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# Efficient solid-phase synthesis of perfluoroalkylated dimerizable cationic detergents for gene delivery

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ABSTRACT

A new straightforward solid-phase synthesis of perfluoroalkylated dimerizable detergents used as gene delivery systems is presented. This route using Fmoc-Cys(SASRIN™-®)-OH resin as solid support affords the target products in almost quantitative yields and high purity.



Gene delivery mediated with synthetic vectors requires the formulation of nanometric multifunctional DNA complexes mimicking viruses (e.g., 'artificial' or 'synthetic' viruses).[1–6] Such multicomponent systems were designed in order to take advantages of viral vector properties such as their small size (⩽100 nm), capability to escape the immune system, cell tropism, cytoplasm delivery and/or nuclear targeting, and penetration. This requires the assembly of various components around DNA, which must further be condensed into very small- and homogeneously-sized 'stealth' as well as ligand-functionalized nanoparticles. Indeed, a small nanoparticle size is critical for its in vivo fate, for facilitating its extravasation across blood vessels and diffusion through cytoplasm and nucleus, and for benefiting from the enhanced permeability and retention effect (passive accumulation into tumors through the fenestrated blood vasculature).[7] Our group succeeded in obtaining very tiny, monomolecular, and cationic DNA nanoparticles from perfluoroalkylated dimerizable detergents containing a monocationic aminotriethyleneglycol or a tricationic linear spermine polar head (e.g., **1** and **2** in Fig. 1).[8,9] This could be achieved in a highly controllable and reproducible fashion by a monomolecular DNA condensation process.[8–15] Owing to reversible DNA/detergent and detergent/micelle interactions, and owing to entropy, DNA compaction with such detergents used at a concentration close to their cmc ensures monodispersity, and the largest number of DNA particles, each particle being ultimately made of a single molecule or copy of condensed DNA.[14,15] Once the monomolecular detergent/DNA nanoparticles had thus formed, the particles were then stabilized by oxidation of the thiol detergents into disulfide lipids on the DNA matrix.[8–12] The resulting DNA particles formulated with these two detergents exhibited remarkable in vitro transfection properties with an efficiency at least one order of magnitude higher than that of polyethyleneimine (PEI)-based polyplexes.[8,9]

Two different synthetic routes to these compounds, which were both carried out in liquid phase, were previously described.[14,15] One route utilized cysteine and/or cystine protected scaffold-based chemistry and led to the desired derivatives in overall poor yields due to solubility problems as well as to cumbersome and time consuming purifications.[8] As for the second route, which proved more efficient (nearly 50% yields), it was based on the key dimethylthiazolidine-cysteine synthon, which allowed a simple way to protect the thiol residue of cysteine and, in the meanwhile, elongation onto its N-extremity.[9]

Still looking for synthetic improvements, a very versatile and efficient solid-phase methodology based on the commercially available Fmoc-Cys(SASRIN™-®)-OH resin (from BACHEM, Germany) is proposed here for preparing detergents **1** and **2**. The Fmoc-Cys(SASRIN™-®)-OH resin **3** is used as a solid support as well as a protecting group of the thiol residue of cysteine. The synthetic route outlined on Scheme 1 led to the target derivatives in four steps in almost quantitative yields and high purity. This straightforward route is inspired from the solid-phase synthesis reported for analogous hydrocarbon dimerizable detergents, which was performed in seven steps starting from the NOVASYN-MMT chloride resin onto which Fmoc/alloc-protected cysteine (though not commercially available) was grafted.[11]

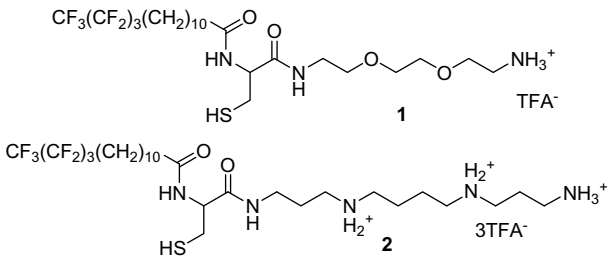


**Figure 1.** Perfluoroalkylated cationic dimerizable detergents for monomolecular DNA condensation.[8,9]

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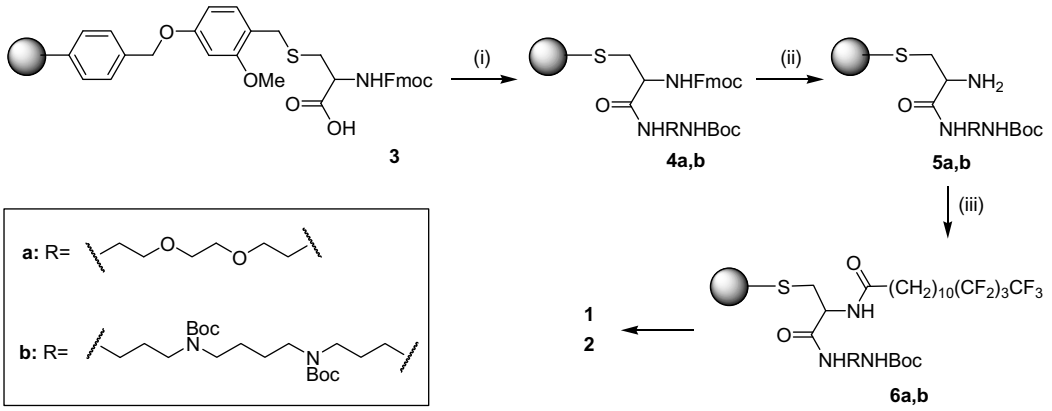


**Scheme 1.** Reagents and conditions: (i) (1-(*N*-Boc)-3,6-dioxa-8-octaneamine for **1** or *N,N′,N″*-triBoc-spermine for **2** (1.1 equiv), HBTU (2 equiv), DMAP (2 equiv), NMP, 20 min (repeat step × 2); (ii) 20% piperidine in DMF, 2 × 10 min; (iii) $CF_3(CF_2)_3(CH_2)_{10}COOH$ **7** (2 equiv), HBTU (2 equiv), DIPEA (4 equiv), 1 h; (repeat step × 2); (iv) $TFA/CH_2Cl_2$ (1/3).

The Fmoc-Cys(SASRIN™-®)-OH resin **3** (0.4 mmol $g^{-1}$ capacity) was first preactivated with a twofold molar excess of dimethylaminopyridine (DMAP) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HBTU) in *N*-methylpyrrolidone (NMP). Then, protected amine (1-(*N*-Boc)-3,6-dioxa-8-octaneamine for **1** or *N,N′,N″*-triBoc-spermine[16] for **2**) was added and left to react for 20 min. This elongation step was repeated twice and its completion was confirmed by a negative Malachite Green test[17] attesting the absence of any unreacted carboxylic residue. The *N*-Fmoc protecting group was then cleaved from the cysteinyl group using 20% piperidine in DMF. The perfluoroalkylated acid **7**,[18] preactivated with HBTU and diisopropylethylamine (DIPEA) in DMF, was then added to the resin slurry containing the cysteinyl free amino group. This condensing step was repeated twice and the excess perfluoroalkylated acid **7** was filtrated and easily recycled. Completion of the coupling reaction (absence of free amino groups on the resin) was assessed and confirmed by a negative Kaiser test.[19] Final cleavage of the target detergents from the solid support was performed using a 1/3 (v/v) solution of TFA in dichloromethane. The filtrate was carefully concentrated in vacuo and TFA was eliminated by co-evaporation with dioxygen-free ethanol to afford the detergents (as TFA salts) as white solids in almost quantitative yields. Indeed, using 100 mg of resin ($4 \times 10^{-2}$ mmol), we isolated 24 mg of detergent **1** ($4 \times 10^{-2}$ mmol, MW = 638.3 g $mol^{-1}$). The purity of the material was assessed by HPLC and the analytical data ($^1H$, $^{13}C$, and $^{19}F$ NMR, MS) collected on these materials were fully identical to those already published.[8,9]

In conclusion, we have elaborated a novel, versatile, straightforward solid-phase methodology for the production of perfluoroalkylated dimerizable detergents containing a monocationic or a tricationic polar head. Besides, it should be emphasized that bead-grafted derivatives of type **6a** (e.g., deriving for example from a α,ω-diaminopolyethylene-glycol) constitute very useful synthons for the conjugation with various ligands. Such conjugates are needed for the formulation of 'stealth', functionalized detergent/DNA nanoparticles, and aimed at specific ligand-receptor mediated transfection for in vivo purposes.

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