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 fluorous-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 fluorous group was deprotected by TBAF treatment and oligomers were easily purified by CH_3CN/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 planed 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 fluorous phase in a liquid–liquid extraction between an organic solvent and a fluorous solvent.[6,7] One or more reactions are then conducted and the fluorous 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 fluorous labels are cleaved and the product rendered organic. Furthermore, fluorous solvents are much robust than most polymers and linkers in current use for solid-phase synthesis.

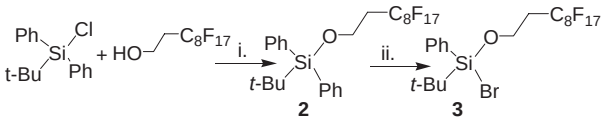
Silicon-based methodology for the protection of alcohols has made a major contribution in organic synthesis.[8] The ability to modulate selectivity and reactivity by varying the steric and electronic requirements of the substituents on silicon has been demonstrated by such reagents as *tert*-butyldimethylsilyl chloride[9] 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 fluorous alkoxy silyl ethers were tried. These are reported to impart acid stability to the silane protecting groups.[11,12] The fluorous 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 fluorous reverse phase silica gel.[13,14] $(C_6F_{13}CH_2CH_2)_3Si$ group, the classical fluorous silyl tag is quite acid sensitive. Since, the bis-alkoxysilyl ethers have shown improved acid stability therefore, Wipf et al.[15] have tried fluorous alkoxysilyl 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 $(C_6F_{13}CH_2CH_2)_3Si$ group. Here, the BPFOS group is used for protection of 5′-hydroxy group of nucleosides, an example of primary hydroxy group.

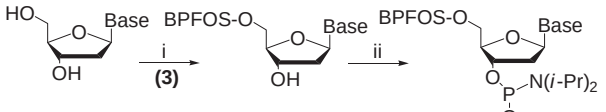
A general method for the preparation of 5′-protected deoxyribonucleosides has been out in Schemes 1 and 2. The alkoxysilyl ethers (BPFOS) were obtained by the reaction of decanol with 1.1–1.3 equiv. of the appropriate silyl chloride and 1.5 equiv imidazole at rt. in CH_2Cl_2 as solvent. Excellent yields of alkoxysilyl ethers results in all cases, except for the hydrolytically. This alkoxysilyl 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 fluorous-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 fluorous-tagged sequences with tetrabutylammonium fluoride (TBAF).



Scheme 1. Synthesis of fluorous alkoxysilyl ether: Reagent and conditions; i). DCM, DMAP, imidazole, rt. ii) Br_2 DCM, 0 °C, rt.



Series	Base	Yield of 4	Yield of 5
a	Thymine	75%	79%
b	N^6-benzoyladenine	89%	68%
c	N^4-benzoylcytidine	91%	65%
d	N^2-isobutyrylguanine	94%	81%

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

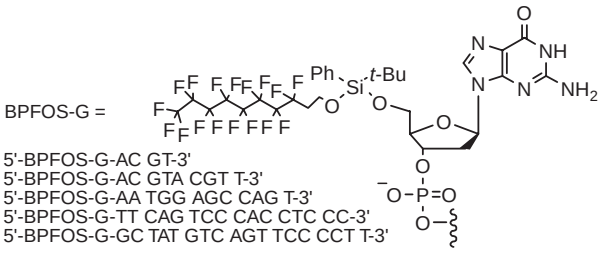


Figure 1. Examples of fluorous-tagged oligodeoxyribonucleotides.[20,21]

The present communication to use fluorous 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 fluorous alkoxysilyl-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 fluorous 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 fluorous 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.

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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}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*-perfluorodecyloxysilyl 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}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*-perfluorodecyloxysilyl 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*-perfluorodecyloxysilyl 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 fluorous-tagged oligonucletides. 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*-perfluorodecyloxysilyl 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 fluorous tagged oligonucletides. After the cleavage from solid support the fluorous-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 (fluorous solvent). To deprotect the fluorous 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.

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