Polymerization- and Solvent-Triggered Cooperativity Between Copper(II) Ions in the Catalysis of the Hydrolysis of Amino Esters by Pyridine-Based Ligands

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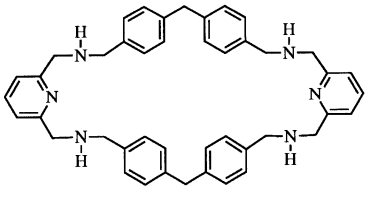
Polymeric (**2**) and oligomeric (**4**, **5**) materials made of repeating units of 2,6-diaminomethylpyridine and 4,4′-diphenylmethane have been synthesized with the number of monomeric units (*n*) ranging from 2 to 29. In 1:1 DMSO/water solutions, these materials are fully soluble and strongly bind Cu^{II} ions. The complexes catalyze to different extents the hydrolysis of the *p*-nitrophenyl esters of α-, β-, and γ-amino acids. Only Cu^{II} complexes of polymeric **2** ($n \geq 10$) are more effective catalysts than free Cu^{II} ions in the cleavage of β-amino esters. Such enhanced reactivity, which in the case of β-alanine *p*-nitrophenyl ester (β-AlaPNP) amounts to almost two orders of magnitude when the comparison is made with the Cu^{II} complex of monomeric ligand (*N,N′*-benzyl)-2,6-aminomethylpyridine (**3**), is observed in 1:1 (v/v) DMSO/H_2O only when a certain degree of polymerization is reached ($6 < n < 10$). In 1:1 (v/v) CH_3CH_2OH/H_2O the kinetic benefits of the complexes of polymer **2** ($n = 10$) diminishes and vanishes in 9:1 (v/v) CH_3CH_2OH/H_2O. Analysis of rate data suggests that two neighboring Cu^{II} ions bound to the polymeric ligands cooperate for the occurrence of the hydrolytic process: one of them coordinates the amino group of the substrate so that the carbonyl of the carboxylate faces the second metal ion which delivers a bound hydroxyl acting as the nucleophilic species. The selectivity toward β-amino ester is likely associated with a rather rigid conformation of these metallopolymers which places two metal centers at the appropriate distance one from the other. It is suggested that the onset of the metal ion cooperativity is connected to a conformational change of the metallopolymer from an extended to a globular structure, likely triggered by hydrophobic forces.

Introduction

Many hydrolytic enzymes (phosphatases are pertinent examples) rely, for activity, on the key role of two or more transition metal ions (typically Zn^{II}) in their active site.[1] Furthermore, metal ions in proteins may be not only involved in catalytic sites but also in the organization of tertiary structures and in the recognition of substrates.[2] A few years ago, with the aim to mimick key features of metalloenzymes, we synthesized macrocycle **1** able to bind two Cu^{II} ions with its pyridine subunits and reported[3] clear evidence of cooperativity between the two metal centers in



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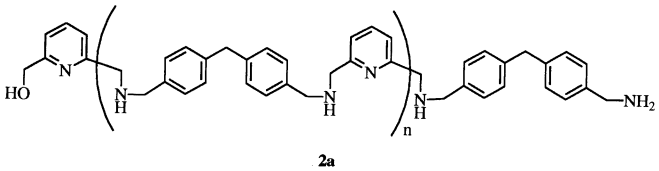

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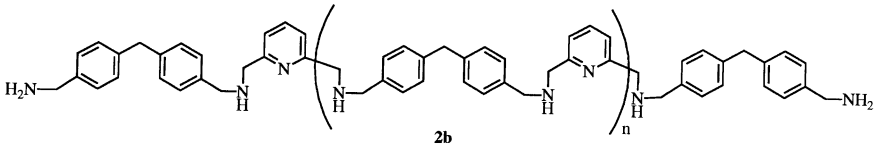

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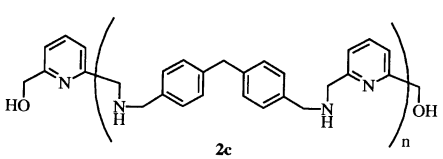

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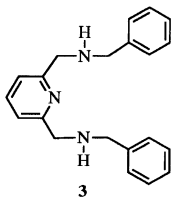

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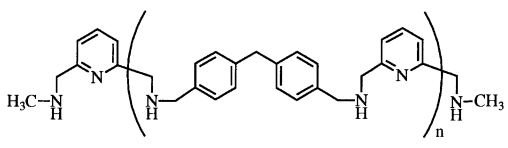

4: n = 2
5: n = 5

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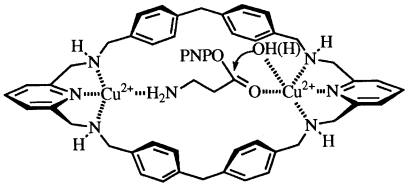
the hydrolytic cleavage of a β-amino ester. The suggested mechanism is depicted in Figure 1.

Figure 1. Suggested mechanism for the cleavage of β-AlaPNP by the dinuclear complex **1**·2 Cu^{II}



Macrocycle **1** was synthesized in a one-pot process by mixing, in equimolar amounts, 2,6-pyridinedicarboxyaldehyde and 4,4′-(diaminomethyl)diphenylmethane and reducing the imine derivative with $NaBH_4$. A side product in the synthesis of **1** was a polymeric material (**2a−c**) which, depending on the solvent chosen to carry out the reaction, may become the only isolated product. This polymer shows ^{1}H-NMR signals very similar to those of **1** but for the presence of a small singlet at δ = 4.70 attributable to the terminal hydroxymethylpyridine unit (structures **2a** and **2c**) and considerable line broadening (due to the relatively high molecular weight). To our surprise, complexes of this material with Cu^{II}, when saturation of all binding units is achieved, showed even higher activity than the dicopper complex of macrocycle **1** in the hydrolysis of the *p*-nitrophenyl esters of β-alanine and other β-amino acids. This activity is totally absent in the case of the 1:1 complex with Cu^{II} of the monotopic ligand **3** and is observed only when a critical degree of polymerization is reached in the ligand depending also on the solvent employed.

This paper reports in full our efforts to ascertain the critical degree of polymerization which causes the onset of the activity of the polymeric complexes with Cu^{II} and to clarify the source of activity of the system. For this purpose we have synthesized oligomeric compounds **4** and **5** and polymeric materials of different molecular weight and tested their Cu^{II} complexes as catalysts of the cleavage of the *p*-nitrophenyl ester of β-alanine (β-AlaPNP) and other α-, β-, and γ-amino esters as well.

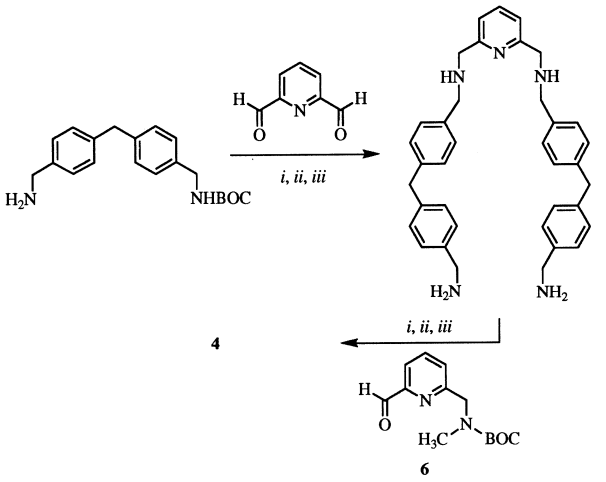
Results and Discussion

Syntheses

Ligands:

a) *Polymeric Compounds:* Polymeric compounds **2** of different molecular weight were obtained by reaction of 2,6-pyridine dicarboxyaldehyde with equimolar amounts of 4,4′-di(aminomethyl)diphenylmethane in different solvents followed by the reduction of the polymeric imine derivative with $NaBH_4$. Assuming[4] that the major product of the polymerization has structure **2a**, while **2b** and **2c** constitute only a minor component of the polymeric mixture, the degree of polymerization can be obtained from the ratio of the integrals of the signals of methylene CH_2OH (δ = 4.70) bound to the terminal pyridine, and the most downfield protons of pyridine in the ^{1}H-NMR spectrum. This analysis gives an average molecular weight of 3,600 Daltons when the reaction is carried out in acetonitrile (*n* = 10) and a molecular weight of 6,000 Daltons (*n* = 17) or 10,000 Daltons (*n* = 29) when the reaction is carried out in a 1:1 acetonitrile/toluene or a 1:1 toluene/*n*-hexane mixture, respectively. Crude materials have been purified by elution through a silica gel column and, in the case of **2** (*n* = 17), also through a Sephadex LH 20 column. All attempts to get molecular weights of the polymers by MS (FAB, MALDI, and ESI) were unsuccessful.

Scheme 1. Synthetic route to oligomer **4**. Reagents: i) Benzene, reflux; ii) $NaBH_4$, $EtOH/CH_2Cl_2$; iii) CF_3COOH/CH_2Cl_2; Na_2CO_3 10%



Scheme 2. Synthetic route to oligomer **5**. Reagents: i) CH_2Cl_2, molecular sieves; ii) $NaBH_4$, $EtOH/CH_2Cl_2$; iii) CF_3COOH/CH_2Cl_2; iv) Benzene, reflux

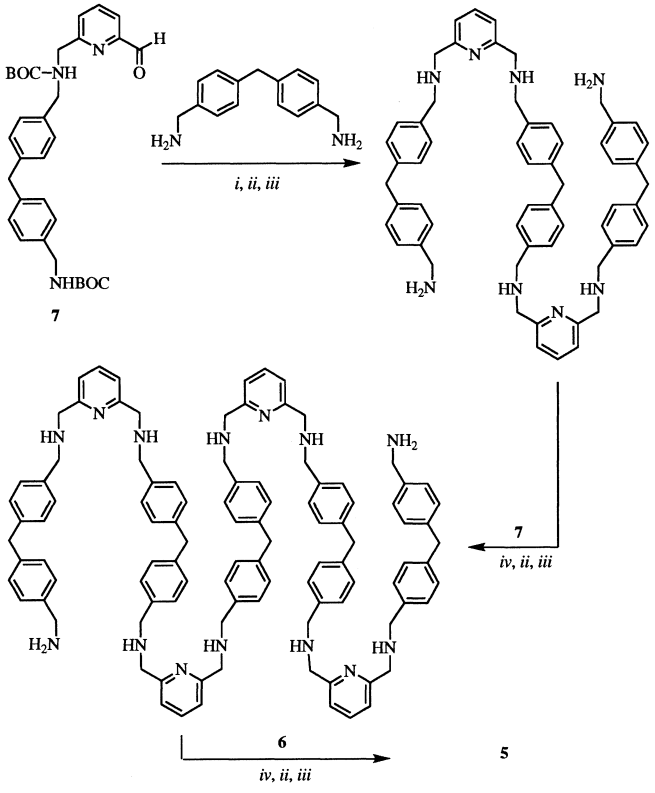
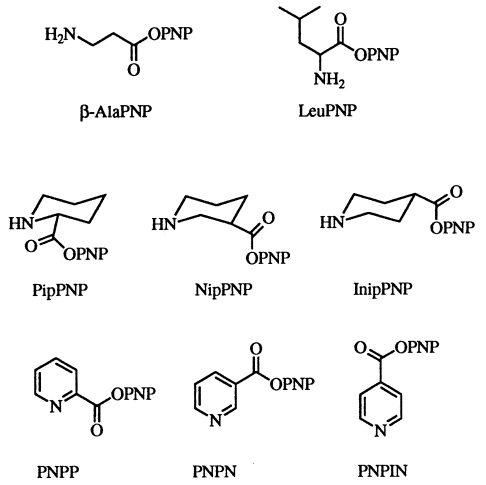


Chart 1



b) *Oligomeric Compounds:* Oligomeric compounds **4** and **5** were obtained following the strategies depicted in Schemes 1 and 2, respectively. In the case of **4**, Boc-monoprotected 4,4′-di(aminomethyl)diphenylmethane was treated with 2,6-pyridinedialdehyde. After reduction and deprotection, the resulting derivative was treated with 2-formyl-6-(*N*-methyl-*N*-butoxycarbonyl)aminomethylpyridine, **6**, and eventually converted to the final product. In the case of **5**, the synthetic sequence comprises the use in two subsequent steps of pyridine monoaldehyde **7** functionalized with a 4,4′-di(aminomethyl)diphenylmethane unit. The final product was obtained, as for **4**, after reaction with **6**. Details of the synthetic procedure and characterization of the products are reported in the Experimental Section.

Substrates. New amino acid *p*-nitrophenyl esters of racemic pipecolic acid (PipPNP), nipecotic acid (NipPNP), and isonipecotic acid (InipPNP) were obtained following conventional procedures for the preparation of amino acid esters.[5] Structures and abbreviation for all substrates used are reported in Chart 1.

Solubilization of Ligands and Complexation of Cu^{II} Ions

Polymeric as well as oligomeric compounds are not soluble in neutral water and are only little soluble at pH < 3. However, they are soluble in a 1:1 (v/v) mixture DMSO/water. For this reason this solvent was used throughout this investigation. It should be pointed out that, if solvophobic forces are involved in any of the processes studied, these should be similar,[6] in DMSO/water mixtures, to those in aqueous solution. This point is important for the arguments we will discuss below. Whenever buffered solutions were used, the pH refers to that of the aqueous component prior to the mixing and no correction has been made for the change of solvent composition.

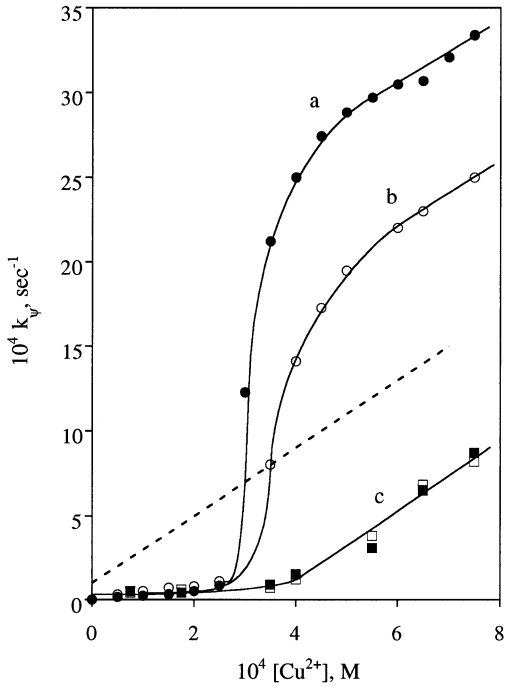
Upon addition of $Cu(NO_3)_2$ to solutions in the above solvent of the ligands at pH>4, a new absorption band is observed in the 260−290 nm region due to the formation of the copper complexes. In this regard the present ligands do not differ from macrocycle **1** or monomer **3**. Although we have not determined the affinity constants for Cu^{II} of these ligands, they may be assumed to be quite large if one takes into account that the reported[7] $\log K_{Cu}$ for the 1:1 complex of this metal ion with 2,6-diaminomethylpyridine is 15.7. The single pyridine units in the oligomers and polymers are separated by the relatively rigid spacer diphenylmethane and, hence, there should not be a strong interaction between the binding sites. However, it is expected that as the ligands are progressively loaded with Cu^{II} ions the affinity of the pyridine sites for the metal ion decreases and hence the average affinity constant is likely slightly lower than that of monomeric **3**. We have recently reported[8] that in the limiting case of amphiphilic ligands which form micellar or vesicular aggregates in aqueous solution, thus forcing the metal centers in close proximity one to the other on the aggregate/water interface, there may be a decrease of up to two order of magnitude of the binding constant when the aggregates are cationic. At any rate large binding constants are expected. Job plots with the two oligomers **4** and **5** reveal the formation of 1:1 complexes for each pyridine unit. The same stoichiometry of complexation characterizes the polymeric ligands. Furthermore, the very sharp maximum of the curves support a very strong affinity constant for these polymers, in accord with the arguments discussed above.

Kinetics

When solutions of polymer **2** ($n = 10$) are progressively loaded with Cu^{II} ions in the presence of substrate β-AlaPNP, a β-amino ester, the observed rate constant, $k\psi$, for its hydrolysis shows a sigmoidal dependence on the Cu^{II} concentration, as shown in Figure 2 (trace a). Almost identical behavior is observed for higher molecular weight polymers (curves not shown) as well as for macrocycle **1** (trace b). In the presence of oligomers **4** and **5** the rate constant (trace c) remains well below that observed in the presence of Cu^{II} alone (dashed line). If we consider that the concentration of the different ligands expressed in terms of binding units (i.e. pyridine moieties) was $4.0 \cdot 10^{-4}$ M in these experiments, the behavior of the polymeric material indicates that this is much less active than Cu^{II} alone when not all binding sites are filled with Cu^{II} ions. This occurs up to $[Cu^{II}] = 2 \cdot 10^{-4}$ M when only half of the pyridine subunits are complexed with Cu^{II} ions. Statistical arguments and electrostatic factors make quite reasonable the assumption that each Cu^{II}-bound pyridine is close to unbound neighbors under these conditions. This situation is equivalent to that found with the 1:1 complex of macrocycle **1** where only one binding subunit is complexed to the metal ion. As further metal ion is added and any complexed pyridine starts to have as the next neighbor another complexed pyridine, the activity of the system becomes higher reaching its maximum at the concentration of Cu^{II} ($4.0 \cdot 10^{-4}$ M) at which all binding sites of the polymer are saturated with metal ions. Above this concentration the rate constant still increases but with the slope similar to that observed for solutions containing Cu^{II} ions alone: it is in fact parallel to the

dashed line and indicates the presence of free Cu^{II} as all the metal-binding subunits are filled with metal ions. Thus the behavior of the metallopolymer suggests cooperativity[9] between neighboring, polymer-bound Cu^{II} ions as in the case of the dinuclear complex of macrocycle **1**. The failure of oligomers **4**·3 Cu^{II} and **5**·6 Cu^{II} to show such an enhanced reactivity indicates that with these systems this cooperativity between Cu^{II} centers is not attained. Their reactivity never becomes larger than that of Cu^{II} alone and the behavior is very similar to that of mononuclear **3**·Cu^{II} (curve not shown).

Figure 2. Dependence of the observed rate constant, $k\psi$, for the cleavage of β-AlaPNP from the concentration of Cu^{II} in the presence of different ligands in 1:1 DMSO/water at pH 6.3. [Ligand's pyridine subunits] = $4\cdot10^{-4}$M

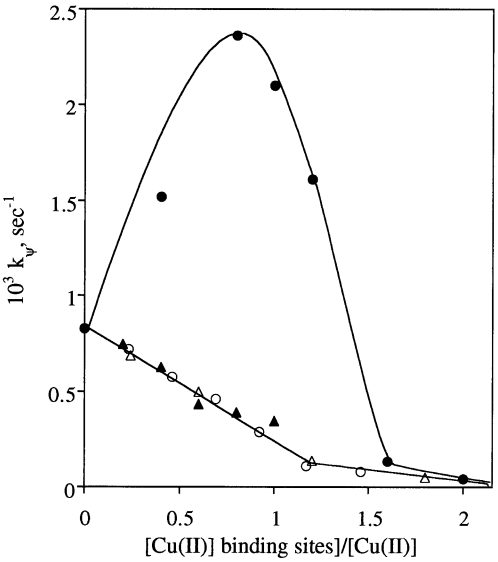


Curve a: **2** (n =10); curve b: **1**; curve c: ■ = **4**, □ = **5**; the dashed line shows the effect of Cu^{II} alone

The same evidence of cooperativity is obtained when kinetics are carried out by keeping constant the concentration of metal ions and adding increasing amounts of ligand (Figure 3). In this particular case, the addition of polymers **2** as well as macrocycle **1** (although to a lower extent) causes the increase of the rate constant with respect to the Cu^{II}-catalyzed process up to a 1:1 ratio between copper ions and pyridine binding units. As the concentration of binding sites becomes larger than the concentration of metal ions available in solution, the rate acceleration decreases and eventually the rate becomes slower than that in the presence of aquo Cu^{II}. With oligomers **4** and **5**, and monomer **3** as well, the rate constants are always lower than those measured with Cu^{II} alone.

All the above experimental results strongly suggest that only in polymeric complexes and not in those of oligomers **4** and **5**, Cu^{II} sites act cooperatively in the cleavage of β-AlaPNP in the same way as they do with macrocycle **1** (Figure 1). Thus, polymers are better catalysts than the aquo ion whereas oligomers are worse.

Figure 3. Dependence of the observed rate constant, $k\psi$, for the cleavage of β-AlaPNP from the binding sites (i.e. the number of pyridine subunits) over Cu^{II} concentrations ratio in 1:1 DMSO/water at pH 6.3. [Cu^{II}] is constant = $5\cdot10^{-4}$M



Ligands used: ● = **2** (n = 10); ▲ = **3**, △ = **4**, ○ = **5**

What triggers this enhanced reactivity which is absent in oligomers **4** and **5**? In the oligomers, and polymers as well, there are repeating units with quite different solvation requirements. The Cu^{II}-bound diaminopyridine subunits are highly hydrophilic, soluble in water and, being positively charged, repell each other. This repulsion can be explained on the basis of simple electrostatic arguments. On the contrary, the diphenylmethane subunits are rather hydrophobic, poorly solvated in polar environments and likely tend to aggregate in the polar medium (1:1 DMSO/H_2O) used for the hydrolysis experiments. It is quite reasonable to assume that, when a critical number of monomeric units comprised between six and ten is reached, the hydrophobic interactions[10] take over the electrostatic repulsion and the system switches from an extended conformation to a globular one. This situation is very similar to the micellization of amphiphiles[11] or, more appropriately, to conformational changes of copolymers made of hydrophilic and hydrophobic units as a function of the length of the polymer and/or change of solvent.[12] Consequently, above this critical number of the monomeric units the system self-assembles into an active catalyst which exhibits cooperativity between Cu^{II} ions in the hydrolysis of β-amino ester β-AlaPNP. This hypothesis is indirectly confirmed by experiments carried out in 1:1 (v/v) and 9:1 (v/v) CH_3CH_2OH/H_2O: as the polarity of the solvent decreases the extra activity of the Cu^{II}-loaded polymer decreases eventually vanishing. For comparison, observed rate constants for the cleavage of β-AlaPNP by metallopolymer **2** (n = 10) in the three different solvents are reported in Table 1 (compare entries 4−6 with 7, 10, and 11). Studies carried out with surfactant cetyltrimethylammonium bromide[13] (CTABr) have shown that in

mixed DMSO/water solutions the amphiphile forms micellar aggregates up to 70% (v/v) of DMSO while micellization is no longer possible in mixed ethanol/water solutions when the amount of ethanol is larger than 15% (v/v). The specific role suggested[13] for ethanol (and other short alcohols as well) is the decrease of the solvophobic effect due to the interaction of the cosolvent with water that leads to the destruction of the original structure of water itself with the formation of new hydrogen bonds between water and alcohol. Thus in the present case, when the solvent is 9:1 CH_3CH_2OH/H_2O the solvophobic interactions within the metallopolymer likely vanish and, with them, its extra reactivity. As a possible explanation for the fact that the amount of ethanol needed to disaggregate the metallopolymer is larger than that required for CTABr, one may suggest a lower enthropic price to be paid for the aggregation of the polymer than for the surfactant.

Table 1. Rate constants determined for the cleavage of the various substrates by different catalysts[a]

	Substrate	Catalyst[b]	$k\psi$ [s^{-1}]	$k\psi/k_o$	T [°C]
1	LeuPNP	–	1.7	1	25
2	LeuPNP	**2**[c]	0.4	0.23	25
3	LeuPNP	**3**	0.2	0.12	25
4	β-AlaPNP	–	$8.3\cdot10^{-4}$	1	25
5	β-AlaPNP	–	$1.1\cdot10^{-3}$[d]	1[d]	25
6	β-AlaPNP	–	$1.1\cdot10^{-3}$[e]	1[e]	25
7	β-AlaPNP	**2**[c]	$2.7\cdot10^{-3}$	3.2	25
8	β-AlaPNP	**2**[f]	$2.5\cdot10^{-3}$	2.9	25
9	β-AlaPNP	**2**[g]	$2.3\cdot10^{-3}$	2.8	25
10	β-AlaPNP	**2**[c]	$1.3\cdot10^{-3}$[d]	1.29[d]	25
11	β-AlaPNP	**2**[c]	$4.1\cdot10^{-4}$[g]	0.36[g]	25
12	β-AlaPNP	**4**	$1.0\cdot10^{-4}$	0.12	25
13	β-AlaPNP	**5**	$0.9\cdot10^{-4}$	0.11	25
14	β-AlaPNP	**3**	$1.1\cdot10^{-4}$	0.13	25
15	β-AlaPNP	**3**	$1.9\cdot10^{-4}$[d]	0.18[d]	25
16	β-AlaPNP	**3**	$3.2\cdot10^{-4}$[g]	0.28[g]	25
17	PipPNP	–	5.5	1	50
18	PipPNP	**2**[c]	1.8	0.33	50
19	PipPNP	**3**	1.5	0.27	50
20	NipPNP	–	$1.5\cdot10^{-5}$	1	50
21	NipPNP	**2**[c]	$3.5\cdot10^{-5}$	2.3	50
22	NipPNP	**3**	$1.7\cdot10^{-5}$	1.13	50
23	InipPNP	–	$1.8\cdot10^{-5}$	1	50
24	InipPNP	**2**[c]	$1.0\cdot10^{-5}$	0.55	50
25	InipPNP	**3**	$1.0\cdot10^{-5}$	0.55	50
26	PNPP	–	$8.5\cdot10^{-1}$	1	50
27	PNPP	**2**[c]	$3.0\cdot10^{-1}$	0.35	50
28	PNPP	**3**	$2.5\cdot10^{-1}$	0.29	50
29	PNPN	–	$1.3\cdot10^{-5}$	1	50
30	PNPN	**2**[c]	$3.0\cdot10^{-5}$	2.3	50
31	PNPN	**3**	$1.0\cdot10^{-5}$	0.77	50
32	PNPIN	–	$7.0\cdot10^{-5}$	1	50
33	PNPIN	**2**[c]	$1.5\cdot10^{-4}$	2.1	50
34	PNPIN	**3**	$5.0\cdot10^{-5}$	0.71	50

[a] Conditions: $DMSO/H_2O$ (1:1, v/v); pH of the aqueous component was 6.3 in all experiments (0.05 M MES buffer). – [b] As Cu^{II} complexes; [pyridine units] = $[Cu^{II}] = 5\cdot10^{-4}$ M. – [c] Polymer used was **2** (n = 10). – [d] In 1:1 (v/v) CH_3CH_2OH/H_2O. – [e] In 9:1 (v/v) CH_3CH_2OH/H_2O. – [f] **2** (n = 17). – [g] **2** (n = 29).

Substrate Selectivity

The similarity of behavior between the metallopolymers and metallomacrocycle raises the question whether polymers **2** are also selective in the catalysis of the cleavage of substrates with different separation between the amino and carboxylate ester group. An important feature of the reactivity of macrocycle **1**·2 Cu^{II} was its selectivity toward β-amino esters. With reference to the Cu^{II}-catalyzed hydrolysis the dinuclear complex *accelerated* the process when the substrate was β-AlaPNP but *inhibited* it when the ester was LeuPNP, an α-amino ester. Table 1 reports the rate effects exerted by the different ligands in the presence of α- (LeuPNP, PipPNP, PNPP), β- (β-AlaPNP, NipPNP, PNPN), and γ-amino esters (InipPNP, PNPIN).[14] Analysis of Table 1 provides the following information. a) Cu^{II}-loaded polymer **2** (n = 10) is a better catalyst than Cu^{II} only in the case of all β-amino esters and γ-amino ester PNPIN (compare entries 4 and 7, 20 and 21, 29 and 30, 32 and 33). b) For all α-amino esters and the γ-amino ester InipPNP it is a worse catalyst than Cu^{II} (compare entries 1 and 2, 17 and 18, 23 and 24, 26 and 27). c) The rate acceleration with respect to aquo Cu^{II} is modest (2–3 fold). However, if comparison is made with the Cu^{II} complex of monomeric ligand **3**, i.e. a Cu^{II} ion with a coordination sphere quite similar to that experienced in the polymer, the rate acceleration becomes higher (25-fold with β-AlaPNP, compare entries 7 and 14) and even higher if the comparison is made with the complexes of oligomers **4** and **5** (ca. 28-fold, compare entries 7 and 12 or 13). d) The complexes of oligomers **4** and **5** and monomer **3** are worse catalysts than aquo Cu^{II}.[15]

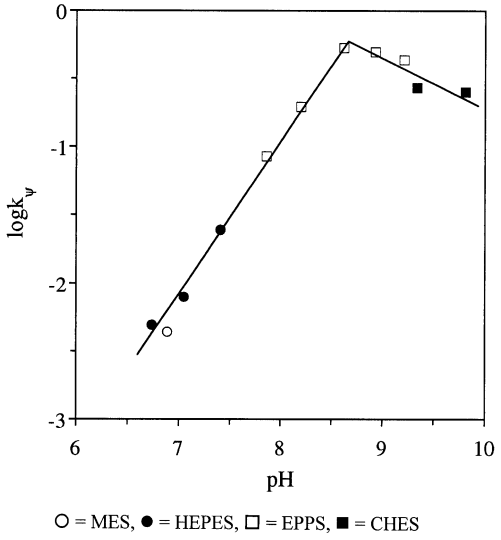
Thus, all pieces of evidence point toward a selectivity for β-amino esters quite similar to that observed for the binuclear complex of macrocycle **1**. This supports the idea that the conformation of the Cu^{II}-loaded polymer is not random but such as to place neighboring Cu^{II} centers at suitable distance for their cooperative involvement in the hydrolysis of a β-amino ester. The only exception to this type of selectivity was found in the case of β- and γ-amino esters of pyridine carboxylates which react at similar rates (entries 30 and 33). This lack of selectivity may tentatively be attributed to the planar structure of these two substrates that minimizes the difference in distance between the amino nitrogen and the carbonyl group of the two esters.

Mechanism

As we have suggested above, a possible mechanism for the Cu^{II}-loaded polymer **2** acceleration of the hydrolytic cleavage of the β-amino esters could imply the formation of a supramolecular complex very similar to that depicted in Figure 1 for **1**·2 Cu^{II}. If this is the case we expect binding of the substrate to the metallopolymer and dependence of the rate constant on the pH. It is well known that Cu^{II}-bound water molecules become particularly acidic,[16] their pK_a ranging from 6 to 9. We have reported[17] that in the case of 2,6-di(methyl)aminomethylpyridine the pK_a of a coordinated water molecule is ca. 8. Hence, if the nucleophilic species in the present system is a Cu^{II}-bound hydroxyl we expect an increase of the rate constant up to the pH at which deprotonation of the Cu^{II}-bound water is complete.[18] The pH may also affect, in opposite ways, the binding of the substrate if this involves the coordination of the β-amino group to one of the four strong binding posi-

tions of the Cu^{II} ion. On one hand, as the pH increases, deprotonation of this amino group occurs[19] and binding to the metal center is favored; on the other hand, deprotonation of the Cu^{II}-bound water molecule implies the displacement of a more tightly bound OH^- and this may disfavor the binding of the substrate. Figure 4 reports the pH dependence of the observed rate constant for the hydrolysis of β-AlaPNP in the presence of Cu^{II}-loaded **2**. The curve shows a maximum at pH = 8.6 which is consistent with the opposite effects on binding discussed above and the involvement, as the nucleophile, of a Cu^{II}-bound hydroxyl.

Figure 4. pH Dependence of the rate constant for the cleavage of β-AlaPNP in 1:1 DMSO/water by Cu^{II}-saturated polymer **2** (n = 10). Symbols indicate the different buffers used; for buffer abbreviations see the Experimental Section



Clear evidence for the effect of the pH on the binding of β-AlaPNP may be obtained from the analysis of the dependence of the observed rate constant on increasing complex concentration at pH 6.3 and 8.5 (Figure 5). At the lower pH the curve is almost a straight line indicating a very low binding constant of the substrate to the catalyst. At pH = 8.5 (close to the maximum of Figure 4) the curvature of the plot indicates strong binding. Since more than one molecule of substrate can bind to the Cu^{II}-loaded polymer and all evidence suggests involvement of two neighboring metal centers in the catalytic process, in the profiles of Figure 6 the concentration reported is that of the "active" complex, i.e. the concentration of binuclear repeating units in the polymer. Conventional analysis[20] of the curve obtained at pH 8.5 gives an affinity constant, K_b, of $4.3 \cdot 10^3$ M^{-1} per "active" site. At pH 6.3 it may be evaluated as lower than 10 M^{-1}. We may also estimate the rate constant of the fully bound β-AlaPNP (k_{lim}) which is $8.2 \cdot 10^{-1}$ s^{-1}, almost two order of magnitude larger than that observed for the uncatalyzed hydrolysis of this substrate.

Table 2 reports these results and those of a similar analysis carried out with the substrates for which rate acceleration was observed. We note that for pyridine derivatives the binding constant is very low even at the higher pH studied. This is consistent with the lower strength of coordination of pyridine to a Cu^{II} center compared with that of an aliphatic amine.[21]

Figure 5. Dependence of the observed rate constant, $k\psi$, for the cleavage of β-AlaPNP from the concentration of the Cu^{II}-saturated polymer **2** (catalyst) in 1:1 DMSO/water

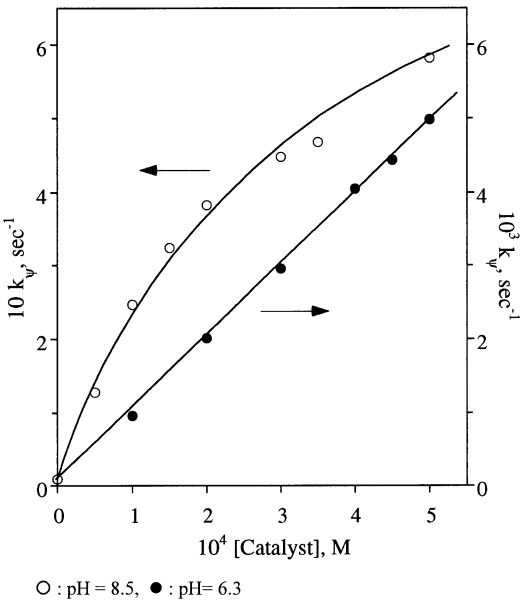


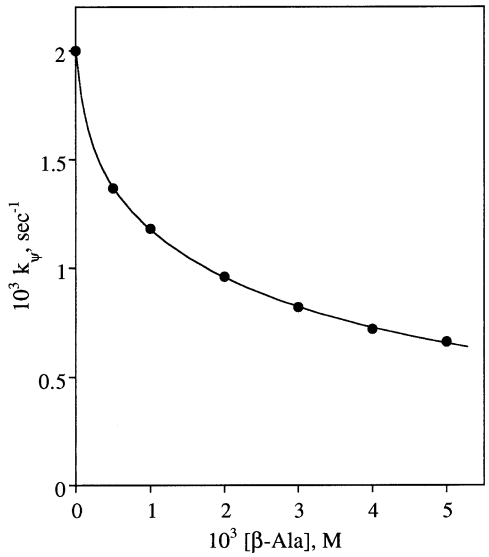
Table 2. Binding constants, K_b, and rate constants for fully bound substrates, k_{lim}, determined for Cu^{II}-loaded polymer **2** (n = 10)[a]

Substrate	pH	K_b[b] [M^{-1}]	$10^3 k_{lim}$ [s^{-1}]	k_{lim}/k_0[d]
β-AlaPNP	6.3	<10[c]	–	–
β-AlaPNP	8.5	$4.3 \cdot 10^3$	820	92
NipPNP	8.5	$3.0 \cdot 10^3$	8.8	63
PNPN	8.5	<10[c]	–	–
PNPIN	8.5	ca. 10[c]	–	–

[a] Conditions: 1:1 DMSO/H_2O; 25°C; pH refers to the aqueous component before mixing; buffer used (0.05 M): pH = 6.3, MES; pH = 8.5, EPPS. – [b] Concentration of catalyst used in the calculation is that of the "active" component (see text for details). – [c] Uncertainity in the determination of these constants is too high to give reliable values. The figures reported provide only the order of magnitude of the binding constant. – [d] k_0 is the observed rate constant for the cleavage in the absence of catalyst all other conditions being unchanged. Its value is $8.96 \cdot 10^{-3}$ s^{-1} for β-AlaPNP and $1.4 \cdot 10^{-4}$ s^{-1} for NipPNP.

Last, the question concerning the real catalytic behavior of the metallopolymer has to be addressed. In all kinetic experiments described so far the concentration of substrate is at least one order of magnitude lower than that of the "active" complex. Under these conditions the formation of a inactive intermediate such as an acylated ligand, although rather unlikely, cannot be detected. For this reason we run kinetic experiments using excess substrate β-AlaPNP and found no evidence of biphasic kinetics as it is typically observed when less active intermediates are formed.[22] This is again consistent with the mechanism proposed: nucleophilic attack of the Cu^{II}-bound OH^- leads to the full cleavage of the ester with formation of *p*-nitrophenol (or *p*-nitrophenolate, depending on the pH) and the amino acid without affecting the complex. Not surprisingly, using very large con-

Figure 6. Dependence of the observed rate constant, $k\psi$, for the cleavage of β-AlaPNP from the concentration of added β-alanine (β-Ala) in the presence of Cu^{II}-loaded polymer **2** (n = 10). Conditions: 1:1 DMSO/water, pH = 6.3, 25°C, $[Cu^{II}] = 4 \cdot 10^{-4}$ M



centrations of β-AlaPNP we observed inhibition of the hydrolytic process. This inhibition can be associated with the competition of the product, β-alanine, with the substrate for binding to the metallopolymer as independently proved by running the hydrolysis experiments in the presence of increasing amounts of the β-amino acid (Figure 6). Incidentally, we note that these experiments, carried out at pH = 6.3, provide indirect evidence for the binding of the substrate to the metallopolymer as a requisite for the occurrence of an efficient hydrolytic process. As discussed above (see Figure 5 and Table 2) at this pH the binding constant is too low and cannot be directly determined with precision.

Thus with Cu^{II}-loaded polymer **2**, as it was for the dicopper complex of macrocycle **1**, the rate accelerations of the hydrolysis may be ascribed to a mechanism in which one Cu^{II} ion is involved in the binding of the substrate and another in the delivery of the nucleophilic species, a Cu^{II}-bound hydroxyl. This second metal ion is the closest neighbor of the first one and the correct positioning of the two cooperating Cu^{II} ions is critical for the occurrence of the catalytic process.

Conclusion

In this paper we have reported the first evidence of solvent- and polymerization-triggered cooperativity between Cu^{II} ions in a hydrolytic process.[23] The number of repeating metal-chelating and diphenylmethane units in this system as well as the choice of the solvent is critical for the achievement of the kinetic benefits. The suggested explanation is the switch, driven by hydrophobic forces, from an extended to a globular conformation of the polymer in which the metal ions are held at a relatively fixed distance because of the rigidity of the diphenylmethane units. Such a distance is well suited to allow productive binding of a β-amino ester (like β-AlaPNP) between two metal centers. Force field calculations indicate that in β-AlaPNP the separation between amino and C=O groups is 4.6 Å. Allowing for the average coordination distance from the metal (2−2.5 Å), the separation between the two pyridine units should be ca. 9−10 Å.

Kinetic evidence indicates that: a) the hydrolytic process for the active metallopolymer requires the involvement of a Cu^{II} ion and its nearest neighbor; b) the cleavage of the esters occurs on the polymer-bound substrate; c) the system is catalytic although inhibition by one of the products, the amino acid, is observed using high substrate concentrations.

In the case of the best substrate studied (β-AlaPNP) the gain in reactivity due to the second metal center with respect to monomeric complex **3**·Cu^{II}, taken as reference, amounts to a 25-fold acceleration. Recent findings in the laboratories of Chin[24] and Reinhoudt[25] have shown that the kinetic contribution to the cleavage of a phosphate diester by a second metal ion can be quantified in a ca. 50-fold acceleration, close to what we have found in the present system.

There is a quite interesting lesson that we learn by comparing the reactivity of the dinuclear Cu^{II} complex of macrocycle **1** with metallopolymer **2**: flexibility is better than rigidity. As a matter of fact, at pH = 6.3 the metallopolymer is twice more active than **1**·2 Cu^{II} against β-AlaPNP, a result that we attribute to the relative conformational flexibility of the globular metallopolymer compared to that of the macrocycle. This has certainly to be considered when designing new supramolecular catalysts in the future.

The authors acknowledge the finacial support by the *Ministry of University, Scientific, and Technological Research* (MURST) and by the *National Research Council* (CNR). They are also indebted to Drs. *A. Veronese, M. Verzini*, and *G. Cremonese* for experimental work.

Experimental Section

General Methods and Materials: Melting points are uncorrected. − ^{1}H-NMR spectra were recorded on a Bruker AC 250 F spectrometer operating at 250 MHz. 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.[25] The buffer components were used as supplied by the manufacturers: 2-morpholinoethanesulfonic acid (MES, Fluka), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS, Aldrich), 2-(cyclohexylamino)ethanesulfonic acid (CHES, Aldrich). The synthesis of macrocycle **1** and of 2,6-bis[(phenyl)aminomethyl-]pyridine (**3**) has been reported.[3] The *p*-NO_2-phenyl esters of L-leucine,[3] β-alanine,[3] picolinic,[23a] nicotinic,[23a] and isonicotic acid,[23a] used as substrates, were prepared as described.

General Procedure for the Synthesis of the p-Nitrophenyl Esters of 2-, 3-, and 4-Piperidinecarboxylic Acids: The proper carboxylic acid (2.0 g, 15.48 mmol, 2-, 3-, or 4-piperidinecarboxylic acid) was dissolved in a mixture of 80 ml of 1,4-dioxane and 15 ml of water. To this solution were added 2.15 ml of triethylamine (15.48 mmol) and di-*tert*-butyl dicarbonate (3.80 g, 17.41 mmol). After stirring

at room temperature overnight the solvent was evaporated, the residue was dissolved in CH_2Cl_2 and washed first with a 1 N solution of HCl (3× 50 ml) and then with water. The evaporation of the dried (Na_2SO_4) organic solvent afforded the Boc-protected derivative which was used without further purification.

The proper Boc-*N*-protected piperidinecarboxylic acid (3.21 g, 14 mmol) was dissolved in 100 ml of dry CH_2Cl_2. To this solution were added *p*-nitrophenol (1.95 g, 14 mmol), 1,3-dicyclohexylcarbodiimide (2.90 g, 14 mmol), and 0.06 g of 4-dimethylaminopyridine. The reaction mixture, protected from moisture with a $CaCl_2$ tube, was stirred at room temperature overnight. After cooling in a ice-water bath, a white precipitate was filtered off, washed with CH_2Cl_2 and the combined organic solvents were evaporated to leave a crude which was purified by column chromatography on silica gel (toluene/ethyl acetate, 7:3). The Boc-protected ester (2 g, 6.46 mmol) was deprotected by dissolving it in 100 ml of a 2 M solution of HBr in acetic acid. After stirring at room temperature for 1 h, 300 ml of Et_2O were added. The white precipitate formed was collected by filtration, washed with Et_2O and dried. The following pure esters were obtained:

4-Nitrophenyl 2-Piperidinecarboxylate · HBr (PipPNP): Yield 26%. − M. p. 189−190°C. - ^{1}H NMR ($CDCl_3$): δ = 1.70−2.17 and 2.55 (2m, 6 H, H^{3,4,5}Piperidine), 3.57−3.19 (2m, 2 H, H^{6}Piperidine), 4.48 (dd, *J* = 11.60 and 3.36 Hz, 1 H, H^{2}Piperidine), 7.52 (d, *J* = 8.02 Hz, 2 H, H^{2}Ph), 8.41 (d, *J* = 8.02 Hz, 2 H, H^{3}Ph). − $C_{12}H_{14}N_2O_4$·HBr·0.5 H_2O (340.18): calcd. C 42.36, H 4.74, N 8.27; found C 42.79, H 4.58, N 8.10.

4-Nitrophenyl 3-Piperidinecarboxylate · HBr (NipPNP): Yield 33%. − M. p. 173−175°C. − ^{1}H NMR ($CDCl_3$): δ = 1.89, 2.12, and 2.43 (3m, 4 H, H^{4,5}Piperidine), 3.00 and 3.19 (2m, 2 H, H^{6}Piperidine), 3.51 (m, 2 H, H^{2}Piperidine), 3.82 (dd, *J* = 13.12 and 3.66 Hz, 1 H, H^{3}Piperidine), 7.30 (d, *J* = 8.02 Hz, 2 H, H^{2}Ph), 8.28 (d, *J* = 8.02 Hz, 2 H, H^{3}Ph). − $C_{12}H_{14}N_2O_4$·HBr (331.17): calcd. C 43.52, H 4.57, N 8.46; found C 43.31, H 4.53, N 8.25.

4-Nitrophenyl 4-Piperidinecarboxylate · HBr (InipPNP): Yield 32%. − M. p. > 230°C. − ^{1}H NMR ($CDCl_3$): δ = 2.07 and 2.40 (2m, 4 H, H^{3,5}Piperidine), 3.19 and 3.52 (2m, 5 H, H^{2,4,6}Piperidine), 7.46 (d, *J* = 8.35 Hz, 2 H, H^{2}Ph), 8.36 (d, *J* = 8.35 Hz, 2 H, H^{3}Ph). − $C_{12}H_{14}N_2O_4$·HBr (331.17): calcd. C 43.52, H 4.57, N 8.46; found C 43.12, H 4.57, N 8.20.

Compound **2**: Two solutions of the same volume and concentration (150 ml, $5.97\cdot10^{-2}$ M) were prepared dissolving 4,4′-di(aminomethyl)diphenylmethane[3] (2.02 g, 8.95 mmol) and 2,6-pyridinedicarboxaldehyde (1.21 g, 8.95 mmol) in the proper solvent [CH_3CN for **2** (*n* = 10), CH_3CN/toluene 1:1 for **2** (*n* = 17), and toluene/hexane 5:1 for **2** (*n* = 29)]. These two solution were dropped at the same rate during 5 hours to a flask containing 60 ml of the same solvent. After the addition was completed the resulting slurry was stirred at room temperature for 16 hours. A white precipate was filtered off, washed with the same solvent, and dried. This solid was dissolved in 50 ml of CH_2Cl_2 and, to this solution, a suspension of $NaBH_4$ (0.67 g, 17.8 mmol) in 350 ml of EtOH was added. After stirring at room temperature for 24 hours 10 ml of water was added and the solvent was evaporated under reduced pressure. The residue was taken up with 100 ml of water and extracted with CH_2Cl_2 (4 × 10 ml). The evaporation of the dried (Na_2SO_4) organic phase afforded a crude which was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH/NH_4OH, 89:10:1). In the case of **2** (*n* = 17) the product was further purified by chromatography on Sephadex LH 20 column using CH_2Cl_2 as eluant. The purification afforded the polimeric materials **2** (*n* = 10), **2** (*n* = 17), **2** (*n* = 29) in a 53%, 28%, and 41% yield, respectively. The shorter polimer is solid (m.p. 85−87°C) while the other two are viscous oils.

2 (*n* = 10): ^{1}H NMR ($CDCl_3$/CD_3OD, 9:1): δ = 2.90 (bs, NH), 3.78 (s, 44 H, NH*CH*$_2$Ph and Ph*CH*$_2$NH$_2$), 3.90 (s, 64 H, PhCH$_2$Ph e NHCH$_2$Py), 4.70 (s, 2 H, Py*CH*$_2$OH), 6.90−7.18 (m, 110 H, Ph and H^{3,5}Py), 7.53 (m, 11 H, H^{4}Py). − $C_{242}H_{255}N_{33}O\cdot16\ H_2O$ (3930.19): calcd. C 73.96, H 7.36, N 11.76; found C 73.26, H 7.12, N 11.20.

2 (*n* = 17): the ^{1}H-NMR spectra of this polimeric material closely resemble that of **2** (*n* = 10) but for the integral values which are in agremeent of a molecular weight of ca. 6000 Daltons. − $C_{396}H_{416}N_{54}O\cdot22\ H_2O$ (6344.43): calcd. C 74.97, H 7.31, N 11.92; found C 74.32, H 7.08, N 11.50.

2 (*n* = 29): the ^{1}H-NMR spectra of this polimeric material closely resemble that of **2** (*n* = 10) but for the integral values which are in agremeent of a molecular weight of ca. 10000 Daltons. $C_{660}H_{692}N_{90}O\cdot36\ H_2O$ (10550.03): calcd. C 75.14, H 7.30, N 11.95; found C 74.62, H 7.02, N 11.58. This analysis is not complitely satisfactory but could not be repeated because of the little amount of material available.

2-(Formyl)-6-[(Boc-methylamino)methyl]pyridine (**6**): To a solution of 6-[(methylamino)methyl]-2-(hydroxymethyl)pyridine[11e] (3.7 g, 24.3 mmol) in 100 ml of dioxane were added 3.75 ml of triethylamine (26.9 mmol) and di-*tert*-butyl dicarbonate (5.87 g, 26.9 mmol). The reaction mixture protected from moisture with a $CaCl_2$ tube was stirred at room temperature for 2 h. The solvent was evaporated and the residue was taken up with 100 ml of a 10% solution of Na_2CO_3 and extracted with $CHCl_3$ (3× 50 ml). Evaporation of the dried (Na_2SO_4) organic solvent afforded a crude product which was purified by column chromatography on silica gel ($CHCl_3$/CH_3OH, 20:1) to give 3.5 g (57%) of pure 6-[(Boc-methylamino)methyl]-2-(hydroxymethyl)pyridine as a clear oil. − ^{1}H NMR ($CDCl_3$): δ = 1.41, 1.49 [2s, 9 H, C(CH$_3$)$_3$]; 2.92 (m, 3 H, CH$_3$N), 3.96 (m, 1 H, OH), 4.55 (m, 2 H, CH$_2$N), 4.73 (m, 2 H, CH$_2$OH), 7.10 (m, 2 H, H^{3,5}Py), 7.66 (t, *J* = 7.68 Hz, 1 H, H^{4}Py).

To the previous protected compound (3.15 g, 12.5 mmol) dissolved in 100 ml of dioxane was added SeO_2 (0.83 g, 7.5 mmol). The reaction mixture was stirred under heating at 70°C for 5 h. After cooling at room temperature it was filtered through a thin celite pad and the solvent was evaporated to afford a crude oil. This was purified by column chromatography on silica gel ($CHCl_3$) to give 1.57 g (50%) of pure **6** as a colorless oil. − ^{1}H NMR ($CDCl_3$): δ = 1.41, 1.50 [2s, 9 H, C(CH$_3$)$_3$]; 2.96 (m, 3 H, CH$_3$N), 4.63 (m, 2 H, CH$_2$N), 7.44 (m, 1 H, H^{5}Py), 7.86 (m, 2 H, H^{3,4}Py), 10.04 (s, 1 H, CHO). − $C_{13}H_{18}N_2O_3$ (250.30): calcd. C 62.38, H 7.25, N 11.19; found C 62.16, H 7.33, N 11.14.

Compound **4**: To a solution of 4,4′-di(aminomethyl)diphenylmethane[3] (3.33 g, 14.7 mmol) in 280 ml of distilled DMF was added 2.6 ml of diisopropylethylamine (14.9 mmol). The solution was heated at 40°C with a oil bath and a solution of di-*tert*-butyl dicarbonate (1.60 g, 7.35 mmol) in 100 ml of DMF was slowly added dropwise. After the addition was completed the reaction mixture was stirred at room temperature overnight. A white precipitate of unreacted diamine was filtered off, washed with few a ml of DMF and the combined organic solvents were evaporated at reduced pressure giving a solid residue. This was dissolved in 200 ml of a $CHCl_3$/MeOH (8:2) mixture and washed first with a 10% solution of Na_2CO_3 and then several times with water. The evaporation of the dried (Na_2SO_4) organic phase afforded a crude product which was purified by column chromatography on silica gel ($CHCl_3$/CH_3OH/NH_4OH, 84:15:0.5) to give 1.24 g (26%) of pure 4′-(Boc-

aminomethyl)-4-aminomethyldiphenylmethane. – ^{1}H NMR ($CDCl_3$): δ = 1.51 [s, 9 H, $C(CH_3)_3$]; 3.87 (s, 2 H, PhCH_2NH_2), 3.95 (s, 2 H, PhCH_2Ph), 4.30 (m, 2 H, PhCH_2NHBoc), 6.78 (m, 4 H, *Ph*CH_2NHCO), 7.17 (m, 4 H, *Ph*CH_2NH).

To the previous monoprotected diamine (1.17 g, 3.58 mmol) dissolved in 250 ml of benzene was added 2,6-pyridinedicarboxaldehyde (0.242 g, 1.79 mmol). The reaction mixture was heated at reflux for 2 h distilling azeotropically the water formed during the reaction. The solvent was evaporated at reduced pressure and the residue dissolved in 100 ml of CH_2Cl_2. To this solution a a suspension of $NaBH_4$ (0.344 g, 9.1 mmol) in 100 ml of EtOH was added. After stirring overnight at room temperature the solvent was evaporated and the residue dissolved in 100 ml of a 8:2 $CHCl_3/CH_3OH$ mixture and washed with water. The evaporation of the dried (Na_2SO_4) organic solvent afforded 1.18 g (90%) of 2,6-bis{[4′-(Boc-aminomethyl)-4-diphenylenemethane]methyl}aminomethylpyridine which was used without further purification. – ^{1}H NMR ($CDCl_3$): δ = 1.45 [s, 18 H, $C(CH_3)_3$]; 3.80 (s, 4 H, PhCH_2NH), 3.89 (s, 4 H, PyCH_2NH), 3.93 (s, 4 H, PhCH_2Ph), 4.25 and 4.28 (2s, 4 H, PhCH_2NHBoc), 7.08–7.22 (m, 16 H, Ph), 7.28 (d, *J* = 7.68 Hz, 2 H, $H^{3,5}$Py), 7.58 (t, *J* = 7.68 Hz, 1 H, H^4Py).

The above compound (1.18 g, 1.57 mmol) was dissolved in 10 ml of distilled CH_2Cl_2 and 5 ml of trifluoroacetic acid was added. After stirring for 1 h, 100 ml of a 10% solution of Na_2CO_3 was added and the two phases were separated. The water phase was extracted with a 8:2 CH_2Cl_2/CH_3OH mixure (3× 50 ml), the combined organic phases were dried and the solvent was evaporated at reduced pressure to leave 0.81 g (93%) of 2,6-bis{[(4′-aminomethyl)-4-diphenylenemethane]methyl}aminomethylpyridine. – ^{1}H NMR ($CDCl_3$): δ = 1.74 (bs, NH), 3.80 (s, 4 H, PhCH_2NH), 3.82 (s, 4 H, PhCH_2NH_2), 3.89 (s, 4 H, PyCH_2NH), 3.94 (s, 4 H, PhCH_2Ph), 7.09–7.30 (m, 18 H, Ph and $H^{3,5}$Py), 7.58 (t, *J* = 7.31 Hz, 1 H, H^4Py).

To a solution of the previous material (0.81 g, 1.46 mmol) in 100 ml of benzene was added **6** (0.99 g, 3.96 mmol). The reaction mixture was heated at reflux for 3 h distilling azeotropically the water formed during the reaction. The solvent was then evaporated at reduced pressure and the residue dissolved in 50 ml of CH_2Cl_2. To this solution a suspension of $NaBH_4$ (0.35 g, 9.25 mmol) in 50 ml of EtOH was added. After stirring overnight at room temperature the solvent was evaporated and the residue dissolved in 100 ml of a 8:2 $CHCl_3/CH_3OH$ mixture and washed with water. The evaporation of the dried (Na_2SO_4) organic solvent afforded 1.27 g of Boc-protected **4** which was deprotected in CH_2Cl_2/TFA as described above. The crude product thus obtained was purified by column chromatography on silica gel ($CHCl_3/CH_3OH/NH_4OH$, 85:15:1.5) to give 0.51 g (42%) of pure **4** as a pale yellow oil. – ^{1}H NMR ($CDCl_3$): δ = 2.04 (bs, NH), 2.48 (s, 6 H, CH_3N), 3.80 (s, 8 H, PhCH_2NH), 3.84 (s, 4 H, PyCH_2NHCH_3), 3.88 (s, 8 H, PyCH_2NH), 3.94 (s, 4 H, PhCH_2Ph), 7.10–7.19 (m, 16 H, Ph) 7.23–7.30 (m, 6 H, $H^{3,5}$Py), 7.56 (t, *J* = 7.68 Hz, 1 H, H^4Py), 7.59 (t, *J* = 7.68 Hz, 2 H, H^4Py). – FAB-MS (NBA) *m/z* = 823 [M^+]. – $C_{53}H_{61}N_9 \cdot 2\ H_2O$ (860.17): calcd. C 74.01, H 7.62, N 14.66; found C 73.42, H 7.66, N 14.31.

Compound **7**: 4′-(Boc-aminomethyl)-4-aminomethyldiphenylmethane (0.98 g, 3.0 mmol) was dissolved in 150 ml of distilled CH_2Cl_2. To this solution, heated at 40°C, were added 6-formyl-2-(carboxymethyl)pyridine[11c] (0.49 g, 3.0 mmol) and 5 g of activated 4-Å molecular sieves. After stirring at room temperature for 6 h the reaction mixure was filtered and the solvent was evaporated to leave the crude imine derivative. To this crude was added a suspension of $NaBH_4$ (0.58, 15 mmol) in 100 ml of EtOH and the reaction mixture was stirred, protected from moisture, for 72 h at room temperature. A few ml of water were then added and the solvent was evaporated. The residue was dissolved in 100 ml of a 8:2 $CHCl_3/CH_3OH$ mixture and washed with a 10% solution of Na_2CO_3. The evaporation of the dried (Na_2SO_4) organic phase afforded 1.24 g (92%) of 2-{[4′-(Boc-aminomethyl)-4-diphenylenemethane]-methyl}aminomethyl-6-(hydroxymethyl)pyridine as a clear oil which was used as obtained. – ^{1}H NMR ($CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$]; 3.81 (s, 2 H, PhCH_2NHCH_2), 3.92 (s, 2 H, PyCH_2NH), 3.94 (s, 2 H, PhCH_2Ph), 4.26 and 4.28 (2s, 2 H, PhCH_2NHBoc), 4.72 (s, 2 H CH_2OH), 7.08–7.28 (m, 10 H, Ph and $H^{3,5}$Py), 7.62 (t, J = 7.68 Hz, 1 H, H^4Py).

The previous derivative (1.24 g, 2.77 mmol) was dissolved in 50 ml of dioxane. To this solution were added triethylamine (0.4 ml, 2.87 mmol) and a solution of di-*tert*-butyl dicarbonate (0.610 g, 2.79 mmol) in 40 ml of dioxane. The reaction mixture, protected from moisture with a $CaCl_2$ tube, was stirred at room temperature for 2 h. The solvent was evaporated and the residue was taken up with 100 ml of a 10% solution of Na_2CO_3 and extracted with a 8:2 $CHCl_3/CH_3OH$ mixture (3× 50 ml). Evaporation of the dried (Na_2SO_4) organic solvent afforded 1.43 g (93%) of 2-[Boc-{[4′-(Boc-aminomethyl)-4-diphenylenemethane]methyl}]aminomethyl-6-(hydroxymethyl)pyridine as a yellow oil. – ^{1}H NMR ($CDCl_3$): δ = 1.45 and 1.53 [2s, 18 H, $C(CH_3)_3$]; 3.94 (s, 2 H, PhCH_2Ph), 4.26 and 4.29 (2s, 2 H, PhCH_2NHBoc), 4.44 (s, 2 H, Ph$CH_2NBocCH_2$), 4.50 and 4.52 (2s, 2 H, PyCH_2NBoc), 4.70 (s, 2 H CH_2OH), 4.80 (bs, 1 H, NHBoc), 7.05–7.22 (m, 10 H, Ph and $H^{3,5}$Py), 7.61 (t, *J* = 7.68 Hz, 1 H, H^4Py).

To the previous protected compound (1.42 g, 2.59 mmol) dissolved in 80 ml of dioxane was added SeO_2 (0.15 g, 1.36 mmol). The reaction mixture was stirred and heated at 70°C for 3 h. After cooling at room temperature the reaction mixture was filtered through a thin celite pad and the solvent was evaporated to afford a crude material. This was purified by column chromatography on silica gel ($CHCl_3/CH_3OH$, 75:1) to give 0.68 g (48%) of pure **7** as a yellowish oil. – ^{1}H NMR ($CDCl_3$): δ = 1.45 [bs, 18 H, $C(CH_3)_3$]; 3.92 (s, 2 H, PhCH_2Ph), 4.27 and 4.29 (2s, 2 H, PhCH_2NHBoc), 4.48 and 4.54 (2bs, 2 H, Ph$CH_2NBocCH_2$), 4.63 (bs, 2 H, PyCH_2N-Boc), 7.08–7.22 (m, 8 H, Ph) 7.35 and 7.48 (2m, 1 H H^3Py), 7.78 (m, 2 H, $H^{4,5}$Py), 10.03 (s, 1 H, CHO). – $C_{32}H_{39}N_3O_5$ (545.68): calcd. C 70.44, H 7.20, N 7.70; found C 70.05, H 7.42, N 7.55.

Compound **5**: To **7** (0.40 g, 0.73 mmol) dissolved in 80 ml of benzene was added 4,4′-di(aminomethyl)diphenylmethane (0.083 g, 0.36 mmol). The reaction mixture was heated at reflux for 24 h distilling azeotropically the water formed during the reaction. The solvent was then evaporated at reduced pressure and to the residue was added a suspension of $NaBH_4$ (0.090 g, 2.38 mmol) in 50 ml of EtOH. After stirring overnight at room temperature the solvent was evaporated and the residue dissolved in 100 ml of a 8:2 $CHCl_3/CH_3OH$ mixture and washed first with a 2% solution of NaOH and then with water. The evaporation of the dried (Na_2SO_4) organic solvent afforded a crude which was purified by column chromatography on silica gel ($CHCl_3/CH_3OH$, 50:1) to give 0.26 g (55%) of pure Boc-protected dimer. – ^{1}H NMR ($CDCl_3$): δ = 1.41 and 1.45 [2s, 36 H, $C(CH_3)_3$]; 3.78 (s, 4 H, PhCH_2NHCH_2), 3.86 (s, 4 H, PyCH_2NH), 3.92 and 3.94 (2s, 4 H and 2 H, PhCH_2Ph), 4.25 and 4.28 (2s, 4 H, PhCH_2NHBoc), 4.41 (bs, 4 H, Ph$CH_2NBocCH_2$), 4.49 (bs, 4 H, PyCH_2NBoc), 7.06–7.17 (m, 24 H, Ph), 7.20–7.27 (m, 4 H, $H^{3,5}$Py), 7.60 (m, 2 H, H^4Py).

The previous Boc-protected derivative was dissolved in 12 ml of distilled CH_2Cl_2 and 4 ml of CF_3COOH were added. After stirring at room temperature for 1 h a 10% solution of Na_2CO_3 was added.

The two phases were separated and the aqueous one was extracted twice with 20 ml of CH_2Cl_2. The combined organic solvents were dried (Na_2SO_4) and evaporated to afford 0.15 g (85%) of dimer which was used without further purification. – ^{1}H NMR ($CDCl_3$): δ = 1.82 (bs, NH); 3.79 (s, 8 H, PhCH_2NHCH$_2$), 3.81 (s, 4 H, PhCH_2NH$_2$), 3.89 (s, 8 H, PyCH_2NH), 3.94 (s, 6 H, PhCH_2Ph), 7.11–7.18 (m, 24 H, Ph), 7.24 –7.27 (m, 4 H, H^{3,5}Py), 7.57 (t, J = 7.68 Hz, 2 H, H^{4}Py).

To the amine thus obtained (0.15 g, 0.17 mmol) dissolved in 40 ml of benzene was added **7** (0.19 g, 0.34 mmol). The reaction mixture was heated at reflux for 5 h distilling azeotropically the water formed during the reaction. The solvent was then evaporated at reduced pressure and to the residue was added a suspension of $NaBH_4$ (0.052 g, 1.37 mmol) in 40 ml of EtOH. After stirring overnight at room temperature the solvent was evaporated and the residue dissolved in 100 ml of a 8:2 $CHCl_3$/CH_3OH mixture and washed first with a 10% solution of Na_2CO_3 and then with water. The evaporation of the dried (Na_2SO_4) organic solvent afforded a crude which was purified by column chromatography on Sephadex LH 60 (CH_2Cl_2) to give 0.31 g (91.5%) of pure Boc-protected tetramer. – ^{1}H NMR ($CDCl_3$): δ = 1.41 and 1.45 [2s, 36 H, C(CH$_3$)$_3$]; 1.84 (bs, NH), 3.79 (s, 12 H, PhCH_2NH), 3.86 and 3.88 (2s, 4 H and 8 H, PyCH_2NH), 3.91 and 3.94 (2s, 4 H and 6 H, PhCH_2Ph), 4.25 and 4.27 (2s, 4 H, PhCH_2NHBoc), 4.41 (bs, 4 H, PhCH_2NBocCH$_2$), 4.48 (bs, 4 H, PyCH_2NBoc), 7.06–7.17 (m, 40 H, Ph), 7.20–7.27 (m, 8 H, H^{3,5}Py), 7.56 (t, J = 7.68 Hz, 2 H, H^{4}Py), 7.59 (t, J = 7.68 Hz, 2 H, H^{4}Py).

The previuos protected amine was deprotected using CF_3COOH/CH_2Cl_2 as described above. The crude was purified by column chromatography on silica gel ($CHCl_3$/CH_3OH/NH_4OH, 90:10:1) to give 0.024 g (10%) of pure tetramer. – ^{1}H NMR ($CDCl_3$): δ = 1.96 (bs, NH); 3.79 (s, 20 H, PhCH_2NH), 3.88 (s, 16 H, PyCH_2NH), 3.93 (s, 10 H, PhCH_2Ph), 7.11–7.19 (m, 40 H, Ph), 7.24 –7.27 (m, 8 H, H^{3,5}Py), 7.56 (t, J = 7.68 Hz, 4 H, H^{4}Py).

To the amine thus obtained (0.024 g, 0.016 mmol) dissolved in 6 ml of distilled CH_2Cl_2 were added **6** (0.016 g, 0.063 mmol) and 1g of activated 4-Å molecular sieves. The reaction mixture, protected from moisture, was stirred at room temperature for 20 h. The reaction mixture was filtered through a short celite pad and the solvent was evaporated at reduced pressure. To the residue obtained was added a suspension of $NaBH_4$ (0.030 g, 0.8 mmol) in 10 ml of EtOH. After stirring overnight at room temperature the solvent was evaporated and the residue dissolved in 100 ml of a 8:2 $CHCl_3$/CH_3OH mixture and washed first with a 10% solution of Na_2CO_3 and then with water. The evaporation of the dried (Na_2SO_4) organic solvent afforded 0.024 mg of crude Boc-protected **5** which was deprotected by treatment with CF_3COOH/CH_2Cl_2 as described above. The purification of crude oligomer **5** was performed first by by column chromatography on silica gel ($CHCl_3$/CH_3OH/NH_4OH, 90:10:1) and then by column chromatography on Sephadex LH 60 (CH_2Cl_2) to afford 7.5 mg of pure material as a viscous oil. The small amount of product obtained did not allow combustion analysis. – ^{1}H NMR ($CDCl_3$): δ = 1.25 (bs, NH); 2.48 (s, 6 H, NCH$_3$), 3.79 (s, 20 H, PhCH_2NH), 3.84 (s, 4 H, PyCH_2NCH$_3$), 3.88 (s, 20 H, PyCH_2NCH$_2$), 3.93 (s, 10 H, PhCH_2Ph), 7.11–7.16 (m, 40 H, Ph), 7.24 –7.27 (m, 12 H, H^{3,5}Py), 7.56 (t, J = 7.68 Hz, 6 H, H^{4}Py).

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 solutions were prepared in water. The reactions were run in a mixture 1:1 DMSO/water (unless otherway stated) 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 $1-2\cdot10^{-3}$ 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 $2-4\cdot10^{-5}$ M solution of substrate with the solution of ligands, additives and buffer both prepared in DMSO/water. The final concentration of substrate was $1-2\cdot10^{-5}$ 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[27] or the software package provided with the SF.17MV stopped-flow work station) and the fit error on the rate constant was always less than 1%. Reproducibility of different runs were within 5%.

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