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Linear synthesis of the hexasaccharide related to the repeating unit of the O-antigen from *Shigella flexneri* serotype 1d (I: 7,8)

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ABSTRACT

Total synthesis of the hexasaccharide repeating unit of the O-antigen from *Shigella flexneri* serotype 1d (I: 7,8), α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-β-D-Glc*p*NAc, is reported by following a linear strategy. The target hexasaccharide was synthesized by sequential glycosylations of suitably protected monosaccharide derivatives prepared from commercially available monosaccharides through rational protecting group manipulations. Stereoselective glycosylations were accomplished by the activation of thioglycoside using N-iodosuccinimide and H_2SO_4-silica. The use of H_2SO_4-silica in place of traditional promoters like TfOH or TMSOTf was proved to be a better option for the NIS-mediated thioglycoside activation.

1. Introduction

Bacterial O-antigens are responsible for their adhesion to the host cells that cause the infection. They are important part of the cell-surface lipopolysaccharide of the Gram-negative bacteria. The O-antigen consists of several repeats of an oligosaccharide named as O-units. *Shigella flexneri* (*S. flexneri*) is an important Gram-negative bacteria that is responsible for shigellosis in human. The strains of *S. flexneri* are classified into 19 serotypes[1,2] and the structures of the O-antigens of all serotypes have been elucidated[2,3] except that of serotype 1d until recently. Shashkov *et al.*[4] have elucidated the structure of the O-antigen of *S. flexneri* isolated from the stool specimen of diarrheal patients in China.[5] It is established that this particular O-antigen structure possesses a unique combination of type O-factor I and group O-factor 7,8. Herein, we report the linear synthesis of the hexasaccharide repeating unit of the O-antigen from *S. flexneri* serotype 1d (I: 7,8) in the form of its 4-methoxyphenyl glycoside (**1**, Fig. 1). The 4-methoxyphenyl group at the reducing end would be cleaved selectively from the per-acetate derivative of the target hexasaccharide and allow further conjugation with suitable aglycons using trichloroacetimidate chemistry. Although, synthesis of the same structure was reported previously by Misra and co-workers[6] through a different strategy, the linear route has its practical advantage in terms of its reproducibility. The chemical route will provide the scope of preparing the O-antigen structure with high purity and adequate quantity essential for the biological experiments to understand its role in the pathogenic cycle.

2. Results and discussion

Careful adjudication of the retrosynthetic analysis for the target hexasaccharide suggested that a linear strategy involving sequential glycosylations of the required monosaccharide unit will suit best. 4-Methoxyphenyl group was chosen as the reducing end glycoside. Once the target oligosaccharide is ready, the OPMP group may be cleaved selectively and glycoconjugates may be formed with suitable aglycons using trichloroacetimidate chemistry. Chloroacetate group was used as a participating 1,2-*trans* directing protection as well as a temporary protecting group that can be selectively deprotected to pave the path for the next saccharide addition. Simple acetate group could have been an alternative. However, the chance of the decomposition of phthalimido group during de-O-acetylation steps prompted us to choose chloroacetate group instead. All monosaccharide derivatives may be obtained from their respective commercially available sugars through rational protecting group manipulations (Fig. 2).

The synthesis started with the known monosaccharide derivative, 4-methoxyphenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**).[7] It was chloroacetylated at the 3-OH position using chloroacetic anhydride in pyridine[8] to give the fully protected derivative **3** in 92% yield. Subsequently, the benzylidene acetal was regioselectively opened by using triethylsilane in the

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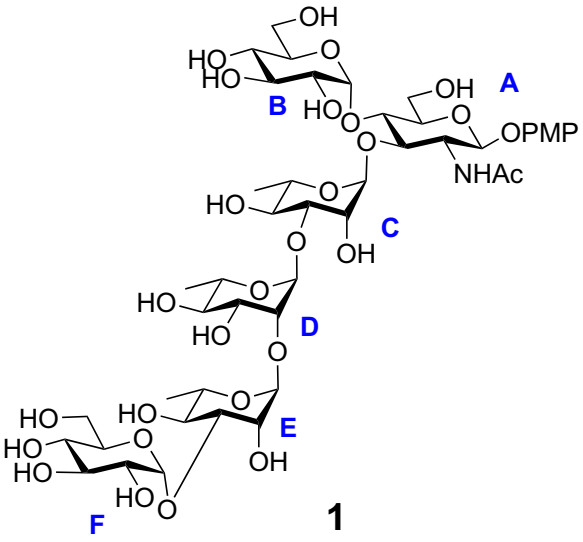
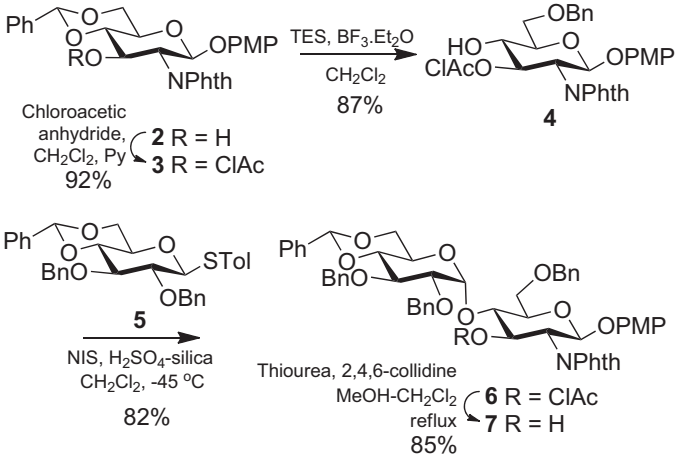


Fig. 1. Structure of the target hexasaccharide **1**.

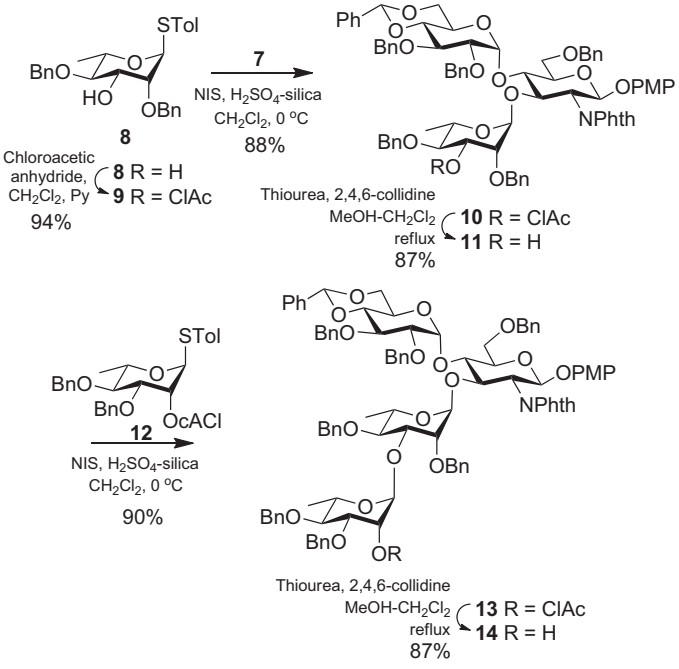
presence of $BF_3.Et_2O$[9] to afford the desired monosaccharide acceptor **4** in 87% yield. Glycosylation of the acceptor **4** with the known monosaccharide donor, 4-tolyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**5**),[10] using NIS in the presence of H_2SO_4-silica[11] furnished the α-disaccharide **6** only in 82% yield. It is worth noting that the same glycosylation using 4,6-di-O-acetyl-2,3-di-O-benzyl-α-D-glucopyranosyl trichloroacetimidate donor as used by Misra *et al*[6] resulted in α/β mixture of disaccharide in 3:1 ratio. Formation of the desired 1,2-*cis* glycoside was evident from the ^1H signal at δ 5.02 (d, 1H, $J_{1',2'}$ 3.2 Hz, H-1′) and ^{13}C signal at δ 96.4 assigned to the newly formed glycosidic linkage. Possibly the structural rigidity imposed by the benzylidene acetal in the donor molecule is the driving force for this stereo-selective glycosylation. It is important to note that when the glycosylation between compound **4** and **5** was carried out using NIS in the presence of TMSOTf, the desired disaccharide **6** was formed only in 73% yield. Careful chromatography revealed the formation of the corresponding hemiacetal of the donor and disaccharide devoid of the benzylidene group. This result suggests that the use of H_2SO_4-silica as the promoter for NIS-mediated activation of thioglycoside is particularly beneficial. Further, the removal of the chloroacetate group using thiourea[12] gave the disaccharide acceptor **7** in 85% yield (Scheme 1).



Scheme 1. Synthesis of the disaccharide acceptor **7**.

In a separate experiment, known rhamnose derivative, 4-tolyl 2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**8**),[13] was chloroacetylated using chloroacetic anhydride in pyridine[8] to give the corresponding fully protected donor **9** in 94% yield. Glycosylation of the donor **9** with the disaccharide acceptor **7** using NIS in the presence of H_2SO_4-silica afforded the trisaccharide **10** in 88% yield. ^1H signal at δ 5.81 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1′) and ^{13}C signal at δ 96.2 assigned to the newly formed linkage. The exclusive formation of the desired 1,2-*trans* linkage was further confirmed by the $J_{C,H}$ value of 169.7 ppm. Further, selective removal of the chloroacetate group of the trisaccharide **10** using thiourea afforded the trisaccharide acceptor **11** in 87% yield. Glycosylation of the trisaccharide acceptor **11** with the known rhamnosyl donor, 4-tolyl 3,4-di-O-benzyl-2-O-chloroacetyl-1-thio-α-L-rhamnopyranoside (**12**),[14] using NIS in the presence of H_2SO_4-silica furnished the protected tetrasaccharide **13** in 90% yield. Next, the thiourea-mediated selective removal of the chloroacetate group gave the tetrasaccharide acceptor **14** in 87% yield (Scheme 2).

Next, glycosylation of the tetrasaccharide acceptor **14** with the rhamnosyl donor **9** using NIS in the presence of H_2SO_4-silica gave the protected pentasaccharide **15** in 91% yield. ^1H signal at δ 5.08 (d, 1H, $J_{1''',2'''}$ 1.5 Hz, H-1‴) and ^{13}C signal at δ 98.8 assigned to the newly formed linkage. The exclusive formation of the desired 1,2-*trans* linkage was further confirmed by the $J_{C,H}$ value of 170.3 ppm. It was found that the replacement of chloroacetate group by 4-methoxybenzyl group in the rhamnose donor (*ca*. Misra *et al*.[6]) makes the synthon too reactive and thus, the yield of the glycosylations reactions drops drastically. Moreover, glycosylation with the highly reactive rhamnose donor at very low temperature yielded traces of the 1,2-*cis* glycoside. Further, selective removal of the chloroacetate group in the presence of thiourea furnished the pentasaccharide acceptor **16** in 85% yield. Finally, glycosylation of the pentasaccharide acceptor **16** with the known glucosyl donor **17**[15] using NIS in the presence of H_2SO_4-silica afforded the protected hexasaccharide **18** in 81% yield. It is worth mentioning that the per-O-benzylated glucose donor was found to be too reactive and resulted in very low yield. Majority of the donor converted to the corresponding hemiacetal during glycosylation reaction. The



Scheme 2. Synthesis of the tetrasaccharide acceptor **14**.