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First Zn^{II} Bowl-Complexes Modeling the Tris(histidine) Metallo-Site of Enzymes

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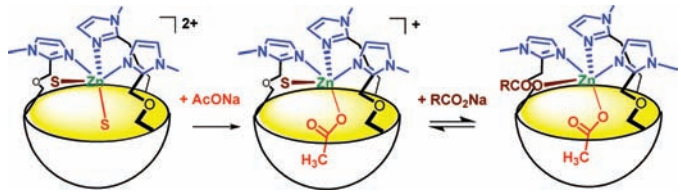
ABSTRACT



The bowl-shaped resorcin[4]arene-based ligand was prepared as a model of the trihistidine coordination core present in many mononuclear metalloenzymes. The $-CH_2-O-CH_2-$ linkers connecting the imidazoles to the cavity allow three imidazoles to simultaneously bind a metal ion, and favor cis-coordination of two exchangeable ligands. The corresponding mononuclear Zn^{II} complexes were shown to be capable of the selective guest binding and exchange at both endo and exo positions.

Modeling the active site of metallo-enzymes[1] is of fundamental importance for both the understanding of the catalysis, and the design of new artificial catalysts. Classical models aim at reproducing the first coordination sphere of the metal ion. Cavity effects are more difficult to mimic, while they obviously play a major role in substrate recognition, product release, but also in the intimate mechanism (e.g., associative vs dissociate ligand exchange, protection of reactive intermediates, control of solvent access, etc.). To evaluate such cavity effects, the design of model systems must associate a cavity to a biomimetic coordination core in such a way that, once bound, the metal ion will retain a labile coordination site either embedded in, or oriented toward the cavity. Hence, whereas the commonly encountered poly-histidine motif has guided the concept of a number of poly aza ligands of low molecular weight able to reproduce the first coordination sphere of the metal in metalloenzymes,[2] very few biomimetic complexes have combined a metal ion and a hydrophobic cavity.[3] Over the past decade, we have developed three generations of calix[6]arene-based supramolecular biomimetic zinc[4] or copper[5] containing systems. These metal complexes not only reproduce the first coordination sphere of the trishistidine core in the active site of a mononuclear metalloenzyme, but also provide the hydrophobic channel for the specific substrate access. The Zn^{II} calix-complexes are exceptionaly stable dicationic tetrahedral complexes that behave as selective receptors for neutral coordinating guests. However, they possess a single endo binding site with no possible cis-

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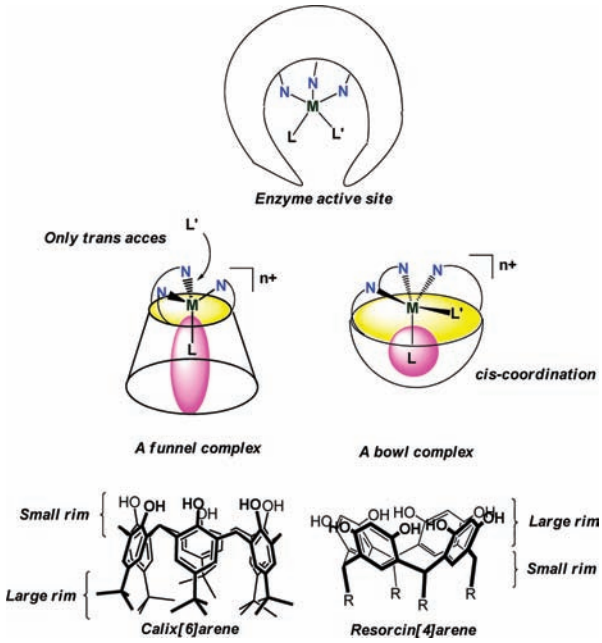
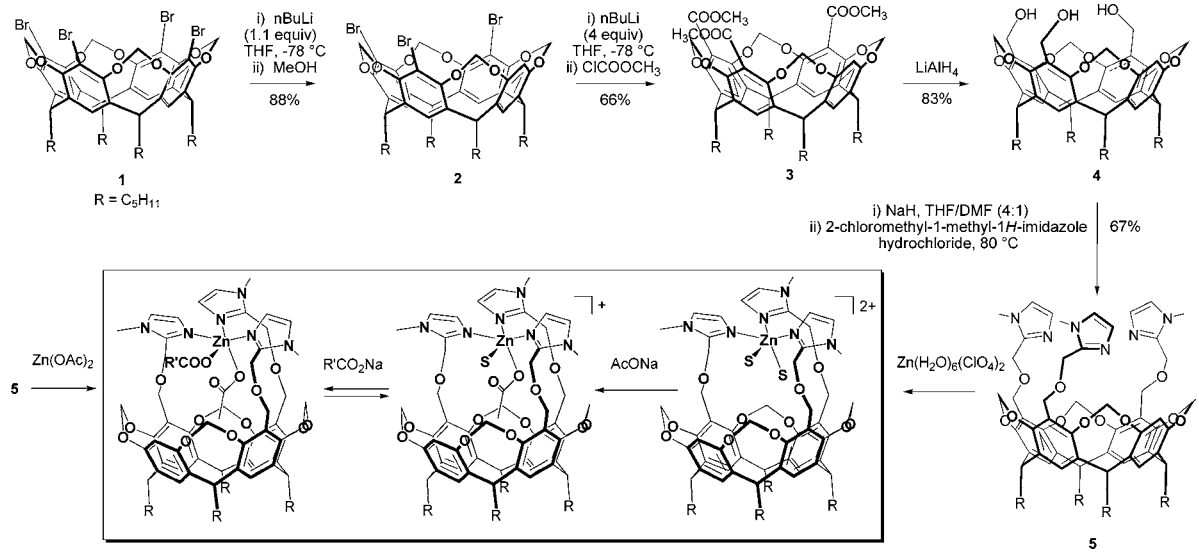


Figure 1. Schematized active site of a mononuclear metallo-enzyme and two families of cavity-based model complexes with their corresponding macrocyclic scaffold.

coordination of two guests at the level of the small rim, which is too narrow. The only way for a second ligand to interact with the metal center is in exo position, trans to the guest[4b,5b,c] (Figure 1). Another specific feature is their reluctance to anion binding at the endo site due to the oxygen-rich environment of the rim.[4b,5c] Many enzymes, however, involve in their catalytic cycles the formation of intermediates where two metal labile sites are occupied by the reactants, intermediates (often anionic) or products in cis-position. This is typically the case of proteases and nucleases.[6] Bearing this in mind, we wanted to design a novel system where the metal ion has two open coordination sites in cis position with at least one of them being oriented to and controlled by a cavity (Figure 1). Resorcinarene macrocycles are interesting scaffolds that can be rigidified into bowl-shaped cavities.[7,8] Some resorcinarenes have also been used as platforms to preorganize a set of ligands for metal ion binding.[9] None, however, has been designed for the coordination of a single metal ion possessing an introverted labile site. Also, although a few resorcinarene-based phospho-ligands have been described, very few amino-ligands have been reported,[10] and none of these can be considered as biomimetic.[11]

Here we describe the synthesis and coordination properties of the first resorcin[4]arene-based ligand featuring a biomimetic tris-imidazole coordination core. The two keys for the design of the ligand were: (i) decrease of the 4-fold symmetry of the resorcinarene scaffold in order to obtain a tripodal ligand and (ii) a wise choice of the length of the linkers between the imidazol units and the bowl-shaped cavity. These linkers must be long enough to allow all three imidazoles to bind to the same metal ion, but short enough to favor an intracavity coordination. The first requirement was achieved by selective debromination of one out of four aromatic units of the tetra-bromo resorcinarene **1** (Scheme 1) as described by Sherburn for a closely related compound.[12] The bowl-shaped macrocycle **1**,[13] obtained within three steps from resorcinol according to previously reported procedures, was reacted with 1 equiv of BuLi to yield, after protolysis, the new tribromo derivative **2**. In the following step, the three remaining bromo-substituted aromatic units of **1** were lithiated with *n*-BuLi, and treated with methylchloroformate to give triester **3**. Reduction of **3** with $LiAlH_4$ yielded triol **4**. Finally, reaction of **4** with 2-chloromethyl-1-methyl-1*H*-imidazole in the presence of NaH gave the desired N_3 ligand **5**. An overall yield of 32% (23% starting from resorcinol) was obtained, and the synthesis has been performed on a gram scale.

Scheme 1. Synthesis and Complexation of the Resorcinarene-Based Tris-Imidazole Ligand



A Zn^{II} complex was obtained by reacting ligand **5** with one equivalent of Zn^{II} perchlorate in ethanol. The corresponding complex spontaneously precipitated out of the solution as a white powder (86% yield). The elemental analysis was in agreement with a 1:1:2 ligand/Zn/perchlorate stoichiometry and 1 equiv each of water and EtOH. ESI mass analyses in MeCN showed $[Zn(\mathbf{5})(ClO_4)]^+$ as the principal ion, together with peaks corresponding to $[Zn(\mathbf{5})](HCO_2)]^+$, and $[Zn(\mathbf{5})Cl]^+$. No peak corresponding to a dication has ever been observed. The presence of the chloro- and formiato-complexes are to be ascribed to residual traces of chloride ions and formic acid in the mass apparatus, which highlights the high affinity of Zn^{II} for these small anions in the environment provided by the resorcinarene-ligand. The ^1H NMR analysis of the complex in $CDCl_3$ showed relatively broad resonances at both 250 and 500 MHz. However, a sharp spectrum was obtained in CD_3CN and all resonances could be assigned thanks to 2-D experiments. A comparison of the spectra recorded for the free ligand and the isolated Zn^{II} complex is presented in Figure 2. Both free ligand **5** and complex molecule are bisected by a mirror plane. This is attested by the presence of three resonances for the aromatic units in a 2:1:1 ratio for the four small rim protons together with one extra resonance for the single remaining large rim proton. The coordination of Zn^{II} to all three nitrogenous arms is best demonstrated by the splitting and shifts ($\Delta\delta \cong 0.6$ ppm) of the corresponding imidazole proton resonances.[14] The protons of the methylene group to which the imidazoles are connected also become well differentiated, two sets being downfield shifted, while one is upfield shifted. All these observations are consistent with the formation of a complex possessing a symmetry plane, with two imidazole arms bound at the two equivalent (opposite) positions, at both sides of the mirror plane, and one imidazole bound in the plane. Adding CH_3CO_2Na as a solid in excess into the CD_3CN solution of the complex, remarkably changed the spectrum (Figure 2). All protons belonging to the imidazole core underwent an important shift, thus denoting a change in the proximal environment of Zn^{II} in spite of the symmetry conservation. Most interestingly, a new sharp singlet was observed at −2.36 ppm accounting for 3H. This indicates the solubilization of one molar equivalent of acetate that is coordinated in endo position, ie. inside the bowl cavity, thus experiencing the shielding effect of the four aromatic units of the resocinarene macrocycle. When excess of solid acetate was solubilized, (adding a drop of D_2O), all resonances broadened with the exception of those corresponding to the free solvent, the pentyl feet (R in Scheme 1) and two sharp singlets emerging in the aromatic region, corresponding to one proton of each imidazole. The resonance broadening can be attributed to the chemical exchange between free and bound acetate, which was evidenced by saturation transfer experiments. Very similar NMR signatures, either in MeCN or $CDCl_3$/MeOD, were obtained with the complex synthesized in THF/MeOH through the stoichiometric reaction of $Zn(OAc)_2$ and ligand **5** (see the SI).

In a noncoordinating solvent such as $CDCl_3$, the addition of aliquots of various neutral coordinating molecules affected the acetato resonance (Figure 3). This indicates that the endobound acetato ligand senses the presence of additional exogenous ligands: upon addition of water, MeOH or MeCN, its environment is slightly changed. The splitting of the acetato resonance into two peaks in spectra c and d of Figure 3 is best explained by competitive binding of two different ligands in exo position (likely MeCN and water in c, MeCN and MeOH in e), whereas acetate occupies the endoposition. Importantly, this confirms that a fifth coordination site is accessible for the binding of a second exogenous ligand.

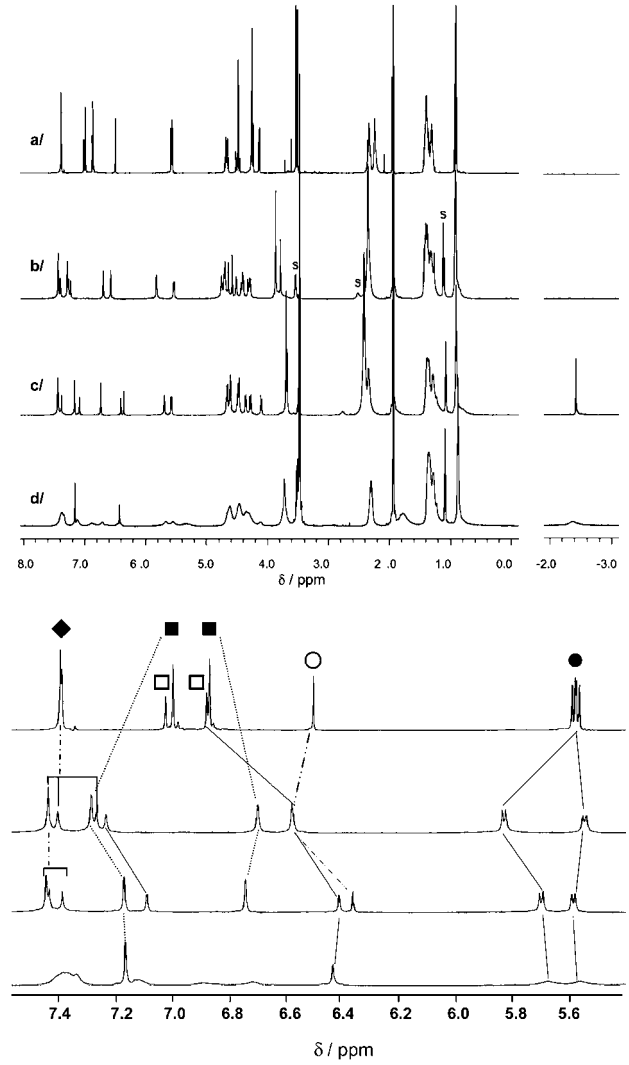


Figure 2. ^1H NMR spectra in CD_3CN of free ligand **5** (a/, 300 K, 500 MHz) and the corresponding perchlorato-Zn-complex (600 MHz) before (b/, 300 K) and after (c/, 253 K) the addition of solid NaOAc, and after further addition of D_2O (d/, 10% v/v, 253 K). s stands for one equiv of free EtOH. (Bottom) Enlargement of the aromatic region. $H_{aromatic\ down}$, ◆; $H_{imidazole\ external}$, ■; $H_{imidazole\ internal}$, □; $H_{aromatic\ up}$, ○; $O-CH_2-O_{out}$, ●.

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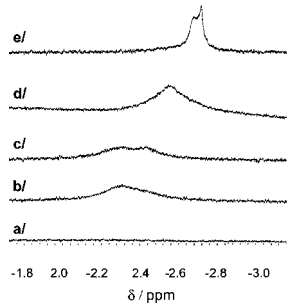

Figure 3. ^{1}H NMR (500 MHz) resonances of the endobound acetate of the acetato-Zn complex in $CDCl_3$ (confirmed by saturation transfer experiments). From bottom to top: (a) 300 K, (b) + CH_3CN (4% v/v, ca. 8 equiv, 263 K), (c) + D_2O (4% v/v, ca. 20 equiv, 263 K), (d) + CD_3CN (10% v/v, 263 K), (e) + CD_3OD (10% v/v, 263 K).

The selectivity of the endo site appeared to be very tight. Addition of sodium propionate to either the perchlorato dicationic complex or to the acetato-complex did not lead to the appearance of the corresponding endobound propionate derivative as analyzed by ^{1}H NMR at various T. Likewise, whereas coordination of acetate and smaller anions was readily observed by ESI mass spectroscopy, no peak corresponding to trifluoroacetate, propionate and at fortiori larger ones have ever been detected, even with a large excess relative to the Zn^{II} complex itself or relative to acetate added to the solution (up to 50 molar equiv). Nevertheless, the addition of propionate to the acetato complex led to the progressive broadening of the high-field resonance in the ^{1}H NMR spectra. This suggests that propionate cannot bind in endo position due to the dimensions of the cavity, but that it does interact in exoposition, thus labilizing the endobound acetate.

All presented observations are consistent with the formation of a 5-coordinate Zn complex as depicted in Scheme 1. Upon the addition of one equivalent of Zn, the three imidazole arms wrap the metal center to produce a species exhibiting a pseudoplanar symmetry with the additional coordination of one or two solvent or cosolvent molecule(s). Upon addition of 1 equiv of acetate, a monocationic species is produced, with one acetato ligand included in the bowl-shaped cavity. A fifth coordination site is then accessible in exo position and can be occupied by a neutral or an anionic ligand such as another carboxylate. The endo site is highly selective relative to the size of the guest as it is readily occupied by acetate but not by propionate. Intriguingly, the fifth ligand in exo position appears to tune the exchange rate of the endoligand. Simple modeling shows that the only way to construct such a 5-coordinate species is to consider the two labile sites in cis position relative to each other, as illustrated in Scheme 1. It is important to note that the successful design of the first member of a new cavity-based ligand family with a bowl shape fullfilling the requirements described in the introduction, opens the route toward a wide range of interesting studies: a small reactive species embedded in and protected by the bowl may react selectively with large substrates bound in exo position, or the converse: small substrate, large reactant. We are now exploring the coordination of other metal ions such as copper and the reactivity of the bowl-complexes toward various reactants and substrates.

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Supporting Information Available: Synthetic procedures, spectroscopic data and supplementary spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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