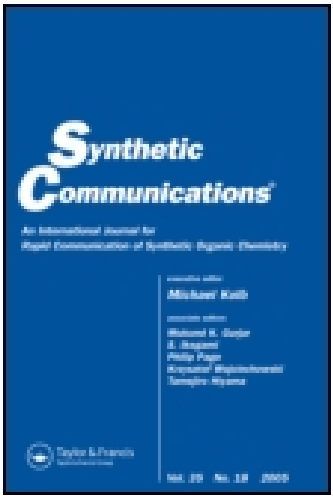

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Synthesis of a DI- and A Trisaccharide Related to the Antigen From Klebsiella Type 16

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SYNTHESIS OF A DI- AND A TRISACCHARIDE RELATED TO THE ANTIGEN FROM *KLEBSIELLA* TYPE 16

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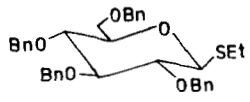
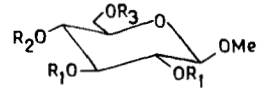
ABSTRACT : Using methyl triflate as promoter, methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate) and methyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galacto-pyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate) have been synthesised. Removal of protecting groups gave the di- and trisaccharide in the form of their methyl ester methyl glycoside related to the antigen of *Klebsiella* type 16.

The structure of the repeating unit of capsular polysaccharide from *Klebsiella* type 16 has been established[1] . We have recently reported[2] the synthesis of tetrasaccharide repeating unit of the antigen from *Klebsiella* type 16. In continuation of our efforts to determine the relationship between the structure and immunological specificity of various saccharides, we now report the synthesis of a di- and a trisaccharide fragment related to the repeating unit of this antigen.

Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[3] (**2**) obtained from methyl β-D-glucopyranoside was benzoylated by conventional method[7] to afford methyl 2,3-di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (**3**). Compound **3** had ^{1}H NMR signal at δ 5.43 for $CH_3OC_6H_4CH$. Regioselective opening of the 4-methoxybenzylidene ring[4] with $NaCNBH_3$ and trifluoroacetic acid gave the 4-hydroxy compound **4** . Compound **4** gave ^{1}H NMR signals for $CH_3OC_6H_4CH_2$, OCH_3 and anomeric protons but there was no signal at δ 5.43 indicating the absence of benzylidene ring . The acceptor **4** was then allowed to react with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[8] (**1**) in presence of methyl triflate using Et_2O as solvent to afford the disaccharide derivative **8** in 66.6% yield. Compound **8** had ^{1}H NMR peaks for $CH_3OC_6H_4CH_2$, OCH_3 together with signals at δ 4.68 and 4.96 corresponding to β-glucosidic and α-glucosidic moities .



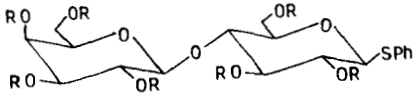
1



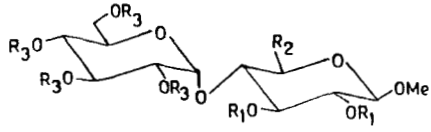
2. R_1=H, R_2R_3=CH-(C$_6$H$_4$)-OCH_3

3. R_1=Bz, R_2R_3=CH-(C$_6$H$_4$)-OCH_3

4. R_1=Bz, R_2=H, R_3=CH_2-(C$_6$H$_4$)-OCH_3=MBn

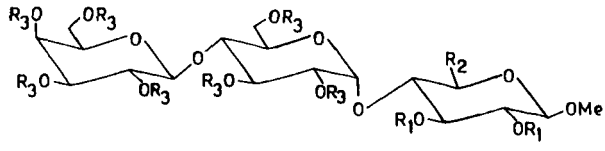


5. R= AC
6. R= H
7. R= Bn



8. R_1= Bz, R_2= CH_2OMBn , R_3= Bn
9. R_1= Bz , R_2= CH_2OH , R_3= Bn
10. R_1= Bz , R_2= COOH , R_3= Bn
11. R_1= Bz , R_2= COOMe , R_3= Bn
12. R_1= R_3= H , R_2= COOMe

In a separate experiment the thioglycoside **5** was prepared from lactose octaacetate and thiophenol in presence of boron trifluoride etherate. Deacetylation of **5** according to Zemplen and benzylation of the resulting product **6** afforded the thioglycoside donor **7** . The acceptor **4** was allowed to react with **7** in presence of methyl triflate using 14:1 ethylether-dichloromethane as solvent to afford the trisaccharide derivative **13** in 70.4% yield. The ^1H NMR spectrum of **13** showed the presence of three anomeric protons in addition to other characteristic peaks.



13. R_1 = Bz , R_2 = CH_2OMBn , R_3 = Bn
14. R_1 = Bz , R_2 = CH_2OH , R_3 = Bn
15. R_1 = Bz , R_2 = COOH , R_3 = Bn
16. R_1 = Bz , R_2 = COOMe , R_3 = Bn
17. R_1 = R_3 = H , R_2 = COOMe

Oxidative removal of 4-methoxybenzyl groups from **8** and **13** with ceric ammonium nitrate[4] gave the 6-hydroxy compounds **9** and **14** respectively. The primary hydroxyl group of **9** was oxidised with CrO_3[5] and treatment of the resulting acid **10** with CH_2N_2 afforded the disaccharide pseudomaltouronic acid derivative as its methyl ester **11** in 71.5% overall yield . The structure of **11** was confirmed by ^1H and ^{13}C NMR spectra . Removal of protecting groups from **11** gave pseudomaltouronic acid as its methyl ester methyl glycoside **12** in 76.5% yield . In the similar manner, the trisaccharide **14** was oxidised and the resulting acid **15** was esterified to give the trisaccharide derivative as its methyl ester methyl glycoside **16** . Removal of protecting groups from **16** gave the desired trisaccharide methyl

ester methyl glycoside **17**. The structure of **17** was confirmed by ^1H and ^{13}C NMR spectra.

EXPERIMENTAL

Optical rotations measured at 25 ^0C with a Perkin-Elmer 241 MC polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded (internal standrad tetramethylsilane) with a Jeol FX-100 or a Bruker 200 MHz spectrometer, using $CDCl_3$ as the solvent unless stated otherwise.

Methyl 2,3-di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside(3). Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (**2**) (1.8 g, 5.74 mmol) was benzoylated[7] with benzoyl chloride and pyridine. The product was crystallised from EtOH giving **3** (2.6 g, 86.9%); mp 88-90 ^0C ; $[\alpha]_D^{25}$ -6.98^0 (*c* 0.81, $CHCl_3$). ^1H NMR : δ 7.97-6.93 (m, 14H, aromatic protons), 5.43 (s, 1H, $CHC_6H_4OCH_3$), 4.66 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 3.73 (s, 3H, $CHC_6H_4OCH_3$), 3.50 (s, 3H, OCH_3). Anal. Calcd for $C_{29}H_{28}O_9$: C, 66.91; H, 5.42. Found : C, 67.10; H, 5.63.

Methyl 2,3-di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (4) . A solution of **3** (2.1 g, 4.03 mmol) in dry DMF (15 mL) was stirred with $NaCNBH_3$ (1.27 g) and MS 3A for 1h at 0^0 C. A solution of trifluoroacetic acid (3.1 mL) in dry DMF (5 mL) was then added and the mixture was stirred for 10h. The reaction mixture was worked up in the usual way and concentrated. Column chromatography with 6:1 toluene-EtOAc gave **4** as a syrup (1.70 g, 80.6%) ; $[\alpha]_D^{25}$ +51.8 ^{0} (*c* 0.42, $CHCl_3$). ^1H NMR : δ 8.0-6.9 (m, 14H, aromatic protons), 4.60 (d, 1H, $J_{1,2}$=9.0 Hz, H-1), 3.75 (s, 3H, $CH_2C_6H_4OCH_3$), 3.48 (s, 3H, OCH_3). Anal. Calcd for $C_{29}H_{30}O_9$: C, 66.65; H, 5.78. Found : C, 66.82; H, 6.00.

Phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (5). β-Lactose octaacetate (2.4

g, 3.62 mmol) was allowed to react with thiophenol (0.45 mL, 4.34 mmol) by using the same reaction condition used for the preparation of ρ-tolyl thioglycoside of lactose[2]. Column chromatography of the crude product with 6:1 toluene-EtOAc afforded a syrup which was crystallised from EtOH-EtOAc giving **5** (2.3g, 87%) ; mp 160-162 ^{0}C ; $[\alpha]_D^{25}$ -15.38^0 (*c* 2.0, $CHCl_3$). ^{1}H NMR: δ 7.33 (m, 5H, aromatic protons), 4.86 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.50 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1), 2.17-1.97 (7s, 21H, 7OAc) . Anal. Calcd for $C_{32}H_{40}O_{17}S$: C, 52.74; H, 5.53. Found : C, 52.58; H, 5.93.

Phenyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (7). Compound **5** (2.1 g, 2.88 mmol) was de-*O*-acetylated with 0.5 M NaOMe[6] and the product **6** (1.2 g, 2.76 mmol) was benzylated in the usual way to afford **7** which crystallised from EtOH-EtOAc to give **7** (2.1 g, 71.4%) ; mp 100-102 ^{0}C ; $[\alpha]_D^{25}$ -3.87 ^{0} (*c* 1.96, $CHCl_3$) . ^{1}H NMR : δ 7.28 (m, 40H, aromatic protons), 4.78 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.35 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1). Anal. Calcd for $C_{67}H_{68}O_{10}S$: C, 75.53; H, 6.43. Found : C, 75.74; H, 6.60.

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (8) . Methyl triflate was added to a stirred mixture of **1**[8] (400 mg, 0.68 mmol), **4** (300 mg, 0.57 mmol), Et_2O (6 mL), and MS 4A (0.5 g) at 22 ^{0}C . After stirring for 16h, the reaction was quenched with Et_3N, filtered and the filtrate concentrated to a syrup. Column chromatography with 6:1 toluene-EtOAc gave **8** as white foam (400 mg, 66.6%) ; $[\alpha]_D^{25}$ + 12.3^0 (*c* 0.16, $CHCl_3$) . ^{1}H NMR : δ 7.98-6.86 (m, 34H, aromatic protons), 4.96 (d, 1H, $J_{1',2'}$ = 3.75 Hz, H-1'), 4.70 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 3.80 (s, 3H, $CH_2C_6H_4OC\mathit{H}_3$), 3.52 (s, 3H, OCH_3). Anal. Calcd for $C_{63}H_{64}O_{14}$: C, 72.39; H, 6.17. Found : C, 72.13; H, 6.40.

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranoside (9). Compound **8** (0.4 g, 0.38 mmol) was allowed to react with ceric ammonium nitrate and the reaction mixture was worked up in the usual way[4]. Column chromatography of the product with 5:1 toluene-EtOAc afforded **9** as white foam (276 mg, 76.5%) ; $[\alpha]_D^{25}$ + 7.5^0 (*c* 0.4, $CHCl_3$) . ^{1}H NMR : δ 7.98-7.32 (m, 30H, aromatic protons), 5.04 (d, 1H, $J_{1',2'}$ = 4.0 Hz, H-1'), 4.68 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.52 (s, 3H, OCH_3). Anal. Calcd for $C_{55}H_{56}O_{13}$: C, 71.41; H, 6.10 . Found : C, 71.59 ; H, 5.96.

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate) (11). Jones reagents [1 mL, from CrO_3 (227 mg), in 3.5 M H_2SO_4 (1 mL)] was added to **9** (210 mg, 0.28 mmol) in 3:2 acetone-CH_2Cl_2 (3 mL) at 0 ^{0}C with stirring. After 15 min the temperature was raised to room temperature and stirring was continued for 5h . The reaction was then quenched with EtOH and worked up in the usual way[2] to give the acid **10**. Treatment of **10** with CH_2N_2 in dichloromethane then gave the ester **11**. Column chromatography with 5:1 toluene-EtOAc gave syrupy **11** (155 mg, 71.5%) ; $[\alpha]_D^{25}$ + 9.47^0 (*c* 0.42, $CHCl_3$). ^{1}H NMR : δ 7.98-7.30 (m, 30H, aromatic protons), 4.88 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 4.58 (d, 1H, $J_{1,2}$ = 12.0 Hz, H-1), 3.68 (s, 3H, $COOCH_3$), 3.52 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) : δ 168.1-165.3 ($COOCH_3$, 2 COC_6H_5), 138.8-125.3 (aromatic carbons), 102.3 (C-1), 99.1 (C-1'), 81.3, 79.0, 77.7, 77.1, 76.5, 75.5, 74.9, 73.5, 73.2, 73.0, 72.5, 71.8, 67.9 (C-6'), 57.3 ($COOCH_3$), 52.8 (OCH_3). Anal. Calcd for $C_{56}H_{56}O_{14}$: C, 70.57; H, 5.92 . Found : C, 70.32; H, 6.10 .

Methyl *O*-(α-D-glucopyranosyl)-(1→4)-(methyl β-D-glucopyranosyluronate) (12) . A solution of **11** (130 mg, 0.14 mmol) in acetic acid (5

mL) was hydrogenolysed over 10% Pd-C (100 mg) for 2 days. The product was isolated and treated with 0.1M NaOMe[6] in MeOH by the conventional way. The product was chromatographed with 3:1 EtOAc-MeOH to give **12** (40 mg, 76.5%); $[\alpha]_D^{25}$ + 66.6^0 (*c* 0.1, MeOH). ^{1}H NMR (D_2O): δ 5.37 (d, 1H, $J_{1',2'}$ = 4.0 Hz, H-1'), 4.43 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.80 (s, 3H, $COOCH_3$), 3.53 (s, 3H, OCH_3).

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (13). Methyl triflate was added to a stirred mixture of **7** (370 mg, 0.35 mmol), **4** (150 mg, 0.29 mmol) and MS 4A (0.5 g) in 14:1 Et_2O-CH_2Cl_2 (5 mL) . Stirring was continued for 18h and the reaction was quenched with Et_3N then filtered and concentrated . Column chromatography with 6:1 toluene-EtOAc gave **13** as syrup (300 mg, 70.8%) ; $[\alpha]_D^{25}$ + 16.7 ^{0} (*c* 0.24, $CHCl_3$). ^{1}H NMR : δ 7.98-6.82 (m, 49H, aromatic protons), 4.92 (d, 1H, $J_{1',2'}$ = 3.0 Hz, H-1'), 4.70 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.64 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.78 (s, 3H, $CH_2C_6H_4OCH_3$), 3.52 (s, 3H, OCH_3). Anal. Calcd for $C_{90}H_{92}O_{19}$: C, 73.15; H, 6.27. Found : C,72.90; H, 6.42 .

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranoside (14) . Treatment of **13** (207 mg, 0.14 mmol) with ceric ammonium nitrate as described for **9**, gave **14** . column chromatography with 5:1 toluene-EtOAc gave syrupy **14** (155 mg, 81.6%) ; $[\alpha]_D^{25}$ + 14.1 ^{0} (*c* 0.21, $CHCl_3$). ^{1}H NMR : δ 7.96-7.28 (m, 45H, aromatic protons), 4.98 (d, 1H, $J_{1',2'}$ = 2.0 Hz, H-1'), 4.82 (d, 1H, $J_{1'',2''}$ = 6.0 Hz, H-1''), 4.64 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 3.52 (s, 3H, OCH_3). Anal. Calcd for $C_{82}H_{84}O_{18}$: C, 72.54; H, 6.23 . Found : C, 72.19; H, 6.47 .

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate) (16) . Oxidation of **14** (295 mg, 0.22 mmol) with Jones reagent[4] and the product **15** was treated with CH_2N_2 as described for the preparation of **11**. Column chromatography with 6:1 toluene-EtOAc afforded syrupy **16** (185 mg, 61.3%) ; $[\alpha]_D^{25}$ + 16.6 ^{0} (*c* 0.2, $CHCl_3$) . ^{1}H NMR : δ 7.98-7.30 (m 45H, aromatic protons), 4.90 (d, 1H, $J_{1',2'}$ = 4.0 Hz, H-1'), 4.78 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.66 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.52 (s, 3H, $COOCH_3$), 3.48 (s, 3H, OCH_3) . Anal. Calcd for $C_{83}H_{84}O_{19}$: C, 71.94; H, 6.11. Found : C, 71.66; H, 6.13 .

Methyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-(α-D-glucopyranosyl)-(1→4)-(methyl β-D-glucopyranosyluronate) (17) . Compound **16** (60 mg, 0.04 mmol) was converted to **17** as described for the preparation of **12**. The product was chromatographed with 2:1 EtOAc-MeOH to afford **17** (15 mg, 63.8%) ; $[\alpha]_D^{25}$ + 24.75^0 (*c* 1.2, MeOH) . ^{1}H NMR (D_2O) : δ 5.24 (d, 1H, $J_{1',2'}$ = 3.0 Hz, H-1'), 4.72 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.49 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.38 (s, 3H, $COOCH_3$), 3.37 (s, 3H, OCH_3). ^{13}C NMR (D_2O, internal standard 1,4-dioxane) : δ 170.0 (*C*$OOCH_3$), 102.9 (C-1''), 102.7 (C-1), 98.2 (C-1'), 66.3 (C-6''), 60.5 (C-6'), 57.0 (COO*C*H_3), 53.0 (OCH_3).

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