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# Fluorous Linker Facilitated Synthesis of Teichoic Acid Fragments

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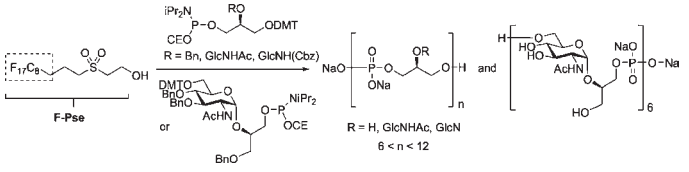
## ABSTRACT




**The use of perfluorooctylpropylsulfonylethanol as a new phosphate protecting group and fluorous linker is evaluated in the stepwise solution phase synthesis of a number of biologically relevant (carbohydrate substituted) glycerol teichoic acid fragments. Teichoic acid fragments, up to the dodecamer level, were assembled by means of phosphoramidite chemistry, using a relatively small excess of the building blocks and a repetitive efficient purification procedure of the protected intermediates by fluorous solid phase extraction (F-SPE).**

The synthesis of fragments of biopolymers, and functionalized derivatives thereof, is essential for progress in the life sciences and the development of ever more efficient synthetic procedures for their assembly, which continues to be an active field of research. The generation of biopolymers is pursued via solution phase or (automated) solid phase approaches, both of which have advantages and disadvantages. Solution phase synthesis protocols are labor- and time-consuming due to the intermediate isolation and purification steps but can be executed on a small and large scale employing a stoichiometric amount or small excess of reagents. (Automated) solid phase synthesis strategies are time and labor efficient by postponing product purification to the final stage of the synthesis. They do, however, require a relatively large excess of reagents (including expensive building blocks) and are mostly executed on a relatively small scale.[1] Alternative synthetic strategies have recently emerged that are based on the use of soluble supports. In an ideal situation, these strategies combine the "best of both worlds": the soluble support allows the application of (a relatively small) excess of reagents to drive the reactions to completion; it also enables the rapid isolation and purification of intermediates and is readily adapted to different reaction scales. Several supports have been recommended over the years, including polyethylene glycol polymers (PEG),[2] lipophilic tails,[3] and ionic tags.[4] With the advent of fluorous solid phase extraction (F-SPE) methodologies, a technique known as light fluorous synthesis[5] has become popular for the construction of biopolymers, especially in the area of carbohydrate chemistry.[5e,6] Applications in the assembly of oligopeptides[5e] have further been reported, but the use of fluorous chemistry in oligonucleotide synthesis has been restricted to tagging techniques,[7] in which a fluorous

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building block is employed at the end of a solid phase oligonucleotide synthesis to discriminate the target full length oligomers from unwanted, capped deletion sequences.

Teichoic acids are biopolymers comprised of repeating alditol phosphate residues, seemingly randomly decorated with carbohydrate and/or D-alanyl substituents. They are prime constituents of the Gram-positive bacterial cell wall and, as such, interact with their surroundings and play an important role in immunology.[8] We have recently reported on both the solution and solid phase synthesis of teichoic acid fragments of *Enterococcus faecalis*,[9] built up from repeating glycerol phosphate residues. Using the former technique we assembled a kojibiose containing glycerol phosphate hexamer, and with the latter technique, we generated a small library of teichoic acid fragments, varying in both size and substitution pattern.[10] We here report on a fluorous phase synthesis strategy to assemble teichoic acid structures. To this end, we implemented a new fluorous phosphate protective group and developed synthetic protocols compatible with F-SPE extraction techniques. We show that a light fluorous synthesis strategy is an efficient approach to assemble medium sized teichoic acid fragments.

Our first objective was the selection of a suitable light fluorous phosphate protecting group. To date only one such protecting group has been reported, which has been applied in the synthesis of a disaccharide.[11] We have recently reported on the use of fluorous sulfonylethyl-based groups to protect both amino and hydroxyl functions, in the form of a carbamate and carbonate respectively (see Figure 1).[12] We deemed this group suitable to protect phosphate functions since it can be removed at the end of the synthesis by base catalyzed $\beta$-elimination. The effective use of the 2-(methylsulfonyl)-ethyl (MSc) group in solid phase oligonucleotide synthesis bodes well for this approach. Whereas the use of the fluorous version of the MSc group, the [1*H*,1*H*,2*H*,2H]-perfluorodecylsulfonylethoxycarbonyl (F-Msc, **1**), worked very well as a nitrogen protecting group, it turned out to be too base labile in use as a hydroxyl protecting group (as in **2**). Thus, for the fluorous version of the Msc carbonate, we incorporated an extra methylene moiety between the fluorous part and the sulfonyl group to provide extra insulation for the $C_8F_{17}$ tail, giving the [1*H*,1*H*,2*H*,2H,3*H*,3*H*]-perfluoro-octylpropylsulfonylethoxycarbonyl (F-Psc, **3**).[12b] Based on these findings we decided to explore the F-Pse group **4** as a fluorous linker and phosphate protecting group. With this linker, synthesized as reported previously, we set out to establish the scope and limitations of fluorous teichoic acid synthesis. As depicted in Scheme 1, we elongated F-Pse alcohol **5** in a stepwise manner with glycerol phosphoramidite **6**.[10a] Each elongation cycle consisted of four steps: (1) reaction of the alcohol with phosphoramidite **6** under the agency of dicyanoimidazole

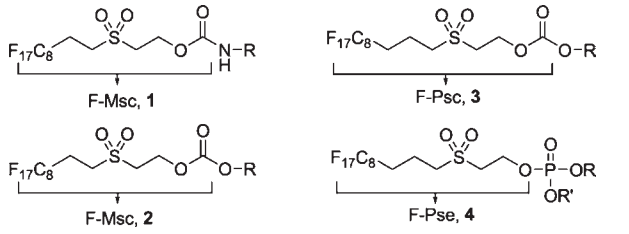
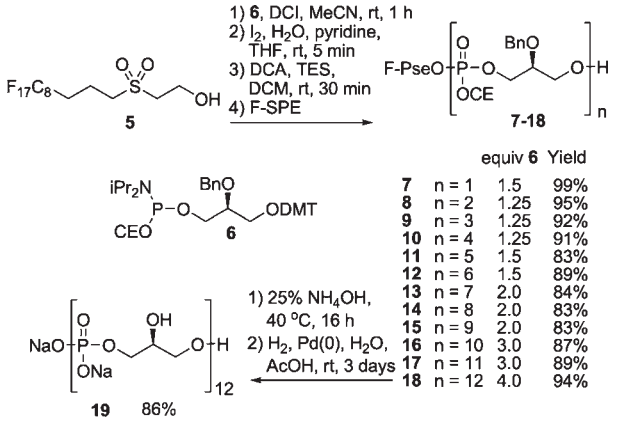



**Figure 1.** Fluorous versions of the MSc type protecting group.

**Scheme 1.** Synthesis of Glycerolphosphate Dodecamer **19**




(DCI); (2) oxidation of the intermediate phosphite with $I_2$; (3) removal of the DMT protecting group using dichloroacetic acid (DCA) in the presence of triethylsilane (TES); and (4) F-SPE purification. It proved to be essential that, before each F-SPE purification, the crude reaction mixtures were partitioned between MeCN/water (80/20) and hexane to remove most of the 4,4′-dimethoxytriphenylmethane and excess TES, since we found that these could not be separated from the target compounds by F-SPE when present in relatively large amounts. Using this protocol we were able to rapidly and efficiently

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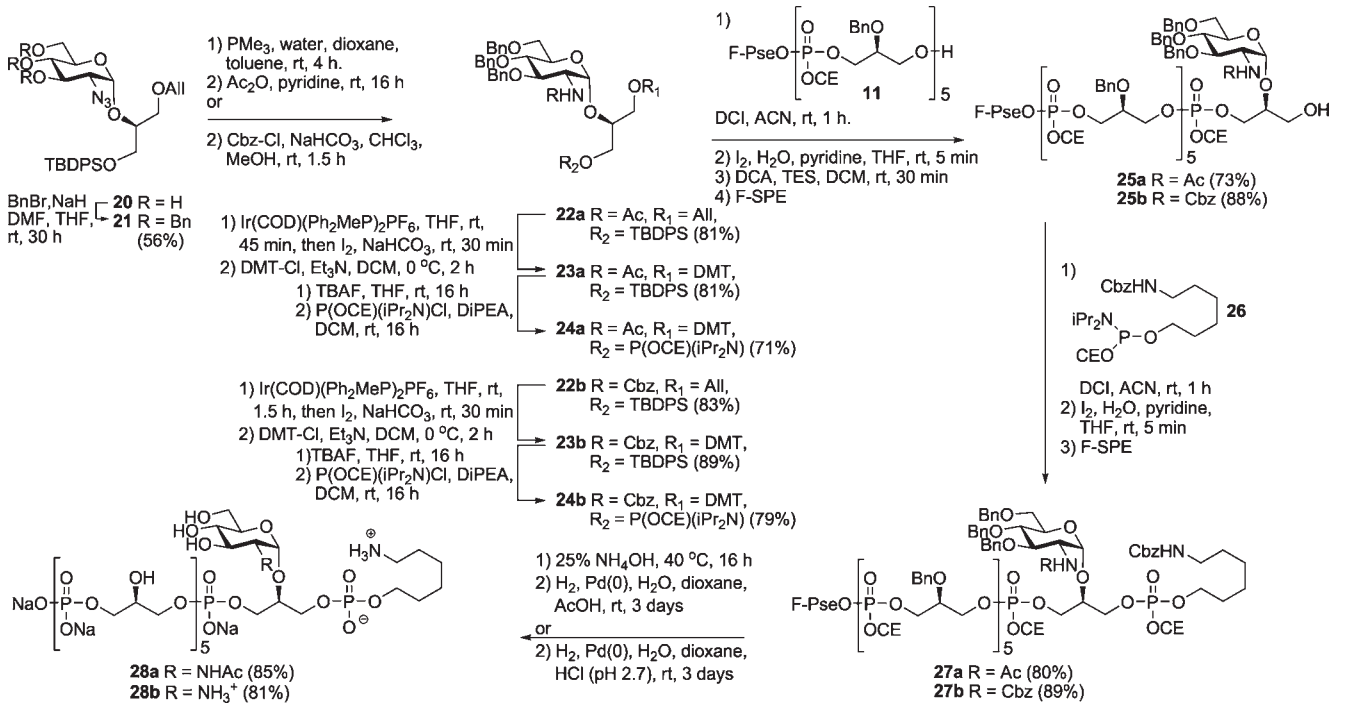
**Scheme 2.** Synthesis of Glucosamine Containing Hexamers **28a** and **28b**




generate protected oligoglycerolphosphates up to the dodecamer level. Although the relative fluorous content in compounds **7**–**18** significantly decreases with increasing oligomer length,[13] this had little or no effect on the purification efficiency and a single fluorous silica column (2 or 4 g) was sufficient to purify all oligomers (0.1–0.25 mmol). We did observe that a larger excess of **6** was required to push the coupling reactions to completion when going for larger oligomers. Up to 4 equiv of the phosphoramidite were required in the final coupling step to the dodecamer (see Scheme 1). We argue that for longer oligomers the automated solid phase approach becomes competitive, and therefore, we did not explore the synthesis of longer oligomers.

Deprotection of the fully protected dodecamer started with removal of the F-Pse and cyanoethyl groups using 25% aqueous ammonia solution. In a model deprotection experiment (see Supporting Information) we observed that the F-Pse group at the terminal phosphotriester was selectively cleaved with respect to the cyanoethyl group. Elimination of the remaining cyanoethyl group on the obtained phosphodiester required slightly elevated temperatures (40 °C) and prolonged reaction times (typically overnight) to ensure complete unmasking to the target phosphomonoester. Applying this protocol to dodecamer **18**, and ensuing hydrogenolysis of the partially protected oligomer and gel filtration, led to 30 mg of fully deprotected dodecamer **19** in 86% yield over the last two steps.

Having established that F-Pse alcohol **5** can be used for the efficient assembly of glycerol phosphate teichoic acids, we next investigated the incorporation of glycosyl substituents in the TA chains through the assembly of two teichoic acid hexamers. TA **28a** carries a GlcNAc residue, as present in TA chains of *Staphylococcus aureus*,[14] whereas a positively charged glucosamine is grafted on hexamer **28b**, a structural element found in several *Streptomyces* species.[15] The required glucosaminyl glycerol phosphoramidites were obtained as depicted in Scheme 2. Triol **20** was benzylated to give intermediate **21**[16] from which both building blocks **24a** and **24b** were assembled. Reduction of the azide functionality in **21** and subsequent acetylation gave *N*-acetyl glucosamine derivative **22a**, while protection with a benzyloxycarboxyl group led to **22b**. Both glucosaminyl glycerol building blocks were then transformed into the required phosphoramidites **24a** and **24b** following a well-established sequence of reactions, involving deallylation, dimethoxytritylation, desilylation, and phosphitylation. With building blocks **24a/b** the target hexamers **28a/b** were assembled starting from fluorous pentamer **11**. Thus, condensation of **11** and

(13) The relative fluorine content ranges from 37% in compound **7** to 7% in hexaGlcNHAc-glycerol phosphate **38**.

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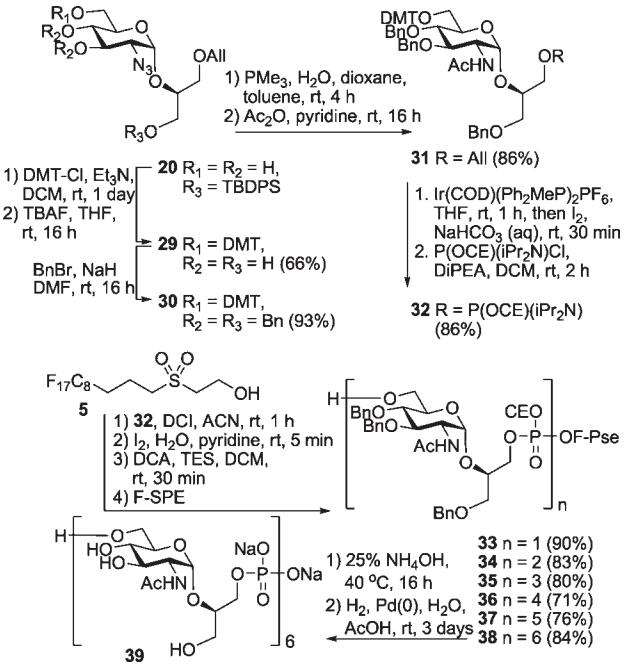
**24a/b** and subsequent oxidation and removal of the DMT-group gave crude **25a/b**. Also in this case F-SPE purification proceeded uneventfully and hexamers **25a/b** were obtained in 73% and 88% respectively. The hexamers were then equipped with a hexanolamine spacer using phosphoramidite **26** to give fully protected TA structures **27a/b** in pure form after F-SPE. Global deprotection using the deprotection scheme described above led to both target hexamers **28a/b**, the successful assembly of which indicates that the fluorous synthesis strategy can also be applied to substituted oligoglycerolphosphates.

As a final research goal we sought to explore the assembly of more complex structures as exemplified by the synthesis of teichoic acid fragment **39**, which is characterized by the [→6)-α-glucosamine-(1→2)-*sn*-glycerol-1-phosphate-] repeating unit and is found in *Spirilliplanes yamanashiensis* (Scheme 3).[17] The synthesis of the required GlcNHAc-glycerol phosphoramidite building block **32** commenced with dimethoxytritylation of the primary alcohol in **20** (Scheme 3). Subsequent desilylation and benzylation of the resulting triol gave the fully protected GlcNHAc-glycerol **30**, which was transformed into required phosphoramidite **32** through azide reduction and acetylation followed by a deallylation–phosphitylation reaction sequence. For the assembly of hexamer **39**, F-Pse linker **5** was elongated in a stepwise manner with **32** using the chemistry described above. As can be seen in Scheme 3 all elongation steps proceeded efficiently. Although **32** is a more lipophilic building block than the above-described glycerol phosphates, this did not pose any problems in the purification of the oligomers. Notably the single $C_8F_{17}$-tail sufficed for the easy purification of fully protected hexamer **38**, having a molecular mass of ~4.7 kD. Finally deprotection of **38** was accomplished by *β*-elimination of the F-Pse linker and cyanoethyl groups and global debenzylation led to 32 mg of target compound **39** in 76% yield.

In conclusion, we have developed an efficient fluorous synthesis strategy for the assembly of teichoic acid fragments, based on the application of perfluorooctylpropylsulfonylethanol as a new fluorous phosphate protecting group. The strategy is especially useful for the assembly of multimilligram quantities of medium sized TA fragments, featuring 6–12 repeating units. As displayed by the assembly of teichoic acid fragment **39**, complex glycerol phosphate building blocks can also be used, indicating that our strategy is a valuable asset for the construction of various classes of phosphate ester containing biomolecules.

**Scheme 3.** Synthesis of Complex Hexamer **39**

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**Supporting Information Available.** Full experimental procedures and copies of $^1$H, $^{13}$C, and $^{31}$P NMR spectra for **7**–**19**, **21**–**25**, and **27**–**39**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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