Activation of Oximic Nucleophiles by Coordination of Transition Metal Ions

Fabrizio Mancin,*[a] Paolo Tecilla,[b] and Umberto Tonellato*[a]

Keywords: Hydrolysis / Nucleophilicity / α-Effect / Metal ion complexes / Biomimetic models

A kinetic study of the reactivity in the cleavage of *p*-nitrophenyl acetate of a series of 2-pyridineoximes, **1**−**4**, and their complexes with Ni^{II}, Zn^{II} and Cu^{II} is reported. Complexation of the oximic ligands leads to a remarkable increase in the acidity of the oximic group, following the order $Cu^{II} > Ni^{II} > Zn^{II}$. The oximates of free ligands and their metal complexes, being α-nucleophiles, are quite effective in promoting the cleavage of PNPA. However, the reactivity, as defined by the second-order rate constants, is not predictably related to the acidity of the oximic function. The corresponding Brønsted plot shows a linear behaviour up to a p*K*a value of ca. 8, above which the reactivity of the uncomplexed oximic functions levels off to a limiting value. In the case of the metal ion complexes, a large effect of the ligand structure on the reactivity was observed. As a consequence, complexes of appropriate ligands and metal ions show a reactivity that exceeds the apparent limiting one of the oximates. The origin of such effects and their possible implications in the development of new reactivators of phosphorylated acetylcholinesterase are discussed.

Introduction

A key role in the activity of hydrolytic metalloenzymes is played by the "essential" metal ion,[1] most often Zn^{II}, which activates available nucleophilic functions such as hydroxy groups or water molecules, mainly by inducing their deprotonation at physiological pH values. Aimed at mimicking such a fundamental role in the catalytic mode of enzymes and at realising efficient artificial catalysts, much effort has been devoted to the design, synthesis and study of metallocomplexes in which a nucleophilic function is located in the proximity of the chelating subsite of the ligand, so that it may be activated through coordination with the metal ion.[2]

Among the several nucleophilic functions that can be introduced in the structure of the ligand, the oximic one, unnatural as it appears in the realm of enzymes, is a choice of great interest for artificial models. Oximate anions are powerful α-nucleophiles[3] that can be exploited in the hydrolytic cleavage of carboxylic or phosphoric acid esters or amides. As a matter of fact, pyridinium carbaldoximates, such as 2-PAM (**6**), to name the best known of them, rank among the most efficient reactivators of the phosphorylated acetylcholinesterase poisoned by organophosphorus inhibitors (nerve gas).[4][5] Reactivation implies the cleavage of a phosphorylated serine at the site of the inactivated enzyme: in the case of 2-PAM the effectiveness of the oximic function is ascribed to its enhanced acidity, the pK_a being in the range 7−8 (down from 10−12) as a consequence of the positive charge present in the pyridinium moiety.

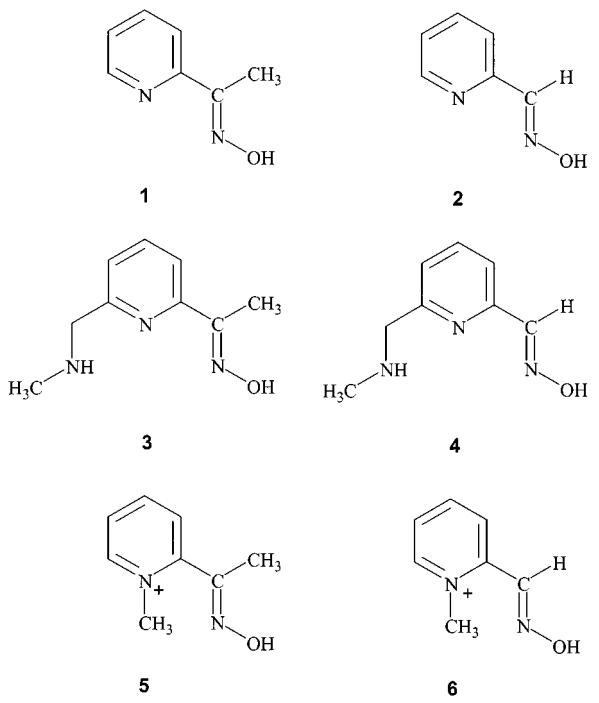
[a] University of Padova, Department of Organic Chemistry and Centro CNR Meccanismi di Reazioni Organiche,
Via Marzolo 1, I-35131 Padova, Italy
Fax: (internat.) + 39-049/827-5239
E-mail: tonum@chor.unipd.it, mancin@chor.unipd.it
[b] University of Trieste, Department of Chemical Sciences,
Via Giorgieri 1, I-34127 Trieste, Italy
Fax: (internat.) + 39-040/676-3903
E-mail: tecilla@dsch.univ.trieste.it

Besides the electrostatic effects at work in the case of the above pyridinium derivatives, the pK_a of the oximic function may dramatically decrease upon coordination with metal ions[6][7] − up to seven orders of magnitude in the case of Cu^{II} − and, in fact, coordinated 2-pyridineoximes have received attention as hydrolytic catalysts.[7][8] Some published studies concerning their reactivity indicate that in the case of complexes of transition metal ions with oximic ligands, at variance with the analogous pyridinium compounds, the decrease of the apparent pK_a of the nucleophilic function does not always imply a related decrease of the reactivity of the complexed oximate.[7d,7g]

In order to confirm this interesting phenomenon and gain more insight into the reactivity of the complexed oximate functions, which could open the way to very efficient catalysts and drugs, we undertook a study of the nucleophilic reactivity and its relationship with the acidity for the complexes of Cu^{II}, Ni^{II}, and Zn^{II} ions with the 2-pyridineoxime based ligands **1**−**4** and, for comparison purposes, for metal ion-free **1**−**4** and 2-pyridiniumoximes **5**−**6** (Scheme 1).

Oximes **1** and **2** are the basic ligands often employed in studies on the hydrolytic reactivity of coordinated oximes,[7] while **3** and **4** were synthesized in order to obtain ligands that are predictably stronger than the others under investigation. The substrate of choice was *p*-nitrophenyl acetate (PNPA) and kinetic measurements were carried out to define the second-order rate constants for its cleavage in aqueous solutions. The study was also stimulated by the results of our previous studies on the hydrolytic reactivity of Ni^{II} complexes with lipophilic 2-pyridineoximes in micellar aggregates, which brought to light a rather intriguing lack of sensitivity to pH over an extended range (3.5 to 8).[8] The results reported here confirm that activation of oximes by complexation with transition metal ions can lead to an increased reactivity, and indicate that an important role in this effect is played by the structure of the ligand.

  1434−193X/00/0306−1045 $ 17.50+.50/0



Scheme 1. Oximic ligands and pyridinium oximes used in this work

Results and Discussion

The Oximes and Their Complexes

Besides the commercially available compounds **2** and **6**, the oximes investigated here were synthesized by reaction of hydroxylamine with the corresponding ketones or aldehydes. The pyridinium derivative **5** was obtained by reaction of **1** with methyl iodide.[9] The (*E*) configuration for each oxime was confirmed by the observed Nuclear Overhauser Effect between the hydroxy proton and the hydroxyimino C*H* or methyl.[10] All oximic ligands are soluble in water up to 2 mM and form complexes with Cu^{II}, Zn^{II}, and Ni^{II} as highlighted by the spectral changes observed for their aqueous solutions upon addition of the metal ions as nitrate salts.

Several complexation stoichiometries are possible, as reported,[6] depending on the metal ion and the ligand. In order to avoid complications arising from the presence of different complexes and ensure the presence of the sole 1:1 species, all the experiments were carried out using solutions containing at least a 10-fold molar excess of metal ion over the ligand.[11]

Acidity of the Oximic Group

The acidity of oximes **1**−**6**, if not reported in the literature, and of the complexes of **1**−**4** with the transition metal ions, was measured by spectrophotometric or potentiometric methods. The UV spectra of the solutions of free and complexed ligands appear to be sensitive to pH and therefore the acid dissociation constants of the oximic functions were measured at 25°C by standard spectrophotometric titrations. In the case of free ligand **4**, the pK_a values for the acid dissociation of the ammonium and of the oximic groups were too close to allow the determination with this method and their values, as well as those of pyridinium oximes **5** and **6**, were determined by potentiometric titrations.

Table 1 shows all the acidity data for **1**−**6** and the metal complexes of **1**−**4**. Inspection of these data shows that the pK_a values of the oxime function of the free ligands are in the range 10−11, and that they decrease by two to three units in the case of pyridinium oximes, and by up to seven units in that of the metal ion complexes. A closer look at the acidity of the oximic function observed in the case of the complexes, reveals that a major role is played by the nature of the metal ion: the pK_a values for Cu^{II} complexes are in the range 3−5, those for Ni^{II} complexes in the range 6−7 and those for Zn^{II} complexes in the range 6.5−8. Minor, but still remarkable, effects are due to the structure of the ligand. Thus, the introduction of the methyl group in ligands **1** and **3** (i.e., on moving from aldoximes to the corresponding ketoximes) decreases the acidity in the free ligands, in their metal complexes and also in the pyridinium oximes. The presence of a third coordinating site, namely the methylamino moiety such as in ligands **3** and **4**, does not affect the acidity of the free ligand but brings about a remarkable decrease in the acidity of its complexes.

Interestingly, the acidity order in all the complexes, $Cu^{II} > Ni^{II} > Zn^{II}$, is different from that reported for the aqua complexes, $Cu^{II} > Zn^{II} > Ni^{II}$.[12] This clearly indicates that the activation of the oximic function is not due only to electrostatic effects.

Table 1. Second-order rate constants, k_2, for the cleavage of PNPA by the oximate ions of **1**−**6** and their metal complexes, formation constants, log K_f, for the metal complexes, and the acid dissociation constants, pK_a, for the corresponding oximic functions, in water at 25°C

Entry	Oxime	Metal	log K_f [M]	pK_a	k_2 [$M^{-1}s^{-1}$][a]
1	1	–	–	10.8[b]	22 ± 1
2	1	Cu^{II}	5.9[c]	3.5[c]	0.2 ± 0.1
3	1	Ni^{II}	5.0[d]	6.1	6.5 ± 0.5
4	1	Zn^{II}	2.6[b]	7.0[b]	200 ± 5 (400[b])
5	2	–	–	10.0[e]	45 ± 1
6	2	Cu^{II}	4.7[c]	3.2[c]	0.003 ± 0.001
7	2	Ni^{II}	4.2[f]	6.2 (6.3[g])	0.46 ± 0.03
8	2	Zn^{II}	2.2[e]	6.5[e]	9.4 ± 0.1 (10[b])
9	3	–	–	11.1	45 ± 2
10	3	Cu^{II}	>7[h]	4.4	–
11	3	Ni^{II}	6.0[h]	6.9	0.20 ± 0.01
12	3	Zn^{II}	3.2[h]	7.8	7.1 ± 0.1
13	4	–	–	9.9	71 ± 1
14	4	Cu^{II}	>7[h]	5.1	0.001 ± 0.0005
15	4	Ni^{II}	5.3[h]	6.5	0.10 ± 0.01
16	4	Zn^{II}	2.7[h]	7.7	2.5 ± 0.1
17	5	–	–	9.0 (9.0[i])	55 ± 1
18	6	–	–	7.7 (7.7[l])	21 ± 1 (25[l])

[a] [Ox] = $1\cdot10^{-4}$−$1\cdot10^{-3}$ M, [M^{2+}] = $1\cdot10^{-2}$ M, [Buffer] = $5\cdot10^{-2}$ M. – [b] Ref.[7d] – [c] Ref.[7e] – [d] Ref.[6c] – [e] Ref.[7a] –[f] Ref.[6b] – [g] Ref.[7f] – [h] Apparent value at pH = 7.0. – [i] Ref.[9] – [l] Ref.[5]

Nucleophilicity

The reaction of choice for the assessment of the nucleophilicity of the oximate function was the cleavage of PNPA. The accepted mechanism[7d,8b] of the reaction involves nucleophilic attack of the oximate on the carbonyl carbon atom of the ester, followed by the release of *p*-nitrophenol and the formation of an acylated intermediate. This intermediate, in a subsequent step, is hydrolysed by water, which is quite likely to be coordinated to the metal ion.

The kinetic studies were carried out by following the appearance of *p*-nitrophenol at 25°C at a pH value chosen to ensure at least partial dissociation of the oximic function. The pseudo-first-order rate constants, k_ψ, were thus obtained for different concentrations of the catalyst. Under the conditions used, the k_ψ value may be expressed in terms of Equation 1 in the case of free ligands and pyridinium

$$k_\psi = k_0 + k_2[Ox]_0 \frac{1}{1 + \frac{[H^+]}{K_a}} \quad (1)$$

$$k_\psi = k_o + k_2[Ox]_0 \frac{1}{1 + \frac{[H^+]}{K_a}\left(1 + \frac{1}{[M]_0 K_f}\right)} \quad (2)$$

oximes and in terms of Equation 2 for the metal complexes.

In these equations, k_0 is the rate contribution of all possible hydrolytic pathways in the absence of the oximes or their complexes, k_2 is the second-order rate constant for the reaction between PNPA and the oximate anions, $[Ox]_0$ and $[M]_0$ are the total oxime and metal concentrations, K_a is the acidity constant reported in Table 1 of the oximic group (in Equation 2 it is the acidity constant of the complexed oxime), and K_f is the formation constant of the complex. The K_f values were taken from the literature or measured (under the experimental conditions, see Table 1). In most cases, the K_f value was sufficiently high to neglect the term $(1 + 1/K_f[M]_0)$ in Equation 2.

The k_ψ values obtained from the kinetic experiments in most cases fit nicely with Equation 1 or 2, thus allowing the evaluation, with confidence, of the second-order rate constants, k_2, reported in Table 1. Only in the case of the Cu^{II} complexes of oximes **1**−**4** the rate constants could not be measured with satisfactory accuracy (see Experimental Section) and, in one case (the Cu^{II} complex of ligand **3**), reliable kinetic data could not be obtained by any analytical method.

The values obtained compare well with those available (within parentheses) except for that of the Zn complex with **1** reported by Suh[7d] (see entry 4), which is twice as large as the value reported here. At first sight, the rate constants given in Table 1 indicate that, while there is not much change on moving from free oxime **1** to **6**, complexation of **1**−**4** with the metal ions leads to a general (vide infra, however) decrease in the k_2 value, with the effect following the same order $Cu^{II} > Ni^{II} > Zn^{II}$ for each ligand and being of quite different size depending on the structure of the oxime (compare entries 1−2 and 5−6 in Table 1).

Acidity-Nucleophilicity Relationship

The Brønsted catalysis law, relating rate and acidity and predicting a linear plot of log k_2 versus log K_a with slope β_{nuc}, in the present case is not obeyed for the whole set of data in Table 1. This fact is quite evident from Figure 1.

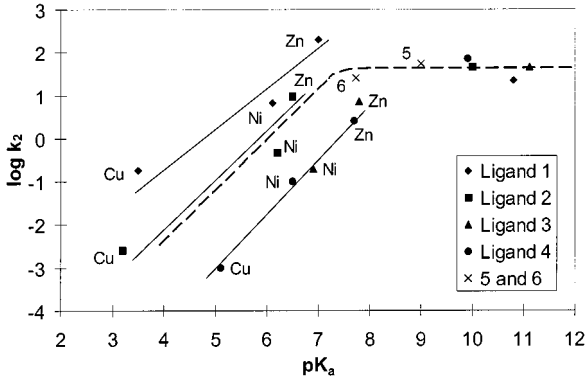


Figure 1. log k_2 vs. pK_a (Brønsted plot) for the reactions of oximate ions with PNPA in water at 25°C; for comparison purposes, the dashed line shows the behaviour of free oximates as reported in ref.[5]

As reported by Terrier and co-workers,[5] the Brønsted plots for oximate ions are generally defined by two different linear regions. In the first region, for pK_a values lower than 8, log k_2 increases in a linear fashion with the pK_a of the oximic function, the β_{nuc} value being close to 0.7. Structurally different oximates describe parallel lines but show slightly different reactivities for any given acidity constant. In the second region, for pK_a values greater than 8, the reactivity of all the different oximate series tends to level off to approximately the same limiting value.

This behaviour is also shown by the compounds under investigation here. The pK_a values for *free* ligands and pyridinium oximes lie in the region of the levelled-off reactivity and show second-order rate constants comparable with those of other basic oximate species previously reported. On the other hand, in the case of *metal ion complexes* whose pK_a values lie in the nonzero slope region of the general Brønsted plot for the oximates, the reactivity increases with the basicity of the function for each ligand series, describing an approximate linear behaviour that allows the evaluation of β_{nuc} values in the range 0.8−1. The linear tracts are well separated and this implies that the reactivity of the complexed oximates depends not only on the nature of the bound metal ion, but also on the structure of the oxime-functionalized ligand. For example, the ketoximes (**1** and **3**) are more reactive than the corresponding aldoximes (respectively, **2** and **4**), whereas the introduction of the methylamino moiety in the ligands (**3** and **4**) leads to a decrease in the reactivity. Although analogous effects have been reported for free oximes, as mentioned above, the extent of such an effect is much larger in the metal complexes

investigated here, in spite of the relatively small differences in the structures of the ligands. For instance, the Ni^{II} complexes of the ligands **1** and **4** have similar pK_a values (6.1−6.5), but their reactivities are two orders of magnitude apart. A very interesting consequence of such reactivity modulation due to ligand structure, is that the reactivity of the Zn^{II} complex with ligand **1** is 4−5 times larger than the apparently limiting reactivity of the free oximates.

That the reactivity of complexed oximates exceeds that of the uncomplexed ones is not unprecedented and has indeed been recorded in one case by Suh et al.[7d] and, more recently, in a study by Yatsimirsky et al.[7g] According to Yatsimirsky, the enhanced reactivity is the result of the stabilization of the carbonyl oxygen atom in the acylation transition state by interaction with the metal ion acting as a Lewis acid. However, this explanation, although generally acceptable, is of little help in explaining the effect of the ligand structure on the enhanced reactivity. The rationale offered by Suh is based on the assumption that there is an electron-donating effect of the methyl group in **1**. These arguments, however, can hardly be used to fit the whole reactivity sequence **1** > **2** > **3** > **4** in the current series of complexes (see Figure 1).

The effects observed in this study and, particularly, the effects of the metal ion-complexed oxime structure and the "exceedingly" high reactivity of their anions relative to that of other oximates, are quite intriguing as it is the limiting reactivity and the very source of the α-effect. Ground-state destabilization, transition-state stabilization, solvation, and inductive or field effects, which are currently assumed to be responsible, in a subtle interplay, for the α-nucleophilicity,[4] are further complicated in the case of the metal ion complexes. We suggest here that their enhanced reactivity is likely to arise from electronic effects due to ligand structure (**1** > **2** and **3** > **4**) and from the specific geometry that is allowed by the ligand (probably less flexible in the case of the tridentate **3** and **4** than in the bidentate ligands **1** and **2**) in response to the requirements (tetrahedral, square bipyramidal) of the of the metal ions.

Admittedly, there is not much room for further speculation and one may also argue that the magnitude of the effects due to the ligand structure is not sufficiently spectacular to stimulate it. However, there is a definite trend, as indicated in Figure 1: once complexed with the appropriate metal ion the oximate group of simple pyridine oximates, available at physiological pHs, may break the intrinsic nucleophilic barrier of the uncomplexed systems.

Conclusion

The design of more effective acetylcholinesterase reactivators using oximes that are more reactive than those currently in use (2-PAM and TMB-4, with pK_a values around 7.8) has been discouraged either by the levelling effect discussed above[5] and by the early report by Bolton and Beckett[13] of the limited effectiveness of complexes of **1** with Cu^{II} and Ni^{II} as compared to that of 2-PAM. This pioneering published work, in our opinion, deserves to be reconsidered and extended to assess the effect of other metal ions, such as Zn^{II}, which, from this study, appears to be better suited than the other metals tested for the enhancement of esterolytic reactivity of structurally simple 2-pyridineoximes in the development of reactivators of poisoned acetylcholinesterase.

Experimental Section

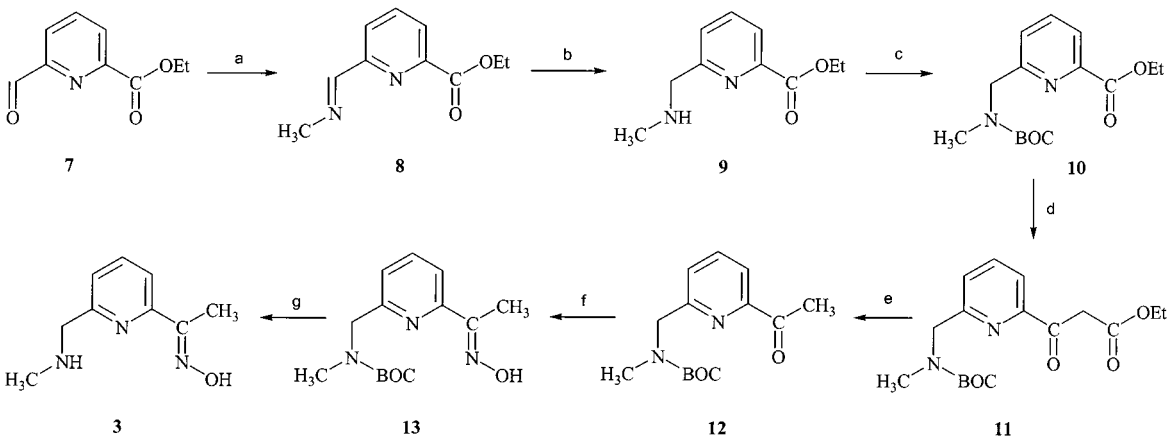
General Methods and Materials: Melting points are uncorrected. − ^{1}H-NMR spectra were recorded with a Bruker AC250F spectrometer operating at 250 MHz and chemical shifts are reported relative to internal Me_4Si. − Elemental analyses were performed by the Laboratorio di Microanalisi of the Inorganic and Analytical Chemistry Department of the University of Padova. − UV/Vis spectra and kinetic traces were recorded with a Perkin−Elmer Lambda 16 spectrophotometer equipped with a thermostatted cell holder. − $Cu(NO_3)_2$, $Zn(NO_3)_2$, and $Ni(NO_3)_2$ were analytical-grade products. Metal ion stock solutions were titrated against EDTA following standard procedures.[14] The buffer components were used as supplied by the manufacturers: acetic acid (Aldrich), 2-morpholinoethanesulfonic acid (MES, Fluka), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPES, Sigma), 2-(cyclohexylamino)ethanesulfonic acid (CHES, Aldrich), 3-(cyclohexylamino)propanesulfonic acid (CAPS, Aldrich). The *p*-nitrophenyl acetate (Sigma) was used as received. The 2-[(hydroxyimino)methyl]pyridine (**2**) and the 2-[(hydroxyimino)methyl]-1-methylpyridinium iodide (**6**) (both Aldrich) were used as received.

Syntheses: The syntheses of the 2-[(1-hydroxyimino)ethyl]pyridine (**1**),[9] the 2-[1-(hydroxyimino)ethyl]-1-methylpyridinium iodide (**5**)[9] and the 6-[(methylamino)methyl]-2-[(hydroxyimino)methyl]-pyridine (**4**)[8c] were performed as reported. The synthesis of 6-[(methylamino)methyl]-2-[(1-hydroxyimino)ethyl]pyridine (**3**) was carried out starting from ethyl 6-formyl-2-pyridinecarboxylate (**7**)[15] following the sequence shown in Scheme 2.

Ethyl 6-[(Methylimino)methyl]-2-pyridinecarboxylate (8): To a solution of **7** (7.0 g, 39 mmol) in 1 L of CH_2Cl_2, were added 6.0 mL of a 33% solution of methylamine in ethanol (8.0 M, 48 mmol) and molecular sieves (4 Å). The reaction mixture was stirred for 1 h, filtered and the solvent removed under vacuum, yielding 7.1 g (95%) of **8** as a pale yellow solid. − ^{1}H NMR ($CDCl_3$): δ = 1.43 (t, *J* = 5 Hz, 3 H, CH_3), 3.56 (s, 3 H, NCH_3), 4.48 (q, *J* = 5 Hz, 2 H, OCH_2), 7.87 (t, *J* = 7.5 Hz, 1 H, H_4Py), 8.13 (m, 2 H, H_5Py and H_3Py), 8.51 [s, 1 H, CH(=N)].

Ethyl 6-[(Methylamino)methyl]-2-pyridinecarboxylate (9): To a solution of **8** (4.8 g, 25 mmol) in 480 mL of ethanol, was added 0.48 g of palladium on activated carbon (10%). The reaction mixture was stirred for 1 h under hydrogen and then filtered through a short Celite pad. The solvent was evaporated to yield 4.8 g (99%) of **9**. − ^{1}H NMR (CD_3OD): δ = 1.45 (t, *J* = 5 Hz, 3 H, CH_3), 2.45 (s, 3 H, NCH_3), 3.94 (s, 2 H, NCH_2Py), 4.49 (q, *J* = 5 Hz, 2 H, OCH_2), 7.66 (d, *J* = 7.5 Hz, 1 H, H_5Py), 7.99 (t, *J* = 7.5 Hz, 1 H, H_4Py), 8.07 (d, *J* = 7.5 Hz, 1 H, H_3Py).

Ethyl 6-{[*tert*-Butoxycarbonyl(methyl)amino]methyl}-2-pyridinecarboxylate (10): To a solution of **9** (4.8 g, 25 mmol) in 500 mL of CH_2Cl_2, were added di-*tert*-butyl carbonate (6.5 g, 30 mmol) and triethylamine (4.5 mL, 30 mmol). The reaction mixture was stirred overnight at room temperature and the material obtained after removal of the solvent under vacuum was dissolved in 200 mL of



Scheme 2. (a) methylamine, CH_2Cl_2, room temp.; (b) Pd/C 10%, H_2, EtOH, room temp.; (c) di-*tert*-butyl dicarbonate, triethylamine, CH_2Cl_2, room temp.; (d) sodium ethoxide, ethyl acetate, EtOH, reflux; (e) Al_2O_3, H_2O, dioxane, reflux; (f) hydroxylamine hydrochloride, Na_2CO_3, EtOH/H_2O, 60°C; (g) trifluoroacetic acid, CH_2Cl_2, room temp.

CH_2Cl_2. The organic solution was extracted with a 5% solution of $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL) and then dried (Na_2SO_4). Evaporation of the solvent gave 7.0 g (95%) of **10** as a yellowish oil. − ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, *J* = 5 Hz, 3 H, CH_3), 1.4−1.5 [m, 9 H, $C(CH_3)_3$], 2.91 (br. s, 3 H, NCH_3), 4.47 (q, *J* = 5 Hz, 2 H, OCH_2), 4.67 (br. s, 2 H, NCH_2Py), 7.36 (br. m, 1 H, H_5Py), 7.81 (t, *J* = 7.5 Hz, 1 H, H_4Py), 7.99 (d, *J* = 7.5 Hz, 1 H, H_3Py).

Ethyl 3-(6-{[*tert*-Butoxycarbonyl(methyl)amino]methyl}-2-pyridyl)-3-oxopropanoate (11): Sodium (0.42 g, 18 mmol) was added to 5 mL of dry ethanol and the mixture was stirred. After all the sodium had reacted, **10** (1.0 g, 3.4 mmol) and 9 mL of ethyl acetate were added and the reaction mixture was stirred at reflux for 4 h. 20 mL of water was added and the aqueous phase was extracted with $CHCl_3$ (3 × 20 mL). The organic phase was dried (Na_2SO_4) and the solvent evaporated, yielding 0.8 g (70%) of **11** as a yellow oil. − ^{1}H NMR ($CDCl_3$): δ = 1.35 (t, 3 H, *J* = 5 Hz, CH_3), 1.4−1.5 [m, 9 H, $C(CH_3)_3$], 2.96 (br. s, 3 H, NCH_3), 4.2 [m, 4 H, OCH_2 + $CH_2C(=O)$], 4.59 (br. s, 2 H, NCH_2Py), 7.38 (br. m, 1 H, H_5Py), 7.82 (t, *J* = 7 Hz, 1 H, H_4Py), 7.98 (d, *J* = 7 Hz, 1 H, H_3Py).

2-Acetyl-6-{[*tert*-butoxycarbonyl(methyl)amino]methyl}pyridine (12): To a solution of **11** (0.80 g, 2.4 mmol) in 80 mL of dioxane, containing 1.2 mL of water, was added 25.6 g of aluminum oxide (basic, type E, Merck).[16] The reaction mixture was stirred overnight at reflux under nitrogen. The mixture was cooled to room temperature, filtered, and the solvent evaporated. The crude material was purified by flash column chromatography (silica gel, toluene/AcOEt, 7:3), giving 0.17 g (27%) of **12** as a yellow oil. − ^{1}H NMR ($CDCl_3$): δ = 1.4−1.5 [m, 9 H, $C(CH_3)_3$], 2.68 [s, 3 H, $C(=O)CH_3$], 2.96 (br. s, 3 H, NCH_3), 4.58 (br. s, 2 H, NCH_2Py), 7.4 (br. m, 1 H, H_5Py), 7.78 (t, *J* = 7 Hz, 1 H, H_4Py), 7.95 (d, *J* = 7 Hz, 1 H, H_3Py).

6-{[*tert*-Butoxycarbonyl(methyl)amino]methyl}-2-[1-(hydroximino)-ethyl]pyridine (13): To a solution of **12** (0.17 g, 0.64 mmol) in 3 mL of EtOH, was added firstly a solution of hydroxylamine hydrochloride (0.06 g, 0.86 mmol) in 2 mL of water and then a solution of $Na_2CO_3 \cdot 10H_2O$ (0.13 g, 0.45 mmol) in 2 mL of water. The reaction mixture was stirred and heated at 60°C for 1 h. After the mixture was cooled to room temperature, 10 mL of water was added and the aqueous phase was extracted with $CHCl_3$ (3 × 15 mL). The organic phase was dried (Na_2SO_4) and the solvent evaporated. The crude material was purified by a flash column cromatography (silica gel, toluene/AcOEt, 7:3), affording 0.16 g (90%) of **13** as a white solid. − ^{1}H NMR ($CDCl_3$): δ = 1.4−1.5 [m, 9 H, $C(CH_3)_3$], 2.38 [s, 3 H, $C(=N)CH_3$], 2.93 (br. s, 3 H, NCH_3), 4.53 (br. s, 2 H, NCH_2Py), 7.15 (br. s, 1 H, H_5Py), 7.6−7.7 (m, 2 H, H_3Py and H_4Py).

6-[(Methylamino)methyl]-2-[1-(hydroximino)ethyl]pyridine (3): To a solution of **13** (0.16 g, 0.6 mmol) in 6 mL of CH_2Cl_2, was added 0.9 mL of trifluoroacetic acid. The reaction mixture was stirred for 2 h (monitoring by TLC, toluene/AcOEt, 7:3) and the solvent was removed under vacuum to give 0.29 g (97%) of **3** as the tris(trifluoroacetate) (brown solid). − M.p. 78−80°C. − ^{1}H NMR (CD_3OD): δ = 2.38 [s, 3 H, $C(=N)CH_3$], 2.87 (s, 3 H, NCH_3), 4.42 (s, 2 H, NCH_2Py), 7.42 (d, *J* = 7.5 Hz, 1 H, H_5Py), 7.87 (t, 1 H, *J* = 7.5 Hz, H_4Py), 7.96 (d, 1 H, *J* = 7.5 Hz, H_3Py). − $C_{15}H_{16}F_9N_3O_7$ (521.29): calcd. C 34.56, H 3.09, N 8.06; found C 34.09, H 2.78, N 7.48.

Kinetic Measurements: The reactions were followed on a Perkin−Elmer Lambda 16 spectrophotometer equipped with a thermostated cell holder ensuring a temperature of 25±1°C. Reactions were started by addition of 20 μL of a solution of a $(1-2)\cdot10^{-3}$ M solution of PNPA in CH_3CN to a 2-mL solution of oxime and additives in the appropriate buffer and monitored by following the formation of *p*-nitrophenol. No changes in pH were observed during the kinetic runs. The initial concentration of substrate was $(1-2)\cdot10^{-5}$ M and the kinetics were, in each case, first-order up to 90% of the reaction. The rate constants were obtained by nonlinear regression analysis of the absorbance versus time data[17] and the fit error on the rate constant was less than 1% (except in the case of the Cu^{II} complexes, see infra). Second-order rate constants were obtained by nonlinear regression analysis of the $k_{\psi;}$ versus oxime concentration data according to Equations 1 and 2.[17] In the case of Cu^{II} complexes, rate measurements following the described procedure were complicated by the fact that, on the one hand, the use of rather acidic solutions meant that the *p*-nitrophenol was in its undissociated form and thus partly masked by the absorbance of other species present in solution and, on the other hand, that employing slightly basic solutions led to turbidity of the solutions. In particular, in the case of the complexes of **3** and **4** this problem forced us to work at pH = 7.0 (to detect the formation of *p*-nitrophenolate) and with a metal/ligand ratio of 1:1 (to avoid precipitation of copper). However, with the exception of the Cu^{II} complex

of ligand **3**, the observed rate constants were reasonably reproducible in a number of independent kinetic analyses and their values can be taken as reliable within a rather large experimental error.

Apparent Formation Constants: The apparent formation constants for the metal complexes of the ligands **3**−**4** were determined at the pH values of the kinetic experiments. To a buffered ($5\cdot10^{-2}$ M) solution of the ligand ($5\cdot10^{-5}$ M), small volumes of concentrated metal ion solutions (which also contained the ligand at the same concentration of the sample) were added and the UV/Vis spectra were recorded. From the spectral changes observed upon addition of the metal ion, the apparent K_f values were obtained by nonlinear regression analyses of the absorbance (at a selected wavelength) versus metal ion concentrations, based on models for 1:1 or 1:2 stoichiometries.[18]

Acidity Constants: Acidity constants of the NOH group for free and complexed oximes were obtained from the UV/Vis spectra recorded for several buffered solutions of oxime ($5\cdot10^{-5}$ M) and, when present, metal ion (at least $5\cdot10^{-4}$ M) on the basis of the absorption band observed at 300−330 nm pertaining to the oximate function. The K_a values were obtained by nonlinear regression analysis of the absorbance versus pH data.[17] In the case of ligand **4**, and of pyridinium oximes **5** and **6**, the pK_a values were determined by potentiometric titrations (25°C, 0.10 M NaCl) of $2\cdot10^{-3}$ M solutions of these compounds using a 0.1 M sodium hydroxide solution. The electrode system was calibrated by titrating a 0.01 M solution of HCl so that the pK_w value was 13.78. The pH and the volume of added NaOH were fitted with the computer program BEST[19] to obtain the desired pK_a values.

Acknowledgments

Financial support for this research has been partly provided by the Ministry of the University and Scientific and Technlogical Research (MURST) under the framework of the "Supramolecular Devices" project. The authors thank Mr. B. Toulorge, a Socrates student from the University of Versailles, for his participation.

[1] S. J. Lippard, J. M. Berg, *Principles of Bioinorganic Chemistry*, Univ. Science Books, Mill Valley, **1994**.
[2] [2a] J. Chin, *Acc. Chem. Res.* **1991**, *24*, 145−152. − [2b] T. H. Fife in *Perspective on Bioinorganic Chemistry* (Eds: R. W. Hay, J. R. Dilworth, K. B. Nolan), JAI Press, London, **1991**, vol. 1, p. 43. − [2c] T. Koike, E. Kimura, *J. Am. Chem. Soc.* **1991**, *113*, 8935−8941. − [2d] J. Suh, *Acc. Chem. Res.* **1992**, *25*, 273−279. − [2e] P. Scrimin, P. Tecilla, U. Tonellato, *J. Phys. Org. Chem.* **1992**, *5*, 619−627 and references cited therein.
[3] [3a] J. March, *Advanced Organic Chemistry*, 4th ed., Wiley, New York, **1992**, p. 351. − [3b] G. Moutiers, E. Le Guevel, L. Villien, F. Terrier, *J. Chem. Soc., Perkin Trans. 2* **1997**, 7−13 and references cited therein.
[4] [4a] C. D. Bedford, R. W. Harris, R. A. Howd, A. Miller, H. W. Nolen III, R. A. Kenley, *J. Med. Chem.* **1984**, *27*, 1431−1438. − [4b] R. A. Kenley, C. D. Bedford, O. D. Dailey, R. A. Howd, A. Miller, *J. Med. Chem.* **1984**, *27*, 1201−1211. − [4c] C. D. Bedford, M. Miura, J. C. Bottaro, R. A. Howd, H. W. Nolen III, *J. Med. Chem.* **1986**, *29*, 1689−1696.
[5] F. Terrier, P. MacCormack, E. Kizilian, J. Halle, P. Demerseman, F. Guir, C. Lion, *J. Chem. Soc., Perkin Trans. 2* **1991**, 153−158.
[6] [6a] J. Faucherre, C. Petitfaux, *Bull. Soc. Chim. Fr.* **1965**, *2*, 347−359. − [6b] M. Orama, H. Saarinen, J. Korveranta, *Acta Chem. Scand.* **1989**, *43*, 407−412. − [6c] M. Orama, H. Saarinen, *Acta Chem. Scand.* **1998**, *52*, 1209−1213.
[7] [7a] R. Breslow, D. Chipman, *J. Am. Chem. Soc.* **1965**, *87*, 4195−4196. − [7b] C.-M. Hsu, B. S. Cooperman, *J. Am. Chem. Soc.* **1976**, *98*, 5652−5657. − [7c] K. Ogino, K. Shindo, T. Minami, W. Tagaki, T. Eiki, *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1101−1106. − [7d] J. Suh, M. Cheong, H. Han, *Bioorg. Chem.* **1984**, *12*, 188−196. − [7e] J. Suh, M. P. Suh, J. D. Lee, *Inorg. Chem.* **1985**, *24*, 3088−3090. − [7f] J. Suh, B. Chang, *Bioorg. Chem.* **1987**, *15*, 167−182. − [7g] A. K. Yatsimirsky, P. Gomez-Tagle, S. Escalante-Tovar, L. Ruiz-Ramirez, *Inorg. Chim. Acta* **1998**, *273*, 167−174.
[8] [8a] J. Budka, F. Hampl, F. Liska, P. Scrimin, P. Tecilla, U. Tonellato, *J. Mol. Cat. A: Chemical* **1996**, *104*, 201−204. − [8b] F. Hampl, F. Liska, F. Mancin, P. Tecilla, U. Tonellato, *Langmuir* **1999**, *15*, 405−412. − [8c] F. Mancin, P. Tecilla, U. Tonellato, *Langmuir*, **2000**, *16*, 227−233.
[9] S. Ginsburg, I. B. Wilson, *J. Am. Chem. Soc.* **1957**, *79*, 481−485.
[10] G. Heinisch, W. Holzer, *Tetrahedron Lett.* **1990**, *31*, 3109−3112.
[11] Except in the case of the kinetic experiments for the complexes **3**·Cu^{II} and **4**·Cu^{II}, in which it was necessary to use a metal/ligand ratio of 1:1 (see Experimental Section). The formation constants of these complexes are, however, sufficiently high (Table 1) to ensure complete formation of 1:1 complexes also under these conditions.
[12] F. Basolo, R. G. Pearson, *Mechanism of Inorganic Reactions*, 2nd ed., Wiley, New York, **1967**, p. 32.
[13] S. Bolton, A. Beckett, *J. Pharm. Sci.* **1964**, *53*, 55−60.
[14] Z. Holzbecher, *Handbook of Organic Reagents in Inorganic Analysis*, Wiley, Chichester, **1976**.
[15] G. Quenginer, P. Pastour, *Bull. Soc. Chim. Fr.* **1969**, *6*, 3678−3681.
[16] A. E. Greene, A. Cruz, P. Crabbe, *Tetrahedron Lett.* **1976**, *31*, 2707−2708.
[17] The fit of the data was made using the sofware package Enzfitter: R. J. Leatherbarrow, *Enzfitter*, Elsevier, Amsterdam, **1987**.
[18] The fit of the data was made using the sofware package Scientist: *Scientist 2.01*, MicroMath Scientific Software, Salt Lake City, Utah, **1995**.
[19] A. E. Martell, R. J. Motekaitis, *Determination and Use of Stability Constants*, 2nd ed., VCH, New York, **1992**.

Received August 6, 1999
[O99487]