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Synthesis of disaccharide methyl glycosides related to the polysaccharide from *Klebsiella* serotype 40 and a study of their inhibition in the precipitin reaction

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ABSTRACT

The methyl glycosides of α-D-Man*p*-(1→4)-α-L-Rha*p* (**3**), α-L-Rha*p*-(1→3)-β-D-Gal*p* (**4**), β-L-Rha*p*-(1→3)-β-D-Gal*p* (**5**), β-D-Gal*p*-(1→2)-α-L-Rha*p* (**6**), and β-D-Glc*p*A-(1→2)-α-L-Rha*p* (**7**) have been synthesised and their inhibition reactions in the *Klebsiella* serotype 40 immune system have been studied. The results obtained accord with only one of two structures proposed for the repeating unit of the K40 antigen.

INTRODUCTION

The structures **1** and **2** of the repeating unit of the capsular polysaccharide from *Klebsiella* serotype 40 have been published by Ray *et al.*[1] and Chakraborty *et al.*[2], respectively.

→4)-α-D-Glc*p*A-(1→4)-β-D-Gal*p*-(1→2)-α-D-Man*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→
3
↑
1
β-D-Gal*p*-(1→2)-β-L-Rha*p*

1

→4)-β-D-Glc*p*A-(1→2)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→2)-α-L-Rha*p*-(1→
4
↑
1
α-D-Man*p*

2

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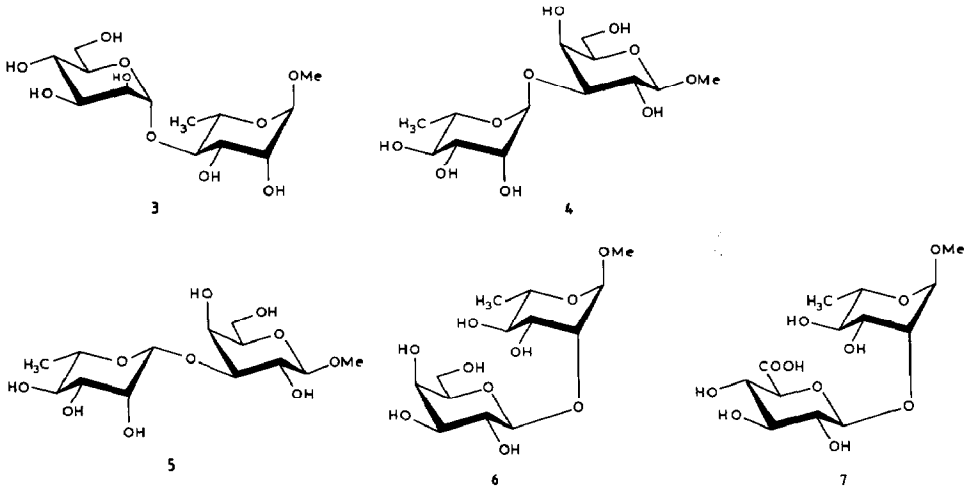
Both the structures were based on chemical evidence only. In order to obtain more information on the structure of the polysaccharide and its immunodominant fragments, the following disaccharide methyl glycosides related to **1** and **2** have been synthesised and their inhibition in the precipitin reaction studied: methyl 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranoside (**3**), methyl 3-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**4**), methyl 3-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**5**), methyl 2-*O*-β-D-galactopyranosyl-α-L-rhamnopyranoside (**6**), and methyl 2-*O*-(β-D-glucopyranosyluronic acid)-α-L-rhamnopyranoside (**7**).

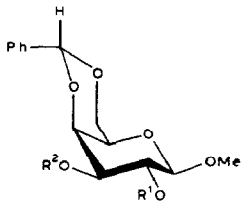


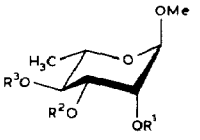
RESULTS AND DISCUSSION

The glycoside **3** was prepared as described[3]. In order to synthesise **4**, methyl 4,6-*O*-benzylidene-β-D-galactopyranoside[4] (**8**) was benzylated by the phase-transfer technique[5] to give methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**9**, 42%). Condensation of **9** with tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[6] in the presence of mercury(II) cyanide in acetonitrile[5] gave the disaccharide derivative **10** from which protecting groups were removed[7] to afford the glycoside **4**.

Condensation of **9** with ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-rhamnopyranoside[7] in the presence of methyl triflate in dichloromethane[8] gave methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl)-β-D-galactopyranoside (**11**). Hydrogenation[7] (Pd–C) of **11** then gave **5**.

Methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[3] (**12**) was benzylated[8] with benzyl bromide and sodium hydride in *N*,*N*-dimethylformamide to give the 4-*O*-benzyl derivative **13**. The isopropylidene group was removed from **13**, and the product **14** was allylated by the phase-transfer method[5] to give **15**. Benzylation[9] of **15** gave methyl 2-*O*-allyl-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**16**), *O*-deallylation[10] of which with SeO_2 gave methyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**17**). Condensation of **17** with tetra-*O*-acetyl-α-D-galactopyranosyl bromide[11] in the presence of mercury(II)





8 $R^1 = R^2 = H$
9 $R^1 = Bn, R^2 = H$
10 $R^1 = Bn, R^2 =$ 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl
11 $R^1 = Bn, R^2 =$ 2,3,4-tri-*O*-benzyl-β-L-rhamnopyranosyl

12 $R^1, R^2 =$ Ip, $R^3 = H$
13 $R^1, R^2 =$ Ip, $R^3 = Bn$
14 $R^1 = R^2 = H, R^3 = Bn$
15 $R^1 =$ allyl, $R^2 = H, R^3 = Bn$
16 $R^1 =$ allyl, $R^2 = R^3 = Bn$
17 $R^1 = H, R^2 = R^3 = Bn$
18 $R^1 =$ 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl
19 $R^1 =$ methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl-uronate

cyanide in acetonitrile[9] gave methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside (**18**, 39%). Removal of the protecting groups from **18** then gave **6**.

Reaction of **17** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[12] in the presence of mercury(II) cyanide and mercury(II) bromide[13] for 24 h gave methyl 3,4-di-*O*-benzyl-2-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-α-L-rhamnopyranoside (**19**). Removal of protecting groups from **19** then gave **7**.

The antiserum used in the homologous precipitin reaction was raised[14] against killed whole cells of *Klebsiella* Type 40 in rabbits. The serum was brought to pH 6 with dilute hydrochloric acid and set up with increasing amounts of saline. After 5 days at 2°, the precipitates were removed by centrifugation and the clear supernatant solutions were examined for antibody excess by the Ouchterlony double diffusion method[15]. The results showed that, at the equivalence point, 480 μg of the polysaccharide precipitated 1820 μg of the antibody nitrogen from 1 mL of the antiserum. The inhibition study was conducted with four methyl glycosides and **3**–**7**, and the results are summarised in Table I. It was observed that methyl α-D-mannopyranoside, methyl α-L-rhamnopyranoside, **3**, **4**, **6**, and **7**, which simulated parts of the structure **2**, did not inhibit the precipitin reaction significantly. The disaccharide glycoside **5**, which is related to a part of the structure **1**, inhibited the precipitin reaction to the extent of 86% and was effective at low concentration. Methyl β-D-galactopyranoside and D-glucuronic acid inhibited the precipitin reaction to ~50% at a quite high concentration. Inhibition to this extent reflects the presence of β-D-galactopyranosyl and D-glucuronic acid residues in the repeating unit of the K40 polysaccharide. All these results support the assignment of **1** as the structure for the repeating unit of the K40 polysaccharide, and the unit related to methyl 3-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**5**) is probably the immunodominant group.

TABLE I

Inhibition of antigen–antibody precipitation in the *Klebsiella* type 40 immune system

	Concentration (μM) needed for maximum inhibition[a]	*Antibody nitrogen precipitated (μg)*	*Inhibition*[b] *(%)*	*Concentration (μM) needed for 50% inhibition*[c]
None		1820		
Methyl β-D-galactopyranoside	7.0	917	50	7.0
Methyl α-D-mannopyranoside	8.0	1330	27	
Methyl α-L-rhamnopyranoside	9.0	1281	30	
D-Glucuronic acid	4.0	840	54	3.0
Methyl 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranoside (**3**)	5.0	1540	37	
Methyl 3-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside (**4**)	3.5	1015	44.5	
Methyl 3-*O*-β-L-rhamnopyranosyl-β-D-galactopyranoside (**5**)	1.8	259	86	1.1
Methyl 2-*O*-β-D-galactopyranosyl-α-L-rhamnopyranoside (**6**)	4.5	1344	26.5	
Methyl 2-*O*-(β-D-glucopyranosyluronic acid)-α-L-rhamnopyranoside (**7**)	3.0	1435	21.5	

[a] Experiment involved 1 mL of undiluted serum. [b] Average of duplicate runs. [c] Obtained directly from the graph.

EXPERIMENTAL

General methods. — All reactions were monitored by t.l.c. on Silica Gel G (Merck). All solvents were distilled before use, and all evaporations were conducted at 50° under diminished pressure unless otherwise stated. Column chromatography was performed on silica gel (SRL 100–120 mesh). Melting points were determined on 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 a Jeol FX-100 spectrometer for solutions in $CDCl_3$ (internal Me_4Si).

Methyl 2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (**9**). — A mixture of methyl 4,6-O-benzylidene-β-D-galactopyranoside (**8**; 6.5 g, 23 mmol), tetrabutylammonium bromide (1.5 g, 4.6 mmol), benzyl bromide (4.8 g; 28 mmol), aqueous 10% sodium hydroxide (30 mL), and dichloromethane (500 mL) was stirred for 24 h at room temperature. The organic layer was separated, washed with water (3 × 10 mL), dried (Na_2SO_4), and concentrated. T.l.c. (1:1 benzene–ether) revealed compounds that corresponded to the 2,3-di-*O*-, 2-*O*- (**9**), and 3-*O*-benzyl derivatives. Column chromatography using the same solvent system gave **9** (3.6 g, 42%), m.p. 110° (from ethanol), $[\alpha]_D^{27}$ +15.5° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 3.40 (s, 3 H, OMe), 4.46–4.49 (s, 2 H, PhCH_2), 4.82 (d, 1 H, *J* 7.5 Hz, H-1), 7.32–7.46 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{21}H_{24}O_6$: C, 67.73; H, 7.84. Found: C, 67.66; H, 7.91.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**10**). — A solution of **9** (1 g, 2.7 mmol) in dry acetonitrile (15 mL) was stirred with tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[6] (2.05 g, 5.8 mmol), mercury(II) cyanide (1.3 g, 5.2 mmol), and 3 Å molecular sieves (1 g) for 24 h at room temperature. The mixture was diluted with chloroform (500 mL), filtered through Celite, washed successively with aqueous 5% KI, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Column chromatography (4:1 benzene– ether) of the residue and crystallisation from ethanol gave **10** (1.17 g, 68%), m.p. 152–153°, $[\alpha]_D$ −19° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 1.18 (d, 3 H, *J* 6 Hz, H-6′,6′,6′), 2.00, 2.05, and 2.09 (3 s, 9 H, 3 OAc), 3.38 (s, 3 H, OMe), 4.32 (d, 1 H, *J* 7.5 Hz, H-1), 5.06 (d, 1 H, *J* 1 Hz, H-1′), 7.32–7.58 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{33}H_{40}O_{13}$: C, 61.48; H, 6.25; Found: C, 61.64; H, 6.51.

Methyl 3-O-α-L-rhamnopyranosyl-β-D-galactopyranoside (**4**). — A solution of **10** (300 mg) in ethyl acetate (910 mL) was stirred with 10% Pd–C (100 mg) under hydrogen at room temperature for 24 h, filtered, and concentrated to dryness. The glassy product was *O*-deacetylated with methanolic 0.1M sodium methoxide (5 mL). After 3 h, the solution was decationised with Amberlite IR-120 (H^+) resin and concentrated to dryness to give **4** (145 mg, 90%), $[\alpha]_D^{25}$ −14° (*c* 1.2, water). ^{1}H-N.m.r. data (D_2O): δ 1.34 (d, 3 H, *J* 6 Hz, H-6′,6′,6′), 3.42 (s, 3 H, OMe), 4.40 (d, 1 H, *J* 7.6 Hz, H-1), 4.96 (d, 1 H, *J* 1.5 Hz, H-1′).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.05; Found: C, 45.97; H, 7.20.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2,3,4-tri-O-benzyl-β-L-rhamnopyranosyl)-β-D-galactopyranoside (**11**). — To a stirred mixture of **9** (112 mg, 0.3 mmol), ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside[7] (145 mg, 0.3 mmol), and 4 Å molecular sieves (500 mg) in dichloromethane (5 mL) was added methyl trifluoromethanesulfonate (100 μL, 6 mmol). The mixture was stirred under argon for 48 h, triethylamine (170 μL, 1.2 mmol) was added, and stirring was continued for 10 min. The mixture was filtered through Celite and concentrated. Column chromatography (4:1 benzene–ether) of the resulting mixture and crystallisation from ethanol gave **11** (55 mg, 24%), m.p. 204–206°, $[\alpha]_D$ −37° (*c* 0.3, chloroform). ^{1}H-N.m.r. data: δ 1.28 (d, 3 H, *J* 6 Hz, H-6′,6′,6′), 3.46 (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′), 5.50 (s, 1 H, PhC*H*), 7.22–7.30 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{48}H_{52}O_{10}$: C, 73.08; H, 6.64. Found: C, 72.90; H, 6.91.

Methyl 3-O-β-L-rhamnopyranosyl-β-D-galactopyranoside (**5**). — To a solution of **11** (500 mg) in ethyl acetate (10 mL) was added 10% Pd–C (250 mg). The mixture was stirred for 24 h under hydrogen, then filtered through Celite, and concentrated to give **5** (208 mg, 95%), $[\alpha]_D^{25}$ −1.1° (*c* 1.1, water), R_{Glc} 0.76 (9:2:2 ethyl acetate–acetic acid–water). ^{1}H-N.m.r. data (D_2O): δ 1.30 (d, 3 H, *J* 6 Hz, H-6′,6′,6′), 3.40 (s, 3 H, OMe), 4.46 (d, 1 H, *J* 7.6 Hz, H-1), 4.60 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.05; Found: C, 45.72; H, 7.16.

Methyl 4-O-benzyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (**13**). — To a solution of methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**14**; 2.28 g, 10.5 mmol) in

N,N-dimethylformamide (30 mL) were added sodium hydride (50% oil, 3 g) and benzyl bromide (2.5 g, 15 mmol). The mixture was stirred for 3 h at room temperature, then diluted with dichloromethane (30 mL), washed with cold water (2 × 25 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (15:1 benzene–ether) of the residue gave **13** as a thick syrup (2.71 g, 88%), $[\alpha]_D^{25}$ −21° (*c* 2, chloroform). ^{1}H-N.m.r. data: δ 1.08 (d, 3 H, *J* 6 Hz, H-6,6,6), 1.30 and 1.40 (2 s, each 3 H, Me_2C), 3.38 (s, 3 H, OMe), 4.95 (d, 1 H, *J* 1.5 Hz, H-1), 7.24–7.32 (m, 5 H, Ph).

Anal. Calc. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.40; H, 7.58.

Methyl 4-O-benzyl-α-L-rhamnopyranoside (**14**). — Compound **13** (2 g) was heated with aqueous 80% acetic acid for 2 h at 85°. The solvents were evaporated to give **14** (1.65 g, 95%), $[\alpha]_D$ −39° (*c* 1.9, chloroform).

Anal. Calc. for $C_{14}H_{20}O_5$: C, 62.58; H, 7.46. Found: C, 62.75; H, 7.62.

Methyl 2-O-allyl-4-O-benzyl-α-L-rhamnopyranoside (**15**). — To a solution of **14** (1.65 g, 6.2 mmol) in dichloromethane (25 mL) were added allyl bromide (0.8 mL, 6.2 mmol), tetrabutylammonium bromide (0.43 g, 1.3 mmol), and aqueous 10% sodium hydroxide[5] (10 mL). The mixture was stirred vigorously for 2 days, dichloromethane (25 mL) was added, and the organic layer was separated, washed with water, dried (Na_2SO_4), and concentrated. T.l.c. (6:1 benzene–ether) revealed the 2,3-di-, 2- (**15**), and 3-*O*-allyl derivatives. Column chromatography using the same solvent gave **15** (1.10 g, 58%) together with the 3- (10%) and 2,3-di-*O*-allyl derivative (20%). Compound **15** had $[\alpha]_D^{25}$ −12° (*c* 2, chloroform). ^{1}H-N.m.r. data: δ 3.4 (s, 3 H, OMe), 5.1 (d, 1 H, *J* 1.5 Hz, H-1), 5.8–6.02 (m, 1 H, $CH_2=CH$), 7.3 (m, 5 H, 1 Ph).

Methyl 2-O-allyl-3,4-di-O-benzyl-α-L-rhamnopyranoside (**16**). — Compound **15** (1.5 g) was benzylated[5] as described in the preparation of **13**. Column chromatography (15:1 benzene–ether) of the product gave **16** (1.32 g, 68%), isolated as a syrup, $[\alpha]_D^{25}$ −6° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 1.12 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.4 (s, 3 H, OMe), 5.1 (d, 1 H, *J* 1.5 Hz, H-1), 6.0–6.22 (m, 1 H, $CH_2=CH$), 7.3 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{24}H_{30}O_5$: C, 72.33; H, 7.60. Found: C, 72.24; H, 7.63.

Methyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (**17**). — A solution of **16** (1.3 g, 3.27 mmol) in 1,4-dioxane (20 mL) and acetic acid (0.42 mL, 7.2 mmol) was stirred[10] unde reflux with SeO_2 (0.8 g, 7.2 mmol) for 40 min, then filtered, and concentrated to dryness. Column chromatography (15:1 benzene–ether) of the residue gave **17** (1.05 g, 90%), isolated as a syrup, $[\alpha]_D^{25}$ −4° (*c* 2, chloroform). ^{1}H-N.m.r. data: δ 1.12 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.4 (s, 3 H, OMe), 5.1 (d, 1 H, *J* 1.5 Hz, H-1), 7.3 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{21}H_{26}O_5$: C, 70.39; H, 7.26. Found: C, 70.17; H, 7.36.

Methyl 3,4-di-O-benzyl-2-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-α-L-rhamnopyranoside (**18**). — A solution of **17** (1 g, 2.8 mmol) in dry acetonitrile[13] (10 mL) was stirred under argon with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.8 g, 4.7 mmol), mercury(II) cyanide (1.26 g, 5 mmol), and molecular sieves 3 Å (1.5 g) for 24 h at room temperature. The mixture was filtered through Celite, diluted with chloroform (200 mL), washed successively with aqueous 5% KI, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Column chromatography (4:1 benzene–ether) of the residue gave **18** (0.76 g, 39%), isolated as a syrup, $[\alpha]_D^{25}$ −23° (*c*

1.2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.34 (d, 3 H, J 6 Hz, H-6,6,6), 3.42 (s, 3 H, OMe), 5.00 (d, 1 H, J 2 Hz, H-1), 1.96–2.10 (4 s, 12 H, 3 OAc), 4.6 (d, 1 H, J 7.5 Hz, H-1'), 7.43–7.74 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{35}H_{44}O_{14}$: C, 61.04; H, 6.44. Found: C, 61.02; H, 6.35.

Methyl 2-O-β-D-galactopyranosyl-α-L-rhamnopyranoside (**6**). — A mixture of **18** (300 g), 10% Pd–C (100 mg), and ethyl acetate (15 mL) was stirred under hydrogen for 24 h, then worked-up as described above. The product, obtained as a thick glass, was *O*-deacetylated as described above to give **6** (116 mg, 81%), $[\alpha]_D$ −33.5°. ^{1}H-N.m.r. data ($CDCl_3$): δ 1.30 (d, 3 H, J 6 Hz, H-6,6,6), 3.40 (s, 3 H, OMe), 4.52 (d, 1 H, J 8 Hz, H-1'), 4.98 (d, 1 H, J 1.5 Hz, H-1).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.06. Found: C, 45.69; H, 7.21.

Methyl 3,4-di-O-benzyl-2-O-[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl) uronate]-α-L-rhamnopyranoside (**19**). — A solution of **17** (1 g, 2.8 mmol) in dry acetonitrile at room temperature under argon was stirred with methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl bromide)uronate (0.64 g, 1.6 mmol), mercury(II) cyanide (1.26 g, 5 mmol), mercury(II) bromide (1.8 g, 5 mmol), and molecular sieves 3 Å (1.5 g) for 24 h at room temperature[13]. The mixture was filtered through Celite, diluted with chloroform (200 mL), washed successively with aqueous 5% KI, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Column chromatography (4:1 benzene–ether) of the residue gave syrupy **19** (0.817 g, 44%), $[\alpha]_D^{24}$ +82° (*c* 1.2 chloroform). ^{1}H-N.m.r. data: δ 1.28 (d, 3 H, J 6 Hz, H-6,6,6), 1.84-1.98 (3 s, 9 H, 3 OAc), 3.31 (s, 3 H, OMe), 3.64 (s, 3 H, COOMe), 4.60 (d, 1 H, J 8 Hz, H-1'), 5.02 (d, 1 H, J 1.5 Hz, H-1), 7.32–7.68 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{34}H_{42}O_{14}$: C, 60.53; H, 6.27. Found: C, 60.26; H, 6.41.

Methyl 2-O-(β-D-glucopyranosyluronic acid)-α-L-rhamnopyranoside (**7**). — Compound **19** (700 mg, 1.3 mmol) was *O*-debenzylated and *O*-deacetylated as described above. A few drops of water were added to the *O*-deacetylation mixture after 2 h in order to saponify the methyl ester. After 1 h, the mixture was worked-up in the usual way to give **7** (318 mg, 85%), $[\alpha]_D^{25}$ +14° (*c* 1.1, water). ^{1}H-N.m.r. data (D_2O): δ 1.19 (d, 3 H, J 6 Hz, H-6,6,6), 3.40 (s, 3 H, OMe), 4.58 (d, J 7.6 Hz, H-1'), 5.05 (d, 1 H, J 1.5 Hz, H-1).

Anal. Calc. for $C_{13}H_{22}O_{11}$: C, 44.07; H, 6.21. Found: C, 44.16; H, 6.30.

Quantitative precipitin reactions. — The polysaccharide was added in increasing amounts (10–100 μg) to portions (0.1 mL) of homologous K-40 antiserum and the volume was made up to 0.5 mL with normal saline. Each mixture (in duplicate) was kept for 1 h at 37° and then, together with blanks containing serum only, 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, using Ouchterlony plates. Each precipitate was washed thrice with chilled aqueous 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. The 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, one containing the same amount of antigen and antibody as in the other tubes and the other containing only the serum, were also included. The tubes were kept at 0–2° for 96 h and the amounts of precipitated nitrogen were calculated as described above. The results are given in Table I.

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