

Expedient Synthesis of the Pentasaccharide Repeating Unit of the Polysaccharide O-Antigen of *Escherichia coli* O11

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A convergent [3+2] block synthetic strategy was developed for the synthesis of the pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O11 strain in excellent yield in a minimum number of steps. Several suitably functionalized thioglycoside derivatives were used as glycosyl donors during the synthesis of the target compound. A thioglycoside was the glycosyl donor used to couple with another thioglycoside derivative in a highly stereoselective manner exploiting the difference of their reactivity profile. A combination of *N*-iodosuccinimide (NIS) and perchloric acid supported over silica gel (HClO₄-SiO₂) was used as a thiophilic glycosylation activator system in all stereoselective glycosylation reactions. HClO₄-SiO₂ acted as a user-friendly solid acid catalyst. Yields were very good in all glycosylation steps with a high stereoselective outcome. The synthetic pentasaccharide could be coupled to an appropriate protein to furnish a glycoconjugate derivative for its use in immunochemical studies.

Diarrheal infections are frequently encountered enteric diseases all over the world, particularly in developing countries that lack adequate sanitation.^[1] Gastrointestinal infections are the leading cause of death in children, the elderly, and immunocompromised patients.^[2] Among several bacterial species associated with the enteric infections, *Escherichia coli* (*E. coli*) is predominant.^[3,4] Most commonly, *E. coli* strains are found as the commensal microorganism in the intestine with a beneficial symbiotic relationship with the host.^[5] However, some strains become virulent and cause several serious infections to the host.^[6] In general, pathogenic *E. coli* strains are responsible for a) diarrheal infections,^[3] b) urinary tract infections,^[7] and c) sepsis/meningitides,^[8] as examples.

The diarrheagenic *E. coli* strains are divided into several subclasses based on the pathogenic nature of infections,^[3,6] such as a) enteropathogenic *E. coli* (EPEC), b) enterohemorrhagic *E. coli* (EHEC), c) enterotoxigenic *E. coli* (ETEC), d) enteroaggre-

gative *E. coli* (EAEC), e) enteroinvasive *E. coli* (EIEC), etc. Among the subclasses of diarrheagenic *E. coli* strains, ETEC is responsible for traveller's diarrhea^[9] and enteric disorders in children and the immunocompromised, as well as for causing outbreaks.^[10] Bacterial cell wall polysaccharide O-antigens play a vital role in the pathogenicity of the virulent strains.^[11] As a consequence, several reports have appeared on the structural aspects of cell wall polysaccharides of various bacterial species.^[12-14] Recently, Perapelov et al. reported^[15] the structure of the pentasaccharide repeating unit of the cell wall O-antigen of *E. coli* O11, which is a member of ETEC strains^[3] and associated with diarrheal outbreaks. In the current scenario, bacterial infections caused by multidrug-resistant strains are a serious concern and require alternative approaches to conventional therapeutics for controlling the diseases.^[16]

Cell wall polysaccharides of bacterial strains have been used to develop polysaccharide conjugate vaccines against a number of bacterial infections.^[17,18] However, use of polysaccharides isolated from the natural sources suffers from several drawbacks such as a) handling of live bacterial strains for the production and collection of polysaccharides, b) batch-to-batch variation in the length of the polysaccharides, c) lack of adequate purity and presence of biological impurities (e.g. lipids, nucleic acids), and d) tedious isolation protocols. In the recent past, it has been reported that the use of chemically synthesized glycoconjugate derivatives could act as vaccine candidates similar or somewhat superior to the conventional polysaccharide vaccines.^[19,20] Therefore, it could be useful to develop glycoconjugate derivatives for their use as therapeutics to control diarrheal infections. The first step for the development of a glycoconjugate is to develop an efficient chemical synthetic strategy for the synthesis of the oligosaccharide components. As a part of the ongoing program for the synthesis of complex oligosaccharides, a convergent synthetic strategy for the synthesis of the pentasaccharide repeating unit of the cell wall O-antigen of *E. coli* O11 is reported herein.

Since the target compound **1** contains four α -glycosidic linkages and one β -glycosidic linkage in the molecule, judiciously functionalized monosaccharide intermediates were chosen to couple them together with an appropriate stereochemical outcome at the glycosidic linkages. Application of a series of sequential glycosylation reactions in a multistep synthetic scheme often requires a large number of reaction steps resulting in a low overall yield. Alternatively, the number of steps can be decreased with the significant improvement of the overall yield by using a block glycosylation strategy. Therefore, in the present study, a convergent [3+2] block glycosylation

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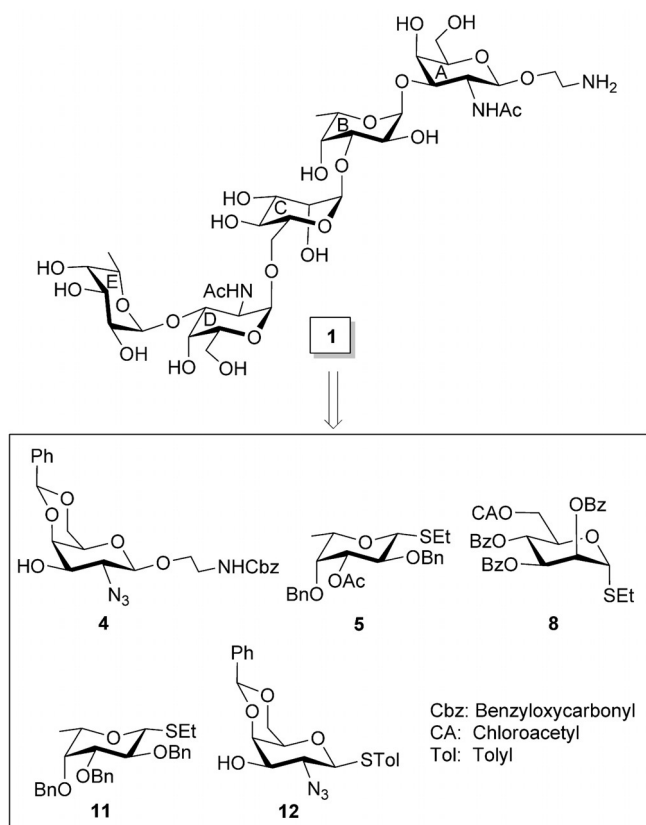


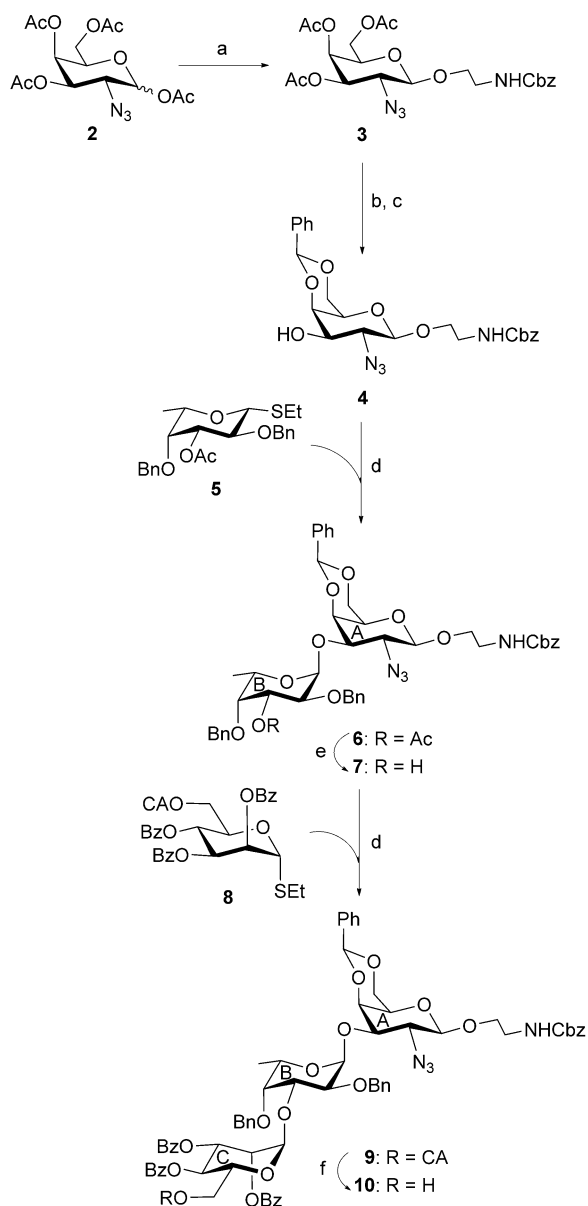
Figure 1. Structure of the synthesized pentasaccharide corresponding to the cell wall O-antigen of *E. coli* O11 strain as its 2-aminoethyl glycoside.

approach was adopted to achieve the target compound. A set of suitably functionalized monosaccharide intermediates **4**, **5**,^[21] **8**,^[22] **11**,^[23] and **12**^[24] were prepared from the reducing sugars, applying the reaction conditions reported in the literature (Figure 1). The trisaccharide acceptor **10** was synthesized using sequential glycosylations and protecting group manipulations using the monosaccharide intermediates **4**, **5**, and **8**. The disaccharide thioglycoside **13** was prepared by the glycosylation of two thioglycoside derivatives (**11** and **12**) in which compound **11** acted as glycosyl donor, allowing compound **12** to act as glycosyl acceptor by tuning their relative reactivity profile. The trisaccharide acceptor **10** was allowed to couple stereoselectively with the disaccharide thioglycoside **13** under a recently reported glycosylation condition^[25] to give the pentasaccharide derivative **14** in fairly good yield. The 2-aminoethyl group at the reducing terminal of the pentasaccharide **1** could be used to conjugate the glycan moiety with an appropriate protein.

Stereoselective 1,2-*trans* glycosylation of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy- α/β -D-galactopyranose (**2**)^[26] with 2-(*N*-benzyloxycarbonyl)amino ethanol in the presence of borontrifluoride diethyletherate in acetonitrile utilizing the concept of nitrile effect^[27] of the solvent in β -glycosylation furnished compound **3** in 69% yield. A small quantity (~10%) of the 1,2-*cis* glycoside was also formed, which was separated by repeated column chromatography. Appearance of a doublet at δ = 4.30 ppm (J = 8.0 Hz, H-1) in the ¹H NMR spectrum of com-

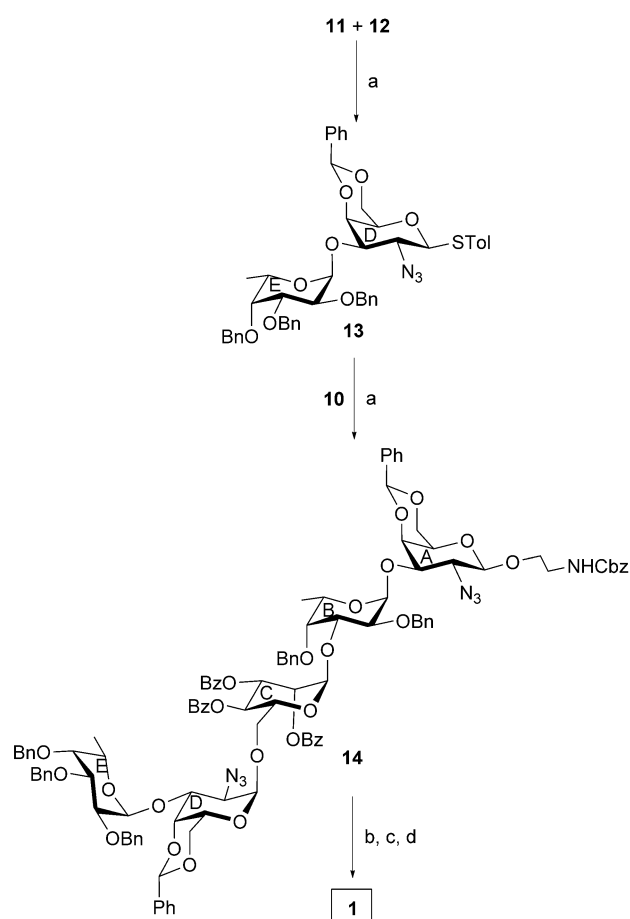
pound **3** confirmed the formation of the 1,2-*trans* glycoside. Treatment of compound **3** with sodium methoxide^[28] gave the de-*O*-acetylated product, which on treatment with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid^[29] led to the formation of compound **4** in 76% overall yield. Iodonium-ion-mediated stereoselective 1,2-*cis* glycosylation of compound **4** with L-fucosyl thioglycoside donor **5** in the presence of a combination^[25] of *N*-iodosuccinimide (NIS) and perchloric acid supported over SiO₂ (HClO₄-SiO₂)^[30] in a mixed solvent (CH₂Cl₂-Et₂O; 1:2) resulted in the formation of the disaccharide derivative **6** in 70% yield. The spectral analysis of compound **6** confirmed its formation: signals at δ = 5.05 (d, J = 3.5 Hz, H-1_B) and 4.28 ppm (d, J = 9.0 Hz, H-1_A) in the ¹H NMR and at 102.7 (C-1_A) and 100.3 ppm (C-1_B) in the ¹³C NMR spectra. Treatment of compound **6** with sodium methoxide furnished the disaccharide acceptor **7** in 90% yield. Following the similar reaction condition as in the preparation of compound **6**, compound **7** was stereoselectively coupled with D-mannose-derived thioglycoside donor **8** in the presence of a combination^[25] of NIS and HClO₄-SiO₂ in CH₂Cl₂-Et₂O (1:2) to produce trisaccharide derivative **9** in 71% yield. Exclusive formation of compound **9** was confirmed from its spectral analysis: signals at δ = 5.71 (br s, H-1_C), 5.10 (d, J = 3.5 Hz, H-1_B), and 4.42 ppm (d, J = 8.5 Hz, H-1_A) in the ¹H NMR and at 102.7 (C-1_A), 100.1 (C-1_B), and 99.1 ppm (C-1_C) in the ¹³C NMR spectra. The chloroacetyl group in compound **9** was selectively removed^[31] by the treatment with thiourea to give the trisaccharide acceptor **10** in 70% yield without affecting the benzoyl group present in it (Scheme 1).

In another experiment, stereoselective orthogonal glycosylation^[32] of thioglycoside **11** and thioglycoside **12** in the presence of a combination^[25] of NIS and HClO₄-SiO₂ in a mixed solvent (CH₂Cl₂-Et₂O; 1:4) afforded the disaccharide thioglycoside derivative **13** in 68% yield. The presence of a benzyl group at the C-2 position of compound **11** improved the nucleophilicity of the anomeric sulfur atom, whereas the azido group at the C-2 position of compound **12** together with the tolyl moiety linked to the sulfur atom deactivated the nucleophilicity of the anomeric sulfur atom. As a result, compound **11** was activated faster than compound **12** in the presence of the thiophilic activator and behaved as a glycosyl donor. Having a hydroxy group present in the molecule, compound **12** played the role of a glycosyl acceptor. The spectral analysis of compound **13** confirmed its formation: signals at δ = 4.91 (d, J = 3.5 Hz, H-1_E) and 4.31 ppm (d, J = 9.5 Hz, H-1_D) in the ¹H NMR and 100.8 (C-1_E) and 85.4 ppm (C-1_D) in the ¹³C NMR spectra. Finally, glycosylation of the trisaccharide acceptor **10** with the disaccharide thioglycoside donor **13** in the presence of a combination^[25] of NIS and HClO₄-SiO₂ in a mixed solvent (CH₂Cl₂-Et₂O; 1:4) resulted in the formation of the pentasaccharide derivative **14** in 70% yield. The stereochemistry at the glycosidic linkages in compound **14** was confirmed from its spectral analysis: signals at δ = 5.64 (br s, 1H, H-1_C), 5.14 (d, J = 3.5 Hz, H-1_D), 5.05 (d, J = 3.5 Hz, H-1_B), 5.01 (d, J = 3.5 Hz, H-1_E), and 4.25 ppm (d, J = 8.5 Hz, H-1_A) in the ¹H NMR and 102.8 (C-1_A), 100.6 (C-1_D), 99.6 (C-1_C), 99.4 (C-1_E), and 98.9 ppm (C-1_B) in the ¹³C NMR spectra. Compound **14** was subjected to a sequence of functional-



Scheme 1. Reagents and conditions: a) 2-(*N*-benzyloxycarbonyl)amino ethanol, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_3CN , $0^\circ\text{C} \rightarrow \text{rt}$, 36 h, 69%; b) 0.1 M CH_3ONa , CH_3OH , rt, 3 h; c) $\text{PhCH}(\text{OCH}_3)_2$, *p*-TsOH, mol. sieve 3 Å, CH_3CN , rt, 8 h, overall 76%; d) NIS, $\text{HClO}_4\text{-SiO}_2$, mol. sieve 4 Å, $\text{CH}_2\text{Cl}_2\text{-Et}_2\text{O}$ (1:2), -15°C , 25 min, **6**: 70% and **9**: 71%; e) 0.1 M CH_3ONa , CH_3OH , rt, 1 h, 90%; f) thiourea, $\text{CH}_2\text{Cl}_2\text{-CH}_3\text{OH}$ (1:1), 50°C , 2 h, 70%.

group transformations consisting of a) direct conversion of azido group into acetamido group using thioacetic acid,^[33] b) removal of *O*-benzoyl group using sodium methoxide, and c) removal of benzyl ethers and benzylidene acetal by catalytic transfer hydrogenation^[34] using triethylsilane in the presence of $\text{Pd}(\text{OH})_2\text{-C}$ to give the target pentasaccharide **1** as its 2-aminoethyl glycoside in 54% overall yield. The structure of compound **1** was unambiguously confirmed from its spectral analysis: signals at $\delta = 5.10$ (br s, H-1_C), 5.04 (d, $J = 3.0$ Hz, H-1_B), 5.03 (d, $J = 3.0$ Hz, H-1_E), 4.92 (d, $J = 3.5$ Hz, H-1_D), and 4.59 ppm (d, $J = 8.5$ Hz, H-1_A) in the ^1H NMR and 102.6 (C-1_C), 101.2 (C-1_B),



Scheme 2. Reagents and conditions: a) NIS, $\text{HClO}_4\text{-SiO}_2$, $\text{CH}_2\text{Cl}_2\text{-Et}_2\text{O}$ (1:4), -10°C , 30 min, **13**: 68% **14**: 70%; b) CH_3COSH , pyridine, rt, 16 h; c) 0.1 M CH_3ONa , CH_3OH , rt, 6 h; d) Et_3SiH , $\text{Pd}(\text{OH})_2\text{-C}$ (20%), CH_3OH , rt, 18 h, overall 54%.

101.0 (C-1_E), 100.9 (C-1_A), and 97.2 ppm (C-1_D) in the ^{13}C NMR spectra (Scheme 2).

In summary, a pentasaccharide corresponding to the repeating unit of the cell wall polysaccharide of *E. coli* O11 was synthesized in good yield using a convergent [3+2] block glycosylation approach. During the synthesis of the target molecule, similar reaction conditions were used in all intermediate glycosylation reaction steps with satisfactory yield and stereoselectivity. A stereoselective glycosylation was carried out between thioglycoside donor and thioglycoside acceptor by tuning their relative reactivity profile.

Experimental Section

For details of the experimental conditions and characterization data for the compounds reported, see the Supporting Information.

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