

Synthesis and Application of Fluorous-tagged Oligonucleotides

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Nucleoside phosphoramidites bearing a adsorbent *tert*-butylphenyl-1*H*,1*H*,2*H*,2*H*-heptafluorodecyloxysilyl (BPFOS) group were used to synthesize fluororous-tagged oligonucleotides, viz. 5-mer, 10-mer, 13-mer, 17-mer, and 19-mer, which were subjected to liquid-phase extraction. The labeled oligomers were found to be highly hydrophobic in nature, become precipitated in water, therefore allowing easy purification from the failure sequences. The silyl fluororous group was deprotected by TBAF treatment and oligomers were easily purified by CH₃CN/FC-72 liquid–liquid extraction. Fluorous-tagged oligonucleotides have high selectivity for the removal of failure sequences, high recoveries (typically 70–100%).

The purification and yield improvement are the two much challenged jobs in the field of oligonucleotide synthesis. To avoid chromatographic purification, reactions should be planned such that the phase of the desired product is different from phases of all the other reaction components and undesired products. The “fluorous phase”^{1–5} has been recently used to advantage in traditional organic synthesis. The new techniques rely on the ability of a “fluorous” (highly fluorinated) molecule to partition into the fluororous phase in a liquid–liquid extraction between an organic solvent and a fluororous solvent.^{6,7} One or more reactions are then conducted and the fluororous components of the reaction are subsequently separated from all non-fluorous (organic, inorganic, solid or volatile) components by an appropriate phase-separation technique. At the desired stage, the fluororous labels are cleaved and the product rendered organic. Furthermore, fluororous solvents are much robust than most polymers and linkers in current use for solid-phase synthesis.

Silicon-based methodology for the protection of alcohols has made a major contribution in organic synthesis.⁸ The ability to modulate selectivity and reactivity by varying the steric and electronic requirements of the substituents on silicon has been demonstrated by such reagents as *tert*-butyldimethylsilyl chloride⁹ and *tert*-butyldiphenylsilyl chloride.¹⁰ Therapeutic uses of antisense oligonucleotides warrant efficient synthesis and purification. But on solid-support synthesis, however, the final yield of the desired sequence is greatly affected by purification cycles.

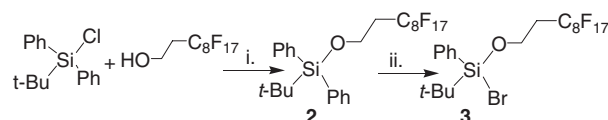
The idea nurtured in this communication was to carry out the solid-support synthesis in such a way that the desired sequences after cleavage from the support may be easily separated from the failed ($n - 1$) sequences by liquid–liquid extraction or by simple precipitation, thus tedious chromatographic separations may be avoided. To implement the idea, the fluororous alkoxy silyl ethers were tried. These are reported to impart acid stability to the silane protecting groups.^{11,12} The fluororous synthesis schemes allow for easy purification or removal of highly fluorinated intermediates or reagents e.g. via liquid–liquid extraction (3 phase extraction) between organic solvents, fluorinated solvents

and water or via solid-phase extraction with fluororous reverse phase silica gel.^{13,14} (C₆F₁₃CH₂CH₂)₃Si group, the classical fluororous silyl tag is quite acid sensitive. Since, the bis-alkoxy-silyl ethers have shown improved acid stability therefore, Wipf et al.¹⁵ have tried fluororous alkoxy-silyl groups and they found it to be more acid stable and with primary and secondary alcohols the yields were found to be fair to excellent. They reported *tert*-butylphenyl-1*H*,1*H*,2*H*,2*H*-heptafluorodecyloxysilyl (BPFOS) to be more acid stable than (C₆F₁₃CH₂CH₂)₃Si group. Here, the BPFOS group is used for protection of 5'-hydroxy group of nucleosides, an example of primary hydroxy group.

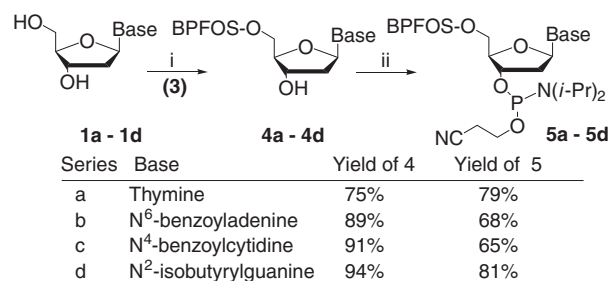
A general method for the preparation of 5'-protected deoxyribonucleosides has been out in Schemes 1 and 2. The alkoxy-silyl ethers (BPFOS) were obtained by the reaction of decanol with 1.1–1.3 equiv. of the appropriate silyl chloride and 1.5 equiv imidazole at rt. in CH₂Cl₂ as solvent. Excellent yields of alkoxy-silyl ethers results in all cases, except for the hydrolytically. This alkoxy-silyl ether (**2**)¹⁶ was brominated in 1,2-dichloroethane at 0 °C to yield (BPFOSiBr) (**3**)¹⁷ (Scheme 1).

Therefore, the *tert*-butylphenyl-1*H*,1*H*,2*H*,2*H*-heptafluorodecyloxysilyl bromide (BPFOSiBr) group was used for protection of 5'-hydroxy group of nucleosides, an example of primary hydroxy group, the 5'-O-protected nucleosides (**4a–4d**)¹⁸ were phosphitylated using 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (bis-reagent) and activator pyridinium trifluoroacetate (Py-TFA) in dry DCM (Scheme 2).¹⁹

The fluororous-tagged sequences were readily precipitated out when dissolved in deionized water. This may be due to enhancement in hydrophobicity. Fluorous tag from 5'-OH of oligonucleotides sequences was removed by treating fluororous-tagged sequences with tetrabutylammonium fluoride (TBAF).



Scheme 1. Synthesis of fluororous alkoxy-silyl ether: Reagent and conditions; i) DCM, DMAP, imidazole, rt. ii) Br₂ DCM, 0 °C, rt.



Scheme 2. Synthesis of 5'-O-BPFOS nucleoside phosphoramidites; Reagent and conditions; bis-reagent /DCM, Py-trifluoroacetate.

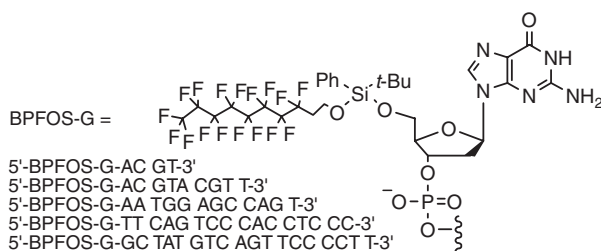


Figure 1. Examples of fluorinated-tagged oligodeoxyribonucleotides.^{20,21}

The present communication to use fluorinated group as efficient purification tag will be helpful over the existing purification protocols, which mainly comprise of the tedious chromatographic techniques. Thus, if a sequence is synthesized by conventional phosphoramidite chemistry and is tagged at the end with this fluorinated alkoxy-silyl-protected nucleoside then after cleavage from the CPG it is supposed to be separated easily. Different length of sequences were chosen to prove the viability of the fluorinated protecting groups and to see that how much long sequence it can separate easily in liquid–liquid extraction. The maximum length used over here is of 19-mer (18-mer tagged with the fluorinated tagged dG) and it shows easy precipitation, 100% recovery after deprotection in acetonitrile and the ability to purify small as well as long oligonucleotides.

Thus, the desired sequences may be purified from the rest of the ($n - 1$) failure sequences by suspending the sequence in water after deprotecting them from the solid support. The desired sequences precipitated out and were separated by simple vacuum filtration.

References and Notes

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16. **2:** *tert*-Butyldiphenyl-1*H*,1*H*,2*H*,2*H*-perfluorodecylsilyl ether. A solution of *tert*-butyldiphenylsilyl chloride (4.39 mmol, 1.20 g), 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decanol (4 mmol, 1.586 g), DMAP (0.2 mmol, 25 mg), and imidazole (5.6 mmol, 380 mg) in CH_2Cl_2 (15 mL) was stirred at rt for overnight. 25 mL CH_2Cl_2 was added and the solution was washed with water, 1 M HCl and brine. Drying the organic layer over Na_2SO_4 and evaporation of the solvent yielded the TBDPOS ether as colorless oil. Yield: 88%, 2.472 g; $^1\text{H NMR}$ (CDCl_3) 7.69–7.66 (m, 4H), 7.45–7.38 (m, 6H), 3.96 (t, 2H), 2.45–2.25 (m, 2H), 1.07 (s, 9H).
17. **3:** *tert*-Butylphenyl-1*H*,1*H*,2*H*,2*H*-perfluorodecylsilyl bromide. Bromine (3.0 mmol, 1.43 mL) was added drop-wise to a solution of the TBDPS ether (2.5 mmol, 1.755 g) in 1,2-dichloroethane (15 mL) at 0 °C. The reaction mixture was stirred at rt for overnight. Distillation at reduced pressure yielded the product as colorless oil. Yield: 71% (2.003 g); $^1\text{H NMR}$ (CDCl_3): 7.69–7.65 (m, 2H), 7.48–7.39 (m, 3H), 4.11–4.06 (m, 2H), 2.47–2.35 (m, 2H), 1.01 (s, 9H).
18. **4a–4d:** Synthesis of 5'-*O*-*tert*-butylphenyl-1*H*,1*H*,2*H*,2*H*-perfluorodecylsilyl deoxyribonucleosides. Above compound (**3**) (1.1 mmol) was dissolved in CH_2Cl_2 (15 mL). The four deoxyribonucleosides viz. (dA/dC/dG/T; 1.42 mmol) and DMAP (0.05 mmol) were added and the reaction mixture was stirred at rt. overnight. CH_2Cl_2 (15 mL) was added and the mixture was washed with NaHCO_3 solution. The organic phase was dried over Na_2SO_4 , and DCM was evaporated in vacuo. The product was filtered through silica (DCM:hexane; 60:40) The desired batches were pooled and evaporated to get product.
19. **5a–5d:** 5'-*O*-*tert*-butylphenyl-1*H*,1*H*,2*H*,2*H*-perfluorodecylsilyl deoxyribonucleosides-3'-*O*-phosphoramidite. The products **4a–4d** (1 mmol) each was suspended in dry DCM and bis-reagent (2'-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphoramidite–phosphoramidite) (1.2 mmol) was added to it at ambient temperature. Pyridinium trifluoroacetate (1.2 mmol) was added to reaction mixture and the solution was stirred for 4 h. Upon complete consumption of the starting material (tlc), the entire reaction mixture was transferred directly on the top of a short silica column. The product was eluted with DCM:hexane, 60:40. The appropriate fractions were collected and pooled and the solvent was evaporated to furnish the product.
20. Synthesis of fluorinated-tagged oligonucleotides. All the oligomers were synthesized at 0.2 mmol scale on an ABI 392 model DNA/ RNA synthesizer using nucleoside analogues (**5a–5d**) with only benzoyl and isobutyryl group on exocyclic amino functions. The last coupling cycle was increased by 10 min. All the oligomers were mixed type. Five (4-mer, 9-mer, 12-mer, 16-mer, and 18 mer) sequences were synthesized using phosphoramidite chemistry. Modified 5'-*O*-*t*-butylphenyl-1*H*,1*H*,2*H*,2*H*-perfluorodecylsilyl deoxyribonucleosides-3'-*O*-phosphoramidite (of thymidine, cytidine, adenosine, and guanosine) were added in last coupling cycle (Figure 1). All the oligomers were treated with 30% aqueous ammonia for 16 h at 55 °C to remove protecting groups from the bases and internucleotide phosphates and also to cleave oligomer from the solid support. The ammoniacal solution was concentrated under a vacuum in a speed vac, and the residue was subjected to desalting on a reverse phase silica gel column.
21. Solubility pattern of fluorinated tagged oligonucleotides. After the cleavage from solid support the fluorinated-tagged oligonucleotides were readily precipitated when suspended in deionized water due to enhanced hydrophobicity. They are sparingly soluble in CH_3CN (organic solvent) and insoluble in FC-72 (fluorinated solvent). To deprotect the fluorinated tag the sequences were added to a solution of TBAF (0.6 M) in 0.5 mL of THF. After 3 h the deprotected oligonucleotides was extracted in acetonitrile. The organic layer was pooled and evaporated and the residue was partitioned in FC-72 and fresh CH_3CN . The sequences are retained in organic solvent layer while the protecting group passes to FC-72 layer. The organic phase on evaporated in vacuo gave the desired sequences in better yield. The purity of desired oligonucleotide were analyzed on reverse phase HPLC with a Merck lichrosphere RP-18 column using 0.1 M ammonium acetate buffer at pH 7.1 and acetonitrile solvent.