THIOGLYCOSIDES HAVING *O*-BENZYL BLOCKING GROUPS AS INTERMEDIATES FOR THE SYSTEMATIC, SEQUENTIAL SYNTHESIS OF OLIGOSACCHARIDES. SYNTHESIS OF ISOMALTOSE

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ABSTRACT

A series of *O*-benzylated 1-thio-D-glucosides was prepared, by the condensation of *O*-benzylglucosyl halides with thiols, and the behavior of these products was examined under the conditions of the coupling and deblocking steps of an oligosaccharide synthesis. Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**9**), obtained via the 6-acetate **8**, was coupled with 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**7**). The yield of blocked disaccharide **11** (+ β-anomer ?) was better than the yield of the isomaltoside **17**, which resulted from the coupling of **7** with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**6**). Under the conditions of *O*-debenzylation by sodium–liquid ammonia, the phenylthio group of the tetra-*O*-benzylthioglucoside **3** was cleaved to a considerable extent, with reduction of the anomeric carbon. The 3-phenylpropyl thioglucosides **4** (tetra-*O*-benzyl) and **10** (2,3,4-tri-*O*-benzyl) were smoothly deblocked to 3-phenylpropyl 1-thio-β-D-glucopyranoside (**5**), which was hydrolyzed to D-glucose by aqueous mercuric chloride. The condensation of **7** and **10** gave, in 67% yield, a disaccharide product (**12**+**13**, after deacetylation **14** and **15**) which was 72% α-anomer (isomaltoside), 28% β-anomer (gentiobioside). The isomaltoside was *O*-debenzylated (to **16**) and hydrolyzed to isomaltose in good yield.

INTRODUCTION

The preparation, by chemical means, of a complex oligosaccharide in which the identities of the constituent sugars, their anomeric configurations, and the positions of the intersugar linkages may vary from residue to residue requires methods for the stepwise construction of the desired molecule (sequential synthesis). An important element in the design of such stepwise syntheses is the selection of the blocking groups used to restrict glycoside-bond formation to the desired positions. These must be chosen to allow for several cycles of selective, partial deblocking, then be removable by a minimal number of operations at the end of the sequence. Three

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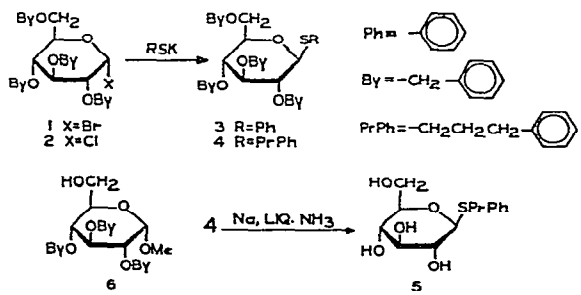
types are required — a persistent blocking group for the anomeric center of the eventual reducing residue, persistent hydroxyl-blocking groups for the positions that will not be involved in glycoside-bond formation, and temporary hydroxyl-blocking groups for the positions where successive sugar residues will be coupled.

In this paper we consider the thioglycoside function as a means of blocking the anomeric position, specifically in conjunction with *O*-benzyl groups as persistent hydroxyl-blocking groups. The *O*-benzyl group is well suited to this latter role, and recently several investigators,[1–4] especially Hasegawa *et al.*[5] in Nakajima's laboratory, have demonstrated the utility of *O*-benzyl-blocked glycosyl halides in glycoside and "pseudoöligosaccharide" synthesis. To determine whether the thioglycoside function would be stable to the conditions of the modified Koenigs–Knorr reaction of Hasegawa *et al.*, and to the reagents used in the removal of the *O*-benzyl groups at the end of the synthetic sequence, several model compounds were prepared and subjected to these respective conditions. The investigation was restricted to 1-thioglycosides having phenyl groups in their aglycone moieties, so that the results would be applicable to eventual work in the solid-phase mode, with a suitably modified polystyrene as the support material. Finally, isomaltose was prepared by a sequence involving all of the reactions that would be used in the synthesis of a larger oligosaccharide, except for *repeated* partial deblocking and coupling steps.

RESULTS AND DISCUSSION

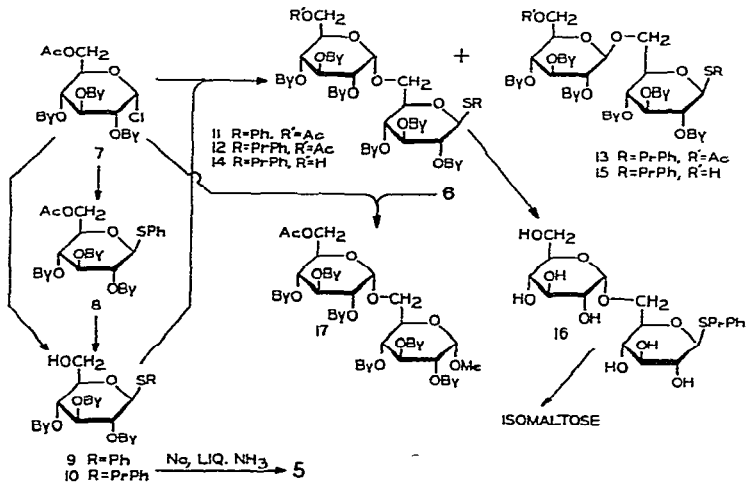
The first compound of the "model" series was obtained by condensation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide (**1**) with potassium thiophenoxide, which readily gave a crystalline thioglucoside. Since one can suppose that sulfur nucleophiles would have a higher probability than other common nucleophiles of reacting with glycosyl halides by an SN2 process[6], it was provisionally assumed that inversion of configuration had occurred at the anomeric center to give phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3**). The low specific rotation



([α]$_D$ <1°) of the compound accords with this assumption. The assignment of the β-anomeric configuration to **3** is also supported by the definite characterization of the phenylpropyl homolog (**4**) and the related 6-*O*-acetyl compound **10** as β-glucosides (see below). The method of preparation of **4** and **10** was essentially the same as that used for **3**.

An *O*-benzyl protected thioglucoside having a free 6-hydroxyl group (**9**) was readily obtained *via* reaction of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**7**) with potassium thiophenoxide, followed by saponification of the intermediate 6-acetate **8**. The acetate **8**, and hence also **9**, is formulated as the 1-thio-β-D-glucoside for the same reasons as given for **3**.

In the coupling procedure of Hasegawa *et al.*[5] benzene or chloroform with 10 to 20% *p*-dioxane is used as the reaction medium, and silver perchlorate and silver carbonate as catalysts. This procedure had been shown to give high yields of α-glycosides with *O*-benzylated glucosyl chlorides carrying an acetamido group in the 6- (or 3-) position, but it was not known whether a glycosyl halide with a similarly situated *acetoxy* substituent would give similar results. To test this point in a system where coupling could be expected to proceed readily, the 6-*O*-acetyl glycosyl chloride **7** was condensed with an equimolar portion of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**6**). From the reaction mixture we isolated, in 47% yield, a crystalline, protected disaccharide which, on benzylation, gave the known[7] methyl hepta-*O*-benzyl-α-isomaltoside. The coupling product is therefore the 6′-*O*-acetylisomaltoside **17**.*



When the phenyl 6-hydroxy-1-thioglycoside **9** was tried in the coupling with **7**, chromatographic workup of the reaction mixture gave a disaccharide fraction in 62% yield, and a 14% recovery of unchanged **9**. The positive specific rotation of the condensation product suggests it was largely the α-linked disaccharide **11**. Because of the unlikelihood of deblocking it successfully (see later) this product was not studied further. Nevertheless, it was shown that the free hydroxyl group of the thioglycoside

*More-recent work has shown that *p*-dioxane is essential for α-selectivity with tetra-*O*-benzyl-D-glucopyranosyl chloride, but not with the acetamido- and acetoxy-substituted chlorides mentioned here (N. Kurihara, personal communication; L. Anderson, unpublished work). The α-selectivity is however a function of the Koenigs–Knorr conditions, since the solvolysis of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**7**) gives preponderantly the methyl β-glucoside[8].

is an excellent glycosyl acceptor, and the thioglycoside function is stable to the conditions of the modified Koenigs–Knorr reaction.

The behavior of an *O*-benzylated phenyl thioglucoside, namely the tetrabenzyl ether **3**, was, however, less satisfactory when it was debenzylated with sodium–liquid ammonia. Under some conditions (ether and toluene as co-solvents) the major product was a substance having the chromatographic and spectroscopic properties of 1,5-anhydroglucitol. With tetrahydrofuran as co-solvent, cleavage of the thioglycoside function was greatly diminished, but not entirely suppressed.* It thus appeared necessary to turn to the aralkyl series of *O*-benzylated thioglucosides, having aglycones of the structure $Ph(CH_2)_n$–.

Since benzyl thioglycosides ($n = 1$) would undoubtedly be cleaved at the *S*-benzyl linkage by sodium–liquid ammonia,** and 2-phenylethyl thioglycosides ($n = 2$) might be subject to cleavage by β-elimination, we elected to use 3-phenylpropyl thioglucosides ($n = 3$) for further investigation. These (compounds **4** and **10**) were prepared by reaction of the *O*-benzylated glucosyl chlorides **2** and **7** with potassium 3-phenylpropanethiolate. Because of the greater alkalinity of the reagent, as compared with potassium thiophenoxide, the 6-*O*-acetyl glucosyl chloride **7** gave the deacetylated thioglucoside **10** directly. The β-anomeric configuration was provisionally assigned to **4** and **10** on the basis of their low specific rotations.

Both the phenylpropyl thioglucosides **4** and **10** were smoothly debenzylated in sodium–liquid ammonia–tetrahydrofuran. The glassy, debenzylated product, obtained in essentially quantitative yield, could be shown by its u.v. and p.m.r. spectra, optical rotation, and elemental analysis to be 3-phenylpropyl 1-thio-β-D-glucopyranoside (**5**). In the particular the β-anomeric configuration of **5**, and thus of the precursors **4** and **10**, was confirmed by the p.m.r. signal for H-1, which occured at relatively high field (τ 5.51) and showed a large value for $J_{1,2}$ (9 Hz). Cleavage of the thioglycoside function of **5** was readily effected with aqueous mercuric chloride in the presence of barium carbonate[9]. After deionization of the reaction mixture, the only product detectable by t.l.c. was glucose.

The foregoing results suggested that the phenylpropyl 6-hydroxy thioglycoside **10** would be a suitable starting unit for sequential oligosaccharide synthesis. Accordingly, **10** was coupled, by the Hasegawa procedure, with the 6-*O*-acetyl glucosyl chloride **7**. A disaccharide product was obtained in 65% yield. T.l.c. of this product indicated two components, but these were not fully separable in any of the several systems tried. Better separation was achieved with the deacetylated mixture, and this was accordingly chromatographed on a silica gel column, which gave a major, high-rotating fraction (72% of the total) and a minor, low-rotating fraction (28% of the total). A p.m.r. examination of a sample of the mixture, after deacetylation and debenzylation, gave signals for anomeric protons at τ 5.00 (H-1'α) and 5.48 (H-1β + H-1'β) with intensity ratios 0.72:1.28. On the basis of this observation, and the respec-

*Data collected by Messrs. A. H. Conner and J.-Y. Tang.

**Such a cleavage may not be disadvantageous. Its incorporation into an oligosaccharide synthesis scheme is currently being explored.

tive optical rotations, the major fractions were assigned the isomaltoside structures **12** (acetylated) and **14** (deacetylated). The minor fractions then have the corresponding gentiobioside structures **13** and **15**.

Tests of the deblocking procedures were carried out on the isomaltoside **14**. The insolubility of this compound in sodium–liquid ammonia at −33° made a search for a suitable co-solvent[10] necessary. After considerable preliminary experimentation, tetrahydrofuran, which is tolerated in fairly high proportions by the liquid ammonia–solvated-electron reagent, was selected as most suitable. With this system about 40% of the benzyl residues were converted into bibenzyl. The debenzylation of **14** proceeded in essentially quantitative yield, giving a glass that could be characterized as 3-phenylpropyl 1-thio-β-D-isomaltoside **16** by its optical rotatory and spectral properties.

Without purification, the thioisomaltoside was treated with mercuric chloride–barium carbonate. This reagent appeared to cause some alteration (oxidation ?) of the liberated disaccharide, and so it was deemed best to stop the reaction when about 5% uncleaved thioglycoside was still present. An amorphous sugar could then be isolated from the reaction mixture in 78% overall yield from the protected thioglycoside **14**. After purification by chromatography on charcoal this sugar and its octaacetate were shown to be identical in all respects to isomaltose and β-isomaltose octaacetate.

The coupling of additional sugar residues to the partially deblocked (at position 6′) disaccharide thioglycosides **14** and **15** was not attempted. We preferred to effect removal of the *O*-benzyl and thioglycoside groups to demonstrate the general utility of this combination of blocking groups in oligosaccharide synthesis. Nevertheless, it is evident that **14** and **15** would be suitable intermediates for the extension of the synthesis to the tri- or tetrasaccharide stage.

The size of the α-linked glucoöligosaccharides that could be prepared by the Hasegawa coupling procedure is limited by the less than complete stereoselectivity of this reaction. In our hands it indeed gives a high portion (75–80%) of α-linkages with a variety of glycosyl acceptors, but the presence of 20–25% of β-linked product makes it necessary to separate the anomers chromatographically at each stage. Coupling reactions that are more nearly stereospecific will need to be used for the construction of α-linkages in large, complex oligosaccharides.

EXPERIMENTAL

General. — Compounds were purified to homogeneity as judged by t.l.c. on Silica Gel G (E. Merck). The following solvent systems were used (all ratios are *v/v*): *A*, acetone–benzene 1:20; *B*, chloroform–methanol–acetic acid 35:14:1, and *C*, 30:19:1; *D*, acetone–water–acetic acid 83:15:2; *E*, heptane–ethyl acetate 7:3 and *F*, 1:1. Column chromatography was done on E. Merck's silica gel.

Melting points designated "hot stage" were determined on a Monoscop model IV apparatus. Optical rotations were recorded with a Perkin–Elmer model 141 polarimeter and p.m.r. spectra with Varian 60 MHz instruments (A-60, T-60). Ultraviolet

spectroscopy was used to check the number of benzyl (including phenylpropyl) groups per molecule in compounds containing these groups. The value 186 was taken as the expected molar absorbancy per benzyl group at 258 nm[11]. Elemental analyses were performed by the Galbraith Laboratories.

Starting materials. — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose was prepared from methyl α-D-glucopyranoside by modifying the procedure of Perrine *et al.*[12] so that weaker acid (0.5M H_2SO_4 in acetic acid–water 8:2 *v/v*) and a shorter heating time (15 min at reflux) were used in the hydrolysis of the intermediate methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**2**) was made by the thionyl chloride method[1]. The precursor, tetra-*O*-benzyl-D-glucopyranose, was added portionwise to the thionyl chloride and the mixture was stirred for 48 h at room temperature. 6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**7**) was prepared from 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose[13] by the method of Pravdić and Keglević[14].

*Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside* (**3**). — The 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide[15] (**1**) from 3.31 g (4.80 mmole) of 2,3,4,6-tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-α-D-glucopyranose was taken up in 10 ml of chloroform and added to a solution of 0.54 ml (5.3 mmole) of thiophenol and 11.1 ml 0.43M methanolic potassium hydroxide. The reaction mixture was refluxed for 30 min on a steam bath, cooled, diluted with 20 ml water, and extracted twice with chloroform. The combined chloroform layers were washed thrice with 10% sodium carbonate solution, and then water, dried ($MgSO_4$), filtered, and evaporated under diminished pressure to an oil. Crystallization from ethanol gave 2.20 g (72%) of the title compound, m.p. (capillary) 93.5–94.5°, $[\alpha]_D^{25}$ +0.65°, $[\alpha]_{436}^{25}$ +1.08° ($CHCl_3$); p.m.r. ($CDCl_3$) τ 2.13–2.95 (m, 25, Ph–*H*), 4.92–5.63 (m, 9, H-1, PhCH_2), and 6.14–6.70 (m, 6, H-2–H-6). Found: C, 76.37; H, 6.27; S, 4.80. $C_{40}H_{40}O_5S$ (632.79) requires: C, 75.92; H, 6.37; S, 5.07.

*Phenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside* (**8**) — A solution of 2.12 g (4.1 mmole) of crude **7** in 8.3 ml of chloroform was added to a solution of 0.43 ml (4.2 mmole) of benzenethiol and 11.4 ml 0.365M potassium hydroxide in methanol. The reaction mixture was refluxed for 30 min on a steam bath, and then cooled, diluted with water, and extracted with chloroform. The chloroform layer was washed thrice with 10% sodium carbonate solution and once with water, and then dried ($MgSO_4$), filtered and evaporated under diminished pressure to an oil. The oil was chromatographed on a silica gel column (2.5-cm diameter, 150 g, acetone–benzene (1:50 *v/v*), 1 ml/min). The fractions containing material having R_F 0.53 in t.l.c. system *A* gave on evaporation 1.43 g (59%) of the oily title compound. A portion of this, dried in vacuum and stored, crystallized after several months. It was recrystallized from methanol; m.p. (hot stage) 69–73°; $[\alpha]_D^{25}$ +11.7°, $[\alpha]_{436}^{25}$ +23.4° ($CHCl_3$); p.m.r. ($CDCl_3$) τ 2.32–2.83 (m, 20, Ph–*H*), 4.84–6.68 (m, 13, PhCH_2, H-1–H-6), and 7.98 (s, 3, $COCH_3$). Found: C, 72.14; H, 6.22; S, 5.36. $C_{35}H_{36}O_6S$ (584.70) requires: C, 71.89; H, 6.21; S, 5.48.

*Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside* (**9**). — A solution of

0.687 g (1.18 mmole) of **8** in 5 ml of dry methanol containing 0.07 ml of 0.6M methanolic sodium methoxide was refluxed for 40 min on a steam bath. When the solution was allowed to cool, crystals of the product formed. Recrystallization from ethanol gave 0.51 g (80%) of the pure title compound; m.p. (capillary) 121.5–122°; $[\alpha]_D^{25}$ +7.7° (*c* 3.38, $CHCl_3$); t.l.c.: system *A*; p.m.r. ($CDCl_3$) τ 2.36–3.02 (m, 20, Ph–*H*), 4.90–6.81 (m, 13, PhCH_2, H-1–H-6), and 8.00 (broad s, 1, O*H*). Found: C, 73.09; H, 6.20; S, 5.83. $C_{33}H_{34}O_5S$ (542.67) requires: C, 73.03; H, 6.32; S, 5.91.

*3-Phenylpropyl 2,3,4-tri-*O*-benzyl-1-thio-β-*D*-glucopyranoside* (**10**). — The syrupy glucosyl chloride (**7**) from 5.35 g (10 mmoles) of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose, in 5.1 ml of benzene, was added over a period of 1 h to a stirred solution of 1.94 g (30 mmoles) of KOH and 7.53 ml (50 mmoles) of 3-phenylpropanethiol in 20 ml of 1-propanol, and stirring was continued overnight at 25°. Benzene (40 ml) and water (20 ml) were added, the mixture was neutralized to pH 8 with CO_2, and separated. The benzene layer was washed twice with water (20 ml ea.) and concentrated *in vacuo*.

Material pure enough to crystallize spontaneously was obtained by preparative t.l.c. (silica gel PF_{254}, benzene–methanol 49:1 *v/v*) of a portion of the residue. Attempted crystallization of the remainder from cyclohexane–heptane (1:2 *v/v*) initially gave a gel, but this was converted into a fine powder by seeding and stirring at 0°. Isolation at 0° gave 4.57 g (78%) of product melting at 63–68°. This was recrystallized from cyclohexane (140 ml) with seeding and keeping overnight at 25°, followed by the addition of 0.1 vol. of heptane and slow cooling to 0° to give fine needles, m.p. (hot stage) 68–70°; $[\alpha]_D^{25}$ −2.0°, $[\alpha]_{436}^{25}$ +1.9° (*c* 1.56, $CHCl_3$); λ_{max}^{EtOH} 258 nm (ε 785); p.m.r. ($CDCl_3$) τ 2.7 (d, 20, Ph–*H*), 4.8–5.7 (m, 7, PhCH_2, H-1), 6.0–6.8 (m, 6, H-2–H-6), 7.24 (t, 4, S–CH_2–C–CH_2–Ph), and 8.02 (m, 3, 1 exchangeable with D_2O, C–CH_2–C, O*H*). Found: C, 73.86; H, 6.76; S, 5.35. $C_{36}H_{40}O_5S$ (584.75) requires: C, 73.94; H, 6.90; S, 5.48.

*3-Phenylpropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-*D*-glucopyranoside* (**4**) was obtained by treating the glucosyl chloride (**2**) from 541 mg (1 mmole) of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with 3-phenylpropanethiol and KOH. The procedure was essentially as described for the preceding thioglycoside, except that an attempt was made to extract excess thiol from the benzene–propyl alcohol solution of the reaction product with 2M KOH. The crude product was chromatographed on 1-mm layers of silica gel PF_{254}, with benzene as irrigant. It was eluted with chloroform–ethyl acetate (9:1 *v/v*) from a broad, ultraviolet-absorbing zone having R_F 0.2–0.5. On concentration and storage at 0° it crystallized. Recrystallization, after dissolution in hexane at 60°, required seeding and slow cooling from 25° down to 0°. Isolation at 0° gave 206 mg (31%) of the pure compound; m.p. (hot stage) 64°, $[\alpha]_D^{25}$ −1.4°, $[\alpha]_{436}^{25}$ −1.6° (*c* 2.06, $CHCl_3$); λ_{max}^{EtOH} 258 nm (ε 926); p.m.r. ($CDCl_3$) τ 2.75 (m, 25, Ph–*H*), 5.0–5.7 (m, 9, PhCH_2, H-1), 6.1–6.8 (m, 6, H-2–H-6), 7.24 (t, 4, S–CH_2–C–CH_2–Ph), and 8.0 (m, 2, C–CH_2–C). Found: C, 76.38; H, 6.77; S, 4.69. $C_{43}H_{46}O_5S$ (674.86) requires: C, 76.52; H, 6.87; S, 4.75.

*3-Phenylpropyl 1-thio-β-*D*-glucopyranoside* (**5**). — Compound **4** (67.5 mg,

0.1 mmole) and compound **10** (58.5 mg, 0.1 mmole) were treated with sodium in liquid ammonia–tetrahydrofuran as described for the debenzylation of 3-phenylpropyl hexa-*O*-benzyl-β-isomaltoside (**14**) (see later). The product **5** was obtained as a glass in 95% yield; $[\alpha]_D^{25}$ −47.7°, $[\alpha]_{436}^{25}$ −100° (*c* 2.20, H_2O); t.l.c.: system *B*; λ_{max}^{EtOH} 258 nm (ε 340); p.m.r. (CD_3COOD) τ 2.79 (s, 5, Ph–*H*), 5.51 (d, 1, *J* 9 Hz, H-1), 5.9–6.9 (m, 6, H-2–H-6), 7.25 (t, 4, S-CH_2–C–CH_2–Ph), and ~8.1 (m, partly obscured by the solvent, C–CH_2–C). Found: C, 57.64; H, 7.24. $C_{15}H_{22}O_5S$ (314.39) requires: C, 57.30; H, 7.05.

Methyl 6′-O-acetyl-2,3,4,2′,3′,4′-hexa-O-benzyl-α-isomaltoside (**17**). — A mixture of 0.456 g (0.98 mmole) of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside[7] (**6**), 10 ml of dry benzene, 4 ml of dry *p*-dioxane, and 4 g of crushed Drierite was stirred for 2 h at room temperature in a flask wrapped with aluminum foil. Silver carbonate (2.18 g, 7.9 mmoles) was then added, and the mixture stirred for an additional h. A solution of 0.500 g (0.98 mmole) of crude 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**7**) in 2 ml of dry benzene was added to the mixture, followed by 0.0236 g (0.114 mmole) of anhydrous silver perchlorate. The reaction mixture was stirred for 20 h and filtered (washing the solid well with benzene). Evaporation of the filtrate under diminished pressure left an oil that on crystallization from ethanol gave 0.431 g (47%) of the title compound; m.p. (capillary) 99.5–100.5° (air dried), 109–109.5° (dried 4 hr. *in vacuo* at 60°), $[\alpha]_D^{25}$ +66° (*c* 1.5, $CHCl_3$); t.l.c.: system *A*; p.m.r. ($CDCl_3$) τ 2.55–2.90 (m, 30, Ph–*H*), 4.86–6.76 (m, 29, PhCH_2, H-1–H-6, H-1′–H-6′, s at 6.65, OCH_3), and 8.05 (s, 3, COCH_3). Found: C, 72.36; H, 6.43. $C_{57}H_{62}O_{12}$ (939.07) requires: C, 72.90; H, 6.66.

Deacetylation and benzylation of a portion of the compound gave, in 70% yield, crystalline methyl hepta-*O*-benzyl-α-isomaltoside, $[\alpha]_D$ +61.6°, $[\alpha]_{436}$ +114° (*c* 1.16, $CHCl_3$) (reported[7] +59°, +109.5°); p.m.r. identical with that of an authentic sample.

Coupling of 6-O-acetyl glucosyl chloride **7** *with phenyl thioglycoside* **9**. — The reaction was conducted as for the synthesis of **17**, by using 0.366 mmole each of phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**9**) and 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**7**). On concentration, the filtered reaction mixture gave an oil, which was chromatographed on a silica gel column (2.5-cm diameter, 150 g, acetone–benzene 1:50 *v/v*, 0.67 ml/min). A disaccharide fraction of 0.231 g (62%) was characterized by the following properties: $[\alpha]_D$ +38.1°, $[\alpha]_{436}$ +74.3° (*c* 2.25, $CHCl_3$); p.m.r. ($CDCl_3$) τ 2.33–3.00 (m, 35, Ph–*H*), 4.80–7.00 (m, 26, PhCH_2, H-1–H-6, H-1′–H-6′), and 8.02 (s, 3, COCH_3). The positive optical rotation indicates a preponderance of *6′-O-acetyl-2,3,4,2′,3′,4′-hexa-O-benzyl-1-thio-β-isomaltoside* (**11**). A later fraction from the column consisted of 0.028 g (14%) of unreacted **9**. T.l.c.: system *A*.

Coupling of 6-O-acetyl glycosyl chloride **7** *with phenylpropyl thioglucoside* **10**. — To a solution of 2.92 g (5 mmoles) of 3-phenylpropyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**10**) in 30 ml of abs. benzene was added 1.72 g (6.2 mmoles) of silver carbonate, 0.104 g (0.5 mmole) of silver perchlorate, 5.0 g of powdered Drierite, and 20 ml of abs. *p*-dioxane. The mixture, in a flask wrapped to exclude light and

protected by a drying tube, was stirred overnight at room temperature. The crude glucosyl chloride (**7**) (2.84 g, ~5 mmoles), which had been dissolved in 50 ml absolute benzene and stirred overnight with Drierite, was now added. Stirring was continued for 24 h. A solution of 5 g of sodium chloride and 0.5 g of potassium carbonate in 50 ml of water was then added, the mixture was shaken, and the mixed phases filtered. The residue on the filter was washed with 10 ml of benzene, which was added to the original filtrate, and 50 ml of acetone, which was collected separately. Benzene (5 ml) and water (5 ml) were added, with shaking, to the residue from the acetone wash, and the benzene layer was united with the main filtrate.

After a further washing with water, the benzene solution of the reaction product was evaporated to give 6.5 g of an oil that partially solidified on standing. This was dissolved in 265 ml of methanol at 50°, and the solution filtered. The filtrate was seeded and stirred, first for 2 h at 25°, whereupon extensive flocculation occurred, and then overnight at 0°. The precipitated mixed disaccharide (**12**+**13**) was filtered off at 0°, washed with methanol (25 ml), and dried to a white powder weighing 3.47 g (65%). The components were partially separated on t.l.c. in system *E*; m.p. (hot stage) ~85°, $[\alpha]_D^{25}$ +24.7°, $[\alpha]_{436}^{25}$ +48.4° (*c* 3.60, $CHCl_3$); λ_{max}^{EtOH} 258 nm (ε 1350); λ_{max}^{KBr} 1740 (C=O), 737 and 698 cm^{-1} (mono-substituted benzene); p.m.r. ($CDCl_3$) τ 2.7 (d, 35, Ph–*H*), 4.7–6.9 (m, 26, PhCH_2, H-1–H-6, H-1′–H-6′), 7.29 (t, 4, S–CH_2–C–CH_2–Ph), and ~8.0 (m, 5, $COCH_3$, C–CH_2–C).

3-Phenylpropyl 2,3,4,2′,3′,4′-hexa-O-*benzyl-1-thio-β-gentiobioside* (**15**). — The above mixed disaccharide product (2.0 g) was dissolved in 10 ml of tetrahydrofuran, 2 ml each of methanol and 2M aqueous KOH were added, and the mixture was stirred for 2 h at room temperature. Water (10 ml) and benzene (20 ml) were added, and the mixture was neutralized to pH 8 with CO_2. After two washings of the benzene layer, concentration followed by vacuum drying of the residue gave 1.95 g (quantitative) of the mixed, deacetylated disaccharides as a colorless syrup. T.l.c. in system *F* showed two well separated spots having R_F 0.46 and 0.37, respectively.

Separation of the mixture was accomplished by four runs on a single column of silica gel (3.7-cm diameter, 600 g, ether–pentane 3:1 *v/v*, 8 ml/min). The high flow-rate was used because the disaccharide products showed some tendency to decompose on the column. The gentiobioside (**15**) was eluted first, followed by the isomaltoside (**14**). At high loading (600 mg) **14** and **15** overlapped slightly, but the mixed fractions from the first three runs were separated by rechromatography at low loading on the fourth run. The yield of gentiobioside, which crystallized spontaneously, was 0.526 g (28% of the disaccharide recovered). It was recrystallized from 16 ml of warm cyclohexane, with seeding at 35°, keeping for 4 h at room temperature, slow cooling to 6°, adding 4 ml of heptane, and keeping overnight at 0°. Filtration at 0° gave 0.449 g of pure product; m.p. (hot stage) ~90°, $[\alpha]_D^{25}$ −0.40°, $[\alpha]_{436}^{25}$ −0.6° (*c* 1.82, $CHCl_3$); λ_{max}^{EtOH} 258 nm (ε 1330); p.m.r. ($CDCl_3$) τ 2.7 (d, 35, Ph–*H*), 4.75–5.72 (m, 14, PhCH_2, H-1, H-1′), 5.72–6.91 (m, 12, H-2–H-6, H-2′–H-6′), 7.34 (t, 4, S–CH_2–C–CH_2–Ph), 8.07 (s, 1, exchangeable with D_2O, O*H*), and 8.13 (m, 2, C–CH_2–C). Found: C, 74.32; H, 6.85; S, 2.97. $C_{63}H_{68}O_{10}S$ (1017.24) requires: C, 74.38; H, 6.74; S, 3.15.

3-Phenylpropyl 2,3,4,2',3',4',-hexa-O-benzyl-1-thio-β-isomaltoside (**14**). — The isomaltoside fraction from the above chromatogram weighed 1.355 g (72% of the disaccharide recovered). Seed crystals were obtained from a concentrated solution in ether–pentane (3:1 *v/v*) at 0°. The main batch was crystallized from 41 ml of warm methanol by seeding at room temperature and stirring until the formation of fluffy crystals was extensive (4 h). The mixture was then cooled slowly, and kept overnight with stirring at 0°. Isolation at 0° yielded 1.056 g of pure **14**; m.p. (hot stage) ~50°; $[\alpha]_D^{25}$ +36.7°, $[\alpha]_{436}^{25}$ +70.3° (*c* 1.65, $CHCl_3$); λ_{max}^{EtOH} 258 nm (ε 1325); p.m.r. ($CDCl_3$) τ 2.7 (d, 35, Ph–*H*), 4.77–5.76 (m, 14, PhCH_2, H-1, H-1'), 5.76–7.02 (m, 12, H-2–H-6, H-2'–H-6'), 7.28 (t, 4, S–CH_2–C–CH_2–Ph), 8.06 (m, 2, C–CH_2–C), and 8.32 (s, 1, exchangeable with D_2O, O*H*). Found: C, 74.57; H, 6.87; S, 2.88. $C_{63}H_{68}O_{10}S$ (1017.24) requires: C, 74.38; H, 6.74; S, 3.15.

Isomaltose and its octaacetate. — The hexa-*O*-benzyl thioglycoside **14** (1.02 g, 1.00 mmole) in 12 ml of abs. tetrahydrofuran was placed in a flask equipped with a cold finger-type condenser filled with Dry Ice–acetone slurry. Ammonia gas dried by passage over KOH pellets was run in and condensed until the volume of the solution was 36 ml. Freshly cut sodium was added in 50-mg portions to the vigorously stirred (glass-coated magnet), refluxing solution until it stayed deep blue for 0.5 h (278 mg, 12 mg-atoms required). After evaporation of the ammonia at room temperature 3 ml of methanol was added to decompose excess sodium, and the solution was concentrated to dryness. The residue was taken up in 12 ml of water and the solution neutralized to pH 9 with CO_2, and then extracted thrice (12 ml ea.) with benzene.

To deionize the water solution it was treated with Dowex 50-X8 resin (pyridine form, washed with water, 24 mequiv exchange capacity), and the suspension was degassed by a brief application of vacuum. It was then poured into a column over an additional 12 mequiv of resin. The effluent from this column (water, 90 ml) on concentration gave glassy *3-phenylpropyl 1-thio-β-isomaltoside* (**16**). T.l.c. in systems *C* and *D* indicated the presence of some minor impurities in this preparation, which had $[\alpha]_D^{25}$ +9.2°, $[\alpha]_{436}^{25}$ +12.7° (*c* 3.14, H_2O); λ_{max}^{EtOH} 258 nm (ε 425); p.m.r. (CD_3COOD) τ 2.75 (s, 5, Ph–*H*), 5.00 (d, 1, *J* 2 Hz, H-1'), 5.48 (d, 1, *J* 8 Hz, H-1), 5.8–6.8 (m, 12, H-2–H-6, H-2'–H-6'), 7.24 (t, 4, *J* 7 Hz, S–CH_2–C–CH_2–Ph), and ~8.1 (m, partly obscured by the solvent, C–CH_2–C).

To the crude phenylpropyl thioisomaltoside dissolved in 4.77 ml of water were added 0.953 g of mercuric chloride and 0.953 g of barium carbonate. The mixture was stirred for 3 days at room temperature, after which time t.l.c. (system *C*) showed the reaction to be ~90% complete. Pyridine (0.95 ml) and water (4.77 ml) were then added. The resulting slurry was transferred to a glass column and drained, and then the residual barium carbonate and precipitated mercury complex was washed with 95 ml of water. The combined effluent was concentrated to 5 ml. Deionization was accomplished as in the preceding paragraph, with Amberlite MB-3 (10 mequiv exchange capacity) that had been stirred in pyridine–water (1:1 *v/v*) with a stream of CO_2 for 2 h, and then washed with water for 4 days and re-treated with CO_2. The first two fractions (42 ml total) from the ion-exchange column contained the product;

unchanged thioglycoside was slowly eluted in the succeeding fractions. Crude isomaltose·0.5 EtOH[16] (266 mg) was obtained by concentrating the aqueous solution, taking the residue up in ethanol, and precipitating with ethyl acetate; p.m.r. (D_2O:CD_3CO_2D,1:3) τ 4.67 (d, 0.4, J 3.2 Hz, H-1α), 5.01 (d, 1, J 2.6 Hz, H-1'), 5.26 (d, 0.6, J 6.6 Hz, H-1β), and 5.8–6.8 (m, 12, H-2–H-6, H-2'–H-6'). On t.l.c. in systems *C* and *D*, and paper chromatography in water–pyridine–butyl alcohol (3:4:6), the R_F values of the product were identical with those of authentic isomaltose.

The residue from the ethanol–ethyl acetate mother liquor was chromatographed on charcoal (Darco G-60, 9 g, packed as a slurry in water). After passage of 43 ml of water through the column, the solvent was changed to water–ethanol (85:15 *v/v*), which eluted the pure disaccharide, again isolated as isomaltose·0.5 EtOH; $[\alpha]_D^{25}$ (equil.) +119°, ethanol-free basis (*c* 0.34, H_2O). Reported[16,17] $[\alpha]_D^{25}$ +122°, +120°. The overall yield from the benzyl-protected thioglucoside **14** was 78%.

The octaacetate[18] of the synthetic disaccharide had m.p. (hot stage) 143–146°, $[\alpha]_D^{25}$ +107.5°, and $[\alpha]_{436}^{25}$ +187° (*c* 0.4, $CHCl_3$). An authentic sample of β-isomaltose octaacetate had identical constants, within experimental error, and an identical p.m.r. spectrum.

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