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Synthesis of the repeating trisaccharide unit of the cell wall lipopolysaccharide of *Escherichia coli* type 8

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ABSTRACT

NIS/TfOH mediated glycosidation of methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside with phenyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside furnished the corresponding disaccharide derivative in excellent yield and α-selectivity. Zémplen deacetylation of the same followed by reaction with BSP/Tf_2O-preactivated phenyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside generated methyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside in very good yield and excellent β-selectivity. Pd/C catalyzed hydrogenation of the latter finally afforded the repeating trisaccharide of *Escherichia coli* 8 O-antigen as its methyl glycoside.

Escherichia coli type 8 strain F492 is a gram-negative enterotoxic and enteropathogenic bacterium. The structure of the repeating unit **1** of the O-antigen is a mannose trisaccharide (Fig. 1) containing (1→2)-β-linked mannoside at the non-reducing end.[1] In continuation of our research based on carbohydrates[2] and also as part of our ongoing research programme on the synthesis of oligosaccharides related to bacterial antigens, we have synthesized the trisaccharide repeating unit of the O-antigen of *E. coli* 8 strain 492 and report herein the synthesis of **1** as its methyl glycoside (Scheme 2). Surprisingly, the synthesis of **1** has not yet been reported and to the best of our knowledge this is the first report of this repeating unit.

The O-specific polysaccharide of the lipopolysaccharide is a mannan.

Retrosynthetic analysis of **1** gives probable protected building blocks **3, 4** and **7** (Scheme 1). The glycosyl acceptor **3**[3] and the glycosyl donor **4**[4] were prepared from the common building block **2**[3] following literature procedures (Scheme 2). NIS/TfOH mediated glycosidation[5] of **3** with **4** produced the corresponding α-glycoside **8** in a better yield than that reported earlier.[6] The next glycosyl acceptor **9** was generated from **8** by Zémplen deacetylation. We then adopted Crich's β-mannosidation technique[7] for the next glycosidation step. Although Crich's general approach of direct β-mannosidation[7] has been applied widely to synthesize various complex oligosaccharides,[8] remarkably, the application of this methodology to the synthesis of β-(1→2)-mannosides is virtually absent from the literature except those by Crich et al.[7e,f] or that of Mallet and co-workers.[9] Unlike these reports, we have used a thioglycoside donor in place of the corresponding sulfoxide.

β-D-Manp-(1→2)-α-D-Manp-(1→2)-D-Manp.
1

Figure 1. The trisaccharide repeating unit of the O-antigen of *E. coli* type 8.

Thus, the glycosyl donor **7**[10] was synthesized from phenyl 1-thio-β-D-mannoside (**6**). Benzylidination of **6** with dimethoxytoluene in the presence of $NaHSO_4$–SiO_2 catalyst[11] followed by benzylation with NaH and benzyl bromide produced **7**. This glycosyl donor was then preactivated with 1-benzenesulfinyl piperidene (BSP)[12] and Tf_2O at –60 °C and the resulting mixture was reacted with the glycosyl acceptor **9** at –78 °C to afford the corresponding trisacchharide **10** in very good yield.[13] We could not detect the formation of any α-anomer in the product; 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-glucose, however, was also formed as a side product. The reaction proceeds with in situ generation of the corresponding glycosyl triflate[7c,d,g,14] from **7**, which as an intimate ion pair gives preferred displacement via the acceptor from the β-face, giving finally β-mannoside. The appearance of a single proton multiplet at δ 2.97 confirmed the generation of β-mannoside.[7c–f] The structure of **10** was also confirmed by ^{13}C NMR, COSYGP, TOCSY, HMBC, HSQC and NOESY techniques. The three consecutive anomeric protons of **10** from the non-reducing end appeared at δ

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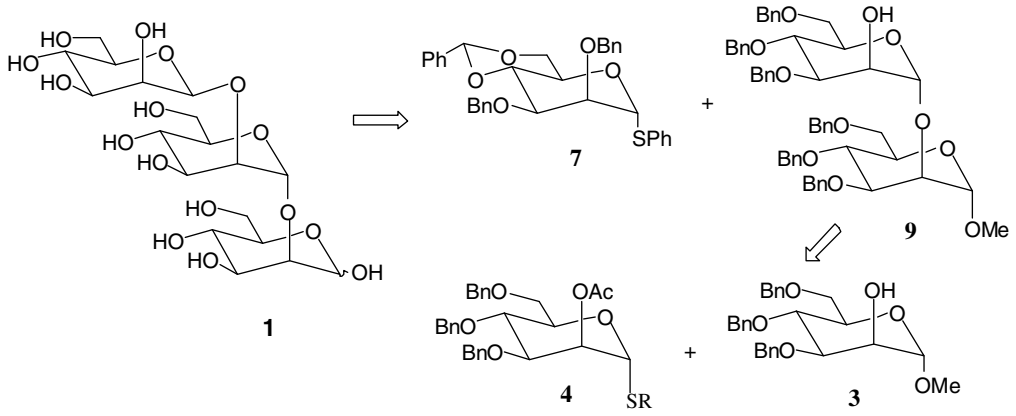
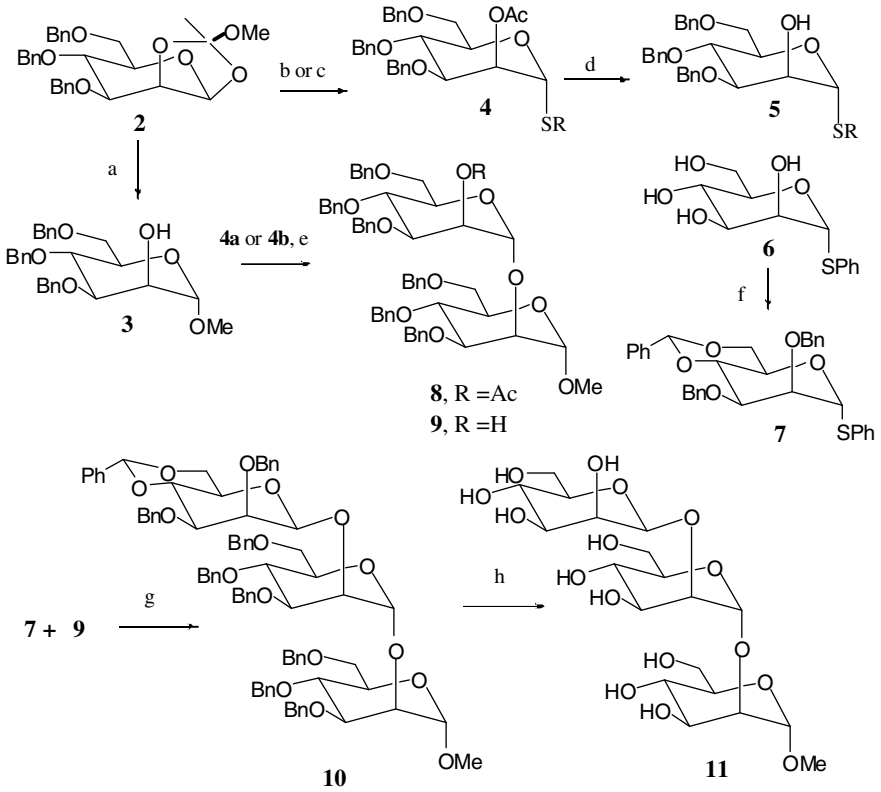


Scheme 1. Retrosynthetic analysis of **1**.



Scheme 2. Reagents and conditions: (a) MeOH/CH_3COCl (5 equiv)/reflux/10 h, 88%; (b) $HgBr_2$ (0.1 equiv)/PhSH/CH_3CN/4 Å MS/Ar/60 °C/4 h, 92%; (c) $BF_3{\cdot}Et_2O$ (2 equiv)/EtSH/0 °C/30 min, 88%; (d) NaOMe (0.02 M)/MeOH/5 h, quantitative; (e) TfOH (15 mol %)/NIS (1 equiv)/DCM/4 Å MS/0 °C/Ar/30 min, 84–88%; (f) (i) dimethoxytoluene/$NaHSO_4{\cdot}SiO_2$/MeCN/rt/2 h, 65%; (ii) NaH/BnBr (1.2 equiv)/DMF/2 h, 98%; (g) BSP/Tf_2O/TTBP/4 Å MS/Ar/−78 °C/2 h, 78%; (h) 10% Pd/C/H_2/AcOH/MeOH/24 h, 86%. Synthesis of the repeating unit of the O-antigen of *E. coli* type 8.

4.35, 5.09 and 4.76, respectively. The chemical shifts of the two successive non-reducing end anomeric carbons merged at δ 99.98 and the reducing end anomeric carbon appeared at δ 99.9. Interestingly, COSYGP and TOCSY did not show any correlation of H-1″ with any other proton, probably due to very small coupling between H-1″ and H-2″ and consequent magnetization transfer. However, H-1″ could be identified from the NOESY and HSQC of **10**. As expected, the NOESY experiment showed a distinct correlation between protons H-1″ and H-5″ (which again established the β-glycoside linkage of the non-reducing mannose) and also with H-2″, 3″ and 1′ of compound **10**; H-1″ and H-1′ are thus also within the NOE spin cross-relaxation distance.

Catalytic hydrogenation of **10** with Pd/C finally afforded the target trisaccharide as its methyl glycoside **11**. Compound **11** showed three equally strong anomeric proton signals appearing successively from the non-reducing end at δ 4.62, 5.02 and 4.88 with corresponding anomeric ^{13}C-chemical shifts at δ 99.8 ($J_{C,H}$ 158.8 Hz), 100.7 ($J_{C,H}$ 171.6 Hz) and 101.5 ($J_{C,H}$ 170.7 Hz), respectively; the J values indicated[15] the presence of one β-linkage and two α-linkages in the final trisaccharide glycoside.

In summary, the methyl glycoside of the repeating trisaccharide unit of the O-antigen of *E. coli* type 8 was synthesized starting from the reducing end. The crucial step was the β-(1→2)-mannosidation on the (1→2)-linked disaccharide derived acceptor using BSP-pre-

activated thioglycoside donor, which proceeded in very good yield and with excellent β-selectivity.

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Supplementary data

The ^{1}H and ^{13}C NMR spectra of compounds **10** and **11** and the COSYGP, NOESY, HSQCGP, TOCSY and HMBC spectra of compound **10** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.07.089.

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13. *Methyl 2,3-Di-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**10**). A stirred solution of thioglycoside **7** (236 mg, 0.437 mmol), BSP[14] (101 mg, 0.483 mmol), TTBP (217.2 mg, 0.874 mmol) and activated 4Å powdered molecular sieves in dichloromethane (10 mL) under an argon atmosphere was kept at −60 °C for 30 min. Then, Tf_2O (88.4 μL, 0.525 mmol) was added, and after 5 min acceptor **9** (353 mg, 0.394 mmol) in dry dichloromethane (2 mL) was added via syringe and the reaction mixture was cooled down to −78 °C and stirred for an additional 2 h before the molecular sieves were filtered off. The organic layer was washed sequentially with saturated aqueous $NaHCO_3$ solution, brine and dried (Na_2SO_4). The organic layer was concentrated under reduced pressure. Purification by silica gel column chromatography (10% ethyl acetate in hexane) afforded compound **10** as foamy material (406 mg, 78%), $[\alpha]_D^{25}$ −38.90 (*c* 1.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.47–7.46 (m, 4H, Ph-H), 7.40–7.17 (m, 36H, Ph-H), 7.09–7.03 (m, 5H, Ph-H), 5.54 (s, 1H, PhCH), 5.09 (d, *J* = 2.4 Hz, 1H, H-1′), 4.99 (d, *J* = 12.0 Hz, 1H, $PhCH_2$), 4.85 (d, *J* = 10.8 Hz, 1H, $PhCH_2$), 4.78 (d, *J* = 11.4 Hz, 1H, $PhCH_2$), 4.77 (s, 1H, H-1), 4.76 (d, *J* = 11.4 Hz, 1H, $PhCH_2$), 4.68–4.63 (m, 3H, 3 × $PhCH_2$), 4.59–4.42 (m, 8H, 8 × $PhCH_2$), 4.35 (br s, 1H, H-1″), 4.23 (t, *J* = 2.4 Hz, 1H, H-2'), 4.20 (d, *J* = 10.8 Hz, 1H, $PhCH_2$), 4.17 (dd, *J* = 4.8, 9.6 Hz, 1H, H-6″b), 4.11–4.07 (m, 2H, H-2 and H-4″), 3.94 (dd, *J* = 3.0, 7.8 Hz, 1H, H-3′), 3.89–3.86 (m, 2H, H-3 and H-5′), 3.79–3.63 (m, 9H, H-2″, 4, 4′, 5, 6a, 6b, 6′a, 6′b, and 6″a), 3.28 (dd, *J* = 3.6, 10.2 Hz, 1H, H-3″), 3.23 (s, 3H, OMe), 2.97 (m, 1H, H-5″); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.79, 138.59, 138.44, 138.29, 138.18, 138.07, 137.80, 128.77, 128.58, 128.53, 128.34, 128.30, 128.27, 128.23, 128.12, 128.04, 127.99, 127.78, 127.71, 127.61, 127.55, 127.44, 127.38, 127.32, 127.25, 126.13, 101.42, 99.98, 99.90, 80.27, 78.48, 77.77, 77.22, 76.32, 75.08, 74.41, 74.16, 73.50, 73.34, 73.28, 71.69, 71.63, 71.36, 69.52, 69.25, 68.54, 67.17, 54.63; ESI-HRMS (TOF), calcd for $C_{82}H_{86}O_{16}Na$ ($[M+Na]^+$): 1349.5814; found: 1349.5413. Anal. Calcd for $C_{82}H_{86}O_{16}$: C, 74.19; H, 6.53. Found: C, 74.25; H, 6.58. *Methyl β-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside* (**11**). A mixture of trisaccharide derivative (**10**, 80 mg, 0.154 mmol) and 10% Pd/C (100 mg) in MeOH–AcOH (10:1, 3 mL) was stirred under an atmosphere of H_2 for 24 h. The reaction mixture was then filtered through Celite and the filtrate was concentrated. The product was purified by reverse phase HPLC to give **11** (27 mg, 86%), $[\alpha]_D^{25}$ −61.02 (*c* 0.48, H_2O); ^{1}H NMR (300 MHz, CD_3OD): δ 5.02 (d, *J* = 1.44 Hz, 1H), 4.88 (d, *J* = 1.39 Hz, 1H), 4.62 (br s, 1H), 4.16 (q, *J* = 3.0 Hz, 1H), 3.84–3.37 (m, 17H), 3.31 (s, 3H, OMe), 3.19 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD–D_2O: 4:1): δ 101.48, 100.76, 99.81, 80.13, 78.32, 77.97, 74.58, 74.13, 72.26, 71.79, 71.37, 68.73, 68.54, 68.14, 62.47, 62.41 (2C), 55.54; ESI-HRMS (TOF), calcd for $C_{19}H_{34}O_{16}Na$ ($[M+Na]^+$): 541.1745; found: 541.1743.
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