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SYNTHESIS OF SOME DI- AND TRISACCHARIDES RELATED TO THE REPEATING UNIT OF THE ANTIGEN FROM *KLEBSIELLA* TYPE 20

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ABSTRACT : Starting from D-galactose, D-glucurono-lactone, and D-mannose, two trisaccharides and two disaccharides related to the repeating unit of *Klebsiella* type 20 have been synthesised using methyl triflate as promoter with success.

The structure of the repeating unit of the antigen from *Klebsiella* type 20 has been established by Choy and Dutton[1]. In our effort to determine the relationship between the structure and immunological specificity of the carbohydrate moieties it was necessary to synthesise some oligosaccharide fragments related to the repeating unit. The synthesis of methyl 2-*O*-β-D-galactopyranosyl-3-*O*-α-D-galactopyranosyl-α-D-mannopyranoside (**4**), methyl 3-*O*-α-D-galactopyranosyl-α-D-mannopyranoside (**9**), methyl 3-*O*-[3-*O*-(methyl β-D-glucopyranosyluronate)-α-

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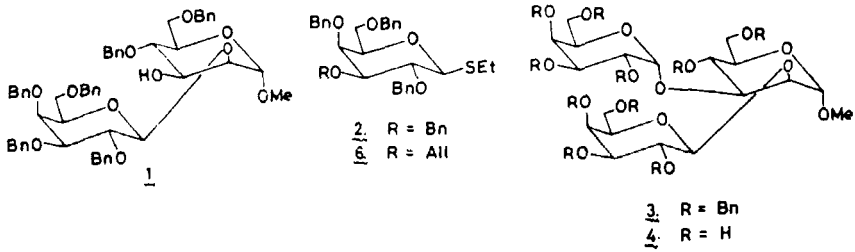
D-galactopyranosyl]-α-D-mannopyranoside (12) and methyl 3-*O*-β-D-glucopyranosyluronic acid-α-D-galactopyranoside (17) were therefore taken up.

Methyl 4,6-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside[2] (1) was allowed to condense with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[3] (2) in presence of methyl triflate[4] as promoter to provide methyl 4,6-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside (3) in 70.4% yield. Compound 3 was hydrogenolysed to give methyl 2-*O*-β-D-galactopyranosyl-3-*O*-α-D-galactopyranosyl-α-D-mannopyranoside (4), which was characterised by its ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum exhibited a one proton broad singlet at δ 4.73 (H-1) for α-mannosidic, a doublet at δ 4.33 (J=7 Hz, H-1') for β-galactosidic and a doublet at δ 5.2 (J=3.75 Hz, H-1") for α-galactosidic linkages. The ^{13}C NMR spectrum showed the presence of signals at δ 99.58 (C-1), 103.14 (C-1') and 101.04 (C-1") confirming the anomeric assignments of the sugar units.

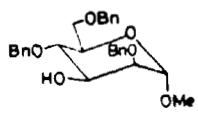


Methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside[5,6] (5) was allowed to condense with ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[7] (6) using

methyl triflate[4] as promoter to afford methyl 3-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**7**) in 83% yield. Removal of allyl group[8] from **7** gave methyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside (**8**). Hydrogenolysis of **8** gave the methyl 3-*O*-α-D-galactopyranosyl-α-D-mannopyranoside (**9**). The ^{1}H NMR spectrum exhibited the expected signals. The ^{13}C NMR spectrum showed the presence of 13 characteristic carbon atoms with two anomeric carbons appearing at the same position at δ 101.60 and having intensity twice as much as that compared to other carbons.

Compound **8** was condensed with methyl (ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranoside)uronate[9] (**10**) in the presence of methyl triflate[4] to give methyl 3-*O*-[3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl]-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**11**) in 61.8% yield. Hydrogenolysis of **11** followed by deacetylation of the product gave methyl 3-*O*-[3-*O*-(methyl β-D-glucopyranosyluronate)-α-D-galactopyranosyl]-α-D-mannopyranoside (**12**). ^{1}H NMR spectrum of the compound showed the presence of three anomeric protons together with one methoxyl and one methyl ester group. The ^{13}C NMR spectrum exhibited 20 carbon signals. The anomeric linkages were confirmed by the signals at δ 101.45 (C-1'),101.60 (C-1) and 104.50 (C-1").

Methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside[10] was benzylated using benzyl bromide[11] to give methyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (**13**). Removal of isopropylidene group[12] from **13** gave methyl 2,6-di-*O*-benzyl-α-D-galactopyranoside[13]



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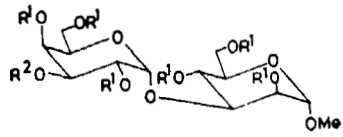
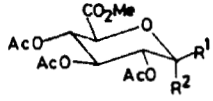
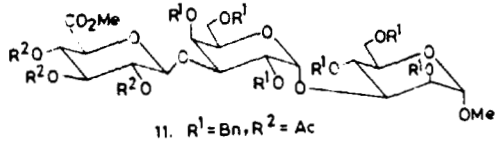


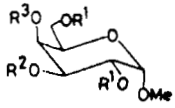
7. $R^1 = Bn, R^2 = All$
8. $R^1 = Bn, R^2 = H$
9. $R^1 = R^2 = H$



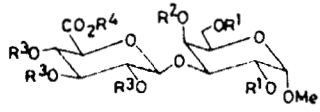
10. $R^1 = SEt, R^2 = H$
15. $R^1 = H, R^2 = Br$



11. $R^1 = Bn, R^2 = Ac$
12. $R^1 = R^2 = H$



13. $R^1 = Bn, R^2, R^3 = CMe_2$
14. $R^1 = Bn, R^2 = R^3 = H$
18. $R^1 = Bn, R^2 = All, R^3 = H$
19. $R^1 = R^3 = Bn, R^2 = All$
20. $R^1 = R^3 = Bn, R^2 = H$



16. $R^1 = Bn, R^2 = H, R^3 = Ac, R^4 = Me$
17. $R^1 = R^2 = R^3 = R^4 = H$
21. $R^1 = R^2 = Bn, R^3 = Ac, R^4 = Me$

(14). Compound 14 was allowed to condense with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[14] (15) in presence of Ag_2CO_3 and iodine to give methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-galactopyranoside (16) in 62.6% yield. Compound 16 was hydrogenolysed, deacetylated[15] and saponified in succession to give methyl 3-*O*-β-D-glucopyranosyluronic acid-α-D-galactopyranoside (17). Its structure was confirmed by ^1H and ^{13}C NMR spectra. That the D-glucose moiety is linked to the 3-position of galactose was confirmed by methylation analysis of 17 where gas liquid chromatographic analysis exhibited the presence of 2,4,6-trimethyl galactose.

The disaccharide **17** was also synthesised by an alternative route. The compound **14** was selectively allylated at the 3-position using stannylidene complexation technique[16] to produce methyl 3-*O*-allyl-2,6-di-*O*-benzyl-α-D-galactopyranoside (**18**) which on benzylation[11] gave methyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranoside[17] (**19**). Deallylation[8] of **19** afforded methyl 2,4,6-tri*O*-benzyl-α-D-galactopyranoside[17,18] (**20**). This compound **20** was allowed to condense with **10**[9] in CH_2Cl_2 in the presence of methyl triflate[4] to give methyl 3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (**21**) in 66% yield. Compound **21** on hydrogenolysis followed by deacetylation and saponification gave the same disaccharide **17**.

EXPERIMENTAL

All reactions were monitored by TLC on Silica Gel G (Merck). Column chromatography was performed on 100-200 mesh silica gel (SRL, India). All solvents were dried and/distilled before use. All solvents were removed under reduced pressure at 40°C unless otherwise stated. The organic layers were dried over anhydrous Na_2SO_4. Optical rotations were measured with a Perkin-Elmer model 241 MC polarimeter. Melting points were determined on a paraffin oil bath and are uncorrected. The NMR spectra were recorded with either Jeol FX-100 or Bruker 300 MHz instrument using chloroform-d as solvent and TMS as internal standard, unless stated otherwise.

Preparation of **3**.- To a mixture of **1** (300 mg, 0.33 mmol) and **2** (293 mg, 0.5 mmol) in ether (10 ml) 4A molecular sieves (1 g) were added and the mixture was stirred under N_2 for 45 min. Methyl triflate (0.39 mL) was injected into

the mixture and stirring was continued at 22°C for 98 h. The reaction was then quenched with Et_3N, filtered through a celite bed and concentrated. The crude mixture was chromatographed using 9:1 toluene-ether to give **3** (330 mg, 70.4%); $[\alpha]_D^{24}$ +53.49° (*c* 0.3, $CHCl_3$). ^1H NMR: δ 3.24 (s, 3H, OMe), 4.75 (d, 1H, J=7.0 Hz, H-1'), 4.98 (br s, 1H, H-1), 5.18 (d, 1H, J=4.0 Hz, H-1"), 7.16-7.40 (m, 50H, 10Ph); Elemental analysis; calc. for $C_{89}H_{94}O_{16}$: C: 75.30; H: 6.67; found C: 75.21; H: 6.68.

Preparation of **4**.- Compound **3** (314 mg, 0.22 mmol) was hydrogenolysed in acetic acid (7 mL) in the presence of 10% Pd-C (133 mg) for 72 h. The reaction mixture was filtered through a celite bed and concentrated. Column chromatography (5:2:0.4 $CHCl_3$-MeOH-H_2O) gave pure **4** (95 mg, 83%); $[\alpha]_D^{24}$ +63.6° (*c* 0.3, H_2O). ^1H NMR (D_2O): δ 3.28 (s, 3H, OMe), 4.33 (d, 1H, J=7.0 Hz, H-1'), 4.73 (br s, 1H, H-1), 5.2 (d, 1H, J=3.75 Hz, H-1"). ^{13}C NMR (D_2O, internal standard 1,4-dioxane): δ 55.76 (OCH_3), 61.02 (C-6"), 61.73 (C-6), 62.20 (C-6'), 99.58 (C-1), 101.04 (C-1"), 103.14 (C-1').

Preparation of **7**.- Compound **5** (0.7 g, 1.5 mmol) was allowed to condense with **6** (1.2 g, 2.3 mmol) in ether (20 mL) in presence of methyl triflate (1.28 mL) as described above. Column chromatography afforded **7** (1.17 g, 83%); $[\alpha]_D^{27}$ +39.14° (*c* 0.8, $CHCl_3$). ^1H NMR: δ 3.32 (s, 3H, OMe), 4.86 (br s, 1H, H-1), 5.22 (d, 1H, J=3.0 Hz, H-1'), 5.76-6.14 (m, 1H, CH_2-C*H*=CH_2), 7.20-7.30 (m, 30H, 6Ph); Elemental analysis; calc. for $C_{58}H_{64}O_{11}$: C: 74.33; H: 6.88; found C: 74.05; H: 6.99.

Preparation of **8**.- To a solution of **7** (1.1 g, 1.2 mmol) in methanol (15 mL), $PdCl_2$ (104 mg, 0.5 mmol) was added and the mixture was stirred for 3 h. After

evaporation of the solvent, the crude product was immediately chromatographed with 7:1 toluene-ether to give pure **8** (589 mg, 56%); $[\alpha]_D$ +59.2° (*c* 1.7, $CHCl_3$). ^1H NMR: δ 3.33 (s, 3H, OMe), 4.88 (br s, 1H, H-1), 5.26 (d, 1H, J=3.5 Hz, H-1'), 7.20-7.40 (m, 30H, 6Ph); Elemental analysis; calc. for $C_{55}H_{60}O_{11}$: C: 73.64; H: 6.74; found C: 73.59; H: 6.88.

Preparation of **9**.- Compound **8** (150 mg, 0.18 mmol) was hydrogenolysed in ethanol (10 mL) in presence of 10% Pd-C (125 mg) at room temperature for 50 h. The mixture was filtered through a celite bed and the concentrated mixture was chromatographed using 10:5:1 $CHCl_3$-MeOH-H_2O to give **9** (44.4 mg, 71%); $[\alpha]_D^{27}$ +74.0° (*c* 0.12, H_2O). ^1H NMR (D_2O): δ 3.41 (s, 3H, OMe), 4.75 (d, 1H, J=1.6 Hz, H-1), 5.24 (d, 1H, J=3.96 Hz, H-1'). ^{13}C NMR (D_2O): δ 55.62 (OCH_3), 61.70 (C-6), 62.03 (C-6'), 66.88, 69.56, 70.11, 70.17, 70.53, 72.22, 73.47, 79.55, 101.60 (C-1 and C-1').

Preparation of **11**.- Compound **8** (219 mg, 0.24 mmol) was allowed to react with methyl (ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranoside)uronate (**10**; 139 mg, 0.36 mmol) in ether (6 mL) in the presence of methyl triflate (0.21 mL), as described for the preparation of **3**. The chromatographic yield of **11** was 183.2 mg (61.8%); $[\alpha]_D^{25}$ +22.31° (*c* 0.8, $CHCl_3$). ^1H NMR: δ 1.99 and 2.00 (2s, 9H, 3Ac), 3.32 (s, 3H, OMe), 3.61 (s, 3H, COOMe), 4.57 (d, 1H, J=8.0 Hz, H-1"), 4.75 (d, 1H, J=2.0 Hz, H-1'), 4.9 (d, 1H, J=3.0 Hz, H-1), 7.20-7.38 (m, 30H, 6Ph); Elemental analysis; calc. for $C_{68}H_{76}O_{20}$: C: 67.31; H: 6.31; found C: 67.10; H: 6.41.

Preparation of **12**.- Compound **11** (98 mg, 0.08 mmol) was hydrogenolysed as described before. The product was stirred with 0.05 M NaOMe (1 mL) for 3 h, decationised with

Dowex 50W-X8 (H^+) resin and concentrated. Column chromatography using 10:5:1 $CHCl_3$-MeOH-H_2O gave pure **12** (36.2 mg, 82%); $[\alpha]_D$ +31.63° (*c* 0.3, H_2O). ^1H NMR: δ 3.41 (s, 3H, OMe), 3.74 (s, 3H, COOMe), 4.68 (d, 1H, J=7.7 Hz, H-1"), 4.75 (d, 1H, J=1.44 Hz, H-1), 5.26 (d, 1H, J=3.88 Hz, H-1'). ^{13}C NMR (D_2O, internal standard 1,4-dioxane): δ 55.62 (OCH_3), 57.42 ($COOCH_3$), 61.72 (C-6), 62.05 (C-6'), 66.91, 68.56, 69.73, 70.50, 72.03, 72.66, 73.47, 74.03, 76.20, 79.42, 80.17, 101.45 (C-1'), 101.60 (C-1), 104.50 (C-1"), 172.29 ($COOCH_3$).

Preparation of **13**.- Methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside (3 g, 13.4 mmol) was benzylated using benzyl bromide and NaH in *N,N*-dimethylformamide according to the method of Brimacombe[11]. Chromatographic yield of **13** was 4.3 g (81%); crystallised from CH_2Cl_2-Et_2O-petroleum ether (40-60°); m.p. 167-168°C; $[\alpha]_D^{24}$ + 36.65° (*c* 2.0, $CHCl_3$). ^1H NMR: δ 1.4 and 1.48 (2s, 6H, CMe_2), 3.35 (s, 3H, OMe), 4.67 (d, 1H, J=3.75 Hz, H-1), 7.28-7.42 (m, 10H, 2Ph); Elemental analysis; calc. for $C_{24}H_{30}O_6$: C: 69.55; H: 7.29; found C: 69.29; H: 7.30.

Preparation of **14**.- Compound **13** (4 g, 9.65 mmol) was stirred with 85% acetic acid (30 mL) at 70°C for 3 h. Removal of solvents gave **14** (3.18 g, 88%); $[\alpha]_D^{27}$ +80.7° (*c* 1.5, $CHCl_3$)(lit.[13] $[\alpha]_D^{22}$ +74.9°, *c* 1.68, $CHCl_3$). ^1H NMR: δ 3.32 (s, 3H, OMe), 4.69 (d, 1H, J=3.5 Hz, H-1), 7.25-7.35 (m, 10H, 2Ph); Elemental analysis; calc. for $C_{21}H_{26}O_6$: C: 67.36; H: 7.00; found C: 67.30; H: 7.05.

Preparation of **16**.- Compound **14** (436 mg, 1.16 mmol) was dissolved in CH_2Cl_2 (10 mL) and Ag_2CO_3 (504 mg), I_2 (a pinch) and 4A molecular sieves were added and stirred under N_2 for 1h. Compound **15** (603 mg, 1.52 mmol) in CH_2Cl_2 (5 mL) was then added dropwise during 3h. Stirring was

continued for 48 h at room temperature. The mixture was filtered through a celite bed and evaporated to dryness. Column chromatography using 4:1 toluene-ether gave **16** which was crystallised from EtOH; (503 mg, 62.6%); m.p. 97-98°C; $[\alpha]_D^{27}$ +0.99° (*c* 0.6, $CHCl_3$). ^1H NMR: δ 2.00 (s, 9H, 3Ac), 3.36 (s, 3H, OMe), 3.7 (s, 3H, COOMe), 4.65 (d, 1H, J=6.5 Hz, H-1'), 4.97 (d, 1H, J=3.0 Hz, H-1), 7.28-7.40 (m, 10H, 2Ph); Elemental analysis; calc. for $C_{34}H_{42}O_{15}$: C: 59.12; H: 6.13; found C: 58.81; H: 6.33.

Preparation of **17**.- Compound **16** (300 mg, 0.43 mmol) was hydrogenolysed in EtOH (10 mL) in presence of 10% Pd-C (135 mg) at room temperature for 6 days. Worked up as usual and the dry material was treated with 0.05 M NaOMe (4 mL) and stirred for 4 h at room temperature. A few drops of water were then added and the stirring was continued for another 2 h. The solution was decationised with Dowex 50W-X8 (H^+) resin, filtered and the filtrate was evaporated to dryness. Column chromatography using 100:50:10:1 $CHCl_3$-MeOH-H_2O-AcOH gave pure **17** (143 mg, 89.2%); $[\alpha]_D^{27}$ +39.6° (*c* 1.7, H_2O). ^1H NMR (D_2O): δ 3.42 (s, 3H, OMe), 4.39 (d, 1H, J=7.0 Hz, H-1'), 4.87 (br s, 1H, H-1). ^{13}C NMR (D_2O, internal standard 1,4-dioxane) : δ 55.87 (OCH_3), 62.28 (C-6), 100.10 (C-1), 104.31 (C-1'), 171.90 (COOH).

Preparation of **18**.- Compound **14** (414 mg, 1.1 mmol) and Bu_2SnO (330 mg, 1.7 mmol) were taken in benzene and refluxed for 12 h with azeotropic removal of water. Allyl bromide (0.1 mL) and Bu_4NBr (360 mg) were added and stirred for 6 h at 63°C. The solvent was removed by evaporation, unwanted solids precipitated on addition of cold methanol were filtered off and the filtrate was evaporated to dryness. The residue was chromatographed using 5:3 toluene-ether as eluent to give pure **18** (377.5 mg, 82.4%); $[\alpha]_D^{28}$ +46.8° (*c* 1.0, $CHCl_3$). ^1H NMR : δ 3.38 (s, 3H, OMe),

4.68 (d, 1H, J=2.0 Hz, H-1), 5.79-6.11 (m, 1H, CH_2-C*H*=CH_2), 7.30-7.42 (m, 10H, 2Ph); Elemental analysis; calc. for $C_{24}H_{30}O_6$: C: 69.54; H: 7.29; found C: 69.47; H: 7.33.

Preparation of **19**.- Compound **18** (350 mg, 0.84 mmol) was benzylated using benzyl bromide in the usual way to give **19** as syrup (396 mg, 93%); $[\alpha]_D^{27}$ +18.85° (*c* 1.2, $CHCl_3$)(lit.[17] $[\alpha]_D^{20}$ +18.7°, *c* 1.6, $CHCl_3$). ^1H NMR: δ 3.36 (s, 3H, OMe), 4.7 (d, 1H, J=2.0 Hz, H-1), 5.80-6.18 (m, 1H, CH_2-C*H*=CH_2), 7.29-7.42 (m, 15H, 3Ph).

Preparation of **20**.- Compound **19** (500 mg, 1.07 mmol) was deallylated as described for the preparation of **8** to obtain **20** (450.2 mg, 80.3%); $[\alpha]_D^{27}$ +45.44° (*c* 2.0, $CHCl_3$)(lit.[17] $[\alpha]_D^{20}$ +45.8°, *c* 1.6, $CHCl_3$; lit.[18] $[\alpha]_D^{25}$ +46.6°, *c* 1.4, $CHCl_3$). ^1H NMR: δ 3.35 (s, 3H, OMe), 4.7 (d, 1H, J=2.0 Hz, H-1), 7.32-7.42 (m, 15H, 3Ph).

Preparation of **21**.- Compound **20** (241 mg, 0.52 mmol) was allowed to react with methyl (ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranoside)uronate (270 mg) in CH_2Cl_2 in presence of MeOTf (0.4 mL) and 4A molecular sieves as described for the preparation of **3**. The product was crystallised from ethanol giving pure **21** (268 mg, 67%); m.p. 131-132°C; $[\alpha]_D^{25}$ -3.97° (*c* 0.85, $CHCl_3$). ^1H NMR: δ 1.97 and 2.01 (2s, 9H, 3Ac), 3.33 (s, 3H, OMe), 3.71 (s, 3H, COOMe), 4.59 (d, 1H, J=6.5 Hz, H-1'), 4.92 (br s, 1H, H-1), 7.28-7.44 (m, 15H, 3Ph); Elemental analysis; calc. for $C_{41}H_{48}O_{15}$: C: 63.07; H: 6.2; found C: 63.10; H: 6.20.

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