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SYNTHESIS OF DI- AND TRI-SACCHARIDES RELATED TO THE POLYSACCHARIDE FROM *Streptococcus pneumoniae* TYPE 23 AND A STUDY OF THEIR INHIBITION IN THE PRECIPITIN REACTION

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ABSTRACT

Syntheses of methyl 2-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**9**), methyl 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**13**), and methyl 4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**16**) in good yields are described. Both **13** and **16** significantly inhibit antigen–antibody precipitation in the *S. pneumoniae* Type 23 immune system. The results indicate that the rhamnosyl group in the side chain of the repeating unit of the antigen is α and not β as reported previously, and that the trisaccharide related to **16** is the immunodominant group.

INTRODUCTION

In the structure (**1**) of the repeating unit of the capsular polysaccharide from *Streptococcus pneumoniae* Type 23 published previously[1], both the L-rhamnose residues were designated as β on the basis of the results of oxidation with chromium trioxide. In a subsequent n.m.r. study[2], it was concluded that the side chain contained an α-L-rhamnosyl unit linked to D-galactose. Heidelberger and Nimmich[3] found the side-chain L-rhamnose residue to be immunodominant and it is therefore pertinent that its configuration be verified and further information obtained on the immunodominant group in this antigen. Methyl 2-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**8**), methyl 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**13**), and methyl 4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**16**) have therefore been synthesised in order to ascertain their effect in the *S. pneumoniae* Type 23 immune system.

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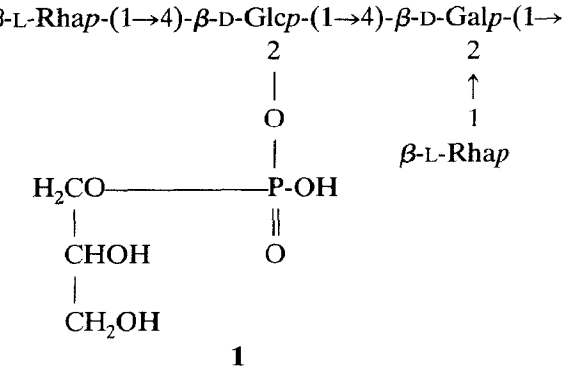

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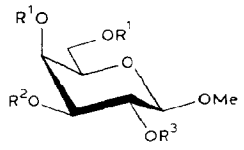
RESULTS AND DISCUSSION

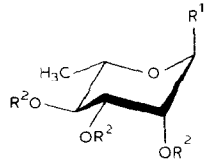
Methyl 3-*O*-benzyl-β-D-galactopyranoside (**3**), obtained by one-step benzylation[4] of methyl β-D-galactopyranoside (**2**) in the presence of dibutyltin oxide and tetraethylammonium bromide, was reacted with α,α-dimethoxytoluene[5] to give methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**4**).



2 $R^1 = R^2 = R^3 = H$

3 $R^1 = R^3 = H, R^2 = Bn\ (CH_2Ph)$

4 $R^1, R^1 = CHPh, R^2 = Bn, R^3 = H$



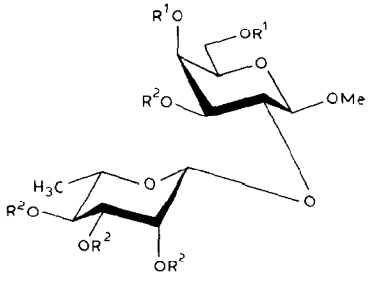
5 $R^1 = SEt, R^2 = Ac$

6 $R^1 = SEt, R^2 = Bn$

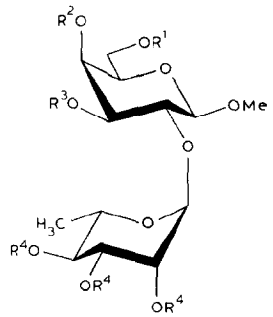
7 $R^1 = Br, R^2 = Bn$

Condensation of **4** with 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl bromide (**7**), prepared by treatment of ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**6**) with bromine in the presence of silver carbonate and silver oxide in dichloromethane, gave methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-β-D-galactopyranoside (**8**). The $[\alpha]_D^{24}$ value (+52°) of **8** indicated the new linkage to be β. Hydrogenolysis of **8** gave methyl 2-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**9**), $[\alpha]_D^{24}$ +17°.

Condensation of **4** with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[6] in the presence of mercury(II) cyanide in acetonitrile[8] gave methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**10**). Benzylation[7] of **10** gave methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**11**), $[\alpha]_D^{24}$ −20°. Removal of the benzylidene group from **11** yielded methyl 3-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**12**), and hydrogenolysis of **12** then gave methyl 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**13**), $[\alpha]_D^{24}$



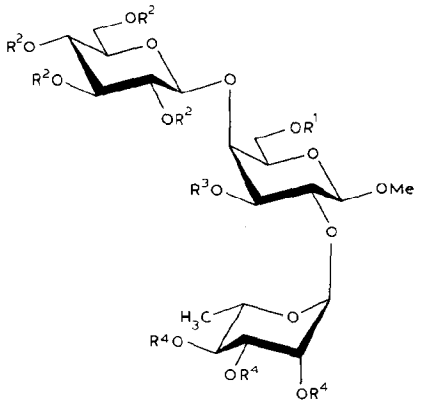
8 $R^1, R^1 = CHPh, R^2 = Bn$
9 $R^1 = R^2 = H$



10 $R^1, R^2 = CHPh, R^3 = Bn, R^4 = Ac$
11 $R^1, R^2 = CHPh, R^3 = R^4 = Bn$
12 $R^1 = R^2 = H, R^3 = R^4 = Bn$
13 $R^1 = R^2 = R^3 = R^4 = H$
14 $R^1 = R^3 = R^4 = Bn, R^2 = H$

$-11°$. Selective benzylation of **12** by the phase-transfer method[8,9], using benzyl bromide and tetrabutylammonium hydrogensulfate, gave methyl 3,6-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**14**). Condensation of **14** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[10] in the presence of silver triflate and tetramethylurea[11] in dichloromethane gave methyl 3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**15**). Debenzylation of **15** and deacetylation of the product gave methyl 4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**16**). The structures of **8, 13,** and **16** were confirmed by acid hydrolysis and methylation analysis.

The antiserum used in the homologous precipitin reaction was kindly



15 $R^1 = R^3 = R^4 = Bn, R^2 = Ac$
16 $R^1 = R^2 = R^3 = R^4 = H$

supplied by Professor M. Heidelberger. The pH of the serum was adjusted to 6 with dilute hydrochloric acid, and the serum was used with increasing amounts of antigen in saline solution. After 5 d at 2°, the precipitates were collected by centrifugation and the clear supernatant solutions were examined for antibody excess by the Öuchterlony double-diffusion method[12]. The results showed that, at the equivalence point, 460 μg of the polysaccharide precipitated 1064 μg of the antibody nitrogen from 1 mL of the antiserum. The effects of seven different inhibitors on the precipitin reaction are summarized in Table I. Methyl 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**13**) and methyl 4-*O*-β-D-glucopyranosyl-2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**16**) inhibited the precipitin reaction to extents of 71% and 78.5%, respectively, whereas methyl 2-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**8**) and methyl α-L-rhamnopyranoside (**17**) did not inhibit the reaction significantly. These results indicate that, contrary to the previous conclusion[1], the side-chain L-rhamnosyl unit has the α anomeric configuration and the trisaccharide related to **16** is the immunodominant group.

EXPERIMENTAL

Materials and methods. — Reactions were monitored by t.l.c. on Silica Gel G (Merck), solvents were distilled before use, and evaporations were conducted at 50° under diminished pressure unless otherwise stated. Column chromatography was performed under normal pressure with Silica Gel 60 (Merck). G.l.c. of alditol

TABLE I

INHIBITION OF ANTIGEN-ANTIBODY PRECIPITATION IN THE *S. pneumoniae* TYPE 23 IMMUNE SYSTEM

Inhibitor	*Inhibitor needed[b] for maximum inhibition[a] (μM)*	*Antibody nitrogen precipitated (μg)*	*Inhibition[b] (%)*	*Inhibitor needed for 50% inhibition[c] (μM)*
None	—	1064	—	—
Methyl β-D-galactopyranoside	38	848	20.0	—
Methyl β-D-glucopyranoside	32	788	26.0	—
Methyl α-D-rhamnopyranoside	32	700	34.2	—
Methyl 4-*O*-β-D-galactopyranosyl-α-L-rhamnopyranoside[18]	34	632	40.5	—
Methyl 2-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**9**)	36	616	42.1	—
Methyl 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**13**)	24	308	71.1	16.0
Methyl 2-*O*-α-L-rhamnopyranosyl-4-*O*-β-D-glucopyranosyl-β-D-galactopyranoside (**16**)	24	228	78.5	8.4

[a]Diluted (1:1) serum was used, but the calculations were done on the basis of 1 mL of undiluted serum. [b]Average of duplicate runs. [c]Obtained directly from the graph.

acetates[13] was performed with a Hewlett–Packard 5730A gas chromatograph fitted with a flame-ionisation detector, and a glass column (1.83 m × 6 mm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 180° for neutral sugars and 170° for methylated sugars.

Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were recorded with a Perkin–Elmer 241MC polarimeter. ^{1}H-N.m.r. spectra were recorded with Varian XL-200 and Jeol FX-100 spectrometers for solutions in $CDCl_3$ (internal Me_4Si). Absorbances were measured with Hitachi 100-60 spectrophotometers. The glycoside syntheses were performed under dry nitrogen.

Methyl 3-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (**4**). — A solution of methyl 3-*O*-benzyl-β-D-galactopyranoside[4] (**3**; 8 g, 28.14 mmol) in *N,N*-dimethylformamide (50 mL) and α,α-dimethoxytoluene[5] (5.1 mL, 34 mmol) containing toluene-*p*-sulfonic acid (100 mg) was kept at 70°/12 mmHg in a rotary evaporator for 1.5 h. Solid $NaHCO_3$ (1 g) was then added, and the mixture was concentrated to small volume, diluted with chloroform (200 mL), washed with water, dried (Na_2SO_4), and concentrated. Crystallisation of the residue from ethanol gave **4** (9.8 g, 93%), m.p. 198–199°, $[\alpha]_D^{24}$ +58° (*c* 2.2, chloroform); lit.[14] m.p. 200–201°, $[\alpha]_D^{24}$ +56° (*c* 1, chloroform).

Ethyl 2,3,4-tri-O-acetyl-1-thio-α-L-rhamnopyranoside (**5**). — A mixture of dry α-L-rhamnopyranose tetra-acetate (10 g), ethanethiol (100 mL), and anhydrous zinc chloride[15] (3 g) was stirred at 0° for 16 h, then poured into saturated aq. $NaHCO_3$. The precipitate was collected and extracted with boiling chloroform (300 mL), and the extract was washed with water, dried (Na_2SO_4), and concentrated to dryness. Ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside (**5β**; 2.1 g, 18%) crystallized rapidly on the addition of ether; m.p. 121°, $[\alpha]_D^{24}$ +63.5° (*c* 2.1, chloroform). ^{1}H-N.m.r. data: δ 1.16 (d, 3 H, *J* 6 Hz, H-6,6,6), 1.20 (t, 3 H, SCH_2CH_3), 2.02–2.24 (3 s, 9 H, 3 OAc), 2.78 (q, 2 H, SCH_2CH_3), 4.48 (s, 1 H, H-1).

Anal. Calc. for $C_{14}H_{22}O_7S$: C, 50.28; H, 6.63. Found: C, 50.02; H, 6.76.

Column chromatography (4:1 benzene–ether) of the non-crystalline material gave **5** (7.6 g, 65%), $[\alpha]_D^{24}$ −98° (*c* 2.5, chloroform). ^{1}H-N.m.r. data: δ 1.14 (d, 3 H, *J* 6 Hz, H-6,6,6), 1.18 (t, 3 H, SCH_2CH_3), 2.00–2.20 (3 s, 9 H, 3 OAc), 2.76 (q, 2 H, SCH_2CH_3), 4.88 (d, 1 H, H-1).

Anal. Found: C, 50.42; H, 6.82.

2,3,4-Tri-O-benzyl-α-L-rhamnopyranosyl bromide (**7**). — Compound **5** (5 g) was treated[7] with benzyl chloride and potassium hydroxide in 1,4-dioxane to give ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**6**; 6.6 g, 92%) which, after column chromatography (15:1 benzene–ether), had $[\alpha]_D^{24}$ −58.5° (*c* 2, chloroform). ^{1}H-N.m.r. data: δ 1.12 (d, 3 H, *J* 6 Hz, H-6,6,6), 1.16 (t, 3 H, SCH_2CH_3), 2.72 (q, 2 H, SCH_2CH_3), 4.54–4.78 (m, 6 H, 3 $PhCH_2$), 4.84 (d, 1 H, H-1), 7.32–7.58 (m, 15 H, 3 Ph).

To a cooled solution of **6** (3.5 g) in dichloromethane (20 mL) was added bromine[16] (0.35 mL), and the mixture was stirred for 15 min at 0°, then concentrated to give **7** which was used immediately in the next step.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl-β-L-rhamnopyranosyl)-β-D-galactopyranoside (**8**). — A solution of **4** (2 g, 5.4 mmol) in dichloromethane (50 mL) was stirred with Ag_2CO_3 (2.8 g), Ag_2O (2.3 g), and molecular sieves 4 Å (3 g) for 1 h at room temperature. A solution of **7**, prepared from **6** (3.5 g, 7.3 mmol), in dichloromethane (10 mL) was added. The mixture was stirred at room temperature for 20 h in the dark, then filtered, and concentrated to dryness. Column chromatography (10:1 benzene–ether) of the residue gave **8** (2.6 g, 61%), which, on crystallisation from ethanol, gave material (1.9 g, 46%) with m.p. 166°, $[\alpha]_D^{24}$ +52° (*c* 2.1, chloroform). ^{1}H-N.m.r. data: δ 1.34 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.52 (s, 3 H, OMe), 4.38 (d, 1 H, *J* 7.6 Hz, H-1), 4.56–4.76 (m, 8 H, 4 PhC*H*$_2$), 4.58 (d, 1 H, *J* 3 Hz, H-1′), 5.50 (s, 1 H, PhC*H*), 7.30–7.60 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{48}H_{52}O_{10}$: C, 73.04; H, 6.64. Found: C, 72.96; H, 6.78.

Methyl 2-O-β-L-rhamnopyranosyl-β-D-galactopyranoside (**9**). — A solution of **8** (280 mg) in methanol (10 mL) was stirred in the presence of 10% Pd/C (100 mg) under hydrogen for 20 h at room temperature, then filtered, and concentrated to dryness, to give **9** (110 mg, 94%), $[\alpha]_D^{24}$ +17° (*c* 1.2, methanol). ^{1}H-N.m.r. data (D_2O): δ 1.34 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.44 (s, 3 H, OMe), 4.50 (d, 1 H, *J* 7.6 Hz, H-1), 4.62 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.11. Found: C, 45.70; H, 7.25.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**10**). — A mixture of **4** (6 g, 16.2 mmol), tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[6] (6.3 g, 17.8 mmol), 3 Å molecular sieves (4 g), mercury(II) cyanide (4.55 g, 18 mmol), and acetonitrile (60 mL) was stirred at room temperature for 24 h, then filtered through Celite, concentrated, and diluted with chloroform (200 mL). The solution was washed with aq. 10% KI, saturated aq. $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (4:1 chloroform–ether) of the residue followed by crystallisation from ethanol gave **10** (7.5 g, 72%), m.p. 98–100°, $[\alpha]_D^{24}$ −8.6° (*c* 2.1, chloroform). ^{1}H-N.m.r. data: δ 1.20 (d, 3 H, *J* 6 Hz, H-6,6,6), 2.02–2.08, 2.12 (3 s, 9 H, 3 OAc), 3.70 (s, 3 H, OMe), 4.34 (d, 1 H, *J* 7.6 Hz, H-1), 4.70 (s, 2 H, PhC*H*$_2$), 5.08 (d, 1 H, *J* 1 Hz, H-1′), 5.48 (s, 1 H, PhC*H*), 7.30–7.42 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{33}H_{40}O_{13}$: C, 61.48, H, 6.25. Found: C, 61.21; H, 6.21.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**11**). — Compound **10** (7 g) was treated[7] with benzyl chloride and KOH in 1,4-dioxane. Column chromatography (10:1 benzene–ether) of the product (8.1 g, 95%) and crystallisation from ethanol gave **11** (7.2 g, 84%), m.p. 149–151°, $[\alpha]_D^{24}$ −20° (*c* 2.4, chloroform). ^{1}H-N.m.r. data: δ 1.30 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.61 (s, 3 H, OMe), 4.32 (d, 1 H, *J* 7.6 Hz, H-1), 4.56–4.74 (m, 8 H, 4 PhC*H*$_2$), 4.92 (d, 1 H, *J* 1 Hz, H-1′), 5.48 (s, 1 H, PhC*H*), 7.32–7.58 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{48}H_{52}O_{10}$: C, 73.04; H, 6.64. Found: C, 73.12; H, 6.75.

Methyl 3-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galac-

topyranoside (**12**). — Compound **11** (6.5 g) was stirred with aq. 90% acetic acid (25 mL) for 2 h at 85°. The solvents were removed by evaporation and the residue was crystallised from ethanol to give **12** (5.2 g, 92%), m.p. 136–138°, $[\alpha]_D^{24}$ −11° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 1.28 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.58 (s, 3 H, OMe), 4.40 (d, 1 H, *J* 7.6 Hz, H-1), 4.58–4.79 (m, 8 H, 4 PhCH_2), 4.94 (d, 1 H, *J* 1 Hz, H-1′), 7.30–7.64 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{41}H_{48}O_{10}$: C, 70.26; H, 6.90. Found: C, 70.09; H, 7.06.

Methyl 2-O-α-L-rhamnopyranosyl-β-D-galactopyranoside (**13**). — A solution of **12** (500 mg) in methanol (10 mL) was debenzylated, as described for **9**, to give **13** (191 mg, 92%), $[\alpha]_D^{24}$ −46° (*c* 1, methanol). ^{1}H-N.m.r. data (D_2O): δ 1.30 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.48 (s, 3 H, OMe), 4.42 (d, 1 H, *J* 7.6 Hz, H-1), 4.92 (d, 1 H, *J* 2 Hz, H-1′).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.11. Found: C, 45.72; H, 7.30.

Methyl 3,6-di-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**14**). — To a solution of **12** (5 g, 7.13 mmol) in dichloromethane (60 mL) was added benzyl bromide (1.02 mL, 8.56 mmol), tetrabutylammonium hydrogensulfate (0.5 g, 1.5 mmol), and aq. 5% sodium hydroxide (10 mL). The suspension was boiled for 48 h under reflux, cooled, washed with water, dried (Na_2SO_4), and concentrated to dryness. Column chromatography (10:1 benzene–ether) of the residue gave **14** (3.52 g, 62%), m.p. 142°, $[\alpha]_D^{24}$ −54° (*c* 1.3, chloroform). ^{1}H-N.m.r. data: δ 1.30 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.51 (s, 3 H, OMe), 4.42 (d, 1 H, *J* 8 Hz, H-1), 4.54–4.76 (m, 10 H, 5 PhCH_2), 4.98 (d, 1 H, *J* 1 Hz, H-1′), 7.32–7.62 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{48}H_{54}O_{10}$: C, 72.89; H, 6.88. Found: C, 72.61; H, 6.96.

Methyl 3,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**15**). — A mixture of **14** (2.5 g, 1.97 mmol), dichloromethane (25 mL), 4 Å molecular sieves (2 g), and tetramethylurea[11] (0.3 mL) was stirred at room temperature for 30 min. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1 g, 2.45 mmol) was added, the mixture was cooled to −20°, silver triflate (643 mg, 2.5 mmol) was added, and stirring was continued for 12 h at −20° in the dark. The mixture was then filtered through Celite, washed with saturated aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (5:1 benzene–ether) of the residue and crystallisation from ethanol gave **15** (1.8 g, 51%), m.p. 163°, $[\alpha]_D^{24}$ −38° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 1.28 (d, 3 H, *J* 6 Hz, H-6,6,6), 2.01–2.14 (4 s, 12 H, 4 OAc), 3.56 (s, 3 H, OMe), 4.39 (d, 1 H, *J* 7.6 Hz, H-1), 4.56 (d, 1 H, *J* 7.9 Hz, H-1′), 4.60–4.66 (m, 10 H, 5 PhCH_2), 4.98 (d, 1 H, *J* 1 Hz, H-1″), 7.28–7.46 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{62}H_{72}O_{19}$: C, 66.65; H, 6.50. Found: C, 66.95; H, 6.48.

Methyl 4-O-β-D-glucopyranosyl-2-O-α-L-rhamnopyranosyl-β-D-galactopyranoside (**16**). — Compound **15** (500 mg) was debenzylated as described for **9**. The product was deacetylated with methanolic 0.1M sodium methoxide (10 mL) for 3 h. The solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and con-

centrated to dryness, to give **16** (195 mg, 87%), $[\alpha]_D^{24}$ −42° (*c* 1.2, water). ^{1}H-N.m.r. data (D_2O): δ 1.30 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.48 (s, 3 H, OMe), 4.42 (d, 1 H, *J* 7.6 Hz, H-1), 4.50 (d, 1 H, *J* 7.9 Hz, H-1′), 4.92 (d, 1 H, *J* 2 Hz, H-1″).

Anal. Calc. for $C_{19}H_{34}O_{15}$: C, 45.42; H, 6.82. Found: C, 45.50; H, 7.00.

Acid hydrolysis and methylation analysis of **9**, **13**, *and* **16**. — These methods were carried out as described[13].

Quantitative precipitin reaction. — The polysaccharide was added in increasing amounts (10–100 μg) to portions (0.1 mL) of homologous S-23 antiserum, and the volume was made up to 0.5 mL with normal saline. The mixtures (in duplicate) were kept for 1 h at 37°, then, together with blanks containing serum only, were kept for 96 h at 0–2°. Each mixture was centrifuged for 1 h at 3000 r.p.m. at 0–2° and the supernatant solution was tested for excess of antibody in Öuchterlony plates. Each precipitate was washed three times with chilled aq. 0.9% sodium chloride and then dissolved in 0.25M acetic acid (3 mL), and the absorbance was determined at 280 nm. The amount of antibody nitrogen precipitated was calculated from a standard curve calibrated by using rabbit IgG.

Inhibition studies. — To 0.1 mL of antiserum, in duplicate, were added increasing amounts of inhibitors, and the mixtures were stored at 0–2° for 1 h. An amount of antigen solution needed to bring the system to equivalence was then added to each mixture. The volume was made up to 0.5 mL with normal saline and the mixture was kept for 1 h at 37°. Two sets of controls involved the same amount of antigen and antibody as in the other tubes and serum only. The tubes were kept for 96 h at 0–2° and the amounts of precipitated nitrogen were calculated as described above. The same procedure was repeated for each inhibitor used. The results are given in Table I.

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