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PARTIALLY BENZYLATED OXAZOLINE DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE AS "STANDARDIZED INTERMEDIATES" FOR OLIGOSACCHARIDE SYNTHESIS. PREPARATION OF DISACCHARIDES HAVING THE SEQUENCES β-D-Glc*p*NAc(1→x)-D-Gal AND β-D-Glc*p*NAc(1→4)-D-GlcNAc

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ABSTRACT

Three benzyl tri-*O*-benzyl-1-thio-β-D-galactopyranosides (**5**, **6**, and **7**) were prepared from the corresponding *O*-acyltri-*O*-benzyl-D-galactopyranosyl bromides (**1a–c**) *via* the benzylxanthates **2a–c** and the fully protected benzyl thiogalactosides **3a–c**. The α anomer (**4**) of **5** resulted from the reaction of bromide **1a** with α-toluenethiol. Conditions were found for the successful coupling of *O*-acetyl-di-*O*-benzyl derivatives (**8** and **13**) of 2-methyl-(1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline to the thiogalactosides **5–7**, and to a partially protected glycoside (**16**) of 2-acetamido-2-deoxy-D-glucose. The products were fully substituted disaccharides of 2-acetamido-2-deoxy-β-D-glucose linked 1→3 (**9a**), 1→6 (**11a**), 1→4 (**14a**), and 1→4 (**17a**), respectively. Cleavage of the single, temporary protecting group (*O*-acetyl) from these compounds gave partially deblocked disaccharides capable of chain extension from position 3′ (**9b** and **11b**) or 4′ (**14b** and **17b**).

INTRODUCTION

Syntheses of the position-isomeric mono-*O*-acetyl di-*O*-benzyl derivatives of 2-methyl-(1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline have been developed in our laboratory[1,2], and in the laboratories of Jeanloz[3] and Sinaÿ[4]. These oxazolines were designed as precursors of β-linked, interior 2-acetamido-2-deoxy-D-glucopyranose ("*N*-acetyl-D-glucosamine") residues in oligosaccharides, and preliminary explorations of their use for this purpose have been reported. Thus, Warren, Shaban, and Jeanloz[3] prepared differentially protected, β-(1→6)-linked disaccharides of 2-acetamido-2-deoxy-D-glucose by coupling the 4-*O*-acetyl-3,6-di-*O*-benzyl derivative **13**

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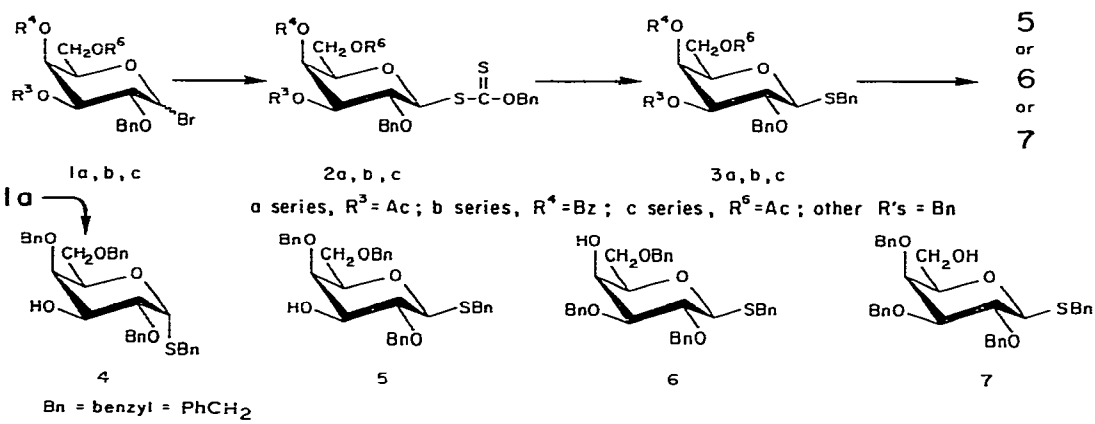
and its 6-*O*-acetyl-3,4-di-*O*-benzyl isomer to allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranose. Similarly, Rollin and Sinaÿ[4] coupled **13** to 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and obtained the protected, β-(1→6)-linked disaccharide in good yield. And, finally, a protected form of "*N*,*N*'-diacetylchitobiose" [β-D-Glc*p*NAc-(1→4)-D-GlcNAc] was prepared in this laboratory and elaborated to the trisaccharide β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-GlcNAc by the Harvard group[5].

In the present paper we describe the successful coupling of the 3-*O*-acetyl-4,6-di-*O*-benzyl (**8**) and the 4-*O*-acetyl-3,6-di-*O*-benzyl (**13**) -oxazolines to partially protected benzyl 1-thio-β-D-galactopyranosides, to give protected β-(1→3)-, β-(1→4)-, and β-(1→6)-linked disaccharides.* The use of **13** to prepare an additional chitobiose derivative is also reported.

RESULTS AND DISCUSSION

Synthesis of the acceptor sugars. — A number of tri-*O*-benzyl-D-galactopyranose derivatives have been prepared in this laboratory for use as building blocks in systematic oligosaccharide synthesis[6-8]. It therefore seemed desirable to employ compounds of this series in work with oxazolines **8** and **13**. Specifically, we wished to couple the oxazolines to protected benzyl 1-thio-D-galactopyranosides (**5**, **6**, and **7**) that could be regarded as models of acceptor sugars bonded to a solid support[9].

The most direct route to **5**, **6**, and **7** would appear to be the reaction of the *O*-acetyltri-*O*-benzyl-D-galactopyranosyl bromides **1a–c** (or the corresponding chlorides) with α-toluenethiol in the presence of potassium hydroxide or carbonate[10]. In our hands, however, this reaction failed to give consistent results. In some cases only the thio-β-glycoside was formed, but in others anomeric mixtures were obtained. Indeed, in one experiment with the 3-*O*-acetyl-2,4,6-tri-*O*-benzyl halide **1a**, the thio-α-glycoside **4** was the major product.



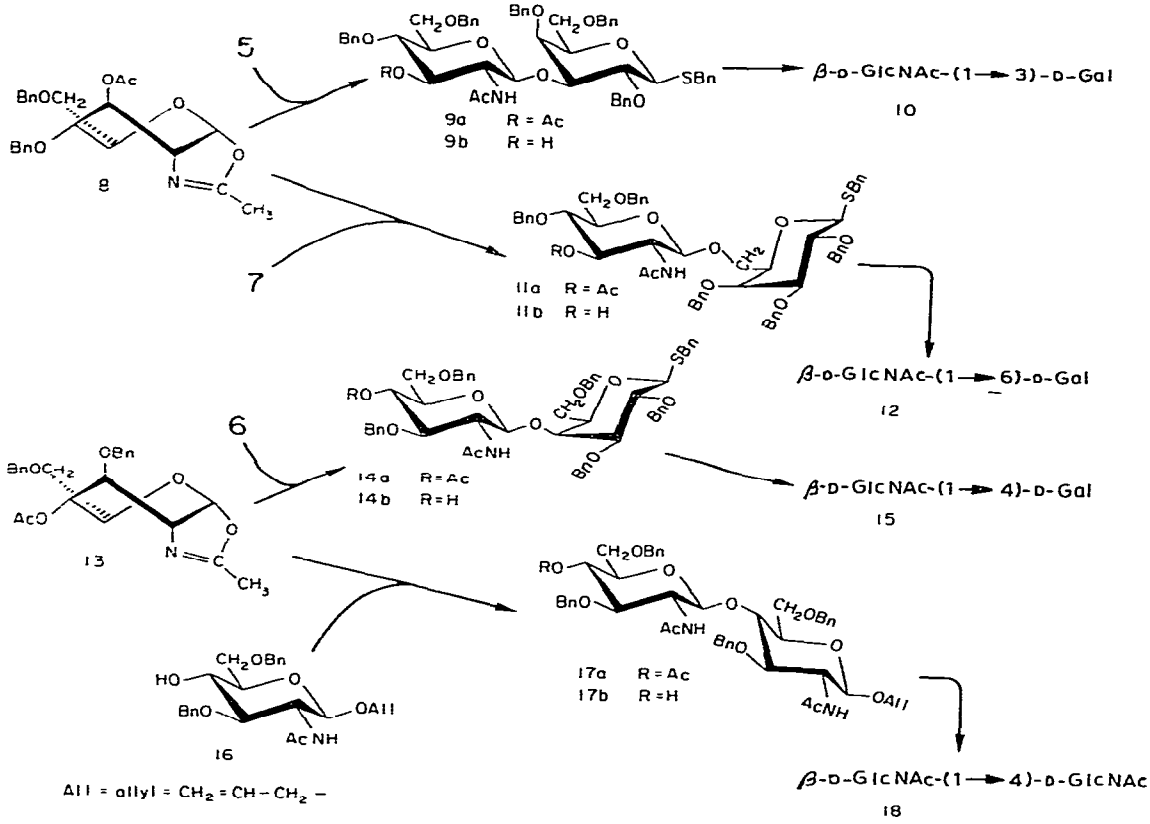
*A preliminary account of this work was presented at the IXth International Symposium on Carbohydrate Chemistry, London, April 1978; abstract B63.

A more-reliable way of obtaining the anomerically pure benzyl thioglycosides was found in the work of Sakata, Haga, and Tejima[11], who observed that *O*-substituted *β*-glycosyl benzyl- and methyl-xanthates rearrange to the corresponding thioglycosides, with the loss of carbonyl sulfide, on brief warming in certain solvents. To exploit this reaction, we prepared the *O*-acyltri-*O*-benzyl-*β*-D-galactopyranosyl benzylxanthates **2a–c** by treating the halides **1a–c** with potassium benzylxanthate in ethanol–benzene. The new xanthates are analogs of the ethylxanthates already described by us[6,7], and they are interchangeable with the ethylxanthates as precursors of the position-isomeric tri-*O*-benzyl-1-thio-*β*-D-galactopyranoses[6,7]. The latter may be obtained by saponification of the xanthates with methanolic sodium methoxide. When instead the benzylxanthates were heated for 5–10 min in pyridine solution[11], they were quantitatively converted into the benzyl thioglycosides **3a–c**. Removal of the *O*-acyl groups by ester exchange furnished **5**, **6**, and **7**, having free hydroxyl groups in positions 3, 4, and 6, respectively.

Coupling conditions. — In experiments on the acid-catalyzed coupling of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-*α*-D-glucopyrano)-[2,1-*d*]-oxazoline (the tri-*O*-acetyl analog of **8** and **13**) to an unreactive acceptor (the *α* anomer of **16**), Warren and Jeanloz[12] found 1,2-dichloroethane superior to the more-commonly used toluene–nitromethane[13–15] as a solvent for the reaction. However, in their later work with the more reactive oxazolines **13** and its 3,4-di-*O*-benzyl analog, using the 1,2-dichloroethane–*p*-toluenesulfonic acid system, Warren, Shaban, and Jeanloz[3] achieved coupling to a useful extent only when the free hydroxyl group of the acceptor sugar was in the primary position. Because of these conflicting results, we studied the coupling reaction carefully, varying solvent, temperature, reactant concentration, and *p*-toluenesulfonic acid concentration. Confirming the original observation of Warren and Jeanloz[12], we obtained our best results with dichloroethane at temperatures near its boiling point (83°). Rigorous control of the catalyst concentration was found essential. The possibility was considered that the ratio of *p*-toluenesulfonic acid to oxazoline is a more important parameter than the absolute concentration of the acid, but this point was not clarified.

Under the best conditions, decomposition of the oxazolines–probably to 1,2-unsaturated compounds–was decreased, but was still substantial. Hence an excess of oxazoline was always required. With oxazoline:acceptor ratios of 2:1, and a 0.01M concentration of *p*-toluenesulfonic acid, we achieved excellent yields (70–85%) in couplings to the 3 or 6 position of our protected thiogalactosides. As expected, couplings to the 4 position of acceptor sugars were more difficult. To obtain usable yields in these instances, we used three molar portions of oxazoline (added in increments).

Disaccharide products. — The fully substituted disaccharides **9a** (1→3) and **11a** (1→6) were the products of the reaction of the 3-*O*-acetyloxazoline **8** with the benzyl thiogalactosides **5** and **7**, respectively. Similarly, **14a** (1→4) resulted from coupling the 4-*O*-acetyl oxazoline **13** to the benzyl thiogalactoside **6**. In addition,



13 gave the fully protected chitobioside* **17a** when coupled to allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**16**). One of the substituted disaccharides (**9a**) could be crystallized directly from the crude mixture of products of the coupling procedure. The other three were amorphous, and were separated by chromatography on columns of silica gel.

The conversion of the primary coupling-products to the selectively deprotected disaccharides **9b**, **11b**, **14b** and **17b** was accomplished by conventional catalytic de-esterification.

The initial characterization of the coupling products as disaccharides was based on their ^{1}H-n.m.r. spectra. These showed signals for two (**9a**, **11a**, and **14a**) or three (**17a**) acetyl groups, and the expected ratios of aromatic protons to acetyl methyl protons. In each case, treatment with methanolic sodium methoxide caused the loss of one acetyl signal from the spectrum.

The positions of the intersugar linkages in **9**, **11**, **14**, and **17** could be assumed from the known structures of the acceptor sugars **5**, **6**, **7**, and **16**, as each of these compounds has only one free hydroxyl group, and carries stable protecting groups

*The α anomer (at C-1) of this compound was obtained, in low yield, by Warren *et al.*[3]

at the other positions. However, it was necessary to establish the β-anomeric configurations of the (non-reducing) 2-amino-2-deoxy-D-glucose residues. To this end, first the glycosidic protecting groups and then the *O*-benzyl groups were removed from **9b**, **11b**, **14b**, and **17b**. The optical rotations of the resulting, unsubstituted disaccharides agreed satisfactorily with the respective literature values for β-D-GlcNAc-(1→3)-D-Gal[16,17] (**10**), β-D-GlcNAc-(1→6)-D-Gal[18] (**12**), β-D-GlcNAc-(1→4)-D-Gal[19] (**15**), and β-D-GlcNAc-(1→4)-D-GlcNAc[20] (**18**). Each of the free disaccharides was then converted into its alditol, and the alditols were examined by n.m.r. spectroscopy. The ^{1}H-spectra all showed doublets for β-anomeric protons at δ 4.4–4.6 p.p.m. (*J* 7.5–8.8 Hz), but no signals for α-anomeric protons were visible. The number of scans was such that, in test mixtures of cellobiitol and maltitol, 2% of the latter could be detected. In the ^{13}C spectra, no signals attributable to α-anomeric carbon atoms were found.

Discussion. — The results of the present work suggest that *O*-benzyl oxazoline derivatives may be used to attach a 2-acetamido-2-deoxy-D-glucose group in β-linkage to any desired position of a "following" sugar, given the availability of a suitably protected derivative of that sugar. Such derivatives could, of course, include partially constructed oligosaccharides. The coupling of the *O*-benzylated oxazolines to acceptors evidently proceeds with a high degree of stereoselectivity, for pure, β-linked disaccharides were readily isolated in the instances reported here. As no evidence was found for α-linked compounds, the "wrong anomers" cannot have been formed in any substantial amounts. The presence of minor quantities in the crude reaction-products cannot, of course, be excluded. The conversion of a portion of the starting oxazolines into decomposition products did not seriously interfere with our isolation of the disaccharides. However, as already noted, conditions giving fully satisfactory yields in couplings to the 4-position of hexopyranoses were not found. The search for improved conditions should be continued.

O-Deacylation after the coupling of oxazoline to acceptor gives protected oligosaccharides (such as **9b**, **11b**, **14b**, and **17b**) in which the (non-reducing terminal) 2-acetamido-2-deoxy-D-glucose residue carries a single, free hydroxyl group. The coupling of an additional sugar, or block of sugars, to this hydroxyl group will lead in a straightforward and systematic way to products containing β-linked, interior 2-acetamido-2-deoxy-D-glucose residues. Such components occur, for example, in the ABO–Lewis blood-group determinants, and in the *N*-linked oligosaccharides of glycoproteins. The synthesis of these oligosaccharides, or segments thereof, has been the focus of intensive efforts during the past decade.

Although benzyl thioglycosides were employed in the present work, sugars to be used as acceptors in coupling with oxazolines may have their anomeric positions protected in a variety of ways. In many cases, ordinary *O*-benzyl glycosides will be the derivatives of choice. When selective deprotection of the reducing function will be required at a later stage of the synthesis, this can be accomplished as readily with benzyl thioglycosides as with the alternative allyl or 2,2,2-trichloroethyl glycosides.

EXPERIMENTAL

Instrumental and chromatographic procedures. — These were as described in the previous paper in this series[2]. The ^{1}H-n.m.r. data recorded here were taken from spectra determined on a Bruker WH 270 instrument, with decoupling as required. Line assignments, except those that could be made unambiguously by inspection, are based on the decoupling experiments.

O-Acyltri-O-benzylgalactosyl benzylxanthates: general procedure. — *O*-Acyltri-*O*-benzyl-D-galactopyranosyl bromide, freshly prepared from the corresponding 1-*O*-acetyl derivative, was dissolved in 1:1 (v/v) benzene–ethanol (20 mL per g of 1-acetate), and potassium benzylxanthate[21] (1.2 molar portions) was added. After being stirred for 1.5 h at room temperature, the mixture was poured into water, and the benzene layer was separated. The benzene solution was washed twice with water, dried, and evaporated under diminished pressure. The residual, crude xanthate was purified on a column of silica gel.

Conversion of benzylxanthates to benzyl 1-thiogalactosides. — The galactosyl benzylxanthate was dissolved in 1 volume (1 mL per g) of pyridine, and the mixture was heated for 5 min on a steam bath[11]. It was then poured into chloroform, and the chloroform solution was washed with 5% hydrochloric acid, water, 5% sodium hydrogencarbonate, and water. Evaporation of the dried (magnesium sulfate) chloroform solution under diminished pressure gave the crude thiogalactoside.

O-Deacylation. — Compounds to be *O*-deacylated were kept in methanolic sodium methoxide at room temperature until the reaction was complete, as judged by t.l.c. The solution was then deionized (sulfonic resin) and evaporated to dryness.

3-O-Acetyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl benzylxanthate (**2a**). — The requisite glycosyl bromide (**1a**) was obtained[6] from 1,3-di-*O*-acetyl-2,4,6-tri-*O*-benzyl-α,β-D-galactopyranose[6] (5 g, 9.35 mmol), which was in turn prepared[6] from 2,4,6-tri-*O*-benzyl-α,β-D-galactopyranose[8]. Treatment of the bromide with potassium benzylxanthate by the general procedure gave **2a** in 97% yield; syrup, $[\alpha]_D^{25}$ +41.2°, $[\alpha]_{436}^{25}$ +118.2° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.34–7.23 (m, 20 H, Ph-H), 5.61, 5.53 [AB, 2 H, *J* 12.5 Hz, PhCH_2OC(S)], 5.37 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.98 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 2.9 Hz, H-3), 4.75–4.38 (m, 6 H, 3 PhCH_2), 4.06–3.53 (m, 5 H, sugar C*H* and CH_2), and 1.87 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{37}H_{38}O_7S_2$ (658.83): C, 67.45; H, 5.81; S, 9.73. Found: C, 67.76; H, 5.78; S, 9.28.

Benzyl 3-O-acetyl-2,4,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (**3a**). — The conversion of the benzylxanthate **2a** (4 g, 6.1 mmol) by the general procedure afforded crude **3a**. Crystallization from ether–hexane and recrystallization from methanol gave 3.1 g (85%) of the thioglycoside as long needles, m.p. 54–54.5°, $[\alpha]_D^{25}$ −14.8°, $[\alpha]_{436}^{25}$ −27.2° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **2a** except: upfield shift of PhCH_2OC(S) (δ now <5, overlapped) and H-1 (δ now 4.31, d, $J_{1,2}$ 9.7 Hz).

Anal. Calc. for $C_{36}H_{38}O_6S$ (598.76): C, 72.22; H, 6.40; S, 5.36. Found: C, 71.98; H, 6.43; S, 5.37.

*Benzyl 2,4,6-tri-*O*-benzyl-1-thio-α-*D*-galactopyranoside* (**4**). — Crude glycosyl bromide **1a** (1.5 mmol) was dissolved in benzene (5 mL) and added to a stirred solution of potassium hydroxide (20 mmol) and α-toluenethiol (18 mmol) in 1-propanol (7 mL). The mixture was stirred overnight at room temperature. Benzene was added, and the organic layer was washed with 2M potassium hydroxide and then with water. The dried benzene was evaporated and the syrupy residue left was taken up in diethyl ether. The title compound **4** crystallized out on addition of hexane to the ether solution and was recrystallized from methanol as needles, m.p. 81–82°, $[\alpha]_D^{25}$ +213.8°, $[\alpha]_{436}^{25}$ +447.0° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.67–7.28 (m, 20 H, Ph-H), 5.31 (d, 1 H, $J_{1,2}$ 5.2 Hz, H-1), and 4.95–3.45 (m, 14 H, ring protons and 3 PhCH_2).

Anal. Calc. for $C_{34}H_{36}O_5S$ (556.72): C, 73.35; H, 6.52; S, 5.76. Found: C, 73.30; H, 6.62; S, 5.44.

*Benzyl 2,4,6-tri-*O*-benzyl-1-thio-β-*D*-galactopyranoside* (**5**). — *O*-Deacetylation of **3a** gave compound **5** as a syrup in 95% yield, $[\alpha]_D^{25}$ −37.7°, $[\alpha]_{436}^{25}$ −78.3° (*c* 2, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **3a** except: upfield shift of H-3, appearance of δ 2.30 (d, 1 H, *J* 5.4 Hz, D_2O-exchangeable, O*H*), and loss of CH_3CO.

Anal. Calc. for $C_{34}H_{36}O_5S$ (556.72): C, 73.35; H, 6.52; S, 5.76. Found: C, 72.93; H, 6.52; S, 5.89.

*4-*O*-Benzoyl-2,3,6-tri-*O*-benzyl-β-*D*-galactopyranosyl benzylxanthate* (**2b**). — The galactosyl bromide **1b** generated[7] from 2 g (3.35 mmol) of 1-*O*-acetyl-4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α,β-D-galactopyranose[7] was treated with potassium benzylxanthate according to the general procedure to give **2b** in ~70% yield; syrup, $[\alpha]_D^{25}$ +19.8°, $[\alpha]_{436}^{25}$ +93.4° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.08–7.15 (m, 25 H, Ph-H), 5.91 (bd, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.58 [AB, 2 H, *J* 12.1 Hz, PhCH_2-OC(S)], 5.41 (dd, 1 H, $J_{1,2}$ 8.3 and $J_{1,x}$ 1.7 Hz, H-1), 4.90–4.36 (3 AB, 6 H, 3 PhCH_2), and 3.90–3.50 (m, 5 H, sugar C*H* and CH_2).

Anal. Calc. for $C_{42}H_{40}O_7S_2$ (720.90): C, 69.98; H, 5.59; S, 8.90. Found: C, 69.79; H, 5.66; S, 8.62.

*Benzyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-1-thio-β-*D*-galactopyranoside* (**3b**). — The conversion of the benzylxanthate **2b** by the general procedure furnished crude **3b** in almost quantitative yield. The syrup, after purification on silica gel, had $[\alpha]_D^{25}$ −11.2°, $[\alpha]_{436}^{25}$ −24.4° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **2b** except: upfield shift of PhCH_2OC(S) (δ now <5, overlapped) and H-1 (δ now 4.35, d, $J_{1,2}$ 9.2 Hz).

Anal. Calc. for $C_{41}H_{40}O_6S$ (660.83): C, 74.52; H, 6.10; S, 4.85. Found: C, 74.14; H, 6.26; S, 4.81.

*Benzyl 2,3,6-tri-*O*-benzyl-1-thio-β-*D*-galactopyranoside* (**6**). — *O*-Debenzoylation of **3b** afforded syrupy **6** in almost quantitative yield, $[\alpha]_D^{25}$ −37.6°, $[\alpha]_{436}^{25}$ −89.6° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **3b** except: loss of the benzoyl signals (δ 8.1–7.4), upfield shift of H-4, appearance of δ 2.50 (bs, 1 H, D_2O-exchangeable, O*H*).

Anal. Calc. for $C_{34}H_{36}O_5S$ (556.72): C, 73.35; H, 6.52; S, 5.76. Found: C, 73.12; H, 6.51; S, 5.81.

To obtain **6** for use as a glycosyl acceptor, the steps **1b** → **2b** → **3b** → **6** were carried out without purification of intermediates. The resulting crude **6** was then chromatographed on a column of silica gel.

6-O-Acetyl-2,3,4-tri-O-benzyl-β-D-galactopyranosyl benzylxanthate (**2c**). — 1,6-Di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α,β-D-galactopyranose[6] (3 g, 5.6 mmol) was converted[6] into the bromide **1c**. The latter, on reaction with potassium benzylxanthate, afforded the title compound, which was isolated in 76% yield (2.8 g) by chromatography on a column of silica gel. After recrystallization from methanol, it had m.p. 84–85°, $[\alpha]_D^{25}$ +16.5°, $[\alpha]_{436}^{25}$ +78.0° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.37–7.27 (m, 20 H, Ph-H), 5.62, 5.54 [AB, 2 H, *J* 12.1 Hz, PhCH_2OC(S)], 5.32 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.96, 4.64 (AB, 2 H, *J* 11.4 Hz, PhCH_2), 4.80, 4.72 (AB, 2 H, *J* 10.7 Hz, PhCH_2), 4.77 (s, 2 H, PhCH_2), 4.20–3.60 (m, 6 H, sugar C*H* and CH_2), and 1.95 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{37}H_{38}O_7S_2$ (658.83): C, 67.45; H, 5.81; S, 9.73. Found: C, 67.36; H, 5.81; S, 9.83.

Benzyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio-β-D-galactopyranoside (**3c**). — When subjected to the general procedure for conversion, compound **3b** (1.5 g) gave crude **3c**, which crystallized from methanol. The yield was 1.08 g (79%), m.p. 99.5–100.5°, $[\alpha]_D^{25}$ −56.8°, $[\alpha]_{436}^{25}$ −117.8° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **2c** except: upfield shift of PhCH_2OC(S) (δ now <5, overlapped) and H-1 (δ now 4.23, d, $J_{1,2}$ 9.6 Hz).

Anal. Calc. for $C_{36}H_{38}O_6S$ (598.76): C, 72.22; H, 6.40; S, 5.36. Found: C, 72.01; H, 6.31; S, 5.12.

Benzyl 2,3,4-tri-O-benzyl-1-thio-β-D-galactopyranoside (**7**). — *O*-Deacetylation of compound **3c** (9.16 g) gave **7**. The compound crystallized from ether–hexane as fibrous needles (8.2 g, 96%), m.p. 102.5–103°, $[\alpha]_D^{25}$ −27.6°, $[\alpha]_{436}^{25}$ −56.3° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **3c** except: appearance of δ 1.57 (s, 1 H, D_2O-exchangeable, O*H*), and loss of CH_3CO.

Anal. Calc. for $C_{34}H_{36}O_5S$ (556.72): C, 73.35; H, 6.52; S, 5.76. Found: C, 73.02; H, 6.56; S, 5.35.

Coupling. — A solution containing acceptor sugar (thioglycoside) (0.2M) and anhydrous *p*-toluenesulfonic acid (0.01M) in dry 1,2-dichloroethane was prepared. (When small quantities are involved, the *p*-toluenesulfonic acid is best dispensed as an aliquot of a stock solution of known concentration.) The oxazoline (2 molar portions with respect to acceptor) was dissolved in the acceptor–catalyst solution, and the mixture was heated at 70° (bath temperature). When t.l.c. showed complete disappearance of the oxazoline, a few drops of pyridine were added to the solution, and it was evaporated to dryness. A chloroform extract of the residue was washed sequentially with water, 5% hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, and then dried. Evaporation of the chloroform gave a residue from which the coupling product was separated by appropriate means.

Removal of protecting groups from the substituted disaccharide thioglycosides. — Operation on the 0.5-mmol scale is described for the sake of example. The *O*-deacylated coupling-product (470 mg) was dissolved in acetone (15 mL), and water (4 mL) and methyl iodide (4 mL) were added[22]. The mixture was boiled for ~24 h under reflux, then the solvents were evaporated off. The residue was taken up in chloroform, the solution was washed with 5% sodium thiosulfate and with water, and dried with magnesium sulfate. The syrup obtained by evaporation of the chloroform was chromatographed on a column of silica gel to remove any traces of sulfur-containing by-products.

The sulfur-free, *O*-benzylated disaccharide was dissolved in 25 mL of 90% aqueous ethanol, 25 mg of 10% palladium-on-charcoal was added, and the solution was stirred under hydrogen at 1 atm pressure. When the *O*-debenzylation was complete (1–3 days), the catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness.

Benzyl 3-O-(2-acetamido-3-O-acetyl-4,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (**9a**). — The oxazoline[2] **8** was coupled to compound **5** (118 mg, 0.2 mmol) by the procedure just described. The reaction period was 4 h. The crude, syrupy residue was taken up in dichloromethane and, on the addition of ether and hexane, the coupling product crystallized out. Recrystallization from methanol gave 118 mg of pure **9a** as prisms, and an additional 25 mg was isolated from the mother liquors by column chromatography. Thus the total yield was 143 mg (69%), m.p. 137–138°, $[\alpha]_D^{25}$ −51.7°, $[\alpha]_{436}^{25}$ −106.2° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.31–7.10 (m, 30 H, Ph-H), 5.00–3.43 (m, 27 H, N*H*, ring protons, and 6 PhCH_2), and 1.89 and 1.55 (2s, 3 H each, 2CH_3CO).

Anal. Calc. for $C_{58}H_{63}NO_{11}S$ (982.20): C, 70.93; H, 6.47; N, 1.43; S, 3.26. Found: C, 70.70; H, 6.34; N, 1.13; S, 3.45.

Benzyl 3-O-(2-acetamido-4,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (**9b**). — *O*-Deacetylation of **9a** (500 mg) afforded a quantitative yield of the title compound as an amorphous solid, $[\alpha]_D^{25}$ −36.5°, $[\alpha]_{436}^{25}$ −68.3° (*c* 0.3, chloroform): ^{1}H-n.m.r. ($CDCl_3$) similar to **9a** except: upfield shift of H-3′, resolved signals at δ 5.47 (d, 1 H, $J_{NH,2}$ 4.4 Hz, D_2O-exchangeable, N*H*), and loss of the lower field CH_3CO.

Anal. Calc. for $C_{56}H_{61}NO_{10}S$ (940.17): C, 71.54; H, 6.54; N, 1.49; S, 3.41. Found: C, 70.93; H, 6.58; N, 1.23; S, 3.60.

3-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**10**). — Removal of the protecting groups from **9b** (270 mg, 0.3 mmol) yielded 93.5 mg (85%) of the title compound **10**. When crystallized from methanol, it had m.p. 155–160° (lit.[16,17] m.p. 131–133°, 132–134°), $[\alpha]_D^{20}$ +37.6°, $[\alpha]_{436}^{20}$ +67.8° (24 h) (*c* 0.5, water) (lit.[16,17] $[\alpha]_D^{20}$ +35.7°, +35°).

Benzyl 6-O-(2-acetamido-3-O-acetyl-4,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-1-thio-β-D-galactopyranoside (**11a**). — Oxazoline[2] **8** (0.67 g, 1.57 mmol) was condensed with compound **7** (0.44 g, 0.79 mmol) by the general procedure for coupling. Chromatography of the reaction residue on silica gel yielded

0.64 g (82%) of disaccharide **11a**. Recrystallization from methanol gave solid material containing varying proportions of short needles, and varying in m.p. from 153 to 161°. The lower figure was observed with preparations consisting preponderantly of needles; $[\alpha]_D^{25}$ −47.2°, $[\alpha]_{436}^{25}$ −102.1° (*c* 1.0, chloroform): ^{1}H-n.m.r. ($CDCl_3$): δ 7.34–7.13 (m, 30 H, Ph-H), 5.44 (d, 1 H, $J_{NH,2}$ 9.5 Hz, N*H*), 5.01–3.41 (m, 26 H, sugar C*H* and CH_2, PhCH_2), 1.94 (s, 3 H, CH_3CO), and 1.84 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{58}H_{63}NO_{11}S$ (982.20): C, 70.93; H, 6.47; N, 1.43; S, 3.26. Found: C, 70.82; H, 6.36; N, 1.24; S, 3.15.

*Benzyl 6-O-(2-acetamido-4,6-di-O-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,4-tri-O-benzyl-1-thio-β-*D*-galactopyranoside* (**11b**). — *O*-Deacetylation of **11a** (640 mg) yielded 0.59 g (96.3%) of **11b**. Crystallization and recrystallization from methanol produced very fine needles, m.p. 136.5–138°, $[\alpha]_D^{25}$ −44.4°, $[\alpha]_{436}^{25}$ −98.2° (*c* 1.0, chloroform): ^{1}H-n.m.r. ($CDCl_3$) similar to **11a** except: small $J_{NH,2}$ 5.0 Hz at δ 6.07 (d, 1 H, D_2O-exchangeable, N*H*), and loss of 1 CH_3CO.

Anal. Calc. for $C_{56}H_{61}NO_{10}S$ (940.17): C, 71.54; H, 6.54; N, 1.49; S, 3.41. Found: C, 71.19; H, 6.27; N, 1.15; S, 3.32.

*6-O-(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)-*D*-galactopyranose* (**12**). — Removal of the protecting groups from **11a** (588 mg, 0.626 mmol) gave amorphous **12** in 95% yield, $[\alpha]_D^{25}$ +8.6° (*c* 0.5, water) (lit.[18] $[\alpha]_D^{20}$ +9.2°).

*Benzyl 4-O-(2-acetamido-4-O-acetyl-3,6-di-O-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,6-tri-O-benzyl-1-thio-β-*D*-galactopyranoside* (**14a**). — Thiogalactoside **6** (300 mg, 0.54 mmol) was dissolved in 1 mL of dry 1,2-dichloroethane containing 20 μmol of *p*-toluenesulfonic acid. Oxazoline[2] **13** (658 mg, 1.55 mmol) was separately dissolved in 3 mL of dry dichloroethane. After the addition of one mL of the latter solution to the solution of acceptor and catalyst, the mixture was boiled under reflux, and the remainder of the oxazoline was added in two portions at intervals of 1 h. Heating was continued for a total of 5 h. Extractive processing as prescribed in the general procedure for coupling, followed by chromatographic separation, gave the title compound. After recrystallization from dichloromethane–ether–hexane, the yield was 280 mg (53%), m.p. 99.5–100°, $[\alpha]_D^{25}$ −16.6°, $[\alpha]_{436}^{25}$ −40.8° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.41–7.14 (m, 30 H, Ph-H), 5.65 (d, 1 H, $J_{NH,2}$ 7.7 Hz, N*H*), 5.01–3.47 (m, 26 H, ring protons and 6 PhCH_2), 1.81 (s, 3 H, CH_3CO), and 1.73 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{58}H_{63}NO_{11}S$ (982.20): C, 70.93; H, 6.47; N, 1.43; S, 3.26. Found: C, 71.19; H, 6.32; N, 1.36; S, 3.25.

*Benzyl 4-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,6-tri-O-benzyl-1-thio-β-*D*-galactopyranoside* (**14b**). — *O*-Deacetylation of **14a** gave the title compound **14b** in almost quantitative yield. After recrystallization from dichloromethane–ether–hexane, it had m.p. 101–102°, $[\alpha]_D^{25}$ −27.6°, $[\alpha]_{436}^{25}$ −53.5° (*c* 0.49, chloroform): ^{1}H-n.m.r. ($CDCl_3$) similar to **14a** except: loss of the lower-field CH_3CO.

Anal. Calc. for $C_{56}H_{61}NO_{10}S$ (940.17): C, 71.54; H, 6.54; N, 1.49; S, 3.41. Found: C, 71.40; H, 6.59; N, 1.21; S, 3.78.

4-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**15**). — Removal of the protecting groups from **14b** gave amorphous **15**, $[\alpha]_D^{25}$ +8.1→+25.1°, $[\alpha]_{436}^{25}$ +22.5→+42.6° (24 h) (*c* 0.5, water) (lit.[19] $[\alpha]_D$ +8°).

Allyl 2-acetamido-4-O-(2-acetamido-4-O-acetyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**17a**). — The coupling of the oxazoline[2] **13** (350 mg, 0.82 mmol) to the allyl glycoside[2] **16** (150 mg, 0.34 mmol) was accomplished by the same procedure as used for the coupling of **13** to **6**. Chromatographic separation afforded 88.5 mg (30%) of the title compound, which was recrystallized from ethanol; m.p. 197–198°, $[\alpha]_D^{25}$ −20.6°, $[\alpha]_{436}^{25}$ −54.8° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.10 (m, 20 H, Ph-H), 6.25 (d, 1 H, *J* 8.5 Hz, N*H*), 5.78 (m, 1 H, -C*H*=CH_2), 5.20–5.00 (m, 4 H, -CH=CH_2, H-4′, and N*H*), 4.70–3.40 (m, 23 H, 4 PhCH_2, OCH_2-CH=, and ring protons), 1.79, 1.90, and 1.94 (3 s, 9 H, 3 CH_3CO).

Anal. Calc. for $C_{49}H_{58}N_2O_{12}$ (867.01): C, 67.88; H, 6.74; N, 3.23. Found: C, 68.02; H, 6.86; N, 2.96.

Allyl 2-acetamido-4-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**17b**). — *O*-Deacetylation of **17a** gave **17b** (quantitative), which was chromatographed on silica gel and then crystallized from ethanol; m.p. 174.5–175.5°, $[\alpha]_D^{25}$ −38.2°, $[\alpha]_{436}^{25}$ −94.6° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$) similar to **17a** except: upfield shift of H-4′, addition of δ 3.06 (bs, 1 H, O*H*), and loss of CH_3CO.

Anal. Calc. for $C_{47}H_{56}N_2O_{11}$ (824.97): C, 68.43; H, 6.84; N, 3.40. Found: C, 68.35; H, 6.84; N, 3.13.

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (**18**). — For isomerization of the allyl group, compound **17b** (60 mg) was treated with potassium *tert*-butoxide in methyl sulfoxide[23]. The isolated 1-propenyl glycoside was hydrolyzed in acetone–hydrochloric acid[23], and the resulting tetra-*O*-benzyl disaccharide was chromatographed on silica gel to remove sulfur compounds. Hydrogenolysis then gave **18** as an amorphous solid, $[\alpha]_D^{25}$ +17°, $[\alpha]_{436}^{25}$ +25° (equilibrium) (*c* 0.56, water); lit.[20] $[\alpha]_D^{25}$ +18.5° (equilibrium).

Preparation and ^{1}H-n.m.r. examination of disaccharide alditols. — A portion (20 mg) of each of the fully deprotected disaccharides **10**, **12**, **15**, and **18** was dissolved in water (1–2 mL) and treated with sodium borohydride (40 mg). After 4 h, acetic acid was added, then the solution was deionized with Rexyn 101 (H^+) resin. The filtrate from removal of the resin was evaporated to dryness, and several portions of methanol were evaporated from the residue. Finally the residue was dissolved in 0.2–0.3 mL of deuterium oxide containing sodium 4,4-dimethyl-4-silapentanoate as internal standard, and the ^{1}H spectrum was recorded with a Bruker 270-MHz spectrometer at room temperature or 60°, as appropriate. Scanning was continued until the base-line noise was less than 1% of the height of the lines arising from the anomeric proton.

The anomeric protons (H-1′) gave doublet signals as follows: alditol from **10**, δ 4.53, $J_{1',2'}$ 7.5 Hz; alditol from **12**, δ 4.57, $J_{1',2'}$ 8.7 Hz; alditol from **15**, δ ~4.5,

$J_{1',2'}$ 8.8 Hz; and alditol from **18**, δ ~4.4, $J_{1',2'}$ 8.4 Hz. There were no discernible signals at lower field.

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