

Convergent synthesis of the tetrasaccharide repeating unit of the cell wall lipopolysaccharide of *Escherichia coli* O40

Abhijit Sau and Anup Kumar Misra*

Full Research Paper

Open Access

Address:
Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII-M, Kolkata-700054, India; FAX: +91-33-2355 3886

Email:
Anup Kumar Misra* - akmisra69@gmail.com

* Corresponding author

Keywords:
Escherichia coli; glycosylation; lipopolysaccharide; O-antigen; tetrasaccharide

Beilstein J. Org. Chem. **2012**, *8*, 2053–2059.
doi:10.3762/bjoc.8.230

Received: 30 August 2012
Accepted: 01 November 2012
Published: 22 November 2012

Associate Editor: S. Flitsch

© 2012 Sau and Misra; licensee Beilstein-Institut.
License and terms: see end of document.

Abstract

A tetrasaccharide repeating unit corresponding to the cell-wall lipopolysaccharide of *E. coli* O40 was synthesized by using a convergent block glycosylation strategy. A disaccharide donor was coupled to a disaccharide acceptor by a stereoselective glycosylation. A 2-aminoethyl linker was chosen as the anomeric protecting group at the reducing end of the tetrasaccharide. All glycosylation steps are significantly high yielding and stereoselective.

Introduction

Infantile diarrhoea is one of the major causes of morbidity and mortality in infancy in developing countries [1]. Among several factors, *Escherichia coli* (*E. coli*) infection is one of the major causes of diarrhoeal disease in the developing countries [2]. *E. coli* are Gram-negative opportunistic pathogens and belong to the genus *Enterobacteriaceae*. In general, *E. coli* is considered as a friendly organism present in the normal intestinal flora of humans and animals and can kill harmful bacteria by producing vitamins and other immunostimulants [3]. However, a number of *E. coli* strains acquire virulence factors and cause severe intestinal and urinary-tract infections [4,5]. *E. coli* serotypes are generally classified based on the somatic, flagella

and capsular antigens [6]. Diarrhoea-causing *E. coli* strains are broadly classified in four categories: (a) Enteropathogenic *E. coli* infects through the production of heat-labile and heat-stable toxins; (b) enteroinvasive *E. coli* acts through the invasion of the host body; (c) enteropathogenic *E. coli* infects by adhering to the membrane of the host intestine; and (d) verotoxin *E. coli* infects by the production of verotoxin or shiga toxin [7]. Recently, Zhao et al. reported the structure of the repeating unit of the cell-wall antigenic lipopolysaccharide of *E. coli* O40 [8], which contains two D-galactosyl moieties with alpha and beta linkage, one beta-linked D-glucosamine and one beta-linked D-mannosyl moiety (Figure 1).

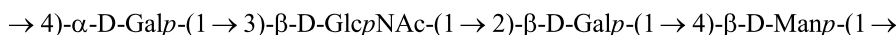


Figure 1: Structure of the tetrasaccharide repeating unit of the cell-wall lipopolysaccharide of *Escherichia coli* O40.

Although several therapeutics have appeared in the past to control the diarrheal epidemics caused by *E. coli* infections, emergence of resistant strains is a serious concern in the development of therapeutics against this organism. Since, bacterial cell-wall lipopolysaccharides play important roles in the pathogenicity of the virulent strains, it would be pertinent to develop glycoconjugate therapeutics based on the cell-wall oligosaccharide haptens to reduce the number of infections [9–12]. In order to evaluate the therapeutic efficacy of the glycoconjugate derivatives it is essential to have a significant quantity of oligosaccharides, which is difficult to isolate from natural sources. Therefore, the development of a chemical synthetic strategy for the synthesis of the oligosaccharides and their close analogues can add momentum towards the preparation of glycoconjugate-based therapeutics. In this perspective, we report herein a concise chemical synthesis of the tetrasaccharide repeating unit of the cell-wall lipopolysaccharide of *E. coli* O40, using a convergent block synthetic strategy.

Results and Discussion

The target tetrasaccharide **1** as its 2-aminoethyl glycoside was synthesized by a stereoselective glycosylation of a disaccharide acceptor **8** and a disaccharide thioglycoside donor **9** using a [2 + 2] block synthetic strategy. The disaccharide intermediates were synthesized from the suitably protected monosaccharide derivatives **2** [13], **3** [14], **4** [15] and **5** [16], which were prepared from the commercially available reducing sugars, by applying a series of functional group protection–deprotection methodologies (Figure 2). The synthetic strategy has a number of notable features, which include (a) stereoselective [2 + 2] block glycosylation; (b) application of general glycosylation reactions by using thioglycosides as glycosyl donors and a combination of *N*-iodosuccinimide (NIS) and perchloric acid supported over silica ($\text{HClO}_4\text{-SiO}_2$) [17,18] as glycosyl activator; (c) exploitation of the armed–disarmed glycosylation

concept for the orthogonal activation of thioglycoside during the synthesis of disaccharide derivative **9** [19]; (d) use of aminoethyl linker as the anomeric protecting group; (e) removal of benzyl groups using a combination of triethylsilane and $\text{Pd}(\text{OH})_2\text{-C}$ [20]; and (f) preparation of β -D-mannosidic moiety from the β -D-glucoside [13].

Benylation of 2-azidoethyl 3-*O*-benzyl-4,6-*O*-benzylidene- β -D-mannopyranoside (**2**) [13] (prepared from D-glucose in nine steps) by using benzyl bromide and sodium hydroxide [21] followed by reductive ring opening of the 4,6-*O*-benzylidene acetal with triethylsilane and iodine [22] furnished compound **6** in 82% yield. Stereoselective glycosylation of compound **6** with thioglycoside derivative **3** in the presence of a combination of *N*-iodosuccinimide (NIS) and $\text{HClO}_4\text{-SiO}_2$ [17] gave disaccharide derivative **7** in a 77% yield. Formation of compound **7** was confirmed from its spectral analysis [signals at δ 4.73 (d, $J = 8.0$ Hz, H-1_B), 4.41 (br s, H-1_A) in the ¹H NMR and at δ 101.6 (C-1_A), 100.7 (C-1_B) in the ¹³C NMR spectra respectively]. Saponification of compound **7** by using sodium methoxide followed by 3,4-*O*-isopropylideneation with 2,2-dimethoxypropane and *p*-toluenesulfonic acid [23] furnished disaccharide derivative **8** in 74% yield (Scheme 1).

In a separate experiment, stereoselective glycosylation of thioglycoside derivative **4** with the thioglycoside acceptor **5** in the presence of a combination of NIS and $\text{HClO}_4\text{-SiO}_2$ [17] in dichloromethane–diethyl ether furnished disaccharide thioglycoside derivative **9** in a 74% yield together with a minor quantity of its other isomer ($\approx 5\%$), which was separated by column chromatography. Formation of compound **9** was confirmed from its spectral analysis [δ 5.51 (d, $J = 3.5$ Hz, H-1_D), 5.37 (d, $J = 10.5$ Hz, H-1_C) in the ¹H NMR and δ 97.4 (C-1_D), 83.0 (C-1_C) in the ¹³C NMR spectra, respectively]. During the synthesis of compound **9**, thioglycoside **4** acted as glycosyl donor

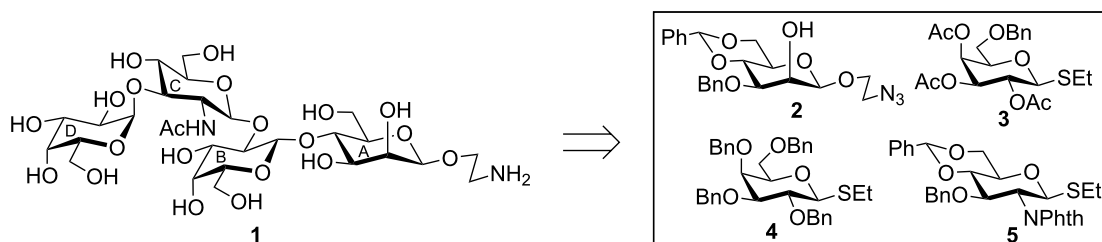
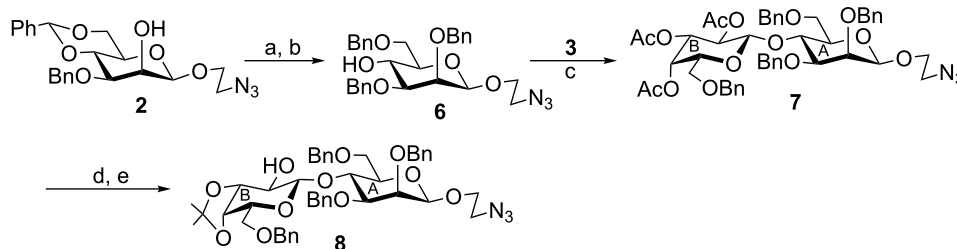
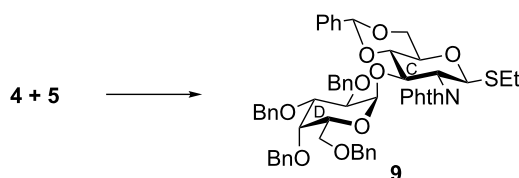


Figure 2: Structure of the synthesized tetrasaccharide **1** and its synthetic precursors.



Scheme 1: Synthesis of disaccharide derivative **8**. Reagents and conditions: (a) benzyl bromide, NaOH, DMF, room temperature, 1 h; (b) Et_3SiH , I_2 , CH_3CN , 0°C , 1 h, 82%; (c) NIS, $\text{HClO}_4\text{-SiO}_2$, MS 4A, CH_2Cl_2 , -25°C , 1 h, 77%; (d) 0.1 M CH_3ONa , CH_3OH , room temperature, 3 h; (e) 2,2-dimethoxypropane, *p*-TsOH, DMF, room temperature, 5 h, 74%.

and thioglycoside **5** acted as orthogonal glycosyl acceptor because of the difference in their reactivity following the “armed–disarmed glycosylation” concept [19,24] (Scheme 2).



Scheme 2: Synthesis of disaccharide derivative **9**. Reagents and conditions: (a) NIS, $\text{HClO}_4\text{-SiO}_2$, MS 4A, $\text{CH}_2\text{Cl}_2\text{-Et}_2\text{O}$, -25°C , 1 h, 74%.

Iodonium ion promoted [2 + 2] stereoselective glycosylation of compound **8** and compound **9** in the presence of NIS and $\text{HClO}_4\text{-SiO}_2$ [17] furnished tetrasaccharide derivative **10** in 71% yield. Formation of compound **10** was confirmed by its spectral analysis [signals at δ 101.6 (C-1_B), 101.5 (PhCH), 100.8 (C-1_C), 100.2 (C-1_A), 97.3 (C-1_D) in the ^{13}C NMR spectrum]. Compound **10** was subjected to a sequence of reactions involving (a) removal of *N*-phthalimido group by using hydrazine hydrate [25]; (b) *N*-acetylation by using acetic anhydride and pyridine; (c) removal of isopropylidene ketal and benzylidene acetal by acid hydrolysis; and finally (d) removal of benzyl ethers by using triethylsilane and 20% $\text{Pd}(\text{OH})_2\text{-C}$

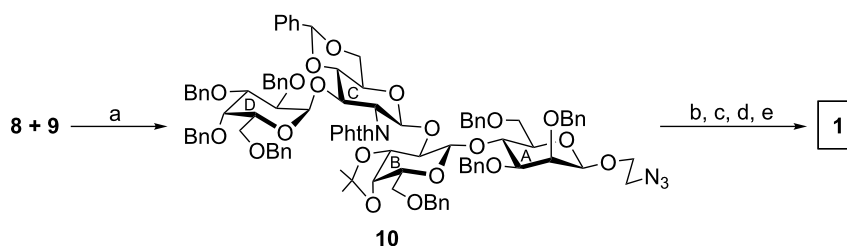
[20] to furnish target compound **1**, which was purified through a Sephadex[®] LH-20 column to give pure compound **1** in 60% overall yield. Spectral data of compound **1** confirmed its formation [signals at δ 5.31 (d, $J = 8.5$ Hz, H-1_C), 5.15 (d, $J = 3.5$ Hz, H-1_D), 4.63 (br s, H-1_A), 4.34 (d, $J = 8.5$ Hz, H-1_B) in the ^1H NMR and at δ 100.7 (C-1_B), 100.6 (C-1_B), 99.6 (2 C, C-1_A, C-1_C) in the ^{13}C NMR] (Scheme 3).

Conclusion

In summary, synthesis of a tetrasaccharide repeating unit corresponding to the cell-wall lipopolysaccharide of *E. coli* O40 was achieved by using a convergent [2 + 2] block synthetic strategy. The yields are excellent in all reactions. A general reaction condition was used in all glycosylation reactions. All intermediates and final compounds were characterized by their spectral analysis. The armed–disarmed glycosylation concept was applied for the synthesis of disaccharide derivative **9**. A 2-Aminoethyl linker was used as the anomeric protecting group.

Experimental

General methods: All reactions were monitored by thin-layer chromatography over silica-gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% $\text{Ce}(\text{SO}_4)_2$ 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 Bruker Avance 500 MHz by using



Scheme 3: Synthesis of target tetrasaccharide **1**. Reagents and conditions: (a) NIS, $\text{HClO}_4\text{-SiO}_2$, CH_2Cl_2 , -15°C , 1 h, 71%; (b) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, EtOH, 90°C , 5 h; (c) acetic anhydride, pyridine, room temperature, 2 h; (d) 80% aq AcOH, 80°C , 1.5 h; (e) triethylsilane, 20% $\text{Pd}(\text{OH})_2\text{-C}$, CH_3OH , 6 h, room temperature, overall 60%.

CDCl₃ as solvent and TMS as internal reference, unless stated otherwise. Chemical shift values are expressed in δ ppm. MALDI-MS were recorded on a Bruker Daltronics mass spectrometer. Commercially available grades of organic solvents of adequate purity were used in all reactions. HClO₄-SiO₂ was prepared following the method reported in the literature [18].

2-Azidoethyl 2,3,6-tri-*O*-benzyl- β -D-mannopyranoside (6):

To a solution of compound **2** (2.0 g, 4.68 mmol) in dry DMF (10 mL) were added benzyl bromide (1.2 mL, 10.09 mmol) and powdered NaOH (750.0 mg, 18.75 mmol) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with water (100 mL) and extracted with CH₂Cl₂ (100 mL). The organic layer was washed with H₂O, dried (Na₂SO₄) and concentrated. The crude product was passed through a short pad of SiO₂ by using hexane-EtOAc (5:1) as eluant to give the *O*-benzylated product (2.2 g, 91%). A solution of the *O*-benzylated product (2.2 g, 4.25 mmol) in dry CH₃CN (20 mL) was cooled to 0 °C. To the cooled reaction mixture were added Et₃SiH (1.4 mL, 8.76 mmol) and I₂ (250.0 mg, 0.98 mmol), and the reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and the organic layer was successively washed with saturated NaHCO₃ and H₂O, and then dried (Na₂SO₄) and concentrated. The crude product was purified over SiO₂ by using hexane-EtOAc (4:1) as eluant to give pure compound **6** (1.7 g, overall 82%). White solid; mp 89–90 °C; $[\alpha]_D^{25}$ –97 (*c* 1.0, CHCl₃); IR (KBr): 3293, 2845, 2110, 1497, 1454, 1365, 1310, 1119, 1065, 779, 659, 599 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.43–7.23 (m, 15H, Ar-H), 4.98 (d, *J* = 12.5 Hz, 1H, PhCH₂), 4.75 (d, *J* = 12.5 Hz, 1H, PhCH₂), 4.61, 4.58 (2 d, *J* = 12.0 Hz, 2H, PhCH₂), 4.48 (br s, 1H, H-1), 4.45 (d, *J* = 12.0 Hz, 1H, PhCH₂), 4.32 (d, *J* = 12.0 Hz, 1H, PhCH₂), 4.15–4.11 (m, 1H, -OCH₂-), 3.96 (br s, 1H, H-2), 3.94 (t, *J* = 9.5 Hz each, 1H, H-4), 3.85 (dd, *J* = 10.5, 3.5 Hz, 1H, H-6_a), 3.75 (dd, *J* = 10.5, 6.5 Hz, 1H, H-6_b), 3.66–3.62 (m, 1H, OCH₂-), 3.58–3.53 (m, 1H, CH₂N₃), 3.46–3.42 (m, 1H, H-5), 3.34–3.30 (m, 1H, CH₂N₃), 3.29 (dd, *J* = 10.0, 3.5 Hz, 1H, H-3); ¹³C NMR (125 MHz, CDCl₃) δ 138.6–127.4 (Ar-C), 101.8 (C-1), 81.3 (C-3), 75.4 (C-5), 74.4 (PhCH₂), 73.7 (PhCH₂), 73.5 (C-5), 71.1 (PhCH₂), 70.7 (C-6), 68.6 (OCH₂), 68.0 (C-2), 50.9 (CH₂N₃); ESI-MS: 542.2 [M + Na]⁺; Anal. calcd for C₂₉H₃₃N₃O₆: C, 67.04; H, 6.40; found: C, 66.90; H, 6.58.

2-Azidoethyl *O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl- β -D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl- β -D-mannopyranoside (7):

To a solution of compound **3** (1.4 g, 3.18 mmol) and compound **6** (1.5 g, 2.88 mmol) in anhydrous CH₂Cl₂ (10 mL) was added MS 4Å (2.0 g), and the reaction mixture was stirred at room temperature for 30 min under

argon. The reaction mixture was cooled to –25 °C, and *N*-iodosuccinimide (NIS; 0.8 g, 3.55 mmol) and HClO₄-SiO₂ (25.0 mg) were added to it. After being stirred at same temperature for 1 h the reaction mixture was filtered through a Celite[®] bed and washed with CH₂Cl₂ (100 mL). The organic layer was successively washed with 5% Na₂S₂O₃, saturated NaHCO₃ and water, and then dried (Na₂SO₄) and concentrated under reduced pressure to give the crude product. The crude product was purified over SiO₂ by using hexane-EtOAc (7:1) as eluant to give pure compound **7** (2.0 g, 77%). Yellow oil; $[\alpha]_D^{25}$ –13 (*c* 1.0, CHCl₃); IR (neat): 3087, 2956, 2153, 1605, 1487, 1345, 1254, 1183, 1142, 1045, 999, 774, 734, 647, 542 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.35–7.16 (m, 20H, Ar-H), 5.33 (d, *J* = 3.0 Hz, 1H, H-4_B), 5.06 (t, *J* = 8.0 Hz each, 1H, H-2_B), 4.87 (d, *J* = 12.0 Hz, 1H, PhCH₂), 4.84 (dd, *J* = 10.5, 3.5 Hz, 1H, H-3_B), 4.73 (d, *J* = 8.0 Hz, 1H, H-1_B), 4.72–4.71 (2 d, *J* = 12.0 Hz each, 2H, PhCH₂), 4.59 (d, *J* = 12.0 Hz, 1H, PhCH₂), 4.47, 4.45 (2 d, *J* = 12.0 Hz each, 2H, PhCH₂), 4.41 (br s, 1H, H-1_A), 4.39 (d, *J* = 12.0 Hz, 1H, PhCH₂), 4.19 (d, *J* = 12.0 Hz, 1H, PhCH₂), 4.14 (t, *J* = 9.0 Hz each, 1H, H-4_A), 4.10–4.05 (m, 1H, OCH₂), 3.92 (d, *J* = 3.0 Hz, 1H, H-2_A), 3.76–3.70 (m, 2H, H-6_{abA}), 3.65–3.60 (m, 1H, OCH₂), 3.54–3.49 (m, 2H, H-5_B, CH₂N₃), 3.47 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3_A), 3.42–3.38 (m, 1H, H-5_A), 3.32–3.22 (m, 3H, H-6_{abB}, CH₂N₃), 1.99, 1.95, 1.90 (3 s, 9H, 3 COCH₃); ¹³C NMR (125 MHz, CDCl₃) δ 169.9, 169.8, 169.4 (3 COCH₃), 138.5–126.8 (Ar-C), 101.6 (C-1_A), 100.7 (C-1_B), 80.6 (C-5_B), 75.7 (C-5_A), 74.7 (C-2_A), 74.3 (2 C, C-4_A, PhCH₂), 73.7 (PhCH₂), 73.3 (PhCH₂), 71.7 (C-5_B), 71.5 (PhCH₂), 71.3 (C-3_B), 70.0 (C-2_B), 68.6 (C-6_A), 68.5 (OCH₂), 67.4 (C-4_B), 66.9 (C-6_B), 50.8 (CH₂N₃), 20.7, 20.6, 20.5 (COCH₃); MALDI-MS: 920.3 [M + Na]⁺; Anal. calcd for C₄₈H₅₅N₃O₁₄: C, 64.20; H, 6.17; found: C, 64.06; H, 6.35.

2-Azidoethyl *O*-(6-*O*-benzyl-3,4-*O*-isopropylidene- β -D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl- β -D-mannopyranoside (8):

A solution of compound **7** (1.8 g, 2.0 mmol) in 0.1 M CH₃ONa (25 mL) was stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex 50W X8 (H⁺) resin, filtered and concentrated. To a solution of the de-*O*-acetylated product in dry DMF (10 mL) was added 2,2-dimethoxypropane (0.7 mL, 5.69 mmol) followed by *p*-TsOH (0.2 g) and the reaction mixture was stirred at room temperature for 5 h. The reaction was quenched with Et₃N (1 mL), the solvents were removed under reduced pressure, and the crude reaction mixture was diluted with CH₂Cl₂ (100 mL). The organic layer was washed with saturated NaHCO₃, dried (Na₂SO₄) and concentrated to give the crude product, which was purified over SiO₂ by using hexane-EtOAc (2:1) as eluant to give pure compound **8** (1.2 g, 74%). Yellow oil; $[\alpha]_D^{25}$ –21 (*c* 1.0, CHCl₃); IR (neat): 3418, 3030, 2926, 2198, 1743, 1711, 1646, 1390, 1253, 1099, 1053, 864, 754, 667, 531 cm⁻¹;

^1H NMR (500 MHz, CDCl_3) δ 7.30–7.14 (m, 20H, Ar-H), 4.84 (d, $J = 12.5$ Hz, 1H, PhCH_2), 4.63–4.40 (m, 6H, PhCH_2), 4.38 (d, $J = 8.0$ Hz, 1H, H-1_B), 4.33 (br s, 1H, H-1_A), 4.30 (d, $J = 12.5$ Hz, 1H, PhCH_2), 4.22 (t, $J = 9.5$ Hz each, 1H, H-4_A), 4.02–3.98 (m, 2H, H-2_A, OCH_2), 3.91 (dd, $J = 10.0$, 3.5 Hz, H-3_B), 3.83 (dd, $J = 12.0$, 5.5 Hz, 1H, H-6_{aB}), 3.81 (d, $J = 2.0$ Hz, 1H, H-4_B), 3.76 (dd, $J = 12.0$, 2.0 Hz, 1H, H-6_{bB}), 3.71 (br s, 1H, H-5_B), 3.70–3.68 (m, 1H, OCH_2), 3.62–3.58 (m, 1H, H-6_{aA}), 3.56–3.51 (m, 1H, CH_2N_3), 3.49 (dd, $J = 8.0$ Hz each, 1H, H-2_B), 3.47–3.43 (m, 1H, H-6_{bA}), 3.42–3.37 (m, 2H, H-3_A, H-5_A), 3.25–3.19 (m, 1H, CH_2N_3), 1.42, 1.25 (2 s, 6H, 2 $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (125 MHz, CDCl_3) δ 138.7–127.3 (Ar-C), 109.8 ($\text{C}(\text{CH}_3)_2$), 102.5 (C-1_B), 101.9 (C-1_A), 80.6 (C-2_B), 78.9 (C-3_B), 75.2 (C-5_A), 74.3 (PhCH_2), 74.2 (C-3_A), 74.0 (C-4_B), 73.8 (C-2_A), 73.5 (PhCH_2), 73.4 (2C, C-4_A, PhCH_2), 72.4 (C-5_B), 71.3 (PhCH_2), 69.4 (OCH_2), 69.3 (C-6_B), 68.5 (C-6_A), 50.8 (CH_2N_3), 28.2, 26.4 ($\text{C}(\text{CH}_3)_2$); MALDI-MS: 834.3 [$\text{M} + \text{Na}$] $^+$; Anal. calcd for $\text{C}_{45}\text{H}_{53}\text{N}_3\text{O}_{11}$: C, 66.57; H, 6.58; found: C, 66.42; H, 6.75.

Ethyl *O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio- β -D-glucopyranoside (9): To a solution of compound **4** (1.4 g, 2.39 mmol) and compound **5** (1.0 g, 2.26 mmol) in anhydrous CH_2Cl_2 – Et_2O (10 mL; 1:1 v/v) was added MS 4 \AA (2.0 g), and reaction mixture was stirred at room temperature for 30 min under argon. The reaction mixture was cooled to -25 $^\circ\text{C}$ and NIS (550.0 mg, 2.44 mmol) and HClO_4 – SiO_2 (15.0 mg) were added. After being stirred at same temperature for 1 h the reaction mixture was filtered through a Celite[®] bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% $\text{Na}_2\text{S}_2\text{O}_3$, saturated NaHCO_3 and water, and then dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product. The crude product was purified over SiO_2 by using hexane– EtOAc (7:1) as eluant to give pure compound **9** (1.6 g, 74%). White solid; mp 67–68 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{25} +39$ (c 1.0, CHCl_3); IR (KBr): 3417, 3063, 2870, 1774, 1715, 1610, 1495, 1485, 1385, 1216, 1099, 1023, 914, 753, 719 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 7.76–6.91 (m, 29H, Ar-H), 5.51 (d, $J = 3.5$ Hz, 1H, H-1_D), 5.37 (d, $J = 10.5$ Hz, 1H, H-1_C), 5.32 (s, 1H, PhCH), 4.84 (t, $J = 9.5$ Hz each, 1H, H-3_C), 4.77–4.58 (3 d, $J = 12.0$ Hz each, 3H, PhCH_2), 4.46 (t, $J = 9.5$ Hz each, 1H, H-2_C), 4.44 (d, $J = 11.5$ Hz, 1H, PhCH_2), 4.34 (d, $J = 11.5$ Hz, 1H, PhCH_2), 4.29 (t, $J = 9.5$ Hz each, 1H, H-4_C), 4.19 (d, $J = 11.5$ Hz, 1H, PhCH_2), 3.86 (br s, 2H, PhCH_2), 3.85 (br s, 1H, H-4_D), 3.81 (dd, $J = 10.5$, 3.0 Hz, 1H, H-2_D), 3.72–3.67 (m, 3H, H-3_D, H-5_C, H-6_{aD}), 3.58 (br s, 1H, H-5_D), 3.33–3.31 (m, 1H, H-6_{bD}), 3.23–3.19 (m, 1H, H-6_{aC}), 2.80–2.77 (m, 1H, H-6_{bC}), 2.67–2.56 (m, 2H, SCH_2CH_3), 1.12 (t, $J = 7.5$ Hz each, 3H, SCH_2CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 168.1, 167.9 (PhthCO), 138.9–123.1 (Ar-C), 101.7 (PhCH), 97.4 (C-1_D),

83.0 (C-1_C), 81.7 (C-4_D), 78.1 (C-3_D), 75.4 (C-2_D), 74.8 (C-5_D), 74.7 (PhCH_2), 73.3 (2 C, C-3_C, PhCH_2), 72.8 (PhCH_2), 71.8 (PhCH_2), 70.1 (C-5_C), 69.4 (C-4_C), 68.8 (C-6_D), 67.7 (C-6_C), 54.2 (C-2_C), 24.0 (SCH_2CH_3), 14.9 (SCH_2CH_3); MALDI-MS: 986.3 [$\text{M} + \text{Na}$] $^+$; Anal. calcd for $\text{C}_{57}\text{H}_{57}\text{NO}_{11}\text{S}$: C, 71.01; H, 5.96; found: C, 70.88; H, 6.13.

2-Azidoethyl *O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido- β -D-glucopyranosyl)-(1 \rightarrow 2)-*O*-(6-*O*-benzyl-3,4-*O*-isopropylidene- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-*O*-benzyl- β -D-mannopyranoside (10): To a solution of compound **8** (1.0 g, 1.23 mmol) and compound **9** (1.3 g, 1.35 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4 \AA (2.0 g), and reaction mixture was stirred at room temperature for 30 min under argon. The reaction mixture was cooled to -25 $^\circ\text{C}$ and NIS (350.0 mg, 1.55 mmol) and HClO_4 – SiO_2 (10.0 mg) were added to it. After being stirred at same temperature for 1 h the reaction mixture was filtered through a Celite[®] bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% $\text{Na}_2\text{S}_2\text{O}_3$, saturated NaHCO_3 and water, and then dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product. The crude product was purified over SiO_2 by using hexane– EtOAc (7:1) as eluant to give pure compound **10** (1.5 g, 71%). White solid; mp 65–66 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{25} +34$ (c 1.0, CHCl_3); IR (KBr): 3423, 3063, 3030, 2871, 2105, 1776, 1744, 1715, 1497, 1454, 1389, 1239, 1102, 1060, 874, 737, 721, 697, 600, 530 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 7.76–6.96 (m, 49H, Ar-H), 5.52 (d, $J = 3.5$ Hz, 1H, H-1_D), 5.42 (d, $J = 8.0$ Hz, 1H, H-1_C), 5.25 (s, 1H, PhCH), 4.86–4.45 (m, 13H, PhCH_2), 4.41 (d, $J = 9.5$ Hz, 1H, H-1_B), 4.37 (t, $J = 8.0$ Hz, 1H, H-2_C), 4.29 (d, $J = 11.5$ Hz, 1H, PhCH_2), 4.24–4.20 (m, 4H, H-1_A, H-3_C, H-4_A, OCH_2), 4.13–4.08 (m, 2H, H-2_A, OCH_2), 3.91 (br s, 2H, PhCH_2), 3.89–3.85 (m, 3H, H-2_D, H-3_B, H-3_D), 3.84–3.75 (m, 3H, H-2_B, H-4_C, H-4_D), 3.70 (t, $J = 10.5$ Hz each, 1H, H-6_{aB}), 3.64–3.60 (m, 4H, H-4_B, H-5_B, H-5_D, H-6_{bB}), 3.58–3.43 (m, 5H, H-3_A, H-6_{aA}, H-6_{aC}, H-6_{abD}), 3.42–3.36 (m, 4H, H-5_A, H-6_{bA}, H-6_{bC}, CH_2N_3), 3.34–3.26 (m, 2H, H-5_C, CH_2N_3), 1.27, 1.25 (2 s, 6H, 2 CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 138.6–126.3 (Ar-C), 109.5 ($\text{C}(\text{CH}_3)_2$), 101.6 (C-1_B), 101.5 (PhCH), 100.8 (C-1_C), 100.2 (C-1_A), 97.3 (C-1_D), 82.9 (C-4_C), 82.7 (C-5_A), 80.1 (C-5_C), 78.7 (C-3_A), 78.1 (C-4_D), 76.2 (C-2_D), 75.5 (C-2_B), 74.9 (C-3_B), 74.8 (C-5_D), 74.7 (PhCH_2), 74.6 (C-3_D), 73.9 (PhCH_2), 73.5 (C-3_C), 73.4 (2 C, 2 PhCH_2), 73.2 (PhCH_2), 72.8 (PhCH_2), 72.6 (C-2_A), 72.0 (PhCH_2), 71.7 (2 C, C-4_B, PhCH_2), 69.3 (C-4_A), 68.9 (C-6_C), 68.8 (OCH_2), 68.7 (C-6_A), 68.4 (OCH_2), 67.7 (2 C, C-6_B, C-6_D), 65.4 (C-5_B), 56.0 (C-2_C), 50.8 (CH_2N_3), 27.6, 25.7 ($\text{C}(\text{CH}_3)_2$); MALDI-MS: 1735.7 [$\text{M} + \text{Na}$] $^+$; Anal. calcd for $\text{C}_{100}\text{H}_{104}\text{N}_4\text{O}_{22}$: C, 70.08; H, 6.12; found: C, 69.94; H, 6.30.

2-Aminoethyl (α -D-galactopyranosyl)-(1 \rightarrow 3)-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-(1 \rightarrow 2)-(β -D-galactopyranosyl)-(1 \rightarrow 4)- β -D-mannopyranoside (1): To a solution of compound **10** (500.0 mg, 0.29 mmol) in EtOH (5 mL) was added $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (0.1 mL) and the reaction mixture was stirred at 90 °C for 5 h. The solvents were removed under reduced pressure, and a solution of the crude product in acetic anhydride–pyridine (2 mL, 1:1 v/v) was kept at room temperature for 2 h and then concentrated. A solution of the crude product in 80% aq AcOH (10 mL) was stirred at 80 °C for 1.5 h and then concentrated. To a solution of the crude product in CH_3OH (5 mL) were added Et_3SiH (1.5 mL, 9.39 mmol) and 20% $\text{Pd}(\text{OH})_2\text{-C}$ (100.0 mg) and the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was filtered through a Celite[®] bed and washed with $\text{CH}_3\text{OH-H}_2\text{O}$ (2:1). The solvents were removed under reduced pressure and the product was passed through a Sephadex[®] LH-20 column by using $\text{CH}_3\text{OH-H}_2\text{O}$ (3:1) as eluant to furnish pure compound **1** (135.0 mg, 60%). Glass; $[\alpha]_{\text{D}}^{25} +29$ (c 1.0, H_2O); IR (KBr): 3436, 2948, 1619, 1369, 1162, 669 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 5.31 (d, $J = 8.5$ Hz, 1H, H-1_C), 5.15 (d, $J = 3.5$ Hz, 1H, H-1_D), 4.63 (br s, 1H, H-1_A), 4.48 (t, $J = 10.5$ Hz each, 1H, H-3_C), 4.34 (d, $J = 8.5$ Hz, 1H, H-1_B), 4.09 (t, $J = 10.0$ Hz each, 1H, H-2_C), 4.05–3.92 (m, 4H, H-2_A, H-4_D, H-5_D, OCH_2a), 3.90–3.80 (m, 4H, H-2_B, H-3_A, H-6_{ab}, OCH_2b), 3.78–3.57 (m, 11H, H-3_D, H-4_B, H-4_C, H-6_{abA}, H-6_{bB}, H-6_{abC}, H-6_{abD}), 3.55–3.47 (m, 2H, H-2_D, H-3_B), 3.45–3.40 (m, 2H, H-5_A, H-5_B), 3.35–3.33 (m, 1H, H-5_C), 3.20–3.15 (m, 2H, CH_2NH_2), 2.06 (s, 3H, COCH_3); ^{13}C NMR (125 MHz, D_2O) δ 171.5 (COCH_3), 100.7 (C-1_B), 100.6 (C-1_B), 99.6 (2C, C-1_A, C-1_C), 81.7 (C-3_B), 79.0 (C-3_C), 78.5 (C-4_D), 77.0 (C-3_D), 75.5 (C-4_A), 75.1 (C-4_B), 73.5 (C-2_D), 73.3 (C-5_A), 71.3 (C-2_A), 70.7 (C-4_C), 70.6 (C-3_A), 69.8 (2 C, C-5_C, C-5_D), 68.3 (2C, C-2_B, C-5_B), 65.5 (OCH_2), 60.6 (C-6_B), 60.5 (C-6_C), 60.2 (C-6_A), 59.2 (C-6_D), 55.7 (C-2_C), 39.5 (CH_2NH_2), 23.1 (COCH_3); MALDI-MS: 799.2 $[\text{M} + \text{Na}]^+$; Anal. calcd for $\text{C}_{28}\text{H}_{48}\text{N}_4\text{O}_{21}$: C, 43.30; H, 6.23; found: C, 43.14; H, 6.45.

Supporting Information

Supporting Information File 1

1D and 2D NMR spectra of compounds **1** and **6–10**.

[<http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-8-230-S1.pdf>]

References

- Hisham, N. J. *Trop. Pediatr.* **1982**, *28*, 1–4.
- Vila, J.; Gene, A.; Vargas, M.; Gascon, J.; Latorre, C.; Jimenez De Anta, M. T. *J. Med. Microbiol.* **1998**, *47*, 889–891. doi:10.1099/00222615-47-10-889
- Kaper, J. B.; Nataro, J. P.; Mobley, H. L. T. *Nat. Rev. Microbiol.* **2004**, *2*, 123–140. doi:10.1038/nrmicro818
- Russo, T. A.; Johnson, J. R. *J. Infect. Dis.* **2000**, *181*, 1753–1754. doi:10.1086/315418
- Johnson, J. R. *Clin. Microbiol. Rev.* **1991**, *4*, 80–128.
- Stenutz, R.; Weintraub, A.; Widmalm, G. *FEMS Microbiol. Rev.* **2006**, *30*, 382–403. doi:10.1111/j.1574-6976.2006.00016.x
- Beutin, L.; Aleksic, S.; Zimmermann, S.; Gleier, K. *Med. Microbiol. Immunol.* **1994**, *183*, 13–21. doi:10.1007/BF00193627
- Zhao, G.; Perepelov, A. V.; Senchenkova, S. N.; Shashkov, A. S.; Feng, L.; Li, X.; Knirel, Y. A.; Wang, L. *Carbohydr. Res.* **2007**, *342*, 1275–1279. doi:10.1016/j.carres.2007.03.005
- Roy, R. *Drug Discovery Today: Technol.* **2004**, *1*, 327–336. doi:10.1016/j.ddtec.2004.10.005
- Pozsgay, V. *Curr. Top. Med. Chem.* **2008**, *8*, 126–140. doi:10.2174/156802608783378864
- Ada, G.; Isaacs, D. *Clin. Microbiol. Infect.* **2003**, *9*, 79–85. doi:10.1046/j.1469-0691.2003.00530.x
- Vliegthart, J. F. *FEBS Lett.* **2006**, *580*, 2945–2950. doi:10.1016/j.febslet.2006.03.053
- Mukherjee, C.; Ranta, K.; Savolainen, J.; Leino, R. *Eur. J. Org. Chem.* **2012**, 2957–2968. doi:10.1002/ejoc.201200041
- Mandal, P. K.; Misra, A. K. *Bioorg. Chem.* **2010**, *38*, 56–61. doi:10.1016/j.bioorg.2010.01.001
- Basu, S.; Pal, J. N. *Carbohydr. Res.* **1990**, *208*, 241–245. doi:10.1016/0008-6215(90)80103-A
- Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863. doi:10.1021/jo00296a055
- Mukhopadhyaya, B.; Collet, B.; Field, R. A. *Tetrahedron Lett.* **2005**, *46*, 5923–5925. doi:10.1016/j.tetlet.2005.06.119
- Chakraborti, A. K.; Gulhane, R. *Chem. Commun.* **2003**, 1896–1897. doi:10.1039/b304178f
- Fraser-Reid, B.; Lopez, J. C. *Top. Curr. Chem.* **2011**, *301*, 1–29. doi:10.1007/128_2010_105
- Mandal, P. K.; McMurray, J. S. *J. Org. Chem.* **2007**, *72*, 6599–6601. doi:10.1021/jo0706123
- Madhusudan, S. K.; Agnihotri, G.; Negi, D. S.; Misra, A. K. *Carbohydr. Res.* **2005**, *340*, 1373–1377. doi:10.1016/j.carres.2005.03.007
- Panchadhayee, R.; Misra, A. K. *Synlett* **2010**, 1193–1196. doi:10.1055/s-0029-1219798
- Bergonzi, M. C.; Catelani, G.; D'Andrea, F.; De Rensis, F. *Carbohydr. Res.* **1998**, *311*, 231–234. doi:10.1016/S0008-6215(98)00211-0
- Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12073–12074. doi:10.1021/ja00105a066
- Lee, H.-H.; Schwartz, D. A.; Harris, J. F.; Carver, J. P.; Krepinsky, J. J. *Can. J. Chem.* **1986**, *64*, 1912–1918. doi:10.1139/v86-315

Acknowledgements

A. S. thanks CSIR, New Delhi for providing a Senior Research Fellowship. This work was supported by DST, New Delhi (Project No. SR/S1/OC-83/2010).

License and Terms

This is an Open Access article under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The license is subject to the *Beilstein Journal of Organic Chemistry* terms and conditions: (<http://www.beilstein-journals.org/bjoc>)

The definitive version of this article is the electronic one which can be found at:
[doi:10.3762/bjoc.8.230](https://doi.org/10.3762/bjoc.8.230)