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SYNTHESIS OF THE METHYL AND 1-OCTYL GLYCOSIDES OF THE P-ANTIGEN TETRASACCHARIDE (GLOBOTETRAOSE)

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ABSTRACT

The methyl and 1-octyl β-glycosides of the P-antigen tetrasaccharide [globotetraose, β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Glc] were synthesised from a tetrasaccharide precursor, prepared using methyl disaccharide 1-thioglycosides as intermediates. In the key glycosidation with silver triflate, HO-2 was used as an α-directing group in the glycosyl bromide.

INTRODUCTION

Globotetraosyl ceramide [β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-ceramide] occurs non-covalently bound to the outside of membranes of various types of human cells. Glycolipids on uroepithelial cells containing the structural element α-D-Gal*p*-(1→4)-β-D-Gal*p* (of which the above is one) are responsible for the specific adhesion of P-fimbriated *E. coli* cells. These glycolipids are therefore involved in the pathogenesis of urinary tract infections[1–3]. Globotetraose also has P antigenic activity in the P blood-group system[4].

Globotetraose with a free reducing end has been synthesised by a block-type route[5]. We now report a simpler (block type) synthesis of the protected globotetraose derivative **11** and its conversion into the methyl (**16**) and 1-octyl (**17**) β-glycosides.

RESULTS

The glycosyl acceptor disaccharide **7** was prepared starting from 2,3,6-tri-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide[6]. Treatment of this bromide with thiourea in acetone and saponification of the resulting pseudothiourea hydrobromide **1** gave the acetylated 1-thiosugar **2**. Alkylation of **2** with methyl iodide gave the methyl 1-thioglycoside **3** (87%, based on the bromide), treatment of which with methanolic sodium methoxide gave

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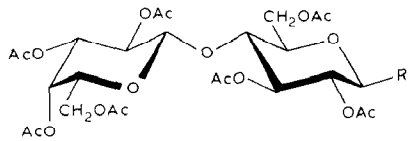
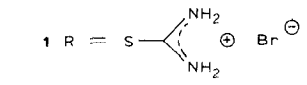
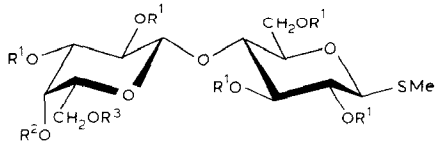
methyl 1-thio-β-lactoside (**4**, 96%). Reaction of **4** with benzaldehyde and formic acid gave the 4′,6′-benzylidene acetal **5** (95%), which, with benzoyl chloride in pyridine, gave **6** (67%). Reductive opening of the acetal ring in **6** with sodium cyanoborohydride–hydrogen chloride[7] gave the 6′-benzyl ether **7** (52%).





1 R = S–C(NH$_2$)=NH$_2$ ⊕ Br$^{\ominus}$

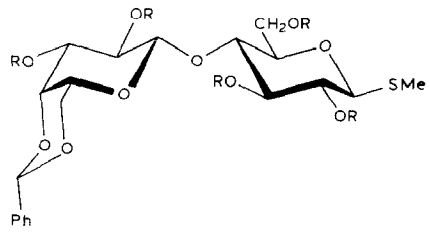
2 R = SH



3 R^1 = R^2 = R^3 = Ac

4 R^1 = R^2 = R^3 = H

7 R^1 = Bz, R^2 = H, R^3 = Bn



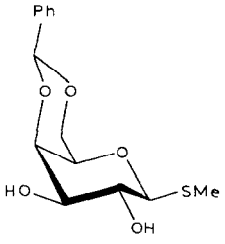
5 R = H

6 R = Bz

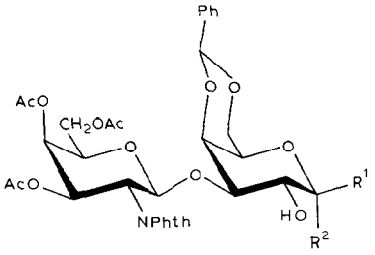
The glycosyl donor disaccharide **10** was prepared from methyl 1-thio-β-D-galactopyranoside[8], which was treated with benzaldehyde and formic acid to give the 4,6-benzylidene acetal **8** (52%). Silver triflate-promoted partial glycosidation of **8** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide[9] gave **9** (55%). The position of the glycosidic linkage in **9** was verified by methylation analysis, and the anomeric configuration was evident from the ^{1}H-n.m.r. spectrum. A minor product (11%) was also isolated, which, according to the n.m.r. data, was the (1→2)-β-linked isomer of **9**. Treatment of **9** with bromine gave the bromide **10**, which was unstable and was used immediately.

Silver triflate-promoted condensation of **7** and **10** gave the key tetrasaccharide derivative **11** (66%). The α configuration of the newly formed glycosidic bond was evident from the ^{1}H-n.m.r. spectrum of **11**. Treatment of **11** with methyl triflate[10] and either methanol or 1-octanol gave the glycosides **12** (69%) or **13** (72%), respectively. These glycosides were treated with hydrazine hydrate followed by acetic anhydride–pyridine to give the peracetylated derivatives **14** (65%) and **15** (75%), both having di-*N*-acetyl groups in the 2-amino-2-deoxy-galactose residue. *O*-Deacetylation with methanolic sodium methoxide followed by catalytic hydrogenation gave the tetrasaccharide glycosides **16** (50%) and **17** (61%),

the structures of which were verified by methylation analysis and n.m.r. spectroscopy.

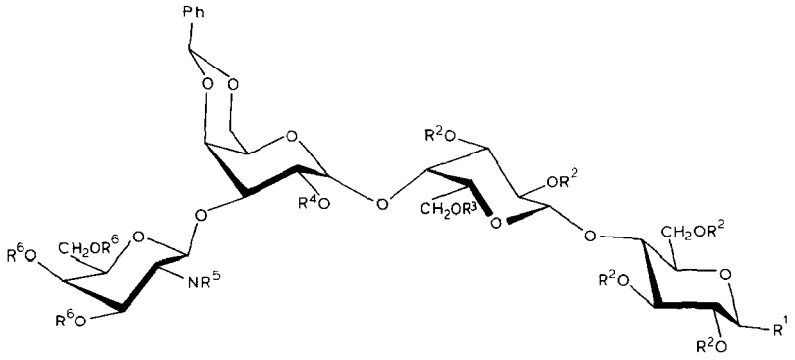


8



9 $R^1 = SMe, R^2 = H$

10 $R^1 = H, R^2 = Br$



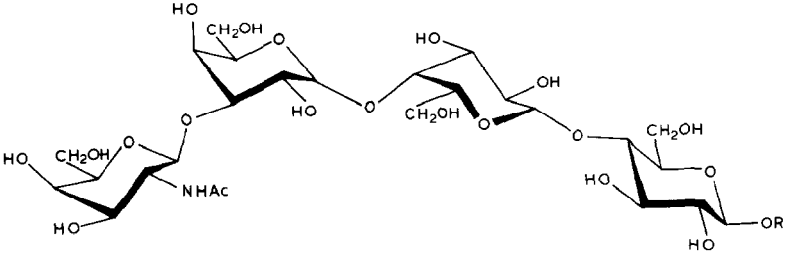
11 $R^1 = SMe, R^2 = Bz, R^3 = Bn, R^4 = H, R^5 = Phth, R^6 = Ac$

12 $R^1 = OMe, R^2 = Bz, R^3 = Bn, R^4 = H, R^5 = Phth, R^6 = Ac$

13 $R^1 = O(CH_2)_7CH_3, R^2 = Bz, R^3 = Bn, R^4 = H, R^5 = Phth, R^6 = Ac$

14 $R^1 = OMe, R^2 = R^4 = R^6 = Ac, R^3 = Bn, R^5 = (Ac)_2$

15 $R^1 = O(CH_2)_7CH_3, R^2 = R^4 = R^6 = Ac, R^3 = Bn, R^5 = (Ac)_2$



16 R = Me

17 R = $(CH_2)_7CH_3$

DISCUSSION

Successful oligosaccharide synthesis requires efficient glycosidation procedures and suitable protecting-groups. In block synthesis of larger oligosaccharides, temporary protection of the glycosidic function is especially important; in this context, thioglycosides[11,12] are useful since they are stable under glycosidation reaction conditions and those associated with the manipulation of most protecting groups. When desired, they can be converted into glycosyl-donating halides by mild treatment with halogen[13,14] or used as glycosyl donors in methyl triflate-promoted glycosidations[10]. The present investigation further exemplifies the usefulness of thioglycosides in oligosaccharide synthesis.

Oligosaccharides intended for biological studies should preferably be "extendable" at the reducing end. Various derivatives of the same oligosaccharide may then be prepared, such as methyl or ethyl glycosides (for inhibition studies) and spacer-arm glycosides (for the preparation of glycoconjugates) or glycolipids. Bromoethyl oligosaccharide-glycosides[15] have been used successfully in this regard. However, a strategy based on thioglycoside protection at the reducing end offers greater flexibility since it allows attachment of the oligosaccharide not only to spacer arms and lipids but also to other sugars, as exemplified by the glycosidation of the disaccharide **7** with the thioglycoside-derived disaccharide bromide **10**. Also, the protected tetrasaccharide thioglycoside **11** could be converted in good yield into the glycosides **12** and **13** by methyl triflate-promoted glycosidation of the parent alcohols.

The present work was undertaken to find a simpler route to globotetraose than that published[5]. This was facilitated by the finding that the diol **8** could be partially glycosidated at position 3 in reasonable yield (55%) and that the HO-2 of the product **9** could be left unprotected in the subsequent glycosidation with the derived bromide **10**. Whether such an approach is generally applicable for the synthesis of α-glycosides remains to be investigated. Reports on the use of an unprotected HO-2 of a glycosylating halide have appeared[16].

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed at 1–2 kPa at <40° (bath). Optical rotations were recorded for 0.4–1.0% solutions (22–24°) in chloroform, unless otherwise stated, using a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded for solutions in $CDCl_3$ ($CHCl_3$ δ 7.25 for ^1H, $CDCl_3$ δ 77.0 for ^{13}C) unless otherwise stated, using a JEOL JNM FX-100 or GX 270 instrument. N.m.r. spectra, recorded for all new compounds, were in agreement with the postulated structures, and only selected data are reported. T.l.c. was performed on Silica Gel F_{254} (Merck) with detection by u.v. light when applicable or by charring with sulfuric acid. Column chromatography was performed on Silica Gel 60 (0.04–0.063 mm, Merck) with loading in the range

1/25–1/100 and elution with toluene–ethyl acetate mixtures unless otherwise stated. Organic solutions were dried over $MgSO_4$. Molecular sieves (3 or 4Å, Union Carbide) were desiccated in a vacuum at 300° overnight and ground immediately before use. Dowex 50 (H^+) resin (20–50 mesh) was washed thoroughly with methanol before use. Elemental analyses were not obtained for syrupy and amorphous products. These products were purified by chromatography and characterised by n.m.r. specctroscopy.

*Methyl 4-*O*-β-*D*-galactopyranosyl-1-thio-β-*D*-glucopyranoside* (**4**). — A solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide[6] (40.0 g) and thiourea (14.0 g) in acetone (120 mL) was boiled under reflux for 1 h and then concentrated. A solution of the residue (63 g), sodium carbonate (anhydrous, 17.6 g), and sodium sulfite (anhydrous, 11.0 g) in water (300 mL) was stirred with dichloromethane (300 mL) at room temperature for 1 h. The organic layer was dried, and methyl iodide (13.5 mL) and *N*-ethyldiisopropylamine (18.4 mL) were added. After 1 h at room temperature, the mixture was washed twice with water, dried, and concentrated. Column chromatography of the residue gave syrupy methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (**3**; 33.0 g, 87%), a solution of which in methanolic 0.1M sodium methoxide (50 mL) was kept at room temperature for 4 h, then neutralised with Dowex 50 (H^+) resin, and concentrated. Crystallisation of the solid residue (17.7 g, 96%) from ethanol gave **4**, m.p. 207°, $[\alpha]_{578}$ +9° (*c* 0.5, water). ^{13}C-N.m.r. data (D_2O, internal 1,4-dioxane δ 67.4): δ 12.2 (MeS), 61.0, 61.9 (C-6,6'), 69.4, 71.8, 72.1, 73.4, 76.2, 76.6, 79.0, 79.6 (C-2,3,4,5, C-2',3',4',5'), 86.2 (C-1), 103.7 (C-1').

Anal. Calc. for $C_{13}H_{24}O_{10}S$: C, 41.9; H, 6.5; S, 8.6. Found: C, 41.7; H, 6.6; S, 8.4.

*Methyl 4-*O*-(4,6-*O*-benzylidene-β-*D*-galctopyranosyl)-1-thio-β-*D*-glucopyranoside* (**5**). — A mixture of **4** (6.0 g), benzaldehyde (12 mL), and formic acid (12 mL) was stirred at room temperature for 40 min, ether (200 mL) was then added, and the precipitated **5** (7.0 g, 95%) was collected and washed with ether. Crystallisation from methanol–ether gave material having m.p. 224–225°, $[\alpha]_{578}$ −27° (*c* 0.6, pyridine). ^{13}C-N.m.r. data (pyridine-d_5, C-2 δ 149.9): δ 12.3 (MeS), 61.6 (C-6), 67.9, 70.0, 71.7, 73.7, 73.8, 77.6, 78.0, 80.1, 80.7 (C-2,3,4,5, C-2',3',4',5',6'), 87.3 (C-1), 101.7 (PhCH), 105.3 (C-1'), and 127.2, 128.5, 129.1, 139.4 (aromatic C).

Anal. Calc. for $C_{20}H_{28}O_{10}S \cdot H_2O$: C, 50.2; H, 6.3. Found: C, 50.3; H, 6.2.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-1-thio-β-*D*-glucopyranoside* (**6**). — Benzoyl chloride (17.5 mL) was added dropwise to a stirred solution of **5** (7.0 g) in pyridine (50 mL) at 0°. The mixture was stirred at room temperature for 2 h, then water (0.5 mL) was added, and stirring was continued for 15 min. Dichloromethane (200 mL) was added, the mixture was washed with water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate, dried, and concentrated. Crystallisation of the residue from ether gave **6**

(10.0 g, 67%), m.p. 235–236°, $[\alpha]_{578}$ +112°. N.m.r. data: ^{13}C, δ 11.5 (MeS), 62.5 (C-6), 66.5 (C-5'), 68.0 (C-6'), 69.5 (C-2'), 70.4 (C-2), 72.6 (C-3'), 73.0 (C-4'), 74.9 (C-3), 76.5 (C-4), 76.8 (C-5), 83.0 (C-1), 100.6 (PhCH), 101.4 (C-1'), 126.3–137.4 (aromatic C), 164.8–166.1 (C=O); ^1H, δ 2.99 (s, H-5'), 3.58 (d, $J_{6a'6b'}$ 12.3 Hz, H-6a'), 3.76 (d, H-6b'), 3.86 (m, H-5), 4.21 (t, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 4.30 (d, $J_{3',4'}$ 3.5 Hz, H-4'), 4.39 (dd, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.61 (d, $J_{1,2}$ 9.9 Hz, H-1), 4.62 (dd, $J_{5,6b}$ 2.1 Hz, H-6b), 4.84 (d, $J_{1',2'}$ 7.9 Hz, H-1'), 5.15 (dd, $J_{2',3'}$ 10.4 Hz, H-3'), 5.28 (s, PhCH), 5.38 (t, $J_{2,3}$ 9.2 Hz, H-2), 5.77 (dd, H-2'), 5.85 (t, H-3).

Anal. Calc. for $C_{55}H_{48}O_{15}S$: C, 67.3; H, 4.9. Found: C, 66.8; H, 4.9.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-*D*-galacto-pyranosyl)-1-thio-β-*D*-glucopyranoside* (**7**). — Sodium cyanoborohydride (2.0 g) followed by hydrogen chloride in ether (saturated, enough to make the mixture acidic) was added to a stirred mixture of **6** (3.0 g) and molecular sieves in dry tetrahydrofuran (45 mL) at 0°. The mixture was stirred at room temperature for 3 h, dichloromethane (10 mL) and water (5 mL) were added, and the mixture was filtered through Celite. The organic layer was washed with water, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. Column chromatography of the residue gave **7** (1.55 g, 52%). Recrystallisation from ether gave material having m.p. 197–198°, $[\alpha]_{578}$ +75°. N.m.r. data: ^{13}C, δ 11.4 (MeS), 62.6 (C-6), 67.0 (C-4'), 67.4 (C-6'), 69.9 (C-2'), 70.0 (C-2), 73.0 (C-5'), 73.2 (CH_2Ph), 74.2 (C-3'), 74.4 (C-3), 76.0 (C-4), 77.0 (C-5), 82.9 (C-1), 101.2 (C-1'), 127.1–137.7 (aromatic C), 165.0–165.7 (C=O); ^1H, δ 3.07 (m, H-6a',6b'), 3.49 (t, $J_{4',5'}$ 6.1, $J_{5',6'}$ 6.1 Hz, H-5'), 3.87 (m, H-5), 4.15–4.29 (m, H-4,4', CH_2Ph), 4.44 (dd, $J_{5,6a}$ 4.4, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.60 (d, $J_{1,2}$ 9.7 Hz, H-1), 4.60 (d, H-6b), 4.78 (d, $J_{1',2'}$ 7.9 Hz, H-1'), 5.13 (dd, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.1 Hz, H-3'), 5.45 (t, $J_{2,3}$ 9.7 Hz, H-2), 5.72 (dd, H-2'), 5.78 (t, $J_{3,4}$ 9.2 Hz, H-3).

Anal. Calc. for $C_{55}H_{50}O_{15}S$: C, 67.2; H, 5.1. Found: C, 67.1; H, 5.2.

*Methyl 4,6-*O*-benzylidene-1-thio-β-*D*-galactopyranoside* (**8**). — This compound was prepared from methyl 1-thio-β-D-galactopyranoside[8] (2.7 g), benzaldehyde (5.4 mL), and formic acid (5.4 mL) as described for the preparation of **5**. Precipitation with ether (150 mL) and recrystallisation of the precipitate from ethanol gave **8** (2.5 g, 57%), m.p. 149–151°, $[\alpha]_{578}$ −34° (*c* 0.5, water). ^{13}C-N.m.r. data: δ 10.8 (MeS), 68.8, 69.1, 69.8, 73.5, 75.6 (C-2,3,4,5,6), 84.8 (C-1), 101.2 (PhCH), 126.3–137.6 (aromatic C).

Anal. Calc. for $C_{14}H_{18}O_5S \cdot 0.5$ EtOH: C, 56.1; H, 6.6; S, 10.0. Found: C, 55.2; H, 6.4; S, 10.2.

Before use in the next step, crystal-bound ethanol was removed by codistillations with acetone.

*Methyl 4,6-*O*-benzylidene-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-1-thio-β-*D*-galactopyranoside* (**9**). — A solution of silver triflate (725 mg) and 2,4,6-trimethylpyridine (380 μL) in dichloromethane–toluene (3:2, 5 mL) was added to a stirred mixture of **8** (420 mg), 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl bromide[7] (700 mg), and molecular sieves in dry

dichloromethane (20 mL) at −15° under dry nitrogen. Stirring was continued for 30 min, aqueous sodium thiosulfate (10%, 20 mL) was added, and the mixture was allowed to attain room temperature. Dichloromethane (2 mL) was added, the mixture was filtered through Celite, and the organic layer was washed with water, dried, and concentrated. Column chromatography of the residue gave a main fraction (806 mg). Rechromatography of this material, using chloroform–ethyl acetate (65:35), gave first a minor fraction (115 mg), which, according to the n.m.r. data, was the (1→2)-β-linked isomer of **9**. The main fraction was amorphous **9** (555 mg, 55%), $[\alpha]_{578}$ −33°. N.m.r. data: ^{13}C, δ 10.4 (MeS), 20.5 and 20.7 (3 C, acetyl Me), 51.5 (C-2′), 61.5 (C-6′), 66.7 (C-4′), 67.0 (C-2), 68.0 (C-3′), 69.1 (C-6), 70.1 (C-5), 70.9 (C-5′), 75.8 (C-4), 81.5 (C-3), 85.0 (C-1), 99.7 (C-1′), 100.9 (PhCH), 123.4–137.9 (aromatic C), 169.7, 170.3, 170.4 (C=O); ^1H, δ 3.44 (m, H-5), 3.65 (dd, $J_{2,3}$ 9.3, $J_{3,4}$ 3.1 Hz, H-3), 3.75 (dt, $J_{1,2}$ 9.3, $J_{2,OH}$ 1.8 Hz, H-2), 3.96 (dd, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.06–4.30 (m, H-1,4,6b and H-5′,6a′,6b′), 4.60 (dd, $J_{1',2'}$ 8.4, $J_{2',3'}$ 11.5 Hz, H-2′), 5.46 (s, PhCH), 5.48 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, H-4′), 5.66 (d, H-1′), 5.82 (dd, H-3′).

Mild, acid hydrolysis (aqueous 80% acetic acid, 90°, 2 h) of **9**, methylation, and then acid hydrolysis (M trifluoroacetic acid, 100°, 16 h) gave 2,4,6-tri-*O*-methylgalactose and 2-deoxy-3,4,6-tri-*O*-methyl-2-methylaminogalactose, identified by g.l.c.–m.s. as their alditol acetates.

Methyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-(1→3)-*O*-(4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-1-thio-β-*D*-glucopyranoside)* (**11**). — A solution of bromine (54 μL) in dry dichloromethane (50 mL) was added, under dry nitrogen, to a stirred mixture of **9** (740 mg) and molecular sieves in dry dichloromethane at 0°. After 20 min, the mixture was filtered and concentrated, and toluene was evaporated twice from the residue. To a stirred solution of the resulting crude bromide **10** in dry dichloromethane at −15° (20 mL) containing molecular sieves and **7** (510 mg) under dry nitrogen was added a solution of silver triflate (270 mg) and 2,4,6-trimethylpyridine (140 μL) in dichloromethane–toluene (3:2, 5 mL). After 30 min, aqueous sodium thiosulfate (10%, 20 mL) was added and the mixture was allowed to attain room temperature. Dichloromethane (20 mL) was added, the mixture was filtered through Celite, and the organic layer was washed with water, dried, and concentrated. Column chromatography of the residue gave amorphous **11** (564 mg, 66%), $[\alpha]_{578}$ +49°. N.m.r. data: ^{13}C, δ 11.5 (MeS), 20.6–20.8 (acetyl Me), 51.8 (C-2‴), 61.5–77.8 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, C-2″,3″,4″,5″,6″, C-3‴,4‴,5‴,6‴), 82.8 (C-1), 99.0, 99.9, 100.7, 101.7 (C-1′, C-1″, C-1‴, PhCH), 123.4–137.9 (aromatic C), 165.1–165.8 (C=O benzoyl), 169.8–170.5 (C=O acetyl); ^1H, δ 4.64 (d, $J_{1,2}$ 10.1 Hz, H-1), 4.76 (d, $J_{1',2'}$ 7.7 Hz, H-1′), 5.56 (d, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.91 (d, $J_{1''',2'''}$ 8.2 Hz, H-1‴).

Methyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-(1→3)-*O*-(4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside)*

(**12**). — Methyl triflate (82 μL) was added, under dry nitrogen, to a stirred mixture of **11** (250 mg), dry methanol (61 μL), and molecular sieves in dry toluene (15 mL). After stirring and heating at 50° for 4 h under nitrogen, triethylamine (210 μL) was added and the mixture was stirred for 20 min at room temperature. The mixture was filtered through Celite, washed with water, dried, and concentrated. Column chromatography of the residue gave amorphous **12** (170 mg, 69%), $[\alpha]_{578}$ +46°. ^{13}C-N.m.r. data: δ 20.6–20.8 (acetyl Me), 51.8 (C-2‴), 57.0 (MeO), 61.6–77.9 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, C-2″,3″,4″,5″,6″, C-3‴,4‴,5‴,6‴), 99.1, 99.9, 100.7, 101.5, 101.7 (C-1, C-1′, C-1″, C-1‴, PhCH), 126.0–137.9 (aromatic C), 165.0–165.8 (C=O benzoyl), 169.8–170.5 (C=O acetyl).

1-Octyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-(1→3)-*O*-(4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside)* (**13**). — Using **11** (229 mg), dry 1-octanol (181 mg), dry toluene (20 mL), methyl triflate (76 μL), and molecular sieves essentially as described for the preparation of **12**, reaction for 8 h at 55° gave crude **13**. Column chromatography gave syrupy **13** (174 mg, 72%), $[\alpha]_{578}$ +41°. ^{13}C-N.m.r. data: δ 14.0 (Me), 21.4, 22.6, 25.8, 29.1, 29.4, 31.7 (CH_2), 51.9 (C-2‴), 61.6–78.0 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, C-2″,3″,4″,5″,6″, C-3‴,4‴,5‴,6‴, CH_2-O), 99.1, 100.0, 100.7, 101.7 (C-1, C-1′, C-1″, C-1‴, PhCH), 123.4–137.8 (aromatic C), 165.1–165.8 (C=O benzoyl), 169.8–170.5 (C=O acetyl).

Methyl O-[*3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-*D*-galactopyranosyl]-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside)* (**14**). — A solution of **12** (220 mg) and hydrazine hydrate (100%, 2 mL) in aqueous 90% ethanol (20 mL) was boiled under reflux for 16 h and then concentrated, and water was evaporated from the residue, which was then partitioned between water and chloroform. The aqueous layer was concentrated, and a solution of the residue in pyridine–acetic anhydride (1:1, 10 mL) was kept at 110° for 2 h, and then concentrated. Column chromatography of the residue gave **14** (116 mg, 65%). Recrystallisation from ether gave material having m.p. 162–164°, $[\alpha]_{578}$ +42°. N.m.r. data: ^{13}C, δ 20.4–21.1 (acetyl Me), 24.9, 27.8 (*N*-acetylacetamido Me), 57.0 (MeO), 59.1 (C-2‴), 61.3–77.7 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, C-2″,3″,4″,5″,6″, C-3‴,4‴,5‴,6‴), 99.9, 100.4, 101.1, 101.5 (C-1, C-1′, C-1″, C-1‴, PhCH), 126.0–137.9 (aromatic C), 169.3–170.4 (C=O acetyl), 174.3 (C=O *N*-acetylacetamido); ^{1}H, δ 1.94, 1.95, 2.02, 2.027, 2.031, 2.04, 2.08, 2.10, 2.15, 2.26, 2.32 (acetyl Me).

Anal. Calc. for $C_{61}H_{77}NO_{31}$: C, 55.4; H, 5.7; N, 1.1. Found: C, 55.3; H, 5.8; N, 1.1.

1-Octyl O-[*3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-*D*-galactopyranosyl]-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside)* (**15**). — Compound **13** (154 mg) was treated essentially as

described for the preparation of **14** (partitioning between chloroform and water was omitted) to give solid **15** (95 mg, 75%), $[\alpha]_{578}$ +35°. ^{13}C-N.m.r. data: δ 14.1 (Me), 20.4–21.1 (acetyl Me), 22.7 (CH_2), 24.9 (*N*-acetylacetamido Me), 25.8 (CH_2), 27.8 (*N*-acetylacetamido Me), 29.2, 29.4, 31.8 (CH_2), 59.1 (C-2‴), 61.4–77.3 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, C-2″,3″,4″,5″,6″, C-3‴,4‴,5‴,6‴, CH_2-O), 100.0, 100.4, 100.8, 101.1 (C-1, C-1′, C-1″, C-1‴, Ph*C*H), 123.2–138.0 (aromatic C), 169.3–170.4 (C=O acetyl), 174.3, 174.6 (C=O *N*-acetylacetamido).

Methyl O-*(2-acetamido-2-deoxy-β-*D-*galactopyranosyl)-(1→3)-*O-*α-*D-*galactopyranosyl-(1→4)-*O-*β-*D-*galactopyranosyl-(1→4)-β-*D-*glucopyranoside* (**16**). — A solution of **14** (115 mg) in methanolic 0.05M sodium methoxide (15 mL) was kept at room temperature for 16 h, neutralised with Dowex 50 (H^+) resin, and concentrated. A solution of the residue in aqueous 80% acetic acid (4 mL) was hydrogenated over Pd/C (10%, 200 mg) at 400 kPa for 48 h, then filtered, and concentrated. The residue was partitioned between chloroform and water, the aqueous layer was concentrated, and the residue was eluted from a column of Biogel P-2 with water to give amorphous **16** (31 mg, 50%), $[\alpha]_{578}$ +72° (*c* 0.4, water). N.m.r. data (D_2O; Me_2CO, δ_H = 2.22; 1,4-dioxane, δ_C = 67.4): ^{13}C, δ 23.7 *N*-acetyl Me), 54.1 (C-2‴), 58.6 (MeO), 61.6 (C-6), 61.8 (C-6′), 61.9 (C-6″), 62.5 (C-6‴), 69.0 (C-4‴), 69.3 (C-2″), 70.4 (C-4″), 71.8 (C-3‴), 72.3 (C-5″), 72.4 (C-2′), 73.7 (C-3′), 74.3 (C-2), 76.0 (C-3), 76.2 (C-5), 76.4 (C-5‴), 76.8 (C-5′), 78.8 (C-4′), 80.2 (C-4), 80.4 (C-3″), 101.9 (C-1″), 104.5 (C-1), 104.6 (C-1′), 104.8 (C-1‴); ^{1}H, δ 3.28 (dd, $J_{1,2}$ 7.5, $J_{2,3}$ 9.0 Hz, H-2), 3.55 (s, MeO), 3.55 (m, H-5), 3.55 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2′), 3.65 (m, H-3,4,5‴), 3.67 (dd, $J_{5'',6b''}$ 6.8, $J_{6a'',6b''}$ 12.0 Hz, H-6b″), 3.70 (dd, $J_{5'',6a''}$ 6.0 Hz, H-6a″), 3.72 (dd, $J_{3',4'}$ 3.4 Hz, H-3′), 3.72 (dd, $J_{2''',3'''}$ 10.0, $J_{3''',4'''}$ 3.5 Hz, H-3‴), 3.72 (dd, $J_{5''',6b'''}$ 4.5, $J_{6a''',6b'''}$ 11.0 Hz, H-6b‴), 3.76 (m, H-5′), 3.78 (dd, $J_{5''',6a'''}$ 7.0 Hz, H-6a‴), 3.80 (dd, $J_{5,6b}$ 5.6, $J_{6a,6b}$ 12.0 Hz, H-6b), 3.88 (dd, $J_{5',6a'}$ 7.7, $J_{6a'6b'}$ 11.0 Hz, H-6a′), 3.90 (dd, $J_{1'',2''}$ 3.6, $J_{2'',3''}$ 9.8 Hz, H-2″), 3.90 (dd, $J_{1''',2'''}$ 8.2 Hz, H-2‴), 3.92 (dd, $J_{3'',4''}$ 3.5 Hz, H-3″), 3.92 (dd, $J_{4''',5'''}$ 0.8 Hz, H-4‴), 3.98 (dd, $J_{5,6a}$ 2.1 Hz, H-6a), 4.02 (dd, $J_{4',5'}$ 0.8 Hz, H-4′), 4.22 (dd, $J_{3'',4''}$ 3.5, $J_{4'',5''}$ 0.8 Hz, H-4″), 4.34 (m, H-5″), 4.38 (d, H-1), 4.49 (d, H-1′), 4.60 (d, H-1‴), and 4.92 (d, H-1″).

Methylation followed by hydrolysis with strong acid gave a mixture of 2-deoxy-3,4,6-tri-*O*-methyl-2-*N*-methylacetamidohexose, 2,4,6-tri-*O*-methylhexose, and two 2,3,6-tri-*O*-methylhexoses, all identified as their alditol acetates by g.l.c.–m.s.

1-Octyl O-*(2-acetamido-2-deoxy-β-*D-*galactopyranosyl)-(1→3)-*O-*α-*D-*galactopyranosyl-(1→4)-*O-*β-*D-*galactopyranosyl-(1→4)-β-*D-*glucopyranoside* (**17**). — Treatment of **15** (95 mg), essentially as described for the preparation of **16**, gave amorphous **17** (41 mg, 75%), $[\alpha]_{578}$ +68° (*c* 0.4, water). ^{13}C-N.m.r. data (D_2O, internal 1,4-dioxane δ 67.4): δ 14.2 (Me), 22.8 (CH_2), 23.1 (*N*-acetyl Me), 25.9, 29.2, 29.3, 29.6, 31.9 (CH_2), 53.5 (C-2‴), 61.0, 61.2, 61.3, 61.8 (C-6,6′,6″,6‴), 68.5–79.7, C-2,3,4,5, C-2′,3′,4′,5′, C-2″,3″,4″,5″, C-3‴,4‴,5‴, CH_2O), 101.3, 102.8, 104.0, 104.1 (C-1, C-1′, C-1″, C-1‴), and 176.0 (C=O *N*-acetyl).

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