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THE SYNTHESIS OF METHYL 4-*O*-(4-*O*-α-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSYL)-β-D-GLUCOPYRANOSIDE: THE METHYL β-GLYCOSIDE OF THE TRISACCHARIDE RELATED TO FABRY'S DISEASE

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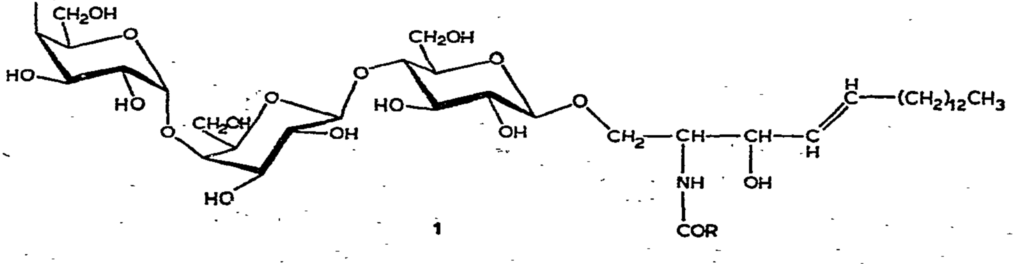
ABSTRACT

As part of a program to synthesize the ceramide trisaccharide (**1**) related to Fabry's disease, methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**) was prepared. Methyl β-lactoside (**2**) was converted into methyl 4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**7**) was synthesized from **4** through the intermediates methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**) and methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**). The halide-catalyzed condensation of **7** with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide (**8**) gave methyl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**10**). Stepwise deprotection of **10** led to **12**, the methyl β-glycoside of the trisaccharide related to Fabry's disease.

DISCUSSION

Fabry's disease is a deficiency of the α-D-galactosidase that catalyzes cleavage of the terminal α-D-galactopyranosyl group from the 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranosylceramide[1] (**1**). In order to study some aspects of this disease, we have devised a partial synthesis of **1** that is amenable to



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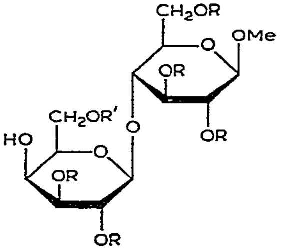
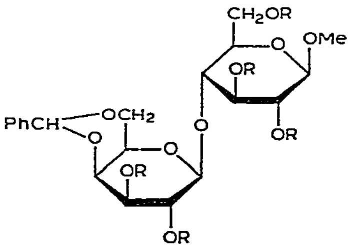
labelling with ^{14}C in the terminal α-D-galactopyranosyl moiety. The trisaccharide portion of **1** is potentially the product of the glycosidic condensation of a suitably protected galactopyranosyl halide with a suitably protected β-lactoside that offers a free 4′-hydroxyl group. This synthetic route to the trisaccharide presents several challenges. First, lactose must be selectively substituted so that only the 4′-hydroxyl group is free. For condensation to occur, this 4′-hydroxyl group must be reasonably nucleophilic, although it adopts in the less reactive[2,3] axial disposition. Finally, the condensation must produce a 1,2-*cis* linkage between the terminal galactose and the lactose moieties.

The selective substitution of lactose or of alkyl lactosides has been studied by several groups previously[3-7]. In a systematic study of the selective benzoylation of methyl β-lactoside (**2**), Bhatt *et al.*[4] determined the order of reactivity of the hydroxyl groups in **2** to be 6′ > 3′ > 6 > 2 > 2′,4′ > 3. Treatment of **2** with 6.5 equivalents of benzoyl chloride in pyridine gave 33% of a hexabenzoate identified as methyl 2,6,2′,3′,4′,6′-hexa-*O*-benzoyl-β-lactoside[4]. Similarly, Valashek[5] reported a 31% yield of benzyl 2,6,2′,3′,4′,6′-hexa-*O*-benzoyl-β-lactoside from benzyl β-lactoside and 7.2 equivalents of benzoyl chloride in pyridine. A second hexabenzoate, possibly benzyl 2,3,6,2′,3′,6′-hexa-*O*-benzoyl-β-lactoside, was also detected by Valashek[5] in the product mixture (in less than 13% yield), but it was not isolated or characterized. Vazquez *et al.*[6] prepared 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-β-lactose by the selective benzoylation of lactose with benzoyl chloride in 20% sodium hydroxide solution. Beith-Halahmi *et al.*[3] synthesized benzyl 2,3,6,2′,6′-penta-*O*-acetyl-β-lactoside (**3**) via benzyl 3′,4′-*O*-isopropylidene-β-lactoside. Lactoside **3**, which contains both a free, axial 4′-hydroxyl group and a free, equatorial 3′-hydroxyl group, could be selectively galactosylated at the 3′-hydroxyl group. In this manner, Beith-Halahmi prepared 4-*O*-(3-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl)-D-glucopyranose[3].



2 R = R′ = H
6 R = Bz; R′ = H
7 R = R′ = Bz



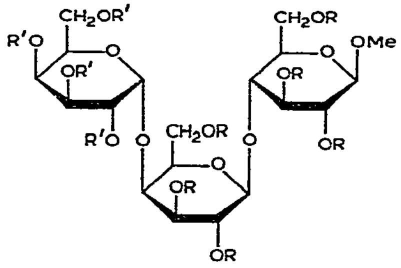
4 R = H
5 R = Bz

For our trisaccharide synthesis, a lactose derivative having seven protected hydroxyl groups (1,2,3,6,2′,3′,6′) and only one free (4′) was desired. As direct acylation had failed as a practical approach to this moiety[4-6] a stepwise synthesis was adopted. As a precursor to benzyl 2,3,6,2′,3′,4′-hexa-*O*-benzyl-β-lactoside, Liptak[7] synthesized benzyl 4′,6′-*O*-benzylidene-β-lactoside from benzyl β-lactoside. Similarly, the azeotropic removal of water from a mixture of *p*-toluenesulfonic acid, **2**, and benzaldehyde in refluxing *N*,*N*-dimethylformamide–benzene gave methyl 4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). Pure **4** could be isolated from the mixture, but the yield was not consistent. Alternatively, the crude mixture was neutralized with triethylamine and evaporated. Benzoylation of this crude **4** in pyridine gave methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**). After partial purification of **5**, the benzylidene group was removed with 90% trifluoroacetic acid to give **6**. Pure methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**) was obtained in 17% yield from methyl β-lactoside (**2**). The pentabenzoate **6** contained a free, primary 6'-hydroxyl and a free, axial, secondary 4'-hydroxyl group. Selective benzoylation of **6** with benzoyl chloride in pyridine gave methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**7**) in 62% yield.

The selectivity of benzoylation of **6** was confirmed by ^{13}C-n.m.r. spectroscopy. The spectrum of **6** included single lines at δ 67.04, 63.61, and 60.91. The line at δ 67.04, which was 4 p.p.m. upfield from any other line attributable to a secondary carbon-atom, was assigned to C-4', the only secondary carbon atom bearing a free hydroxyl group. The line of the primary carbon atom having a free hydroxyl group (C-6') was at δ 60.91, 2.7 p.p.m. upfield from the line (δ 63.61) of C-6, which was *O*-benzoylated. Selective benzoylation of **6** at the 6'-hydroxyl group gave **7**. The ^{13}C-n.m.r. spectrum of **7** revealed that the two C-6 lines had become coincident at δ 63.55, as both C-6 and C-6' are *O*-benzoylated in **7**. In contrast, the C-4' line (δ 67.27) of **7** was shifted only slightly downfield relative to the C-4' line (δ 67.04) of **6**. The C-4' line was still significantly upfield (3.8 p.p.m.) from the nearest other line attributable to a secondary carbon atom. Thus, in the conversion of **6** into **7**, the 6'-hydroxyl group had become benzoylated whereas the 4'-hydroxyl group had remained free.

In a model-disaccharide synthesis[8], the halide-catalyzed condensation of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide (**8**) with methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**9**) gave the fully protected, α-linked disaccharide in 83% yield from **9**. Similarly, the hexabenzoate **7** and compound **8** (3 equiv.) were dissolved in a dichloromethane solution of tetraethylammonium bromide containing a few drops of *N*,*N*-dimethylformamide. The solution was boiled for 5 days under reflux over molecular sieves. After preparative t.l.c. a 34% yield (based on **7**) of the fully protected trisaccharide, methyl 2,3,6-tri-*O*-benzoyl-4-*O*-[4-*O*-(2,3,4,6-tetra-*O* benzyl-α-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl]-β-D-glucopyranoside (**10**), was recovered.

Compound **10** was debenzoylated catalytically with methanolic sodium methoxide to give methyl 4-*O*-[4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**11**) in 92% yield. Catalytic hydrogenolysis (palladium-on-charcoal) of **11** gave a 94% yield of methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**). The yield of **12** from methyl β-lactoside (**2**) was 3%. Acetylation of **12** in acetic anhydride–pyridine gave a 70% yield of methyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-



10 R = Bz; R′ = CH_2Ph
11 R = H; R′ = CH_2Ph
12 R = R′ = H
13 R = R′ = Ac

tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**13**).

The trisaccharide nature of **10** was evident from its ^{13}C-n.m.r. spectrum, which showed three lines attributable to C-1 anomeric carbon atoms: δ 102.11, 101.72, and 101.34. The anomeric region of the spectra of the deprotected trisaccharide derivative **12** and of the corresponding decaacetate **13** could be assigned more definitely. The spectrum of **13** revealed two closely-spaced lines (δ 101.90 and 101.63) corresponding to the β-anomeric C-1 and C-1′, and an upfield peak (δ 99.89) attributable to the α-anomeric C-1″. Similarly, the spectrum of **12** had two, nearly coincident lines (δ 104.13 and 103.90), assigned to the β-anomeric C-1 and C-1′, in addition to an upfield line (δ 101.22) corresponding to the α-anomeric C-1″. The complete ^{13}C-n.m.r. spectrum of **12** has been assigned and analyzed in detail in a separate paper[9]. The complete ^{13}C-n.m.r. data[9] provided substantial confirmation that the synthetic trisaccharide **12** was indeed methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside.

Halide-catalyzed, glycosidation condensations have been shown to give a high percentage of 1,2-*cis*-linked products[8,10,11]. Similarly, within the limits of ^{13}C-n.m.r. analysis, the fully protected trisaccharide **10** isolated by preparative t.l.c. appeared anomerically pure. In addition, none of the other prominent t.l.c. bands contained a trisaccharide product*. Thus, the halide-catalyzed condensation of **8** with **7** also resulted in the highly selective formation of a new, 1,2-*cis*-glycosidic linkage.

Stepwise protection of methyl β-lactoside (**2**) gave the trisaccharide precursor **7**, having a free 4′-hydroxyl group, in 11% yield. The halide-catalyzed condensation of **7** with **8** produced, after deprotection, a 3% yield (based on **2**) of methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**), the methyl β-glycoside of the trisaccharide related to Fabry's disease.

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*Chromatographic fractions were checked by ^{1}H-n.m.r. to determine whether both benzyl and benzoyl groups were present, as would be expected for a fraction that contained trisaccharide product.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points were determined in capillary tubes with a Thomas–Hoover melting point apparatus and are uncorrected. Proton-decoupled^{13}C magnetic resonance spectra were recorded with a Varian XL-100-15 Fourier Transform Spectrometer modified for multinuclear operation[12] and equipped with a Varian 620L computer for data handling. Column chromatography was performed on silica gel reagent (90–200 mesh), Accurate Chemical and Scientific Corp. EM Silica gels GF254 and PF254 were used for t.l.c. Solutions were evaporated or concentrated under diminished pressure unless otherwise indicated.

Methyl 4-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). — A 500-ml round-bottomed flask was charged with methyl β-lactoside[13] (**2**, 4.0 g, 14 mmol), anhydrous *p*-toluenesulfonic acid (1.5 g, 14 mmol), benzaldehyde (10 ml, 100 mmol), dry *N,N*-dimethylformamide (40 ml), and dry benzene (200 ml). The mixture was stirred and heated to reflux under nitrogen. Benzene and its azeotropes were allowed to distil, and the distillate was collected in a graduated flask. Dissolution occurred slowly in the reaction flask and was complete when the volume of distillate reached 160 ml. When this volume reached 170 ml, heating was discontinued. The flask was opened briefly, and triethylamine (3 ml, 21 mmol) was added to the solution, which turned from pale to dark yellow. When the solution had cooled to room temperature under nitrogen, it was evaporated to a mixture of solids and oil. This residue was dissolved in a mixture of ether (100 ml) and water (100 ml). The layers were shaken and separated, and the aqueous layer was washed twice with ether (2 × 50 ml). Evaporation of the aqueous layer gave crude **4** as a solid foam (9.4 g). This crude mixture was used subsequently for the preparation of **5**.

An analytical sample of **4** was prepared as follows. The foregoing procedure was followed, except that no triethylamine was added to the mixture before it was cooled. The flask was stoppered and placed in a freezer compartment overnight. The powder that precipitated was collected by filtration and air-dried. Crude **4** (2.28 g, 37%) melted* at 170°. Recrystallization from abs. ethanol (40 ml) containing a few drops of triethylamine gave 1.52 g (24%) of pure (**4**) as tiny, colorless needles, m.p. 220°, $[\alpha]_D^{21}$ −25° (*c* 1.0, water).

Anal. Calc. for $C_{20}H_{28}O_{11}$: C, 54.05; H, 6.35. Found: C, 54.00; H, 6.25.

Methyl 2,3,6-tri-O-benzoyl-4-O-(4,6-O-benzylidene-2,3-di-O-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**). — Compound **4** (9.4 g of crude foam, ~14 mmol) was dissolved in dry pyridine (200 ml), and the solution was stirred and cooled to 0° under nitrogen. Benzoyl chloride (10.0 ml, 86 mmol, 6.2 equiv.) was added by syringe in 1-ml portions during 5 min. When addition was complete, the

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*The yield of precipitate was not consistent; it ranged from 15% to 50%, and was approximately inversely proportional to the scale of the reaction. The m.p. of solid, crude **4** varied from 150 to 180° in several preparations.

solution was allowed to warm to room temperature. The nitrogen inlet was then removed, and the dark-orange solution was protected with a Drierite drying-tube. After 2 days at room temperature, the mixture was poured into a stirred mixture of chloroform (200 ml), saturated sodium hydrogencarbonate solution (200 ml), and ice (200 ml). The flask was washed with chloroform (2 × 50 ml), and the combined mixture was stirred for 2 h as it warmed to room temperature. The layers were separated, and the chloroform layer was washed with water (2 × 250 ml) and with brine (250 ml). Each aqueous wash was back-extracted with a small amount of chloroform. The combined chloroform solution was dried (sodium sulfate) and evaporated, and the residue was dissolved in toluene. Evaporation of the toluene solution gave a solid residue (13.9 g). This crude solid was dissolved in abs. ethanol (450 ml) and benzene (180 ml). The solution, concentrated on a hot plate to 400 ml, was kept for several h at room temperature and then overnight in a refrigerator. The glassy solid that separated was collected by filtration, powdered, and air-dried to give crude **5**, 6.3 g, m.p. 214–218°. Recrystallization from benzene–ethanol gave 4.2 g (31%) of **5**, m.p. 219–222°.

Crude, solid **4** (m.p. 150°, see footnote, p. 143) was also benzoylated as just described for benzoylation of the crude, foamy **4**. Crystallization of the crude benzoylation product from benzene–ethanol gave an analytical sample of **5** in 57% yield as off-white needles, m.p. 238–239°, $[\alpha]_D^{21}$ +133° (c 1.0, chloroform).

Anal. Calc. for $C_{55}H_{48}O_{16}$: C, 68.46; H, 5.01. Found: C, 68.51; H, 4.96.

Methyl 2,3,6-tri-O-benzoyl-4-O-(2,3-di-O-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**). — Powdered compound **5** (3.91 g, 4.05 mmol, m.p. 219–222°) was treated with 90% aqueous trifluoroacetic acid (25 ml), and the mixture was stirred until dissolution was complete (10 min). After an additional 20 min, the solution was poured into a stirred mixture of water (200 ml) and chloroform (150 ml), and the flask was rinsed with chloroform (2 × 25 ml). After 30 min, the layers were shaken and separated, and the chloroform solution was washed successively with portions (150 ml) of water, saturated sodium hydrogencarbonate solution, water, and brine. Each aqueous wash was back-extracted with a small amount of chloroform. The combined chloroform solution was dried (sodium and magnesium sulfates) and evaporated to a solid foam that was triturated three times with low-boiling petroleum ether (75 ml) to give 3.84 g of crude **6**. Crystallization from boiling ethanol (150 ml) gave tiny needles of **6**, 1.78 g, m.p. 195–196°. Concentration of the mother liquor produced an additional 0.48 g, m.p. 194–196°; total yield of **6**, 2.26 g (64%). Recrystallization from ethanol gave an analytical sample; yield 1.91 g (54%), m.p. 202–203°, $[\alpha]_D^{20}$ +91° (c 1.0, chloroform).

Analytically pure **5** (8.4 g) was also treated with 90% trifluoroacetic acid to give analytically pure **6** (7.0 g), m.p. 201–204°, in 91% yield after crystallization from ethanol; ^{13}C-n.m.r. (acetone-d_6): δ 102.10 and 101.89 (β-anomeric carbon atoms C-1 and C-1′) 67.04 [C-4′ (-OH)], 63.61 [C-6 (-OBz)], 60.91 [C-6′ (-OH)].

Anal. Calc. for $C_{48}H_{44}O_{16}$: C, 65.75; H, 5.06. Found: C, 65.70; H, 5.02.

Methyl 2,3,6-tri-O-benzoyl-4-O-(2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-β-D-

glucopyranoside (**7**). — A solution of compound **6** (5.65 g, 6.44 mmol) in dry pyridine (125 ml) was stirred under nitrogen at room temperature. To this solution was added by syringe redistilled benzoyl chloride (0.75 ml, 6.44 mmol). The reaction was monitored by t.l.c. with 2:1 ethyl ether–petroleum ether (30–60°) as developing solvent. After 3 h, the solution contained three components (t.l.c.). At the origin was a spot corresponding to benzoic acid (from residual water in the original solution). Spots at R_F 0.10 (pentabenzoate) and R_F 0.45 (hexabenzoate) were of about equal intensity by fluorescence and charring. Therefore, additional benzoyl chloride (0.35 ml, 3.01 mmol) was added by syringe to the solution. An hour later, t.l.c. revealed no starting pentabenzoate, but a new, very faint spot at R_F 0.55, corresponding to heptabenzoate, was present. The spot for the desired hexabenzoate was prominent by fluorescence and charring. The solution was then poured into a stirred mixture of chloroform (250 ml), saturated sodium hydrogencarbonate solution (125 ml), and ice (125 ml). The flask was washed with two 25-ml portions of chloroform. When the mixture had reached room temperature, the layers were shaken and separated. The aqueous layer was extracted with a small amount of chloroform. The chloroform extracts were washed with water (2 × 200 ml) and brine (200 ml); each washing was back-extracted with a small amount of chloroform. The chloroform solution was dried (sodium sulfate) and concentrated to a pyridine-rich solution. This solution was treated with toluene (300 ml), and the resulting solution was evaporated. The toluene treatment and evaporation were repeated to give a colorless, viscous syrup (7.21 g). This syrup was dissolved in hot ethanol (225 ml), and the solution was allowed to cool. Filtration of the crystals that formed gave, after air-drying, 3.91 g (62%) of pure **7**; m.p. 120° $[\alpha]_D^{21}$ +65° (*c* 1.0, chloroform); ^{13}C-n.m.r. (acetone-d_6): δ 102.12 and 101.68 (β-anomeric carbon atoms C-1 and C-1′), 67.27 [C-4′ (-OH)], 63.55 [C-6 (-OBz)], 63.55 [C-6′ (-OBz)].

Anal. Calc. for $C_{55}H_{48}O_{17}$: C, 67.34; H, 4.93. Found: C, 67.14; H, 4.89.

Methyl 4-O-(4-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**) *via* **10** *and* **11**. — A 10-ml round-bottomed flask was charged with compound **7** (130 mg, 0.133 mmol), tetraethylammonium bromide (85 mg, 0.4 mmol), and molecular sieves (5Å, 0.5 g). To this mixture was added a dichloromethane solution (3 ml) of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide[8,14] (**8**, ~0.39 mmol). The mixture was stirred, and 3 drops of dry *N*,*N*-dimethylformamide were added. The flask was topped with a reflux condenser, and the mixture, protected from light, was brought to reflux under nitrogen. Refluxing was continued for 5 days and then the mixture was filtered through fine-sintered glass, and the reaction flask was washed with four 5-ml portions of dichloromethane. The dichloromethane solution was washed with water, saturated sodium hydrogencarbonate solution, water, and brine. Each of the aqueous washings was back-extracted with dichloromethane. The organic layers were pooled, dried (sodium sulfate), and evaporated to give a nearly colorless oil (334 mg). This oil was purified by preparative t.l.c. with 10:1 benzene–ethyl ether as developer. The fraction having R_F 0.40–0.47 was eluted with chloroform. Filtration and evaporation gave 67 mg (34%) of **10** as a colorless oil; ^{13}C-n.m.r. (acetone-d_6): δ 102.11, 101.72, and 101.34 (anomeric carbon atoms).

Oily **10** (67 mg, 0.044 mmol) was dissolved in a mixture of anhydrous ether (10 ml) and abs. methanol (10 ml). A small piece of sodium was added, and when the bubbling ceased, the flask was stoppered and kept in the dark for 2.5 days. The solution was then evaporated, and the residue was dissolved in a mixture of chloroform (50 ml), ether (10 ml), and water (50 ml). The layers were shaken and separated, and the organic layer was washed with saturated sodium hydrogencarbonate solution, water, and brine. The organic solution was dried (sodium sulfate) and evaporated to a colorless oil that smelled of methyl benzoate. Trituration of the oil with low-boiling petroleum ether removed the methyl benzoate, and the residual, colorless oil was compound **11**; yield 36 mg (92%).

To a solution of **11** (36 mg, 0.041 mmol) in abs. ethanol (180 ml) was added 10% palladium-on-carbon (0.5 g) and distilled water (20 ml). The mixture was shaken on a Parr apparatus for 2 days under hydrogen (48 lb.in^{-2}). The mixture was then filtered through Celite, and the bottle was washed with water. Evaporation of the solution gave **12** as a colorless glass yield; 20 mg (94%), $[\alpha]_D^{20}$ +63° (*c* 1.2, water); ^{13}C-n.m.r. (D$_2$O)[9]: δ 104.13 and 103.90 (β-anomeric carbon atoms), 101.22 (α-anomeric carbon atom).

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.61. Found: C, 43.80; H, 6.44.

Methyl 2,3,6-tri-O-acetyl-4-O-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranoside (**13**). — Compound **12** was converted into the decaacetate by treatment with 1:1 acetic anhydride–pyridine for 2 days at room temperature. Preparative t.l.c. of the crude product gave a 70% yield of the decaacetate **13** as a colorless glass; $[\alpha]_D^{21}$ +42° (*c* 0.85, chloroform); ^{13}C-n.m.r. (acetone-d_6): δ 101.90 and 101.63 (β-anomeric carbon atoms), 99.89 (α-anomeric carbon atom).

Anal. Calc. for $C_{39}H_{54}O_{26}$: C, 49.89; H, 5.80. Found: C, 49.66; H, 5.78.

Decaacetate **13** could be reconverted into **12** in 95% yield by treatment with methanolic sodium methoxide.

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