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Convergent Synthesis of the Hexasaccharide Repeating Unit of the *O*-antigenic OPS of *Escherichia coli* O133

Ankita Mitra,[a] and Balaram Mukhopadhyay*[a]

Abstract: Synthesis of the hexasaccharide repeating unit of the O-antigen from *E. coli* O133 has been accomplished with rational protecting group manipulations on commercially available monosaccharides and stereoselective glycosylations through a convergent protocol. A late stage TEMPO mediated oxidation is used to install the required uronic acid moiety. Chloroacetate group is used extensively as a temporary protecting group.

Introduction

Escherichia coli (*E. coli*) is a group of gram-negative bacteria which has both commensal and pathogenic forms. The pathogenic class of *E. coli*bacteria also happens to be the biggest cause of diarrhoea with life threatening complications, for example, haemorrhagic colitis (HC) and haemolytic–uraemic syndrome (HUS).[1] The other general clinical syndromes caused by *E. coli* are urinary tract infection and meningitis.[2] Although till date antibiotics are the main therapeutic agent to treat the infections caused by *E. coli*, increasing drug resistance to the bacterial infection are making the vaccines a more attractive choice of therapeutics. The *O*-specific polysaccharide (OPS) or *O*-antigens in the outer membrane of the bacteria is highly immunogenic and nontoxic in general[3] and thus can be utilized to develop vaccine in the form of glycoconjugates. Though currently more than 180 *O*-antigens from *E. coli* has been recognized, isolation of them rendered the vaccine too costly and development of the vaccine ends in the early stages of clinical trials.[4] As it turns out, scalable chemical synthesis of these *O*-antigens becomes the inevitable route to achieve the glycoconjugate vaccine. In the recent years, number of articles[5]has appeared in the literature describing both the structure elucidation and the synthesis of different *O*-antigenic oligosaccharides from different *E. coli* strains. Recently Knirel and his co-workers reported[6] the structure of the *O*-specific polysaccharide (OPS) of *E. coli* O133. In the endeavour to develop glyco-conjugate vaccine against *E. coli*, herein we report the total synthesis of the hexasaccharide repeating unit of the *O*-antigenic OPS of *Escherichia coli* O133 in the form of its aminoethyl glycoside (**1**, **Figure 1**).

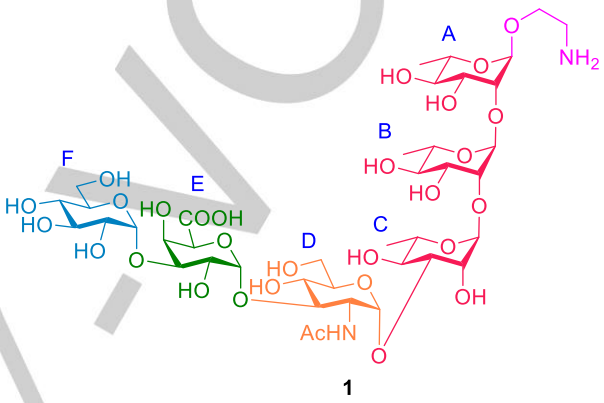

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Figure 1. Structure of the target hexasaccharide (**1**)

Results and Discussion

The retrosynthetic analysis (**Figure 2**) for the hexasaccharide indicated a [2 + 2 + 1 + 1] glycosylation strategy will be the best suitable approach to achieve the target structure. 2-Aminoethyl glycoside[7] serve as an ideal choice for the aglycone at the reducing end of the hexasaccharide **1** as it can facilitate glycoconjugate formation using the free amine functionality without hampering the anomeric stereochemistry.

The synthesis would start with the preparation of two disaccharide building blocks, the reducing end disaccharide **4** and the disaccharide donor **10**. All the stereoselective glycosylations were achieved by the activation of thioglycoside using NIS and H_2SO_4-silica. The metal free and environmental friendly H_2SO_4-silica serves as an efficient alternative to traditional toxic and hygroscopic promoters like TfOH and TMSOTf. H_2SO_4-silica is particularly beneficial as (a) it is solid material and (b) it also acts as a desiccant during the glycosylation reactions thus improving the yields. Glycosylation between the reducing end disaccharide **4** and the thio-disaccharide **10** would furnish the protected tetrasaccharide**12**. Rational protecting group manipulations and stereoselective glycosylation of the tetrasaccharide acceptor **13** with D-galactosyl donor **17** would then furnish the fully protected pentassacharide **18**. Further cleavage of the temporary 4-methoxybenzyl group followed by glycosylation with suitably protected D-glucosyl donor **21** will give the hexasaccharide **22**. Hydrolysis of the 6-*O*-chloroacetate group followed by TEMPO-mediated oxidation was planned for the installation of the

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required uronic acid moiety. Finally, global de-protection would furnish the target hexasaccharide **1** (**Figure 2**).

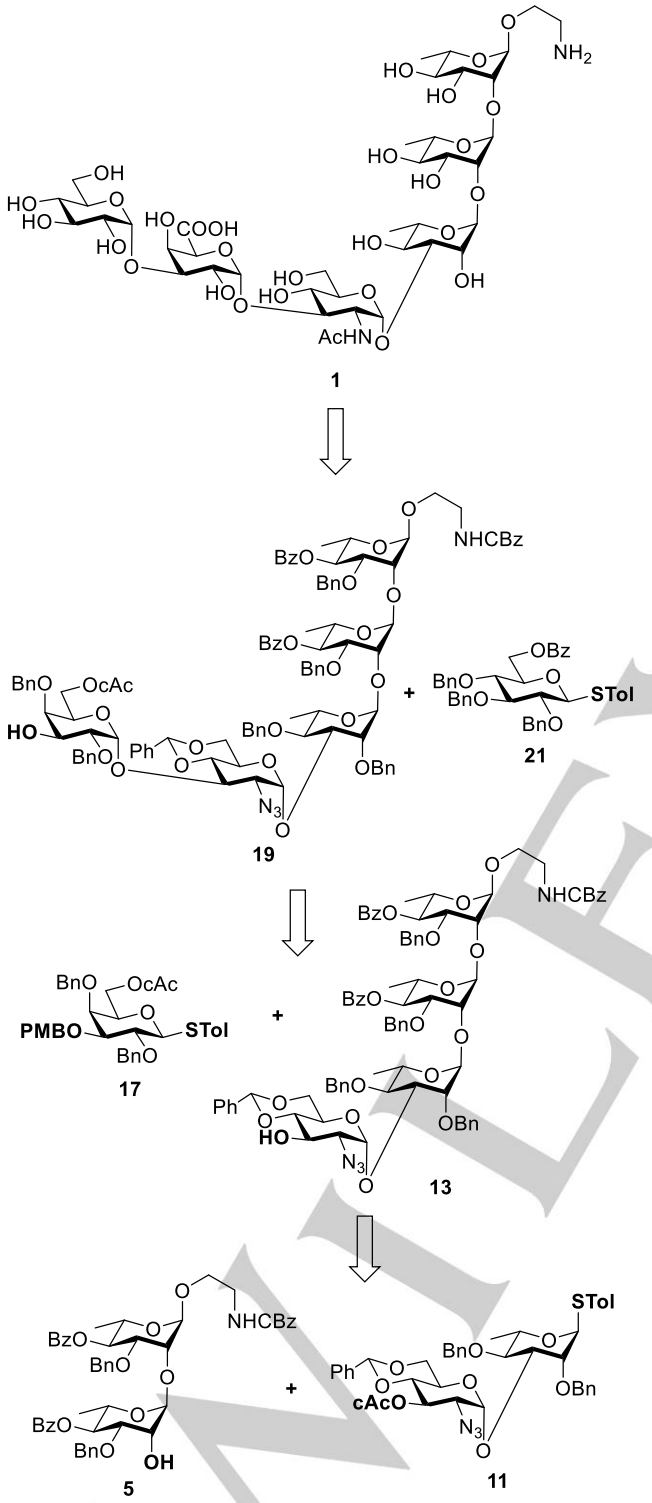
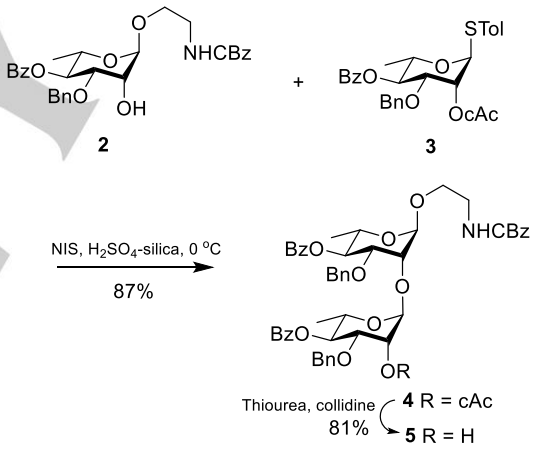


Figure 2. Retrosynthetic analysis for the synthesis of the target hexasaccharide **1**

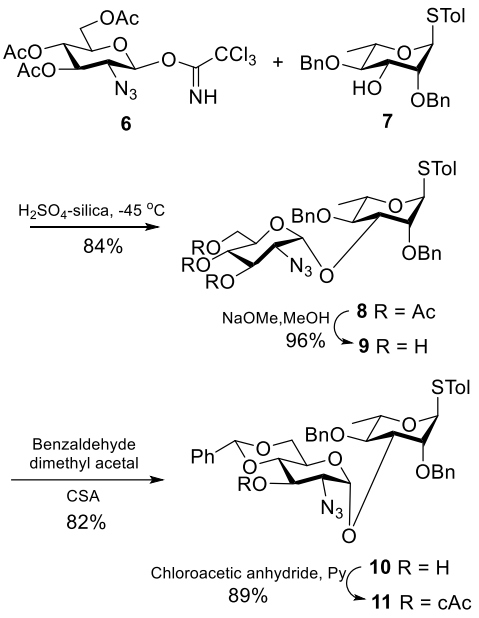
It was envisaged that the tetrasaccharide **12** can be most conveniently synthesized by performing a [2+2] glycosylation between the disaccharide acceptor **5** and thio-disaccharide donor **11**. In order to prepare disaccharide **4**, the known linker-equipped rhamnose acceptor **2**[8] was coupled with known rhamnose donor **3**[14] through activation of the thioglycoside by NIS in the presence of H_2SO_4-silica[15] to furnish the disaccharide **4** in 87% yield. This disaccharide was essentially prepared in the same way as reported in the literature.[8] However, since the required disaccharide acceptor is different from that of the reported one, we had to change the donor rhamnose derivative accordingly. The formation of the disaccharide was confirmed by NMR spectroscopy showing ^{1}H NMR signals at 5.05 ppm ($J_{1',2'}$ 1.5 Hz, H-1′) for the newly formed 1,2-*trans* glycoside and at 4.89 ppm ($J_{1,2}$< 1.0 Hz, H-1) for the other anomeric proton.The^{13}C NMR signals for the two anomeric carbons at 99.5 ppm (C-1′) and 99.0 ppm (C-1) further affirmed the formation of the desired disaccharide. The chloroacetate group in donor **3** was strategically used to provide anchimeric assistance as well as a temporary protecting group. It was selectively cleaved using thiourea and 2,4,6-collidine[16] to afford the required disaccharide acceptor **5** in 81% yield (**Scheme 1**).



Scheme 1. Synthesis of the disaccharide acceptor **5**

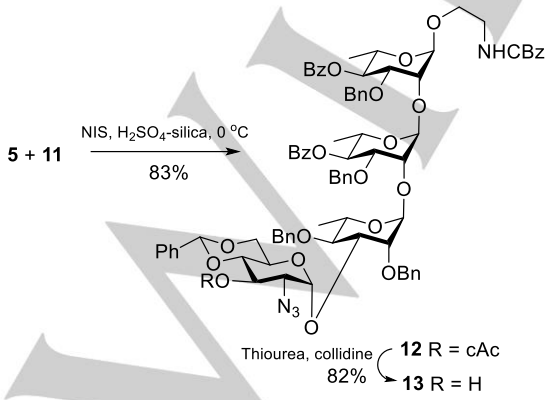
A chemoselective glycosylation between known trichloroacetimidate donor **6**[17] and thioglycoside acceptor **7**[18] at -45 °C in the presence of H_2SO_4-silica furnished the disaccharide **8** in 84% yield. The presence of a 6-OAc group on the D-glucosamine donor **6** was expected to provide a distant neighbouring group participation leading to the required 1,2-*cis* linked glycoside **8** which is clearly evident from ^{1}H NMR peaks at 5.01 ppm (H-1′) and 5.44 ppm ($J_{1,2}$ 3.0 Hz, H-1). The ^{13}C NMR peaks for the two anomeric carbons at 92.8 ppm (C-1′)and 85.2 ppm (C-1) also confirmed the formation of the desired disaccharide. The acetyl groups were then removed by trans-esterification using NaOMe in MeOH to afford the disaccharide derivative **9** in 96% yield. Further, reaction with benzaldehyde dimethyl acetal in the presence of CSA[19] afforded the disaccharide derivative **10** in 82% yield. Chloroacetylation of the

free 3-OH group in **10** using chloroacetic anhydride and pyridine[20] afforded the fully protected disaccharide donor **11** in 89% yield (**Scheme 2**).



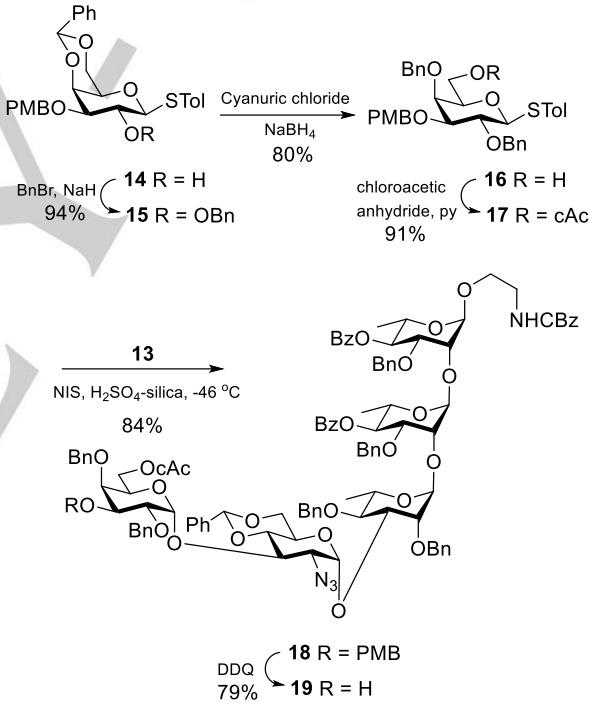
Scheme 2. Synthesis of the disaccharide donor **11**

With the two disaccharide building blocks in hand, NIS/H_2SO_4-silica mediated glycosylation was performed between the disaccharide acceptor **5** and disaccharide donor **11** that ultimately furnished the fully protected tetrasaccharide **12** in 83% yield. ^{1}H NMR signal at 5.06 ppm ($J_{1''',2'''}$ 3.0 Hz, H-1‴) and ^{13}C NMR signal at 93.6 ppm (C-1‴) were assigned to the newly formed glycosidic linkage. The exclusive formation of the 1,2-*trans* glycoside was also evident from the J_{CH} coupling value of 169.7 ppm. Further, the chloroacetyl ester group in the tetrasaccharide **12** was selectively cleaved using thiourea and 2,4,6-collidine[16] to furnish the required tetrasaccharide acceptor **13** in 82% yield (**Scheme 3**).



Scheme 3. Synthesis of the tetrasaccharide acceptor **13**

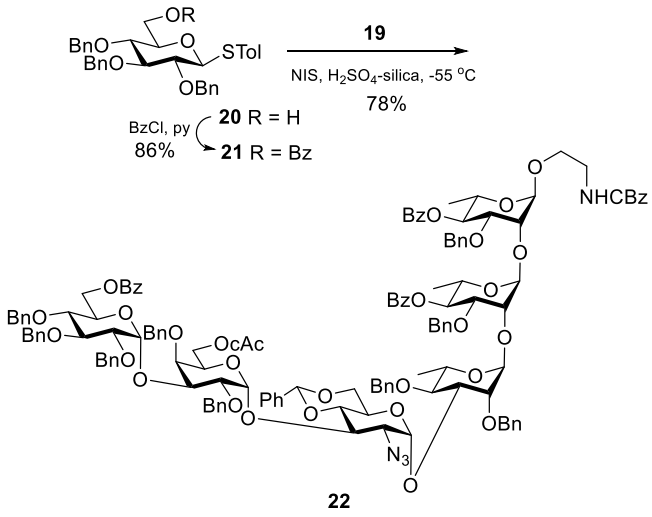
In a separate experiment, known galactose derivative **14**[21] was benzylated using BnBr and NaH[22] to give compound **15** in 94% yield.Further,regio-selective ring opening of the benzylidene acetalusing cyanuric chloride (TCT)[23] and $NaBH_4$ gave the galactosyl derivative **16** in 80% yield. Subsequently, the 6-OH group was chloroacetylated to give the desired galactose donor**17** in 91% yield. Glycosylation of donor **17** with tetrasaccharide acceptor **13** using NIS and H_2SO_4-silica at -45 °C afforded the desired pentasaccharide **18** in 84% yield. Low reaction temperature and presence of non-participating 2-OBn adjacent to the anomeric position resulted the reaction towards α-selectivity. The ^{1}H and ^{13}C NMR analysis showing ^{1}H NMR peak at 5.72 ppm ($J_{1'''',2''''}$ 2.0 Hz, H-1‴) and ^{13}C NMR signal at 96.9 ppm (C-1‴), clearly suggests the exclusive formation of 1,2-*cis* glycosidic linkage in the newly formed pentasaccharide **18**. Selective oxidative cleavage of the 4-methoxybenzyl ether in compound **18** using DDQ[24] furnished the pentasaccharide acceptor **19** in 79% yield (**Scheme 4**).



Scheme 4. Synthesis of the pentasaccharide acceptor **19**

The known compound **20**[25] was treated with benzoyl chloride and pyridine[11] in order to protect the 6-OH with benzoyl ester and thus furnishing the final monosaccharide donor **21** in 86% yield. Subsequently, the pentasaccharide acceptor **19** was coupled with the donor **21** in presence of NIS and H_2SO_4-silica at -55 °C to afford the desired hexasaccharide **22** in 78% yield (**Scheme 5**). Here, in addition to the presence of non-participating 2-OBn group in D-glucosyl moiety and distant participation[26] of the 6-OBz group likely have facilitated the exclusive formation of 1,2-*cis* glycosidic linkage. The newly

formed α-glycosidic bond in **22** was confirmed by NMR analysis which shows ^{1}H NMR peak at 5.15 ppm ($J_{1''''',2''''}$ 2.5 Hz, H-1''''') and ^{13}C NMR peak at 96.8 ppm (C-1''''').

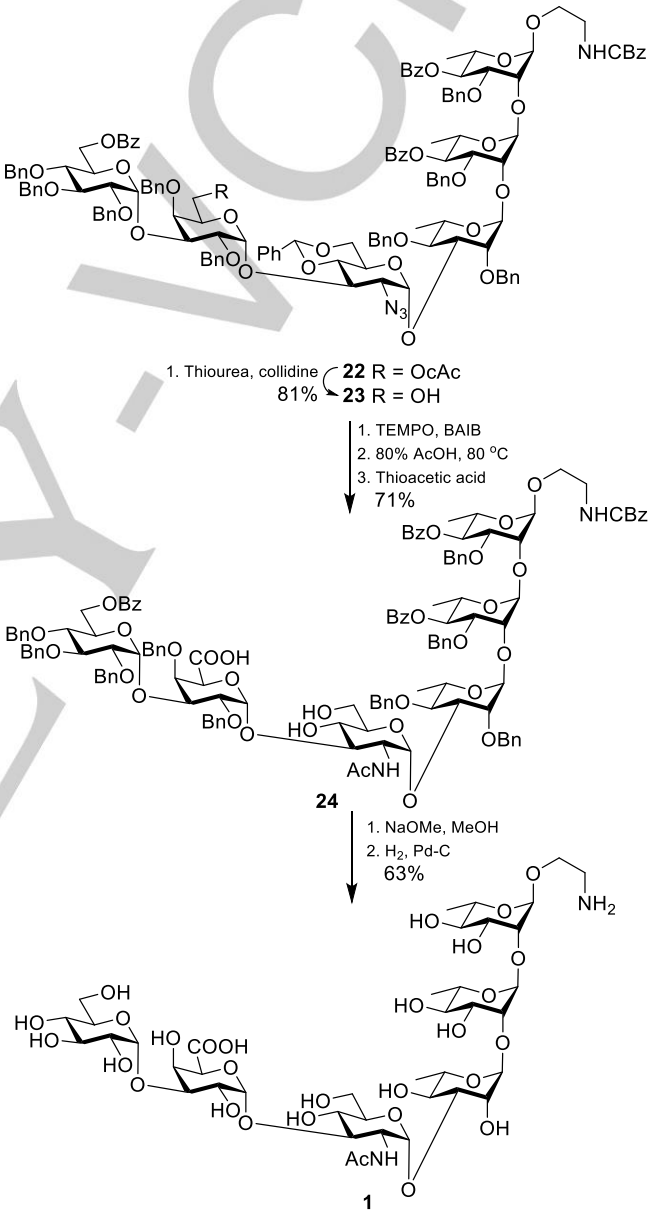


Scheme 5. Synthesis of the protected hexasaccharide **22**

Once the hexasaccharide was obtained the chloroacetate group was cleaved using thiourea and 2,4,6-collidine furnishing 6-OH free derivative **23** in 81% yield which was immediately oxidized to the corresponding uronic acid derivative using 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) together with (diacetoxyiodo)benzene (BAIB)[27] as co-oxidant. The 6-O-chloroacetyl moiety was strategically installed so that it can be selectively cleaved to furnish a free primary hydroxyl group that can undergo TEMPO mediated oxidation to give the desired uronic acid derivative. In the subsequent steps global deprotection was performed to obtain the target hexasaccharide **1**. At first,the benzylidine group was hydrolyzed using aqueous AcOH[12] at 80 °C to afford the di-ol derivative which was directly treated with thioacetic acid[28] for 72 hours at room temperature to permit the conversion of azido to the corresponding acetamido derivative **24** in 71%yield.In the next step, Zemplen de-*O*-acetylation[9] unmasked all the ester protections on the hexasaccharide derivative. Finally, Hydrogenolysis using 10% Pd-C cartridge on a ThalesNano continuous flow hydrogenation assembly removed the benzyl and NHCBz protection and furnished the target hexasaccharide **1** in 63% overall yield (**Scheme 6**).

Conclusions

Convergent total synthesis of the hexasaccharide repeating unit of the *O*-antigen from *E. coli* O133 is achieved in the form of its 2-aminoethyl glycoside. The 2-aminoethyl glycoside is useful for further glycoconjugate formation utilizing the free terminal amine without disturbing the anomeric stereochemistry. The challenging 1,2-*cis* glycosylations required in the total synthetic route were successfully achieved by rational protecting group manipulations and utilizing the remote participation of the 6-O-acyl groups. A late stage TEMPO-mediated oxidation successfully installed the uronic acid moiety in the target hexasaccharide structure.



Scheme 6. Synthesis of the target hexasaccharide **1**

Experimental Section

General Methods

All solvents and reagents were dried prior to use according to standard methods.[29] 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. ^{1}H and ^{13}C NMR were recorded at 500 MHz and 125 MHz respectively on a Bruker Avance 500 MHz spectrometer. The assignments of ^{1}H and ^{13}C peaks were done with the help of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC spectra. In the target hexasaccharide structure **1**, the ^{1}H 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**, H′′′′ for the unit **E** and H**′′′′′** for the unit **F** as marked in the **Figure 1**.

Preparation of H_2SO_4-Silica

To slurry of silica gel (10 g, 230-400 mesh) in dry diethyl ether (50 mL) was added commercially available concentrated H_2SO_4 (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 ^{o}C for 3 hours and then used for reactions.

2-(benzyloxycarbonyl)-aminoethyl 4-*O*-benzoyl-3-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (4):

A mixture of compound **2** (1.1 g, 2.1 mmol), known compound **3**[14] (1.4 g, 2.7 mmol) and activated MS 4Å (2.0 g) in dry CH_2Cl_2 (20 mL) was stirred in ice-water bath for 1 hour. NIS (790 mg, 3.5 mmol) was added and the reaction mixture was stirred under nitrogen for 20 min. H_2SO_4-silica (45 mg) was then added to the reaction vessel and allowed to stir until complete consumption of the acceptor as evident by TLC using (*n*-hexane-EtOAc; 3:1). Within 10 minutes, the entire acceptor was consumed and the 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). The organic layer was collected, dried over $NaSO_4$ and evaporated to syrup. The crude product thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (2:1) to afford the disaccharide **4** (1.7 g, 87%) as white foam. $[\alpha]_D^{25}$ = +116° (*c* 1.1, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.15-7.18 (m, 25H, Ar*H*), 5.73 (t, 1H, $J_{1',2'}$, $J_{2',3'}$ 2.5 Hz, H-2′), 5.46 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 5.29 (t, 1H, $J_{3,4}$,$J_{4,5}$ 9.5 Hz, H-4), 5.20 (d, 2H, COOCH_2Ph), 5.16 (bs, 1H, N*H*), 5.05 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.89 (d, 1H, $J_{1,2}$< 1.0 Hz, H-1), 4.71, 4.57 (ABq, 2H, J_{AB} 12.5 Hz, CH_2Ph), 4.65 (s, 2H, CH_2Ph), 4.22, 4.14 (ABq, 2H, J_{AB} 15.5 Hz, COCH_2Cl), 4.11-4.05 (m, 3H, H-2, H-3′, H-5), 4.01 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.5 Hz, H-3), 3.94 (m, 1H, H-5′), 3.84 (m, 1H, OCH_2), 3.61 (m, 1H, OCH_2), 3.55 (m, 1H, CH_2NH), 3.46 (m, 1H, CH_2NH), 1.33 (d, 1H, $J_{5,6}$ 6.5 Hz, C-CH_3), 1.29 (d, 1H, $J_{5',6'}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 166.3, 165.7, 165.5 (3 ×*C*OPh), 156.3 (*C*OOCH$_2$Ph), 137.6, 137.2, 136.2, 133.7, 133.1, 129.9 (2), 129.8, 129.7 (2), 129.6, 128.5 (2), 128.4 (4), 128.3 (4), 128.2 (4), 128.1, 127.7 (3), 127.6 (Ar*C*), 99.5 (C-1′), 99.0 (C-1), 76.2, 76.1, 73.4, 73.1, 72.4 (2), 71.1, 70.0, 67.3, 67.1, 66.9 (2), 40.8 (*C*OCH$_2$Cl), 40.7 (*C*H$_2$NH), 17.6 (C-*C*H$_3$), 17.5 (C-*C*H$_3$). HRMS calcd for $C_{52}H_{54}ClNO_{14}Na$ (M+Na)$^+$:974.3131, found 974.3129.

2-(benzyloxycarbonyl)-aminoethyl 4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (5):

A mixture of pure disaccharide **4** (1.6 g, 1.7 mmol), thiourea (1.2 g, 17.0 mmol) and 2,4,6-collidine (2.3 mL, 17.0 mmol) was stirred in 25 mL CH_2Cl_2-MeOH (2:3) and refluxed for 10 hours. The completion of the reaction was evident by a complete conversion of the starting material to a slower moving spot as evident from the TLC using (*n*-hexane-EtOAc; 3:2). The solvents were evaporated *in vacuo* and the solid residue obtained was dissolved in CH_2Cl_2 and washed with brine (2 × 30 mL). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated *in vacuo* to obtain a syrupy residue. This was further subjected to flash chromatography using *n*-hexane-EtOAc (1:1) as eluent to furnish the pure disaccharide acceptor **5** (1.2 g, 81%) as colorless foam. $[\alpha]_D^{25}$ = +98° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.16-7.27 (m, 25H, Ar*H*), 5.48 (m, 2H, H-4, H-4′), 5.23 (s, 3H, COOCH_2Ph, H-1′), 4.94 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1), 4.78, 4.61 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.70, 4.66 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.43 (d, 1H, $J_{1',2'}$< 1.0 Hz, $J_{2',3'}$ 3.0 Hz, H-2′), 4.17 (d, 1H, $J_{1,2}$< 1.0 Hz, $J_{2,3}$ 2.5 Hz, H-2), 4.09 (m, 1H, H-5), 4.06 (dd, 1H, $J_{2',3'}$3.0 Hz, $J_{3',4'}$ 9.5 Hz, H-3′), 4.03 (dd, 1H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 10.0 Hz, H-3), 3.95 (m, 1H, H-5′), 3.88, 3.63 (m, 2H, OCH_2), 3.57, 3.50 (m, 2H, CH_2NH), 2.90 (bs, 1H, O*H*), 1.35 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3), 1.32 (d, 3H, $J_{5',6'}$6.5 Hz, C-CH_3).^{13}C NMR ($CDCl_3$, 125 MHz) δ: 165.8, 165.7 (2×*C*OPh), 156.3 (*C*OOCH$_2$Ph), 137.5, 137.3, 136.2, 133.1 (2), 129.8 (2), 129.7 (2), 128.5 (2), 128.4 (2), 128.3 (8), 128.2, 128.1, 128.0 (3), 127.7 (4) (Ar*C*), 101.2 (C-1′), 99.2 (C-1), 76.1, 76.0, 75.0, 73.1, 72.7, 72.0, 71.9, 67.9, 67.1, 66.9, 66.8 (2), 17.6 (C-*C*H$_3$), 17.4 (C-*C*H$_3$). HRMS calcd for $C_{50}H_{53}NO_{13}Na$ (M+Na)$^+$: 898.3415, found: 898.3410.

***p*-Tolyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (8)**:

A mixture of known acceptor **7**[18](1.1 g, 2.3 mmol), trichloroacetamidate donor **6**(1.4 g, 3.0 mmol) and activated MS 4Å (2.0 g) in anhydrous CH_2Cl_2 (20 mL) was stirred under N_2 for 15 min. The reaction mixture was cooled to -45 ^{o}C and H_2SO_4-Silica (55 mg) was added followed by stirring the reaction mixture at same temperature. Complete consumption of the acceptor within few minutes was evident from the TLC (*n*-hexane-EtOAc; 3:1). The reaction mixture was immediately filtered through a pad of Celiteand 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 **8**(1.6 g, 84%) as amorphous solid. $[\alpha]_D^{25}$ = +112° (*c*0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.43-7.11 (m, 14H, Ar*H*), 5.56 (dd, 1H, $J_{2',3'}$10.0 Hz, $J_{3',4'}$ 6.0 Hz, H-3′), 5.44 (dd, 1H, $J_{1,2}$ 3.0 Hz, H-1), 5.07-4.97 (m, 2H, H-1′, H-4′), 4.91, 4.61 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.72(d, 2H, CH_2Ph), 4.21-4.17 (m, 2H, H-5, H-5′), 4.08-4.02 (m, 3H, H-2, H-3, H-6a′), 3.92 (dd, 1H, $J_{5',6a'}$ 2.0 Hz,$J_{6a',6b'}$ 12.5 Hz, H-6b′), 3.73 (t, 1H, $J_{3,4}$,$J_{4,5}$ 9.5 Hz, H-4), 3.34 (dd, 1H, $J_{1',2'}$ 3.5 Hz,$J_{2',3'}$ 10.0 Hz, H-2′), 2.34 (s, 3H, $SC_6H_4CH_3$), 2.09, 2.07, 1.91 (3s, 9H, 3× COCH_3), 1.35 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.6, 169.8, 169.6 (3×*C*OCH$_3$), 137.9, 137.8, 132.8, 132.1 (2), 129.4, 130.0 (2), 128.4 (2), 128.3 (4), 128.0, 127.6 (3) (Ar*C*), 92.8 (C-1′), 85.2 (C-1), 79.2, 75.5, 74.4, 74.0, 71.7, 70.4, 69.2, 68.1, 67.4, 61.6, 60.6, 21.0 ($SC_6H_4CH_3$), 20.7, 20.6, 20.4 (3×*C*OCH$_3$), 17.7 (C-*C*H$_3$). HRMS calcd for $C_{39}H_{45}N_3NaO_{11}S$ (M+Na)$^+$:786.2672, found 786.2669.

***p*-Tolyl 2-azido-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (9)**:

To a solution of pure disaccharide **8** (1.5 g, 1.9 mmol) in MeOH (25 mL), NaOMe in MeOH (2 mL, 0.5 M) was added and the reaction was allowed

to stir at room temperature for 5 hours. Complete consumption of the starting material to a slower moving spot as evident from TLC (*n*-hexane-EtOAc; 1:2) ensures the formation of the product. The reaction mixture was neutralized with Dowex 50W X8 H^+ resin, filtered and evaporated *in vacuo.* The crude product was further purified by flash chromatography to afford the desired compound **9**(1.2 g, 96%) as amorphous powder. $[\alpha]_D^{25}$ = +98° (*c* 1.0, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.40-7.09 (m, 14H, Ar*H*), 5.45 (d, 1H, $J_{1,2}$ 1.0 Hz, H-1), 4.95 (d, 1H, $J_{1',2'}$< 1.0 Hz, H-1′), 4.81, 4.62 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.70, 4.64 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.16 (m, 1H, H-5), 4.07 (m, 1H, H-2), 4.02-3.99 (m, 2H, H-3, H-3′), 3.81 (m, 1H, 6a′), 3.71-3.67 (m, 4H, H-4, H-4′, H-5′, H-6b′), 3.61 (dd, 1H, $J_{1',2'}$< 1.0 Hz, $J_{2',3'}$ 7.0 Hz, H-2′), 2.33 (s, 3H, $SC_6H_6CH_3$), 1.31 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 138.0, 137.8, 137.4,132.1 (3), 130.4, 129.9 (2), 128.5 (2), 128.4 (3), 128.2 (2), 128.1, 127.9 (Ar*C*), 93.7 (C-1′), 85.6 (C-1), 79.5, 75.4, 74.8, 74.7, 72.0, 71.7, 71.0, 70.8, 69.2, 62.5, 61.5, 21.1 ($SC_6H_4CH_3$), 17.8 (C-CH_3). HRMS calcd for $C_{33}H_{39}N_3O_8Na$ (M+Na)$^+$:660.2356, found 660.2351.

***p*-Tolyl 2-azido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (10)**:

To a solution of pure compound **9** (1.2 g, 1.8 mmol) in dry CH_3CN (20 mL), benzaldehyde dimethyl acetal (0.4 mL, 2.3mmol) and CSA (50 mg) was added and stirred at room temperature. The reaction mixture was continued to stir for 3 hours till the TLC showed complete consumption of the starting material (*n*-hexane-EtOAc; 3:1). Et_3N was added to neutralise the reaction mixture and the solvent was evaporated *in vacuo*. The crude residue obtained was subjected to flash chromatography using *n*-hexane-EtOAc (2:1) as eluent to furnish the pure disaccharide derivative **10** (1.1 g, 82%) as colorless foam. $[\alpha]_D^{25}$ = +110° (*c* 1.0, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.43-7.12 (m, 19H, Ar*H*), 5.53 (s, 1H, C*H*Ph), 5.50 (d, 1H, $J_{1,2}$< 1.0 Hz, H-1), 4.97 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1′), 4.91, 4.61 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.73, 4.64 (ABq, 2H, J_{AB}11.5 Hz, CH_2Ph), 4.27 (m, 2H, H-3′, H-6a′), 4.21 (m, 1H, H-5), 4.10 (m, 3H, H-2, H-3, H-5′), 3.75-3.68 (m, 2H, H-4, H-6b′), 3.54 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 3.32 (dd, 1H, $J_{1',2'}$ 4.0 Hz,$J_{2',3'}$ 10.0 Hz, H-2′), 2.35 (s, 3H, $SC_6H_4CH_3$), 1.35 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 137.8, 137.7, 137.3, 136.9, 132.2 (2), 130.5, 129.9 (2), 129.3, 128.5 (2), 128.4 (2), 128.3 (2), 128.2 (2), 128.1 (2), 127.9, 127.8, 126.4 (2) (Ar*C*), 102.0 (CHPh), 94.1 (C-1′), 85.4 (C-1), 81.8, 79.7, 75.9, 74.8, 74.6, 71.9, 69.2, 68.8, 68.7, 62.8, 62.5, 21.1 ($SC_6H_4CH_3$), 17.7 (C-CH_3). HRMS calcd for $C_{40}H_{43}N_3O_8Na$ (M+Na)$^+$:748.2669, found 748.2662.

***p*-Tolyl 2-azido-2-deoxy-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (11)**:

To a solution of pure disaccharide derivative **10** (1.0 g, 1.4 mmol) in dry CH_2Cl_2 (20 mL), Pyridine (0.5 mL, 5.6 mmol) was added and the reaction mixture was stirred at -5 °C. After stirring for 10 minutes, chloroacetic anhydride (479 mg, 2.8 mmol) was added to the reaction vessel and the reaction was stirred for 1 hour at same temperature. After complete conversion of the starting material (TLC in *n*-hexane-EtOAc; 3:1), the solvent was evaporated *in vacuo*. The crude residue thus obtained, was purified by flash chromatography using *n*-hexane-EtOAc (4:1) as eluent to afford the desired disaccharide derivative **11** (983 mg, 89%) as white powder. $[\alpha]_D^{25}$ = +126° (*c* 1.0, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.42-7.12 (m, 19H, Ar*H*), 5.74 (t, 1H, $J_{2',3'}$,$J_{3',4'}$ 9.5 Hz, H-3′), 5.53 (d, 1H, $J_{1,2}$< 1.0 Hz, H-1), 5.48 (s, 1H, C*H*Ph), 5.01 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.94, 4.64 (ABq, 2H, J_{AB} 10.5 Hz, CH_2Ph), 4.73, 4.57 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.26 (dd, 1H, $J_{5',6a'}$ 5.0 Hz,$J_{6a',6b'}$ 10.5 Hz, H-6a′), 4.20 (m, 1H, H-5), 4.15 (s, 2H, COCH_2Cl), 4.13-4.08 (m, 3H, H-2, H-3, H-5′), 3.75 (t, 1H, $J_{3',4'}$,$J_{4',5'}$9.5 Hz, H-4′), 3.69 (m, 2H, H-4, H-6b′), 3.24 (dd, 1H, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 2.34 (s, 3H, $SC_6H_4CH_3$), 1.38 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3).^{13}C NMR ($CDCl_3$, 125 MHz) δ: 166.4 (*C*OCH$_2$Cl), 137.8, 137.4, 137.1, 136.8, 132.2 (3), 130.4, 130.0 (2), 129.1, 128.5 (5), 128.3 (2), 128.1 (2), 128.0, 127.8, 126.4 (2) (Ar*C*), 101.7 (*C*HPh), 94.0 (C-1′), 85.1 (C-1), 79.5, 79.0, 76.3, 74.6, 74.1, 71.7, 70.9, 69.2, 68.5, 62.9, 61.2, 40.5 (CO*C*H$_2$Cl), 21.1 ($SC_6H_4CH_3$), 17.7(C-*C*H$_3$). HRMS calcd for $C_{39}H_{45}ClN_3O_{11}Na$ (M+Na)$^+$: 824.2384, found: 824.2381.

2-(benzyloxycarbonyl)-aminoethyl 2-azido-2-deoxy-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (12):

A suspension of the disaccharide acceptor **5** (810 mg, 0.9 mmol), the disaccharide donor **11** (935 mg, 1.2 mmol) and MS 4Å (2.0 g) in dry CH_2Cl_2 (20 mL) was stirred under N_2 for 30 minutes. NIS (263 mg, 1.2 mmol) was added and the reaction mixture was cooled to -5 ºC followed by addition of H_2SO_4-silica (45 mg). The reaction mixture was stirred at the same temperature for 20 minutes when TLC (*n*-hexane-EtOAc; 5:2) revealed complete consumption of the disaccharide acceptor **5**. The reaction mixture was immediately filtered through a pad of Celite and washed with CH_2Cl_2. The combined filtrate obtained was washed successively with $Na_2S_2O_3$ (2×30 mL), $NaHCO_3$ (2×30 mL) and brine (30 mL). The organic layer was separated, dried over anhydrous $NaSO_4$, filtered and evaporated *in vacuo*. The residue was then purified by flash chromatography using *n*-hexane-EtOAc (4:1) as eluent to afford the desired tetrasaccharide **12** (1.2 g, 83%) as white powder. $[\alpha]_D^{25}$ = +108° (c0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.08-7.10 (m, 40H, Ar*H*), 5.75 (t, 1H, $J_{2''',3'''}$, $J_{3''',4'''}$ 10.0 Hz, H-3‴), 5.48 (s, 1H, C*H*Ph), 5.37 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 5.33 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5Hz, H-4), 5.25 (d, 1H, $J_{1'',2''}$< 1.0 Hz, H-1″), 5.13 (d, 2H, COOCH_2Ph), 5.10 (bs, 1H, N*H*), 5.06 (d, 1H, $J_{1''',2'''}$ 3.0 Hz, H-1‴), 5.04 (d, 1H, $J_{1',2'}$< 1.0 Hz, H-1′), 4.92, 4.54 (ABq, 2H, J_{AB} 11.5 Hz , CH_2Ph), 4.82 (d, 1H, $J_{1,2}$ <1.0 Hz, H-1), 4.64, 4.61 (d, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.59, 4.48 (d, 2H, J_{AB} 12.0Hz, CH_2Ph), 4.42, 4.29 (d, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.40 (m, 1H, H-2′), 4.30 (m, 1H, H-6a‴), 4.18 (dd, 1H, $J_{2''',3'''}$2.5 Hz, $J_{3''',4'''}$10.0 Hz, H-3‴), 4.16 (s, 2H, COCH_2Cl), 4.11 (m, 1H, H-5‴), 4.06 (m, 1H, H-2″), 4.02-3.94 (m, 3H, H-2, H-3′, H-5), 3.90 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 10.0 Hz, H-3), 3.80 (m, 2H, H-5′, H-6b‴), 3.75 (m, 1H, OCH_2), 3.72-3.64 (m, 3H, H-4″, H-4‴, OCH_2), 3.47, 3.41 (m, 2H, CH_2NH), 3.21 (dd, 1H, $J_{1''',2'''}$3.0 Hz, $J_{2''',3'''}$ 10.5 Hz, H-2‴), 3.08 (m, 1H, H-5″), 1.38 (d, 3H, $J_{5'',6''}$ 6.5 Hz, C-CH_3), 1.27 (d, 3H, $J_{5',6'}$ 6.5 Hz, C-CH_3), 1.18 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH_3).^{13}C NMR ($CDCl_3$, 125 MHz) δ: 166.3 (*C*OCH$_2$Cl), 165.7, 165.6 (2×*C*OCH$_3$), 156.3 (*C*OOCH$_2$Ph), 137.6, 137.5, 137.4, 136.9, 136.2, 133.2, 133.1, 129.9, 129.8 (3), 129.0, 128.5 (2), 128.4 (8), 128.3 (6), 128.2 (4), 128.1 (4), 128.0 (3), 127.8 (4), 127.6 (2), 126.3 (3) (Ar*C*), 101.6 (*C*HPh), 101.2 (C-1′), 99.2 (C-1), 97.9 (C-1″), 93.6 (C-1‴), 79.3, 79.0, 76.4, 76.1, 75.7, 75.4, 73.8, 73.6, 73.2, 73.0, 72.3, 72.2, 71.8, 71.6, 71.0, 68.5, 68.4, 67.5, 67.1, 66.9 (2), 62.6, 61.1, 45.7 (*C*H$_2$NH), 40.6 (CO*C*H$_2$Cl), 17.8 (C-*C*H$_3$), 17.6 (C-*C*H$_3$).HRMS calcd for $C_{85}H_{89}ClN_4O_{22}Na$ (M+Na)$^+$: 1575.5555, found: 1575.5553.

2-(benzyloxycarbonyl)-aminoethyl 2-azido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (13):

A mixture of tetrasaccharide **12** (1.0 g, 0.6 mmol) in 20 mL of CH_2Cl_2-CH_3OH (2:3), thiourea (456 mg, 6.0 mmol) and 2,4,6-collidine (0.8 mL, 6.0 mmol) was refluxed at 50 ºC for 8 hours till the TLC (*n*-hexane-EtOAc; 2:1) showed complete conversion of the starting material to a slower moving spot. The solvents were evaporated *in vacuo*, the solid residue thus obtained was dissolved in CH_2Cl_2 (50 mL) and washed with brine (2 × 20 mL). The organic layer was collected, dried ($NaSO_4$), filtered and evaporated *in vacuo*. The crude product was further purified

by flash chromatography using *n*-hexane-EtOAc (2:1) as eluent to afford the pure tetrasaccharide acceptor **13** (780 mg, 82%) as foam. $[\alpha]_D^{25}$ = +114° (*c*0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.10-7.15 (m, 40H, Ar*H*), 5.54 (s, 1H, C*H*Ph), 5.38 (m, 2H, H-4, H-4′), 5.25 (d, 1H, $J_{1'',2''}$< 1.0 Hz, H-1″), 5.15 (d, 2H, COOCH_2Ph), 5.08 (m, 2H, H-1′, H-1‴), 4.93, 4.51 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.85 (d, 1H, $J_{1,2}$< 1.0 Hz, H-1), 4.62 (m, 2H, CH_2Ph), 4.56 (m, 2H, CH_2Ph), 4.44 (s, 2H, CH_2Ph), 4.40 (m, 1H, H-2′), 4.30 (m, 2H, H-5‴, H-6a″), 4.21 (m, 1H, H-3″), 4.10-3.98 (m, 5H, H-2, H-2″, H-3′, H-3‴, H-5), 3.92 (m, 1H, H-3), 3.84 (m, 2H, H-5′, H-5″), 3.74 (m, 2H, H-6b‴, OCH_2), 3.65 (t, 1H, $J_{3''',4'''}$,$J_{4''',5'''}$ 9.5 Hz, H-4‴), 3.55 (m, 2H, H-4″, OCH_2), 3.47, 3.42 (m, 2H, CH_2NH), 3.30 (dd, 1H, $J_{1''',2'''}$ 3.5 Hz, $J_{2''',3'''}$ 10.0 Hz, H-2‴), 2.94 (bs, 1H, O*H*), 1.28 (m, 6H, 2×C-CH_3), 1.22 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 165.8, 165.7 (2×*C*OPh), 156.4 (*C*OOCH_2Ph), 138.0,137.9, 137.6(2), 137.3, 136.4, 133.2 (2), 130.1, 130.0 (2), 129.9, 129.2, 128.6 (2), 128.5 (6), 128.4 (5), 128.3 (6), 128.2 (10), 127.9 (2), 127.6 (2), 126.5 (2) (Ar*C*), 102.0 (*C*HPh), 101.2 (C-1'), 99.3 (C-1), 98.4 (C-1"), 94.0 (C-1'"), 81.9,79.7, 76.5, 75.9, 75.8, 75.6, 74.3, 73.7, 73.3, 73.2, 73.1, 72.3, 72.0 (2), 69.0, 68.8, 68.6, 67.7, 67.3, 67.0 (2), 63.1, 62.5, 40.8 (*C*H_2NH), 18.0 (2×C-*C*H_3), 17.7 (C-*C*H_3). HRMS calcd for $C_{83}H_{88}N_4O_{21}Na$ $(M+Na)^+$:1499.5839, found: 1449.5834.

_p_-Tolyl 4-*O*-benzyl-3-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (15):

To a solution of known compound **14** (750 mg, 1.5 mmol) in DMF at 5ºC, NaH (108 mg, 4.5 mmol) was added and stirred for 10 minutes. Thereafter, BnBr (0.3 mL, 2.3 mmol) was added to the reaction mixture and it was stirred at room temperature for 3 hours until the TLC (*n*-hexane: EtOAc; 5:2) showed complete consumption of the starting material to a faster moving spot. Immediately, the excess NaH was quenched with MeOH (5 mL) and DMF was evaporated *invacuo.* The mixture was diluted with CH_2Cl_2 (20 mL) and successively washed with H_2O (2×30 mL) and brine (2×30 mL). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated *invacuo*. The residue was purified by flash chromatography using using*n*-hexane-EtOAc (3:2) to afford the pure compound **15** (833 mg, 94%) as colourless gel.$[\alpha]_D^{25}$ = +89° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.68-6.86 (m, 18H, Ar*H*), 5.52 (s, 1H, C*H*Ph), 4.77, 4.72 (m, 4H, H-6a, H-6b, CH_2Ph), 4.61 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 4.38, 3.99 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.15 (d, $J_{3,4}$ 2.5 Hz, H-4), 3.89 (t, 1H, $J_{1,2}$,$J_{2,3}$ 9.5 Hz, H-2), 3.81 (s, 3H, $CH_2C_6H_4OCH_3$), 3.65 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 2.5 Hz, H-3), 3.39 (m, 1H, H-5), 2.35 (s, 3H, $SC_6H_4CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 159.1, 138.5, 137.8, 137.4, 133.2 (2), 130.0, 129.7, 129.5 (2), 129.3 (2), 128.8, 128.1 (2), 128.0 (2), 127.9 (2), 127.6, 127.5, 126.5 (2), 113.6, 101.1 (CHPh), 86.4 (C-1), 80.9, 75.2, 73.4, 71.2, 69.6, 69.2, 55.1 ($CH_2C_6H_4OCH_3$), 21.0 ($SC_6H_4CH_3$). HRMS calcd for $C_{28}H_{30}O_6SNa$ $(M+Na)^+$: 517.1661, found: 517.1659.

_p_-Tolyl 2,4-di-*O*-benzyl-3-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (16):

To a solution of known compound **15** (650 mg, 1.1 mmol) in anhydrous CH_3CN (15 mL), $NaBH_4$ (1.6 g, 8.8 mmol) was slowly added at 0ºC. After 10 min, TCT (416 mg, 11.0 mmol) was added and the reaction mixture was stirred at room temperature for 8 hours till the TLC showed (*n*-hexane-EtOAc; 3:2) complete consumption of the starting material to a slower moving spot. The reaction mixture was immediately filtered through a pad of Celite followed by evaporation of the filtrate in *vacuo*. The crude compound thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (1:2) to afford the pure compound **16** (522 mg, 80%) as white foam. $[\alpha]_D^{25}$ = +112° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.48-6.87 (m, 18H, Ar*H*), 4.97, 4.62 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.85, 4.76 (ABq, 2H, J_{AB} 10.5 Hz, CH_2Ph), 4.69 (s, 2H, CH_2Ph), 4.60 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 3.91 (t, 1H, $J_{1,2}$,$J_{2,3}$ 9.5 Hz, H-2), 3.86 (m, 1H, H-6a), 3.82 (d, 1H, $J_{3,4}$ 2.5 Hz, H-4), 3.81 (s, 3H, $CH_2C_6H_4OCH_3$), 3.59 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 2.5 Hz, H-3), 3.53 (m, 1H, H-6b), 3.43 (m, 1H, H-5), 2.31 (s, 3H, $SC_6H_4CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 159.3, 138.3, 137.3, 132.1 (2), 129.9, 129.6 (2), 129.3 (2), 128.4, 128.3 (6), 128.2 (2), 128.1 (2), 127.7 (2), 127.5, 113.8 (2) (Ar*C*), 88.0 (C-1), 83.9, 78.7, 77.4, 75.6, 74.1, 73.3, 72.6, 62.2, 55.2 ($CH_2C_6H_4OCH_3$), 21.0 ($SC_6H_4CH_3$). HRMS calcd for $C_{35}H_{38}O_6SNa$ $(M+Na)^+$: 609.2287, found: 609.2283.

6-*O*-chloroacetyl-2,4-di-*O*-benzyl-3-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (17):

Compound **16** (480 mg, 0.8 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and pyridine (0.3 mL, 3.2 mmol) was added to the reaction mixture and continued to stir at -5ºC for 15 minutes. Chloroacetic anhydride (272 mg, 1.6 mmol) was then added and the reaction was stirred at the same temperature for 2 hours till the TLC in *n*-hexane-EtOAc (5:2) confirmed complete consumption of the starting material. The solvents were evaporated and co-evaporated with toluene for complete removal of pyridine. The crude residue thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (2:1) as eluent to afford compound **17** (493 mg, 91%) as white powder. $[\alpha]_D^{25}$ = +132° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.52-6.91 (m, 18H, Ar*H*), 5.05, 4.66 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.90, 4.82 (ABq, 2H, J_{AB} 10.0 Hz, CH_2Ph), 4.74 (s, 2H, CH_2Ph), 4.61 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 4.41 (dd, 1H, $J_{5,6a}$, 7.5 Hz, $J_{6a,\ 6b}$ 11.0 Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b}$ 5.5 Hz, $J_{6a,6b}$ 11.0 Hz, H-6b), 3.96 (m, 3H, H-2, COCH_2Cl), 3.84 (m, 4H, H-4, $CH_2C_6H_4OCH_3$), 3.64 (m, 2H, H-3, H-5), 2.35 (s, 3H, $SC_6H_4CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 166.7 (*C*OCH$_2$Cl), 159.2, 138.2, 138.1, 137.3, 132.3 (2), 130.0, 129.9, 129.4 (2), 129.2 (2), 128.4, 128.2 (4), 128.1 (4), 127.6 (2), 127.4, 88.0 (C-1), 83.6, 77.2, 75.5, 75.4, 74.0, 73.0, 72.7, 64.7, 55.1 ($CH_2C_6H_4OCH_3$), 40.5 (*C*OCH$_2$Cl), 21.0. HRMS calcd for $C_{37}H_{39}ClO_7SNa$ $(M+Na)^+$: 685.2003, found: 685.2000.

2-(benzyloxycarbonyl)-aminoethyl 6-*O*-chloroacetyl-2,4-di-*O*-benzyl-3-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (18):

A mixture of tetrasaccharide acceptor **13** (750 mg, 0.5 mmol), monosaccharide donor **17** (430 mg, 0.7 mmol) and MS 4Å (2.0g) in dry CH_2Cl_2 (30 mL) was stirred under N_2 for 10 min at 0ºC. NIS (190 mg, 0.8 mmol) was added followed by addition of H_2SO_4-silica (50 mg) at 0°C. The reaction mixture was stirred for 10 minutes at same temperature when TLC *n*-hexane-EtOAc (5:2) indicated complete consumption of the acceptor **13**. The mixture was immediately filtered through a pad of Celite. The filtrate was diluted with CH_2Cl_2 (30 mL) 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 over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (2:1) to afford pure pentasaccharide **18** (866 mg, 84%) as colourless foam. $[\alpha]_D^{25}$ = +126° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.12-6.91 (m, 54H, Ar*H*), 5.72 (d, 1H, $J_{1''',2'''}$2.0 Hz, H-1‴), 5.42 (s, 1H, C*H*Ph), 5.38 (m, 2H, H-4, H-4′), 5.29 (d, 1H, $J_{1'',2''}$<1.0 Hz, H-1″), 5.14 (d, 2H, COOCH_2Ph), 5.07 (d, 1H, $J_{1',2'}$< 1.0 Hz, H-1′), 4.99 (d, 1H, $J_{1''',2'''}$ 3.5 Hz, H-1‴), 4.94, 4.91 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.85 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1), 4.84, 4.71 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.67-4.59 (m, 6H, 3×CH_2Ph), 4.57, 4.52 (ABq, 2H, J_{AB} 12.5 Hz, CH_2Ph), 4.48, 4.45 (ABq, 2H, J_{AB} 12.5 Hz, CH_2Ph), 4.42 (m, 2H, H-2′, H-6a''''), 4.29 (m, 2H, H-6a‴, H-6b''''), 4.22-4.14 (m, 4H, H-3", H-3‴, H-5‴, H-5''''), 4.07, 3.75 (ABq, 2H, J_{AB} 14.5 Hz, COCH_2Cl), 4.05-3.98

(m, 5H, H-2, H-2", H-2"", H-3', H-5), 3.93 (m, 3H, H-3, H-5', H-5"), 3.87-3.70 (m, 5H, H-3"", H-4‴, H-4"", H-6b‴, OCH_2), 3.84 (s, 3H, $CH_2C_6H_4OCH_3$), 3.63 (t, 1H, $J_{3"4"}J_{3"4"}$ 9.5 Hz, H-4"), 3.55 (m, 1H, OCH_2), 3.50.3.42 (m, 3H, H-2‴, CH_2NH), 3.43 (m, 1H, OCH_2), 1.32 (d, 3H, $J_{5",6"}$6.5 Hz, C-CH_3), 1.28 (d, 3H, $J_{5',6'}$6.5 Hz, C-CH_3), 1.20 (d, 3H, $J_{5, 6}$ 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 167.4 (COCH$_3$), 165.6 (2) (COPh), 156.3 (COOCH$_2$Ph), 138.1, 138.0, 137.8, 137.5, 137.4 (2), 134.0, 136.3, 133.1, 133.0, 130.7, 130.0, 129.8, 129.7 (2), 129.1 (2), 128.9, 128.5 (10), 128.3 (12), 128.2 (6), 128.1 (2), 128.0 (2), 127.9, 127.7 (5), 127.6, 127.5, 127.4, 127.3, 127.2 (2), 126.4 (2), 113.7 (2) (Ar*C*), 101.9 (*C*HPh), 101.2 (C-1'), 99.2 (C-1), 97.7 (C-1"), 96.9 (C-1""), 92.9 (C-1‴), 82.9, 79.4, 78.1, 76.5, 75.8 (2), 75.5, 75.3, 74.6, 74.4, 73.5, 73.3 (2), 73.1, 72.3, 72.1, 71.9 (2), 71.8, 71.6, 71.5, 68.8 (2), 68.5, 67.5, 67.1, 66.9, 66.8, 65.4, 62.5, 62.1, 55.2 ($CH_2C_6H_4OCH_3$), 40.9 (COCH_2Cl), 40.7 (CH_2NH), 17.9 (C-CH_3), 17.6 (2×C-CH_3). HRMS calcd for $C_{113}H_{119}ClN_4O_{28}Na$ (M+Na)$^+$: 2037.7597, found: 2037.7593.

2-(benzyloxycarbonyl)-aminoethyl 6-*O*-chloroacetyl-2,4-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (19):

To a 20 mL solution of pure pentasaccharide **18** (842 mg, 0.4 mmol) in CH_2Cl_2-H_2O (4:1), DDQ (182 mg, 0.8 mmol) was added and the reaction mixture was vigorously stirred at room temperature. After 2 hours, the starting material was fully consumed (TLC in *n*-hexane-EtOAc; 2:1) and a slower moving spot was generated. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with H_2O (2×20 mL) and brine (2×20 mL) successively. The organic layer was then collected, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The crude syrupy residue was subjected to flash chromatography (*n*-hexane-EtOAc; 1.4:1) to afford the desired pentasaccharide acceptor **19** (666 mg, 79%) as white foam. $[\alpha]_D^{25}$ = +148° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.23-7.33 (m, 50H, Ar*H*), 5.87 (d, 1H, $J_{1"",2""}$ 3.5 Hz, H-1""), 5.57 (m, 2H, H-4, H-4′), 5.52 (s, 1H, C*H*Ph), 5.45 (d, 1H, $J_{1",2"}$ < 1.0 Hz, H-1"), 5.33 (m, 2H, COOCH_2Ph), 5.25 (d, 1H, $J_{1',2'}$ < 1.0 Hz, H-1′), 5.19 (d, 1H, $J_{1",2"}$ 3.5 Hz, H-1‴), 5.12, 5.08 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 5.03 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1), 4.88-4.70 (m, 10H, 5×CH_2Ph), 4.60 (m, 3H, H-2′, H-3‴, H-6a""), 4.49-4.34 (m, 5H, H-3"", H-5‴, H-5"", H-6a‴, H-6b""), 4.24, 3.93 (ABq, 2H, J_{AB} 15.0 Hz, COCH_2Cl), 4.19-4.15 (m, 6H, H-2, H-2", H-3, H-3", H-5, H-5"), 4.11 (m, 1H, H-3′), 4.01 (m, 2H, H-2"", OCH_2), 3.89 (m, 4H, H-4‴, H-4"", H-5′, H-6b‴), 3.81 (t, 1H, $J_{3",4"}$ $J_{4",5"}$ 9.5 Hz, H-4"), 3.72 (m, 1H, OCH_2), 3.67, 3.60 (m, 2H, CH_2NH), 3.59 (m, 1H, H-2‴), 1.50 (d, 3H, $J_{5", 6"}$6.5Hz, C-CH_3), 1.46 (d, 3H, $J_{5',6'}$ 6.5Hz, C-CH_3), 1.39 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 167.2 (COCH_2Cl), 165.6 (2×COPh), 156.2 (COOCH_2Ph), 138.0, 137.9, 137.6, 137.5, 137.4, 136.9, 136.3, 133.1 (2), 130.0, 129.8 (2), 129.2, 128.5 (6), 128.4 (2), 128.3 (12), 128.2 (2), 128.1 (7), 128.0 (2), 127.7 (10), 127.4 (4), 126.5 (2) (Ar*C*), 102.2 (*C*HPh), 101.3 (C-1'), 99.2 (C-1), 97.8 (C-1"), 96.3 (C-1""), 93.0 (C-1‴), 82.9, 79.4, 76.5, 75.8, 75.5, 75.4, 75.3, 74.8,73.6, 73.3, 73.2, 73.1, 72.3, 72.2, 72.1, 71.9 (2), 71.5, 70.8, 69.8, 68.8, 68.7, 68.5, 67.6, 67.2, 66.9 (2), 65.2, 62.4, 62.2, 40.9 (COCH_2Cl), 40.7 (CH_2NH), 17.9 (C-CH_3), 17.6 (2×C-CH_3). HRMS calcd for $C_{105}H_{111}ClN_4O_{27}Na$ (M+Na)$^+$: 1917.7022, found: 1917.7020

***p*-Tolyl 6-*O*-Benzoyl-2,3,4-tri-*O*-Benzyl-β-D-glucopyranoside (21):**

To a solution of compound **20**(350 mg, 0.6 mmol) in pyridine (10 mL), benzoyl chloride (0.1 mL, 0.9 mmol) was added and the reaction mixture was stirred at room temperature for 4 hours until the TLC (*n*-hexane: EtOAc; 4:1) showed complete consumption of the starting material to a faster moving spot. The solvent was then evaporated and co-evaporated with toluene to ensure complete removal of pyridine. The crude compound was then subjected to flash silica gel chromatography using *n*-hexane-EtOAc (3:1) as eluent to furnish the desired compound **21**(357 mg, 86%) as colorless foam. $[\alpha]_D^{25}$ = +95° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.20-6.94 (m, 24H, Ar*H*), 4.99, 4.77 (ABq, 2H, J_{AB} 10.5 Hz, CH_2Ph), 4.92, 4.66 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.95, 4.90 (ABq, 2H, J_{AB} 10.5 Hz, CH_2Ph), 4.69 (m, 1H, H-6a), 4.64 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 4.46 (dd, 1H, $J_{5,6b}$ 4.6 Hz, $J_{6a, 6b}$ 11.5 Hz, H-6b), 3.79 (t, 1H, $J_{2,3}J_{3,4}$ 8.5 Hz, H-3), 3.67 (m, 2H, H-4, H-5), 3.52 (t, 1H, $J_{1,2}J_{2,3}$ 9.5 Hz, H-2), 2.29 ($SC_6H_4CH_3$).^{13}C NMR ($CDCl_3$, 125 MHz) δ: 166.1 (COPh), 162.3, 138.1, 138.0, 137.8, 137.5, 134.5 (2), 133.0 (2), 130.5 (2), 129.8 (2), 129.6 (2), 128.8 (2), 128.5 (2), 128.4 (2), 128.3, 128.1 (2), 128.0 (2), 127.9 (2), 127.8 (Ar*C*), 87.4 (C-1), 86.7, 80.7, 77.6 (2), 75.9, 75.4, 75.1, 63.5, 21.1 ($SC_6H_4CH_3$) HRMS calcd for $C_{41}H_{40}O_6SNa$ (M+Na)$^+$: 683.2443, found: 683.2446.

2-(benzyloxycarbonyl)-aminoethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→3)-6-*O*-chloroacetyl-2,4-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (22):

A solution of known glucose donor **21** (257 mg, 0.4 mmol), pentasaccharide acceptor **19** (610 mg, 0.3 mmol) and MS 4Å (2.0 g) in dry $CH_2Cl_2$was stirred under N_2 for 20 min. NIS (114 mg, 0.5 mmol) was added and the reaction mixture was cooled to -55 °C followed by addition of H_2SO_4-silica (35 mg). After 30 minutes, TLC in *n*-hexane-EtOAc (5:2) indicated complete consumption of the acceptor **19.** The reaction mixture was immediately filtered through a pad of Celite and the filtrate was successively washed 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) and evaporated *in vacuo*. The residue thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (2:1) and afforded the pure hexasaccharide **22** (609 mg, 78%) as colorless foam. $[\alpha]_D^{25}$ = +122° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.13-7.04 (m, 70H, Ar*H*), 5.71 (d, 1H, $J_{1"",2""}$ 3.5 Hz, H-1""), 5.40 (m, 2H, H-4, H-4′), 5.30 (d, 1H, $J_{1",2"}$ < 1.0 Hz, H-1"), 5.24 (s, 1H, C*H*Ph), 5.15 (m, 3H, COOCH_2Ph, H-1‴"), 5.11, 4.74 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 5.07 (d, 1H, $J_{1',2'}$ < 1.0 Hz, H-1'), 5.00 (d, 1H, $J_{1"",2""}$ 3.5 Hz, H-1"'), 4.95, 4.90 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.87 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1), 4.85 (m, 4H, 2×CH_2Ph), 4.67 (m, 2H, CH_2Ph), 4.63-4.54 (m, 4H, 2×CH_2Ph), 4.54, 4.50 (ABq, 2H, J_{AB} 11.5 Hz, CH_2Ph), 4.43 (m, 2H, CH_2Ph), 4.38 (2H, H-2', H-3‴), 4.29-4.22 (m, 4H, H-3", H-5‴, H-6a"", H-6a‴"), 4.19-4.13 (m, 5H, H-3"", H-3‴", H-6a‴, H-6b‴", H-6b‴"), 4.07 (m, 3H, H-2", H-4‴", H-5"), 4.05, 3.81 (m, 2H, COCH_2Cl), 3.99 (m, 2H, H-2, H-2""), 3.92 (m, 3H, H-5, H-5', H-4""), 3.87 (m, 1H, H-6b‴), 3.78 (m, 2H, H-3, H-5""), 3.74, 3.57 (m, 2H, OCH_2), 3.71-3.61 (m, 5H, H-2‴", H-3', H-4", H-4‴, H-5‴"), 3.49, 3.43 (m, 2H, CH_2NH), 3.37 ((dd, 1H, $J_{1",2"}$ 3.5 Hz, $J_{2",3"}$ 10.0 Hz, H-2‴), 1.32 (d, 3H, $J_{5",6"}$ 6.5 Hz, C-CH_3), 1.28 (d, 3H, $J_{5',6'}$ 6.0 Hz, C-CH_3), 1.21 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 167.3 (COCH$_2$Cl), 166.1, 165.6 (3×COPh), 156.2 (COOCH$_2$Ph), 139.2, 138.5, 138.4, 138.2, 138.0, 137.9, 137.8, 137.6 (2), 137.5 (2), 136.9, 136.3 (2), 133.1, 133.0, 132.9, 130.2, 130.1, 129.9, 129.8, 129.7 (4), 129.6 (2), 129.1 (2), 128.5 (4), 128.4 (4), 128.3 (10), 128.2 (10), 128.1 (5), 127.9 (4), 127.8 (4), 127.7 (2), 127.6 (4), 127.4, 126.4 (2) (Ar*C*), 102.1 (*C*HPh), 101.3 (C-1'), 99.3 (C-1), 97.8 (C-1"), 96.8 (C-1‴"), 96.4 (C-1""), 93.0 (C-1‴), 83.0, 82.0, 80.5, 79.5, 77.9, 76.6, 76.4, 75.8 (2), 75.7, 75.6, 75.0, 74.9, 74.8, 74.4, 73.9, 73.6, 73.3, 73.2, 72.4, 72.3, 72.0, 71.7, 71.6, 71.0, 70.5, 69.1, 68.9, 68.8, 68.5, 67.6, 67.2, 67.0, 66.9, 65.3, 63.1, 62.4, 62.2, 40.9 (COCH_2Cl), 40.8 (CH_2NH), 18.0 (C-CH_3), 17.6 (2×C-CH_3). HRMS calcd for $C_{139}H_{143}ClN_4NaO_{33}$ (M+Na)$^+$:2453.9221, found: 2453.9218.

2-(benzyloxycarbonyl)-aminoethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-galactopyranosyl-

(1→3)-2-azido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (23):

To a solution of pure hexasaccharide **22** (552 mg, 0.2 mmol) in 25 mL of CH_2Cl_2-MeOH (2:3), thiourea (152 mg, 2.0 mmol) and collidine (0.3 mL, 2.0 mmol) was added. The reaction mixture was refluxed for 10 hours at 55ºC until the TLC (*n*-hexane-EtOAc; 1.8:1) showed consumption of the hexasaccharide to a slower moving spot. The solvent mixture was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 (25 mL). It was then successively washed with H_2O (25 mL) and brine (25 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to a syrupy residue which was further purified by flash chromatography using n-hexane-EtOAc (3:2) as eluent to obtain the pure hexasaccharide derivative **23** (433 mg, 81%) as foam. $[\alpha]_D^{25}$ = +94° (*c*0.8, $CHCl_3$).^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.09-7.11 (m, 70H, Ar*H*), 5.75 (d, 1H, $J_{1''',2'''}$ 3.5 Hz, H-1'''), 5.35 (m, 2H, H-4, H-4′), 5.25 (m, 2H, H-1", C*H*Ph), 5.15 (m, 3H, H-1''''', COOCH_2Ph), 5.11, 4.74 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 5.02 (m, 2H, H-1', H-1'''), 4.93-4.82 (m, 6H, 3×CH_2Ph), 4.84 (m, 1H, H-1), 4.62 (m, 4H, 2×CH_2Ph), 4.55 (m, 4H, 2×CH_2Ph), 4.46 (m, 2H, CH_2Ph), 4.44 (m, 2H, H-2′, H-3'''), 4.38-4.31 (m, 3H, H-3", H-5''', H-5''''), 4.23 (m, 2H, H-3'''', H-6a''''), 4.17-4.09 (m, 3H, H-2, H-6a''', H-6b''''), 4.03-3.96 (m, 6H, H-2", H-2''', H-3′, H-4''', H-4'''', H-5), 3.89 (m, 1H, H-3), 3.83 (m, 1H, H-5"), 3.76 (m, 2H, H-5′, OCH_2), 3.68 (m, 4H, H-2'''', H-5''', H-6a'''', H-6b''), 3.63 (m, 4H, H-3''', H-4", H-4''', H-6b''''), 3.54 (m, 1H, OCH_2), 3.45, 3.40 (m, 2H, CH_2NH), 3.28 (dd, 1H, $J_{1'',2''}$ 3.5 Hz, $J_{2'',3''}$ 10.0 Hz, H-2'''), 1.25 (m, 6H, 2×C-CH_3), 1.20 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 166.1, 165.7, 165.6 (3×*C*OPh), 156.3 (*C*OOCH$_2$Ph), 139.2, 138.7, 138.4, 138.2, 138.0 (2), 137.8 (2), 137.6, 137.5, 136.9, 136.3, 133.1, 133.0, 132.9, 130.2, 130.1, 129.9, 129.8 (3). 129.7 (2), 129.1, 128.7, 128.5 (4), 128.4 (8), 128.3 (8), 128.2 (10), 128.1 (3), 128.0, 127.9 (4), 127.8 (8), 127.7 (2), 127.6 (4), 127.5 (2), 127.4, 127.1, 126.4 (2), 126.0 (Ar*C*), 102.0 (*C*HPh), 101.4 (C-1'), 99.3 (C-1), 98.2 (C-1"), 96.7 (C-1''''), 96.4 (C-1'''), 94.1 (C-1'''), 83.1, 82.0, 80.6, 79.7, 76.6, 76.5, 75.8, 75.7 (2), 75.6 (2), 75.0, 74.8, 74.4, 74.2 (2), 74.1, 73.4, 73.3, 73.2 (2), 73.1, 72.3, 72.0, 71.9 (2), 71.8, 71.3, 71.1, 70.9, 69.1, 68.8, 67.6, 67.2, 67.0, 66.9, 40.8 (*C*H$_2$NH), 17.9 (C-*C*H$_3$), 17.7 (2×C-*C*H$_3$). HRMS calcd for $C_{137}H_{140}N_4NaO_{33}$ $(M+Na)^+$:2391.9298, found 2391.9295.

2-(benzyloxycarbonyl)-aminoethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-galactopyranosyluronic acid-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzoyl-3-*O*-benzyl-α-L-rhamnopyranoside (24):

Compound **23** (387 mg, 0.2 mmol) was dissolved in a biphasic-mixture of CH_2Cl_2-H_2O (1.5:1; 25 mL) followed by addition of TEMPO (21.0 mg, 0.1 mmol) and iodosobenzene diacetate (IBDA) (343 mg, 1.1 mmol). The reaction mixture was vigorously stirred at 5 ºC for 8 hours till the TLC (*n*-hexane-EtOAc; 1:3) showed complete consumption of the starting material to a slower moving spot. 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 crude oxidized residue that was directly treated with 80% AcOH (15 mL) at 80 ºC for 4 hours to selectively cleave the benzylidene acetal protection. After the starting material was fully consumed (TLC in *n*-hexane-EtOAc; 1:6) and a slower moving spot was generated, the solvent was evaporated and co-evaporated with toluene. The crude diol residue thus obtained was dissolved in CH_3COSH (10 mL) and allowed to stir at room temperature for 48 hours until TLC (*n*-hexane-EtOAc; 1:9) showed complete consumption of the starting material. Solvents were removed under reduced pressure followed by purification of the crude compound using flash chromatography (*n*-hexane-EtOAc; 1:9) to furnish the pure hexasaccharide derivative **24** (266 mg, 71%) as colorlessfoam.$[\alpha]_D^{25}$ = +106° (*c*0.8, $CHCl_3$).8.06-7.11 (m, 65H, Ar*H*), 5.35 (m, 2H, H-4, H-4′), 5.24 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1''''), 5.13 (m, 3H, H-1''''', COOCH_2Ph), 5.04 (m, 2H, H-1′, H-1'''), 5.04, 4.74 (ABq, 2H, 11.5 Hz, CH_2Ph), 4.98 (d, 1H, $J_{1'',2''}$ < 1.0 Hz, H-1"), 4.96, 4.92 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.90-4.80 (m, 4H, 2×CH_2Ph), 4.82 (m, 1H, H-1), 4.68-4.52 (m, 6H, 3×CH_2Ph), 4.49 (m, 1H, H-2'), 4.50, 4.46 (ABq, 2H, J_{AB} 11.0 Hz, CH_2Ph), 4.38 (m, 2H, H-3", H-3'''), 4.42, 4.32 (ABq, 2H, J_{AB} 12.0 Hz, CH_2Ph), 4.29 (m, 2H, H-5''', H-5''''), 4.23 (m, 2H, H-2'''', H-3''''), 4.12 (m, 2H, H-3', H-4''''), 4.04 (m, 1H, H-2"), 4.01-3.93 (m, 5H, H-2, H-4''''', H-5, H-6a''''', H-6b''''), 3.89 (m, 2H, H-3, H-5"), 3.82 (m, 3H, H-2''''', H-5', H-6a'''), 3.76-3.69 (m, 5H, OCH_2, H-3'''', H-4'''', H-5'''', H-6b'''), 3.61 (t, 1H, $J_{3'',4''}$, $J_{4'',5''}$ 9.5 Hz, H-4"), 3.54 (m, 2H, H-4''', OCH_2), 3.47, 3.41 (m, 2H, CH_2NH), 2.86 (dd, 1H, $J_{1'',2''}$ 3.0 Hz, $J_{2'',3''}$ 10.0 Hz, H-2'''), 1.94 (s, 3H, NHCOCH_3), 1.25 (d, 3H, $J_{5'',6''}$6.5 Hz, C-CH_3), 1.21 (d, 3H, $J_{5',6'}$ 6.0 Hz, C-CH_3), 1.19 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH_3).^{13}C NMR ($CDCl_3$, 125 MHz) δ: 177.7 (*C*OOH), 170.6 (NH*C*OCH$_3$), 166.1, 166.0, 165.6 (3×*C*OPh), 156.3 (*C*OOCH$_2$Ph), 138.4, 138.3 (2), 137.9 (4), 137.7, 137.6, 136.6, 133.4, 133.1, 133.0, 130.0, 129.9 (4), 129.8, 129.7 (2), 129.0 (2), 128.6 (8), 128.5 (6), 128.4 (8), 128.2 (8), 128.1 (2), 128.0 (8), 127.9 (6), 127.8 (6), 127.6 (2), 127.5 (Ar*C*), 101.4 (2) (C-1', C-1"), 99.3 (C-1), 98.7 (C-1''''), 95.8 (C-1'''), 93.5 (C-1'''), 82.4, 82.0, 80.4, 79.4, 78.0, 76.3 (2), 75.8, 75.7 (2), 75.4, 75.1, 75.0, 74.8, 74.6 (2), 74.4, 73.4 (2), 73.2, 72.6, 72.1 (2), 71.9, 71.7, 71.4, 71.2, 69.6, 68.7, 67.6, 67.2, 67.0, 66.9, 63.2, 62.4, 62.0, 61.4, 40.8 (*C*H$_2$NH), 21.1 (NHCO*C*H$_3$), 18.0, 17.7, 17.5 (3×C-*C*H$_3$). HRMS calcd for $C_{132}H_{140}N_2NaO_{34}$ $(M+Na)^+$:2319.9185, found 2319.9182.

2-amino β-D-glucopyranosyl-(1→3)-α-D-galactopyranosyluronic acid-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (1):

A solution of the pure hexasaccharide (223 mg, 0.1 mmol) derivative **24** in NaOMe (0.05 M solution in MeOH; 20 mL) was stirred at room temperature for 8 hours to remove all the ester protections. The reaction mixture was neutralized with Dowex 50W X8 H^+ resin, filtered and evaporated *in vacuo*. The crude product 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.Thehydrogenolysis of the benzyl groups was complete after 3 such cycles as evident from mass spectrometry. This process also removed the NHCBz protection liberating the free amino group.Finally, the residue 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** (64 mg, 63%) as white amorphous mass. $[\alpha]_D^{25}$ = +76° (*c*0.5, MeOH). Partial ^{1}H NMR (MeOD, 500 MHz) δ: 5.12 (d, 1H, $J_{1',2'}$ < 1.0 Hz, H-1'), 5.06(d, 1H, $J_{1''',2'''}$ 3.0 Hz, H-1'''''), 4.97(d, 1H, $J_{1,2}$ <1.0 Hz, H-1), 4.95 (d, 1H, $J_{1'',2''}$ < 1.0 Hz, H-1"), 4.91 (d, 1H, $J_{1'''',2''''}$ 3.5 Hz, H-1''''), 4.81 (d, 1H, $J_{1''',2'''}$ 3.0 Hz, H-1'''), 1.99 (s, 3H, NHCOCH_3), 1.40, 1.36, 1.12 (3d, 9H, *J* 6.0 Hz, 3×CH_3). ^{13}C NMR (125 MHz, MeOD) δ: 177.8 (*C*OOH), 172.1 (NH*C*OCH$_3$), 101.9 (C-1'''') 101.7 (C-1''''), 100.5 (C-1'), 99.4 (C-1"), 98.2 (C-1), 97.7 (C-1'''), 80.7, 78.5, 77.3, 76.2, 75.6, 75.2, 74.8, 74.2, 73.9, 73.6, 73.3, 73.0, 72.6, 72.3, 71.9, 71.5, 71.1, 70.6, 70.2, 69.8, 69.2, 68.4, 67.4, 66.9, 65.9, 65.2, 52.6 (OCH$_2$), 41.8 (*C*H$_2$NH), 21.3 (NHCO*C*H$_3$), 18.2, 18.1, 18.0 (3×C-*C*H$_3$). HRMS calcd for $C_{40}H_{68}N_2O_{29}Na$ $(M+Na)^+$1063.3805, found 1063.3802.

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Keywords:O-antigen • total synthesis • TEMPO oxidation • glycosylation • H_2SO_4-silica

[1] (a) L. Beutin, S. Aleksic, S. Zimmermann, K. Gleier, *Med. Microbiol. Immunol.* **1994**, *183*, 13-21; (b) H. Russmann, E. Kothe, H. Schmidth, S. Franke, D. Harmsen, A. Caprioli, H. Karch, *J. Med. Microbiol.* **1995**, *42*, 404-410.

[2] I. Ørskov, F. Ørskov,B. Jann, K.Jann, *Bacteriol. Rev.* **1977**, *41*, 667-710.

[3] S. J. Cryz, E. Furer, R. Germanier, *Infect. Immun.* **1985**, *150*, 225-230.

[4] R. Follador, E. Heinz, K. L. Wyres, M. J. Ellington, M. Kowarik, K. E. Holt, N. R. Thomson, *Microb. Genomics* **2016**, *2*, e000073.

[5] (a) I. Bhaumik, A. K. Misra, *Glycoconj. J.* **2016**, *33*, 887-896; (b) T. Ghosh, A. K. Misra, *Chemistry Open* **2015**, *5*, 43-46; (c) K. B. Pal, B. Mukhopadhyay, *ChemistrySelect* **2017**, *2*, 7378-7381.

[6] A. S. Shashkov, Y. Zhang, Q. Sun, Xi. Guo, S. N. Senchenkova, A. V. Perepelov, Y. A. Knirel, *Carbohydr. Res.* **2016**, *430*, 82-84.

[7] R. Šardzik, G. T. Noble, M. J. Weissenborn, A. Martin, S. J. Webb, S. L. Flitsch, *Beilstein J. Org. Chem.* **2010**, *6*, 699-703.

[8] V. Sarkar, B. Mukhopadhyay, *Carbohydr. Res.* **2018**, *470*, 13-18.

[9] G. Zemplén, *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1254-1266.

[10] K. Aït-Mohand, C. Lopin-Bon, J. C. Jacquinet, *Carbohydr. Res.* **2012**, *353*, 33-48.

[11] J. M. Williams, A. C. Richardson, *Tetrahedron* **1967**, *23*, 1369-1378.

[12] P. A. Gent, R. J. Gigg, *Chem. Soc. Perkin Trans. 1* **1974**, 1446-1455.

[13] V. K. Rajput, B. Mukhopadhyay, *J. Org. Chem.* **2008**, *73*, 6924-6927.

[14] V. Sarkar, B. Mukhopadhyay, *RSC Adv.* **2016**, *6*, 40147-40154.

[15] (a) P. R. Verma, B. Mukhopadhyay, *Carbohydr. Res.* **2010**, *345*, 432-436. (b) A. Mitra, B. Mukhopadhyay, *Synthesis* **2015**, *47*, 3061-3066. (c) P. R. Verma, B. Mukhopadhyay,*RSC Adv.* **2013**, *3*, 201-207. (d) R. Das, B. Mukhopadhyay, *ChemistryOPEN* **2016**, *5*, 401-433.

[16] K. Takeo, K.Maki, Y. Wada, S. Kitamura, *Carbohydr. Res.* **1993**, *245*, 81-96.

[17] G. Grundler, R. R. Schmidt, *Liebigs Ann. Chem.* **1984**, *11*, 1826-1847.

[18] A. Mitra, B. Mukhopadhyay, *Carbohydr. Res.* **2016**, *426*, 1-8.

[19] S. Dasgupta, B. Mukhopadhyay, *Eur. J. Org. Chem.* **2008**, *34*, 5770-5777.

[20] M. J. Bertolini, C. P. J. Glaudemans, *Carbohydr. Res*. **1970**, *15*, 263-270.

[21] C. -H. Hsu, K. -C. Chu, Y. -S. Lin, J. -L. Han, Y. -S. Peng, C. -T. Ren, C. -Y. Wu, C. -H. Wong, *Chem. Eur. J.* **2010**, *16*, 1754-1760.

[22] B. Thollas, C. Biosset, *Synlett* **2007**,*11*, 1736-1738.

[23] M. Tatina, S. K. Yousuf, D. Mukherjee, *Org. Biomol. Chem.* **2012**, 10, 5357-5360.

[24] Y. Oikawa, T. Yoshioka, O. Yonemitsu, *Tetrahedron Lett.* **1982**, *23*, 885-888.

[25] Y.Zhao,S. Niu, L.Huang, J.Wang, Z. Yin, Q. Li, Z. Li, *Tetrahedron* **2013**, *69*, 5022-5028.

[26] G. Ngoje, Z. Li, *Org. Biomol. Chem.* **2013**, *11*, 1879-1886.

[27] L. J.van den Bos, J. D. C. Codee, J. C.van der Toorn, T. J.Boltje, J. H. van Boom, H. S. Overkleeft, G. A. van der Marel, *Org. Lett.* **2004**, *6*, 2165-2168.

[28] Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1996**, *292*, 71-81.

[29] D. D. Perrin, W. L. Amarego, D. R. Perrin *Purifcation of Laboratory Chemicals,* Pergamon, London, **1996**.

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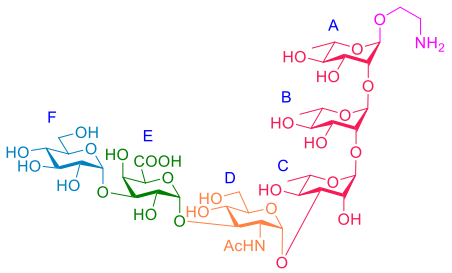
Entry for the Table of Contents

Total Synthesis



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Text for Table of Contents: A convergent synthetic route is established for the total synthesis of the hexasaccharide repeating unit of the *O*-antigen from *E. coli* O133. The desired glycosidic linkages were achieved through activation of either thioglycosides or trichloroacetimidates in stereoselective manner. The required uronic acid moiety was installed at the hexasaccharide late-stage by using TEMPO mediated oxidation of the primary hydroxyl group.