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Synthesis of tri- and tetra-saccharide derivatives related to *Klebsiella* type 57

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2,4,6-Tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-1,6-anhydro-2-*O*-benzyl-β-D-galactopyranose **12** has been prepared from D-galactose. The disaccharide **12** reacts with ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside in the presence of NIS/TfOH as promoter to give the tri- and tetra-saccharide derivatives 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→3)-1,6-anhydro-2-*O*-benzyl-β-D-galactopyranose **15** and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-galactopyranosyl-(1→3)- [2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→4)] -1,6-anhydro- 2-*O*-benzyl-β-D-galactopyranose **16** respectively in moderate yield.

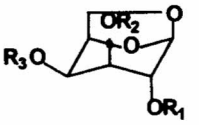
Klebsiella type 57 is a heteropolysaccharide composed[1] of the tetrasaccharide repeating unit **A**. It is a gram-negative, opportunistic pathogen[2] causing a variety of specific infections that can give rise to bacterioma, acute broncho-pneumonia and also more chronic destructive lesions with multiple abscess formation in lungs. As a part of our programme to synthesize the repeating unit of the antigen from *Klebsiella* type 57, the stable 1,6-anhydro derivatives in the form of tri- and tetra-saccharides were synthesized starting from D-galactose and D-mannose using a convenient synthetic strategy.

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

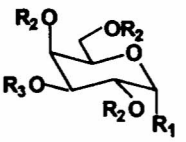
(A)

1,6-Anhydro-β-D-galactopyranose[3] **1** prepared from D-galactose, was treated with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in DMF to give the 3,4-*O*-isopropylidene derivative **2**[4]. Compound **2** was benzylated at its 2-position to give **3**[5]. Removal of isopropylidene[6] group from **3** followed by selective allylation of the resulting **4** using phase transfer method[7] afforded 4-*O*-allyl-1,6-anhydro-2-*O*-benzyl-β-D-galactopyranose **5**.

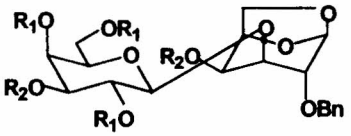
In another series of experiments methyl, 3-*O*-allyl-α-D-galactopyranoside **6**[8] on acetylation[9] followed by acetolysis[10] with 2% sulfuric acid in acetic anhydride gave **8** which was brominated to afford 2,4,6-tri-*O*-acetyl-3-*O*-allyl-galactopyranosyl bromide **9**. Reaction of **5** with the donor **9** in acetonitrile using mercury(II) cyanide[11] as promoter, gave the disaccharide derivative **10** in 54% yield. Compound **10** after deacetylation[12] and benzylation, afforded 2,4,6-tri-*O*-benzyl-3-*O*-allyl-β-D-galactopyranosyl-(1→3)-4-*O*-allyl-1, 6-anhydro-2-*O*-benzyl-β-D-galactopyranose **11**. The two *O*-allyl groups were selectively removed with $PdCl_2$[13] to afford **12** with two free hydroxyl groups. Compound **12** exhibits the characteristic ^{1}H NMR signals at δ 4.6 (d, J = 7Hz, 1H, H-1′), 5.36 (bs, 1H, H-1), 7.34 (m, 20H, 4 Ph) and ^{13}C NMR signals at δ 99.6 (C-1) and 104.1 (C-1′). The acceptor **12** was allowed to react with ethyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside **14** prepared from ethyl 1-thio-α-D-mannopyranoside[14] **13** in the presence of NIS/TfOH[15] to give a trisaccharide derivative 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2, 4, 6-tri-*O*-benzyl-β-D-galactopyra-nosyl-(1→3)-*O*-1,6- anhydro-2-*O*-



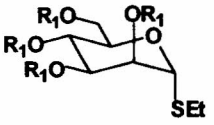
1. $R_1 = R_2 = R_3 = H$
2. $R_1 = H$; $R_2,R_3 = CMe_2$
3. $R_1 = Bn$; $R_2,R_3 = CMe_2$
4. $R_1 = Bn$; $R_2 = R_3 = H$
5. $R_1 = Bn$; $R_2 = H$; $R_3 = All$



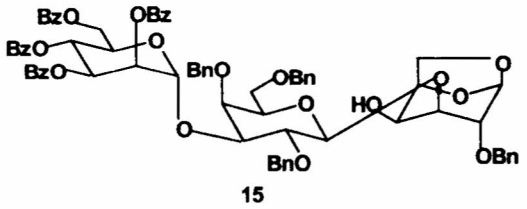
6. $R_1 = OMe$; $R_2 = H$; $R_3 = All$
7. $R_1 = OMe$; $R_2 = Ac$; $R_3 = All$
8. $R_1 = OAc$; $R_2 = Ac$; $R_3 = All$
9. $R_1 = Br$; $R_2 = Ac$; $R_3 = All$



10. $R_1 = Ac$; $R_2 = All$
11. $R_1 = Bn$; $R_2 = All$
12. $R_1 = Bn$; $R_2 = H$



13. $R_1 = H$
14. $R_1 = Bz$



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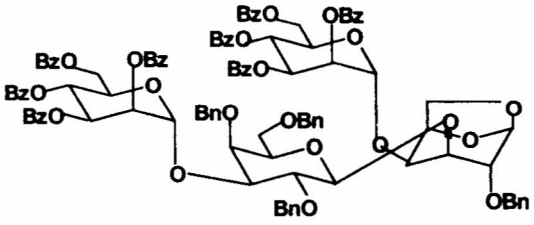

16

benzyl-l-β-D-gal-acetopyranose **15** in 50% yield, together with the tetrasaccharide derivative **16** in about 20% yield. Compounds **15** and **16** were characterized by ^1H NMR spectra showing characteristic signals for 1,6-anhydro-β-D-galactose and aromatic protons. Compound **15** shows the characteristic ^{13}C NMR signals at δ 99.6 (C-1), 104.7 (C-1') and 94.2 (C-1'') while compound **16** exhibits the characteristic ^{13}C NMR signals at δ 94.1 (C-1''), 97.4 (C-1'''), 99.2 (C-1) and 104.1 (C-1').

The yield of the tetrasaccharide derivative could not be improved by changing the reaction conditions. The blocked tri- and tetrasaccharides could be utilised for the preparation of glyco-conjugates.

Experimental Section

General. All reactions were monitored by TLC on silica gel G (E. Merck). Column chromatography was performed on 100-200 mesh silica gel (SRL, India). All solvents were distilled and/ or dried before use and all evaporations were conducted below 40°C under reduced pressure unless stated otherwise. Optical rotations were measured on a Perkin-Elmer model 241 MC polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Jeol FX-100 or Bruker 300 Spectrometer using $CDCl_3$ as solvent (internal standard TMS) unless otherwise stated. Melting points were determined on a paraffin oil bath and are uncorrected.

Compound 2. 1,6-Anhydro-β-D-galactopyranose **1** (2.50 g, 15.4 mmoles) was allowed to react with 2,2-dimethoxypropane (2.1 mL, 17 mmoles) and *p*-TsOH (catalytic amount) in DMF (25 mL) at room temperature for 1 hr. The reaction was quenched with Et_3N (0.2 mL) and partitioned between water and dichloromethane (3x20 mL). The dichloromethane extract was washed successively with water, aq. $NaHCO_3$ and water, dried and concentrated. The product was crystallized from chloroform-hexane giving **2** (3.4 g, 95%), m.p. 153-54°, $[\alpha]_D^{25}$ – 73.2 (*c* 1, $CHCl_3$) [Lit .[3a, 4] m.p. 151-

52°; $[\alpha]_D^{25}$ - 73° (*c* 1.7. $CHCl_3$)]; ^1H NMR :δ 1.28, 1.46 [2s, 6H, $(CH_3)_3$ C], 3.84 (q, $J_{6,6}$ = 7.00, $J_{6,5}$ = 12 Hz, 1H, H-6_{exo}), 4.08 (d, *J* = 7 Hz, H-6_{endo}), 4.16 (bs, 1H, H-5), 4.32-4.44 (m, 2H, H-3, H-4), 5.32 (bs, 1H, H-1). Anal. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.40; H, 7.02%.

Compound 3. A mixture of **2** (3 g, 12.8 mmoles), benzyl bromide (2.0 mL, 15.3 mmoles), sodium hydride (60% oil coated, 1.2 g, 25.2 mmoles) and *N,N*-dimethylformamide (15 mL) was stirred at room temperature for 2 hr. Methanol (1 mL) was added to decompose the excess reagents and the mixture was then diluted with CH_2Cl_2 (50 mL). The organic layer was washed with water, dried and concentrated. The crude product was column chromatographed, using first light petroleum ether (40-60°C) and then light petroleum ether - toluene (1:3) as eluents, giving **3** which was crystallized from absolute ethanol (3.5 g, 85.5%), m.p. 81-82°; $[\alpha]_D^{25}$ – 81.9° (*c* 1, $CHCl_3$) [Lit [5]: m.p. 84°C; $[\alpha]_D^{25}$ - 81.9 (*c* 0.87, $CHCl_3$)]; ^1H NMR: δ 1.28,1.46 [2s, 6H, $(CH_3)_3$], 4.04 (q, 1H, H-6_{exo}), 4.32-4.48 (m, 2H, H-3, H-4), 4.62 (dd, *J* = 3 Hz, 2H, PhCH_2), 5.4 (bs, 1H, H-1), 7.28-7.36 (m, 5H, Ph). Anal. Calcd for $C_{16}H_{20}O_5$: C,66.02; H, 6.95. Found: C, 65.74; H, 6.89%.

Compound 4. A solution of **3** (3 g, 9.25 mmoles) in 80% acetic acid (30 mL) was heated at 80°C for 3 hr. The mixture was concentrated to dryness and the product crystallized from ether - dichloromethane giving **4** (2.2 g, 94.7%), m.p. 102-03°; $[\alpha]_D^{25}$ – 75° (*c* 1, $CHCl_3$) [Lit[16] m.p. 104-05°; $[\alpha]_D^{25}$ – 76.2° (*c* 0.5, $CHCl_3$)]; ^1H NMR: δ 3.63 (q, $J_{6,6}$ = 7 Hz, $J_{6,5}$ = 12 Hz, 1H, H-6_{exo}), 4.18 (dd, 2H, PhCH_2), 5.42 (bs, 1H, H-1), 7.32-7.46 (m, 5H, Ph). Anal. Calcd for $C_{13}H_{16}O_5$: C, 61.89; H, 6.39. Found: C, 62.02; H, 6.52%.

Compound 5. A mixture of **4** (3.0 g, 12 mmoles) and dibutyltin oxide (3.3 g, 13.4 mmoles) in benzene (40 mL) was refluxed for 16 hr. Allyl bromide (1.2 mL, 13.4 mmoles) and tetrabutylammonium bromide (3.5 g, 13.4 mmoles) were then added and the temperature was kept at 63-65°C with stirring. After 5 hr, solvents were evaporated and the residue was taken up in methanol. After cooling, the crystalline precipitate formed was filtered off. The filtrate was concentrated and the crude product chromatographed with toluene-ethyl acetate (3:1) as eluent to give **5** (2.5 g, 75%), $[\alpha]_D^{25}$ –37.4°(*c* 1.3, $CHCl_3$); ^1H NMR : δ 5.40 (s, 1H, H-1), 6.0 (m, 1H, CH_2=C*H*–CH_2), 4.64 (dd, 2H, PhCH_2). Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.68; H, 7.01%.

Compound 7. Compound **6** (2.9 g, 12.4 mmoles) was acetylated with acetic anhydride (16 mL) in pyridine (32 mL) for 4 hr at room temperature. Solvents were coevaporated with toluene. Column chromatography of the residue with toluene-ether (3:1) as eluent gave **7** as a syrup (4.5 g, 99%), $[\alpha]_D^{25}$ +142.4 (*c* 1, $CHCl_3$); ^1H NMR: δ 2.07, 2.08, 2.14 (3s, each 3H, 3 OAc), 3.4 (s, 3H, OCH_3), 3.88 and 3.76 (2 m, each 1H, CH_2=CH-CH_2), 5.08-5.24 (2 m, each 1H, CH_2=CH-CH_2), 5.44 (d, *J* = 3Hz, 1H, H-1), 5.78 (m, 1H, CH_2=C*H*-CH_2). Anal. Calcd for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 53.22; H, 6.79%.

Compound 8. To a solution of **7** (4 g, 11.1 mmoles) in acetic anhydride (40 mL) at 0°C was added a solution of concentrated H_2SO_4 (1.6 mL) in acetic anhydride (36 mL). After 1.5 hr at 0°C the mixture was poured into aq. $NaHCO_3$ and the product extracted with CH_2Cl_2 The organic layer was washed with water, dried and concentrated to afford **8** (3.1 g, 70%), $[\alpha]_D^{25}$ +111.82 (*c* 0.8, $CHCl_3$); ^1H NMR : δ 2.04, 2.06, 2.16 (3s, 9H, 3 OAc), 5.12-5.4 (2 m, 2H, CH_2=CH-CH_2), 5.8 (m, 1H, CH_2=C*H*-CH_2). Anal. Calcd for $C_{17}H_{22}O_{10}$: C, 52.57; H, 6.23. Found : C, 52.38; H, 6.65%.

Compound 9. Compound **8** (2.4 g, 6.2 mmoles) was brominated with brominating reagent (6 mL) for 2 hr at room temperature following the conventional method to give **9** (2.2 g) as a syrup. The product was dried under vacuum and used directly in the next step.

Compound 10. A mixture of **5** (300 mg, 1.03 mmoles), **9** (637 mg, 1.55 mmoles), MS-3Å (1 g) and mercury(II) cyanide (300 mg, 1.2 mmoles) in acetonitrile (4 mL) was stirred at room temperature for 24 hr, then filtered through Celite, concentrated and diluted with chloroform (12 mL). The solution was washed with aq. 10% KI, aq. $NaHCO_3$ and water, dried and concentrated. Column chromatography (3:1 toluene-ether) of the residue gave **10** (345 mg, 55%), $[\alpha]_D^{24}$ +55.3° (*c* 0.9, $CHCl_3$); ^1H

NMR: δ 2.0, 2.04, 2.12 (3s, 9H, 3 OAc). 3.55 (q, $J_{6,6}$= 7 Hz, $J_{6,5}$ = 12 Hz, 1H, H-6_{exo}), 4.62 (d, *J*= 7Hz, 1H, H-1'), 4.58 (dd, *J* = 3Hz, 2H, PhCH_2), 5.14-5.28 (2 m, 4H, 2CH_2CH=CH_2), 5.32 (bs, 1H, H-1), 5.80-6.12 (2 m, 2H, 2CH_2C*H*=CH_2), 7.28-7.48 (m, 5H, Ph). Anal. Calcd for $C_{31}H_{40}O_{13}$: C, 59.99; H, 6.49. Found: C, 59.91; H, 6.60%.

Compound 11. Compound **10** (487 mg, 0.8 mmole) was deacetylated with methanolic 0.1 *M* sodium methoxide (10 mL) for 4 hr. The solution was neutralized with Dowex 50W X8(H^+) resin, filtered and concentrated to dryness (380 mg, 98%). To a solution of the product (380 mg, 0.77 mmole) in DMF were added benzyl bromide (0.1 mL, 0.8 mmole) and sodium hydride (60% oil coated, 250 mg) and the mixture was stirred for 3 hr at room temperature. Methanol (0.1 mL) was added to decompose the excess reagent and the mixture was then diluted with CH_2Cl_2 (10 mL). The organic layer was washed with water, dried and concentrated to give **11** (520 mg, 92%), $[\alpha]_D^{25}$ – 12.3° (*c* 1, $CHCl_3$); ^{1}H NMR: δ 3.80 (q, $J_{6,6}$ = 7Hz, $J_{6,5}$ = 12Hz, 1H, H-6_{exo}), 4.4-4.6 (m, 8H, 4 PhCH_2), 4.64 (d, *J* = 7Hz, 1H, H-1'), 5.36 (s, 1H, 1H, H-1), 4.96-5.24 (2 m, 4H, 2CH_2CH=CH_2), 5.72-6.08 (2 m, 2H, 2 CH_2C*H*=CH_2), 7.12-7.84 (m, 20H, 4Ph). Anal. Calcd for $C_{46}H_{51}O_{10}$: C, 72.41; H, 6.74. Found : C, 72.29; H, 6.83%.

Compound 12. A mixture of **11** (400 mg, 0.5 mmole), $PdCl_2$ (124 mg, 0.7 mmole) and NaOAc (168 mg, 2.05 mmoles) in 20:1 AcOH-H_2O (4 mL) was stirred for 12 hr. at 20°, and then diluted with EtOAc and filtered through Celite. The filtrate was washed successively with aq. $NaHCO_3$ and aq. NaCl, dried and evaporated. Column chromatography of the product with 4:1 toluene-Et_2O gave **12** (178.5 mg, 50%), $[\alpha]^{25}_D$ + 7.8° (*c* 0.8, $CHCl_3$); ^{1}H NMR: δ 4.6 (d, *J* = 7Hz, 1H, H-1'), 5.36 (bs, 1H, H-1), 7.34 (m, 20H, 4 Ph); ^{13}C NMR: δ 99.6 (H-1), 104.1 (H-1') 63.2, 62.7, 71.44, 71.85, 72.84, 73.20, 73.7, 74.13, 74.48, 74.78. Anal. Calcd for $C_{40}H_{44}O_{10}$: C, 70.16; H, 6.47. Found: C, 70.02; H, 6.77%.

Compound 14. Benzoylation of compound **13** (5 g, 22.7 mmoles) was carried out with benzoyl chloride (12.8 mL, 108.9 mmoles) in pyridine (60 mL) following the conventional method to give a syrup which was crystalized from ethanol to give **14** (13 g, 95%), m.p.124-25°; $[\alpha]_D^{25}$ –15.8° (*c* 0.8, $CHCl_3$); ^{1}H NMR: δ1.35 (t, 3H, SCH_2CH_3), 2.75(q, 2H, SCH_2CH_3), 5.57 (bs, 1H, H-1), 7.25-7.60 and 7.83-8.1(m, 20H, 4 Ph). Anal. Calcd for $C_{36}H_{32}O_9S$: C, 71.05; H, 5.26. Found: C, 71.01; H, 5.30%.

Compound 15. A mixture of **12** (150 mg, 0.2 mmole), **14** (380 mg, 0.6 mmole) and MS- 4Å (1 g) in CH_2Cl_2 (10 mL) was stirred under Ar for 18 hr. The mixture was then cooled to 0°C and NIS (190 mg. 0.86 mmole) and TfOH (1μL) were added to it. Temperature was then raised to 25° and stirring continued for 2 hr. The solids were filtered off and the filtrate was diluted with CH_2Cl_2, washed with 10% $Na_2S_2O_3$ and 1*M* $NaHCO_3$, dried and concentrated. Column chromatography of the syrupy product with toluene-ether (3:1) as eluent gave **15** (135 mg, 50%); $[\alpha]_D^{25}$ + 15.29 (*c* 1.4, $CHCl_3$); ^{1}H NMR : δ 4.03 (q, 1H, H-6), 4.70 (d, 1H, *J* = 7Hz, H-1'), 5.27 (bs, 1H, H-1), 5.57 (bs, 1H, H-1''); ^{13}C NMR: δ61.8, 62.0, 63.4, 64.4, 66.3, 66.8, 67.9, 68.9, 69.0, 69.9, 70.3, 70.6, 72.0, 72.7, 73.5, 74.0, 74.5, 75.5, 76.3, 76.7, 76.9, 99.6 (C-1), 104.7 (C-1'), 94.2 (C-1''), 127.2-137.9 (aromatic protons), 165.3-166.0 (*C*OPh). Anal. Calcd. for $C_{74}H_{70}O_{19}$: C, 70.35; H, 5.58. Found: C, 70.29; H, 5.63%.

Compound 16. A mixture of **12** (150 mg, 0.2 mmole), **14** (380 mg, 0.6 mmole) and MS-4Å (1 g) in CH_2Cl_2 (10 mL) was treated in the same way as described for **15** to give **16** (78 mg, 20%); $[\alpha]_D^{25}$ –43.06 (*c* 0.6, $CHCl_3$); ^{1}H NMR: δ 5.29 (bs, 1H, H-1), 4.04 (q, 1H, H-6), 4.72 (d, 1H, *J*=7Hz, H-1'), 5.57 (bs, 1H, H-1''), 5.7 (bs, 1H, H-1'''), ^{13}C NMR: δ 61.9, 62.0, 62.7, 63.3, 64.4, 66.3, 68.4, 68.7, 69.0, 69.7, 69.9,70.2, 70.3,70.4, 70.5,71.8, 72.0, 72.7, 73.2, 74.8, 75.0, 76.5, 76.9, 94.2, 94.1 (C-1''), 97.4 (C-1'''), 99.2 (C-1), 104.1 (C-1'),127.2-137.7 (aromatic protons), 164.8 166.1 (*C*OPh). Anal. Calcd for $C_{108}H_{96}O_{28}$: C, 70.4; H, 5.25. Found: C, 69.7; H, 5.35%.

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