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BLOCK-SYNTHESIS OF HIGHER OLIGOSACCHARIDES: SYNTHESIS OF HEXA- AND NONA-SACCHARIDE FRAGMENTS OF THE O-ANTIGENIC POLYSACCHARIDE OF *Salmonella newington*

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ABSTRACT

Successive condensation of derivatives of the trisaccharide, biological repeating-unit of the O-antigenic polysaccharide of *Salmonella newington*, followed by removal of protecting groups, has given the hexa- and nona-saccharides. The structures of these oligosaccharides were confirmed chemically and by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

O-Antigenic bacterial polysaccharides are regarded as being composed of oligosaccharide repeating-units that contain 2–6 monosaccharide residues[1]. The immunological specificity of these antigens is governed by immunodeterminant groups that are located inside the repeating units or at their junctions, and are multiply repeated along the polysaccharide chain[2]. Improved methods of oligosaccharide synthesis have yielded the complete repeating-units of the specific polysaccharides of some groups of *Salmonella*[3–5] and *Shigella*[6,7]. Moreover, a polycondensation reaction has been developed and applied[8] to synthesise a polysaccharide identical, both in chemical and serological respects, with that from *S. newington*.

However, in spite of progress in the synthesis of oligo- and poly-saccharides, individual oligosaccharides consisting of several biological repeating-units of the antigenic chain have not been obtained. Such higher oligosaccharides retain the immunological information of the native antigen and are of importance in bacterial immunology[9,10]. Large fragments of O-antigenic polysaccharides have been obtained recently by specific depolymerisation with bacteriophage glycosidases[9–12]. However, since the resulting oligosaccharides usually were not multiples of the biological repeating-units, but only of the chemical one, and the corresponding phages are not readily available, this approach is limited and the need for effective methods for the synthesis of polysaccharide fragments of defined structure is emphasised.

One approach involves the successive condensation of suitable derivatives of the repeating units, and we now report on the chemical synthesis of hexasaccharide **1**

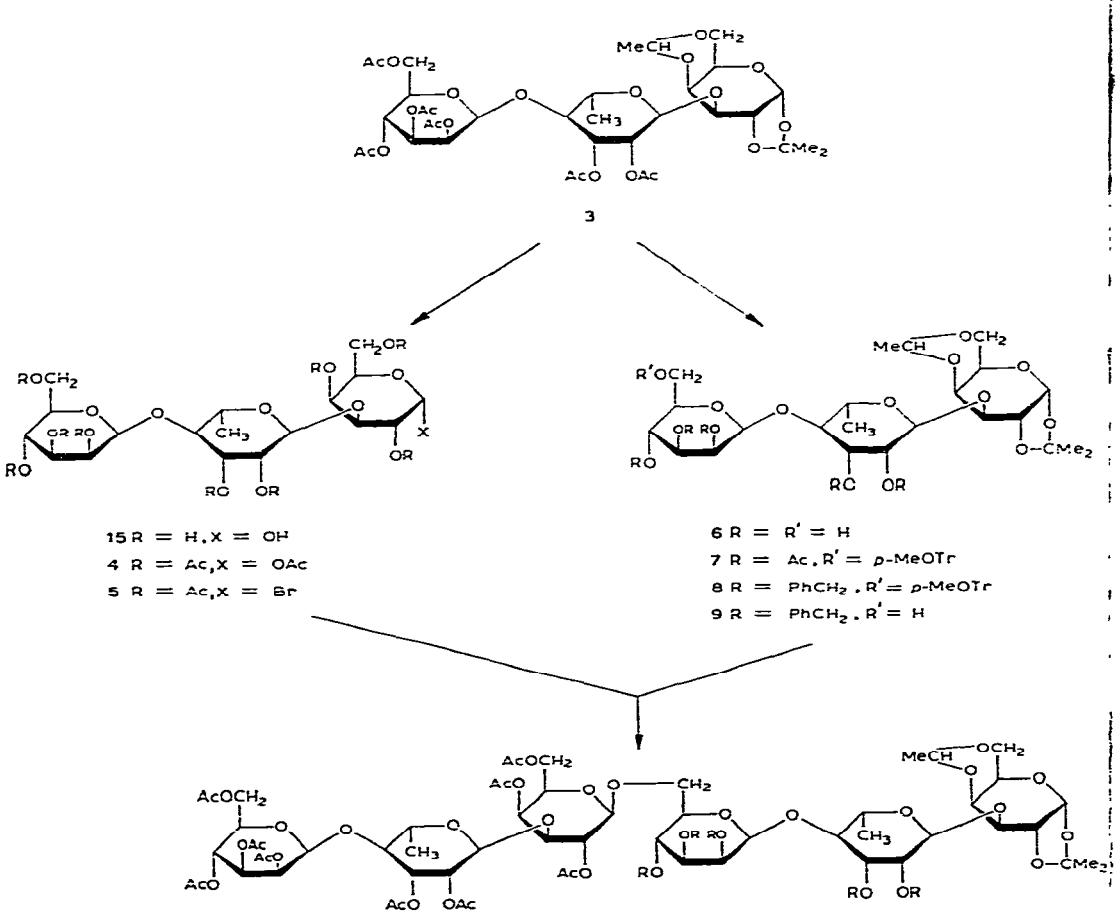
and nonasaccharide **2**, which are a dimer and trimer, respectively, of the biological repeating-unit of the O-specific polysaccharide of *Salmonella newington*.

β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→$_n$

1 $n = 1$ (hexasaccharide)
2 $n = 2$ (nonasaccharide)
Polysaccharide of *S. newington*[13], n = 14–15.

RESULTS AND DISCUSSION

Oligosaccharides **1** and **2** have regular structures and their synthesis was approached by successive condensation of derivatives of the trisaccharide repeating-unit suitable for glycosylation. Earlier, we described[14] the synthesis of the trisaccharide unit β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal.

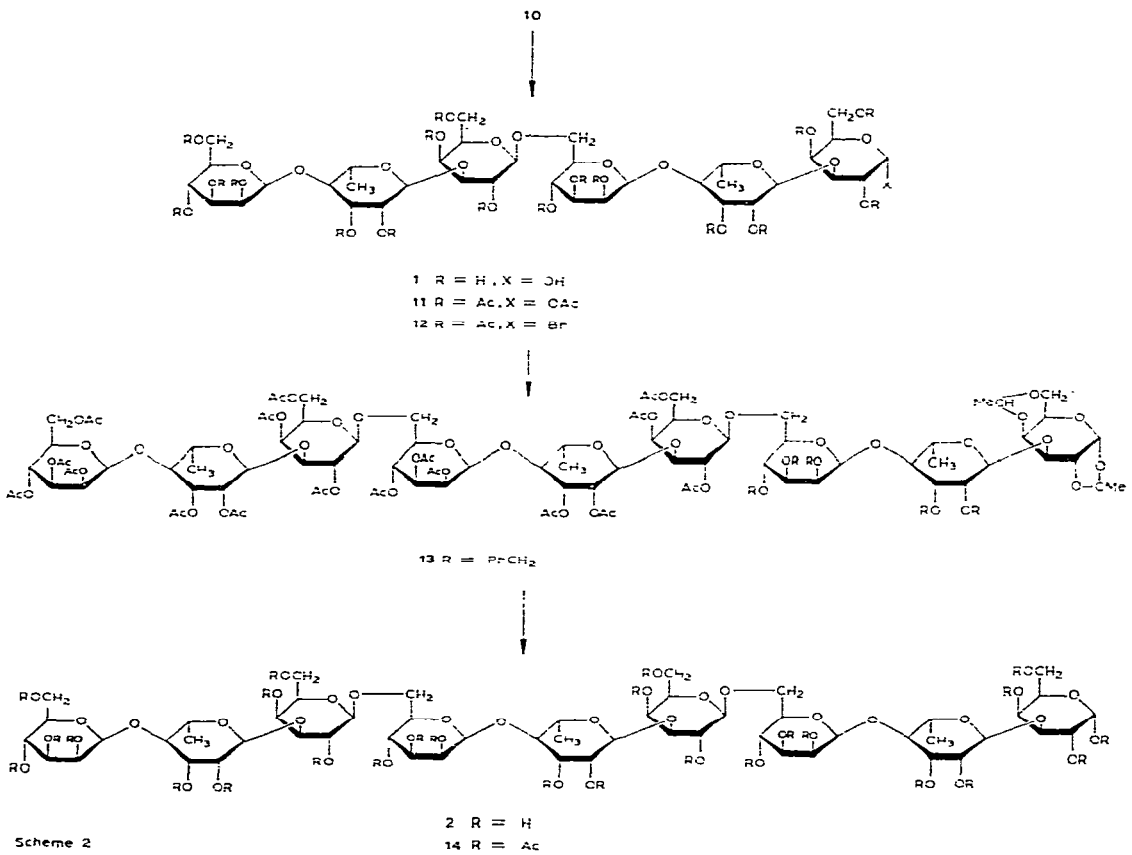


Scheme 1

Block-synthesis has been employed for the production of tetra- and penta-saccharide repeating-units of O-antigenic polysaccharides[3-6], higher oligosaccharides of the isomaltose (octasaccharide, 4 + 4)[15] and gentiobiose (hexasaccharide, 3 + 3)[16] series, and also a hexasaccharide analogue of a fragment of the antigenic chain of *S. newington*[17].

Hexasaccharide **1** was synthesised using the recently described[18], crystalline trisaccharide derivative **3** as the starting compound for both the glycon and aglycon components. Compound **3** was converted[19] into the deca-acetate **4**, treatment of which with hydrogen bromide in chloroform–acetic acid at 0° gave an almost quantitative yield of the glycosyl bromide **5**, which was used as the glycosylating agent. The $[\alpha]_D$ value (+35°) of **5** indicated the α configuration of the galactose residue.

A suitable derivative of **3** with HO-6 of the mannose residue unsubstituted, for use as the aglycon, was obtained as follows. Deacetylation of **3** with methanolic triethylamine followed by *p*-methoxytritylation and acetylation gave **7** (94%). Saponification of **7** and treatment of the product with benzyl bromide* in the presence



Scheme 2

*To avoid acetyl migration[17], benzyl groups were used.

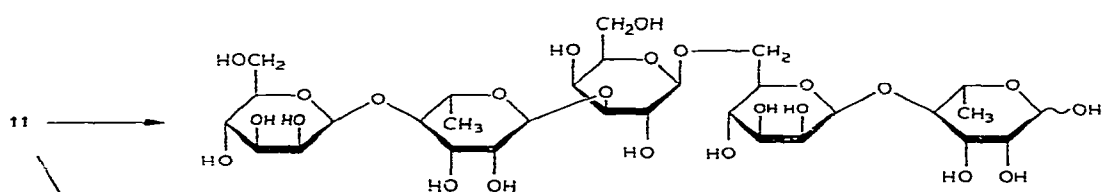
of methylsulfinyl anion[17,20] gave the trisaccharide derivative **8**. Treatment of **8** with 1% trifluoroacetic acid in dichloromethane removed the *p*-methoxytrityl group, to give the crystalline alcohol **9** (87%). The structures of **6**–**9** were confirmed by their p.m.r. spectra and elemental-analysis data.

The aglycon **9** and the glycosyl bromide **5** were allowed to react in acetonitrile in the presence of $Hg(CN)_2$, using the vacuum technique[17,21]. The yield of the resulting hexasaccharide derivative **10** was very dependent on the pre-treatment conditions of the reagents, but conditions were developed to give a yield of 84%. The structure of **10** was confirmed by p.m.r. spectroscopy and the analytical data.

Compound **10** was subjected in sequence to hydrogenolysis over Pd/C, acetylation, mild acetolysis[19], hydrolysis with 80% acetic acid, and acetylation, to give the octadeca-acetate **11** (overall yield, 81%). The α configuration of the terminal galactose residue was indicated by the $J_{1,2}$ value of 4 Hz. Treatment of **11** with hydrogen bromide in chloroform–acetic acid at 0° gave the glycosyl bromide **12**, which was slightly contaminated with decomposition products (t.l.c.) and was used directly for glycosylation.

The condensation of **12** and **9** under the conditions described above for the synthesis of **10** gave the nonasaccharide derivative **13** (57%). The structure of **13** was confirmed by p.m.r. spectroscopy. The doublet for H-1 of the terminal galactose residue at δ 5.70–5.87 ($J_{1,2}$ ~4 Hz) was also observed in the spectra of **3**, **6**, and **7**–**10**, each of which contains a 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactopyranose residue. The nonasaccharide derivative **13** was subjected, in sequence, to hydrogenolysis, acetylation, acetolysis, hydrolysis, and acetylation (as described for **10**), to give the acetylated derivative **14** (overall yield, 60%). The latter can be converted into a nonasaccharide glycosyl bromide for further extension of the oligosaccharide chain, and this work is in progress.

Conventional deacetylation of **11** and **14** is complicated by the β-elimination



16

β-D-Manp-(1→4)-α-L-Rhap-(1→3)-β-D-Galp-(1→6)-β-D-Manp-(1→4)-α-L-Rhap-(1→3)-D-Gal

2-OAc(?)

Scheme 3

1a

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS FOR OLIGOSACCHARIDES **1**, **2**, AND **16** (δ, p.p.m.)

	β-D-*Man*-(1→4)-L-*Rha*			α-L-*Rha*-(1→3)-D-*Gal*		
	β-Man	*α-Rha*	*β-Rha*	*α-Rha*	*α-Gal*	*β-Gal*
C-1	101.75	95.05	94.5	103.7	93.9	98.0
C-2	71.8	72.2	71.8	71.7	70.6	72.85
C-3	74.25	71.2	74.0	71.7	78.45	81.85
C-4	67.95	80.8	80.4	73.6	70.0	69.25
C-5	77.5	68.2	72.8	70.4	71.7	76.4
C-6	62.2	18.3	18.3	18.0	62.4	62.2

	β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-D-*Gal* (**15**)			
	β-Man	*α-Rha*	*α-Gal*	*β-Gal*
C-1	101.9	103.5	93.6	97.6
C-2	71.9	71.45	70.4	72.5
C-3	74.4	72.1	78.7	82.0
C-4	68.1	80.9	69.8	68.9
C-5	77.5	69.15	71.9	75.4
C-6	62.3	18.2	62.3	62.2

	β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-β-D-*Gal*-(1→6)-β-D-*Man*-(1→4)-L-*Rha* (**16**)				
	β-Man	*α-Rha*	*β-Gal*	*β-Man*	*α-Rha*
C-1	101.95	103.5	104.5	101.8	95.2
C-2	71.8	71.5	71.5	71.8	72.5
C-3	74.5	71.9	81.8	74.5	71.9
C-4	68.1	80.75	69.7	68.1	80.75
C-5	77.5	69.0	76.3	76.6	68.1
C-6	62.2	18.2	62.0	69.7	18.5

	β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-β-D-*Gal*-(1→6)-β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-D-*Gal* (**1**)						
	β-Man	*α-Rha*	*β-Gal*	*β-Man*	*α-Rha*	*α-Gal*	*β-Gal*
C-1	101.9	103.5	104.4	101.9	103.5	93.6	97.7
C-2	71.8	71.45	71.45	71.8	71.45	70.25	72.55
C-3	74.4	71.9	81.8	74.4	71.9	78.7	81.8
C-4	68.05	80.8	69.75	68.05	80.8	69.75	69.1
C-5	77.5	69.1	76.3	76.3	69.1	71.9	76.6
C-6	62.2	18.2	62.2	70.25	18.2	62.2	62.2

	β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-β-D-*Gal*-(1→6)-β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-β-D-*Gal*-(1→6)-β-D-*Man*-(1→4)-α-L-*Rha*-(1→3)-D-*Gal* (**2**)									
	β-Man	*α-Rha*	*β-Gal*	*β-Man*	*α-Rha*	*β-Gal*	*β-Man*	*α-Rha*	*α-Gal*	*β-Gal*
C-1	101.8	103.45	104.3	101.8	103.45	104.3	101.8	103.45	93.5	97.6
C-2	71.7	71.4	71.4	71.7	71.4	71.4	71.7	71.4	70.25	72.5
C-3	74.25	71.7	81.85	74.25	71.7	81.85	74.25	71.7	78.6	81.85
C-4	68.05	80.8	69.7	68.05	80.8	69.7	68.05	80.8	69.7	69.1
C-5	77.4	69.1	76.25	76.25	69.1	76.25	76.25	69.1	71.7	76.6
C-6	62.2	18.3	62.1	70.25	18.3	62.1	70.25	18.3	62.2	62.2

TABLE II

METHYLATION ANALYSIS DATA FOR OLIGOSACCHARIDES **1**, **2**, **15**, AND **16** (G.L.C. DATA FOR THE MIXTURES OF PARTIALLY METHYLATED ALDITOL ACETATES)

Alditols	*Relative amounts*				T
	1	**2**	**16**	**15**	
3-*O*-Acetyl-1,2,4,5,6-penta-*O*-methylgalactitol-*l*-*d*	0.54[a]	0.58[a]	—	0.67[a]	0.91
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol	1.94	3.05	1.99	1.00	0.96
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol	1.00	1.00	1.00	1.00	1
1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol	0.90	1.77	0.94	—	1.12
1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methylmannitol	0.87	1.67	0.78	—	1.15

[a]Amounts diminished because of losses due to high volatility[24] and partial *O*-demethylation during acid hydrolysis[26]. Methylation analysis[14] of reduced **15** also gave a mixture of alditols with a diminished content of 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylgalactitol-*l*-*d*.

reaction typical of 3-substituted reducing-sugars. Thus, treatment of **11** with methanolic sodium methoxide (0.05 equiv. per acetyl group) at room temperature for 16 h gave a mixture of hexasaccharide **1** (56%) and pentasaccharide **16** (40%).

When the deacetylation was performed under milder conditions[18,22] (at 0°), **11** gave mixture of **1** (minor) and its mono-acetate **1a** (major)*. The p.m.r. spectrum of **1a** contained an *O*-acetyl singlet with an integrated intensity half of that of the rhamnose *C*-methyl doublet.

When the mixture of **1** and **1a** was treated with aqueous triethylamine followed by preparative p.c., 75% of **1** could be isolated. The reaction mixture also contained small proportions of **1a** and the pentasaccharide **16**. Analogous, two-step deacetylation of **14** followed by preparative p.c. gave 70% of the nonasaccharide **2**. The reaction mixture also contained several minor components of higher chromatographic mobility, which were not studied.

The oligosaccharides **1**, **2**, and **16** were homogeneous in ion-exchange chromatography in borate buffer[3,18] (automatic carbohydrate analysis), and the configurations of the newly formed glycosidic bonds were indicated by the ^{13}C-n.m.r. data. Assignments of the signals were made by comparison with the spectra of the structurally related disaccharides β-D-Man*p*-(1→4)-L-Rha[5] and α-L-Rha-(1→3)-D-Gal[18], and trisaccharide **15**[18] (see Table I).

The presence of low-field signals (69.7 and 70.25 p.p.m.) for C-6 of the mannose residue in **1**, **2**, and **16** proved the non-terminal mannose residue to be substituted at position 6. Also, the total, integrated intensity of the peaks at 62 p.p.m. (due to

*Mild saponification of 3-*O*-glycosylated allyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside with methanolic sodium methoxide gives the corresponding 2-acetate. The location of the acetyl group was determined by ^{13}C-n.m.r. spectroscopy. It is assumed that the acetyl group in **1a** is at position 2 of the non-reducing galactose residue and that its stability is due to the absence of vicinal hydroxyl groups.

-CH_2OH) corresponded to two carbon atoms for pentasaccharide **16**, three for hexasaccharide **1**, and four for nonasaccharide **2**. The β configuration of the galactosidic bonds was supported by the signals of C-1, C-2, C-3, and C-5 of the non-reducing galactose residues at 104.3–104.5, 71.4–71.5, 81.8, and 76.3 p.p.m., respectively (for the α configuration, these atoms should resonate at 100.5, 70.6, 78.5, and 71.8 p.p.m., respectively[23]). The types and configurations of the remaining glycosidic bonds in the oligosaccharides were in agreement with the ^{13}C-n.m.r. data (Table I).

Oligosaccharides **1** and **2** were also subjected to methylation analysis[24], after reduction with $NaBD_4$ in the presence of boric acid[25], to give the corresponding galactitol derivatives (pentasaccharide **16** was methylated as such). The resulting mixtures of partially methylated alditol acetates were analysed by g.l.c. (see Table II) and g.l.c.–m.s. The results obtained accord with the structures assigned to **1**, **2**, and **16**. These oligosaccharides were strongly inhibitory in the passive haemagglutination reactions with anti-3 and anti-15 antisera, and these results will be discussed elsewhere.

EXPERIMENTAL

Melting points were determined with a Kofler apparatus, and optical rotations with a Perkin–Elmer 141 polarimeter. G.l.c. was performed on an LKHM-8MD chromatograph, with a glass column (50 m × 27 mm) packed with OV-1, nitrogen as the carrier gas, an evaporator temperature of 350°, and column and detector temperatures of 227°. G.l.c.–m.s. was performed on a Varian MAT-111 Gnom spectrometer and a 3-m steel column packed with 5% of SE-30 on Chromaton N-AW. The homogeneity of oligosaccharides was determined with an Automatic Carbohydrate Analyser type 71100 A (Czechoslovakia), using a column (0.6 × 11 cm) of Durrum DA X4 resin and 0.7M borate buffer (pH 7.9) at 70° and an elution rate of 20 mL/h.

P.m.r. spectra (internal Me_4Si) were recorded with Varian DA-60-IL and Tesla BS-497 (100 MHz, C.S.S.R.) instruments. The ^{13}C-n.m.r. spectra of **1**, **2**, and **16** were measured at 15.08 MHz with a Bruker WP-60 spectrometer, employing a ^{2}H-lock and solutions in deuterium oxide (internal MeOH). The chemical shift of MeOH relative to that of Me_4Si (50.15 p.p.m.) was confirmed separately. Proton-decoupled F.t.-spectra were measured with a repetition time of 1.1 s, a pulse width of 5.5 μs (45°), 3750-Hz sweep width, and 4K real data points.

Solutions were concentrated *in vacuo* at $\leqslant$40°. T.l.c. was performed on silica gel LS 5/40 μm (Czechoslovakia) +6% of gypsum, with detection by charring with H_2SO_4, and, for p.l.c., with H_2O. Column chromatography was performed on silica gel L 100/250 μm and L 40/100 μm (Czechoslovakia) by elution with ether or ethyl acetate gradients (0→100%) in benzene. T.l.c. was effected with *A*, acetone–chloroform (15:85); *B*, ethyl acetate–methanol (1:1); *C*, methanol–chloroform (1:4); *D*, acetone–chloroform (3:97); and ethyl acetate–toluene, *E* (1:1) and *F* (2:1). P.c. was performed on Filtrak FN 11 paper with 1-butanol–pyridine–water (4:6:3) and detection with KIO_4–$AgNO_3$–NaOH or aniline hydrogen phthalate.

To determine monosaccharide composition, the oligosaccharide sample was hydrolysed with 2M HCl (2 h, 100°), the hydrolysate was concentrated, and the residue was assayed on the sugar analyser.

2,4,6-Tri-O-acetyl-3-O-[2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-α-D-galactopyranosyl bromide (**5**). — To a solution of **4** (200 mg, obtained[19] from **3**) in chloroform (20 mL) at 0° was added a mixture (4 mL) of 33% HBr in glacial AcOH containing 4% of Ac_2O (v/v). After storage for 2 h at 0°, the mixture was diluted with $CHCl_3$, washed with ice–water and cold, aqueous $NaHCO_3$, dried, and concentrated, to give syrupy **5** (200 mg), $[\alpha]_D^{20}$ +35° (*c* 1, chloroform), R_F 0.59 (solvent *A*), that was used directly for glycosylation.

4,6-O-Ethylidene-1,2-O-isopropylidene-3-O-(4-O-β-D-mannopyranosyl-α-L-rhamnopyranosyl)-α-D-galactopyranose (**6**). — To a solution of **3** (806 mg) in MeOH (27 mL) was added dry triethylamine (3 mL), and the mixture was stirred for 16 h at 20° and then concentrated, to give chromatographically homogeneous **6** (554 mg, ~100%), $[\alpha]_D^{20}$ −17° (*c* 1, methanol), R_F 0.58 (solvent *B*) and 0.14 (solvent *C*). P.m.r. data (CD_3OD): δ 1.18–1.55 (12 H, C-Me) and 5.79 d (1 H, $J_{1,2}$ 3.5 Hz, H-1 of galactose) (Calc. for $C_{23}H_{38}O_{15}$: C, 49.82; H, 6.91. Found: C, 49.85; H, 6.91%).

3-O-[2,3-Di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-O-p-methoxytrityl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-galactopyranose (**7**). — A solution of **6** (760 mg, 1.37 mmol) and *p*-anisylchlorodiphenylmethane (850 mg, 2.74 mmol) in dry pyridine (30 mL) was kept for 24 h at 20°. T.l.c. then revealed only one product, R_F 0.45 (solvent *C*). Ac_2O was added, and the mixture was kept for 24 h at 20°, poured into ice–water, and extracted with $CHCl_3$. The extract was washed with aqueous $NaHCO_3$ and water, dried, and concentrated. Column chromatography of the residue gave **7** (1.33 g, 94%) as a solid, $[\alpha]_D^{20}$ −17° (*c* 1, chloroform), R_F 0.62 (solvent *A*). P.m.r. data (CCl_4): δ 1.10–1.57 (12 H, C-Me), 1.65, 2.05, 2.13 (3 s, 9 H, 3 OAc), 1.88 s (6 H, 2 OAc), 3.74 (s, 3 H, OMe), 5.77 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1 of galactose), and 6.63–7.63 (14 H, aromatic protons including 2 d for the *p*-methoxyphenyl ring at δ 6.80 and 7.34, J_{AB} 8 Hz) (Calc. for $C_{53}H_{64}O_{21}$: C, 61.38; H, 6.22. Found: C, 61.30; H, 6.00%).

3-O-[2,3-Di-O-benzyl-4-O-(2,3,4-tri-O-benzyl-6-O-p-methoxytrityl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-galactopyranose (**8**). — Sodium hydride (140 mg of a 50% suspension in mineral oil) under argon was washed with pentane (3 × 10 mL), mixed with methyl sulfoxide (8 mL), and stirred at 70° to complete dissolution (1–1.5 h).

A solution of **7** (240 mg) in 0.2M methanolic MeONa (5 mL) was kept for 1 h at 20° and then concentrated, and the dry residue was dissolved in methyl sulfoxide (4 mL). To this solution, stirred under argon, was added the above solution of sodium methylsulfinylmethanide at 20°, and, after 40 min and cooling with ice–water, benzyl bromide (0.6 mL). The mixture was stirred for 20 h at 20°, poured into ice–water, and extracted with $CHCl_3$. The extract was washed with aqueous $NaHCO_3$ and water, dried, and concentrated. The methyl sulfoxide and benzyl bromide were

distilled off *in vacuo* at 70°. Column chromatography of the residue gave homogeneous, syrupy **8** (270 mg, 91%), $[\alpha]_D^{20}$ −3.5° (*c* 1, chloroform), R_F 0.57 (solvent *D*). P.m.r. data (CCl_4): δ 1.20–1.57 (12 H, CMe), 3.60 (s, 3 H, OMe), 5.76 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1 of galactose), and 6.54–7.54 (39 H, aromatic protons including 2 d for the *p*-methoxyphenyl ring at δ 6.64 and 7.38, J_{AB} 8 Hz) (Calc. for $C_{78}H_{84}O_{16}$: C, 73.33; H, 6.63. Found: C, 73.10; H, 6.68%).

3-O-[2,3-Di-O-benzyl-4-O-(2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-galactopyranose (**9**). — A solution of **8** (600 mg) in CH_2Cl_2 (25 mL) was treated with 2% CF_3COOH in CH_2Cl_2 (25 mL) for 1 min at 0°, washed with cold, aqueous $NaHCO_3$ and water, dried, and concentrated. Column chromatography of the residue gave **9** (410 mg, 87%), m.p. 154–155° (from ether), $[\alpha]_D^{20}$ +3.5° (*c* 1, chloroform), R_F 0.53 (solvent *A*) and 0.43 (solvent *E*). P.m.r. data ($CDCl_3$): δ 1.20–1.60 (12 H, 4 CMe), 2.15 (s, 1 H, OH), 5.86 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of galactose), and 6.80–7.76 (25 H, aromatic protons) (Calc. for $C_{58}H_{68}O_{15}$: C, 69.30; H, 6.82. Found: C, 69.15; H, 6.89%).

2,3,4,6-Tetra-O-acetyl-β-D-Manp-(1→4)-2,3-di-O-acetyl-α-L-Rhap-(1→3)-2,4,6-tri-O-acetyl-β-D-Galp-(1→6)-2,3,4-tri-O-benzyl-β-D-Manp-(1→4)-2,3-di-O-benzyl-α-L-Rhap-(1→3)-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-Galp (**10**). — The synthesis was performed by the high-vacuum technique[17,21]. A solution of **5** (110 mg, 0.12 mmol) in dry benzene (2.5 mL) was placed in one arm of a two-armed ampoule, and a solution of **9** (100 mg, 0.10 mmol) in benzene (2.5 mL), together with $Hg(CN)_2$ (27 mg, 0.11 mmol) and a magnet, in the other. The ampoule was connected to a vacuum system, and the contents were freeze-dried at 3×10^{-3} mmHg. Then CH_3NO_2 (2.5 mL, degassed and twice-distilled over CaH_2 at 3×10^{-3} mm Hg) was distilled into the ampoule, reactants **5** and **9** were dissolved in the distillate, and the resulting solutions were freeze-dried again. Acetonitrile (2.5 mL, prepared as for CH_3NO_2) was distilled into the ampoule, which was then disconnected without disturbing the vacuum, and the contents of the two arms were mixed and stirred at 20° for 16 h. The mixture was then diluted with $CHCl_3$, washed with M KBr and water, dried, and concentrated. Homogeneous **10** (155 mg, 84%) as a solid was isolated from the residue by column chromatography and had $[\alpha]_D^{20}$ −13.5° (*c* 1, chloroform), R_F 0.35 (solvent *E*). P.m.r. data ($CDCl_3$): δ 1.16–1.60 (15 H, 5 CMe), 1.92–2.24 (27 H, 9 OAc), 5.89 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of terminal galactose), and 7.00–7.49 (25 H, aromatic protons) (Calc. for $C_{94}H_{116}O_{38}$: C, 60.89; H, 6.31. Found: C, 60.71; H, 6.49%).

β-D-Manp-(1→4)-α-L-Rhap-(1→3)-β-D-Galp-(1→6)-β-D-Manp-(1→4)-α-L-Rhap-(1→3)-α-D-Galp octadeca-acetate (**11**). — A solution of **10** (280 mg) in MeOH (16 mL) was hydrogenated for 10 h at 40° over Pd/C, and then filtered, and concentrated. The residue was treated with Ac_2O (2 mL) and pyridine (2 mL) for 16 h at 20°. The mixture was co-concentrated with toluene to dryness, and a solution of the residue in $CHCl_3$ was washed with cold water, cold 0.5M HCl, aqueous $NaHCO_3$, and water, dried, and concentrated. A solution of the dry residue in Ac_2O (2 mL) was mixed with a solution (0.5 mL) of H_2SO_4 (0.1 mL) in glacial AcOH (25 mL).

The mixture was stored for 16 h at 20°, diluted with water (1 mL), kept for 45 min at 85°, and then co-concentrated with toluene. The residue was treated with Ac_2O (2 mL) for 4 h at 20°, and the solution was then diluted with $CHCl_3$, washed with ice-water, aqueous $NaHCO_3$, and water, dried, and concentrated. Column chromatography of the residue gave **11** (210 mg, 81%) as a solid, $[\alpha]_D^{20}$ −20° (*c* 2, chloroform), R_F 0.35 (solvent *A*) and 0.23 (solvent *F*). P.m.r. data ($CDCl_3$): δ 1.30–1.34 (2 d, 6 H, 2 CMe), 1.94–2.26 (54 H, 18 OAc), and 6.34 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of terminal galactose).

2,3,4,6-Tetra-O-*acetyl*-β-D-*Man*p-(*1*→*4*)-*2,3-di*-O-*acetyl*-α-L-*Rha*p-(*1*→*3*)-*2,4,6-tri*-O-*acetyl*-β-D-*Gal*p-(*1*→*6*)-*2,3,4-tri*-O-*acetyl*-β-D-*Man*p-(*1*→*4*)-*2,3-di*-O-*acetyl*-α-L-*Rha*p-(*1*→*3*)-*2,4,6-tri*-O-*acetyl*-β-D-*Gal*p-(*1*→*6*)-*2,3,4-tri*-O-*benzyl*-β-D-*Man*p-(*1*→*4*)-*2,3-di*-O-*benzyl*-α-L-*Rha*p-(*1*→*3*)-*4,6*-O-*ethylidene*-*1,2*-O-*isopropylidene*-α-D-*Gal*p (**13**). — To a solution of **11** (200 mg) in $CHCl_3$ (20 mL) at 0° was added a solution (4 mL) of 33% HBr in glacial AcOH containing 4% of Ac_2O (v/v), and the mixture was kept for 2 h at 0°, diluted with $CHCl_3$, washed with ice–water and cold aqueous $NaHCO_3$, dried, and concentrated. The resulting, syrupy glycosyl bromide **12** (200 mg), $[\alpha]_D^{20}$ +3° (*c* 1, chloroform), R_F 0.45 (solvent *A*), contained traces of decomposition products (t.l.c.) and was used immediately in the following glycosylation reaction.

A solution of **12** (200 mg, 0.115 mmol) in benzene (3 mL) was placed in one arm of a two-armed ampoule, and a solution of **9** (100 mg, 0.1 mmol) in benzene (3 mL), together with $Hg(CN)_2$ (30 mg, 0.119 mmol) and a magnet, in the other. The further operations were performed as described above. Column chromatography of the products gave **13** (150 mg, 57%) as a solid, $[\alpha]_D^{20}$ −12° (*c* 1, chloroform), R_F 0.40 (solvent *F*). P.m.r. data ($CDCl_3$): δ 1.20–1.58 (18 H, 6 CMe), 1.80–2.30 (51 H, 17 OAc), 5.79 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of terminal galactose), and 7.00–7.48 (25 H, aromatic protons).

β-D-*Man*p-(*1*→*4*)-α-L-*Rha*p-(*1*→*3*)-β-D-*Gal*p-(*1*→*6*)-β-D-*Man*p-(*1*→*4*)-α-L-*Rha*-(*1*→*3*)-β-D-*Gal*p-(*1*→*6*)-β-D-*Man*p-(*1*→*4*)-α-L-*Rha*-(*1*→*3*)-α-D-*Gal*p *hexacosa-acetate* (**14**). — A solution of **13** (70 mg) in MeOH (10 mL) was hydrogenated for 10 h at 40° over 10% Pd/C, filtered, and concentrated, and the residue was treated with Ac_2O (2 mL) and pyridine (2 mL) for 16 h at 20°. After co-concentration of the solution with toluene, a solution of the residue in $CHCl_3$ was washed with water, cold 0.3M HCl, cold aqueous $NaHCO_3$, and water, dried and concentrated. The dry residue was dissolved in Ac_2O (2 mL) and mixed with a solution (0.5 mL) of H_2SO_4 (0.1 mL) in glacial AcOH (25 mL). The mixture was kept for 16 h at 20°, water (1 mL) was then added, and the mixture was heated for 45 min at 85°. After co-concentration of the mixture with toluene, the residue was treated with Ac_2O (2 mL) for 4 h at 20°, and the solution was diluted with $CHCl_3$, washed with ice–water, cold aqueous $NaHCO_3$, and water, dried, and concentrated. P.l.c. of the residue gave homogeneous **14** (40 mg, 60%) as a solid, $[\alpha]_D^{20}$ −16° (*c* 1, chloroform), R_F 0.24 (solvent *A*). P.m.r. data ($CDCl_3$): δ 1.20–1.47 (9 H, 3 CMe) and 1.92–2.40 (78 H, 26 OAc).

Saponification of **11**. — (*a*) A solution of **11** (20 mg) in 5mM methanolic MeONa (2 mL, 0.048 equiv. per OAc) was kept for 12.5 h at 0°, deionised with Amberlite IR-120 (H^+) resin, and concentrated. A solution of the residue in water (10 mL) was mixed with triethylamine (1.5 mL) for 1.5 h at 20°, and then concentrated. Preparative p.c. of the residue gave β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal (**1**; 8.4 mg, 75%), $[\alpha]_D^{20}$ −32.5° (*c* 1, water), R_{Gal} 0.26. The product was homogeneous, as shown by ion-exchange chromatography with an automatic carbohydrate analyser (time of elution, *T* 90 min), and contained mannose, rhamnose, and galactose in the ratios 1:1:0.9.

(*b*) A solution of **11** (80 mg) in 0.01M methanolic MeONa (4 mL) was kept for 16 h at 20°, deionised with Amberlite IR-120 (H^+) resin, and concentrated. Preparative p.c. of the residue gave **1** (25 mg, 56%), R_{Gal} 0.26, and β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-L-Rha (**16**; 15 mg, 40%), $[\alpha]_D^{20}$ −38° (*c* 1, water), R_{Gal} 0.45. Pentasaccharide **16** was shown by ion-exchange chromatography to be homogeneous (*T* 34 min), and contained mannose, rhamnose, and galactose in the ratios 0.9:1:0.5.

(*c*) A solution of **11** (30 mg) in 5mM methanolic MeONa (3 mL) was stirred for 30 min at 20°, kept for 12 h at 0°, deionised with Amberlite IR-120 (H^+) resin, and concentrated. Preparative p.c. of the residue gave **1** (24%, R_{Gal} 0.26) and its monoacetate **1a** (11 mg, 63%; R_{Gal} 0.41). The latter contained mannose, rhamnose, and galactose in the ratios 0.8:0.9:1.

β-D-*Man*p-(*1*→*4*)-α-L-*Rha*p-(*1*→*3*)-β-D-*Gal*p-(*1*→*6*)-β-D-*Man*p-(*1*→*4*)-α-L-*Rha*p-(*1*→*3*)-β-D-*Gal*p-(*1*→*6*)-β-D-*Man*p-(*1*→*4*)-α-L-*Rha*p-(*1*→*3*)-D-*Gal*p (**2**). — To a solution of **14** (30 mg) in MeOH (3 mL) was added 0.1M methanolic MeONa (0.13 mL), and the mixture was kept for 12 h at 0°, deionised with Amberlite IR-120 (H^+) resin, and concentrated. A solution of the residue in water (20 mL) was stirred with triethylamine (1.4 mL) for 1.5 h at 20° and then concentrated. Preparative p.c. of the residue gave **2** (11.9 mg, 70%), $[\alpha]_D^{20}$ −32° (*c* 1, water), R_{Gal} 0.09. The product was shown by ion-exchange chromatography to be homogeneous (*T* 150 min), and contained mannose, rhamnose, and galactose in the ratios 0.9:1:0.9.

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