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Note

Blood-group Ii-active oligosaccharides. Synthesis of *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-D-mannose

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In a preceding paper[1], we described the synthesis of the trisaccharide *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-D-mannose; this sequence had been observed[2] in the carbohydrate chain of some glycopeptides isolated from calf-thymocyte, plasma membranes, where two, outer lactosamine-chains are linked to C-3 and C-6 of the same outer D-mannose residue. These structures may be postulated to be present also in the major sialoglycoprotein of human erythrocyte membranes and be responsible for some blood-group Ii activity[3]. These considerations prompted us to synthesize oligosaccharides that could be used in hemagglutination-inhibition studies. We now report the synthesis of the trisaccharide *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-D-mannose (**7**). A similar sequence where the terminal, D-mannose reducing unit is replaced by a D-galactose residue has been shown[4] to be recognized by two anti-I sera (Ma and Woj).

In 1972, Matta and Bahl[5] coupled 2-methyl-[3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline with 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose in 26% yield. When the condensation of the disaccharide oxazoline, 2-methyl-[3,6-di-*O*-acetyl-1,2-dideoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline[6] (**4**) was attempted with the same D-mannopyranosyl derivative, intractable mixtures were always obtained. We observed that prolonged heating at 60° of the D-mannopyranosyl derivative alone, in the presence of catalytic amounts of *p*-toluenesulfonic acid, gave rise to many compounds susceptible of coupling with the glycosylating agent.

An acid-stable derivative of D-mannopyranose was therefore required for condensation with a disaccharide oxazoline. Benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**3**) was easily prepared by *O*-benzylation of benzyl 6-*O*-trityl-α-D-mannopyranoside[1] (**1**), followed by acid hydrolysis of the trityl group. Compound 3

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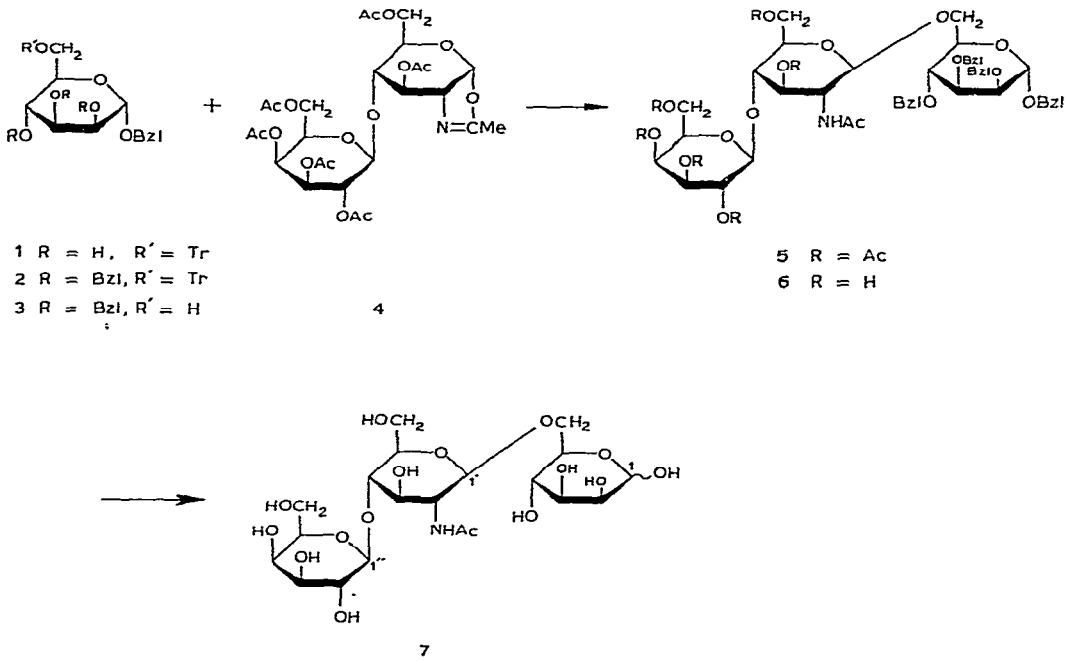

was found to be identical with the compound obtained[7] by hydrogenolysis of benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside with lithium aluminium-hydride–aluminium chloride.

Condensation of **3** with oxazoline **4** was conducted in 1,2-dichloroethane for 60 h at 60°, in the presence of *p*-toluenesulfonic acid, and gave the protected trisaccharide **5** as a syrup in 52% yield. *O*-Deacetylation afforded **6**, crystallized as a hemihydrate. Removal of the *O*-benzyl groups by catalytic hydrogenolysis gave the free trisaccharide **7**, obtained as an amorphous solid. Its 250-MHz ^{1}H-n.m.r. spectrum showed two signals, at δ 5.12 and 4.85, corresponding to the anomeric proton of the reducing D-mannose unit, which exists as two α- and β-D-pyranose configurations in a ratio of ~1 : 1. The β-D configuration of the 2-acetamido-2-deoxy-D-glucopyranosyl residue was confirmed by the signal of the anomeric proton (δ 4.56, $J_{1',2'}$ 7.5 Hz). The anomeric proton of the terminal D-galactose residue gave a signal at the usual location[4] (δ 4.45, $J_{1'',2''}$ 7.5 Hz).

EXPERIMENTAL

General methods. — Optical rotations were measured at 20° with a Roussel–Jouan electronic, digital micropolarimeter. N.m.r. spectra were recorded at 250 MHz with a Cameca model STN 250 spectrometer, equipped with a Fourier-transform unit for solutions in [^{2}H]chloroform and with tetramethylsilane as internal standard, or in deuterium oxide with tetramethylsilane (0.2% solution in [^{2}H]chloroform) as external reference. T.l.c. was performed on plates of silica gel (with fluorescence

indicator; layer thickness 0.25 mm; E. Merck, Darmstadt, Germany). The compounds were detected by spraying the plates with 1:19 (v/v) conc. sulfuric acid–ethanol. Silica gel Merck (70–325 mesh; E. Merck) was used for column chromatography. Paper chromatography was performed on Whatman No. 1 paper. Free sugars were detected with the aniline hydrogenphthalate reagent. Microanalyses were performed by the Laboratoire Central de Micro-Analyse du C.N.R.S.

*Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-trityl-α-*D*-mannopyranoside* (**2**). — A solution of benzyl 6-*O*-trityl-α-D-mannopyranoside (**1**) (5.12 g, 10 mmol) in *N,N*-dimethylformamide (60 mL) was treated with sodium hydride (1.20 g, 50 mmol) and benzyl bromide (6 mL 50 mmol) for 20 h at room temperature. The excess of hydride was decomposed by the addition of methanol to the ice-cooled mixture. Ether (600 mL) was added, and the solution was washed with water, dried (magnesium sulfate), and evaporated. The residue was chromatographed on silica gel with 1:9 (v/v) ether–light petroleum, to give **2** as a syrup (5.2 g, 67%), $[\alpha]_D^{20}$ +36° (*c* 0.84, chloroform).

Anal. Calc. for $C_{53}H_{50}O_6$: C, 81.30; H, 6.44; O, 12.26. Found: C, 81.13; H, 6.46; O, 12.09.

*Benzyl 2,3,4-tri-*O*-benzyl-α-*D*-mannopyranoside* (**3**). — A solution of **2** (2.20 g, 2.8 mmol) in acetone (36 mL) and M hydrochloric acid (4 mL) was heated under reflux for 2 h. The cooled mixture was neutralized with solid sodium hydrogencarbonate, and the acetone was evaporated. The residue was extracted with chloroform; the extract was washed with water, dried (magnesium sulfate), and evaporated. The resulting material was chromatographed on silica gel with 1:9 (v/v) ethyl acetate–toluene to give **3** as a syrup (1.14 g, 75%), $[\alpha]_D^{20}$ +55° (*c* 1.48, chloroform); lit.[6] $[\alpha]_D$ +54° (*c* 0.3, chloroform).

Anal. Calc. for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71; O, 17.76. Found: C, 75.27; H, 6.73; O, 17.92.

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**). — To a solution of **3** (0.727 g, 1.35 mmol) in dry 1,2-dichloroethane (25 mL) were added anhydrous *p*-toluenesulfonic acid (30 mg) and a solution of the oxazoline[6] **4** (0.850 g, 1.38 mmol) in dry 1,2-dichloroethane (8.5 mL). The mixture was stirred under nitrogen for 60 h at 60°, further addition of **4** (0.850 g, 1.38 mmol) and *p*-toluenesulfonic-acid (40 mg) being made after 24 h. T.l.c. in 7:7:1 (v/v) benzene–ether–methanol showed the presence of unchanged alcohol **3** (R_F 0.92), a major new compound (R_F 0.60), and only traces of oxazoline **4** (R_F 0.48). The reaction was stopped at this stage, as many decomposition products arising from the oxazoline had begun to appear. The solution was cooled, diluted with dichloromethane, washed with a saturated solution of sodium hydrogencarbonate and then with water, dried (magnesium sulfate), and evaporated. The residue was chromatographed on silica gel with 1:2 (v/v) ethyl acetate–toluene to give **5** as a syrup (0.805 g, 52%), $[\alpha]_D^{20}$ +9° (*c* 1.51, chloroform); n.m.r.: δ 7.36–7.20 (20 H, 4 Ph), 5.56 (d, 1 H, *J* 9 Hz, NH), 5.36 (d, 1 H, $J_{3'',4''} = J_{4'',5''}$ 3.5 Hz, H-4″), 2.16, 2.06, 2.04, 2.02, and 1.96 (18 H, 6 OAc), and 1.82 (s, 3 H, NAc).

Anal. Calc. for $C_{60}H_{71}NO_{22}$: C, 62.22; H, 6.17; N, 1.21; O, 30.40. Found: C, 62.10; H, 6.30; N, 1.04; O, 29.80.

Benzyl O-β-D-*galactopyranosyl*-(*1→4*)-O-(*2-acetamido-2-deoxy-β-D-glucopyranosyl*)-(*1→6*)-*2,3,4-tri-O-benzyl-α-D-mannopyranoside* (**6**). — Compound **5** (570 mg) was *O*-deacetylated overnight at room temperature with 50mM sodium methoxide in methanol (20 mL). Methanol (110 mL) was added to dissolve a white precipitate, and the solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin, filtered, and evaporated. The residue crystallized from methanol to give **6** (315 mg, 70%), m.p. 210–211°, $[\alpha]_D^{20}$ +11° (*c* 1.38, chloroform).

Anal. Calc. for $C_{48}H_{59}NO_{16} \cdot 0.5\ H_2O$: C, 63.01; H, 6.61; N, 1.53; O, 28.85. Found: C, 62.98; H, 6.55; N, 1.39; O, 29.02.

O-β-D-*Galactopyranosyl*-(*1→4*)-O-(*2-acetamido-2-deoxy-β-D-glucopyranosyl*)-(*1→6*)-D-*mannose* (**7**). — A solution of **6** (490 mg) in glacial acetic acid (40 mL) was hydrogenated catalytically in the presence of 10% palladium-on-charcoal (500 mg) for 3 days at room temperature and atmospheric pressure. The mixture was evaporated to dryness, without removal of the catalyst. The residue was applied to a column of silica gel. Elution with 1:3:3, and then 1:2:2 (v/v) water–ethyl acetate–2-propanol afforded pure trisaccharide **7** as an amorphous solid (175 mg, 50%), $[\alpha]_D^{20}$ −12° (at equilibrium, *c* 1.72, water). This material showed a single spot in t.l.c. on silica gel with 3:3:2 (v/v) water–ethyl acetate–2-propanol (R_{Glc} 0.46), and in paper chromatography with 2:1:2 (v/v) (upper layer) ethyl acetate–pyridine–water (R_{Glc} 0.50 and $R_{lactose}$ 0.76); n.m.r. (D_2O): δ 5.12 (s, 0.5 H, 1-Hβ), 4.85 (s, 0.5 H, 1-Hα); 4,56 (d, 1 H, $J_{1',2'}$ 7.5 Hz, 1′-H), 4.45 (d, 1 H, $J_{1'',2''}$ 7.5 Hz, 1″-H), and 2.03 (s, 3 H, NAc).

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