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Synthesis of *Haemophilus influenzae* carbohydrate surface antigens

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Abstract

The pathogenic bacteria *Haemophilus influenzae*, causing, i.a., meningitis and otitis, contain both capsular and lipopolysaccharide surface antigens. The syntheses of several oligosaccharides corresponding to native *H. influenzae* polysaccharide structures is outlined with an emphasis on synthetically challenging features. Hence, the synthesis of a branched inner core lipopolysaccharide tetrasaccharide structure, α-L,D-Hep*p*-(1∅3)-[β-D-Glc*p*-(1∅4)]-α-L,D-Hep*p*-(1∅5)-αKdo, containing the unusual higher carbon sugars L-*glycero*-D-*manno*-heptose and Kdo is described, as well as the assembly of di- and trimers of the repeating unit of the capsular polysaccharides of serotype c,[−4)-3-OAc-β-D-Glc*p*NAc-(1∅3)-α-D-Gal*p*-(1-PO_3^-−] and serotype f[−3)-β-D-Gal*p*NAc-(1∅4)-3-OAc-α-D-Gal*p*NAc-(1-PO_3^-], both linked via anomeric phospodiester linkages. Also efforts towards the synthesis of the repeating unit of the capsular polysaccharide of serotype e,∅3)-β-D-Glc*pNAc*-(1∅4)-[β-D-Fru*f*-(2∅3)]-β-D-Man*p*NAcA-(1∅, containing a β-fructofuranosidic residue, is discussed. All synthetic derivates are spacer-equipped to allow formation of glycoconjugates for biological applications. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Capsular polysaccharide; Lipopolysaccharide; *Haemophilus influenzae*

1. Introduction

The surface of bacteria is generally covered by carbohydrate structures. Capsular polysaccharides (CPSs) surround many bacteria and if it is a Gram-negative bacterium there are always lipopolysaccharides (LPSs) anchored in the outer membrane (Kenne & Lindberg, 1983). Because of their abundance and surface position the polysaccharides are important bacterial antigens. Furthermore, since most structures are bacteria specific, they are used routinely as a base for the serotyping of bacteria and might also be used to construct effective (and specific) vaccines against the bacterium. Especially efficient vaccines are obtained after the conjugation of the carbohydrate structures to protein carriers, so-called glycoconjugate vaccines. Commercial glycoconjugate vaccines against *Haemophilus influenzae* type b, causing, i.a. meningitis, especially in small children, have been available for almost a decade and have more or less wiped out the disease in vaccinated populations (Garpenholt et al., 1996; Lindberg & Pillai, 1996; Peeters et al., 1996).

Well-defined synthetic oligosaccharides and glycoconjugates corresponding to the native bacteria structures are helpful and often necessary tools for more detailed investigations of the immunological response against the polysaccharides. The synthetic derivatives can be employed, i.a. to map immunodominant motifs, to determine the specificity of the antibody response obtained from native structures, to investigate the processing of glycoproteins by the immune system, and as vaccine candidates when native substances cannot be utilized.

Haemophilus influenzae is a Gram-negative bacterium, which is divided into six different serogroups a–f, all corresponding to a different CPS structure (Kenne & Lindberg, 1983). In this article, we review our efforts to synthesize structures from the *H. influenzae* types c, d, e, and f CPSs as well as structures from the LPS of *H. influenzae*. The emphasis will be on specific synthetic features, the formation of 3,4-branched heptose structures in the LPS core, the construction of the phosphodiester linkages in the types c and f CPSs, and the introduction of the β-fructofuranosyl residue found in the type e CPS.

2. Synthesis of branched LPS core structures via a 1,6-anhydroheptose intermediate

The LPS of *H. influenzae* is truncated (it lacks the O-antigen part) and shows considerable heterogeneity. Due to this, analysis of the structures has been most difficult, but during recent years a number of papers on suggested structures have been published (e.g. Phillips, Apicella, Griffiss & Gibson, 1993, 1996; Risberg, Schweda & Jansson,

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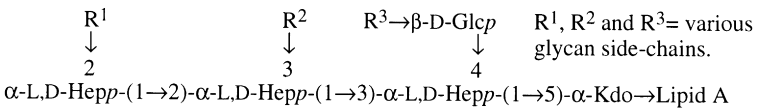
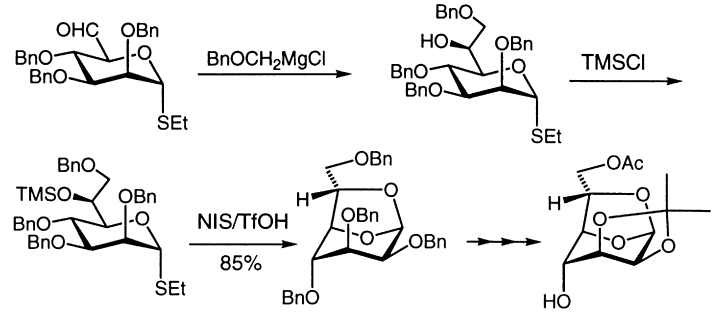
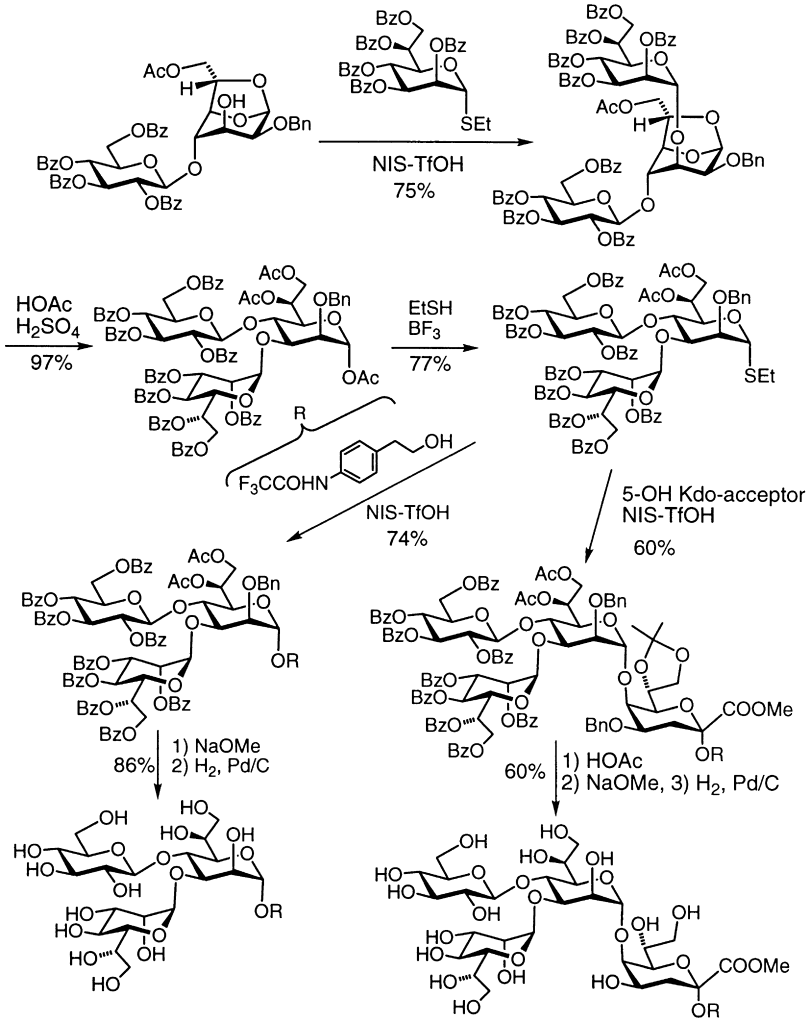


Fig. 1. Generalized structure of the dephosphorylated LPS of *H. influenzae* without the Lipid A moiety



Scheme 1.



Scheme 2.

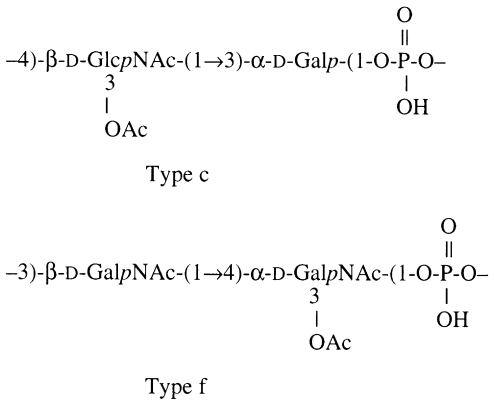


Fig. 2. The repeating units of *H. influenzae* types c and f CPSs.

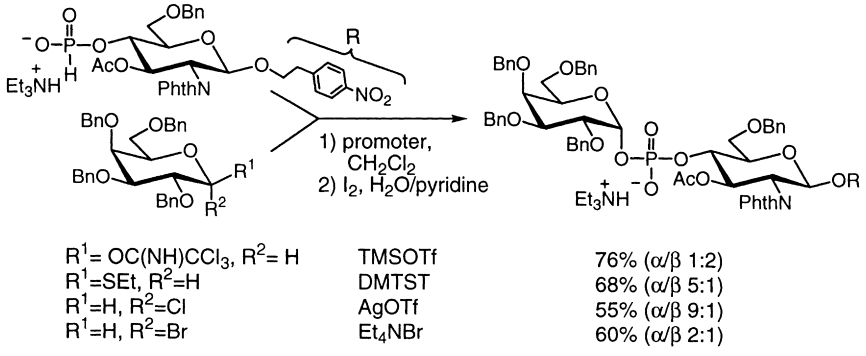
1997; Schweda et al., 1993). A schematic structure is shown in Fig. 1.

Because of the heterogeneity, native LPS material as such is not suitable for use as vaccines and synthetic structures is an alternative, if available. A number of linear structures were synthesized (Bernlind & Oscarson, 1996), i.a. the triheptoside core, but problems were encountered when trying to construct the 3,4-branched heptose structure. Irrespective of whether the glucosyl moiety or the heptosyl moiety was first introduced, 3,4-branched structures could not be obtained (Bernlind, 1998; Oscarson, 1997). Since this probably was because of steric crowding between the equatorial 3- and 4-substituents, we reasoned that a change of ring conformation of the central heptose might solve the problem. Therefore a 1,6-anhydro heptose derivative was synthesized (Scheme 1), suitably protected to allow glycosylation at OH-4 and (later) OH-3, which now were both axial and thus far apart.

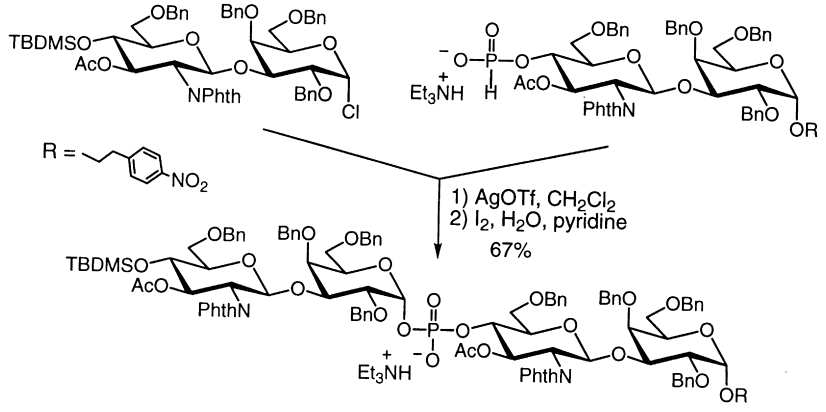
Introduction of the glycosyl moiety at OH-4 followed by protecting group manipulation afforded a 3-OH acceptor, which in a coupling with a heptosyl donor now smoothly produced the 3,4-branched trisaccharide (Scheme 2). Acetolysis of the anhydro bridge and subsequent transformation of the anomeric acetate into a thioglycoside yielded an effective trisaccharide donor, as proven by successful couplings to a spacer and a known unreactive 5-OH Kdo-derivate. Deprotection then finally gave the desired 3,4-branched tri- and tetrasaccharides present in the LPS core of *H. influenzae* and also of various other bacteria, e.g. *Neisseria menigitidis* (Bernlind & Oscarson, 1998).

3. Formation of the phosphodiester linkages in serotype c and f

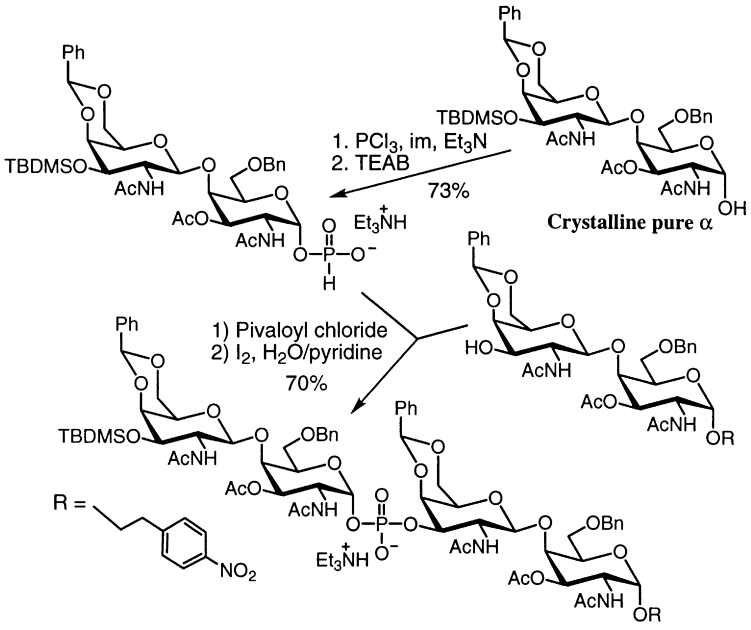
The structures of the types c and f CPSs are shown in Fig. 2



Scheme 3.



Scheme 4.



Scheme 5.

(Kenne & Lindberg, 1983). As in serogroups a and b the repeating units are connected by a phosphodiester linkage, but, in contrast to types a and b, the phosphodiester bridge in types c and f is anomeric, which complicates the synthesis, since anomeric phosphates are more unstable and also have to be constructed in a stereospecific manner.

Early model studies using an anomeric α-galactosyl H-phosphonate monoester, a 4-OH GlcPhth acceptor and pivaloyl chloride as coupling reagent, in an attempt to construct the type c structure, surprisingly gave no phosphodiester products at all (Helland, 1991). To overcome this problem a new concept in the formation of anomeric phosphodiesters from H-phosphonates was tried. The H-phosphonate monoester moiety was instead introduced into the GlcPhth acceptor and used as a nucleophile in a glycosylation reaction with galactosyl donors (Scheme 3). Using this approach the phosphodiester bridge was efficiently formed, however, as an α/β-mixture. Various donors and promoters could be employed all giving comparable yields, but differing in α/β-ratio. Also the type f disaccharide structure was effectuated using this approach (Garegg, Hansson, Helland & Oscarson, 1999).

With these model studies as a base, the synthesis of dimer (tetrasaccharide) structures was then attempted. Selecting the optimum conditions, with regard to the α/β-ratio, from the model studies, a chloro sugar donor and silver triflate as promoter was used in the type c synthesis to give exclusively the desired α-linked product in a 67% yield (Scheme 4) (Hansson, 1998). However, in the type f synthesis, a thioglycoside donor and dimethyl(methylthio)sulfonium triflate (DMTST) as promoter resulted in a 72% product yield, but with a disappointing α/β-ratio of 1:1.

Therefore, a return to the earlier pathway with anomeric H-phosphonate monoesters was made in the type f synthesis. The anomerically pure disaccharide H-phosphonate could be obtained easily, since luckily the α-hydroxyl precursor crystallized out in a pure form from an anomeric mixture (Scheme 5). In contrast to type c, the structure of type f could be constructed conveniently by this approach, resulting in a dimeric tetrasaccharide structure in 70% yield. Both the types c and f dimers are designed to allow further elongation to trimers and higher oligomers, using the same elongating monomer, after the removal of the temporary silyl protecting group. Alternatively the dimers have been deprotected for use in biological studies.

4. Introduction of the β-fructofuranosyl moiety in type e

The structures of *H. influenzae* CPSs types d and e are shown in Fig. 3 (Kenne & Lindberg, 1983). They both have a disaccharide repeating unit built up by the same two monosaccharides, β-D-GlcNAc and the synthetically challenging β-D-ManNAcA, but differ in their linkage positions. We have earlier synthesized the two repeating unit

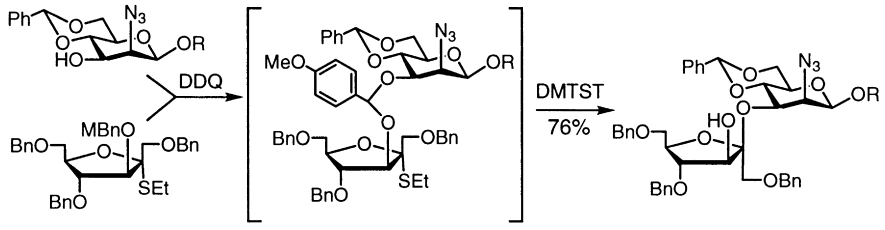
→4)-β-D-Glc*pNAc*-(1→3)-β-D-Man*p*NAcA-(1→

Type d

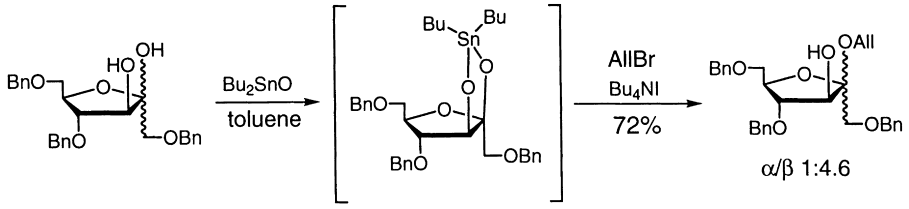
→3)-β-D-Glc*pNAc*-(1→4)-β-D-Man*p*NAcA-(1→
3
|
β-D-Fru*f*-2

Type e

Fig. 3. The repeating units of *H. influenzae* types d and e CPSs.



Scheme 6.



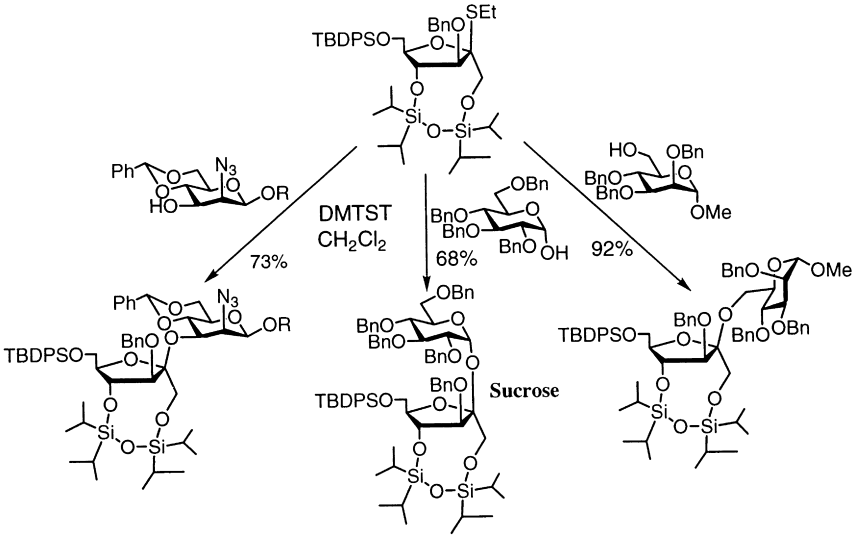
Scheme 7.

disaccharides as spacer derivatives (Classon, Garegg, Oscarson & Tidĕn, 1991; Garegg, Oscarson & Tidĕn , 1992), where the β-D-ManNAcA motif was manufactured by a displacement reaction with sodium azide on a β-D-glucopyranoside 2-O-triflate derivative followed by a pyridinium dichromate (PDC) oxidation of the primary 6-OH group later in the synthesis. However, some strains of type e also contain a β-D-fructofuranosyl moiety linked to the 3-position of the ManNAcA unit. In order to introduce this residue into the target structure, new methodology had to be invented, since there were no synthetic methods to construct the β-linked fructofuranosides available.

The first successful attempt was performed using the internal acceptor delivery approach known to work well in pyranosidic systems, i.e. in the formation of β-mannosides (Barresi & Hindsgaul, 1994; Ito & Ogawa, 1994; Stork & Kim, 1992). A thiofructofuranoside with a 3-O-*p*-methoxybenzyl group was oxidized by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of an acceptor to give the intermediate mixed acetal, which was treated with DMTST (Scheme 6). The activation of the thioglycoside with concomitant cleavage of the acetal allowed the efficient and stereospecific internal delivery of the acceptor to the anomeric site to give the β-linked disaccharide in 76% yield, proving the concept also in furanosidic systems (Krog-Jensen & Oscarson, 1996, 1998).

Although the formation of the β-linked fructofuranoside was successful, problems were encountered both in the later formation of the branched trisaccharide target structure and if the acceptor in the internal delivery coupling was of a more complex or labile type (Krog-Jensen, 1997), and the search for other β-directing fructofuranosyl donors was continued.

Anomeric 1,2-stannylidene acetals have worked well as



Scheme 8.

nucleophiles in displacement reactions with halides and triflates as electrophiles to give β-linked manno- and rhamnopyranosides in good yields (Hodosi & Kovãc, 1997). When this approach was tried on fructofuranosides, the result was fairly good with reactive electrophiles, e.g. allyl bromide (Scheme 7), but failed when monosaccharide halides or triflates were used.

Then, another idea including the construction of an internal (cleavable) bridge between the 4- and the 1-OH groups in fructose was considered; this would lock the C1–C2 bond in the α-position, and, thus, inevitably force any incoming acceptor to the β-side of the anomeric position. A thioglycoside donor was constructed according to this idea, with a tetraisopropyldisiloxane (TIPS) acetal as the internal bridge (Scheme 8). Coupling of this donor with various monosaccharide acceptors using DMTST as promotor gave efficiently and anomerically pure β-linked fructofuranosides; i.a. sucrose was for the first time stereospecifically synthesized, a coupling that failed completely when using the internal delivery approach.

5. Conclusions

Together with the structures corresponding to types a and b CPSs earlier synthesized in our laboratory (Classon, Garegg & Lindh, 1988; Garegg, Johansson, Lindh & Samuelsson, 1986), we now have access to dimers of the repeating unit of types c and f as well as the repeating disaccharide units of types d and e, all as spacer derivatives allowing the formation of immunogenic glycoconjugates. Furthermore, we have the methodology and precursors to prepare oligomers of all the phosphodiester linked structures and also to introduce the β-fructofuranosyl residue and construct the complete trisaccharide repeating unit of type e. Presently, with all these synthetic derivatives in hand, a comprehensive immunological study of all the six serogroups of *H. influenzae* will be started in collaboration with biologists at the Karolinska Institute in Stockholm.

A number of synthetic structures from the core of the *H. influenzae* LPS, both linear and branched, are now available. These will be tested as vaccine candidates against bacteria lacking CPS (NTHi = Non-Typable *H. influenzae*), a major cause of otitis media. More complex structures, i.a. containing various phosphate groups, will be synthesized in the near future. The biological testing will be part of an international collaboration, including groups from Canada, Great Britain, France and Sweden.

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