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ORIGINAL ARTICLE

Straightforward synthesis of the pentasaccharide repeating unit of the cell wall *O*-antigen of *Escherichia coli* O43 strain

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Abstract

A concise synthetic strategy has been developed for the synthesis of the pentasaccharide repeating unit of the cell wall *O*-antigen of *Escherichia coli* O43 strain involving stereoselective β-D-mannosylation and α-L-fucosylation using corresponding trichloroacetimidate intermediates and perchloric acid supported over silica ($HClO_4$-SiO_2) as glycosylation promoter. The yield and stereoselectivity of the glycosylations were very good.

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

Introduction

Diarrhoeal outbreaks associated with gastrointestinal infections are serious health concern in the developing countries due to the lack of adequate sanitation [1]. In the recent past, it became severe health hazard in the developed countries also due to the intake of contaminated food and water [2]. Most frequently found bacteria causing enteric infections are *Shigella* [3], *Salmonella* [4], *Vibrio Cholerae* [5], enteropathogenic *Escherichia coli* (*E. coli*) [6], *Campylobacter jejuni* [7] etc. Among several enteropathogenic microbes causing diarrhoeal infections, pathogenic *Escherichia coli* (*E. coli*) strains are important. *E. coli* is a Gram-negative, facultative anaerobic pathogen predominantly found in the gastrointestinal tract of humans [8]. Despite their harmless existence in the gut flora and beneficial contribution, a certain species of *E. coli* acquired virulence factors and causes severe intestinal and urinary infections in humans and animals [9, 10]. They are associated with gastrointestinal infections, particularly "traveller's diarrhoea" [11] and classified in several sub-types such as, enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenix *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) etc. [12]. Among different sub-classes, shiga-toxin (ST) producing EHEC strains are associated with diarrhoeal diseases with life threatening complications such as haemorrhagic colitis [13]. Shiga-toxin producing *E. coli* O43 strain belongs to EHEC and has been responsible for several diarrhoeal outbreaks in the past [14]. Because of the important role of the cell wall polysaccharides of the pathogenic bacterial strains during the initial stage of infection to the host, a large number of polysaccharides from several bacterial strains have been characterized. Recently, the structure of the repeating unit of the *O*-antigenic polysaccharide of *E. coli* O43 has been reported by Perepelov *et al* [15]. It is a pentasaccharide composed of one β-*N*-acetyl-D-galactosamine, two α-L-fucose, one α-D-mannose and one β-D-mannose moieties. Bacterial cell wall polysaccharides have been used in the preparation of glycoconjugate vaccines because of their notable antigenic properties [16–20]. Development of alternative approaches for the control of bacterial infections is the thrust area in medicinal chemistry to overcome the failure of the antibiotic activities on the multi-drug-resistant (MDR) bacterial strains [21]. Isolation of oligosaccharides from the natural bacterial culture is tedious as well as suffers from several shortcomings such as difficulties associated with the removal of biological impurities, handling of live bacterial strains, heterogeneity in the isolated oligosaccharide fragments, lack of sufficient quantity etc. Alternatively, significant quantities of pure oligosaccharide fragments with conserved structure and free from biological impurities can be obtained by efficient chemical synthesis. Significant quantities of oligosaccharides are required for their detailed biological studies. In this context,

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a straightforward synthesis of the pentasaccharide repeating unit of the *O*-antigenic polysaccharide of *E. coli* O43 is presented herein.

Results and discussion

The target pentasaccharide **1** contains two L-fucose moieties having α-linkages, which require stereoselective 1,2-*cis*-glycosylations for their incorporations. There is a β-linked D-mannose moiety also present in the molecule whose incorporation is considered as a difficult task even after development of a variety of β-D-mannosylation techniques till date. Initially, It was decided to adopt a block glycosylation strategy to carry out stereoselective glycosylation between a disaccharide acceptor with a trisaccharide thioglycoside donor minimizing the number of reaction steps. For this purpose a number of suitably protected monosaccharide intermediates **2** [22], **3** [23], **4, 5** [24], **6** and **7** [25] have been selected, which either were known in the literature or prepared following earlier literature reports (Fig. 1). In order to achieve better yield and stereoselectivity in the glycosylation steps, it was decided to use glycosyl trichloroacetimidate derivatives [26] as glycosyl donors in most of the cases and perchloric acid supported over silica ($HClO_4$-SiO_2) [27] as a solid acid glycosylation promoter for the activation of glycosyl trichloroacetimidate derivatives [28].

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**8**) [29], prepared from D-mannose following earlier reported reaction conditions was treated with levulinic acid in the presence of diisopropyl dicarbodimide (DIC) [30] and DMAP to furnish levulinic acid ester (**9**) in 74% yield. Regioselective reductive ring opening of the benzylidene acetal in compound **9** using a combination of triethylsilane and trifluoroacetic acid (TFA) [31] followed by acetylation of the free hydroxyl group furnished ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-levulinyl-1-thio-α-D-mannopyranoside (**6**) in 68% over all yield (Scheme 1).

Stereoselective 1,2-*cis*-glycosylation of *p*-methoxyphenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (**2**) with 3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-fucopyranosyl trichloroacetimidate (**3**) in the presence of perchloric acid supported over silica ($HClO_4$-SiO_2) [27, 28] as the glycosylation activator in a mixed solvent, CH_2Cl_2–Et_2O (2:3; *v*/v) to enhance the 1,2-*cis* glycosylation [32], furnished the disaccharide derivative, which was subsequently de-*O*-acetylated using sodium methoxide to give the disaccharide acceptor **10** in 67% over all yield. A minor quantity (~5%) of 1,2-*trans* glycosylation product was also formed, which was separated by column chromatography. The NMR spectroscopic analysis

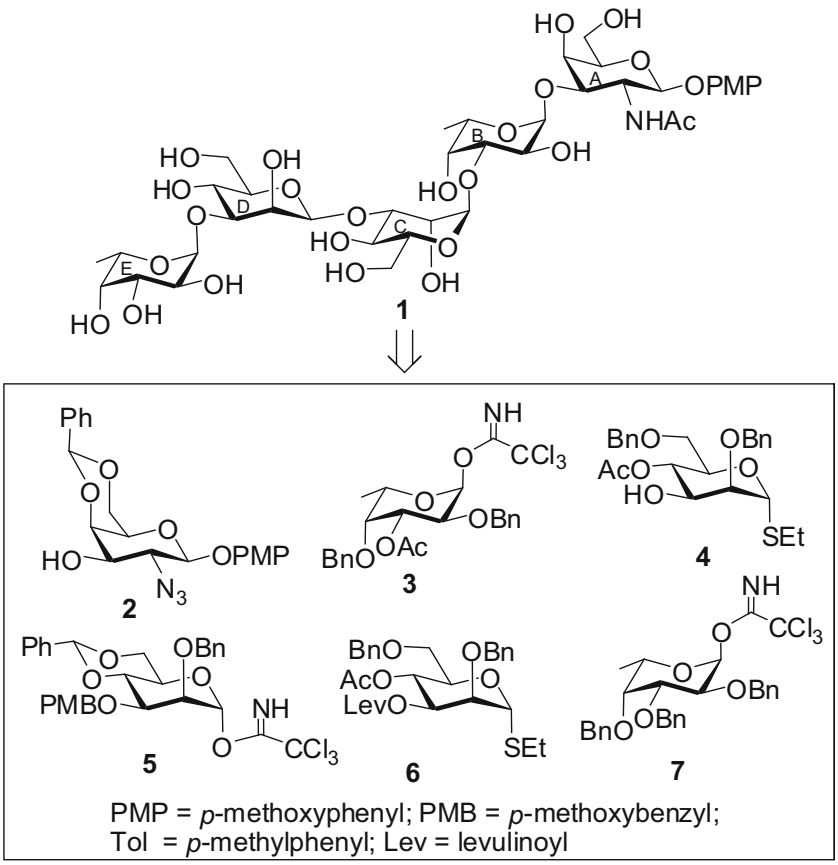
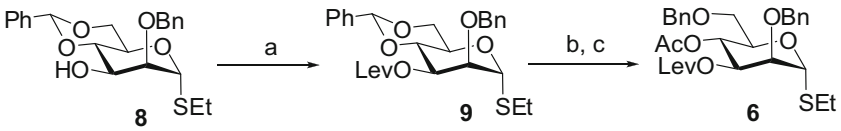


Fig. 1 Structure of the synthesized pentasaccharide repeating unit and its synthetic intermediates



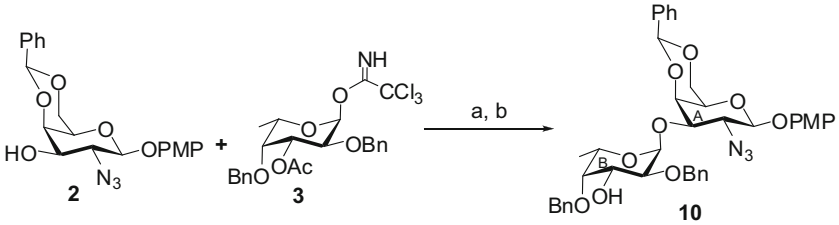
Scheme 1 Reagents and conditions: (a) levulinic acid, DIC, DMAP, CH_2Cl_2, r t, 5 h, 74%; (b) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 3 h; (c) Ac_2O, pyridine, r t, 2 h, 68%

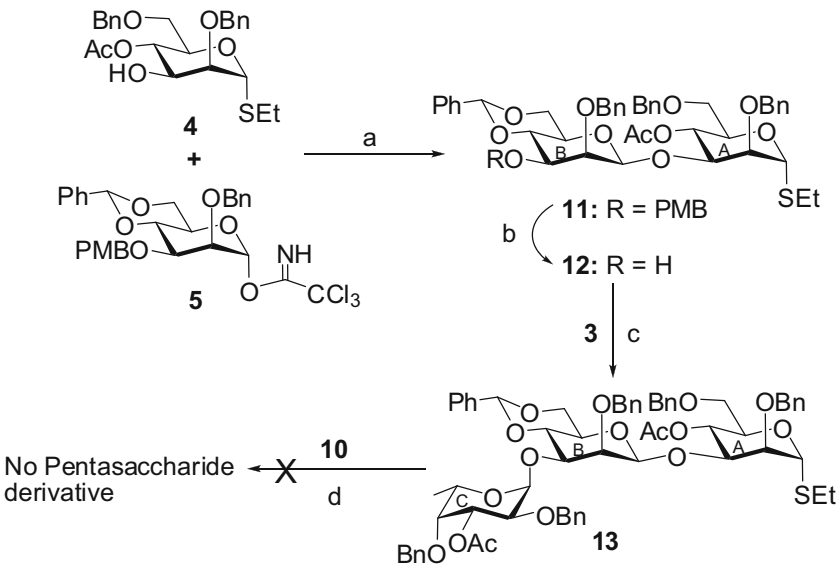
of compound **10** confirmed its formation [signals at δ 5.46 (s, PhC*H*), 5.06 (d, $J = 3.5$ Hz, H-1_B), 4.72 (d, $J = 8.0$ Hz, H-1_A) in ^{1}H NMR and at δ 102.3 (C-1_A), 101.1 (Ph*C*H), 99.9 (C-1_B) in ^{13}C NMR spectra] (Scheme 2)

After having the disaccharide acceptor (**10**), attempts were made to obtain the trisaccharide thioglycoside derivative (**13**). Following a recently reported reaction condition for functional group influenced stereoselective β-mannosylation [24], an orthogonal glycosylation [33] of the D-mannosyl thioglycoside acceptor (**4**) with 3-*O*-*p*-methoxybenzyl (PMB) protected D-mannosyl trichloroacetimidate donor (**5**) [24] in the presence of $HClO_4$-SiO_2 furnished β-mannosidic linkage containing disaccharide thioglycoside derivative **11** in 65% yield. The anomeric thioglycosidic group in the acceptor remained unaffected under the reaction condition. The stereochemistry of the newly formed β-mannosidic linkage was characterized by its NMR spectroscopic analysis [signals at δ 5.54 (s, PhC*H*), 5.47 (br s, H-1_A), 4.01 (br s, H-1_B) in ^{1}H NMR and at δ 101.4 (Ph*C*H), 98.0 ($J_{H1,C1} = 158.5$ Hz, C-1_B, β), 80.8 ($J_{H1,C1} = 174.5$ Hz, C-1_A, α) in ^{13}C NMR spectra]. The incorporation of the β-mannosidic linkage in compound **11** was unambiguously confirmed from its $J_{H1,C1}$ value (158.5 Hz) in the ^{1}H coupled ^{13}C NMR spectrum. The $J_{H1,C1}$ value in ^{1}H coupled ^{13}C NMR spectrum appears in the region of <160 Hz for β-glycosidic linkage whereas, $J_{H1,C1}$ value appears in the region of >170 Hz for α-glycosidic linkage [34, 35]. Oxidative removal of the PMB group from compound **11** using DDQ [36] furnished the disaccharide acceptor **12** in 73% yield, which was allowed to couple with a L-fucosyl trichloroacetimidate derivative (**3**) in the presence of $HClO_4$-SiO_2 in a mixed solvent CH_2Cl_2–Et_2O (2:3; *v*/v) [32] under orthogonal 1,2-*cis*-glycosylation approach [33] without affecting the thioglycoside to furnish trisaccharide thioglycoside derivative **13** in 68% yield together with a minor quantity (~7%) of 1,2-*trans* glycosylation product, which was separated by column chromatography. Spectroscopic analysis of compound **13** confirmed its formation [signals at δ 5.48 (s, PhC*H*), 5.47 (br s, H-1_A), 4.93 (d, $J = 3.5$ Hz, H-1_C), 4.00 (br s, H-1_B) in ^{1}HNMR and at δ 101.7 (Ph*C*H), 97.6 ($J_{H1,C1} = 159.0$ Hz, C-1_B, β), 96.0 ($J_{H1,C1} = 172.0$ Hz, C-1_C, α), 80.7 ($J_{H1,C1} = 174.0$ Hz, C-1_A, α) in ^{13}C NMR spectra]. Next the trisaccharide derivative **13** was allowed to react with the disaccharide acceptor **10** in the presence of a combination of NIS and $HClO_4$-SiO_2 [37] to provide desired pentasaccharide derivative following a block glycosylation strategy. Unfortunately, the expected pentasaccharide derivative was not formed and the decomposition of the trisaccharide donor **13** was observed leaving the disaccharide acceptor **10** unaffected. Application of other thiophilic glycosylation activators such as DMTST [38], NIS-TfOH [39], methyl triflate [40] also did not lead to the formation of the desired product except decomposition of the glycosyl donor **13**. Such unexpected outcome of the block glycosylation strategy forced to identify a different approach for the synthesis of the desired pentasaccharide **1** (Scheme 3).

In this endeavor, a sequential glycosylation strategy has been adopted for the synthesis of the pentasaccharide **1**. Stereoselective glycosylation of the disaccharide acceptor **10** with D-mannosyl thioglycoside donor **6** in the presence of a combination [37] of NIS and $HClO_4$-SiO_2 furnished a trisaccharide derivative, which on subsequent treatment with hydrazine acetate [41] resulted the trisaccharide acceptor **14** in 72% over all yield. NMR spectroscopic analysis of compound **14** supported its formation [signals at δ 5.41 (s, PhC*H*), 5.30 (br s, H-1_C), 5.07 (d, $J = 3.5$ Hz, H-1_B), 4.68 (d, $J = 8.0$ Hz, H-1_A) in ^{1}H NMR and at δ 102.0 (C-1_A), 101.2 (PhCH), 99.9 (C-1_B), 98.8 (C-1_C) in ^{13}C NMR spectra]. Following the recently reported reaction condition [24] on the functional group induced β-mannosidic glycosylation as well as removal of PMB group in one-pot [42] by tuning the reaction condition, treatment of trisaccharide acceptor **14** with D-mannosyl trichloroacetimidate

Scheme 2 Reagents and conditions: (a) $HClO_4$-SiO_2, CH_2Cl_2 –Et_2O (2:3, *v*/v), −10 °C, 2 h; (b) 0.1 M CH_3ONa, CH_3OH, r, t, 2 h, 67%.





Scheme 3 Reagents and conditions: (a) $HClO_4$-SiO_2, CH_2Cl_2, −40 °C, 1 h, 65%; (b) DDQ, CH_2Cl_2-H_2O, r t, 5 h, 73%; (c) $HClO_4$-SiO_2, CH_2Cl_2 –Et_2O (2:3, *v*/v), −10 °C, 1 h, 68%; (d) NIS, $HClO_4$-SiO_2, MS 4 Å, CH_2Cl_2, −10 °C, 2 h, 0%

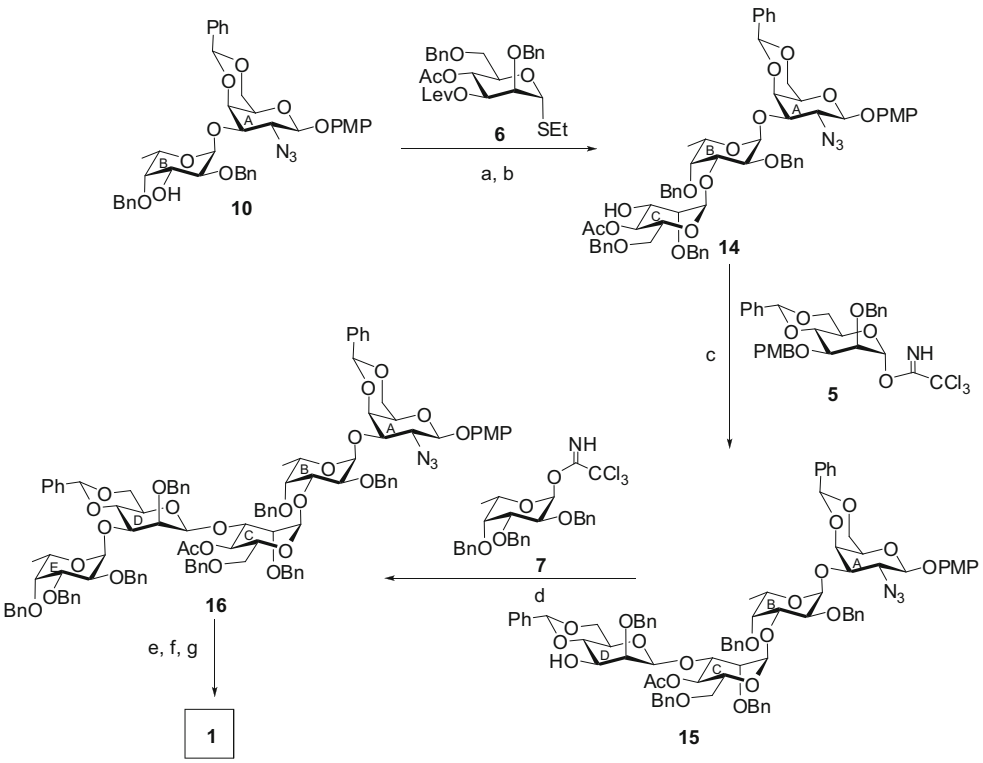
donor (**5**) in the presence of $HClO_4$-SiO_2 furnished tetrasaccharide derivative **15** in 65% yield. The NMR spectroscopic analysis unambiguously established the formation of the newly formed β-mannosidic linkage together with other glycosidic linkages [signals at δ 5.41 (s, PhC*H*), 5.40 (br s, H-1_C), 5.11 (d, J = 3.5 Hz, H-1_B), 4.69 (d, J = 8.0 Hz, H-1_A), 4.15 (br s, H-1_D) in ^{1}H NMR and at δ 102.1 ($J_{H1,C1}$ = 156.0 Hz, C-1_A, β), 101.9 (Ph*C*H), 101.2 (Ph*C*H), 99.7 ($J_{H1,C1}$ = 171.0 Hz, C-1_B, α), 98.9 ($J_{H1,C1}$ = 159.0 Hz, C-1_D, β), 98.6 ($J_{H1,C1}$ = 173.5 Hz, C-1_C, α) in ^{13}C NMR spectra]. The incorporation of the β-mannosidic linkage in compound **15** was unambiguously confirmed from its $J_{H1,C1}$ value (159.0 Hz) in the ^{1}H coupled ^{13}C NMR spectrum [34, 35]. Compound **15** was then allowed to couple with L-fucosyl trichloroacetimidate derivative **7** under 1,2-*cis* glycosylation condition using $HClO_4$-SiO_2 as glycosylation activator in a mixed solvent CH_2Cl_2-Et_2O (2:3, *v*/v) [32] to furnish pentasaccharide derivative **16** in 70% yield together with a minor quantity (~5%) of 1,2-*trans* glycosylation product, which was separated using column chromatography. The NMR spectroscopic analysis of compound **16** confirmed the formation of a new α-glycosidic linkage together with existing glycosidic bonds [signals at δ 5.48 (br s, H-1_C), 5.05 (d, J = 3.5 Hz, H-1_B), 4.88 (d, J = 3.5 Hz, H-1_E), 4.73 (d, J = 8.0 Hz, H-1_A), 3.91 (br s, H-1_D) in ^{1}H NMR and at δ 101.9 (PhCH), 101.4 (C-1_A), 100.3 (C-1_B), 98.5 (C-1_C), 97.5 (C-1_D), 96.6 (C-1_E) in ^{13}C NMR spectra]. Finally, compound **16** was subjected to a sequence of reactions comprising (a) transformation of azido group in acetamide using thioacetic acid [43]; (b) removal of acetyl group using sodium methoxide and (c) hydrogenolysis over Pearlman's catalyst [44] to furnish compound **1** in 60% over all yield. NMR spectroscopic analysis confirmed the formation of compound **1** [signals at δ 5.24 (d, J = 1.5 Hz, H-1_C), 5.06 (d, J = 4.0 Hz, H-1_E), 4.97 (d, J = 8.5 Hz, H-1_A), 4.94 (d, J = 4.0 Hz, H-1_B), 4.78 (br s, H-1_D) in ^{1}H NMR and at δ 101.4 ($J_{H1,C1}$ = 174.0 Hz, C-1_C, α), 101.3 ($J_{H1,C1}$ = 171.0 Hz, C-1_B, α), 100.8 ($J_{H1,C1}$ = 157.0 Hz, C-1_A, β), 96.7 ($J_{H1,C1}$ = 158.5 Hz, C-1_D, β), 95.9 ($J_{H1,C1}$ = 171.0 Hz, C-1_E, α) in ^{13}C NMR spectra]. The anomeric configuration of the glycosidic bonds were unambiguously confirmed from the $J_{H1,C1}$ values in the ^{1}H coupled ^{13}C NMR spectrum, particularly in case of the β-mannosidic linkage ($J_{H1,C1}$ = 158.5 Hz). In addition, appearance of C-5 carbons at δ 77.1 (C-5_D) of the β-mannosidic moiety and at δ 73.3 (C-5_C) of the α-mannosidic moiety in the deprotected pentasaccharide **1** also confirmed the presence of β-mannose in the molecule (Scheme 4).

Conclusion

In summary, a straightforward synthetic strategy has been developed for the synthesis of the pentasaccharide repeating unit of the cell wall *O*-antigen of *Escherichia coli* O43 using sequential glycosylation strategy after a failed attempt to achieve the target pentasaccharide using a block synthetic scheme. Satisfactory yield with appropriate stereochemistry has been achieved applying PMB group influenced β-D-mannosylation reaction using D-mannosyl trichloroacetimidate as glycosyl donor. 1,2-*Cis* glycosylation of L-fucose moieties have also been achieved in very good yield. The yields of the glycosylation steps were very good with satisfactory stereochemistry at the newly formed glycosidic linkages.

Experimental

General methods All reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on



Scheme 4 Reagents and conditions: (a) NIS, $HClO_4$-SiO_2, MS 4 Å, CH_2Cl_2, −15 °C, 2 h; (b) AcOH, $NH_2NH_2{\cdot}H_2O$, CH_3OH-CH_2Cl_2 (1:1), 0 °C-r t, 2 h, 72%; (c) $HClO_4$-SiO_2, CH_2Cl_2, −40 °C, 2 h, then at 20 °C for 30 min, 65%; (d) $HClO_4$-SiO_2, CH_2Cl_2-Et_2O (2:3, *v*/v), −20 °C, 1 h, 70%; (e) CH_3COSH, pyridine, r t, 12 h; (f) 0.1 M CH_3ONa, CH_3OH, r, t, 3 h; (g) H_2, 20% $Pd(OH)_2$-C, CH_3OH, r t, 24 h, 62% in three steps

TLC were visualized by warming ceric sulphate (2% $Ce(SO_4)_2$ in 2 N H_2SO_4) sprayed plates on hot plate. Silica gel 230–400 mesh was used for column chromatography. NMR spectra were recorded on Brucker Avance 500 MHz using $CDCl_3$ as solvent and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. ESI-MS were recorded on a Thermo Scientific Orbitrap Velos Pro TM mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer. Commercially available grades of organic solvents of adequate purity are used in all reactions. $HClO_4$-SiO_2 was prepared following the report of Chakraborti *et al.* [27]

Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-levulinyl-1-thio-α-D-mannopyranoside (6) To a solution of compound **9** (3 g, 5.99 mmol) in dry CH_2Cl_2 (25 mL) were added triethylsilane (4.8 mL, 30 mmol) at 0 °C followed by the addition of trifluoroacetic acid (TFA) (1.75 mL, 22.8 mmol) and the reaction mixture was stirred at the same temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ solution (2 × 50 mL), dried (Na_2SO_4), and concentrated. To the solution of the crude product in pyridine (10 mL) was added Ac_2O (10 mL) and the reaction mixture was stirred at room temperature for 2 h. The solvents were removed and co-evaporated with toluene (2 × 25 mL) under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (6:1) as eluant to give pure compound **6** (2.2 g, 68%). Yellow oil; $[\alpha]_D$ + 11 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.22 (m, 10 H, Ar-H), 5.38 (t, *J* = 10.0 Hz, 1 H, H-4), 5.29 (br s, 1 H, H-1), 5.10 (dd, *J* = 9.5 Hz, 3.0 Hz, 1 H, H-3), 4.66 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.56 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.54 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.47 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.28–4.20 (m, 1 H, H-5), 3.87 (br s, 1 H, H-2), 3.59–3.50 (m, 2 H, H-$6_{a,b}$), 2.64–2.60 (m, 4 H, 2 CH_2), 2.57–2.41 (m, 2 H, SCH_2CH$_3$), 2.11 (s, 3 H, CH_3CO), 1.93 (s, 3 H, CH_3COO), 1.26 (t, *J* = 7.4 Hz, 3 H, SCH$_2$CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.5 (*C*O), 171.5, 169.6 (2 *C*O), 138.0–127.7 (Ar-C), 81.6 (C-1), 77.0 (C-2), 73.4 (Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 71.9 (C-5), 70.1 (C-4), 69.2 (C-6), 67.2 (C-3), 37.6 (*C*H$_2$), 29.6 (*C*H$_3$CO), 27.9 (*C*H$_2$), 25.0 (S*C*H$_2$CH$_3$), 20.7 (CO*C*H$_3$), 14.8 (SCH$_2$*C*H$_3$); HRMS (ESI) for $C_{29}H_{36}O_8S$ $[M + H]^+$: Calcd. 545.2209; found, 545.2200.

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-levulinyl-1-thio-α-D-mannopyranoside (9) To a solution of compound **8** (4 g, 9.94 mmol) in anhydrous CH_2Cl_2 (30 mL) were added levulinic acid (1.3 mL, 12.76 mmol), DIC (1.7 mL, 10.85 mmol) and DMAP (1.2 g, 9.82 mmol) and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with 2 M HCl (50 mL), H_2O (100 mL), 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 **9** (3.7 g, 74%); Yellow oil; $[\alpha]_D$ + 6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.12 (m, 10 H, Ar-H), 5.49 (s, 1

H, PhC*H*), 5.20 (br s, 1 H, H-1), 5.12 (dd, *J* = 9.5 Hz, 3.0 Hz, 1 H, H-3), 4.61 (br s, 2 H, PhCH_2), 4.20–4.10 (m, 3 H, H-5, H-$6_{a,b}$), 3.99 (d, *J* = 1.2 Hz, 1 H, H-2), 3.80 (t, *J* = 10.0 Hz, 1 H, H-4), 2.67–2.47 (m, 6 H, 2 CH_2, SCH_2CH$_3$), 2.07 (s, 3 H, CH_3CO), 1.22 (t, *J* = 7.4 Hz, 3 H, SCH$_2$CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.9 (*C*O), 171.9 (*C*O), 137.7–126.3 (Ar-C), 101.8 (Ph*C*H), 83.1 (C-1), 77.9 (C-2), 76.3 (C-5), 73.4 (Ph*C*H$_2$), 71.0 (C-4), 68.6 (C-6), 64.5 (C-3), 37.8 (*C*H$_2$), 29.8 (*C*H$_3$CO), 27.9 (*C*H$_2$), 25.3 (S*C*H$_2$CH$_3$), 14.9 (SCH$_2$*C*H$_3$); HRMS (ESI) for $C_{27}H_{32}O_7S$ $[M + H]^+$: Calcd. 501.1947; found, 501.1940.

p-Methoxyphenyl (2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (10) A solution of compound **2** (1 g, 2.50 mmol) and compound **3** (1.6 g, 3.01 mmol) in anhydrous CH_2Cl_2-Et_2O (15 mL; 2:3 *v*/v) was cooled to - 10 °C under argon. To the cooled reaction mixture was added $HClO_4$-SiO_2 (50 mg) and it was allowed to stir at same temperature for 2 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (25 mL). The organic layer was successively washed with satd. $NaHCO_3$ (25 mL) and H_2O (25 mL), dried (Na_2SO_4) and concentrated. A solution of the product in 0.1 M CH_3ONa in CH_3OH (15 mL) was stirred at room temperature for 2 h and neutralized with Amberlite IR-120 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **10** (1.2 g, 67%). Yellow oil; $[\alpha]_D$ - 8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31–7.17 (m, 15 H, Ar-H), 7.07 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.79 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.46 (s, 1 H, PhC*H*), 5.06 (d, *J* = 3.5 Hz, 1 H, H-1_B), 4.90 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.80 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.72 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.62 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.59 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.33 (d, *J* = 12.5 Hz, 1 H, H-6_{aA}), 4.23 (d, *J* = 3.0 Hz, 1 H, H-4_A), 4.12–4.09 (m, 4 H, H-2_A, H-3_B, H-5_B, H-6_{bA}), 3.83 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-2_B), 3.77 (s, 3 H, OCH_3), 3.58 (d, *J* = 2.0 Hz, 1 H, H-4_B), 3.42 (br s, 1 H, H-5_A), 3.36 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-3_A), 1.08 (d, *J* = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.8–114.5 (Ar-C), 102.3 (C-1_A), 101.1 (Ph*C*H), 99.9 (C-1_B), 80.2 (C-3_A), 78.6 (C-4_B), 76.6 (C-2_B), 75.2 (Ph*C*H$_2$), 74.8 (C-4_A), 72.0 (Ph*C*H$_2$), 70.5 (C-3_B), 68.9 (C-6_A), 67.1 (C-5_B), 66.5 (C-5_A), 61.5 (C-2_A), 55.5 (O*C*H$_3$), 16.9 (C*C*H$_3$); HRMS (ESI) for $C_{40}H_{43}N_3O_{10}$ $[M + H]^+$: Calcd. 726.3026; found, 726.3017.

Ethyl [2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(*p*-methoxybenzyl)-β-D-mannopyranosyl]-(1 → 3)-4-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (11) A solution of compound **4** (1.2 g, 2.69 mmol) and compound **5** (2 g, 3.21 mmol) in anhydrous CH_2Cl_2 (25 mL) was cooled to - 40 °C under argon. To the cooled reaction mixture was added $HClO_4$-SiO_2 (80 mg) and it was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with satd. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **11** (1.6 g, 65%). Yellow oil; $[\alpha]_D$ - 13 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–6.80 (m, 22 H, Ar-H), 6.80 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.54 (s, 1 H, PhC*H*), 5.47 (br s, 1 H, H-1_A), 5.40 (t, *J* = 10.0 Hz, 1 H, H-4_A), 4.86 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.76 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.68 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.59–4.43 (m, 5 H, 5 PhC*H*), 4.26–4.20 (m, 2 H, H-5_A, H-6_{aA}), 4.08 (dd, *J* = 9.5 Hz, 3.5 Hz, 1 H, H-3_A), 4.05 (t, *J* = 10.0 Hz, 1 H, H-4_B), 4.01 (br s, 1 H, H-1_B), 3.83–3.79 (m, 1 H, H-6_{bA}), 3.78 (s, 3 H, OCH_3), 3.77 (br s, 1 H, H-2_A), 3.64–3.60 (m, H-2_B, H-6_{aB}), 3.56 (dd, *J* = 12.5 Hz, 2.5 Hz, 1 H, H-6_{bB}), 3.26 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-3_B), 3.03–2.99 (m, 1 H, H-5_B), 2.76–2.58 (m, 2 H, SCH_2CH$_3$), 1.87 (s, 3 H, COCH_3), 1.32 (t, *J* = 7.4 Hz, 3 H, SCH$_2$CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.7 (*C*O), 159.2–113.7 (Ar-C), 101.4 (Ph*C*H), 98.0 ($J_{H1,C1}$ = 158.5 Hz, C-1_B), 80.8 ($J_{H1,C1}$ = 174.5 Hz, C-1_A), 78. 6 (C-4_B), 76.9 (C-3_B), 75.7 (C-2_B), 74.3 (Ph*C*H$_2$), 74.0 (C-2_A), 73.8 (C-3_A), 72.3 (Ph*C*H$_2$), 71.6 (Ph*C*H$_2$), 71.2 (Ph*C*H$_2$), 70.4 (C-5_A), 69.4 (C-6_B), 68.5 (C-6_A), 67.7 (C-5_B), 67.6 (C-4_A), 55.1 (O*C*H$_3$), 25.4 (S*C*HCH$_3$), 20.9 (CO*C*H$_3$), 14.9 (SCH$_2$*C*H$_3$); HRMS (ESI) for $C_{52}H_{58}O_{12}S$ $[M + H]^+$: Calcd. 907.3727; found, 907.3720.

Ethyl (3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1 → 3)-4-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (13) To a solution of compound **11** (1.5 g, 1.65 mmol) in CH_2Cl_2 (20 mL) was added a solution of DDQ (680 mg, 2.99 mmol) in H_2O (5 mL) and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with satd. $NaHCO_3$ (25 mL), dried (Na_2SO_4) and concentrated to give the crude product, which was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure product **12** (950 mg, 73%). A solution of compound **12** (900 mg, 1.14 mmol) and compound **3** (730 mg, 1.37 mmol) in CH_2Cl_2–Et_2O (15 mL; 2:3, *v*/v) was cooled to - 10 °C under argon. To the cooled reaction mixture was added $HClO_4$-SiO_2 (50 mg) and it was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with satd. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **13** (900 mg, 68%). Yellow oil; $[\alpha]_D$ + 5 (*c* 1.0, $CHCl_3$); ^{1}H

NMR (500 MHz, $CDCl_3$): δ 7.43–7.17 (m, 30 H, Ar-H), 5.48 (s, 1 H, PhC*H*), 5.47 (br s, 1 H, H-1_A), 5.35 (t, *J* = 10.0 Hz, 1 H, H-4_A), 5.33 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-3_C), 4.93 (d, *J* = 3.5 Hz, 1 H, H-1_C), 4.78–4.43 (m, 10 H, 10 PhC*H*), 4.23–4.18 (m, 3 H, H-3_A, H-5_A, H-6_{aA}), 4.07–4.01 (m, 2 H, H-2_C, H-5_C), 4.00 (br s, 1 H, H-1_B), 3.96 (t, *J* = 10.0 Hz, 1 H, H-4_B), 3.79 (br s, 1 H, H-2_A), 3.78–3.75 (m, 1 H, H-6_{bA}), 3.63 (d, *J* = 2.0 Hz, 1 H, H-4_C), 3,62–3.50 (m, 4 H, H-2_B, H-3_B, H-$6_{a,bB}$), 3.05–2.98 (m, 1 H, H-5_B), 2.70–2.63 (m, 2 H, SC*H*$_2$CH$_3$), 1.90 (s, 3 H, COC*H*$_3$), 1.74 (s, 3 H, COC*H*$_3$), 1.28 (t, *J* = 7.4 Hz, 3 H, SCH$_2$C*H*$_3$), 0.80 (d, *J* = 6.5 Hz, 3 H, CC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0 (2 *C*O), 138.2–126.2 (Ar-C), 101.7 (Ph*C*H), 97.6 ($J_{H1,C1}$ = 159.0 Hz, C-1_B), 96.0 ($J_{H1,C1}$ = 172.0 Hz, C-1_C), 80.7 ($J_{H1,C1}$ = 174.0 Hz, C-1_A), 78.9 (C-4_C), 76.8 (C-4_B), 76.5 (C-2_B), 75.9 (C-3_B), 75.6 (Ph*C*H$_2$), 75.2 (Ph*C*H$_2$), 74.1 (C-2_A), 73.9 (2 C, C-4_A, C-5_C), 73.6 (C-2_C), 73.4 (Ph*C*H$_2$), 73.3 (Ph*C*H$_2$), 71.2 (Ph*C*H$_2$), 70.5 (C-3_A), 69.4 (C-6_B), 68.5 (C-6_A), 67.9 (C-3_C), 67.5 (C-5_B), 65.9 (C-5_A), 25.3 (S*C*H$_2$CH$_3$), 20.7, 20.6 (2 CO*C*H$_3$), 15.9 (C*C*H$_3$), 14.9 (C*C*H$_3$); HRMS (ESI) for $C_{66}H_{74}O_{16}S$ $[M+H]^+$: Calcd. 1155.4776; found, 1155.4766.

***p*-Methoxyphenyl (4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (14)** To a solution of compound **10** (1 g, 1.38 mmol) and compound **6** (900 mg, 1.65 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS 4 Å (1 g) and it was cooled to - 15 °C under argon. To the cooled reaction mixture were successively added NIS (370 mg, 1.64 mmol) and $HClO_4$-SiO_2 (10 mg) and it was allowed to stir at same temperature for 2 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (25 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$ (25 mL), satd. $NaHCO_3$ (25 mL) and H_2O (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product. To a solution of the crude product in CH_3OH-CH_2Cl_2 (15 ml; 1:1 *v*/v) were added AcOH (300 μL, 5.25 mmol) and $NH_2NH_2{\cdot}H_2O$ (255 μL, 5.25 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL), successively washed with 2 N HCl (25 mL), H_2O (25 mL), 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 **14** (1.1 g, 72% in two steps). Yellow oil; $[\alpha]_D$ + 9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–7.06 (m, 27 H, Ar-H), 6.80 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.41 (s, 1 H, PhC*H*), 5.30 (br s, 1 H, H-1_C), 5.07 (d, *J* = 3.5 Hz, 1 H, H-1_B), 4.98 (t, *J* = 10.0 Hz, 1 H, H-4_C), 4.85 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.77 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.68 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.60 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.50 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.46 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.38 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.35–4.23 (m, 2 H, H-6_{aA}, PhC*H*), 4.22 (d, *J* = 3.0 Hz, 1 H, H-4_A), 4.20 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-3_A), 4.15–4.08 (m, 2 H, H-2_A, H-5_B), 4.00 (d, *J* = 12.5 Hz, 1 H, H-6_{bA}), 3.98 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-2_B), 3.94 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 3.93–3.90 (m, 1 H, H-5_C), 3.81–3.77 (m, 1 H, H-3_C), 3.76 (s, 3 H, OC*H*$_3$), 3.71–3.70 (m, 1 H, H-2_C), 3.57 (d, *J* = 1.5 Hz, 1 H, H-4_B), 3.52 (dd, *J* = 12.5 Hz, 6.0 Hz, 1 H, H-6_{aC}), 3.47 (dd, *J* = 12.5 Hz, 2.5 Hz, 1 H, H-6_{bC}), 3.41 (br s, 1 H, H-5_A), 3.39 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-3_B), 1.97 (s, 3 H, COC*H*$_3$), 0.99 (d, *J* = 6.5 Hz, 3 H, CC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3 (*C*OCH$_3$), 155.7–114.4 (Ar-C), 102.0 (C-1_A), 101.2 (PhCH), 99.9 (C-1_B), 98.8 (C-1_C), 80.6 (C-3_B), 79.9 (C-4_B), 77.9 (C-2_C), 77.1 (C-2_B), 76.7 (C-3_A), 75.1 (Ph*C*H$_2$), 74.6 (C-4_A), 73.3 (Ph*C*H$_2$), 72.3 (Ph*C*H$_2$), 71.9 (Ph*C*H$_2$), 70.5 (C-4_C), 70.4 (C-5_C), 69.6 (C-6_A), 69.5 (C-3_C), 68.9 (C-6_C), 67.6 (C-5_B), 66.5 (C-5_A), 61.3 (C-2_A), 55.5 (O*C*H$_3$), 20.97 (CO*C*H$_3$), 16.8 (C*C*H$_3$); HRMS (ESI) for $C_{62}H_{67}N_3O_{16}$ $[M+H]^+$: Calcd. 1110.4599; found, 1110.4590.

***p*-Methoxyphenyl (2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1 → 3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (15)** A solution of compound **14** (1 g, 0.90 mmol) and compound **5** (650 mg, 1.04 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to - 40 °C under argon. To the cooled reaction mixture was added $HClO_4$-SiO_2 (40 mg) and it was allowed to stir at same temperature for 2 h. After consumption of the starting materials the reaction mixture was allowed to stir at 20 °C for 30 min. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (25 mL). The organic layer was successively washed with satd. $NaHCO_3$ (25 mL) and H_2O (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **15** (850 mg, 65%). Yellow oil; $[\alpha]_D$ - 17 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–7.05 (m, 37 H, Ar-H), 6.80 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.41 (s, 1 H, PhC*H*), 5.40 (br s, 2 H, H-1_C, PhC*H*), 5.33 (t, *J* = 10.0 Hz, 1 H, H-4_C), 5.11 (d, *J* = 3.5 Hz, 1 H, H-1_B), 4.94 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.90 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.82 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.69 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.65 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.60 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.55 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.50–4.38 (m, 4 H, 4 PhC*H*), 4.30 (d, *J* = 12.5 Hz, 1 H, H-6_{aA}), 4.26 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-3_A), 4.23 (d, *J* = 2.0 Hz, 1 H, H-4_A), 4.15 (br s, 1 H, H-1_D), 4.13–4.07 (m, 3 H, H-2_A, H-3_C, H-5_B), 4.04 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, H-2_B), 3.99 (d, *J* = 12.5 Hz, 1 H, H-6_{bA}), 3.98–3.92 (m, 1 H, H-5_C), 3.90–3.85 (m, 1 H, H-6_{aD}), 3.75 (s, 3 H, OC*H*$_3$), 3.72–3.71 (m, 1 H, H-2_C), 3.61–3.55 (m, 4 H, H-3_D, H-4_D, H-6_{aC}, H-6_{bD}), 3.52 (d, *J* = 3.0 Hz, 1 H, H-4_B), 3.50

(dd, J = 12.5 Hz, 2.5 Hz, 1 H, H-6_{bC}), 3.43–3.37 (m, 3 H, H-2_D, H-3_B, H-5_A), 2.96–2.89 (m, 1 H, H-5_D), 1.85 (s, 3 H, COCH_3), 1.04 (d, J = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (*C*OCH$_3$), 155.7–114.5 (Ar-C), 102.1 ($J_{H1,C1}$ = 156.0 Hz, C-1_A), 101.9 (PhCH), 101.2 (PhCH), 99.7 ($J_{H1,C1}$ = 171.0 Hz, C-1_B), 98.9 ($J_{H1,C1}$ = 159.0 Hz, C-1_D), 98.6 ($J_{H1,C1}$ = 173.5 Hz, C-1_C), 80.5 (C-3_B), 79.4 (C-3_D), 79.3 (C-4_D), 78.1 (C-4_B), 76.7 (C-3_A), 76.1 (C-4_A), 75.1 (Ph*C*H$_2$), 75.0 (Ph*C*H$_2$), 74. 7 (C-3_C), 74.0 (C-2_B), 73.7 (Ph*C*H$_2$), 73.3 (C-2_C), 71.7 (Ph*C*H$_2$), 71.6 (Ph*C*H$_2$), 71.0 (C-5_C), 70.5 (C-2_D), 69.8 (C-6_C), 68.9 (C-6_A), 68.4 (C-6_D), 68.1 (C-4_C), 68.0 (C-5_B), 67.0 (C-5_D), 66.5 (C-5_A), 61.3 (C-2_A), 55.5 (O*C*H$_3$), 20.9 (CO*C*H$_3$), 16.8 (C*C*H$_3$); HRMS (ESI) for $C_{82}H_{87}N_3O_{21}$ $[M+H]^+$: Calcd. 1450.5910; found, 1450.5902.

***p*-Methoxyphenyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1 → 3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-(2,4-di-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (16)** A solution of compound **15** (750 mg, 0.51 mmol) and compound **7** (330 mg, 0.57 mmol) in anhydrous CH_2Cl_2-Et_2O (10 mL; 2:3 *v*/v) was cooled to -20 °C under argon. To the cooled reaction mixture was added $HClO_4$-SiO_2 (40 mg) and it was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (25 mL). The organic layer was successively washed with satd. $NaHCO_3$ (25 mL) and H_2O (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **16** (665 mg, 70%). Yellow oil; $[\alpha]_D$ - 3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44–7.09 (m, 52 H, Ar-H), 6.83 (d, J = 9.0 Hz, 2 H, Ar-H), 5.50 (s, 1 H, PhC*H*), 5.48 (br s, 2 H, H-1_C, PhC*H*), 5.29 (t, J = 10.0 Hz, 1 H, H-4_C), 5.05 (d, J = 3.5 Hz, 1 H, H-1_B), 4.92 (d, J = 11.5 Hz, 1 H, PhC*H*), 4.88 (d, J = 3.5 Hz, 1 H, H-1_E), 4.83–4.77 (m, 3 H, 3 PhC*H*), 4.73 (d, J = 8.0 Hz, 1 H, H-1_A), 4.71–4.58 (m, 7 H, 7 PhC*H*), 4.55–4.48 (m, 3 H, 3 PhC*H*), 4.37–4.33 (m, 2 H, H-6_{aA}, PhC*H*), 4.30–4.26 (m, 2 H, H-3_A, H-4_A), 4.23–4.16 (m, 2 H, H-5_C, H-6_{aD}), 4.12–4.00 (m, 6 H, H-2_A, H-2_B, H-3_C, H-3_D, H-5_B, H-6_{bA}), 3.98–3.93 (m, 3 H, H-4_D, H-5_E, PhC*H*), 3.91 (br s, 1 H, H-1_D), 3.83–3.80 (m, 2 H, H-2_C, H-2_E), 3.78 (s, 3 H, OCH_3), 3.77–3.74 (m, 3 H, H-4_B, H-4_E, H-6_{bD}), 3.59–3.55 (m, 1 H, H-6_{aC}), 3.53–3.44 (m, 5 H, H-2_D, H-3_B, H-3_E, H-5_A, H-6_{bC}), 2.98–2.90 (m, 1 H, H-5_D), 2.13 (s, 3 H, COCH_3), 1.18 (d, J = 6.5 Hz, 3 H, CCH_3), 0.75 (d, J = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6 (*C*O), 155.8–114.5 (Ar-C), 102.0 (PhCH), 101.9 (PhCH), 101.4 (C-1_A), 100.3 (C-1_B), 98.5 (C-1_C), 97.5 (C-1_D), 96.6 (C-1_E), 80.9 (C-3_B), 78.4 (C-3_D), 77.8 (C-4_D), 77.2 (C-4_B), 76.9 (2 C, C-4_A, C-4_E), 76.4 (C-3_A), 76.2 (C-3_E), 75.2 (Ph*C*H$_2$), 75.1 (C-2_E), 74.7 (C-2_D), 74.6 (Ph*C*H$_2$), 74.0 (Ph*C*H$_2$), 73.4 (2 C, 2 Ph*C*H$_2$), 73.1 (C-2_B), 72.7 (C-3_C), 72.4 (Ph*C*H$_2$), 71.9 (Ph*C*H$_2$), 71.2 (C-2_C), 71.0 (C-5_E), 70.9 (Ph*C*H$_2$), 69.8 (C-6_C), 69.0 (C-6_A), 68.7 (C-6_D), 67.6 (C-5_D), 67.1 (2 C, C-4_C, C-5_C), 66.5 (C-5_A), 64.7 (C-5_B), 61.4 (C-2_A), 55.5 (O*C*H$_3$), 21.0 (CO*C*H$_3$), 17.4 (C*C*H$_3$), 15.7 (C*C*H$_3$); HRMS (ESI) for $C_{109}H_{115}N_3O_{25}$ $[M+H]^+$: Calcd. 1866.7898; found, 1866.7890.

***p*-Methoxyphenyl (α-L-fucopyranosyl)-(1 → 3)-(β-D-mannopyranosyl)-(1 → 3)-(α-D-mannopyranosyl)-(1 → 3)-(α-L-fucopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranoside (1)** To a solution of compound **16** (500 mg, 0.27 mmol) in pyridine (2 mL) was added CH_3COSH (0.5 mL) and the reaction mixture was stirred at room temperature for 12 h. The solvents were removed and co-evaporated with toluene (3 × 20 mL) under reduced pressure and the crude product was passed through a short pad of SiO_2. A solution of the N-acetylated product in 0.1 M CH_3ONa in CH_3OH (10 mL) was stirred at room temperature for 3 h, neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. To a solution of the de-*O*-acetylated product in CH_3OH (5 mL) was added 20% $Pd(OH)_2$-C (50 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of H_2 for 24 h. The reaction mixture was filtered through a Celite bed, washed with CH_3OH-H_2O (30 mL; 2:1 *v*/v) and concentrated under reduced pressure. The deprotected product was passed through a Sephadex LH-20 column using CH_3OH-H_2O (3:1) as eluant to give pure compound **1** (160 mg, 62%). White powder; $[\alpha]_D$ + 11 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 6.99 (d, J = 9.0 Hz, 2 H, Ar-H), 6.90 (d, J = 9.0 Hz, 2 H, Ar-H), 5.24 (d, J = 1.5 Hz, 1 H, H-1_C), 5.06 (d, J = 4.0 Hz, 1 H, H-1_E), 4.97 (d, J = 8.5 Hz, 1 H, H-1_A), 4.94 (d, J = 4.0 Hz, 1 H, H-1_B), 4.78 (br s, 1 H, H-1_D), 4.27 (br s, 1 H, H-5_E), 4.20–4.16 (m, 3 H, H-2_A, H-2_C, H-2_D), 4.10–4.02 (m, H-3_D, H-5_B), 4.00–3.90 (m, 4 H, H-3_A, H-3_E, H-4_A, H-4_E), 3.88–3.84 (m, 1 H, H-6_{aC}), 3.82–3.78 (m, 4 H, H-3_C, H-6_{aA}, H-6_{aD}, H-6_{bC}), 3.78 (s, 3 H, OCH_3), 3.77–3.62 (m, 9 H, H-2_E, H-3_B, H-4_B, H-4_C, H-4_D, H-5_A, H-5_C, H-6_{bA}, H-6_{bD}), 3.55–3.47 (m, 1 H, H-2_B), 3.36–3.32 (m, 1 H, H-5_D), 1.94 (s, 3 H, COCH_3), 1.20 (d, J = 6.5 Hz, 3 H, CCH_3), 1.02 (d, J = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, D_2O): δ 175.8 (*C*OCH$_3$), 155.4–114.8 (Ar-C), 101.4 ($J_{H1,C1}$ = 174.0 Hz, C-1_C), 101.3 ($J_{H1,C1}$ = 171.0 Hz, C-1_B), 100.8 ($J_{H1,C1}$ = 157.0 Hz, C-1_A), 96.7 ($J_{H1,C1}$ = 158.5 Hz, C-1_D), 95.9 ($J_{H1,C1}$ = 171.0 Hz, C-1_E), 78.8 (C-3_A), 78.5 (C-3_C), 78.2 (C-3_B), 77.1 (C-5_D), 76.3 (C-3_D), 75.3 (2 C, C-4_B, C-5_A), 74.4 (C-4_E), 73.3 (C-5_C), 70.1 (C-3_E), 68.2 (2 C, C-2_E, C-4_A), 67.8 (2 C, C-2_B), 67.6 (C-5_E), 67.0 (C-5_B), 66.5 (C-2_C), 65.7 (C-4_C), 65.0 (C-4_D), 64.9 (C-2_D), 61.1 (C-6_D), 60.7 (2 C, C-6_A, C-6_C), 55.7 (O*C*H$_3$), 51.4 (C-2_A), 22.2 (CO*C*H$_3$), 16.4 (C*C*H$_3$), 15.0 (C*C*H$_3$); HRMS (ESI) for $C_{39}H_{61}NO_{25}$ $[M+H]^+$: Calcd. 944.3611; found, 944.3602.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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