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Review Commentary

Mechanisms of the substitution reactions of phosphoramidites and their congeners

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ABSTRACT: Mechanistic research on the nucleophilic substitution of trivalent phosphorus compounds is reviewed with emphasis on the reactions of phosphoramidites. The reactivity of these compounds towards hydroxyl groups is utilized, for instance, in the coupling step of the modern automated solid-support synthesis of oligonucleotides. Growing interest in the large-scale synthesis of oligonucleotides as antisense-strategy drugs has increased the demand for relevant mechanistic research that has considerably improved our understanding of phosphoramidite alcoholysis reaction during the last few years. The review also covers phosphites that are formed as products of this reaction and also phosphorohalidites, azolyl phosphonites and P(III) azolidites that serve as its intermediates. Mechanisms of reactions of phosphoramidites with azoles and carbozylic acids are included together with those of halogenation and transamidation. Alternative reaction mechanisms published in the literature for substitution reactions of P(III) compounds range from dissociative to concerted and associative pathways while different types of activation have been suggested. Reactions of phosphoramidites with alcohols have been shown to be subject to both nucleophilic and acid catalysis and most likely proceed with a concerted mechanism of dissociative character. The consequences of these conclusions for the development of the phosphoramidite approach of oligonucleotide synthesis are also discussed. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: phosphoramidites; trivalent phosphorus; nucleophilic substitution; reaction mechanisms; oligonucleotides

INTRODUCTION

Automated machine-assisted synthesis of oligonucleotides on a solid support is nowadays everyday laboratory routine. The most extensively applied protocol, the so-called phosphoramidite approach, is based on the phosphite triester method of Letsinger and Ogilvie[1] developed to the currently used form by Beaucage and Caruthers[2] (Scheme 1). The breakthrough of the methodology has largely resulted from the replacement of the originally employed P(III) chloridite building blocks with significantly more stable phosphoramidites. When activated with 1*H*-tetrazole, the appropriately protected nucleoside 3′-phosphoramidites may readily be reacted with the 5′-hydroxy of the growing solid-supported oligonucleotide chain.

It has been well established that the expression of a given gene may be inhibited in a highly selective manner with structurally modified, so-called antisense oligonucleotides.[3] This finding has brought oligonucleotides among the potential chemotherapeutic agents, the first of which has already entered the market. Clinical phase screening of various antisense oligonucleotides has, in turn, created the demand for increasing amounts of pure oligonucleotides via minimal purification steps. Accordingly, although the existing phosphoramidite chemistry is satisfactory for laboratory-scale synthesis, many of its steps are currently under reinvestigation from the point of view of safety, convenience and cost-effectiveness crucial for scaling-up the synthesis. Alternative activators for the coupling step have, for example, been searched because 1*H*-tetrazole is relatively expensive, hygroscopic, sparingly soluble in acetonitrile and even explosive. Substituted 1*H*-tetrazoles[4–7] still share the shortcomings of the parent compound, and hence the search for better alternatives has been directed to acidic azoles,[8–12] azolium salts,[7,13–17] pyridinium salts,[18–21] anilinium salts,[2,17,21,22] phenols[23] and combinations of these.[20,24]

Mechanisms of the substitution reactions of P(III) compounds have not been very intensively studied, compared with their pentavalent analogues, undoubtedly largely owing to the experimental difficulties. Sensitivity towards water and polar impurities necessitates rigorous

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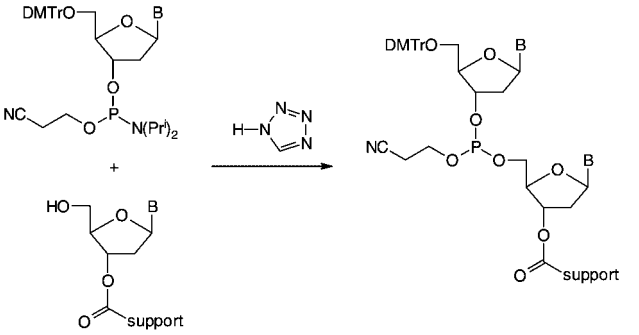
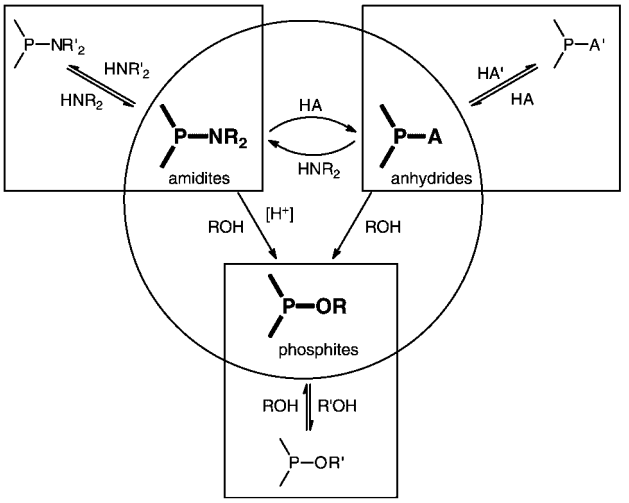


Scheme 1. Coupling step in oligonucleotide synthesis by the phosphoramidite approach

purification and continuous protection from the moisture, and further challenges are involved in the monitoring of very fast reactions and interpretation of the usually complex reaction kinetics. Only fairly recently have the new demands for more effective oligonucleotide synthesis urged scientists to overcome these thresholds.

The mechanistic significance of many studies has been rather limited, owing to their highly applied nature. While experiments on DNA synthesizers may well demonstrate the efficiency of various activators, they tell us little about the reasons behind the observed behaviour. Furthermore, basic physical chemical factors, such as the effect of solvent on the relative acidity of the solutes, are often insufficiently emphasised in the discussions.

The phosphoramidite coupling reaction actually is a nucleophilic substitution reaction by an alcohol on a trivalent phosphorus centre (Scheme 2). This kind of nucleophilic substitutions are typical reactions for most P(III) compounds. The P(III) halidites, mixed anhydrides, phenyl esters and azolidites (also called azolyl phosphonites) all contain a good leaving group, and they react with acids, alcohols and amines without additional activation. Phosphoramidites, in turn, are less reactive, requiring an acidic promoter for reactions with nucleophiles. The



Scheme 2. Substitution reactions of trivalent phosphorus compounds

amino ligand must be protonated to become displaced. The alkoxy ligand is displaced only reluctantly.

Hydrolysis of P(III) compounds yields hydrogen phosphonates. In addition, they may undergo oxidation, acid-catalysed dealkylation and Arbusov reaction giving pentavalent products. Accordingly, dry organic, preferably aprotic solvents, such as dioxane ($\varepsilon = 2.2$), $CHCl_3$ (4.7), THF (7.4), CH_2Cl_2 (9.1) or MeCN (36.7), have to be employed.

PHYSICO-CHEMICAL PROPERTIES OF P(III) COMPOUNDS IN ORGANIC MEDIA

Protolytes and salts in aprotic solvents

Salt effects and association in aprotic media. Phosphite esters and phosphoramidites are commonly synthesized from the corresponding phosphorochloridites in the presence of amines as HCl acceptors. Consequently, P(III) compounds not carefully purified are contaminated by an ammonium chloride salt that may result in substantial effects on reaction rates, especially in apolar solvents.[25] The magnitude of the influence that ammonium salts have on the reactivity of phosphoramidites[26] was not fully understood until in 1985, when methods for the removal of the salt and an assay for the detection its traces were presented.[27,28] The ability of ion pairs to polarize the medium and stabilize developing charge during the reaction may even surpass that of acid or base catalysts,[29] hence kinetic studies ignoring the careful control of such issues should be considered with certain caution.

Protic reagents may participate in the solvation of salts in organic medium, and this may affect their own reactivity. Simultaneously, the solubility of the salt is increased, hence the salt effect is enforced. In solvents of low polarity, salts and protolytes often prefer association to ion pairs, hydrogen-bonded dimers and higher aggregates,[29] as has been reported, for instance, for azolium salts in MeCN.[30] This may lead to altered kinetic orders, as observed for tetrazole-promoted phosphoramidite alcoholysis.[31] Accordingly, the mechanistic interpretation of an observed reaction order is not always straightforward.

Protolytic equilibria in acetonitrile. Although aqueous pK_a values may be applicable in alcohol solutions, they certainly do not provide a useful basis for discussion in aprotic solvents. The use of appropriate dissociation constants is crucial since even the order of acidity of the solutes may be changed on going from one solvent to another. Tables 1 and 2 summarize the pK_a values for some neutral and cationic acids in an aqueous solution and in MeCN, the solvent most extensively used in phosphoramidite chemistry.

As far as compounds with the same functionality are concerned, the pK_a values in MeCN and in aqueous

Table 1. pK_a values for neutral acids in acetonitrile and water

	Acid	pK_a(MeCN)[a]	pK_a(H_2O)[b]
Alcohols	ROH	41–46[c]	15–20
	2,2,2-Trifluoroethanol	33.4[c]	12.4
Amines	Aniline	41.1[c]	27.7
	Diphenylamine	37.1[c]	22.4
Azoles	Pyrrole	34.6[c]	16.5[f]
	Imidazole	29.4[c]	14.5[f]
	Benzimidazole	(26.7)[d]	12.9
	1,2,4-Triazole	(22.4)[d]	10.3
	1*H*-Tetrazole	14.5[e]	4.9
Phenols	Phenol	27.2	9.99
	4-Nitrophenol	20.7	7.14
	2,4-Dinitrophenol	16.0	4.1
	2,4,6-Trinitrophenol	10.9	0.3
Carboxylic acids	Acetic acid	22.3	4.76
	Chloroacetic acid	18.8	2.86
	Dichloroacetic acid	15.8	1.35
	Trichloroacetic acid	10.6	−0.5
	Trifluoroacetic acid	12.7	−0.6
Halogen acids	Hydrochloric acid	8.9	
	Hydrobromic acid	5.5	
	Perchloric acid	1.6	
Sulphonic acids	Methanesulphonic acid	8.4	−1.9
	Triflic acid	2.6	−5.1

[a] Ref. 32a.
[b] Ref. 32b.
[c] Ref. 32c.
[d] Estimated based on the fact for azoles (in water) $pK_a(HA) - pK_a(BH^+) =$ constant[32d] and that for imidazole (in MeCN) $pK_a(HA) - pK_a(BH^+) = 12.35$.
[e] Ref. 33.
[f] Ref. 32e.

Table 2. pK_a values for the conjugate acids of some nitrogen bases and alcohols in acetonitrile and water

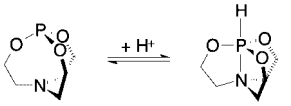
	Base	pK_a(MeCN)[a]	pK_a(H_2O)[b]
Amines	Tetramethylguanidine	23.3[c]	13.6
	Triethylamine	18.5	10.75
	N,*N*-Dimethylbenzylamine	16.5[e]	8.91[f]
	N-Methylmorpholine	15.6	7.38[f]
	Triallylamine	15.2[e]	8.31
	N,*N*-Diisopropylaniline	14.5[e]	7.37[f]
	Aniline	10.7	4.6
	N,*N*-Dimethylaniline	8.6[e]	5.1[f]
Azoles	Imidazole	17.1[e]	7.0
	N-Methylimidazole	17.1[e]	7.95[f]
	Benzimidazole	14.3[e]	5.5
	N-Methylbenzimidazole	14.1[e]	5.54[i]
	1,2,4-Triazole	10.0[e]	2.3
Pyridines	2,6-Lutidine	14.4[h]	6.60[f]
	Pyridine	12.6[h]	5.23
	3-Chloropyridine	10.0[h]	2.84[f]
	2-Chloropyridine	6.8[h]	0.49[f]
Alcohols	Methanol	2.3	−2.05
	tert-Butyl alcohol	3.4	−3.8[f]
	Phenol	0.4	−6.4

[a–c,e,f] As in Table 1.
[h] Ref. 32g.
[i] Ref. 32d.

solution are linearly related ($r > 0.92$). Interestingly, the pK_a values of azoles, and also those of amines, fall on a single correlation line irrespective of whether the proton donor is a neutral molecule or an azolium (ammonium) ion. These correlations are useful when pK_a values in MeCN are approximated on the basis of aqueous data. Concerning the other protolytes, carboxylic acids are much weaker acids in MeCN than in water, while the opposite is true for ammonium ions. In other words, the stability of ionic species compared with neutral molecules is much lower in MeCN than in an aqueous solution.

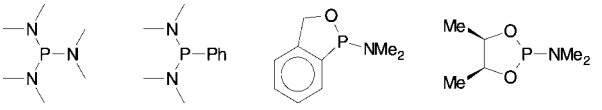
Protonation of trivalent phosphorus compounds.

Trialkyl phosphites have been protonated on the phosphorus atom ($^1J_{PH} \approx 700$ Hz) with sulphuric acid,[34] triflic acid or HCl,[35] although concurrent dealkylation has sometimes been observed. Protonation of the bicyclic phosphite **1** with HBF_4 has been shown to yield a pentacoordinative species **2** (x-ray crystallography).[36]



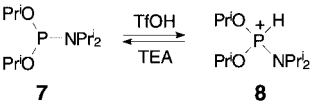
1 **2**

Protonated P(III) amides were first studied by Dahl.[37] When compounds **3–6** were treated with triflic acid in chloroform, protonation took place mainly on phosphorus ($\delta_P \approx 45$ ppm). Additional signals ($\delta_P \approx 153.0$, 168.3 and 142.9 ppm) were tentatively assigned to *N*-protonated species. Nifant'ev and co-workers managed to isolate and purify P(III) trisamidites and phosphinamidites as *P*-protonated salts obtained by treatment with HBF_4.[38] The surprisingly high stability of the protonated structures allowed their crystallisation from alcohol and characterisation by x-ray crystallography.[28]



3 **4** **5** **6**

P-protonation of phosphoramidites was first proposed on the basis of an IR absorption band at 2450 cm^{-1}, but treatment with HBF_4 was later found to yield a complex mixture of products.[28] These were identified to be fluorinated P(III) structures in another study[39] in which phosphoramidite **7** was treated with triflic acid in MeCN. This afforded a relatively stable *P*-protonated product (**8**, $\delta_P = 25.9$ ppm) that was easily deprotonated on addition of a base [triethylamine (TEA)], but it reacted reluctantly with nucleophiles (propan-2-ol, tetrazole). Similar *P*-protonation of **7** was resulted in by sufficiently acidic (p$K_a < 10$) ammonium and pyridinium triflates.[33]



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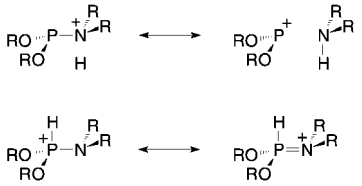
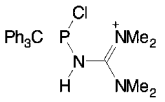


Scheme 3. Resonance structures of *N*- and *P*-protonated phosphoramidite

Protonation of a simple model structure, $H_2P—NH_2$, has been studied with molecular modelling. Calculations by Korkin and Tsvetkov[40] indicate that protonation of the phosphorus atom shortens and strengthens the P—N bond. As a result, the p character of the unshared electron pair of the nitrogen atom is increased and the NH_2 group becomes more flattened. Protonation of the nitrogen atom, in turn, lengthens and weakens the P—N bond. The s character of the phosphorus lone pair is increased, and the resulting structure resembles an adduct of phosphenium ion and ammonia (Scheme 3).

N-Protonation has been detected (^{31}P NMR spectroscopy and x-ray crystallography) only on treating exceptionally resonance-stabilized tetramethylguanidinyl P(III) amides, such as **9**, with HCl in $CDCl_3$.[41]

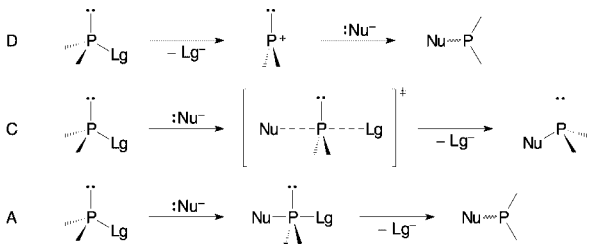


9

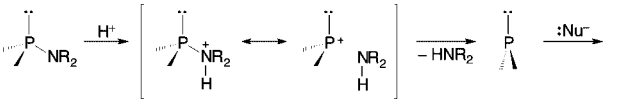
Mechanistic alternatives of P(III) substitutions

Associative vs dissociative mechanisms. The classical categorization of substitution mechanisms on the basis of the relative timing of the nucleophilic attack and the leaving group departure gives the following alternatives for P(III) substitutions: (1) dissociative S_N1-type reaction via a phosphenium cation, (2) concerted S_N2-type mechanism and (3) associative mechanism via a pentacoordinated intermediate (Scheme 4).

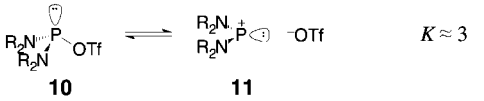
An S_N1-type process is initiated by rate-determining departure of a good leaving group, and the resulting phosphenium cation is then trapped by the entering nucleophile (Scheme 5). A good leaving group, such as an anion of a strong acid, is sufficiently stable to depart spontaneously, whereas a poor leaving group, such as an



Scheme 4. Dissociative (D), concerted (C) and associative (A) mechanisms for P(III) substitution



Scheme 5. P(III) amidite replacement by a dissociative S_N1 mechanism



Scheme 6. Observed equilibrium between covalent and ionic forms of P(III) triflate[42]

amino group, requires protonation either prior to or concerted with the bond cleavage.

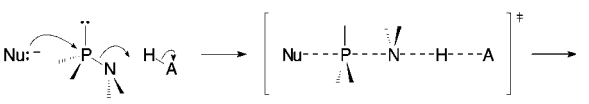
Consistent with this, phosphenium cations have been observed ($\delta_P \approx 270$ ppm), for instance, on treatment of phosphorotrisamidites with triflic acid.[37] Furthermore, in the absence of a nucleophile, P(III) triflate **10** has been found to exist mainly as the phosphenium triflate **11** (Scheme 6).[42]

The concerted mechanism is basically an S_N2 process obeying second-order kinetics and leading to inversion of configuration. Also in this mechanism, the leaving group has to be a good one or activated by protonation either prior to or concerted with its departure (Scheme 7).

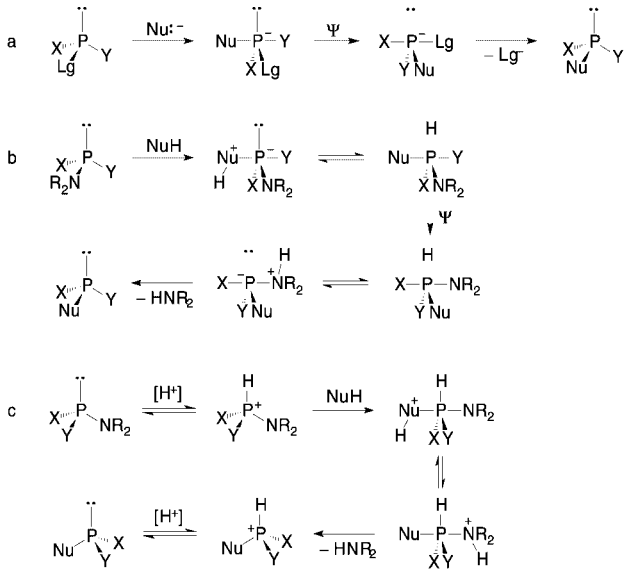
Inversion of configuration resulting from in-line attack of the nucleophile is observed, for instance, for P(III) halide substitutions.[43] Under acid-catalysed conditions, the measurements have been complicated by racemization taking place during the substitution: for ammonium halide-catalysed alcoholysis and transamidation of P(III) amides in $CDCl_3$, Nielsen and Dahl observed inversion of stereochemistry during early stages of the reaction, although retention dominated at the final equilibrium.[44] An S_N2 mechanism has also been proposed for reactions of acyl phosphites with alcohols and amines.[45]

An associative mechanism proceeds via a phosphorane-like pentacoordinated intermediate having a trigonal bipyramid (TBP) geometry with three shorter, equatorial bonds and two longer, apical bonds. In most cases, these species obey the so-called Westheimer guidelines, according to which the ligands may interchange positions by pseudorotation (Ψ) and nucleophiles enter and depart only through apical positions.

Attack of an anionic nucleophile yields a phosphoranide intermediate [Scheme 8(a)] whose pseudorotation may result in retention of configuration not possible for the S_N2 mechanism.[46] In case of protic nucleophiles, true pentacovalent species are usually presented as intermediates, owing to the possibility of proton transfer to



Scheme 7. P(III) amidite replacement with a concerted S_N2 mechanism



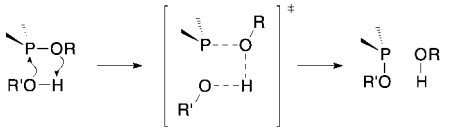
Scheme 8. Associative P(III) substitutions by (a) an anionic nucleophile through a phosphoranide anion, (b) by neutral, protic nucleophile through a pentacoordinated intermediate and (c) by initial protonation of the phosphorus atom

phosphorus [Scheme 8(b)].[47] When the —NH_2 group is less apicophilic than —Y, it originally takes an equatorial position and departs only after pseudorotation accompanied by a proton transfer from the phosphorus atom to the leaving nitrogen atom. Since *P*-protonation is known to promote the formation of a pentacoordinated structure,[36] it is also possible that an acid-catalytic step precedes the formation of the pentacoordinated intermediate [Scheme 8(c)].

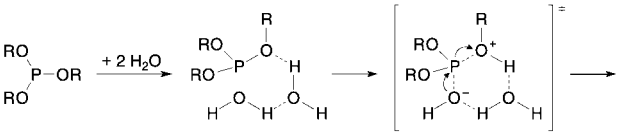
According to Westheimer's rules, the TBP species is stabilized by an equatorial–apical ring and phosphoranes with cyclic or bicyclic structure are well known. Pentacoordinative species have been detected as intermediates (^{31}P NMR) in reactions where alcohol replaces an alkoxy[48] or phenoxy[49] group.

The reaction through a four-membered cyclic transition state (Scheme 9) has been regarded as a viable mechanistic alternative for protic nucleophiles. This mechanism involves binding of the proton of the nucleophile concerted with coordination of the phosphorus atom to the lone electron pair of the nucleophile.[50]

The four-membered ring mechanism has been proposed for substitutions of phosphites,[51] phenyl phosphites,[35] phosphoramidites[52] and phosphorochloridites.[53] In a modified version of the mechanism, two nucleophile molecules participate, giving a six-membered cyclic transition state, as proposed for the hydrolysis of phos-



Scheme 9. Mechanism for concerted P(III) ester transesterification involving a four-membered cyclic transition state



Scheme 10. Phosphite hydrolysis through a six-membered cyclic transition state

phites (Scheme 10).[54] Related mechanisms have been described for the Wittig reaction and the ligand-exchange reactions between two P(III) compounds.

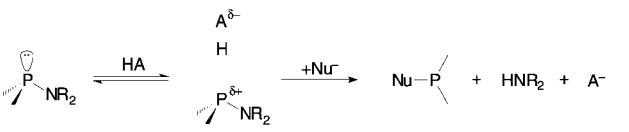
Catalysis alternatives. Substitution reactions of P(III) amides and esters are subject to acid catalysis that is also considered to be a prerequisite for these displacements.[55] Nevertheless, the mechanism of the acid catalysis, above all the site of protonation, has remained a subject to debate and speculations.

pK_a data suggest protonation of the —NR_2 group before its departure, which should lengthen and weaken the P—N bond,[40] and hence promote the substitution by a dissociative or concerted mechanism. With phosphite esters, proton transfer concerted with the leaving group departure appears more likely, owing to the extremely high acidity of the *O*-protonated species. The *P*-protonated P(III) amides have *a priori* been postulated to be highly reactive, but experimental findings have proved otherwise.[28,39] *P*-Protonation that promotes pentacoordination[36] and shortens and strengthens the P—N bond of phosphoramidites,[40] should, however, accelerate the reaction by an associative mechanism via a pentacoordinated intermediate.

A catalytic H-complex with only partial *P*-protonation has been presented as a mechanistic alternative for the phosphoramidite alcoholysis (Scheme 11).[28] The suggestion was supported by low reactivity of the *P*-protonated species and the Brønsted α value (0.05–0.65)[28,56] taken as a measure of the degree of proton transfer in the transition state.[57] Proton transfer to the departing amine was assumed to take place only after the nucleophilic attack.

Assistance by a base may be envisaged in P(III) substitutions where a good leaving group is replaced by a protic nucleophile and the otherwise resulting unfavourable *N*- or *O*-protonated structure can be stabilized by deprotonation. Base catalysis has been observed in alcoholysis of a phenyl phosphite,[49] P(III) halides,[58] acyl phosphites[45,59] and tetrazolidite.[31]

The catalytic role of acids or bases is often complicated by their protolytic reaction with the released leaving group. For instance, the amine liberated in acid-catalysed



Scheme 11. Acid-catalysed P(III) amide substitution via a catalytic H-complex

phosphoramidite alcoholysis deprotonates the activator that hence has to be applied in a stoichiometric rather than catalytic amount. The protolytic removal of the product, in turn, drives the substitution equilibrium towards completion.

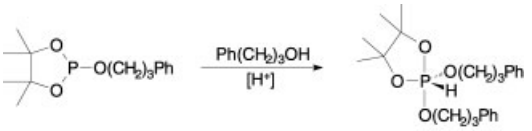
P(III) substitutions have often been suspected to be subject to nucleophilic catalysis, and hence to consist of two consecutive displacements. This complicates the mechanistic discussion, since both reactions must proceed by some of the mechanistic alternatives discussed above. Accordingly, evidence for the nucleophilic catalysis would still not reveal the detailed mechanisms of the partial reactions. Furthermore, experimental verification of nucleophilic catalysis is not easy since the intermediate may be too unstable to be detected, even less subjected to direct kinetic studies. It may also be formed via a reactive species that could also to some extent be attacked directly by the final stoichiometric nucleophile.

MECHANISMS OF THE SUBSTITUTION REACTIONS OF P(III) ESTERS, ANHYDRIDES, AZOLIDES AND HALIDES

RO— as a leaving group

The pK_a values of alcohols in MeCN are >40, which makes the alkoxy group a poor leaving group that has to be protonated during the displacement.[60] Moreover, the remarkable stability of the P(III)—O bond limits the potential of RO— as a leaving group. Transesterification of phosphite esters, for example, usually requires elevated temperature and removal of the liberated alcohol to proceed. Reaction via a pentacoordinated intermediate [Scheme 8(c)] obtained by a nucleophilic attack on a *P*-protonated phosphite has been suggested.[61]

Direct NMR spectroscopic evidence for a phosphorane-like intermediate has been reported by Watanabe *et al.* in tetrazole-promoted transesterification of phosphite **12** (CH_2Cl_2–$CHCl_3$).[48] A ^{31}P NMR signal at −24.95 ppm ($^1J_{PH} = 666.5$ Hz) was assigned to pentacovalent species **13** stabilized by a five-membered apical–equatorial ring (no analogous acyclic structures were detected).



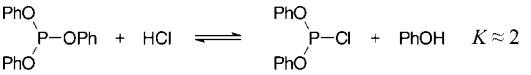
12 **13**

The pentacoordinated reaction path has also been disputed and a four-membered ring mechanism (Scheme 9) suggested instead.[51] Consistent with this suggestion, a trimolecular six-membered cyclic transition state has been proposed for phosphite hydrolysis (Scheme 10) on the basis of the second-order dependence of the rate on the concentration of water.

ArO— and RCOO— as leaving groups

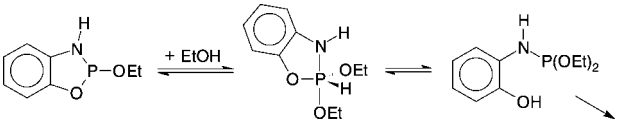
Phenoxy [pK_a(MeCN) = 10.9–26.6] and acetoxy [pK_a(MeCN) = 10.5–22.3] groups are more potent leaving groups than alkoxy groups, and they are not susceptible to acid-catalysed dealkylation. Acyl phosphites give P(III) substitution products with alcohols, phenols and amines,[62] the reactions being catalysed by bases rather than acids.[59] An S_N2-type mechanism is supported by first-order kinetics in nucleophiles and their reactivity order ($EtO^- > Et_2NH > H_2O > EtOH$).[45] The base-catalysed displacement of the acetyl group is faster than that of phenyl group.

The conversion of phenyl phosphite **14** to chloridite **15** in dioxane or ether has been proposed to proceed by a four-membered ring mechanism (Scheme 9).[35]



14 **15**

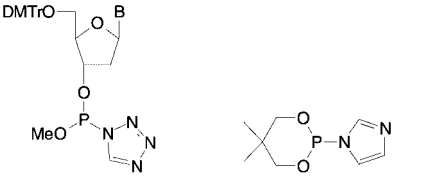
A cyclic pentacoordinated intermediate **17** (δ_P = 34–44 ppm) was observed during the base-catalysed alcoholysis of **16**. The superiority of phenoxide ion as a leaving group is indicated by the release of phenol (pK_{HA} = 26.6) rather than aniline (pK_{HA} = 41.1) in both the alcoholysis and aminolysis of **16**.[49]



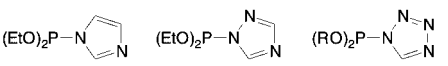
16 **17**

Azoles or azolide ions as leaving groups

Both acidic (e.g. tetrazole) and basic azoles (e.g. benzimidazole and imidazole) have proved to be efficient leaving groups in P(III) substitutions. Tetrazolidite **18** was found to react with alcohols faster than the corresponding chloridite.[63] Reaction of imidazolidite **19** in the presence of imidazolium fluoride had $t_{1/2} < 2$ min.[58] Rapid alcoholysis of azolidites **20** and **22** has been verified by ^{31}P NMR spectroscopy.[64]



18 **19**



20 **21** **22**: R = Et
23: R = Pr^i

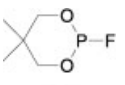
Alcoholysis and aminolysis of a series of P(III) azolidites have been studied in dioxane by Nifant'ev *et al.*, who found that alcoholysis is faster than aminolysis, the reactivity order of the leaving groups being triazolide < benzimidazolide < imidazolide.[65] The reaction rate also depends on the identity of the other substituents on phosphorus, increasing in the series $R_2P— < (R_2N)_2P— < (RO)_2P—$.

A kinetic study on substitution reactions of tetrazolidite **23** in THF revealed a first-order dependence of the rate on the nucleophile concentration, alcoholysis being faster than aminolysis.[31] Alcoholysis was found to be subject to acid, base and salt catalysis (in order of increasing efficiency), the latter being attributed to ion-pair catalysis. Diisopropylammonium tetrazolide salt that easily contaminates tetrazolidites had a significant effect on the reactivity of **23** even at the low concentration limited by its poor solubility in THF.

The tetrazolyl group may also be displaced by other azoles: addition of 5-(4-nitrophenyl)tetrazole, 5-ethylthiotetrazole or 3-nitro-1,2,4-triazole to a solution of **23** in THF resulted in fast azole exchange,[31] whereas the corresponding triethylammonium azolide salts gave no such reaction. Undoubtedly, **23** undergoes in the presence of tetrazole a similar exchange as first suggested by Stec and co-workers on the basis of the lack of stereoselectivity in the tetrazole-catalysed phosphoramidite alcoholysis.[66] Later, **23** has been observed (^{31}P NMR spectroscopy) to exist as *N*1 and *N*2 isomers that slowly equilibrate in THF,[31] but collapse into one averaged signal in more polar MeCN, owing to faster exchange.

Halide ions as leaving groups

As anions of strong acids (pK_a of HCl = 8.9 and that of HBr = 5.5 in MeCN), halide ions are excellent leaving groups, as indicated by the frequent use of PCl_3 as a starting material for the synthesis of P(III) esters and amides. The phosphorus–halogen bond strength increases in the series $I^- < Br^- < Cl^- < F^-$, the phosphorus–fluorine bond being in fact considerably stable [ΔH(P—F) = 490, while ΔH(P—O) = 407, ΔH(P—Cl) = 319 and ΔH(P—N) = 290 kJ mol^{-1}]. Consequently, phosphorofluoridite **24** has been found to be unreactive with amines in spite of its fast base-catalysed alcoholysis, the rate of which depended on the strength and concentration of the base.[58]



24

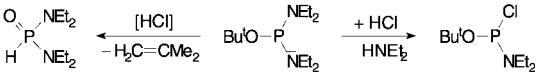
Studies on substitution reactions of P(III) chloridites revealed complete inversion of stereochemistry, which allowed the authors to propose an S_N2 mechanism (Scheme 7).[43] A four-membered ring mechanism has also been suggested.[53] Szewczyk and Quin reported the halogen displacement to proceed by retention of configuration, with the exception of aminolysis.[46] This was explained by an associative mechanism whose tendency to undergo pseudorotation determines the stereochemistry. Nielsen and Dahl also found that displacement of chloride at P(III) in $CDCl_3$ gives retention products already at the early stages of the reaction and proposed that non-stereoselectivity of the actual substitution step rather than racemisation of the starting material or product was responsible for this.[44]

MECHANISMS OF THE SUBSTITUTION REACTIONS OF P(III) AMIDES

Substitutions of P(III) amides by departure of the amino group as an amide anion is very unfavourable (pK_a of amines in MeCN >40). A proton has to be transferred to the leaving group by an acidic activator before or during the P—N bond cleavage.[60] Sometimes acidic impurities are sufficient to result in the displacement. Phosphoramidites have been the subject of many studies. Their use as phosphitylating agents has been reviewed by Nifant'ev and co-workers[67] and the applications in solid-support synthesis of oligonucleotides by Beaucage and Iyer.[68]

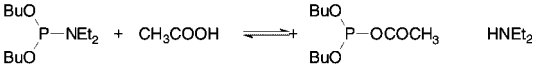
Reactions with acids

Replacement by an acid. Sufficiently strong acids replace the amino function of phosphoramidites unless their conjugate bases are too weakly nucleophilic. Triflic acid (in MeCN, $pK_a = 2.6$), for example, results in *P*-protonation rather than substitution, while methanesulphonic acid ($pK_a = 8.4$) affords mesyl phosphite.[33] Treatment of bisamidite **25** with HCl leads either to P(III) substitution (**26**, in light petroleum) or dealkylation (**27**, in CH_2Cl_2), the main product depending on the reaction conditions:[69] *N*-protonation promotes the P(III) substitution and *P*-protonation dealkylation.



27 **25** **26**

P(III) amides react with phenols and carboxylic acids giving phenyl and acyl phosphites, respectively. In the absence of solvent, **28** reacts instantly with acetic acid or trifluoroacetic acid,[59] and the reaction with phenols takes place without catalysts, which are able to result in only a modest acceleration.[70] The reactivity of these nucleophiles has been attributed to *N*-protonation, which enhances the displacement by a dissociative pathway (cf. Scheme 3). A four-center mechanism (Scheme 9) has also been discussed as a possible alternative.[50]



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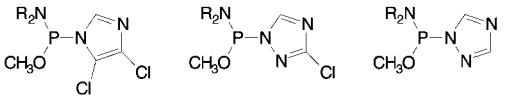


30 **31** **32**

Scheme 12. Azolidites observed by Moore and Beaucage.[8] R_2N = *N*,*N*-dimethylamino, *N*,*N*-diisopropylamino, morpholino or pyrrolidino

In spite of fast substitutions in protic systems, the reactions do not necessarily proceed at all in MeCN, in which solvent AcOH and PhOH are weak acids ($pK_a = 22.3$ and 27.2, respectively). TFA[71] ($pK_a = 12.4$) and 2,4-dinitrophenol[23] ($pK_a = 16.0$), in turn, are fairly reactive even in MeCN.

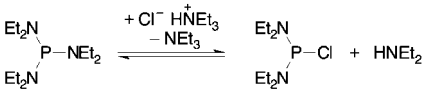
Reactions of acidic azoles with P(III) amides have been studied for their applications in oligonucleotide synthesis. In THF, the rate of displacement [products **30**–**32** (Scheme 12), ^{31}P NMR spectroscopy] increased in the activator series 1,2,4-triazole < 2,3-dichloroimidazole ≤ 3-chloro-1,2,4-triazole and in the leaving group series pyrrolidine < dimethylamine < morpholine.[8] The authors suggested a mechanism via an *N*-protonated species that was assumed to equilibrate rapidly with both the starting material and product. Still, no direct evidence for the existence of the *N*-protonated species could be obtained even at −50 °C. It is worth noting that bisamidites gave only monosubstituted products even when activators were used in 4-fold excess.

The reaction between diethyl *N*,*N*-diisopropylphosphoramidite (**29**) and tetrazole gives the corresponding tetrazolidite **22**, as proved by Berner *et al.*,[64] who identified the product by comparing its NMR spectra with those of an authentic sample. Whereas the reaction of **29** with tetrazole in MeCN was complete in <2 min, those with 1,2,4-triazole and imidazole gave **21** and **22** in only 30 and 10% yield, respectively. The authors attributed this difference to decreasing activator acidity on the basis of the $pK_a(H_2O)$ values for tetrazole, triazole and imidazole (4.76, 10.26 and 14.0, respectively).

The reaction of diisopropyl *N*,*N*-diisopropyl phosphoramidite **7** with 3-nitro-1,2,4-triazole, 5-(4-nitrophenyl) tetrazole and 5-methylthiotetrazole also yielded azolidites in THF. The reaction rate increased with the decreasing pK_a of the azole.[72] By contrast with earlier studies,[64] triazole ($pK_a = 22.4$) and imidazole ($pK_a = 29.4$) were not observed to react with **7** in MeCN.[33] However, in the presence of triethylammonium triflate ($pK_a = 18.5$), imidazole, benzimidazole and triazole did slowly convert **7** to the corresponding azolidites.

The reaction of tetrazole with amidite **7** in THF proved to be second-order in tetrazole concentration,[71] which was tentatively explained by a mechanism involving *N*-protonation by a tetrazole dimer. In THF, association of azoles has been observed to yield hydrogen-bonded associated chains[30b] and this may also account for the observed higher kinetic orders.

Reactions with salts of acids. Ammonium salts of nucleophilic acids allow substitution of P(III) amides under milder conditions than the acids themselves. Weakly acidic ammonium chlorides promote the reversible conversion of amidites to the corresponding chloridites.[74] The equilibrium can be shifted from, for instance, **33** towards **34** by removal of the liberated TEA.[75]

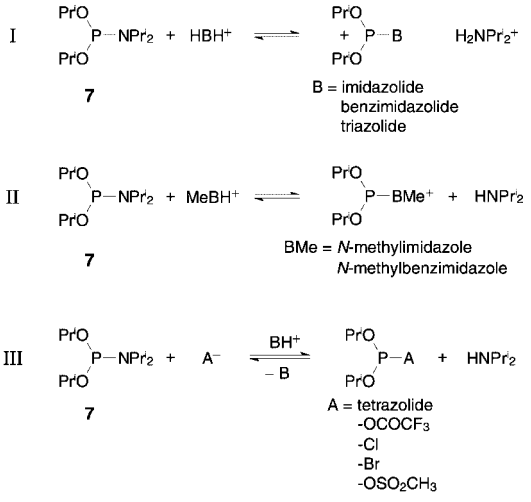


33 **34**

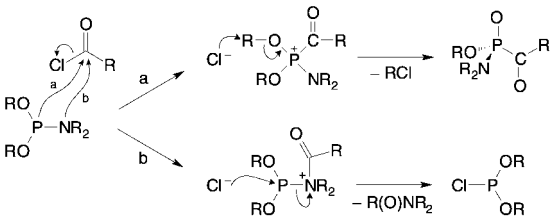
Sufficiently acidic ammonium salts drive the substitution to completion by protolytic trapping of the departed amine: 2 equiv. of *N*,*N*-dimethylanilinium chloride convert a phosphoramidite to the corresponding chloridite in $CDCl_3$.[2] Ammonium chlorides also permit the selective monosubstitution of bisamidites whereas HCl results in a disubstitution.[76] It has been suggested that the ammonium salts are unable to *N*-protonate the monosubstitution product that is a weaker base than the bisamidite.

Ammonium carboxylates and suphonates convert amidite **7** to acyl and sulphonyl phosphites, respectively.[74] In MeCN, the reactivity of ammonium trifluoroacetates and mesylates correlates with the pK_a value of the ammonium ion, while the corresponding triflates are unreactive.[33] Tetrazolide salts give tetrazolidites almost instantly, which is indicative of the high nucleophilicity of azoles towards trivalent phosphorus.

The salts of basic azoles, such as imidazolium and benzimidazolium triflates ($pK_a = 17.1$ and 14.3, respectively), also yield azolidites when reacted with **7** (Scheme 13).[33] Azolium salts with a potentially nucleophilic anion, such as a trifluoroacetate ion, invariably give an



Scheme 13. Displacement of the diisopropylamino group of **7** with a protonated azole (I), protonated *N*-methylazole (II) and an acid anion (III). The overall stoichiometry is determined by both the actual displacement and the subsequent protolytic equilibrium



Scheme 14. Proposed mechanisms of Arbusov (a) and P(III) substitution (b) reactions between phosphoramidites and carboxylic acid chlorides[78]

azolidite rather than an acyl phosphite as a product. Even salts of *N*-methylimidazole and *N*-methylbenzimidazole yield analogous P(III) substitution products in spite of the inability of these adducts to undergo deprotonation after the displacement.

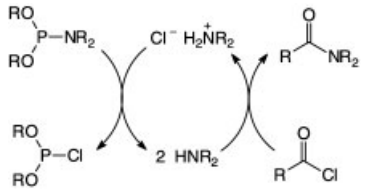
Reactions with other acid derivatives. Acid chlorides react with P(III) amides giving a carboxylic amide and phosphorochloridite (route b in Scheme 14) or a pentavalent Arbusov product (route a in Scheme 14), the favoured path depending on the reaction conditions and reagents.[77,78] A four-membered ring mechanism (Scheme 9)[77] and an addition–elimination process have been considered to be possible mechanistic alternatives.[78]

Batyeva *et al.*[79] suggested that ammonium chloride impurities catalyse the reaction of acid chlorides with P(III) amides. Accordingly, a P(III) substitution between the amidite and ammonium chloride is assumed to yield the phosphorochloridite, while subsequent reaction of the released amine with acid chloride regenerates the catalyst (Scheme 15).

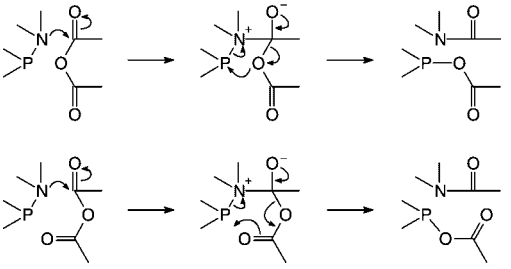
Similarly, the reaction of P(III) amides with carboxylic acid anhydrides yields acyl phosphites and carboxylic amides. Four-membered[80] and six-membered[78] ring mechanisms have been proposed for the transformation (Scheme 16).

Transamidation

Transamidation of P(III) amides has been studied mostly in the presence of ammonium halides that evidently act as acid catalysts: the reaction rate is increased with the increasing concentration and acidity of the salt, and a carefully purified amidite does not react. For instance, the

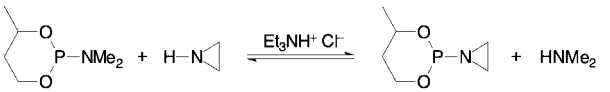


Scheme 15. Suggested nucleophilic catalysis of ammonium halides in halogenation of phosphoramidites with acid chlorides[79]



Scheme 16. Four- and six-membered ring mechanisms proposed for the reaction of phosphoramidites with carboxylic acid anhydrides

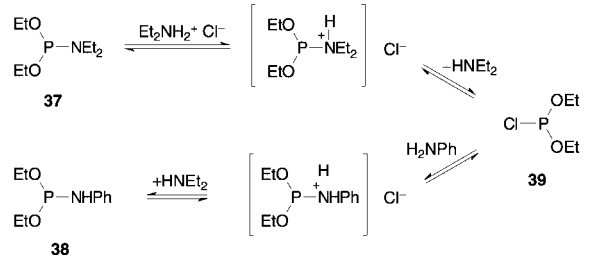
ammonium halide catalysis in the reaction of **35** with aziridine (**36**) has plausibly been explained by a *P*-protonation based acid catalysis mechanism.[81]



35 **36**

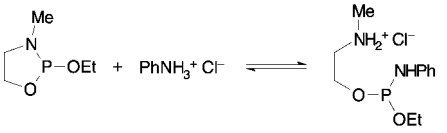
In addition, nucleophilic participation of the halide anion has been considered possible.[55,82] Phosphoramidites are in equilibrium with the corresponding chloridites[74,75] that may react either with the added amine or the one liberated from the original amidite. For instance, in the presence of diethylammonium chloride, amidite **37** has been shown to be converted to **38**, since aniline is more reactive than diethylamine towards chloridite **39** (Scheme 17).

Consequently, Batyeva *et al.*[82] distinguished two pathways: (1) transamidation proper (energetically unfavoured), proceeding via an attack of the more basic amine on the phosphorus atom and cleavage of the less basic one by a four-center mechanism, and (2) transamidation with ammonium chlorides that proceeds via phosphorochloridites, formed by *N*-protonation. The reversibility of the reaction has been demonstrated by the temperature-dependent equilibration between **40** and **41**.[83] The driving force of the substitution evidently is the formation of a more stable ammonium salt. Consistent with this, anilinium chloride, when added to a mixture of **40** and TEA, has not been observed to give any substitution product (triethylammonium chloride was formed), while deprotonation of **41** with TEA has been shown to



Scheme 17. Mechanism for the ammonium chloride-catalysed phosphoramidite transamidation[82]

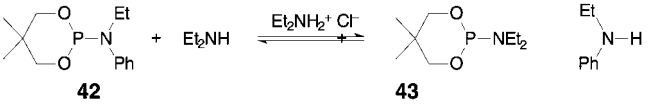
yield **40**. Pudovik *et al.*[83] suggested that after *N*-protonation either aniline or chloride ion may act as a nucleophile, giving the transamidation product either directly or via a chloridite intermediate, respectively.



40 **41**

Nulceophilic catalysis has also been observed in tetrazole-activated transamidation of phosphoramidites.[84] The two amidites, bearing diisopropyl and 5′-O-dimethoxytrityl-3′-aminothymidine groups on phosphorus, were both found to be in equilibrium with corresponding P(III) tetrazolidite. The overall equilibrium between the amidites was found to proceed according to the principle of the cleavage of the more basic amine, the equilibrium constant being in good agreement with the (aqueous) dissociation constants of the applied amines.

Nifantyev *et al.* have reported the transamidation of **42** to obey zero-order kinetics, the rate being dependent only on the catalyst concentration.[85] An equilibrium between the two amidites occurs, and the same equilibrium composition is reached on using **43** as a starting material. The slow reaction rates in $CHCl_3$ (as compared with those in MeCN) have been observed to increase with decreasing concentration. This has been attributed to rate-retarding association of the reactants in a weakly polar solvent, especially at higher concentrations.



42 **43**

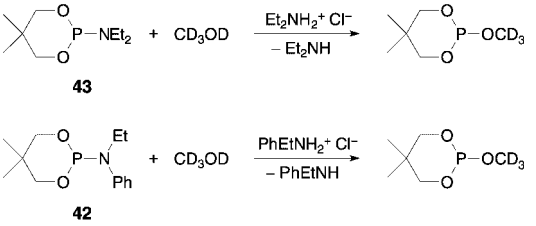
Alcoholysis

The pioneering research in the 1960s already revealed that the alcoholysis of phosphoramidites was affected by ammonium chlorides,[26] acetic acid[86] and phenol.[70] The presence of a sufficiently strong acid was realised to be a prerequisite for the reaction[55] and the idea of acid catalysis was then introduced by Nifant'ev and co-workers.[26] Theories of nucleophilic catalysis originated from the observation that phosphoramidites reacted readily with acidic nucleophiles and gave displacement products that exhibited high reactivity towards alcohols. Evdakov *et al.* proposed a nucleophilic contribution of carboxylic acids and phenols,[59] and Batyeva *et al.* suggested a similar mode of action for ammonium chlorides.[55] Nucleophilicity of tetrazole and other azoles was considered to be an important factor since their introduction as promoters for oligonucleotide synthesis.[63] This catalytic role was actually taken as a fact[8] well before any solid evidence for it had been published.

Effect of the entering alcohol, leaving group and other substituents on P(III). In the absence of an acidic activator, phosphoramidites react only with acidic alcohols in a protic medium. The rate of this unassisted reaction depends on the pK_a value of the alcohol; among the aliphatic alcohols, the acidity of 1*H*,1*H*-heptafluorobutan-1-ol [$pK_a(H_2O) \approx 12$] is comparable to that of phenol [$pK_a(H_2O) = 10$].[70] Ethylene glycol [$pK_a(H_2O) = 15.4$] has been suggested to react by intramolecular assistance of the vicinal hydroxyl group, but it is also significantly more acidic than *tert*-butyl alcohol [$pK_a(H_2O) = 19.2$] that remained unreacted under the same conditions.[86]

Formal kinetics of the alcoholysis with respect to the alcohol concentration have often been obscured by the usually applied pseudo-first-order conditions. A study on tetrazole-activated alcoholysis if **1c** in THF has, however, revealed a zero-order dependence of the rate on the alcohol concentration, consistent with nucleophilic catalysis by tetrazole.[71]

It has been shown that an aniline ligand is displaced faster than a more basic aliphatic amine. Apparently, this might argue against mechanisms involving *N*-protonation,[87] but the observation may as well be accounted for by the fact that the anilinium salt contaminating the aniline derivative is more acidic than the ammonium salt contaminating the amidite. The latter explanation receives support from more recent results reporting $t_{1/2} = 50$ min for the alcoholysis of **43** catalysed by diethylammonium fluoride, but only $t_{1/2} < 2$ min for the corresponding reaction of **42** in the presence of *N*-ethylanilinium fluoride.[58]



43

42

Similar results have been reported by Dahl and co-workers. On using 0.02 equiv. of dimethylammonium chloride as an activator, the alcoholysis rate of phosphoramidites in $CHCl_3$ is increased with decreasing leaving group basicity: $—NPr^i_2 < —NMe_2 < —NMePH < —NPh_2$.[4] In contrast, in the presence of 1 equiv. of tetrazole in MeCN an opposite trend has been observed.[88] In the presence of a weakly acidic dimethylammonium ion ($pK_a = 18.7$), especially in a solvent of low polarity, the effect of the salt presumably present as a contaminant may become significant and account for the first trend. On the other hand, an equimolar amount of tetrazole ($pK_a = 14.5$) should result in such an efficient rate acceleration that the possible salt effects may be neglected.

The identity of the alkoxy groups on the phosphorus atom has also been found to affect the alcoholysis rate

that is increased in the series 2-chlorophenyl $\ll$—$C(CH_3)_2CH_2CN$ < —$CH(CH_3)CH_2CN$ < —CH_2CH_2CN < —CH_3. The sensitivity of the reaction to steric hindrance appears to be modest compared with the inductive effect of the aryl group.

General acid catalysis. Kinetic studies have proved that phosphoramidite alcoholysis is subject to general acid catalysis. Nifant'ev *et al.* followed (^{31}P NMR spectroscopy) the reaction in *tert*-butyl alcohol in the presence of various ammonium salts, the pK_a values of which ranged from 5 to 11 (aqueous pK_a values used in alcohol solutions).[89] Pseudo-first-order rate constants (k_1^{Ψ}) were determined and used for the calculation of catalytic constants (k_c) of each salt:

$$\frac{dP}{dt} = k_1^{\Psi}[\text{amidite}] = (k_0 + k_c[\text{catalyst}])[\text{amidite}] \quad (1)$$

$$\log\left(\frac{k_c}{S}\right) = \log G_A - \alpha pK_a \quad (2)$$

The logarithmic plot of k_c vs –pK_a [Eqn (2)] gave a value of 0.05 for the Brønsted α coefficient, interpreted as a measure of the degree of proton transfer in the transition state.[57] More extensive studies in *tert*-butyl alcohol gave a Brønsted α of 0.13[28] and those in methanol a value of 0.65.[56]

General acid catalysis has later been confirmed in MeCN with the reaction of amidite **7** with propan-2-ol in the presence of ammonium triflates, mesylates and trifluoroacetates falling in the pK_a range 14.5–16.5, the Brønsted α values obtained being 0.6, 0.7 and 0.9, respectively (see Fig. 1 and Table 3). Triethylammonium salts to (pK_a = 18.5) have been reported to be too weak acids to permit the alcoholysis.

Studies on the alcoholysis of phosphonamidite **44** have provided evidence for intramolecular general acid catalysis.[90] The reaction rate was found to be independent on the pK_a value of the added ammonium halides that became deprotonated by the basic amino function of the starting material. The alcoholysis was 300 times faster than that of the analogue **45** that cannot be activated in an intramolecular manner. With **45**, the normal dependence of rate on the activator acidity was observed.

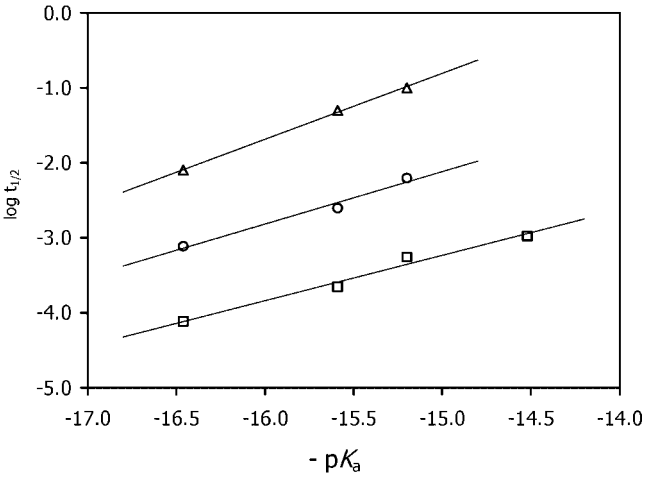
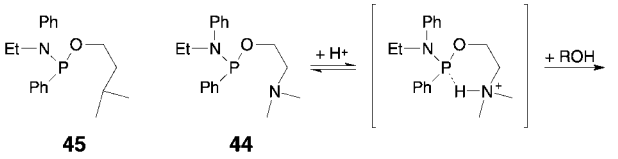


Figure 1. Reaction of **7** with Pr^{i}OH in the presence of ammonium trifluoroacetates (△), mesylates (○) and triflates (□) in MeCN. Brønsted dependence of the half-life of the reaction on acidity of the activator[33]



Nucleophilic catalysis by azoles. The first observation lending support to nucleophilic catalysis of tetrazole (TH) was the appearance of a ^{31}P NMR signal ($\delta_P \approx 126$ ppm), tentatively assigned to a tetrazolidite.[88,91] This allowed Moore and Beaucage to suggest a mechanism in which *N*-protonated amidite was attacked either by the azole or alcohol,[8] and any azolidite formed reacted subsequently with the alcohol. Dahl *et al.*,[88] in turn, proposed a fast proton transfer followed by a slow equilibrium between the *P*-protonated species and the tetrazolidite that acts as the phosphitylating agent. The identity of tetrazolidite intermediate **22** was later verified, lending further support to the nucleophilic role of tetrazole.[64]

Kinetic work by Dahl *et al.* in MeCN showed that the alcoholysis was faster if tetrazole and amidite had been mixed before the introduction of alcohol.[88] (Reaction samples were quenched with TEA, which would convert any tetrazolidite formed to amidite and hence [amidite]$_{obs}$ = [amidite] + [tetrazolidite].) This is in agreement

Table 3. Half-lives (s) for the reaction of **7** (0.1 mol dm^{-3}) with Pr^{i}OH (1.0 mol dm^{-3}) in acetonitrile on using trialkylammonium salts (0.1 mol dm^{-3}) as activators[33]

Ammonium salts Cations	Anions pK_a	Triflate 2.6	Mesylate 8.4	Trifluoroacetate 12.7	Tetrazolide 14.5
Triethylammonium	18.5	∞	∞	∞	∞
N,*N*-Dimethylbenzylammonium	16.5	13 000	1300	125	<5
N-Methylmorpholinium	15.6	4500	400	20	<5
Triallylammonium	15.2	1800	120	10	<5
N,*N*-Diisopropylanilinium	14.5	950	—	—	

with the nucleophilic contribution of tetrazole, but alternative explanations, such as rate-retarding association of tetrazole with the alcohol, are still worth considering. Furthermore, the reaction rate was dramatically reduced by addition of even 0.1 equiv. of diisopropylammonium tetrazolide to the system. The applied method gives two alternative explanations for this: retardation of the formation of tetrazolidite could be explained by rate-retarding tetrazole–salt association, while the alcoholysis of tetrazolidite might be slowed either by a similar alcohol–salt association or by a common ion mass effect.

The strongest evidence for nucleophilic catalysis by tetrazole (TH) was obtained in a kinetic study on amidite **7** in THF, in which solvent the moderate reaction rates (^{31}P NMR spectroscopy) allowed calculation of the rate constants on the basis of the initial rates. The key observation was that the reaction of **7** with alcohol in the presence of tetrazole was kinetically equivalent to the reaction of **7** with tetrazole: both reactions were observed to be second order in TH [Eqn. (3), $n = 2$] with the rate constants 0.33 and 0.31 $dm^6\,mol^{-2}\,s^{-1}$, respectively.

$$v_0 = \lim_{t\to 0}\left(\frac{d[7]}{dt}\right) = k_1\,[7]_0\,[TH]_0^n \qquad (3)$$

The formal kinetics for the alcoholysis of **23** were also determined and the model of two consecutive reactions tested by a simulation program. The reasonably good fit supported the nucleophilic catalysis, but some systematic deviation occurred, leaving a possibility for the existence of a minor reaction path bypassing **23** as intermediate. One explanation for the incomplete fit was presented later: the reactivity of **23** in THF is very sensitive to the ammonium tetrazolide salt formed during the reaction.[31] The solubility of this salt, in turn, has been observed to be affected by the presence of tetrazole. Consequently, the high tetrazole concentration at early stages of the reaction enhances the salt concentration and thus accelerates the reaction of **23** more than predicted by the tested model.

Nucleophilic catalysis by ammonium salts. The early suggestions,[55] according to which the halide ion of ammonium halides served as a nucleophilic catalyst in the alcoholysis of phosphoramidites, were questioned by a study on the alcoholysis of phosphoramidite **42** and the corresponding fluoridite **24**.[58] In the absence of alcohol, **42** and *N*-ethylanilinium fluoride yielded a mixture of **24** and *N*-methylaniline. These were found to react with alcohol more slowly than the original reactants, which was interpreted to argue against the role of **24** as an intermediate. The pitfall of this logic, however, is that the substantial amount of the salt activator still present after the formation of the fluoridite **24** may have a huge effect on the reactivity of **24**, and hence the relevance of comparisons of two separate reactions may be questioned.[31] Furthermore, the affinity of F towards P(III) and the remarkable stability of the P—F bond impede extrapolation of fluoridite reactivity to the other halidites. For example, diethylammonium chloride and bromide have been observed to be twice as effective activators of the phosphoramidite alcoholysis as the corresponding fluoride.

Evidence for the nucleophilic contribution of ammonium salts has been presented in a recent kinetic study. The reaction of amidite **7** with nucleophilic salts in the absence of alcohol was faster than its alcoholysis in the presence of a weakly nucleophilic salt. For salts of equal acidity, the alcoholysis rate was found to depend on their nucleophilicity (Table 3). The nucleophilicity of the anions increased in the order $CF_3SO_3^- < CH_3SO_3^- \approx Br^- < Cl^- \approx CF_3COO^-$ < tetrazolide anion. If the basicity of the anion is taken as a measure of its nucleophilicity, β_{nucl} value of 0.2 is obtained for the applied ammonium salts.

The nucleophilic contribution of the triflate anion remained uncertain, since these salts were unable to give displacement products in the absence of alcohol. Nevertheless, the appearance of a highly unstable P(III)–triflate intermediate cannot be ruled out, since the corresponding stable structures have been reported to exist.[42] It has also been argued that under weakly nucleophilic conditions, traces of H-phosphonate, formed as a hydrolysis product, could act as a nucleophile, enabling alcoholysis to occur via dialkylphosphite anhydride, the ^{31}P NMR signal of which has been detected during many reactions.

Nucleophilic catalysis by azolium and pyridinium salts. Kinetic results (MeCN) have lent support to the suspected nucleophilic role of azolium salts.[33] Alcoholyses promoted by benzimidazolium and imidazolium salts ($pK_a = 14.3$ and 17.1, respectively) took place almost instantaneously. Even the corresponding *N*-methylazoles were equally effective as activators, while less nucleophilic salts of similar acidity gave lower reaction rates. Triethylammonium salts ($pK_a = 18.5$) were too weakly acidic to catalyse the alcoholysis when the anion was a poor nucleophile, such as a triflate ion. The reaction, however, took place when a good nucleophile was added. Neutral nucleophiles proved to be superior to anionic ones, since even the tetrazolide anion remained unreactive while imidazole and benzimidazole favoured the reaction. Azolium salts have been studied by Hayakawa *et al.*, who also explained the observed reactivity by a nucleophilic contribution.[14]

The absence of phosphorochloridite (^{31}P NMR signals, MeCN) after the reaction of pyridinium chloride with a phosphoramidite led Beier and Pfleiderer to suggest nucleophilic catalysis by pyridine.[18] Products resonating at 10–20 ppm, most likely hydrogen phosphonates formed by hydrolysis or dealkylation of the 2-cyanoethoxy group, were observed instead. However, pyridinium chloride and 2,6-di-*tert*-butylpyridinium chloride successfully served as promoters on a synthesizer.

Degradation to the product at 10–20 ppm was slower with the less acidic *tert*-butyl derivative.[18]

^{15}N NMR observations of Sanghvi *et al.* indicated that no pyridinium species were formed in a reaction of phosphoramidite with a pyridinium salt, since pyridine seemed to exist in a neutral form.[19] Indeed, the anions of pyridinium salts, rather than pyridine, have later been observed to displace the amino group of phosphoramidite **1c**.[33] The evident thermodynamic stability of these products is not, however, a sufficient proof for their kinetic significance: the pyridinium salt-promoted substitutions by salt anions as well as alcohol could still take place via the debated P(III)–pyridinium species.

In P(V) chemistry, the intermediacy of pyridinium structures has been suspected since their observation by ^{31}P NMR spectroscopy.[92] More recently, pyridine adducts have been detected even for neutral P(V) diesters[93] and kinetic evidence has been presented that H-phosphonate alcoholysis proceeds via such species.[94] For trivalent phosphorus, the phosphenium ion character of P(III) triflates has been shown to be enforced by the introduction of 4-aminopyridine, which has been attributed to the formation of P(III)–pyridinium species.[95] Furthermore, kinetic results on pyridinium activators are best explained by the nucleophilic contribution of the deprotonated pyridine:[33] For example, in MeCN, the alcoholysis promoted by 2,6-lutidinium triflate (pK_a = 14.4) was considerably faster than that in the presence of *N*,*N*-diisopropylanilinium triflate ($pK_a = 14.5$). Like azoles, pyridine and 2,6-lutidine promoted the alcoholysis when they were added to an otherwise unreactive mixture of **1c**, triethylammonium triflate and propan-2-ol in MeCN. This suggests a reaction via a P(III)–pyridinium species that is too unstable to be observed directly.

Concise mechanism of the P(III) amide substitution

Acid catalysis. The applicable promoters of phosphoramidite alcoholysis fall in the pK_a range (in MeCN) 12.5–18.5. Acids weaker than this are unable to promote the reaction (in MeCN), whereas ammonium ions with $pK_a = 18.5$ are effective only in the presence of good neutral nucleophiles. About 1.5 units more acidic imidazolium salts are already effective activators. The acidity of many activators (tetrazole and benzimidazolium salts, $pK_a = 14.5$) is surprisingly high compared with that of dichloroacetic acid (DCA, 15.8) used as an acid catalyst in the detritylation step of oligonucleotide synthesis. Even more acidic pyridinium salts (12.6) could be expected to be less compatible with the normal protocol of oligonucleotide synthesis. In spite of this, these activators have been reported to give good results.

The acid catalysis of the activators refers in all likelihood to *N*-protonation of the leaving group. The *N*-protonated species is too unstable to be detected, but indirect evidence for its existence has been presented: *P*-protonation has only been observed with non-nucleophilic acids having a $pK_a < 10$.[39] While weaker acids are unable to result in *P*-protonation, nucleophilic salts of similar acidity still are good activators, and the reaction hence must proceed by another route, namely *N*-protonation taking place as part of the rate-limiting step, as discussed below in more detail.

According to molecular modelling, *N*-protonation promotes the reaction by a dissociative mechanism, while a *P*-protonated phosphoramidite reacts by an associative reaction path.[40] Apparently, protonation of the phosphorus atom is the thermodynamically favoured alternative, but it does not lead to fast alcoholysis. Since *P*-protonation strengthens the P—N bond, it prevents the other possible reaction, viz. *N*-protonation of the leaving group concerted with the P—N bond cleavage. Accordingly, relatively strong acids [pK_a(MeCN) < 10] capable of quantitative protonation of the starting material are not very efficient activators (Fig. 2). Weaker acids that leave the starting material largely unprotonated permit P—N bond elongation facilitated by a concerted proton transfer from the activator. Evidently, this mechanism for P—N bond cleavage is considerably more favoured than that via the *P*-protonated species.[39]

Concerted mechanism. The phosphoramidite substitution reaction is above all a process of P—N bond cleavage, whose immediate products, the amide anion

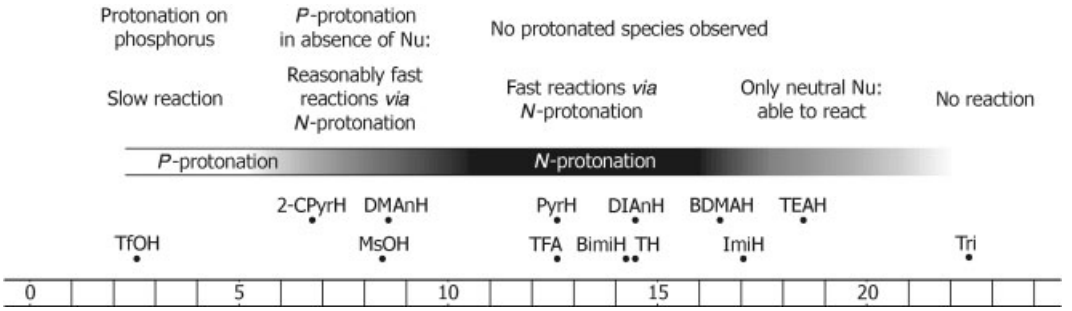


Figure 2. Effect of acids on phosphoramidites as a function of the acid strength. 2-CPyrH = 2-chloropyrinium ion; DMAnH = *N*,*N*-dimethylanilinium ion; PyrH = pyridinium ion; DIAnH = diisopropylanilinium ion; BDMAH = *N*,*N*-dimethylbenzylammonium ion; BimiH = benzimidazolium ion; ImiH = imidazolium ion; Tri = triazole

and phosphenium cation, need to be stabilized by an acid and a nucleophile, respectively. The observed Brønsted $\alpha = 0.6$–0.9 and $\beta_{nucl} = 0.2$ values indicate that the nucleophile and the acid enter the reaction in a concerted manner, but in the transition state, proton transfer is well advanced compared with the modest degree of the nucleophilic attack.[33] This means that the reaction mechanism must have a dissociative character. Brønsted α values can be taken as measure of degree of proton transfer of the partial charge of the protonated transition state.[56,57] Since *N*-protonation of phosphoramidites induces the positive charge on phosphorus and promotes P—N bond cleavage,[40] the high α values also indicate advanced departure of the cleaving group compared with the phosphorus–nucleophile bond formation.

Consequently, a mechanism commencing with stretching of the P—N bond and proton transfer to the nitrogen, followed by concerted, dissociative nucleophilic attack, has been proposed.[33] The identity of the nucleophile has been observed to affect the reaction rate, in which case the nucleophile naturally has to be present in the transition state. However, excess of imidazole used as a nucleophile has been observed not to accelerate substitution reactions in the presence of ammonium ions as acids. This has been explained by a hypothesis stating that for a very good nucleophile used in excess, the barrier of the nucleophilic attack may become lower than that of the acid catalytic step, which then alone remains rate-limiting (Fig. 3).

A concerted mechanism is in good agreement with stereochemical results of phosphoramidite alcoholysis. In the presence of a non-nucleophilic activator such as trialkylammonium fluoborate, phosphoramidite alcoholysis has been observed to proceed with inversion of stereochemistry attributable to direct in-line attack of the alcohol.[96] Nucleophilic promoters have frequently resulted in racemisation of any *P*-enantiopure phosphoramidite.[66] However, in some cases use of diastereomeric phosphoramidite with chiral phosphorus moiety or an amidite bearing a chiral auxiliary have selectively yielded retention products, presumably as a result of two consecutive in-line replacements on the phosphorus centre.[97]

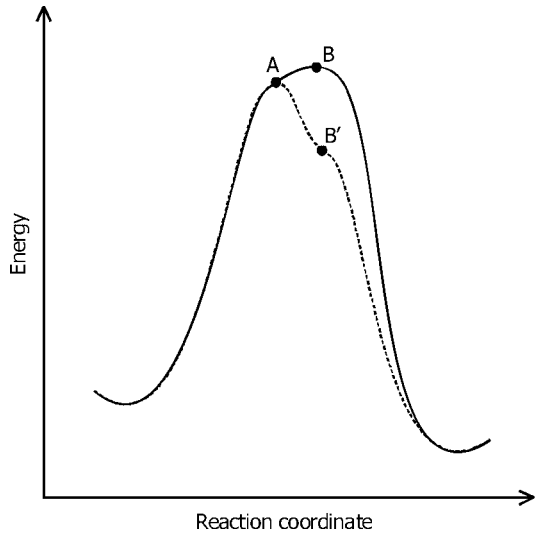


Figure 3. Energy profiles for the acid-catalysed phosphoramidite alcoholysis with a poor (solid line) and a good (dashed line) nucleophile. The barrier for the nucleophilic attack (B) is of comparable height to that of the protonation step (A) in the case of a poor nucleophile, but with a good nucleophile it is significantly lower than the protonation barrier

Nucleophilic catalysis and effect of the leaving group. The paradox of phosphoramidite alcoholysis lies in the considerably high activation energy required to obtain the thermodynamically stable product. The barrier that is partially attributable to the poor protolytic properties of alcohols in an aprotic medium is easily overcome if the leaving group is good, but amino groups that require activation are alcoholysed reluctantly. Hence nucleophilic catalysis is crucial for fast alcoholysis of phosphoramidites.

Nucleophilic attack on P(III) seems to require a sufficiently well developed electron deficiency on phosphorus, the amount of which depends mainly on the nature of the leaving group. An easily polarizable bond to a good leaving group, such as tetrazolide, trifluoroacetate of chloride, ensures advanced charge formation on P(III) and hence results in a fast displacement. For phosphoramidites, the required polarization can only be obtained by proton transfer to the departing amino group. Since *N*-protonated phosphoramidites are only marginally stable, phosphoramidites react readily only with nucleophiles capable of attacking before complete protonation (cf. the α values). The short lifetime of the activated species hinders or completely blocks reactions with nucleophiles whose reactivity is reduced by unfavoured immediate products. The positive charge of P(III)–pyridinium species and especially that of P(III) *N*-methylazolidites is stabilized by resonance that increases their reactivity. While these leaving groups clearly depart as neutral species, it is easily overlooked that this must also apply to the non-methylated P(III) azolidites of similar basicity. They become excellent leaving groups on protonation that is facilitated by the topological fact that it does not take place on the nitrogen atom bonded to phosphorus.

Interestingly, the observed outcome of most reactions is surprisingly well explained by viewing them solely as protolytic equilibria. In other words, the equilibrium constant of the actual substitution may be insignificant to the reaction that is controlled by the protolytic interests of its components.

Conclusion: Catalyst alternatives in the past and the future

Table 4 lists activators reported in the literature together with data for their acidity and nucleophilicity. For

Table 4. List of activators reported in literature, and the nucleophiles and acids responsible for their reactivity together with their pK_a values in MeCN, with activity indicated as follows: —, not as good as TH; + as good as TH; ++ better than TH; +++, the highest activity observed thus far

Activator	Acid	pK_a	Nucleophile	Activity	Ref.
1*H*-Tetrazole	TH	14.5	T^-	+	
5-(*p*-Nitrophenyl)-1*H*-tetrazole	NO_2-Ph-TH	13.3[a]	NO_2-Ph-T^-	++	5, 14
5-(Ethylthio)-1*H*-tetrazole	EtS-TH	12.9[a]	EtS-T^-	++	6
5-Trifluoromethyl-1*H*-tetrazole	CF_3-TH	—	CF_3-T^-	++	7
3-Chloro-1,2,4-triazole	Cl-Tri	—	Cl-Tri^-		8
4,5-Dichloroimidazole	Di-Cl-Imi	—	Di-Cl-Imi^-	+	8
4,5-Dicyanoimidazole	Di-CN-Imi	—	Di-CN-Imi^-	++/−	10, 14, 19
2-Bromo-4,5-dicyanoimidazole	Br-di-CN-Imi	—	Br-di-CN-Imi^-	+++	11, 14
2-Mesityl-4,5-dicyanoimidazole	Mes-di-CN-Imi	—	Mes-di-CN-Imi^-	++	12
Benzimidazolium triflate	$BimiH^+$	14.3	Bimi	++	13, 14
Benzimidazolium fluoborate	$BimiH^+$	14.3	Bimi	++	13, 14
Imidazolium triflate	$ImiH^+$	17.1	Imi	++/−	14, 15, 19
Imidazolium perchlorate	$ImiH^+$	17.1	Imi	++	14, 15
Imidazolium fluoborate	$ImiH^+$	17.1	Imi	++	14, 15
Imidazolium chloride	$ImiH^+$	17.1	Imi	−	19
N-Methylbenzimidazolium triflate	*N*-Me-$BimiH^+$	14.1	*N*-Me-Bimi	+++	14
N-Methylimidazolium triflate	*N*-Me-$ImiH^+$	17.1	*N*-Me-Imi	++	14, 16
N-Methylimidazolium chloride	*N*-Me-$ImiH^+$	17.1	*N*-Me-Imi	++	7
N-Methylimidazolium trifluoroacetate	*N*-Me-$ImiH^+$	17.1	*N*-Me-Imi	−	17
N-Phenylimidazolium triflate	*N*-Ph-$ImiH^+$	16.0[a]	*N*-Ph-Imi	+++	14
N-Phenylimidazolium perchlorate	*N*-Ph-$ImiH^+$	16.0[a]	*N*-Ph-Imi	+++	14
N-Phenylimidazolium fluoborate	*N*-Ph-$ImiH^+$	16.0[a]	*N*-Ph-Imi	+++	14
Pyridinium chloride	$PyrH^+$	12.6	Pyr	+/−	18–20
Pyridinium bromide	$PyrH^+$	12.6	Pyr	+	18
Pyridinium fluoborate	$PyrH^+$	12.6	Pyr	+++/−	14, 17
Pyridinium tosylate	$PyrH^+$	12.6	Pyr	+	18
Pyridinium triflate	$PyrH^+$	12.6	Pyr	−	18
Pyridinium trifluoroacetate	TFA/$PyrH^+$	12.7	Pyr	+/−	19, 20
Pyridinium dichloroacetate	DCA	15.8	Pyr	+	19
4-Chloropyridinium chloride	4-Cl-$PyrH^+$	10.9[a]	4-Cl-Pyr	−	18
2,6-Di(*tert*-butyl)pyridinium chloride	di-Bu^t-$PyrH^+$	—	Cl^-	+	18
N,*N*-Dimethylanilinium chloride	HCl/$DMAnH^+$	8.9	Cl^-	+	2
N-Methylanilinium trifluoroacetate	TFA	12.7	CF_3COO^-	+/−	21, 17
N-Methylanilinium trichloroacetate	TCA	10.6	CCl_3COO^-	+	22
Pyridinium chloride and imidazole	$ImiH^+$	17.1	Imi	+	24
Pyridinium salts and *N*-methylimidazole	*N*-Me-$ImiH^+$	17.1	*N*-Me-Imi	+	20
Pyridinium bromide and *N*-methylimidazole	*N*-Me-$ImiH^+$	17.1	*N*-Me-Imi	−	20
2,4-Dinitrophenol	Di-NO_2-PhOH	16.0	Di-NO_2-PhO^-	+	23

[a] Values estimated from aqueous constants using the appropriate pK_a(H_2O) − pK_a (MeCN) dependences illustrated in Table 1.

catalysts with several possible nucleophiles, the one found to be the most effective[33] is suggested. It seems that some salt activators may have been chosen without an exact knowledge of their acidity, since they do not exist as ions in MeCN (although they can still be efficient activators). Interestingly, the catalyst concentration may also affect its success; for instance, pyridinium tetrafluoroborate (p$K_a = 12.6$) has given excellent results when used in a 1:1 ratio with the amidite, but resulted in detritylation when applied in excess.[14,17]

Acid strength as modest as 17.1 of imidazolium ion has proved to be sufficient for activation as long as a good neutral nucleophile is present.[14,15,33] Since the detritylation risk increases together with pK_a, there seems to be little reason to use acids stronger than tetrazole (14.5) or, preferably, DCA (15.8). Imidazolium, *N*-methylimidazolium and the recently introduced *N*-phenylimidazolium salts[14] (p$K_a \approx 16.0$) seem to be good activator candidates. Alternatively, a combination of a neutral azole and a suitable acid can be applied, in which case the nucleophilicity and the acidity of the activator to be adjusted in an independent manner.

The choice of an optimum catalyst is a complicated issue depending on the oligomer being synthesized, and many factors contributing to the outcome are still unclear. For instance, TFA used alone resulted in detritylation whereas TFA–pyridine mixture of equal acidity was a successful activator.[19] The activity of the azolium activators has been reported to depend on the identity of the anion,[14] which, according to other results, should not participate in the reaction.[33] It seems that a deeper understanding of the interactions between the activators and phosphoramidites, and research assessing the kinetics of the really fast processes involved in the

activation, are required to complete the mechanistic picture of P(III) amide substitution and give us keys to master the reaction.

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