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Note

Synthesis of *p*-trifluoroacetamidophenyl *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→4)-α-L-rhamnopyranoside

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The *Salmonella* lipopolysaccharides belonging to serogroups A, B, and D[1] have the following repeating-unit in the O-specific side-chains:

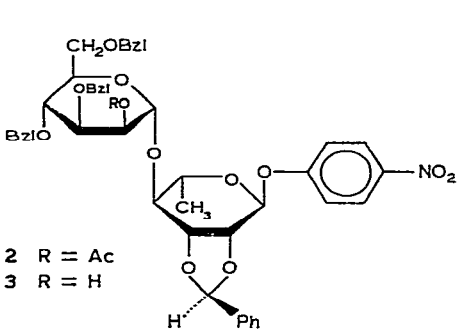
3,6-Dideoxy-α-hex*p*
1
↓
3
→2)-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-α-D-Gal*p*-(1–

in which the 3,6-dideoxyhexose is paratose, abequose, or tyvelose. Additional structural features may include acetylation of some sugar residues and α-D-glucopyranosyl groups attached to some of the galactose residues[2].

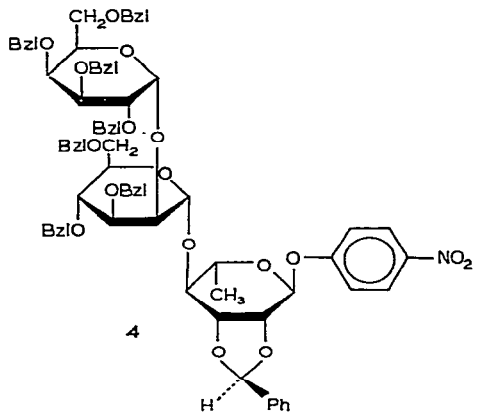
For immunological purposes, it is desirable to evaluate the importance, in the antibody response, of the main chain in these three serogroups. The disaccharide *p*-nitrophenyl 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranoside has been synthesised[3] and, after reduction of the nitro group to an amino group, attached to bovine serum albumin[4]. Only a low antibody-response was obtained in biological tests for this antigen[5]. For this reason, the above galactosylmannosylrhamnoside trisaccharide unit was needed. Another reason for making this compound was the fact that phage hydrolysis of these lipopolysaccharides cleaves the rhamnosylgalactosyl bond, releasing oligomers terminated by a galactosyl group[6]. Again, the immunological importance of the main chain, terminated by a galactosylmannosylrhamnosyl unit, had to be evaluated as part of a programme directed towards the production of improved diagnostics and vaccines for *Salmonella* infections. We now describe the synthesis of the trisaccharide derivative *O*-α-D-Gal*p*-(1→2)-*O*-α-D-Man*p*-(1→4)-*O*-α-L-Rha*p*-(1→O)-C_6H_4-*p*-NHCOCF_3 (**1**). The *p*-trifluoroacetamidophenyl group is readily hydrolysed to a *p*-aminophenyl group, which can be used for the formation of a linkage to proteins[4].

p-Nitrophenyl 2,3-*O*-(*S*)-benzylidene-α-L-rhamnopyranoside[3] was glycosylated

at O-4 by using 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[7,8] with silver triflate as promoter, to give the α-linked disaccharide **2**. This was deacetylated with methanolic sodium methoxide, to give **3**. Attempted galactosylation at O-2 of the mannose residue in **3** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide, using the halide-assisted method[10] or the triflate modification thereof[11], gave low yields of the required trisaccharide **4**. However, the silver triflate-promoted condensation[12] of **3** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride yielded the α-linked trisaccharide[4] in 76% yield. The nitro group in **4** was converted into the corresponding trifluoroacetamido group by hydrogenation over Adams' catalyst, followed by *N*-trifluoroacetylation. The remaining benzyl and benzylidene groups were removed by catalytic hydrogenolysis over palladium-on-carbon, to give the trisaccharide **1**. A convenient preparation of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[9] is given in the Experimental section. The overall yield of **1** from *p*-nitrophenyl 2,3-*O*-(*S*)-benzylidene-α-L-rhamnopyranoside was 44%.



2 R = Ac
3 R = H



4

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at a bath temperature below 40°. Melting points are corrected. Optical rotations were measured at 20–22° with a Perkin–Elmer 241 polarimeter. 99.55-MHz ^{1}H- and 25.05-MHz ^{13}C-n.m.r. spectra were recorded on a Jeol JNM FX 100 instrument in the Fourier-transform mode. For solutions in $CDCl_3$, chemical shifts were recorded in p.p.m. downfield from that of internal tetramethylsilane. For solutions in D_2O, the chemical shifts are given in p.p.m. downfield from that of external tetramethylsilane. T.l.c. was performed on precoated, Silica Gel F_{254} plates (Merck) with detection by charring with H_2SO_4. Column chromatography was performed on Merck prepacked columns of silica gel or on Merck silica gel (0.040–0.063 mm). G.l.c. was performed with a Perkin–Elmer 990 instrument equipped with an OV-225 column (3% on Gas-Chrom Q), and g.l.c.–m.s. with Perkin–Elmer 270 or Varian-MAT instruments equipped with OV-225 columns (3% on Gas-Chrom Q). Mass spectra were recorded at 70 eV.

The purity of new compounds, for which elemental analysis was not performed, was carefully ascertained by t.l.c. in solvent systems giving R_F values of ~0.5, and the substances were re-chromatographed until pure. N.m.r. data were recorded for all new compounds, and were invariably in agreement with the postulated structures. The anomeric purity of the glycosides and di- and oligo-saccharides was carefully ascertained by ^{13}C-n.m.r. spectroscopy, in which spectra having high signal-intensity in the anomeric region were recorded. Only especially significant n.m.r. data are presented.

p-*Nitrophenyl 4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-O-(S)-benzylidene-α-L-rhamnopyranoside* (**2**). — A solution of 3,4,6-tri-*O*-benzyl-1,2-*O*-(methoxyethylidene)-β-D-mannopyranose[7,8,13] (2.00 g) and trimethylsilyl chloride (0.5 g) in dry dichloromethane (20 ml) was boiled under reflux for 2 h, cooled, and concentrated. A solution of the resulting 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[7,8] in dry dichloromethane (10 ml) was added with stirring to a mixture of *p*-nitrophenyl 2,3-*O*-(*S*)-benzylidene-α-L-rhamnopyranoside (1.00 g), silver triflate (1.02 g), and 2,4,6-collidine in dry dichloromethane (15 ml) at −50°. The mixture was allowed to attain room temperature (30 min), diluted with dichloromethane, and filtered. The filtrate was washed successively with aqueous sodium thiosulfate and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue on silica gel (toluene–ethyl acetate, 8:1) afforded **2** (1.55 g, 68%), $[\alpha]_D$ −22.6° (*c* 0.75, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.28 (d, 3 H, $J_{5,6}$ 4.6 Hz, C-Me, Rha), 2.16 (s, 3 H, OAc), 5.04 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1, Man), 5.82 (s, 1 H, H-1, Rha), and 6.09 (s, 1 H, PhC*H*); ^{13}C-n.m.r. ($CDCl_3$) data: δ 17.4 (C-6, Rha), 21.1 (*C*H$_3$CO), 95.6 (C-1, Rha), 98.8 (C-1, Man), and 103.1 (benzylidene C). Sugar analysis[14,15] of **2** yielded equimolecular amounts of hexa-*O*-acetylmannitol and penta-*O*-acetylrhamnitol. Methylation analysis[16] of **2** gave equimolecular amounts of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol.

p-*Nitrophenyl 2,3-O-(S)-benzylidene-4-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl-α-L-rhamnopyranoside* (**3**). — A catalytic amount of sodium was added to a solution of **2** (1.00 g) in methanol (30 ml). The solution was kept at room temperature for 1 h, neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography of the residue on silica gel (toluene–ethyl acetate, 2:1) afforded **3** (910 mg, 96%), $[\alpha]_D$ − 12° (*c* 1.1, chloroform). 100-MHz, ^{1}H-n.m.r. data ($CDCl_3$): δ 1.18 (d, 3 H, $J_{5,6}$ 4.6 Hz, C-Me, Rha), 5.00 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1, Man), 5.75 (s, 1 H, H-1, Rha), and 5.09 (s, 1 H, PhC*H*); ^{13}C-n.m.r. data ($CDCl_3$): δ 17.4 (C-6, Rha), 95.4 (C-1, Rha), 100.5 (C-1, Man), and 103.0 (benzylidene C).

Anal. Calc. for $C_{46}H_{47}NO_2$: C, 68.6; H, 5.84; N, 1.73. Found: C, 68.5; H, 5.95; N, 1.68.

2,3,4,6-Tetra-O-benzyl-D-galactose[9]. — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.5 g) in chloroform (10 ml) was added with stirring during 20 min at room temperature to a solution of thiophenol (0.74 g) and potassium hydroxide (0.34 g) in ethanol (10 ml). The reaction was monitored by t.l.c. (chloro-

form–acetone, 10:1). After 20 min, the reaction mixture was diluted with chloroform, washed successively with aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. The crude product was dissolved in methanol (70 ml) and deacetylated by adding a catalytic amount of sodium. After 1 h at room temperature, the reaction mixture was concentrated and toluene was distilled several times from the residue to remove moisture. The crude product was dissolved in dry *N,N*-dimethylformamide (50 ml) and added to sodium hydride (0.65 g). After 30 min at room temperature, benzyl bromide (4.6 g) was added dropwise with stirring. After stirring for 5 h at room temperature, methanol was added and then excess of chloroform. The chloroform solution was washed with water, dried (Na_2SO_4), filtered, and concentrated, to afford thiophenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (2.7 g, 71%), m.p. 88–89°, $[\alpha]_D$ +1° (*c* 1.0, chloroform). Water (1 ml) and silver nitrate (140 mg) were added to a stirred solution of the galactoside (0.5 g) in acetone (5 ml) at room temperature. After 12 h, the mixture was filtered and concentrated, and the product was dissolved in dichloromethane. The solution was washed successively with aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. The 2,3,4,6-tetra-*O*-benzyl-D-galactose thus obtained was sufficiently pure for use in the next step. An aliquot was recrystallised from diethyl ether–hexane; m.p. 67–69°, $[\alpha]_D$ +70° (*c* 1.0, benzene); lit. m.p. 67–69°, $[\alpha]_D$ +74°.

p-*Nitrophenyl* O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*galactopyranosyl*)-(*1→2*)-O-(*3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl*)-(*1→4*)-[*2,3*-O-(S)-*benzylidene*-α-L-*rhamnopyranoside*] (**4**). — Oxalyl chloride (0.5 ml) was added dropwise at 0° to a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactose (250 mg) in dry *N,N*-dimethylformamide. After 1 h at room temperature, t.l.c. (toluene–ethyl acetate) showed that no starting-material remained. The mixture was diluted with dichloromethane and washed successively with water, aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), filtered, and concentrated. A solution of the resulting, crude 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[9] in toluene (1.5 ml) was added with stirring to a mixture of **3** (161 mg), silver triflate (132 mg), and 2,4,6-collidine (62 mg) in toluene (3.5 ml) at −70°. The mixture was allowed to attain room temperature, diluted with toluene, filtered, washed successively with aqueous sodium thiosulfate and water, dried (Na_2SO_4), filtered, and concentrated. Column chromatography of the residue on silica gel [light petroleum (b.p. 40–60°)–ethyl acetate–chloroform, 4:1:1] afforded **4** (203 mg, 76%), $[\alpha]_D$ +16° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.19 (d, 3 H, $J_{5,6}$ 6.0 Hz, C-Me, Rha), 5.06 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1, Man), 5.50 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1, Gal), 5.80 (s, 1 H, H-1, Rha), and 6.16 (s, 1 H, PhC*H*); ^{13}C-n.m.r. data ($CDCl_3$): δ 17.2 (C-6, Rha), 95.3 (C-1, Rha), 97.8 (C-1, Gal), 100.2 (C-1, Man), and 103.0 (benzylidene C).

Anal. Calc. for $C_{80}H_{81}NO_{17}$: C, 72.3; H, 6.10; N, 1.06. Found: C, 72.2; H, 6.19; N, 1.01.

p-*Trifluoroacetamidophenyl* O-α-D-*galactopyranosyl*-(*1→2*)-O-α-D-*mannopyranosyl*-(*1→4*)-α-L-*rhamnopyranoside* (**1**). — A solution of **4** (200 mg) in ethyl acetate (2 ml) was hydrogenated at room temperature and atmospheric pressure over

Adams' catalyst (40 mg). When sufficient hydrogen had been consumed ($NO_2 \rightarrow NH_2$), trifluoroacetic anhydride (0.5 ml) and pyridine (1.2 ml) were added, and the mixture was kept at 60° for 30 min, filtered, and concentrated. A solution of the residue in chloroform was extracted with water, dried (Na_2SO_4), filtered, and concentrated. The product was purified by column chromatography on silica gel (toluene–ethyl acetate, 12:1), to give a chromatographically pure syrup (t.l.c., same solvent; yield 195 mg, 93%), $[\alpha]_D$ +36° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.09 (d, 3 H, $J_{5,6}$ 6.0 Hz, C-Me, Rha), 5.00 (s, 1 H, H-1, Man), 5.45 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1, Gal), 5.62 (s, 1 H, H-1, Rha), and 6.08 (s, 1 H, PhC*H*); ^{13}C-n.m.r. data ($CDCl_3$): δ 17.3 (C-6, Rha), 95.4 (C-1, Rha), 97.73 (C-1, Gal), 100.2 (C-1, Man), and 102.9 (benzylidene C).

A solution of the product (150 mg) in acetic acid (50 ml) was hydrogenated at room temperature and atmospheric pressure over 10% palladium-on-carbon (20 mg). When t.l.c. (ethyl acetate–methanol–water, 6:3:1) showed no remaining starting-material, the mixture was filtered and the filtrate concentrated. Purification of the residue by chromatography on a column of Biogel P-2 yielded **1** (70 mg, 96%) as a chromatographically homogeneous syrup, $[\alpha]_D$ +18° (*c* 0.8, water). ^{1}H-N.m.r. data (D_2O): δ 1.22 (d, 3 H, $J_{5,6}$ 6.1 Hz, C-Me, Rha), 7.12 and 7.45 (2 d, each 2 H, $J_{H,H}$ 9.0 Hz, aromatic H); ^{13}C-n.m.r. data (D_2O): δ 18.2 (C-6, Rha), 99.09, 100.91, and 102.5 (C-1 of Rha, Man, and Gal).

Sugar analysis[14,15] of **1** yielded equimolecular amounts of hexa-*O*-acetylgalactitol, hexa-*O*-acetylmannitol, and penta-*O*-acetylrhamnitol. Methylation analysis[16] gave equimolar amounts of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylmannitol, and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol.

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