PARTIALLY SUBSTITUTED CALIX[4]ARENEDERIVATIVES: SYNTHESIS, CHARACTERISATION
AND BINDING PROPERTIES

by

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ABSTRACT

This thesis concerns the synthesis, characterisation and binding properties of partially substituted calix[4]arene amino derivatives in non-aqueous media.

Thus, 5,11,17,23 - tetrakis - (1,1-dimethyl ethyl) - 25,27 - bis - [2 -(diethyl amino) ethoxy] calix[4]arene (L3) was structurally (1H NMR and X-ray crystallography) characterised and its interaction with metal cations and the proton has been investigated by several techniques.

Information about the active sites of interaction of this ligand with ionic species was obtained from 1H NMR measurements. Conductance measurements were used to establish the composition of the metal-ion complexes in protic and dipolar aprotic solvents. Potentiometric measurements using two electrodes systems (mercury and glass electrodes) were carried out to determine quantitatively the strength of ligand-ion interactions in acetonitrile at 298.15 K.

2D NMR techniques were used to investigate the predominant processes (complexation or protonation) taking place in solution.

The medium effect on the interaction of L3 with metal cations and the proton was assessed using a dipolar aprotic (acetonitrile) and a protic (methanol) solvent. In acetonitrile, L3 interacts with mercury (II), lead (II), cadmium (II), and magnesium (II) forming complexes of 1:1 stoichiometry while two protons are taking up per unit of ligand. No interaction was observed between L3 and alkali-metal cations in this solvent. In CD3OD the 1H NMR data show that the interaction is limited to mercury (II), lead (II) and magnesium (II) and protonation of L3 also occurs in methanol. In both solvents, the stoichiometry of the interaction of aluminium (III) and L3 was not clearly defined.

It is concluded that both processes, complexation and protonation are taking place during the interaction of partially substituted calix[4]arene amino derivatives with metal cations in acetonitrile.
THIS THESIS IS DEDICATED TO

Jesús, for allowing me to progress further, to serve better.

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1. INTRODUCTION

1.1. Supramolecular Chemistry.

Supramolecular Chemistry is one of the fastest growing areas of chemical research mainly because of its wider applications in chemical, biological and industrial processes. In addition, this field is highly interdisciplinary and involves from chemists to mathematicians, passing through biologists and biochemists. This subject goes further than the simple definition of a molecule and the exclusive chemistry of carbon because it includes almost every element of the Periodic Table.

Supramolecular Chemistry has been defined in the literature in several ways. One of the initial definitions is that proposed by Jean-Marie Lehn, the 1987 Nobel Prize winner for his work in this field. He defined Supramolecular Chemistry as the “chemistry of molecular assemblies and of the intermolecular bond”. Due to the fact that most of these supramolecular assemblies involve interactions between hosts and guests, the definition of host - guest chemistry is also being widely used.

Supramolecular Chemistry started to develop faster in the late 60s, although its roots are much older. Important advances in this area are those by Donald Cram for his work on the cyclophanes, Charles Pedersen with the discovery of the crown ethers, Jean-Marie Lehn with the synthesis of the first cryptand and Gokel and Okahara for the development of lariat ethers as a subclass of hosts. Representative examples of these hosts are shown in Figure 1.1.

Supramolecular host-guest compounds can be classified taking into account either the type of interaction between the host and the guest (ion-dipole, hydrogen bonds, van der Waals forces, etc) or the position of the guest with respect to the host (capsular, nesting, sandwich, wrapping, etc). (Figure 1.2). The selective behaviour of a given host for a particular ionic or neutral species can be quantitatively assessed from the ratio between the thermodynamic stability constants of a given ligand and a guest in a given solvent and at a specified temperature relative to another guest in the same solvent and temperature.
The design of a synthetic host with high selectivity for a given cation is a very challenging task because of the various factors to be considered. Some of them are listed as follows,

- The size match between cation and host cavity or hole,
- The electrostatic charge,
- The solvent (polarity, hydrogen bonding, coordination ability),
- The degree of host pre-organisation,
- The enthalpic and entropic contributions to the stability of complex formation.
Chapter 1 - Introduction

- The solvation of the reactants and the products,
- The nature of the counter-anion and its interactions with the solvent and the cation,
- The kinetics of the binding process, and
- The chelate ring size.

Then, following the chronological developments in the field of Supramolecular Chemistry, a brief review of the most important groups of supramolecular assemblies will be given according to the following sequence: i) crown ethers, ii) lariat ethers and podands, iii) cryptands and iv) calixarenes. A description of each class of compounds will be given with particular emphasis on calixarene derivatives, which are the main subject of this thesis.

1.1.1. Crown ethers

Crown ethers were synthesised by Pedersen in 1967.\(^3\) Dibenzo-18-crown-6 was the first of the crown compounds synthesised according to the method shown in Scheme 1.1. They have the basic structural unit \(-(\text{CH}_2\text{CH}_2\text{O})-\). Several ligands derived from this unit have been prepared with ring sizes varying between 12 and 60 atoms including four to thirty oxygen atoms.

![Scheme 1.1: Synthesis of crown ethers](image)

A representative crown ether and its potassium complex are shown in Figure 1.3. The trivial names for crown ethers consist of the number and the kind of substituent groups,
the number of atoms in the polyether ring, the class name crown and the number of oxygen atoms in the ring.

Figure 1.3  18-crown-6 and its potassium complex

Replacement of oxygen donor atoms by sulphur, nitrogen, and other heteroatoms has been explored extensively and led to the synthesis of compounds such as azacrown, thiacrown ethers and other derivatives. (Figure 1.4) Among the crown ethers, 18-crown-6 is the most widely investigated ligand. Different substituents have been introduced in their structures. Crown ethers are characterised by the presence of holes rather than cavities and they are able to interact with metal cations through ion-dipole interactions and with ammonium ions and amino acids through hydrogen bond formation.

Figure 1.4  Representative examples of azacrown and thiacrown ethers.

1.1.2. Podands and Lariat Ethers.

Podands are acyclic hosts, analogues of the crown ethers with pendant binding sites. Representative examples are shown in Figure 1.5. They show less affinity than crown ethers for metal cations because of the unfavourable entropic effects derived from their less rigid conformations. On the other hand, the extra flexibility of these ligands gives
them the possibility of producing more complex arrangements. The term podand was first used by Vogtle and Weber in 1979 and they formulated the important end group concept. This is related to the great flexibility of these ligands that, in some cases, allows them to adopt non-binding open conformations. Then the introduction of a rigid functionality at the ends of the chain of the podands will improve the binding properties because the extra degree of ligand's organisation.

![Figure 1.5 Representative examples of podands and lariat ethers.](image)

Other macrocyclic ligands with a similar structure to that of the crown ethers are the lariat ethers. The synthetic steps for the preparation of these ligands are shown in Scheme 1.2. The main feature of these ligands is the presence of appendages especially used to give them three dimensional characteristics.

![Scheme 1.2 Synthesis of lariat ethers](image)
A comparison of the affinity (reflected in the stability constant expressed as log $K_s$) of crown ethers, azacrown ethers, podands and lariat ethers for potassium in methanol at 298.15 K is shown in Figure 1.6.

![Diagram of molecular structures with log $K_s$ values]

Figure 1.6  Comparison of the affinity of crown ethers, azacrown ethers, podands and lariat ethers for the potassium cation in methanol at 298.15 K.

1.1.3. Cryptands

Based on Pedersen’s work, Lehn\(^4\) designed three dimensional macrocycles known as cryptands. Scheme 1.3 shows the synthetic procedure used for the preparation of cryptand 222.

![Scheme 1.3: Synthesis of cryptand 222]

Depending on the orientation of the lone pair of electrons of the nitrogen atoms, the diazabicycloalkane molecules display different conformations in the solid state. These
are known as \textit{exo-exo}, \textit{exo-endo} and \textit{endo-endo}\textsuperscript{10} as shown in Figure 1.7. However, NMR studies suggest the same different conformations in solution\textsuperscript{11}.

![Figure 1.7 Solid state conformations of cryptands.\textsuperscript{12}]

Having given a brief description on the most widely known synthetic macrocycles, the main emphasis will be centred on calixarene derivatives since these are the ligands investigated in this thesis.

### 1.2. Calixarene derivatives

Calixarenes are a popular and a versatile class of macrocycles. Their historical development started in 1872 with the reaction between phenol and formaldehyde to give several polymeric tar-or cement-like materials which were hard to characterise due to the lack of suitable techniques. In 1902, Leo Baekeland, turned his attention to this reaction hoping to find a commercial use for the phenol formaldehyde resins. He carried out the reaction in the presence of very carefully controlled amounts of base being able to produce a homogeneous material which he called Bakelite.

In 1942, Alois Zinke was reviewing the bakelite process and decided to simplify the reaction by examining the condensation reaction involving \textit{p}-substituted phenols and formaldehyde. Zinke\textsuperscript{13} was able to isolate a crystalline product of empirical formula C\textsubscript{31}H\textsubscript{40}O\textsubscript{3}, from the \textit{p}-\textit{tert}-butylphenol - formaldehyde reaction. Because this substituted phenol can only react at the \textit{ortho} position and based on previous reports, he postulated a cyclic tetrameric structure. However, he was unable to support his theory because of the low purity of the compound obtained. In 1955, John Cornforth\textsuperscript{30} using a combination of preliminary X-ray crystallographic evidence and molecular weight determinations corroborated the tetrameric structure proposed by Zinke.
Calixarenes are formally members of the cyclophane family and it was not until 1972 that C. David Gutsche\(^1\)\(^{14}\) revived the chemistry of these cyclic oligophenol products in the hope of producing a range of cavity-containing substances suitable for the construction of enzyme mimics. They offer many interesting possibilities in Supramolecular Chemistry, particularly in ion complexation processes. There are several reasons for the current widespread interest in calixarenes. An important one is the remarkably simple way (single-step procedure) used for the synthesis of the parent compounds. These are prepared by the condensation reaction between para-substituted phenols and formaldehyde in basic conditions (Scheme 1.4)\(^1\)\(^5\),\(^16\) They can be obtained in large scale from inexpensive starting materials.

Parent calix[4]arenes in the ‘cone’ conformation are said to have a lower rim (where the hydroxyl groups are) and an upper rim (where the p-substituents are) (Figure 1.8).

![Scheme 1.4 Single step synthesis of calixarenes](image)

In 1978 Gutsche\(^1\)\(^{14}\) proposed naming these cyclic oligomers calixarenes. This nomenclature is derived from the Greek-Latin calix, which means ‘chalice’, due to the similarity between the conformation of the cyclic tetramer and a Greek vase. The last part of the name ‘arene’ is related to the presence of aromatic rings in the structure of these compounds. A bracketed number, indicating the number of benzene units, is inserted between ‘calix’ and ‘arene’. The position and the type of substituents are indicated by prefixes positioned before the word ‘calix’. A representative example of a calixarene structure named in both, the abbreviated and the systematic way respectively is presented in Figure 1.9.

### 1.2.1. Physical properties of calixarenes.

As far as the melting point of these substances is concerned, higher values can be observed specially for the compounds with even number of units, due to the extensive
hydrogen bond formation between the hydroxyl groups. So, \( p-\text{tert-butyl calix}[4]\)arene and \( p-\text{tert-butyl calix}[5]\)arene, have melting points of 342-344 °C\(^{17}\) and 310-312 °C\(^{18,19}\) respectively. By derivatisation of the phenolic hydrogens, the melting points fall dramatically. According to Asfari and Vicens,\(^{20}\) some calix[6]arenes derived from \( p-n\)-alkyl-phenols (ranging from \( p-n\)-octyl to \( p-n\)-octadecyl) have melting points as low as 110 °C.

\[
\begin{align*}
R &= H, \text{Alkyl } n=4-8
\end{align*}
\]

Figure 1.8  Calix\([n]\)arenes.

Upper rim

Lower rim

Figure 1.9  Structure and numbering of \( p-\text{tert-butylcalix}[4]\)arene or 5,11,17,13-tetra-(1,1-dimethylethyl)-25,26,27,28-tetrahydroxy-calix[4]arene.
Another characteristic property of calixarenes is their low solubility in most solvents (see Table 1.1), which is mainly attributed to the extensive intermolecular hydrogen bonding as reflected in their melting points. The solubility of calixarenes can vary significantly according to the nature of the \( p \)-substituent in a similar fashion to that observed with their melting points. The substituents that lower the melting point of these compounds generally increase their solubility in organic solvents.\(^{21}\)

**Table 1.1** Solubilities of \( p \)-tert-butylcalix[4]arene and \( p \)-tert-butyl calix[8]arene in various solvents at 298.15 K.\(^{22,23}\)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( p \text{-tert}-\text{butylcalix[4]} \text{arene} )</th>
<th>( p \text{-tert}-\text{butylcalix[8]} \text{arene} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>( 5.90 \times 10^{-4} )</td>
<td>(&lt; 10^{-5} )</td>
</tr>
<tr>
<td>Ethanol</td>
<td>( 3.30 \times 10^{-4} )</td>
<td>( &gt; 10^{-6} )</td>
</tr>
<tr>
<td>N,N-dimethylformamide</td>
<td>( 1.10 \times 10^{-3} )</td>
<td>( 2.20 \times 10^{-3} )</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>( 4.73 \times 10^{-5} )</td>
<td>( 1.68 \times 10^{-5} )</td>
</tr>
<tr>
<td>Hexane</td>
<td>( 2.12 \times 10^{-4} )</td>
<td>( 2.51 \times 10^{-5} )</td>
</tr>
<tr>
<td>Chloroform</td>
<td>( 4.34 \times 10^{-3} )</td>
<td>( 6.23 \times 10^{-3} )</td>
</tr>
<tr>
<td>Benzonitrile</td>
<td>( 9.47 \times 10^{-4} )</td>
<td>( 1.14 \times 10^{-3} )</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>( 1.83 \times 10^{-2} )</td>
<td>( 2.57 \times 10^{-3} )</td>
</tr>
</tbody>
</table>

A wide variety of calixarene derivatives have now been prepared by incorporating suitable substituents in their structure and these led to compounds which are more soluble in organic solvents and also in water. Water soluble calixarenes in particular were first synthesised in 1984 by Ungaro and co-workers\(^ {24}\) who prepared the carboxymethyl ether of the cyclic tetramer. Later on, in 1984, Shinkai and co-workers\(^ {25,26}\) prepared the sulfonated calixarenes (upper rim substitution) which were considerably more soluble in water than the carboxy calixarenes, whilst Gutsche \textit{et al.}\(^ {27,28}\) synthesised aqueous acid-soluble amino calixarenes and aqueous base-soluble carboxy calixarenes (by upper rim substitution in both cases).

### 1.2.2. Stereochemical properties of calixarenes

X-ray crystallographic studies have been extensively used in calixarene chemistry in order to provide proof of their structure and conformation in the solid state whilst \textit{NMR} studies provide structural information on the behaviour of these compounds in
Parent calixarenes are highly flexible molecules being characterised by a distinctive capability of undergoing complete ring inversions. The transformation between the different conformations is accompanied by the aryl groups rotating through the centre (annulus) of the macrocycle. This interconversion of the aryl units between 'up' and 'down' orientations depends mainly on the number of phenolic units present in their structure as well as on the nature of the substituents in the aromatic ring, although temperature and solvent polarity can also play important roles.

In the case of p-tert-butylcalix[4]arene, four conformations are possible as initially perceived by Cornforth, with the different numbers of aryl groups projecting upwards or downwards relative to an average plane defined by the bridging methylene groups as shown in Figure 1.10. Gutsche has introduced the terminology 'cone', 'partial cone', '1-2 alternate' and '1-3 alternate to indicate their conformation. Due to strong intramolecular hydrogen bonding however, p-tert-butylcalix[4]arene exists almost entirely as the 'cone' conformer.

![Figure 1.10 Conformations of p-tert-calix[4]arenes](image-url)
By increasing the number of phenolic units in the structure of calixarenes, the flexibility and variety of possible conformations escalate considerably. Thus, calix[5]arenes can assume four 'up/down conformations' (the same number as calix[4]arenes), while calix[6] and calix[8]arenes can adopt eight and sixteen conformations. X-ray studies carried out by Andreetti and co-workers have shown that p-tert-butylcalix[8]arene in the solid state consists of two arrays of three hydroxyl groups looking like a large cone pinched at two opposite methylene groups, a shape which Gutsche called 'winged conformation'.

Similar studies on the cyclic octamer have revealed that p-tert-butylcalix[8]arene exists in a 'pleated loop conformation' in which the eight OH groups lie in a circle which takes the undulating form of a 'pleated loop' kept in place by circular hydrogen bonding. To summarise, the flexibility of parent calixarenes is enhanced by an increase in the number of aryl groups and therefore this is accompanied by a loss of the 'cone' conformation. Additional orientations departing from the true 'up/down' conformations are also present in all calixarenes (e.g. the aryl ring projecting outwards). The likelihood of these orientations however increases with increasing flexibility of the system. By appropriate functionalisation of the system at the upper and lower rims, conformational mobility can be curtailed, freezing the molecule into one or more of the available conformations.

1.2.3. Spectral properties of calixarenes

1.2.3.1. NMR spectra

Due to the symmetry of calixarenes, their $^{13}$C and $^1$H NMR spectra are simple regardless of their ring size. Thus, the $^1$H NMR spectrum of p-tert-butylcalix[4]arene at room temperature (Figure 1.13), consists of three singlets corresponding to the aromatic, the tert-butyl and the hydroxyl protons, as well as a pair of doublets due to the bridging methylene protons.

Temperature dependent $^1$H NMR studies first carried out by Kämmerer and co-workers on p-tert-butylcalix[4]arene in CDCl$_3$ have shown that at around 20°C, the methylene hydrogens appear as a pair of doublets which collapse to a singlet when the temperature is raised to about 60°C. This behaviour can be explained in terms of a 'cone conformation' that interconverts slowly on the NMR time-scale at low temperature but rapidly at high temperature.
NMR studies provide a valuable tool for investigating the conformational behaviour of calixarenes by enabling the measurement of the rate of conformational conversion of mobile as well as establishing the conformation of immobile calixarenes. These can be achieved by observing the position of the resonance of the bridging methylene protons in the $^{13}$C spectrum and the distinctive pattern of their resonance in the $^1$H NMR spectrum as shown in Table 1.2. Thus, De Mendoza and co-workers have shown that in the $^{13}$C spectra of calix[4]arenes the resonance of the CH$_2$ carbon is near 31 ppm when the attached phenol groups are in the syn orientation (i.e. both groups 'up' or both groups 'down') and at about 37 ppm when these are in the anti orientation (i.e. one group 'up' and one group 'down'). The suggestion put forward by De Mendoza has also been applied with reasonable success in the case of calix[5] and calix[6]arenes.

Table 1.2 $^1$H NMR spectral patterns for the CH$_2$ protons of the various conformations of calix[4]arenes at 298 K.

<table>
<thead>
<tr>
<th>Conformation</th>
<th>$^1$H NMR pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone</td>
<td>One pair of doublets ($J = 12$ Hz)</td>
</tr>
<tr>
<td>Partial cone</td>
<td>Two pairs of doublets ($J = 12$ Hz) (ratio 1:1) or one pair of doublets ($J = 12$ Hz) and one singlet (ratio 1:1)</td>
</tr>
<tr>
<td>1,2-Alternate</td>
<td>One singlet and two doublets ($J = 12$ Hz) (ratio 1:1)</td>
</tr>
<tr>
<td>1,3-Alternate</td>
<td>One singlet</td>
</tr>
</tbody>
</table>
The resonance arising from the OH groups in the $^1$H NMR spectra varies with the ring size. For the parent calixarenes listed in Table 1.3, the value of the OH resonance ($\delta_{OH}$) gives an indication of the strength of the intramolecular hydrogen bonding. Thus, the greater the value, the stronger is the hydrogen bond. Intramolecular hydrogen bonding is therefore strong in calix[4] and calix[6]arenes and weak in calix[5] and calix[8]arenes as shown in Table 1.3.

Table 1.3 $^1$H NMR resonances of the OH protons in parent calixarenes at 298 K.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^1$H NMR resonance ($\delta_{OH}$ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-tert-butylcalix[4]arene$^{17}$</td>
<td>10.34</td>
</tr>
<tr>
<td>$p$-tert-butylcalix[5]arene$^{18}$</td>
<td>9.64</td>
</tr>
<tr>
<td>$p$-tert-butylcalix[6]arene$^{17}$</td>
<td>10.53</td>
</tr>
<tr>
<td>$p$-tert-butylcalix[7]arene$^{18}$</td>
<td>10.34</td>
</tr>
<tr>
<td>$p$-tert-butylcalix[8]arene$^{17}$</td>
<td>9.60</td>
</tr>
</tbody>
</table>

1.2.3.2. Infrared spectra

The most characteristic feature of the infrared spectra of all parent calixarenes is the unusually low frequency of the OH stretching band occurring in the region between 3100 - 3500 cm$^{-1}$. Thus, the stretching vibration of the OH groups was found around 3150 cm$^{-1}$ for the cyclic tetramer and 3300 cm$^{-1}$ for the cyclic pentamer with the other oligomers falling between these limits. The significantly low position of the OH stretch vibrations is again attributed to the intramolecular hydrogen bonding being stronger for calix[4], calix[6] and calix[8]arenes. Calix[5]arene exhibits a more open 'cone' conformation than calix[4]arene. As far as calix[7]arene is concerned, this macrocycle shows an interrupted pleated loop conformation as compared with the completed loop conformation of calix[8]arene. These findings lead to weaker intramolecular hydrogen bonding for the former relative to the latter. By increasing the number of aryl units in the calixarene structure (n>8) its flexibility increases giving rise to a decrease in hydrogen bond formation. Table 1.4 lists values for the infrared stretching frequencies of the OH bond in calixarenes.
Characterisation of calixarenes using the 'fingerprint' region has limited possibilities since a similar spectrum is recorded for all calixarenes between 1500 and 900 cm\(^{-1}\) although some variations do exist in the 500-900 cm\(^{-1}\) region.

### Table 1.4 Infrared frequencies of the OH stretching vibrations in calixarenes

<table>
<thead>
<tr>
<th>Number of phenol units</th>
<th>R group</th>
<th>(v_{\text{OH}}(\text{cm}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>4(^{17}) tert-butyl</td>
<td></td>
<td>3179</td>
</tr>
<tr>
<td>4(^{39}) SO(_3)H</td>
<td></td>
<td>3232, 3411</td>
</tr>
<tr>
<td>6(^{17}) tert-butyl</td>
<td></td>
<td>3120</td>
</tr>
<tr>
<td>6(^{39}) SO(_3)H</td>
<td></td>
<td>3393</td>
</tr>
<tr>
<td>8(^{17}) tert-butyl</td>
<td></td>
<td>3190</td>
</tr>
<tr>
<td>8(^{39}) SO(_3)H</td>
<td></td>
<td>3242, 3426</td>
</tr>
</tbody>
</table>

#### 1.2.3.3 Ultraviolet spectra

Apart from thin layer chromatography, TLC; high performance liquid chromatography, HPLC, and proton nuclear magnetic resonance, \(^1\)H NMR, measurements, UV spectroscopy provides a useful tool occasionally employed in macrocyclic ligand chemistry for monitoring the course of a reaction as well as investigating the interactions taking place. Calixarenes show two absorption maxima at 280 and 288 nm in the ultraviolet region. The ratio of the intensity at these two wavelengths is a function of the ring size, ranging from 1.3 for cyclic tetramers to 0.75 for cyclic octamers as shown in Table 1.5.

#### 1.2.4 Interactions with metal cations, organic substances and the proton.

A wide range of lower rim calixarene derivatives has been prepared. These include esters, amides, ketones, acids, amines and others, which are able to complex cations. Roundhill et al.\(^{40}\) and McKervey et al.\(^{41}\) have given an account on cation complexation by calixarenes. Stability constants for the complexation of cations by calix[n]arenes
functionalised at the lower rim have been reported mainly for alkali-metal cations and to a lesser extent for alkaline-earth metal cations. The most detailed thermodynamic information on calixarene derivatives and alkali-metal cations is that involving the esters.\textsuperscript{42}

<table>
<thead>
<tr>
<th>R group</th>
<th>Ring size</th>
<th>280 (\pm 1) nm</th>
<th>288 (\pm 1) nm</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tert-butyl\textsuperscript{43}</td>
<td>4</td>
<td>9,800</td>
<td>7,700</td>
<td>CHCl\textsubscript{3}</td>
</tr>
<tr>
<td>AH methyl\textsuperscript{16}</td>
<td>4</td>
<td>10,500</td>
<td>8,300</td>
<td>Dioxane</td>
</tr>
<tr>
<td>Me &amp; tert-butyl\textsuperscript{44}</td>
<td>5</td>
<td>14,030</td>
<td>14,380</td>
<td>Dioxane</td>
</tr>
<tr>
<td>All tert-butyl\textsuperscript{43}</td>
<td>6</td>
<td>15,500</td>
<td>17,040</td>
<td>CHCl\textsubscript{3}</td>
</tr>
<tr>
<td>Me &amp; tert-butyl\textsuperscript{16}</td>
<td>6</td>
<td>17,210</td>
<td>17,600</td>
<td>Dioxane</td>
</tr>
<tr>
<td>All tert-butyl\textsuperscript{43}</td>
<td>7</td>
<td>18,200</td>
<td>20,900</td>
<td>CHCl\textsubscript{3}</td>
</tr>
<tr>
<td>Me &amp; tert-butyl\textsuperscript{16}</td>
<td>7</td>
<td>19,800</td>
<td>20,900</td>
<td>Dioxane</td>
</tr>
<tr>
<td>All tert-butyl\textsuperscript{43}</td>
<td>8</td>
<td>23,100</td>
<td>32,000</td>
<td>CHCl\textsubscript{3}</td>
</tr>
</tbody>
</table>

Sometimes the term stability constant is used to describe association rather than complexation.\textsuperscript{41} Therefore, it is important to define carefully the parameters relating to the complexation process. The stability constants, \(\log K_s\) and derived standard Gibbs energies, \(\Delta\text{G}^\circ\), enthalpies, \(\Delta\text{H}^\circ\) and entropies, \(\Delta\text{S}^\circ\) of 1:1 (metal cation:ligand) complexation of lower rim calixarene derivatives, \(L\), and cations, \(M^{n+}\) in various solvents(s) refer to the process described in Eq. 1,

\[
M^{n+}_{(s)} + L_{(s)} \rightleftharpoons M^{n+}L_{(s)}
\]

The thermodynamic stability constant, \(K_s\) (molar scale) is defined by Eq. 2 in which \(a\) and \(\gamma\) denote activity and activity coefficients, respectively

\[
K_s = \frac{a_{M^{n+}L}}{a_{M^{n+}} \cdot a_{L}} = \frac{[M^{n+}L] \gamma_{M^{n+}} \cdot \gamma_{L}}{[M^{n+}] \gamma_{M^{n+}} \cdot [L] \gamma_{L}} \approx \frac{[M^{n+}L]}{[M^{n+}] [L]}
\]
The inequality of Eq. 2 holds provided that the solutions are relatively dilute, in which case \( \gamma_{\text{lig}} \equiv 1 \) and \( \gamma_{\pm M\text{lig}^{\pm}} = \gamma_{\pm M^{\pm}} \). For processes involving charged ligands, particularly multi-charged ligands, it may be necessary to consider explicitly the activity coefficients of the species involved in the equilibrium. In solvents of low dielectric constants, such as chloroform or water-saturated chloroform, where ion pair formation becomes important, it is common to define an 'association constant', \( K_{\text{ass}} \), which refers to the following 1:1 (salt:ligand) process:\(^{4141}\)

\[
M^+X^{-}_{(s)} + L_{(s)} \rightarrow ML^+X^{-}_{(s)}
\]

involving ion-pairs rather than free ions. It is important not to confuse \( K_{\text{ass}} \) and \( K_a \) as they refer to two different processes. Interpretation of association constants determined in low dielectric media should be taken prudently, particularly in chloroform and tetrahydrofuran as they relate to ion-pairs as well as the binding process. Therefore these constants do not refer to a well-characterised thermodynamic process and do not reflect 'true' stability constants.\(^{42}\)

Several methods have been used to determine stability constants including potentiometry, ultraviolet, visible and fluorescence spectrophotometry, NMR spectroscopy, titration calorimetry and conductimetry. Of these methods, potentiometry is often considered the most accurate, where particular stable metal ligand complexes are studied, due to the proportionality of the measured potential difference to the logarithm of the metal-ion activity. As a result, measurements are far more sensitive to changes in the extent of complexation when the process is nearly complete and the free metal ion activity is low.\(^{42}\)

This last method has been extensively used by Cox and Schneider\(^{46,47}\) and others to measure the activity of free silver ions in the presence of a competitive equilibrium involving a second metal cation in order to calculate the stability constants of the relevant metal-ion cryptate in a variety of solvents. A condition to apply this method is that the ligand complexes with the silver cation.\(^{48}\) This is not always the case, as for calix[4]arene esters in solvents like acetonitrile and benzonitrile. Using silver electrodes, a double competition method involving cryptands and calix[4]arene esters has been introduced by Danil de Namor and co-workers\(^{49}\) for the potentiometric determination of stability constants of highly stable complexes of calixarene esters and metal cations (Li\(^+\) and Na\(^+\)) in acetonitrile and benzonitrile. This approach can be
applied to systems in which the first ligand has a high affinity for silver while the second has low or no affinity.

1.2.5. Applications

Calixarenes have developed into a valuable class of macrocyclic host molecules with numerous applications within a short period of time. Well over 100 patents have been issued describing the various practical applications of calixarene-based molecules. According to Perrin and Harris the growing interest in these compounds is due to the following reasons.

i) Parent calixarenes can be easily prepared in a simple one-step procedure. In addition large quantities of product can be readily obtained from cheap starting materials.

ii) Parent compounds can be chemically modified in various ways and this has led to a vast number of calixarene derivatives able to target a wide range of guests.

iii) Calixarenes form a series of cyclic oligomers ranging from the tetrameric calix[4] to the octameric calix[8]arene. They are therefore characterised by a variety of cavity sizes and shapes and this allows molecular recognition to be attained.

Perrin and co-workers as well as Gutsche have given detailed accounts of the various industrial applications of these macrocycles. Thus, this section gives a review on the main ways in which calixarenes have been or might be put to use as reflected in published papers and existing patents.

1.2.5.1. Ion sequestration

Metal sequestration is one of the most important industrial applications involving calixarenes. Patents for the recovery of caesium, uranium, lanthanides are now explained in detail.

i) Recovery of caesium

The first patent concerning calixarenes for a practical application was issued in 1984 to Izatt et al. This patent describes the use of p-tert-butylcalix[8]arene for the recovery of caesium from nuclear waste materials. The process involves three liquid phases (see Figure 1.12). The first one, the aqueous phase, contains the degradation products of uranium splitting including caesium. The second
dissolved in a mixture of carbon tetrachloride and dichloromethane whilst the third one is distilled and deionised water. The second phase acts as a liquid membrane transporting caesium from the first phase to the third. Various aqueous metal hydroxides were used as the source phase in this system, which showed a transport rate for Cs⁺ about 100 times higher than for K⁺, Na⁺ and Li⁺.

![Diagram](image)

**Figure 1.12** Schematic representation of the apparatus used for the recovery of caesium using calixarenes.

**ii) Recovery of uranium**

The selective extraction of uranium from seawater has attracted considerable attention, due to its importance in relation to energy problems. One of the main difficulties in the extraction of uranium is that the ligand to be used must strictly discriminate the uranyl ion (UO₂²⁺) from other metal ions present in great excess in sea water (concentration of UO₂²⁺ is 3 ppb whilst those of the competing metal cations are in the range of 10 ppm). Thus, calixarene derivatives are required which have a selectivity factor (stability constant, $K_s$, ratio $K_{s\text{UO}_2^{2+}} / K_{s\text{M}^{2+}}$; M²⁺ = Ni²⁺, Cu²⁺, Zn²⁺, etc.) greater than $10^4$. Most of the recent work in this field comes from Japan with at least six patents issued to Shinkai's group. A polymer-bound calixarene (Figure 1.13) has also been reported by Shinkai et al. This was obtained by treating p-(chlorosulfonyl)calix[6]arene with polyethyleneimine which resulted in the formation of gel-like hexamer derivatives immobilised in a polymeric framework.

**iii) Lanthanide sequestration**

Bünzli and Harrowfield have suggested that p-tert-butylcalix[8]arene may be used in solvent extraction and purification of lanthanides as well as in lanthanide ion catalysis of reactions carried out in apolar solvents. This suggestion is based on the ability of
calixarenes to form complexes with all lanthanide ions, which are soluble in organic solvents.

Figure 1.13 Structure of Shinkai’s calixarene derivative used for the selective extraction of uranium.

1.2.5.2. Ion and molecule selective electrodes

Calixarene derivatives have been used for the design of ion selective membrane electrodes. Amongst these, electrodes selective for alkali and alkaline-earth cations have received particular attention because of their medical importance (e.g. in blood analysis). Thus, McKervey and Diamond and co-workers have developed ion selective electrodes for Na\(^+\), K\(^+\), Cs\(^+\), and Ca\(^{2+}\) using calixarene derivatives containing hard oxygen donor atoms such as tetra esters and tetra ethers. Softer donor substituents on the lower rim of calixarenes including thioether and thioamide groups have been used for sensing cations such as Ag\(^+\), Cu\(^{2+}\), and Pb\(^{2+}\). Recently Danil de Namor and co-workers\(^1\) have reported a detailed study of the interaction of ester and ketone calix[4]arene derivatives with bivalent cations (alkaline-earth, transition and heavy metal) in several solvents (methanol, N,N-dimethylformamide and acetonitrile) showing that this interaction just takes place in acetonitrile and the role played by cation desolvation and ligand binding energy in complex formation was highlighted.

The development of anion selective electrodes has not been characterised with comparable success. More recently, Pb\(^{2+}\) complexes of calix[4]arene with thioamide groups on the lower rim (see Figure 1.16) have been reported.\(^2\) These complexes when incorporated into membranes without lipophilic salts showed high selectivity for perchlorates over other anions with linear responses in the range of pClO\(_4\) 1-5 with a
slope of 58.6 mV x decade. In addition to ion selective electrodes, more recently, calixarene derivatives have also been used, for the development of electrodes sensing a variety of molecules including heptanal,\textsuperscript{73} glucose,\textsuperscript{74} ammonium and pyridinium surfactants\textsuperscript{75,76}, as well as carboxylic acids.\textsuperscript{77}

![Figure 1.14 X-ray structure of the complex of lead and calix[4]arene thioamide derivative used in the design of anion selective membrane electrodes.\textsuperscript{78}]

One of the recent applications of calixarenes include their uses as sensors for monitoring the activity of chemical and biochemical species. Thus, calixarene-based devices have been designed in order to detect the species being monitored. The chemical response which is at the molecular level is subsequently converted into an electrical or optical signal at the macroscopically observable and measurable level. Within this context, calixarenes have been employed in a variety of interesting ways including as detectors of toxic chemicals,\textsuperscript{79} optical amine sensors\textsuperscript{80} and calcium sensors.\textsuperscript{81} Additional uses of calixarenes are described in the following section.

1.2.5.3. Separation and purification of neutral molecules

Due to their ability to host neutral molecules, calixarenes have been used in the following processes,

i) Purification of fullerenes

Fullerenes are synthesised by arc discharging or vaporisation of graphite. This produces the fullerite or C\textsubscript{60} containing soot, which also includes some bigger
fullerenes such as C\textsubscript{70}. Separation techniques based on chromatography have not been successful since these are characterised by low efficiency and high cost.

Williams and co-workers,\textsuperscript{82} Atwood and co-workers\textsuperscript{83} and Shinkai and co-workers\textsuperscript{84} almost simultaneously isolated a solid calixarene complex of C\textsubscript{60} by mixing toluene solutions of p-tert-butylcalix[8]arene and C\textsubscript{60} containing soot. Two recrystallizations of the complex (initial composition 89 \% C\textsubscript{60} and 11 \% C\textsubscript{70}) yielded a material of 99.5\% purity from which C\textsubscript{60} was obtained. In 1995, two patents were issued to Atwood’s\textsuperscript{85} and Shinkai’s\textsuperscript{86} groups.

More recent studies reported by Isaacs and co-workers from Atwood’s group,\textsuperscript{87} have investigated the complexation of C\textsubscript{60} with p-benzylcalix[5]arene in toluene. Using high-precision densitometry they measured the change in partial molar volume on complexation. Their results have shown that two toluene molecules are displaced from the cavity of the host by C\textsubscript{60} upon complexation.

\textit{ii) Removal of halogenated hydrocarbons from water supplies}

In 1974 it was discovered that the chlorination of water supplies (for improving their microbiological quality) is accompanied by undesirable side reactions, \textit{(i.e., reaction of chlorine with naturally occurring humic and fulvic acids resulting in the formation of trihalomethanes)}. This resulted in the presence of contaminants in water supplies with carcinogenic properties such as chloroform as well as dichloromethane, chlorodibromomethane and bromoform if bromide ions are also present. Wainwright\textsuperscript{88} has patented the use of non-solvated calixarene compounds \textit{e.g.} a tert-butylcalix[6]arene for the removal of trihalomethane molecules with high rates of reaction, leading to the formation of highly stable inclusion complexes.

\textbf{1.2.5.4. Calixarenes as catalysts}

Several patents have been issued describing the catalytic properties of modified calixarenes in hydrolysis reactions. Some examples of this include:

\textit{i.) The hydrolysis of 2,4-dinitrophenyl phosphate which is moderately catalysed by calixarenes carrying p-trimethylammonium groups.}\textsuperscript{89}

\textit{ii.) The base-induced hydrolysis of p-nitrophenyl dodecanoate which is dramatically catalysed by calix[4] and calix[6]arene salts bearing trimethylamino groups in the \textit{para} position (Figure 1.15a).}\textsuperscript{90} The hydrolysis reaction in the latter case is
catalysed approximately 105-fold more effectively by the (a1) than by the (a2) derivative.

![Structures of calixarene derivatives with catalytic properties.](image)

Figure 1.15 Structures of calixarene derivatives with catalytic properties.

Harris et al.\(^9\) has patented the catalytic properties of p-tert-butylethoxy calix[4] and calix[6]arene esters (Figure 1.15b) in a free radical polymerisation reaction. In this process, the calixarene derivative acts as a complexing agent in the polymerisation reaction of acrylic monomers using the metal salt as a free radical initiator. A good example of a well-defined calixarene catalysed reaction is the addition of water to 1-benzyl-1,4-dihydronicotinamide. This reaction has been studied by Shinkai and co-workers\(^9\) (who tested the use of p-sulfonatocalix[6]arene derivatives of Figure 1.15c) and Gutsche and Alam\(^9\) (who investigated the entire series of p-carboxycalix[n]arenes from the cyclic tetramer through the cyclic octamer, see Figure 1.15d). The results revealed the superiority of p-sulfonatocalix[6]arene (Figure 1.15c) over p-carboxycalix[n]arene (Figure 1.15d) as a catalyst for this reaction. It was suggested that this was due to the greater concentration of negative charge at the upper rim of

\(^{a1} n = 6; R = \text{Methyl or Octyl} \\
^{a2} n = 4; R = \text{Methyl} \\
^{b} n = 4 \text{ or } 6 \\
^{c} R = \text{H, CH}_2\text{CO}_2\text{H, CH}_3, \text{n-C}_6\text{H}_{13, n-C}_{12}\text{H}_{25} \\
^{d} n = 5, 6, 7, 8\)
the p-sulfonatocalix[6]arene resulting in the six sulfonato-groups being held more tightly in place than the more flexible carboxyethyl moieties.

1.2.5.5. Miscellaneous applications

i) **Langmuir-Blodgett films**

Hard and heat resistant films have been obtained with modified methylcalix[n]arenes.\textsuperscript{94} Acetylation of methylcalixarenes (which are practically insoluble in all solvents) gives rise to derivatives (Figure 1.16) highly soluble in organic solvents which are hard and very resistant to heat. The highest solubilities in various organic solvents are exhibited by the hexamer. The heat-resistant film (up to 400°C) can be easily removed partially or entirely by organic solvents since the film-forming process does not involve any chemical modification of the calixarene.

![Calixarene derivative](image)

**Figure 1.16** Calixarene derivatives used in the formation of hard and heat resistant films.

ii) **Accelerators for instant adhesives**

Several patents issued to Harris et al.\textsuperscript{95,96} claim that certain calixarene derivatives can be used as accelerants for cyanoacrylate instant adhesives. Thus, calixarene derivatives shown in **Figure 1.17** are able to reduce bonding times on porous substrates such as paper, leather, fabrics and woods from minutes to seconds. These compounds are employed at levels between ~0.1 and 1 % by weight of the cyanoacrylate composition.

The mode of action is not entirely clear in these patents, but it is believed to involve the generation of 'naked' anionic initiators for the polymerisation involving complexation with counter-cations.
iii) Ion scavengers for electronic devices

Calixarene derivatives bearing ester and ketone substituents able to complex with alkali-metal cations have been shown to be useful as metal cation-immobilising additives for electronic encapsulants such as epoxides and silicones.\textsuperscript{97} Ions such as Na\textsuperscript{+}, K\textsuperscript{+}, and Cl\textsuperscript{−} can cause corrosion of metal components or malfunction of the electronic devices. The development of advanced, highly specific and sensitive devices in the electronic industry also necessitates the availability of compounds with suitable alkali-metal sequestering properties. According to the results reported in the patent literature,\textsuperscript{97} the above mentioned modified calixarenes are at least as effective as 18-crown-6\textsuperscript{98} in extracting unwanted metal cations. Using 1-5 \% by weight of a calixarene derivative in epoxides resulted in a drop of the Na\textsuperscript{−} levels from 240 to less than 30 ppm.

iv) Therapeutic and biologically active calixarene derivatives

The exploration of calixarene based compounds with therapeutic properties dates back to 1955 when Sir Cornforth and co-workers,\textsuperscript{30} studied the use of cyclic oligomers (later named calixarenes) as tuberculostatic agents. They used oxyalkylated derivatives called Macrocyclons. Forty-one years later D'Arcy Hart \textit{et al.}\textsuperscript{99} reported that the \textit{in vivo} inhibition of \textit{Mycobacterium tuberculosis} can be induced inside macrophages using a calixarene derivative bearing short polyethyleneoxy chains on the lower rim. Recent patents\textsuperscript{100,101} have further investigated the use of calixarene based molecules as therapeutic and biologically active agents and have shown that certain derivatives exhibit anti-bacterial, anti-fungal, anti-cancer, anti-viral and particularly anti-HIV activities.
Other potential industrial applications of calixarenes include their uses as electrophotographic photoreceptors,\textsuperscript{102} photographic toners,\textsuperscript{103} hair dyes,\textsuperscript{104} diesel fuel additives,\textsuperscript{105} antistatic agents,\textsuperscript{106} antioxidants,\textsuperscript{107} curing agents,\textsuperscript{108} temperature sensing devices,\textsuperscript{109} safety glass compositions\textsuperscript{110} and flame proofing compounds.\textsuperscript{111}

As mentioned before, the characterization of calixarenes interactions with several guests can be analysed by instrumental techniques, then a brief review of two of the main techniques used in the present research work is given in the following section.

1.3. Potentiometry

Potentiometry is the field of electroanalytical chemistry in which the potential is measured under the conditions of no current flow. The measured potential may then be used to determine the analytical quantity of interest, generally the concentration of some component of the analyte solution. The potential that develops in the electrochemical cell is the result of the Gibbs energy change, $\Delta G$, that would occur if the chemical phenomena were to proceed until the equilibrium condition has been satisfied.\textsuperscript{112}

$$\Delta G = nFE$$ \hfill (4)

In Eq. 4, $n$, $F$ and $E$, denote the number of electrons taking part in the reaction, the Faraday constant and the potential developed during the reaction, respectively. This concept is typically introduced in quantitative analysis courses in relation to electrochemical cells that contain an anode and a cathode. For these electrochemical cells, the potential difference between the cathode and the anode electrode potentials is the potential of the electrochemical cell, $E_{\text{cell}}$, as shown in Eq. 5.

$$E_{\text{cell}} = E_{\text{cathode}} - E_{\text{anode}}$$ \hfill (5)

If the reaction is conducted under standard state conditions, this equation allows the calculation of the standard cell potential. When the reaction conditions are not in the standard state, however, the Nernst equation must be used to determine the cell potential, (Eq. 6)

$$E_{\text{cell}} = E^\circ - \frac{RT}{nF} \ln K_{eq}$$ \hfill (6)
In Eq. 6, $E$ denotes the electrode potential, while $E^o$, $R$, $T$, $F$ (96487 C.mol$^{-1}$) and $n$, are the standard electrode potential, the gas constant, the Faraday constant and the number of electrons involved in the reaction taking place in the electrochemical cell, respectively. Then, at 298.15 K the Nernst equation can be expressed as follows,

$$E_{cell} = E^o - \frac{0.059}{n} \log K_{eq}$$  \hspace{1cm} (7)

In Eq. 7, $RT/F \times 2.303 = 0.059$ V, physical phenomena which do not involve explicit redox reactions, but whose initial conditions have a non-zero Gibbs energy, also will generate a potential. An example of this would be the ion concentration gradients across a semi-permeable membrane. This can also be a potentiometric phenomenon, and the basis of measurements that use ion-selective electrodes.

$$E_{mem} = C - \frac{RT}{nF} \ln a_i$$  \hspace{1cm} (8)

In Eq. 8, $C$ is a constant and $n$, and $a_i$ denote the charge and the activity of the ion under study. Potentiometric measurements require an indicator and a reference electrode, and a potentiometer (millivoltmeter). It is used for the quantitative determination and for monitoring many species in solution over a wide range of concentrations ($10^{-7}$ to 1 mol.dm$^{-3}$). Its relative precision is 0.1 to 5 %. On the other hand, it has the disadvantage of being a slow and a time consuming, method unless this is automated.$^{112-114}$

1.3.1. Electrode Systems.

1.3.1.1. Reference Electrodes

Potentiometric measurements relating potential changes to activities of ions in solution rely on the response of one electrode (indicator electrode) only, the other, ideally is independent of the solution composition and the conditions. The latter is known as the reference electrode. Two of the electrode systems currently used are the calomel and the silver-silver chloride electrodes whose potentials are well established against the standard hydrogen electrode (SHE). There are practical problems with the latter. This is the reason why secondary reference electrodes have been introduced, formed by metal/sparsely soluble salt of metal/fully ionised salt with common anion.
The Calomel reference electrode is based on the redox couple between $\text{Hg}_2\text{Cl}_2$ and Hg (calomel is a common name for $\text{Hg}_2\text{Cl}_2$),\textsuperscript{112,115}

$$\text{Hg}_2\text{Cl}_2(s) + 2e^- \rightleftharpoons 2\text{Hg}^0(l) + 2\text{Cl}^-(aq)$$

The Nernst equation applied to Eq. 9 is formulated as follows,

$$E = E_{\text{Hg}_2\text{Cl}_2/Hg}^0 - \frac{0.05916}{2} \log [\text{Cl}]^2$$

The potential of a calomel electrode, therefore, is determined by the activity of Cl$^-$ ions in solution. The saturated calomel electrode (SCE), which uses an aqueous saturated solution of KCl, has a potential at 298.15 K of +0.2444 V. A typical SCE consists of an inner tube, packed with a paste of Hg, $\text{Hg}_2\text{Cl}_2$, and saturated KCl, situated within a second tube filled with a saturated solution of KCl. A small hole connects the two tubes, and an asbestos fiber serves as a salt bridge to the solution in which the SCE is immersed. The stopper in the outer tube may be removed when additional saturated KCl is needed. The shorthand notation for this half cell is,

$$\text{Hg}(l) \mid \text{Hg}_2\text{Cl}_2(sat\text{d}), \text{KCl}(aq, sat\text{d}.\text{soln}) \mid \mid$$

The SCE has the advantage that the activity of Cl$^-$ ions and, therefore, the potential of the electrode, remains constant even if the KCl solution partially evaporates. On the other hand, a significant disadvantage of the SCE is that the solubility of KCl is sensitive to a change in temperature. At higher temperatures the concentration of Cl$^-$ increases, and the electrode’s potential decreases. For example, the potential of the SCE at 308.15 K is +0.2376 V. Electrodes containing unsaturated solutions of KCl have potentials that are less temperature-dependent, but experience a change in potential if the concentration of KCl increases due to evaporation. On the other hand, calomel electrodes cannot be used at temperatures above 80°C.\textsuperscript{116}

Another common reference electrode based on the same principle as the calomel electrode is the silver/silver chloride electrode, which is based on the redox couple between AgCl and Ag,\textsuperscript{112,115}

$$\text{AgCl}(s) + e^- \rightleftharpoons \text{Ag}^0(s) + \text{Cl}(aq)$$
Like the saturated calomel electrode, the potential of the Ag/AgCl electrode is
determined by the activity of KCl used in its preparation,

\[ E = E^{\circ}_{\text{AgCl}/\text{Ag}} - 0.05916 \log a_{\text{Cl}^-} \]

When this electrode is prepared using a saturated solution of KCl, the Ag/AgCl
electrode has a potential of +0.197 V at 298.15 K. Another common Ag/AgCl electrode
uses a solution of 3.5 mol.dm\(^{-3}\) KCl and has a potential of +0.205 at 298.15 K. The
Ag/AgCl electrode prepared with saturated KCl, of course, is more

temperature-sensitive than the one prepared with an unsaturated solution of KCl. A typical Ag/AgCl
electrode consists of a silver wire, the end of which is coated with a thin film of AgCl.
The wire is immersed in a solution that contains the desired concentration of KCl and
that is saturated with AgCl. A porous plug serves as the salt bridge. The shorthand
notation for the half cell is,

\[ \text{Ag(s)} \mid \text{AgCl (satd), KCl (x M)} \]

In Eq. 14 x is the concentration of KCl. In comparison to the SCE the Ag/AgCl
electrode has the advantage of being useful at higher temperatures. On the other
hand, the Ag/AgCl electrode is more prone to reacting with solutions to form insoluble
silver complexes that may plug the salt bridge between the electrode and the
solution.\(^{116}\)

1.3.1.2. Indicator Electrodes

The potential of the indicator electrode is related to the activities of one or more of the
components of the solution and therefore, it determines the overall cell potential.
Ideally, its response to changes in activity should be rapid, reversible and governed by
the Nernst equation. There are two types of indicator electrodes which possess the
desired characteristics, and these are described below.

1.3.1.3. Metallic Indicator Electrodes

Metals such as silver, copper, mercury, lead and cadmium answer to variations in the
activities of their own ions in a Nernstian and reproducible way, e.g. for silver, the
electrode reaction is,
and the electrode potential is given by:

\[ E_{\text{cell}} = E^o + 0.059 \log \alpha_{Ag^+} \]

Metal electrodes that respond directly to solutions of their own ions are called Class I or first order electrodes.

Metals which form sparingly soluble salts will also respond to changes in the activity of the relevant anion, e.g. for silver in contact with a saturated solution of silver chloride and containing solid silver chloride the electrode reaction is,

\[ AgCl(s) + e^- \rightarrow Ag^0(s) + Cl^-(aq) \]

and the electrode potential is given by,

\[ E_{\text{cell}} = E^o - 0.059 \log \alpha_{Cl^-} \]

Such electrodes are described as Class II or second order electrodes. For titrations involving a change in the oxidation state, an inert electrode material such as platinum is used. The proportions of oxidised and reduced forms present in the solution determine the potential adopted by the electrode. Thus, for the Fe\(^{3+}/Fe^{2+}\) redox system,

\[ Fe^{3+}(aq) + e^- \rightarrow Fe^{2+}(aq), \]

the potential adopted by the electrode is a function of the Fe\(^{3+}/Fe^{2+}\) ratio as shown in Eq. 20.

\[ E_{\text{cell}} = E^o + 0.059 \log \frac{\alpha_{Fe^{3+}}}{\alpha_{Fe^{2+}}} \]

1.3.2. Membrane or Ion-Selective Electrodes

Ion-selective electrodes (ISEs) are the chemical sensors of longest history and the ones which still offer the largest number of applications. More recently, optical sensors
based on partitioning of the analyte between the sample and the bulk of the sensing film (bulk optodes) are exhibiting a rapid development. Given the closely related detection mechanism, many of the characteristics such as selectivities and detection limits are comparable for both types of transduction.\textsuperscript{111} Also the possibility of miniaturization is similar with the two transduction techniques. The recent advances of fluorescence based bulk optodes\textsuperscript{112} have led to dimensions comparable to those of ion-selective microelectrodes introduced decades ago\textsuperscript{113} for activity measurements in cells or in some cases in cell nuclei. So far, ISEs have been described for about 60 analytes, about twice as many as optodes\textsuperscript{114}. However, it would be a relatively straightforward task to develop bulk optodes for the remaining 30 analytes on the basis of their potentiometric counterparts. As to ruggedness and response time, especially at submicromolar activities, ISEs have clear advantages.

1.3.3. The Mercury Electrode

The mercury electrode is a Class I electrode. Therefore, its potential depends on the activity of mercury (II) ions in the solution,

\[
\text{Hg}^{2+} + 2e^- \rightarrow \text{Hg}^0, \quad E^0 = 0.850 \text{ V vs SHE at 298.15 K}
\]

Applying the Nernst equation to this reaction, it follows that,

\[
E_{\text{cell}} = E^0_{\text{Hg}^{2+}/\text{Hg}} + \frac{0.059}{2} \log a_{\text{Hg}^{2+}} \quad 22
\]

However, the mercury electrode can be used to measure the activity or concentration of other cations as Cd\textsuperscript{2+}, Pb\textsuperscript{2+} etc. if a small quantity of a suitable mercury complex is added to the solution. Under these conditions a third class electrode is obtained, namely

\[
\text{Hg} \mid \text{Hg}^{2+}; \quad \text{HgY}^2; \quad \text{MY}^{(n-4)+}; \quad \text{M}^{n+}
\]

The potential of the cell (E) can therefore be expressed as follows,

\[
E = E^0 - \frac{0.059}{2} \log \left( \frac{K_{\text{HgY}}}{a_{\text{Hg}^{2+}}} \right) - \frac{0.059}{2} \log \left( \frac{a_{\text{MY}^{(n-4)+}}}{K_{\text{MY}}} \right)
\]

\[
24
\]
For the general case in which the stability constant of the mercury complex, $K_{HgY}$, is greater than that for the metal complex, $K_{MY}$, then the concentration of the mercury complex, $[HgY^2-]$, will remain practically constant and the first of the two logarithmic terms in Eq. 24 would also be constant. Then, the electrode potential is dependent on the metal cation activity and it is possible to determine the stability constant of metal ion complexes, with cations other than mercury. Interfering ions are halides, $X^-$, sulphides, $S^{2-}$, and cyanides, $CN^-$ ions, because of their possibility to interact with mercury. The presence of $O_2$ dissolved in the solvent is also a problem. The suitable range of pH is from 2 to 11 and ligands such as $2,2',2''$-(ethane-1,2-diyldinitrilo) tetraacetic acid, EDTA; $N,N'$-bis(2-aminoethyl)ethane-1,2-diamine, TRIEN; $N,N'$-(azanediylidethane-2,1-diyl) bis (ethane-1,2-diamine), TETREN; $2,2',2''$-[ethane-1,2-diylibis(oxyethane-2,1-diylnitrilo)] tetraacetato, EGTA, etc. can be used. All these characteristics of the mercury electrode have been widely studied in water. Although this electrode is a good analytical tool in aqueous medium, its behaviour in organic solvents has not been tested.\textsuperscript{112-114}


The enormous growth in available NMR pulse methods over the last two decades may leave one wondering just where to start or how best to make use of these new developments. The answer to this is not straightforward since it strongly depends on the system under study and on the information required.\textsuperscript{117}

Most of the research on NMR should begin with the analysis of the proton spectra of the sample, followed by the usual analysis of the chemical shifts, coupling constants and relative signal intensities. Beyond this, a wide range of NMR methods can be used. The key factor for the selection of the appropriate experiments for a given problem is an appreciation of the type of information that NMR techniques can provide. Although a huge number of pulse sequences are available, the number of ‘core’ experiments, from which others are derived by minor variations is indeed very small. Most NMR methods exploit only three basic phenomena; which are briefly described.\textsuperscript{117}

- Through-bend interactions: scalar ($J$) coupling via bonding electrons,
- Through-space interactions: the nuclear Overhauser effect mediated through dipole coupling and spin relaxation.
• Chemical exchange: the physical exchange of one spin for another at a specific location.

In an attempt to analyse the structure of a molecule and/or its behaviour in solution by NMR spectroscopy, these phenomena can be exploited to gain the desired information, and therefore to select the appropriate techniques. Then, these sequential steps can be carried out as follows,

i) Search for evidence of scalar coupling between nuclei in order to find the location of chemical bonds. Once these are established, the gross structure of the molecule is defined and often this is enough to confirm it.

ii) Spatial proximities between nuclei and between protons in particular, can be used to define the stereochemical relationship within a molecule and thus address questions regarding configuration and conformation of molecules.

The unique feature of NMR spectroscopy, and the principal reason for its superiority over any ether solution-state technique for structure elucidation, is its ability to define relationships between specific nuclei within a molecule or even between molecules. The principal spin interactions and the main techniques used to meet these are summarised in Table 1.6.\textsuperscript{117}

The homonuclear correlation experiment, known as COSY, identifies those nuclei that share a J-coupling, which, for protons, operate over two, three and less frequently, four bonds. This information can therefore be used to indicate the presence of the bonding pathway. The correlation of protons that exists within the same-coupled network chain of spins, but do not themselves share a J-coupling can be made with TOCSY experiments.\textsuperscript{118} This can be used to identify groups of nuclei that sit within the same isolated spin system.

One-bond heteronuclear correlation methods (HMQC or HSQC) identify the heteroatoms to which the protons are directly attached and can provide, for example, carbon assignments from previously established proton assignments. Proton chemical shifts can also be dispersed according to the shift of the attached heteroatom, so aiding the assignment of the proton spectrum itself. Long-range heteronuclear correlations over typically two or three bonds (HMBC) provide a wealth of information on the skeleton of the molecule and can be used to infer the location of carbon-carbon
or carbon-heteroatom bonds. These correlations can be particularly valuable when proton-proton correlations are not available. The experiment known as INADEQUATE identifies connectivity between like nuclei of low natural abundance, for which it is favoured over COSY.\textsuperscript{117}

Table 1.6 Principal correlations established through NMR techniques

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Principal Technique</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>H H</td>
<td>(^1\text{H}-\text{H COSY})</td>
<td>Proton J-coupling typically over 2 or 3 bonds</td>
</tr>
<tr>
<td>H H H</td>
<td>(^1\text{H}-\text{H TOCSY})</td>
<td>Relayed proton J-couplings within a coupled spin system. Remote protons may be correlated provided there is a continuous coupling network in between them.</td>
</tr>
<tr>
<td>H H</td>
<td>(^1\text{H}-\text{X HMBC})</td>
<td>One-bond heteronuclear couplings via proton observation.</td>
</tr>
<tr>
<td>H H</td>
<td>(^1\text{H}-\text{X HMBC})</td>
<td>Long-range heteronuclear couplings via proton observation. Typically over 2 or 3 bonds when (X=^{13}\text{C}).</td>
</tr>
<tr>
<td>1X 1X</td>
<td>(^1\text{X}-\text{X COSY})</td>
<td>COSY only used when X-spin natural abundance &gt; 20%. Sensitivity problems when X has low natural abundance.</td>
</tr>
<tr>
<td>X X</td>
<td>(^1\text{H}-\text{X NOE diff.})</td>
<td>Through-space correlations. ROESY most applicable to &quot;mid-sized&quot; molecules with masses ca. 1-2 kDa.</td>
</tr>
<tr>
<td>X X</td>
<td>(^1\text{H}-\text{X NOE diff.})</td>
<td>Selectivity limited by X-spin observation. Care required to make NOEs specific in presence of proton decoupling.</td>
</tr>
<tr>
<td>X X</td>
<td>(^1\text{H}-\text{X EXSY})</td>
<td>Interchange of spins at chemically distinct locations. Exchange must be slow on NMR timescale for separated resonances to be observed. Intermediate to fast exchange requires lineshape analysis.</td>
</tr>
<tr>
<td>X X</td>
<td>2D (\text{HOESY})</td>
<td></td>
</tr>
<tr>
<td>X X</td>
<td>2D (\text{INADEQUATE})</td>
<td></td>
</tr>
<tr>
<td>A B</td>
<td>1D saturation or inversion transfer.</td>
<td></td>
</tr>
</tbody>
</table>

Measurements based on the nuclear Overhauser effect (NOE) are most often applied after the gross structure is defined and NMR assignments established in order to identify the 3D stereochemistry of a molecule since this effect maps through space proximity between nuclei. The vast majority of such experiments investigate proton-proton NOEs, although in exceptional cases heteronuclear NOEs involving a proton
and a heteroatom have been applied successfully. The final group of experiments correlates nuclei involved in chemical exchange processes that are slow on the NMR timescale and thus give rise to distinct resonances for each exchanging species or sites.\textsuperscript{117}

The greatest use of NMR in chemical research is in the routine characterisation of synthetic starting materials, intermediates and final products. Routine analysis follows a general procedure similar to that summarised in Table 1.7. This general protocol has been greatly influenced by developments which have taken place over the last decade, such as,

- The indirect observation of heteronuclides via proton detection.
- The routine application of 2D methods on a daily basis.
- The application of pulsed field gradients for clean signal selection.
- The wider use of sophisticated data processing procedures which enhance the information content or quality of spectra.

| Table 1.7 . A typical protocol for the routine structure confirmation of synthetic organic materials.\textsuperscript{117} |
|------------------|------------------|------------------|
| Procedure        | Technique        | Information                                              |
| 1D $^1$H spectrum| 1D               | Information from chemical shifts, coupling constants, integrals. |
| 2D $^1$H-$^1$H correlation | COSY | Identify J-coupling relationships between protons |
| 1D $^{13}$C (with spectrum editing) | 1D, (DEPT or APT) | Carbon count and multiplicity determination (C, CH, CH$_2$, CH$_3$). Can be often avoided by using proton-detected heteronuclear 2D experiments |
| 1D heteronuclide spectra (31P, 19F) | 1D | Chemical shifts and homonuclear/heteronuclear coupling constants |
| 2D 1H-$^{13}$C one-bond correlation (with spectrum editing) | HMQC or HSQC (with editing) | Carbon assignments transposed from proton assignments. Proton spectrum dispersed by $^{13}$C shifts. Carbon multiplicities from edited HSQC (faster than above 1D approach). |
| 2D 1H-$^{13}$C long range correlation | HMBC | Correlation identified over two and three bonds. Correlations established across heteroatoms e.g. N and O. Structural fragments pieced together. |
| Through-space NOE correlation | 1D or 2D NOE | Stereochemical analysis: configuration and conformation. |
The first item of this list arises from the near universal adoption of techniques based on proton observation whenever possible. This is mainly to help in overcoming the limitations from the relatively low sensitivity associated with NMR observations. As a result, data are provided in smaller sample quantities and/or provide data in shorter times. The second significant feature is the increased use of a range of 2D methods for routine structural characterisation. The third issue is the development of pulsed field gradients and the enormous impact these have had on the practical implementation of modern techniques.

1.5. Aims of the Thesis.

As stated in the preceding sections, calixarenes have a wide range of applications in environmental chemistry, biology and other fields. Then the synthesis and chemical characterisation of new calixarene derivatives and the analysis of their interaction with polluting neutral and ionic species are matters of considerable interest in the field of Supramolecular Chemistry. On the other hand, the development of tools to clarify the phenomena taking place between host, calixarenes, and guest (ionic or neutral species), specially to overcome inherent problems with existing techniques is always a challenge in this field.

Previous work carried out at the Thermochemistry Laboratory reported the synthesis and characterisation of fully substituted calix[4]arene amino derivatives and their potential as anion and cation binders in solvents such as methanol. The resonance positions of some of the protons was altered by the addition of Pb$^{2+}$, Hg$^{2+}$ and Cd$^{2+}$ producing significant deshielding effects relative to the free ligand. On the other hand, the possibility, via a pH switching mechanism, of rapid unloading of the metal cation from the ligand has been stressed.

This project explores the synthesis of 5,11,17,23-tetrakis(1,1-dimethylethyl)-25,27-bis-[2-(ethylamine)ethoxy] calix[4]arene (L1), 5,11,17,23-tetrakis(1,1-dimethylethyl)-25,27-bis[2-(N-diethylphosphorominate)ethoxy] calix[4]arene (L2) and 5,11,17,23-tetrakis(1,1-dimethylethyl)-25,27-bis-[2-(diethylamino)ethoxy] calix[4]arene (L3), partially substituted calix[4]arene derivatives. These structures are shown in Figure 1.18. Then the interaction of these new ligands and those shown in Figure 1.19 towards metal cations and to protons in acetonitrile at the standard temperature has been explored.
Figure 1.18 New ligands investigated in the present work

Figure 1.19 Calix[4]arene derivatives used to evaluate the metallic mercury electrode in acetonitrile at 298.15 K.
Finally, the interactions taking place between these hosts and ionic guests will be explored using 1D and 2D NMR techniques. The possibility of differentiating between metal cation and proton interactions with these ligands will be evaluated in detail.

Therefore the aims of this thesis are,

1. To synthesise and to characterise partially substituted calix[4]arene derivatives instead of the fully substituted derivatives in order to simplify the system under study.

2. To investigate the cation receptor properties of L3, including their interaction with protons. Thus $^1$H NMR measurements will be carried out to establish the active sites of interaction of these ligands with a variety of metal cations in solution. The composition of the metal-ion L3 complexes will be determined from conductance and $^1$H NMR measurements.

3. To design, build and characterise a mercury electrode as a tool for measuring stability constants of calix[4]arene-mercury (II) complexes in acetonitrile at 298.15 K.

4. To use i) the glass electrode and ii) 2D NMR techniques for investigating interactions between the partially substituted calix[4]arene with metal cations as well as the protonation processes taking place in acetonitrile at 298.15 K.
2. **EXPERIMENTAL PART**

2.1. **List of chemicals used for the synthesis of calix[4]arene derivatives.**

2.1.1. **Chemicals used.**

2-Diethylaminoethyl chloride hydrochloride (p.a. Sigma) was dried under vacuum at 80° C. Anhydrous potassium carbonate (Aldrich), anhydrous potassium hydrogen carbonate (Fluka), 18-crown-6 (18-C-6) (Fluka), diethyl phosphite, 98% (Aldrich), tetra-n-butylammonium bromide (TBAB), 99% (Fluka), tetra-n-butylammonium hydrogen sulphate (TBAHS), 99% (Fluka) and triethylbenzylammonium bromide (TEBA), 99% (Fluka), ethylamine, 97% (Aldrich), propylamine, 98% (Aldrich), 2-(methylamine) ethanol, 98% (Aldrich), 2-(ethylamine) ethanol, 98% (Aldrich), 1,2-dibromoethane, 99% (Aldrich), thionyl chloride, 99% (Aldrich) and sodium hydride (60%), (Aldrich) were used as purchased.

2.1.2. **Purification of solvents.**

Toluene, A.C.S. reagent (Fisher Chemicals), hexane, A.C.S. (Fisher Chemicals) and tetrahydrofuran (THF), HPLC grade without stabilizer (BDH), were dried by refluxing with a sodium wire followed by distillation through a Vigreux column. Acetonitrile (AN) HPLC grade (Fisher Chemicals) was dried by refluxing with CaH$_2$ followed by distillation.$^{120}$ It was used immediately.

N,N-dimethylformamide, (DMF) (Fisher Chemicals) was stored over molecular sieves (4A) and distilled under reduced pressure before use.$^{120}$

Pyridine, A.C.S. reagent (Aldrich) was dried by refluxing with sodium hydroxide pellets for three hours and then distilled.$^{120}$

Dichloromethane, (DCM) A.C.S. reagent, carbon tetrachloride, A.C.S. reagent, methanol, HPLC grade were bought from Fisher Chemicals. Ethanol, 99.86% was from Hayma. These solvents were use without further purification.
2.2. List of chemicals used for building up the electrode and for stability constant measurements.

Mercury (II) perchlorate, \( \text{Hg(ClO}_4\text{)}_2 \), cadmium (II) perchlorate, \( \text{Cd(ClO}_4\text{)}_2 \), lead perchlorate, \( \text{Pb(ClO}_4\text{)}_2 \), magnesium perchlorate, \( \text{Mg(ClO}_4\text{)}_2 \), aluminium perchlorate, \( \text{Al(ClO}_4\text{)}_3 \), sodium perchlorate, \( \text{NaClO}_4 \), and potassium perchlorate, \( \text{KClO}_4 \) were all reagent grade, purchased from Aldrich. These were dried over phosphorus pentoxide, \( \text{P}_4\text{O}_{10} \), under vacuum for five days. Mercury (Aldrich, A.C.S.) was used without further purification. Tetra-n-butylammonium perchlorate - TBAP - (Fluka, electrochemical grade) and perchloric acid 70\% (Fluka, p.a.) were used without further purification.


2.3. General \( ^1 \text{H} \) NMR measurements

\( ^1 \text{H} \) NMR measurements were recorded at 298 K using a Bruker AC-300E pulsed Fourier Transform NMR spectrometer. Typical operating conditions for routine proton measurements involved 'pulse' or flip angle of 300, spectral frequency (SF) of 300.135 MHz, delay time of 1.60 s, acquisition time (AQ) of 1.819 s, and line broadening of 0.55 Hz. Solutions of the samples in question were prepared by dissolving the solid (5-20 mg) in the deuterated solvents (chloroform, CDCl\(_3\); dichloromethane, CD\(_2\)Cl\(_2\); acetonitrile, CD\(_3\)CN; methanol, CD\(_3\)OD). These were then placed in 5 mm NMR tubes using TMS (tetramethylsilane) as the internal reference.

2.4. X-Ray Crystallography.

X-Ray diffraction studies were performed by Dr. Angel Alvarez-Larena, Departamento de Geologia, Universidad Autonoma de Barcelona, Spain; Dr. Oscar E. Piro, Departamento de Fisica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina; and Professor Eduardo E. Castellano at the Instituto de Fisica de Sao Carlos, Universidade de Sao Paulo, Brazil.
The instruments used were an Enraf-Nonius CAD4 Diffractometer (data collected with CAD4 EXPRESS\textsuperscript{122} and reduced with XCAD4\textsuperscript{123} programs) and KappaCCD Diffractometer (data collected with COLLECT\textsuperscript{124} and reduced with DENZO and SCALEPACK\textsuperscript{125} programs). The structures were solved by direct and Fourier methods using the SHELXS\textsuperscript{126} Program and their non-H atom refined by a full matrix least-square method included in SHELXL\textsuperscript{128} Program. The H-atoms were positioned stereochemically and refined with the riding model.


For the calixarene derivative synthesised in the present work, several procedures were carried out. Thus, a secondary amine calix[4]arene derivative synthesis was attempted. The different pathways (schemes 2.1, 2.2 and 2.3) and characterisation methods used for each product are described in the following section.

2.5.1. Phosphorylation of amines — Synthesis of N-alkyl diethyl phosphoroamidates (2a).\textsuperscript{127,128}

**Method A.** A solution of diethyl phosphite (0.125 mol) and ethylamine (0.1 mol) in dichloromethane (30 ml) was added dropwise and under vigorous stirring to a two-phase system consisting of dichloromethane (30 ml), tetrachloromethane (30 ml), 20% aqueous sodium hydroxide (40 ml) and benzyltriethylammonium bromide (1 g). The temperature was kept at 0 – 5°C by cooling the mixture with an ice bath. Stirring was continued for a period of 1 h at the same temperature, then for the same period of time at room temperature. The mixture was diluted with dichloromethane (25 ml), and the organic phase was separated, washed with hydrochloric acid (5%, 50 ml) and water (2 x 50 ml) and dried over anhydrous magnesium sulphate. The solvent was rotavaporated and then the sample was heated at 30 — 40 °C under high vacuum (0.2 mm of Hg) for a period of 1 h. The product was dissolved in CDCl\textsubscript{3} and analysed by \textsuperscript{1}H NMR.

\textsuperscript{1}H NMR, δ (ppm) (CDCl\textsubscript{3}): 1.14 (t, 3H, NCH\textsubscript{2}CH\textsubscript{3}), 1.32 (t, 6H, OCH\textsubscript{2}CH\textsubscript{3}), 2.95 (m, 2 q overlapped, 2H, NCH\textsubscript{2}CH\textsubscript{3}), 4.06 (m, 2 q overlapped, 4H, OCH\textsubscript{2}CH\textsubscript{3}).
Methods A and B

\[ R-NH_2 + \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{CCl}_4 \rightarrow \text{NaOH/TEBA} \]

\[ \text{CH}_2\text{Cl}_2 \rightarrow \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{HCCl}_3 + 2a \]

Methods C and D

\[ \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{Br} - \text{Br} \rightarrow \text{NaOH/NBu}_4 \text{HSO}_4 \]

\[ \text{C}_6\text{H}_5\text{CH}_3 \rightarrow \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{Br} + \text{Br} + 2b \]

Methods A and B

\[ \text{HO} - \text{NH}_2 + \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{CCl}_4 \rightarrow \text{NaOH/TEBA} \]

\[ \text{CH}_2\text{Cl}_2 \rightarrow \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{HCCl}_3 + 2c \]

Methods C and D

\[ R-X + \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) \rightarrow \text{NaOH/TBAB} \]

\[ \text{C}_6\text{H}_5\text{CH}_3 \rightarrow \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{OH} + 2d \]

Method B

\[ \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{R} - \text{OH} + \text{CCl}_4 \rightarrow \text{KHC}O_2/\text{K}_2\text{CO}_3/TBAB \]

\[ \text{CH}_2\text{Cl}_2 \rightarrow \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{HCCl}_3 + 2e \]

Scheme 2.1 Synthetic approach used for the preparation of phosphoryl protected secondary amine (starting material for L1)

\[ + \text{SOCl}_2 \rightarrow \]

\[ \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{SOCl}_2 \rightarrow \text{Pyridine or DMF} \]

\[ \text{Triphenylphosphine} \rightarrow \left( \begin{array}{c} \text{O} \text{P} \text{O} \\ \text{H} \end{array} \right) + \text{X} + \text{Te-CI} \rightarrow \text{Pyridine} \]

\[ 2f \]
Scheme 2.2 Synthetic approach used for the halogenation of the starting material for the preparation of the secondary amine calix[4]arene derivative, L1.

Method B. The second method is an improved version of Method A in which the system's phases were changed from liquid - liquid to solid - liquid in order to employ the milder conditions required for the preparation of these compounds. In a typical procedure, a solution of diethylphosphite (50 mmol) was prepared in tetrachloromethane (~10 ml). This was dropwise added to a mixture of propylamine (50 mmol), potassium hydrogen carbonate (100 mmol), potassium carbonate (100 mmol), dichloromethane (40 ml) and tetra-n-butylammonium bromide (2.5 mmol) under continuous stirring. The temperature was kept at 10 - 15 °C for two hours and then the mixture was left overnight at room temperature. The remaining inorganic salts were filtered and washed with dichloromethane. The solution combined with the washings was evaporated to give a crude phosphoroamidate. It was purified by heating at 40 - 50 °C under high vacuum (0.1 mm of Hg) for 1 hour. It was analysed by $^1$H NMR, δ (ppm) (CDCl₃): 0.85 (t, 3H, NCH₂CH₂CH₃), 1.26 (t, 6H, OCH₂CH₃), 1.43 (m, 2H, NCH₂CH₂CH₃), 2.78 (m, 2H, NCH₂CH₂CH₃), 3.99 (m, 2 q overlapped, 4H, OCH₂CH₃).
2.5.2. Bromoalkylation of N-alkyl diethyl phosphoroamidates
(2b).\textsuperscript{129,130}

Method C. A mixture of N-propyl diethyl phosphoroamidate 2a (0.05 mol), toluene (30 ml), 1,2-dibromoethane (0.055 mol), an aqueous solution of sodium hydroxide (50 \%, 25 ml), and tetra-n-butyl ammonium hydrogen sulphate (0.85 g) was refluxed under vigorous stirring for a period of 4 h. Then toluene (50 ml) was added, the organic layer was separated, washed with water (20 ml), dried with anhydrous magnesium sulphate and evaporated under vacuum. No N-2-bromoethylene-N-propyl diethyl phosphoroamidate (2b) could be isolated.

Method D. A solution of N-propyl diethyl phosphoroamidate 2a (0.02 mol) in toluene (20 ml) was added dropwise under continuous stirring to a suspension of sodium hydride (0.022 mol, previously washed with n-hexane) in toluene (15 ml) for approximately 15 min at 15 - 20 °C. After the evolution of hydrogen has ceased, 1,2-dibromoethane (0.024 mol) and TBAB (0.001 mol) were added and the mixture was refluxed under stirring for a period of 2 hrs. The resulting mixture was cooled down to room temperature, diluted with toluene (50 ml) and washed with water (3 x 20 ml). The organic phase was then dried with anhydrous magnesium sulphate, evaporated and kept at 50 - 60 °C / 0.1 mm of Hg for 1 hour. No N-2-bromoethyl,N-propyl diethyl phosphoroamidate (2b) could be isolated.

2.5.3. Phosphorylation of amines — Synthesis of N-2-hydroxyethyl diethyl phosphoroamidates (2c).\textsuperscript{127,128}

Method B. A solution of diethylphosphite (50 mmol) was prepared in tetrachloromethane (~10 ml) and it was added to a mixture of 2-ethanolamine (50 mmol), potassium hydrogen carbonate (100 mmol), potassium carbonate (100 mmol), dichloromethane (40 ml) and tetra-n-butylammonium bromide (2.5 mmol) under continuous stirring. The temperature was kept at 10 - 15 °C for two hours and then the mixture was left overnight at room temperature. The inorganic salts were filtered and washed with dichloromethane. The solution combined with the washings, was evaporated to give a crude phosphoroamidate. It was purified by heating at 40 - 50 °C under high vacuum (0.1 mm of Hg) for 1 hour.

It was analysed by \textsuperscript{1}H NMR, \( \delta \) (ppm) (CDCl\textsubscript{3}): 1.32 (t, 6H, OCH\textsubscript{2}CH\textsubscript{3}), 3.05 (m, 2H, NCH\textsubscript{2}CH\textsubscript{2}OH), 3.62 (t, 2H, NCH\textsubscript{2}CH\textsubscript{2}OH), 4.02 (m, 2 q overlapped, 4H, OCH\textsubscript{2}CH\textsubscript{3}).
2.5.4. Alkylation of N-2-hydroxyethyl diethyl phosphoroamidate (2d).\textsuperscript{129,130}

**Method C.** A mixture of N-2-hydroxyethyl diethyl phosphoroamidates, 2c (0.05 mol), toluene (30 ml), bromoethane (0.055 mol), an aqueous solution of sodium hydroxide (50\%, 25 ml), and tetra-n-butyl ammonium hydrogen sulphate (0.85 g) was refluxed under vigorous stirring for a period of 4 h. Then toluene (50 ml) was added, the organic layer was separated, washed with water (20 ml), dried with anhydrous magnesium sulphate and evaporated under vacuum. No diethyl phosphoroamidate derivative, 2d, could be isolated.

**Method D.** A solution of N-2-hydroxyethyl diethyl phosphoroamidate, 2c (0.02 mol) in toluene (20 ml) was added dropwise under continuous stirring to a suspension of sodium hydride (0.022 mol, previously washed with n-hexane) in toluene (15 ml) more or less during 15 mm at 15 - 20 °C. After evolution of hydrogen has ceased, bromoethane (0.024 mol) and TBAB (0.001 mol) were added and the mixture was refluxed under stirring for a period of 2 hrs. The resulting mixture was cooled down to room temperature, diluted with toluene (50 ml) and washed with water (3 x 20 ml). The organic phase was then dried with anhydrous magnesium sulphate and then the organic solvent was removed. The remaining residue was kept at 50 - 60 °C / 0.1 mm of Hg for 1 hour. No N,N-dialkyl diethyl phosphoroamidate (2d) could be isolated.

2.5.5. Phosphorylation of amines - Synthesis of N-alkyl, N-2-hydroxyethyl diethyl phosphoroamidates (2e).\textsuperscript{127,128}

**Method B.** A solution of diethylphosphite (50 mmol) was prepared in tetrachloromethane (~10 ml) and it was added dropwise to a mixture of N-methyl-2-ethanolamine (50 mmol), potassium hydrogen carbonate (100 mmol), potassium carbonate (100 mmol), dichloromethane (40 ml) and tetra-n-butylamonium bromide (2.5 mmol) under continuous stirring. The temperature was kept at 10 - 15 °C for two hours and then the mixture was left overnight at room temperature. The inorganic salts were filtered and washed with dichloromethane. The solution combined with the washings was evaporated to give a crude phosphoroamidate. It was purified by heating at 40 - 50 °C under high vacuum (0.1 mm of Hg) for 1 hour. It was analysed by \textsuperscript{1}H NMR, $\delta$ (ppm) (CDCl\textsubscript{3}): 1.33 (t, 6H, CCH\textsubscript{2}CH\textsubscript{3}), 2.71 (2 s, 3H, CH\textsubscript{3}NCH\textsubscript{2}CH\textsubscript{2}OH), 3.21
Several ways were tested to substitute the hydroxyl groups by a halide functional group with the aim of increasing the yield of the starting material. These are discussed as follows,

a) The N-2-hydroxyethyl-N-methyl diethyl phosphoroamidate, 2e (100 mmol) either alone or mixed with pyridine (100 mmol), was added dropwise to thionyl chloride (200 mmol) at 0 - 10 °C. A vigorous stirring was applied during the addition process. Then, the mixture was heated under reflux for 1 hour, after which, ice was added in order to eliminate the excess of thionyl chloride. Two phases were expected. However, a homogeneous solution was obtained and no halogenated product could be isolated.

b) Thionyl chloride (110 mmol), followed by N-2-hydroxyethyl-N-methyl diethyl phosphoroamidate, 2e (100 mmol) was added dropwise to N,N-dimethylformamide at 0 - 10 °C under continuous stirring at room temperature. Then, the mixture was heated to 100 °C for 1 h. After the reaction had taken place, an excess amount of water was added. The upper layer was then washed, dried with anhydrous potassium carbonate and distilled under high vacuum. No halogenated product could be isolated.

c) In a three necked flask with a magnetic stirrer and a reflux condenser (to which a drying tube containing CaCl₂ was attached), carbon tetrahalide* (110 mmol) and N-2-hydroxyethyl-N-methyl diethyl phosphoroamidate, 2e (100 mmol) were placed. To this solution, triphenylphosphine (130 mmol) was added under continuous stirring and it was heated under reflux for a period of 3 h. The mixture was allowed to cool down at room temperature. Then, dry hexane (100 ml) was added and stirring was continued for an additional period of 5 minutes. A suspension was formed and it was filtered and washed with a hexane: CCl₄ (1:1) mixture (50 ml). The solvent was removed from the combined filtrate with a rotary evaporator under vacuum at 50 °C. The crude product was purified by high vacuum distillation (61 - 62 °C at 0.1 mm of Hg for N-2-chloroethyl-N-methyl diethyl phosphoroamidate and 70 °C at 0.1 mm of Hg for the N-2-bromoethyl-N-methyl diethyl phosphoroamidate).
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The bromide derivative gave the following signals after $^1$H NMR analysis, $\delta$ (ppm) (CDCl$_3$): 1.31 (t, 6H, OCH$_2$CH$_3$), 2.72 (2 5, 3H, CH$_3$NCH$_2$CH$_2$Br), 3.36 (m, 2 t overlapped, 2H, CH$_3$NCH$_2$CH$_2$Br), 3.60 (t, 2H, CH$_3$NCH$_2$CH$_2$Br), 4.04 (m, 2 q overlapped, 4H, OCH$_2$CH$_3$).

* in case of CBr$_4$ 80 ml of acetonitrile was added to the mixture in order to dissolve it.

d) N-2-hydroxyethyl, N-methyl diethyl phosphoroamidate, 2e (0.22 mmol), was placed in an ice-cooled three necked round bottom flask for 10 minutes. Then pyridine (0.44 mmol) was added and the solution was stirred for a further period of 10 minutes until a clear solution was obtained. Tosyl chloride (0.33 mmol) added dropwise to this solution. The mixture was left under continuous stirring for a period of 4 hours. Then the crude product was purified by high vacuum distillation. The $^1$H NMR analysis gave the following signals: $\delta$ (ppm) (CDCl$_3$): 1.18 (t, 3H, NCH$_2$CH$_3$), 1.28 (m, 2 t overlapped, 6H, OCH$_2$CH$_3$), 2.45 (s, 3H, ArCH$_3$), 3.03 (m, 2 q overlapped, 2H, NCH$_2$CH$_3$), 3.30 (m, 2 q overlapped, 2H, NCH$_2$CH$_2$OTs), 4.07 (q, 4H, OCH$_2$CH$_3$), 4.19 (t, 2H, NCH$_2$CH$_2$OTs), 7.36 (d, 2H, ArH-CH$_3$), 7.80 (d, 2H, ArH-SO$_2$).

2.5.7. Attachment of starting material (halogenated or tosyalted diethyl phosphoroamidate) to p-tert-butylcalix[4]arene.$^{132,133}$

In this step, again, several, alternative ways were attempted with the aim of obtaining a substituted calix[4]arene derivative. These are now described,

a) p-tert-Butylcalix[4]arene (0.01 mol), potassium carbonate (0.08 mol) and 18-crown-6 (0.004 mol) were placed in a three-necked round bottom flask containing a magnetic stirrer and a reflux condenser in an inert atmosphere. Acetonitrile (100 ml) was added and the mixture was stirred for 30 ml at room temperature. Then the halogenated or tosyalted diethyl phosphoroamidate starting material, 2e, was slowly added and the mixture was refluxed at 80 °C for 7 days. The reaction was monitored by TLC using dichloromethane:methanol (9:1). The mixture was allowed to cool down and the solvent was removed under vacuum. The solid was extracted with dichloromethane (100 ml), filtered, and washed with dichloromethane (20 ml). The solution afforded was dried with anhydrous sodium sulphate and evaporated under vacuum. The residue was recrystallised from methanol. No evidence of the product 2g was found by $^1$H NMR analysis.
b) A mixture of sodium hydride (0.06 mol) and p-tert-butylcalix[4]arene (0.01 mol) was stirred with N,N-diethylformamide (20 ml) for 15 min. at room temperature in a three-necked round bottom flask with reflux condenser in an inert atmosphere. Then, the halogenated or tosylated diethyl phosphoroamidate derivative, 2e (0.08 mol) was dissolved in THF and dropwise added to the mixture under vigorous stirring. The mixture was refluxed at 80 °C for 5 days. The course of the reaction was monitored by TLC using a dichloromethane:methanol (9:1) mixture as developing solvent. After cooling, the solvent was removed under vacuum. The residue was dissolved in dichloromethane and extracted with HCl (2 mol dm$^{-3}$), saturated solution of NaHCO$_3$ and washed with distilled water. The organic phase was dried with anhydrous magnesium sulphate and the solvent removed under vacuum. The crude product was recrystallised from ethanol. No evidence of substitution was found after $^1$H NMR analysis.


This procedure involves three steps all of them with yields over 70%.


The partially substitution of the phenolic protons with the cyanomethoxy groups was achieved using the K$_2$CO$_3$/18-crown-6/CH$_3$CN system as it is shown in Scheme 2.4,

![Scheme 2.4](image)

Scheme 2.4 Synthesis of 5,11,17,23-tetrakis(1,1-dimethylethyl)-25,27-bis (cyanomethoxy) calix[4]arene
In a typical experiment, \( p \)-tert-butyl calix[4]arene (8 mmol), potassium carbonate anhydrous (32 mmol) and 18-crown-6 (5 mmol %) were dissolved in dry acetonitrile. The solution was stirred for 15 minutes. Then bromoacetonitrile (32 mmol) dissolved in acetonitrile was added dropwise and the mixture was heated at 80°C. The progress of the reaction was followed by TLC (dichloromethane:methanol, 9:1). After the reaction was complete, the mixture was filtered and the residue was washed with dichloromethane. The filtrate was rotary evaporated and the residue was suspended in methanol, refluxed for 1 hour and left overnight. A white solid was recovered by filtration and it was washed with methanol. The product was dried under vacuum at 80°C and used without further purification. \(^1\)H NMR analysis : \( \delta \) (ppm) (CDCl\(_3\)): 0.88 (s, 18H, t-Bu/-OR), 1.33 (s, 18H, t-Bu/-OH), 3.45 (d, 4H, J=13.5, eq-CH), 4.23 (d, 4H, J=12.6, ax-CH), 4.81 (s, 4H, OCH\(_2\)CN), 6.73 (s, 4H, ArH/-OR), 7.12 (s, 4H, ArH/-OH).


The reduction of 3a to 3b was carried out using LiAlH\(_4\) as shown in Scheme 2.5,

![Scheme 2.5 Reduction of the 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-bis-(cyanomethoxy) calix[4]arene](image)

So, LiAlH\(_4\) (40 mmol) was suspended in freshly distilled tetrahydrofuran (100 ml) in a three necked round bottom flask at 0°C in inert atmosphere (N\(_2\)). A solution of 3a (8 mmol) in tetrahydrofuran was added under vigorous stirring for one hour. Then it was stirred for a further period of 4 hours at room temperature. The reaction was stopped by addition of a NaOH solution (5 ml, 20%) and water (25 ml). The precipitate formed was filtered and washed with tetrahydrofuran. The filtrate was rotary evaporated and the yellowish solid obtained was recrystallised from ethanol. \(^1\)H NMR analysis : \( \delta \) (ppm) (CDCl\(_3\)): 1.10 (s, 18H, t-Bu/-OR), 1.25 (s, 18H, t-Bu/-OH), 3.31 (t, 4H, OCH\(_2\)CH\(_2\)NH\(_2\)), 3.32 (t, 4H, OCH\(_2\)CH\(_2\)NH\(_2\)), 4.25 (t, 4H, OCH\(_2\)CH\(_2\)NH\(_2\)), 7.12 (s, 4H, ArH/-OR).
3.37 (d, 4H, J=14, eq-CH), 4.07 (t, 4H, OCH₂CH₂NH₂), 4.33 (d, 4H, J=14, ax-CH), 6.97 (s, 4H, ArH/-OR), 7.04 (s, 4H, ArH/-OH).


The procedure shown in Scheme 2.6 was used to introduce the diethyl phosphoryl group in the partially substituted calixarene derivative.

[Scheme 2.6 Introduction of the diethylphosphoryl group in 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-bis-(2-aminoethoxy) calix[4]arene]

Diethyl phosphite (10 mmol) was dissolved in CCl₄ (10 ml) and placed in a three necked round bottom flask containing the calix[4]arene amino derivative, 3b (4 mmol). Then, potassium carbonate (16 mmol), potassium hydrogen carbonate (16 mmol) and TBAB (0.8 mmol) slurry in dichloromethane (40 ml) at 0 °C were added. The mixture was then stirred at room temperature for 48 hrs and filtered. The solid obtained was washed with 3 portions of dichloromethane and the filtrate was rotary evaporated. The solid obtained was recrystallised from acetonitrile.

$^1$H NMR analysis: δ(ppm) (CDCl₃); 1.14 (5, 18H, t-Bu/-OR), 1.23 (s, 18H, t-Bu/-OH), 1.32 (t, 12H, OCH₂CH₃), 3.37 (d, 4H, J=13, eq-CH), 3.62 (b q, 2 t overlapped, 4H, OCH₂CH₂NH), 4.11 (q, 8H, OCH₂CH₃), 4.15 (t, 4H, OCH₂CH₂NH), 4.34 (d, 4H, J=12, eq-CH), 7.02 (s, 8H, ArH), 8.57 (s, 2H, ArOH).

δ(ppm) (CD₃CN); 1.16 (s, 18H, t-Bu/-OR), 1.21 (s, 18H, t-Bu/-OH), 1.29 (t, 12H, OCH₂CH₃), 3.45 (d, 4H, J=13, eq-CH), 3.52 (m, 2 t overlapped, 4H, OCH₂CH₂NH), 4.07 (m, 2 q overlapped, 8H, OCH₂CH₃), 4.12 (t, 4H, OCH₂CH₂NH), 4.31 (d, 4H, J=13, eq-CH), 7.21 (s, 4H, ArH/-OR), 7.26 (s, 4H, ArH/OH), 8.55 (s, 2H, ArOH).

Partially substituted calix[4]arene derivatives were prepared, in order to reduce the hosting ability of the fully substituted calix[4]arene amino derivatives for mercury (II) and other cations by the formation of 1:1 rather than 2:1 (metal cation:ligand) complexes.

Both, 18-crown-6 and TBAB were used as phase transfer catalysts. Potassium carbonate and potassium carbonate/potassium hydrogen carbonate system were used as bases, and acetonitrile and dichloromethane as solvents, respectively.

\[
\begin{align*}
\text{Scheme 2.7 \ Preparation of 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-} \\
\text{bis-[2-(diethylamino)ethoxy] calix[4]arene}
\end{align*}
\]

Thus, p-tert-butylcalix[4]arene, (12.5 mmol) the phase transfer catalyst, (2.5 mmol) and the base (50.0 mmol) were placed in a three neck round bottom flask with 100 ml of the solvent (acetonitrile or dichloromethane). The mixture was stirred for 30 minutes and then the N-2-chloroethyl diethylamine, (50.0 mmol) previously dissolved in the respective solvent, was dropwise added. Then, the mixture was heated in an oil bath at 80 °C. The reaction was monitored by TLC using both, dichloromethane:methanol (9:1) and hexane:ethylacetate (6:4) mixtures as developing solvents and iodine vapour as the developer. The Rts obtained were compared with those for the pure substances.

At the end of the reaction, the solvent was evaporated under vacuum and the residue was dissolved in a mixture of water/dichloromethane. Then, the organic phase was separated, washed with hydrochloric acid (0.2 mol.dm\(^{-3}\)), sodium hydrogen carbonate (saturated solution) and distilled water. Finally, it was dried with magnesium sulphate.
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The solvent was removed under vacuum and the residue was recrystallised from ethanol and acetonitrile.

\(^1\)H NMR analysis: \(\delta\) (ppm) (CDCl\(_3\)); 0.93 (s, 18H, t-Bu/-OR), 1.10 (t, 12H, NCH\(_2\)CH\(_3\)), 1.29 (s, 18H, t-Bu/-OH), 2.68 (q, 8H, OCH\(_2\)CH\(_3\)), 3.08 (t, 4H, OCH\(_2\)CH\(_2\)N), 3.29 (d, 4H, \(J = 13\), eq-CH), 4.02 (t, 4H, OCH\(_2\)CH\(_2\)N), 4.32 (d, 4H, \(J = 13\), eq-CH), 6.76 (s, 4H, ArH/-OR), 7.05 (s, 4H, ArH/-OH), 7.27 (s, 2H, ArOH).

2.8. \(^1\)H NMR studies on the interaction of partially substituted tertiary amino calix[4]arene derivatives with metal cations and the proton.

\(^1\)H NMR titration experiments were carried out to assess the interaction of partially substituted tertiary amine calix[4]arene derivatives with metal cations and the proton in deuterated acetonitrile and deuterated methanol. In a typical experiment, a solution of \(L3\) (~1.00 x 10\(^{-3}\) mol.dm\(^{-3}\) in the deuterated solvent, 500 \(\mu\)l) was placed in a NMR tube and the spectrum of the ligand was recorded. Then, aliquots of the metal ion-salt solution (as perchlorate, ~1.00 x 10\(^{-2}\) mol.dm\(^{-3}\) in deuterated solvent) were added to the ligand solution in the NMR tube (10 - 25 \(\mu\)l). A new NMR spectrum was recorded after each addition. The list of chemical shifts was printed out and maintained as a hard copy to facilitate the calculations. A spreadsheet was used to calculate the \(\Delta\delta\) for each main signal with respect to those of the free ligand. Then, the next convention (eq. 25) was used to interpret the results,

\[
\Delta\delta_n = \delta_n - \delta_o = \begin{cases} \frac{\Delta\delta_n}{\Delta\delta_o} > 0 \Rightarrow de-shielding \\ \frac{\Delta\delta_n}{\Delta\delta_o} < 0 \Rightarrow shielding \end{cases} \tag{25}
\]

In Eq. 25, \(\delta_o\) and \(\delta_n\) are the proton chemical shifts of the ligand solution, before and after addition of the metal-cation salt solution, respectively. \(\Delta\delta\) denotes the chemical shift change of these protons. A positive \(\Delta\delta\) indicates de-shielding while shielding effects lead to negative \(\Delta\delta\) values.

All \(^1\)H NMR measurements were recorded at 298 K using a Bruker AC-300E pulsed Fourier Transform NMR spectrometer. Typical operating conditions for routine proton measurements involved 'pulse' or flip angle 30\(^\circ\), spectral frequency (SF) of 300.135 MHz, delay time 1.60 s, acquisition time (AQ) of 1.819 s, line broadening 0.55 Hz. The NMR standard used was TMS.
2.9. Conductance Measurements

Conductance measurements were performed using the Wayne-Kerr Autobalance Universal Bridge, type B642. The conductance cell consists of a vessel of about 50 ml of capacity containing two platinum black electrodes inside of a glass support tube. The conductance cell and its contents were maintained at 298.15 ± 0.01 K in a thermostated bath. The cell constant (θ) of the conductivity cell was determined using the method described by Jones and Bradshaw. The cell containing deionised water was kept in a thermostated bath at 298.15 K at least for one hour. A solution of KCl (0.1 mol.dm⁻³) was added by step additions. The corresponding molar conductances, \( \Lambda_m \), were calculated from the equation of Lind, Zwolenik and Fuoss, (Eq. 26),

\[
\Lambda_m = 149.93 - 94.65 c^{1/2} + 58.74 c \log c + 198.4 c
\]

The molar conductances of KCl solutions were used to calculate the specific conductivity, \( \kappa \) (eq. 27);

\[
\Lambda_m = \frac{1000.\kappa}{c}
\]

From the \( \kappa \) values, (\( \kappa = S/\theta \), where S is the reciprocal of the resistance) the cell constant, \( \theta \), was calculated, from Eq. 28;

\[
\theta = \frac{\Lambda_m C}{1000.5S}
\]

Prior to the experimental run, the conductance cell was dried and weighed accurately. It was then filled with the metal cation solution as to cover the platinum electrodes. The cell was inserted in a thermostated bath and left to reach thermal equilibrium while a slow stream of dried nitrogen was passed through the solution. The purpose of using dry nitrogen was i) to prevent contamination of the solvent with CO₂ and moisture from the atmosphere, ii) to facilitate mixing of the solution and iii) to inhibit electrolyte adsorption on the electrodes. To minimise the stirring effect and to obtain consistent resistance readings, the bridge was balanced immediately after the nitrogen flow had been shut off. Then, the conductivity was measured. Small quantities of the stock solution were added from a syringe. Readings were taken once thermal equilibrium was achieved and no changes in conductance were observed.
The experiments were carried out using a solution of the metal-ion salt in the appropriate solvent as the 'titrand' and the ligand solution as the 'titrant'. Thus, the solution containing the metal-cation salt, (concentration range from $1 \times 10^{-4}$ to $3 \times 10^{-4}$ mol.dm$^{-3}$) in the appropriate solvent was titrated with a solution of the ligand, (concentration range from $0.99 \times 10^{-4}$ to $2 \times 10^{-3}$ mol.dm$^{-3}$ in the same solvent). A plot of molar conductance, $\Lambda_{m}$, against the ligand-metal ion concentration ratio ($C_L / C_{M^{n+}}$) was used to determine the stoichiometry of the metal-ion complex.


A Metrohm 716 DMS Titrino potentiometric automatic titrator was used to collect the data of the calibration and titration curves of mercury (II) and its complexes with calix[4]arene derivatives. A set of indicator and reference electrodes was conditioned and used. At the end of the process, the data was transferred to a PC using a DB25-DB9 serial cable and the Microsoft Windows's HyperTerminal Program. The data was processed using Microsoft Excel spreadsheets and the Analysis Tools included in it.

2.10.1. Assembling the metallic mercury electrode

A piece of gold wire (5 cm x 1 mm of diameter, 99.999%, Aldrich) was heat-fitted at the end of a glass body electrode and used as support for a thin layer of mercury. The electrode was prepared by washing the gold electrode with water: nitric acid (1:1), water and acetone, successively. Then a thin layer of mercury was deposited on its surface by an electrolytic process against a platinum electrode (anode) in a Hg(ClO$_4$)$_2$ solution ($0.01$ mol.dm$^{-3}$) as electrolytic bath. In order to obtain accurate and repetitive results, the electrode was prepared on daily basis maintaining constant the electrolytic bath composition, the electrolysis time and the surface of the gold electrode covered. The mercury electrode, designed during the course of these investigations, is shown in Figure 2.1.

2.10.2. Conditioning of the reference electrode

In the experiments carried out in the organic solvent (acetonitrile), two different reference electrodes were used. The first one consisted of a concentration cell with the indicator electrode. Then a small mercury electrode was assembled into a double junction glass body electrode and dipped in a Hg(ClO$_4$)$_2$ solution ($9 \times 10^{-3}$ mol.dm$^{-3}$).
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The second reference electrode was the well known double junction Hg/Hg₂Cl₂/KCl (calomel) electrode whose internal solution was replaced by a saturated solution of LiCl in ethanol (in order to provide compatibility with the organic solvent). The external compartment of these electrodes was used as a salt bridge in order to avoid the interference of chloride ions with the mercury electrode. It was refilled with tetra-n-butylammonium perchlorate solution in acetonitrile.

### 2.10.3. Setting up the experimental array.

In a typical procedure, the apparatus shown in Figure 2.2 was used. Firstly, a calibration curve was constructed. For this purpose, a solution of TBAP (20 ml, 0.1 mol.dm⁻³) in acetonitrile was placed in the titration vessel. Then, the mercury and the reference electrodes were placed in the vessel using a suitable lid and an inert atmosphere was provided by passing N₂ through the solution. The system was left under stirring for one hour to reach equilibrium. Then, twenty additions of a standardised Hg(ClO₄)₂ solution (~0.001 mol.dm⁻³) in TBAP (0.10 mol.dm⁻³ in acetonitrile) were made. Readings were taken each 180 seconds after each addition.
This waiting time was established after carrying out several titrations under the same experimental conditions in order to improve the electrode response to low mercury (II) concentrations in solution. In all cases, the temperature was maintained at 298.15 K. After calibrating the electrode, the vessel was filled with a solution of calix[4]arene derivative (\(\sim 1.0 \times 10^{-4} \text{ mol.dm}^{-3}\)) in TBAP 0.10 mol.dm\(^{-3}\) in acetonitrile. Then, the electrodes were immersed in the solution and the system was left under stirring for 30 minutes. The same solution of the Hg(ClO\(_4\))\(_2\) described above was added using both constant and variable volume increments (10-50 ul).

A mathematical model was designed for the calculation of the stability constant and this is described under the Results and Discussion Chapter.

![Experimental array used for potentiometric measurements](image)

**Figure 2.2** Experimental array used for potentiometric measurements


In order to evaluate the protonation constants of 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-bis-[2-(diethylamine)ethoxy] calix[4]arene (L3) in acetonitrile, perchloric acid and a glass electrode were used in conjunction of the TITRINO 716 DMS interfaced to a PC for data acquisition and processing. A similar experimental array to that for the mercury electrode was used; (Figure 2.3) including a inert atmosphere and controlled temperature.
In a typical procedure, TBAP (20 ml, 0.1 mol.dm\(^{-3}\)) was poured into the titrator cell and left to stand for a few minutes to stabilize the temperature. Then, perchloric acid (2.0 ml, 8.00 \(\times\) \(10^{-3}\) mol.dm\(^{-3}\)) was added in small portions to the solution in the cell and the potential was recorded to establish the calibration curve for the glass electrode. Then, the solution in the cell was replaced by a solution of the ligand (20 ml, \(8.00 \times 10^4\) mol.dm\(^{-3}\)) to be titrated with the same acid as that used during the calibration. The data were recorded and processed to obtain the protonation constant values using a mathematical model described under the Chapter on Results and Discussion.


Using the experimental array shown in Figure 2.3, the interaction between L3 and heavy metal cations was investigated. In a typical experiment, to a solution containing the protonated ligand, a solution of the metal ion salt (\(\sim 8.00 \times 10^{-3}\) mol.dm\(^{-3}\)) was added and the pH was recorded again. Then, both protonation and interaction curves were analysed to determine the stability constants of the metal cation complex in the appropriate solvent.
2.13. 2D Nuclear magnetic resonance studies of calix[4]arene derivative interactions.

In this specific case, it is important to clarify that when two-dimensional techniques are discussed, there are referred to two frequency dimensions, while the so-called one-dimensional methods have only one. This two frequency dimensions may represent any combination of chemical shifts or scalar couplings. These methods find such an extensive use in chemical research due to their ability to map out interactions within, and sometimes between molecules of interest. Through-space coupling provides the basis for the nuclear Overhauser effect (NOE) which is most often employed to identify molecular stereochemistry and conformation.\(^{138}\)

Following the protocol suggested in the previous chapter, some two-dimensional techniques were applied to study the structure of the ligand and its complexes. These are now discussed.

i) Correlations through the chemical bend: Homonuclear shift correlation - Double-Quantum Filtered COSY.

The DQF-COSY\(^ {139}\) pulse sequence consists of three pulses, where the third pulse converts part of the multiple-quantum coherence into observable single-quantum coherence detected during the acquisition period. One advantage of the DQF-COSY experiment is the phase-sensitivity, i.e., the cross peaks can be displayed with pure absorption lineshapes in both the F1 and the F2 dimension. In general, a phase-sensitive spectrum has a higher resolution than an otherwise equivalent magnitude spectrum because the magnitude lineshape is broader than the pure absorption lineshape.\(^ {140}\)

Another advantage is the partial cancellation of the diagonal peaks in a DQF-COSY spectrum: Thus, the diagonal ridge is much less pronounced in a DQF-COSY spectrum than in a normal COSY spectrum. A third advantage of the double quantum
filter is the elimination of strong signals, e.g., the solvent $^1\text{H}$ which does not experience homonuclear J-coupling. In a typical experiment, the acquisition parameters listed in Table 2.1 and the processing parameters of Table 2.2 have been used to analyse free ligand, metal ion complex and protonated ligand solutions.

### Table 2.1 DQF-COSY acquisition parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>cosvrfh</td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>1 k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>16</td>
<td>The number of scans should be $4 \times n$</td>
</tr>
<tr>
<td>DS</td>
<td>4</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td>3 dB</td>
<td>high power level on F1 channel ($^1\text{H}$) as determined in Section 5.2.4</td>
</tr>
<tr>
<td>P1</td>
<td>10.25 usec</td>
<td>$^1\text{H}$ 90° pulse on F1 channel as determined in Section 5.2.4.</td>
</tr>
<tr>
<td>P0</td>
<td>P1*0.5</td>
<td>$^1\text{H}$ 45° pulse</td>
</tr>
<tr>
<td>D0</td>
<td>3 usec</td>
<td>incremented delay (t1); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td>relaxation delay; should be about $1.25 \times T_1(\text{H})$.</td>
</tr>
</tbody>
</table>

### Table 2.2 DQF-COSY processing parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>1024</td>
<td></td>
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<tr>
<td>PH_mod</td>
<td>Pk</td>
<td>determine 0°- and 1°-order phase correction with phasing subroutine.</td>
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<tr>
<td>PKNL</td>
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<td>necessary when using the digital filter.</td>
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<table>
<thead>
<tr>
<th>F1 Parameters</th>
<th>F2 Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>1 k</td>
</tr>
<tr>
<td>PH_mod</td>
<td>Pk</td>
</tr>
<tr>
<td>MC2</td>
<td>QF</td>
</tr>
</tbody>
</table>
Correlations through the space: The Nuclear Overhauser effect - NOESY.

Nuclear Overhauser Effect Spectroscopy is a 2D spectroscopy method whose aim is to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear $^1$H technique.

The basic NOESY sequence consists of three $\pi/2$ pulses. The first pulse creates transverse spin magnetization. This recesses during the evolution time $t_1$, which is incremented during the course of the 2D experiment. The second pulse produces longitudinal magnetization equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for the mixing period $\tau_m$. Note that, for the basic NOESY experiment, $\tau_m$ is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time $t_2$. The NOESY spectrum is generated by a 2D Fourier transform with respect to $t_1$ and $t_2$. Axial peaks which originate from magnetization that has relaxed during $\tau_m$, can be removed by the appropriate phase cycling.

NOESY spectra can be obtained in 2D absorption mode. Occasionally, COSY-type artifacts appear in the NOESY spectrum; however, these are easy to identify by their anti-phase multiplet structure. The NOESY pulse sequence is shown in the next figure.

The delay $d_8$ determines the length of the mixing period, during which NOE build up occurs. In a typical experiment, the acquisition parameters listed in Table 2.3 and the processing parameters of Table 2.4, have been used.
Table 2.3 NOESY acquisition parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>1024</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>16</td>
<td>the number of scans must be 8 * n</td>
</tr>
<tr>
<td>DS</td>
<td>4</td>
<td>number of dummy scans</td>
</tr>
<tr>
<td>PL1</td>
<td>3 dB</td>
<td>high power level on F1 channel (1H).</td>
</tr>
<tr>
<td>PI</td>
<td>10.25 usec</td>
<td>1H 90° pulse</td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td>relaxation delay; should be about 1.25 * T1(1H).</td>
</tr>
<tr>
<td>D8</td>
<td>500 msec</td>
<td>mixing time for NOE build-up; should be on the order of T1(1H).</td>
</tr>
</tbody>
</table>

Table 2.4 NOESY processing parameters

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<td>SF</td>
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<td>spectrum reference frequency (1H).</td>
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<tr>
<td>WDW</td>
<td>QSINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>choose pure cosine wave.</td>
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<tr>
<td>PH_mod</td>
<td>pk</td>
<td>apply 0°- and 1°-order phase correction determined by phase correcting the first row.</td>
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<tr>
<td>PKNL</td>
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<td>necessary when using the digital filter.</td>
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</tbody>
</table>

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<th>Comments</th>
</tr>
</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>SF</td>
<td>500.13</td>
<td>spectrum reference frequency (1H).</td>
</tr>
<tr>
<td>WDW</td>
<td>QSINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>choose pure cosine wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>pk</td>
<td>first determine 0°- and 1°-order phase correction with phasing subroutine.</td>
</tr>
<tr>
<td>MC2</td>
<td>States-TPPI</td>
<td>States-TPPI results in a forward complex FT.</td>
</tr>
</tbody>
</table>
3. RESULTS AND DISCUSSION

Following the same sequence stated in the Experimental Chapter, the synthetic pathway used and the results obtained are reported and discussed in the following section.


Calix[4]arene tertiary amine derivatives have been used in complexation/extraction processes of heavy metal cations for environmental purposes.\textsuperscript{119,121} These are shown in Figure 3.1,

![Figure 3.1 Tertiary Amine calix[4]arene derivatives.](image)

However there are no reports on investigations involving secondary amine derivatives of calix[4]arene (Figure 3.2). These calix[4]arene derivatives have promising characteristics because of the presence of additional protons attached to the nitrogen atoms and hence the difference in basicity with respect to these containing tertiary amine functional groups. It is expected that their complexing properties will be affected although the possibility of recycling these compounds via a pH switching mechanism after loading them with the cation still exists. On the other hand, the possibility of binding anions via hydrogen bond formation has also been considered.
Thus, in schemes 2.1, 2.2 and 2.3, the synthetic approaches designed for the synthesis of L1 have been described. These pathways are supported by the availability, reactivity and low cost of phosphorus derived reagents. The diethyl phosphite has been widely used as starting material in organophosphorous chemistry and its reactivity is well established. Surprisingly, a peculiar $^1$H NMR was obtained (Figure 3.3), where a second signal for the proton attached to the phosphorus was observed. This is attributed to the named ‘phosphite/phosphoryl equilibrium’ shown in the same figure.

As a result of this equilibrium, characteristic signals are found at 4.05 and 1.25 ppm, both as multiplets resulting from the overlapping of two quartets and two triplets, respectively. These are characteristic of the $^1$H NMR spectrum of the diethyl phosphoryl group, which are frequently found in the spectra of their derivatives. A discussion on the methods used and the results found follows.
3.1.1. Phosphorylation of amines - Synthesis of N-alkyl diethyl phosphoro-amidates (2a).

**Method A.** For the liquid - liquid system used (NaOH 20% or 50% / organic solvent) in the reactions (Method A) to obtain 2a; the conditions were relatively strong due to the low stability of the phosphoroamidates in strong alkaline media, As a result the hydrolysis of the compound occurred.

This statement is supported by the $^1$H NMR spectrum of the N-ethyl diethyl phosphoro.amidate in CDCI$_3$ shown in Figure 3.4. This explains why low yields (<50%) were obtained and the sample was always contaminated with side products. The fact that the starting material, products and some of the side products are liquid, and these are characterised by high boiling points led to the use of high vacuum fractional distillation to isolate and purify these compounds. Higher yields for these systems are reported in the literature.$^{127}$

**Method B.** The solid - liquid system (KHCO$_3$, K$_2$CO$_3$ / organic solvent) was found to be most effective, due to the milder conditions used. Consequently, high yields of the compounds 2a were obtained (>95%).

Figure 3.5, shows the $^1$H NMR spectrum of the N-propyl diethyl phosphoroamidate (2a). Characteristic chemical shifts for the propyl group can be observed, ($\delta$(ppm) 2.84, CH$_3$CH$_2$CH$_2$NH, 1.49 CH$_3$CH$_2$CH$_2$NH and 0.90 CH$_3$CH$_2$CH$_2$NH). The signals of the diethyl phosphoryl group at 4.04 and 1.31 ppm are also shown. The signal for the hydrogen attached to the nitrogen atom can be observed at $\delta$=2.56 ppm.
Additional signals due to the impurities in the $^1$H NMR spectrum of 2a are also observed at 3.37, 1.67, 147 and 1.01 ppm. These signals were assigned to the phase transfer catalyst, TBAB, which remain in the sample due to its solubility in 2a. This material was used without further purification in the next step.

![Figure 3.5 $^1$H NMR of N-propyl diethyl phosphoroamide in CDC$_3$ at 298 K](image)

### 3.1.2. Bromoalkylation of the N-alkyl diethyl phosphoroamidates (2b)

**Method C.** The second step, (synthesis of 2b, Scheme 2.1) using 1,2-dibromo ethane, failed, because the final compound, 2-bromoethyl diethylphosphoroamide, 2b, could not be isolated. The high reactivity of the 1,2-dibromoethane and the strong conditions used could be the reason why this product was not obtained. Instead several side products were found. Based on these findings the synthesis of 2c shown in Scheme 2.1 was carried out.

### 3.1.3. Synthesis of N-2-hydroxyethyl diethyl phosphoroamide (2c)

Given that the KHCO$_3$/K$_2$CO$_3$/TBAB system has shown a good performance and selectivity, this system was adopted for the synthesis of 2c. A good yield of 2c (>95%) was obtained and a high selectivity of this base (KHCO$_3$) for the amine against the hydroxyl group was observed. The $^1$H NMR spectrum (Figure 3.6) shows a significant downfield shift of the proton signals of the ethylene bridge situated between the hydroxyl and the amine groups. The high electro-negativity of the oxygen and the nitrogen atoms is the reason why a high de-shielding effect was observed in the spectra. Some impurities, mainly TBAB were shown, but this did not present problems because the same phase transfer catalyst was used in the following step.
3.1.4. Alkylation of the N-2-hydroxyethyl diethyl phosphoroamidate (2d)

This step involves the introduction of a new alkyl residue in the N-2-hydroxyethyl diethyl phosphoroamidate prepared in the last step. The final product could not be isolated after applying both methods, C and D. This could be attributed to the lack of selectivity of the deprotonating agent for the amine or hydroxyl groups due to the relatively strong conditions required in this step. As a result a complex mixture of compounds was obtained. Then, an alternative synthetic procedure was explored.

3.1.5. Synthesis of N-alkyl, N-2-hydroxyethyl diethyl phosphoroamidates (2e).

The commercial availability of N-alkyl ethanol amines made possible the synthesis of 2e. The solid - liquid system, [KHCO₃/K₂CO₃/TBAB]/AN was used. Good yields (>95%) and a shorter synthetic procedure were successfully achieved. The relative high purity of the compound obtained allowed the use of this compound without further purification. Thus, the ¹H NMR spectrum of 2e is shown in Figure 3.7. Characteristic signals of the diethyl phosphoryl group are shown at values of 4.04 and 1.31 ppm. The signals of the ethylene bridge protons are in δ(ppm) 3.21, m, 2 t overlapped, 2H, CH₃NCH₂CH₂OH), 3.72, t, 2H, CH₃NCH₂CH₂OH. On the other hand, the protons of the methyl group attached to the nitrogen atom showed split signals (δ(ppm), 2.71, 2 s, 3H, CH₃NCH₂CH₂OH) due to the resonance of the phosphoroamidate group.
This led to a partial double bond (C=N) and consequently its hindered rotation (Figure 3.8), which affect other groups in the same molecule.\textsuperscript{145}

![Figure 3.7](attachment:image.png)\textbf{Figure 3.7} \textsuperscript{1}H NMR of N-methyl,N-2-hydroxyethyl diethyl phosphoroamidate 2e in CDCl\textsubscript{3} at 298K

![Figure 3.8](attachment:image.png)\textbf{Figure 3.8} Partial double bond in diethyl phosphoroamidate derivatives.

In order to attach the diethyl phosphoroamidate derivative to the parent calix[4]arene it was necessary to introduce a good leaving group (halogen or tosyl groups). The results of these experiments are shown in the following sections.

\textbf{3.1.6. Substitution of the hydroxyl group of the phosphorylated starting material (2e) by halide or tosyl groups (2f).}

The substitution of the hydroxyl groups by halide or tosyl groups was attempted through several ways. The use of thionyl chloride in any of its conventional procedures (alone or in presence of a base such as pyridine or dimethylformamide) was always difficult due to the release of hydrogen chloride\textsuperscript{145} during the reaction, which could hydrolyse the diethyl phosphoryl derivatives. This led to complex mixtures, which were difficult to resolve by conventional methods.
Chapter 3 – Results and Discussion

The use of other phosphorous derivatives, again led to better yields due to the softer reaction conditions used. In this case, the combination of triphenylphosphine (TPP) with carbon tetrahalides gave 2f with yields over 90%. It was found that the isolated compound contained small amounts of triphenylphosphine oxide as impurity. This was removed by distillation under high vacuum (bp. 62-63° C at 0.08 mm Hg for the chlorine derivative). The \(^1\)H NMR spectra for the purified substituted products are shown in Figure 3.9.

![Figure 3.9](image)

Figure 3.9 \(^1\)H NMR of (a) N-2-chloroethyl, N-methyl diethylphosphoroamidate
(b) N-2- bromoethyl, N-methyl diethylphosphoroamidate,
(c) N-methyl, N-2-tosylethoxy diethyl phosphoroamidates,
2f, in CDCl\(_3\) at 298 K
The substitution of hydroxyl by halide or tosyl groups led to the expected chemical shift changes of the protons of the ethylene bridge. This was due to the differences in electro-negativities between oxygen and the halide and tosyl groups.

In Scheme 2.3 several proposed methods to obtain the secondary amine calix[4]arene derivative, L1, are shown, but its isolation was not achieved. This could be explained on the basis of the relative bulky size of the starting material (2f) and the possibility of hydrogen bond formation between the starting material and the hydroxyl groups of the parent calix[4]arene. Other factors to consider are related to the strong conditions and the slow kinetics of the reactions involved which may affect the stability of the phosphoroamidate group of the starting material.

The difficulties found in the synthesis of secondary amino calix[4]arene derivatives led to the preparation of a new phosphorous-containing calix[4]arene derivative which may have interesting complexing properties. In the following section the three step procedure used for the synthesis of 5,11,17,23-tetakis-(1,1-dimethylethyl)-25,27-bis-[2-N-(diethyl phosphoroamidate)ethoxy] calix[4]arene (L2) are described.


The reactions shown in Scheme 3.1 were carried out to obtain L2. Each step is described in the next sections.


The first step was already reported by Collins \textit{et al.}\textsuperscript{134} in 1991 and involves the introduction of the acetonitrile residue in the lower rim of the parent calix[4]arene. In this work, the phase transfer system suggested by Danil de Namor \textit{et al.}\textsuperscript{132} was used to carry out this derivatisation and so the main product of the reaction was the 1,3 partially substituted calix[4]arene derivative in a yield of 89%. The $^1$H NMR of the raw product (used in the next step without further purification) is shown in Figure 3.10.

Figure 3.10 $^1$H NMR of the cyanomethoxy calix[4]arene derivative in CDCl$_3$ at 298K.
Chapter 3 – Results and Discussion

The use of 18-crown-6 (18-C-6) as the phase transfer catalyst confers the soft conditions required and allows the selective introduction of only two pendant arms at the lower rim of the calixarene. On the other hand, the low solubility of this compound in methanol was found to be advantageous to shorten the time of the workup. Indeed the refluxing process in this solvent was sufficient to obtain the desired product with a high degree of purity.


The second step of this synthesis consisted in the reduction of the cyano to an amine group with the introduction of a methylene unit yielding the amine calix[4]arene derivative (92% yield). The characterisation of this compound was achieved using \(^1\)H NMR in deuterated chloroform (Figure 3.11). The 'cone' conformation of this derivative is reflected by the presence of a characteristic pair of doublets assigned to the methylene bridge protons between the aromatic rings.\(^{33}\) It was found that the material had the required purity to be used without further recrystallisation.

![Figure 3.11](image)

Figure 3.11 \(^1\)H NMR of the amine calix[4]arene derivative in CDCl\(_3\) at 298K.


The acquired experience in organophosphorous compounds was then put into play for the phosphoroamidation of the calixamine derivative using the same conditions as described above. The only difference was that in this case, the time of reaction was 48 hours longer than before. This is mainly attributed to steric effects on the 'cone'
conformation of the calix[4]arene derivative. The $^1$H NMR spectrum of the phosphoroamidate derivative, L2, can be observed in Figure 3.12.

![Diagram of molecular structure](image)

Figure 3.12 $^1$H NMR of the 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-bis[2-(N-diethyl phosphoroamidate)ethoxy] calix[4]arene in (a) CDCl$_3$ and (b) CD$_3$CN at 298 K

The presence of a couple of doublets corresponding to the axial (IV) and the equatorial (III) protons of the methylene bridge (Figure 3.12) confirms that this ligand adopts a 'cone' conformation in solution. The difference between the chemical shifts of the signals belonging to the axial and equatorial protons ($\Delta\delta$(ppm) = 0.97) of the methylene bridge strongly suggest a 'quasi' perfect cone conformation for this ligand. The overlapping of other several signals in the $^1$H NMR spectrum made difficult to confirm its structure. In an attempt to overcome this problem a new spectrum in deuterated acetonitrile was obtained. This is also shown in Figure 3.12. Thus, this spectrum shows
the two signals for the aromatic protons (ArH) clearly identified. Unlike in CDCl₃, in CD₃CN the signals of the ethylene bridge protons (situated between the calix[4]arene ring and the phosphoroamidate group) are also identified. These changes could be explained in terms of an interaction between the solvent and the hydrophobic cavity of the calix[4]arene which has been often observed for other calix[4]arene derivatives.⁷¹

Even though this ligand has multiple possibilities due to the presence of different donor atoms in its structure, some disadvantages were envisaged. These are, (i) its instability in acid medium and (ii) the presence of the diethyl phosphoryl groups. These groups could lead to toxic degradation products. Therefore attempts to find a more suitable ligand for the removal of heavy metal cations were made. Previous work performed at the Thermochemistry Laboratory,¹¹⁹•¹²¹ based on tetra substituted tertiary amino calix[4]arenes and their possible interaction with heavy metals encourage the preparation of a ligand with less complexity. The aim was to assess its complexing properties with heavy metal cations. Thus, partially substituted tertiary amine derivatives were prepared and these are now discussed.


This new macrocycle, 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-dihydroxy-26,28-bis-[(diethylamine)ethoxy] calix[4]arene (L3) has two free hydroxyls groups (Figure 3.13) and therefore less donor atoms than the fully substituted ligand. A greater flexibility can be expected for this ligand due to the reduced steric effect at the hydrophilic cavity relative to that involving fully substituted calix[4]arene amino derivatives.

![Figure 3.13 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-dihydroxy-26,28-bis-[(diethylamine)ethoxy] calix[4]arene (L3)](image-url)
In this synthesis, 18-crown-6 was used as phase transfer catalyst and potassium carbonate as the base following the procedure previously developed at Thermochemistry Laboratory.\textsuperscript{132} Thus, L3 was obtained in \(~85\,\%\). The small amount of p-tert-butylcalix[4]arene found made difficult the work-up of the reaction. In order to overcome this problem in the second approach, KHCO\textsubscript{3}/K\textsubscript{2}CO\textsubscript{3}/TBAB were used. However the yield was much lower (35\%) and the amount of p-tert-butylcalix[4]arene found was even greater. This can be attributed to the lower basicity of the system used with respect to K\textsubscript{2}CO\textsubscript{3}/18C6. An important conclusion is that related to the number of moles of base employed in the derivatisation of the main calix[4]arene. In this case a 100\% excess of K\textsubscript{2}CO\textsubscript{3} (50 mmol) with respect to the p-tert-butylcalix[4]arene (12.5 mmol) was added. This excess was necessary to remove the water formed during the reaction which was likely to affect the successful completion of the reaction. On the other hand, the starting material was a protonated amine salt, which in contact with potassium carbonate is likely to release the free amine, consuming a stoichiometric amount of carbonate. By increasing the amount of K\textsubscript{2}CO\textsubscript{3}, the yield of the fully substituted calix[4]arene derivative was found to be much greater, however difficulties were found to separate the components of the mixture.

The structural characterisation of L3 was confirmed by elemental analysis (Table 3.1), \textsuperscript{1}H NMR (Table 3.2) and X-ray diffraction studies (Section 3.4).

<table>
<thead>
<tr>
<th>Table 3.1 Microanalysis data for L3.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Calculated %</td>
</tr>
<tr>
<td>Found 1</td>
</tr>
<tr>
<td>Found 2</td>
</tr>
</tbody>
</table>

The agreement between calculated and found micro analytical data was good. Then \textsuperscript{1}H NMR studies for this ligand were carried out. Thus, \textsuperscript{1}H NMR data for this ligand in various deuterated solvents (acetonitrile, CD\textsubscript{3}CN; methanol, CD\textsubscript{3}OD; dichloromethane, CD\textsubscript{2}Cl\textsubscript{2} and chloroform, CD\textsubscript{3}Cl) at 298 K are shown in Table 3.2. The \textsuperscript{1}H NMR spectra are those presented in Figure 3.14.

Characteristic arrangements of chemical shifts for the partial substitution can be observed. Thus, two signals for the aromatic protons (Ar-H), for the tert-butyl hydrogens and one for the ‘free’ hydroxyl groups are shown. The ‘cone’ conformation
of this calix[4]arene derivative is revealed by the presence of a pair of doublets (AB system with \( J = 12.5 \) Hz in CD3CN and 12.8 Hz in CDCl3)\textsuperscript{33} corresponding to the axial (IV) and equatorial (III) protons of the methylene bridge between the aromatic rings. As far as the shape of the cone is concerned, this information can be extrapolated from the distance in ppm between the signals of the methylene bridge protons, \( \Delta \delta_{\text{ax-eq}} \) which is generally 0.9 ppm for a system adopting a perfect `cone' conformation.\textsuperscript{16} For L3 these values are solvent dependent. Thus, \( \Delta \delta_{\text{ax-eq}} \) (ppm) values of 1.02, 1.06, 0.95 and 1.04 ppm were obtained in CD3CN, CD2Cl2, CD3OD and CDCl3 respectively. The fact that \( \Delta \delta_{\text{ax-eq}} \) values are close to 0.9 ppm indicates that the ligand in solution adopts a quasi cone conformation varying to a small extent from one solvent to another. The perfect `cone' conformation is not frequently observed in fully substituted calix[4]arene derivatives\textsuperscript{147} where larger differences in \( \Delta \delta_{\text{ax-eq}} \) values are observed.

Table 3.2 \( ^1H \) NMR data for L3 in several deuterated solvents at 298 K

<table>
<thead>
<tr>
<th>Deuterated Solvent</th>
<th>CD3CN</th>
<th>CD3OD</th>
<th>CD2Cl2</th>
<th>CDCl3</th>
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</thead>
<tbody>
<tr>
<td>(-\text{OH})</td>
<td>8.21</td>
<td>na</td>
<td>8.17</td>
<td>7.25</td>
</tr>
<tr>
<td>(-\text{ArH} \text{PhOH})</td>
<td>7.20</td>
<td>7.12</td>
<td>7.02</td>
<td>7.05</td>
</tr>
<tr>
<td>(-\text{ArH} \text{PhOR})</td>
<td>7.15</td>
<td>7.03</td>
<td>7.02</td>
<td>6.76</td>
</tr>
<tr>
<td>(-\text{CH}_{\text{ax}})</td>
<td>4.38</td>
<td>4.33</td>
<td>4.38</td>
<td>4.33</td>
</tr>
<tr>
<td>(-\text{OCH}<em>{2}\text{CH}</em>{2})</td>
<td>4.03</td>
<td>4.09</td>
<td>4.01</td>
<td>4.03</td>
</tr>
<tr>
<td>(-\text{CH}_{\text{eq}})</td>
<td>3.36</td>
<td>3.38</td>
<td>3.33</td>
<td>3.29</td>
</tr>
<tr>
<td>(-\text{CH}<em>{2}\text{CH}</em>{2}\text{N}^-)</td>
<td>3.07</td>
<td>3.19</td>
<td>3.09</td>
<td>3.08</td>
</tr>
<tr>
<td>(-\text{NCH}<em>{2}\text{CH}</em>{3})</td>
<td>2.70</td>
<td>2.78</td>
<td>2.69</td>
<td>2.68</td>
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<tr>
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<td>1.20</td>
<td>1.24</td>
<td>1.22</td>
<td>1.29</td>
</tr>
<tr>
<td>(-\text{C(CH}<em>{3})</em>{\text{PhOR}})</td>
<td>1.15</td>
<td>1.06</td>
<td>1.13</td>
<td>0.93</td>
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<td>(-\text{CH}<em>{2}\text{CH}</em>{3})</td>
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<td>1.18</td>
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<td>1.10</td>
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</table>

This quasi perfect cone' conformation can be justified if the possibility of hydrogen bond formation between the hydroxyl and ethereal groups is considered. This phenomenon has been observed in the solid state as discussed later on in this thesis. In any case, even though the \( \Delta \delta_{\text{ax-eq}} \) values are close to 0.9 ppm, further analysis of the data (Table 3.3) shows that the distance between the signals for the protons in the
aromatic ring (ArH) is not constant but changes from one solvent to another in the following sequence: \( \delta_{\text{CD}2\text{Cl}_2} > \delta_{\text{CD}3\text{CN}} > \delta_{\text{CD}3\text{OD}} > \delta_{\text{CD}3\text{Cl}_3} \)

Figure 3.14 \( ^1 \text{H NMR spectra of L3 in several deuterated solvents at 298 K.} \)
The above sequence is in agreement with that found for the $\Delta \delta_{\text{ax-eq}}$ values with the exception of the value for this ligand in CDCl$_3$. For the latter, proton signals of the hydroxyl group in this solvent overlap with those for the solvent at 7.26 ppm. This is an indication that in CDCl$_3$, this proton is more shielded than in CD$_3$CN and CD$_2$Cl$_2$ suggesting that in this solvent the tendency of this proton to enter hydrogen bond formation in the hydrophilic cavity is very low.

Table 3.3 $^1$H NMR $\Delta \delta$ values for selected signals of Li in several deuterated solvents at 298 K

<table>
<thead>
<tr>
<th>$\Delta \delta$</th>
<th>CD$_3$CN</th>
<th>CD$_2$Cl$_2$</th>
<th>CD$_3$OD</th>
<th>CDCl$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ArH</td>
<td>0.06</td>
<td>0.00</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>-CH$_{3x-eq}$</td>
<td>1.02</td>
<td>1.06</td>
<td>0.95</td>
<td>1.04</td>
</tr>
<tr>
<td>(CH$_3$)$_3$C-</td>
<td>0.06</td>
<td>0.09</td>
<td>0.18</td>
<td>0.36</td>
</tr>
</tbody>
</table>

A possible explanation for this finding is the presence of a molecule of solvent in the hydrophobic cavity of the ligand, which is likely to lead to a more symmetrical conformation of the ligand. This statement is corroborated by the significant chemical shift changes observed in the aromatic protons (ArH) (effect of the polarity of the solvent molecule) of this ligand in these solvents relative to those found in CD$_3$Cl.

In an attempt to elucidate further these observations a $^1$H NMR titration of L3 with deuterated acetonitrile in CDCl$_3$ was carried out at 298 K (Figure 3.15). The results obtained show that,

i) The signal for the hydroxyl protons moves to a lower field. This is indicative that a de-shielding effect occurs upon addition of acetonitrile to a solution of L3 in CDCl$_3$, suggesting a stronger hydrogen bond formation between the hydroxyl groups and the ethereal oxygens.

ii) The signals for the aromatic and tert-butyl protons get closer with a tendency to adopt the same position as that found in pure acetonitrile.

These facts provide some evidence about the presence of acetonitrile in the hydrophobic cavity of the ligand. As a result (i) a strong hydrogen bond is formed between the hydroxyl groups and the ethereal oxygens of the pendant arms in the hydrophilic cavity, and (ii) the ligand adopts a more symmetrical structure.
Figure 3.15 1H NMR titration of L3 solution (5 x 10^{-3} mol.dm^{-3}) in CDCl3 with CD3CN at 298 K
Guests such as CD$_2$Cl$_2$ or CD$_3$OD in the hydrophobic cavity of some calix[4]arene derivatives have been also reported.$^{146}$ Comparison of the $^1$H NMR data of L3 in CD$_2$Cl$_2$ with those in CD$_3$CN suggest that like in the latter solvent a molecule of CD$_2$Cl$_2$ may also be hosted in the hydrophobic cavity of the ligand. The unusual equivalence observed in the aromatic protons and the strong evidence of hydrogen bond formation in the hydrophilic rim in contrast with the lack of chemical shift changes for the proton signals of the $p$-tert-butyl group could support this suggestion.

In contrast to these solvents, the possibility of hydrogen bond formation between CD$_3$OD and the nitrogen atoms of L3 can not be excluded. This statement is corroborated by the downfield shifts observed in the protons close to the amine nitrogens.

In order to gain information on the structure of L3 in the solid state, suitable crystals for X-ray structural studies were obtained by slow recrystallisation of this ligand from ethanol. Thus, the X-ray structure was determined,$^{147}$ and this is now discussed.

### 3.4. X-Ray diffraction studies of L3.

The following crystal data was obtained for L3 crystals recrystallised from ethanol saturated solution: L3:EtOH, has as molecular formula: C$_{58}$H$_{88}$N$_2$O$_5$ and a molecular weight of 893.3. It belongs to the triclinic crystal system with the following unit cell lengths, $a = 21.960(4)$ Å, $b = 13.504(5)$ Å, $c = 17.200(1)$ Å, $V = 2745(2)$ Å$^3$, $T = 293(2)$ K, space group P-1 (No. 2), $Z = 2$, $\mu$(MoKα) = 0.067 mm$^{-1}$, 9994 reflections measured, 9639 unique ($R_{int} = 0.013$), 6518 observed [I>2σ(I)], $R_1 = 0.08$ and $wR(F^2) = 0.265$ (observed data).

The X-ray structure (figures 3.16 and 3.17) shows a hydrophobic cavity bounded by the phenyl rings and a hydrophilic pocket defined by alternated OH and pendant O(CH$_2$)$_2$NH(CH$_2$)$_2$(CH$_3$)$_2$ groups. These can be easily distinguished. A pair of strong OH...O bonds [O...O distances of 2.676 and 2.687 Å, and O-H...O angles of 167.6 and 158.5°, respectively, in the lower rim produce a relatively open calix. This affords the inclusion of one unit of ethanol found in the basket to form an intra-molecular complex. This fact could support the presence of other similar solvents (CD$_3$OD, CD$_3$CN, CD$_2$Cl$_2$) in the hydrophobic cavity of L3 in solution, as it was suggested on the basis of $^1$H NMR studies.
Figure 3.16 Side view of L3 intra-molecular complex with ethanol
Figure 3.17  Top view of L3 intra-molecular complex with ethanol
Chapter 3 – Results and Discussion

If the X-ray structures of the partial and the fully substituted calix[4]arene amine derivative,\textsuperscript{132} are compared, it is concluded that while the latter has a flattened 'cone conformation, a regular 'cone conformation is observed for the former. These data are referred to the solid state and therefore only with NMR evidence could this information be extrapolated to the process in solution.

Conventionally the conformation of the calix[4]arene can be defined by the $\delta$ angles at which the plane of the aromatic rings joins the plane defined by the four -CH$_2$-groups linking them.$^{148}$ These dihedral angles are shown in Table 3.4 and they are $\delta_1 = 111.34$, $\delta_2 = 111.08$; $\delta_3 = 112.33$ and $\delta_4 = 111.43$. Values of $\delta$ greater than 90$^\circ$ indicate that the aromatic rings are tilted. The arrangement is such that its tert-butyl groups are away from the hydrophobic cavity. Two opposite rings (1 and 3 or 2 and 4) move away from the parallelism by about 30$^\circ$. Again this phenomenon allows enough space to accommodate a solvent molecule into the hydrophobic cavity.

Table 3.4 Selected data from the X-ray structure of L3

<table>
<thead>
<tr>
<th>Localisation</th>
<th>Atoms</th>
<th>Distance(Å) / Angle(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper bore</td>
<td>C34 - C74</td>
<td>8.271 Å</td>
</tr>
<tr>
<td>(Opposites aromatic carbon atoms)</td>
<td>C14 - C54</td>
<td>8.196 Å</td>
</tr>
<tr>
<td>Lower bore</td>
<td>C31 - C71</td>
<td>5.239 Å</td>
</tr>
<tr>
<td>(Opposites aromatic carbon atoms)</td>
<td>C11 - C51</td>
<td>5.448 Å</td>
</tr>
<tr>
<td>&gt;CH$_2$ bridge angle</td>
<td>C16 - C2 - C32</td>
<td>111.34°</td>
</tr>
<tr>
<td>(C-C-C)</td>
<td>C36 - C4 - C52</td>
<td>111.08°</td>
</tr>
<tr>
<td></td>
<td>C72 - C6 - C56</td>
<td>112.33°</td>
</tr>
<tr>
<td></td>
<td>C12 - C8 - C76</td>
<td>111.43°</td>
</tr>
<tr>
<td>Hydroxyl groups</td>
<td>O41 - O81</td>
<td>3.898 Å</td>
</tr>
<tr>
<td>Ethereal oxygen atoms</td>
<td>O21 - O61</td>
<td>4.118 Å</td>
</tr>
<tr>
<td>Nitrogen atoms</td>
<td>N24 - N64</td>
<td>7.918 Å</td>
</tr>
</tbody>
</table>

Having discussed structural aspects of the ligand in solution ($^1$H NMR) and in the solid state (X-ray crystallography), solubility measurements were carried out. These are discussed in the following section.
3.5. Solubility Measurements.

Solubility measurements were performed as described in the Experimental Section. Thus, solubility data for 5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-bis-[2-(diethylamino)ethoxy] calix[4]arene (L3) in various solvents at 298.15 K are listed in Table 3.5. The data in each solvent are the result of analytical measurements carried out from the same equilibrium mixture. UV/V spectrophotometry was the technique used to analyze the amount of ligand in three aliquots taken of saturated solution. Solvate formation was observed in dichloromethane, chloroform and, tetrahydrofuran.

Table 3.5 Solubility (mol dm⁻³) of L3 in various solvents at 298.15 K

<table>
<thead>
<tr>
<th>Solvent</th>
<th>MeOH a</th>
<th>EtOH a</th>
<th>MeCN a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.78 x 10⁻³</td>
<td>7.39 x 10⁻³</td>
<td>7.7 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>8.35 x 10⁻³</td>
<td>7.57 x 10⁻³</td>
<td>7.9 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>8.27 x 10⁻³</td>
<td>7.66 x 10⁻³</td>
<td>7.7 x 10⁻⁴</td>
</tr>
</tbody>
</table>

(8.13 ± 0.31) x 10⁻³ (7.54 ± 0.14) x 10⁻³ (7.8 ± 0.1) x 10⁻⁴

a Abbreviations: MeOH, methanol; EtOH, ethanol; MeCN, acetonitrile.

Solubility data are referred to the process described in Eq. 9 where the p-tert-butylcalix(4)arene derivative (L) in the solid state (sol) is in equilibrium with its saturated solution (s).

L(sol) ⇌ L(s)

Provided that the composition of the solid in equilibrium with the saturated solution of the ligand is the same, these data can be used to calculate the standard solution Gibbs energy changes, \(\Delta_s G^0\), of the ligand in a given solvent and at a given temperature using the following expression,

\[\Delta_s G^0 = -RT \ln[L]_{\gamma_L}\]

In this equation \(\gamma_L\) is the activity coefficient (molar scale). Provided that low solubilities are involved, \(\gamma_L\) can be considered to be equal to unity. Thus, the solubility of the ligand on the molar scale is referred to the standard state of 1 mol dm⁻³.
The changes in solvation of these compounds in different solvents may be assessed using the standard transfer Gibbs energies, $\Delta_r G^\circ$, from the reference solvent $s_1$ to another $s_2$. The data are referred to the process,

$$L(s_1) \xrightarrow{K_i} L(s_2)$$

Thus, the thermodynamic transfer constant, $K_i$, is given by,

$$K_i = \frac{[L]y_{L}(s_2)}{[L]y_{L}(s_1)} = \frac{[L](s_2)}{[L](s_1)}$$

The $K_i$ value is obtained from the solubility ratio of the ligand in two different solvents and the standard transfer Gibbs energy can be therefore calculated from Eq. 32.

$$\Delta_r G^\circ(L)(s_1 \rightarrow s_2) = -RT \ln K_i = \Delta_r G^\circ(L)(s_2) - \Delta_r G^\circ(L)(s_1)$$

In Eq. 32, $R$ is the universal gas constant (8.31 J.K$^{-1}$.mol$^{-1}$) and $T$ is the absolute temperature (K). Table 3.6 reports the solubilities of L3 in different solvents. Using Eq. 33, the standard Gibbs energies of these ligands in the various media are calculated. Taking acetonitrile as the reference solvent, $K_i$ and derived standard transfer Gibbs energies to the alcohols (MeOH, EtOH) are calculated. These data are also included in Table 3.6.

Its calculation requires the same composition for both, the solid and the saturated solution of the ligand at equilibrium,$^{160}$ $\Delta_r G^\circ$ values shown in the above table give quantitative information about the differences in the solvation of this ligand in two solvents. A negative value for the standard transfer Gibbs energy from $s_1$ to $s_2$ means that the ligand is better solvated in the receiving than in the reference solvent.

As far as the transfer Gibbs energy of this ligand from acetonitrile to protic solvents (MeOH, EtOH) is concerned, the data presented in Table 3.6 show that as the hydrophobic nature of the aliphatic chain of the alcohols in going from MeOH to EtOH, has not much influence. Generally L3 is better solvated in protic than in dipolar aprotic...
solvents. This is due to the ability of the alcohols to interact with the hydroxyl, ethereal oxygens and amino groups through hydrogen bond formation.

Table 3.6 Solubilities, standard Gibbs energies of solution of L3 in various solvents at 298.15K. Derived transfer constant and transfer Gibbs energies from acetonitrile.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility mol.dm$^{-3}$</th>
<th>$\Delta G^\circ$ kJ.mol$^{-1}$</th>
<th>$K_{MeCN\rightarrow s_2}$</th>
<th>$\Delta G^\circ$ kJ.mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeCN</td>
<td>7.76E-04</td>
<td>17.8</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>MeOH</td>
<td>8.13E-03</td>
<td>11.9</td>
<td>10.48</td>
<td>-5.9</td>
</tr>
<tr>
<td>EtOH</td>
<td>7.54E-03</td>
<td>12.1</td>
<td>9.42</td>
<td>-5.7</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>v. soluble</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>v. soluble</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>THF</td>
<td>v. soluble</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: MeCN, acetonitrile, MeOH, methanol; EtOH, ethanol;

After characterisation of L3, its interaction with metal cations was investigated. Thus, $^1$H NMR studies on the interaction of this ligand with metal cations and the proton is discussed in the next section.

3.6. $^1$H NMR Studies of the host - guest interaction of L3.

As previously discussed (Figure 3.14) a typical pattern of $^1$H NMR signals for L3 was found. Therefore, the $^1$H NMR spectrum of the ligand is used as reference to determine any chemical shift changes which are indicative of the strength and sites of host - guest interactions in solution. The solvent used was deuterated acetonitrile (CD$_3$CN) because this was the medium in which most cation-calixarene complexation studies have been previously reported.$^{71,42}$ $^1$H NMR investigations were carried out using different concentrations of metal ion salts. Thus, $^1$H NMR titrations of L3 with different metal cations are shown in figures 3.18 - 3.23. When complexation occurs, the signals showing the greatest chemical shift changes are those corresponding to the hydrogens labelled VI (OCH$_2$CH$_2$N) and VII (NCH$_2$CH$_3$). These findings strongly indicate that in the complexation process, the amine nitrogens are involved.
Figure 3.18 $^1$H NMR titration of L3 with Hg(ClO$_4$)$_2$ in CD$_3$CD at 298 K
Figure 3.19  $^1$H NMR titration of L3 with Pb(ClO$_4$)$_2$ in CD$_3$CN at 298 K
Figure 3.20  $^1$H NMR titration of L3 with Cd(ClO$_4$)$_2$ in CD$_3$CN at 298 K
Figure 3.21  $^1$H NMR Titration of L3 with Mg(ClO$_4$)$_2$ in CD$_3$CN at 298 K
Figure 3.22  $^1$H NMR titration of L3 with Al(ClO$_4$)$_3$ in CD$_3$CN at 298 K
Figure 3.23  $^1$H NMR titration of L3 with HClO$_4$ in CD$_3$CN at 298 K
Thus the inductive effect of a positive charge acquired by the nitrogen atoms upon complexation led to downfield chemical shift changes. The same sequence is found for most of the metal cations with the exception of cadmium. For the latter cation, smaller changes are observed in the $^1$H NMR spectra relative to other cations. This statement is better reflected in figures 3.24 and 3.25, where the $\Delta\delta$(ppm) are plotted against the metal cation - ligand ([M]/[L]) ratio. In both cases $\Delta\delta$(ppm) values are positive, indicating that protons VI and VII are de-shielded upon complexation with the metal cation. Other signals with moderate $\Delta\delta$(ppm) values are those related to protons V (OCH$_2$CH$_2$N), VIII (NCH$_2$CH$_3$) and IV (Hax) although protons V and VIII behave in the same way as protons VI and VII, the de-shielding effect observed in the former protons is smaller than those for the later. This may be attributed to the greater steric distance of these protons from the nitrogen atoms. The similar $\Delta\delta$(ppm) values found for protons V and VIII could be taken as an indication that the phenolic oxygen atoms do not take part in the complexation process. As far as proton IV is concerned, a moderate shielding of the axial protons is observed. This may indicate that upon complexation these protons are moving away from the oxygen atoms of the hydrophilic cavity.

The complex stoichiometry deduced from figures 3.24 and 3.25, for Hg$^{2+}$, Pb$^{2+}$ and Mg$^{2+}$ complexes is 1:1 and this was corroborated by conductance studies.$^{151}$ Selected conductometric titration curves are shown in Figure 3.26. The latter method was used to verify the stoichiometry shown in the NMR titrations.

In Figure 3.25c the interaction between perchloric acid and L3 can be observed. The expected stoichiometry of this interaction can be deducted from this figure. Thus the two amino nitrogen atoms of the pendant arms in L3 are protonated. In order to use these results to compare the affinities of L3 for the different metal cations tested, analysis of variance, ANOVA and least significant differences, LSD were applied and the results are shown in Table 3.7. The two signals chosen for this comparison were those corresponding to the protons labelled as VI and VII (Figure 3.14) because of their pronounced chemical shift changes ($\Delta\delta$). Therefore it can be concluded that L3 has a similar affinity for Hg$^{2+}$, Pb$^{2+}$, Al$^{3+}$, and H$^+$ and to a lesser extent for Mg$^{2+}$. However the less significant chemical shift changes are those observed for the interaction of L3 and Cd$^{2+}$ in CD$_3$CN. In all cases a important fact to consider is that shown by L3 by alkali metal cations such as sodium or potassium. This statement is supported by the lack of change in the chemical shifts of the signals of the $^1$H NMR spectra of L3 upon addition of alkali-metal cation salts dissolved in CD$_3$CN.
Figure 3.24 $^1$H-NMR $\Delta \delta$ (ppm) for the interaction of L3 with (a) Hg$^{2+}$, (b) Pb$^{2+}$, (c) Cd$^{2+}$ in CD$_3$CN at 298 K
Figure 3.25 $^1$H-NMR $\Delta \delta$ (ppm) for the interaction of L3 with (a) Mg$^{2+}$, (b) Al$^{3+}$, (c) H$^+$ in CD$_3$CN at 298 K.
Figure 3.26 Conductometric titration curves of (a) Hg$^{2+}$, (b) Mg$^{2+}$, (c) K$^+$ with L3 in acetonitrile at 298.15 K.
A different picture emerges when acetonitrile is changed to deuterated methanol. Thus, the $^1$H NMR titration spectra obtained in this solvent can be observed in figures 3.27, 3.28, 3.29, 3.30 and 3.31. No complexation of $L_3$ with $Mg^{2+}$ in this solvent is observed. This can be attributed to the higher solvation of this metal cation in methanol relative to acetonitrile. Several conclusions can be drawn from figures 3.32 and 3.33, such as,

1) The interaction of $L_3$ with $Hg^{2+}$, $Pb^{2+}$, $Al^{3+}$, $K^+$ and $H^+$ in CD$_3$OH shows a similar behaviour than that observed in acetonitrile;
A weaker interaction between L3 and Cd$^{2+}$ in CD$_3$OH is observed.

In general terms a varied strength of interactions is found in this solvent relative to those in acetonitrile. This can be concluded from the data shown in Table 3.8.

### Table 3.8 Statistical comparison of the $^1$H NMR $\Delta\delta$ values for the interaction between metal cations and L3 in CD$_3$OD at 298 K

<table>
<thead>
<tr>
<th></th>
<th>H$^+$</th>
<th>Al$^{3+}$</th>
<th>Hg$^{2+}$</th>
<th>Pb$^{2+}$</th>
<th>Cd$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>0.60</td>
<td>0.60</td>
<td>0.57</td>
<td>0.38</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>0.60</td>
<td>0.57</td>
<td>0.41</td>
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</tr>
<tr>
<td></td>
<td>0.60</td>
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<td>0.57</td>
<td>0.43</td>
<td>0.08</td>
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<tr>
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<td>0.60</td>
<td>0.60</td>
<td>0.57</td>
<td>0.44</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean</td>
<td>0.60</td>
<td>0.60</td>
<td>0.57</td>
<td>0.43</td>
<td>0.08</td>
</tr>
<tr>
<td>SD</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>0.01</td>
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</tbody>
</table>

LSD = 0.09

<table>
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<tr>
<th></th>
<th>H$^+$</th>
<th>Al$^{3+}$</th>
<th>Hg$^{2+}$</th>
<th>Pb$^{2+}$</th>
<th>Cd$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>0.79</td>
<td>0.78</td>
<td>0.73</td>
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<td>0.12</td>
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<td>0.73</td>
<td>0.59</td>
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<td>0.73</td>
<td>0.59</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>0.79</td>
<td>0.79</td>
<td>0.73</td>
<td>0.59</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean</td>
<td>0.79</td>
<td>0.79</td>
<td>0.73</td>
<td>0.59</td>
<td>0.13</td>
</tr>
<tr>
<td>SD</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

LSD = 0.06
Figure 3.27 $^1$H NMR titration of L3 with Hg(ClO$_4$)$_2$ in CD$_2$OD at 298 K.
Figure 3.28 $^1\text{H}$ NMR titration of L3 with Pb(ClO$_4$)$_2$ in CD$_3$OD at 298 K
Figure 3.29  $^1$H NMR titration of L3 with Cd(ClO$_4$)$_2$ in CD$_3$OD at 298 K
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Figure 3.30 ¹H NMR titration of L3 with Al(CIO₄)₃ in CD₃OD at 298 K
Figure 3.31 $^1$H NMR titration of L3 with HClO$_4$ in CD$_3$OD at 298 K
Figure 3.32  $^1$H-NMR $\Delta \delta$(ppm) for the interaction of L3 with (a) Hg$^{2+}$, (b) Pb$^{2+}$, (c) Cd$^{2+}$ in CD$_3$OD at 298 K
Figure 3.33 $^1$H-NMR $\Delta \delta$(ppm) for the interaction of L3 with (a) Al$^{3+}$, (b) H$^+$, (c) K$^+$ in CD$_3$OD at 298 K
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After analysing these results, the potentiometric technique was chosen to quantify the interaction between the proton and lower rim calix[4]arene derivatives. Taking into account the importance of establishing the interaction of Hg\(^{2+}\) and lower rim calix[4]arene derivatives and due to the high values expected for this interaction, attempts were made to design and test a mercury selective electrode and this is now discussed.


Under this heading the procedures used to test the mercury electrode as a tool for measuring high stability constants (outside the range of conventional methods, such as microcalorimetry and spectrophotometry), will be presented and discussed in the following section.

3.6.1. Calibration and standardisation of the mercury electrode in water at 298.15 K.

Previously to the calibration of the assembled mercury electrode, the semi automatic burette to be used as dispensing device, was checked in order to assess its performance. Thus, Figure 3.34 (a plot of counts against amount of water in grams) shows the calibration curve for the quantification of the amount of water delivered by the automatic burette used in the present work. A good linear response (R\(^2\) = 0.9999) in a wide range of volumes (0.001 to 3.000 ml) was found. The limit of accuracy of the Autoburette ABU12 was found to be around of 0.002 ml and the equivalence between the counts in the automatic burette and the dispensed volume was of 0.297 counts/ml. From these data, the volume of titrant added to the vessel in function of the counts displayed by the burette was calculated.

In Table 3.9 and Figure 3.35, calibration data (average of three determinations) for the mercury electrode in water at 298.15 K are presented. The experimental array previously described under Material and Methods, using calomel as the reference electrode was used. In all cases good linear responses (R\(^2\) = 0.9998 ± 0.0001) and a Nernstian behaviour were found. Thus, the slope is close to the standard value of 29.5 mV for a cell reaction in which two electrons are transferred (28.97 ± 0.71 mV). Under these conditions, the potential for the mercury electrode was 529 ± 3 mV within the concentration range from 1 x 10\(^{-3}\) to 1 x 10\(^{-5}\) mol.dm\(^{-3}\).
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Figure 3.34  Calibration curve data for Autoburette ABU12A with water at 298.15K

Figure 3.35  Calibration curve for the mercury electrode in water at 298.15 K

Table 3.9  Calibration curve data for mercury electrode in water at 298.15

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>II</th>
<th>Mean</th>
<th>S.D.</th>
<th>%VD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (mV)</td>
<td>506.6</td>
<td>527.8</td>
<td>532.0</td>
<td>522.13</td>
<td>13.59</td>
<td>2.60</td>
</tr>
<tr>
<td>Slope (mV)</td>
<td>-28.4</td>
<td>-28.7</td>
<td>-29.8</td>
<td>-28.97</td>
<td>0.71</td>
<td>2.45</td>
</tr>
<tr>
<td>R²</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9997</td>
<td>0.9985</td>
<td>0.0001</td>
<td>0.01</td>
</tr>
</tbody>
</table>
The mercury electrode had to be prepared on daily basis because of the thin layer of mercury on the gold wire degenerates quickly. This is mainly due to mercury's volatility and the solubility of mercury in the gold wire. In all cases the ionic strength of these solutions was kept constant by the use of a solution of NaClO₄ (1.0 mol.dm⁻³) in water.

3.6.2. Calibration and standardisation of the mercury electrode in acetonitrile at 298.15 K.

Given that the design of this electrode was for the purpose of determining the stability constants of calix[4]arene derivatives and Hg²⁺ in acetonitrile, calibration experiments with the Autoburette ABU12 were carried out in this solvent. The results are shown in Figure 3.36. A very good correlation was found between the counts reported for the Autoburette ABU12 and the volume delivered (R²=0.9999). The smallest volume measured in the burette was around of 0.005 ml, which is greater than that for water because the greater vapour pressure of acetonitrile relative to water. This factor increases the error in the measurements. The equivalence between the counts of the burette and the volume delivered was 0.302 counts/ml, which is slightly greater than that for water.

![Figure 3.36 Calibration of the Autoburette ABU12 using acetonitrile at 298.15 K](image)

Calibration data for the mercury electrode in acetonitrile was obtained using the experimental array shown in the Experimental Chapter (Figure 2.2). This arrangement was required in order to i) avoid evaporation of the solvent and ii) minimise the presence of interfering species such as O₂ and Cl⁻ from the reference electrode.
In Figure 3.37 an average curve for the calibration of the mercury electrode in acetonitrile is given. In this case, a concentration cell was used and the mercury (II) concentration in the reference electrode was of 0.0090 mol.dm$^{-3}$. Under these conditions, the potential of the mercury electrode was of 49.0 mV and some deviation from the Nernst’s Law was observed because the slope obtained (25.6 mV) was relatively low in comparison with the expected value of 29.5 mV.

Using a silver/silver chloride electrode as reference, the potential for the mercury electrode was found to be 878.56 ± 1.84 mV and the slope shows a good Nernstian behaviour (29.68 ± 0.59 mV). This improvement in the behaviour of the electrode when using the latter reference electrode may be due to the elimination of the accumulation error introduced by the former reference electrode. The average of five calibration curves is shown in Figure 3.38. A decay of the response of the electrode was observed after ~24 hrs and calibration curves with no linear shapes were obtained. This was the main reason why the mercury electrode was prepared and calibrated on daily basis.


Upon titration of the different ligands with Hg$^{2+}$ solution in acetonitrile, with the aim of deriving stability constant data, typical titration curves (a plot of potential against [M]/[L])
ratio) were obtained and selected ones are shown in Figure 3.39. Some differences are observed in the shape of the titration curves. This is attributed to i) the different ability of the ligands to interact with mercury (II) in acetonitrile and ii) the relatively low concentrations of the ligands used, due to the low solubility of these ligands in this solvent. In all the cases, typical "S" shaped curves are observed. Titration curves involving the tetradiethylamine calix[4]arene derivative (L4) and mercury (II) showed the higher jump which may be indicative of a strong interaction between this cation and this ligand relative to other calix[4]arene derivatives in this solvent.

![Graph](image)

Figure 3.38 Calibration curve for mercury (II) using a mercury electrode and a Ag/AgCl modified reference electrode in acetonitrile at 298.15 K

Figure 3.39, also shows the stoichiometry of these complexes. In all cases, the 2:1 [M]/[L] ratio was observed. However the fact that only one jump was observed, implies that the stability constants for the two processes (eqs. 33 and 34) must be quite close. It means that the difference between the pKs_1 and pKs_2 values is less than 4 units. The processes involved in the formation of 1:2 (ligand: metal cation) complexes are shown in eqs. 33 and 34. The overall process is given in Eq. 35:

\[
M^{n+} (s) + 2L(s) \rightarrow M_2L^{2n+} (s) \quad 35
\]

\[
M^{n+} (s) + L(s) \rightarrow ML^{n+} (s) \quad 33
\]

\[
ML^{n+} (s) + M^{n+} (s) \rightarrow M_2L^{2n+} (s) \quad 34
\]
Figure 3.39  Potentiometric curves for mercury (II) (perchlorate as counter ion) and calix[4]arene derivatives in acetonitrile at 298.15 K
The equilibrium constants for processes 33, 34 and 35 are shown in eqs. 36, 37 and 38, respectively.

\[
K_1 = \frac{[ML]}{[M][L]} = \beta_1 \tag{36}
\]

\[
K_2 = \frac{[M_2L]}{[M][ML]} \tag{37}
\]

\[
K_1 * K_2 = \frac{[M_2L]}{[M]^2[L]} = \beta_2 \tag{38}
\]

In the last equations, concentrations instead of activities are used because the ionic strength of the solution under study was kept constant by the addition of tetra-n-butylammonium perchlorate. Under this condition, activity coefficients should be constant and the activity values could be replaced by concentrations.

To calculate these stability constants, the following mathematical model was used. In a similar form to the \( \bar{n} \) (number of formation)\textsuperscript{114}, the \( \phi \) function defined in Eq. 39 was used to calculate the conditional stability constants \( K_1 \) and \( K_2 \) of calix[4]arene derivatives and metal cations. A curve-fitting method included in Microsoft Excel (SOLVER) was used for the equation-solving procedure.\textsuperscript{152}

Then, plots of pHg against the \( \phi \) function in acetonitrile at 298.15 K were drawn. The stoichiometry of the reaction can be verified with this plot, given that a linear behaviour is shown between \( \phi \) values from 0 to 2. This linear part is related to the first part of the titration curve, just when the equilibrium is going on and it justifies the mathematical model used. Stability constants values for the various ligands and mercury (II) in acetonitrile at 298.15 K are listed in Table 3.10.

\[
M_T = [M] + [ML] + 2[M_2L]
\]

\[
L_T = [L] + [ML] + [M_2L]
\]

\[
\phi = \frac{M_T - [M]}{L_T} = \frac{[ML] + 2[M_2L]^2}{[L] + [ML] + 2[M_2L]^2}
\]

\[
\phi = \frac{M_T - [M]}{L_T} = \frac{\beta_1[M][L] + 2\beta_2[M]^2[L]}{[L] + \beta_1[M][L] + \beta_2[M]^2[L]}
\]

\[
\phi = \frac{M_T - [M]}{L_T} = \frac{\beta_1[M] + 2\beta_2[M]^2}{1 + \beta_1[M] + \beta_2[M]^2} \tag{39}
\]
Close stability constants are found for the 1:1 and 2:1 (metal-cation:ligand) complexes. As far as the ligands are concerned the selectivity for mercury (II) as assessed from their log $K_s$ values follows the sequence,

$L_4 > L_5 > L_6 > L_7$

This sequence found is opposite to that expected. Indeed ligands containing sulphur donor atoms are known to interact more strongly with $\text{Hg}^{2+}$ than those containing nitrogen donor atoms. Furthermore, the ratio of complexation is showing that two units
of Hg$^{2+}$ are being taken for each ligand unit. The peculiar behaviour observed for these systems could suggest the presence of other equilibria such as protonation or mercury-solvent interactions. Another fact to be taken into account is that during the titration of L4 and mercury (II) perchlorate in the presence of an excess of perchloric acid, no jump was observed as shown in Figure 3.40. As a result of these findings, it was thought that either the protonation of the ligand is stronger than the complexation or both processes are taking place simultaneously.

Several attempts to obtain suitable crystals of L3-metal cation complexes were made, and these are now discussed.

3.7. X-Ray diffraction studies of the protonated L3.\textsuperscript{147}

The following data were obtained for L3H$_2$(ClO$_4$)$_2$.MeOH, C$_{27}$H$_{38}$Cl$_2$N$_2$O$_{13}$, M = 1080.19, monoclinic, a = 15.391(1), b = 15.980(1), c = 24.994(1)Å, $\beta$ = 82.952(4)$^\circ$, V = 6098.3(6) Å$^3$, $T$ = 100(2) K, s.g P2$_1$/c (No. 14), $Z$ = 4, $\mu$(MoK$\alpha$) = 0.166 mm$^{-1}$, 77808 reflections measured, 10244 unique ($R_{int}$ = 0.10), 7378 observed, R1 = 0.154 and wR(F$^2$) = 0.432. In figures 3.41 and 3.42 X-ray structure of the protonated L3 can be observed. As for the X-ray structure of L3.EtOH complex, both hydroxyl groups in the lower rim are forming hydrogen bonds with the ethereal oxygen atom of an adjacent pendant arm, but now with reduced strength.
Figure 3.41 Side view of L3 perchlorate salt

Figure 3.42 Top view of L3 perchlorate salt
In fact, one of the hydrogen bonds, O3-H3...O2, is of medium strength \[d(O3...O2) = 2.744(3) \text{ Å}, d(H3...O2) = 1.967 \text{ Å}, \text{angle(O3-H3...O2) = 158.0°} \] while the other one, O1-H1...O4, is much weaker \[d(O1...O4) = 2.949(3) \text{ Å}, d(H1...O4) = 2.177 \text{ Å}, \text{angle(O1-H1...O4) = 157.0°} \]. Further ionic interactions occur at the periphery of the hydrophilic pocket of the protonated calix[4]arene, involving the two charged O(CH₂)₂NH⁺(CH₂)₂(CH₃)₂ pendant arms in N-H...O bonds with the perchlorate anions \[d(N...O) \text{ distances of 2.887, 3.055 and 3.140 \text{ Å}, and N-H...O angles of 149.4, 164.4 and 134.1°, respectively}. \] L3 in this case shows a flattened 'cone' conformation and this could be the reason why no solvent molecule was observed in the hydrophobic cavity in this system. In fact, a methanol molecule is found outside the calixarene at an interstitial lattice site.

In order to investigate this further a more simple system, a disubstituted calix[4]arene amine derivative (L3), was used. Attempts to determine the stability constants of its complexes with metal cations and its protonation constants were made.

### 3.8. Determination of protonation constants and stability constants of L3 and heavy metal cations using a glass electrode in acetonitrile.

Once L3 was prepared and characterised, studies about its interaction with the proton and heavy metal cations were carried out. As discussed before a strong indication of protonation was found. Therefore the characterisation of the interaction between the proton and L3 was first studied.

Thus, using a double junction pH electrode, previously calibrated with a perchloric acid solution in acetonitrile (Figure 3.43), typical titration curves were obtained (Figure 3.44). From these titration curves a series of values for the protonation constants \(K_p\) of L3 were obtained and they are shown in Table 3.11. The fact that the values for the protonation constants are relatively close clearly indicate that the two pendant arms are independent from each other. The X-ray structure of L3 shows that these are far apart from each other. Although this refers to the solid state, based solely on electrostatic grounds, it is expected that this is also the case in solution. It should be also stressed that the flexibility of the pendant arms also plays an important role in the interaction of these ligands with ionic species in solution.
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Figure 3.43 Calibration curve for the double junction glass electrode with HClO₄ in acetonitrile at 298.15 K

Figure 3.44 Titration curve of L₃ with HClO₄ in acetonitrile at 298.15 K

The equilibrium equations for the above titration are detailed as follows

\[ \begin{align*}
H^+ + L & \rightarrow HL^+ \quad 40 \\
HL^+ + H^+ & \rightarrow H_2L^{2+} \quad 41 \\
2H^+ + L & \rightarrow H_2L^{2+} \quad 42
\end{align*} \]
Table 3.11  Protonation constants for L3 measured in acetonitrile at 298.15 K

<table>
<thead>
<tr>
<th>Log $K_{H1}$</th>
<th>Log $K_{H2}$</th>
<th>$\beta_{H2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.77</td>
<td>14.17</td>
<td>29.94</td>
</tr>
<tr>
<td>15.80</td>
<td>14.49</td>
<td>30.29</td>
</tr>
<tr>
<td>15.54</td>
<td>14.29</td>
<td>29.83</td>
</tr>
<tr>
<td>15.67</td>
<td>14.42</td>
<td>30.09</td>
</tr>
<tr>
<td>15.63</td>
<td>14.38</td>
<td>30.01</td>
</tr>
<tr>
<td>15.69</td>
<td>14.43</td>
<td>30.12</td>
</tr>
</tbody>
</table>

Average ± Standard deviation

15.68 ± 0.09 | 14.36 ± 0.12 | 30.05 ± 0.16

where the equilibrium constants equation for processes in eqs. 40, 41 and 42 are shown in eqs. 43, 44 and 45,

\[
K_{H1} = \frac{[HL^+]}{[H^+][L]} = \beta_1
\]

\[
K_{H2} = \frac{[H_2L^{2+}]}{[H^+][HL^+]}
\]

\[
K_{H1} \times K_{H2} = \frac{[H_2L^{2+}]}{[H^+]^2[L]} = \beta_2
\]

Then using $n$ (number of formation) and the last equation (3.17) the conditional protonation constants $K_{H1}$ and $K_{H2}$ were calculated using a least squares curve-fitting method. Solver, an analysis tool of Microsoft Excel, was used for the equation - solving process.
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\[
H_T = [H^+] + [HL^+] + 2[H_2L^{2+}]
\]
\[
L_T = [L] + [HL^+] + [H_2L^{2+}]
\]
\[
\bar{n} = \frac{H_T - [H]}{L_T} = \frac{[HL^+] + 2[H_2L^{2+}]}{[L] + [HL^+] + [H_2L^{2+}]}
\]
\[
\bar{M} = \frac{M_T - [M]}{L_T} = \frac{\beta_1[H^+L] + 2\beta_2[H^+]^2[L]}{[L] + \beta_1[H^+][L] + \beta_2[H^+]^2[L]}
\]
\[
\bar{n} = \frac{M_T - [M]}{L_T} = \frac{\beta_1[H^+] + 2\beta_2[H^+]^2}{1 + \beta_1[H^+] + \beta_2[H^+]^2}
\]

A typical plot of observed and calculated values of \(\bar{n}\), is shown in Figure 3.45 and a good fitting is observed.

![Image of Figure 3.45](image-url)

Figure 3.45 Observed and calculated data for \(K_H\) calculations

Having established the protonation constants of \(L_3\), a competitive method was used to establish the stability constants of \(L_3 - \) metal cation complexes. The following equations were considered:

\[
L(s) + 2H^+(s) \Leftrightarrow LH_2^{2+}(s)
\]

and,

\[
ML^{2+}(s) + 2H^+(s) \Leftrightarrow H_2L^{2+}(s) + M^{2+}(s)
\]

From eqs. 47 and 48, it follows that,

\[
M^{2+}(s) + L(s) \Leftrightarrow ML^{2+}(s)
\]
Potentiometric titration curves for the processes described in eqs. 47 and 48 are shown in Figure 3.46. In this Figure, the protonation constants, $\beta_2$, the overall constant, $\beta'_2$, and the stability constant for the metal cation - L3 complex, $pK_s$, (Eq. 49) are shown. Several problems with this method were encountered. These are, i) low reproducibility in the different curves. This may be due to the long exposure time of the electrode to the organic solvent, ii) relatively high SSE (square sum errors) for the calculation of the overall constant, $\beta'_2$ (Eq. 48) relative to the protonation process, $\beta_2$ (Eq. 47), and iii) titration curves of different shapes were observed for $\text{Hg}^{2+}$, $\text{Pb}^{2+}$ and $\text{Cd}^{2+}$. These findings suggested the possibility of secondary reactions taking place. Other factors to consider in this competitive method was the presence of water in the solution of perchloric acid solution (70 % w/w). This factor could lead to hydrolysis of metal cation salts used.

Taking into account the basicity of the tertiary amines under study, the simplified process shown in Eq. 50, could be favouring the protonation of this ligand (L3).

$$\text{Hg}^{2+} + 2\text{H}_2\text{O} \rightleftharpoons 2\text{H}^+ + \text{Hg(OH)}_2$$

$$\text{C}(\text{N}R_2)_2 + 2\text{H}^+ \rightleftharpoons \text{C}(\text{N}^+\text{HR}_2)_2$$

$$\text{C}(\text{N}R_2)_2 + \text{Hg}^{2+} + 2\text{H}_2\text{O} \rightleftharpoons \text{C}(\text{N}^+\text{HR}_2)_2 + \text{Hg(OH)}_2$$

(50)

In Eq. 50, $\text{C}(\text{N}R_2)_2$ and $\text{C}(\text{N}^+\text{HR}_2)_2$ are the free and protonated forms of the partially substituted calix[4]arene amino derivative (L3), respectively. As no precipitation was observed during these experiments, the possibility of formation of a colloidal solution can not be excluded. This fact could be justified by the low concentrations of ligand and metal cation salts used.

Based on the latter, a possible explanation for the shape and stoichiometry for the titration curves of L4 and L5 with mercury (II) using the mercury electrode could be drawn.

$$2\text{Hg}^{2+} + 4\text{H}_2\text{O} \rightleftharpoons 4\text{H}^+ + 2\text{Hg(OH)}_2$$

$$\text{C}(\text{N}R_2)_4 + 4\text{H}^+ \rightleftharpoons \text{C}(\text{N}^+\text{HR}_2)_4$$

$$\text{C}(\text{N}R_2)_4 + 2\text{Hg}^{2+} + 4\text{H}_2\text{O} \rightleftharpoons \text{C}(\text{N}^+\text{HR}_2)_4 + 2\text{Hg(OH)}_2$$

(51)

Where $\text{C}(\text{N}R_2)_4$ and $\text{C}(\text{N}^+\text{HR}_2)_4$ are the base and protonated forms of the fully substituted calix[4]arene amino derivative (L4, L5), respectively.
Figure 3.46 Titration curves of (a) L3, (b) L3-Hg (1:1), (c) L3-Pb (1:1), and (d) L3-Cd (1:1) complexes with HClO₄ in acetonitrile at 298.15 K
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From Eq. 51, the ratio observed in these titrations curves (Figure 3.39), could be justified. But in the case of L5 and L6 (Figure 3.39), where just 2 nitrogen atoms are found in their structure, both processes, protonation and complexation could be taking place simultaneously. This fact is supported for the high affinity of mercury for the sulphur donor atoms. On the other hand, even in the absence of water the following processes (eqs. 52-55) may take place and therefore the stoichiometry of the titration curves obtained with the mercury electrode could be justified.

\[
2\text{CH}_3\text{C} = \text{N}: \rightleftharpoons \text{CH}_3\text{C} = \text{N}:\text{H} + \cdot:\text{CH}_2\text{C} = \text{N} \tag{52}
\]

\[
2\text{CH}_3\text{C} = \text{N} + \text{C}(\text{NR}_2)_2 \rightleftharpoons 2\cdot:\text{CH}_2\text{C} = \text{N} + \text{C}(\text{NHR}_2)_2 \tag{53}
\]

\[
\text{Hg}^{2+} + 2\cdot:\text{CH}_2\text{C} = \text{N} \rightarrow \text{N} = \text{CCH}_2\text{-Hg-CH}_2\text{C} = \text{N} \tag{54}
\]

\[
2\text{CH}_2\text{C} = \text{N} + 2\text{H}^+ \rightleftharpoons 2\text{CH}_3\text{C} = \text{N}:\text{H} \tag{55}
\]

In Eq. 52 the autoprotolysis equilibrium of acetonitrile is represented as a reference of the processes shown in eqs. 53 and 54 supported by the possibility to form mercury-carbon bonds. Thus, taking in account the difference between carbon and mercury electro-negativities, the Hg-C bond will be 91-93% covalent with strength of 200 kJ mol\(^{-1}\) leading in a quite inert and then stable bond.\(^{133}\) Obviously, the presence of an acid will inhibit these processes as shown in Eq. 55. However, a basic compound such as amine could lead to the equilibrium shown in Eq. 53, which will improve the possibility of mercury (II) - solvent interaction.

In order to get a deeper understanding of the processes taking place between the partially substituted calix[4]arene amine derivative, the metal cations and the protons, 2D NMR studies were carried out and these are now discussed.

3.9. 2D NMR studies of the interaction of L3 with metal cations and the proton.

The \(^{13}\text{C}\) NMR and the \(^1\text{H}\)-\(^{13}\text{C}\) NMR correlation spectra for L3 are shown in Figure 3.47. The one-dimensional 500 MHz \(^1\text{H}\) NMR spectrum is shown at the top edge and the 125 MHz \(^{13}\text{C}\) NMR spectrum at the right-hand edge. The signals that can be assigned with confidence are marked accordingly. The peak assignment was confirmed with both techniques.
Figure 3.47 $^{13}$C and $^1$H-$^{13}$C NMR correlation for L3 in CDCl$_3$ at 298
In Figure 3.48, part of the 500 MHz COSY-90 spectrum of L3 is shown as a contour plot. At the top edge is the $^1$H NMR spectrum with assignments. The diagonal and cross peaks joined by dashed construction lines indicate the protons having a mutual scalar coupling. Thus, correlations between i) the axial (IV) and equatorial (III) protons; ii) the protons of the ethylene bridge (V y VI) and iii) protons of the terminal ethyl group (VII and VIII) are observed. This spectrum will be used as a reference for the nuclear Overhauser enhancement spectroscopy studies (NOESY) where these signals will appear again and must be differentiated.

![Figure 3.48](image)

Figure 3.48 $^1$H-$^1$H NMR correlation spectrum for L3 in CD$_3$CN at 298 k

In Figure 3.49, the 500 MHz NOESY correlation spectrum of L3 in deuterated acetonitrile is shown. Several new spots appear in comparison with the COSY spectrum showing through-space correlations (coupling between protons not directly related). Thus, in the extended spectrum (Figure 3.49, lower part), coloured squares are indicating the through-space correlation taking place in the hydrophilic cavity. The stronger the signals are the closer is the distance between the nuclei involved.
Figure 3.49  NOESY spectrum of L3 in CD$_3$CN at 298 K
From Figure 3.50, further conclusions can be drawn. These are based on the last statement. The most important one is to assign the couple of doublets to the protons of the methylene bridge ($H_{eq} = \text{III}$ and $H_{ax} = \text{IV}$). So, the assignment of the signal at downfield (IV) to the $H_{ax}$ can be confirmed by the presence of several spots showing the correlation between the signal for proton IV and those for protons V, VI and VII. This phenomenon is not observed for the signal of proton III (highfield). In this case, only COSY correlation spots are observed.

![Diagram showing proton assignments and correlations](image)

**Figure 3.50** Selected NOESY spectra of L3 in CD$_3$CN for the $H_{ax}$ (IV) and $H_{eq}$ (III) proton signals at 298 K

On the other hand, this NOESY experiment reveals that a molecule of solvent is interacting with the hydrophilic cavity. This is shown in Figure 3.51. The presence of just one spot (A) showing correlation with one $p$-tert-butyl group could indicate the non-symmetrical position of this molecule of solvent in the hydrophobic cavity. In Figure 3.52, the NOESY couplings for the aromatic proton signals at the hydrophobic cavity with the solvent signals (B) are shown. These have a lower intensity than the spot A for
the coupling between the \( p\text{-}\text{tert-butyl} \) groups signals and the solvent, then a higher distance between the protons of the solvent molecule and the aromatic protons than that for \( p\text{-}\text{tert-butyl} \) group protons can be deducted. Thus the orientation of the solvent molecule can be inferred. In the same drawing (Figure 3.52) the close correlation between the phenolic group at the hydrophilic cavity and the water molecules of the solvent (spot C) can be observed.

Figure 3.51 NOESY correlation between the solvent signal (top-edge) and \( p\text{-}\text{tert-butyl} \) proton signals (right-hand edge) of L3 in \( \text{CD}_3\text{CN} \) at 298 K.

Figure 3.52 NOESY correlation between the solvent signal (right-hand edge) and aromatic proton signals (top-edge) of L3 in \( \text{CD}_3\text{CN} \) at 298 K.
The possibilities of using the quantitative information extracted from the intensity of the spots generated by the NOESY correlation experiments are now discussed. The main goal of this procedure is to get more information about the protonation and complexation processes taking place in the hydrophilic cavity of L3. Thus, in figures 3.53, 3.54 and 3.55, partial NOESY spectra for free L3, L3 in the presence of an excess of HClO₄ and L3 in an excess of CdClO₄₂, respectively, are shown. It should be noted that different scales are used for the former relative to the two latter. Not only the distribution of the peaks but the intensity of the signals varied from one spectrum to another. Thus, a different arrangement of the pendant arms may be expected. In order to verify the latter statement, a series of integration values for selected correlation peaks (H-H through space correlations) were calculated and tabulated in tables 3.12 and 3.13. On the left side of these tables, raw integration data for each selected peak are shown. Some values could not be calculated due to the overlapping of the peaks (blank spaces). On the right side of these tables, normal-colour values are the average of each couple of integration values of the corresponding cell at the left side of these tables. The gray shaded cell on the right side is the “reference distance” value. It belongs either to the COSY coupling of the signals of the protons axial and equatorial or to the signals of the protons of the ethylene bridge between the ethereal oxygen and the amino nitrogen of the pendant arm. The latter values were selected because they belong to protons on adjacent carbons and their inter-atomic distances are not expected to be significantly affected during the host-guest interaction process. These values will be used to normalize the data from the three spectra and for comparison purposes.

As it was previously explained, some data were lost due to the overlapping of the peaks. Therefore, only three values (the last column on the right side of tables 3.12 and 3.13) remain constant in all the spectra. These are used to proceed with the final calculation. The differences between the reference (gray shaded value) and the average integration values for the signals belonging to protons IV, V and VI are reported (values in bold). These normalized difference values can be considered as relative distances between the proton VII and protons IV, V and VI.

These relative distances are plotted and shown in figures 3.56 and 3.57. An expected behaviour is shown for the free ligand and the protonated ligand. However for the ligand in the presence of Cd²⁺, both curves show a different behaviour indicating a different folding of the pendant arms in this case.
Figure 3.53  NOESY spectrum of L3 in CD$_3$CN at 298 K
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Figure 3.54  NOESY spectrum of L3 with an excess of HClO₄ in CD₃CN at 298 K

Figure 3.55  NOESY spectrum of L3 with an excess of Cd(ClO₄)₂ in CD₃CN at 298 K
### Table 3.12 Relative distances (H\textsubscript{ax}→H\textsubscript{eq} as reference) from the NOESY correlation peaks of the spectra of free L3, H\textsuperscript{+}-L3 and Cd\textsuperscript{2+}-L3 in CD\textsubscript{3}CN at 298 K

<table>
<thead>
<tr>
<th></th>
<th>Hax</th>
<th>-OCH\textsubscript{2}</th>
<th>Heq</th>
<th>-CH\textsubscript{2}-N</th>
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Table 3.13  Relative distances (-OCH$_2$→-CH$_2$N as reference) from the NOESY correlation peaks of the spectra of free L3, H$^+$-L3 and Cd$^{2+}$-L3 in CD$_3$CN at 298 K

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Figure 3.56 Comparison of relative distances (H\text{ax} \rightarrow H\text{eq} as reference) between selected protons of L3, H\text{'}-L3 and Cd\text{2+}-L3 in CD\textsubscript{3}CN at 298 K.
Figure 3.57  Comparison of relative distances (-OCH$_2$→CH$_2$-N as reference) between selected protons of L3, H$^+$-L3 and Cd$^{2+}$-L3 in CD$_3$CN at 298 K
Thus, in presence of Cd\(^{2+}\) ions, the distance between protons VI and VII of L3 becomes shorter than that for the free ligand as well as for the ligand with an excess of acid. This fact could be explained on the bases of the geometry resulting from the sp\(^3\) hybridization of the nitrogen atom. This is trigonal pyramidal structure due to the lone pair of electrons (angle of C-N-C of \(~113.5^\circ\) for triethylamine). Upon complexation with the metal cation, this structure becomes less flattened and the C-N-C angle is shortened. This fact will reduce the distance between these carbon atoms. This statement could be indicative of complexation between L3 and Cd\(^{2+}\) ions.

In Figure 3.58, a hypothetical representation of Cd\(^{2+}\)-L3 complex is shown. The idea is to demonstrate the reduction in distances between protons VII y IV when the metal cation interacts with the pendant arms of the ligand.

![Figure 3.58: Hypothetical representation of the Cd\(^{2+}\)-L3 complex](image)

This is statement is corroborated by the difference between the chemical shift (\(\Delta\delta\)) for the axial and equatorial protons, \(\Delta\delta_{\text{ax-eq}}\) arising from the methylene bridge of L3. Thus, the following \(\Delta\delta_{\text{ax-eq}}\) values (ppm) were calculated, 1.0, 0.7 and 0.6, for L3, Cd\(^{2+}\)-L3, and H\(^+\)-L3, respectively. It was shown by Gutsche\(^{16}\) that a \(\Delta\delta_{\text{ax-eq}}\) value of 0.9 \(\pm\) 0.2 ppm correspond to a calix[4]arene ligand in its cone conformation and smaller \(\Delta\delta_{\text{ax-eq}}\) values (0.5 \(\pm\) 0.1 ppm) are observed for a ligand in a flattened cone conformation.

The suggestion that the phenolic oxygens participate in the complexation process as shown in Figure 3.60 does not fit the experimental data which clearly indicate that the ligand adopts a cone conformation upon complexation with the metal cation.
Figure 3.59 Conformational changes in calix[4]arene derivatives\textsuperscript{16}

Figure 3.60 Hypothetical representation of the Cd\textsuperscript{2+}-L3 complex
4. CONCLUSIONS.

From the above discussion, the following conclusions are drawn,

i) The hydrophobic cavity of calix[4]arene derivatives, due to their hydrophobic cavity formed by four phenol units, displays significant affinities towards neutral guests. Thus, 1D and 2D NMR studies on L3 suggested that interactions take place between these derivatives and solvent molecules such as acetonitrile, methanol and dichloromethane. A molecule of ethanol was found in the X-ray structure of L3 (solid state). These studies have demonstrated that these ligands undergo solvent-induced conformational changes, leading to more symmetrical cones which appear to be better ‘pre-organised’ to complex metal cations.

ii) $^1$H NMR data show that L3 interacts with the Hg$^{2+}$, Pb$^{2+}$, Cd$^{2+}$ and Mg$^{2+}$ metal cations forming 1:1 complexes in CD$_3$CN. These studies also revealed the sites of complexation. At the same time, the interaction with the proton was also observed in this solvent. Two protons were taken up by calix[4]arene unit. No interaction was observed between this ligand and alkali metal cations such as Na$^+$ and K$^+$. In CD$_3$OD, the $^1$H NMR data show that L3 interacts with Hg$^{2+}$, Pb$^{2+}$ and Mg$^{2+}$. However this ligand is unable to complex Cd$^{2+}$ and alkali metal cations. Protonation of L3 also occurs in methanol. Thus, two protons are taking up per unit of ligand. In both cases, the main sites of interaction are provided by the amino nitrogens of the pendant arms.

iii) Through conductance measurements the stoichiometry of complex formation between L3 and metal cations (Hg$^{2+}$, Pb$^{2+}$, Cd$^{2+}$ and Mg$^{2+}$) and the proton was established. Semi quantitative information on the strength of complexation was also obtained from conductometric titration data. Thus, well defined breaks in the titration curves demonstrated the formation of 1:1 complexes between L3 and these metal cations in acetonitrile at 298.15 K.

iv) The mercury electrode has proved to be a good analytical tool for monitoring Hg$^{2+}$ in organic solvents such as acetonitrile. Other processes
have been identified and it is concluded that protonation and solvolysis must be taken into account in the derivation of data.

v) Potentiometric studies using the glass electrode indicate that the protonation constant values for L3 are very close. From the titration of mixtures containing L3 and the metal cation (Hg^{2+}, Pb^{2+}, Cd^{2+}) salt solutions it was possible to calculate the stability constants of cation complexes. Then, both processes, complexation and protonation, must be considered to take place during the interaction of partially substituted calix[4]arene amino derivatives with metal cations. Solvents and ion water interactions taking place during the complexation processes, must be also considered.

vi) 2D NMR studies provided structural information in solution on host-guest interactions in acetonitrile. The complexation process was verified using relative distances calculated from NOESY correlation peaks. Data for L3, H^{+}-L3 and Cd^{2+}-L3 were compared and the different folding up of the pendant arms during metal cation-ligand interactions was demonstrated.
Suggestions for further work:

i) 2D NMR has proved to be a valuable tool to establish the conformational changes that the receptor undergoes upon complexation and protonation. These investigations should be extended to other metal cations and other ligands to compare the data obtained. The use of other 2D techniques such as ROESY and TROESY should be explored.

ii) Other techniques, such as voltammetry, should be used to corroborate the stability constant data obtained in this work.

iii) To explore the practical applications of this ligand for a) the production of ion selective electrodes, b) as anchor group in solid supports, c) the removal of chlorophenoxy acids (herbicides) from water.
REFERENCES.


123. XCAD4 - CAD4 Data Reduction. K Harms and 3 Wocadlo, University of Marburg, Marburg, Germany, 1995.


