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Convergent synthesis of the pentasaccharide repeating unit of the *O*-antigenic polysaccharide of enterohaemorrhagic *Escherichia coli* O113

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Abstract An acidic pentasaccharide repeating unit corresponding to the O-antigenic polysaccharide of enterohaemorrhagic *Escherichia coli* O113 as its *p*-methoxyphenyl glycoside has been synthesized in a convergent manner by adopting a [3+2] block glycosylation strategy. During the synthetic endeavor a one-pot reaction condition for stereoselective glycosylation and protecting group manipulation has been applied. All glycosylation steps are highly stereoselective with good to excellent yield.

Keywords Enterohaemorrhagic · *Escherichia coli* O113 · Pentasaccharide · O-antigen · Glycosylation

Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) are one of the major causative agents of diarrhoea with life-threatening complications *e.g.* haemorrhagic colitis and haemorrhagic uremic syndrome [1, 2]. EHEC acquire their virulent action due to the release of vero-toxin or Shiga-toxin and hence are also called Verotoxigenic *E. coli* (VTEC) [3]. The action of the Shiga-toxin released by the EHEC on the endothelial cells of the host leads to the pathological lesions associated with haemorrhagic colitis and haemorrhagic uremic syndrome [4]. The young and elderly people are susceptible for the EHEC infections and suffer from diarrhoea, and haemorrhagic uremic syndrome. The most cited EHEC strain is O157:H7, which is associated with several diarrhoeal outbreaks in the developed countries [5, 6]. Besides this, several EHEC strains have been identified for their pathogenic potential to cause severe diarrhoral infections, which include *E. coli* O4, O5, O16, O26, O46, O48, O55, O91, O98, O111ab, O113, O117, O118, O119, O125, O126, O128, O145 *etc.* [7]. It has been established that the bacterial virulence comes up from the *O*-specific polysaccharides (*O*-antigens) present in their cell membrane. As a consequence, a large number of reports appeared on the structural characterization of the *O*-antigens of various pathogenic bacteria as well as their application in the development of glycoconjugate based therapeutics [8–10]. The structure of the pentasaccharide repeating unit of the *O*-specific lipopolysaccharide of *E. coli* O113 has been established by Parolis *et al.* (Fig. 1) [11]. It is an acidic oligosaccharide having a D-galacturonic acid moiety with three 1,2-*cis* glycosyl linkages present in it.

Cell wall *O*-antigens are considered as important class of molecules for the development of glycoconjugate based therapeutics. It would be pertinent to carry out several biological experiments using a pure pentasaccharide fragment to establish its pathological implications in the diarrhoeal infections. Development of a chemical synthetic strategy for the preparation of pure pentasaccharide would always be welcome to avoid the troublesome isolation and purification of the pentasaccharide from the natural source. In this context, most of the currently available vaccines contain attenuated live strains of bacteria or isolated polysaccharides, there could be a chance of self infection or contaminations respectively. Replacement of the live strains or natural polysaccharides with synthetic oligosaccharides or glycoconjugates could reduce the abovementioned shortcomings as well as could improve the access to the vaccines

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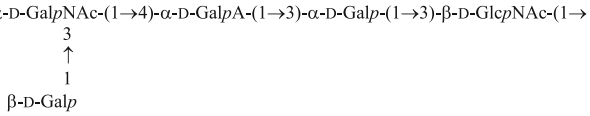


Fig. 1 Structure of the repeating unit of the *O*-antigenic lipopolysaccharise of *E. coli* O113

with higher potency. As a part of our ongoing program for the synthesis of complex oligosaccharides from microbial origin, an efficient chemical synthesis of the pentasaccharide corresponding to the *O*-antigen of the enterohaemorrhagic *E. coli* O113 is presented (Fig. 2). *p*-Methoxyphenyl group could act as a temporary anomeric protecting group and can be removed as and when necessary.

Results and discussion

The synthesis of the pentasaccharide **1** as its *p*-methoxyphenyl glycoside was carried out using a convergent [3+2] block glycosylation strategy. A disaccharide glycosyl acceptor (**7**) was stereoselectively condensed with a trisaccharide glycosyl donor (12). The disaccharide derivative (**7**) and the trisaccharide derivative (**12**) were prepared from suitably protected monosaccharide intermediates **2** [12], **3** [13], **4** [14], **5** [15] and **6** [16] prepared from the commercially available reducing sugars using reported literature (Fig. 2). The presence of α-D-galactosamine, α-D-galacturonic acid and α-D-galactose moieties make the synthesis of the target molecule challenging. A number of noteworthy features in the present synthetic strategy, such as: (a) application of *p*-methoxybenzyl ether as a temporary protecting group and its removal by tuning the glycosylation reaction condition in one-pot increases overall yield by reducing the number of steps [17]; (b) stereoselective [3+2] block glycosylation reduces the number of steps and improves overall yield; (c) application of perchloric acid supported over silica ($HClO_4$-SiO_2) as a heterogeneous acid catalyst in the glycosylation of trichloroacetimidate derivative and thioglycoside; benzylidene acetal formation and direct conversion of benzylidene acetal to *O*-acetylated derivative allowed to avoid the use of corrosive, moisture sensitive triflic acid or other protic acids and harsh reaction conditions; (d) the D-galactosamine moiety has been derived from tri-*O*-acetyl-D-galactal using azido-nitration technique reported earlier [18]; (e) preparation of the α-D-galacturonic acid moiety at the late stage of the synthetic strategy using a TEMPO mediated selective oxidation protocol under phase transfer reaction condition.

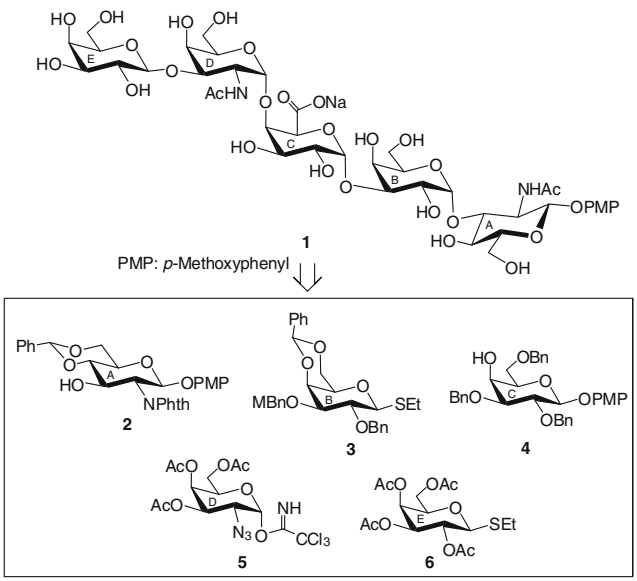
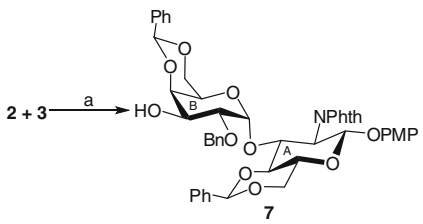


Fig. 2 Structure of the synthesized pentasaccharide (1) and its synthetic precursors

p-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (**2**) was allowed to couple with ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**3**) in the presence of a combination of *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TfOH) [17, 19, 20]. Controlling the reaction condition initially at low temperature and then at high temperature furnished the disaccharide derivative (7) in a 72 % yield, in which stereoselective glycosylation and removal of *p*-methoxybenzyl group was achieved in one pot. A minor quantity (~8 %) of 1,2-*trans* isomer of compound 7 was also formed, which was separated by column chromatography. It is worth mentioning that after stereoselective glycosylation at low temperature, the elevation of temperature resulted in the clean removal of *p*-methoxybenzyl group by TfOH present in the reaction medium. Appearance of signals in the NMR spectra confirmed the formation of compound **7** [signals at δ 5.70 (d, J=8.5 Hz, H-1_A), 5.48 (d, J=3.5 Hz, H-1_B) in ^{1}H NMR and δ 98.1 (C-1_B), 97.8 (C-1_A) in the ^{13}C NMR spectra] (Scheme 1).

In another set of experiments, stereoselective glycosylation of *p*-methoxyphenyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**) with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (**5**) in the presence



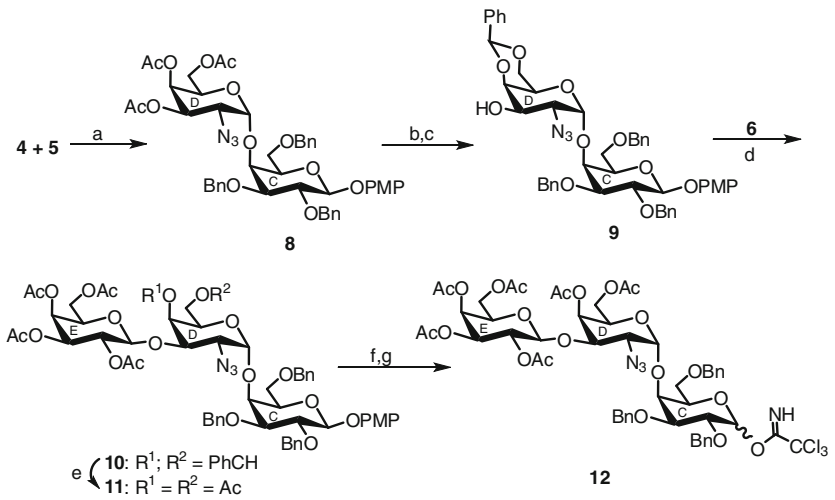
Scheme 1 Reagents: (a) NIS, TfOH, CH_2Cl_2, MS 4 Å, −30 °C for 45 min, then 0 °C for 30 min, 72 %

of perchloric acid supported over silica ($HClO_4$-SiO_2) [21] in a mixture of dichloromethane-diethyl ether furnished the disaccharide derivative (**8**) in a 64 % yield. The moderate yield of the reaction may be explained considering the less reactivity of the 4-hydroxyl group of the D-galactose derivative (**4**). The formation of compound 8 was supported by spectral analysis [signals at δ 4.95 (d, J=3.5 Hz, H-1_D), 4.72 (d, J=7.5 Hz, H-1_C) in the ^{1}H NMR and δ 103.2 (C-1_C), 98.5 (C-1_D) in the ^{13}C NMR spectra]. Saponification of compound 8 using sodium methoxide followed by 4,6-*O*-benzylidene acetal formation using benzaldehyde dimethyl acetal in the presence of $HClO_4$-SiO_2 [22] furnished disaccharide derivative **9** in a 77 % yield. Stereoselective glycosylation of compound **9** with ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**6**) in the presence of a combination of NIS and $HClO_4$-SiO_2 in dichloromethane [23] afforded trisaccharide derivative **10** in a 78 % yield. Appearance of signals in the NMR spectra confirmed the formation of compound **10** [signals at δ 5.01 (d, J=3.5 Hz, H-1_D), 4.78 (d, J=8.0 Hz, H-1_E), 4.76 (d, J=7.5 Hz, H-1_C) in the ^{1}H NMR and δ 103.4 (C-1_E), 103.4 (C-1_C), 99.6 (C-1_D) in the ^{13}C NMR spectra]. In a one-pot de-benzylidenation and *O*-acetylation reaction condition [24], compound **10** was treated with acetic anhydride in the presence $HClO_4$-SiO_2 to give compound **11** in 80 % yield. Oxidative removal of *p*-methoxyphenyl group in compound **11** using ceric ammonium (IV) nitrate (CAN) [25] followed by the reaction of the resulting hemiacetal with trichloroacetonitrile in the presence of DBU [26] led to the formation of a α/β mixture of trisaccharide trichloroacetimidate derivate **12** in 74 % yield, which was immediately used in the next step (Scheme 2).

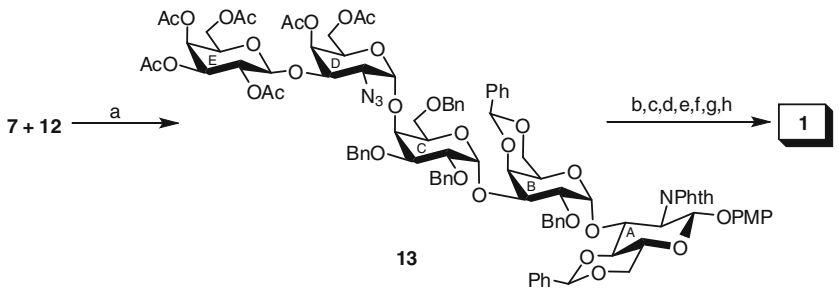
Stereoselective glycosylation of compound **7** with compound **12** in the presence of $HClO_4$-SiO_2 [21] as a heterogeneous catalyst in a mixture of dichloromethane-diethyl ether furnished pentasaccharide derivative **13** in a 68 % yield together with minor quantity (~10 %) of it's another isomer, which was separated by column chromatography. Spectral analysis of compound **13** confirmed its formation [signals at δ 6.76 (d, J=8.5 Hz, H-1_A), 5.48 (d, J=3.5 Hz, H-1_B), 5.26 (d, J=3.0 Hz, H-1_C), 5.08 (d, J=3.5 Hz, H-1_D), 4.52 (d, J=8.0 Hz, H-1_E) in the ^{1}H NMR and δ 100.7 ($J_{C\text{-}1/H\text{-}1}$=158.0 Hz) (C-$1_E$), 98.3 ($J_{C\text{-}1/H\text{-}1}$=170.0 Hz) (C-$1_B$), 98.1 ($J_{C\text{-}1/H\text{-}1}$=160.0 Hz) (C-$1_A$), 98.0 ($J_{C\text{-}1/H\text{-}1}$=169.0 Hz) (C-$1_D$), 91.7 ($J_{C\text{-}1/H\text{-}1}$=172.0 Hz) (C-$1_C$) in the ^{13}C NMR spectra]. Appearance of $J_{C\text{-}1/H\text{-}1}$ values 158.8 and 160.0 Hz indicated the presence of two equatorial or β-glycosyl linkages and $J_{C\text{-}1/H\text{-}1}$ values 169.0, 170.0 and 172.0 Hz indicated the presence of three axial or α-glycosyl linkages in the compound **13** [27, 28]. Compound **13** was subjected to a series of reactions involving (a) reduction of azido group and selective removal of benzyl groups by controlled hydrogenation [12, 29] over $Pd(OH)_2$-C followed by *N*-acetylation; (b) selective TEMPO mediated oxidation of the primary hydroxyl group to the carboxylic group under a phase transfer reaction condition [30, 31]; (c) removal of *N*-phthalimido group by hydrazinolysis [32] followed by acetylation using acetic anhydride and pyridine; (d) removal of benzylidene acetal by hydrogenation and finally (e) de-*O*-acetylation using sodium methoxide to furnish target pentasaccharide 1, which was purified over Sephadex® LH-20 column using (CH_3OH-H_2O; 4:1) as eluant to give pure compound **1** in a 51 % yield. Spectral analysis of compound **1** unambiguously confirmed its formation [signals at δ 5.15 (br s, H-1_C), 4.90 (br s, H-1_B, H-1_D), 4.80–4.78 (m, H-1_A), 4.32 (d, J=8.0 Hz, H-1_E) in the ^{1}H NMR and δ 104.9 (C-1_E), 102.6 (C-1_A), 99.1 (2 C, C-1_B, C-1_D), 96.1 (C-1_C) in the ^{13}C NMR spectra] (Scheme 3).

Conclusion

In conclusion, a straight forward convergent synthetic strategy has been developed for the synthesis of the pentasaccharide repeating unit corresponding to the *O*-antigen of



Scheme 2 Reagents: (a) $HClO_4$-SiO_2, CH_2Cl_2-Et_2O, −20 °C, 1 h, 64 %; (b) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h; (c) benzaldehyde dimethylacetal, $HClO_4$-SiO_2, CH_3CN-DMF, room temperature, 5 h, 77 % in two steps; (d) NIS, $HClO_4$-SiO_2, CH_2Cl_2, MS 4 Å, −40 °C, 45 min, 78 %; (e) acetic anhydride, $HClO_4$-SiO_2, room tempearature, 30 min, 80 %; (f) CAN, CH_3CN-H_2O, room temperature, 2 h; (g) CCl_3CN, CH_2Cl_2, DBU, −20 °C, 1 h, 74 % in two steps



Scheme 3 Reagents: (a) $HClO_4$-SiO_2, CH_2Cl_2-Et_2O, −10 °C, 1 h, 68 %; (b) H_2, 20 % $Pd(OH)_2$-C, CH_3OH, room temperature, 4 h; (c) acetic anhydride, CH_3OH, room temperature, 1 h; (d) (i) TEMPO, NaBr, TBAB, $NaHCO_3$, NaOCl, CH_2Cl_2, H_2O, 5 °C, 3 h; (ii) $NaClO_2$, *tert*-butanol, 2-methyl-2-butene, NaH_2PO_4, room temperature, 3 h; (e) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 7 h; (f) acetic anhydride, pyridine, room temperature, 2 h; (g) H_2, 20 % $Pd(OH)_2$-C, CH_3OH, room temperature, 24 h; (h) 0.1 M CH_3ONa, CH_3OH, room temperature, 3 h, 51 %

enterohaemorrhagic *E. coli* O113. A one-pot reaction for the glycosylation and removal of *p*-methoxybenzyl ether has been adopted. $HClO_4$-SiO_2 has been used as an effective acid catalyst in a number of reaction steps such as benzylidene acetal formation, direct conversion of benzylidene acetal to *O*-acetylated derivative and to activate glycosyl trichloroacetimidate derivative and thioglycoside in combination with NIS avoiding the use of moisture sensitive protic acids. A [3+2] block glycosylation technique has been used. A late stage selective TEMPO mediated oxidation protocol has been applied for the preparation of D-galacturonic acid moiety. Over all, the target compound **1** was efficiently synthesized in a minimum number of steps.

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 2N 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 COSY, HMQC and gated ^1H coupled ^{13}C NMR spectra were recorded on Brucker Avance DRX 500 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 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. Perchloric acid supported over silica ($HClO_4$-SiO_2) was prepared following the protocol reported by Chakraborty *et al.* [33].

p-Methoxyphenyl (2-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (7) To a solution of compound **2** (1.0 g, 1.98 mmol) and compound 3 (1.2 g, 2.29 mmol) in anhydrous CH_2Cl_2 (8 mL) was added MS 4 Å (1 g) and the reaction mixture was stirred at room temperature under argon for 30 min. The reaction mixture was cooled to −30 °C and *N*-iodosuccinimide (NIS; 570.0 mg, 2.53 mmol) and TfOH (20 μL) were added to it. After stirring the reaction mixture at −30 °C for 45 min the temperature was raised to 0 °C and the reaction mixture was allowed to stir at 0 °C for 30 min. The reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (150 mL). The organic layer was successively washed with 5 % $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **7** (1.2 g, 72 %). Colourless oil; $[\alpha]_D^{25}$ +92.6 (*c* 1.2, $CHCl_3$); IR (neat): 3033, 2980, 2933, 2876, 1780, 1742, 1717, 1502, 1459, 1400, 1099, 1031, 996, 699, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.82–6.89 (m, 19 H, Ar-H), 6.74 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.64 (d, *J*=9.0 Hz, 2 H, Ar-H), 5.70 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.48 (d, *J*=3.5 Hz, 1 H, H-1_B), 5.25 (s, 1 H, PhC*H*), 5.14 (s, 1 H, PhC*H*), 4.87 (t, *J*=9.5 Hz, 1 H each, H-3_A), 4.54 (t, *J*=9.0 Hz each, 1 H, H-2_A), 4.38 (d, *J*=12.5 Hz, 1 H, PhCH_2), 4.28–4.25 (m, 1 H, H-5_A), 4.10 (d, *J*=12.5 Hz, 1 H, PhCH_2), 3.86 (dd, *J*=9.5, 3.0 Hz, 1 H, H-3_B), 3.82 (t, *J*=9.0 Hz each, 1 H, H-4_A), 3.79 (d, *J*=3.0 Hz, 1 H, H-4_B), 3.76–3.70 (m, 2 H, H-6_{abA}), 3.64 (s, 3 H, OCH_3), 3.57 (dd, *J*=10.0, 3.5 Hz, 1 H, H-2_B), 3.36 (d, *J*=12.0 Hz, 1 H, H-6_{aB}), 2.95 (br s, 1 H, H-5_B), 2.93 (d, *J*=12.0 Hz, 1 H, H-6_{bB}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.7–114.5 (Ar-C), 102.1 (PhCH), 100.9 (PhCH), 98.1 (C-1_B), 97.8 (C-1_A), 82.3 (C-4_A), 75.5 (C-4_B), 74.9 (C-2_B), 73.8 (C-3_A), 71.2 (PhCH_2), 68.7 (C-6_B), 68.5 (C-5_A), 67.4 (C-3_B), 66.1(C-6_A), 62.9 (C-5_B), 55.6 (C-2_A), 55.3 (OCH_3); ESI-MS: 866.2 $[M+Na]^+$; Anal. Calcd. for $C_{48}H_{45}NO_{13}$ (843.29): C, 68.32; H, 5.37; found: C, 68.10; H, 5.60.

p-Methoxyphenyl (3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-

*galactopyranoside (**8**)* A solution of compound **4** (1.4 g, 2.51 mmol) and compound **5** (1.9 g, 4.0 mmol) in anhydrous CH_2Cl_2-Et_2O (12 mL, 1:1 v/v) was cooled to −20 °C. To the cooled reaction mixture was added $HClO_4$-SiO_2 (200 mg) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was filtered and washed with CH_2Cl_2 (100 mL). The combined organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure compound **8** (1.4 g, 64 %). Colourless oil; $[\alpha]_D^{25}$ +73.8 (*c* 1.2, $CHCl_3$); IR (neat): 3024, 2936, 2362, 2100, 1751, 1374, 1212, 1043, 767, 670 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.30–7.15 (m, 15 H, Ar-H), 6.90 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.70 (d, *J*= 9.0 Hz, 2 H, Ar-H), 5.36 (d, *J*=2.0 Hz, 1 H, H-4_D), 5.30 (dd, *J*=10.5, 3.5 Hz, 1 H, H-3_D), 4.95 (d, *J*=3.5 Hz, 1 H, H-1_D), 4.94 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.82 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.72 (d, *J*=7.5 Hz, 1 H, H-1_C), 4.70–4.65 (m, 2 H, H-5_D), 4.45 (m, 1 H, PhCH_2), 4.08 (d, *J*=3.0 Hz, 1 H, H-4_C), 3.90–3.83 (m, 3 H, H-5_C, H-6_{aC}, H-6_{aD}), 3.67 (s, 3 H, OCH_3), 3.60 (dd, *J*=11.0, 4.0 Hz, 1 H, H-2_D), 3.58–3.52 (m, 2 H, H-2_C, H-6_{bD}), 3.48–3.45 (m, 1 H, H-6_{bC}), 3.40 (dd, *J*= 10.0, 3.0 Hz, 1 H, H-3_C), 2.02, 1.97, 1.80 (3 s, 9 H, 3 COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (2 C), 169.8 (3 *C*OCH$_3$), 155.7–114.5 (Ar-C), 103.2 (C-1_C), 98.5 (C-1_D), 79.7 (C-3_C), 78.7 (C-2_C), 75.2 (Ph*C*H$_2$), 73.8 (C-4_C), 73.5 (Ph*C*H$_2$), 73.3 (Ph*C*H$_2$), 72.9 (C-5_C), 68.8 (C-3_D), 67.3 (C-4_D), 67.0 (C-6_D), 66.2 (C-5_D), 60.6 (C-6_C), 58.0 (C-2_D), 55.6 (O*C*H$_3$), 20.2, 20.6 (2 C) (3 CO*C*H$_3$); ESI-MS: 892.3 $[M+Na]^+$; Anal. Calcd. for $C_{46}H_{51}N_3O_{14}$ (869.34): C, 63.51; H, 5.91; found: C, 63.28; H, 6.15.

*p-Methoxyphenyl (4,6-O-benzylidene-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**9**)* A solution of compound **8** (1.2 g, 1.38 mmol) in 0.1 M CH_3ONa in CH_3OH (20 mL) was allowed to stir at room temperature for 2 h. The reaction mixture was neutralized with Dowex 50 W X8 (H^+) resin, filtered and concentrated under reduced pressure. To a solution of the deacetylated product in anhydrous CH_3CN-DMF (10 mL; 1:1 v/v) was added benzaldehyde dimethyl acetal (0.4 mL, 2.66 mmol) followed by $HClO_4$-SiO_2 (50 mg) and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was filtered and the solvents were removed under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure compound **9** (880.0 mg, 77 %). Colourless oil; $[\alpha]_D^{25}$ +86.8 (*c* 1.2, $CHCl_3$); IR (neat): 3336, 2926, 2121, 1737, 1716, 1514, 1467, 1226, 1170, 1067, 756, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.38–7.17 (m, 20 H, Ar-H), 6.97 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.72 (d, *J*=9.0 Hz, 2 H, Ar-H), 5.34 (s, 1 H, PhC*H*), 4.97 (d, *J*=3.5 Hz, 1 H, H-1_D), 4.95 (d, *J*= 11.5 Hz, 1 H, PhCH_2), 4.82 (d, *J*=11.5 Hz, 1 H, PhCH_2), 4.78 (d, *J*=8.0 Hz, 1 H, H-1_C), 4.66–4.61 (2 d, *J*=12.0 Hz each, 2 H, PhCH_2), 4.51–4.43 (2 d, *J*=11.5 Hz each, 2 H, PhCH_2), 4.15 (d, *J*=2.5 Hz, 1 H, H-4_D), 5.30 (dd, *J*=10.5, 3.0 Hz, 1 H, H-3_D), 4.07 (br s, 1 H, H-5_C), 4.05 (d, *J*= 3.0 Hz, 1 H, H-4_C), 3.95–3.92 (m, 1 H, H-6_{aC}), 3.78 (t, *J*= 10.0 Hz each, 1 H, H-2_C), 3.69 (s, 3 H, OCH_3), 3.60–3.51 (m, 4 H, H-2_D, H-5_D, H-6_{aD}, H-6_{bC}), 3.43 (dd, *J*=10.0, 3.0 Hz, 1 H, H-3_C), 3.33–3.30 (m, 1 H, H-6_{bD}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.3–114.5 (Ar-C), 103.3 (C-1_C), 101.0 (Ph*C*H), 99.5 (C-1_D), 80.6 (C-3_C), 78.1 (C-2_C), 75.6 (C-4_C), 75.0 (Ph*C*H$_2$), 73.6 (Ph*C*H$_2$), 73.3 (C-4_D), 73.1 (C-5_D), 72.7 (Ph*C*H$_2$), 68.9 (C-6_C), 67.8 (C-3_D), 67.0 (C-6_D), 62.7 (C-5_C), 61.4 (C-2_D), 55.7 (O*C*H$_3$); ESI-MS: 854.3 $[M+Na]^+$; Anal. Calcd. for $C_{47}H_{49}N_3O_{11}$ (831.34): C, 67.86; H, 5.94; found: C, 67.65; H, 6.18.

*p-Methoxyphenyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-(4,6-O-benzylidene-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**10**)* To a solution of compound **9** (800.0 mg, 0.96 mmol) and compound **6** (450.0 mg, 1.14 mmol) in anhydrous CH_2Cl_2 (5 mL) was added MS 4 Å (1.0 g) and the reaction mixture was stirred at room temperature under argon for 30 min. The reaction mixture was cooled to −40 °C and NIS (280.0 mg, 1.24 mmol) and $HClO_4$-SiO_2 (15.0 mg) were added to it and the reaction mixture was allowed to stir at same temperature for 45 min. The reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with 5 % $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure compound **10** (870.0 mg, 78 %). Colourless oil; $[\alpha]_D^{25}$ +80 (*c* 1.2, $CHCl_3$); IR (neat): 3474, 2932, 2868, 2117, 1757, 1502, 1226, 1100, 1079, 1056, 737, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.42–7.17 (m, 20 H, Ar-H), 6.94 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.72 (d, *J*=9.0 Hz, 2 H, Ar-H), 5.33 (d, *J*=3.5 Hz, 1 H, H-4_E), 5.31 (s, 1 H, PhC*H*), 3.40 (dd, *J*=8.0 Hz each, 1 H, H-2_E), 5.01 (d, *J*=3.5 Hz, 1 H, H-1_D), 4.97 (d, *J*=11.5 Hz, 1 H, PhCH_2), 4.95 (dd, *J*=10.5, 3.0 Hz, 1 H, H-3_E), 4.84 (d, *J*= 11.0 Hz, 1 H, PhCH_2), 4.78 (d, *J*=8.0 Hz, 1 H, H-1_E), 4.76 (d, *J*=7.5 Hz, 1 H, H-1_C), 4.70–4.60 (2 d, *J*=11.5 Hz each, PhCH_2), 4.49–4.41 (2 d, *J*=11.0 Hz each, PhCH_2), 4.20 (d, *J*=3.0 Hz, 1 H, H-4_D), 4.17 (d, *J*=3.5 Hz, 1 H, H-4_C), 4.12–4.10 (m, 1 H, H-6_{aE}), 4.07–4.04 (m, 1 H, H-6_{bE}), 4.03 (br s, 1 H, H-5_C), 3.99 (dd, *J*=10.5, 3.0 Hz, 1 H, H-3_D), 3.95–3.88 (m, 2 H, H-5_E, H-6_{aC}), 3.79–3.76 (m, 2 H, H-2_C, H-2_D), 3.69 (s, 3 H, OCH_3), 3.60–3.55 (m, 3 H, H-5_D, H-6_{aD}, H-6_{bC}), 3.44 (dd, *J*=10.0, 3.0 Hz, 1 H, H-3_C), 3.37–3.35 (m, 1 H, H-6_{bD}), 2.06, 2.00, 1.93, 1.90 (4 s, 12 H, 4 COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.5 (4 *C*OCH$_3$), 155.4–114.5 (Ar-C), 103.4 (C-1_E), 103.4 (C-1_C), 100.5 (Ph*C*H),

99.6 (C-1_D), 80.6 (C-3_C), 78.5 (C-2_C), 76.6 (C-4_D), 75.6 (C-3_D), 75.0 (Ph*C*H_2), 73.7 (Ph*C*H_2), 73.2 (C-4_C), 73.1 (C-5_D), 72.7 (Ph*C*H_2), 71.2 (C-3_E), 70.8 (C-5_E), 69.0 (C-6_C), 68.8 (C-2_E), 67.0 (C-4_E), 66.9 (C-6_D), 62.9 (C-5_C), 61.5 (C-6_E), 59.4 (C-2_D), 55.6 (O*C*H_3), 20.9, 20.8, 20.7, 20.6 (4 CO*C*H_3); ESI-MS: 1184.4 $[M+Na]^+$; Anal. Calcd. for $C_{61}H_{67}N_3O_{20}$ (1161.43): C, 63.04; H, 5.81; found: C, 62.82; H, 6.04.

*p-Methoxyphenyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**11**)* To a solution of compound **10** (850.0 mg, 0.73 mmol) in acetic anhydride (2 mL) was added $HClO_4$-SiO_2 (50.0 mg) and the reaction mixture was allowed to stir at room temperature for 30 min. The reaction mixture was filtered and washed with EtOAc (25 mL). The organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was passed through a short pad of SiO_2 using hexane-EtOAc (3:1) to give pure compound **11** (675.0 mg, 80 %). Colourless oil; $[\alpha]_D^{25}$ +58 (*c* 1.2, $CHCl_3$); IR (neat): 3484, 3036, 2957, 2931, 1757, 1508, 1378, 1244, 1179, 1093, 977, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.29–7.17 (m, 15 H, Ar-H), 6.90 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.71 (d, *J*=9.0 Hz, 2 H, Ar-H), 5.41 (d, *J*=2.0 Hz, 1 H, H-4_E), 5.30 (d, *J*=3.0 Hz, 1 H, H-4_D), 5.12 (dd, *J*=8.0 Hz each, 1 H, H-2_E), 4.96 (dd, *J*=9.5, 3.0 Hz, 1 H, H-3_E), 4.94 (d, *J*=11.0 Hz, 1 H, PhC*H*$_2$), 4.91 (d, *J*=3.5 Hz, 1 H, H-1_D), 4.80 (d, *J*=11.0 Hz, 1 H, PhC*H*$_2$), 4.72 (d, *J*=7.5 Hz, 1 H, H-1_C), 4.70 (d, *J*=11.0 Hz, 1 H, PhC*H*$_2$), 4.78 (d, *J*=8.0 Hz, 1 H, H-1_E), 4.63 (d, *J*=11.0 Hz, 1 H, PhC*H*$_2$), 4.48–4.45 (m, 1 H, H-5_D), 4.43 (br s, 2 H, PhC*H*$_2$), 4.10–4.00 (m, 4 H, H-3_D, H-4_C, H-6_{abE}), 3.90–3.85 (m, 3 H, H-6_{aC}, H-6_{abD}), 3.75 (dd, *J*=7.5 Hz each, 1 H, H-2_C), 3.68 (s, 3 H, OC*H*$_3$), 3.59 (dd, *J*=9.5, 3.0 Hz, 1 H, H-2_D), 3.57–3.50 (m, 3 H, H-5_C, H-5_E, H-6_{bC}), 3.39 (dd, *J*=10.0, 3.0 Hz, 1 H, H-3_C), 2.09, 2.00, 1.95, 1.90, 1.87 (6 s, 18 H, 6 CO*C*H_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 170.1, 169.7, 169.5 (6 *C*OCH$_3$), 155.3–114.5 (Ar-C), 103.2 (C-1_C), 100.6 (C-1_E), 98.8 (C-1_D), 79.8 (C-3_C), 78.6 (C-2_C), 75.1 (C-5_E), 75.0 (Ph*C*H_2), 73.9 (C-3_D), 73.6 (Ph*C*H_2), 73.1 (Ph*C*H_2), 73.0 (C-5_C), 71.0 (C-3_E), 70.9 (C-4_C), 69.3 (C-4_E), 68.3 (C-2_E), 67.0 (C-6_C), 66.8 (C-5_D), 66.7 (C-4_D), 61.5 (C-6_D), 61.0 (C-6_E), 60.2 (C-2_D), 55.6 (O*C*H_3), 21.0, 20.7 (2 C), 20.6 (2 C), 20.5 (6 CO*C*H_3); ESI-MS: 1180.4 $[M+Na]^+$; Anal. Calcd. for $C_{58}H_{67}N_3O_{22}$ (1157.42): C, 60.15; H, 5.83; found: C, 60.00; H, 6.00.

*(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-α/β-D-galactopyranosyl trichloroacetimidate (**12**)* To a solution of compound **11** (650.0 mg, 0.56 mmol) in CH_3CN-H_2O (15 mL; 1:1 v/v) was added CAN (1.0 g, 1.82 mmol) and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was extracted with EtOAc (50 mL). The organic layer was successively washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was passed through a short pad of SiO_2 using hexane-EtOAc (3:1) to give trisaccharide hemiacetal derivative. To a solution of the hemiacetal derivative in anhydrous CH_2Cl_2 (5 mL) was added CCl_3CN (0.4 mL, 3.98 mmol) and cooled to −20 °C. To the cooled reaction mixture was added DBU (10 μL) and the reaction mixture was stirred at same temperature for 1 h. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane-EtOAc (6:1) as eluant to give pure compound 12 (500.0 mg, 74 %), which was used immediately for the next step without further characterization.

*p-Methoxyphenyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-benzyl-α-D-galactopyranosyl-(1→3)-(2-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**13**)* A solution of compound **7** (320.0 mg, 0.38 mmol) and compound **12** (500.0 mg, 0.42 mmol) in anhydrous CH_2Cl_2-Et_2O (5 mL, 3:1 v/v) was cooled to −10 °C. 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 and washed with CH_2Cl_2 (50 mL). The combined organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **13** (485.0 mg, 68 %). Colourless oil; $[\alpha]_D^{25}$ +92 (*c* 1.2, $CHCl_3$); IR (neat): 3022, 2365, 1746, 1653, 1218, 769, 677 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.91–6.92 (m, 34 H, Ar-H), 6.87 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.76 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.76 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.48 (d, *J*=3.5 Hz, 1 H, H-1_B), 5.44 (d, *J*=3.0 Hz, 1 H, H-4_D), 5.39 (br s, 1 H, H-4_E), 5.38 (s, 1 H, PhC*H*), 5.26 (d, *J*=3.0 Hz, 1 H, H-1_C), 5.22 (dd, *J*=8.0 Hz each, 1 H, H-2_E), 5.18 (s, 1 H, PhC*H*), 5.08 (d, *J*=3.5 Hz, 1 H, H-1_D), 5.02 (dd, *J*=10.0, 3.0 Hz, 1 H, H-3_E), 4.92 (t, *J*=9.0 Hz, 1 H each, H-3_A), 4.60–4.43 (m, 8 H, H-2_A, H-5_D, PhC*H*$_2$), 4.52 (d, *J*=8.0 Hz, 1 H, H-1_E), 4.33–4.22 (m, 3 H, H-5_A, PhC*H*$_2$), 4.08–3.96 (m, 4 H, H-4_C, H-6_{abE}, H-6_{aD}), 3.95–3.88 (m, 4 H, H-3_B, H-3_D, H-5_C, H-6_{bD}), 3.86–3.67 (m, 8 H, H-2_B, H-3_C, H-4_A, H-4_B, H-5_E, H-6_{aA}, H-6_{abC}), 3.64 (s, 3 H, OC*H*$_3$), 3.61–3.58 (m, 1 H, H-2_C), 3.52–3.45 (m, 2 H, H-2_D, H-6_{bA}), 3.38–3.33 (m, 1 H, H-6_{aB}), 2.95–2.88 (m, 2 H, H-5_B, H-6_{bB}), 2.06, 2.00, 1.98, 1.90, 1.89, 1.82 (6 s, 18 H, 6 CO*C*H_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3 (2 C), 170.2 (2 C), 169.6, 169.5 (6 *C*OCH$_3$), 155.6–114.5 (Ar-C), 101.8 (Ph*C*H), 101.2 (Ph*C*H), 100.7 ($J_{C\text{-}1/H\text{-}1}$=

158.0 Hz) (C-1_E), 98.3 ($J_{C\text{-}1/H\text{-}1}$=170.0 Hz) (C-$1_B$), 98.1 ($J_{C\text{-}1/H\text{-}1}$=160.0 Hz) (C-$1_A$), 98.0 ($J_{C\text{-}1/H\text{-}1}$=169.0 Hz) (C-$1_D$), 91.7 ($J_{C\text{-}1/H\text{-}1}$=172.0 Hz) (C-$1_C$), 82.2 (C-$4_A$), 76.0 (C-$4_B$), 75.5 (C-$3_C$), 75.0 (C-$2_B$), 73.8 (C-$3_A$), 73.1 (Ph*C*$H_2$), 72.0 (C-$5_C$), 71.8 (Ph*C*$H_2$), 71.7 (C-$3_D$), 70.9 (C-$5_E$), 70.6 (2 C, C-$3_E$, C-$5_A$), 70.5 (Ph*C*$H_2$), 70.4 (C-$3_B$), 69.1 (C-$4_C$), 68.9 (C-$2_E$), 68.8 (C-$4_D$), 68.7 (2 C, C-$6_B$, Ph*C*$H_2$), 66.7 (C-$2_C$), 66.6 (C-$6_C$), 66.4 (C-$5_D$), 66.1 (C-$4_E$), 62.6 (C-$5_B$), 61.5 (C-$6_A$), 60.9 (2 C, C-$6_D$, C-$6_E$), 60.1 (C-$2_D$), 55.6 (O*C*$H_3$), 55.2 (C-2A), 20.8, 20.7, 20.6 (2 C), 20.5 (2 C) (6 CO*C*H_3); MALDI-TOF-MS: 1899.6 $[M+Na]^+$; Anal. Calcd. for $C_{99}H_{104}N_4O_{33}$ (1876.66): C, 63.32; H, 5.58; found: C, 63.10; H, 5.84.

*p-Methoxyphenyl (β-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→4)-(sodium α-D-galactopyranosyl uronate)-(1→3)-(α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**1**)*

To a solution of compound **13** (450.0 mg, 0.24 mmol) in CH_3OH (8 mL) was added 20 % $Pd(OH)_2$-C (100 mg) and the reaction mixture was allowed to stir under a positive pressure of hydrogen at room temperature for 4 h. The reaction mixture was filtered through a Celite® bed and concentrated to the one third of the reaction volume. To the methanolic solution of the selectively hydrogenated product was added acetic anhydride (1 mL) and the reaction mixture was kept at room temperature for 1 h. The solvents were removed under reduced pressure to give *N*-acetylated and debenzylated product. To a solution of the pentasaccharide tetraol derivative in CH_2Cl_2 (20 mL) and H_2O (5 mL) were successively added aq. NaBr (3 mL; 1 M), aq. TBAB (5 mL; 1 M), TEMPO (150.0 mg, 0.96 mmol), satd. $NaHCO_3$ (15 mL) and 4 % aq. NaOCl (15 mL) and the reaction mixture was allowed to stir at 5 °C for 3 h and neutralized with 1 N HCl. To the reaction mixture were added *tert*-butanol (20 mL), 2-methyl-but-2-ene (20 mL; 2 M solution in THF), aq. $NaClO_2$ (2.0 g/10 mL) and aq. NaH_2PO_4 (2.0 g/10 mL) and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was diluted with satd. aq. NaH_2PO_4 and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water, dried (Na_2SO_4) and concentrated to dryness to give the crude product, which was passed through a short pad of SiO_2. To a solution of the sodium salt of the oxidized product in C_2H_5OH (10 mL) was added hydrazine monohydrate (0.2 mL) and the reaction was allowed to stir at 80 °C for 7 h. The solvents were removed under reduced pressure and the crude product was dissolved in acetic anhydride and pyridine (2 mL, 1:1 v/v) and kept at room temperature for 2 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 acetylated product in CH_3OH (5 mL) was added 20 % $Pd(OH)_2$-C (100 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 concentrated. A solution of the crude product in 0.1 M CH_3ONa in CH_3OH (5 mL) was allowed to stir at room temperature for 3 h, neutralized with Dowex 50 W X8 (H^+) resin and then treated with Dowex 50 W X8 (Na^+) resin, filtered and evaporated to dryness. The sodium salt of the pentasaccharide was purified through a Sephadex® LH-20 column using CH_3OH-H_2O (3:1) as eluant to give pure compound **1** (130.0 mg, 51 %) as white powder. $[\alpha]_D^{25}$ +67 (*c* 1.2, CH_3OH); IR (KBr): 2364, 2343, 1727, 1646, 1567, 1382, 1074, 838, 770, 676 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 6.79 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.73 (d, *J*=9.0 Hz, 2 H, Ar-H), 5.15 (br s, 1 H, H-1_C), 4.90 (br s, 2 H, H-1_B, H-1_D), 4.80–4.78 (m, 1 H, H-1_A), 4.32 (d, *J*=8.0 Hz, 2 H, H-1_E), 4.27–3.89 (m, 7 H, H-2_A, H-2_D, H-4_A, H-4_C, H-4_D, H-4_E, H-5_C), 3.90–3.72 (m, 8 H, H-2_C, H-3_A, H-3_B, H-3_C, H-3_D, H-5_B, H-6_{abE}), 3.70–3.54 (m, 8 H, H-2_E, H-6_{abA}, H-6_{abD}, O*C*H_3), 3.52–3.30 (m, 7 H, H-2_B, H-3_E, H-5_A, H-4_D, H-5_E, H-6_{abB}), 1.92 (br s, 6 H, 2 CO*C*H_3); ^{13}C NMR (125 MHz, CD_3OD): δ 174.6 (*C*OONa), 172.7, 172.6 (2 *C*OCH$_3$), 155.2–114.1 (Ar-C), 104.9 (C-1_E), 102.6 (C-1_A), 99.1 (2 C, C-1_B, C-1_D), 96.1 (C-1_C), 78.8 (C-4_C), 77.5 (C-3_A), 76.5 (C-3_D), 76.3 (C-3_B), 75.3 (2 C, C-5_A, C-5_E), 73.3 (2 C, C-3_E, C-4_D), 71.3 (2 C, C-2_E, C-4_A), 71.1 (C-5_D), 69.7 (C-4_E), 68.9 (2 C, C-2_C, C-3_C), 68.6 (C-2_B), 67.7 (C-5_B), 65.2 (C-5_C), 63.0 (C-4_B), 61.2 (3 C, C-6_A, C-6_B, C-6_E), 60.2 (C-6_D), 55.8 (C-2_D), 55.4 (C-2_A), 54.7 (O*C*H_3), 21.5 (2 C, 2 CO*C*H_3); ESI-MS: 1053.3 $[M+1]^+$; Anal. Calcd. for $C_{41}H_{61}N_2NaO_{28}$ (1052.33): C, 46.77; H, 5.84; found: C, 47.0; H, 6.12.

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