DEVELOPMENT AND PHYSICOCHEMICAL CHARACTERIZATION OF CALIX[6]ARENE-BASED CHEMICAL RECOGNITION SYSTEMS

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ABSTRACT

Synthetic molecular receptors find applications in the selective extraction, transport and detection of neutral or charged species and the study of these systems is an important facet of supramolecular chemistry. In this thesis, we focused our attention on a specific family of molecular receptors called calix[6]arenes. These receptors possess a hydrophobic cavity formed by 6 aromatic rings that can accommodate small organic molecules. They can furthermore be easily functionalized and give rise to for example ditopic receptors or sensing systems. We worked with two families of calix[6]arenes but also looked at the complexation properties of some related compounds: a homooxacalix[3]arene and a resorcin[4]arene derivatives.

The first part of this thesis is devoted to the study of the complexation properties of a fluorescent calix[6]tris-pyrenylurea. The binding of anions, ion pairs, ion triads and phospholipids was monitored by $^1$H Nuclear Magnetic Resonance (NMR) and Emission Spectroscopy. Our results showed that the receptor exhibits a remarkable selectivity for the sulfate anion in DMSO for which a binding constant of the order of $10^3$ M$^{-1}$ was found. In chloroform the affinity for sulfate is of the order of $10^5$ M$^{-1}$ and the selective recognition of ammonium-TBASO$_4$ triads was observed (TBA = tetra-$n$-butylammonium; ammonium = PrNH$_3^+$, HexNH$_3^+$ or DodNH$_3^+$). This work has been reported in the paper “Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity” Emilio Brunetti, Jean-François Picron, Karolina Flidrova, Gilles Bruylants, Kristin Bartik and Ivan Jabin J. Org. Chem. 2014, 79, 6179–6188.

We also showed that calix[6]tris-pyrenylurea displays a remarkable selectivity in chloroform for phospholipids bearing a phosphatidylcholine head (PCs) over those bearing a phosphoethanolamine head (PEs). We were able to show that this fluorescent receptor is able to extract PCs from a water solution enabling their quantification. This work has been reported in the paper “A Selective Calix[6]arene-based Fluorescent Chemosensor for Phosphatidylcholine Type Lipids” Emilio Brunetti, Steven Moerkerke, Johan Wouters, Kristin Bartik and Ivan Jabin Org. Biomol. Chem. 2016. Accepted Manuscript. DOI: 10.1039/C6OB01880G.

The second part of this thesis is devoted to the evaluation of the binding properties of different receptors incorporated into dodecylphosphocholine (DPC) micelles. This strategy was used to make the hydrophobic molecular receptors “water-compatible” without having to undertake
synthetic modifications. Our results showed that a calix[6]azacryptand-based receptor can be incorporated into DPC micelles, either as a zinc complex or as a polyammonium at low pH. We observed that the zinc complex incorporated in the micelles is able to bind small and long linear primary amines in its cavity and we were able to highlight that complexation is driven by the hydrophobic effect. This work has been reported in the paper “Primary Amine Recognition in Water by a Calix[6]aza-cryptand Incorporated in Dodecylphosphocholine Micelles” Emilio Brunetti, Alex Inthasot, Flore Keymeulen, Olivia Reinaud, Ivan Jabin and Kristin Bartik Org. Biomol. Chem. 2015, 13, 2931-2938.

We also validated the micellar incorporation strategy with a homooxacalix[3]tris-acid and with a resorcin[4]arene zinc complex bearing four methyl-imidazole moieties. Once incorporated into DPC micelles, we showed that the two receptors can bind small organic guests: the homooxacalix[3]arene derivative can bind tert-butylammonium or adamantylammonium, albeit with low affinity and the resorcin[4]arene-based zinc complex can bind acetate and acetylacetone.

The final part of this thesis is devoted to the work undertaken in order to try and elucidate the guest exchange mechanism of calix[6]arene-zinc complexes where the zinc is tri-coordinated to the calixarene-based ligand and coordinates a guest molecule inside the calixarene cavity. The hypothesis that we put forward is that when the zinc is only tri-coordinated to the calixarene ligand, the guest exchange mechanism involves a zinc penta-coordinated intermediate where the zinc atom is simultaneous coordinated to an endo-complexed guest (inside the cavity) and an exo-complexed molecule (outside the cavity). 1D EXchange SpectroscopY experiments (EXSY) were undertaken with two calix[6]arene complexes where the zinc is tri-coordinated to the calixarene ligand and with a calix[6]arene-zinc complex where the zinc is tetra-coordinated to the calixarene ligand. The exchange of different guests (ethanol, dimethylformamide and acetonitrile) was monitored in deuterated dichloromethane. We observed that in all cases water accelerates guest exchange but that the guest residence times are highly dependent on the acidity of the metal center and on the nature of the guest buried inside the cavity.

Keywords: supramolecular chemistry, molecular receptors, calix[6]arenes, Nuclear Magnetic Resonance (NMR), Emission Spectroscopy, chemosensors, micelle, 1D EXchange SpectroscopY (EXSY).
“A chemist who does not know mathematics is seriously handicapped”

Irving Langmuir
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LIST OF ABBREVIATIONS

|=|Included
|AcO⁻|Acetate
|ArH|Aromatic protons
|CIS|Complex Induced Shift
|δ|Chemical shift
|DCM-d₂|Deuterated dichloromethane
|DMF|Dimethylformamide
|DOSY|Diffusion-Ordered Spectroscopy
|D₂O|Deuterium oxide
|DPC|Dodecylphosphocholine
|DPC-d₃₈|Deuterated dodecylphosphocholine
|DOPC|1,2-Dioleoyl-sn-glycero-3-phosphocholine
|Equiv. (eq.)|Equivalent
|EtOH|Ethanol
|EXSY|EXchange Spectroscopy
|F|Fluorescence intensity
|G|Guest
|H|Host
|Hepes|4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid
|HG|Host-Guest complex
|H-bond|Hydrogen bond
|IMI|Imidazolidin-2-one
|K|Kinetic constant
|K (Kₐ)|Binding constant
|λ|Wavelength
|OMe|Methoxy group
|NMR|Nuclear Magnetic Resonance
|T₁|Longitudinal relaxation time
|TBA|Tetra-n-butylammonium
|tBu|Tert-butyle
|τₙ|Mixing time
|TREN|Tris(2-aminoethyl)amine
|UV-vis|Ultra-Violet visible
|XRD|X-Ray Diffraction
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CHAPTER 1.
GENERAL INTRODUCTION
The work presented in this thesis falls in the field of supramolecular chemistry, the area of chemistry that focuses on chemical systems made up of a discrete number of assembled molecular subunits or components. In this thesis, we focused more particularly on calix[6]arenes receptors. In this chapter, we will first introduce the context of supramolecular chemistry and present calix[6]arenes and their binding properties. We will then introduce in more detail the two main families of calixarenes we worked with.

1.1. SUPRAMOLECULAR CHEMISTRY

Supramolecular chemistry focuses essentially on the weaker and reversible non-covalent interactions between molecules. In 1894, Emil Fischer proposed the “Lock and Key” model to visualize the interaction between an enzyme and its substrate. Even though successive studies have shown that not all enzymatic reactions can be explained with this model, it laid the foundations for the concept of molecular complementarity in biochemistry, further taken up in supramolecular chemistry. The importance of supramolecular chemistry was recognized by the 1987 Nobel Prize for Chemistry, which was awarded to Donald J. Cram, Jean-Marie Lehn, and Charles J. Pedersen for their “development and use of molecules with structure-specific interactions of high selectivity”. The development of selective “host-guest” complexes in which a host molecule (receptor) recognizes and selectively binds a guest was cited as an important part of their work (Figure 1-1).

![Figure 1-1. Schematic representation of the interactive between a host and a guest molecule.](image_url)
plusieurs espèces chimiques, les complexes, et de la liaison intermoléculaire”.1 More recently, supramolecular chemistry has been once again put forward by the 2016 Nobel Prize for Chemistry awarded to Jean-Pierre Sauvage, J. Fraser Stoddart and Ben Feringa for their “design and production of molecular machines with controllable movements”.

In a supramolecule, the different entities interact through low energy (compared to covalent interactions) “non-covalent interactions”, such as coordination bonds, H-bonds, ionic, or Van der Waals interactions (Table 1-1).2 We can also cite more unconventional non-covalent interactions, such as anion-π or halogen-bonds.3 The supramolecule is characterized by the spatial arrangement of its different components and by its stability.4 The individual molecular entities are not modified in their chemical structure, but the new system acquires new properties in comparison to the molecules composing it.

Table 1-1. Examples of non-covalent interactions.

<table>
<thead>
<tr>
<th>Type of interaction</th>
<th>Energy (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van der Waals</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>H-bond</td>
<td>1 to 120</td>
</tr>
<tr>
<td>CH-π, cation-π, π-π</td>
<td>1 to 80</td>
</tr>
<tr>
<td>Ion-Ion</td>
<td>200 to 300</td>
</tr>
<tr>
<td>Ion-Dipole</td>
<td>50 to 200</td>
</tr>
<tr>
<td>Dipole-Dipole</td>
<td>5 to 50</td>
</tr>
<tr>
<td>Coordination (metal-ligand)</td>
<td>200 to 350</td>
</tr>
</tbody>
</table>

Non-covalent interactions are of prime-importance in biological systems,5 such as for the forming of the DNA double helix or the maintenance of the phospholipids bilayer in cellular membranes.6 Inspired by nature, chemists have been interested in developing synthetic supramolecular systems able to recognize different chemical species. Such systems, called molecular receptors, find applications in many different fields such as catalysis, environmental monitoring or medical diagnostics. Many different types of receptors, which can complex

---

cations, anions or neutral species, have been developed over the past thirty years. Much work has focused on the development of systems, which are selective and even more importantly stable in water. A class of macrocyclic synthetic receptors is the one composed by those bearing a cavity (thus also called cavitands). Examples of these are cucurbit[n]uriles, cyclodextrines, cyclotrimeratilenes, cryptophanes, homocalix[n]arenes, pillar[n]arenes, resorcin[n]arenes (the list is not exhaustive, Figure 1-2). In the context of this thesis, we are interested in a particular class of macrocyclic receptors called calix[6]arenes, which possess a cavity that can accommodate small organic molecules.

![Diagram of macrocyclic molecular receptors bearing a cavity](image)

**Figure 1-2.** Examples of macrocyclic molecular receptors bearing a cavity.

### 1.2. CALIX[6]ARENES

Calix[n]arenes are macrocyclic organic molecules composed of $n$ para-substituted phenolic units (4 to 16) linked by methylene bridges. Their name comes from the Greek *calix* (flask) as the structure of a calixarene molecule in its cone conformation resembles a flask, and *arene* for the aromatic units. These molecules were discovered in the early 40’s by Zinke\(^7\) but calixarene

chemistry really developed with the work of Gutsche in the 80’s who managed to describe a simple procedure for the synthesis of the p-tBu-calix[n]arene (good yields were obtained for n=4, 6 and 8).\(^8\) The calixarenes are obtained via a macrocyclisation reaction of p-tBu-phenol with formaldehyde in the presence of a base (Figure 1-3).

![Figure 1-3. Synthesis of the p-tBu-calix[n]arene.](image)

Calix[6]arenes exhibit conformational flexibility as the inversion of the phenolic units is possible. This inversion involves a rotation around the methylene bridges where either the hydroxyl group (OH group) or the tertio-butyl group can toggle into the calix cavity (Figure 1-4).

![Figure 1-4. Inversion modes of the phenolic units of calix[6]arenes.](image)

A consequence of the conformational flexibility is that 8 different specific conformations are possible for calix[6]arenes (Figure 1-5).

When all the phenolic units point in the same direction, the calixarene is said to be in its cone conformation and a wide rim (composed of the tBu groups) and a small rim (composed of the OH groups) can be distinguished. In their cone conformation, calix[6]arenes have a cavity, which can accommodate small organic molecules. Thus, they can be envisaged for the inclusion of different guests. Different strategies have been developed so as to prevent the conformational flexibility of calix[6]arenes and ensure that they adopt their cone conformation and have thus a well-defined cavity. Three strategies are described below.

Coordination chemistry

A first possibility is to use coordination interactions involving metal cations (e.g. Zn$^{2+}$, Cu$^{n+}$) that can bind to nitrogen containing groups grafted onto the narrow rim. These types of systems are called “funnel complexes” because the free coordination site of the metal cation is within a hydrophobic cavity, which can act as a molecular funnel for neutral organic guest molecules (Figure 1-6). The guest molecules bind to the metal by coordination interactions.\(^9\)

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CHAPTER 1. GENERAL INTRODUCTION

Figure 1-6. Rigidification strategy of calix[6]arenes via the coordination of a metallic cation.\textsuperscript{10}

In the same context, another strategy reported is the introduction of groups on the small rim of the calixarene that allows the coordination to an anion (Figure 1-7).

Figure 1-7. Rigidification strategy of calix[6]arenes via the coordination to an anion.\textsuperscript{11}

Self-assembling\textsuperscript{12}

A second possibility is to exploit the self-assembly of complementary organic functions. For example, an ion-paired cap can close the cavity efficiently and restricts the calixarene in its cone conformation. Examples of such self-assembled systems with calix[6]arenes have been


reported in order to form capsules\textsuperscript{13} and rotaxanes\textsuperscript{14} in solution. Such strategy, based on a calix[6]tris-amine, has been developed in our laboratory in the past years (Figure 1-8).\textsuperscript{15}

\textbf{Figure 1-8.} Calix[6]arenic receptor obtained by rigidification via self-assembly.

**Grafting of covalent bonds**

A third possibility is to close the narrow rim with covalent bridges between the phenolic units. Examples are calix[6]arenes bearing an aza-cryptand cap (Figure 1-9).

\textbf{Figure 1-9.} Rigidification of calix[6]arenes by covalent grafting of an aza-cryptand cap.\textsuperscript{16}

In the next two sections, we will focus on the two main families of calixarene we have worked with: (i) calix[6]arenes-zinc complexes, known as “funnel complexes” and (ii) calix[6]arenes functionalized with urea moieties.


1.3. **FUnnel Complexes**

Calix[6]arenes that are functionalized on alternate phenolic positions (positions 1,3,5) by three amino arms that can complex a metallic cation are known as “funnel complexes”. The binding of the metal ion constrains the calix[6]arene into its cone conformation, leaving a single metal coordination site accessible for guest binding inside the cavity (Figure 1-6).

One of the reasons for the interest in calix[6]arene funnel complexes, is that they can be seen as models for the active site of metallo-enzymes. Indeed, many enzymes contain a metallic cation, which can have a structural role, be involved in the recognition process, or be active in the catalytic process. The analysis of the mechanisms of these enzymes is not straightforward as they are very unstable when out of their biological environment. Over the years, chemists have therefore been interested in the design of synthetic model systems that can be used to elucidate the functioning of metallo-enzymes.

Zinc is the second most abundant transition metal cation found in biological systems and zinc metallo-enzymes play many important roles in the human body. The most commonly observed binding geometry at the level of the coordination sphere of the zinc cation is tetrahedral. The zinc cation present in the active site of carbonic anhydrase is for example coordinated to three histidine moieties and to a water molecule. There are also many enzymes, such as astacins or β-lactamase, where a penta-coordinated trigonal bipyramidal coordination geometry is observed. Accurate models for the active site of metallo-enzymes must present the following characteristics:

- coordinating arms, which reproduce the direct environment near the metal (first coordination sphere);
- groups that reproduce aminoacids residues which are in proximity with the metallic center that stabilize the substrate (second coordination sphere). The selectivity for the substrate is also given by the arranging of these residues in the active site;
- an access funnel (hydrophobic cavity that plays the role of the enzymatic pocket) that controls the arrival of the substrate. This funnel allows the protection of the active site.

---


from the external media permitting the approach of the substrate and the expulsion of the product after the catalysis (third coordination sphere).

Calix[6]arenes, such as those being developed in the laboratories of Prof. Reinaud and Prof. Jabin, are good models as they present a cavity, which can host organic molecules, and a binding site for metallic cations. They do not only imitate the “first coordination sphere” thanks to the ligands present on the narrow rim but also the “second and third coordination sphere” thanks to the hydrophobic cavity. Three families of calix[6]arenes have been developed: calix[6]N₃, calix[6]N₃Z and the calix[6]aza-cryptands (Figure 1-10). In this thesis, we are mainly interested in the calix[6]N₃ and calix[6]aza-cryptands that are described in more detail in the next sections.

**Figure 1-10.** Supramolecular approach for the mimic of the active site metallo-enzymes.

### 1.3.1. **Calix[6]N₃**

The first generation of models developed bear three nitrogen arms in position 1-3-5 at the level of the small rim of the calixarene. Different ligands have been developed (Figure 1-11).

**Figure 1-11.** Schematic representation of the family of receptors calix[6]N₃.¹⁰

The most studied receptor for copper (II) and zinc complexation is the one with 3 methyl-imidazole moieties grafted on the small rim that for the sake of convenience we will call calix[6]tris-imidazole or host 1. In the 1.Zn²⁺ complex, the cation coordinated to the imidazole moieties acquires a tetrahedral geometry. XRD and ¹H NMR spectroscopy studies in organic
solvents have shown that, in the absence of any coordinating guest, two water molecules are included in the calixarene cavity: the first molecule is linked to the metal, whereas the second one is linked to the first water molecule and to a phenolic oxygen by H-bonding interactions (Figure 1-12).\textsuperscript{19,20}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1-12.png}
\caption{Host 1 Zn$^{2+}$ with two water molecules buried in the cavity.\textsuperscript{19}}
\end{figure}

Host 1 Zn$^{2+}$ is known to bind neutral guest molecules at the level of the metal center. The affinity for some neutral guest was determined against water and DMF, and the relative affinity constants are given in Table 1-2.\textsuperscript{21} Results showed that:

- the guest is exchangeable;
- a selectivity is observed in terms of the affinity of the guest for the metal and of its size and shape;
- the system formed is stabilized by H-bonds and CH-\(\pi\) interactions;
- anionic guests are not recognized inside the cavity due to the electrostatic repulsion generated by the second coordination sphere (oxygen atoms present at the small rim).


Table 1-2. Relative affinity constants $K_{L/water}$ and $K_{L/DMF}$ at 298 K for guest exchange for $1.Zn^{2+}$ in CDCl₃.¹⁹

<table>
<thead>
<tr>
<th>Guest (G)</th>
<th>$K_{L/water}$ (M)</th>
<th>$K_{L/DMF}$ (M⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptylamine</td>
<td>&gt;2</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2-Methylbutylamine</td>
<td>&gt;2</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2-Butylylamine</td>
<td>0.78</td>
<td>9</td>
</tr>
<tr>
<td>DMF</td>
<td>0.082</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.39</td>
<td>4.7</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>0.013</td>
<td>0.16</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>0.0004</td>
<td>0.005</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.031</td>
<td>0.38</td>
</tr>
<tr>
<td>Propionitrile</td>
<td>0.011</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The selectivity for the different endo-complexed guests is conditioned by steric hindrance at the level of the coordinating atom (O or N) and at its α-position which explains why primary amines and primary alcohols are preferred guest molecules.²⁰ Another important property of $1.Zn^{2+}$, highlighted by XRD, is that the imidazole arms twist around the metal center forming a helix. This induces chirality in the complex. The helix can twist in two opposite directions, giving rise to two enantiomeric conformers, which are at equilibrium. Passing from one conformer to another is called the “helical inversion” (Figure 1-13).²²

![Figure 1-13. Equilibrium between the two enantiomeric conformers with an acetonitrile molecule buried inside the cavity.](image)

1.3.2. CALIX[6]AZA-CRYPTANDS

The calix[6]aza-cryptand family (Figure 1-14), with its covalently linked nitrogen arms, presents advantages compared to the systems with three independent nitrogen arms.²²

---

The systems with three independent arms generally exhibit a low affinity for the metal and the metallic complexes have been shown to exhibit a lack of stability in the presence of coordinating species such as hydroxyl group or DMSO. Moreover, the metal is exposed giving rise to an exo-coordination site accessible in the apical position for an exogenous molecule (Figure 1-15).

A grafted cap gives rise to the following advantages:

- a higher stability of the metal complexes formed thanks the chelate effect;
- the metal center is protected from the bulk and no exo-coordination is possible.

The most studied aza-cryptand complex is the calix[6]tren 6 (Figure 1-16). This macrocycle is functionalized by a tris(2-aminoethyl)amine cap called tren. Its host-guest properties have been intensively studied in organic solvents. Results show that this host can accommodate neutral guests in chloroform (Figure 1-16A). Recognition is possible thanks to the multiple H-bond interactions between the ammonium head and the calixarene, but also thanks to CH-π interactions.
interactions between the organic part of the ammonium and the aromatic unities of the cavity of the calixarene.

![Figure 1-16](image)

**Figure 1-16.** (A) the neutral ligand can accommodate a primary ammonium; (B) the tetra-protonated ligand can accommodate neutral polar molecules such as an amide or an alcohol; (C) the metallic complex (Zn$^{2+}$, Cu$^{2+}$ etc.) can complex a molecule with a coordinating group.

Affinities of host 6 for different guests relative to ethylammonium (EtNH$_3^+$) have been measured by NMR. Results (in CDCl$_3$ at 298 K) showed that the affinity is dependent on the length of the ammonium chain and its degree of substitution: propylammonium ($K_{\text{PrNH}_3^+/\text{EtNH}_3^+} = 3$) and butylammonium ($K_{\text{BuNH}_3^+/\text{EtNH}_3^+} = 0.1$). For (CH$_3$)$_2$NH$^+$ the relative affinity constant determined was 0.05 and no interaction was observed for hindered quaternary ammoniums ((CH$_3$)$_4$N$^+$).

The tetra-protonated system 6.4H$^+$ exhibits good recognition properties for neutral polar guests, such as alcohols, nitriles, aldehydes amides and DMSO, in CDCl$_3$ (Figure 1-16B). The complexes are stabilized by dipole-charge interactions between the polar guest and the protonated cap of the receptor but also thanks to H-bond and CH–π interactions.

When a metal cation such as (Zn$^{2+}$ or Cu$^{2+}$) is coordinated to 6, the system can *endo*-complex different types of guests (Figure 1-16C). The affinities relative to DMF of 6.Zn$^{2+}$ for different guests have been determined by NMR (in CDCl$_3$ at 298 K) and have showed that amines are preferred over alcohols and amides and that the affinity is dependent on the length of the amine chain: $K_{\text{PrNH}_2/\text{DMF}} = 1600$, $K_{\text{OctylNH}_2/\text{DMF}} = 0.9$, $K_{\text{MeCONH}_2/\text{DMF}} = 0.4$ and $K_{\text{EtOH/DMF}} = 0.4$. It has also been shown that when 6.Zn$^{2+}$ is in the presence of PrNH$_2$ and DMF only 6.Zn$^{2+}$⊂PrNH$_2$
is observed but that, upon addition of TFA, a de-metalation occurs and the formation of $6\cdot 4{H^+}\subset{\text{DMF}}$ is observed. The addition of NEt$_3$ reestablishes $6\cdot Zn^{2+}\subset{\text{PrNH}_2}$.

1.4. CALIX[6]UREAS-BASED RECEPTORS

The complexation of a cation or an anion by a neutral receptor is always in competition with the association of the guest with its counter-ion. In the context of host-guest chemistry this is problematic as it can lead to the precipitation of the ion-pair or it can even inhibit the complexation of the charged guest. In order to override this problem, the classical strategy is the use of non-coordinating counter-ions (TBA$^+$, Pic$^-$, BF$_4^-$, PF$_6^-$, ClO$_4^-$ etc.). Another strategy, which has come under considerable spotlight in the last years,\textsuperscript{25} is the development of systems that can simultaneously complex the two partners of the ion-pair. This strategy can indeed lead to a minimization of the competition due to the formation of the free ion-pair but can also find interesting applications in the recognition of zwitterionic types of guest such as phospholipids.

1.4.1. COMPLEXATION OF ION-PAIRS: THE STRATEGY

A considerable amount of work has been devoted to the design of ditopic molecular receptors capable of complexing ion-pairs. Three strategies are reported for the recognition of ion-pairs as described below (Figure 1-17).

![Figure 1-17. The three strategies used for ion-pair recognition: A “Dual Host” strategy, B ditopic receptors for dissociated ion-pairs; C ditopic receptors for contact ion-pairs.\textsuperscript{26}]

**The Dual-Host strategy**

This strategy involves two different receptors: one for each partner of the ion-pair. As it requires two receptors this strategy cannot give rise to cooperative processes (Figure 1-17A).


\textsuperscript{26} McConnell, A.J.; Beer, P. D. *Angew. Chem Int. Ed.* 2012, 5, 2-12.
Ditopic receptors for dissociated ion-pair

This approach allows the complexation of the two partners of the ion-pair within the same receptor, at the level of two recognition sites that are spatially distinct. The complexation of the first partner can lead to a modification of the receptor conformation that can favor or disfavor the complexation of the second partner. Thus this strategy can lead to allostERIC processes (Figure 1-17B).

Ditopic receptors for contact ion-pairs

This third strategy permits the complexation of the two partners of the ion-pair in close proximity within the receptor. This close proximity can allow the establishment of an ionic interaction between the two partners. This is energetically favorable as it avoids the dissociation of the ion-pair and can possibly lead to a cooperative process (Figure 1-17C). As calix[6]arenes can be designed as molecular receptors for ditopic guests, in this thesis we are particularly interested in this strategy since we want to exploit them for the recognition of different types of analytes.

1.4.2 CALIX[6]UREAS FOR CONTACT ION-PAIR RECOGNITION

Calix[6]arenes can be easily functionalized at the small rim with moieties capable of recognizing an anion. The cavity, for its part, can host a cationic molecule. These systems are undoubtedly suitable candidates for recognizing ion-pairs.

Neutral anions receptors typically contain H-bond donor groups such as sulfonamides,27 amides,28 (thio)ureas,29 pyrroles,30 carbazoles31 or indoles.32 The grafting of arms bearing such moieties on the small rim of calix[6]arenes make the systems suitable candidates for the recognition of ion-pairs as the calixarene cavity can potentially accommodate a cationic species.

The calix[6]arenes bearing two (thio)ureas arms developed by Yang et al. and those bearing

three urea arms developed by Reinhoudt et al. (Figure 1-18) have been shown to efficiently complex, in organic solvents, anions at the level of the urea groups. The recognition of ion-pairs was however not evaluated by these groups with these systems.

![Figure 1-18](image)

**Figure 1-18.** Left: Yang’s receptors; Right: Reinhoudt’s receptors.

A calixarene-based receptor, similar to the ones of Yang and Reinhoudt, has been synthetized in the Jabin laboratory (calix[6]tris-phenylurea, host 10). The NMR study of the complexation capabilities of host 10 has shown that this is an excellent heteroditopic receptor for contact in pairs such as ammonium halides like PrNH$_3^+$Cl$^-$ (Figure 1-19).

---

35 Which possess two distinct sites of recognition.
Recognition of the anion via H-bonding interactions at the level of the urea moieties polarizes the receptor promoting the *endo*-complexation of the ammonium inside the cavity. The ammonium is stabilized by an ionic interaction with the anion and by non-covalent interactions (CH-π and H-bonding interactions) with the calix structure. A cooperative effect is observed in the sense that the complexation of an ammonium ion is possible only if the anion is simultaneously bound at the level of the urea groups. It was furthermore observed that the anions with the higher binding affinity are not those that best promote the inclusion of the ammonium. This was rationalized as a consequence of the size of the anion and by the fact that the stronger the interaction with the urea moieties, the more the charge density will be weakened at the level of the anion and consequently the ionic interaction with the ammonium. The remarkable properties of this receptor are the consequence of the close proximity of the two binding sites that enables the complexation of contact ion-pairs.

Calix[6]arenes with a cryptamide or cryturea cap have also been developed in the Jabin’s laboratory for ion-pair recognition. Receptor 11, shown in Figure 1-20, has for example been shown to complex various ammonium fluorides with good affinity. The chelate effect is of course beneficial. Calixcrypturea 12 shows similar characteristics to its cryptamide

---

**Figure 1-19.** Recognition mode for contact ion-pairs with receptor 10.\(^{31}\)

analog (Figure 1-20). It complexes not only linear ammonium halides but also biological ammoniums.

![Complexation of a contact ion-pair with receptors 11 and 12.](image)

**Figure 1-20.** Complexation of a contact ion-pair with receptors 11 and 12.

Other systems synthesized and studied in the Jabin’s group, in this case for the binding of ion triads, are the calix[6]tube-(thio)ureas 13a-b, which display two divergent cavities linked by three (thio)urea arms. It was shown that 13a-b exhibit efficient binding properties for contact ion triads and a selectivity for primary or secondary ammoniums associated to a divalent anion (ex. : SO$_4^{2-}$) (Figure 1-21). 13b was also shown to be able to bind larger ammoniums in protic solvents. XRD results showed that the cascade complexes are stabilized through a solid arrangement of hydrogen-bonding interactions. It was also observed that ammonium nitrate salts can be selectively extracted from water to chloroform as the nitrate anion combines weak hydration with good geometrical complementarity with the tris-thiourea binding site.

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Figure 1-21. Complexation of a contact ion with a calix[6]tube-(thio)ureas 13a-b.

Very recently, it has been reported that the thio(urea) derivative 13b, is able to selectively bind phosphatidylcholines (PCs) over phosphatidylethanolamines (PEs).\(^{40}\) The binding of the PCs is made possible thanks to multiple H-bonding interactions between their anionic moiety and the tris-thiourea groups of the host, as well as through cation-\(\pi\) and CH-\(\pi\) interactions between their cationic \((\text{CH}_3)\text{N}^+\) group and one of the two polyaromatic calixarene cavities. Moreover, results showed that the fatty acid chains of the bound lipids do not protrude from the second calixarene cavity but from one of the three macrocycles formed by the thio-urea arms.

1.5. AIM OF THE THESIS

As we have seen in this introduction, calix[6]arenes are versatile scaffolds to build molecular hosts that can be exploited in molecular recognition processes. Their interesting and polyvalent complexation properties depend on their functionalization. Different calix[6]arene based systems have been studied in this thesis, using mainly Nuclear Magnetic Resonance (NMR), UV-visible and emission spectroscopies.

The calixarene cavity offers recognition selectivity, via form and size complementarity, and a first goal of our work is to see if systems with grafted chromophores could be used for the selective sensing of different guests. We report in chapter 2 our studies undertaken with fluorescent receptors calix[6]tris-pyrenylurea 14a-b (Figure 1-22).

We first completed the work that Dr. Picron initiated in the framework of its PhD thesis under the supervision of Prof. Jabin\textsuperscript{41}, devoted to the recognition of ions and ammonium salts. We also studied the receptors for the sensing of phospholipids.

![Figure 1-22. Structure of receptors 14a-b.](image)

Another goal of the thesis is to evaluate the possibility to transfer calixarenes and related hydrophobic receptors into aqueous media using micelles. Hydrophobic receptors incorporated into micelles can indeed be exploited for the recognition of analytes in an aqueous environment. The studies undertaken on the binding capacities of the calix[6]tren zinc complex (6.Zn\textsuperscript{2+}) incorporated in doceylphosphocholine (DPC) micelles are reported in chapter 3. The

binding potential of a homooxacalix[3]arene derivative (15) and a resorcin[4]arene-based zinc complex (16.Zn\textsuperscript{2+}) incorporated in DPC micelles is also evaluated and the results are also described in chapter 3 (Figure 1-23).

![Figure 1-23. Structure of 6.Zn\textsuperscript{2+}, 15 and 16.](image)

The last goal of the thesis is to try and elucidate the mechanism of guest exchange of the funnel complex (1.Zn\textsuperscript{2+}). The hypothesis put forward is that the exo-coordination of a ligand (solvent or another coordinating molecule) at the level of the zinc cation is required for exchange to occur. In other words, guest exchange requires an intermediate step. The model behind this hypothesis is described in detail in chapter 4. In order to validate it, we measure the residence time of various guests in the cavity of 1.Zn\textsuperscript{2+} as a function of the concentration of different possible exo-ligands. Similar receptors calix[6]tris-benzimidazole zinc (2.Zn\textsuperscript{2+}) and calix[6]tren zinc (6.Zn\textsuperscript{2+}) are also studied in this context.

![Figure 1-24. Structures of 1.Zn\textsuperscript{2+}, 2.Zn\textsuperscript{2+} and 6.Zn\textsuperscript{2+}.](image)
CHAPTER 2.

STUDY OF A FLUORESCENT CALIX[6]ARENE FOR THE RECOGNITION OF ANIONS, CONTACT ION-PAIRS AND PHOSPHOLIPIDS
In this chapter we discuss the work that we have undertaken with a fluorescent calix[6]arene receptor bearing urea moieties. The binding of anions, contact ion-pairs and phospholipids was monitored by NMR and emission spectroscopies.

2.1. COMPLEXATION OF ANIONS AND CONTACT ION-PAIRS

2.1.1. CONTEXT OF THE RESEARCH

As presented in the Introduction (section 1.4.2), calix[6]tris-phenylurea (host 10) (Figure 2-1) is an excellent heteroditopic receptor for contact ion-pairs such as ammonium halides. It indeed combines:

- a site composed of three urea moieties which can bind anions;
- a cavity adapted to the endo-complexation of small organic molecules.

A remarkable positive cooperativity has been reported as the complexation of the ammonium is only observed if the anion is complexed at its recognition site. The recognition process is induced fit driven, involving a conformational reorganization of the calixarene structure.

![Figure 2-1. Structures of calix[6]tris-phenylurea 10a-b and calix[6]tris-pyrenylurea 14a-b.](image)

In order to monitor the complexation process by UV-visible absorption and emission spectroscopies, Dr. Jean-François Picron synthesized and studied, in the framework of his PhD thesis, the calix[6]tris-pyrenylurea 14a, which bears three pyrenyl chromophores (Figure 2-1). He studied the complexation of different anions and monovalent ion-pairs in different organic

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solvents (DMSO, CHCl₃, CHCl₃/CH₃CN 1:1) by NMR and emission spectroscopies. He also undertook preliminary studies with ion triads.

In the framework of this thesis we have undertaken complementary studies with 14a and have also looked at the binding properties of 14b, which was synthesized by Dr. Karolina Flidrova in the Jabin’s laboratory. We rationalized all the acquired data and it is all reported in the paper “Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity” which was published in the Journal of Organic Chemistry (2014, 79, 6179–6188).

2.1.2. SUMMARY OF PUBLISHED RESULTS

In the paper “Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity”, the synthesis of the fluorescent calix[6]tris-pyrenlyurea hosts 14a and 14b is reported. The complexation properties of both systems are also described in detail.

Host 14a exhibits a remarkable selectivity for the sulfate anion in DMSO. An important drop of the fluorescence intensity is indeed observed only upon addition of SO₄²⁻. A significantly weaker drop is observed with AcO⁻ and none with the other anions tested (halides, HSO₄⁻, nitrate). In CDCl₃, the host forms complexes of different stoichiometry with the tested monovalent anions (e.g. Cl⁻, AcO⁻) but forms a 1:1 complex with sulfate, as confirmed by Job Plot experiments. This contrasts with the results obtained with parent receptor 10, where 1:1 complexes were observed for all monovalent anions in CDCl₃. The presence of pyrenes at the level of the urea moieties clearly creates steric hindrance preventing the receptor to adapt to smaller anions.

Host 14a is also able to bind ammonium ions efficiently in CDCl₃ when a sulfate anion is present at the level of the urea moieties. This cooperative binding has already been observed for host 10. The studies undertaken with SO₄²⁻-PrNH₃⁺ (with TBA⁺ as a counter-ion) show that guest exchange is slow on the NMR chemical shift timescale. Signals for the NH protons of the receptors appear downfield upon titration attesting of the interaction of the sulfate anion with the urea moieties via H-bonding interactions. The inclusion of the PrNH₃⁺ inside the cavity is attested by the presence of high field signals in the NMR spectrum, due to the anisotropy cones of the aromatic cycles. Binding constants were too high to be determined by ¹H NMR (log $K > 4$ for SO₄²⁻-PrNH₃⁺), but were determined by UV-vis absorption and emission spectroscopies. Upon addition of the guest to a solution of 14a, a new absorption band, signature of the emergence of a new complex, is observed. An increase in the monomer emission and a decrease
in the excimer emission is also observed, suggestive of the separation of the urea groups upon anion coordination. Affinity for $\text{SO}_4^{2-}$-$\text{PrNH}_3^+$ of the order of $\log K \sim 5$ was determined.

The interaction of the TBA$^+$ counter-ion with the receptor at the level of the pyrene units was also highlighted. The complexation of this quaternary ammonium, which is in fast exchange on the chemical shift timescale, was evidenced through $^1$H DOSY NMR and a $\log K \sim 2$ was determined. CH-$\pi$ interactions between the TBA$^+$ and the pyrenes are probably at the origin of this favorable interaction.

The binding of bigger ammonium salts (e.g. TBA$^+$-$\text{SO}_4^{2-}$-$\text{HexNH}_3^+$ or TBA$^+$-$\text{SO}_4^{2-}$-$\text{DodNH}_3^+$) was evaluated with 14a and also receptor 14b (Figure 2-1). The latter receptor only presents three $t$Bu groups at the level of the large rim of the calix[6]arene. The $t$Bu groups bound to the phenyl rings that bear the urea moieties have been removed as it was observed with 14a that they point inside the cavity upon anion coordination disturbing the potential complexation of guests that protrudes out of the cavity. Upon addition of TBA$^+$-$\text{SO}_4^{2-}$-$\text{HexNH}_3^+$ or TBA$^+$-$\text{SO}_4^{2-}$-$\text{DodNH}_3^+$, the formation of the corresponding inclusion complexes was observed with 14b. With 14a we observe a competition between the inclusion complex (long chain ammonium in the cavity) and the complex with only the sulfate. Furthermore, only host 14b was able to bind 3,4-O-dimethyldopammonium sulfate salt.

In conclusion calix[6]arene-based receptors have been synthetized and have shown to bind ion-pairs via urea moieties grafted on their small rim and their cavity. Moreover, a third recognition site was observed at the level of the pyrenyl groups, which are able to weakly bind the counter-ion TBA$^+$. The complexes formed are stabilized by H-bonding interactions, ionic interactions and CH-$\pi$ interactions (Figure 2-2 for $14 \supset \text{TBAPrNH}_3\text{SO}_4$).
Figure 2-2. Representation of the recognition mode for the complexation of TBA$^+$-PrNH$_3^+$-SO$_4^{2-}$ by 14.

In this context, the present work opens interesting perspectives for the elaboration of unique fluorescent cavity-based systems for the selective sensing of anions or biologically relevant ammonium salts such as neurotransmitters.
CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity

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Supporting Information

ABSTRACT: The association of a concave macrocyclic compound to one or multiple fluorophores is an appealing strategy for the design of chemosensors. Indeed, as with biological systems, a cavity-based selectivity can be expected with such fluorescent receptors. Examples of calix[6]arene-based systems using this strategy are rare in the literature, and to our knowledge, no examples of fluorescent receptors that can bind organic contact ion pairs have been reported. This report describes the straightforward synthesis of fluorescent calix[6]arene-based receptors 4a and 4b bearing three pyrenyl subunits and the study of their binding properties toward anions and ammonium salts using different spectroscopies. It was found that receptor 4a exhibits a remarkable selectivity for the sulfate anion in DMSO, enabling its selective sensing by fluorescence spectroscopy. In CDCl3, the receptor is able to bind ammonium ions efficiently only in association with the sulfate anion. Interestingly, this cooperative binding of ammonium sulfate salts was also evidenced in a protic environment. Finally, a cavity-based selectivity in terms of size and shape of the guest was observed with both receptors 4a and 4b, opening interesting perspectives on the elaboration of fluorescent cavity-based systems for the selective sensing of biologically relevant ammonium salts such as neurotransmitters.

INTRODUCTION

The design of artificial receptors that can selectively bind charged or neutral species with high affinity is a major objective in supramolecular chemistry. Indeed, such receptors could find many applications in various areas and can for example be envisaged for the sensing of chemical species in the fields of biological and environmental analyses. A classical strategy for the design of such chemosensors consists of grafting one or multiple fluorophores onto a molecular receptor that displays a high selectivity for a given guest. Indeed, fluorescence spectroscopy is particularly sensitive and allows the detection of chemical species at nanomolar concentrations. For the elaboration of the molecular receptor, the use of cavity-based macrocyclic compounds is particularly attractive because, in strong relation to natural systems, it can be expected that the cavity will ensure very high selectivity. Calixarenes are well-known concave macrocyclic compounds that have been widely used for the development of fluorescent chemosensors. However, these fluorescent systems have been obtained quasi-exclusively using calix[4]-arenes, whose cavities are too small for the recognition of organic guests, and therefore, they have been mostly used as a platform for the preorganization of a recognition site outside of the cavity. Surprisingly, only a few papers have described the use of fluorescent calix[6]arenes, despite the fact that these larger oligomers can accommodate organic guests in their cavities. Moreover, to our knowledge, there are no examples of fluorescent receptors that can bind organic contact ion pairs as most of the systems are devoted to the recognition of cations, anions, or neutral guests.

As part of our continuous interest in the synthesis and study of calix[6]arene-based receptors for neutral or charged species, we wanted to exploit the cavity-based selectivity of these receptors for the design of highly selective fluorescent systems. In this regard, we were interested in the elaboration of fluorescent derivatives of calix[6]tris(urea) compounds 1a and 1b (Figure 1). Indeed, it was reported that these receptors can efficiently bind either anions or, in a cooperative way, organic contact ion pairs. The three converging urea groups allow strong binding to anions, which in turn can lead to the strong

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binding of an ion paired ammonium accommodated in the calix[6]arene cavity. The proximity of the two binding sites is crucial in the recognition process as it circumvents the highly energetically unfavorable separation of the cobound ions.\textsuperscript{19} It is noteworthy to mention that calix[6]tris(urea) receptors 1a and 1b constitute one of the rare examples of molecular receptors able to bind organic contact ion pairs.\textsuperscript{20}

With the aim of studying the potential of transforming system 1a into a sensor, we have grafted fluorophores in close proximity to the tris(urea) binding site. The pyrenyl group was chosen as the fluorophore\textsuperscript{21} as the variation of the excimer to monomer emission intensity ratio can yield information on conformational changes of the receptor upon binding.\textsuperscript{22} Besides the synthesis of new fluorescent calix[6]arene-based receptors, the aims of this study were to see (i) whether the binding properties of the receptors were preserved despite the introduction of bulky fluorophores in close proximity to the urea groups, (ii) whether it was possible to give rise to an effective anion or ion-pair sensor in terms of detection by fluorescence spectroscopy, and (iii) whether cavity-based selectivity, notably in terms of size and shape complementarities, could be associated with the sensing process. Herein we describe the synthesis of these new fluorescent calix[6]arene-based receptors and the study of their binding properties toward anions and ammonium salts using NMR, UV–vis absorption, and fluorescence spectroscopy.

### RESULTS AND DISCUSSION

**Synthesis and Characterization of Calix[6]tris-(pyrenylurea) Receptors 4a and 4b.** The synthesis of two receptors that offer cavities of different size and shape, 4a and 4b, was undertaken. Calix[6]tris(azido) 3b was first obtained in 77% yield through allylation of the partially de-tet-butylated 1,3,5-tris(methoxy)calix[6]arene 2\textsuperscript{23} with an excess of 2-azidethyl-4-methylbenzenesulphonate.\textsuperscript{24} A one-pot, two-step procedure consisting of a domino Staudinger/aza-Wittig reaction (PPh\textsubscript{3}/CO\textsubscript{2}H) and a subsequent addition of pyren-1-amine afforded the desired calix[6]tris(pyrenylurea) compounds 4a and 4b in 70% and 35% overall yields from calix[6]tris(azido) precursors 3a\textsuperscript{25} and 3b, respectively (Scheme 1).

The \textsuperscript{1}H NMR spectra of calix[6]tris(pyrenylurea) compounds 4a and 4b in CDCl\textsubscript{3}, show broad signals characteristic of C\textsubscript{2v}-symmetric species (for 4a, see Figure 2a). When the spectra were recorded in a competing solvent (DMF-d\textsubscript{7}), sharp NMR signals characteristic of a major C\textsubscript{2h}-symmetric flattened cone conformation were observed for both systems (\textDelta\delta\textsubscript{iso} = 0.70 and 0.72 ppm for 4a and 4b, respectively) with the methoxy groups pointing inside the cavity (\textDelta\delta\textsubscript{iso} = 2.12 and 2.60 ppm for 4a and 4b, respectively; for 4a, see Figure 2b). In strong analogy with the parent receptors 1a and 1b, this solvent-dependent conformational behavior indicates intramolecular self-association of the urea groups through hydrogen-bonding interactions in apolar
solvents such as CDCl₃ (see the structure displayed in Figure 2a), the exchange process between the three nonequivalent urea groups being fast on the NMR chemical shift time scale) and, on the contrary, separation of the bulky pyrenyleurea groups in competing solvents (see the structure displayed in Figure 2b). It should be noted that in the particular case of 4a and 4b, the self-association of the urea arms could also be due in part to π-stacking interactions between the pyrenyl moieties. Calix[6]-tris(pyrenyleurea) receptors 4a and 4b exhibit UV–vis absorption bands at 280, 346, and 389 nm in chloroform and at 279, 348, and 390 nm (4a) or 285, 354, and 390 nm (4b) in DMSO. Anion Complexation Properties of 4a. The ability of calix[6]-tris(pyrenyleurea) 4a to bind anions of various geometries (i.e., Cl⁻, AcO⁻, HSO₄⁻, and SO₄²⁻) was first investigated in DMSO-d₆ by NMR spectroscopy through the progressive addition of the corresponding tetra-n-butylammonium salts (TBA⁺). Except for a weak downfield shift of the urea NH signals, the NMR spectrum of 4a remained quasi-unchanged upon the addition of a large excess (up to 20 equiv) of either Cl⁻, AcO⁻, or HSO₄⁻. The absence of a conformational reorganization of the calixarene core suggests an extremely weak binding of these anions by only one of the three ureido groups. In strong contrast, upon the progressive addition of (TBA⁺)₂SO₄·6H₂O, downfield shifts of the CH₂CO, CH₂NH, and OCH₃ signals and upfield shifts of some of the pyrenyleurea signals were observed (Figure 2c). Significant downfield shifts of the urea NH signals (δ NH upfield = 1.60 and 2.18 ppm) were also observed, which clearly indicates strong hydrogen-bonding interactions between the urea groups and the anion. All of these NMR data are compatible with strong binding of the sulfate anion by the convergent NH groups of the pyrenyleurea arms, which are thus projected above the small rim upon anion complexation. The resulting host–guest complex 4a·SO₄²⁻ displays a major flattened cone conformation (Δδ NH = 0.63 ppm and Δδ NH = 0.57 ppm) with the methoxy groups pointing inside the cavity (δ NH = 2.55 ppm), as illustrated in Figure 2c. It is noteworthy to mention that only one set of signals is apparent over the course of the titration, evidencing fast host–guest exchange on the NMR chemical shift time scale. Job’s plot experiments²⁸ by UV–vis spectroscopy indicate a 1:1 binding stoichiometry.²⁷ An association constant of log K = 3.4 ± 0.3 [Table 1] was determined for the binding of the sulfate anion by monitoring the concentration-induced shifts (CISs) of appropriate signals of host 4a (i.e., signals displaying significant shifts upon complexation and no overlap).²⁷ UV–vis and fluorescence titrations in DMSO afforded K values on the same order of magnitude (log K = 3.6 ± 0.4 and 3.9 ± 0.4, respectively).²⁷ The fluorescence spectra show that the monomer emission (402 and 420 nm) decreases and slightly shifts to higher wavelengths while the excimer emission (506 nm) increases slightly upon successive addition of (TBA⁺)₂SO₄·6H₂O (Figure 3 left). These spectral changes are highly compatible with the anion

Table 1. Affinities of Hosts 4a and 4b toward Different Charged Species at 298 K

<table>
<thead>
<tr>
<th>complex formed</th>
<th>solvent</th>
<th>log K°[H₂O]</th>
<th>log K°[HCl]</th>
<th>log K°[HNO₃]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a·SO₄²⁻</td>
<td>DMSO-d₆</td>
<td>3.4 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>4a·Cl⁻</td>
<td>CDCl₃</td>
<td>&gt;4</td>
<td>5.3 ± 0.6</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>4a·SO₄²⁻</td>
<td>CDCl₃</td>
<td>&gt;4</td>
<td>4.0 ± 0.3</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>4a·PyreneNH₃⁺ ·SO₄²⁻</td>
<td>CDCl₃</td>
<td>&gt;4</td>
<td>4.1 ± 0.5</td>
<td>4.8 ± 0.2</td>
</tr>
</tbody>
</table>

"TBA" was used as the counterion in all cases. K = [complex]/([host][guest]). K°[H₂O], K°[HCl], and K°[HNO₃] refer to binding constants determined by NMR, UV–vis absorption, and fluorescence spectroscopy, respectively.
CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

Figure 3. Left: Fluorescence spectra of 4a (~10 μM) upon the addition of TBA\(^+\)SO\(_4\)\(^-\) (0, 20, 100, and 500 equiv) in DMSO (excitation at 348 nm). Right: fluorescence intensity changes \([100\% \times (I - I_0)/I_0]\) for 4a (~10 μM) in DMSO upon the addition of various anions (100 equiv) (excitation at 348 nm). \(I_0\) is the fluorescence emission intensity at 402 nm for free host 4a, and \(I\) is the fluorescence emission intensity after the addition of the anion.

Figure 4. Left: UV-vis spectra of 4a upon the addition of (TBA\(^+\))\(_2\)SO\(_4\)\(^-\) (0 to 21 equiv) in chloroform. \([4a]_0 = 4.8 \times 10^{-5} \text{ M}\) The inset shows the variation of the absorbance at 391 nm upon the addition of (TBA\(^+\))\(_2\)SO\(_4\)\(^-\). Right: fluorescence spectra of 4a upon the addition of (TBA\(^+\))\(_2\)SO\(_4\)\(^-\) (0 to 21 equiv) in chloroform. \([4a]_0 = 4.8 \times 10^{-5} \text{ M}\), \(\lambda_{ex} = 350 \text{ nm}\). The inset shows the variation of the fluorescence intensity at 402 nm upon the addition of (TBA\(^+\))\(_2\)SO\(_4\)\(^-\).

coordination at the level of the urea groups resulting in a greater proximity between the pyrene moieties.

To verify that receptor 4a is indeed a selective sensor for the sulfate anion in DMSO\textsuperscript{3a}, the binding of common anions of various geometries (i.e., SO\(_4\)\(^-\), HSO\(_3\)\(^-\), NO\(_3\)\(^-\), AcO\(^-\), Cl\(^-\), Br\(^-\), I\(^-\)) was investigated by fluorescence spectroscopy. To our delight, only the sulfate anion caused a significant quenching in the emission spectrum of 4a, confirming the high selectivity for this anion (Figure 3 right).

In a second set of experiments, the binding behavior of 4a toward anions was evaluated in CDCl\(_3\). Again, NMR-monitored titration experiments and Job’s plot experiments confirmed the 1:1 complexation of the sulfate anion. The association constant for the host–guest complex 4a\(\times\)SO\(_4\)\(^-\) \((\log K > 4)\) was too high to be determined accurately by NMR spectroscopy but could however be determined by absorbance and fluorescence titrations (Figure 4), which afforded log \(K\) values of 5.1 ± 0.5 and 5.4 ± 0.4, respectively (Table 1). The fluorescence spectra clearly showed that in this solvent the monomer emission (395 and 415 nm) increases while the excimer emission (484 nm) decreases upon successive additions of (TBA\(^+\))\(_2\)SO\(_4\)\(^-\). These spectral changes are in accordance with a separation of the intramolecularly self-associated urea groups bearing the pyrene moieties upon anion coordination.

In comparison with the results obtained in DMSO, the host–guest properties of 4a in chloroform are slightly different. The NMR signals of the calixarene, and in particular those of the urea NH\(_2\), are significantly affected upon the successive addition of anions other than sulfate (i.e., Cl\(^-\), Br\(^-\), AcO\(^-\), HSO\(_3\)\(^-\), H\(_2\)PO\(_4\)\(^-\)), indicating that these anions also bind at the level of the urea groups. However, for these anions the C\(\text{ISS}\) as well as the Job’s plot experiments undertaken by NMR spectroscopy were not compatible with a 1:1 binding stoichiometry but more likely corresponded to the formation of mixtures of complexes with different binding ratios.\textsuperscript{30} Another surprising difference observed was that the chemical shifts of the protons of the TBA\(^+\) counterion were strongly affected during the titrations with all of the tested anions. In the case of (TBA\(^+\))\(_2\)SO\(_4\)\(^-\), a binding constant with log \(K\) = 2.6 ± 0.2 was determined by monitoring the upfield shift of the N’CH\(_3\) signal of TBA\(^+\) (Table 2).\textsuperscript{27} From this titration, it was possible to evaluate the chemical shift for these protons when the TBA\(^+\) cation is complexed, and the obtained value (\(\delta_{N’CH\(_3\)} = 1.62 ± 0.02 \text{ ppm}\)) suggests that this
CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

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Table 2. Affinities of 4a, 4aSO₂⁻, and 4aPnNH₂SO₂⁻ toward TBA⁺ (CDCl₃, 298 K) and Estimated Chemical Shifts of the N’CH₂ Protons of the Bound TBA⁺ Ion

<table>
<thead>
<tr>
<th>Host</th>
<th>log K⁺</th>
<th>δNCH₂ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>1.9 ± 0.1</td>
<td>3.31 ± 0.01</td>
</tr>
<tr>
<td>4aSO₂⁻</td>
<td>2.6 ± 0.2</td>
<td>1.62 ± 0.05</td>
</tr>
<tr>
<td>4aPnNH₂SO₂⁻</td>
<td>2.4 ± 0.2</td>
<td>1.74 ± 0.16</td>
</tr>
</tbody>
</table>

"K⁺ was determined by following the variation of the chemical shift of the N’CH₂ protons of TBA⁺ (vide infra) as a function of log K⁺ = 1.9 ± 0.1 and a chemical shift of 3.11 ± 0.01 ppm for the N’CH₂ protons of the bound TBA⁺ were determined (Table 2). The value of log K⁺ was confirmed through DOSY experiments. The higher binding constant observed for the system in the presence of sulfate can be explained by the favorable electrostatic interaction between the ammonium ion and the cationic TBA⁺ also by the fact that sulfate complexation at the level of the urea units induces a collapse of the pyrenyl units, which form a π-electron donor cavity favorable for cation binding. This hypothesis is confirmed by the fact that the chemical shift for the N’CH₂ protons of the bound TBA⁺ ion is significantly more upfield in the presence of sulfate (1.62 ± 0.05 vs 3.11 ± 0.01 ppm). In other words, the complexation of the anion reorganizes the binding site for the TBA⁺ cation, leading to a positive cooperativity. It is important to point out that in the absence of the receptor, no significant chemical shift changes were observed for the TBA⁺ signals in CDCl₃ as a function of [TBA⁺]SO₂⁻ or TBA⁺BF₄⁻ concentration (up to 50 mM), confirming that the observed change in chemical shift is indeed due to the interaction with the receptor.

Altogether, the results obtained with receptor 4a stand in contrast to those obtained with the parent receptors 1a and 1b, which exhibit a much lower selectivity for the sulfate anion. Indeed, 1a and 1b strongly recognize a large variety of anions with a 1:1 binding stoichiometry (e.g., log K = 2.2 for Br⁻ and log K > 3.9 for AcO⁻, HSO₄⁻, and SO₄²⁻ in the case of 1a in CDCl₃). This difference in the behaviors of the two families of receptors may be rationalized by the presence of steric interactions between the bulky pyrenylemeru subunits when the three urea arms have to come in close proximity upon anion complexation. In the case of 4a, the high selectivity for SO₂⁻ could be due to the fact that this anion is large and doubly charged and displays good complementarity with the tris(urea) binding site.

Ammonium Salt Complexation. The simultaneous complexation of an ammonium ion in the calixarene cavity and an anion at the level of the tris(urea) binding site was investigated by NMR, UV–Vis, and fluorescence spectroscopy in chloroform. In a first set of experiments, PnNH₄⁺ was chosen as the cationic partner as it is known to display a high affinity for the calix[6]arene cavity. The addition of PnNH₄⁺ associated with a low-coordinating anion (i.e., picrate, Pic⁻) did not have an effect on the NMR spectrum of 4a in CDCl₃, which highlights the poor ability of 4a to bind ammonium ions independently of a coordinating anion. In contrast, upon the addition of ca. 3 equiv of PnNH₄⁺X⁻ (X = Cl⁻, AcO⁻, HSO₄⁻), the intracavity binding of the ammonium ion was evidenced by the presence of high-field signals in the NMR spectra (<0 ppm). However, in addition to the NMR signals for the complex 4aPnNH₄⁺X⁻, broad signals corresponding to another calixarene species were observed. Even with a larger excess of the ammonium salt (ca. 10 equiv), it was not possible to obtain only the 4aPnNH₄⁺X⁻ complex. The second species could correspond to binding of the ion pair PnNH₄⁺X⁻ with exocomplexation of the ammonium ion (i.e., with the ammonium ion outside of the calixarene cavity at the level of the pyrenyl units). It is noteworthy to mention that the simultaneous addition of ca. 3 equiv of PnNH₄⁺Pic⁻ and TBA⁺HSO₄⁻ did not yield the same NMR spectrum as obtained upon the addition of ca. 3 equiv of PnNH₄⁺HSO₄⁻, as the proportion of complex 4aPnNH₄⁺HSO₄⁻ was even lower in this case. This is probably due to the concomitant complexation of TBA⁺HSO₄⁻ by the calixarene (vide supra) and highlights the importance of the counterion in the case of receptor 4a. In other words, all of these data indicate a lack of selectivity for the binding process of ammonium salts PnNH₄⁺X⁻.

In view of the high selectivity of 4a for the sulfate anion, the ability of the receptor to bind this anion when it is associated with an ammonium ion that can be endocomplexed was evaluated in chloroform. Receptor 4a was unable to extract (PnNH₄⁺)₂SO₄²⁻ in CDCl₃, but to our delight, the progressive addition of PnNH₄⁺TBA⁺SO₄⁻ led to a new and unique NMR pattern displaying sharp signals characteristic of a C₃v symmetric calixarene species (Figure 5a).

After the addition of 1 equiv of the salt PnNH₄⁺TBA⁺SO₄⁻, only the signals of this new species were observed, and the spectrum was not influenced by the further addition of a large excess of the salt (ca. 20 equiv), indicating strong and selective 1:1 binding. All of the signals of this new species were assigned by 2D NMR spectroscopy (i.e., COSY and HMBC), and it was thus possible to make the following observations:

(i) The calixarene core adopts a flattened cone conformation (ΔNCH₂ = 0.55 ppm, and ΔNCH₃ = 0.58 ppm), and the significant downfield shift of the OMe protons (δOMe = 4.13 ppm) shows that these groups have been expelled from the cavity.

(ii) The NH protons of the ureido groups experience a significant downfield shift (δNH(pyr) = 10.48 ppm and δNH(ure) = 8.88 ppm), highlighting the binding of the sulfate anion through hydrogen bonding interactions.

(iii) High-field signals belonging to the alkyl chain of PnNH₄⁺ (δ = −1.26 and −1.92 ppm) attest to the inclusion of the ammonium ion into the cavity with a 1:1 binding ratio. This host–guest exchange process is slow on the NMR chemical shift time scale. The CIS of the NCH₂ protons of the ammonium ion is quite moderate (i.e., −0.47 ppm) in comparison with those of the rest of the alkyl chain (i.e., −3.03 and −2.84 ppm). This result is highly compatible with the presence of the sulfate anion in close proximity to the ammonium ion and thus with the binding of the two ionic partners as a contact ion pair.

(iv) The HMBC spectrum clearly indicates that the fluoro groups of the aniste moieties are directed toward the outside of the cavity (see the structure displayed in Figure 5a).
Figure 5. (a) $^1$H NMR spectrum (600 MHz, 298 K) of 4a in CDCl$_3$, in the presence of 1 equiv of PrNH$_3^+$TBA$^+$SO$_4^{2-}$. (b) $^1$H NMR spectrum (300 MHz, 298 K) of 4b in CDCl$_3$, in the presence of ca. 2 equiv of DopaNH$_3^+$TBA$^+$SO$_4^{2-}$. Labels: G, TBA$^+$; V, RNH$_3^+$; t, residual solvent; w, residual water.

Figure 6. Host–guest properties of calix[6]tris(pyrenylurea) 4a toward ammonium sulfate salts. Inset: CDss determined by $^1$H NMR spectroscopy in CDCl$_3$.

(v) The chemical shifts of the TBA$^+$ protons are strongly affected during the course of the titration as a result of complexation of the TBA$^+$ cation at the level of the pyrenyl units. The exchange is fast on the NMR chemical shift time scale, and monitoring of the upfield shift of the N'CH$_3$ signal yielded a binding constant of $K = 2.4 \pm 0.2$ and an estimated chemical shift of 1.74 $\pm$ 0.16 ppm for the N'CH$_3$ protons of the bound TBA$^+$ ion. These values are comparable to those observed in the presence of the sole sulfate ion (Table 2).

Altogether, these observations show the formation of the ternary host–guest complex 4a$\cdot$PrNH$_3^+$TBA$^+$SO$_4^{2-}$ with a weak interaction of the TBA$^+$ counterion at the level of the pyrenyl units that leads ultimately to the complex 4a$\cdot$PrNH$_3^+$TBA$^+$SO$_4^{2-}$ (Figure 6). In other words, 4a behaves as a heterotopic receptor.
CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

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Figure 7. Left: UV–vis spectra of 4a upon the addition of PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} (0 to 22 equiv) in chloroform. [4a]\textsubscript{o} = 10\textsuperscript{–} M. The inset shows the variation of the absorbance at 391 nm. Right: fluorescence spectra of 4a upon the addition of PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} (0 to 19 equiv) in chloroform. [4a]\textsubscript{o} = 4.8 \times 10\textsuperscript{–} M, [Pr\textsubscript{2}H\textsubscript{3}] = 346 nm. The inset shows the variation of the fluorescence intensity at 402 nm.

Numerical data and graphical representation of UV–vis and fluorescence spectra support the study of a fluorescent calix[6]arene in the presence of ammonium salt. The absorbance and fluorescence intensities showed a decrease with the addition of ammonium salt, indicating a possible interaction between the calixarene and ammonium ions.

For sulfuric ammonium salts, binding with the anion of the urea groups being a prerequisite for the inclusion of the ammonium ion into the calixarene cavity. This positive cooperativity is clearly due to the fact that two ions can be bound as a contact ion pair, thus avoiding the highly energetically unfavorable dissociation of the ion pair. The association constant for PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} was estimated to be log K > 4 by NMR spectroscopy (Table 1). Absorbance and fluorescence titrations in chloroform afforded association constants of log K = 5.3 ± 0.6 and 5.4 ± 0.1, respectively (Figure 7). Again, the spectral changes observed in the fluorescence spectra, namely, an increase of the monomer emission and a decrease of the excimer one, are suggestive of the separation of the self-associated urea groups upon anion coordination.

The affinities of receptors 1a and 4b for PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} were also evaluated in CDCl\textsubscript{3} in order to make a comparison with host 4a.\textsuperscript{37} Similarly to 4a, a high affinity constant for the formation of the complexes 1a•PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} and 4b•PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} was determined by 1H NMR spectroscopy (log K > 4). In the case of 4b, absorbance and fluorescence titrations afforded association constants of log K = 5.0 ± 0.2 and 4.8 ± 0.2, respectively, suggesting that 4a and 4b display similar recognition properties toward this ammonium salt. Surprisingly, a weak interaction of the TBA\textsuperscript{+} counterion at the level of the phenyl units was also observed in the case of 1a (log K = 2.3 ± 0.2),\textsuperscript{27} but a much higher chemical shift for the CH\textsubscript{3}CN protons of the bound TBA\textsuperscript{+} (2.94 ± 0.02 vs 1.74 ± 0.16 for 4a vs 4a, respectively) was estimated and no binding of the TBA\textsuperscript{+} was observed when this cation was associated with a low-coordinating anion (i.e., BF\textsubscript{4}–). Again, this positive cooperativity shows that the binding of the ion pair PrN\textsubscript{2}H\textsubscript{3}, TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–} preorganizes the binding site for the TBA\textsuperscript{+} cation by forming an electron-rich tris(phenyl) cavity.

All of these findings prompted us to investigate the ability of the fluorescent receptor 4a to bind larger ammonium ions associated with TBA\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2–}. To this end, additions of hexyl-dodecyl-, and (+)-sc-butylammonium salts as well as the pyrrolidinium salt to CDCl\textsubscript{3} solutions of 4a were performed. In all cases, the formation of the inclusion complex was clearly evidenced by NMR spectroscopy, and the CIs of these ammonium ions were compatible with their inclusion into the calixarene cavity (Figure 6). In particular, all of the methylene protons of the included allyl chain of HexNH\textsubscript{2}H\textsubscript{3} appeared as well-separated resonances and, in the case of DodNH\textsubscript{4}H\textsubscript{3}, the CIs decreased dramatically from the middle of the allyl chain, indicating that the dodecyl chain protrudes out of the cavity. For the pyrrolidinium cation (PyrrNH\textsubscript{2}H\textsubscript{3}), accurate determinations of the association constants by absorbance and fluorescence titrations afforded log K values of 4.1 ± 0.5 and 4.5 ± 0.2, respectively (Table 1). In the case of the HexNH\textsubscript{2}H\textsubscript{3} and DodNH\textsubscript{4}H\textsubscript{3} salts, accurate determinations of the associations constant were not possible because of the competitive formation of 4a•SO\textsubscript{4}\textsuperscript{2–}.\textsuperscript{37} This poor selectivity is likely due to a lower affinity of the calixarene for HexNH\textsubscript{2}H\textsubscript{3} and DodNH\textsubscript{4}H\textsubscript{3} that can be rationalized by the fact that guests with an allyl chain longer than propyl lead to a steric clash with the introverted flb groups that close the cavity of the host, forcing the calx[6]arene skeleton to adopt an energetically unfavorable straight conformation.\textsuperscript{33} This induced-fit process is clearly evidenced in the NMR spectra of the 4a•HexNH\textsubscript{2}H\textsubscript{3}, SO\textsubscript{4}\textsuperscript{2–} and 4a•DodNH\textsubscript{4}H\textsubscript{3}, SO\textsubscript{4}\textsuperscript{2–} complexes. Indeed, the calixarene aromatic units bearing the urea groups adopt a conformation more parallel to the C\textsubscript{2} axis with their flb groups expelled from the cavity by the allyl chain of the included ammonium ion (\Delta\delta_{H\textsubscript{2}H\textsubscript{3}H\textsubscript{3}} = 0.41 and 0.40 ppm, respectively. Moreover, the inclusion of the bulkier and nonlinear 3,4-O-dimethylidopammonium (DopNH\textsubscript{2}H\textsubscript{3}), (±)-α-ethylbenzylammonium, and O-methylserotonin (SerOH\textsubscript{3}H\textsubscript{3}) ions was not observed, again certainly because of the conformational energy penalty resulting from the steric clash with the flb groups (Figure 6). Steric hindrance at the level of the ammonium group and its α-position also appeared to be a major selectivity factor because of a steric clash with the small rim of the calixarene. Indeed, the inclusion of tert-butylammonium and diisopropylammonium ions was not detected (Figure 6), and the complexation of the (±)-sc-butylammonium salt was found to be at least 20 times weaker than that of PrN\textsubscript{2}H\textsubscript{3}, SO\textsubscript{4}\textsuperscript{2–}. Interestingly, the selective formation of 4a•PrN\textsubscript{2}H\textsubscript{3}, SO\textsubscript{4}\textsuperscript{2–} was obtained in the presence of a large excess of these ammonium salt sulfates.\textsuperscript{27} All of these results clearly show that the calixarene cavity controls the recognition of the cationic guest on the basis of its size and geometry. It was thus of particular interest to investigate the binding of the bulky salts by receptor 4b, which
displays an enlarged and more open cavity (Scheme 1). With this receptor, additions of the SeroNH$_3^+$ (z)-methylbenzylammonium, tBuNH$_2^-$, and (tBu)$_2$NH$_2^-$ ions associated with TBA$^{2-}$SO$_4^{2-}$ did not yield any inclusion complexes, certainly because of poor shape complementarity between the cationic guest and the cavity. However, to our delight, the addition of DodeNH$_3^+$TBA$^{2-}$SO$_4^{2-}$ and DopaNH$_2^-$TBA$^{2-}$SO$_4^{2-}$ in CDCl$_3$ led to the exclusive formation of the corresponding complexes 4b$\simeq$DodeNH$_3^+$TBA$^{2-}$SO$_4^{2-}$ and 4b$\simeq$DopaNH$_2^-$TBA$^{2-}$SO$_4^{2-}$ (Figure 5b). All in all, these results show that both fluorescent receptors 4a and 4b can ensure a cavity-based selectivity at the level of the recognition of the ammonium ion and also that fine-tuning of the recognition process can be obtained through modification of the large-nim substituents.

Studies in a Protic Environment. The next challenge was to evaluate the ability of 4a to bind ammonium salts in a protic environment. The $^1$H NMR spectrum of receptor 4a in a 1:11 CD$_3$OD/CDCl$_3$ mixture remained unchanged upon the addition of either (TBA)$^+$SO$_4^{2-}$ or PrNH$_2^-$Pic$^-$. This highlights the extremely weak anion or ammonium ion recognition properties of 4a in a protic and thus highly competitive environment. Furthermore, the $^1$H NMR spectrum of receptor 4a in a 1:11 CD$_3$OD/CDCl$_3$ mixture remained unaffected upon the addition of a large excess of (PrNH$_2^-$)$_2$SO$_4^{2-}$ (8 equiv), but the subsequent addition of (TBA)$^+$SO$_4^{2-}$ (ca. 1.5 equiv) led by the quantitative formation of the complex 4a$\simeq$(PrNH$_2^-$)$_2$SO$_4^{2-}$. It is noteworthy to mention that an identical NMR pattern was obtained upon the addition of PrNH$_2^-$TBA$^{2-}$SO$_4^{2-}$ to 4a. This remarkable result highlights once again the importance of the counterion and shows that the presence of a dissociated cation such as TBA$^+$ is required for the complexation of ammonium sulfate salts by host 4a. The association constant for PrNH$_2^-$TBA$^{2-}$SO$_4^{2-}$ (log $K = 3.9 \pm 0.1$) was determined by integration of the $^1$H NMR spectrum recorded after the addition of 1 equiv of PrNH$_2^-$TBA$^{2-}$SO$_4^{2-}$ (Table 1). A similar log $K$ value of 4.0 $\pm$ 0.3 was obtained by UV--vis titration. It should be noted that in this solvent the binding constant for the complexation of the TBA$^+$ was too small to be determined accurately. Considering the inertness of the receptor toward (TBA)$^+$SO$_4^{2-}$ and PrNH$_2^-$Pic$^-$ in a 1:11 CD$_3$OD/CDCl$_3$ mixture, the strong and simultaneous binding of both partners (i.e., PrNH$_2^-$ and SO$_4^{2-}$) in this protic environment highlights a remarkable mutual cooperativity. Indeed, the complexation of the sulfate anion can only proceed when an ammonium ion is present in the calixarene cavity, and conversely, without SO$_4^{2-}$ the receptor 4a is inefficient at binding the ammonium ion. Finally, a certain affinity of receptor 4b for DopaNH$_2^-$TBA$^{2-}$SO$_4^{2-}$ in 1:11 CD$_3$OD/CDCl$_3$ was also observed (log $K \sim 1.5$ obtained by NMR spectroscopy), showing a remarkable behavior of this receptor for the recognition of biological ammonium salts in a protic environment.

CONCLUSION

The straightforward syntheses of the first fluorescent calix[6]-tris(urea) hosts 4a and 4b was achieved efficiently. In comparison to the parent receptors 1a and 1b, the introduction of bulky pyrenyl groups in close proximity to the anion binding site did not inhibit the binding properties of the receptors toward ammonium and contact ion pairs. In contrast to 1a and 1b, it was observed that receptor 4a exhibits a remarkable selectivity for the sulfate anion in DMSO, enabling its selective sensing by fluorescence spectroscopy. In CDCl$_3$, 4a was able to bind ammonium ions efficiently only in association with the sulfate anion.

To our delight, this cooperative binding of ammonium sulfate salts was also observed in a protic environment. The binding constants determined by UV--vis absorbance, fluorescence, and NMR titrations were found to be in agreement in all cases. Corroborating conformational changes of the receptor upon binding were evidenced by fluorescence and NMR spectroscopies. Interestingly, cavity-based selectivity in terms of the size and shape of the guest was observed with both receptors 4a and 4b. While 4a displays a strong affinity for small or linear ammonium salts, 4b was found to have the ability to recognize the 3,4-Dimethylammonium sulfate salt and not the corresponding serotonin derivative. In other words, this work opens interesting perspectives on the elaboration of unique fluorescent cavity-based systems for the selective sensing of anions or biologically relevant ammonium salts such as neurotransmitters. Current efforts are now being directed toward the design of water-soluble fluorescent calix[6]arene-based receptors.

EXPERIMENTAL SECTION

General Experimental Methods. All of the reactions were performed under an inert atmosphere. Anhydrous THF was obtained through distillation over Na/benzophenone. Silica gel (230–400 mesh) was used for flash chromatography purifications. Chloroform (both dehydrated and18)18 and use was filtered prior to use. A short of the amount of an aqueous column of basic alumina to remove traces of HCl/DCM. $^1$H NMR spectra were recorded on a 600, 400, or 300 MHz spectrometer, and $^1$C NMR spectra were recorded on the 300 or 400 MHz spectrometer at 75 or 100 MHz, respectively. 2D NMR spectra (COSY, HMQC, HMBC) were obtained to complete signal assignments. DOSY experiments were recorded on the 600 MHz spectrometer. NMR parameters (acquisition time, recycling times, and signal accumulation) were chosen to ensure that quantitative data could be obtained from signal integration in the 1D $^1$H spectra. Traces of residual solvent were used as an internal chemical shift reference. Chemical shifts are quoted on the $^1$H $^1$H scale. The NMR, UV, and fluorescence spectra were recorded at 298 K, unless otherwise stated. De-tether butylated 1,3,5-tris(methoxy)calix[6]arene 24)

calix[6](tris(1-azido) 3b). De-tether butylated 1,3,5-tris(methoxy)calix[6]arene 2 (1.81 g, 2.14 mmol) was dissolved in freshly distilled THF (100 mL), and NaH (60 wt % in oil, 0.51 g, 12.82 mmol) was added. The mixture was heated to reflux for 30 min. Then 2-azidoethyl-4-methylbenzenesulfonate (2.00 g, 8.24 mmol) was added, and the heating was maintained for 48 h. After the mixture was cooled to room temperature, methanol (15 mL) was added, and the solution was stirred for 30 min. Then the solvents were evaporated, and the residue was dissolved in a mixture of CH$_2$Cl$_2$ (80 mL) and HCl (80 mL, 1 M). The organic layer was separated, and the aqueous layer was extracted twice with CH$_2$Cl$_2$. The combined organic layers were washed twice with brine, filtered through a WA filter, and evaporated under vacuum. The crude product was then purified by flash chromatography on silica gel (CH$_2$Cl$_2$/CH$_3$OH, 100:0 then 95:5) to give calix[6](tris(azido) 3b (1.750 g, 77% yield). Mp: 125–130°C (dec.). IR (KBr): 2956, 2105, 1455 cm$^{-1}$. $^1$H NMR (CD$_3$OD, 300 MHz): δ (ppm) 1.28 (s, 27H), 2.80 (s, 9H, OCH$_2$), 3.46 (m, 6H, CH$_2$N$_2$H$_2$), 3.75 (m, 6H, OCH$_2$), 4.02 (s, 12H, ACH$_2$), 6.33 (s, 9H, ArH), 7.31 (s, 6H, ArH). $^1$C NMR (CD$_3$OD, 75 MHz, 298 K): δ (ppm) 30.6, 31.5, 34.2, 51.2, 93.8, 70.9, 133.9, 126.8, 128.3, 134.5, 146.1, 153.9, 154.2. ESI-HRMS (CH$_3$OH/CH$_2$Cl$_2$): [M+H]$^+$ = 1071.6184, found 1071.6182.

Calix[6](tri(pyrenylurea) 4a. Calix[6](tris(azido) 3a (0.350 g, 0.29 mmol) was dissolved in anhydrous THF (10 mL), and triphenylphosphine (0.450 g, 1.72 mmol) was added. Then Co$_2$ was bubbled through the solution for 5 min. The solution was stirred for 19 h at room temperature and kept under a CO$_2$ atmosphere. The medium was then purged with argon for 5 min, and 1-aminoipropene (0.273 g, 1.26 mmol) was added. The mixture was then stirred under argon for 48 h at 50°C. The solvent was evaporated under vacuum, and acetone
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Fluorescent Chemosensors for Anions and Contact Ion Pairs with a Cavity-Based Selectivity

Emilio Brunetti, Jean-François Picron, Karolina Flidrova, Gilles Bruylants, Kristin Bartik* and Ivan Jabin*

Supporting information

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V. 1D and 2D NMR spectra of 3b, 4a, 4b .............................................................................. 20
I. UV-visible and fluorescence studies of 4a and 4b

**Figure S1.** Left: UV-vis spectrum of 4a in chloroform; [4a]₀ = 1.1 × 10⁻⁵ M. Right: UV-vis spectrum of 4a in DMSO; [4a]₀ = 1.1 × 10⁻⁵ M.

**Figure S2.** Left: UV-vis spectrum of 4b in chloroform; [4b]₀ = 1.0 × 10⁻⁵ M. Right: UV-vis spectrum of 4b in DMSO; [4b]₀ = 9.7 × 10⁻⁶ M.
**Figure S3.** Top: variation of absorbance at 392 nm upon the addition of (TBA$^+$)$_3$SO$_4^{2-}$ in DMSO; [4a]$_0$ = 1.0 × 10$^{-5}$ M. Bottom: variation of fluorescence intensity at 402 nm upon the addition of (TBA$^+$)$_3$SO$_4^{2-}$; [4a]$_0$ = 1.0 × 10$^{-5}$ M. $\lambda_{ex} = 348$ nm. Parametric adjustment performed with a 1:1 binding model.
Figure S4. Variation of absorbance at 391 nm of 4a upon the addition of PrNH$_3$$^+$TBA$^+$SO$_4$$^{2-}$ (0 to 50 equiv.) in CH$_3$OH/CHCl$_3$ 1:11; [4a]$_0$ = 0.7 × 10$^{-5}$ M. Parametric adjustment performed with a 1:1 binding model.
II. Job’s plot analysis of the association between 4a and (TBA\(^+\))\(_2\)SO\(_4\)\(^-\)

![Chemical structures](image)

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![Graph showing Job's plot](image)

**Figure S5.** A Job Plot carried out between 4a and (TBA\(^+\))\(_2\)SO\(_4\)\(^-\) in DMSO. The observed maximum around 0.5 is consistent with the proposed 1:1 complex between 4a and SO\(_4\)\(^-\): [4a] + [(TBA\(^+\))\(_2\)SO\(_4\)\(^-\)] = 1.0 \times 10^{-5} \text{ M}.
III. $^1$H NMR study of the complexing properties of 4a and 4b

![Chemical structures of 4a and 4b with data](image)

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**Figure S6.** Variation of the chemical shift of the tBu$_{out}$ of 4a upon the addition of (TBA$^+$)$_2$SO$_4^{2-}$ (0 to 6 equiv.) in DMSO-d6 (300 MHz); [4a]$_0$ = 2.53 $\times$ 10$^{-3}$ M. Parametric adjustment performed with a 1:1 binding model.
Figure S7. Variation of the chemical shift of TBA$^+$ (CH$_3$N$^+$) upon the addition of (TBA$^+$)$_2$SO$_4$$^-$ (0 to 12 equiv.) to 4a in CDCl$_3$ (600 MHz); [4a]$_0$ = 2.39 $\times$ 10$^{-3}$ M. Parametric adjustment performed with a 1:1 binding model.
CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

Figure S8. Variation of the diffusion coefficient of TBA$^+$ normalized by the diffusion coefficient of CHCl$_3$ upon the addition of TBA$^+$BF$_4^-$ (0 to 80 equiv.) to 4a in CDCl$_3$ (600 MHz); [4a]$_0$ = $2.82 \times 10^{-3}$ M. Parametric adjustment performed with a 1:1 binding model.

Parameters of the DOSY experiment: 15 values of the magnitude of the gradient pulses ranging between approximately 2 G/cm to 50 G/cm, diffusion delay 100 ms, time-length of the gradient 5 ms, acquisition time 3 s, relaxation delay 10 s, 16 transients.
Figure S11. $^1$H NMR spectrum (300MHz) of 4b in CDCl$_3$ in the presence of ~1 equiv. of DodNH$_3^+$TBA$^+$SO$_4^{2-}$; O = TBA$^+$; ▽ = DodNH$_3^+$ in; s: residual solvent; w: residual water.
Figure S12. COSY spectrum (300MHz) of 4b in CDCl₃ in the presence of ~2 equiv. of DopaNH₃⁺TBA⁺SO₄²⁻.
Figure S13. HSQC spectrum of 4b in CDCl₃ in the presence of ~2 equiv. of DopaNH₃⁺TBA⁺SO₄²⁻.
Figure S14. $^1$H NMR spectrum (300MHz) of a) 4a in CD$_3$OD/CDC$_3$l 1:1; b) after the subsequent addition of ~8 equiv. of (PrNH$_3$)$_2$$^+$SO$_4$$^-$$^-$; c) after the subsequent addition of ~1.5 equiv. of (TBA)$_3$$^+$SO$_4$$^-$; ○ = TBA$^+$; ▽ = PrNH$_3^+$ out; ▼ = PrNH$_3^+$ in; s: residual solvent.
Figure S15. COSY spectrum (300 MHz) of receptor 4a in the presence of ~4 equiv. of PrNH$_3^+$TBA$^+$SO$_4^{2-}$ in CDCl$_3$; ▼ = PrNH$_3^+$ in.
Figure S16. HMBC spectrum of receptor 4a in the presence of ~4 equiv. of PrNH₃⁺TBA⁺SO₄²⁻ in CDCl₃.
Figure S17. $^1$H NMR spectrum (600MHz) of a) 4a in CDCl$_3$; b) after the subsequent addition of ~4 equiv. of TBA$^+\text{HSO}_4^-$ and ~4 equiv. of (±)-α-methylbenzylamine; c) after the subsequent addition of ~4 equiv. of TBA$^+\text{HSO}_4^-$ and ~4 equiv. of $t$BuNH$_2$; d) after the subsequent addition of ~4 equiv. of TBA$^+\text{HSO}_4^-$ and ~4 equiv. of (iPr)$_2$NH; e) after the subsequent addition of ~1 equiv. of TBA$^+\text{HSO}_4^-$ and ~1 equiv. of PrNH$_2$; ▼ = PrNH$_3^+$ in; s: residual solvent.

Note that the changes observed from a) to d) are due to an interaction between the sole sulfate anion and the urea groups.
IV. \(^1\text{H}\) NMR study of the complexing properties of 1a

Figure S18. \(^1\text{H}\) NMR spectrum (600MHz) of 1a in CDCl\(_3\) in the presence of ~1 equiv. of PrNH\(_3\)^+TBA\(^-\)SO\(_4\)^2-; O = TBA\(^-\); ▽ = PrNH\(_3\)^+ in; s: residual solvent.
Figure S19. Variation of the chemical shift of the CH$_2$N$^+$ (TBA$^+$) upon the addition of PrNH$_3^+$TBA$^+$SO$_4^{2-}$ (0 to 23 equiv.) to 4a in CDCl$_3$ (600 MHz); [1a]$_0$ = 3.32 $\times$ 10$^{-3}$ M. Parametric adjustment performed with a 1:1 binding model.
V. 1D and 2D NMR spectra of 3b, 4a, 4b

Figure S20. $^1$H NMR spectrum (400 MHz, DMSO-d6) of compound 4a. s: residual solvent; w: water.

Figure S21. $^{13}$C NMR spectrum (100 MHz, DMSO-d6) of compound 4a. s: residual solvent.
Figure S22. HSQC spectrum (DMSO-d6) of compound 4a.

Figure S23. HMBC spectrum (DMSO-d6) of compound 4a.
Figure S24. $^1$H NMR spectrum (300 MHz, DMSO-d6) of compound 4b. s: residual solvent; w: water.

Figure S25. $^{13}$C NMR spectrum (75 MHz, DMSO-d6) of compound 4b. s: residual solvent.
Figure S26. COSY spectrum (300 MHz, DMSO-d6) of compound 4b.

Figure S27. HSQC spectrum (DMSO-d6) of compound 4b.
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Figure S28. $^1$H NMR spectrum (300 MHz, CDCl$_3$) of compound 3b. s: residual solvent; w: water.

Figure S29. $^{13}$C NMR spectrum (75 MHz, CDCl$_3$) of compound 3b. s: residual solvent.
**Figure S30.** COSY spectrum (300 MHz, CDCl₃) of compound 3b.

**Figure S31.** HSQC spectrum (CDCl₃) of compound 3b.
2.2. COMPLEXATION OF PHOSPHOLIPIDS

2.2.1. CONTEXT OF THE RESEARCH

Considering the results obtained with anions and contact ion-pairs, we wanted to see whether receptor 14a could be exploited for the sensing of zwitterionic phospholipids. Phospholipids are major components in cell membranes and are very important for the functioning of living systems. They are composed of a hydrophobic tail and a hydrophilic head linked by a glycerol unit. The hydrophilic head is composed in the case of phosphatidylycholines (PCs) and phosphatidylethanolamines (PEs) of a phosphate group and a choline or ethanolamine moiety (Figure 2-3).

Figure 2-3. Structures of some phospholipids.

In biological systems PCs and PEs are the most abundant phospholipids and are the major components of lipid bilayers. Natural protein receptors recognize and bind PCs and PEs with a high selectivity via specific interactions with their zwitterionic head. Typically, the phosphate group of the phospholipid is coordinated to a metal center or interacts with a positively charged Arg or Lys residue through ionic and H-bonding interactions, while the ammonium group interacts with a polyaromatic binding pocket through cation-π interactions. A possible biomimetic approach for the selective sensing of phospholipids could consist in the use of heteroditopic receptors that associate a polar recognition site to a polyaromatic cavity. For example, Rebek and co-workers have developed a Zn salen-based cavitand that efficiently binds

---

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) reminiscent of the binding motif of the enzyme phospholipase (Figure 2-4).

![Figure 2-4](image)

**Figure 2-4.** A bifunctional Zn-salen cavitand that binds DOPC (figure taken from reference 7).

As presented in the Introduction (section 1.4.2), host 13b (Figure 2-5) presents interesting properties for phospholipids recognition. It is indeed able to selectively bind PCs over PEs in chloroform with the fatty acid chains of the bound lipids protruding from one of the three macrocycles formed by the thio-urea arms.⁹

![Figure 2-5](image)

**Figure 2-5.** Energy minimized structures of 13b-DOPC.⁹

In this thesis, we wished to see if it was possible to give rise to a lipid sensor in terms of detection by fluorescence spectroscopy. We studied the interaction of different phospholipids with host 14a, using ¹H and ³¹P NMR and fluorescence spectroscopy. Our results have been

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2.2.2. SUMMARY OF PUBLISHED RESULTS:

In the manuscript “A Selective Calix[6]arene-based Fluorescent Chemosensor for Phosphatidylcholine Type Lipids” we report on the binding studies of host calix[6]tris-pyrenlyurea 14a with different phospholipids.

Studies undertaken in CDCl₃ by ¹H NMR showed that host 14a displays a remarkable selectivity for lipids bearing a phosphatidylcholine head (PCs) over those bearing a phosphoethanolamine head (PEs) (Figure 2-3). Indeed, upon addition of a few equivalents of different PCs, the formation of a new C₃V species is observed. The metoxy groups are expelled from the cavity (δOMe > 3.87 ppm) and the receptor NH are shifted downfield, signature of an H-bonding interaction with the phosphate group. Guest exchange is slow on the chemical shift timescale and EXSY experiments enabled us to show that the cationic group of the lipids exhibit a signal at high-field which confirms their inclusion in the cavity. With 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) for example, inversion of the signal of the free CH₃N⁺ of the lipid (3.27 ppm) gave rise to a signal at 1.54 ppm. The 14a-PC complexes are thus stabilized by interactions with the zwitterionic head of the guest. No change in the NMR spectra are observed when adding PEs attesting that they are not recognized by 14a.

Titrations of 14a with PCs and PEs in CHCl₃ were also monitored by emission spectroscopy. Upon addition of the PCs, an increase of the monomer band and a decrease of the excimer band are observed, signature of a complexation at the level of the urea moieties. Depending on the lipid, values ranging from log K ~ 4 to log K ~ 5 were determined for a 1:1 binding model. Not surprisingly, upon addition of PEs no spectral changes were observed.

Titrations by ³¹P NMR were also undertaken to corroborate these results. Upon addition of host 14a to a solution of 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC) in CDCl₃, the signal of bound POPC was clearly detected as the phosphorus signals are in slow exchange on the ³¹P chemical shift time scale. An affinity log K ~ 4 could also be determined.

Finally, extraction experiments were conducted in order to evaluate the possibility of using host 14a as a sensor for lipids in an aqueous environment. Remarkably, results showed that 14a
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dissolved in CHCl₃ could be used to quantify, by emission spectroscopy, the amount of DOPC extracted from the aqueous phase.

A selective calix[6]arene-based fluorescent chemosensor for phosphatidylcholine type lipids†

Emilio Brunetti,a b Steven Moerkerke,a Johan Wouters,c Kristin Bartik,b and Ivan Jabin*a

The development of chemosensors that can selectively detect phosphatidylcholines (PCs) in biological samples is of medical relevance considering the importance of these phospholipids in cell growth and survival. Their selective sensing over phosphatidyethanolamines (PEs) is however a challenging task. We report here on the chemosensing capacities of calix[6]tris-pyrenylurea 1, which is able to selectively interact with phosphatidylcholine-type lipids in organic media. Host 1 also binds them in a biphasic chloroform/water solution, opening the way to the design of selective chemosensors for these lipids in biological media. The results obtained by NMR, fluorescence spectroscopy and modelling studies show that the selectivity is the result of the high degree of complementarity between the lipids’ zwitterionic phosphatidylcholine headgroup and the receptor’s H-bonding donor site and hydrophobic pocket. The mode of recognition is reminiscent of natural systems, such as human phosphatidylcholine transfer proteins (PC-TPs), validating the biomimetic approach adopted in our work.

Introduction

Phospholipids are a major class of zwitterionic lipids, present predominantly in cellular membranes.1 These amphiphilic lipids possess a hydrophobic domain and a hydrophilic head linked together, either by a glycerol unit (glycerophospholipids) or by a sphingosine (sphingolipids) (Fig. 1). Their polar head is composed of a phosphodiester group and typically a nitrogenous moiety such as choline, ethanolamine or serine. Phosphatidylcholines (PCs) and phosphatidyethanolamines (PEs) (Fig. 1) are generally the most abundant membrane phospholipids and the ratio of PCs to PEs in the plasma membrane is, for example, reported as crucial for maintaining cellular growth and survival.2 The quantification of these lipids in biological samples is thus of medical importance and is classically achieved by gravimetric3 or colorimetric techniques.4 Recently, a NMR method for phospholipids quantification has been reported.5 The selective sensing of PCs over PEs remains however a challenge.

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4Electronic supplementary information (ESI) available: 1H NMR spectra, 13C NMR spectra and fluorescence studies of the complexation properties of 1. See DOI: 10.1039/c6ob01800g

Natural protein receptors recognize and bind PCs and PEs with a high selectivity, thanks to specific interactions with the zwitterionic head. Typically, the phosphate group of the phospholipid is coordinated to a metal center6 or interacts with a positively charged Arg or Lys residue through hydrogen and H-bonding interactions,7 while the ammonium group interacts with a polyanionic binding pocket through cation-π interactions.8 A possible biomimetic approach for the selective sensing of phospholipids could consist in the use of heteroditopic receptors that associate a polar recognition site to a polyanionic cavity.9

We have developed calix[6]arene-based systems bearing thiourea or urea moieties, that behave as heteroditopic receptors toward charged or neutral species, such as zwitterions, which could be interesting in the context of phospholipid sensing.10 Very recently, we have reported that a calix[6]arene possessing two divergent hydrophobic cavities connected via three thiourea linkages (Fig. 2, right) is able to selectively bind PCs over PEs in nonpolar solvents.11 The binding of the phospholipids proceeds through multiple H-bonding interactions between their anionic moieties and the tris-thiourea groups of the host, as well as through cation-π and CH–π interactions between their cationic (CH)3N+ group and one of the two polyanromatic calixarene cavities. It is to be noted that the fatty acid chains of the bound lipids do not protrude from the second calixarene cavity but from one of the three macrocycles formed by the (thiourea) arms. Also of interest is our recently developed fluorescent calix[6]tris-urea receptor 1 (Fig. 2, left) that can be used for the sensing of anions and organic ion-pairs, and
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A) PCs: R = CH₂, R₁=H; R₂=R₃=R₄=alkyl chain
PEs: R=H; R₂=R₃=R₄=alkyl chain
DOPC: R=CH₂, R₁=H; R₂=R₃=R₄=alkyl chain
DPPC: R=CH₂, R₁=H; R₂=R₃=R₄=alkyl chain
POPC: R=CH₂, R₁=H; R₂=R₃=R₄=palmityl chain; R₅=palmityl chain
DOPE: R=H; R₁=R₂=alkyl chain
PE: R=H; R₁=COO; R₂=R₃=R₄=alkyl or stearil chain

B) SPH: R=CH₂, R₁=H

C) DPC: R=CH₂, R₁=dodecyl chain
MPC: R=CH₂, R₁=H

Fig. 1. Structure of (A) various glycerophospholipids, (B) a sphingolipid and (C) non-natural phosphocholines.

Fig. 2. Structure of calix[6](tris-pyrenylures 1 (left) and calix[6]tube (right).

for which a cavity-based selectivity, in terms of size and shape of the guests, was observed with ammonium salts. Considering these previous results, we wished to see if receptor 1 could be exploited for the chemosensing of zwitterionic phospholipids and in particular if it could distinguish between PCs and PEs.

Herein, we report on the study by NMR and fluorescence spectroscopy of the interaction of chemosensor 1 with different phospholipids.

Results and discussion

The ability of calix[6](tris-pyrenylures 1 to bind different phospholipids was investigated in CDCl₃ via ¹H NMR titration experiments. As shown previously, the ¹H NMR spectrum of 1 in CDCl₃ displays a broad NMR signature due to intra-molecular self-association of the urea moieties (Fig. 3a). The quantitative formation of a new species that displays well-defined NMR signals was however observed upon the addition of a few equivalents of either 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) [see Fig. 3b for the ¹H NMR spectrum of DOPC], 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), N-acetyl-4-sphinganyl-1-O-phosphocholine (SPH), dodecylphosphocholine (DPC) or 2-methacycloxyethyl phosphocholine (MPC) [see Fig. 1 for the structures of the lipids]. As a representative example, the ¹H spectrum of host 1 in presence of ca. 3 equiv. of DOPC is shown in Fig. 3c.

The following common features were observed for all the newly formed host-guest species:

(i) The calixarene adopts a flattened C₁₆ symmetrical cone conformation (∆δ₁₆₆₃ > 0.62 ppm and ∆δ₁₆₃₉ > 0.61 ppm) with the OMe groups expelled from the cavity (∆δ₁₆₃₉ > 3.87 ppm);
(ii) The presence of strong H-bonding interactions between the urea groups NH₃⁺ and the bound lipid, as evidenced by the significant down-field shift of the NH₃⁺ signal;
(iii) Intracavity complexion of the cationic head of the lipid, as clearly evidenced through HSQC and 1D ESY experiments. For instance, a signal at 1.54 ppm corresponding to the bound Me₃N⁺ group is observed upon inversion of the Me₃N⁺ signal of free DOPC (selective pulse excitation at 3.27 ppm) (Fig. 4).

These NMR data suggest that host 1 behaves as a heteroditopic receptor capable of binding phosphatidylcholine type lipids through the establishment of specific interactions with their zwitterionic head. In all cases, two sets of signals were apparent over the course of the titration, indicating slow host-guest exchanges on the ¹H NMR chemical shift timescale. In the case of 1 + DOPC, all the ¹H signals belonging to the free and bound DOPC were assigned by 2D-NMR experiments (COSY, HSQC and ROESY), and the compound-induced shifts (CISs) could be calculated (values given in the inset of Fig. 3). The signals corresponding to the α(CH₃)₂N⁺ and β(CH₂)₂N⁺CH₂
protons display negative CIsS, which is coherent with the inclusion of the cationic part of DOPC in the heart of the calixarene cavity. In contrast, the γ, δ and ε protons exhibit positive CIsS, in accordance with H-bonding interactions between the H-bond acceptor groups of DOPC (phosphate and esters) and the urea groups of 1. The negative CIsS of the γ, δ and θ protons suggest that they are positioned at the level of the pyrene moieties. All the other protons of DOPC experience negligible CIsS, showing that the long oleyl chains protrude from the calixarene-based receptor.

Very interestingly, the 1H spectrum of 1 remained unaffected upon the addition of either 1,2-diacyl-sn-glycero-3-phosphoethanolamine (DOPE) or 1,2-diacyl-sn-glycero-3-phosphoyl-1-serine (PS).13 This inertness toward PE type lipids implies that the presence of a quaternary ammonium group at the level of the polar head is mandatory for the recognition process, which would be the result of (i) the establishment of CH–x and x-cationic interactions between the Me3N+ group and the aromatic walls of the calixarene and (ii) the stronger self-association of PE type lipids in nonpolar solvents.14

The binding affinities toward the different PC type lipids in CDCl3 were estimated via integration of the Me3N+ signal of the free lipid and of the PyrH signals of the host–guest complex. In all cases, the association constants (log Kassb) were too high to be determined accurately by NMR (Table 1).15 Upon addition of ca. 4% of CD3OD to a solution of 1 in the presence of 3 equiv. of DOPC in CDCl3, ca. 5% of the host–guest complex 1 + DOPC was still detected.15 In DMSO-d6, a more competitive solvent, DOPC and DPC were however not recognized.15

The binding of POPC by receptor 1 was also investigated in CDCl3, by 31P NMR spectroscopy. The 31P NMR spectrum of POPC exhibits a signal at ~0.88 ppm (Fig. 5a). Upon progressive addition of host 1, the signal corresponding to the bound POPC was clearly detected at ~2.18 ppm (Fig. 5b–d). The significant CIS (~1.30 ppm) confirms the formation of H-bonding


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Table 1  Affinity of host 1 towards different lipids at 298 K

<table>
<thead>
<tr>
<th>Complex</th>
<th>Solvent</th>
<th>( \log K_{\text{assoc}}^{*} )</th>
<th>( \log K_{\text{eff}}^{*} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ⊗ DOPE</td>
<td>CDC15</td>
<td>4.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ DPPC</td>
<td>CDC15</td>
<td>4.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ POPC</td>
<td>CDC15</td>
<td>4.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ SPH</td>
<td>CDC15</td>
<td>4.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ DPC</td>
<td>CDC15</td>
<td>4.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ MPE</td>
<td>CDC15</td>
<td>4.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ DOPE</td>
<td>DMSO_d6</td>
<td>nd 6</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ DPPC</td>
<td>DMSO_d6</td>
<td>nd 6</td>
<td></td>
</tr>
<tr>
<td>1 ⊗ DPC</td>
<td>DMSO_d6</td>
<td>nd 6</td>
<td></td>
</tr>
</tbody>
</table>

* \( K = [\text{Complex}][\text{[Host]}]/[\text{[Guest]}] \) *\( K_{\text{assoc}} \) and \( K_{\text{eff}} \) refer to binding constants determined by \(^1\)H NMR spectroscopy and fluorescence spectroscopy respectively. \(^{12} \)No binding detected.

Fig. 5  \(^{31}\)P NMR spectra (400 MHz, 298 K, CDC) of: (a) POPC (6.22 mM); (b) after addition of ca. 0.1 equiv. of host 1; (c) after addition of ca. 0.3 equiv. of host 1; (d) after addition of ca. 0.6 equiv. of host 1; triphenylphosphine used as internal reference.

Fig. 6  Energy minimized structure of 1 ⊗ DOPE. Hydrogen bonds are indicated by dashed lines. Selected distances (Å): N(host)−O(DOPC): 2.97, 3.00, 3.01, 3.37, 3.36, 3.51; H(host)−H(centroid): 4.24, 4.29, 4.52. With the exception of the NH of the host, all the hydrogen atoms of 1 and of DOPC are omitted for clarity. Two perpendicular orientations of the structure of 1 ⊗ DOPE are presented.

Fig. 7  Emission spectra of 1 upon addition of DOPE (0 to 85 equiv.) in chloroform. \( I_{0} = 5.0 \mu M \) and \( I_{0} = 345 \text{ nm} \). Inset: variation of fluorescence intensity at 420 nm upon the addition of DOPE. Solid line: fitting to a 1:1 binding model.

Interactions between the phosphate group and the urea moieties. As expected, a high affinity was estimated through integration of the two signals [\( \log K_{\text{assoc}} \geq 4 \)] and no change in the \(^{31}\)P NMR spectrum was observed upon addition of 1 to a DOPE solution. \(^{12} \)

To gain insight into the recognition process between host 1 and PC type lipids, the geometry of complex 1 ⊗ DOPE was optimized by molecular mechanics using the CHARMM force field and the SmartMinimizer algorithm (RMS gradient 0.1). \(^{12} \)

The resulting energy minimized structure is in good agreement with the NMR results (Fig. 6). The calixarene exhibits a flattened conformation with the OMe groups directed toward the outside of the cavity and interactions between host 1 and the zwitterionic head of the lipid are observed. Indeed, the MeN− group is buried inside the poly-aromatic cavity enabling it to establish \( \pi \)-cationic and CH−π interactions while the convergent urea groups stabilize the edifice through multiple H-bonding interactions. The two oly chains of DOPE are located between the three pyrenyl units where they are stabilized through CH−π interactions. From a biomimetic point of view, 1 ⊗ DOPE shows strong similarities with the complexes formed between PCs and their natural receptors such as the human phosphatidylethanolamine transfer protein (PC-TP). \(^{17} \)

The complexation of the different lipids by host 1 was also investigated by fluorescence spectroscopy. No significant changes were observed in the fluorescence spectra of host 1 during the titrations with the PE type lipids (DOPG and PS). \(^{13} \)

With the PC type lipids, an increase of the monomer emission (at 398 and 420 nm) and a decrease of the excimer emission (at 446 nm) were clearly observed over the course of the titrations (see Fig. 7 for DOPE). \(^{13} \) These spectral changes are the signature of the separation of the self-associated pyrenyl urea groups upon lipid complexation. Association constants (\( \log K_{\text{eff}} \)) of the same order of magnitude were determined.
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Experimental section

General experimental methods

DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), SPH (N-acetyl-sphingenyl-1-O-phosphocholine), MPC (2-methacyclopropylphosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) and PS (1,2-dilinoleoyl-sn-glycero-3-phospho-L-serine) (all ≥99%) were purchased from Sigma-Aldrich (Missouri, USA). DPPC (dodecyphosphocholine) (≥99%) was purchased from Avanti Polar Lipids (Alabena, USA). DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) (≥99%) from Buchem (Bubenordof, Switzerland). Chloroform (both deuterated and non-deuterated) was filtered, prior to use, over a short column of basic alunina to remove traces of HCl and DCI. 1H spectra were recorded at 400 MHz (9.4 T) or 600 MHz (14.1 T). 2D NMR spectra (COSY, HSQC, and ROESY) were recorded to complete signal assignments. 1D ESSY experiments were recorded with an ibupfl pulse sequence. NMR parameters (acquisition time, receling times, and signal accumulation) were chosen to ensure that quantitative data could be obtained from signal integration in the 1D 1H spectra. Trace of residual solvent was used as an internal chemical shift reference. Chemical shifts were quoted on the δ scale. The NMR and fluorescence spectra were recorded at 298 K unless otherwise stated.

1H NMR titration experiments

All experiments were undertaken following a similar protocol: a known volume (ca. 600 μL) of solution of known concentration of host (ca. 2 mM) was placed in a NMR tube and the 1H NMR spectrum was recorded. Aliquots (ca. 5 μL, corresponding to 0.25 equiv. of host) of a stock solution of guest were successively added and a 1H NMR spectrum was recorded after each addition. Aliquots were added until no change was observed for the host signals.

31P NMR titration experiment

A known volume (ca. 600 μL) of solution of known concentration of POPC (ca. 6 mM) was placed in a NMR tube and the

Conclusion

Calix[6]iris-pyrenylurea 1 behaves as an efficient heteroditopic chemosensor able to strongly interact with lipids in organic media (log K > 4). A high selectivity for phosphatidylcholine type lipids over the closely related phosphatidylethanolamines is observed, highlighting the importance of specific interactions between the receptor and the zwitterionic head of the lipid. This mode of recognition is reminiscent of natural systems such as human phosphatidylcholine transfer proteins (PC-TPs), validating the biomimetic approach adopted in our work, that consists in designing receptors that associate a H-bonding donor site to a hydrophobic pocket in order to achieve a high degree of complementarity with the two ionic groups of zwitterions. Interestingly, liquid-liquid extraction experiments showed that host 1 can still bind phosphatidylcholine type lipids in a biphasic chloroform/water solution, opening the way to the design of selective chemosensors for lipids in biological media.
$^{31}$P NMR spectrum was recorded. An external capillary tube containing a known concentration of triphenylphosphine was used to provide the reference peak for quantification. Aliquots (ca. 5 μL corresponding to 0.25 equiv. of guest) of a stock solution of host 1 were successively added and a $^{31}$P NMR spectrum was recorded after each addition.

Fluorescence titration experiments

All experiments were undertaken following a similar protocol: a known volume (ca. 2 mL) of solution of known concentration of host (ca. 5.0 × 10^{-6} M) was placed in a cell and the absorbance and emission spectra were recorded. Aliquots (ca. 5 μL corresponding to 0.5 equiv. of host) of a stock solution of guest were successively added and a spectrum was recorded after each addition. Aliquots were added until no change was observed in the spectrum. The values obtained for emission were corrected for dilution.

$^1$H NMR characterization of the guest-host complex 1 → DOPC

$^1$H NMR (CDCl₃), 298 K, 600 MHz, δ (ppm): 0.70-0.81 (m, 27H, fluor., 0.87 (m, 6H, DOPC), 1.17 (m, 4H, DOPC), 1.21-1.45 (m, 4H, DOPC), 1.35-1.44 (m, 27H, fluor.), 1.54 (m, 9H, DOPC), 1.80 (m, 4H, DOPC), 2.00 (m, 4H, DOPC), 2.15 (m, 2H, DOPC), 3.46 (m, 6H, ArCH₂), 5.77 (br s, 6H, CH₂), 5.90 (m, 9H, OMe), 3.06 (m, 1H, DOPC), 4.06 (br s, 6H, CH₂O), 4.21 (m, 1H, DOPC), 4.30 (m, 2H, DOPC), 4.48 (m, 6H, ArCH₂), 4.66 (m, 2H, DOPC), 5.34 (m, 1H, DOPC), 5.34 (m, 4H, DOPC), 6.64 (s, 6H, Ar=CH₂), 7.30 (s, 6H, Ar=CH₂), 7.41-8.14 (m, 21H, PyrH), 7.44 (s, 3H, NHCH₃), 8.57 (m, 6H, PyrH), 9.31 (s, 3H, NHPy). The majority of the signals of DOPC were assigned thanks to the COSY, HSQC and 2D ROESY spectra.

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References


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13 See the ESI.


15 It is noteworthy that the signal corresponding to the \((\text{CH}_3)_2 \text{N}^+\) protons of the free lipid shift downfield as the lipid concentration increases. This suggests that the free lipid is in exchange between different environments. This is not simply the signature of a lipid monomer-dimer equilibrium as this signal shifts upfield with lipid concentration in the absence of any host.

16 Structure of host 1 was built on the basis of previous structures of related calix[6]arene molecules (ref. 10a and c). Starting conformation for the \(1 \cdot \text{DOPC}\) complex has been manually built using Discovery Studio 2016 (BIOVIA, Dassault Systèmes). Geometry was further optimized with the same suite of programs using the CHARM force field and the Smart Minimizer algorithm (RMS gradient 0.1).

Supporting information

A Selective Calix[6]arene-based Fluorescent Chemosensor for Phosphatidylcholine Type Lipids

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CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

I. Complexation studies between host 1 and DOPC

Figure S1. COSY spectrum (600 MHz, 298 K, CDCl₃) of compound 1 in the presence of ca. 3 equiv. of DOPC.

Figure S2. 2D ROESY spectrum (600 MHz, 298 K, CDCl₃, mixing time = 300 ms) of compound 1 in the presence of ca. 3 equiv. of DOPC.
CHAPTER 2. STUDY OF A FLUORESCENT CALIX[6]ARENE

Figure S3. HSQC spectrum (600 MHz, 298 K, CDCl₃) of compound 1 in the presence of ca. 3 equiv. of DOPC.

Figure S4. ¹H NMR spectra (600MHz, 298 K) of 1 in presence of ca. 3 equiv. of DOPC in: a) CDCl₃; b) in a mixture CDCl₃/CD₃OD ca. 98:2; c) in a mixture CDCl₃/CD₃OD ca. 96:4; s: solvent.
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II. Complexation studies between host 1 and POPC

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Figure S9. Top: fluorescence spectra of 1 upon the addition of POPC (0 to 185 equiv.) in chloroform, [1]₀ = 1.9 × 10⁻⁶ M, λₑₓ = 345 nm. Bottom: variation of fluorescence intensity at 420 nm upon the addition of POPC.
III. Complexation studies between host \textbf{1} and DPPC

![NMR spectra](image)

**Figure S10.** $^1$H NMR spectra (600MHz, 298 K, CDCl$_3$) of: a) \textbf{1}; b) \textbf{1} in the presence of ca. 3 equiv. of DPPC; s: solvent.

![NMR spectra](image)

**Figure S11.** NMR spectra (600MHz, 298 K, CDCl$_3$) of host \textbf{1} in the presence of ca. 3 equiv. of DPPC: a) 1D EXSY spectrum (mixing time = 25 ms) after selective excitation of the $^4$NMe$_3$ signal at 3.24 ppm; b) $^1$H NMR spectrum. ▼: Pulse excitation.
Figure S12. Top: fluorescence spectra of 1 upon the addition of DPPC (0 to 54 equiv.) in chloroform, [I]₀ = 2.6 × 10⁻⁶ M, λₑₓₙ = 345 nm. Bottom: variation of fluorescence intensity at 420 nm upon the addition of DPPC.
IV. Complexation studies between host 1 and SPH

Figure S13. a) $^1$H NMR spectra (600MHz, 298 K, CDCl$_3$) of: a) 1; b) 1 in the presence of ca. 11 equiv. of SPH; s: solvent.

Figure S14. NMR spectra (600MHz, 298 K, CDCl$_3$) of host 1 in the presence of ca. 11 equiv. of SPH: a) 1D EXSY spectrum (mixing time = 25 ms) after selective excitation of the $^3$NMe$_3$ signal at 3.32 ppm; b) $^1$H NMR spectrum; $\blacktriangledown$: Pulse excitation.
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Figure S18. HSQC spectrum (600 MHz, 298 K, CDCl₃) of compound 1 in the presence of ca. 6 equiv. of DPC.

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VI. Complexation studies between host 1 and MPC

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Figure S23. 2D ROESY spectrum (600 MHz, 298 K, CDCl$_3$, mixing time = 300 ms) of compound 1 in the presence of ca. 4.5 equiv. of MPC.
Figure S24. HSQC spectrum (600 MHz, 298 K, CDCl₃) of compound 1 in the presence of ca. 4.5 equiv. of MPC.
Figure S25. Top: fluorescence spectra of 1 upon the addition of MPC (0 to 71 equiv.) in chloroform. [1]₀ = 2.9 \times 10^{-6} \text{ M}, \lambda_{ex} = 345 \text{ nm}. Bottom: variation of fluorescence intensity at 420 nm upon the addition of MPC.
VII. Complexation studies between host 1 and DOPE

Figure S26. $^1$H NMR spectra (600MHz, 298 K, CDCl$_3$) of: a) 1; b) 1 in the presence of ca. 3 equiv. of DOPE; s: solvent; *: DOPE signals.

Figure S27. Fluorescence spectra of 1 upon the addition of DOPE (0 to 140 equiv.) in chloroform. [1]$_0$ = 2.5 x $10^{-9}$ M, $\lambda_{ex}$ = 345 nm.
VIII. $^{31}$P NMR measurements

**Figure S28.** $^{31}$P NMR spectra (400MHz, 298 K, CDCl$_3$) of: a) a solution of DOPE (ca. 2.9 mM); b) to f) a solution of DOPE (ca. 2.9 mM) after addition of host I up to 0.6 equiv.

**Figure S29.** $^{31}$P NMR spectra (400MHz, 298 K, CDCl$_3$) of: a) a solution of POPC (ca. 4.3 mM); b) to d) a solution of POPC (ca. 4.3 mM) diluted up to a concentration of 0.7 mM.
Figure S30. $^{31}$P NMR spectra (400 MHz, 298 K, CDCl$_3$) of: a) a solution of MPC (ca. 16 mM); b) to d) a solution of MPC (ca. 16 mM) diluted up to a concentration of 0.3 mM.
IX. Extraction experiments

1. Two liquid-liquid extraction experiments were conducted preparing two different 20μL water solutions of 2 known DOPC concentration. After adding these solutions to a solution of 1 in chloroform, the resulting fluorescence intensity was monitored.

![Figure S31](image-url)

*Figure S31.* Red cross: fluorescence intensity of a solution of 1–DOPC upon addition of a solution of DOPC in water (ca. 5.5 μM) to a solution of 1 (ca. 2 μM in 2 mL) in CHCl₃. Green triangle: fluorescence intensity of a solution of 1–DOPC upon addition of a solution of DOPC in water (ca. 60 μM) to a solution of 1 (ca. 2 μM in 2 mL) in CHCl₃, $λ_{ex} = 345$ nm. Errors estimated of ± 10% for the equivalents of DOPC and ± 5 % for the fluorescence intensity.
2. NMR experiments

Figure S32. $^1$H NMR spectrum (600MHz, 298 K, CDCl$_3$) of: a) 1.1 mM solution of 1 in CDCl$_3$ (600 µL); b) after addition of 100 µL of a 12 mM solution of DOPC in D$_2$O after mixing; s: solvent.

Figure S33. $^1$H NMR spectra (600MHz, 298 K, CDCl$_3$) of: a) 1; b) 1 after addition of a solution of DOPC liposomes prepared D$_2$O; c) 1 after addition of a solution of DOPC liposomes prepared D$_2$O and after heating and stirring for 16h at 50°C; s: solvent; w: water.
3. **Fluorescence titration**

![Graph showing fluorescence intensity](image)

**Figure S34.** Variation of fluorescence intensity at 397 nm upon the addition of DOPC in chloroform to a 1.9 × 10⁻⁵ M solution of 1 in 2 mL chloroform in the presence of 20 μL of water. Solid line corresponds to 1:1 binding which yields a log $K = 4.5 \pm 0.2$; $\lambda_{ex} = 345$ nm.
2.3. CONCLUSIONS

The results presented in this chapter show that calixarene receptors bearing fluorescent units are very sensitive molecular chemosensors. The fluorescent calix[6]tris-pyrenylurea 14a and its analog 14b, which has a more open cavity, are furthermore remarkably selective.

Receptor 14a exhibits a remarkable selectivity for the sulfate anion in DMSO, enabling its selective sensing by fluorescence spectroscopy. In CDCl₃, it is able to efficiently bind ammonium ions only in association with the sulfate anion. This cooperative binding of ammonium sulfate salts was also observed in a more protic environment (1:11 CD₃OD/CDCl₃). The binding constants determined by UV−vis absorbance, fluorescence, and NMR titrations were found to be in agreement in all cases. Corroborating conformational changes of the receptor upon binding were observed by fluorescence and NMR spectroscopies. Interestingly, a cavity-based selectivity, in terms of the size and shape of the guest, was observed for both receptors. While 14a displays a strong affinity for small or linear ammonium salts, 14b was found to have the ability to recognize the 3,4-O-dimethyldopammonium sulfate salt and not the corresponding serotonin derivative. This work opens interesting perspectives on the elaboration of unique fluorescent cavity-based systems for the selective sensing of anions or biologically relevant ammonium salts such as neurotransmitters.

Receptor 14a is also an efficient heteroditopic chemosensor able to strongly interact with lipids in organic media (log K > 4). A high selectivity for phosphatidylcholine type lipids over the closely related phosphatidylethanolamines was observed, highlighting the importance of specific interactions between the receptor and the zwitterionic head of the lipid. This mode of recognition is reminiscent of natural systems such as human phosphatidylcholine transfer proteins (PC-TPs). This validates the biomimetic approach adopted in our work, that consists in designing receptors that combine a H-bonding donor site to a hydrophobic pocket in order to achieve a high degree of complementarity with the two ionic groups of zwitterions. We also observed, via extraction experiments, that host 14a can still bind phosphatidylcholine type lipids in a biphasic chloroform/water solution, opening the way to the use of calix[6]arenes as selective chemosensors for lipids in biological media.
CHAPTER 3.
INCORPORATION AND STUDY OF CAVITANDS IN MICELLES
3.1. INTRODUCTION

One of the goals of our work, as mentioned in the introduction, is to evaluate the possibility of transferring molecular receptors, in particular calix[6]arenes, into aqueous media and to study their binding properties in water. The development of molecular receptors, that are able to recognize neutral or charged species with a high affinity and selectivity in water, is indeed an important field of research as many medical and environmental applications can be envisaged.¹

Most studies with molecular receptors are carried out in organic media as only few synthetic receptors are water-soluble (e.g. cucurbiturils, cyclodextrines). Most synthetic receptors are only soluble in organic media, which is the case of calixarenes. Moreover, water is a highly competitive medium as water molecules, which are good H-bond donors/acceptors, can actively interfere in the recognition process by efficiently solvating the different binding partners. Water is also a solvent with a high dielectric constant and polar interactions are consequently screened. On the other hand, an attractive feature of working in water is that molecular recognition can benefit from the hydrophobic effect.²

Two strategies exist for transferring hydrophobic receptors into an aqueous medium. A first strategy is the grafting of hydrophilic moieties onto the receptor. The solubilization in water of hydrophobic receptors has been achieved, for example, via the grafting of sulfonates, carboxylates, ammoniums, PEG, sugars or peptides.³ For calix[4]arenes, we can mention water-soluble calix[4]tetra-sulfonates⁴, which are able to recognize small neutral molecules or metallic cations or sugar bearing calix[4]arenes, which are able to recognize specific proteins.⁵ Reinaud’s group has shown that a water-soluble calix[6]tris-imidazole bearing three Me₃N⁺ cationic moieties on the large rim can complex Zn²⁺ at the level of the imidazole groups and hydrophobic amines in the calixarene cavity in a cooperative way at pH ~ 7 (Figure 3-1).⁶

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¹ Lehn, J.-M. La chimie supramoléculaire concepts et perspectives; 1997.
CHAPTER 3. INCORPORATION AND STUDY OF CAVITANDS IN MICELLES

Figure 3-1. The hydrosoluble zinc calix[6]arene-based receptor and its recognition properties in water (figure taken from reference 6).

The Reinaud’s group has also shown that similar systems can complex Cu\(^+\) and Cu\(^{2+}\) in water.\(^7\) Water soluble calix[6]tren derivatives, obtained via grafting of cationic moieties or PEG chains, have recently been synthesized and their complexing properties in water explored.\(^8\) A copper calix[6]tren with grafted cationic moieties was observed to be stable on a large pH range showing similar host-guest properties to its analogue soluble in organic solvents, recognizing guests, such as nitriles, amines and alcohols.

An alternative solution that can be considered in order to transfer hydrophobic molecular receptors into water is the use of micelles. Micelles are aggregates of amphiphilic molecules (surfactants) composed of a hydrophilic head and a hydrophobic tail that self-assemble when placed in an aqueous environment in order to limit their contact with water (Figure 3-2).

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CHAPTER 3. INCORPORATION AND STUDY OF CAVITANDS IN MICELLES

Figure 3-2. Schematic representation of a micelle.

The transfer of hydrophobic compounds into an aqueous medium, via their incorporation into micelles, is a widely used strategy for various applications. For example, in decontamination processes, chelating agents incorporated into micelles are used for the extraction of heavy metals from water.\(^9\) Micelles are also used for drug solubilization and in catalysis where the proximity of the different reactants and high concentration in the small micelles volume can have a very beneficial effect of reaction rate.\(^10,11\) Lipophilic molecules can be solubilized in the core of the micelle and thus transferred into an aqueous environment without the need of any tedious synthetic step (Figure 3-3).

Figure 3-3. Schematic representation of lipophilic receptor incorporated into a micelle.

Different types of micelles exist depending on the nature of the surfactant: ionic bearing either a positive or negative charge at the level of their head group, zwitterionic or non-ionic.\(^10\) Examples of these surfactants are presented in Figure 3-4: a cationic surfactant cetyltrimethylammonium bromide (CTABr) composed of an aliphatic chain, a quaternary ammonium polar head and bromide as counter-ion; an anionic surfactant, sodium

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dodecylsulfate (SDS), composed of an aliphatic chain with a sulfate group as polar head with sodium as a counter-ion; non-ionic surfactant, Triton X-100, composed of an aromatic hydrocarbon tail and a polyethylene oxide polar head and finally a zwitterionic surfactant dodecylphosphocholine (DPC), composed of an aliphatic chain and a phosphocholine head.

![Structures of different types of surfactants.](image)

DPC was the surfactant used in this thesis. It is characterized by an aggregation number of 56 (the number of surfactant monomers composing one micelle) at 20 mM and 298 K and a critical micellar concentration (cmc) of 1.1 mM (the surfactant concentration above which micelles can be formed).\(^{10}\)

### 3.2. EXAMPLES OF MICELLAR INCORPORATION OF MOLECULAR RECEPTORS

Rebek and co-workers have reported on a resorcinarene-based cavitand, insoluble in water that once incorporated into DPC micelles adopts a bowl conformation, which offers the possibility of recognizing small neutral molecules such as cycloalkanes or adamantanes.\(^{12}\) They have also observed that the conformational reorganization, after incorporation into DPC micelles, of a water-soluble resorcinarene-based cavitand increases significantly its binding capacity toward different guests (Figure 3-5).\(^{12}\)

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CHAPTER 3. INCORPORATION AND STUDY OF CAVITANDS IN MICELLES

![Diagram](image)

Figure 3-5. Resorcinarene-based cavitand for recognition of small molecules (figure taken from reference 12).

This receptor is able to simultaneously bind the head of the DPC surfactant and other guests, such as adamantane or quinuclidinium derivatives. The determined binding constants are higher in the micellar media than in water showing the power of micelles as protecting environment for recognition. Another example of the effectiveness of micellar incorporation is given by the study of the uranyl-salophen-based receptors in cationic micelles (CTABr and CTACl)

![Structures](image)

Figure 3-6. Structures of two uranyl-salophenes.

Indeed, with the collaboration of Prof. Dalla Cort from the University La Sapienza in Rome, the EMNS laboratory has reported that this hydrophobic receptor is capable to efficiently and selectively recognize fluoride, once incorporated in the micellar media. Remarkably, the micellar system exhibits an affinity for fluoride (log $K \sim 4$), which is two orders of magnitude higher than the one observed with the water-soluble version of the receptor with grafted sugar moieties (Figure 3-6). These results highlight once again the important role played by the micellar environment in the recognition process.

To our knowledge, there are only very few examples reported in the literature which concern the binding capacities of calixarene-based systems incorporated into micelles. Calix[4]arene-

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crown ethers dissolved in dodecyl sulfate micellar solutions have been reported to be able to complex cesium or potassium in a selective way.\textsuperscript{15} A few years ago, Hu and co-workers prepared a Cu\textsuperscript{2+}-selective self-assembled fluorescent calix[4]arene-based chemosensor by solubilizing the receptor and a fluorescent reporter, perylene, in Triton X-100 micelles. Upon the binding of Cu\textsuperscript{2+}, the fluorescence emission of perylene was quenched. The authors also showed that Cu\textsuperscript{2+} ions can be detected selectively and quantitatively in the presence of other metal ions at concentration in the sub-micromolar range.\textsuperscript{16} It has also been reported that calix[6]arenes bearing long aliphatic chains on their small rim can self-assemble via a co-micellization with the cationic surfactant dodecyltrimethylammonium bromide.\textsuperscript{17} No binding studies have however been undertaken with these systems.

3.3. AIM OF THE WORK

In this thesis, we focused our attention on the micellar incorporation in DPC micelles of a calix[6]tren zinc complex (6.Zn\textsuperscript{2+}), which has already been described in the introduction of this thesis. We also had the opportunity to look at the micellar incorporation of receptors which are related to calixarenes: a homooxacalix[3]arene tris-acid (15) and a resorcin[4]arene-based zinc complex (16.Zn\textsuperscript{2+}).

The binding properties of calix[6]tren 6 in organic media have been detailed in the introduction. It is able to complex Zn\textsuperscript{2+} at the level of the tren cap, leaving a single coordination site accessible for a neutral guest that is incorporated inside the calix[6]arene cavity.\textsuperscript{18,19} 6.Zn\textsuperscript{2+} proved to be remarkably selective regarding the recognition of neutral molecules in organic solvents.

Considering the results in organic media, we wished to evaluate the binding properties of complex 6.Zn\textsuperscript{2+} in an aqueous medium. In order to transfer this hydrophobic metal complex into water, we incorporated it into DPC micelles. This system was extensively studied using \textsuperscript{1}H NMR and our results have been published in the journal Organic and Biomolecular Chemistry: "Primary Amine Recognition in Water by a Calix[6]aza-cryptand Incorporated in

\textbf{References}

Dodecylphosphocholine Micelles” (2015, 13, 2931-2938). The results are summarized in the section 3.4 of this chapter.

The homooxacalix[3]arene derivative has not yet been studied for its binding properties despite its already known synthesis. On the other hand, the resorcin[4]arene derivative has already been the object of studies in the laboratory of Prof. Reinaud. This two receptors were incorporated into DPC micelles and their binding properties evaluated. The results reported in section 3.5 have not yet been the object of a publication.
3.4. SUMMARY OF PUBLISHED RESULTS


$6.Zn^{2+}$ was successfully incorporated into DPC micelles by simple mixing it with the surfactant (20 mM) in D$_2$O at neutral pH (~7.6). Concentrations up to 0.5 mM of $6.Zn^{2+}$ were obtained. The NMR spectrum of the complex incorporated into DPC micelles was very similar to the one observed for the complex in chloroform. The downfield shift of the OMe signal ($\delta > 3.9$ ppm) suggests that these groups have been expelled from the cavity and probably replaced by water molecules. The ArH and tBu protons appear as two very distinct sets of signals highlighting that the complex adopts a flattened cone conformation. DOSY experiments, confirmed the incorporation into the micelles as the receptor and the surfactant molecules exhibit the same diffusion coefficients.

The binding properties of $6.Zn^{2+}$ incorporated in the DPC micelles was compared to those previously reported for the receptor in chloroform. We observed that $6.Zn^{2+}$ incorporated in DPC recognizes propylamine (PrNH$_2$), as evidenced by the presence of high field signals for the propyl chain in the $^1$H NMR spectrum of the system. An apparent affinity constant of 5000

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M⁻¹ at pH~8 was determined. The host-guest complex 6.Zn²⁺⇌PrNH₂ was observed to be stable in the pH range 3–11 which is interesting as 6.Zn²⁺ incorporated in the micelles is only stable in the pH range 6–9. It was also observed that ethylamine (EtNH₂) and heptylamine (HeptylNH₂) are recognized with $K_{EtNH₂} > K_{PrNH₂} > K_{HeptylNH₂}$. The fact that the HeptylNH₂ chain protrudes outside the cavity forcing the calix[6]arene to adopt an energetically less favorable straight conformation certainly explains the lower affinity for this guest. The relative affinity for HeptylNH₂ vs. PrNH₂ observed in the micellar environment compared to CDCl₃ (0.5 vs. 0.00025) highlights the importance of the hydrophobic effect in the recognition process. No inclusion of alcohols or amino-alcohols was observed.

The calix[6]tren ligand 6 was also successfully incorporated in the DPC micelles but only at low pH (~3), showing that the system needs to be positively charged in order to be incorporated into the micelle. It seems that a favorable interaction with the buried negatively charged part of the zwitterionic surfactant favors incorporation. Analysis of the $^1$H NMR spectrum highlights that the OMe groups are pointing inside the cavity ($δ$ ~2.4 ppm) and that consequently the cavity is deprived of any guest.

The binding of the neutral guest 2-imidazolidininone (Imi) by protonated ligand was confirmed by the presence of high-field signals for bound Imi ($δ$ ~0.22 ppm for the methylene groups) and by the fact that the OMe signal is shifted downfield attesting that the groups have been expelled from the cavity following guest inclusion. A pH driven shift with 6.Zn²⁺ in the presence of both PrNH₂ and Imi was also observed: at high pH, the complexation of the amine by 6.Zn²⁺ is observed while at low pH the inclusion of Imi by the protonated ligand is observed.
Primary amine recognition in water by a calix[6]aza-cryptand incorporated in dodecylphosphocholine micelles†

Emilio Brunetti,a,b,* Alex Inthasot,a,c Flore Keymeulen,b Olivia Reinaud,b Ivan Jabin,a and Kristin Bartikb

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]arene 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 cavands 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 cavands 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 cavand incorporated into dodecylphosphocholine (DPC) micelles increases significantly its binding capacity towards different guests.9 It has also been reported that unmodified resorcinarenes incorporated into cetyltrimethylammonium bromide (CTAB) 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[n]arenes are a well-known class of corecave 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[n]arene ligands bearing nitrogenous coordinating arms on the narrow rim have been developed.14 In particular,
CHAPTER 3. INCORPORATION AND STUDY OF CAVITANDS IN MICELLES

the calix[4]trene receptor \(1,1^{10}\) belonging to the third generation has been shown to display unique and versatile binding properties, notably toward metal ions and neutral guests (Fig. 1).\(^{11}\) Indeed, the trene cup offers a strong binding site for a metal ion \((\text{e.g. } \text{Zn}^{2+}, \text{Cu}^{2+})\). 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 \(1\text{-Zn}^{2+}\) (Fig. 1) can bind with a high selectivity various neutral organic molecules \((\text{e.g. } \text{amines, alcohols, amides, etc.})\) inside the calix[4]trene core in different organic solvents.\(^{12}\)

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 \(1\text{-Zn}^{2+}\) 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[4]trene \(1\) and of its corresponding metal complex \(1\text{-Zn}^{2+}\) into DPC micelles (Fig. 1). Characterization of the calix[4]trene/arene/micelle systems and evaluation of the binding properties of the incorporated receptors were achieved by NMR spectroscopy.

Results and discussion

Incorporation of complex \(1\text{-Zn}^{2+}\) and ligand \(1\) into DPC micelles. The receptor \(1\text{-Zn}^{2+}\) was successfully incorporated into DPC micelles by simple mixing of the receptor and the surfactant \((20 \text{ mM in } \text{D}_2\text{O at neutral pH } \approx 7.6)\) and concentrations of up to 0.5 mM of \(1\text{-Zn}^{2+}\) could be obtained.\(^{13}\) The \(^1\text{H}\) NMR spectrum of the incorporated receptor \(1\text{-Zn}^{2+}\) in DPC-\(d_{25}\) 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 \(^1\text{H}\) 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 \(C_1\) calix[4]trene-Zn complex.\(^{14}\) Similarly to \(1\text{-Zn}^{2+}\) in CDCl\(_3\), the downfield shift of the \(\text{OMe}\) signals of the incorporated complex \((\delta_{\text{OMe}} \approx 3.9 \text{ ppm in CDCl}_3\) and in D\(_2\)O\(^{15}\)) suggests that these groups have been expelled.

Fig. 2. \(^1\text{H}\) NMR spectra (298 K) of (a) \(1\text{-Zn}^{2+}\) in CDCl\(_3\) (600 MHz); (b) \(1\text{-Zn}^{2+}\) in DPC-\(d_{25}\) (20 mM in \(\text{D}_2\text{O at pH } \approx 7.6, 600 \text{ MHz})\); (c) \(1\text{-Zn}^{2+}\) in CDCl\(_3\) after the addition of \(-1\) equiv. of PrNH\(_2\) (400 MHz); (d) \(1\text{-Zn}^{2+}\) in DPC-\(d_{25}\) (20 mM in \(\text{D}_2\text{O at pH } \approx 8.6\)) after the addition of 5 equiv. of PrNH\(_2\) (600 MHz). \(\text{PrNH}_2\) in; \(\text{PrNH}_2\) out; \(\ast\) residual DPC; s: solvent; w: water.
from the cavity by a coordinated solvent water molecule. Two very distinct sets of signals can be observed for the tBu as well as for the ArPh protons, indicating that the calixarene adopts a major flattened cone conformation. The incorporation of 1-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 ~2 nm. Moreover, knowing that the micellar aggregation number is around 50, it is reasonable to estimate that there is 1 molecule of complex 1-Zn
\(^{2+}\) per micelle.

In order to gain information about the position of 1-Zn
\(^{2+}\) in the micelle, paramagnetic relaxation enhancement (PRE) experiments were undertaken. 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. 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 tBu protons of 1-Zn
\(^{2+}\) have values similar to those of the tail of the surfactant but very different from those of the hydrophilic part. It was thus possible to conclude that the calixarene positions itself in the hydrophobic core. Interestingly, the spectrum of 1-Zn
\(^{2+}\) in DPC micelles was not significantly modified by the addition of DCl or NaOH in the pH range from 6 to 9, highlighting the stability of the system in this pH window.

The calix[4]aren ligand 1 was also successfully incorporated into DPC micelles, by simple mixing in D,
\(_2\)O of the receptor and the surfactant, as attested by the NMR spectrum. It was however necessary to lower the pH to ~3, showing that the ligand must be protonated at the level of its basic tenn cap (1-nH
\(^{+}\)) in order to be incorporated. This result suggests that the favorable electrostatic interaction of the positively charged 1-nH
\(^{+}\) and 1-Zn
\(^{2+}\) 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
\(^{+}\) are pointing inside the cavity (Δ\(\delta_{\text{H}}\) = 2.44 ppm), clearly highlighting the absence of a guest molecule inside the cavity. The efficient incorporation of 1-nH
\(^{+}\) and 1-Zn
\(^{2+}\) into DPC micelles encouraged us to undertake host-guest complexation studies with these systems.

**Guest complexation by 1-Zn
\(^{2+}\) in DPC micelles**

Propylamine (PrNH
\(_2\)) is known to display a high affinity for 1-Zn
\(^{2+}\) in CDCl
\(_3\). A titration with PrNH
\(_2\) of the receptor in DPC micelles was monitored by \(^1\)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 PrNH
\(_2\) (pH = 8.6) (Fig. 2d):

(i) High-field signals for the protons of the alkyl chain of PrNH
\(_2\) (i.e., –1.62 and –1.95 ppm) attest of its inclusion inside the cavity and thus of the formation of the complex 1-Zn
\(^{2+}\) \(\supseteq\) PrNH
\(_2\). These chemical shifts are similar to those obtained with the same system in CDCl
\(_3\) 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 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 tBu or ArPh protons pointing inside and pointing outside the cavity (Δ\(\delta_{\text{H}}\) = 0.65 ppm and Δ\(\delta_{\text{ArPh}}\) = 0.67 ppm).

These NMR data show that the host-guest properties of 1-Zn
\(^{2+}\) toward amines are maintained in the micellar environment (Fig. 3). An apparent binding constant for propylamine (K\(_{b,\text{app}}\)) of ~3 \times 10
\(^{5}\) M
\(^{-1}\) for a 1:1 binding was determined by signal integration at pH = 8.2. At pH ~10, this constant was estimated to be >10
\(^{5}\) M
\(^{-1}\). The complex 1-Zn
\(^{2+}\) \(\supseteq\) PrNH
\(_2\) was observed on a larger pH window (pH from ~7 to ~11) in the micelle than complex 1-Zn
\(^{2+}\) devoid of organic guest coordinated to the Zn atom (vide supra). From these data, a pseudo pKa of ~4.3 for complexed propylamine could be esti-
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This value can be compared to the pKₐ value of free porphyrin, 10.6. This remarkable result shows the extraordinary stabilization of the amine due to its complexation to the metal cation inside the calixarene cavity. This pKₐ shift is comparable to the one estimated for heptylamine with a related water-soluble Zn-calixarene receptor bearing imidazole moieties.²³ It is interesting to point out that this related receptor does not complex PrNH₂ in water, which highlights the role played by the micellar environment on the complexation properties of the calixarene-based system.

Complex 1-Zn²⁺ in DPC micelles also binds ethylamine (EtNH₂) and heptylamine (HeptylNH₂) as evidenced by the presence of high field signals for their allyl chain protons in the ²H NMR spectra.²¹ The affinity constants for these amines relative to PrNH₂ were evaluated by competition experiments (Table 1)²⁶ and the following sequence of affinities was observed at pH ~ 11.1 (±0.1): PrNH₂ > HeptylNH₂ > HeptylNH₂Et. 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 βu substituents at the large rim. Indeed, with other related βu-substituted calix[6]arene-based metal complexes studied in our laboratory, the allyl chain length of 4 and 4-atom lengths that are fully encapsulated in the cavity (as shown here by the high-field shifted ²H-NMR signals), display the highest affinities. In contrast, the allyl chain of the larger amino guest has to protrude outside the cavity and, in order to avoid a steric clash with the βu groups, the calix[6]-arene skeleton is forced to adopt an energetically less favourable straight conformation.²⁸ Finally, it is interesting to note that the relative affinity for HeptylNH₂ (vs. PrNH₂) in the micellar solution is spectacularly much higher than the one measured in CDCl₃ (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 βuNH₂ as well as the secondary amine (Et₂NH) was not observed (from pH ~ 8 to pH ~ 11) despite the fact that these amines are less hydrophilic than EtNH₂ or PrNH₂. This result shows that steric hindrance at the level of an amino group is a major factor of selectivity and thus highlights the cavity-based selectivity ensured by the incorporated receptor. The complexation of other families of guests (hexan-1-ol, butanol, octanol, and aminoalcohols (ethanolamine, (+)-1-amino-2-propanol, (+)-1-amino-3-propanol, and 6-amino-1-hexanol) was also studied. Again, no sign of inclusion was evidenced for any of these guests (Fig. 3). The fact that octanol, which should like heptylamine benefit from the hydrophobic effect, is not complexed by the calixarene suggests that the amino group plays a key role for complexation. It has indeed been shown that the affinity of 1-Zn²⁺ for ethanol in CDCl₃ is four orders of magnitude lower than for propylamine (KaffoCDCl₃ = 2.5 x 10⁻³).²⁷ In order to understand why the aminoalcohols, and in particular the small ethanolamine, are not recognized by 1-Zn²⁺ incorporated into DPC micelles, the affinity for ethanolamine was measured in CDCl₃. The relative affinity compared to PrNH₂ was determined to be close to 1 (KaffoCDCl₃/ethanolamine ~ 0.8, see Table 1). Such a discrepancy between the results obtained in water/micelle conditions and in an organic solvent is also assignable to the hydrophobic effect that obviously plays a major role in the host-guest recognition processes and in the observed selectivities. In this context, it is worth pointing out that DOSY experiments undertaken with DPC solutions showed that PrNH₂ does not incorporate into the DPC micelles but that HeptylNH₂ does. The diffusion coefficient of PrNH₂ in the presence of DPC micelles is indeed identical to the diffusion coefficient of the amine in D₂O and this is not the case for HeptylNH₂ which clearly partitions itself between the azeo-and micellar environments. This difference can be rationalized in terms of the hydrophobicity of the amines which are more hydrophobic with increasing chain length.

Guest complexation by 1-nH⁺ into DPC micelles

We have previously shown that calix[6]arene-based receptors bearing either a polyanion,²¹ polymido²² or polyureido cap²³ 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,²⁴ the high affinity for Imi stems from a complementary DADA–ADDÀ quadruple H-bonding array between the urea guest and the calixarene host. Imi is also recognized by the protonated calixarene derivative 1-nH⁺ in CDCl₃ and, to date, behaves as the best “key” for this receptor.²⁵ The binding of this neutral guest was thus evaluated in the micellar environment by NMR spectroscopy. It was observed that Imi (~250 equiv.) is not recognized by 1-Zn²⁺ incorporated in DPC micelles (from pH ~ 8 to ~ 11). However, 1-nH⁺ 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 (~2.0 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

| Table 1. Affinities, relative to PrNH₂ (pKₐ = 10.58), of various guests G for host 1-Zn²⁺ | Relative affinities²⁶ |
| G (pKₐ)²⁶ | DPC (20 mM in D₂O) | CDCl₃ |
|EtNH₂ (10.67) | 1.3 (pH ~ 11.1) | — |
|HeptylNH₂ (10.65) | 0.5 (pH ~ 11.2) | ~0.00025 |
|HO(CH₂)₂NH₂ (9.30) | n.d. (pH ~ 9.4) | 0.8 |

¹Defined as [G]₀/[PrNH₂] × [PrNH₂][G]₀ and measured at 298 K. The subscript “o” stands for “observed”. Errors estimated ±20%. ²For the pKₐ values, see ref. 30. ³Not detected with up to 50 equiv. of HO(CH₂)₂NH₂.
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-Zn\(^{2+}\)·PrNH\(_2\) and 1-nH\(^+\)·Imi

In CDCl\(_3\), the quantitative acid–base induced interconversion of complexes 1-Zn\(^{2+}\)·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-Zn\(^{2+}\)·PrNH\(_2\) and 1-nH\(^+\)·Imi host-guest adducts incorporated into DPC micelles was tested (Fig. 4). First, PrNH\(_2\) (4.5 equiv.) and Imi (4 equiv.) were added to a solution of 1-Zn\(^{2+}\) in DPC micelles and the resulting NMR spectrum (pH = 10.5) clearly showed the exclusive formation of 1-Zn\(^{2+}\)·PrNH\(_2\) (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-Zn\(^{2+}\)·PrNH\(_2\) and the formation of 1-nH\(^+\)·Imi together with some free protonated receptor 1-nH\(^+\). The subsequent addition of NaOD (to reach pH = 8.4) restored very easily the initial NMR profile corresponding to 1-Zn\(^{2+}\)·PrNH\(_2\) (Fig. 4e). 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]zaeacyclpentand (I) can be incorporated into DPC micelles either as a Zn\(^{2+}\) complex in the [6–9] pH window or as a polyammonium at low pH. The 1-Zn\(^{2+}\) 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.\(^{30}\) Interestingly, the host–guest complex 1-Zn\(^{2+}\)·PrNH\(_2\) was detected in the micelles in an even larger pH window (3–11) than was the 1-Zn\(^{2+}\) 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 pK\(_a\) shift of propylamine recognised by 1-Zn\(^{2+}\) 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 Zn\(^{2+}\) but also the environment provided by the calixarene cavity and the micelle which protects the system from the water. The 1-Zn\(^{2+}\) complex preferentially binds small amines and this size selectivity stands in strong contrast with the previously reported water-soluble calixarene-based Zn\(^{2+}\) complex for which hydrophobic amine binding could be detected, but not with small hydrophilic ones.\(^{22}\) 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-Zn\(^{2+}\) in the presence of both an amine and Imi: at high pH, the Zn\(^{2+}\) 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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Experimental section

Chemicals

DPC and DPC-d38 were purchased from Avanti Lipids and used with no further purification. D2O and CDCl3 were purchased from VWR BDH PIOLABO. Compounds 1 and 1-Zn2+ were synthesized according to the literature.11,16

General procedures

1H NMR spectra were recorded on a 600 MHz, 400 MHz or a 300 MHz spectrometer. For the 1D 1H 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 internalchemical shift references. Chemical shifts are quoted on the δ scale. The NMR spectra were recorded on 298 K unless otherwise stated.

NMR solutions preparation

All solutions for NMR studies were prepared following a similar protocol: 1-Zn2+ (−4 mg) and DPC (−35 mg, deuterated or not depending on the experiments) were added to 5 mL of D2O in order to have, respectively, concentrations of ~0.5 mM and ~20 mM. Solutions were stirred until a clear solution was obtained.

1H NMR characterization of various complexes 1-Zn2+ + nH2O

1H NMR (DPC 20 mM in D2O, 298 K, 600 MHz): δ (ppm) 0.79 (bs, 27H, tBu), 1.44 (bs, 27H, tBu), 3.97 (bs, 9H, OMe), 2.87-4.61 (m, 24H, ArCH2Ph,ArCH2C6H4/CH2N(CH3)2O), 6.19-8.81 (m, 6H, ArHPh), 7.40 (bs, 6H, ArHPh). 1-Zn2+ + PrNH2- 1H NMR (DPC 20 mM in D2O, 298 K, 600 MHz): δ (ppm) −1.95 (bs, 3H, CH2N2PrNH2), −1.63 (bs, 2H, CH2N2PrNH2), 0.74-0.87 (m, 27H, tBu), 1.36-1.57 (m, 27H, tBu), 2.95-4.68 (m, 33H, ArCH2Ph,ArCH2C6H4/CH2N(CH3)2O/Me), 6.19-6.99 (m, 6H, ArHPh). * The chemical shift of the CH2N2 of PrNH2 (in) was not observed due to overlapped signals.

Determination of the apparent affinity of PrNH2 toward complex 1-Zn2+ through 1H NMR titration in DPC (20 mM in D2O)

Different aliquots of a solution of amine (PrNH2) were progressively added to 300 μL of a DPC solution (20 mM in D2O) containing the complex 1-Zn2+. Non-deuterated DPC was used and the integration of the CH2 and CH3 signals were used as internal reference to determine the concentration of 1-Zn2+. The integrations of the free guests and of the included guests were used to calculate the apparent affinity defined as [PrNH2]free/[1-Zn2+][1- [PrNH2]free][PrNH2] (errors estimated ±20%).

Determination of the relative affinities of heptyl- and ethylamine compared to propylamine through 1H NMR competitive binding studies in DPC (20 mM in D2O)

At room temperature, ~7 equivalents of heptylamine (HeptyNHNH2) or ~3 equivalents of ethylamine (EthylNHNH2) were added to 500 μL of a DPC solution (20 mM in D2O) containing the complex 1-Zn2+. Propylamine (PrNH2) was then added (~5 equivalent). The 1H NMR spectrum showed the guest resonances of both complexes 1-Zn2+ + PrNH2 and 1-Zn2+ + HeptyNHNH2 or 1-Zn2+ + EthylNHNH2 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 [Gfree][PrNH2]free/[PrNH2]free [Gfree] (errors estimated ±20%).

Determination of the relative affinity of ethylamine compared to propylamine in CDCl3 via 1H NMR competitive binding studies

At room temperature, ~30 equivalents of propylamine (PrNH2) and ~15 equivalents of ethylamine (EthylNHNH2) were added to 500 μL of a CDCl3 solution of complex 1-Zn2+. A 1H NMR spectrum showed the guest resonances of both complexes 1-Zn2+ + PrNH2 and 1-Zn2+ + EthylNHNH2 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 [Gfree][PrNH2]free/[PrNH2]free [Gfree] (errors estimated ±20%).

Determination of the relative affinity of heptylamine compared to DMF in CDCl3 via 1H NMR competitive binding studies

At room temperature, ~40 equivalents of dimethylformamide (DMF) and ~100 equivalents of heptylamine (HeptyNHNH2) were added to 500 μL of a CDCl3 solution of complex 1-Zn2+. A 1H NMR spectrum showed the guest resonances of both complexes 1-Zn2+ + DMF and 1-Zn2+ + HeptyNHNH2 and the signals corresponding to free DMF and HeptyNHNH2. The integrations of the signals corresponding to the free and included guests were used to calculate relative affinity defined as [Gfree][HeptyNHNH2]free/[Gfree][DMF]free (errors estimated ±20%). In order to obtain the value corresponding to Keq, the value of Kc,eq,HeptyNHNH2 was used for the value for Kc,eq,DMF from ref. 17 (1610).

Reversible interconversion of host-guest adducts

1-Zn2+ + PrNH2 and 1-H+ + Imi

PrNH2 (4.5 equiv.) and Imi (4 equiv.) were added to a ~0.5 mM solution of 1-Zn2+ in DPC-d38 micelles (pH ~9.0). In a second stage, DCl 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.
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Preliminary experimental parameters
For the PRE experiments, aliquots of 5 μl of a 5 mM solution of K₂[Cr(NCS)₄] in D₂O were added to the 600 μl solution under study. T₁ measurements were undertaken using the classical inversion recovery (180° – τ – 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 (ΔT₁; 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 (cme = 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 ESI?.
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. Canany, J. Xu, J. M. Castangetto, D. Rentepero 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 the absence of buffer in order to avoid any interaction with the complex and micelles that could alter the results.


Supporting information

Primary Amine Recognition in Water by a Calix[6]aza-cryptand Incorporated in Dodecylphosphocholine Micelles

*Emilio Brunetti, Alex Inthasot, Flore Keymeulen, Olivia Reinaud, Ivan Jabin* and Kristin Bartik*

S11. 2D DOSY experiment (298K, 600MHz, D₂O) with 1.Zn²⁺ in DPC

S12. NMR PRE experiment (298K, 600MHz, D₂O) with 1.Zn²⁺ in DPC

S13. ¹H NMR (298K, 300MHz, D₂O) spectrum of 1.nH⁺ in DPC

S14. Determination of the pseudo pKₐ for PrNH₂ with 1.Zn²⁺ in DPC

S15. ¹H NMR (298K, 600MHz, D₂O) spectra of 1.Zn²⁺ in the absence and presence of EtNH₂ in DPC-d₃8

S16. ¹H NMR (298K, 600MHz, D₂O) spectra of 1.Zn²⁺ in the absence and presence of HeptylNH₂ in DPC

S17. Experimental conditions for titrations of 1.Zn²⁺ with amines, alcohols and aminoalcohols in DPC (298 K, D₂O)
SII. 2D DOSY experiment (298K, 600MHz, D₂O) with \( \text{Zn}^{2+} \) in DPC

The x- and y- axis represent the regular \(^1\)H chemical shift and the diffusion coefficient, respectively. DCM: residual dichloromethane; HDO: solvent signal chosen as reference for diffusion coefficient determination (\( D = 19.02 \times 10^{-10} \text{ m}^2/\text{s} \) at 298 K).
SI2. PRE NMR experiments (298K, 600MHz, D₂O) with \(1\text{Zn}^{2+}\) in DPC.

Normalized relaxivity (measured relaxivity divided by relaxivity of \(\alpha\text{CH}_2\) protons of the surfactant; \(\varphi\); mM⁻¹s⁻¹; error < 15%): values for the nuclei of DPC (20 mM) and for the nuclei of the incorporated complex \(1\text{Zn}^{2+}\) (0.5 mM).

<table>
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<th>β</th>
<th>γ</th>
<th>δ</th>
<th>Tail</th>
<th>ω</th>
<th>(\text{ArH}^{\text{COP}})</th>
<th>(\text{ArH}^{\text{OMe}})</th>
<th>(\text{tBu}^{\text{OMe}})</th>
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</table>
SI3. $^1$H NMR (298K, 300MHz, D$_2$O) spectrum of $\text{LnH}^+$ in DPC

$^1$H NMR spectrum (300 MHz, 298 K) of $\text{LnH}^+$ in DPC (20 mM in D$_2$O at pH ~3.1); s: solvent.
SI4. Determination of the *pseudo* pKa shift for PrNH₂

The formation constant $K$ and $K'_{\text{pH}}$ are defined according to the following equilibrium:

$$1.\text{Zn}^{2+} + \text{PrNH}_3^+ + \text{H}_2\text{O} \leftrightarrow \text{Zn}^{2+} + \text{PrNH}_2 + \text{H}_3\text{O}^+$$

$$K = \frac{[1.\text{Zn}^{2+} + \text{PrNH}_3^+][\text{H}_3\text{O}^+]}{[1.\text{Zn}^{2+} + \text{PrNH}_2][\text{H}_2\text{O}]}$$

$$K'_{\text{pH}} = \frac{[1.\text{Zn}^{2+} + \text{PrNH}_3^+]}{[1.\text{Zn}^{2+} + \text{PrNH}_2]}$$

From analysis and signal integration in the $^1$H NMR spectra (see experimental section of article for details), $K$ was found to be $\sim 5 \times 10^{-5}$ at pH $\sim 8$ and $K'_{\text{pH}} \sim 5000$ M$^{-1}$. From these data, we can estimate a *pseudo* pKa $(-\log K)$ of $\sim 4.3$.  

5
S15. $^1$H NMR (298K, 600MHz, D$_2$O) spectra of 1.Zn$^{2+}$ in the absence and presence of EtNH$_2$ in DPC-$\alpha$38

$^1$H NMR spectra (600 MHz, 298 K) of (a) 1.Zn$^{2+}$ in DPC-$\alpha$38 (20 mM in D$_2$O at pH −7.6); (b) 1.Zn$^{2+}$ in DPC-$d38$ (20 mM in D$_2$O) after the addition of −3 equiv. of EtNH$_2$. ▼: EtNH$_2$ in; s: solvent.
SI6. $^1$H NMR (298K, 600MHz, D$_2$O) spectra of 1.Zn$^{2+}$ in the absence and presence of HeptylNH$_2$ in DPC micelles.

$^1$H NMR spectra (600 MHz, 298 K) of (a) 1.Zn$^{2+}$ in DPC (20 mM in D$_2$O at pH ~7.6); (b) 1.Zn$^{2+}$ in DPC (20 mM in D$_2$O) after the addition of ~7 equiv. of HeptylNH$_2$: s: solvent; a: acetone.
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S17. Experimental conditions for titrations of $\text{Zn}^{2+}$ with amines, alcohols and aminoalcohols in DPC

The potential binding of different guests was monitored via $^1$H NMR titration experiments at room temperature with ~0.5 mM solutions of $\text{Zn}^{2+}$ in DPC (20 mM in D$_2$O). Progressive additions of the investigated potential guest, until the final concentration indicated below, were undertaken (pH monitored are also indicated). No signals for included guest were observed in the $^1$H NMR spectra for the following molecules:

(i) $\text{tBuNH}_2$ up to 6 mM (pH ~7.8, ~10.4 and 11);
(ii) (Et)$_2$NH up to 15 mM (pH ~7.8 and 11). This amine added after the experiment undertaken in (i);
(iii) ethanol up to 365 mM (pH ~7.6);
(iv) propanol up to 340 mM (pH ~7.6);
(v) butanol up to 18 mM (pH ~7.6). This alcohol added after the experiment undertaken in (iv);
(vi) octanol up to 50 mM (pH ~7.6);
(vii) ethanolamine up to 25 mM (pH ~7.6);
(viii) (±)-1-amino-2-propanol up to 3.5 mM (pH ~6.0 and pH ~10.4);
(ix) (4)-2-amino-1-propanol up to 3.7 mM (pH ~5.5 and pH ~10.5);
(x) 6-amino-1-hexanol up to 10 mM (no pH value available);
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3.5. MICELLAR INCORPORATION OF OTHER SYSTEMS

3.5.1. INCORPORATION OF A HOMOXACALIX[3]ARENE INTO DPC MICELLES

Homooxacalixarenes are closely related to calixarenes: the phenolic units are linked by CH₂OCH₂ groups instead of by methylene bridges. The most studied member of this class of compounds is the hexahomotrioxacalix[3]arene shown in Figure 3-8.

![Figure 3-8](image)

*Figure 3-8. Structure of the p-tert-butyl-hexahomotrioxacalix[3]arene.*

We synthesized, in collaboration with Sara Zahim (Jabin’s laboratory), the homooxacalix[3]tris-acid 15 shown in Figure 3-9, using a protocol described in the literature.

![Figure 3-9](image)

*Figure 3-9. Structure of host 15.*

Previous results with a tris-acid calix[6]arene in chloroform have shown that ammonium ions can be bound in *endo* and *exo* and that the driving force is the favorable electrostatic interaction between the ammonium head and the carboxylate groups of the receptor. Upon titration with

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PrNH₂, deprotonation of the carboxylic groups was observed and after three equivalent all the carboxylic groups were deprotonated; one PrNH₃⁺ was complexed inside the cavity (endo) and two others in exo (Figure 3-10).²⁶

![Diagram](image)

**Figure 3-10.** Endo-complexation of ammonium ions by the tri-deprotonated calix[6]tris-acid in CDCl₃ and NMR complexation induced shifts of various ammonium guests (figure taken from reference 27).

15 was successfully incorporated into DPC micelles by simple mixing of the receptor and the surfactant (20 mM) in D₂O (pH ~8.6). Concentrations of up to 0.3 mM were obtained. The incorporation of 15 inside the DPC micelles was confirmed by DOSY NMR experiments as both receptor and surfactant exhibit the same diffusion coefficient.²⁷ As the system is incorporated in the micellar core, it is possible that it is only partly deprotonated which we will call 15⁻ⁿH⁺. A titration of the system would be needed to determine the exact pKa of the three carboxylic groups. The ¹H NMR spectrum of 15⁻ⁿH⁺ incorporated in DPC micelles shows a pattern very similar to the one recorded in CDCl₃ (Figure 3-11a-b). A single set of signals is observed for the tBu as well as for the ArH protons, indicating that the host adopts a C₃ᵥ symmetry both in CDCl₃ and in DPC micelles.

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²⁷ The DOSY spectrum is presented in Appendix 2 (7.2).
The binding properties of $15^{-nH^+}$ incorporated in DPC micelles, and more particularly the affinity of the system for ammonium ions, were investigated by NMR. The spectra recorded after the addition of tert-butylamine ($tBuNH_2$) to the system are shown in Figure 3-12.

![Figure 3-11](image)

Figure 3-11. $^1$H NMR spectra (298 K, 600 MHz) of: a) $15^{-nH^+}$ in DPC (20 mM in D$_2$O at pH ~8.6); b) $15$ in CDCl$_3$ (2.74 mM); *: DPC signals; s: solvent; g: grease; i: impurities.

![Figure 3-12](image)

Figure 3-12. $^1$H NMR spectra (298 K, 20 mM DPC-d$_{38}$ in D$_2$O, 600 MHz, 11 mM HEPES) of $15^{-nH^+}$: a) in the absence of $tBuNH_2$; b) in the presence of ~4.5 equiv. of $tBuNH_2$; c) in the presence of ~23 equiv. of $tBuNH_2$; c) in the presence of ~80 equiv. of $tBuNH_2$ at pH ~10.2; s: solvent; g: grease; *: residual traces of non-deuterated DPC; °: HEPES.
The poor buffering conditions meant that the pH of the solution increased from 4.8 to 10.2 upon addition of ~80 equiv. of the amine. The high-field signals for the protons of the alkyl chain of \( \text{tBuNH}_3^+ \) (-0.37 ppm) in the \(^1\)H NMR spectra (Figure 3-12), attest of its inclusion inside the cavity and thus of the formation of the complex \( 15\text{H}^+ \rightleftharpoons \text{tBuNH}_3^+ \). The inclusion of the guest was confirmed by a 1D EXSY experiment (Figure 3-13).

Upon addition of 0 to 11 equiv. of the guest, the signal of the host CH protons next to the carboxylic/carboxylate groups shifts significantly to higher field (from 4.41 to 4.27 ppm) and then no longer shift, indicating complete deprotonation of the acid groups. Certain signals of the host are in fast exchange on the chemical shift timescale and essentially do not shift during the titration: \( \text{ArCH}_{\text{eq}} \) (\( \Delta \delta \approx 0.01 \) ppm and \( \Delta \delta \approx 0.08 \) ppm); \( \text{tBu} \) groups (\( \Delta \delta \approx 0.03 \) ppm). This suggests that the conformation of the receptor does not change much upon guest inclusion. In contrast, the \( \text{ArH} \) protons and the \( \text{ArCH}_{\text{ax}} \) of the host appear in slow exchange (\( \Delta \delta_{\text{ArHfree-ArHbound}} \approx 0.3 \) ppm and \( \Delta \delta_{\text{ArCHax}} \approx 0.59 \) ppm). By integrating these signals, we were able to determine an apparent affinity constant (\( K_{\text{app}} \)) of \( \approx 13 \text{ M}^{-1} \) for \( \text{tBuNH}_3^+ \) for a 1:1 binding at pH ~10.2. The affinity constant was determined based on the following equilibrium:

\[
15\text{H}^+ + \text{tBuNH}_3^+ \rightleftharpoons 15\text{H}^+ \text{tBuNH}_3^+
\]

However, even after the addition of 80 equivalent of guest the cavity was not completely filled. The included ammonium guest is certainly stabilized by a favorable electrostatic interaction with the carboxylates. The charge balance could be maintained, as for the calixarene system.

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28 The pKA of \( \text{tBuNH}_2 \) is of ~10.7 at 298 K. In the bulk, under these experimental conditions, the amine appears under the ammonium form.

29 Under these conditions, it is likely to suppose that system 15 is totally deprotonated.
studied in CDCl$_3^{26}$ by other tBuNH$_3^+$ which are in exo, or perhaps by ammonium groups of DPC or guest interacting with the carboxylate moieties.

The recognition of other ammoniums was also studied (Figure 3-14): D/L sec-Butylammonium (sec-ButylnH$_3^+$), 1-adamantylammonium (AdamNH$_3^+$)$^{30}$ and heptylammonium (HeptNH$_3^+$). The titration for AdamNH$_3^+$ is shown in Figure 3-15.

**Figure 3-14.** Guests studied with host 15.

![Diagram showing different guests: NH$_3^+$, PrNH$_3^+$, fBuNH$_3^+$, AdamNH$_3^+$, HeptNH$_3^+$, sec-ButylnH$_3^+$](image)

**Figure 3-15.** $^1$H NMR spectra (298 K, 20 mM DPC-d$_{38}$ in D$_2$O, 600 MHz, 11 mM HEPES) at pH ~7.3 of 15$^{-}$aH$_3$: a) in the absence of AdamNH$_3^+\text{Cl}^-$; b) in the presence of ~2 equiv. of AdamNH$_3^+\text{Cl}^-$; c) in the presence of 48 equiv. of AdamNH$_3^+\text{Cl}^-$; d) in the presence of ~290 equiv. of AdamNH$_3^+\text{Cl}^-$; s: solvent; g: grease; singlets are residual traces of non-deuterated DPC; °: HEPES; a: acetone.

$^{30}$ The titration was performed by adding the chlorine salt of adamantylamine (AdamNH$_3^+\text{Cl}^-$). This explains why in this case the pH did not shift upon addition of the guest.
The NMR spectra of $^{15}\text{H}^+\rightarrow\text{AdamNH}_3^+$, $^{15}\text{H}^+\rightarrow\text{sec-ButylNH}_3^+$ and $^{15}\text{H}^+\rightarrow\text{HeptylNH}_3^+$ exhibit very similar patterns as $^{15}\text{H}^+\rightarrow\text{tBuNH}_2^+$ (Figure 3-16a-b).\(^{29}\) Guest inclusion was in each case evidenced by the presence of high field signals in the $^1\text{H}$ NMR spectra. The apparent binding constants ($K_{\text{app}}$) for AdamNH$_3^+$ (~80 M$^{-1}$ at pH ~7.3) and for sec-ButylNH$_3^+$ (~18 M$^{-1}$ at pH ~11.3) could be determined by integration.\(^{31}\)

![Figure 3-16](image-url)

Figure 3-16. $^1\text{H}$ NMR spectra (298 K, 20 mM DPC-$d_{38}$ in D$_2$O, 600 MHz, 11 mM HEPES) of $^{15}\text{H}^+$ in the presence of: a) 80 equiv. of tBuNH$_2$ at pH ~10.2; b) 48 equiv. of AdamNH$_3^+$Cl$^-$ at pH ~7.3; s: solvent; g: grease; singlets are residual traces of non-deuterated DPC; °: HEPES.

In the case of heptylammonium, bound signal overlap made integration difficult. The addition of ~83 equiv. of PrNH$_2$ to the solution containing $^{15}\text{H}^+\rightarrow\text{sec-ButylNH}_3^+$ (at pH ~11.3) did not lead to the inclusion of PrNH$_3^+$. The complex $^{15}\text{H}^+\rightarrow\text{sec-ButylNH}_3^+$ was observed in the pH range 2-12, i.e. at pH values where the carboxylate groups are expected to be protonated and at pH values where one expects the ammonium to be in its amine form.\(^{32}\)

For comparison purposes, the ability of system 15 to complex ammonium ions was also evaluated in deuterated chloroform. Propylamine (PrNH$_2$), D/L sec-Butylamine (sec-ButylNH$_2$), tBuamine (tBuNH$_2$) and heptylamine (HeptylNH$_2$) are all recognized in their ammonium form in CDCl$_3$ as evidenced by the presence of high field signals for their alkyl chain protons in the $^1\text{H}$ NMR spectra. The signal for the CH$_2$COO protons of the host shifts to higher field during the titration (from 4.95 to 4.82 ppm and then remains constant), indicating that the host undergoes a complete deprotonation. The absolute affinities were not evaluated for the different guests but, after addition of ~3 equivalents of each guest, the cavities were

\(^{31}\) The binding constant were determined following the same equilibrium presented above for tBuNH$_2$. The signals used to determine the binding constant were the Ar$_{\text{H}}$free and Ar$_{\text{H}}$bound and free guest.

\(^{32}\) For the other host-guest complex we did not study the stability over pH.

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almost completely filled. The affinity constant for D/L sec-ButylNH₂ relative to PrNH₂ was however evaluated by a competition experiment: $K_{\text{PrNH₂/sec-ButylNH₂}} \approx 0.3$. The fact that the two affinity constants are of the same order of magnitude in CDCl₃, while in water (at pH ~11.3) the affinity for sec-ButylNH₃⁺ seems to be considerably higher than for PrNH₃⁺, is certainly assignable to the hydrophobic effect as PrNH₂ is much more hydrophilic than D/L sec-ButylNH₂.

3.5.2. **INCORPORATION OF A RESORCIN[4]ARENE DERIVATIVE INTO DPC MICELLES**

Resorcinarenes are cavitands composed of 4 to 6 resorcinol units. They are structurally very close to calixarenes and are obtained by the condensation of a resorcinol and an aldehyde.³³ They are bowl shaped and can be functionalized at the level of their wide rim in order to build a structure able to bind a metal or recognize an organic molecule (Figure 3-17).

![Figure 3-17. Structure of a resorcin[4]arene.](image)

We looked into the possible micellar incorporation of the resorcinarene-based host 16 developed by Dr. Parrot during its PhD thesis in the Reinaud’s group (Figure 3-18).³⁴ This resorcinarene is functionalised by 4 methylimidazole moieties and can bind a zinc cation at the level of the imidazole nitrogens and, simultaneously, a small molecule in its cavity.

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Dr. Parrot observed that 16.Zn$^{2+}$ exhibits a high binding affinity for the acetate anion in acetonitrile. He also developed a hydro-soluble derivative W16, by grafting cationic moieties on the small rim of 16, and its corresponding zinc complex W16.Zn$^{2+}$ (Figure 3-19). A similar system with three imidazole arms and its hydro-soluble equivalent (Rim$_3$ and WRim$_3$) have also been developed and studied in Reinaud’s group (Figure 3-19). The zinc complex WRim$_3$.Zn$^{2+}$ was observed to be stable in the pD range 6.5-9.5.

We successfully incorporated ligand 16 into DPC micelles by simple mixing it with the surfactant (20 mM) in D$_2$O at neutral pH. The incorporation of 16 was confirmed by DOSY NMR. Concentrations up to ~0.7 mM were obtained. The $^1$H NMR spectra of incorporated 16 (Figure 3-20) is similar to the one observed for W16 in water. It was observed with W16 that its imidazole signals shift with pH, due to changes in the protonation state of the host, and the highest pKa of the system was estimated to be in the 5.2 to 7.6 range at 300 K.

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37 Rat, S; Gout, J.; Bistri, O.; Reinaud, O. Org Biomol Chem. 2015, 13, 3194-3197.
16. Zn$^{2+}$ was also successfully incorporated into DPC micelles by simple mixing it with the surfactant (20 mM) in D$_2$O at neutral pH. Concentrations up to $\sim$0.5 mM were obtained. (Figure 3-21a).

The titration of the system with sodium acetate was monitored by $^1$H NMR spectroscopy (Figure 3-21b-d). The pH of the solution increased during the titration due to the lack of buffering capacity. The analysis of the $^1$H spectra however clearly shows that acetate has been

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38 The solution in this case was prepared by adding $\sim$ 3 mL of NaOD ($\sim$ 1M) to a solution of 16.Zn$^{2+}$ (pH $\sim$6.3, 10 mM HEPES) and 20 mM DPC-d$_{38}$ in D$_2$O; The pH increased to $\sim$7.3.
included but also that demetalation occurs.\textsuperscript{39} Indeed, after addition of ~3 equiv. of acetate, the pH is ~9.9 and only the signals characteristic of the neutral ligand 16 in DPC micelles are observed (Figure 3-21d).

When performing the same titration under more acidic and controlled conditions (pH ~6.3, 10 mM HEPES)\textsuperscript{40}, two clear overlapping signals are observed for included acetate (Figure 3-22, see expansions): a sharp signal (δ = -2.27 ppm) and a broader one (δ ~-2.32 ppm).

![Figure 3-22. $^1$H NMR spectra (298 K, 600MHz) of 16.Zn$^{2+}$ (0.49 mM in 20 mM DPC-d$_3$8 in D$_2$O, HEPES 10mM, pH ~6.3): a) in the absence of sodium acetate; b) in the presence of 0.5 equiv. of sodium acetate; c) in the presence of 2.8 equiv. of sodium acetate; s: solvent; singlets are residual traces of non-deuterated DPC; R= alkyl chain of 16; °: HEPES signals.](image)

At this pH (~6.3) it is possible that we are in the presence of two different inclusion complexes 16.Zn$^{2+}$›CH$_3$COO$^-$, 16H$^+$.Zn$^{2+}$›CH$_3$COO$^-$ but also some free protonated ligand 16H$^+$.\textsuperscript{39}

Protonation would occur at the level of one of the imidazole, which would then no longer be able to coordinate to the metal cation. A solvent molecule would then be coordinated to the

\textsuperscript{39} It is important to point that the assignments shown in the figure did not derive from NMR characterization but were made by comparison with the results obtained with 16.Zn$^{3+}$ in acetonitrile and W.16.Zn$^{2+}$ described in Parrot’s thesis.

\textsuperscript{40} In this case no NaOD was added and the pH did not shift during the titration.
metal in *exo* to complete the coordination sphere of the metal. Due to the presence of multiple species, it was unfortunately not possible to determine affinity constants.

**Figure 3-23.** Schematic representation of $16H^+\cdot Zn^{2+}\rightarrow CH_3COO^-$ (left) and $16Zn^{2+}\rightarrow CH_3COO^-$ (right) (figures taken from Parrot’s thesis).

The presence of two inclusion complexes was also observed and rationalized by Dr. Parrot when working in CD$_3$CN: when titrating $16Zn^{2+}\rightarrow CH_3COO^-$ with water, he observed the formation of a second inclusion complex and its proportion was related to the concentration of water in the organic solution.$^{34}$

Spectra of $16Zn^{2+}$ incorporated in DPC micelles with ~3 equivalents of acetate were recorded at 278, 298 and 323 K at pH ~6.3 (Figure 3-24). Very surprisingly, we observed that at low temperature the species giving rise to the broad high field signal for included acetate in the NMR spectrum is favored while at high temperature it is the species giving rise to the sharp signal which is favored.$^{41}$ We know from the literature that the pKa of imidazole varies from ~7.4 at 278 K to ~6.6 at 323 K (~ 1mM).$^{42}$ It is therefore possible that, at high temperature, the 4-imidazole zinc complex $16Zn^{2+}\rightarrow CH_3COO^-$ would therefore be favored whereas at lower temperatures the protonated zinc complex $16H^+Zn^{2+}\rightarrow CH_3COO^-$ would be favored.$^{39}$

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$^{41}$ Complex Induced Shifts determined for the sharp included signal of acetate: 4.14 ppm at 323 K and 4.18 ppm at 298 K.

$^{42}$ Hochachka, P. W.; Somero, G. N; Biochemical Adaptation 1984 Princeton Legacy Library.
At pH ~6.3 and 323 K, where we are in the presence of only one host-guest species, an apparent binding constant for acetate of log \( K \approx 2.9 \) could be determined by signal integration for the following equilibrium:

\[
16\text{Zn}^{2+} + \text{CH}_3\text{COO}^- \rightleftharpoons 16\text{Zn}^{2+}\text{CH}_3\text{COO}^-
\]

This value is relatively large for the complexation of a hydrophilic anion like acetate in water and is very close to the one reported for the water-soluble system WRim: log \( K \approx 2.8 \) (pD \( \approx 7.4 \)).\(^{37}\) The ability of this complex to coordinate acetate in aqueous media is quite remarkable. Indeed, whereas many receptors for acetate have been described in the literature, most only work in organic solvents. Those reported to bind acetate in water are uncommon and the corresponding binding constants are relatively moderate.\(^{43}\)

When adding 6 equiv. of acetate to \( 16\text{Zn}^{2+} \) incorporated in DPC micelles at pH ~8, a mixture of \( 16\text{Zn}^{2+}\text{CH}_3\text{COO}^- \) and of free ligand 16 is observed (Figure 3-25a). Integration of the included acetate signal shows that only \( \approx 50 \% \) of acetate is included. When zinc perchlorate was added to the solution (1.6 equiv.), the signals characteristic of the free ligand disappear (for


example the CH$_2$O at 5.69 ppm) and only the signature of $^{16}$Zn$^{2+}$CH$_3$COO$^-$ is observed with in this case 100% of acetate inclusion (Figure 3-25b). The addition of zinc perchlorate shifts the equilibrium between 16 and $^{16}$Zn$^{2+}$ towards the metal complex, and the amount of guest that can be included increases.

The binding constant for acetate at pH~8 was determined from signal integration and a value slightly higher than at pH 6.3 was determined ($\log K \sim 3.3$ at 323 K). This is also in accordance with the results obtained by Reinaud and co-workers with WRim$_3$ where a bell curved dependence as a function of pH was observed for the formation of the system WRim$_3$-Zn-acetate (detectable in a pD range 5.5-10.5). The optimal pD was determined to be around 7.4.

![Figure 3-25. $^1$H NMR spectra (600MHz, 323K) of $^{16}$Zn$^{2+}$ (0.57 mM) at pH ~7.9 with in a 20 mM DPC in D$_2$O HEPES 40mM solution with: a) 6 equiv. of sodium acetate; b) 6 equiv. of sodium acetate after the addition of 1.6 equiv. of zinc perchlorate; s: solvent; *: DPC; R= alkyl chain of 16; g: grease.](image)

$^{16}$Zn$^{2+}$ incorporated in DPC micelles was also titrated with acetylacetone at pH ~7.3 and guest inclusion was evidenced by the changes in the NMR spectra and in particular the presence of high field signals (Figure 3-27a-d). The coordination of the enolate form of the guest (acetylacetone: C$_5$H$_7$O$_2^-$), at the level of the metal, has been highlighted by Dr. Parrot with this system in CD$_3$CN. Acetylacetone is complexed with one acetyl group in endo-position and the other in exo-position.
Figure 3-26. Schematic representation of $16H^+.Zn^{2+} \rightarrow C_5H_7O_2^-$ (figure taken from Parrot’s thesis).

The bidentate coordination of $C_5H_7O_2^-$ to the metal cation and the simultaneous protonation of one of the imidazoles means that we can only be in the presence of $16H^+.Zn^{2+} \rightarrow C_5H_7O_2^-.

Figure 3-27. $^1H$ NMR spectra (298 K, 600MHz, 20 mM DPC in D$_2$O, HEPES 40mM) of: a) $16.Zn^{2+}$ (0.57 mM) at pH ~7.3; b) $16.Zn^{2+}$ after the addition of 0.4 equiv. of acetylacetone; c) $16.Zn^{2+}$ after the addition of 1.3 equiv. of acetylacetone; d) $16.Zn^{2+}$ after the addition of 1.8 equiv. of acetylacetone at pH~7.3; s: solvent; *: DPC; R= alkyl chain of 16; g: grease.

The quantitative formation of the inclusion complex $16H^+.Zn^{2+} \rightarrow C_5H_7O_2^-$ is observed and an affinity of log K > 5 at pH~7.3 could be determined. The signal of included $C_5H_7O_2^-$ is broad at 298 K but neither the increase nor the decrease of the temperature sharpened the signal.
3.6. CONCLUSIONS

In this chapter we investigated the strategy of transferring molecular receptors into water via micellar incorporation. This approach is indeed a very elegant solution to overcome the solubility problem of organic receptors. We studied hydrophobic receptors, which are only soluble in organic solvents.

We have shown that a calix[6]azacryptand based receptor (host 6) can be incorporated into DPC micelles either as a Zn$^{2+}$ complex in the 6–9 pH window or as a its multi-protonated form at low pH. The zinc complex maintains its host-guest properties already observed in organic solvent and is able to complex small or long linear primary amines inside the calixarene cavity. The inclusion complex $6.Zn^{2+} \rightarrow Pr\text{NH}_2$ was observed even at very low pH where the free amine is fully protonated in water. This shows that the environment provided by the calixarene cavity and the micelle stabilizes PrNH$_2$ in its basic form. The fact that incorporated $6.Zn^{2+}$ binds small amines and longer amines with a similar affinity stands in strong contrast with the results obtained with a water-soluble calix[6]tris-imidazole zinc complex where the binding of long hydrophobic amines was observed but not the binding of small hydrophilic ones. It also stands in contrast with the results obtained in CDCl$_3$ where smaller amines are much better recognized than the longer chain ones. This shows that the hydrophobic effect plays a key role in driving guests from water into the calixarene cavity incorporated in DPC micelles.

We also successfully used the micellar incorporation strategy with a homooxacalix[3]tris-acid derivative, 15, and a resorcin[4]arene zinc complex bearing four methylimidazole moieties, $16.Zn^+$. We were able to show that 15 incorporated into DPC micelles can bind different ammoniums, albeit with low affinity. Regarding $16.Zn^{2+}$ incorporated in DPC micelles, we observed that it can recognize small organic molecules such as acetate and acetylacetone.

Our results highlight that the use of micelles to transfer lipophilic receptors into an aqueous environment is a valuable method and, furthermore, that NMR spectroscopy is a very powerful technique to study the incorporation and the binding properties of micellar based supramolecular receptors.
CHAPTER 4.
FUNNEL COMPLEXES: STUDY OF EXCHANGE DYNAMICS
4.1. INTRODUCTION

As presented in the general introduction, calix[6]tris-imidazole zinc (Figure 4-1) can be considered as a model for the active site of metallo-enzymes. In order to contribute to a better understanding of this system, we wanted to investigate the mechanism of guest exchange which has to date not yet been elucidated.

More often, when looking at host-guest recognition processes, a dissociative mechanism is considered: the guest can leave the host without the need to be replaced by another guest (Figure 4-2).

The change in the concentration of the host-guest complex can be expressed as a function of $k_{\text{out}}$, the first order dissociation rate constant and $k_{\text{in}}$, the second order association rate constant:

$$\frac{d[H\supset G]}{dt} = -k_{\text{out}}[H \supset G] + k_{\text{in}}[H_{\text{free}}][G_{\text{free}}]$$

At equilibrium $\frac{d[HG]}{dt} = 0$ and the following equation holds:

$$k_{\text{in}}[H_{\text{free}}][G_{\text{free}}] = k_{\text{out}}[H \supset G]$$
A pseudo-first order rate constant can be defined for the association process, $k_{in}' = k_{in} [H_{free}]$.

The residence times are defined as follow:

$$\tau_{in} = \frac{1}{k_{out}} \quad \text{and} \quad \tau_{out} = \frac{1}{k_{in}[H_{free}]}$$

In the case of system $1.Zn^{2+}$, since it is not possible for the zinc cation to be tri-coordinated, the outgoing guest cannot simply vacate cavity before the incoming guest arrives.\(^1\) Due to steric constraints, it is also not possible for the incoming and outgoing guests to be simultaneously included in the cavity. The exchange of the guest can therefore neither be a simple dissociative mechanism nor endo-associative mechanism.

The hypothesis that we put forward is that the guest exchange mechanism is an associative mechanism and that the exo-coordination of a ligand is required to enable the guest to leave the cavity (Figure 4-3). In this context it is interesting to point out that previous studies have shown, that a dicationic metal calix[6]arene system presents a coordination site inside the calixarene cavity (for an endo molecule) but also a second coordination site accessible for an exo-molecule outside the cavity.\(^2\)

![Figure 4-3](image_url)

**Figure 4-3.** Hypothetical mechanism presented for guest departure from the cavity of $1.Zn^{2+}$.

The following equilibria represent the systems shown in Figure 4-3:

**Equilibrium 1:** $\text{Zn}^{2+} + G \rightleftharpoons 1.\text{Zn}^{2+} \supset G$

---


Equilibrium 2: $1. \text{Zn}^{2+} \rightleftharpoons \text{G} + \text{L} \rightleftharpoons 1. \text{Zn}^{2+} \text{L} + \text{G}$

The change in the concentration of the host-guest complex can be expressed as a function of the different rate constants:

$$\frac{d[1. \text{Zn}^{2+} \rightleftharpoons \text{G}]}{dt} = -k_{out}[1. \text{Zn}^{2+} \rightleftharpoons \text{G}] + k_{in}[1. \text{Zn}^{2+}][\text{G}] - k^{*}_{out}[1. \text{Zn}^{2+} \rightleftharpoons \text{G}][\text{L}] + k^{*}_{in}[1. \text{Zn}^{2+} \text{L}][\text{G}]$$

At equilibrium, the concentration in host-guest complex does not change and the following equation holds:

$$(k_{out} + k^{*}_{out}[\text{L}])[1. \text{Zn}^{2+} \rightleftharpoons \text{G}] = (k_{in}[1. \text{Zn}^{2+}] + k^{*}_{in}[1. \text{Zn}^{2+} \text{L}])[\text{G}]$$

We can see that the $(k_{out} + k^{*}_{out}[\text{L}])$ characterizes the processes corresponding to the exit of the guest G from the cavity. We can thus write:

$$\tau_{in} = \frac{1}{(k_{out} + k^{*}_{out}[\text{L}]})$$

$$\frac{1}{\tau_{in}} = k_{tot} = (k_{out} + k^{*}_{out}[\text{L}])$$

where $k_{tot}$ represents the sum of all first order (or pseudo-first order) rate constants. It is linearly dependent on the concentration of the ligand L that can be exo-coordinate to the metal ($a$ and $b$ are two constants):

$$k_{tot} = a + b * [\text{L}]$$

If a more complete scheme is considered for guest exchange following an associative mechanism, an intermediate step where zinc is penta-coordinated should be considered as shown in Figure 4-4 where all rate constants are indicated. As an empty cavity is not favored the equilibrium between species A and B is shifted towards species B where, the zinc is tetra-coordinated. With the advent of a ligand L, a trigonal bipyrimidal geometry is established where the metal cation is penta-coordinated with G inside the cavity and L outside the cavity (species C). The endo-coordinated ligand G is then able to leave the cavity leaving the metal once again in a tetrahedral geometry but in this case with an exo-ligand (species D). A new guest molecule
G can be then enter the cavity and the metal will once again be in a trigonal bipyramidal geometry. The de-coordination of the exo-ligand L will bring the system back to species B. The residence time ($\tau_{in}$) of the guest G inside the cavity should thus be influenced by the nature and the concentration of L.

**Figure 4-4.** Hypothetical mechanism presented for guest exchange for system 1.Zn$^{2+}$.

The different equilibria shown in Figure 4-4 are:

**Equilibrium 1:** 1. Zn$^{2+}$ + G $\Leftrightarrow$ 1.Zn$^{2+} \supset$ G

**Equilibrium 2:** 1. Zn$^{2+} \supset$ G + L $\Leftrightarrow$ 1.Zn$^{2+}$L $\supset$ G

**Equilibrium 3:** 1. Zn$^{2+}$L $\supset$ G $\Leftrightarrow$ 1.Zn$^{2+}$L + G

If we consider independently the process corresponding to the guest leaving the cavity of species B or C we can write:

$$\frac{1}{\tau_{in}^{B}} = k_{out} \quad \text{and} \quad \frac{1}{\tau_{in}^{C}} = k'_{out}$$

The inverse of the mean life-time of G in a cavity would be given by the sum of the inverse of the residence time of the guest in state B and the inverse of the residence time of the guest in state C. The equilibrium between species B and C must however be considered as it will influence the proportions of the two species. If we make the hypothesis that the concentrations of species A and D are negligible we can write that the sum of the molar fraction of species B and C is equal to 1:

$$x_{B} + x_{C} = 1$$

The inverse of the mean life-time of G in a cavity would then be given by:
\[
\frac{1}{\tau_{in}} = \frac{x_B}{\tau^B_{in}} + \frac{x_C}{\tau^C_{in}}
\]

\[
\frac{1}{\tau_{in}} = k_{out} \times x_B + k'_{out} \times x_C
\]

Considering the equilibrium constant which characterizes equilibrium B-C:

\[
K = \frac{[1. \text{Zn}^{2+} \L \Rightarrow G]}{[1. \text{Zn}^{2+} \Rightarrow G] \times [\L]}
\]

we have:

\[
K = \frac{x_C}{x_B \times [\L]} \quad \text{and} \quad x_C = \frac{K[\L]}{1 + K[\L]} \quad \text{and} \quad x_B = \frac{1}{1 + K[\L]}
\]

we thus have:

\[
\frac{1}{\tau_{in}} = k_{out} \times \left( \frac{1}{1 + K[\L]} \right) + k'_{out} \times \left( \frac{K[\L]}{1 + K[\L]} \right)
\]

If we make the hypothesis that \( x_C \ll x_B \) we have that:

\[
\frac{K[\L]}{1 + K[\L]} \approx K[\L] \quad \text{and} \quad \frac{1}{1 + K[\L]} \approx 1
\]

and we come to the following expression:

\[
\frac{1}{\tau_{in}} = k_{out} + k'_{out} \times K \times [\L]
\]

If the equilibrium B-C is shifted towards B, which will be the case in the absence of an exo-ligand, the residence time of G will simply be given by the inverse of \( k_{out} \). Following the mathematical development presented above, \( k_{tot} \), like in the case of the previous simpler model will be linearly dependent on the concentration of the ligand \( \L \) that can be exo-coordinate to the metal, \((a' \text{ and } b' \text{ being constants})\):

\[
k_{tot} = \frac{1}{\tau_{in}} = a' + b' \times [\L]
\]
To generalize, if different species in solution can exo-coordinate and enable the guest to leave the cavity, including the guest G itself, the following more general equation should be considered (where A and Bs are constants):

\[ k_{tot} = \sum_{i=1}^{n} \frac{1}{\tau_{in}G} \approx A + (B[L_i] + B_{ii}[L_{ii}]+..+B_n[L_n]) \]

Measuring the residence time of a guest as a function the nature and concentration of L in order to validate these equations will be the main goal of this research. In this context, 1D EXchange Spectroscopy (EXSY) NMR experiments will be very valuable as we will be able to have access to kinetic constants, and therefore to residence times.\(^3\)\(^4\)

### 4.2. 1D-EXSY NMR

When considering a simple isomerization equilibrium between A and B, the exchange can be characterized by the kinetic constants \(k_{AB}\) and \(k_{BA}\), where \(k_{AB}\) represents the jump rate from site A to site B and mutually for \(k_{BA}\) (Figure 4-5). The inverse of these kinetic constants, \(\tau_A\) and \(\tau_B\), represent the residence time in sites A and B respectively.

![Figure 4-5. Exchange between a site A and a site B.](image)

The chemical environments in site A and site B being different, the nuclei of the system in these two environments will have different resonance frequencies and relaxation times, and we talk about chemical exchange. Different regimes can be considered when comparing the lifetimes to the NMR chemical shift timescale which is the difference between the resonance frequencies in sites A and B: \(|\nu_A - \nu_B|\). For the simple A-B equilibrium, the two regimes that can be distinguished are:

- \(\tau_A\) and \(\tau_B \gg |\nu_A - \nu_B|^{-1}\): the exchange is slow compared to the NMR chemical shift timescale. Two signals are observed in the spectrum, one for each site;

---


• $\tau_A$ and $\tau_B << |\nu_A - \nu_B|^{-1}$: the exchange is fast compared to the NMR chemical shift timescale. A unique averages signal is observed.

Information on the rate constants characterizing the exchange process can be obtained via EXchange SpectroscopY (EXSY) experiments. EXSY is an incoherent transfer of longitudinal magnetization that allows qualitative observations or quantitative measurements of exchange processes. EXSY measurements can be used to quantify dynamic processes in the 10-5000 ms time window (corresponding to $k_{ex} \sim 0.2$-$100$ s$^{-1}$) for systems in slow exchange on the NMR chemical shift timescale. Physical process in this time window include slow conformational changes, such as ligand binding or cis-trans isomerization. To obtain quantitative information from EXSY experiments, as a rule of thumb, Equation 4-1 has to be satisfied:

$$\Delta \nu \gg \frac{1}{k_{exchange}} \gg \frac{1}{T_1}$$

Equation 4-1

where $k_{exchange} = k_A + k_B$ in the case of a simple two site exchange; $T_1$ is the longitudinal relaxation time and $\Delta \nu$ the frequency difference between the signals of the species in exchange. Indeed, exchange must be slow on the chemical shift timescale and relaxation must not be too fast or the signal will not be observed throughout the EXSY experiment.

If we consider the exchange between sites A and B, the exchange can be monitored, via 1D EXSY experiments, by selectively inverting the signal of the species (site A or B) by a 180°-pulse. The pulse sequence used is showed below (Figure 4-6).

![Figure 4-6. General scheme of the 1D EXSY sequence.](image)

Spectra are recorded after different mixing times ($\tau_m$). Exchange between the two sites and relaxation will occur during the mixing time. An example of spectra that would be obtained after selective inversion at site B is shown in Figure 4-7.
As it can be observed in Figure 4-7, the signal which was selectively inverted (B) is negative for short mixing times but returns to its initial intensity as the mixing time increases (longitudinal relaxation characterized by $T_{1B}$). For the signal that corresponds to the species in site A, its intensity is function of the exchange process ($k_{BA}$) and of its relaxation ($T_{1A}$). This explains why it initially decreases before returning to its equilibrium intensity for long mixing times. If difference spectra are recorded (difference with the spectrum for the system at equilibrium), the inverted signal returns to zero while the other signal is negative and first increases in absolute intensity before returning to zero.

It is noteworthy that the theory for the determination of the kinetic constants in EXSY measurement presented here is derived from the development made for kinetic NOE measurements. In order to determine the kinetic constants associated with the exchange, signals A and B must be integrated for the different mixing times. The fractional enhancement (f) can be computed:

$$f = \frac{I_\tau - I_0}{I_0}$$

where $I_\tau$ corresponds to the integration of the signal after a mixing time $\tau$ and $I_0$ corresponds to the integration at equilibrium. When B is selectively inverted, the ratio of the fractional enhancements $R_B$ is defined as:

---

\[ R_B(\tau) = \frac{f_A(\tau)}{f_B(\tau)} \]

**Equation 4-3**

In order to obtain the kinetic constant \( k_{BA} \), \( R_B \) is determined for different \( \tau \). The kinetic constant \( k_{BA} \) is the initial slope of the \( R_B(\tau) \) curve (Equation 4-4). For \( k_{AB} \), the same procedure is followed by inverting A.

\[ k_{BA} \approx \lim_{\tau \to 0} \frac{dR_B(\tau)}{d\tau} \]

**Equation 4-4**

Many examples have been reported in the literature on the use of EXSY experiments (1D or 2D experiments which we have not described here) to measure of residence time of guests complexed by molecular receptors. For the study of the dynamics of guest complexation in the cavity of calix[6]arenes, we can mention the work on a receptor composed of a calixarenic and a CTV unit reported by Jabin and co-workers (Figure 4-8). 1D EXSY experiments were performed in order to determine the residence time of the guest (imidazolidinone) in the two complexation sites.

**Figure 4-8.** Supramolecular assembly of a calixarene and a CTV with 2 imidazolidinone as guest (figure taken from reference 8).

Naruta et al. used 2D EXSY experiments to evaluate kinetic and thermodynamics parameters related to the process of encapsulation of small hydrocarbons (e.g. methane) into a cavitation-

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porphyrin.\(^9\) Turro et al. described kinetic and thermodynamic properties of water encapsulation by a [60]fullerene (Figure 4-9) and used 2D EXSY measurements to determine association and dissociation constants as well as the activation energies.\(^{10}\)

![Figure 4-9. Water encapsulation by an open cage [60]fullerene derivative (figure taken from reference 10).](image)

### 4.3. RESULTS AND DISCUSSION

To verify our hypothesis that we described in the introduction to this chapter i.e. that an exo-ligand is needed in order for guest exchange to occur in calixarene-Zn\(^{2+}\) based systems where the zinc is only tri-coordinated to the calixarene, we undertook 1D EXSY experiments with three different systems \(1.\text{Zn}^{2+}\), \(2.\text{Zn}^{2+}\) and \(6.\text{Zn}^{2+}\) (Figure 4-10). We looked at the exchange of different guests (dimethylformamide, acetonitrile and ethanol) and focused our work mostly on investigating the role of water in the exchange process (water as the possible exo-ligand).

![Figure 4-10. Structure of \(1.\text{Zn}^{2+}\), \(2.\text{Zn}^{2+}\) and \(6.\text{Zn}^{2+}\).](image)

#### 4.3.1. EFFECT OF WATER ON GUEST EXCHANGE WITH SYSTEM \(1.\text{Zn}^{2+}\)

The NMR spectra of calix[6]tris-imidazole zinc \(1.\text{Zn}^{2+}\) recorded in DCM-d\(_2\) in the presence of a large excess of dimethylformamide (DMF), acetonitrile (CH\(_3\)CN) or ethanol (EtOH) are

---


shown in Figure 4-11. All the cavities are filled with corresponding guest. The three nitrogen arms of the host coordinate the zinc cation and the guest molecule in the cavity completes the tetrahedral coordination sphere of the metal.

![Figure 4-11](image-url)

The following common features are observed in the spectra of all three host-guest complexes: all NMR profiles are sharp and characteristic of a flattened \( \text{C}_3 \) symmetrical cone conformation (\( \Delta \delta_{\text{ArH}} > 0.56 \text{ ppm} \) and \( \Delta \delta_{\text{tBu}} > 0.66 \text{ ppm} \)); the methoxy protons are shifted downfield confirming that they are expelled from the cavity (\( \delta_{\text{OMe}} > 3.55 \text{ ppm} \)); upfield signals are present for the guest confirming their inclusion. Guest complexation is in slow exchange on the NMR chemical shift timescale and the intra-cavity complexation of the different guests was clearly evidenced through 1D EXSY experiments. For instance, a signal at 2.92 ppm, corresponding to a \( \text{CH}_3 \) group of free DMF, is observed upon inversion of the signal at 0.26 ppm corresponding to one of the \( \text{CH}_3 \) group of bound DMF. The 1D EXSY spectra recorded for different mixing times are shown in Figure 4-12.

---

11 The entire \( ^1\text{H} \) NMR characterization of the complexes is presented in the chapter Material and methods.

1D EXSY experiments were undertaken to monitor the exchange of the three different guests in the presence of different amounts of water. In each case, the signal(s) of the included guest was inverted. There are three signals for included DMF and, when experiments were undertaken with each of these signals, the results were not significantly different. The fractional enhancements “R”, as defined in Equation 4-3, are shown in Figure 4-13 as a function of the mixing time for the $1\text{.Zn}^{2+}$$\rightarrow$DMF system and for different water concentrations. The fractional enhancements reported are the average of the values obtained when inverting each of the signals corresponding to the methyl groups of included guest. Upon water addition, only a very small broadening of the signals of the bound guest is observed in the $^1\text{H}$ NMR spectra and the signature of the system did not change. Experiments were only recorded for short mixing times where a linear relationship is observed. The slopes of the lines yield $k_{tot}$.

Figure 4-12. 1D EXSY spectra (600MHz, 298 K, DCM-d$_2$) of $1\text{.Zn}^{2+}$ in the presence of 32 equiv. of DMF and ~8.3 equiv. of water at different $\tau_m$ (25 to 1500 ms) by inverting the signal of low field bound DMF.

Figure 4-13. Evolution with mixing time $\tau_m$ of the fractional enhancement determined for the more downfield CH$_3$ signal of bound DMF. Data showed for ~6.3 (yellow), ~12.5 (orange) and ~61 (green) equiv. of H$_2$O and 32 equiv. of DMF.
The evolution of $k_{\text{tot}}$ as a function of the number of equivalents of water (relative to $1.Zn^{2+}$) is given in Figure 4-14 for the $1.Zn^{2+} \rightarrow$ DMF system.\textsuperscript{13} The following can be derived from analysis of the data:

- without water, no guest exchange is observed;
- $k_{\text{tot}}$ increases linearly with water concentration for a relatively large window.

This suggests that the model described in the introduction to this chapter could be valid with water as \textit{exo}-ligand, L. As guest exchange does not occur in the absence of water even when there is a large excess of DMF (the experiments were undertaken in the presence of 32 equiv. of DMF) this suggests that DMF does not seem to play the role of \textit{exo}-ligand L.

\begin{table}[h]
\centering
\begin{tabular}{cccc}
Entry & Equiv. water & $k_{\text{tot}}$(s\textsuperscript{-1}) (298K) & DMF$^c$ \\
1 & 1.5 & 0 & \\
2 & 6.3 & $0.0026 \pm 0.0002$ & \\
3 & 8.3 & $0.0028 \pm 0.0002$ & \\
4 & 12.5 & $0.0045 \pm 0.0003$ & \\
5 & 61.0$^{14}$ & $0.0226 \pm 0.0021$ & \\
\end{tabular}
\caption{Rate constant ($k_{\text{tot}}$), characterizing the exchange of DMF at 298 K, as a function of the number of equivalents of water in the DCM-d$_2$ solution. The errors for $k_{\text{tot}}$ are fitting errors. Error for number of equivalents of water was estimated to be 10 \%.}
\end{table}

Similar EXSY experiments were undertaken with ethanol as guest. They were undertaken at lower temperature (253 K instead of 278 K in the case of DMF), as at room temperature, the signal of the included guest was too broad to be efficiently inverted during the EXSY experiment.\textsuperscript{15} Upon addition of water, a significant broadening of the signals of bound ethanol is observed showing that water significantly modifies the kinetic of the buried guest (cf. expansions for the CH$_3$ and OH groups in Figure 4-15). The $k_{\text{tot}}$ derived from the EXSY experiments undertaken in the presence of different number of equivalents of water are shown in Figure 4-16. As in the case of DMF, a linear dependence of $k_{\text{tot}}$ is observed with water concentration.

\textsuperscript{13} For the sake of convenience, when $k_{\text{ex}}$ is too small to be measured we will give it a value of 0.

\textsuperscript{14} In these really wet conditions we observe $\sim$50 \% $1.Zn^{2+} \rightarrow$ DMF and $\sim$50 \% of $1.Zn^{2+} \rightarrow$ (H$_2$O)$_2$.

\textsuperscript{15} The VT study of $1.Zn^{2+} \rightarrow$ EtOH is presented in Appendix 2 (7.2).
CHAPTER 4. FUNNEL COMPLEXES: STUDY OF EXCHANGE DYNAMICS

Figure 4-15. $^1$H NMR spectrum (600MHz, 253 K) of 1.Zn$^{2+}$ in DCM-d$_2$ with 53 equiv. of EtOH in the presence of: a) ~0.3 equiv. of water; b) 9 equiv. of water.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equiv. water</th>
<th>$k_{tot}$(s$^{-1}$) EtOH (253K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>0.0126 ± 0.0017</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.0258 ± 0.016</td>
</tr>
<tr>
<td>3</td>
<td>2.6</td>
<td>0.0753 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>0.465 ± 0.052</td>
</tr>
</tbody>
</table>

Figure 4-16. Rate constant ($k_{tot}$), characterizing the exchange of EtOH at 253 K, as a function of the number of equivalents of water in the DCM-d$_2$ solution. The errors for $k_{tot}$ are fitting errors. Error for number of equivalents of water was estimated to be 10 %.

The results of the EXSY experiments undertaken with acetonitrile as a guest are shown in Figure 4-17. Experiments were run at 298K, like in the case of DMF, as the guest signals were not broad at this temperature, even in the presence of a large excess of water. As in the case of DMF and EtOH a linear dependence of $k_{tot}$ is observed with water concentration.
**Table 4.1.** Rate constant ($k_{tot}$), characterizing the exchange of CH$_3$CN at 298 K, as a function of the number of equivalents of water in the DCM-d$_2$ solution. The errors for $k_{tot}$ are fitting errors. Error for number of equivalents of water was estimated to be 10%.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equiv. water</th>
<th>$k_{tot}$ (s$^{-1}$) (298K)</th>
<th>CH$_3$CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.0047 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>0.0065 ± 0.0013</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>0.0108 ± 0.0009</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-17. Rate constant ($k_{tot}$), characterizing the exchange of CH$_3$CN at 298 K, as a function of the number of equivalents of water in the DCM-d$_2$ solution. The errors for $k_{tot}$ are fitting errors. Error for number of equivalents of water was estimated to be 10%.

The value of $k_{tot}$ clearly depends on the nature of the guest buried inside the cavity (Figure 4-18). For a same number of equivalents of water, $k_{tot}$ of CH$_3$CN is significantly larger than for DMF at 298K. The $k_{tot}$ for ethanol is even larger, as at 253 K it is already one order of magnitude larger than the $k_{tot}$ for CH$_3$CN at 298K.

Figure 4-18. Dependence of $k_{tot}$ at 298 K with water concentration in the case of: DMF (blue triangles) and CH$_3$CN (yellow dots).

The linear dependence observed for the $k_{tot}$ of all three guest of 1.Zn$^{2+}$, as a function of the number of equivalents of water in solution, clearly suggests that the model described in the introduction to this chapter could be valid with water as exo-ligand, L. All three guest furthermore do not seem to be able to play the role of the exo-ligand necessary to allow guest to occur. Indeed, all experiments were undertaken with a large excess of guest and when there is no water present, guest exchange is not observed. A hypothesis that can be put forward at this stage is that EtOH, DMF and CH$_3$CN are sterically too hindered to be able to interact with the zinc via exo-coordination at the level of the imidazole moieties.

The addition of ethanol was monitored to see if it could allow guest exchange. After addition of 75 equivalents of ethanol to a solution of 1.Zn$^{2+} \rightarrow$ DMF with 1 equiv. of water no exchange...
was observed for DMF at this water concentration. Increasing water concentration to 29 equiv. lead to an increase of $k_{\text{tot}}$ for DMF ($k_{\text{tot,DMF}} \sim 0.004 \text{ s}^{-1}$). However, at same water concentration, it seems that the presence of EtOH in solution rather than increasing $k_{\text{tot}}$ for DMF it slightly decreases it (Figure 4-19left).

![Figure 4-19.](image)

The effect of the addition of EtOH was also evaluated in the case of a solution of $1.\text{Zn}^{2+\rightleftharpoons\text{EtOH}}$. After addition of 37 equiv. of EtOH with 11 equiv. of water present in solution we observed a significant decline of $k_{\text{tot}}$ (from 0.86 to 0.33 Figure 4-19 right).

The fact that the residence time ($1/k_{\text{tot}}$) increases with ethanol concentration for a given water concentration clearly suggests that ethanol cannot play the role of the $\text{exo}$-ligand. Concerning the observation that, for a given water concentration, increasing the ethanol concentration slows down the exchange, it can perhaps be put forward that ethanol solvates the water molecules that they are thus less available to be $\text{exo}$-complexed to zinc, slowing down the exchange as a penta-coordinated zinc is crucial in the mechanism of guest exchange. The effect seems to disappear when the concentration of water is lower (Figure 4-19 right).

Based on our results it seems that in the case of system $1.\text{Zn}^{2+}$ the expression given in Equation 4-5 can be simplified as follows (where $b$ is a constant):

$$k_{\text{tot}} = b \times [\text{H}_2\text{O}]$$

Equation 4-5.
4.3.2. **STERIC HINDRANCE**

In order to investigate if steric hindrance at the level of the zinc coordination could be a key parameter governing the *exo*-coordination of a ligand L at the level of the zinc atom, guest exchange with the calix[6]benzimidazole zinc, \(2.\text{Zn}^{2+}\), was investigated (structure of \(2.\text{Zn}^{2+} \rightarrow \text{EtOH}\) in Figure 4-20). The steric hindrance of the benzimidazole groups could make the *exo*-complexation of water more difficult slowing down guest exchange. This could help us to confirm our hypothesis on the existence of an intermediate penta-coordinated species in the exchange process.

![Structure of 2.Zn^{2+} \rightarrow EtOH.](image)

**Figure 4-20.** Structure of \(2.\text{Zn}^{2+} \rightarrow \text{EtOH}\).

The NMR spectra \(2.\text{Zn}^{2+}\) recorded in DCM-\(d_2\) in the presence of a large excess of acetonitrile (CH\(_3\)CN at 298K) or ethanol (EtOH at 253K) are shown in Figure 4-21.\(^{16}\) Under these experimental conditions all the cavities are filled.

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\(^{16}\) The entire \(^1\)H NMR characterization of the complexes is presented in the chapter Material and methods.
As in the case of $1\text{Zn}^{2+}$, each complex can exist as a pair of enantiomers which are in conformational equilibrium. It has however been reported that the helical inversion in system $2\text{Zn}^{2+}$ is characterized by a smaller kinetic constant than in $1\text{Zn}^{2+}$ and this is probably related to the steric hindrance near the metal center.$^{12}$

Guest exchange is slow on the NMR chemical shift timescale and 1D EXSY experiments were undertaken. As in the case of $1\text{Zn}^{2+}$ we monitored the exchange of both guests as a function of water concentration. The $k_{\text{tot}}$ determined for ethanol (at 253 K) and acetonitrile (at 298 and 253 K) as a function of the number of equivalents of water (relative to the host concentration) are reported in Figure 4-22 and Figure 4-23 respectively.
<table>
<thead>
<tr>
<th>Entry</th>
<th>equiv. H$_2$O</th>
<th>$k_{tot}$(s$^{-1}$) CH$_3$CN (298K)</th>
<th>$k_{tot}$(s$^{-1}$) CH$_3$CN (253K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4.3</td>
<td>0.025 ± 0.003</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5.1</td>
<td>0.041 ± 0.003</td>
<td>0.009 ± 0.001</td>
</tr>
<tr>
<td>4</td>
<td>7.2</td>
<td>0.061 ± 0.003</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>10.5</td>
<td>0.090 ± 0.007</td>
<td>0.030 ± 0.002</td>
</tr>
</tbody>
</table>

Figure 4-23. Rate constant ($k_{tot}$), characterizing the exchange of CH$_3$CN at 298 K (left, green) and 253 K (right, blue), as a function of the number of equivalents of water in the DCM-d$_2$ solution. The errors for $k_{tot}$ are fitting errors. Error for number of equivalents of water was estimated to be 10 %.
As with $1.\text{Zn}^{2+}$ a linear dependence of $k_{tot}$ with water concentration is observed and in the absence of any water guest exchange is negligible. The values of $k_{tot}$ for systems $1.\text{Zn}^{2+}$ and $2.\text{Zn}^{2+}$ are furthermore of the same order of magnitude for ethanol at 253 K and for acetonitrile at 298 K. The benzimidazole arms consequently do not seem to introduce a significant steric hindrance for the exo-coordination of water. When decreasing the temperature from 298K to 253K with the system $2.\text{Zn}^{2+} \rightarrow \text{CH}_3\text{CN}$, $k_{tot}$ decreases significantly.

For all the systems that we have look at so far, $1.\text{Zn}^{2+} \rightarrow \text{DMF}$, $1.\text{Zn}^{2+} \rightarrow \text{EtOH}$, $1.\text{Zn}^{2+} \rightarrow \text{CH}_3\text{CN}$, $2.\text{Zn}^{2+} \rightarrow \text{EtOH}$ and $2.\text{Zn}^{2+} \rightarrow \text{CH}_3\text{CN}$, the following common features have been observed:

- the residence time of the guest decreases with increasing equivalents of water, and therefore water concentration;
- under dry conditions, guest residence time is too high to be measured by EXSY experiments;
- the guest itself does not play the role of L.

This leads us to conclude that for systems $1.\text{Zn}^{2+}$ and $2.\text{Zn}^{2+}$, water can play the role of the exo-ligand and that the data fits with the theoretical model described in section 4.1 (where $\alpha$ is a constant).

$$\frac{1}{\tau_{in}} = k_{tot} = \alpha [H_2O]$$

It is interesting to point out that an X-Ray structure of a similar system (a calix[6]tris-ethylimidazole zinc) with acetonitrile inside the cavity has been observed with a $H_2O$ water molecule exo-coordinated to the Zn cation.

![Schematic representation of the penta-coordinated complex (left) and XRD structure taken from Sénéque PhD thesis (right)](image)

**Figure 4-24.** Schematic representation of the penta-coordinated complex (left) and XRD structure taken from Sénéque PhD thesis (right).
4.3.3. **PENTA-COORDINATED ZINC COMPLEX**

The studies described in the previous sections were undertaken with zinc complexes belonging to the N₃ generation of hosts, **1.Zn²⁺** and **2.Zn²⁺**. Results seem to corroborate the hypothesis proposed in the introduction for the guest exchange mechanism: an associative mechanism with an intermediate penta-coordinated zinc complex. Water was also identified as an *exo*-ligand which can enable guest exchange to occur.

In the calix[6]tren zinc complex, **6.Zn²⁺** (Figure 4-25) the metal center is tetra-coordinated in the absence of a guest due to the coordination of the apical nitrogen to the metallic center, and penta-coordinated in the presence of an *endo*-guest. A different guest exchange mechanism could therefore be expected. With **6.Zn²⁺**, the guest should be able to leave the cavity without the help of an *exo*-ligand; we will then expect a dissociative mechanism instead of an associative mechanism (Figure 4-25).

![Figure 4-25](image)

**Figure 4-25.** Schematic representation of a dissociative mechanism with **6.Zn²⁺**.

The NMR spectra of calix[6]tren zinc, **6.Zn²⁺** recorded in DCM-d₂ in the presence of an excess of acetonitrile (DMF, at 298K) or ethanol (EtOH, at 253K)¹⁷ are shown in Figure 4-26.¹⁸ Under these conditions all cavities will be filled with a guest and the zinc metal is coordinated to the 4 nitrogens of the tren cap and to the guest molecule. The exchange of the guest is slow on the NMR timescale. Analysis of the spectra show that the calixarene adopts a flattened alternate cone conformation with the three tert-butyl groups belonging to the anisole units directed towards the inside of the cavity. The geometry around the Zn cation is trigonal bipyramidal and the three amino arms wrapping the zinc cation adopt an asymmetric conformation with two

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¹⁷ The VT study of **6.Zn²⁺→EtOH** is presented in Appendix 2 (7.2).
¹⁸ The entire ¹H NMR characterization of the complexes is presented in the chapter Material and methods.
coordinated arms presenting a relative helical orientation opposite to each other. The $^1$H NMR signature of the host structure attested to a dissymmetric conformation as three signals are observed for the methoxy groups.$^{19}$

For the sake of comparison with the previous systems, 1D EXSY experiments were carried out at 298 K for DMF and at 253 K for EtOH. A clear broadening of the signals of buried EtOH upon addition of water is observed (cf. Figure 4-27a-b). This broadening is without any doubt signature of a change in the kinetics of the exchange process of the guest inside the cavity.

![Figure 4-26. $^1$H NMR spectra (600MHz) of 6.Zn$^{2+}$ in DCM-d$_2$ in the presence of: a) 33 equiv. of EtOH and ~0.3 equiv. of water at 253 K; b) 11 equiv. of DMF and ~0.2 equiv. of water at 298 K; s: solvent; i: impurities.](image)

Figure 4-27. $^1$H NMR spectra (600MHz, 253 K) of 6.Zn$^{2+}$ in DCM-$d_2$ in the presence of 33 equiv. of EtOH and: a) ~0.3 equiv. of water; b) 7.0 equiv. of water; s: solvent; i: impurities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equiv. water</th>
<th>$k_{tot}$(s$^{-1}$) (253K)</th>
<th>EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>0.013 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.028 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>0.073 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>1.807 ± 0.374</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>2.168 ± 0.629</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-28. Rate constant ($k_{tot}$), characterizing the exchange of EtOH at 253 K, as a function of the number of equivalents of water in the DCM-$d_2$ solution. The errors for $k_{tot}$ are fitting errors. Error for number of equivalents of water was estimated to be 10%.
Figure 4-29. Rate constant (k_{tot}), characterizing the exchange of DMF at 298 K, as a function of the number of equivalents of water in the DCM-d$_2$ solution. The errors for k_{tot} are fitting errors. Error for number of equivalents of water was estimated to be 10 %.

In order to simplify reading, the determined kinetic constants will also be called k_{tot}. The determined k_{tot} from 1D EXSY experiments for ethanol (at 253 K) and acetonitrile (at 298 K), as a function of the number of equivalents of water (relative to the host concentration), are reported in Figure 4-28 and Figure 4-30 respectively. The following observations can be made:

- contrary to what we had expected, guest exchange with 6.Zn$^{2+}$ is also influenced by the concentration of water in solution;
- the dependence of the kinetic constant seems to be linear with the number of equivalents of water, as observed with systems 1.Zn$^{2+}$ and 2.Zn$^{2+}$;
- the exchange rate depends significantly on the guest buried inside the cavity; the values obtained for EtOH and DMF are quite different;
- the kinetic constants are much higher in the case of 6.Zn$^{2+}$ compared to 1.Zn$^{2+}$ or 2.Zn$^{2+}$;
- exchange occurs even in the absence of water (see for example expansion Figure 4-29).

The fact that guest exchange for system 6.Zn$^{2+}$ is also affected by water concentration suggests, that the exchange could be favored by the coordination of water molecule to the zinc ensuring that the zinc it is at all times at least penta-coordinated. The coordination of the water molecule would take place through the cap and not in the apical position as for 1.Zn$^{2+}$ and 2.Zn$^{2+}$ (position occupied by a nitrogen in system 6.Zn$^{2+}$) (Figure 4-30).
Figure 4-30. Schematic representation of the water access in system 6.Zn$^{2+}$ with a molecule of DMF buried inside the cavity.
4.3.4. DISCUSSION AND INTERPRETATION

The results presented in the previous section are summarized in Table 4-1 where we present the slopes of the regression lines for $k_{tot}$ vs. the number of equivalents of water.

Table 4-1. Slopes of the regression lines obtained for the dependence of $k_{tot}$ with the equivalents of H$_2$O. Errors: maximum 10 %.

<table>
<thead>
<tr>
<th>System</th>
<th>Temp (K)</th>
<th>Slope $k_{tot}$ vs. #H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EtOH</td>
</tr>
<tr>
<td>1.Zn$^{2+}$</td>
<td>298</td>
<td>-</td>
</tr>
<tr>
<td>1.Zn$^{2+}$</td>
<td>253</td>
<td>0.05</td>
</tr>
<tr>
<td>2.Zn$^{2+}$</td>
<td>298</td>
<td>-</td>
</tr>
<tr>
<td>2.Zn$^{2+}$</td>
<td>253</td>
<td>0.03</td>
</tr>
<tr>
<td>6.Zn$^{2+}$</td>
<td>298</td>
<td>-</td>
</tr>
<tr>
<td>6.Zn$^{2+}$</td>
<td>253</td>
<td>0.35</td>
</tr>
</tbody>
</table>

It is noteworthy that for systems 1.Zn$^{2+}$ and 2.Zn$^{2+}$ the intercept of the regression line is essentially zero. The kinetic constants that we have determined from our 1D EXSY experiments all relate to processes which enable the guest to leave a cavity and its inverse can be considered as the residence time of the guest inside the cavity ($\tau_{in}$). For 1.Zn$^{2+}$ and 2.Zn$^{2+}$ in the absence of water the guest would have an infinite $\tau_{in}$. For system 6.Zn$^{2+}$ at the intercept we have a non-zero values (0.013 s$^{-1}$ for EtOH at 253 K and 0.019 s$^{-1}$ at 298 K for DMF).

Results with EtOH at 253 K (Figure 4-31left) and with DMF at 298 K (Figure 4-31right) show that the dependence of the kinetic constant ($k_{tot}$) with water concentration increases with the donor character of the calixarene complex: steeper slopes are observed for 6.Zn$^{2+}$ compared to 1.Zn$^{2+}$ or 2.Zn$^{2+}$. Zinc is a Lewis acid and its donor or acceptor character depends on the different ligands bound to it. It is more acidic when its coordination is higher and water coordination is favored by a poorer metallic center. Upon water coordination, the acidity of the zinc is lowered and the guest is more labile.
Our results are in agreement with those reported by Dr. Sénèque in his PhD thesis. Indeed he observed that the degree of acidity of the zinc metal center has an important influence on the exchange kinetics of the guest buried inside the cavity. The introduction of a 4th coordinating group such as phenol or phenate on the calix[6]tris-imidazole increased the kinetics of guest exchange, and thus its lability. The more acidic the zinc, the greater the effect of the coordination of water on its acidity. Our results also show that for each system the kinetics are highly dependent on the nature of the guest. The exchange of ethanol is faster in all cases (cf. Table 4-1). Comparison between EtOH and CH$_3$CN for 2.Zn$^{2+}$ is shown in Figure 4-32.

Water coordination to the metal, changes not only its acidity but also its geometry of coordination and the host-guest complex conformation. The change in conformation...
destabilizes the guest inside the calixarene cavity. It is possible that the consequence is more important for ethanol than for the other guests as the H-bond interaction between its hydroxyl groups and the oxygens of the small rim, as observed by XRD with systems \(1.\text{Zn}^{2+} \rightarrow \text{EtOH}\) and \(6.\text{Zn}^{2+} \rightarrow \text{EtOH}\) by Reinaud and Jabin,\(^2,19\) is essential for the stabilization of the host-guest complex. This H-bond interaction could be easily lost following a change in geometry.

In conclusion, water exo-coordination which plays a key role in the exchange process, seems to be governed by (i) the accessibility to the metal which depends on the geometry and the conformation of the calixarene and by (ii) the acidity of the metal which depends on its number of coordinating group and the nature of the guest inside the cavity. In order to complete our knowledge of the studied systems we determined by competition experiments the affinities of EtOH and CH\(_3\)CN relative to DMF for each system (Table 4-2).

**Table 4-2.** Affinities of EtOH and CH\(_3\)CN relative to DMF determined by \(^1\)H NMR in DCM-d\(_2\). Estimated error of ~20 \% due to integration of signals of the free and bound guest.

<table>
<thead>
<tr>
<th>System</th>
<th>Temp. (K)</th>
<th>(K_{\text{EtOH/DMF}})</th>
<th>(K_{\text{CH3CN/DMF}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.\text{Zn}^{2+}</td>
<td>253</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>2.\text{Zn}^{2+}</td>
<td>298</td>
<td>0.7</td>
<td>0.21</td>
</tr>
<tr>
<td>6.\text{Zn}^{2+}</td>
<td>298</td>
<td>1.6</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

We were however not able to rationalize these results in relation to guest exchange dynamics.
4.4. CONCLUSIONS

The guest exchange mechanism of calix[6]arene zinc complexes had to date not been elucidated. The hypothesis we put forward is that when the zinc cation is tri-coordinated to the calix[6]arene ligand an exo-coordination of a molecule is required to allow the endo-guest to exchange. Using 1D EXSY experiments we monitored the exchange of different guest from the cavity of the calix[6]tris-imidazole zinc complex 1.Zn$^{2+}$. We showed that: (i) the residence time of the guests decreases linearly with increasing equivalents of water; (ii) under dry conditions, guest residence time is too high to be measured by EXSY experiments; (iii) the guest itself does not accelerate the exchange process. This suggest that water plays the role of exo-ligand enabling the guest exchange.

In order to investigate if steric hindrance at the level of the zinc coordination could be a key parameter governing the eventual exo-coordination of a ligand L at the level of the zinc atom, experiments were undertaken with the calix[6]benzimidazole zinc, 2.Zn$^{2+}$. Results with this system are not significantly different than the ones obtained with 1.Zn$^{2+}$. The benzimidazole arms consequently do not seem to introduce a significant steric hindrance for the exo-coordination of water.

Experiments were also undertaken with a calix[6]tren zinc complex 6.Zn$^{2+}$ where the zinc cation is tetra-coordinated to the calix[6]tren. Despite the fact that we expected that exchange would not be influenced by the presence of water, we observed also with this system that guest exchange is highly dependent on water concentration in solution. Exchange with this system is however significantly faster than with the other two systems and furthermore also possible in the absence of water.

The fact that guest exchange with system 6.Zn$^{2+}$ is faster than with systems 1.Zn$^{2+}$ and 2.Zn$^{2+}$ can be explained by the higher acidity of the zinc, which makes the guest more labile. We also observed that the exchange of EtOH is always faster that of DMF and CH$_3$CN for each system. This could be explained by water coordination to the metal inducing a change in the geometry of the system, breaking the hydrogen bond that stabilizes the ethanol-host complex.
CHAPTER 5.
CONCLUSIONS
The study of synthetic molecular receptors is an important facet of supramolecular chemistry as these receptors can find applications in the selective extraction, transport or detection of different species. In this thesis we focused our attention on the study of different types of receptors bearing a cavity capable of accommodating small organic molecules. We looked at the binding properties of two families of calix[6]arenes and also at the complexation properties of some related compounds: a homooxacalix[3]arene and a resorcin[4]arene derivatives.

Our work focused on the following three themes:

(i) **the study of the binding properties of a fluorescent calixarene.**

We conducted NMR and emission binding studies with the fluorescent calix[6]tris-pyrenylureas 14a and 14b (Figure 5-1). We could show that both receptors are able to bind ammonium sulfate salts with a cavity-based selectivity. While 14a displays a strong affinity for small or linear ammoniums, 14b, which has a more open cavity, was able to recognize an ammonium derivate of the dopamine family in chloroform. We also showed that 14a is a highly selective chemosensor for the sulfate anion in DMSO and for phosphatidylcholine type lipids in chloroform.

![Figure 5-1. Structure of receptors 14a-b.](image)

Moreover, our results put in evidence that the strategy of combining a calix[6]arene skeleton to chromophores for the design of multitopic sensitive receptors is a valid and appropriate way to obtain selective chemosensors. Indeed, the complexation observed for these different guests leads to significant changes in the spectrophotometric properties of the host and it is furthermore possible to estimate binding constants. The results were in agreement with those obtained from the NMR studies of the systems.
(ii) the use of micelles to transfer the hydrophobic molecular receptors into water.

We studied in micellar media three receptors, which are only soluble in organic solvents. We showed that the calix[6]azacryptand receptor 6 can be incorporated into DPC micelles, either as a Zn$^{2+}$ complex in the 6-9 pH range or as a polyammonium at low pH. We were able to show that the zinc complex maintains its host-guest properties as observed in organic solvent and is able to complex small or long linear primary amines inside the calixarene cavity (Figure 5-2). The stability of the ternary complex $6.Zn^{2+}\rightleftarrows PrNH_2$ over a large pH range showed that the presence of the amine consolidates the complex even at very low pH where the free amine is fully protonated in water. This highlights that the environment provided by the calixarene cavity and the micelle protects the system from water, stabilizing PrNH$_2$ in its basic form. The results also showed that the hydrophobic effect plays a key role in driving guests from water into the calixarene cavity incorporated in DPC micelles.

![Figure 5-2. Schematic representation of the Host-Guest properties of the studied systems incorporated into DPC micelles.](image-url)

We also achieved the incorporation into DPC micelles of the homooxacalix[3]tris-acid 15 and of the resorcin[4]arene zinc complex $16.Zn^{2+}$. We demonstrated that 15 incorporated into DPC micelles can bind different ammoniums, albeit with low affinity (Figure 5-2). On the other hand, $16.Zn^{2+}$ showed interesting binding properties towards small carboxylates and small diketones (Figure 5-2). Our work shows that the micellar incorporation of hydrophobic receptors is a very elegant solution to overcome the solubility problem of organic receptors in water and that, under these conditions, the receptors maintain binding properties.
NMR studies were undertaken in order to try and elucidate the mechanism of guest exchange in system 1.Zn\(^{2+}\). The hypothesis put forward is that an *exo*-coordination of a molecule is required to allow the *endo*-guest to leave the cavity when the zinc cation is tri-coordinated to the calix[6]arene ligand. 1D EXSY experiments were performed in order to determine the residence time of ethanol, acetonitrile and DMF in the calixarene cavity as a function of water concentration. We showed that **the residence time of the guests decreases linearly with increasing equivalents of water. Without water in solution, guest residence time is too long to be evaluated through EXSY experiments. We also observed that the guest itself does not accelerate the exchange process. This suggest that water plays the role of *exo*-ligand enabling guest exchange** (Figure 5-3).

The study of a similar system with benzimidazole arms, system 2.Zn\(^{2+}\), showed that steric hindrance at the level of the metal does not hinder in a significant way the exchange process in the presence of water. Experiments were also undertaken with the calix[6]tren zinc complex 6.Zn\(^{2+}\), where the zinc cation is tetra-coordinated to the calix[6]tren. We expected that the exchange would not be influenced by the presence of water but, on the contrary, we observed that, with this system, guest exchange is also highly dependent on water concentration in solution. Exchange with this system is significantly faster than with the other two systems and also possible in the absence of water. The explanation put forward is that the acidity of the zinc governs in part the exchange rate. We also observed that the exchange of ethanol is always faster than the other studied guests with each system which could be explained by the fact that water coordination to the metal induces a change in the geometry of the system, breaking the hydrogen bonds that stabilize the ethanol-host complex.
In conclusion, the results reported in this thesis clearly show that rightly functionalized calix[6]arene can be remarkable selective and versatile receptors in order to work as efficient chemosensors. The development of molecular receptors combining the benefits of a sensing system with the “water-compatibility” achieved via micellar incorporation would be of particular interest for the detection, for instance, of biologically relevant molecules.
CHAPTER 6.
MATERIALS AND METHODS

In this section we only give the experimental conditions for the experiments reported in section 3.5 and in chapter 4. Receptor 15 was synthetized following a protocol described in the literature in collaboration with Sara Zahim of the group of Prof. Jabin. 16 and 16.Zn²⁺ were synthetized and provided by the group of Prof. Reinaud. 1.Zn²⁺ and 6.Zn²⁺ were synthetized and provided by the group of Prof. Jabin. 2.Zn²⁺ was synthetized and provided by the group of Prof. Reinaud.

**CHEMICALS**

- DPC (C₁₇H₃₈NO₄P), Avanti Polar Lipids >99%
- DPC-d₃₈ (C₁₇D₃₈NO₄P), Avanti Polar Lipids 99%
- D₂O (99.9 %), CD₂Cl₂ (99.8 %) and CDCl₃ (99.8 %), VWR BDH PROLABO
- CHCl₃, Alfa Aesar ≥ 99%
- Tert-Butylamine (C₄H₁₁N), Sigma-Aldrich 98%
- D-L sec-Butylamine (C₄H₁₁N), Sigma-Aldrich 99%
- Adamantylamine hydrochloride (C₁₀H₁₈NC), Sigma-Aldrich ≥ 99%
- Propylamine (C₃H₉N), Sigma-Aldrich ≥ 99%
- Sodium deuterioxide solution (NaOD, 40% wt), Sigma-Aldrich 99.5 %
- Acetylacetone (C₅H₈O₂), Sigma-Aldrich 99%
- Ethanol (C₂H₅OH) absolute, VWR BDH PROLABO 99.5%
- Acetonitrile (CH₃CN) Extra dry, Acros Organics 99.9 %
- Dimethylformamide (C₃H₇NO), Fisher Chemical ≥ 99.8 %
- Sodium acetate (C₂H₃NaO₂), Fluka ≥ 99 %
- Molecular sieve, Fluka
- Hepes (C₈H₁₈N₂O₄S) Free acid, Sigma-Aldrich ≥ 99.5 %

**GENERAL PROCEDURES FOR ¹H SPECTRA**

Chloroform (both deuterated and non-deuterated) was filtered, prior to use, over a short column of basic alumina to remove traces of HCl/DCl. ¹H NMR spectra were recorded at 400 MHz (9.4 T) or 600 MHz (14.1 T). For the 1D ¹H 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. 2D NMR spectra (COSY, HSQC and ROESY) were recorded to complete signal assignments.

**Micellar solutions preparation**

All solutions for NMR studies were prepared following a similar protocol: the host (a few 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 with 15**

$^{15}\text{NH}_3^+$: $^1$H NMR (DPC 20 mM in D$_2$O, 298K, 600 MHz): $\delta$ (ppm) 1.15 (s, 27H, tBu), 4.43 (bs, J~12.6 Hz, 6H, CH$_2$O), 4.53 (d, J~12.6 Hz, 6H, ArCH$_2^{\text{eq}}$), 4.96 (d, 6H, ArCH$_2^{\text{ax}}$), 7.00 (s, 6H, ArH);

$^{15}\text{NH}_3^-$–$\text{BuNH}_3^+$: $^1$H NMR (DPC-d$_{38}$ 20 mM in D$_2$O, 298K, 600 MHz): $\delta$ (ppm) -0.37 (s, 9H, tBuNH$_3^{+}\text{CH}_3^{+}$, in), 1.18 (s, 27H, tBu), 4.27 (bs, 6H, CH$_2$O), 4.42 (d, 6H, ArCH$_2^{\text{eq}}$), 5.54 (bs, 6H, tBuNH$_2$ ArCH$_2^{\text{ax}}$), 7.30 (s, 6H, ArH);

$^{15}\text{NH}_3^-$–AdamNH$_3^+$: $^1$H NMR (DPC-d$_{38}$ 20 mM in D$_2$O, 298K, 600 MHz): $\delta$ (ppm) -0.07 (s, 6H, AdamNH$_3^{+}\alpha^{+}$, in), 0.95 (bs, 3H, AdamNH$_3^{+}\beta^{+}$, in), 1.13 (bs, 6H, AdamNH$_3^{+}\gamma^{+}$, in), 1.22-1.29 (m, 27H, tBu), 4.26 (bs, 6H, CH$_2$O), 4.43 (m, 6H, ArCH$_2^{\text{eq}}$), 5.55 (bs, 6H, tBuNH$_2$ ArCH$_2^{\text{ax}}$), 7.27 (s, 6H, ArH).

**Determination of the apparent affinity of tBuNH$_3^+$, sec-ButylNH$_3^+$ or AdamNH$_3^+$ toward complex 15 through $^1$H NMR titration in DPC-d$_{38}$ (20 mM in D$_2$O).** Different aliquots of a solution of guest (tBuNH$_3^+$, sec-ButylNH$_3^+$ or AdamNH$_3$Cl) were progressively added to 500 µL of a DPC-d$_{38}$ solution (20 mM in D$_2$O) containing 15. The integrations of the free guests and of the included guests were used to calculate the apparent affinity at a given pH defined as [R-NH$_3^+$ bound]/([1-R-NH$_3^+$ bound][R-NH$_3^+$ free][15-$^{15}$H$_3^+$]). (errors estimated ±20%).

**Determination of the relative affinity of sec-Butylamine compared to propylamine in CDCl$_3$ via $^1$H NMR competitive binding studies.** At room temperature, ~4 equivalents of propylamine (PrNH$_2$) and ~1 equivalent of sec-Butylamine (sec-ButylNH$_2$) were added in 500 µL of a CDCl$_3$ solution of 15. A $^1$H NMR spectrum showed the guest resonances of both complexes 15-$^{3}$H$_3^+$–PrNH$_3^+$ and 15-$^{3}$H$_3^+$–sec-ButylNH$_3^+$ 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 \([\text{PrNH}_3^+]/[\text{sec-ButylNH}_2\text{in}] \times [\text{secButylNH}_2\text{Free}]/[\text{PrNH}_2\text{Free}](\text{errors estimated} \pm 20\%).

**Determination of the apparent affinity of CH$_3$COO$^-$ toward complex 16.Zn$^{2+}$ through $^1$H NMR titration in DPC (20 mM in D$_2$O).** Different aliquots of a solution of sodium acetate were progressively added to 500 µL of a DPC solution (20 mM in D$_2$O) containing 16.Zn$^{2+}$. The integrations of the free guests and of the included guests were used to calculate the apparent affinity at a given pH defined as \([\text{CH}_3\text{COO}^\text{bound}]/(1-[\text{CH}_3\text{COO}^\text{bound}][\text{CH}_3\text{COO}^\text{free}][\text{16.Zn}^{2+}]).(\text{errors estimated} \pm 20\%).

**Determination of the relative affinity of EtOH compared to DMF in DCM-d$_2$ via $^1$H NMR competitive binding studies.** A few equivalents of EtOH and a few equivalents of DMF were added in 600 µL of a DCM-d$_2$ solution of 1.Zn$^{2+}$. A $^1$H NMR spectrum showed the guest resonances of both complexes 1.Zn$^{2+}$EtOH and 1.Zn$^{2+}$DMF and signals corresponding to the free guests. The integrations of the signals corresponding to free guests and the included guests were used to calculate relative affinity defined as \([\text{DMF}_\text{in}]/[\text{EtOH}_\text{in}] \times [\text{EtOH}_\text{Free}]/[\text{DMF}_\text{Free}]\) (errors estimated \pm 20\%). We thus determine $K_{\text{DMF/EtOH}}$. The same procedure was undertaken for CH$_3$CN. The relative affinities toward the other complex 2.Zn$^{2+}$ and 6.Zn$^{2+}$ were also determined following the same procedure described above.

**General DOSY experimental parameters:** 15 values for the magnitude of the gradient pulses (ranging from 2 G/cm to 50 G/cm), diffusion delay 50 ms, time-length of the gradient 5 ms, acquisition time 2 s, relaxation delay 7 s, 64 transients.

**General procedure for 1D EXSY experiments**

Prior to the determination of the kinetic constants in the exchange, it was necessary to determine the relaxation times. The longitudinal relaxation times $T_1$ were determined using the Inversion-Recovery sequence: the magnetization is inverted through the application of a 180° pulse, then the magnetization vector initially aligned with the $z$-axis relaxes at a rate determined by $T_1$. The return to equilibrium was monitored by integrating spectra after a delay $\tau$.

1D EXSY experiments were recorded with iburp2 shaped pulses with selective excitation of the bound guest (150 Hz). Evolution with mixing time $\tau_m$ of the normalized integrated intensity
of the signal of bound guest and free guest. The integrated intensities at equilibrium $I_{in}^\circ$ and $I_{out}^\circ$ were measured in a 1D spectrum obtained with identical gain (generally 32 transients with acquisition time and relaxation delay tailored for each different experiment). The mixing time, was increased (generally 8 increments were used 20-160 ms, 100-800 ms or 400-3200 ms depending on each experiment) The rate constants $k_{tot}$ were determined as the initial slope of the signal intensity ratio $I_{out}^\circ/I_{in}^\circ$ measured as a function of mixing time (cf. Figure-4-14 for an example).
**CHAPTER 6. MATERIALS AND METHODS**

**1H NMR CHARACTERIZATION OF VARIOUS COMPLEXES WITH 1.Zn²⁺, 2.Zn²⁺ AND 6.Zn²⁺**

1H NMR (253K, 600 MHz, CD₂Cl₂): \( \delta = -1.46 \) (t, 3H, EtOHCH₃ bound); 0.71 (s,27H, tBu); 1.39 (s, 27H, tBu); 1.18 (t, 3H, EtOHCH₃ free); 1.35 (s, 1H, EtOHCH² free); \( \delta = 1.90-2.11 \) (m, 2H, EtOHCH² bound); 2.83 (s, 9H, OCH₃); 3.33 (d, \( J = 15.6 \) Hz, 3H, Ar-\( \alpha \)CH₉); 3.46 (d, \( J = 15 \) Hz, 3H, Ar-\( \alpha \)CH₉); 3.57 (d, \( J = 15.6 \) Hz, 3H, Ar-\( \alpha \)CH₉); 3.65 (m, 2H, EtOHCH² free); 3.99 (s, 9H, NCH₃); 4.39 (d, \( J = 15 \) Hz, 3H, Ar-\( \alpha \)CH₉); 4.64 (dd, 1H, EtOHCH² free); 5.46 (s, 6H, Bz-\( \alpha \)CH₂); 6.26 (s, 3H, ArH₁); 6.53 (d, 3H, ArH₁); 6.86 (dd, \( J = 8.4 \) Hz, 3H, BzH); 7.28 (d, \( J = 2.4 \) Hz, 3H, ArH₂); 7.49 (d, \( J = 8.4 \) Hz, 3H, BzH); 7.86 (d, \( J = 2.4 \) Hz, 3H, ArH₂); 7.69 (t, \( J = 8.4 \) Hz, 3H, BzH); 7.91 (t, \( J = 8.4 \) Hz, 3H, BzH).

![Figure 6-1](image-url) *Figure 6-1.* ¹H NMR spectrum (600MHz, 253 K) of 2.Zn²⁺ in DCM-d₂ in the presence of ~ 12 equiv. of EtOH.
\[ \text{H NMR (298K, 600 MHz, CD}_2\text{Cl}_2): \delta = -0.66 \text{ (s, 3H, CH}_3\text{CN bound); 0.73 \text{ (s, 27H, } t\text{Bu)}; 1.40 \text{ (s, 27H, } t\text{Bu)}; 1.96 \text{ (s, 3H, CH}_3\text{CN free); 2.90 \text{ (s, 9H, } OCH}_3); 3.27 \text{ (d, } J = 15.6 \text{ Hz, 3H, Ar-} \alpha\text{CH}_\text{eq}); 3.46 \text{ (d, } J = 15 \text{ Hz, 3H, Ar-} \alpha\text{CH}_\text{eq}); 3.61 \text{ (d, } J = 15.6 \text{ Hz, 3H, Ar-} \alpha\text{CH}_\text{ax}); 3.97 \text{ (s, 9H, } NCH}_3); 4.48 \text{ (d, } J = 15 \text{ Hz, 3H, Ar-} \alpha\text{CH}_\text{ax}); 5.42 \text{ (s, 6H, Bz-} \alpha\text{CH}_2); 6.27 \text{ (s, 3H, ArH)}; 6.58 \text{ (s, 3H, ArH)}; 6.94 \text{ (d, } J = 8.4 \text{ Hz, 3H, BzH}); 7.27 \text{ (d, } J = 2.4 \text{ Hz, 3H, ArH}); 7.47 \text{ (t, } J = 8.4 \text{ Hz, 3H, BzH}); 7.38 \text{ (d, } J = 2.4 \text{ Hz, 3H, ArH}); 7.67 \text{ (t, } J = 8.4 \text{ Hz, 3H, BzH}); 7.86 \text{ (d, } J = 8.4 \text{ Hz, 3H, BzH}). \]

**Figure 6-2.** $^1$H NMR spectrum (600 MHz, 298 K) of \(2\cdot\text{Zn}^{2+}\) in DCM-$d_2$ in the presence of ~5 equiv. of CH$_3$CN.
$^1$H NMR (253K, 600 MHz, CD$_2$Cl$_2$): $\delta = -0.71$ (s, 3H, CH$_3$CN bound); 0.71 (s, 27H, tBu$_1$); 1.39 (s, 27H, tBu$_2$); 2.00 (s, 3H, CH$_3$CN free); 2.86 (s, 9H, OCH$_3$); 3.39 (d, $J = 15.6$ Hz, 3H, Ar-$\alpha$CH$_{eq}$); 3.46 (d, $J = 15$ Hz, 3H, Ar-$\alpha$CH$_{ax}$); 3.62 (d, $J = 15.6$ Hz, 3H, Ar-$\alpha$CH$_{ax}$); 3.99 (s, 9H, NCH$_3$); 4.46 (d, $J = 15$ Hz, 3H, Ar-$\alpha$CH$_{ax}$); 5.40 (s, 6H, Bz-$\alpha$CH$_2$); 6.24 (s, 3H, ArH$_1$); 6.56 (s, 3H, ArH$_1$); 6.96 (d, $J = 8.4$ Hz, 3H, BzH); 7.27 (d, $J = 2.4$ Hz, 3H, ArH$_2$); 7.47 (t, $J = 8.4$ Hz, 3H, BzH); 7.37 (d, $J = 2.4$ Hz, 3H, ArH$_2$); 7.67 (t, $J = 8.4$ Hz, 3H, BzH); 7.89 (d, $J = 8.4$ Hz, 3H, BzH).

Figure 6-3. $^1$H NMR spectrum (600MHz, 253 K) of 2.Zn$^{2+}$ in DCM-d$_2$ in the presence of ~ 5 equiv. of CH$_3$CN.
CHAPTER 6. MATERIALS AND METHODS

$^{1}$H NMR (253K, 600 MHz, CD$_2$Cl$_2$): $\delta = -0.72$ (s, 3H, CH$_3$CN bound); 0.73 (s, 27H, tBu); 1.39 (s, 27H, tBu); $\delta = 1.96$ (s, 3H, CH$_3$CN free); 3.41 (d, $J = 15$ Hz, 6H, Ar-$\alpha$CH$_{eq}$); 3.61 (s, 9H, OCH$_3$); 3.76 (s, 9H, NCH$_3$); 4.14 (d, $J = 15$ Hz, 6H, Ar-$\alpha$CH$_{ax}$); 5.27 (s, 6H, Im-$\alpha$CH$_2$); 6.43 (s, 6H, ArH$_1$); 7.14 (d, $J = 1.5$ Hz, 3H, ImH); 7.31 (s, 6H, ArH$_2$); 7.39 (d, $J = 1.5$ Hz, 3H, ImH).

Figure 6-4. $^{1}$H NMR spectrum (600MHz, 298 K) of $^{1}$Zn$^{2+}$ in DCM-d$_2$ in the presence of ~42 equiv. of CH$_3$CN.
(1.Zn$^{2+}$)($\text{DMF}$)(TfO)$_2$

$^1$H NMR (298K, 600 MHz, CD$_2$Cl$_2$); $\delta = 0.02$ (s, 3H, DMF bound); 0.25 (s, 3H, DMF bound); 0.83 (s, 27H, tBu$_1$); 1.39 (s, 27H, tBu$_2$); 2.81 (s, 3H, DMF free); 2.89 (s, 3H, DMF free); 3.39 (d, $J = 15$ Hz, 6H, Ar-$\alpha$CH$_{eq}$); 3.55 (s, 9H, OCH$_3$); 3.84 (s, 9H, NCH$_3$); 4.21 (d, $J = 15$ Hz, 6H, Ar-$\alpha$CH$_{ax}$); 5.37 (s, 6H, Im-$\alpha$CH$_2$); 6.51 (s, 1H, DMF$^{COH}$ bound); 6.66 (s, 6H, ArH$_1$); 7.19 (d, $J = 1.5$ Hz, 3H, ImH); 7.32 (s, 6H, ArH$_2$); 7.43 (d, $J = 1.5$ Hz, 3H, ImH); 7.95 (s, 1H, DMF$^{COH}$ free).

Figure 6-5. $^1$H NMR spectrum (600MHz, 298 K) of 1.Zn$^{2+}$ in DCM-d$_2$ in the presence of 32 equiv. of DMF.
\(1^1\)H NMR (253K, 400 MHz, CD\(_2\)Cl\(_2\)): \(\delta = -1.53\) (t, 3H, EtOH\(^{\text{CH}}\) bound); 0.71 (s, 27H, \(t\text{Bu}_1\)); 1.38 (s, 27H, \(t\text{Bu}_2\)); 1.18 (t, 3H, EtOH\(^{\text{CH}}\) free); 1.55 (s, 1H, EtOH\(^{\text{OH}}\) free); 1.46-1.76 (m, 2H, EtOH\(^{\text{CH}}_2\) bound); 3.59 (s, 9H, OCH\(_3\)); 3.23-3.79 (bs, 6H, Ar-\(\alpha\text{CH}\_eq\)); 3.65 (m, 2H, EtOH\(^{\text{CH}}_2\) free); 3.83 (s, 9H, NCH\(_3\)); 4.54 (bs, 6H, Ar-\(\alpha\text{CH}\_ax\)); 4.08 (t, 1H, EtOH\(^{\text{OH}}\) bound); 5.12 (bs, 3H, Im-\(\alpha\text{CH}\_2\)); 5.57 (bs, 3H, Im-\(\alpha\text{CH}\_2\)); 6.08-6.72 (s, 6H, ArH\(_1\)); 7.14 (s, 3H, ImH); 7.20-7.42 (s, 6H, ArH\(_2\)); 7.43 (s, 3H, ImH).

**Figure 6-6.** \(^1\)H NMR spectrum (600MHz, 253 K) of 1.Zn\(^{2+}\) in DCM-d\(_2\) in the presence of 53 equiv. of EtOH.
CHAPTER 6. MATERIALS AND METHODS

(6.Zn$^{2+}$→DMF)(ClO$_4$)$_2$

$^1$H NMR (298K, 600 MHz, CD$_2$Cl$_2$): $\delta$ = -0.28 (s, 3H, DMF bound); 0.04 (s, 3H, DMF bound); 0.78 (s, 9H, tBu$_1$); 0.82 (s, 9H, tBu$_1$); 0.83 (s, 9H, tBu$_1$); 1.38 (bs, 18H, tBu$_2$); 1.39 (bs, 9H, tBu$_2$); 2.80 (s, 3H, DMF free); 2.89 (s, 3H, DMF free); 2.92-3.93 (m, 24H, NHCH$_2$CH$_2$N-NHCH$_2$CH$_2$N-OCH$_2$CH$_2$N-Ar-αCH$_{eq}$); 3.81 (s, 3H, OCH$_3$); 3.88 (s, 3H, OCH$_3$); 3.95 (s, 3H, OCH$_3$); 4.06-4.49 (m, 12H, OCH$_2$CH$_2$NH-Ar-αCH$_{ax}$); 6.54 (br. s, 1H, ArH$_1$); 6.55 (br. s, 1H, ArH$_1$); 6.68 (d, Hz, 1H, ArH$_1$); 6.72 (d, Hz, 1H, ArH$_1$); 6.76 (d, 2H, ArH$_1$); 6.99 (s, 1H, DMF$^{COH}$ bound); 7.29 (b s 2H, ArH$_2$); 7.31 (d, J ~ 2 Hz, 1H, ArH$_2$); 7.36 (d, J ~ 2 Hz, 1H, ArH$_2$); 7.34 (m, 2H, ArH$_2$); 7.46 (d, 1H, ArH$_2$); 7.94 (s, 1H, DMF$^{COH}$ free).

Figure 6-7. $^1$H NMR spectrum (600MHz, 298 K) of 6.Zn$^{2+}$ in DCM-d$_2$ in the presence of 12 equiv. of DMF.
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\[ \text{Zn}^{2+} \text{EtOH} \text{(ClO}_4\text{)}_2 \]

\( ^1H \) NMR (253K, 600 MHz, CD\textsubscript{2}Cl\textsubscript{2}): \( \delta = 0.66 \) (s, 9 H, tBu\textsubscript{1}); 0.74 (s, 9 H, tBu\textsubscript{1}); 0.76 (s, 9 H, tBu\textsubscript{1}); 1.18 (t, 3H, EtOH\textsuperscript{CH3} free); 1.38 (bs, 18 H, tBu\textsubscript{2}); 1.39 (bs, 9 H, tBu\textsubscript{2}); 1.47 (t, 1H, EtOH\textsuperscript{OH} free); 1.53-1.80 (m, 2H, EtOH\textsuperscript{CH2} bound); 2.92-3.58 (m, 24 H, NHCH\textsubscript{2}CH\textsubscript{2}N-NHCH\textsubscript{2}CH\textsubscript{2}N-OCH\textsubscript{2}CH\textsubscript{2}N-Ar-\alpha\text{CH}_{eq}); 3.65 (m, 2H, EtOH\textsuperscript{CH2} free); 3.80 (s, 3H, OCH\textsubscript{3}); 3.87 (s, 3 H, OCH\textsubscript{3}); 3.94 (s, 3 H, OCH\textsubscript{3}); 3.99-4.43 (m, 12 H, OCH\textsubscript{2}CH\textsubscript{2}NH-Ar-\alpha\text{CH}_{ax}); 4.47 (t, 1H, EtOH\textsuperscript{OH} bound); 6.34 (s, 1H, ArH\textsubscript{1}); 6.40 (m, 2 H, ArH\textsubscript{1}); 6.52 (s,1H, ArH\textsubscript{1}); 6.57 (s, 1 H, ArH\textsubscript{1}); 6.65 (s, 1H,ArH\textsubscript{1}); 7.27 (s, 1H, ArH\textsubscript{2}); 7.30 (s, 1H,ArH\textsubscript{2}); 7.33 (bs , 2H, ArH\textsubscript{2}); 7.43 (bs, 2H, ArH\textsubscript{2}).

Figure 6-8. \(^1H\) NMR spectrum (600MHz, 253K) of \( \text{Zn}^{2+} \) in DCM-d\textsubscript{2} in the presence of 33 equiv. of EtOH.
CHAPTER 7.
APPENDICES
7.1. 1:1 BINDING MODEL

The 1:1 interaction between a host (H) and a guest (G) forming the complex HG can be described by the following equilibrium, which is characterized by a binding constant \( \Ka \):

\[
H + G \rightleftharpoons HG
\]

\[
\Ka = \frac{[HG]}{[H] \cdot [G]}
\]

Concentrations in host and guest can be written as a function of the initial concentrations and of the concentration of complex.

\[
[H] = [H]_0 - [HG]
\]

\[
[G] = [G]_0 - [HG]
\]

By replacing these expressions in the equation describing the binding affinity we can write:

\[
\Ka = \frac{[HG]}{([H]_0 - [HG]) \cdot ([G]_0 - [HG])}
\]

The concentration in complex can be isolated\(^1\):

\[
[HG] = \frac{[H]_0 \cdot \left(1 + \frac{[G]_0}{[H]_0} + \frac{1}{\Ka \cdot [H]_0}\right)}{2} - \frac{[H]_0 \cdot \sqrt{(1 + \frac{[G]_0}{[H]_0} + \frac{1}{\Ka \cdot [H]_0})^2 - 4 \cdot \frac{[G]_0}{[H]_0}}}{2}
\]

In order to determine \( \Ka \), a property of the systems which changes during the titration has to be monitored. Binding affinities have been determined using Nuclear Magnetic Resonance, UV-vis absorption spectroscopy or Emission spectroscopy.

---

TITRATIONS BY UV-VIS ABSORPTION SPECTROSCOPY

Beer-Lambert law expresses that for a given wavelength ($\lambda$), the absorption of a species is function of its concentration:

$$A = \varepsilon \ast l \ast C$$

Where $A$ is the absorbance of the solution, $l$ the optical pathway in cm, $\varepsilon$ the molar extinction coefficient in $\text{M}^{-1}\text{cm}^{-1}$ and $C$ the concentration of a species in $\text{M}^{-1}$. In the case of a solution containing $n$ species, the total absorption of the solution can be express as the sum of the absorption of each species:

$$A = A_1 + A_2 + \cdots + A_n$$

In the case of a titration, we can write when the guest does not absorb:

$$A = [H] \ast \varepsilon_H \ast l + [HG] \ast \varepsilon_{RI} \ast l$$

where $\varepsilon_H$ and $\varepsilon_{HG}$ are respectively the molar extinction coefficients of the host and the complex formed.

$$A = ([H]_0 - [HG]) \ast \varepsilon_H \ast l + [HG] \ast \varepsilon_{HG} \ast l$$

$$A = [H]_0 \ast \varepsilon_H \ast l + (\varepsilon_{HG} - \varepsilon_H) \ast [HG] \ast l$$

Writing that $A_0 = [H]_0 \ast \varepsilon_H \ast l$ et $\Delta \varepsilon = \varepsilon_{HG} - \varepsilon_H$, the total absorbance can be rewritten:

$$A = A_0 + \Delta \varepsilon \ast [HG] \ast l$$

Finally, by replacing the expression of the complex concentration by the one obtained before, we obtain:
The values of $K_a$ and $\Delta \varepsilon$ can be determined by parametric adjustment to the experimental by the least-squares method.

**TITRATIONS BY EMISSION SPECTROSCOPY**

Fluorescence intensity of a species $C$ is given by the equation:

$$F = k_c \times [C]$$

Where $k_c$ corresponds to the fluorescence constant, which includes the yield detection of the fluorimeter, the molar extinction coefficient, the fluorescence quantic yield and the intensity of the incident beam.

In the case of a solution containing $n$ fluorescent species, the total fluorescence of the solution can be expresses as the sum of the fluorescence of each species:

$$F = F_1 + F_2 + \cdots + F_n$$

In our case, during a titration, if we monitor the formation of a complex $HG$ from a host $H$ and a guest $G$ (which does not fluoresce), we can write:

$$F = k_H \times [H] + k_{HG} \times [HG]$$

We can write:

$$F = (k_{HG} - k_H) \times [HG] + k_H \times [H]_0$$

By writing that $F_0 = k^0_H[H]_0$, $\Delta k = (k_{HG} - k_H)$ and after assuming that the modification of the fluorescence intensity is only due to the complexation of $G$ by host $H$ ($k_H = k^0_H$), we obtain:
Finally, replacing the expression of the complex concentration obtained before, we obtain

\[ F = k_0 + \Delta k \times [HG] \]

The values of \( K_a \) and \( \Delta k \) can be determined by parametric adjustment to the experimental by the least-squares method.

**TITRATIONS BY NMR SPECTROSCOPY**

During a titration followed by NMR spectroscopy, the complexation equilibrium can be in slow or fast exchange compared to the chemical shift timescale.

In the case of slow exchange, three different sets of signals are observed during the titration (the signals of the free host and guest, and the signals of the formed complex). By simple integration at equilibrium of the different signals, the binding constant can be determined.

In the case of fast exchange, the observed chemical shift is a weighted average of the chemical shift of the free species and the chemical shift of the complex ones. Thus, the binding affinity constant can be determined by parametric adjustment to the experimental given by these equations:

\[ \delta = x_H \delta_H + x_{HG} \delta_{HG} \]

Where \( \delta \) correspond to the observed chemical shift, \( \delta_H \) to the chemical shift of free host and \( \delta_{HG} \) to the chemical shift of the formed complex. \( x_{HG} \) corresponds to the molar fraction of the complex. Finally, binding constant can be determined through this equation as the molar fraction depends on \( K_a \).
7.2. ADDITIONAL SPECTRA

Figure 7-1. DOSY experiment (600 MHz, 298 K, DPC 20 mM) with 15 incorporated into DPC micelles.

Figure 7-2. $^1$H NMR spectra (600MHz, DCM-d$_2$) of 1.Zn$^{2+}$ in the presence of ~120 equiv. of ethanol and ~8.5 equiv. of water at: a) 298 K; b) 283 K; c) 273 K; d) 263 K a) 253 K.
Figure 7.3. $^1$H NMR spectra (600MHz, DCM-d$_2$) of 6.Zn$^{2+}$ in the presence of ~74 equiv. of EtOH and ~7 equiv. of water at: a) 263 K; b) 253 K; c) 248 K.
7.3. COMMUNICATIONS, POSTERS AND PUBLICATIONS

Oral communications


COST Action CM1005 Meeting "Supramolecular gels and micelles as potential environments for molecular recognition and catalysis in water", 10-11 May 2013, Rome, Italy.

Posters:

11th International Symposium on Macrocyclic and Supramolecular Chemistry (ISMSC 2016), 10-14 July 2016, Seoul, South Korea.

13th International Conference on Calixarenes (Calix 2015), 05-09 July 2015, Giardini Naxos, Italy.


The poster presented won the "Best Poster Prize".

The poster presented won the "COST Poster Prize".

12th edition of the Young Belgian Magnetic Resonance Scientist Symposium (YBMRS 2013), 02-03 December 2013, Blanckenberge, Belgium.


Publications:


7.4. LIST OF RECEPTORS USED

1. Zn$^{2+}$

2. Zn$^{2+}$

6. Zn$^{2+}$

14a : R = fBu
14b : R = H

15

16 (R = C$_6$H$_{13}$)