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Total synthesis of the bacillosamine containing α-L-serine linked trisaccharide of *Neisseria meningitidis*

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ABSTRACT

Total synthesis of the bacillosamine containing L-serine linked *O*-trisaccharide of *Neisseria meningitidis* is described. The synthesis entails installation of two consecutive α-linkages including the coupling of bacillosamine with L-serine derivative.

1. Introduction

Neisseria meningitidis (menigococcus) is a causative bacterium of the highly contagious disease meningitis which involves inflammation of the protective membranes (meninges) of the brain and spinal cord.[1] Meningitis is most common in children aged 2–18 and has a high mortality rate. It is estimated that about 5–10% of the total population may be asymptomatic carriers. Most cases are acquired through the exposure to these carriers. The onset of symptoms is sudden and death can occur in a few hours. In a meningitis pandemic, about 10% of patients die, while about 15% of the survivors develop serious neurological disorders. Thus, novel and more effective vaccines[2] are required to control the periodic outbreak of this deadly disease.

It has been well established that meningococcus pili, which are long polymeric filamentous glycoproteins produced from the surface of pathogenic *N. meningitidis* are a key virulence factor.[3] Pili play crucial roles as essential adhesins in colonization of this capsular bacterium and contribute to the specificity for the human host.[4] In 1995, Stimson et al.[5] showed that the pili are post-translationally modified by glycosylation of serine 63 with a unusual trisaccharide Gal-(β 1-4)-Gal(α 1-3)-2,4-diacetimido-2,4,6-trideoxyhexose [Gal(β 1-4)Gal(α 1-3) DATDH] (Fig. 1). Since the structure of the trisaccharide was proposed based on the linkage analysis by acid hydrolysis and mass spectroscopic studies, the stereochemistry at C4 of the rare sugar (DATDH) could not be defined.

The specific function of glycosylation in meningococcus infection remains obscure.[6] It is suggested that glycosylation may constitute bacterial cloaking devices against the host immune reponses.[7] More importantly, Marceau and Nassif demonstrated that the presence of the glycosylation at serine 63 can influence the amount of soluble and secreted form of pilin (S-pilin), a molecule which is crucial in establishing infections.[8] Since the pilin *O*-glycan contains unique deoxy amino sugars which are not present on the host cell surfaces, the structural differences can be exploited for the development of target specific therapeutics and vaccines.[9]

Given the biological importance and the problems associated with the isolation of the glycoproteins in acceptable amounts and purity, it is imperative to synthesize the serine-linked trisaccharide that can be incorporated into the glycoprotein to expedite the studies probing the exact role of pilin *O*-glycans in meningitis and further vaccine development.

The structure of the target *O*-trisaccharide comprises a digalactosyl moiety, a rare deoxy amino sugar DATDH and L-serine. Since the stereochemistry at C4 of the rare sugar is not defined, two putative trisaccharides **1** and **2** are possible, one with a 2,4-diacetamido 2,4,6-trideoxy D-galactose (DATDG), and one containing a 2,4-diacetamido 2,4,6-trideoxy D-glucose (bacillosamine, Bac), respectively. The main challenges involved in the synthesis of the deceptively simple looking trisaccharides are the synthesis of the rare, deoxyamino glycans[10–13] (Bac[11] and DATDG[12]) and installation of two consecutive α-glycosyl linkages. Moreover, obtaining

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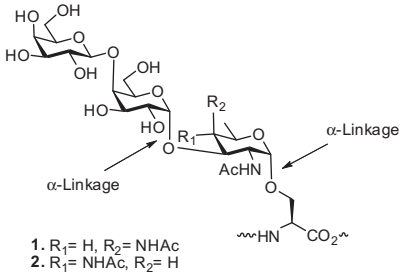


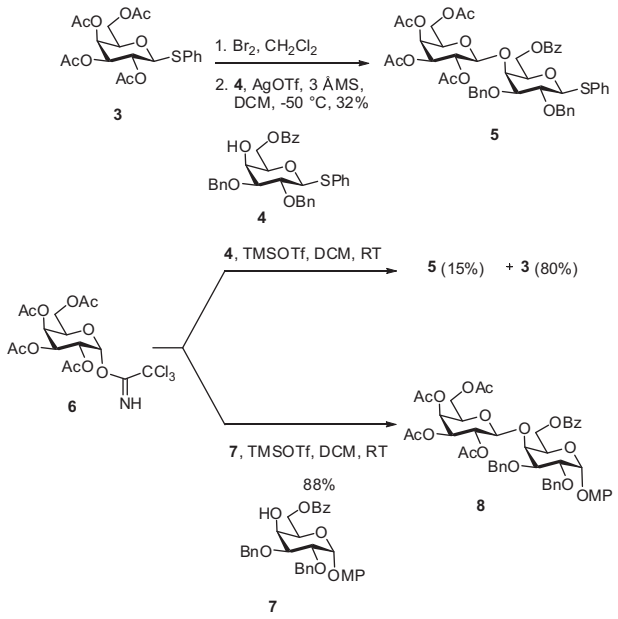
Figure 1. Structures of pilin glycans of *Neisseria meningitidis*.

complete α-stereoselectivity in the coupling of L-serine with the rare trideoxy amino sugars is difficult. Towards this goal, we recently established a methodology to access the rare bacterial deoxy amino sugars including DATDH and thereby accomplished the first synthesis of trisaccharide **1**.[14] In this article we describe the total synthesis of the bacillosamine containing trisaccharide **2**.

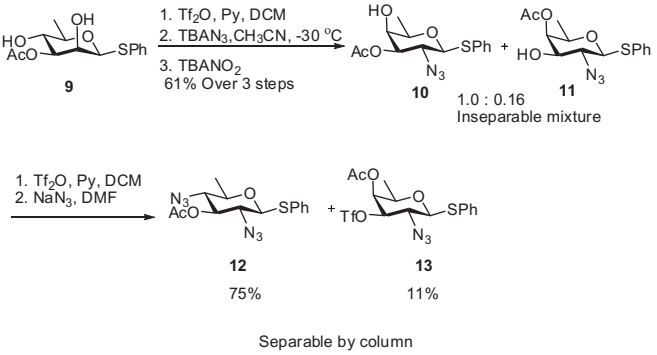
2. Results and discussions

2.1. Synthesis of the left hand disaccharide

For the synthesis of the left hand disaccharide (di-gal), we first prepared the known thioglycoside donor **3**[15] and acceptor **4**[16] using reported procedures. First, thioglycoside **3** was treated with bromine in CH_2Cl_2 and the so formed glycosyl bromide was activated with AgOTf and orthogonally coupled with acceptor **4** to afford the desired disaccharide **5**, albeit in a modest 32% yield (Scheme 1). The low yield encountered in this reaction was attributed to the simultaneous formation of the corresponding orthoester[17] which could not be rearranged to **5** even after prolonged stirring in the presence of excess TMSOTf. Since, we were not able to improve the yield of **5** using glycosyl bromide under various conditions, we decided to try out the corresponding known imidate derivative **6**,[18] with the hope to circumvent the orthoester formation. Glycosylation of imidate **6** with **4** using TMSOTf as an activator at rt[19] did furnish disaccharide **5**, but again as a minor



Scheme 1. Synthesis of the left hand side disaccharide unit.



Scheme 2. Synthesis of bacillosamine thioglycoside donor **12**.

product (15%). In this case, although we did not encounter the orthoester, instead thioglycoside **3** was obtained as a major side product (80%), formed presumably via the aglycon transfer of SPh from acceptor **4** to donor **6**. Such aglycon transfers of thioglycosides are well documented in the literature.[20] To obviate the aglycon transfer, the anomeric thiophenyl group was replaced by a stable methoxy phenyl group (OMP). For this purpose, the corresponding OMP glycoside **7** was prepared in a manner very much similar to **4** from the known α-OMP galactoside[21] through sequential 4,6-O-benzylidenation ($PhCH(OMe)_2$, CSA), 2,3-di-O-benzylation (NaH, BnBr), benzylidene hydrolysis (80% AcOH reflux) and selective O6 benzoylation (Et_3N, Bz_2O), in 68% overall yields. Gratifyingly, glycosylation of imidate **6** with acceptor **7** under TMSOTf activation at rt furnished the desired β-linked disaccharide **8** in 88% yields.[14b]

2.2. Synthesis of the Bac building block

The rare Bac building block **12** was prepared from **9** following our recently established protocol to synthesize rare bacterial sugars (Scheme 2).[14] For this purpose, we were required to carry out a double inversion at C4 of a D-rhamnose derivative to achieve azide substitution with retention of configuration. First, diol **9** was subjected to triflation followed by a one-pot double serial inversion which involved a highly regioselective azide displacement of the C2-OTf followed by a nitrite ion mediated displacement (Lattrell-Dax reaction[22]) of the remaining C4-OTf. The reaction smoothly delivered the D-fucosamine derivative **10** accompanied by a small amount of acetate migration product **11** in a ratio 1:0.16 (as judged by ^1H NMR). Since this side product was inseparable on TLC and by column chromatography, the mixture was subjected as such to a similar C4-triflation and azide displacement sequence, to afford the desired Bac derivative **12** in 75% isolated yield over two steps. At this stage, the unreacted C3-OTf **13** (well separated on TLC) was isolated in 11% yield.

2.3. Stereoselective glycosylation of bacillosamine donor with L-serine acceptor

With the appropriate building blocks in hand, we turned our attention to the synthesis of the right hand unit. As anticipated, the installation of 1,2-*cis* glycosidic linkage in the coupling of the 6-deoxy monosaccharide with the primary OH of L-serine was very difficult. In this case, although the C2-azido group, being a non-participating group, is expected to facilitate the formation of α-linkage, remote participation is not available from the C4 and C6 functionalities.[23] So, we were mindful that various donors and conditions may need to be tested to achieve selectivity. Strategically, our rare sugar building blocks being stable thioglycosides,