45.
(20) Chu, C. K.; Reichman, U.; Watanabe, K. A.; Fox, J. J. *J. Heterocycl. Chem.* **1977**, *14*, 1119.
(21) Still, W. C.; Kahn, N.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

the condition of deprotection.

Compounds **4a** and **19a** were characterized by 500-MHz ^{1}H NMR spectroscopy in D_2O. The anomeric proton (H-1′) signals for **4a** and **19a** were observed at δ 4.825 (t; $J_{1',2a'} = J_{1',2b'} = 7.0$ Hz) and 4.925 (t; $J_{1',2a'} = J_{1',2b'} = 7.0$ Hz), respectively. On the basis of the difference in chemical shift of H-1′, we assigned the β-anomeric configuration to **4a** and the α-configuration to **19a**.[22] This assignment was supported by the downfield position of the H-4′ resonance for compound **19a** (δ 4.30) compared to **4a** (δ 4.1). Correct combustion analysis could be obtained only for crystalline *O,N*-diacetates **4b** and **19b**. The acetylated derivatives **4b** and **19b** were extensively investigated by NMR spectroscopy at 500 MHz in DMSO-d_6. All proton resonances were unambiguously assigned by 2D double-quantum-filtered, phase-sensitive shift correlation (COSY) spectroscopy. The assignments and coupling constants are listed in Tables I and II, respectively. They are in accordance with the aforementioned anomeric configurations.

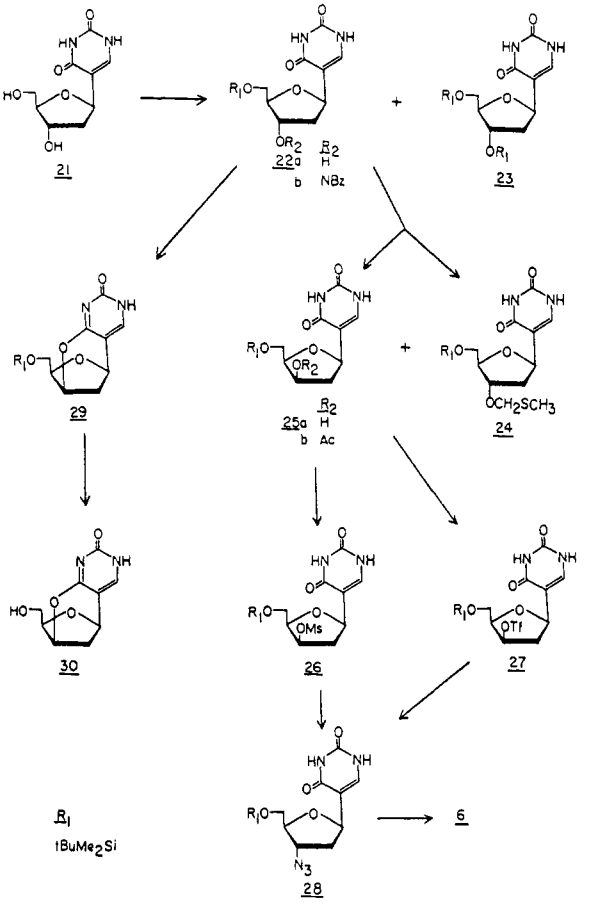
2′,3′-Dideoxy-2′,3′-didehydropseudoisocytidine (**5**) was prepared from **13** in a sequence involving ring transformation and 5′-de-O-silylation as described for **4a** and **19a**. This procedure gave the desired unsaturated nucleoside **5** in poor yield (ca. 8% based on **13**).

Compound **5** was characterized by 1D and 2D NMR spectroscopy at 500 MHz. The numerical value of the configurationally dependent coupling constant $J_{1',4'}$ could not be ascribed[23] due to the complexity of the coupling pattern of H_1' and H_4'. Nevertheless, we assigned the β-configuration to **5** since it is rather unlikely that total anomerization took place during the conversion of **13** into **5**. The ^{1}H NMR parameters for the compound **5** are included in Tables I and II.

3′-Azido-2′,3′-dideoxypseudouridine (**6**), a C-nucleoside analogue of 3′-azido-2′,3′-dideoxyuridine (CS-87, **3**),[6] was synthesized from 2′-deoxypseudouridine (**21**)[15,20] as shown in Scheme II. 5′-Silyl-protected compound **22a** was obtained according to the literature method[12] in good yield (71%) along with a di-O-silylated byproduct **23** (6.6%). Compound **22a** was mesylated and then treated with ethanolic sodium hydroxide to give a 4,3′-anhydro derivative **29**, which was reacted with lithium azide in DMF in order to open the anhydro ring. However, only the 5′-deblocked compound **30** was obtained from this reaction. Compound **30** thus prepared was identical with that obtained from treatment of **29** with tetra-*n*-butylammonium fluoride.

Therefore, alternate route for the synthesis of **6** was devised via 2′-deoxyxylopseudouridine derivative (**25a**). This compound was prepared by oxidation of **22a** with DMSO–Ac_2O followed by reduction with sodium borohydride, which is a known method of inverting configuration in carbohydrate[24] and nucleoside[25] chemistry. (Methylthio)methylene ether **24**, resulting from Pummerer rearrangement,[26] was isolated as a byproduct. Alcohol **25a** was either mesylated, yielding **26**, or triflated, yielding **27**.

Scheme II



Without characterization, **26** and **27** were reacted with an excess of lithium azide to give the desired product **28**. The procedure via mesylate gave a better yield (73%) than the procedure via triflate (41%). Final deblocking of the 5′-*O*-silyl group in **28** afforded the nucleoside **6**. Compound **6** was characterized by 1D and 2D NMR spectroscopy at 500 MHz in DMSO-d_6. The NMR data obtained for this compound are included in Tables I and II.

Compounds **4a**, **5**, and **6** were evaluated in phytohemagglutinin-stimulated human peripheral blood mononuclear cells infected with human immunodeficiency virus type-1 (LAV strain), but no significant antiviral activity was detected.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a JEOL FX 90Q (90 MHz) or Bruker AM-500 (500 MHz) for DMSO-d_6 solutions unless otherwise stated. Tetramethylsilane was the internal standard for organic solvents and sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) was the internal standard for deuterium oxide. Chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet). Signals of *tert*-butyldimethylsilyl group (ca. 0.81 ppm $(CH_3)_3CSi$, ca. 0 ppm $(CH_3)_2Si$), and of methyl groups protecting N-1(3) atoms (3.28–3.25 and 3.14–3.11 ppm) are characteristic for compounds described below. Ultraviolet spectra were recorded on a Bausch & Lomb Spectronic 2000 spectrometer. The TLC analysis was performed on Uniplates purchased from Analtech Co. or precoated TLC sheets (silica gel 60 F-254) by EM Laboratories, Inc. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

5′-*O*-(*tert*-Butyldimethylsilyl)-1,3-dimethylpseudouridine (11a). 1,3-Dimethylpseudouridine (**10**)[11] (7.82 g, 28.73 mmol) was dissolved in DMF (50 mL) and treated with imidazole (4.7 g, 69

(22) Chu, C. K.; El-Kabbani, F. M.; Thompson, B. B. *Nucleosides Nucleotides* **1984**, *3*, 1.

(23) (a) Lemieux, R. V.; Watanabe, K. A.; Pavia, A. A. *Can. J. Chem.* **1969**, *47*, 4413. (b) Hacksell, V.; Daves, G. D., Jr. *J. Org. Chem.* **1983**, *48*, 2870. (c) Cheng, J. C. Y.; Kacksell, V.; Daves, G. D., Jr. *Ibid.* **1986**, *51*, 3093. (d) Matsuda, A.; Chu, C. K.; Reichman, V.; Pankiewicz, K.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1981**, *46*, 3603.

(24) (a) Stevens, J. D. *Methods Carbohydr. Chem.* **1972**, VI, 123. (b) Miljkovic, N.; Sligarjevic, N.; Milkovic, D. *J. Org. Chem.* **1974**, *39*, 2118.

(25) (a) Wu, J. C.; Barin, M.; Chattopadhyaya, J. *Tetrahedron* **1987**, *43*, 2355. (b) Gosselin, G.; Bengogne, M. C.; DeRudder, J.; DeClerq, E.; Imbach, J. L. *J. Med. Chem.* **1987**, *30*, 982.

(26) (a) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165. (b) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.

mmol) and *tert*-butylchlorodimethylsilane (5.2 g, 34.5 mmol) with exclusion of moisture. After the mixture was stirred overnight, the solvent was evaporated and the residual oil was purified by chromatography ($CHCl_3$–MeOH, 200:1, then 100:1) to give **11a**, 9.75 g (88%), as a thick oil: ^1H NMR δ 7.51 (s, 1 H, H-6), 4.90 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6 Hz), 4.65 (d, 1 H, 2′(3′)-OH, J = 4.9 Hz), 4.50 (d, 1 H, 3′(2′)-OH, J = 3.3 Hz), 4.00–3.48 (unresolved, 5 H, remaining skeletal protons). Anal. Calcd for $C_{17}H_{30}N_2O_6Si$: C, 52.83; H, 7.83; N, 7.25. Found: C, 52.73; H, 7.84; N, 7.17.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-di-*O*-acetyl-1,3-dimethylpseudouridine (11b). The above compound **11a** was acetylated with a mixture of Ac_2O–pyridine and catalytic amount of 4-(dimethylamino)pyridine to give **11b** as an oil: ^1H NMR δ 7.67 (s, 1 H, H-6), 5.39–5.12 (m, 2 H, H-2′,3′), 4.68 (d, 1 H, H-1′, $J_{1',2'}$ = 4.8 Hz), 4.08–3.85 (m, 1 H, H-4′), 3.81–3.65 (m, 2 H, 2 H-5′), 1.97 (s, 6 H, 2 OAC). Anal. Calcd for $C_{21}H_{34}N_2O_8Si$: C, 53.59; H, 7.28; N, 5.95. Found: C, 53.48; H, 7.34; N, 5.89.

5′-*O*-(*tert*-Butyldimethylsilyl)-1,3-dimethyl-2′,3′-*O*-thiocarbonylpseudouridine (12). Diol **11a** (5.1 g, 13.2 mmol) in DMF (15 mL) was reacted with thiocarbonyldiimidazole (3.8 g, 19.2 mmol; 90% purity) overnight. The solvent was evaporated and the residue was taken up in $CHCl_3$ and washed twice with water. The organic layer was dried and evaporated. Addition of EtOH (2 mL) to the residual oil resulted in an immediate crystallization. Petroleum ether was added, and the crystals were filtered and washed with the same solvent to remove yellow color. **12**: yield 4.6 g (81.3%); mp 184.5–185 °C (from EtOH); ^1H NMR δ 7.78 (s, 1 H, H-6), 5.65–5.34 (m, 2 H, H-2′,3′), 4.88 (d, 1 H, H-1′, $J_{1',2'}$ = 2.8 Hz), 4.32–4.09 (m, 1 H, H-4′), 3.74 (d, 2 H, 2 H-5′, $J_{4',5'}$ = 5.9 Hz). Anal. Calcd for $C_{18}H_{28}N_2O_6SSi$: C, 50.44; H, 6.58; N, 6.54. Found: C, 50.51; H, 6.62; N, 6.52.

Reduction of 2′,3′-Thiocarbonyl Derivative 12 with Tri-*n*-butyltin Hydride. A solution of compound **12** (4.6 g, 10.7 mmol) in boiling toluene (50 mL) was treated with α,α-azobis-2-methylpropionitrile (1.1 g, 6.7 mmol) and tri-*n*-butyltin hydride (11 mL, 41 mmol) in toluene (80 mL) added dropwise during 45 min. Boiling was maintained for a total period of 4 h. TLC (hexanes–acetone, 3:1) showed the presence of three compounds: R_f 0.51 being 5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-1,3-dimethylpseudouridine (**13**), R_f 0.40 being 5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-1,3-dimethylpseudouridine (**15a**), and R_f 0.32 being 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-2′,3′-dimethylpseudouridine (**14a**). Toluene was evaporated and the residue was separated chromatographically with hexanes–acetone (3:1) to give 0.055 g (1.4%) of **13**, 1.12 g (28.1%) of **15a**, and 1.87 g (45.6%) of **14a**.

13: oil, ^1H NMR (benzene-d_6) δ 6.96 (d, 1 H, H-6, $J_{1',6}$ = 1.1 Hz), 6.26 (dq, 1 H, H-2′(3′), J = 1.5 and 2.1 Hz, $J_{2',3'}$ = 6.2 Hz), 5.94 (octet, 1 H, H-1′, $J_{1',6}$ = 1.4 Hz, $J_{1',2'}$, $J_{1',3'}$, $J_{1',4'}$ = 2.5, 2.7, 2.7 Hz), 5.52 (dq, 1 H, H-3′(2′), J = 1.4 and 2.3 Hz, $J_{2',3'}$ = 6.0 Hz), 4.83 (m, 1 H, H-4′), 3.61 (dd, 2 H, 2 H-5′, J = 2.8 and 4.6 Hz), 3.24 and 2.76 (s, 3 H each, NCH_3). Anal. Calcd for $C_{17}H_{28}N_2O_4Si$: C, 57.82; H, 8.01; N, 7.85. Found: C, 57.84; H, 8.04; N, 7.92.

15a: mp 74–75.5 °C (Et_2O–hexanes); ^1H NMR δ 7.41 (s, 1 H, H-6), 5.06 (d, 1 H, OH, J = 4.0 Hz), 4.48 (br s, 1 H, H-1′), 4.23–3.95 (m, 2 H, H-2′,4′), 3.69 (apparent d, 2 H, 2 H-5′, $J_{4',5'}$ = 4.1 Hz), 1.80–1.52 (m, 2 H, 2 H-3′). Anal. Calcd for $C_{17}H_{30}N_2O_5Si$: C, 55.11; H, 8.18; N, 7.56. Found: C, 55.02; H, 8.19; N, 7.54.

14a: oil; ^1H NMR δ 7.45 (s, 1 H, H-6), 4.99 (d, 1 H, OH, J = 3.8 Hz), 4.83 (dd, 1 H, H-1′, $J_{1',2'}$ = 10.0 and 6.2 Hz), 4.08 (br s, 1 H, H-3′), 3.79–3.45 (unresolved, 3 H, H-4′ and 2 H-5′), 2.05–1.48 (unresolved, 2 H, 2 H-2′). Anal. Calcd as for **15a**. Found: C, 54.64; H, 8.19; N, 7.37.

2′-*O*-Acetyl-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-1,3-dimethylpseudouridine (15b). Alcohol **15a** was acetylated with Ac_2O and pyridine: mp 90–91 °C (Et_2O–hexanes); ^1H NMR δ 7.54 (s, 1 H, H-6), 5.19–4.98 (m, 1 H, H-2′), 4.60 (apparent d, 1 H, H-1′, $J_{1',2'}$ = 1.9 Hz), 4.16–3.86 (m, 1 H, H-4′), 3.79–3.54 (three peaks, 2 H, 2 H-5′), 2.10–1.60 (m, 2 H, 2 H-3′), 2.00 (s, 3 H, OAc). Anal. Calcd for $C_{19}H_{32}N_2O_6Si$: C, 55.31; H, 7.82; N, 6.79. Found: C, 55.30; H, 7.83; N, 6.75.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-1,3-dimethyl-3′-*O*-(*p*-nitrobenzoyl)pseudouridine (14b). Compound **14a** was reacted with *p*-nitrobenzoyl chloride in pyridine: mp 103–104 °C (Et_2O–hexanes); ^1H NMR δ 8.43–8.08 (m, 4 H, aromatic), 7.55 (s, 1 H, H-6), 5.42 (apparent d, 1 H, H-3′, J = 4.1 Hz), 4.93 (dd, 1 H, H-1′, $J_{1',2'}$ = 9.0 and 4.6 Hz), 4.25–4.02 (unresolved, 1 H, H-4′), 3.88–3.59 (unresolved, 2 H, 2 H-5′), 2.56–1.91 (m, superimposed on solvent peak, 2 H-2′). Anal. Calcd for $C_{24}H_{33}N_3O_8Si$: C, 55.47; H, 6.40; N, 8.09. Found: C, 55.33;, H, 6.41; N, 8.04.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-dideoxy-2′,3′-didehydro-1,3-dimethylpseudouridine (13). Method A.[16] Diol **11a** (0.47 g 1.22 mmol) in DMSO (7 mL) and CS_2 (4 mL, 66 mmol) was cooled to ca. 15 °C. Aqueous sodium hydroxide (5 N, 4 mL) was added with continuous stirring. After 10 min CH_3I (4 mL, 64 mmol) was added. Reaction was completed after 40 min with formation of a less polar product (R_f 0.63; benzene–EtOAc, 4:1). The reaction mixture was transferred to a separatory funnel charged with water. Extraction was made with EtOAc. The organic layer was dried and evaporated. Dixanthate thus obtained was dissolved in toluene (20 mL), boiled to reflux, and treated with a solution of α,α'-azobis-2-methylpropionitrile (0.1 g, 0.61 mmol) and Bu_3SnH (1.5 mL, 5.6 mmol) in toluene (15 mL) dropwise. Boiling was maintained for 2 h. TLC showed a single product; R_f 0.33 (benzene–EtOAc, 4:1). After evaporation of toluene, chromatography with the same solvent system as for TLC gave 0.327 g (76.3%) of **13**.

Method B.[17] Diol **11a** (2.5 g, 6.5 mmol) in toluene (40 mL) was vigorously stirred with triphenylphosphine (6.8 g, 25.9 mmol), imidazole (0.88 g, 72.9 mmol), and iodoform (5.1 g, 72.9 mmol) at boiling point. After 40 min, the toluene layer was consecutively washed with aqueous solutions of sodium thiosulfate and sodium carbonate. After being washed with water, the toluene layer was dried and evaporated. TLC showed a product having R_f 0.35 (benzene–EtOAc, 4:1) identical with the compound described above. Chromatography with the same solvent system gave 0.91 g (39.9%) of olefin **13**.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-dideoxy-1,3-dimethylpseudouridine (17). Phenoxythiocarbonyl chloride (1.3 mL, 9.4 mmol) was added to a mixture of compounds **14a** and **15a** (2.43 g, 6.56 mmol) in CH_2Cl_2 (8 mL) and pyridine (2.5 mL) under nitrogen. After 6 h TLC showed formation of one compound (R_f 0.35 hexanes–acetone, 7:1) which is less polar than both substrates. The reaction mixture was taken up in $CHCl_3$ and washed with dilute HCl and water, dried, and evaporated to give a yellowish oil.[27] Esters **16** thus obtained were used in the next step without further purification. Conversion into **17** was done under conditions described above with *n*-Bu_3SnH (6 mL, 22.3 mmol), α,α-azobis-2-methylpropionitrile (0.6 g, 3.6 mmol) in 15 mL of toluene, substrate **16** in toluene (30 mL). The product (R_f 0.38, hexanes–acetone, 7:1) was isolated after usual workup and chromatography; yield 2.14 g (92%) of **17** as an oil; ^1H NMR δ 7.43 (s, 1 H, H-6), 4.63 (t, 1 H, H-1′, $J_{1',2'}$ = 6.7 and 6.2 Hz), 4.02–3.74 (m 1 H, H-4′), 3.62 (d, 2 H, 2 H-5′, $J_{4',5'}$ = 5.1 Hz), 2.31–1.40 (m, 4 H, 2 H-2′ and 2 H-3′). Anal. Calcd for $C_{17}H_{30}N_2O_4Si$: C, 57.59; H, 8.53; N, 7.90. Found: C, 57.34; H, 8.58; N, 7.82.

Conversion of Olefin 13 into 17 via Diimide Reduction of a Carbon–Carbon Double Bond.[19] A solution of sodium periodate (0.094 g, 0.44 mmol) in 1 mL of water was added dropwise to a mixture of **13** (0.062 g, 0.17 mmol) in DMSO (4 mL), 85% hydrazine (0.2 mL), one drop of saturated aqueous solution of copper sulfate, and one drop of glacial acetic acid. TLC showed a very slow formation of a more polar compound. After 2 h, additional sodium periodate (0.094 g) and hydrazine (0.2 mL) were added, and stirring was continued overnight. The reaction mixture was filtered and the filtrate was transferred to a separatory funnel charged with water and extracted with EtOAc. Organic layer was washed with water, dried ($MgSO_4$), and evaporated. Chromatography on a preparative TLC plate (benzene–EtOAc, 3:1) gave 0.0096 g (14.9%) of **17**.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-dideoxypseudoisocytidine (18). Compound **17** (2.1 g, 5.9 mmol) and guanidine [freshly prepared from 8.16 g (85.5 mmol) of guanidine hydrochloride and 1.85 g (80.4 mmol) of sodium in 75 mL of absolute EtOH] were heated under nitrogen at 92–95 °C for 1.5 h. The reaction mixture was passed through a short bed of Amberlite

(27) Chromatography on a preparative TLC plate gave an analytical sample. Calcd for $C_{24}H_{34}N_2O_6SSi$: C, 56.89; H, 6.76; N, 5.53. Found: C, 56.65; H, 6.80; N, 5.48.

IRC 50S with MeOH as solvent. Fractions containing the product (R_f 0.38, $CHCl_3$–MeOH, 15:1) were pooled together and evaporated. Chromatography on silica gel with the same solvent system gave 0.655 g (34%) of **18** as a mixture of β- and α-anomers (55:45). This material spontaneously crystallized; mp 189–192 °C (MeOH); ^1H NMR δ 10.93 (br s, 1 H, H-1), 7.54 and 7.40 (s, relative integration 55:45, H-6), 6.47 (br s, 2 H, NH_2), 4.78–4.50 (m, 1 H, H-1′), 4.18–3.67 (m, 1 H, H-4′), 3.57 and 3.45 (2 d, 2 H-5′, $J_{4',5'}$ = 6.4 Hz), 2.20–1.40 (m, 4 H, 2 H-2′ and 2 H-3′). Anal. Calcd for $C_{15}H_{27}N_3O_3Si·0.5H_2O$: C, 53.86; H, 8.44; N, 12.56. Found: C, 54.05; H, 8.15; N, 12.51.

2′,3′-Dideoxypseudoisocytidine (4a) and Its α-Anomer (19a). Compound **18** (0.167 g, 0.512 mmol) in THF (5 mL) was treated with tetra-*n*-butylammonium fluoride (1.75 mL, 1 mol solution) for 2 h. TLC ($CHCl_3$–MeOH–H_2O, 62:10:1) showed two spots: R_f 0.39 (major) and R_f 0.29. Solvent was evaporated and the yellow oil was chromatographically separated with the above solvent system to give 0.041 g of a less polar compound (β-anomer) (**4a**) and 0.008 g of a more polar one (α-anomer) (**19a**). Total yield: 45.2%.

4a: ^1H NMR (500 MHz, D_2O) δ 7.655 (s, 1 H, H-6), 4.825 (t, 1 H, H-1′, $J_{1',2a'} = J_{1',2b'}$ = 7.0 Hz), 4.100 (m, 1 H, H-4′), 3.666 (m, 2 H, 2 H-5′, $J_{4',5a'}$ = 3.7 Hz, $J_{4',5b'}$ = 5.6 Hz, J_{AB} = 12.0 and 56.3 Hz), 2.263–2.199 (m, 1 H), 2.094–2.027 (m, 1 H), 1.867–1.617 (m, 2 H, H-2a′,2b′,3a′,3b′); UV (MeOH) λ_{max} (pH 6.5) 288.3 nm (ε 5980), (pH 1.2) 260.3 (5780), (pH 12.4) 276.1 (5230).

19a: ^1H NMR (500 MHz, D_2O) δ 7.651 (s, 1 H, H-6), 4.925 (t, 1 H, H-1′, $J_{1',2a'} = J_{1',2b'}$ = 7.0 Hz), 4.30 (m, 1 H, H-4′, $J_{4',5a'}$ = 3.8 Hz, $J_{4',5b'}$ = 7.1 Hz, $J_{4',3a'}$ = 13.2 Hz), 3.615 (m, 2 H, 2 H-5′, J_{AB} = 11.9 and 36.2 Hz), 2.310–2.248 (m, 1 H), 2.124–2.061 (m, 1 H), 1.942–1.879 (m, 1 H), 1.823–1.751 (m, 1 H, H-2a′,2b′,3a′,3b′); UV (MeOH) λ_{max} (pH 6.5) 288.7 nm (6590), (pH 1.4) 260.3 (6540), (pH 12.3) 276.7 (5850).

Compounds **4a** and **19a** were acetylated with Ac_2O–pyridine overnight at 4 °C to give **4b** and **19b**, respectively. **4b**: mp 203–204 °C (methanol). Anal. Calcd for $C_{13}H_{17}N_3O_5$: C, 52.88; H, 5.80; N, 14.23. Found: C, 52.62; H, 5.87; N, 14.15. **19b**: mp 194–200 °C (methanol). Anal. Found: C, 52.66; H, 5.85; N, 14.16. For ^1H NMR data, see Tables I and II.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-didehydro-2′,3′-dideoxypseudoisocytidine (20). Dideoxydidehydronucleoside (**13**) (0.20 g, 0.57 mmol) was heated at 96 °C with guanidine [freshly made from 5.85 g (61.2 mmol) of guanidine hydrochloride and 1.3 g (56 mmol) of sodium in 75 mL of absolute EtOH] for 45 min. TLC ($CHCl_3$–MeOH, 15:1) showed the presence of three spots: not charring compound with blue fluorescence (R_f 0.51), the main product (R_f 0.44), and the compound that chars very poorly (R_f 0.36). The reaction mixture was applied on a silica gel column and eluted with $CHCl_3$–MeOH (15:1). Fractions containing the product (contaminated with aforementioned compounds) were pooled together, evaporated, and rechromatographed to give 0.050 g (ca. 27%) of **20**, still contaminated with a less polar byproduct. After two crystallizations from MeOH–Et_2O, an analytical sample was obtained: mp 197 °C dec; ^1H NMR δ 10.95 (br s, 1 H, H-1), 7.40 (s, 1 H, H-6), 6.65 (br s, 2 H, NH_2), 6.01–5.78 (unresolved, 2 H, H-2′,3′), 5.60 (d, 1 H, H-1′, $J_{1',2'}$ = 3.6 Hz), 4.90–4.61 (m, 1 H, H-4′), 3.60 (d, 2 H, 2 H-5′, $J_{4',5'}$ = 4.4 Hz). Anal. Calcd for $C_{15}H_{25}N_3O_3Si$: C, 55.69; H, 7.79; N, 12.99. Found: C, 55.73; H, 7.81; N, 12.97.

2′,3′-Didehydro-2′,3′-dideoxypseudoisocytidine (5). Deprotection of **20** (0.090 g of crude product, ca. 0.28 mmol) was accomplished by reaction with tetra-*n*-butylammonium fluoride (1 mL of 1 M solution) in THF (6 mL) for 1.5 h. The solvent was evaporated. The product having R_f 0.38 ($CHCl_3$–MeOH–H_2O, 44:10:1) was isolated chromatographically. After crystallization from MeOH, 0.017 g (29%) of analytically pure compound **5** was obtained, which does not give a sharp melting point; decomposition started at ca. 197 °C: ^1H NMR, see Tables I and II; UV (H_2O–MeOH, 1:1) λ_{max} (pH 5.7) 289.5 nm (ε 6800), (pH 1.4) 259.5 (7700), (pH 12.1) 276.5 (7000). Anal. Calcd for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.29; N, 20.08. Found: C, 51.79; H, 5.34; N, 20.04.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxypseudouridine (22a) and 3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxypseudouridine (23). 2′-Deoxypseudouridine[15,20] (**21**) (0.428 g, 1.87 mmol), *tert*-butylchlorodimethylsilane (0.34 g, 2.25 mol), and imidazole (0.31 g, 4.5 mmol) in 15 mL of DMF were stirred overnight. TLC (hexanes–acetone, 10:12) showed two products: di-*O*-silyl derivative **23** (R_f 0.63) and the main compound **22a** (R_f 0.37). The solvent was evaporated and the residual oil was chromatographed to give 0.057 g (6.6%) of **23** and 0.456 g (71%) of **22a**. **22a**: mp 194 °C softening, 208 °C dec (EtOH–EtOAc); ^1H NMR δ 11.00 (s, 1 H, H-3), 10.73 (d, 1 H, H-1, $J_{1,6}$ = 5.1 Hz), 7.17 (d, 1 H, H-6), 4.94 (d, 1 H, OH, J = 3.8 Hz), 4.76 (dd, 1 H, H-1′, $J_{1',2'}$ = 10.0 and 6.1 Hz), 4.06 (br s, 1 H, H-3′), 3.79–3.33 (unresolved, 3 H, H-4′ and 2 H-5′), 2.18–1.34 (m, 2 H, 2 H-2′). Anal. Calcd for $C_{15}H_{26}N_2O_5Si$: C, 52.61; H, 7.65; N, 8.18. Found: C, 52.50; H, 7.68; N, 8.09.

23: mp 193–197 °C (EtOH–hexanes); ^1H NMR δ 11.00 and 10.73 (s, 1 H each, H-1 and H-3), 7.18 (s, 1 H, H-6), 4.72 (dd, 1 H, H-1′, $J_{1',2'}$ = 8.9 and 5.8 Hz), 4.23 (br s, 1 H, H-3′), 3.77–3.34 (unresolved, 3 H, H-4′ and 2 H-5′), 2.08–1.69 (m, 2 H, 2 H-2′). Anal. Calcd for $C_{21}H_{40}N_2O_5Si$: C, 55.20; H, 8.76; N, 6.14. Found: C, 55.13; H, 8.85; N, 6.10.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′-*O*-(*p*-nitrobenzoyl)pseudouridine (22b). Compound **22a** was reacted with *p*-nitrobenzoyl chloride in pyridine to give **22b**: mp 227–229 °C ($CHCl_3$–EtOH); ^1H NMR δ 8.48–8.03 (m, 4 H, aromatic), 7.24 (s, 1 H, H-6), 5.38 (d, 1 H, H-3′, $J_{3',2'}$ = 5.6 Hz), 4.86 (dd, 1 H, H-1′, $J_{1',2'}$ = 10.0 and 5.1 Hz), 4.07 (irregular s, 1 H, H-4′), 3.71 (d, 2 H, 2 H-5′, $J_{4',5'}$ = 4.1 Hz), 2.49–1.85 (m, superimposed on solvent peak, 2 H-2′), signals of the H-1 and H-3 protons are not observed due to the presence of water. Anal. Calcd for $C_{22}H_{29}N_3O_8Si$: C, 53.75; H, 5.95; N, 8.55. Found: C, 53.76; H, 5.92; N, 8.52.

4,3′-Anhydro-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxypseudouridine (29). Compound **22a** (0.546 g, 1.59 mmol) was dissolved in pyridine (5 mL) and methanesulfonyl chloride (0.16 mL, 2.07 mmol). The mixture was then left in a refrigerator overnight. MeOH (1 mL) was added to destroy unreacted methanesulfonyl chloride. After 1 h the mixture was taken up in $CHCl_3$ and washed with water. The organic layer was dried ($MgSO_4$) and evaporated to give 3′-mesylated compound as a yellowish oil (0.62 g), which was not further characterized. The above compound (0.38 g, 0.9 mmol) was dissolved in EtOH (10 mL) and heated under reflux with dropwise addition of 3 mL of 1 N sodium hydroxide in ethanol–water (4:1) during 4 h. TLC showed a slow formation of a more polar product. Boiling was maintained for 15 h, and then the solvent was evaporated. Silica gel chromatography ($CHCl_3$–MeOH, 14:1) gave 0.223 g (76%) of the title compound; mp 204–205 °C (EtOH–hexanes); ^1H NMR δ 11.17 (br s, 1 H, H-1), 7.62 (s, 1 H, H-6), 5.05 (d, 1 H, H-3′, J = 2.4 Hz), 4.82 (apparent d, 1 H, H-1′, $J_{1',2'}$ = 4.1 Hz), 4.00 (dt, 1 H, H-4′, $J_{4',5'}$ = 6.3 Hz, $J_{4',3'}$ = 3.0 Hz), 3.51 (d, 2 H, 2 H-5′), 2.25–1.94 (m, 2 H, 2 H-2′). Anal. Calcd for $C_{15}H_{24}N_2O_4Si$: C, 55.53; H, 7.45; N, 8.63. Found: C, 55.59; H, 7.49; N, 8.59.

Attempted Opening of the 4,3′-Anhydro Ring in 29 with Azide Ions: Synthesis of 4,3′-Anhydro-2′-deoxypseudouridine (30). Compound **29** (0.103 g, 0.317 mmol) in DMF (5 mL) was stirred with lithium azide (0.3 g, 6.1 mmol) at 98–105 °C during 8 h, after which TLC indicated the starting material was no longer present. DMF was evaporated and the product was isolated chromatographically ($CHCl_3$–MeOH, 3:1) to give 0.042 g (63%) of 5′-*O*-desilylated compound **30**. Compound **30** shows no sharp melting point; decomposition started at 192 °C after crystallization from ethanol; ^1H NMR 7.68 (s, 1 H, H-6), 5.13 (dd, 1 H, H-3′, $J_{3',4'}$ = 2.6 Hz, $J_{3',2'}$ = 0.6 Hz), 4.87 (d, 1 H, H-1′, $J_{1',2'}$ = 3.8 Hz), 4.04 (dt, 1 H, H-4′, $J_{4',5'}$ = 6.4 Hz), 3.40 (d, 2 H, 2 H-5′), 2.30–2.01 (m, 2 H-2′), signals of H-1 and 5′-OH are not seen due to the presence of water in the sample. Anal. Calcd for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.79; N, 13.33. Found: C, 51.31; H, 4.84; N, 13.16.

Deprotection of 5′-*O*-(*tert*-Butyldimethylsilyl) Derivative 29 with Fluoride Ions. Compound **29** (0.020 g, 0.06 mmol) in THF (2 mL) was treated with 9 drops of Bu_4NF (1 mol in THF). After 1 h, the solution was applied on a preparative TLC plate and developed with $CHCl_3$–MeOH (3:1) to yield 0.009 g (69%) of **30**.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-D-threopseudouridine (25a) and 5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′-*O*-[(methylthio)methylene]pseudouridine (24). Alcohol **22a** (0.577 g, 1.68 mmol) was dissolved in DMSO (12 mL) and Ac_2O (3 mL). After overnight reaction, the starting material reacted completely (TLC, $CHCl_3$–MeOH, 15:1) with formation

of a new product migrating with the solvent front. The reaction mixture was transferred to a separatory funnel. Water was added and exhaustive extraction was performed with EtOAc. Combined organic layers were evaporated. The resulting yellow oil was dissolved in MeOH (25 mL), and after cooling in ice-cooled water, sodium borohydride (0.4 g, 10.5 mmol) was added portionwise during 20 min. Three compounds were present in the reaction mixture as seen on a TLC plate ($CHCl_3$–MeOH, 15:1): compound **24** (R_f 0.58), compound **25a** (R_f 0.37), and compound **22a** (R_f 0.28). Chromatography ($CHCl_3$–MeOH, 15:1) gave 0.206 g (30%) of **24**, 0.215 g (37%) of **25a**, and 0.048 g of **22a**.

25a: mp 249–251 °C (EtOH–hexanes); ^{1}H NMR δ 10.92 (br s, 2 H, H-1 and H-3), 7.28 (s, 1 H, H-6), 4.85 (br s, 1 H, OH), 4.57 (dd, 1 H, H-1′, $J_{1',2a'}$ = 5.1 Hz, $J_{1',2b'}$ = 9.2 Hz), 4.12 (br s, 1 H, H-3′ (4′)), 3.92–3.48 (unresolved, 3 H, H-4′(3′) and 2 H-5′), 2.57–2.15 (superimposed on solvent peak, 1 H, H-2b′), 1.60 (ddd, 1 H, H-2a′, $J_{2a',2}$ = −13.7 Hz, $J_{2',3'}$ = 1.7 Hz). Anal. Calcd for $C_{15}H_{26}N_2O_5Si$: C, 52.61; H, 7.65; N, 8.18. Found: C, 52.52; H, 7.67; N, 8.11.

24: mp 203–204 °C (EtOH); ^{1}H NMR δ 11.03 and 10.76 (s, 1 H, H-1 and H-3), 7.20 (s, 1 H, H-6), 4.67 (dd, superimposed on the signal of OCH_2SCH_3, 1 H, H-1′, $J_{1',2'}$ = 10.7 and 5.2 Hz), 4.62 (s, 2 H, OCH_2SCH_3), 4.75 (d, 1 H, H-3′(4′), J = 4.9 Hz), 3.92–3.68 (m, 1 H, H-4′(3′)), 3.52–3.44 (m, 2 H, 2 H-5′), 2.31–1.96 (m, 2 H, 2 H-2′), 2.41 (s, 3 H), SMe).

Anal. Calcd for $C_{17}H_{30}N_2O_5SSi$: C, 50.72; H, 7.51; N, 6.96. Found: C, 50.82; H, 7.53; N, 6.95.

3′-*O*-Acetyl-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-D-threopseudouridine (25b). Compound **25a** was acetylated with Ac_2O–pyridine (2:1) to give **25b**: mp 228–230 °C (EtOH–hexanes); ^{1}H NMR δ 11.06 and 10.72 (br s, 1 H each, H-1 and H-3), 7.29 (s, 1 H, H-6), 5.33–5.10 (m, 1 H, H-3′), 4.67 (t, 1 H, H-1′, $J_{1',2'}$ = 6.5 Hz), 4.02–3.59 (m, 3 H, H-4′ and 2 H-5′), 2.77–2.38 (superimposed on solvent peak, 1 H, H-2a′), 1.68 (ddd, 1 H, H-2b′, $J_{2a',2b'}$ = −13.9 Hz, $J_{2',3'}$ = 2.7 Hz). Anal. Calcd for $C_{17}H_{28}N_2O_6Si$: C, 53.10; H, 7.34; N, 7.28. Found: C, 52.96; H, 7.40; N, 7.20.

3′-Azido-5′-(*tert*-butyldimethylsilyl)-2′,3′-dideoxypseudouridine (28). Method A. Via 3′-*O*-Triflate 27. Compound **25a** (0.0541 g, 0.16 mmol) in CH_2Cl_2 (5 mL) and pyridine (0.3 mL) was cooled in an ice–salt bath. To this solution was added triflic anhydride (0.08 mL, 0.47 mmol), and the cooling bath was removed. After 0.5 h, the reaction mixture was diluted with $CHCl_3$, transferred to a separatory funnel, and washed with ice-cold dilute HCl. The organic layer was washed with water, dried (Na_2SO_4), and evaporated. The brownish residue became crystalline during final drying on an oil pump. The resulting triflate was dissolved in DMF (4.5 mL) and heated at 50 °C with lithium azide (0.0422 g, 0.86 mmol). After 20 min, TLC shows that triflate **27** had reacted with formation of four compounds having R_f values of 0.59, 0.49, 0.37, and 0.28 (in hexanes–acetone, 12:10). The solvent was removed under vacuum and the least polar product was isolated on a preparative TLC plate to give 0.0236 g (40.6%) of **28**. Three remaining byproducts were not isolated.

26: mp 210 °C dec, crystallized from acetone–hexanes; ^{1}H NMR (500 MHz) δ 7.259 (d, 1 H, H-6, $J_{6,1'}$ = 1.1 Hz), 4.729 (dd, 1 H, H-1′, $J_{1',2a'}$ = 6.0 Hz, $J_{1,2b'}$ = 9.2 Hz), 4.192 (quintet, 1 H, H-3′, $J_{3',2a'}$ = 3.2 Hz, $J_{3',4'}$ = 3.2 Hz, $J_{3',2b'}$ = 6.6 Hz), 3.81 (m, 1 H, H-4′), 3.64 (m, 2 H, 2 H-5′, $J_{5a',4'}$ = 4.2 Hz, $J_{5b',4'}$ = 5.5 Hz, J_{AB} = 10.8 and 25.6 Hz), 2.035 (m, 2 H, 2 H-2′, $J_{2a',3'}$ = 2.9 Hz, $J_{2a',1'}$ = 6.0 Hz, $J_{2b',1'}$ = 9.3 Hz, $J_{2b',3'}$ = 6.7 Hz, J_{AB} = 13.2 and 60.5 Hz), 0.854 (s, 9 H, *t*-$BuMe_2Si$). Anal. Calcd for $C_{15}H_{25}N_5O_4Si$: C, 49.03; H, 6.86; N, 19.06. Found: C, 49.00; H, 6.89; N, 19.01.

Method B. Via Mesylated Compound 26. Mesyl chloride (0.3 mL, 3.87 mmol) was added to a solution of a compound **25a** (0.0573 g, 0.17 mmol) in CH_2Cl_2 (5 mL) and pyridine (0.75 mL). After 4 h, MeOH (1 mL) was added. Thirty minutes later, the reaction mixture was worked up as for triflate **27**. Displacement with LiN_3 (0.042 g, 0.86 mmol) in DMF (4 mL) at 65 °C was accomplished during an overnight reaction. TLC showed a single product being less polar than **26**. Preparative TLC ($CHCl_3$–MeOH, 15:1) gave 0.045 g (73.2%) of **28**.

3′-Azido-2′,3′-dideoxypseudouridine (6). 5′-*O*-Silyl derivative **28** (0.063 g, 0.17 mmol) in THF (4 mL) was deprotected with Bu_4NF (1.5 mL, 1 M) in THF. After 30 min, TLC showed a single product having R_f 0.43 ($CHCl_3$–MeOH–H_2O, 120:15:1). The solvent was evaporated and the residue was passed through a short bed of Amberlite 45–pyridinium form. Elution was done with pyridine–methanol–water (3:1:1). UV-active fractions were pooled together and evaporated. The product spontaneously crystallized. After recrystallization from MeOH–H_2O, 0.026 g (60%) of **6** was obtained: mp 236 °C dec; ^{1}H NMR, see Tables I and II; UV (MeOH–H_2O, 1:1) λ_{max} (pH 5.6) 261.3 nm (ϵ 8750), (pH 1.4) 260.9 (8130), (pH 12.3) 285.7 (7430). Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.66. Found: C, 42.78; H, 4.39; N, 27.61.

Acknowledgment. This research was supported by the U.S. Public Health Service Grants AI 26055 and AI 25899 from the National Institute of Allergy and Infectious Diseases. We are grateful to Dr. Raymond F. Schinazi of Emory University/VA Hospital for HIV screening. We also express our appreciation to Kyowa Hakko Co., Tokyo, Japan, for a generous gift of pseudouridine.

Isolation and Identification of Eight New Polyhydroxylated Sterols from the Sponge *Dysidea etheria*[1]

Robert R. West and John H. Cardellina, II*

Natural Products Laboratory, Department of Chemistry, Montana State University, Bozeman, Montana 59717

Received December 8, 1987

The isolation and characterization of eight new polyhydroxylated sterols from the Bermudian sponge *Dysidea etheria* are reported. Details of the isolation procedure, structure elucidation, and biological testing of the compounds are presented. These compounds, one of which has been shown to be cytotoxic, are interrelated in that they share a common 5α-cholest-7-ene-2α,3β,5α,6β,9α,11α,19-heptol framework.

Many new sterols have been isolated from marine organisms in the last 2 decades.[2-4] Most of these compounds have been mono- or dihydroxylated, but an increasing number have been found with multiple oxygen functionalities. Some authors have postulated that future studies

(1) Contribution no. 1122 from the Bermuda Biological Station. Portions of this work have been presented at the 193rd National Meeting of the American Chemical Society, Denver, April, 1987 and the 28th Annual Meeting of the American Society of Pharmacognosy, Kingston, July, 1987. Taken, in part, from the Ph.D. Disseration of R. R. West, Montana State University, 1988.

(2) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 155; **1986**, *3*, 1.

(3) Schmitz, F. J. Marine Natural Products; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, Chapter 5.

(4) *Handbook of Marine Science: Compounds from Marine Organisms*; Baker, J. T., Murphy, V., Eds.; CRC Press: Cleveland, 1976; Vol. I.

0022-3263/88/1953-2782$01.50/0