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Note

Synthesis of a di- and a trisaccharide related to the O-antigen of *Escherichia coli* O83:K24:H31☆

Geetanjali Agnihotri and Anup Kumar Misra*

Medicinal and Process Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226001, UP, India

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Abstract—Di- and trisaccharide fragments related to the repeating unit of the O-antigen of *Escherichia coli* O83:K24:H31 have been synthesized as their methyl glycoside analogs starting from readily available monosaccharides.

Keywords: Colinfant; Vaccine; Glycosylation; *E. coli*; Disaccharide; Trisaccharide

'Colinfant', a commercially used oligosaccharide vaccine, contains the bacterial strain *Escherichia coli* O83:K24:H31.[1] In the gut, the bacteria stimulates the production of specific and nonspecific antibodies both at the local level within the gut and in the saliva as well as in the blood. The 'Colinfant' oligosaccharide vaccine decreased the number of nosocomial infections, mortality rate in connection with infection, and the need for antibiotics.[2,3] The structure of the O-antigen of *E. coli* O83:K24:H31 has been established.[4] In the light of increasing drug resistance of pathogenic bacterial infections and the potential importance of artificial antigens, the selective chemical synthesis of immunodominant fragments of bacterial O-antigens, which could be used to study the potential of synthetic antigens for diagnosis and protection, has gained considerable interest. To understand the structure–activity relationship and antigenic properties of the pentasaccharide repeating unit of this polysaccharide, β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-α-D-Glc*p*-, we prepared di- and trisaccharide fragments of it, namely β-D-Glc*p*A-(1→6)-β-D-Gal*p*-OCH_3 and β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-α-D-Glc*p*-OCH_3.

Recently, perchloric acid supported on silica ($HClO_4$–SiO_2) has been successfully used by us and others in several carbohydrate transformations and glycosylation reactions.[5,6] As a part of our continuing efforts to synthesize biologically important carbohydrate haptens in a concise manner, we disclose here a concise approach for the synthesis of the aforementioned di- and trisaccharides using $HClO_4$–SiO_2-mediated transformations.

The synthesis of the disaccharide fragment is shown in Scheme 1. Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (**2**) was prepared from methyl β-D-galactopyranoside (**1**) in three steps involving conventional tritylation,[7] acetylation, and de-tritylation using $HClO_4$–SiO_2[8] in 85% overall yield. Initial attempts to glycosylate **2** with methyl (ethyl 2,3,4-tri-*O*-acetyl-l-thio-β-D-glucopyranoside)uronate (**3**) using either *N*-iodosuccinimide (NIS) and $HClO_4$–SiO_2[6c] or NIS–TfOH did not result in any glycosylated product, which may be explained due to the poor reactivity of glucuronic acid donor. Therefore, following the classical approach, glycosylation of **2** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**4**)[9] using silver trifluoromethanesulfonate as the activator[10] was carried out, which yielded the expected product, **5**, in 85% yield. Deacetylation followed by hydrolysis of the methyl ester in **5** using 0.05 M sodium methoxide and a few drops of water furnished the target disaccharide **6** in 90% yield. The presence of two doublets at δ 4.82

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* Corresponding author. Tel.: +91 522262411; fax: +91 5222623405; e-mail: akmisra69@rediffmail.com

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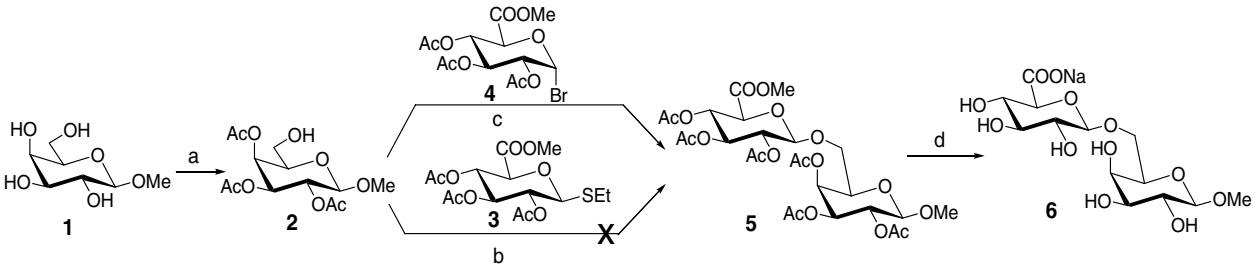
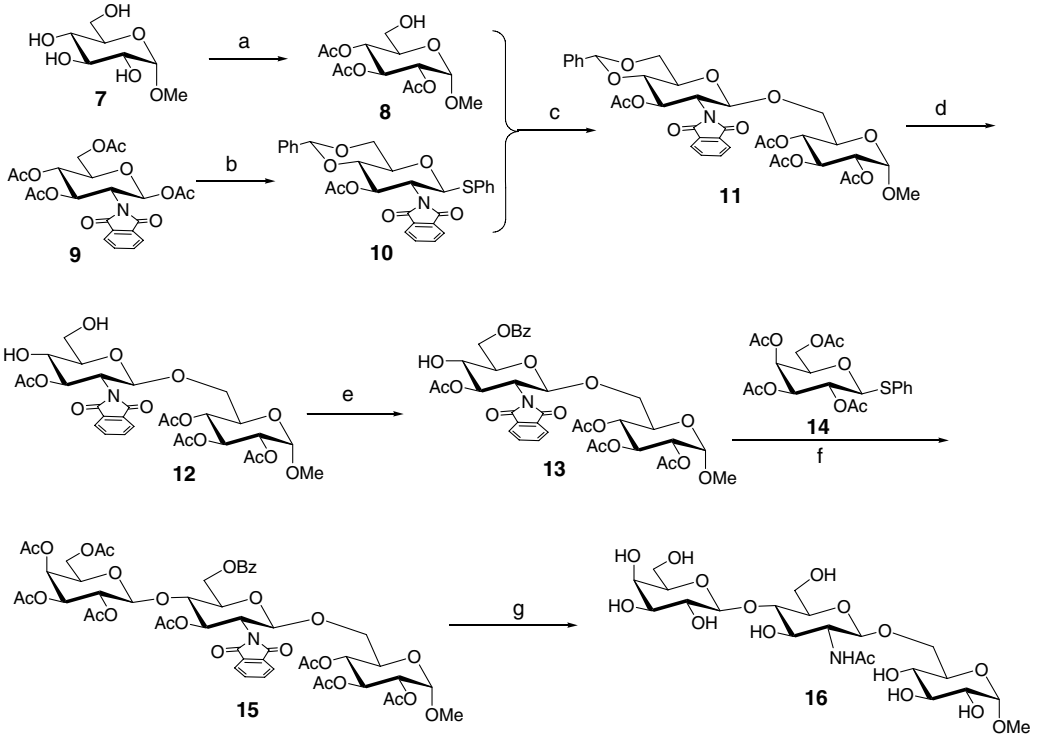


Scheme 1. Reagents and conditions: (a) (1) TrCl, pyridine, rt, 48 h; (2) Ac_2O, pyridine, 0 °C, 5 h; (3) $HClO_4$–SiO_2, rt, 1 h; 85% in three steps; (b) NIS, $HClO_4$–SiO_2, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 1 h; (c) AgOTf, 4 Å molecular sieves, CH_2Cl_2, −20 °C, 4 h, 85%; (d) 0.05 M $NaOCH_3$, CH_3OH, rt, 6 h, then a few drops of water, rt, 12 h, 90%.

($J = 8.1$ Hz) and 4.44 ($J = 8.1$ Hz) in the ^{1}H NMR spectrum and two anomeric signals at δ 102.9 (C-1) and 101.6 (C-1′) in the ^{13}C NMR spectrum of **6** confirmed the structure of the product.

The trisaccharide target was prepared as illustrated in Scheme 2. Thus, methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (**8**)[11] was prepared from methyl α-D-glucopyranoside (**7**) in three steps: tritylation, acetylation, and de-tritylation in 80% overall yield. Glycosylation of **8** with phenyl thioglycoside **10**,[12] which was prepared from **9** in four steps, using NIS and $HClO_4$–SiO_2[6c] provided disaccharide **11** in 80% yield. This product was transformed into disaccharide acceptor **13** in two steps. First, the benzylidene acetal was removed using $HClO_4$–SiO_2 to give disaccharide diol **12** and then selective benzoylation[13] of the primary hydroxyl using benzoyl cyanide afforded **13**. Glycosylation of **13** with thioglycoside **14**[14] using NIS and $HClO_4$–SiO_2 furnished the trisaccharide derivative **15** in 82% yield. Signals at δ 5.48 (d, $J = 8.4$ Hz), 5.28 (d, $J = 10.2$ Hz) and 4.44 (d, $J = 3.3$ Hz) in the ^{1}H NMR and at δ 98.4 (C-1′) and 96.1 (C-1″ and C-1) in ^{13}C NMR spectra of **15** confirmed the formation of desired trisaccharide derivative. Removal of the phthalimido group using hydrazine hydrate[15] followed by N-acetylation and global deacetylation afforded the target trisaccharide **16** in 70% yield



Scheme 2. Reagents and conditions: (a) (1) TrCl, pyridine, rt, 48 h; (2) Ac_2O, pyridine, 0 °C, 5 h; (3) $HClO_4$–SiO_2, rt, 1 h, 80% in three steps; (b) (1) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C–rt, 5 h, 90%; (2) 0.05 M $NaOCH_3$, CH_3OH, rt, 20 min, quantitative; (3) $PhCH(OCH_3)_2$, $HClO_4$–SiO_2, CH_3CN, rt, 2 h, then Ac_2O, 0 °C, 1 h, 85% in two steps; (c) NIS, $HClO_4$–SiO_2, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 1.5 h, 80%; (d) $HClO_4$–SiO_2, CH_3CN, rt, 30 min, 95%; (e) BzCN, pyridine, CH_2Cl_2, rt, 12 h, 90%; (f) NIS, $HClO_4$–SiO_2, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 2 h, 82%; (g) (1) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 8 h, (2) Ac_2O, pyridine, rt, 2 h; (3) 0.05 M $NaOCH_3$, CH_3OH, rt, 12 h, 70% in three steps.

(Scheme 2). The structure of **16** was confirmed by NMR and mass spectroscopy.

In summary, we have developed a concise synthesis of di- and trisaccharide methyl glycoside fragments of the pentasaccharide repeating unit of the O-antigen of *E. coli* O83:K24:H31, which is present in the carbohydrate vaccine 'Colinfant'.

1. Experimental

1.1. General experimental methods

All reactions were monitored by thin layer chromatography on 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. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance DPX 300 MHz using TMS as the internal reference. Chemical shift values are expressed in ppm. Elemental 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 the reactions.

1.2. Preparation of $HClO_4$–SiO_2 catalyst

$HClO_4$ (1.8 g, 12.5 mmol, as a 70% aq solution) was added to a suspension of SiO_2 (230–400 mesh, 23.7 g) in Et_2O (70.0 mL). The mixture was concentrated and the residue was heated at 100 °C for 72 h under vacuum to furnish $HClO_4$–SiO_2 (0.5 mmol/g) as a free flowing powder. **Caution!** Although no explosions were reported under these conditions, special care should be taken for large-scale preparation of this reagent.

1.3. Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (2)

To a solution of **1** (5.0 g, 25.7 mmol) in pyridine (50.0 mL) was added trityl chloride (10.0 g, 35.8 mmol) and the reaction mixture was stirred at rt for 48 h in the dark. After cooling the reaction mixture to 0 °C, acetic anhydride (40.0 mL) was added and the solution was stirred at rt for 12 h. The solvents were removed under reduced pressure and the crude reaction mixture was re-dissolved in CH_3CN (50.0 mL). To this solution was added $HClO_4$–SiO_2 (2.0 g, 1.0 mmol) and the mixture was stirred at rt for 30 min. After completion of the reaction by TLC, the mixture was filtered through Celite and the filtrate was concentrated to dryness. Column chromatography of the crude product over SiO_2 (hexane–EtOAc, 1:1) furnished **2** (6.9 g) as a yellow oil in 85% overall yield. $[\alpha]_D^{25}$ +4.8 (*c* 1.0, $CHCl_3$); IR (Neat): 1748, 1372, 1226, 1061, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.38 (d, $J = 3.3$ Hz, 1H, H-4), 5.22 (dd, $J = 8.1$ and 10.5 Hz, 1H, H-2), 5.04 (dd, $J = 3.3$ and 10.5 Hz, 1H, H-3), 4.41 (d, $J = 7.8$ Hz, 1H, H-1), 3.79–3.71 (m, 2H, H-6_{ab}), 3.59–3.54 (m, 1H, H-5), 3.52 (s, 3H, OCH_3), 2.45–2.39 (br s, 1H, OH), 2.17, 2.08, 1.99 (3s, 9H, 3 COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 170.0, 169.5, 102.1 (C-1), 73.47, 71.0, 69.1, 67.9, 60.5, 56.9 (OCH_3), 20.68, 20.59, 20.49; ESIMS: *m*/*z* 343.1 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found: C, 48.58; H, 6.50.

1.4. Methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (5)

To a solution of **2** (650.0 mg, 2.03 mmol) in CH_2Cl_2 (10.0 mL) were added 4 Å molecular sieves (1.0 g) and silver trifluoromethanesulfonate (1.2 g, 4.67 mmol) and the mixture was stirred under argon at −20 °C for 30 min. A solution of **4** (1.40 g, 3.5 mmol) in CH_2Cl_2 (5.0 mL) was added and the mixture was stirred at −20 °C for 4 h, before being filtered and washed with CH_2Cl_2 (25 mL). The filtrate was washed with satd aq $NaHCO_3$, and water in succession before being dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 (hexane–EtOAc, 2:1) to give **5** (1.1 g) as a yellow oil in 85% yield. $[\alpha]_D^{25}$ −4.2 (*c* 1.0, $CHCl_3$); IR (Neat): 2362, 1752, 1595, 1380, 1228, 1044, 559 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.36 (d, $J = 3.3$ Hz, 1H, H-4), 5.23 (dd, $J = 8.1$ and 2.4 Hz, 1H, H-2′), 5.21–5.18 (m, 1H, H-4′), 5.14 (dd, $J = 7.8$ and 10.5 Hz, 1H, H-2), 4.99 (dd, $J = 10.3$ and 3.3 Hz, 1H, H-3), 4.98–4.92 (m, 1H, H-3′), 4.62 (d, $J = 7.8$ Hz, 1H, H-1′), 4.37 (d, $J = 8.1$ Hz, 1H, H-1), 3.85 (m, 2H, H-6_{ab}), 3.80–3.72 (m, 2H, H-5, H-5′), 3.76 (s, 3H, COOCH_3), 3.52 (s, 3H, OCH_3), 2.14, 2.05, 2.03, 2.02, 2.01, 1.96 (6s, 18H, 6COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (3C), 169.6 (2C), 169.5 (2C), 102.3 (C-1), 100.6 (C-1′), 72.7, 72.4, 72.2, 71.2 (2C), 69.5, 69.1, 67.8, 67.7, 57.2, 53.1, 21.0 (2C), 20.8 (2C), 20.7 (2C); ESIMS: *m*/*z* 659.2 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 48.83; H, 5.95.

1.5. Methyl (β-D-glucopyranosyluronic acid)-(1→6)-β-D-galactopyranoside (6)

A solution of **5** (500 mg, 0.78 mmol) in 0.05 M $NaOCH_3$ in CH_3OH (5.0 mL) was stirred at rt for 6 h and then five drops of water were added and the mixture was stirred for another 12 h at rt. The solution was neutralized with Dowex 50W-X8 (H^+), filtered, and the filtrate was evaporated to dryness to give a glassy solid, which was further dissolved in aqueous CH_3OH and passed through a short column of Dowex 50W-X8 (Na^+) and concentrated under reduced pressure. The crude product

was further purified through Sephadex LH-20 using 80% aqueous EtOH as eluant to give pure disaccharide **6** as its sodium salt (275 mg) in 90% yield. Glassy solid; $[\alpha]_D^{25}$ −10.0 (*c* 1.0, H_2O); IR (KBr): 2351, 1685, 1198, 787 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 4.82 (d, $J = 8.1$ Hz, 1H, H-1′), 4.44 (d, $J = 8.1$ Hz, 1H, H-1), 4.12–3.95 (m, 2H), 3.90–3.85 (m, 3H), 3.80 (s, 1H), 3.62–3.60 (m, 2H), 3.60 (s, 3H, OCH_3), 3.95–3.16 (m, 2H); ^{13}C NMR (75 MHz, D_2O): δ 173.0, 102.9 (C-1), 101.6 (C-1′), 74.0, 72.3, 71.5 (2C), 70.3 (2C), 70.0, 68.5, 67.0, 57.0; ESIMS: *m/z* 415.2 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{21}NaO_{12}$: C, 39.80; H, 5.40. Found: C, 39.52; H, 5.72.

1.6. Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (8)

To a solution of **7** (5.0 g, 25.7 mmol) in pyridine (50 mL) was added trityl chloride (10 g, 35.8 mmol) and the reaction mixture was stirred at rt for 48 h in the dark. After cooling the reaction mixture to 0 °C, acetic anhydride (40 mL) was added and the reaction mixture was stirred at rt for 12 h. The solvent was removed under reduced pressure and the crude product was re-dissolved in CH_3CN (50 mL) before $HClO_4$–SiO_2 (2.0 g, 1.0 mmol) was added. After stirring for 30 min, the reaction was filtered through Celite and concentrated to dryness. Column chromatography of the crude product over SiO_2 (hexane–EtOAc, 1:1) furnished **8** (6.5 g) as a yellow oil in 80% overall yield. $[\alpha]_D^{25}$ +123.6 (*c* 1.0, $CHCl_3$); IR (Neat): 2941, 1750, 1372, 1226, 1041, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 5.54 (t, $J = 9.9$ Hz, 1H, H-3), 5.03 (t, $J = 9.9$ Hz, 1H, H-4), 4.97 (d, $J = 3.6$ Hz, 1H, H-1), 4.87 (dd, $J = 10.5$ and 3.6 Hz, 1H, H-2), 3.81–3.70 (m, 2H, H-6_{ab}), 3.62–3.57 (m, 1H, H-5), 3.41 (s, 3H, OCH_3), 2.08, 2.06, 2.02 (3s, 9H, $3COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 170.4, 170.2, 96.9 (C-1), 71.2, 70.8, 70.0, 69.4, 61.4, 55.6, 20.9 (2C), 20.8; ESIMS: *m/z* 343.1 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found: C, 48.64; H, 6.52.

1.7. Phenyl 3-*O*-acetyl-2-deoxy-4,6-*O*-benzylidene-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (10)

A solution of **9** (5.0 g, 10.47 mmol) and thiophenol (1.6 mL, 15.71 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C. To this solution was added $BF_3{\cdot}OEt_2$ (3.3 mL, 26.0 mmol) and the reaction mixture was stirred at 0 °C for 5 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with water, satd aq $NaHCO_3$, and water in succession. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give crude thiophenyl glycoside, which was purified over SiO_2 (hexane–EtOAc, 2:1) to give phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside as white crystals (4.97 g, 90%). A solution of this compound (4.97 g, 9.42 mmol) in 0.05 M sodium methoxide in CH_3OH (100 mL) was stirred at rt for 20 min. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered and evaporated to dryness. The dried mass thus obtained was re-dissolved in anhydrous CH_3CN (30 mL) and benzaldehyde dimethylacetal (1.7 mL, 11.3 mmol) was added followed by $HClO_4$–SiO_2 (300 mg). After stirring at rt for 2 h, the reaction mixture was cooled to 0 °C and acetic anhydride (5.0 mL) was added and stirring continued at 0 °C for 1 h. The solvent was removed under reduced pressure and the crude mass was purified over SiO_2 (hexane–EtOAc, 2:1) to afford **10** as a pale yellow solid (4.25 g, 85%). Mp 114 °C; $[\alpha]_D^{25}$ +39.8 (*c* 1.0, $CHCl_3$); IR (KBr): 2363, 2340, 1717, 1594, 1379, 1224, 1099, 754 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.85–7.80 (m, 2H, aromatic protons), 7.76–7.70 (m, 2H, aromatic protons), 7.42–7.27 (m, 10H, aromatic protons), 5.89–5.80 (t, $J = 9.5$ Hz, 1H, H-3), 5.80 (d, $J = 9.0$ Hz, 1H, H-1), 5.50 (s, 1H, CHC_6H_5), 4.40 (t, $J = 10.1$ Hz, 1H, H-4), 4.30 (t, $J = 10.1$ Hz, 1H, H-2), 3.82–3.72 (m, 3H, H-5 and H-6_{ab}), 1.87 (s, 3H, $3COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 168.2, 166.6, 137.3, 134.8, 134.6, 133.5 (2C), 132.1, 131.6, 129.5 (2C), 129.4 (2C), 128.7 (2C), 128.6 (2C), 126.6 (2C), 124.1, 102.1 (CHC_6H_5), 84.3, 79.5, 70.9 (2C), 69.0, 54.7, 20.9; ESIMS: *m/z* 554 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{25}NO_7S$: C, 65.52; H, 4.74. Found: C, 65.36; H, 4.86.

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

To a solution of **8** (1.0 g, 3.12 mmol) and **10** (2.0 g, 3.76 mmol) in anhydrous CH_2Cl_2 (40 mL) was added powdered 4 Å molecular sieves (3.0 g) and the mixture was stirred under argon for 1 h. After cooling the reaction mixture to 0 °C, NIS (1.27 g, 5.64 mmol) and $HClO_4$–SiO_2 (300 mg) were added and stirring was continued for 1.5 h at 0 °C. The reaction mixture was filtered through Celite. The filtrate was washed with 5% aq $Na_2S_2O_3$, satd aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 (hexane–EtOAc, 2:1) afforded **11** (1.85 g, 80%) as a glassy solid; $[\alpha]_D^{25}$ +40.2 (*c* 1.0, $CHCl_3$); IR (KBr): 1752, 1718, 1629, 1594, 1368, 1224, 1080, 1037, 757, 722 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.86–7.83 (m, 2H, aromatic protons), 7.74–7.71 (m, 2H, aromatic protons), 7.46–7.44 (m, 2H, aromatic protons) 7.36–7.26 (m, 3H, aromatic protons), 5.85 (t, $J = 9.9$ Hz, 1H, H-3′), 5.54 (s, 1H, CHC_6H_5), 5.48 (d, $J = 8.4$ Hz, 1H, H-1′), 5.30 (t, $J = 9.6$ Hz, 1H, H-3), 4.80 (t, $J = 9.9$ Hz, 1H, H-4), 4.70 (dd, $J = 10.2$ and 3.6 Hz, 1H, H-2), 4.53 (d, $J = 3.3$ Hz, 1H, H-1), 4.43 (dd,

$J = 9.6$ and 3.6 Hz, 1H, H-4′), 4.28 (t, $J = 8.7$ Hz, 1H, H-2′), 3.94–3.71 (m, 5H, H-6_{ab}, H-$6'_{ab}$, H-5′), 3.50 (dd, $J = 10.5$ and 6.6 Hz, 1H, H-5), 3.07 (s, 3H, OCH_3), 2.01, 1.92, 1.89, 1.85 (4s, 12H, 4 COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.4 (2C), 169.7, 168.2 (2C), 137.2, 134.5 (2C), 132.0, 129.6, 128.6 (3C), 126.6 (2C), 123.8 (2C), 102.0 (CHC_6H_5), 99.6 (C-1′), 96.7 (C-1), 79.6, 71.2, 70.3, 70.1, 69.5, 69.1, 69.0, 68.0, 66.8, 55.5, 55.3, 21.0, 20.9 (2C), 20.8; ESIMS: m/z 764 $[M+Na]^+$. Anal. Calcd for $C_{36}H_{39}NO_{16}$: C, 58.30; H, 5.30. Found: C, 58.10; H, 5.53.

1.9. Methyl 3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (12)

To a solution of **11** (1.5 g, 2.02 mmol) in CH_3CN (20 mL) was added $HClO_4$–SiO_2 (400 mg) and the mixture was stirred at rt for 30 min. The reaction mixture was filtered and concentrated to give disaccharide diol **12** (1.25 g, 95%). Glassy solid; $[\alpha]_D^{25}$ +88.2 (*c* 1.0, $CHCl_3$); IR (KBr): 1749, 1718, 1662, 1386, 1226, 1039, 765 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84–7.82 (m, 2H, aromatic protons), 7.73–7.71 (m, 2H, aromatic protons), 5.62 (t, $J = 9.0$ Hz, 1H, H-3′), 5.42 (d, $J = 8.4$ Hz, 1H, H-1′) 5.31 (t, $J = 9.9$ Hz, 1H, H-3), 4.80 (t, $J = 9.9$ Hz, 1H, H-4), 4.68 (dd, $J = 10.2$ and 3.3 Hz, 1H, H-2), 4.52 (d, $J = 3.3$ Hz, 1H, H-1), 4.19 (dd, $J = 10.5$ and 8.7 Hz, 1H, H-4′), 4.0–3.85 (m, 3H), 3.81–3.75 (m, 2H), 3.65–3.61 (m, 1H), 3.51 (dd, $J = 10.3$ and 6.3 Hz, 1H), 3.05 (s, 3H, OCH_3), 1.99 (s, 3H, COCH_3), 1.90 (s, 6H, 2COCH_3), 1.88 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.5, 170.4 (2C), 170.0, 168.3 (2C), 134.5 (2C), 132.0, 123.8 (3C), 98.8 (C-1′), 96.7 (C-1), 76.3, 73.9, 71.2, 70.4, 70.1, 69.5, 69.1, 68.0, 62.3, 55.3, 54.9, 21.0, 20.9 (2C), 20.8; ESIMS: m/z 676.2 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{35}NO_{16}$: C, 53.29; H, 5.40. Found: C, 53.14; H, 5.68.

1.10. Methyl 3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (13)

To a solution of **12** (1.25 g, 1.91 mmol) in CH_2Cl_2 (10 mL) were added pyridine (5.0 mL) and benzoyl cyanide (275 μL, 2.3 mmol) and the reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated and purified by column chromatography over SiO_2 (hexane–EtOAc, 2:1) to give **13** (1.3 g, 90%) as a glassy solid; $[\alpha]_D^{25}$ +87.0 (*c* 1.0, $CHCl_3$); IR (KBr): 3783, 2922, 2364, 1750, 1386, 1226, 1043, 759 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.08 (m, 2H, aromatic protons), 7.86–7.83 (m, 2H, aromatic protons), 7.75–7.72 (m, 2H, aromatic protons), 7.60–7.58 (m, 1H, aromatic protons), 7.51–7.46 (m, 2H, aromatic protons), 5.68 (t, $J = 10.2$ Hz, 1H, H-3′), 5.44 (d, $J = 8.4$ Hz, 1H, H-1′), 5.32 (t, $J = 9.6$ Hz, 1H, H-3), 4.78–4.62 (m, 4H), 4.47 (br s, 1H, H-1), 4.26 (t, $J = 9.0$ Hz, 1H, H-4), 3.94–3.71 (m, 4H), 3.49 (t, $J = 9.0$ Hz, 1H), 3.34 (br s, 1H, O*H*), 3.02 (s, 3H, OCH_3), 2.00 (s, 3H, COCH_3), 1.92 (s, 6H, 2COCH_3), 1.86 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6, 170.4 (2C), 169.8, 168.2, 167.6, 167.5, 134.6 (2C), 133.8, 132.0, 130.3 (2C), 130.2, 128.9 (3C), 123.8 (2C), 99.3 (C-1′), 96.6 (C-1), 74.8, 73.6, 71.2, 70.4, 70.1, 69.6, 69.4, 68.1, 63.8, 55.2, 54.9, 21.0 (2C), 20.8 (2C); ESIMS: m/z 780.3 $[M+Na]^+$. Anal. Calcd for $C_{36}H_{39}NO_{17}$ (757): C, 57.07; H, 5.19. Found: C, 58.94; H, 5.33.

1.11. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (15)

To a solution of **13** (500 mg, 0.66 mmol) and **14** (440 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (10 mL) was added powdered 4 Å molecular sieves (2.0 g) and the mixture was stirred under argon at rt for 1 h. The reaction mixture was cooled to 0 °C and NIS (340 mg, 1.5 mmol) and $HClO_4$–SiO_2 (50 mg) were added. After stirring at 0 °C for 2 h, the reaction mixture was filtered through Celite and washed with 5% aq $Na_2S_2O_3$, satd aq $NaHCO_3$, and water in succession, before being dried (Na_2SO_4) and concentrated to give a crude syrupy mass. Column purification of the crude product over SiO_2 (hexane–EtOAc, 2:1) afforded **15** (590 mg, 82%) as a yellow oil; $[\alpha]_D^{25}$ +56 (*c* 1.0, $CHCl_3$); IR (Neat): 3021, 2366, 1216, 764, 670 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 8.09–8.07 (m, 2H, aromatic protons), 7.86–7.83 (m, 2H, aromatic protons), 7.76–7.70 (m, 2H, aromatic protons), 7.62–7.58 (m, 1H, aromatic protons), 7.53–7.50 (m, 2H, aromatic protons), 5.76 (t, $J = 9.0$ Hz, 1H, H-3′), 5.51 (m, 1H, H-2″), 5.48 (d, $J = 8.4$ Hz, 1H, H-1′), 5.40 (d, $J = 1.8$ Hz, 1H, H-4″), 5.30 (t, $J = 9.6$ Hz, 1H, H-3), 5.28 (d, $J = 10.2$ Hz, 1H, H-1″), 5.12 (dd, $J = 11.1$ and 3.6 Hz, 1H, H-2), 4.79–4.70 (m, 1H, H-4), 4.66 (dd, $J = 10.2$ and 3.6 Hz, 1H, H-3″), 4.54 (dd, $J = 12.3$ and 4.5 Hz, 1H, H-2′), 4.44 (d, $J = 3.3$ Hz, 1H, H-1), 4.25–4.15 (m, 3H), 4.01–3.96 (m, 3H), 3.89–3.76 (m, 3H), 3.48 (dd, $J = 10.5$ and 7.2 Hz, 1H), 2.98 (s, 3H, OCH_3), 2.09 (s, 3H, COCH_3), 1.98 (s, 6H, 2COCH_3), 1.97 (s, 6H, 2COCH_3), 1.90 (s, 3H, COCH_3), 1.86 (s, 3H, COCH_3), 1.82 (s, 3H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 170.2 (2C), 170.3 (3C), 169.8, 169.3, 168.1, 168.0, 166.0, 134.2 (2C), 133.5 (2C), 131.7 (2C), 129.7 (2C), 129.4, 128.6 (2C), 123.4, 98.4 (C-1′), 96.1 (C-1″ and C-1), 73.9, 73.3, 72.5, 70.7, 69.9, 69.1, 68.8, 67.6 (2C), 67.3, 67.1, 66.8, 63.6, 61.1, 55.0, 54.7, 20.6 (3C), 20.5 (3C), 20.3 (2C); ESIMS: m/z 1110.4 $[M+Na]^+$. Anal. Calcd for $C_{50}H_{57}NO_{29}$: C, 55.20; H, 5.28. Found: C, 54.97; H, 5.54.

1.12. Methyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-α-D-glucopyranoside (16)

To a solution of **15** (500 mg, 0.46 mmol) in EtOH (5.0 mL) was added hydrazine hydrate (1.0 mL) and the mixture was stirred at 80 °C for 8 h. The solvent was removed and the crude mass was treated with pyridine (5.0 mL) and acetic anhydride (5.0 mL) at rt for 2 h and then concentrated. The residue was purified over SiO_2 (toluene–EtOAc, 1:1). The trisaccharide derivative thus obtained was dissolved in 0.05 M $NaOCH_3$ in CH_3OH (5.0 mL) and the mixture was stirred at rt for 12 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+), filtered, and concentrated. Purification through Sephadex LH-20 using 80% aq EtOH furnished pure trisaccharide **16** as a white powder (180 mg, 70%). $[\alpha]_D^{25}$ +30 (*c* 1.0, H_2O); IR (KBr): 3026, 2340, 1213 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 4.86 (d, $J = 9.0$ Hz, 1H″), 4.69 (d, $J = 3.0$ Hz, 1H, H-1), 4.62 (d, $J = 7.8$ Hz, 1H, H-1′), 4.16 (d, $J = 10.5$ Hz, 1H, H-2′), 3.99–3.85 (m, 5H), 3.81–3.70 (m, 6H), 3.66–3.63 (m, 3H), 3.58–3.48 (m, 3H), 3.34 (s, 3H, OCH_3), 1.96 (s, 3H, NHAc); ^{13}C NMR (75 MHz, D_2O): δ 173.0, 101.3 (C-1′ and C-1″), 99.8 (C-1), 80.4, 78.6, 76.7 (2C), 75.1, 74.8, 74.0 (2C), 73.5, 72.1, 71.8, 70.7 (2C), 61.4, 61.2, 54.4, 29.0; ESIMS: *m*/*z* 582 $[M+Na]^+$. Anal. Calcd for $C_{21}H_{37}NO_{16}$: C, 45.08; H, 6.67. Found: C, 44.80; H, 6.92.

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