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UNCONVENTIONAL ACTIVATORS IN THE SYNTHESIS OF OLIGONUCLEOTIDES AND THEIR STRUCTURAL ANALOGUES

Wojciech Dabkowski and Jan Michalski

Centre of Molecular and Macromolecular Studies Polish Academy of Sciences
Sienkiewicza 112, 90-363 Lodz, Poland

E-mail: wdabkow@bilbo.cbmm.lodz.pl; jmich@bilbo.cbmm.lodz.pl

Abstract – Activation of phosphoramidites as phosphitylating reagents in the synthesis of biophosphates and their structural analogues is of great importance. Tetrazole a customary activator can be conveniently replaced by trimethylchlorosilane (TMCS) or 2,4-dinitrophenol. Advantages of use of these reagents in the synthesis of biophosphates and their mechanism of action are discussed. Phosphites containing 4-nitrophenoxy leaving group are activated by DBU which role is revealed. Readily accessible P(III)-F structures, can be “activated” by their facile transformation into the corresponding bromides using trimethylbromosilane (TMBS). Application of phosphoramidites in synthesis of phosphates of biological interest and their activation by azolides and acids salts, including and stereochemical aspects was described in 'Topics in Current Chemistry'.⁴

R. L. Letsinger's original nucleosidyl phosphite synthesis was based on chlorophosphites and was too difficult for general use as it required special precautions.¹ The method was modified by M. H. Caruthers who used phosphoroamidites which are relatively stable compounds.² There was a need to activate the amide leaving group, and tetrazole became the most widely used activator. In spite of impressive progress in this field there are still important problems to be solved. When the necessity arises to produce phosphates of alcohols of biological importance in large quantities, better procedures are required for activation of phosphoroamidites. It also seems to be desirable to find leaving groups other than amide groups at the tricoordinate phosphorus center which are suitable for coupling procedures under alkaline conditions. In this area P(III)-compounds containing an aryloxy group or a fluorine ligand are likely to act as valuable phosphitylating reagents. As expected, these systems show chemical and stereochemical

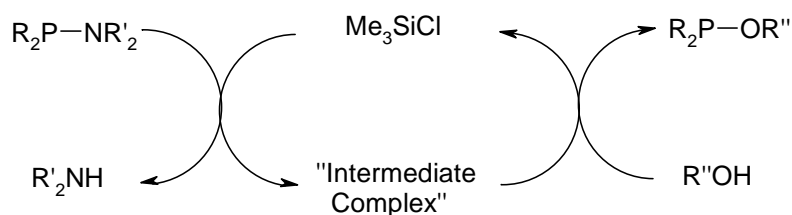
stability. Such P(III)-compounds are of great interest for application in the synthesis of modified oligonucleotides which contain a chiral center at the phosphorus atom.

ACTIVATION OF PHOSPHORAMIDITES BY TRIMETHYLCHLOROSILANE

Phosphoramidites are the phosphitylating reagents most often used in the synthesis of various biomolecules such as oligonucleotides, sugar phosphates, phosphoinositols, and phospholipids.^{3,4} An indispensable part of the phosphoramidite strategy is the employment of a proper activator and a suitable protecting group at the phosphorus centre. Coupling with alcohols is controlled by steric and electronic effects. Bulky substituents in the amido group can help to control selectivity of a coupling, preventing formation of a symmetrical structure. This can be achieved by employing *N,N*-diisopropylamino derivatives which are most frequently used because of their comparatively high stability. Relatively low reactivity of *N,N*-diisopropylamidites is caused by electronic and steric factors which can be compensated by appropriate activation. Tetrazole is by far the most commonly used activator in coupling reactions of phosphoramidites with alcohols and other nucleophiles. However, this activator suffers from some disadvantages. Tetrazole is expensive, explosive, hygroscopic and sparingly soluble in acetonitrile, the solvent most frequently used in the coupling reaction. It may induce transesterification of trialkylphosphites.⁵ Tetrazole is not effective, or must be used in large excess, when strongly electronegative substituents are attached to the P^{III} center.⁶ These problems have become even more pronounced in the synthesis of oligonucleotide phosphorothioates at production scale.

Our earlier work on interaction of P(III) amides with halogenosilanes suggested that phosphoramidites would react with alcohols in the presence of trimethylchlorosilane (TMCS) as catalyst.⁷ Indeed, the reaction of P(III) amidites with an equivalent amount of nucleoside proceeds in the presence of TMCS in very high yield and at rates comparable or higher than those when tetrazole is used. Phosphitylations activated by TMCS proceed at room temperature in solvents such as THF, CH₂Cl₂ or MeCN. On average, the amount of activator required for an efficient coupling is ca. 30-60% of the stoichiometric ratio.⁸

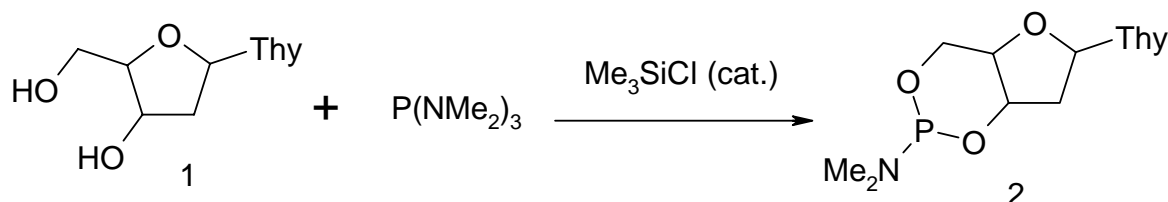
The mechanism of activation by TMCS is presumed to involve its reaction with P(III) amide (Scheme 1). The first hypothetical step produces the salt-like species R₂P⁺(SiMe₃)NR'₂Cl⁻ or R₂PN⁺R'₂(SiMe₃)Cl⁻ which react either directly with alcohol to give ester R₂POR or *via* intermediate formation of R₂PCl. Attempts to evidence formation of salt-like species by ³¹P NMR spectroscopy failed. Formation of R₂PCl was confirmed by ³¹P NMR spectroscopy. In both cases TMCS is regenerated. The following cycle involved in the activation is proposed.



Scheme 1

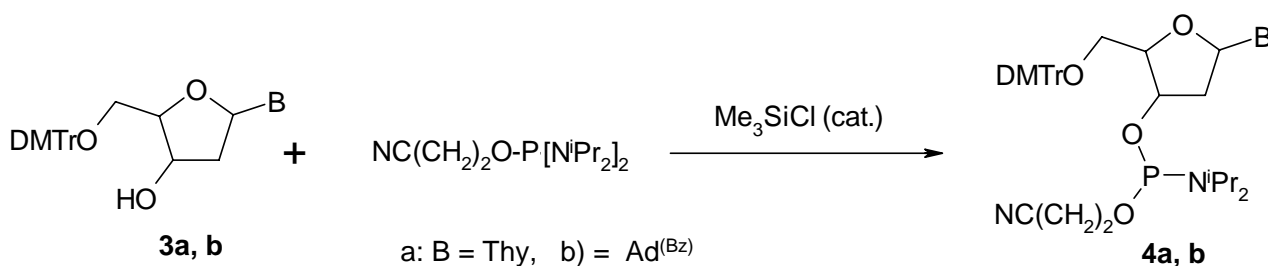
An alternative mechanistic path may be considered in which TMCS reacts with alcohol to form hydrogen chloride which then activates an P(III)-amide *in situ*. But it is well known that TMCS reacts very slowly with alcohols unless a catalyst is present.⁹

An example of activation in the presence of a catalytic amount of TMCS (0.6 equiv.) is the reaction of thymidine (**1**) with tris(dimethylamino)phosphine to give thymidine 3',5'-cyclic dimethylphosphoramidite (**2**) in 95% yield (Scheme 2).⁸ Activation by tetrazole is visibly less effective.¹⁰



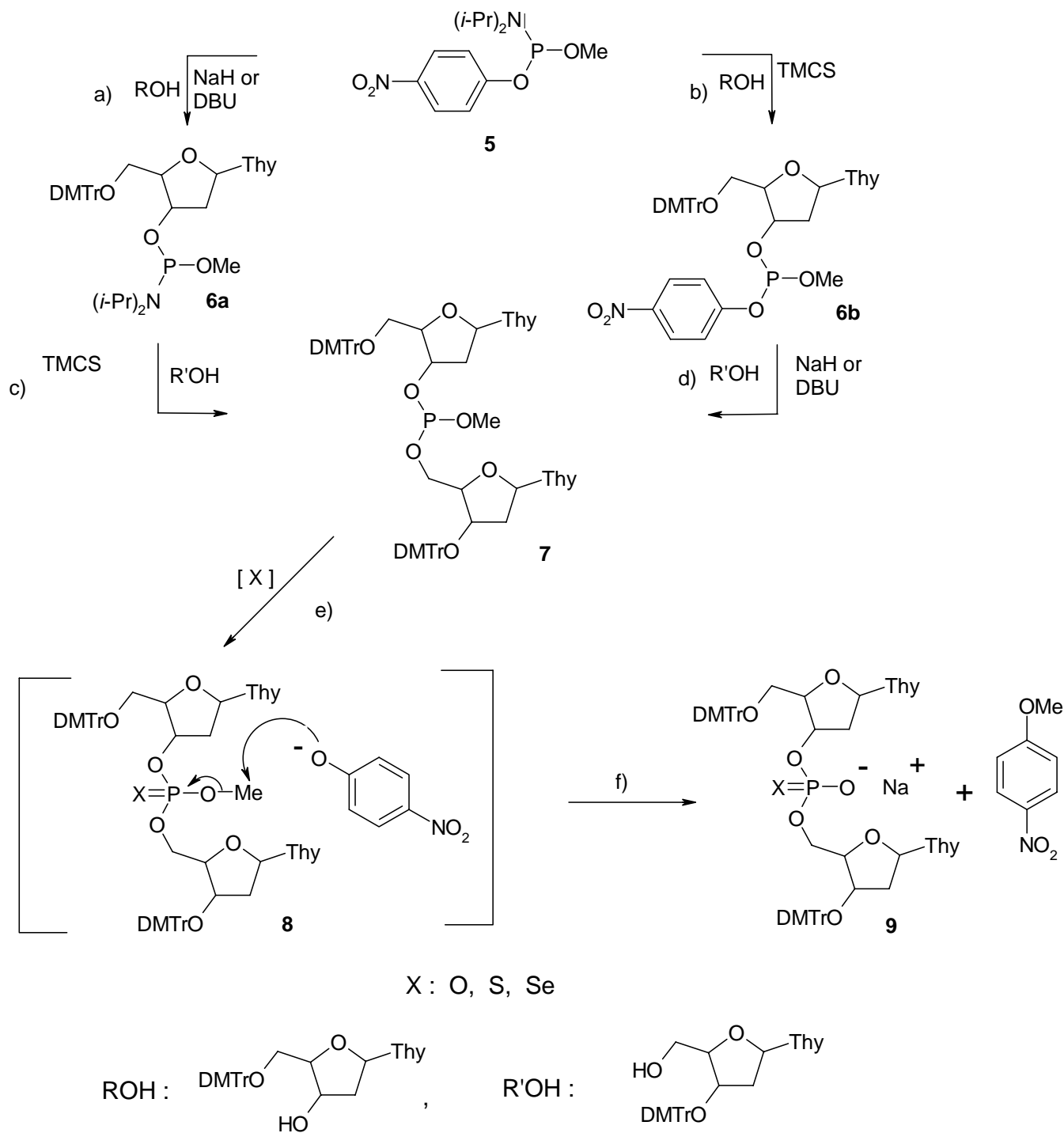
Scheme 2

Commercially available bis(diisopropylamino)-2-cyanoethoxyphosphine reacts selectively in the presence of TMCS (0.6 equiv.) with 5'-O-DMTr-nucleoside (**3a, b**) to form 5'-O-DMTr-thymidine 3'-O-(2-cyano-*N,N*-diisopropyl)phosphoramidite or N6-benzoyl-5'-O-DMTr-deoxyadenosine 3'-O-(2-cyanoethyl-*N,N*-diisopropyl)phosphoramidite (**4b**) in over 97% yield (Scheme 3). The yield and purity of amidites are identical to those obtained by activation with tetrazole.¹¹ This type of procedure has been applied to a wide variety of alcohols of biological interest. The activation works well with sterically hindered alcohols. Formation of hydrogen chloride would effect the removal of the acid labile dimethoxytrityl DMTr protection group used in the synthesis of oligonucleotides. This is actually observed when commercial TMCS is contaminated with HCl. When TMCS is free of hydrogen chloride the DMTr group is retained.¹²



Scheme 3

O-Methyl-*O*-4-nitrophenyl-*N,N*-diisopropylphosphoramidite, which can be qualified as a bidentate phosphitylating reagent having two different leaving groups, is readily available and shows high stability. Michalski *et al.* have applied this reagent in the synthesis of natural and modified dinucleotides.¹³ An example of its use in a synthetic procedure is given in Scheme 4.



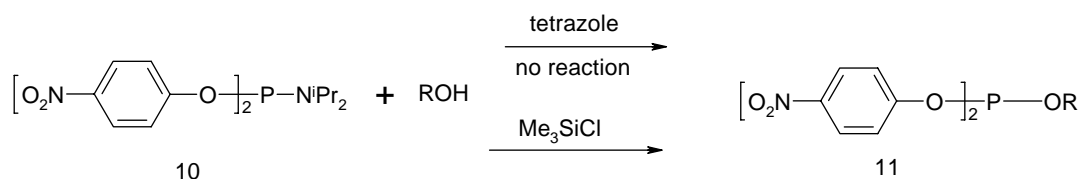
Scheme 4

The intermediate mononucleoside phosphites (6a, b) are formed *via* activation either of the 4-nitrophenoxy group of (5) by DBU (step a) or of the diisopropylamido group by TMCS (step b).

Dinucleoside methylphosphite (**7**) was prepared from the corresponding monophosphites (**6a, b**) in a similar manner (step c and d).

Fast oxidation by dry air or elemental sulfur (selenium) was observed and attributed to the presence of 4-nitrophenoxide anion (step e). Even more remarkable is high capability of the 4-nitrophenolate anion to demethylate the intermediate phosphate (**8**) (step f). This step is so rapid that we were unable to observe the intermediate methylphosphate or corresponding sulfur or selenium analogues (**8**). This series of reactions can be performed as a one-flask procedure with a total yield of (**9**) in over 90%. The involvement of DBU 4-nitrophenolate in the oxidation of methylphosphonites R_2P-OMe and dealkylation of $R_2P(X)OMe$ structures has been confirmed on several model compounds.¹⁴

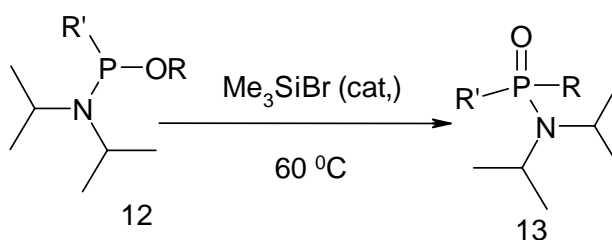
Phosphoramidites containing a strongly electronegative group are more efficiently activated by TMCS than by tetrazole (Scheme 5). For example, bis(*O*-4-nitrophenyl)-*N,N*-diisopropylphosphoramidite (**10**) is an efficient phosphitylating reagent via exchange of the aryloxy group. Exchange of the amido group is not practicable with tetrazole activation but can be readily performed in the presence of TMCS to give **11**.¹⁵



Scheme 5

It has been also demonstrated that activation by tetrazole may give rise to some side reactions which can be avoided when TMCS is used.¹⁶

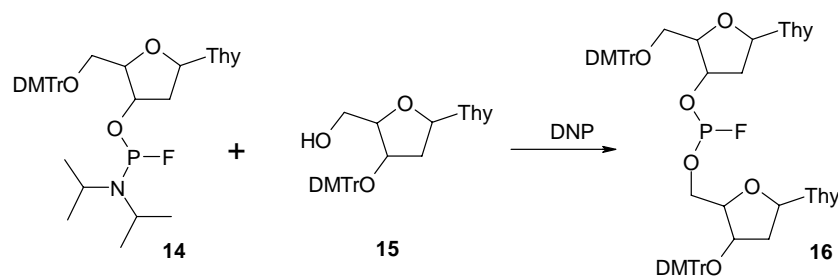
Trimethylbromosilane (TMBS) is a more potent activator of phosphoramidites than TMCS (Scheme 6). However its application raises additional problems. TMBS may cause the rearrangement of an P(III) amidoester (**12**) into a $>P(O)$ structure (**13**) with formation of a P-C bond.¹⁷ TMCS is inert to this type of reaction.



Scheme 6

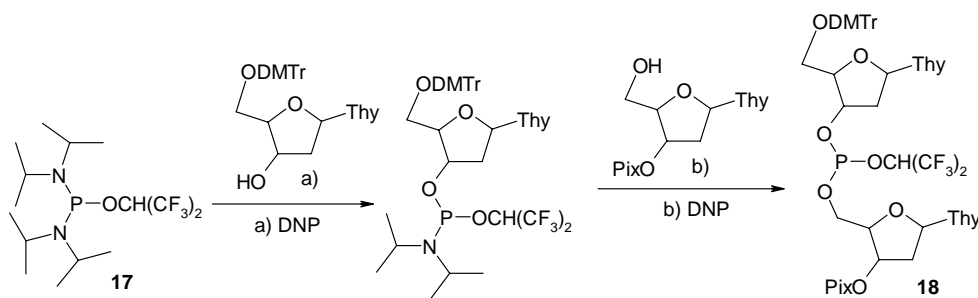
ACTIVATION OF PHOSPHORAMIDITES BY 2,4-DINITROPHENOL.

Dabkowski *et al.* have found that 2,4-dinitrophenol (DNP), whose pKa value 4.1 is close to that of tetrazole (4.9), acts as an efficient activator of phosphate synthesis *via* the phosphoramidite procedure.¹⁶ The reaction of P(III) amidites with an equivalent amount of nucleoside in the presence of DNP proceeds in very high yield and at rates comparable or higher than those when tetrazole is used. Phosphitylations activated by DNP take place at room temperature in aprotic solvents like THF, CH₂Cl₂ or MeCN. On average the optimal amount of activator required for efficient coupling is *ca* 1.5 equivalents of the stoichiometrical ratio. As in the case of TMCS, phosphoramidites (**14**) containing a strongly electronegative group like fluorine at the P(III) center are more efficiently activated by DNP than by tetrazole and react with 3'-O-DMTr-thymidine (**15**) yielding the nucleoside phosphorofluoridite (**16**) (Scheme 7).



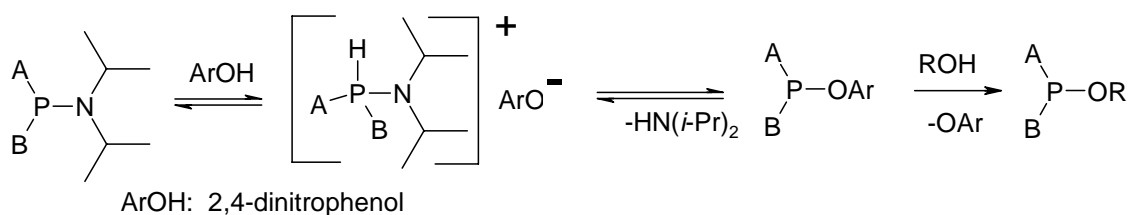
Scheme 7

Similar situations involving the electronegative leaving group OCH(CF₃)₂ have been observed in the phosphitylating procedure introduced by Tataka *et al.* (Scheme 8).¹⁹ Both steps *a* and *b* are highly selective and final dinucleotides (**18**) are formed in over 95% yield from amidite (**17**) at room temperature in THF solution.



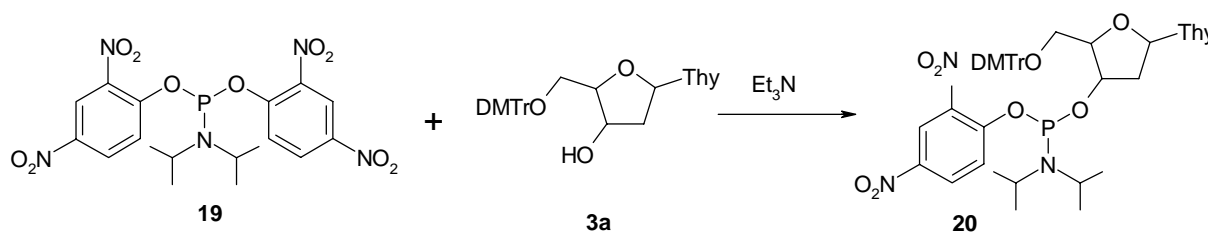
Scheme 8

Mechanistic features of the DNP activation are shown in Scheme 9.



Scheme 9

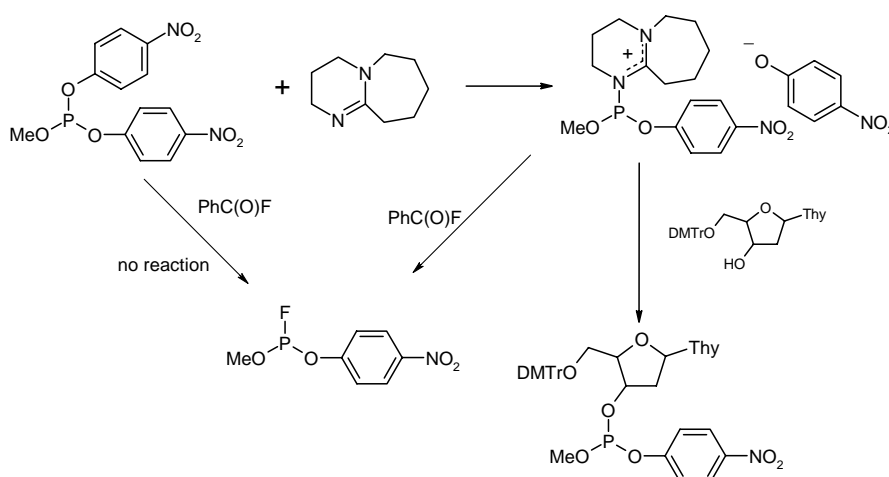
Studies on activation by DNP have paved the way for a novel type of phosphitylating reagent. Spontaneous displacement of the 2,4-dinitrophenoxy group in the reaction of the reagent with alcohols liberates DNP. The latter activates the amino group allowing further ligand exchange. However if phosphitylation of 3a by the bis(2,4-dinitrophenyl)phosphoroamidite (**19**) is performed in the presence of one equivalent of triethylamine, high chemoselectivity is observed, and the nucleosidyl (2,4-dinitrophenyl)phosphoramidite is formed in over 92% yield (Scheme 10).



Scheme 10

ROLE OF DBU IN THE REACTION OF P(III)-OAR SYSTEMS WITH ALCOHOLS

DBU is often referred to as non-nucleophilic strong base. On the other hand the efficiency of DBU in some transformations is related to its nucleophilicity. The procedure involving displacement of a 4-nitrophenoxy ligand by a nucleosidyl-OH group may qualify for the latter category. The involvement of a $(=P-N\equiv)^+$ cationic species has been demonstrated in phosphitylation of nucleosides in the presence of DBU by ³¹P NMR studies and its reaction with benzoyl fluorides (Scheme 11).²⁰

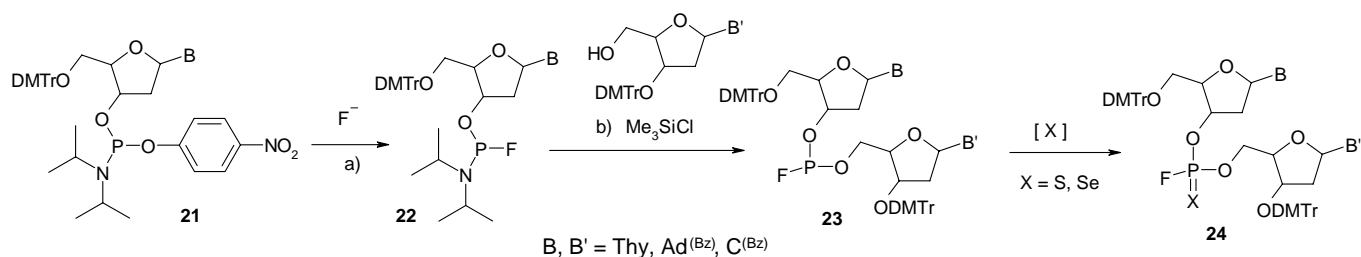


Scheme 11

PHOSPHITYLATING REAGENTS CONTAINING A PHOSPHORUS-FLUORINE BOND

Easy access to P(III)-OAr compounds allowed us to synthesize a number of P(III)-F structures derived from nucleosides and other alcohols of biological interest.

The synthetic pathway leading to the dinucleosidyl phosphorofluorides (**24**) is shown in Scheme 12.²¹



Scheme 12

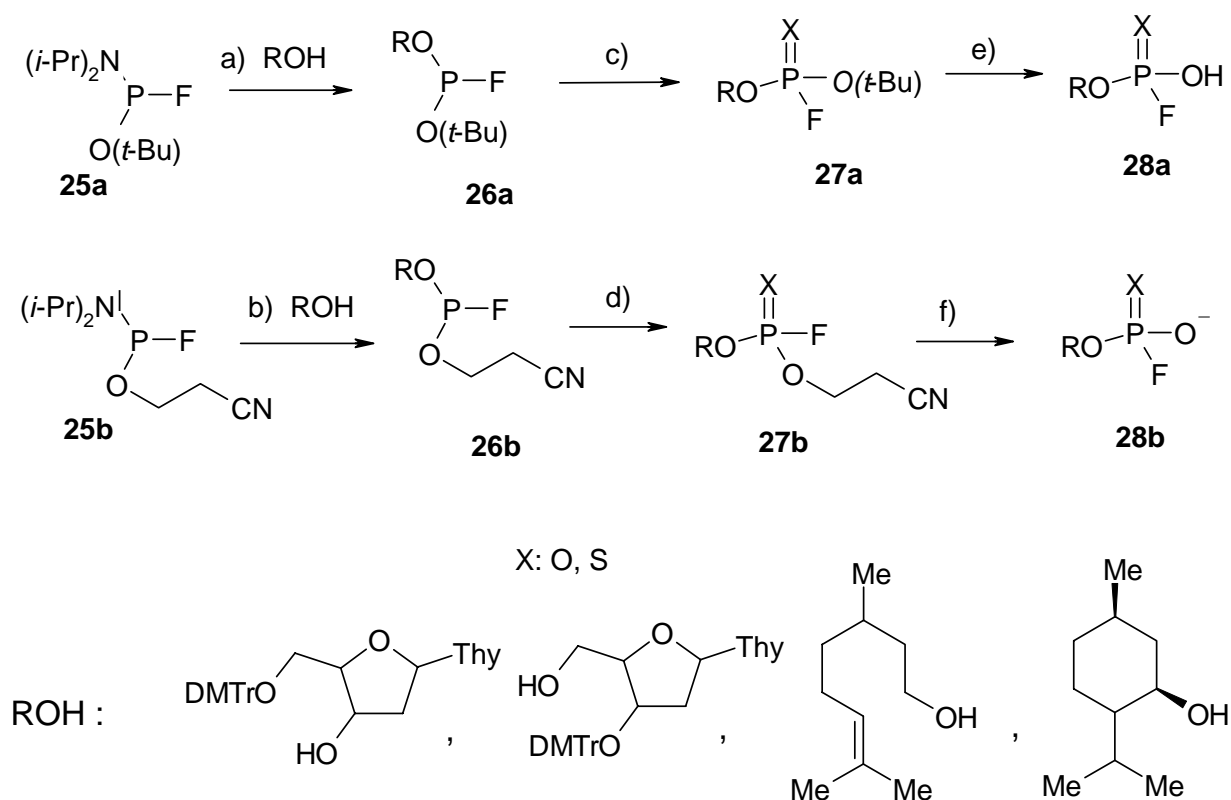
The mono nucleosidyl phosphoroamidofluoridites (**22**) are formed from **21** with some degree of stereoselectivity and can be separated into pure diastereoisomers. However, their coupling reaction with 3'-*O*-protected nucleosides in the presence of TMCS affords dinucleosidyl phosphorofluoridite (**22**) as 1:1 mixtures of diastereomers in almost quantitative yield. It is noteworthy that this coupling does not affect the phosphorus-fluorine bond. Chromatography of phosphorofluoridites on silica gel gives pure "fast" isomers of high configurational stability. This is the first case in nucleotide chemistry of obtaining relative stable P(III)-F diastereoisomers with a chirality center at the phosphorus atom. Relatively little is known about the chemistry and stereochemistry of organophosphorus compounds containing the P(III)-F functional centre. *trans*-2-Fluoro-4-methyl-1,3,2-dioxaphosphorinane has been prepared by Mikolajczyk *et al.*²² The first resolution of a free fluorophosphane MePhPF has been achieved only recently.²³ Interestingly, the optical stability of this phosphane was much lower than of those of dinucleosidyl phosphorofluorides.

The configurational stability of phosphorofluoridites can be explained by the presence of the strong P-F bond and steric hindrance exerted by the nucleosidyl groups. Diastereoisomeric P(III)-F nucleotide compounds racemize in the presence of F^- donors via achiral intermediates containing two fluorine ligands in apical positions.²⁴ Pure diastereoisomers separated by chromatography were transformed in a stereospecific way into the corresponding P(X)-F ($X=S, Se$) compounds (**24**). It is most likely that the transformations presented above proceed with retention of configuration at the stereogenic center connected to the phosphorus atom.

As expected, compounds containing a thiophosphoryl centre are distinctly more resistant towards hydrolysis and other nucleophilic displacements than their oxo analogues. The hydrolytic susceptibility of phosphorofluoridates and phosphorofluoridothionates is strongly influenced by the presence of fluoride ions.²⁵ The relatively high stability of phosphorus(III) phosphorofluoridites towards hydrolysis is somewhat surprising.

Two new readily available amidite reagents containing a fluorine group have been synthesized in our laboratory. Both phosphitylating reagents (**25a, b**) containing *tert*-butyl or 2-cyanoethyl protecting group react with alcohols in the presence of TMCS at ambient temperature (Scheme 13, steps a and d).

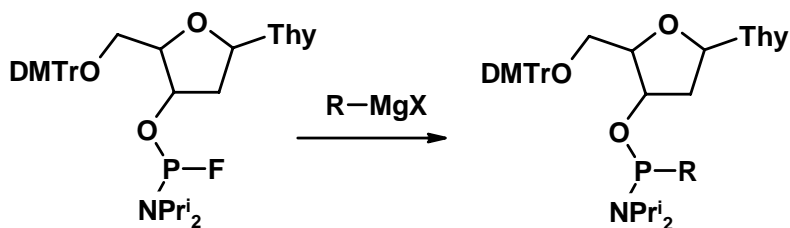
The latter reagent proved to be the superior activating reagent. The corresponding fluorophosphates (**26a, b**) were formed in very high yield. Oxidation by hydrogen *tert*-butyl peroxide or addition of elemental sulfur (selenium) (steps c and d) proceed in very high yield to give the intermediate phosphorofluoridates (**27a, b**) or their sulfur and selenium analogues. The final steps e and f involve thermal elimination of 2-methyl-1-propene or acetonitrile respectively to give the desired fluorophosphorus, fluorothio and fluoroseleno acids in very high yield. Reactions described in Scheme 13 are best performed as one flask procedures with overall yield above 95%. It is noteworthy that in the above coupling procedure activated by TMCS, the *tert*-butyl protective group is unaffected. The method described in Scheme 13 is more convenient than the one described by us earlier²⁶ and also by other authors.²⁷



Scheme 13. *Reagents and conditions:* a, b) ROH, TMCS, THF, 1 h; c) *tert*-butylhydroperoxide, THF, 1 h; d) sulfur in $(C_3H_7)_2NH$, THF, 2 h; e) MeCN, reflux, 2h; f) Et_3N , THF, 6 h

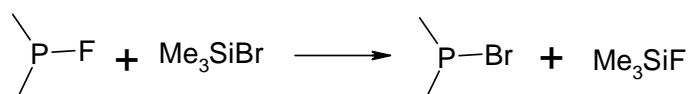
THE ACTIVATION OF P(III)-F GROUP

Compounds containing the P(III)-F moiety are visibly inert towards alcohols. They react selectively with organometallic reagents to form P(III)-C bonds without affecting the nucleosidyloxy residue.¹⁴



Scheme 14

Our recent studies show that P(III)-F structures containing a fluorine ligand can be very readily converted into P(III)-Br bromides by reaction with TMBS or trimethyliodosilane (Scheme 15).¹⁴



Scheme 15

The driving force of these reactions seems to be a high affinity of fluorine toward the silicon center. Me₃SiF is inert to alcohols. This type of activation of P(III)-F structures opens a new perspective for a variety of phosphitylation procedures.¹⁴

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Wojciech. Dabkowski was born in Lodz, Poland. He received his M.Sc. degree from the University of Lodz and his Ph.D. from the Centre of Molecular and Macromolecular Studies of Polish Academy of Sciences with of Professor Jan Michalski, and held postdoctoral research fellowships at the Max-Planck-Institute of Experimental Medicine (Goettingen, Germany) with Professor Friedrich Cramer. Dr. Dabkowski has edited 3 book chapters and 60 research papers. His research interest include synthesis and stereochemistry of organophosphorus derivatives.



Jan J. Michalski was born in Lodz, Poland. He received his M.Sc. degree from Cracov University and Ph.D. degree from Cambridge University (Christ's College) with Lord Tood who inspired him to pursue phosphorus chemistry and biochemistry. He is founding Director of Centre of Molecular and Macromolecular Studies of Polish Academy of Science. At present he is emeritus professor. For over 40 years Michalski has been one of leaders organophosphorus chemistry. Michalski's research covers synthetic and structural studies of organophosphorus compounds, stereochemistry of P-chiral compounds. Application of spectral method in elucidation of reaction mechanisms of organophosphorus compounds, phosphorus anhydrides. Organophosphorus pseudohalogens. Organosulfur and organoselenophosphorus compounds. Modified nucleotides containing phosphorus-fluorine bond. Methods of phosphorylation of alcohols of biological interest. He has published approximately 400 papers and co-authored six books. He served on Editorial Boards: *Tetrahedron*, *Tetrahedron Letters*, *Tetrahedron Asymmetry*, *Physical Organic Chemistry*, *Chemical Communications*, *Topics in Stereochemistry*, and others. His research has been recognized with numerous Distinguished Professorships. Michalski is a Fellow of Polish, French and Berlin Academy of Sciences, he is also a fellow of Royal Chemical Society (London). He is recipient of Humboldt Foundation Prize and Arbuzov Prize.