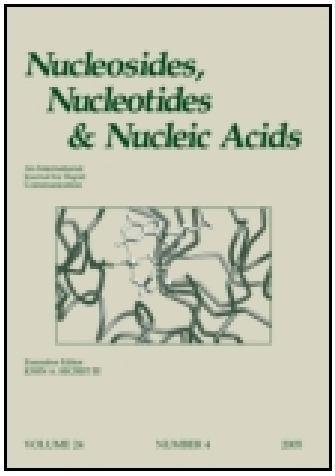

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Novel Unique Catalytic Activating Reagents in Synthesis of Biophosphates via the Phosphoramidite Route[1#]

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Abstract: *Trimethylchlorosilane (TMSCl) and 2,4-dinitrophenol are remarkably efficient activators in reaction of P(III) amides with nucleosides to give P(III) esters in excellent yield.*

Our recent studies on nucleosidyl phosphorofluoridates and nucleosidyl phosphorofluoridites led us to explore unconventional activators in the reaction of phosphoroamidites with alcohols of biological interest. Some years ago we were able to observe that the reaction of tris(dimethylamino)phosphine with trimethylsilyl halides leads to bis(dimethyloamino)halido-phosphine[2]

$$(Me_2N)_3P + Me_3SiX \rightleftharpoons (Me_2N)_2PX + Me_2NSiMe_3$$

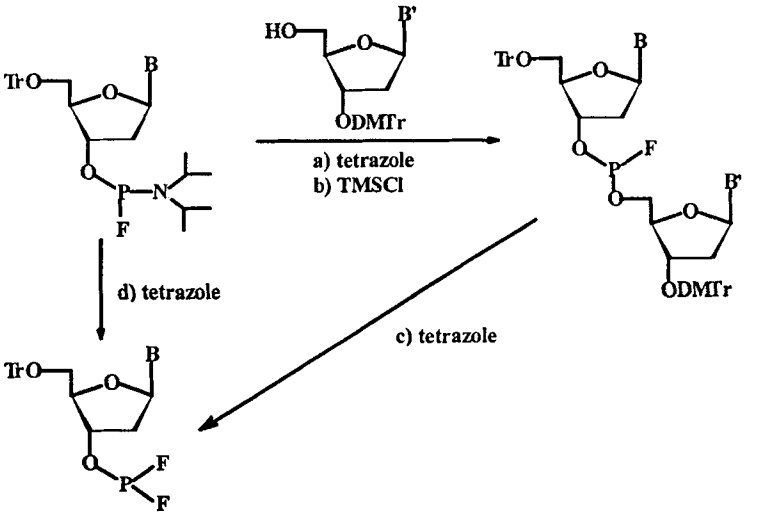
X = Br, I

At ambient temperature this reaction occurs immediately in methylene chloride solution. But at -50°C no formation of the aminohalidophosphine was observed. However, the ^{31}P NMR signals of $P(NMe_2)_3$ were drastically changed following addition of trimethylhalidosilane, indicating a strong interaction of the species. At temperatures below -50°C ^{31}P NMR spectra as well as conductometric measurements indicate an equilibrium. When X = Cl the equilibrium is shifted towards substrates.

$$(Me_2N)_3P + Me_3SiX \rightleftharpoons \left[(Me_2N)_3\overset{\oplus}{P}SiMe_3\right]\overset{\ominus}{X} \rightleftharpoons \left[(Me_2N)_2P{-}\overset{\oplus}{N}Me_2SiMe_3\right]\overset{\ominus}{X}$$

X = Br, I, Cl

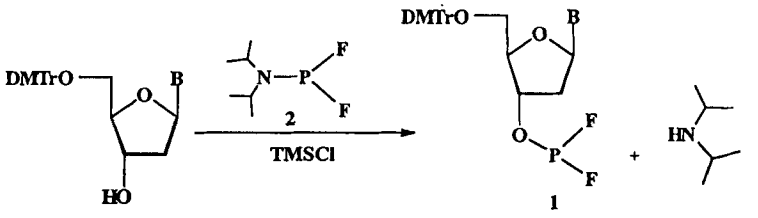
Thus taking into consideration the rapid reversibility of the reaction and the fact that trimethylchlorosilane does not react with alcohols, unless a catalyst is present [3], we anticipated



Scheme 1

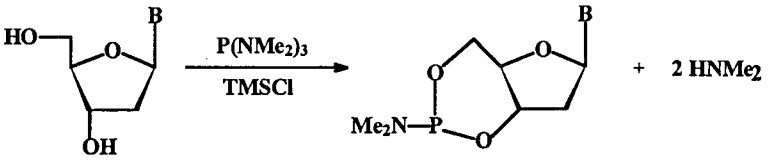
that it would activate the coupling reaction of phosphoroamidites with alcohols. This proved to be so, and was highly efficacious from preparative point of view.

There are several reasons for replacing tetrazole by more convenient activators. This compound is expensive, must be very pure, and needs to be used in large excess.[4] In our experience tetrazole may cause some side reactions of phosphoroamidites other than the simple coupling with alcohols leading to phosphites. For example the reaction shown on Scheme 1 may proceed in two directions depending on the reaction conditions and the amount of tetrazole used. In the absence of alcohols the reaction (d) takes place and leads to the nucleosidyl difluoridites **1**. The formation of **1** has been explained by J.Engels and et al. [5] as reaction a followed by the reaction (c). In contras the reaction (b) proceeds in the presence of TMSCl (0.6 molar equivalent) without formation of the difluoridite **1**. The difluoridite compound can be prepared by an independent route:



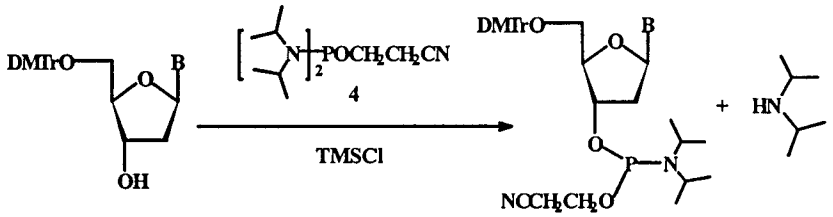
The diisopropylaminodifluorophosphine **2** was prepared in situ in CH_3CN solution from diisopropylaminodi(4-nitrophenoxy)phosphine in the reaction with TBAF at 20°C.

A remarkable example of activation in the presence of a catalytic amount of TMSCl (0.6 eq) is the reaction of thymidine with tris(dimethylamino)phosphine to give thymidine 3',5'-cyclic dimethylphosphoramidite **3** in over 95% yield. Activation by tetrazole is less effective in this case. The yield of cyclic amidite **3** is poor in the absence of TMSCl.[6]



3

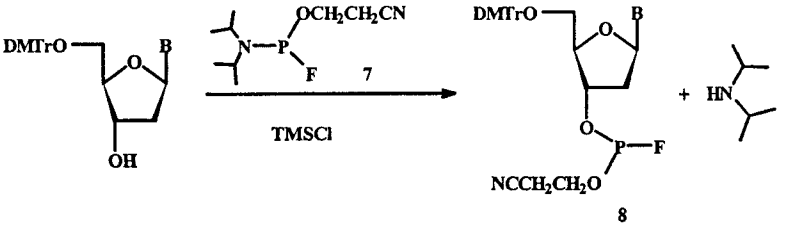
Commercially available bis(diisopropylamino)-2-cyanoethoxyphosphine **4** reacts in the presence of TMSCl (0.6 eq) with 5'-O-DMTr-nucleoside **5** in a highly selective way to form 5'-O-DMTr-thymidine 3'-O-(2-cyano-N,N-diisopropyl)-phosphoramidite **6a** and N^6-Benzoyl-5'-O-DMTr-deoxyadenosine 3'-O-(2-cyanoethyl-N,N-dissopropyl) phosphoramidite **6b** in over 97% yield. In this case the yield and purity of amidites **6a,b** are identical to those obtained by activation with tetrazole.[7]



5a: B=Th
5b: B=Ad(Bz)

6a,b

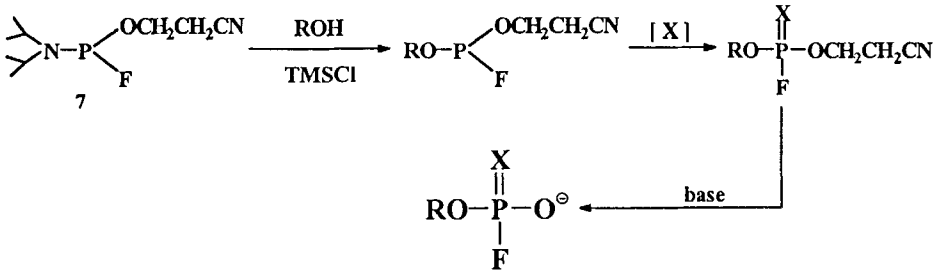
Excellent coupling procedures activated by TMSCl were noted for P(III) amides containing a fluorine ligand. For example, fluoro(diisopropylamino)-2-cyanoethoxyphosphine **7** reacts with 5'-O-DMTr-nucleoside **5a,b** in the presence of TMSCl (0.6 eq) to give 5'-O-DMTr-thymidine 3'-O-(2-cyanoethyl)fluorophosphite **8a** and N^6-Benzoyl-5'-O-DMTr-adenosine 3'-O-(2-cyanoethyl)-fluorophosphite **8b** in almost quantitative yield. The analogous reaction activated by tetrazole requires a 5-fold excess of activator and proceeds distinctly slower.



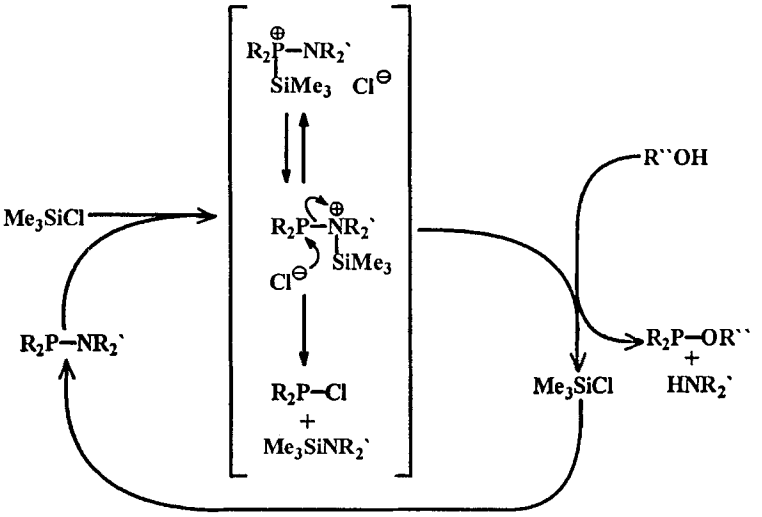
8

8a: B=Th
8b: B=Ad(Bz)

The fluoroamidite **7** was prepared in over 90% yield from chloro(diisopropylamino)-2-cyanoethoxyphosphine via 2-cyanoethoxy diisopropylo-4-nitrophenoxyphosphine by standard ligand exchange procedures. [8] This compound is highly useful in the synthesis of Wittman type nucleosidyl phosphorofluoridates ROP(O)(OH)F and their thio ROP(S)(OH)F and seleno ROP(Se)(OH)F analogues. This procedure is more convenient than that described earlier.[9]



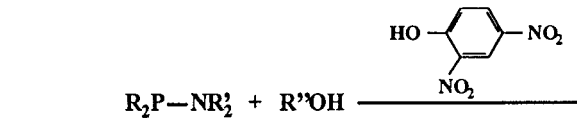
The tentative mechanism of activation by TMSCl is presumed to involve its reaction with P(III) amide. This type of interaction has been discussed in our earlier paper[2] and more recently by Nifantyev.[10] The first step produces salt-like species $R_2P^+(SiMe_3)NR''_2Cl^-$ and $R_2P\text{-}N^+R''_2(SiMe_3)Cl^-$ which react either directly with alcohol to give ester $R_2P\text{-}OR'$ or via intermediate formation of $R_2P\text{-}Cl$. In both cases TMSCl is regenerated.



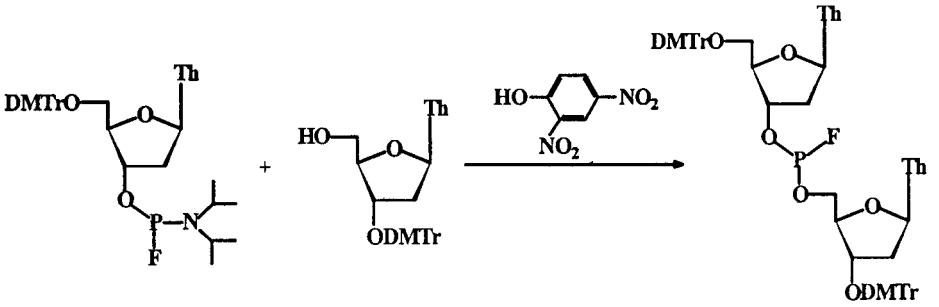
In both cases TMSCl is regenerated. A mechanistic path may be considered in which TMSCl reacts with alcohol to form hydrogen chloride which then activates an P(III) amide in situ. But it is well known that TMSCl reacts very slowly with alcohols unless a catalyst is present.[3] Formation of hydrogen chloride would effect the removal of acid labile DMTr-group. This is actually observed when the commercial TMSCl contaminated with HCl is used. However when hydrogen chloride free TMSCl is utilized, this course is not observed. But even without a full understanding of all the details, the activation procedure by TMSCl works remarkably. Work is

currently in progress aimed at utilizing the TMSCl activation for the synthesis of oligonucleotides and at getting a better understanding of its mechanistic features.

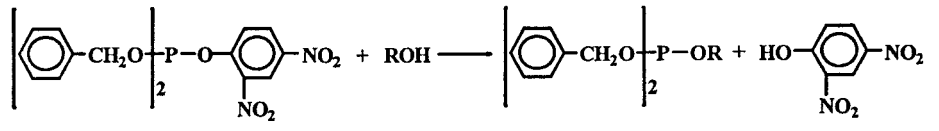
Looking for other unconventional activators we decided to investigate phenols containing electron withdrawing substituents as promotors of the phosphoroamidite coupling with alcohols. These studies led us to the discovery that 2,4-dinitrophenol can be employed as an excellent activator in coupling reactions between phosphoroamidites and alcohols.



Activation of phosphoroamidites by 2,4-dinitrophenol is at least as efficient as in the case of tetrazole or trimethylchlorosilane. The optimal amount of this activator in coupling procedures varies from 30% to 100% of the stoichiometric ratio. In the majority of cases coupling is completed within 5 min. It seems certain that the activation by 2,4-nitrophenol is connected with its high acidity and the excellence of the 2,4- dinitrophenoxy leaving group. Applications of this expedient activation are illustrated below.

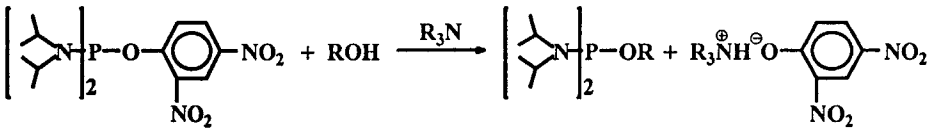


Assuming that the activation by 2,4-dinitrophenol involves the transient formation of the 2,4-dinitrophenylphosphites we designed phosphitylating reagents containing the 2,4-dinitrophenoxy leaving group e.g. dibenzyl-2,4-dinitrophenylphosphite **9** and 2,4-dinitrophenoxy-bis-(N,N-diisopropylamino)phosphine **10**. Both reagents can be readily prepared in excellent yield from commercially available starting materials and are stable, easy to handle and can be stored under moisture free -conditions at 5°C. In contrast to P(III) compounds having mono-nitrophenoxy ligands,[8,11] these compounds react with alcohols at 20°C in aprotic solvents such as dichloromethane or tetrahydrofurane without needing activation.



The reaction is usually complete in a few minutes. Its progress can be followed colorimetrically and this feature could be useful in monitoring phosphorylation procedures leading to polymeric structures. 2,4-Dinitrophenol does not interfere with subsequent oxidation of phosphites to phosphates structures from which benzyl groups can be removed by hydrogenolysis. After oxidation the phosphates formed can be separated from 2,4-dinitrophenol by flash chromatography or by washing-up into the aqueous phase in the presence of mild bases.

The reaction with the diamidite 9 requires trapping of 2,4-dinitrophenol released during the reaction by the equivalent amount of triethylamine in order to avoid activation leading to the diesters $Pr^{i}_{2}NP(OR)_2$.



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