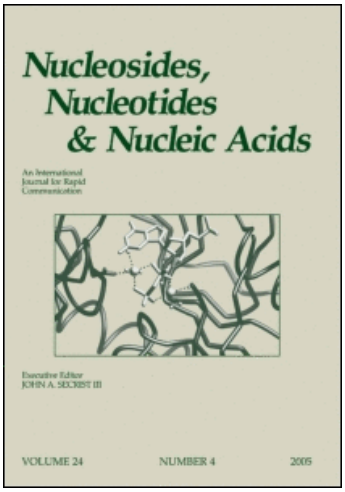

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Acyclic Nucleosides. Synthesis and Antiherpetic Activity of 9-[[[2-Hydroxy-1-(Hydroxymethyl)Ethyl]Thio]Methyl]Guanine and 1-[[[2-Hydroxy-1-(Hydroxymethyl)Ethyl]Thio]Methyl]Cytosine

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ACYCLIC NUCLEOSIDES. SYNTHESIS AND ANTIHERPETIC ACTIVITY OF 9-[[[2-HYDROXY-1-(HYDROXYMETHYL)ETHYL]THIO]METHYL]GUANINE AND 1-[[[2-HYDROXY-1-(HYDROXYMETHYL)ETHYL]THIO]METHYL]CYTOSINE

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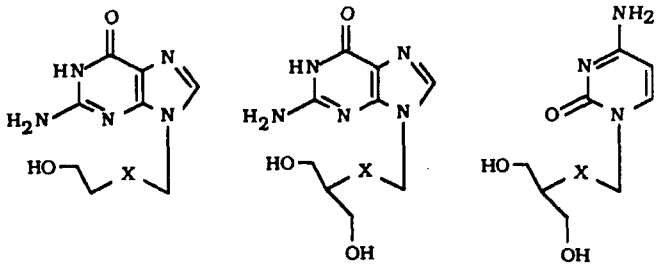
Abstract: The synthesis and antiherpetic activity of 9-[[[2-hydroxy-1-(hydroxymethyl)ethyl]thio]methyl]guanine (4) and 1-[[[2-hydroxy-1-(hydroxymethyl)ethyl]thio]methyl]cytosine (6), the side-chain thio analogues of ganciclovir (3) and BW A1117U (5), are described. The side-chain synthon 1,3-bis(benzyloxy)-2-[(chloromethyl)thio]propane (11) was prepared in four steps from 1,3-bis(benzyloxy)-2-propanol (7). Alkylation of 2-amino-6-chloro-9H-purine with 11 provided the intermediate 9-substituted-2-amino-6-chloropurine 12, which was conveniently converted to 4 in two steps. Reaction of a fivefold excess of cytosine with 11 provided the desired 1-isomer 14, which was debenzylated to give 6. In contrast with ganciclovir (3) and BW A1117U (5), neither 4 nor 6 had significant in vitro activity against human cytomegalovirus.

Acyclic nucleosides have served as a rich source of agents with selective activity against the herpes viruses.[1,2] Acyclovir [9-[(2-hydroxyethoxy)methyl]guanine (1), Zovirax®] is a potent antiherpetic agent with activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV).[3-5] A hydroxymethyl analogue of acyclovir, 9-[[2-hydroxy-1-(hydroxymethyl)-ethoxy]methyl]guanine (3, BW B759U, ganciclovir) has a broader spectrum of antiherpetic activity.[6-9] Its in vitro activity against HSV-1, HSV-2, and VZV is similar to that of acyclovir, but ganciclovir also has good activity against human cytomegalovirus (HCMV) and is about sixfold more active than acyclovir against EBV.[5,7,10-12]



1. X = O
2. X = S

3. X = O
4. X = S

5. X = O
6. X = S

The unique spectrum of antiherpetic activity of 1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]cytosine (5, BW A1117U) was recently reported.[13,14] This cytosine acyclic nucleoside has good activity against HCMV and EBV, but it is essentially inactive against HSV-1 and HSV-2 and is only moderately active against VZV.

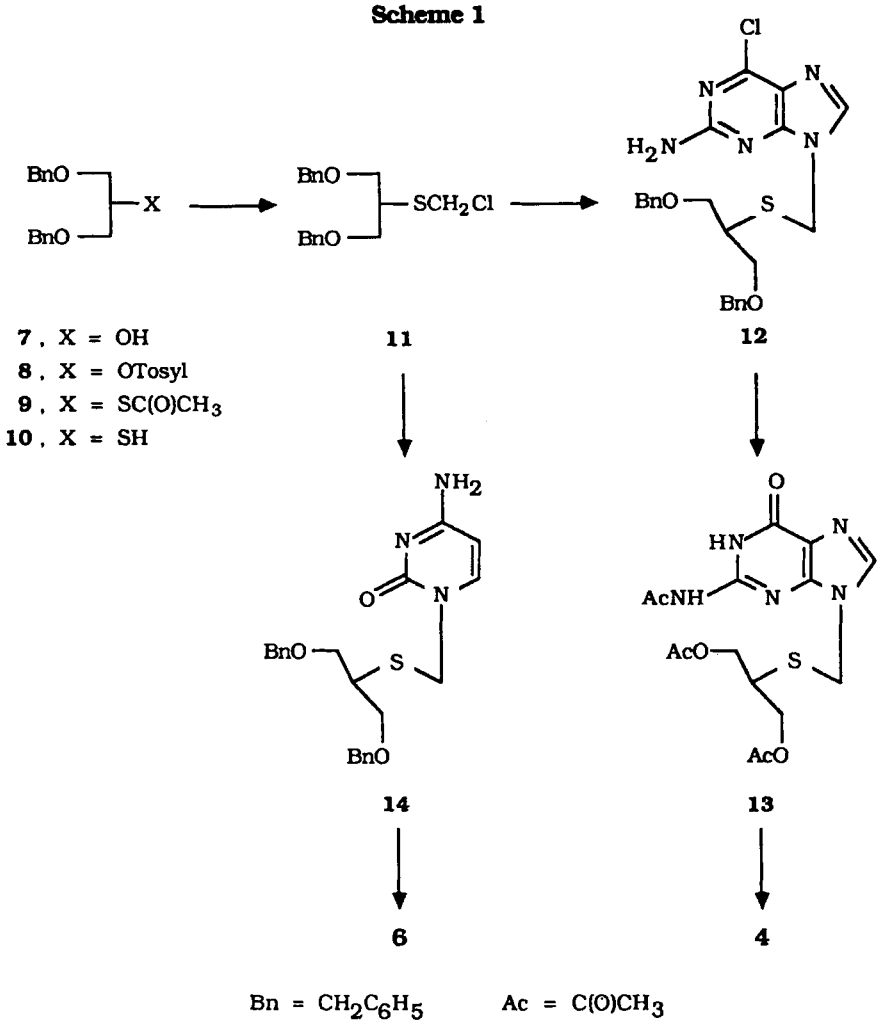
A variety of linear side-chain analogues of acyclovir were synthesized and tested for antiviral activity.[15] The thio analogue 2 was the most active congener with one-fifth the activity of acyclovir against HSV-1.[15] To find other acyclic nucleosides with a unique spectrum of antiviral activity, we prepared 4 and 6, which are the side-chain sulfur analogues of ganciclovir (3) and 5. The synthesis and antiviral activity of these acyclic nucleosides are described herein.

CHEMISTRY

Soon after we prepared 4, a similar synthesis was reported by McGee, et al.[16] Although the same general approach to preparation of the side-chain synthon was used, our synthesis utilized 2-amino-6-chloro-9H-purine rather than guanine as a source of the purine moiety, which facilitated separation of the 9- and 7-isomers. Earlier efforts by McCormick and McElhinney to prepare 4 were not successful.[17]

The side-chain synthon 11 was prepared in four steps from 1,3-bis(benzyloxy)-2-propanol (7). Tosylation of 7[18] with p-toluenesulfonyl chloride gave an 87% yield of tosylate 8,[16,19] which was

Scheme 1



reacted with potassium thioacetate to provide thioacetate 9[16] as an oil in 99% yield. Thioacetate 9 was deacetylated in ethanolic potassium hydroxide to provide a 47% yield of thiol 10.[16] Chloromethylation of 10 with paraformaldehyde and hydrogen chloride gave chloromethyl sulfide 11 as a moderately stable oil.

Alkylation of tris(trimethylsilyl)guanine[20] with 11 gave a mixture of the guanine N-9 and N-7 isomers, which were not easily separated by chromatography. As an alternative approach,[21] the silylated derivative of

2-amino-6-chloro-9H-purine was alkylated with 11. This reaction provided a mixture of 12 and its N-7 isomer, but these were easily separated by flash chromatography to provide pure 12, albeit in low yield. Cleavage of the O-benzyl blocking groups with ferric chloride in acetic anhydride[22] was accompanied by solvolysis of the 6-chloro substituent to give 13 in 41% yield. Deacetylation of 13 with methylamine provided 4, which had melting point and spectral properties similar to 4 prepared from guanine.[16]

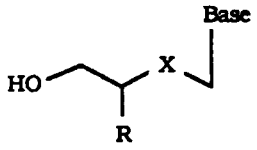
Initial efforts to prepare the cytosine derivative 14 using the trimethylsilylation approach[23,24] resulted in a bis-alkylated product. When the stoichiometry of the reactants was changed to a fivefold excess of silylated cytosine to 11, the desired 1-isomer 14 was isolated in 58% yield. Although the uv spectrum of 14 did not match the λ_{max} reported for 5[13] or 1-[[2-hydroxyethoxy]methyl]cytosine,[24] the NMR spectrum of 14 supported the assigned structure. In an NOE experiment, irradiation of the methylene signal at 4.89 δ caused an enhancement of the C-6 proton signal at 7.62 δ. Initial attempts to cleave the O-benzyl blocking groups with ferric chloride in acetic anhydride[22] or boron trifluoride etherate in acetic anhydride[16] produced a mixture of products. However, use of boron trichloride in methylene chloride[25] followed by ion exchange desalting and column chromatography gave 6 in 32% yield.

BIOLOGICAL RESULTS AND DISCUSSION

The compounds were tested for *in vitro* antiherpetic activity against HSV-1, VZV, and HCMV. The activity of the thio analogues 4 and 6 and three reference compounds are listed in Table I. The IC_{50}s were measured by the plaque reduction assay;[12,26] for a few reference compounds or viruses the IC_{50} values are as previously reported.

The thio analogue 4 of ganciclovir (3) was substantially less active than 3 against HSV-1, VZV, and HCMV with IC_{50}s of 6.9 μM (HSV-1), 26 μM (VZV), and >100 μM (HCMV). This trend is comparable to the decreased

TABLE 1. Antiviral Activity of Acyclic Nucleosides in Cell Culture



Compound	R	X	IC_{50}, μM[a] HSV-1[b]	VZV[c]	HCMV[d]
	BASE = Guanine-9-yl				
1[e]	H	O	1.8	3.2	108[f]
3[g]	CH_2OH	O	0.9	2.8[h]	3.4[h]
4	CH_2OH	S	6.9	26	>100
	BASE = Cytosine-1-yl				
5	CH_2OH	O	140[h]	17[h]	1.9-14[h]
6	CH_2OH	S	_ [i]	_ [i]	>100

[a]The IC_{50}s were measured by the plaque reduction assay as described in references 13, 26 and 27. [b]Strain ICI in Vero H. cells. [c]Strain 6350 in MRC-5 cells. [d]Strain AD169 in HFF cells. [e]Acyclovir. [f]As reported by K.K. Biron, et al. in reference 12. [g]Ganciclovir. [h]As reported by L. Beauchamp, et al. in reference 13. [i]Inactive at 50 μg per disc by the plaque inhibition assay.

activity of the thio analogue 2[15] of acyclovir (1) and is similar to the results reported by McGee, et al.[16] Although the cytosine acyclic nucleoside 5 has good activity against HCMV, its thio analogue 6 was not active (IC_{50} > 100 μM), nor was 6 active against HSV-1 or VZV.

Thus, although the bioisosteric replacement of sulfur for oxygen in potential chemotherapeutic agents is sometimes successful, this structural modification of the potent antiherpetic agents ganciclovir (3) and 5 resulted in a substantial loss in antiviral activity.

EXPERIMENTAL SECTION

NMR spectra were recorded on a Varian XL-300 (^1H NMR, 300 MHz; ^{13}C NMR, 75.43 MHz), a Varian XL-200 (^1H NMR, 200 MHz), a Varian FT-80A

(^{1}H NMR, 80 MHz), a Varian T-60 (^{1}H NMR, 60 MHz), and a Hitachi Perkin-Elmer R-24 spectrometer (^{1}H NMR, 60 MHz). Chemical shift values are reported in parts per million on the δ scale with tetramethylsilane as the internal reference. UV spectra were recorded on a Unicam SP 800 or a Perkin-Elmer 571 spectrophotometer. Data from the latter was analyzed by a Digital Specialties Microcomputer. Mass spectra (~50 eV) were obtained from Oneida Research Services, Whitesboro, NY, using a Finnegan 4500 TFQ mass spectrometer. Elemental microanalyses were determined by Atlantic Microlabs, Atlanta, GA, and gave combustion values for C,H,N,S within 0.4% of theoretical values. Preparative flash chromatography[27] was done using Silica Gel 60 (40-63 μm, E. Merck No. 9385). Analytical thin-layer chromatography was done using silica gel (200 μ) MK GF (Whatman) plates. Melting points were determined with a Thomas Hoover or Mel-Temp capillary melting point apparatus and are uncorrected.

2-(Benzyloxy)-1-[(benzyloxy)methyl]ethyl 4-toluenesulfonate (8).

Purified[28] p-toluenesulfonyl chloride (84.8 g, 0.445 mol) was added in portions to a solution of 1,3-bis(benzyloxy)-2-propanol[18] (7) (120.0 g, 0.441 mol) in dry pyridine (600 ml) that was cooled to 0°C. After 66 h at 0°C the solution was decanted from the residual solid into ice H_2O (2.4 L). The mixture was extracted with Et_2O (2 x 1.0 L), and the combined extract was washed with cold 1 N HCl (4 x 250 ml). The organic layer was dried and spin evaporated *in vacuo* at ambient temperature at aspirator pressure and then at ~1 mm Hg with an oil pump to give 164.0 g (87%) of 8 as an oil:[16] NMR (60 MHz, DMSO-d_6) δ 7.04-7.78 (m,14H,ArH), 4.73 (m,1H,CH), 4.39 (s,4H,$ArCH_2$), 3.64 (d,4H,OCH_2C), 2.37 (s,3H,CH_3).

2-(Benzyloxy)-1-[(benzyloxy)methyl]-S-ethyl thioacetate (9).

A solution of potassium thioacetate (110.0 g, 0.961 mol), 8 (164.0 g, 0.384 mol), and dry DMF (500 ml) was stirred at 80-90°C for

2 h. The reaction was cooled to ambient temperature and poured into ice H_2O (2.5 L). The resultant mixture was extracted with Et_2O (6 x 1.0 L). The combined Et_2O extract was divided into two portions, and each was washed with H_2O (3 x 500 ml) and dried (anhydrous Na_2SO_4). The solution was spin evaporated in vacuo at approximately 40°C to give 126.0 g (99%) of 9 as a dark oil:[16] NMR (60 MHz, DMSO-d_6) δ 7.31 (s,10H,ArH), 4.60 (s,4H,$ArCH_2$), 3.55-4.20 (overlapping multiplets, 5H,OCH_2C and CH), 2.42 (s,3H,CH_3).

1,3-Bis(benzyloxy)-2-propanethiol(10).

A mixture of 20% aqueous KOH (300 ml) in EtOH (300 ml) and 9 (123.0 g, 0.372 mol) was refluxed with stirring for 1.5 h under a nitrogen atmosphere. The reaction solution was cooled to ambient temperature, and the excess EtOH was removed by spin evaporation. The resultant mixture was diluted with H_2O, and the pH of the solution was adjusted to 6 with AcOH. The mixture was extracted with Et_2O (4 x 600 ml), and the combined extract was divided into two portions. Each portion was washed with H_2O (2 x 500 ml) and dried (anhydrous Na_2SO_4). The dried Et_2O solution was spin evaporated in vacuo at 40°C at aspirator pressure and then at ~1 mm Hg with an oil pump to give 103.0 g (96%) of 10 as a crude oil. The oil was dissolved in 300 ml of EtOAc:hexane - 1:5, and the solution was added to a column (9.5 cm x 28 cm) of Silica Gel 60 wetted with EtOAc:hexane - 1:6. The column was eluted with EtOAc:hexane - 1:6, and the highest Rf component was collected in seven 200 ml fractions. The combined fractions were spin evaporated in vacuo to give 50.0 g (47%) of 10 as a yellow oil:[16] NMR (60 MHz, DMSO-d_6) δ 7.30 (s,10H,ArH), 4.56 (s,4H,$ArCH_2$), 3.71 (d,4H,OCH_2C), 3.25 (m,1H,CH), 1.98 (d,1H,SH).

1,3-Bis(benzyloxy)-2-[(chloromethyl)thio]propane (11).

A mixture of 10 (25.0 g, 86.7 mmol), 95% paraformaldehyde (2.74 g, 28.9 mmol), and dry 1,2-dichloroethane (200 ml) was stirred at 0°C on an

ice-salt bath. A stream of HCl gas was bubbled through the reaction mixture for 45 min. The reaction was dried with $CaCl_2$. After 1.5 h the mixture was filtered, and the solids were washed with 1,2-dichloroethane. The filtrate was spin evaporated in vacuo to give 29.0 g (94%) of 11 as a yellow oil, which contained 5% residual dichloroethane: NMR (60 MHz, DMSO-d_6) δ 7.27 (s,10H,ArH), 4.82 (s,2H,CH_2Cl), 4.52 (s,4H,$ArCH_2$), 3.78 (d, 4H,OCH_2C), 3.45 (s,1H,CH).

2-Amino-9[[[2-benzyloxy-1-(benzyloxymethyl)ethyl]thio]methyl]-6-chloro-9H-purine (12).

A mixture of 2-amino-6-chloro-9H-purine (3.62 g, 21.3 mmol), ammonium sulfate (0.362 g, 27.4 mmol), and hexamethyldisilazane (50 ml) was refluxed with stirring under a N_2 atmosphere for 18 h. The excess hexamethyldisilazane was removed by distillation under aspirator vacuum and in the presence of a $CaCl_2$ drying tube. The residue was cooled and dissolved in dry toluene (75 ml) under an N_2 atmosphere. Mercuric cyanide (5.79 g, 22.9 mmol) and 11 (7.90 g, 23.5 mmol) in dry toluene (50 ml) was added to the solution, and the reaction was heated at 80°C for 3 h. The reaction mixture was spin evaporated in vacuo, and CH_2Cl_2 (300 ml) was added to the syrup. The mixture was stirred for 0.5 h and filtered. The solids were washed with CH_2Cl_2 (50 ml), and the combined filtrate and wash were washed with 30% aqueous KI (2 x 75 ml). The organic phase was washed with 10% aqueous K_2CO_3 (2 x 75 ml) and H_2O (2 x 75 ml), and dried with Na_2SO_4. The combined aqueous phase was extracted with CH_2Cl_2 (4 x 300 ml), and the organic phase was dried (Na_2SO_4). The combined solutions were evaporated to give an oil (11.0 g) that was dissolved in CH_2Cl_2 (200 ml). Silica Gel 60 was added to the solution, and the volatiles were removed by spin evaporated in vacuo. The residual solids were added to a column (6 cm x 18 cm) of Silica Gel 60 wetted with methylene chloride. The column was eluted

with CH_2Cl_2 (1 L), CH_2Cl_2:MeOH - 98:2 (1 L), CH_2Cl_2:MeOH - 96:4 (2 L), and CH_2Cl_2:MeOH - 90:10 (1 L) by flash chromatography; 75-100 ml fractions were collected. Fractions 31-49, which contained the highest R_f major spot, were combined and concentrated to give 1.27 g (13%) of 12 as an oil. The oil was rechromatographed with hexane:EtOAc - 1:1. Fractions 19-49, which contained the highest R_f major spot, were combined and concentrated to give 1.27 g (13%) of 12 as an oil; TLC, hexane:EtOAc - 3:2, one spot with R_f = 0.64; UV (pH 1,7,13) λ_{max} 308, λ_{min} 270 nm; ^{1}H-NMR (200 MHz, DMSO-d_6): δ 8.20 (s,1H,H-8), 7.19-7.30 (s,10H, ArH), 6.98 (br s, 2H, NH_2), 5.29 (s,2H,NCH_2S), 4.39 (m,4H,$ArCH_2$), 3.51-3.55 (m,4H,OCH_2C), 3.38 (m,1H,CH); (MS): m/e 379 (M^+-C_7H_7), 215 (MH^+-$C_{17}H_{19}O_2$), 183 (MH^+-$C_{17}H_{19}O_2S$), 91 ($C_7H_7^+$). Anal. ($C_{23}H_{24}ClN_5O_2S \cdot 0.5\ H_2O$) C,H,N,S.

2-[[(2-Acetamido-1,6-dihydro-6-oxo-9H-purin-9-yl)methyl]thio]-1,3-propanediyl diacetate (13).

A mixture of 12 (1.02 g, 2.18 mmol), ferric chloride (0.50 g, 3.08 mmol), and acetic anhydride (50 ml) was heated at 80-90°C with stirring for 4 h and then at ambient temperature for 18 h. The reaction mixture was filtered through a pad of Celite, and the pad was washed with MeOH (75 ml). (The filtration was exothermic - the receiver flask should be cooled.) The filtrate was spin evaporated in vacuo to a dark oil that was dissolved in CH_2Cl_2:MeOH (150 ml:5 ml). Silica gel (10 g) was added to the solution, and the mixture was spin evaporated in vacuo. The residual solids were introduced on a column (5 cm x 20 cm) of Silica Gel 60 wetted with CH_2Cl_2. The column was eluted with MeOH:CH_2Cl_2 - 5:95 by the flash chromatography technique. The fractions containing the major component were collected and spin evaporated in vacuo to give 0.356 g (41%) of 13. Recrystallization from EtOAc:EtOH gave 0.203 g (23%) of analytically pure 13, mp 225-226°C; TLC, MeOH: CH_2Cl_2 (10:90),

one spot with R_f = 0.44; UV (pH 1) λ_{max} 260.5, λ_{min} 230.5, sh 275 nm; (pH 7) λ_{max} 259, λ_{min} 232, sh 279 nm; (pH 13) λ_{max} 264, λ_{min} 240.5 nm; ^{1}H-NMR (80 MHz, DMSO-d$_6$): δ 11.95 and 11.57 (br s, 2H, N(1)H, NHAc), 8.10 (s,1H,H-8), 5.32 (s, 2H, NCH_2S), 4.12 (d,4H,OCH_2C), 3.45 (m,1H,CH), 2.19 (s,3H,$NCOCH_3$), 1.96 (s,6H,$COCH_3$); (MS): m/e 397 (M^+), 337 (M^+-HOAc), 238 (M^+-$C_7H_{11}O_4$), 206 ($C_8H_8N_5O_2^+$), 43 ($COCH_3^+$). Anal. ($C_{15}H_{19}N_5O_6S$) C,H,N,S.

9-[[[2-Hydroxy-1-(hydroxymethyl)ethyl]thio]methyl]guanine (4).

A mixture of 13 (0.175 g, 0.440 mmol), 40% aqueous methylamine (10 ml), and EtOH (10 ml) was heated on a steam bath for 15 min. Additional 40% aqueous methylamine (10 ml) and EtOH (10 ml) was added to the reaction. After 15 min the volatiles were spin evaporated in vacuo, and the residue was triturated in Et_2O (50 ml). Ethanol (5 ml) was added to the residue, and the solids were collected and washed with Et_2O to give 0.117 g (98%) of 4. Recrystallization from H_2O gave 0.096 g (81%) of analytically pure 4, mp 224-226°C (ref. 16 reports mp 221-222°C); TLC, MeOH:CH_2Cl_2 (3:7), one spot with R_f = 0.54; UV (pH 1) λ_{max} 258 (ε11,000), λ_{min} 230 (ε2700) nm, sh 281 (ε7500) nm; (pH 7) λ_{max} 256 (ε12,000), λ_{min} 226 (ε3300), sh 271 (ε9300) nm; (pH 13) λ_{max} 268 (ε11,000), λ_{min} 234 (ε4100) nm; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ 10.59 (br s,1H,NH), 7.78 (s,1H,H-8), 6.49 (br s,2H,NH_2), 5.14 (s,2H,NCH_2S), 4.78 (t,2H,OH), 3.51 (t,4H,OCH_2C), 2.88 (m,1H,CH); (MS): m/e 271 (M^+), 241 (M^+-CH_2O), 196 (M^+-$C_3H_7O_2$), 165 (MH^+-$C_3H_7O_2S$), 151 (guanine$^+$). Anal. ($C_9H_{13}N_5O_3S \cdot 1.5\ H_2O$) C,H,N,S.

1-[[[2-(Benzyloxy)-1-[(benzyloxy)methyl]ethyl]thio]methyl]cytosine (14).

A mixture of cytosine (5.00 g, 45.0 mmol), ammonium sulfate (0.025 g, 0.19 mmol), and hexamethyldisilazane (100 ml) was refluxed with stirring under an N_2 atmosphere for 2 h. The excess

hexamethyldisilazane was removed by distillation at atmospheric pressure, and the residue was diluted with toluene (50 ml). A solution of 11 (3.03 g, 8.99 mmol) in toluene (5 ml) was added, and the solution was refluxed for 18 h. The volatiles were removed by spin evaporation in vacuo, and EtOH (150 ml) was added to the residue. The mixture was refluxed for 1 h, and the solvent was removed by spin evaporation in vacuo. The residue was dispersed in CH_2Cl_2 (250 ml), stirred for 15 min, and filtered. The solids were redispersed in CH_2Cl_2 (250 ml) and refiltered. The combined filtrates were spin evaporated to give 3.95 g of a waxy foam. This residue was dissolved in MeOH:CH_2Cl_2 (25 ml:125 ml), Silica Gel 60 (30 g) was added to the solution, and the solvent was removed under reduced pressure. The residual solids were added to a column (6.5 cm diameter) of Silica Gel 60 and eluted with MeOH:CH_2Cl_2 (5:95 v/v) to remove the major product. The appropriate fractions were combined and spin evaporated in vacuo to give 2.15 g (58%) of 14, mp 126-129°C; TLC, MeOH:CH_2Cl_2 (10:90), one spot with R_f = 0.56; UV (pH 1) λ_{max} 286 (ε11,700), λ_{min} 244 (ε1600) nm; (pH 7) λ_{max} 277 (ε9100), λ_{min} 254 (ε6300); (pH 13) λ_{max} 277 (ε8600) nm, λ_{min} 253 (ε5500) nm; ^{1}H-NMR (200 MHz, DMSO-d_6): δ 7.62 (d,1H,J=7.3 Hz,H-6), 7.28 (complex multiplet, 10H,Ar), 7.14 (br d,2H,NH_2), 5.67 (d,1H,J=7.3 Hz, H-5), 4.89 (s,2H,NCH_2S), 4.44 (s,4H,CH_2Ar), 3.56 (d,4H,OCH_2C), 3.40 (m,1H,CH); (MS): m/e 412 (M^++1), 156 ($C_5H_6N_3OS^+$), 124 ($C_5H_6N_3O^+$), 107 ($C_7H_7O^+$), 91 ($C_7H_7^+$). Anal. ($C_{22}H_{25}N_3O_3S$) C,H,N.

1-[[[2-Hydroxy-1-(hydroxymethyl)ethyl]thio]methyl]cytosine (6).

A mixture of 14 (1.74 g, 4.23 mmol) in CH_2Cl_2 (25 ml) was cooled to -78°C in a dry ice-acetone bath. A solution of boron trichloride (1 M) (50 ml, 50 mmol) in CH_2Cl_2 was added, and the mixture was stirred at -78°C for 3 h. The reaction was quenched by dropwise addition of MeOH:CH_2Cl_2 (50 ml:50 ml) at -78°C. The pH of the mixture was adjusted

to 7-8 with Et_3N (21 ml), and the reaction was brought to ambient temperature. The volatiles were removed by spin evaporation, H_2O (15 ml) was added to the residue, and the volatiles were evaporated. Addition of H_2O and spin evaporation was repeated twice. The residue was diluted with $CHCl_3$ (100 ml) and stirred for 16 h. The solution was filtered through Celite, the pad was rinsed with $CHCl_3$ (2 x 50 ml), and the combined filtrate and wash were spin evaporated in vacuo. The residue was dissolved in 50% aqueous EtOH (100 ml), and then stirred with hydroxide ion exchange resin (Rexyn 201) until a silver nitrate test for chloride ion was negative. The resin was removed by filtration, and the filtrate was evaporated to give 0.83 g of an oil. The oil was dissolved in MeOH and adsorbed on Silica Gel 60 (10 g). The residual solids were added to a silica gel column (5 cm diameter) wetted with CH_2Cl_2. The column was eluted with 1 L of MeOH:CH_2Cl_2 (10:90), with 1 L of MeOH:CH_2Cl_2 (25:75), and finally with 1 L of MeOH:CH_2Cl_2 (30:70). Fractions containing the lower R_f, major product were combined and spin evaporated in vacuo to give 0.68 g of a clear oil. The oil was triturated with a mixture of MeOH (minimum), acetone, and Et_2O to afford 0.578 g of a white solid, mp 138-141°C. The solids were recrystallized from EtOH (50 ml) to give 0.316 g (32%) of 6, mp 158-161°C; TLC, MeOH:CH_2Cl_2 (3:7), one spot with R_f = 0.47; UV (pH 1) λ_{max} 283 (ε12,200), λ_{min} 242 (ε1500) nm; (pH 7) λ_{max} 274 (ε8890), λ_{min} 251 (ε5320) nm; (pH 13) λ_{max} 276.5 (ε9170), λ_{min} 253.5 (ε5560) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.65 (d,1H,H-6), 7.13 (br d,2H,NH_2), 5.70 (d,1H,H-5), 4.87 (s,2H,NCH_2S), 4.76 (t,2H,OH), 3.52 (d,4H,CH_2O), 2.92 (m,1H,CH); ^{13}C-NMR (75.43 MHz, DMSO-d_6): δ 165.76 (C-4), 155.36 (C-2), 144.90 (C-6), 94.03 (C-5), 61.31 (C-4'), 49.39 (C-3'), 48.62 (C-1'); (MS): m/e 232 (M^++1), 156 (M^+-$C_3H_7O_2$), 124 (M^+-$C_3H_7O_2S$). Anal. ($C_8H_{13}N_3O_3S$) C,H,N,S.

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Appendix

Analytical Data

	Calc'd.				Found			
No.	C	H	N	S	C	H	N	S
4	36.24	5.41	23.48	10.75	36.34	5.39	23.48	10.70
6	41.55	5.67	18.17	13.86	41.43	5.72	18.14	13.80
12	57.67	5.26	14.62	6.69	57.92	5.25	14.63	6.71
13	45.34	4.82	17.62	8.07	45.41	4.86	17.59	8.03
14	64.21	6.12	10.21	-	64.18	6.15	10.20	-

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