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Note

Synthesis of *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-D-mannopyranose

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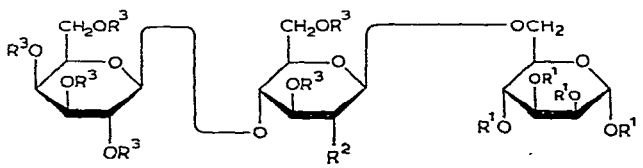
Several syntheses of oligosaccharides derived from the complex type (*N*-acetyl-D-lactosamine type) of carbohydrate portions[1] of glycoproteins have been reported[2–9]. We now report on the synthesis of *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-D-mannopyranose (**1**). This trisaccharide is part of the most highly branched, complex type of carbohydrate portions[1].

Benzyl 6-*O*-triphenylmethyl-α-D-mannopyranoside[10] was benzylated by using sodium hydride–benzyl bromide, to give, after chromatography on silica gel, benzyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (**2**) in 78% yield.

3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide[3,4] (**3**) and mannoside **2** were condensed in nitromethane by using silver trifluoromethanesulfonate as promoter under conditions similar to those described by Bredereck and co-workers[11]. After chromatography on silica gel, the trisaccharide derivative **4** was obtained in 63% yield. The 2-deoxy-2-phthalimido group in **4** was exchanged for a 2-acetamido-2-deoxy group by treatment with hydrazine hydrate[12] followed by acetic anhydride–pyridine, to give the trisaccharide derivative **5** (93% yield). *O*-Deacetylation of **5** followed by treatment with hydrogen over palladium-on-charcoal gave, after gel filtration, the amorphous trisaccharide **1**, $[\alpha]_D$ −10°, in 83% yield. Methylation analysis[13] of the alditol of **1**



1 R^1 = H, R^2 = NHAc, R^3 = H
4 R^1 = Bzl, R^2 = NPhth, R^3 = Ac
5 R^1 = Bzl, R^2 = NHAc, R^3 = Ac

gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, and 1,2,3,4,5-penta-*O*-methyl-D-mannitol.

The ^{1}H- and ^{13}C-n.m.r. spectra for trisaccharide **1** were in good agreement with the postulated structure, and similar to those obtained from related natural[14] and synthetic[4,5] compounds.

Biological experiments performed with this trisaccharide will be reported elsewhere.

EXPERIMENTAL

General methods. — These were as described earlier[4].

Benzyl 2,3,4-tri-O-benzyl-6-O-triphenylmethyl-α-D-mannopyranoside (**2**). — A solution of benzyl 6-*O*-triphenylmethyl-α-D-mannopyranoside (1.4 g) in *N*,*N*-dimethylformamide (50 mL) was added dropwise to sodium hydride (0.42 g) under nitrogen. The suspension was stirred for 1 h at room temperature. Benzyl bromide (2.1 mL) was added dropwise, the mixture was stirred for 3.5 h at room temperature, and then methanol (25 mL) followed by dichloromethane (100 mL) were added. After work-up, the product was purified on silica gel with toluene–light petroleum–ethyl acetate (4:3:1), to yield **2** (1.8 g, 78%) as a syrup, $[\alpha]_D^{25}$ +31° (*c* 1, chloroform); t.l.c. (solvent as above): R_F 0.66; ^{13}C-n.m.r. (25 MHz, $CDCl_3$): δ 62.9–80.3 (CH_2Ph, ring C, C-6), 86.2 (CPh_3), 96.6 (C-1), and 125.2–144.1 (aromatic).

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**4**). — A mixture of mannoside **2** (2.4 g), powdered molecular sieves (4 Å, 0.5 g), and Drierite (0.5 g) in anhydrous nitromethane (20 mL) containing bromide[3,4] **3** (3.6 g) was cooled to 0° under nitrogen. Silver trifluoromethanesulfonate (1.3 g) was added. The mixture was stirred for 10 min, filtered, diluted with dichloromethane, and washed with saturated, aqueous sodium hydrogencarbonate and water. The product was purified on silica gel with light petroleum–ethyl acetate (1:1), to yield trisaccharide **4** as a syrup (2.4 g, 63%). Crystallisation from ethyl acetate–light petroleum gave small flakes, m.p. 146–147°, $[\alpha]_D^{25}$ +29° (*c* 1, chloroform); ^{13}C-n.m.r. (25 MHz, $CDCl_3$): δ 20.2–20.9 (OAc), 55.0 (C-2′), 60.1–80.0 (CH_2Ph, ring C, C-6,6′,6″), 96.7 (C-1), 98.4 (C-1′), 100.9 (C-1″), 123.3–138.3 (aromatic), and 167.5–170.2 (*C*=O).

Anal. Calc. for $C_{66}H_{71}NO_{23}$: C, 63.60; H, 5.74; N, 1.12. Found: C, 63.57; H, 5.73; N, 1.15.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**5**). — Compound **4** (1.7 g) was dissolved in 90% aqueous ethanol (100 mL), hydrazine hydrate (20 mL) was added, and the solution was boiled under reflux for 17 h. After cooling, the solution was concentrated to dryness, and the residue was treated with acetic anhydride–pyridine (1:1, 30 mL) at room temperature overnight. After concentration, the product was purified on silica gel with light petroleum–ethyl

acetate (1:4), to yield trisaccharide **5** as a syrup (1.5 g, 93%), $[\alpha]_D^{21}$ +8° (*c* 1, chloroform); t.l.c. (solvent as above): R_F 0.67; ^{13}C-n.m.r. (25 MHz, $CDCl_3$): δ 20.5–24.8 (NHAc, OAc), 53.1 (C-2'), 60.9–80.1 (CH_2Ph, ring C, C-6,6',6"), 96.9 (C-1), 100.7 (2 C, C-1',1"), 126.8–138.4 (aromatic), and 169.2–170.3 (*C*=O).

O-β-D-Galactopyranosyl-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-D-mannopyranose (**1**). — A catalytic amount of sodium was added to a solution of **5** (1.3 g) in methanol (20 mL). The mixture was left at room temperature overnight, neutralised with acetic acid, and concentrated to dryness. The product was dissolved in 90% aqueous acetic acid (85 mL) and hydrogenated at 400 kPa over 10% palladium-on-charcoal (200 mg) overnight. After filtration and concentration, the product was de-salted on a column (2.5 × 80 cm) of Sephadex G-15 by irrigation with water. After freeze-drying, trisaccharide **1** was obtained as an amorphous powder (510 mg, 83%), $[\alpha]_D^{21}$ −10° (*c* 1, water); ^{1}H-n.m.r. (200 MHz, D_2O): δ 2.06 (s, 3 H, NHAc), 4.48 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1"), 4.60 (d, 1 H, $J_{1,2}$ ~8 Hz, H-1'), 4.88 (bs, 0.3 H, H-1β), and 5.15 (d, 0.7 H, $J_{1,2}$ 1.3 Hz, H-1α); ^{13}C-n.m.r. (25 MHz, D_2O): δ 23.5 (NHAc), 56.2 (C-2'), 61.2–79.6 (C-6,6',6", ring C), 94.9 (0.3 C, C-1β), 95.2 (0.7 C, C-1α), 102.7 (C-1'), 104.0 (C-1"), and 175.9 (*C*=O).

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