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Synthesis of terminal disaccharide unit of *Klebsiella pneumoniae* ssp. R20

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Abstract—Synthesis of the terminal disaccharide unit of a novel α-(1→2) linked heptoglycan of *K. pneumoniae* ssp. strain R20 from methyl α-D-mannopyranoside has been presented. Central to the strategy is the application of Sharpless asymmetric dihydroxylation to introduce a new center at C-6 position of mannopyranoside. The coupling of two heptoglycans (**12** and **13**) was accomplished by a Lewis acid catalyst.

1. Introduction

Klebsiella pneumonia is an important gram-negative pathogenic bacterium associated with nosocomial infections.[1] *K. pneumonia* is most commonly the cause of pneumonia, or hospital-acquired urinary tract or burn wound infections. *Klebsiella* species seems to have resistant plasmids (R-plasmids), which imparts resistance to antibiotics such as ampicillin and carbenicillin.[2] The capsular polysaccharides and lipopolysaccharides (LPS) are thought to participate in many physiological process and play a key role in the pathogenesis and manifestation of infection.[3] The *O*-polysaccharide and core oligosaccharide moieties of LPS is immunogenic, which can give antibodies having specific serological properties and that can also be of diagnostic importance. Antibodies can be directed against the conserved region of LPS, which might provide an useful approach to chemotherapy for infections from *K. pneumoniae*.[4] Such applications require a detailed knowledge of the molecular structure of the targeted LPS molecules the enormous structural variations. Structural investigation of LPS core region began only recently. In a preliminary investigation[5] of LPS from the rough mutant *K. pneumonia* ssp. *pneumonia* R20,[6] a major fraction of the carbohydrate backbone was isolated and its structure was established. The structure (Fig. 1) is unique with regard to the presence of a novel heptoglycan of α-(1→2) linkage and does not contain phosphate substituent in the core region. As a part of our on going studies to synthesize various carbohydrate units present in the LPS of *K. pneumonia*, herein, we report the synthesis of the terminal disaccharide unit of the heptoglycan of α-(1→2) linkage present in *K. pneumonia* ssp. *pneumonia* strain R20.

2. Results and discussion

The literature procedure to introduce a new centre at C-6 involved the reaction of α-D-manno-hexodialdo-1,5-pyranoside and the Grignard complex of benzyloxymethyl-chloride[7] or isopropoxydimethylsilyl-methyl chloride followed by oxidative cleavage of the carbon–silicon bond.[8] We envisaged a new protocol to achieve the objective by employing the Sharpless asymmetric dihydroxylation[9] of the 6-methylene sugar derivative with an appropriate ligand.

The synthesis started from methyl α-D-mannopyranoside (**1**) (Scheme 1), which was converted into the dibenzyl derivative **2** by following the reported procedure.[10] The primary hydroxyl group of **2** was first protected as its TBS ether on treatment with TBS-Cl and imidazole in DMF and subsequently the free OH group at C-2 was blocked with MPM-Br in the presence of NaH in THF to afford **4**. Removal of TBS group with *n*-Bu_4NF in THF gave **5**. Successive oxidation by Swern oxidation reaction[11] and Wittig reaction with PPh_3CH_3I and $NaNH_2$ in anhydrous ether gave olefin **6**.[12]

The Sharpless asymmetric dihydroxylation of **6** (Scheme 2) with $(DHQ)_2PYR$ ligand, $K_3Fe(CN)_6$, K_2CO_3 and catalytic OsO_4 in *tert*-BuOH–H_2O (1:1 v/v) afforded an inseparable (9:1, chiral HPLC) mixture of diastereomers **7**. However the corresponding acetonide derivatives (**8a** and **8b**) prepared by treating **7** with DMP and PPTS, were conveniently separated by silica gel chromatography. The stereochemistry at C-6 of **8b** was confirmed converting it into

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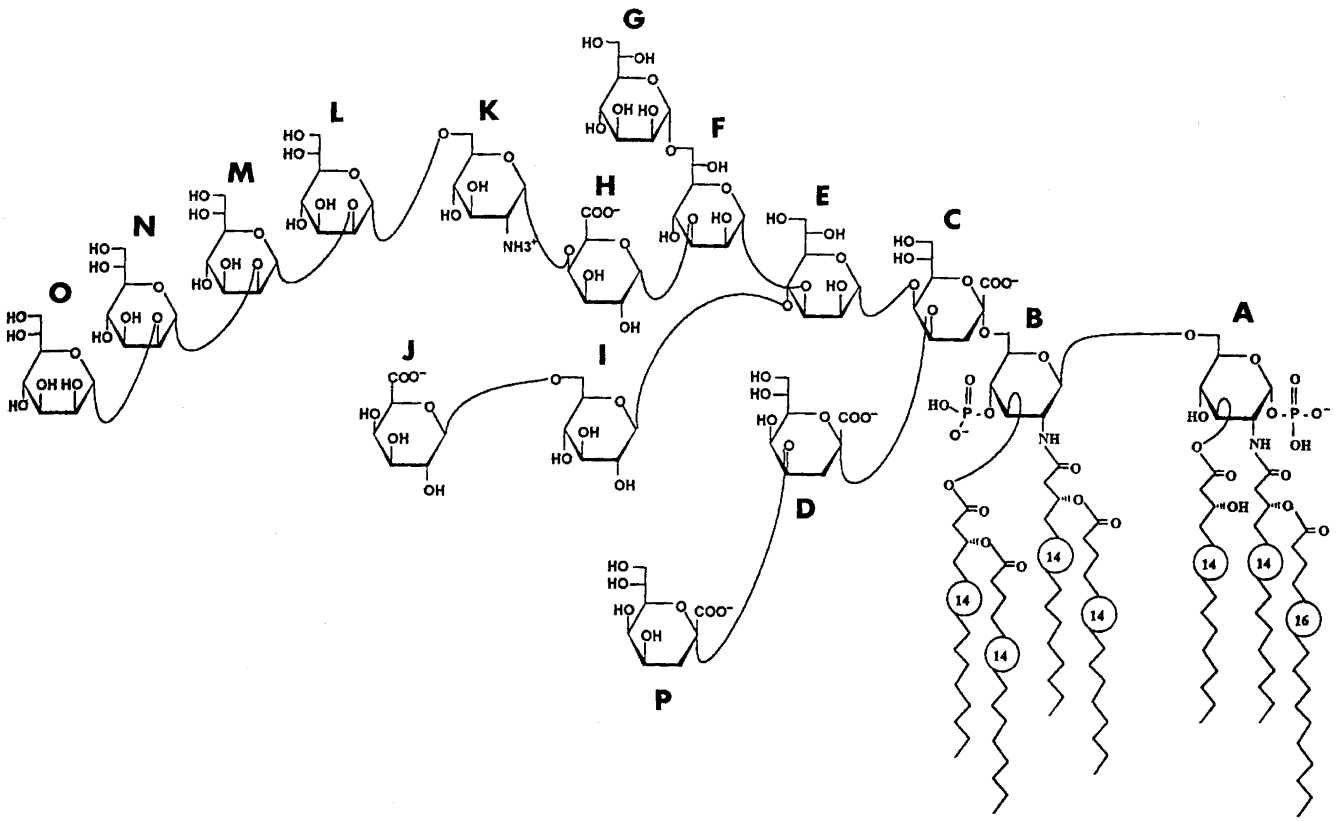


Figure 1. Structure of *K. pneumoniae* lipopolysaccharide.

the known compounds: **9** {$[\alpha]_D$=+22.4 (*c* 0.98, $CHCl_3$); lit.[13] $[\alpha]_D$+23 (*c* 1, $CHCl_3$)} and **10** {$[\alpha]_D$=+27.3 (*c* 0.85, $CHCl_3$), lit.[13] $[\alpha]_D$=+27 (*c* 1, $CHCl_3$)}. These studies indirectly ensured the stereochemical assignment of compound **8a** as indicated.

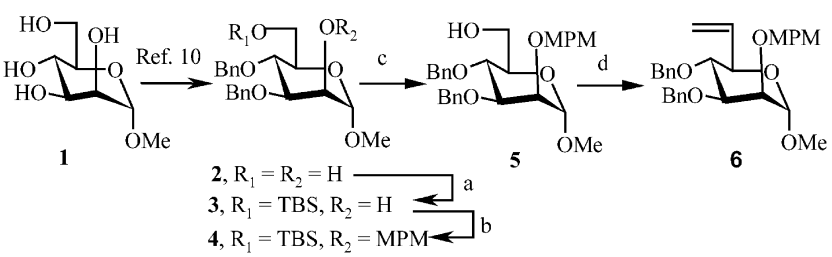
The diol **7a** on treatment with BnBr and NaH in THF provided the benzylated product **11** (Scheme 2). The MPM group at C-2 was then deprotected with DDQ[14] to furnish **12** with a free OH group suitable for glycosylation reaction.

Subsequently, compound **12** was converted into glycosyl donor **13** under acetolysis condition[15] at 0 °C. The ^{1}H NMR spectrum of **13** showed two characteristic singlets at 2.05 and 2.11 ppm.

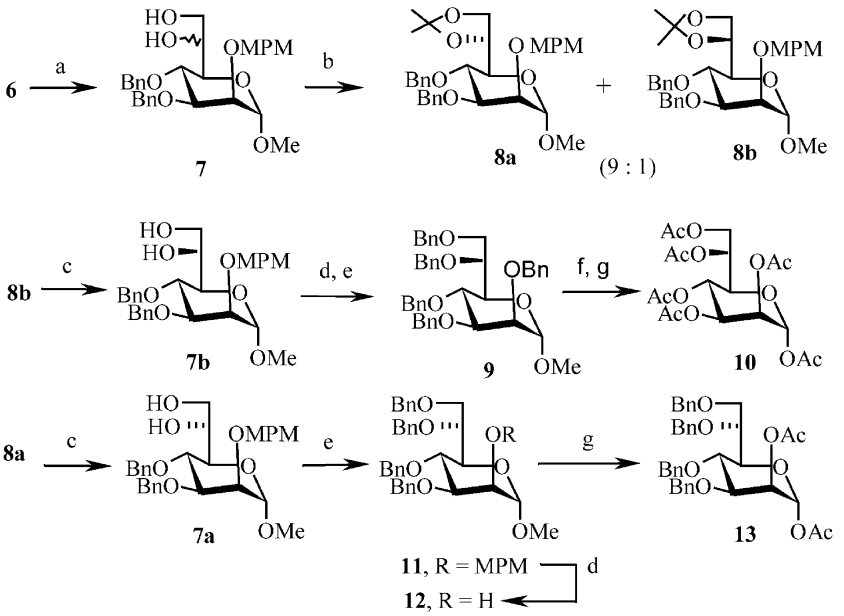
The final coupling between **12** and **13** was conducted[16] (Scheme 3) in presence of catalytic $BF_3{\cdot}OEt_2$ and 4 Å molecular sieves in dry CH_2Cl_2 to provide the disaccharide **14**. The ^{1}H NMR spectrum of **14** showed resonance due to the methyl of C(2)-OAc and anomeric methoxyl as two singlets respectively at 2.09 and 3.29 ppm. A doublet at 5.22 ppm was assigned to H-2 proton. In the ^{13}C NMR spectrum, two anomeric carbon signals were visible at 99.5 and 99.8 ppm, confirming α-configuration at both the anomeric carbons.

Compound **14** on deacetylation (Scheme 3) under Zemplen conditions[17] provided **15**, which in principle can behave as a building block to synthesize higher homologous saccharide. Hydrogenolysis[18] of **15** using 10% $Pd(OH)_2$–C in MeOH gave the required disaccharide **16**. In the ^{1}H NMR spectrum of **16**, signals due to two anomeric protons were observed at 4.77 and 4.90 ppm as doublet (*J*=1.7 Hz). The characteristic coupling constants observed in ^{1}H NMR along with the ^{13}C NMR signals for the anomeric carbons, revealed α-configuration at C-1 as well as C-1′.

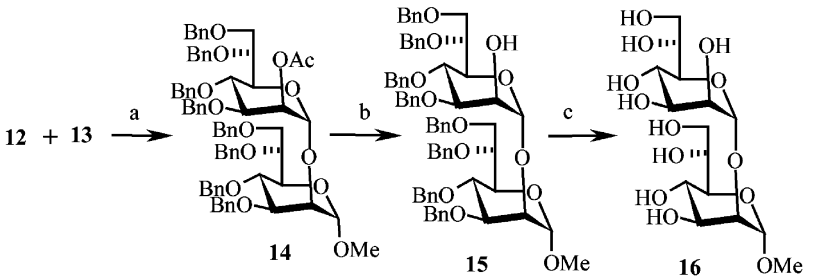
In conclusion, an efficient linear synthesis of terminal



Scheme 1. Reagents and conditions: (a) Imidazole, TBDMS-Cl, DMF, 0 °C to rt, 1 h, 82%; (b) NaH, MPM-Br, THF, 4 h, 73%; (c) *n*-Bu_4NF, THF, 1 h, 74%; (d) (i) $(COCl)_2$, DMSO, Et_3N, −78 °C; (ii) PPh_3CH_3I, $NaNH_2$, Et_2O, −40 °C, 30 min, 32%.



Scheme 2. Reagents and conditions: (a) $(DHQ)_2PYR$, $K_3Fe(CN)_6$, K_2CO_3, cat. OsO_4, *tert*-BuOH–H_2O (1:1 v/v), 0 °C, 6 h; (b) DMP, PPTS, 4 h; (c) PPTS, MeOH, 6 h, 65%; (d) DDQ, CH_2Cl_2–H_2O (9:1), 3 h, 70%; (e) NaH, BnBr, DMF, 30 min, 57%; (f) H_2, Pd/C, MeOH, 30 h (g) AcOH–Ac_2O–H_2SO_4 (5:2:0.3), 60%.



Scheme 3. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, 4 Å mol sieves, CH_2Cl_2, 0 °C to rt, 12 h, 26%; (b) MeONa, MeOH, 10 min, 72%; (c) H_2, $Pd(OH)_2$–C, MeOH, 40 h, 52%.

disaccharide unit of the hepto-glycan of α (1→2) linkage has been accomplished from methyl α-D-mannopyranoside.

3. Experimental

3.1. General

Chemicals used in this study were purchased from Aldrich, Fluka or Lancaster and used as received. Moisture-sensitive reactions were performed in an inert atmosphere of either N_2 or Ar using dry solvents. The elemental analysis was recorded on Elmentar-Vario-EL (Heraeus Company Ltd. Germany). IR spectra were obtained on a Perkin–Elmer FT-IR spectrometer. The NMR spectra were obtained on a Bruker 200 Fourier transform spectrometer. Optical rotations were measured with a JASCO DIP 370 digital polarimeter. Reactions were monitored by Thin Layer chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV, I_2 and anisaldehyde in ethanol as development reagents.

3.1.1. Methyl 3,4-di-*O*-benzyl-6-*O*-(*tert*-butyldimethyl-silyl)-D-mannopyranoside (3). A solution of **2** (15.0 g, 40.1 mmol), imidazole (8.2 g, 120 mmol) and TBS-Cl (6.0 g, 40.0 mmol) in CH_2Cl_2 (120 mL) was stirred for 1 h, concentrated and purified on silica gel using EtOAc–light petroleum (1:9) to afford **3** (16.0 g, 82%) as a colourless syrup; $[\alpha]_D$=+33.9 (*c* 0.49, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) data: δ 0.2 (s, 6H), 0.80 (s, 9H), 2.27 (br s, 1H), 3.33 (s, 3H), 3.71 (m, 6H), 4.66 (m, 5H), 7.29 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.5, 17.7, 23.5, 54.3, 62.0, 70.4, 71.3, 72.3, 74.6, 74.9, 99.1, 127.5, 127.6, 128.2, 128.4, 137.9. Anal. calcd for $C_{27}H_{40}O_6Si$: C, 66.36; H, 8.25. Found: C, 66.69; H, 8.26.

3.1.2. Methyl 3,4-di-*O*-benzyl-6-*O*-(*tert*-butyldimethyl-silyl)-2-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (4). A solution of **3** (11.0 g, 22.5 mmol) and NaH (1.8 g, 45.0 mmol, 60% dispersion in oil) in THF (75 mL) was stirred for 30 min and then MPM-Br (5.0 g, 24.9 mmol) was added. After 4 h, the reaction was quenched with ice and concentrated. The residue was partitioned between EtOAc–water, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc–light petroleum (0.5:9.5) to give **4** (10.0 g, 73%) as a colourless syrup; $[\alpha]_D$=+30.2 (*c* 0.9, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) data: δ 0.2 (s, 6H), 0.82 (s, 9H), 3.20 (s, 3H), 3.41 (m, 1H), 3.96 (s, 3H), 3.74 (m, 5H), 4.51

(m, 6H), 4.82 (d, 1H, J=10.8 Hz), 6.76 (d, 2H, J=8.8 Hz), 7.21 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.4, 18.1, 25.7, 54.1, 54.6, 62.6, 71.8, 71.9, 72.9, 74.4, 74.7, 76.7, 80.0, 98.6, 113.4, 127.2, 127.3, 127.6, 128.0, 129.0, 130.4, 138.5, 138.7. Anal. calcd for $C_{35}H_{48}O_7Si$: C, 69.04; H, 7.95. Found: C, 68.91; H, 7.66.

3.1.3. Methyl 3,4-di-*O*-benzyl-2-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (5). A solution of **4** (10.0 g, 16.4 mmol) and *n*-Bu_4NF (33.0 mL, 33.0 mmol, 1 M) was stirred for 1 h and concentrated. The residue was partitioned between EtOAc–water, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc and light petroleum ether (1:4) to give **5** (6.0 g, 74%) as colourless oil; $[\alpha]_D$=+28.6 (*c* 0.7, $CHCl_3$); IR (cm^{-1}): 3359, 3032, 2868, 1602, 1211, 1155; ^{1}H NMR (200 MHz, $CDCl_3$) data: δ 2.05 (brs, 1H), 3.21 (s, 3H), 3.55 (m, 1H), 3.77 (s, 3H), 3.81 (m, 5H), 4.57 (m, 7H), 6.76 (d, 2H, J=8.7 Hz), 7.23 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 55.1, 62.4, 72.2, 72.6, 74.4, 74.9, 75.1, 80.2, 99.5, 113.8, 127.5, 128.0, 128.3, 129.4, 130.3, 138.6. Anal. calcd for $C_{29}H_{34}O_7$: C, 70.43; H, 6.93. Found: C, 70.59; H, 7.11.

3.1.4. Methyl 3,4-di-*O*-benzyl-6-eno-2-*O*-(*p*-methoxybenzyl)-α-D-mannoheptopyranoside (6). A solution of DMSO (2.8 mL, 32.4 mmol) and oxalyl chloride (1.4 mL, 16.2 mmol) in CH_2Cl_2 (50 mL) at −78 °C was stirred for 30 min and then **5** (4.0 g, 8.1 mmol) was added. After 45 min, Et_3N (6.8 mL, 48.6 mmol) was added and the reaction slowly brought to RT. The CH_2Cl_2 layer was washed with water, dried (Na_2SO_4) and concentrated to obtain the aldehyde (3.4 g), which was dissolved in dry ether (30 mL), cooled to −40 °C and $Ph_3P{=}CH_2$ {generated from PPh_3CH_3I (11.2 g, 27.6 mmol) and sodamide (1.0 g, 25.6 mmol)} was added. After 30 min, reaction mixture was concentrated and the residue purified on silica gel using EtOAc–light petroleum (0.07:0.93) to give **6** (1.3 g, 32%) as a colourless syrup; $[\alpha]_D$=+24.4 (*c* 1, $CHCl_3$); IR (cm^{-1}): 3025, 2890, 1638, 1604, 1205, 1137; ^{1}H NMR (200 MHz, $CDCl_3$) data: δ 3.29 (s, 3H), 3.71 (m, 3H), 3.78 (s, 3H), 3.91 (t, 1H, J=6.9 Hz), 4.64 (m, 7H), 5.25 (d, 1H, J=8.8 Hz), 5.43 (d, 1H, J=14.4 Hz), 5.96 (m, 1H), 6.80 (d, 2H, J=8.4 Hz), 7.28 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 55.2, 72.5, 72.5, 72.9, 74.7, 75.1, 79.0, 80.1, 99.4, 113.9, 117.7, 127.6, 128.0, 128.3, 128.4, 129.5, 130.6, 135.8. Anal. calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.98. Found: C, 73.68; H, 6.92.

3.1.5. Methyl 3,4-di-*O*-benzyl-6,7-*O*-isopropylidine-2-*O*-(*p*-methoxybenzyl)-D-glycero-α-D-mannopyranoside (8a) and methyl 3,4-di-*O*-benzyl-6,7-*O*-isopropylidine-2-*O*-(*p*-methoxybenzyl)-L-glycero-α-D-mannopyranoside (8b). A solution of K_2CO_3 (1.1 g, 8.0 mmol), $K_3Fe(CN)_6$ (2.6 g, 8.0 mmol), OsO_4 (27 mg, 0.1 mmol) and $(DHQ)_2$ PYR (23 mg, 0.026 mmol) in *t*-BuOH–H_2O (16.0 mL, 1:1) was added to **6** (1.3 g, 2.65 mmol). After 6 h at 0 °C, the reaction was quenched with solid sodium sulphite and extracted with EtOAc, dried (Na_2SO_4) and concentrated to give **7a/7b** (1.0 g), which was treated with DMP (5 mL) and PPTS (0.53 g) for 4 h. The reaction mixture was neutralized with Et_3N, concentrated and chromatograph on silica gel using EtOAc–light petroleum (1:9) to give **8a** (0.71 g, 47%); $[\alpha]_D$=+30.8 (*c* 0.8, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) data: δ 1.20 and 1.30 (2s, 6H), 3.18 (s, 3H), 3.56 (m, 5H), 3.65 (s, 3H), 3.85 (t, 1H, J=7.9 Hz), 4.18 (m, 1H), 4.47 (m, 6H), 4.83 (d, 1H, J=11.0 Hz), 6.67 (d, 2H, J=8.7 Hz), 7.17 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 27.2, 54.9, 55.6, 65.1, 69.5, 72.3, 72.6, 73.0, 74.5, 74.8, 75.2, 80.2, 99.7, 127.6, 127.7, 127.8, 128.0, 128.4, 128.6, 129.8, 138.5. Anal. calcd for $C_{33}H_{40}O_8$: C, 70.19; H, 7.14. Found: C, 69.92; H, 7.18. Further elution gave **8b** (0.078 g, 5.2%) as a colourless oil; $[\alpha]_D$=+19.4 (*c* 1.05, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) data: δ 1.38 and 1.44 (2s, 6H), 3.32 (s, 3H), 3.80 (s, 3H), 3.83 (m, 3H), 4.01 (m, 3H), 4.41 (dd, 1H, J=2.3, 6.1 Hz), 4.62 (m, 6H), 4.98 (d, 1H, J=8.3 Hz), 6.83 (d, 2H, J=7.2 Hz), 7.29 (m, 12H). Anal. Calcd for $C_{33}H_{40}O_8$: C, 70.19; H, 7.14. Found: C, 70.04; H, 7.26.

3.1.6. Methyl 2,3,4,6,7-penta-*O*-benzyl-L-glycero-α-D-mannoheptopyranoside (9). A solution of **8b** (1.0 g, 1.8 mmol) and DDQ (0.47 g, 2.1 mmol) in CH_2Cl_2:H_2O (9:1, 7 mL) was stirred for 3 h, concentrated and purified on silica gel using EtOAc–light petroleum (1:4). The resulting product (0.5 g) and NaH (0.2 g, 4.9 mmol, 60% dispersion in oil) in dry DMF (5 mL) were stirred for 30 min followed by addition of BnBr (0.45 mL, 3.7 mmol). After 12 h, the reaction was worked up as usual to afford a residue which was purified on silica gel using EtOAc–light petroleum (1:10) to give **9** (0.68 g, 57%) as a colourless syrup; $[\alpha]_D$=+22.4 (*c* 0.98, $CHCl_3$); IR (cm^{-1}): 3012, 2866, 1611, 1219, 1131; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.26 (s, 3H), 3.81 (m, 7H), 4.55 (m, 11H), 7.25 (m, 25H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 70.9, 72.1, 72.4, 72.6, 73.2, 74.8, 75.0, 75.2, 78.5, 80.5, 98.8, 127.2, 127.3, 127.5, 127.6, 128.2, 138.5, 138.6. Anal. calcd for $C_{43}H_{46}O_7$: C, 76.53; H, 6.87. Found: C, 76.30; H, 6.78.

3.1.7. Methyl 3,4,6,7-tetra-*O*-benzyl-2-*O*-(*p*-methoxybenzyl)-D-glycero-α-D-mannoheptopyranoside (11). A solution of **8a** (0.68 g, 1.2 mmol) and PPTS (0.33 g, 1.3 mmol) in MeOH (10 mL) was stirred for 6 h, neutralized by Et_3N and concentrated. The resulting compound **7a** (0.5 g, 1.0 mmol) was dissolved in dry THF (7 mL) and NaH (0.15 g, 3.8 mmol, 60% dispersion in oil) was added. After 30 min, BnBr (0.3 mL, 2.4 mmol) was introduced, stirred for another 12 h and worked up as usual to afford a residue which was purified on silica gel using EtOAc–light petroleum (1:10) to give **11** (0.55 g, 65%) as a colourless syrup; $[\alpha]_D$=+29.2 (*c* 1.07, $CHCl_3$); IR (cm^{-1}): 3022, 2875, 1612, 1216, 1140; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.31 (s, 3H), 3.70 (m, 5H), 3.77 (m, 3H), 3.96 (s, 2H), 4.65 (m, 11H), 6.76 (d, 2H, J=7.3 Hz), 7.26 (m, 22H); ^{13}C NMR (50 MHz, $CDCl_3$) data: δ 54.6, 55.1, 63.2, 72.0, 72.3, 73.2, 74.7, 74.9, 75.0, 80.5, 99.7, 113.6, 127.4, 127.5, 127.9, 128.3, 129.5, 138.7, 138.9. Anal. calcd for $C_{44}H_{48}O_8$: C, 74.98; H, 6.86. Found: C, 74.69; H, 7.08.

3.1.8. Methyl 3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-mannoheptopyranoside (12). A solution of **11** (1.3 g, 1.8 mmol) and DDQ (0.46 g, 2.0 mmol) in CH_2Cl_2–H_2O (9:1) was stirred for 3 h, concentrated and the residue purified on silica gel using EtOAc–light petroleum (1:4) to give **12** (0.76 g, 70%) as a colourless oil; $[\alpha]_D$=+17.3 (*c* 0.9, $CHCl_3$); IR (cm^{-1}): 3376, 3035, 2892, 1615, 1210, 1190; ^{1}H NMR (200 MHz, $CDCl_3$) data: δ 3.35 (s, 3H), 3.85 (m, 7H), 4.65 (m, 9H), 7.31 (m, 20H); ^{13}C NMR (50 MHz,

$CDCl_3$): δ 54.8, 68.2, 70.8, 72.0, 72.6, 73.3, 74.4, 78.3, 80.8, 100.2, 127.5, 127.6, 127.7, 127.9, 128.3, 128.5, 138.0, 138.6. Anal. calcd for $C_{36}H_{40}O_7$: C, 73.95; H, 6.89. Found: C, 73.81; H, 6.93.

3.1.9. 1,2-Di-*O*-acetyl-3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-mannoheptopyranoside (13). A mixture of acetic acid, acetic anhydride and sulfuric acid (3.0 mL; 25:5:1.5) and **12** (0.3 g, 0.5 mmol) was stirred for 1 h at 0 °C. The reaction was neutralized with $NaHCO_3$ solution, extracted with EtOAc, dried (Na_2SO_4), evaporated and purified on silica gel using EtOAc–light petroleum (1:9) to give **13** (0.2 g, 60%) as a colourless oil; $[\alpha]_D$=+22.5 (*c* 0.9, $CHCl_3$); IR (cm^{-1}): 3015, 2912, 1710, 1611, 1145; ^1H NMR (200 MHz, $CDCl_3$): δ 2.05, 2.11 (2s, 6H), 3.94 (m, 4H), 4.24 (m, 2H), 4.64 (m, 8H), 5.28 (br.d, 1H, *J*=5.6 Hz), 6.05 (d, 1H, *J*=2.0 Hz), 7.32 (m, 20H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.4, 64.3, 71.8, 72.1, 72.3, 73.6, 74.4, 74.8, 79.1, 91.5, 127.1, 127.3, 127.5, 127.7, 127.9, 128.1, 128.7, 137.7, 137.8, 138.0, 138.2, 168.2, 169.8. Anal. calcd for $C_{39}H_{42}O_9$: C, 71.54; H, 6.46. Found: C, 71.41; H, 6.25.

3.1.10. Methyl 2-*O*-acetyl-3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-mannoheptopyranosyl-(1→2)-3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-mannoheptopyranoside (14). To a mixture of **12** (0.14 g, 0.2 mmol), **13** (0.2 g, 0.3 mmol) and activated 4 Å molecular sieves (0.1 g) in dry CH_2Cl_2 (3 mL), $BF_3{\cdot}OEt_2$ (0.05 mL) was added. After 12 h, the reaction was neutralized with Et_3N, filtered, concentrated and purified on silica gel using EtOAc–light petroleum (1:10) to give the disaccharide **14** (0.07 g, 26%) as a colourless syrup; $[\alpha]_D$=+28.9 (*c* 1, $CHCl_3$); IR (cm^{-1}): 3041, 2882, 1725, 1600, 1208, 1180; ^1H NMR (200 MHz, $CDCl_3$) data: δ 2.09 (s, 3H), 3.29 (s, 3H); 3.76 (m, 13H), 4.59 (m, 18H), 5.22 (brd, 1H, *J*=5.4 Hz), 7.21 (m, 40H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.0, 54.7, 68.8, 72.0, 72.3, 72.4, 75.2, 75.3, 78.5, 78.6, 79.5, 99.5, 99.8, 127.5, 127.7, 128.0, 128.4, 135.1, 135.6, 138.5, 169.8. Anal. calcd for $C_{73}H_{78}O_{14}$: C, 74.34; H, 6.66. Found: C, 74.51; H, 6.92.

3.1.11. Methyl 3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-mannoheptopyranosyl-(1→2)-3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-mannoheptopyranoside (15). A solution of **14** (0.06 g, 0.05 mmol) and MeONa (3 mg) in MeOH (2 mL) was stirred for 10 min, quenched by adding solid CO_2 and concentrated. The residue purified on silica gel using EtOAc–light petroleum (1:9) to give **15** (0.042 g, 72%) as a colourless syrup; $[\alpha]_D$=+32.7 (*c* 1.12, $CHCl_3$); IR (cm^{-1}): 3390, 3027, 2870, 1613, 1217, 1163; ^1H NMR (200 MHz, $CDCl_3$) data: δ 3.41 (s, 3H), 3.86 (m, 14H), 4.72 (m, 18H), 7.26 (m, 40H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 54.6, 65.5, 68.8, 72.6, 73.0, 73.5, 75.4, 78.1, 78.4, 79.8, 98.7, 98.9, 127.4, 127.7, 127.8, 128.0, 128.2, 128.4, 135.2, 135.8, 138.0. Anal. calcd for $C_{71}H_{76}O_{13}$: C, 74.98; H, 6.73. Found: C, 75.18; H, 6.99.

3.1.12. Methyl D-glycero-α-D-mannoheptopyranosyl-(1→2)-D-glycero-α-D-mannoheptopyranoside (16). A suspension of 10% $Pd(OH)_2$–C (10 mg) and **15** (0.035 g, 0.03 mmol) in MeOH (5 mL) was hydrogenolyzed at rt for 40 h. Filtration of the catalyst followed by concentration gave **16** (0.007 g, 52%) as a colourless oil; $[\alpha]_D$=+65 (*c* 0.8, H_2O); IR (cm^{-1}): 3390, 2871, 1209, 1161; ^1H NMR (200 MHz, D_2O) data: δ 3.41 (s, 3H), 3.79 (m, 14H), 4.77 (d, 1H, *J*=1.7 Hz), 4.90 (d, 1H, *J*=1.7 Hz); ^{13}C NMR (50 MHz, D_2O): δ 54.3, 62.1, 62.2, 67.6, 67.8, 69.9, 70.1, 71.0, 72.2, 72.4, 74.2, 74.2, 101.9, 102.4. Anal. Calcd for $C_{15}H_{28}O_{13}$: C, 43.27; H, 6.78. Found: C, 43.41; H, 6.93.

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