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Note

Synthesis of a tetrasaccharide related to the repeating unit of the antigen from *Klebsiella* type 55

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The structure of the repeating unit of the capsular polysaccharide of *Klebsiella* type 55 has already been established[1]. In continuation of our effort to determine the relation between structure and immunological specificity, we synthesised the tetrasaccharide related to the repeating unit of the antigen from *Klebsiella* type 55. This tetrasaccharide has all the features of the repeating unit of the K55 polysaccharide except the acetyl group at the 2-position of the L-rhamnose moiety. We now report the details of the synthesis.

Methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[2] (**1**) was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[3] in the presence of mercury(II) cyanide in acetonitrile[4] to give methyl 2,3-*O*-isopropylidene-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**2**) in 81% yield. Zemplén deacetylation[5] of **2** followed by benzylation[6] of the product **3** gave methyl 2,3-*O*-isopropylidene-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**4**). Removal of the isopropylidene group from **4** followed by selective benzylation of **5** by a phase transfer method[7] yielded methyl 2-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**6**). The glycosyl acceptor **6** was allowed to condense with ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[8] (**7**) in the presence of methyl triflate[9] in ethyl ether as solvent to afford methyl 3-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-2-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**8**) in 74% yield. Deallylation of **8** with selenium dioxide[10] gave methyl 2-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-L-rhamnopyranoside (**9**). Hydrogenolysis of **9** in the presence of Pd–C gave methyl 3-*O*-α-D-galactopyranosyl-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (**10**). The trisaccharide derivative **9** was allowed to react with

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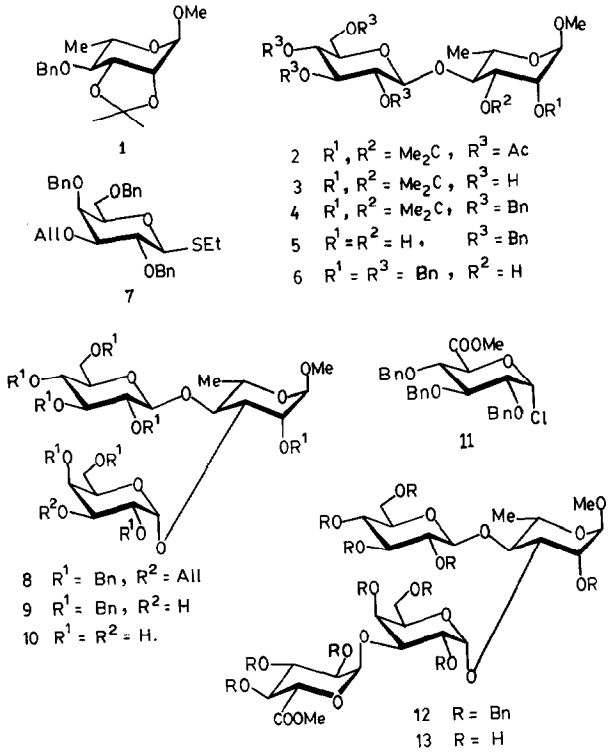

Scheme 1.

methyl (2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride)uronate[11] (**11**), prepared from methyl 1-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranuronate[12], in presence of mercury(II) bromide in 1,2-dichloroethane to give methyl 2-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (**12**) together with some β-glycoside as revealed by its NMR spectrum. The β-glycoside could not be separated by column chromatography at that stage. Removal of protecting groups from this mixture gave a product from which the desired tetrasaccharide **13** and its corresponding β isomer were isolated as their methyl ester methyl glycoside by column chromatography. The tetrasaccharides were characterised by their ^{13}C and ^{1}H NMR spectra.

EXPERIMENTAL

General.—All reactions were monitored by TLC on Silica Gel G (Merck). Column chromatography was performed using 100–200 mesh silica gel (SRL, India). The weight of silica gel taken for individual separations was approximately 10 to 25 times that of the crude reaction mixture, depending on the extent of separation. All solvents were dried and/or distilled before use, and all evapora-

tions were conducted below 50°C unless otherwise stated. Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter. ^{1}H NMR spectra were recorded (internal standard tetramethylsilane) with a Jeol FX-100 or Varian XL-300 spectrometer, using $CDCl_3$ as solvent unless stated otherwise.

Methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**2**).—To a solution of methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[2] (**1**; 3.0 g, 13.8 mmol) and mercury(II) cyanide (4.3 g, 17.2 mmol) in acetonitrile[4] (32.5 mL) containing 4A molecular sieves (5.0 g) under Ar was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[3] (6.45 g, 15.5 mmol) in MeCN (3.5 mL), and the mixture was kept stirring at room temperature. After 24 h, the mixture was diluted with CH_2Cl_2 and filtered through Celite. The organic layer was washed sequentially with water, aq 5% KI, and water, dried (Na_2SO_4), and evaporated to dryness. The residual syrup was chromatographed on a column with 3:1 toluene–ether to give pure **2** (6.97 g, 81%). The product was crystallised from ether–CH_2Cl_2–petroleum ether (bp 40–60°C), giving fine needles; mp 156–157°C; $[\alpha]_D^{25}$ −31.2° (*c* 1.0, $CHCl_3$). ^{1}H NMR data: δ 1.26 (d, 3 H, $J_{5,6}$ 6.0 Hz, CMe), 1.34 and 1.52 (2 s, 6 H, CMe_2), 2.00, 2.02, 2.06 (3 s, 12 H, OAc), 3.38 (s, 3 H, OMe), 5.0 (d, 1 H, $J_{1',2'}$ 7.0 Hz, H-1'), 5.18 (d, $J_{1,2}$ 2.0 Hz, H-1). Anal. Calcd. for $C_{24}H_{36}O_{14}$: C, 52.55; H, 6.62. Found: C, 52.35; H, 6.67.

Methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**4**).—Compound **2** (3.0 g, 5.5 mmol) was stirred with methanolic 0.05 M NaOMe (26 mL) for 5 h. The solution was decationised with Dowex 50W-X8 (H^+) resin, filtered, and concentrated to dryness to give **3** (quantitative yield). To a cold solution of **3** in *N*,*N*-dimethylformamide (18.0 mL) were added NaH (1.57 g, 33.0 mmol, 50% oil-coated) and benzyl bromide (3.51 mL, 29.6 mmol), and the mixture was stirred at room temperature for 6 h. MeOH (1.25 mL) was added to decompose excess of NaH. The mixture was diluted with CH_2Cl_2, and the organic layer was washed thrice with water, dried (Na_2SO_4), and concentrated to a syrup. The product was purified on a column with 6:1 toluene–ether, giving pure **4** (3.2 g, 79%); $[\alpha]_D^{25}$ −14.0° (*c* 2.5, $CHCl_3$). ^{1}H NMR data: δ 1.27 (d, 3 H, $J_{5,6}$ 6.0 Hz, CMe), 1.25 and 1.42 (2 s, 6 H, CMe_2), 3.38 (s, 3 H, OMe), 4.6 (d, 1 H, $J_{1',2'}$, 7.0 Hz, H-1'), 4.98 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 7.23–7.36 (m, 20 H, 4 Ph). Anal. Calcd. for $C_{44}H_{52}O_{10}$: C, 71.33; H, 7.07. Found: C, 71.23; H, 6.80.

Methyl 2-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**6**).—A solution of **4** (2.5 g, 3.4 mmol) in aq 85% acetic acid (10.4 mL) was stirred at 90°C for 2 h. Acetic acid was removed by co-evaporation first with water and then with toluene to give **5** (2.35 g, quantitative yield). The product was stirred vigorously with CH_2Cl_2 (40 mL), aq 10% NaOH (4.75 mL), benzyl bromide (0.6 mL, 5.1 mmol), and tetrabutylammonium bromide (0.32 g, 1.7 mmol) at room temperature for 60 h. The organic layer was washed with water, dried (Na_2SO_4), and concentrated to a syrup. Column chromatography with 5:1 toluene–ether gave **6** (1.71 g, 65%); $[\alpha]_D^{25}$ −6.6° (*c* 2.6, $CHCl_3$). ^{1}H NMR data: δ 1.39 (d, 1 H, $J_{5,6}$ 6.0 Hz, CMe), 2.33 (s, 1 H, OH), 3.33 (s, 3 H, OMe), 4.61 (d, 1 H, $J_{1',2'}$ 6.0 Hz,

H-1′), 4.84 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 7.24–7.36 (m, 25 H, 5 Ph). Anal. Calcd. for $C_{48}H_{54}O_{10}$: C, 72.89; H, 6.88. Found: C, 72.65; H, 6.97.

Methyl 3-O-(3-O-allyl-2,4,6-tri-O-benzyl-α-D-galactopyranosyl)-2-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**8**).—To a mixture of **6** (1.5 g, 1.9 mmol) and ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[8] (**7**; 1.55 g, 2.8 mmol) in ether (25 mL) containing 4A molecular sieves (4 g) under Ar was added methyl triflate[9] (2.2 mL), and the mixture was stirred at 22°C for 25 h. The mixture was worked up in the usual way. Column chromatography of the product with 3:1 hexane–ether yielded pure **8** (1.7 g, 74%) as a syrup; $[\alpha]_D^{25}$ +37.0° (*c* 0.5, $CHCl_3$). ^1H NMR data: δ 1.38 (d, 3 H, $J_{5,6}$ 6.0 Hz, CMe), 3.22 (s, 3 H, OMe), 4.62 (d, 1 H, $J_{1',2'}$ 6.0 Hz, H-1′), 4.94 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.04–5.96 (m, 6 H, 5 allyl and vinyl H, and H-1″), 7.24–7.36 (m, 40 H, 8 Ph). Anal. Calcd. for $C_{78}H_{86}O_{15}$: C, 74.15; H, 6.86. Found: C, 74.44; H, 6.83.

Methyl 2-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-3-O-(2,4,6-tri-O-benzyl-α-D-galactopyranosyl)-α-L-rhamnopyranoside (**9**).—To a solution of **8** (1.7 g, 1.3 mmol) in 1,4-dioxane (14 mL) were added SeO_2 (145 mg, 1.3 mmol) and glacial acetic acid (0.12 mL). The mixture was stirred under reflux for 30 min and then filtered through Celite. The filtrate was concentrated to a glassy material which was purified by column chromatography with 1:8 EtOAc–petroleum ether (bp 60–80°C) to give **9** (1.03 g, 64%); $[\alpha]_D^{25}$ +38.9° (*c* 0.9, $CHCl_3$). ^1H NMR data: δ 1.4 (d, 3 H, $J_{5,6}$ 6.5 Hz, CMe), 2.24 (br s, 1 H, OH), 3.26 (s, 3 H, OMe), 4.66 (d, 1 H, $J_{1',2'}$ 6.5 Hz, H-1′), 4.92 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.4 (d, 1 H, $J_{1'',2''}$ 3.0 Hz, H-1″), 7.22–7.33 (m, 40 H, 8 Ph). Anal. Calcd. for $C_{75}H_{82}O_{15}$: C, 73.63; H, 6.76. Found: C, 73.49; H, 6.88.

Methyl 3-O-α-D-galactopyranosyl-4-O-β-D-glucopyranosyl-α-L-rhamnopyranoside (**10**).—A solution of **9** (93 mg, 76 μmol) in 7:3 EtOH–toluene (10 mL) was hydrogenolysed for 12 h in the presence of 10% Pd–C (31.5 mg) at 24°C and the mixture was worked up in the usual way, to yield pure **10** as revealed by TLC (10:5:1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ +39.4° (*c* 1.0, H_2O). ^1H NMR (D_2O) data: δ 1.37 (d, 3 H, $J_{5,6}$ 6.2 Hz, CMe), 3.43 (s, 3 H, OMe), 4.76 (d, 1 H, $J_{1',2'}$ 7.11 Hz, H-1′), 4.82 (br s, 1 H, H-1), 5.28 (d, 1 H, $J_{1'',2''}$ 3.42 Hz, H-1″). ^{13}C NMR (D_2O, internal standard 1,4-dioxane) data: δ 18.03 (C-CH_3), 55.85 (O-CH_3), 61.77 (C-6″), 62.19 (C-6′), 94.64 (C-1″), 101.42 (C-1), 103.18 (C-1′). Anal. Calcd. for $C_{19}H_{34}O_{15}$: C, 45.42; H, 6.82. Found: C, 45.29; H, 6.92.

Methyl 2-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-3-O-[2,4,6-tri-O-benzyl-3-O-(methyl 2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (**12**).—To a solution of methyl (2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride)uronate[11] (**11**; 800 mg, 1.6 mmol) in 1,2-dichloroethane (30 mL) were added 4A molecular sieves (5 g) and mercury(II) bromide (1.43 g, 4.0 mmol), and the mixture was stirred under Ar for 30 min. A solution of **9** (800 mg, 662.5 μmol) in 1,2-dichloroethane (5 mL) was then injected into the flask and the mixture was stirred at 24°C for 96 h. The contents of the flask were then diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with

aq 5% KI, satd aq $NaHCO_3$, and water in succession, dried (Na_2SO_4), and concentrated in vacuo. Column chromatography of the product with 25:1 toluene–ether gave a mixture of tetrasaccharide derivatives (441 mg, 40%) as a syrup; $[\alpha]_D^{25}$ +47.8° (*c* 1.0, $CHCl_3$). The ^1H NMR spectrum of the compound showed that it contained **12** together with ca. 10–15% of its β isomer which could not be separated by TLC or column chromatography. Anal. Calcd. for $C_{92}H_{110}O_{21}$: C, 71.21; H, 7.14. Found: C, 71.08; H, 7.24.

Methyl 4-O-β-D-glucopyranosyl-3-O-[3-O-(methyl α-D-glucopyranosyluronate)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (**13**).—Compound **12** (400 mg, 0.24 mmol) in 5:3 EtOH–toluene (24.6 mL) was hydrogenolysed for 14 h in the presence of 10% Pd–C (160 mg) at 25°C. The mixture was then filtered through Celite and concentrated in vacuo. An aqueous solution of the product was again filtered through a millipore membrane and concentrated to dryness (138 mg, 85%). TLC of this material in 10:5:1 $CHCl_3$–MeOH–H_2O showed two clear spots which were separated by column chromatography using the same solvent system. The major component (123 mg) was characterised as **13**; $[\alpha]_D^{25}$ +71.0° (*c* 1.0, H_2O). ^1H NMR data (D_2O): δ 1.30 (d, 3 H, $J_{5,6}$ 5.89 Hz, CMe), 3.36 (s, 3 H, OMe), 3.78 (s, 3 H, COOMe), 4.7 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.84 (d, 1 H, $J_{1,2}$ 2.96 Hz, H-1), 5.11 (d, 1 H, $J_{1''',2'''}$ 3.67 Hz, H-1‴), 5.21 (d, 1 H, $J_{1'',2''}$ 3.59 Hz, H-1″). ^{13}C NMR data (D_2O, internal standard 1,4-dioxane): δ 17.96 (C-CH_3), 53.93 (O-CH_3), 55.73 (COOCH_3), 61.46 (C-6″), 62.14 (C-6′), 93.87 (C-1″), 97.12 (C-1‴), 101.38 (C-1), 103.29 (C-1′), 171.96 (COOCH$_3$). Anal. Calcd. for $C_{26}H_{44}O_{21}$ (**13**): C, 45.09; H, 6.40. Found: C, 45.00; H, 6.54.

The minor component (14 mg) was the corresponding β isomer of **12**; $[\alpha]_D^{25}$ +31.9° (*c* 0.9, H_2O). ^{13}C NMR data (D_2O, internal standard 1,4-dioxane): δ 17.97 (C-CH_3), 54.00 (O-CH_3), 55.72 (COOCH_3), 61.46 (C-6″), 62.14 (C-6′), 93.8 (C-1″), 101.39 (C-1), 103.29 (C-1′), 104.84 (C-1‴), 172.89 (COOCH$_3$).

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