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Convergent chemical synthesis of the pentasaccharide repeating unit of the O-antigen from *E. coli* O158

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Synthesis of the pentasaccharide repeating unit of the O-antigen from *E. coli* O158 through a convergent [3+2] strategy is reported. Synthesis of the crucial β-ManNAc moiety was achieved from a β-Glc derivative through inversion of the 2-position by an azide nucleophile in excellent yield. The non-reducing end disaccharide α-D-Gal-(1→3)-β-D-GlcNAc was formed through activation of the thioglycoside Gal donor in chemoselective manner. A late stage TEMPO-mediated oxidation was used to install the desired uronic acid moiety. The required glycosylations were accomplished through activation of thioglycosides using NIS in the presence of H_2SO_4-silica with good to excellent yields and stereoselectivity.

Introduction

Escherichia coli (*E. coli*) is an anaerobic Gram-negative facultative bacterium commonly found in the gut flora.[1] Although most of the *E. coli* strains are harmless, some of their serotypes acquire virulence and cause serious infections in human.[2] These infections can be classified in three major categories: (i) diarrhea; (ii) sepsis/meningitis and (iii) urinary tract infection.[3] *E. coli* strains that are associated with diarrheal infections are generally classified into five subgroups depending on their mode of actions;[4] enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC). EHEC strains are also termed as Shiga-toxin producing *E. coli* (STEC) and verotoxin producing *E. coli* (VTEC), which acts through the production of Shiga-like toxin during the initial stage of infection to the host.[5] Both pathogenic and commensal *E. coli* strains are identified by their somatic (O), capsular (K) or flagellar (H) antigens.[3] The O-antigens are vital part of the lipopolysaccharide consisting of several repeats of a particular oligosaccharide having 2-8 sugar units. This is the most variable cell constituent owing to the presence of various sugar units with different stereochemistry. More than 180 O-antigen variants are already known in the literature for *E. coli*.[4] These diversities resulted from the genetic variation in the O-antigen gene cluster that contains the genes involved in sugar-nucleotide biosynthesis, O-antigen processing and sugar transferases regulation.[6] Recently, through their studies on the O-antigen gene cluster of *E. coli* O158 belong to the EPEC family, Perepelov *et. al* reported the structure of the pentasaccharide repeating unit containing a β-ManNAc residue.[7] Here, we report the chemical synthesis of that pentasaccharide repeating unit attached with a suitable linker that can facilitate further glycoconjugate formation (**1**, **Figure 1**).

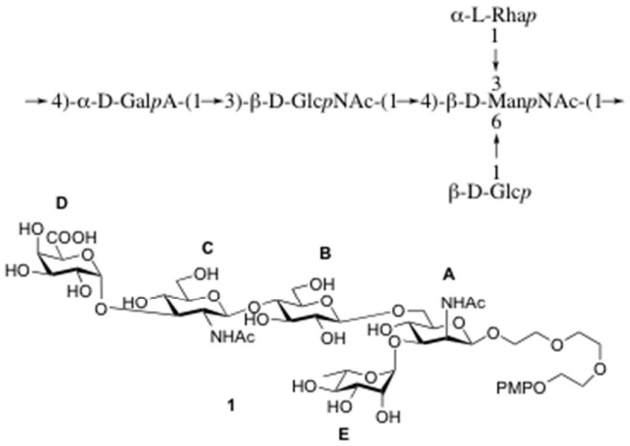

Figure 1: Reported structure of the pentasaccharide repeating unit and the target pentasaccharide **1**

Results and discussion

Study of the retrosynthetic analysis for the target pentasaccharide indicated a [3+2] strategy as the best choice to achieve the structure. Glycosylation between the trisaccharide acceptor **12** and the disaccharide donor **15** would furnish the protected pentasaccharide. It was necessary to attach a suitable aglycon at the reducing end of the target pentasaccharide to leave the scope for further glycoconjugate formation. Taking the cue from the literature,[8] a triethylene glycol linker was chosen at the anomeric position of the reducing end sugar. Once the final target oligosaccharide is prepared and converted to its corresponding per-*O*-acetyl derivative, the *p*-methoxyphenyl group at the terminus of the triethylene glycol may be cleaved selectively and the primary OH group thus obtained can be utilized for conjugation with suitable aglycons. Synthesis of the β-linked ManNAc residue at

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the reducing end was planned from the corresponding β-glucoside **4** through inversion of the 2-position with azide nucleophile. Rational protecting group manipulations and glycosylations with rhamnose donor **7** and glucose donor **10** were expected to furnish the trisaccharide acceptor **12**. For the synthesis of the non-reducing end disaccharide **15**, chemo-selective glycosylation of the armed galactose donor **14** with the disarmed thioglycoside acceptor **13** was postulated (**Figure 2**).

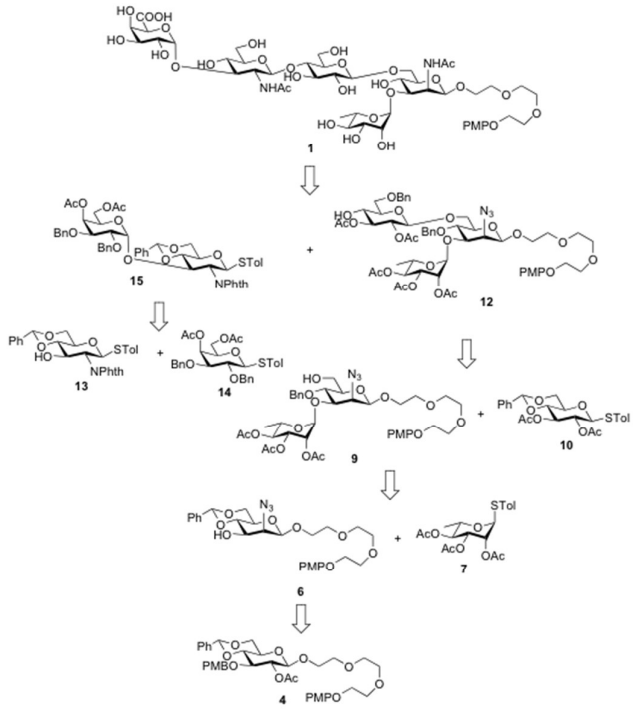
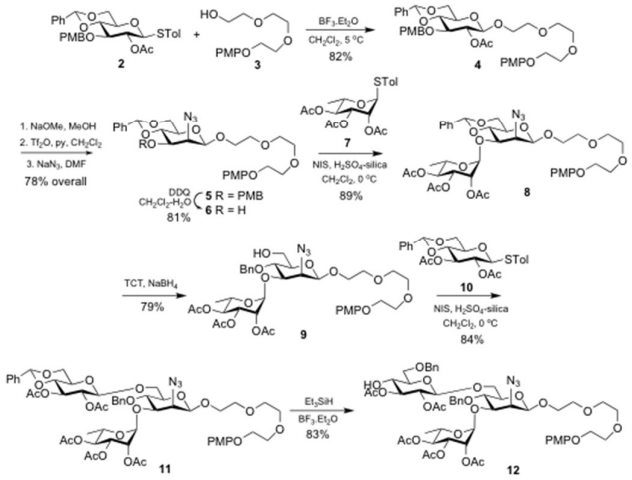


Figure 2: Retrosynthetic analysis for the synthesis of the target pentasaccharide **1**

Synthesis of the trisaccharide acceptor **12** was started with the known glucose donor **2**.[9] Glycosylation of **2** with the known triethylene glycol derivative **3**[10] through activation of the thioglycoside using *N*-Iodosuccinimide (NIS) in the presence of H_2SO_4-silica[11] gave the derivative **4** in 82% yield. The presence of the acetate group at the 2-position of the glucose donor moiety ensured exclusive formation of the 1,2-*trans* glycoside as evident from the ^{1}H NMR peak at δ 4.61 ppm (1H, $J_{1,2}$ = 8.0 Hz) and the ^{13}C NMR peak at δ 101.2 ppm (C-1). The J_{CH} coupling value of 161.5 Hz confirms the formation of the desired product. Selective removal of the 2-*O*-acetyl group using NaOMe in MeOH,[12] formation of the corresponding trifluoromethanesulfonate derivative using Tf_2O in the presence of pyridine[13] and subsequent reaction with NaN_3 in dry DMF[14] enabled us to achieve the mannose derivative **5** in 78% overall yield. The structure was confirmed by 1D and 2D NMR spectra. ^{1}H NMR peak at δ 4.65 ppm (1H, $J_{1,2}$ = 1.5 Hz), the ^{13}C NMR peak at δ 100.1 ppm (C-1) and the J_{CH} coupling value of 162.8 Hz affirmed the formation of the desired derivative. Next, oxidative cleavage of the *p*-methoxy benzyl (PMB) group using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone[15] (DDQ) afforded the required acceptor **6** in 81% yield. Glycosylation of the acceptor **6** with the known rhamnose donor **7**[16] through activation of the thioglycoside using NIS in the presence of H_2SO_4-silica resulted the disaccharide **8** in 89% yield. The newly formed 1,2-*trans* glycoside was confirmed by the ^{1}H NMR peak at δ 4.90 ppm (^{1}H, $J_{1,2}$ = 1.5 Hz), the ^{13}C NMR peak at δ 94.6 ppm (C-1) and the J_{CH} coupling value of 170.3 Hz. Further, regio-selective opening of the benzylidene acetal using 2,4,6-Trichloro-1,3,5-triazine (TCT) and $NaBH_4$[17] furnished the primary-OH acceptor **9** in 79% yield. Glycosylation of the known donor **10**[18] with the disaccharide acceptor **9** using similar NIS-mediated thioglycoside activation gave the protected trisaccharide **11** in 84% yield. The formation of the 1,2-*trans* glycoside was confirmed by the ^{1}H NMR peak at δ 4.67 ppm (^{1}H, $J_{1,2}$ =7.5Hz) and the ^{13}C NMR peak at δ 100.9 ppm (C-1). The J_{CH} coupling value of 162.0 Hz further confirms the formation of the 1,2-*trans* glycosidic linkage. Finally, regio-selective reductive opening of the benzylidene acetal using Et_3SiH in the presence of $BF_3.Et_2O$[19] furnished the trisaccharide acceptor **12** in 83% yield (**Scheme 1**).



Scheme 1: Synthesis of the trisaccharide acceptor **12**

For the synthesis of the disaccharide donor **15**, a chemo-selective glycosylation strategy was adopted. Thus, known disarmed thioglycoside acceptor **13**[20] was treated with armed thioglycoside donor **14**[21] using NIS in the presence of H_2SO_4-silica at -45 °C to afford the desired disaccharide **15** in 83% yield (**Scheme 2**). Formation of the required 1,2-*cis* glycoside was confirmed by the ^{1}H NMR peak at δ 5.57 ppm (1H, $J_{1,2}$ = 4.0Hz) and the ^{13}C NMR peak at δ 97.2 ppm assigned for the newly formed glycosidic linkage with a J_{CH} coupling value of 169.0 Hz.

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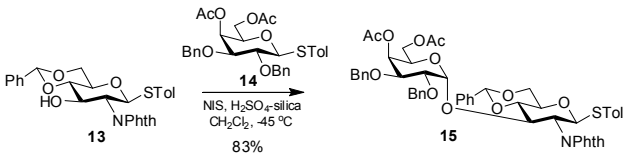
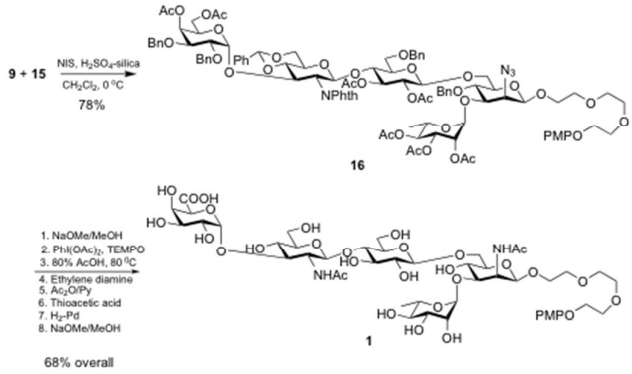


Scheme 2: Synthesis of the disaccharide donor **15**

Final glycosylation of the trisaccharide acceptor **12** with the disaccharide donor **15** was accomplished by NIS-mediated activation of the thioglycoside at 0 °C to give the protected pentasaccharide **16** in 78% yield. Compound **16** was de-*O*-acetylated using NaOMe in MeOH followed by the selective oxidation of the sole primary-OH group at the non-reducing end using 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO) in the presence of iodosobenzene diacetate (IBDA).[22] Further, the phthalimido group was de-protected with ethylene diamine[23] followed by acetylation using Ac_2O in dry pyridine to afford the desired acetamido group. Subsequent treatment with thioacetic acid[24] for 3 days at room temperature enabled to convert the azido group into the required acetamido group. Next, catalytic hydrogenation using 10% Pd-C cartridge on a ThalesNano flow hydrogenation assembly followed by Zemplen de-*O*-acetylation afforded the target pentasaccharide **1** in 68% overall yield (**Scheme 3**). During the protecting group transformations on the pentasaccharide derivative, the polar intermediates were not subjected to chromatographic purifications to avoid potential loss of the materials in silica gel matrix. The final compound was dissolved in water and washed with CH_2Cl_2 to remove the organic impurities. The aqueous layer was separated and freeze dried to get the pure target material.



Scheme 3: Synthesis of the target pentaccharide **1**

Conclusions

We have successfully established a convergent [3+2] strategy for the synthesis of the pentasaccharide repeating unit of the O-antigen from *E. coli* O158 fitted with a masked triethylene glycol linker (**1**). The reducing end β-mannose residue was achieved through inversion of the 2-OH position of a rationally protected glucose precursor with azide nucleophile. Chemo-selective glycosylations strategy was utilized to construct the non-reducing end disaccharide to simplify the total synthesis. A TEMPO-mediated late stage oxidation enabled us to install the required uronic acid moiety. After de-protection of the terminal *p*-methoxyphenyl group in the triethylene glycol linker from the per-*O*-acetylated oligosaccharide one can utilize the free OH group for the attachment of suitable aglycon to form further glycoconjugate without hampering the stereochemistry at the reducing end of the target pentasaccharide.

Experimental section

General Methods

All solvents and reagents were dried prior to use according to standardized methods.[25] The commercially purchased reagents 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 sodium-line at ambient temperature. ^1H and ^{13}C NMR spectra were recorded on Bruker AVANCE 500 MHz spectrometer. ^1H NMR values were denoted as H for the reducing end unit **A**, H′ for the unit **B**, H′′ for the unit **C**, H′′′ for the unit **D** and H′′′′ for the unit **E** as marked in the **Figure 1**.

Preparation of H_2SO_4-Silica[18]

To slurry of silica gel (10 g, 230–400 mesh) in dry diethyl ether (50 mL) was added commercially available concentrated H_2SO_4-Silica (1 mL), and the slurry was shaken for 5 min. The solvent was evaporated under reduced pressure, resulting in free flowing H_2SO_4-Silica, which was dried at 110 °C for 3 hours and then used for the reactions.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethanol (3):[10] To a mixture of 4-iodoanisole (234 mg, 1.0 mmol), potassium carbonate (414 mg, 3.0 mmol) and CuCl (6.7 mg, 0.05 mmol), triethylene glycol (1 mL) was added under N_2. The reaction mixture was stirred for 24 hours at 130 °C followed by treatment with 1N HCl solution to maintain the pH at 3. The resulting solution was extracted in EtOAc (20 mL) and the organic layer obtained was successively washed with H_2O (30 mL) and brine (30 mL). The organic phase was collected, dried over anhydrous Na_2SO_4 and filtered followed by evaporation of the solvent in *vacuo*. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (1:2) as eluent to afford the pure compound **3** (249 mg, 97%) as yellow oil. ^1H NMR ($CDCl_3$, 500 MHz) δ: 6.82-6.76 (2d, 4H, $OC_6H_4OCH_3$), 4.03(m, 2H, OCH_2), 3.78 (m, 2H, OCH_2), 3.70 (s, 3H, $OC_6H_4OCH_3$), 3.68-3.65 (m, 4H, 2×OCH_2), 3.63 (m, 2H, OCH_2), 3.55 (m, 2H, OCH_2), 3.01 (bs, O*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 153.8, 152.7, 115.5 (2), 114.4 (2), 72.4 (OCH_2), 70.5

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(OCH_2), 70.1 (OCH_2), 69.6 (OCH_2), 67.9 (OCH_2), 61.4 (OCH_2), 55.5 ($OC_6H_4OCH_3$). HRMS calcd for $C_{13}H_{20}O_5Na$ $(M+Na)^+$ 279.1208, found 279.1205.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (4): A mixture of known compound **2** (2.3 g, 4.2 mmol), compound **3** (835 mg, 3.3 mmol) and activated MS 4Å (2 g) in dry CH_2Cl_2 (30 mL) was stirred under nitrogen for 1 hour. NIS (1.2 g, 5.5 mmol) was added to the mixture in ice-water bath. After stirring for 15 min, H_2SO_4–silica (25 mg) was added and the mixture was allowed to stir until complete consumption of the acceptor **3** was evident by TLC using *n*-hexane-EtOAc (1:1) in about 15 minutes. The mixture was immediately filtered through a pad of Celite and the filtrate was washed successively with $Na_2S_2O_3$ (2×30 mL), $NaHCO_3$ (2×30 mL) and brine (30 mL). Organic layer was collected, dried over anhydrous Na_2SO_4 and evaporated to syrup. The crude product thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (1:1) to afford pure compound **4** (1.8 g, 82 %) as colorless gel. $[\alpha]_D^{25}$ +88 (*c* 1.0; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.51-6.80 (m, 13H, Ar*H*), 5.56 (s, 1H, C*H*Ph), 5.00 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2), 4.79, 4.60 (ABq, 2H, $J_{A\text{-}B}$ 12.0Hz, $OCH_2C_6H_4OCH_3$), 4.61 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.38 (dd, 1H, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.08 (m, 2H, OCH_2) 3.92 (m, 1H, H-6b), 3.82 (m, 2H, OCH_2), 3.78 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.76 (m, 1H, H-3), 3.75 (s, 3H, $OC_6H_4OCH_3$) 3.74-3.68 (m, 5H, H-4, 2×OCH_2), 3.66-3.62 (m, 4H, 2×OCH_2), 3.42 (m, 1H, H-5), 2.01 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.2 ($COCH_3$), 153.9, 152.9, 137.2, 130.3, 129.4(2), 128.9, 128.2(2), 126.0 (3), 115.6(2), 114.6(2), 113.7(2) (Ar*C*), 101.6 (*C*HPh), 101.2 (C-1), 81.4 (C-3), 78.0 (C-4), 73.6 ($OCH_2C_6H_4OCH_3$), 72.8 (C-2), 70.8 (OCH_2), 70.7 (OCH_2), 70.3 (OCH_2), 69.8 (OCH_2), 69.0 (OCH_2), 68.6 (C-6), 68.1 (OCH_2), 66.2 (C-5), 55.6 ($OC_6H_4OCH_3$), 55.2 ($OCH_2C_6H_4OCH_3$), 20.8 ($COCH_3$). HRMS calcd for $C_{36}H_{44}O_{12}Na$ $(M+Na)^+$ 691.2730, found 691.2726.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-mannopyranoside (5): To a solution of compound **4** (1.6 g, 2.4 mmol) in MeOH (20 mL), NaOMe in MeOH (2 mL, 0.5M) was added and the solution was stirred at room temperature for 2 hours. After complete conversion of the starting material to a slower moving spot, the solution was neutralized with DOWEX 50W H^+ resin, filtered and evaporated to an amorphous mass. The compound was then suspended in CH_2Cl_2 (20 mL) and pyridine (0.2 mL) was added followed by Tf_2O (0.4 mL) and the mixture was allowed to stir at room temperature till entire starting material was consumed. Solvents were evaporated *in vacuo* and the residual pyridine was co-evaporated with toluene. The resulting thick syrup was dissolved in CH_2Cl_2 (30 mL), poured into a separating funnel and washed successively with water (50 mL), and brine (50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was then dissolved in DMF (15 mL) and sodium azide (1.3 g, 20.4 mmol) was added to it. The reaction mixture was stirred overnight at 90 ^{0}C. The complete conversion of the starting material to a slower moving spot confirms the formation of the product. Solvents were evaporated *in vacuo*, the residue was dissolved in CH_2Cl_2 (25 mL) and washed with brine (30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*.The crude product thus obtained was further purified by flash chromatography using *n*-hexane-EtOAc (1:1) to afford the pure compound **5** (1.3 g, 78%) as yellow syrup. $[\alpha]_D^{25}$ +97 (*c* 0.9; $CHCl_3$)^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.50-6.79 (m, 13H, Ar*H*), 5.58 (s, 1H, C*H*Ph), 4.77, 4.66 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.65 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.29 (dd, 1H, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 10.0 Hz, H-6a), 4.06 (m, 2H, OCH_2), 4.01-3.96 (m, 3H, H-2, OCH_2), 3.85 (t, 1H, $J_{5,6b,}$ 5.0 Hz, $J_{6a,6b}$ 10.0 Hz, H-6b), 3.80 (m, 2H, OCH_2), 3.78 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.74 (m, 1H, H-3), 3.72 (s, 3H, $OC_6H_4OCH_3$), 3.71-3.66 (m, 7H, 3×OCH_2, H-4), 3.30 (m, 1H, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 159.2, 153.7, 152.7, 137.2, 129.6, 129.1 (2), 128.7 (2), 128.0, 125.8 (2), 115.4 (2), 114.4 (2), 113.7 (2) (Ar*C*), 101.3 (*C*HPh), 100.1 (C-1), 78.3 (C-2), 75.8 (C-4), 72.3 (CH_2Ph), 70.5 (2×OCH_2), 70.2 (OCH_2), 69.6 (OCH_2), 68.8 (C-3), 68.2 (C-6), 67.9 (OCH_2), 67.1 (C-5), 63.2 (OCH_2), 55.4 ($OC_6H_4OCH_3$), 55.0 ($OCH_2C_6H_4OCH_3$). HRMS calcd for $C_{34}H_{41}N_3O_{10}Na$ $(M+Na)^+$ 674.2690, found 674.2688.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside (6): Compound **5** (1.2 g, 1.8 mmol) was dissolved in 30 mL CH_2Cl_2:MeOH (4:1) followed by addition of DDQ (696 mg, 3.1 mmol) and the mixture was allowed to stir at room temperature till the complete consumption of the starting material was evident from TLC using *n*-hexane-EtOAc (1:1) as eluent. The mixture was diluted with CH_2Cl_2 (15 mL) and washed successively with water (2×40 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash chromatography using *n*-hexane-EtOAc (4:5) to afford the desired acceptor **6** (793 mg, 81%) as colorless gel. $[\alpha]_D^{25}$ +74 (*c* 0.9; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.47-6.80 (m, 9H, Ar*H*), 5.51 (s, 1H, C*H*Ph), 4.70 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.77, 4.29 (dd, 1H, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 10.0 Hz, H-6a), 4.07 (m, 2H, OCH_2), 4.05 (dd, 1H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 4.0 Hz, H-2), 3.99 (m, 1H, H-3), 3.83-3.79 (m, 4H, 2×OCH_2), 3.77 (m, 1H, H-6b), 3.74 (s, 3H, $OC_6H_4OCH_3$), 3.72-3.67 (m, 7H, 3×OCH_2, H-4), 3.29 (m, 1H, H-5), 2.76 (d, 1H, *J* 5.5 Hz, O*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 153.9, 152.8, 137.0, 129.2, 128.2 (2), 126.2 (2), 115.7 (2), 114.6 (2) (Ar*C*), 102.0 (*C*HPh), 100.7 (C-1), 78.5 (C-4), 70.7 (2×OCH_2), 70.4 (OCH_2), 70.1 (OCH_2), 69.8 (OCH_2), 69.2 (OCH_2), 68.3 (C-6), 68.1 (C-2), 67.0 (C-5), 64.5 (C-3), 55.6 ($OC_6H_4OCH_3$). HRMS calcd for $C_{26}H_{33}N_3O_9Na$ $(M+Na)^+$ 554.2114, found 554.2113.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2,3,4-tri-*O*-acetyl- α-L-rhamnopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside (8):
A mixture of acceptor **6** (700 mg, 1.3 mmol), donor **7** (467 mg, 1.7 mmol) and activated MS 4Å in anhydrous CH_2Cl_2 was stirred under N_2 for 30 min. The reaction was cooled to 0 ^{o}C followed by addition of NIS (500 mg, 2.2 mmol) and H_2SO_4-silica (20 mg). Within 5 minutes the acceptor was consumed as

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evident from the TLC using *n*-hexane-EtOAc (1:1) as eluent. The reaction mixture was immediately filtered through a pad of Celite. The filtrate was collected and washed successively with $Na_2S_2O_3$ (2×30 mL), $NaHCO_3$ (2×30 mL) and brine (30 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 using *n*-hexane-EtOAc (1:1) to afford pure disaccharide **8** (942 mg, 89%) as colorless gel. $[\alpha]_D^{25}$ +101 (*c* 0.8; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.46-7.29 (m, 5H, Ar*H*), 6.83, 6.79 (2d, 4H, $OC_6H_4OCH_3$), 5.57 (s, 1H, C*H*Ph), 5.34 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.24 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.5Hz, H-2′), 4.99 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 4.90 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.68 (d, 1H, $J_{1,2}$ <1.0 Hz, H-1), 4.29 (dd, 1H, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 10.0 Hz, H-6a), 4.17 (m, 1H, H-5′), 4.10 (dd, 1H, $J_{1,2}$ <1.0 Hz, $J_{2,3}$ 1.0 Hz, H-2), 4.06 (m, 2H, OCH_2), 4.00-3.90 (m, 2H, H-3, H-4), 3.93, 3.68 (m, 2H, OCH_2), 3.85 (dd, 1H, $J_{5,6b}$ 8.5 Hz, $J_{6a,6b}$ 10.0 Hz, H-6b), 3.81 (m, 2H, OCH_2), 3.72 (s, 3H, $OC_6H_4OCH_3$), 3.71-3.65 (m, 6H, 3×OCH_2), 3.33 (m, 1H, H-5), 2.11, 1.96, 1.95 (3s, 9H, 3×COCH_3), 0.88 (d, 3H, $J_{5',6'}$ 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.9, 169.8, 169.7 (3×*C*OCH$_3$), 153.8, 152.7, 137.1, 128.8, 127.9 (2), 126.0 (2), 115.5 (2), 114.5 (2) (Ar*C*), 101.6 (*C*HPh), 100.0 (C-1), 94.6 (C-1′), 78.4 (OCH_2), 76.1 (C-3), 74.0 (C-4), 70.8 (C-4′), 70.6 (2×OCH_2), 70.3 (OCH_2), 69.7 (OCH_2), 69.6 (C-2′), 69.1 (OCH_2), 68.7 (C-3′), 68.2 (C-6), 68.0 (OCH_2), 67.4 (C-5), 66.3 (C-5′), 61.6 (C-2), 55.5 ($OC_6H_4OCH_3$), 20.7, 20.6 (3×COCH_3), 16.7 (C-CH_3). HRMS calcd for $C_{38}H_{49}N_3O_{16}Na$ (M + Na)$^+$ 826.3011, found 826.3009.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2,3,4-tri-*O*-acetyl- α-L-rhamnopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-β-D-mannopyranoside (9): The dissacharide **8** (900 mg, 1.1 mmol) was dissolved in anhydrous CH_3CN (20 mL) followed by slow addition of $NaBH_4$ (423 mg, 11.2 mmol) at 0 °C. After stirring the reaction mixture at this temperature for 10 min, TCT (1.7 g, 8.9 mmol) was added. The reaction was continued to stir for another 10 hours. After the starting material was completely consumed, the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated and evaporated *in vacuo*. The crude compound was purified by flash chromatography using *n*-hexane-EtOAc (1:2) to afford the pure disaccharide acceptor **9** (713 mg, 79%) as colorless foam. $[\alpha]_D^{25}$ +79 (*c* 0.8; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.37-7.25 (m, 5H, Ar*H*), 6.84, 6.79 (2d, 4H, $OC_6H_4OCH_3$), 5.38 (dd, 1H, $J_{2',3'}$ 3.5Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.30 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.5 Hz, H-2′), 4.99 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 4.97 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.88, 4.68 (ABq, 2H, $J_{A\text{-}B}$ 11.0 Hz, CH_2Ph), 4.65 (d, 1H, $J_{1,2}$ <1.0 Hz, H-1), 4.10 (dd, 1H, $J_{1,2}$ <1.0 Hz, $J_{2,3}$ 2.5 Hz, H-2), 4.07 (m, 2H, OCH_2), 4.03-3.96 (m, 2H, H-3, H-5′), 3.88 (m, 1H, H-6a), 3.86, 3.69 (m, 2H, OCH_2), 3.84-3.81(m, 3H, H-4, OCH_2), 3.77 (m, 1H, H-6b), 3.74 (s, 3H, $OC_6H_4OCH_3$), 3.73-3.65 (m, 6H, 3×OCH_2), 3.31 (m, 1H, H-5), 2.49 (bs, 1H, O*H*),2.14, 1.99, 1.96 (3s, 9H, 3×COCH_3), 1.06 (d, 3H, $J_{5',6'}$ 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.7, 169.8 (2) (3×*C*OCH$_3$), 153.9, 152.8, 137.5, 128.3 (2), 127.9 (2), 127.8, 115.6 (2), 114.5 (2) (Ar*C*), 99.6 (C-1), 93.2 (C-1′), 75.9 (C-5), 75.6 (C-4), 75.4 (*C*H$_2$Ph), 72.9 (OCH_2), 70.7 (C-4′), 70.6 (2×OCH_2), 70.4 (OCH_2), 69.8 (OCH_2), 69.6 (C-2), 69.0 (C-3), 68.7 (C-3′), 68.0 (OCH_2), 66.8 (C-5′), 61.6 (C-6), 59.8 (C-2), 55.6 ($OC_6H_4OCH_3$), 20.8, 20.6 (2) (3×COCH_3), 17.2 (C-CH_3). HRMS calcd for $C_{38}H_{51}N_3O_{16}Na$ (M+Na)$^+$ 828.3167, found 828.3164.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-[2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl]-β-D-mannopyranoside (11): A mixture of the disaccharide acceptor **9** (700 mg, 0.9 mmol), donor **10** (387 mg, 1.2 mmol) and activated MS 4Å in anhydrous CH_2Cl_2 (15 mL) was stirred under N_2 for 30 minutes. The reaction was cooled to 0 °C and NIS (337 mg, 1.5 mmol) was added followed by H_2SO_4-silica (30 mg) and the mixture was stirred at 0 °C for 30 minutes when TLC (*n*-hexane:EtOAc, 3:1) showed complete conversion of the starting materials. The mixture was filtered through a pad of Celite, diluted with CH_2Cl_2 (20 mL) and the combined filtrate was washed successively with aq. $Na_2S_2O_3$ (2×30 mL), $NaHCO_3$ (2×30 mL) and brine (30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and filtered. The solvents were evaporated *in vacuo*. The crude residue was purified by flash chromatography using *n*-hexane-EtOAc (1:1) to afford pure trisaccharide **11** (832mg, 84%) as white foam. $[\alpha]_D^{25}$ +68 (*c* 0.7; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.42-7.26 (m, 10H, Ar*H*), 6.84, 6.80 (2d, 4H, $OC_6H_4OCH_3$), 5.50 (s, 1H, C*H*Ph), 5.38 (dd, 1H, $J_{2',3'}$ 3.5Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.30 (dd, 1H, $J_{1',2'}$1.5 Hz, $J_{2',3'}$ 3.5Hz, H-2′), 5.26 (t, 1H, $J_{2'',3''}$, $J_{3'',4''}$ 9.0 Hz, H-3″), 5.05 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 5.01 (dd, 1H, $J_{1'',2''}$ 7.5 Hz, $J_{2'',3''}$ 9.0 Hz, H-2″), 4.96 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.89, 4.62 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.67 (d, 1H, $J_{1'',2''}$7.5 Hz, H-1″), 4.68 (d, 1H, $J_{1,2}$ 1.0 Hz, H-1), 4.33 (dd, 1H, $J_{5,6a}$ 5.0 Hz, $J_{6a'',6b''}$ 10.0 Hz, H-6a″), 4.10 (dd, 1H, $J_{1,2}$ <1.0 Hz, $J_{2,3}$ 3.5 Hz, H-2), 4.07 (2H, OCH_2), 4.01- 3.94 (m, 3H, H-5′, H-6a, H-6b), 3.86 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.0 Hz, H-3), 3.80 (m, 2H, OCH_2), 3.76 (m, 2H, H-4″, H-6b″), 3.74 (s, 3H, $OC_6H_4OCH_3$), 3.73-3.68 (m, 8H, 4×OCH_2), 3.65 (dd, 1H, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 8.0 Hz, H-4), 3.52 (m, 1H, H-5″), 3.44 (m, 1H, H-5), 2.15, 2.06, 2.02, 2.00, 1.95 (s, 15H, 5×COCH_3), 1.05 (d, 3H, $J_{1',2'}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.0, 169.9, 169.8, 169.7, 169.3 (5× *C*OCH3), 153.9, 152.9, 137.5, 136.8, 129.0, 128.3 (2), 128.2 (2), 127.8, 127.7 (2), 126.1 (2), 115.6 (2), 114.6 (2) (Ar*C*), 101.4 (*C*HPh), 100.9 (C-1″), 99.5 (C-1), 93.0 (C-1′), 78.2 (C-4″), 75.6 (C-3), 75.4 (*C*H$_2$Ph), 75.3 (C-5), 73.4 (C-4), 72.2 (C-2″), 71.9 (C-3″), 70.7 (2×OCH_2), 70.6 (C-4′), 70.4 (OCH_2), 69.8 (OCH_2), 68.8 (C-5′), 68.5 (C-6″), 68.1 (OCH_2), 67.9 (OCH_2), 66.9 (C-6), 66.2 (C-5″), 59.5 (C-2), 55.6 ($OC_6H_4OCH_3$), 20.8, 20.7 (2), 20.6 (2) (5× COCH_3), 17.2(C-*C*H3). HRMS calcd for $C_{55}H_{69}N_3O_{23}Na$ (M + Na)$^+$ 1162.4220, found 1162.4219.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 2,3,4-tri-*O*-acetyl- α-L-rhamnopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-[2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl]-β-D-mannopyranoside (12): To a solution of trisaccharide **11** (800 mg, 0.7 mmol) and Et_3SiH (1.34 ml, 8.4 mmol) in anhydrous CH_2Cl_2 (20 mL), $BF_3.Et_2O$ (0.18 ml, 1.4 mmol) was added at 0 °C. The reaction mixture was stirred at the same temperature till the TLC (*n*-hexane-EtOAc; 1:2) showed

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complete consumption of the starting material to a slower moving spot. The reaction mixture was washed successively with H_2O (30 mL), $NaHCO_3$ (2×30 mL) and brine (30 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The crude residue was purified by flash chromatography using *n*-hexane-EtOAc (1:2) to afford the pure trisaccharide acceptor **12** (665 mg, 83%) as colorless foam. $[\alpha]_D^{25}$ +81 (*c* 0.7; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.42-7.26 (m, 10H, Ar*H*), 6.84, 6.80 (2d, 4H, $OC_6H_4OCH_3$), 5.36 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.0 Hz, H-3'),5.30 (dd, 1H, $J_{1',2'}$1.5 Hz, $J_{2',3'}$ 3.5Hz, H-2'), 5.05 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4'), 5.01 (dd, 1H, $J_{1'',2''}$7.0 Hz, $J_{2'',3''}$ 9.0 Hz, H-2''), 4.96 (d, 1H, $J_{1',2'}$1.5 Hz, H-1'), 5.26 (dd, 1H, $J_{2'',3''}$ 9.0 Hz, $J_{3'',4''}$ 8.0 Hz, H-3''), 4.89, 4.62 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.61-4.57 (m, 4H, H-1, H-1'', 2×CH_2Ph), 4.09-4.06 (4H, 2×OCH_2),4.01-3.98 (m, 1H, H-2), 3.94 (m, 1H, H-5'),3.85-3.80 (m, 3H, H-3, OCH_2), 3.78 (m, 2H, H-6a, H-6a''), 3.75 (s, 3H, $OC_6H_4OCH_3$), 3.73(m, 3H, H-4'', H-6b, H-6b''), 3.72-3.66 (m, 6H, 3×OCH_2), 3.58 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{4,5}$ 9.0 Hz, H-4), 3.54-3.50 (m, 1H, H-5''), 3.46-3.42 (m, 1H, H-5), 3.01 (d, 1H, *J* 5.0 Hz, O*H*), 2.15, 2.06, 2.02, 2.00, 1.95 (s, 15H, 5×COCH_3), 1.05 (d, 3H, $J_{1',2'}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 171.1, 170.0, 169.8(2), 169.4 (5×*C*OCH_3), 153.9, 152.9, 137.5(2), 128.5 (2), 128.3(2), 127.9, 127.8(3), 127.7(2), 115.7(2), 114.6(2) (Ar*C*), 100.7 (C-1''), 99.5 (C-1), 93.1 (C-1'), 75.7 (C-3), 75.6(2) (C-2'', C-5), 75.4 (*C*H_2Ph), 73.9 (C-5''), 73.8 (*C*H_2Ph), 73.5 (C-4), 71.3 (C-3''), 70.9 (O*C*H_2) , 70.7 (2×O*C*H_2), 70.6 (C-4'), 70.4 (C-4''), 70.2 (C-6), 69.8 (C-6''), 69.6 (C-2'), 68.8 (2) (O*C*H_2, C-3'), 68.1 (O*C*H_2), 67.9 (O*C*H_2), 66.9 (C-5'), 59.5 (C-2), 55.7 ($OC_6H_4OCH_3$), 20.8(2), 20.7(2), 20.6 (5×CO*C*H_3), 17.2 (C-*C*H_3). HRMS calcd for $C_{55}H_{71}N_3O_{23}Na$ $(M+Na)^+$ 1164.4376, found 1164.4373.

***p*-Tolyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (15)**: A mixture of known acceptor **13** (700mg, 1.4 mmol)and known donor **14** (994mg, 1.8 mmol) and activated MS 4Åin anhydrous CH_2Cl_2 (20 mL)was stirred under nitrogen for 30 min. The reaction was cooled to – 45 °C, NIS (528 mg, 2.4 mmol) was added followed by addition of H_2SO_4-Silica (25 mg). Complete consumption of the acceptor within 15 minutes was evident from the TLC (*n*-hexane-EtOAc; 2:1). The reaction mixture was immediately filtered through a pad of Celite. The filtrate was successively washed with $Na_2S_2O_3$ (2×30 mL), $NaHCO_3$ (2×30 mL) and brine (30 mL). Organic layer was collected, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The crude product thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (3:1) to afford pure compound **15** (1.07 g, 83%) as amorphous solid. $[\alpha]_D^{25}$ +62 (*c* 0.7; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.94-6.98 (m, 23H, Ar*H*), 5.66 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), 5.57 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.41 (s, 1H, C*H*Ph), 5.15 (bd, 1H, $J_{3',4'}$ 2.0 Hz, H-4'), 4.84 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.63, 4.54 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.50, 4.39 (ABq, 2H, J_{AB} 12.5 Hz, CH_2Ph), 4.47 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.5 Hz, H-2), 4.37 (m, 1H, H-6a'), 3.88 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.0 Hz, H-4), 3.83-3.74 (m, 3H, H-3', H-5, H-6b'), 3.62 (dd, 1H, $J_{1',2'}$ 4.0 Hz, $J_{2',3'}$ 10.0 Hz, H-2'), 3.57 (dd, 1H, $J_{5,6a}$ 8.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.48 (m, 1H, H-5'), 3.35 (dd, 1H, $J_{5,6b}$ 6.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6b), 2.33(s, 3H, $OC_6H_4CH_3$), 1.93 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3) . ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.0, 169.8 (2×*C*OCH_3), 167.7, 167.0 (2*C*×phthalimido), 138.5, 138.1, 136.8, 134.4, 134.3, 133.4 (2), 131.4, 131.1, 129.7 (2), 129.3, 128.3 (2), 128.1 (2), 127.9 (3), 127.8 (2), 127.4, 127.2, 127.1, 126.9 (2), 126.2 (2), 123.8, 123.4 (Ar*C*), 101.7 (CHPh), 97.2 (C-1'), 84.1 (C-1), 82.7 (C-4), 74.9 (C-5), 74.3 (C-2'), 73.6 (C-3), 72.4 (*C*H_2Ph), 71.9 (*C*H_2Ph), 70.0 (C-3'), 68.6 (C-6'), 66.9 (C-4'), 66.6 (C-5), 60.8 (C-6), 54.3 (C-2), 21.1 ($OC_6H_4CH_3$), 20.6, 20.5 (2×CO*C*H_3). HRMS calcd for $C_{52}H_{51}NO_{13}SNa$ $(M+Na)^+$ 952.2979, found 952.2976.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-2-azido-4-*O*-benzyl-2-deoxy-3-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl]-β-D-mannopyranoside (16): A mixture of disaccharide donor **15** (609 mg, 0.7 mmol), trisaccharide acceptor **12** (575 mg, 0.5 mmol) and activated MS 4Å in anhydrous CH_2Cl_2 (20 mL) was stirred under N_2 for 30 min. The reaction mixture was cooled to 0 °C, NIS (190 mg, 0.9 mmol) was added followed by addition of H_2SO_4-Silica (25 mg). The reaction was stirred for 20 minutes till the TLC (*n*-hexane-EtOAc; 1:2) showed complete consumption of the acceptor. The reaction mixture was immediately filtered through a pad of Celite. The filtrate was washed successively with aqueous $Na_2S_2O_3$(2×30 mL), $NaHCO_3$ (2×30 mL) and brine (30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and filtered. The solvents were evaporated *in vacuo*. The crude residue was purified by flash chromatography using *n*-hexane–EtOAc (0.6:1) to afford pure pentasaccharide **16**(764mg, 78%) as colorless foam. $[\alpha]_D^{25}$ +116 (*c* 0.7; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.35- 6.98 (m, 29H, Ar*H*), 6.83, 6.79 (2d, 4H, $OC_6H_4OCH_3$), 5.52 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1'''), 5.37-5.28 (m, 4H, H-1'', H-2''', H-3''', C*H*Ph), 5.10 (m, 2H, H-3', H-4'''), 5.01 (t, 1H, $J_{3''',4'''}$, $J_{4''',5'''}$ 9.5 Hz, H-4'''), 4.93 (d, 1H, $J_{1''',2'''}$ <1.0 Hz, H-1'''), 4.84 (m, 2H, H-2', H-3), 4.76 (dd, 1H, $J_{2'',3''}$ 8.5 Hz, $J_{3'',4''}$ 10.0 Hz, H-3''), 4.62-4.40 (m, 9H, H-1, H-1', H-6a''', 3×CH_2Ph), 4.30-4.27 (m, 4H, H-2'', H-6b''', CH_2Ph), 4.08-4.05 (m, 2H, OCH_2), 3.98 (m, 2H, H-3''', H-6a), 3.96-3.84 (m, 4H, H-2, H-5''', H-5''', H-6b), 3.81 (m, 3H, H-4'', OCH_2), 3.75 (s, 3H, $OC_6H_4OCH_3$), 3.73 (m, 1H, H-4'), 3.71-3.67 (m, 8H, 4×OCH_2), 3.61-3.58 (m, 2H, H-2''', H-6a''), 3.56-3.46 (m, 3H, H-4, H-5', H-6a'), 3.40 (m, 2H, H-5'', H-6b'), 3.31 (m, 2H, H-5, H-6b''),2.14, 2.05, 1.99, 1.94, 1.92, 1.84 (7×COCH_3), 1.02 (d, 3H, $J_{5''',6'''}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 171.2, 171.1 (2*C*×phthalimido), 170.1, 169.8(3), 169.6, 169.5 (7×*C*OCH_3), 138.2, 138.0, 137.9, 137.4, 136.8, 134.4, 131.2, 129.3, 128.5, 128.3(4), 128.2(6), 128.0(2), 127.9(2), 127.8, 127.5(2), 127.3(2), 127.2(2), 127.0(2), 126.2(2), 124.0, 123.5, 115.6(2), 114.6(2) (Ar*C*), 101.7 (*C*HPh), 100.5 (C-1'), 99.5 (C-1), 97.9 (C-1''), 97.2 (C-1'''), 93.0 (C-1'''), 82.7 (C-4''), 75.6 (C-5''), 75.5 (C-3''), 74.9 (C-4'), 74.4 (C-2''', C-6''), 74.2 (C-4), 72.9 (*C*H_2Ph), 72.6 (C-6'''), 72.5 (C-3), 72.4 (*C*H_2Ph), 72.1 (*C*H_2Ph), 71.7 (C-2'), 71.3 (*C*H_2Ph), 71.1 (O*C*H_2), 70.7 (C-4''', O*C*H_2), 70.6 (O*C*H_2), 70.3 (O*C*H_2), 70.1 (O*C*H_2), 69.8 (C-6'), 69.6 (C-2'''), 68.7 (C-2'', C-3'''), 68.4 (C-5'''), 68.1 (O*C*H_2),

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67.8 (C-6), 67.6 (C-5), 66.9(C-3′, C-4‴), 66.6 (C-5‴′), 65.5 (C-5′), 60.8 (C-5‴), 59.5 (C-2), 55.7 ($OC_6H_4OCH_3$), 21.1, 20.8, 20.6(4), 20.5 (7×$COCH_3$), 17.2 (C-CH_3). HRMS calcd for $C_{100}H_{114}N_4O_{36}Na$ $(M + Na)^+$ 1969.7110, found 1969.7108.

2-[2-[2-(4-methoxyphenoxy)ethoxy]ethoxy]-ethyl α-D-galactopyranosyl uronic acid-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2 acetamido-2-deoxy-*O*-[α-L-rhamnopyranosyl]-β-D-mannopyranoside (1): To a solution of pure compound **16** (750 mg, 0.4 mmol) in MeOH (20 mL), NaOMe (2 mL, 0.5M) was added and strirred at room temperature for 8 hr. The reaction mixture was neutralized with Dowex 50W X8 (H+) resin, filtered and evaporated *in vacuo.* The compound was then dissolved in CH_2Cl_2–H_2O (1.5:1; 25 mL) followed by addition of TEMPO (32 mg, 0.2 mmol) and iodosobenzene diacetate (IBDA) (402 mg, 1.56 mmol). The mixture was vigorously stirred at 5 °C for 8 hr till the TLC showed complete consumption of the starting material to a slower moving spot (CH_2Cl_2:MeOH; 9:1). Aqueous saturated $Na_2S_2O_3$ (5 mL) was added to quench the reaction. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with brine (2× 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated in vacuo to give the oxidized product which was directly used for the next step. The crude compound was then treated with 80% AcOH (20 mL) at 80 ^{0}C for 2 hr to cleave the benzylidine ring. The solvents were evaporated using toluene as co-solvent and dried. The compound was then dissolved in *n*-butanol (15 mL) and ethylene diamine (1.5 mL) was added followed by subsequent stirring at 110 °C for 24 hr. The solvent was then evaporated and the crude product was acetylated using Ac_2O (10 mL) and pyridine (10 mL). The starting material was entirely consumed after stirring at room temperature for 10 hr (evident from TLC). The reaction mixture was then co-evaporated with toluene to give the pentasaccharide in crude form. The crude compound was then dissolved in CH_3COSH (10 mL) and allowed to stir at room temperature for 48 hr until TLC showed complete consumption of the starting material. Solvents were removed under reduced pressure. The dried residue thus obtained was dissolved in MeOH (50 mL) and passed through a 10% Pd/C cartridge in a ThalesNano flow hydrogenation assembly under continuous flow of H_2 at atmospheric pressure. The hydrogenolysis of the benzyl groups was complete after 2 such cycles as evident from mass spectroscopy. Finally, a solution of the product in NaOMe (0.1 M solution in MeOH; 20 mL) was stirred at room temperature for 8 hours followed by neutralizing the reaction mixture with Dowex 50W X8 H^+ resin. The reaction mixture was filtered and evaporated *in vacuo*. The residue thus obtained was dissolved in water and washed with CH_2Cl_2 to remove the organic impurities. The aqueous layer was separated and freeze dried to get the pure target compound **1** (300 mg, 68%) as white amorphous mass. The crude product was purified by HPLC on a Waters Nova-Pak C18 column (3.9 × 300 mm; 4 µm particle size) with a flow rate of 1mL/min and using a gradient of CH_3CN in water (Conditions: 0 to 10% A in B, A = CH_3CN, B = water, over 40 min.). The sample of the target compound **1** showed 96.7% purity (area %) with the retention time 25.8 min. $[\alpha]_D^{25}$ +57 (*c* 0.6; CH_3OH) ^{1}H NMR (MeOD, 500 MHz) δ: 6.83, 6.78(2d, 4H, *J* 8.5 Hz, $OC_6H_4OCH_3$), 5.37 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1), 5.30 (d, 1H, $J_{1,2}$ <1.0 Hz, H-1‴), 5.18 (d, 1H, $J_{1,2}$ 7.0 Hz, H-1′), 5.13 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1″), 4.93 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1⁗), 2.03, 2.00 (2s, 6H, 2× $NHCOCH_3$), 1.25 (s, 3H, $J_{5‴,6‴}$ 6.0 Hz, C-CH_3). ^{13}C NMR (MeOD, 125 MHz) δ: 174.8 (*C*OOH), 174.0, 173.9 (2×$COCH_3$), 156.6, 153.9, 116.6 (2), 115.7 (2), 102.6 (C-1′), 100.8 (C-1″), 99.0 (C-1), 98.8 (C-1‴), 98.1 (C-1⁗), 81.7, 79.5, 78.6, 78.0, 77.5, 76.9, 76.5, 76.3, 75.1, 74.0, 73.8, 73.7, 73.5, 73.2, 73.0, 72.5, 72.3, 72.1, 72.0, 71.9, 71.7, 71.6, 71.4, 70.8, 70.0, 69.0, 68.3, 66.8, 65.1, 55.8 ($OC_6H_4OCH_3$), 22.6, 21.3 (2×$COCH_3$), 18.0 (C-CH_3). HRMS calcd for $C_{47}H_{74}N_2NaO_{30}$ $(M+Na)^+$ 1169.4224, found 1169.4220.

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Convergent chemical synthesis of the pentasaccharide repeating unit of the O-antigen from *E. coli* O158

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