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SYNTHESIS OF *O*-α-D-MANNOPYRANOSYL-(1→2)-*O*-α-D-MANNOPYRANOSYL-(1→2)-D-MANNOSE, THE REPEATING UNIT OF THE O8-ANTIGEN OF *Escherichia coli**

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ABSTRACT

The trisaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man was synthesised by using a stepwise method. A key reaction in the preparation of the intermediates was the selective hydrogenolysis of mannopyranoside derivatives having both dioxane- and dioxolane-type benzylidene acetals, resulting in the exclusive cleavage of the former acetal rings.

INTRODUCTION

Escherichia coli strains belonging to serogroups O8 and O9 are particularly important, in that only these two groups form capsular K-antigens and their O-specific polysaccharides are D-mannans.

The investigation of Reske and Jann[1] indicated that the mannan is a carrier rather than an antigen. When the O8 antigenic lipopolysaccharide was treated with mild acid, its serological specificity was lost: although the polysaccharide was not hydrolysed, it is possible that an acid-labile substituent is necessary for full antigenic activity.

Based on the results of methylation analysis, periodate oxidation, and treatment with α- and β-D-mannosidases, an α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man repeating-unit connected by (1→3)-α linkages was proposed.

The proposed trisaccharide repeating-unit was isolated by two different groups[2,3] after acetolysis of yeast mannan, but a chemical synthesis has not been reported. Interest in the synthesis of D-mannose-containing oligosaccharides[4–6] has been promoted by the fact that several glycopeptides isolated from the intestinal fluids of human patients suffering from various inherited disorders contain cores that are complex D-manno-oligosaccharides[7].

Two approaches to the title trisaccharide were considered, involving the reaction between (*a*) an appropriate mannobiose derivative having HO-2′ unsubstituted and a

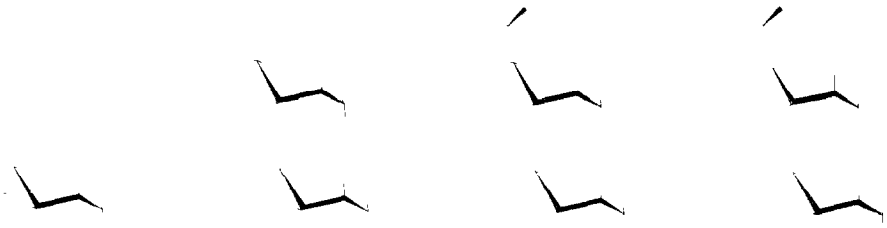
*After the submission of this paper, a synthesis of the title compound was reported by T. Ogawa and H. Yamamoto, *Carbohydr. Res.*, 104 (1982) 271–283.

C-1-activated D-mannose derivative, or (*b*) an appropriate C-1-activated mannobiose derivative and a suitable mannopyranose/mannopyranoside derivative having HO-2 unsubstituted. Since a glycosyl substituent at position 2 is a non-participating group, the glycosyl halide derived from a (1→2)-linked mannobiose derivative would be expected to give a mixture of anomers in the Koenigs–Knorr reaction[8,9], and the first route was therefore chosen.

RESULTS AND DISCUSSION

The dioxolane ring of 2,3:4,6-di-*O*-benzylidene-α- and -β-D-mannopyranoside can be selectively cleaved[10–12] with the $LiAlH_4$–$AlCl_3$ reagent, to give 2-*O*-benzyl-4,6-*O*-benzylidene- or 3-*O*-benzyl-4,6-*O*-benzylidene-mannopyranoside, depending on the *endo*- or *exo*-configuration of the phenyl substituent of the dioxolane ring. This reaction selectively distinguishes between the 5- and 6-membered acetal rings[12].

Benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[10] (**1**), prepared by the hydrogenolysis of benzyl *exo*-2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside[10], was treated with tetra-*O*-acetyl-α-D-mannopyranosyl bromide, using the Helferich method, to afford the crystalline disaccharide derivative **2**, in which only the non-reducing end carries base-sensitive blocking groups. The structure of **2** was confirmed by its ^{13}C-n.m.r. spectrum, and the assignments were based on the spectra[13] of **1** and methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside. The α linkage was indicated by the $J_{C-1,H-1'}$ value of 172.0 Hz.



Zemplén saponification of **2** yielded crystalline **3** which, with benzaldehyde–$ZnCl_2$ or α,α-dimethoxytoluene–toluene-*p*-sulphonic acid, gave a ~1:1 mixture of the tri-*O*-benzylidene derivatives **4** and **5**. The pure, crystalline isomers were isolated by column chromatography. The similar solubility and ability to crystallise of **4** and **5** explains the formation of a ~1:1 mixture (*cf.* the behaviour of benzyl α-D-mannopyranoside[10,12]).

The structures of **4** and **5** were assigned on the basis of ^{1}H- and ^{13}C-n.m.r. data. In the *exo*-isomer **4**, the 4,6- and 4′,6′-*O*-benzylidene acetal protons resonated at 5.45 p.p.m. and the 2′,3′-*O*-benzylidene acetal proton at 6.07 p.p.m., whereas, in

TABLE I

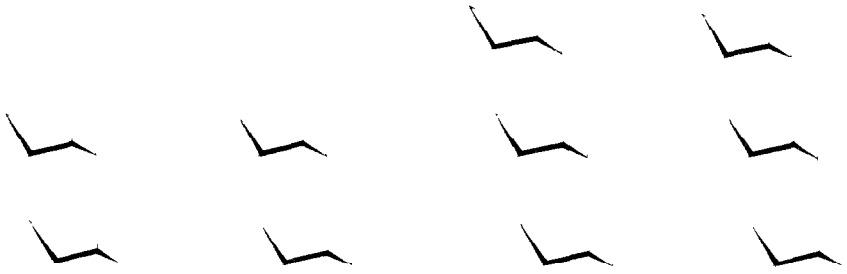
^{13}C-N.M.R. DATA[a] (CHEMICAL SHIFTS IN P.P.M. AND, IN BRACKETS, $^{1}J_{C,H}$ VALUES IN Hz)

Carbon atom	A[b]	**1**	**2**	**4**	**5**	**6**	**8**	**10**
1	98.8 (172.5)	99.5	99.2	100.1	100.1	99.1	99.1 (171.2)	92.5
1′			99.6 (172.0)	99.1	99.2	99.5	99.6 (174.0)	100.5
1″							99.6 (174.0)	102.1
2	69.3	70.1	77.1	76.0	76.0	77.0	76.9	79.1
2′			69.3	75.5	78.4	70.2	77.1	78.3
2″							69.4	70.4
3	68.8	75.9	75.9	76.6	76.6	76.0	75.5	69.9
3′			68.7	77.6	74.1	76.0	76.2	69.9
3″							69.0	69.9
4	66.6	79.0	79.4	79.3	79.4	79.3	79.5	66.8
4′			66.6	75.5	80.6	79.0	79.4	67.1
4″							66.5	67.1
5	69.8	63.7	64.5	64.4	64.4	64.6	64.5	73.2
5′			69.4	61.0	61.0	63.9	64.9	73.2
5″							69.4	72.5
6	62.8	68.9	69.3	69.2	69.2	69.2	69.4	61.0
6′			62.7	68.9	68.9	68.9	69.1	61.0
6″							62.5	61.0
1-*O*-Benzyl CH_2		69.4	69.4	69.2	69.2	69.1	69.6	
q		(137.1)	(137.1)	(137.0)	(137.0)	(137.2)	(137.0)	
3-*O*-Benzyl CH_2		73.1	73.3	73.4	73.4	73.2	73.5	
q						73.4	76.2	
		(138.1)	(138.7)	(138.8)	(138.8)	(138.1)	(138.1)	
						(138.3)	(138.3)	
2,3-*O*-Benzylidene *C*H				103.1	104.1			
q				(139.1)	(137.6)			
4,6-*O*-Benzylidene *C*H		101.6	101 6	101.7	101.8	101.9	101.6	
q				102.0	101.8	102.0	101.7	
		(137.7)	(138.0)	(138.0)	(137.9)	(138.0)	(138.1)	
				(137.6)	(137.5)	(137.8)	(138.1)	

[a]In $CDCl_3$ for *A* and **1**–**8**; in D_2O for **10**. [b]Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside.

the *endo*-isomer **5**, the singlets at 5.47 and 5.31 p.p.m. were assigned to the dioxane rings, and the singlet at 5.73 p.p.m. was assigned[14] to the dioxolane ring. A similar situation[15] was found with the ^{13}C-n.m.r. data (Table I).

Hydrogenolysis of the *exo*-isomer **4** occurred under mild conditions and afforded benzyl 3-*O*-benzyl-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-4,6-*O*-benzylidene-α-D-mannopyranoside (**6**). The position of the benzyl group in **6** was determined on the basis of n.m.r. data. That the two dioxane rings were intact was indicated by the ^{1}H signals at 5.61 and 5.57 p.p.m. 2-*O*-Benzyl-α-D-mannopyranosides show characteristic negative β-shifts[12], the anomeric carbons resonating



at higher field (~97–98 p.p.m.) than those of the 3-*O*-benzyl isomers (~99–100 p.p.m.); since C-1′ of **6** resonated at 99.5 p.p.m., the benzyl group was located at position 3.

At higher temperature or on prolonged reaction time, not only the dioxolane ring of **4** but also one of the two dioxane rings was cleaved to yield a crystalline mono-*O*-benzylidene derivative (**7**). Methylation of **7**, followed by catalytic hydrogenolysis (Pd/C) and hydrolysis, gave D-mannose and 2,6-di-*O*-methyl-D-mannose; thus, **7** was benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside.

Reaction of **6** with tetra-*O*-acetyl-α-D-mannopyranosyl bromide, using the Helferich procedure, gave the syrupy trisaccharide derivative **8** in which the α configuration of the new inter-glycosidic bond was indicated by the $J_{C\text{-}1,H\text{-}1}$ value of 174 Hz. Saponification of **8** gave crystalline **9** which, on catalytic hydrogenolysis (Pd/C) in ethanol–acetic acid, gave the title trisaccharide (**10**) as an amorphous powder.

The ^{13}C-n.m.r. spectra of many (1→2)-linked di- or oligo-saccharides are complex[16,17], due to the fact that the reducing unit may exist as a mixture of anomers and/or pyranoid and furanoid forms which, in turn, markedly affects the signal for C-1′. In some cases, the doubling of signals of other carbons of the first non-reducing unit was observed[18,19]. The spectrum of **10** was simple (Table I), because the α anomer preponderated and only traces of the β anomer could be detected in solution.

EXPERIMENTAL

General methods. – Melting points (uncorrected) were determined with a Kofler apparatus. Reactions were monitored by t.l.c. on DC Alurolle Kieselgel 60 F 254 (Merck) with detection by charring with sulphuric acid. Kieselgel G (Reanal) was used for short-column chromatography. Optical rotations were measured with a Perkin–Elmer 241 automatic polarimeter. ^{1}H-N.m.r. spectra were recorded with a Jeol MH-100 (100 MHz) instrument (internal Me_4Si), and ^{13}C-n.m.r. spectra with

Varian XL-100-15 FT or Bruker WP-200 SY spectrometers for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal 1,4-dioxane).

Benzyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**2**). — To a stirred solution of **1**[10] (4 g) in a 1:1 mixture (125 mL) of dry benzene and nitromethane were added mercuric cyanide (4 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (4 g), and stirring was continued for 2 h at 50°, when more mercuric cyanide (1 g) and glycosyl bromide (1 g) were added. After 16 h, the mixture was cooled, filtered, and concentrated. A solution of the residue in dichloromethane (100 mL) was filtered, washed successively with 5% aqueous potassium iodide (3 × 20 mL) and water (5 × 20 mL), dried (Na_2SO_4), and concentrated. Crystallisation of the syrupy product from ethanol (50 mL) gave **2** (3.67 g, 52.8%), m.p. 106–107°, $[\alpha]_D$ +58° (*c* 0.9, chloroform), R_F 0.82 (dichloromethane–acetone, 19:1).

Anal. Calc. for $C_{41}H_{46}O_{15}$: C, 63.23; H, 5.95. Found: C, 62.94; H, 5.98.

Benzyl 3-O-benzyl-4,6-O-benzylidene-2-O-α-D-mannopyranosyl-α-D-mannopyranoside (**3**). — Compound **2** (3.42 g) was stirred with dry methanol (100 mL) containing sodium methoxide (50 mg). The solution was then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated, to give **3** (2.5 g, 93.3%), m.p. 170–172°, $[\alpha]_D$ +68° (*c* 0.5, chloroform), R_F 0.22 (chloroform–methanol, 9:1). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.30–6.90 (m, 15 H, 3 Ph), 5.44 (s, 1 H, PhC*H*), 4.96 and 4.80 (2 s, 2 H, H-1,1′), 4.66-4.24 (m, 4 H, 2 PhCH_2), 4.12–3.30 (m, 12 H, skeleton protons), 2.64 and 2.50 (2 bs, 4 H, 4 OH).

Anal. Calc. for $C_{33}H_{38}O_{11}$: C, 64.91; H, 6.27. Found: C, 65.10; H, 6.14.

Benzyl 3-O-benzyl-4,6-O-benzylidene-2-O-(exo-2,3:4,6-di-O-benzylidene-α-D-mannopyranosyl)-α-D-mannopyranoside (**4**) *and its* endo-*isomer* (**5**). — A mixture of **3** (2.4 g), freshly fused $ZnCl_2$ (2.4 g), and freshly distilled benzaldehyde (20 mL) was shaken for 48 h at room temperature, diluted with dichloromethane (200 mL), and washed with aqueous 5% sodium hydrogencarbonate (3 × 20 mL). The organic layer was concentrated and the residue was steam distilled in the presence of a small amount of sodium hydrogencarbonate to remove the excess of benzaldehyde. The residue was extracted with dichloromethane (3 × 50 mL), and the combined extracts were washed with water (3 × 20 mL), dried, and concentrated. T.l.c. (chloroform) of the residue revealed two products, R_F 0.70 and 0.55, in the ratio 3:2.

Column chromatography gave, first, the syrupy *exo*-isomer **4**, which was crystallised from ethanol to give a product (810 mg, 26.2%) having m.p. 161–163°, $[\alpha]_D$ +22° (*c* 0.66, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.50–6.90 (m, 25 H, 5 Ph), 6.07 (s, 1 H, dioxolane PhC*H*), 5.45 (s, 2 H, 2 dioxane PhC*H*), 5.27 (s, 1 H, H-1′), 4.80–4.35 (m, 5 H, H-1 and 2 PhCH_2), and 4.32–3.48 (m, 12 H, skeleton protons).

Anal. Calc. for $C_{47}H_{46}O_{11}$: C, 71.74; H, 5.89. Found: C, 71.49; H, 5.93.

Eluted second was the syrupy *endo*-isomer (**5**), which was crystallised from ethanol to yield a product (710 mg, 23%) having m.p. 162–163°, $[\alpha]_D$ −2° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.50–6.90 (m, 25 H, 5 Ph), 5.73 (s, 1 H,

dioxolane PhC*H*), 5.47 and 5.31 (2 s, 2 H, 2 dioxane PhC*H*), 5.26 (s, 1 H, H-1′), 4.80–4.26 (m, 5 H, H-1 and 2 PhCH_2), and 4.22–3.50 (m, 12 H, skeleton protons).

Anal. Calc. for $C_{47}H_{46}O_{11}$: C, 71.74; H, 5.89. Found: C, 71.98; H, 5.87.

Reaction of **4** *with the Li*AlH_4–$AlCl_3$ *reagent.* — (*a*). To a solution of **4** (810 mg) in dichloromethane (10 mL) and ether (5 mL) were added lithium aluminium hydride (80 mg) and an ethereal solution (5 mL) of aluminium chloride (275 mg), and the mixture was kept for 1 h at room temperature. The excess of reagent was decomposed by the addition of ethyl acetate (10 mL) and water (10 mL). The mixture was then diluted with dichloromethane (200 mL), filtered, washed with water (5 × 20 mL), dried, and concentrated. The major product, contaminated by a by-product (1–2%, t.l.c.), was purified by column chromatography (7:3 light petroleum–ethyl acetate), to give **6** (415 mg, 51.1%) as a syrup, $[\alpha]_D$ +43° (*c* 0.6, chloroform), R_F 0.47 (7:3 light petroleum–ethyl acetate). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60–7.10 (m, 25 H, 5 Ph), 5.61 and 5.57 (2 s, 2 H, 2 PhC*H*), 5.14 (d, 1 H, $J_{1',2'}$ 0.7 Hz, H-1′), 4.95–4.38 (m, 7 H, H-1 and 3 PhCH_2), 4.30–3.60 (m, 12 H, skeleton protons), and 2.81 (b, 1 H, HO-2′).

Anal. Calc. for $C_{47}H_{48}O_{11}$: C, 71.56; H, 6.13. Found: C, 71.24; H, 6.02.

(*b*) To a solution of **4** (0.6 g) in dichloromethane (15 mL) and ether (5 mL) were added lithium aluminium hydride (200 mg) and an ethereal solution (5 mL) of aluminium chloride (600 mg). The mixture was stirred under reflux and then worked-up as described in (*a*). The product was purified by column chromatography and then by recrystallisation from ether–light petroleum, to give **7** (430 mg, 71.3%), m.p. 72.5–73.5°, $[\alpha]_D$ +51° (*c* 0.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.50–7.18 (m, 25 H, 5 Ph), 5.59 (s, 1 H, PhC*H*), 5.14 (s, 1 H, H-1′), 4.96–3.76 (m, 21 H, 3 PhCH_2 and skeleton protons), 2.76 (s, 1 H, HO-2′), and 2.00 (t, 1 H, HO-6′).

Anal. Calc. for $C_{47}H_{50}O_{11}$: C, 71.38; H, 6.37. Found: C, 71.28; H, 6.35.

*Benzyl 3-*O*-benzyl-2-*O*-[3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranosyl]-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**8**). — To a stirred solution of **6** (415 mg) in 1,1 benzene–nitromethane (20 mL) were added mercuric cyanide (100 mg) and 2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl bromide (100 mg). After stirring for 2 h at 55°, second portions of the above reagents were added and stirring was continued for 4 h. After work-up, as described for **2**, the crude product was purified by column chromatography (13:7 hexane–ethyl acetate), to give **8** (390 mg, 67%), as a syrup, $[\alpha]_D$ +34° (*c* 0.6, chloroform). R_f 0.64 (13:7 hexane–ethyl acetate).

Anal. Calc. for $C_{61}H_{66}O_{20}$: C, 65.47; H, 5.95. Found: C, 66.00; H, 5.88.

*Benzyl 3-*O*-benzyl-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-α-*D*-mannopyranosyl-α-*D*-mannopyranosyl)-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**9**). — Deacetylation of **8** (390 mg), as described above for **3**, gave **9** (320 mg, 96.4%), m.p. 100–102° (from ethanol), $[\alpha]_D$ +48° (*c* 0.8, chloroform), R_f 0.38 (dichloromethane–methanol, 9:1).

Anal. Calc. for $C_{53}H_{58}O_{16}$: C, 66.94; H, 6.15. Found: C, 67.08; H, 6.05.

O-α-*D*-*Mannopyranosyl*-(1→2)-O-α-*D*-*mannopyranosyl*-(1→2)-*D*-*mannose* (**10**).

— To a solution of **9** (320 mg) in ethanol (10 mL) and acetic acid (5 mL) was added 10% Pd/C (100 mg) suspended in ethanol (15 mL), and the mixture was hydrogenolysed for 16 h. The mixture was filtered and concentrated, to give amorphous **10** (133 mg, 80.1%), $[\alpha]_D$ +48° (*c* 0.8, water), R_F 0.28 (1-butanol–ethanol–water, 2:1:1); lit.[2] $[\alpha]_D$ +55° (*c* 1.5, water).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 9.39. Found: C, 42.78; H, 6.51.

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