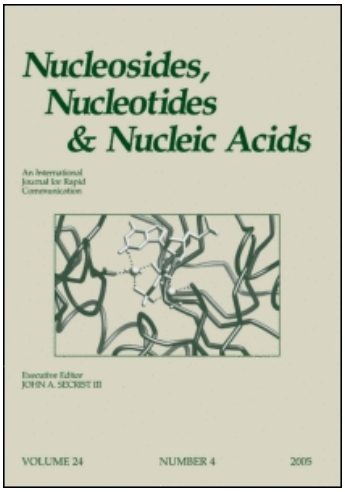


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthetic, Structural, and Conformational Studies of Methylated Ring-Expanded Nucleosides Containing the Ihidazo[4, 5-e][1, 4]Diazepine Ring System

Ramachandra S. Hosmane[a]; Anila Bhan[a]; Martin Hulce[a]; Hongming Zhang[b]; Narayan S. Hosmane[b]
[a] Laboratory for Chemical Dynamics Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland [b] Department of Chemistry, Southern Methodist University, Dallas, Texas

To cite this Article Hosmane, Ramachandra S. , Bhan, Anila , Hulce, Martin , Zhang, Hongming and Hosmane, Narayan S.(1991) 'Synthetic, Structural, and Conformational Studies of Methylated Ring-Expanded Nucleosides Containing the Ihidazo[4, 5-e][1, 4]Diazepine Ring System', Nucleosides, Nucleotides and Nucleic Acids, 10: 4, 819 — 836
To link to this Article: DOI: 10.1080/07328319108046664
URL: http://dx.doi.org/10.1080/07328319108046664

NUCLEOSIDES & NUCLEOTIDES, 10(4), 819-836 (1991)

SYNTHETIC, STRUCTURAL, AND CONFORMATIONAL STUDIES OF METHYLATED RING-EXPANDED NUCLEOSIDES CONTAINING THE IMIDAZO[4,5-e][1,4]DIAZEPINE RING SYSTEM

Ramachandra S. Hosmane,* Anila Bhan, and Martin Hulce

Laboratory for Chemical Dynamics
Department of Chemistry and Biochemistry
University of Maryland Baltimore County
Baltimore, Maryland 21228

and

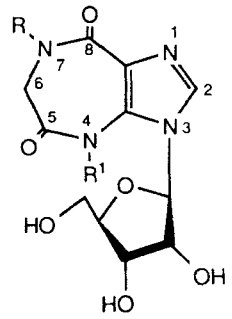
Hongming Zhang and Narayan S. Hosmane

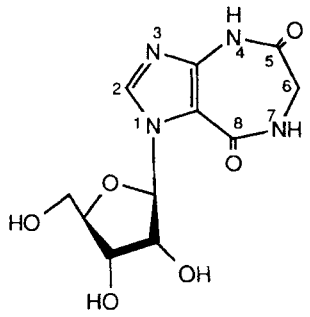
Department of Chemistry, Southern Methodist University
Dallas, Texas 75275

ABSTRACT: Syntheses of 4- and 7-methyl 4,5,7,8-tetrahydro-6H-3-(β-D-ribofuranosyl)imidazo[4,5-e][1,4]diazepine-5,8-dione, **3** and **4**, respectively, are reported. Single-crystal X-ray diffraction analysis of the aglycon of **3** aided in confirming the site of methylation in **3**, and that of **4** in elucidating the solid state conformation of **4**. Solution conformations of **3** and **4**, along with their parent nucleoside **1** and the latter's 1-glycosyl regioisomer **2**, were investigated by NOE and CD measurements.

We have recently described the synthesis[1] and conformational studies[2,3] of two novel regioisomeric ring-expanded nucleosides (**1** and **2**), their 5'-mono- and -diphosphate derivatives,[2] and homopolymers derived enzymatically from the diphosphates.[3] While the base-ribose conformational relationship of **1** in both solid state (X-ray) and solution (CD, NMR) is found to be *syn*, that of **2** is *anti* in both states. Likewise, the sugar pucker in **1**, C2'-*endo*-C3'-*exo* in solid state, is reversed in **2**. Other contrasting salient features[3] of **1** and **2** are their respective ease of enzymic polymerization and the subsequent helical structure, stability, and conformation of the

resultant homopolymers. The polymerization process is more facile with **2** than **1**, and the homopolymer derived from **2** exhibits considerable secondary structure absent in that derived from **1**. The syn conformation of **1** is apparently due in part to the strong intramolecular hydrogen bonding between the 5'-OH group and the N^4-H of the diazepine moiety.





1; R = R^1 = H

2

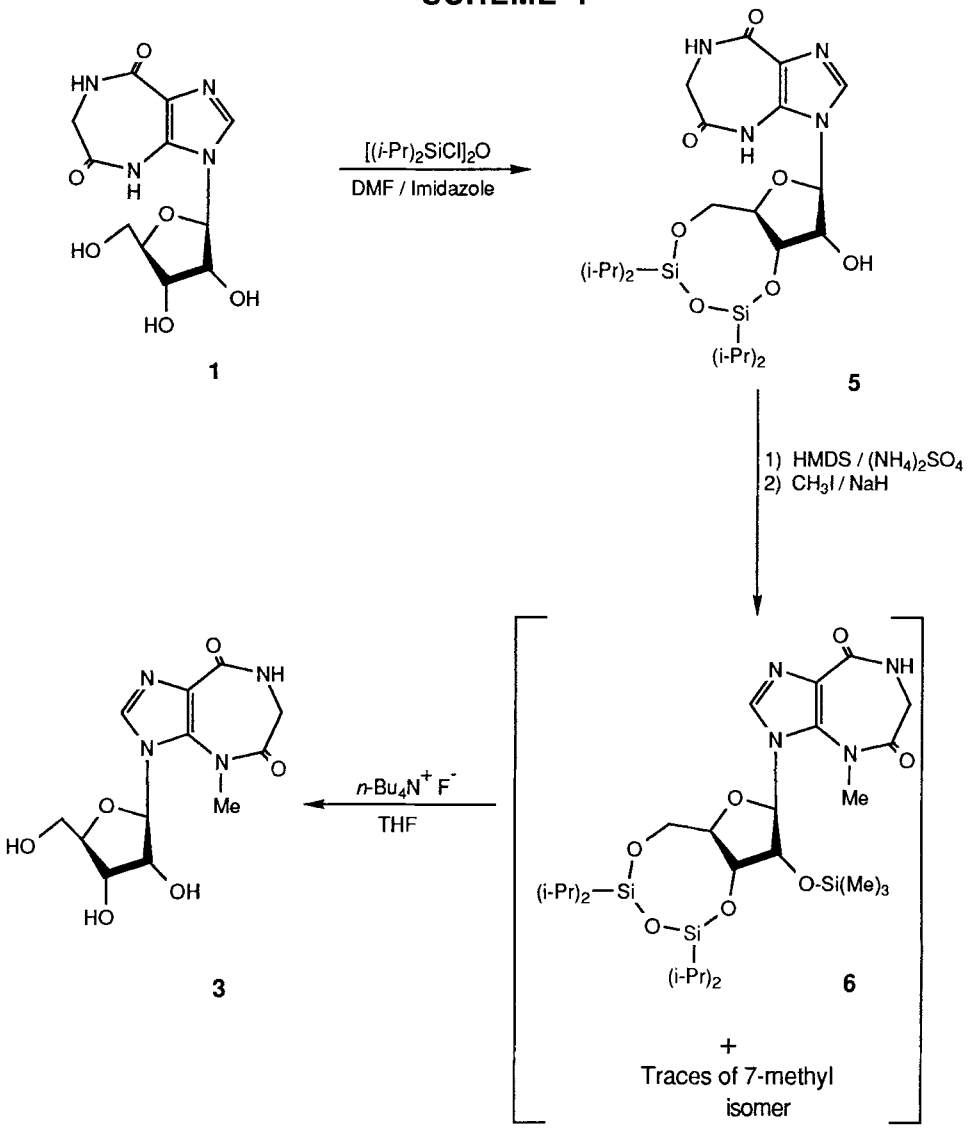
3; R = H, R^1 = Me

4; R = Me, R^1 = H

In this context, methyl derivatives **3** and **4** would be excellent probes to explore the possible correlation between conformations of these ring-expanded nucleosides and biophysical properties of the corresponding polynucleotides. Methylation at N-4 of **1**, while eliminating the above presumed intramolecular hydrogen bonding, would be anticipated to cause severe steric interactions with the sugar moiety, forcing the base-ribose conformation of **3** to an anti orientation. On the other hand, conformational consequences of N-7 methylation in **4** would be difficult to predict a priori. We report here our studies on the synthesis, structure, and conformation of **3** and **4**, employing X-ray, CD, and ^{1}H NMR techniques.

Nucleoside **3** was synthesized from **1** (Scheme I) via protection of the 3',5'-hydroxyl groups with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[4,5] which gave **5**. In situ protection of the remaining 2'-hydroxyl group with hexamethyldisilazane (HMDS), followed by treatment with methyl iodide in the presence of sodium hydride provided mostly **6** with traces of what is apparently the N^7-methyl isomer, isolation of

SCHEME I



which was not attempted. Deprotection of the silyl group of **6** with tetrabutylammonium fluoride afforded **3**.

The structure of **3** was confirmed by hydrolysis to the aglycon **7** by treatment with Dowex-50 [H^+] resin. The structure of **7**, prepared separately by alkylation of **8**[7] with methyl iodide (Scheme II), was confirmed by single-crystal X-ray diffraction analysis (Figure 1).

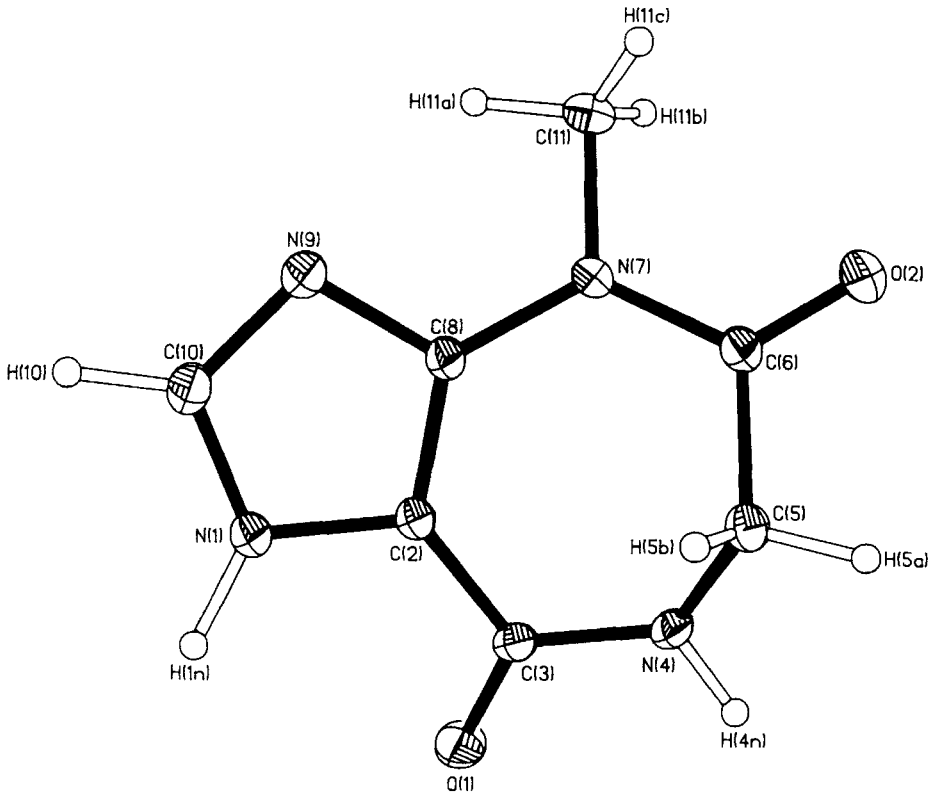


Fig. 1: ORTEP view of 7 with atom numbering scheme.

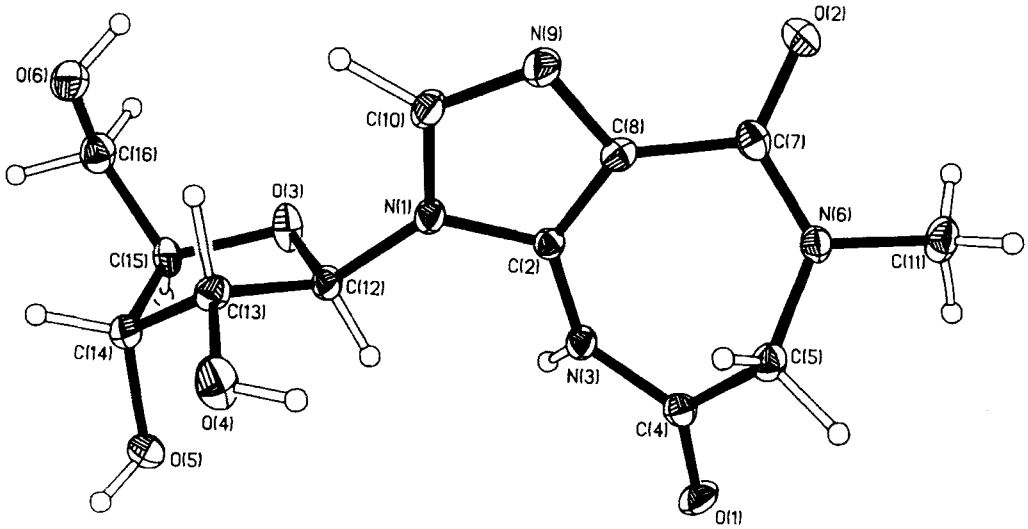
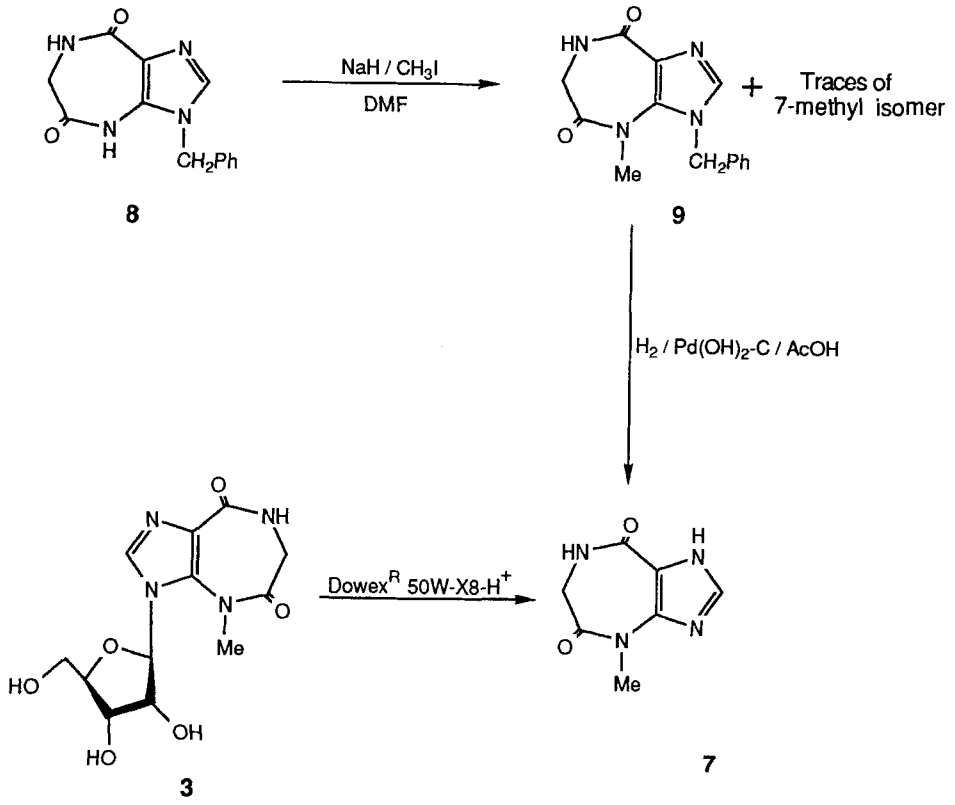


Fig. 2: ORTEP view of 4 with atom numbering scheme.

SCHEME II

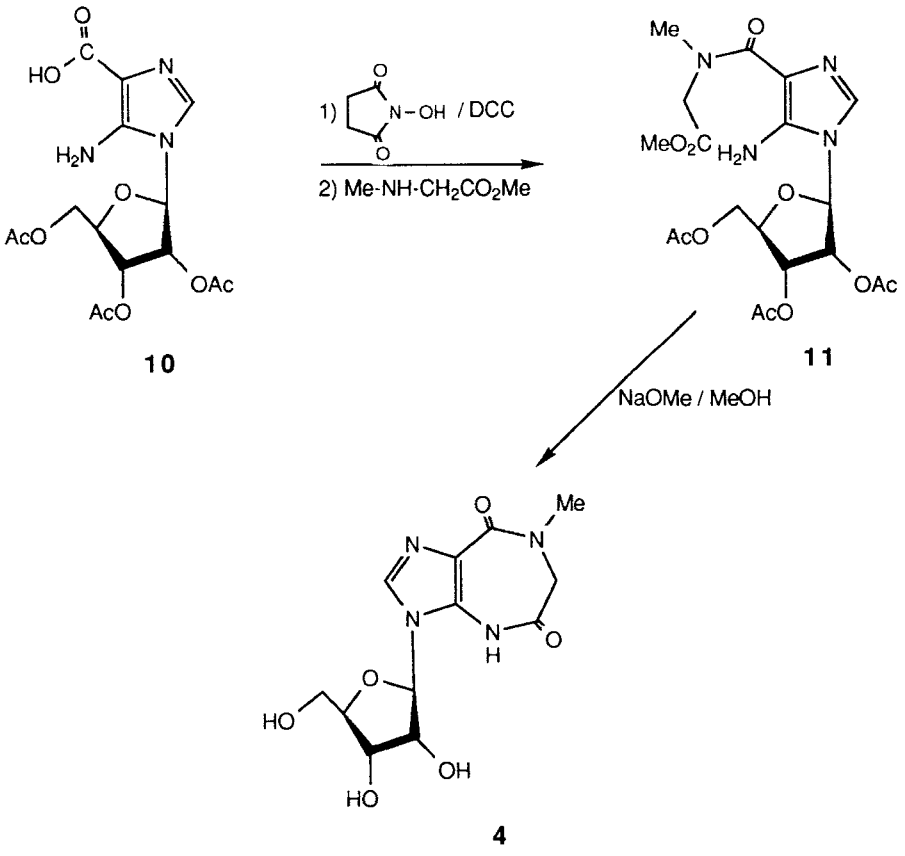


Nucleoside **4** was synthesized (Scheme III) starting from 2′,3′,5′-tri-O-acetyl-1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxylic acid (**10**).[6] Condensation of **10** with sarcosine methyl ester, mediated by N-hydroxysuccinimide/1,3-dicyclohexylcarbodiimide (DCC), gave **11**, which upon ring-closure with sodium methoxide/methanol, accompanied by hydroxyl deprotection, produced **4**.

Single-crystal X-ray diffraction analysis of **4** (Figure 2) revealed that it, unlike **1**, exists in an anti conformation in the solid state despite the presence of free N^4-H available for hydrogen bonding with the sugar 5′-OH. The sugar pucker geometry of **4**, however, is the same as that of **1**.

Conformations of **3** and **4** in solution were assessed by nuclear Overhauser effect (NOE) studies, and were compared with those of **1**, **2**,

SCHEME III



10 11 4

and xanthosine (Table I). Significant NOE enhancements were oberved in **1** and **4** between the imidazole H-2 and the sugar H-1′ and H-2′, the enhancements being relatively more pronounced in **1**. Nucleosides **2** and **3**, on the other hand, exhibited little, if any, NOE between H-2 and H-1′ or between H-2 and H-2′. In addition, there was a considerable NOE between H-1′ and the methyl hydrogens of **3**. The observed relative NOE's for each pair of hydrogens in **1-4** were consistent with the calculated relative distance between each pair by energy minimization employing ALCHEMY II™.[8] These data are consistent[9] with the predominantly syn conformations of **1** and **4** and anti conformations of **2** and **3** in solution.

The circular dichroism (CD) spectra of **3** and **4** in water (Figure 3) exhibited remarkably different Cotton effects. We had earlier observed such a phenomenon in the CD spectra of **1** and **2** in water, which

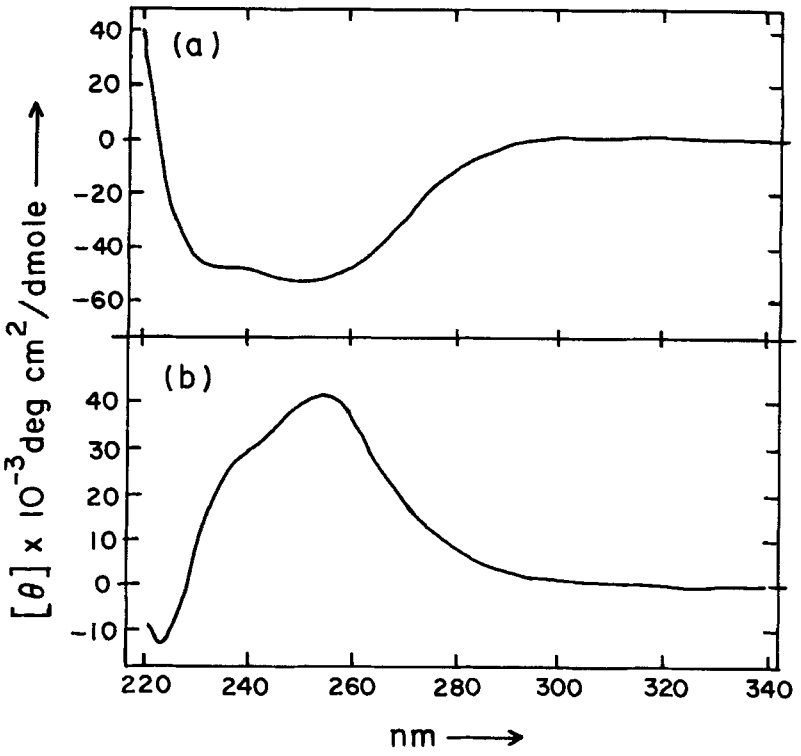


Fig. 3: Circular dichroism (CD) spectra of (a) 3 and (b) 4.

Table I: NOE enhancements (%) of nucleosides 1-4 and xanthosine in a mixture of DMSO-d_6 and D_2O at 25 °C.

Compound Number	Protons Observed	Protons Irradiated: imid-H	H-1′	H-2′	N-Me
Xanthosine	H-1′	14.8	- -	- -	- -
	H-2′	0.0	- -	- -	- -
	imid-H	- -	26.0	<4.0	- -
1	H-1′	39.8	- -	- -	- -
	H-2′	19.7	- -	- -	- -
	imid-H	- -	36.4	21.1	- -
2	H-1′	0.0	- -	- -	- -
	H-2′	0.0	- -	- -	- -
	imid-H	- -	0.0	0.0	- -
3	H-1′	0.0	- -	- -	19.4
	H-2′	0.0	- -	- -	15.8
	imid-H	- -	0.0	0.0	- -
	N-Me	- -	7.5	6.5	- -
4	H-1′	11.2	- -	- -	- -
	H-2′	10.7	- -	- -	- -
	imid-H	- -	29.1	28.4	- -

Table II: CD and UV spectral data of nucleosides 3, 4, and xanthosine in water at 25 °C

Compound Number	UV λmax nm	ε	CD λmax nm	θ m deg	$_2$[θ] λmax cm^2 deg/dmole	Δε
Xanthosine	262.5	9000	265.0	-3.1	-1484	-0.45
3	260.0	5560	254.0	-53.14	-24,717	-7.40
4	258.0	5680	254.0	+41.86	+15,796	+4.70

exhibited predominantly syn and anti conformations, respectively. The CD and UV spectral data of 3 and 4, and for comparison, those of xanthosine are collected in Table II.

We are currently investigating the consequences of these methylation-induced conformational variations upon the biochemical and biophysical properties of polynucleotides derived from 3 and 4.

EXPERIMENTAL SECTION

^{1}H NMR spectra were recorded at 80 or 500 MHz, on an IBM NR/80 or a General Electric GN-500 spectrometer, respectively. The reported spectral data are relative to Me_4Si as an internal reference standard. Nuclear Overhauser effect (NOE) studies were performed on the above 500 MHz instrument. Electron impact (EI) or chemical ionization (CI) mass spectra were recorded at 70 eV on a Hewlett Packard 5988A mass spectrometer. Isobutane was the reagent gas used in CI mass spectral determinations. Circular dichroism measurements were made on an AVIV 60DS spectrometer using a pathlength of 1 cm. X-Ray diffraction analyses were carried out at the Department of Chemistry, Southern Methodist University, Dallas, TX on an automatic Nicolet R3m/V diffractometer. Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. Melting points are uncorrected. Dry solvents were prepared as follows: methanol, ether, toluene, and xylene were distilled over sodium; acetonitrile was distilled from CaH_2, followed by distillation over P_2O_5; DMF and DMSO were distilled under reduced pressure from CaH_2; THF was first dried over KOH and then distilled over sodium. All dry solvents were stored over 3 or 4 Å molecular sieves.

4,5,7,8-Tetrahydro-6H-3-[[3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)]-β-D-ribofuranosyl]imidazo[4,5-e][1,4]diazepine-5,8-dione (5). To a solution of dry nucleoside 1 (1.0 g, 3.35 mmol), obtained by repeated co-evaporations with anhydrous xylene, and imidazole (0.98 g, 16.8 mmol) in DMF (20 mL), was added dropwise under a stream of N_2, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.1 g, 3.52 mmol). The reaction mixture was stirred at room temperature for 4-5 h, then quenched with MeOH, and evaporated to dryness on a rotary evaporator. The gummy residual mass was stirred with ice-water to obtain a white powder which was recrystallized from CH_3CN, yield 1.63 g (90%), mp 160-163 °C: ^1H NMR ($CDCl_3$) δ 8.2 (br s, 1 H, exchangeable with D_2O, NH), 8.1 (s, 1 H, imidazole CH), 5.7 (br s, 1 H, anomeric CH), 4.48 (br s, 1 H), 4.31 (d, J = 5.5 Hz, 2 H, ring CH_2), 4.19 (m, 1 H, CH), 4.02 (m, 1 H, CH), 3.9 (br s, 2 H, ribose CH_2), 0.99 (br s, 28 H, i-Pr groups); IR (KBR) 1700, 1650 (C=O) cm^{-1}; MS (CI) m/z 541 (M^+ + 1), 375, 315, 261, 167; UV (MeOH) λ_{max} 260 nm, (pH 13) 295.5, 250.5.

Anal. Calcd for $C_{23}H_{40}O_7N_4Si_2 \cdot \frac{1}{2} H_2O$: C, 50.20; H, 7.45; N, 10.18. Found: C, 49.87; H, 7.57, N, 10.09.

4,5,7,8-Tetrahydro-4-methyl-6H-3-(β-D-ribofuranosyl)imidazo[4,5-e][1,4]diazepine-5,8-dione (3). A mixture of 5 (0.5 g, 0.92 mmol), 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (15 mL), and $(NH_4)_2SO_4$ (15 mg) was heated at 50 °C for 2 h under a stream of N_2. The reaction mixture was evaporated to dryness on a rotary evaporator and the residue was co-evaporated with dry xylene (2 x 7 mL) to remove residual HMDS. The pale yellow syrup obtained was added to a cooled flask (ice water-salt bath), containing 80% NaH (31 mg) in dry DMF (10 mL), and maintained under a steady stream of N_2. Methyl iodide (0.6 mL, 0.96 mmol) was introduced through a syringe needle and the contents were stirred for 3 h. The reaction mixture was quenched with MeOH and rotary evaporated to dryness. To the residual syrup was added THF (15 mL) and a 1 M solution in THF of tetrabutylammonium fluoride (1.5 mL, 1.5 mmol). After 50 min, the solution was diluted with a mixture of pyridine-MeOH-H_2O (3:1:1, 25 mL). The resin Dowex^R-50 (pyridinium form, 10 g) was added and the mixture was stirred for 20 min. The resin was filtered off, the filtrate was concentrated to a volume of 10-15 mL, and neutralized with NH_4OH (10%, ≈20 mL). The reaction mixture was stirred for 10 min, and rotary evaporated to dryness. The residue was co-

evaporated with dry toluene, and then loaded onto a Chromatotron^R disc [silica gel (kieselgel 60 GF_{254}), 2-mm thickness]. Elution with $CHCl_3$-MeOH (5:1) and evaporation of appropriate fractions afforded **3** as a white powder which was recrystallized from CH_3CN, yield 170 mg (60%), mp 145-148 °C: ^1H NMR (DMSO-d_6) δ 8.1 (br s, 1 H, exchangeable with D_2O, NH), 8.0 (s, 1 H, imidazole CH), 5.5 (d, J = 5.5 Hz, 1 H, anomeric CH), 5.46 (s, 1 H, exchangeable with D_2O, OH), 5.16 (d, 1 H, exchangeable with D_2O, OH), 5.01 (t, 1 H, exchangeable with D_2O, OH), 4.35 (m, 1 H, CH), 4.07 (m, 1 H, CH), 3.90 (m, 2 H, two CH), 3.64 (br s, 2 H, CH_2), 3.58 (br s, 2 H, CH_2), 3.24 (s, 3 H, N-Me); IR (KBr) 3500-3300 (br, NH + OH), 1650 (C=O) cm^{-1}; MS (CI) m/z 313 ($M^+ + 1$), 219, 195, 181, 142, 136, 133; UV (MeOH) λ_{max} 259.5 nm (ε 5.6 x 10^3), (pH 12.3) 263 (ε 8.7 x 10^3), (pH 1.2) 259.5 sh (ε 5.0 x 10^3), 236.5 sh (ε 7.6 x 10^3).

Anal. Calcd for $C_{12}H_{16}N_4O_6$: C, 46.16; H, 5.16; N, 17.94. Found: C, 46.08; H, 5.17: N, 17.99.

3-Benzyl-4,5,7,8-tetrahydro-4-methyl-6*H*-imidazo[4,5-*e*][1,4]-diazepine-5,8-dione (9). A mixture of **8** (0.8 g, 3.1 mmol), 80% NaH (100 mg, 3.3 mmol), and CH_3I (0.21 mL, 3.3 mmol) in dry DMF (10 mL) was heated to reflux for 3.5 h. A TLC (silica gel, $CHCl_3$-MeOH, 4:1) indicated a major UV-absorbing spot along with a minor spot with higher R_f. The reaction mixture was cooled, quenched with MeOH (5 mL), and treated with 1 N HCl to adjust the pH to ≈6.5. The mixture was evaporated to dryness on a rotary evaporator, and the residue was chromatographed on a flash chromatography[10] column packed with silica gel and eluted with $CHCl_3$-MeOH (20:1). Evaporation of appropriate UV-absorbing fractions yielded a solid which was recrystallized from acetone into colorless crystals of **9**, yield 0.43 g (50%), mp 224-226 °C: ^1H NMR (DMSO-d_6) δ 8.08 (t, 1 H, exchangeable with D_2O, NH), 7.98 (s, 1 H, imidazole CH), 7.42 (m, 5 H, Ph-H), 5.32 (s, 2 H, benzyl CH_2), 3.60 (br s, 2 H, ring CH_2), 3.1 (s, 3 H, N-Me); IR (KBr) 1680, 1650 (C=O) cm^{-1}; MS (CI) m/z 271 ($M^+ + 1$), 237, 219, 195, 181; UV (MeOH) λ_{max} 257.5 nm, 216, (pH 12) 258, 218, (pH 0.9) 234.5, 212.

Anal. Calcd for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.21; N, 20.72. Found: C, 62.30; H, 5.20; N, 20.65.

4,5,7,8-Tetrahydro-4-methyl-6*H*-imidazo[4,5-*e*][1,4]diazepine-5,8-dione (7). Method A. Debenzylation of 9. A mixture of **9** (0.40 g,

1.48 mmol), glacial CH_3CO_2H (20 mL), and 20% $Pd(OH)_2/C$ (100 mg) was hydrogenated in a Parr apparatus at 40 psi for 18 h. The mixture was filtered through a pad of Celite^R, and the filtrate was evaporated to dryness. The residue was repeatedly co-evaporated with MeOH to remove residual acetic acid. The white solid obtained was recrystallized from MeOH-H_2O into colorless needles of 7, yield 0.2 g (75%), mp 275 °C: ^1H NMR (DMSO-d_6) δ 13.02 (br s, 1 H, exchangeable with D_2O, imidazole NH), 8.06 (t, J = 5.0 Hz, 1 H, exchangeable with D_2O, N^7-H), 7.79 (s, 1 H, imidazole CH), 3.75 (d, J = 5.0 Hz, 2 H, ring CH_2), 3.33 (s, 3 H, N-Me); IR (KBr) 1670, 1650 (C=O) cm^{-1}; MS (CI) m/z 181 (M^+ + 1), 167, 133; UV (MeOH) λ_{max} 269.5 nm, 210, (pH 12) 282.5, 215.5, (pH 0.9) 258.5, 234.5, 216.5

Anal. Calcd for $C_7H_8N_4O_2$: C, 46.67; H, 4.47; N, 31.09. Found: C, 46.71; H, 4.48; N, 31.01.

Method B. Deglycosylation of 3. Dowex^R-50 (H^+ form, 20-50 mesh) (5 g) was thoroughly washed successively with EtOH and H_2O, and suspended in 50% aqueous MeOH (100 mL). To this was added nucleoside 3 (100 mg, 0.32 mmol), and the mixture was stirred at room temperature for 15 min. The resin was filtered off and the filtrate evaporated to dryness. The light brown solid obtained was dissolved in MeOH, treated with decolorizing charcoal, and filtered. Evaporation of the filtrate yielded a colorless solid, yield 35 mg (61%). By both ^1H NMR and mixture melting point determination, this compound was found to be identical with 7 obtained by Method A.

5-Amino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-(N-methoxycarbonylmethyl-N-methyl)carboxamide (11). To a mixture of compound[6] **10** (1.5 g, 3.9 mmol) and N-hydroxysuccinimide (0.5 g, 4.3 mmol) in dry THF (30 mL) was added dicyclohexylcarbodiimide (DCC) (1.0 g, 4.8 mmol). The reaction mixture was stirred at room temperature for 24 h under anhydrous conditions. The precipitated dicyclohexylurea (DCU) was filtered off, washed with dry THF (10 mL), and the filtrate was evaporated to dryness at 35-40 °C. The residual pale yellow syrup was directly used in the next step.

To an ice-cold solution of sarcosine methyl ester hydrochloride (1.1 g, 7.8 mmol) in dry methylene chloride (20 mL) was added freshly distilled triethylamine (1.1 mL, 7.8 mmol). The mixture was stirred at 0 °C for 5-8 min. The $Et_3N \cdot HCl$ which separated was filtered, and the

filtrate was added to the above syrup taken in 15 mL of THF. The reaction mixture was stirred at room temperature for 24 h, and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 (100 mL) and the solution was transferred to a separatory funnel, washed with H_2O (100 mL), and dried over anhydrous $MgSO_4$. Filtration, evaporation, and purification by silica gel flash chromatography[10] using $CHCl_3$ as the eluting solvent afforded **11** as a semi-solid, yield 1.2 g (66%): ^1H NMR (DMSO-d_6) δ 7.4 (s, 1 H, imidazole CH), 6.3 (br s, 2 H, exchangeable with D_2O, NH_2), 5.90 (d, J = 7.2 Hz, 1 H, anomeric CH), 5.45 (m, 2 H, ribose CH's), 4.29 (br s, 3 H, CH_2 + CH), 4.06 (s, 1 H), 3.68 (s, 1H), 3.62 (s, 3 H, O-Me), 3.19-3.05 (br, 3 H, N-Me), 2.10 (s, 3 H, one OAc), 2.05 (s, 6 H, two OAc); IR (KBr) 3420 (NH_2), 1750 (C=O) cm^{-1}; MS (CI) m/z 471 (M^+ + 1), 399, 289, 186; UV (MeOH) λ_{max} 271.5 nm, (pH 13) 249.5, 282.5, (pH 0.9) 268, 248.

Anal. Calcd for $C_{19}H_{26}N_4O_{10}\cdot\frac{1}{4}H_2O$: C, 48.05; H, 5.62; N, 11.80. Found: C, 48.07; H, 5.64; N, 11.24

4,5,7,8-Tetrahydro-7-methyl-6H-3-(β-D-ribofuranosyl)imidazo[4,5-e][1,4]diazepine-5,8-dione (4). A three-neck flask, maintained under a stream of N_2, was charged with dry MeOH (30 mL), followed by freshly cut sodium metal (100 mg, 4.3 mg-atom). The mixture was stirred to form a clear solution. Compound **11** (1.0 g, 2.1 mmol) was added, and the reaction mixture was heated to reflux for 3 h. It was cooled, neutralized with 0.5 N HCl, and rotary evaporated to dryness. The residue was purified by flash chromatography[10] on silica gel, using $CHCl_3$-MeOH (3:1) as the eluting solvent system. The solid obtained was recrystallized from CH_3CN into white crystals of **4**, yield 0.55 g (84%), mp 187-190 °C: ^1H NMR (DMSO-d_6) δ 10.73 (br s, 1 H, exchangeable with D_2O, N^4-H), 7.88 (s, 1 H, imidazole CH), 5.65 (d, J = 5.5 Hz, 1 H, anomeric CH), 5.41 (br s, 2 H, exchangeable with D_2O, two OH's), 5.21 (d, J =4.2 Hz, 1 H, exchangeable with D_2O, OH), 4.21 (d, J = 4.5 Hz, 1 H, CH), 4.06 (d, J = 3.5 Hz, 1 H, CH), 3.91 (m, 1 H, CH), 3.87 (br s, 2 H, ring CH_2), 3.60 (br s, 2 H, ribose CH_2), 3.0 (s, 3 H, N-Me); IR (KBr) 3400, 1700, 1628, 1540 cm^{-1}; MS (CI) m/z 313 (M^+ + 1), 223, 181, 133; UV (MeOH) λ_{max} 260 nm (ε 5.7 x 10^3), (pH 12.5) 290.5 (ε 6.5 x 10^3), 246 (8.8 x 10^3), (pH 1.2) 226.5

Anal. Calcd for $C_{12}H_{16}N_4O_6$: C, 46.16; H, 5.16; N, 17.94. Found: C, 45.98; H, 5.12; N, 18.19.

NOE Studies. The values for NOE enhancements, collected in Table I, were obtained by performing NOE difference spectroscopy at 500 MHz, using the method of Hall and Sanders.[11] Data were collected with 5-10 mg samples, using a mixture of 0.4 mL of 99.8% atom D DMSO-d_6 and 0.1 mL of D_2O as the solvent. Samples were freeze-pump-thaw degassed and sealed with N_2. Pre-irradiation times of 25 s, resulting in 90% reduction of intensity, were used to transfer polarization; data acquisition commenced after a delay of 500 μs. The resulting free-induction decays were subtracted and transformed.

Single Crystal X-ray Diffraction Analyses of Compounds 4 and 7. Suitable crystals were grown through slow crystallization from the appropriate solvents (see pertinent experimental data above). The unit cell dimensions were obtained by a least-squares fit of the angles of 24 centered reflections in the range of $20° < 2\theta < 30°$. Intensity data were collected by using a $\theta/2\theta$ scan type in the range of $3° \leq 2\theta \leq 54°$ for **4** and $2.5° \leq 2\theta \leq 56°$ for **7** at -53 °C, using graphite monochromated Mo Kα (λ = 0.71073 Å) radiation. Three standard reflections monitored after every 100 reflections did not show any significant change in intensity during data collections. Intensities were corrected for Lorentz and polarization effects but not for absorption. The structure was solved and all non-hydrogen atoms were found by using results of SHELXTL-PLUS.[12] Full-matrix least-squares refinement was performed. Neutral atom scattering factors and anomalous scattering correction terms were taken from the International Tables for X-ray Crystallography.[13] The structure of **4** was determined based on the configuration of the known chiral centers. The hydrogen atoms were located from difference Fourier maps and were included in the final refinement with fixed isotropic thermal parameters. The weights had the form, $\underline{w} = [\sigma^2(F_0) + g(F_0)^2]^{-1}$ with $g = 1.1 \times 10^{-4}$ for **4** and $g = 8 \times 10^{-4}$ for **7**. Final cycles of refinements converged at the discrepancy indices $\underline{R} = \Sigma||F_0| - |F_c||/\Sigma|F_0|$, and $R_w = [\Sigma\underline{w}(|F_0| - |F_c|)^2/\Sigma\underline{w}(|F_0|)^2]^{1/2}$ whose values are given in the crystallographic data collected below. The largest parameter shifts were less than 0.01 of their estimated standard deviations for **4** and <0.002 for **7**. A final difference Fourier map showed no features greater than 0.29 $e^-/Å^3$ for **4** and 0.27 $e^-/Å^3$ for **7**. The final atomic coordinates, bond lengths, bond angles, and selected torsion angles for **4** and **7** are collected in Tables III-VIII.

Table III. Atomic coordinates ($x10^4$) and equivalent isotropic displacement parameters ($Å^2x10^3$) for **4**

	x	y	z	U(eq)
O(1)	2289(4)	3443(2)	4355(2)	28(1)
O(2)	-5422(4)	3278(2)	2618(2)	31(1)
N(1)	-2187(5)	662(2)	3891(2)	19(1)
N(3)	270(5)	2126(2)	3879(2)	19(1)
N(6)	-2839(5)	3824(2)	3500(2)	20(1)
N(9)	-5423(5)	1311(2)	3477(2)	25(1)
C(2)	-1807(5)	1674(3)	3744(2)	17(1)
C(4)	456(6)	3088(3)	4176(2)	19(1)
C(5)	-1741(6)	3674(3)	4255(2)	22(1)
C(7)	-4149(6)	3102(3)	3177(2)	21(1)
C(8)	-3809(5)	2062(3)	3488(2)	19(1)
C(10)	-4393(6)	494(3)	3716(2)	25(1)
C(11)	-2756(9)	4845(3)	3149(2)	29(1)
O(3)	270(4)	-647(2)	3530(1)	26(1)
O(4)	-1682(6)	-499(2)	5528(2)	36(1)
O(5)	2509(5)	-1242(2)	5099(2)	29(1)
O(6)	-2264(5)	-2641(2)	3540(2)	30(1)
C(12)	-526(6)	-70(2)	4174(2)	20(1)
C(13)	-1366(6)	-853(3)	4757(2)	20(1)
C(14)	560(6)	-1616(3)	4700(2)	21(1)
C(15)	1080(6)	-1624(3)	3823(2)	21(1)
C(16)	50(6)	-2474(3)	3359(2)	26(1)

Table IV. Atomic coordinates ($x10^4$) and equivalent isotropic displacement parameters ($Å^2x10^3$) for **7**

	x	y	z	U(eq)
O(1)	4011(1)	5451(1)	2367	30(1)
O(2)	412(1)	3832(1)	1156(6)	36(1)
N(1)	4176(1)	3673(1)	6074(6)	24(1)
C(2)	3342(1)	4009(1)	4554(7)	19(1)
C(3)	3276(1)	4978(1)	3089(6)	19(1)
N(4)	2359(1)	5303(1)	2508(6)	22(1)
C(5)	1505(1)	4878(1)	4121(7)	23(1)
C(6)	1187(1)	3919(1)	2607(7)	21(1)
N(7)	1801(1)	3145(1)	2979(6)	20(1)
C(8)	2726(1)	3212(1)	4423(6)	18(1)
N(9)	3164(1)	2415(1)	5788(6)	24(1)
C(10)	4033(1)	2731(1)	6761(7)	28(1)
C(11)	1481(1)	2202(1)	1679(7)	25(1)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

Table V Bond lengths (Å) and bond angles (°) for **4**

O(1)-C(4)	1.221 (4)	O(2)-C(7)	1.238 (4)
N(1)-C(2)	1.373 (4)	N(1)-C(10)	1.359 (5)
N(1)-C(12)	1.459 (4)	N(3)-C(2)	1.386 (4)
N(3)-C(4)	1.367 (4)	N(6)-C(5)	1.457 (5)
N(6)-C(7)	1.344 (4)	N(6)-C(11)	1.470 (5)
N(9)-C(8)	1.375 (4)	N(9)-C(10)	1.302 (5)
C(2)-C(8)	1.363 (5)	C(4)-C(5)	1.519 (5)
C(7)-C(8)	1.479 (5)	O(3)-C(12)	1.417 (4)
O(3)-C(15)	1.460 (4)	O(4)-C(13)	1.408 (4)
O(5)-C(14)	1.428 (5)	O(6)-C(16)	1.422 (5)
C(12)-C(13)	1.516 (5)	C(13)-C(14)	1.523 (5)
C(14)-C(15)	1.528 (5)	C(15)-C(16)	1.501 (5)

C(2)-N(1)-C(10)	106.0(3)	C(2)-N(1)-C(12)	126.1(3)
C(10)-N(1)-C(12)	127.9(3)	C(2)-N(3)-C(4)	122.0(3)
C(5)-N(6)-C(7)	121.6(3)	C(5)-N(6)-C(11)	118.0(3)
C(7)-N(6)-C(11)	119.9(3)	C(8)-N(9)-C(10)	105.3(3)
N(1)-C(2)-N(3)	122.0(3)	N(1)-C(2)-C(8)	106.2(3)
N(3)-C(2)-C(8)	131.7(3)	O(1)-C(4)-N(3)	121.2(3)
O(1)-C(4)-C(5)	123.1(3)	N(3)-C(4)-C(5)	115.7(3)
N(6)-C(5)-C(4)	111.9(3)	O(2)-C(7)-N(6)	122.3(3)
O(2)-C(7)-C(8)	122.1(3)	N(6)-C(7)-C(8)	115.3(3)
N(9)-C(8)-C(2)	109.9(3)	N(9)-C(8)-C(7)	124.4(3)
C(2)-C(8)-C(7)	125.4(3)	N(1)-C(10)-N(9)	112.7(3)
C(12)-O(3)-C(15)	108.4(3)	N(1)-C(12)-O(3)	108.7(3)
N(1)-C(12)-C(13)	116.4(3)	O(3)-C(12)-C(13)	104.8(3)
O(4)-C(13)-C(12)	115.6(3)	O(4)-C(13)-C(14)	112.2(3)
C(12)-C(13)-C(14)	99.2(3)	O(5)-C(14)-C(13)	110.4(3)
O(5)-C(14)-C(15)	107.8(3)	C(13)-C(14)-C(15)	102.6(3)
O(3)-C(15)-C(14)	105.3(3)	O(3)-C(15)-C(16)	109.9(3)
C(14)-C(15)-C(16)	116.1(3)	O(6)-C(16)-C(15)	113.1(3)

Table VI. Bond lengths (Å) and bond angles (°) for **7**

O(1)-C(3)	1.232 (2)	O(2)-C(6)	1.217 (2)
N(1)-C(2)	1.375 (2)	N(1)-C(10)	1.338 (2)
C(2)-C(3)	1.460 (2)	C(2)-C(8)	1.382 (2)
C(3)-N(4)	1.351 (2)	N(4)-C(5)	1.459 (2)
C(5)-C(6)	1.517 (3)	C(6)-N(7)	1.363 (2)
N(7)-C(8)	1.396 (2)	N(7)-C(11)	1.465 (2)
C(8)-N(9)	1.365 (2)	N(9)-C(10)	1.324 (2)

C(2)-N(1)-C(10)	107.2(1)	N(1)-C(2)-C(3)	122.6(1)
N(1)-C(2)-C(8)	104.9(1)	C(3)-C(2)-C(8)	131.9(2)
O(1)-C(3)-C(2)	121.7(1)	O(1)-C(3)-N(4)	122.8(2)
C(2)-C(3)-N(4)	115.4(1)	C(3)-N(4)-C(5)	122.2(2)
N(4)-C(5)-C(6)	113.3(2)	O(2)-C(6)-C(5)	122.0(1)
O(2)-C(6)-N(7)	120.9(2)	C(5)-C(6)-N(7)	117.1(2)
C(6)-N(7)-C(8)	123.6(1)	C(6)-N(7)-C(11)	117.7(1)
C(8)-N(7)-C(11)	118.7(1)	C(2)-C(8)-N(7)	128.4(2)
C(2)-C(8)-N(9)	110.6(1)	N(7)-C(8)-N(9)	120.9(1)
C(8)-N(9)-C(10)	104.6(1)	N(1)-C(10)-N(9)	112.7(2)

Table VII. Selected torsion angles (°) for 4

C10	N1	C2	N3	178.2(0.3)	C10	N1	C2	C8	0.0(0.4)
C12	N1	C2	N3	-2.1(0.5)	C12	N1	C2	C8	179.8(0.3)
C2	N1	C10	N9	-0.4(0.4)	C12	N1	C10	N9	179.9(0.3)
C2	N1	C12	O3	-100.1(0.4)	C2	N1	C12	C13	142.0(0.3)
C10	N1	C12	O3	79.6(0.4)	C10	N1	C12	C13	-38.3(0.5)
C4	N3	C2	N1	-141.4(0.3)	C4	N3	C2	C8	36.2(0.5)
C2	N3	C4	O1	173.6(0.3)	C2	N3	C4	C5	-7.0(0.4)
C7	N6	C5	C4	83.1(0.4)	C11	N6	C5	C4	-105.8(0.4)
C5	N6	C7	O2	166.1(0.3)	C5	N6	C7	C8	-19.3(0.5)
C11	N6	C7	O2	-4.9(0.5)	C11	N6	C7	C8	169.7(0.3)
C10	N9	C8	C2	-0.5(0.4)	C10	N9	C8	C7	172.9(0.3)
C8	N9	C10	N1	0.5(0.4)	N1	C2	C8	N9	0.3(0.4)
N1	C2	C8	C7	-173.0(0.3)	N3	C2	C8	N9	-177.6(0.3)
N3	C2	C8	C7	9.1(0.6)	O1	C4	C5	N6	117.9(0.4)
N3	C4	C5	N6	-61.5(0.4)	O2	C7	C8	N9	-31.0(0.5)
O2	C7	C8	C2	141.4(0.4)	N6	C7	C8	N9	154.4(0.3)
N6	C7	C8	C2	-33.2(0.5)	C15	O3	C12	N1	-155.0(0.3)
C15	O3	C12	C13	-30.0(0.3)	C12	O3	C15	C14	3.6(0.3)
C12	O3	C15	C16	129.4(0.3)	N1	C12	C13	O4	-76.4(0.4)
N1	C12	C13	C14	163.5(0.3)	O3	C12	C13	O4	163.5(0.3)
O3	C12	C13	C14	43.4(0.3)	O4	C13	C14	O5	-47.8(0.4)
O4	C13	C14	C15	-162.5(0.3)	C12	C13	C14	O5	74.8(0.3)
C12	C13	C14	C15	-39.9(0.3)	O5	C14	C15	O3	-92.8(0.3)
O5	C14	C15	C16	145.3(0.3)	C13	C14	C15	O3	23.7(0.3)
C13	C14	C15	C16	-98.1(0.3)	O3	C15	C16	O6	-73.8(0.4)
C14	C15	C16	O6	45.5(0.4)					

Table VIII. Selected torsion angles (°) for 7

C10	N1	C2	C3	-172.4(0.2)	C10	N1	C2	C8	0.0(0.3)
C2	N1	C10	N9	0.3(0.3)	N1	C2	C3	O1	19.0(0.4)
N1	C2	C3	N4	-162.7(0.2)	C8	C2	C3	O1	-151.2(0.2)
C8	C2	C3	N4	27.1(0.4)	N1	C2	C8	N7	-176.7(0.2)
N1	C2	C8	N9	-0.4(0.3)	C3	C2	C8	N7	-5.3(0.4)
C3	C2	C8	N9	171.1(0.2)	O1	C3	N4	C5	-163.2(0.2)
C2	C3	N4	C5	18.5(0.3)	C3	N4	C5	C6	-78.0(0.3)
N4	C5	C6	O2	-113.5(0.2)	N4	C5	C6	N7	67.5(0.3)
O2	C6	N7	C8	175.6(0.2)	O2	C6	N7	C11	-2.1(0.4)
C5	C6	N7	C8	-5.3(0.3)	C5	C6	N7	C11	176.9(0.2)
C6	N7	C8	C2	-27.3(0.4)	C6	N7	C8	N9	156.7(0.2)
C11	N7	C8	C2	150.5(0.2)	C11	N7	C8	N9	-25.6(0.3)
C2	C8	N9	C10	0.6(0.3)	N7	C8	N9	C10	177.2(0.2)
C8	N9	C10	N1	-0.6(0.3)					

Crystallographic Data. A. Compound 4: $C_{12}H_{16}N_4O_6$, M_r = 312.28, space group $P2_12_12_1$, orthorhombic, a = 5.925 (1) Å, b = 13.150 (4) Å, c = 17.070 (5) Å, V = 1330.0 (7) Å^3, Z = 4, D_x = 1.56 g cm^{-3}, (Mo Kα) = 0.71073 Å, μ = 1.36 cm^{-1}. Final R = 3.9%, R_w = 4.2% for 1664 unique reflections with $I \geq 3\sigma(I)$ = 1362.

B. Compound 7: $C_7H_8N_4O_2$, M_r = 180.17, space group $Pna2_1$, a = 13.675 (4) Å, b = 13.735 (4) Å, c = 4.046 (2) Å, V = 759.9 (4) Å^3, Z = 4, D_x = 1.57 g cm^{-3}, (Mo Kα) = 0.71073 Å, μ = 1.29 cm^{-1}. Final R = 3.1%, R_w = 4.5% for 1059 unique reflections with $I \geq 3\sigma(I)$ = 998.

Acknowledgments. This investigation was supported by the National Institutes of Health (R.S.H. # CA 36154), the American Cancer Society Institutional Research Grant (A.B. # IN-147H), MD Cancer Program/Univ. of Maryland, the American Cancer Society-Maryland Div., Inc. (M.H.), and the National Science Foundation (N.S.H. # CHE-88-00328).

REFERENCES

1. Hosmane, R. S.; Bhan, A. Nucleosides and Nucleotides **1990**, 9, 913.
2. Hosmane, R. S.; Bhan, A. Biochem. Biophys. Res. Commun. **1989**, 165, 106.
3. Hosmane, R. S.; Bhan, A.; Karpel, R.; Siriwardane, U.; Hosmane, N. S. J. Org. Chem. **1990**, 55, 5882.
4. Markiewicz, W. T.; Wiewiorowski, M. Nucleic Acids Res., Spec. Publ. No. 4 **1978**, s185.
5. Markiewicz, W. T. J. Chem. Res. (S) **1979**, 24.
6. Srivastava, P. C.; Mancuso, R. W.; Rousseau, R. J.; Robins, R. K. J. Med. Chem. **1974**, 17, 1207.
7. Hosmane, R. S.; Bhan, A. J. Heterocycl. Chem., in press.
8. "ALCHEMY II", Molecular Modeling Software, Tripos Associates, Inc., St. Louis, Missouri, 1988.
9. (a) Schirmer, R. E.; Davis, J. P.; Noggle, J. H.; Hart, P. A. J. Am. Chem. Soc. **1972**, 94, 2561. (b) Hart, P. A.; Davis, J. P. J. Am. Chem. Soc. **1972**, 94, 511. (c) Hart, P. A.; Davis, J. P. J. Am. Chem. Soc. **1971**, 93, 753. (d) For a recent review on NOE, see Derome, A. E., "The Nuclear Overhauser Effect," in "Modern NMR Techniques for Chemistry Research," Pergamon Press, Oxford, U.K., 1987. (e) For a detailed treatment on NOE, see Neuhaus, D. and Williamson, M., "The NOE in Structural and Conformational Analysis," VCH Publishers, New York, 1989.
10. Still, W. C.; Kahn, M.; Mitra, A. J. Org. Chem. **1978**, 43, 2923.

11. Hall, L. S.; Sanders, J. K. M. J. Am. Chem. Soc. **1980**, 102, 5703.
12. Sheldrick, G. M. "SHELXTL-Plus88: Structure Determination Software Programs," Nicolet Instrument Corporation, Madison, Wisconsin.
13. International Tables for X-Ray Crystallography, Vol. IV, Kynoch Press, Birmingham, England, 1974.

Received September 12, 1990.