# Hydrolytic Reactions of Nucleoside Phosphoramidates: Kinetics and Mechanisms

Tuomas Lönnberg, Mikko Ora and Harri Lönnberg*

*Department of Chemistry, University of Turku, FIN-20014 Turku, Finland*

**Abstract:** Nucleoside phosphoramidates have recently received considerable interest as pro-drugs of antiviral analogs of nucleoside monophosphates and as constituents of antisense oligonucleotides. For these reasons, the hydrolytic stability of phosphoramidate linkages also is of interest. The present paper gives a survey of the kinetic and mechanistic studies with phosphoramidates, above all the studies with nucleoside phosphoramidates.

**Keywords:** Phosphoramidates, hydrolysis, kinetics, mechanims, nucleotides.

## INTRODUCTION

Nucleoside phosphoramidates are congeners of nucleotides having one of the phosphate oxygen atoms, either a bridging or non-bridging one, replaced with an amino group. While the chemistry of nucleotides has been an object of continuous interest ever since the description of DNA structure in early 1950s, their phosphoramidate analogs have received marked attention only during the past decade. This recent interest largely originates from promising chemotherapeutic applications of phosphoramidates. Most of the antiviral drugs in clinical use are structural analogs of nucleosides. In some cases, the antiviral influence has experienced a marked increase on using, instead of an underivatized nucleoside analog, its appropriately protected 5´-phosphate as a drug [1]. Nucleoside 5´-phosphoramidates derived from α-amino acids constitute one such class of pro-drug candidates [2]. Their increased therapeutic potency has been attributed to enhanced cellar uptake and subsequent intracellular release of the nucleoside 5´-monophosphate [3]. Accordingly, phosphoramidates are believed to offer a by-pass to the conversion of nucleoside analogs to their monophosphates by thymidine kinase. In addition, the recent studies suggest that nucleoside phosphoramidates may also serve as substrates of HIV-1 reverse transcriptase [4]. Besides applications as monomeric antiviral agents, nucleoside phosphoramidates have shown promise as constituents of antisense oligonucleotides [5].

The obvious therapeutic potential of phosphoramidates has aroused interest in the kinetics and mechanisms of their solvolytic reactions. Since the amino ligand of phosphoramidates is a much stronger Brönsted base and much weaker Brönsted acid than the hydroxy group of phosphate esters, the protolytic equilibria of these two classes of compounds are quite different, which has a profound effect on their solvolytic stability. The present review briefly summarizes what is known about the course of the solvolytic reactions of nucleoside phosphoramidates. To provide a sufficient mechanistic background, the kinetic studies on the hydrolysis of structurally simple phosphoramidates are first surveyed.

## HYDROLYSIS OF PHOSPHORAMIDIC ACID

Phosphoramidates are mono- or di-esters of phosphoramidic acid, the phosphoryl amino ligand of which may additionally be substituted or incorporated in a ring structure, either an aliphatic or aromatic one. Unsubstituted phosphoramidic acid has two p$K_a$ values in the normal pH-range: p$K_{a1}$ = 3.00 and p$K_{a2}$ = 8.15 for the deprotonation of the neutral (**1b**) and monoanionic (**1c**) species, respectively [6]. Both of these species may in principle occur as a nitrogen or oxygen protonated tautomer ($O^-/NH_3^+$ *vs*. $OH/NH_2$), as indicated in Scheme 1. However, in contrast to carboxylic amides, the tautomers through which the solvolytic reactions proceed most likely are the nitrogen protonated species. Hydrolysis of the P-N bond is pH-independent over a pH range 4 - 7, the first-order rate constants being $k^C = 6.9\times10^{-5}$ s$^{-1}$ ($\tau_{½}$ = 2.8 h) at 36.8 °C ($I$ = 0.2 mol L$^{-1}$). The reactive ionic form under these conditions is the zwitterionic monoanion (**1c**), on which water attacks. Evidently, the reaction represents a borderline case between an associative and dissociative mechanism: the cleavage of the P-N bond is rather advanced, but not yet complete, upon the attack of water (Reaction **C** in Scheme **1**). This conclusion receives support from the fact that the entropy of activation is only slightly negative (-7 J K$^{-1}$ mol$^{-1}$) and the attack of the more nucleophilic alcohol is somewhat favored in water-alcohol mixtures [6].

On the acidic side of p$K_{a1}$, the reaction turns acid-catalyzed. Around pH 3, attack of water on the neutral zwitterion **1b** (Reaction **B**) predominates, but on going to more acidic solutions, hydrolysis of the monocationic conjugate acid **1a** (Reaction **A**) gradually takes over. The rate constants for these partial reactions are $k^B = 1.17\times10^{-4}$ s$^{-1}$ ($\tau_{½}$ = 1.6 h) and $k^A = 9.3\times10^{-3}$ L mol$^{-1}$ s$^{-1}$, respectively. The clearly negative values of the entropy of activation (-76 and -88 J K$^{-1}$ mol$^{-1}$ for **B** and **A**, respectively) suggest that the transition states are more associative in nature than that of reaction C [6], but a dissociative mechanism has also received support [7]. The dianionic form (**1d**) is much less reactive than the monoanion **1c**; a rate-retardation takes place on going to alkaline solutions.

The hydrolysis of phosphoramidic acid monoanion (**1c**) is catalyzed by tertiary amines, such as pyridines, imidazoles and their congeners [6,8]. The catalytic constants vary from $3\times10^{-3}$ to $6.7\times10^{-2}$ L mol$^{-1}$ s$^{-1}$ (39 °C, $I$ = 1.0 mol L$^{-1}$) with amines having p$K_a$ values in the range 2.8 - 9.7. Accordingly, the susceptibility to the basicity of the catalyst is quite low, the $\beta_{nuc}$ value being 0.22 [8]. The reaction, in all likelihood, proceeds by two consecutive nucleophilic displacements; the amine ligand of **1c** is first displaced with the attacking amine and this is then displaced of with water. In addition to amines, formaldehyde and hypochlorite ion serve as catalysts [9]. Evidently, hydroxymethylation or chlorination converts the amino group to a better leaving group.

## HYDROLYSIS OF THE MONO- AND DI-ESTERS OF PHOSPHORAMIDIC ACID

The monoalkyl esters of phosphoramidic acid, such as **2b,** are hydrolyzed under acidic conditions by the same mechanisms as the neutral zwitterionic phosphoramidic acid (Reactions **A** and **B** in Scheme **1**). The p$K_{a1}$ value for the neutral methyl ester (**2b**) is 2.50. The rate constants for the attack of water on the zwitterionic tautomer of this species (Reaction **B**) and on its conjugate acid **2a** (Reaction **A**) are $k^B = 1.61\times10^{-4}$ s$^{-1}$ ($\tau_{½}$ = 1.2 h) and $k^A = 1.56\times10^{-3}$ L mol$^{-1}$ s$^{-1}$ (36.8 °C, $I$ = 0.2 mol L$^{-1}$), respectively. In other words, the spontaneous cleavage is almost 40% faster and the hydronium ion catalyzed cleavage 80% slower than the corresponding reactions of phosphoramidic acid (**1b**) [10]. Both reactions most likely proceed via an associative transition state [10], although the value close to unity obtained for the kinetic $k(N^{14})/k(N^{15})$ isotope effect in ethanol rather suggests a metaphosphate-like transition state [11]. The monoanionic form (**2c**), predominating at pH > 2.5, is hydrolyzed only very slowly (2% in 26 h at pH 6.58), analogously to the

*Address correspondence to this author at the Department of Chemistry, University of Turku, FIN-20014 Turku, Finland; E-mail: harlon@utu.fi

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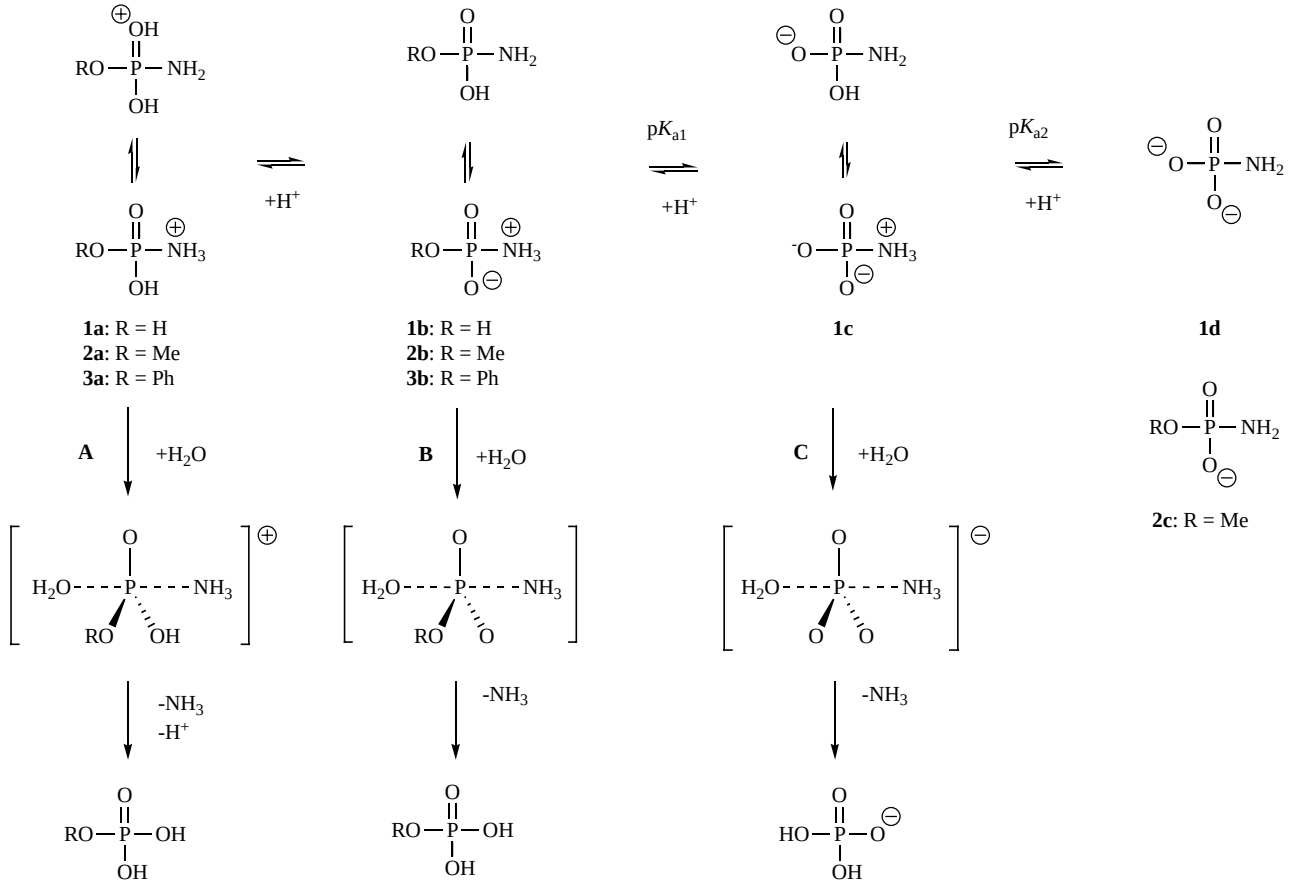
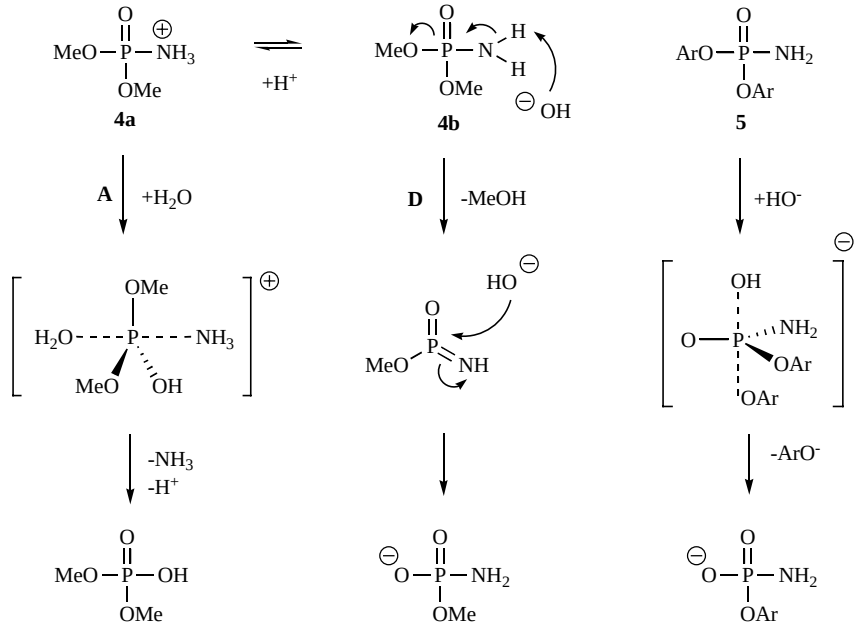



**Scheme 1.**




**Scheme 2.**

dianion of the free acid (**1d**). With both **2c** and **1d**, the amino group should depart as an amide ion and this obviously is very difficult. With the phenyl ester (**3b**), hydrolysis by the hydronium ion catalyzed route (Reaction **A**) is favored: $k^A/k^B$ = 170 L mol$^{-1}$ with **3b** [12] and 9.7 L mol$^{-1}$ with **2b** [10].

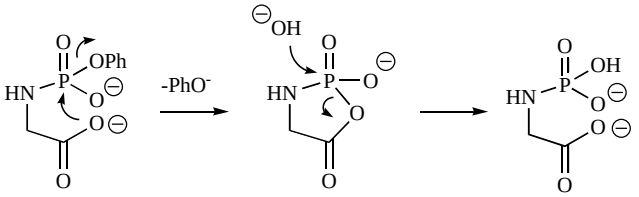
The dimethyl ester of phosphoramidic acid (**4b**) is hydrolyzed approximately as fast as the monomethyl ester (**2b**) at pH < 1, i. e. when the hydronium ion catalyzed reaction *via* the monocation, **4a** or **2a**, predominates: $k^A$ = 1.54×10$^{-3}$ L mol$^{-1}$ s$^{-1}$ ($I$ = 0.8 mol L$^{-1}$) with **4a** [12] (Scheme **2**) and 1.56×10$^{-3}$ L mol$^{-1}$ s$^{-1}$ ($I$ = 0.2 L mol$^{-1}$ s$^{-1}$) with **2a** (Scheme **1**) [10] at 36.8 $^o$C. Replacement of the phosphoryl hydroxy ligand with a methoxy ligand expectedly has only a minor effect on the reactivity. In striking contrast to the monomethyl ester (**2b**), the dimethyl ester (**4b**) does not undergo a spon-

taneous cleavage (Reaction **B** in Scheme **1**), since the reactive zwitterionic structure is impossible with **4b**. Another marked difference compared to monoesters is that the dialkyl and diaryl esters (**5**) of phosphoramidic acid undergo a hydroxide ion catalyzed hydrolysis of the P-OR bond (Reaction **D** in Scheme **2**). With the dialkyl esters (**4b**), deprotonation of the amino ligand is essential; the *N*,*N*-dimethyl analog reacts $3.8\times10^4$ times more slowly [10]. The mechanism, hence, seems to be dissociative. For the diaryl esters (**5**), nucleophilic displacement through an associative transition state has, in turn, been suggested on the basis of clearly negative entropies of activation [13].

## HYDROLYSIS OF *N*-SUBSTITUTED PHOSPHORAMIDIC ACIDS AND ESTERS

*N*-Alkylation has a moderate rate-retarding effect on the hydrolytic reactions of phosphoramidic acid. With *N*-butylphosphoramidic acid, for example, the hydrolysis is at 55 °C even somewhat slower than the hydrolysis of unsubstituted phosphoramidic acid at a 20 °C lower temparature [14]. The product distribution in mixtures of water and alcohol is not markedly influenced by the *N*-alkylation, which may be taken as an indication of similar transition states. Cleavage of the P-N bond by an external nitrogen nucleophile proceeds via a transition state where the departure of the alkylamino group is more advanced than the bond formation to the entering amine [15]. Hydrolysis of *N*-arylphosphoramidic acids is up to $10^3$-fold faster than the hydrolysis of their *N*-alkyl counterparts [14,16]. The rate is insensitive to the nature of polar substituents on the phenyl ring [16]. An associative rather than dissociative mechanism is followed [16,17]; the entropies of activation are negative and alcohols are preferred as entering nucleophiles in mixtures of alcohol and water. 2-Carboxy substituent on the phenyl ring accelerates the cleavage of *N*-phenylphosphoramidic acid at pH < 4 by a factor of 2-4 [17]. The group most likely serves as an intramolecular general acid catalyst donating a proton to the departing nitrogen atom.

Phenyl *N*-carboxymetylphosphoramidate (**6**) undergoes at pH > 4 an intramolecular pH-independent attack of the carboxylate group on the phosphorus atom with concomitant departure of phenoxide ion (Scheme **3**) [12]. The cyclic acylphosphoramidate intermediate obtained is rapidly hydrolyzed to *N*-carboxymethylphosphoramidic acid (**7**) by an attack of water or hydroxide ion on the phosphorus atom. The first-order rate constant for this reaction is $1.5\times10^{-4}$ $s^{-1}$ ($I$ = 0.2 mol $L^{-1}$) at 75 °C and $2.0\times10^{-6}$ $s^{-1}$ at 35 °C. The existence of the acylphosphate intermediate has been verified by hydroxylamine trapping that has given a small but still detectable amount (8 ± 2% of theoretical) of the expected hydroxamic acid.




**6** **7**

**Scheme 3.**

Diesters of *N*-alkylated phosphoramidic acid are hydrolyzed under acidic conditions by rapid initial protonation of the nitrogen atom and subsequent $S_N2(P)$-substitution of the protonated alkylamino ligand by water [18,19], analogously to their *N*-unsubstituted analog **4b** (Reaction **A** in Scheme **2**). Consistent with this mechanism, increasing electronegativity of the *N*-alkyl substituent accelerates and increasing size decelerates the reaction. The entropy of activation is clearly negative (around 120 J $K^{-1}$ $mol^{-1}$) and the kinetic solvent isotope effect, $k_D/k_H$ = 2, is typical for an A-2 reaction. The diaryl [13,20,21] and alkyl aryl [22,23] esters of *N*-alkyl- and *N*-arylphosphoramidic acids undergo, as their unsubstituted counterparts (**5**), a hydroxide ion catalyzed hydrolysis by a $S_N2(P)$-mechanism. The effect of the *N*-substitution on the hydrolytic stability is modest. The $\beta_{lg}$-value is rather small (0.41) [22] consistent with the assumed bimolecular nature of the transition state. As mentioned above, the hydroxide ion catalyzed hydrolysis of the diesters of *N*,*N*-dialkylphosphoramidic acid is exceedingly slow [10]. The lack of a dissociable proton on the nitrogen atom renders the dissociative mechanism followed by **4b** (reaction **D** in Scheme **2**) impossible, and a $S_N2(P)$-type displacement of alkoxy group is difficult, owing to high basicity of the departing alkoxide ion.

## HYDROLYSIS OF CYCLIC PHOSPHORAMIDATES

Cyclization of phosphoramidates to 2-aryloxy (**8**,**9**) or 2-alkoxy (**10**,**13**) substituted 1,3,2-oxazaphospholan-2-ones has a marked effect on their hydrolytic stability under alkaline conditions (Fig. **1**) [23]. In acid, these 5-membered cyclic phosphoramidates [24,25] and their 6-membered counterparts [26] are hydrolyzed approximately as fast as acyclic phosphoramidates. In alkaline solution, the cyclic phosphoramidates are cleaved much faster. Compounds **8** and **9** offer a good example. They are hydrolyzed under alkaline conditions $1.8\times10^4$ times as fast as their acyclic counterparts **11** and **12** (Fig. **1**) [23]. Cleavage of the exocyclic P-O bond predominates (91 and 71% with **8** and **9**, respectively), but endocyclic P-O (4 and 29% with **8** and **9**, respectively) and P-N cleavage (5% with **8**) also takes place. When the phenoxy ligand is replaced with a poor leaving group, such as a methoxy group (**10**), only endocyclic P-O (60%) and P-N (40%) bond ruptures occur [27]. Exocyclic dimethylamino group (**14**) departs approximately as readily (30%) as the endocyclic PO bonds (P-O1 50%, P-O3 20%) are cleaved. Evidently, an attack of hydroxide ion on the phosphorus atom initially gives a pentacoordinated phosphorane intermediate. According to quantum chemical calculations, the marked acceleration of the hydrolysis compared to acyclic compounds results from more favorable solvation of the transition leading to the cyclic phosphorane [28]. Within the cyclic phosphorane intermediate, the attacking hydroxide ion takes an apical position, the remaining apical position being occupied by the ring oxygen since, according to Westheimer´s rules [29], one of the P-bonded heteroatoms of a 5-membered ring must always be apical and the other equatorial. Oxygen atom is more electronegative, and hence more apicophilic, than nitrogen. This intermediate may then decompose by three alternative routes (Scheme **4**) [27]. Firstly, an endocyclic P-O fission may take place by departure the apical ring oxygen as an alkoxide ion (Reaction **A**). Secondly, a proton may be transferred from the hydroxyl ligand to the nitrogen atom. This triggers pseudorotation, since the oxyanion ligand tends to take an equatorial position and the protonated nitrogen an apical position. Consequently, the P-N bond is cleaved (Reaction **B**). Thirdly, the same pseudorotation process allows the exocyclic phenoxy ligand to take an apical position and depart (Reaction **C**). It should be, however, noted that the exocyclic methoxy group does not depart, only the phenoxy group. Evidently, methoxide ion is too poor a leaving group to compete with the departure of the protonated ring oxygen. When the ring nitrogen bears a phenyl substituent, no P-N bond rupture is detected (**9**,**13**). The basicity of the aniline nitrogen is low compared to aliphatic amines, and hence, the nitrogen is not protonated, but remains equatorial.

## HYDROLYSIS OF *N*-ACYLPHOSPHORAMIDATES

*N*-Acylphosphoramidic acids differ from their *N*-alkyl or *N*-aryl congeners discussed above in the sense that the nitrogen atom is not basic, but the carbonyl oxygen rather than the amido nitrogen is preferred as the site of protonation. Hydrolysis of these compounds exhibit bell-shaped pH-rate profiles, the rate-maximum falling on a slightly acidic region (pH 3-6), where the monoanionic form pre-

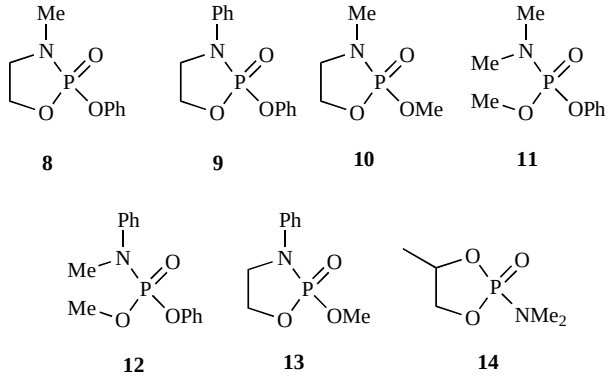
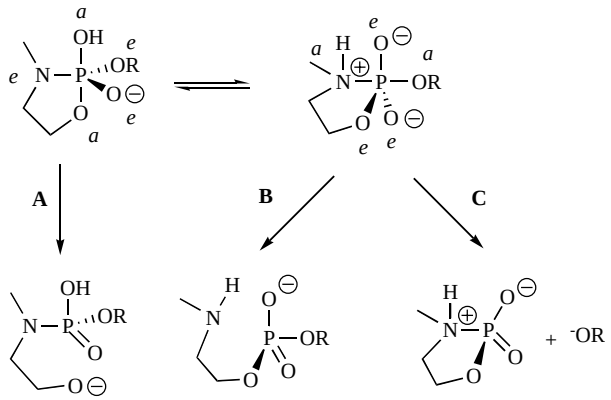


**Fig. (1).** Structures of 2-methoxy and 2-phenoxy substituted 1,3,2-oxazaphospholan-2-ones, their acyclic analogs and 2-dimethylamino-1,3,2-dioxaphospholan-2-one.



**Scheme 4.**

dominates [30,31]. Under these conditions, the hydrolysis is approximately as fast as the hydrolysis of the monoanion of unsubstituted phosphoramidic acid, while the neutral form is 2-3 times and the dianionic form 900 times more stable [30,32,33]. With *N*-benzoylphosphoramidic acid (**15**), for example, the first-order rate constants at 37 °C are $8.65\times10^{-5}$ $s^{-1}$, $1.84\times10^{-4}$ $s^{-1}$ and $2.1\times10^{-7}$ $s^{-1}$ for hydrolysis of the neutral, monoanionic and dianionic form, respectively [31]. With *N*-ethoxycarbonylphosphoramidic acid (**16**), the corresponding values are $8.25\times10^{-5}$ $s^{-1}$, $2.12\times10^{-4}$ $s^{-1}$ and $2.4\times10^{-7}$ $s^{-1}$ in the same order [34]. One should, however, bear in mind that while under neutral conditions only the P-N bond is cleaved, the cleavage of the C-N bond tends to compete in acidic solutions, and at low pH this reaction may even predominate [35]. The situation is similar with the dimethyl diester of *N*-acetyl-*N*-methylphosphoramidic acid (**17**) and evidently with other dialkyl esters of *N*-acylphosphoramidates as well.

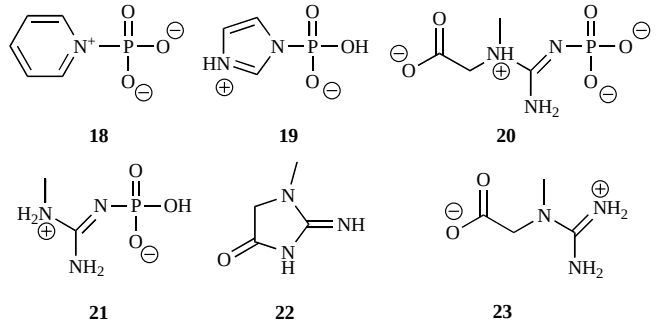


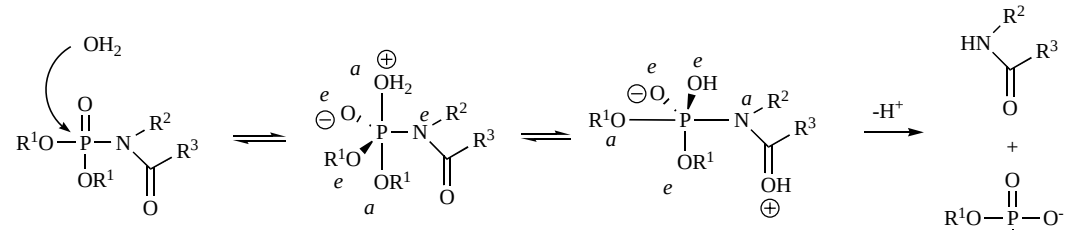
**Fig. (2).** Structures of phosphoramidates derived from heteroaromatic amines, phosphoguanidines and hydrolysis products of the latters.

The cleavage of the P-N bond has been suggested to proceed via a pentacoordinated intermediate obtained by an attack of water on the phosphorus atom (Scheme **5**). Proton transfer from the aqua ligand to the carbonyl oxygen, concomitant pseudorotation that poses the amido ligand into an apical position and cleavage of the P-N bond then completes the reaction. Formation of the phosphorane intermediate has been suggested to be the rate-limiting step. Under acidic conditions, hydronium ion catalyzed hydrolysis of the N-C(O) bonds starts to compete [35].

## HYDROLYSIS OF PHOSPHORAMIDATES DERIVED FROM HETEROAROMATIC AMINES

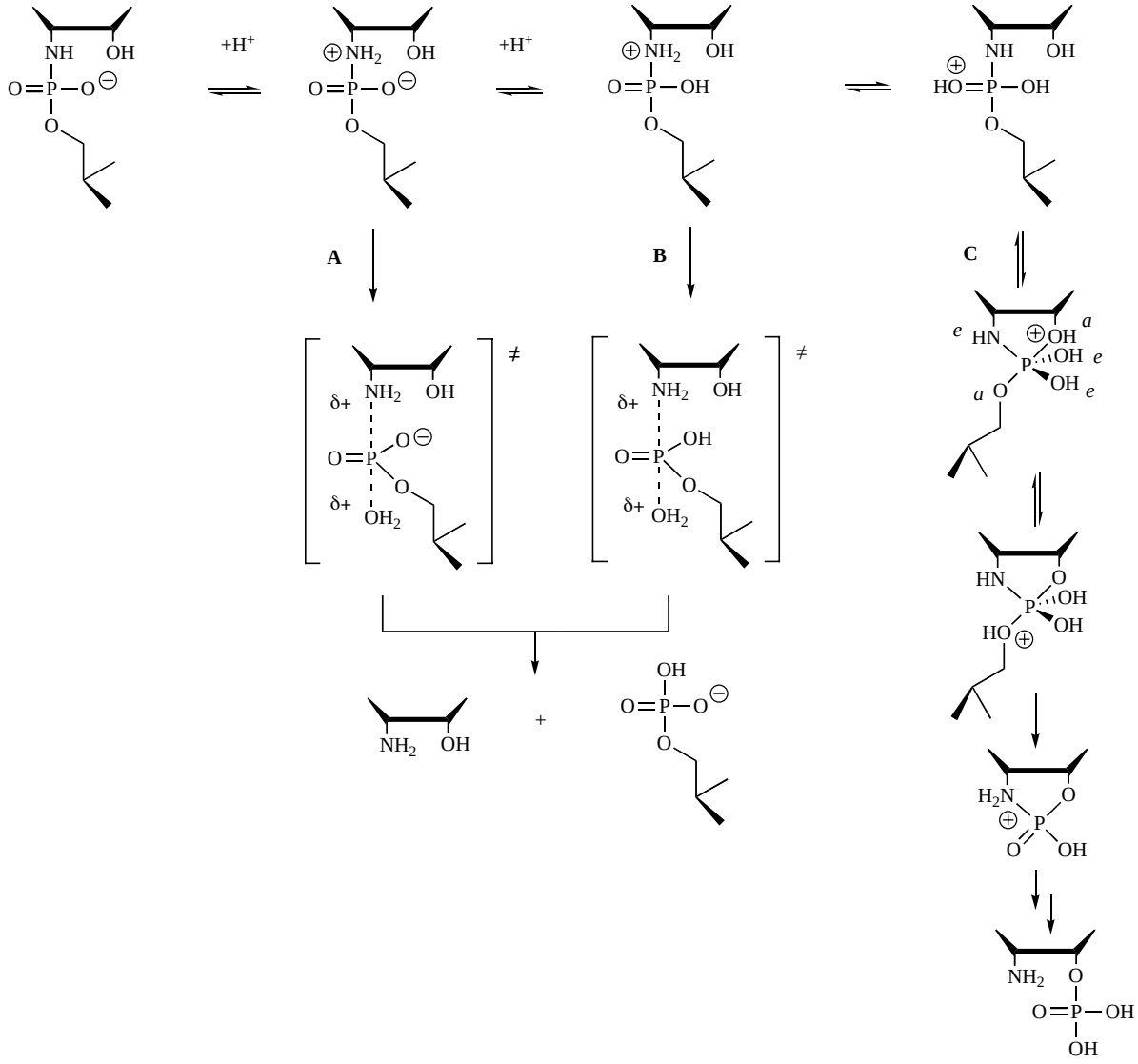
Pyridinio-*N*-phosphonates (**18**) (Fig. **2**), i. e. phosphoramidates derived from pyridine, eventually bear a positive charge on the leaving quaternary nitrogen atom and they, hence, undergo a rapid pH-independent hydrolysis to pyridine and orthophosphate at pH > 3 via the zwitterionic monoanion [36]. The half-life of the reaction is only about 30 s at 25 °C ($I$ = 1.0 mol $L^{-1}$). At pH < 3, the reaction turns acid-catalyzed proceeding, depending on the acid concentration, via a neutral zwitterion or a monocation. Studies on transfer of the phosphoryl group between two differently substituted pyridines suggest that both the entering and leaving nucleophiles are present in the transition state, the P-N bond formation being less advanced than the P-N bond cleavage [37].

1*H*-Imidazol-ylphosphonic acid (**19**) (Fig. **2**) and its monoesters are, in turn, stable between pH 7 and 12 [38]. Protonation of the imidazole ring is required for reasonably fast hydrolysis, but even for the hydrolysis of the neutral zwitterionic monoester, the half-life is more than 1 hour at 37 °C. The reaction most likely is associative by nature, proceeding by an attack of water on the phosphorus atom and concomitant departure of neutral imidazole. Hydroxide ion catalyzed displacement of imidazolide ion takes over at pH > 12.



**15**: $R^1 = R^2 = H$, $R^3 = Ph$
**16**: $R^1 = R^2 = H$, $R^3 = OEt$
**17**: $R^1 = R^2 = R^3 = Me$

**Scheme 5.**



**Scheme 6.**

## HYDROLYSIS OF PHOSPHOGUANIDINES

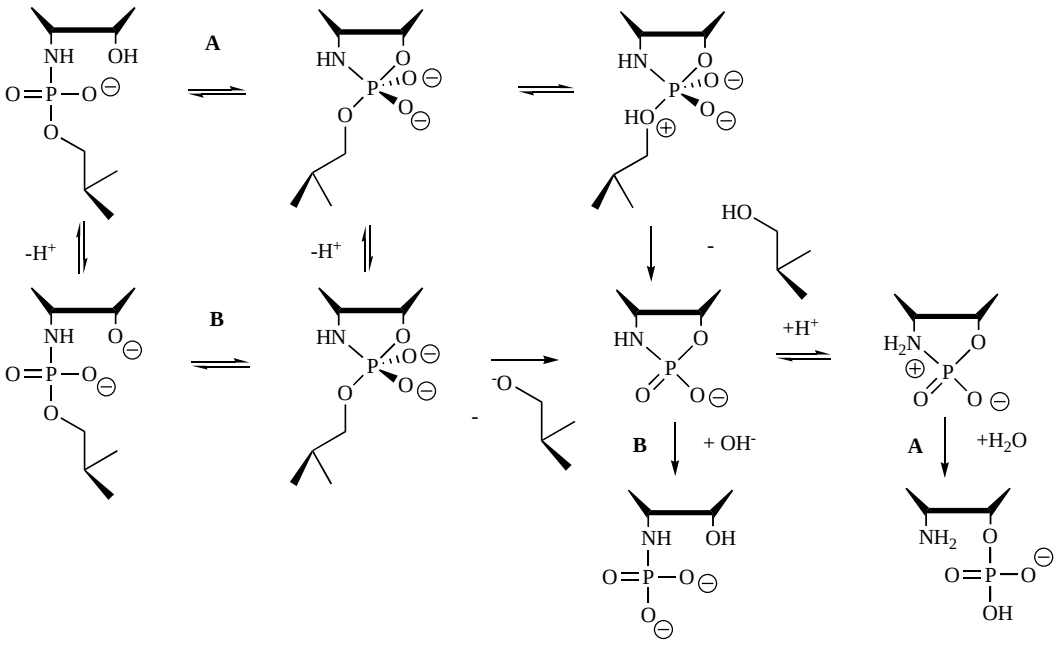
The best known representative of phosphoguanidines is phosphocreatine (**20**) (Fig. **2**) that phosphorylate ADP to ATP during periods of rapid consumption of ATP. Under physiological conditions, phosphoguanidines occur as zwitterions having the positive charge delocalized between the carbon and nitrogen atoms of the guanidinium moiety. The p$K_a$ values for the first and second dissociation of the phosphoryl hydroxy ligands are -0.4 and 4.5 (30.5 $^oC$, $I$ = 0.2 mol $L^{-1}$), respectively, and the p$K_a$ for the dissociation of the carboxy group is 2.8 [39]. The guanidinium group loses its dissociable proton at pH 11.2.

Phosphoguanidines are hydrolyzed solely by the P-N bond cleavage. With the decarboxy analog of phosphocreatine (**21**) (Fig. **2**), the neutral zwitterionic form is the reactive species. Accordingly, the hydrolysis is almost pH-independent between pH 1 and 3 ($k$ = 6.2×$10^{-4}$ $s^{-1}$ at 30.5 $^oC$, $I$ = 0.2 mol $L^{-1}$) [40]. The reaction has been suggested to proceed by a dissociative mechanism, giving a metaphosphate ion that then serves as the actual phosphorylating agent. Consistent with the unimolecular nature of the rate-limiting step, the solvent isotope effect is close to unity and the entropy of activation close to zero. The neutral and monoanionic species are $10^4$ times less reactive. Phosphocreatine undergoes, in addition to the pH-independent hydrolysis at pH 1-3 ($k$ = 2.6×$10^{-4}$ $s^{-1}$ at 30.5 $^oC$, $I$ = 0.2 mol $L^{-1}$), an acid-catalyzed reaction at pH < 1 [39]. The latter reaction yields creatinine (**22**) instead of creatine (**23**) (Fig. **2**), but the mechanistic details of its formation remains obscure.

## HYDROLYSIS OF INTERNUCLEOSIDIC PHOSPHORAMIDATE LINKAGES

As mentioned above, phosphoramidate analogs of oligonucleotides having the 3´-oxygen atom replaced with nitrogen show promise as antisense oligonucleotides, with which gene expression may be inhibited in a very selective manner. They are resistant towards enzymatic degradation and form stable double and triple helixes with natural nucleic acids [41]. Owing to these attractive properties, the hydrolytic stability of internucleosidic 3´-*N*-phosphoramidate linkages of both oligodeoxyribonucleotides and oligoribonucleotides is of interest.

The 3´,5´-(3´-*N*-phosphoramidate) linkages of oligoribonucleotides are hydrolyzed, depending on pH, by cleavage of either the P-N3´ or P-O5´ bond [41c,42,43]. Over a wide pH range 2-6, hydronium ion catalyzed hydrolysis of the P-N3´ bond predominates, the second-order rate constant being 1.0 L $mol^{-1}$ $s^{-1}$ at 90 $^oC$ and 9.2×$10^{-4}$ L $mol^{-1}$ $s^{-1}$ at 25 $^oC$ ($I$ = 0.1 mol $L^{-1}$) [42]. Under such conditions, the prevailing ionic form of the phosphoramidate linkage is monoanion. The reactive species, hence, is neutral phosphorami-



**Scheme 7.**

date, evidently the zwitterionic form [12,42,43]. As for the hydrolysis of neutral zwitterionic phosphoramidic acid [6], the entropy of activation is moderately negative, (-65 ± 5) J $K^{-1}$ $mol^{-1}$, suggesting that the attack of water on the phosphorus atom is markedly advanced in the transition state (Reaction **A** in Scheme **6**) [42]. Interestingly, at pH < 2, i. e. under conditions where the neutral zwitterion is the predominant ionic form and the phosphoramidate monocation the reactive species, cleavage of the P-O5´ starts to compete with the P-N3´ bond rupture, although no deviation from the first-order dependence of the rate on hydronium ion concentration occurs [42,43]. In addition to the 3´-aminonucleoside, its 2´-phosphate is now formed, which may be taken as an indication of nucleophilic attack of the 2´-OH on the phosphorus atom. Evidently, the phosphoramidate monocation is hydrolyzed by two parallel routes, by a nucleophilic attack of a water molecule (Reaction **B**) or the 2´-OH on the phosphorus atom (Reaction **C**). While the former reaction releases 3´-aminonucleoside, the intramolecular participation of the 2´-OH leads to formation of 3´-aminonucleoside 2´-phosphate by a multistep pathway.

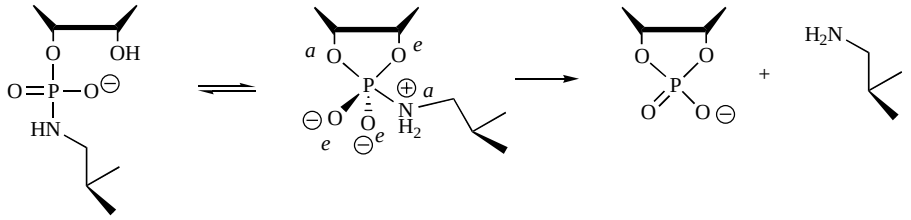
Under neutral and alkaline conditions, cleavage of the P-O5´ bond prevails. Over a narrow pH range 6-8, the main product is 3´-aminonucleoside 2´-phosphate and the formation of this species is pH-independent, the first-order rate constant being $1.4\times10^{-6}$ $s^{-1}$ at 90 °C ($I$ = 0.1 mol $L^{-1}$) [42]. Most likely 2´-OH attacks on the phosphorus atoms of the predominant ionic form, viz. the phosphoramidate monoanion, concerted with a water-mediated proton transfer from the attacking oxygen to a phosphoryl oxygen (Reaction **A** in Scheme **7**). The monoanionic phosphorane intermediate obtained then decomposes by departure of the 5´-linked nucleoside concerted with a proton transfer from the phosphoryl oxygen to the departing oxygen atom. No P-N3´ bond cleavage takes place. Isomerization to a 2´,5´-phosphodiester has never been observed under these or less acidic conditions. The nitrogen atom, as a member of a five-membered ring incorporating the entering 2´-O nucleophile, initially adopts an equatorial position. Evidently, proton transfer to the 5´-O and concomitant departure of the 5´-linked nucleoside is a more facile process than pseudorotation that is a prerequisite for the rupture of the P-N3´ bond. The much faster hydrolysis of the $O^{2´},N^{3´}$-cyclic phosphoramidate obtained then gives the 2´-phosphate [42,44]. The reactive species most likely is neutral zwitterionic cyclic phosphoramidate, which naturally prefers P-N bond cleavage.

Under more alkaline conditions (pH > 8), the reaction is first-order in hydroxide ion concentration, the main product being 3´-*N*-phoshoramidate (Reaction **B** in Scheme **7**) [42,44]. This product is most likely formed via the same $O^{2´},N^{3}$-cyclic phosphoramidate intermediate as the 2´-phosphate. At pH > 8, the intermediate remains anionic. Upon attack of hydroxide ion on the phosphorus atom, a marginally stable dianionic phosphorane is formed. While either 3´-N or 2´-O may in principle adopt an apical position, and hence depart, 2´-$O^-$ is a better leaving group than 3´-$NH^-$. Accordingly, the phosphate group remains bonded to N3´.

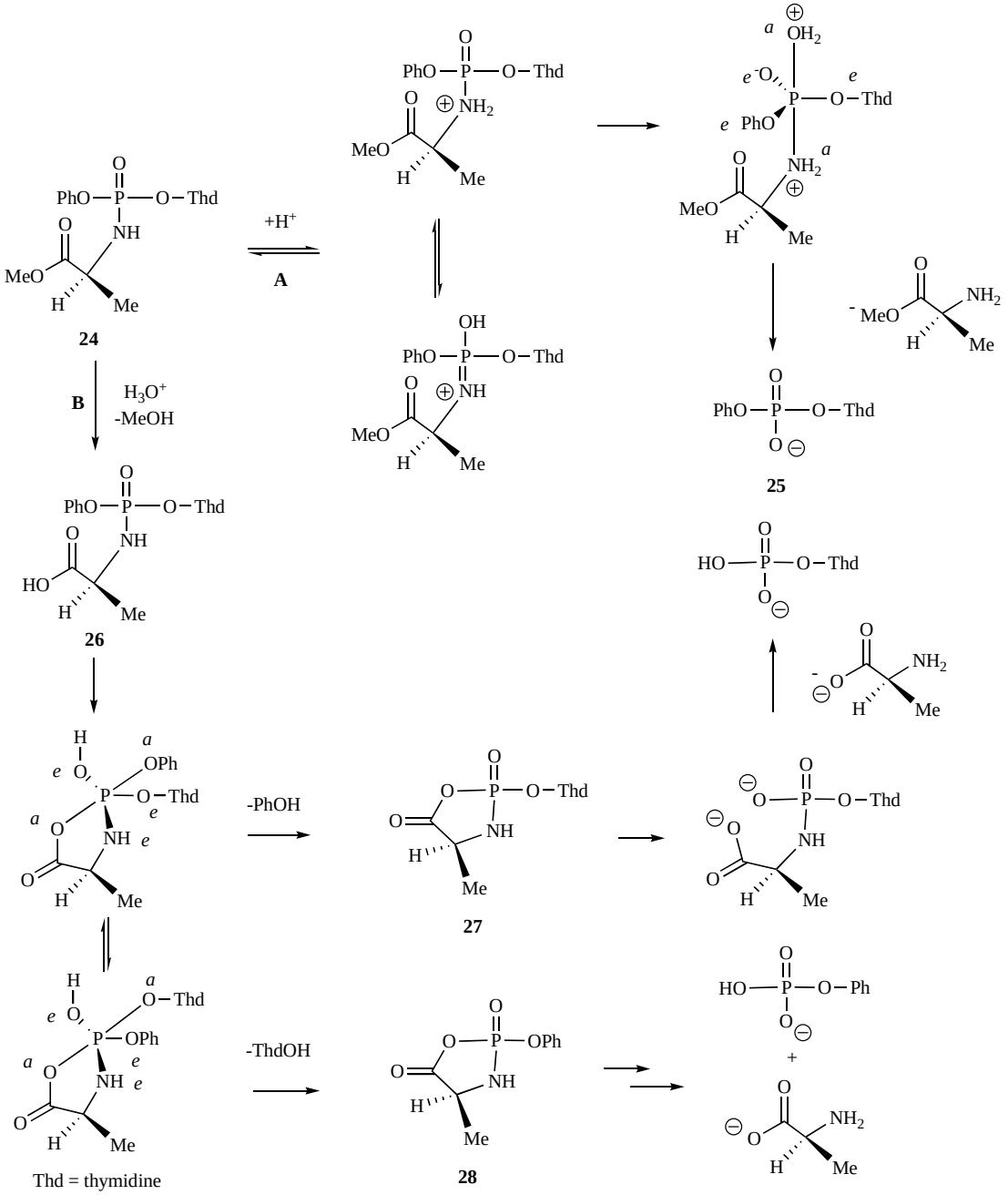
Di(ribonucleoside)-3´,5´-(5´-*N*-phosphoramidates) are hydrolyzed exclusively by cleavage of the P-N5´ bond over the pH range 5-12 [44]. The reaction is first-order in hydronium ion concentration at pH < 6 and pH-independent at a higher pH, the first-order rate constant being $1.4\times10^{-4}$ $s^{-1}$ at 37 °C. $Zn^{2+}$ and $Cd^{2+}$ ions at a concentration of 10 mmol $L^{-1}$ have been observed to accelerate the cleavage by one order of magnitude. Since the corresponding phosphoramidates derived from 2´-deoxyribonucleosides are hydrolytically stable under neutral conditions, the hydrolysis is in all likelihood initiated by a nucleophilic attack of the 2´-hydroxy group on the phosphorus atom, concerted with a water-mediated proton transfer from the attacking hydroxy group to the 5´-nitrogen atom, which leads to departure of the 5´-aminonucleoside. Formation rather than breakdown of the phosphorane intermediate is the rate-limiting step (Scheme **8**). The mechanism of the metal ion promoted reaction remains unkown.

Di(ribonucleoside)-3´,5´-phosphoramidates containing a non-bridging amino group are hydrolytically unstable, giving in neutral aqueous buffers a mixture of 2´- and 3´-phosphoramidates [45]. Evidently, the 5´-linked nucleoside is displaced by an attack of the 2´-OH on the phosphorus atom. Subsequent attack of water on the resulting cyclic phosphoramidate then results in an endocyclic bond fission, either the P-O2´ or P-O3´ bond.

Dinucleoside-3´,5´-(3´-*N*- and 5´-*N*-phosphoramidate)s derived from 2´-deoxyribonucleosides undergo hydronium ion catalyzed hydrolysis of the P-N3´ bond at pH < 5 [46]. The reaction is ap-

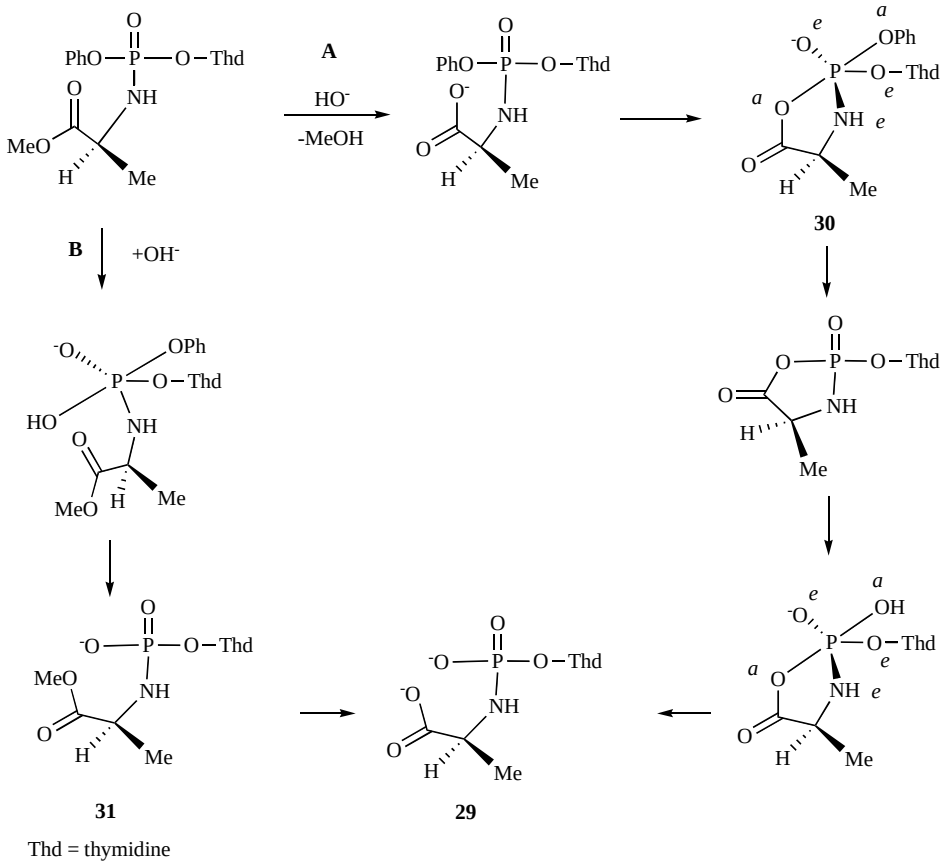


**Scheme 8.**




**Scheme 9.**

proximately as fast as with the corresponding ribonucleoside derivative; the 2´-OH does not play any role in this reaction. At higher pH, the disappearance of the starting material becomes pH-independent and the cleavage of the *N*-glycosidic bond gradually becomes the predominant reaction. The 5´-*N*-phosphoramidate linkages undergo a similar hydronium ion catalyzed hydrolysis of the P-N bond under acidic conditions [47,48]. In striking contrast to di(ribonucleoside)-3´,5´-(5´-*N*-phosphoramidates), their 2´-deoxyribonucleoside counterparts, both the 3´-*N*- and 5´-*N*-phosphoramidates, are hydrolytically stable under neutral and alkaline conditions [46,48]. The di(thymidine) derivatives, for example, have been shown to withstand 1 h treatment with 0.5 mol $L^{-1}$ aq sodium hydroxide at 98 $^{o}$C [48].



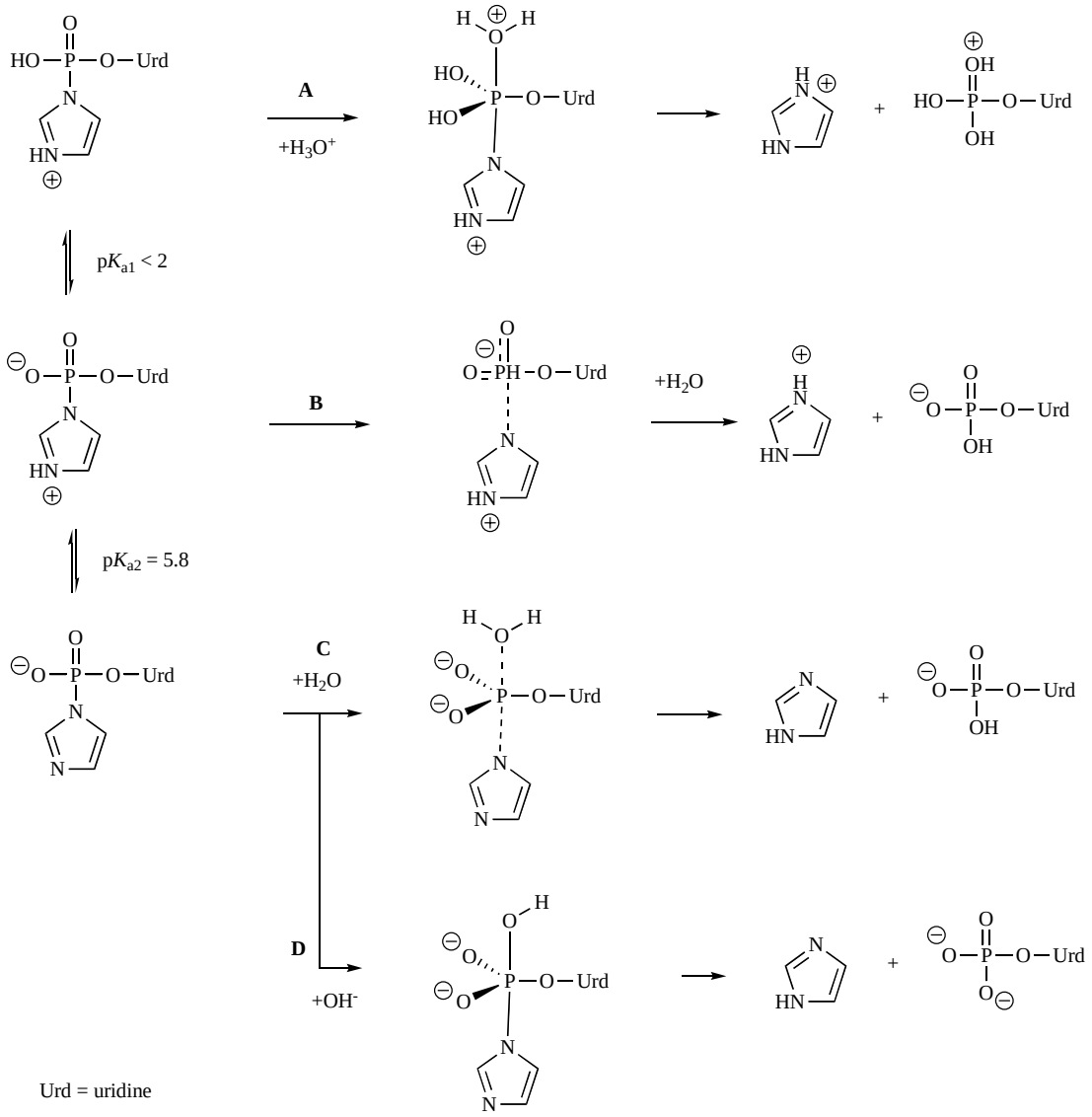

**Scheme 10.**

3′,5′-Cyclic *N,N*-dimethylphosphoramidates of purine ribonucleosides are hydrolyzed under acidic conditions to the corresponding cyclic phosphates [49]. The half-life of the reaction is 3 h at pH 1 and 37 °C. No mechanistic studies on the reaction have been carried out.

## HYDROLYSIS OF NUCLEOSIDE 5′-(*O*-ARYLPHOSPHORAMIDATES)

Another class of nucleosidic phosphoramidates that has received considerable interest is *O*-aryl substituted nucleoside 5′-phosphoramidates, regarded as potential pro-drugs of antiviral nucleoside 5′-monophosphates. The hydrolytic reactions of such compounds have been studied over a wide pH-range using thymidine 5′-(*O*-phenylphosphoramidate) derived from L-alanine methyl ester (**24**) as a model compound [50]. The $R_P$ and $S_P$ diastereomers of the compound are hydrolyzed approximately as rapidly at pH < 4, both exhibiting first-order dependence of the rate on hydronium ion concentration. The second order rate constants for the disappearance of the diastereomers are $4.9\times10^{-3}$ L mol$^{-1}$ s$^{-1}$ and $5.5\times10^{-3}$ L mol$^{-1}$ s$^{-1}$ at 90 °C. Two reactions compete, each representing approximately half of the disappearance of the starting material. One of them involves a rapid pre-equilibrium protonation of the phosphoramido moiety and subsequent attack of water molecule on the phosphorus atom (Reaction **A** in Scheme **9**). The pentacoordinated phosphorane intermediate (or transition state) obtained is then decomposed by cleavage of the P-N bond, giving the phenyl ester of thymidine 5′-monophosphate (**25**). The initial protonation probably gives a mixture of *N*- and *O*-protonated species [18], but the former is the reactive ionic form. The alternative reaction pathway consists of hydrolysis of the methyl ester linkage, giving **26**, followed by the attack of the resulting carboxy group on the phosphorus atom (Reaction **B**). Within the phosphorane intermediate obtained, the attacking carboxy oxygen takes one of the apical positions, the other one being occupied by either the phenoxy oxygen or the O5′ of thymidine. Cleavage of the apical P-O bond gives a thymidin-5′-yl (**27**) or phenyl (**28**) substituted cyclic phosphoramidate, respectively. These compounds actually are mixed anhydrides of a carboxylic and phosphoric acid, which are readily decomposed by hydrolysis of the anhydro bridge. The resulting acyclic phosphoramidate monoesters are finally hydrolyzed to the final products, thymidin-5′-yl or phenyl phosphate, by the P-N bond cleavage. It should be noted that the hydronium ion catalyzed hydrolysis of phosphoramidate monoesters is two orders of magnitude faster than the corresponding reaction of their diester counterparts. As discussed in the early part of the review, with phosphoramidic acid monoesters, a dianionic metaphosphate-like structure may be developed upon departure of the amine ligand. This increases the electron density at the phosphorus atom, and hence, the cleavage of the P-N bond is facilitated.

At pH > 5, hydrolysis of **24** becomes hydroxide-ion catalyzed [50]. The first-order rate constants for the disappearance of the two diastereomeric forms at pH 8.0 (90 °C, $I$ = 0.1 mol L$^{-1}$) are $1.2\times10^{-3}$ s$^{-1}$ and $1.4\times10^{-3}$ s$^{-1}$. The main product is alaninyl phosphoramidate **29**, which probably is obtained by initial hydrolysis of the carboxy ester bond and subsequent intramolecular displacement of phenoxide ion by the carboxylate group (Reaction **A** in Scheme **10**). The highly unstable cyclic phosphorane intermediate (**30**) then undergoes fast subsequent hydrolysis by an attack of hydroxide ion on the phosphorus atom. Endocylic oxygen adopts, as a more electronegative atom than nitrogen, an apical position within the resulting phosphorane intermediate, and hence, only the P-O bond is cleaved. Accordingly, a similar mechanism as in the alkaline cleavage of *O*-phenylphosphoramidic acid operates [11]. The attack of carboxylate ion on the negatively charged phosphoramidate group is so slow




**Scheme 11.**

that **29** is accumulated as a stable product at pH 7-8. This reaction represents about 90% of the disappearance of **24**. The rest 10% results from direct intermolecular displacement of the phenoxide ion by hydroxide ion, leading to intermediary accumulation of **31** (Reaction **B** in Scheme **10**). Hydrolysis of the alaninyl methyl ester linkage of **31** then finally gives **29**. The first-order rate constant for the latter reaction is $2.3\times10^{-4}$ $s^{-1}$ at pH 8.0. Nucleoside 5´-[*N*-(phenylalaninyl)phosphoramidate] has been reported to be stable for 24 h in 0.1 mol $L^{-1}$ aq hydrogen chloride and 0.1 mol $L^{-1}$ aq sodium hydroxide at ambient temperature [51].

## HYDROLYSIS OF NUCLEOSIDE 5´-PHOSPHORIMIDAZOLIDES

Nucleoside 5´-phosphorimidazolides are extensively used as starting materials for the synthesis of di-, tri- and oligophosphate bridges. Therefore, their hydrolytic stability is of interest. The reaction is pH-independent between pH 2-5, proceeding by P-N bond cleavage of the predominating neutral zwitterionic species (Reaction **B** in Scheme **11**) [53]. With the uridine derivative, the first-order rate constant for this reaction is $9.4\times10^{-5}$ $s^{-1}$ at 27 °C. The rate is buffer-independent excluding catalysis by general acids or bases. Most likely, the transition state is dissociative rather than associative. The rate is sensitive to the basicity of the departing imidazole ($\beta_{lg}$ = -1.52), suggesting that the P-N bond is largely cleaved in the transition state. At pH < 2, the monocationic form of the starting material starts to predominate and this species undergoes a hydronium ion catalyzed hydrolysis. Evidently, the reaction proceeds through a dicationic phosphorane obtained by an attack of a water molecule on the substrate dication bearing a proton on both non-bridging phosphoryl oxygens in addition to the imidazole ring (Reaction **A**).

The monoanionic form that predominates at pH > 6, underogoes a pH-independent hydrolysis over a narrow pH range around pH 10. The reaction is 1500 times slower than the corresponding reaction of the zwitterionic species. The transition state of this reaction (Reaction **C**) may well be associative, but this has not been rigorously studied. Whether this reaction really consists of an attack of water on the phosphorimidazolide monoanion [52], or a kinetically equivalent attack of hydroxide ion on phosphoimidazolide zwitterion [38, 53] still seems to be open to various interpretations. At pH > 11, the reaction becomes hydroxide ion catalyzed. Tentatively, attack of hydroxide ion on the substrate monoanion gives a dianionic phosphorane intermediate, which is decomposed by deprotonation of the hydroxo ligand and protonation of the departing imi-

dazolide ion concerted with the P-N bond cleavage. The second-order rate constant is $4.2\times10^{-5}$ L mol$^{-1}$ s$^{-1}$ at 27 $^{o}$C (Reaction **D**).

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