Synthesis of the pentasaccharide repeating unit of the O-antigen of *E. coli* O117:K98:H4

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Full Research Paper

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Abstract

The pentasaccharide repeating unit of the O-antigen of *E. coli* O117:K98:H4 strain has been synthesized using a combination of sequential glycosylations and [3 + 2] block synthetic strategy from the suitably protected monosaccharide intermediates. Thioglycosides and glycosyl trichloroacetimidate derivatives have been used as glycosyl donors in the glycosylations.

Introduction

*Escherichia coli* becomes an important human pathogen in recent years owing to the emergence of new pathogenic strains [1]. Several diseases, such as meningitis and sepsis [2], diarrhoeal outbreaks [3] and urinary tract infections [4] are associated with pathogenic *Escherichia coli* (*E. coli*) strains. *E. coli* strains have been found to produce the Shiga toxin (Stx), heat-labile (LT) or heat-stable (ST) enterotoxins, cytotoxic necrotizing factors (CNF1 and CNF2) and hemolysins (α-Hly and E-Hly) [5,6] and are responsible for hemorrhagic colitis and haemolytic-uremic syndroms in humans [7]. The different strains of *E.coli* as well as bacteria belonging to different genera, e.g., *Shigella*, *Salmonella*, and *Klebsiella* show serological cross-reactions within the species [8]. The *E. coli* O117 strain emerged as a significant cause for septicaemia, bovine diarrhoea in new born children and human [9]. Together with other *E. coli* strains *E. coli* 0117 strains are responsible for pyelonephritis which is sexually transmitted by a woman that spread up to 60 to 80% of community acquired urinary tract or travelled through the bloodstream to the kidneys [10,11]. The O-specific polysaccharide of *E. coli* O117:K98:H4 is a linear pentasaccharide repeating unit consisting of D-galactosamine, D-glucose, D-galactose, and L-rhamnose (Figure 1) [12].

![Figure 1: Structure of the pentasaccharide repeating unit of the O-specific polysaccharide of E. coli O117:K98:H4.](image-url)
Vaccination is the recent thrust in the drug discovery program to prevent bacterial infections. Several bacterial O-antigens have been chosen for the development of glycoconjugate vaccine candidates against infectious diseases [13-16]. As a consequence, a significant quantity of oligosaccharides is required to evaluate their immunological properties for detailed understanding of the role of O-antigens in the pathogenicity of the E. coli strains. Development of chemical synthetic strategies would be useful to get large quantities of the oligosaccharides. As a part of the ongoing studies on the synthesis of bacterial cell wall oligosaccharides [17-19], a straightforward synthesis of the pentasaccharide repeating unit of the O-specific polysaccharide of E. coli O117:K98:H4 as its 3-aminopropyl glycoside is presented herein (Figure 2). The 3-aminopropyl group would be suitable for attachment of the pentasaccharide to any surface or carrier proteins.

Results and Discussion
The target pentasaccharide 1 has been synthesized as its 3-aminopropyl glycoside using a combination of sequential and [3 + 2] block glycosylation strategy. A trisaccharide acceptor 11 and a disaccharide trichloroacetimidate donor 14 were synthesized from the appropriately protected monosaccharide intermediates 2 [20], 3 [21], 4 [22], 5 and 6 [23] (Figure 2) derived from the commercially available aldoses. Trisaccharide acceptor 11 was then glycosylated with disaccharide trichloroacetimidate donor 14 to form pentasaccharide derivative 15, which was finally deprotected to give target pentasaccharide 1 (see below Scheme 2). Some of the notable features of this synthetic strategy are (a) application of iodonium ion mediated general glycosylation conditions; (b) nitrosyl tetrafluoroborate (NOBF₄) mediated activation of glycosyl trichloroacetimidate donor; (c) the attachment of an aminopropyl linker at the anomic center; (d) glycosylation and removal of the p-methoxybenzyl (PMB) group in one-pot.

Treatment of p-methoxyphenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (7) [24] (prepared from D-galactosamine hydrochloride in six steps) with acetic anhydride in pyridine followed by regioselective reductive opening of the benzylidene acetal on treatment with sodium cyanoborohydride in the presence of HCl/Et₂O [25] furnished p-methoxyphenyl 3-O-acetyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (5) in 77% yield over two steps (Scheme 1).

![Scheme 1: Reagents and conditions: (a) (i) acetic anhydride, pyridine, room temperature, 2 h; (ii) NaBH₃CN, HCl/Et₂O, 5 °C, 2 h, 77% overall yield.](image)

![Figure 2: Structure of the synthesized pentasaccharide (1) and its precursor intermediates.](image)
Trisaccharide acceptor 11 could be synthesized following the reaction pathway depicted in Scheme 2. Glycosylation of 3-azidopropyl 2,3,6-tri-O-benzyl-β-D-galactopyranoside (2) with the thioglycoside donor 3 in the presence of N-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) [26,27] gave disaccharide derivative 8 in 72% yield. NMR spectroscopy confirmed the formation of compound 8 [δ 5.04 (d, J = 3.6 Hz, 1H, H-1B), 4.38 (d, J = 7.6 Hz, 1H, H-2B), 4.07 (d, J = 7.6 Hz, 1H, H-3B), 3.85 (d, J = 7.6 Hz, 1H, H-4B), 3.74 (d, J = 7.6 Hz, 1H, H-5B), 3.62 (d, J = 7.6 Hz, 1H, H-6B)] with 1H, H-1B, 4.38 (d, J = 7.6 Hz, 1H, H-2B), 4.07 (d, J = 7.6 Hz, 1H, H-3B), 3.85 (d, J = 7.6 Hz, 1H, H-4B), 3.74 (d, J = 7.6 Hz, 1H, H-5B), 3.62 (d, J = 7.6 Hz, 1H, H-6B) with 1H, H-1B, 4.38 (d, J = 7.6 Hz, 1H, H-2B), 4.07 (d, J = 7.6 Hz, 1H, H-3B), 3.85 (d, J = 7.6 Hz, 1H, H-4B), 3.74 (d, J = 7.6 Hz, 1H, H-5B), 3.62 (d, J = 7.6 Hz, 1H, H-6B)
acetates were removed by Zemplén de-O-acetylation [37] using hydrogenation using Pearlman's catalyst [36]. Finally the removal of the benzyl group was accomplished by forming was acetylated using acetic anhydride in pyridine [35]. The removal of the benzyl group was accomplished using hydrazine hydrate and the free amine thus formed was supported by NMR spectroscopic analysis [signals at \( \delta 1H NMR \) and at \( \delta 13C NMR \) spectra]. Following an earlier report [28], cleavage of the benzylidene acetal from compound 8 catalyzed by perchloric acid on silica (HClO\(_4\)/SiO\(_2\)) [28,29] afforded 3-azidopropyl (2,3-di-O-benzyl-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-D-galactopyranoside (9) in 85% yield. Selective 6-O-benzoylation of compound 9 was accomplished with benzyl cyanide [30] to furnish disaccharide acceptor 10 in 80% yield. Compound 10 was reacted with 3-O-PMB protected L-rhamnosylthioglycoside donor 4 and NIS/TfOH [26,27] to yield the trisaccharide derivative by an iodonium ion catalyzed glycosylation. Participation of the 2-0-acetyl group in donor 4 ensured the \( \alpha \)-selectivity of the glycosylation. Following an earlier report [19], raising the temperature of the reaction mixture after the glycosylation led to the removal of the PMB group in the same pot [31] to furnish trisaccharide acceptor 11 in 77% yield. The formation of compound 11 was supported by NMR spectral analysis [signals at \( \delta 4.85 (d, J = 2.4 Hz, 1H, H-1_H) \), 4.84 (b, 1H, H-1_C), 4.18 (d, \( J = 7.4 Hz, 1H, H-1_A \)) and at \( \delta 104.1 (C-1_A) \), 98.7 (C-1_B), 97.6 (C-1_C) in the \( 1H \) and \( 13C \) NMR spectra respectively]. In another experiment, coupling of 2-azido-6-D-galactosyl trichloroacetimidate derivative 6 and compound 5 in the presence of NOBF\(_4\) [32] in Et\(_2\)O/CH\(_2\)Cl\(_2\) gave disaccharide derivative 12 in 75% yield. The formation of compound 12 was confirmed by NMR spectroscopic analysis [signals at \( \delta 5.92 (d, J = 7.7 Hz, 1H, H-1) \), 5.06 (d, \( J = 2.7 Hz, 1H, H-1\) ) in the \( 1H NMR \) and at \( \delta 99.1 (C-1) \), 97.7 (C-1) in the \( 13C \) NMR spectra, respectively]. Reduction of the azido groups were carried out by treatment with triphenylphosphine [33], then the product was acetylated using acetic anhydride and pyridine to give disaccharide derivative 13 in 84% overall yield in two steps. The anomeric PMP group of compound 13 was oxidatively cleaved using ceric(IV) ammonium nitrate (CAN) [15] and the hemeacetal thus obtained was reacted with trichloroacetateonitrile in the presence of DBU [34] to afford the desired disaccharide trichloroacetimidate derivative 14 in 77% yield. It was used directly without further purification [17] (Scheme 2). Finally, glycosylation of trisaccharide acceptor 11 with the trichloroacetimidate donor 14 in the presence of NOBF\(_4\) [32] in \( CH2Cl2 \) furnished pentasaccharide derivative 15 in 70% yield. Formation of compound 15 was supported by NMR spectral analysis [signals at \( \delta 5.10 (d, J = 3.3 Hz, 1H, H-1) \), 5.00 (d, \( J = 2.4 Hz, 1H, H-1) \), 4.92 (b, 1H, H-1_C), 4.69 (d, \( J = 7.7 Hz, 1H, H-1) \), 4.35 (d, \( J = 7.8 Hz, 1H, H-1\) ) and at \( \delta 104.0 (C-1) \), 101.2 (C-1), 98.9 (C-1_B), 98.3 (C-1_C), 97.4 (C-1_F) in the \( 1H \) and \( 13C \) NMR spectra, respectively]. The N-phthaloyl group was removed using hydrizide hydrate and the free amine thus formed was acetylated using acetic anhydride in pyridine [35]. Then the removal of the benzyl group was accomplished by hydrogenation using Pearlman’s catalyst [36]. Finally the acetates were removed by Zemplén de-O-acetylation [37] using sodium methoxide to afford the target pentasaccharide 1 in 58% overall yield (Scheme 2).

**Conclusion**

In summary, a \([3 + 2]\) block glycosylation strategy has been developed to synthesize a pentasaccharide 3-aminopropyl glycoside (1) corresponding to the \( O \)-antigen of \( E. coli \) O117:H98:H4 strain. The in situ removal of the PMB ether in one-pot following the glycosylation reaction reduced the overall number of steps.

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**References**

30. See for a preparation of HClO₄-SiO₂.

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