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Concise synthesis of two pentasaccharides corresponding to the α-chain oligosaccharides of *Neisseria gonorrhoeae* and *Neisseria meningitidis*☆

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ABSTRACT

Two pentasaccharides containing a common tetrasaccharide (lacto-*N*-neotetraose) core, and D-galactosamine and *N*-acetyl neuraminic acid in the non-reducing ends, respectively, corresponding to the lipooligosaccharides of *Neisseria gonorrhoeae* and *Neisseria meningitidis* were synthesized in a very concise manner from a common trisaccharide derivative using minimum number of steps. Thioglycosides and glycosyl trichloroacetimidate have been used as glycosyl donors for glycosylations and yields were excellent in every step.

1. Introduction

Neisseria gonorrhoeae and *Neisseria meningitidis* are two important human pathogens causing gonorrhea, meningitis, and septicemia.[1] Although, gonorrhea infection is more common in comparison to meningococcal meningitis, meningitis and induced septicemia are of much more serious concern because of the associated mortality.[2] The pathogenicity of *N. gonorrhoeae* and *N. meningitidis* originated from the antigenic lipooligosaccharides (LOSs) present in the outer surface of their cell membranes.[3] In general, LOSs are a family of complex oligosaccharides[3] found in the cell-wall glycolipids of Gram-negative bacteria and possess a number of antigenic haptens responsible for natural and acquired immunity.[3,4] The intensity of infections caused by these two species is proportional to the levels of circulating gonococcal and meningococcal LOSs in the endotoxins released from their cell surfaces.[5] The LOS induces a proinflammatory response in the host, which influence the colonization to cross the epithelial barrier to induce the clinical outcome of gonorrhea and meningitis infections.[6] Serologically meningococcal LOS has been classified into 12 immunotypes of which 8 have been structurally characterized.[7] Recent structural and immunochemical analysis of gonococcal[2,8] and meningococcal[9] LOSs established that both LOSs are multiantennary and contain a pentasaccharide α-chain having a common core of lacto-*N*-neotetraose moiety. The α-chain of gonococcal LOS terminated with a D-galactosamine moiety whereas in the case *N. meningitidis* it is *N*-acetyl neuraminic acid (sialic acid).

Development of antibacterial vaccines is one of the thrust areas of research in the medicinal chemistry. Due to the established antigenic properties of LOSs, biologists have focused extensive efforts toward the study of bacterial oligosaccharides as potential antibacterial glycoconjugate vaccine candidates and a number of reports appeared in the literature for the development of the glycoconjugate vaccine candidates against several pathogenic bacteria.[10] Although, LOSs can be isolated from the natural sources, their limited availability cannot always meet the required quantity for their extensive biological evaluation. Therefore, only option left for the large-scale production of the oligosaccharides is to develop efficient chemical synthetic strategies. In this report, we describe concise synthesis of two pentasaccharides corresponding to the α-chain of *N. gonorrhoeae* and *N. meningitidis* as their 4-methoxyphenyl glycosides (Fig. 1).

2. Results and discussion

Two target pentasaccharides as their 4-methoxyphenyl glycosides (**1** and **2**) was synthesized using a combination of sequential glycosylations and block synthetic strategy. A common trisaccharide derivative (**13**) was used as a glycosyl acceptor in both cases minimizing protective group manipulations. Suitably functionalized monosaccharide derivatives (Fig. 2) used in the synthesis of target molecules were prepared from the commercially available

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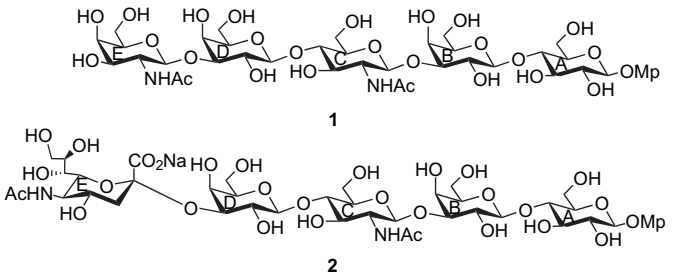


Figure 1. Structure of the synthesized pentasaccharides corresponding to the α-chain of *Neisseria gonorrhoeae* (**1**) and *Neisseria meningitidis* (**2**) as their 4-methoxyphenyl glycosides.

reducing sugars using the literature reported methodologies. Compound **5** was prepared in 80% overall yield from 4-methoxyphenyl 2,3,4,6 tetra-*O*-acetyl-β-D-galactopyranoside (**9**)[11] following a sequence of reactions comprising deacetylation, isopropylidenation,[12] benzoylation, and acid catalyzed removal of isopropylidene ketal[13] followed by selective acetylation via orthoesterification using triethyl orthoacetate and *p*-TsOH[14] (Scheme 1).

After having the access to a series of suitably protected monosaccharide derivatives, synthesis of target pentasaccharides (**1** and **2**) has been attempted. For the preparation of compound **1**, a disaccharide trichloroacetimidate glycosyl donor (**11**) was coupled with a trisaccharide glycosyl acceptor (**13**) using Schmidt's trichloroacetimidate glycosylation method.[15] Glycosylation of compound **5** with thioglycoside derivative **6**[16] in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[17] provided the disaccharide derivative **10** in 82% yield. Presence of signals in the ^{1}H and ^{13}C NMR spectra confirmed the formation of **10**. Oxidative removal of the anomeric 4-methoxyphenyl group of compound **10** using ammonium ceric nitrate (CAN)[18] furnished disaccharide hemiacetal, which was treated with trichloroacetonitrile in the presence of DBU[19] to generate the disaccharide trichloroacetimidate derivative **11** in 85% yield, which was used directly without further purification (Scheme 1).

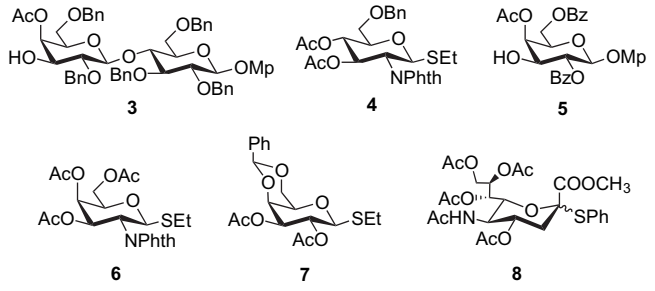
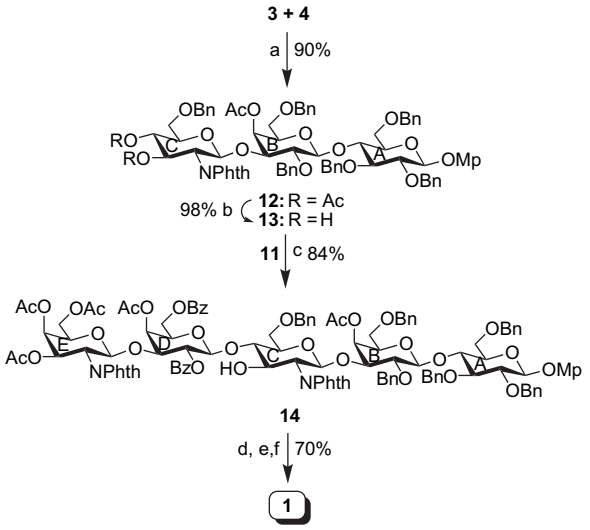


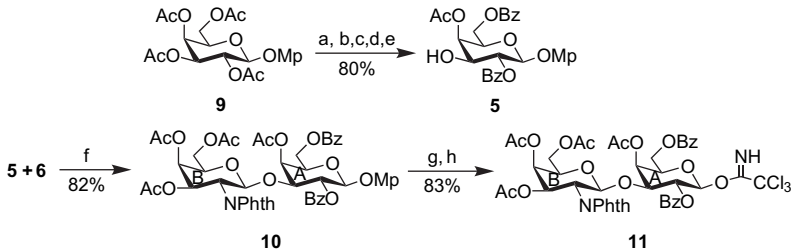
Figure 2. Suitably functionalized monosaccharide intermediates used for the synthesis of pentasaccharides (**1** and **2**).

In another experiment, iodonium ion promoted glycosylation of thioglycoside **4**[20] with disaccharide acceptor **3**[21] using NIS–TMSOTf furnished trisaccharide derivative **12**, which was deacetylated to give trisaccharide glycosyl acceptor **13** in excellent yield. Formation of compound **12** was confirmed from its spectral analysis. Selective glycosylation of disaccharide donor **11** with trisaccharide diol acceptor **13** in the presence of TMSOTf[15] afforded the pentasaccharide derivative **14** in 86% yield, which was supported by its NMR spectra. Deprotection of protecting groups involving hydrazinolysis,[22] N-acetylation, saponification, and hydrogenolysis[23] furnished target pentasaccharide **1** as its 4-methoxyphenyl glycoside in 78% overall yield. Presence of signals at δ 5.08 (d, H-1$_D$), 4.74 (d, H-1$_C$), 4.66 (d, H-1$_E$), 4.51 (2d, H-1$_A$ and H-1$_B$) in the ^{1}H NMR and at δ 103.3 (C-1$_E$), 102.9 (2C, C-1$_A$ and C-1$_B$), 102.7 (C-1$_C$), 101.0 (C-1$_D$) in the ^{13}C NMR spectra confirmed the formation of compound **1** (Scheme 2).



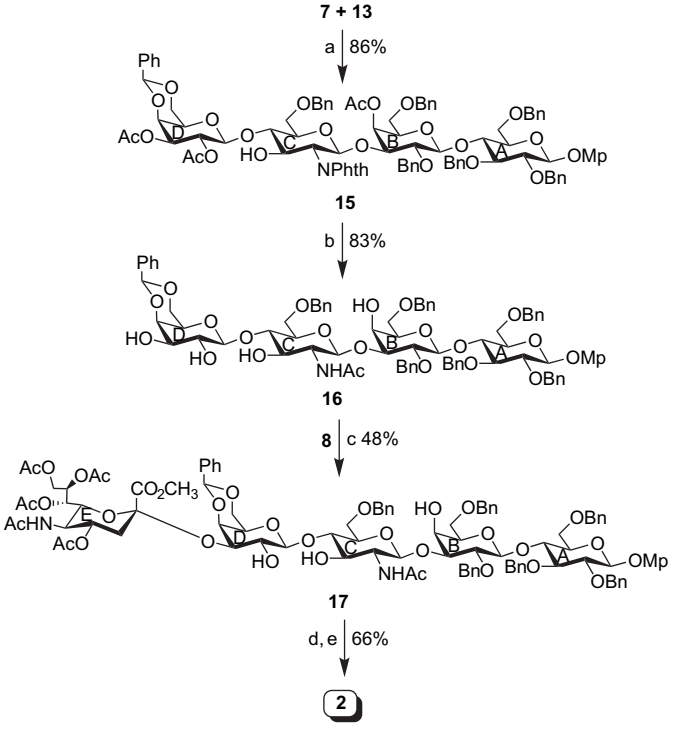
Scheme 2. Reagents: (a) *N*-iodosuccinimide, TMSOTf, MS-4 Å, CH_2Cl_2, −30 °C, 1 h; (b) CH_3ONa, CH_3OH, rt, 30 min; (c) TMSOTf, CH_2Cl_2, −10 °C, 1 h; (d) (i) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 6 h; (ii) Ac_2O, pyridine, rt, 6 h; (e) CH_3ONa, CH_3OH, rt, 10 h; (f) H_2, 20% $Pd(OH)_2$–C, CH_3OH, rt, 24 h.

In another experiment, selective glycosylation of compound **7**[24] with trisaccharide diol acceptor **13** furnished tetrasaccharide derivative **15** in 87% yield. Spectral data of compound **15** supported its formation. On treatment with hydrazine hydrate[22] followed by N-acetylation and saponification afforded tetrasaccharide tetraol **16** acceptor in 82% yield. In order to achieve stereo- and regioselective glycosylation of compound **16** with sialic acid thioglycoside donor **8**,[25] a number of literature reported glycosylation condition were explored.[26] After a series of unsuccessful attempts, pentasaccharide derivative **17** was obtained in moderate yield (48%) by condensation of compound **16** with compound **8** using NIS–TMSOTf as



Scheme 1. Reagents: (a) CH_3ONa, CH_3OH, rt, 5 h; (b) 2,2-dimethoxypropane, *p*-TsOH, DMF, rt, 12 h; (c) benzoyl chloride, pyridine, rt, 6 h; (d) 80% AcOH, 80 °C, 2 h; (e) (i) $CH_3C(OC_2H_5)_3$, *p*-TsOH, DMF, 2 h; (ii) 80% AcOH, rt, 1 h; (f) *N*-iodosuccinimide, TMSOTf, MS-4 Å, CH_2Cl_2, −30 °C, 1 h; (g) CAN, CH_3CN, H_2O, rt, 1.5 h; (h) CCl_3CN, DBU, CH_2Cl_2, −10 °C, 1 h.

thioglycoside activator in a mixed solvent (CH_3CN–CH_2Cl_2 5:1 v/v). Formation of compound **17** was confirmed from its spectral analysis. The presence of α-linked sialic acid moiety in the compound **17** was supported by its ^1H NMR spectrum, which was comparable with the NMR signals of α-linked sialic acid moiety in earlier reports.[26,27] In the ^1H NMR spectrum of compound **17**, appearance of a signal at δ 2.74 (dd, J=12.0, 4.8 Hz, H-3_{eE}) indicated the α-stereochemistry of the C-2 of sialic acid moiety. In case of β-linked sialic acid this signal generally appears in upfield position. In addition, ^1H NMR signals for H-4_E (δ 4.95–4.86), H-7_E and H-8_E (δ 5.47–5.39) confirmed the presence of α-linked sialic acid moiety.[27] Hydrogenolysis followed by saponification of pentasaccharide derivative **17** furnished target pentasaccharide **2** in 74% yield (Scheme 3). Presence of signals at δ 4.82 (2d, H-1_A, H-1_C), 4.46 (2d, H-1_B, H-1_D), 2.82 (dd, H-3_{eE}), 1.70 (t, H-3_{aE}) in the ^1H NMR and δ 104.9 (2C, C-1_B, C-1_D), 103.1 (2C, C-1_A, C-1_C), 101.0 (C-2_E) in the ^{13}C NMR spectra supported the structure of compound **2** (Scheme 3). The stereocontrol of the glycosylation reactions was achieved using judicious choice of protecting groups in the glycosyl donors and acceptors.



Scheme 3. Reagents: (a) *N*-iodosuccinimide, TMSOTf, MS-4 Å, CH_2Cl_2, −30 °C, 1 h; (b) (i) $NH_2NH_2 \cdot H_2O$, EtOH, 80 °C, 6 h; (ii) Ac_2O, pyridine, rt, 6 h; (iii) CH_3ONa, CH_3OH, rt, 2 h; (c) *N*-iodosuccinimide, TMSOTf, MS-4 Å, CH_3CN–CH_2Cl_2 (5:1), −10 °C, 16 h; (d) H_2, 20% $Pd(OH)_2$–C, CH_3OH, rt, 24 h; (e) CH_3ONa, CH_3OH, rt, 8 h then few drops of water, 12 h.

3. Conclusions

In summary, efficient syntheses of two pentasaccharides (**1** and **2**) corresponding to the α-chain of lipooligosaccharides of *N. gonorrhoeae* and *N. meningitidis* have been achieved in excellent yield. Thioglycosides were used as glycosyl donors in most of the glycosylation reactions. Synthesis of compound **1** was carried out following a block synthetic strategy and compound **2** was prepared using sequential glycosylations. Both pentasaccharides contain 4-methoxyphenyl group as a temporary anomeric protecting group, which can be removed using standard reaction protocol for the preparation of glycoconjugates.

4. Experimental

4.1. General procedure

General methods. All the 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 in hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR, 2D COSY, HSQC spectra were recorded on Brucker Advance DPX 300 and 400 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ (ppm). ESI-MS were recorded on a MICROMASS QUTTRO II triple quadrupole mass spectrometer. Elementary analysis was carried out on Carlo ERBA-1108 analyzer. Optical rotations were measured at 25 °C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity are used in many reactions.

*4.1.1. 4-Methoxyphenyl 4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranoside (**5**)*

A solution of compound **9** (10 g, 22 mmol) in 0.1 M CH_3ONa (150 mL) was allowed to stir at room temperature for 5 h. The reaction mixture was neutralized with Amberlite-IR 120 (H^+) resin, filtered, and concentrated. To a solution of the deacetylated product in anhydrous DMF (30 mL) were added 2,2-dimethoxypropane (4 mL, 33 mmol) and *p*-TsOH (0.5 g), 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. To a solution of the dry mass in pyridine (60 mL) was added benzoyl chloride (8 mL, 69 mmol) at 0 °C and the reaction mixture was allowed to stir at room temperature for 6 h. The excess reagents were quenched with CH_3OH (5 mL) and the solvents were removed under reduced pressure. A solution of the crude mass in 80% AcOH (150 mL) was allowed to stir at 80 °C for 2 h and the solvents were removed under reduced pressure to give the crude diol, which was passed through a short pad of SiO_2. To a solution of the diol derivative in dry DMF (25 mL) were added triethyl orthoacetate (15 mL, 82 mmol) and *p*-TsOH (0.5 g), and the reaction mixture was allowed to stir at room temperature for 2 h. The solvents were removed under reduced pressure and a solution of the crude mass in 80% AcOH (100 mL) was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness and the crude mass was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to furnish pure **5** (9.5 g, 80%). R_f 0.4 (hexane–EtOAc 3:1); white solid; mp 150–51 °C; $[\alpha]_D^{25}$ −4 (*c* 1.2, $CHCl_3$); IR (KBr): 2961, 1727, 1601, 1509, 1451, 1380, 1269, 1220, 1110, 1074, 829, 710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.07–8.0 (m, 4H, Ar–H), 7.59–7.38 (m, 6H, Ar–H), 6.90 (d, J=9.0 Hz, 2H, Ar–H), 6.60 (d, J=9.0 Hz, 2H, Ar–H), 5.54 (br s, 1H, H-4), 5.51 (t, J=8.0 Hz, 1H, H-2), 5.03 (d, J=8.0 Hz, 1H, H-1), 4.50–4.43 (m, 2H, H-$6_{a,b}$), 4.15–4.06 (m, 2H, H-3 and H-5), 3.68 (s, 3H, OCH_3), 2.22 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8 (*C*OCH$_3$), 166.7, 165.9 (2*C*OPh), 155.6–114.4 (Ar–C), 100.8 (C-1), 73.2 (C-5), 71.5 (C-2), 71.4 (C-4), 69.8 (C-3), 62.4 (C-6), 55.4 (O*C*H$_3$), 20.7 (CO*C*H$_3$); ESI-MS: m/z 559.1 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{28}O_{10}$ (536.17): C, 64.92; H, 5.26. Found: C, 64.75; H, 5.50.

*4.1.2. 4-Methoxyphenyl (3,4,6-tri-O-acetyl-2-deoxy-2-N-phthalimido-β-D-galactopyranosyl)-(1→3)-4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranoside (**10**)*

To a solution of compound **5** (3 g, 5.6 mmol) and thioglycoside donor **6** (3.2 g, 6.7 mmol) in CH_2Cl_2 (30 mL) was added MS-4 Å (3 g) and the reaction mixture was allowed to stir at room temperature under argon for 30 min. The reaction mixture was cooled to −30 °C and *N*-iodosuccinimide (1.8 g, 8 mmol) and TMSOTf (50 μL) were

added to it. After stirring the reaction mixture at the same temperature for 1 h, it was filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), satd $NaHCO_3$ (100 mL), and water (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to furnish pure **10** (4.4 g, 82 %). R_f 0.3 (hexane–EtOAc 3:1); white solid; mp 181–83 °C; $[\alpha]_D^{25}$ +35 (*c* 1.52, $CHCl_3$); IR (KBr): 2924, 2142, 1727, 1593, 1379, 1226, 1114, 855, 773, 505 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.07–8.05 (m, 2H, Ar–H), 7.65–7.25 (m, 12H, Ar–H), 6.74 (d, *J*=9.0 Hz, 2H, Ar–H), 6.50 (d, *J*=9.0 Hz, 2H, Ar–H), 5.67 (dd, *J*=11.5, 3.3 Hz, H-3_B), 5.63 (d, *J*=3.6 Hz, 1H, H-4_A), 5.51 (dd, *J*=8.1, 8.1 Hz, 1H, H-2_A), 5.45 (d, *J*=8.3 Hz, 1H, H-1_B), 5.41 (d, *J*=3.1 Hz, H-4_B), 4.87 (d, *J*=8.0 Hz, 1H, H-1_A), 4.58–4.36 (m, 3H, H-2_B and H-$6_{a,bA}$), 4.25–4.18 (m, 2H, H-$6_{a,bB}$), 4.15–4.02 (m, 3H, H-3_A, H-5_A, and H-5_B), 3.63 (s, 3H, OCH_3), 2.24, 2.20, 2.05, 1.76 (4s, 12H, 4COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 170.7, 170.5, 170.1 (4COCH_3), 168.0, 167.4 (*C*OPhth), 166.4, 165.0 (2*C*OPh), 155.8–114.2 (Ar–C), 100.9 (C-1_A), 98.8 (C-1_B), 77.3 (C-5_A), 71.8 (C-5_B), 70.8 (2C, C-2_A and C-3_A), 69.2 (C-3_B), 67.5 (C-4_A), 66.4 (C-4_B), 62.8 (C-6_A), 61.1 (C-6_B), 55.4 (OCH_3), 51.2 (C-2_B), 20.7, 20.6 (2C), 20.3 (4$COCH_3$); ESI-MS: *m*/*z* 976.2 $[M+Na]^+$. Anal. Calcd for $C_{49}H_{47}NO_{19}$ (953.27): C, 61.70; H, 4.97. Found: C, 61.51; H, 5.20.

4.1.3. 4-Methoxyphenyl (3,4-di-O-acetyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**12**)

To a solution of compound **3** (5 g, 5.3 mmol) and thioglycoside donor **4** (3.4 g, 6.4 mmol) in anhydrous CH_2Cl_2 (60 mL) was added MS-4 Å (5 g) and the reaction mixture was allowed to stir at room temperature under argon for 30 min. The reaction mixture was cooled to −30 °C and *N*-iodosuccinimide (1.7 g, 7.5 mmol) and TMSOTf (50 μL) were added to it. After stirring the reaction mixture at the same temperature for 1 h, it was filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), satd $NaHCO_3$ (100 mL), and water (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to furnish pure **12** (6.7 g, 90%). R_f 0.3 (hexane–EtOAc 3:1); colorless syrup; $[\alpha]_D^{25}$ +7.3 (*c* 1.2, $CHCl_3$); IR (neat): 3021, 2925, 1749, 1720, 1488, 1385, 1219, 1064, 757, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.48–7.0 (m, 34H, Ar–H), 6.90–6.62 (m, 4H, Ar–H), 5.81 (t, *J*=9.2 Hz, 1H, H-3_C), 5.51 (d, *J*=8.4 Hz, 1H, H-1_C), 5.43 (br s, 1H, H-4_B), 5.18 (t, *J*=9.8 Hz, 1H, H-4_C), 4.95–4.70 (m, 3H, PhCH_2), 4.66 (d, *J*=7.3 Hz, 1H, H-1_A), 4.65–4.42 (m, 5H, PhCH_2), 4.30–4.14 (m, 2H, H-1_B and PhCH_2), 4.10–4.03 (m, 1H, H-2_C), 3.92–3.80 (m, 3H, H-3_A, H-4_A, and H-5_C), 3.78 (s, 3H, OCH_3), 3.66–3.62 (m, 2H, H-$6_{a,bC}$), 3.55–3.49 (m, 1H, H-3_B), 3.46–3.36 (m, 3H, H-2_A, H-5_B, and H-6_{aA}), 3.33–3.22 (m, 4H, H-2_B, H-6_{bA}, and H-$6_{a,bB}$), 3.02–2.97 (m, 1H, H-5_A), 2.05, 1.91, 1.81 (3s, 9H, 3COCH_3); ^{13}C NMR (75 MHz, CDCl3): δ 169.9, 169.6, 169.3 (3COCH_3), 167.5, 167.3 (*C*OPhth), 154.0–110.9 (Ar–C), 102.5 (C-1_A), 101.9 (C-1_B), 98.5 (C-1_C), 82.4 (C-2_A), 81.2 (C-3_B), 79.3 (C-5_C), 78.7 (C-5_A), 77.2 (C-5_B), 75.5 (C-2_B), 75.2, 74.8, 74.4, 73.6 (2C), 73.4 (6PhCH_2), 73.3 (C-3_A), 73.1 (C-4_A), 72.6 (C-4_C), 70.6 (C-3_C), 69.9 (C-6_C), 69.8 (C-4_B), 68.9 (C-6_B), 68.3 (C-6_A), 56.6 (OCH_3), 54.9 (C-2_C), 20.7, 20.6, 20.4 (3COCH_3); ESI-MS: *m*/*z* 1428.5 $[M+Na]^+$. Anal. Calcd for $C_{81}H_{83}NO_{21}$ (1405.55): C, 69.17; H, 5.95. Found: C, 68.96; H, 6.20.

4.1.4. 4-Methoxyphenyl (6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**13**)

A solution of compound **12** (6.5 g, 4.6 mmol) in 0.05 M CH_3ONa (130 mL) was allowed to stir at room temperature for 30 min and neutralized with Amberlite-IR 120 (H^+) resin. The reaction mixture was filtered and evaporated to dryness to give the crude product, which was passed through a short column of SiO_2 using hexane–EtOAc (2:1) as eluant to give pure **13** (6 g, 98%). R_f 0.3 (hexane–EtOAc 1:1); colorless syrup; IR (neat): 2923, 2372, 2142, 1661, 1591, 1055, 611 cm^{-1}; $[\alpha]_D^{25}$ −10 (*c* 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.0 (m, 34H, Ar–H), 6.92–6.60 (m, 4H, Ar–H), 5.42 (d, *J*=2.0 Hz, 1H, H-4_B), 5.28 (d, *J*=8.1 Hz, 1H, H-1_C), 4.95–4.65 (m, 4H, PhCH_2), 4.63 (d, *J*=7.6 Hz, 1H, H-1_A), 4.59–4.50 (m, 2H, PhCH_2), 4.46–4.30 (m, 2H, PhCH_2), 4.27–4.15 (m, 4H, H-1_B, H-3_C, and PhCH_2), 4.10–3.95 (m, 4H, H-2_C, H-4_C, and PhCH_2), 3.90–3.80 (m, 2H, H-3_A and H-6_{aA}), 3.76 (s, 3H, OCH_3), 3.75–3.71 (m, 1H, H-6_{bA}), 3.59–3.49 (m, 4H, H-2_A, H-3_B, and H-$6_{a,bC}$), 3.46–3.33 (m, 4H, H-2_B, H-5_B, and H-$6_{a,bB}$), 3.28–3.22 (m, 2H, H-4_A and H-5_C), 3.15–3.0 (m, 1H, H-5_A), 2.01 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2 (*C*OCH_3), 167.9 (2C, *C*OPhth), 155.0–110.9 (Ar–C), 102.6 (C-1_A), 101.9 (C-1_B), 98.7 (C-1_C), 82.5 (C-2_A), 81.2 (C-3_B), 78.9 (C-5_A), 78.1 (C-5_C), 77.3 (C-5_B), 75.5 (C-2_B), 75.2 (C-3_A), 75.1, 74.9, 74.5, 73.5, 73.4, 73.0 (6PhCH_2), 72.4 (2C, C-3_C and C-4_C), 71.0 (C-4_A), 70.5 (C-4_B), 69.9 (C-6_C), 68.1 (2C, C-6_A and C-6_B), 56.9 (C-2_C), 56.6 (OCH_3), 20.7 (COCH_3); ESI-MS: *m*/*z* 1344.6 $[M+Na]^+$. Anal. Calcd for $C_{77}H_{79}NO_{19}$ (1321.52): C, 69.93; H, 6.02. Found: C, 69.74; H, 6.25.

4.1.5. 4-Methoxyphenyl (3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1→3)-(4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1→4)-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**14**)

To a solution of compound **10** (3 g, 3.14 mmol) in CH_3CN–H_2O (25 mL, 4:1 v/v) was added ammonium cerium nitrate (CAN, 2.6 g, 4.7 mmol) and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the organic layer was washed with satd $NaHCO_3$ (2×100 mL) and water (100 mL), dried (Na_2SO_4), and evaporated to dryness to give disaccharide hemiacetal. To a solution of the hemiacetal in anhydrous CH_2Cl_2 (30 mL) was added trichloroacetonitrile (2.5 mL, 24.9 mmol) and the reaction mixture was cooled to −10 °C. To the cooled reaction mixture was added DBU (0.2 mL, 1.3 mmol) and it was allowed to stir at −10 °C for 1 h. The reaction mixture was evaporated to dryness and the crude product was passed through a short pad of SiO_2 to furnish 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate (**11**, 2.6 g, 83%). A solution of compound **13** (1.5 g, 1.1 mmol) and compound **11** (1.4 g, 1.4 mmol) in anhydrous CH_2Cl_2 (25 mL) was cooled to −10 °C. To the cooled reaction mixture was added TMSOTf (100 μL) and it was allowed to stir at −10 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with satd $NaHCO_3$ (100 mL) and water (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure **14** (2 g, 84%). R_f 0.5 (hexane–EtOAc 1:1); white solid; mp 106–108 °C; IR (KBr): 2922, 1721, 1459, 1371, 1230, 1070, 766, 717 cm^{-1}; $[\alpha]_D^{25}$ +24 (*c* 1.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.99–7.96 (m, 2H, Ar–H), 7.65–7.0 (m, 44H, Ar–H), 6.96–6.60 (m, 6H, Ar–H), 5.67 (dd, *J*=11.5, 3.3 Hz, 1H, H-3_E), 5.57 (d, *J*=3.1 Hz, 1H, H-4_D), 5.49–5.37 (m, 3H, H-1_E, H-4_B, and H-4_E), 5.34–5.28 (m, 1H, H-2_D), 5.20 (d, *J*=8.4 Hz, 1H, H-1_C), 5.0–4.83 (m, 2H, PhCH_2), 4.76–4.52 (m, 5H, H-1_A, H-1_D, H-$6_{a,bD}$, and PhCH_2), 4.44–4.28 (m, 6H, H-2_E and PhCH_2), 4.25–4.10 (m, 8H, H-1_B, H-2_C, H-4_C, H-6_{aC}, H-$6_{a,bE}$, and PhCH_2), 4.08–3.94 (m, 7H, H-3_C, H-3_D, H-5_D, H-5_E, H-6_{bC}, and PhCH_2), 3.89–3.79 (m, 1H, H-3_A), 3.77 (s, 3H, OCH_3), 3.64–3.59 (m, 1H, H-2_A), 3.53–3.32 (m, 5H, H-2_B, H-3_B, H-4_A, H-5_B, and H-5_C), 3.28–3.19 (m, 4H, H-$6_{a,bA}$ and H-$6_{a,bB}$), 3.09–2.98 (m, 1H, H-5_A), 2.20, 2.18, 2.04, 1.97, 1.74 (5s, 15H, 5COCH_3); ^{13}C NMR (75 MHz,

$CDCl_3$): δ 170.3, 170.2, 169.7 (2C), 169.5 (5*C*OCH$_3$), 167.7 (2C), 166.8 (2C) (2*C*OPhth), 166.1, 164.2 (2*C*OPh), 153.9–110.9 (Ar–C), 102.4 (C-1$_A$), 101.7 (C-1$_B$), 101.4 (C-1$_D$), 98.6 (2C, C-1$_C$ and C-1$_E$), 82.3 (C-2$_B$), 82.1 (C-5$_C$), 81.2 (C-5$_B$), 78.8 (2C, C-2$_A$ and C-3$_B$), 77.2 (C-3$_A$), 75.0 (PhCH$_2$), 74.7 (C-4$_A$), 74.4 (PhCH$_2$), 73.7 (C-4$_C$), 73.3 (2C), 72.9, 72.6 (4PhCH$_2$), 72.5 (C-3$_D$), 72.2 (C-3$_C$), 70.8 (C-4$_E$), 70.5 (C-4$_B$), 69.9 (C-2$_D$), 69.2 (C-5$_D$), 69.0 (C-5$_A$), 68.2 (C-6$_B$), 67.7 (C-6$_A$), 67.3 (2C, C-3$_E$ and C-4$_D$), 66.3 (C-5$_E$), 62.9 (C-6$_D$), 61.2 (C-6$_C$ and C-6$_E$), 56.6 (OCH$_3$), 56.0 (C-2$_C$), 51.1 (C-2$_E$), 20.6 (2C), 20.5 (2C), 20.2 (5COCH$_3$); ESI-MS: *m*/*z* 2173.8 $[M+Na]^+$. Anal. Calcd for $C_{119}H_{118}N_2O_{36}$ (2150.75): C, 66.41; H, 5.53. Found: C, 66.20; H, 5.77.

4.1.6. 4-Methoxyphenyl (2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**15**)

To a solution of compound **13** (2.5 g, 1.9 mmol) and thioglycoside donor **7** (900 mg, 2.3 mmol) in anhydrous CH_2Cl_2 (25 mL) was added MS-4 Å (2 g) and the reaction mixture was allowed to stir at room temperature under argon for 30 min. The reaction mixture was cooled to −30 °C and *N*-iodosuccinimide (625 mg, 2.7 mmol) and TMSOTf (10 μL) were added to it. After stirring the reaction mixture at the same temperature for 1 h, it was filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (50 mL), satd $NaHCO_3$ (100 mL), and water (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to furnish pure **15** (2.7 g, 86%). R_f 0.4 (hexane–EtOAc 2:1); white solid; mp 95–97 °C; IR (KBr): 2924, 2856, 2363, 1654, 1649, 1515, 1460, 1218, 1072, 759, 671 cm^{-1}; $[\alpha]_D^{25}$ +16.3 (*c* 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.48–7.11 (m, 39H, Ar–H), 6.92–6.69 (m, 4H, Ar–H), 5.44 (d, *J*=3.3 Hz, 1H, H-4$_B$), 5.39 (s, 1H, PhC*H*), 5.35 (d, *J*=8.0 Hz, 1H, H-1$_C$), 5.30 (dd, *J*=7.8 Hz, 1H, H-2$_D$), 4.92–4.84 (m, 3H, H-3$_D$ and PhC*H*$_2$), 4.82–4.75 (m, 2H, PhC*H*$_2$), 4.71 (d, *J*=8.7 Hz, 1H, H-1$_A$), 4.68–4.57 (m, 2H, PhC*H*$_2$), 4.55 (d, *J*=7.9 Hz, 1H, H-1$_D$), 4.53–4.38 (m, 3H, PhC*H*$_2$), 4.29 (d, *J*=7.5 Hz, 1H, H-1$_B$), 4.29–4.23 (m, 2H, PhC*H*$_2$), 4.21–4.17 (m, 3H, H-3$_C$ and H-6$_{a,bD}$), 4.16–4.04 (m, 2H, H-2$_C$ and H-4$_D$), 3.98–3.80 (4H, H-2$_A$, H-3$_A$, H-4$_C$, and H-6$_{aA}$), 3.73 (s, 3H, OC*H*$_3$), 3.67–3.57 (m, 2H, H-3$_B$ and H-6$_{bA}$), 3.55–3.49 (m, 2H, H-2$_B$ and H-6$_{aC}$), 3.47–3.40 (m, 4H, H-5$_B$, H-6$_{bC}$, and H-6$_{a,bB}$), 3.37–3.26 (m, 3H, H-4$_A$, H-5$_C$, and H-5$_D$), 3.12–2.98 (m, 1H, H-5$_A$), 2.07, 2.06, 2.0 (3s, 9H, 3COC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 169.7, 168.6 (3*C*OCH$_3$), 167.7 (2C, *C*OPhth), 155.3–114.4 (Ar–C), 102.7 (C-1$_A$), 101.9 (C-1$_B$), 101.2 (C-1$_D$), 100.9 (Ph*C*H), 98.8 (C-1$_C$), 82.5 (C-2$_A$), 81.4 (C-3$_B$), 80.8 (C-3$_D$), 79.2 (C-5$_B$), 78.8 (C-5$_D$), 75.7 (C-2$_D$), 75.1, 75.0 (2PhC*H*$_2$), 74.8 (C-5$_C$), 74.4 (PhC*H*$_2$), 74.3 (C-2$_B$), 73.6, 73.4 (2PhC*H*$_2$), 72.9 (2C, C-3$_A$ and PhC*H*$_2$), 72.6 (C-3$_C$), 71.6 (C-5$_A$), 69.9 (C-4$_C$), 68.9 (C-4$_A$), 68.6 (C-4$_B$), 68.2 (2C, C-6$_C$ and C-6$_D$), 67.8 (C-6$_A$), 67.6 (C-6$_B$), 66.5 (C-4$_D$), 56.3 (C-2$_C$), 55.4 (OC*H*$_3$), 20.7 (2C), 20.6 (3COC*H*$_3$); ESI-MS: *m*/*z* 1673.4 $[M+NH_4]^+$. Anal. Calcd for $C_{94}H_{97}NO_{26}$ (1655.63): C, 68.15; H, 5.90. Found: C, 67.94; H, 6.14.

4.1.7. 4-Methoxyphenyl (4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**16**)

To a solution of compound **15** (2.5 g, 1.5 mmol) in EtOH (70 mL) was added hydrazine monohydrate (0.4 mL, 8.2 mmol) 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 mass in acetic anhydride–pyridine (10 mL, 1:1 v/v) was kept at room temperature for 6 h and evaporated to dryness. A solution of the acetylated product in 0.1 M CH_3ONa (60 mL) was allowed to stir at room temperature for 2 h and neutralized with Amberlite-IR 120 (H^+) resin. The reaction mixture was filtered and concentrated to give the crude product, which was purified over SiO_2 using hexane–EtOAc (1:2) as eluant to furnish pure **16** (1.8 g, 83%). R_f 0.2 (hexane–EtOAc: 1:2); white solid; mp 86–88 °C; IR (KBr): 2922, 2864, 2361, 1711, 1649, 1511, 1460, 1389, 1229, 1069, 750, 699 cm^{-1}; $[\alpha]_D^{25}$ +8 (*c* 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.48–7.13 (m, 35H, Ar–H), 6.93–6.66 (m, 4H, Ar–H), 5.42 (s, 1H, PhC*H*), 5.36 (d, *J*=8.3 Hz, 1H, H-1$_C$), 4.93 (d, *J*=9.0 Hz, 1H, H-1$_A$), 4.89 (d, *J*=11.2 Hz, 1H, PhC*H*$_2$), 4.76–4.56 (m, 5H, PhC*H*$_2$), 4.48–4.38 (m, 4H, H-1$_B$, H-1$_D$, and PhC*H*$_2$), 4.32–4.26 (m, 4H, H-3$_C$, H-4$_C$, and PhC*H*$_2$), 4.22–4.11 (m, 5H, H-3$_A$, H-6$_{a,bA}$, and PhC*H*$_2$), 4.08–4.04 (m, 2H, H-2$_D$ and H-3$_D$), 3.95–3.82 (m, 5H, H-2$_C$, H-4$_B$, H-4$_D$, and H-6$_{a,bD}$), 3.73 (s, 3H, OC*H*$_3$), 3.72–3.58 (m, 4H, H-2$_A$, H-3$_B$, and H-6$_{a,bC}$), 3.56–3.42 (m, 4H, H-2$_B$, H-5$_D$, and H-6$_{a,bB}$), 3.39–3.30 (m, 3H, H-4$_A$, H-5$_B$, and H-5$_C$), 3.15–3.07 (m, 1H, H-5$_A$), 2.05 (s, 3H, COC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9 (NHCOCH$_3$), 167.7 (2C, *C*OPhth), 155.2–114.4 (Ar–C), 103.7 (C-1$_A$), 102.8 (C-1$_B$), 101.9 (C-1$_D$), 101.0 (Ph*C*H), 98.7 (C-1$_C$), 82.5 (C-2$_A$), 81.9 (C-3$_B$), 81.4 (C-3$_D$), 78.9 (2C, C-5$_B$ and C-5$_D$), 75.7 (C-2$_D$), 75.1 (PhCH$_2$), 75.0 (2C, C-5$_A$ and PhCH$_2$), 74.9 (C-5$_C$), 74.5 (PhCH$_2$), 74.0 (C-2$_B$), 73.4 (2C, PhCH$_2$), 73.0 (PhCH$_2$), 72.6 (C-3$_A$), 72.5 (C-4$_C$), 71.1 (C-3$_C$), 70.1 (C-4$_A$), 69.0 (C-4$_B$), 68.8 (C-6$_D$), 68.6 (C-6$_C$), 68.2 (C-6$_A$), 67.7 (C-6$_B$), 66.7 (C-4$_D$), 56.5 (C-2$_C$), 55.4 (OCH$_3$), 20.8 (NHCOCH$_3$); ESI-MS: *m*/*z* 1464.6 $[M+Na]^+$. Anal. Calcd for $C_{82}H_{91}NO_{22}$ (1441.60): C, 68.27; H, 6.36. Found: C, 68.06; H, 6.55.

4.1.8. 4-Methoxyphenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**17**)

To a solution of compound **16** (1.5 g, 1 mmol) and thioglycoside donor **8** (1.1 g, 1.9 mmol) in anhydrous CH_3CN–CH_2Cl_2 (20 mL, 5:1 v/v) was added MS-3 Å (2 g) and the reaction mixture was allowed to stir at room temperature under argon for 30 min. The reaction mixture was cooled to −10 °C and *N*-iodosuccinimide (500 mg, 2.2 mmol) and TMSOTf (15 μL) were added to it. After stirring the reaction mixture at the same temperature for 16 h, it was filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), satd $NaHCO_3$ (100 mL), and water (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified over SiO_2 using toluene–EtOAc (1:2) as eluant to furnish pure **17** (920 mg, 48%). R_f 0.2 (toluene–EtOAc 1:3); colorless syrup; IR (neat): 2925, 2339, 1750, 1663, 1595, 1440, 1373, 1222, 1048, 760 cm^{-1}; $[\alpha]_D^{25}$ +10 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.42–7.14 (m, 35H, Ar–H), 6.95–6.69 (m, 4H, Ar–H), 5.47–5.39 (m, 2H, H-7$_E$ and H-8$_E$), 5.33 (s, 1H, PhC*H*), 5.30 (d, *J*=9.3 Hz, 2H, H-1$_C$ and N*H*COCH$_3$), 4.95–4.86 (m, 2H, H-4$_E$ and PhC*H*$_2$), 4.80 (d, *J*=10.8 Hz, 1H, H-1$_A$), 4.77–4.62 (m, 4H, PhC*H*$_2$), 4.52 (d, *J*=7.5 Hz, 1H, H-1$_B$), 4.50–4.36 (m, 3H, PhC*H*$_2$), 4.32 (d, *J*=8.6 Hz, 1H, H-1$_D$), 4.30–4.15 (m, 8H, H-3$_C$, H-4$_C$, H-6$_{a,bA}$, H-9$_{aE}$, and PhC*H*$_2$), 4.13–4.08 (m, 4H, H-3$_A$, H-4$_B$, H-9$_{bE}$, and PhC*H*$_2$), 4.06–4.04 (m, 2H, H-2$_D$ and H-5$_E$), 4.02–3.86 (m, 5H, H-3$_D$, H-4$_D$, H-6$_E$, and H-6$_{a,bD}$), 3.83–3.76 (m, 2H, H-2$_C$ and H-3$_B$), 3.74 (s, 3H, OC*H*$_3$), 3.59 (s, 3H, OC*H*$_3$), 3.54–3.50 (m, 2H, H-2$_A$ and H-6$_{aB}$), 3.48–3.38 (m, 4H, H-2$_B$, H-5$_D$, and H-6$_{a,bC}$), 3.36–3.28 (m, 4H, H-4$_A$, H-5$_B$, H-5$_C$, and H-6$_{bB}$), 3.18–3.06 (m, 1H, H-5$_A$), 2.74 (dd, *J*=12.0, 4.8 Hz, 1H, H-3$_{eE}$), 2.16, 2.14, 2.04, 2.02, 2.0, 1.89 (6s, 18H, 6COC*H*$_3$), 1.99–1.97 (m, 1H, H-3$_{aE}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (2C), 170.3 (2C), 170.1 (2C) (6*C*OCH$_3$), 168.3 (*C*OOCH$_3$), 155.3–114.4 (Ar–C), 103.6 (C-1$_B$), 102.6 (C-1$_A$), 101.8 (C-1$_D$), 100.7 (Ph*C*H), 98.7 (C-1$_C$), 97.2 (C-2$_E$), 82.5 (C-2$_A$), 82.0 (C-3$_B$), 81.5 (C-3$_D$), 78.8 (C-5$_B$), 78.7 (C-5$_D$), 78.7 (C-2$_D$), 75.6 (C-5$_A$), 75.1 (2C, PhCH$_2$), 74.6 (2C, C-2$_B$ and C-5$_C$), 74.2 (2C, C-3$_A$ and PhCH$_2$), 73.3 (2C, PhCH$_2$), 72.9 (2C, C-4$_B$ and PhCH$_2$), 72.6 (2C, C-4$_D$ and C-5$_E$), 70.8 (C-4$_C$), 70.1 (C-3$_C$), 69.0 (C-4$_E$), 68.7 (2C, C-6$_D$ and C-7$_E$), 68.5 (2C, C-6$_C$ and C-8$_E$), 68.3 (2C, C-6$_A$

and C-6_B), 67.1 (C-4_A), 62.4 (C-9_E), 56.4 (C-6_E), 55.5 (O*C*H_3), 52.8 (COO*C*H_3), 49.5 (C-2_C), 38.3 (C-3_E), 23.0, 22.6, 20.7 (4C) (6CO*C*H_3); ESI-MS: *m*/*z* 1937.7 $[M+Na]^+$. Anal. Calcd for $C_{102}H_{118}N_2O_{34}$ (1914.76): C, 63.94; H, 6.21. Found: C, 63.76; H, 6.45.

4.1.9. 4-Methoxyphenyl (2-acetamido-2-deoxy-β-D-galacto-pyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (**1**)

To a solution of compound **14** (1.5 g, 0.7 mmol) in EtOH (50 mL) was added hydrazine monohydrate (1 mL, 20.6 mmol) 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 mass in acetic anhydride–pyridine (10 mL, 1:1 v/v) was kept at room temperature for 6 h and evaporated to dryness. A solution of the acetylated product in 0.1 M CH_3ONa (50 mL) was allowed to stir at room temperature for 10 h and neutralized with Dowex 50W X8 (H^+) resin. The reaction mixture was filtered and concentrated. To a solution of the deacetylated product in CH_3OH (20 mL) was added 20% $Pd(OH)_2$–C (400 mg) and the reaction mixture was allowed to stir at room temperature for 24 h under positive pressure of hydrogen. The reaction mixture was filtered through a Celite® bed, concentrated, and purified by passing through a column of Sephadex LH-20 using CH_3OH–H_2O (4:1) as eluant to give compound **1** (500 mg, 70%). R_f 0.3 ($CHCl_3$–CH_3OH–H_2O 2:1:0.4); white powder; $[\alpha]_D^{25}$ −10 (*c* 1.2, H_2O); IR (KBr): 3445, 2361, 1670, 1649, 1541, 1462, 1026, 767 cm^{-1}; ^1H NMR (400 MHz, D_2O): δ 7.20 (d, *J*=9.0 Hz, 2H, Ar–H), 7.04 (d, *J*=9.0 Hz, 2H, Ar–H), 5.08 (d, *J*=7.8 Hz, 1H, H-1_D), 4.74 (d, *J*=8.4 Hz, 1H, H-1_C), 4.66 (d, *J*=8.1 Hz, 1H, H-1_E), 4.51 (2d, *J*=7.7 Hz, 2H, H-1_A and H-1_B), 4.19 (br s, 2H, H-4_B and H-4_D), 4.07–3.95 (m, 4H, H-2_E, H-3_C, H-4_E, and H-6_{aE}), 3.90–3.86 (m, 2H, H-2_C and H-6_{bE}), 3.85 (s, 3H, O*C*H_3), 3.84–3.72 (m, 15H, H-3_A, H-3_B, H-3_D, H-3_E, H-4_A, H-4_C, H-5_C, H-$6_{a,bA}$, H-$6_{a,bB}$, H-$6_{a,bC}$, and H-$6_{a,bD}$), 3.71–3.67 (m, 2H, H-5_A and H-5_E), 3.66–3.60 (m, 5H, H-2_A, H-2_B, H-2_D, H-5_B, and H-5_D), 2.07 (s, 6H, 2NHCOCH_3); ^{13}C NMR (100 Hz, D_2O): δ 174.2 (2C, 2NH*C*OCH$_3$), 155.0–115.1 (Ar–C), 103.3 (C-1_E), 102.9 (2C, C-1_A and C-1_B), 102.7 (C-1_C), 101.0 (C-1_D), 82.0 (C-5_A), 81.8 (C-5_E), 78.2 (C-3_D), 78.1 (C-3_B), 75.0 (C-4_C), 74.9 (3C, C-3_A, C-4_A, and C-5_C), 74.6 (C-5_D), 74.2 (C-5_B), 72.6 (C-2_A), 72.2 (C-3_C), 70.7 (C-4_E), 70.1 (C-2_D), 69.9 (C-2_B), 68.5 (2C, C-4_B and C-4_D), 67.8 (C-3_E), 60.9 (4C, C-6_A, C-6_B, C-6_C, and C-6_D), 59.9 (C-6_E), 55.8 (O*C*H$_3$), 55.2 (C-2_C), 52.5 (C-2_E), 22.2, 22.1 (2NHCO*C*H$_3$); ESI-MS: *m*/*z* 1039.4 $[M+Na]^+$. Anal. Calcd for $C_{41}H_{64}N_2O_{27}$ (1016.37): C, 48.42; H, 6.34. Found: C, 48.20; H, 6.55.

4.1.10. 4-Methoxyphenyl (sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (**2**)

To a solution of the pentasaccharide derivative **17** (900 g, 0.47 mmol) in methanol (20 mL) was added 20% $Pd(OH)_2$–C (500 mg) and the reaction mixture was allowed to stir at room temperature for 24 h under a positive pressure of hydrogen. The reaction mixture was filtered through a Celite® bed and concentrated under reduced pressure. The crude mass was dissolved in 0.1 M sodium methoxide (30 mL) and the reaction mixture was allowed to stir at room temperature for 8 h and then a few drops of distilled water was added to the reaction mixture and allowed to stir for overnight. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered, and evaporated to dryness and again passed through a short pad of Dowex 50W X8 (Na^+) resin. The crude product was purified by passing through a column of Sephadex LH-20 using CH_3OH–H_2O (4:1) as eluant to give pentasaccharide **2** as its sodium salt (350 mg, 66%) as a white powder. R_f 0.2 (CH_3CN–CH_3OH–H_2O 1:1:0.5); $[\alpha]_D^{25}$ −9.0 (*c* 1.1, H_2O); IR (KBr): 3021, 2361, 1730, 1217, 1046, 763 cm^{-1}; ^1H NMR (400 MHz, D_2O): δ 7.06 (d, *J*=8.8 Hz, 2H, Ar–H), 6.84 (d, *J*=8.8 Hz, 2H, Ar–H), 4.82 (2d, *J*=7.5 Hz, 2H, H-1_A, H-1_C), 4.46 (2d, *J*=7.8 Hz, 2H, H-1_B, H-1_D), 4.18–4.0 (m, 4H, H-3_C, H-4_B, H-4_D, H-8_E), 3.98–3.80 (m, 9H, H-3_A, H-3_B, H-3_D, H-4_A, H-4_C, H-$6_{a,bA}$, H-6_E, H-7_E), 3.79–3.70 (m, 8H, H-4_E, H-5_E, H-$6_{a,bB}$, H-6_{aC}, OCH_3), 3.68–3.57 (m, 8H, H-2_C, H-2_D, H-5_A, H-6_{bC}, H-$6_{a,bD}$, H-$9_{a,bE}$), 3.56–3.43 (m, 5H, H-2_A, H-2_B, H-5_B, H-5_C, H-5_D), 2.82 (dd, *J*=12.2, 3.3 Hz, 1H, H-3_{eE}), 2.02 (s, 6H, 2NHCOCH_3), 1.70 (t, *J*=12.2 Hz, 1H, H-3_{aE}); ^{13}C NMR (100 Hz, D_2O): δ 175.5 (2C, *C*OONa, NHCOCH$_3$), 174.8 (NH*C*OCH$_3$), 156.6–115.7 (Ar–C), 104.9 (2C, C-1_B, C-1_D), 103.1 (2C, C-1_A, C-1_C), 101.0 (C-2_E), 80.5 (2C, C-2_D, C-5_A), 77.5 (C-2_A), 77.0 (C-2_B), 76.5 (4C, C-3_C, C-4_A, C-5_B, C-5_D), 76.1 (C-5_C), 74.8 (C-3_A), 74.6 (2C, C-3_B, C-4_B), 72.9 (C-6_E), 71.2 (C-4_C), 70.6 (C-7_E), 70.0 (2C, C-3_D, C-8_E), 69.6 (C-4_D), 69.2 (C-4_E), 64.4 (C-9_E), 62.7 (C-6_A), 62.4 (C-6_C), 61.7 (2C, C-6_B, C-6_D), 56.1 (O*C*H$_3$), 53.6 (2C, C-2_C, C-5_E), 42.1 (C-3_E), 22.1 (2C, NHCO*C*H$_3$); ESI-MS: *m*/*z* 1127.2 $[M+1]^+$. Anal. Calcd for $C_{44}H_{67}N_2NaO_{30}$ (1126.37): C, 46.89; H, 5.99. Found: C, 46.67; H, 6.25.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.07.004.

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