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Note

Synthesis of a hexasaccharide that forms part of an alveolar glycoprotein

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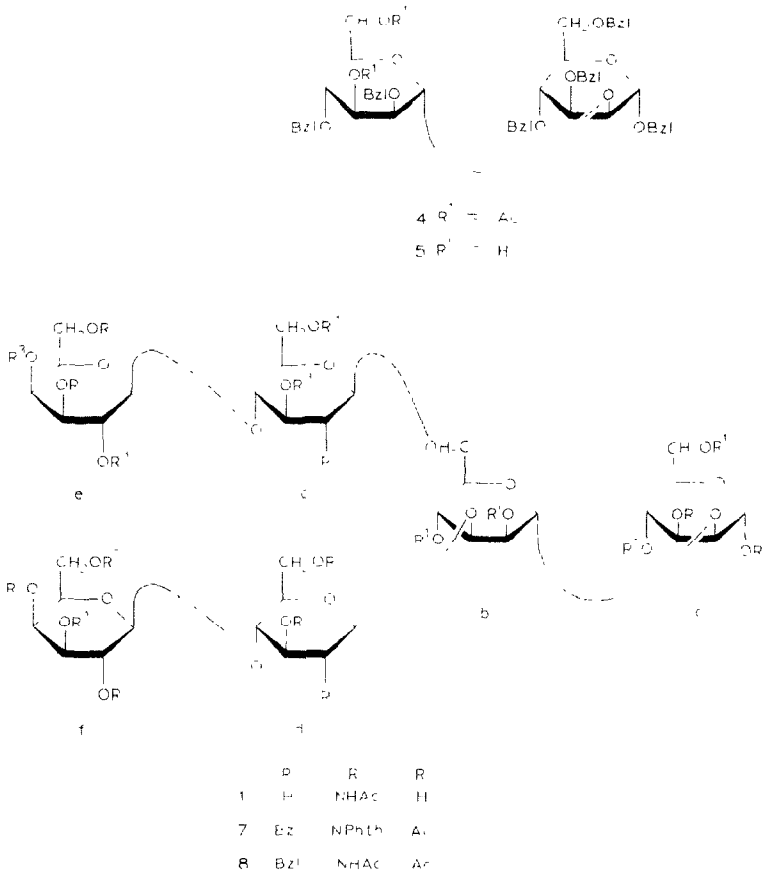
The carbohydrate portion of a glycoprotein isolated from patients with alveolar proteinosis is related[1] to that of the normal complex (*N*-acetyl-D-lactosaminic) type of glycoprotein[2]. In the former, however, two *N*-acetyl-β-D-lactosaminyl residues are linked directly to O-3 and O-6 of a branching α-D-mannopyranosyl residue, a substitution pattern not found in normal glycoproteins. We now report the synthesis of 2-*O*-{3,6-di-*O*-[*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranosyl}-D-mannose (**1**), which is part of the alveolar glycoprotein. This hexasaccharide should be useful in studies of certain carbohydrate-binding proteins (lectins).

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl chloride[3] was condensed with benzyl alcohol, using silver trifluoromethanesulfonate(silver triflate)–2,4,6-trimethylpyridine as promoter[4,5]. Benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[6] (**2**) was obtained in 80% yield and was then deacetylated to give benzyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside[6] (**3**, 88%).

3,6-Di-*O*-acetyl-2,4-di-*O*-benzyl-D-mannopyranosyl chloride[7] was similarly condensed[4,5] with **3** and, after chromatography on silica gel, the α-linked disaccharide derivative **4** was obtained in 91% yield. Compound **4** was deacetylated to give **5** (74%).

Compound **5** was condensed with 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[8,9] (**6**), using silver triflate–2,4,6-trimethylpyridine as promoter, conditions known to give β-glycosides[4,5,8,9]. After chromatography on silica gel, **7** was obtained in 39% yield. The 2-deoxy-2-phthalimido groups in **7** were exchanged for 2-acetamido-2-deoxy groups by treatment with hydrazine hydrate[5] followed by acetic anhydride–pyridine, to give, after chromatography, compound **8** (73%).

Finally, **8** was de-protected by *O*-deacetylation followed by catalytic hydrogenolysis (Pd/C) to give, after gel filtration and freeze-drying, amorphous **1** in 78% yield. Methylation analysis[10] of the alditol of **1** gave 2,3,4,6-tetra-*O*-methyl-D-galactose,



2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose, 2,4-di-*O*-methyl-D-mannose, and 1,3,4,5,6-penta-*O*-methyl-D-mannitol. The ^{1}H- and ^{13}C-n.m.r. spectra of **1** were in agreement with those from related natural[11] and synthetic compounds[12].

EXPERIMENTAL

General methods. — These were as described earlier[9]. Elemental analyses were not obtained for syrupy and amorphous products, but their purity was established by chromatography and n.m.r. spectroscopy.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**2**). — A mixture of silver triflate (3.85 g), 2,4,6-trimethylpyridine (0.85 g), benzyl alcohol (0.93 mL), and ground molecular sieves (5 g, 3Å) in dry dichloromethane (20 mL) was cooled to −40° under nitrogen. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl chloride (5.04 g) in dry dichloromethane (10 mL) was added during 30 min with stirring. The mixture was allowed to attain room temperature (2 h) and pyridine (0.8 g) was added. The mixture was filtered and the solution was washed with dilute aqueous sodium

thiosulfate and water. The product was purified on silica gel with toluene–ethyl acetate (6:1), to yield **2** as a syrup (4.2 g, 80%), $[\alpha]_{578}^{22}$ +33° (*c* 1.5, chloroform); lit.[6] $[\alpha]_D$ +38.2°; R_F 0.67 (t.l.c., toluene–ethyl acetate, 4:1); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 20.9 (OAc), 68.7 (2 C, C-2, Ph*C*H_2), 69.2 (C-6), 71.6 (2 C, C-5, Ph*C*H_2), 73.3 (Ph*C*H_2), 74.3 (C-4), 75.1 (Ph*C*H_2), 78.3 (C-3), 97.0 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 169 Hz)[13], 127.5–138.4 (aromatic), and 170.1 (*C*=O).

Benzyl 3,4,6-tri-O-*benzyl*-α-D-*mannopyranoside* (**3**). — A catalytic amount of sodium was added to a solution of **2** (4.0 g) in dry methanol (100 mL). The mixture was left at room temperature overnight, neutralised with Dowex 50 (H^+) resin, filtered, and concentrated to dryness. The product was purified on silica gel with toluene–ethyl acetate (4:1), to yield **3** as a syrup (3.26 g, 88%), $[\alpha]_{578}^{22}$ +48° (*c* 0.6, chloroform); lit.[6] $[\alpha]_D$ +35°; R_F 0.37 (solvent as above); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 68.3 (C-2), 69.0 (2 C, C-6, Ph*C*H_2), 71.4 (C-5), 71.8 (Ph*C*H_2), 73.3 (Ph*C*H_2), 74.4 (C-4), 75.0 (Ph*C*H_2), 80.2 (C-3), 98.4 (C-1), and 127.4–138.3 (aromatic).

Benzyl 3,4,6-tri-O-*benzyl-2*-O-(*3,6-di*-O-*acetyl-2,4-di*-O-*benzyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranoside* (**4**). — Compound **4** was prepared from **3** (1.01 g) analogously to **2** by using 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-D-mannopyranosyl chloride[7] (1.29 g), molecular sieves (3 g, 3Å), and silver triflate (0.83 g)–2,4,6-trimethylpyridine (0.39 g) in dry toluene. The product was purified on silica gel with light petroleum–ethyl acetate (4:1), to yield **4** as a syrup (1.65 g, 91%), $[\alpha]_{578}^{22}$ +20° (*c* 1.4, chloroform); R_F 0.71 (toluene–ethyl acetate, 3:1); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 20.7, 20.9 (OAc), 63.4 (C′-6), 69.0–80.1 (ring C, Ph*C*H_2, C-6), 98.1 (C-1, $^1J_{C\text{-}1H\text{-}1}$ 169 Hz)[13], 99.5 (C′-1, $^1J_{C\text{-}1,H\text{-}1}$ 172 Hz)[13], 127.3–138.6 (aromatic), 169.7, and 170.5 (*C*=O).

Benzyl 3,4,6-tri-O-*benzyl-2*-O-(*2,4-di*-O-*benzyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranoside* (**5**). — Compound **4** was deacetylated as described for **2** and the product was purified on silica gel with toluene–ethyl acetate (6:1), to yield **5** as a syrup (0.98 g, 74%), $[\alpha]_{578}^{22}$ +32° (*c* 1, chloroform); R_F 0.37 (toluene–ethyl acetate, 3:1); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 62.0 (C′-6), 69.0–80.0 (ring C, Ph*C*H_2, C-6), 98.0 (C-1), 98.7 (C′-1), and 127.3–138.4 (aromatic).

Benzyl 3,4,6-tri-O-*benzyl-2*-O-{*2,4-di*-O-*benzyl-3,6-di*-O-[*3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-4*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-α-D-*mannopyranosyl*}-α-D-*mannopyranoside* (**7**). — A mixture of silver triflate (766 mg), 2,4,6-trimethylpyridine (360 mg), compound **5** (390 mg), and ground molecular sieves (2 g, 3Å) in dry dichloromethane (30 mL) was cooled to −40° under nitrogen. Bromide **6**[8,9] (1.57 g) in dichloromethane (10 mL) was added dropwise during 1 h with stirring. The mixture was allowed to attain room temperature, kept overnight, and then worked-up as described above. The product was purified on silica gel with toluene–ethyl acetate (3:2), to yield **7** as a syrup (398 mg, 39%), $[\alpha]_{578}^{22}$ +0.5° (*c* 0.9, chloroform); R_F 0.17 (solvent as above); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 20.5 (OAc), 54.8, 55.1 (C-2^e, C-2^d), 60.7–80.4 (ring C, Ph*C*H_2, C-6^{a-f}), 96.1 (C-1^a), 97.7 (C-1^b), 98.3, 98.4 (C-1^c, C-1^d), 101.0 (C-1^{e,f}), 120.9–138.7 (aromatic), and 167.4–170.3 (*C*=O).

Benzyl 3,4,6-tri-O-benzyl-2-O-{3,6-di-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-di-O-benzyl-α-D-mannopyranosyl}-α-D-mannopyranoside (**8**). — A catalytic amount of sodium was added to a solution of **7** (381 mg) in methanol (30 mL); the mixture was left at room temperature overnight, neutralised with acetic acid, and concentrated to dryness. The product was dissolved in ethanol (30 mL), hydrazine hydrate (1.4 mL) was added, and the solution was boiled under reflux for 6 h, cooled, and concentrated to dryness. The product was then acetylated with acetic anhydride–pyridine (1:1, 30 mL) at 100° for 30 min. After concentration, the product was purified on silica gel with chloroform–acetone (2:1), to yield **8** as a syrup (256 mg, 73%), $[\alpha]_{578}^{22}$ +3 (*c* 1, chloroform); R_F 0.39 (solvent as above); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 20.6, 20.8, 23.0, 24.9 (NHAc, OAc), 53.5, 54.5 (C-2^c, C-2^d), 60.9–79.9 (ring C, $PhCH_2$, C-6^{a-f}), 98.4 (C-1^a), 99.6 (C-1^b), 99.9 (C-1^d), 100.6, 100.9, 101.1 (3 C, C-1^c, C-1^e, C-1^f), 127.7–138.9 (aromatic), and 169.1–171.6 (C=O).

2-O-{3,6-Di-O-[O-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranosyl}-D-mannose (**1**). — A catalytic amount of sodium was added to a solution of **8** (235 mg) in dry 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 (20 mL) and hydrogenolysed at 400 kPa over 10% palladium-on-charcoal (420 mg). After filtration, the product was desalted on a column (2.5 × 90 cm) of Sephadex G-15 by elution with water. After freeze-drying, **1** was obtained as an amorphous powder (93 mg, 78%), $[\alpha]_{578}^{22}$ −7 (*c* 0.9, water); R_F 0.20 (ethyl acetate–acetic acid–methanol–water, 4:3:3:2); ^{1}H-n.m.r. (99.60 MHz, D_2O, 85°) δ 2.06 (s, 6 H, NHAc), 4.49 (d, 2 H, $J_{1,2}$ 7.5 Hz, H-1^{e,f}), 4.61 (broad d, 1 H, $J_{1,2}$ ~8 Hz, H-1^c), 4.71 (broad d, 1 H, $J_{1,2}$ ~8 Hz, H-1^d), 5.04 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^b), 5.30 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^a); ^{13}C-n.m.r. (25.05 MHz, D_2O): δ 23.5 (2 C, NHAc), 56.3, 56.4 (2 C, C-2^c, C-2^d), 59.1–80.4 (ring C, C-6^{a-f}), 93.5 (C-1^a), 100.3 (C-1^b), 102.7 (C-1^d), 103.3 (C-1^c), and 104.1 (2 C, C-1^{e,f}). No signals which could be assigned to the β-form of **1** were discernible.

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