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Synthesis of the trisaccharide outer core fragment of *Burkholderia cepacia* pv. *vietnamiensis* lipooligosaccharide

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ABSTRACT

The synthesis of β-Gal-(1→3)-α-GalNAc-(1→3)-β-GalNAc allyl trisaccharide as the outer core fragment of *Burkholderia cepacia* pv. *vietnamiensis* lipooligosaccharide was accomplished through a concise, optimized, multi-step synthesis, having as key steps three glycosylations, that were in-depth studied performing them under several conditions. The target trisaccharide was designed with an allyl aglycone in order to open a future access to the conjugation with an immunogenic protein *en route* to the development of a synthetic neoglycoconjugate vaccine against this *Burkholderia* pathogen.

1. Introduction

Burkholderia cepacia complex (*Bcc*) is a group of closely related Gram-negative bacteria, that comprises at least fifteen species. Originally it was identified as a plant pathogen in the 1950s, when it was recognized as the causative agent of soft onion rot.[1] Its discovery as an opportunistic pathogen in patients with cystic fibrosis (CF) occurred in the 1980s.[2] CF patients colonized with *Bcc* experience a more rapid decline than those colonized with the more commonly acquired pathogen *Pseudomonas aeruginosa*.[3] Actually, the infection by *Bcc* determine, in approximately 20% of CF patients infected with *Bcc*, the 'cepacia syndrome', characterized by fever, pneumonia and bacteraemia.[4] In addition, *Bcc* is inherently resistant to antibiotic treatment,[5] to antimicrobial peptides[6] and increased resistance is observed on formation of *Bcc* biofilms in vitro. Overall, infection with *Bcc* in CF patients correlates with poorer prognosis, longer hospital stays and an increased risk of death. Another typical feature of the *Bcc* strains is their ability to spread among patients. When the use of antibiotics in chronic infections determine the selection of multiple-antibiotic-resistant strains, as usually occurs in CF patients, the development of convenient vaccines represents a desirable resource to prevent infection and for the necessary therapeutic approach. This problem is common to all bacterial infections, but for *Bcc* strains it is more serious due to their inherently acquired resistance to antibiotic treatment.

Among the many types of vaccines, extensive use of polysaccharide carbohydrates has been made against several human pathogens, such as *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae*. An improvement was achieved by covalently coupling the polysaccharide to carrier proteins, thus generating a long-lasting immunity, and making the vaccine effective even on infants and immunocompromised individuals. In the last decade, a higher level of sophistication was brought with the preparation of synthetic neoglycoconjugate vaccines (or vaccine candidates), where a haptenic oligosaccharide epitope is linked by a spacer to an immunogenic protein.[7] The sources of oligosaccharides for vaccines usually arise from bacterial surface carbohydrates as capsular polysaccharides or lipopolysaccharides (LPSs). LPSs are the major component of the outer leaflet of almost all Gram-negative bacteria and are one of virulence factors of pathogenic bacteria. They usually consist of a polysaccharide region, named O-chain, that is linked to an oligosaccharide part—termed core and usually divided into an outer and an inner core—in turn bonded to a glycolipid region, lipid A, which is anchored to the lipid external membrane of bacteria and represents the toxic part of LPSs.[8] The O-chain polysaccharide, also known as O-antigen, is responsible for the serotype classification of the strains. The repeating unit of capsular polysaccharides as well as of the O-chain region of LPSs is usually taken as oligosaccharide hapten for the construction of a vaccine candidate. In the case of *P. aeruginosa* an in-depth investigated glycoconjugate vaccine candidate was

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built as the whole O-chain conjugated to a toxin, showing significant results also on humans.[9] Furthermore, the synthesis of the O-chain repeating unit of the LPS of a *B. cepacia* strain isolated from CF patients *en route* to a potential synthetic neoglycoconjugate vaccine was recently reported.[10]

Some bacteria present no O-chain and the outer core region results as the most external saccharide portion of such lipooligosaccharides (LOSs) on the bacterial surface. It is usually thought that the core can play some roles in place of the O-chain in LOSs and in particular the outer moiety of core can be the source of hapten oligosaccharides. Indeed, the synthesis of some oligosaccharides arising from the outer core of *P. aeruginosa* LOSs has been performed for the future preparation of potential vaccines.[11] Furthermore, the synthesis of some neoglycoconjugates containing *Bcc* inner core epitopes was very recently accomplished.[12] In this work it is reported for the first time a concise, high-yielding synthesis of an outer core fragment of LOS from *Burkholderia cepacia* pv. *vietnamiensis*—the third most prevalent species of the *Bcc* found among CF patients—[13] in order to study its antigenic properties *en route* to the development of a synthetic neoglycoconjugate vaccine candidate.

2. Results and discussion

Full structural characterization of *B. vietnamiensis* LOS was very recently reported (Fig 1).[14] The inner core part showed typical heptose and oct-2-ulosonic acid (Kdo and Ko) constituents, whereas the outer region was constituted of GalNAc and Gal residues.

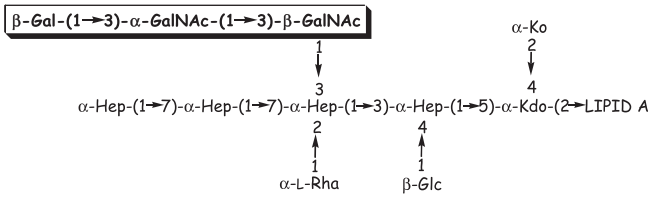
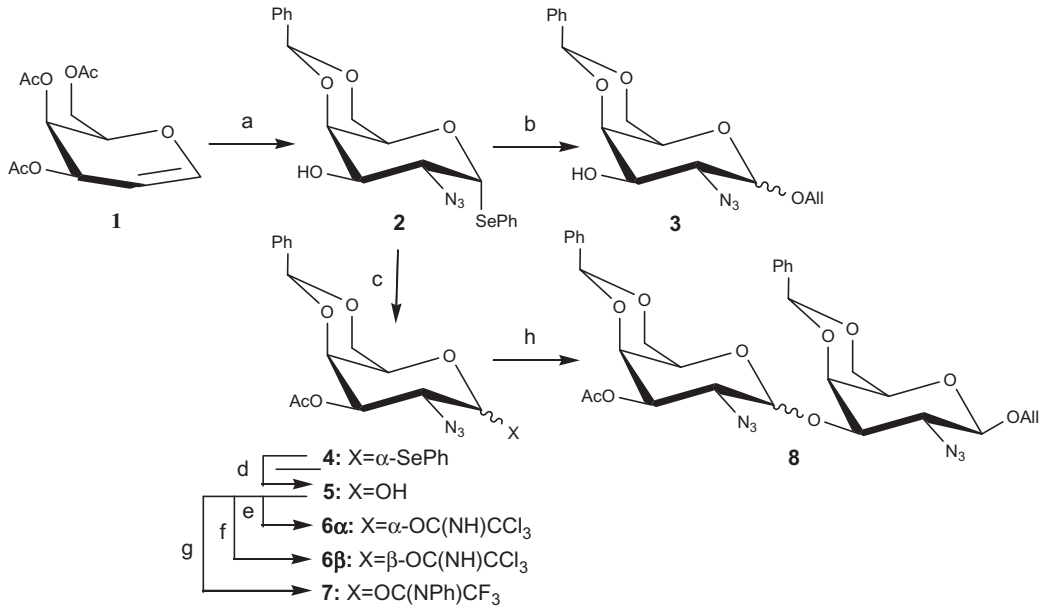


Figure 1

Figure 1. Structure of the core part of LOS from *B. vietnamensis*.

The trisaccharide target of this synthetic work (highlighted in Fig 1) presented two GalNAc units with different stereochemistry at the anomeric position. In order to set up a concise and high-yielding synthetic strategy, in the retrosynthetic analysis we focused on a GalN glycosyl donor, that could be glycosylated with either α- or β-stereoselectivity by a suitable change of reaction conditions. 2-Azido-2-deoxy-GalN[15] and 2-deoxy-2,3-oxazolidinone-GalN donors[16] have been reported in both α- and β-stereoselective glycosylations. Several protocols are known for the straightforward synthesis of 2-azido-2-deoxy-GalN donors from commercially available tri-*O*-acetyl-galactal.[17] We selected **2**[18] as key compound for the synthesis of GalN donor and acceptor. It was obtained from tri-*O*-acetyl-galactal **1** through homogeneous azidophenylselenylation, followed by de-*O*-acetylation and 4,6-benzylidenation (Scheme 1). The synthesis of GalN acceptor required the insertion of a β-linked allyl aglycon for future derivatizations (conjugation with an antigenic protein, coupling with inner core fragments, etc.). We screened several selenoglycoside activation conditions[19] in order to achieve a good yield together with a satisfying β-stereoselectivity (Table 1). The best result was obtained with NBS/$Bi(OTf)_3$ activation system[19a] affording **3β** in 57% yield (Table 1, entry 6). Acetylation of **2** afforded glycosyl donor **4** (99%), that unfortunately gave no coupling product when subjected to glycosylation with **3β** under NBS/$Bi(OTf)_3$ activation (Table 2, entries 1 and 2). Thus, a leaving group exchange at anomeric position was effected in two steps by hydrolysis of selenoglycoside **4** with NBS in aqueous THF and subsequent conversion of the obtained hemiacetal **5** (81%) into trihaloacetimidates **6α** (Cl_3CCN/DBU, 96%) and **7** ($CF_3C(NPh)Cl/Cs_2CO_3$, 79%). Glycosylation of **6α** and **3β** under TMSOTf catalysis in CH_2Cl_2 gave disaccharide **8** in 62% yield but almost no stereoselectivity (Table 2, entry 3) in spite of the α-stereoselectivity reported for Gal trichloroacetimidates with non-participating protecting groups at *O*-2 and *O*-3 and a benzylidene ring system at positions 4 and 6 hindering the attack of the acceptor from the β-face.[20] Attempts to enhance the α-stereoselectivity by conducting the reaction in ethereal solvents lowered the global yield of the glycosylation (entries 4 and 5).



Scheme 1. Synthesis of 2-azido-2-deoxy-GalN donors and acceptor. Reagents and conditions: (a) see Ref. 18; (b) see Table 1; (c) Ac_2O, py, rt, 99%; (d) NBS, 1:1 v/v THF–H_2O, rt, 81% (α/β = 2:1); (e) Cl_3CCN, DBU, CH_2Cl_2, rt, 96%; (f) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 60%; (g) $CF_3C(NPh)Cl$, Cs_2CO_3, acetone, rt, 79% (α/β = 1.4:1); (h) see Table 2.