thin layer chromatography using ether as the developer. The band containing the product could be seen readily under ultraviolet light. This was scraped off, and the product was extracted from the silica gel with chloroform in a Soxhlet extractor. The chloroform was evaporated and the residue was sublimed at 100° (0.04 mm.) to give 0.086 g. (55%) of pure material with m.p. 126–128°; infrared absorption in potassium bromide: 1610 cm.$^{-1}$ (aromatic); ultraviolet absorption in 95% ethanol (max.): 295 mμ (log ε 3.96), 254 (4.34), and 230 (4.05); ultraviolet absorption spectrum of 2-phenylnaphthalene in 95% ethanol (max.)[32]: 288 mμ (log ε 4.1), 259 (4.1), and 251 (4.8).

Anal. Calcd. for $C_{14}H_{10}N_2$: C, 81.53; H, 4.89; N, 13.58. Found: C, 81.76; H, 4.81; N, 13.59.

A **monopicrate**, m.p. 210–211°, was prepared in ethanol and recrystallized from the same solvent.

Anal. Calcd. for $C_{20}H_{13}N_5O_7$: C, 55.18; H, 3.01; N, 16.09. Found: C, 55.19; H, 3.18; N, 16.06.

A **monopicrolonate**, m.p. 255–256° dec. (cap.), was prepared in ethanol and recrystallized from the same solvent.

Anal. Calcd. for $C_{24}H_{18}N_6O_5$: C, 61.27; H, 3.86; N, 17.87. Found: C, 61.13; H, 3.95; N, 18.19.

8-Oxo-6-phenyl-5,6,7,8-tetrahydroisoquinoline (18).—Sodium (287.5 mg., 0.0125 g.-atom) was dissolved in 35 ml. of anhydrous ethanol. Ethyl malonate (2.0 g., 0.0125 mole) was added, followed by 2.060 g. (0.01 mole) of 3-cyano-4-stilbazole (**8**). The red solution was stirred for 8 hr. at room temperature. Subsequent examination by thin layer chromatography, using ether as developer, showed the absence of any starting material. The ethanol was evaporated under vacuum and the oily red residue was heated under reflux for 6 hr. with 60 ml. of 3 *N* hydrochloric acid. The cooled acid solution was extracted once with 25 ml. of ether, made slightly alkaline with solid sodium bicarbonate, and extracted six times with 35-ml. portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate. The chloroform was evaporated to give 2.5 g. of oil. The oil was dissolved in ether, evaporated on 5 g. of deactivated neutral alumina, and chromatographed on 100 g. of neutral alumina, activity grade IV (Brockmann). The column was developed using gradient elution. The first solvent was 1 l. of *n*-pentane followed by 1 l. of ether–*n*-pentane (25:75), followed by 1 l. of ether–*n*-pentane (40:60). Fractions of 15 ml. were collected and examined by thin layer chromatography using ether–*n*-pentane (75:25) as the developer. Fractions **1–72** contained nothing. Fractions **73–80** contained an impurity. Fractions **81–100** contained impurity and a trace of the product. Fractions **101–150** contained pure product. The latter were combined and evaporated to yield 1.433 g. (64% yield) of 8-oxo-6-phenyl-5,6,7,8-tetrahydroisoquinoline (**15**). After sublimation (110° at 0.04 mm.) and three recrystallizations from benzene–hexane (1:1), the ketone had m.p. 83–84°; infrared absorption in potassium bromide: 1680 cm.$^{-1}$ (ArCO—).

Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27. Found: C, 80.93; H, 5.91; N, 6.37.

An **oxime**, m.p. 228–229° (cap.), was prepared in aqueous ethanol and recrystallized from the same solvent.

Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.34; H, 6.06; N, 11.71.

A **picrate**, m.p. 154–157° (cap.), was prepared in ethanol and recrystallized from the same solvent.

Anal. Calcd. for $C_{21}H_{16}N_4O_8$: C, 55.76; H, 3.57; N, 12.38. Found: C, 55.84; H, 3.70; N, 12.39.

A **styphnate**, m.p. 190–191° (cap.), was prepared in ethanol and recrystallized from the same solvent.

Anal. Calcd. for $C_{21}H_{16}N_4O_9$: C, 53.85; H, 3.44; N, 11.96. Found: C, 53.80; H, 3.60; N, 12.03.

(32) L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, **60**, 940 (1938).

Thio Sugars. III. Synthesis and Rearrangement of 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea Dihydrobromide and Analogs[1]

M. L. WOLFROM, W. A. CRAMP, AND D. HORTON

Department of Chemistry, The Ohio State University, Columbus 10, Ohio

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Condensation of 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl bromide hydrobromide (I) with thiourea in acetone solution gave 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea dihydrobromide (II) crystallizing with 1 mole of acetone and further characterized as the diflavianate; condensation in a 2-propanol medium gave predominantly isopropyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranoside hydrobromide (IV). Improved preparative directions are cited for the D-*gluco* analog (V) of II and it is shown that some isopropyl tetra-*O*-acetylglycoside is likewise formed when V is prepared in 2-propanol solution. Substance V was further characterized as its diflavianate. Substance II, an analog of the radiation protective agent 2-(2-aminoethyl)-2-thiopseudourea (AET), undergoes rearrangement in neutral solution to 3,4,6-tri-*O*-acetyl-2-deoxy-2-guanidino-1-thio-β-D-galactose hydrobromide (III). Determination of III and its D-glucose analog (VI) by thiol assay is described.

In the first paper[2] in this series we reported the preparation of 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea dihydrobromide (V), and a subsequent paper[3] described the rearrangement undergone by V at pH 7 in aqueous solution to give 3,4,6-tri-*O*-acetyl-2-deoxy-2-guanidino-1-thio-β-D-glucose (VI). Systems of this type are of interest as potential radiation protective agents, since they incorporate into a carbohydrate matrix the functional groups of 2-(2-aminoethyl)-2-thiopseudourea (AET). The latter[4] is one of the most effective agents known for the protection of biological systems against ionizing radiation,[5] but its high toxicity is a disadvantage. It was hoped that carbohydrate derivatives would function as protective compounds with low toxicity. The present work describes the synthesis of the D-galactose analog (II) of V, together with further studies on V. A study of the rearrangement of II and V to the corresponding guanidino thiols (III and VI) is also described. It was considered that the D-galactose derivative (II) would be less readily metabolizable than the D-glucose analog

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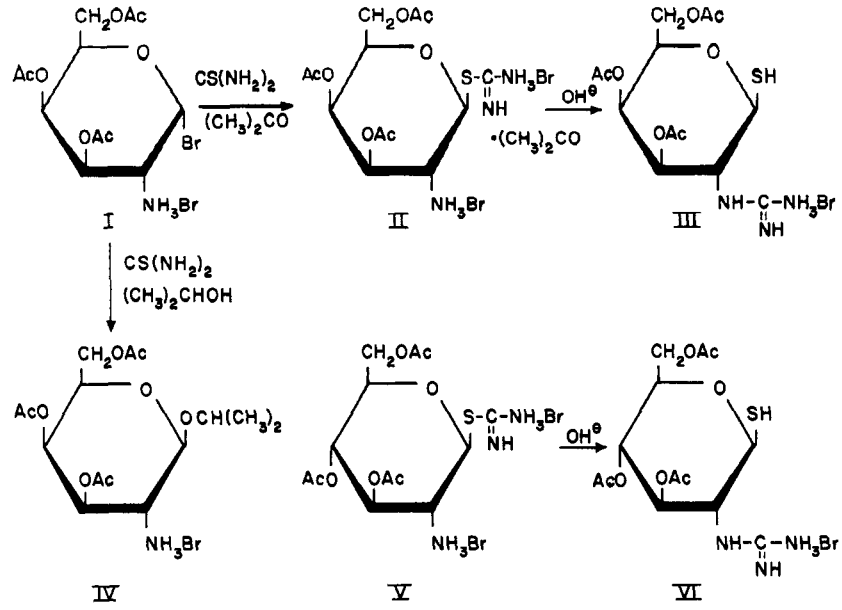
(2) D. Horton and M. L. Wolfrom, *J. Org. Chem.*, **27**, 1794 (1962).

(3) M. L. Wolfrom, D. Horton, and D. H. Hutson, *ibid.*, **28**, 845 (1963).

(4) J. X. Khym, R. Shapira, and D. G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957); D. G. Doherty, R. Shapira, and W. T. Burnett, Jr., *ibid.*, **79**, 5667 (1957).

(5) D. G. Doherty, in "Radiation Protection and Recovery," A. Hollaender, Ed., Pergamon Press, London, 1960, p. 45.



(V), and might thus be more effectively transported *in vivo* to the site of action.

3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl bromide hydrobromide[6] (I), prepared from 2-amino-2-deoxy-D-galactose hydrochloride[7] in one step, underwent condensation with thiourea smoothly in boiling acetone solution, to give 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea dihydrobromide (II) as a well-crystallized product in high yield; its infrared spectrum was closely similar to that of the D-glucose analog[2] (V) in the region 2.5–9.0 μ, and its specific rotation (−8°) was indicative of the β-D configuration. The crystalline product was a solvate, having 1 mole of acetone of crystallization, as determined by elemental analysis and by quantitative determination of acetone as the crystalline 2,4-dinitrophenylhydrazone. The acetone was tenaciously retained, even on recrystallization from different solvents. Treatment of an aqueous solution of II with flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid) gave a crystalline diflavianate salt analog of II in high yield as a monohydrate. Attempted preparation of II from I and thiourea, in a 2-propanol medium, a procedure effective[2] for preparation of the D-glucose analog (V), was found to give, as major product, a nonreducing substance having the analysis, specific rotation, and infrared spectrum required for isopropyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranoside hydrobromide (IV). Only a small proportion of the thiourea condensation product (II) was formed under these reaction conditions.

A re-examination of the reaction conditions[2] leading to 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea dihydrobromide (V) indicated that improvements could be made by substitution of acetone in place of 2-propanol as the condensation medium, with precipitation of the product in microcrystalline form instead of isolation by slow crystallization. The X-ray powder diffraction pattern attested to the crystallinity of the precipitated product. Aqueous flavianic acid converted V into the crystalline diflavianate salt, isolated in high yield as a monohydrate. Numerous preparations of V were made, by the original procedure[2] with 2-propanol as the reaction medium, and also with acetone as solvent; better yields were obtainable in the latter solvent. On some occasions, a small proportion (about 10%) of a side product separated directly from the reaction mixture when 2-propanol was used as solvent. Analytical data indicated this product to be isopropyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide. Its structure was proved by acetylation to isopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside, synthesized independently by the condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[2,8] with 2-propanol in benzene solution in the presence of mercuric cyanide.[9] No crystalline products were obtained on attempts to deacetylate V or to prepare the disulfide of its rearranged product (VI).

When an aqueous solution of 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea dihydrobromide (II) was treated with base to bring the pH to 7, or if II was dissolved in a phosphate buffer (pH 7.0), a very rapid rearrangement to the guanidino thiol (III) took place, as shown by the fact that the product gave positive reactions for thiol and for the guanidino function. The behavior of II in undergoing the rearrangement is thus closely similar to that of the D-glucose analog[3] (V).

The release of free thiol on rearrangement of II and V was determined by the thiol assay procedure of Basford and Hunnekens,[10] as used by Doherty and associates.[4] Reference thiol derivatives, also determined by this procedure, were 2-guanidinoethanethiol (prepared by rearrangement of 2-(2-aminoethyl)-2-thiopseudourea hydrobromide in a neutral buffer solution), and 1-thio-D-glucose (prepared in solution by saponification of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucose[2]).

(6) M. L. Wolfrom, W. A. Cramp, and D. Horton, *J. Org. Chem.*, **28**, 3231 (1963).

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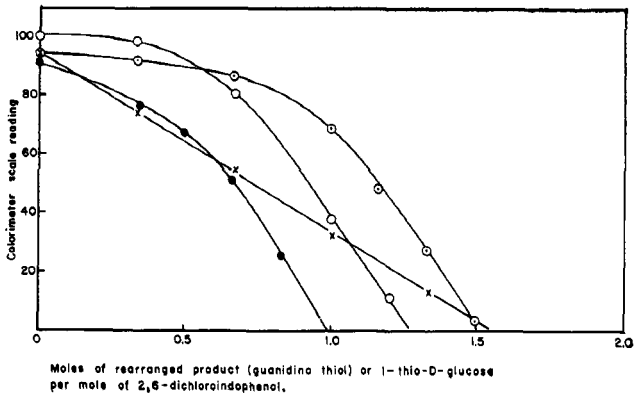

Fig. 1.—Thiol assay on products as rearranged to ×, 2-guanidinoethanethiol; O, III; ⊙, VI, and ●, 1-thio-β-D-glucopyranose. See Experimental section for details.

The determination[10] utilizes the decoloration of the blue dye 2,6-dichloroindophenol by free thiol, and the amount of decoloration, determined photocolorimetrically against a standard, gives the desired titre. It can be seen (Fig. 1) that, for 2-guanidinoethanethiol, the decoloration of the dye is linearly related to the amount of thiol added, and complete decoloration occurred when 1.6 moles of thiol per mole of dye had been added. In the case of the sugar thiol derivatives the relation was not linear (Fig. 1); the extrapolated values for complete decoloration gave, per mole of dye, thiol consumptions of 1.3, 1.5, and 1.0 moles of III, VI, and 1-thio-D-glucose, respectively. Basford and Hunnekens[10] postulate two alternative mechanisms for the reduction of 2,6-dichloroindophenol by thiols, one requires 1 molar equiv., the second requires 2 molar equiv. of thiol per mole of dye. The observed values for III, VI, and 2-guanidinoethanethiol would suggest the involvement of both reaction pathways.

Experimental[11]

2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea Dihydrobromide Monoacetonate (II).—To a solution of thiourea (0.18 g.) in warm anhydrous acetone (30 ml.) was added 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl bromide hydrobromide[6] (I, 1.00 g.), and the mixture was refluxed 30 min. Refrigeration overnight gave crystalline II, yield 0.95 g. (82%), m.p. 159–161° dec. Recrystallization was readily effected from methanol–ethyl acetate or from acetone to give analytically pure material which contained 1 molar equiv. of acetone of crystallization. It had m.p. 161–163° dec., $[\alpha]^{23}D$ −8 ± 2° (*c* 0.89, methanol); λ_{max}^{KBr} 5.70 (OAc), 6.1 (C=N) μ; X-ray powder diffraction data[12]: 15.19 s, 11.63 vs (1), 7.56 s, 6.56 w, 6.19 m, 5.64 s, 4.87 s, 4.67 w, 4.48 vs (2,2), 4.29 vs (2,2), 4.00 s, 3.65 vs (3) Å.

Anal. Calcd. for $C_{13}H_{23}Br_2N_3O_7S \cdot (CH_3)_2CO$: C, 32.94; H, 5.01; Br, 27.39; N, 7.25; S, 5.51; $(CH_3)_2CO$, 9.95. Found: C, 32.97; H, 5.05; Br, 27.04; N, 7.24; S, 5.43; $(CH_3)_2CO$, 9.82.

The acetone of crystallization in II was determined quantitatively by precipitation of the 2,4-dinitrophenylhydrazone. The precipitating reagent was prepared by dissolving 2,4-dinitrophenylhydrazine (2 g.) in methanol (30 ml.), adding water (10 ml.) and concentrated sulfuric acid (4 ml.), then filtering. To a solution of crystalline II (200 mg.) in water (3 ml.) was added an excess of the 2,4-dinitrophenylhydrazine reagent. The orange precipitate was filtered after 12 hr. and dried to constant weight at room temperature. Analysis of the precipitate gave acceptable values for acetone 2,4-dinitrophenylhydrazone.

Anal. Calcd. for $C_9H_{10}N_4O_4$: C, 45.37; H, 4.23. Found: C, 45.32; H, 4.48.

Product II tenaciously retained 1 mole of acetone of crystallization, even after repeated recrystallization from methanol–ethyl acetate; the product showed no change in melting point or X-ray powder diffraction pattern. Removal of the acetone by heating or by repeated codistillation with another solvent gave noncrystallizable sirups.

2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea Diflavianate Monohydrate.—A 0.5 *M* aqueous solution of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid) was added dropwise to a solution of 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea dihydrobromide (II, 100 mg.) in water (5 ml.) until no further precipitation was observed. The oil which separated solidified on refrigeration, and the solid was washed by decantation with water at 0°. Recrystallization from absolute ethanol gave pure material, as a lemon yellow monohydrate, yield 145 mg. (85%), m.p. 182–184° dec.

Anal. Calcd. for $C_{33}H_{33}N_7O_{23}S_3 \cdot H_2O$: C, 39.29; H, 3.56; N, 9.74; S, 9.16. Found: C, 39.11; H, 3.78; N, 9.85; S, 9.14.

Thin layer chromatography[11] of the product, with 3:1 ethanol–water eluent, revealed the product as a single discrete zone, R_f 0.8.

Isopropyl 3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranoside Hydrobromide (IV).—This compound was the major product when condensation of I with thiourea was attempted, with 2-propanol as solvent. To a solution of thiourea (0.18 g.) in warm 2-propanol (30 ml.) was added 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl bromide hydrobromide[6] (I, 1.00 g.), and the mixture was refluxed 30 min. The isopropyl tri-*O*-acetylglycoside crystallized on cooling, yield 0.57 g. (60%), m.p. 248–252°, $[\alpha]^{24}D$ −27.5 ± 2° (*c* 2.1, methanol); X-ray powder diffraction data[12]: 15.19 s, 12.52 vs (1), 8.19 w, 5.34 s, 5.16 m, 4.84 w, 4.69 s, 4.53 s, 4.33 m, 4.21 w, 3.90 vs (2,2), 3.71 vs (2,2), 3.58 w, 3.47 m, 3.40 vs (3), 3.20 m Å.

Anal. Calcd. for $C_{15}H_{26}BrNO_8$: C, 42.07; H, 6.12, Br, 18.67; N, 3.27. Found: C, 42.41; H, 6.43; Br, 19.37; N, 3.44.

The product was nonreducing to Benedict solution. Thin layer chromatography of the residual mother liquors indicated that little of the condensation product II had been formed in the reaction.

Preparation of 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea Dihydrobromide (V).—This compound is formed readily under the reaction conditions described by Horton and Wolfrom[2] but crystallization is often slow, even on nucleation. It was found that substitution of dry acetone as the reaction medium, instead of 2-propanol, gave the most consistent results in the reaction. Evaporation of the reaction solution, dissolution of the sirup in 2-propanol, followed by cautious addition of ethyl acetate, then petroleum ether (b.p. 30–60°), caused the separation of V as microcrystalline material in yields of 55–80%. This product had X-ray powder diffraction pattern, specific rotation, and infrared spectrum identical with those of the product prepared by the original procedure[2]; the melting point (167–174° dec.) was about 9° lower than that of the slowly crystallized material. Attempts to deacetylate V by acid-catalyzed methanolysis or to obtain the disulfide of VI by iodine oxidation following rearrangement did not lead to definitive products of predictable analysis.

On some occasions, when V was prepared by the procedure of Horton and Wolfrom,[2] a small proportion (10%) of product separated directly from the cooled 2-propanol reaction medium. Recrystallization of this side product from 2-propanol gave fine needles, m.p. 238–240° dec., $[\alpha]^{24}D$ −22 ± 2° (*c* 3.03, methanol); λ_{max}^{KBr} 5.72 (OAc) μ, NHAc absent; X-ray powder diffraction data[12]: 11.52 vs (1), 8.12 w, 5.31 s, 4.98 w, 4.62 vs (3), 4.17 w, 3.92 vs (2), 3.36 s, 2.89 m Å. This product was identified, by con-

(11) Melting points were taken on a Fisher-Johns apparatus. The $[\alpha]D$ values were measured with a 2-dm. polarimeter tube. Microanalyses were performed by W. N. Rond. Infrared spectra were measured on a Perkin-Elmer Infracord Model 137 infrared spectrophotometer. The potassium bromide pellets were pressed from a finely ground mixture of the substance with dried A.R. grade potassium bromide. Thin layer chromatography was carried out with silica gel G (E. Merck, Darmstadt, Germany) activated at 100°, and zones were revealed by spraying with concentrated sulfuric acid, with subsequent heating at 100°.

(12) Interplanar spacing Å., Cu Kα radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. First few strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

versions shown below, as **isopropyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide.**

Anal. Calcd. for $C_{15}H_{26}BrNO_8$: C, 42.07; H, 6.12; Br, 18.67; N, 3.27. Found: C, 42.06; H, 6.28; Br, 19.9: N, 3.27.

2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea Diflavianate Monohydrate.—This derivative was prepared from the dihydrobromide salt V (100 mg.) by a procedure essentially similar to that used in preparation of the D-*galacto* analog. The yellow product crystallized from absolute ethanol, yield 0.150 g. (88%), m.p. 179–180° dec. The melting point was not changed by further recrystallization.

Anal. Calcd. for $C_{33}H_{33}N_7O_{23}S_3 \cdot H_2O$: C, 39.29; H, 3.56; N, 9.74; S, 9.16. Found: C, 39.68; H, 3.85; N, 9.63; S, 8.96.

The product showed a single zone, R_f 0.8, on thin layer chromatography with 3:1 ethanol–water developer.

Isopropyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside. A. From 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl Chloride.—To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[2,8] (4.0 g.) in dry benzene (75 ml.) was added 2-propanol (3.0 ml.) and mercuric cyanide (3.6 g.). The mixture was stirred overnight, chloroform (150 ml.) was added, and the solution was extracted with three 20-ml. portions of water, dried over anhydrous magnesium sulfate, and evaporated to a sirup, which crystallized from methylene chloride–ether as fine needles, yield 2.68 g. (63%), m.p. 169–170°, $[\alpha]^{22}D$ +22 ± 2° (*c* 0.65, chloroform); λ_{max}^{KBr} 5.75 (OAc), 6.07, 6.45 (NHAc) μ; X-ray powder diffraction data[12]: 11.63 vs (1), 8.50 s, 7.97 w, 7.25 s, 6.70 m, 6.24 m, 5.83 w, 5.25 m, 4.72 vs (2), 4.51 s, 4.37 vs (3), 4.23 m Å.

Anal. Calcd. for $C_{17}H_{27}NO_9$: C, 52.43; H, 6.99; N, 3.60. Found: C, 52.40; H, 7.13; N, 3.72.

B. From Isopropyl 2,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside Hydrobromide.—A solution of isopropyl 2,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide (0.3 g.) in pyridine (5 ml.) and acetic anhydride (2.5 ml.) was maintained at room temperature for 15 hr., poured on ice, and the product extracted with chloroform. The washed and dried extract was evaporated and the residue was crystallized from methanol–ether to give needles, yield 0.21 g. (63%), m.p. 169–170°, $[\alpha]^{22}D$ +24 ± 2° (*c* 0.77, chloroform), identical by mixture melting point, elemental analysis, X-ray powder diffraction pattern, and infrared spectra with the product isolated under A above. Both samples migrated as a single zone, R_f 0.45, on thin layer chromatography with 4:1 benzene–methanol as developer.

Rearrangement of 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea Dihydrobromide (II).—Aqueous solutions of II gave a negative thiol reaction, but, after adjusting to pH 7 by the addition of sodium hydroxide solution or phosphate buffer, the solution gave strong thiol reactions with sodium nitroprusside or 2,6-dichloroindophenol, and also gave a strong Sakaguchi reaction.[13] These data indicate that II had undergone rearrangement to the guanidino thiol III.

(13) R. A. B. Bannard, A. A. Casselman, W. F. Cockburn, and G. M. Brown, *Can. J. Chem.*, **36**, 1541 (1958).

Thiol Assay Procedure.—Free thiol was determined[10] by adding a solution of the thiol to a solution of the dye 2,6-dichloroindophenol, and determining photocolorimetrically the extent to which the dye was decolorized. A Klett-Summerson photoelectric colorimeter, with a Wratten No. 70 (dark red) filter transmitting at 650–700 mμ, was used. A 0.4 *M* phosphate buffer solution, pH 7.0, was prepared from potassium dihydrogen phosphate (9.1 g.) and disodium hydrogen phosphate (18.9 g.) made up to 1 l. with distilled water. A stock solution of the dye was prepared by dissolving sodium 2,6-dichloroindophenol dihydrate (4 mg.) in buffer (200 ml.). For each determination, a blank was prepared from the stock dye solution (5.00 ml.) and 0.2 *N* hydrochloric acid (1.00 ml.). The resultant 5×10^{-5} *M* solution showed no significant change in pH, and it gave a colorimeter scale reading of 100 ± 4 units; the zero reading was made with distilled water. Solutions for thiol assay were prepared in 0.2 *N* hydrochloric acid at a concentration of 1×10^{-3} *M*, and the determination was performed by adding to an aliquot of this solution sufficient 0.2 *N* hydrochloric acid to bring the volume to 1.00 ml., and subsequently adding 5.00 ml. of the stock dye solution. The colorimeter reading was taken, in stoppered tubes, 2–3 min. after mixing, when decoloration of the dye was at a maximum. Colorimeter readings were plotted against thiol concentration, and the curves were extrapolated to zero reading, the point which corresponded to complete bleaching of the dye.

Rearrangement of Amino 2-Thiopseudourea Derivatives to Guanidino Thiols and Determination of Thiol. A. 2-(2-Aminoethyl)-2-thiopseudourea (AET).—A 10^{-3} *M* solution of 2-(2-aminoethyl)-2-thiopseudourea in 0.2 *N* hydrochloric acid was used, the rearrangement taking place on addition to the neutral buffer system. A linear plot of sample added against dye bleached, was obtained (Fig. 1), and extrapolation to total bleaching of dye showed that approximately 1.6 moles of substance per mole of dye was required.

B. 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea Dihydrobromide (V).—A 10^{-3} *M* solution of V in 0.2 *N* hydrochloric acid was used in the determination. A nonlinear plot of sample added against dye bleached was observed (Fig. 1), and extrapolation to total bleaching of dye indicated consumption of approximately 1.5 moles of rearranged V (VI) per mole of dye.

C. 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranosyl)-2-thiopseudourea Dihydrobromide (II).—Under the conditions of the above determination (B) the rearranged D-galactose derivative (III) gave a nonlinear plot (Fig. 1), with an extrapolated value for total bleaching of approximately 1.3 moles of III per mole of dye.

Thiol Assay on 1-Thio-D-glucose.—A solution of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose[2] (20.0 mg.) in dry methanol (5.0 ml.) was treated, under nitrogen, with 1 *N* methanolic sodium methoxide (0.5 ml.), and after a short time the solution was made up to 100 ml. with 0.2 *N* hydrochloric acid. The resultant solution was used in the thiol assay, and a nonlinear plot was observed (Fig. 1). The extrapolated value for total bleaching of the dye was 1 mole of 1-thio-D-glucose per mole of dye.