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Concise synthesis of the pentasaccharide *O*-antigen corresponding to the Shiga toxin producing *Escherichia coli* O171

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ABSTRACT

An expedient total synthesis of a pentasaccharide as its 4-methoxyphenyl glycoside corresponding to the Shiga toxin producing *Escherichia coli* O171 has been achieved for the first time in excellent yield. Most of the glycosylation steps are highly stereoselective. Stereoselective glycosylation of sialic acid derivative was obtained exploiting the nitrile effect of the solvent used.

1. Introduction

Escherichia coli is a group of Gram-negative bacteria that colonize in the infant's gastrointestinal tract within hours of birth [1]. *Escherichia* has been classified by a series of physiological and morphological traits. Based on the immunogenicity of the surface oligosaccharides [2], *E. coli* have been divided into a number of serotypes. There are three types of *E. coli* antigens which include: (i) somatic (*O*) antigen, (ii) capsular (*K*) antigen and (iii) flagellar (*H*) antigen. Although *E. coli* is generally confined to the intestinal lumen, in an immunosuppressed host "nonpathogenic" strains of *E. coli* can also cause infections [3]. In general, three common infections, e.g. (i) enteric/diarrhoeal, (ii) urinary tract infections and (iii) septicaemia/meningitis caused by virulent *E. coli* strains [4]. Pathogenic *E. coli* strains are divided in six classes, which are: (i) enteropathogenic *E. coli* (EPEC), (ii) enteroinvasive *E. coli* (EIEC), (iii) enterotoxigenic *E. coli* (ETEC), (iv) enteroaggregative *E. coli* (EAEC), (v) diffusely adherent *E. coli* (DAEC), and (vi) enterohemorrhagic *E. coli* (EHEC) [5].

Enterohemorrhagic *E. coli* (EHEC) strains act as the etiological agent of diarrhoea with lifethreatening complications like haemorrhagic colitis (HC) and haemolytic–uraemic syndrome (HUS). EHEC strains show considerable toxic effect on the cultured Vero cells and also known as "verotoxigenic *E. coli*" (VTEC) strains. They are also termed as "Shiga toxin producing *E. coli*" (STEC) because of their ability to produce bacteriophage-mediated Shiga-like toxin [6]. The well documented Shiga toxin producing EHEC strain is *E. coli* O157:H7, which is responsible for the frequent cause of serious intestinal infections and associated with several outbreaks of disease in the developed countries [7,8]. Besides *E. coli* O157:H7, several other *E. coli* serotypes have been reported to be associated with the STEC class [9]. Recent structural analysis of the *O*-antigen of Shiga toxin producing *E. coli* O171 showed that it contains an acidic pentasaccharide repeating unit having an *N*-acetylneuraminic acid (sialic acid) at the non-reducing terminus [10]. Several studies in the past have established that the oligosaccharide epitopes of the bacterial *O*-antigens have strong influence on the immunochemical activities of the glycoconjugate vaccines derived from them. Therefore, bacterial *O*-antigens have been chosen for the development of glycoconjugate vaccine candidates against infectious diseases [11]. Because of the limited availability of the desired cell-wall pentasaccharide of *E. coli* O171 from the natural source, it is highly essential to develop a concise chemical synthetic strategy for the preparation of the target pentasaccharide for its ready availability in the biological studies. In the immunochemical studies, most often it is required to conjugate the oligosaccharide moiety with a carrier protein through a spacer linker. Therefore, synthesis of the target pentasaccharide with a temporary protecting group at the reducing end would be beneficial for its ready removal after construction of the pentasaccharide. As a part of our ongoing program on the synthesis of complex oligosaccharides corresponding to the microbial cell-wall polysaccharides [12], we describe herein an efficient chemical synthesis of the sialic acid containing pentasaccharide repeating unit of the *O*-antigen of Shiga toxin producing *E. coli* O171 as its 4-methoxyphenyl glycoside involving a block synthetic strategy (Fig. 1). 4-Methoxyphenyl group has been chosen as the temporary anomeric protecting group because of its easy removal after construction of the pentasaccharide.

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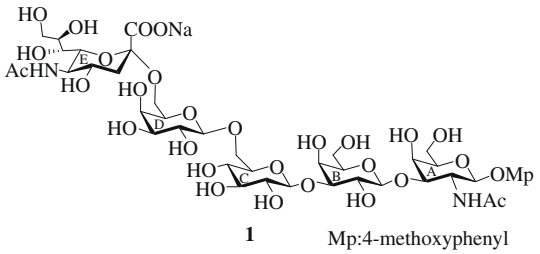


1 Mp:4-methoxyphenyl

Fig. 1. Chemical structure of the synthesized pentasaccharide repeating unit of *E. coli* O171 as its 4-methoxyphenyl glycoside.

2. Experimental

2.1. General

All reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (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, 2D COSY, HMQC spectra were recorded on Brucker Avance DRX 500 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 Perkin Elmer 341 polarimeter. Commercially available grades of organic solvents of adequate purity are used in many reactions.

2.2. 4-Methoxyphenyl (2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranoside (**7**)

To a solution of compound **2** (2 g, 3.97 mmol) and thioglycoside donor **3** (2 g, 5.04 mmol) in CH_2Cl_2 (25 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 −25 °C and *N*-iodosuccinimide (1.3 g, 5.77 mmol) and TMSOTf (20 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 **7** (2.8 g, 84%): R_f (0.4, toluene–EtOAc: 4:1); colorless oil; $[\alpha]_D^{25}$ +41.3 (*c* 1.5, $CHCl_3$); IR (neat): 3443, 2841, 1714, 1510, 1390, 1269, 1089, 999, 704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.89–7.23 (m, 14H, Ar-H), 6.81 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.64 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.64 (d, *J* = 8.5 Hz, 1H, H-1_A), 5.47 (s, 1H, PhC*H*), 5.19 (s, 1H, PhC*H*), 4.98 (dd, *J* = 10.8, 3.6 Hz, 1H, H-3_B), 4.88–4.83 (m, 2H, H-2_B, H-2_A), 4.69 (dd, *J* = 11.2, 3.3 Hz, 1H, H-3_A), 4.60 (d, *J* = 8.0 Hz, 1H, H-1_B), 4.32–4.25 (m, 2H, H-4_B, H-6_{aB}), 4.06–3.99 (m, 2H, H-4_A, H-6_{bB}), 3.71–3.68 (m, 1H, H-6_{aA}), 3.64 (s, 3H, OCH_3), 3.57 (brs, 1H, H-5_A), 3.47–3.43 (m, 1H, H-6_{bA}), 3.17 (brs, 1H, H-5_B), 1.92, 1.50 (2s, 6H, 2COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.2 (2*C*OCH$_3$), 168.7, 167.3 (*C*OPhth), 155.4–114.5 (Ar-C), 101.1 (C-1_B), 100.9 (PhCH), 100.6 (PhCH), 97.9 (C-1_A), 74.9 (C-4_B), 73.3 (C-4_A), 72.5 (C-3_A), 69.0 (C-6_B), 68.8 (C-6_A), 67.8 (2C, C-2_B, C-3_B), 66.8 (C-5_A), 62.6 (C-5_B), 55.5 (OCH$_3$), 54.6 (C-2_A), 20.8, 20.2 (2COCH$_3$); ESI-MS: *m/z* 860.8 $[M+Na]^+$; Anal. Calcd for $C_{45}H_{43}NO_{15}$ (837.26): C, 64.51; H, 5.17. Found: C, 64.32; H, 5.43.

2.3. 4-Methoxyphenyl (4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranoside (**8**)

A solution of compound **7** (2.5 g, 2.98 mmol) in 0.1 M CH_3ONa in CH_3OH (100 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 **8** (2.2 g, quantitative): R_f (0.2, toluene–EtOAc: 2:1); Yellow oil; $[\alpha]_D^{25}$ +27.5 (*c* 1.5, $CHCl_3$); IR (neat): 3502, 2926, 1751, 1716, 1508, 1388, 1369, 1222, 1080, 1043, 952, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.85–7.23 (m, 14H, Ar-H), 6.88 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.69 (d, *J* = 9.1 Hz, 2H, Ar-H), 5.69 (d, *J* = 8.3 Hz, 1H, H-1_A), 5.51 (s, 1H, PhC*H*), 5.19 (s, 1H, PhC*H*), 4.75 (t, *J* = 8.3 Hz, 1H, H-2_A), 4.73 (dd, *J* = 10.8, 3.2 Hz, 1H, H-3_A), 4.59 (d, *J* = 8.0 Hz, 1H, H-1_B), 4.34 (brs, 1H, H-4_A), 4.23 (d, *J* = 12.0 Hz, 1H, H-6_{aA}), 3.95 (d, *J* = 11.8 Hz, 1H, H-6_{bA}), 3.77 (brs, 1H, H-4_B), 3.74–3.71 (m, 1H, H-6_{aB}), 3.67 (s, 3H, OCH_3), 3.56–3.47 (m, 2H, H-6_{bB}, H-3_B), 3.17–3.16 (m, 1H, H-5_B), 3.12–2.82 (m, 1H, H-2_B), 2.40–2.36 (m, 1H, H-5_A); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 167.4 (*C*OPhth), 155.5–114.3 (Ar-C), 101.0 (PhCH), 100.7 (PhCH), 99.3 (C-1_B), 97.8 (C-1_A), 75.5 (C-4_B), 74.3 (C-4_A), 72.5 (C-3_A), 69.2 (C-2_B), 68.9 (2C, C-3_B, C-6_B), 68.1 (C-6_A), 66.6 (C-5_A), 63.8 (C-5_B), 55.6 (OCH$_3$), 52.2 (C-2_A); ESI-MS: *m/z* 776.8 $[M+Na]^+$; Anal. Calcd for $C_{41}H_{39}NO_{13}$ (753.24): C, 65.33; H, 5.22. Found: C, 65.10; H, 5.46.

2.4. Phenyl (2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside (**10**)

To a solution of compound **4** (2.5 g, 5.67 mmol) in CH_3CN–H_2O (30 mL; 20:1 v/v) was added *N*-iodosaccharin (3.5 g, 11.32 mmol) and the reaction mixture was allowed to stir for 15 min at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and successively washed with 5% aq. $Na_2S_2O_3$ and water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to give pure hemiacetal derivative, which was used immediately for the next step. To a solution of the hemiacetal derivative (1.9 g, 4.79 mmol) in anhydrous CH_2Cl_2 (25 mL) was added CCl_3CN (3 mL, 29.91 mmol) and the reaction mixture was cooled to −10 °C. To the cold reaction mixture was added DBU (100 μL, 0.67 mmol) and the reaction mixture was stirred at 5 °C for 6 h and evaporated to dryness under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (6:1) as eluant to give pure compound **9**, which was used immediately for the glycosylation. To a solution of compound **9** (2.3 g, 4.25 mmol) in anhydrous CH_2Cl_2 (25 mL) were added compound **5** (1.4 g, 3.51 mmol) and MS-4 Å (3 g) and the reaction mixture was allowed to cool at −30 °C. To the cold reaction mixture was added TMSOTf (30 μL) and the reaction mixture was allowed to stir at −20 °C for 1 h. The reaction was quenched with Et_3N (0.1 mL), filtered and washed with CH_2Cl_2 (100 mL). The organic layer was washed with satd. aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure compound **10** (2.1 g, 77%): R_f (0.3, hexane–EtOAc: 2:1); colorless oil; $[\alpha]_D^{25}$ −29.8 (*c* 1.5, $CHCl_3$); IR (neat): 2953, 1761, 1712, 1379, 1382, 1223, 1069, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.39–7.26 (m, 10H, Ar-H), 5.45 (d, *J* = 2.9 Hz, 1H, H-4_D), 5.19 (t, *J* = 9.4 Hz, 1H, H-2_D), 5.17 (dd, *J* = 10.4, 8.0 Hz, 1H, H-3_C), 5.00 (dd, *J* = 10.0, 3.4 Hz, 1H, H-3_D), 4.98 (t, *J* = 9.7 Hz, 1H, H-2_C), 4.89 (t, *J* = 9.6 Hz, 1H, H-4_C), 4.55 (d, *J* = 11.9 Hz, 1H, PhCH_2), 4.51 (d, *J* = 7.9 Hz, 1H, H-1_D), 4.46 (d, *J* = 10.0 Hz, H-1_C), 4.42 (d, *J* = 11.9 Hz, 1H, PhCH_2), 3.89 (dd, *J* = 11.0, 2.2 Hz, 1H, H-6_{aC}),

3.84–3.83 (m, 1H, H-5_D), 3.70–3.67 (m, 1H, H-5_C), 3.60–3.53 (m, 2H, H-6_{bC}, H-6_{aD}), 3.48 (dd, J = 10.0, 6.6 Hz, 1H, H-6_{bD}), 2.07, 2.05, 2.04, 1.99, 1.98, 1.97 (6s, 18H, 6COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5 (2C), 170.4, 170.0 169.9, 169.8 (6$COCH_3$), 137.9–128.3 (Ar-C), 101.7 (C-1_D), 83.5 (C-1_C), 77.7 (C-5_C), 74.3 (C-2_D), 73.9 (PhCH_2), 72.6 (C-5_D), 71.5 (C-3_D), 70.3 (C-2_C), 69.5 (C-3_C), 69.3 (C-4_C), 68.9 (C-6_C), 67.9 (C-4_D), 67.8 (C-6_D), 21.2, 21.1, 21.0, 20.9, 20.8 (2C, 6$COCH_3$); ESI-MS: m/z 799.3 $[M+Na]^+$; Anal. Calcd for $C_{37}H_{44}O_{16}S$ (776.24): C, 57.21; H, 5.71. Found: C, 57.02; H, 6.0.

2.5. 4-Methoxyphenyl (2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranosyl-(1 → 6)-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 3)-(2-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranoside (**11**)

To a solution of the disaccharide acceptor **8** (1.7 g, 2.25 mmol) and the disaccharide thioglycoside donor **10** (2 g, 2.57 mmol) in CH_2Cl_2 (20 mL) was added MS-4 Å (2 g) and the reaction mixture was allowed to stir at room temperature for 1 h. The reaction mixture was cooled to −40 °C and NIS (650 mg, 2.88 mmol) and TMSOTf (10 μL) were added in succession. The reaction mixture was allowed to stir at same temperature for 30 min and diluted with CH_2Cl_2 (100 mL). The reaction mixture was filtered through a Celite® bed and the organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness. The crude mass was acetylated using acetic anhydride (5 mL) and pyridine (5 mL) at room temperature. The acetylated crude mass was purified over SiO_2 using hexane–EtOAc (3:1) as eluant to furnish pure **11** (2.6 g, 79%): R_f (0.5, hexane–EtOAc: 1:1); colorless oil; $[\alpha]_D^{25}$ +12.5 (c 1.5, $CHCl_3$); IR (neat): 2933, 1755, 1716, 1508, 1388, 1369, 1247, 1220, 1051, 754, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.63–7.24 (m, 19H, Ar-H), 6.87 (d, J = 9.1 Hz, 2H, Ar-H), 6.72 (m, J = 9.1 Hz, 2H, Ar-H), 5.72 (d, J = 8.4 Hz, 1H, H-1_A), 5.55 (s, 1H, PhCH), 5.47 (d, J = 3.3 Hz, 1H, H-4_D), 5.33 (s, 1H, PhCH), 5.20 (dd, J = 10.8, 3.4 Hz, 1H, H-3_C), 5.07 (t, J = 9.4 Hz, 1H, H-2_D), 5.02–4.99 (m, 2H, H-3_D, H-2_B), 4.94–4.91 (m, 2H, H-2_C, H-2_A), 4.87 (t, J = 8.1 Hz, 1H, H-4_C), 4.81 (dd, J = 10.8, 3.2 Hz, 1H, H-3_A), 4.63 (d, J = 7.9 Hz, 1H, H-1_B), 4.60 (d, J = 8.0 Hz, 1H, H-1_D), 4.52 (d, J = 11.9 Hz, 1H, PhCH_2), 4.46 (d, J = 8.0 Hz, 1H, H-1_C), 4.40 (d, J = 11.9 Hz, 1H, PhCH_2), 4.34 (d, J = 12.2 Hz, 1H, 6_{aC}), 4.25 (d, J = 3.2 Hz, 1H, H-4_B), 4.11 (d, J = 11.0 Hz, 1H, 6_{bC}), 4.02 (d, J = 3.2 Hz, 1H, H-4_A), 3.94 (dd, J = 10.9, 3.5 Hz, 1H, H-3_B), 3.90 (d, J = 8.7 Hz, 1H, H-6_{aD}), 3.84–3.82 (m, 1H, H-5_D), 3.76 (brs, 1H, H-5_B), 3.71 (s, 3H, OCH_3), 3.62–3.44 (m, 6H, H-6_{bD}, H-6_{abA}, H-6_{abB}, H-5_C), 3.17 (brs, 1H, H-5_A), 2.06, 2.03, 2.00, 1.97, 1.95, 1.87, 1.72 (7s, 21H, 7COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.6, 170.5, 170.4, 169.9, 169.8, 169.3 (7$COCH_3$), 168.9, 167.9 (COPhth), 155.9–114.8 (Ar-C), 101.9 (C-1_C), 101.8 (PhCH), 101.5 (C-1_D), 100.7 (PhCH), 98.5 (C-1_A), 96.1 (C-1_B), 75.8 (C-4_A), 73.9 (2C, PhCH_2, C-3_A), 73.7 (C-3_B), 73.4 (C-2_D), 73.2 (C-3_D), 72.9 (C-4_B), 72.6 (C-4_C), 71.6 (2C, C-2_B, C-4_D), 70.5 (C-6_C), 69.6 (C-6_A), 69.4 (C-6_B), 69.1 (C-2_C), 68.6 (C-6_D), 68.0 (C-3_C), 67.9 (C-5_D), 67.1 (C-5_C), 63.7 (C-5_A), 57.2 (C-5_B), 55.9 (OCH_3), 52.7 (C-2_A), 21.2, 21.1 (2C), 21.0, 20.9 (2C), 20.8 (7COCH_3); ESI-MS: m/z 1484.5 $[M+Na]^+$; Anal. Calcd for $C_{74}H_{79}NO_{30}$ (1461.47): C, 60.78; H, 5.44. Found: C, 60.57; H, 5.67.

2.6. 4-Methoxyphenyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyl-(1 → 6)-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 3)-(2-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside (**12**)

To a solution of compound **11** (2.5 g, 1.71 mmol) in n-butanol (80 mL) was added ethylene diamine (0.5 mL, 7.47 mmol) and the reaction mixture was allowed to stir at 100 °C for 6 h. The solvents were removed under reduced pressure and co-evaporated with toluene (3 × 30 mL). To a solution of the crude product in pyridine (10 mL) was added acetic anhydride (10 mL) and the reaction mixture was stirred at room temperature for 3 h. The solvents were removed under reduced pressure and co-evaporated with toluene (3 × 30 mL). To a solution of the acetylated product in CH_3OH (30 mL) was added 20% $Pd(OH)_2$–C (100 mg) and the reaction mixture was allowed to stir under a positive pressure of hydrogen at room temperature for 4 h. The reaction mixture was filtered through a Celite® bed and evaporated to dryness. The crude product was passed through a short pad of SiO_2 using hexane–EtOAc (1:1) as eluant to give pure **12** (1.4 g, 64%); R_f (0.2, hexane–EtOAc: 1:2); colorless oil; $[\alpha]_D^{25}$ +13.2 (c 1.5, $CHCl_3$); IR (neat): 3481, 2924, 1753, 1714, 1508, 1371, 1246, 1222, 1047, 754 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80–7.27 (m, 10H, Ar-H), 6.87 (d, J = 9.1 Hz, 2H, Ar-H), 6.72 (d, J = 9.1 Hz, 2H, Ar-H), 5.71 (d, J = 8.4 Hz, 1H, H-1_A), 5.56 (s, 1H, PhCH), 5.35 (d, J = 3.4 Hz, 1H, H-4_D), 5.31 (s, 1H, PhCH), 5.20 (dd, J = 10.4, 3.3 Hz, 1H, H-3_C), 5.08 (t, J = 9.4 Hz, 1H, H-2_D), 5.03–4.97 (m, 3H, H-2_B, H-3_D, H-4_C), 4.91–4.87 (m, 2H, H-2_C, H-2_A), 4.77 (dd, J = 10.8, 3.4 Hz, 1H, H-3_A), 4.68 (d, J = 8.0 Hz, 1H, H-1_B), 4.66 (d, J = 8.0 Hz, 1H, H-1_D), 4.47 (d, J = 8.0 Hz, 1H, H-1_C), 4.34 (d, J = 12.3 Hz, 1H, H-6_{aC}), 4.25 (d, J = 3.4 Hz, 1 H, H-4_B), 4.12 (d, J = 11.2 Hz, 1H, H-6_{bC}), 4.00–3.97 (m, 2H, H-4_A, H-3_B), 3.93–3.90 (m, 1H, H-6_{aD}), 3.71 (s, 3H, OCH_3), 3.69–3.56 (m, 4H, H-6_{abB}, H-6_{aA}, H-6_{bD}), 3.47–3.39 (m, 1H, H-6_{bA}), 3.37–3.32 (m, 3H, H-5_C, H-5_D, H-5_B), 3.15 (brs, 1 H, H-5_A), 2.16, 2.15, 2.04, 2.02, 2.01, 1.96, 1.89 (7s, 21 H, 7COCH_3), 1.78 (s, 3 H, NHCOCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.2, 170.8, 170.7, 170.5, 170.1, 169.9, 169.3 (7$COCH_3$), 168.9 (NH$COCH_3$), 156.0–114.8 (Ar-C), 101.9 (PhCH), 101.5 (C-1_C), 101.4 (C-1_D), 100.7 (PhCH), 98.5 (C-1_A), 96.6 (C-1_B), 75.9 (C-4_A), 74.4 (C-3_A), 74.2 (C-5_D), 73.5 (C-3_B), 73.4 (C-2_D), 73.2 (C-4_B), 73.1 (C-2_C), 71.6 (C-2_B), 71.5 (C-3_D), 70.5 (C-4_C), 69.7 (C-3_C), 69.6 (C-6_C), 69.2 (C-5_C), 68.6 (C-6_B), 68.5 (C-6_D), 68.4 (C-4_D), 67.1 (C-5_B), 63.8 (C-5_A), 61.2 (H-6_A), 55.9 (OCH_3), 52.7 (C-2_A), 21.3, 21.2 (2C), 21.1 (2C), 21.0, 20.9 (7COCH_3), 20.8 (NHCOCH_3); ESI-MS: m/z 1301.2 $[M+NH_4]^+$; Anal. Calcd for $C_{61}H_{73}NO_{29}$ (1283.43): C, 57.05; H, 5.73. Found: C, 56.90; H, 5.94.

2.7. 4-Methoxyphenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 6)-(2,3,-4-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 6)-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 3)-(2-O-acetyl-4,6-O-benzylidene-β-D-galacto-pyranosyl)-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside (**13**)

To a solution of compound **12** (1 g, 0.78 mmol) and thioglycoside donor **6** (800 mg, 1.37 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 (350 mg, 1.55 mmol) and TfOH (5 μL) were added to it. After stirring the reaction mixture at the same temperature for 20 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 **13** (725 mg, 53%); R_f (0.3, toluene–EtOAc: 1:4); colorless oil; $[\alpha]_D^{25}$ +19.5 (c 1.5, $CHCl_3$); IR (neat): 3481, 2928, 1749, 1716, 1541, 1373, 1222, 1045, 756, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.62–7.29 (m, 10H, Ar-H), 6.91 (d, J = 9.0 Hz, 2H, Ar-H), 6.69 (d, J = 9.0 Hz, 2H, Ar-H), 5.73 (d, J = 8.5 Hz, 1H, H-1_A), 5.56 (s, 1H, PhCH), 5.49 (d, J = 3.4 Hz, 1H, H-4_D), 5.35 (s, 1H, PhCH), 5.34–5.26 (m, 3H, H-7_E, H-8_E and NHCOCH$_3$), 5.17 (dd, J = 10.1, 3.5 Hz, 1H, H-3_C), 5.09–5.05 (m, 2H, H-2_D, H-2_C), 5.03–4.97 (m, 2H, H-4_E, H-3_D), 4.93–4.84 (m, 3H, H-2_B, H-4_C, H-2_A), 4.78 (dd, J = 10.8, 3.4 Hz, 1H, H-3_A), 4.67 (d, J = 8.0 Hz, 1H, H-1_B), 4.58 (d, J = 8.0 Hz, 1H, H-

1_D), 4.55 (d, J = 7.9 Hz, 1H, H-1_C), 4.42–4.37 (m, 2H, H-9_{aE}, H-6_{aC}), 4.37 (dd, J = 10.8, 3.5 Hz, 1H, H-3_B), 4.29 (d, J = 3.4 Hz, 1H, H-4_B), 4.16–4.09 (m, 2H, H-9_{bE}, H-6_{bC}), 4.06–4.01 (m, 4H, H-5_E, H-6_E, H-4_A, H-6_{aD}), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.95–3.68 (m, 6H, H-6_{abA}, H-6_{abB}, H-6_{bD}, H-5_C), 3.59–3.57 (m, 1H, H-5_B), 3.18 (brs, 1H, H-5_A), 2.71 (dd, J = 12.1, 3.8 Hz, 1H, H-3_{eE}), 2.19, 2.16, 2.13, 2.06, 2.05, 2.04, 2.03, 2.02, 2.01, 1.97, 1.95, 1.89, 1.88 (13s, 39H, 13COCH_3), 1.86–1.84 (m, 1H, H-3_{aE}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 171.1, 170.9 (2C), 170.8 (2C), 170.7, 170.6, 170.5, 170.3, 170.2, 170.0 169.5 (13COCH$_3$), 168.3 (COOCH$_3$), 155.3–114.5 (Ar-C), 101.9 (C-2_E), 101.8 (PhCH), 101.7 (C-1_C), 101.6 (C-1_D), 100.6 (PhCH), 99.5 (C-1_A), 98.2 (C-1_B), 75.7 (C-4_A), 73.8 (C-3_A), 73.5 (C-2_B), 73.2 (C-5_D), 73.0 (2C, C-3_B, C-2_D), 72.3 (C-4_B), 71.6 (C-5_C), 71.5 (C-4_C), 69.6 (C-2_C), 69.3 (C-3_C), 69.1 (C-3_D), 69.0 (C-5_E), 68.6 (C-7_E), 68.3 (C-8_E), 68.4 (2C, C-4_E and C-6_D), 68.2 (C-6_B), 67.9 (C-6_A), 67.6 (2C, C-5_B and C-4_D), 67.1 (C-6_C), 63.8 (C-9_E), 63.0 (C-5_A), 57.2 (COOCH_3), 54.2 (C-6_E), 53.7 (OCH_3), 52.2 (C-2_A), 38.3 (C-3_E), 23.2 (2C), 21.4, 21.3 (2C), 21.2 (3C), 21.1, 21.0 (2C), 20.9, 20.8 (13COCH_3); ESI-MS: m/z 1774.6 $[M+NH_4]^+$; Anal. Calcd for $C_{81}H_{100}N_2O_{41}$ (1756.58): C, 55.35; H, 5.73. Found: C, 55.13; H, 5.97.

*2.8. 4-Methoxyphenyl (sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 6)-(β-D-galactopyranosyl)-(1 → 6)-(β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranoside (**1**)*

To a solution of the pentasaccharide derivative **13** (700 mg, 0.4 mmol) in methanol (20 mL) was added 20% $Pd(OH)_2$–C (150 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 12 h and then a few drops of distilled water was added to the reaction mixture and allowed to stir for 6 h. The reaction mixture was neutralized with Dowex 50 W X8 (H^+) resin, filtered and evaporated to dryness and again passed through a short pad of Dowex 50 W 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 **1** as its sodium salt (330 mg, 73%); R_f (0.3, CH_3CN–CH_3OH–H_2O: 2:1:0.5); White powder; $[\alpha]_D^{25}$ −9.7 (c 1.1, H_2O); IR (neat): 3441, 3021, 1712, 1464, 1230, 767, 669 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ 6.99 (d, J = 8.8 Hz, 2H, Ar-H), 6.86 (d, J = 8.8 Hz, 2H, Ar-H), 5.09 (d, J = 8.4 Hz, 1H, H-1_A), 4.59 (d, J = 7.8 Hz, 1H, H-1_C), 4.48–4.45 (m, 1H, H-5_E), 4.29 (d, J = 8.1 Hz, 1H, H-1_B), 4.26 (d, J = 7.7 Hz, 1H, H-1_D), 4.25–4.22 (m, 2H, H-7_E, H-8_E), 4.16–4.12 (m, 1H, H-3_A), 4.09–3.87 (m, 5H, H-4_A, H-4_B, H-4_D, H-4_E, H-6_E), 3.85–3.59 (m, 12H, H-5_D, H-4_C, H-2_A, H-6_{abA}, H-6_{abB}, H-9_{abE}, H-6_{abC}, H-6_{aD}), 3.69 (s, 3H, OCH_3), 3.57–3.37 (m, 6H, H-5_B, H-6_{bD}, H-2_D, H-3_D, H-2_B, H-3_B), 3.35–3.22 (m, 4H, H-2_C, H-3_C, H-5_A, H-5_C), 2.67 (dd, J = 12.2, 3.3 Hz, 1H, H-3_{eE}), 1.97 (s, 3H, NHCOCH_3), 1.96 (s, 3H, NHCOCH_3), 1.69 (t, J = 12.2 Hz, 1H, H-3_{aE}); ^{13}C NMR (125 MHz, D_2O): δ 175.2 (2C, COONa, NHCOCH$_3$), 174.6 (NHCOCH$_3$), 118.4–115.5 (Ar-C), 104.1 (C-1_C), 104.0 (C-1_D), 103.9 (C-1_B), 103.8 (C-1_A), 101.5 (C-2_E), 75.8 (C-3_C), 75.6 (C-3_B), 75.4 (C-3_A), 74.8 (C-5_A), 73.7 (2C, C-3_D, C-4_E), 73.1 (C-5_B), 73.0 (C-5_D), 72.8 (C-6_E), 71.2 (C-2_C), 71.1 (C-5_C), 70.8 (C-2_B), 70.6 (2C, C-4_B, C-4_D), 69.3 (2C, C-2_D, C-4_C), 68.6 (C-7_E), 67.2 (C-4_A), 63.6 (C-8_E), 63.5 (C-9_E), 61.4 (2C, C-6_A, C-6_C), 61.2 (2C, C-6_B, C-6_D), 56.2 (OCH$_3$), 52.5 (C-2_A), 51.6 (C-5_E), 39.4 (C-3_E), 22.5 (2C, NHCOCH$_3$); ESI-MS: m/z 1127.5 $[M+1]^+$; Anal. Calcd for $C_{44}H_{67}N_2NaO_{30}$ (1126.37): C, 46.89; H, 5.99. Found: C, 46.68; H, 6.25.

3. Results and discussion

In order to synthesize the target pentasaccharide **1** as its 4-methoxyphenyl glycoside, a block synthetic strategy has been adopted in which a disaccharide thioglycoside (**10**) was stereoselectively coupled to a disaccharide acceptor (**8**) under iodonium ion promoted thioglycoside activation condition. The tetrasaccharide derivative (**11**) thus obtained was transformed to a tetrasaccharide acceptor (**12**) for the final stereoselective glycosylation with sialic acid derived thioglycoside donor towards the formation of a sialylated tetrasaccharide derivative (**13**), which was deprotected using standard reaction condition to furnish pentasaccharide **1** as its 4-methoxyphenyl glycoside. For this purpose a series of suitably functionalized monosaccharide derivatives **2** [13], **3** [14], **4** [15], **5** [16] and **6** [17] were prepared from the commercially available reducing sugars (Fig. 2).

Stereoselective glycosylation of compound **2** with the thioglycoside derivative **3** under iodonium ion mediated thioglycoside activation condition using *N*-iodosuccinimide (NIS)–trimethylsilyl trifluoromethanesulfonate (TMSOTf) [18] furnished disaccharide derivative **7** in 84% yield, which was deacetylated using sodium methoxide to give disaccharide diol derivative (**8**) in quantitative yield. Appearance of signals at δ 5.64 (d, J = 8.5 Hz, H-1_A), 5.47 (s, PhCH), 5.19 (s, PhCH), 4.60 (d, J = 8.0 Hz, 1H, H-1_B) in the ^{1}H NMR and at δ 101.1 (C-1_B), 100.9 (PhCH), 100.6 (PhCH), 97.9 (C-1_A) in the ^{13}C NMR spectra confirmed the stereoselective formation of disaccharide derivative **7**. In another experiment, compound **4** was treated with *N*-iodosaccharin in moist acetonitrile [19] to give hemiacetal derivative, which was converted to trichloroacetimidate [20] derivative **9** in 89% yield. Compound **9** was allowed to glycosylate with thioglycoside acceptor **5** in the presence of TMSOTf [21] to give disaccharide thioglycoside donor **10** in 77% yield. In this case thioglycoside **5** has been used as orthogonal glycosyl donor as it acts as a glycosyl acceptor under trichloroacetimidate activation condition (Scheme 1).

After preparing a disaccharide glycosyl acceptor **8** and a disaccharide thioglycoside donor **10**, attempts were made towards the synthesis of target pentasaccharide **1** using stereoselective glycosylations. Iodonium ion mediated regio and stereoselective glycosylation of compound **8** with compound **10** using NIS-TMSOTf [18] as glycosylation activator furnished (1 → 3)-β-linked tetrasaccharide derivative, which was purified after acetylation of the glycosylation product to give the tetrasaccharide derivative **11** in 79% yield. Presence of signals at δ 5.72 (d, J = 8.4 Hz, H-1_A), 5.55 (s, PhCH), 5.33 (s, PhCH), 4.63 (d, J = 7.9 Hz, H-1_B), 4.60 (d, J = 8.0 Hz, H-1_D), 4.46 (d, J = 8.0 Hz, H-1_C) in the ^{1}H NMR and at δ 101.9 (C-1_C), 101.8 (PhCH), 101.5 (C-1_D), 100.7 (PhCH), 98.5 (C-1_A), 96.1 (C-1_B) in the ^{13}C NMR spectra supported the stereoselective forma-

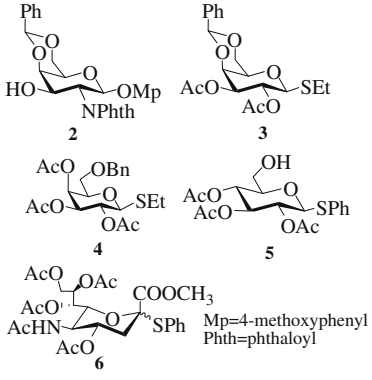
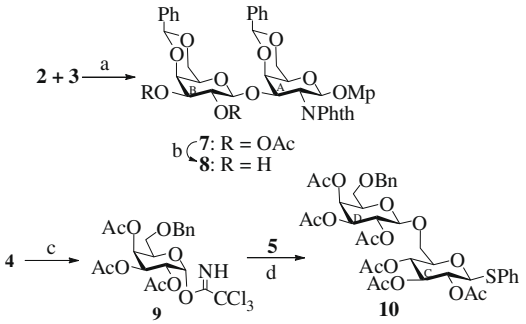
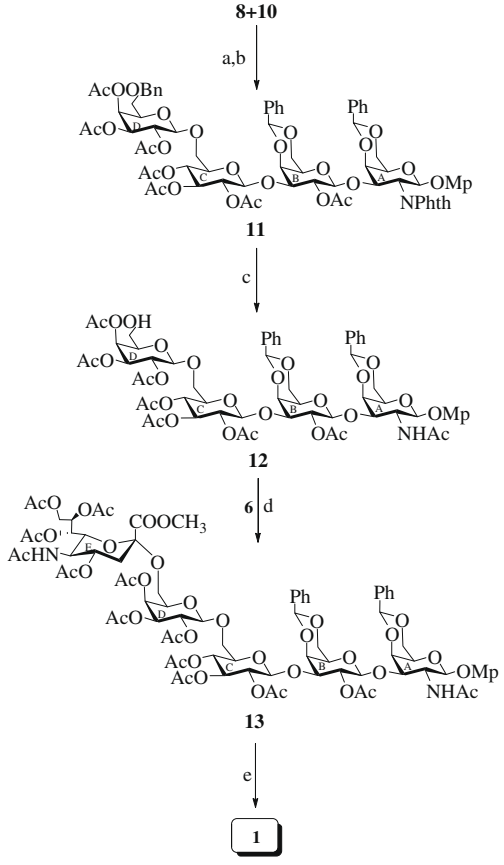


Fig. 2. Suitably functionalized monosaccharide intermediates used for the construction of the target pentasaccharide (**1**).



Scheme 1. Reagents: (a) *N*-iodosuccinimide, TfOH, CH_2Cl_2, MS-4 Å, −25 °C, 1 h, 84%; (b) CH_3ONa, CH_3OH, room temperature, 30 min, quantitative; (c) (i) *N*-iodosaccharin, CH_3CN–H_2O, room temperature, 15 min; (ii) CCl_3CN, DBU, CH_2Cl_2, 5 °C, 6 h, 89% and (d) TMSOTf, CH_2Cl_2, MS-4 Å, −20 °C, 1 h, 77%.

tion of tetrasaccharide derivative **11**. *N*-Phthaloyl group of the tetrasaccharide derivative **11** was converted to the *N*-acetyl group on treatment with ethylenediamine [22] followed by *N*-acetylation and the *N*-acetylated product was subjected to a controlled hydrogenation condition [12i,23] using H_2 over Pearlmans catalyst [24] to furnish 6-*O*-debenzylated tetrasaccharide acceptor **12** in 64% yield having benzylidene acetals unaffected. Besides the ring protons, presence of two singlets at δ 5.56 (s, PhC*H*), 5.31 (s, 1 H, PhC*H*) in the ^{1}H NMR and two signature signals at δ 101.9 (Ph*C*H), 100.7



Scheme 2. Reagents: (a) *N*-iodosuccinimide, TMSOTf, CH_2Cl_2, MS-4 Å, −40 °C, 30 min, 79%; (b) acetic anhydride, pyridine, room temperature, 3 h, quantitative; (c) (i) ethylenediamine, *n*-butanol, 100 °C, 6 h; (ii) acetic anhydride, pyridine, room temperature, 3 h; (iii) H_2, 20% $Pd(OH)_2$–C, CH_3OH, room temperature, 4 h, 64% in three steps; (d) NIS, TfOH, MS-3 Å, CH_3CN–CH_2Cl_2 (5:1), −10 °C, 20 h, 53% and (e) (i) H_2, 20% $Pd(OH)_2$–C, CH_3OH, room temperature, 24 h; (ii) 0.1 M CH_3ONa, CH_3OH, room temperature, 12 h then five drops of H_2O, 6 h, room temperature, 73%.

(Ph*C*H) corresponding to the benzylidene acetal in the ^{13}C NMR spectra confirmed the removal of 6-*O*-benzyl ether leaving benzylidene acetal intact in the compound **12**. Finally iodonium ion promoted stereoselective glycosylation of the tetrasaccharide derivative **12** with sialic acid derived thioglycoside derivative **6** using CH_3CN–CH_2Cl_2 as solvent exploiting nitrile effect [25] of the solvent afforded pentasaccharide derivative **13** in 53% yield, in which the *N*-acetyl neuraminic acid moiety was linked through a α-(2 → 6)-linkage. Exclusive formation compound **13** was confirmed from the signature proton and carbon signals in its NMR spectra. Prolonged hydrogenolysis followed by saponification of the pentasaccharide derivative **13** furnished target pentasaccharide **1** as its 4-methoxyphenyl glycoside in 73% yield. Formation of compound **1** was confirmed from its 1D and 2D NMR and mass spectral analysis. Signals at δ 5.09 (d, J = 8.4 Hz, H-1_A), 4.59 (d, J = 7.8 Hz, H-1_C), 4.29 (d, J = 8.1 Hz, H-1_B), 4.26 (d, J = 7.7 Hz, H-1_D), 4.25–4.22 (m, 2 H, H-7_E, H-8_E), 2.67 (dd, J = 12.2, 3.3 Hz, H-3_{eE}), 1.69 (t, J = 12.2 Hz, H-3_{aE}) in the ^{1}H NMR and at δ 104.1 (C-1_C), 104.0 (C-1_D), 103.9 (C-1_B), 103.8 (C-1_A), 101.5 (C-2_E) in the ^{13}C NMR spectra supported the formation of compound **1** (Scheme 2).

In summary, a concise chemical synthesis of the sialic acid containing pentasaccharide repeating unit of the *O*-antigen corresponding to the Shiga toxin producing *E. coli* O171 as it 4-methoxyphenyl glycoside has been carried out successfully involving [2+2] glycosylation. All glycosylation steps are highly stereoselective and reproducible for scale-up preparation. Thioglycoside derivative was used as orthogonally protected glycosyl donor. Regioselective glycosylation using disaccharide diol acceptor reduced the number of protecting group manipulation steps. Final stereoselective incorporation of *N*-acetylneuraminic acid (sialic acid) in the target pentasaccharide derivative was achieved using nitrile effect of the solvent used in the glycosylation. 4-Methoxybenzyl group has been chosen as the temporary anomeric protecting group at the reducing end.

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