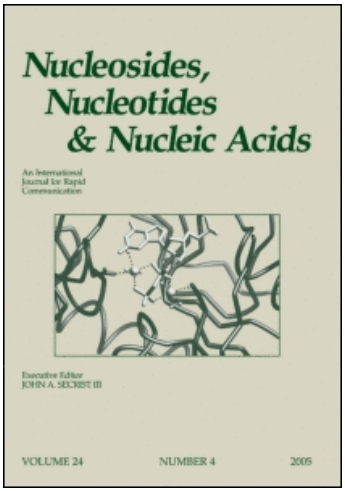

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Synthesis of 5-Mercaptocytosine Nucleosides and Nucleotides

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SYNTHESIS OF 5-MERCAPTOCYTOSINE NUCLEOSIDES AND NUCLEOTIDES

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Abstract: The synthesis of a series of new nucleosides and nucleotides, including ribo-, 2-deoxyribo- and arabinofuranosides of 5-sulfur-substituted cytosines, is described. The synthetic methods employed involve 5-thiolation of the appropriate cytosine or 5-bromocytosine nucleosides and nucleotides, or alternatively, 4-thiation followed by amination of the corresponding protected 5-(S-benzyl)mercaptouracil nucleosides and subsequent deblocking with sodium and liquid ammonia.

In view of the interesting chemical and biological properties of some 5-mercaptopyrimidine nucleosides[1-5] and of polynucleotides containing these moieties [6-8]; it was of interest to investigate the syntheses and properties of the still unreported monomeric 5-mercaptocytosine nucleosides and nucleotides by several alternative procedures.

5-Mercaptocytidine (7a) and 2'-deoxy-5-mercaptocytidine (7b) were first prepared *via* thiation of the S-benzyl, O-(p-chlorobenzoyl) protected 5-mercaptouridine and 5-mercapto-2'-deoxyuridine, respectively, followed by amination of the corresponding 4-thio derivatives according to the general method of Fox *et. al.*[9]. Subsequent reaction with sodium in liquid ammonia removed all blocking groups without reducing the 5,6-double bond of the cytosine moiety. However, this four-step synthesis was tedious, and it proved to be difficult to purify the final products from contamination with ammonium salts (Scheme 1).

The above method was then modified by using the 4-silyl intermediate (10) obtained in an attempted synthesis of 2'-deoxy-5-mercaptouridine *via* the S-benzyl derivative as described by Kotick et. al.[1] (Scheme 1/a). However, when

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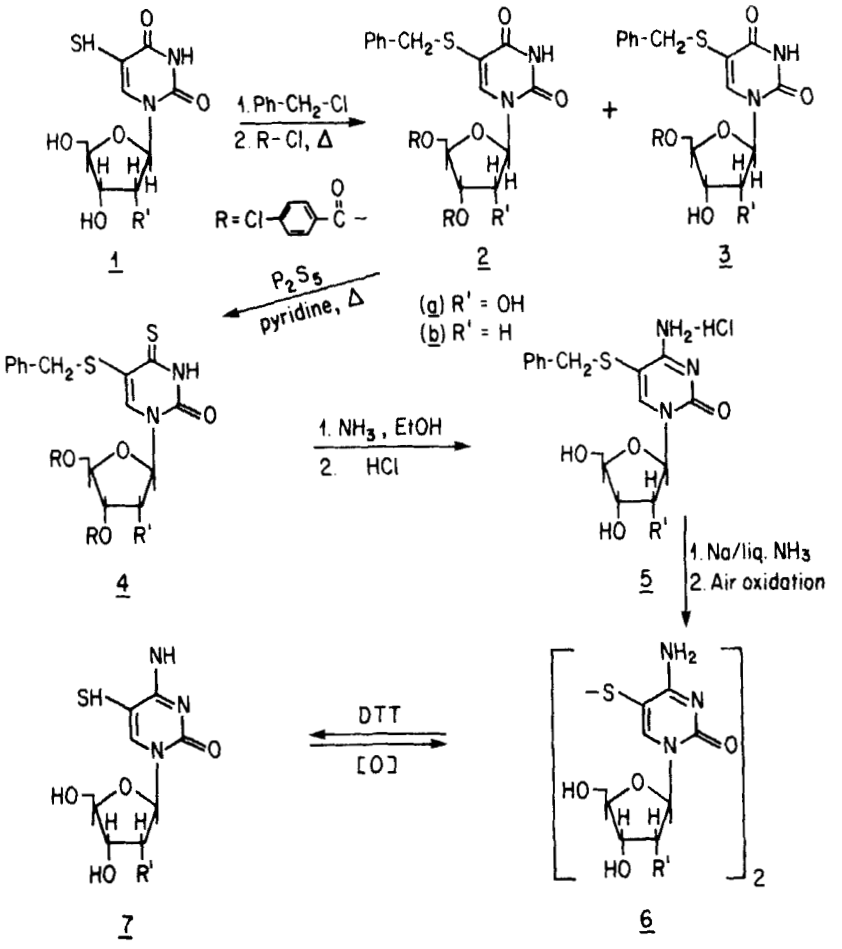
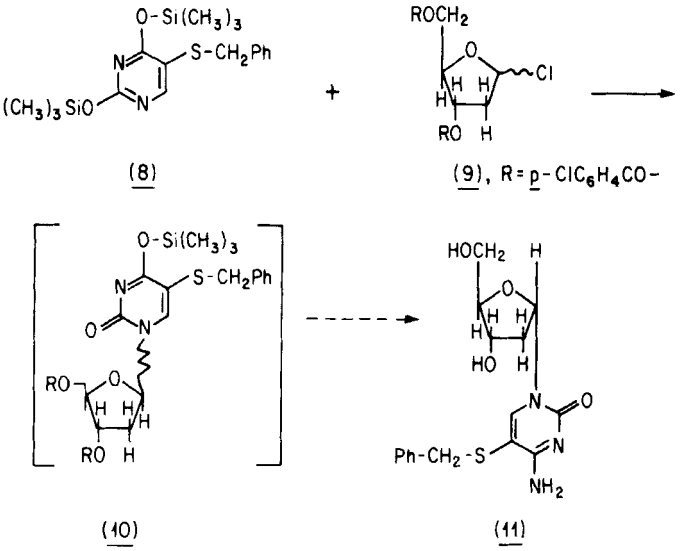


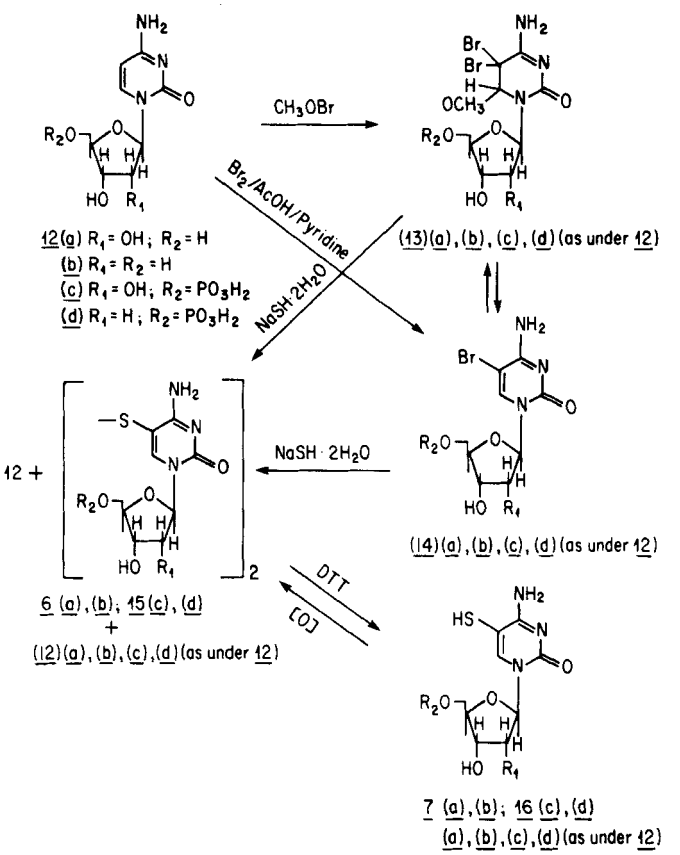
Scheme 1

compound 10 was treated with liquid ammonia in a steel bomb at 145°C for 16 hours, it gave a very low yield (< 1%) of the α-anomer of the desired 5-sulfur substituted 2'-deoxycytidine, in the form of the crystalline hydrochloride salt (11) as the only isolatable nucleoside (Scheme 1/a).

Subsequently, we tried a different route for the synthesis of the desired 5-mercaptocytosine nucleosides by applying the general method developed in this laboratory for the partial "5-thiolation" of uracil and/or cytosine-containing polynucleotides[7,12]. Essentially, this method consists of two steps: addition of methyl hypobromite in methanol at 5°C, followed by reaction with sodium hydrosulfide in dimethylacetamide (Scheme 2). In the case of uracil nucleotides, one mole-equivalent of methyl hypobromite was found to add quantitatively to the 5,6-double bond; the resulting adduct, a 5-



Scheme 1/a



Scheme 2

bromo-6-methoxy-5,6-dihydrouracil derivative, was then treated with sodium hydrosulfide, or preferably with sodium disulfide[13]. Each of these sulfide reagents converted the adduct in part to the corresponding 5-mercaptouracil derivative (via nucleophilic displacement of the bromine followed by the elimination of methanol), while the remaining part of the adduct was reverted to the uracil starting material (via the elimination of methyl hypobromite)[13]. However, in the case of cytidine (12a), one mole-equivalent of methyl hypobromite caused partial bromination, yielding a small amount of 5-bromocytidine (14a) as the only isolatable product, while treatment with two mole-equivalents of methyl hypobromite resulted in the apparently quantitative conversion of 12a to 5,5-dibromo-6-methoxy-5,6-dihydrocytidine (13a), based on the UV and NMR spectra. The analogous product derived from 2'-deoxycytidylic acid, 13d, was fully characterized (Scheme 2).

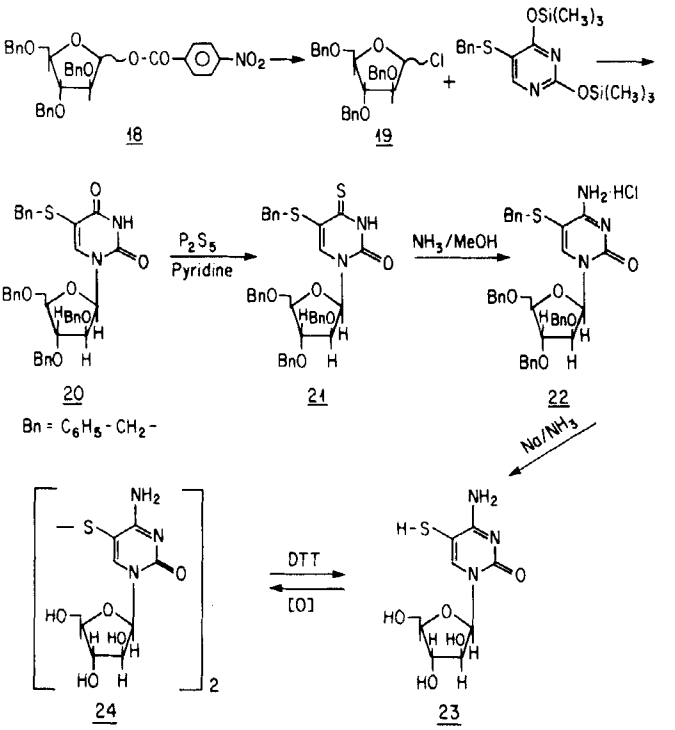
In contrast to the methyl hypobromite adducts of N_1-substituted uracil derivatives[13], those of the 5-bromocytosine nucleosides and nucleotides, 13(a-d), upon reaction with sodium disulfide ($Na_2S_2 \cdot 5H_2O$) were converted to the corresponding 5,6-unsaturated 5-bromocytosine derivatives, 14(a-d). On the other hand, excess sodium hydrosulfide ($NaSH \cdot 2H_2O$) converted each of the 5-bromocytosine adducts 13(a-d) to a mixture of the desired 5-mercaptocytosine derivative 6(a,b) or 15(c,d) with the corresponding cytosine nucleoside 12(a-d), in a similar manner as in the case of the analogous uracil derivatives[13]. It was established that the 5-bromocytidine adduct 13a was first converted with one mole-equivalent of sodium hydrosulfide to 5-bromocytidine (14a), being identical with an authentic sample of this compound (tlc, UV). Subsequently, the reaction proceeded with 14a and two additional mole-equivalents of sodium hydrosulfide presumably via a similar sequence of addition-substitution-elimination reactions as it was proposed in the case of the analogous reaction of N_1-methyl-5-bromouracil, the mechanism of which had been studied in detail[13].

As in the case of the 5-mercaptouracil derivatives, the 5-mercaptocytosines 15(a-d) were obtained in the form of the corresponding disulfides. Unfortunately, their separation from the reaction mixtures was quite difficult and the yields of analytically pure products were low. Therefore, in order to avoid the presence of methyl hypobromite and/or of its reaction products with sodium hydrosulfide in the reaction mixture, the above procedure was modified in that the appropriate 5-bromocytosine derivatives 14(a-d) were prepared from the corresponding unsubstituted cytidines 12(a-d) by direct bromination in acetic acid-pyridine. The 5-bromocytosine derivatives were isolated and then treated with sodium hydrosulfide, as above. This resulted in cleaner reaction mixtures and somewhat better separation of

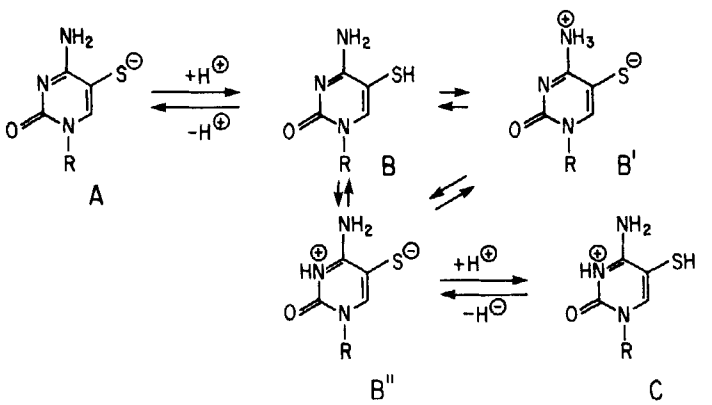
the desired products than in the in situ reaction from the methyl hypobromite adducts. Furthermore, it was observed that the use of about 5 mole-equivalents of sodium hydrosulfide in dimethylacetamide at 50°C for 7-8 hours, or alternatively, at room temperature for 1-3 days (under nitrogen atmosphere), gave the optimal yields of 5-mercaptocytosine derivatives.

While the above "5-thiolation" procedure proved to be eventually more successful for the synthesis of 5-mercaptocytidine, 5-mercapto-2'-deoxycytidine, and their 5'-phosphates, than the first mentioned method involving 4-thiation followed by amination of the corresponding 5-mercaptouracil derivatives, the latter method was found to be much superior for the synthesis of the 5-mercaptocytosine arabinoside (see Scheme 3). Its starting material, the fully benzyl-protected 5-mercaptouracil-β-D-arabinoside (20) was obtained by the condensation of 2,3,5-tri-O-benzyl-α-D-arabinofuranosyl chloride (19) with silylated 5-(S-benzyl)-mercaptouracil in very high yield (90%) and anomeric purity. (The remarkable stereoselectivity of the reactions of this particular benzyl-protected halogenose has been studied by Szekeres;[14] see also ref. 11). Treatment of the blocked nucleoside 20 with threefold excess of phosphorus pentasulfide in pyridine gave 1-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-5-(S-benzyl)mercapto-4-thiouracil (21) in 76% yield. Treatment with saturated methanolic ammonia at 95°C for 20 hours gave the hydrochloride salt of 1-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-5-(S-benzyl)-mercapto-cytosine (22) in 89% yield. Removal of the benzyl groups with sodium and liquid ammonia gave the disulfide of N_1-(β-D-arabinofuranosyl)-5-mercaptocytosine (24) in a yield of 64% (Scheme 3).

The ionization constants of the 5-mercaptocytosine nucleosides were determined by spectrophotometric titration according to the classical method of Shugar and Fox[15], using N_1-ethyl-5-mercaptocytosine (25) as a model compound (which also served as a model in some of the synthetic studies). Since all 5-mercaptocytosine derivatives were found to undergo air oxidation in neutral or basic solution as readily as the 5-mercaptouracil derivatives[16,17] to the corresponding disulfides, and the latter were reduced again by the addition of dithiothreitol (DTT) with similar ease to the free 5-mercapto compounds, the titrations of 25 were conducted in the presence of DTT. It was found that, at neutral and basic pH, the ionized 5-mercapto group of A (see Scheme 4) gives rise to an absorption maximum at 333 nm (ε=3945), just as in the case of the 5-mercaptouracils[16]. However, in contrast to the latter, lowering of the pH shifts this absorption maximum toward longer wavelength; between 4-5, the λ_{max} is at 363 nm, which is presumably due to a zwitter-ionic species such as B' or B". Further lowering of the pH shifts the λ_{max} toward lower wavelength; at pH 1.44, λ_{max}=293 nm (ε=7681) corresponding to the cationic species C (see Scheme 4).



Scheme 3



Scheme 4

The pK_a values of the two reaction equilibria corresponding to the protonation steps were calculated[14] from the results of measuring the change in optical density at 363 nm as a function of varying pH. Maximal absorption at this wavelength is observed at pH 4.33 (ε=3544) indicating the isoelectric point where the compound appears to exist predominantly in the zwitter-ionic form. The two pK_a values were found to be 5.72 and 2.64 respectively. Thus, the thiol group of the 5-mercaptocytosines, as that of the 5-mercaptouracil derivatives, is essentially ionized at physiologic pH.

The three 5-mercaptocytosine nucleosides, in the form of the disulfides, were tested for biological activity in vitro in the leukemia L1210 cell culture assay of Bloch[18]. Both the 2'-deoxyriboside (6b) and the riboside (6a) exhibited moderate but significant inhibitory effect, with $I_{50} = 5 \times 10^{-5}$ M and 2×10^{-5} M, respectively, The arabinoside (5-mercapto-ara-C-disulfide, 24) showed only 30% inhibition at 10^{-4} M.

EXPERIMENTAL SECTION

All melting points were taken on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord or Beckman IR 8 spectrophotometer. NMR spectra were recorded on a Varian Model A-60 spectrophoto-meter in $CDCl_3$ unless otherwise indicated, with TMS as an internal standard. UV spectra were obtained on Beckman Model 25 and Gilford 2400 S spectrophotomers. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA, and Galbraith Laboratories, Knoxville, TN. 5-bromo-2'-deoxy-cytidine and 2'-deoxycytidine-5'-monophosphoric acid were purchased from Sigma Chemical Co., St. Louis, MO.

Synthesis of bis [1-(2-deoxy-β-D-ribofuranosyl)-cytosine-5-yl] disulfide (6b).

Method A: from 5-mercapto-2'-deoxyuridine (1b: Scheme 1).

N_1-[3,5-Di-O-(p-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-5-(S-benzyl)-mercaptouracil (2b). To a solution of 5-mercapto-2'-deoxyuridine[1] (1.56 g, 6 mmol) in anhydrous dimethylformamide (DMF; 15 ml) was added benzyl chloride (0.69 g, 6 mmol) and triethylamine (1.05 ml, 7.5 mmol), and the mixture was stirred under N_2 atmosphere for 12 hours. The crystalline Et_3N^+HCl was filtered off and the DMF evaporated in vacuo to give an oil. This was dissolved in anhydrous pyridine (50 ml), and after the addition of p-chlorobenzoyl chloride (2.62 g, 15 mmol), the mixture was refluxed for 3.5 hours. After cooling, the dark colored solution was poured on ice-water (200

g). The aqueous solution was extracted with $CHCl_3$ (3 x 80 ml), and the $CHCl_3$ layer was washed with water, saturated $NaHCO_3$ (3x), saturated ice cold Na_2CO_3, and again with water. After drying over $MgSO_4$, the chloroform was evaporated *in vacuo* and the residual pyridine was co-evaporated with ethanol. The oily residue was dissolved in $CHCl_3$ (20 ml), and after standing at room temperature, deposited crystals of a partially acylated product which was identified as analytically pure N_1-[5-O-(p-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-5-(S-benzyl)-mercaptouracil. (3b; 0.787 g, 26.8%); mp 170-171°C; $[\alpha]_D$ -15.5° (c0.5, EtOAc). Anal. Calcd. for $C_{23}H_{21}ClN_2O_6S$: C, 56.50; H, 4.33; Cl, 7.25; N, 5.73; S, 6.56. Found: C, 56.70; H, 4.31; Cl, 7.40; N, 5.74; S, 6.47.

The chloroform mother liquor was applied to a silica gel column (25 x 1.5 cm) in $CHCl_3$ and was eluted with $CHCl_3$. Fractions containing the fully blocked 2'-deoxy-5-mercaptouridine (2b) were collected and concentrated to give a solid foam (1.227 g, 32.7%). Crystallization from ethanol resulted in a pure (tlc) semi-crystalline product, 2b; $[\alpha]_D$ -32.4° (c1.1, $CHCl_3$). NMR ($CDCl_3$) δ 8.36 ppm (C-6, 1H); 7.5-8.15 ppm m(8H) exhibiting a typical *para* splitting pattern for the two p-chlorobenzoyl blocking groups; 7.22 s(5H) phenyl group; 6.20 ppm t (anomeric H); 5.62 ppm s(1H); 4.61 ppm (5H) sugar moiety; 3.28 ppm (2H) benzylic-CH_2 group. Anal. Calcd. for $C_{30}H_{24}Cl_2N_2O_7S$: C, 57.42; H, 3.80; Cl, 11.30; N, 4.46; S, 5.11. Found: C, 56.91; H, 3.79; Cl, 11.89; N, 4.40; S, 5.09. This preparation of 2b was used for the next step in the synthetic scheme.

N_1-[3,5-Di-O-(p-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-4-thio-5-(S-benzyl)-mercaptouracil (4b). To a solution of 2b (0.627 g, 1 mmol) in anhydrous pyridine (20 ml) was added phosphorus pentasulfide (P_2S_5; 0.720 g, 12 mmol), and the mixture was refluxed (oil bath) 6 hours. After cooling, it was poured on ice-water (100 g) and extracted with $CHCl_3$ (3 x 70 ml). After drying over $MgSO_4$, the $CHCl_3$ was evaporated *in vacuo* and the residual pyridine was removed by co-evaporation with ethanol. The solid residue was crystallized from ethanol, yielding long needles of the product 4b (0.42 g, 65.6%); mp 166-168°C. Recrystallization from EtOH raised the mp to 171-172°C. $[\alpha]_D$ -44.28° (c1.5, $CHCl_3$). NMR (CD_3OD): δ 6.22 ppm pseudo t (anomeric H); 7.65 s(C-6H).

Alternatively, an anomeric mixture of N_1-[3,5-di-O-(p-chlorobenzoyl)-2-deoxy-D-ribofuranosyl]-5-(S-benzyl)-mercaptouracils, prepared by the condensation of the blocked chlorosugar with 2,4-bis-O-(trimethylsilyl)-5-(S-benzyl)-mercaptouracil[1], was subjected to thiation with P_2S_5 in anhydrous pyridine (as described above), and the α and β anomers of the blocked 4-thio

nucleotides were subsequently separated by chromatography on a silica gel column (40 x 5 cm, packed in benzene) with benzene-chloroform (85:15) as eluent. Each fraction was checked by tlc (silica gel plates, EtOAc-$CHCl_3$ 20:80; in this system, the β anomer had R_f = 0.87, the α anomer R_f = 0.71). Only fractions containing the pure anomers were combined and concentrated to dryness. Both pure anomers were crystallized from ethanol; 2.68 g (29.8%) yield of the β anomer, mp 169-171°C, and 0.807 g (10.4%) yield of the α anomer, mp 169-171°C were obtained. The β anomer was identical with the sample 4b derived from authentic 5-mercapto-2'-deoxyuridine (see above) and their mixed mp showed no depression. For the α-anomer, $[\alpha]_D$ -101.81° (c1.6 $CHCl_3$), and the NMR spectrum showed a pseudo-quartet for the anomeric proton at δ 6.2 ppm. Anal. Calcd. for $C_{30}H_{24}Cl_2N_2O_6S_2$: C, 55.99; H, 3.75; Cl, 11.02; N, 4.35; S, 9.96. Found, for β-anomer (4b): C, 55.82; H, 3.68; Cl, 11.15; N, 4.38; S, 10.19. Found, for α-anomer: C, 56.10; H, 3.79; Cl, 11.03; N, 4.46; S, 10.09.

N_1-(2-Deoxy-β-D-ribofuranosyl)-5-(S-benzyl)-mercaptocytosine hydrochloride (5b). A solution of 4b (0.643 g, 1 mmol) in ethanol (20 ml), previously saturated with NH_3 gas at 0°C, was heated at 95°C (oil bath) for 24 hours. After cooling, the ethanol was evaporated *in vacuo* and the residue was subjected to high vacuum at 80-90°C for 2 hours to remove side products. The residue was then treated with hot water (100 ml) and the aqueous filtrate was extracted with $CHCl_3$ (3 x 25 ml). The aqueous layer was concentrated *in vacuo* and dried by repeated additions and evaporations of ethanol. The residue was dissolved in ethanol (12 ml), cooled in ice-water, and HCl gas was bubbled through for 3 minutes. Addition of ether (90 ml) caused separation of a white precipitate which became crystalline after 12 hours at 5°C (0.32 g, 83%); mp 129-132°C (dec.). Recrystallization from ethanol-ether furnished analytically pure product (5b): mp 133-134°C (dec.) $[\alpha]_D$ -53.05° (c1.2, EtOH). NMR (CD_3OD): δ 7.65 ppm S(C-6H), 5.42 ppm pseudo t (anomeric H). Anal. Calcd. for $C_{16}H_{20}ClN_3O_4S$: C, 49.80; H, 5.22; Cl, 9.19; N, 10.89; S, 8.31. Found: C, 49.82; H, 5.16; Cl, 9.11; N, 11.03; S, 8.16.

Bis[1-(2-Deoxy-β-D-ribofuranosyl)-cytosine-5-yl]-disulfide (6b). Compound 5b (1.898 g, 4.92 mmol) was dissolved in liquid NH_3 (100 ml) and sodium metal was added (0.230 g; 10 mg-atom). After complete dissolution of the sodium, NH_4Cl (0.535 g; 10 mmol) was added, and the liquid NH_3 was allowed to evaporate. The residue was washed with Et_2O, and was then dissolved in EtOH (150 ml) and stirred overnight while exposed to air. The EtOH solution containing some insoluble material was heated to boiling, and some insoluble NaCl was removed by filtration. The ethanol solution was concentrated several times and the product (6b) was crystallized from ethanol but was contaminated

with NH_4Cl. Repetitive fractional crystallization in water gave 6b (0.910 g, 71.6%) which appeared pure by tlc, and its UV spectral properties were similar to those of the analytical sample obtained by Method B (see below). However, this product was still contaminated with NH_4Cl as shown by its somewhat lower ε value and unsatisfactory elemental analysis.

Method B: from 5-bromo-2'-deoxycytidine (Scheme 2).

To a solution of 5-bromo-2'-deoxyctidine (14b) (0.612 g, 2 mmol) in anhydrous dimethylacetamide (10 ml), finely ground sodium hydrosulfide dihydrate ($NaSH \cdot 2H_2O$) (0.92 g, 10 mmol) was added under nitrogen. The reaction mixture was stirred at 50°C for 7 to 8 hours at which time the disappearance of 5-bromo-2'-deoxycytidine (tlc) was complete. Ether (3 x 30 ml) was added to the reaction mixture and the resulting greenish gummy residue was dissolved in 15 ml water, and the solution was adjusted to pH 4.0. The precipitated sulfur was removed by filtration, and the pH of the filtrate was brought to 7.5. The disulfide formed by air oxidation was reduced with 0.1 M of 2-mercaptoethanol, and the solution was applied to a DEAE cellulose column (40 x 6 cm, HCO_3^- form) equilibrated with 0.01 M triethylammonium bicarbonate buffer containing 0.01 M 2-mercaptoethanol. The column was washed with 0.01 M triethylammonium bicarbonate buffer (TEAB) containing 0.01 M mercaptoethanol. The 5-mercapto-2'-deoxycytidine bound to the column was eluted with a linear gradient of 0.01 M to 0.2 M TEAB at pH 7.5. The fractions containing the mercaptonucleoside were pooled and evaporated to dryness in vacuo. The residue was dissolved in a minimum amount of H_2O and applied to a Bio-Gel P-2 column (110 x 3 cm). The product was eluted with H_2O. The fractions containing the thiolated product were pooled, concentrated and lyophilized. In some cases, the Bio-Gel column step was repeated to remove traces of impurities. The lyophilized powder was twice recrystallized from water to give needles of the product (0.211 g; 20.5%); mp 290°C (dec); NMR (DMSO) δ 5.77 (t, C'_1-H); 7.72 (s, C_6-H); UV (pH 8); λ_{max} 272 nm (ε 14.9 x 10^3); λ_{min} 250 nm (ε 13.9 x 10^3). On addition of DTT: λ_{max} 335nm (ε 4.17 x 10^3) and λ_{min} 292 nm (ε 1.83 x 10^3) where the ε values are based on the MW of the reduced (monomeric) thiol 7b. Anal. Calcd. for $C_{18}H_{24}N_6O_8S_2 \cdot (0.5\ H_2O)$: C, 41.14; H, 4.79; N, 15.99; S, 12.20. Found: C, 40.82; H, 4.22; N, 15.84; S, 12.03.

Synthesis of bis[N_1-(β-D-ribofuranosyl)cytosine-5-yl]-disulfide (6a).

Method A: from 5-mercaptouridine (Scheme 1).

This synthesis of 6a, which was analogous to that of 6b by Method A (see above) will not be described here. The final product, after extensive purification, still contained a trace of NH_4Cl.

Method B: from 5-bromocytidine (Scheme 2).

Compound 6a was synthesized from 5-bromocytidine [19] (14a) and purified in an analogous manner as described under Method B for the corresponding 2'-deoxy analog, 6b (see above). However, after the purification on Bio-Gel P-2 column, various attempts to crystallize the compound were unsuccessful. The compound was dissolved in hot anhydrous methanol. On cooling, white amorphous powder was obtained which was dissolved in water and lyophilized, giving 91 mg of 6a (16.6%). mp 175-180°C (dec); $[\alpha]_D^{25}$ + 293.6° (c0.52, H_2O); UV (pH 8) λ_{max} 275 nm (ε 15.0 x 10^3, based on the MW of the thiol 7a). Anal. Calcd. for $C_{18}H_{24}N_6O_{10}S_2$: C, 39.41; H, 4.41; N, 15.32; S, 11.69. Found: C, 39.25; H, 4.45; N, 15.15; S, 11.50.

Method C: from the methyl hypobromite-adduct of 5-bromocytidine (13a, Scheme 2).

The adduct, 5,5-dibromo-6-methoxy-5,6-dihydrocytosine 13a was prepared in an analogous manner as described for the systhesis of 13d, below. UV: λ_{max} 237 nm; NMR (CD_3COCD_3): δ 5.65 (s, 1H, C-6), 3.4 (s, 3H, CH_3O).

To a cold suspension of $NaHS \cdot H_2O$ (5.3 g) in anhydrous DMF (20 ml) at 15°C was added under N_2 a solution of the adduct 13a in dry DMF (30 ml). The reaction mixture was stirred under N_2 for 2 hours at 15°C, then at room temperature for another 36 hours. After addition of ether (300 ml) and cooling of the reaction mixture, a brown gummy precipitate was obtained. This was dissolved in water (20 ml) and chromatographed on a Bio-Rad AG1-X2 (OH^-) column (50 x 2.5 cm). The column was eluted with 50% aqueous methanol followed by 0.2 N NH_4Cl solution. Fractions absorbing at λ_{max} 335 nm (with DTT) were collected and lyophilized, giving a white material which was desalted by twice applying it to a Sephadex G-25 column (78 x 2.8 cm) and eluting with water. The fractions containing the product were collected, lyophilized and dried in vacuo over P_2O_5 to give 0.21 g (3.3%) of the analytically pure product (6a), mp 175°C (dec), having identical spectra as described under Method B (see above).

Synthesis of Bis N_1-(β-D-arabinofuranosyl)cytosine-5-yl disulfide (24; Scheme 3).

2,3,5-Tri-O-benzyl-1-O-(p-nitrobenzoyl)-D-arabinofuranose anomers (18). Anomeric 2,3,5-tri-O-benzyl-1-O-(p-nitrobenzoyl)-D-arabinofuranose (5.0 g, 8.7 mmol), prepared according to the procedure of Barker and Fletcher[20], was dissolved in refluxing n-pentane-Et_2O (4:5) mixture, and the volume was reduced to 350 ml by boiling off some of the solvent. After standing at 5°C for 12 hours, the crystals were collected by filtration (1.825 g); mp 95-98°C.

Recrystallization of this compound from n-pentane-Et_2O (1:1) gave pure α anomer (1.3 g); mp 98-100°C, $[\alpha]_D$ +59.71° (c2.5, $CHCl_3$). The C_1 proton appeared as a somewhat broadened singlet at δ 6.32 ppm. Gradual concentration of the original mother liquor gave rise to a second, third, and fourth crystal crop. The second crop (0.594 g) was almost pure β-anomer, mp 78-80°C; $[\alpha]_D$ -41.66°. The third crystal crop (0.78 g) was a mixture of the two anomers; mp 73-75°C; $[\alpha]_D$ -9.9°. The fourth crystal crop consisted of pure β-anomer (0.423 g); mp 79-80°C; $[\alpha]_D$ -43.65° (c2.4, CH_2Cl_2). The C_1 proton appeared as a poorly resolved doublet at δ 6.55 ppm. Anal. Calcd. for $C_{33}H_{31}NO_8$: C, 69.58; H, 5.49; N, 2.46. Found, α-anomer: C, 69.78; H, 5.68; N, 2.46. Found, β-anomer: C, 69.67; H, 5.63; N, 2.45.

2,3,5-Tri-O-benzyl-α-D-arabinofuranosyl chloride (19). Samples (0.250 g) of each of the pure anomers of the fully blocked sugar (18) and of the anomeric mixture as well, were separately dissolved in CH_2Cl_2 (5 ml) previously saturated with HCl gas at 0°C. The solutions were kept at 0°C for 2 hours, then p-nitrobenzoic acid was removed by filtration, and the CH_2Cl_2 was evaporated to give the syrupy halogenose[19]. The NMR spectra of all three samples obtained in this manner were identical; δ 6.15 ppm, s(C-1 H).

N_1-(2,3,5-Tri-O-benzyl-β-D-arabinofuranosyl)-5-(S-benzyl)-mercaptouracil (20). To a solution of 19 (5.696 g, 10 mmol) in CH_2Cl_2 (50 ml) was added 2,4-bis-(O-trimethylsilyl)-5-(S-benzyl)-mercaptouracil[1] (3.68 g, 10 mmol) in 50 ml of CH_2Cl_2. The solution was evaporated under reduced pressure, and the residual mixture was stirred vigorously at 110°C in vacuo (6-12 mm Hg). After 30 minutes, when gas evolution stopped completely, the resulting homogeneous melt was dissolved in C_6H_6 (60 ml), H_2O (1-2 ml) was added and allowed to stand for 12 hours. The solvent was removed in vacuo, and the residue was treated with $CHCl_3$ (200 ml). Insoluble 5-(S-benzyl)-mercaptouracil (mp 295-300°C) was removed by filtration (0.402 g, 17.2% recovery), and the $CHCl_3$ solution was concentrated to 30-40 ml. This was purified on a column of silica gel (30 x 3.2 cm in $CHCl_3$) by eluting with $CHCl_3$. Fractions containing a single reaction product by tlc ($CHCl_3$:EtOAc 9:1) were combined and concentrated to dryness. The resulting viscous oil obtained was rechromatographed in an analogous manner to give 4.61 g (90.5%, based on the reacted silyl pyrimidine) of the product as an oil; $[\alpha]_D$ -12.79° (c4.4, $CHCl_3$). In the NMR spectrum the C-1' proton appeared as a doublet at 6.25 ppm; J = 5.0 Hz. This product (20) was used without further purification for the next step.

N_1-(2,3,5-Tri-O-benzyl-β-D-arabinofuranosyl)-4-thio-5-(S-benzyl)-mercaptouracil (21). The fully-benzyl protected arabinoside 20 was converted to the corresponding 4-thio analog in the same manner as described above for

the conversion of 2b to 4b (Method A). After evaporation of the pyridine, the oily residue was dissolved in small amount of C_6H_6 and applied to a silica gel column (30 x 3.2 cm) packed in benzene. The product, collected by eluting with benzene-chloroform (1:1) (3.44 g, 76.2%), showed a single spot on silica gel plate (R_f = 0.3, $CHCl_3$). It was rechromatographed to give an analytically pure sample of 21 as an oil: $[\alpha]_D$ +44.55° (c2.2, $CHCl_3$); NMR: the anomeric proton showed a doublet at 6.18 ppm (J = 5.0 Hz). Anal. Calcd. for $C_{37}H_{36}N_2O_5S_2$: C, 68.07; H, 5.56; N, 4.29; S, 9.82. Found: C, 68.20; H, 5.55; N, 4.60; S, 10.10.

N_1-(2,3,5-Tri-O-benzyl-β-D-arabinofuranosyl)-5-(S-benzyl)-mercaptocytosine hydrochloride (22). To a solution of 21 (2.7 g, 4.13 mmol) in EtOH (40 ml) was added 60 ml of saturated (0°C) ethanolic ammonia solution and the mixture was heated at 90°C in a steel bomb for 24 hours. After cooling, the ethanol was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$ (80 ml). The $CHCl_3$ solution was extracted with water, and after drying over $MgSO_4$ concentrated to dryness. The semisolid residue was dissolved in EtOH (45 ml), cooled in ice-water, and HCl gas bubbled through for 5 minutes. Addition of ether (300 ml) caused immediate crystallization of the hydrochloride salt (22) (2.478 g, 89.4%); mp 137-139°C. This was recrystallized from EtOH:Et_2O to give an analytically pure sample; mp 140.5-141.5°C. $[\alpha]_D$ +42.52° (c2.1, EtOH). NMR: C-1' proton appeared as a doublet at 6.20 ppm; J = 5.0 Hz. Anal. Calcd. for $C_{37}H_{38}ClN_3O_5$: C, 66.10; H, 5.70; Cl, 5.27; N, 6.25; S, 4.77. Found: C, 66.30; H, 5.65; Cl, 5.30; N, 6.05; S, 4.60.

Bis-[N_1-(β-D-arabinosyl)-cytosine-5-yl]-disulfide (24). The procedure used for the debenzylation of 22 (1.14 g, 1.7 mmol) was essentially the same as described above for the deblocking of 5b. It yielded a crystalline product (0.298 g, 64%). Recrystallization from ethanol furnished an analytically pure sample of 24. UV (pH 9.1): λ_{max} 274 nm; after addition of DTT, λ_{max} 333 nm (ε 4.2 x 10^3, calculated for the thiol 23). Optical rotation of the disulfide form[21] (24): $[\alpha]_D$ +438.0° (c0.35, H_2O), and that of the reduced form, 23 (determined in EDTA buffer of pH 8.8 containing a large excess of 2-mercaptoethanol): $[\alpha]_D$ +39.40° (c0.52, H_2O). Anal. Calcd. for $C_{18}H_{24}N_6O_{10}S_2$: C, 39.41; H, 4.41; N, 15.32; S, 11.69. Found: C, 39.35; H, 4.70; N, 15.10; S, 11.50.

Synthesis of Bis[1-(5-O-phosphono-2-deoxy-β-D-ribofuranosyl)-cytosine-5-yl]-disulfide (15d) (Scheme 2).

A fresh and cold (-15°C) solution of MeOBr[22] (7 mmol in 30 ml MeOH) was filtered directly through a Celite pad into a solution of 2'-deoxycytidine-5'-

monophosphoric acid disodium salt (dCMP $Na_2 \cdot 2.5\ H_2O$: 1 g, 2 mmol) in methanol (20 ml) with stirring at -10° (CCl_4 with dry ice). Stirring and cooling were continued for 30 minutes. The reaction was followed by UV until the solution showed λ_{max} only at 237 nm in methanol. The pale yellow solution was concentrated to approximately 4 ml in vacuo at 20°C. The residue was treated with cold ether (125 ml, 0°C) and allowed to stand for 6 hours at 4°C. A white precipitate formed which was filtered, and dried in vacuo over P_2O_5 for 2 hours at 4°C, to yield 1.37 g (92.6%) of 5,5-dibromo-6-methoxy-5,6-dihydro-2'-deoxycytidine-5'-phosphate (13d). In the melting point tube, 13d foamed at 45°C and the remaining white solid decomposed at 135-145°C. UV λ_{max} (pH 2) 236 nm (ε 11.2 x 10^3) λ_{max} (MeOH) 237 nm (ε 10.5 x 10^3) NMR (DMSO-d_6) δ 3.18 (s, CH_3O-6). Anal. Calcd. for $C_{10}H_{14}Br_2N_3O_8P_1Na_2 \cdot 2.5H_2O$: C, 20.50; H, 3.27; Br, 27.27; N, 7.17. Found: C, 20.70; H, 3.15; Br, 27.40; N, 7.05.

A concentrated solution of sodium methoxide (0.6 mmol) in methanol (5 ml) was dissolved in dimethylacetamide (15 ml), flushed with N_2, and H_2S gas was bubbled through slowly for 2 hours, during which time the mixture became deep blue in color. Excess of methanol and H_2S were removed in vacuo, and the powdered 13d (1.2 g; 2mmol) was added at -5°C, with vigorous stirring under N_2. Stirring was continued under N_2 for 24 hours at room temperature. Ether (250 ml) was added, and the tan colored precipitate formed was filtered and dried in vacuo. This was dissolved in water (30 ml), filtered through a Celite pad to remove the insoluble material. The filtrate was diluted to 350 ml with 0.005 M TEAB solution (pH 7.8) and applied to a Cellex-D (HCO^-) column (26 x 2.5 cm)[23] which was equilibrated with the same buffer. The column was first washed with 0.005 M TEAB solution (200 ml) which was followed by a linear gradient of TEAB solution (pH 7.8) from 0.005 M to 0.3 M (800 ml each). The first peak fractions contained 2'-deoxycytidine 5'-monophosphate (21.8% recovery). The second peak fractions containing the desired product were combined and evaporated in vacuo at 35°C. The residue was dissolved in water (10 ml) and lyophilized to give 0.145 g of a yellow solid; λ_{max} 275 nm (pH 8 buffer); upon addition of DTT: λ_{max} 332 nm. The lyophilized product was dissolved in water, and the aqueous solution was adjusted to pH 7.85 with saturated aqueous barium hydroxide solution. The barium salt was precipitated by the addition of ethanol, filtered and dried, to give the analytically pure Ba salt of 15d as a white solid which decomposed at about 180°C. Anal. Calcd. for $C_{18}H_{22}N_6O_{14}S_2P_2Ba_2 \cdot 3H_2O$: C, 21.60; H, 2.80; N, 8.40; S, 6.40; P, 6.20. Found: C, 21.65; H, 2.99; N, 8.19; S, 6.51; P, 6.15.

Synthesis of Bis[1-(5-O-phosphono-β-D-ribofuranosyl)-cytosine-5-yl]-disulfide (15c).

This was synthesized as described for the corresponding deoxycytidylic acid derivative except that NaSH was added instead of generated in situ.

After isolation and purification, the yield of the product was 15%. UV (pH 8): λ_{max} 279 nm (ε 14.0 x 10^3). After addition of DTT: λ_{max} 334 nm (ε 4.0 x 10^3, calculated for the thiol 16c). Anal. Calcd. for the barium salt $C_{18}H_{22}N_6O_{16}S_2P_2Ba_2 \cdot 2H_2O$: C, 21.24; H, 2.75; N, 8.26; P, 6.09. Found: C, 21.54; H, 2.90; N, 8.49; P, 6.13.

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