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Convergent synthesis of a common pentasaccharide corresponding to the *O*-antigen of *Escherichia coli* O168 and *Shigella dysenteriae* type 4

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Abstract A convenient synthetic strategy of the common acidic pentasaccharide repeating unit corresponding to the *O*-antigen of enterotoxigenic *E. coli* O168 and *Shigella dysenteriae* type 4 has been successfully developed. A stereoselective [2+3] block glycosylation method has been exploited to get the target pentasaccharide derivative. Most of the synthetic intermediates were solid and prepared in high yields from commercially available reducing sugars following a series of protection-deprotection reactions. A α-D-mannose moiety has been used as the source of α-D-glucosamine moiety. A late-stage TEMPO mediated selective oxidation reaction finally resulted in the pentasaccharide containing a glucuronic acid unit.

Keywords Lipopolysaccharides · Glycosylations · Antigens · *Escherichia coli* · *Shigella dysenteriae*

Introduction

Diarrhoea is a common infectious disease in infants and children in tropical countries and in places where sanitary conditions are poor. The major causative agents for the diarrhoeal infections are enteropathogenic *Escherichia coli* (*E. coli*) strains. Although, *E. coli* serves a useful function in human body by suppressing the growth of harmful bacteria and by synthesising considerable amounts of vitamins [1], a number of virulent *E. coli* strains are responsible for severe enteric/diarrhoeal infections [2, 3]. In general, the enteropathogenic strain of *E. coli* have been classified in several classes [4, 5], which include (a) enteropathogenic *E. coli* (EPEC), (b) enteroinvasive *E. coli* (EIEC), (c) enterotoxigenic *E. coli* (ETEC), (d) enteroaggregative *E. coli* (EAEC), (e) diffusely adherent *E. coli* (DAEC), and (f) enterohemorrhagic *E. coli* (EHEC) *etc*. ETEC strains are the major cause of traveller's diarrhoea. The pathogen adheres to the mucosa in the small intestine and produce toxins that act on mucosal cells to cause diarrhoea. In general, two types of toxins are produced by ETEC strains, heat-labile (LT) and heat-stable (ST) [6, 7]. ETEC colonizes the surface of the small bowel mucosa and elaborates enterotoxins, which give rise to intestinal secretion.

Similar to ETEC, *Shigella dysenteriae* is the most virulent among the pathogenic bacilli of the genus *Shigella* [8]. They are responsible for severe intestinal diseases including dysentery and have the potential for causing disastrous health problems in developing countries [9, 10]. Virulent *E. coli* and *Shigella* strains have long been known to be closely related in terms of their gene sequences and pathogenic actions [11, 12]. Recently, Perepelove *et al*. reported [13] the structure of a common oligosaccharide repeating unit corresponding to the *O*-polysaccharide of enterotoxigenic *E. coli* O168 and revised structure of the *O*-antigen of *Shigella dysenteriae* type 4 (Fig. 1). Earlier Dmitriev *et al*. [14] reported a structure of the *O*-antigen of *Shigella dysenteriae* type 4, which was partially incorrect (Fig. 2). Both strains are highly associated with several diarrhoeal pandemic.

Although, several therapeutics have appeared to control these infections, emergance of the drug resistant strains necessitates the development of alternative approaches for

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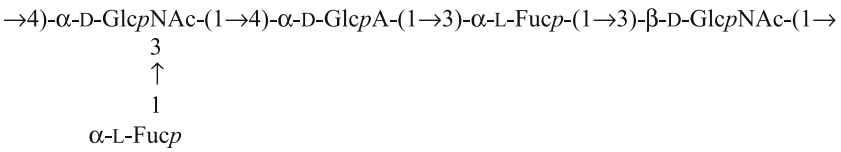


Fig. 1 Common pentasaccharide repeating unit of the *O*-antigen of *E. coli* O168 and *Shigella dysenteriae* type 4 (revised structure) [13]

controlling these infections [15]. Since, *O*-polysaccharide is highly immunogenic and important virulence factor, earlier several attempts have been made to develop *O*-polysaccharide derived glycoconjugates for their use as antibacterial vaccine candidates [16–19]. For a detailed understanding of the pathogenic role of the *O*-antigens, several biological experiments are necessary demanding large quantities of oligosaccharides in hand. Although, oligosaccharides can be isolated from the natural source, concise chemical synthesis only provide the access to a large quantity of a particular oligosaccharide. Since, both enterotoxigenic *E. coli* O168 and *Shigella dysenteriae* type 4 have common structure of their *O*-antigens as well as their pathogenic actions, it would be beneficial to synthesize this oligosaccharide for its use in the development of glycoconjugate for biological evaluation as anti-diarrhaeal agents. Although, a synthesis of the incorrect structure of the *O*-antigen of *Shigella dysenteriae* type 4 has been reported earlier [20], development of a synthetic strategy for the revised structure is quite essential to validate biological function of the *O*-antigen. In this context, we report herein convenient synthesis of a common pentasaccharide as its 4-methoxyphenyl glycoside (**1**) corresponding to the *O*-antigen of enterotoxigenic *E. coli* O168 and revised structure of *Shigella dysenteriae* type 4 using sequential and [2+3] block glycosylation strategies (Fig. 3). 4-Methoxyphenyl (PMP) group can be easily removed under oxidative condition to provide the pentasaccharide hemiacetal for its further use.

Results and discussion

The synthesis of the target pentasaccharide poses a number of challenges because of the presence of several 1,2-*cis* glycosidic linkages between the monosaccharide units. The final compound has been synthesized applying sequential and block glycosylation strategies. Following features can be found in this convenient synthetic strategy: (a) use of 1,2-*trans* linked α-D-mannosidic moiety as the precursor of 1,2-*cis* linked α-D-glucosamine unit in pentasaccharide derivative **12**, (b) late stage TEMPO mediated selective oxidation of primary hydroxyl group to the carboxylic functionality, (c) stereoselective 1,2-*cis* glycosylation using L-fucose derivatives, (d) use of 4-methoxyphenyl group as the anomeric protection for its easy removal under oxidative condition. In order to construct the target pentasaccharide (**1**), a series of suitably protected monosaccharide intermediates **2** [21], **3** [22] and **4** were prepared from the commercially available reducing sugars using literature reported protection-deprotection methodologies. Ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**5**) was synthesized from L-fucose derivative and D-mannose derivatives through a stereoselective "armed-disarmed" glycosylation following the method reported earlier [20] (Fig. 3).

Ethyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**6**) [23] was allowed to react with 4-methoxybenzyl chloride in the presence of sodium hydroxide [24] to give ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside (**4**) in 89% yield. Stereoselective 1,2-*cis*-glycosylation of compound **2** with L-fucose derived thioglycoside derivative (**3**) in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) [25, 26] furnished disaccharide derivative **7** in 85% yield. Appearance of characteristic signal in the NMR spectra [δ 4.82 (d, J=3.4 Hz, H-1_B) in the ^{1}H NMR and δ 99.5 ($J_{C\text{-}1/H\text{-}1}$=171 Hz, C-$1_B$) in the ^{13}C NMR spectra] confirmed its stereoselective formation. Compound **7** was subjected to a sequence of reactions involving saponification using sodium methoxide and selective acetylation via orthoesterification [27] using triethyl orthoacetate in the presence of *p*-toluenesulfonic acid followed by hydrolysis to give disaccharide derivative **8** in 90% yield. NMR spectra of compound **8** supported its formation. Stereoselective 1,2-*cis* glycosylation of compound **8** with thioglycoside derivative **4** in the presence of NIS-TMSOTf [25, 26] furnished

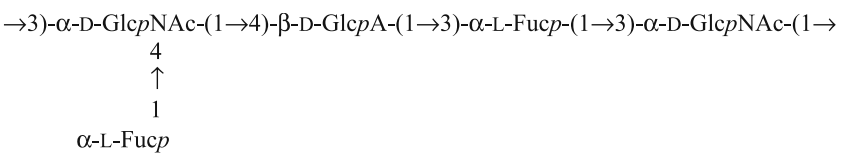


Fig. 2 Incorrect structure of the pentasaccharide repeating unit of the *O*-antigen of *Shigella dysenteriae* type 4 reported earlier (Dmitriev *et al.*) [14]

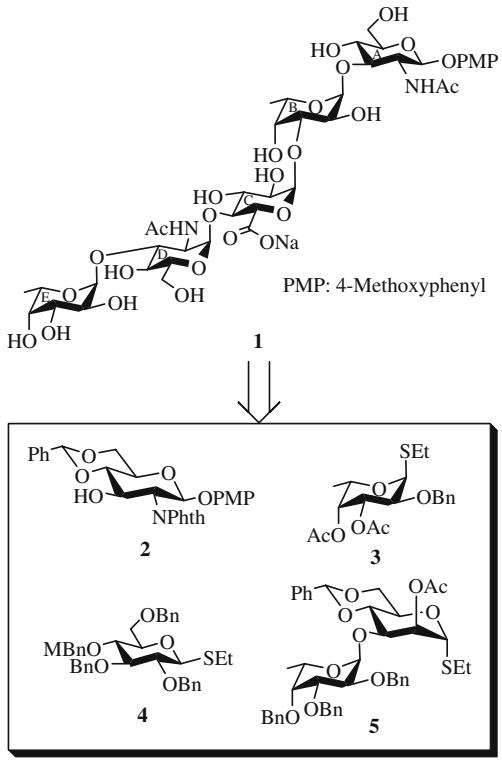


Fig. 3 Structure of the synthesized pentasaccharide (**1**) as its 4-methoxyphenyl glycoside corresponding to the common *O*-antigen of *E. coli* O168 and *Shigella dysenteriae* type 4

trisaccharide derivative **9** in 81% yield together with minor quantities of 1,2-*trans* glycosylated product (~10%), which was confirmed from its spectral analysis [appearance of characteristic signal at δ 4.83 (d, J=3.6 Hz, H-1_C) in the ^{1}H NMR and δ 99.0 ($J_{C\text{-}1/H\text{-}1}$=172 Hz, C-$1_C$) in the ^{13}C NMR spectra]. Oxidative removal [28] of 4-methoxybenzyl group from compound **9** using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) afforded trisaccharide acceptor **10** in 75% yield. Stereoselective block glycosylation of compound **10** with disaccharide thioglycoside **5** in the presence of NIS-TMSOTf [25, 26] furnished pentasaccharide derivative **11** in 80% yield. Appearance of new characteristic signals at δ 5.34 (br s, H-1_D), 4.94 (d, J=2.8 Hz, H-1_E) in the ^{1}H NMR and δ 99.8 ($J_{C\text{-}1/H\text{-}1}$=174 Hz, C-$1_D$), 94.0 ($J_{C\text{-}1/H\text{-}1}$=172 Hz, C-$1_E$) in the ^{13}C NMR spectra of compound **11** supported its formation. The 2-*O*-acetyl group of the D-mannosyl moiety in compound **11** was chemoselectively removed using dilute sodium methoxide keeping the 4-*O*-acetyl group in the L-fucosyl moiety intact exploiting the difference of reactivity of two *O*-acetyl groups. The selective deacetylated product was allowed to react with trifluoromethanesulfonic anhydride in the presence of pyridine and the resulting triflate derivative was treated with sodium azide to furnish compound **12** in 76% over all yield [29]. Formation of compound **12** was confirmed from its spectral analysis [signals at δ 5.44 (br s, 1 H, H-1_D), 4.81–4.79 (m, H-2_D) in ^{1}H NMR and δ 97.8 (C-1_D) in the ^{13}C NMR spectra]. Compound **12** was subjected to a set of reactions involving (a) treatment with ethylene diamine followed by *N*-acetylation to transform *N*-phthalimido group to acetamido group [30], (b) selective hydrogenolysis [31] to remove benzyl ethers and reduction of azido group to amine keeping benzylidene acetals unaffected followed by *N*-acetylation using conventional acetylation and de-*O*-acetylation, (c) selective TEMPO mediated oxidation [32–34] of the primary hydroxyl group to the carboxylic group under a phase-transfer condition and finally (d) removal of benzylidene acetals under hydrogenolysis [31] to furnish target pentasaccharide (**1**) as its 4-methoxyphenyl glycoside in 57% over all yield. Spectral analysis of compound **1** confirmed its formation [signals at δ 5.18 (d, J=3.7 Hz, H-1_B), 5.16 (br s, H-1_D), 5.15 (br s, H-1_E), 5.05 (d, J=3.4 Hz, H-1_C), 4.69 (br s, H-1_A) in the ^{1}H NMR and δ 100.5 (2 C, C-1_D, C-1_E), 100.1 (C-1_A), 99.9 (C-1_B), 99.5 (C-1_C) in the ^{13}C NMR spectra] (Fig. 4, Scheme 1).

Conclusion

In conclusion, a convergent chemical synthesis of a common pentasaccharide repeating unit of the *O*-antigen of *E. coli* O168 and *Shigella dysenteriea* type 4 as its 4-methoxyphenyl glycoside has been successfully developed using a block synthetic strategy. Most of the intermediates are solid and all glycosylation steps are highly stereoselective and reproducible for scale-up preparation. A α-D-mannosyl moiety has been used as the source of α-D-glucosaminyl moiety to get exclusively 1,2-*cis* linked α-D-glucosaminyl unit in the pentasaccharide. Selective TEMPO mediated oxidation of a primary hydroxyl group in a pentasaccharide derivative was achieved using a two-step, one-pot phase transfer oxidation protocol without affecting other secondary hydroxyl groups present in the molecules. 4-Methoxyphenyl group has been chosen as the temporary protecting group at the reducing end.

Experimental section

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 sulphate (2% $Ce(SO_4)_2$ in 2N H_2SO_4) sprayed plates in hot plate. Silica gel 230-400 mesh was used for column chromatography. ^{1}H and ^{13}C NMR, 2D COSY, HMQC spectra were recorded on Brucker Avance DRX 500 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 mass spectrometer. Elementary analysis was

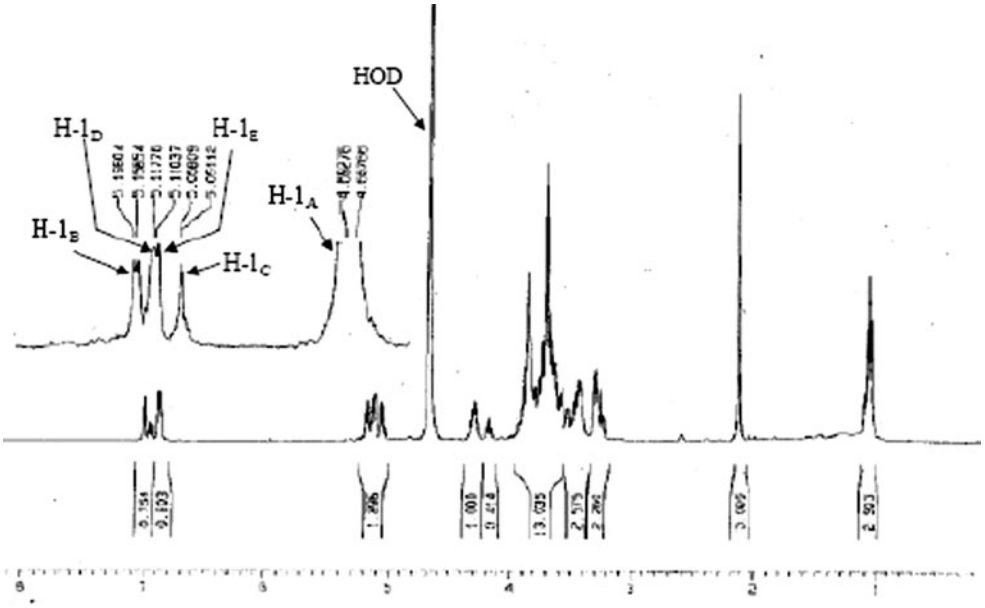
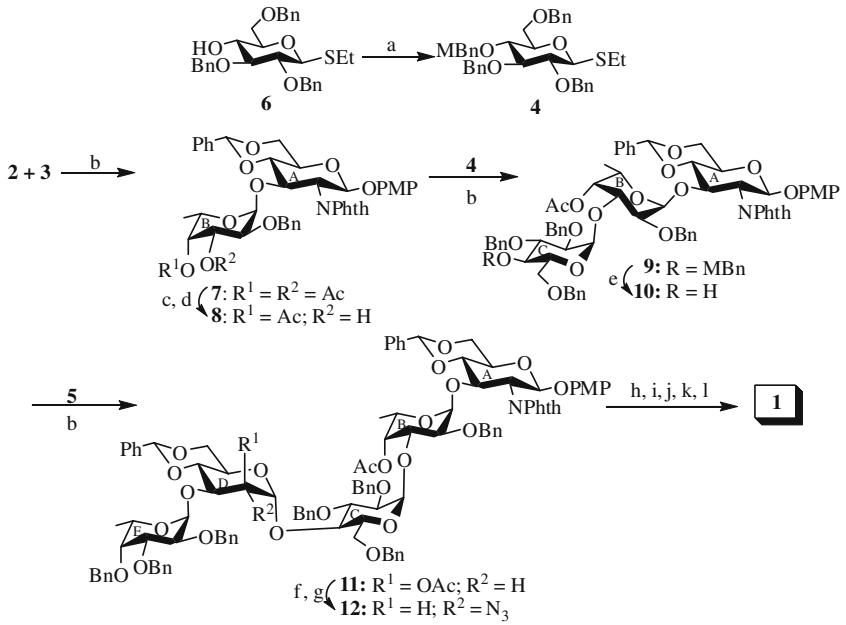


Fig. 4 ^{1}H NMR spectrum of synthesized pentasaccharide as its 4-methoxyphenyl glycoside (**1**)

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.

Ethyl 2,3,6-tri-O-benzyl-4-O-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside (4) To a solution of compound **6** (2.0 g, 4.04 mmol) in dry DMF (10 mL) were added powdered NaOH (0.5 g, 12.5 mmol) and 4-methoxybenzyl chloride (1.0 mL, 7.37 mmol) and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was poured into water and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was



Scheme 1 Reagents: (a) 4-Methoxybenzyl chloride, NaOH, DMF, room temperature, 3 h, 89%; (b) *N*-iodosuccinimide (NIS), TMSOTf, CH_2Cl_2, −30°C, 1 h, 85% for **7**, 81% for **9** and 80% for **11**; (c) 0.05 M CH_3ONa, CH_3OH, room temperature, 1 h; (d) (i) triethylorthoacetate, *p*-TsOH, DMF, room temperature, 1 h; (ii) 80% AcOH, room temperature, 30 min, 90% in two steps; (e) DDQ, CH_2Cl_2, H_2O, room temperature, 2 h, 75%; (f) 0.05 M CH_3ONa, CH_3OH, room temperature, 2 h; (g) (i) triflic anhydride, pyridine, CH_2Cl_2, 0°C, 8 h; (ii) NaN_3, DMF, 70°C, 3 h, 76% in two steps; (h) (i) ethylene diamine, *n*-BuOH, 90°C, 6 h; (ii) acetic anhydride, pyridine, room temperature, 2 h; (i) H_2, 20% $Pd(OH)_2$-C, CH_3OH-EtOAc (1:1 v/v), room temperature, 6 h; (j) (i) acetic anhydride, pyridine, room temperature, 2 h; (ii) 0.1 M CH_3ONa, CH_3OH, room temperature, 5 h; (k) (i) NaBr, CH_2Cl_2, H_2O, TBAB, TEMPO, $NaHCO_3$, NaOCl, 0–5°C, 3 h; (ii) *tert*-butanol, 2-methyl-but-2-ene, $NaClO_2$, NaH_2PO_4, room temperature, 3 h; (l) H_2, 20% $Pd(OH)_2$-C, CH_3OH, room temperature, 24 h, 57% in seven steps

purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure **4** (2.2 g, 89%) as white solid. m.p. 82°C; $[\alpha]_D^{25}$ −5.2 (*c* 1.6, $CHCl_3$); IR (KBr): 3436, 3061, 3030, 2954, 2905, 2788, 1962, 1759, 1614, 1587, 1515, 1496, 1452, 1401, 1362, 1250, 1083, 990, 950, 737, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.25–7.18 (m, 15 H, Ar-H), 6.97 (d, *J*=8.6 Hz, 2 H, Ar-H), 6.68 (d, *J*=8.6 Hz, 2 H, Ar-H), 4.82 (2 d, *J*= 11.0 Hz, 2 H, PhCH_2), 4.76 (d, *J*=11.0 Hz, 1 H, PhCH_2), 4.63 (2 d, *J*=11.0 Hz, 2 H, PhCH_2), 4.51 (d, *J*=12.0 Hz, 1 H, PhCH_2), 4.45 (d, *J*=12.0 Hz, 1 H, PhCH_2), 4.40 (d, *J*= 10.5 Hz, 1 H, PhCH_2), 4.35 (d, *J*=9.7 Hz, 1 H, H-1), 3.67 (s, 3 H, OCH_3), 3.64 (dd, *J*=10.9, 1.8 Hz, 1 H, H-6_a), 3.58–3.53 (m, 2 H, H-3, H-6_b), 3.49 (t, *J*=9.4 Hz, 1 H, H-4), 3.35–3.32 (m, 2 H, H-2, H-5), 2.69–2.64 (m, 2 H, SC$H_2$$CH_3$), 1.25 (t, *J*=7.4 Hz, 3 H, SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.7–114.2 (Ar-*C*), 87.1 (C-1), 85.5 (C-5), 82.2 (C-2), 79.5 (C-3), 78.0 (C-4), 76.0 (Ph*C*H_2), 75.8 (Ph*C*H_2), 75.0 (Ph*C*H_2), 73.8 (Ph*C*H_2), 69.5 (C-6), 55.6 (O*C*H_3), 25.3 (S*C*$H_2$$CH_3$), 15.6 ($SCH_2$*C*$H_3$); ESI-MS: 637.2 $[M+Na]^+$; Anal. Calcd. for $C_{37}H_{42}O_6S$ (614.27): C, 72.28; H, 6.89%; found: C, 72.10; H, 7.12%.

*4-Methoxyphenyl (3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**7**)* To a solution of compound **2** (2.0 g, 3.97 mmol) and compound **3** (1.8 g, 4.70 mmol) in anhydrous CH_2Cl_2 (20 mL) was added MS 4Å (5 g) and the reaction mixture was allowed to stir at room temperature for 1 h under argon. The reaction mixture was cooled to −30°C and *N*-iodosuccinimide (NIS; 1.3 g, 5.77 mmol) followed by trimethylsilyltrifluoromethane sulfonate (TMSOTf; 25 μL) were added to it and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was quenched with Et_3N (0.1 mL), filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated to dryness. The crude product was purified over SiO_2 using hexane-EtOAc (6:1) as eluant to give pure **7** (2.8 g, 85%) as white solid. m.p. 79°C; $[\alpha]_D^{25}$ −26.7 (*c* 1.6, $CHCl_3$); IR (KBr): 3476, 3064, 3033, 2981, 2932, 2873, 1778, 1738, 1714, 1507, 1455, 1390, 1243, 1222, 1099, 1031, 997, 965, 755, 722, 699, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.68–7.19 (m, 14 H, Ar-H), 6.84 (d, *J*=9.1 Hz, 2 H, Ar-H), 6.72 (d, *J*=9.0 Hz, 2 H, Ar-H), 5.75 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.57 (s, 1 H, PhC*H*), 5.12 (dd, *J*=10.6, 3.2 Hz, 1 H, H-3_B), 5.07–5.06 (m, 1 H, H-4_B), 4.82 (d, *J*=3.4 Hz, 1 H, H-1_B), 4.78 (dd, *J*=8.8, 8.8 Hz, 1 H, H-3_A), 4.61 (dd, *J*=8.5, 8.5 Hz, 1 H, H-2_A), 4.43–4.40 (m, 1 H, H-6_{aA}), 4.28–4.24 (m, 1 H, H-5_B), 4.06 (d, *J*=12.9 Hz, 1 H, PhCH_2), 3.94 (d, *J*=12.9 Hz, 1 H, PhCH_2), 3.90–3.86 (m, 1 H, H-6_{6A}), 3.82 (t, *J*=9.1 Hz, 1 H, H-4_A), 3.79–3.74 (m, 1 H, H-5_A), 3.70 (s, 3 H, OCH_3), 3.52 (dd, *J*=10.5, 3.5 Hz, 1 H, H-2_B), 1.93 (s, 3 H, COCH_3), 1.69 (s, 3 H, COCH_3), 0.54 (d, *J*=6.4 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.1 (2 *C*OCH_3), 168.2 (2 C, Phth), 156.0–114.9 (Ar-*C*), 102.5 (Ph*C*H), 99.5 ($J_{C\text{-}1/H\text{-}1}$=171 Hz; C-$1_B$), 98.7 ($J_{C\text{-}1/H\text{-}1}$= 163.2 Hz; C-$1_A$), 81.6 (C-$5_A$), 75.6 (C-$3_A$), 73.2 (C-$2_B$), 73.1 (Ph*C*$H_2$), 72.1 (C-$4_B$), 70.8 (C-$3_B$), 69.1 (C-$6_A$), 67.0 (C-$4_A$), 65.5 (C-$5_B$), 56.1 (C-$2_A$), 56.0 (O*C*$H_3$), 20.9 (2 C, CO*C*$H_3$), 15.5 (C*C*$H_3$); ESI-MS: 846.2 $[M+Na]^+$; Anal. Calcd. for $C_{45}H_{45}NO_{14}$ (823.28): C, 65.61; H, 5.51%; found: C, 65.39; H, 5.76%.

*4-Methoxyphenyl (4-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**8**)* A solution of compound **7** (2.7 g, 3.28 mmol) in 0.05 M CH_3ONa in CH_3OH (50 mL) was allowed to stir at room temperature for 1 h and neutralized with Dowex-50W X8 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure. To a solution of the crude product in dry DMF (20 mL) was added triethylorthoacetate (3.0 mL, 16.36 mmol) followed by *p*-TsOH (250 mg) and the reaction mixture was allowed to stir at room temperatute for 1 h. The solvents were removed under reduced pressure and a solution of the crude orthoester derivative in 80% aq. AcOH (50 mL) was allowed to stir at room temperature for 30 min. The reaction mixture was evaporated and co-evaporated with toluene (3×100 mL) to give the crude product, which was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure **8** (2.3 g, 90%) as white solid. m.p. 81°C; $[\alpha]_D^{25}$ −26.7 (*c* 1.6, $CHCl_3$); IR (KBr): 3480, 3061, 3023, 2961, 2932, 1778, 1738, 1714, 1507, 1455, 1390, 1243, 1222, 1099, 1031, 997, 965, 754, 722, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.57–7.13 (m, 14 H, Ar-H), 6.74 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.64 (d, *J*= 9.1 Hz, 2 H, Ar-H), 5.70 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.48 (s, 1 H, PhC*H*), 4.93–4.92 (m, 1 H, H-4_B), 4.78 (d, *J*=3.3 Hz, H-1_B), 4.64 (dd, *J*=8.5, 8.5 Hz, 1 H, H-3_A), 4.49 (dd, *J*=8.5, 8.5 Hz, 1 H, H-2_A), 4.36–4.33 (m, 1 H, H-6_{aA}), 4.17–4.12 (m, 2 H, H-5_B, PhCH_2), 3.87 (dd, *J*=10.0, 3.5 Hz, 1 H, H-3_B), 3.82 (d, *J*=12.5 Hz, 1 H, PhCH_2), 3.79–3.65 (m, 2 H, H-4_A, H-5_A), 3.63 (s, 3 H, OCH_3), 3.27 (dd, *J*=10.0, 3.3 Hz, 1 H, H-2_B), 1.90 (s, 3 H, COCH_3), 0.63 (d, *J*=6.5 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.2 (*C*OCH_3), 168.8 (2 C, Phth), 156.0–114.9 (Ar-*C*), 102.1 (Ph*C*H), 98.8 (C-1_B), 98.6 (C-1_A), 81.9 (C-5_A), 76.0 (C-2_B), 75.5 (C-3_A), 73.9 (C-4_B), 72.7 (Ph*C*H_2), 69.0 (C-6_A), 68.4 (C-3_B), 66.9 (C-4_A), 65.9 (C-5_B), 56.2 (C-2_A), 55.9 (O*C*H_3), 21.1 (CO*C*H_3), 16.0 (C*C*H_3); ESI-MS: 804.2 $[M+Na]^+$; Anal. Calcd. for $C_{43}H_{43}NO_{13}$ (781.27): C, 66.06; H, 5.54%; found: C, 65.83; H, 5.80%.

*4-Methoxyphenyl [2,3,6-tri-O-benzyl-4-O-(4-methoxybenzyl)-α-D-glucopyranosyl]-(1→3)-(4-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**9**)* To a solution of

compound **8** (2.1 g, 2.68 mmol) and compound **4** (2.0 g, 3.25 mmol) in anhydrous CH_2Cl_2 (20 mL) was added MS 4Å (4 g) and the reaction mixture was allowed to stir at room temperature for 1 h under argon. The reaction mixture was cooled to −30°C and NIS (0.9 g, 4.0 mmol) followed by TMSOTf (10 μL) were added to it and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was quenched with Et_3N (0.1 mL), filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated to dryness. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure **9** (2.9 g, 81%) as white solid. m.p. 86°C; $[\alpha]_D^{25}$ +43.7 (*c* 1.6, $CHCl_3$); IR (KBr): 3450, 3063, 3031, 2932, 2869, 1778, 1741, 1715, 1507, 1455, 1390, 1371, 1222, 1100, 1028, 969, 965, 755, 722, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.36–7.05 (m, 33 H, Ar-H), 6.78 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.73 (d, *J*=9.1 Hz, 2 H, Ar-H), 5.75 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.51 (s, 1 H, PhC*H*), 5.05 (br s, 1 H, H-4_B), 4.83 (d, *J*= 3.6 Hz, 1 H, H-1_C), 4.80 (d, *J*=11.8 Hz, 1 H, PhCH_2), 4.75 (d, *J*=11.8 Hz, 1 H, PhCH_2), 4.67 (d, *J*=3.4 Hz, 1 H, H-1_B), 4.70–4.64 (m, 4 H, H-3_A, PhCH_2), 4.57 (dd, *J*=8.5, 8.5 Hz, 1 H, H-2_A), 4.50–4.39 (m, 6 H, H-6_{aA}, PhCH_2), 4.30 (d, *J*= 12.9 Hz, 1 H, H-6_{aC}), 4.15–4.13 (m, 1 H, H-5_B), 4.07 (dd, *J*= 10.0, 3.5 Hz, 1 H, H-3_B), 3.86–3.78 (m, 1 H, H-6_{bA}), 3.75 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 3.78–3.68 (m, 5 H, H-3_C, H-4_A, H-5_A, H-5_C, H-6_{bC}), 3.59 (t, *J*=9.1 Hz, 1 H, H-4_C), 3.50 (dd, *J*=10.2, 3.3 Hz, 1 H, H-2_B), 3.35 (dd, *J*=9.8, 3.6 Hz, 1 H, H-2_C), 1.99 (s, 3 H, COCH_3), 0.58 (d, *J*= 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8 (*C*OCH_3), 168.7 (2 C, Phth), 159.4–114.0 (Ar-*C*), 102.2 (Ph*C*H), 99.9 ($J_{C\text{-}1/H\text{-}1}$=171 Hz; C-$1_B$), 99.0 ($J_{C\text{-}1/H\text{-}1}$= 172 Hz; C-$1_C$), 98.6 ($J_{C\text{-}1/H\text{-}1}$=164 Hz; C-$1_A$), 81.9 (C-$5_A$), 81.8 (C-$5_C$), 80.0 (C-$2_C$), 77.7 (C-$4_C$), 76.0 (C-$3_A$), 75.7 (Ph*C*$H_2$), 75.0 (C-$2_B$), 74.6 (Ph*C*$H_2$), 74.5 (C-$4_B$), 74.4 (C-$3_C$), 73.8 (Ph*C*$H_2$), 72.89, 72.82 (2 Ph*C*$H_2$), 71.4 (C-$3_B$), 69.1 (C-$6_A$), 69.0 (C-$6_C$), 66.9 (C-$4_A$), 66.3 (C-$5_B$), 56.1 (C-$2_A$), 55.9, 55.5 (2 OC$H_3$), 21.3 (CO*C*$H_3$), 16.1 (C*C*$H_3$); ESI-MS: 1356.5 $[M+Na]^+$; Anal. Calcd. for $C_{78}H_{79}NO_{19}$ (1333.52): C, 70.20; H, 5.97%; found: C, 70.00; H, 6.22%.

*4-Methoxyphenyl (2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (***10***)* To a solution of **9** (2.5 g, 1.87 mmol) in CH_2Cl_2 (30 mL) was added a solution of 2,3-dicholoro-5,6-dicyano-*p*-benzoquinone (DDQ, 850 mg, 3.74 mmol) in H_2O (15 mL) and the biphasic reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (100 mL). The organic layer was successively 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 **10** (1.7 gmg, 75%) as white solid. m.p. 84°C; $[\alpha]_D^{25}$ +40.2 (*c* 1.6, $CHCl_3$); IR (KBr): 3476, 3087, 3063, 3032, 2933, 2870, 1778, 1740, 1715, 1507, 1454, 1390, 1372, 1222, 1099, 1058, 1027, 969, 965, 829, 755, 738, 722, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.38–7.13 (m, 29 H, Ar-H), 6.82 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.71 (d, *J*=9.1 Hz, 2 H, Ar-H), 5.76 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.48 (s, 1 H, PhC*H*), 5.05 (br s, 1 H, H-4_B), 4.85 (d, *J*=11.4 Hz, 1 H, PhCH_2), 4.80 (d, *J*=3.3 Hz, 1 H, H-1_C), 4.73 (d, *J*=11.4 Hz, 1 H, PhCH_2), 4.67 (d, *J*=3.3 Hz, 1 H, H-1_B), 4.64–4.63 (m, 4 H, PhCH_2), 4.57 (dd, *J*=8.5, 8.5 Hz, 1 H, H-2_A), 4.44–4.35 (m, 3 H, H-3_A, PhCH_2), 4.25 (d, *J*=12.9 Hz, 1 H, H-6_{aA}), 4.15–4.13 (m, 1 H, H-5_B), 4.10 (dd, *J*=10.0, 3.5 Hz, 1 H, H-3_B), 3.85–3.72 (m, 6 H, H-3_C, H-4_C, H-5_A, H-6_{bA}, H-6_{abC}), 3.68 (s, 3 H, OCH_3), 3.59–3.58 (m, 2 H, H-4_A, H-5_C), 3.50 (dd, *J*=10.2, 3.3 Hz, 1 H, H-2_B), 3.32 (dd, *J*=9.8, 3.6 Hz, 1 H, H-2_C), 2.00 (s, 3 H, COCH_3), 0.62 (d, *J*= 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.1 (*C*OCH_3), 167.9 (2 C, Phth), 156.0–114.9 (Ar-*C*), 102.2 (Ph*C*H), 99.8 (C-1_B), 98.7 (C-1_C), 98.6 (C-1_A), 81.9 (C-5_A), 81.3 (C-5_C), 79.6 (C-2_C), 76.1 (C-2_B), 75.4 (Ph*C*H_2), 75.0 (C-4_C), 74.5 (Ph*C*H_2), 73.7 (C-4_B), 72.9, 72.8 (2 Ph*C*H_2), 71.8 (C-3_B), 71.7 (C-3_C), 70.1 (C-6_C), 69.1 (C-6_A), 67.0 (C-4_A), 66.1 (C-5_B), 56.1 (C-2_A), 55.9 (OCH_3), 21.3 (CO*C*H_3), 16.1 (C*C*H_3); ESI-MS: 1236.4 $[M+Na]^+$; Anal. Calcd. for $C_{70}H_{71}NO_{18}$ (1213.46): C, 69.24; H, 5.89%; found: C, 69.00; H, 6.15%.

*4-Methoxyphenyl (2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-(2-O-acetyl-4,6-O-benzylidene-α-D-mannopyranosyl)-(1→4)-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (***11***)* To a solution of compound **10** (1.5 g, 1.23 mmol) and compound **5** (1.1 g, 1.42 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS 4Å (3 g) and the reaction mixture was allowed to stir at room temperature for 1 h under argon. The reaction mixture was cooled to −30°C and NIS (380 mg, 1.68 mmol) followed by TMSOTf (5 μL) were added to it and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was quenched with Et_3N (0.1 mL), filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated to dryness. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure **11** (1.9 g, 80%) as white solid; m.p. 80°C; $[\alpha]_D^{25}$ −6.7 (*c* 1.6, $CHCl_3$); IR (KBr): 3473, 3088, 3063, 3031, 2973, 2933, 2870, 1778, 1744, 1716, 1609, 1508, 1454, 1389, 1232, 1101, 1053, 1027, 971, 965, 829, 738, 722, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ

7.37–7.14 (m, 49 H, Ar-H), 6.83 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.71 (d, *J*=9.1 Hz, 2 H, Ar-H), 5.76 (d, *J*=8.5 Hz, 1 H, H-1_A), 5.53 (s, 1 H, PhC*H*), 5.51 (s, 1 H, PhC*H*), 5.40–5.39 (m, 1 H, H-2_D), 5.34 (br s, 1 H, H-1_D), 5.04 (br s, 1 H, H-4_B), 4.94 (d, *J*=2.8 Hz, H-1_E), 4.83 (d, *J*=3.3 Hz, 1 H, H-1_C), 4.90–4.87 (m, 2 H, PhCH_2), 4.79 (d, *J*=11.8 Hz, 1 H, PhCH_2), 4.78–4.63 (m, 6 H, PhCH_2), 4.69 (d, *J*=3.6 Hz, 1 H, H-1_B), 4.57–4.52 (m, 5 H, H-2_A, H-3_A, PhCH_2), 4.45-4.36 (m, 1 H, H-3_E), 4.35–4.33 (m, 2 H, PhCH_2), 4.32 (d, *J*= 12.9 Hz, 1 H, H-6_{aA}), 4.21 (dd, *J*=10.0, 3.5 Hz, 1 H, H-3_B), 4.16 (m, 1 H, H-5_B), 4.12 (m, 1 H, H-5_E), 4.07–4.06 (m, 1 H, H-4_D), 3.97–3.78 (m, 8 H, H-2_E, H-3_C, H-3_D, H-4_C, H-4_E, H-6_{bA}, H-6_{abD}), 3.75–3.67 (m, 5 H, H-4_A, H-5_A, H-5_C, H-6_{abC}), 3.69 (s, 3 H, OCH_3), 3.55 (dd, *J*=10.2, 3.3 Hz, 1 H, H-2_B), 3.52 (m, 1 H, H-5_D), 3.32 (dd, *J*=9.8, 3.6 Hz, 1 H, H-2_C), 2.00 (s, 3 H, COCH_3), 1.70 (s, 3 H, COCH_3), 0.97 (d, *J*= 6.5 Hz, 3 H, CCH_3), 0.63 (d, *J*=6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.0 (2 *C*OCH$_3$), 167.7 (2 C, Phth), 156.0–114.9 (Ar-*C*), 102.2 (Ph*C*H), 101.9 (Ph*C*H), 99.9 (($J_{C\text{-}1/H\text{-}1}$=171 Hz; C-$1_B$), 99.8 ($J_{C\text{-}1/H\text{-}1}$= 174 Hz; C-$1_D$), 98.7 (2 C, C-$1_A$, C-$1_C$), 94.0 ($J_{C\text{-}1/H\text{-}1}$= 172 Hz; C-$1_E$), 82.0 (C-$5_A$), 81.4 (C-$5_C$), 80.2 (C-$2_E$), 79.8 (C-$2_C$), 78.5 (C-$5_D$), 77.2 (C-$3_D$), 76.4 (3 C, C-$2_B$, C-$2_C$, C-$4_C$), 76.3 (C-$3_E$), 75.3 (Ph*C*H$_2$), 75.2 (Ph*C*H$_2$), 75.0 (C-$3_A$), 74.7 (C-$4_B$), 74.6 (Ph*C*H$_2$), 74.3 (C-$3_C$), 73.9 (Ph*C*H$_2$), 73.5 (Ph*C*H$_2$), 73.4 (Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 70.8 (C-$2_D$), 69.3 (C-$6_A$), 69.1 (2 C, C-$6_D$, C-$6_C$), 68.8 (C-$4_D$), 67.0 (C-$5_E$), 66.6 (C-$4_A$), 66.3 (C-$5_B$), 65.3 (C-$4_E$), 56.1 (C-$2_A$), 55.9 (O*C*H$_3$), 21.2, 20.9 (2 *C*OCH$_3$), 16.9, 16.2 (2 C*C*H$_3$); MALDI-MS: 1944.7 $[M+Na]^+$; Anal. Calcd. for $C_{112}H_{115}NO_{28}$ (1921.76): C, 69.95; H, 6.03%; found: C, 69.72; H, 6.28%.

*4-Methoxyphenyl (2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-(2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (***12***)* A solution of compound **11** (1.6 g, 0.83 mmol) in 0.05 M CH_3ONa in CH_3OH (50 mL) was allowed to stir at room temperature for 2 h and neutralized with Dowex-50W X8 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure. To a solution of the crude product in anhydrous CH_2Cl_2 (20 mL) was added pyridine (5 mL) and the reaction mixture was cooled to 0°C. Trifluoromethanesulfonic anhydride (triflic anhydride; 300 μL, 1.78 mmol) was added to the cold reaction mixture and it was allowed to stir at 0°C for 8 h. The solvents were removed under reduced pressure and the crude product was used directly for the next step. To a soultion of the crude product in dry DMF (5 mL) was added NaN_3 (1.3 g, 20 mmol) and the reaction mixture was allowed to stir at 70°C for 3 h. The reaction mixture was poured into cold water and extracted with EtOAc (100 mL). The organic layer was washed with satd. aq. $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **12** (1.2 g, 76%) as yellow oil. $[\alpha]_D^{25}$ +11.2 (*c* 1.6, $CHCl_3$); IR (neat): 3474, 3089, 3063, 3033, 2975, 2933, 2870, 1778, 1745, 1716, 1609, 1508, 1455, 1371, 1312, 1232, 1132, 1101, 1053, 1027, 971, 965, 829, 738, 722 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32–7.14 (m, 49 H, Ar-H), 6.83 (d, *J*=9.0 Hz, 2 H, Ar-H), 6.71 (d, *J*=9.1 Hz, 2 H, Ar-H), 5.75 (d, *J*= 8.5 Hz, 1 H, H-1_A), 5.52 (s, 1 H, PhC*H*), 5.50 (s, 1 H, PhC*H*), 5.44 (br s, 1 H, H-1_D), 5.31 (d, *J*=3.2 Hz, 1 H, H-1_E), 5.08 (br s, 1 H, H-4_B), 4.94 (d, *J*=11.5 Hz, 1 H, PhCH_2),), 4.87 (d, *J*=11.3 Hz, 1 H, PhCH_2), 4.82 (d, *J*= 11.3 Hz, 1 H, PhCH_2), 4.81–4.79 (m, 2 H, H-1_C, H-2_D), 4.69–4.64 (m, 6 H, H-1_B, PhCH_2), 4.62–4.55 (m, 5 H, H-2_A, PhCH_2), 4.39–4.36 (m, 3 H, H-3_A, PhCH_2), 4.26 (d, *J*= 12.9 Hz, 1 H, H-6_{aA}), 4.17 (m, 1 H, H-5_B), 4.11–4.08 (m, 3 H, H-3_B, H-4_D, H-5_E), 4.01 (m, 1 H, H-2_E), 3.98–3.95 (m, 2 H, H-3_E, H-4_A), 3.83–3.81 (m, 2 H, H-3_C, H-3_D), 3.79-3.68 (m, 9 H, H-4_C, H-4_E, H-5_A, H-5_C, H-6_{bA}, H-6_{abC}, H-6_{abD}), 3.71 (s, 3 H, OCH_3), 3.55 (br s, 1 H, H-5_D), 3.51 (dd, *J*=10.0, 3.3 Hz, 1 H, H-2_B), 3.3 (m, 1 H, H-2_C), 2.01 (s, 3 H, COCH_3), 1.07 (d, *J*=6.5 Hz, 3 H, CCH_3), 0.58 (d, *J*=6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6 (*C*OCH$_3$), 167.9 (2 C, Phth), 156.0–114.9 (Ar-*C*), 102.3 (Ph*C*H), 102.2 (Ph*C*H), 99.9 (C-1_B), 98.7 (C-1_A), 98.4 (C-1_C), 97.8 (C-1_D), 95.3 (C-1_E), 82.1 (C-5_A), 81.9 (C-5_C), 80.3 (C-2_E), 79.3 (2 C, C-2_C, C-5_D), 78.3 (C-3_D), 76.1 (2 C, C-2_B, C-3_B), 76.0 (C-4_C), 75.6 (Ph*C*H$_2$), 75.3 (Ph*C*H$_2$), 74.9 (C-3_E), 74.6 (C-3_A), 74.3 (C-4_B), 74.2 (C-2_D), 73.8 (2 C, 2 Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 71.1 (C-3_C), 69.5 (C-C_{6A}), 69.4 (C-6_C), 69.1 (2 C, C-4_D, C-6_D), 67.9 (C-5_E), 67.0 (C-4_A), 66.3 (C-5_B), 64.6 (C-4_E), 56.1 (C-2_A), 55.9 (O*C*H$_3$), 21.2, (CO*C*H$_3$), 16.9, 16.1 (2 C*C*H$_3$); MALDI-MS: 1927.7 $[M+Na]^+$; Anal. Calcd. for $C_{110}H_{112}N_4O_{26}$ (1904.75): C, 69.31; H, 5.92%; found: C, 69.07; H, 6.18%.

*4-Methoxyphenyl (α-L-fucopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-(sodium α-D-glucopyranosyl uronate)-(1→3)-(α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (***1***)* To a solution of compound **12** (1.0 g, 0.52 mmol) in *n*-butanol (20 mL) was added ethylene diamine (0.2 mL, 3.0 mmol) and the reaction mixture was allowed to stir at 90°C for 6 h and the solvents were removed under reduced pressure. A solution of the crude mass in acetic anhydride-pyridine (3 mL, 1:1 v/v) was kept at room temperature for 2 h and solvents were removed under reduced pressure and the crude reaction product was passed through a short pad of SiO_2 using hexane-EtOAc (1:1) as eluant. To a solution of the acetylated product in CH_3OH-EtOAc (10 mL, 1:1 v/v)

was added 20% $Pd(OH)_2$-C (150 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of hydrogen for 6 h. The reaction mixture was filtered through a Celite® bed and the washed with CH_3OH (100 mL). A solution of the hydrogenolyzed product in acetic anhydride-pyridine (3 mL, 1:1 v/v) was kept at room temperature for 2 h and solvents were removed under reduced pressure. A solution of the crude mass in 0.1 M sodium methoxide (30 mL) was allowed to stir at room temperature for 5 h and neutralized with Dowex-50W X8 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure. To a solution of the crude product in CH_2Cl_2 (30 mL) and H_2O (5 mL) were added aq. solution of NaBr (2 mL; 1 M), aq. solution of TBAB (2 mL; 1 M), TEMPO (80 mg, 0.6 mmol), satd. aq. solution of $NaHCO_3$ (10 mL) and 4% aq. NaOCl (15 mL) in succession and the reaction mixture was allowed to stir at 0–5°C for 3 h. The reaction mixture was neutralized with the addition of 1 N aq. HCl solution. To the reaction mixture were added *tert*-butanol (20 mL), 2-methyl-but-2-ene (20 mL; 2 M solution in THF), aq. solution of $NaClO_2$ (2 g in 10 mL) and aq. solution of NaH_2PO_4 (2 g in 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. The crude product was passed through a short pad of SiO_2 using CH_2Cl_2-CH_3OH (10:1) as eluant. To a solution of the oxidized product in CH_3OH (10 mL) was added 20% Pd $(OH)_2$-C (150 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 the washed with CH_3OH-H_2O (60 mL; 1:1 v/v) and concentrated under reduced pressure. The crude product was purified through a Sephadex LH-20 column using CH_3OH-H_2O (2:1) as eluant to give pure compound **1** (300 mg, 57%) as a sodium salt as white powder. $[\alpha]_D^{25}$ +8.36 (*c* 1.6, H_2O); IR (KBr): 3410, 2928, 2855, 1709, 1646, 1585, 1566, 1508, 1445, 1392, 1221, 1149, 1082, 1030, 969, 926, 756, 700 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.18 (d, J=3.7 Hz, 1 H, H-1_B), 5.16 (br s, 1 H, H-1_D), 5.15 (br s, 1 H, H-1_E), 5.05 (d, J=3.4 Hz, 1 H, H-1_C), 4.69 (br s, 1 H, H-1_A), 4.32–4.28 (m, 2 H, H-5_B, H-5_E), 4.18–4.10 (m, 1 H, H-2_A), 3.89–3.79 (m, 7 H, H-2_E, H-3_A, H-3_D, H-3_E, H-4_D, H-6_{abA}), 3.75–3.60 (m, 9 H, H-2_D, H-3_B, H-3_C, H-4_A, H-4_C, H-6_{abC}, H-6_{abD}), 3.69 (s, 3 H, OCH_3), 3.45–3.41 (m, 4 H, H-4_B, H-4_E, H-5_C, H-5_D), 3.31–3.28 (m, 3 H, H-2_B, H-2_C, H-5_A), 2.12 (s, 6 H, 2 COCH_3), 1.07–1.04 (m, 6 H, 2 CCH_3), ^{13}C NMR (125 MHz, D_2O): δ 177.3 (*C*OONa), 173.2 (*C*OCH$_3$), 172.4 (*C*OCH$_3$), 100.5 (2 C, C-1_D, C-1_E), 100.1 (C-1_A), 99.9 (C-1_B), 99.5 (C-1_C), 78.0 (C-3_A), 77.1 (C-4_D), 76.4 (C-4_C), 76.1 (C-3_C), 73.3 (3 C, C-2_C, C-3_D, C-5_A), 72.7 (2 C, C-4_B, C-5_C), 72.2 (4 C, C-3_E, C-4_A, C-4_E, C-5_D), 70.1 (3 C, C-2_B, C-2_E, C-3_B), 67.4 (C-5_B), 66.8 (C-5_E), 61.3 (C-2_D), 61.1 (2 C, C-6_A, C-6_D), 56.2 (O*C*H$_3$), 54.9 (C-2_A), 23.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 15.7, 15.6 (2 C*C*H$_3$); ESI-MS: 1021.3 $[M+1]^+$; Anal. Calcd. for $C_{41}H_{61}N_2NaO_{26}$ (1020.34): C, 48.24; H, 6.02%; found: C, 48.00; H, 6.29%.

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