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Metal Ions Co-operativity in the Catalysis of the Hydrolysis of a β-amino Ester by a Macrocyclic Dinuclear Cu(II) Complex

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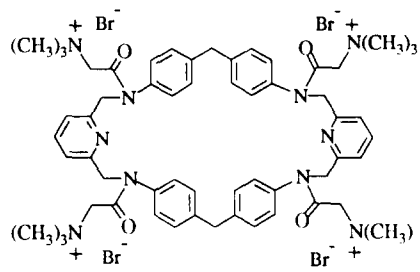
Abstract: The macrocyclic receptor **3**, featuring two diaminomethylpyridine moieties as ligand subunits and two diphenylmethane lipophilic spacers was synthesized and the crystal structure of its dinuclear Cu(II) complex, **3**·2Cu, defined by X-ray analysis. From a kinetic study of the catalytic activity of **3**·2Cu in the hydrolysis of the *p*-nitrophenyl ester of β-alanine (AlaPNP), a β-amino acid, clear evidence of co-operativity of the two metal ions was obtained. Such an allosteric effect was not observed in the hydrolysis of the *p*-nitrophenyl ester of leucine (LeuPNP), an α-amino acid. The reactivity of **3**·2Cu was compared with that of the mononuclear complex of the acyclic ligand **4** having a single diaminomethylpyridine subunit and to that of Cu(II) alone. At pH=6.3 in a 1:1 water/DMSO mixture, being [Cu(II)]=$6x10^{-4}$ M, a 80-fold acceleration was observed employing **3**·2Cu compared with a 35-fold rate increase with Cu(II) alone and a 17-fold increase with **4**·Cu. The crystal structure of the dinuclear Cu(II) complex gives a distance between the two Cu(II) centers of 9.9 Å, suitable for the co-ordination of the β-amino ester by both Cu(II) ions with the nitrogen of the amino group and the oxygen of the C=O. Although significant, the rate accelerations observed employing **3**·2Cu are rather modest and this is likely due to the orientation of the two pyridine moieties in the macrocycle which does not allow the most favorable inclusion mode of the substrate.

INTRODUCTION

Several metal ions play a crucial role in many biological systems such as the metalloproteins or the metalloenzymes[1]. Quite often, as in the superoxide dismutases[2], copper oxidases, alkaline phosphatase[3], just to mention a few, two or more metal ions properly located in the active site of the enzymes, effectively co-operate in the process of recognition and/or transformation of given substrates. Thus, it is not surprising that the idea of putting together two (or more) metal ions in a well defined structure has attracted the attention and stimulated the synthetic imagination and skill of chemists in order to realize supramolecular[4] structures for the selective recognition and, possibly, modification of proper substrates. The goal is not only that of modeling biological processes[5] but also creating completely new systems with particular functions. Early ideas and a few interesting structures were laid out already 15 years ago by Lehn[6]. Since then, many molecules able to interact with more than one metal ion have been synthesized and studied as possible molecular receptors. For instance Drew has reported the X-ray structure of a μ-imidazolato complex involving a binuclear Cu(II) complex of a 24-membered macrocycle[7]; Martell and Lehn have reported on a bis-tren cryptate forming in the presence of two Co(II) ions a relatively stable dioxygen complex[8]. Dinuclear Cu(II) complexes of macrocycles able to interact with different anions has also been reported[9] as well as cofacial dinuclear Cu(II) complexes forming a macrocyclic structure

which binds bidentate Lewis bases[10]. Lehn has also shown how a supramolecule made of properly assembled azacrown ethers and porphyrin may produce a long-lived charge-separated state in a photoinduced electron transfer from the singlet excited zinc porphyrin[11]. Molecular recognition by co-facial ligands (mainly porphyrins) has been reported by Sutherland[12] while rather complicated polynuclear macrocyclic receptors have been synthesized by Vögtle[13]. Catalytic activity in the chemical trasformation of substrates by a dicopper macrocycle containing two 2,6-bis(thiomethyl)pyridine units has been shown to be possible by Burrows[14]. The activation of small molecules by dinuclear complexes of copper and other metals has been recently reviewed[15].

Following our interest in the use of transition-metal ions to activate nucleophilic functions under mild conditions either in micellar agregates[16] or in polytopic receptors,[17] we are currently pursuing the realization of a molecule capable of binding two transition-metal ions and acting as a selective receptor and catalyst. Recently, the catalytic properties in dephosphorylation or transphosphorylation reactions of binuclear (Cu(II) or Co(II)) complexes, which indicate co-operativity between the two metal ions, have independently been reported by Czarnik[18] and Chin[19]. Our early attempts led to the synthesis[17a] of macrocycle **1** which is quite soluble in water at neutral pH, binds hydrophobic substrates but has only a modest ability to bind transition metal ions. More recently, we have shown[17b] that receptor **2**, quite soluble in water at neutral pH as a complex with two Cu(II) ions, is a catalyst of the cleavage of a activated β-amino acid ester. We suggested that the process involved the co-ordination of the nitrogen of the amino ester to one Cu(II) ion and the catalytic action of the second ion acting as a Lewis acid. On these premises we thought that a properly designed macrocyclic molecule could be more selective and, possibly, more active than compound **2** in the hydrolysis of a β-amino ester on the simple argument of the reduction of the degrees of freedom in a closed structure. Accordingly, we designed macrocycle **3**, as a suitable candidate for our investigation. We here report on its synthesis, its complexation of Cu(II) ions and its effect on the cleavage of the *p*-nitrophenyl esters of leucine (LeuPNP), an α-amino acid, and β-alanine (AlaPNP), a β-amino acid. We have also studied, for comparison purposes, ligand **4** which is an open structure, half of macrocycle **3**, and, consequently, with only one binding site for Cu(II) ions.



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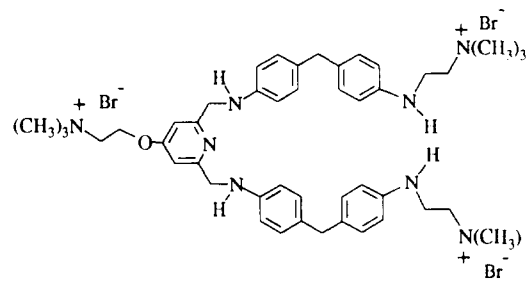

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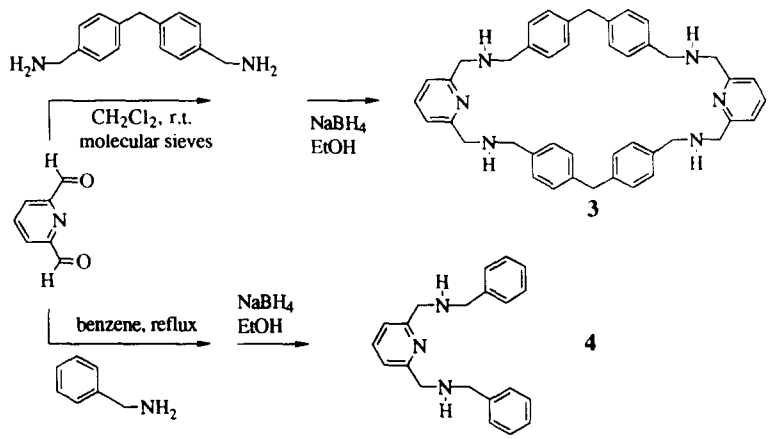
RESULTS AND DISCUSSION

Synthesis of the ligands

Macrocycle **3** was synthesized as outlined in the Scheme. The macrocyclization was achieved in a single step mixing together, in CH_2Cl_2 in the presence of molecular sieves, 2,6-pyridinedicarboxaldehyde and 4,4'-di(aminomethyl)-diphenylmethane. Subsequent reduction of the tetra-imine derivative with $NaBH_4$ gave

compound **3** in 23% overall yield. Model compound **4** was obtained by reacting 2,6-pyridine carboxaldehyde with benzylamine in toluene under Dean-Stark conditions and, subsequently, reducing the di-imine derivative with $NaBH_4$. Details of the syntheses are reported in the Experimental. The two diaminomethylpyridine subunits of the macrocycle define two sites for the co-ordination of a transition metal ion, like Cu(II), while only one of these units is present in the model compound **4**. The diphenylmethane subunits present in the macrocycle are expected to define a lipophilic cavity to exploit possible lipophilic interactions with a guest substrate. Diphenylmethane-based cyclophanes have been described, among others by Diederich[20], while polyaza macrocyclic ligands incorporating pyridine units have been reported by Lehn[21]. But for our macrocycle **1**, these two frameworks have never been assembled together in a single receptor molecule. The rigidity of the resulting molecule should allow location of the metal ions at a defined distance.



Scheme

The two ligands are quite lipophilic and not soluble in neutral water. Solubilization is only possible in moderately acidic aqueous solutions (pH < 3) or in mixtures of neutral water and a proper organic solvent. Among these solvents DMSO proved particularly suitable because of its negligible interaction with Cu(II) ions; furthermore it has been claimed[22] that, in the case of an involvement of "lyophobic" forces in the recognition of a substrate, these should be similar, in DMSO-water mixtures, to those in aqueous solution. Therefore, all experiments have been performed in a 1:1 water/DMSO solution. The pH reported for these solutions is that of the aqueous component before mixing and no correction has been made for the addition of DMSO.

Cu(II) binding and structure of the complexes

When $Cu(NO_3)$ is added to a pH=6.3 solution of ligands **3** and **4** a new absorption band is observed with maxima at *ca.* 260 and *ca.* 290 nm. From the Job plot analysis[23], ligand **4** binds only one Cu(II) ion while ligand **3** binds two Cu(II). The Job plot for this latter ligand is illustrated in Figure 1. We did not determine the strength of the binding constants with Cu(II) and assumed that they should be very close to those reported for 2,6-diaminomethylpyridine[24], $\log K_{Cu}$ = 15.7 (1:1 complex) at least in the case of ligand **4** and in the case of macrocycle **3** for the first Cu(II) ion, assuming a negligible effect of the substituents on the two nitrogens. For the second Cu(II) ion in **3** a decreased value of the affinity constant is conceivable because of the negative electrostatic interaction with the first Cu(II) ion as reported[25] for a macrocycle having the two

aminomethylpyridine units connected by two flexible C_4 hydrocarbon chains. It is however quite important to mention that macrocycle **3** is much less flexible and, hence, the influence of the first Cu(II) on the complexation of the second should be less important. At any rate, the two ligands form quite stable complexes with Cu(II) as indicated also by the sharp maxima in the Job plots.

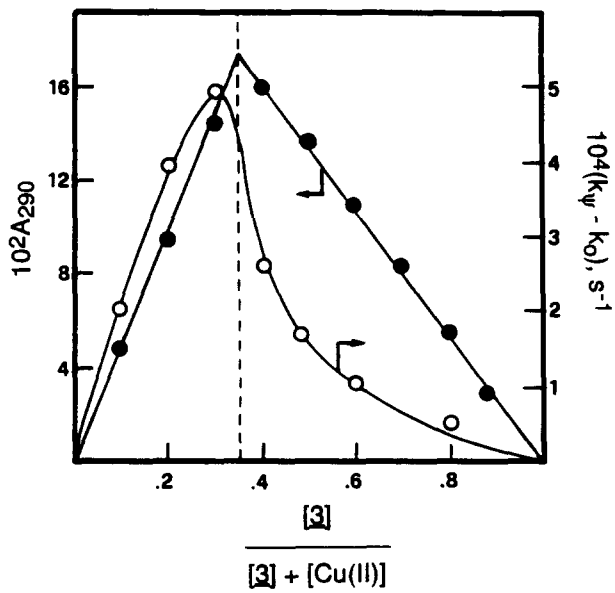


Figure 1: Job plot for the complex formed by **3** and Cu(II) obtained from UV-Vis measuraments (•) and from kinetic data (○). Conditions: [**3**] +[Cu(II)] = 5.0 x 10^{-5} M for the UV-Vis experiment and 5.0 x 10^{-4} M for the kinetic experiment; DMSO/water 1:1; pH 6.3 (MES buffer 0.05 M); 25 °C.

Single crystals of a L_2Cu complex of ligand **4** and of a LCu_2 complex of ligand **3** where grown in CH_3CN solutions (see the Experimental for details) and their structures defined by X-ray analysis. These are reported in Figures 2 and 3, respectively. In the case of **4**·Cu the elongated octahedral co-ordination geometry around Cu(II) shows the four planar positions occupied by the three nitrogens of one ligand and by the pyridine nitrogen of a second ligand molecule. The two weaker apical positions are occupied by the two amine nitrogens of the second ligand. This is a quite usual co-ordination geometry for Cu(II) ions[26] and it is conceivable that, in solution, the forth strong co-ordination position in the plane may be occupied by a water molecule or by other donors, when present. The co-ordination geometry around the two Cu(II) ions in the macrocyclic complex **3**·2Cu is different: it is a square pyramidal arrangement with the three nitrogens of each ligand subunit and a

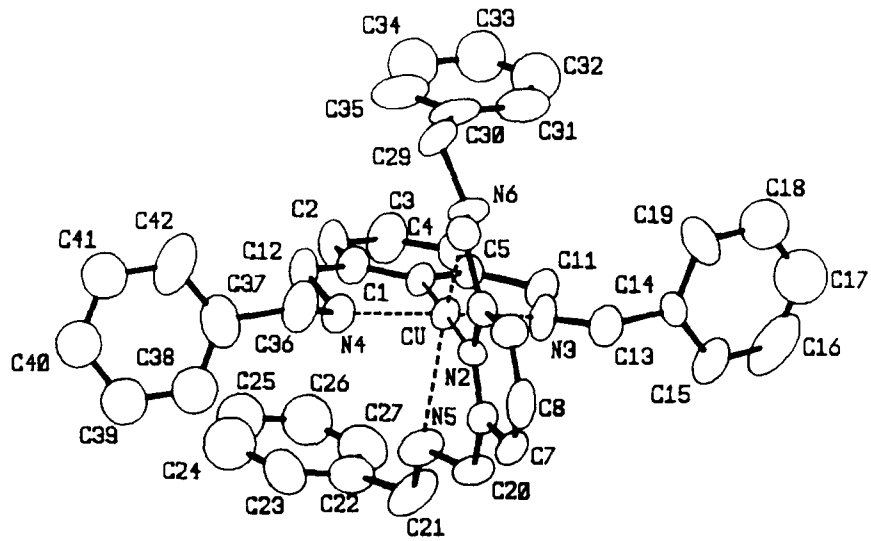


Figure 2: ORTEP view of the strucure of 24·Cu^{2+}·2ClO_4^-. The ClO_4^- counterions and the label of N1 are omitted. Relevant atomic distances in Å: Cu-N1 = 1.91(1), Cu-N2 = 2.01(1), Cu-N3 = 2.10(2), Cu-N4 = 2.08(2), Cu-N5 = 2.58(2), Cu-N6 = 2.38(2).

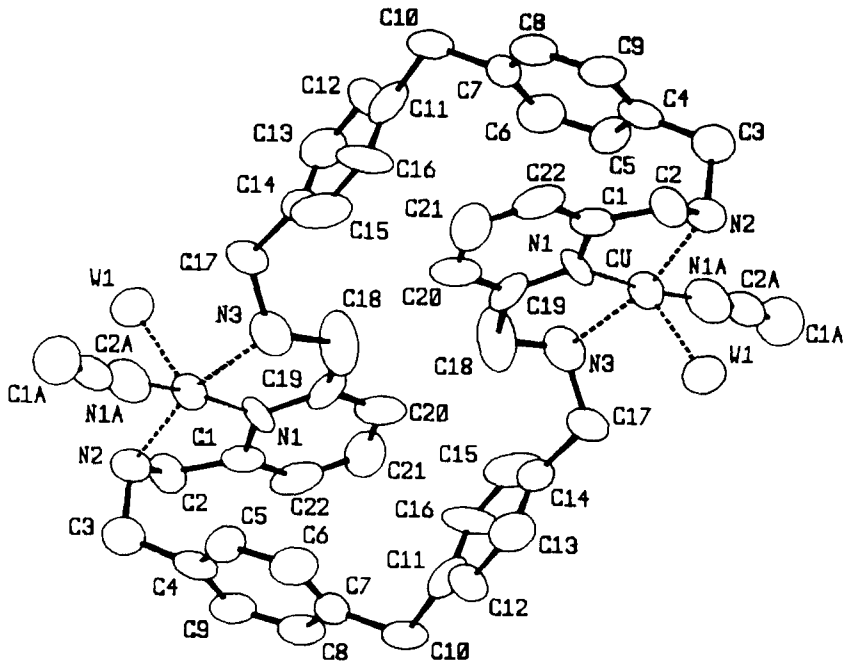


Figure 3: ORTEP view of the strucure of 3·2Cu^{2+}·4ClO_4^-. The ClO_4^- counterions are omitted. Relevant atomic distances in Å: Cu-N1 = 1.90(1), Cu-N2 = 2.06(1), Cu-N3 = 2.06(1), Cu-N1A = 1.94(2), Cu-W1 = 2.35(1).

CH_3CN molecule occupying the fourth strong co-ordination positions in the plane. The weaker apical position of the pyramid is occupied by a water molecule. The structure of this complex reveals that the two pyridines are oriented in such a way that the two CH_3CN molecules point outside the cavity defined by the two diphenylmethane spacers. The two water molecules occupying the weak apical position lie in the correct plane for being inserted into the cavity but do not point inside it. The distance between the two Cu(II) ions in the macrocycle is 9.9 Å: a molecule that would take advantage of the co-ordination to both ions within the cavity (assuming an average co-ordination distance of 2.4 Å) should have donors atoms ca. 5 Å apart. Quite clearly, in solution, the structure of the macrocyclic complex **3**·2Cu can gain a much larger flexibility than in the solid state so that the above distances can be taken as reference values for the selection of proper substrates. More to the point, the crystal structure would indicate that a substrate with two donor atoms cannot bind to the strong planar positions of the two Cu(II) ions in the complex and reside within the cavity unless at least one of the pyridine residues is tilted in such a way that its nitrogen atom points toward the cavity. Inspection of CPK models indicate that such an orientation can be easily achieved although it is probably higher in energy than that defined for the crystal structure, in the absence of interactions with the solvent and other solutes.

Kinetics

On the grounds of the above arguments, we thought that the ester of a β-amino acid could be a proper substrate to be accommodated within the cavity of the complex **3**·2Cu(II) in such a way to take advantage of the interaction with both ions. Force field calculations[27] of the structure of a β-amino acid ester give a distance between the nitrogen and the carbonyl oxygen of 4.6 Å, slightly shorter than the distance evaluated from the crystal structure for a correct fitting to the macrocycle, as said above, but well within the range of variability of the distances found for the co-ordination to Cu(II) ions in complexes whose structure has been reported[26]. Accordingly, we studied the hydrolysis of AlaPNP in the presence of the Cu(II) complexes of ligands **3** and **4** and compared the data with those obtained for the hydrolysis of LeuPNP to evaluate if our expectation of co-operativity in the binuclear Cu(II) complex of macrocycle **3** could be fulfilled.

Figure 4a shows the observed rate constants determined for the hydrolysis of AlaPNP with **3**·2Cu and **4**·Cu compared with those obtained in the presence of Cu(II) only. All systems accelerate the rate of hydrolysis of this substrate. However, while the mononuclear complex of ligand **4** is less efficient than Cu(II) alone, the binuclear complex of the macrocycle is more efficient than the metal ion. Furthermore, from experiments performed at a fixed ligand concentration ([L] = $2x10^{-4}$ M) and changing [Cu(II)], we obtain for the two ligands the rate data reported in Figure 4b. In the case of **4** the rate constants are very low up to [Cu(II)] $2x10^{-4}$ M (*i.e.* the complete formation of the 1:1 complex) and at higher [Cu(II)] linearly increase with a slope very close to that observed in the presence of Cu(II) only (dotted line). In the case of ligand **3** the rate is also very slow up to [Cu(II)] sligthly higher than $2x10^{-4}$ M (*i.e.* the complex with one Cu(II) ion) but, when the second copper ion starts to fill the second co-ordination site of the macrocycle, the rate constants become much faster. When [Cu(II)] = 2x[**3**], the dependence of the rate constants on [Cu(II)] becomes less pronounced eventually increasing with the slope observed in the presence of free Cu(II) (*i.e.* when all the binuclear complex is formed). This behavior of the macrocyclic ligand is clearly indicative of co-operativity between the two metal centers in the acceleration of the hydrolytic process. Similar kinetic profiles are also shown in allosteric systems[28]. The higher efficiency of the macrocyclic LCu_2 complex is also highlighted by the kinetic version of the Job plot reported in Figure 1 since the maximum of this curve is very close to a molar fraction of 0.33, that

is expected for such a stoichiometry of complexation. Under the conditions of the experiments shown in Figure 4a, the very large binding constants of these ligands for Cu(II) allows us to assume that there is no free Cu(II) when the ligands are present. Accordingly, the ratio between the slopes of the two stright lines obtained with the complexes of ligand **4** and **3** give a direct evaluation of the relative efficiency of the catalysts: this amounts to a factor of *ca.* 5 in favor of the macrocycle. This value takes into account the statistical factor since it has been obtained under conditions [**4**·Cu] = 2 [**3**·2Cu].

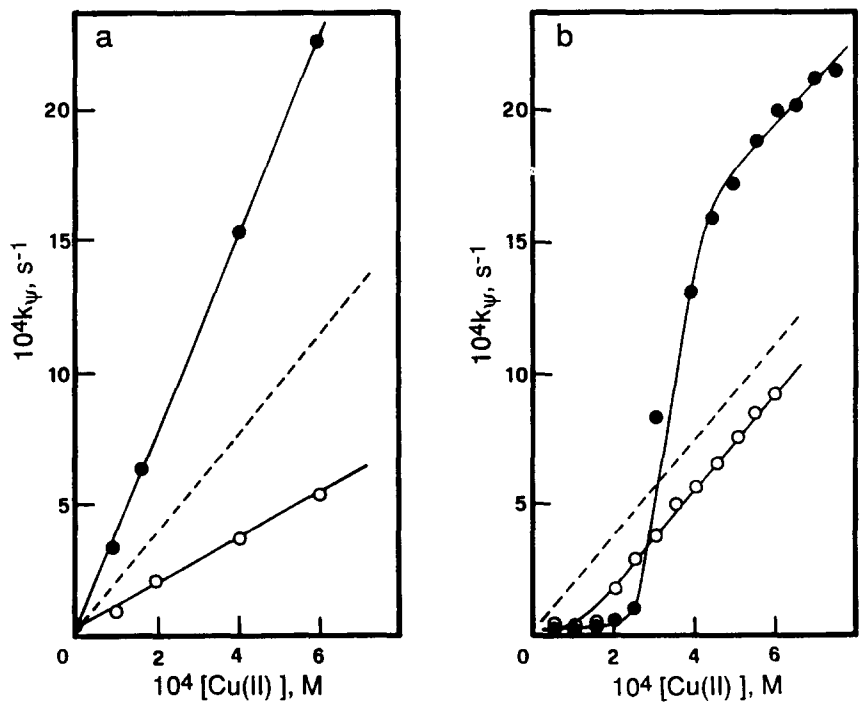


Figure 4: a) k_ψ *vs* concentration profiles for the hydrolysis of AlaPNP in the presence of **3**·2Cu(•), Cu(II) (dashed line), and **4**·Cu (○) in DMSO/water 1:1, pH 6.3 (MES buffer 0.05 M); 25 °C. b) k_ψ *vs* Cu(II) concentration profiles for the hydrolysis of AlaPNP in the presence of **3** (•) and **4** (○) at a fixed concentration of 2.0 x 10^{-4} M in DMSO/water 1:1, pH 6.3 (MES buffer 0.05 M); 25 °C. The dashed line is the rate of hydrolysis in the absence of ligand.

The kinetic behavior of the α-amino ester LeuPNP is quite different. First, Figure 5a, both complexes are less efficient than Cu(II) in the acceleration of the hydrolytic process; second, the kinetic profile obtained by increasing [Cu(II)] while keeping constant the concentration of ligand is very similar for the two ligands. In both cases the rate constants are very low up to the Cu(II) concentration of the binding sites present in the ligand and then increase with the slope of the Cu(II)-catalyzed process. In no case is the hydrolytic process in the presence of the two ligands faster than that in the presence of Cu(II) alone.

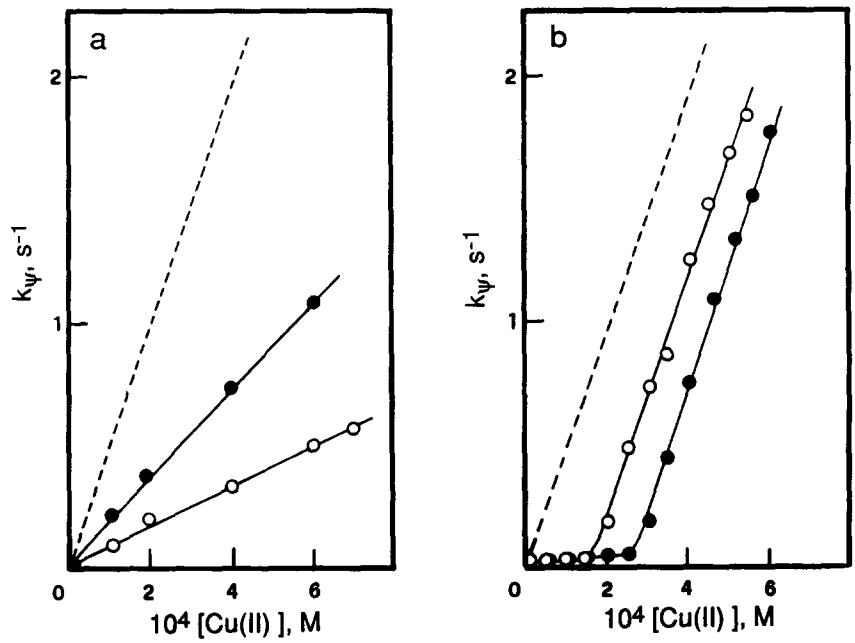


Figure 5: a) k_ψ *vs* concentration profiles for the hydrolysis of LeuPNP in the presence of **3**·2Cu(•), Cu(II) (dashed line), and **4**·Cu (○) in DMSO/water 1:1, pH 6.3 (MES buffer 0.05 M); 25 °C. b) k_ψ *vs* Cu(II) concentration profiles for the hydrolysis of LeuPNP in the presence of **3** (•) and **4** (○) at a fixed concentration of 2.0 x 10^{-4} M in DMSO/water 1:1, pH 6.3 (MES buffer 0.05 M); 25 °C. The dashed line is the rate of hydrolysis in the absence of ligand.

The above kinetic behavior of the complex of the macrocyclic ligand **3** with two Cu(II) ions can be explained assuming the formation, in the case of the β-amino ester, of a supramolecular complex in which the amino group of the substrate is bound to one Cu(II) ion while the carbonyl of the ester may approach the other one. This "second" Cu(II) ion would act as a catalyst of the hydrolytic process acting as a Lewis acid and activating the carbonyl toward the nucleophilic attack of an external or of a metal-bound water molecule. These are the most accepted mechanisms for metal ion catalyzed ester hydrolysis[29]. The fact that the curves of Figure 4a are straight lines indicates that the formation constant of the complexes of the substrate with the metal ion are modest: in fact, if the affinity constants were high, a curved profile with tendency toward saturation would have been observed. This is also confirmed by experiments carried out using excess substrate over the LCu_2 complex. These experiments show a single monoexponential increase of the absorbance during the kinetics with no appreciable inhibition due to product formation. A tight catalyst-substrate complex would result in an even tighter catalyst-product complex with a significant inhibition of the hydrolysis rate. Furthermore this kinetic behaviour is clear evidence that our system is a real catalyst.

The failure of the LCu_2 complex of macrocycle **3** to show a similar co-operative behavior of the two metal ions in the hydrolysis of the α-amino ester may be attributed, among others, to: a) the shorter distance, 3.5 Å, as evaluated from force field calculations, between the amine nitrogen and the carbonyl; b) the known tendency of these compounds to form chelates in the co-ordination to transition metal ions[30]. The formation of chelates with transition metal ions is well documented only in the case of α-amino acids; no evidence has been reported for β-amino derivatives[31]. Indirect evidence of the involvement of chelates in the case of α-amino acid derivatives comes from the higher acceleration in the hydrolysis of LeuPNP (*ca.*250-fold over the reaction without Cu(II) at [Cu(II)] = $6x10^{-4}$ M) as compared with that of AlaPNP (*ca.* 35-fold).

On analyzing Figures 4a and 5a it is also evident that, with the exception of the dicopper complex of macrocycle **3** and AlaPNP, the two complexes are less effective than free Cu(II) as catalysts of the hydrolysis. This may be due: a) to a less electrophilic character of the metal ion when bound to the complex; b) to a less productive substrate-metal ion arrangement within such a complexes; c) to the competitive formation of complexes involving two ligands for single Cu(II) ion (like that of the crystal structure of Figure 2) in which the first co-ordination sphere of the metal ion is completely saturated and the substrate is unable to reach the metal center. The latter argument may be at the source of the lower reactivity of the mononuclear complex **4**·Cu than the binuclear complex **3**·2Cu in the cleavage of LeuPNP.

CONCLUSION

To the best of our knowledge the kinetic effects of the complex **3**·2Cu in the hydrolysis of a β-amino acid ester provide the first clear example of co-operativity between the metal centers of a dinuclear complex receptor in the catalysis of such a reaction. Though the hydrolysis reaction in the presence of the dicopper complex of the macrocycle is ca. 80 times faster (at [**3**·2Cu]=$3x10^{-4}$ M) than that in the absence of metal ions, the acceleration is only slightly less than 5 when the comparison is made with the mononuclear **4**·Cu complex (at [**4**·Cu]=$6x10^{-4}$ M). The modest acceleration observed can be ascribed to the fact that the structure of **3**·2Cu in the solid state is the most stable one also in solution. Thus, for a proper substrate, such as the β-amino acid ester, in order to take advantage of the interaction with both transition metal ions two thermodynamically unfavorable options are available: a) to occupy weak apical positions in the co-ordination to the Cu(II) ions allowing the pyridines to be oriented perpendicularly with respect to the cavity (as in the crystal structure of Figure 3); b) to interact with the metal ions *via* the strong co-ordination positions forcing the pyridines to be oriented with their nitrogens pointing toward the interior of the cavity. Quite likely, less productive modes of binding are thermodynamically more favorable and, as a consequence, the overall rate effects are relatively small. On these bases, we may anticipate that a binuclear complex with a geometry such as to allow the co-ordination of the substrate to the strong co-ordination positions of both metal ions would be much more efficient than the one we have described here. On the other hand, if we compare the results obtained with the macrocycle **3** and the acyclic molecule **2** with the analogous mononucleating ligands, the above mentioned 5-fold acceleration in the first case, becomes a 3-fold acceleration in the latter. Though these data are far from impressive, they clearly indicate that the macrocyclic system is more efficient, as expected from the reduction of the degrees of freedom of the system.

Work is in progress in our laboratory aimed to the realization of a dinucleating macrocycle analogous with **3** but devoid of the flaws that we think impair the catalytic efficiency of the system descibed in this paper.

EXPERIMENTAL

General Methods and Materials. Melting points are uncorrected. NMR spectra were recorded on a Bruker AC 250 F spectrometer operating at 250 MHz for ^{1}H-NMR spectra and at 62.9 MHz for ^{13}C-NMR spectra. Chemical shifts in ppm are reported relative to internal Me_4Si. UV-Vis spectra were recorded on a Perkin Elmer Lambda 5 spectrophotometer. Microanalyses were performed by the Laboratorio di Microanalisi of our department. $Cu(NO_3)_2$ was an analytical grade product. Metal ion stock solutions were titrated against EDTA following standard procedures[32]. The *p*-NO_2-phenylesters of L-leucine and β-alanine, used as substrates, were prepared using literature methods[33].

2,6-Bis((N-benzyl)aminomethyl)pyridine 4. Benzylamine (0.79 g, 7.4 mmol, Aldrich) was added to a solution of 2,6-pyridinedicarboxaldehyde (0.50 g, 3.7 mmol, Aldrich) in 100 mL of toluene. The solution was heated at reflux for 5 h distilling azeotropically the water formed during the reaction. The solvent was then evaporated leaving the imine derivative in quantitative yield. This was dissolved in 100 mL of EtOH containing $NaBH_4$ (0.28 g, 7.4 mmol). After stirring at room temperature overnight the solvent was evaporated, the residue was treated with water and extracted with $CHCl_3$ (3 x 50 mL). The evaporation of the dried (Na_2SO_4) organic solvent afforded the crude amine that was purified by crystallization and obtained as the hydrochloride from acetone and HCl. The salt was filtered, washed with Et_2O, dissolved in $CHCl_3$ and treated with a saturated solution of $NaHCO_3$ (3 x 20 mL). The evaporation of the dried (Na_2SO_4) organic solvent afforded 0.93 g (79 %) of pure **4** as a white solid: m.p. 45-46 °C. ^{1}H-NMR ($CDCl_3$) δ 2.92 (bs, 2H, NH), 3.92 (s, 4H, $PhCH_2$), 3.94 (s, 4H, $PyCH_2$), 7.17 (d, J = 7.63 Hz, 2H, $H_{3,5}$ Py), 7.32 (m, 10H, Ph), 7.60 (t, J = 7.63 Hz, 1H, H_4Py); ^{13}C-NMR (DMSO-d_6) δ 52.7 (CH_2Ph), 54.1 (CH_2Py), 119.9 (C_3Py), 126.8 (C_4Ph), 128.2 and 128.3 ($C_{2,3}$Ph), 137.0 (C_4Py), 140.9 (C_1Ph), 159.7 (C_2Py). Anal. Calcd. for $C_{21}H_{23}N_3$: C, 79.46; H, 7.30; N, 13.24. Found: C, 79.32; H, 7.30; N, 13.16.

8,16,28,36,49,50-exazaeptacyclo[36.2.2.2^{3,6}.2^{18,21}.2^{23,26}.1^{10,14}.1^{30,34}] pentaconta-3,5,10,12,18,20, 23,25,30,32,38,40,41,43,45,47,49,50-octadecaene 3. 4,4'-Di-(aminomethyl)diphenylmetane[34] (0.5 g, 2.2 mmol) was dissolved in 150 mL of dry CH_2Cl_2 containing 5g of activated molecular sieves. To this solution 2,6-pyridinedicarboxaldehyde (0.3 g, 2.2 mmol) dissolved in 150 mL of dry CH_2Cl_2 was slowly added drop by drop. After the addition was completed (ca. 4 h) the reaction mixture was stirred at room temperature for 15 h. The molecular sieves were filtered off on a short celite pad and the organic solvent was evaporated leaving a crude imine derivative that was dissolved in 50 mL of dry CH_2Cl_2. To this solution a suspension of $NaBH_4$ (0.5 g, 13 mmol) in 50 mL of EtOH was added. After stirring at room temperature overnight the reaction mixture was diluted with 100 mL of water and the two phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organic phases were dried (Na_2SO_4) and evaporated to give a crude that was purified first by chromatography on silica gel column ($CHCl_3$/CH_3OH/NH_4OH 89:10:1) and then on Sephadex LH 20 column (CH_2Cl_2) to yield 0.34 g (23%) of pure **3**: m.p. 102-104 °C. ^{1}H-NMR ($CDCl_3$) δ 2.03 (bs, 4H, NH), 3.75 (s, 8H, $PhCH_2N$), 3.91 (s, 12H, $PyCH_2$ and $PhCH_2Ph$), 7.06 (d, J = 8.1 Hz, 8H, $H_{2,6}$Ph), 7.12 (d, J = 7.63 Hz, 4H, $H_{3,5}$ Py), 7.20 (d, J = 8.1 Hz, 8H, H3,5Ph), 7.57 (t, J = 7.63 Hz, 2H, H_4Py); ^{13}C-NMR ($CDCl_3$) δ 41.2 ($PhCH_2Ph$), 53.1 (NCH_2Ph), 54.2 (CH_2Py), 120.7 (C_3Py), 128.3 and 128.9 ($C_{2,3}$Ph), 136.7 (C_4Ph), 137.8 (C_4Py), 139.7 (C_1Ph), 158.9 (C_2Py). FAB-MS (NBA) m/z 658 (M^+). Anal. Calcd. for $C_{44}H_{46}N_6 \cdot 3H_2O$: C, 74.13; H, 7.35; N, 11.79. Found: C, 74.62; H, 7.25; N, 11.64.

Preparation of the complexes for X-ray analysis. Suitable crystals for X-ray analysis were obtained for the complex **3**·2Cu^{2+}·4ClO_4^- by slow evaporation of a CH_3CN solution of **3** and $Cu(ClO_4)_2$ in 1:2 molar ratio. The triclinic crystal system was obtained with an automatic Philips PW 1100 diffractometer with standard

software. Cell parameters: a = 13.9200 (0.0020) Å, b = 19.7950 (0.0020) Å, c = 10.6840 (0.0020) Å, α = 97.00° (0.20), β = 105.90° (0.20), γ = 86.40° (0.20); space group $P1^-$, Z = 4. The structure was solved with the Patterson method and refined to R = 0.103 by using 2840 observed reflections with F>4σF. The crystals for the complex **24**·Cu^{2+}·$2ClO_4^-$ were grown by slow deposition of a $Cu(ClO_4)_2$ solution in CH_3CN over a equimolar solution of **4** in CH_3CN. Suitable crystals for X-ray analysis growed at the pseudointerphase of the solutions. The monoclinic crystal system was obtained as above. Cell parameters: a = 11.4220 (0.0020) Å, b = 11.5840 (0.0020) Å, c = 16.9580 (0.0030) Å, β = 92.60° (0.20), space group $P2_1$, Z = 2. The structure was solved with the Patterson method and refined to R = 0.094 by using 1983 observed reflections with F>6σF. The high values of R obtained for both structures are due to disorder of the ClO_4^- ions.

Kinetic Studies. Slower reactions were followed on a Perkin Elmer Lambda 5 spectrophotometer equipped with a thermostatted cell holder and faster reactions on an Applied Photophysics SF.17MV stopped flow spectrometer. Solution of the ligands were prepared in DMSO while metal ions and buffers stock solutions were prepared in water. The reactions were run in a mixture 1:1 DMSO/water at a 0.05 M total buffer concentration The pH given is that of the water phase before mixing. Reaction temperature was maintained at 25 ± 0.1 °C. Slower reactions were started by addition of 20 μL of a 1-2 x 10^{-3} M solution of substrate in CH_3CN to 2 mL of solution of ligand, additives and buffer in DMSO/water and faster reactions were started by mixing equal volumes of a 2-4 10^{-5} M solution of substrate with the solution of ligands, additives and buffer both prepared in DMSO/water. The final concentration of substrate was 1-2 x 10^{-5} M and the kinetics follow in each case a first order law up to 90% of reaction. The rate constants were obtained by non linear regression analysis of the absorbance *vs* time data (using the software package Enzfitter: Leatherbarrow, R. J. ***Enzfitter***; Elsevier: Amsterdam, 1987 or the software package SF.17MV provided with the stopped-flow work station) and the fit error on the rate constant was always less than 1%.

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