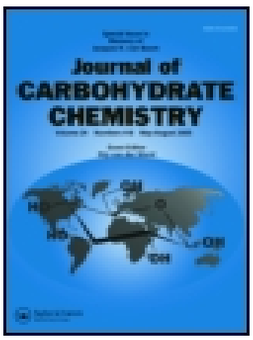

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One-pot iterative glycosylations toward a tetrasaccharide related to the *O*-specific polysaccharide from *Escherichia coli* O132

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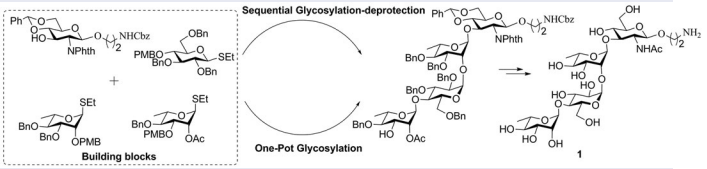
ABSTRACT

An efficient synthetic strategy has been developed for the synthesis of a tetrasaccharide related to the *O*-specific polysaccharide from *Escherichia coli* O132 as their 2-aminoethyl glycosides in a very good yield by adopting sequential glycosylation followed by *in-situ* removal of the *p*-methoxybenzyl (PMB) group in the same reaction pot. Furthermore, the synthetic route was adapted by carrying out three stereoselective iterative glycosylations followed by in situ removal of the PMB group in one pot. The stereochemical outcomes of all the glycosylation steps were excellent with satisfactory yields.

GRAPHICAL ABSTRACT



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KEYWORDS
Escherichia coli; oligosaccharide; one-pot; glycosylation; H_2SO_4-silica

Introduction

Escherichia coli is a facultative Gram-negative bacterium that presents predominantly in the guts of human and other warmblooded animals.[1,2] Although most of the *E. coli* strains are usually harmless and beneficial to the host's body, there are some *E. coli* variants having virulence factors and contributing to a variety of infections in humans and animals.[3,4] These pathotypes cause mainly three type of diseases, i.e., enteric/diarrheal disease, sepsis/meningitis and urinary tract infections (UTIs), and are also

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→2)-α-D-Gal*f*-(1→3)-α-L-Rha*p*2Ac-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→

Figure 1. Structure of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O132.

responsible for losing of life for more than two million humans per year due to both intraintestinal and extraintestinal diseases.[5,6]

Being a major component of the cell wall of Gram-negative bacteria, the *O*-antigens often play important roles during host infections, subsequent immune responses in the host and in controlling its virulence property. The virulent *E. coli* strains causing diarrhea are classified into six subgroups based on their mechanisms of action, which include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), and enterohaemorrhagic *E. coli* (EHEC).[7]

Recently, Knirel et al.[8] reported the structure of the *O*-polysaccharide repeating unit (*O*-unit) of *E. coli* O132. It is a Shiga toxin-producing *E. coli*, and its pathogenic strain is reported to be the causative agent for septicemia in chicken,[9] diarrhea in children under three years of age,[10] diarrhea in calves and rabbits.[11,12] The structure of *E. coli* O132 comprises a linear repeating unit of pentasaccharide (Fig. 1), which consists of a D-glucosamine (GlcNAc*p*), two L-rhamnose (Rha*p*), a D-glucose (Glc*p*), and a D-galactofuranose (Gal*f*) sugar residues.

The recent thrust in the drug discovery program is to develop newer approaches to control bacterial infections due to the emergence of multi-drug resistant bacterial strains. It is well recognized that bacterial antigenic character regulates the immunochemical activity of glycoconjugate vaccines, making them more attractive targets for the design of potential vaccine candidates.[13] For the study of the involvement of these *O*-antigens in the pathogenicity and the immunological properties, desirable amount of the *O*-polysaccharide is required. Whereas the *O*-antigen from naturally occurring biological sources is difficult to isolate and very expensive to produce appropriate quantities of material with sufficient purity. Subsequently, chemical synthetic strategies for the preparation of oligosaccharides as well as their glycoconjugate derivatives are beneficial to get significant quantity of pure compounds.

The stepwise oligosaccharide syntheses demand extensive protecting group manipulation and purification after each step making them expensive, time consuming, and tedious. The oligosaccharide synthesis related to the *O*-antigen of *E. coli* O132 repeating unit was reported only by Mukhopadhyay et al.[14] using a [3 + 2] convergent strategy. However, the introduction of an expeditious, step-economic, one-pot total synthesis is necessary to be relatively environmentally friendly and cost-effective. We report herein a concise one-pot synthesis of the tetrasaccharide fragment **1**

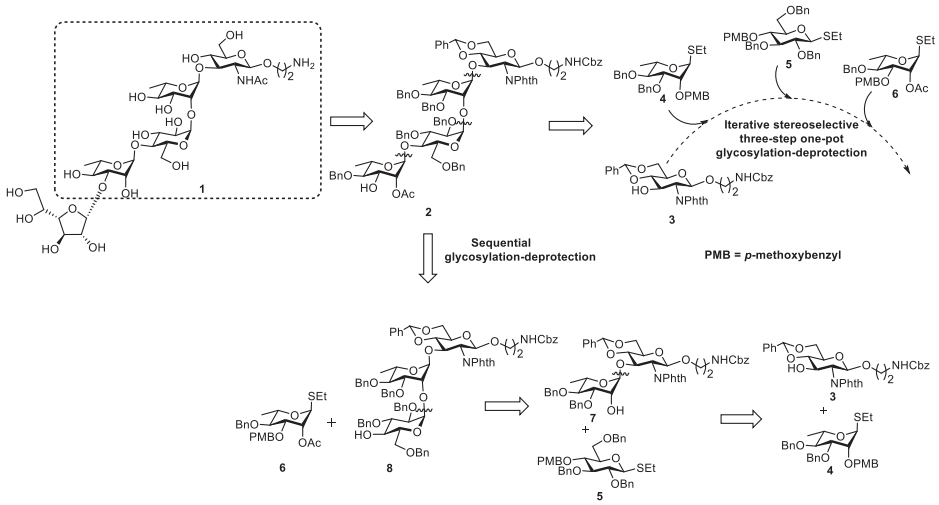


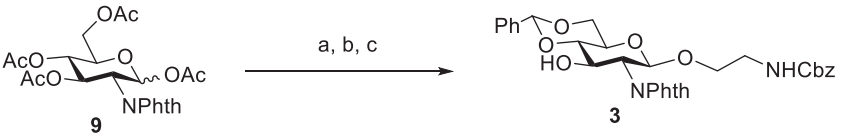
Figure 2: Retrosynthetic strategy for target tetrasaccharide **1** as its 2-aminoethyl glycoside and related building blocks.

(Fig. 2), which is part of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O132. It was achieved by using a linear and one-pot iterative glycosylations approach. The 2-aminoethyl moiety was applied as a protecting group at the reducing end, which will facilitate further glycoconjugate formation without hampering the stereochemistry of the anomeric center. Its terminally free amine can provide glycoconjugates to elucidate further biological implications of the *O*-antigen and vaccine potential.

Results and discussion

Tetrasaccharide **1** as its 2-aminoethylglycoside could be obtained from protected tetrasaccharide **2** through global deprotection. The retrosynthetic disconnection of tetrasaccharide **2** revealed that it could be prepared either via a sequential glycosylation-deprotection technique or by one-pot sequential glycosylation. The sequential disconnection approach indicates that protected tetrasaccharide **2** can be achieved by stereoselective glycosylation of trisaccharide acceptor **8** with rhamnosyl donor **6**, whereas trisaccharide **8** can be prepared with thioglucosyl donor **5** and disaccharide acceptor **7**, which is achievable with monosaccharide acceptor **3** and rhamnosyl donor **4** (Fig. 2).

We have designed to have all the thioglycoside donors with a suitable *in situ* removable protecting group like *p*-methoxybenzyl (PMB) group. This serves two purposes. Firstly, most glycosylation reactions rely on the same activation methods, and secondly, *in-situ* removable of the PMB group by increasing the temperature of the same reaction mixture, which can reduce the number of reaction steps by providing glycosyl acceptor. This was



Scheme 1. Synthesis of glycosyl acceptor **3**. ***Reagents and conditions:*** (a) $HO(CH_2)_2NHCbz$ (Cbz = benzyloxycarbonyl), $BF_3{\cdot}OEt_2$, $(CH_2Cl)_2$, 55 °C, 12 h, 89%; (b) CH_3ONa, CH_3OH, rt, 1 h; (c) $PhCH(OCH_3)_2$, *p*-TsOH, DMF, rt, 12 h, 82% over two steps.

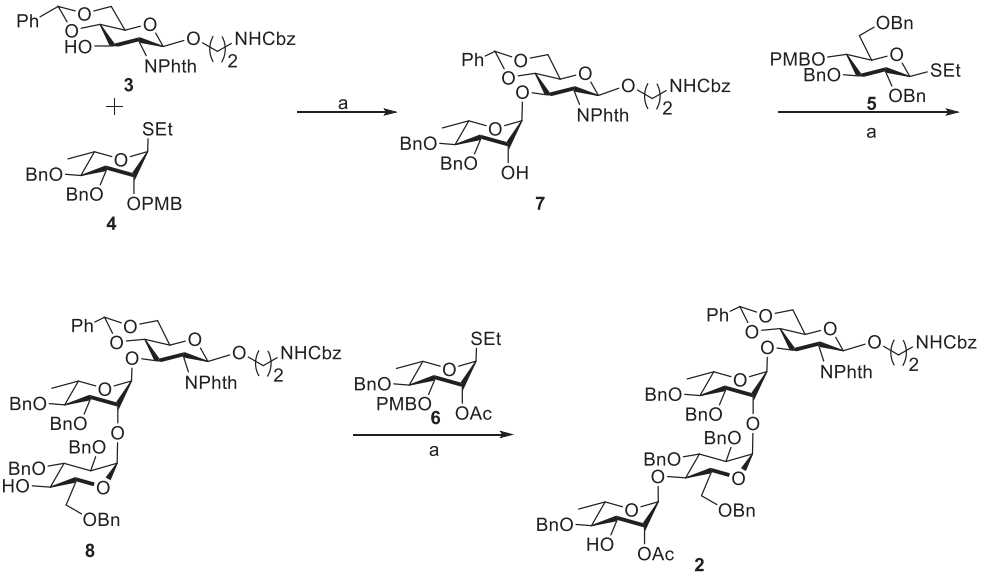
achieved by using a combination of *N*-iodosuccinimide (NIS) and silica-supported sulfuric acid (H_2SO_4-SiO_2) as the promoter.[15,16] Here, H_2SO_4-silica has been used successfully as a noncorrosive solid acid promoter for all glycosylation reaction.

One-pot sequential glycosylation was achieved from the same suitable protected monosaccharide building blocks, **3**[17], **4**[18], **5**[19] and **6**,[20] derived from commercially available D-glucosamine hydrochloride, L-rhamnose, and D-glucose (Fig. 2). For each pathway, a retro-synthetic analysis of the fully protected tetrasaccharide derivative **2** led to common building blocks.

Firstly, 2-(benzyloxycarbonylamino)ethyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside **3** was prepared from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside **9**[21] in a 76% yield using a three-step reaction sequence involving glycosylation with 2-N-carboxybenzylamino ethanol with the promotion of boron trifluoride-diethyl ether ($BF_3{\cdot}OEt_2$) and then deacetylation using sodium methoxide and benzylidene acetal formation using benzaldehyde dimethylacetal[22] in the presence of *p*-toluenesulfonic (Scheme 1).

Synthesis of 2 via a sequential glycosylation-deprotection strategy

The stereoselective glycosylation of acceptor **3** with L-rhamnosyl thioglycoside donor **4** in the presence of NIS and H_2SO_4-silica[15,16] followed by removal of the PMB group[23] from the product in the same pot by tuning the reaction conditions led to the formation of disaccharide **7** in an 82% yield (Scheme 2). The quantity of H_2SO_4-silica present in the reaction mixture was very low, which allowed the selective deprotection of the highly acid labile PMB group without affecting the benzylidene acetal in the molecule. The stereochemical outcome of the newly formed glycosidic linkage was confirmed by NMR spectroscopic analysis [appearance of signals at δ 5.52 (s, 1 H, PhC*H*), 5.26 (d, $J = 8.5$ Hz, 1 H, H-1_A), 4.60 (d, $J = 1.6$ Hz, 1 H, H-1_B) in ^{1}H NMR spectrum, and δ 102.0 (PhCH), 99.5 (C-1_B), 98.9 (C-1_A) in the ^{13}C NMR spectrum]. Although the C-2 position of the L-rhamnosyl donor **4** was protected with a nonparticipating PMB group, the



Scheme 2. Synthesis of tetrasaccharide **2** by a sequential glycosylation-deprotection strategy. ***Reagents and conditions:*** (a) NIS, H_2SO_4-silica, CH_2Cl_2/Et_2O (2:1), MS 4 Å, −30 °C, 30 min, then 0 °C, 30 min, 82% for compound **7**, 71% for compound **8**, and 77% for compound **2**.

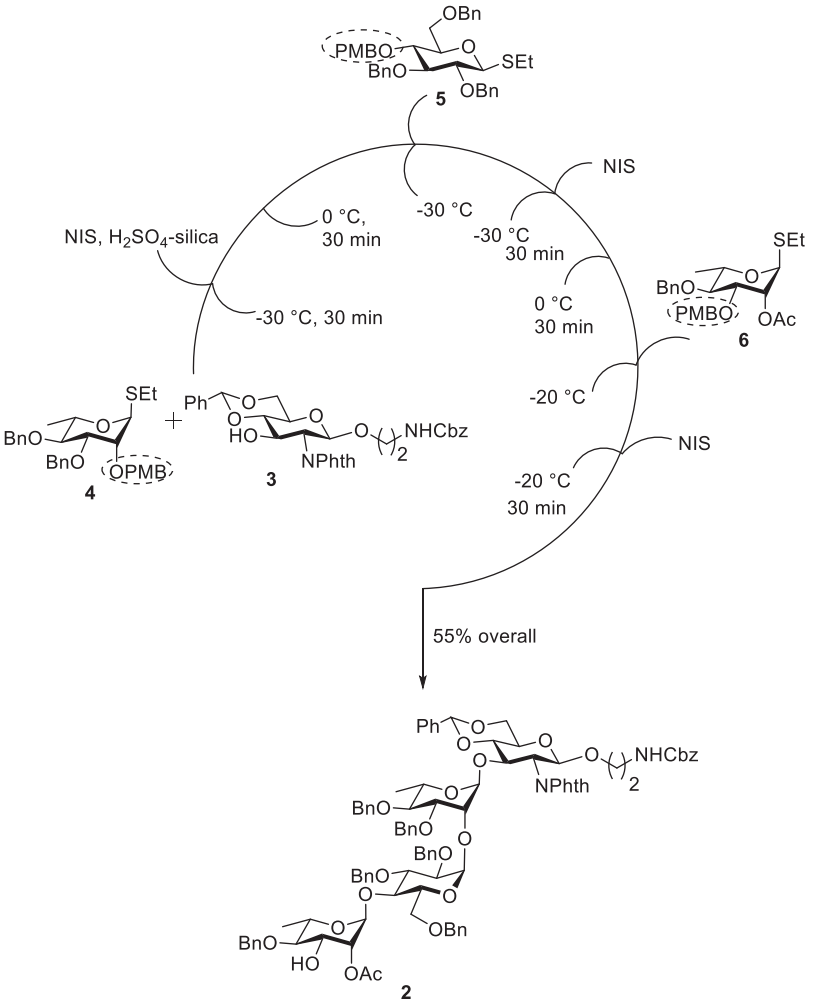
glycosylation reactions furnished exclusively 1,2-*trans*-glycosidic linkages under the control of anomeric effect.[24] Subsequently, the disaccharide acceptor **7** was reacted with 4-*O*-PMB protected thioglycoside donor **5** in the presence of NIS and H_2SO_4-silica[15,16] followed by removal of the PMB group from the product in the same pot by raising the temperature of the reaction mixture to furnish trisaccharide **8** in a 71% yield (Scheme 2) together with a minor quantity (∼1%) of its β-isomer, which was separated by column chromatography. Gratifyingly, compound **8** was obtained in satisfactory yield with good stereochemical outcome. The reaction temperature and promoter combination might have an influence on the stereochemical outcome of the glycosylation. Compound **8** formation was confirmed by spectroscopic study [signals at δ 5.29 (d, $J = 8.5$ Hz, 1 H, H-1_A), 4.56 (d, $J = 1.6$ Hz, 1 H, H-1_B), 4.01 (d, $J = 2.4$ Hz, 1 H, H-1_C) in ^{1}H NMR spectrum and δ 98.8 (C-1_A), 97.8 C-1_B), 96.2 (C-1_C) in ^{13}C NMR spectrum, respectively]. The stereoselective glycosylation of trisaccharide acceptor **8** with compound **6** followed by the removal of the PMB group from the intermediate products was repeated by the same protocols to furnish tetrasaccharide **2** in a 77% yield (Scheme 2). The structure of compound **2** was confirmed by spectroscopic data [signals at δ 5.27 (d, $J = 8.52$ Hz, 1 H, H-1_A), 4.79 (brs, 1 H, H-1_D), 4.60 (brs, 1 H, H-1_B), 3.94

(d, $J = 3.8$ Hz, 1 H, H-1_C) in the ^{1}H NMR spectrum and δ 98.0 (C-1_A), 97.0 (C-1_D), 96.9 (C-1_B), 93.6 (C-1_C) in the ^{13}C NMR spectrum, respectively].

Synthesis of 2 via one-pot iterative glycosylations

Having obtained the protected tetrasaccharide **2** in a minimum number of steps and a very good yield by the sequential synthetic glycosylation strategy, we decided to explore the possibility of achieving compound **2** by carrying out three iterative glycosylation steps and *in situ* removal of the temporary protecting PMB group from the glycosylation products in the same flask. For this purpose, temporary protecting groups that are sensitive to acidic conditions, e.g., TBS,[25] PMB,[26] and TBDPS,[27] are typically employed for glycosylation – deprotection – glycosylation in one-pot.[28] For example, Hung and co-workers[29] employed a TBS[26] protecting group as a temporary masking group for one-pot glycosylation. Similarly, Nilsson and co-workers[26] employed PMB ether as an *in situ* removable protecting group in the one-pot glycosylation. In that case, glycosylations were usually conducted at subzero temperatures under acidic conditions. The temporary protecting group is removed postglycosylation either by warming up the reaction mixture or by adding more acid such as TfOH, TMSOTf, or $BF_3{\cdot}Et_2O$.

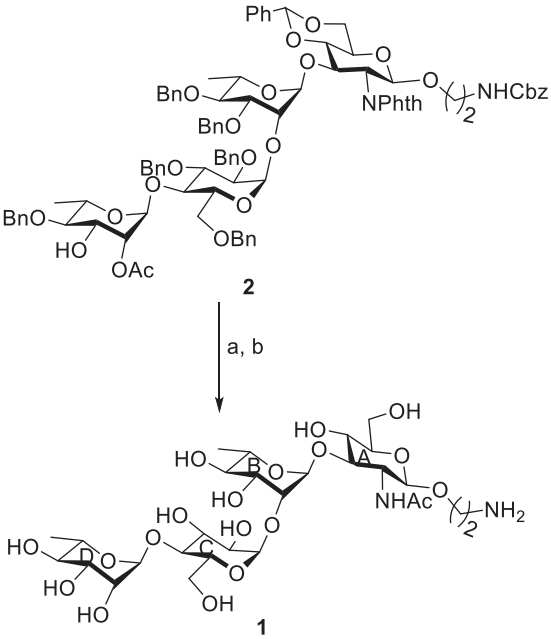
Here, we explored the synthesis of tetrasaccharide **2** via a one-pot glycosylation technique using the same building-blocks as above. For this purpose, compound **3** was first glycosylated with 2-PMB protected L-rhamnosyl thioglycoside donor **4** via the previously standardized procedure using NIS and H_2SO_4-silica as the promoter in dichloromethane-diethyl ether at −30 °C for 30 min to consume all of the donor **4** as indicated by TLC. Once the donor **4** was completely consumed, the temperature of the reaction mixture was raised to 0 °C for *in-situ* removal of *p*-methoxybenzyl ether in 30 min according to TLC results, and produced the desired disaccharide acceptor. The reaction mixture was again cooled to −30 °C, and to this cold reaction mixture, 4-PMB protected glucothioglycoside donor **5** and fresh NIS was added with the reaction kept at that temperature for 30 min similar as previously standardized procedure. Then, the temperature of reaction mixture was raised again to 0 °C for 30 min for *in-situ* removal of *p*-methoxybenzyl ether group, resulting in the desired trisaccharide acceptor. Thereafter, the same reaction vessel was again cooled to −30 °C, and 3-PMB protected L-rhamnosyl thioglycoside donor **6** in dry dichloromethane-diethyl ether solution was injected into the cold reaction vessel together with fresh NIS. The reaction was allowed at this temperature for 30 min, which followed by rising the temperature to 0 °C for 30 min for *in-situ* removal of *p*-methoxybenzyl ether group to produce the desired



Scheme 3. Synthesis of tetrasaccharide **2** via a one-pot glycosylation-deprotection-glycosylation technique.

tetrasaccharide **2**. Eventually, we got the target tetrasaccharide **2** in a 55% overall yield via the one-pot glycosylation strategy (Scheme 3). Compound **2** formation was confirmed by NMR spectroscopy. Carrying out the glycosylation-deprotection reactions in a one pot setup significantly reduced the number of purification steps. The PMB ether acted as an *in-situ* removable temporary protecting group for the hydroxyl functionality for next glycosylation reaction. Important advantages of our one-pot glycosylation strategy are that only one class of glycosyl donors (simple and stable thioglycosides) and one promotor system (NIS/H_2SO_4-silica) are required.

Finally, tetrasaccharide **2** was subjected to a series of deprotection reactions, (a) elimination of the phthalimido group through hydrazinolysis,[30] followed by *N*-acetylation and *O*-deacetylation with sodium methoxide[31]



Scheme 4. Synthesis of the target compound **1**. ***Reagents and conditions:*** (a) (i) $NH_2NH_2{\cdot}H_2O$, C_2H_5OH, 80 °C, 6 h; (ii) acetic anhydride, pyridine, rt, 2 h; (iii) NaOMe/MeOH; (b) H_2, 20% $Pd(OH)_2$- C, CH_3OH, rt, 24 h, 56% overall yield.

and (b) removal of all benzyl groups through hydrogenolysis using Pearlman's catalyst,[32] to furnish the desired free tetrasaccharide **1** in an overall yield of 56% (Scheme 4). Compound **1** has been confirmed by its spectral data and its anomeric protons have been identified at δ **5.10 (d, *J* = 7.7 Hz, 1 H, H-1$_A$), 4.92 (brs, 1 H, H-1$_B$) and 4.56 (brs, 2 H, H-1$_D$, H-1$_C$)**) ppm, and their corresponding carbon signals are at **102.8 (C-1$_A$), 101.9 (C-1$_D$), 100.4 (C-1$_B$), and 98.4 (C-1$_C$)** ppm, respectively (See Supporting information).

Conclusions

In summary, we have developed an expeditious synthetic strategy for a tetrasaccharide related to the *O*-antigen of *E. coli* O132 as its 2-aminoethyl glycoside (**1**) through one-pot glycosylation-deprotection and sequential glycosylation-deprotection techniques. The synthetic strategy relies on *in-situ* removable PMB ether that serves as a temporary protecting group for hydroxyl functionality undergoing glycosylation reaction in one pot, which greatly reduced the number of product purification steps. A general iodonium ion-mediated glycosylation condition was applied to all glycosylation steps.

Experimental section

General methods

All reactions were monitored by using a thin layer chromatography (TLC) over silica gel-coated TLC plates. The spots on TLC were visualized with UV when there is an active UV group or by warming ceric sulfate [2% $Ce(SO_4)_2$ in 5% H_2SO_4 in EtOH]-sprayed plates on a hot plate if there is no UV active moiety. The spots on TLC were also confirmed by the latter method even when there is an active UV group. Silica gel (230–400 mesh) was used for purification by column chromatography. All NMR spectra, ^1H and ^{13}C NMR, DEPT 135, 2 D COSY and HSQC, were recorded on Brucker DPX 400 MHz spectrometers using $CDCl_3$ and D_2O as solvents and TMS used as an internal reference unless stated otherwise. Chemical shift values are represented in δ ppm. ESI-MS data were recorded on a JEOL spectrometer. Optical rotations were determined on an Autopol III polarimeter. IR spectra were recorded on Shimadzu Spectrophotometers. Commercially available grades of organic solvents of adequate purity were used in all reactions.

Preparation of H_2SO_4-silica

To a 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 for uniformly acidic strength. Then the solvent was evaporated under reduced pressure resulting in free-flowing H_2SO_4-silica, which was dried at 110 °C for 3 h and used for the reactions.

2-(Benzyloxycarbonylamino)ethyl 4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (3)

To a solution of compound **9** (5 g, 10.4 mmol) in DCE (100 mL) were added 2-N-carboxybenzylamino ethanol (2.5 g, 12.6 mmol) and $BF_3{\cdot}Et_2O$ (1.8 mL, 12.6 mmol) at 0 °C and and the reaction mixture was allowed to stir at 55 °C for 12 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (2:1) as eluant to give pure compound. To a solution of the pure compound in 0.1 M CH_3ONa (50 mL) was allowed to stir at room temperature for 1 h. The re-action mixture was neutralized with Amberlite-IR 120 (H^+) resin,filtered, and concentrated. To a solution of the deacetylated product (3.9 g, 8.16 mmol) in anhydrous DMF (20 mL) were added benzaldehyde dimethyl acetal (1.40 mL, 9.24 mmol) and *p*-TsOH (300 mg, 1.6 mmol), and the reaction

mixture was allowed to stir at room temperature for 12 h. The reaction mixture was neutralized with Et_3N (1.5 mL) and evaporated to dryness under reduced pressure. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (1:1) as eluant to give pure compound **3** (3.7 g, 82%) as semi solid, $[\alpha]_D^{25}$ −30 (*c* 1.0, $CHCl_3$); IR (KBr): 3400, 3020, 1716, 1389, 1215, 1084, 761, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.82–7.79 (m, 2 H, Ar-H), 7.66–7.65 (m, 2 H, Ar-H), 7.50–7.48 (m, 2 H, Ar-H), 7.39–7.31 (m, 6 H, Ar-H), 7.29–7.26 (m, 2 H, Ar-H), 5.55 (s, 1 H, PhC*H*), 5.27 (d, $J = 8.5$ Hz, 1 H, H-1), 5.01–4.90 (m, 3 H, N*H*, COOCH_2Ph), 4.65–4.60 (m, 1 H, H-6_A), 4.36 (dd, $J = 4.2$, 10.2 Hz, 1H, H-6_B), 4.23 (dd, $J = 8.5$, 10.5 Hz, 1H, H-2), 3.82–3.78 (m, 2 H, H-4, –OCH_2), 3.66–3.57 (m, 3 H, H-3, H-5, –OCH_2), 3.28-3.27 (m, 2 H, NCH_2), 2.57 (brs, 1 H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 168.1 (*C*OPhth), 156.3–123.6 (Ar-C), 101.9 (Ph*C*H), 99.1 (C-1), 82.0 (C-3), 69.1 (-O*C*H_2), 68.6 (2 C, C-6, C-4), 66.6 (NHCOOCH_2Ph), 66.2 (C-5), 56.6 (C-2), 40.8 (N*C*H2-); HRMS (ESI-TOF) calcd for $C_{31}H_{31}N_2O_9^+$ $[M+H]^+$ 575.2024, found 575.2020.

2-(Benzyloxycarbonylamino)ethyl (3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (7)

To a solution of compound **3** (2.5 g, 4.35 mmol) and thioglycoside donor **4** (2.93 g, 5.22 mmol) in anhydrous CH_2Cl_2/Et_2O (2:1 v/v; 10 mL) was added flame-activated MS-4 Å (1.5 g) and the reaction mixture was cooled to −30 °C under N_2. To the cold reaction mixture was added NIS (1.4 g, 6.25 mmol) followed by H_2SO_4-SiO_2 (30 mg), and the reaction mixture was stirred at the same temperature for 30 min. After complete consumption of compound **4** [TLC; hexane/EtOAc 2:1], the reaction mixture was allowed to stir at 0 °C for 30 min, filtered and washed with CH_2Cl_2 (30 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$ (25 mL), satd. $NaHCO_3$ (25 mL), and H_2O (25 mL), dried (Na_2SO_4), and concentrated to give the crude product, which was purified on SiO_2 using hexane/EtOAc 4:1 as eluent to give pure compound **7** (3.5 g, 82%) as a colorless oil. $[\alpha]_D^{25}$ +18 (*c* 1.0, $CHCl_3$); IR (neat): 3401, 2926, 1713, 1386, 1219, 1090, 769 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.83–7.81 (m, 2 H, Ar-H), 7.67–7.66 (m, 2 H, Ar-H), 7.50–7.48 (m, 2 H, Ar-H), 7.38–7.25 (m, 16 H, Ar-H), 7.22–7.20 (m, 2 H, Ar-H), 5.52 (s, 1 H, PhC*H*), 5.26 (d, $J = 8.5$ Hz, 1 H, H-1_A), 5.01–4.91 (m, 3 H, N*H*, COOCH_2Ph), 4.73 (d, $J = 11$ Hz, 1 H, PhCH_2), 4.66–4.61 (m, 1 H, H-4_A), 4.60 (d, $J = 1.6$ Hz, 1 H, H-1_B), 4.56–4.46 (m, 3 H, PhCH_2), 4.37 (dd, $J = 4.16$, 10.24 Hz, 1 H, H-6_A), 4.24

(dd, $J = 8.56$, 10.2 Hz, 1 H, H-2_A), 3.95–3.88 (m, 1 H, H-5_B), 3.82–3.76 (m, 2 H, H-6_B, OC*H*2), 3.74–3.57 (m, 5 H, OCH_2, H-3_B, H-2_B, H-5_A, H-3_A), 3.27–3.20 (m, 3 H, NCH_2, H-4_B), 0.78 (d, $J = 6.1$ Hz, 3 H, CH_3);^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3 (*C*OPhth), 156.2–123.8 (Ar-C), 102.0 (PhCH), 99.5 (C-1_B), 98.9 (C-1_A), 80.7 (H-3_A), 79.9 (C-4_B), 79.7 (C-3_B), 75.0 (PhCH_2), 74.1 (C-4_A), 71.8 (2 C, PhCH_2), 69.2 (-OCH_2), 68.6 (2 C, C-6_A, C-2_B), 67.7 (C-5_B), 66.6 (NHCOOCH_2Ph), 66.5 (C-5_A), 56.7 (C-2_A), 40.8 (NCH_2-), 17.3 (*C*H_3); HRMS (ESI-TOF) calcd for $C_{51}H_{56}N_3O_{13}^+$ $[M + NH_4]^+$ 918.3808, found 918.3808.

2-(Benzyloxycarbonylamino)ethyl (2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (8)

To a solution of compound **7** (3 g, 3.32 mmol) and compound **5** (2.45 g, 3.99 mmol) in anhydrous CH_2Cl_2/Et_2O (2:1 v/v; 40 mL) was added flame-activated MS 4 Å (1.5 g) and the mixture was allowed to stir under N_2 at room temperature for 1 h. The reaction mixture was cooled to −30 °C and NIS (1.07 g, 4.78 mmol) and H_2SO_4-SiO_2 (40 mg) were added to it and stirred at same temperature for 30 min. After complete consumption of compound **5** [TLC; hexane/EtOAc 2:1], the reaction mixture was allowed to stir at 0 °C for 30 min, filtered and washed with CH_2Cl_2 (30 mL). Then the organic layer was successively washed with $Na_2S_2O_3$ (5%), $NaHCO_3$ (satd. aq.), and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (4:1) as eluent to give pure compound **8** (3.4 g, 71%) as a colorless oil. $[\alpha]_D^{25}$ +14 (*c* 1.0, $CHCl_3$); IR (neat): 3740, 3443, 3020, 2926, 1716, 1516, 1385, 1216, 1091, 766, 669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.46 (m, 4 H, Ar-H), 7.36–7.19 (m, 35 H, Ar-H), 5.53 (s, 1 H, PhC*H*), 5.26 (d, $J = 8.52$ Hz, 1 H, H-1_A), 5.00–4.89 (m, 3H, N*H*, COOCH_2Ph), 4.81–4.75 (m, 2 H, PhCH_2), 4.67–4.62 (m, 3 H, H-4_A, H-1_B, PhCH_2), 4.55–4.48 (m, 4 H, PhCH_2), 4.38–4.34 (m, 3 H, PhCH_2), 4.28–4.23 (m,1 H, H-2_A), 4.11–4.07 (m, 3 H, PhCH_2, H-1_C), 3.91–3.86 (m, 2 H, H-5_B), 3.79–3.76 (m, 3 H, OCH_2, H-4_C, H-5_C), 3.66–3.62 (m, 4 H, H-5_A, H-6_A, H-3_A, H-4_B), 3.52–3.46 (m, 1H, H-2_B), 3.38 (d, $J = 9.7$ Hz, 1 H, H-2_C), 3.27–3.24(m, 3 H, NCH_2, H-6_{abC}), 3.18–3.14 (m, 1 H, H-6_{abC}), 3.10–3.07 (m, 1 H, H-3_B), 0.90 (d, $J = 6.3$ Hz, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4 (*C*OPhth), 138.8–126.4 (Ar-C), 101.9 (PhCH), 98.9 (C-1_A), 97.4 (C-1_B), 95.1 (C-1_C), 80.6 (C-3_A) 80.5 (C-4_B), 80.0 (C-2_C), 79.1 (C-3_B), 78.4 (C-5_C), 74.9 (PhCH_2), 73.7 (C-4_A), 73.5 (PhCH_2), 73.3 (C-4_C), 71.8 (PhCH_2), 71.4 (PhCH_2), 70.8 (C-2_B), 69.8 (C-6_{aA}), 69.3 (C-6_{abC}), 69.2 (–OCH_2), 68.6 (C-5_B), 66.6 (C-5_A), 67.8 (C-2_B), 66.7 (2 C, C-5_A, NHCOOCH_2Ph), 56.6 (C-2_A), 40.8 (NCH_2-), 17.4

(CH_3); HRMS (ESI-TOF) calcd for $C_{78}H_{84}N_3O_{18}^+$ $[M+NH_4]^+$ 1350.5744, found 1350.5734.

2-(Benzyloxycarbonylamino)ethyl (2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→4)- (2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (2)

Flame-activated molecular sieves (4 Å; 1.5 g) were added to a solution of compound **8** (3 g, 2.25 mmol) and compound **6** (1.24 g, 2.70 mmol) in CH_2Cl_2/Et_2O (2:1 v/v; 50 mL), and the reaction mixture was stirred at room temperature under N_2 for 1 h. The reaction mixture was cooled to −25 °C, and NIS (0.73 g, 3.23 mmol) and H_2SO_4-SiO_2 (30 mg) were added. The reaction mixture was stirred at the same temperature for 30 min, then the temperature was raised to 0 °C, and it was stirred at 0 °C for another 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with $Na_2S_2O_3$ (5%), $NaHCO_3$ (satd. aq.), and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (3:1) as eluent to give pure compound **2** (2.9 g, 77%) as a colorless oil; $[\alpha]_D^{25}$ +24 (*c* 1.0, $CHCl_3$); IR (neat): 3395, 3020, 1392, 1215, 1044, 761, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.51–7.49 (m, 2 H, Ar-H), 7.36–7.21 (m, 37 H, Ar-H), 7.18–7.14 (m, 5 H, Ar-H), 5.54 (s, 1 H, PhC*H*), 5.27 (d, $J = 8.52$ Hz, 1 H, H-1_A), 5.01–4.89 (m, 4 H, N*H*, COOCH_2Ph, H-4_A), 4.84-4.80 (m, 4 H, PhCH_2, H-2_D), 4.79 (brs, 1 H, H-1_D), 4.72-4.51 (m, 6 H, PhCH_2, H-1_B), 4.48–4.34 (m, 4 H, PhCH_2, H-5_D), 4.26 (dd, $J = 8.7$, 10.0 Hz, 1 H, H-2_A), 4.06–4.00 (m, 2 H, PhCH_2, H-5_B), 3.95–3.88 (m, 4 H, H-1_C, H-2_B, H-3_D, H-3_B), 3.80–3.76 (m, 5 H, OCH_2, H-4_C, H-5_C, H-3_C), 3.67–3.60 (m, 4 H, H-5_A, H-6_{abA}, H-2_C), 3.46–3.41 (m, 1 H, H-4_B), 3.27–3.18 (m, 5 H, NCH_2, H-3_A, H-6_{abC}), 3.10–3.06 (m, 1 H, H-4_D), 2.04 (s, 3 H, COCH_3), 0.91 (d, $J = 6.2$ Hz, 3 H, CH_3), 0.89 (d, $J = 6.2$ Hz, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (*C*OCH$_3$), 167.9, 167.7 (*C*OPhth), 156.2–126.3 (Ar-C), 101.8 (PhCH), 98.0 (C-1_A), 97.0 (C-1_D), 96.9 (C-1_B), 93.6 (C-1_C), 82.1 (C-3_A), 80.5 (C-4_D), 80.1 (2 C, C-4_B, C-5_C), 78.9 (C-2_C), 78.4 (C-3_D), 75.0 (2 C, C-3_B, Ph*C*H$_2$), 74.4 (C-3_C), 73.7 (PhCH$_2$), 72.9 (PhCH$_2$), 72.8 (2 C, PhCH$_2$), 72.2 (2 C, C-2_D, C-4_C), 72.1 (2 C, C-4_A, PhCH$_2$), 71.5 (C-5_D), 69.8 (C-5_B), 69.2 (C-6_A), 68.6 (C-2_B), 68.1 (-OCH$_2$), 67.7 (C-6_{aBC}), 66.6 (2 C, C-5_A, NHCOOCH$_2$Ph), 56.6 (C-2_A), 40.8 (NCH$_2$-), 17.6 (*C*H$_3$); HRMS (ESI-TOF) calcd for $C_{93}H_{99}N_2O_{23}^+$ $[M+H]^+$ 1611.6633, found 1611.6628.

2-(Benzyloxycarbonylamino)ethyl (2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→4)-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (2). (One-Pot procedure)

A mixture of **3** (300 mg, 0.52 mmol), **4** (232.0 mg, 0.57 mmol), and flame-activated 4 Å molecular sieves were stirred anhydrous CH_2Cl_2/Et_2O (2:1 v/v; 20 mL) for 1 h at room temperature under N_2 atmosphere. The reaction mixture was cooled to −30 °C, and NIS (142 mg, 0.63 mmol) and H_2SO_4-SiO_2 (100 mg) were added in succession. After 30 min, the total consumption of both starting materials was observed (checked by TLC). Then the reaction mixture was slowly raised to 0 °C, and the mixture was stirred for 30 min. The temperature of the reaction mixture was again lowered to −30 °C, and the donor **5** (369 mg, 0.60 mmol) and NIS (148 mg, 0.66 mmol) were added to the same vessel. The reaction mixture was stirred at the same temperature for 30 min. After full consumption of starting materials (checked by TLC), the reaction mixture was slowly raised to 0 °C, and the mixture was stirred for 30 min. Then the reaction mixture was slowly brought to −30 °C and thioglycoside donor **6** (164 mg, 0.36 mmol) in dry CH_2Cl_2/Et_2O (5 mL) and NIS (88 mg, 0.39 mmol) was added. The reaction was completed within 30 min (indicated by TLC). After that, the reaction mixture was slowly raised to 0 °C, and the mixture was stirred for 30 min. After full consumption of starting materials (checked by TLC), the reaction mixture was quenched by Et_3N and then filtered through a celite bed followed by washing with CH_2Cl_2 (50 mL). The combined organic layer was successively washed with 5% $Na_2S_2O_3$, satd. $NaHCO_3$, water, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **2** (293 mg, 55%). Colorless foam. Spectral data match that of the substrate synthesized previously.

2-Aminoethyl (α-L-rhamnopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

To a solution of compound **2** (200 mg, 0.12 mmol) in EtOH (20 mL) was added hydrazine monohydrate (200 µl) and the reaction mixture was allowed to stir at 80 °C for 6 h. The solvents were removed under reduced pressure and a solution of the crude product in acetic anhydride-pyridine (5 mL; 1:1 v/v) was allowed to stir at room temperature for 2 h. The solvents were removed under reduced pressure and the crude product in 0.1 M sodium methoxide (10 mL) was allowed to stir at room temperature for 2 h and neutralized with Dowex 50 W X-8 (H^+). The reaction mixture was filtered and passed through a short pad of SiO_2. To a solution of

purified compound in methanol (20 mL) was added 20% $Pd(OH)_2/C$ (200 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite® bed and evaporated to dryness. The solution of the crude mass in methanol was passed through a column of Dowex 50 W-X8 (Na+) and evaporated to give tetrasaccharide **1** (51 mg, 56%) as a white powder, which was further purified by passing through a column of Sephadex-LH 20 using $CH_3OH–H_2O$ (4:1) as eluant. $[\alpha]_D^{25}$ −16 (*c* 1.0, H_2O); IR (KBr): 3419, 2910, 1302, 1142, 988, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.10 (d, $J = 7.7$ Hz, 1 H, H-1_A), 4.92 (brs, 1 H, H-1_B), 4.56 (brs, 2 H, H-1_D, H-1_C), 4.22–4.08 (m, 3 H, H-5_D, H-5_B, H-5_C). 3.94–3.21 (m, 17 H, H-2_D, H-2_B, H-3_D, H-3_B, H-3_C, H-6_{abC}, O*C*H_2, H-3_A, H-2_A, H-4_C, H-6_{abA}, H-4_D, H-2_C, H-4_A), 2.88–2.84 (m, 2 H, H-4_B, H-5_A), 2.25–2.22 (m, 2 H, NCH_2), 2.11 (s, 3 H, COCH_3), 1.28 (s, 6 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.0 (*C*OCH_3), 102.8 (C-1_A), 101.9 (C-1_D), 100.4 (C-1_B), 98.4 (C-1_C), 78.2 (C-3_A), 77.2 (C-4_C), 75.7 (C-2_B), 75.4 (C-3_D), 75.0 (C-5_A), 74.3 (C-2_D), 73.4 (C-4_B), 72.5 (C-4_D), 71.6 (C-2_C), 71.1 (C-3_B), 70.9 (C-3_C), 70.4 (C-5_B), 69.2 (C-5_D), 68.6 (C-5_C), 67.1 (OCH_2), 65.8 (C-4_A), 61.1 (C-6_A), 60.1 (C-6_C), 54.0 (C-2_A), 40.5 (NCH_2), 22.0 ($COCH_3$), 18.1 (CH_3), 17.9 (CH_3).; HRMS (ESI-TOF) calcd for $C_{28}H_{51}N_2O_{19}^+$ $[M+H]^+$ 719.3081, found 719.3087.

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