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EDGE ARTICLE

Recognition of primary amines in water by a zinc funnel complex based on calix[6]arene†

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A water-soluble calix[6]arene-based tris(imidazole) ligand behaves as a highly selective receptor for primary amines in the presence of Zn(II) in water near physiological pH. It represents the first compound of this family of ligands that binds a Zn dication and an organic guest in water, thus giving rise to a stable host–guest adduct in spite of the highly competitive medium. The herein described self-assembly process displays a remarkable set of biomimetic properties. The ternary system (calix/Zn/amine) is formed in a very synergistic and allosteric manner and stabilizes the neutral form of the amino guest with a spectacular pseudo-pK_a shift of *ca.* 7 units. This system constitutes an interesting structural model of metalloenzymes in aqueous solution.

Introduction

Natural receptors such as enzymes and antibodies show strong and selective host–guest complexation through multiple weak, non covalent interactions between the binding partners. These natural systems provide the inspiration for the rational design of synthetic receptors that can be used to gain an understanding of the binding forces that contribute to the formation of complexes. One of the goals of supramolecular chemistry is the creation of synthetic receptors that have both a high affinity and a high selectivity for the binding of guests.[1] For several decades now, very sophisticated and efficient receptors have been designed based on multiple electrostatic interactions or their recognition properties. However, most of the synthetic receptors have so far been studied in organic solvents, although recognition events in nature take place in aqueous media. Water-soluble receptors are rare. The design of synthetic receptors which can be used in water presents a special challenge.[2] First, the host needs to be soluble in water. This severely limits the type of building blocks which can be used for its construction. Second, special interactions and approaches have to be chosen to overcome the competitive influence of water. Indeed, water is both a H-bond donor and acceptor, and provides a medium of high polarity that stabilizes polar guests and decreases host–guest electrostatic attractive interactions. The hydrophobic effect, however, can drive an apolar guest into an apolar cavity, provided that enthalpic/entropic compensation is overcome.

Calix[*n*]arenes are among the most versatile and useful building blocks in supramolecular chemistry.[3] Unfortunately, unlike some other cavitands, parent calixarenes are not soluble in water. Water-soluble calixarenes have been obtained by attachment of sulfonates, carboxylic acids, phosphonates, amines, guanidinium groups, peptides and saccharides either directly or through linkers to the small or large rim. This chemistry has been mainly applied to calix[4]arenes.[4] The small size of their cavity, however, restrains their use as a molecular host and they have usually been exploited as platforms in order to gather and pre-organize weak interactions or functional groups. Calix[6]arenes are larger but highly flexible molecules. Their water-soluble sulfonate derivatives have been extensively studied.[5] Introduction of six charges at the large rim of the calixarene macrocycle leads to alternate conformations due to charge repulsion and prevents the formation of a cone conformation more suitable for guest inclusion.[6] Yet, interesting biological applications have been described, such as complexation of small bioactive molecules,[7] complexation with proteins,[8] ion channel mimics,[9] drug delivery[10] or enzyme inhibition.[11,12]

For the past decade, we have been developing biomimetic receptors based on calix[6]arenes.[13,14] The calix[6]arene macrocycles are selectively functionalized at the small rim by three nitrogen coordinating groups mimicking the first coordination sphere encountered in many metallo-enzyme active sites.[15] Upon binding to a metal cation, the conformational freedom of the calixarene is restricted and the cone conformation is favored. These so-called "funnel complexes" take advantage of the conic shape of the calix[6]arene: the geometry of the system constrains the metal center in a tetrahedral environment, orienting the fourth labile coordination site toward the inside of the cavity provided by the calixarene macrocycle. The hydrophobic cavity

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is reminiscent of the enzyme active site pocket governing the recognition event. These funnel complexes behave as remarkable biomimetic receptors for neutral molecules in organic solvents. Recently, we have dedicated part of our research to evaluate the properties of these systems in water. The selective functionalisation of the large rim of a tris(imidazolyl)calixarene by three sulfonate groups allowed the stabilisation of the first water-soluble copper(I) calix[6]arene funnel complex.[16] A related trisulfonato ligand was shown to self-assemble into "smart" vesicles.[17] More recently, we used the Copper-Catalysed-Alkyne-Azide-Cycloaddition (CuAAC)[18] methodology to achieve the functionalization of the large rim of a calix[6]arene *via* the tris(azido)calix[6]arene **1** (Scheme 1).[19] The thoughtful choice of the alkyne engaged in the reaction can lead to very attractive macrocycles bearing various functionalities at their large rim. As far as water solubilization is concerned, a tris(amino)calix[6]arene was prepared by this methodology. It was shown to form spontaneously very stable vesicles upon protonation by 3 equivalents of a sugar bearing a carboxylic acid group.[20]

The next step was to exploit the host–guest properties of our systems in water. We herein describe the synthesis of a new water-soluble derivative of a calix[6]arene-based tris(imidazolyl) ligand *via* the CuAAC methodology and its capability to behave as a receptor for primary amines in the presence of zinc(II) in water.

Results

Synthesis

In previous studies, we tackled the problem of water-solubilization of our systems *via* selective tris(*ipso*-sulfonation) at the large rim of a tris(imidazolyl)calix[6]arene (Fig. 1).[21] Whereas the water-solubility of the ligand was good, the corresponding Zn(II) complexes precipitated out of the solution, which was ascribed to charge neutralisation with this metal dication.[22]

A cationic tris(ammonium)calix[6]arene was synthesized *via* sequential *ipso*-nitration, reduction and alkylation.[23] However, whereas the corresponding Zn(II) complex displayed a nice C_{3v} symmetrical conic structure in CD_3CN, it fell apart in the presence of even trace water.

Reasoning that such a disappointing lack of stability in water was somehow related to the proximity of three bulky and positively charged tris(methyl)ammonium substituents at the calixarene large rim once the cone was shaped by metal ion complexation, we thought of introducing a spacer between the calixarene core and the cationic hydrophilic groups. For this purpose, a CuAAC reaction performed with compound **1** in the presence of a hydrophilic alkyne was selected, the newly formed triazole playing the role of the spacer. Among the potential hydrophilic groups substituting the alkyne moiety, a positively charged trimethylammonium group was selected in order to prevent charge neutralisation and thus precipitation of the corresponding Zn(II) complexes.[24]

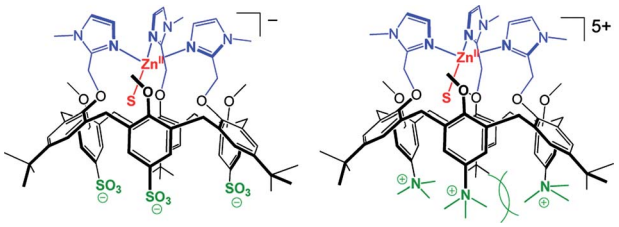
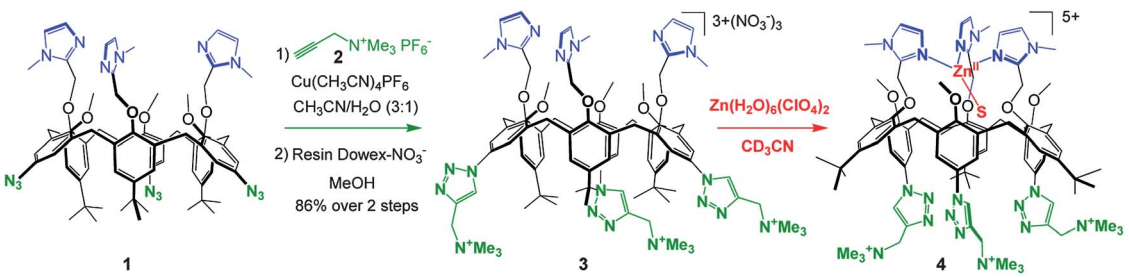


Fig. 1 Previous work: calix[6]arene Zn(II) complexes with hydrophilic substituents at their large rim.

Tris(azido)calix[6]arene **1** was readily obtained through the selective nitration of the corresponding hexa(*t*Bu)calixarene parent compound followed by a reduction–diazotation sequence as previously described.[19] Compound **1** was then treated with a slight excess of alkyne **2** in the presence of a Cu(I) salt and 2,6-lutidine in a mixture of acetonitrile and water (Scheme 1). An ion-exchange resin was used to change the counter-anions of the tricationic macrocycle into nitrate ions. This straightforward procedure efficiently yielded a water-soluble compound, the IR-spectrum of which confirmed the complete exchange of hexafluorophosphate ions for nitrate. Thanks to this two-step procedure, water-soluble calix[6]arene **3** was obtained in 86% yield from tris(azido)calix[6]arene **1**. This new tricationic ligand was fully characterized in acetonitrile as its mono-Zn(II) adduct **4** displaying a ^{1}H NMR C_{3v} signature typical of tris(imidazolyl) calix[6]arene-based tetrahedral complexes (see Scheme 1 and ESI†).

Complexation in water

In pure deuterated water at 300 K, the ^{1}H NMR spectrum of calixarene **3** displayed extremely broad resonances (Fig. 2a). This observation is in agreement with ^{1}H NMR spectra usually obtained with similar triazole-derivatives of calix[6]arene ligands in organic solvent because of relatively slow conformational motions of the calixarene core on the NMR time scale.[19] After addition of a stoichiometric amount of $Zn(NO_3)_2$, the spectrum remained disappointingly unchanged. This stands in contrast to the behavior of ligand **3** in acetonitrile: in this organic solvent,



Scheme 1 Synthesis of the water-soluble calix[6]arene **3** and complexation with $Zn(H_2O)_6(ClO_4)_2$ in CD_3CN (S stands for the solvent, here CD_3CN).

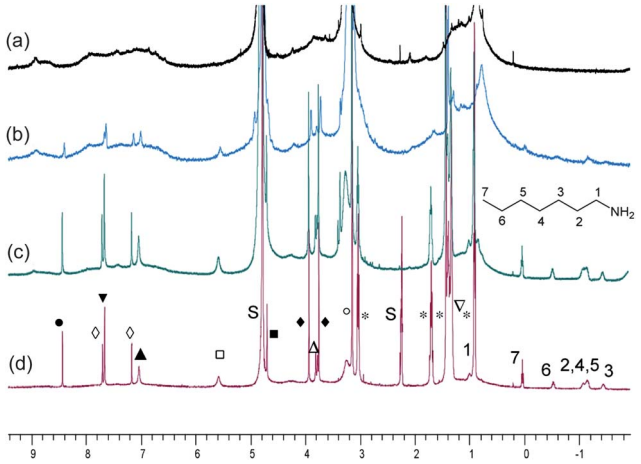


Fig. 2 ^{1}H NMR of calixarene **3** ($c = 3$ mM) in D_2O in the presence of Zn$(NO_3)_2$ hydrate before (a) and after (b)–(d) subsequent addition of heptylamine (300 K, 500 MHz). From top to bottom: (a) 1 equiv. of Zn$(NO_3)_2$; (b) 1 equiv. of Zn$(NO_3)_2$ and 0.1 equiv. of heptylamine; (c) 1 equiv. of Zn$(NO_3)_2$ and 1 equiv. of heptylamine; (d) 3 equiv. of Zn$(NO_3)_2$ and 15 equiv. of heptylamine: (●) H_{tria}, (◇) H_{Im}, (▼) H_{ArtBu}, (▲) $H_{ArTriaz}$, (□) CH_2Im, (■) $triaCH_2$, (◆) OCH_3 and NCH_3, (△) $ArCH_2$, (○) N^+Me_3, S: residual solvents, (*) free heptylamine, (▽) *t*Bu. Numbered resonances stand for protons of the encapsulated heptylamine.

the mononuclear Zn(II) complex **4** is quantitatively obtained under the same experimental conditions.

However, upon addition of 0.1 equiv. of heptylamine to the aqueous solution of **3** and Zn(II), sharp resonances emerged from the ill-defined baseline. Further additions of heptylamine led to the intensification of these sharp resonances (Fig. 2), thus attesting to the formation of a new well-defined species having a rigidified calixarene core. Most interestingly, small resonances in the high-field region of the spectrum were clearly distinguished, revealing the concomitant *endo*-complexation of heptylamine.

Under stoichiometric conditions (1 : 1 : 1 **3** : Zn : amine) at 3.9 mM in D_2O, integration of the NMR signals indicated the formation of *ca.* 15% of complex (based on the relative intensity of free and coordinated heptylamine). Upon further addition of Zn(II) and heptylamine, full complexation of ligand **3** was almost obtained with heptylamine bound in its aromatic cavity (see Fig. 2d). In this new complex, the conformation adopted by the calixarene structure is similar to that described for the Zn(II) complex in deuterated acetonitrile (see ESI†). The sharp resonances have chemical shifts that are characteristic of a C_{3v} symmetrical complex with methoxy groups expelled from the cavity. The large splitting of the protons of the imidazolyl donors attests to their coordination to a Zn(II) dication. Finally, all resonances belonging to the heptylamine ligand coordinated inside the aromatic cavity have been assigned through 2D-NMR experiments, confirming its inclusion in the calix-cone.

It is interesting to note that neither the formation of a Zn(II) complex in the absence of amine, nor the inclusion of the amine in the absence of Zn(II) has ever been observed, even with a large excess of metal ion or guest. This suggests that the three species – calixarene, metal ion and amine – interact synergistically with each other to form the *endo*-complex (see Scheme 2).

pH dependence

The formation of the ternary complex is pH-sensitive. Indeed, all three partners (calix[6]arene **3**, Zn(II) and heptylamine) are involved in acid–base processes. Therefore, we undertook a study in order to determine the optimal pH for the amine encapsulation. The pK_a values associated to the three imidazole groups of calix[6]arene **3** were determined by potentiometric titration (pK_a = 4.76, 4.89, 6.09, see ESI†). The optimal pD for the formation of the ternary complex was determined by ^{1}H NMR spectroscopy in D_2O ($c_0 = 2.9$ mM) in the presence of 1 equiv. of Zn$(NO_3)_2$ and 3 equiv. of heptylamine (C_7NH_2). The integration value of one of the encapsulated amine signals ($I_{complex}$) in comparison to the acetone reference peak (I_{ref}) was measured as a function of pD (Fig. 3a).

Under these experimental conditions, the ternary complex [**3**·Zn(II)·heptylamine] was observed from pD = 6.4 to 10.8. It is important to note that at this concentration, heptylamine remains fully water-soluble in this range of pD values. At lower pD values, no trace of complex was detected by ^{1}H NMR, which can be assigned to the competitive protonation of heptylamine (pK_a = 10.7) and of the imidazolyl units of the calixarene. At higher pD values, formation of zinc hydroxide species (pK_a = 10.0)[25] competes with the formation of the dicationic complex. Indeed, bound hydroxide is known to decrease the Lewis acidity of the metal ion and can lead to the formation of multinuclear metal complexes devoid of hosting properties.[26] Finally, the maximal amount of ternary complex was observed at a pD around 7.8.

At constant pH = 7.8 in protiated water (0.2 M Hepes buffer), gradual addition of heptylamine to an equimolar mixture of calixarene **3** and Zn$(NO_3)_2$ ($c_0 = 2.2$ mM) led to an increase of the observed amount of ternary complex (Fig. 3b). It reached a maximal value when 7 equiv. of heptylamine were added. Above this value, no significant change occurred when the amount of heptylamine was increased.

The formation constants K and K'_{pD} of the ternary complex are defined according to equilibrium (1):

$$Zn^{2+} + \mathbf{3}^{3+} + RNH_3^+ + H_2O \rightleftharpoons [Zn(\mathbf{3})(RNH_2)]^{5+} + H_3O^+ \quad (1)$$

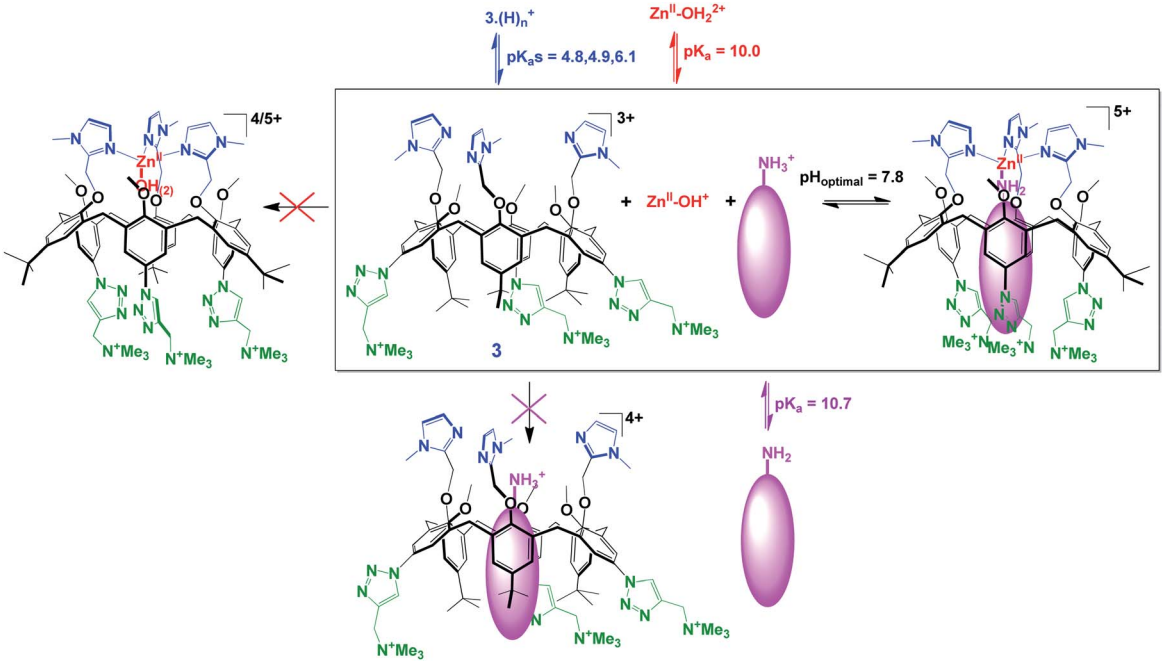
$$K = K_{aeff} = \frac{[[Zn(\mathbf{3})(RNH_2)]^{5+}][H_3O^+]}{[Zn^{2+}][\mathbf{3}^{3+}][RNH_3^+]}$$

$$K'_{pH} = \frac{[[Zn(\mathbf{3})(RNH_2)]^{5+}]}{[Zn^{2+}][\mathbf{3}^{3+}][RNH_3^+]}$$

From the ^{1}H NMR studies, K was found to be in the order of 10^{-4} L mol^{-1} and $K'_{(pD\ =\ 7.8)} = 5200 \pm 1400$ L^2 mol^{-2} at T = 300 K. From these data a pseudo-pK_a, pK_{aeff}, of 4.0 ± 0.3 for heptylamine as defined above was estimated. This value can be compared to the pK_a value of free heptylamine of 10.7 at 300 K. The tuning of the acidity corresponding to a pseudo-pK_a shift of 7 units, accounts for the impressive stabilisation of the free amine base due to its complexation to Zn^{2+} inside the calixarene cone of **3**.

Temperature dependence

From 275 to 310 K, at pH = 7.8, in Hepes buffer (H_2O), the ^{1}H NMR spectrum of calixarene **3** displayed broad resonances and



Scheme 2 Water-soluble biomimetic receptors – Illustration of the synergistic interaction of calixarene **3**, heptylamine and Zn(II) for the complex formation.

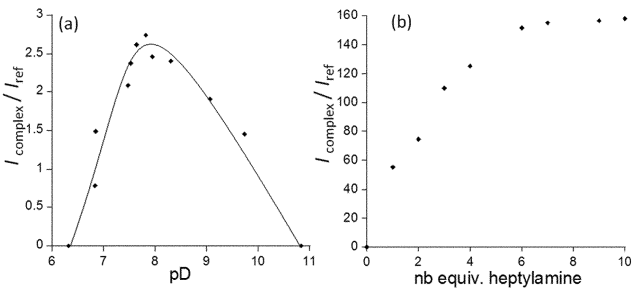


Fig. 3 (a) pD-dependance of the ternary complex formation in D_2O [c_0 = 2.9 mM, **3** : Zn(II) : C_7NH_2 (1 : 1 : 3)]; (b) Formation of ternary complex as a function of the number of equiv. of heptylamine at constant pH = 7.8 [c_0 = 2.2 mM, **3** : Zn(II) (1 : 1), Hepes buffer (c = 0.2 M) in H_2O]; $I_{complex}/I_{ref}$ refers to the integration values of one of the encapsulated amine signal relative to a reference peak.

the spectra remained unchanged after the addition of a stoichiometric amount of $Zn(NO_3)_2$. In this temperature range, ligand **3** is not properly organized and no trace of complex could be detected. Under the same experimental conditions and temperature range, but in the presence of one equivalent of heptylamine, formation of the ternary complex $[Zn(\mathbf{3})(RNH_2)]^{5+}$ was observed. However, above 310 K, some irreversible degradation of the complex was observed, possibly due to the partial de-*O*-alkylation of the ligand itself as suggested by ESI MS analysis of the heated solution. A van't Hoff plot for the formation constant of the ternary complex could be obtained in the 275–300 K temperature range where the complex was stable and could be properly quantified (Fig. 4).

Very interestingly, the plot reveals that the complex formation is entropically driven ($\Delta_r S^{\circ\prime}_{7.8} = 103 \pm 9$ J mol^{-1} K^{-1}), in spite of the [1 + 1 + 1] association process. Such an entropy increase is attributable to the desolvation of all three partners. The associated enthalpy variation is slightly positive ($\Delta_r H^{\circ\prime}_{7.8} = 10.2 \pm 2$ kJ mol^{-1}) in spite of the formation of four coordination bonds between the imidazolyl arms, the amine and the Zn(II) center. This is likely due to the very competitive water coordination to Zn(II) and protonation of the amine. These thermodynamic observations are actually in full agreement with a calorimetric study recently carried out on the coordination of tris(imidazolyl) calix[6]arene ligands to Zn(II) ion in various organic solvents.[27] This study revealed that the stability of such four-coordinate host–guest complexes in solution is highly dependent on solvation–desolvation processes. On the one hand, the complexation in aprotic solvents such as acetonitrile or THF was found to be

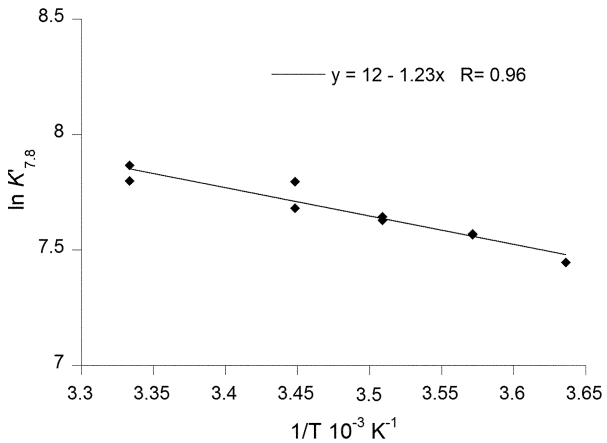


Fig. 4 Van't Hoff plot for the formation of the ternary complex at pH = 7.8 [c_0 = 5.8 mM, **3** : Zn(II) : C_7NH_2 (1 : 1 : 2), Hepes buffer (c = 0.58 M) in H_2O].

enthalpically driven due to an exothermic process associated to a negative entropy change. Under these conditions, the formation of stable coordination links to the three imidazole arms and the reduction in the flexibility of the calixarene host-ligand are the main factors that determine the thermodynamic parameters. On the other hand, in the presence of a trace of water or in a protic solvent such as methanol, the complexation switched into an endothermic and entropically driven process due to the much more extensive desolvation of the host and the metal ion.

Guest screening and relative affinity

Other substrates capable of inducing the formation of ternary complexes in water were screened. However, no trace of coordination was observed with nitriles, alcohols, amides or sulfoxides. The more sterically hindered secondary or tertiary amines were not bound either, nor highly hydrophilic primary amines such as *n*-propylamine, 5-aminobutanol, spermidine and histamine. Hence, the hydrophobic character of the amine seems to be crucial since in acetonitrile all these primary amines do form the zinc complex.[13c] In strong contrast, the more lipophilic primary amines such as dimethyldopamine, tyramine and tryptamine (Fig. 5) behaved similarly to heptylamine. In each case, the water-soluble ternary complex was observed by ^{1}H NMR in D_2O as soon as 1 equiv. of amine was added to a 1 : 1 solution of calix[6]arene **3** and $Zn(NO_3)_2$ in D_2O (c = 2.5 mM). It is noteworthy to emphasise that, under these experimental conditions, these amines are fully water-soluble,[28] which demonstrates the high-efficiency of the encapsulation process.

The relative capacity of these amines (RNH_2) to displace heptylamine was evaluated through competition experiments monitored by ^{1}H NMR spectroscopy in D_2O at pD = 7.8 (see ESI†). The constant K_{rel} is defined by equilibrium (2):

$$[Zn(\mathbf{3})(nC_7H_{15}NH_2)]^{5+} + RNH_3^+ \rightleftharpoons [Zn(\mathbf{3})(RNH_2)]^{5+} + nC_7H_{15}NH_3^+ \quad (2)$$

$$K_{rel} = \frac{[[Zn(\mathbf{3})(RNH_2)]^{5+}][nC_7H_{15}NH_3^+]}{[[Zn(\mathbf{3})(nC_7H_{15}NH_2)]^{5+}][RNH_3^+]}$$

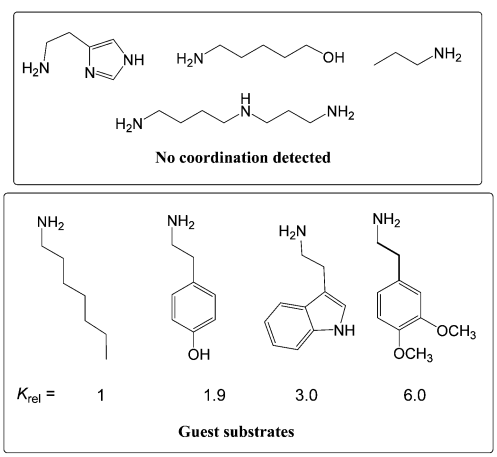


Fig. 5 Guest screening and relative affinities of primary amines; K_{rel} is defined as K(guest-amine)/K(heptylamine) according to eqn (2).

The relative constants K_{rel} obtained for tyramine, tryptamine and dimethyldopamine in competition with heptylamine are 1.9 ± 0.4, 3.0 ± 0.1, 6.0 ± 0.2, respectively. The three equilibria are thus shifted in favor of the encapsulation of the aromatic amines. Obviously, the lipophilicity of the guest plays a key role in the formation of the complex. This is ascribable to the hydrophobic effect, where desolvation of the alkyl or aromatic group drives the equilibria towards the encapsulation of the guest, together, possibly, with an increased enthalpic contribution of the London interactions between the guest and the elongated aromatic walls of the calixarene cavity.

Insights into the host–guest interactions

All ^{1}H NMR spectra of the ternary complexes with heptylamine, dimethyldopamine, tryptamine or tyramine displayed similar profiles. However, their in-depth comparison provides interesting information (Fig. 6).

The imidazolyl protons, as well as the aromatic protons belonging to the *t*BuAr units of the calixarene core are little affected by the nature of the guest. This is in accordance with their spatial position relative to the guest: indeed, their protons are far away from the inner space where the guest is embedded. In strong contrast, an important upfield shift of the resonance of the triazolyl protons (H_{tria}) and the related methylene substituents is observed when aromatic amines are used in comparison to heptylamine [$\Delta\delta(H_{tria}) \cong 1.2$ ppm with tryptamine]. This

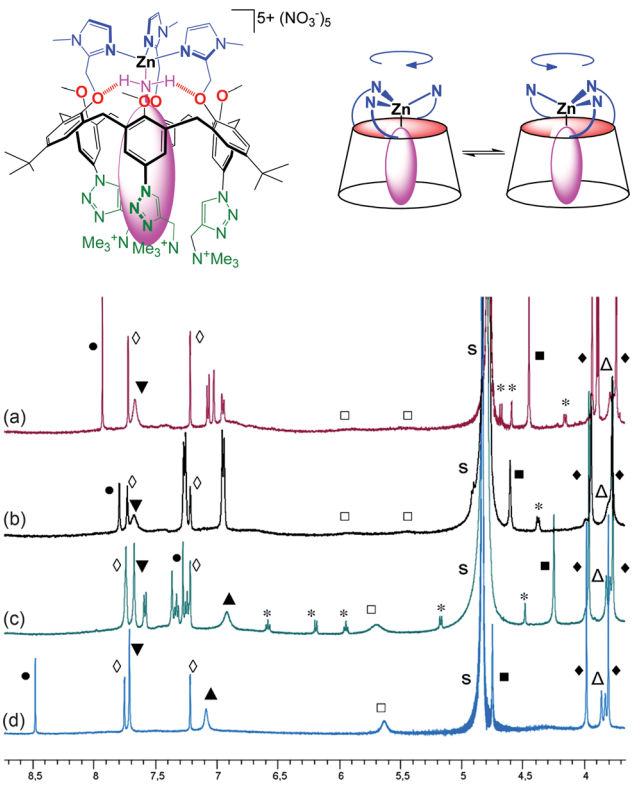


Fig. 6 ^{1}H NMR spectra in the 8.5–3.5 ppm region of [Zn(**3**)(amine)]$(NO_3)_5$ (D_2O, 300 K, 500 MHz). From top to bottom: amine = (a) dimethylDOPA, (b) tyramine, (c) tryptamine and (d) heptylamine: (●) H_{tria}, (◇) H_{Im}, (▼) H_{ArtBu}, (▲) $H_{ArTriaz}$, (□) CH_2Im, (■) $triaCH_2$, (◆) OCH_3 and NCH_3, (△) $ArCH_2$, * encapsulated amine. Non-attributed signals correspond to free amine resonances.

indicates that the triazole moieties are sitting in front of the guest aromatic group and suggests the existence of additional C–H/π interactions participating to the stabilization of the host–guest adduct.

For heptylamine and tryptamine, the resonances of the aromatic protons belonging to the calix-anisole moieties (that are in *in* position relative to the other calix-aromatic units as schematized in Fig. 6) and those corresponding to the methylene groups connected to the imidazolyl arms exhibit a single, although broad, resonance at *ca.* 7.0 and 5.7 ppm, respectively. On the contrary, the same aromatic protons are hardly visible with dimethyldopamine and tryptamine and the methylene ones are split into two very broad peaks also centered around 5.7 ppm. These observations are easily explained by the helical shape of the system. The imidazolyl arms coordinating the metal center form a helix that twists the calixarene cavity (Fig. 6). As a result, the diastereotopic protons that are the most affected can be differentiated if the left/right equilibrium is slow compared to the NMR analysis time scale.[29] With dimethyldopamine and tyramine, the helical movement is obviously slower than with the other two amines. In view of the different relative affinity of these two guests, this slow helical movement cannot be attributed to their complexation strength. Nevertheless, these two amines are the only ones possessing oxygenated substituents facing the large rim, which can potentially favor additional weak interactions at the large rim, thus slowing down the helical twisting process.

Discussion and conclusion

Amine recognition in water

Examples of amine encapsulation in water-soluble supramolecular systems are rare[2] and almost all of them describe the complexation of quaternary or tertiary ammoniums with ion–ion, ion–dipole, cation–π interactions and hydrogen-bonding within the organic host as major driving forces for their extraction from water.[30] Due to their higher hydrophilicity, primary ammoniums are much more difficult to extract. For example, Raymond's supramolecular assemblies of polyaromatic walls sealed by metal ions define hydrophobic anionic capsules that allow hosting ammoniums: protonated tertiary amine guests and to a lesser extent protonated secondary amines have been observed inside the cage but protonated primary amines, which are more highly solvated in water, are not encapsulated at all.[31] Indeed, rare are the receptors capable of binding primary amines in water. Open hydrophobic cavities with highly polar rims such as cucurbit[6]uril,[32] β-cyclodextrin[33] or *p*-sulfonatocalix[4]arene[5c,34] are known to host α-substituted methylammoniums provided the alkyl substituent fits accurately to the cavity size and shape. Recently, Rebek's deep cavitand was made water-soluble over a large pH window and shown to efficiently bind lipophilic polycyclic primary ammoniums, based on the same principle.[35] Even rarer are the receptors capable of binding in water the *neutral* form of primary amines: one based on a water-soluble zinc porphyrin equipped with a hydrophobic arch that binds butylamine but only at basic pH,[36] and β-cyclodextrin.[33b]

The present work extends the principle of recognition in water by using a functionalized calix[6]arene-based receptor that actually gathers all the binding properties of the above-described receptors: a well-defined hydrophobic cavity, a polar rim and a metal-ion binding site. The selectivity of the receptor for lipophilic primary amines as well as the stability of the corresponding ternary complexes in water is the result of many different factors. First, the variable-temperature study has revealed that the process is entropically driven: desolvation of the calixarene core, the Zn(II) dication and the ammonium/amine dominates the thermodynamics of the equilibria. This is in good accordance with the hydrophobic effect that favors inclusion of lipophilic amines over hydrophilic ones. Second, there are many factors that contribute to the enthalpic component. The costly ones are mainly decoordination of the solvent (water/hydroxide) from the Zn(II) ion and deprotonation of the amine. Those favorable are (i) first coordination sphere: formation of coordination bonds to four good amino σ-donors in a well-suited tetrahedral geometry; (ii) second coordination sphere: the phenoxyl units allow multiple hydrogen bonding with the guest amino moiety[13a] (as schematized in Fig. 6) and disfavor *endo*-binding of a hydroxide ion due to multiple repulsive anion/dipole interactions;[13b,26,37] (iii) third coordination sphere: the calix-cavity provides weak but multiple CH/π and possibly also π/π interactions between the phenoxyl units elongated by the triazole substituents of the host and the lipophilic part of the guest. Finally, like the organo-soluble version of the receptor,[13a] the system is selective over secondary and tertiary amines because of over-sterical crowding inside the cone at the small rim if the amino-guest were to carry a second alkyl substituent.

Guest pK_a shift

Large pK_a perturbations of substrate and enzymatic groups have been evidenced in many enzyme active sites employing general acid/base catalysis.[38] With artificial receptors open to the solvent and deprived of metal ion, only small pK_a shifts were observed upon their binding: for example, Nau reported a pK_a shift from 9.2 to 8.1 for 1-aminomethyl-2,3-diazabicyclo[2.2.2]oct-2-ene bound to β-cyclodextrin[33b] and a pK_a shift from 10.50 to 11.75 for the cucurbit[6]uril encapsulation of cyclohexylammonium.[32c] With Raymond's metallo-host, a Δ(pK_a) as high as 4.5 units for a tertiary ammonium has been obtained, the anionic cage and its π-basic walls greatly stabilizing the acid and the positively charged form of the guest.[31] It is also very well known that coordination to a Lewis metal ion plays a key role for decreasing the pK_a of a ligand as it stabilizes its basic form through the coordination link. pK_a values as low as 7 have been reported for the coordinated water in Zn enzymes[39] and related model compounds.[40] The reduced pK_a of the zinc-bound water is not only due to Zn(II) coordination as the pK_a for $[Zn(OH_2)_6]^{2+}$ is 10. It is also due to the environment around the protic ligand at the active site, *i.e.* the amino-acid residues that define a second and third coordination sphere. Such an effect has also been reported for various inhibitors such as carboxylic and hydroxamic acids bound to the Zn(II) active site of proteases.[41] With receptor **3**, the resulting pseudo-pK_a shift of the amine associated to the formation of the ternary complex with Zn(II) is quite remarkable, as exemplified by the case of heptylamine (from 10.7 to 4.0). Such an impressive stabilisation of the basic form of the amine at neutral pH is obviously the result of both the coordination to Zn(II) and the environment provided by the calixarene pocket (*vide supra*).

Allostery and synergism

It is worth noticing that the calix[6]arene receptor **3** is a highly flexible macrocycle displaying allosteric properties: the metal ion binding to the imidazole arms favors the calixarene-shaping into a cone conformation necessary for guest inclusion. Conversely, guest inclusion greatly favors Zn(II) binding as it provides a tetrahedral environment with a fourth good donor (the amine) completing the tetrahedral core around Zn(II). Hence, the three self-assembled partners interact in a highly synergistic manner through the formation of a well-defined ternary host–guest adduct. As a result, calix[6]arene **3** behaves as a good ligand for Zn(II) provided a lipophilic amine is present in the medium, and it also behaves as a remarkably selective receptor for lipophilic primary amines provided Zn(II) is present in water. Such a cooperative behavior has been previously reported by Nau *et al.* for the ternary system associating *p*-sulfonatocalix[4]arene, a diazo guest and Zn(II).[34] In their case however, both binary adducts (calix-Zn(II) and calix-diazo) did form in the absence of the third partner. With receptor **3** such binary adducts do not form, at least at a detectable level, which highlights a much higher synergism for the ternary system.

Biomimetism

The simple and efficient synthetic pathway herein described has led to a water-soluble calix[6]arene-based receptor, compound **3**, that displays a remarkable set of biomimetic properties: (i) it provides a hydrophobic pocket at the bottom of which a tris (imidazole) core binds a Zn(II) ion, very much like Zn-enzymes, (ii) it behaves as a highly *selective* receptor, the selectivity being based on guest function, shape and lipophilicity, iii) it works in *water at pH near 7*, (iv) the guest binding leads to a spectacular tuning of its acidity (measured as a pseudo-pK_a shift of almost 7 units, like for Zn-enzymes), (v) the three actors of the ternary system (organic host, metal ion, guest substrate) interact with each other in a cooperative manner, and (vi) the flexibility of the host allows *allosteric* behaviors.

The use of the same synthetic strategy to water-solubilize other calix[6]arene scaffolds such as the calix-azacryptands[14] as well as the implementation of a fluorescent reporter for biological applications are now underway.

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Notes and references

1 J. M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, 1995.
2 G. V. Oshovsky, D. N. Reinhoudt and W. Verboom, *Angew. Chem., Int. Ed.*, 2007, **46**, 2366.
3 (*a*) C. D. Gutsche, in Calixarenes An introduction, *Monographs in Supramolecular Chemistry*, ed. J. F. Stoddart, The Royal Society of Chemistry, Cambridge, 2nd edn, 2008; (*b*) C. D. Gutsche, in Calixarenes Revisited, *Monographs in Supramolecular Chemistry*, ed. J. F. Stoddart, The Royal Society of Chemistry, Cambridge, 1998.
4 (*a*) A. Casnati, D. Sciotto and G. Arena, in *Calixarenes 2001*, ed. Z. Asfari, V. Böhmer, J. Harrowfield and J. Vicens, Kluwer, Dordrecht, 2001, p. 440; (*b*) M. Segura, F. Sansone, A. Casnati and R. Ungaro, *Synthesis*, 2001, 2105; (*c*) C. D. Gutsche and I. Alam, *Tetrahedron*, 1988, **44**, 4689; (*d*) F. Sansone, E. Chierici, A. Casnati and R. Ungaro, *Org. Biomol. Chem.*, 2003, **1**, 1802; (*e*) A. Dondoni, A. Marra, M. C. Sherrmann, A. Casnati, F. Sansone and R. Ungaro, *Chem.–Eur. J.*, 1997, **3**, 1774.
5 (*a*) X. S. Shinkai, S. Mori, T. Tsubaki, T. Sone and O. Manabe, *Tetrahedron Lett.*, 1984, **25**, 5315; (*b*) F. Perret, A. N. Lazar and A. W. Coleman, *Chem. Commun.*, 2006, 2425; (*c*) D.-S. Guo, K. Wang and Y. Liu, *J. Inclusion Phenom. Macrocyclic Chem.*, 2008, **62**, 1.
6 (*a*) J. L. Atwood, D. L. Clark, R. K. Juneja, G. W. Orr, K. D. Robinson and R. L. Vincent, *J. Am. Chem. Soc.*, 1992, **114**, 7558; (*b*) R. Castro, L. A. Godinez, C. M. Criss, S. M. Bott and A. E. Kaifer, *Chem. Commun.*, 1997, 935; (*c*) R. Castro, L. A. Godinez, C. M. Criss and A. E. Kaifer, *J. Org. Chem.*, 1997, **62**, 4928.
7 (*a*) J. S. Millership, *J. Inclusion Phenom. Macrocyclic Chem.*, 2001, **39**, 327; (*b*) J. M. Lehn, R. Meric, J. P. Vigneron, M. Cesario, J. Guilherm, C. Pascard, Z. Asfari and J. Vicens, *Supramol. Chem.*, 1995, **5**, 97.
8 (*a*) L. Memmi, A. Lazar, A. Brioude, V. Ball and A. W. Coleman, *Chem. Commun.*, 2001, 2474; (*b*) E. Da Silva, C. F. Rousseau, I. Zanella-Cléon, M. Becchi and A. W. Coleman, *J. Inclusion Phenom. Macrocyclic Chem.*, 2006, **54**, 53.
9 G. Droogman, J. Prenens, J. Eggermont, T. Voets and B. Nilius, *Am. J. Physiol.*, 1998, **275**, 646.
10 E. Da Silva, A. Lazar and A. W. Coleman, *STP Pharma. Sci.*, 2004, **14**, 3.
11 E. Aubert-Foucher, A. W. Coleman and D. J. S. Hulmes, *Fr. Pat.*, FR 60252J, France, 1999.
12 Interestingly, toxicological studies on the sulfonato calixarenes and their derivatives suggest their innocuous nature at doses of less than 50 mM. See: E. Da Silva, P. Shahgaldian and A. W. Coleman, *Int. J. Pharm.*, 2004, **273**, 57.
13 For host–guest systems based on tris(imidazolyl)calix[6]arene Zn(II) complexes, see: (*a*) O. Sénèque, M.-N. Rager, M. Giorgi and O. Reinaud, *J. Am. Chem. Soc.*, 2000, **122**, 6183; (*b*) D. Coquière, S. Le Gac, U. Darbost, O. Sénèque, I. Jabin and O. Reinaud, *Org. Biomol. Chem.*, 2009, **7**, 2485 (Perspective); and most recent developments; (*c*) D. Coquière, A. de la Lande, O. Parisel, T. Prangé and O. Reinaud, *Chem.–Eur. J.*, 2009, **15**, 11912; (*d*) D. Coquière, A. de la Lande, S. Martí, O. Parisel, T. Prangé and O. Reinaud, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 10449; (*e*) C. Monnereau, J.-N. Rebilly and O. Reinaud, *Eur. J. Org. Chem.*, 2011, 166.
14 For host–guest complexes based on calix[6]aza-cryptands, see: (*a*) U. Darbost, M.-N. Rager, S. Petit, I. Jabin and O. Reinaud, *J. Am. Chem. Soc.*, 2005, **127**, 8517; (*b*) X. Zeng, D. Coquière, A. Alenda, E. Garrier, T. Prangé, Y. Li, O. Reinaud and I. Jabin, *Chem.–Eur. J.*, 2006, **12**, 6393; (*c*) D. Over, A. de la Lande, X. Zeng, O. Parisel and O. Reinaud, *Inorg. Chem.*, 2009, **48**, 4317.
15 Reviews of bioinorganic enzymology: *Chem. Rev.*, 1996, **96**, 2237–3042.
16 Y. Rondelez, G. Bertho and O. Reinaud, *Angew. Chem., Int. Ed.*, 2002, **41**, 1044.
17 S. Houmadi, D. Coquière, L. Legrand, M. C. Fauré, M. Goldmann, O. Reinaud and S. Rémita, *Langmuir*, 2007, **23**, 4849.
18 (*a*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057; (*b*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596.
19 B. Colasson, M. Save, P. Milko, J. Roithová, D. Schröder and O. Reinaud, *Org. Lett.*, 2007, **9**, 4987.
20 C. Bize, J. C. Garrigues, M. Blanzat, I. Rico-Lattes, O. Bistri, B. Colasson and O. Reinaud, *Chem. Commun.*, 2010, **46**, 586.
21 D. Coquière, H. Cadeau, Y. Rondelez, M. Giorgi and O. Reinaud, *J. Org. Chem.*, 2006, **71**, 4059.
22 David Coquière, PhD thesis, Université Paris Descartes, 2006.
23 (*a*) S. Redon, Y. Li and O. Reinaud, *J. Org. Chem.*, 2003, **68**, 7004; (*b*) D. Coquière, J. Marrot and O. Reinaud, *Org. Lett.*, 2007, **9**, 3271.
24 E. H. Ryu and Y. Zhao, *Org. Lett.*, 2005, **7**, 1035.

25 I. Bertini and C. Luchinat, in *Bioinorganic Chemistry*, ed. I. Bertini, H. B. Gray, S. J. Lippard and J. Valentine, University Science Books, Mill Valley, CA, 1994.

26 (*a*) G. Izzet, Y. M. Frapart, T. Prangé, K. Provost, A. Michalowicz and O. Reinaud, *Inorg. Chem.*, 2005, **44**, 9743; (*b*) G. Izzet, H. Akdas, N. Hucher, M. Giorgi, T. Prangé and O. Reinaud, *Inorg. Chem.*, 2006, **45**, 1069.

27 K. Kano, M. Kondo, H. Inoue, H. Kitagishi, B. Colasson and O. Reinaud, *Inorg. Chem.*, 2011, **50**, 6353.

28 (*a*) N. Nishino and M. Nakamura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 1617; (*b*) *Handbook of Chemistry and Physics*, ed. D. R. Lide, CRC Press, 75th edn.

29 (*a*) S. Blanchard, M.-N. Rager, A. F. Duprat and O. Reinaud, *New J. Chem.*, 1998, **22**, 1143; (*b*) Y. Rondelez, O. Sénèque, M.-N. Rager, A. Duprat and O. Reinaud, *Chem.–Eur. J.*, 2000, **6**, 4218; (*c*) O. Sénèque, Y. Rondelez, L. Le Clainche, C. Inisan, M.-N. Rager, M. Giorgi and O. Reinaud, *Eur. J. Inorg. Chem.*, 2001, 2597.

30 Selected references: (*a*) G. Arena, S. Gentile, F. G. Gulino, D. Sciotto and C. Sgarlata, *Tetrahedron Lett.*, 2004, **45**, 7091 (calix[4] and calix[5]arenes); (*b*) M. Fokkens, C. Jasper, T. Schrader, F. Koziol, C. Ochsenfeld, J. Polkowska, M. Lobert, B. Kahlert and F. G. Klärner, *Chem.–Eur. J.*, 2005, **11**, 477 (molecular clip); (*c*) F. Corbellini, L. Di Costanzo, M. Crego-Calama, S. Geremia and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2003, **125**, 9946; (*d*) F. Corbellini, R. Fiammengo, P. Timmerman, M. Crego-Calama, K. Versluis, A. J. R. Heck, I. Luyten and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2002, **124**, 6569 (capsules); (*e*) A. V. Davis, D. Fiedler, G. Seeber, A. Zahl, R. van Eldik and K. N. Raymond, *J. Am. Chem. Soc.*, 2006, **128**, 1324 (metallo-cages).

31 M. D. Pluth, R. G. Bergman and K. N. Raymond, *J. Am. Chem. Soc.*, 2007, **129**, 11459.

32 (*a*) J. W. Lee, S. Samal, N. Selvapalam, H. J. Kim and K. Kim, *Acc. Chem. Res.*, 2003, **36**, 621; (*b*) L. Isaacs, *Chem. Commun.*, 2009, 619; (*c*) C. Marquez and W. M. Nau, *Angew. Chem., Int. Ed.*, 2001, **40**, 3155.

33 (*a*) M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, 1998, **98**, 1875; (*b*) X. Zhang, G. Gramlich, X. Wang and W. M. Nau, *J. Am. Chem. Soc.*, 2002, **124**, 254.

34 H. Bakirci, A. L. Koner, M. H. Dickman, U. Kortz and W. M. Nau, *Angew. Chem., Int. Ed.*, 2006, **45**, 7400.

35 A. Lledó and J. Rebek, *Chem. Commun.*, 2010, **46**, 8630.

36 H. Imai, H. Munakata, A. Takahashi, S. Nakagawa, Y. Ihara and Y. Uemori, *J. Chem. Soc., Perkin Trans. 2*, 1999, 2565.

37 G. Izzet, X. Zeng, H. Akdas, J. Marrot and O. Reinaud, *Chem. Commun.*, 2007, 810.

38 N.-M. Ha, M.-S. Kim, W. Lee, K. Y. Choi and B.-H. Oh, *J. Biol. Chem.*, 2000, **275**, 41100.

39 W. N. Lipscomb and N. Strater, *Chem. Rev.*, 1996, **96**, 2375.

40 G. Parkin, *Chem. Rev.*, 2004, **104**, 699.

41 J. B. Cross, J. S. Duca, J. J. Kaminski and V. S. Madison, *J. Am. Chem. Soc.*, 2002, **124**, 11004.

