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Note

Synthesis of a novel pentasaccharide core component from the lipooligosaccharide of *Moraxella catarrhalis*

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ABSTRACT

The novel pentasaccharide [*p*-(trifluoroacetamido)phenyl]ethyl 3-*O*-β-D-glucopyranosyl-4-*O*-β-D-glucopyranosyl-6-*O*-[2-*O*-(α-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranoside (**1**), which includes a linker moiety to enable facile coupling to an antigenic protein, was synthesised as a component of a potential vaccine candidate against the Gram-negative bacterium *Moraxella catarrhalis*. This microorganism is one of three principal causative agents of otitis media in children. The pentasaccharide represents a common cross-serotype (A, B and C) structure from the lipooligosaccharides of *Moraxella catarrhalis*.

Moraxella catarrhalis is a Gram-negative pathogen that, along with *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*, is responsible for 15–25% of cases of otitis media (middle ear infection) in children.[1] There is a high incidence of otitis media cases in infants in developed countries with at least 80% contracting the disease before the age of 3 years. *M. catarrhalis* also contributes to a range of respiratory tract infections in adults and exacerbates serious conditions such as chronic obstructive pulmonary disease (COPD), a leading cause of mortality in the US.[2] Currently, there are no licensed *M. catarrhalis* vaccines available for prevention of otitis media.[3]

The major surface lipid of Gram-negative bacteria is lipopolysaccharide. *M. catarrhalis* lacks the O-antigenic repeating component of lipopolysaccharide and therefore its bacterial outer membrane is covered in lipooligosaccharide (LOS), containing core oligosaccharide and lipid A components. Three major serotypes of *M. catarrhalis* have been identified—A, B and C.[4] As shown in Figure 1, it is the heterogeneity in the central β-D-Glc-(1,4)-linked chain of the core oligosaccharide that determines the specific serotype. Details of the composition and biosynthesis of the core oligosaccharide structures of the serotypes have been studied.[5]

Previous studies into the utility of LOS as a vaccine candidate have demonstrated that exposure to *M. catarrhalis* LOS results in the production of LOS-specific antibodies.[6] Several studies using serotype-specific LOS derivatives have shown serotype specific protection on subsequent bacterial challenge.[7] These studies highlight the requirement to include LOS from all serotypes to provide effective protection against all serotypes of *M. catarrhalis*.

A major limitation of including full length oligosaccharide in a potential vaccine candidate is that a conserved region of the LOS structure in all serotypes (see Fig. 1) is identical to the P^K antigen found on human erythrocyte cells.[8] If included in a vaccine, this structure could potentially stimulate an auto immune response. Excluding the structure of the P^K antigen reveals a less specific conserved structure common to all *M. catarrhalis* LOS serotypes A, B and C highlighted in Figure 1.

In an attempt to synthesise a truncated OS common to all *M. catarrhalis* serotypes but excluding the structure of the P^K antigen that could be linked to an antigenic protein as a vaccine candidate, we embarked on the synthesis of the novel pentasaccharide **1** and the tetrasaccharide **2**,[9] as shown in Scheme 1. While a synthesis of **2** has been previously reported, we employed a different approach in part for the synthesis of **2**, to give us ready access to both **2** and the novel target compound **1** (see Fig. 2). In addition, full NMR assignment of **2** is provided. The design of **1** and **2** includes [*p*-trifluoroacetamido)phenyl]ethyl linker moiety, which Ekelöf and Oscarson[9] have also employed previously, that facilitates the convenient attachment of an antigenic protein. Herein the synthesis and full NMR characterisation of **1** and **2** are presented. It is expected that when linked to a carrier protein such as diphtheria toxin, **1** will elicit an immune response that protects mice against

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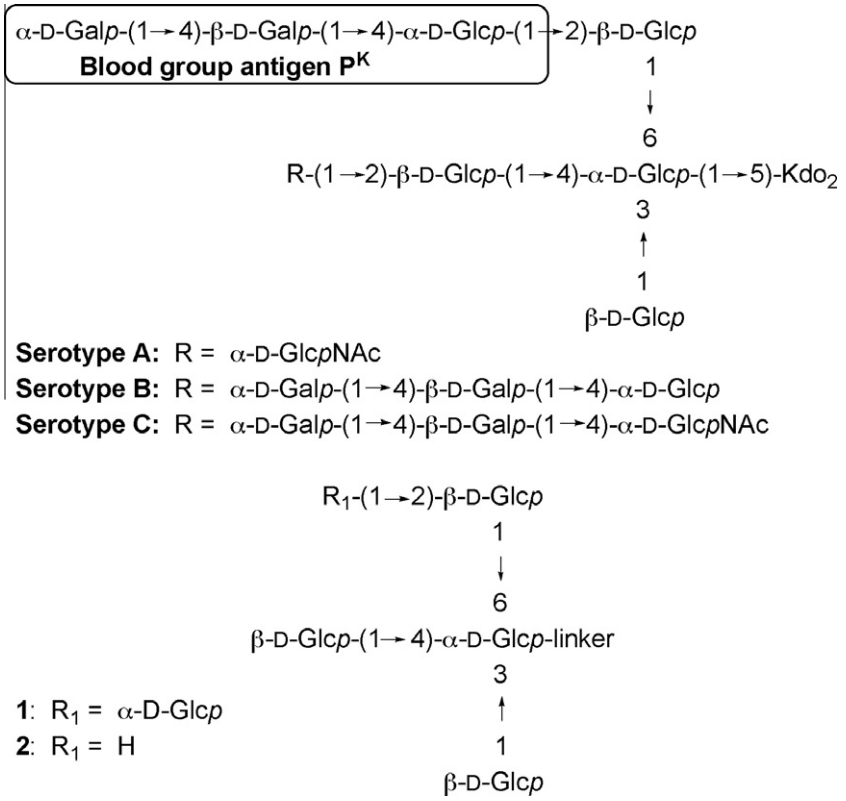

Figure 1. *Moraxella catarrhalis* serotypes A, B and C oligosaccharide structures (top), along with a conserved-oligosaccharide core minus the terminal α-D-Gal*p*-(1,4)-β-D-Gal*p* of the P^K antigen (bottom).

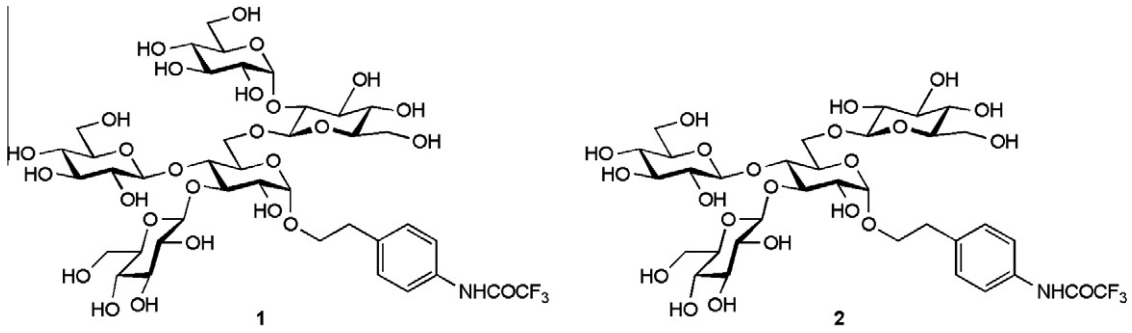

Figure 2. The structure of the pentasaccharide-linker (**1**) and tetrasaccharide-linker (**2**).

all serotypes of *M. catarrhalis*. Although **2** is a reduced common core in relation to **1**, it also could be a component of a potential vaccine candidate.

The chosen route for the synthesis of compound **1** is shown in Scheme 1. The known trisaccharide derivative **3**,[10] which contained the α-[*p*-(trifluoroacetamido)phenyl]ethyl glycosidic linkage, was chosen as the key intermediate and was synthesised through 11 steps. The silyl protecting group TBDMS was removed from **3** to afford the novel trisaccharide **4** in 75% yield. Trisaccharide **4** then underwent a silver-triflate-promoted coupling in dichloromethane with the chloroacetyl glucose derivative **5**[10] (synthesised in 7 steps from D-glucose) which had first been converted to the bromosugar, to afford the novel tetrasaccharide **6** in 82% yield. The chloroacetyl group installed to direct β-glycosylation was removed from **6** by treatment with thiourea and $NaHCO_3$ in methanol to afford **7** in 72% yield. The introduction of the benzylated glucosyl bromide donor **8**[11] was achieved by silver triflate-promoted glycosidation in diethyl ether. Conditions were optimised to obtain the α-glycoside and afford the fully protected novel pentasaccharide **9** in 53% yield. Full NMR assignment of **6**, **7** and **9** including labelling of the sugar residues is provided in Table 1.

Protected tetrasaccharide **7** and pentasaccharide **9** were then deprotected by treatment with methoxide, followed by hydrogenolysis to afford deprotected compounds **2** and **1**, respectively. For **9**, the initial methoxide treatment failed to remove the benzoate, and the subsequent hydrogenolysis was slow to remove the benzyl ethers (4 days). However, upon successful hydrogenolysis, the single benzoate was easily removed by standard methoxide treatment.

Full NMR assignment of **1** and **2** including labelling of the sugar residues is provided in Table 2. Characterisation of **2** was achieved by NMR analysis. Examination of the ^1H 1D spectrum revealed only three anomeric signals at 4.88, 4.56 and 4.41 ppm. The signals at 4.56 and 4.41 ppm were due to β-Glc residues with $^3J_{H1H2}$ of 7.86