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Synthesis of the repeating unit of the lipoteichoic acid of *Streptococcus pneumoniae*

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ABSTRACT

The lipoteichoic acid repeating unit of *Streptococcus pneumoniae* is a complex pseudopentasaccharide (**3**). It consists of one ribitol-phosphate, one 2-acetamino-4-amino-2,4,6-trideoxy-galactose, one glucose and two galactosamine residues each differently linked, but both carrying one phosphocholine substituent, at position 6. Suitable building blocks (**6**–**10**) for efficient and diastereocontrolled ligations were designed, thus providing, after complete deprotection, the target molecule in high purity. Biological tests revealed that repeating unit **3**, lacking the lipid moiety, did not stimulate a pro-inflammatory response in human monocytes (hMNCs).

1. Introduction

During the onset of bacterial infections, recognition of microbial cell wall constituents occurs via pattern recognition receptors (PRRs) of the innate immune system. The recognition of these molecules of microbial origin, the so called pathogen-associated molecular patterns (PAMPs), triggers signalling pathways that activate transcription of pro-inflammatory cytokines, which participate in the generation of a rapid but nevertheless specific immune response. The most important conserved PAMPs in Gram-negative bacteria are the lipopolysaccharides (LPS, endotoxin). They are found in the outer leaflet of the outer membrane in the Gram-negative bacterial cell wall. Their potency to activate pro-inflammatory reactions in cells of the myeloid lineage has been known for a long time as it is extremely high.[1,2]

The corresponding immunostimulatory component of Gram-positive bacteria was not clear for a long time. Yet, a structural counterpart to LPS called lipoteichoic acid (LTA) was found in the cell wall of Gram-positive bacteria. As LPS, LTA shares its amphiphilic nature consisting of a lipid anchor, a core oligosaccharide and the so called 'repeating unit', which is generally a negatively charged, hydrophilic glycerophosphate or ribitolphosphate residue, respectively.[3,4]

Streptococcus pneumoniae, one of the most common Gram-positive pathogens also causes severe infections like otidis media, sinusitis and others.[5–7] When reaching the lower respiratory tract or bloodstream, *S. pneumoniae* infections may result even in more life-threatening diseases like pneumonia, bacteraemia and meningitis.[5] The cell wall of *S. pneumoniae* consists of several layers of peptidoglycan covalently linked to teichoic acid, and of lipoteichoic acid, that is anchored in the cell membrane.[3,4,8] Structural analysis of pneumococcal LTA of the R6 strain (Scheme 1, **1**) revealed that it contains phosphodiester interlinked pseudopentasaccharide repeating units each carrying two phosphocholine residues (**3**) and a glycolipid core structure **2** comprising a trisaccharide linked to diacylglycerol.[9,10] This structural analysis was confirmed by our recent total synthesis of **1** with R=H, X=NH_3^+ and n=1;[11] also details of the synthesis of the core structure **2** were reported.[12] Biological studies with **1** and **2** showed that both compounds stimulate interleukin-8 (IL-8) release in human monocytes (hMNCs). Since this activity was not mediated via toll-like receptor 2 (TLR2), the investigation of the biological properties of the repeating unit **3** became of interest. Hence, the overall strategy and execution of the synthesis

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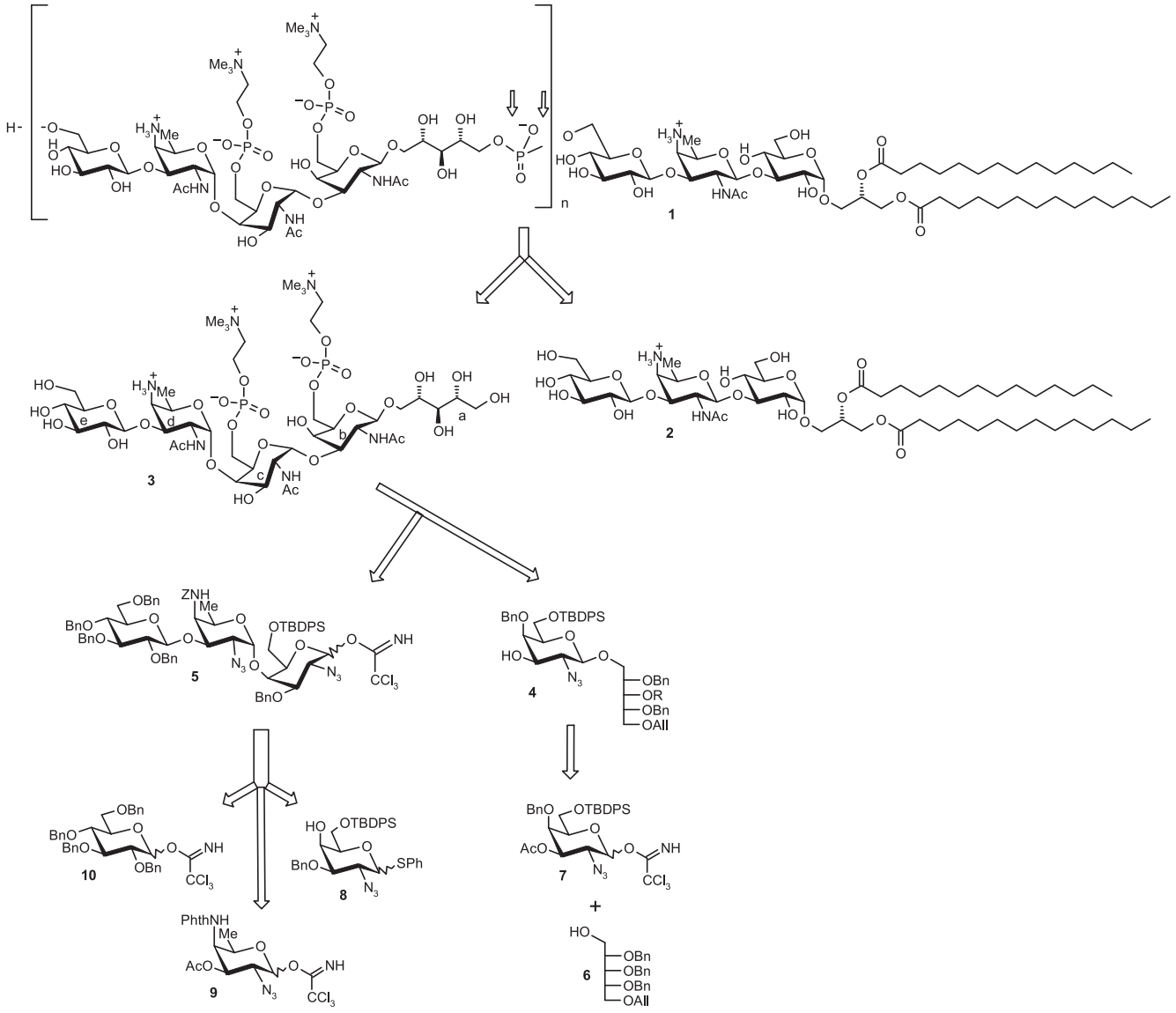

Scheme 1. Structure of the LTA of *S. pneumoniae* (**1**), the derived core structure **2** and the repeating unit **3**. A retrosynthetic scheme for the synthesis of **3**.

of **3** based on monosaccharide intermediates having different anomeric configurations as well as some biological results are reported in the present paper. Evidence is provided that, for biological activity of LTA in hMNCs, the lipid anchor is indispensable, whereas the pseudopentasaccharide **3** expresses no such pro-inflammatory activity.

2. Results and discussion

The retrosynthesis of pseudopentasaccharide **3** is displayed in Scheme 1. For a convergent synthesis strategy, disconnection between sugar residues b and c was chosen leading to pseudodisaccharide **4** and trisaccharide donor **5**. Hence, *tert*-butyldiphenylsilyl (TBDPS) groups at 6-*O* of sugar residues b and c were introduced for the regioselective attachment of the choline phosphate residues and the 5-*O*-allyl group at ribitol residue a was chosen for an eventual regioselective attachment of a phosphate residue as required for the total synthesis of **1**.[11] The introduction of the 2-acetylamino groups in sugar residues b, c and d is based on concomitant reduction of three azido groups and their subsequent N-acetylation. Thereafter the amino group in sugar residue d can be liberated by hydrogenolysis, thus also cleaving all other *O*-benzyl protecting groups. Hence, pseudodisaccharide **4** should be available from known ribitol derivative **6**[13] and 2-azidogalactosyl donor **7** and trisaccharide **5** from previously prepared glycosyl donors **9**[12] and **10**[14] and 4-*O*-unprotected 2-azido-galactosyl thioglycoside **8** as acceptor, that is, readily available from galactosamine (vide infra). After the assembly of building blocks **8**–**10**, transformation of the resulting trisaccharide into the corresponding trichloroacetimidate based glycosyl donor **5** will be performed.[14]

For the synthesis of galactose derived intermediates **7** and **8**, galactosamine was transformed into tetra-*O*-acetyl-2-azido derivative **11** following a reported procedure (Scheme 2).[15] Treatment with thiophenol in the presence of boron trifluouride ether complex afforded known phenyl thioglycoside **12**[16] as a 9:7 α/β-mixture. Removal of the *O*-acetyl groups with sodium methoxide in methanol and then treatment with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid (*p*-TsOH) furnished 4,6-*O*-benzylidene protected derivatives **13α**,β that could be readily separated. Subjecting the **13α,β** mixture to different reaction sequences transformed it into the required donor **7** and into the **8α,β** mixture, which was subsequently used to prepare