SYNTHESIS AND BINDING PROPERTIES OF AN INHERENTLY CHIRAL CALIX[6]ARENE.

THESIS

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ABSTRACT

SYNTHESIS AND BINDING STUDIES OF AN INHERENTLY CHIRAL CALIX[6]ARENE

by

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August 2005

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Calixarenes are macrocyclic compounds composed of phenol and formaldehyde units and have been utilized extensively as supramolecular host molecules. Formation of chiral calixarenes for recognition of chiral guest species has been accomplished by two general methods. The first method involves the covalent attachment of a chiral auxiliary to the calixarene framework while the second method involves desymmetrization of the substitution pattern (inherent chirality) of the phenol rings. Of the two methods, the former is much more common and only limited examples of inherently chiral calixarenes exist to date. Due to their smaller size and number of phenol rings, inherently chiral

calix[4]arenes have been explored more and few examples of inherently chiral calix[5]arenes have been reported. To date, only one example of an inherently chiral calix[6]arene has been reported.

We have recently synthesized a racemic mixture of an inherently chiral calix[6]arene-crown-6 compound with an (A,B,H) substitution pattern by monobridging a disubstituted calix[6]arene with penta(ethylene glycol) di-*p*-tosylate in a diagonal-transannular-distal motif. X-ray crystallography confirms that the molecule adopts a cone conformation and provided the relevant dimensions of compound. When the racemic mixture was treated with 2 and 5 equivalents of Pirkle's chiral reagent, two diastereomeric complexes were formed as evidenced by shifts in certain ^{1}H NMR resonances for the host. Unfortunately attempts to optically resolve the racemic mixture into separate enantiomers by either chromatographic methods or via attachment of a chiral auxiliary have been unsuccessful. Efforts in this area are ongoing as well as chiral recognition studies.

The alkali metal binding properties of the chiral calixcrown were probed by electrospray ionization mass spectrometry. The results clearly demonstrate that the compound forms a 1:1 complex with both sodium and cesium ions, but has a large preference for cesium. The binding properties with the same metal ions were also studied by liquid-liquid extraction experiments followed by UV analysis of the picrate salts extracted into chloroform. Those experiments contradicted the ESI/MS experiments in that no apparent selectivity was observed for Na^+, K^+, Rb^+ or Cs^+ ions which all showed percent extraction ranging from 5-11%. In addition, the ammonium ion affinity of the

calixcrown was briefly examined via extraction experiments using ammonium picrate and the picrate salt of a chiral amine and both of these species formed 1:1 complexes.

1.0. INTRODUCTION

1.1 Calixarenes

There has been a rejuvenated interest in the field of calixarenes due to a need for supramolecular molecules capable of various functions. Calix[n]arenes are split into 2 major groups, phenol-derived calixarenes and resorcinol-derived calixarenes. Phenol-derived calix[n]arenes consist of phenolic residues connected by methylene carbons at the ortho position, and [n] represents the number of phenolic units in the macrocycle. The high reactivity of the aromatic groups on calixarenes makes them easily manipulated and molded into complex "baskets". In addition, calixarenes may be synthesized in adequate yields. These characteristics make calixarenes prime candidates for conformational studies as well as excellent host molecules for a variety of guest species.

Calixarenes containing 4, 6, and 8 phenolic units in the cycle are easily isolated, and therefore the predominant compounds studied. These macrocycles are synthesized from a combination of t-butyl phenol and formaldehyde with various alkali metal hydroxides in a base induced reaction[1]. The size of the metal cation involved determines the size of the macrocycle that will be formed. In other words, a larger cation correlates to a larger macrocycle. The metal cations promote a "template effect" that is responsible for the size of the calixarene. Basically, the macrocycle is formed around the cation, and this determines the size. For example, it is well established that NaOH consistently yields calix[4]arenes.[1] RbOH will yield a mixture of calix[5]arene and calix[6]arene with the latter being more predominant.[1]

1.2 Nomenclature and Orientation

Figure 1.1 illustrates the method by which the carbons in calix[6]arene are numbered.

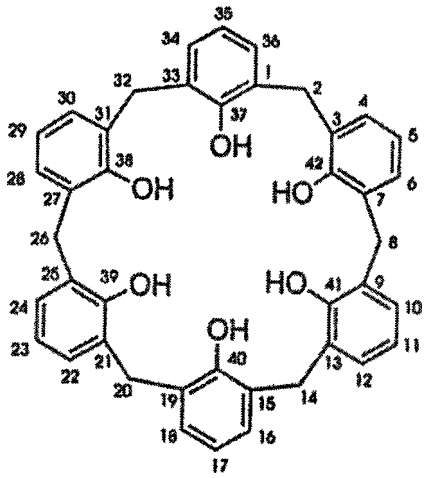


Figure 1.1 *Carbon Numbering of Calix[6]arene-37,38,39,40,41,42-hexol*

Calixarenes also have a degree of conformational mobility that increases with the size of the macrocyclic framework. Therefore, a calix[6]arene will possess more conformational mobility than a calix[4]arene. The conformational mobility is an important factor in the process of forming a suitable supramolecular host. Therefore, it is prudent to devise a method of depicting the orientation of calix[6]arenes beyond simply numbering the carbons. The first step is to arbitrarily label the rings A, B, C, *etc.* This also aids in specifying where certain substituents are attached. Figure 1.2 below illustrates a calix[6]arene with the carbons numbered and the rings labeled as well.

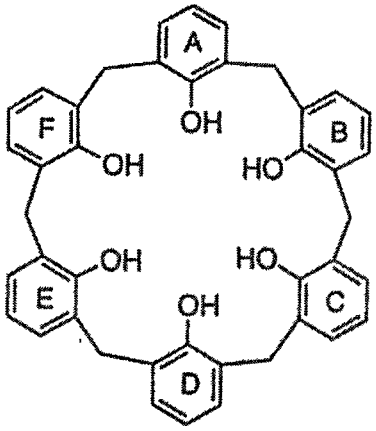


Figure 1.2 *Ring Labeling of Calix[6]arene-37,38,39,40,41,42-hexol*

The next step involves designation of the "faces" of the calixarene. Calixarenes are often structurally compared to vases, and their name is derived from the Greek word for vase. Much like a vase, calixarenes are often depicted as standing on a base. The face of the calixarene bearing the hydroxyl groups pointing downward is referred to as the 'lower rim'. Similarly, the face of the calixarene bearing the *para* substituents is called the 'upper rim'. In addition, there is a macrocyclic ring defined by the methylene units, the ortho ring carbons, and the substituted carbon. For example, in calix[6]arene the annulus is a 24 membered macrocyclic ring. A representation of the upper and lower rims on a calix[4]arene is shown in Figure 1.3.

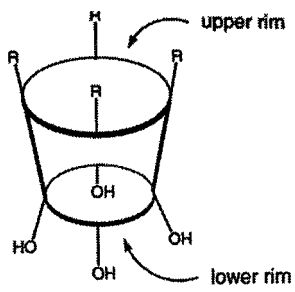


Figure 1.3 *Representation of the faces on calix[4]arene*

Finally, we are ready to introduce a method to describe the conformational orientation of the calixarene. The various aryl groups on a calixarene may project upward or downward with respect to the macrocyclic annulus. A reference ring is designated as 'up' or 'down', and each remaining ring is assigned an up or down designation relative to the reference ring. The designations are abbreviated u = up, d = down, o = out, di = down in, uo = up out, *etc.* The most common conformation encountered in calixarene research is referred to as the 'cone' conformation. A calixarene in the cone conformation will contain all rings in the macrocycle in the 'up' position and syn to each other (figure 1.4).

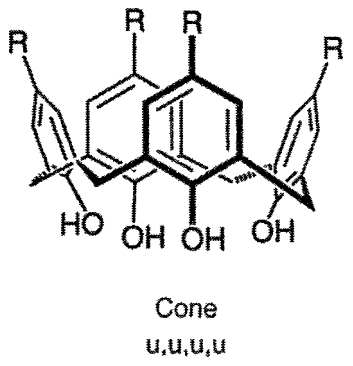


Figure 1.4 *'Cone' Calix[4]arene*

1.2.1 Dihedral Angles

There is a more definitive way to determine and describe the conformation of a calixarene. X-ray crystallography can be used to obtain a picture of a calixarene's solid state conformation, but an electronic or printed image can be unreliable. However, the atomic coordinates of a crystal structure can be used to determine the diagnostic angles, φ

and χ. The angles are determined by the sequence of carbons where $\varphi = C_1\text{-}C_2\text{-}C_3\text{-}C_4$ and $\chi = C_2\text{-}C_3\text{-}C_4\text{-}C_5$, respectively, shown in figure 1.5.[1]

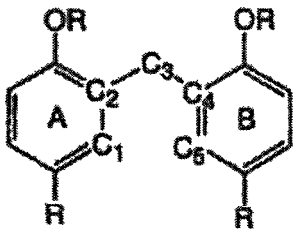


Figure 1.5 *Aryl groups involved in determining dihedral angles*

Figure 1.6 illustrates 2 possible orientations for rings A and B. When the rings are like that of orientation-A, referred to as the 'syn' orientation, coordinates φ and χ are opposite in sign. When the angles are in orientation-B, known as the 'anti' orientation, the coordinates have the same sign. All these angles must be determined in order to accurately determine the conformation of a calixarene.

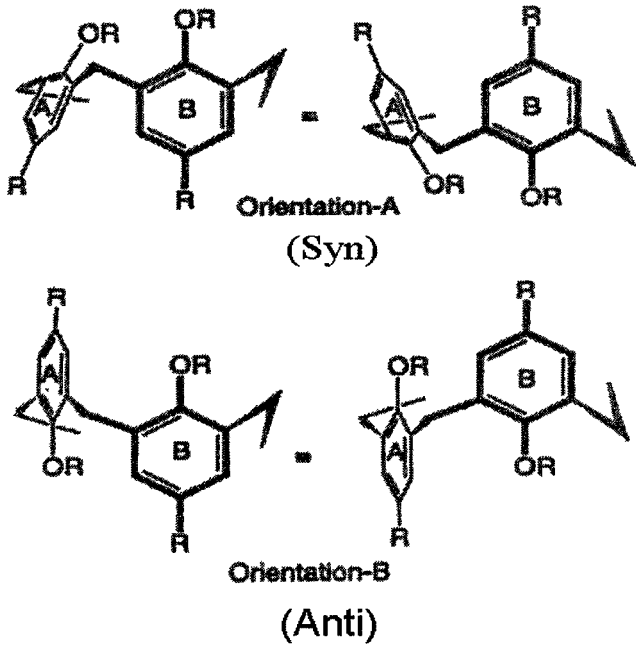


Figure 1.6 *Aryl orientations of rings A and B*

1.3 Chiral Calixarenes

1.3.1 Chirality and Enantiomers

Recently, there has been increasing interest in the synthesis and separation of enantiomers of organic compounds due to their importance in the biochemical and pharmaceutical industries. Enantiomers are chemically identical, but exist as two non-superimposable mirror images. Enantiomers are a special variety of stereoisomers. The chirality of a pair of enantiomers is caused by the absence of symmetry, or chiral elements (chiral axis, plane, center, etc.) in their structure. Optical activity of the enantiomers is determined by the spatial arrangement of their atoms in the molecule. The Cahn-Ingold-Prelog (R, S) system is used to designate the configuration of enantiomers[2]. The evidence for the existence of inherent chirality can be deduced by NMR spectra in the absence or presence of a chiral shift reagent. The NMR spectrum exhibits splitting of various signals due to formation of diastereomeric complexes. In some cases it can be attested through circular dichroisim symmetric images of a pair of enatiomers. Optical resolution can also be achieved via HPLC using a chiral column. Yet another approach to separate enantiomers is indirect enantiomeric resolution. This technique involves the coupling of enantiomers with an auxillary chiral reagent to convert them into diastereomers. The diastereomers can then be separated by any achiral separation technique.

1.3.2 Synthesis of Chiral Calixarenes Using a Chiral Moiety.

There are two possible methods of synthesizing a chiral calixarene compound. The majority of chiral calixarenes are made by attaching a chiral moiety, or a chiral auxiliary compound to the upper or lower rim of an achiral calixarene.[1]

Yuan *et al.*[3] combined achiral 1,3,5-trimethoxy-*p-tert*-butylcalix[6]arene with N-chloroacetyl amino acid ester, and various other amino acid esters in the presence of K_2CO_3. The results show chiral modification of the calix[6]arene, increased conformational stability, and the chiral centers provided an asymmetric environment for discrimination of chiral guest (Figure 1.7).

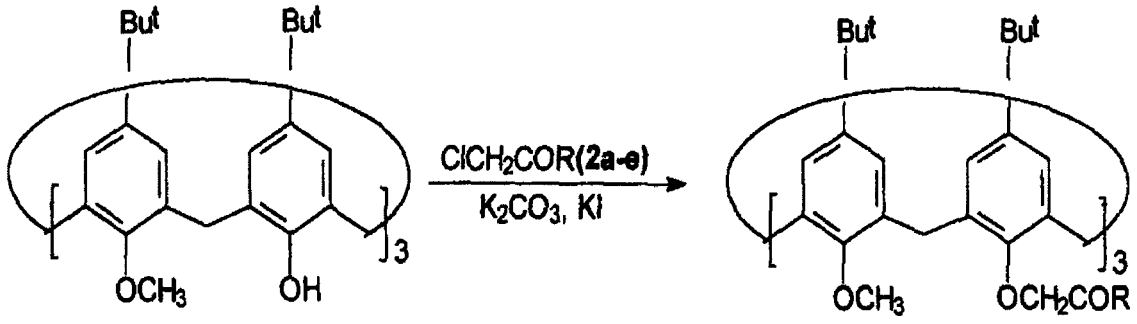


Figure 1.7 *Calix[6]arene containing a chiral moiety.*

1.3.3 Synthesis of an Inherently Chiral Calixarene

The second preparative method of chiral calixarenes is much less practical due to synthetic processes. This is done by simply establishing an asymmetric pattern of substitution after the calixarene ring has been established. There are 3 possible substitution patterns in an inherently chiral calix[4]arene (Figure 1.8).[4]

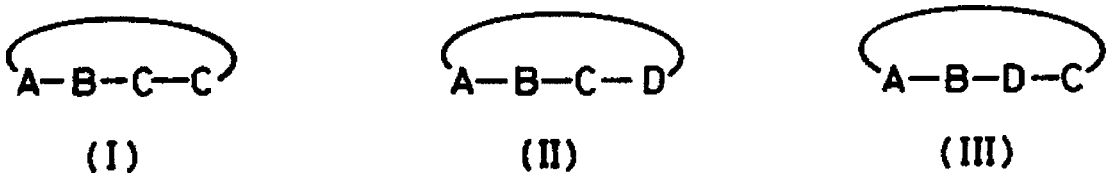


Figure 1.8 *Substitution patterns for chiral calix[4]arenes*

For example, Bohmer *et al*[4] developed 2 different synthetic schemes in order to synthesize a single chiral calix[4]arene with the 'A-B-C-D' pattern (Figure 1.9).

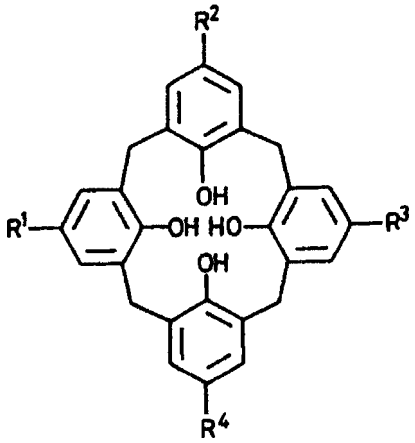


Figure 1.9 *'A-B-C-D' substituted chiral calix[4]arene*

Vysotsky *et al*[5] also synthesized a series of inherently chiral calix[4]arenes using asymmetrical combinations of substituents at the lower and upper rims (figure 1.10). Vysotsky was able to prove the existence of a racemic mixture by the doubling of signals in the ^{1}H and ^{31}P NMR spectra after addition of chiral solvating Pirkle's reagent.

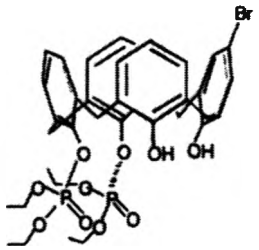


Figure 1.10 *Diethoxyphosphoryl calix[4]arene*

Cao *et al*[6] developed the synthesis of racemic inherently chiral calix[4]crown derivatives by *o*-monoalkylation of calix[4]crowns. The compounds were successfully resolved using (S)-BINOL followed by diaestereomer separation using preparative TLC (Figure 1.11).

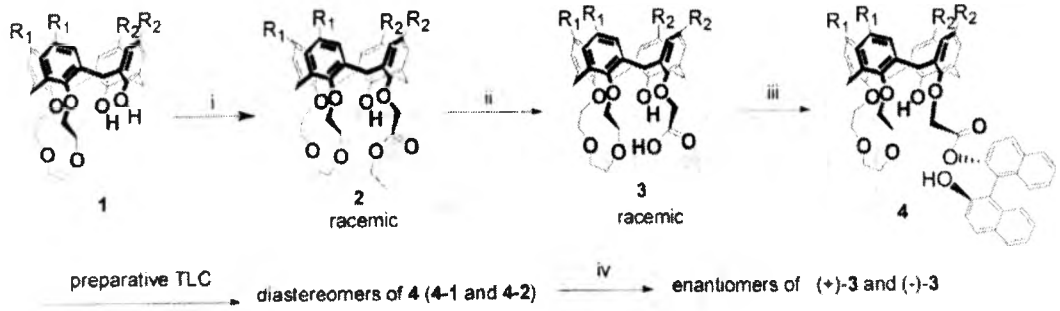


Figure 1.11 *Synthesis of diastereomeric calix[4]crowns*

Verboom *et al*[7] has established a novel method for preparation of inherently chiral calix[4]arenes by direct introduction of a substituent at the meta position of the phenol rings of a calix[4]arene (Figure 1.12)

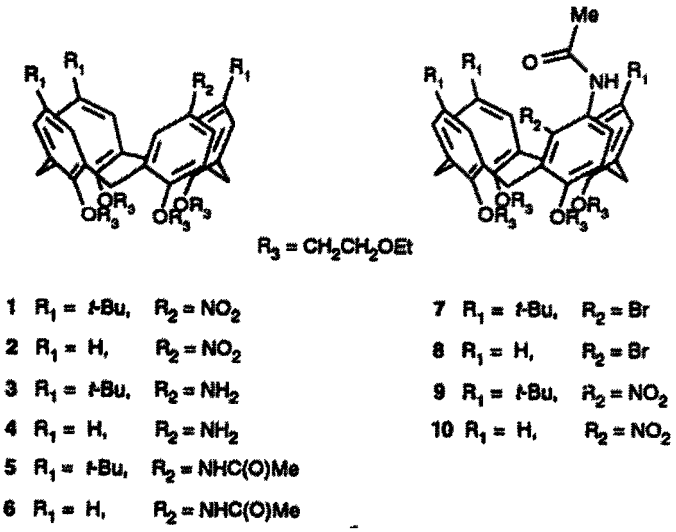


Figure 1.12 *Inherently chiral calix[4]arene with meta substituent*

However, some problems arise from transformation of achiral compounds in this manner. Formation of a chirality element in this manner often results in the product being a mixture of the enantiomers, or a racemate. Racemates are optically inactive, and separating the enantiomers is a difficult problem in stereochemical research as well as drug research.

1.3.4 Chiral Calix[4]arenes

We've now seen 2 examples of 2 ways of introducing chirality into calixarenes in compounds, and can now turn our attention to the remaining examples of chiral calix[4]arenes.

Shinkai *et al*[8] synthesized a cone shaped, asymmetrically substituted calix[4]arene, and optically resolved the enantiomers successfully, (figure 1.13).

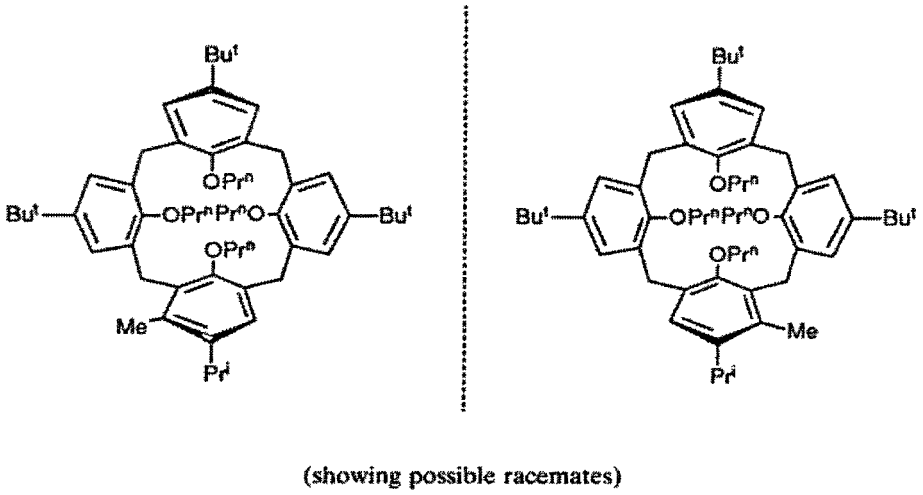


Figure 1.13 *Racemates from Shinkai*

Shinkai validated that his compound was chiral by NMR titration with Pirkle's reagent The splitting in the 1H NMR spectrum is apparent upon closer inspection of the *m*-methyl protons of the calix4arene (figure 1.14).

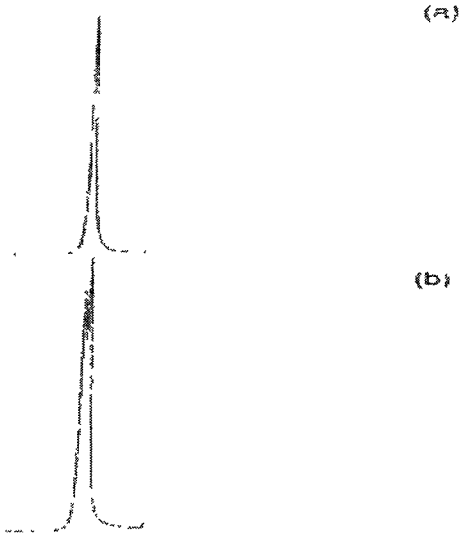


Figure 1.14 *^{1}H NMR Stack plot of m-methyl protons of a) racemic mixture, b) racemic + Pirkle's reagent.*

Pappalardo *et al*[9] reported a series of inherently chiral calix[4]arenes that existed in the cone conformation. The synthetic strategy involved modifying the substitution pattern on the lower rim of the calixarene with oligoethylene glycol ditosylates. The purpose of using these ethylene glycols was not only to introduce asymmetry and structural stability, but to create a binding site as well. NMR analysis was able to confirm that the calix[4]arene in figure 1.15 successfully binds either chiral alkyl ammoniums or Zn^{2+} species. Similar crown ethers have been shown to be very effective molecular receptors for alkali and primary ammonium cations[10]. Recently, calixarenes have even been constructed to serve as hosts for neutral and anionic species[11].

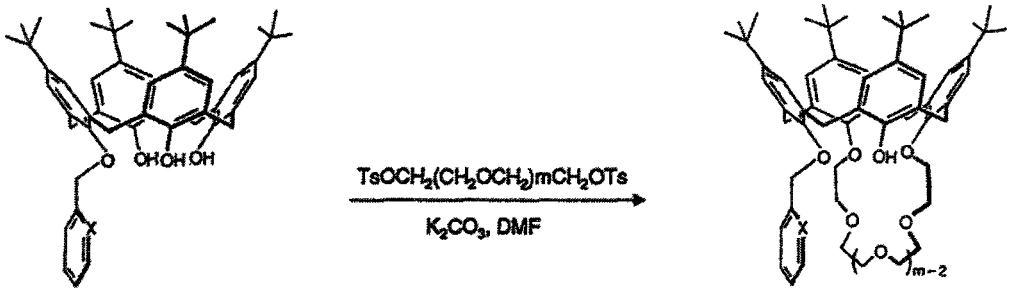
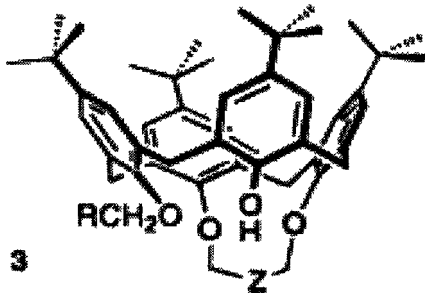


Figure 1.15 *Inherently chiral calix[4]arene in cone conformation*

Arnaud-Neu *et al.*[12] also reported the synthesis of several inherently chiral calix[4]crown ethers (figure 1.16). Once again, evidence of chirality was provided by the interaction with enantiopure alkyl ammonium salts using NMR analysis. The complexing ability of the compound was also tested using extraction studies of alkali, alkaline earth and heavy metal picrates. Also, this synthetic process is taken one step further by the enantiomeric resolution by enantioselective HPLC. These racemic

calix[4]crown ethers were successfully resolved on a Chiralpak AD CSP[amylase tris(3,5-dimethylphenylcarbamate)] column.



3-5	R	Z
a	2-Py	$(CH_2OCH_2)_2$
b	2-Py	$(CH_2OCH_2)_3$
c	2-Py	$(CH_2OCH_2)_4$
d	Ph	$(CH_2OCH_2)_4$

Figure 1.16 *Inherently chiral calix[4]crown ether*

1.3.5 Chiral Calix[5]arenes

The creation of inherently chiral calix[4]crowns for use as molecular receptors has been fairly well established. So, the next step is to move to larger calix[5]crowns. Arnecke[13] reported the preparation of a series of inherently chiral o-alkyl derivatives of calix[5]crown ethers(figure 1.17).

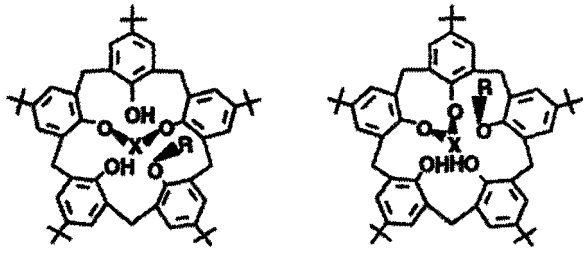


Figure 1.17 *Inherently chiral calix[5] crown ethers*

The strategy employed here is identical to previous reported studies involving calix[4]crowns. Multiple residues have been attached to the phenolic oxygens on the lower rim of the calix[6]arene, and the compound is also simultaneously locked into its cone conformation.

1.3.6 Chiral Calix[6]arenes

Inherently chiral calix[6]arenes are still extraordinarily novel and there have not been many reported. Shinkai reported a xylenyl-bridged chiral calixarene[14] (figure 1.18).

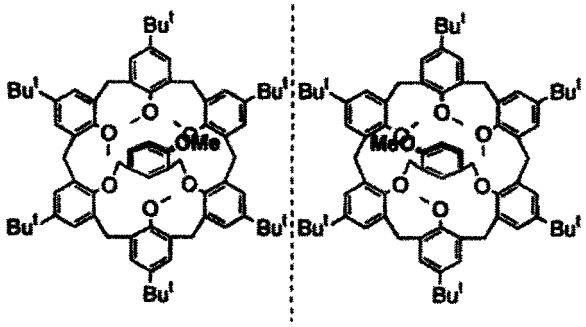


Figure 1.18 *Inherently chiral calix[6]arene*

Once again, the chirality was substantiated by observation of the ^{1}H NMR spectrum in the presence of Pirkle's chiral shift reagent.

1.4 Thesis Proposal

An abundance of inherently calix[6]arenes have not been synthesized due to the large ring size and complexity. The need for novel, chiral calixarenes is endless due to their cation binding properties and potential to serve as biomimetic receptors.

The purpose of this research was to synthesize a rigid, inherently chiral calix[6]arene crown ether, and investigate the binding properties with a series of cationic guests. An A, D-dibenzyl calix[6]arene was used in order to selectively monobridge the calixarene on the C and F rings in order to create an asymmetric compound. In addition, we attempted to separate the two enantiomers of the calix[6]crown.

Conformational and structural arrangement of the host was established using x-ray crystallography. Binding studies were carried out using NMR titrations, UV metal picrate extractions, and mass spectrometry.

1.5 Cation Binding Studies

An inherently chiral calix[6]crown ether has been synthesized in the cone conformation. Liquid extractions were carried out with aqueous picrate salts of K^+, Na^+, Rb^+, Cs^+, NH_4^+, (S)-methyl benzylamine along with the host dissolved in organic solution. The methods used were borrowed from previously reported studies[15].

2.0 PROPOSED STRATEGY FOR RESOLUTION OF RACEMIC MIXTURES

2.1 Mono-bridged Calix[6]arenes

2.1.1 Bridging Motifs

We have seen examples of inherently chiral calix[4]arenes, calix[5]arenes, and how the lower rim can be modified to introduce asymmetry. The first challenge of our research was to find a strategy to introduce asymmetry into a calix[6]arene by modifying the lower rim. The most obvious method was to attach substituents on the lower rim, and modify the substitution pattern in the dealkylated calix (H-H-H-H-H-H) to A-B-H (Figure 2.1). The A-B-H-A-B-H pattern will be inherently chiral for reasons described below.

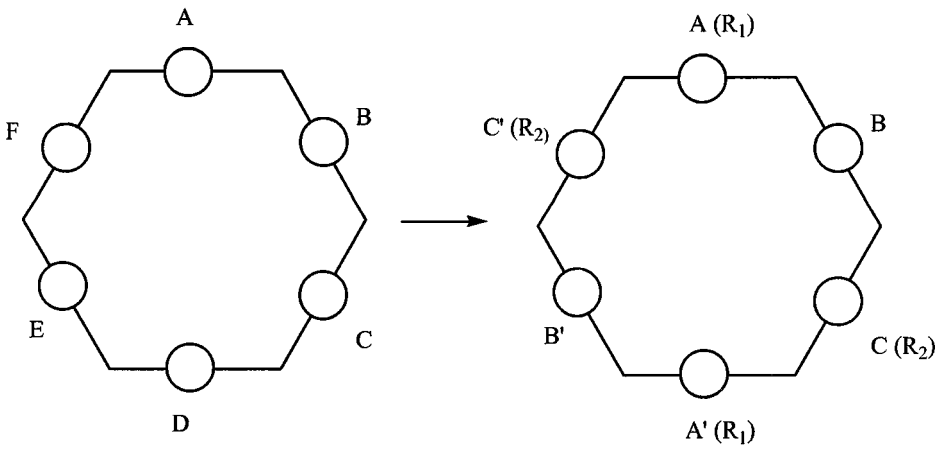


Figure 2.1 *A-B-H substitution of calix[6]arene*

The first step was to place alkyl groups on the A and D rings of the calixarene usually in the form of a benzyl or allyl (figure 2.2). When this occurs, the A/D rings become mirror images of each other and are designated as A/A'.

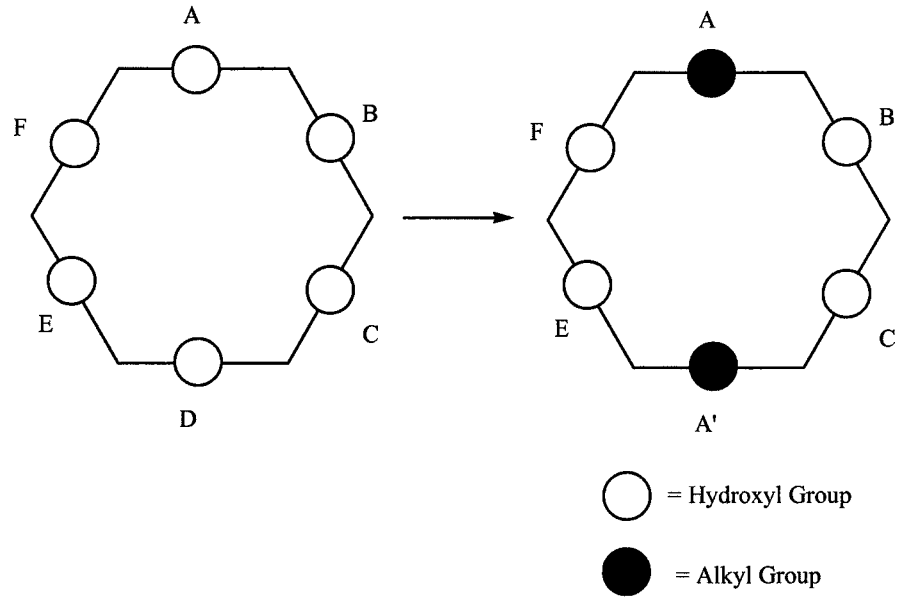


Figure 2.2 *Alkyl groups on rings A and A'*

After the first dialkylation step, bridging is only possible on rings B, C, E, and F. However, there are still several configurations possible for the bridging process. The first pattern is known as the 'proximal' motif in which two adjacent rings (C to B in figure 2.3) are bridged. However this does not produce an inherently chiral compound due to the presence of a σ plane within the structure.

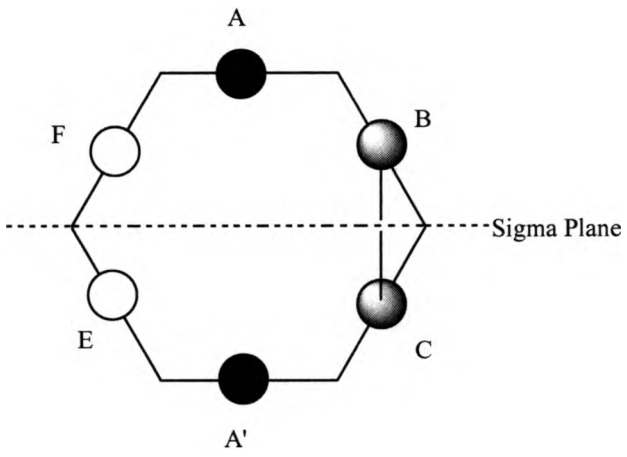


Figure 2.3 *Proximal bridging*

The next possibility is known as 'transannular distal' motif (C to E) shown in figure 2.4. The bridge connects in a straight line across the calix[6]arene annulus. Again this will not produce a chiral molecule due to the internal mirror plane system.

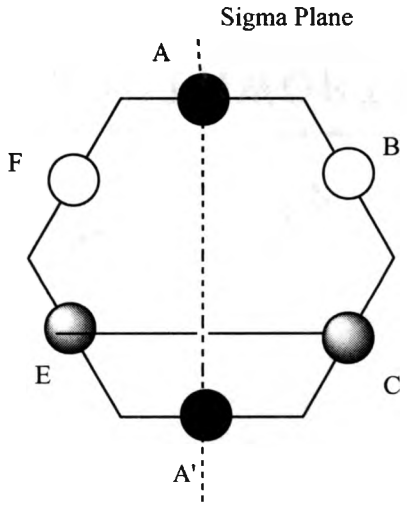


Figure 2.4 *Transannular distal bridging*

Finally, the last motif possible, and the variety of bridging pertinent to our research is the 'transannular-distal-diagonal' motif. This motif is similar to transannular distal,

but the bridge is a diagonal line across the calix[6]arene annulus (C to F) (Figure 2.5). Even though there is an inversion center and C_2 axis, this pattern will produce a chiral structure due to the absence of the internal σ plane. Moreover, a racemic mixture of enantiomers is anticipated.

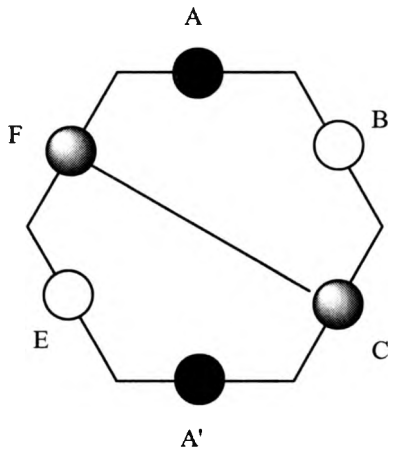


Figure 2.5 *Transannular distal diagonal bridging*

The final two rings could be left unfunctionalized or functionalized with a group other than those on the A/D rings or C/F rings. Transannular distal diagonal bridging coupled with the 'A-B-H' substitution pattern mentioned earlier gave us the inherently chiral calix[6]arene we desired. Actually, the transannular-distal-diagonal bridging may be favored due to better geometric complementarity between the pentaethyleneoxy bridge and the annulus. Figure 2.6 illustrates a pair of superimposible enantiomers, (transannular distal diagonal motif).

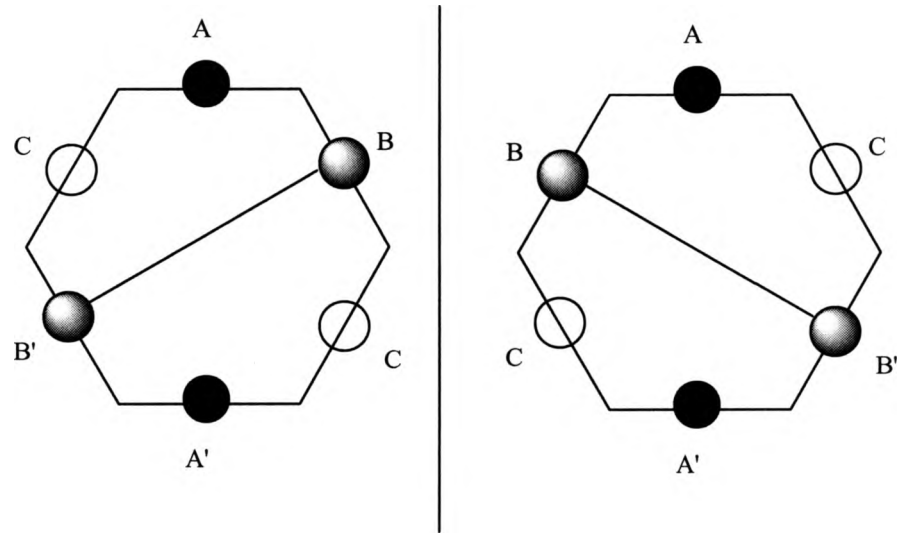


Figure 2.6 *Monobridged calix[6]arene enantiomers*

The following is a summary of the specific reactions used to synthesize the chiral calix[6]arene described above.

[2.2] 5, 11, 17, 23, 29, 35-hexa-*p-tert*-butyl-37,38,39,40,41,42-hexahydroxycalix[6]arene synthesis (1)

Compound **1** was synthesized using the method established by Gutsche[1] (figure 2.7). Yields for compound **1** are generally in the 88 to 93% range and are carried out on a 100 g scale with minimal purification.

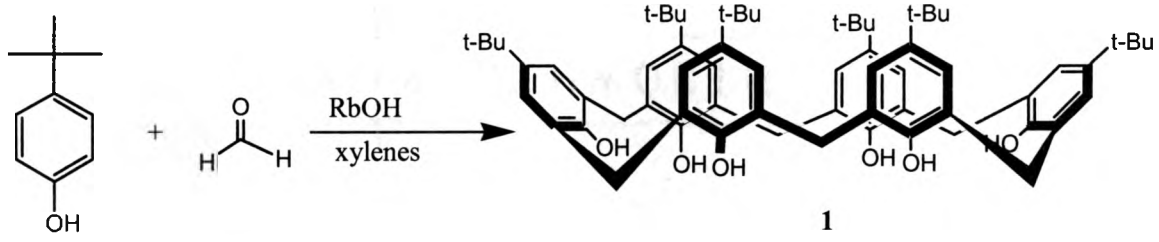
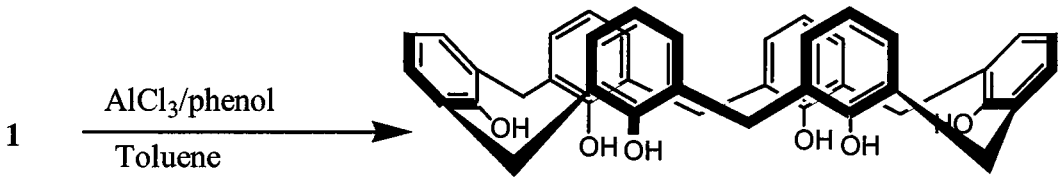


Figure 2.7 *Synthesis of compound* **1**

There is a significant amount of conformational mobility associated with compound **1.** The phenolic rings are capable of rotating through the annulus of the calixarene.

[2.3] 37,38,39,40,41,42-hexahydroxycalix[6]arene synthesis (2)

Compound **2** was synthesized with a reverse Friedel-Crafts alkylation also developed by Ungaro[16] (figure 2.8). The removal of the alky groups from the upper rim of the calixarene increases the amount of conformational mobility with respect to compound **1**. Therefore, there is more rotation of the rings through the annulus.



2

Figure 2.8 *Synthesis of compound* **2**

Yields for **2** range from 80 to 85% and are usually carried out on a 20 g scale. No further purification is required after workup.

[2.4] 37,40-Dibenzyloxy-38,39,41,42-tetrahydroxycalix[6]arene synthesis (3)

Compound **3** was obtained by selective dibenzylation on the phenolic oxygens of the A and D rings of the compound **2** using benzyl bromide in the presence of potassium trimethyl silanoate, (Figure 2.9)[17]. The dibenzylation selectivity is observed due to the stabilization of the phenolate ions by 2 flanking hydrogen bonding interactions.

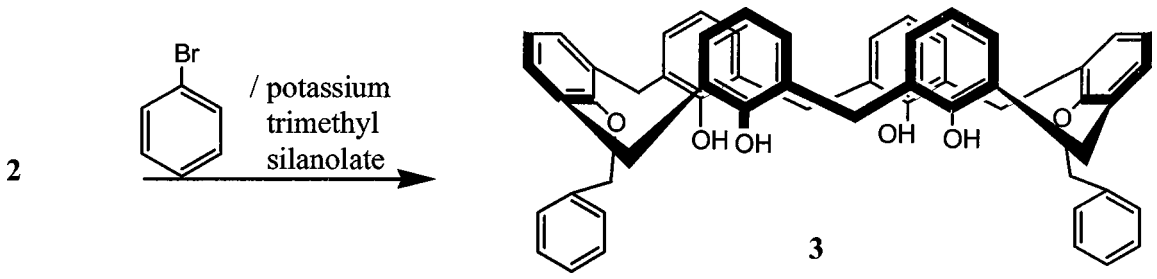


Figure 2.9 *Synthesis of compound* **3**

These alkylating groups will only allow bridging between the B and F rings and C and E rings, respectively, and the conformational mobility of **3** is significantly reduced at A and D due to the alkyl groups. However, there is still rotation through the annulus at the remaining rings. Compound **3** is synthesized in 90 to 95% yields and required minimal purification.

[2.5] 37,40-Dibenzyloxy-(39-42)-crown-6-calix[6]arene (4)

When compound **3** was treated with penta(ethylene glycol)-di-p-tosylate and NaH in acetone, compound **4** was isolated (figure 2.10). Conditions for this bridging reaction were taken from Blanda et al[18]. Compound **4** is generally synthesized in 50 to 55% yields and required purification by column chromatography after workup.

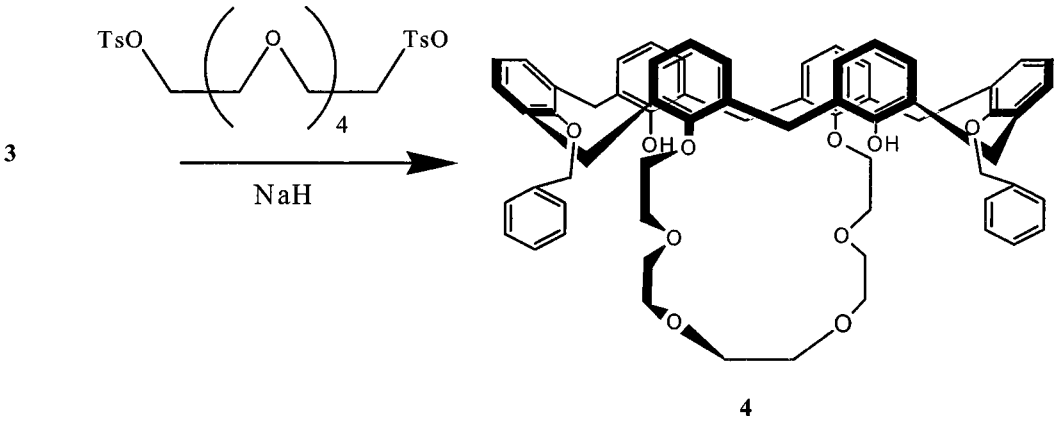


Figure 2.10 *Synthesis of compound* **4**

The addition of the pentaethlyene oxy bridge significantly inhibits the rotation of the rings through the annulus. Rotation is now only possible at the free phenolic rings, (B and E). Also, the oligoethylene chain creates a potential binding site for cations and other guest species. Attempts to resolve the enantiomers of compound **4** using chromatographic methods were unsuccessful. Therefore, alternative synthetic methods were attempted in order to resolve the enantiomers.

[2.6] 37,40-Dibenzyloxy-(38,41)-diester-(39-42)-crown-6-calix[6]arene (5)

Next, we attempted to resolve the enantiomers via attachment of a chiral auxiliary. The attachment of a chiral auxiliary results in the creation of diastereomers. Enantiomers have identical physical properties except for the direction in which they polarize light. Diastereomers, on the other hand, generally have different physical properties and are separated more easily than enantiomers. We attempted to attach (S)-(-) methyl butyric anhydride to the free phenolic groups on compound **4**, but we were unsuccessful.

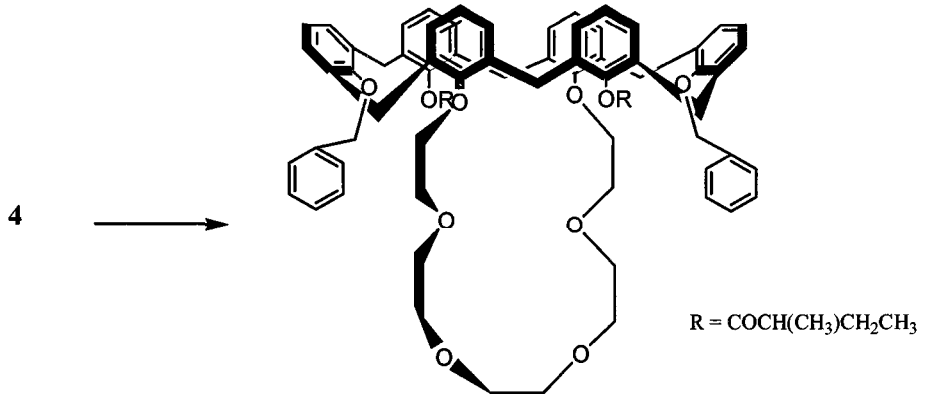


Figure 2.11 *Attachment of (S)-(-) methyl butyric anhydride to form* **5**

[2.7] 37,40-Dibenzyloxy-(38-41)-dipyridinal-(39,42)-crown-6-calix[6]arene (6)

We attached pyridinal rings with the goal of enhancing the binding site with the lone pairs on the nitrogen atoms. Compound **6** is synthesized on a 1 g scale and is synthesized in 25-30% yields. The product requires purification via column chromatography after workup.

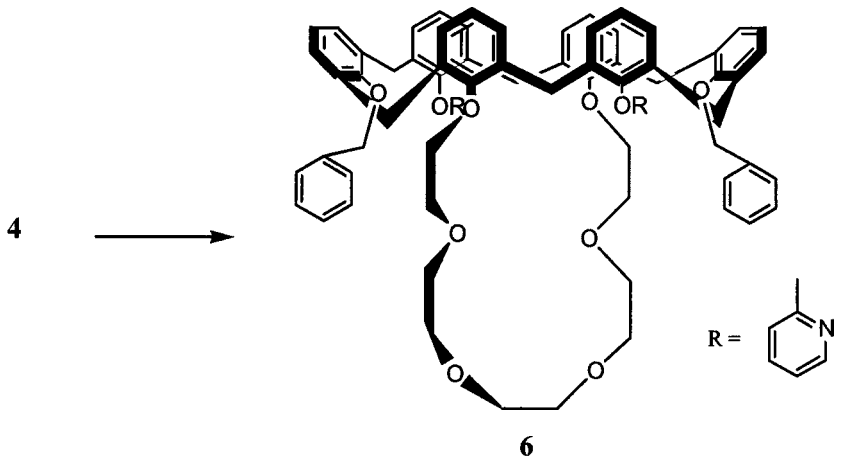


Figure 2.12 *Synthesis of compound* **6**

3.0 STRUCTURAL ANALYSIS OF COMPOUND 4

3.1.1 Mass Spectrometry (4)

The first piece of evidence that compound **4** was a monobridged calix6arene was provided by electrospray ionization mass spectrometry. The spectrum of compound **4** showed a strong peak at molecular mass of 1041 g/mol (figure 3.1) which corresponds to the sodium complex of 4^*Na^+.

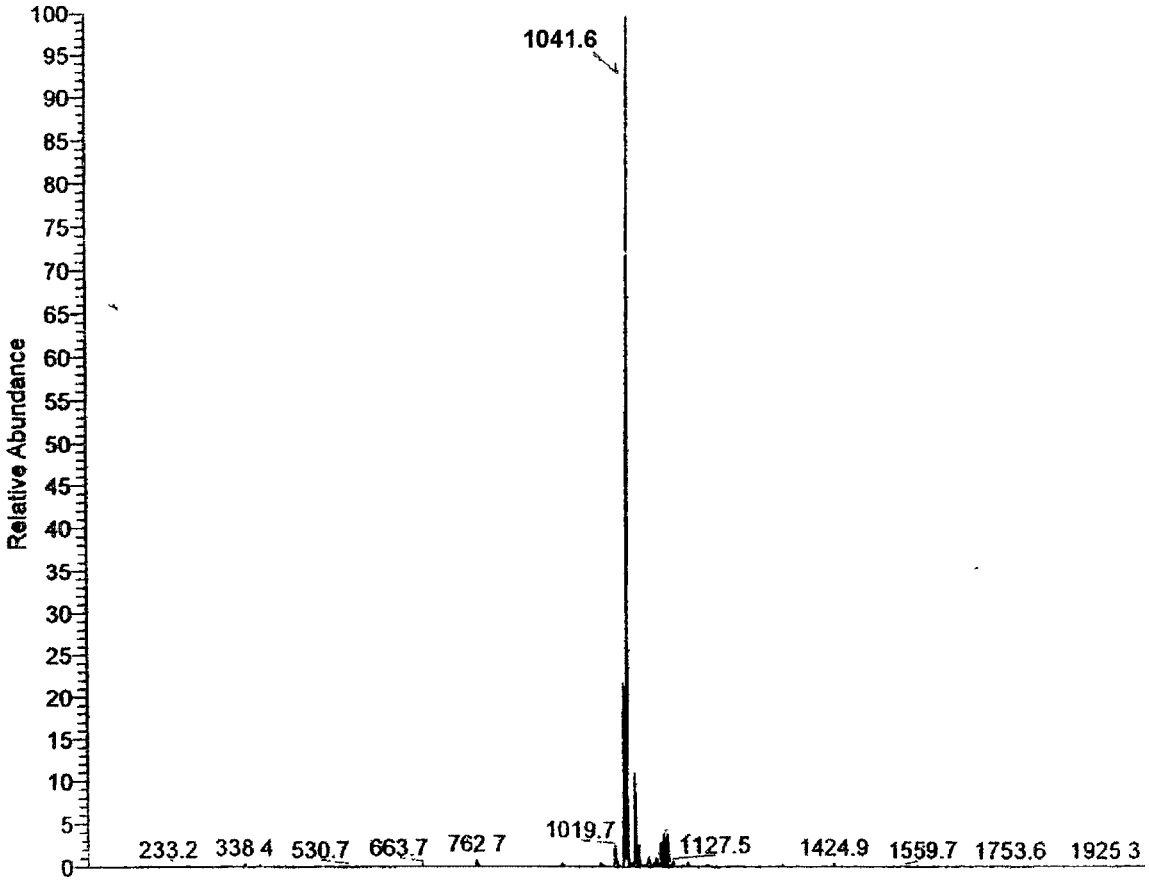


Figure 3.1 *Mass spectrum of compound* 4^*Na^+

Since sodium ions are ubiquitous in ESI-MS methods the sodium complex was formed due to the cation affinity of the polyether bridge in **4.** If we subtract the weight of the sodium ion from the molecular weight of the complex we are left with a molecular weight for uncomplexed **4** of 1018 g/mol. From this molecular mass we predicted that compound **4** contained a single pentaethylene glycol bridge. Because there are several bridging patterns (as discussed in chapter 2) a solid state structure was necessary in order to accurately determine the bridging motif.

3.1.2 X-ray Crystal Structure (4)

The solid state crystal structure of compound **4** was confirmed using x-ray crystallography. Figures 3.2 and 3.3 verified that rings C-F were in fact bridged in a transannular diagonal distal motif. The polyether bridge is situated below the plane of the calixarene rings at the lower rim.

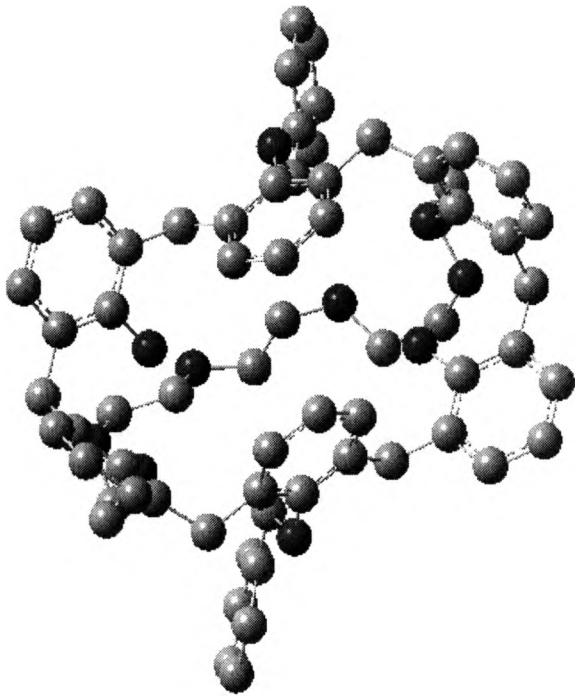


Figure 3.2 *Side view of crystal structure of compound* **4**

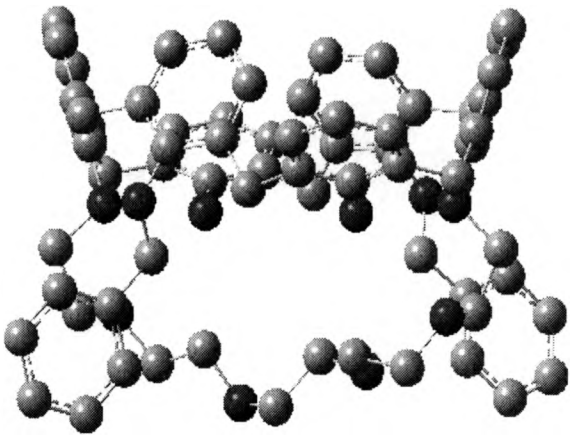


Figure 3.3 *Top view of crystal structure of compound* **4**

The B and E rings containing the free phenolic groups are oriented up and out (uo) relative to the benzyl substituted A/D rings which are up/in and the bridged C/F rings are up/out. The stereochemical conformation can be described as (uo, ui, uo, uo, ui, uo) which describes the cone conformation.[1]

The transannular distance between rings A and D is 4.03 Å at the upper rim. Similarly, the distance between rings B and E is 11.8 Å and 13.4 Å between rings C

and F. Therefore, the structure resembles a rectangle as shown in figure 3.3. We were able to measure the distances around the perimeter of the upper rim of the calixarene. The B, C, E, and F rings have a rectangular shape with average dimensions of 11.10 Å long and 6.08 Å wide.

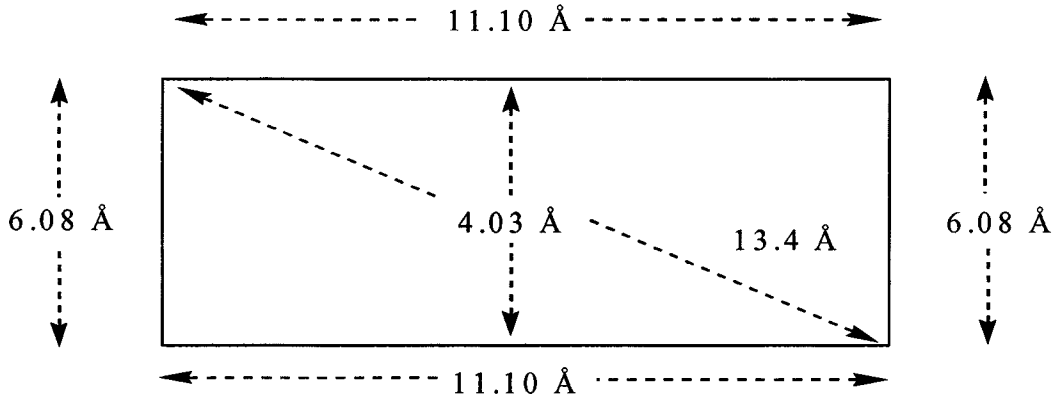
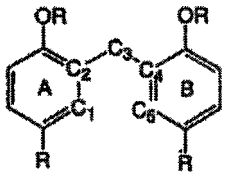


Figure 3.4 *Upper rim dimensions of* **4**

Next, we were able to obtain selected distances at the lower rim. The distance between rings A and D was 7.15 Å. The transannular distances at the upper rim of compound **4**, on average, are larger than the distances at the lower rim. In other words, the compound is wider at the upper rim and becomes narrower at the lower rim, taking on a conical shape.

3.1.3 Dihedral Angles (4)

The final pieces of data extracted from the x-ray structure were the dihedral angles, φ and χ. The angles are determined by the sequence of carbons previously discussed in section 1.2.2 and illustrated in figure 3.4.



φ = C1-C2-C3-C4

χ = C2-C3-C4-C5

Figure 3.5 *Aryl groups involved in determining dihedral angles*

In a calix6arene there are six distinct φ and χ. The values of φ and χ for compound **4** are summarized in table 3.1.

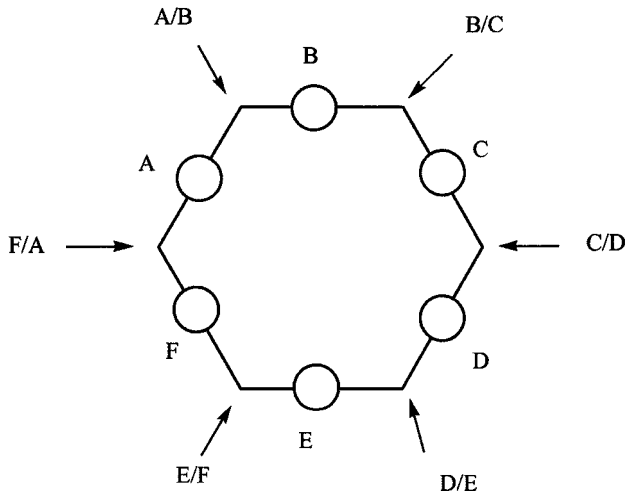


Figure 3.6 Location of *φ and χ dihedral angles of compound* **4**

Table 3.1 *φ and χ values for compound 4*

Ring Position	φ	χ
A/B	- 15.162	+ 103.643
B/C	- 101.473	+ 81.460
C/D	- 78.526	+ 7.966
D/E	- 15.109	+ 103.639
E/F	- 101.485	+ 81.409

The most important structural aspect of the dihedral angles is to note that the algebraic signs of φ and χ are opposite in all cases. This indicates that all the aryl rings in compound **4** have the same relative orientation with respect to the macrocyclic annulus and the compound is in the cone conformation.[1]

3.1.4 ^{13}C NMR Analysis (4)

The only symmetry elements present in the molecule are C_2 axes, thus the structure belongs to the C_2 point group.

As such, even though there are 64 total carbon atoms, the ^{13}C contains only half that number of signals. For example, there are 23 signals for the aromatic rings in calix6arene in the region from 120 to 156 ppm, 5 signals for the pentaethylene oxy

bridge in the region from 68 to 74 ppm, 1 signal for the benzylic carbon, and 3 signals for the Ar-CH_2-Ar carbons in the 28 to 32 ppm region.

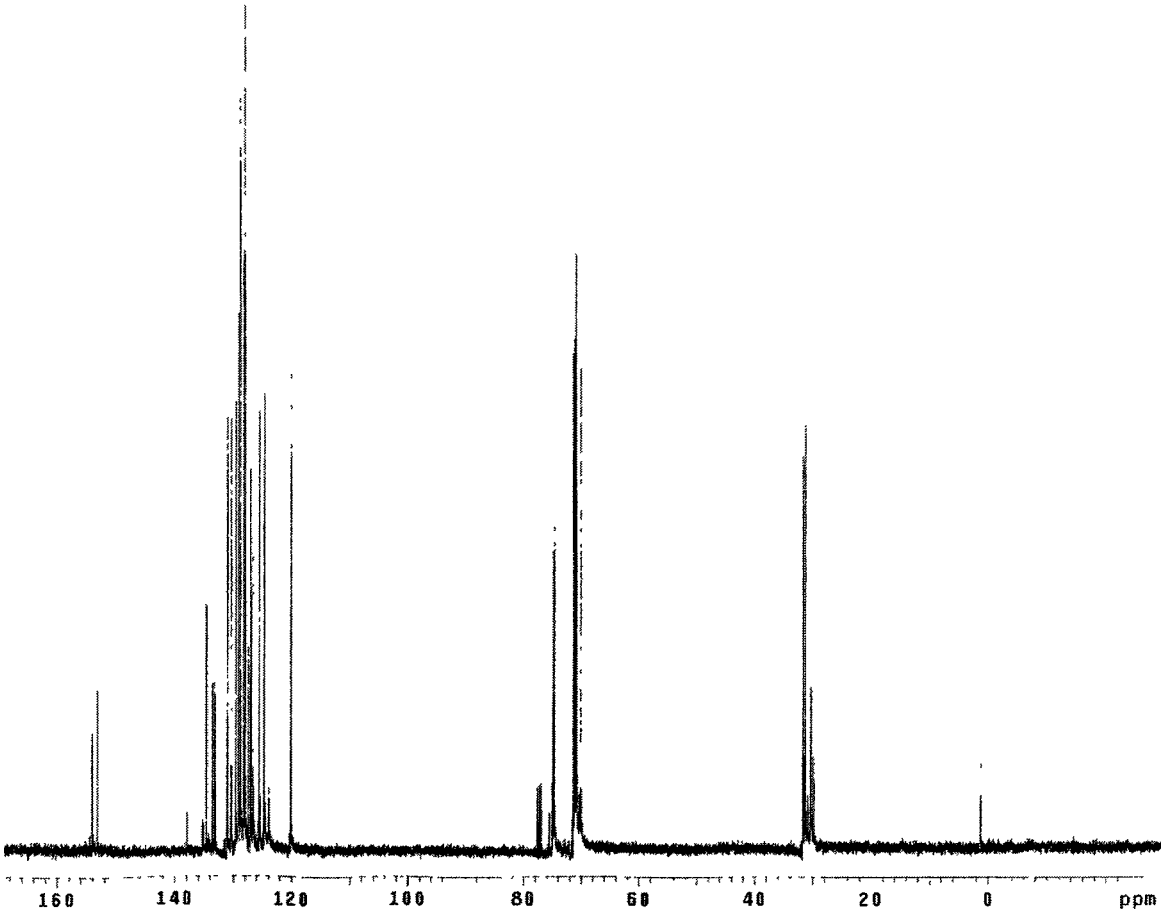


Figure 3.7 *^{13}C NMR Spectrum of* **4**

3.1.5 ^{1}H NMR Spectrum (4)

The ^{1}H nmr spectrum is more difficult to interpret due to the presence of several overlapping signals and complicated splitting patterns. However, integration of the various types of protons present in the structure (i.e. aromatic, pentaethlyene

oxy, benzylic, and Ar-CH_2-Ar) confirmed the presence of the key structural elements in the correct ratio.

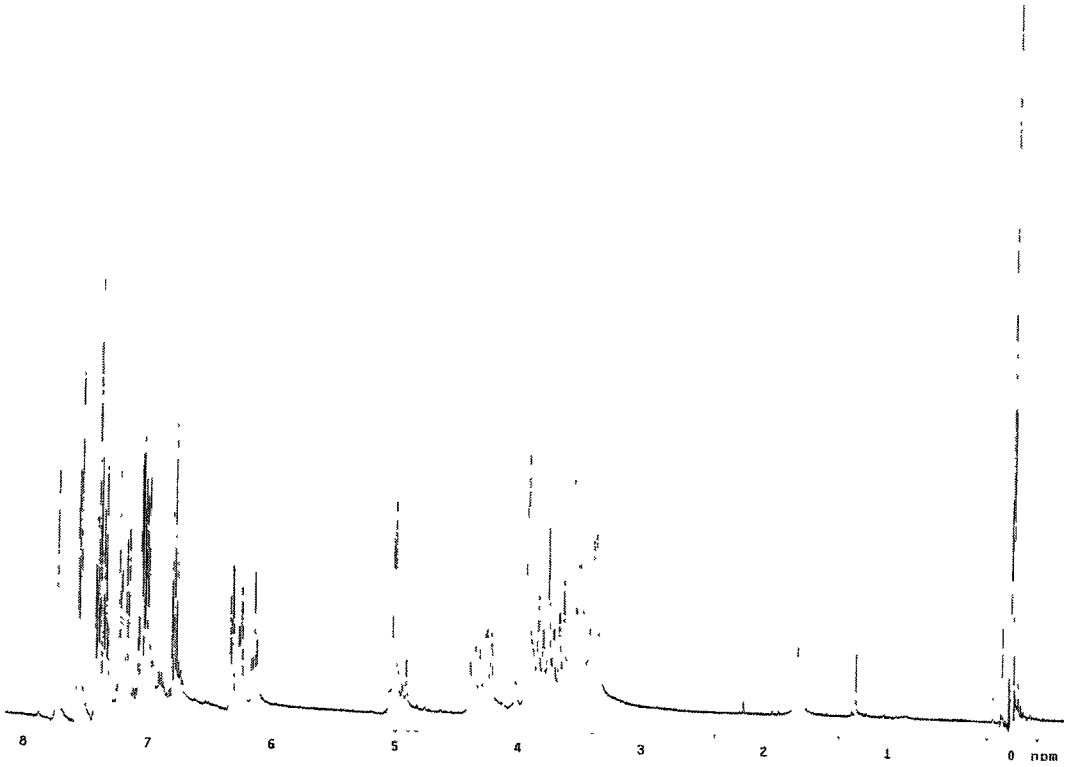


Figure 3.8 *^1H Spectrum of* **4**

In addition, there is still some conformational mobility in the unbridged rings (A/D and B/E) and this contributes to the presence of broadened signals and/or additional smaller signals.

4.0 Binding Properties of Compound 4

4.1 ^{1}H NMR Spectra of 4-Chiral Reagent Complex

Enantiotopic protons (belonging to the R- and S-form) can only be distinguished

in the presence of a chiral environment. Such a chiral environment may, for example, be a chiral solvent but could also be a chiral molecule that complexes to the molecule of interest. We performed an NMR titration with compound **4** and Pirkle's reagent, (1-(9-Antryl)-2,2,2-trifluorethanol), in $CDCl_3$ (figure 4.1).

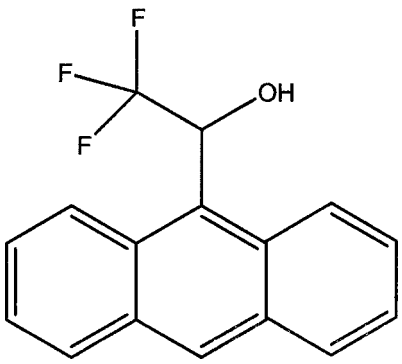


Figure 4.1 *Pirkle's Reagent*

Subtle changes were observed in the ^{1}H NMR spectrum of compound **4** as the mass of Pirkle's reagent present was increased (figure 4.2).

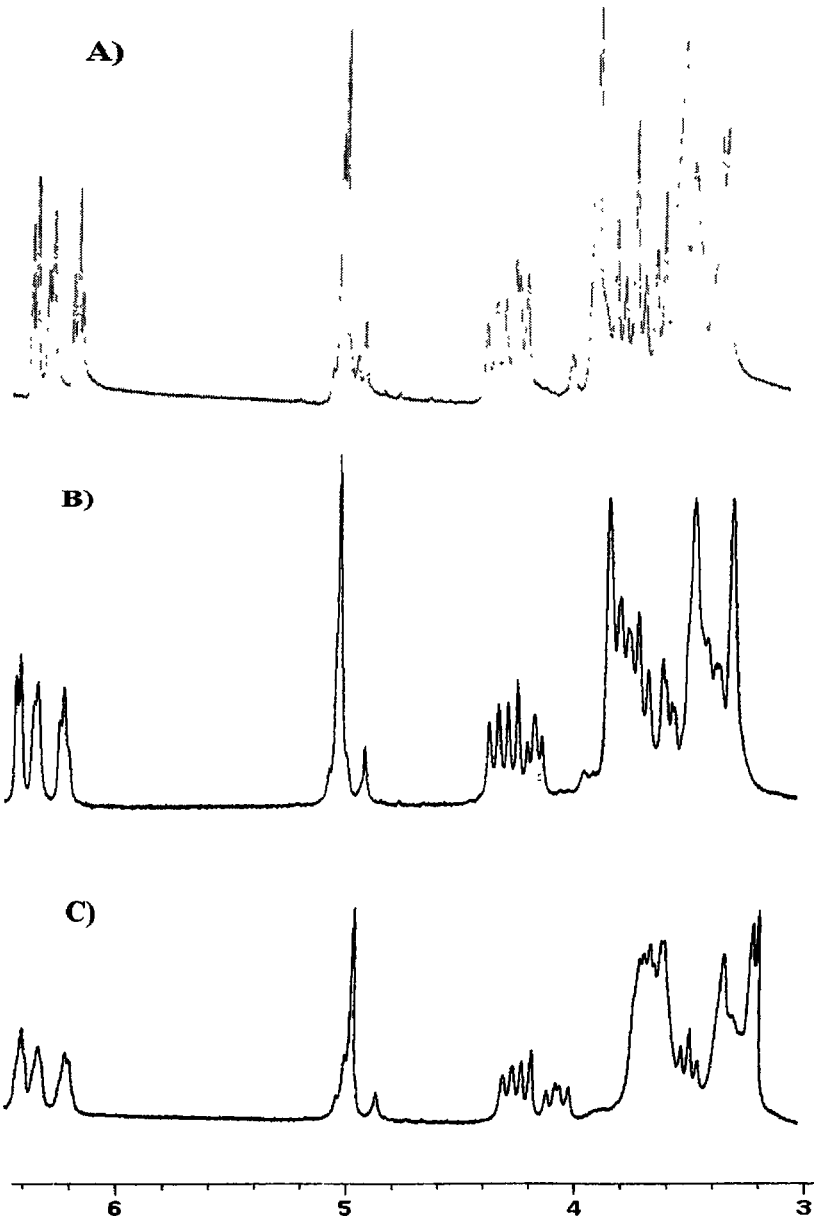


Figure 4.2 *Stack plot of compound* **4** *with a) Free Host b) 2 equiv Pirkle, c) 5 equiv Pirkle*

Broadening of certain signals and upfield shift are indicative of rapid exchange of the guest (Pirkle's reagent) on the NMR time scale into and out of the binding site. Since

the host in a racemic mixture (R/S) and the chiral reagent is optically pure (R/S) then a pair of diastereomeric complexes should be formed. If the exchange rate of the guest is slow, then separate sets of proton resonances should be observed for each diastereomeric complex. However, in the case here, the exchange rate is rapid. Thus a time-averaged set of signals for both diastereomeric complexes is observed. Generally, rapid exchange is associated with weak binding interactions between the host and guest.

4.2 Alkali Metal Binding Properties

In section 3.1 we saw that compound **4** is normally observed as a 1:1 complex with Na^+. However, **4*** Na^+ was also placed in competition with Cs^+ in ESI/MS experiments. The peak at 1151 m/z correlates to a 4*Cs^+ complex and clearly proves that **4** prefers to form a 1:1 complex with Cs^+ over Na^+ due to the higher concentration, (3:1), of Cs^+ versus Na^+ (figure 4.2).

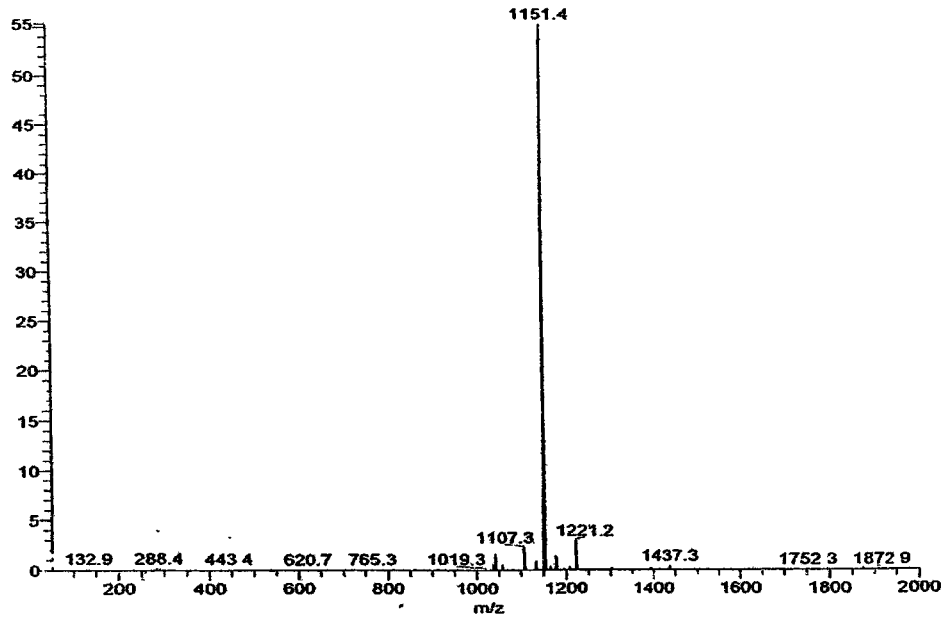


Figure 4.3 *Mass Spectrum of* **4***Cs^+ *Complex*

The two ESI/MS spectra of **4** with Na^+ and Cs^+ ions demonstrates that **4** has an appreciable affinity for alkali metal cations, since a signal for the free host (MW = 1018 g/mol) is not observed in either case. The mass spectra only show qualitatively that **4** will bind to both small and large cations. To ascertain any selectivity exhibited by the host, more qualitative extraction experiments were conducted.

The binding properties of **4** with alkali metal picrate salts were studied by liquid-liquid extraction experiments and followed by UV analysis of the picrate salts extracted into chloroform. The concentrations of the aqueous picrate salts and the host compound **4** were all approximately 1.0×10^{-3}. Table 4.1 shows the percent extraction, extraction constant (K_e), association constant (K_a), and the distribution constant (K_d) for the various metal picrate salts.

Table 4.1 *Percent extraction, K_e, K_a, and K_d values for compound* **4**

Metal Picrate	% Extraction	K_e	K_d (M^{-1})	K_a (M^{-1})
K^+	11	65.2	2.55×10^{-3}	25,568
Cs^+	8.4	50.7	5.41×10^{-3}	9,371
Na^+	7.9	52.1	1.74×10^{-3}	29,942
Rb^+	5.1	40.5	4.57×10^{-3}	8,862

The results demonstrate that the host has an affinity for all of the metal cations examined. In addition, the values for K_a suggest that the host has a slight preference (3:1) for the smaller Na^+ and K^+ ions over the larger Rb^+ and Cs^+ ions. This is most likely due to a better complementary fit between the diameter of the cations and the

crown ether site dimensions. When compared to calix[4]crown 6 ethers[19] locked in the cone conformation **4** demonstrates a much higher rate of extraction.

4.3 Ammonium Ion Binding Properties (4)

In addition to alkali metals we also probed **4** with various NH_4^+ species. The first ion was simply the picrate salt of ammonium hydroxide. The second guest used was (S)-(-)-α-methylbenzylamine (figure 4.3).

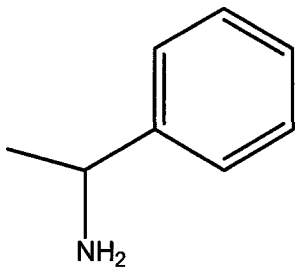


Figure 4.4 *(S)-(-)-α-methylbenzylamine*

The appropriate absorptivity constants had been previously reported for the alkali metals as well as for the ammonium hydroxide species. However, the constant was not available for (S)-(-)-α-methylbenzylammonium picrate, and had to be determined experimentally by UV-Vis. We created a calibration curve by measuring the absorbances of 4 stock solutions of concentrations 5.0×10^{-5}, 2.5×10^{-5}, 1.5×10^{-5}, and 5×10^{-6} M. The respective absorbances were plotted against concentration and a linear relationship was observed. The molar absorptivity ε was equal to 11641 $M^{-1}cm^{-1}$, or the slope of the trendline (figure 4.4).

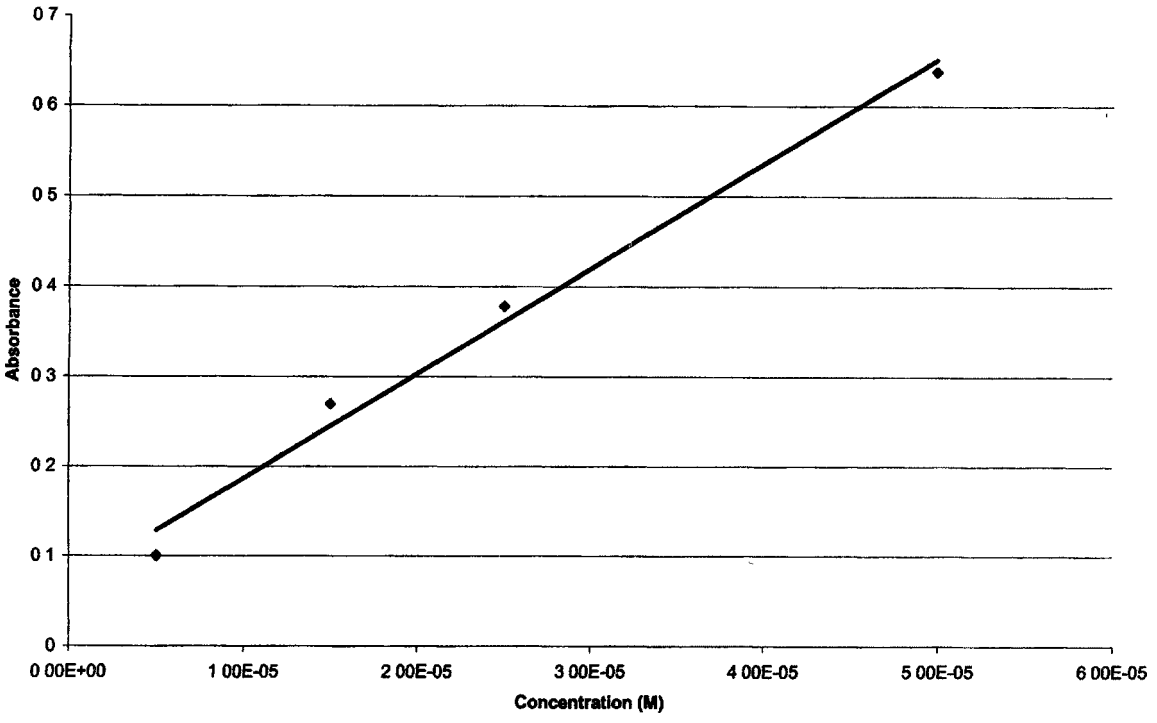


Figure 4.5 *Calibration curve of (S)-(-)-α-methylbenzylammonium picrate*

The host's binding ability for ammonium ions was also investigated using the picrate salt extraction methods and the results are displayed in Table 4.2.

Table 4.2 *Extraction data for compound* **4**

Ammonium Picrate	% Extraction	K_e	K_d (M^{-1})	K_a (M^{-1})
(S)-(-)-α-methylbenzylamine picrate	17.2	105	230×10^{-3}	456
NH_4^+	10.4	64.1	4.02×10^{-3}	15,945

The binding of ammonium (NH_4^+) ion is comparable to that of alkali metals and intermediate between the smaller (Na^+, K^+) and larger (Rb^+ and Cs^+) ions. When the chiral guest was studied the percent extraction was the highest for any of the guests,(including the metal ions) but that is because of the lipophilicity of the α-methylbenzylamine ion. Thus, its K_d is also the highest. The K_a for the chiral guest was actually the smallest measured and this is one reason why the NMR titration experiments were negative. However, the extraction experiments do confirm that the host can bind ammonium ions, albeit weakly. Now, that the basic binding characteristics of **4** have been defined future studies may be focused primarily on chiral recognition of amino acid esters. Preliminary investigation of several amino acid esters (valine, alanine, leucine, tryptophan, and phenylalanine) indicated a high selectivity for phenylalanine methyl ester.

4.4 Determination of Ke and Ka Values

The extraction constants (K_e) were calculated from experimental data and the distribution constants (K_d) used have been previously reported.[20] The values of K_a, the host-guest association constant, were calculated from K_e and K_d and the definitions and relationships in the following equations 1-4 wherein H represents the host were used.

$$(1) \qquad [M^+Pic^-]_{org} + [H]_{org} \overset{Ka}{\rightleftharpoons} [M^+*H*Pic^-]_{org}$$

$$(2) \qquad [H]_{org} + [M^+]_{aq} + [Pic^-]_{aq} \overset{Ke}{\rightleftharpoons} [M^+*H*Pic^-]_{org}$$

$$(3) \qquad [M^+]_{aq} + [Pic^-]_{aq} \overset{Kd}{\rightleftharpoons} [M^+Pic^-]_{org}$$

(4) $\mathbf{K_a} = K_e/K_d$

The physical measurements that were utilized in the calculations of Ka values were as follows:

(5) $*[M^+*H*Pic^-]_{org} = \{[Pic^-,M^+]_{aq}i - AD/\Sigma\ L\ \}$

(6) $[Pic^-, H,M^+]_{org} = AD/\Sigma\ L$

(7) $[Pic^-,M^+]_{aq} = \{[Pic^-,M^+]_{aq}i - AD/\Sigma\ L\ \}$

(8) $K_e = [Pic^-, H,M^+]_{org} / \gamma_{\pm}^2\ ([Pic^-,M^+]_{aq})\ \{(H_{org}i]) - ([Pic^-, H,M^+]_{org})\}$

The formalizations are as follows: K_a and K_d are determined by eq 1 and 3; Pic^- and M^+ are the picric and the metal ions of the picric; $[Pic^-, H,M^+]_{org}$ is the host picrate complex concentration in $CHCl_3$ calculated at equilibrium from direct measurements. $[Pic^-,M^+]_{aq}$ represents the alkali cation and picric anion in aqueous phase; $[Pic^-,M^+]_{aq}$ represents the initial picrate concentration in the aqueous phase; H is the host; A is the observed absorbance; D is the factor at which the aliquots taken from the $CHCl_3$ or H_2O layers were appropriately diluted; $\gamma^2 = .95$ is the activity coefficient of the picrate in water[21]; L is the light pathlength for the UV cell; Σ is the extinction coefficient.

5.0 EXPERIMENTAL

5.1 Materials

All solvents and chemicals were purchased from Aldrich, and used without further purification. ^{1}H-NMR and ^{13}C-NMR data were collected on a 400 MHz Varian NMR. Chemical shifts (δ) are expressed in ppm relative to internal tetramethyl silane (TMS). All melting points were obtained in unsealed capillary tubes and are uncorrected. All reactions were carried out in a dry argon atmosphere. Analytical TLC was performed on precoated silica gel plates (Silica Gel IB2-F) and column chromatography was performed with Silica Gel Geoduran IB2-F 150, 60-200 Mesh (63 -200 micron).

5.2 Preparation of 5, 11, 17, 23, 29, 35-hexa-*p-tert*-butyl-37,38,39,40,41,42-hexahydroxycalix[6]arene synthesis (1)

Compound **1** was synthesized in 88-95% yields using previously reported methods.[1]

5.3 Preparation of 37,38,39,40,41,42-hexahydroxycalix[6]arene synthesis (2)

Compound **2** was synthesized from in 65-85% yields using previously reported methods.[1]

5.4 Preparation of 37,40-Dibenzyloxy-38,39,41,42-tetrahydroxycalix[6]arene synthesis (3)

To a flame dried 1.0 L one necked, round bottom flask with a magnetic stir bar 10.0 g (15.7 mmol) of compound **2** and 14.1 g (109 mmol) of potassium trimethyl-silanolate, $K^+(CH_3)_3SiO^-$, was dissolved in 450 mL of anhydrous THF and 50 mL of DMF. After the reaction was allowed to stir for 5 min at room temperature, 5.59 mL (47.1 mmol) of benzyl bromide was added and the reaction was allowed to stir for 24 h at room temperature. The reaction solvent was removed under reduced pressure to remove the THF, and then a mechanical vacuum pump to remove the DMF. The residue was dissolved in $CHCl_3$ (1 L) and washed 3 X with equal volumes (200 mL) of 2 M HCl. The organic solution was then dried over anhydrous $MgSO_4$, filtered, and the volume was reduced to approximately 50 mL and poured into 1 L of methanol. The product was collected by filtration to provide 11.3 g of **3** in 88% yield. mp 258 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ = 7.94 (s, 4H), 7.01-6.98 (m, 14 H,), 6.71 (t, 4 H, J= 1.9), 5.02 (s, 4 H) 3.91 and 3.73 (two s, 12 H). ^{13}C NMR (100 Mhz, $CdCl_3$) δ = 186.7, 184.7, 165.2, 150.8, 133.6, 129.3, 129.2, 128.8, 128.2, 125.9, 120.4, 117.9, 77.673, 31.9, and 31.6

5.5 37,40-Dibenzyloxy-(39-42)-crown-6-calix[6]arene (4)

NaH, 1.95g (81.2 mmol), was placed in a glass fritted funnel and washed 3 X (20 mL) with hexane (caution: NaH can become flammable when dry). The hexane was then filtered off under reduced pressure.

Next, to a flame dried 2.0 L two necked, round bottom flask with a magnetic stir bar 4.0 g (4.9 mmol) of compound **3** and the previously washed NaH were suspended in 500 mL of

acetone. A solution of 5.31 ml (12.2 mmol) of pentaethylene glycol di-*p*-tosylate and 100 mL of acetone was placed in a 100 mL addition funnel. The funnel was attached to a neck of the round bottomed flask and was allowed to drip into the reaction mixture over 1h. After addition of the tosylate was completed the addition funnel was removed, and the flask was fitted with a water condenser. The reaction was heated to reflux for 48 hours. The reaction solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (1 L) and washed 2 M HCl (3 x 200 mL). The organic solution was then dried over $MgSO_4$, filtered, and removed under reduced pressure to provide a viscous, brown liquid. The brown liquid was purified before precipitation by column chromatography on a silica gel column (70% EtOAc/30% Hexane). Eluent fractions containing the product were concentrated and precipitated from 1 L of methanol to yield 1.5 g (30%). mp = 177 ^{O}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (s, 2H), 7.54 (d, 4H, J=7.6), 7.40 (t, 4H, J=6.8), 7.35 (d, 2H, J=6.8), 7.23 (d of d, 2H, J=6.2), 7.16 (d of d, 2H, J=6.8), 7.07-6.99 (m, 6H), 6.78 (t, 2H, J=7.6), 6.32 (d, 2 H, J=7.2), 6.24 (d, 2H, J=7.2), 6.141 (t, 2H, J= 7.2), 5.0 (d of d, 4H, J= 4.8), 4.43-4.18 (m, 6h), 4.02-3.30 (m, 26H).). ^{13}C NMR (100 Mhz, $CdCl_3$) δ = 153.2, 137.8, 134.5, 131.0, 130.3, 129.6, 129.1, 128.8, 128.2, 127.9, 127.4, 126.9, 126.5, 125.6, 124.7, 123.9, 120.1, 74.9, 74.5, 71.4, 71.0, 70.8, 70.1, 31.7, 31.3, 30.2.

5.6 37,40-Dibenzyloxy-(38-41)-dipyridinal-(39-42)-crown-6-calix[6]arene (5)

To a flame dried 500 mL one necked, round bottom flask with a magnetic stir bar 1.0 g (0.98 mmol) of compound **4** and 0.960 g (2.9 mmol) of cesium carbonate was placed in 250 mL of acetonitrile. After the reaction was allowed to stir for 5 min 0.74 g (2.9 mmol) of 2-(bromomethyl) pyridine was added and the flask was fitted with a

condenser with argon inlet. The reaction was brought to a boil and allowed to reflux for 48 h. The reaction solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (.5 L) and washed 2 M HCl (3 x 200mL). The organic layer was then drawn off and washed 3 X with equal volumes of a saturated NaCl solution. The solution was dried over $MgSO_4$, filtered, and the volume was reduced to minimal solvent. The product was a viscous, brown liquid. The brown liquid was purified before precipitation by column chromatography on silica gel column (90% $CHCl_3$/10%Acetone). Eluent fractions containing the product were concentrated and precipitated from 1 L of methanol to yield 100g (8%).

5.7 Alkali Metal Picrate Extractions

All glassware used in the extractions was washed with acetone, dried and then flame dried. $CHCl_3$ was washed with a 2 M solution of NaOH. Double distilled water was used in making the aqueous metal picrate solutions. Picric acid was neutralized with the LiOH, NaOH, KOH, RbOH, CsOH, NH_4OH, and (S)-(-)-methyl benzylamine in ultrapure water (1:1). The solutions were stored in stoppered volumetric flasks. Absorbance measurements were taken on a Beckman 7000 UV-Vis using an absorptivity coefficient ($\varepsilon_{380\ nm}$ = 18,000 cm^{-1} M^{-1}) in $CHCl_3$ and CH_3CN. In 8 mL glass vials, 2.0 mL of a 1.0 x 10^{-3} M (**4**) solution of host dissolved in $CHCl_3$ was delivered (via 2.00 mL glass pipette) along with 2.00 mL (via pipette also) of prepared aqueous metal picrates having concentrations of 2.57 x 10^{-3} M, 2.35 x 10^{-3} M, 1.56 x 10^{-3} M, 2.04 x 10^{-3}, 2.19 x 10^{-3}, 2.09 x 10^{-3} M, for Na^+, K^+ Rb^+, Cs^+, NH_4^+, and methyl benzyl amine ions,

respectively. The vials were sealed and shaken vigorously for 2 min. and allowed to sit for 5 min. prior to UV analysis.

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