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Synthesis of trisaccharide and a tetrasaccharide repeating unit corresponding to the *O*-antigen of Shiga toxin producing *Escherichia coli* O177

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ABSTRACT

A trisaccharide and a tetrasaccharide repeating unit corresponding to the cell wall *O*-antigen of verotoxin producing *Escherichia coli* O177 strain have been synthesized in excellent yield using sequential stereoselective glycosylation strategy. A common trichloroacetimidate intermediate has been used for the incorporation of two L-fucosamine moieties in the target molecules.

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1. Introduction

Diarrhoeal outbreaks in the developing countries due to the bacterial infections are serious concern in the present days.[1] Intake of contaminated food and water as well as lack adequate sanitation were found to be the cause of diarrhoea in most of the cases.[2,3] Among several pathogenic bacteria responsible of food borne diseases, *Escherichia coli* (*E. coli*) are predominant.[3] *E. coli* is a Gram-negative, facultative anaerobic rod and present in the intestinal microflora as commensal organism. However, in many instances they become virulent and cause several infections such as (a) diarrhoea, (b) urinary tract infections and (c) septicaemia.[4–6] Among several diarrhoeagenic *E. coli* strains verotoxin producing *E. coli* (VTEC) are considered as most important human pathogens in the developing countries.[7] They are also termed as Shiga-toxin producing *E. coli* (STEC) because of their ability to produce Shiga like toxins.[8] Among several STEC strains identified till date, *E. coli* O157 is well documented.[9] However, there are several other STEC strains, which are associated with the gastrointestinal infections. *E. coli* O177 is a member of STEC, found in soil and water and responsible for diarrhoeal infections in human.[10] Since, cell wall *O*-antigens of the bacteria are directly responsible for their virulent activities, structure of a number of *E. coli O*-antigens have been reported.[11] Recently, the structure of the repeating unit of the cell wall *O*-antigen of *E. coli* O177 has been established by Svensson et al.,[12] which is composed of L-rhamnose, D-glucosamine and relatively rare sugar, L-fucosamine moieties. Development of a glycoprotein conjugate based vaccine candidate against the bacterial infections using cell wall *O*-antigen polysaccharide fragment would be pertinent. However, isolation of polysaccharide fragments in significant quantity from the bacterial cell wall with adequate purity and free from biological impurities is troublesome. Chemical synthesis of the polysaccharide fragments corresponding to the *O*-antigen of a particular bacterial strain with distinct structural identity would be a better alternative. The synthetic oligosaccharide can be easily modified for its coupling to a suitable protein. In an ongoing program for the synthesis of the oligosaccharides related to bacterial cell wall *O*-antigens, synthesis of a tri- and a tetrasaccharide corresponding to the repeating unit of the *O*-antigen of *E. coli* O177 is presented herein (Fig. 1). The 2-aminoethyl group present at the anomeric center of the reducing end could provide ready availability of amine functionality for the modification of the glycan during the conjugation with a protein.

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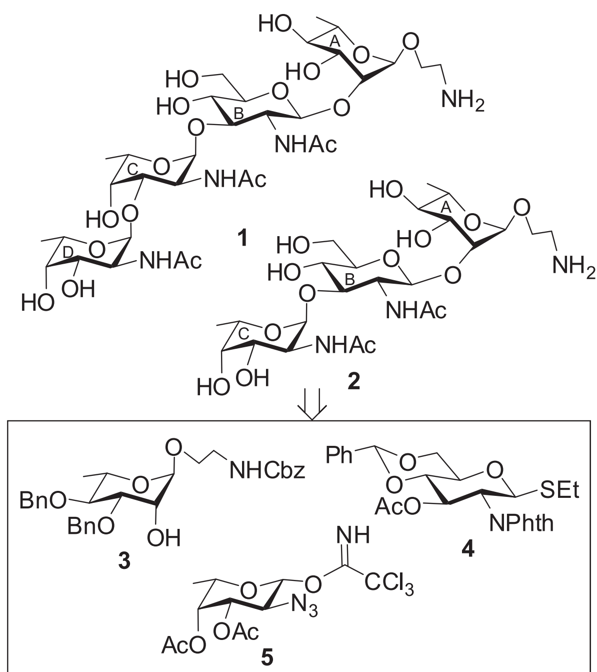

Fig. 1. Structure of the synthesized tetra- and trisaccharide fragments corresponding to the cell wall *O*-antigen of *Escherichia coli* O177 and their synthetic intermediates.

2. Results and discussion

The compounds **1** and **2** were synthesized using sequential glycosylation strategies by stereoselective glycosylation of the monosaccharide intermediates **3**,[13] **4**[14] and **5**,[15] which were prepared from the commercially available reducing sugars using earlier reported reaction conditions. The trisaccharide derivative **8** and tetrasaccharide derivative **11** were synthesized using similar glycosylation reaction conditions, which on deprotection of the functional groups furnished target compounds **1** and **2** in satisfactory yields. In this context, it is noteworthy that synthesis of a similar trisaccharide derivative **8** has been reported by Roy and co-workers[15] using compound **5** as the glycosyl donor in the presence of triethylsilyl trifluoromethanesulfonate as activator. Earlier nitrosyl tetrafluoroborate ($NOBF_4$) has been found to act as an efficient non-metallic glycosylation promoter for the activation of glycosyl trichloroacetimidate derivative replacing conventionally used strong protonic or Lewis acids.[16] In order to establish the versatility of this catalyst, $NOBF_4$ has been applied in this synthetic strategy for the activation of 2-azido-L-fucosyl trichloroacetimidate derivative (**5**) in the stereoselective glycosylation. Iodonium ion mediated stereoselective glycosylation of the L-rhamnosyl acceptor **3** with thioglycoside derivative **4** in the presence of a combination of *N*-iodosuccinimide (NIS) and $HClO_4$–SiO_2 (a solid supported acid)[17,18] at low temperature furnished disaccharide derivative **6** in 78% yield, which was confirmed from its NMR spectral analysis (signals at δ 5.42 (d, J=8.5 Hz, H-1_B), 4.65 (br s, H-1_A) in ^{1}H NMR and δ 100.3 (C-1_B), 99.0 (C-1_A) in ^{13}C NMR spectra). De-O-acetylation of compound **6** using a dilute solution of sodium methoxide[19] afforded the disaccharide acceptor **7** in 92% yield. $NOBF_4$ mediated stereoselective glycosylation[16] of compound **7** with compound **5** in CH_2Cl_2–Et_2O (1:1) mixed solvent furnished trisaccharide derivative **8** in 68% yield. NMR spectral analysis of compound **8** unambiguously confirmed the presence of appropriate glycosyl linkages in compound **8** (signals at δ 5.14 (d, J=9.5 Hz, H-1_B), 4.62 (d, J=3.5 Hz, H-1_C), 4.58 (br s, H-1_A) in ^{1}H NMR and δ 100.8 (C-1_B), 99.0 (C-1_A), 98.8 (C-1_C) in ^{13}C NMR spectra). Removal of the *O*-acetyl groups in compound **8** using a dilute solution of sodium methoxide afforded the trisaccharide diol **9** in 90% yield. Treatment of compound **9** with triethyl orthoacetate in the presence of camphorsulfonic acid resulted in the formation of an orthoester intermediate,[20] which on acidic hydrolysis selectively furnished mono-acetylated trisaccharide acceptor **10** in 78% yield. Repeated $NOBF_4$ mediated[16] stereoselective glycosylation of compound **10** with compound **5** in CH_2Cl_2–Et_2O (1:1) mixed solvent furnished tetrasaccharide derivative **11** in 66% yield. NMR spectral analysis of compound **11** unambiguously confirmed the presence of appropriate glycosyl linkages in compound **11** (signals at δ 5.25 (d, J=8.5 Hz, H-1_B), 5.12 (d, J=3.5 Hz, H-1_D), 4.75 (d, J=3.5 Hz, H-1_C), 4.65 (br s, H-1_A) in ^{1}H NMR and δ 100.7 (C-1_B), 99.3 (C-1_A), 98.9 (C-1_C), 93.3 (C-1_D) in ^{13}C NMR spectra). The presence of 1,2-*cis* glycosyl linkages in compound **11** was unambiguously confirmed from the ^{1}H coupled ^{13}C NMR spectral analysis. Appearance of $J_{C\text{-}1/H\text{-}1}$ values 170 Hz, 170 Hz, 169 Hz for three α-glycosidic bonds and 156 Hz for one β-glycosidic bond confirmed the formation of required glycosyl linkages.[21] Compound **11** was subjected to a series of reactions consisting of (a) treatment with hydrazine monohydrate[22]; (b) N- and O-acetylation using acetic anhydride and pyridine; (c) conversion of azido group to acetamido group using thioacetic acid[23]; (d) removal of benzyl ethers and benzylidene acetal under a catalytic transfer hydrogenation condition[24] using triethylsilane and Pearlman's catalyst and finally (e) saponification using sodium methoxide to give compound **1** in overall 54% yield. NMR spectral analysis of compound **1** confirmed its formation (signals at δ 5.18 (d, J=2.5 Hz, H-1_C), 4.92 (br s, H-1_A), 4.60 (br s, H-1_D), 4.59 (d, J=8.5 Hz, H-1_B) in ^{1}H NMR and δ 95.9 (2C, C-1_B, C-1_D), 94.0 (C-1_A), 92.1 (C-1_C) in ^{13}C NMR spectra).

In a separate experiment, compound **2** was prepared from compound **9** in overall 58% yield using similar reaction conditions as used in the preparation of compound **1** from compound **11**. NMR spectral analysis of compound **2** confirmed its formation (signals at δ 5.04 (d, J=2.5 Hz, H-1_C), 4.97 (br s, H-1_A), 4.66 (d, J=8.5 Hz, H-1_B) in ^{1}H NMR and δ 102.7 (C-1_B), 98.8 (C-1_A), 97.6 (C-1_C) in ^{13}C NMR spectra) (Scheme 1).

3. Conclusions

In summary, straightforward synthetic strategies have been developed for the synthesis of trisaccharide and tetrasaccharide repeating unit corresponding to the cell wall *O*-polysaccharide of *Escherichia coli* O177 in very good yield. Two target compounds were synthesized using common intermediates and similar reaction conditions. Application of $NOBF_4$ mediated activation of 2-azido-L-fucosyl trichloroacetimidate derivative in the glycosylation reactions resulted in the formation of 1,2-*cis* glycosyl linkages in good yield.

4. Experimental

4.1. 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% $Ce(SO_4)_2$ in 2N H_2SO_4) sprayed plates in hot plate. Silica gel 230–400 mesh was used for column chromatography. NMR spectra were recorded on Bruker Avance 500 MHz using $CDCl_3$ as solvent and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. The complete assignment of proton and carbon spectra was carried out by using a standard set of NMR experiments, e.g., ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135, 2D COSY and 2D HSQC etc. MALDI-MS were recorded on a Bruker Daltonics mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer.

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