Trimethylchlorosilane: a novel activating reagent in nucleotide synthesis *via* the phosphoramidite route

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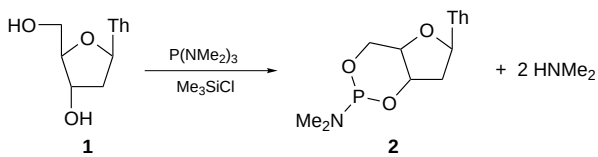
Trimethylchlorosilane (TMSCl) is a remarkably efficient activator in the reaction of phosphorus(III) amides with nucleosides to give phosphorus(III) esters in excellent yield.

Phosphoramidites are among the most important reagents in the synthesis of phosphates of biological interest by the 'phosphite approach'.[1] Tetrazole, tetrazole salts with amines and structural analogues of this heterocycle are generally used as activators of the reaction between alcohols and phosphoramidites.[2] Tetrazole, the most widely employed activator, must often be used in excess and be of high purity, which involves hazardous sublimation. Other activators like amine hydrochlorides[3] and acyl chlorides that act *via* intermediate formation of phosphorchloridites[4] have been used only occasionally.

In our recent studies on the synthesis of modified nuclotides we were confronted with the challenge of finding an alternative mode of activation without using tetrazole or similar compounds. Our earlier work on interaction of P^{III} amides with halogenosilanes[5] suggested to us that phosphoramidites would react with alcohols in the presence of trimethylchlorosilane (TMSCl) as catalyst. This proved to be so, and was highly efficacious from a preparative point of view. The reaction of P^{III} amidites with an equivalent amount of nucleoside proceeds in the presence of TMSCl in very high yield and at rates comparable or higher than those when tetrazole is used. Phosphitylations activated by TMSCl proceed at room temperature in solvents like THF, CH_2Cl_2 or MeCN. On average, the amount of activator required for an efficient coupling is *ca.* 30–60% of the stoichiometrical ratio. Most of our experiments were performed with P^{III} amides derived from diisopropylamine in order to conform to the most popular phosphitylation procedures. Selected examples illustrating our metholology are chosen from nucleotide chemistry.†

A remarkable example of activation in the presence of a catalytic amount of TMSCl (0.6 equiv.) is the reaction of thymidine **1** with tris(dimethylamino)phosphine to give thymidine 3′,5′-cyclic dimethylphosphoramidite **2** in 95% yield. Activation by tetrazole is less effective in this case.The yield of cyclic amidite **2** is poor in the absence of TMSCl.[6]

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



1 **2**

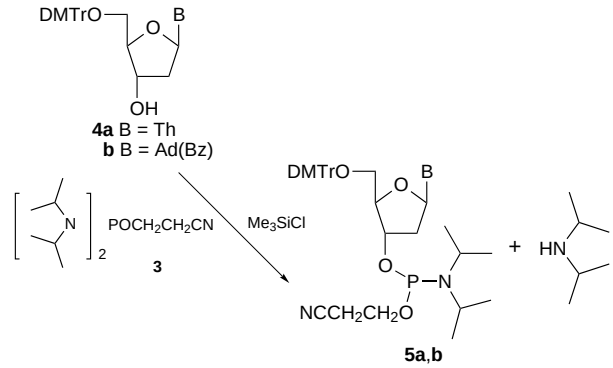
Scheme 1 *Reagents and conditions*: **1** (1.0 equiv.), $P(NMe_2)_3$ (1.0 equiv.), TMSCl (0.6 equiv.), THF, room temp., 2 h

Excellent coupling procedures activated by TMSCl were noted for P^{III} amides containing a fluorine ligand. For example, fluoro(diisopropylamino)-2-cyanoethoxyphosphine **6**§ reacts with 5′-*O*-DMTr-nucleoside **4a,b** in the presence of TMSCl (0.6 equiv.) to give 5′-*O*-DMTr-thymidine 3′-*O*-(2-cyanoethyl)-fluorophosphite **7a** and N^6-benzoyl-5′-*O*-DMTr-adenosine 3′-*O*-(2-cyanoethyl)fluorophosphite **7b**¶ in almost quantitative yield. The analogous reaction activated by tetrazole requires a 5-fold excess of activator and proceeds distinctly more slowly.

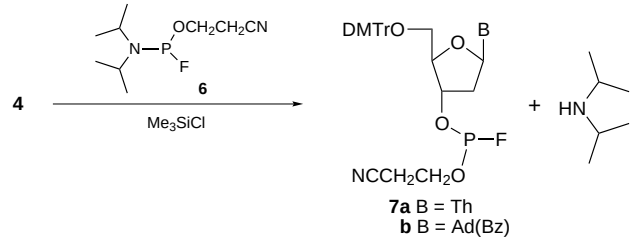
Efficient synthesis of 5′-*O*-DMTr-thymidine (3′-5′) 3′-*O*-DMT-thymidine phosphorfluoridite **10a** and N^6-benzoyl-5′-*O*-DMT-2′-deoxyadenosine (3′-5′)-3′-*O*-DMTr-thymidine phosphofluoridite **10b**‖ was achieved by coupling of 3′-*O*-nucleosidyl-phosphorfluoroamidites **8**[8] with a 3′-*O*-protected nucleoside in the presence of TMSCl (0.3 equiv.). The analogous reaction activated by tetrazole proceeds more slowly and requires a large excess of the activator.[9]

Synthesis of the 5′-*O*-DMTr-thymidine difluorophosphine **12**** was achieved by the coupling of diisopropylamino-difluorophosphine **11**[8] with 3′-*O*-protected nucleosides in the presence of TMSCl activator.

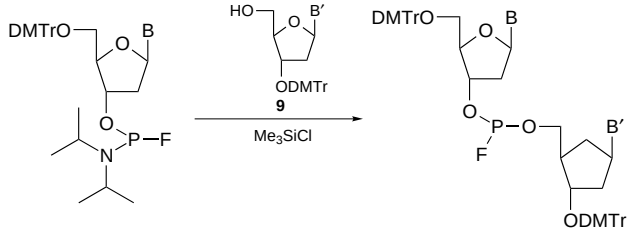
The mechanism of activation by TMSCl is presumed to involve its reaction with P^{III} amide. This type of interaction has been discussed in our earlier paper[5] and more recently by Nifantyev.[10] The first step produces salt-like species $R_2P^+(SiMe_3)NR''_2Cl^-$ and $R_2PN^+R''_2(SiMe_3)Cl^-$ which react either



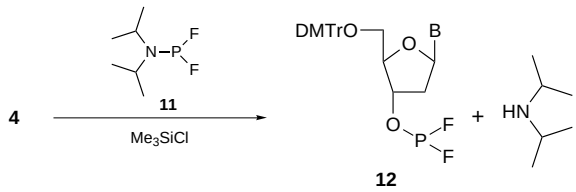
Scheme 2 *Reagents and conditions*: **4** (1.0 equiv.), **3** (1.0 equiv.), TMSCl (0.6 equiv.), THF, room temp., 1 h



Scheme 3 *Reagents and conditions*: **4** (1.0 equiv.), **6** (1.0 equiv.), TMSCl (0.6 equiv.), THF, room temp., 1 h



Scheme 4 *Reagents and conditions*: **8** (1.0 equiv.), **9** (1.0 equiv.), TMSCl (0.30 equiv.), THF, room temp., 1 h



Scheme 5 *Reagents and conditions*: **4** (1.0 equiv.), **11** (1.1 equiv.), TMSCl (0.6 equiv.), room temp., 1 h

directly with alcohol to give ester R_2POR' or *via* intermediate formation of R_2PCl. 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.[11] Formation of hydrogen chloride would effect the removal of the 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 is not observed. Work is currently in progress aimed at utilizing the TMSCl activation for the synthesis of oligonucleotides on solids supports and better understanding its mechanistic features.

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Footnotes

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† All manipulations were performed under argon and all solvents dried prior to use. NMR spectra were recorded on a Bruker AC 200 spectrometer (^{31}P 81.014 MHz, H_3PO_4 external standard; ^{19}F 188.15 MHz, $CFCl_3$ external standard). TMSCl was freshly distilled.

‡ *Selected data* for **5a**: yield 97%; δ_P ($CDCl_3$): 147.37, 147.93. For **5b** yield 98%; δ_P ($CDCl_3$): 147.49, 147.37.

§ Compound **6** was prepared in 90% yield from chloro(diisopropylamino)-2-cyanoethoxyphosphine *via* 2-cyanoethoxy(diisopropyl)amino-4-nitrophenoxyphospine by standard ligand exchange procedures (ref. 8). δ_P ($CDCl_3$): 162.8, 148.9 ($J_{P–F}$ 111.8.1 Hz); δ_F ($CDCl_3$): −75.1, −81.06 ($J_{P–F}$ 1118.4 Hz).

¶ *Selected data* for **7a**: yield 97%; δ_P (C_6D_6): 138.02, 123.03, 137.78, 122.78; δ_F (C_6D_6): −53.07, −59.51 ($J_{P–F}$ 1214.07 Hz), −53.44, −59.90 ($J_{P–F}$ 1216.05 Hz). For **7b**: yield 95%; δ_P (C_6D_6): 138.90 , 123.47; δ_F (C_6D_6): −52.99, −59.46 ($J_{P–F}$ 1216.9 Hz), −53.19, −59.58 ($J_{P–F}$ 1216.8 Hz).

‖ *Selected data* for **10a**: yield 98%; δ_P ($CDCl_3$): 138.42, 123.35, 139.72, 124.61; δ ($CDCl_3$): −53.91, −60.21 ($J_{P–F}$ 1220.63 Hz), −53.61, −60.07 ($J_{P–F}$ 1224.03 Hz). For **10b**: yield 98%, δ_P ($CDCl_3$): 138.50, 123.47, 140.04, 124.90; δ_P ($CDCl_3$): −52.18, −58.66 ($J_{P–F}$ 1221.24 Hz), −52.77, −59.29 ($J_{P–F}$ 1226.99 Hz).

** *Selected data* for **12**: yield 95%; δ_P ($CDCl_3$): 128.58, 111.77, 95.79 ($J_{P–F}$ 1295.4, $J_{P–F}$ 1294.9 Hz); δ_F ($CDCl_3$): −44.18, −51.06, −44.36, −51.63 ($J_{P–F}$ 1296.9, $J_{P–F}$ 1293.6 Hz).

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