Synthesis of 5-S-Substituted 2'-Deoxyuridines. Study of the Factors Influencing the Stereoselectivity of the Silyl Modification of the Hilbert–Johnson Reaction[1]

MICHAEL P. KOTICK,[2a] CSABA SZANTAY, AND THOMAS J. BARDOS[2b]

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214

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Coupling of bis-*O*-(trimethylsilyl)-*S*-substituted 5-mercaptouracils (**3b–f**) with 2-deoxy-3,5-di-*O*-(*p*-chlorobenzoyl)-α-D-ribofuranosyl chloride (**4**) under various reaction conditions allowed stereoselective synthesis of the β and α anomers of the corresponding fully protected 2'-deoxyribonucleosides. The anomeric configuration of the nucleosidic products was found to be dependent on the presence or absence of trimethylsilyl chloride during the condensation; reaction conditions allowing rapid removal of the trimethylsilyl chloride formed lead to the β anomer as the only isolatable nucleosidic product, while continued presence of the trimethylsilyl chloride in the reaction mixture favors formation of the α anomer. The halogenose **4**, on standing in benzene solution, was found to undergo changes in optical rotation, with eventual degradation to *p*-chlorobenzoic acid, furfuryl-*p*-chlorobenzoate, and a disaccharide, **14**. This process is prevented in the presence of trimethylsilyl chloride. The coupling reactions with the silylpyrimidines, leading to either β or α nucleosidic products, are viewed as proceeding uniformly *via* an SN2 mechanism, with inversion of configuration at the anomeric carbon of the α or β halogenose. The free nucleosides were obtained by sodium methoxide catalyzed deacylation. The mercapto nucleosides are rapidly oxidized to the corresponding disulfides, which exhibit very high optical rotation and Cotton effects.

In a preliminary communication,[3] we reported the synthesis of the anomeric 2'-deoxyribonucleosides of 5-mercaptouracil. These compounds have interesting physico-chemical[4] and biological[5] properties. We now wish to report further studies relating to the stereoselectivity of the coupling reaction used in the synthesis of these anomeric nucleosides and to describe the preparation and some physico-chemical properties of several new compounds in this series.

5-*S*-Benzylmercaptouracil (**2b**), originally obtained by Johnson and Guest[6] by cyclization of nonpyrimidine components, was prepared by the reaction of 5-mercaptouracil (**1**)[7] with benzyl chloride in the presence of base. Preliminary experiments indicated that treatment of **2b** with excess sodium in liquid ammonia yielded the debenzylated compound **1** without reduction of the 5,6 double bond. Reaction of **1** with acetic anhydride in refluxing pyridine gave 5-*S*-acetylmercaptouracil (**2c**) in good yield. 5-*S*-Methylmercaptouracil (**2d**) was prepared by reaction of the thiolate anion of **1** with methyl iodide in aqueous solution. In a similar manner, the 5-*S*-ethyl- (**2e**) and 5-*S*-butylmercaptouracils (**2f**) were prepared by reaction of **1** with the appropriate alkyl halide. Carpenter and Shaw[8] have recently reported the synthesis of several 5-*S*-alkylmercaptouracils by a similar route. In addition, the formation of 5-*S*-methylmercaptouracil (**2d**) by the reaction of uracil with dimethyl sulfoxide and monochloromethyl ether has been reported.[9]

The silyl ethers (**3a–f**) used in this work were prepared by reaction of the appropriate pyrimidine (**2a–f**) with 2 mol equiv of trimethylsilyl chloride in refluxing benzene and triethylamine as the acid acceptor.[10] Several of these silyl ethers were obtained as crystalline solids upon prolonged standing at low temperatures; however, in most cases, the freshly distilled viscous silylpyrimidines were used directly in the condensation reactions (Scheme I).

Condensation of bis-*O*-(trimethylsilyl)-5-*S*-benzylmercaptouracil (**3b**) with 2-deoxy-3,5-di-*O*-(*p*-chlorobenzoyl)-α-D-ribofuranosyl chloride[3,11] (**4**) was conducted under fusion conditions. Although the mixed anomers **6b** and **7b** could be purified by column chromatography, separation of the anomeric blocked nucleosides was not achieved and crystalline material could not be obtained. Condensation of the *S*-acetyl derivative **3c** with 1 mol equiv of the blocked deoxyribosyl halide **4**, however, provided the α and/or β anomers of the corresponding fully protected nucleoside (**7c** and/or **6c**) which could be isolated in an anomerically pure state by fractional crystallization. Preliminary work[3] indicated variations in the anomeric composition of the nucleosidic product when the condensation (coupling reaction) was conducted under differing reaction conditions.

In our preliminary studies,[3] using small quantities of the reactants, when the coupling reaction of the silylpyrimidine with the glycosyl halide **4** was conducted in refluxing benzene solution (method A) or by fusion at 100° (method B), only the β anomer of the blocked nucleoside was isolated. In contrast, when the reaction was conducted in benzene solution at 37° (method C), the α anomer was the only isolated product. Therefore, it was initially concluded that the steric course of the coupling reaction was controlled by the temperature at which it was conducted.[3] However, in subsequent preparations which were usually conducted at somewhat larger scales, the products obtained by use of either of these methods contained varying ratios of the α and β anomers, and it became apparent that, in addition to the temperature, some other factors play an important role in determining the stereoselectivity of the coupling reaction.

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(2) (a) Abstracted in part from the Ph.D. dissertation of M. P. K., State University of New York at Buffalo, Feb 1968. (b) To whom inquiries should be addressed.

(3) T. J. Bardos, M. P. Kotick, and C. Szantay, *Tetrahedron Lett.*, 1759 (1966).

(4) T. I. Kalman and T. J. Bardos, *J. Amer. Chem. Soc.*, **89**, 1171 (1967).

(5) K. Baranski, T. J. Bardos, A. Bloch, and T. I. Kalman, *Biochem. Pharmacol.*, **18**, 347 (1969).

(6) T. B. Johnson and H. H. Guest, *Amer. Chem. J.*, **42**, 271 (1909).

(7) (a) T. J. Bardos, R. R. Herr, and T. Enkoji, *J. Amer. Chem. Soc.*, **77**, 960 (1955); (b) T. J. Bardos, R. R. Herr, and T. Enkoji, *ibid.*, **78**, 401 (1956).

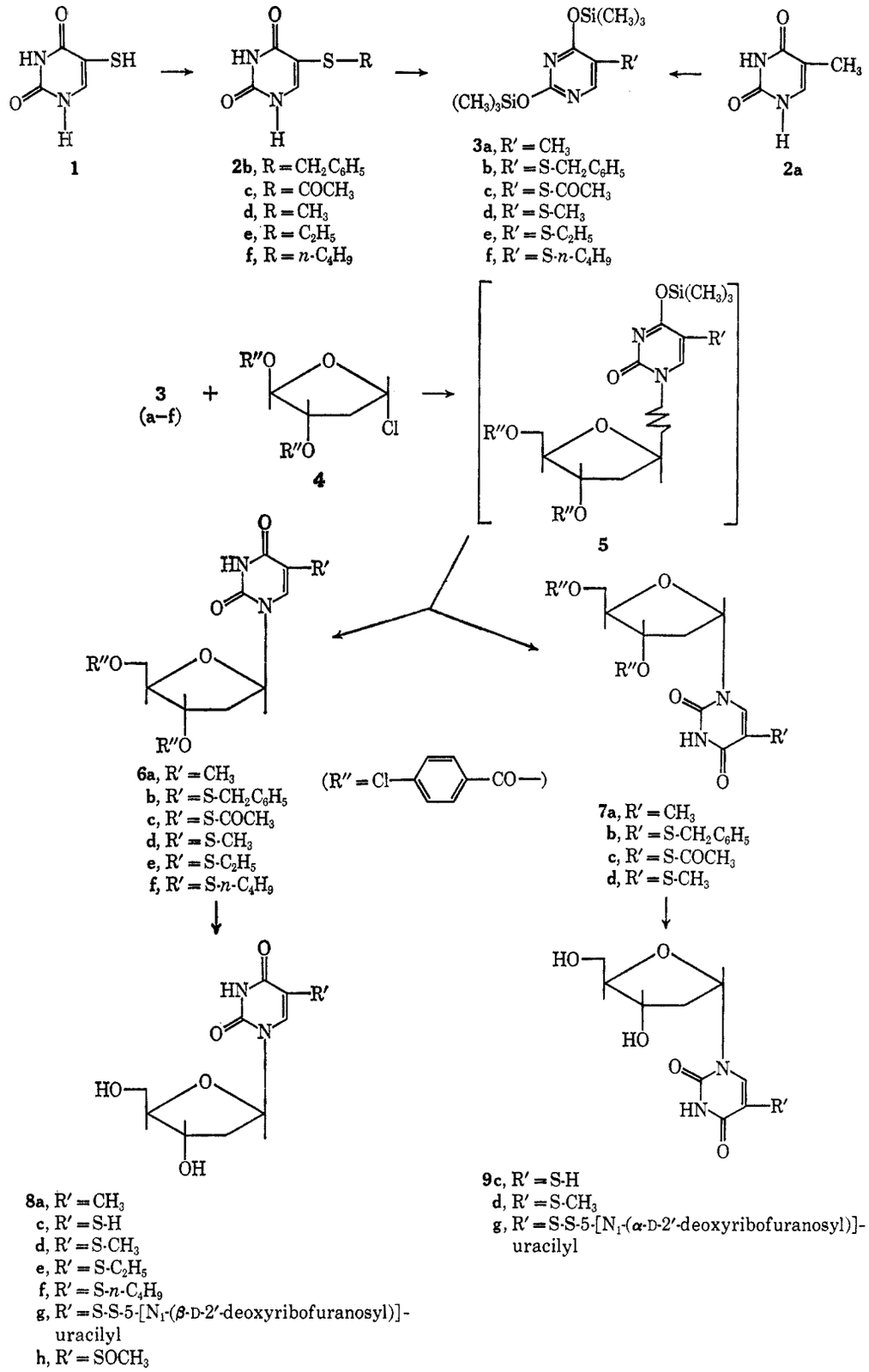
(8) J. M. Carpenter and G. Shaw, *J. Chem. Soc.*, 3987 (1965).

(9) K. Anzai and S. Suzuki, *Agr. Biol. Chem.* (Tokyo), **30**, 597 (1966).

(10) T. Nishimura and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 352 (1964).

(11) J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, *J. Amer. Chem. Soc.*, **83**, 4066 (1961).

SCHEME I



For this reason, a series of experiments was conducted using various silylpyrimidines (**3a–d**) under varying reaction conditions (see Table I). The results indicate that conditions promoting rapid removal of the trimethylsilyl chloride by-product lead to the predominant or exclusive formation of the β nucleoside (see methods D, F, and G). In contrast, addition of trimethylsilyl chloride to the reaction mixture results in the predominant formation of the α anomer (see method E). Thus, the varying results obtained when the original methods A, B, and C were employed at different reaction scales appear to be due to the fact

TABLE I

CONDENSATION OF SILYLPYRIMIDINES WITH THE HALOGENOSE 4 UNDER VARIOUS CONDITIONS

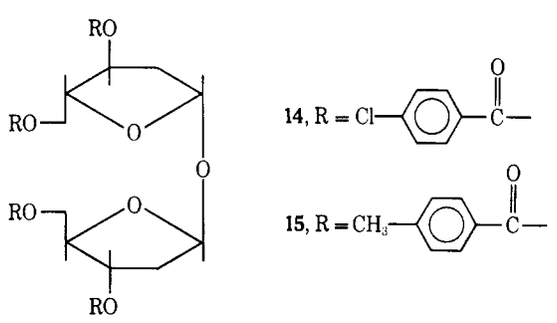
Expt no.	Method[a]	Silyl-pyrimidine	Scale, mmol	Yield,[b] %	Yield,[c] %	Predominant anomer	Ratio[d] of α/β	Remarks
1	A	**3c**	2	10	16	β	...	
2	A	**3c**	5	45	62	α	...	
3	A	**3d**	3	51	92	β	1:4.6	
4	A	**3a**	5	67	91	α	1:0.6	
5	B	**3c**	43	39	48	α	...	
6	B	**3c**	10	36	40	β	...	
7	B	**3a**	1	36	39	β	1:3.2	
8	C	**3c**	4	11	15	α	...	110 hr, 37°[e]
9	C	**3d**	5	60	87	α	1:0.8	300 hr, 37°[e]
10	C	**3d**	10	49	86	β	1:4.0	150 hr, 25°[e]
11	C	**3a**	5	30	57	β	1:2.0	215 hr, 25°[e]
12	D	**3c**	8	43	63	β	1:4.0	1.1 ml/min[f]
13	D	**3c**	4	29	56	β	...	0.6 ml/min
14	D	**3d**	7	36	56	β	...	3.0 ml/min
15	D	**3a**	5	34	80	β	1:3.2	1.5 ml/min
16	E	**3c**	7	51	67	α	1:0.4	1.1 ml/min
17	E	**3d**	7	47	71	α	1:0.3	2.5 ml/min
18	F	**3c**	10[g]	41	50	β	...	
19	F	**3c**	4	38	45	β	...	
20	F	**3d**	10	58	75	β	...	
21	F	**3e**	8	50	61	β	...	
22	F	**3f**	8	58	66	β	...	
23	G	**3c**	35	60	76	β	...	
24	G	**3c**	41	52	62	β	...	

[a] For description of methods A–G, see Experimental Section. [b] Yield of total purified protected nucleosides (6 + 7), based on silylpyrimidine (**3**). [c] Yield of same, based on reacted (unrecovered) pyrimidine. [d] Ratio of anomers in total nucleosidic products; where no ratio is indicated, the "predominant anomer" was the only isolable product. [e] Reaction time and temperature. [f] Rate of distillation of solvent under azeotropic conditions, while maintaining a constant (25-ml) reaction volume. [g] Larger scale reactions required stirring (method G) to give consistent results.

that in these experiments the amount of trimethylsilyl chloride present in the reaction mixture was not controlled and represented an unknown variable.[12]

If the coupling reaction proceeds by nucleophilic attack of the silylpyrimidine on the glycosyl halide **4**, with a single Walden inversion (SN2 mechanism), then the reaction of a pure α halogenose should lead to the formation of a β nucleoside. This was, indeed, the only isolable product in most experiments except when trimethylsilyl chloride was present in the reaction mixture. Therefore, it appears that trimethylsilyl chloride may cause anomerization of the halogenose under the reaction conditions, presumably by chloride exchange. Reaction of the β halogenose with the silylpyrimidine *via* an SN2 mechanism would result in the formation of α nucleoside.

To study the effect of trimethylsilyl chloride on the glycosyl halide reactant, **4**, alone, the latter (which we obtained with a higher melting point than previously reported[11] and believed to be the essentially pure α anomer[13]) was allowed to stand in benzene solution at room temperature in the presence and absence of trimethylsilyl chloride. Suprisingly, a substantial decrease within a few hours in the optical rotation of **4** was obtained only in the absence of trimethylsilyl chloride, while, in the presence of the latter, the optical rotation of **4** appeared to be stabilized (see Figure 1). On prolonged standing in benzene solution, *p*-chlorobenzoic acid (**12**) and furfuryl-*p*-chlorobenzoate (**13**)[14] could be isolated in addition to a small amount of **14**. If triethylamine was added to the benzene solution, a similar decrease in rotation was observed, and, in addition to the above products, triethylamine hydrochloride was isolated. Molecular weight determination of **14** indicated a dimeric structure. Similarity of the nmr spectrum and specific rotation of **14** to those reported by Keller, *et al.*,[15] for the disaccharide **15** served to confirm the structure of this product.



Since a carbonium ion intermediate (**10**) (see Scheme II) would presumably be involved in the formation of all of these degradation products (**12**, **13**, and **14**) of the glycosyl halide **4**, it appears that the presence of trimethylsilyl chloride either prevents or reverses the

(12) This is particularly evident if one compares expt no. 5 (Table I) with all the other "fusion" reactions (methods B, F, and G), which generally favor formation of the β anomer. Experiment 5 was run on a relatively large (43-mmol) scale, without evacuation or mechanical stirring, so that the trimethylsilyl chloride could not escape from the thick, viscous melt. Characteristically, this experiment yielded the α anomer as the only isolable product.

(13) A. K. Battacharya, R. K. Ness, and H. G. Fletcher, *J. Org. Chem.*, **28**, 428 (1963).

(14) M. Prystas and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 2960 (1965).

(15) F. Keller, J. E. Bunker, and L. H. Brown, *J. Org. Chem.*, **31**, 3840 (1966).

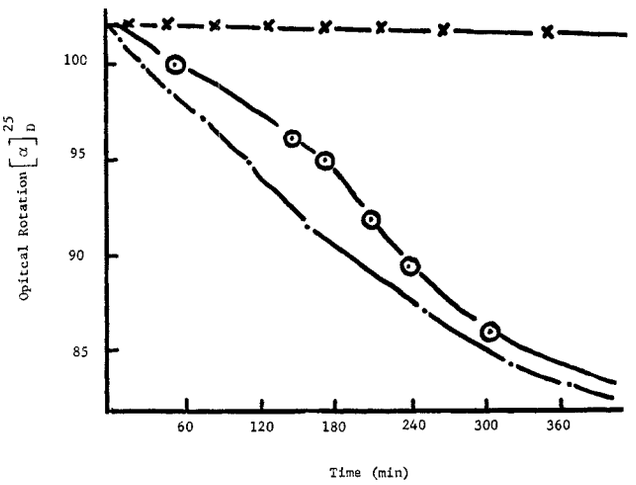


Figure 1.—Changes in the optical rotation of the halogenose **4**, on standing at room temperature: —·— in dry benzene; —⊙— in dry benzene, with 1 mol equiv of triethylamine; —x— in dry benzene, with 1 mol equiv of trimethylsilyl chloride.

formation of this carbonium ion and thus stabilizes **4**, presumably in the form of its α–β anomeric equilibrium mixture. That no change could be detected in the optical rotation of **4** in the presence of trimethylsilyl chloride could be explained on the basis that the "equilibrium mixture" of the α and β anomers of **4** probably contains only a very small percentage of the generally less stable[13] β anomer, which may have been already present in our original preparation of the "pure α" glycosyl halide **4**.[16] In the coupling reaction, the β halogenose (**4**β) could presumably react much more rapidly with the silylpyrimidine than the α anomer (**4**α), and, as the former is consumed in the reaction, the trimethylsilyl chloride present could serve to reestablish the equilibrium with the conversion of more **4**α into **4**β.

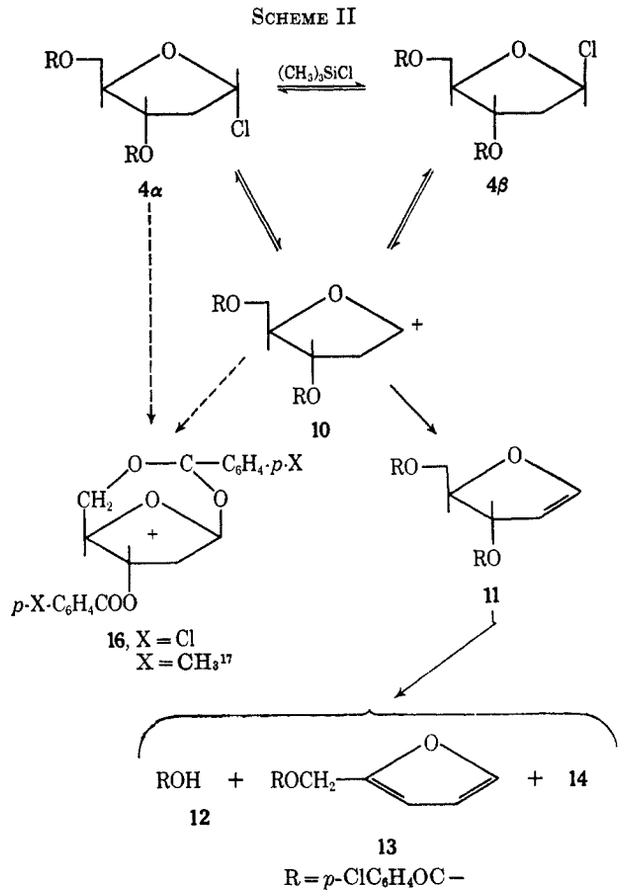
Thus, it is possible to explain the stereoselective formation of either the α or the β nucleosides in the coupling reactions on the basis of a uniform, SN2 mechanism. This explanation is contrary to that proposed by Šorm and coworkers[17] to explain the predominant formation of the α anomer in the condensation of α-2-deoxydi-*O*-(*p*-substituted)benzoylribofuranosyl halides with 2,4-dialkoxypyrimidines at room temperature. These workers regard the reaction as proceeding through the carbonium ion intermediate **10**, which is attacked by the pyrimidine from the less hindered α face, suggesting that the C_5 *O*-benzoyl group sterically hinders the attack on the C_1 carbon from the β side, or may participate with the formation of a cyclic ion (**16**) which would react with the pyrimidine in a stereospecific manner, leading to α nucleoside.[17]

Whatever the mechanism of the observed stereoselectivity may be, it must account for the fact that reactions which are conducted under conditions permitting rapid removal of trimethylsilyl chloride from the reaction mixture lead to the β anomer as the only

(16) The alternative possibility cannot be excluded that trimethylsilyl chloride catalyzes the anomerization of **4** only in the presence of the silylpyrimidine reactant, and that in the latter's absence it merely inhibits the ionization of **4**, *e.g.*, by diminishing the ion-solvating power of the medium (competing for trace H_2O).

(17) M. Prystas, J. Farkas, and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 3123 (1965).

SCHEME II



isolable product, whereas reactions which proceed in the presence of trimethylsilyl chloride allow isolation of the α anomer as the predominant product. In order to examine the possibility, though improbable, that trimethylsilyl chloride might facilitate anomerization of the already formed nucleoside, pure **6a** was refluxed for 5 hr with 15% trimethylsilyl chloride. No change in optical rotation of the β nucleoside occurred.

On the basis of the above studies, larger quantities of the blocked (β and α) anomeric 2′-deoxyribosides of 5-*S*-acetylmercaptouracil (**6c** and **7c**) and their new corresponding *S*-alkyl derivatives, **6d–f** and **7d**, could be obtained in a stereoselective manner by methods G (F) and E, respectively. Hydrolysis of these protected nucleoside derivatives was carried out by sodium methoxide catalyzed transesterification in methanol, leading to the completely deacylated nucleosides (Scheme I). Some variations in the physical constants of the free mercapto nucleosides **8c** and **9c** were noted on recrystallization owing to their partial oxidation to the corresponding disulfides.[18] Changes in optical rotation of the anomeric 5-mercapto-2′-deoxyuridines were observed upon dissolution and standing in distilled water in a closed polarimeter tube, as indicated for the α anomer, **9c**, in Table II. The optical rotation of these compounds could also be influenced by changes in pH, owing to ionization of the sulfhydryl group. Thus, the $[\alpha]^{25}D$ value of **9c** in a "stabilizing" EDTA buffer (which prevents autoxidation),[4] is +2.2 at pH 2.8 for the un-ionized thiol, +5.9 at pH 5.0 (equal to pK_a)[18] for a 1:1 mixture

(18) T. J. Bardos and T. I. Kalman, *J. Pharm. Sci.*, **55**, 606 (1966).

TABLE II

CHANGE IN ROTATION OF THE MERCAPTO NUCLEOSIDE[a] 9c WITH TIME

(+0.1 ml of 30% hydrogen peroxide solution, −228°)

Time, hr	$[\alpha]^{25}D$
0.0	+7.9
0.5	+4.4
1.7	−4.0
3.5	−5.2
25.0	−25.0
27.0	−29.7
45	−43.5
50	−49.5

[a] Concentration, 2% in distilled water.

of the un-ionized mercapto compound and the thiolate anion, and +17.3 at pH 7.5 for the thiolate anion.

Oxidation of the mercapto nucleosides **8c** and **9c** was complete in 24 hr. These, on standing in concentrated aqueous solutions adjusted to pH 8–9 with ammonium hydroxide, yielded the disulfides **8g** and **9g**. Oxidation of the methylmercapto nucleoside **8d** with hydrogen peroxide in glacial acetic acid gave the corresponding sulfoxide, N_1-(2′-deoxy-β-D-ribofuranosyl)-5-methylsulfinyluracil (**8h**).

In view of the unusually high optical rotation values obtained at the sodium D line for the anomeric disulfides **8g** and **9g**, compared with the corresponding mercapto and *S*-alkylmercapto nucleosides, it was of interest to determine the optical rotatory dispersion of these compounds. The ORD curves (Figure 2) and the data in Table III indicate that the α and β

TABLE III

ORD SPECTRA OF 5-*S*-SUBSTITUTED 2′-DEOXYURIDINES[a]

	1st extremum		2nd extremum		Amplitude,
Compd	λ, mμ	[Φ][b]	λ, mμ	[Φ][b]	$[a]^c \times 10^{-2}$
8d	270	+1,210	246	−1,100	+23
8e	285	+1,440	248	−2,880	+43
8f	275	+1,390	246	−3,030	+44
8g	285	+34,200	210	−127,700	+1,620
8h	278	+2,320	252	−3,830	+61
9d	285	−1,100	246	+6,580	−77
9g	274	−38,900	220	+154,600	−1,936

[a] Concentration, 0.5 mg/ml in distilled water; 20°. [b] [Φ] = [α] × mol wt/100. [c] [α] = $[\Phi]_1 - [\Phi]_2$.

anomers of all of these 5-*S*-substituted uracil nucleosides exhibit negative and positive Cotton effects, respectively, in line with the conclusions derived from the studies of other pyrimidine nucleosides,[19] but that the values for the disulfides are almost two orders of magnitude higher than those for the monomeric *S*-alkylmercapto nucleosides. Thus, if the empirical rule of Ulbricht and coworkers[19] (which attempts to correlate the Cotton effects of pyrimidine nucleosides with their conformations in solution) holds true for the dimeric nucleosides **8g** and **9g**, the ORD data of these compounds seem to indicate that, for each anomer, (1) both nucleoside units of the molecule are in the *anti* conformation, and (2) the two base and two sugar moieties in the dimeric nucleoside must have similar orientations; *i.e.*, the two halves of the molecule would have to overlap with each other to a considerable

(19) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, **6**, 843 (1967).

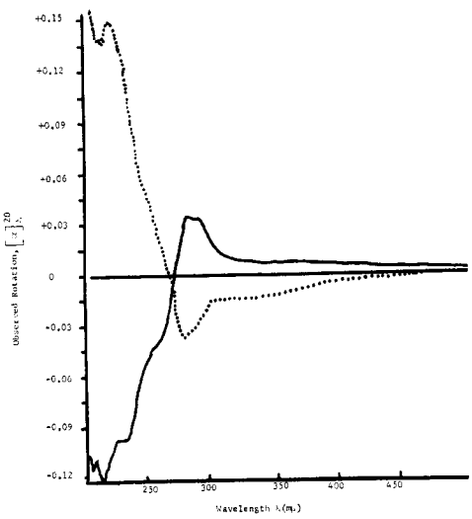


Figure 2.—ORD spectra of anomeric disulfides: ———, β anomer (**8g**); . . . . . , α anomer (**9g**).

degree. Such conformations would provide for substantial interaction (*e.g.*, hydrogen bonding) between the two nucleoside units. The previously noted temperature dependence of the optical rotatory power of these disulfides was attributed to hindered rotation around the S–S bond.[20] In addition to the characteristic electronic structure of the disulfide linkage, indicated by X-ray analysis of **9g** in the crystal state,[21] the above conclusions from the ORD data, relating to the conformations of these compounds in solution, seem to corroborate this interpretation.

Experimental Section[22]

***S*-Benzyl-5-mercaptouracil (2b).**—To a solution of 5-mercaptouracil[7] (1, 5.76 g, 0.04 mol) and sodium methoxide (2.16 g, 0.04 mol) in methanol (200 ml) was added benzyl chloride (5.20 g, 0.04 mol), and the solution was refluxed with stirring under anhydrous conditions for 2.5 hr. After the solution had cooled, water (300 ml) and 3 *N* HCl (22 ml) were added. The precipitated solid was collected, washed with water, washed with acetone, and dried *in vacuo* to give 8.71 g (92.9%) of crude **2b**. Several recrystallizations from 50% DMF–water gave an analytical sample: mp 296–297° (lit.[6] mp 290°); $\lambda_{max}^{0.1\ N\ NaOH}$ 294 mμ (ε 7070); $\lambda_{inflection}^{0.1\ N\ HCl}$ 258 mμ.

Anal. Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.91. Found: C, 56.29; H, 4.27; N, 11.97.

***S*-Acetyl-5-mercaptouracil (2c).**—5-Mercaptouracil (1, 5.0 g) and acetic anhydride (5.0 ml) in dry pyridine (50 ml) were refluxed for 2 hr under anhydrous conditions. The solution was evaporated *in vacuo* (bath 50°) to give a tan, solid residue which was dissolved in boiling methanol (400 ml), treated with charcoal, and filtered through Celite. From the filtrate, upon standing at 5° overnight, a light tan crystalline material precipitated. The product was collected by filtration, washed with cold methanol, and dried to give **2c** in 71.5% yield: mp 253–255°. Re-

(20) C. Szantay, M. P. Kotick, and T. J. Bardos, *J. Pharm. Sci.*, 1293 (1967).

(21) E. Schefter, M. P. Kotick, and T. J. Bardos, *ibid.*, **56**, 1293 (1967).

(22) All melting and boiling points are uncorrected. The former were taken in open capillary tubes on a Mel-Temp apparatus. Nmr spectra were recorded on a Varian Model A-60 spectrophotometer in the indicated solvent with TMS as internal standard. Ultraviolet spectra were obtained on a Beckman DB recording spectrophotometer and the molar extinction coefficients were determined on a Gilford multiple sample recorder utilizing the optical system of a Beckman DU spectrophotometer. Optical rotations were measured in a 1-dm tube using a Perkin-Elmer Model 141 automatic polarimeter at 589 mμ. ORD spectra were determined on a Durram–Jasco ORD-CD-5 instrument. Evaporations were carried out *in vacuo* with bath temperatures kept below 45° unless otherwise stated. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., Dr. S. Nagy, Massachusetts Institute of Technology, and Dr. A. Bernhardt, Max Planck Institute, Mülheim, Germany.

crystallization from methanol gave white needles: mp 254–255.5°; λ_{max}^{EtOH} 270 mμ (ε 8400); λ_{min}^{EtOH} 242 mμ (ε 4000).

Anal. Calcd for $C_6H_6N_2O_3S$: C, 38.70; H, 3.25; N, 15.05; S, 17.22. Found: C, 39.01; H, 3.38; N, 14.80; S, 17.20.

S-Methyl-5-mercaptouracil (2d).—To 1 (14.4 g, 0.1 mol) in water (300 ml) containing sodium hydroxide (4.0 g) was added methyl iodide (6.85 ml) and the solution was stirred vigorously at room temperature. After 2.5 hr, the suspension was acidified to pH 1 with concentrated HCl. The separated yellow material was collected and dissolved with warming in 0.5 *N* NaOH (250 ml) and, after the addition of charcoal, the solution was filtered. The cooled filtrate was acidified with concentrated HCl to pH 1, and the precipitate was collected and recrystallized from water (1.5 l.). The aqueous solution, after standing overnight at 5°, yielded the product as yellow crystals which were collected and dried *in vacuo* to give **2d** in 68.7% yield: mp 307–308° (lit.[8] mp 300°); uv as reported previously.[18]

S-Ethyl-5-mercaptouracil (2e).—This compound was prepared in the same manner as described above for **2d**, but using ethyl bromide. Recrystallization from water gave **2e** in 83.1% yield: mp 255–257° (lit.[8] mp 258°).

S-Butyl-5-mercaptouracil (2f).—This compound was prepared by reaction of **1** with butyl bromide under the conditions described above, except for the addition of 95% ethanol (10 ml) and stirring at room temperature for 18 hr. Recrystallization of the crude material from 50% dimethylformamide–water gave **2f** in 72.5% yield: mp 258–260°. A sample for analysis was recrystallized from the same solvent mixture to give white crystals: mp 260–262°; $\lambda_{max}^{0.1\ N\ NaOH}$ 292 mμ (ε 6900); $\lambda_{min}^{0.1\ N\ NaOH}$ 262 mμ (ε 2700).

Anal. Calcd for $C_8H_{12}N_2O_2S$: C, 48.04; H, 6.05; N, 14.08. Found: C, 48.13; H, 6.32; N, 14.08.

2,4-Bis-*O*-(trimethylsilyl)-5-Substituted Uracils (3a–f).—These compounds were all prepared in an analogous manner. Thus, to 0.110 mol of trimethylsilyl chloride in dry benzene (300 ml) were added 0.054 mol of the appropriate pyrimidine (**2a–f**) and 0.110 mol of triethylamine. The resulting thick suspension was refluxed with stirring for 3 hr. After cooling, the mixture was filtered to remove triethylamine hydrochloride and unreacted pyrimidine, and the combined filtrate and washings (benzene) were evaporated *in vacuo* to a thick oil. Fractional distillation of the latter gave the silylpyrimidines with the properties indicated in Table IV. In some cases, satisfactory analytical results could not be obtained owing to the extreme sensitivity of these compounds toward atmospheric moisture.

TABLE IV
2,4-BIS-*O*-(TRIMETHYLSILYL)-5-SUBSTITUTED URACILS

Compd	R	Bp, °C (mm)	Mp, °C	Yield, %
3a	CH_3	85–89[a] (0.05)	74.5–75[a]	81.0
3b	$SCH_2C_6H_5$	147–149 (0.15)	Oil[b]	70.9
3c	$SCOCH_3$	110–111 (0.35)	Oil[c]	75.5
3d	SCH_3	98–99 (0.30)	36–37[d]	63.3
3e	SCH_2CH_3	96–97 (0.20)	Oil[b]	86.3
3f	$S(CH_2)_3CH_3$	109–111 (0.20)	Oil[b]	81.7

[a] T. Nishimura, B. Shimuzu, and I. Iwai [*Chem. Pharm. Bull.* (Tokyo), **11**, 1470 (1963)] report bp 124° (14 mm); mp 63–65°. *Anal.* Calcd for $C_{11}H_{19}N_2O_2Si_2$: C, 48.83; H, 7.90; N, 10.35. Found: C, 48.41; H, 7.89; N, 10.97. [b] Not analyzed but used directly in condensation reactions. [c] Solidified in several cases on prolonged standing at −15°; mp 36–37°. *Anal.* Calcd for $C_{12}H_{22}N_2O_3SSi_2$: C, 43.60; H, 6.66; N, 8.49. Found: C, 43.89; H, 6.38; N, 8.26. [d] *Anal.* Calcd for $C_{11}H_{22}N_2O_2SSi_2$: C, 40.40; H, 7.34; N, 9.28. Found: C, 40.42; H, 7.24; N, 10.10.

2-Deoxy-3,5-di-*O*-(*p*-chlorobenzoyl)-α-D-ribofuranosyl Chloride (4).—Essentially the method described by Fox, *et al.*,[11] was followed in the preparation of this compound. 2-Deoxy-D-ribose[23] (10.0 g) was dissolved in anhydrous methanol (190 ml) and treated with 10.0 ml of a 0.271 *N* solution of anhydrous hydrogen chloride in methanol. The clear solution was allowed to stand at room temperature (24°) for 45 min. Dry pyridine (50 ml) was added and the solution was concentrated *in vacuo* (bath 35°) to a thick syrup. Additional pyridine (25 ml) was added and evaporation was repeated to give a thick syrup, which was diluted with pyridine (60 ml), and treated dropwise (below 20°) with *p*-chlorobenzoyl chloride (25 ml). The solution was stirred overnight at room temperature, water (150 ml) and methylene chloride (250 ml) were added, and the organic phase was separated and washed successively twice each with cold sodium bicarbonate solution, water, cold 3 *N* sulfuric acid, and water (150 ml each). The organic phase was dried over sodium sulfate and, after filtration from salts, evaporated to dryness. The thick residue was dissolved in ether (75 ml) and filtered from undissolved crystalline material. The clear ether solution was cooled to 0° and added to glacial acetic acid (100 ml) which had been previously saturated with dry hydrogen chloride at 0°. With continued cooling and stirring, more hydrogen chloride was rapidly added and, after 6–8 min, the product began to crystallize. Addition of hydrogen chloride was continued for an additional 2 min; the product was then collected by suction on a sintered-glass funnel and washed with cold ether (100 ml). This crude material (mp 118–122°) was dissolved in warm carbon tetrachloride (600–800 ml), and the solution was filtered and kept at 5° for 2 hr. A fluffy, crystalline material separated which was dried *in vacuo* over P_2O_5 at room temperature, to give 15.0–16.5 g of deoxyribosyl chloride **4** (46.7–51.4% of theoretical): mp 131–132°, resolidification, mp 238–241° dec (lit.[11] mp 118–120°); $[\alpha]^{25}D$ + 109° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 2.70 (s, 2, $H_{2,2'}$), 4.5 (s, 3, $H_{5,5',4}$), 5.38 (m, 1, H_3), 6.27 (m, 1, H_1), 7.55 (broad d, 4, J = 8 cps, aromatic), 7.62 and 7.75 (both overlapping d, 4, J = 8 cps, aromatic).

Anal. Calcd for $C_{19}H_{15}Cl_3O_5$: C, 53.07; H, 3.52; Cl, 24.71. Found: C, 52.84; H, 3.40; Cl, 25.26.

This material could be kept for 2 weeks, over P_2O_5 *in vacuo*, without significant decomposition.

Preparation of the Protected Anomeric Nucleosides 6a–f and 7a–d.—These compounds were obtained by condensation reactions using a variety of conditions as described below (methods A–G). In each reaction, equimolar amounts of the silylpyrimidine **3 a–f** and the deoxyribosyl chloride **4** were used. Representative reactions and their results are given in Table I.

Method A.—Refluxing in benzene solution for 1.5 hr.

Method B.—Fusion at 100–110°, 15 min. None of the reactants or products was removed from the reaction flask during the reaction period. The escape of trimethylsilyl chloride was largely prevented by the use of an air condenser topped by a calcium chloride guard tube.

Method C.—Stirring in benzene solution; reaction time and temperature are as indicated for each experiment in Table I.

Method D.—Azeotropic distillation with the addition of benzene.[24] The average rate of distillation and addition of solvent, to maintain a constant volume of 25 ml, is indicated for each experiment in Table I.

Method E.—Azeotropic distillation with the addition of 15% trimethylsilyl chloride in benzene. Average number of milliliters of solution per minute added and distilled, while maintaining the constant reaction volume of 25 ml, is indicated in Table I.

Method F.—Fusion *in vacuo* (10–20 mm) but without stirring, 100–110°, 15 min.

Method G.—Fusion *in vacuo* (10–20 mm), with stirring of the thick, viscous melt by means of a motor-powered stirring blade, 100–110°, 15–20 min.

After the reaction was completed, the reaction mixture was taken up in benzene, and water (1–5 ml) was added. The nonhomogeneous mixture was evaporated to dryness and taken up in toluene. The mixture was heated to boiling and filtered while hot to remove the insoluble pyrimidine **2** formed from unreacted starting material. Fractional crystallization of the toluene filtrate (with repeated filtration, if needed, to remove additional insoluble pyrimidine) gave the anomeric blocked nucleosides. In cases where high anomeric purity was obtained in the reaction (see Table I), the anomerically pure nucleoside could be isolated after three to eight crystallizations. In certain cases, it was difficult to separate small amounts of the anomeric nucleosides, although they could be obtained free from other materials present

(23) Purchased from Nutritional Biochemical Corp., Cleveland, Ohio.

(24) Trimethylsilyl chloride can be azeotropically distilled with benzene under these conditions.

TABLE V

PROPERTIES OF BLOCKED ANOMERIC NUCLEOSIDES

Compd	Mp,[a] °C	λ_{max}^{EtOH}, mμ	ε × 10⁻³	[α]²⁵D[b]	Formula		Analysis, % C	H	Cl	N	S
6a	193–194[c]	242	34.6	−44.4 (*c* 1)	$C_{24}H_{20}Cl_2N_2O_7$	Calcd	55.45	3.85	13.88	5.39	
						Found	55.44	4.09	13.57	5.19	
7a	179–180[d]	242	34.8	−15.6 (*c* 1)	$C_{24}H_{20}Cl_2N_2O_7$	Found	55.29	3.97	13.60	5.17	
6c	169–170	272	10.8	−53.9	$C_{25}H_{20}Cl_2N_2O_8S$	Calcd	52.72	3.49	12.22	4.82	5.50
		241	36.6	(*c* 1)		Found	52.41	3.56	12.54	4.86	5.72
7c	168–168.5	272	11.2	+53.3	$C_{25}H_{20}Cl_2N_2O_8S$	Found	52.56	3.66	12.40	4.90	5.63
		241	36.0	(*c* 1.1)							
6d	196	242	40.8	−58.3	$C_{24}H_{20}Cl_2N_2O_7S$	Calcd	52.32	3.66	12.69	5.08	5.80
						Found	52.31	3.52	12.70	5.20	5.81
7d	185–186	242	41.2	+38.6 (*c* 1.1)	$C_{24}H_{20}Cl_2N_2O_7S$	Found	52.61	3.62	12.81	5.15	5.62
6e	152–153[e]	242	37.6	−61.5	$C_{25}H_{22}Cl_2N_2O_7S$	Calcd	53.09	3.92	12.36	4.95	
						Found	53.17	4.06	12.40	5.01	
6f	85[f]	242	39.5	−60.8 (*c* 1.2)	$C_{27}H_{26}Cl_2N_2O_7S$	Calcd	54.64	4.41	11.95	4.72	
						Found	54.90	4.63	12.20	4.69	

[a] Compounds crystallized and recrystallized from toluene, except where noted. [b] Optical rotations in $CHCl_3$ at given concentrations. [c] Lit.[14] mp 192.5–194° (ethanol). [d] Lit.[14] mp 154–156°, resolidification, and mp 177–179° (ethanol). [e] Crystallized from toluene and recrystallized from ethanol. [f] Crystallized and recrystallized from ethanol.

in the reaction mixture by crystallization from toluene. The ratio of anomeric products in these cases was established by the optical rotation of the mixture, after the absence of other impurities was checked by thin layer chromatography on silica gel (silica gel HF_{254}, Brinkman Instruments, spread on microscope slides and detected by uv light). The results given in Table I represent reactions which gave relatively good yields and could be satisfactorily resolved by crystallization. Purity in each case was checked by melting point, mixture melting point, and optical rotation.

Analyses and physical properties of the blocked nucleosides **6a**, **c**–**f**, and **7a**, **c**, and **d** are given in Table V.

*N*₁-(**2′-Deoxy-β-D-ribofuranosyl**)-**5-mercaptouracil** (**8c**).—To a suspension of protected *S*-acetyl nucleoside **6c** (2 mmol) in methanol (50 ml) under a nitrogen atmosphere was added a solution of sodium (3-g atoms) in methanol (10 ml), and the resulting solution was stirred at room temperature under nitrogen for 2 hr. Dowex 50 W resin (H^+ form, 10 ml, previously washed with methanol) was added and, after an additional 5 min of stirring, the solution was filtered from the resin and glacial acetic acid (0.2 ml) was added to the filtrate. The solution was evaporated *in vacuo* (bath 30°) to *ca.* 2-ml volume, and the product was precipitated with the addition of ether (100 ml). After standing for several hours at room temperature, the product was collected and washed wth ether to give **8c**, 407 mg, mp 152–154°. Evaporation of the ether filtrate to *ca.* 20 ml yielded additional product, 47 mg, mp 163–164°. The total yield of **8c** over several runs varied from 87.5 to 90.4%. The melting point and optical rotation values obtained for **8c** varied depending upon the percentage of disulfide **8g** formed during work-up of the reaction; the product with mp 152–154° contains *ca.* 95% thiol and 5% disulfide, as estimated from the ultraviolet spectrum (in "stabilizing" EDTA buffer, with the use of DTT),[4,18] [α]²⁵D + 26.4° (*c* 2, H_2O); uv data at various pH values were reported.[18] A sample for analysis was quickly recrystallized from methanol–ether.

Anal. Calcd for $C_9H_{12}N_2O_5S$: C, 41.53; H, 4.61; N, 10.76; S, 12.32. Found: C, 41.44; H, 4.49; N, 10.44; S, 12.12.

*N*₁-(**2′-Deoxy-α-D-ribofuranosyl**)-**5-mercaptouracil** (**9c**).—In a similar manner, the α anomer was obtained from **7c**, in 87.2 to 94.5% total yield over several runs. The melting point and optical rotation values varied depending upon the extent of oxidation; the product with mp 183–185°, [α]²⁵D +7.9° (*c* 2.0, H_2O), contains 95% thiol and 5% disulfide as estimated from the uv spectra.[4,18] A sample for analysis was recrystallized from methanol–ether.

Anal. Calcd for $C_9H_{12}N_2O_5S$: C, 41.53; H, 4.61; N, 10.76; S, 12.32. Found: C, 41.70; H, 4.80; N, 10.58; S, 12.11.

*N*₁-(**2′-Deoxy-β-D-ribofuranosyl**)-*S*-**methyl-5-mercaptouracil** (**8d**).—To a suspension of protected nucleoside **6d** (2 mmol) in methanol (20 ml) was added a solution of sodium (0.5 g-atom) in methanol (5 ml), and the resulting solution was stirred at room temperature for 2 hr. Dowex 50 W resin (H^+ form, 5 ml, previously washed with methanol) was added and stirring was continued for 5 min. The solution was filtered from the resin and the filtrate was evaporated *in vacuo* to dryness. Ether (50 ml) was added to the residual material and the crystalline product was collected by filtration in 96.7% yield: mp 179–180°. Recrystallization from methanol gave an analytical sample: mp 179–180°; [α]²⁵D +25.1° (*c* 0.95, H_2O); $\lambda_{max}^{0.1\ N\ NaOH}$ 282 mμ (ε 4610); $\lambda_{min}^{0.1\ N\ NaOH}$ 265 mμ (ε 4000); $\lambda_{max}^{0.1\ N\ HCl}$ 282 (ε 5150) and 232 mμ (ε 6710); $\lambda_{min}^{0.1\ N\ HCl}$ 265 (ε 3860) and 225 mμ (ε 6620).

Anal. Calcd for $C_{10}H_{14}N_2O_5S$: C, 43.84; H, 5.15; N, 10.23; S, 11.70. Found: C, 43.66; H, 5.05; N, 10.20; S, 11.70.

*N*₁-(**2′-Deoxy-α-D-ribofuranosyl**)-*S*-**methyl-5-mercaptouracil** (**9d**).—In a manner analogous to that described above for the β anomer **8d**, the α blocked nucleoside, **7d**, was deacylated in 87.5% yield to give the product **9d**: mp 182–183° after recrystallization from methanol; [α]²⁵D +32.1° (*c* 0.95, H_2O); uv identical with that of **8d**. On admixture with the β anomer, the melting point was depressed by 10–15°.

Anal. Calcd for $C_{10}H_{14}N_2O_5S$: C, 43.84; H, 5.15; N, 10.23; S, 11.70. Found: C, 43.61; H, 5.09; N, 10.29; S, 11.50.

*N*₁-(**2′-Deoxy-β-D-ribofuranosyl**)-*S*-**ethyl-5-mercaptouracil** (**8e**).—This was obtained in an overall yield of 89.9% from the blocked nucleoside **6e**: mp 138–139°. Recrystallization from methanol–ether gave an analytical sample: mp 139–139.5°; [α]²⁵D +27.0° (*c* 1.0, H_2O); λ_{max}^{EtOH} 288 (ε 5160) and 233 mμ (ε 6560); λ_{min}^{EtOH} 270 mμ (ε 5070).

Anal. Calcd for $C_{11}H_{16}N_2O_5S$: C, 45.83; H, 5.59; N, 9.72; S, 11.12. Found: C, 45.80; H, 5.56; N, 9.82; S, 11.12.

*N*₁-(**2′-Deoxy-β-D-ribofuranosyl**)-*S*-**butyl-5-mercaptouracil** (**8f**).—This was obtained by hydrolysis of the protected nucleoside **6f** in an over-all yield of 81.5%: mp 117–118°. After recrystallization from ethyl acetate–benzene, the analytical sample had mp 125–126°; [α]²⁵D +21.8° (*c* 1.03, H_2O); λ_{max}^{EtOH} 292 (ε 4950) and 235 mμ (ε 5470); λ_{min}^{EtOH} 274 mμ (ε 4930).

Anal. Calcd for $C_{13}H_{20}N_2O_5S$: C, 49.38; H, 6.37; N, 8.85; S, 10.10. Found: C, 49.53; H, 6.21; N, 8.74; S, 10.25.

5-[1-(2′-Deoxy-β-D-ribofuranosyl)uracilyl] Disulfide (**8g**).—A solution of **8c** (100 mg) in water (20 ml) was adjusted to pH 8–9 with ammonium hydroxide solution and the solution was allowed to stand for 24 hr at room temperature. The solution was then evaporated to dryness *in vacuo*, methanol (5 ml) and ether (15 ml) were added, and the solution was kept at −5°. The white precipitate which formed on standing overnight was collected and washed with ether to give 96 mg of **8g**, mp 202–214°. Several recrystallizations from methanol (1 part)–ether (6 parts) gave an analytical sample: mp 214–215°; [α]²⁵D +215.4° (*c* 0.50, H_2O); uv as reported.[18]

Anal. Calcd for $C_{18}H_{22}N_4O_{10}S_2$: C, 41.70; H, 4.28; N, 10.82; S, 12.38. Found: C, 41.62; H, 4.37; N, 10.90; S, 12.47.

5-[1-(2′-Deoxy-α-D-ribofuranosyl)uracilyl] Disulfide (9g).—This compound was prepared from **9c** (100 mg) as described for the β anomer **8g**, except that, after evaporation of the ammoniacal solution to dryness, water (2 ml) was added. This solution, after standing at 5° overnight, gave yellow crystals which were collected, washed with water, and dried to give **9g**, 70 mg, mp 202–203°. After recrystallization from water, **9g** had mp 202–203°; $[\alpha]^{25}D$ −262.2° (*c* 0.51, H_2O); uv as reported for **8g**.[7]

Anal. Calcd for $C_{18}H_{22}N_4O_{10}S_2$: C, 41.70; H, 4.28; N, 10.82; S, 12.38. Found: C, 41.52; H, 4.17; N, 10.85; S, 12.18.

Decomposition of the Halogenose 4 in Xylene.—Recrystallized deoxyribosyl chloride **4** (1.0 g, 2.32 mmol) was dissolved in dry xylene (3 ml) and the solution was refluxed on an oil bath for 20 min, during which time evolution of hydrogen chloride was noted. The solution was cooled in an ice bath and filtered from the crystalline *p*-chlorobenzoic acid (**12**), 320 mg, mp 243–244°, identified by mixture melting point and infrared spectra with an authentic sample. The filtrate was evaporated to a thick oil from which an additional 17 mg of **12** could be obtained by addition of petroleum ether (bp 30–60°) followed by filtration. The overall yield of *p*-chlorobenzoic acid (**12**) was 92.5%. The petroleum ether solution was evaporated to an oil (532 mg, 98.5%) which was distilled *in vacuo* to give furfuryl-*p*-chlorobenzoate (**13**), bp 108° (0.18 mm), which solidified on standing at −5° to give crystals, mp 36–37° [lit.[14] bp 150–152° (0.4 mm), mp 36–37°].

1,1′-(2-Deoxy-D-ribofuranosyl-2′-deoxy-D-ribofuranoside)-tetra-*p*-chlorobenzoate (14). A. From the Halogenose 4 in Benzene.—A solution of **4** (1.72 g, 4.0 mmol) in dry benzene (100 ml) in a stoppered flask was placed in an incubator at 37°. After standing for 16 days, the solution was filtered to remove *p*-chlorobenzoic acid (**12**), 300 mg. Concentration of the benzene solution gave additional **12**, 96 mg (for an overall yield of 6.35%). Complete evaporation of the solvent gave a residue which contained some **13** (not isolated). Crystallization of this residue from methanol–dioxane gave **14**: 65 mg (4% based on **4**); mp 168°; $[\alpha]^{25}D$ −13.0° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 2.30 (s, 2, $H_{2,2'}$), 4.37 (s, 3, $H_{4,5,5'}$), 5.5 (broad, 2, $H_{1,3}$), 7.13 (broad d, 4, *J* = 8 cps, aromatic), 7.63 and 7.78 (overlapping d, 4, *J* = 8 cps, aromatic) (integration for ½ of the symmetrical molecule).

Anal. Calcd for $C_{38}H_{30}Cl_4O_{11}$: C, 56,77; H, 3.76; Cl, 17.64; mol wt, 804.2. Found: C, 56.43, 56.90; H, 3.98; 3.80; Cl, 17.24, 16,69; mol wt, 850 ($CHCl_3$).

B. From the Halogenose 4 in Benzene, in the Presence of Triethylamine.—A solution of chloro sugar **4** (2.0 g, 4.65 mmol) and dry triethylamine (0.467 g, 4.62 mmol) in dry benzene (115 ml) in a stoppered flask was placed in an incubator at 37°. After this stood for 13 days, the crystalline triethylamine hydrochloride (345 mg, 54.4%) was collected by filtration. The filtrate was extracted with potassium bicarbonate solution (three 30-ml portions) and dried over sodium sulfate. After filtration from salts, the solution was evaporated to dryness and the residue was crystallized from methanol–dioxane to give **14**, 50 mg (2.7% based on **4**), mp 168°, identical by mixture melting point with the material obtained above.

***N*1-(2′-Deoxy-β-D-ribofuranosyl)-5-methylsulfinyluracil (8h).**—To a solution of **8d** (0.5 mmol) in glacial acetic acid (15 ml) was added 30% hydrogen peroxide solution (0.5 mmol 0.06 ml) and the solution was stirred at room temperature. After this stirred for 20 hr, the resulting suspension was evaporated to dryness *in vacuo* (bath below 40°). After extensive drying of the residual syrup, methanol (1 ml) and ether (0.5 ml) were added, and the solution was kept at −15° overnight to give white crystals, which were collected, washed with a few drops of ether, and dried to give **8h** in 68.6% yield: mp 164–165°. A sample for analysis was prepared by recrystallization from methanol–ether: mp 164–165°; λ_{max}^{EtOH} 273 mμ (ε 8140); λ_{min}^{EtOH} 240 mμ (ε 2390).

Anal. Calcd for $C_{10}H_{14}N_2O_6S$: C, 41.37; H, 4.89; N, 9.64; S, 11.05. Found: C, 41.29; H, 4.64; N, 9.54; S, 11.22.

Registry No.—**2b**, 21736-44-7; **2c**, 6984-56-1; **2f**, 21736-46-9; **3a**, 7288-28-0; **3b**, 21779-12-4; **3c**, 6984-57-2; **3d**, 21736-49-2; **3e**, 21779-13-5; **3f**, 21736-50-5; **4**, 21740-23-8; **6a**, 21740-24-9; **6c**, 6984-58-3; **6d**, 21740-25-0; **6e**, 21740-26-1; **6f**, 21740-27-2; **7a**, 21740-28-3; **7c**, 7085-53-2; **7d**, 21740-30-7; **8c**, 7085-54-3; **8d**, 14985-32-1; **8e**, 21740-33-0; **8f**, 21740-34-1; **8g**, 14985-33-2; **8h**, 21740-36-3; **9c**, 6984-59-4; **9d**, 14795-28-9; **9g**, 14795-27-8; **14**, 21740-40-9.

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