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SYNTHESIS OF DI-, TRI-, AND TETRA-SACCHARIDES CORRESPONDING TO RECEPTOR STRUCTURES RECOGNISED BY *Streptococcus pneumoniae*

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ABSTRACT

Syntheses are described for methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside, methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl-β-D-galactopyranoside, methyl 3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside, and methyl 4-*O*-[3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside.

INTRODUCTION

Oligosaccharides on epithelial cell surfaces function as bacterial receptors[1]. *Streptococcus pneumoniae* is a constituent of the flora in the healthy nasopharynx as well as a cause of localised or invasive infection and is believed to cause some 30% of bacterial otitis media[2]. Preliminary results have shown that lacto-*N*-tetraose [β-D-Gal-(1→3)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc] and lacto-*N*-neotetraose [β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc] act as receptors for pneumococci[2]. In order to define the minimum oligosaccharide fragment required for recognition by these bacteria, a series of synthetic glycosides were needed. The synthesis of methyl β-glycosides of lacto-*N*-neotetraose and several other di- and tri-saccharides that constitute parts of this tetrasaccharide were therefore undertaken. We now report the synthesis of methyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-α-D-glucopyranoside (**18**), methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**20**), methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**24**), methyl 3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**27**), and methyl 4-*O*-[3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyra-

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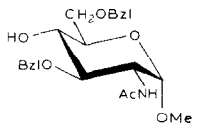
nosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**30**). The results of the biological tests on some of these synthetic glycosides have been reported[3].

RESULTS AND DISCUSSION

Reaction of tetra-*O*-acetyl-α-D-galactopyranosyl bromide with methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[4] (**1**) in dry benzene–nitromethane in the presence of mercuric cyanide afforded the fully protected disaccharide **16** (63%). Deacetylation (→**17**) followed by debenzylation gave methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**18**, 54%).



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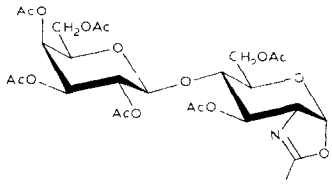

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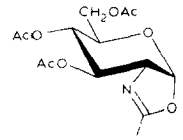

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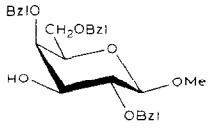

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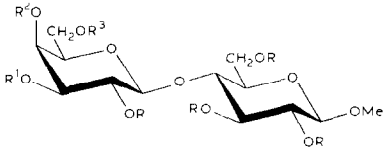
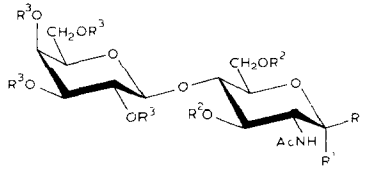
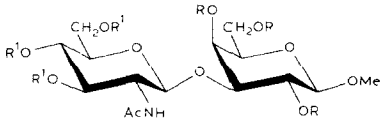


5 R = R^1 = R^2 = R^3 = Ac
6 R = R^1 = R^2 = R^3 = H
7 R = R^3 = H, R^1, R^2 = CMe_2
8 R = R^3 = Bzl, R^1, R^2 = CMe_2
9 R = R^3 = Bzl, R^1 = R^2 = H
10 R = R^3 = Bzl, R^1, R^2 = CHPh
11 R = R^1 = R^3 = Bzl, R^2 = H
12 R = R^2 = R^3 = Bzl, R^1 = H
13 R = R^1 = R^3 = Bzl, R^2 = Ac
14 R = R^2 = R^3 = Bzl, R^1 = Ac
15 R = R^2 = R^3 = Bzl, R^1 = Bz



16 R = H, R^1 = OMe, R^2 = Bzl, R^3 = Ac
17 R = H, R^1 = OMe, R^2 = Bzl, R^3 = H
18 R = R^2 = R^3 = H, R^1 = OMe
19 R = OMe, R = H, R^2 = R^3 = Ac
20 R = OMe, R^1 = R^2 = R^3 = H

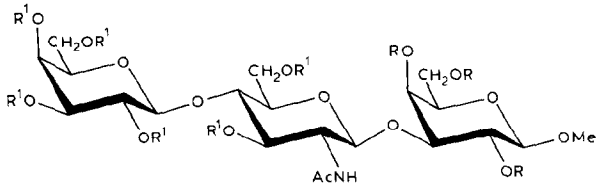


21 R = Bzl, R = Ac
22 R = H, R = Ac
23 R = R^1 = Ac
24 R = R^1 = H

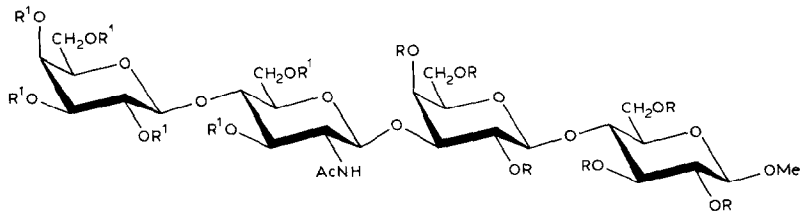
The β-glycoside **19** was prepared (77%) by a slight modification of the method reported by Tejima and co-workers[5]. Deacetylation of **19** gave methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**20**, 92%).

Trimethylsilyl trifluoromethanesulphonate(triflate)-catalysed[7] glycosidation of methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**, prepared by partial benzylation of methyl β-D-galactopyranoside)[8,9] with the oxazoline **3**[10] gave the fully protected disaccharide **21** (60%). One of the acetyl groups in **21** gave a signal at unusually high field (δ 1.52) in the ^{1}H-n.m.r. spectrum. The shielding is presumably due to an anisotropic effect caused by the benzyl group(s). Nashed *et al.*[11] have recently observed such a shielding effect in the ^{1}H-n.m.r. spectrum of a similar compound. Catalytic hydrogenolysis of **21** gave **22** (82%). The ^{1}H-n.m.r. data for **23**, obtained by acetylation of **22**, combined with the ^{13}C chemical shift of the signal for C-6 (68.9 p.p.m.) in **21** clearly showed that the galactose unit was glycosylated at position 3. Deacetylation of **22** then gave crystalline methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**24**, 93%).

Trimethylsilyl triflate-catalysed condensation of the oxazoline **2** with **4** gave the trisaccharide derivative **25** (18%), which was debenzylated to give **26** (52%; isolated by chromatography on silica gel). The 2,2,2-trichloroethyl and 8-(methoxycarbonyl)octyl glycosides corresponding to the methyl glycoside **26** have been prepared by Lemieux and co-workers[12] *via* a different route, also in low yield. Deacetylation of **26** gave methyl 3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**27**, 79%).



25 R = Bzl, R^1 = Ac
26 R = H, R^1 = Ac
27 R = R^1 = H



28 R = Bzl, R^1 = Ac
29 R = H, R^1 = Ac
30 R = R^1 = H

The tetrasaccharide-glycoside **28** was prepared in an analogous manner from the oxazoline **2** and methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**) which, in turn, was prepared from methyl β-D-lactoside (**6**). Isopropylidenation[13] of **6** in 2,2-dimethoxypropane in the presence of toluene-*p*-sulphonic acid gave **7** (84%) which was benzylated to give **8** (89%). Removal of the isopropylidene group from **8** with hot aqueous 80% acetic acid gave crystalline **9** (82%) which was then benzylidenated to give **10** (89%). Reductive ring-opening of the benzylidene acetal[14] gave the partially protected lactoside **12** (54%; 30% from **6**) as the major product together with the isomeric lactoside **11** (8%). The structures of **11** and **12** were determined *via* the respective acetylated derivatives **13** and **14**. The ^{1}H-n.m.r. spectra of **13** and **14** contained 3-proton singlets at δ 2.03 and 1.91, respectively, indicating the presence of one acetyl group in each compound. In addition, the H-4′ signal for **13** appeared as a doublet shifted downfield to a unique position in the spectrum (δ 5.55). The H-3′ signal for **14**, however, did not show such a large deshielding effect and remained masked by the signals of the benzyl groups. The benzoylated derivative **15**, on the other hand, permitted the identification of the H-3′ signal (δ 5.10).

Trimethylsilyl triflate-catalysed condensation of the oxazoline **2** with the partially protected lactoside **12** gave the fully protected tetrasaccharide derivative **28**. Catalytic hydrogenolysis of **28** gave the partially protected tetrasaccharide **29** (29% from **12**). Deacetylation then gave methyl 4-*O*-[3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**30**, 59%).

Finally, we would like to comment on our strategy for the syntheses of the methyl β-glycosides of trisaccharide **27** and tetrasaccharide **30**. The synthesis of the 2,2,2-trichloroethyl and 8-(methoxycarbonyl)octyl analogues of **27** by Lemieux *et al.*[12] has already been mentioned. The synthesis of the benzyl analogue of **30** has been achieved by Ponpipom *et al.*[15] Both groups employed 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl chloride as the glycosyl donor. In addition, Ponpipom *et al.*[15] used acetyl groups for partial protection of the glycosyl acceptor. Because of the availibility of large amounts of *N*-acetyl-lactosamine (by fermentation), the oxazoline **2** was chosen instead of the phthaloylated derivative mentioned above. Benzyl protecting-groups were also chosen for the glycosyl acceptor since they are much less prone to migration. Furthermore, benzyl groups activate near-by hydroxyl groups in glycosidation reactions[16].

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded with a Varian XL 200 spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or D_2O [internal sodium 3-(trimethylsilyl)propionate-d_4 (TSP)].

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**16**). — A suspension of **1**[4] (317 mg, 0.6 mmol) and mercuric cyanide (418 mg, 1.6 mmol) in dry benzene–nitromethane (35 mL; 1:1) was heated (100°) until 10 mL of the solvent had been distilled off. A solution of freshly recrystallised tetra-*O*-acetyl-α-D-galactopyranosyl bromide (678 mg, 1.6 mmol) in benzene (10 mL) was added dropwise with stirring during 3 h at 60°. The temperature was then adjusted to 40°, and the mixture was stirred for a further 16 h, cooled to room temperature, washed with saturated aqueous sodium hydrogencarbonate and saturated aqueous sodium chloride, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (SiO_2; iso-octane–ethyl acetate, 1:2) of the residue gave amorphous **16** (281 mg, 63%), which contained a small amount of the α isomer. ^{1}H-N.m.r. data ($CDCl_3$): δ 5.26 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ <1.0 Hz, H-4'), 5.12 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.3 Hz, H-2'), 4.72 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.47 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), and 3.32 (s, 3 H, OMe). This product was used in the next step without further characterisation.

Methyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-α-D-glucopyranoside (**18**). — A solution of **16** (281 mg, 0.4 mmol) in dry methanol (10 mL) containing a catalytic amount of sodium methoxide was left at room temperature for 2 h. T.l.c. (chloroform–methanol, 6:1) then showed complete conversion. The mixture was neutralised with Duolite C26 (H^+) resin, filtered, and concentrated to give syrupy **17** which, without further characterisation, was dissolved in methanol (10 mL) and hydrogenated (10% Pd/C; 150 mg) at ambient temperature and pressure for 20 h. The mixture was filtered through Celite, the solvent was removed, and the crude product was subjected to column chromatography (SiO_2; chloroform–methanol–water, 65:35:10) to give **18** as an amorphous solid (87 mg, 54%), $[\alpha]_D^{21}$ +80° (*c* 0.5, water); lit.[4] $[\alpha]_D$ +98.4° (methyl sulphoxide). N.m.r. data (D_2O): ^{1}H, δ 4.78 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.63 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 3.39 (s, 3 H, OMe), and 2.03 (s, 3 H, NAc); ^{13}C, δ 177.2 (CO), 105.8 (C-1'), 100.6 (C-1), 81.6 (C-4), 63.9 (C-6'), 62.8 (C-6), 58.1 (OMe), 56.1 (C-2), and 24.7 ($NCOCH_3$).

Anal. Calc. for $C_{15}H_{27}NO_{11}$: C, 45.34; H, 6.85; N, 3.52. Found: C, 45.41; H, 6.92; N, 3.44.

Methyl 2-acetamido-3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**19**). — A solution of the oxazoline **2**[6] (151 mg, 0.24 mmol) in dry methanol (6 mL) containing anhydrous toluene-*p*-sulphonic acid (10 mg) was stirred at 60° for 15 min, cooled, neutralised with pyridine (0.5 mL), and co-concentrated with toluene. The syrupy residue was subjected to column chromatography (SiO_2; chloroform–methanol, 20:1) to afford **19** (97 mg) and a slower-moving product. Acetylation of the latter (acetic anhydride–pyridine) gave more **19** (total yield, 122 mg, 77%) as an amorphous solid, $[\alpha]_D^{25}$ −18° (*c* 1.3, $CDCl_3$); lit.[5] $[\alpha]_D^{25}$ −9° (chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.64 (d, 1 H, $J_{NH,2}$ 9.3 Hz, NH), 5.36 (dd, 1 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ <1.0 Hz, H-4'), 4.50 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.36 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1'), and 3.46 (s, 3 H, OMe); ^{13}C, δ 101.8, 101.0 (C-1,1'), 62.3, 60.8 (C-6,6'), 56.6 (OMe), 53.0 (C-2), and 23.3 ($NCOCH_3$).

Methyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranoside (**20**). — A solution of **19** (77 mg) in dry methanolic sodium methoxide (5 mL) was left at room temperature overnight. The precipitated product was dissolved by the addition of a few drops of water, and the solution was neutralised with Duolite C26 (H^+) resin and filtered. The resin was washed thoroughly with aqueous methanol. The combined filtrate and washings were co-concentrated with methanol several times and the residue was dried over phosphorous pentaoxide under vacuum to afford **20** (43 mg, 92%) as an amorphous solid, $[\alpha]_D^{25}$ −25° (*c* 1.5, D_2O); lit.[5] $[\alpha]_D$ −16.7° (water); lit.[17] $[\alpha]_D^{23}$ −23.1° (water). N.m.r. data (D_2O): ^1H, δ 4.45 (d, 2 H, *J* 7.6 Hz, H-1,1′), 3.50 (s, 3 H, OMe), and 2.02 (s, 3 H, NAc); ^{13}C, δ 177.5 (CO), 105.7 (C-1′), 104.7 (C-1), 81.3 (C-4), 63.9 (C-6′), 62.9 (C-6), 59.9 (OMe), 57.8 (C-2), and 25.0 ($NCOCH_3$).

Methyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**21**). — A solution of the oxazoline **3**[10] (5.02 g, 15.3 mmol) and the partially protected galactoside **4**[8] {m.p. 60.5–62°, $[\alpha]_D^{25}$ +1.4° (*c* 1.2, $CDCl_3$); 3.53 g, 7.62 mmol} in dry 1,2-dichloroethane (50 mL) containing tetramethylurea (1.77 g, 15.3 mmol) and trimethylsilyl triflate (1.99 g, 9.1 mmol) was heated at 80° (bath) under nitrogen. After 30 min, more **3** (1.30 g, 2.8 mmol) in dichloromethane (5 mL) was added and heating was continued for 3 h. The solution was then left to attain ambient temperature and, after 16 h, diluted with dichloromethane, and washed with cold water, cold saturated aqueous sodium hydrogencarbonate, and water. The organic phase was dried (Na_2SO_4), filtered, and concentrated to a brown syrup which was subjected to column chromatography (SiO_2; toluene–ether–methanol, 14:14:1). The fractions containing **21** were re-chromatographed (toluene–ethyl acetate, 1:1) to give pure **21** (3.65 g, 60.4%), m.p. 160.5–161.5° (from 2-propanol), $[\alpha]_D^{25}$ −28° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 3.52 (s, 3 H, OMe), 2.03, 2.02, 1.98, and 1.52 (4 s, each 3 H, 3 OAc and NAc); ^{13}C, δ 104.8 (C-1), 101.8 (C-1′), 74.4, 74.3, 73.5 (3 CH_2Ph), 68.9 (C-6), 62.1 (C-6′), 56.9 (OMe), 54.2 (C-2′), and 22.8 ($NCOCH_3$).

Anal. Calc. for $C_{42}H_{51}NO_{14}$: C, 63.54; H, 6.48; N, 1.78. Found: C, 63.61; H, 6.51; N, 1.88.

Methyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**22**). — A solution of **21** (2.3 g, 2.9 mmol) in ethyl acetate (70 mL) and methanol (20 mL) was hydrogenated (10% Pd/C; 1.0 g; 3.5 atm.) overnight, then filtered through Celite, and concentrated. The residue was subjected to column chromatography (SiO_2; chloroform–methanol, 8:1) to give **22** (1.24 g, 82.2%) as an amorphous powder, $[\alpha]_D^{25}$ −12° (*c* 1.1, methanol). N.m.r. data (CD_3OD): ^1H, δ 5.23 (dd, 1 H, $J_{2',3'}$ 8.3, $J_{3',4'}$ 10.4 Hz, H-3′), 4.86 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.14 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), and 3.52 (s, 3 H, OMe); ^{13}C, δ 106.0 (C-1), 103.6 (C-1′), 63.3, 62.3 (C-6,6′), 57.3 (OMe), 55.6 (C-2′), and 22.9 ($NCOCH_3$).

Conventional acetylation (acetic anhydride–pyridine) of **22** gave **23**, $[\alpha]_D^{25}$ +21° (*c* 1, chloroform). ^1H-N.m.r. data ($CDCl_3$): δ 5.53 (dd, 1 H, $J_{2',3'}$ 10.6, $J_{3',4'}$

9.2 Hz, H-3′), 5.48 (d, 1 H, $J_{NH,2'}$ 7.6 Hz, NH), 5.38 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ <1.0 Hz, H-4), 5.12 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2), 5.08 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.31 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.83 (dd, 1 H, $J_{3,4}$ 3.5, $J_{2,3}$ 10.0 Hz, H-3), 3.50 (s, 3 H, OMe), and 3.29 (m, 1 H, H-2′).

Methyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**24**). — A solution of **22** (2.18 g) in dry methanol (50 mL) containing a catalytic amount of sodium methoxide was stirred at ambient temperature overnight. The precipitated product was dissolved by the addition of water (20 mL), and the solution was neutralised with Duolite C26 (H^+) resin, filtered, and co-concentrated with methanol. The residue was recrystallised from aqueous ethanol to give **24** (1.22 g). More **24** was obtained by column chromatography (chloroform–methanol–water, 65:35:10) of the mother liquor followed by crystallisation (total yield, 1.52 g, 93%). Compound **24** had m.p. 264° (Kofler hot-plate), $[\alpha]_D^{27}$ +3° (*c* 1.1, D_2O). N.m.r. data (D_2O): ^1H, δ 4.69 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1′), 4.30 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.14 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 3.56 (s, 3 H, OMe), and 2.03 (s, 3 H, NAc); ^{13}C, δ 177.8 (CO), 106.7 (C-1), 105.5 (C-1′), 85.3 (C-3), 63.7, 63.3 (C-6,6′), 60.0 (OMe), 58.5 (C-2′), and 25.0 ($NCOCH_3$).

Anal. Calc. for $C_{16}H_{27}NO_{11}$: C, 45.34; H, 6.85; N, 3.52. Found: C, 45.30; H, 6.81; N, 3.39.

Methyl 3-O-[2-acetamido-3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**25**). — A solution of the oxazoline **2** (617 mg, 1 mmol), **4** (464 mg, 1 mmol), trimethylsilyl triflate (218 mg, 1 mmol), and tetramethylurea (232 mg, 2 mmol) in dry 1,2-dichloroethane (15 mL) was heated at 80° for 2 h and then left at room temperature for 16 h. T.l.c. (toluene–ethyl acetate–methanol, 7:7:1) then revealed unreacted **2**. The mixture was heated at 80° for a further 3 h, cooled, diluted with chloroform (150 mL), washed with saturated aqueous sodium hydrogencarbonate and water, dried, and concentrated. The residue was subjected to column chromatography (SiO_2; toluene–ethyl acetate–methanol, 7:7:1) which gave **4** (320 mg, 69% recovery) and a fraction (420 mg) containing **25**. Re-chromatography (SiO_2; toluene–ethyl acetate–2-propanol, 16:8:1) afforded pure **25** (195 mg, 18% based on **4**) as a foam, $[\alpha]_D^{23}$ −14° (*c* 1, $CDCl_3$). N.m.r. data ($CDCl_3$): ^1H, δ 5.35 (dd, 1 H, $J_{3'',4''}$ 3.2, $J_{4'',5''}$ <1.0 Hz, H-4″), 5.13 (dd, 1 H, $J_{1'',2''}$ 8.0, $J_{2'',3''}$ 10.4 Hz, H-2″), 4.73 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.51 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.23 (m with virtual coupling[18], H-1), 3.52 (s, 3 H, OMe), 2.16, 2.07, 2.05, 2.02, 1.98, and 1.51 (6 s, 21 H, 6 OAc and NAc); ^{13}C, δ 104.8, 101.9, 101.2 (C-1,1′,1″), 62.0 (C-6″), 60.7 (C-6′), 56.9 (OMe), and 53.8 (C-2′).

Methyl 3-O-(2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**27**). — Compound **25** (614 mg) was hydrogenated (10% Pd/C, 300 mg) as in the preparation of **22**. The product was subjected to column chromatography (SiO_2; chloroform → chloroform–methanol, 10:1) to afford **26** (195 mg, 52%) as a syrup, $[\alpha]_D^{23}$ −5° (*c* 0.8, chloroform). N.m.r. data ($CDCl_3$–CD_3OD): ^1H, δ 6.95 (d, 1 H, $J_{NH,2'}$ 8.9 Hz, NH), 5.35 (dd, 1 H, $J_{3'',4''}$ 3.2,

$J_{4'',5''}$ <1.0 Hz, H-4''), 4.72 (d, 1 H, $J_{1'',2''}$ 8.3 Hz, H-1''), 4.56 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), and 3.55 (s, 3 H, OMe); ^{13}C, δ 104.0, 101.8, 101.3 (C-1,1',1''), 62.05, 62.01, 60.69 (C-6,6',6''), 57.0 (OMe), and 54.1 (C-2).

A solution of **26** (156 mg) in dry methanol (10 mL) containing a catalytic amount of sodium methoxide was left at room temperature for 20 h. The precipitated product was dissolved by addition of water, and the solution was neutralised with Duolite C26 (H^+) resin, filtered, and concentrated. Gel filtration (Sephadex G10) of the residue and freeze-drying gave **27** (85 mg, 79%), $[\alpha]_D^{25}$ +4° (*c* 0.6, water). N.m.r. data (D_2O, 80°): δ 4.79, 4.50, 4.30 (each 1 H, m with virtual coupling[18], d with *J* 7.5 Hz, and d with *J* 7.6 Hz, H-1,1',1''), 3.56 (s, 3 H, OMe), and 2.03 (s, 3 H, NAc); ^{13}C, δ 177.7 (CO), 106.7, 105.6, 105.4 (C-1,1',1''), 85.1 (C-3), 80.9 (C-4'), 63.8, 63.7, 62.6 (C-6,6',6''), 60.0 (OMe), 58.0 (C-2'), and 24.8 p.p.m. ($NCOCH_3$).

Anal. Calc. for $C_{21}H_{37}NO_{16}$: C, 45.08; H, 6.67; N, 2.50. Found: C, 45.05; H, 6.63; N, 2.60.

*Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**5**). — A solution of freshly prepared acetobromolactose[19] (72 g, 103 mmol) in dry toluene (250 mL) and dichloromethane (250 mL) was added to a cooled (−55°), stirred solution of methanol (28.5 g, 890 mmol), tetramethylurea (12.9 g, 110 mmol), and silver triflate (28.5 g, 110 mmol) in dry toluene (250 mL) during 1 h. The mixture was left for 3.5 h to attain ambient temperature. T.l.c. (iso-octane–ethyl acetate, 1:2) then revealed some acetobromolactose. The mixture was cooled to −15°, and more silver triflate (5.1 g, 20 mmol) and tetramethylurea (3.1 g, 27 mmol) were added. After 1 h, t.l.c. showed that the reaction was complete. The mixture was then diluted with dichloromethane (700 mL), filtered through Celite, washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. The residue was subjected to chromatography (SiO_2; iso-octane–ethyl acetate, 2:1 → 3:2) to afford **5** (55.8 g, 78%) as a syrup, $[\alpha]_D^{25}$ −11.5° (*c* 1.3, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.35 (dd, 1 H, $J_{4',5'}$ 1.0, $J_{3',4'}$ 3.4 Hz, H-4'), 4.49 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.40 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), and 3.49 (s, 3 H, OMe); ^{13}C, δ 101.3, 101.0 (C-1,1'), 61.9, 60.7 (C-6,6'), and 57.0 (OMe).

*Methyl 4-*O*-β-*D*-galactopyranosyl-β-*D*-glucopyranoside* (**6**). — A solution of **5** (53.8 g, 82.8 mol) in methanolic sodium methoxide (600 mL) was stirred at 20° for 3 h. The precipitated product was recovered and washed several times with methanol to give **6** (28.2 g, 96%), $[\alpha]_D^{25}$ +2° (*c* 1.8, D_2O); lit.[20] $[\alpha]_D^{23}$ +6.3° (*c* 3.5, water). N.m.r. data (D_2O): ^1H, δ 4.44, 4.40 (2 d, each 1 H, *J* 7.4 and 8.4 Hz, H-1,1'), and 3.34 (s, 3 H, OMe); ^{13}C, δ 105.9, 105.8 (C-1,1'), 63.9, 62.9 (C-6,6'), and 60.1 (OMe).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 43.90; H, 6.75.

*Methyl 4-*O*-(3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**7**). — A suspension of **6** (2.0 g, 5.6 mmol) in 2,2-dimethoxypropane (45 mL)

containing anhydrous toluene-*p*-sulphonic acid (250 mg) was stirred at room temperature for 20 h and then diluted with methanol. Water (5 mL) was added, and the clear solution was neutralised with triethylamine (5 mL) and concentrated. The residue was subjected to column chromatography (SiO_2; chloroform–methanol, 6:1) to obtain **7** (1.87 g, 84%), m.p. 222–223° (from methanol), $[\alpha]_D^{25}$ +19° (*c* 1.2, D_2O). ^{1}H-N.m.r. data (D_2O): δ 4.51 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 4.42 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.38 (dd, 1 H, $J_{3',4'}$ 5.3, $J_{4',5'}$ 1.9 Hz, H-4'), 4.22 (dd, 1 H, $J_{2',3'}$ 7.5 Hz, H-3'), 3.59 (s, 3 H, OMe), 1.55 and 1.40 (s, each 3 H, CMe_2).

Anal. Calc. for $C_{18}H_{28}O_{11}$: C, 48.48; H, 7.12. Found: C, 48.90; H, 7.06.

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyransyl)-β-*D*-glucopyranoside* (**8**). — Sodium hydride (1.98 g, 50% dispersion in oil) was added with stirring to a solution of **7** (1.36 g, 3.4 mmol) in *N,N*-dimethylformamide (25 mL). The mixture was stirred at room temperature for 30 min, and benzyl bromide (7.05 g, 41.2 mmol) was added. The mixture was stirred for 64 h, excess of sodium hydride was decomposed by dropwise addition of methanol (15 mL), the mixture was diluted with ethyl acetate, washed three times with water, dried (Na_2SO_4), and concentrated. The residue was subjected to column chromatography (SiO_2; iso-octane–ethyl acetate, 2:1) to obtain **8** (2.50 g, 89%) as a syrup, $[\alpha]_D^{25}$ +23° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 3.56 (s, 3 H, OMe), 1.40, 1.35 (2 s, each 3 H, CMe_2); ^{13}C, δ 109.8 (CMe_2), 104.8 (C-1'), 101.9 (C-1), 69.0 and 68.3 (C-6,6'), 57.2 (OMe), 28.1, and 26.6 [$C(CH_3)_2$].

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**9**). — A solution of **8** (15.04 g, 17.8 mmol) in aqueous 80% acetic acid (450 mL) was heated (100°) for 30 min, and then co-concentrated with toluene several times. The residue was recrystallised from ether–light petroleum (b.p. 60–80°) to give **9** (11.7 g, 82%), m.p. 111.5–112.5°, $[\alpha]_D^{24}$ +23° (*c* 1.3, $CDCl_3$). N.m.r. data ($CDCl_3$): ^{1}H, δ 3.56 (s, 3 H, OMe), 2.53 (bd, 1 H, OH), and 2.44 (bs, 1 H, OH); ^{13}C, δ 104.7 (C-1'), 102.6 (C-1), 68.7, 68.3 (C-6,6'), and 57.1 (OMe).

Anal. Calc. for $C_{48}H_{54}O_{11}$: C, 71.44; H, 6.45. Found: C, 71.18; H, 6.76.

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-benzylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**10**). — A solution of **9** (11.5 g, 14.3 mmol) and α,α-dimethoxytoluene (10.53 g, 69.3 mmol) in dry tetrahydrofuran (150 mL) containing toluene-*p*-sulphonic acid monohydrate (0.86 g) was stirred at room temperature overnight, neutralised with triethylamine (10 mL), and concentrated. The residue was subjected to column chromatography (SiO_2; iso-octane–ethyl acetate, 4:1 → 2.5:1) to give **10** (11.37 g, 89%) as a syrup. ^{1}H-N.m.r. data ($CDCl_3$): ^{1}H, δ 5.96 (s, 1 H, PhC*H*) and 3.57 (s, 3 H, OMe). The product contained a trace of a slightly faster-moving substance (t.l.c.), presumably an isomer, and was used in the next step without further purification.

Anal. Calc. for $C_{55}H_{58}O_{11}$: C, 73.81; H, 6.53. Found: C, 73.75; H, 6.50.

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**11**) *and methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**12**). — To a solution of **10** (11.37 g, 12.7

mmol) and sodium cyanoborohydride (7.19 g, 114.5 mmol) in dry tetrahydrofuran (170 mL) containing powdered molecular sieves (3Å) at 0° was added saturated ethereal hydrogen chloride until the solution was acidic. After 1 h, t.l.c. (iso-octane–ethyl acetate, 1:2) showed complete reaction. The mixture was diluted with ethyl acetate, filtered though Celite, washed with aqueous 10% hydrochloric acid, water, and saturated aqueous sodium hydrogencarbonate, and then stirred with silica gel (to destroy unreacted sodium cyanoborohydride) for 24 h. The residue was subjected to column chromatography (SiO_2; iso-octane–ethyl acetate, 3:1 → 2:1) to give, first, syrupy **11** (0.90 g, 8%), $[\alpha]_D^{25}$ +25° (*c* 1, chloroform). Conventional acetylation gave **13**, $[\alpha]_D^{29}$ +20° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.56 (d, 1 H, $J_{3',4'}$ 2.2 Hz, H-4'), 3.56 (s, 3 H, OMe), and 2.03 (s, 3 H, OAc).

Eluted second was syrupy **12** (6.18 g, 54%), $[\alpha]_D^{27}$ +6° (*c* 1.3, chloroform). N.m.r. data ($CDCl_3$): ^{13}C, δ 104.6 (C-1'), 102.6 (C-1), 68.2, 67.6 (C-6,6'), 57.0 (OMe).

Conventional acetylation of **12** gave **14**, $[\alpha]_D^{27}$ +23° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 3.56 (s, 3 H, OMe), 1.91 (s, 3 H, OAc); ^{13}C, δ 170.4 (CO), 104.7 (C-1'), 102.7 (C-1), 68.2, 67.6 (C-6,6'), 57.1 (OMe), and 21.0 ($OCOCH_3$).

Benzoylation (benzoyl chloride–pyridine) of **12** gave **15** as a syrup, $[\alpha]_D^{25}$ +23° (*c* 1.7, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.10 (dd, 1 H, $J_{3',4'}$ 3.1, $J_{2',3'}$ 10.1 Hz, H-3'), 3.57 (s, 3 H, OMe); ^{13}C, δ 165.8 (CO), 104.7 (C-1'), 102.7 (C-1), 68.1, 67.6 (C-6,6'), and 57.1 (OMe).

*Methyl 4-*O*-{3-*O*-[2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl}-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**28**). — A mixture of **12** (4.29 g, 3.84 mmol), oxazoline **2**[6] (3.97 g, 6.43 mmol), trimethylsilyl triflate (1.40 g, 6.43 mmol), and tetramethylurea (1.11 g, 9.57 mmol) in dry 1,2-dichloroethane (200 mL) under nitrogen was heated at 70° for 3 h. More **2** (2.00 g, 3.24 mmol), trimethylsilyl triflate (0.62 g, 2.82 mmol), and tetramethylurea (0.50 g, 4.31 mmol) were then added and heating (60°) was continued for 19 h. The mixture was cooled, diluted with dichloromethane (300 mL), washed with cold, saturated aqueous sodium hydrogencarbonate and cold water, dried (Na_2SO_4), filtered, and concentrated. The residue was subjected to column chromatography (SiO_2; toluene–ether–methanol, 14:14:1) to give **12** (1.60 g, 37%), **2** (1.10 g, 18%), and a fraction containing impure **28**. The last fraction was re-chromatographed (SiO_2; iso-octane–ethyl acetate, 1:2) to give pure **28** (2.50 g) as a foam, $[\alpha]_D^{25}$ −9° (*c* 1.6, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.35 (d, 1 H, $J_{3''',4'''}$ 3.2 Hz, H-4'''), 3.52 (s, 3 H, OMe), 2.15, 2.08, 2.04, 2.02, 1.97, 1.96, and 1.46 (7 s, each 3 H, 6 OAc and NAc); ^{13}C, δ 104.6, 102.5, 102.3, 101.2 (C-1,1',1'',1'''), 62.1, 60.7 (C-6'',6'''), 57.0 (OMe), 53.9 (C-2''), 22.7 ($NCOCH_3$).

*Methyl 4-*O*-{3-*O*-[2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside* (**29**). — A suspension of **28** (2.50 g) and 10% Pd/C (2.50 g) in ethyl acetate (50 mL) and methanol (50 mL) was hydrogenolysed for 24 h at 30

p.s.i., filtered through Celite, and concentrated. The residue was subjected to column chromatography (SiO_2; chloroform–methanol, 8:1 → 6:1) to give pure **29** (1.20 g, 29% from **12**), $[\alpha]_D^{25}$ −2° (*c* 1.1, $CDCl_3$) and −5° (*c* 0.8, chloroform). N.m.r. data ($CDCl_3$ + CD_3OD): ^1H, δ 5.36 (dd, 1 H, $J_{3''',4'''}$ 3.2, $J_{4''',5'''}$ <1.0 Hz, H-4'''), 4.70, 4.54, 4.35, 4.25 (4 d, each 1 H, *J* 8.5, 7.7, 7.9, and 7.7 Hz, H-1,1′,1″,1‴), and 3.56 (s, 3 H, OMe); ^{13}C, δ 103.9, 103.7, 101.9, 101.2 (C-1,1′,1″,1‴), 62.2, 61.8, 61.4, 60.8 (C-6,6′,6″,6‴), 57.2 (OMe), 54.1 (C-2″), and 22.7 ($NCOCH_3$).

Anal. Calc. for $C_{39}H_{59}NO_{27}$: C, 48.10; H, 6.11; N, 1.44. Found: C, 48.16; H, 6.07; N, 1.44.

Methyl 4-O-[3-O-(2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-gluco-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**30**). — A solution of **29** (650 mg) in methanolic sodium methoxide (40 mL) was left at room temperature for 18 h. T.l.c. (ethyl acetate–acetic acid–water, 2:1:1) then indicated incomplete deacetylation. The precipitate was dissolved in distilled water (15 mL), sodium methoxide was added, and the mixture was left at 7° for 48 h. The mixture was neutralised with Duolite C26 (H^+) resin, filtered, and concentrated to give a glass which was treated with hot aqueous ethanol. The precipitated amorphous product was collected, and a solution in distilled water was filtered through a millipore membrane (0.5 μm) and freeze-dried to give **30** (328 mg, 68%), $[\alpha]_D^{27}$ +4° (*c* 0.9, D_2O). N.m.r. data (D_2O): ^1H, δ 4.71, 4.49, 4.44, 4.41 (4 d, each 1 H, *J* 7.6, 7.6, 7.8, and 7.9 Hz, H-1,1′,1″,1‴), 3.58 (s, 3 H, OMe), and 2.04 (s, 3 H, NAc); ^{13}C, δ 177.7 (CO), 105.9, 105.8, 105.7, 105.6 (C-1,1′,1″,1‴), 63.83, 63.78, 62.8, 62.6 (C-6,6′,6″,6‴), 60.0 (OMe), 58.0 (C-2″), and 25.0 ($NCOCH_3$).

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