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Concise synthesis of a pentasaccharide repeating unit corresponding to the *O*-antigen of *Escherichia coli* O102

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ABSTRACT

An efficient synthetic strategy has been developed for the synthesis of a pentasaccharide repeating unit of the *O*-antigen of *Escherichia coli* O102 strain. The target pentasaccharide **1** has been synthesized using a [2+3] block glycosylation strategy. All glycosylation steps are highly stereoselective and high yielding. Concept of armed-disarmed and orthogonal glycosylation strategies has been applied during the synthesis. The target compound has been synthesized using the minimum number of steps.

1. Introduction

Diarrheal infections in developing countries are a serious problem.[1,2] In general, enteric infections and their associated problems such as hemorrhagic colitis and hemorrhagic uremic syndrome, spread through contaminated food and water.[3,4] The major causative agent of the diarrheal disease is enteropathogenic *Escherichia coli* (EPEC).[5] Among several pathogenic strains of *E. coli*, Shiga toxin producing *E. coli* (STEC) strains is most virulent.[6] Among several STEC strains, *E. coli* O157 is most often cited for its association with several diarrheal outbreaks in developed countries.[7,8] Recently, *E. coli* O102 strain has been found to produce the Shiga toxin and act as the causative agent of avian colibacillisis[9] and STEC infections in humans.[10] *E. coli* O102 strain has also been identified as a multidrug-resistant pathogenic strain causing infections in hospitals and social communities.[11,12]

The *O*-antigens of pathogenic Gram-negative bacteria exist in the lipopolysaccharide chains of the cell wall and play important roles in the initial stage of bacterial adhesion with the host.[13,14] Therefore, the development of glycoconjugate based therapeutics would be important to combat the battle against such infections. A number of reports have appeared dealing with glycoconjugate based vaccine preparations.[15–19] Recently, Perepelov et al.[20] reported on the structure of the *O*-antigen of *E. coli* O102, which is a pentasaccharide repeating unit consisting of D-galactosamine, D-glucose, D-galactose, and L-rhamnose (Fig. 1). The development of glycoconjugate derivatives corresponding to the *O*-antigen of *E. coli* O102 and its biological studies requires large quantities of pentasaccharide, which is difficult to isolate from a natural source. Therefore, chemical synthesis of the pentasaccharide repeating unit with the required stereochemistry at the glycosyl linkages has been undertaken. A convergent synthesis of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O102 is reported herein (Fig. 2).

α-L-Rha*p*-(1→4)-β-D-Gal*p*NAc
1
↓
3
→4)-α-D-Gal*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→

Figure 1. Structure of the pentasaccharide repeating unit of the *O*-antigen of *Escherichia coli* O102.

2. Results and discussion

The target pentasaccharide **1** protected as its *p*-methoxyphenyl glycoside has been synthesized by the stereoselective glycosylation of a trisaccharide acceptor **11** with a disaccharide thioglycoside donor **12** using a [2+3] block glycosylation strategy (Fig. 2). The trisaccharide derivative **11** and the disaccharide derivative **12** were prepared from suitably protected monosaccharide intermediates **2**, **3**,[21] **4**,[22] **5**, and **6**,[23] which were prepared from commercially available reducing sugars using reported reaction methodologies (Fig. 2).

p-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-galactopyranoside **7**[24] (prepared from D-galactosamine hydrochloride in six steps) was treated with sodium cyanoborohydride in the presence of HCl–ether[25] to furnish *p*-methoxyphenyl 6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-galactopyranoside **2** in 82% yield. Ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactopyranoside **8**[26] (prepared from D-galactosamine hydrochloride in six steps) was subjected to acid hydrolysis using 80% aq acetic acid to give diol derivative, which

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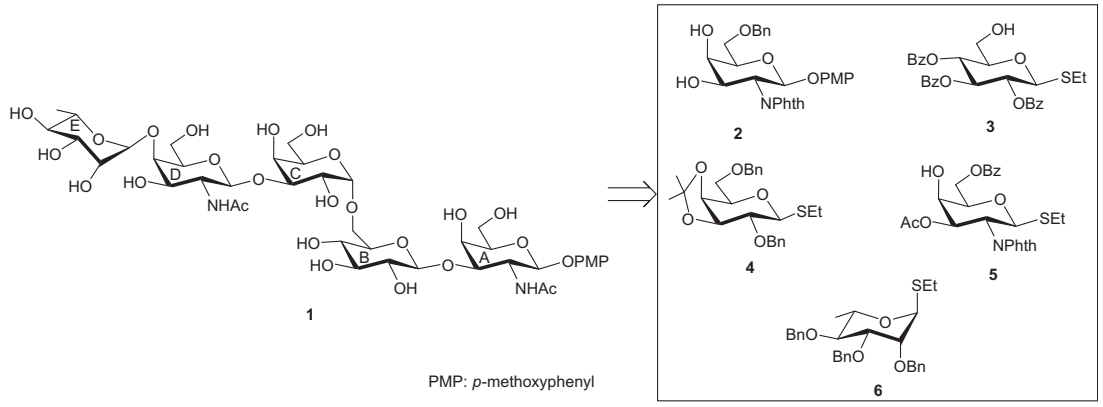
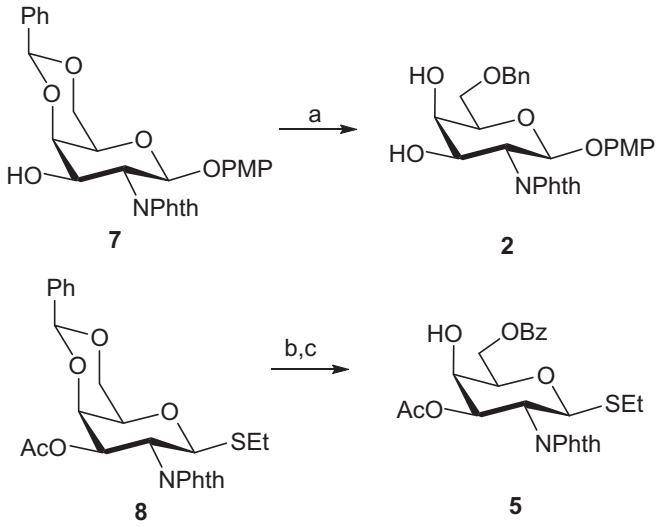


Figure 2. Structure of the synthesized pentasaccharide **1** as its *p*-methoxyphenyl glycoside and synthetic intermediates.

was selectively benzoylated using benzoyl cyanide[27] to furnish compound **5** in 80% yield (Scheme 1).

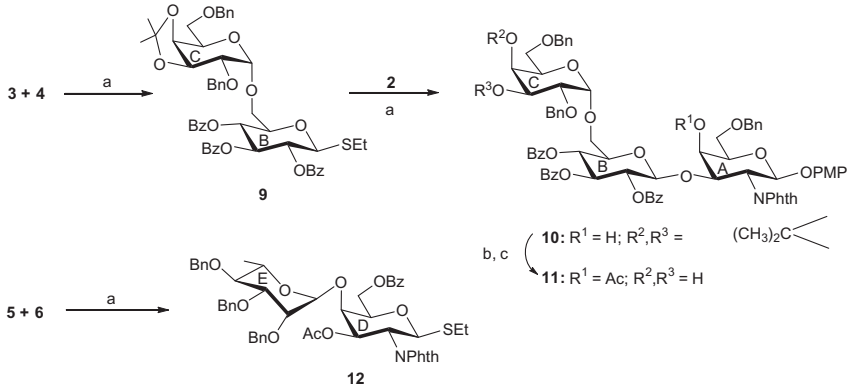


Scheme 1. Reagents and conditions: (a) $NaBH_3CN$, HCl–Et_2O, 5 °C, 2 h, 82%; (b) 80% aq AcOH, 80 °C, 1.5 h; (c) benzoyl cyanide, pyridine, CH_2Cl_2, room temperature, 2 h, 80% in two steps.

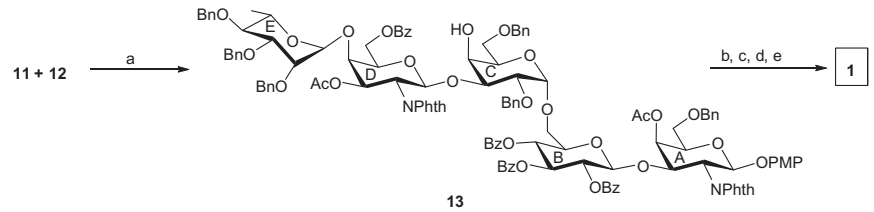
The stereoselective glycosylation of thioglycoside derivative **3** with thioglycoside derivative **4** in the presence of a combination of N-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[28,29] exploiting the 'armed-disarmed' glycosylation strategy[30] furnished disaccharide thioglycoside derivative **9** in 73% yield. The anomeric thioethyl group of compound **3** also acted as an orthogonal anomeric protecting group, which can be activated in a later glycosylation reaction.[31] The formation of compound **9** was confirmed by spectroscopic analysis [signals at δ 4.79 (br s, H-1_C), 4.75 (d, J = 10 Hz, H-1_B) and δ 97.4 (C-1_C), 83.5 (C-1_B) in the ^{1}H and ^{13}C NMR spectra, respectively]. Compound **9** was allowed to couple regio- and stereoselectively with D-galactosamine derived diol derivative **2** in the presence of a combination of NIS-TMSOTf[28,29] to furnish trisaccharide derivative **10** in 71% yield. Spectroscopic analysis of compound **10** supported its formation [signals at δ 5.64 (d, J = 8.5 Hz, H-1_A), 5.06 (d, J = 8.0 Hz, each, 1 H, H-1_B), 4.76 (br s, 1 H, H-1_C) and δ 101.3 (C-1_B), 97.5 (C-1_A), 97.2 (C-1_C) in the ^{1}H and ^{13}C NMR spectra respectively]. Acetylation of compound **10** using acetic anhydride and pyridine followed by acidic hydrolysis using 80% aq acetic acid led to the formation of trisaccharide diol derivative **11** in 93% yield (Scheme 2).

In another experiment, thioglycoside derivative **5** was allowed to condense with thioglycoside derivative **6** in the presence of a combination of NIS-TMSOTf[28,29] exploiting 'armed-disarmed' glycosylation to furnish disaccharide thioglycoside derivative **12** in 74% yield (Scheme 2). The stereoselective formation of compound **12** was supported by its spectroscopic analysis [signals at δ 5.52 (d, J = 10.5 Hz, H-1_D), 5.14 (d, J = 2.5 Hz, H-1_E) and δ 99.9 (C-1_E), 81.3 (C-1_D) in the ^{1}H and ^{13}C NMR spectra, respectively].

Finally, iodonium ion promoted regio- and stereoselective glycosylation of disaccharide glycosyl donor **12** with the trisaccharide glycosyl acceptor **13** in the presence of NIS-TMSOTf furnished pentasaccharide derivative **13** in 74% yield. The formation of



Scheme 2. Reagents and conditions: (a) *N*-iodosuccinimide (NIS), TMSOTf, MS-4Å, CH_2Cl_2, −30 °C, 45 min, 73% for compound **9**, 71% for compound **10** and 74% for compound **12**; (b) acetic anhydride, pyridine, room temperature, 2 h; (c) 80% aq AcOH, 80 °C, 1.5 h, 93% over two steps.



Scheme 3. Reagents and conditions: (a) NIS, TMSOTf, MS-4Å, CH_2Cl_2, −30 °C, 45 min, 74%; (b) $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH, 80 °C, 8 h; (c) acetic anhydride, pyridine, room temperature, 3 h; (d) Et_3SiH, 10% Pd-C, CH_3OH, room temperature, 12 h; (e) 0.1 M CH_3ONa, CH_3OH, room temperature, 4 h, 60% overall yield.

compound **13** was confirmed by its spectroscopic analysis [signals at δ 5.77 (d, J = 8.5 Hz, H-1_A), 5.47 (d, J = 8.5 Hz, H-1_D), 5.01 (br s, H-1_E), 4.87 (d, J = 8.0 Hz, H-1_B), 4.47 (d, J = 3.5 Hz, H-1_C) and δ 101.4 (C-1_A), 100.3 (C-1_C), 98.7 (C-1_D), 97.9 (C-1_E), 97.2 (C-1_B) in the ^{1}H and ^{13}C NMR spectra, respectively]. Compound **13** was subjected to a series of reactions involving (a) removal of *N*-phthalimido group using hydrazine hydrate;[32] (b) acetylation using acetic anhydride and pyridine; (c) catalytic transfer hydrogenation using triethylsilane and Pd-C[33] and (d) de-O-acetylation using sodium methoxide to furnish compound **1**, which was purified using Sephadex® LH-20 gel to give pure compound **1** in 60% yield. Spectroscopic analysis of compound **1** unambiguously confirmed its formation [signals at δ 5.12 (br s, H-1_E), 5.05 (d, J = 8.5 Hz, H-1_A), 4.90 (d, J = 8.5 Hz, H-1_D), 4.87 (d, J = 3.0 Hz, H-1_C), 4.62 (d, J = 7.5 Hz, H-1_B) and 104.0 (C-1_B), 103.1 (C-1_D), 101.7 (C-1_E), 100.6 (C-1_A), 98.6 (C-1_C) in the ^{1}H and ^{13}C NMR spectra, respectively] (Scheme 3).

3. Conclusion

In conclusion, a convenient synthesis for the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O102 has been successfully developed using a [2+3] block glycosylation strategy. The 'arm-disarmed' glycosylation and 'orthogonal' protecting group concept has been applied during the synthesis of the target compound. The yields and stereoselectivity of the glycosylation were excellent.

4. Experimental

4.1. General

All reactions were monitored by thin layer chromatography over silica gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate [2% $Ce(SO_4)_2$ in 2 N H_2SO_4]-sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. ^{1}H and ^{13}C NMR, DEPT 135, 2D COSY, and 2D HSQC NMR spectra were recorded on Brucker Avance DRX 500 MHz spectrometers using $CDCl_3$ and D_2O as solvents and TMS as the internal reference unless stated otherwise. Chemical shift values are expressed in δ ppm. MALDI-MS were recorded on a Bruker Daltronics mass spectrometer. Elementary analysis was carried out on a Carlo Erba analyzer. Optical rotations were measured at 25 °C on a Jasco P-2000 polarimeter. Commercially available grades of organic solvents of adequate purity are used in all reactions.

4.1.1. *p*-Methoxyphenyl 6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-galactopyranoside 2

To a solution of compound **7** (4 g, 7.94 mmol) in anhydrous THF (25 mL) were added $NaBH_3CN$ (3 g, 47.74 mmol) and MS-3Å (5 g) and the reaction mixture was allowed to stir at 0 °C for 15 min. To the cooled reaction mixture was added dropwise HCl–Et_2O (~10 mL) until the pH of the solution became ~2. After stirring the reaction mixture at 5 °C for 1 h, it was poured into a satd. $NaHCO_3$ solution and extracted with CH_2Cl_2 (150 mL). The organic layer was washed with water, dried (Na_2SO_4), and concentrated to give the crude product, which was purified over SiO_2 using hexane/EtOAc (3:1) as eluant to give pure compound **2** (3.3 g, 82%). White solid; mp 147–148 °C; $[\alpha]_D^{25} = +6$ (c 1.0, $CHCl_3$); IR (KBr): 3418, 3064, 2870, 1776, 1717, 1600, 1500, 1478, 1217, 1099, 914 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79–7.25 (m, 9 H, Ar-H), 6.83 (d, J = 9.0 Hz, 2 H, Ar-H), 6.68 (d, J = 9.0 Hz, 2 H, Ar-H), 5.67 (d, J = 8.0 Hz, 1 H, H-1), 4.60 (d, J = 12.0 hz, 1 H, PhCH2), 4.55 (d, J = 12.0 Hz, 1 H, PhCH_2), 4.36–4.30 (m, 2 H, H-6_{ab}), 3.80–3.75 (m, 2 H, H-4, H-5), 3.69 (s, 3 H, OCH_3), 3.67–3.62 (m, 2 H, H-2, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.3 (PhthCO), 155.4–114.4 (Ar-C), 97.5 (C-1), 74.4 (C-3), 73.7 (PhCH_2), 73.3 (C-4), 71.7 (C-5), 70.0 (C-6), 56.3 (OCH_3), 55.5 (C-2); ESI-MS: 528.1 [M+Na]$^+$; Anal. Calcd for $C_{28}H_{27}NO_8$ (505.17): C, 66.53; H, 5.38. Found: C, 66.40; H, 5.60.

4.1.2. Ethyl 3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactopyranoside 5

A solution of compound **8** (4 g, 8.27 mmol) in 80% aq AcOH (100 mL) was allowed to stir at 80 °C for 1.5 h. The solvents were then removed under reduced pressure and the reaction mixture was co-evaporated with toluene. To a solution of the crude product in CH_2Cl_2 (30 mL) were added pyridine (5 mL) and benzoyl cyanide (1.1 g, 8.38 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and successively washed with 1 M HCl, satd $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane/Et_2O (3:1) as eluant to give pure compound **5** (3.3 g, 80%). Yellow oil; $[\alpha]_D^{25} = +29$ (c 1.0, $CHCl_3$); IR (neat): 3443, 2926, 1718, 1638, 1219, 1080, 770 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08–7.40 (m, 9 H, Ar-H), 5.73 (dd, J = 11.0, 3.0 Hz, 1 H, H-3), 5.47 (d, J = 10.5 Hz, 1 H, H-1), 4.68 (t, J = 11.0 Hz each, 1 H, H-2), 4.65 (dd, J = 11.5, 6.0 Hz, 1 H, H-6_a), 4.55 (dd, J = 11.5, 6.0 Hz, 1 H, H-6_b), 4.24 (d, J = 3.0 Hz, 1 H, H-4), 4.11–4.09 (m, 1 H, H-5), 2.73–2.60 (m, 2 H, SCH_2CH$_3$), 1.96 (s, 3 H, COCH_3), 1.25 (t, J = 7.4 Hz each, 3 H, SCH$_2$CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.7 (COCH$_3$), 167.9, 167.8 (PhthCO), 166.3 (PhCO), 134.2–123.6 (Ar-C), 81.3 (C-1), 76.1 (C-3), 71.2 (C-4), 66.8 (C-5), 63.1 (C-6), 50.1 (C-2), 24.2 (SCH$_2$CH$_3$), 20.7 (COCH$_3$), 14.9 (SCH$_2$CH$_3$); ESI-MS: 522.1 [M+Na]$^+$; Anal. Calcd for $C_{25}H_{25}NO_8S$ (499.13): C, 60.11; H, 5.04. Found: C, 59.93; H, 5.20.

4.1.3. Ethyl (2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside 9

To a solution of compound **3** (2 g, 3.72 mmol) and compound **4** (1.7 g, 3.82 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS-4Å (2 g) and the reaction mixture was stirred at −30 °C for 30 min under argon. To the cooled reaction mixture was added NIS (0.9 g, 4.0 mmol) followed by TMSOTf (5 μL) and then allowed to stir at the same temperature for 45 min. The reaction mixture was poured into a 5% $Na_2S_2O_3$ solution 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 (7:1) as eluant to give pure

compound **9** (2.5 g, 73%). White solid; mp 55–56 °C; $[\alpha]_D^{25} = +30$ (*c* 1.0, $CHCl_3$); IR (KBr): 3442, 3064, 2930, 1736, 1602, 1494, 1452, 1380, 1315, 1260, 1092, 872, 755, 709, 617, 510 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.95–7.20 (m, 25 H, Ar-H), 5.85 (t, *J* = 9.5 Hz each, 1 H, H-3_B), 5.53 (t, *J* = 10.0 Hz, each, 1 H, H-2_B), 5.49, (t, *J* = 10.0 Hz each, 1 H, H-4_B), 4.81 (d, *J* = 12.5 Hz, 1 H, PhCH_2), 4.79 (br s, 1 H, H-1_C), 4.75 (d, *J* = 10 Hz, 1 H, H-1_B), 4.67 (d, *J* = 12.5 Hz, 1 H, PhCH_2), 4.56 (d, *J* = 12.5 Hz, 1 H, PhCH_2), 4.49 (d, *J* = 12.5 Hz, 1 H, PhCH_2), 4.32 (dd, *J* = 9.0, 3.0 Hz, 1 H, H-3_C), 4.26–4.24 (m, 1 H, H-5_C), 4.09–4.05 (m, 2 H, H-4_C, H-5_B), 3.90 (dd, *J* = 12.0, 6.0 Hz, 1 H, H-6_{aC}), 3.65–3.58 (m, 3 H, H-6_{bC}, H-6_{abB}), 3.48 (dd, *J* = 10.0, 3.5 Hz, 1 H, H-2_C), 2.79–2.71 (m, 2 H, SC$H_2$$CH_3$), 1.38, 1.32 (2 s, 6 H, 2 COCH_3), 1.23 (t, *J* = 7.4 Hz each, 3 H, SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7, 165.1, 165.1, 165.0, (3 PhCO), 138.4–127.4 (Ar-C), 109.0 (C$(CH_3)_2$), 97.4 (C-1_C), 83.5 (C-1_B), 77.4 (C-4_C), 76.6 (C-2_C), 75.8 (C-3_C), 74.4 (C-3_B), 73.8 (C-5_B), 73.1 (PhCH_2), 72.4 (PhCH_2), 70.5 (C-4_B), 69.6 (C-2_B), 69.4 (C-6_C), 67.1 (C-6_B), 66.5 (C-5_C), 28.2 (CH_3), 26.4 (CH_3), 23.9 (SC$H_2$$CH_3$), 14.9 ($SCH_2CH_3$); ESI-MS: 941.3 $[M+Na]^+$; Anal. Calcd for $C_{52}H_{54}O_{13}S$ (918.32): C, 67.96; H, 5.92. Found: C, 67.80; H, 6.15.

4.1.4. *p*-Methoxyphenyl (2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-galactopyranoside 10

To a solution of compound **2** (1 g, 1.97 mmol) and compound **9** (2 g, 2.17 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS-4Å (2 g) and the reaction mixture was stirred at −30 °C for 30 min under argon. To the cooled reaction mixture was added NIS (0.5 g, 2.22 mmol) followed by TMSOTf (3 μL) and then allowed to stir at the same temperature for 45 min. The reaction mixture was poured into a 5% $Na_2S_2O_3$ solution 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 (7:1) as eluant to give pure compound **10** (1.9 g, 71%). Yellow oil; $[\alpha]_D^{25} = +35$ (*c* 1.0, $CHCl_3$); IR (neat): 3457, 3254, 2897, 2453, 1756, 1709, 1645, 1576, 1487, 1324, 1245, 1176, 1095, 765, 710 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.92–7.21 (m, 34 H, Ar-H), 6.77 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.62 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.68 (t, *J* = 9.5 Hz each, 1 H, H-3_B), 5.64 (d, *J* = 8.5 Hz, 1 H, H-1_A), 5.24 (t, *J* = 8.5 Hz each, 1 H, H-2_B), 5.20 (t, *J* = 8.5 Hz, each, 1 H, H-4_B), 5.06 (d, *J* = 8.0 Hz, each, 1 H, H-1_B), 4.78 (d, *J* = 10.5 Hz, 1 H, PhCH_2), 4.76 (br s, 1 H, H-1_C), 4.64–4.61 (m, 3 H, PhCH_2), 4.55–4.48 (m, 2 H, PhCH_2), 4.45 (dd, *J* = 8.5 each 1 H, H-2_A), 4.41–4.35 (m, 1 H, H-5_A), 4.27–4.24 (m, 2 H, H-3_C, H-5_C), 4.15 (d, *J* = 2.5 Hz, 1 H, H-4_A), 3.98–3.95 (m, 2 H, H-4_C, H-6_{aC}), 3.87–3.81 (m, 2 H, H-6_{aA}, H-6_{aB}), 3.75–3.70 (m, H-5_B, H-6_{bC}), 3.68–3.66 (m, 1 H, H-6_{bB}), 3.65 (s, 3 H, OCH_3), 3.54 (dd, *J* = 10.0, 3.5 Hz, 1 H, H-3_A), 3.51–3.47 (m, 1 H, H-6_{bA}), 3.46 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-2_C), 1.31, 1.09 (2 s, 6 H, 2 CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7, 165.5, 165.0 (3 PhCO), 155.6–114.4 (Ar-C), 109.0 (C$(CH_3)_2$), 101.3 (C-1_B), 97.5 (C-1_A), 97.2 (C-1_C), 77.3 (C-4_A), 76.6 (C-3_C), 75.9 (C-2_C), 73.9 (C-4_C), 73.7 (C-3_B), 73.4 (PhCH_2), 73.1 (C-5_B), 73.0 (C-2_B), 72.7 (C-4_B), 72.4 (PhCH_2), 72.3 (PhCH_2), 69.5 (C-5_C), 69.4 (C-6_C), 68.9 (C-6_A), 68.6 (C-3_A), 66.9 (C-6_B), 66.1 (C-5_A), 55.4 (OCH_3), 54.6 (C-2_A), 28.2 (CH_3), 26.3 (CH_3); ESI-MS: 1384.4 $[M+Na]^+$; Anal. Calcd for $C_{78}H_{75}NO_{21}$ (1361.48): C, 68.76; H, 5.55. Found: C, 68.58; H, 5.70.

4.1.5. *p*-Methoxyphenyl (2,6-di-*O*-benzyl-α-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-galactopyranoside 11

A solution of compound **10** (1.5 g, 1.10 mmol) in acetic anhydride (3 mL) and pyridine (3 mL) was allowed to stir at room temperature for 2 h. The solvents were removed under reduced pressure to give the acetylated product. A solution of the acetylated product in 80% aq AcOH (mL) was stirred at 80 °C for 1.5 h and the solvents were removed under reduced pressure. The crude product was purified over SiO_2 using hexane/EtOAc (4:1) as eluant to give pure compound **11** (1.4 g, 93%). Yellow oil; $[\alpha]_D^{25} = +12$ (*c* 1.0, $CHCl_3$); IR (neat): 3480, 3064, 3017, 2926, 2854, 1778, 1720, 1602, 1585, 1507, 1453, 1386, 1316, 1281, 1094, 830, 759, 710 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.05–7.23 (m, 34 H, Ar-H), 6.76 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.61 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.86 (t, *J* = 9.0 Hz each, 1 H, H-3_B), 5.73 (d, *J* = 2.5 Hz, 1 H, H-4_A), 5.64 (d, *J* = 8.5 Hz, 1 H, H-1_A), 5.56 (t, *J* = 10.0 Hz each, 1 H, H-4_B), 5.42 (t, *J* = 9.0 Hz each, 1 H, H-2_B), 5.02 (d, *J* = 7.5 Hz, 1 H, H-1_B), 4.70 (dd, *J* = 8.5 Hz, each, 1 H, H-2_A), 4.62 (br s, 2 H, PhCH_2), 4.53–4.45 (dd, *J* = 11.5 Hz, each, 2 H, PhCH_2), 4.43 (br s, 2 H, PhCH_2), 4.31 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_A), 3.93–3.87 (m, 5 H, H-4_C, H-5_A, H-5_C, H-6_{abA}), 3.84–3.76 (m, 3 H, H-5_B, H-6_{aB}, H-6_{aC}), 3.69 (dd, *J* = 9.5, 3.0 Hz, 1 H, H-2_C), 3.66 (s, 3 H, OCH_3), 3.57–3.54 (m, 3 H, H-3_C, H-6_{bB}, H-6_{bC}), 1.69 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (COCH_3), 165.7, 165.4, 165.0 (3 PhCO), 155.6–114.3 (Ar-C), 100.9 (C-1_B), 97.5 (C-1_C), 97.3 (C-1_A), 76.6 (C-2_C), 74.3 (C-4_C), 73.5 (C-5_C), 73.4 (2 C, 2 PhCH_2), 73.2 (2 C, C-2_B, C-3_A), 72.8 (PhCH_2), 72.7 (C-3_B), 70.4 (C-4_A), 70.0 (C-6_C), 69.9 (C-4_B), 69.8 (C-6_A), 69.6 (C-5_A), 69.0 (C-3_C), 68.7 (C-5_B), 66.7 (C-6_B), 55.4 (OCH_3), 51.4 (C-2_A), 20.3 (COCH_3); ESI-MS: 1386.4 $[M+Na]^+$; Anal. Calcd for $C_{77}H_{73}NO_{22}$ (1363.46): C, 67.78; H, 5.39. Found: C, 67.60; H, 5.60.

4.1.6. Ethyl (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactopyranoside 12

To a solution compound **5** (2 g, 4.0 mmol) and compound **6** (2 g, 4.17 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS-4Å (2 g) and the reaction mixture was stirred at −30 °C for 30 min under argon. To the cooled reaction mixture was added NIS (950 mg, 4.22 mmol) followed by TMSOTf (5 μL) and it was allowed to stir at the same temperature for 45 min. The reaction mixture was poured into 5% $Na_2S_2O_3$ solution 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 (7:1) as eluant to give pure compound **12** (2.7 g, 74%). Yellow oil; $[\alpha]_D^{25} = +10$ (*c* 1.0, $CHCl_3$); IR (neat): 3080, 2980, 1750, 1654, 1345, 1437, 1324, 1256, 1123, 1098, 1076, 789, 745, 710 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.05–7.26 (m, 24 H, Ar-H) 5.77 (dd, *J* = 11.0, 3.0 Hz, 1 H, H-3_D), 5.52 (d, *J* = 10.5 Hz, 1 H, H-1_D), 5.14 (d, *J* = 2.5 Hz, 1 H, H-1_E), 4.87–4.71 (m, 6 H, PhCH_2), 4.60–4.56 (m, 2 H, H-2_D, H-6_{aD}), 4.43 (dd, *J* = 11.5, 6.0 Hz, 1 H, H-6_{bD}), 4.32 (d, *J* = 2.0 Hz, 1 H, H-4_D), 4.15–4.12 (m, 1 H, H-5_D), 4.09–4.08 (m, 1 H, H-2_E), 3.96 (dd, *J* = 8.0, 2.5 Hz, 1 H, H-3_E), 3.84–3.78 (m, 1 H, H-5_E), 3.58 (t, *J* = 8.5 Hz each, 1 H, H-4_E), 2.78–2.62 (m, 2 H, SC$H_2$$CH_3$), 1.76 (s, 3 H, COC$H_3$), 1.26 (d, *J* = 6.0 Hz, 3 H, CH_3), 1.23 (t, *J* = 7.4 Hz each, 3 H, SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.4 (COCH_3), 165.9 (PhCO), 138.5–123.6 (Ar-C), 99.9 (C-1_E), 81.3 (C-1_D), 80.3 (C-4_E), 79.5 (C-3_E), 75.9 (C-2_E), 75.8 (C-5_D), 74.7 (PhCH_2), 73.1 (PhCH_2), 72.5 (C-4_D), 72.3 (PhCH_2), 71.9 (C-3_D), 69.5 (C-5_E), 63.1 (C-6_D), 50.3 (C-2_D), 24.2 (SC$H_2$$CH_3$), 20.5 (COC$H_3$), 18.1 (C$H_3$), 15.0 ($SCH_2CH_3$); ESI-MS: 938.3 $[M+Na]^+$; Anal. Calcd for $C_{52}H_{53}NO_{12}S$ (915.32): C, 68.18; H, 5.83. Found: C, 68.00; H, 6.00.

4.1.7. *p*-Methoxyphenyl (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-2-*N*-phthalimido-β-D-galactopyranosyl)-(1→3)-(2,6-di-*O*-benzyl-α-D-galactopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-galactopyranoside 13

To a solution of compound **11** (1 g, 0.73 mmol) and compound **12** (0.7 g, 0.76 mmol) in anhydrous CH_2Cl_2 (10 mL) was added

MS-4Å (2 g) and the reaction mixture was stirred at −30 °C for 30 min under argon. To the cooled reaction mixture was added NIS (190 mg, 0.84 mmol) followed by TMSOTf (2 μL) and then allowed to stir at the same temperature for 45 min. The reaction mixture was poured into a 5% $Na_2S_2O_3$ solution and extracted with CH_2Cl_2 (50 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 (7:1) as eluant to give pure compound **13** (1.2 g, 74%). Yellow oil; $[\alpha]_D^{25} = +8$ (*c* 1.0, $CHCl_3$); IR (neat): 3272, 3064, 3017, 2930, 1778, 1721, 1602, 1507, 1453, 1430, 1585, 1507, 1452, 1430, 1385, 1282, 1261, 1218, 1177, 1028, 919, 755, 710, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.07–6.97 (m, 58 H, Ar-H), 6.77 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.48 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.86 (br s, 1 H, H-4_A), 5.84 (t, *J* = 10.5 Hz each, 1 H, H-3_B), 5.77 (d, *J* = 8.5 Hz, 1 H, H-1_A), 5.64 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_D), 5.47 (d, *J* = 8.5 Hz, 1 H, H-1_D), 5.43 (t, *J* = 9.5 Hz each, 1 H, H-2_B), 5.35 (t, *J* = 9.5 Hz each, 1 H, H-4_B), 5.01 (br s, 1 H, H-1_E), 4.87 (d, *J* = 8.0 Hz, 1 H, H-1_B), 4.82–4.76 (m, 5 H, PhCH_2), 4.74–4.70 (m, 2 H, H-2_A, H-2_D), 4.59–4.51 (m, 4 H, PhCH_2), 4.47 (d, *J* = 3.5 Hz, 1 H, H-1_C), 4.41 (d, *J* = 11.5 Hz, PhCH_2), 4.34 (d, *J* = 11.5 Hz, 1 H, PhCH_2), 4.28–4.17 (m, 4 H, H-4_D, H-6_{aB}, H-6_{aD}, PhCH_2), 4.12–4.07 (m, 3 H, H-6_{abA}, H-6_{bD}), 4.06 (br s, 1 H, H-2_E), 3.95–3.89 (m, 4 H, H-5_A, H-5_E, H-6_{bB}, H-6_{aC}), 3.87–3.75 (m, 2 H, H-3_A, H-3_E), 3.74–3.70 (m, 1 H, H-5_B), 3.67–3.65 (m, 2 H, H-2_C, H-4_C), 3.57 (s, 3 H, OCH_3), 3.56–3.51 (m, 2 H, H-5_C, H-5_D), 3.40 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_C), 3.35–3.27 (m, 2 H, H-4_E, H-6_{bC}), 1.77, 1.70 (2 s, 6 H, 2 COCH_3), 0.87 (d, *J* = 6.0 Hz, 3 H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.0, (2 *C*OCH$_3$), 167.9, 167.8 (2 Phth*C*O), 165.6, 165.5, 165.3, 165.1 (4 Ph*C*O), 155.6–114.2 (Ar-C), 101.4 (C-1_A), 100.3 (C-1_C), 98.7 (C-1_D), 97.9 (C-1_E), 97.2 (C-1_B), 80.7 (C-5_A), 80.5 (C-5_D), 78.9 (C-3_A), 76.7 (C-3_D), 76.1 (C-2_E), 75.6 (C-4_D), 74.7 (C-3_E), 74.6 (Ph*C*H$_2$), 74.0 (C-4_C), 73.7 (Ph*C*H$_2$), 73.4 (C-3_C), 73.2 (2 C, 2 Ph*C*H$_2$), 73.1 (Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 72.8 (C-4_A), 72.3 (C-2_B), 71.9 (C-4_B), 70.0 (C-4_E), 70.6 (C-6_A), 70.3 (C-3_B), 69.7 (C-6_D), 69.5 (C-5_C), 68.7 (C-5_B), 68.5 (C-5_E), 66.7 (C-6_C), 62.8 (C-6_B), 55.4 (OCH_3), 51.4 (C-2_A), 51.1 (C-2_D), 20.5 (COCH_3), 20.4 (COCH_3), 18.1 (CH_3); MALDI-MS: 2239.7 $[M+Na]^+$; Anal. Calcd for $C_{127}H_{120}N_2O_{34}$ (2216.77): C, 68.76; H, 5.45. Found: C, 68.57; H, 5.66.

4.1.8. *p*-Methoxyphenyl (α-L-rhamnopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→3)-(α-D-galactopyranosyl)-(1→6)-(β-D-glucopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside 1

To a solution of compound **13** (1 g, 0.45 mmol) in EtOH (10 mL) was added $NH_2NH_2{\cdot}H_2O$ (0.5 mL, 10.3 mmol) and the reaction mixture was allowed to stir at 80 °C for 8 h. The reaction mixture was then evaporated to dryness and a solution of the crude mass in acetic anhydride (2 mL) and pyridine (2 mL) was kept at room temperature for 3 h and concentrated under reduced pressure. To a solution of the crude acetylated product in CH_3OH (10 mL) was added 10% Pd-C (150 mg) followed by the dropwise addition of Et_3SiH (1 mL, 6.26 mmol) and the reaction mixture was allowed to stir at room temperature for 12 h. The reaction mixture was filtered through a Celite® bed and the filtrate was concentrated under reduced pressure. A solution of the crude product in 0.1 M CH_3ONa (10 mL) was allowed to stir at room temperature for 4 h, neutralized with Dowex 50 W X8 (H^+) resin, filtered, and concentrated. The crude product was purified over Sephadex® LH-20 gel using CH_3OH/H_2O (4:1) as eluant to give pure compound **1** (270 mg, 60%). Glass; $[\alpha]_D^{25} = +4$ (*c* 1.0, H_2O); IR (KBr): 3020, 2929, 1742, 1367, 1214, 1066, 799 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 6.99 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.68 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.12 (br s, 1 H, H-1_E), 5.05 (d, *J* = 8.5 Hz, 1 H, H-1_A), 4.90 (d, *J* = 8.5 Hz, 1 H, H-1_D), 4.87 (d, *J* = 3.0 Hz, 1 H, H-1_C), 4.62 (d, *J* = 7.5 Hz, 1 H, H-1_B), 4.20 (t, *J* = 8.5 Hz each, 1 H, H-2_A), 4.17–4.13 (m, 2 H, H-4_A, H-4_D), 4.10 (br s, 1 H, H-4_C), 4.08–4.00 (m, 1 H, H-2_D), 3.98–3.91 (m, 3 H, H-2_E, H-3_C, H-5_C), 3.88–3.78 (m, 6 H, H-3_A, H-3_D, H-4_B, H-5_B, H-5_D, H-6_{aC}), 3.76–3.55 (m, 9 H, H-3_E, H-5_E, H-6_{abA}, H-6_{abB}, H-6_{abD}, H-6_{bC}), 3.69 (s, 3 H, OCH_3), 3.44–3.32 (m, 5 H, H-2_B, H-2_C, H-3_B, H-4_E, H-5_A), 2.11, 1.80 (2 s, 6 H, 2 COCH_3), 1.19 (d, *J* = 6.0 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, D_2O): δ 175.8, 173.7 (2 *C*OCH$_3$), 154.8–115.0 (Ar-C), 104.0 (C-1_B), 103.1 (C-1_D), 101.7 (C-1_E), 100.6 (C-1_A), 98.6 (C-1_C), 76.7 (C-4_A), 75.7 (C-5_A), 75.1 (C-3_C), 74.8 (C-2_E), 74.5 (C-3_D), 74.4 (C-2_B), 73.7 (C-2_C), 72.1 (C-3_E), 71.7 (C-4_E), 71.4 (C-5_C), 70.5 (C-5_E), 70.2 (C-3_B), 70.0 (C-3_A), 69.4 (C-4_B), 69.2 (C-4_C), 69.0 (C-4_D), 67.2 (C-5_B), 66.2 (C-5_D), 61.4 (C-6_C), 61.0 (2 C, C-6_B, C-6_D), 60.7 (C-6_A), 55.8 (OCH_3), 53.7 (C-2_D), 53.4 (C-2_A), 22.4, 20.0 (2 COCH_3) 16.6 (CCH_3); ESI-MS: 1023.3 $[M+Na]^+$; Anal. Calcd for $C_{41}H_{64}N_2O_{26}$ (1000.37): C, 49.20; H, 6.44. Found: C, 49.03; H, 6.66.

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