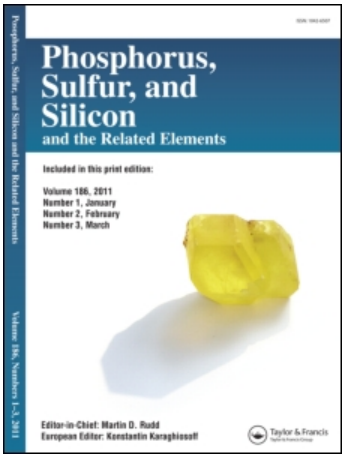

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NOVEL CHEMISTRY AND STEREOCHEMISTRY OF P-F MODIFIED OLIGONUCLEOTIDES

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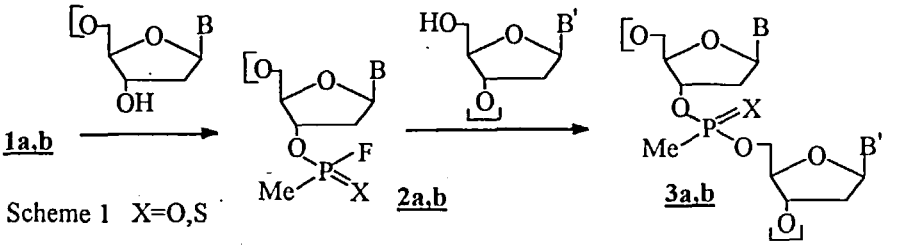
This communication deals with further progress in chemistry and stereochemistry of deoxynucleosidylphosphorofluoridates and fluoridites [1].

Methylphosphonate oligonucleotides are important antisense inhibitors of gene expression and possess the criteria to become useful as therapeutic agents [2]. Synthesis of methylphosphonates oligonucleotides via P(III) intermediates has proved costly, and inefficient to conduct on a large scale. In our search for an improved synthesis of these compounds we concluded that a route via P(IV) coordinate phosphorus compounds was feasible. It is known that replacement of the first fluorine ligand in compounds $RP(O)F_2$ and $RP(S)F_2$ is faster than that of the second. This should be even more pronouced in the reactions with 3'-OH nucleosides. Our recent work showed that these expectations are correct. Methylphosphonodifluoridate $MeP(O)F_2$ **1a** and its sulfur analogue $MeP(S)F_2$ **1b** can be readily prepared in one-flask procedures from the commercially available dichlorides $MeP(S)Cl_2$ or $MeP(S)Cl_2$ in over 90% yield. [1]



Scheme 1 X=O,S

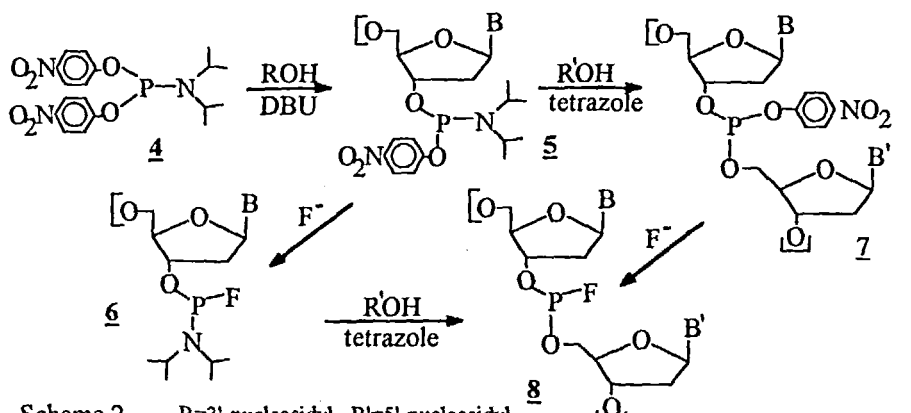
When the 5'-O-protected deoxynucleosides were allowed to react with difluorides **1a,b** in the presence of triethylamine in the proportion 1:1, the 3'-

deoxynucleosidylphosphonofluoridates **2a** or the 3'-deoxynucleosidylfluoridomethylphosphonothionates **2b** are formed in over 95% yield [3].

Both fluorides **2a,b** are formed as 1:1 mixture of diastereoisomers. High reactivity of **2a** prevented their separation into pure diastereoisomers. In contrast thioanalogues **2b** are more stable and were separated into „slow" and „fast" diastereomers by silica-gel column chromatography. Coupling of the fluoridate **2a** or **2b** in the presence of DBU or NaH with another nucleosides led to di(deoxynucleosidyl)phosphonates **3a** or their thioanalogues **3b** respectively. As expected difluoride **1b** and fluoride **2b** containing the P(S) group undergo condensation reactions with nucleosides more slowly than **1a** and **2a** [3]. Work is in progress to use pure diastereomers **2b** in stereoselective preparation of compounds **3b** and other derivatives.

Relatively little is known about the chemistry and stereochemistry of compounds containing a P(III)-F functional center. Cyclic diastereomeric phosphorofluoridite has been prepared by Mikołajczyk et al.[4], and the first resolution of free fluorophosphane MePhPF has been achieved only recently [5]. In the past the chemistry of modified nucleotides containing P(III)-F bond was a void field. Recently this type of compounds became of great importance in our studies[6].

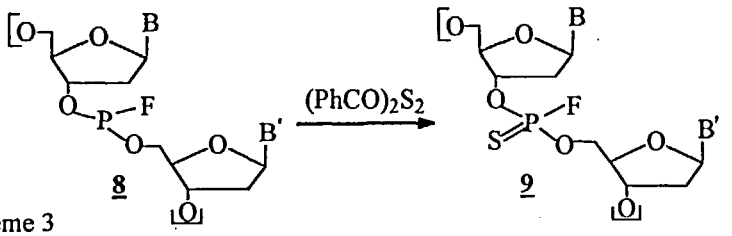
In connection with this chemistry new phosphitylating reagents containing the 4-nitrophenoxy leaving group have been devised. Our strategy can be exemplified by phosphitylation reactions employing amidophosphite **4** [7].



Scheme 2 R=3'-nucleosidyl, R'=5'-nucleosidyl

The reaction of **4** with a nucleoside in the presence of DBU allows quantitative formation of the amidite **5** which reacts in a highly selective way with $Bu_4N^+F^-$ to give the nucleosidyl phosphoroamidofluoridite **6**. There are two pathways leading to dimers **8** as shown in Scheme 2. Surprisingly, the monofluoride **6** can be transformed into the dimer **8** without affecting the P-F bond. The nucleosidyl phosphoroamidofluoridites **6** are formed with some degree of stereoselectivity and can be separated into pure diastereoisomers. However, their coupling reaction with nucleosides in the presence of tetrazole under normal conditions affords diastereoisomers **8** as 1:1 mixtures in almost quantitative yield. The high configurational and chemical stability of phosphorofluorides **8** can be explained by the presence of the electronegative fluorine ligand and steric hindrance exerted by the nucleosidyl groups [7].

To underline the desirable features of phosphorofluoridites **8**, their use in stereospecific synthesis of phosphorofluoridothionates and their hydrolytic stability can be cited. The reaction of the phosphorofluoridite **8** with bis(benzoyl)disulfide leads to a single diastereomer **9** (Scheme 3).



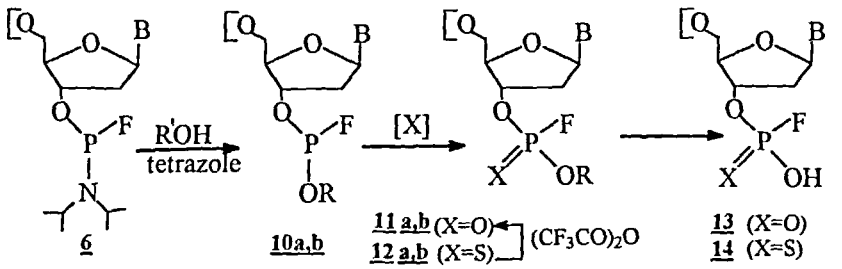
8 **9**

Scheme 3

Compounds **9** containing a thiophosphoryl group are distinctly more resistant towards hydrolysis and other nucleophilic displacements than their oxo analogues. Hydrolytic susceptibility of phosphorofluoridates and phosphorofluoridothionates is strongly influenced by the presence of fluoride ions. The same phenomenon was also observed in our studies [7]. Unexpected high stability of phosphorofluoridites **8** towards hydrolysis is somewhat surprising.

Another example of our strategy is a novel approach towards synthesis of ionic 3'- or 5'-nucleosidylphosphorofluoridates RO-P(O)(OH)F **13** and 3'- or 5'-nucleosidyl phosphorofluoridothionates RO-P(S)(OH)F **14** via P(III)-intermediates **6**. Thioacids **14** were unknown within nucleotide chemistry.

Thiophosphoroamidofluoridites **6** react smoothly at room temperature in the presence of tetrazole with an equivalent amount of tert-butanol or 2-cyanoethanol to give the corresponding fluoridites **10**.



Scheme 4 **a**:R=Bu^t **b**: R=CH_2CH_2CN B: Th,Ad(Bz)

Oxidation of phosphorofluoridities **10a,b** by tert-butylhydroperoxide or addition of elemental sulfur gave the fluoridates **11a,b** or fluoridothionates **12a,b**. Both compounds **11a,b** and **12a,b** were converted by elimination of 2-methyl-1-propen or vinyl cyanide into the desired final products **13** and **14**. Phosphorofluoridothionates **12a,b** were converted by trifluoroacetyl anhydride into the corresponding oxo derivatives **11a,b**. Compounds **14** have a chiral phosphorus center and their separation into pure diastereoisomers is currently being studied.

We believe, that further developments in the field of P(III)-F modified nucleotides may lead up to even more significant discoveries of general importance in bioorganic chemistry.

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