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Kinetics and Mechanism of the Cu^{2+} Induced Hydrolysis of Nitrile Groups in the Side Chain of Tetraazamacrocycles. Models for Nitrilases

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A series of mono-N-functionalized tetraaza macrocycles having a nitrile group in their side chain have been synthesized and the kinetics and mechanism of the Cu^{2+} induced hydrolysis has been studied. Two factors were systematically varied: the length of the side chain and thus the distance between Cu^{2+} and the nitrile group, as well as the rigidity of the macrocycle by introducing an additional ethylene bridge.

The mechanism of the hydrolysis proceeds by an intramolecular attack of a coordinated OH^- onto the nitrile group in a five or six center transition state. The intramolecular nature of the reaction has been proven (a) by the pH dependence of the hydrolysis, which in some cases has a plateau at high pH values, (b) by the competitive inhibition with SCN^-, and (c) by the spectral changes observed at high pH.

The sequence of Cu^{2+} induced hydrolysis rates is the following: flexible macrocycle with a short chain > rigid macrocycle with a short chain > flexible macrocycle with a longer chain ~ rigid macrocycle with a longer chain. The length of the side chain, which determines whether a five or six center transition state is formed, is the most important factor. The fastest hydrolysis has a half-life time of about 50 ms at pH 12.5 and 25°C and indicates the efficiency of the metal ion. The rigidity of the macrocycle also influences the reactivity since in the rigid complexes on one side the Cu^{2+} ion is less accessible for OH^- to give the reactive intermediate and on the other side the transition state is less reactive because of topological aspects.

Keywords: Cu^{2+} complexes; Tetraazamacrocycles; Hydrolysis; Nitrile; Kinetics; Mechanism

INTRODUCTION

Nature uses many hydrolytic enzymes (esterases, peptidases, phosphatases, urease and nitrilases), which have a metal ion in their active site [1]. In the field of bioinorganic chemistry models for such metallo enzymes are a subject of continued interest and have been studied in detail [2–4]. The aim of model compounds is to clarify the mechanism of such reactions and to find structure reactivity relationships, which of course cannot be studied in the natural systems. A lot of information about hydrolysis reactions has been obtained from studies on Co^{3+} complexes [3], which are kinetically inert and thus allow to correlate the reactivity with the structure. Two main pathways have thereby been found: (a) attack of external nucleophiles on the coordinated and thus polarized substrate,

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or (b) attack of an internal coordinated nucleophile onto the organic substrate.

In the case of labile metal ions, which of course are more relevant for biological systems, the differentiation between the two mechanism is more difficult, since rapid equilibria between the different forms of the complex are present and thus the exact nature of the reactive species is more difficult to proof. One way to overcome this difficulty is to incorporate the labile metal ion into a macrocycle, which mostly gives a kinetically inert complex with a fixed structure [4]. In addition by functionalizing the macrocycle with a side chain carrying the reactive group, it can be brought close to the metal ion under controlled structural conditions. We have previously reported on such reactions with esters [5], phosphonate esters [5] and nitriles [6] as functional groups and shown the potential of this approach.

We now present results of the nitrile hydrolysis in a series of mono-N-functionalized tetraazamacrocyclic Cu^{2+} complexes. In this study we have modified the length of the side chain and the rigidity of the macrocyclic unit (**2**, **3**, **5**, **6**) in order to investigate their effect on the rate of the nitrile hydrolysis.

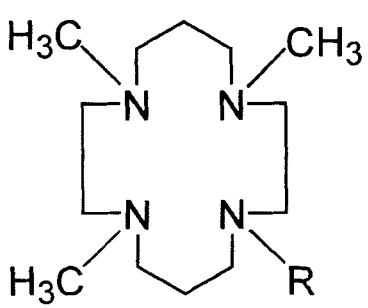
EXPERIMENTAL

Materials

1,4,8-Trimethyl-1,4,8,11-tetraazacyclotetradecane (**1**) [7], 3-(4,8,11-trimethyl-1,4,8,11-tetraazacyclotetradec-1-yl)propionitrile (**3**) [8], and 5-methyl-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (**4**) [9] were prepared according to the literature.

Physical Measurements

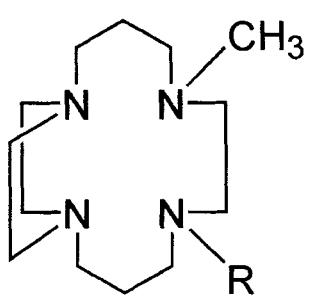
^{1}H- and ^{13}C-NMR spectra: *Varian Gemini 300*. δ relative to $SiMe_4$ as internal standard for $CDCl_3$ or to sodium-(3-trimethylsilyl)propansulfonate for D_2O solution. GC-MS: *Hewlett Packard* (mass selective detector 5971 series, gas chromatograph 5890 series II; column Me_2Si, 25 m). Mp.: *Büchi* 535 not corrected. FAB-MS: VG 70–250 with nitrobenzylalkohol as matrix. Elemental analysis were performed by the analytical laboratory of *CIBA AG*, Basel. IR-spectra were run on a Perkin-Elmer 1600 using KBr pellets or in D_2O using IRTRAN cells.



1 R = H

2 R = CH_2CN

3 R = CH_2CH_2CN



4 R = H

5 R = CH_2CN

6 R = CH_2CH_2CN

Preparations

2-(4,8,11-Trimethyl-1,4,8,11-tetraazacyclotetradec-1-yl)acetonitrile (2)

To a solution of **1** (1.21 g, 5 mmol) and formaldehyde (37%, 0.45 ml) in water (10 ml), a solution of KCN (0.34 g, 5.2 mmol) in water (2 ml) and acetic acid (1 ml) were added dropwise from two dropping funnels at 0° C. After complete addition the solution was stirred for 1.5 h. Then the mixture was made alkaline by adding NaOH and extracted with $CHCl_3$ (2 × 100 ml). The organic phase was dried over Na_2SO_4 and evaporated. The residue was crystallized from petrol ether giving the pure product (1.25 g, 89%). M.p. 79.5–81° C. IR (KBr): 2240 cm^{-1} (CN). Elemental analysis: found C 63.80, H 10.99, N 24.80%, calculated for $C_{15}H_{31}N_5$ (281.25) C 64.01, H 11.10, N 24.88%; ^{1}H-NMR ($CDCl_3$): 1.58 (q, C—CH_2—C), 2.14 (s, N—CH_3), 3.00–2.13 (m, N—CH_2—), 3.60 (s, N—CH_2—CN).

5-Cyanomethyl-8-methyl-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (5)

To **4** (2.30 g, 9.6 mmole) in H_2O (25 ml) a formaldehyde solution (1.4 ml, 35%) and after cooling to 0° C KCN (15 ml, 1.5 M) and acetic acid (3.5 ml) were added. The solution was warmed to RT and stirred for 22 h. To the mixture KOH (11.3 ml, 30%) and solid KOH (5.95 g) were given. The solution was then extracted with CH_2Cl_2 (2 × 140 ml, 5 × 70 ml), from which after drying and evaporation a colorless oil (2.62 g, 98%) was obtained. IR (KBr): 2228 cm^{-1} (CN); ^{1}H-NMR ($CDCl_3$) 1.65 (m, C—CH_2—C), 2.19 (s, CH_3N), 2.38–3.15 (m, —CH_2N), 3.74 (s, CH_2CN). ^{13}C-NMR: 23.09, 23.79 (CCH_2C), 40.91, 45.31, 47.22, 49.57, 51.84, 52.89, 53.25, 55.09, 55.61 (CH_2—N), 41.87 (CH_3—N, ATP), 115.17 (CN).

5-(2-Cyanoethyl)-8-methyl-1,5,8,12-tetraazbicyclo[10.2.2]hexadecane (6)

To a solution of **4** (205 mg, 0.84 mmole) in abs. EtOH (5.6 ml) acrylonitrile (1.38 ml) was added. After 24 h at RT the solution was filtered and evaporated giving the product (231 mg, 94%) as colorless oil. IR (KBr): 2244 cm^{-1} (CN); ^{1}H-NMR ($CDCl_3$) 1.68 (q, C—CH_2—C), 2.18 (s, CH_3N), 2.39–2.69, 2.81–2.91, 3.03–3.19 (m, —CH_2N). ^{13}C-NMR: 15.03 (CH_2—CN), 23.88, 24.33 (CCH_2C), 41.49 (CH_3N, ATP) 45.89, 46.98, 49.35, 50.20, 51.96, 50.20, 51.96, 52.34. 52.54, 54.54, 56.39, 57.12 (CH_2—N), 119.09 (CN).

Cu^{2+}-Complexes

The Cu^{2+}-complexes were prepared by mixing equimolar amounts (4 or 7 mmol) of ligand and $Cu(NO_3)_2 \cdot 3H_2O$ or $Cu(ClO_4)_2 \cdot 6H_2O$ in EtOH/MeOH (4 ml or 30 ml) and gently heating. [Cu(**3**)]$(ClO_4)_2$. The complex precipitates from MeOH. Yield 48.0%. IR (KBr): 2120 cm^{-1} (CN). Elemental analysis: found C 33.83, H 5.70, N 12.40%, calculated for $C_{16}H_{33}Cl_2CuN_5O_8 \cdot 0.5$ H_2O (566.92), C 33.86, H 5.99, N 12.40%.

[Cu(**5**)]$(PF_6)_2 \cdot CH_3CN$. The EtOH solution was evaporated and the residue taken up with DMSO to which a saturated solution of NH_4PF_6 in EtOH was added. The solid was collected and recrystallized from MeOH/CH_3CN yielding blue violet crystals (24% yield). IR (KBr): 2309 and 2277 cm^{-1} (CN). Elemental analysis: found C 30.10, H 4.74, Cu 9.84, N 12.26%, calculated for $C_{15}H_{29}CuF_{12}N_5P_2 \cdot CH_3CN$ (673.95) C 30.30, H 4.79, Cu 9.43, N 12.47%.

[Cu(**6**)]$(ClO_4)_2$. The precipitate was isolated and recrystallized from EtOH/H_2O giving blue crystals (54%). IR (KBr): 2233 cm^{-1} (CN). Elemental analysis: found C 34.27, H 5.80, Cl 13.02, Cu 11.40, N 12.51, H_2O 1.24%, calculated for $C_{16}H_{31}CuCl_2N_5O_8 \cdot 0.4$ H_2O (562.93) C 34.14, H 5.69, Cl 12.60, Cu 11.29, N 12.44, H_2O 1.25%.

Crystals for the X-ray structure determination were obtained by evaporation of an aqueous solution of the complex.

X-ray Diffraction

The crystal data and parameters of the data collection for [Cu(**6**)]$(ClO_4)_2$ are given in Table I.

Data collection has been carried out at 293° using an *Enraf-Nonius* CAD4 diffractometer. Unit cell parameters were determined by accurate centering of 11 independent strong reflections by the least-squares method. Three standard reflections monitored every 2 h during data collection showed no significant variation of the intensity. The raw data set was corrected for polarization effects. The structure was solved by the direct method [10]. Anisotropc least-square refinements were carried out on all no-H-atoms using the program *CRYSTALS* [11]. Scattering factors are taken from *International Tables for Crystallography, Vol. IV* [12].

Kinetics

The kinetics of the hydrolysis reaction were studied using a stopped-flow *DurrumD110* instrument with a 2 cm cell or a *Hitech* stopped-flow unit with a 1 cm cell equipped with a *J* and *M* photodiode array or a *VarianE635* spectrophotometer equipped with a hand-stopped-flow unit and a 1 cm cell. Aqueous solutions of the Cu^{2+} complexes (10^{-4}–5.10^{-4} M) at pH 6 and I = 0.5 (KNO_3) were

TABLE I Crystal data and parameters of data collection for the Cu^{2+}-complex of **6**

Formula	$(C_{16}H_{31}CuN_5)(ClO_4)_2$
Mole weight [gmol^{-1}]	555.90
Crystal system	orthorhombic
Space group	$P2_1cn$[a]
a [Å]	9.938 (2)
b [Å]	14.520 (4)
c [Å]	16.155 (3)
α [°]	90.00
β [°]	90.00
γ [°]	90.00
V [Å^3]	2331.3 (9)
Z	4
F (000)	1156
Density [gcm^{-3}]	1.58
μ [mm^{-1}]	3.92
Crystal size [mm]	0.14/0.26 /0.34
Temperature [K]	293
Radiation	Cu K_α ($\lambda = 1.54180$)
Scan type	$\omega/2\Theta$
Θ_{max} [°]	77.50
No. of measured reflexes	2147
No. of independent reflexes	2070
No. of reflexes in refinement	1437
No of variables	326
Final R-value	5.04
R_W	5.68
Weighting scheme	Chebychev polynomial-weighting[b]
Last Max/Min in differ. Fourier map	0.60 / −0.26

[a] Non-Standard Setting of $Pna2_1$ (Nr.33).
[b] Carruthers, J. R.; Watkin, D. J.; *Acta Crystallogr. Sect. A* 1979, 35, 698.

mixed with buffer solutions (0.1 M t-butylaminoethanol) or KOH to reach pH values between 7 and 13, at which the hydrolysis could be followed. In the case of the very reactive complex with **2** the Cu^{2+} complex was first prepared in DMF to give a 10^{-2} M stock solution and then diluted with an aqueous solution of KNO_3 ($I = 0.5$ M) to the desired concentration. With the *Durrum* instrument the reactions were measured at 643, 690, 850 and 850 nm for **2**, **3**, **5**, and **6**, respectively. With the hand-stopped-flow instrument the reactions were measured at 320 nm for **3**. With the *Hitech* photodiode spectrometer spectra were taken from 400 to 750 nm. For **2** and **3** the inhibition by SCN^- (0.002 – 0.02 M) was studied by adding it to the solution of the complex. The kinetics was fitted either with one or two exponentials using the program KINFIT [13]. The so obtained k-values are mean values of at least five experiments.

In a few experiments with the photodiode array instrument very fast series of spectra were measured in order to obtain the spectrum of the initial product just after mixing. For **2** and **5** spectral changes at high pH were observed, indicating that the starting complex underwent a rapid reaction, whereas for **3** and **6** no such changes could be detected.

In the case of the Cu^{2+} complex with **3** the kinetics were also studied by IR spectroscopy. A D_2O solution of the Cu^{2+} complex with **3** ($5 \cdot 10^{-2}$ M) adjusted to pH = 11.25 with NaOD was followed between 2000 – 1600 cm^{-1} every 5 min. At 1625 cm^{-1} a band slowly appeared with a half life time of about 10 min.

Spectrophotometric Titrations

The Cu^{2+} complexes of **2** and **3** ($2.6 \cdot 10^{-3}$ M) kept at pH 6.2 (2,4,6-collidine buffer; $KNO_3 = 0.5$ M) were titrated with KSCN (0.167 M), which was added in small portions, using our fully automated titration system [14]. The measurements were evaluated using the program SPECFIT [15].

RESULTS AND DISCUSSION

Structures in the Solid State and in Solution

The crystal structure of **6** shows a polymeric assembly of molecules which are bridged by the nitrile group of one molecule to the next one. In each unit the Cu^{2+} ion is pentacoordinated by the four nitrogens of the macrocycle and the nitrile group of the next unit (Fig. 1). The metal ion is 0.33 Å out of the best plane of the four nitrogen atoms (± 0.008 or ± 0.009 Å) in the direction of the axial ligand. The equatorial Cu—N bonds are 2.01 – 2.04 Å, whereas the axial Cu—N is 2.307 Å. Interesting is the observation that it is not the nitrile of the pendant arm which coordinates to the metal ion, but that of a neighboring molecule. The angle Cu(1)—N(5)′—C(15)′ is 175.1° indicating that the nitrile group binds through a σ-bond to the metal ion.

The macrocycle is in a N-configuration in which the two substituents at N(1) and N(2) are on the same side of the N_4 plane. Because of the steric requirements of the piperazine ring the angle N(3)—Cu(1)—N(4) is very small with 72.4°.

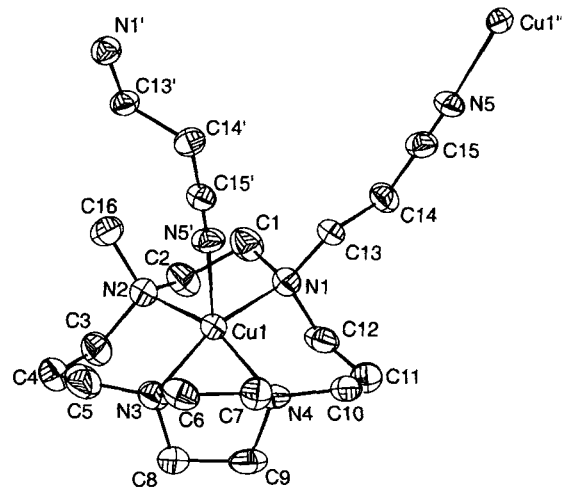


FIGURE 1 ORTEP plot of the Cu^{2+} complex with **6**. Selected bond lengths: Cu(1)—N(1) 2.049(8); Cu(1)—N(2) 2.012(8); Cu(1)—N(3) 2.048(8); Cu(1)—N(4) 2.031(8); Cu(1)—N(5) 2.367(8).

TABLE II Spectral properties of the Cu^{2+} complexes with **1–6** in aqueous solution

Complex with	λ_{max}(nm)	$\varepsilon(M^{-1}\,cm^{-1})$
1	640	252 [8]
2	643	256 [8]
3	646	256 [8]
4	556	328
5	592	279
6	579	380

The discussion of the solution structure of the Cu^{2+} complexes with the macrocycles carrying a nitrile group is based on their UV-VIS spectra (Tab. II).

The complexes with **1–3** all show absorption maxima typical for a five coordinated Cu^{2+}, which has four nitrogen atoms in equatorial positions and a H_2O in the axial position [8]. Similarly the absorption characteristics of the complexes with the reinforced macrocycles **5** and **6** also indicate pentacoordination of the metal ion [16].

The spectral changes observed at high pH for **2** and **4** just after mixing indicate that hydroxo species must be present Eq. (1), but because of the following nitrile hydrolysis these equilibria could not be quantitatively evaluated.

$$CuL^{2+} + OH^- \rightleftharpoons CuL(OH)^+ ; K_{OH} \qquad (1)$$

At lower pH, however, one can study the coordination of SCN^- at the axial position (Eq. (2)), which also indicates, that five coordination is typical for these species [8].

$$CuL^{2+} + SCN^- \rightleftharpoons CuL(SCN)^+ ; K_{SCN} \qquad (2)$$

The stability constants for this equilibrium were determined by spectrophotometric titrations and gave log $K_{SCN} = 2.40(2)$ and log $K_{SCN} = 1.88(2)$ for the Cu^{2+} complex of **2** and **3**, respectively.

Nitrile Hydrolysis

When the nitrile complexes are brought to high pH hydrolysis of the nitrile to the corresponding amide takes place [6]. In the case of **2** we observe kinetics which can be fitted with two exponentials. The first and faster step has a relatively small amplitude, whereas the second slower one exhibits a large amplitude. In order to find out by which chemical process the two observed rates can be accounted for, we have studied the reactivity of the Cu^{2+} complex of the amide ligand. This complex shows the typical O- to N-coordination change [17], when the pH is increased from 7 to higher values. The rate of this interconversion exactly fits the results of the second phase of the measurements with the Cu^{2+} complex of the nitrile derivative. Thus the first step corresponds to the nitrile hydrolysis giving the amide, which then rearranges in the typical way. The pH dependence in this step is linear below pH 12 and gives a plateau at higher pH values, indicating that a maximal rate is then reached (Fig. 2). The same reaction in the presence of SCN^- shows that up to 10^{-3}M SCN^- has no effect, but at higher values it inhibits the reaction (Fig. 3). To explain all these points we propose *Scheme* 1.

The starting complex $CuL(H_2O)^{2+}$ is a five coordinate species as discussed above. The *Scheme* implies that the Cu^{2+} complex of **2** exists depending on the pH also as a hydroxo species (Eq. (1)), in which the hydroxo group is axially bound to the Cu^{2+}. When SCN^- is added the axial position is occupied by this ion (Eq. (2), K_{SCN}), giving a ternary species. Assuming that $CuLOH^+$ is the reactive form we obtain Eq. (3) for the rate expression, with which all the observed dependencies of the first step can be explained (Tab. III). k_1 describes the hydrolysis

$$k_{1,obs} = k_1 \cdot K_{OH}[OH^-] / (K_{OH} \cdot K_{SCN} + K_{OH}[OH^-] + K_{SCN}[SCN^-]) \qquad (3)$$

of the nitrile complex $CuLOH^+$ and is with a half life time of about 50 ms an extremely fast

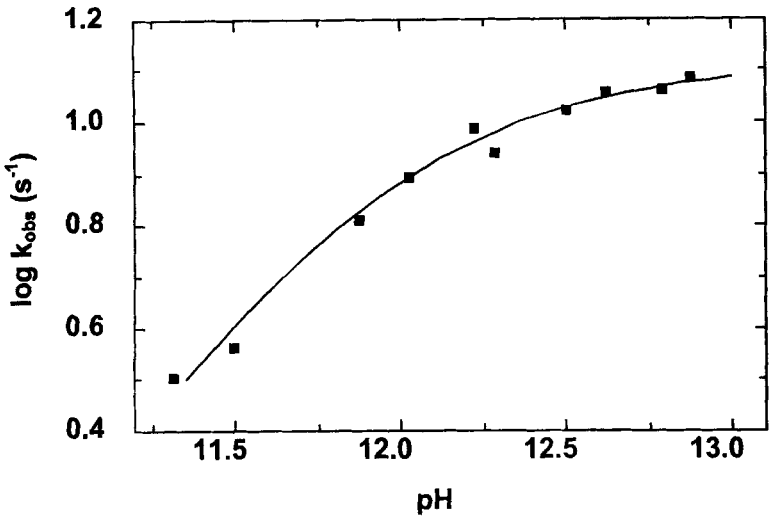


FIGURE 2 pH dependence of the nitrile hydrolysis of the Cu^{2+} complex with 2. Experimental conditions $[CuL^{2+}] = 1.5 \cdot 10^{-4}$M.

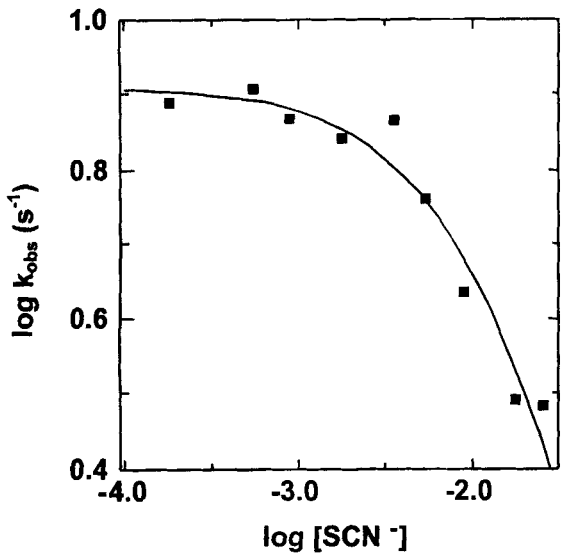
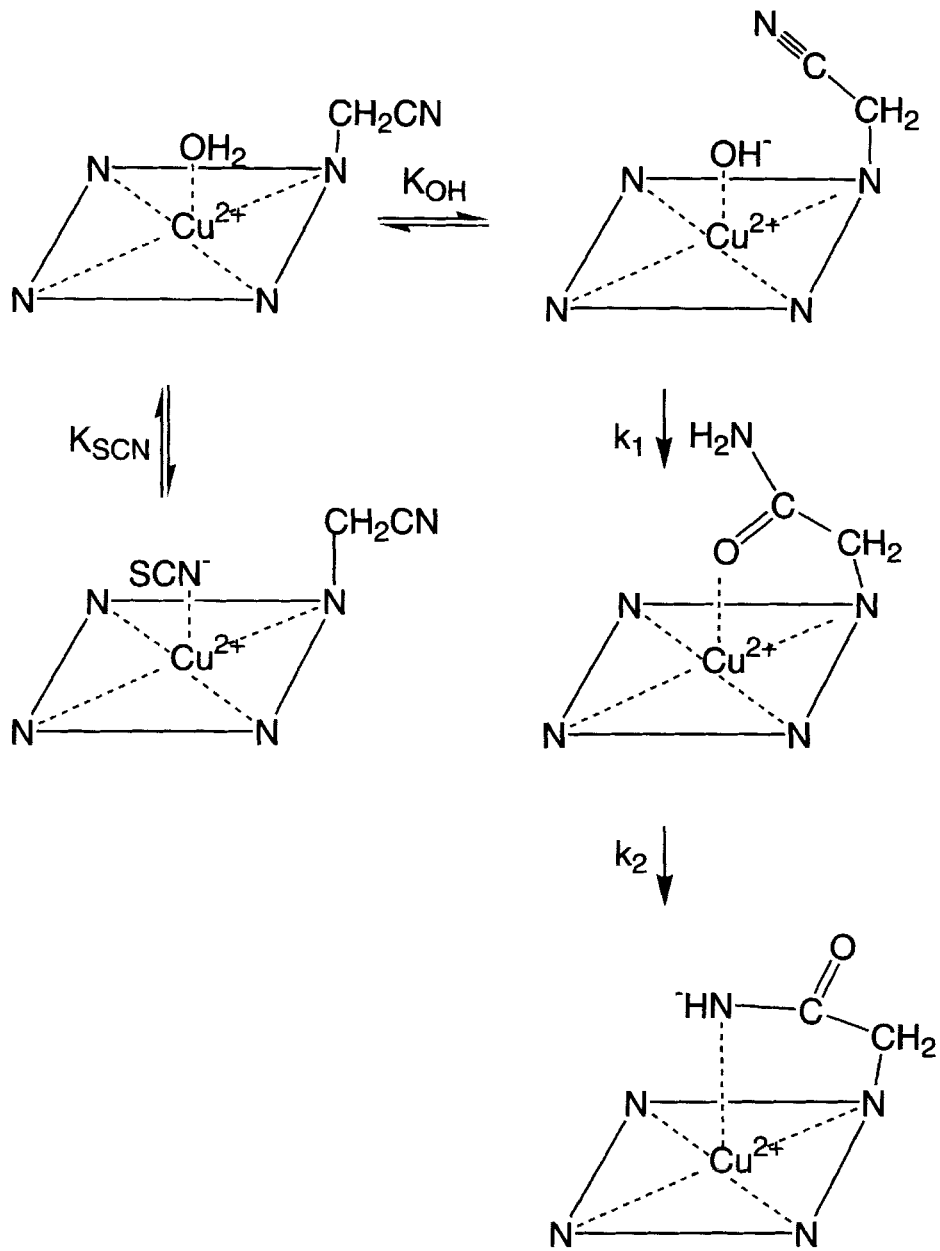


FIGURE 3 SCN^- inhibition of the nitrile hydrolysis of the Cu^{2+} complex with 2. Experimental conditions $[CuL^{2+}] = 2 \cdot 10^{-4}$M, pH = 12.05.

reaction. For it we propose the intramolecular process depicted in Figure 4, in which a five membered transition state takes place when the coordinated OH^- group attacks as a nucleophile the nitrile function of the side chain. Thus the role of the metal ion is to organize the reactants in such a way that they are in close vicinity to each other and so oriented that an effective reaction can take place.

Interesting is the observation that the hydrolysis can be inhibited by SCN^-. SCN^- in fact competes for the same axial position as OH^- and once this is blocked the intramolecular hydrolysis by OH^- is not possible any more. The kinetically determined value $K_{SCN} = 1.9(2) \cdot 10^2$ (Tab. III) nicely corresponds to that obtained by spectrophotometric titration $K_{SCN} = 2.5 \cdot 10^2$ (see above). So the three experimental results: plateau in the pH dependence, competitive inhibition by SCN^- and the spectral changes observed at high pH consistently indicate that a hydroxo species is formed and that an intramolecular process is responsible for the nitrile hydrolysis in this complex, which thus mimics the reactivity of metal ions as found in many metallo enzymes. The resulting product of the hydrolysis is the O-coordinated form of the amide, which then interconverts in a second step (k_2) into the N-coordinated form after deprotonation of the amide.

For ligand 3 the situation is somewhat different since we observe only a monophasic



SCHEME 1 Reaction sequence of the nitrile hydrolysis of the Cu^{2+} complex with **2**.

TABLE III Reactivity and equilibrium constants for the nitrile hydrolysis in the Cu^{2+} complexes with **2**, **3**, **5**, and **6**

Cu^{2+} complex	k_1 (s^{-1})	K_{OH} (M^{-1})	$k_1 K_{OH}$ ($M^{-1} s^{-1}$)	K_{SCN} (M^{-1})
2	13.0 (5)	$1.0(1) \cdot 10^2$	$1.3 \cdot 10^3$	$1.9(2) \cdot 10^2$
3	$< 3 \cdot 10^{-3}$	$< 1 \cdot 10^2$	0.29(1)	$5.3(5) \cdot 10^1$
5	0.15 (2)	$4.6(6)\ 10^1$	6.9	
6	$< 7 \cdot 10^{-3}$	$< 5 \cdot 10^1$	0.35(1)	

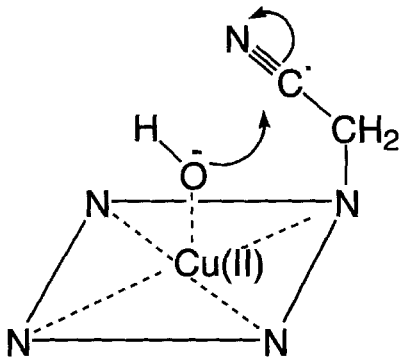


FIGURE 4 Transition state of the intramolecular nitrile hydrolysis in the macrocyclic Cu^{2+} complex of 2.

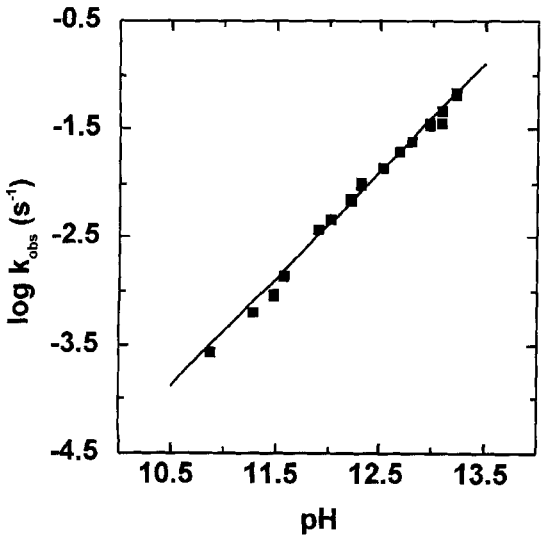


FIGURE 5 pH dependence of the nitrile hydrolysis of the Cu^{2+} complex with 3. Experimental conditions $[CuL^{2+}] = 2.2 \cdot 10^{-4}$M.

kinetics and log k_{obs} is linear up to pH 13.5 (Fig. 5). This rises the question which chemical process is measured in this case. To clarify this point we have taken a solution of the Cu^{2+} complex with the nitrile 3, which had already reacted and given the amide, and after adjusting its pH to about 7 mixed again with base at pH 12. No kinetical change could be observed. A spectrophotometric pH-titration of the amide also shows no spectral change between pH 7 and 12, thus indicating that in this instance deprotonation of the amide does not take place. In addition we have qualitatively followed the kinetical process by IR-spectroscopy in D_2O. At neutral pD no amide band is found. However, once the pD is increased to 11.2 the typical band for the amide at 1625 cm^{-1} begins to appear and increases with a half time of about 10 min, which corresponds to a rate constant of about 10^{-3} s^{-1}, a value found in the quantitative stopped flow study of this pH (Fig. 5). All this indicates that the process we observe is the nitrile hydrolysis. As for 2 the process can also be inhibited by SCN^- (Fig. 6) and here again the kinetically determined value $K_{SCN} = 53$ compares well to that measured by spectrophotometry $K_{SCN} = 76$. The fact that the pH dependence does not show a plateau at high pH values must be due to the non-existence of the hydroxo species under our experimental conditions. The longer side chain being sterically more demanding reduces the ability of the metal ion to axially bind ligands. This is not only true for OH^- but also for SCN^-. For the Cu^{2+} complex with 3 we can therefore only determine the product $k_1 \cdot K_{OH}$, which is about 4000 times smaller than that of the Cu^{2+}

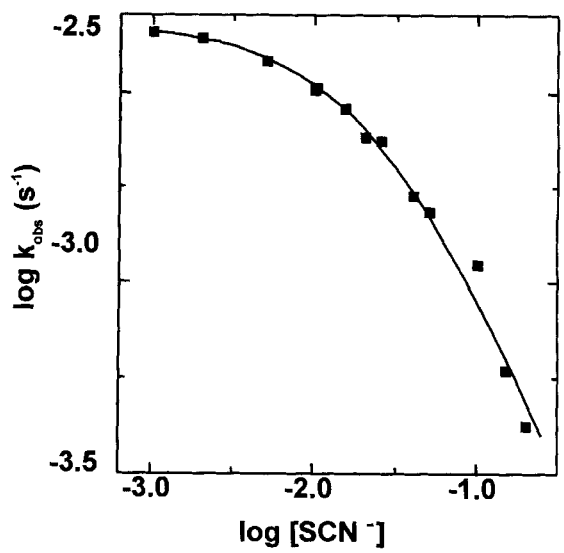


FIGURE 6 SCN^- inhibition of the nitrile hydrolysis of the Cu^{2+} complex with 3. Experimental conditions $[CuL^{2+}] = 4.5 \cdot 10^{-4}$M, pH = 12.1.

complex with ligand **2** (Tab. III). Since we cannot separate the two terms describing the reactivity (k_1) and the stability of the hydroxo species (K_{OH}) we must guess them. K_{OH} is certainly smaller than $100\,M^{-1}$, which gives k_1 at best $3\cdot10^{-3}\,s^{-1}$.

The hypothesis of entatic states for metalloenzymes is still a very attractive idea to explain the high reactivity of such systems [18]. So we tried to put strain onto the metal ion by including an additional bridge between two nitrogen atoms of the macrocycle, which makes it more rigid. The reactivity of the Cu^{2+} complexes with the two ligands **5**, **6** are in part similar to the previous ones, the complex with the shorter chain reacting faster than that with the longer chain, but in part different since they show only a one reaction step. For **5** we find a non linear pH dependence with a plateau at high pH (Fig. 7), which allows to separate k_1 and K_{OH} so that a comparison with **2** can be made. Although the tendency for axial coordination in general [16] and for OH^- in particular is lower, the main difference is found in k_1, which is about 100 times smaller (Tab. III). This means that the less flexible ligand **5** does not allow to form an ideal transition state as does **2**. The side chain of the more rigid ligand is probably oriented in such a way, that the distance between the coordinated OH^- and the nitrile group is larger than for the more flexible system **2**.

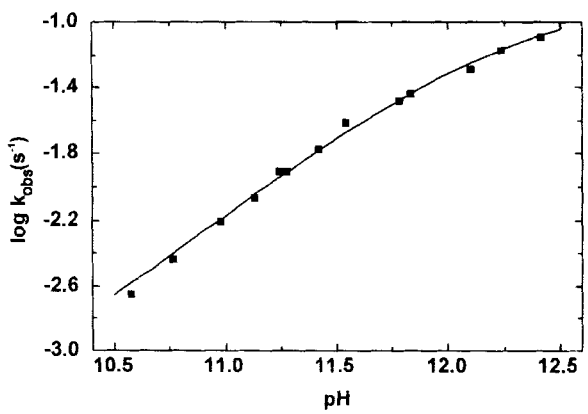


FIGURE 7 pH dependence of the nitrile hydrolysis of the Cu^{2+} complex with **5**. Experimental conditions $[CuL^{2+}] = 5\cdot10^{-4}$ M.

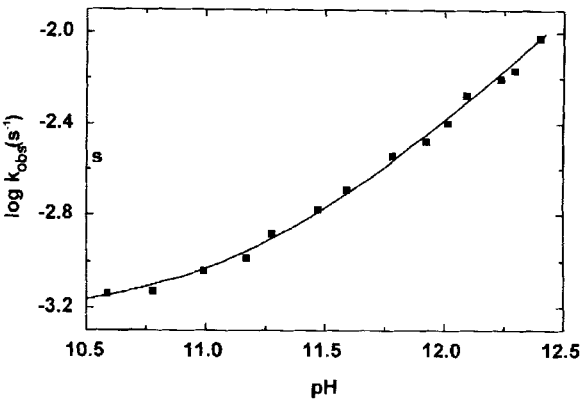


FIGURE 8 pH dependence of the nitrile hydrolysis of the Cu^{2+} complex with **6**. Experimental conditions $[CuL^{2+}] = 6\cdot10^{-4}$ M.

The Cu^{2+} complex with ligand **6** gives a more complicated pH dependence (Fig. 8), which allows to determine, as in the case of **3**, $k_1\cdot K_{OH}$ (Tab. III), but also a pH independent term $k_0 = 5.7(3)\cdot10^{-4}\,s^{-1}$, which probably describes the reactivity of the aquo complex.

Summarizing the results we can state that the complex with ligand **2** seems to be the best of this series. On one side the tendency to bind OH^- in the axial position is somewhat higher than for the other complexes. On the other side the reactivity for the intramolecular attack of the coordinated OH^- onto the nitrile group is at least 100 times higher than for any of the other systems. This indicates that the short chain and the less rigid macrocycle allow the best arrangement of the reactants for the intramolecular reaction.

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References

[1] Kaim, W. and Schwederski, B., In: *Bioanorganische Chemie*, Treuber Verlag, Stuttgart 1991; Lippard, S. J. and Berg, J. M., In: *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, 1994.

[2] Breslow, R., Fairweather, R. and Keana, J. (1967). *J. Amer. Chem. Soc.*, **89**, 2135; Gultneh, Y., Allwar, A., Ahvazi, B., Blaise, D., Butcher, R. J. and Jasinski, J. (1996). *Inorg. Chim. Acta*, **241**, 31; Itoh, T., Hisada, H., Sumiya, T., Hosono, M., Usui, Y. and Fujii, Y. (1997). *J. Chem. Soc. Chem. Commun.*, pp. 677–678; Jenkins, L. A., Bashkin, J. K., Pennock, J. D., Florian, J. and Warshel, A. (1999). *Inorg. Chem.*, **38**, 3215; McCue, K. P., Voss, D. A., Marks, C. and Morrow, J. R. (1998). *J. Chem. Soc., Dalton Transactions*, p. 2961.

[3] Baraniak, E., Buckingham, D. A., Clark, C. R., Moynihan, B. H. and Sargeson, A. M. (1986). *Inorg. Chem*, **25**, 3466; Buckingham, D. A. and Clark, C. R. (1986). *Inorg. Chem.*, **25**, 3478; Buckingham, D. A., Foster, D. M. and Sargeson, A. M. (1969). *J. Amer. Chem. Soc.*, **91**, 3451; Buckingham, D. A., Morris, P., Sargeson, A. M. and Zanella, A. (1977). *Inorg. Chem.*, **16**, 1910; Kim, J. H., Britten, J. and Chin, J. (1993). *J. Am. Chem. Soc.*, **115**, 3618.

[4] Bazzicalupi, C., Bencini, A., Berni, E., Bianchi, A., Fedi, V., Fusi, V., Giorgi, C., Paoletti, P. and Valtancoli, B. (1999). *Inorg. Chem.*, **38**, 4115; Beissel, T., Burger, K. S., Voigt, G., Wieghardt, K., Butzlaff, C. and Trautwein, A. X. (1993). *Inorg. Chem.*, **32**, 124; Burstyn, J. N. and Deal, K. A. (1993). *Inorg. Chem.*, **32**, 3585; Chapman, W. H. and Breslow, R. (1995). *J. Am. Chem. Soc.*, **117**, 5462; Deal, K. A. and Burstyn, J. N. (1996). *Inorg. Chem.*, **35**, 2792; Hay, R. W. and Govan, N. (1998). *Polyhedron*, **17**, 463–468; Hegg, E. L., Deal, K. A., Kiessling, L. L. and Burstyn, J. N. (1997). *Inorg. Chem.*, **36**, 1715; Jurek, P. and Martell, A. E. (1999). *Inorg. Chim. Acta*, **287**, 47; Kimura, E., Hashimoto, H. and Koike, T. (1996). *J. Am. Chem. Soc.*, **118**, 10963; Koike, T., Kajitani, S., Nakamura, I., Kimura, E. and Shiro, M. (1995). *J. Am. Chem. Soc.*, **117**, 1210; Suh, J. H., Son, S. J. and Suh, M. P. (1998). *Inorg. Chem.*, **37**, 4872; Wendelstorf, C., Warzeska, S., Kovari, E. and Kramer, R. (1996). *J. Chem. Soc., Dalton Transactions*, p. 3087.

[5] Tschudin, D., Riesen, A. and Kaden, Th. A. (1989). *Helv. Chim. Acta*, **72**, 131; Kaden, Th. A., Tschudin, D., Studer, M. and Brunner, U. (1989). *Pure Appl. Chem.*, **61**, 879.

[6] Schibler, W. and Kaden, Th. A. (1981). *J. Chem. Soc. Chem. Commun.*, p. 603.

[7] Barefield, E. K., Fogler, K. A., Freeman, G. M. and Hodges, K. B. (1986). *Inorg. Chem.*, **25**, 4663.

[8] Tschudin, D., Basak, A. and Kaden, Th. A. (1988). *Helv. Chim. Acta*, **71**, 100.

[9] Kolinski, R. A. (1995). *Polish J. Chem.*, **69**, 1039.

[10] Altomare, A., Cascarano, G., Giacovazzo, G., Guagliardi, A., Burla, M. C., Polidori, G. and Camalli, M. (1994). *J. Appl. Cryst.*, **27**, 435.

[11] Watkin, D. J., Carruthers, R. J. and Betteridge, P., *CRYSTALS*, Chemical Crystallography Laboratory, Oxford, 1985.

[12] *International Tables for Crystallography*, Kynoch Press, Birmingham, 1974, Vol. IV, Table 2.2 B.

[13] Jung, B., *Thesis*; Universität Basel, 1995.

[14] Hänisch, G., Kaden, Th. A. and Zuberbühler, A. D. (1979). *Talanta*, **26**, 563.

[15] Gampp, H., Maeder, M., Meyer, Ch. J. and Zuberbühler, A. D. (1985). *Talanta*, **32**, 257.

[16] Kowallick, R., Neuburger, M., Zehnder, M. and Kaden, Th. A. (1997). *Helv. Chim. Acta*, **80**, 948.

[17] Fairlie, D. P., Angus, P. M., Fenn, M. D. and Jackson, W. G. (1991). *Inorg. Chem.*, **30**, 1564; Hancock, R. D., Maumela, H. and Desousa, A. S. (1996). *Coord. Chem. Rev.*, **148**, 315; Zuberbühler, A. D. and Kaden, Th. A. (1982). *Helv. Chim. Acta*, **651**, 753; Kaden, Th. A. (1984). *Topics Curr. Chemistry*, **121**, 157.

[18] Vallee, B. L. and Williams, R. J. P. (1968). *Proc. Nat. Acad. Sciences*, **59**, 498.