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The Ph.D thesis of "Chemistry and Stereochemistry of Nucleosidyl Phosphorofluoridates and Their Structural Analogues"

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Introduction

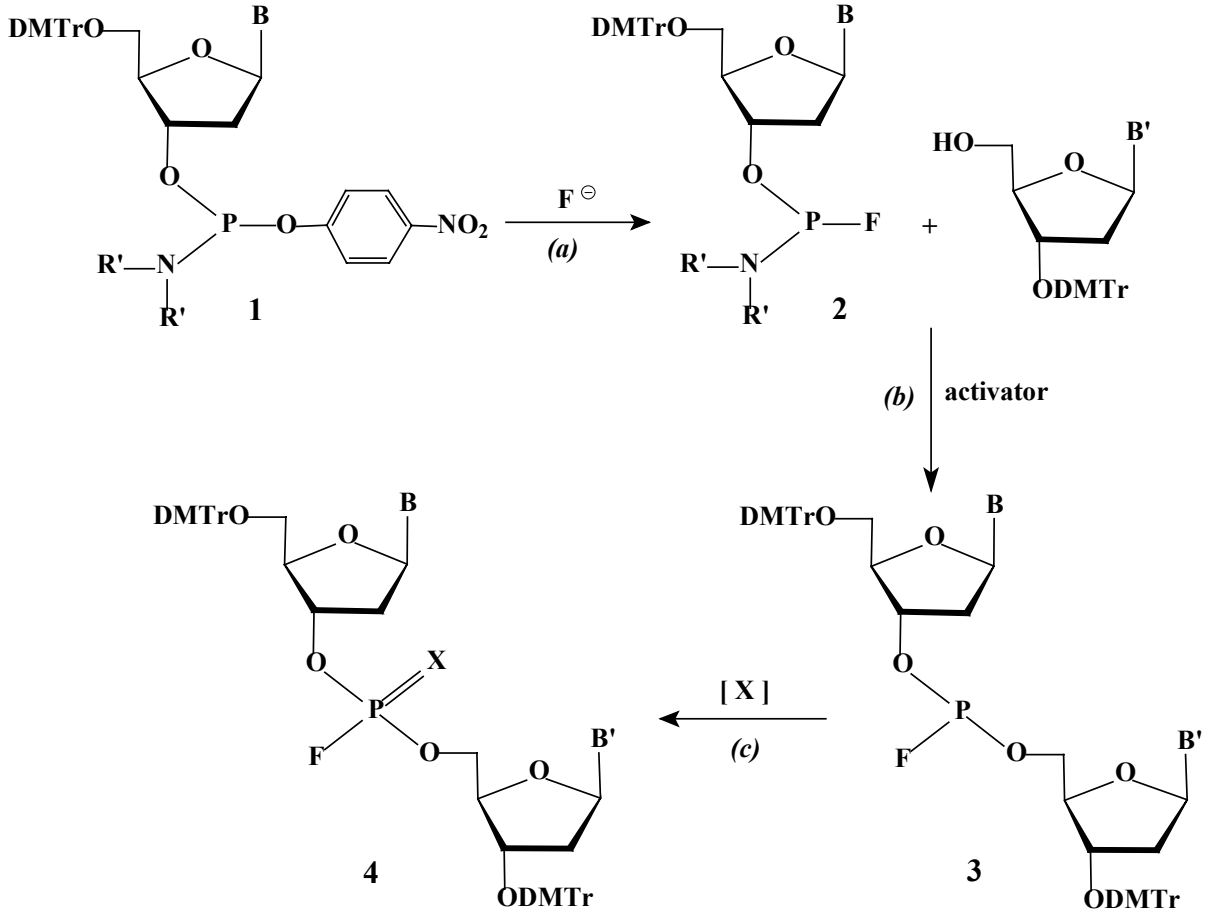
Oligonucleotides containing modified internucleotide bond have drawn my attention owing to their potential biochemical and therapeutical usage.[1] An introduction of a fluorine group into a phosphoroorganic compounds causes remarkable changes of their chemical and biochemical properties. It was reported that 5'-fluorophosphates inhibit the phosphorylation reaction catalyzed by adenylate kinases and inhibit HIV reproduction in cell cultures.[2] I anticipated that phosphorofluoridates incorporated into oligonucleotides could be useful in the studies of biological functions of nucleic acids.

Results and discussion

My work described in the Ph.D. thesis was focused on the synthesis of nucleotides modified at the phosphorus center. In this essay I present only part of my result. I have found very efficient and general procedures leading to the synthesis of tricoordinate phosphorus compounds containing P-F bond derived from biologically important alcohols. Consequently the first synthesis of nucleosidyl phosphorfluoridites, dinucleosidyl phosphorofluoridates and phosphorofluoridothio-nates *via* phosphoramidite approach has been achieved. The success connected with this approach was accomplished by constructing of phosphitylating reagents containing P-F bond. Known methods leading to phosphorfluoridites were not suitable for my aim.

For example the synthesis of modified nucleotides *via* nucleosidyl chlorophosphites is not practical because of their low availability and stability.

The following sequence of reactions leading to dinuclesidyl phosphorfluoridites **3** was invented as presented in Scheme 1.



B, B' : T, A^{Bz}, C^{Bz}, G^{iBu}

R, R' : $(CH_3)_2CH$-, CH_3CH_2-

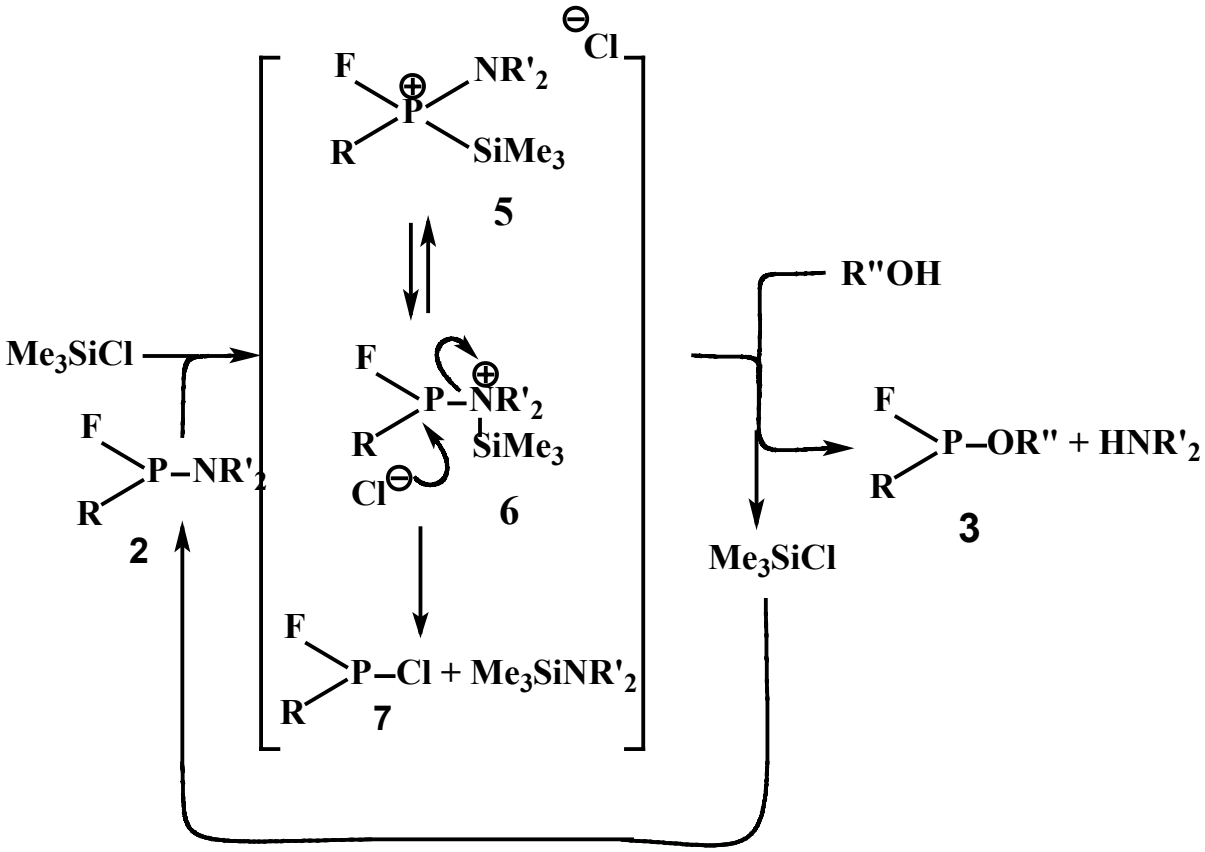
X = O, S

Scheme 1

Earlier in this laboratory it was found that 4-nitrophenoxy group attached to tricoordinate phosphorus center acts as an excellent leaving group.[3] This observation enabled me to obtain nucleosidyl *N,N*-dialkylfluorophosphoramidites **2** (step *a*, Scheme 1) *via* reaction with fluoride anion derived from *tert*-butylammonium fluoride (TBAF) or other nucleophilic fluoride donors.[4] Under properly chosen conditions conversion of nucleosidyl *O*-aryl-*N,N*-dialkylphosphoramidites **1** into nucleosidyl phosphorfluoridites **2** proceeds

in almost quantitative yield with some stereoselection. It was also possible to separate compounds **1** and **2** and into single diastereoisomers by a standard gel silica chromatography.

Activation of phosphoramidites is the crucial step of the synthesis of modified nucleotides. Tetrazole, amine hydrochloride, tetrazole salts with amine are "classic" activators used in phosphitylating reactions. These compounds have many disadvatages. The most frequently employed activator- tetrazole -must be used in very large excess and be purified by hazardous sublimation. Therefore it was necessary to find suitable activating reagent. I have found that coupling of phosphoramidites with alcohols can be performed very efficiently in the presence of trimethylchlorosilane (TMCS) as catalytic activator (Scheme 2). The amount of TMCS required for an efficient coupling is 30-60% of the stoichiometrical ratio.[5] The first step is interaction of phosphorfluoridite **2** and TMCS leading to the salt-like species **5** and **6**. These intermediates react either with alcohol to give phosphorofluoridite **3** or *via* formation of chlorophosphite **7.** In both cases TMCS is regenerated. Formation of intermediate **7** was confirmed by ^{31}P NMR spectroscopy.



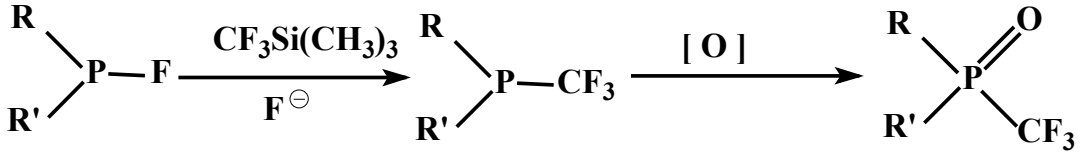
Scheme 2

Dinucleosidyl phosphorfluoridites **3** are formed in this procedure as 1:1 mixture of diastereoisomers. Fortunately chromatography of dinucleosidyl phosphorfluoridites on standard silica gel led to pure diastereoisomers of dinucleotides. The high stereochemical stability of phosphorfluoridites is to be explained by the presence of the electronegative fluorine ligand and steric hindrance due to nucleosidyl groups.

In my Ph. D. thesis another unconventional coupling reagent 2,4-dinitrophenol has been described. Its potency is comparable and in many cases higher than TMCS and tetrazole.[6] I was able to confirm that both activators can be use in the synthesis of other oligonucleotides also on the preparative scale.

I made also another significant observation that pure diasteroisomers of **3** undergo stereospecific oxidation and sulfurization reactions leading to dinucleosidyl phosphorofluoridates **4** and their sulfur analogues [7]. I have found that pure diasteroisomers of phosphorfluoridites **3** react with *tert*-butyl hydroperoxide to form in the stereospecific manner dinucleosidyl phosphorofluoridates **4** (X=O). To obtain pure diastereoisomers of the phosphorofluoridothionates **4** (X=S) I used *bis*-benzyol disulfide as the stereoselective sulfurization reagents. This reaction most likely proceeds with retention of configuration at the chiral phosphorus atom.

Another objective of my recent studies was to find a selective reaction allowing the replacement of the fluorine ligand. I have demonstrated that phosphorfluoridites P(III)-F act as phosphitylating reagents in reaction with alcohols in the presence of base to obtain phosphotriester P(III)-OR. I have also find out a general and highly efficient procedure for conversion of P(III)-F compounds into corresponding P(III)-CF_3 by reaction with $(CH_3)_3SiCF_3$ reagent.[8] This reaction requires the presence of fluorine anion as catalyst (Scheme 3). This significant recent result was achieved outside the range of my Ph.D. work thesis.



R, R' : alkyl, alkoxyl, nucleosid-*3'*-yl, nucleosid-*5'*-yl

Scheme 3

In conclusion:

1. I achieved the first synthesis of diastereomerically pure dinucleosidyl phosphorfluoridites, dinucleosidyl phosphoro-fluoridates and phosphorofluoridothionates.
2. I have found highly efficient and general activators of phosphitylating reactions (trimethylchlorosilane and 2,4-dinitrophenol) and explained their action by the corresponding mechanistic schemes.
3. I have demonstrated that phosphorfluoridites act as efficient phosphitylating reagents with leaving fluorine ligand.

References

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