Protonation of phosphoramidites. The effect on nucleophilic displacement

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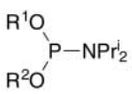
Phosphoramidites (**1**) have been protonated on the phosphorus atom by treatment with triflic acid and the properties and reactivity of the formed *P*-protonated species (**2**) have been studied. Cation **2** was found to react with alcohol more reluctantly than **1** in the presence of weak acids, which suggests that the fast acid-catalyzed alcoholysis of **1** must take place *via N*-protonation. Mechanisms of the nucleophilic displacement are discussed.

Introduction

Phosphoramidites are widely used as phosphitylating agents in automated solid-support DNA synthesis; a nucleoside 3′-phosphoramidite (**1c**) is repeatedly reacted with the 5′-hydroxy function of the growing support-bound oligonucleotide chain.[1]



1a: $R^1 = R^2 = Pr^i$
1b: $R^1 = R^2 = Me$
1c: R^1 = nucleotid-3'-yl, $R^2 = -CH_2CH_2CN$

An acidic activator is needed to promote the displacement of the nitrogen ligand with the entering hydroxy group. Usually azoles, such as tetrazole, are used for this purpose. Investigations on the nature of the catalysis have shown that tetrazole acts as an acid catalyst, but eventually also as a nucleophile since tetrazolylphosphonite is accumulated during the alcoholysis.[2] Although the kinetic results suggest that the starting material must undergo protonation either prior to or concerted with the P–N bond rupture, no direct observation on the protonation of phosphoramidites exists. Molecular modelling with a compound bearing two hydrogens as substituents at both the phosphorus and nitrogen atoms suggests that *P*-protonation should shorten and strengthen the P–N bond, whereas *N*-protonation lengthens and weakens it, the positive charge being localized on the phosphorus atom rather than on the protonated nitrogen.[3] *P*-Protonated phosphorous triamide and phosphinous amides have been prepared and characterized,[4] and P(III)-compounds with tetramethylguanidino groups at phosphorus have been shown to undergo both *P*- and *N*-protonation.[5] None of these results, however, justifies any firm conclusions concerning the site of protonation on **1**, since the electronic structure of the compounds studied significantly differs from that of **1**.

Results

Protonation of phosphoramidites

We now wish to report preparation and characterization of *P*-protonated phosphoramidites (**2a** and **2b**). These compounds were obtained *in situ* in an NMR tube by adding triflic acid (TfOH) to solutions of *N*,*N*,*O*,*O*-tetraisopropylphosphoramidite (**1a**) or *O*,*O*-dimethyl *N*,*N*-diisopropylphosphoramidite (**1b**) in acetonitrile or THF, respectively. The ^{31}P NMR spectroscopic data for **2a** and **2b** are shown in Table 1 together with those detected for the other phosphorus compounds studied in the present work. The chemical shifts of **2a**,**b** match those published for other protonated P(III) species[4] and their P–H coupling of about 700 Hz is a clear sign of a proton directly bonded to phosphorus. Protonation was also detected after treatment of **1a** with perchloric acid, indicating that the acid anion is not covalently bonded to **2a**,**b**. Consistent with this, the ^{13}C NMR signal of the trifluoromethyl carbon showed a single quartet due to C–F coupling, but no additional splitting resulting from covalent bonding between phosphorus and the triflate group. Compounds **2a**,**b** were easily converted back to **1a**,**b** with triethylamine (TEA).

1 **2a**: R = Pr^i **2b**: R = Me

The stability of **2** depends on its alkyl groups and the purity and amount of TfOH added. Protonation with 1 equiv. of TfOH from a newly opened ampoule gave **2a** that remained unchanged for several hours in a septum-sealed NMR tube, while the lifetime of **2b** was less than 30 minutes. Use of impure TfOH, or excess of it, decreased the stability of **2**. Upon prolonged treatment, **2a** decomposed to the corresponding protonated phosphonate (**5a**) and an unknown compound ($\delta_P = 113$ ppm). The formation of **5a** probably occurs by an oxidative reaction between **2a** and the triflate anion rather than by hydrolysis, since the amount of water present in the solution (< 0.05 equiv.), is not enough to account for more than 0.5 equiv. of **5a** formed from **2a**.

Earlier attempts to protonate phosphoramidites with tetrafluoroboric acid, HBF_4, had failed, yielding an unidentifiable mixture of compounds.[4] Phosphoramidites seem to exhibit high affinity to the fluorine atoms of this strong acid. In our hands addition of HBF_4 to the acetonitrile solution of **1b** gave a mixture of $MeOP(F)NPr^i_2$, $(MeO)_3P$, $(MeO)_2PF$, $MeOPF_2$ and several unidentified minor components.

Reactivity of the *P*-protonated phosphoramidites

Compounds **2a**,**b** undergo nucleophilic substitution reactions (Scheme 1). Since the amine displaced in the process is a

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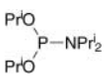
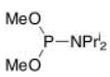
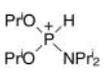
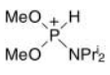
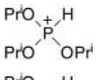
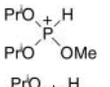
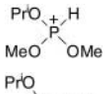
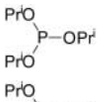
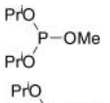
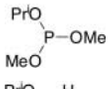
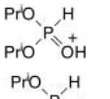
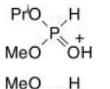
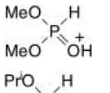
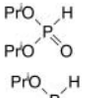
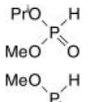
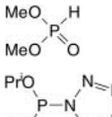
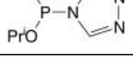
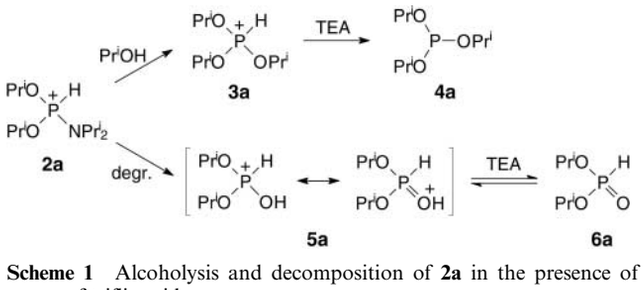
Table 1 NMR spectroscopic data of the phosphorus compounds identified in the present work

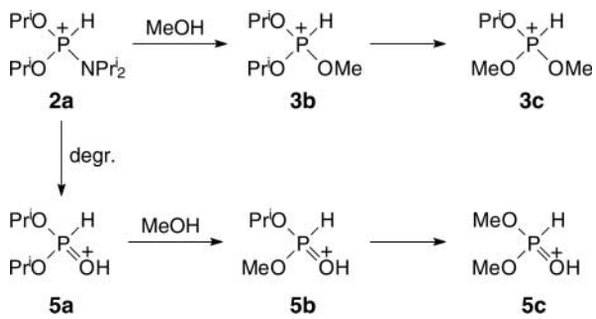
	Compound	δ_P (ppm)	Signal form	$^1J_{PH}$/ Hz	$^3J_{PH}$/ Hz
1a		143.1	br m		—
1b		150.3	br m		—
2a		25.9	d t t	702	8.0 18.9
2b		38.6	d t sept	738	13.0 18.7
3a		17.8	d q	807	6.7
3b		21.3	d q t	819	12.5 6.3
3c		29.9	d m	819	—
4a		138.6	q		8.8
4b		139.8	sext		9.5
4c		141.4	oct		10.7
5a		13.5	d br	777	—
5b		15.1	d br	775	—
5c		15.8	d br	763	—
6a		5.5	d q	685	8.2
6b		8.8	d q d	695	12.1 9.0
6c		12.0	d sept	700	12.0
7		126.0	br		—



Scheme 1 Alcoholysis and decomposition of **2a** in the presence of excess of triflic acid.

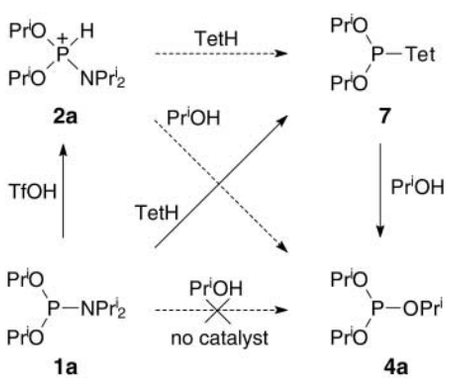
relatively strong base, the product of the reaction depends on the amount of acid used for the protonation of **1**. Reaction between **2a** protonated using 1 equiv. of acid and propan-2-ol yielded a neutral phosphite, **4a**, whereas on using 2 equiv. of acid, the reaction product was the protonated phosphite, **3a**, that could be further deprotonated by addition of base. Similarly, the decomposition of **2a** gave as a byproduct either the corresponding phosphonate **6a**, or its *P*-protonated conjugate acid, **5a**. However, while the other phosphorus compounds still remain protonated, an equilibrium between **6a** and **5a** seems to exist: an averaged ^{31}P NMR signal between 13.5 and 5.5 ppm (depending on the degree of protonation) was observed.

The reaction of **2a** with methanol gave both alcoholysis and transesterification products (Scheme 2), $(Pr^iO)_2POMe$ (**4b**),



Scheme 2 Transesterification of protonated phosphite **3a** and phosphonate **5a**.

$(MeO)_2POPr^i$ (**4c**), $(Pr^iO)_2P(O)H$ (**6a**), $Pr^iO(MeO)P(O)H$ (**6b**) and $(MeO)_2P(O)H$ (**6c**). Phosphonates **6** showed a significantly higher degree of transesterification than phosphites **4**. All the products were obtained in protonated form if excess TfOH had been added. The reaction of **2a** with tetrazole (TetH) yielded the corresponding tetrazolylphosphonite, **7**. Similarly, imidazole, benzimidazole and pyridine reacted with **2a**, giving substitution products with a covalent phosphorus–heterocycle bond. The progress of the reactions of **2a** with tetrazole and propan-2-ol was followed in a semiquantitative manner, and the results were compared with those observed for **1a** (see Scheme 3). In acetonitrile at 20 °C, the protonation of **1a** is a very fast process (completed within 20 s, the time required for the



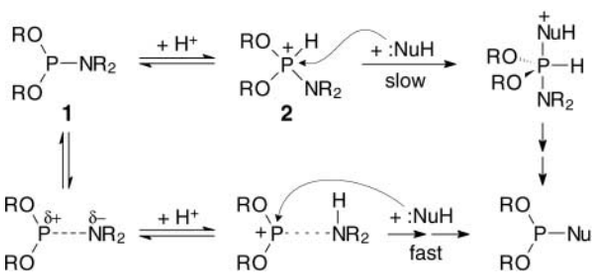
Scheme 3 Reactions of **1a** and **2a** with tetrazole and propan-2-ol in acetonitrile at 20 °C. Solid lines indicate fast reactions (completed within 20 s) and dotted lines slow ones ($t_{\frac{1}{2}} > 15$ min).

recording of the first NMR spectrum after mixing of the reagents). Tetrazolysis and the tetrazole-promoted alcoholysis of **1a** are rapid as well, but the reaction of **2a** with 1 equiv. of tetrazole is surprisingly slow ($t_{1/2} = 15$ min) and that with 1 equiv. of propan-2-ol proceeds hardly at all. Nevertheless, reasonably fast alcoholysis is observed if the alcohol is used in excess (< 3 equiv.) or if ammonium triflate is present. Since the latter is formed in the alcoholysis, a rate profile of an autocatalytic reaction is observed. By contrast, the reaction of **2a** with tetrazole was not affected by the presence of triflate salts. All the reactions were even slower if excess of TfOH had been used for protonation. The only really fast reactions of **2a** were those with imidazole, benzimidazole and pyridine.

Discussion

The key observation of the present study is that the *P*-protonated amidite **2a** reacts with nucleophiles more reluctantly than the unprotonated amidite **1a** in the presence of weak acids. This strongly suggests that the tetrazole-activated reactions of **1a** cannot take place *via P*-protonation. The low reactivity of **2a** is not entirely unexpected. Nifant'ev *et al.* have observed that protonated phosphinamidites are even more passive and can be crystallized from alcohol.[4] Nevertheless, the paradox between high catalytic efficiency of weak acids and the inactivity of the protonated species calls for an explanation.

Let us consider the stretching vibration of the P–N bond. At its normal length, the bond is only slightly polarized and the phosphorus atom is more basic than the nitrogen. Weak acids are not able to protonate this ground state, but treatment with really strong acids results in *P*-protonation. On stretching the bond, the electron density of the nitrogen atom is increased until at some stage it becomes more basic than the phosphorus. For sufficiently weak acids, the proton transfer to the starting material takes place only at this stage, concerted with the departure of the leaving group. This is in agreement with the result of Korkin and Tsvetkov according to which *N*-protonation lengthens and weakens the P–N bond.[3] This protonation of the already partially departed leaving group accelerates the reaction proceeding by a dissociative mechanism, whereas protonation of the phosphorus promotes the associative reaction proceeding *via* a pentacoordinated intermediate (or transition state). One may assume that the dissociative mechanism is inherently preferred by phosphoramidites and weak acids are therefore able to selectively trigger the faster reaction path. Strong acids, in turn, enable the otherwise slow associative substitution pathway, but block the dissociative one, thus resulting in only a modest catalytic effect (see Scheme 4). The fast reactions of **2** with imidazole, benzimidazole and pyridine are due to the ability of these bases to deprotonate **2**, to give a mixture of **1** and a weak acid. This allows the dissociative reaction path to be utilized and leads to a rapid reaction.



Scheme 4 Proposed associative (higher) and dissociative (lower) reaction pathways triggered by strong and weak acids, respectively.

Two mechanistic suggestions may be given for the observed acceleration of the dissociative reaction by weak acids. Firstly, the reaction could be simply susceptible to general acid catalysis, the conjugate base of the catalyst becoming bonded to phosphorus after the rate-limiting bond rupture. Alternatively, the reaction is essentially unimolecular, but the P–N bond rupture takes place only when the developing amide anion is stabilized by protonation and the phosphanylium cation is immediately trapped by a nucleophile appropriately preassociated with the starting material. The concentration of the nucleophile is therefore not necessarily kinetically visible and the selectivity of the reaction towards any particular nucleophile may be low. These consequences are in agreement with our earlier results on reactions of phosphoramidites.[2]

Experimental

The syntheses of **1a**,**b** have been published previously.[2] Triflic acid was stored in vials sealed with airtight septa after opening of the ampoule. The experiments showed sensitivity to the quality of TfOH used, and reliable and repeatable results were obtained only when the purity of the acid used was constantly high. Acid showing any sign of decomposition (cloudiness or darkened color) was discarded. Alcohols and amines were distilled and stored over molecular sieves (4 Å) and KOH pellets, respectively. Solvents were dried with CaH_2 and stored over it. In kinetic runs, pure **1a**,**b** and TfOH were added, other reagents as solutions stored over 4 Å molecular sieves. Reactions were performed in oven-dried, septum-sealed NMR tubes, into which the reagents were introduced using syringes. The NMR spectra were recorded on a 500 MHz spectrometer magnet (202.35 MHz for ^{31}P).

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