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Synthesis of *Staphylococcus aureus* lipoteichoic acid derivatives for determining the minimal structural requirements for cytokine induction

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Abstract—For the investigation of the minimal structural requirements for cytokine induction, *Staphylococcus aureus* lipoteichoic acid derivatives with two, three, four, and five glycerophosphate backbone moieties, carrying each a D-alanyl residue, were needed. Based on two different glycerophosphate building blocks and 6b-*O*-phosphitylated gentiobiosyl diacylglycerol the desired target molecules (compounds **1**–**4**) could be readily obtained and provided for biological studies.

Keywords: Bacteria; Gram-positive; Lipoteichoic acid; Synthesis; Modifications

1. Introduction

During infection, the recognition of conserved bacterial structures called pathogen-associated molecular patterns occurs via receptor recognition on immune cells and leads to the activation of the innate immune system resulting in the release of a variety of cytokines. Lipopolysaccharides (LPS) have been known as the most important conserved bacterial structures of Gram-negative bacteria inducing cytokine release for more than 50 years.[1] Immune recognition takes place by the binding of LPS to the toll-like receptor 4 (TLR4) involving also other co-factors.[2–4]

The immunostimulatory component of Gram-positive bacteria was not clear for a long time, although a structural counterpart of LPS called lipoteichoic acid (LTA) was found in the bacterial membrane; this LTA is also an amphiphilic molecule with a lipid anchor and a generally negatively charged glycerophosphate backbone. An improved preparation procedure applied to the isolation of LTA from *Staphylococcus aureus* led to biologically active LTA,[5–7] whose structure could be assigned by NMR and MS data.[5,8] The receptor for LTA recognition is TLR2[9] accompanied by the co-factors TLR6,[7] CD14,[10,11] and CD36.[12]

Modifications of the LTA structure gave information on the prerequisites for the induction of cytokine release. For instance, a complete deacylation led to less active material and selective removal of the D-alanyl residues from the glycerophosphate backbone which strongly reduced the immunostimulatory potency.[8] This result indicated that the lipid anchor and also the D-alanyl residues are essential for the immunostimulatory potency. The chemical synthesis of a truncated LTA, based on the native LTA structure of *S. aureus*, confirmed these results.[13,14] To determine the key components for immune cell activation, several LTA derivatives were synthesized starting from a molecule with two lipid anchors (=two gentiobiosyl-diacylglycerol anchors) and a backbone with six glycerophosphate units substituted

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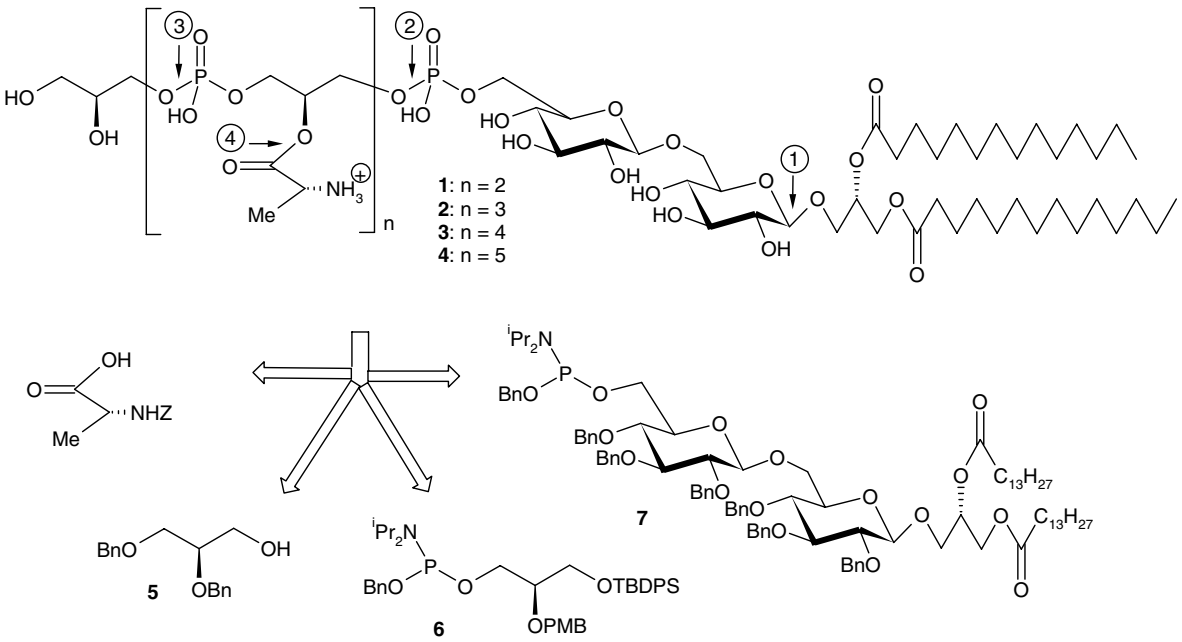

Scheme 1. Retrosynthetic scheme for target molecules **1**–**4**.

by four D-alanine residues and one *N*-acetyl-D-glucosamine residue which was more potent than natural LTA.[15] Neither the absence of the gentiobiose residue nor the loss of *N*-acetyl-D-glucosamine altered the ability of LTA with one lipid anchor to induce cytokine release; only replacement of D-alanine by L-alanine blunted the cytokine-inducing potency.[16,17] These results approximated the crucial pattern required for the immune recognition of LTA and prompted us to synthesize further LTA derivatives with a reduced structure down to the synthetic anchor, namely gentiobiosyl-diacylglycerol, in order to determine the minimal structural requirements for cytokine induction. For these studies compounds **1**–**4** (Scheme 1) were needed. The synthesis of these compounds is described in this paper.

2. Results and discussion

The retrosynthesis of compounds **1**–**4** is shown in Scheme 1. Disintegrations ①–④ lead to building blocks **5**–**7** and to *N*-benzyloxy-carbonyl (Z)-protected D-alanine. These building blocks consider the presence of gentiobiosyl diacylglycerol, *O*-(D-alanyl)-glycerol, and 2-*O*-nonsubstituted glycerol residues, respectively, and their sequence specific linkage via mixed phosphorous diester bonds. Also the most important aspect, the hydrolytic lability of the D-alanyl residues, which are readily cleaved at pH 8.5,[13] is taken into account: as temporary protecting group for building block **6**, the *p*-methoxylbenzyl (PMB) group is chosen which can be selectively cleaved after completion of the backbone synthesis. Following the attachment of D-alanyl residues with Z-protected D-alanine and then a complete O-debenzylation will provide the target molecules.

The synthesis of the previously designed building blocks **5**–**7** and also the sequence specific synthesis of oligomers **8**–**10** (Scheme 2) has been already reported.[14,15,17] From gentiobiose building block **7**[15] and glycerophosphate oligomer **8**,[17] possessing the required protecting group for regioselective chain extension and following D-alanyl residue attachment, the synthesis of target molecule **1** could be readily accomplished. Ligation of **7** and **8** in the presence of tetrazole and then oxidation with *tert*-butylhydroperoxide gave phosphate linked intermediate **13**, which contains the backbone of the target molecule with $n = 2$. The treatment of **13** with ceric(IV) ammonium nitrate (CAN)[18] liberated two of the glycerol hydroxy groups affording compound **14**. Attachment of the Z-protected D-alanyl residue was performed with excess Z-protected D-alanine in the presence of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)[19]/*N*-methylimidazole (Me–Im) as condensing agent to give the fully protected target molecule **15**. Hydrogenolysis with Pearlman's catalyst[20] in a mixture of dichloromethane/methanol/water gave the desired final product **1** with two D-alanyl residues.

Similarly, from **7** and **9**[17] or **10**[17] compounds **16** and **19**, respectively, were obtained, which were transformed into the partially deprotected compounds **18** and **21** and then into target molecules **2** and **3**, having three or four D-alanyl residues. For the synthesis of target molecule **4**, compound **10** had to be chain extended with building block **6**.[17] The activation of **6** with tetrazole led to the desired phosphite intermediate which on oxidation with