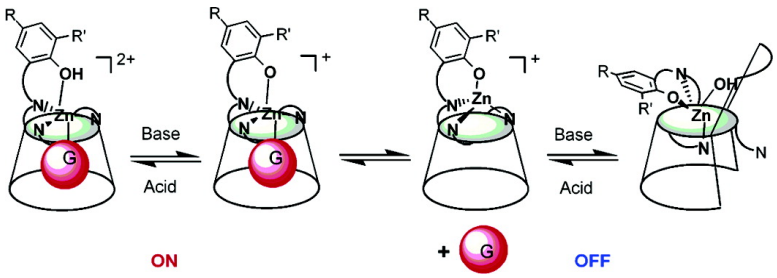
Article

Biomimetic Zinc Funnel Complexes Based on Calix[6]*N*Ar*O* Ligands: An Acid–Base Switch for Guest Binding

Olivier Snque, Marie-Nolle Rager, Michel Giorgi, Thierry Prang, Alain Tomas, and Olivia Reinaud

J. Am. Chem. Soc., **2005**, 127 (42), 14833-14840• DOI: 10.1021/ja053620e • Publication Date (Web): 04 October 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 6 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 10/04/2005

Biomimetic Zinc Funnel Complexes Based on Calix[6]N_3ArO Ligands: An Acid−Base Switch for Guest Binding

Olivier Sénèque,[†,⊥] Marie-Noëlle Rager,[‡] Michel Giorgi,[§] Thierry Prangé,[||] Alain Tomas,[||] and Olivia Reinaud*[,†]

Contribution from the Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR CNRS 8601, Université René Descartes, 45 rue des Saints Pères, 75270 Paris Cedex 06, France, Service de RMN, Ecole Nationale Supérieure de Chimie de Paris (ENSCP), 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France, Laboratoire de Cristallochimie, Centre Scientifique Saint-Jérôme, Université Paul Cézanne Aix-Marseille III, av. Escadrille Normandie-Niemen, 13397 Marseille Cedex 20, France, and Laboratoire de Cristallographie et RMN Biologiques, UMR CNRS 8015, Université René Descartes, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France

Received June 2, 2005; E-mail: olivia.reinaud@univ-paris5.fr

Abstract: The coordination chemistry of Zn in an N_3ArOH environment has been explored. The ligands are based on calix[6]arenes that present two imidazole arms and an amino phenol moiety at the narrow rim. Three different types of complexes have been characterized. One is dicationic with Zn^{2+} coordinated to the three nitrogen atoms and to the oxygen of the phenol group of the calix[6]ligand. This complex is very sensitive to exogenous coordinating molecules and exists as a 5-coordinate species due to the endo-complexation of a guest. The second species is a monocationic complex for which the phenol group has been deprotonated. The resulting N_3ArOZn complex can also bind a guest ligand albeit with a lower affinity than the dicationic complex. The third species is neutral. It can be obtained upon reaction with a base to yield a hydroxo complex or with an anion such as a chloride that coordinates the metal center from the outside of the calixarene cavity. The simultaneous binding of two anionic donors decreases the Zn Lewis acidity, allowing an impressive conformational reorganization of the system. One imidazole arm is released by the metal center. The other one undergoes self-inclusion into the π-basic calixarene cavity because the low affinity of the metal center for neutral ligand does not allow the endo-coordination of an exogenous guest. Hence, the calix[6]N_3ArOH-based Zn complexes act as an acid−base switch for guest binding. Several aspects of this system appear reminiscent of Zn-peptidases of the astacin and serralisin families.

Introduction

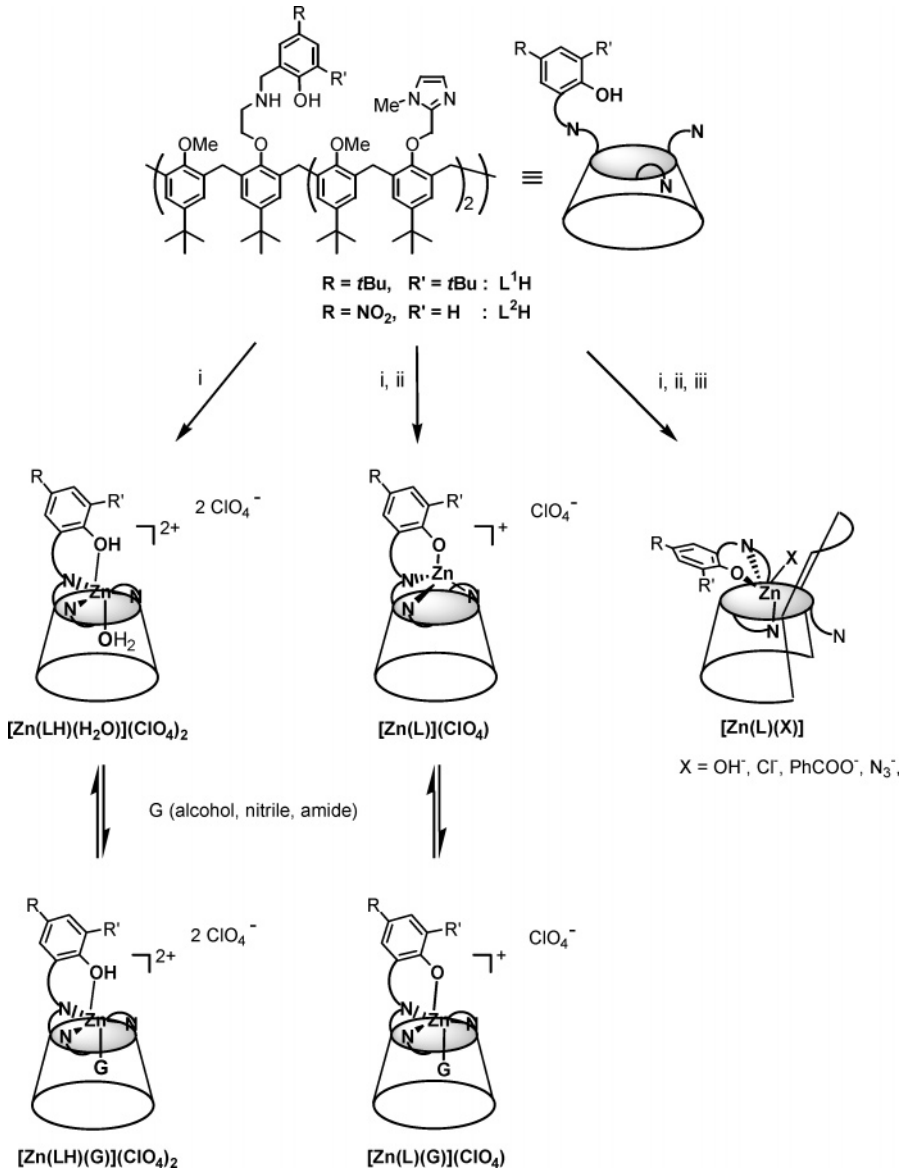
Zinc enzymes constitute an important class of metalloproteins.[1] They are mainly involved in hydrolytic reactions. A recurrent motif in mononuclear enzymes is a Zn ion coordinated by three histidine residues and either a water $\{[ZnN_3(OH_2)]^{2+}\}$ or a hydroxide $\{[ZnN_3(OH)]^{+}\}$. Many model complexes have been synthesized to elucidate the protonation state at the active site during a catalytic cycle.[2] Interestingly, some zinc enzymes such as astacin[1,3] and serralysin[4] possess a fourth amino acid residue coordinated to Zn, a tyrosine, which is deprotonable. Hence, in these enzymes, three different protonation states become accessible, a dicationic $[Zn(N_3ArOH)(OH_2)]^{2+}$, a monocationic $[Zn(N_3ArO)(OH_2)]^{+}$, and a neutral $[Zn(N_3ArO)(OH)]$ one. Up to now, the precise role of the coordinated tyrosine remains unknown.

We have previously described a system based on calix[6]arene-N_3 ligands to mimic mononuclear Cu and Zn enzymes active sites with their substrate binding pockets.[5−7] The corresponding complexes behaved as remarkable molecular receptors, binding small organic neutral molecules in the heart of the hydrophobic cavity provided by the calixarene. In the particular case of Zn complexes, we found that "weak", that is, nonco-

[†] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université René Descartes.
[‡] ENSCP.
[§] Université Paul Cézanne Aix-Marseille III.
[||] Laboratoire de Cristallographie et RMN Biologiques, Université René Descartes.
[⊥] Present address: Laboratoire de Physicochimie des Métaux en Biologie, DRDC, UMR 5155 CEA-CNRS-UJF, 17 rue des Martyrs, 38054 Grenoble Cedex 9, France.

(1) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375.
(2) Parkin, G. *Chem. Rev.* **2004**, *104*, 699.
(3) (a) Gomis-Ruth, F.-X.; Stöcker, W.; Huber, R.; Zwilling, R.; Bode, W. *J. Mol. Biol.* **1993**, *229*, 945. (b) Grams, F.; Dive, V.; Yiotakis, A.; Yiallouros, I.; Vassiliou, S.; Zwilling, R.; Bode, W.; Stöker, W. *Nat. Struct. Biol.* **1996**, *3*, 671. (c) Ik Park, H.; Ming, L.-J. *J. Inorg. Biochem.* **1998**, *72*, 57. (d) Yiallouros, I.; Grosse Berkhoff, E.; Stöcker, W. *FEBS Lett.* **2000**, *484*, 224. (e) Stöcker, W.; Yiallouros, I. In *Handbook of metalloproteins*; Messerschmidt, A., Bode, W., Cygler, M., Eds.; Wiley: Chichester, 2004; Vol. 3, pp 116.
(4) (a) Baumann, U. *J. Mol. Biol.* **1994**, *242*, 244. (b) Mock, W. L.; Yao, J. *Biochemistry* **1997**, *36*, 4949. (c) Hege, T.; Baumann, U. *J. Mol. Biol.* **2001**, *314*, 187. (d) Park, H. I.; Ming, L.-J. *J. Biol. Inorg. Chem.* **2002**, *7*, 600 and refs cited therein.
(5) Blanchard, S.; Le Clainche, L.; Rager, M.-N.; Chansou, B.; Tuchagues, J.-P.; Duprat, A. F.; Le Mest, Y.; Reinaud, O. *Angew. Chem., Int. Ed.* **1998**, *37*, 2732.
(6) Le Clainche, L.; Giorgi, M.; Reinaud, O. *Inorg. Chem.* **2000**, *39*, 3436.
(7) Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2000**, *122*, 6183.

Scheme 1. Synthesis of Zn^{2+} Complexes with the Calix[6]N_3Ar*OH* Ligands L^1H and L^2H[a]



[a] Reagents: (i) $Zn(H_2O)_6(ClO_4)_2$, (ii) Et_3N, (iii) NaX.

valent interactions within the calixarene structure were of major importance for the stabilization of tetrahedral dicationic complexes. This allowed the first example of such an aqua-complex to be structurally characterized.[8] More recently, we have described the synthesis of a second generation of calix[6]arene-based ligands bearing three *N*-donors and a phenol group.[9] Here, we report on their zinc complexes. The three different protonation states accessible to this novel supramolecular system have been characterized. Interestingly, comparative host/guest studies show that they behave very differently. As a result, this calix[6]N_3Ar*O* system acts as an acid−base switch for guest binding.

Results

Dicationic $[Zn(N_3ArOH)(G)]^{2+}$ Complexes. The calix[6]arene-based N_3Ar*O*H ligand L^1H (Scheme 1, R = *t*Bu and R′ = *t*Bu)[9] was reacted with 1 equiv of $Zn(H_2O)_6(ClO_4)_2$ in THF. The IR spectrum and elemental analysis of the isolated solid were in agreement with the presence of two perchlorate counterions per calix-ligand, thereby suggesting the formation of a dicationic N_3Ar*O*H−Zn^{2+} complex. Its ^{1}H NMR spectra, recorded at room temperature (rt) either in a noncoordinating solvent ($CDCl_3$) or in acetonitrile, displayed broad signals indicative of some conformational motion. When a few molar equivalents of a small neutral ligand G (ca. 10 equiv of either ethanol, acetonitrile propionitrile, or dimethylformamide) was added to a $CDCl_3$ solution (3 mM) of the complex, changes were observed in the NMR spectra for the calixarene resonances and new peaks appeared in the high field region (δ < 0 ppm). This attested to the presence of the guest ligand in the heart of the calixarene cone, surrounded by the aromatic walls of the cavity. These high-field shifted resonances were broad at room temperature (rt) but sharpened at lower T. This allowed the clear identification of the coordinated guest (G) sitting in the cavity[7] (Figure 1A) and attested to the formation of complexes $[Zn(L^1H)(G)]^{2+}$ (Scheme 1).[10]

(8) Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2001**, *123*, 8442.
(9) Sénèque, O.; Reinaud, O. *Tetrahedron* **2003**, *59*, 5563.

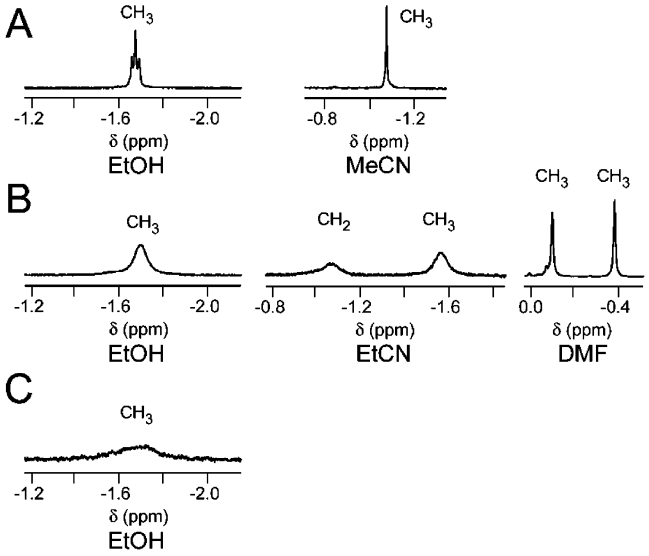


Figure 1. Representative examples of ^{1}H NMR spectra high-field regions (400 MHz, $CDCl_3$, 243 K) of complexes $[Zn(L^1H)(G)]^{2+}$ (A), $[Zn(L^2)(G)]^+$ (B), and $[Zn(L^1)(G)]^+$ (C).[15]

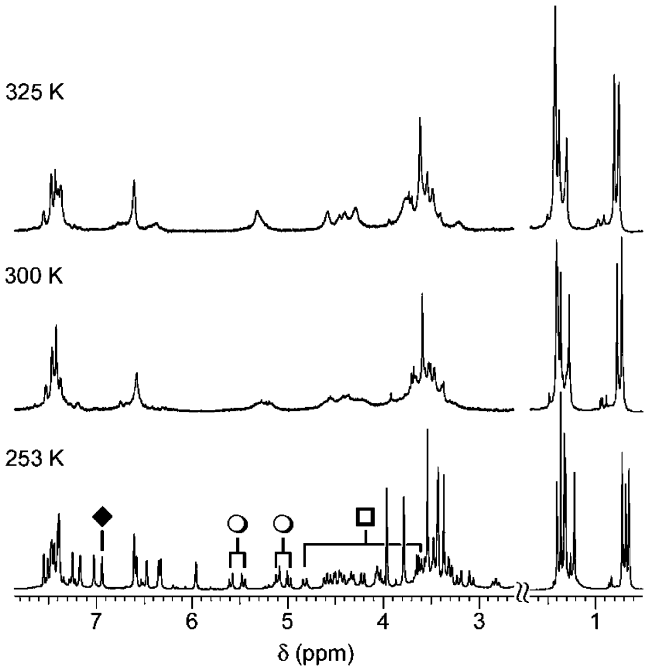


Figure 2. ^{1}H NMR spectra of complex $[Zn(L^1H)(CD_3CN)](ClO_4)_2$ in CD_3CN. Spectra at 325 and 300 K were recorded at 250 MHz, and a spectrum at 253 K was recorded at 400 MHz. The intensity of the signals in the *t*Bu region has been reduced. ◆, ArO*H*; ○, OC*H*$_2$Im; □, NC*H*$_2$ArO.

At 253 K, the spectrum in CD_3CN was very well defined, with narrow resonances (Figure 2). All protons of the calix-ligand were differentiated. Six peaks were observed for the *t*Bu groups of the calixarene units (three at around 0.7 ppm and three at around 1.3 ppm). The three methoxy resonances at 3.36, 3.42, and 3.53 ppm indicated that they were not situated in the anisotropy cone of the calixarene walls, as previously observed for other calix[6]arene-based Zn complexes.[7,11] All of this indicated that the calix[6]arene adopted a flattened cone conformation with the *t*Bu substituents in alternate in/out positions and the methoxy groups rejected away from the center of the cavity, in an out position. The OCH_2CH_2N resonances (4.08 and 4.35 ppm for OCH_2, 2.84 and 3.32 ppm for NCH_2) showed that this arm also lay in an out position (vide infra). The methylene protons belonging to the imidazole arms and to the NCH_2ArOH group were differentiated as AB systems. This indicated that the metal ion was coordinated to all three nitrogen atoms and to the phenol site. The ArO*H* resonance was located at 6.94 ppm (saturation transfer with H_2O), thus confirming that the coordinated phenolic oxygen atom was protonated. Finally, 12 resonances were observed for the aromatic protons belonging to the calixarene phenoxyl units and all bridging methylene protons were differentiated as well. This showed that, at low temperature, the helical twisting[11,12] of the coordinating arms around the metal ion was frozen at the NMR time scale. As a result, the C_s symmetry of the calixarene ligand[9] was broken, no point of symmetry remaining. Hence, although coordinated to a tetradentate ligand, this novel Zn complex presented supramolecular features that were similar to those displayed by the first generation of funnel complexes based on tridentate ligands.

With the calix[6]N_3Ar*OH* ligand L^2H that presents a nitro-substituted phenol unit in place of the di-*t*Bu-phenol one (Scheme 1, R = NO_2 and R′ = H),[9] the same synthetic procedure led to the zinc−phenolate complex $(N_3ArO{-}Zn)^+$ (vide infra). Indeed, the higher acidity of the phenol ligand due to the presence of the electron-withdrawing substituent led to its spontaneous deprotonation upon reaction with Zn(II) perchlorate. As a result, the corresponding dicationic Zn−phenol species was not stabilized.

Monocationic $[Zn(N_3ArO)]^+$ and $[Zn(N_3ArO)(G)]^+$ Complexes. In the presence of a base such as triethylamine, L^1H and L^2H reacted with $Zn(H_2O)_6(ClO_4)_2$ in MeOH to yield a white and a yellow solid compound, respectively. The IR spectra and elemental analyses of these complexes showed a 1:1:1 Zn/ligand/ClO_4 stoichiometry. This corresponds to complexes of general formula $[Zn(L)](ClO_4)$ with a deprotonated, that is, phenolate ligand. X-ray quality crystals of $[Zn(L^1)](ClO_4)$ were grown out of a methanol solution of the complex. The crystal structure (Figure 3) displays a distorted tetrahedral Zn ion coordinated to both imidazole arms and to the nitrogen and oxygen donors of the amino-phenolate arm of ligand L^1. The calixarene stands in a cone conformation. The complex crystallized with a methanol molecule that is buried in the calixarene cavity but not coordinated to the Zn ion. This solvate molecule is connected to a hydrogen bond network involving two calixarene oxygen atoms and the NH of the amino-phenolate arm [d(O2,N1) = 3.16 Å, d(N1,O1w) = 2.95 Å, d(O1w,O1) = 2.72 Å]. This stands in strong contrast with all of the other structures of calix[6]arene-based complexes we have reported so far, for which the included molecule was coordinated to the metal ion. In the present structure, the OCH_2CH_2N arm of the

(10) In the absence of an organic guest, the NMR spectrum of the dicationic complex in $CDCl_3$ displays very broad peaks whatever the temperature (243−328 K). Therefore, we cannot determine the precise nature of the complex in solution ($\{[Zn(L^1H)(H_2O)](ClO_4)_2\}$ with a water molecule coordinated to the zinc ion or $\{[Zn(L^1H)](ClO_4)_2\}$ with an empty cavity. In the case of complexes $\{[Zn(L^1H)(G)](ClO_4)_2\}$, the resonances of the calixarene ligand were either broad (G = DMF) or sharp (S = acetonitrile, ethanol), depending on the included guest. This may be related to the kinetics of the helical twisting of the binding arms, as observed for complexes with N_3 ligands (see ref 11).

(11) Sénèque, O.; Rondelez, Y.; Le Clainche, L.; Inisan, C.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *Eur. J. Inorg. Chem.* **2001**, 2597.
(12) Blanchard, S.; Rager, M.-N.; Duprat, A. F.; Reinaud, O. *New J. Chem.* **1998**, 1143.

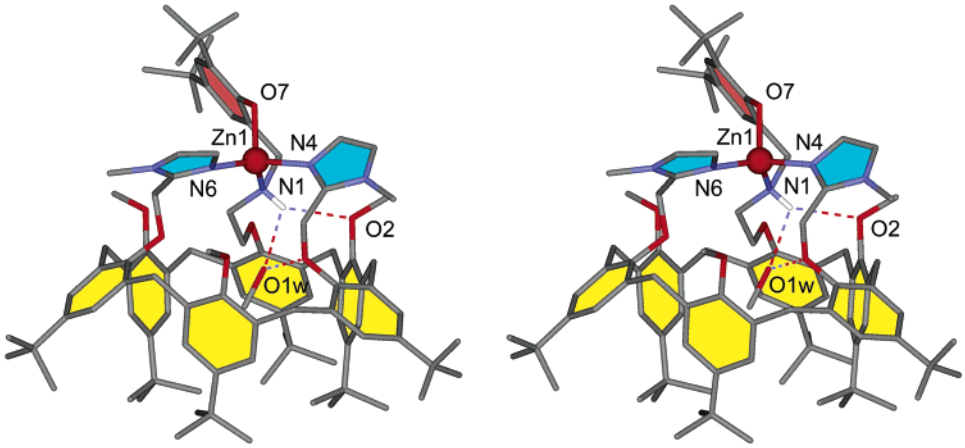


Figure 3. Stereoview of the X-ray structure of complex $[Zn(L^1)](ClO_4)\cdot CH_3OH$. Hydrogen atoms (excepting NH), the perchlorate counterion, and solvate molecules of crystallization have been omitted for clarity. Selected bond lengths (Å) and angles (deg): Zn1−O7 1.929(6), Zn1−N6 1.997(8), Zn1−N4 2.052(9), Zn1−N1 2.062(6), O7−Zn1−N6 106.9(4), O7−Zn1−N4 102.8(4), N6−Zn1−N4 109.4(3), O7−Zn1−N1 100.4(3), N6−Zn1−N1 128.6(3), N4−Zn1−N1 105.7(3).

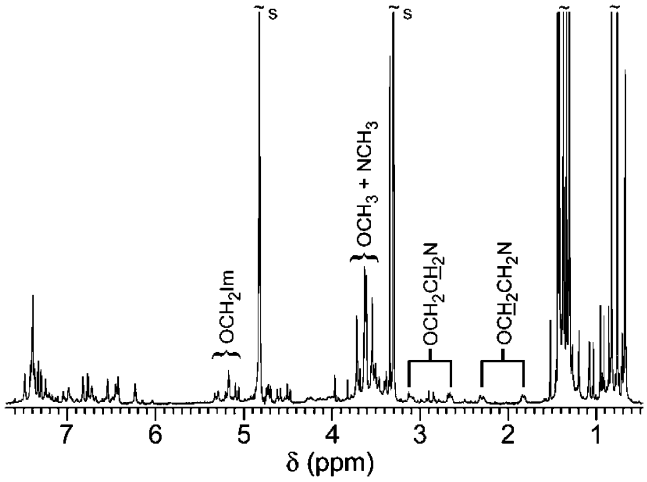


Figure 4. ^1H NMR (400 MHz, $CD_3OD/CDCl_3$ 5:1, 298 K) spectrum of complex $[Zn(L^1)]$. The solvent peaks are labeled "s". The minor species, particularly visible in the *t*Bu region, was not identified.

ligand is actually partially located in the aromatic cavity [the distances between OCH_2 and two aromatic rings of the calixarene (C···Ar = 3.34 and 3.51 Å) denote CH/π interactions].

The ^1H NMR spectra recorded either in pure $CDCl_3$ or in a $CD_3OD/CDCl_3$ mixture (Figure 4) were very similar. They showed *t*Bu groups in an alternate in/out position and methoxy groups rejected away from the conic cavity (δ > 3.5 ppm). The OCH_2 group belonging to the amino-phenolate arm displayed two resonances (multiplets at 1.84 and 2.30 ppm) that are upfield shifted as compared to both the free calix-ligand (singlet at 4.03 ppm) and the dicationic complex. This indicated that the complex underwent partial self-inclusion of its amino arm with the Zn ion in a 4-coordinate N_3ArO environment in agreement with the solid-state structure. In contrast, the spectrum recorded in pure CD_3CN showed no methylene signal at around 2 ppm, indicating that the OCH_2CH_2N arm has been expelled from the cavity, which advocates the binding of MeCN within the calixarene cavity and thus formation of $[Zn(L^1)(MeCN)]^+$.[13] The host−guest behavior of $[Zn(L^1)]^+$ was further investigated in $CDCl_3$ (3 mM in complex) with various coordinating molecules. At room temperature, no change in the NMR profile of the complex could be observed upon the addition of up to 20 molar equiv of a small coordinating molecule such as EtOH, DMF, MeOH, or MeCN. At 243 K, binding of the formers two to the metal center was evidenced by the appearance of characteristic upfield shifted resonances attesting to the formation of $[Zn(L^1)(G)]^+$ with G = EtOH or DMF as a very minor species (less than 10%) (Scheme 1, Figure 1). Hence, the anionic environment provided by the deprotonated ligand L^1 allows the stabilization of two different species. One is a 4-coordinate Zn complex with the amino-arm in endo-position and the cavity likely occupied by a solvent molecule that does not bind to the metal center and is in fast exchange with the bulk. The other is a 5-coordinate complex with a guest ligand that has expelled the amino-arm. The ratio of these two species depends on the "quality" of the guest, that is, its relative affinity for the calixarene cavity and its donor ability.[14]

With ligand L^2, both 4-coordinate $[Zn(L^2)]^+$ and 5-coordinate $[Zn(L^2)(MeOD)]^+$ species were observed in a $CD_3OD/CDCl_3$ 5:1 mixture. Indeed, two doublets at 2.46 and 1.95 ppm attested to the presence of complex $[Zn(L^2)]^+$ with the amino-phenolate arm partially included in the cavity, as for $[Zn(L^1)]^+$, beside multiple resonances that accounted for the coexistence of the 5-coordinate complex. In contrast, upon the addition of only a few molar equivalents of a guest (ca. 10 equiv of G = EtOH, MeCN, EtCN, DMF; see Figure 1) in $CDCl_3$, the formation of endo-complexes $[Zn(L^2)(G)]^+$ was clearly evidenced by the disappearance of the 2.46 and 1.95 ppm signals at room temperature, associated with the appearance of guest resonances below 0 ppm observable at 243 K.[14]

The host/guest behaviors of complexes $[Zn(L^1H)]^{2+}$, $[Zn(L^2)]^+$, and $[Zn(L^1)]^+$ were then compared at 243 K, the temperature at which quantification of the coordinated guest was possible for all complexes due to slow exchange. First, a ^1H NMR study showed that the addition of 10 molar equiv of DMF to a 3 mM $CDCl_3$ solution of each complex led to the formation of 100%, 35%, and 1% of DMF adducts, $[Zn(L^1H)(DMF)]^{2+}$, $[Zn(L^2)(DMF)]^+$, and $[Zn(L^1)(DMF)]^+$, respectively.

(13) This spectrum is nearly identical to that of $[Zn(L^2)(MeCN)]^+$ in CD_3CN, a complex which was readily formed in $CDCl_3$ with only a few molar equivalents of MeCN.

(14) H_2O and MeOH are good donors but lack a lipophilic chain. Therefore, they are not "good" guests (see refs 7, 8).

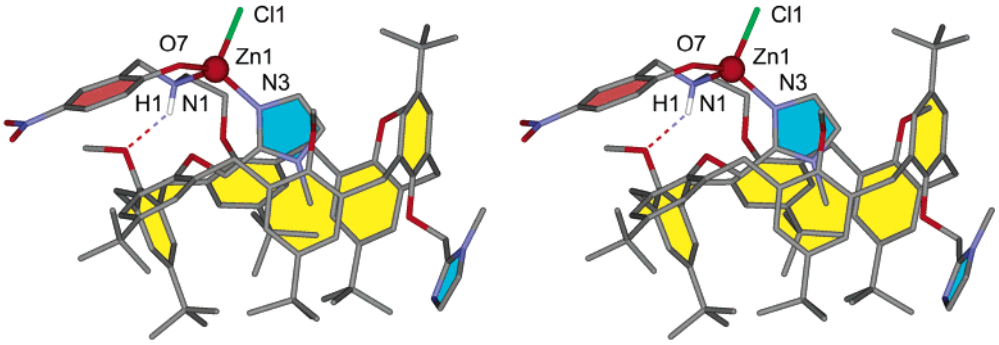


Figure 5. Stereoview of the X-ray structure of complex [Zn(L^2)(Cl)]. Hydrogen atoms (excepting NH) and solvate molecules of crystallization have been omitted for clarity. Selected bond lengths (Å) and angles (deg): Zn1−Cl1 2.228(2), Zn1−N1 2.037(3), Zn1−N3 1.979(4), Zn1−O7 1.943(3), N1−Zn1−N3 112.2(2), N1−Zn1−O7 97.7(2), N1−Zn1−Cl1 109.0(2), N3−Zn1−O7 117.9(2), N3−Zn1−Cl1 109.4(2), O7−Zn1−Cl1 109.0(2).

Hence, from a thermodynamic point of view, [Zn(L^2)]$^+$ behaved as a better receptor than did [Zn(L^1)]$^+$, but not as good as the dicationic [Zn(L^{1}H)]$^{2+}$. A similar experience, conducted with EtOH as a guest, allowed the comparison of the line widths $\Delta\nu$ of the high-field shifted CH_3 resonance of included EtOH for each of the three complexes (243 K, Figure 1). Hence, the measured $\Delta\nu$ values, which were not dependent on the guest concentration (in the 5−50 mM range), were 3.8, 22, and 80 Hz for complexes [Zn(L^{1}H)(EtOH)]$^{2+}$, [Zn(L^2)(EtOH)]]$^+$, and [Zn(L^1)(EtOH)]]$^+$, respectively. Assuming a dissociative mechanism for the guest exchange,[15] this indicates that the exchange rate increases in the order [Zn(L^{1}H)(G)]$^{2+}$ < [Zn(L^2)(G)]$^+$ < [Zn(L^1)(G)]$^+$, that is, following an increase of the donor ability of the calixarene-based coordination core. Indeed, the stronger is the Zn Lewis acidity, the stronger is the Zn−guest bond and the slower is the exchange rate.

Neutral [*N*$_3$*ArO*ZnX] Complexes. Addition of excess triethylamine to a $CD_3OD/CDCl_3$ (5:1) solution of [Zn(L^2)](ClO$_4$) yielded a new species whose ^{1}H NMR spectrum was totally different from those of both the di- and the monocationic Zn complexes with an intriguing singlet integrating for three H at around 0 ppm. The same spectrum was obtained upon the addition of 1 equiv of NaOH, which suggests the formation of a zinc-hydroxo (or methoxo) complex [Zn(L^2)(OH)] (Scheme 1). Reaction of [Zn(L^2)](ClO$_4$) with NaCl, NaN_3, or $PhCO_2Na$ under similar conditions led to species displaying the same ^{1}H NMR profiles with only slightly different chemical shifts. All of them presented the singlet in the 0.0−0.5 ppm region. This was indicative of the formation of complexes [Zn(L^2)(X)] with X = Cl, N_3, and $PhCO_2$, respectively (Scheme 1). In the latter case, the resonances of the bound $PhCO_2^-$ anion were located at 8.07 (*p*), 7.43 (*m*), and 7.35 ppm (*o*), showing that the coordinated anion (X^- = $PhCO_2^-$) lay outside of the cavity. These complexes were quite resistant as no decoordination of Zn occurred in the presence of anion excess (except when using NaOH).

Slow evaporation of a methanol/chloroform solution of complex [Zn(L^2)(Cl)] yielded yellow crystals suitable for X-ray analysis. The molecular structure of [Zn(L^2)(Cl)] displays a mononuclear complex with a tetrahedral Zn^{2+} ion coordinated by the nitrogen and oxygen atoms of the amino-phenolate arm, one imidazole of the calixarene ligand, and a chloride ion (Figure 5). The Zn−N$_{Im}$ bond length [1.979 (3) Å] is slightly shorter than the Zn−N$_{amine}$ bond length [2.037(3)]. Zn−O and Zn−Cl distances are similar to those observed in other tetrahedral zinc−phenolate[16] and zinc−chloride complexes.[17] The calix[6]arene stands in a partial cone conformation with the aromatic ring bearing the noncoordinated imidazole arm turned upside down. The Zn-bound imidazole is buried in the heart of the calixarene cavity with its NCH_3 group at the level of the centroids of the five aromatic rings delineating the walls of the cavity. The structure is thus stabilized by a CH/π interaction between the included imidazole and the upside down aromatic ring [*d*(C···Ar) = 3.6 Å and *d*(H···Ar) = 2.8 Å]. The coordinated phenol ring is parallel to the aromatic unit bearing the included imidazole, revealing a π/π interaction (the distance between the two C=C bonds involved in this interaction is 3.4 Å). Finally, the NH group of the amino-phenolate arm is hydrogen-bonded to a methoxy group [*d*(N1,O2) = 2.869 Å and *d*(H1,O2) = 2.139 Å, −(N1,H1,O2) = 131.9°].

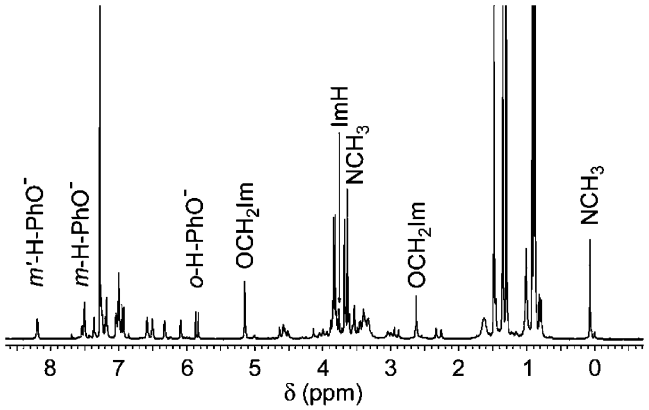


Figure 6. ^{1}H NMR (250 MHz, $CDCl_3$, 300 K) spectrum of complex [Zn(L^2)(Cl)].

In light of the X-ray structure, we investigated in detail the solution structure of complex [Zn(L^2)(Cl)] by ^{1}H (Figure 6) and ^{13}C NMR spectroscopy. Attribution of all signals was performed by COSY, HMBC, HMQC, and ROESY (τ_m = 200 ms) experiments. The ^{13}C δ chemical shifts of the two bridging methylene groups linked to one $ArOCH_2Im$ unit are ca. 36 ppm, whereas the four others display resonances at around 30 ppm. This indicates a partial cone conformation[18] for the calixarene

(15) An associative mechanism implying the coordination of a sixth ligand to zinc is highly unlikely, due to sterical overcrowding at the metal center in such an environment.

(16) (a) Abufarag, A.; Vahrenkamp, H. *Inorg. Chem.* **1995**, *34*, 3279. (b) Trösch, A.; Vahrenkamp, H. *Inorg. Chem.* **2001**, *40*, 2305.
(17) Orpen, A. G.; Brammer, L.; Allen, F. H.; Kennard, O.; Watson, D. G.; Taylor, R. *J. Chem. Soc., Dalton. Trans.* **1989**, S1.
(18) Kanamatharedy, S.; Gutsche, C. D. *J. Org. Chem.* **1994**, *59*, 3871.

structure, with the corresponding aromatic unit upside down as compared to the others. This was confirmed by the presence of ROE cross-peaks between the protons of the imidazolyl arm (OCH_2Im) and the aromatic and *t*Bu protons belonging to the adjacent anisole groups. The self-inclusion of the other imidazole ring was inferred from the high-field chemical shifts of all H atoms belonging to the OCH_2Im moiety: the singlet at 0.05 ppm corresponded to the NCH_3 group located in the heart of the calixarene cavity ($\Delta\delta \approx -3.5$ ppm), and the singlet at 2.61 ppm was attributed to the OCH_2 group ($\Delta\delta \approx -2.4$ ppm).

Hence, in solution, as in the solid state, neutral complexes $[Zn(L^2)(X)]$ adopted a rare[19] 1-alternate conformation [(u,u,u-,u,u,d) type in the Gutsche nomenclature],[20] which was stabilized thanks to the self-inclusion of one imidazole unit into the calix-[6]cavity.[21] $[Zn(L^1)](ClO_4)$ presented the same reactivity as $[Zn(L^2)](ClO_4)$ toward anions leading to $[Zn(L^1)(X)]$ complexes that, according to their ^1H NMR spectra, had a structure close to $[Zn(L^2)(X)]$. Finally, [Zn(L)(X)] complexes appeared absolutely insensitive to small neutral guests, according to various ^1H NMR investigations. Thus, in contrast to the di- and monocationic complexes based on ligands L^i, these neutral complexes did not behave as molecular receptors. Finally, and quite interestingly, when aliquots of NaOH were added to a $CD_3OD/CDCl_3$ (5:1) solution of $[Zn(L^1H)(DMF)]^{2+}$ (obtained upon the addition of ca. 40 molar equiv of DMF), the zinc complex progressively evolved toward the monocationic and then the neutral form with final ejection of the DMF guest. Subsequent addition of $HClO_4$ (or HPF_6) completely reversed the process, with the progressive appearance of the monocationic and then the dicationic species and back endo-complexation of DMF (Figure 7).

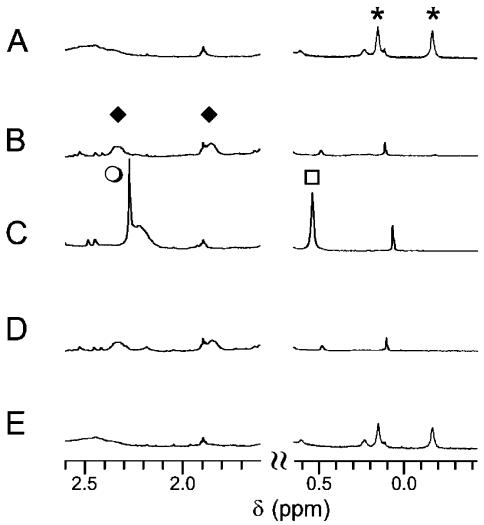


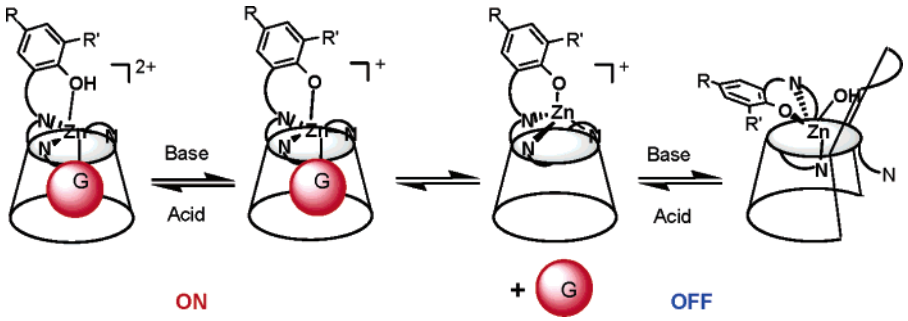
Figure 7. Titration of complex $[Zn(L^1H)(DMF)]^{2+}$ by NaOH and back-titration by $HClO_4$. Only characteristic regions of ^1H NMR spectra (500 MHz, $CD_3OD/CDCl_3$ 5:1, 298 K) are displayed. The initial solution contained $[Zn(L^1H)](ClO_4)_2$ (3 mM) and DMF (40 equiv). (A) Initial spectrum showing $[Zn(L^1H)(DMF)]^{2+}$ (*: C*H*$_3$ of the included DMF), (B) spectrum after addition of 1 equiv of NaOH (solution in CD_3OD) showing $[Zn(L^1)]^+$ (◆: OC*H*$_2$CH$_2$N), (C) spectrum after addition of a second equivalent of NaOH showing $[Zn(L^1)(OH)]$ (□, NC*H*$_3$; ○, OC*H*$_2$Im), (D) spectrum after subsequent addition of 1 equiv of $HClO_4$ (solution in CD_3OD) showing $[Zn(L^1)]^+$, and (E) spectrum after final addition of a second equivalent of $HClO_4$ (showing $[Zn(L^1H)(DMF)]^{2+}$).

Discussion and Conclusion

The calixarene-based N_3Ar*O*H ligands LH allowed the characterization of three different mononuclear complexes, a dicationic, a monocationic, and a neutral one. In the dicationic phenol complex $[Zn(L^1H)(G)]^{2+}$, Zn(II) displays a 5-coordinate environment with an exchangeable guest ligand G (a small amide, alcohol, or nitrile) confined in the heart of the calixarene cavity. It behaves as a good molecular receptor for neutral molecules such as its parent tetrahedral calix[6]N_3−Zn complexes.[7,8] The monocationic zinc−phenolate complexes exist as an equilibrated mixture of a 4-coordinate form $[Zn(L)]^+$ with no endo-coordination and a 5-coordinate form $[Zn(L)(G)]^+$ with an included coordinated guest G. Hence, deprotonation of the phenol binding site leads to a decrease of the receptor ability of the metal center. Subsequent deprotonation of a water molecule (or addition of an anion X^-) drives the calixarene system to undergo an impressive structural reorganization. One imidazole arm is released by the metal center, opening thereby a coordination site for the anion that points away from the calixarene structure. The other imidazole arm, still bound to the metal center, undergoes self-inclusion in the calixarene π-basic pocket, to fill the cavity. Hence, the resulting neutral complex [Zn(L)(X)] appears reluctant to endo-binding of a guest as neither OH^- nor a halide ($X^- = Cl^-$) nor a neutral ligand G have ever been detected. From this, three points may be emphasized: (i) as previously observed with other calixarene-based systems, the π-basic cavity of calixarenes appears reluctant to anionic guests,[21,22] which stands in contrast to nonaromatic molecular receptors such as cyclodextrins;[23] (ii) the binding of two anions (ArO^- and X^-) decreases the Zn Lewis acidity down to a point that the metal center refuses to bind a fifth donor, which leads to the loss of the receptor properties of the system; (iii) this calix[6]N_3Ar*O*H-based host−guest system is thermodynamically and kinetically tunable. On one hand, the dicationic zinc−phenol complex is a better receptor than the monocationic zinc−phenolate one, whereas the uncharged complex does not respond to neutral guests at all. On the other hand, the electron-donating ability of the ArO-(H) ligand allowed the tuning of the Lewis acidity of the metal center. Indeed, the *p*NO_2 substituted one (L^2) yields a monocationic complex that is more sensitive to the binding of a guest than the *o*,*p*-*t*Bu-substituted one (L^1). Hence, the Zn affinity for a guest increased in the order $[Zn(L^i)(X)] \ll < [Zn(L^1)]^+ < [Zn(L^2)]^+ < [Zn(L^1H)]^{2+}$. The kinetics of these host−guest exchange processes appear to be tuned in the same way as is thermodynamics, in agreement with a dissociative process. The more electron-rich is the metal center, the more labile is the Zn−G link. Thus, the additional phenol/phenolate coordinating site present in these novel calix[6]N_3Ar*O*(H) ligands as compared to the former calix[6]N_3 systems allowed the tuning of the receptor properties.

(19) A rare example has been recently reported. See: Akine, S.; Goto, K.; Kawashima, T. *Tetrahedron Lett.* **2003**, *44*, 1171.
(20) Gutsche, C. D. In *Calixarenes, Monographs in Supramolecular Chemistry*; Stoddart, J. F., Ed.; The Royal Society of Chemistry: Cambridge, U.K., 1989.
(21) The self-inclusion of one imidazole unit into the calix[6]cavity has been previously observed in a tetranuclear Cu(II) complex that was characterized by X-ray diffraction analysis. Sénèque, O.; Campion, M.; Douziech, B.; Giorgi, M.; Rivière, E.; Journaux, Y.; Le Mest, Y.; Reinaud, O. *Eur. J. Inorg. Chem.* **2002**, 2007.
(22) This has also been observed with other calixarene-based complexes. See, for example: Darbost, U.; Zeng, X.; Rager, M.-N.; Giorgi, M.; Jabin, I.; Reinaud, O. *Eur. J. Inorg. Chem.* **2004**, 4371.
(23) Engendilger, E.; Armspach, D.; Matt, D. *Chem. Rev.* **2004**, *104*, 4147.

Scheme 2. Acid−Base Switch for Guest Binding by the Calix[6]N_3Ar*O*H-Based Zn Complexes[a]



[a] G is either a water molecule or a small organic ligand such as DMF, EtOH, or MeCN.

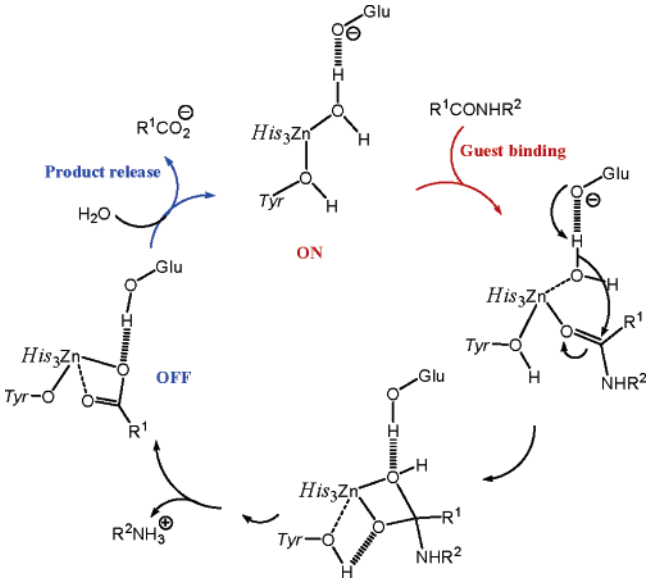
Interestingly, the system behaves as an acid−base switch (Scheme 2 and Figure 7). First, the dicationic form that presents the strongest Lewis acidic Zn center stands for the "on" state as it will host a small guest ligand G. Second, deprotonation of the phenol site labilizes the Zn−Guest bond, and the resulting monocationic complex will either remain in an "on" state or become in an "off" state with the release of the guest ligand depending on its nature and concentration. Third, further addition of base drives the equilibrium to a full "off" state with the self-inclusion of the imidazole arm. Conversely, addition of aliquots of acid to the neutral complex ("off" state) drives back the equilibria to the "on" states in the presence of a guest G.

Additional Comments on the Role of a Coordinated Tyrosine Residue in Hydrolytic Zn Enzymes. In the proteases of the astacin[3] and serralysin[4] families, the active site Zn(II) is coordinated to three histidine residues, a tyrosine side chain, and a water molecule that is hydrogen bonded to a glutamate. The activity of these enzymes presents a bell-shaped pH profile involving at least two ionizable groups, one being the coordinated tyrosine. This tyrosine residue has been shown to play a significant role in the action of the enzyme, stabilizing the enzyme−substrate and the transition state complexes, most likely via H-bonding with the substrate and the developing tetrahedral oxy-anion intermediate, respectively.

This study nicely illustrates the effect of the protonation state of an ArOH ligand on substrate binding. The $(Zn-ArOH)^{2+}$ core clearly favors substrate binding relative to the deprotonated one, $(Zn-ArO)^{+}$, which conversely plays in favor of product release. This point is consistent with a mechanism where TyrOH also acts as a general acid in the transition state, as recently proposed.[4d] Our system also highlights the sharp off-switch effect on substrate binding of the simultaneous coordination of a phenolate and a hydroxide to the Zn^{2+} ion. This second point substantiates the proposal according to which the inactive state of the enzyme at high pH corresponds to the neutral core [Zn(ArO)(OH)].[4d] Hence, one of the roles of the Tyr residue encountered in these Zn enzymes would be to accurately control the activity as the pH varies, acting as an off-switch upon a pH raise.

Still, the mechanism of the hydrolytic catalysis remains controversial and the precise nature of the active state is unknown. This is due to the high number (3) of acid−base couples involved in the catalytic process (Glu, Tyr, and water). Our study plays in favor of a dicationic $[Zn(His)_3(ArOH)(H_2O)]^{2+}$ active state as (i) it displays, by far, the highest Lewis acidity for substrate binding, (ii) the H-bonded Glu residue can play its role of general base and activate the coordinated water molecule, and (iii) the protonated Tyr residue can act as a general acid, hence lowering the activation energy required for the formation of the tetrahedral intermediate. Taken together, these three statements lead us to propose an alternative mechanism for the peptidase activity of this family of enzymes as schematized Scheme 3.

Scheme 3. Proposed Mechanism for the Peptidase Activity of Zn-Enzymes of the Astacin and Serralysin Families



Substrate binding first occurs to the dicationic core $[Zn(His)_3(ArOH)(H_2O)]^{2+}$ ("on" state for substrate binding). Subsequent attack of H_2O assisted by the general base Glu leads to a tetrahedral intermediate that is stabilized by the general acid TyrOH. The amino group departure leaves a $[Zn(TyrO)(RCO_2)]$ core that is neutral ("off" state). The latter evolves toward product release (the carboxylate group) with regeneration of the dicationic state after proton exchange.[24,25]

Experimental Section

Material and Methods. All solvents and reagents were obtained commercially. THF was distilled over sodium/benzophenone under argon. CH_2Cl_2 was distilled over CaH_2 under argon. ^1H NMR spectra were recorded either on a Bruker ARX 250, Avance 400, or Avance 500 spectrometer. IR spectra were recorded on a Perkin-Elmer 783 spectrometer. Elemental analyses were performed at the Institut de

(24) Finally, in our proposed catalytic cycle, inactivation of the enzyme at low pH could stem from the protonation of the glutamate residue.
(25) Such a mechanism cannot be efficient in our calixarene-based system as the narrow rim of the macrocycle precludes the stabilization of a tetrahedral intermediate in the endo-position for sterical reasons.

Chimie des Substances Naturelles, Gif s/Yvette, France. Ligands L^iH ($i = 1$ and 2) were synthesized according to the procedure previously described.[9]

Safety Note. Caution! Although we have not encountered any problem, it is noteworthy that perchlorate salts of metal complexes with organic ligands are potentially explosive and should be handled only in small quantities with appropriate precautions.

Synthesis of $[Zn(L^1H)(H_2O)](ClO_4)_2$. Under an argon atmosphere, THF (1 mL) was added to a mixture of $[Zn(H_2O)_6](ClO_4)_2$ (17.1 mg, 0.046 mmol) and L^1H (67.3 mg, 0.046 mmol). The solution was stirred for 30 min at room temperature and precipitated with pentane. The solid was separated from the supernatant by centrifugation, washed twice with pentane, and dried under vacuum (55 mg). Yield: 67%. IR (KBr): $\nu = 3502$ (H_2O), 1505, 1481, 1463, 1416, 1394, 1363, 1115 (ClO_4^-), 625 (ClO_4^-) cm^{-1}. Anal. Calcd for $[Zn(C_{96}H_{129}N_5O_7)(H_2O)](ClO_4)_2 \cdot 5H_2O$: C, 62.75; H, 7.73; N, 3.81. Found: C, 62.58; H, 7.31; N, 3.44.

Synthesis of $[Zn(L^1)](ClO_4)$. Triethylamine (19.3 μL, 0.138 mmol) was added to a solution of de L^1H (101.5 mg, 0.069 mmol) and $[Zn(H_2O)_6](ClO_4)_2$ (25.8 mg, 0.069 mmol) in MeOH (2 mL). After 10 min, a white precipitate appeared. The mixture was further stirred for 1 h. The solid was separated from the supernatant by centrifugation, washed with MeOH (5 × 0.5 mL), and dried under vacuum (85 mg). Yield: 74%. IR (KBr): $\nu = 3503$ (H_2O), 1502, 1481, 1465, 1416, 1393, 1362, 1108 (ClO_4^-), 623 (ClO_4^-) cm^{-1}. Anal. Calcd for $[Zn(C_{96}H_{128}N_5O_7)](ClO_4) \cdot 2H_2O$: C, 69.25; H, 7.99; N, 4.21. Found: C, 69.29; H, 7.95; N 4.09.

Synthesis of $[Zn(L^2)](ClO_4)$. Triethylamine (18.3 μL, 0.132 mmol) was added to a solution of de L^2H (92 mg, 0.066 mmol) and $[Zn(H_2O)_6](ClO_4)_2$ (24.5 mg, 0.066 mmol) in MeOH (1 mL) and CH_2Cl_2 (0.5 mL). The mixture was slowly concentrated to one-third, and a precipitate appeared. The yellow solid was separated from the supernatant by centrifugation, washed with MeOH (2 × 0.5 mL) and then pentane (2 × 0.5 mL), and dried under vacuum (82.7 mg). Yield: 80%. IR (KBr): $\nu = 3530$ (H_2O), 1602 (NO_2), 1505, 1492, 1482, 1420, 1397, 1365, 1100 (ClO_4^-), 624 (ClO_4^-) cm^{-1}. Anal. Calcd for $[Zn(C_{88}H_{111}N_6O_9)](ClO_4) \cdot 4H_2O$: C, 64.69; H, 7.34; N, 5.14. Found: C, 64.57; H, 7.01; N, 5.14.

Synthesis of $[Zn(L^2)(Cl)]$. This complex can be generated by addition of NaCl to a solution of $[Zn(L^2)](ClO_4)$ in MeOH. It can be obtained with ~5% $[Zn(L^2)](ClO_4)$ impurity by the following procedure. NaCl (30 mg) was added to a solution of $[Zn(L^2)](ClO_4)$ (30 mg, 0.019 mmol) in $CH_3OH/CHCl_3$ 9:1. The reaction mixture was stirred for 4 h, filtered over Celite, and evaporated. The solid was dissolved in MeCN, filtrated over Celite, and evaporated to yield a yellow powder. ^1H NMR (500 MHz, $CDCl_3$): *t*Bu, $\delta = 0.86$, 0.88, 0.90, 1.28, 1.34, 1.46 (6 s, 9H each); $ArCH_2Ar$, $\delta = 2.28$ and 3.36 (^{13}C $\delta = 28.2$), 2.90 and 3.42 (^{13}C $\delta = 30.1$), 3.35 and 3.55 (^{13}C $\delta = 29.8$), 3.40 and 4.59 (^{13}C $\delta = 31.4$), 3.50 and 3.67 (^{13}C $\delta = 35.8$), 3.81 and 3.88 (^{13}C $\delta = 36.3$) (12 d ($J \approx 15$ Hz), 1H each); ArH, $\delta = 6.07$ and 7.19, 6.31 and 7.03, 6.49 and 6.57, 6.97 and 6.99, 6.98 and 7.22, 7.34 and 7.48 (12 d ($J \approx 2$ Hz), 1H each); OCH_3, $\delta = 3.65$, 3.79, 3.81 (3 s, 3H each); OCH_2Im, $\delta = 2.61$ and 5.13 (2 s, 2H each); NCH_3, 0.05 and 5.13 (2 s, 2H each); ImH, $\delta = 6.93$ and 7.16, 3.74 and 6.91 (4 d ($J \approx 1.5$ Hz), 1H each); OCH_2CH_2N, $\delta = 3.32$ and 3.98 (2 m, 1H each); OCH_2CH_2N, $\delta = 3.00$ et 3.30 (2 m, 1H each); NH, $\delta = 4.03$ (m, 1H); NCH_2Ar, 3.58 and 4.52 (2 m, 1H each); *p*-NO_2-phenolate, 5.81 (*ortho*, d ($^3J = 9.2$ Hz), 1H), 7.51 (*meta*, dd ($^3J = 9.2$ Hz and $^4J = 2.9$ Hz), 1H), 8.17 (*meta*, d ($^4J = 2.9$ Hz), 1H).

X-ray Structures. X-ray quality crystals of $[Zn(L^1)](ClO_4)$ were grown by slow evaporation of a 3 mM solution of this complex generated in situ by addition of excess triethylamine into a methanol solution of complex $[Zn(L^1H)](ClO_4)_2$.[26] X-ray quality crystals of $[Zn(L^2)](Cl)$ were grown by slow evaporation of a 3 mM solution of this complex generated in situ by addition of NaCl into a chloroform/methanol solution of complex $[Zn(L^2)](ClO_4)$.[26] Crystallographic data and experimental details are given in the Supporting Information.

Supporting Information Available: Crystallographic data and experimental details for $[Zn(L^1)](ClO_4)$ and $[Zn(L^2)](ClO_4)$. This material is available free of charge via the Internet at http://pubs.acs.org.

JA053620E

(26) $[Zn(L^1H)](ClO_4)_2$: $R[F > 4\sigma F] = 0.0830$; $wR[F^2 > 4\sigma F^2] = 0.2672$. $[Zn(L^2)](ClO_4)$: $R[F > 4\sigma F] = 0.0711$; $wR[F^2 > 4\sigma F^2] = 0.2124$. See Table S1 in the Supporting Information.