Primary amine recognition in water by a calix[6]aza-cryptand incorporated in dodecylphosphocholine micelles†

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Water is a unique solvent and the design of selective artificial hosts that can efficiently work in an aqueous medium is a challenging task. It is known that the calix[6]tren zinc complex can recognize neutral guests in organic solvents. This complex was incorporated into dodecylphosphocholine micelles (DPC) and studied by NMR. The incorporated complex is able to extract selectively primary amines from the aqueous environment driven by an important hydrophobic effect which also affects the selectivity of the complex for these amines. This work shows how the incorporation of organo-soluble receptors in micelles can be an elegant and very efficient strategy to obtain water compatible nanosized supramolecular recognition devices which can be prepared via a straightforward self-assembly process.

Introduction

The design of artificial receptors that can selectively bind, with a high affinity, charged or neutral species is one of the major objectives of supramolecular chemistry.1 Indeed, such receptors have potential applications in the sensing of chemical species in the fields of biological and environmental analysis.2 The development of such receptors is nevertheless challenging because water is a very competitive solvent.3 Exploiting the hydrophobic effect which can drive apolar guests into apolar cavities is an interesting strategy. In this context, many examples of neutral guest complexation have been reported with water-soluble cavitands having a hydrophobic inner space such as cyclodextrins4 and cucurbiturils.5 These macrocycles, however remain difficult to modify selectively6 which limits the design of sophisticated receptors. In the case of the other families of cavitands such as calixarenes, fastidious syntheses are often required in order to make them water-soluble.

Micelles are extensively used in a wide variety of industrial and recovery processes to incorporate lipophilic compounds7 and are thus a possible solution to overcome the solubility problem of organic receptors in water. This strategy is interesting, as no synthesis needs to be undertaken in order to introduce hydrophilic groups on the receptor. A few examples of receptors and sensors incorporated in micelles have been reported in the literature8 and in some cases their binding properties have been investigated and shown to be enhanced by the micellar environment. Rebek and co-workers have, for instance, observed that a conformational reorganization of a resorcinarene cavatand incorporated into dodecylphosphocholine (DPC) micelles increases significantly its binding capacity towards different guests.9 It has also been reported that uranyl-salophen receptors incorporated into cetyltrimethylammonium bromide (CTABr) micelles have an affinity for fluoride which is two order of magnitude larger than the affinity of the related water-soluble receptor.10,11

Calix[6]arenes are a well-known class of concave macrocyclic compounds presenting a cavity well adapted for the inclusion of organic guests and which can be selectively modified.12,13 Depending on their functionalization, they have revealed to be efficient receptors for a variety of neutral or charged species. In this context, different generations of biomimetic receptors based on calix[6]arene ligands bearing nitrogenous coordinating arms on the narrow rim have been developed.14 In particular,
The calix[6]tren receptor belonging to the third generation has been shown to display unique and versatile binding properties, notably toward metal ions and neutral guests (Fig. 1). Indeed, the tren cap offers a strong binding site for a metal ion (e.g., Zn$^{2+}$, Cu$^{2+}$). Once coordinated, the metal ion presents a single coordination site accessible for an exogenous neutral guest, which is fully controlled by the hydrophobic funnel. Hence, complex 1·Zn$^{2+}$ (Fig. 1) can bind with a high selectivity various neutral organic molecules (e.g., amines, alcohols, amides, etc.) inside the calix[6]arene core in different organic solvents.

With the aim of developing selective molecular receptors that exhibit high affinity for specific guests in water, we were interested in evaluating the binding properties of complex 1·Zn$^{2+}$ in an aqueous medium. In order to transfer this lipophilic metal complex into water, we thus envisioned its incorporation into micelles. Herein, we report the incorporation of calix[6]tren 1 and of its corresponding metal complex 1·Zn$^{2+}$ into DPC micelles (Fig. 1). Characterization of the calix[6]arene/micelle systems and evaluation of the binding properties of the incorporated receptors were achieved by NMR spectroscopy.

Results and discussion

Incorporation of complex 1·Zn$^{2+}$ and ligand 1 into DPC micelles. The receptor 1·Zn$^{2+}$ was successfully incorporated into DPC micelles by simple mixing of the receptor and the surfactant (20 mM) in D$_2$O at neutral pH (∼7.6) and concentrations of up to 0.5 mM of 1·Zn$^{2+}$ could be obtained. The $^1$H NMR spectrum of the incorporated receptor 1·Zn$^{2+}$ in DPC-d$_{38}$ micelles shows a NMR pattern which is very similar to the one recorded in CDCl$_3$ (Fig. 2a vs. 2b). No significant changes were observed in the $^1$H NMR spectrum between 283 K and 330 K. As shown previously, the complexity of the NMR profile arises from the heterochirality of the three nitrogen stereocenters which leads to a nonsymmetrical C$_1$ calixtren-Zn complex.

Similarly to 1·Zn$^{2+}$ in CDCl$_3$, the downfield shift of the OMe signals of the incorporated complex (δOMe > 3.9 ppm in CDCl$_3$ and in D$_2$O) suggests that these groups have been expelled.

![Fig. 1](image_url) Structures of ligand 1, complex 1·Zn$^{2+}$, G, and DPC surfactant.

![Fig. 2](image_url) $^1$H NMR spectra (298 K) of (a) 1·Zn$^{2+}$ in CDCl$_3$ (600 MHz); (b) 1·Zn$^{2+}$ in DPC-d$_{38}$ (20 mM in D$_2$O at pH ~7.6, 600 MHz); (c) 1·Zn$^{2+}$ in CDCl$_3$ after the addition of ~1 equiv. of PrNH$_2$ (400 MHz); (d) 1·Zn$^{2+}$ in DPC-d$_{38}$ (20 mM in D$_2$O at pH ~8.6) after the addition of 5 equiv. of PrNH$_2$ (600 MHz). ▼: PrNH$_2$ in; ▲: PrNH$_2$ out; *: residual DPC; s: solvent; w: water.
from the cavity by a coordinated solvent water molecule.\textsuperscript{20} Two very distinct sets of signals can be observed for the \( \text{tBu} \) as well as for the \( \text{Ar} \) protons, indicating that the calixarene adopts a major flattened cone conformation. The incorporation of \( 1·\text{Zn}^{2+} \) inside the DPC micelles was unambiguously confirmed by DOSY NMR experiments as both receptor and micelle exhibit the same diffusion coefficient, corresponding to a hydrodynamic radius of \( \sim 2 \) nm.\textsuperscript{21} Moreover, knowing that the micellar aggregation number is around 50,\textsuperscript{22} it is reasonable to estimate that there is 1 molecule of complex \( 1·\text{Zn}^{2+} \) per micelle.

In order to gain information about the position of \( 1·\text{Zn}^{2+} \) in the micelle, paramagnetic relaxation enhancement (PRE) experiments were undertaken.\textsuperscript{23} It is indeed possible to obtain reliable information on the localization of receptors in micelles by comparing the PRE data obtained for the receptor protons with those for the surfactant protons.\textsuperscript{10} When plotting the relaxation rate enhancement as a function of paramagnetic species concentration, a linear relationship was obtained and the slope, known as the relaxivity, was extracted. The normalized relaxivities for the aromatic and \( \text{tBu} \) protons of \( 1·\text{Zn}^{2+} \) have values similar to those of the tail of the surfactant but very different from those of the hydrophilic part.\textsuperscript{21} It was thus possible to conclude that the calixarene positions itself in the hydrophobic core. Interestingly, the spectrum of \( 1·\text{Zn}^{2+} \) in DPC micelles was not significantly modified by the addition of DCl or NaOD in the pH range from 6 to 9, highlighting the stability of the system in this pH window.\textsuperscript{24}

The calix[6]tren ligand 1 was also successfully incorporated into DPC micelles, by simple mixing in \( \text{D}_2\text{O} \) of the receptor and the surfactant, as attested by the NMR spectrum.\textsuperscript{21} It was however necessary to lower the pH to \( \sim 3 \), showing that the ligand must be protonated at the level of its basic tren cap (1·nH\textsuperscript{+}) in order to be incorporated.\textsuperscript{25} This result suggests that the favourable electrostatic interaction of the positively charged 1·nH\textsuperscript{+} and 1·Zn\textsuperscript{2+} with the phosphate group of the zwitterionic surfactant is a driving force for their incorporation (Fig. 3). It is noteworthy to mention that the OMe groups of the incorporated receptor 1·nH\textsuperscript{+} are pointing inside the cavity (\( \delta_{\text{OMe}} = 2.41 \) ppm), clearly highlighting the absence of a guest molecule inside the cavity. The efficient incorporation of 1·nH\textsuperscript{+} and 1·Zn\textsuperscript{2+} into DPC micelles encouraged us to undertake host–guest complexation studies with these systems.

**Guest complexation by 1·Zn\textsuperscript{2+} in DPC micelles**

Propylamine (\( \text{PrNH}_2 \)) is known to display a high affinity for 1·Zn\textsuperscript{2+} in CDCl\textsubscript{3}.\textsuperscript{17} A titration with \( \text{PrNH}_2 \) of the receptor in DPC micelles was monitored by \( ^1\text{H} \) NMR spectroscopy. As the DPC solution was not buffered, the addition of the amine increased the pH somewhat during the titration (from 7.6 to 8.6). The following observations can be made from the spectrum recorded with the addition of 5 equiv. of \( \text{PrNH}_2 \) (pH = 8.6) (Fig. 2d):

(i) High-field signals for the protons of the alkyl chain of \( \text{PrNH}_2 \) (i.e. \( \sim 1.62 \) and \( \sim 1.95 \) ppm) attest of its inclusion inside the cavity and thus of the formation of the complex 1·Zn\textsuperscript{2+} \( \Leftrightarrow \) PrNH\textsubscript{2}. These chemical shifts are similar to those obtained with the same system in CDCl\textsubscript{3} suggesting a similar positioning of the guest in the cavity (Fig. 2c vs. 2d). The exchange process is slow on the NMR chemical shift timescale.

(ii) There are only small changes in the chemical shifts of the aromatic and \( \text{tBu} \) calixarene protons, which shows that the conformation of the receptor essentially does not change upon inclusion of a small amine such as propylamine. The receptor maintains a flattened cone conformation clearly evidenced by the large difference between the resonances of the \( \text{tBu} \) or \( \text{Ar} \) protons pointing inside and pointing outside the cavity (\( \Delta \delta_{\text{tBu}} \sim 0.65 \) ppm and \( \Delta \delta_{\text{Ar}} \sim 0.67 \) ppm).

These NMR data show that the host–guest properties of 1·Zn\textsuperscript{2+} toward amines are maintained in the micellar environment (Fig. 3). An apparent binding constant for propylamine (\( K_{\text{app}} \)) of \( \sim 5 \times 10^3 \) M\textsuperscript{-1} for a 1:1 binding was determined by signal integration at pH \( \sim 8 \).\textsuperscript{26} At pH \( \sim 10 \), this constant was estimated to be \( >10^4 \) M\textsuperscript{-1}. The complex 1·Zn\textsuperscript{2+} \( \Leftrightarrow \) PrNH\textsubscript{2} was observed on a larger pH window (pH from \( \sim 3 \) to \( \sim 11 \)) in the micelle than complex 1·Zn\textsuperscript{2+} devoid of organic guest coordinated to the Zn atom (\textit{vide supra}). From these data, a pseudo p\( K_a \) of \( \sim 4.3 \) for complexed propylamine could be esti-
amine binding in water. The secondary amine (Et)2NH was not observed (from pH \( \sim 11.1 \)).

Complex 1·Zn\(^{2+}\) in DPC micelles also binds ethylamine (EtNH\(_2\)) and heptylamine (HeptylNH\(_2\)) as evidenced by the presence of high field signals for their alkyl chain protons in the \(^1\)H NMR spectra.\(^{21}\) The affinity constants for these amines relative to PrNH\(_2\) were evaluated by competition experiments (Table 1)\(^{18}\) and the following sequence of affinities was observed at pH \( \sim 11.1 \) (±0.1): EtNH\(_2\) > PrNH\(_2\) > HeptylNH\(_2\). They are all of the same order of magnitude, with, however, a significant advantage to the smallest amine, in spite of its lower lipophilicity. This may be explained by a better fit of the guest considering the volume of the calix[6]arene cone as defined by the tBu substituents at the large rim. Indeed, with other related p-tBu-substituted calix[6]arene-based metal complexes studied in chloroform, it has been shown that 3- and 4-atom length guests that are fully encapsulated in the cavity (as shown here by the high-field shifted \(^1\)H-NMR signals), display the highest affinities. In contrast, the alkyl chain of the larger amino guest has to protrude outside the cavity and, in order to avoid a steric clash with the tBu groups, the calix[6]-arene skeleton is forced to adopt an energetically less favourable straight conformation.\(^{29}\) Finally, it is interesting to note that the relative affinity for HeptylNH\(_2\) (vs. PrNH\(_2\)) in the micellar solution is spectacularly much higher than the one measured in CDCl\(_3\) (i.e. 0.5 vs. 0.00025). Such a difference may well reflect the benefit of the hydrophobic effect for lipophilic amine binding in water.

Very interestingly, the binding of tBuNH\(_2\) as well as the secondary amine (Et)\(_2\)NH was not observed (from pH \( \sim 8 \) to \( \sim 11 \)) despite the fact that these amines are less hydrophilic than EtNH\(_2\) or PrNH\(_2\). This result shows that steric hindrance at the level of amino group is a major factor of selectivity and thus highlights the cavity-based selectivity.

<table>
<thead>
<tr>
<th>G (pK(_a))</th>
<th>Relative affinities(^a)</th>
<th>DPC (20 mM in D(_2)O)</th>
<th>CDCl(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtNH(_2) (10.67)</td>
<td>1.3 (pH ( \sim 11.1 ))</td>
<td>—</td>
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</tr>
<tr>
<td>HeptylNH(_2) (10.65)</td>
<td>0.5 (pH ( \sim 11.2 ))</td>
<td>0.000025</td>
<td>—</td>
</tr>
<tr>
<td>HO(CH(_2))(_2)NH(_2) (9.50)</td>
<td>n.d. (pH ( \sim 9.4 ))</td>
<td>0.8</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^{a}\) Defined as \[ \frac{[G\text{in}][\text{PrNH}_2]}{[\text{PrNH}_2\text{Free}]} \times \frac{[\text{PrNH}_2\text{Free}]}{[G\text{Free}]} \] and measured at 298 K. The subscript “in” stands for “included”. Errors estimated ±20%.

For the pK\(_a\) values, see ref. 30. \(^{+}\) Not detected with up to 50 equiv. of HO(CH\(_2\))\(_2\)NH\(_2\).

### Guest complexation by 1·H\(^+\) into DPC micelles

We have previously shown that calix[6]arene-based receptors bearing either a polyammonium,\(^{31}\) polyamido,\(^{32}\) or polyureido cap\(^{33}\) can bind with a high affinity the neutral guest 2-imidazolidinone (Imi) in their cavity. As shown on the XRD structure of one of these host-guest systems,\(^{34}\) the high affinity for Imi stems from a complementary DAAD-ADDA quadruple H-bonding array between the urea guest and the calixarene host. Imi is also recognized by the protonated calix[6]arene derivative 1·H\(^+\) in CDCl\(_3\) and, to date, behaves as the best “key” for this receptor.\(^{35}\) The binding of this neutral guest was thus evaluated in the micellar environment by NMR spectroscopy. It was observed that Imi (\( \sim 230 \) equiv.) is not recognized by 1·Zn\(^{2+}\) incorporated into DPC micelles (from pH \( \sim 8 \) to \( \sim 11 \)). However, 1·H\(^+\) incorporated in DPC micelles at low pH complexes Imi under a slow exchange regime on the NMR chemical shift timescale. A singlet at 0.22 ppm is observed for the two methylene groups of the included Imi and an impressive downfield shift of the signal corresponding to the OMe groups (\( \Delta\delta\text{OMe} = 1.35 \) ppm) attests to the ejection of these groups from the cavity upon guest inclusion. A large excess of Imi (40 equiv.), which is very soluble in water, was however required to observe the binding. Unfortunately, the addition of Imi also leads to a pH increase and ultimately to a partial

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**Table 1** Affinities, relative to PrNH\(_2\) (pK\(_a\) = 10.58), of various guests G for host 1·Zn\(^{2+}\)

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For the pK\(_a\) values, see ref. 30. \(^{+}\) Not detected with up to 50 equiv. of HO(CH\(_2\))\(_2\)NH\(_2\).
deprotonation of receptor 1. As a result, quantitative formation of the complex 1·nH+ □ Imi was difficult to achieve. From signal integration, the binding constant is estimated to be lower than 10 M⁻¹ at pH = 5.3.

**Reversible interconversion of host–guest adducts 1·Zn²⁺ □ PrNH₂ and 1·nH⁺ □ Imi**

In CDCl₃, the quantitative acid-base induced interconversion of complexes 1·Zn²⁺ □ EtOH and 1·nH⁺ □ EtOH was previously reported.¹⁷ In order to see if such a supramolecular switching process can be transposed into water and triggered by a pH change, the inter-conversion of the 1·Zn²⁺ □ PrNH₂ and 1·nH⁺ □ Imi host–guest adducts incorporated into DPC micelles was tested (Fig. 4). First, PrNH₂ (4.5 equiv.) and Imi (4 equiv.) were added to a solution of 1·Zn²⁺ in DPC micelles and the resulting NMR spectrum (pH = 10.5) clearly showed the exclusive formation of 1·Zn²⁺ □ PrNH₂ (Fig. 4a). Fig. 4b shows the spectrum of the same solution at pH 3.1 after the addition of DCl and a large excess of Imi (~106 equiv.) It evidences the disappearance of complex 1·Zn²⁺ □ PrNH₂ and the formation of 1·nH⁺ □ Imi together with some free protonated receptor 1·nH⁺. The subsequent addition of NaOD (to reach pH = 8.8) restored very easily the initial NMR profile corresponding to 1·Zn²⁺ □ PrNH₂ (Fig. 4c). This experiment highlights that the host–guest properties of receptor 1 can be nicely tuned and controlled by the pH.

**Conclusion**

In summary, we have shown that a calix[6]azacryptand (1) can be incorporated into DPC micelles either as a Zn²⁺ complex in the [6–9] pH window or as a polyammonium at low pH. The 1·Zn²⁺ complex, incorporated into DPC micelles, is able to host selectively small or long linear primary amines with high affinity constants. This corresponds to one of the rare examples of systems capable of recognising primary amines in water media.¹⁶ Interestingly, the host–guest complex 1·Zn²⁺ □ PrNH₂ was detected in the micelles in an even larger pH window (3–11) than was the 1·Zn²⁺ host itself, which shows that the presence of the amine strengthens the complex even at very low pH where the free amine is fully protonated in water. Moreover, we observed a quite remarkable pseudo pKₐ shift of propylamine recognised by 1·Zn²⁺ in DPC micelles (from 10.6 to 4.3). This impressive stabilisation of the basic form of propylamine is the result of its coordination to Zn²⁺ but also the environment provided by the calixarene cavity and the micelle which protects the system from the water. The 1·Zn²⁺ complex preferentially binds small amines and this size selectivity stands in strong contrast with the previously reported water-soluble calixarene-based Zn²⁺ complex for which hydrophobic amine binding could be detected, but not with small hydrophilic ones.²⁷ With free receptor 1, no amine binding was detected even at low pH. However, an entrapped urea guest (i.e. Imi) could be observed, though only at a high concentration. Interestingly, a pH driven guest switch was evidenced with 1·Zn²⁺ in the presence of both an amine and Imi: at high pH, the Zn²⁺ complex binds the amine, at low pH the amino arms are protonated, which leads to the decoordination of the metal ion and the embedment of Imi. All in all, these results validate the very simple strategy that consists of incorporating an organo-soluble receptor in micelles to obtain water compatible nanosized supramolecular recognition devices. Efforts are now being directed towards the design of calixarene-based sensing systems incorporated in micelles.

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Fig. 4 $^1$H NMR spectra (600 MHz, 298 K, DPC-d38 20 mM in D₂O) of (a) complex 1·Zn²⁺ in the presence of ~4.5 equiv. of PrNH₂ and ~4 equiv. of Imi (pH = 10.5); (b) after the subsequent addition of DCI and ~106 equiv. of Imi (pH = 3.1); (c) after the subsequent addition of NaOD (pH = 8.8). ▼: PrNH₂ in; ▽: PrNH₂ out; ▢: Imi in; □: Imi out; ▪: 1·nH⁺ □ Imi; ○: 1·nH⁺; s: solvent.
Experimental section

Chemicals

DPC and DPC-d38 were purchased from Avanti Lipids and used with no further purification. D$_2$O and CDCl$_3$ were purchased from VWR BDH PROLABO. Compounds 1 and 1·Zn$^{2+}$ were synthesized according to the literature.$^{15,16}$

General procedures

$^1$H NMR spectra were recorded on a 600 MHz, 400 MHz or a 300 MHz spectrometer. For the 1D $^1$H spectra, parameters (acquisition time, recycling times, and signal accumulation) were chosen so as to ensure that quantitative data could be obtained from signal integration. Traces of residual solvents were used as internal chemical shift references. Chemical shifts are quoted on the δ scale. The NMR spectra were recorded at 298 K unless otherwise stated.

NMR solutions preparation

All solutions for NMR studies were prepared following a similar protocol: 1·Zn$^{2+}$ (~4 mg) and DPC (~35 mg, deuterated or not depending on the experiments) were added to 5 mL of D$_2$O in order to have, respectively, concentrations of ~0.5 mM and ~20 mM. Solutions were stirred until a clear solution was obtained.

$^1$H NMR characterization of various complexes 1·Zn$^{2+}$ ⊗ nH$_2$O

$^1$H NMR (DPC 20 mM in D$_2$O, 298 K, 600 MHz): δ (ppm) 0.79 (bs, 27H, tBu), 1.44 (bs, 27H, tBu), 3.97 (bs, 9H, OMe), 4.61 (m, 24H, ArCH$_2$), 1.36 (bs, 27H, tBu), 2.99–4.68 (m, 33H, ArCH$_2$)$_{eq}$/ArCH$_2$)$_{ax}$/ArCH$_2$O/CH$_2$N/CH$_2$O), 6.49–6.81 (m, 6H, ArH$_{cap}$), 7.40 (bs, 6H, ArH$_{cap}$). 1·Zn$^{2+}$ ⊗ PrNH$_2$: $^1$H NMR (DPC 20 mM in D$_2$O, 298 K, 600 MHz): δ (ppm) –1.95 (bs, 3H, PrNH$_2$-in), –1.63 (bs, 2H, CH$_2$ PrNH$_2$-in), 0.74–0.87 (m, 27H, tBu), 1.36–1.57 (m, 27H, tBu), 2.99–4.68 (m, 33H, ArCH$_2$)$_{eq}$/ArCH$_2$)$_{ax}$/ArCH$_2$O/CH$_2$N/CH$_2$O/OMe), 6.49–6.99 (m, 6H, ArH$_{OMe}$), 7.22–7.51 (m, 6H, ArH$_{cap}$). * The chemical shift of the CH$_2$N of PrNH$_2$ (in) was not observed due to overlapped signals.

Determination of the apparent affinity of PrNH$_2$ toward complex 1·Zn$^{2+}$ through $^1$H NMR titration in DPC (20 mM in D$_2$O)

Different aliquots of a solution of amine (PrNH$_2$) were progressively added to 500 µL of a DPC solution (20 mM in D$_2$O) containing the complex 1·Zn$^{2+}$. Non-deuterated DPC was used and the integration of the CH$_3$ and CH$_2$ signals were used as internal reference to determine the concentration of 1·Zn$^{2+}$. The integrations of the free guests and of the included guests were used to calculate the apparent affinity defined as [PrNH$_2$-in]/(1 – [PrNH$_2$-in] [PrNH$_2$Free]/[1·Zn$^{2+}$]) (errors estimated ±20%).

Determination of the relative affinities of heptyl- and ethylamine compared to propylamine through $^1$H NMR competitive binding studies in DPC (20 mM in D$_2$O)

At room temperature, ~7 equivalents of heptylamine (HeptylNH$_2$) or ~3 equivalents of ethylamine (EthylNH$_2$) were added to 500 µL of a DPC solution (20 mM in D$_2$O) containing the complex 1·Zn$^{2+}$. Propylamine (PrNH$_2$) was then added (~5 equivalent). The $^1$H NMR spectrum showed the guest resonances of both complexes 1·Zn$^{2+}$ ⊗ PrNH$_2$ and 1·Zn$^{2+}$ ⊗ HeptylNH$_2$ or 1·Zn$^{2+}$ ⊗ EthylNH$_2$ and signals corresponding to the free amines. The integrations of the signals corresponding to the free guests and included guests were used to calculate relative affinity defined as [G$_{in}$/][PrNH$_2$-in] × [PrNH$_2$Free]/[G$_{free}$] (errors estimated ±20%).

Determination of the relative affinity of ethylamine compared to propylamine in CDCl$_3$ via $^1$H NMR competitive binding studies

At room temperature, ~30 equivalents of propylamine (PrNH$_2$) and ~15 equivalents of ethylamine (EthylNH$_2$) were added in 500 µL of a CDCl$_3$ solution of complex 1·Zn$^{2+}$. A $^1$H NMR spectrum showed the guest resonances of both complexes 1·Zn$^{2+}$ ⊗ PrNH$_2$ and 1·Zn$^{2+}$ ⊗ EthylNH$_2$ and signals corresponding to the free amines. The integrations of the signals corresponding to free guests and the included guests were used to calculate relative affinity defined as [EtNH$_2$-in]/[PrNH$_2$-in] × [PrNH$_2$Free]/[EtNH$_2$Free] (errors estimated ±20%).

Determination of the relative affinity of heptylamine compared to DMF in CDCl$_3$ via $^1$H NMR competitive binding studies

At room temperature, ~40 equivalents of dimethylformamide (DMF) and ~100 equivalents of heptylamine (HeptylNH$_2$) were added in 500 µL of a CDCl$_3$ solution of complex 1·Zn$^{2+}$. A $^1$H NMR spectrum showed the guest resonances of both complexes 1·Zn$^{2+}$ ⊗ DMF and 1·Zn$^{2+}$ ⊗ HeptylNH$_2$ and the signals corresponding to free DMF and HeptylNH$_2$. The integrations of the signals corresponding to the free and included guests were used to calculate relative affinity defined as [DMF$_{in}$/][HeptylNH$_2$-in] × [HeptylNH$_2$Free]/[DMF$_{free}$] (errors estimated ±20%). In order to obtain the value corresponding to $K_{PrNH_2/HeptylNH_2}$, we used the value for $K_{PrNH_2/DMF}$ from ref. 17 (1610).

Reversible interconversion of host–guest adducts

1·Zn$^{2+}$ ⊗ PrNH$_2$ and 1·nH$^+$ ⊗ Imi

PrNH$_2$ (4.5 equiv.) and Imi (4 equiv.) were added to a ~0.5 mM solution of 1·Zn$^{2+}$ in DPC-d38 micelles (pH ~10.5). In a second stage, DCI and a large excess of Imi (~106 equiv.) were added to the system (pH ~3.1). In the final stage, NaOD was added to reach pH = 8.8.

DOSY experimental parameters

15 values for the magnitude of the gradient pulses (ranging from 2 G cm$^{-1}$ to 50 G cm$^{-1}$), diffusion delay 50 ms, time-length of the gradient 5 ms, acquisition time 2 s, relaxation delay 7 s, 32 transients.
PRE experimental parameters

For the PRE experiments, aliquots of 5 μl of a 5 mM solution of K₃[Cr(CN)₆] in D₂O were added to the 600 μl solution under study. T₁ measurements were undertaken using the classical inversion recovery (180° – t – 90° – acquisition) sequence, with 15 points and the delay varying between 0 and 7 s. For each signal monitored, the increase in the longitudinal relaxation rate induced by the paramagnetic species (Δ₁/Τ₁); the difference between the longitudinal relaxation rate measured in the presence and in the absence of the paramagnetic species) was plotted as a function of the concentration of paramagnetic species. A linear regression was undertaken with the experimental data points from which the relaxivity value was derived (slope of the line).

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Notes and references


18 This value is far above the critical micellar concentration (cmc = 1.1 mM). See: R. Stafford, T. Fanni and E. Dennis, Biochemistry, 1989, 28, 5113–5120.

19 The assignment of the OMe signal was confirmed by a NMR HSQC experiment.


21 See the ESL†


24 Note that all pH values have been measured in D₂O solutions.

25 Note that studies undertaken with the tren ligand in water have shown that, at pH ~ 3, the ligand is protonated at least three times. See: J. W. Canary, J. Xu, J. M. Castagnetto, D. Rentzeperis and L. A. Marky, J. Am. Chem. Soc., 1995, 117, 11545–11547.

26 As the DPC solution was not buffered, it is therefore difficult to discuss this binding constant quantitatively.

All measurements were conducted in pure water, i.e. in absence of buffer in order to avoid any interaction with the complex and micelles that could alter the results.


