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Convergent synthesis of the tetrasaccharide repeating unit corresponding to the *O*-antigen of the verotoxin-producing *Escherichia coli* O176

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Abstract A convergent synthesis of the tetrasaccharide repeating unit of the *O*-antigen of the verotoxin producing *E. coli* O176 has been achieved in excellent yield adopting a [2+2] block glycosylation strategy. The β-D-mannosidic moiety of the tetrasaccharide was prepared from β-D-glucoside and α-D-galactosamine moiety was derived from D-galactal. The tetrasaccharide was synthesized as its 2-trimethylsilylethyl glycoside in excellent yield. All intermediate steps are high yielding.

Keywords *E. coli* · Oligosaccharide · Glycosylation · *O*-antigen · Diarrhea

Introduction

Escherichia coli (*E. coli*), a Gram-negative, facultative anaerobic pathogen can be predominantly found in the gastrointestinal tract of humans [1]. Although, most of the *E. coli* strains found in the gut flora are harmless, a certain species of *E. coli* acquired virulence factors and causes severe intestinal and urinary infections in humans and animals. Most frequently encountered *E. coli* infections are (a) diarrhea, (b) urinary tract infections and (c) meningitis [2]. Verocytotoxin producing *E. coli* strains (VTEC) are mostly responsible for several diarrheal outbreaks in the developed and developing countries [3]. The VTEC strains are also associated with the diarrhoeal diseases with life threatening complications *e.g.* haemorrhagic colitis and haemolytic uraemic syndrome and termed as enterohaemorrhagic *E. coli* (EHEC) [4]. The VTEC strains are mostly present in cattle and transmitted to humans by contaminated food and water. The best characterized VTEC serotype is *E. coli* O157:H7, which is responsible for fatal intestinal disorders of humans in Europe, America and Japan [5, 6]. Besides, *E. coli* O157:H7, several other VTEC strains have also been well characterized during last few years [7]. Recently, Widmalm *et al*. reported the tetrasaccharide structure of the repeating unit of the *O*-antigenic polysaccharide of the verotoxin-producing *E. coli* O176, which contains a β-D-mannose and a α-D-galactosamine moiety (Fig. 1) [8]. Cell wall *O*-antigenic polysaccharides are attractive targets for the development of carbohydrate based therapeutics, which is reflected in several reports in the recent past [9, 10]. For a detailed understanding of the role of *O*-antigenic polysaccharide of *E. coli* O176 in the pathogenicity, a large quantity of the tetrasaccharide is required for its use in several immunochemical experiments. Since the natural source can not provide the required amount of the tetrasaccharide, it is essential to develop a concise chemical synthetic strategy for its preparation. We report herein a convergent synthesis of the tetrasaccharide repeating unit of the *O*-antigenic polysaccharide of *E. coli* O176 as its 2-trimethylsilylethyl glycoside using block synthetic strategy (Fig. 2). 2-Trimethylsilylethyl group (SE) has been used as a temporary anomeric protecting group, which could be removed under acidic condition to furnish tetrasaccharide hemiacetal derivative [11].

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Results and discussion

The convergent synthesis of the target tetrasaccharide **1** as its 2-trimethylsilylethyl glycoside was achieved using a

→3)-α-D-GalpNAc-(1→4)-α-D-Manp-(1→2)-α-D-Manp-(1→2)-β-D-Manp-(1→

Fig. 1 Structure of the tetrasaccharide repeating unit of the *O*-antigen of the verotoxin-producing *Escherichia coli* O176

stereoselective [2+2] block glycosylation strategy. The tetrasaccharide contains synthetically challenging β-D-mannose and α-D-galactosamine moieties. The β-D-mannoside acceptor (**2**) was prepared from a D-glucose derivative using oxidation-reduction at the C-2 position. The D-galactosamine donor (**4**) for the construction of α-D-galactosamine moiety has been constructed from a D-galactal derivative. The key features of the synthetic strategy are: (a) stereoselective [2+2] block glycosylation; (b) application of perchloric acid supported over silica ($HClO_4$-SiO_2) as a solid acid catalyst in the glycosylation of trichloroacetimidate derivative [12] and thioglycoside [13] as well as in *N*- and *O*-acetylation [14]; (c) use of D-galactal as a precursor of α-D-galactosamine moiety (**4**); (d) activation of glycosyl trichloroacetimidate derivative (**4**) in the presence of thioglycoside (**5**) tuning the orthogonal property of anomeric thioethyl group of compound **5** [15]; (e) use of 2-trimethylsilylethyl group as temporary anomeric protecting group *etc*.

2-Trimethylsilylethyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**2**) [11], derived from D-glucose in seven steps was allowed to stereoselectively couple with ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**3**) [16] in the presence of a combination of *N*-iodosuccinimide (NIS) and $HClO_4$-SiO_2 [13] to furnish 2-trimethylsilylethyl (2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**6**) in 87% yield. Presence of signals in the NMR spectra of compound **6** confirmed its formation [δ 5.68 (s, PhC*H*), 5.61 (s, PhC*H*), 5.27 (br s, H-1_B), 4.53 (br s, H-1_A) in the ^{1}H NMR and δ 101.6 (Ph*C*H), 101.5 (Ph*C*H), 100.2 (C-1_A), 99.9 (C-1_B) in the ^{13}C NMR spectra]. Deacetylation of compound **6** using sodium methoxide in methanol furnished the disaccharide acceptor 2-trimethylsilylethyl (3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**7**) in quantitative yield. In another experiment, $HClO_4$-SiO_2 promoted stereoselective glycosylation [12] of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (**4**) [17] and thioglycoside acceptor, ethyl 2,3-di-*O*-acetyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**5**) [18] furnished ethyl (3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**8**) in 78% yield. Presence of signals in the NMR spectra of compound **8** confirmed its formation [δ 5.28 (br s, H-1_D), 5.24 (br s, H-1_C) in the ^{1}H NMR and δ 99.0 (C-1_D), 81.9 (C-1_C) in the ^{13}C NMR spectra]. Stereoselective glycosylation of disaccharide thioglycoside donor (**8**) with disaccharide acceptor (**7**) in the presence of a combination of NIS and $HClO_4$-SiO_2 [13] furnished 2-trimethylsilylethyl (3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-6-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**9**) in 77% yield. Spectral analysis of compound **9** unambiguously confirmed its stereoselective formation [δ 5.66 (s, PhC*H*), 5.57 (s, PHC*H*), 5.33 (br s, H-1_B), 5.31 (d, *J*=2.4 Hz, H-1_D), 5.10 (br s, H-1_C), 4.43 (br s, H-1_A) in the ^{1}H NMR and δ 101.4 (Ph*C*H), 101.3 (Ph*C*H), 100.5 (C-1_D), 100.2 (C-1_A), 99.2 (C-1_C), 98.5 (C-1_B) in the ^{13}C NMR spectra]. Tetrasaccharide derivative **9** was subjected to a sequence of reactions involving (a) hydrogenolysis over Pearlman's catalyst [19] for the reduction of azido group and removal of *O*-benzyl ether and 4,6-*O*-benzylidene acetal; (b) *N*- and *O*-acetylation using acetic anhydride and $HClO_4$-SiO_2 [14] and (c) removal of *O*-acetyl groups using sodium methoxide to give target tetrasaccharide **1** as its 2-trimethylsilylethyl glycoside in 63% over all yield. The structure of the tetrasaccharide **1** was unambiguously confirmed using spectral analysis [δ 5.32 (br s, H-1_B), 5.17 (d, *J*=3.6 Hz, H-1_D), 5.00 (br s, H-1_C), 4.52 (br s, H-1_A) in the ^{1}H NMR and δ 103.5 (C-1_C), 101.5 (C-1_B), 101.1 (2 C, C-1_A, C-1_D) in the ^{13}C NMR spectra] (Scheme 1). In order to establish the potential of 2-trimethylsilylethyl group as a temporary anomeric protecting group, compound **1** was subjected to a two step reaction sequence involving (a) per-*O*-acetylation using acetic

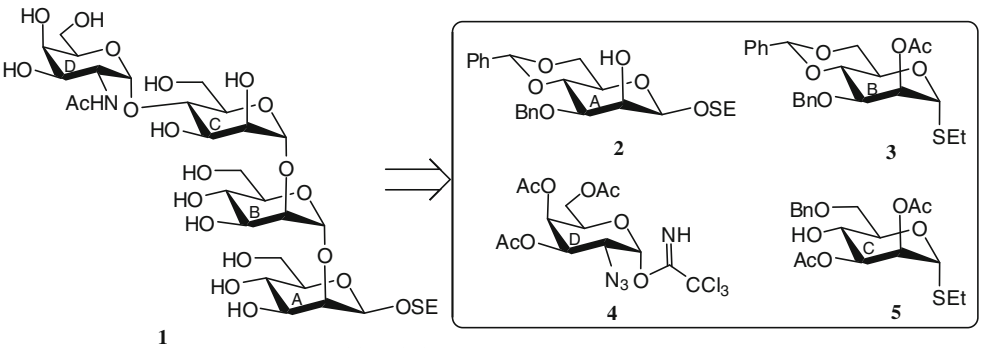
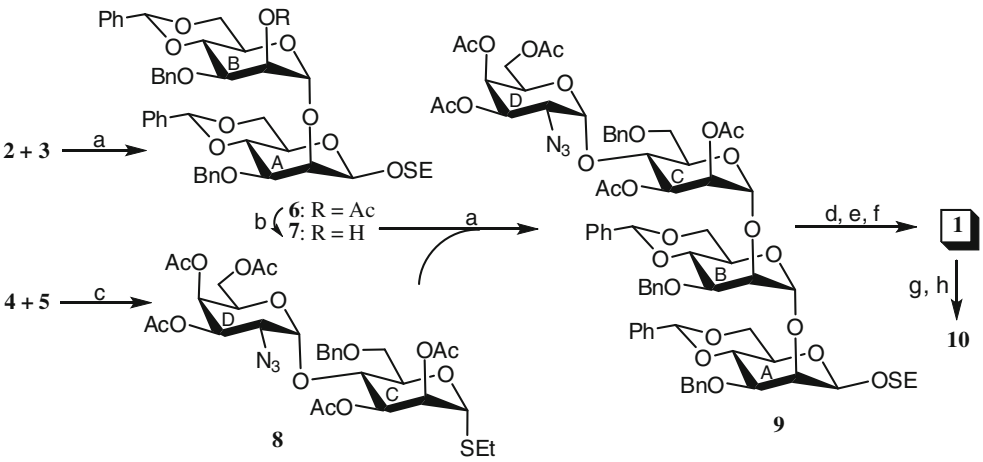


Fig. 2 Chemical structure of the synthesized tetrasaccharide as its 2-trimethylsilylethyl glycoside (**1**)

SE: 2-Trimethylsilylethyl



Scheme 1 Reagents: **a** *N*-Iodosuccinimide (NIS), $HClO_4$-SiO_2, CH_2Cl_2, MS 4Å, −30°C, 1 h, 87% for **6** and 77% for **9**; **b** CH_3ONa, CH_3OH, room temperature, 2 h, quantitative; **c** $HClO_4$-SiO_2, CH_2Cl_2, −10°C, 1 h, 78%; (d) H_2, 20% $Pd(OH)_2$-C, CH_3OH, room temperature, 24 h; **e** acetic anhydride, $HClO_4$-SiO_2, room temperature, 1 h; **f** CH_3ONa, CH_3OH, room temperature, 6 h, 63% in three steps; **g** acetic anhydride, $HClO_4$-SiO_2, room temperature, 3 h; **h** TFA, CH_2Cl_2, room temperature, 8 h, 58% in two steps

anhydride and $HClO_4$-SiO_2 and (b) treatment of the per-*O*-acetylated tetrasaccharide derivative with trifluoroacetic acid (TFA) to furnish per-*O*-acetylated tetrasaccharide hemiacetal derivative (**10**) in 58% yield, which was confirmed from its spectral analysis.

Experimental

General methods All reactions were monitored by thin layer chromatography over silica gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% $Ce(SO_4)_2$ in 1 M H_2SO_4)-sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR, DEPT 135, 2D NMR spectra were recorded on Brucker Avance DRX 400, 500 and 600 MHz spectrometers using $CDCl_3$ and CD_3OD as solvents and TMS as internal reference unless stated otherwise. Chemical shift values are expressed in δ ppm. ESI-MS were recorded on a Micromass Quttro mass spectrometer. Elementary analysis was carried out on Carlo Erba-1108 analyzer. Optical rotations were measured at 25°C on a Jasco P-2000 polarimeter. Commercially available grades of organic solvents of adequate purity are used in all reactions. Silica supported perchloric acid ($HClO_4$-SiO_2) was prepared following the earlier report [14].

2-Trimethylsilylethyl (2-O-acetyl-3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranosyl)-(1→2)-3-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (6) To a solution of compound **2** (1.2 g, 2.61 mmol) and compound **3** (1.3 g, 2.92 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4 Å (2.0 g) and reaction mixture was cooled to −30°C. To the cooled reaction mixture were added NIS (700.0 mg, 3.11 mmol) and $HClO_4$-SiO_2 (25.0 mg) and it was allowed to stir for 1 h at the same temperature. The reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% aq. $Na_2S_2O_3$, $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure compound **6** (1.9 g, 87%). White solid; m.p. 65°C; $[\alpha]_D^{25}$ −15.3 (*c* 1.1, $CHCl_3$); IR (KBr): 3465, 3091, 3065, 3034, 2953, 2927, 2871, 1751, 1606, 1497, 1455, 1373, 1312, 1284, 1233, 1142, 1096, 1047, 970, 860, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.54–7.25 (m, 20 H, Ar-*H*), 5.71–5.70 (m, 1 H, H-2_B), 5.68 (s, 1 H, PhC*H*), 5.61 (s, 1 H, PhC*H*), 5.27 (br s, 1 H, H-1_B), 4.92 (d, *J*=12.4 Hz, 1 H, PhCH_2), 4.76–4.67 (m, 3 H, PhCH_2), 4.64–4.56 (m, 1 H, H-5_B), 4.53 (br s, 1 H, H-1_A), 4.37–4.33 (dd, *J*=10.4, 4.8 Hz, 1 H, H-6_{aA}), 4.27–4.24 (dd, *J*=10.4, 4.6 Hz, 1 H, H-6_{aB}), 4.22–4.17 (m, 2 H, H-2_A, H-3_B), 4.15 (t, *J*=9.6 Hz each, 1 H, H-4_A), 4.04 (t, *J*=9.6 Hz each, 1 H, H-4_B), 4.00–3.95 (m, 1 H, OCH_2), 3.93 (t, *J*=10.0 Hz each 1 H, H-6_{bA}), 3.79 (t, *J*=10.0 Hz each, 1 H, H-6_{bB}), 3.67 (dd, *J*=10.0, 2.8 Hz, 1 H, H-3_A), 3.60–3.52 (m, 1 H, OCH_2), 3.40–3.33 (m, 1 H, H-5_A), 2.14 (s, 3 H, COCH_3), 1.20–1.05 (m, 2 H, CH_2), 0.01 (br s, 9 H, Si(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (*C*OCH$_3$), 138.4–126.1 (Ar-*C*), 101.6 (PhCH), 101.5 (PhCH), 100.2 (C-1_A), 99.9 (C-1_B), 79.2 (C-3_B), 78.5 (C-4_B), 77.9 (C-3_A), 74.5 (C-4_A), 74.4 (C-2_A), 73.1 (PhCH$_2$), 72.4 (PhCH$_2$), 69.5 (C-2_B), 68.7 (C-6_A), 68.6 (C-6_B), 67.7 (C-5_A), 67.3 (OCH$_2$), 63.4 (C-5_B), 21.1 (COCH$_3$), 18.3 (CH$_2$), −1.4 (Si(CH$_3$)$_3$); ESI-MS: 863.3 $[M+Na]^+$; Anal. Calcd. for $C_{47}H_{56}O_{12}Si$ (840.35): C, 67.12; H, 6.71%; found: C, 66.93; H, 6.95%.

2-Trimethylsilylethyl (3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranosyl)-(1→2)-3-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (7) A solution of compound **6** (1.8 g, 2.14 mmol) in 0.1 M CH_3ONa in CH_3OH (25 mL) was allowed to stir at room temperature for 2 h and neutralized with Dowex 50 W X8 (H^+) resin. The reaction mixture was filtered, concentrated and passed through a short pad of SiO_2 to give pure compound **7** (1.7 g, quantitative). Yellow

oil; $[\alpha]_D^{25}$ −4.3 (*c* 1.0, $CHCl_3$); IR (neat): 3470, 3089, 3064, 3033, 2953, 2928, 2336, 2109, 1955, 1887, 1727, 1497, 1378, 1215, 1098, 917, 837, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.56–7.29 (m, 20 H, Ar-*H*), 5.66 (s, 1 H, PhC*H*), 5.62 (s, 1 H, PhC*H*), 5.41 (br s, 1 H, H-1_B), 4.91–4.73 (m, 4 H, PhCH_2), 4.62–4.55 (m, 1 H, H-5_B), 4.54 (br s, 1 H, H-1_A), 4.38 (dd, *J*=10.4, 4.0 Hz, 1 H, H-6_{aA}), 4.30 (br s, 1 H, H-2_B), 4.29–4.25 (m, 1 H, H-6_{aB}), 4.24 (br s, 1 H, H-2_A), 4.15–4.08 (m, 3 H, H-3_B, H-4_A, H-4_B), 4.08–3.98 (m, 1 H, OCH_2), 3.92 (t, *J*=10.0 Hz each, 1 H, H-6_{bA}), 3.87 (t, *J*=10.0 Hz each, 1 H, H-6_{bB}), 3.70 (dd, *J*=10.0, 2.8 Hz, 1 H, H-3_A), 3.60–3.52 (m, 1 H, OCH_2), 3.40–3.32 (m, 1 H, H-5_A), 2.77 (br s, 1 H, O*H*), 1.26–1.07 (m, 2 H, CH_2), 0.04 (br s, 9 H, Si(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.4–126.1 (Ar-*C*), 101.6 (Ph*C*H), 101.5 (Ph*C*H), 100.9 (C-1_B), 100.4 (C-1_A), 79.1 (C-4_B), 79.0 (C-3_B), 78.0 (C-3_A), 75.9 (C-4_A), 74.1 (C-2_A), 73.1 (Ph*C*H_2), 73.0 (Ph*C*H_2), 69.9 (C-2_B), 68.8 (C-6_A), 68.7 (C-6_B), 67.7 (O*C*H_2), 67.3 (C-5_A), 62.9 (C-5_B), 18.3 (*C*H_2), −1.4 (Si(*C*H_3)$_3$); ESI-MS: 821.3 $[M+Na]^+$; Anal. Calcd. for $C_{45}H_{54}O_{11}Si$ (798.34): C, 67.65; H, 6.81%; found: C, 67.42; H, 7.00%.

Ethyl (3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-6-O-benzyl-1-thio-α-D-mannopyranoside (8) A solution of compound **4** (1.3 g, 2.72 mmol) and compound **5** (1.0 g, 2.51 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to −10°C under argon. To the cooled reaction mixture was added $HClO_4$-SiO_2 (50.0 mg) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite® bed and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to furnish pure compound **8** (1.4 g, 78%). Yellow oil; $[\alpha]_D^{25}$+17.8 (*c* 1.2, $CHCl_3$); IR (neat): 3481, 3031, 2873, 2113, 1752, 1498, 1373, 1237, 1130, 1045, 970, 850, 746, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.24 (m, 5 H, Ar-*H*), 5.32–5.31 (m, 2 H, H-2_C, H-3_C), 5.30 (br s, 1 H, H-4_D), 5.28 (br s, 1 H, H-4_D), 5.24 (br s, 1 H, H-1_C), 5.19 (dd, *J*=10.6, 3.4 Hz, 1 H, H-3_D), 4.65–4.59 (2 d, *J*=12.1 Hz, 2 H, PhCH_2), 4.32–4.29 (m, 1 H, H-5_C), 4.11 (t, *J*=9.6 Hz, 1 H, H-4_C), 4.04–4.01 (m, 1 H, H-5_D), 3.90–3.85 (m, 2 H, H-6_{abC}), 3.83 (dd, *J*=11.5, 5.2 Hz, 1 H, H-6_{aD}), 3.72 (dd, *J*=11.5, 1.8 Hz, 1 H, H-6_{bD}), 3.66 (dd, *J*=10.7, 3.7 Hz, 1 H, H-2_D), 2.68–2.58 (m, 2 H, SCH_2CH$_3$), 2.09, 2.01, 1.98, 1.97 (4 s, 15 H, 5 COCH_3), 1.25 (t, *J*=7.4 Hz each, 3 H, SCH$_2$CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 169.7, 169.5, 5 *C*OCH$_3$, 138.3–127.6 (Ar-*C*), 99.0 (C-1_D), 81.9 (C-1_C), 73.2 (Ph*C*H_2), 73.0 (C-4_C), 72.6 (C-4_D), 71.6 (C-2_C), 70.8 (C-5_C), 69.2 (C-6_D), 68.1 (C-3_D), 67.5 (C-3_C), 67.4 (C-5_D), 61.6 (C-6_C), 57.6 (C-2_D), 25.6 (S*C*H$_2$CH$_3$), 21.1, 21.0, 20.9, 20.8, 20.7 (5 CO*C*H$_3$), 14.8 (SCH$_2$*C*H$_3$); ESI-MS: 734.2 $[M+Na]^+$; Anal. Calcd. for $C_{31}H_{41}N_3O_{14}S$ (711.23): C, 52.31; H, 5.81%; found: C, 52.10; H, 6.05%.

2-Trimethylsilylethyl (3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2,3-di-O-acetyl-6-O-benzyl-α-D-mannopyranosyl)-(1→2)-(3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranosyl)-(1→2)-3-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (9) To a solution of compound **7** (1.0 g, 1.25 mmol) and compound **8** (980 mg, 1.37 mmol) in anhydrous CH_2Cl_2 (5 mL) was added MS 4Å (1.0 g) and reaction mixture was cooled to −30°C. To the cooled reaction mixture were added NIS (350.0 mg, 1.55 mmol) and $HClO_4$-SiO_2 (15.0 mg) and it was allowed to stir for 1 h at the same temperature. The reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% aq. $Na_2S_2O_3$, $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **9** (1.4 g, 77%). White solid; m.p. 83°C; $[\alpha]_D^{25}$=+ 42.3 (*c* 1.2, $CHCl_3$); IR (KBr): 3482, 3034, 2954, 2929, 1753, 1498, 1371, 1241, 1177, 1093, 972, 861, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.49–7.19 (m, 25 H, Ar-*H*), 5.66 (s, 1 H, PhC*H*), 5.57 (s, 1 H, PHC*H*), 5.48–5.46 (m, 2 H, H-2_C, H-3_C), 5.33 (br s, 1 H, H-1_B), 5.32 (br s, 1 H, H-4_D), 5.31 (d, *J*=2.4 Hz, 1 H, H-1_D), 5.20 (dd, *J*=10.6, 3.0 Hz, 1 H, H-3_D), 5.10 (br s, 1 H, H-1_C), 4.90 (d, *J*=12.0 Hz, 1 H, PhCH_2), 4.76–4.70 (m, 2 H, PHCH_2), 4.60 (d, *J*=12.4 Hz, 1 H, PhCH_2), 4.52 (d, *J*=12.4 Hz, 1 H, PhCH_2), 4.51–4.48 (m, 1 H, H-5_B), 4.45 (d, *J*=12.0 Hz, 1 H, PhCH_2), 4.43 (br s, 1 H, H-1_A), 4.33–4.29 (m, 1 H, H-6_{aA}), 4.24 (br s, 1 H, H-2_B), 4.23–4.20 (m, 1 H, H-6_{aB}), 4.18 (t, *J*=9.6 Hz each, 1 H, H-4_B), 4.14 (t, *J*=9.7 Hz each, 1 H, H-4_C), 4.12–4.10 (m, 1 H, H-3_B), 4.09 (br s, 1 H, H-2_A), 3.99 (t, *J*=9.6 Hz each, 1 H, H-4_A), 3.96–3.93 (m, 3 H, H-5_C, H-5_D, OCH_2), 3.91–3.84 (m, 3 H, H-6_{aC}, H-6_{bA}, H-6_{bB}), 3.80 (dd, *J*=11.5, 5.0 Hz, 1 H, H-6_{bC}), 3.69 (dd, *J*=10.8, 3.4 Hz, 1 H, H-2_D), 3.56 (dd, *J*=10.0, 2.8 Hz, 1 H, H-3_A), 3.54–3.49 (m, 3 H, H-6_{abD}, OCH_2), 3.31–3.28 (m, 1 H, H-5_A), 2.10, 2.05, 2.04, 2.01, 1.94 (5 s, 15 H, 5 COCH_3), 1.19–1.02 (m, 2 H, CH_2), 0.00 (br s, 9 H, Si(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 169.6, 169.5 (5 *C*OCH$_3$), 139.0–126.0 (Ar-*C*), 101.4 (Ph*C*H), 101.3 (Ph*C*H), 100.5 (C-1_D), 100.2 (C-1_A), 99.2 (C-1_C), 98.5 (C-1_B), 79.3 (C-4_B), 78.9 (C-4_A), 78.1 (C-3_A), 77.1 (C-2_B), 75.8 (C-3_B), 74.4 (C-2_A), 73.2 (Ph*C*H_2), 72.9 (Ph*C*H_2), 72.7 (Ph*C*H_2), 72.1 (C-3_C), 71.9 (C-4_C), 70.7 (C-5_D), 69.6 (C-2_C), 68.8 (C-6_A), 68.7 (C-6_B), 68.6 (C-6_D), 68.0 (C-3_D), 67.6 (O*C*H$_2$), 67.2 (C-5_A), 67.1 (C-4_D), 66.9 (C-5_C), 63.6 (C-5_B), 61.2 (C-6_C), 57.4 (C-2_D), 20.8, 20.7, 20.6, 20.5 (2 C) (5 CO*C*H$_3$), 18.2 (*C*H_2), −1.50 (Si(*C*H_3)$_3$); ESI-MS: 1470.5 $[M+Na]^+$; Anal. Calcd. for

$C_{74}H_{89}N_3O_{25}Si$ (1447.56): C, 61.36; H, 6.19%; found: C, 61.15; H, 6.43%.

2-Trimethylsilylethyl (2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→4)-(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-β-D-mannopyranoside (1) To a solution of compound **9** (1.0 g, 0.69 mmol) in CH_3OH-EtOAc (20 mL; 10:1 v/v) was added 20% $Pd(OH)_2$-C (200.0 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite® bed and evaporated to dryness. To a solution of the crude product in acetic anhydride (3 mL) was added $HClO_4$-SiO_2 (50.0 mg) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered, and solvents were removed under reduced pressure. A solution of the acetylated product in 0.1 M CH_3ONa in CH_3OH (25 mL) was allowed to stir at room temperature for 6 h and neutralized with Dowex 50 W X8 (H^+) resin. The reaction mixture was filtered, concentrated and passed through a column of Sephadex® LH-20 using CH_3OH-H_2O (80 mL; 4:1 v/v) as eluant to give pure compound **1** (350.0 mg, 63%). White powder; $[\alpha]_D^{25}$+30.4 (*c* 1.0, CH_3OH); IR (KBr): 3392, 2940, 2510, 2106, 1643, 1379, 1068, 1027, 792, 697 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.32 (br s, 1 H, H-1_B), 5.17 (d, *J*=3.6 Hz, 1 H, H-1_D), 5.00 (br s, 1 H, H-1_C), 4.52 (br s, 1 H, H-1_A), 4.25 (dd, *J*=10.8, 3.6 Hz, 1 H, H-2_D), 4.07–4.04 (m, 1 H, H-5_C), 4.02–4.01 (m, 1 H, H-2_B), 3.97–3.96 (m, 1 H, H-2_A), 3.95–3.92 (m, 3 H, H-2_C, H-5_B, OCH_2), 3.91–3.89 (m, 2 H, H-3_B, H-4_C), 3.87–3.85 (m, 2 H, H-3_D, H-6_{aA}), 3.84–3.79 (m, 2 H, H-4_A, H-6_{ac}), 3.78–3.68 (m, 6 H, H-4_B, H-5_D, H-6_{abD}, H-6_{bA}), 3.67–3.65 (m, 3 H, H-6_{bC}, H-6_{abB}), 3.56–3.50 (m, 3 H, H-3_A, H-3_C, OCH_2), 3.22–3.18 (m, 1 H, H-5_A), 1.99 (s, 3 H, COCH_3), 0.96–0.94 (m, 2 H, CH_2), 0.00 (m, 9 H, (Si(CH_3)$_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 174.8 (*C*OCH$_3$), 103.5 (C-1_C), 101.5 (C-1_B), 101.1 (2 C, C-1_A, C-1_D), 79.2 (C-2_B), 78.8 (C-5_A), 77.4 (C-2_A), 77.3 (C-4_A), 76.1 (C-3_C), 73.8 (C-5_C), 73.7 (C-5_D), 73.4 (C-3_B), 72.6 (C-5_B), 72.3 (2 C, C-2_C, C-4_C), 70.8 (C-4_D), 70.5 (C-3_D), 69.1 (C-3_A), 68.6 (C-4_B), 68.1 (O*C*H$_2$), 63.1 (C-6_A), 63.0 (C-6_D), 62.7 (C-6_B), 62.5 (C-6_C), 52.3 (C-2_D), 23.0 (CO*C*H$_3$), 19.3 (*C*H$_2$); ESI-MS: 830.3 $[M+Na]^+$; Anal. Calcd. for $C_{31}H_{57}NO_{21}Si$ (807.32): C, 46.09; H, 7.11%; found: C, 46.30; H, 7.37%.

(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-acetyl-α,β-D-mannopyranose (10) To a solution of compound **1** (100 mg, 0.12 mmol) in acetic anhydride (1.5 mL) was added $HClO_4$-SiO_2 (20 mg) and the reaction mixture was kept at room temperature for 3 h. The reaction mixture was filtered and solvents were removed and co-evaporated with toluene (3×10 mL) under reduced pressure. To a solution of the acetylated product in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (0.3 mL) and the reaction mixture was allowed to stir at room temperature for 8 h. The solvents were removed and co-evaporated with toluene (3×10 mL) under reduced pressure. The crude product was passed through a short pad of SiO_2 to give pure compound **10** (85 mg, 58%) as a mixture of α- and β-isomers (2:1). ^1H NMR ($CDCl_3$, 400 MHz): α/β-isomers: δ 5.43–5.40 (m, 3 H), 5.39–5.29 (m, 6 H), 5.27–5.16 (m, 3 H), 5.14–5.05 (m, 3 H), 4.94–4.92 (m, 1.5 H), 4.67–4.62 (m, 1.5 H), 4.51–4.45 (m, 2 H), 4.31–3.95 (m, 22 H), 2.14, 2.11, 2.10, 2.09, 2.07, 2.06, 2.04 (7 s, 39 H, 13 COC$H_{3\alpha}$), 2.02, 2.01, 2.00, 1.99, 1.97, 1.96, 1.94 (7 s, 19.5 H, 13 COC$H_{3\beta}$); ^{13}C NMR ($CDCl_3$, 100 MHz): α/β-isomer: δ 171.1–169.9 (CH$_3$*C*O), 99.3 (C-$1_{D\alpha}$), 99.1 (C-$1_{D\beta}$), 98.9 (C-$1_{B\beta}$), 98.8 (C-$1_{C\beta}$), 98.7 (2 C, C-$1_{B\alpha}$, C-$1_{C\alpha}$), 98.3 (C-$1_{A\beta}$), 93.1 (C-$1_{A\alpha}$), 77.4 (2 C, α), 76.2 (2 C, β), 76.1 (2 C, β), 73.4 (β), 72.3 (β), 71.7 (2 C, β), 71.6 (α), 71.5 (2 C, α,β), 70.4 (β), 70.3 (α), 70.2 (α), 69.6 (2 C, β), 69.5 (2 C, α), 69.3 (α), 68.7 (α), 68.3 (β), 67.9 (2 C, α), 67.8 (α), 67.7 (3 C, β), 67.5 (2 C, α), 67.4 (β), 66.9 (β), 66.8 (α), 63.8 (α), 63.5 (β), 62.8 (α), 62.7 (α), 62.5 (β), 62.0 (α), 20.9–20.7 (CO*C*H$_3$).

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