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Concise synthesis of the tetrasaccharide repeating unit of the *O*-polysaccharide isolated from *Edwardsiella tarda* PCM 1156 strain

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ABSTRACT

A convergent strategy has been developed for the synthesis of the tetrasaccharide repeating unit of the *O*-antigen from *Edwardsiella tarda* PCM 1156. Sequential glycosylations of a series of rationally protected monosaccharide intermediates were achieved either by the activation of thioglycosides using N-iodosuccinimide (NIS) in conjunction with H_2SO_4–silica or by activation of trichloroacetimidate by H_2SO_4–silica only. All glycosylation reactions resulted in the formation of the desired linkage with absolute stereoselectivity and yielded the required derivatives in good to excellent yields. Both azido and phthalimido groups have been used as the precursor of the desired acetamido group depending on the requirement of 1,2-*cis*- or 1,2-*trans*-glycosidic linkage.

1. Introduction

Lipopolysaccharides are the essential and characteristic components of the outer membranes of Gram negative bacteria. The *O*-polysaccharides are the constituents of these lipopolysaccharides which are in turn responsible for determining and regulating various biological conditions of the organisms. The *O*-antigenic polysaccharide chain is made up of oligosaccharide repeating units and remains exposed on the outer surface of bacterial cell walls. By virtue of their orientation, the *O*-polysaccharides are responsible for binding with the receptors of the host cells and play a pivotal role in infections.[1] Due to the presence of different sugar residues, the *O*-antigens demonstrate a varied character and act as the elicitor of innate immune responses. There have been extensive studies with these bacterial *O*-antigens as potential anti-microbial agents and vaccine-targets.[2–4] However, difficult isolation and cumbersome purification process limit the scope of accumulating large quantities of these oligosaccharides from natural sources. Therefore, the chemical synthesis of these *O*-antigen repeating units becomes pertinent to explore their vivid biological roles and potential as vaccine targets.

Edwardsiella tarda (*E. tarda*) is a Gram-negative bacterium belonging to the Endobacteriaceae family.[5,6] This microorganism is an opportunistic pathogen, which is responsible for haemorrhagic septicaemia, also known as Edwardsiellosis among freshwater fish. It inhabits and infects freshwater and marine fishes as well as a broad range of other animals.[7,8] This species is also responsible for gastroenteritis, colitis and dysentery like diseases in humans.[9] Several strains of *E. tarda* including MT108,[10] PCM 1153,[11] PCM 1150,[12] PCM 1145, PCM 1151 and PCM 1158[13] have been elucidated and studied. Recently, Knirel et al. reported a new structure of the *O*-polysaccharide of *E. tarda* PCM 1156.[14] The structural analysis of this oligosaccharide revealed the presence of a glucosamine, a fucose, a mannose and a galactosamine unit. The biological implications of the *O*-antigens of the strain trigger an interest to build a strategy for the synthesis of the tetrasaccharide repeating unit. Moreover, the presence of two acetamido sugars in the tetrasaccharide structure makes it synthetically challenging. Herein, we report the total chemical synthesis of the tetrasaccharide repeating unit in the form of its *p*-methoxyphenyl glycoside (Fig. 1).

2. Results and discussion

Adjudication of the retrosynthetic analysis suggests that sequential glycosylations of rationally protected monosaccharide

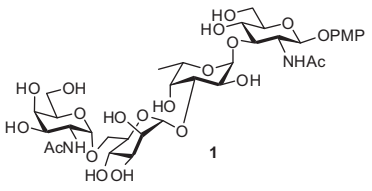

Figure 1. Structure of the target tetrasaccharide related to the repeating unit of the *O*-antigen from *E. tarda* PCM 1156 in the form of its PMP glycoside.

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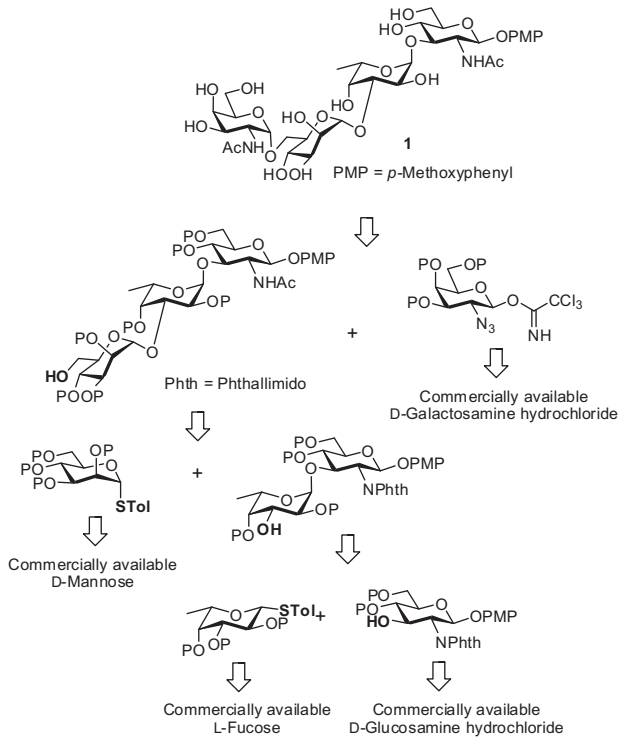


Figure 2. Retrosynthetic analysis for the target tetrasaccharide **1**.

synthons are the best bet to achieve the linear tetrasaccharide **1** (Fig. 2). The *p*-methoxyphenyl glycoside was preferred at the reducing end of the target **1**. It can be cleaved selectively from the per-O-acetylated derivative of the target tetrasaccharide and allow the introduction of relevant aglycon using trichloroacetimidate chemistry.

Thus, the known *p*-tolyl 2-*O*-benzyl-1-thio-β-L-fucopyranoside **2**[15] was treated with trimethyl orthobenzoate in the presence of catalytic amount of 10-camphorsulfonic acid (CSA) to yield the corresponding orthobenzoate that was subsequently rearranged by the treatment of aqueous HCl (1 M)[16] to give the required fucose derivative **3** in 87% yield. The remaining free hydroxyl group in the 3-position was protected with the chloroacetyl group using chloroacetic anhydride in the presence of pyridine[17] to get the required fucose donor **4** in 91% yield. Glycosylation between known acceptor **5**[18] and donor **4** was achieved by the activation of thioglycoside using N-iodosuccinimide (NIS) in the presence of H_2SO_4–silica[19–21] at −40 °C resulting in the required disaccharide **6** in 85% yield. The use of H_2SO_4–silica for the activation of thioglycoside in conjunction with NIS was found to be beneficial over the traditional use of trifluoromethanesulfonic acid (TfOH) or trimethylsilyl trifluoromethanesulfonate (TMSOTf) for this purpose. The solid acid catalyst is easy to handle and can be weighed exactly per the requirement. It is moisture tolerant and, moreover, the silica acts as a desiccant to facilitate the anhydrous condition required for successful glycosylation. Indeed, the same glycosylation reactions using TfOH and TMSOTf ended with lower yields, 71% and 74%, respectively, suggesting the better performance of H_2SO_4–silica. The phthalimido group was preferred as the acetamido precursor in order to have 1,2-*trans*-glycoside at the reducing end. Selective removal of the chloroacetyl group of the disaccharide **6** using thiourea in the presence of 2,4,6-collidine[22] yielded the disaccharide acceptor **7** in 80% yield (Scheme 1).

In a separate experiment, the known *p*-tolyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside **8**[23] was treated with chloroacetic anhydride in the presence of pyridine to get our required mannose donor **9** in 92% yield. The presence of the benzoyl group in the C-2 position ensures the stereospecific formation of 1,2-*trans*-glycoside. Next, glycosylation of the disaccharide acceptor **7** with the donor **9** using NIS in the presence of H_2SO_4–silica at 0 °C yielded the desired trisaccharide **10** in 82% yield. Selective removal of the chloroacetyl group using thiourea produced the required trisaccharide acceptor **11** in 80% yield. Final glycosylation between the trisaccharide acceptor **11** and the known trichloroacetamide donor **12**[24] using H_2SO_4–silica at −55 °C furnished the protected tetrasaccharide **13** in 73% yield. Once the protected tetrasaccharide **13** was obtained, the benzylidene group was selectively cleaved using 80% acetic acid (AcOH) at 80 °C.[25] Then, the azido group was converted to the desired acetamido functionality by using thioacetic acid[26] at room temperature. Next, the phthalimido group was cleaved using ethylene diamine[27] followed by the global acetylation using acetic anhydride (Ac_2O) in pyridine. Removal of the benzyl groups through catalytic hydrogenation using Pd–C in the presence of H_2 followed by de-O-acetylation using NaOMe in methanol[28] yielded the target tetrasaccharide **1** in 62% yield (Scheme 2).

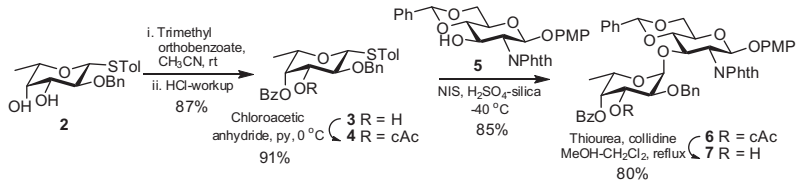
3. Conclusion

In conclusion, the total chemical synthesis of the tetrasaccharide repeating unit of the *O*-antigen from *E. tarda* has been achieved by a linear strategy using rationally protected monosaccharide synthons. The azido precursor was successfully used for 1,2-*cis* glycosylation whereas the phthalimido precursor lead to the formation of the required 1,2-*trans* linkage. Both azido and phthalimido groups were successfully converted to the desired acetamido functionality to produce the target tetrasaccharide. Glycosylation reactions were accomplished by our in-house methodologies of NIS/H_2SO_4–silica mediated activation of thioglycosides or H_2SO_4–silica mediated activation of glycosyl trichloroacetimidates resulting in good to excellent yield of the required glycosides. The final target having the PMP glycoside at the reducing end leaves the scope for its selective removal from the per-O-acetylated tetrasaccharide and further conjugation with suitable aglycon per the biological need.

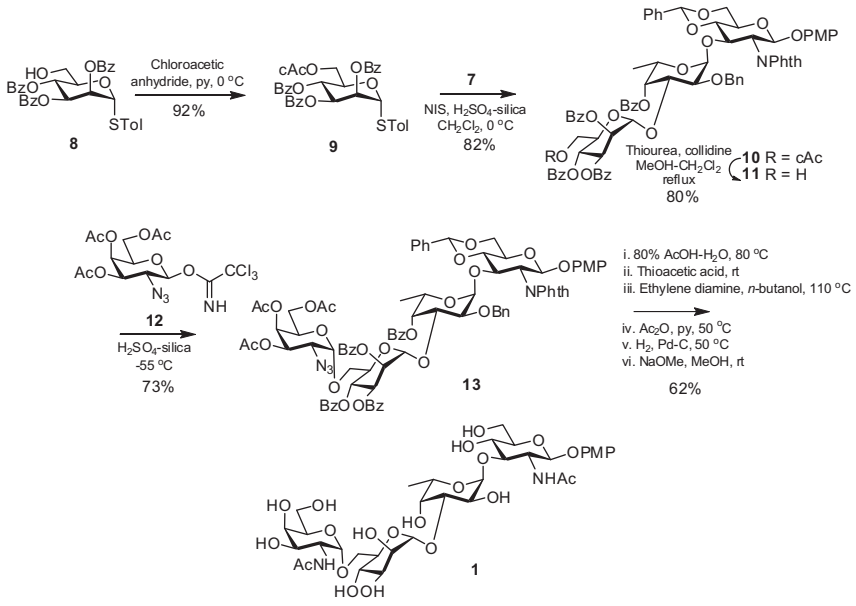
4. Experimental

4.1. General

All solvents and reagents were dried prior to use according to standardized methods.[29] The commercially purchased reagents



Scheme 1. Synthesis of the disaccharide acceptor **7**.



Scheme 2. Synthesis of the target tetrasaccharide **1**.

were used without any further purification unless mentioned otherwise. Dichloromethane was dried and distilled over P_2O_5 to make it anhydrous and moisture-free. All reactions were monitored by thin layer chromatography (TLC) on Silica-Gel 60-F_{254} with detection by fluorescence followed by charring after immersion in 10% ethanolic solution of H_2SO_4. Flash chromatography was performed with silica gel 230–400 mesh. Optical rotations were measured on a sodium-line at ambient temperature. ^1H and ^{13}C NMR were recorded on a Bruker 500 MHz spectrometer. In the case of the tetrasaccharide, ^1H NMR values are denoted as H for the reducing end glucosamine unit, H′ for the fucose unit, H″ for the mannose unit and H‴ for the remaining galactosamine unit.

4.2. Preparation of H_2SO_4–silica

To a slurry of silica gel 230–400 mesh (10 g) in dry Et_2O (20 mL) was added concentrated H_2SO_4 (1 mL) and the mixture was hand shaken for couple of minutes. Then the solvents were evaporated in vacuo and the residue was dried at 100 °C for 3 h. The dried material was kept in an airtight bottle for further use.[30]

4.3. *p*-Tolyl 4-*O*-benzoyl-2-*O*-benzyl-1-thio-β-L-fucopyranoside (3)

To a suspension of known *p*-tolyl 2-*O*-benzyl-1-thio-β-L-fucopyranoside **2** (3.5 g, 9.7 mmol) in dry CH_3CN (20 mL), trimethyl orthobenzoate (2.2 mL, 12.6 mmol) was added followed by the addition of CSA (25 mg). The reaction mixture was allowed to stir at room temperature for 2 h until the TLC (*n*-hexane–EtOAc; 2:1) showed complete conversion of the starting material. Then the solvents were evaporated in vacuo and the crude mixture was diluted with CH_2Cl_2 (25 mL) and washed with 1 M HCl (2 × 25 mL). The organic layer was then collected, dried (Na_2SO_4) and filtered. The solvents were then evaporated and the crude mixture was purified by flash chromatography to give the pure product **3** (3.9 g, 87%) as a colourless syrup. $[\alpha]_D^{25}$ −34 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.09–7.21 (m, 14H, ArH), 5.38 (d, 1H, $J_{3,4}$, $J_{4,5}$ 3.0 Hz, H-4), 4.96 (ABq, 1H, J_{AB}11.5 Hz, CH_2Ph), 4.72 (ABq, 1H, J_{AB}11.5 Hz, CH_2Ph), 4.64 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 3.93 (dd, 1H, $J_{2,3}$ 6.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 3.78 (m, 1H, H-5), 3.67 (t, 1H, $J_{1,2}J_{2,3}$ 10.0 Hz, H-2), 2.44 (s, 3H, S-$C_6H_4CH_3$) and 1.31 (d, 3H, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.6 (COC_6H_5), 138.1, 137.7, 133.2, 132.9 (2), 129.9(2), 129.6(2), 129.5, 129.3, 128.4(2), 128.3(2), 128.1(2), 127.9 (ArC), 86.8 (C-1), 75.1, 74.0, 73.6, 73.3, 21.1 (S-$C_6H_4CH_3$) and 16.7 (C-CH_3). HRMS calcd for $C_{27}H_{28}O_5SNa$ $(M+Na)^+$: 464.1657, found: 464.1661.

4.4. *p*-Tolyl 4-*O*-benzoyl-2-*O*-benzyl-3-*O*-chloroacetyl-1-thio-β-L-fucopyranoside (4)

To a mixture of compound **3** (3.9 g, 8.5 mmol) in dry CH_2Cl_2 (20 mL), pyridine (5 mL) was added followed by the addition of chloroacetic anhydride (1.6 g, 9.3 mmol) at 0 °C. The reaction mixture was allowed to stir at the same temperature for 1 h until the TLC (*n*-hexane–EtOAc; 2:1) showed complete conversion of the starting material to a faster moving spot. The solvents were evaporated and co-evaporated with toluene. The crude mixture was dissolved in CH_2Cl_2 (20 mL) and washed successively with 1 M HCl (2 × 25 mL), H_2O (25 mL), aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was collected, dried (Na_2SO_4), filtered and evaporated in vacuo. The crude mixture thus obtained was purified by flash chromatography by using *n*-hexane–EtOAc; 2:1 as the eluent to yield the pure product **4** (4.1 g, 91%) as a white solid. $[\alpha]_D^{25}$ −45 (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.04–7.19 (m, 14H, ArH), 5.48 (dd, 1H, $J_{3,4}$ 3.0 Hz, $J_{4,5}$1.5 Hz, H-4), 5.19 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 4.84 (ABq, 1H, J_{AB}11.0 Hz, CH_2Ph), 4.69 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 4.57 (ABq, 1H, J_{AB}11.0 Hz, CH_2Ph), 3.91 (m, 1H, H-5), 3.79 (ABq, 2H, J_{AB} 15.0 Hz, COCH_2Cl), 3.78 (t, 1H, $J_{1,2}J_{2,3}$ 10.0 Hz, H-2), 2.41 (s, 3H, S-$C_6H_4CH_3$) and 1.30 (d, 3H, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 138.1, 137.9, 133.5, 133.4(2), 129.9(2), 129.7(2), 129.2, 128.7, 128.4(2), 128.3(2), 127.9(2), 127.8 (ArC), 87.0 (C-1), 75.3, 74.5, 73.0, 71.1, 40.4 (COCH_2Cl), 21.2 (S-$C_6H_4CH_3$) and 16.7 (C-CH_3). HRMS calculated for $C_{29}H_{29}O_6SClNa$ $(M+Na)^+$: 540.1373, found: 540.1379.

4.5. *p*-Methoxyphenyl 4-*O*-benzoyl-2-*O*-benzyl-3-*O*-chloroacetyl-α-L-fucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (6)

A mixture of known *p*-methoxyphenyl 4,6-*O*-benzylidene-2 deoxy-2-phthalimido-β-D-gucopyranoside **5** (1.5 g, 3.0 mmol), donor **4** (1.90 g, 3.5 mmol) and MS 4 Å (2 g) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen atmosphere for 20 min. Then NIS (1.1 g, 4.7 mmol) was added followed by the addition of

H_2SO_4–silica (75 mg) and the reaction mixture was stirred at −40 °C for 20 min. The TLC (*n*-hexane–EtOAc 2:1) showed the complete consumption of the donor **4**. The reaction was then neutralized by the addition of Et_3N and filtered on a bed of Celite® (Merck, Germany) and the filtrate was washed successively with saturated aqueous $Na_2S_2O_3$ solution (2 × 25 mL), saturated aqueous $NaHCO_3$ solution (2 × 25 mL) and brine (25 mL). The organic layer was separated, dried (Na_2SO_4) filtered and evaporated in vacuo. The crude mixture obtained was purified by flash chromatography using *n*-hexane–EtOAc (2:1) as the eluent to yield the disaccharide **6** (2.3 g, 85%) as a white amorphous solid. $[\alpha]_D^{25}$ +53 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.87–6.74 (m, 23H, Ar*H*), 5.80 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.62 (s, 1H, C*H*Ph), 5.31 (m, 2H, H-3′, H-4′), 4.87 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.82 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 9.0 Hz, H-3), 4.67 (dd, 1H, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 4.45 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.39 (m, 1H, H-5′), 4.10 (ABq, ^{1}H J_{AB}12.5 Hz, CH_2Ph), 3.87 (m, 4H, H-5, H-6b, H-4, CH_2Ph), 3.72 (s, 3H, $C_6H_5OCH_3$), 3.70 (m, 1H, H-2′), 3.56 (dd, 2H, *J*15.0 Hz, COCH_2Cl) and 0.62 (d, 3H, *J* 6.5 Hz, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.0 (*C*OCH$_2$Cl), 155.5, 150.5, 137.5, 137.0, 133.9, 133.1, 129.6(2), 129.3, 129.2, 128.3(2), 128.2(3), 128.1(3), 127.5, 127.4(2), 126.4(3), 118.5(3), 114.4(3), (Ar*C*), 102.1 (*C*HPh), 99.3 (C-1′), 98.2 (C-1), 81.1, 75.5, 72.7, 72.5, 72.2, 71.8, 68.6, 66.5, 65.2, 55.6, 55.5 ($C_6H_5OCH_3$), 40.3 (COCH_2Cl) and 15.1 (C-*C*H$_3$). HRMS calculated for $C_{50}H_{46}O_{14}$NClNa (M+Na)$^+$: 919.2607, found: 919.2604.

4.6. *p*-Methoxyphenyl 4-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalim ido-β-D-glucopyranoside (7)

To a solution of the disaccharide **6** (2.3 g, 2.5 mmol) in MeOH/CH_2Cl_2 (3:2; 50 mL), thiourea (1.1 g, 15.0 mmol) was added followed by the addition of 2,4,6-collidine (1.7 mL, 12.5 mmol). The reaction mixture was stirred under reflux for 24 h until the TLC (*n*-hexane–EtOAc 1.5:1) showed complete consumption of the starting material. The reaction mixture was transferred into a separating funnel, diluted with CH_2Cl_2 (10 mL) and washed successively with H_2O (3 × 30 mL). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated in vacuo, and the crude product thus obtained was purified by flash chromatography to yield the disaccharide acceptor **7** (1.7 g, 80%) as an amorphous white solid. $[\alpha]_D^{25}$ +65 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.85–6.73 (m, 23H, Ar*H*), 5.80 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.59 (s, 1H, C*H*Ph), 5.24 (m, 1H, H-4′), 4.90 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.81 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 9.0 Hz, H-3), 4.65 (dd, 1H, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 4.44 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.34 (m, 1H, H-5′), 4.27 (ABq, 1H J_{AB}12.5 Hz, CH_2Ph), 4.13 (m, 1H, H-3′), 3.86 (m, 4H, H-5, H-6b, H-4, CH_2Ph), 3.72 (s, 3H, $C_6H_5OCH_3$), 3.51 (dd, 1H, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10.5 Hz, H-2′) and 0.70 (d, 3H, *J* 6.5 Hz, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.5 (*C*OC_6H_5), 155.6, 150.6, 137.5, 137.0, 134.1, 132.9, 131.7, 129.7(2), 129.2, 128.3(3), 128.2(2), 127.8(2), 127.7, 126.2(2), 118.6(2), 114.5(2), (Ar*C*), 102.0 (*C*HPh), 98.5 (C-1′), 98.2 (C-1), 81.3, 75.0, 74.9, 68.7, 68.3, 66.5, 65.7, 55.9, 55.5 ($C_6H_5OCH_3$) and 15.6 (C-*C*H$_3$). HRMS calculated for $C_{48}H_{45}O_{13}$NNa(M+Na)$^+$: 843.2891, found: 843.2895.

4.7. *p*-Tolyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-mannopyranoside (9)

To a mixture of *p*-tolyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside **8** (1.9 g, 3.2 mmol) in dry CH_2Cl_2 (20 mL), pyridine (5 mL) was added followed by the addition of chloroacetic anhydride (600 mg, 3.5 mmol) at 0 °C. The reaction mixture was allowed to stir at the same temperature for 1 h until the TLC (*n*-hexane–EtOAc 3:1) showed complete conversion of the starting material to a faster-moving spot. The solvents were evaporated and co-evaporated with toluene. The crude mixture thus obtained was dissolved in CH_2Cl_2 (20 mL) and washed successively with 1 M HCl (2 × 25 mL), H_2O (25 mL), aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was collected, dried (Na_2SO_4), filtered and evaporated in vacuo. The crude product thus obtained was purified by flash chromatography by using *n*-hexane–EtOAc (3:1) as the eluent to yield the pure **9** (2.0 g, 92%) as a colourless syrup. $[\alpha]_D^{25}$ +103 (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.13–7.15 (m, 19H, Ar*H*), 5.98 (t, 1H, $J_{3,4}J_{4,5}$ 10.0 Hz, H-4), 5.96 (m, 1H, H-2), 5.87 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.71 (d, 1H, $J_{1,2}$ 1.0 Hz, H-1), 4.9 (m, 1H, H-5), 4.52 (dd, 1H, $J_{5,6a}$ 5.5 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.38 (dd, 1H, $J_{5,6b}$ 2.5 Hz, $J_{6a,6b}$ 12.0 Hz, H-6b), 4.04 (ABq, 2H, J_{AB} 15.0 Hz, COCH_2Cl) and 2.34 (s, 3H, S-$C_6H_4CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.9 (*C*OCH$_2$Cl), 165.5, 165.4, 165.3 (*C*OC_6H_5), 138.6, 133.7(2), 133.6(2), 133.3, 132.6(2), 130.1(2), 130.0(2), 129.9(2), 129.8(2), 129.7(2), 129.3, 129.1, 128.6(2), 128.5, 128.4, 128.3 (Ar*C*), 86.1 (C-1), 71.7, 70.1, 69.3, 66.9, 64.2, 40.5 (*C*OCH$_2$Cl) and 21.1 (S-$C_6H_4CH_3$). HRMS calculated for $C_{36}H_{31}O_9$ClSNa(M+Na)$^+$: 674.1377, found: 674.1380.

4.8. *p*-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-mannopyranosyl-(1→3)-4-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalim ido-β-D-glucopyranoside (10)

A mixture of the disaccharide acceptor **7** (1.7 g, 2.0 mmol), donor **9** (1.75 g, 2.6 mmol) and MS 4 Å (2.0 g) in dry CH_2Cl_2 (20 mL) was stirred under a nitrogen environment for 20 min. Then to the reaction mixture, NIS (2.3 g, 3.4 mmol) was added followed by the addition of H_2SO_4–silica (75 mg). The reaction mixture was allowed to stir at 0 °C for 20 min until the TLC (*n*-hexane–EtOAc 3:1) showed complete conversion of the donor **9**. The reaction mixture was immediately filtered on a bed of Celite® and the filtrate was washed successively with saturated aqueous $Na_2S_2O_3$ (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL) and brine (25 mL). The organic layer was collected, dried (Na_2SO_4) and filtered, and the solvents were evaporated in vacuo. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (2:1) as the eluent to yield the pure trisaccharide **10** (2.35 g, 82%) as a white amorphous solid. $[\alpha]_D^{25}$ +78 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.17–6.74 (m, 38H, Ar*H*), 5.92 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.83 (t, 1H, $J_{3'',4''}J_{4'',5''}$ 10.0 Hz, H-4″), 5.64 (s, 1H, C*H*Ph), 5.53 (dd, 1H, $J_{2'',3''}$ 3.0 Hz, $J_{3'',4''}$ 10.0 Hz, H-3″), 5.48 (m, 2H, H-4′, H-2″), 4.95 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.64 (d, 1H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.62 (m, 4H, H-6a, H-3, H-6a″, H-2), 4.46 (m, 3H, H-6b″, H-5′, H-5″), 4.33 (ABq, 2H, J_{AB} 15.0 Hz, COCH_2Cl), 4.24 (ABq, 1H, J_{AB} 12.0 Hz, CH_2Ph), 4.19 (dd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{3',4'}$3.5 Hz, H-3′), 3.93 (m, 2H, CH_2Ph, H-6b), 3.83 (t, 1H, $J_{3,4}J_{4,5}$ 9.0 Hz, H-4), 3.80 (m, 1H, H-5), 3.74 (dd, 1H, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10.0 Hz, H-3′), 3.72 (s, 3H, $C_6H_5OCH_3$) and 1.03 (d, 3H, *J* 6.5 Hz, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.6, 150.5, 137.3, 137.1, 133.8, 133.4, 133.2, 132.9, 131.5, 129.9(2), 129.8(2), 129.7, 129.2(2), 128.9(3), 128.7(2), 128.5(2), 128.2(2), 128.1(2), 127.8, 126.1(2), 118.7(2), 114.5(2)(Ar*C*), 101.4 (*C*HPh), 99.2 (C-1′), 98.5 (C-1″), 98.0 (C-1), 82.1, 77.2, 75.5, 73.9, 73.8, 73.0, 69.7, 69.5, 68.7, 68.6, 66.7, 66.4, 66.1, 64.4, 55.5 ($C_6H_5OCH_3$), 55.3, 40.9 (COCH_2Cl) and 16.0 (C-*C*H$_3$). HRMS calculated for $C_{77}H_{68}O_{22}$ClNNa(M+Na)$^+$: 1393.3922, found: 1393.3928.

4.9. *p*-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4-*O*-benzoyl-2-*O*-benzyl-α-L-fuco pyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (11)

To a solution of the trisaccharide **10** (2.35 g, 1.7 mmol) in MeOH/CH_2Cl_2 (3:2) (50 mL), thiourea (770 mg, 10.1 mmol) was

added followed by the addition of 2,4,6-collidine (1.1 mL, 8.5 mmol). The reaction mixture was stirred under reflux for 48 h until the TLC (*n*-hexane–EtOAc 1.5:1) showed complete conversion of the starting material to a slower moving spot. The mixture was diluted with CH_2Cl_2 (10 mL) and washed successively with H_2O (3 × 30 mL). The organic layer was then separated, dried (Na_2SO_4) and filtered, and the solvents were evaporated in vacuo. The crude product thus obtained was purified by flash chromatography to get the pure trisaccharide acceptor **11** (1.8 g, 80%) as an amorphous white solid. $[\alpha]_D^{25}$ +101 (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.16–6.73 (m, 38H, Ar*H*), 5.93 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.74 (t, 1H, $J_{3'',4''}J_{4'',5''}$ 10.0 Hz, H-4″), 5.69 (dd, 1H, $J_{2'',3''}$ 3.0 Hz, $J_{3'',4''}$ 10.0 Hz, H-3″), 5.63 (s, 1H, C*H*Ph), 5.57 (m, 1H, H-4′), 5.43 (m, 1H, H-2″), 4.96 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.66 (d, 1H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.61 (m, 2H, H-3, H-2), 4.48 (m, 2H, H-6a, H-5′), 4.37 (m, 1H, H-5″), 4.32 (dd, 1H, $J_{2',3'}$3.5 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 4.21 (ABq, 1H, J_{AB} 12.0 Hz, CH_2Ph), 3.93 (m, 4H, CH_2Ph, H-6b, H-6a″, H-6b″), 3.78 (m, 3H, H-2′, H-4, H-5), 3.71 (s, 3H, $C_6H_5OCH_3$) and 1.09 (d, 3H, *J* 6.5 Hz, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.6, 150.5, 137.3, 137.1, 133.7, 133.4, 133.1, 132.9, 131.3, 130.0(2), 129.9(2), 129.8(2), 129.5(2), 129.3, 129.1(2), 128.9(3), 128.8, 128.5(2), 128.4(2), 128.1(2), 127.8, 126.0(2), 118.7(2), 114.5(2) (Ar*C*), 101.2 (*C*HPh), 99.1 (C-1′), 97.8 (C-1), 97.6 (C-1″), 82.3, 77.3, 76.4, 73.7, 73.0, 71.9, 71.2, 70.2, 69.4, 68.6, 67.5, 66.4, 66.3, 62.5, 55.5 ($C_6H_5OCH_3$), 55.4 and 16.0 (C-CH_3). HRMS calculated for $C_{75}H_{67}O_{21}NNa$ $(M+Na)^+$: 1317.4206, found: 1317.4203.

4.10. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mann opyranosyl-(1→3)-4-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthali mido-β-D-glucopyranoside (13)

A mixture of trisaccharide acceptor **11** (1.8 g, 1.4 mmol), donor **12** (536 mg, 1.6 mmol) and MS 4 Å (1.5 g) in dry CH_2Cl_2 (15 mL) was allowed to stir at −55 °C under a nitrogen atmosphere for 30 min. H_2SO_4–silica (50 mg) was added to the reaction mixture and it was allowed to stir at the same temperature for 12 h. Then the reaction was quenched by adding Et_3N and immediately filtered over a bed of Celite®. The solvents were evaporated in vacuo and the crude product was purified by flash chromatography using *n*-hexane–EtOAc (2:1) to give the pure tetrasaccharide **13** (1.6 g, 73%) as an amorphous solid. $[\alpha]_D^{25}$ +82 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.20–6.74 (m, 38H, Ar*H*), 5.95 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.84 (t, 1H, $J_{3'',4''}J_{4'',5''}$ 10.0 Hz, H-4″), 5.82 (dd, 1H, $J_{2''',3'''}$11.0 Hz, $J_{3''',4'''}$ 3.0 Hz, H-3‴), 5.66 (s, 1H, C*H*Ph), 5.65 (m, 1H, H-4‴), 5.59 (dd, 1H, $J_{2'',3''}$ 3.0 Hz, $J_{3'',4''}$ 10.0 Hz, H-3″), 5.56 (d, 1H, $J_{3',4'}$ 3.5 Hz, $J_{4',5'}$ <1.0 Hz, H-4′) , 5.49 (dd, 1H, $J_{1'',2''}$ 1.5 Hz, $J_{2'',3''}$ 3.0 Hz, H-2″), 5.21 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.90 (d, 1H, $J_{1''',2'''}$ 3.0 Hz, H-1‴), 4.75 (d, 1H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.73 (m, 1H, H-5″), 4.61 (m, 3H, H-2, H-3, H-5′), 4.47 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.32 (dd, 1H, $J_{2',3'}$ 10.0, Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 4.15 (m, 2H, CH_2Ph, H-6a″), 4.06 (m, 3H, H-6a‴, H-6b″, H-4), 3.90 (t, 1H, $J_{5,6b}$ Hz, $J_{6a,6b}$ 12.0 Hz, H-6b), 3.77 (m, 5H, H-6b‴, CH_2Ph, H-5‴, H-2‴, H-5), 3.72 (s, 3H, $C_6H_5OCH_3$), 3.70 (m, 1H, H-2′), 2.16, 2.14, 1.84 (3s, 9H, 3 × COCH_3) and 1.04 (d, 3H, *J* 6.5 Hz, C-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.3, 169.9, 169.5 (*C*OCH$_3$), 166.8 (*C*OCH$_2$Cl), 165.6, 164.9, 164.8 (*C*OC$_6$H$_5$), 155.4, 150.6, 137.5, 137.2, 133.6(2), 133.3, 133.0, (3), 132.8, 131.6, 130.0(3), 129.9(2), 129.8(2), 129.5(2), 129.3, 129.2, 129.1, 128.8, 128.4(2), 128.2(2), 128.1(2), 127.9(2), 127.7(2), 126.1(3), 122.9, 118.5(2), 114.4(2) (Ar*C*), 101.2 (*C*HPh), 99.4(C-1‴), 98.3 (C-1″), 98.0 (C-1′), 97.6 (C-1), 81.7, 77.5, 76.1, 74.0, 73.0, 72.8, 70.0, 69.7, 69.5, 68.7, 68.5, 68.1, 67.1, 66.8, 66.7, 66.3, 66.2, 62.2, 57.9, 55.6 ($C_6H_5OCH_3$), 55.2 and 15.8 (C-CH_3). HRMS calculated for $C_{87}H_{82}O_{28}N_4Na$ $(M+Na)^+$: 1630.5116, found: 1630.5119.

4.11. *p*-Methoxyphenyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→6)-α-D-mannopyranosyl-(1→3)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-acetamido-β-D-gluco pyranoside (1)

Compound **13** (1.6 g, 1.0 mmol) was dissolved in AcOH/H_2O (8:1) (20 mL) and the reaction mixture was stirred at 80 °C for 4 h until the starting material was completely consumed to form a more polar compound as observed from TLC (*n*-hexane–EtOAc 1:1). Then the solvents were evaporated and co-evaporated with toluene. The residue was dried, dissolved in thioacetic acid (10 mL) and stirred at room temperature in the dark for 72 h, until the starting material was completely consumed. The solvents were evaporated and co-evaporated with toluene. The residue was dried and was further dissolved in *n*-butanol (15 mL) followed by the addition of ethylene diamine (250 μL). The reaction mixture was allowed to stir at 110 °C for 24 h until the TLC showed complete consumption of the starting material. The solvents were evaporated and co-evaporated with toluene and dried. The residue was dissolved in pyridine (5 mL) followed by the addition of Ac_2O (5 mL) and the mixture was allowed to stir at 50 °C for 10 h. Then the solvents were evaporated and co-evaporated with toluene. It was then dissolved in CH_2Cl_2 (20 mL) and washed successively with H_2O (25 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and filtered. The solvents were evaporated and the residue was dried. A dilute solution of the residue in MeOH (30 mL) and AcOH (1 mL) was passed through flow-hydrogenation assembly fitted with a Pd–C cartridge at 50 °C at normal atmospheric pressure of hydrogen. The starting material was completely consumed to yield a more polar compound as evident from TLC (CH_2Cl_2/MeOH 7:1) after four cycles. The solvents were then evaporated in vacuo and the residue was dissolved in MeOH (5 mL) followed by the addition of freshly prepared NaOMe in MeOH (5 mL). The reaction mixture was allowed to stir at 50 °C for 10 h until TLC (CH_2Cl_2/MeOH 5:1) showed complete conversion of the starting material. The reaction was neutralized using DOWEX 50-W H^+ resin. The mixture was then filtered through a cotton plug to remove DOWEX and the filtrate was evaporated in vacuo to yield the pure tetrasaccharide **1** (510 mg, 62%) as a white sticky compound. $[\alpha]_D^{25}$ +36 (*c* 0.8, CH_3OH). ^1H NMR (500 MHz, CD_3OD) δ: 6.95, 6.82 (2d, 4H, *J* 9.0 Hz, $C_6H_4OCH_3$), 5.11 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.96 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.94 (d, 1H, $J_{1''',2'''}$ 3.5 Hz, H-1‴), 4.85 (s, 1H, H-1″), 3.73 (s, 3H, $C_6H_4OCH_3$), 2.01, 1.98 (2s, 6H, 2 × NHCOCH_3) and 1.26 (d, 3H, *J* 6.5 Hz, C-CH_3). ^{13}C NMR (125 MHz, CD_3OD) δ: 174.6, 174.4 (2 × NH*C*OCH$_3$), 156.8, 153.1, 119.2(2), 115.6(2) (ArC), 103.8 (C-1′), 101.8 (C-1), 101.6 (C-1‴), 99.1 (C-1″), 83.0, 79.3, 78.1, 73.5, 73.2, 72.5, 72.4, 72.0, 70.4, 70.3, 70.2, 69.1, 68.3, 68.2, 67.5, 62.7, 62.5, 56.9, 56.1 (C_6H_4-OCH$_3$), 52.6, 51.8, 23.0, 22.6 (2 × NHCOCH$_3$) and 16.6 (C-CH_3). HRMS calculated for $C_{35}H_{54}O_{21}N_2Na$ $(M+Na)^+$: 861.3117, found: 861.3121.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2014.08.004.

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