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Concise synthesis of the pentasaccharide *O*-antigen of *Escherichia coli* O83:K24:H31 present in the Colinfant vaccine

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Abstract A block synthetic approach is presented for the synthesis of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O83:K24:H31 strain, present in the "Colifant" vaccine. The target pentasaccharide has been synthesized by coupling a disaccharide with a trisaccharide in excellent yield. Yields are quite satisfactory in all intermediate steps.

Keywords Oligosaccharides · Glycosylation · Antigen · Vaccine · *E. coli*

Escherichia coli (*E. coli*) is a group of gram-negative bacteria that colonizes at infant's gastrointestinal tract within hours of life [1]. Although, *E. coli* is generally confined to the intestinal lumen, it causes infections in a debilitated or immunosuppressed host [2]. *E. coli* infections may be limited to the mucosal surfaces or can disseminate throughout the body. The three general clinical syndromes caused by the pathogenic *E. coli* strains are urinary tract infection, sepsis/meningitis, and enteric/diarrheal disease [3]. The *E. coli* O83:K24:H31 strain is used in the form of a vaccine under the name of "Colinfant" in Czech Republic [4]. This particular strain presents in the gut and stimulates the production of specific and non-specific antibodies both at the local level within the gut and in the blood circulation. The "Colinfant" vaccine has been found to be effective to reduce the requirement of the antibiotic treatment for several infections [5, 6]. In the light of increasing drug resistance to the bacterial infections, chemical synthesis of glycoconjugate vaccines has gained considerable interest to induce protective immune response for the precise diagnosis and protection. Furthermore, replacement of the attenuated live strains of bacteria present in several currently used vaccines by synthetic glycoconjugates can reduce the chance of self infections. Bacterial *O*-antigens are highly responsible for the antigenic action of the glyco-vaccines, which make them attractive targets for the development of synthetic glycoconjugate vaccines. In the recent past, a number of reports appeared in the literature for the synthesis and evaluation of glycoconjugate vaccines against bacterial infections [7–18]. The *O*-antigen of *E. coli* O83: K24: H31 consists of a repeating unit of an acidic pentasaccharide and its structure has been reported by Jann *et al*. (Fig. 1) [19]. It is essential to have a higher quantity of the pentasaccharide for the detailed study on its antigenic properties and other biochemical behaviors. However, oligosaccharides isolated from the natural sources can not meet the quantity required for their biological studies. Concise chemical syntheses always offer the access to the large quantities of oligosaccharides in their natural structure as well as several analogues. In order to induce a specific immune response in the host it is often required to conjugate the oligosaccharides with a carrier protein through a spacer arm. Under conventional reductive amination protocol conjugation of the oligosaccharides isolated from the natural sources with the protein generally destroys the cyclic structure of the monosaccharide of the reducing end. This can be avoided by pre-linking a hydroxyalkyl or aldehydoalkyl side chain at the anomeric position of the reducing end of the oligosaccharide using a precise chemical synthesis. Synthesis of an oligosaccharide

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→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-α-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→

Fig. 1 Pentasaccharide repeating unit of the *O*-antigen of *E. coli* O83:K24:H31

moiety with a temporary protecting group (*e.g.* 4-methoxyphenyl) at the reducing end is considered to be beneficial for its removal at the later stage to make a hydroxyalkyl glycoside for conjugation with a carrier protein. We describe herein a concise chemical synthesis of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O83:K24:H31 present in the "Colinfant" vaccine as its 4-methoxyphenyl glycoside using a block synthetic approach (Fig. 2).

The synthesis of the pentasaccharide **1** as its 4-methoxyphenyl glycoside was achieved using a block synthetic approach. For this purpose a disaccharide donor **8** and a trisaccharide acceptor **17** were prepared from the suitably protected monosaccharide derivatives (Fig. 3) derived from the commercially available reducing sugars. Glycosylation of disaccharide donor **8** with trisaccharide acceptor **17** furnished pentasaccharide derivative, which was transformed to the target pentasaccharide **1** applying a late stage oxidation under a phase transfer reaction condition followed by deprotections (Scheme 1, 2 and 3).

The preparation of disaccharide thioglycoside donor (**8**) is presented in Scheme 1. Glycosylation of ethyl 6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**) [20] with trichloroacetimidate donor (**6**) [21] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) [22] furnished disaccharide thioglycoside derivative **7**, which on conventional acetylation gave disaccharide thioglycoside donor (**8**) in over all 76% yield in two steps. Presence of signals at δ [5.44 (d, J=10.6 Hz, H-1_D) and 4.49 (d, J=8.0 Hz, H-1_E)] in the ^{1}H NMR and at δ [100.3 (C-1_E) and 80.9 (C-1_D)] in the ^{13}C NMR spectra supported its formation. Although, glycosylation was carried out using a diol acceptor (**5**) formation of only (1→4)β-linked disaccharide derivative 7 was observed, as confirmed from the NMR spectral analysis of the acetylated product **8** [δ 5.70 (dd, J=9.2 Hz, H-3_D)].

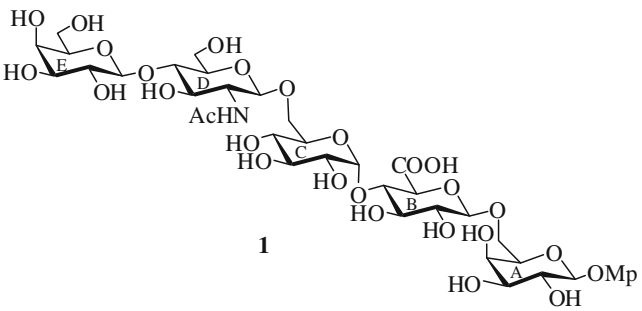

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Fig. 2 Chemical structure of the synthesized pentasaccharide repeating unit of *E. coli* O83:K24:H31 as it 4-methoxyphenyl glycoside

In another experiment, trisaccharide acceptor (**17**) was prepared following a set of reactions presented in Scheme 2. Glycosylation of 4-methoxyphenyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**2**) [23] with the thioglycoside donor **3** [24] in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) [25, 26] gave disaccharide derivative **9** in 91% yield. Formation of compound **9** was confirmed from its spectral data [δ 5.47 (s, PhC*H*), 4.90 (d, J=8.5 Hz, H-1_B), 4.69 (d, J=7.5 Hz, H-1_A) in ^{1}H NMR and at δ 103.7 (C-1_A), 101.9 (C-1_B), 101.4 (Ph*C*H) in ^{13}C NMR spectra]. Removal of benzylidene acetal from compound **9** using silica supported perchloric acid ($HClO_4$–SiO_2) [27, 28] afforded 4-methoxyphenyl (2,3-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**10**). Compound **10** was treated with *tert*-butyldiphenylchloro silane in pyridine [29] to furnish 4-methoxyphenyl (2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**11**). Unfortunately, NIS–TfOH catalyzed glycosylation of compound **11** with the thioglycoside donor **4** [30] went in vain and no trace of trisaccharide derivative was formed. We presumed that presence of *O*-TBDPS group and *O*-benzoyl group at the C-6 and C-3 position respectively make the C-4 position of the glycosyl acceptor sterically overcrowded. Following a different strategy, compound **9** was converted into disaccharide derivative **12** following a one-pot benzylation protocol [31]. Removal of the benzylidene acetal using $HClO_4$–SiO_2 furnished disaccharide diol **13**. However, repeated attempts for the selective silylation of compound **13** using *tert*-butyldiphenyl chlorosilane under literature reported reaction conditions did not produce any silylated derivative. After second strategic failure, compound **13** was selectively 6-*O*-benzoylated using benzoyl cyanide [32] to give disaccharide acceptor **14** in excellent yield. Iodonium ion catalyzed glycosylation of compound **14** with thioglycoside donor **4** in the presence of NIS–TfOH furnished exclusively trisaccharide derivative **15** in 82% yield without formation of its β-anomer. The exclusive stereo outcome can be explained by considering the presence of a non-participating benzyl group at the C-2 position of the thioglycoside donor **4**. Removal of the benzoyl group from compound **15** followed by protection with *tert*-butyldiphenylsilyl group afforded trisaccharide derivative **16**, which on removal of benzylidene acetal using $HClO_4$–SiO_2 furnished trisaccharide diol acceptor **17** (Scheme 2). Selective glycosylation of the trisaccharide acceptor **17** with disaccharide thioglycoside donor **8** in the presence of NIS–TfOH [24, 26] furnished pentasaccharide derivative **18** in 76% yield. Presence of signals at δ [102.9 (C-1_E), 102.8 (C-1_A),

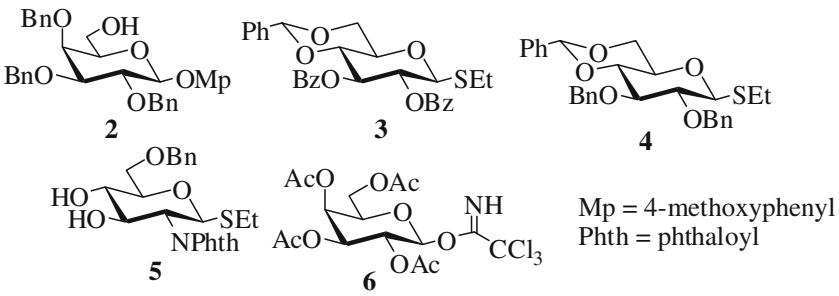


Fig. 3 Suitably functionalized monosaccharide intermediates used for the synthesis of the target pentasaccharide (**1**)

100.2 (C-1_B), 98.6 (C-1_D), 97.0 (C-1_C)] in the ^{13}C NMR spectra confirmed the formation of required pentasaccharide derivative **18**. Following a reaction sequence involving removal of phthalimido group under hydrazinolysis [33], *N*-acetylation, fluoride ion mediated desilylation [34] and TEMPO mediated oxidation of the primary hydroxyl group [35, 36], compound **18** was converted into pentasaccharide acid derivative **19**. Selective formation of (1→6) *β*-linkage in the pentasaccharide derivative **18** was reconfirmed from the spectral analysis of compound **19** [*e.g.* δ 4.90–4.80 (H-1_E, H-4_C and PhCH_2)]. It is worth noting that TEMPO mediated oxidation of the primary hydroxyl group to acid was achieved in excellent yield using a phase transfer reaction protocol [35, 36]. Complete deprotection of the pentasaccharide derivative **19** by hydrogenolysis followed by saponification furnished pure target pentasaccharide repeating unit (**1**) as its 4-methoxyphenyl glycoside, which was characterized from its 1D and 2D NMR and mass spectral analyses (Scheme 3). Signals at δ [5.36 (d, *J*= 3.0 Hz, H-1_C), 4.92 (d, *J*=7.3 Hz, H-1_A), 4.66 (d, *J*=7.6 Hz, H-1_E), 4.47 (d, *J*=7.8 Hz, H-1_B), 4.42 (d, *J*=8.7 Hz, H-1_D)] in the ^{1}H NMR and at δ [103.2 (C-1_B), 102.8 (C-1_D), 102.0 (C-1_A), 101.4 (C-1_E), 99.1 (C-1_C)] in the ^{13}C NMR spectrum confirmed the formation of target pentasaccharide **1** as its 4-methoxyphenyl glycoside. Assignments of proton and carbon signals of the intermediates and target pentasaccharide have been made by analyzing 1D and 2D NMR spectra of all compounds as well as stepwise correlation of the NMR signals of the intermediates.

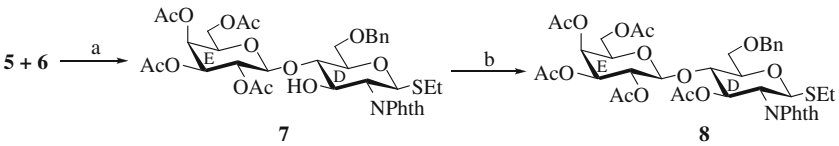
Conclusion

In summary, the synthesis of a pentasaccharide repeating unit of the *O*-antigen of *E. coli* O83:K24:H31 strain, present in the "Colinfant" vaccine as its 4-methoxyphenyl glycoside has been achieved in a concise manner following a block synthetic strategy. All glycosylation steps were high yielding and reproducible for scale-up preparation. A general iodonium ion mediated glycosylation condition has been applied in all glycosylation steps. Oxidation of a primary hydroxyl group in a pentasaccharide derivative was achieved using a two-step, one-pot phase transfer oxidation protocol. 4-Methoxybenzyl group at the reducing end can be removed under established reaction condition for making a hydroxyalkyl glycoside to be used for the conjugation of the pentasaccharide moiety with a carrier protein.

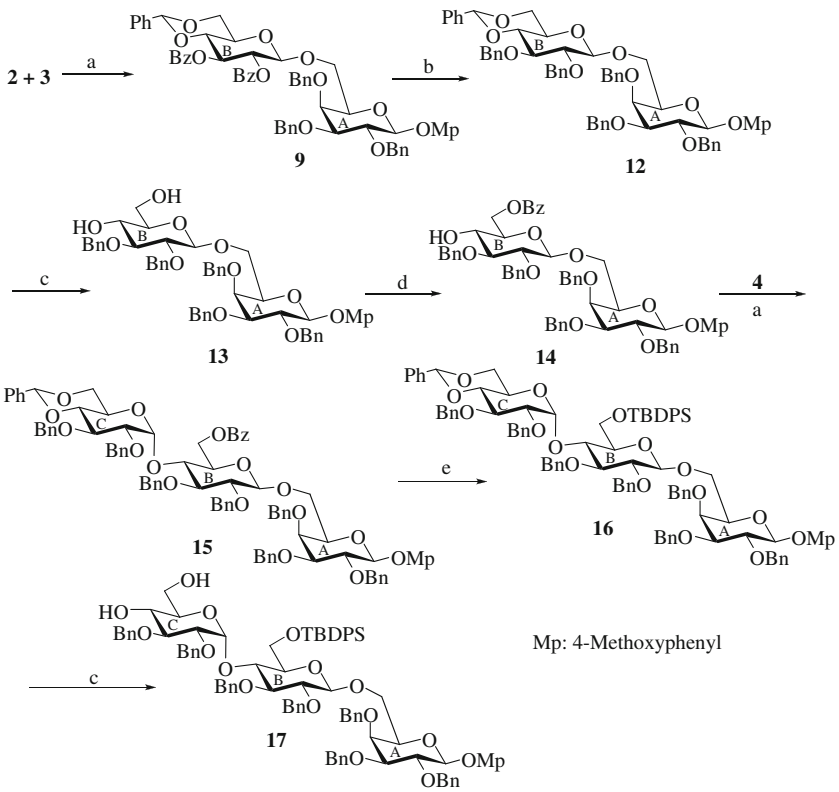
Experimental section

General methods All the reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% $Ce(SO_4)_2$ in 2N H_2SO_4) sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. ^{1}H and ^{13}C NMR, 2D COSY, HSQC spectra were recorded on Brucker Advance DPX 200 and 300 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. ESI-MS were recorded on a MICROMASS QUTTRO II triple quadrupole mass spectrometer. Elementary analysis was carried out on Carlo ERBA-1108 analyzer. Optical rotations were measured at 25°C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity are used in many reactions.

Ethyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (8) To a solution of compound **5** (3 g, 6.8 mmol) and compound **6** (6.7 g, 13.6 mmol) in dry



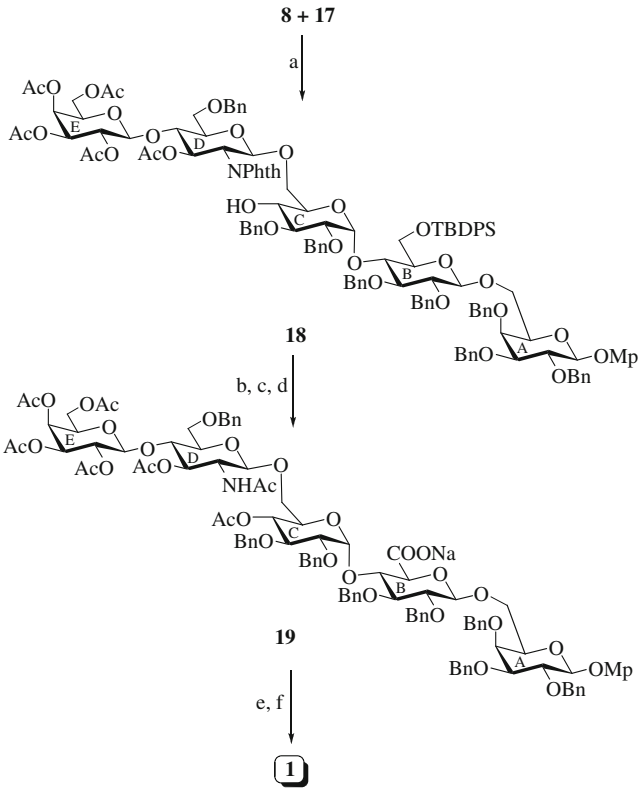
Scheme 1 Reagents: (**a**) TMSOTf, CH_2Cl_2, −10°C, 45 min; (**b**) acetic anhydride, pyridine, rt, 2 h, 76% in two steps



Scheme 2 Reagents: (**a**) *N*-Iodosuccinimide, TfOH, CH_2Cl_2, MS-4 Å, −30°C; 1 h, (91% for **9**, 82% for **15**); (**b**) benzyl bromide, NaOH, THF, rt, 5 h, 85%; (**c**) $HClO_4$-SiO_2, CH_3CN, rt, 20 min, 85% for **13**, 82% for **17**; (**d**) benzoyl cyanide, pyridine, 60°C, 10 h, 81%; (**e**) (1) CH_3ONa, CH_3OH, rt, 2 h; (2) TBDPS-Cl, pyridine-$(CH_2Cl)_2$, 80°C, 10 h, 84%

CH_2Cl_2 (30 ml) was added MS-4 Å (5 g) and the reaction mixture was cooled to −10°C. To the cold reaction mixture was added TMSOTf (200 μl) and it was allowed to stir at the same temperature for 45 min. The reaction mixture was diluted with CH_2Cl_2 (100 ml) and filtered through a Celite® bed. The organic layer was washed with satd. aq. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness to give crude compound **7**. To a solution of the crude product in pyridine (10 ml) was added acetic anhydride (10 ml) and the reaction mixture was kept at room temperature for 2 h. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane–EtOAc (3:1) as eluent to give pure **8** (4.2 g, 76%); White solid, m. p. 94–95°C; $[\alpha]_D^{25}$+17.8 (*c* 1.5, $CHCl_3$); IR (KBr): 3429, 2927, 2369, 1752, 1717, 1635, 1574, 1377, 1228, 1070, 906 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.86–7.71 (m, 4 H, Ar–H), 7.40–7.35 (m, 5 H, Ar–H), 5.70 (dd, *J*=9.2 Hz, 1 H, H-3_D), 5.44 (d, *J*=10.6 Hz, 1 H, H-1_D), 5.23 (d, *J*= 2.8 Hz, 1 H, H-4_E), 4.99 (dd, *J*=8.0 Hz, 1 H, H-2_E), 4.80 (dd, *J*=10.3, 3.4 Hz, 1 H, H-3_E), 4.78 (d, *J*=12.0 Hz, 1 H, PhCH_2), 4.51 (d, *J*=12.0 Hz, 1 H, PhCH_2), 4.49 (d, *J*= 8.0 Hz, 1 H, H-1_E), 4.28 (t, *J*=10.4 Hz, 1 H, H-2_D), 4.06–3.98 (m, 3 H, H-4_D and H-$6_{a,bD}$), 3.80–3.75 (m, 2 H, H-$6_{a,bE}$), 3.66–3.62 (m, 2 H, H-5_D and H-5_E), 2.69–2.63 (m, 2 H, SC$H_2$$CH_3$), 2.11, 2.06, 1.96, 1.95, 1.87 (5 s, 15 H, 5 COCH_3), 1.22 (t, *J*=7.4 Hz, 3 H, SCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0 (2 C), 169.8, 169.7, 168.6 (5 *C*OCH_3), 167.5, 167.2 (2 *C*OPhth), 137.7–123.5 (Ar–C), 100.3 (C-1_E), 80.9 (C-1_D), 78.8 (C-5_D), 75.3 (C-4_D), 73.6 (Ph*C*H_2), 71.8 (C-3_D), 70.9 (C-3_E), 70.4 (C-5_E), 69.1 (C-2_E), 67.5 (C-6_E), 66.7 (C-4_E), 60.7 (C-6_D), 53.9 (C-2_D), 24.0 (S*C*H_2CH_3), 20.7 (2 C), 20.6 (2 C), 20.5 (5 CO*C*H_3), 14.9 (SCH_2*C*H_3); ESI–MS: *m/z* 838.3 $[M+Na]^+$; Anal. Calcd. for $C_{39}H_{45}NO_{16}S$ (815.24): C, 57.42; H, 5.56; found: C, 57.25; H, 5.75.

4-Methoxyphenyl (2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (9) To a solution of compound **2** (3 g, 5.4 mmol) and compound **3** (3.4 g, 6.5 mmol) in dry CH_2Cl_2 (20 ml) was added MS-4 Å (3 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. *N*-Iodosuccinimide (NIS; 1.8 g, 8 mmol) was added to the reaction mixture and it was cooled to −30°C. To the cooled reaction mixture was added TfOH (100 μl) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 ml) and the organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) as eluent to furnish pure compound **9** (5 g, 91%); yellow oil; $[\alpha]_D^{25}$+5 (*c* 1.5,



Scheme 3 Reagents: (**a**) *N*-Iodosuccinimide, TfOH, CH_2Cl_2, MS-4 Å, −40°C; 25 min, 76%; (**b**) (1) $NH_2NH_2.H_2O$, C_2H_5OH, 80°C, 6 h; (2) acetic anhydride, pyridine, rt, 3 h; (**c**) TBAF, AcOH, THF, 60°C, 6 h; (**d**) TEMPO, NaBr, NaOCl, TBAB, $NaClO_2$, 2-methyl-but-2-ene, NaH_2PO_4, $NaHCO_3$, CH_2Cl_2, 0°C-rt, 5 h, 77%; (**e**) H_2, 20% $Pd(OH)_2$-C, CH_3OH, rt, 24 h; (**f**) CH_3ONa, CH_3OH, rt, 8 h, then few drops of H_2O, rt, 6 h, 75% in two steps

$CHCl_3$); IR (neat): 2869, 2366, 1731, 1506, 1452, 1272, 1098, 1069, 708 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.93–7.90 (m, 2 H, Ar–H), 7.82–7.79 (m, 2 H, Ar–H), 7.45–7.24 (m, 26 H, Ar–H), 7.0 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.88 (d, *J*=9.0 Hz, 2 H, Ar–H), 5.67 (t, *J*=9.6 Hz, 1 H, H-3_B, 5.47 (s, 1 H, PhC*H*), 5.35 (t, *J*=8.0 Hz, 1 H, H-2_B), 4.98 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.90 (d, *J*=8.5 Hz, 1 H, H-1_B), 4.89 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.77 (d, *J*= 11.8 Hz, 1 H, PhCH_2), 4.69 (d, *J*=7.5 Hz, 1 H, H-1_A), 4.67 (d, *J*=11.8 Hz, 1 H, PhCH_2), 4.63 (d, *J*=11.8 Hz, 1 H, PhCH_2), 4.55 (d, *J*=11.8 Hz, 1 H, PhCH_2), 4.32 (dd, *J*= 10.5, 10.5 Hz, 1 H, H-4_B), 3.97 (dd, *J*=7.7, 7.7 Hz, 1 H, H-2_A), 3.87–3.77 (m, 4 H, H-$6_{a,bA}$ and H-$6_{a,bB}$), 3.73 (s, 3 H, OCH_3), 3.72 (d, *J*=2.9 Hz, 1 H, H-4_A), 3.54–3.46 (m, 1 H, H-5_B), 3.43–3.39 (m, 1 H, H-5_A), 3.34 (dd, *J*=9.8, 2.7 Hz, 1 H, H-3_A); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.7, 165.4 (2 *C*OPh), 156.0–115.0 (Ar–C), 103.7 (C-1_A), 101.9 (C-1_B), 101.4 (PhCH), 82.3 (C-5_A), 79.4 (C-2_A), 79.3 (C-2_B), 76.2 (C-3_A), 75.9 (PhCH_2), 75.3 (PhCH_2), 74.8 (C-5_B), 73.8 (PhCH_2), 73.0 (C-3_B), 72.5 (C-4_A), 68.9 (C-6_A), 68.2 (C-6_B), 66.9 (C-4_B), 55.9 (OCH_3); ESI–MS: *m/z* 1037.4 $[M+Na]^+$; Anal. Calcd. for $C_{61}H_{58}O_{14}$ (1014.38): C, 72.18; H, 5.76; found: C, 72.0; H, 5.97.

*4-Methoxyphenyl (2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (12)*To a solution of compound **9** (4.5 g, 4.43 mmol) in anhydrous THF (50 ml) were added powdered NaOH (1.4 g, 35 mmol), benzyl bromide (2 ml, 16.84 mmol) and Bu_4NBr (200 mg) and the reaction mixture was stirred briskly at room temperature for 5 h. The solvents were removed under reduced pressure and the reaction product was diluted with CH_2Cl_2 (100 ml). The organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (7:1) as eluent to give pure **12** (3.7 g, 85%); white solid, m.p. 148–150°C; $[\alpha]_D^{25}$ −28 (*c* 1.5, $CHCl_3$); IR (KBr): 3745, 3617, 1742, 1696, 1649, 1517, 1071, 741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.17 (m, 30 H, Ar–H), 6.94 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.65 (d, *J*=9.0 Hz, 2 H, Ar–H), 5.48 (s, 1 H, PhC*H*), 4.99–4.92 (2 d, *J*=11.3 Hz, 2 H, PhCH_2), 4.87 (d, *J*=11.7 Hz, 1 H, PhCH_2), 4.83–4.69 (m, 3 H, PhCH_2), 4.70–4.60 (m, 3 H, PhCH_2), 4.64 (d, *J*=9.3 Hz, 1 H, H-1_A), 4.52 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.43 (d, *J*=7.5 Hz, 1 H, H-1_B), 4.24 (dd, *J*=10.5, 10.5 Hz, 1 H, H-4_B), 4.02 (dd, *J*=7.7, 7.7 Hz, 1 H, H-2_A), 3.81–3.74 (m, 3 H, H-4_A and H-$6_{a,bA}$), 3.64–3.61 (m, 1 H, H-2_B), 3.59 (s, 3 H, OCH_3), 3.57–3.54 (m, 2 H, H-$6_{a,bB}$), 3.52–3.44 (m, 2 H, H-3_A and H-5_B), 3.34–3.22 (m, 2 H, H-3_B and H-5_A); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.3–114.6 (Ar–C), 103.6 (C-1_B), 103.1 (C-1_A), 101.3 (PhCH), 82.4 (C-2_A), 82.0 (C-5_A), 81.7 (C-2_B), 81.0 (C-3_B), 79.2 (C-3_A), 75.3 (2 C, 2 PhCH$_2$), 75.0 (PhCH_2), 74.5 (PhCH_2), 74.4 (C-5_B), 74.1 (C-4_A), 73.3 (PhCH_2), 68.8 (C-6_A), 68.6 (C-6_B), 66.0 (C-4_B), 55.3 (OCH_3); ESI–MS: *m/z* 1009.5 $[M+Na]^+$; Anal. Calcd. for $C_{61}H_{62}O_{12}$ (986.42): C, 74.22; H, 6.33; found: C, 74.06; H, 6.52.

4-Methoxyphenyl (2,3-di-O-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (13) To a solution of compound **12** (3.5 g, 3.55 mmol) in CH_3CN (50 ml) was added $HClO_4$–SiO_2 (0.5 g) and the reaction mixture was allowed to stir at room temperature for 20 min. The reaction mixture was filtered and the filtrate was evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (2:1) to give pure compound **13** (2.7 g, 85%); white solid, m.p. 142–144°C; $[\alpha]_D^{25}$ −18 (*c* 1.5, $CHCl_3$); IR (KBr): 3427, 2927, 2364, 1596, 1506, 1455, 1352, 1226, 1161, 1058, 742, 696 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.35–7.17 (m, 25 H, Ar–H), 6.94 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.61 (d, *J*=9.0 Hz, 2 H, Ar–H), 4.99 (d, *J*=11.3 Hz, 1 H, PhCH_2), 4.91 (d, *J*=12.5 Hz, 1 H, PhCH_2), 4.86 (d, *J*=11.6 Hz, 1 H, PhCH_2), 4.83 (d, *J*= 11.4 Hz, 1 H, PhCH_2), 4.79 (d, *J*=12.0 Hz, 1 H, PhCH_2),

4.74 (d, J=9.5 Hz, 1 H, H-1_A), 4.72 (d, J=12.5 Hz, 1 H, PhCH_2), 4.66 (d, J=12.0 Hz, 1 H, PhCH_2), 4.62–4.56 (3 d, J=11.6 Hz, 3 H, PhCH_2), 4.37 (d, J=7.3 Hz, 1 H, H-1_B), 4.02 (dd, J=7.7 Hz, 1 H, H-2_A), 3.82–3.63 (m, 5 H, H-2_B, H-$6_{a,bA}$ and H-$6_{a,bB}$), 3.57 (s, 3 H, OCH_3), 3.53–3.44 (m, H-3_A, H-4_A and H-4_B), 3.32–3.22 (m, 2 H, H-3_B and H-5_A), 3.11–3.06 (m, 1 H, H-5_B); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.0–114.4 (Ar–H), 103.3 (C-1_A), 102.8 (C-1_B), 83.9 (C-5_A), 82.0 (C-3_B), 81.8 (C-3_A), 79.1 (C-2_A), 75.3 (PhCH$_2$), 75.2 (PhCH$_2$), 75.0 (C-5_B), 74.6 (C-4_A), 74.4 (C-2_B), 74.3 (PhCH$_2$), 73.8 (PhCH$_2$), 73.1 (PhCH$_2$), 70.2 (C-4_B), 68.4 (C-6_B), 62.1 (C-6_A), 55.3 (OCH$_3$); ESI–MS: m/z 921.4 $[M+Na]^+$; Anal. Calcd. for $C_{54}H_{58}O_{12}$ (898.39): C, 72.14; H, 6.50; found: C, 71.95; H, 6.72.

4-Methoxyphenyl (6-O-benzoyl-2,3-di-O-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (14) To a solution of compound **13** (2.6 g, 2.9 mmol) in pyridine (15 ml) was added benzoyl cyanide (400 µl, 3.5 mmol) and the reaction mixture was allowed to stir at 60°C for 10 h. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane–EtOAc (5:1) to give pure **14** (2.3 g, 81%); yellow oil; $[\alpha]_D^{25}$ −10 (*c* 1.5, $CHCl_3$); IR (KBr): 2917, 1721, 1597, 1503, 1451, 1276, 1221, 1066, 750 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05–8.02 (m, 2 H, Ar–H), 7.51–7.24 (m, 28 H, Ar–H), 7.0 (d, J=9.0 Hz, 2 H, Ar–H), 6.69 (d, J=9.0 Hz, 2 H, Ar–H), 5.01 (d, J=11.1 Hz, 1 H, PhCH_2), 4.97–4.84 (4 d, J=11.4 Hz, 4 H, PhCH_2), 4.81 (d, J=7.8 Hz, 1 H, H-1_B), 4.78–4.59 (4 d, J=11.6 Hz, 4 H, PhCH_2), 4.53 (d, J=11.7 Hz, 1 H, PhCH_2), 4.49 (d, J= 7.8 Hz, 1 H, H-1_A), 4.07 (dd, J=7.8 Hz, 1 H-2_A), 3.90–3.79 (m, 3 H, H-2_B, H-$6_{a,bB}$), 3.65 (s, 3 H, OCH_3), 3.57 (dd, J= 8.4, 1.4 Hz, 1 H, H-3_A), 3.55–3.51 (m, 2 H, H-4_A and H-4_B), 3.47–3.35 (m, 3 H, H-3_B and H-$6_{a,bA}$), 3.20–2.90 (m, 2 H, H-5_A and H-5_B); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.7 (*C*OPh), 155.1–114.5 (Ar–C), 103.6 (C-1_A), 102.8 (C-1_B), 83.7 (C-5_A), 81.9 (2 C, C-3_A and C-3_B), 79.2 (C-2_A), 75.5, 75.3, 74.8 (PhCH$_2$), 74.6 (C-5_B), 74.4 (PhCH$_2$), 73.8 (2 C, C-2_B and C-4_A), 73.2 (PhCH$_2$), 69.9 (C-4_B), 68.7 (C-6_B), 63.5 (C-6_A), 55.3 (OCH$_3$); ESI–MS: m/z 1025.6 $[M+Na]^+$; Anal. Calcd. for $C_{61}H_{62}O_{13}$ (1002.41): C, 73.04; H, 6.23; found: C, 72.85; H, 6.44.

4-Methoxyphenyl (2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→4)-(6-O-benzoyl-2,3-di-O-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (15) To a solution of compound **14** (2.2 g, 2.2 mmol) and thioglycoside donor **4** (1.3 g, 2.64 mmol) in anhydrous CH_2Cl_2 (20 ml) was added MS-4 Å (2 g) and the reaction mixture was allowed to stir at room temperature for 1 h. The reaction mixture was cooled to −30°C and NIS (700 mg, 3.1 mmol) and TfOH (40 µl) were added in succession. The reaction mixture was allowed to stir at same temperature for 1 h and diluted with CH_2Cl_2 (50 ml). The reaction mixture was filtered through a Celite® bed and the organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluent to furnish pure compound **15** (2.6 g, 82%); yellow oil; $[\alpha]_D^{25}$+9 (*c* 1.5, $CHCl_3$); IR (neat): 3779, 3405, 2922, 1591, 1354, 1066, 762 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.0 (m, 2 H, Ar–H), 7.44–7.11 (m, 43 H, Ar–H), 6.96 (d, J=9.0 Hz, 2 H, Ar–H), 6.63 (d, J=9.0 Hz, 2 H, Ar–H), 5.49 (s, 1 H, PhCH), 5.04–4.82 (m, 5 H, PhCH_2), 4.80–4.60 (8 H, H-1_B, H-1_C, PhCH_2), 4.58–4.40 (m, 4 H, H-1_A, PhCH_2), 4.13–3.95 (m, 3 H, H-4_C and H-$6_{a,bB}$), 3.88 (t, J=7.8, 7.8 Hz, 1 H, H-2_B), 3.80–3.62 (m, 6 H, H-3_C, H-4_A, H-$6_{a,bA}$ and H-$6_{a,bC}$), 3.59 (s, 3 H, OCH_3), 3.57–3.50 (m, 2 H, H-2_C and H-3_A), 3.48–3.33 (m, 4 H, H-2_A, H-3_B, H-5_A and H-5_C), 3.28–3.21 (m, 1 H, H-4_B), 2.76–2.68 (m, 1 H, H-5_B); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.8 (COPh), 153.7–114.4 (Ar–C), 103.8 (C-1_A), 103.2 (C-1_B), 103.1 (C-1_C), 102.5 (PhCH), 82.4 (2 C, C-3_C and C-5_C), 81.9 (2 C, C-3_A and C-5_A), 81.3 (C-2_A), 79.2 (C-3_B), 78.6 (C-2_C), 75.6 (C-4_C), 75.3 (2 C, PhCH$_2$), 75.0 (2 C, PhCH$_2$), 74.6 (C-5_B), 74.4 (PhCH$_2$), 73.9 (C-2_B), 73.2 (2 C, PhCH$_2$), 72.6 (C-4_A), 68.7 (C-4_B), 66.1 (C-6_B), 64.0 (C-6_A), 58.6 (C-6_C), 56.4 (OCH$_3$); ESI–MS: m/z 1455.6 $[M+Na]^+$; Anal. Calcd. for $C_{88}H_{88}O_{18}$ (1432.59): C, 73.72; H, 6.19; found: C, 73.55; H, 6.40.

4-Methoxyphenyl (2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→4)-(6-O-tert-butyl-diphenylsilyl-2,3-di-O-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (16) A solution of compound **15** (2.5 g, 1.74 mmol) in 0.1 M CH_3ONa (50 ml) was allowed to stir at room temperature for 2 h. The reaction mixture was neutralized with Amberlite-IR 120 (H^+), filtered and evaporated to dryness. To a solution of the dried mass in pyridine–$(CH_2Cl)_2$ (20 ml; 1:1 *v/v*) was added *tert*-butyldiphenyl chlorosilane (675 µl, 2.6 mmol) and the reaction mixture was allowed to stir at 80°C for 10 h. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane–EtOAc (7:1) as eluent to give pure **16** (2.3 g, 84%); yellow oil; $[\alpha]_D^{25}$+11 (*c* 1.5, $CHCl_3$); IR (neat): 2923, 2857, 1590, 1443, 1219, 1088, 749, 700 cm−1; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68–7.11 (m, 50 H, Ar–H), 6.97 (d, J=9.0 Hz, 2 H, Ar–H), 6.64 (d, J=9.0 Hz, 2 H, Ar–H), 5.49 (s, 1 H, PhCH), 5.0 (d, J=11 Hz, 1 H, PhCH_2), 4.92–4.84 (m, 5 H, H-1_C and PhCH_2), 4.82–4.77 (m, 3 H, PhCH_2), 4.76 (d, J=7.7 Hz, 1 H, H-1_B), 4.74–4.57 (m, 6 H, PhCH_2), 4.41 (d, J=7.4 Hz, 1 H, H-1_A), 4.30–4.22 (m, 1 H, H-6_{aC}), 4.20–4.11 (m, 2 H, H-4_C and H-6_{aA}), 4.03 (dd, J= 7.7, 7.7 Hz, 1 H, H-2_B), 3.85–3.70 (m, 4 H, H-4_A, H-6_{bA}

and H-$6_{a,bB}$), 3.61–3.60 (m, 1 H, H-3_A), 3.59 (s, 3 H, OCH_3), 3.58–3.54 (m, 2 H, H-3_B and H-6_{bC}), 3.51–3.43 (m, 3 H, H-3_C, H-5_A and H-5_C), 3.40–3.30 (m, 2 H, H-2_A and H-2_C), 3.27–3.18 (m, 1 H, H-4_B), 3.05–3.01 (m, 1 H, H-5_B), 1.02 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.08–114.5 (Ar–C), 103.2 (C-1_A), 102.8 (C-1_B), 102.5 (C-1_C), 101.1 (PhCH), 82.8 (C-5_A), 82.6 (C-5_C), 82.3 (C-3_C), 81.9 (2 C, C-3_A and C-3_B), 81.2 (C-2_C), 79.1 (C-2_A), 75.8 (C-4_C), 75.7 (PhCH_2), 75.5 (PhCH_2), 75.4 (C-5_B), 75.3 (PhCH_2), 75.0 (PhCH_2), 74.9 (PhCH_2), 74.7 (C-2_B), 74.3 (PhCH_2), 73.7 (C-4_A), 73.2 (PhCH_2), 68.8 (C-6_C), 67.8 (C-6_B), 65.9 (C-4_B), 61.4 (C-6_A), 55.3 (OCH_3), 26.9 (3 C, C(CH_3)$_3$), 19.5 (C(CH$_3$)$_3$); ESI–MS: *m/z* 1589.8 $[M+Na]^+$; Anal. Calcd. for $C_{97}H_{102}O_{17}Si$ (1566.68): C, 74.30; H, 6.56; found: C, 74.12; H, 6.75.

4-Methoxyphenyl (2,3-di-O-benzyl-α-D-glucopyranosyl)-(1→4)-(6-O-tert-butyl-diphenylsilyl-2,3-di-O-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (17) To a solution of compound **16** (2.2 g, 1.4 mmol) in CH_3CN (50 ml) was added $HClO_4$–SiO_2 (300 mg) and the reaction mixture was allowed to stir at room temperature for 20 min. The reaction mixture was filtered and the filtrate was evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (2:1) to give pure compound **17** (1.7 g, 82%); yellow oil; $[\alpha]_D^{25}$+33 (*c* 1.5, $CHCl_3$); IR (neat): 3779, 2924, 2361, 1593, 1218, 1071, 763 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70–7.16 (m, 45 H, Ar–H), 6.98 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.64 (d, *J*= 9.0 Hz, 2 H, Ar–H), 5.01 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.94–4.89 (m, 3 H, PhCH_2), 4.85 (d, *J*=3.4 Hz, 1 H, H-1_C), 4.82 (d, *J*=7.6 Hz, 1 H, H-1_B), 4.81–4.77 (m, 3 H, PhCH_2), 4.74–4.70 (m, 3 H, PhCH_2), 4.68–4.58 (m, 4 H, PhCH_2), 4.43 (d, *J*=7.6 Hz, 1 H, H-1_A), 4.12–4.07 (m, 2 H, H-4_C and H-6_{aA}), 4.04 (dd, *J*=7.7, 7.7 Hz, 1 H, H-2_B), 3.84–3.76 (m, 4 H, H-4_A, H-6_{bA} and H-$6_{a,bC}$), 3.65 (dd, *J*=12.0, 2.9 Hz, 1 H, H-6_{aB}), 3.59 (s, 3 H, OCH_3), 3.58–3.55 (m, 1 H, H-3_B), 3.51–3.40 (m, 4 H, H-3_A, H-3_C, H-5_C and H-6_{bB}), 3.37–3.28 (m, 3 H, H-2_A, H-2_C and H-5_A), 3.18–3.10 (m, 1 H, H-4_B), 3.08–3.05 (m, 1 H, H-5_B), 1.03 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.5–114.5 (Ar–C), 103.2 (C-1_A), 102.8 (C-1_B), 102.4 (C-1_C), 84.4 (C-5_A), 82.8 (C-3_C), 82.6 (C-3_B), 82.2 (C-3_A), 81.9 (C-2_C), 79.1 (C-2_A), 75.8 (C-5_C), 75.6 (PhCH_2), 75.4 (C-4_C), 75.3 (2 C, PhCH_2), 75.1 (C-5_B), 75.0 (PhCH_2), 74.9 (PhCH_2), 74.7 (C-2_B), 74.3 (PhCH_2), 73.8 (C-4_A), 73.2 (PhCH_2), 70.5 (C-4_B), 67.9 (C-6_C), 62.2 (C-6_B), 61.5 (C-6_A), 55.3 (OCH_3), 26.9 (3 C, C(CH_3)$_3$), 19.5 (C(CH$_3$)$_3$); ESI-MS: *m/z* 1502.7 $[M+Na]^+$; Anal. Calcd. for $C_{90}H_{98}O_{17}Si$ (1479.82): C, 73.05; H, 6.68; found: C, 72.86; H, 6.90.

4-Methoxyphenyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-(2,3-di-O-benzyl-α-D-glucopyranosyl)-(1→4)-(6-O-tert-butyl-diphenylsilyl-2,3-di-O-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (18) To a solution of the trisaccharide acceptor **17** (1.5 g, 1 mmol) and the disaccharide thioglycoside donor **8** (980 mg, 1.2 mmol) in CH_2Cl_2 (10 ml) was added MS-4 Å (1 g) and the reaction mixture was allowed to stir at room temperature for 1 h. The reaction mixture was cooled to −40°C and NIS (325 mg, 1.44 mmol) and TfOH (5 μl) were added in succession. The reaction mixture was allowed to stir at same temperature for 25 min and diluted with CH_2Cl_2 (50 ml). The reaction mixture was filtered through a Celite® bed and the organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluent to furnish pure compound **18** (1.7 g, 76%); yellow oil; $[\alpha]_D^{25}$+10 (*c* 1.5, $CHCl_3$); IR (neat): 2922, 1748, 1718, 1507, 1458, 1372, 1225, 1064, 745 cm−1; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.07 (m, 56 H, Ar–H), 6.66 (d, *J*=9.0 Hz, 2 H, Ar–H), 5.70 (t, *J*=8.5, 8.5 Hz, 1 H, H-3_D), 5.50 (d, *J*=3.2 Hz, 1 H, H-1_C), 5.40 (d, *J*=8.5 Hz, 1 H, H-1_D), 5,28 (br s, 1 H, H-4_E), 5.04–4.93 (m, 4 H, H-2_E, PhCH_2), 4.90–4.78 (m, 4 H, H-1_E, H-3_E and PhCH_2), 4.76–4.57 (m, 8 H, PhCH_2), 4.49 (d, *J*=7.6 Hz, 1 H, H-1_B), 4.47–4.38 (m, 4 H, H-1_A and PhCH_2), 4.28 (t, *J*=8.5 Hz, 1 H, H-2_D), 4.11–3.93 (m, 5 H, H-4_B, H-4_C, H-4_D, H-$6_{a,bE}$), 3.90–3.70 (m, 7 H, H-4_A, H-$6_{a,bA}$, H-$6_{a,bC}$, H-$6_{a,bD}$), 3.66–3.57 (m, 6 H, H-3_C, H-5_C, H-5_D, H-5_E and H-$6_{a,bB}$), 3.54 (s, 3 H, OCH_3), 3.50–3.20 (m, 5 H, H-2_B, H-3_B, H-3_A, H-5_A and H-5_B), 3.18–3.10 (m, 2 H, H-2_A and H-2_C), 2.11, 2.04, 1.96, 1.88 (4 s, 15 H, 5 COCH_3), 1.04 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 169.9 (2 C), 169.8, 168.7 (5 *C*OCH$_3$), 155.6–114.3 (Ar–C), 102.9 (C-1_E), 102.8 (C-1_A), 100.2 (C-1_B), 98.6 (C-1_D), 97.0 (C-1_C), 84.5 (C-5_C), 82.3 (C-5_A), 81.8 (2 C, C-2_A and C-2_C), 80.8 (C-5_B), 78.9 (2 C, C-4_C and C-4_D), 75.2 (PhCH_2), 75.1 (PhCH_2), 74.9 (C-4_A), 74.3 (C-4_B), 73.2 (2 C, PhCH_2), 73.9 (C-3_C), 73.8 (C-2_E), 73.6 (C-3_B and C-5_D), 73.3 (2 C, C-3_A and PhCH_2), 72.9 (PhCH_2), 72.8 (PhCH_2), 72.8 (2 C, C-2_B and C-5_E), 70.8 (PhCH_2), 70.7 (C-3_D), 70.6 (C-3_E), 70.3 (C-6_B), 69.2 (C-6_A), 68.9 (C-6_D), 67.0 (C-6_E), 66.6 (C-4_E), 60.8 (C-6_C), 55.2 (OCH_3), 54.3 (C-2_D), 26.6 (3 C, C(CH_3)$_3$), 20.6 (3 C), 20.5 (2 C) (5 COCH_3), 19.2 (C(CH$_3$)$_3$); ESI-MS: *m/z* 2250.0 $[M+NH_4]^+$; Anal. Calcd. for $C_{127}H_{137}NO_{33}Si$ (2231.88): C, 68.29; H, 6.18; found: 68.10; H, 6.40.

4-Methoxyphenyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-(4-O-acetyl-2,3-di-O-benzyl-α-D-glucopyranosyl)-(1→4)-(sodium 2,3-di-O-benzyl-β-D-glucopyranosyluronate)-(1→6)-2,3,4-tri-O-benzyl-β-D-gal-

actopyranoside (19) To a solution of compound **18** (1.6 g, 0.72 mmol) in EtOH (20 ml) was added hydrazine monohydrate (200 μl) and the reaction mixture was allowed to stir at 80°C for 6 h. The solvents were removed under reduced pressure and a solution of the crude product in acetic anhydride-pyridine (10 ml; 1:1 *v/v*) was allowed to stir at room temperature for 3 h. The solvents were removed under reduced pressure and the crude product was passed through a short pad of SiO_2. To a solution of the *N*-acetylated product in AcOH (5 ml) was added Bu_4NF in THF (10 ml) and the reaction mixture was stirred at 60°C for 6 h. The solvents were removed and the crude mass was dissolved in CH_2Cl_2 (50 ml). The organic layer was washed with satd. $NaHCO_3$ and water, dried and concentrated. To a solution of the crude product in CH_2Cl_2 (20 ml) and H_2O (3.5 ml) were added aq. solution of NaBr (1 ml; 1 M), aq. solution of TBAB (2 ml; 1 M), TEMPO (80 mg, 0.5 mmol), satd. aq. solution of $NaHCO_3$ (8 ml) and 4% aq. NaOCl (10 ml) in succession and the reaction mixture was allowed to stir at 0–5°C for 2 h. The reaction mixture was neutralized with the addition of 1 N aq. HCl solution. To the reaction mixture were added *tert*-butanol (25 ml), 2-methyl-but-2-ene (30 ml; 2 M solution in THF), aq. solution of $NaClO_2$ (1 g in 5 ml) and aq. solution of NaH_2PO_4 (1 g in 5 ml) and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was diluted with satd. aq. NaH_2PO_4 and extracted with CH_2Cl_2 (3×50 ml). The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (1:2) to give pure compound **19** (1.1 g, 77%); pale yellow solid; m.p. 83–85°C; $[\alpha]_D^{25}$ −11 (*c* 1.0, $CHCl_3$); IR (KBr): 3057, 2990, 2935, 2829, 1695, 1602, 1508, 1466, 1369, 1341, 1191, 1170, 1125, 1099, 1055, 992, 857, 823, 746 cm−1; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75–7.07 (m, 40 H, Ar–H), 6.98 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.62 (d, *J*=9.0 Hz, 2 H, Ar–H), 5.69 (t, *J*=9.5 Hz, 1 H, H-3_D), 5.53 (d, *J*=9.5 Hz, 1 H, H-1_D), 5.50 (d, *J*=3.2 Hz, 1 H, H-1_C), 5.26 (d, *J*=2.7 Hz, 1 H, H-4_E), 5.05–4.92 (m, 3 H, H-2_E and PhC*H*$_2$), 4.90–4.80 (m, 4 H, H-1_E, H-4_C and PhC*H*$_2$), 4.79–4.60 (m, 7 H, H-3_E and PhC*H*$_2$), 4.57–4.40 (m, 6 H, H-1_A and PhC*H*$_2$), 4.30 (d, *J*=7.7 Hz, 1 H, H-1_B), 4.25 (t, $_J$=9.5 Hz, 1 H, H-2_D), 4.18–4.10 (m, 6 H, H-3_B, H-4_B, H-4_D, H-$6_{a,bE}$ and PhC*H*$_2$), 3.94 (t, *J*=7.8 Hz, 1 H, H-2_B), 3.88–3.75 (m, 6 H, H-3_A, H-4_A, H-5_D, H-5_E and H-$6_{a,bA}$), 3.74–3.57 (m, 6 H, H-3_C, H-4_C, H-5_B, H-$6_{a,bC}$ and H-$6_{a,bD}$), 3.57 (s, 3 H, OC*H*$_3$), 3.54–3.44 (m, 2 H, H-2_A and H-5_C), 3.30–3.19 (m, 2 H, H-2_C and H-5_A), 2.10, 2.08, 1.95, 1.90, 1.85, 1.57 (6 s, 21 H, 6 COC*H*$_3$ and NHCOC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (2 C), 170.2 (2 C), 170.0 (3 C), 168.9 (7 *C*OCH$_3$ and *C*OONa), 155.2–114.3 (Ar–C), 103.3 (C-1_A), 102.7 (C-1_E), 100.2 (C-1_B), 98.2 (C-1_D), 96.6 (C-1_C), 83.7 (C-5_D), 81.9 (C-5_A), 81.5 (C-2_A), 80.5 (C-2_C), 79.1 (2 C, C-4_D and C-5_C), 78.4 (C-3_B), 77.2 (C-3_C), 75.3 (2 C, PhC*H*$_2$), 74.7 (2 C, PhC*H*$_2$), 74.5 (PhC*H*$_2$), 74.3 (2 C, C-4_C and C-5_E), 74.2 (2 C, C-3_A and C-5_B), 73.7 (C-4_B), 73.6 (PhC*H*$_2$), 73.2 (PhC*H*$_2$), 72.9 (PhC*H*$_2$), 72.5 (C-4_B), 71.0 (C-4_A), 70.9 (C-3_D), 70.8 (C-2_B), 70.4 (C-3_E), 69.8 (C-6_C), 69.5 (C-6_A), 66.9 (C-6_D), 66.7 (C-4_E), 60.8 (C-6_E), 55.4 (OC*H*$_3$), 54.5 (C-2_D), 20.6 (3 C), 20.5 (4 C) (6 COC*H*$_3$ and NHCOC*H*$_3$); ESI-MS: *m/z* 1984.0 $[M]^+$; Anal. Calcd. for $C_{107}H_{118}NNaO_{34}$ (1983.74): C, 64.74; H, 5.99; found: C, 64.55; H, 6.22.

4-Methoxyphenyl (β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-(α-D-glucopyranosyl)-(1→4)-(sodium β-D-glucopyranosyluronate)-(1→6)-β-D-galactopyranoside (1) To a solution of compound **19** (1 g, 0.5 mmol) in methanol (20 ml) was added 20% Pd $(OH)_2$/C (300 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. A solution of the crude mass in 0.1 M CH_3ONa in methanol (20 ml) was allowed to stir at room temperature for 8 h, then few drops of water were added to the reaction mixture and it was allowed to stir for another 6 h at room temperature. The reaction mixture was neutralized with Dowex 50 W-X8 (H^+), filtered and evaporated to dryness. The solution of the crude mass in methanol was passed through a column of Dowex 50 W-X8 (Na^+) and evaporated to give pentasaccharide **1** (380 mg, 75%) as a white powder, which was further purified by passing through a column of Sephadex-LH-20 using CH_3OH–H_2O (4:1) as eluant. $[\alpha]_D^{25}$−17 (*c* 1.0, H_2O); IR (KBr): 3427, 2927, 1597, 1353, 1129, 1073, 635 cm^{-1}; ^{1}H NMR (D_2O, 300 MHz): δ 7.08 (d, *J*=8.8 Hz, 2 H, Ar–H), 6.95 (d, *J*=8.8 Hz, 2 H, Ar–H), 5.36 (d, *J*=3.0 Hz, 1 H, H-1_C), 4.92 (d, *J*=7.3 Hz, 1 H, H-1_A), 4.66 (d, *J*=7.6 Hz, 1 H, H-1_E), 4.47 (d, *J*=7.8 Hz, 1 H, H-1_B), 4.42 (d, *J*=8.7 Hz, 1 H, H-1_D), 4.05–3.92 (m, 5 H, H-2_E, H-3_A, H-3_E, H-4_A and H-4_E), 3.90–3.80 (m, 5 H, H-4_B, H-$6_{a,bA}$, H-$6_{a,bD}$), 3.77 (s, 3 H, OC*H*$_3$), 3.75–3.55 (m, 13 H, H-2_A, H-3_B, H-3_D, H-4_D, H-5_A, H-5_B, H-5C, H-5_D, H-5_E, H-$6_{a,bC}$ and H-$6_{a,bE}$), 3.54–3.40 (m, 3 H, H-2_B, H-2_C and H-3_C), 3.35–3.22 (m, 2 H, H-2_D and H-4_C), 1.86 (s, 3 H, NHCOC*H*$_3$); ^{13}C NMR (75 MHz, D_2O): δ 174.2 (2 C, COONa and NH*C*OCH$_3$), 155.2–115.6 (Ar–C), 103.2 (C-1_B), 102.8 (C-1_D), 102.0 (C-1_A), 101.4 (C-1_E), 99.1 (C-1_C), 78.2 (C-5_B), 77.4 (C-4_D), 76.7 (C-3_D), 75.9 (C-3_C), 75.3 (C-4_B), 74.6 (C-2_E), 73.5 (C-4_C), 73.2 (C-2_C), 73.1 (2 C, C-5_C and C-5_D), 72.9 (C-5_E), 72.1 (C-2_A), 71.5 (C-2_B), 71.3 (C-3_B), 70.7 (C-5_A), 69.2 (4 C, C-3_A, C-3_E, C-4_A and C-4_E), 67.8 (C-6_A), 61.6 (2 C, C-6_C and C-6_E), 60.6 (C-6_D), 56.6 (C-2_D), 56.4 (OC*H*$_3$), 22.6 (NHCOC*H*$_3$); ESI-MS: *m/z* 1011.2 $[M]^+$; Anal. Calcd. for $C_{39}H_{58}NNaO_{28}$ (1011.30): C, 46.29; H, 5.78; found: C, 46.10; H, 6.05.

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