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Polyaza[n]paracyclophanes as Synthetic Models of Zn Containing Enzymes. The Role of a non Coordinated Nitrogen Atom in the Proximity of the Metal

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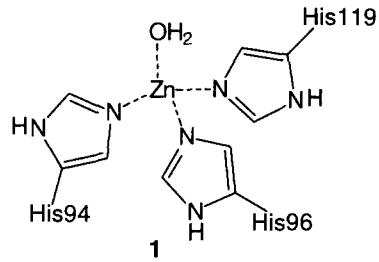
Abstract: 2,6,9,13-Tetraaza[14]paracyclophane (**3**, **B323**) represents a simple model for Zn containing enzymes like HCAII which possess additional reactive groups in the proximity of the metal center. The Zn^{2+}.**3** complex shows notable catalytic activity for the hydrolysis of *p*-nitrophenyl acetate. Structural modifications of **3** which affect to the non coordinated nitrogen atom greatly modify the catalytic activity of the receptor. © 1997 Elsevier Science Ltd.

Much effort has been devoted to the understanding of the chemical mechanisms involved in Zn containing enzymes.[1,2] The carbonic anhydrases (CA) which are able to catalyze the reversible hydration of CO_2 to HCO_3^- and to act as hydrolases are some of the systems more extensively studied in this respect.[3-6] Accordingly, a great deal of work is currently being directed towards the development of synthetic models mimicking the active site of these metalloenzymes. Two main approaches to attain this target have been the synthesis of different imidazol containing receptors[7,8] and the use of macrocyclic polyamines.[9-11] An efficient model for the CA active site should be able to form Zn complexes of reasonable stability and yet to leave an uncompleted coordination of the Zn cation as to allow its participation in catalytic processes. In the HCAII, for instance, Zn^{2+} is coordinated to three histidine residues (His-96, His-94 and His-119) and to a water molecule or a hydroxide ion, as a function of pH, with distorted tetrahedral geometry.[1-6] As a consequence of the low coordination of the Zn^{2+} cation by nitrogen donors, coordinated water is easily deprotonated (pK around 7.5) and the formation of Zn-OH^- species seems to be at the origin of the catalytic activity. However, some other residues besides these three histidines also play an important role in the activity of this enzyme. In particular, Thr-199 which is hydrogen bonded to Zn-bound ligands and to Glu-106, and His-64 which is important in the shuttling of protons out of the active site, are considered essential for the catalytic activity.[1,3-5]

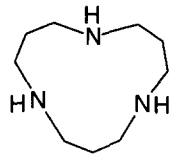
Among macrocyclic polyamines studied as CA mimics, saturated triazamacrocycle [12]aneN_3 (**2**) seems to be one of the best models up to now reported as it reproduces the distorted tetrahedral geometry of the active site and particularly, presents a pK_a value (pK_a = 7.3) of the coordinated water molecule close to the value assigned for the deprotonation of the water molecule in CA.[11] On the other hand, this model also reproduces

some other features of the biocatalyst since its activity is inhibited by appropriate anions able to displace the water molecule or the substrate from the coordination sphere of the metal.

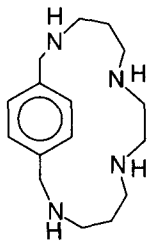
Recently we have shown that polyaza[n]paracyclophanes display some interesting coordination features with different metal cations and, in particular, with Zn^{2+}.[12-15] In the present work we have explored how these coordination features can be advantageously used to model Zn containing enzymes. To that purpose, we have studied the catalytic activity of Zn-polyaza[n]paracyclophane complexes in the hydrolysis of active esters such as *p*-nitrophenylacetate (PNPA), a reaction that has been often used to analyze the activity of CA active center mimics.



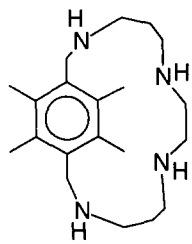
1
CA active center



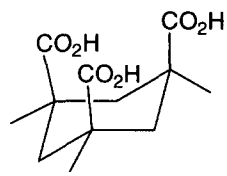
2
[12]ane N_3



3
B323



4
D323



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RESULTS AND DISCUSSION

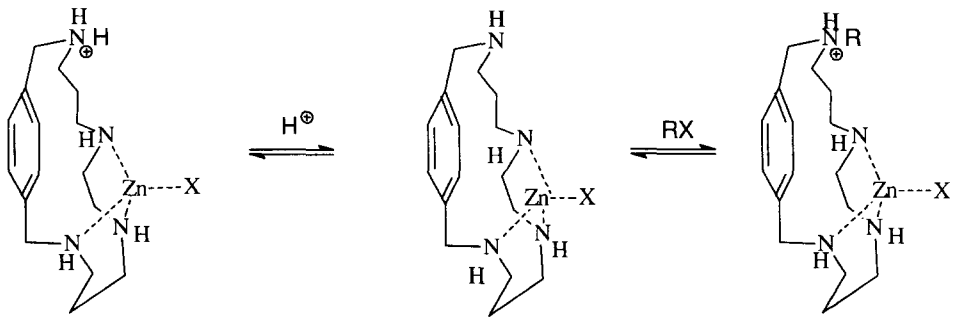
In polyaza[n]paracyclophanes, the presence of the *para*-phenylene subunit linking the ends of a polyamine chain precludes the simultaneous coordination of both benzylic nitrogen atoms to a single metal center. Thus, for 2,6,9,13-tetraaza[14]paracyclophane **3**, (**B323**), only three nitrogen atoms are involved in the interaction with Zn^{2+} yet yielding a complex of appreciable stability (see Table 1). The consequent low symmetry of the resulting complex is clearly denoted by its NMR spectra. In CD_3CN the ^{13}C NMR spectrum consists of thirteen different signals at 24.6, 26.3, 43.9, 46.1, 46.5, 48.9 (two carbon atoms), 52.0, 52.2, 55.1, 131.5 (two carbon atoms), 132.4, 133.3, and 137.6 (two carbon atoms) ppm, while that of the free ligand just displays seven signals, in accordance with the two-fold symmetry of the ligand.[12] This coordination of the metal cation resembles that of the Zn^{2+} in CA and, in agreement with this, the metal would be further coordinated to a water molecule which displays a significant acidity (pK_a = 8.67). Although this pK_a value is not so low as the one reported for the [12]aneN_3 complex (pK_a = 7.3),[11a] and CA itself,[1] the presence of a non-coordinated nitrogen atom could be an interesting feature in order to assist the catalytic process.

Table 1.- *Logarithms of the Stability Constants for the Interaction of Different Macrocyclic Polyamines with Zn^{2+} at 298.1 K in 0.15 mol dm^{-3} $NaClO_4$.*

Reaction[a]	log K				
	2[b]	**3**	**4**	**6**	**8**
Zn + L = ZnL	8.41 (2)	6.54 (1)	6.86 (1)	5.55 (6)	6.06 (1)
ZnL + H = ZnLH		7.74 (2)	---	---	---
ZnL + H_2O = ZnL(OH)	-7.30 (2)	-8.67 (2)	-9.25 (2)	-7.92 (5)	-8.18 (1)

a) Charges have been omitted for clarity. b) 0.10 mol dm^{-3} $NaClO_4$; see ref. 11a.

Further evidences to the proposed structure of the Zn complex of **B323** are provided by the acid-base and nucleophilic properties of the non-coordinated benzylic nitrogen that, additionally, suggest potential ways for the participation of this nitrogen atom in chemical processes catalyzed by the Zn complex. In fact, Zn.**3** is readily protonated to afford Zn.H**3**, the pK_a value for this process (pK_a = 7.74) being comparable with that of the third protonation constant of the uncomplexed receptor (pK_a = 7.31). This reflects the fact that, also in water, one benzylic nitrogen is non-coordinated to the metal cation and no nitrogen-metal bond needs to be broken in this protonation process. Thus, a basic nitrogen atom is appropriately located in the vicinity of the metal center. On the other hand, this benzylic nitrogen is also nucleophilic, being able to react with suitable alkylating agents to yield, with high efficiency and selectivity, different N-monofunctionalized macrocycles.[15] Similar coordination patterns have been characterized in the crystal structure of a Hg^{2+} complex with **3**.[14b]



Scheme 1

As has been found in the enzymic site and in other models, the low coordination of Zn^{2+} in the complex is also reflected in its ability to interact with additional ligands and, in particular with anions.[11] In this respect, the Zn.**3** complex is able, for instance, to form strong intermolecular complexes with carboxylate anions. Thus, values of the constants obtained for the interaction of Zn.**3** with carboxylate anions derived from the *cis,cis*-1,3,5-trimethyl-1,3,5-cyclohexanetricarboxylic acid **5** (Kemp's triacid),[16,17] a polycarboxylic acid having a well defined and preorganized shape, are given in Table 2.

As can be seen in the table, the tricarboxylate forms strong complexes with Zn.**3** and Zn.H**3** species, the values for the constants being two orders of magnitude higher than the ones obtained for the interaction of the tricarboxylate anion with the protonated ligand having the same total charge. An interesting point relevant to the

present discussion is the observation that highly protonated **B323** as well as its complex with Zn^{2+} interacts at an appreciable extent even with protonated species derived from the tricarboxylate. The low coordination of Zn^{2+} in its complex with **B323** allows an efficient behavior of the metal cation as a Lewis acid not only with anions but also with neutral Lewis bases.

Table 2. *Logarithms of the Stability Constants for the Interaction of 3 and its Zn^{2+} complex with Carboxylate anions derived from Kemp's triacid.*[a]

Reaction[b]	log K	Reaction	log K
$LH_2 + A$	2.76 (2)	$ZnL + A$	4.69 (1)
$LH_3 + A$	3.68 (2)	$ZnLH + A$	5.68 (1)
$LH_3 + HA$	3.43 (2)	$ZnLH + HA$	5.99 (1)
$LH_3 + H_2A$	3.77 (2)	$ZnLH + H_2A$	6.37 (2)
$LH_4 + H_2A$	4.27 (2)		
$LH_4 + H_3A$	4.18 (2)		

a) 0.15 mol dm^{-3} $NaClO_4$, 298.1 K. b) Charges have been omitted for clarity.

The existence of a non-coordinated basic group in the vicinity of Zn^{2+} could be reminiscent of the presence of His-64 in the hydrophilic part of the enzyme site of HCAII. Catalytic efficiency of this complex was tested using *p*-nitrophenyl acetate (PNPA) as a substrate. The results obtained (see Table 3), which are graphically presented in Figures 1 and 2, show for Zn.**3** a similar catalytic efficiency to [12]aneN$_3$,[11a] but the curve k_2 *vs.* pH is displaced to higher pH values. The mean point of this *s*-shaped curve is coincident with the pK_a value of the coordinated water molecule, supporting the hypothesis that the catalytically active species is the hydroxo complex.

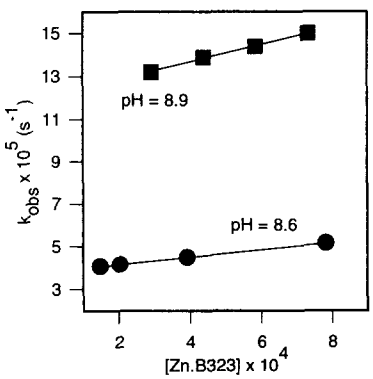


Figure 1. *Acceleration of PNPA hydrolysis in the presence of Zn.**B323** complexes.*

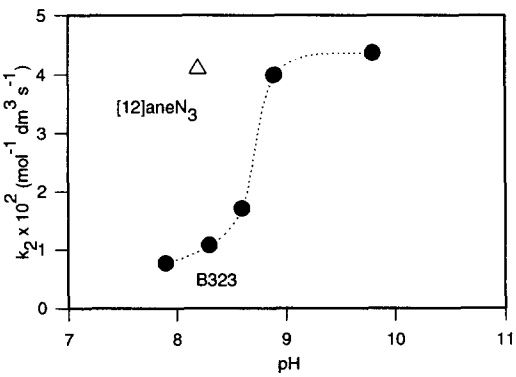


Figure 2. *Second order rate constants of PNPA hydrolysis as a function of pH with the Zn.**B323** complex.*

A similar study was also performed for the durene derivative **4** (**D323**), which differs from **B323** just by the methylation of the aromatic moiety. Polyamine **4** displays similar basicity to **3** and forms Zn^{2+} complexes of similar stability (see Table 1), being the coordinated water slightly less acidic (pK_a = 9.2). According to these data a similar catalytic activity should be expected for Zn^{2+} complexes of **B323** and **D323**, with a small displacement to higher pH values of the sigmoid curve for the later. The results were, however, completely different than expected on that grounds, as the Zn.**4** complex not only did not catalyze the hydrolysis of the *PNPA* but inhibited the process (see Table 3 and Figure 3).

Table 3. *Selected Second-order Rate Constants* $k_{(PNPA)}$ *(mol^{-1} dm^3 s^{-1}) of 4-Nitrophenyl Acetate Hydrolysis at 25°C.*

Compound	pH	k_2
2[a]	8.2	0.041
3	8.3	0.007
4	8.8	-0.042
6	8.6	-0.034
8	8.2	0.234

a) From ref. 11a.

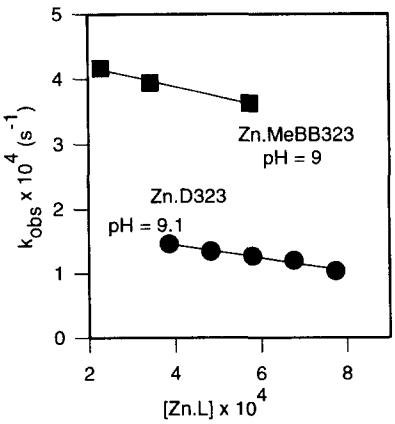


Figure 3. *Inhibition of PNPA hydrolysis in the presence of the Zn.**4** complex (pH = 9.1) and the Zn.**6** complex (pH = 9. 0).*

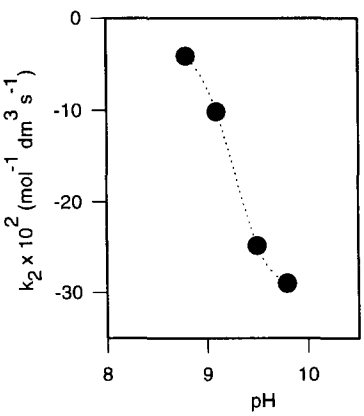
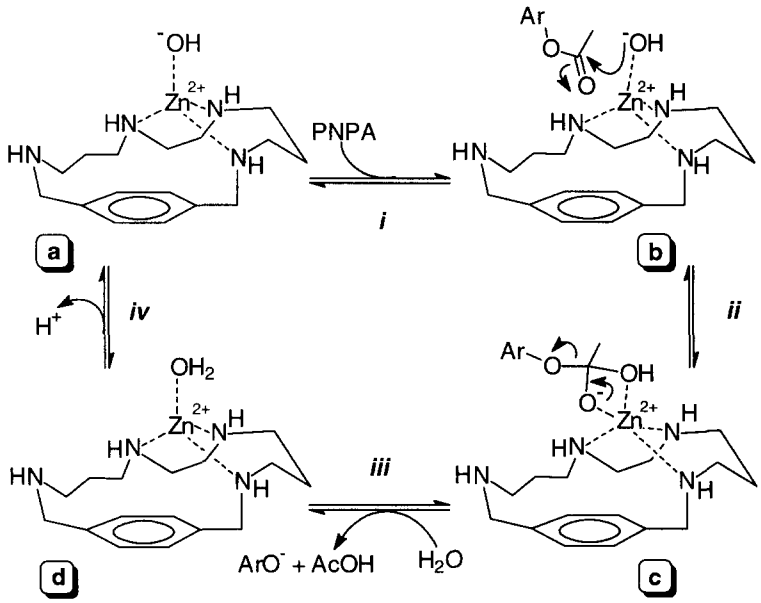


Figure 4. *Second-order rate constants of PNPA hydrolysis as a function of pH with the Zn.**D323** complex.*

These data imply that the acidity of the coordinated water is not the only requirement for catalytic activity to occur in our models. Most likely, the role played by the non-coordinated nitrogen atom has to be taken into account to explain these results.

The catalytic mechanism for the CA activity in the CO_2 to bicarbonate conversion is complex and requires several steps.[3-5] General trends of this mechanism are also applicable to CA hydrolytic activity as well as to

explain the behavior of other hydrolytic metalloenzymes and artificial models.[18-21] From a simplified point of view, the catalytic cycle requires four essential processes as summarized in *Scheme 2*. *(i)* Introduction of the substrate into the active site. For this purpose the metal cation can act as a Lewis acid or some other groups in the active site can participate recognizing the substrate.[18-20] *(ii)* Nucleophilic attack of the Zn-bound hydroxide ion on a carbonyl carbon. *(iii)* Product dissociation with regeneration of the Zn-OH_2 form. *(iv)* Deprotonation of coordinated water to afford the active hydroxo species.



Scheme 2

The basic nitrogen atom in the vicinity of Zn^{2+} could favor the presence of appropriately ordered water molecules, promoting rapid dissociation of the Zn^{2+}-bound ion, and cooperate to the deprotonation of the coordinated water, facilitating the proton transfer out of the active site (steps *iii* and *iv* in Scheme 2). This situation should be quite similar to the one described for the role of His-64 in HCAII.[3-5]

The actuation of the durene derivative as an inhibitor requires the formation of a stable, non productive complex between the Zn.**D323** species and the substrate or a product derived from it, so that no phenolate ion is released from that complex and, at the same time, less free substrate is present in solution, reducing the background hydrolysis by the solvent. This seems to suggest that inhibition affects to the last steps (*iii* and *iv* in Scheme 2, in particular *iii*) of the hydrolytic mechanism. As a matter of fact, when the inhibition of PNPA hydrolysis by the Zn.**4** complex was studied at different pH values, a sigmoid curve was obtained (see Figure 4) with a mean point coincident with the pK_a value of the Zn-bound water. This indicates that, for **D323**, the hydroxo complex (ZnOH.**D323**) is again the active species and inhibition of the hydrolytic process has to take place after interaction of the hydroxo complex with the active ester.

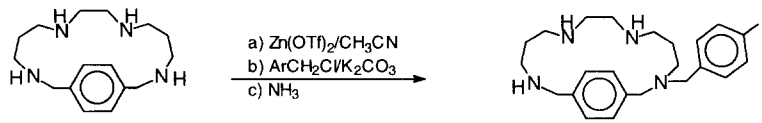
This behavior of the Zn.**D323** complex is not easy to rationalize, and several points need to be considered. The lower conformational mobility of the chain in Zn complexes of these tetramethylated ligands, which has been

inferred from monofunctionalization experiences[15] and molecular mechanics studies,[22] could be of importance. In fact, a high degree of conformational flexibility has been described as one of the most interesting aspects related to His-64.[23] A second point to be considered is the increase in the hydrophobicity of the molecule which should difficult, along with the first factor, the presence of water molecules appropriately ordered as well as the proton transfer step. It is also important, in this respect, that His-64 is located at the hydrophilic part of the active site. Thus, a strong non-productive complex, favored by the hydrophobicity of the ligand, seems to be formed between ZnOH.**D323** and the product formed upon nucleophilic attack on the carbonyl group of PNPA (complex *c* in Scheme 2) and this would explain the inhibition observed in the hydrolytic process. It is important to remark that for HCAII Thr-199 has been described to maximize catalytic efficiency through destabilization of the product complex, providing a way for its rapid dissociation.

One of the main differences between Zn.**3** and Zn.**4** complexes is the acid-base behavior of the non-coordinated nitrogen atom. As can be seen in Table 1, the formation of ZnLH has not been detected under our experimental conditions for **D323**, revealing that the stability constant for the process: Zn.**4** → Zn.H**4** is very low. This is probably related with the hydrophobicity of **D323** as it has been observed that solvation and desolvation processes play an essential role in determining the values for the protonation constants of polyaza[n]paracyclophanes, in particular for protonation steps involving benzylic nitrogen atoms.[13] This could suggest that the decreased basicity of the non-coordinated nitrogen atom is reflected in the formation of the non productive complex between the Zn complex of **D323** and the substrate.

An alternative explanation to the role played by the benzylic nitrogen taking into account its nucleophilic character should be, in principle, discarded. No evidence for the formation of the expected intermediate species has been obtained and, additionally, monofunctionalization experiences have shown how the non-coordinated nitrogen atom in the Zn complex of **D323** can behave as a good nucleophile.[15]

According to the former considerations it is to be expected that structural changes affecting the non-coordinated benzylic nitrogen atom should dramatically affect catalytic activity of the resulting receptors. In this respect, monobenzylated **B323** derivative **6** and the monotosylated compound **8** seem to be ideal candidates to check the validity of this hypothesis.



3 **6**

Scheme 3

Compound **6** (**MeBB323**) was obtained by selective N-functionalization of one of the benzylic nitrogen atoms of **B323** as has been previously described.[15] The presence of the new *p*-methylbenzyl group clearly affects to the properties of receptor **6**. Study of the protonation behavior of this compound reveals that the substituted nitrogen atom is the last one being protonated. Indeed, the constant for the process $H_3L + H \rightarrow H_4L$ was too small as to be determined by potentiometry.[24] In the same way, interaction with Zn^{2+} takes place so that coordination to the metal cation involves preferentially the three non-substituted nitrogen atoms. The structural change is also reflected in a lower value of the stability constant determined for the interaction with the zinc

cation (Zn + L → ZnL, Log K = 5.55). Protonation of the non-coordinated nitrogen in the complex (ZnL + H → HZnL) was not detected, a situation similar to that found for **D323**. The increase in hydrophobicity provided by the presence of the additional benzyl group most likely hinders the protonation of the substituted nitrogen in both the free receptor and the Zn complex. Again, the Zn^{2+} cation completes its coordination sphere with water molecules, the acidity of the coordinated water being higher than for the other cases considered (pK_a = 7.92).

Analysis of the catalytic behavior of the complex Zn.**6** shows similar trends to those observed for the Zn.**D323** complex (see Table 1 and Figure 3). The higher acidity of coordinated water was not reflected in a more efficient catalytic activity, and a clear inhibition of the hydrolysis of *p*-nitrophenyl acetate was observed, the Zn-hydroxo complex being the species acting as an inhibitor. Again the increased hydrophobicity of this receptor as well as a decrease in the ability of the non-coordinated nitrogen to participate in proton transfer steps should be considered to explain these results. Hydrophobic effects could also operate in both receptors **4** and **6** through the formation of aggregates that would difficult the interaction with the substrate or the participation of the non-coordinated nitrogen in the catalytic cycle. Nevertheless, aggregate formation could not be detected under our experimental conditions.

If the basicity of the non-coordinated nitrogen atom was the main factor affecting the catalytic activity, the Zn^{2+} complex of compound **8**, which is neither nucleophilic nor basic, should act as an efficient inhibitor. However, this was not the case.

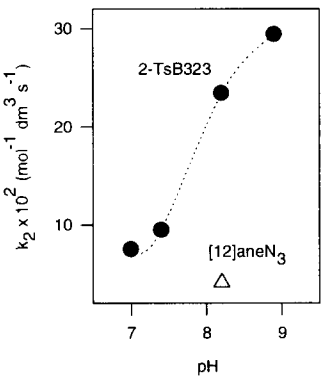
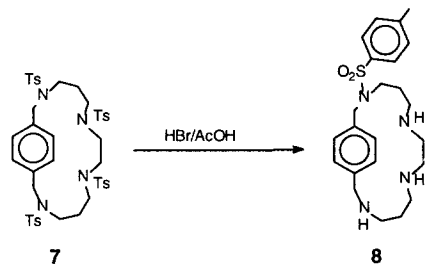


Figure 5. *Second-order rate constants of PNPA hydrolysis as a function of pH wiht the Zn.8 complex.*



Scheme 4

Monotosylated derivative **8** could be obtained in moderate yields by careful detosylation of tetratosylated **B323** (**7**) with PhOH/HBr under mild conditions. Spectroscopic characterization of this product revealed that the remaining tosyl group was on a benzylic nitrogen atom. ^{13}C and ^1H NMR spectra showed similar trends to those observed for compounds obtained via selective monofunctionalization of **B323** at the benzylic position such as **6**.[15] In particular, two different benzylic singlets are observed in the ^1H NMR spectrum, one of them having a similar chemical shift than benzylic protons in **3** and the other being shifted ca. 0.5 ppm downfield to the same chemical shift observed for benzylic protons in tetratosylated compound **7**. Additionally, irradiation of this downfield benzylic signal produced a NOE enhancement of one of the doublets corresponding to the tosyl

group. As can be observed in Table 1, compound **8** forms Zn^{2+} complexes of similar stability (log K = 6.06) to those formed by **3**, **4** and **6**, the coordinated water being slightly less acidic (pK_a = 8.18) than the one in the Zn complex of **6**. It has to be noted that in both cases, **6** and **8**, N-functionalization is reflected in an increase of the acidity of coordinated water, even if the observed value is still smaller than the value measured for [12]aneN_3 (**2**).[25] As a consequence of the presence of the tosyl group, the substituted benzylic nitrogen atom should remain non-coordinated to the metal and protonation of the Zn.**8** complex is not detected by potentiometry.

When the catalytic activity of the Zn.**8** complex was studied for the hydrolysis of PNPA, results obtained (see Table 3 and Figure 5) showed that this complex had the higher catalytic efficiency for the process under study. As a matter of fact, results were better than those reported for receptor **2**. At pH 8.2 the Zn complex of **8** showed a second order constant about 5 times higher than the one for the Zn.**2** species.

This result rules out the possibility that basicity of the non coordinated nitrogen is the only or the main factor affecting the catalytic efficiency. The main structural change from **6** to **8** is the substitution of a -CH_2- group by a -SO_2- group. Overall, both compounds are more hydrophobic than the parent receptor **3**, as is shown by the increased solubility in organic solvents. However, Molecular Mechanics and semiempirical calculations at the PM3 level using the Macromodel[26] 3.5 and Spartan[27] packages reveal that the structural change is reflected in the presence, for **8**, of a highly polar region, due to the -SO_2- group, in the vicinity of the active site. This situation could facilitate the presence of appropriately ordered water molecules to displace the Zn-bound ion after the nucleophilic attack as well as the proton transfer step, in such a way that catalytic efficiency is greatly increased.

When comparing **3** and **8**, two factors have to be taken into account. First, the polarity provided by the -SO_2- group is higher than the one provided by the NH fragment, as shown by molecular mechanics and semiempirical calculations. In this respect it is important to consider that effective participation of His64 in the catalytic process requires its accommodation in an hydrophylic environment so that it is connected to the Zn through hydrogen-bonded water molecules and the proton is not retained by the imidazol group but is immediately transferred to the surrounding medium. The appreciable basicity of the non coordinated nitrogen atom in **3** could make less efficient the last step which releases the proton to the solvent.

CONCLUSIONS

In conclusion, compounds with polyaza[n]paracyclophane structure represent novel and interesting models for the study of the active site of hydrolytic enzymes containing a non-coordinated nitrogen donor atom or additional polar groups in the vicinity of the complexed metal cation. One important advantage of these systems is their simple and easy structural modification which allows the preparation of different receptors having well defined structural characteristics. The analysis of the catalytic activity of polyaza[n]paracyclophanes **3**, **4**, **6** and **8** evidences the important role played by the presence of additional non coordinated groups as well as by the hydrophobicity of the metal site surroundings in the building of catalytic models.[21] Modulation of the hydrophobic and hydrophilic regions in the receptor seems to be at the origin of the important differences found for the catalytic efficiency of these model compounds. It has been shown that even minor structural modifications can produce very important changes in the catalytic activity. Inhibition of the hydrolysis of PNPA has been obtained when the more hydrophobic receptors **4** and **6** were used. Results obtained evidence that the Zn-OH complexes are the active species both for the catalysis and the inhibition of PNPA hydrolysis, and

suggest that, for those systems, the displacement of the anion formed upon nucleophilic attack and the proton transfer are the kinetically more relevant steps, as has been observed for HCAII itself. Catalytic activity of the Zn.**8** complex for the hydrolysis of PNPA is the highest up to now reported for this sort of compounds.

Acknowledgements. We are indebted to DGICYT (project PB93-0700) and IVEI (project CPE/091) for financial support.

EXPERIMENTAL SECTION

Materials. All reagents and solvents used were of analytical grade. *p*-nitrophenylacetate (PNPA) and chemical buffers MES, HEPES, CHES and TAPS were purchased from Fluka and used without further purification. Macrocycles **3**, **4** and **6** were prepared as reported previously[12,15] and handled as their hydroperchlorate salts. All the potentiometric measurements were carried out in 0.15 mol dm^{-3} $NaClO_4$ solutions. Sodium perchlorate was purified according to a procedure already described.[28] Stock solutions of $Zn(ClO_4)_2$ were prepared in doubly distilled water and their concentration determined by standard gravimetric methods. Carbon dioxide free NaOH and $HClO_4$ solutions were prepared following the procedure described in ref. 29.

***Synthesis of 2-Tosyl-2,5,8,12-Tetraaza[14]paracyclophane* (8).** Tetratosylated B323 (**7**) (15 g, 0.0168 mol),[12] and phenol (6 g, 0.0625 mol) in a solution of HBr in AcOH (60 mL, 0.246 mol HBr) were heated at 90°C for 22h. Upon cooling compound **8** crystallized as its trihydrobromide (1.12 g). ^{1}H NMR (200 MHz) ($CDCl_3$, δ): 1.25 (m, 2H), 1.64 (m, 2H), 2.3-2.8 (m, 10H), 2.36 (s, 3H), 3.08 (t, 2H), 3.78 (s, 2H), 4,24 (s, 2H), 7.2-7.4 (m, 6H), 7.75 (d, 2H). ^{13}C NMR (50.3 MHz) ($CDCl_3$, δ): 35.7, 36.2, 39.7, 55.5, 56, 57.4, 58.8, 59.5, 63, 64.6, 68.8, 142, 144.2, 145, 145.8, 146.1, 147, 153.7, 160.5. Anal. Calcd. for $C_{23}H_{34}N_4O_2S.3HBr$: C, 41.0; H, 5.5; N, 8.3; S, 4.8. Found: C, 40.71; H, 5.69; N, 8.02; S, 4.66.

Kinetics. The kinetic study was carried out by a UV-Visible spectral method using a VARIAN CARY 3 spectrophotometer equipped with a thermostated cell compartment. Kinetics of PNPA in a 10 % CH_3CN solution was followed by an increase in absorption maximum at 400 nm (assigned to 4-nitrophenolate anion) at 25.0±0.1 °C in 0.15 mol dm^{-3} $NaClO_4$. Buffered reaction solutions containing 15 mmol dm^{-3} buffer (MES (pH 6-7), HEPES (pH 7-8), TAPS (pH 8-9) and CHES (pH>9)) were used.

Immediately after rapid injection of 50 μL of 5 mmol dm^{-3} PNPA in dry CH_3CN into a buffer solution (2.5 mL, 15 mmol dm^{-3}) containing different concentrations of the Zn^{2+} complexes (0.1-1 mmol dm^{-3}), the absorption increase was recorded over 5 minutes. This hydrolysis showed a good first order behavior . The pseudo-first order rate constants, k_{obs} (PNPA)(s^{-1}), were obtained by a log plot method (correlation coefficients >0.99). From the slopes of all the linear plots of k_{obs} (PNPA) against the Zn^{2+} complex concentrations the second order rate constants k (PNPA) (mol^{-1} dm^3 s^{-1}) were obtained.

e.m.f. Measurements. The potentiometric titrations were carried out in 0.15 mol dm^{-3} solutions at 298.1±0.1 K, by using the equipment (potentiometer, burette, stirrer, microcomputer, etc.) that has been fully described.[30] The acquisition of the emf data was performed with the computer program PASAT.[31] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as an hydrogen concentration probe by titration of well-known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by the Gran´s method,[32] which gives the standard potential of the electrode, $E^{o'}$,

and the ionic product of water. The computer program SUPERQUAD[33] was employed to calculate the protonation and stability constants. DISPO[34] program was used to obtain the distribution diagrams. At least three titration curves were performed for each one of the studied systems. Concentrations of Zn^{2+} were *ca* $1x10^{-3}$ mol dm^{-3} and those of the ligands varied in the range $1x10^{-3}$ - $5x10^{-3}$ mol dm^{-3}, the pH range investigated was 2.5 - 10.0. The titration curves for each system were treated either as a single set or separately without significant variations in the values of the equilibrium constants. Furthermore, the sets of data were merged together to obtain the final values of the stability constants.

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