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Light fluorous synthesis of glucosylated glycerol teichoic acids

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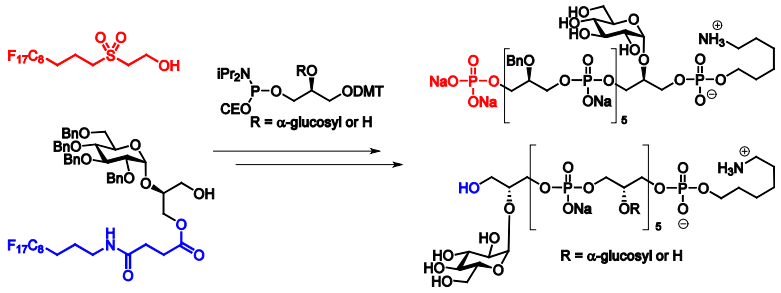
ABSTRACT



We here describe the synthesis of glucosylated teichoic acid (TA) fragments using two complementary fluorous scaffolds. The use of a perfluorooctylpropylsulfonylethyl (F-Pse) linker in combination with (glucosyl)glycerol phosphoramidite building blocks allows for the assembly of TA fragments with a terminal phosphate mono-ester, whereas the use of a perfluorooctylsuccinyl spacer delivers TA oligomers featuring a terminal alcohol functionality. These complementary linker systems have been developed because the nature of the TA chain terminus can play a role in the biological activity of the synthetic TAs. A novel α-glucosylated glycerolphosphoramidite building block is introduced to allow for a robust light fluorous synthetic protocol.

1. Introduction

The cell walls of pathogenic and non-pathogenic Gram-positive bacteria are characterized by a thick peptidoglycan layer, in which polyanionic glycopolymers are present. An important class of these cell surface polymers is comprised by the teichoic acids (TAs), which can be divided in wall teichoic acids (WTAs), covalently linked to the peptidoglycan layer and lipoteichoic acids (LTAs), which are anchored in the bacterial membrane. The repeating units of both types of TA are generally alditol (glycerol, ribitol) phosphates of which the hydroxyl functions are randomly equipped with carbohydrate or D-alanine residues. TAs perform vital functions as exemplified by their role in membrane integrity and permeability and their task as scaffold for various extracytoplasmic enzymes.[1] TAs also mediate extracellular interactions and are recognized by the mammalian adaptive and innate immune system, thus functioning as antigenic structures and immunostimulatory ligands for Toll-like receptors, respectively.[2] To establish structure–activity relationships of teichoic acids we started a programme aimed at the development of synthetic strategies to generate well-defined TA structures to help elucidate their mode of action at the molecular level.[3]

Enterococcus faecalis is a commensal, Gram-positive bacterium, responsible for many hospital related infections and represents a significant health threat especially to immunocompromised patients. The advent of strains resistant against multiple antibiotics is a strong stimulus for the development of alternative prophylactic and therapeutic strategies.[4] *E. faecalis* LTA has been shown to be a target of protective antibodies, and as such it represents a potential candidate for future vaccine development.[5] To identify potent

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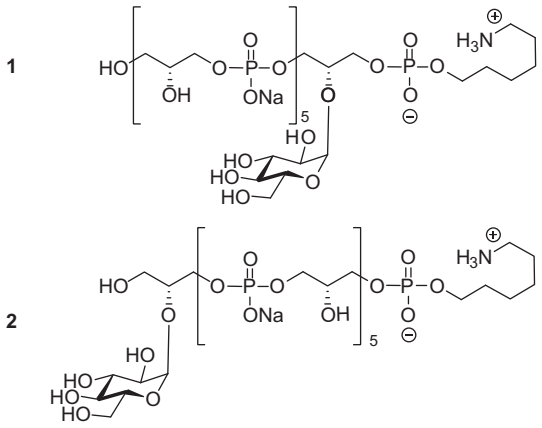


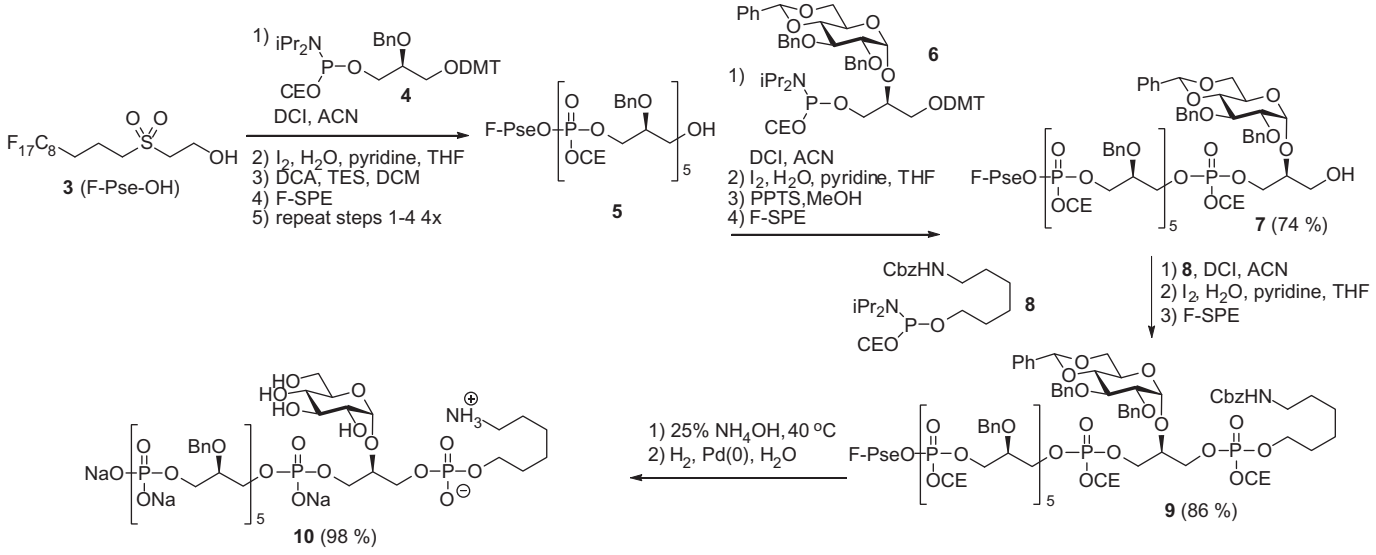
Figure 1. Lead structures **1** and **2**.

antigenic fragments of the microheterogenic *E. faecalis* TA we developed an automated solid phase synthesis approach, in which well-defined TA fragments were assembled on a commercially available DNA synthesizer using suitably protected glycerol phosphoramidite building blocks.[3b] Through this methodology we generated a small library of TA fragments, which allowed us to identify two potent antigenic TA structures in an opsonophagocytic killing inhibition assay (OPIA). In this assay compounds are screened for their ability to inhibit the complement-assisted killing of bacteria though opsonic antibodies.[5] These two compounds, **1** and **2** (Fig. 1), are characterized by the presence of an α-glucosyl substituent on either of the two terminal glycerolphosphate residues. Notably, this type of substitution is common in *Bacillus subtilis*[1c,6] and several *Staphylococcus* species[1c,7] but has not been encountered in native *E. faecalis* LTA. To further investigate these antigenic lead compounds we required an amount of these structures, larger than automated solid phase synthesis could provide us with and therefore we set out to develop a complementary synthesis strategy based on the use of 'light fluorous' chemistry.[3c,8]

2. Results and discussion

We have recently disclosed that perfluorooctylpropylsulfonylethanol (F-Pse) can be used effectively as a phosphate protecting group and concomitantly serve as a fluorous linker in the solution phase synthesis of TA fragments.[3c,8g] Therefore we initially explored the synthesis of hexamer **10**, a phosphorylated analogue of TA-fragment **1**, using this linker-system. Thus, F-Pse **3** was elongated in a stepwise manner (Scheme 1) with glycerol phosphoramidite **4**[3a] in a four-step elongation process, in which: (1) The phosphoramidite was coupled with the alcohol using dicyanoimidazole (DCI) as an activator in acetonitrile. (2) The resulting phosphite intermediate was oxidized using I_2 in a mixture of THF/H_2O/pyridine. (3) From the elongated fragment the 4,4′-dimethoxytrityl (DMT) ether was cleaved using dichloroacetic acid (DCA) and triethylsilane (TES) in DCM. (4) Fluorous solid phase extraction (F-SPE) was employed to separate the fluorous products from the non-fluorous side products. Before the F-SPE purification the mixture was partitioned between acetonitrile/water (80/20) and hexane to remove the bulk of TES and DMT-H to simplify the F-SPE purification, as described previously.[3c] Repeating this process four times led to pentamer **5** which was then elongated with benzylidene protected glucosylglycerol phosphoramidite **6** under the agency of DCI and oxidized. As the 4,6-*O*-benzylidene moiety is unstable towards DCA/TES, detritylation of the intermediate hexamer was effected using the milder PPTS/MeOH cocktail.[3a,9] The presence of the lipophilic carbohydrate moiety did not influence the F-SPE purification and the target compound was obtained uneventfully in 74% yield. At this stage an aminospacer was introduced to allow the conjugation of the target structure to, for instance, a carrier protein. Condensation of hexamer **7** and phosphoramidite **8** was followed by oxidation and F-SPE to give the fully protected construct **9** in 86% yield. Deprotection of hexamer **9** started by removal of the cyanoethyl (CE) and F-Pse groups by overnight treatment with aqueous ammonia at 40 °C. The semi-protected intermediate was separated from the eliminated fluorous scaffold (perfluorooctylpropylsulfonylethene) using a Et_2O/H_2O extraction. Subsequently, the benzylidene moiety, benzyl ethers and benzyl carbamate function were all removed by means of hydrogenolysis (Pd/H_2), leading to the target hexamer **10** in 98% yield. Surprisingly, in sharp contrast to its close analogue **1**, hexamer **10** proved to be inactive in the OPIA inhibition assay, indicating that the presence of the terminal phosphate is detrimental to the antigenicity of the synthetic TA fragment.[10]

To allow the light-fluorous assembly of TA fragments without a terminal phosphate moiety we set out to probe a hydroxyl



Scheme 1. Assembly of TA-fragment **10**.