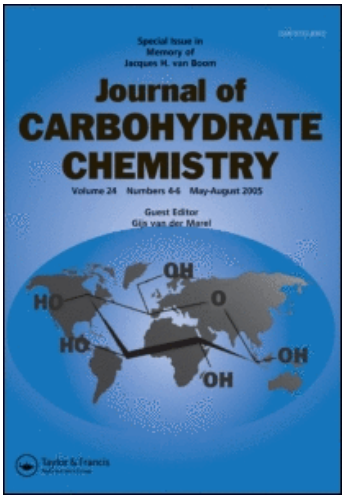

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Synthesis of Methyl *O*-(2-Acetamido-2-Deoxy-α-D-Glucopyranosyl)-(1⟶2)-*O*-α-D-Glucopyranosyl-(2)-α-D-Galactopyranoside and of Methyl *O*-α-D-Glucopyranosyl-(2)-*O*-α-D-Galactopyranosyl-(1⟶3)-O-[α-D-Galactopyranosyl-(1⟶6)]-α-D-Glucopyranoside, Corresponding to Parts of the Outer Core of the *Salmonella* Cell Wall Lipopolysaccharide

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SYNTHESIS OF METHYL *O*-(2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSYL)-(1→2)-*O*-α-D-GLUCOPYRANOSYL-(1→2)-α-D-GALACTOPYRANOSIDE AND OF METHYL *O*-α-D-GLUCOPYRANOSYL-(1→2)-*O*-α-D-GALACTOPYRANOSYL-(1→3)-*O*-[α-D-GALACTOPYRANOSYL-(1→6)]-α-D-GLUCOPYRANOSIDE, CORRESPONDING TO PARTS OF THE OUTER CORE OF THE *SALMONELLA* CELL WALL LIPOPOLYSACCHARIDE

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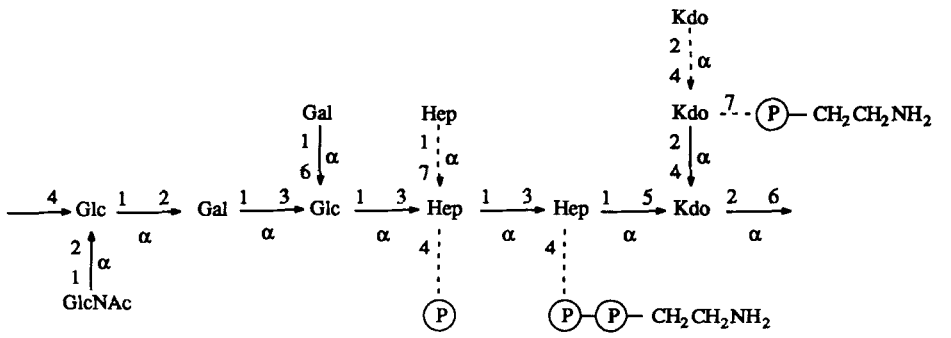
ABSTRACT

In the syntheses of the title oligosaccharides, the glycosyl donors had non-participating groups, either benzyl, *p*-methoxybenzyl or azidodeoxy, in the 2-positions. Glycosylations with glycosyl bromides were performed using halide assistance or silver triflate promotion. Glycosidations using thioglycosides were performed with dimethyl(methylthio)sulfonium triflate as promoter.

INTRODUCTION

The title compounds were required in continued immunological studies of the core structure[1] of the *Salmonella* LPS, shown in **Figure 1**, in particular for studies of monoclonal antibodies raised against R-mutants. These studies will be presented elsewhere.



Hep = L-*glycero*-D-*manno*-heptopyranosyl
Kdo = 3-deoxy-D-*manno*-2-octulosonic acid

Figure 1

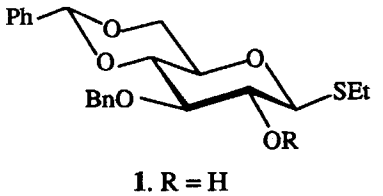
RESULTS AND DISCUSSION

Copper (II) chloride promoted partial benzylation[2,3] of ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[4] gave the corresponding 3-*O*-benzyl ether **1** (57% with recovery of 36% starting material). Temporary protection of the 2-position was then obtained by *p*-methoxybenzylation to give **2** (95%). The latter was used to glycosylate methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-α-D-galactopyranoside.[5] Promotion by dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[6] produced the α-(1→2)-linked disaccharide **3** (84%). Treatment of **3** with cerium(IV) ammonium nitrate (CAN)[7] removed the single *p*-methoxybenzyl group, producing the glycosyl acceptor **4** (74%).

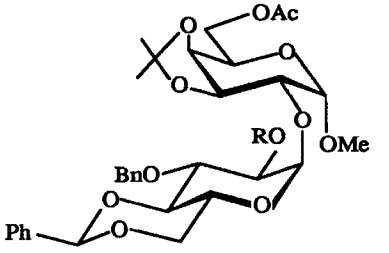
Silver triflate[8,9] promoted glycosylation of **4** with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide[10] gave the trisaccharide **5** (92%). This was taken through routine deprotection to finally yield methyl *O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→2)-*O*- α - D-glucopyranosyl - (1→2) - *O*-α-D-galactopyranoside **6**. The steps were catalytic hydrogenolysis followed by *N*-acetylation (84%), de-isopropylidenation by mild acidic treatment (91%) and finally de-*O*-acetylation (89%).

The tetrasaccharide glycoside **14** was then made as follows: methyl 2,4-di-*O*-benzyl-α-D-glucopyranoside[11] was glycosylated in the 6-position by treatment with 1.1 mole equivalents of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide[12] (made by bromine treatment of the corresponding ethyl thioglycoside[13] and used immediately) in the presence of tetraethylammonium bromide[14] to give **7** (56%)

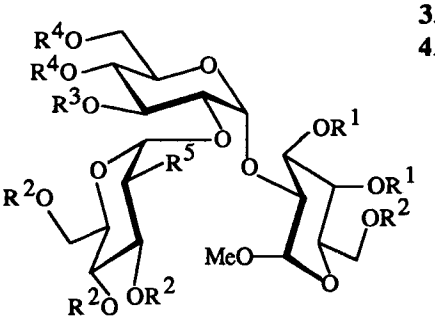
with a free 3-OH group. Next, methyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside[15] was 4,4′-dimethoxytritylated in the 6-position and the product (**8**, 97%) was *p*-methoxybenzylated at *O*-2 (**9**, 96%). Treatment of **9** with formic acid to give the 6-OH compound **10** (76%), was followed by acetylation to give the glycosyl donor (**11**, 98%). Glycosylation of **7** with **11**, promoted by DMTST then gave the trisaccharide **12** (71%), from which the *p*-methoxybenzyl group was removed by treatment with CAN. The trisaccharide (82%) was glycosylated with **2** to give the fully protected tetrasaccharide **13** (85%). This was taken through the requisite deprotection steps. Thus, the *p*-methoxybenzyl group was removed by CAN (73%). This treatment was then followed by de-*O*-acetylation, hydrolytic de-isopropylidenation and catalytic hydrogenolysis to give the final product **14** (73%). An alternative, less satisfactory, route to the trisaccharide glycoside **6**, and also a low-yielding, unsatisfactory, route to the pentasaccharide glycoside methyl *O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl) - (1→2) - *O* - α - D-glucopyranosyl-(1→2)-*O*-α - D-galactopyranosyl - (1→3) - *O* - [α-D-galactopyranosyl- (1→6)] -α-D-glucopyranoside have previously been described.[16]



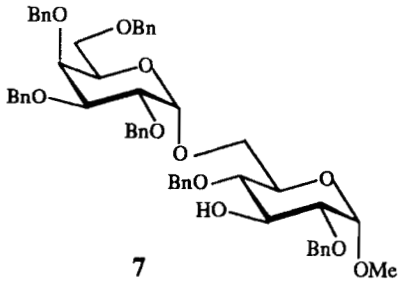
1. R = H
2. R = pMBn



3. R = pMBn
4. R = H



5. R^1 = isopropylidene, R^2 = Ac, R^3 = Bn, R^4 = benzylidene, R^5 = N_3
6. $R^1 = R^2 = R^3 = R^4$ = H, R^5 = NHAc



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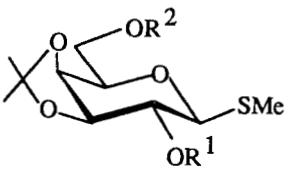
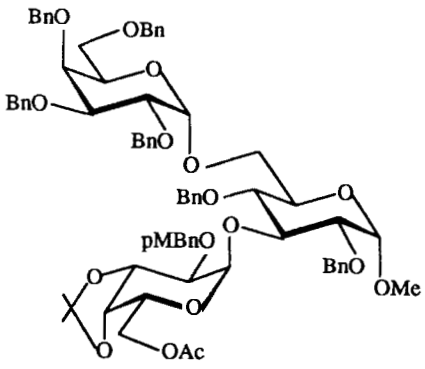


8. $R^1 = H$, $R^2 = DMTr$
9. $R^1 = pMBn$, $R^2 = DMTr$
10. $R^1 = pMBn$, $R^2 = H$
11. $R^1 = pMBn$, $R^2 = Ac$



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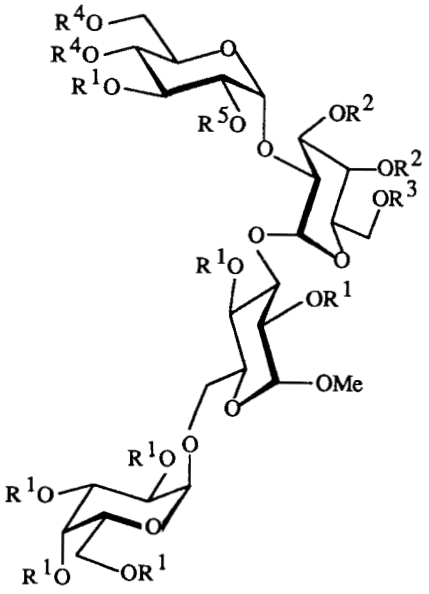

13. $R^1 = Bn$, $R^2 = isopropylidene$, $R^3 = Ac$, $R^4 = benzylidene$, $R^5 = pMBn$
14. $R^1 = R^2 = R^3 = R^4 = R^5 = H$

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed under reduced pressure at < 40 °C (bath). Optical rotations were recorded for 0.2-1.0% solutions at room temperature (22-25 °C) using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25 °C for solutions in $CDCl_3$, using a JEOL GX-270 instrument, and chemical shifts are given in ppm relative to internal tetramethylsilane, unless otherwise stated. All ^1H assignments were based on 2D experiments. NMR spectra recorded for all new compounds were in agreement with the postulated structures and only selected data are reported. TLC was performed on Silica Gel F_{254} HPTLC (Merck) with detection by UV and/or by charring with sulfuric acid. Column chromatography was performed on silica gel (Matrex Silica Si 60A, 35-70μ, Amicon). Organic solutions were dried over magnesium sulfate. Molecular sieves (4Å, Fluka) were desiccated at 300 °C overnight. Elemental analyses were not obtained for some amorphous compounds. These were purified by column chromatography and the purity was ascertained by HPTLC (in two different systems) and by NMR spectroscopy.

Ethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (1). Ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[4] (1.97 g, 6.3 mmol) was stirred with sodium hydride (380 mg, 80%, 12.7 mmol) in dry *N,N*-dimethylformamide (15 mL) at room temperature. When the evolution of hydrogen had ceased, copper (II) chloride (850 mg, 6.3 mmol) was added and the reaction mixture was stirred for another 10 minutes before benzyl bromide (820 μL, 6.9 mmol) was added. After 2 h the reaction mixture was purified on a short silica column (light petroleum-ethyl acetate, 3:2). Crystallization from ethyl acetate-isooctane gave **1** (1.44 g, 3.6 mmol, 57%), mp 136-137 °C, $[\alpha]_{578}$ -53° (*c* 0.5, chloroform), (710 mg, 36% of the starting material was recovered.) NMR data: ^{13}C, δ 15.3 (Me ethyl), 24.6 (CH_2S), 68.7-81.6 (C ring), 86.6 (C-1), 101.2 (PhCH), 126.0-138.4 (aromatic C); ^1H, δ 3.49 (m, H-5), 3.58 (m, $J_{1,2}$=9.5 Hz, $J_{2,3}$=10.4 Hz, $J_{OH,2}$=2.4 Hz, H-2), 3.69 (dd, $J_{3,4}$=9.0 Hz, $J_{4,5}$=8.4 Hz, H-4), 3.72 (dd, $J_{2,3}$=10.4 Hz, H-3), 3.79 (H-6a), 4.36 (dd, $J_{5,6}$=4.9 Hz, $J_{6a,6b}$=10.4 Hz, H-6b), 4.47 (d, H-1). H-2 moved to 4.97 ppm after treatment with trichloroacetyl isocyanate.

Anal. Calcd for $C_{22}H_{26}O_5S$: C, 65.6; H, 6.5. Found: C, 65.8; H, 6.6.

Ethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (2). A solution of **1** (1.02 g, 2.5 mmol) and *p*-methoxybenzyl chloride (500 μL, 3.7 mmol) in dry *N,N*-dimethylformamide (20 mL) was added dropwise to 60% sodium hydride (200 mg, 5 mmol) at 0 °C under nitrogen. The reaction mixture was stirred overnight at room temperature. Ethanol (10 mL) was added and the reaction mixture was partitioned between toluene and aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried and concentrated. Column chromatography (light petroleum-ethyl acetate, 5:2) gave **2** (1.25 g, 2.4 mmol, 95%). Crystallization from ethyl acetate-light petroleum gave material having mp 116-118 °C, $[\alpha]_{578}$ -24° (*c* 0.5, chloroform). NMR data: ^{13}C, δ 15.3 (Me ethyl), 25.3 (CH_2S), 55.4 (*p*-methoxybenzyl), 68.9-83.0 (C ring), 86.0 (C-1), 101.2 (PhCH), 113.9 (aromatic C *p*-methoxybenzyl), 126.1-138.6 (aromatic C), 159.5 (aromatic C *p*-methoxybenzyl).

Anal. Calcd for $C_{30}H_{34}O_6S$: C, 68.9; H, 6.6. Found: C, 68.8; H, 6.6.

Methyl 6-*O*-Acetyl-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl - α - D - glucopyranosyl) - 3,4 - *O* - isopropylidene-α-D-galactopyranoside (3). A solution of DMTST (600 mg, 2.3 mmol) in dry dichloromethane (1 mL) was added to a stirred mixture of **2** (810 mg, 1.6 mmol), methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-α-D-galactopyranoside[5] (340 mg, 1.2 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (500 mg, 2.4 mmol) and molecular sieves in dichloromethane-diethyl ether (1:1, 20 mL) at room temperature under nitrogen. The mixture was stirred for 2 h at room temperature and then triethylamine (1 mL) was added. After 30 min the mixture was filtered through Celite, washed with sodium hydrogencarbonate, dried and concentrated. Column chromatography (toluene-ethyl acetate, 1:1) gave **3** (765 mg, 1.04 mmol, 84%) having $[\alpha]_{578}$ +57° (*c* 0.5, chloroform). NMR data: ^{13}C, δ 21.0 (Me acetyl), 26.4, 28.4 (Me isopropylidene), 55.4, 55.5 (MeO, *p*-methoxybenzyl), 62.4-82.5 (C ring), 96.4, 97.0 (2 C-1, $^1J_{C,H}$=169 Hz and 168 Hz respectively), 101.4 (PhCH), 109.6 (isopropylidene), 113.9 (aromatic C *p*-methoxybenzyl), 126.2-139.0 (aromatic C), 159.4 (aromatic C *p*-methoxybenzyl), 170.9, (C=O acetyl); ^1H, δ 3.54 (dd, $J_{1,2}$=3.9 Hz, $J_{2,3}$=9.2 Hz, H-2′), 3.66 (dd, $J_{3,4}$=9.2 Hz, $J_{4,5}$=9.8 Hz, H-4′), 3.79 (dd, $J_{1,2}$=3.4 Hz, $J_{2,3}$=8.1 Hz, H-2), 4.13 (dd, H-3′), 4.19 (dd, $J_{3,4}$=5.5 Hz, H-4), 4.23 (m, H-5′), 4.37 (dd, H-3), 4.78 (d, H-1), 4.81 (d, H-1′).

Anal. Calcd for $C_{40}H_{48}O_{13}$: C, 65.2; H, 6.6. Found: C, 65.5; H, 6.7.

Methyl 6-*O*-Acetyl-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3,4-*O*-isopropylidene-α-D-galactopyranoside (4). CAN (450 mg, 820 μmol) in acetonitrile (5 mL) was added to a stirred solution of **3** (393 mg, 530 μmol) in dichloromethane saturated with water (25 mL) at 0 °C. After 16 h at room temperature the reaction mixture was partitioned between dichloromethane and aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried and concentrated. Column chromatography (light petroleum-ethyl acetate, 1:1) gave **4** (243 mg, 390 μmol, 74%) having $[\alpha]_{578}$ +136° (*c* 0.5, chloroform). NMR data: ^{13}C, δ 21.0 (Me acetyl), 26.4, 28.1 (Me isopropylidene), 55.7 (MeO), 62.9-81.8 (C ring), 96.9, 98.2 (2 C-1), 101.5 (PhCH), 109.9 (isopropylidene), 125.4-138.8 (aromatic C), 170.9, (C=O acetyl).

Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→2)-*O*-(3-*O* - benzyl - 4,6- *O* - benzylidene - α -D-glucopyranosyl)- (1→2) - 6 - *O* -acetyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (5). A dry solution of silver triflate (40 mg, 160 μmol) in toluene (2 mL) was added to a stirred mixture of **4** (60 mg, 97 μmol), 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide[10] (65 mg, 160 μmol), DTBMP (55 mg, 270 μmol) and molecular sieves in dry dichloromethane (10 mL) at -30 °C under nitrogen. When TLC indicated complete reaction, aqueous sodium thiosulfate (10%, 25 mL) was added, and the reaction mixture was allowed to attain room temperature. The mixture was filtered through Celite, and the organic layer was washed with water, dried and concentrated. Column chromatography (toluene-ethyl acetate, 4:1) of the residue gave **5** (84 mg, 89 μmol, 92%), $[\alpha]_{578}$ +138° (*c* 0.2, chloroform). NMR data: ^{13}C, δ 20.7, 20.8, 20.8, 21.0 (4 Me acetyl), 26.4, 28.2 (Me isopropylidene), 55.5 (MeO), 60.9-82.6 (C ring), 94.3, 95.0, 97.2 (3 C-1, $^1J_{C,H}$=167 Hz, 174 Hz and 172 Hz respectively), 101.5 (PhCH), 109.7 (isopropylidene), 125.4-138.3 (aromatic C), 169.7, 170.0, 170.6, 171.0 (4 C=O acetyl); ^1H (*inter alia*) δ 3.23 (dd, $J_{1,2}$=3.6 Hz, $J_{2,3}$=10.5 Hz, H-2″), 3.65 (dd, $J_{3,4}$=9.4 Hz, $J_{4,5}$=9.5 Hz, H-4′), 3.82 (dd, $J_{1,2}$=3.8 Hz, $J_{2,3}$=9.4 Hz, H-2′), 3.88 (dd, $J_{1,2}$=3.4 Hz, $J_{2,3}$=7.4 Hz, H-2), 4.15 (dd, H-3′), 4.22 (dd, $J_{3,4}$=5.7 Hz, H-4), 4.27 (m, H-5″), 4.39 (dd, H-3), 4.84 (d, H-1), 5.03 (dd, $J_{3,4}$=9.7, $J_{4,5}$=10.4 Hz, H-4″), 5.16 (d, H-1″), 5.18 (d, H-1′), 5.54 (dd, H-3″).

Anal. Calcd for $C_{44}H_{55}N_3O_{19}$: C, 56.8; H, 6.0; N, 4.5. Found: C, 57.1; H, 5.9; N, 4.5.

Methyl *O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-α-D-galactopyranoside (6). A solution of **5** (65 mg, 69 μmol) in acetic

acid (10 mL) was hydrogenolyzed over Pd/C at atmospheric pressure overnight, then filtered and concentrated. The residue was dissolved in pyridine-acetic anhydride (5 mL, 1:1). When TLC indicated complete reaction the reaction mixture was concentrated. Column chromatography (chloroform-acetone, 4:1) of the residue gave methyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-6- *O*-acetyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (51 mg, 58 μmol, 84%). NMR data: ^{13}C, δ 20.7-21.1 (8 Me acetyl), 23.3 (Me *N*-acetyl), 26.3, 28.3 (Me isopropylidene), 52.5, 55.5 (MeO, C´´-2), 62.4-75.6 (C ring), 93.4, 95.6, 97.2 (3 C-1), 110.0 (isopropylidene), 169.6-170.9 (9 C=O acetyl, *N*-acetyl). This compound (45 mg, 51 μmol) was dissolved in 80% aqueous acetic acid, heated at 100 °C for 1h, cooled and concentrated. The residue was purified on a short silica gel column (chloroform-acetone, 4:1) to give methyl *O*-(2-acetamido-3,4,6-tri-*O* - acetyl - 2 - deoxy - α - D - glucopyranosyl) -(1→2) - *O* - (3,4,6 - tri - *O* - acetyl -α-D-glucopyranosyl)-(1→2)-6-*O*-acetyl-α- D- galactopyranoside (39 mg, 46 μmol, 91%). NMR data: ^{13}C, δ 90.6, 93.6, 96.1 (3 C-1). Methanolic sodium methoxide (0.5 mL, 0.5M) was added to a solution of the latter compound (21 mg, 23 μmol) in dichloromethane (3 mL). The reaction mixture was stirred at room temperature for 2 h, then neutralized with Dowex 50 (H^+) resin, and concentrated. The residue was purified on a Biogel P-2 column, using water containing 1% 1-butanol as eluent, giving compound **6** (12 mg, 22 μmol, 89%) having $[\alpha]_{578}$ +158° (*c* 0.5, water). NMR data (D_2O; Me_2CO, δ_H=2.225; external TMS δ_C=0): ^{13}C, δ 23.0 (Me *N*-acetyl), 54.1, 55.3 (MeO, C´´-2), 61.3, 61.4, 62.0, 69.1, 70.0, 70.1, 70.6, 71.1, 71.3, 72.1, 72.1, 72.4, 72.9, 73.5 (C ring), 91.0, 94.0, 96.5 (3 C-1), 174.9 (C=O *N*-acetyl); ^1H (*inter alia*) δ 3.48 (dd, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.1 Hz, H-4´), 3.52 (dd, $J_{3,4}$=9.0 Hz, $J_{4,5}$=9.9 Hz, H-4´´), 3.71 (dd, $J_{1,2}$=3.9 Hz, $J_{2,3}$=9.9 Hz, H-2´), 3.82 (dd, $J_{2,3}$=10.5 Hz, H-3´´), 3.89 (dd, $J_{2,3}$=9.9 Hz, H-3´), 3.93 (dd, $J_{1,2}$=2.8 Hz, H-2), 3.99 (H-3), 4.03 (dd, $J_{1,2}$=3.5 Hz, H-2´´), 5.02 (d, H-1´´), 5.10 (d, H-1), 5.35 (d, H-1´).

Methyl 2,4-di-*O*-Benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranoside (**7**). Bromine (115 μL, 2.2 mmol) was added to ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[13] (1.3 g, 2.2 mmol) in dichloromethane at 0 °C. After 10 minutes the solution was concentrated and the residual bromine was coevaporated with toluene. The residue, in dichloromethane (10 mL), was added to a solution of methyl 2,4-di-*O*-benzyl-α-D-glucopyranoside[11] (750 mg, 2.0 mmol) and

tetraethylammonium bromide (470 mg, 2.2 mmol) in *N,N*-dimethylformamide (20 mL) containing molecular sieves at 0 °C. The mixture was stirred overnight, filtered through a layer of Celite, diluted with toluene, washed with sodium hydrogencarbonate, dried and concentrated. Column chromatography (light petroleum-ethyl acetate, 5:2), followed by crystallization from ethyl acetate-isooctane gave **7** (1.0 g, 1.2 mmol 56%) mp 50-53 °C, $[\alpha]_{578}$ +74° (*c* 0.5, chloroform). NMR data: ^{13}C, δ 55.1 (OMe), 66.5-79.9 (C ring), 97.3 (C-1, $^1J_{C,H}$=170 Hz), 97.9 (C-1′, $^1J_{C,H}$=171 Hz), 127.5-139.0 (aromatic C); ^1H, δ 3.22 (dd, $J_{1,2}$=3.8, $J_{2,3}$=9.6 Hz, H-2), 3.50 (dd, $J_{3,4}$=8.7, $J_{4,5}$=9.8 Hz, H-4), 3.71 (m, H-5), 3.93 (dd, $J_{2,3}$=9.7 Hz, H-3′), 4.03 (dd, H-3), 4.04 (dd, $J_{1,2}$=3.7, $J_{2,3}$=9.7 Hz, H-2′), 4.53 (d, H-1), 5.01 (d, H-1′). H-3 moved to 5.47 ppm after acetylation.

Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.6; H, 6.7. Found: C, 73.4; H, 6.8.

Methyl 6-*O*-(4,4′-Dimethoxytrityl)-3,4-*O*-isopropylidene-1-thio-β-D-galacto-pyranoside (8). 4,4′-Dimethoxytrityl chloride (3.0 g, 8.9 mmol) was added to a solution of methyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside[15] (2.05 g, 8.2 mmol) in pyridine (100 mL). The reaction mixture was stirred at 70 °C. After 2 h the reaction mixture was cooled and concentrated. Column chromatography (light petroleum-ethyl acetate, 5:2, containing 1% pyridine) of the residue gave **8** (4.38 g, 7.9 mmol, 97%). Precipitation from light petroleum gave material having mp 70-72 °C, $[\alpha]_{578}$ +6° (*c* 1, chloroform). NMR data (pyridine-d_5, δ_C=135.5): ^{13}C, δ 12.1 (SMe), 26.6, 28.4 (Me, isopropylidene), 55.1 (dimethoxytrityl), 63.8-81.1 (C ring), 86.7 (C-1), 109.6 (isopropylidene), 113.6-159.0 (aromatic C).

Anal. Calcd for $C_{31}H_{36}O_7S$: C, 67.4; H, 6.6. Found: C, 67.5; H, 6.6.

Methyl 6-*O*-(4,4′-Dimethoxytrityl)-3,4-*O*-isopropylidene-2-*O*-*p*-methoxy-benzyl-1-thio-β-D-galactopyranoside (9). A solution of **8** (2.19 g, 3.9 mmol) and *p*-methoxybenzyl chloride (600 μL, 4.4 mmol) in dry *N,N*-dimethylformamide (20 mL) was added dropwise to 60% sodium hydride (240 mg, 6.0 mmol) at 0 °C under nitrogen. The reaction mixture was stirred overnight at room temperature. Ethanol (5 mL) was added and the reaction mixture was partitioned between toluene and aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried and concentrated. Column chromatography (light petroleum-ethyl acetate, 5:2) gave **9** (2.54 g, 3.8 mmol, 96 %). Precipitation from light petroleum gave material having mp 55-57 °C, $[\alpha]_{578}$ -15° (*c* 1, chloroform). NMR data: ^{13}C, δ 12.8 (SMe), 26.5,

28.1 (Me isopropylidene), 55.3, 55.4 (*p*-methoxybenzyl, dimethoxytrityl), 62.7-79.9 (C ring), 84.6 (C-1), 109.9 (isopropylidene), 113.2-159.4 (aromatic C).

Anal. Calcd for $C_{39}H_{44}O_8S$: C, 69.6; H, 6.6. Found: C, 69.8; H, 6.7.

Methyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-1-thio-β-D-galacto-pyranoside (10). Formic acid (1 mL) was added to a solution of **9** (1.18 g, 1.7 mmol) in dichloromethane (10 mL). The reaction mixture was stirred for 15 minutes, concentrated, coevaporated with toluene and purified by column chromatography (toluene-ethyl acetate, 1:1), to give **10** (497 mg, 1.3 mmol, 76%). Crystallization from ethyl acetate-isooctane gave material having mp 83-85 °C, $[\alpha]_{578}$ +3° (*c* 1, chloroform). NMR data: ^{13}C,δ 12.9 (SMe), 26.4, 27.9 (Me isopropylidene), 55.3 (*p*-methoxybenzyl), 62.6 (C-6), 74.1 (C-4), 76.8 (C-5), 78.3 (C-2), 79.8(C-3), 84.6 (C-1), 110.2 (isopropylidene), 113.8, 130.0, 159.4 (aromatic C); ^{1}H, δ 3.43 (dd, $J_{1,2}$=9.6 Hz $J_{2,3}$=6.2 Hz, H-2), 3.80 (H-5, H-6a), 3.90 (H-6b), 4.18 (dd, $J_{4,5}$=1.9 Hz, H-4), 4.23 (dd, $J_{2,3}$=6.9 Hz, $J_{3,4}$=5.6 Hz, H-3), 4.30 (d, H-1).

Anal. Calcd for $C_{18}H_{26}O_6S$: C, 58.4; H, 7.1. Found: C, 58.5; H, 7.1.

Methyl 6-*O*-Acetyl-3,4-*O*-isopropylidene-2-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (11). Acetyl chloride (200 μL, 2.8 mmol) was added to a solution of **10** (706 mg, 1.9 mmol) and pyridine (1 mL) in dichloromethane (5 mL) at 0 °C. The reaction mixture was stirred at room temperature and after 1 h the mixture was washed with aqueous sodium hydrogencarbonate and water, dried and concentrated. Column chromatography (light petroleum-ethyl acetate, 5:2) of the residue gave **11** (769 mg, 1.9 mmol 98%). Crystallization from ethyl acetate-isooctane gave material having mp 125-126 °C, $[\alpha]_{578}$ +11° (*c* 1, chloroform). NMR data: ^{13}C, δ 12.6 (SMe), 20.7 (Me acetyl), 26.2, 27.7 (Me isopropylidene), 55.1 (*p*-methoxybenzyl), 63.6-79.4 (C ring), 84.1 (C-1), 110.0 (isopropylidene), 113.6, 129.9, 159.3 (aromatic C), 170.6 (C=O); ^{1}H, δ 3.45 (dd, $J_{1,2}$=9.5 Hz $J_{2,3}$=6.0 Hz, H-2), 3.95 (m, H-5), 4.18 (dd, $J_{3,4}$=5.7 Hz $J_{4,5}$=2.0 Hz, H-4), 4.23 (t, J=6.1 Hz, H-3), 4.29 (d, H-1), 4.32 (H-6).

Anal. Calcd for $C_{20}H_{28}O_7S$: C, 58.2; H, 6.8. Found: C, 58.3; H, 6.8.

Methyl 2,4-Di-*O*-benzyl-3-*O*-(6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galacto-pyranosyl)-α-D-glucopyranoside (12). A solution of DMTST (550 mg, 2.1 mmol) in dry dichloromethane (5 mL) was added to a stirred mixture of **7** (626 mg, 700 μmol), **11** (600 mg, 1.4 mmol), DTBMP (550 mg, 2.6 mmol) and molecular sieves in

dichloromethane-diethyl ether (1:1, 20 mL) at room temperature as described for compound **3**. Column chromatography (toluene-ethyl acetate, 1:1) gave **12** (624 mg, 490 μmol, 71%) having $[\alpha]_{578}$ +90° (*c* 0.5, chloroform). NMR data: ^{13}C, δ 21.0 (Me acetyl), 26.4, 28.1 (Me isopropylidene), 54.9, 55.2 (MeO, *p*-methoxybenzyl), 63.7-79.6 (C ring), 97.1, 97.6, 98.2 (3 C-1, $^1J_{C,H}$=170 Hz, 171 Hz and 171 Hz respectively), 109.1 (isopropylidene), 113.6 (aromatic C *p*-methoxybenzyl), 127.0-139.0 (aromatic C), 159.1 (aromatic C *p*-methoxybenzyl), 171.1 (C=O acetyl); ^1H (*inter alia*) δ 3.34 (dd, $J_{1,2}$=3.6 Hz, $J_{2,3}$=9.8 Hz, H-2), 3.45 (dd, $J_{1,2}$=3.7 Hz, $J_{2,3}$=8.2 Hz, H-2′), 3.65 (dd, $J_{3,4}$=8.2 Hz, H-4), 3.76 (dd, $J_{3,4}$=5.2 Hz, $J_{4,5}$=3.0 Hz, H-4′), 3.77 (m, H-5), 3.88 (dd, $J_{2,3}$=9.6 Hz, H-3′′), 4.02 (dd, $J_{1,2}$=3.5 Hz, H-2′′), 4.25 (dd, $J_{2,3}$=9.8 Hz, H-3), 4.28 (dd, H-3′), 4.48 (d, H-1), 4.78 (m, H-5′), 4.95 (d, H-1′′), 5.50 (d, H-1′).

Anal. Calcd for $C_{74}H_{84}O_{18}$: C, 70.5; H, 6.7. Found: C, 69.4; H, 6.7.

Methyl *O*-(3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-(1→2)-*O*-(6-*O*- acetyl - 3,4 - *O* - isopropylidene - α - D-galactopyranosyl) - (1→3) - *O* - 2,4-di - *O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranoside (13). CAN (110 mg, 200 μmol) in acetonitrile (2 mL) was added to a stirred solution of **12** (130 mg, 100 μmol) in dichloromethane saturated with water (15 mL) as described for compound **4**. Column chromatography (toluene-ethyl acetate, 3:2) gave methyl 2,4-di-*O*-benzyl-3-*O*-(6-*O*-acetyl-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranoside (96 mg, 84 μmol, 82%). NMR data: ^{13}C, δ 20.9 (Me acetyl), 26.1, 27.8 (Me isopropylidene), 55.0 (MeO), 63.6-79.0 (C ring), 97.4, 97.7, 97.9 (3 C-1), 109.7 (isopropylidene), 127.3-139.1 (aromatic C), 171.1 (C=O acetyl). A solution of DMTST (17 mg, 66 μmol) in dry dichloromethane (0.5 mL) was added to a stirred mixture of the latter compound (39 mg, 34 μmol), **2** (40 mg, 76 μmol), DTBMP (20 mg, 9.7 μmol) and 4Å molecular sieves in dichloromethane-diethyl ether (1:1, 5 mL) as described for compound **3**. Column chromatography (toluene-ethyl acetate, 1:1) gave **13** (47 mg, 29 μmol, 85%) having $[\alpha]_{578}$ +71° (*c* 0.5, chloroform). NMR data: ^{13}C, δ 21.0 (Me acetyl), 26.4, 28.4 (Me isopropylidene), 55.0, 55.3 (MeO, *p*-methoxybenzyl), 62.7-82.5 (C ring), 96.0, 97.4, 97.8, 97.8 (4 C-1), 101.4 (PhCH), 109.3 (isopropylidene), 113.9 (aromatic C *p*-methoxybenzyl), 126.2-139.0 (aromatic C), 159.3 (aromatic C *p*-methoxybenzyl), 171.1 (C=O acetyl); ^1H (*inter alia*) δ 3.41 (dd, $J_{1,2}$=3.4 Hz, $J_{2,3}$=9.5 Hz, H-2), 3.46 (dd, $J_{1,2}$=3.8 Hz, $J_{2,3}$=9.5 Hz, H-2′′′), 3.47 (dd, $J_{3,4}$=8.8, $J_{4,5}$=10.8 Hz, H-4), 3.67

(dd, $J_{1,2}$=3.1 Hz, $J_{2,3}$=8.1 Hz, H-2′), 3.88 (dd, H-3′′), 3.91 (dd, $J_{3,4}$=5.6, H-4′), 4.01 (dd, $J_{3,4}$=9.4 Hz, H-3′′′), 4.03 (dd, $J_{1,2}$=3.4 Hz, H-2′′), 4.28 (dd, H-3′), 4.43 (dd, H-3), 4.53 (d, H-1), 4.73 (m, H-5′), 4.79 (d, H-1′′′), 4.89 (d, H-1′′), 5.60 (d, H-1′).

Anal. Calcd for $C_{94}H_{104}O_{23}$: C, 70.5; H, 6.5. Found: C, 70.4; H, 6.4.

Methyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-*O*-[α-D-galactopyranosyl-(1→6)]-α-D-glucopyranoside (14). CAN (60 mg, 110 μmol) in acetonitrile (1 mL) was added to a stirred solution of **13** (82 mg, 51 μmol) in dichloromethane saturated with water (10 mL) at 0 °C as described for compound **4**. Column chromatography (toluene-ethyl acetate, 3:2) gave methyl *O*-(3-*O*-benzyl-4,6-*O* - benzylidene - α - D - glucopyranosyl) - (1→2) -*O* - (6 - *O* - acetyl - 3,4 - *O*-isopropylidene-α-D-galactopyranosyl)-(1→3)-*O*-2,4-di-*O*-benzyl - 6 - *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranoside (55 mg, 37 μmol, 73%). NMR data: ^{13}C, δ 21.0 (Me acetyl), 26.4, 28.1 (Me isopropylidene), 55.2 (MeO), 62.3-81.8 (C ring), 97.1, 97.7, 97.7, 98.0 (4 C-1), 101.3 (PhCH), 109.7 (isopropylidene), 126.2-139.2 (aromatic C), 170.9 (C=O acetyl). Methanolic sodium methoxide (0.5 mL, 0.5M) was added to a solution of the latter compound (21 mg, 14 μmol) in dichloromethane (2 mL). The reaction mixture was stirred at room temperature for 1 h, then neutralized with acetic acid (2 mL). The solution was concentrated to 2 mL. Water (200 μL) was added and the reaction mixture was heated at 100 °C for 1 h. Pd/C was added and the reaction mixture was hydrogenolyzed at 400 kPa overnight, then filtered and concentrated. The residue was purified on a Biogel P-2 column, using water containing 1% 1-butanol as eluent, giving compound **14** (7 mg, 11 μmol, 73%) having $[\alpha]_{578}$ +174° (*c* 0.3, water). NMR data (D_2O; Me_2CO, δ_H=2.225; external TMS δ_C=0): ^{13}C, δ 56.0 (MeO), 61.3, 61.4, 61.9, 66.0, 68.4, 69.3, 70.1, 70.1, 70.3, 70.3, 70.6, 70.6, 71.3, 71.8, 72.2, 72.6, 73.4, 73.8, 81.3 (C ring), 96.6, 97.7, 98.9, 100.4 (4 C-1); ^1H (*inter alia*) δ 3.28 (dd, H-4′′′), 3.42 (dd, $J_{1,2}$=3.8 Hz, $J_{2,3}$=9.6 Hz, H-2′′′), 3.51 (dd, $J_{1,2}$=3.8 Hz, H-2), 3.62 (dd, H-3′′′), 3.66 (dd, H-3), 3.66 (dd, $J_{1,2}$=3.3 Hz, H-2′′), 3.84 (dd, $J_{1,2}$=2.8 Hz, H-2′), 3.88 (dd, H-3′), 4.67 (d, H-1), 4.81 (d, H-1′′), 5.01 (d, H-1′′′), 5.37 (d, H-1′).

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