the three compounds on apomorphine-induced emesis in dogs was not observed with doses up to 10 mg/kg (sc), while chlorpromazine was able to block the emesis completely with 1.5 mg/kg.

In summary, among the ten compounds tested, **2** with favorable pharmacological properties seems to be a promising antitussive agent in that it shows approximately equal antitussive effect to codeine with slightly less toxicity. Here also the role of piperidino group in manifestation of antitussive activity was illustrated. This substance is now undergoing clinical trials as an antitussive agent.

Synthesis of 5-Mercaptouridine[1,2]

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Received November 14, 1969

Reaction of 5-acetylmercapto-2,4-bis(trimethylsiloxy)pyrimidine (I) with (anomeric) 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride led to an anomeric mixture of the blocked nucleoside. Reaction of I with 2,3,5-tri-*O*-*p*-anisoyl-D-ribofuranosyl chloride resulted, instead of coupling with the blocked ribosyl group, in *p*-anisoylation of the N_1 position of the pyrimidine. Stereoselective synthesis of 5-mercaptouridine (MUR) was achieved by fusion *in vacuo* of I with 2,3,5-tri-*O*-*p*-chlorobenzoyl-α-D-ribofuranosyl bromide (prepared from the anomerically pure β-1-*p*-nitrobenzoate), followed by removal of the blocking groups. MUR showed inhibitory activity on preliminary testing in bacterial and tissue culture assays.

Among a number of 5-substituted uridine derivatives which showed growth inhibition in bacterial and viral (tissue culture) assay systems, 5-hydroxyuridine was found to be the most effective antimetabolite[4] and to possess significant *in vivo* activity as an antitumor agent.[5] This compound undergoes most of the biochemical reactions of uridine including conversion into the mono-, di-, and triphosphates and minor incorporation into RNA.[6] Its 5′-monophosphate, 5-hydroxyuridylic acid, is a strong inhibitor of orotidylic acid decarboxylase,[6] and its triphosphate, 5-hydroxy-UTP, acts as a competitive inhibitor of UTP in the RNA polymerase reaction.[7] Roy-Burman, *et al.*, demonstrated that the inhibitory effect of 5-hydroxy-UTP is lowest at pH 7.0 and increases with increasing pH values to a maximum at pH 9.0, while the ability of this analog to replace UTP as a substrate for RNA polymerase (*i.e.*, to incorporate into RNA) showed the opposite pH dependence.[7] Thus, the inhibitory effect of the analog is predominant when its 5-hydroxyl group (pK_a 7.2) is in the ionized form, in contrast to its utilization as a substrate which appears to be suppressed by ionization.[7]

In view of the much lower pK_a value (pK_a 5.0)[8] of the previously synthesized 5-mercapto-2′-deoxyuridine (MUdR)[9] which, in its essentially ionized form (pH 7.4), was shown to undergo phosphorylation by thymidine kinase[10] and, subsequently, to act as a potent competitive inhibitor of dUMP in thymidylate synthetase,[11] it appeared of interest to prepare the corresponding uridine analog, 5-mercaptouridine (MUR; XIV). For the synthesis of this compound, the silyl modification of the Hilbert–Johnson reaction[12] appeared to be the method of choice.

Reaction of 5-acetylmercapto-2,4-bis(trimethylsiloxy)pyrimidine[9] (I) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (*II*)[13] yielded, after hydrolysis of the 4-*O*-silyl group, an anomeric mixture of the blocked nucleoside III (Scheme I). The nmr spectrum of this product showed two close but separate peaks for the CH_3 of the *S*-acetyl group, at δ 2.30 and 2.36 ppm. Examination of the nmr spectra of the previously synthesized pure α and β anomers of *S*-acetyl-N_1-[3′,5′-di-*O*-*p*-chlorobenzoyl-2′-deoxy-D-ribofuranosyl]-5-mercaptouracil[9] similarly revealed a difference (of 2 Hz) between the chemical shifts for the *S*-acetyl protons of the two anomers, the corresponding resonance peaks being located at δ 2.38 ppm for the α, and 2.35 ppm for the β anomer. By analogy, in the spectrum of III the resonance peak at the higher field (2.30 ppm) would correspond to the β anomer; integration indicated that the ratio of α and β anomers was approximately 1:2. Separation of the two anomers of III proved to be quite difficult, and therefore, a more stereoselective method for the preparation of the β anomer was desired.

The above result is in agreement with previous reports[14] indicating that the *trans* rule is not applicable

(1) This work was supported by U.S. Public Health Service Research Grant R01-CA 06695-8 from the National Cancer Institute, National Institutes of Health, Bethesda, Md.

(2) A preliminary report on part of this work was presented to the Division of Medicinal Chemistry, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.

(3) To whom inquiries should be addressed.

(4) I. J. Slotnik, D. W. Visser, and S. C. Rittenbert, *J. Biol. Chem.* **203**, 647 (1953).

(5) K. Sugiura and H. J. Creech, *Ann. N. Y. Acad. Sci.*, **63**, 962 (1955).

(6) D. A. Smith and D. W. Visser, *J. Biol. Chem.*, **240**, 446 (1965).

(7) S. Roy-Burman, P. Roy-Burman, and D. W. Visser, *ibid.*, **241**, 781 (1966).

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

(9) (a) T. J. Bardos, M. P. Kotick, and C. Szantay, *Tetrahedron Lett.*, 1759 (1966); (b) M. P. Kotick, C. Szantay, and T. J. Bardos, *J. Org. Chem.*, **34**, 3806 (1969).

(10) T. J. Bardos, J. I. Ambrus, K. Baranski, T. I. Kalman, and C. Ambrus, *Proc. Amer. Ass. Cancer Res.* **8**, 4 (1967).

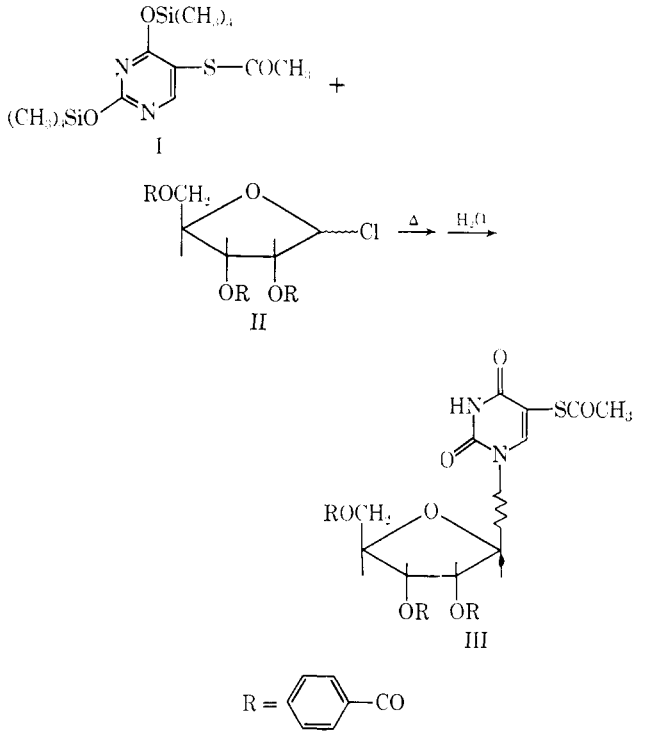
(11) (a) T. I. Kalman and T. J. Bardos, *Fed. Proc.*, **27**, 650 (1968); (b) K. Baranski, T. J. Bardos, A. Bloch, and T. I. Kalman, *Biochem. Pharmacol.*, **18**, 347 (1969).

(12) (a) T. Nishimura, B. Shimuzu, and I. Iwai, *Chem. Pharm. Bull.*, **11**, 1470 (1963); (b) T. Nishimura and I. Iwai, *ibid.*, **12**, 357 (1964); T. Nishimura, B. Shimuzu, and I. Iwai, *ibid.*, **12**, 1471 (1964); (d) E. Wittenburg, *Z. Chem.*, **4**, 303 (1964); and more recent publications.

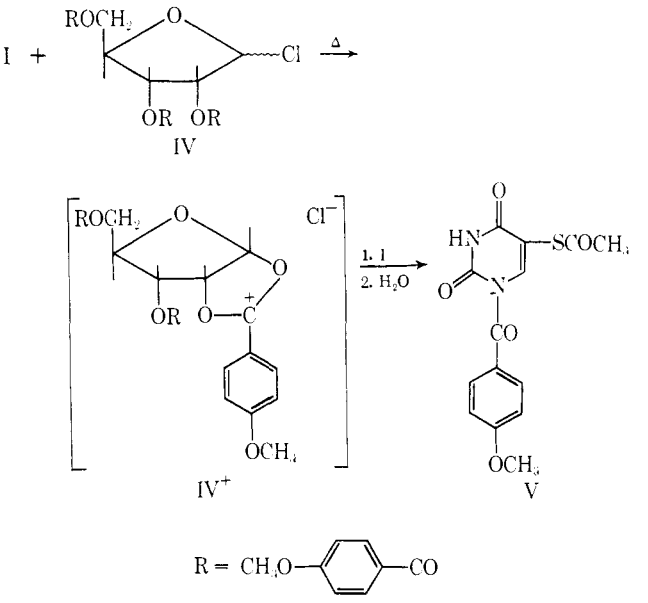
(13) H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Amer. Chem. Soc.*, **77**, 18 (1955).

(14) (a) J. D. Stevens, R. K. Ness, and H. B. Fletcher, Jr., *J. Org. Chem.*, **33**, 1806 (1968); (b) J. Farkas, L. Kaplan, and J. J. Fox, *ibid.*, **29**, 1469 (1964); (c) M. Pyrstas and F. Sorm, *Collect. Czech. Chem. Commun.*, **31**, 1035 (1966).

Scheme I



Scheme II



to the Hilbert–Johnson reaction, or to its silyl modification. Our results in the synthesis of deoxyribosides by the silyl method[9] indicated that these reactions proceed by SN2 mechanisms, with inversion of configuration at C-1. We thought that it might be possible to make the reaction follow the *trans* rule if we could induce carbonium ion formation with increased neighboring group participation[15] by the use of an acyl group having an electron-releasing substituent for the blocking of the C-2 hydroxyl of the halogenose. In this case, the ester carbonyl group at C-2 could anchimerically promote the formation of a carbonium ion in the C-1 position (which may be stabilized as an ortho ester ion),[16] and, by allowing the approach of the pyrimidine only from the β side, it could make the coupling reaction stereospecific. For this reason, 2,3,5-tri-*O*-*p*-anisoyl-D-ribofuranosyl chloride (IV) was prepared, and this was reacted with the silyl pyrimidine I.

Surprisingly, instead of the expected blocked nucleoside, N_1-*p*-anisoyl-*S*-acetyl-5-mercaptouracil (V) was isolated from the reaction mixture (after hydrolysis of the 4-*O*-silyl group) in nearly quantitative yield based on the reacted silyl pyrimidine (Scheme II). That this product did not arise by simple *N*-acylation of the pyrimidine with *p*-anisoyl chloride which conceivably might have been formed *via* degradation of the blocked halogenose IV involving cleavage of an anisoyl ester group, was demonstrated by a control experiment showing the absence of *p*-anisoyl chloride formation from IV under the reaction conditions (see Experimental Section). Thus, it appears that the desired ortho ester carbonium ion (IV^+) was indeed formed from the *p*-anisoyl-blocked halogenose, but the subsequent nucleophilic attack by the silyl pyrimidine occurred directly on the ester-carbon of the *p*-anisoyl group on which the positive charge was localized rather than on the C-1 carbon of the ribose. This interpretation is supported by the observation of Haga and Ness that in 2,3,5-tri-*O*-*p*-anisoyl-D-ribofuranosyl bromide, when dissolved in aq acetone, the *p*-anisoyl group at C-2 undergoes ready migration to the C-1 pos tion, with displacement of the bromide, to give 1,3,5-tri-*O*-*p*-anisoyl-α-D-ribofuranose.[17] The latter reaction must involve the same ortho ester ion intermediate IV^+ which subsequently hydrolyzes at the C-2 ester bond *via* attack of OH^- on the positively changed anisoyl carbon. In our case, the silyl pyrimidine was the attacking nucleophile with resultant acylation of its N_1 position.

Consequently, taking the opposite approach, we sought to avoid carbonium ion formation and aimed to increase the stereoselectivity of the coupling reaction by attempting to prepare a crystallizable, pure α-anomeric blocked halogenose which, in the course of an SN2 displacement (involving a single Walden inversion) would favor the formation of the β-nucleoside (Scheme III). Methyl 2,3,5-tri-(*O*-*p*-chlorobenzoyl-β-D-ribofuranoside (VII) was readily prepared, but the displacement of OMe with Br requires an excess amount of 30% HBr in glacial HOAc which would be expected to yield an anomeric mixture of the halogenose. Therefore, VII was converted by standard methods[18] into 2,3,5-tri-*O*-*p*-chlorobenzoyl-1-*O*-*p*-nitrobenzoyl-D-ribofuranose from which the β anomer X could be crystallized in pure form. The *p*-nitrobenzoate group in the C-1 position is readily displaced by an equivalent amount of HBr in CH_2Cl_2, and the reaction can be followed by the change of optical rotation and by weighing the amount of *p*-nitrobenzoic acid precipitated from the solution.[18] This reaction should proceed with a single Walden inversion yielding the α-halogenose XI; in the absence of excess halide, only minimal amount of anomerization would be expected. The halogenose obtained could be isolated in crystalline form, and its nmr spectrum

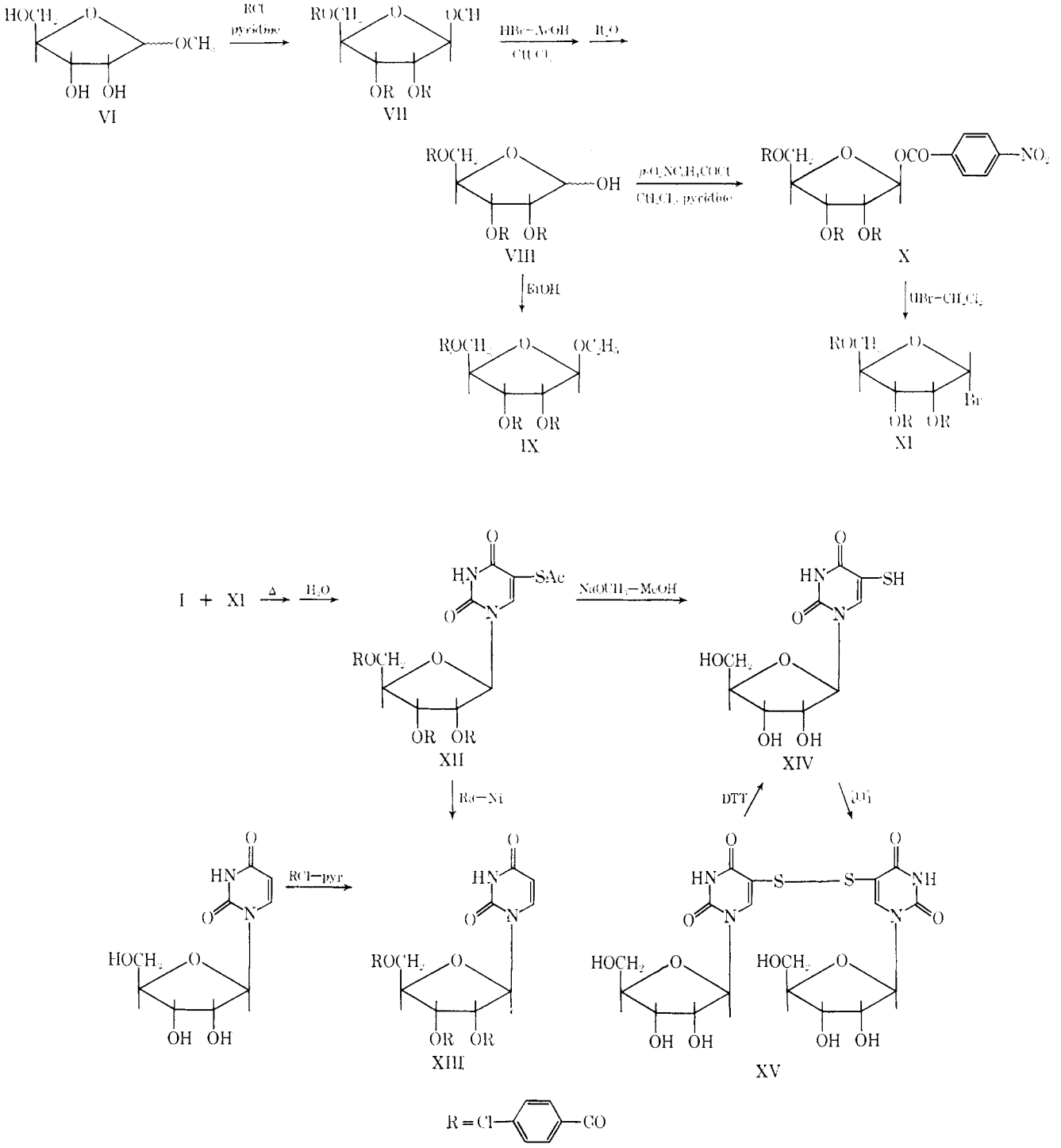
(15) L. J. Haynes, N. A. Hughes, G. W. Kenner, and A. Todd, *J. Chem. Soc.*, 3727 (1957).

(16) B. R. Baker, *Chem. Biol. Purines, Ciba Found. Symp.*, **1956**, 120 (1957).

(17) M. Haga and R. K. Ness, *J. Org. Chem.*, **30**, 158 (1965).

(18) H. G. Fletcher, Jr., *Trans. N. Y. Acad. Sci.*, **30**, 649 (1968).

SCHEME III



showed only the doublet for the α-anomeric proton at δ 6.70; the resonance for the C-1 proton of the β-halogenose (usually a singlet at higher field)[14,19] was absent. Reaction of this halogenose with the silyl pyrimidine I, under fusion conditions *in vacuo*,[9] gave the β-anomeric blocked nucleoside XII in 75–80% yield (based on the reacted pyrimidine). The single, sharp resonance line for the CH_3 of the S-Ac of XII (at δ 2.37 ppm) indicated the anomeric purity of this product. It is interesting to note that a sample of the crude reaction product, before crystallization, showed in its nmr spectrum the same singlet for the CH_3 and the same pattern for the anomeric proton as the purified product; thus, this reaction appears to be entirely stereospecific, yielding only the β anomer.

In order to prove conclusively the anomeric configuration of XII, a sample of this compound was desulfurized with Raney Ni, to yield N_1-(2′,3′,5′-tri-*O*-*p*-chlorobenzoyl-β-D-ribofuranosyl)uracil, XIII. This compound was synthesized by *p*-chlorobenzoylation of uridine and found to be identical with the desulfurization product.

Removal of the blocking groups of XII with NaOMe in MeOH gave 85% yield of the nucleoside, 5-mer-

19) J. D. Stevens and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1799 (1968).

captouridine (XIV) which, however, contained approximately 5% of the oxidized form, bis-(5-uridinyl) disulfide (XV). On recrystallization, the disulfide content increased, due to air oxidation. The uv spectrum of XIV at neutral or alkaline pH shows the characteristic absorption peak of the 5-thiolate ion at λ_{max} 335 mμ which disappears on oxidation to the disulfide, but reappears upon the addition of dithiothreitol (DTT), due to reduction to the thiol.[8] Thus, the relative amount of the disulfide XV in the sample can be estimated on the basis of the absorbancy at 335 mμ in the presence and absence of DTT.

In the preliminary biological testing, 5-mercaptouridine showed significant inhibitory activity in the *Streptococcus faecalis* assay system[20] (I_{50} 2×10^{-6} *M*)[21] but was less active against leukemia L1210 cells in culture (I_{50} 4×10^{-4} *M*).[21] Further studies, relating to the activity spectrum and the mode of action of this analog are in progress.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are uncorrected. Ir spectra (KBr) were recorded on a Perkin-Elmer Infracord or Beckman IR8. Nmr spectra were recorded on a Varian Model A-60 spectrophotometer in $CDCl_3$, unless otherwise indicated, with TMS or *t*-BuOH as an internal standard. Uv spectra were obtained on a Beckman DB recording spectrophotometer. Optical rotations were measured in a dm tube using a Perkin-Elmer Model 141 automatic polarimeter at 589 mμ. Elemental analyses[22] were performed by Galbraith Laboratories, Knoxville, Tenn.

Anomeric *S*-Acetyl-N_1-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)-5-mercaptouracils.—To 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (syrup) prepared from 7.56 g (15 mmol) of 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-β-D-ribofuranose according to Kissman, *et al.*,[13] was added 5-acetylmercapto-2,4-bis(trimethylsiloxy)pyrimidine[9] (I, 5.17 g, 15.7 mmol), and the mixture was mechanically stirred *in vacuo* at 155° (oil bath) for 40 min. The resulting homogeneous melt was dissolved in C_6H_6 (300 ml), and 3 ml of H_2O was added. After standing for 1 hr, the solution containing some ppt was evaporated *in vacuo* and the residue was dried by azeotropic evaporation with C_6H_6 (2×150 ml).

The residue was then dissolved in hot CCl_4 and some insol material was removed by filtration. This was shown to be *S*-acetyl-5-mercaptouracil[9] by mp (251–252°), ir spectrum, and mixture melting point with an authentic sample. The amount of recovered pyrimidine varied between 25 and 35% based on the silyl pyrimidine I used. From the CCl_4 solution, after standing in the cold for 24 hr, the anomeric blocked nucleoside III deposited in the form of somewhat sticky crystals (3.2 g, 50–60% based on *reacted* silyl pyrimidine). Melting range 80–130°, which did not change on repeated attempts of recrystallization from various solvents, nor did the ratio (2:1) of the two nmr peaks at δ 2.30 and 2.36 ppm ($SCOCH_3$) change appreciably on recrystallization. However, the ratio of these two nmr peaks varied somewhat from run to run. The substance gave a single uv-absorbing spot on tlc (silica gel HF) with several eluent systems.

Reaction of the Silylpyrimidine I with 2,3,5-Tri-*O*-*p*-anisoyl-D-ribofuranosyl Chloride (IV).—Methyl 2,3,5-tri-*O*-*p*-anisoyl-β-D-ribofuranoside, prepared according to the method of Haga and Ness,[17] (16.98 g, 30 mmol) was dissolved in ice-cold anhydrous Et_2O (320 ml) which had been saturated with dry HCl at 0°, and the solution was kept at −10° for 1 week. The Et_2O was evaporated *in vacuo*, two 100-ml portions of dry Et_2O were added and again evaporated, to give a syrupy residue of the blocked halogenose IV. To this was added the silyl pyrimidine I (9.90 g, 30 mmol), and the mixture was stirred vigorously and heated *in vacuo* at 110°. A homogeneous melt was obtained within a few min which then gradually solidified. After 30 min the resulting solid was dissolved in C_6H_6 (600 ml) and H_2O (10 ml) was added. After 1 hr, the C_6H_6 solution containing some ppt was concentrated to dryness *in vacuo*, and the residue was dried by repeated addition of C_6H_6 (2×150 ml) followed by evaporation.

The residual solid was treated with boiling C_6H_6 (2 l.) and some insoluble substance was removed by filtration. This was identical with an authentic sample of *S*-acetyl-5-mercaptouracil by mp (251–252°), mixture melting point, and ir spectrum. Recovery of unreacted pyrimidine varied between 30–50% based on the starting material, silyl pyrimidine I. The C_6H_6 filtrate was concentrated and, after 24 hr, a crystallized product V was collected; mp 186–189°; yield 3.8–5.3 g (80% based on the amount of reacted silyl pyrimidine). Two recrystallizations from C_6H_6 yielded an analytically pure sample, mp 192–194°. The nmr spectrum (DMSO-d_6) contained only 3 singlets, at δ 2.4 (3 H, $COCH_3$), 3.9 (3 H, OCH_3), and 8.2 ppm (1 H, C-6), in addition to aromatic protons showing a typical *para* splitting pattern at δ 7.0–8.0 ppm (4 H). All resonance peaks attributable to the protons of the ribose moiety were totally absent. *Anal.* for N_1-*p*-anisoyl-*S*-acetyl-5-mercaptouracil (V) ($C_{14}H_{12}N_2SO_5$): C, H, N, S.

In a control experiment, the syrupy 2,3,5-tri-*O*-*p*-anisoyl-D-ribofuranosyl chloride (IV) was prepared as described above and transferred into a microdistillation apparatus. On attempted vacuum distillation, no distillable material was obtained. Added authentic *p*-anisoyl chloride could be fully recovered by distilling it out from the mixture at 108° (1.5 mm).

Methyl 2,3,5-Tri-*O*-*p*-chlorobenzoyl-β-D-ribofuranoside (VII).—To a solution of D-ribose (15.0 g, 0.1 mol) in dry MeOH(250 ml) was added 1 ml concd H_2SO_4, and the mixture was stirred at 5° for 15 hr. Dry C_5H_5N (50 ml) was then added, and the solution was concentrated *in vacuo* to yield a syrup (anomeric methyl riboside, VI).[23] Addition of dry C_5H_5N (2×40 ml) and evaporation was repeated two more times. The residue was then dissolved in 70 ml of dry C_5H_5N, and the solution was cooled in an ice bath, while *p*-ClC_6H_4COCl (70 g, 0.5 mol) was added gradually. After standing overnight, H_2O (200 ml) and CH_2Cl_2 (250 ml) were added to the partially solidified reaction mixture, and after complete dissolution, two layers were separated. The CH_2Cl_2 layer was washed (H_2O, ice-cold 3.0 *N* H_2SO_4, H_2O saturated $NaHCO_3$), dried ($MgSO_4$), and evaporated *in vacuo*. The residual white solid was crystallized from EtOH to give VII (22.96 g, 40%), mp 102–104°. A small sample for analysis was twice recrystallized from EtOH to give needles, mp 106–108°; $[\alpha]^{20}D$ +89.3° (*c* 5.23, $CHCl_3$).[24] *Anal.* ($C_{27}H_{21}Cl_3O_8$) C, H, Cl.

Anomeric 2,3,5-Tri-*O*-*p*-chlorobenzoyl-D-ribofuranose (VIII) and the Corresponding Ethyl β-Glycoside (IX).—To a solution of VII (2.90 g, 0.005 mol) in CH_2Cl_2 (20 ml) was added a solution of 30% HBr in glacial AcOH (16 ml), and the mixture was stirred for 1 hr. More AcOH (10 ml) was then added, and the soln was kept below 10° while H_2O (10 ml) was gradually added. The two-phase mixture was stirred for an additional 40 min and then poured into ice–water (100 ml) and CH_2Cl_2 (100 ml). The layers were separated, and the CH_2Cl_2 layer was washed several times with $NaHCO_3$ solution, then dried ($MgSO_4$). Evaporation of the CH_2Cl_2 *in vacuo* yielded a syrup which was crystallized from cyclohexane, then recrystallized from petroleum ether, to give the anomeric mixture, VIII, (0.25 g, 8.5%); mp 73–82°. The ir spectrum showed absorption for free (C-1) OH of the ribofuranose at 3500 cm^{-1}, and C=O for the acyl blocking groups at 1735 cm^{-1}. *Anal.* ($C_{26}H_{19}Cl_3O_8$) C, H, Cl.

An attempt to crystallize the originally obtained syrup (crude VIII) from EtOH resulted in the isolation of the corresponding ethyl β-glycoside (IX) in 55% yield. This was purified by repeated recrystallization from EtOH and finally from *n*-hexane; mp 104–107°; $[\alpha]^{20}D$ +85.5° (*c* 1.9; $CHCl_3$). The nmr spectrum showed a doublet for the anomeric proton at δ 5.88 ppm ($J_{1,2}$ = 5.0 Hz).[24] *Anal.* ($C_{28}H_{23}Cl_3O_8$) C, H, Cl.

2,3,5-Tri-*O*-*p*-chlorobenzoyl-1-β-*O*-*p*-nitrobenzoyl-D-ribofuranose (X).—The crude, syrupy anomeric 2,3,5-*p*-chlorobenzoyl-D-ribofuranose (VIII) obtained above was redissolved in dry CH_2Cl_2 (15 ml). To this was added a solution of *p*-nitrobenzoyl chloride (1.86 g, 0.01 mol) in a mixture of dry CH_2Cl_2 (20 ml) and dry C_5H_5N (15 ml) while the reaction flask was kept in ice–water bath. After 4–5 hr stirring, chips of ice were added

(20) A. Bloch, M. H. Fleysher, R. Thedford, R. F. Maule, and R. H. Hall, *J. Med. Chem.*, **9**, 886 (1966).

(21) A. Bloch, personal communication.

(22) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(23) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4605 (1961).

(24) Assignment of anomeric configuration tentative, based on analogies of preparation and comparative optical rotation of similar glycosides.[13,17]

and stirring was continued for additional 30 min. The solution was then transferred into a separatory funnel and washed successively with ice-cold 1.0 *N* H_2SO_4, H_2O, ice-cold saturated $NaHCO_3$, and ice-cold saturated Na_2CO_3 solution, then dried ($MgSO_4$) and evaporated *in vacuo*. The solid residue was crystallized from EtOH (Norite), to give crude X; 0.962 g (26%); mp 140–145°. A sample for analysis was several times recrystallized from EtOH; mp 151–153°; $[\alpha]^{20}D$ +51.7° (*c* 2.0, $CHCl_3$). The nmr spectrum of the recrystallized sample showed an apparent singlet for the C-1 proton, at δ 6.65 ppm, indicating *trans* C$_1$-H/C$_2$-H (*i.e.*, β) configuration (the nmr of crude sample showed, in addition to this singlet, also a small doublet at δ 7.05 ppm ($J_{1,2}$ = 4.0 Hz) for the anomeric proton of the α isomer). *Anal.* ($C_{33}H_{22}Cl_3NO_{11}$) C, H, Cl, N.

2,3,5-Tri-*O*-*p*-chlorobenzoyl-α-D-ribofuranosyl Bromide (XI). To a solution of X (1.43 g, 2 mmol) in dry CH_2Cl_2 (15 ml) was added a saturated solution of HBr gas (2 mmol) in CH_2Cl_2 (5 ml). After 1 hr, the crystallized *p*-$O_2NC_6H_4CO_2H$ was separated by filtration (90–96% of theoretical), and the filtrate was evaporated *in vacuo*. The residual syrup solidified to a crystalline mass on standing for 2 hr; nmr (C-1 proton): a doublet at δ 7.70 ppm ($J_{1,2}$ = 2.7 Hz). This product was used in the subsequent step without further purification. A small sample was twice recrystallized (CCl_4); $[\alpha]^{20}D$ +36.0° (*c* 1.15, $CHCl_3$). *Anal.* ($C_{26}H_{18}BrCl_3O_7$) C, H.

***S*-Acetyl-N_1-[2,3,5-tri-*O*-*p*-chlorobenzoyl)-β-D-ribofuranosyl]-5-mercaptouracil (XII).**—To a solution of 2,3,5-tri-*O*-*p*-chlorobenzoyl-D-ribofuranosyl bromide (XI), freshly prepared from 1.43 g (2 mmol) of X (see above), in dry CH_2Cl_2 (10 ml), was added a solution of 5-acetylmercapto-2,4-bis(trimethylsiloxy)-pyrimidine (I, 0.66 g, 2 mmol) in dry CH_2Cl_2 (5 ml). The solvent was slowly evaporated *in vacuo* while stirring. The flask containing the residual foam was submerged in an oil bath preheated to 110°, and stirring *in vacuo* was continued for 50 min. During this period, evolution of gaseous Me_3SiBr was observed, and a homogenous melt was obtained. The melt was dissolved in 50 ml of hot C_6H_6, and 1 ml of H_2O was added. After standing for 1 hr, the C_6H_6 solution was concentrated *in vacuo*, and the residue was dried by repeated addition of fresh C_6H_6 (2 × 20 ml) followed by evaporation. The thick residue was treated with C_6H_6 (30 ml), and some insoluble substance was removed by filtration. The latter was identified as *S*-acetyl-5-mercaptouracil[9] by mp (251–252°), mixture melting point with an authentic sample, and ir spectrum. Recovery of unreacted pyrimidine varied between 20 and 25% (based on I).

The filtrate was heated to boiling and hot cyclohexane was added until the solution became turbid. After standing for several hours at room temperature, the separated crystalline material was collected. Concentration of the mother liquor yielded more crystalline material, to give a total of 1.10–1.17 g of XII (75–80% based on the ribofuranosyl halide XI and almost 100% based on the reacted amount of silyl pyrimidine I). After several crystallizations from C_6H_6–cyclohexane, an analytical sample was obtained; mp 125–128°; $[\alpha]^{20}D$ +26.0° (*c* 5.23, $CHCl_3$). The nmr spectrum showed a singlet for the $SCOCH_3$ protons at δ 2.37 ppm, and a doublet for the C′-1 proton at 6.67 (J = 5.0 Hz). *Anal.* ($C_{32}H_{23}Cl_3N_2O_{10}S$) C, H, Cl, N, S.

N_1-[(2,3,5-Tri-*O*-*p*-chlorobenzoyl)-β-D-ribofuranosyl]uracil (XIII).—To a solution of uridine (1.220 g, 5 mmol) in dry C_5H_5N (80 ml) was gradually added *p*-chlorobenzoyl chloride (3.500 g, 20 mmol) in dry C_5H_5N (20 ml). After 4 hr refluxing, the C_5H_5N solution was poured on ice (150 g) and CH_2Cl_2 (20 ml) was added. After vigorous shaking in a separatory funnel, the CH_2Cl_2 layer was separated and washed with ice-cold 3 *N* H_2SO_4, H_2O, and saturated $NaHCO_3$, then dried ($MgSO_4$) and the CH_2Cl_2 evaporated *in vacuo*. The residue was crystallized from boiling EtOH (400 ml); yield 2.2 g (72%); mp 231–234°. Several crystallizations from EtOH yielded an analytical sample, mp 237–239°; $[\alpha]^{20}D$ −58.0° (*c* 0.85, $CHCl_3$). *Anal.* ($C_{36}H_{21}Cl_3N_2O_{11}$) C, H, Cl, N.

Desulfurization of *S*-Acetyl-N_1-](2,3,5-tri-*O*-*p*-chlorobenzoyl)-β-D-ribofuranosyl]-5-mercaptouracil (XII).—To a solution of XII (0.367 g) in C_6H_6 (25 ml) was added Raney Ni (W2; 2.2 g) and the mixture was refluxed for 24 hr. The catalyst was then carefully removed by filtration and centrifugation, and the C_6H_6 was evaporated *in vacuo*. The solid residue was crystallized from hot EtOH to yield a crystalline substance (0.170 g), which was shown to be identical with XIII by melting point, mixture melting point, optical rotation, ir spectrum, and tlc (silica gel HF, with EtOH as the eluent).

5-Mercaptouridine (XIV).—To a suspension of XII (0.734 g, 1 mmol) in dry MeOH (25 ml) was added a solution of $NaOCH_3$, freshly prepared by dissolving Na metal (46 mg, 2 mg-atoms) in MeOH (10 ml). The mixture was stirred under N_2 for 2 hr. Dowex 50W-X8 ion-exchange resin (H^+ form, approximately 9 mequiv) was then added and the resulting suspension stirred for 10 min. The ion-exchange resin was removed by filtration and washed with MeOH (10 ml). The combined filtrate and wash was concentrated *in vacuo* to yield a syrup. To this was added MeOH (1.5 ml), then Et_2O (40 ml). After 24 hr standing, the separated white crystalline material XIV was collected by filtration; 0.207 g. Concentration of the mother liquor yielded additional 0.025 g, raising the yield of XIV to 0.232 g (84%). This product contained about 5% of the disulfide XV, based on its uv absorbancy at λ 335 mμ in the presence and absence of DDT.[8] Two crystallizations from MeOH–Et_2O raised the disulfide content to 30%; nmr (D_2O): 6.19 (d, J = 2.6 Hz, C′-1H) and 7.78 ppm (s, C_6-H).

Optical rotation was taken in EDTA buffer (pH 7.6) in the presence of 2-mercaptoethanol (the blank containing the 2-mercaptoethanol showed zero optical rotation) $[\alpha]^{20}D$ +21.8° (*c* 2.18); uv (pH 7.6, in the presence of DTT) λ_{max} 335 mμ (ε 5400).

Anal. ($C_9H_{12}N_2SO_6$) Calcd: C, 39.13; H, 4.38; N, 10.14; S, 11.61. Found: C, 38.63; H, 4.14; N, 9.75; S, 11.24.

Bis[N_1-(β-D-ribofuranosyl)-5-mercaptouracilyl] Disulfide (XV).—The pH of a solution of 5-mercaptouridine (0.100 g) in 50 ml of H_2O was adjusted to 9.0 by dropwise addition of NH_4OH. The solution was allowed to stir for 24 hr exposed to air. The H_2O was then evaporated *in vacuo* and, repeatedly, EtOH was added and evaporated, to remove last traces of H_2O. The solid residue was crystallized from hot EtOH; 0.060 g; mp 236–237°, uv (pH 7.6) λ_{max} 281 mμ; after addition of DTT, λ_{max} 335 mμ (ε 11,200); optical rotation of XV, $[\alpha]^{20}D$ −212.0° (*c* 0.75, MeOH). *Anal.* ($C_{18}H_{22}N_4S_2O_{12}$) C, H, N, S.