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SYNTHESIS OF THE TETRASACCHARIDE REPEATING-UNIT OF THE O-SPECIFIC POLYSACCHARIDE FROM *Salmonella senftenberg*

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ABSTRACT

The oligosaccharide β-D-Man-(1→4)-α-L-Rha (1→3)-D-Gal-(6←1)-α-D-Glc, which is the repeating unit of the O-specific polysaccharide chain of the lipopolysaccharide from *Salmonella senftenberg*, was obtained by glycosylation of benzyl 2,4-di-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside or benzyl 2-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside with 3-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-β-L-rhamnopyranose 1,2-(methyl orthoacetate) followed by removal of protecting groups

INTRODUCTION

O-Specific chains of lipopolysaccharides of Gram-negative bacteria are built of repeating oligosaccharide units, the structures of which are unique for each serological type[1]. The synthesis of these oligosaccharide units is of interest in studies of the biosynthesis and immunochemistry of microbial polysaccharides, and as a first stage in the chemical synthesis of O-specific polysaccharides.

Recently, we reported on the first synthesis of the repeating unit of the O-specific polysaccharide from *Salmonella anatum* and its analogues[2,3], and the β-D-glucopyranosyl analogue of the repeating unit[4] of the polysaccharide from *S. senftenberg*.

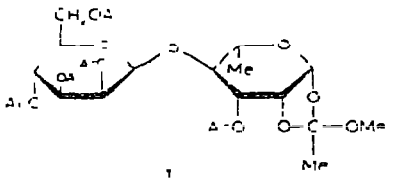
We now describe the synthesis of β-D-Man-(1→4)-α-L-Rha-(1→3)-D-Gal-(6←1)-α-D-Glc, which is the repeating tetrasaccharide unit[5] of the O-specific polysaccharide of *S. senftenberg* and its α-D-*manno* analogue α-D-Man-(1→4)-α-L-Rha-(1→3)-D-Gal-(6←1)-α-D-Glc.

RESULTS AND DISCUSSION

A route of synthesis involving consecutive addition of monosaccharide residues from the non-reducing end of the chain could not be developed due to lack of appropriate protecting groups for the synthesis of the α-D-Glc-(1→6)-D-Gal bond. An

alternative route included the formation of the *O*-α-D-glucopyranosyl-(1→6)-D-galactopyranose fragment, followed by glycosylation at position 3 of the galactopyranose moiety by a β-D-mannopyranosyl-(1→4)-L-rhamnopyranose derivative This scheme is analogous to that used for the synthesis of the glucose analogue of the tetrasaccharide[4]

Glycosylation of benzyl 2,4-di-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside[4] and benzyl 2-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside[4] with 3-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-β-L-rhamnopyranose 1,2-(methyl orthoacetate) (**1**) was carried out with molecular sieve 4 Å as an acceptor of the methanol formed[6], to yield the respective tetrasaccharide derivatives **2** (27%) and **3** (40%)



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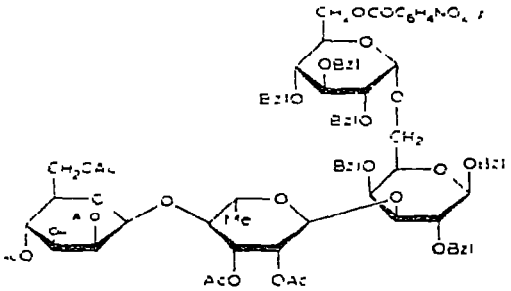

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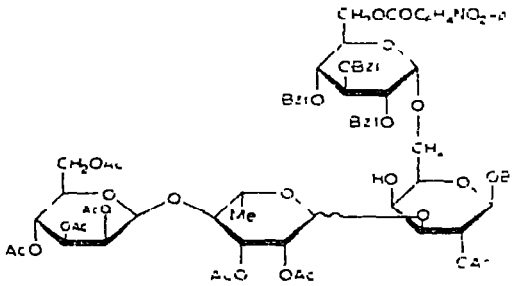

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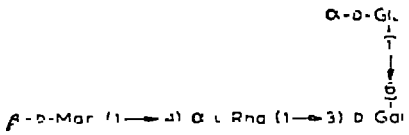

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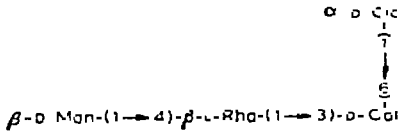

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The elemental-analysis and p m r data of **2** and **3** were in accord with the expected structures Deacylation of **2** followed by hydrogenolysis gave the tetrasaccharide β-D-Man-(1→4)-α-L-Rha-(1→3)-D-Gal-(6←1)-α-D-Glc (**4**), which contained rhamnose, mannose, galactose, and glucose residues in the molar ratios 1·1.1.1 as indicated by sugar analysis

The structure of **2**, expected by the route of synthesis, was proved by identification (g l c –m s) of 3,6-di-*O*-acetyl-1,2,4,5-tetra-*O*-methylgalactitol-*1-d*, which was formed on reduction of **2** with $NaBD_4$ followed by methylation analysis

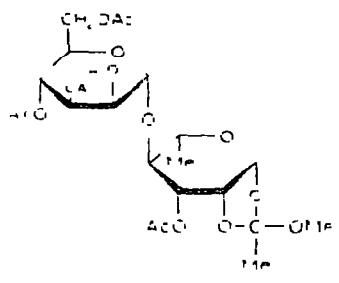
Deacylation of **3** followed by hydrogenolysis afforded a tetrasaccharide which yielded rhamnose, mannose, galactose, and glucose in the molar ratios 1 1 1 1 after acid hydrolysis, and had the same chromatographic mobility as the tetrasaccharide obtained from **2**, but a different optical rotation value The presence of the rhamnopyranosyl-(1→3)-galactopyranose bond in **3** was confirmed by methylation analysis which gave 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylgalactitol (identified by g l c –m s)

Ion-exchange chromatography in borate buffer of the tetrasaccharides derived from **2** and **3** showed the former to be homogeneous and the latter to contain two components (**4** and **5**) in the ratio 5 1, the former possessing the same retention time as **4** The components were isolated by preparative ion-exchange chromatography on Durrum DAx4 resin with borate buffer[7], on acid hydrolysis, each yielded rhamnose, mannose, galactose, and glucose in the molar ratios 1.1 1.1, but the $[\alpha]_D$ values were **4** +27° (water) and **5** +56 (water)

When the acetates of the tetrasaccharides **4** and **5** were subjected to oxidation with CrO_3 in AcOH, with subsequent hydrolysis and sugar analysis[6], the rhamnose and mannose residues in the latter were destroyed, but only the mannose residue in the former Thus, **5** is isomeric with **4** and has a β-L-rhamnopyranosyl bond

The high selectivity of glycosylation at position 3 in the synthesis of **3** is probably due to the shielding of HO-4 by the glucopyranose residue at C-6, and the formation of a minor proportion of β-glycoside in addition to the α anomer may be attributed to HO-4

The α-D-*manno* analogue (**8**) of **4** was prepared *via* glycosylation of benzyl 2,4-di-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-



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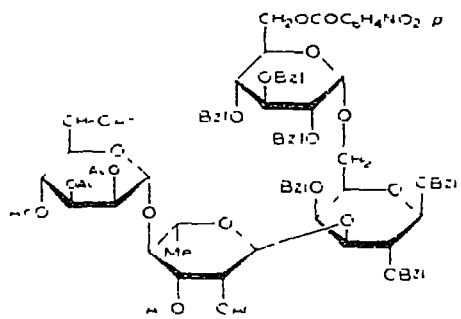

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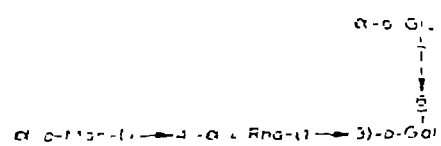

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β-D-galactopyranoside with 3-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-L-rhamnopyranose 1,2-(methyl orthoacetate) (**6**), followed by removal of protecting groups from the product **7**. The structure of **7** and the configuration of the glycosidic bonds were proved in a manner similar to that used for the compounds described above.

EXPERIMENTAL

Melting points were determined with a Kofler apparatus and are uncorrected. P.m.r. spectra were recorded on a Varian DA-60-IL spectrometer with Me_4Si as the internal standard. G.l.c. was carried out with a LHM-8-MD chromatograph and columns of 3% of ECNSS-M on Chromosorb W (1 m) and 5% of SE-30 on Chromaton N-AW (2 m). Mass spectra were obtained with Varian MAT CH-6 and MAT 111 Gnom spectrometers. Optical rotations were determined with a Perkin–Elmer 141 polarimeter. Solutions were concentrated *in vacuo* at 40°. Ion-exchange chromatography of neutral carbohydrates was carried out with a Technicon carbohydrate analyzer on a column (13 × 0.5 cm) of Durrum DA-4 resin with 0.5M sodium borate buffer (pH 8.54) at 55° and 20 ml/h. The orcinol–sulphuric acid reagent was used to monitor separations. T.l.c. was performed on silica gel "KSK", and p.l.c. on silica gel containing 5% of gypsum. p.c. was carried out by the ascending method on Filtrak FN 11 paper with (*1*) chloroform–acetone (95:5), (*2*) 1-butanol–pyridine–water (6:4:3), (*3*) chloroform–acetone (9:1). Methylation analysis of oligosaccharide derivatives was performed conventionally[8].

Synthesis of orthoesters **1** *and* **6**. — The literature procedure[9] was used, but with lyophylisation of AcOH and HBr from the bromide reaction mixture instead of washing with water[3] which gave increased yields (2.5–3 fold) of the orthoesters.

Thus, di-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl bromide[2] (1.7 g) gave **1** (1.2 g, 75%), $[\alpha]_D^{20}$ −4° (*c* 8, chloroform). P.m.r. data ($CDCl_3$): δ 3.2 (3 H, O-Me of orthoester), 2.2–1.8 (15 H, 5 Ac), 1.64 (3 H, C-Me of orthoester), 1.22 (d, 3 H, *J* 5 Hz, C-Me of rhamnose).

Anal. Calc. for $C_{25}H_{36}O_{16}$: C, 50.60; H, 6.09. Found: C, 49.17; H, 6.08.

Di-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranosyl bromide[2] (1.2 g) gave **6** (980 mg, 90%), $[\alpha]_D^{20}$ +50° (*c* 11, chloroform). P.m.r. data ($CDCl_3$): δ 3.2 (3 H, O-Me of orthoester), 2.2–1.8 (15 H, 5 Ac), 1.64 (3 H, C-Me of orthoester), 1.22 (d, 3 H, *J* 5 Hz, C-Me of rhamnose).

Anal. Found: C, 50.20; H, 6.13.

Standard procedure for glycosylation by orthoesters. — A solution of 0.3–0.6 mmol of orthoester and 0.2–0.4 mmol of aglycon in dry, freshly distilled CH_3NO_2 (20 ml) was boiled under reflux for 1 h with exclusion of moisture, in a stream of nitrogen and with an interposed molecular sieve (Linde 4 Å). A solution of $HgBr_2$ (0.1 g) in CH_3NO_2 (10 ml) was added dropwise until reaction was initiated (0.05 mol of $HgBr_2$ per mol of orthoester). Boiling was continued until reaction was complete

(~5 h, negative orthoester test, monitoring by t l c) The mixture was then filtered, and concentrated, and the product was isolated by p l c

Synthesis of tetrasaccharide derivatives — (*a*) *Benzyl 2,4-di-O-benzyl-3-O-[2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-6-O-(2,3,4-tri-O-benzyl-6-O-p-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside* (**2**) Benzyl 2,4-di-*O*-benzyl-6-*O*-(2 3 4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside[4] {0 2 g m p 125–127 , $[\alpha]_D^{20}$ +40° (*c* 2 chloroform)} was glycosylated, as described above, with orthoester **1** (0 2 g) to give **2** (80 mg, 27%), which was isolated by p l c (R_F 0 5, solvent *1*) as a syrup, $[\alpha]_D$ −0 075° (*c* 8, chloroform) P m r data ($CDCl_3$). δ 8 0 (4 H, aromatic), 7 2 (30 H, aromatic), 2 0 (18 H, 6 Ac), 1 3 (d, 3 H *J* 5 Hz, rhamnose C-Me)

Anal Calc. for $C_{89}H_{91}NO_{39}$ C, 64 20, H, 5 84 Found C, 64 23, H, 5 96

(*b*) *Benzyl 2-O-acetyl-3-O-[2,3-di-O-acetyl-4-O-(2,3 4 6-tetra-O-acetyl-β-D-mannopyranosyl)-αβ-L-rhamnopyranosyl]-6-O-(2,3,4-tri-O-benzyl-6-O-p-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside* (**3**) Benzyl 2-*O*-acetyl-6-*O*-(2,3 4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside[4] {0 2 g, m.p 173–175°, $[\alpha]_D^{20}$ +22 5° (*c* 2, chloroform)} was glycosylated as described above with orthoester **1** (0 4 g) to give **3** (0 22 g, 40%), which was isolated by p l c (R_F 0 5, solvent *3*) as a syrup, $[\alpha]_D^{20}$ −9 5° (*c* 2, chloroform) P m r data ($CDCl_3$): δ 8 0 (4 H, aromatic), 7 2 (20 H, aromatic), 2 0 (21 H, 7 Ac) 1 3 (d, 3 H, *J* 5 Hz, rhamnose C-Me)

Anal Calc for $C_{73}H_{82}NO_{30}$ C, 60 03, H, 5 89 Found C, 60 35, H, 5 80

The acetates of 2,3-di-*O*-methylrhamnitol, 2,3,4,6-tetra-*O*-methylmannitol, and 2,4-di-*O*-methylgalactitol were identified (g l c –m.s) after deacetylation and methylation analysis of **3**

(*c*) *Benzyl 2,4-di-O-benzyl-3-O-[2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranosyl]-6-O-(2,3 4-tri-O-benzyl-6 -O- p -nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside* (**7**) Benzyl 2,4-di-*O*-benzyl-6-*O*-(2 3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside[4] (0 2 g) was glycosylated, as described above, with orthoester **6** (0 2 g) to give **7** (80 mg, 27%) which was isolated by t l c (R_F 0 5, solvent *1*) as a syrup, $[\alpha]_D^{20}$ +24° (*c* 4, chloroform) P m r data ($CDCl_3$) δ 8 0 (4 H, aromatic), 7 2 (30 H, aromatic), 2 0 (18 H, 6 Ac), 1 3 (d, 3 H, *J* 5 Hz, rhamnose C-Me)

Anal Calc for $C_{89}H_{93}NO_{39}$ C, 64 20 H 5 84 Found C, 64 30, H, 6 02

Synthesis of tetrasaccharides — (*a*) A methanolic solution of **2** (60 mg) was deacetylated conventionally with 0 1M methanolic sodium methoxide, and then deionized with KU-2 (H^+) resin, filtered, and concentrated The product was debenzylated over palladium-on-charcoal to give 6-*O*-α-D-glucopyranosyl-3-*O*-[4-*O*-(β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-D-galactose (**4**, 18 mg, 80%), $R_{LACTOSE}$ 0 34 (p c , solvent *2*), $[\alpha]_D^{20}$ +27 (*c* 2, water)

(*b*) By a procedure similar to that described in (*a*), **7** (60 mg) was converted into 6-*O*-α-D-glucopyranosyl-3-*O*-[4-*O*-(α-D-mannopyranosyl)-α-L-rhamnopyranosyl]-D-galactose (**8**, 18 mg, 80%), $R_{LACTOSE}$ 0 34 (p c , solvent *2*), $[\alpha]_D^{20}$ +39° (*c* 2, water)

The tetrasaccharides **4** and **8** were homogeneous on assay with the Technicon carbohydrate analyzer, with retention times of 73 and 125 min, respectively, and gave rhamnose, mannose, galactose, and glucose in the molar ratios 1:1:1:1 after acid hydrolysis (0 5M HCl, 16 h, 100°) The acetates of 2,3-di-*O*-methylrhamnitol, 2,3,4,6-tetra-*O*-methylglucitol, and 1,2,4,5-tetra-*O*-methylgalactitol-*1-d* were identified (g l c –m s) after the reduction of **4** and **8** by $NaBD_4$ in borate buffer[10] followed by methylation analysis

(*c*) By a procedure similar to that described in (*a*), **3** (0 2 g) was converted into a mixture of **4** and **5** ($R_{LACTOSE}$ 0 34, p c , solvent *2*), which was fractionated on Durrum DA-4 resin with a borate buffer to give 6-*O*-α-D-glucopyranosyl-3-*O*-[4-*O*-(β-D-mannopyranosyl)-β-L-rhamnopyranosyl)-D-galactose (**5**, 10 mg), $[\alpha]_D^{20}$ +56° (*c* 1, water), and **4** (50 mg), $[\alpha]_D^{20}$ +27° (*c* 5, water), with retention times of 54 and 73 min, respectively

Acid hydrolysis of **4** and **5** as in (*b*) gave rhamnose, mannose, galactose, and glucose in the molar ratios 1 1:1:1.

Oxidation of the acetates of tetrasaccharides **4**, **5**, *and* **8** — Each tetrasaccharide was conventionally acetylated with acetic anhydride–pyridine and then oxidised[11] with CrO_3–AcOH The products were hydrolysed (2M HCl, 16 h, 100°) By this procedure, the following sugar units were destroyed: mannose in **4**, mannose and rhamnose in **5**, none in **8**

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