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Convergent synthesis of a pentasaccharide repeating unit corresponding to the cell wall *O*-antigen of *Salmonella enterica* O44

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ABSTRACT

A convergent synthetic strategy has been developed for the synthesis of a pentasaccharide fragment corresponding to the *O*-antigen of *Salmonella enterica* O44 strain. An intermediate tetrasaccharide derivative was prepared by a [2+2] block glycosylation of two disaccharide derivatives. The *p*-methoxybenzyl (PMB) group has been used as the in situ temporary protecting group minimizing the number of functional group manipulation steps. The application of the armed–disarmed glycosylation concept reduced the number of steps in the synthetic strategy. The glycosylation steps were highly stereoselective and high yielding.

1. Introduction

Salmonellosis is a major cause of food borne illness and diarrheal infections, which spreads through foods and water infected by the *Salmonella* bacteria.[1] Mostly, young and elderly populations as well as individuals with compromised immunity are prone to *Salmonella* infections.[2] Diarrhea with gastrointestinal complication is a serious health concern in tropical countries.[3] Infected poultry products and household pets act as reservoirs of *Salmonella* infections.[4] The common symptoms of salmonellosis include diarrhea (bloody and mucosal), fever, stomach ache, vomiting with dehydration and so on. *Salmonella enterica* strains are mostly responsible for the diarrheal infections among several strains of *Salmonella*.[5] In addition to the available therapeutics for controlling diarrheal infections, the development of novel alternative approaches is highly essential due to the emergence of multi-drug resistant bacteria.[6] The polysaccharide antigens present in the cell wall of *Salmonella* strains are closely associated with its pathogenicity and play crucial roles during the initial stage of bacterial infections. Therefore, glycoconjugates derived from the cell wall polysaccharide could be promising for the eradication or control of gastrointestinal infections.[7] However, the quantity of the polysaccharides or its repeating fragments required for the preparation of glycoconjugates to be used in several biological experiments cannot be accessed from natural sources and hence chemical synthesis is the best option for obtaining significant quantities of polysaccharide fragments with adequate purity.

Recently, Perepelov et al.[8] reported the structure of a pentasaccharide repeating unit of the *O*-polysaccharide present in the cell wall of *Salmonella enterica* O44, which is a pentasaccharide consisting of two alpha linked D-glucose moieties, two beta linked D-glucosamine moieties, and one alpha linked D-galactose moiety. During the course of the synthesis of complex oligosaccharides for their use in the preparation of glycoconjugate derivatives, we herein report a convergent chemical synthesis of the pentasaccharide corresponding to the cell wall polysaccharide of *Salmonella enterica* O44 in the form of its 2-aminoethyl glycosides. The presence of a 2-aminoethyl group at the reducing end of the synthesized pentasaccharide would be useful for its conjugation with a protein or lipid (Fig. 1).

→4)-[β-D-Glc*p*NAc-(1→3)]-α-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→

Structure of the pentasaccharide repeating unit of the cell wall *O*-antigen of *Salmonella enterica* O44 strain.

2. Results and discussion

The pentasaccharide **1** in the form of its 2-aminoethyl glycoside was synthesized using a convergent [2+2] block glycosylation strategy. The presence of three alpha linkages poses an extra challenge during the synthetic endeavor in the stereochemical outcome of the glycosylation steps. Suitably functionalized monosaccharide intermediates **2**,[9] **3**,[10] **4**,[11] **5**, and **6**[12] were prepared from reducing sugars using reported reaction conditions for their use in the construction of the desired pentasaccharide. The use of *p*-methoxybenzyl (PMB) ether as an in situ removable temporary

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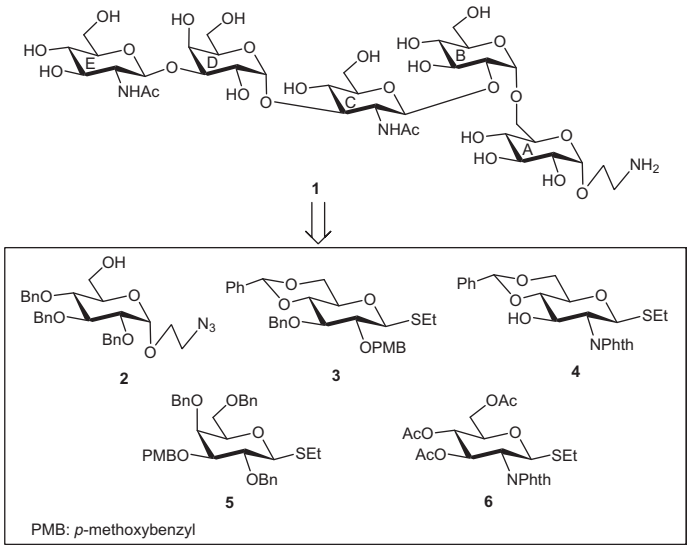
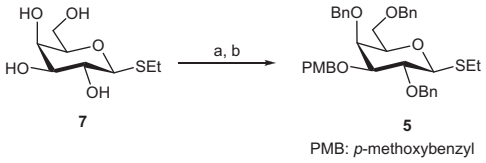


Figure 1. Structure of the synthesized pentasaccharide **1** and its synthetic intermediates.

protecting group[13] allowed to perform the stereoselective glycosylation as well as removal of the PMB group in one pot during the synthesis of the disaccharide intermediate **8** and tetrasaccharide intermediate **10** in excellent yield. During the synthesis, thioglycoside derivatives were used as glycosyl donors as well as acceptors adopting Fraser-Reid's concept of 'armed–disarmed' glycosylation.[14]

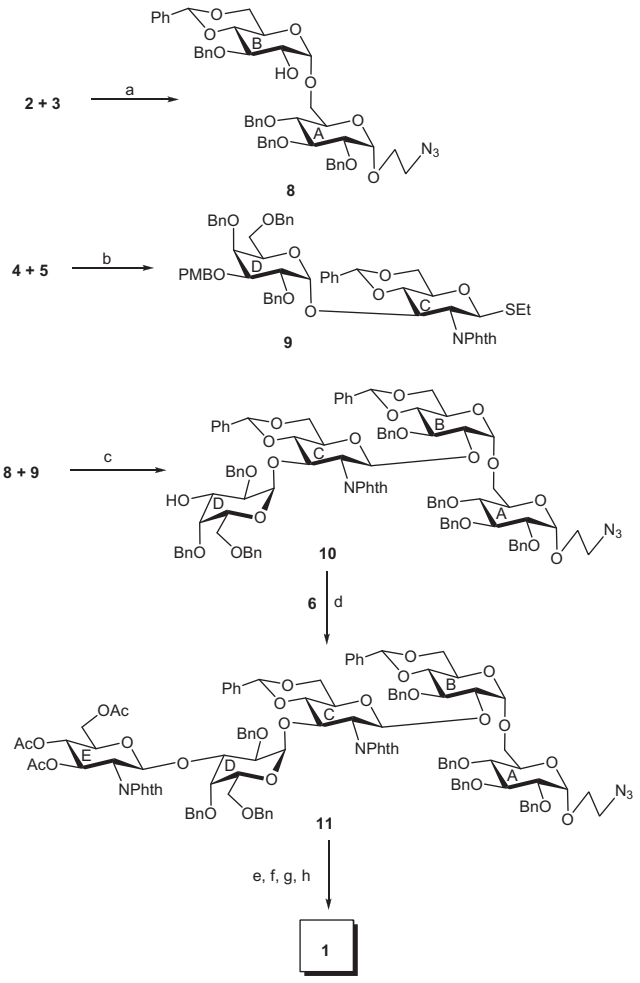
Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside **5** was prepared from ethyl 1-thio-β-D-galactopyranoside **7**[15] in 73% yield using a one-pot multistep reaction sequence involving selective 3-*O*-*p*-methoxybenzylation via the formation of a stannylidene acetal using dibutyltin oxide and its regioselective opening[16] using a combination of *p*-methoxybenzyl chloride and tetra-*n*-butylammonium bromide (TBAB), followed by benzylation of the remaining hydroxyl groups using benzyl bromide and sodium hydroxide[17] (Scheme 1).



Scheme 1. Reagents and conditions: (a) (i) Bu_2SnO, CH_3OH, 80 °C, 3 h; (ii) *p*-methoxybenzyl chloride, TBAB, DMF, 80 °C, 16 h; (b) benzyl bromide, NaOH, DMF, room temperature, 8 h, overall 73%.

The iodonium ion mediated stereoselective 1,2-*cis* glycosylation of compound **2** with thioglycoside derivative **3** in the presence of a combination of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[18,19] in a mixed solvent (CH_2Cl_2–Et_2O; 1:1) followed by tuning the reaction conditions[14] for the removal of the PMB group in one-pot, resulted in the formation of the disaccharide acceptor **8** in 74% yield together with its other isomer in a minor quantity (~5%), which was separated by chromatography. The NMR spectroscopic analysis of compound **8** unambiguously confirmed its formation [signals at δ 4.88 (d, J = 3.0 Hz, H-1_B), 4.72 (d, J = 3.5 Hz, H-1_A) in the ^{1}H NMR and δ 99.4 (J_{C1-H1} = 169.0 Hz; C-1_B), 97.2 (J_{C1-H1} = 168.5 Hz; C-1_A) in the ^{13}C NMR spectra]. The appearance of J_{C1-H1} values 168.5 and 169.0 Hz in the ^{1}H coupled gated ^{13}C NMR spectrum of compound **8** confirmed the presence of two alpha glycosyl linkages.[20] The non-participating ability of the PMB ether at the C-2 position of compound **3** favored the formation of the 1,2-*cis* glycosyl linkage and was removed in the same pot after the glycosylation step. In another experiment, the iodonium ion mediated stereoselective 1,2-*cis* glycosylation of thioglycoside glycosyl donor **5** with a thioglycoside acceptor **4** in the presence of NIS-TfOH[18,19] in a mixed solvent (CH_2Cl_2–Et_2O; 1:1) gave the desired disaccharide thioglycoside derivative **9** in 72% yield together with a minor quantity (~8%) of the 1,2-*trans* glycosyl product, which was separated by chromatography. The presence of a benzyl ether at the C-2 position of compound **5** made it an activated or 'armed' glycosyl donor while compound **4** acted as a 'disarmed' glycosyl acceptor due to the presence of an *N*-phthaloyl group at its C-2 potion following the Fraser-Reid's 'armed–disarmed' glycosylation concept.[14] The stereochemistry at the glycosyl linkages in compound **9** was confirmed from its spectroscopic analysis [signals at δ 5.52 (d, J = 4.0 Hz, H-1_D), 5.40 (d, J = 10.0 Hz, H-1_C) in the ^{1}H NMR and δ 97.4 (J_{C1-H1} = 168.0 Hz, C-1_D), 81.7 (J_{C1-H1} = 155.0 Hz, C-1_C) in the ^{13}C NMR spectra]. The appearance of J_{C1-H1} values 168.0 and 155.0 Hz in the ^{1}H coupled gated ^{13}C NMR spectrum of compound **9** confirmed the presence of one alpha and one beta glycosyl linkage, respectively.[20] Condensation of the disaccharide derivative **8** with the disaccharide thioglycoside derivative **9** in the presence of an NIS-TfOH[18,19] combination followed by removal of the PMB group from the newly formed tetrasaccharide derivative under one-pot reaction conditions[13] furnished the tetrasaccharide acceptor **10** in 69% yield. The stereochemical outcome at the newly formed glycosyl linkages in compound **10** was confirmed from its spectroscopic analysis [signals at δ 5.56 (d, J = 3.5 Hz, H-1_D), 5.54 (d, J = 9.0 Hz, H-1_C), 4.91 (d, J = 3.5 Hz, H-1_B) and 4.85 (d, J = 3.0 Hz, H-1_A) in the ^{1}H NMR and δ 100.0 (C-1_B), 99.7 (C-1_C), 97.0 (C-1_D) and 96.5 (C-1_A) in the ^{13}C NMR spectra]. Finally, glycosylation of compound **10** with compound **6** in the presence of an NIS-TfOH[18,19] combination furnished pentasaccharide derivative **11** in 67% yield. The NMR spectroscopic analysis of compound **11** supported its formation [signals at δ 5.58 (d, J = 8.5 Hz, H-1_C), 5.47 (d, J = 8.5 Hz, H-1_E), 5.35 (d, J = 3.5 Hz, H-1_D), 4.86 (d, J = 3.5 Hz, H-1_B) and 4.62 (d, J = 3.0 Hz, H-1_A) in the ^{1}H NMR and at δ 100.0 (C-1_E), 99.5 (C-1_B), 99.4 (C-1_C), 97.0 (C-1_D) and 96.6 (C-1_A) in the ^{13}C NMR spectra]. Removal of the protecting groups in compound **11** using a set of reactions consisting of; (a) treatment with hydrazine followed by acetylation for the transformation of the *N*-phthaloyl group to an acetamido group;[21] (b)

saponification using sodium methoxide; and (c) removal of the benzyl ethers and benzylidene acetals as well as the reduction of the azido group by catalytic transfer hydrogenation using triethylsilane and Pd-C,[22] resulted in the formation of pentasaccharide **1** in the form of its 2-aminoethyl glycoside in 57% overall yield. The formation of compound **1** was confirmed by its spectroscopic analysis [signals at δ 5.35 (d, J = 3.0 Hz, H-1_D), 5.00 (br s, H-1_A, H-1_B) and 4.88 (2 d, J = 8.0 Hz each, H-1_C, H-1_E) in the ^{1}H NMR and at δ 98.4 (3 C, C-1_C, C-1_D, C-1_E), 97.9 (C-1_A) and 97.8 (C-1_B) in the ^{13}C NMR spectra] (Scheme 2).



Scheme 2. Reagents and conditions: (a) *N*-iodosuccinimide (NIS), TfOH, MS 4 Å, CH_2Cl_2–Et_2O (1:1), −45 °C, 45 min, then 0 °C, 1 h, 74%; (b) NIS, TfOH, MS 4 Å, CH_2Cl_2–Et_2O (1:1), −35 °C, 30 min, 72%; (c) NIS, TfOH, MS 4 Å, CH_2Cl_2, −30 °C, 30 min, then 0 °C, 1 h, 69%; (d) NIS, TfOH, MS 4 Å, CH_2Cl_2, −25 °C, 20 min, 67%; (e) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 8 h; (f) acetic anhydride, pyridine, room temperature, 1 h; (g) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h; (h) Et_3SiH, 20% $Pd(OH)_2$-C, CH_3OH–EtOAc (3:1), room temperature, 10 h, over all 57%.

3. Conclusion

In summary, a pentasaccharide repeating unit of the cell wall *O*-polysaccharide of *Salmonella enterica* O44 strain in the form of its 2-aminoethyl glycoside has been synthesized in excellent yield and the stereochemical outcome was established by using a convergent synthetic scheme involving a one-pot glycosylation and protecting group manipulations as well as 'armed–disarmed' glycosylation. The use of a PMB group as the in situ removable temporary protecting group for the hydroxyl groups reduced the number of steps and increased the overall yield of the synthetic strategy.

4. Experimental

General methods are same as reported earlier.[22]

4.1. Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside 5

To a solution of compound **7** (2 g, 8.92 mmol) in dry CH_3OH (60 mL) was added Bu_2SnO (6 g, 24.10 mmol) and the reaction mixture was stirred at 80 °C for 3 h. The solvents were removed under reduced pressure to give the crude stannylidene acetal derivative. To a solution of the stannylidene acetal derivative in dry DMF (25 mL) were added *p*-methoxybenzyl chloride (2.2 mL, 16.22 mmol) and TBAB (3 g, 9.30 mmol) and the reaction mixture was allowed to stir at 80 °C for 16 h. The reaction mixture was cooled to room temperature and benzyl bromide (6.5 mL, 54.65 mmol) followed by powdered NaOH (4 g, 100 mmol) were added to it. After stirring the reaction mixture at room temperature for 8 h, the solvents were removed under reduced pressure and the crude product was dissolved in EtOAc (150 mL). The organic layer was washed with 1 M HCl, satd $NaHCO_3$ and water in succession, dried (Na_2SO_4), and concentrated to give the crude product, which was purified over SiO_2 using hexane–EtOAc (10:1) as eluant to give pure compound **5** (4 g, 73%). Colorless oil; $[\alpha]_D^{25} = +19$ (*c* 1.0, $CHCl_3$); IR (neat): 3408, 2811, 2331, 1654, 1542, 1498, 1379, 1235, 1198, 1083, 982, 668 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.25 (m, 17H, Ar-H), 6.83 (d, J = 9.0 Hz, 2H, Ar-H), 4.94 (d, J = 12.0 Hz, 1H, PhCH_2), 4.87 (d, J = 11.0 Hz, 1H, PhCH_2), 4.79 (d, J = 11.0 Hz, 1H, PhCH_2), 4.65 (br s, 2H, PhCH_2), 4.59 (d, J = 11.5 Hz, 1H, PhCH_2), 4.44 (d, J = 11.5 Hz, 1H, PhCH_2), 4.41 (d, J = 10.0 Hz, 1H, H-1), 4.39 (d, J = 11.5 Hz, 1H, PhCH_2), 3.92 (d, J = 2.5 Hz, 1H, H-4), 3.80 (s, 3H, OCH_3), 3.82-3.77 (m, 1H, H-2), 3.59–3.52 (m, 4H, H-3, H-5, H-6_{ab}), 2.78 (m, 2H, SCH_2CH$_3$), 1.31 (t, J = 7.5 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.2–113.8 (Ar-C), 85.3 (C-1), 83.8 (C-5), 78.4 (C-3), 77.2 (C-4), 75.7 (PhCH$_2$), 74.4 (PhCH$_2$), 73.6 (C-2), 73.5 (PhCH$_2$), 72.4 (PhCH$_2$), 68.8 (C-6), 55.2 (OCH$_3$), 24.7 (SCH$_2$CH$_3$), 15.1 (SCH$_2$CH$_3$); ESI-MS: 637.2 [M+Na]$^+$; Anal. Calcd for $C_{37}H_{42}O_6S$ (614.27): C, 72.28; H, 6.89; found: C, 72.10; H, 7.10.

4.2. 2-Azidoethyl (3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside 8

To a solution of compound **2** (1.4 g, 2.69 mmol) and compound **3** (1.5 g, 2.87 mmol) in anhydrous CH_2Cl_2–Et_2O (20 mL; 1:1) was added MS 4 Å (2 g) and the reaction mixture was cooled to −45 °C under argon. To the cooled reaction mixture was added NIS (700 mg, 3.11 mmol) followed by TfOH (20 μL) and then allowed to stir at −45 °C for 45 min. The reaction mixture was warmed up to 0 °C and stirred at 0 °C for 1 h. The reaction mixture was filtered through a Celite® bed and washed several times with CH_2Cl_2 (50 mL). The organic layer was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and water in succession, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure compound **8** (1.7 g, 74%). Colorless oil; $[\alpha]_D^{25} + 33$ (*c* 1.0, $CHCl_3$); IR (neat): 3021, 2927, 2847, 1734, 1535, 1323, 1133, 1079, 782, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–7.46 (m, 2H, Ar-H), 7.45–7.23 (m, 23H, Ar-H), 5.52 (s, 1H, PhC*H*), 4.97 (d, J = 11.0 Hz, 1H, PhCH_2), 4.92 (d, J = 12.0 Hz, 2H, PhCH_2), 4.88 (d, J = 3.0 Hz, 1H, H-1_B), 4.79 (dd, J = 12.0 Hz, 3H, PhCH_2), 4.72 (d, J = 3.5 Hz, 1H, H-1_A), 4.65 (d, J = 11.0 Hz, 1H, PhCH_2), 4.57 (d, J = 11.0 Hz, 1H, PhCH_2), 4.19–4.16 (m, 1H, H-6_{aB}), 3.99 (t, J = 9.5 Hz, 1H, H-3_A), 3.85–3.74 (m, 4H, H-3_B, H-5_B, H-6_{aA}, OCH_2), 3.70–3.65 (m, 4H, H-2_B, H-5_A, H-6_{bB}, OCH_2), 3.60–3.49 (m, 3H, H-2_A, H-4_B, H-6_{bA}), 3.48–3.34 (m, 3H, H-4_A, CH_2N$_3$); ^{13}C NMR

(125 MHz, $CDCl_3$): δ 128.9–126.1 (Ar-C), 101.3 (PhCH), 99.4 (C-1_B), 97.2 (C-1_A), 81.8 (C-4_B), 81.7 (C-3_A), 80.2 (C-2_A), 78.6 (C-2_B), 77.4 (C-4_A), 75.7 ($PhCH_2$), 74.9 ($PhCH_2$), 74.5 ($PhCH_2$), 73.3 ($PhCH_2$), 72.6 (C-5_A), 70.1 (C-3_B), 68.9 (C-6_B), 67.2 (C-6_A), 66.8 (OCH_2), 62.9 (C-5_B), 50.6 (CH_2N_3); ESI-MS: 882.3 $[M+Na]^+$; Anal. Calcd for $C_{49}H_{53}N_3O_{11}$ (859.37): C, 68.44; H, 6.21; found: C, 68.27; H, 6.35.

4.3. Ethyl (2,4,6-tri-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside 9

To a solution of compound **4** (1.3 g, 2.94 mmol) and compound **5** (1.9 g, 3.09 mmol) in anhydrous CH_2Cl_2–Et_2O (20 mL; 1:1) was added MS 4 Å (2 g) and the reaction mixture was cooled to −35 °C under argon. To the cooled reaction mixture was added NIS (700 mg, 3.11 mmol) followed by TfOH (15 μL) and then allowed to stir at −35 °C for 30 min. The reaction mixture was filtered through a Celite® bed and washed several times with CH_2Cl_2 (50 mL). The organic layer was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and water in succession, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure compound **9** (2.1 g, 72%). Colorless oil; $[\alpha]_D^{25} = +25$ (*c* 1.0, $CHCl_3$); IR (neat): 3418, 2911, 2769, 2211, 1664, 1498, 1411, 1379, 1255, 1063, 982, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.89–7.77 (m, 2H, Ar-H), 7.56–7.54 (m, 2H, Ar-H), 7.53–6.79 (m, 24H, Ar-H), 5.52 (d, *J* = 4.0 Hz, 1H, H-1_D), 5.40 (d, *J* = 10.0 Hz, 1H, H-1_C), 5.36 (s, 1H, PhC*H*), 4.89–4.85 (m, 1H, PhCH_2), 4.72 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.68 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.57 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.51–4.47 (m, 2H, H-2_C, PhCH_2), 4.39 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.36–4.34 (m, 1H, H-6_{aD}), 4.19 (d, *J* = 11.0 Hz, 1H, PhCH_2), 3.90–3.56 (m, 7H, H-2_D, H-3_C, H-3_D, H-4_C, H-4_D, H-6_{bD}, PhCH_2), 3.78 (s, 3H, OCH_3), 3.56 (br s, 1H, H-5_D), 3.34–3.32 (m, 1H, H-5_C), 3.25–3.23 (m, 1H, H-6_{aC}), 2.81–2.78 (m, 1H, H-6_{bC}), 2.72–2.64 (m, 2H, SC$H_2$$CH_3$), 1.94 (t, *J* = 7.4 Hz, 1H, SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.9, 167.4 (Phth), 159.1–113.5 (Ar-C), 101.7 (PhCH), 97.4 (C-1_D), 83.1 (C-3_D), 81.7 (C-1_C), 77.8 (C-3_C), 75.4 (C-4_D), 74.9 (C-2_D), 74.7 ($PhCH_2$), 73.3 (C-5_D), 72.9 ($PhCH_2$), 72.6 ($PhCH_2$), 71.8 ($PhCH_2$), 70.2 (C-4_C), 69.5 (C-5_C), 68.8 (C-6_D), 67.5 (C-6_C), 55.2 (OCH_3), 54.2 (C-2_C), 23.9 (SCH_2CH_3), 14.9 (SCH_2CH_3); ESI-MS: 1016.3 $[M+Na]^+$; Anal. Calcd for $C_{58}H_{59}NO_{12}S$ (993.38): C, 70.07; H, 5.98; found: C, 69.90; H, 6.16.

4.4. 2-Azidoethyl (2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside 10

To a solution of compound **8** (1.2 g, 1.39 mmol) and compound **9** (1.5 g, 1.51 mmol) in anhydrous CH_2Cl_2 (20 mL) was added MS 4 Å (2 g) and the reaction mixture was cooled to −30 °C under argon. To the cooled reaction mixture was added NIS (360 mg, 1.6 mmol) followed by TfOH (15 μL) and then allowed to stir at −30 °C for 30 min. The reaction mixture was warmed up to 0 °C and stirred at 0 °C for 1 h. The reaction mixture was filtered through a Celite® bed and washed several times with CH_2Cl_2 (50 mL). The organic layer was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and water in succession, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure compound 10 (1.6 g, 69%). Colorless oil; $[\alpha]_D^{25} = +28$ (*c* 1.0, $CHCl_3$); IR (neat): 3087, 2856, 1732, 1719, 1625, 1520, 1375, 1239, 1173, 1097, 1072, 989, 911, 823, 755, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.69–7.24 (m, 35H, Ar-H), 7.14–6.92 (m, 14H, Ar-H), 5.56 (d, *J* = 3.5 Hz, 1H, H-1_D), 5.54 (d, *J* = 9.0 Hz, 1H, H-1_C), 5.34 (s, 1H, PhC*H*), 5.06 (s, 1H, PhC*H*), 5.05 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.92 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.91 (d, *J* = 3.5 Hz, 1H, H-1_B), 4.88–4.86 (m, 2H, PhCH_2), 4.85 (d, *J* = 3.0 Hz, 1H, H-1_A), 4.84 (d, *J* = 11.5 Hz, 2H, PhCH_2), 4.77 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.60 (d, *J* = 1.5 Hz, 1H, PhCH_2), 4.56 (dd, *J* = 12.0 Hz, 3H, PhCH_2), 4.47–4.45 (m, 1H, H-2_C), 4.32–4.28 (m, 2H, H-6_{abB}), 4.20 (dd, *J* = 12.0 Hz, 2H, PhCH_2), 4.19–4.17 (m, 1H, H-6_{aD}), 4.09 (t, *J* = 9.5 Hz, 1H, H-3_A), 4.04 (d, *J* = 11.5 Hz, 1H, PhCH_2), 3.93–3.82 (m, 5H, H-2_D, H-3_C, H-3_D, H-4_D, H-6_{bD}), 3.79–3.60 (m, 10H, H-2_A, H-2_B, H-3_B, H-4_A, H-4_B, H-4_C, H-5_A, H-5_B, H-6_{abA}), 3.53–3.45 (m, 1H, CH_2N_3), 3.44–3.37 (m, 5H, H-5_C, H-5_D, H-6_{abC}, CH_2N_3), 3.40–3.37 (m, 1H, OCH_2), 2.89–2.83 (m, 1H, OCH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.8, 167.4 (Phth), 138.5–126.1 (Ar-C), 101.9 (PhCH), 101.3 (PhCH), 100.0 (C-1_B), 99.7 (C-1_C), 97.0 (C-1_D), 96.5 (C-1_A), 83.0 (C-3_A), 82.3 (C-3_D), 81.9 (C-2_A), 80.2 (C-4_B), 79.7 (C-4_A), 78.1 (C-2_B), 76.5 (C-4_D), 75.8 ($PhCH_2$), 75.7 (C-3_C), 75.6 (C-2_D), 74.9 ($PhCH_2$), 74.8 ($PhCH_2$), 73.6 ($PhCH_2$), 73.1 ($PhCH_2$), 72.7 ($PhCH_2$), 72.4 (C-5_A), 71.0 ($PhCH_2$), 70.3 (C-5_D), 69.4 (C-3_B), 69.2 (C-5_C), 68.9 (C-6_B), 68.7 (C-6_D), 67.9 (C-6_C), 67.7 (C-6_A), 66.8 (OCH_2), 65.6 (C-4_C), 62.4 (C-5_B), 55.4 (C-2_C), 50.6 (CH_2N_3); MALDI-MS: 1693.6 $[M+Na]^+$; Anal. Calcd for $C_{97}H_{98}N_4O_{22}$ (1670.67): C, 69.69; H, 5.91; found: C, 69.52; H, 6.10.

4.5. 2-Azidoethyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside 11

To a solution of compound **10** (1.2 g, 0.72 mmol) and compound **6** (380 mg, 0.79 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4 Å (1 g) and the reaction mixture was cooled to −25 °C under argon. To the cooled reaction mixture was added NIS (190 g, 0.84 mmol) followed by TfOH (5 μL) and then allowed to stir at −25 °C for 20 min. The reaction mixture was filtered through a Celite® bed and washed several times with CH_2Cl_2 (50 mL). The organic layer was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and water in succession, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure compound **11** (1 g, 67%). Colorless oil; $[\alpha]_D^{25} = +18$ (*c* 1.0, $CHCl_3$); IR (neat): 3019, 2418, 2210, 1849, 1628, 1415, 1322, 1122, 1042, 988, 756, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.63–6.93 (m, 51H, Ar-H), 6.62–6.60 (m, 2H, Ar-H), 5.82 (dd, *J* = 10.2, 10.2 Hz, 1H, H-3_E), 5.58 (d, *J* = 8.5 Hz, 1H, H-1_C), 5.47 (d, *J* = 8.5 Hz, 1H, H-1_E), 5.35 (d, *J* = 3.5 Hz, 1H, H-1_D), 5.34 (s, 1H, PhC*H*), 5.10 (t, *J* = 8.5 Hz, 1H, H-4_E), 5.06 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.92 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.87 (s, 1H, PhC*H*), 4.86 (d, *J* = 3.5 Hz, 1H, H-1_B), 4.84 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.79 (d, *J* = 11.5 Hz, 1H, PhCH_2), 4.72 (dd, *J* = 11.0 Hz, 2H, PhCH_2), 4.62 (d, *J* = 3.0 Hz, 1H, H-1_A), 4.55 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.39 (dd, *J* = 8.5 Hz, 1H, H-2_C), 4.35–4.28 (m, 3 H, H-2_E, H-6_{aE}, H-5_E), 4.22 (d, *J* = 11.5 Hz, 1H, PhCH_2), 4.32–3.88 (m, 10H, H-2_D, H-3_C, H-3_D, H-4_D, H-6_{abB}, H-6_{bE}, PhCH_2), 3.83–3.51 (m, 18H, H-2_B, H-3_A, H-3_B, H-4_C, H-5_A, H-5_C, H-5_D, H-6_{abA}, H-6_{abC}, H-6_{abD}, PhCH_2, CH_2N_3), 3.52–3.43 (m, 2H, H-4_A, H-5_B), 3.36–3.33 (m, 2H, H-2_A, H-4_B), 2.98–2.94 (m, 1H, OCH_2), 2.79–2.73 (m, 1H, OCH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9 (3 $COCH_3$), 167.6, 167.3 (Phth), 167.2 (2C, Phth), 148.2–123.0 (Ar-C), 101.3 (PhCH), 100.9 (PhCH), 100.0 (C-1_E), 99.5 (C-1_B), 99.4 (C-1_C), 97.0 (C-1_D), 96.6 (C-1_A), 82.5 (C-3_A), 82.4 (C-4_B), 81.9 (C-2_A), 80.1 (C-4_A), 78.4 (C-3_D), 78.1 (C-2_B), 76.5 (C-5_E), 76.4 (C-4_D), 75.8 ($PhCH_2$), 74.9 ($PhCH_2$), 74.8 ($PhCH_2$), 74.6 (C-2_D), 73.9 (C-3_C), 73.5 ($PhCH_2$), 73.1 ($PhCH_2$), 72.8 ($PhCH_2$), 72.6 (C-5_A), 71.3 (C-5_D), 70.6 (C-3_B), 70.5 ($PhCH_2$), 70.2 (C-3_E), 69.0 (C-4_E), 68.9 (C-6_B), 68.5 (C-6_D), 67.9 (C-6_C), 67.7 (C-6_A), 67.0 (OCH_2), 66.9 (C-5_C), 65.7 (C-4_C), 62.3 (C-5_B), 61.9

(C-6_E), 55.5 (C-2_C), 55.0 (C-2_E), 50.5 (CH_2N_3), 20.8 ($COCH_3$), 20.6 ($COCH_3$), 20.4 ($COCH_3$); MALDI-TOF-MS: 2110.7 $[M+Na]^+$; Anal. Calcd for $C_{117}H_{117}N_5O_{31}$ (2087.77): C, 67.26; H, 5.64; found: C, 67.10; H, 5.82.

4.6. 2-Aminoethyl (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-glucopyranosyl)-(1→6)-α-D-glucopyranoside 1

To a solution of compound **11** (700 mg, 0.34 mmol) in EtOH (15 mL) was added $NH_2NH_2{\cdot}H_2O$ (0.5 mL, 10.31 mmol) and the reaction mixture was stirred at 80 °C for 8 h. The solvents were removed under reduced pressure and a solution of the crude product in acetic anhydride and pyridine (5 mL; 1:1) was kept at room temperature for 1 h. The reaction mixture was evaporated and co-evaporated with toluene (3 × 20 mL) under reduced pressure. A solution of the acetylated product in 0.1 M CH_3ONa in CH_3OH (10 mL) was stirred at room temperature for 2 h, neutralized with Dowex 50W X8 (H^+), filtered, and concentrated. To a solution of the de-*O*-acetylated product and 20% $Pd(OH)_2$-C (100 mg) in CH_3OH–EtOAc (10 mL; 3:1) was added dropwise Et_3SiH (2 mL, 12.52 mmol) over 30 min and the reaction mixture was allowed to stir at room temperature for 10 h. The reaction mixture was filtered through a Celite bed and washed with CH_3OH (50 mL) and the combined filtrate was evaporated under reduced pressure to give a product, which was passed through a Sephadex LH-20 column using CH_3OH–H_2O (3:1) as eluant to give pure compound **1** (185 mg, 57%). Glass; $[\alpha]_D^{25} = +8$ (*c* 1.0, H_2O); IR (KBr): 3435, 2946, 1617, 1397, 1155, 1096, 667 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.35 (d, J = 3.0 Hz, 1H, H-1_D), 5.00 (br s, 2H, H-1_A, H-1_B), 4.88 (2 d, J = 8.0 Hz each, 2H, H-1_C, H-1_E), 4.16–4.00 (m, 2H, H-4_D, H-5_A), 3.96–3.70 (m, 6H, H-2_C, H-2_D, H-3_C, H-5_D, H-6_{aA}, H-6_{aB}), 3.68–3.50 (m, 14H, H-2_E, H-3_A, H-3_B, H-3_D, H-4_C, H-5_B, H-6_{bA}, H-6_{bB}, H-6_{abC}, H-6_{abD}, H-6_{abE}), 3.48–3.15 (m, 8H, H-2_A, H-2_B, H-3_E, H-4_A, H-5_C, H-5_E, OCH_2), 3.14–3.00 (m, 2H, H-4_B, H-4_E), 2.99–2.92 (m, 2H, NCH_2), 2.12, 2.11 (2 s, 6H, 2 $COCH_3$); ^{13}C NMR (125 MHz, D_2O): δ 176.0, 175.9 (2 $COCH_3$), 98.4 (3C, C-1_C, C-1_D, C-1_E), 97.9 (C-1_A), 97.8 (C-1_B), 75.9 (C-2_B), 75.8 (C-3_D), 73.0 (3 C, C-3_C, C-5_D, C-5_E), 71.5 (3 C, C-4_D, C-5_A, C-5_C), 71.1 (3 C, C-2_A, C-2_D, C-3_E), 70.6 (3 C, C-3_A, C-3_B, C-5_B), 69.6 (2 C, C-4_A, C-4_B), 66.1 (C-4_C), 63.7 (C-6_A), 60.7 (3 C, C-6_B, C-6_C, OCH_2), 60.4 (2 C, C-6_D, C-6_E), 59.9 (C-4_E), 56.8 (C-2_C), 54.2 (C-2_E), 39.3 (NCH_2), 23.2 (2 C, 2 $COCH_3$); ESI-MS: 976.3 $[M+Na]^+$; Anal. Calcd for $C_{36}H_{63}N_3O_{26}$ (953.37): C, 45.33; H, 6.66; found: C, 45.16; H, 6.80.

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