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Synthesis of the tetrasaccharide repeating unit of the K-antigen from *Klebsiella* type 57[1]

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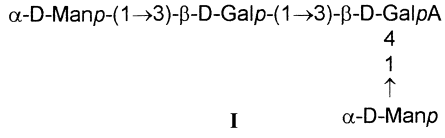
Abstract

Starting from D-galactose and D-mannose two disaccharide blocks, namely 2-(trimethylsilyl)-ethyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl-(1→4)-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside and ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside, were synthesized which were then allowed to react, in the presence of dimethyl(methylthio)sulfonium triflate to give a tetrasaccharide derivative. This compound was converted to 2-(trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→4)]-*tert*-butyl (2-*O*-acetyl-β-D-galactopyranosid)uronate which, on treatment with sodium methoxide followed by hydrogenolysis, afforded the methyl [2-(trimethylsilyl)ethyl glycosid]uronate of the tetrasaccharide repeating unit of the K-antigen from *Klebsiella* type 57.

Keywords: Synthesis; *Klebsiella* type 57; Tetrasaccharide repeating unit

1. Introduction

Klebsiella type 57 is a gram-negative, opportunistic pathogen [1] causing a variety of specific infections that can result in bacterioma, acute broncho-pneumonia and also more chronic destructive lesions with multiple abscess formation in lungs. The polysaccharide from *Klebsiella* type 57 is a heteropolysaccharide composed [2] of the tetrasaccharide repeating unit **I**.



I

This structure was found to be identical to that of the K-antigen from *Escherichia coli* type 36, which is also a gram negative pathogenic bacteria. Serum antibodies to the bacterial polysaccharides may provide the basis for the protection against specific bacterial infection through vaccination [3]. Host-protective antibodies can be elicited by synthetic antigens in which the native polysaccharide antigen is covalently linked to a carrier protein [4].

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It has also been claimed that the immunogenic properties of the bacterial cell-surface polysaccharides can be expressed by carbohydrate structures smaller than the native polysaccharides [5]. As a part of our programme to determine the relationship between structure and immunological specificities, we were interested to synthesize the repeating unit and other related oligomers of the K-antigen from *Klebsiella* type 57. The synthesized oligosaccharide can be used as a molecular probe for studying the immunochemical behaviour of the antigen.

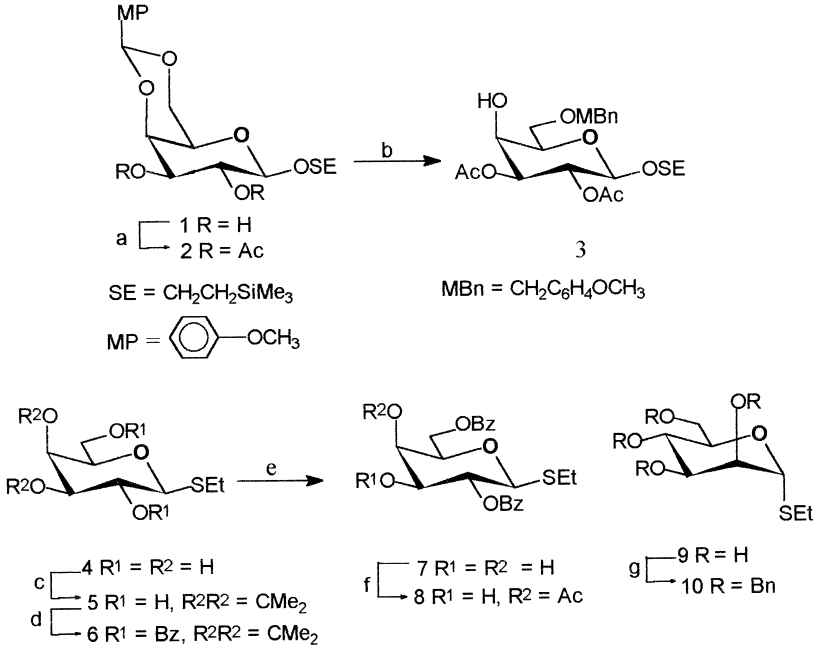
2. Results and discussion

The known 2-(trimethylsilyl)ethyl 4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside (**1**) [6], on acetylation followed by regioselective reductive opening [7] of the 4-methoxybenzylidene acetal of the resulting di-*O*-acetyl-derivative **2** with sodium cyanoborohydride and trifluoroacetic acid afforded 2-(trimethylsilyl)ethyl 2,3-di-*O*-acetyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**3**).

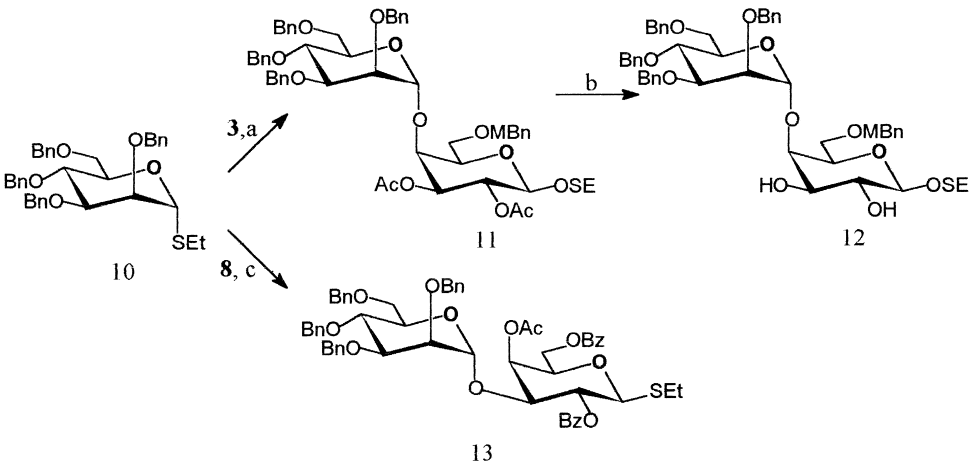
In another experiment, ethyl 1-thio-β-D-galactopyranoside (**4**) [8] was treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in *N*,*N*-dimethylformamide to obtain ethyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (**5**) [9]. Benzoylation [10] of **5** followed by removal of the isopropylidene group [11] from the product **6** with 80% acetic acid gave **7** with two hydroxyl groups. Preparation of the 3,4-orthoester derivative [12] of **7** with triethylorthoacetate, followed by regioselective opening of the orthoester, afforded the acceptor ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside (**8**) (Scheme 1).

Compound **3** was allowed to react with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-mannopyranoside (**10**), prepared from ethyl 1-thio-α-D-mannopyranoside **9** [13], in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) [14] at room temperature for 30 min to give the disaccharide derivative **11** in 95% yield. Compound **11** was characterised by its signals at δ 5.16 (H-2^{I}), 4.97 (H-3^{I}), 4.95 (H-1^{II}) and 4.42 (H-1^{I}) in the ^{1}H NMR spectrum. Removal of the acetyl groups [15] in **11** afforded the acceptor **12** having two hydroxyl groups. The disaccharide **12** has its signals at δ 4.91 (H-1^{II}), 4.47 (H-1^{I}), 4.23 (H-3^{I}) and 4.15 (H-2^{I}) in the ^{1}H NMR spectrum and at δ 102.7 (C-1^{I}) and 100.7 (C-1^{II}) in the ^{13}C NMR spectrum together with the characteristic peaks for $OCH_2CH_2SiMe_3$ and MBn.The observed upfield shift of the signals for H-2^{I} and H-3^{I} in the ^{1}H NMR spectrum in **12** compared to those observed in **11** was expected.

The thioglycoside **8**, being disarmed due to the presence of the 2-*O*-benzoyl group, was allowed to react with the thioglycoside donor **10**, which is



Scheme 1. (a) Ac_2O, pyridine; (b) trifluoroacetic acid, $NaBH_3CN$, DMF; (c) 2,2-dimethoxypropane, *p*-TsOH, DMF; (d) BzCl, pyridine; (e) 80% acetic acid; (f) triethylorthoacetate, benzene, 80% acetic acid; (g) benzyl bromide, NaH, DMF.



Scheme 2. (a) NIS, TfOH, CH_2Cl_2, MS 4Å; (b) NaOMe; (c) IDCP, CH_2Cl_2 , MS 4Å.

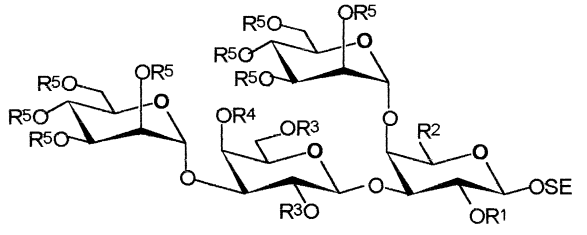
armed due to the 2-*O*-benzyl substitution, in the presence of iodonium dicollidine perchlorate (IDCP) [16] to afford the disaccharide ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→4)-4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**13**) in 70% yield (Scheme 2).

Compound **13** was characterised by its signals at δ 5.63 (H-4^{I}), 5.44 (H-2^{I}), 5.26 (H-1^{II}) and 4.65 (H-1^{I}) in the ^{1}H NMR spectrum and at δ 93.7 (C-1^{II}) and 84.2 (C-1^{I}) together with characteristic signals for SEt and OAc in the ^{13}C NMR spectrum.

The thioglycoside **13** was then used as the donor and was allowed to react with **12** in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) [17] to afford the tetrasaccharide derivative **14** in 81% yield (Scheme 3). Compound **14** had characteristic ^{1}H NMR signals at δ 5.68 (H-4^{III}), 5.63 (H-2^{III}), 5.30 (H-1^{IV}), 4.95 (H-1^{II}), 4.73 (H-1^{III}), 4.48 (H-1^{I}), 4.07 (H-2^{I}) and ^{13}C NMR signals at δ 101.6 (C-1^{I}), 101.3 (C-1^{III}), 100.5 (C-1^{II}), 94.5 (C-1^{IV}). Acetylation of **14** gave the diacetate **15**. The ^{1}H NMR spectrum of **15** revealed the shift of the H-2^{I} signal from δ 4.07 to δ 5.10 indicating that the glycosidic linkage created during the formation of the tetrasaccharide **14** is (1→3) and not (1→2). The structure of **14** was also confirmed by chemical methods. Hydrogenolysis of **14** followed by treatment of the product with sodium methoxide [15] provided the deprotected tetrasaccharide. Methylation analysis [18] of the latter gave, as expected, 2,3,4,6-tetra-*O*-methyl-mannose, 2,4,6-tri-*O*-methyl-galactose and 2,6-di-*O*-methyl-galactose. Removal of the 4-methoxybenzyl group [19] from **15** with ceric ammonium nitrate (CAN) followed by oxidation with pyridinium dichromate (CrO_3-pyridine-Ac_2O-*t*-BuOH) [20] of the primary alcohol **16** gave the *tert*-butyl ester **17**. Compound **17** had characteristic signals in its ^{1}H NMR spectrum at δ 5.22 (H-1^{IV}), 4.91 (H-1^{II}), 4.49 (H-1^{I}), 4.33 (H-1^{III}) and ^{13}C NMR signals at δ 101.3 (C-1^{I}), 100.4 (C-1^{III}), 99.0 (C-1^{II}) and 93.9 (C-1^{IV}) together with the characteristic peak for *tert*-butyl ester. Treatment of **17** with sodium methoxide in methanol gave the methyl ester **18** which was hydrogenolysed [21] over Pd/C to afford the tetrasaccharide repeating unit of the K-antigen from *Klebsiella* type 57 as its methyl ester 2-(trimethylsilyl)ethyl glycoside (**19**). Compound **19** had characteristic signals in its ^{1}H and ^{13}C NMR spectra.

3. Experimental

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



a 14 R^1 = H, R^2 = CH_2OMBn, R^3 = Bz, R^4 = Ac, R^5 = Bn
b 15 R^1 = R^4 = Ac, R^2 = CH_2OMBn, R^3 = Bz, R^5 = Bn
c 16 R^1 = R^4 = Ac, R^2 = CH_2OH, R^3 = Bz, R^5 = Bn
d 17 R^1 = R^4 = Ac, R^2 = $COOBu^t$, R^3 = Bz, R^5 = Bn
e 18 R^1 = R^3 = R^4 = H, R^2 = COOMe, R^5 = Bn
19 R^1 = R^3 = R^4 = R^5 = H, R^2 = COOMe,

Scheme 3. (a) Ac_2O, pyridine; (b) ceric ammonium nitrate; (c) CrO_3, ^{t}BuOH, pyridine, Ac_2O; (d) NaOMe; (e) Pd-C, AcOH.

dried before use and all evaporations were conducted below 40 °C under reduced pressure unless stated otherwise. Optical rotations were measured with a Perkin-Elmer model 241 MC polarimeter. ^{1}H 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.

2-(Trimethylsilyl)ethyl 2,3-di-O-*acetyl-4,6*-O-*(4-methoxybenzylidene)*-β-D-*galactopyranoside* (**2**).—Compound **1** ([6], 2.5 g, 6 mmol) was acetylated with acetic anhydride (2.5 mL, 26.4 mmol) and pyridine (5 mL) in the conventional method to give **2** (2.3 g, 93%); mp 137–139 °C; $[\alpha]_D^{25}$ + 43.4° (*c* 2, $CHCl_3$). ^{1}H NMR: δ 6.80–7.44 (m, 4 H, aromatic protons), 5.4 (s, 1 H, C*H*$C_6H_4OCH_3$), 5.34 (dd, 1 H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 4.92 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 4 Hz, H-3), 4.48 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.76 (s, 3 H, OC*H*$_3$), 3.68 (m, 2 H, OC*H*$_2$CH$_2$Si), 2.0 (s, 6 H, 2 COC*H*$_3$), 1.0 (m, 2 H, CH$_2$C*H*$_2$Si), 0.01 [s, 9 H, Si(CH$_3$)$_3$]. Anal. Calcd for $C_{23}H_{34}O_9Si$: C, 57.24; H, 7.1. Found: C, 57.08; H, 7.41.

2-(Trimethylsilyl)ethyl 2,3-di-O-*acetyl-6*-O-*(4-methoxybenzyl)*-β-D-*galacto-pyranoside* (**3**).—A soln of trifluoroacetic acid (1.96 g, 17.2 mmol) in DMF (10.3 mL) was added dropwise to a mixture of **2** (858 mg, 1.72 mmol), $NaCNBH_3$ (540 mg, 8.6 mmol) and MS 3 Å in DMF (14 mL) at 0 °C with stirring. Stirring was continued for 7 h at room temperature after which the mixture was filtered through a celite bed and poured into cold saturated $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3×40 mL) and the organic layer was washed successively with water, saturated $NaHCO_3$, and water, dried (Na_2SO_4) and filtered. The syrupy product was purified by column chromatography with 3:1 toluene–EtOAc giving pure **3** (1.5 g, 76%); $[\alpha]_D^{25}$ −3.24° (*c* 2, $CHCl_3$). ^{1}H NMR: δ 6.94–7.48 (m, aromatic protons), 5.24 (dd, 1 H, $J_{1,2}$ 7.3 Hz, $J_{2,3}$ 9.0 Hz, H-2), 4.92 (dd, 1H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 2.1 Hz, H-3), 4.46 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.80 (s, 3 H, OC*H*$_3$), 3.60 (m, 2 H, OC*H*$_2$CH$_2$Si), 2.10, 2.04 (2 s, 6 H, 2 COC*H*$_3$), 1.0 (m, 2 H, CH$_2$C*H*$_2$Si), 0.01 [s, 9 H, Si(C*H*$_3$)$_3$]. Anal. Calcd for $C_{23}H_{36}O_9Si$: C, 57.0; H, 7.49. Found: C, 56.92; H, 7.60.

Ethyl 3,4-O-*isopropylidene-1-thio*-β-D-*galacto-pyranoside* (**5**).—A mixture of **4** ([8], 5 g, 22.4 mmol), 2,2-dimethoxypropane (5 mL, 38.1 mmol) and *p*-TsOH (200 mg) in DMF (25 mL) was stirred for 2 h at 40 °C. The reaction was then quenched with Et_3N (0.2 mL) and partitioned between water and CH_2Cl_2 (3×20 mL). The organic layer was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography with 3:1 toluene–EtOAc gave pure **5** (5.4 g, 61%); $[\alpha]_D^{25}$ + 38.4° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{11}H_{20}O_5S$: C, 49.98; H, 7.63. Found: C, 49.82; H, 7.69.

Ethyl 2,6-di-O-*benzoyl-3,4*-O-*isopropylidene-1-thio*-β-D-*galactopyranoside* (**6**).—Benzoyl chloride (5.6 mL, 40 mmol) was added to a soln of **5** (5 g, 19 mmol) in pyridine (15 mL) at 0 °C. The mixture was stirred at room temperature for 2 h and then poured into ice water. The product was extracted with CH_2Cl_2, washed with water, dried (Na_2SO_4) and concentrated. Traces of pyridine were removed by co-evaporation with toluene. Column chromatography of the product with 12:1 toluene–EtOAc gave a syrup which was crystallized from EtOH giving pure **6** (8 g, 90%); mp 141 °C; $[\alpha]_D^{25}$ + 46.8° (*c* 2.2, $CHCl_3$). ^{1}H NMR: δ 7.4–8.1 (m, 10 H, aromatic protons), 5.24 (dd, 1 H, $J_{1,2}$ 11 Hz, $J_{2,3}$, 7.0 Hz, H-2), 4.44 (d, 1 H, $J_{1,2}$ 11 Hz, H-1), 2.68 (q, 2 H, SC*H*$_2$CH$_3$), 1.32, 1.6 [2 s, 6 H, C(C*H*$_3$)$_2$], 1.2 (t, 3 H, SCH$_2$C*H*$_3$). Anal. Calcd for $C_{25}H_{28}O_7S$: C, 63.54; H, 5.97. Found: C, 63.39; H, 6.12.

Ethyl 2,6-di-O-*benzoyl-1-thio*-β-D-*galactopyranoside* (**7**).—A soln of **6** (7 g, 14.8 mmol) in 80% acetic acid (50 mL) was heated at 80 °C for 1 h. Solvents were then evaporated off and the product **7** crystallized from EtOH; mp 147 °C; $[\alpha]_D^{25}$ −3.6° (*c* 1, $CHCl_3$). ^{1}H NMR: δ 7.4–8.08 (m, 10 H, aromatic protons), 5.26 (dd, 1 H, $J_{1,2}$ 11 Hz, $J_{2,3}$ 7 Hz, H-2), 4.43 (d, 1 H, $J_{1,2}$ 11 Hz, H-1), 2.7 (q, 2 H, SC*H*$_2$CH$_3$), 1.2 (t, 3 H, SC*H*$_2$CH$_3$). Anal. Calcd for $C_{22}H_{24}O_7S$: C, 61.09; H, 5.59. Found: C, 61.00; H, 5.68.

Ethyl 4-O-*acetyl-2,6-di*-O-*benzoyl*-β-D-*galacto-pyranoside* (**8**).—To a soln of **7** (6 g, 13.9 mmol) in dry benzene (40 mL), triethylorthoacetate (12.8 mL, 70 mmol) and *p*-TsOH (10 mg) were added and the mixture was stirred at room temperature for 1 h. The reaction was quenched with Et_3N (0.2 mL) and poured into ice water. The mixture was extracted three times with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4) and concd to a syrup. The product was dissolved in 80% acetic acid (100 mL) and the soln was kept at room temperature for 10 min. Solvents were evaporated off and the product was crystallized from EtOH giving pure **8** (6.1 g, 92%); mp 158 °C; $[\alpha]_D^{25}$ −61.7° (*c* 2, $CHCl_3$). ^{1}H NMR: δ

7.45–8.2 (m, 10H, aromatic protons), 5.56 (bs, 1 H, H-4), 5.32 (dd, 1 H, $J_{1,2}$ 10 Hz, $J_{2,3}$ 10 Hz, H-2), 4.70 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 2.74 (q, 2 H, SCH_2CH_3), 2.24 (s, 3 H, $COCH_3$), 1.27 (t, 3 H, SCH_2CH_3). Anal. Calcd for $C_{24}H_{26}O_8S$: C, 60.75; H, 5.52. Found: C, 60.69; H, 5.60.

Ethyl 2,3,4,6-tetra-O-*benzyl-1-thio*-β-D-*mannopyranoside* (**10**).—Benzylation of **9** (9 g, 40.2 mmol) was carried out according to a conventional method [13] to give **10** as a syrup (18.7 g, 80%); $[\alpha]_D^{25}$ +46.3° (*c* 0.7, $CHCl_3$). ^{1}H NMR: δ 7.32–7.40 (m, 20 H, 4 Ph), 5.44 (bs, 1 H, H-1), 2.63 (q, 2 H, SCH_2CH_3), 1.27 (t, 3 H, SCH_2CH_3). Anal. Calcd for $C_{36}H_{40}O_5S$: C, 78.23; H, 7.30. Found: C, 78.01; H, 7.63.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl-(1→4)-2,3-di*-O-*acetyl-6*-O-*(4-methoxybenzyl)*-β-D-*galactopyranoside* (**11**).—To a soln of **3** (1.3 g, 2.2 mmol) and **10** (2 g, 3.3 mmol) in CH_2Cl_2 (30 mL) was added MS 4 Å (3 g) and stirred under Ar for 18 h. The mixture was then cooled to 0 °C and NIS (1 g, 4.3 mmol) and TfOH (5 μL) were added. After 45 min at room temperature, the solids were filtered off, the filtrate was diluted with CH_2Cl_2, washed with 10% $Na_2S_2O_3$ and 1 M $NaHCO_3$, dried (Na_2SO_4) and concd. Column chromatography of the syrupy product with 19:1 toluene–EtOAc gave pure **11** (2.3 g, 95%); $[\alpha]_D^{25}$ +19.2° (*c* 0.7, $CHCl_3$). ^{1}H NMR: δ 6.8–7.8 (m, 25 H, aromatic protons), 5.16 (dd, 1 H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.5 Hz, H-2^I), 4.97 (dd, 1 H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.0 Hz, H-3^I), 4.95 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1^{II}), 4.42 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^I), 3.77 (s, 3 H, $C_6H_4OCH_3$), 3.51 (m, 2 H, OCH_2CH_2Si), 2.03, 1.99 (2 s, 6 H, 2 $COCH_3$), 0.96, (m 2 H, OCH_2CH_2Si), 0.02 [s, 9 H, $Si(CH_3)_3$]. Anal. Calcd for $C_{57}H_{70}O_{14}Si$: C, 67.97; H, 7.00. Found: C, 67.78; H, 7.20.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl-(1→4)-6*-O-*(4-methoxybenzyl)*-β-D-*galactopyranoside* (**12**).—Compound **11** (2 g, 2.17 mmol) was de-*O*-acetylated according to conventional method [15] to give **12** (1.75 g, 96%); $[\alpha]_D^{24}$ +0.46° (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 4.91 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1^{II}), 4.47 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^I), 4.23 (d, 1 H, $J_{2,3}$ 7.2 Hz, H-3^I), 4.15 (dd,1 H, $J_{1,2}$ 8.4 Hz, $J_{2,3}$ 7.2 Hz, H-2^I), 3.73 (s, 3 H, $C_6H_4OCH_3$), 3.57 (m, 2 H, OCH_2CH_2Si), 0.93 (m, 2 H, CH_2CH_2Si), 0.02 [s, 9 H, $Si(CH_3)_3$]. ^{13}C NMR: δ 127.6–138.5 (aromatic carbons), 102.9 (C-1^I), 100.8, 79.29, 76.45, 75.77, 75.18, 74.50, 73.51, 73.44, 73.10, 72.89, 72.62, 71.99, 69.4, 69.16, 67.91, 67.34 (C-1^{II}), 55.16 ($C_6H_4OCH_3$), 18.22 (OCH_2CH_2Si), −1.42 [$Si(CH_3)_3$]. Anal. Calcd for $C_{53}H_{66}O_{12}Si$: C, 68.96; H, 7.21. Found: C, 68.90; H, 7.29.

Ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl-1-thio*-β-D-*galactopyranoside* (**13**).—Freshly prepared IDCP (2.45 g, 7 mmol) was added to a stirred soln of **8** (1.7 g, 3.5 mmol) and **10** (3.1 g, 5.2 mmol) in CH_2Cl_2 (20 mL) containing MS 4 Å (4 g) at −10 °C. The temperature was raised to 24 °C and stirring was continued for 2 h. The mixture was then filtered, washed with 10% $Na_2S_2O_3$, 1 M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The product was purified by column chromatography with 12:1 toluene–EtOAc to give **13** (2.6 g, 72%); $[\alpha]_D^{25}$ +32° (*c* 1.5, $CHCl_3$). ^{1}H NMR: δ 7.2–8.2 (m, 30 H, 2 PhCO, 4 $PhCH_2$), 5.63 (bs, 1 H, H-4^I), 5.44 (dd, 1 H, $J_{1,2}$ 7.2 Hz, $J_{2,3}$ 7.5 Hz, H-2^I), 5.26 (bs, 1 H, H-1^{II}), 4.65 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1^I), 2.75 (q, 2 H, SCH_2CH_3), 2.37 (s, 3 H, $COCH_3$), 1.38 (t, 3 H, SCH_2CH_3). ^{13}C NMR: δ 171.2 ($OCOCH_3$), 165.3, 165.2 (benzoate), 106.9–138.8 (aromatic carbons), 93.7 (C-1^{II}), 84.2, 79.5, 76.4, 74.4, 74.2, 73.2, 72.8, 72.1, 71.9, 69.2, 68.5, 65.4, 62.0 (C-1^I), 24.4 (SCH_2CH_3), 20.75 ($OCOCH_3$), 14.97 (SCH_2CH_3). Anal. Calcd for $C_{58}H_{60}O_{13}S$: C, 69.86; H, 6.06. Found: C, 69.70; H, 6.20.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl*-β-D-*galactopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzyl*-α-D-*mannopyranosyl-(1→4)]-6*-O-*(4-methoxybenzyl)*-β-D-*galactopyranoside* (**14**).—To a soln of **12** (633 mg, 0.61 mmol) and **13** (612 mg, 0.61 mmol) in dry CH_2Cl_2 (3 mL) was added MS 4 Å (1 g) and the mixture was stirred for 12 h. The mixture was then cooled to −30 °C and DMTST (300 mg, 2.4 mmol, 4.4 equiv) was added to it. After stirring for 14 h at −15 °C the mixture was diluted with CH_2Cl_2 and filtered. The filtrate was washed with aq $NaHCO_3$, water, dried (Na_2SO_4) and concd. Column chromatography (19:1 toluene–EtOAc) of the product gave **14** (465 mg, 81%); $[a]_D^{25}$ +1.3° (*c* 0.9, $CHCl_3$). ^{1}H NMR: δ 6.9–8.1 (m, 55 H, aromatic protons), 5.60 (dd, 1 H, $J_{1,2}$ 8.4 Hz, $J_{2,3}$ 7.2 Hz, H-2^{III}), 5.30 (bs, 1 H, H-1^{IV}), 4.95 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1^{II}), 4.73 (d, 1 H, $J_{1,2}$ 6.9 Hz, H-1^{III}), 4.48 (d, 1 H, *J* 8.0 Hz, H-1^I), 4.07 (dd, 1 H, $J_{2,3}$ 7.2 Hz, H-2^I), 3.75 (s, 3 H, OCH_3), 3.45 (m, 2 H, OCH_2CH_2Si), 2.04 (s, 3 H, $COCH_3$), 0.90 (m, 2 H, OCH2CH_2Si), 0.01 [s, 9 H, $Si(CH_3)_3$].

^{13}C NMR: δ 170.13, 165.7, 159.3 (2 *C*OPh, O*C*OCH$_3$), 126.8–138.9 (aromatic carbons), 101.6 (C-1^{I}), 101.3 (C-1^{III}), 100.5 (C-1^{II}), 94.5, 80.04, 79.4, 78.3, 75.7, 75.5, 75.0, 74.7, 74.6, 74.1, 73.2, 73.0, 72.6, 72.2, 71.9, 70.3, 70.1, 69.1, 68.8, 67.7, 67.3, 64.95, 61.1 (C-1^{IV}), 55.12 ($C_6H_4OCH_3$), 20.5 (OCO*C*H$_3$), 18.15 (OCH$_2$C*H*$_2$Si), −1.4 [Si(*C*H$_3$)$_3$]. Anal. Calcd for $C_{109}H_{120}O_{25}Si$: C, 70.59 ; H, 6.53. Found: C, 70.22; H, 6.78.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-*4*-O-*acetyl*-*2,6*-*di*-O-*benzoyl*-β-D-*galactopyranosyl*-(1→3)-[*2,3,4,6-tetra*-O-*benzyl*-α-D-*mannopyranosyl*-(1→4)]-*2*-O-*acetyl*-*6*-O-(*4*-*methoxybenzyl*)-β-D-*galactopyranoside* (**15**).—The tetrasaccharide derivative **14** (400 mg, 0.2 mmol.) was acetylated conventionally with Ac_2O and pyridine. The crude product was purified by column chromatography giving **15** (392 mg, 94%); $[\alpha]_D^{25}$ +19.5° (*c* 0.8, $CHCl_3$). ^{1}H NMR: δ 6.92–8.10 (m, 55 H, aromatic protons), 5.60 (bs, 1 H, H-4^{III}), 5.54 (dd, 1 H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 7.2 Hz, H-2^{III}), 5.28 (bs, 1 H, H-1^{IV}), 5.10 (dd, 1 H, $J_{1,2}$ 7.2 Hz, $J_{2,3}$ 10.2, H-2$^{I)}$, 4.97 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1^{II}), 4.52 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1^{I}), 4.75 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1^{III}), 3.75 (s, 3 H, C_6H_4OC*H*$_3$), 3.64 (m, 2 H, OC*H*$_2$CH$_2$Si), 2.10, 2.08 (2 s, 6 H, 2 COC*H*$_3$), 0.09 (m, 2 H, OCH$_2$C*H*$_2$Si), 0.01 [s, 9 H, Si(C*H*$_3$)$_3$]. Anal. Calcd for C_{111} $H_{122}O_{26}Si$: C, 70.16; H, 6.47. Found: C, 70.08; H, 6.56.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-*4*-O-*acetyl*-*2,6*-*di*-O-*benzoyl*-β-D-*galactopyranosyl*-(1→3)-[*2,3,4,6-tetra*-O-*benzyl*-α-D-*mannopyranosyl*-(1→4)]-*2*-O-*acetyl*-β-D-*galactopyranoside* (**16**).—Ceric ammonium nitrate (95 mg, 0.17 mmol), dissolved in 5:1 MeCN–water (2 mL), was added to a soln of **15** (160 mg, 0.08 mmol) in CH_2Cl_2 (3.5 mL) at 0 °C and stirred at room temperature for 2 h. The mixture was then diluted with CH_2Cl_2 (10 mL) and washed with satd $NaHCO_3$ and water, dried (Na_2SO_4) and concd to a syrup. Column chromatography (4:1 toluene–EtOAc) gave **16** (104 mg, 69%); $[\alpha]_D^{25}$ −12.9° (*c* 0.9, $CHCl_3$). ^{1}H NMR: δ 5.26 (bs, 1 H, H-1^{IV}), 4.96 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1^{II}), 4.72 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{III}), 4.50 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 3.68 (m, 2 H, OC*H*$_2$CH$_2$Si), 2.06, 2.04 (2 s, 6 H, 2 COC*H*$_3$), 0.90 (m, 2 H, OCH$_2$ C*H*$_2$Si), 0.01 [s, 9 H, Si(C*H*$_3$)$_3$]. Anal. Calcd for $C_{103}H_{114}O_{25}Si$: C, 69.64; H, 6.74. Found: C, 69.42; H, 6.89.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-*4*-O-*acetyl*-*2,6*-*di*-O-*benzoyl*-β-D-*galactopyranosyl*-(1→3)-[*2,3,4,6-tetra*-O-*benzyl*-α-D-*mannopyranosyl*-(1→4)]-tert-*butyl* (*2*-O-*acetyl*-β-D-*galactopyranosid*)*uronate* (**17**).—Compound **16** (114 mg, 0.06 mmol), CrO_3 (25.6 mg, 0.26 mmol), pyridine (41.3 μL, 0.51 mmol), Ac_2O (47.6 μL, 0.51 mmol) and *t*-BuOH (120 μL, 1.2 mmol) in CH_2Cl_2 (0.6 mL). The mixture was stirred for 4 h at 0 °C and then applied on the top of a silica gel column in EtOAc with a 5 cm layer of EtOAc on top of the gel. The chromium compound precipitated in the presence of EtOAc. After 15 min the product was eluted with EtOAc, concd to a syrup and purified by column chromatography using 12:1 toluene–EtOAc to afford **17** (94.8 mg, 80%); $[\alpha]_D^{25}$ −5.34° (*c* 0.7, $CHCl_3$). ^{1}H NMR: δ 7.18–8.14 (m, 50 H, aromatic protons), 5.22 (bs, 1 H, H-1^{IV}), 4.93 (bs, 1 H, H-1^{II}), 4.49 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{I}), 4.68 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1^{III}), 3.69 (m, 2 H, OC*H*$_2$CH$_2$Si), 2.37, 2.1 (2 s, 6 H, 2 COC*H*$_3$), 1.28 [s, 9 H, COOC(C*H*$_3$)$_3$], 0.90 (m, 2 H, OCH$_2$C*H*$_2$Si), −0.03 [s, 9 H, Si(C*H*$_3$)$_3$]. ^{13}C NMR: δ 173.3, 170.1, 165.9, 165.2, 126.9–138.8 (aromatic carbons), 101.3 (C-1^{I}), 100.4 (C-1^{III}), 99.0 (C-1^{II}), 93.9, 79.1, 76.4, 75.0, 74.8, 74.4, 74.2, 73.4, 72.9, 72.8, 72.4, 72.2, 71.9, 71.5, 70.2, 70.0, 69.6, 69.1, 65.7, 65.0, 61.8 (C-1^{I}V), 29.6 [COO*C*(CH$_3$)$_3$], 22.2, 20.6 (2 OCO*C*H$_3$), 17.1 (OCH$_2$*C*H$_2$Si), −1.5 [Si(*C*H$_3$)$_3$]. Anal. Calcd for $C_{107}H_{122}O_{26}Si$: C, 69.30; H, 6.64. Found: C, 69.11; H, 6.72.

2-(Trimethylsilyl)ethyl α-D-*mannopyranosyl*-(1→3)-β-D-*galactopyranosyl*-(1→3)-[α-D-*mannopyranosyl*-(1→4)]-*methyl* (β-D-*galactopyranosid*)*uronate* (**19**).—Compound **17** (80 mg, 0.04 mmol) was deacetylated with 0.05 M NaOMe in dry MeOH at room temperature for 6 h. The soln was neutralized with Dowex 50W-X8 (H^+) resin, filtered and concentrated to give **18** as a methyl ester. A soln of the product **18**, in AcOH (3 mL), was hydrogenolysed over 10% Pd-C (100 mg) in a paar apparatus overnight. Column chromatography ($CHCl_3$–MeOH–H_2O; 10:5:1) gave **19** (34.5 mg; 70%); $[\alpha]_D^{25}$ +53.8° (*c* 0.7, H_2O). ^{1}H NMR: δ 5.04 (bs, 1 H, H-1^{IV}), 4.92 (bs, 1 H, H-1^{II}), 4.46 (d, 1 H, $J_{1,2}$ 11 Hz, H-1^{I}), 4.18 (d, $J_{1,2}$ 8.5 Hz, H-1^{III}), 3.78 (m, 2 H, OC*H*$_2$CH$_2$Si), 3.71 (s, 3 H, COOC*H*$_3$), 0.84 (m, 2 H, OCH$_2$-C*H*$_2$Si), −0.09 [s, 9 H, Si(C*H*$_3$)$_3$]. ^{13}C NMR: δ 178.6 (*C*OOCH$_3$), 101.3 (C-1^{I}), 100.6 (C-1^{III}), 97.5 (C-1^{II}), 95.1, 79.1, 75.7, 74.5, 73.5, 72.8, 72.2, 71.8, 71.3, 71.2, 69.3, 68.0, 67.7, 67.2, 66.8, 64.9, 63.1, 62.1, 61.7, 61.2 (C-1^{IV}), 54.1 (COO*C*H$_3$), 17.8 (OCH$_2$-*C*H$_2$Si), −1.3 [Si(*C*H$_3$)$_3$]. Anal. Calcd for $C_{30}H_{54}$-$O_{22}Si$: C, 45.56; H, 6.88. Found: C, 45.37; H, 7.03.

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