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A new route for the synthesis of *Streptococcus pneumoniae* 19F and 19A capsular polysaccharide fragments avoiding the β-mannosamine glycosylation step ☆

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Dedicated to Professor Hans Kamerling on the occasion of his 65th birthday

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ABSTRACT

The recently described [Attolino, E.; Bonaccorsi, F.; Catelani, G.; D'Andrea, F. *Carbohydr. Res.* **2008**, 343, 2545–2556.] β-D-MaNAc*p*-(1→4)-β-D-Glc*p* thiophenyl glycosyl donor **3** was used in α-glycosylation reactions of OH-2 and OH-3 of the suitably protected *p*-MeO-benzyl α-L-rhamnopyranoside acceptors **7** and **8**. Glycosylation of the axial OH-2 of **7** took place in high yield (76%) and with acceptable stereoselectivity (α/β = 3.4) leading to the protected trisaccharide α-**11**, corresponding to the repeating unit of *Streptococcus pneumoniae* 19F. The same reaction on equatorial OH-3 of acceptor **8** gave the trisaccharide α-**15**, a constituent of the repeating unit of *S. pneumoniae* 19A, but in lower yield (41%) and without stereoselection (α/β = 1:1.3). Utilizing the introduced orthogonal protection of OH-1 and OH-4″, the trisaccharide α-**11** was transformed into a trisaccharide building block suitable for the synthesis of its phosphorylated oligomers.

1. Introduction

Encapsulated bacteria present an external carbohydrate coat for protection against the host's immune system and osmotic lysis, that is, in the pathogenic strains, responsible for their virulence. The capsular polysaccharide (CPS) structure defines the serotype, and since the discovery that CPS fragments induce a response of the host's adaptive immune system, research has been focused on developing CPS-based vaccines as an alternative to antibiotic therapy.[2] The first attempts to create such vaccines were made by administration of CPS fragments obtained by purification after controlled lysis of the capsule, and there are still commercial multivalent vaccines produced by this method. More recently, there has been a development of most efficient glycoconjugate vaccines, where the CPS is conjugated to a carrier protein to allow a T-cell-dependent immune response. Also, large efforts have been made to develop synthetic vaccines portrayed by well-defined molecular structures and the complete absence of biological contaminants.[3,4] *Streptococcus pneumoniae* serotypes 19F (*SP* 19F) and 19A (*SP* 19A) are responsible for a large number of infections of the upper respiratory system and meningitis, especially in children and immunodeficient subjects. These infections are associated with a large number of deaths (1.2 million/year just in developing countries). The repeating units[5] of *SP* 19F and *SP* 19A CPS (Fig. 1) are both made up of a trisaccharide containing an *N*-acetyl-D-mannosamine unit (A) linked through a β-1→4 bond to a D-glucose (B) residue that is linked to an L-rhamnose unit (C) through an α-1→2 (*SP* 19F) or an α-1→3 bond (*SP* 19A). The repeating units are linked to each other via an α-1→4 phosphodiester bridge (Fig. 1).

Since the elucidation of these structures, chemists have been involved with their synthesis, especially with that of *SP* 19F. The reported synthetic approaches involve, in all cases, two different glycosidation reactions, and can be classified into two groups: the first is based on the initial synthesis of the A–B fragment and the successive coupling with the unit C,[6] while the second approach involves the coupling of unit A with the B–C fragment.[7] The most challenging task is the introduction of the *N*-acetyl-β-D-mannosamine linkage, because of the difficulties in stereoselective formation of β-D-mannosamine glycopyranosides by direct glycosidation with D-mannosamine donors.[8] The strategy most employed is based on the initial formation of a β-D-glucopyranoside residue, followed by its transformation into

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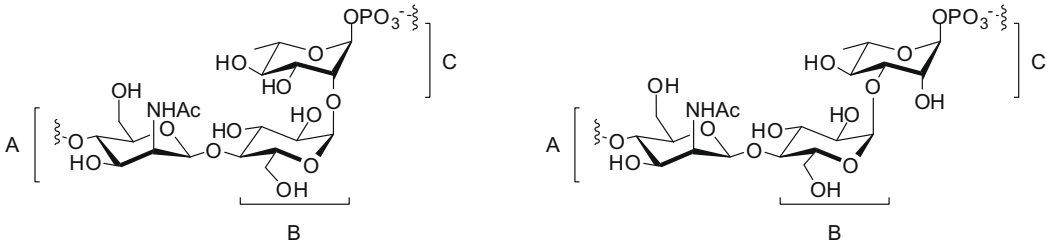


Figure 1. *SP* 19F (**1**) and *SP* 19A (**2**) CPS repeating units.

a β-D-mannosamine moiety by amination with inversion of configuration at C-2′ through an oxidation–oximation, followed by reduction of the oximino derivative[6a] or through an S_N2 displacement with sodium azide on a 2-*O*-sulfonyl intermediate followed by reduction.[7b,6d] Other methods described are direct glycosidations with 2-azido-2-deoxy-D-mannopyranose donors, either the glycosyl bromide activated by silver silicate[7a,6b] or with a C-2 oximino glycosyl donor, followed by stereoselective reduction.[6c] Still, most of these methods suffer from problems related to low reaction yields and stereoselectivity reducing the efficiency of the syntheses.

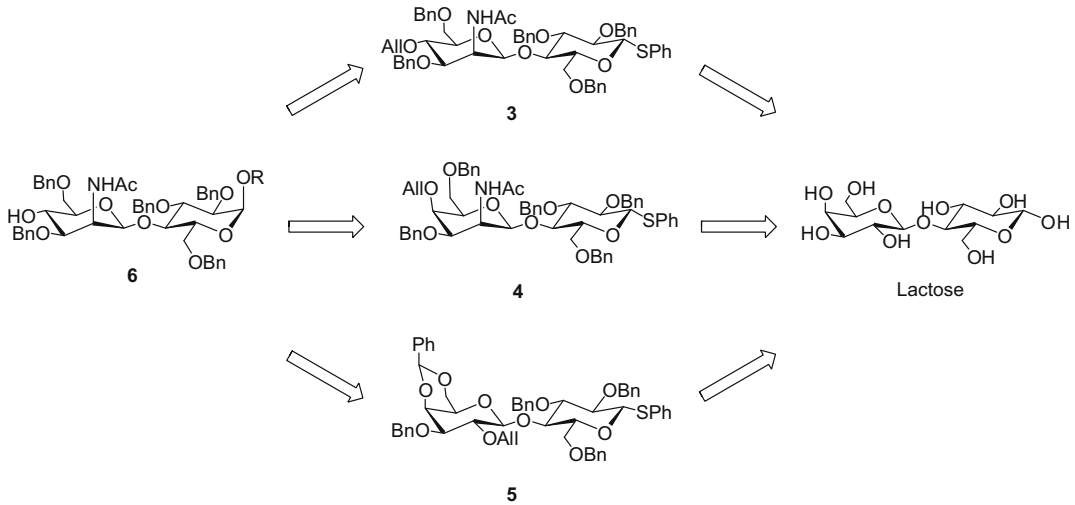
A recently reported method[9] for the synthesis of β-D-mannosaminosides and β-D-mannosides is based on the completely stereoselective elaboration in positions 2 (amination with inversion) and 4 (epimerization) of β-D-galactopyranosides. This suggested the possibility for obtaining α-glycosides of the β-D-ManNAc*p*-(1→4)-D-Glc*p* of type **6** from lactose by converting its nonreducing end into the *N*-acetyl-D-mannosamine moiety. To this end a systematic investigation[10] (Scheme 1) has been performed on the glycosidation properties of three different disaccharide thiophenyl glycosyl donors obtained from lactose, each carrying at the nonreducing end a D-mannosamine (**3**), a D-talosamine (**4**) or a D-galactopyranose (**5**) unit, with a simple alcoholic acceptor.

Using NIS/TfOH or MeOTf as activators, donor **3** gave no glycoside product, whereas donor **4** afforded the desired glycosides but only in a low yield and without any α-stereoselectivity. The best results were obtained with donor **5**. As a continuation of these results, we herein present an investigation for obtaining the trisaccharides of the repeating units of *SP* 19 F and 19A from lactose avoiding the β-mannosaminylation step.

2. Results and discussion

Because of the poor donor properties experienced with compounds **3** and **4**,[10] we initially thought that the best way to obtain the CPS trisaccharide repeating units of *SP* 19F and 19A was to glycosidate acceptors **7**[11] and **8**[12] with donor **5** and subsequently convert the D-galactopyranoside units in the trisaccharides so obtained into D-mannosamine residues (Scheme 2).

Thus, acceptors **7** and **8** were coupled with donor **5** using MeOTf[13] (5 equiv) as promoter in 4:1 CH_2Cl_2–Et_2O leading to anomeric mixtures of **9** (75%, α/β 2:1) and **10** (78%, α/β 1.7:1), respectively (Scheme 2). In both cases, the mixtures obtained were easily separated by chromatographic means, affording pure samples of α-**9** and α-**10**, in 50% and 49% isolated yields, respectively. Successful application of the sequence previously optimized for the analogous isopropyl α-disaccharide[10] to the 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl unit of α-**9** and α-**10** would afford the protected trisaccharide repeating units of *SP* 19F and *SP* 19A CPS. However, realizing the rather long sequence required for the above procedure (20% overall yield over 12 steps in the reported case[10]), we reconsidered the possibility of employing the mannosamine disaccharide **3** as a donor to glycosylate the two rhamnoside acceptors **7** and **8**. Taking into account the completely negative results obtained in the preliminary study using NIS–TfOH or MeOTf as promoter,[10] we decided to explore the glycosidation properties of **3** with other activating systems. Hence, glycosidation reactions between donor **3** and rhamnoside acceptor **7** (Scheme 3) were carried out with the most widely used activating systems of thioglycoside donors,[14] but, as in the case of the previously tried NIS–TfOH and MeOTf, also NIS–TMSOTf, PhIO–TMSOTf, MeOTf–



Scheme 1. Complementary approaches to β-D-ManNAcp-(1→4)-α-D-Glcp glycosides from lactose.