Università degli Studi di Salerno
Dipartimento di Chimica e Biologia
“Adolfo Zambelli”

Corso di Dottorato di Ricerca in Chimica
XIV CICLO NUOVA SERIE

Cavity Filling and Chirality
Effects in Calixarene Threading

Candidato:
Concilio Gerardo
Matr. 8880700196

Relatore: Coordinatore:
Prof. Placido Neri Prof. Gaetano Guerra

INDEX

INTRODUCTION ................................................................. 1

1.1 SUPRAMOLECULAR CHEMISTRY AND MOLECULAR RECOGNITION ........................................ 1

1.2 MACROCYCLIC HOSTS: CALIXARENES ........................................ 5

1.2.1 Calixarenes in molecular recognition ...................................... 8

CHAPTER II ............................................................................ 10

CAVITY FILLING OF THE CALIX[6]ARENE MACROCYCLE WITH DIALKYLAMMONIUM GUESTS .................................................. 10

2.1 RECOGNITION OF ALKYLAMMONIUM GUESTS BY CALIXARENES MACROCYCLES ................................. 10

2.2 AIMS ................................................................................. 13

2.3 RESULT AND DISCUSSION .................................................. 14

2.3.1 Packing Coefficient Calculation ................................................. 29

2.3.2 Contacting Coefficient Calculation .......................................... 34

2.3 CONCLUSION ....................................................................... 38

2.4 EXPERIMENTAL SECTION .................................................. 39

CHAPTER III ........................................................................... 74

CHIRALITY AND CHIRAL MOLECULAR RECOGNITION IN THE CALIXARENE THREADING ............................................. 74

3.1 CHIRAL RECOGNITION IN SUPRAMOLECULAR STRUCTURES ........................................................................ 74

3.2.2 Chiral recognition in gas phase .................................................. 78

3.3 AIMS .................................................................................. 82

3.4 RESULTS AND DISCUSSIONS ............................................. 82
3.4.1 Synthesis of chiral hosts and guest ........................................... 86
3.4.2 Gas-phase study ......................................................................... 96
3.4.3 Synthesis of enantiopure hosts .................................................. 97
3.4.4 Synthesis of labelled enantiopure guest ..................................... 100
3.4.5 MS experiments ......................................................................... 103
3.4.6 Concentration Effect ................................................................. 105
3.4.7 Isotopic Effects ........................................................................ 105
3.4.8 Chiral Recognition ..................................................................... 109
3.5 CONCLUSION .................................................................................. 113
3.6 EXPERIMENTAL SECTION ............................................................... 114

CHAPTER IV .......................................................................................... 155
SYNTHESIS OF NEW CALIXARENE AND RESORCINARENE BASED
CHRIMAL HOSTS .................................................................................. 155

4.1 CHIRALITY AND INHERENT CHIRALITY IN CALIXARENES
AND RESORCINARENES ........................................................................ 155

4.1.1 Inherently chiral calixarenes ....................................................... 156
4.1.2 Meta-functionalization of Calixarenes: the “p-
bromodienone route” ...................................................................... 159
4.1.3 Chiral and inherently chiral resorcinarenes ................................. 163

4.2 AIMS ............................................................................................... 164

4.2 RESULTS AND DISCUSSION ............................................................ 165

4.2.1 Synthesis of calixarene hosts via p-bromodienone route
............................................................................................................. 165
4.2.2 Synthesis of inherently chiral resorcin[4]arene ......................... 176
4.2.3 HPLC-EDC analysis and Computational Spectroscopy
............................................................................................................. 181
CHAPTER I

INTRODUCTION

1.1 SUPRAMOLECULAR CHEMISTRY AND MOLECULAR RECOGNITION

Supramolecular Chemistry has been defined by Jean Marie Lehn as “the chemistry beyond the molecule”,¹ and refers to the study of the complex systems resulting from the aggregation of more chemical entities (molecules or ions) through non-covalent forces such as: ion-ion interactions (100-350 kJ mol⁻¹); ion-dipole (50-200 kJ mol⁻¹); hydrogen bonding (4-120 kJ mol⁻¹); dipole-dipole (5-50 kJ mol⁻¹); cation-π (5-80 kJ mol⁻¹); π-π (1-50 kJ mol⁻¹) Van der Waals (< 5 kJ mol⁻¹).²

Non-covalent interactions are weaker with respect to covalent bonds, however, this lability allows the occurrence of novel phenomena such as self-assembly³, that provide a valid approach to the synthesis of molecular systems on nanometric scale.

In analogy with biological systems like enzyme-substrate Cram defined *host-guest complex*\(^4\), in which a host (receptor) is able to selectively complexes (recognizes) a guest (substrate). This process is a bedrock of supramolecular chemistry and it is called “*molecular recognition*”.

Emil Fisher was the first who gave the interpretation of the molecular recognition phenomenon introducing the *lock-and-key*\(^5\) model in 1894.

The model describes the specificity and the bond strength as the result of complementary interactions between the host and guest structures.

The most important requirements to obtain a high selectivity in the recognition process are:

a) Geometric size and shape complementarity between

---


host and guest.

b) Interactional complementarity which is achieved when an optimal match between the two interacting molecules is accomplished, even via electronic interactions such as van der Waals, ion-ion; etc.;
c) Vast contact areas to enhance the host-guest interactions;
d) Multiple interaction sites;
e) Strong bond energies to realize a good stability of the complex and enhance the selectivity towards a certain guest.

Furthermore the discovery that proteins have an high flexibility and the observation that they can change their conformation during protein-substrate interactions, led to the extension of the lock-and-key model and the formulation of the induced-fit principle.\(^6\)

The induced-fit process was described for the first time by Koshland in 1958 and it was mainly used to describe the interactions that are established between enzymes and substrates in biological systems. In this case the enzymatic action requires a specific orientation of the catalytic groups first and then the substrate causes a significant three-

dimensional modification of the active site in order to bring the catalytic groups in the correct position. (Figure 2)

![Figure 1.2 Difference between: a) lock-and-key model, and b) induced-fit model.](image)

The *induced-fit* model in biological systems was demonstrated by a huge number of experiments and nowadays it is accepted that almost all enzymes show conformational changes after the interaction with substrates. Although in Supramolecular Chemistry, the *induced-fit* process has provided a good model to explain the host-guest interactions.

The substantial difference between the *lock-and-key* model and the *induced-fit* one is that the first model requires rigid molecules, the substrate in particular, whereas the *induced-fit* needs a certain flexibility that allows a conformational change in the molecular architectures to promote the molecular recognition process.
1.2 MACROCYCLIC HOSTS: CALIXARENES

Calixarenes\textsuperscript{7} have been widely studied in molecular recognition processes as versatile hosts because of their conformational properties and synthetic versatility.

![Scheme 1.1 Synthesis of calix[4]arene.](image)

Calix\textit{n}arene are metacyclophanes obtained by condensation of phenols with formaldehyde in different conditions. The name of these compounds has been attributed by C. D. Gutsche because the cyclic tetramer, the \textit{p-terz}-butylcalix[4]arene, adopts both in solution and solid state, a cup-like shape that reminded a Greek vase which name is \textit{“calyx krater”}.

The “n” in brackets means the number of aromatic units of the macrocycle.

Calixarenes co called “major”\(^8\) are those made of 4, 6 and 8\(^9\) phenolic units, they are easy to obtain and for this reason they have been found more applications in many fields. Contrary calixarenes “minor” are composed of 5 and 7 units and they are less studied because of the tediousness of the reactions and the low yields in which these compounds are obtained. However calixarenes having phenolic units from 9 to 20\(^8\) have been fully characterized but are less used.

The calix[6]arene conformation have been described as distorted cone,\(^10\) compressed cone,\(^11\) pinched cone,\(^12\) double

---

partial cone,\textsuperscript{13} winged,\textsuperscript{14} 1,2,3-alternate,\textsuperscript{15} 1,3,5-alternate and distorted 1,2,3-alternate.\textsuperscript{16}

Bott and coworkers demonstrated that the calix[6]arene conformation, in the solid state, depends strongly on the solvent used for the crystallization. Because the solvent is involved in the hydrogen bonds formation that drives the obtaining of a certain structure adopted by the calixarene.

The calixarene functionalization is the key step in the design and realization of a host capable to selectively recognize a certain guest.

Calixarenes can be functionalized by means of a transformation of the substituent at lower rim (endo rim) or at upper rim (eso rim).

The lower rim functionalization can be achieved through a simple etherification and can be extended by considering transformation reactions of phenol in alkoxy esters, alkoxy amides, alkoxy ketones and phosphonates\textsuperscript{17}.

As regards the functionalization at the upper-rim the most

common strategies provide a wide range of electrophilic aromatic substitutions including sulfonation\textsuperscript{18}, acylation\textsuperscript{19}, nitration\textsuperscript{20}, halogenation\textsuperscript{21}, formylation\textsuperscript{22}, chlorosulfonation\textsuperscript{23}, the \textit{Claisen rearrangement route}\textsuperscript{24}, the \textit{p-quinone-methide-route}\textsuperscript{25} and \textit{p-chloromethylation-route}\textsuperscript{26}.

1.2.1 Calixarenes in molecular recognition

The synthetic versatility of the calixarene macrocycles allows to obtain derivatives with significant recognition properties towards cations/anions, organic and biomolecular guests. Thus novel calixarene-hosts have been reported for the development of biosensors,\textsuperscript{27} DNA chip,\textsuperscript{28} and inhibitors in the field of drug discovery.\textsuperscript{29}

---

\textsuperscript{29} a) G. M. Consoli, G. Granata, V. Cafiso, S. Stefani, C. Geraci,
A special attention has been dedicated to the recognition properties of water soluble calixarenes because water represents the natural environment in which biological processes take place.

An example of such molecular recognition is reported below and it shows the particular affinity of $p$-sulfonatocalix[4]arene against several biochemically important analytes (Figure 4).

In detail the inclusion complex between $p$-sulfonatocalix[4]arene (1.4) and 2,3-diazabicyclo[2.2.2]oct-2-ene (1.5) was used as sensor system to sense the binding of choline and carnitine derivatives and tetraalkylammonium cations (1.6) over a large pH range.

![Diagram](image)

**Figure 1.4** $p$-sulfonatocalix[4]arene sensor.

---


CHAPTER II

CAVITY FILLING OF THE CALIX[6]ARENE MACROCYCLE WITH DIALKYLAMMONIUM GUESTS

2.1 RECOGNITION OF ALKYLAMMONIUM GUESTS BY CALIXARENES MACROCYCLES

In 2010, the our group\textsuperscript{31} showed that the calix[6]arene hosts 2.1a,b and 2.2a (Figure 2.1) were able to recognize dialkylammonium axle 2.3a-f·TFPB\textsuperscript{−} when they were coupled with the weakly coordinating Tetrakis[3,5-bis(tri-Fluoromethyl)Phenyl]Borate (TFPB\textsuperscript{−}) “superweak anion” (Figure 2.1) that gives very loose ion-pairs with dialkylammonium cations in solution.

Thus, in presence of dipentylammonium $2.3b^+\cdot\text{TFPB}^-$ or
dibenzylammonium $2.3c^+\cdot\text{TFPB}^-$ guests the calix[6]arene
macrocycle $2.1a$ gave complexes in which the cationic guest
was "threaded" into the calix[6]arene-wheel (Figure 2.2).
These interpenetrated structures have been defined in
supramolecular chemistry as pseudorotaxane and can be
considered as synthetic precursors of catenane and rotaxane
architectures\textsuperscript{32} which have showed appealing properties as

\textsuperscript{32} a) G. Schill, \textit{Catenanes, Rotaxanes, and Knots}, Academic Press, New
York, USA, \textbf{1971}; b) J. P. Sauvage, C. Dietrich-Buchecker, eds.
\textit{Molecular Catenanes, Rotaxanes and Knots: A Journey through the World
of Molecular Topology}, Wiley VCH, Weinheim, \textbf{1999}; c) G. G. Ramírez,
molecular machines\textsuperscript{33}

![Figure 2.2](image)

**Figure 2.2.** Calixarene-based pseudorotaxane structures obtained by *through-the-annulus* threading of calixarenes with TFPB\textsuperscript{−} salts of dialkylammonium cations.

Interestingly, the threading of *directional* alkylbenzylammonium guests \textbf{2.3d-f\textsuperscript{+}} with calix[6]arene wheels, in principle would give rise to two diastereoisomeric pseudorotaxane complexes, one with an *endo*-cavity alkyl chain (*endo*-alkyl stereoisomer) and the other with an *endo*-cavity benzyl moiety (*endo*-benzyl stereoisomer) (Figure 2.3). Thus, the experiments performed with *n*-alkylbenzylammonium cations bearing shorter alkyl chains, such as butyl *2.4d\textsuperscript{+}·TFPB\textsuperscript{−}* and pentyl *2.4e\textsuperscript{+}·TFPB\textsuperscript{−}* , led to an *endo*-alkyl/*endo*-benzyl ratio of 30:1 and 10:1, respectively.

The observed stereo-selectivity brought to the definition of the so called “endo-alkyl rule”: threading of a directional alkylbenzylammonium axle through a hexaalkoxycalix[6]arene occurs with an endo-alkyl preference.\textsuperscript{34-35}

**2.2 AIMS**

On these basis we have studied the recognition abilities of the calix[6]arene derivatives 2.1a toward alkylbenzylammonium guests 2.3\textsuperscript{d+}·TFPB\textsuperscript{-}, 2.3\textsuperscript{f+}·TFPB\textsuperscript{-} and 2.3g-p\textsuperscript{+}·TFPB\textsuperscript{-}

---

\textsuperscript{34} R. Ciao, C. Talotta, C. Gaeta, P. Neri, *Supramolecular Chemistry* 2014, 26, 569-578.

bearing aliphatic chains with different shape and length (Figure 2.4). In particular, we studied the validity of the endo-alkyl rule with alkylbenzylammonium axles 2.3g,h,d and f+ bearing alkyl chains with different lengths with respect to 2.3d,e+ and finally using the guests 2.3i-p+ bearing branched alkyl chains.

![Figure 2.4 Guests studied.](image)

2.3 RESULT AND DISCUSSION

The starting point was the synthesis of the different axles (reported in Figure 2.4). They were synthesised, in most cases, starting from a coupling primary amine/aldehyde or amine/ketone to obtain an imine successively reduced with NaBH4 to obtain the secondary amine. Than the resulting secondary amine was treated with HCl (37%) to obtain the corresponding chloride that was subjected to the anion
exchange with sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) (see experimental section for more details).

Than it was studied the threading ability of the calix[6]arene 2.1a with alkylbenzylammonium cations 2.3d$^+\cdot$TFPB$^-$, 2.3f$^+\cdot$TFPB$^-$, 2.3g$^+\cdot$TFPB$^-$ and 2.3h$^+\cdot$TFPB$^-$ bearing linear aliphatic chain having a number of carbon atoms ranging from 2 to 6.

A mixture of $p$-tert-butylhexametoxycalix[6]arene 2.1a and 1 equivalent of the corresponding axle was prepared dissolving the two compound in 0.5 mL of CDCl$_3$. 
As it can be seen from the $^1$H NMR spectra, in Figure 2.6 the cations $2.3d^{+}\cdot\text{TFPB}^-$, $2.3f^{+}\cdot\text{TFPB}^-$, $2.3g^{+}\cdot\text{TFPB}^-$ and $2.3h^{+}\cdot\text{TFPB}^-$ are able to give endo-alkyl complexation in presence of calix 2.1a. Indeed the NMR signals at negative chemical shifts are related to the axle’s alkyl moiety settled into the calixarene cavity that shields its protons.
The “endo-alkyl rule” was respected also in these cases but the important aspect was in presence of alkylbenzylammonium cations with a shorter alkyl chain $2.3g^+\cdot\text{TFPB}^-$ and $2.3h^+\cdot\text{TFPB}^-$, the selectivity was absolutely in favour of the alkyl moiety, no evidence of endo-benzyl $2.4g-h^+\cdot\text{TFPB}^-$ stereoisomers was observed (Figure 2.6 A and B), while increasing the chain length, with the $2.3d^+\cdot\text{TFPB}^-$ and $2.3f^+\cdot\text{TFPB}^-$ guests also the endo-benzyl-$2.4d-f^+\cdot\text{TFPB}^-$ stereoisomer appeared (Figures 2.6 C and D).
Figure 2.6 Significant portions of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of (A) 1:1 mixture of $2.1a$ (3.8·$10^{-3}$M) and $2.3g^+\cdot$TFPB$^-$ (3.8·$10^{-3}$M); (B) 1:1 mixture of $2.1a$ (3.8·$10^{-3}$ M) and $2.3h^+\cdot$TFPB$^-$ (3.8·$10^{-3}$ M); (C) 1:1 mixture of $2.1a$ (3.8·$10^{-3}$ M) and $2.3d^+\cdot$TFPB$^-$ (3.8·$10^{-3}$ M); (D) 1:1 mixture of $2.1a$ (3.8·$10^{-3}$ M) and $2.3f^+\cdot$TFPB$^-$ (3.8·$10^{-3}$ M).

At this point the association constants in Table 2.1 for pseudorotaxane complexes $2.4d^+\cdot$TFPB$^-$, $2.4f^+\cdot$TFPB$^-$, $2.4g$-
were determined by analysis of the $^1$H NMR spectra of the complexation experiments, which showed slowly exchanging signals for both free and complexed host (see experimental section for the $^1$H NMR complexation experiments).

As reported in Table 2.1 with the loss of selectivity a decrease in the constant value was observed.

<table>
<thead>
<tr>
<th>Guest</th>
<th>Endo-Alkyl</th>
<th>Endo-Benzyl</th>
<th>endo-alkyl/endo-benzyl ratio</th>
<th>$K_{endo-alkyl}$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3g$^+$</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>1.2±0.2 x10$^6$</td>
</tr>
<tr>
<td>2.3h$^+$</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>5.0±0.8 x10$^3$</td>
</tr>
<tr>
<td>2.3d$^+$</td>
<td>Yes</td>
<td>Yes</td>
<td>3:1</td>
<td>6.5±0.9 x10$^4$</td>
</tr>
<tr>
<td>2.3f$^+$</td>
<td>Yes</td>
<td>Yes</td>
<td>1:1</td>
<td>5.5±0.6 x10$^1$</td>
</tr>
</tbody>
</table>

Table 2.1 Linear derivatives.

Successively we have studied the binding abilities of calix[6]arene 2.1a with alkylbenzylammonium cations bearing $\alpha$-branched aliphatic chains 2.3g$^+\cdot$TFPB$^-$, 2.3i$^+\cdot$TFPB$^-$ and 2.3j$^+\cdot$TFPB$^-$. 
Figure 2.7 Complexation experiments between \textit{p-terz}-butylhexametoxycalix[6]arene and \textit{a}-branched guests.

$^{1}$H NMR spectra in Figure 2.7 evidenced that in presence of the \textit{i}-propylbenzylammonium cations \textit{2.3g$^{+}$·TFPB$^{-}$} the calix[6]arene \textit{2.1a} gave exclusively the \textit{endo-alkyl} stereoiosomer. Differently in presence of \textit{t}-butylbenzylammonium cation \textit{2.3j$^{+}$·TFPB$^{-}$} a mixture of \textit{endo-alkyl/endo-benzyl} stereoisomers was obtained in a 3/1 ratio.
Figure 2. Significant portions of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of (A) 1:1 mixture of 2.1a (3.8·10$^{-3}$ M) and 2.3g$^+\cdot$TFPB$^-$ (3.8·10$^{-3}$ M); (B) 1:1 mixture of 2.1a (3.8·10$^{-3}$ M) and 2.3i$^+\cdot$TFPB$^-$ (3.8·10$^{-3}$ M); (C) 1:1 mixture of 2.1a (3.8·10$^{-3}$ M) and 2.3j$^+\cdot$TFPB$^-$ (3.8·10$^{-3}$ M).

For the cations 2.3i$^+\cdot$TFPB$^-$ and 2.3j$^+\cdot$TFPB$^-$ there was also a decrease in the complexation constants values.
Table 2. 2 α-branched derivatives.

<table>
<thead>
<tr>
<th>Guests</th>
<th>Endo-Alkyl</th>
<th>Endo-Benzyl</th>
<th>endo-alkyl/endo-benzyl ratio</th>
<th>$K_{endo-alkyl}$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3g$^+$</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>1.2±0.2 x10$^6$</td>
</tr>
<tr>
<td>2.3i$^+$</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>4.2±0.6 x10$^4$</td>
</tr>
<tr>
<td>2.3j$^+$</td>
<td>Yes</td>
<td>Yes</td>
<td>3:1</td>
<td>3.6±0.5 x10$^2$</td>
</tr>
</tbody>
</table>

Interestingly, $^1$H NMR analysis of the $^1$H NMR spectrum of the 1:1 mixture in CDCl$_3$ of 2.3j$^+\cdot$TFPB$^-$ and calix[6]arene 2.1a led to an association constant of 3.6±0.5x10$^2$ a value lower than that observed for 2.3i$^+\cdot$TFPB$^-$ and 2.3g$^+\cdot$TFPB$^-$. In accord with this, the lowest energy structure of the complex endo-alkyl 2.4j$^+\cdot$TFPB$^-$ obtained by molecular mechanics calculations (OPLS, Macro Model CHCl$_3$) evidenced that the excessive branching allowed the formation of one single hydrogen bonding instead than two as in the endo-alkyl
2.4g,j⁺·TFPB⁻ pseudorotaxanes.

Successively, we studied the binding abilities of the calix[6]arene 2.1a toward alkylbenzylammonium guests 2.3g⁺·TFPB⁻, 2.3i⁺·TFPB⁻ and 2.3j⁺·TFPB⁻ bearing alkyl moiety with branching at the β-position.

\[ \text{Figure 2.10 Complexation experiments between } p\text{-terz-butyllhexametoxycalix[6]arene and } \beta\text{-branched guests.} \]

From the complexation experiments in Figure 2.11 resulted
that the $\beta$-ramification did not lead to the formation of endo-benzyl stereoisomers but influenced the stability of the pseudorotaxane complexes as shown by the decrease of the their complexation constants (Table 2.3).

**Figure 2.11** Significant portions of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of (A) 1:1 mixture of 2.1a (3.8·10$^{-3}$M) and 2.3h$^+$.TFPB$^-$ (3.8·10$^{-3}$M); (B) 1:1 mixture of 2.1a (3.8·10$^{-3}$M) and 2.3k$^+$.TFPB$^-$ (3.8·10$^{-3}$M); (C) 1:1 mixture of 2.1a (3.8·10$^{-3}$M) and 2.3l$^+$.TFPB$^-$ (3.8·10$^{-3}$M).
Table 2.3 β-branched derivatives.

<table>
<thead>
<tr>
<th>Axle</th>
<th>Endo-Alkyl</th>
<th>Endo-Benzyl</th>
<th>endo-alkyl/endo-benzyl ratio</th>
<th>$K_{endo}$-alkyl (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2h^+$</td>
<td>![Image](100x473 to 146x492)</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>$2k^+$</td>
<td>![Image](100x433 to 145x449)</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>$2l^+$</td>
<td>![Image](100x388 to 155x410)</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
</tbody>
</table>

Then with the intention to explore a mixed α,β-ramification the guests $2.3m^+\cdot$TFPB$^-$, $2.3n^+\cdot$TFPB$^-$, $2.3o^+\cdot$TFPB$^-$ and $2.3p^+\cdot$TFPB$^-$, (Figure 2.12) were synthesised and the binding abilities of calix[6]arene $2.1a$ was explored.
In this case it was not observed a significant trend concerning the calixarene cavity selectivity because there were no signals related to \textit{endo}-benzyl\(-2,4^\text{+}\text{TFPB}^-\) stereoisomers in the \textsuperscript{1}H NMR spectra (\textbf{Figure 2.13} and \textbf{Figure 2.14}).
Figure 2.13 Significant portions of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of (A) 1:1 mixture of 2.1a (3.8·$10^{-3}$M) and 2.3m$^+$·TFPB$^-$ (3.8·$10^{-3}$M); (B) 1:1 mixture of 2.1a (3.8·$10^{-3}$M) and 2.3n$^+$·TFPB$^-$ (3.8·$10^{-3}$M); (C) 1:1 mixture of 2.1a (3.8·$10^{-3}$M) and 2.3o$^+$·TFPB$^-$ (3.8·$10^{-3}$M).

However the steric encumbrance of the alkyl chain effects the complexation constant to a point that no complexation was observed for the derivative 2.3p$^+$·TFPB$^-$ (Figure 2.14).
Figure 2. Significant portions of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of 1:1 mixture of 2.1a (3.8•10$^{-3}$M) and 2.3p$^+$·TFPB$^-$ (3.8•10$^{-3}$M).

Table 2. 4 α-, β- mixed branched derivatives

<table>
<thead>
<tr>
<th>Guest</th>
<th>Endo-Alkyl</th>
<th>Endo-Benzyl</th>
<th>endo-alkyl/endo-benzyl ratio</th>
<th>$K_{\text{endo-alkyl}}$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3m$^+$</td>
<td>![image]</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>2.3n$^+$</td>
<td>![image]</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>2.3o$^+$</td>
<td>![image]</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>2.3p$^+$</td>
<td>![image]</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
</tbody>
</table>

Examination of the data in the tables 2.1-2.4 evidenced that the shape and size of the alkyl moiety of the axle influences the stability of the complex. Certainly the ability of the alkyl group to fill the cavity of the calix[6]arene-wheel plays a fundamental role in the stabilization of the pseudorotaxane.
Thus we have envisioned to correlate the association constant of the pseudorotaxanes to two parameters related to cavity filling of the calixarene:

- Packing Coefficient
- Contacting Coefficient

2.3.1 Packing Coefficient Calculation

At this point the data in Tables 2.1-2.4 relative to association constants for the formation of the calixarene-based pseudorotaxanes were correlated with the packing coefficient of the [2]pseudorotaxanes.

The term packing coefficient (PC) was introduced for the first time by Rebek\textsuperscript{36} and it is defined as the ratio between the sum of the van der Waals volumes ($vW$) of the $n$ molecules in a given volume and the volume ($V$)

$$PC = \frac{\sum_{i=1}^{n} v_{w}^{i}}{V} = \frac{V_{w}}{V}$$

(1)

Studying resorcinarene derivatives (Figure 2.15), Rebek

demonstrated that they were able to give self-assembly only when the guest hosted in the cavity occupied a given volume. In particular when PCs fell in the range $0.55 \pm 0.09$ defining the so called “55% rule”.\textsuperscript{37}

When packing coefficients are less than 45% (40% for gaseous guest) the encapsulation does not occur because the guest is characterized by intermolecular interactions with the host that are less strong than those with solvent molecules. From the opposing point of view when the packing coefficient are higher than 65 % the guest is not encapsulated because it pays the price of an unnatural immobilization inside the capsule.

**Figure 2. 15** Rebek’s resorinarene-based capsule
2.3.1.1 Calculation of the packing coefficients of the pseudorotaxanes endo-alkyl-\textbf{2.4g}^+\cdot\textbf{TFPB}^-

Pseudorotaxane structures were built using the MacroModel-Maestro and minimized with the force field OPLS 2005. Subsequently the structures obtained in this way were used as input data to perform DFT calculations. In detail, DFT calculations were performed using B3LYP/6-31G level of the theory and Grimme’s dispersion corrections IOP(3/124 = 3).\textsuperscript{38} The van der Waals molecular volumes were calculated with the program Swiss-PdbViewer\textsuperscript{39} with a probe size of 1.4 Å as diameter.

The volume of the calixarene cavity for each pseudorotaxane system was obtained removing the guest from it and then it was measured the volume of the guest hosted in the cavity. Employing these values, packing coefficients (PCs) were calculated for all pseudorotaxane systems and they are reported in Table 2.5 (see experimental section, pp. 72-73, for more details on the PCs calculation procedure).


\textsuperscript{39} Swiss-PdbViewer application is used to analyze several proteins at the same time. In particular, proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface.
<table>
<thead>
<tr>
<th>(endo-alkyl)-Pseudorotaxane</th>
<th>( K_{\text{ass}} ) (M(^{-1}))</th>
<th>PC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LINEAR DERIVATIVES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 2.4g^+ \cdot \text{TFPB}^- )</td>
<td>1.2±0.2x10(^6)</td>
<td>45</td>
</tr>
<tr>
<td>( 2.4h^+ \cdot \text{TFPB}^- )</td>
<td>5.0±0.8x10(^3)</td>
<td>41</td>
</tr>
<tr>
<td>( 2.4d^+ \cdot \text{TFPB}^- )</td>
<td>6.5±0.9x10(^4)</td>
<td>46</td>
</tr>
<tr>
<td>( 2.4f^+ \cdot \text{TFPB}^- )</td>
<td>5.5±0.6x10(^1)</td>
<td>46</td>
</tr>
<tr>
<td><strong>α-BRANCHED DERIVATIVES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 2.4g^+ \cdot \text{TFPB}^- )</td>
<td>1.2±0.2x10(^6)</td>
<td>45</td>
</tr>
<tr>
<td>( 2.4i^+ \cdot \text{TFPB}^- )</td>
<td>4.2±0.6x10(^4)</td>
<td>41</td>
</tr>
<tr>
<td>( 2.4j^+ \cdot \text{TFPB}^- )</td>
<td>3.6±0.5x10(^2)</td>
<td>45</td>
</tr>
<tr>
<td><strong>β-BRANCHED DERIVATIVES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 2.4h^+ \cdot \text{TFPB}^- )</td>
<td>5.0±0.8x10(^3)</td>
<td>41</td>
</tr>
<tr>
<td>( 2.4k^+ \cdot \text{TFPB}^- )</td>
<td>5.1±0.6x10(^3)</td>
<td>44</td>
</tr>
</tbody>
</table>
Close inspection of data in Table 2.5 evidenced that the packing coefficients of the pseudorotaxanes 2.4g-o+ fall in the range 46-64% in accord with the Rebek’s rule, however they were not closely related to the $K_{ass}$ values. In fact the pseudorotaxanes 2.4g+ and 2.4j+ which have different stability constants ($1.2\pm0.2\times10^6$ and $3.6\pm0.5\times10^2$ M$^{-1}$) showed similar packing coefficients (45 and 45% respectively).

2.3.2 Contacting Coefficient Calculation

At this point, for the first time we have studied the relationship between the association constant of the pseudorotaxanes and the contact surface between the aromatic cavity of the calix[6]arene host and the alkyl moiety inside the cavity. This novel parameters has been defined by us as the
ratio between the guest's surface in close contact with the calixarene cavity surface (portion of the alkylbenzylammonium guest inside the calix-cavity) and the total surface of the guest portion hosted in the cavity (Eq. 2), and should takes account of the ability of the guest to fill the calix-cavity establishing van der Waals and C-H⋯π interactions with it.

\[
CC = \frac{S_{\text{host-guest contact}}}{S_{\text{guest}}} \quad (2)
\]

Thus, through the program Swiss-PdbViewer it was calculated the CC value for the ethyl moiety inside the calixarene cavity for the pseudorotaxane 2.4g⁺. Thus we evidenced that the 97% (Table 2.6) of the surface of the ethyl moiety (see Figure 2.16 left) was in close contact with the surface of the calixarene cavity, a value significantly higher than the CC value of 62% (Table 2.6 and Figure 2.16) measured for the neopentyl moiety inside the calix-cavity of 2.4l⁺.
Figure 2.16 Swiss-PdbViewer representation of the contact surface (in blu) between 2.1a and 2.4g\(^+\) (left) and between 2.1a and 2.4l\(^+\) (right) (in yellow the free guest's surface).

Interestingly the two CC values of 2.4g\(^+\) and 2.4l\(^+\) of 97 and 62 % (Table 2.6) were well related to the association constants of two pseudorotaxanes of 1.2±0.2x10\(^6\) and 1.7±0.2 x10\(^2\) M\(^{-1}\) respectively. Thus to a high CC value corresponds a greater stability of the complex, likely due to the increased contribution of Van der Waals interactions between host and guest. In Figure 2.16 (left) we report the structure of the pseudorotaxane 2.4g\(^+\), in yellow the free surface of the ethyl moiety of the guest not in contact with the calixarene cavity, in blu the portion in contact. Comparison with the analogue structure of 2.4l\(^+\) evidences a higher free surface (yellow) for the neopentyl moiety in 2.4l\(^+\).
Close inspection of the data reported in Table 2.6 evidenced a

<table>
<thead>
<tr>
<th>(endo-alkyl) Pseudorotaxane</th>
<th>$K_{ass}$ (M$^{-1}$)</th>
<th>PC(%)</th>
<th>CC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4g$^+$·TFPB$^-$</td>
<td>1.2±0.2x10$^6$</td>
<td>45</td>
<td>97</td>
</tr>
<tr>
<td>2.4i$^+$·TFPB$^-$</td>
<td>4.2±0.6 x10$^4$</td>
<td>41</td>
<td>88</td>
</tr>
<tr>
<td>2.4m$^+$·TFPB$^-$</td>
<td>6.9±0.8 x10$^3$</td>
<td>38</td>
<td>79</td>
</tr>
<tr>
<td>2.4n$^+$·TFPB$^-$</td>
<td>2.9±0.4 x10$^3$</td>
<td>42</td>
<td>78</td>
</tr>
<tr>
<td>2.4j$^+$·TFPB$^-$</td>
<td>3.6±0.5x10$^2$</td>
<td>45</td>
<td>65</td>
</tr>
<tr>
<td>2.4o$^+$·TFPB$^-$</td>
<td>2.7±0.4 x10$^2$</td>
<td>44</td>
<td>78</td>
</tr>
<tr>
<td>2.4I$^+$·TFPB$^-$</td>
<td>1.7±0.2 x10$^2$</td>
<td>58</td>
<td>62</td>
</tr>
<tr>
<td>2.4f$^+$·TFPB$^-$</td>
<td>5.5±0.6x10$^1$</td>
<td>46</td>
<td>61</td>
</tr>
</tbody>
</table>
good relationship between the association constants and CC values. Thus at high $K_{\text{ass}}$ values corresponds high CC values.

2.3 CONCLUSION

In conclusion there were synthesised alkylbenzylammonium axles differently substituted and the branching effect on calixarene threading was studied. The complexation experiment showed that, in the formation of pseudorotaxane structures the endo-alkyl orientation was always preferred, and endo-benzyl adducts were observed only for the guests 2.3d$^+$·TFPB$^-$, 2.3f$^+$·TFPB$^-$ and 2.3j$^+$·TFPB$^-$ (Figures 2.6 and 2.8). For all pseudorotaxane structures PCs were calculated and it was seen that they were not correlated with the effectiveness of the calixarene threading, represented by complexation constants but they were in agreement with the Rebek’s 55% rule.

In order to find a correlation between guest structure and threading effectiveness, a new parameter was investigated. The new parameter CC, defined as Contacting Coefficient, showed a relationship with the binding constant, so this parameter can be a valid support or an alternative to the study of the recognition properties of the calixarene hosts.
2.4 **Experimental Section**

ESI(+)−MS measurements were performed on a Micromass Bio-Q triple quadrupole mass spectrometer equipped with electrospray ion source, using a mixture of H₂O/CH₃CN (1:1) and 5% HCOOH as solvent. Flash chromatography was performed on Merck silica gel (60, 40-63 μm). All chemicals were reagent grade and were used without further purification. Anhydrous solvents were purchased from Aldrich. When necessary compounds were dried in vacuo over CaCl₂. Reaction temperatures were measured externally. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light, or by sprying with H₂SO₄-Ce(SO₄)₂ or phosphomolybdic acid. Derivatives 2.1a, 2.3c⁺·TFPB⁻ and sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate⁴⁰ were synthesized according to literature procedures and they are not reported here in this section. 1D NMR spectra were recorded on a Bruker Avance-400 spectrometer [400 (1H) and 100 MHz (¹³C)], Bruker Avance-300 spectrometer [300 (1H) and 75 MHz (¹³C)] and Bruker Avance-250 spectrometer [250 (1H) and 63 MHz (¹³C)]; chemical shifts are reported relative to the residual solvent peak (CHCl₃: δ 7.26, CDCl₃: δ 77.23;

---

CD$_3$OH: $\delta$ 4.87, CD$_3$OD: $\delta$ 49.0).

Molecular modeling studies were performed with a combined use of the MacroModel 9/Maestro-4.1\textsuperscript{41} program and the Gaussian-09 software package\textsuperscript{42} and Swiss-PdbViewer.\textsuperscript{43}


\textsuperscript{43} N. Guex, M. Peitsch, T. Schwede, A. Diemand, DeepView Swiss PDB Viewer, Glaxo Smith Klein.
Synthesis of derivative $\text{2.3g}^+\cdot\text{TFPB}^-$

To the benzylamine (0.010 mol) was added acetic anhydride (0.010 mol) and pyridine (0.50 mL) and the reaction mixture was kept overnight under stirring at room temperature. The excess of anhydride was removed under reduced pressure to give compound 2.7 (1.2 g; R= 85%).

In a three-necked flask, under nitrogen atmosphere, the compound 2.7 (7.7 mmol) was dissolved in THF anhydrous (70 mL) and LiAlH$_4$ (0.18 mol) was added at 0° C. The
resulting mixture was stirred at room temperature for 2 h and at reflux for 2 h. The reaction was quenched pouring the mixture in HCl 1.0 N (0.10 L) and the organic compound was extract with AcOEt. The organic layer was washed with water, dried over MgSO₄ and the solvent was removed under reduced pressure to give derivative 2.8g as a yellow viscous liquid. The crude product (8.0 mmol) was dissolved in Et₂O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 8.0 mmol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with Exane/MeOH and dried under vacuum, to give derivative 2.9g⁺·Cl⁻ as a white solid. Derivative 2.9g⁺·Cl⁻ (0.15 mmol) was dissolved in dry MeOH (5.0 mL), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (0.16 mmol) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives 2.3g⁺·TFPB⁻.
Derivative $2.3g^+\cdot\text{TFPB}^-$: (0.1310 g, 0.13 mmol, 80%).

ESI(+) MS: $m/z = 136.14$ (M$^+$).

$^1\text{H NMR}$ (250 MHz, CD$_3$OD, 298 K): $\delta$ 1.33 (t, $J = 7$, 3H, H$_a$), 3.11 (d, $J = 7$, 2H, H$_b$), 4.18 (s, 2H, H$_c$), 7.47 (s, 5H, H$_{d-f}$), 7.59 (overlapped, 12H, ArH$_{\text{TFPB}}$); $^{13}\text{C NMR}$ (100 MHz, CD$_3$OD, 298 K): $\delta$ 10.0, 42.4, 50.6, 117.1, 120.3, 123.0, 125.7, 128.4, 128.6, 128.9, 129.2, 129.3, 129.4, 129.5, 131.2, 134.4, 160.7, 161.2, 161.7, 162.2.
Synthesis of derivative \textbf{2.3h+·TFPB}⁻

\[ \begin{align*}
\text{PhNH}_2 + \text{1-iodopropane} & \quad \xrightarrow{\text{RT, 2h}} \quad \text{PhNH}_2 \text{2.8h} \\
\text{PhN}^+\text{H}_2\text{O}^+ & \quad \xrightarrow{\text{TFPBNa, MeOH, RT 12h}} \quad \text{PhN}^+\text{H}_2\text{Cl}^- \text{2.9h+} \text{Cl}^- 
\end{align*} \]

To the benzylamine (0.010 mol) was added 1-iodopropane (0.010 mol) and the reaction mixture was stirred at room temperature for 2 h.

After 2 h stirring, unreacted benzylamine was removed by crystallization with CH\textsubscript{2}Cl\textsubscript{2}. The resulting crude product was subjected to flash chromatography on silica gel (CHCl\textsubscript{3}/MeOH, 98/2) to give the ammonium iodide intermediate (1.7 g, 4.2 mmol, 42%).

The ammonium iodide (1.7 g, 4.2 mmol) was dissolved in AcOEt (30 mL) and KOH aqueous solution 1.0 N (30 mL) and the resulting mixture was stirred at room temperature for 4 h to give the amine \textbf{2.8h}. The amine (0.15 mmol) was dissolved in Et\textsubscript{2}O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 1.2 eq) was added dropwise. The mixture
was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with acetonitrile and dried under vacuum, to give derivative $2.9h^+\cdot Cl^-$ as a white solid.

Derivative $2.9g^+\cdot Cl^-$ (1 eq.) was dissolved in dry MeOH ($C = 0.20$ M), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (1.1 eq.) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives $2.3h^+\cdot TFPB^-$.

**Derivative $2.3h^+\cdot TFPB^-$**: (0.13 g, 0.13 mmol, 78%). ESI(+) MS: $m/z = 150.14$ (M$^+$). $^1H$ NMR (300 MHz, CD$_3$OD, 298 K): $\delta$ 1.01 (t, $J = 7.5$, 3H, H$_a$), 1.73 (sext, $J = 7.2$ 2H, H$_b$), 2.99 (t, $J = 8$, 2H, H$_c$), 4.18 (s, 2H, H$_d$), 7.46 (overlapped, 5H, H$_e$-g), 7.59 (overlapped, 12H, ArH$_{TFPB}$). $^{13}$C NMR (75 MHz, CD$_3$OD, 298 K): $\delta$ 9.9, 19.3, 48.9, 51.1, 117.2, 119.1, 122.7, 126.3, 129.1, 129.5, 129.6, 129.9, 131.3, 134.5, 160.6, 161.3, 161.9, 162.6.

45
Synthesis of derivatives $2.3_{d,f,l,j,k,m,p}^+ \cdot \text{TFPB}^-$

To the benzaldehyde (0.010 mol) was added the appropriate derivative amine (0.010 mol). The reaction mixture was stirred at room temperature for 2 h to give the imine intermediate in a quantitative yield. The imine was used for the next step without further purification. The imine (0.010 mol) was dissolved in dry MeOH (20 mL) under a nitrogen atmosphere and NaBH$_4$ (0.10 mol) was added at 0 °C and then the mixture was allowed to warm at room temperature.
The solution was kept under stirring for 3 h. The solvent was removed under reduced pressure and the residue partitioned between AcOEt and an aqueous saturated solution of NaHCO₃. The organic layer was dried over MgSO₄ and the solvent was removed, under reduced pressure, to give the secondary amine as a yellow viscous liquid. The amine was used for the next step without further purification. The crude product (0.010 mol) was dissolved in Et₂O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 0.010 mol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with acetonitrile and dried under vacuum, to give the corresponding chloride as a white solid.

The corresponding chloride (1 eq.) was dissolved in dry MeOH (C= 0.20 M), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (1.1 eq) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give the corresponding derivative 2.3d,f,I,j,k,m,p⁺·TFPB⁻.
Derivative 2.3d⁺·TFPB⁻: (0.11 g, 0.10 mmol, 93%). ESI(+) MS: $m/z = 164.16 \text{ (M}^+\text{)}$. $^1\text{H NMR}$ (300 MHz, CD$_3$OD, 298 K): $\delta$ 0.99 (t, $J = 7.5$, 3H, H$_a$), 1.43 (m, 2H, H$_b$), 1.68 (m, 2H, H$_c$), 3.02 (t, $J = 8$, 2H, H$_d$), 4.18 (s, 2H, H$_e$), 7.47 (overlapped, 5H, ArH$_f$-$h$), 7.59 (overlapped, 12H, ArH$_{\text{TFPB}}$); $^{13}\text{C NMR}$ (100 MHz, CDCl$_3$, 298 K): $\delta$ 13.0, 19.2, 28.1, 48.8, 53.6, 117.6, 120.5, 123.2, 125.9, 127.5, 128.5, 128.6, 128.8, 129.0, 129.4, 130.4, 131.7, 134.8, 160.9, 161.4, 161.9, 162.4.

Derivative 2.3f⁺·TFPB⁻: (0.16 g, 0.15 mmol, 99%). ESI(+) MS: $m/z = 192.18 \text{ (M}^+\text{)}$. $^1\text{H NMR}$ (300 MHz, CD$_3$OD, 298 K): $\delta$ 0.91 (s, broad, 3H, H$_a$), 1.36 (s, broad, 6H, H$_{b-d}$), 1.72 (s, broad, 2H, H$_e$), 3.04 (s, broad, 2H, H$_f$), 4.02 (s, broad, 2H, H$_g$), 7.48 (s, broad, 5H, ArH), 7.64 (s, broad, 12H, ArH$_{\text{TFPB}}$); $^{13}\text{C NMR}$ (75 MHz, CD$_3$OD, 298 K): $\delta$ 14.2, 23.4, 27.1, 27.3,
32.3, 52.4, 118.5, 120.4, 123.0, 127.6, 129.9, 130.3, 130.7, 130.9, 131.2, 132.5, 135.8, 161.9, 162.6, 163.2, 163.9.

**Derivative 2.3i⁺·TFPB⁻:** (0.090 g, 0.10 mmol, 88%). ESI(+) MS: *m/z* = 150.15 (M⁺). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD, 298 K): δ 1.39 (d, J = 7, 6H, H<sub>a</sub>), 3.42 (m, 1H, H<sub>b</sub>), 4.19 (s, 2H, H<sub>c</sub>), 7.48 (overlapped, 5H, H<sub>c-e</sub>), 7.61 (overlapped, 12H, ArH<sup>TFPB</sup>); <sup>13</sup>C NMR (65 MHz, CD<sub>3</sub>OD, 298 K): δ 17.0, 47.5, 49.5, 116.2, 117.0, 121.3, 125.6, 127.4, 128.0, 128.3, 128.5, 128.9, 130.0, 130.7, 133.5. DEPT 135° (75 MHz, CD<sub>3</sub>OD, 298 K): δ 17.0, 47.5, 49.5, 116.1, 116.2, 116.3, 128.0, 128.3, 128.5, 133.5.

**Derivative 2.3j⁺·TFPB⁻:** (0.090 g, 0.10 mmol, 88%). ESI(+) MS: *m/z* = 164.15 (M⁺). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, 298 K): δ 1.45 (s, 9H, H<sub>a</sub>), 4.17 (s, 2H, H<sub>b</sub>), 7.48 (overlapped, 5H,
H\textsubscript{c,e}), 7.60 (overlapped, 12H, ArH\textsuperscript{TFPB}); \textsuperscript{13}C NMR (100 MHz, CD\textsubscript{3}OD, 298 K): $\delta$ 24.4, 45.2, 57.1, 117.1, 120.3, 123.0, 125.7, 128.4, 128.6, 129.0, 129.2, 129.5, 131.6, 134.4, 160.7, 161.2, 161.7, 162.2.

Derivative 2.3k$^+$.TFPB$^-$: (0.11 g, 0.11 mmol, 73%). ESI(+)
 MS: $m/z$ = 164.14 (M$^+$). \textsuperscript{1}H NMR (250 MHz, MeOD, 298 K):
 $\delta$ 1.02 (d, $J$ = 7, 6H, H\textsubscript{a}), 2.02 (m, 1H, H\textsubscript{b}), 2.87 (d, $J$ = 7, 2H, H\textsubscript{c}), 4.20 (s, 2H, H\textsubscript{d}), 7.47 (s, 5H, ArH\textsubscript{e-g}), 7.59 (s, 12H, ArH\textsuperscript{TFPB}); \textsuperscript{13}C NMR (100 MHz, CD\textsubscript{3}OD, 298 K): $\delta$ 20.3, 27.2, 52.8, 55.7, 116.5, 121.7, 124.4, 127.1, 129.8, 130.3, 130.6, 130.8, 131.0, 132.4, 135.8, 162.1, 162.6, 163.1, 163.6.

Derivative 2.3m$^+$.TFPB$^-$: (0.11g, 0.10 mmol, 91%). ESI(+)
 MS: $m/z$ =164.18 (M$^+$). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3} 298 K): $\delta$
0.94 (t, J = 7, 3H, H_a), 1.34 (d, J = 6, 3H, H_d), 1.60 (m, broad, 1H, H_b), 1.70 (overlapped, 1H, H_b), 3.30 (s, broad, 1H, H_c), 4.13 (m, broad, 2H, H_e), 5.65-5.76 (m, broad, 2H, H_NH2+), 7.20 (d, J = 7, 2H, ArH) 7.42-7.53 (overlapped, 3H + 4H, ArH+ ArH_TFPB), 7.69 (s, 8H, ArH_TFPB); \(^{13}\)C NMR (75 MHz, CDCl\(_3\), 298 K): δ 27.5, 32.3, 35.9, 54.2, 60.1, 117.7, 119.3, 122.9, 126.5, 128.0, 128.4, 128.9, 129.2, 129.7, 130.1, 130.4, 131.6, 134.9, 160.8, 161.5, 162.2, 162.8.

Derivative 2.3p^+·TFPB^-: (0.096 g, 0.085 mmol, 83%).

ESI(+) MS: \( m/z = 256,23 \) (M^+). \(^1\)H NMR (400 MHz, CDCl\(_3\), 298 K): δ 1.41 (s, 6H, H_a), 1.55 (d, J = 13, 3H, H_c), 1.76 (d, J = 13, 3H, H_c), 2.02 (s, 3H, H_b), 2.72 (m, broad, 2H, H_d), 4.09 (t broad 2H, H_e), 6.23 (s broad 2H, H_NH2), 7.21 (d, J = 7, 2H, H_f), 7.43 (t, J = 7, 2H, H_g); 7.48-7.58 (overlapped, 5H, H_{a-c}), 7.59 (overlapped, 12H, ArH_TFPB); \(^{13}\)C NMR (75 MHz, CDCl\(_3\), 298 K): δ 27.5, 32.3, 35.9, 54.2, 60.1, 117.7, 119.3, 122.9, 126.5, 128.0, 128.4, 128.9, 129.2, 129.7, 130.1, 130.4, 131.6, 134.9, 160.8, 161.5, 162.2, 162.8.
To the benzylamine (0.010 mol) was added 3,3-dimethylbutyraldehyde (0.010 mol) and the reaction mixture was stirred at room temperature for 3 h.

The resulting imine (0.010 mol) was dissolved in dry MeOH (20 mL) under a nitrogen atmosphere and NaBH₄ (0.10 mol) was added at 0 °C and then the mixture was allowed to warm at room temperature. The solution was kept under stirring for 3 h. The solvent was removed under reduced pressure and the residue partitioned between AcOEt (30 mL) and an aqueous saturated solution of NaHCO₃ (30 mL). The organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure, to give derivative 2.8l as a yellow viscous liquid. The compound was used for the next step without further purification. The crude product (0.010 mol) was dissolved in Et₂O (20 mL) at room temperature and an
aqueous solution of HCl (37% w/w, 0.011mol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with Exane/MeOH and dried under vacuum, to give derivative \( 2.9l^+\cdot\text{Cl}^- \) as a white solid. Derivative \( 2.9l^+\cdot\text{Cl}^- \) (0.15 mmol) was dissolved in dry MeOH (5.0 mL), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (0.16 mmol) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives \( 2.3l^+\cdot\text{TFPB}^- \).

Derivative \( 2.3l^+\cdot\text{TFPB}^- \): (0.090 g, 0.22 mmol, 95%). ESI(+)

**MS**: \( m/z = 178.17 \) (M+). **\( ^1\text{H NMR} \)** (250 MHz, CD\(_3\)OD, 298 K): \( \delta \) 1.01 (s, 9H, Ha), 2.78 (s 2H, Hb), 4.24 (s, 2H, Hc), 7.31-7.80 (overlapped, 5H, Hd-f + 12H, ArH\(_\text{TFPB} \); **\( ^{13}\text{C NMR} \)** (65 MHz, CDCl\(_3\), 298 K): \( \delta \) 26.5, 31.3, 54.5, 60.2, 117.6, 117.7, 119.3, 122.9, 126.5, 127.9, 128.4, 128.79, 128.82, 129.2, 129.6, 130.10, 130.4, 131.7, 134.9, 160.8, 161.4, 162.1, 162.8.
Benzylamine (0.010 mol) was dissolved into the corresponding ketone **2.14** (40 mL) and the reaction mixture was stirred at reflux for 18 h. The reaction mixture was then cooled to room temperature and the excess of ketone was removed under reduced pressure.

The resulting imine (0.010 mol) was dissolved in dry MeOH (20 mL) under a nitrogen atmosphere and NaBH₄ (0.10 mol) was added at 0 °C and then the mixture was allowed to warm at room temperature. The solution was kept under stirring for 3 h. The solvent was removed under reduced pressure and the
residue partitioned between AcOEt (30 mL) and an aqueous saturated solution of NaHCO₃ (30 mL). The organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure, to give derivative 2.8n,o as a yellow viscous liquid. The compound was used for the next step without further purification. The crude product (0.010 mol) was dissolved in Et₂O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 0.011 mol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with Exane/MeOH and dried under vacuum, to give derivative 2.9n,o⁺·Cl⁻ as a white solid.

Derivative 2.9n,o⁺·Cl⁻ (1 eq.) was dissolved in dry MeOH (C= 0.20 M), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (1.1 eq) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives 2.3n,o⁺·TFPB⁻.
Derivative 2.3n⁺·TFPB⁻: (0.14 g, 0.14 mmol, 99%). ESI(+) MS: \( m/z = 178.15 \) (M⁺). \(^1\)H NMR (400 MHz, CDCl₃, 298 K): \( \delta \) 0.86-1.00 (overlapped, 6H, \( H_a \)), 1.29 (d, \( J = 7 \), 3H, \( H_d \)), 1.98 (m broad, 1H, \( H_b \)), 3.17 (m, broad 1H, \( H_c \)), 4.08 (m, broad 1H, \( H_e \)), 6.17 (s, broad 1H, \( H_{NH2} \)), 6.88 (s, broad 1H, \( H_{NH2} \)), (overlapped, 5H, \( H_{c-e} \)), 7.48-7.61 (overlapped, 5H, \( H_f \) + \( \text{ArH}_{TFPB} \)) 7.45 (t broad, \( J = 7 \), 2H, \( H_g \)), 7.71 (s, 8H, \( \text{ArH}_{TFPB} \)); \(^{13}\)C NMR (100 MHz, CD₃OD, 298 K): \( \delta \) 10.0, 14.3, 18.2, 29.4, 48.8, 59.0, 117.1, 120.3, 123.0, 125.7, 128.4, 128.6, 128.9, 129.2, 129.3, 129.6, 131.1, 134.4, 160.7, 161.2, 161.7, 162.2.

![Diagram](image)

Derivative 2.3o⁺·TFPB⁻: (0.12 g, 0.11 mmol, 78%). ESI(+) MS: \( m/z = 178.15 \) (M⁺). \(^1\)H NMR (250 MHz, CD₃OD, 298 K): \( \delta \) 1.00 (t, \( J = 7 \), 6H, \( H_a \)), 1.81 (m, broad, 4H, \( H_b \)), 3.08 (m, broad, 1H, \( H_c \)), 4.21 (s, 2H, \( H_d \)), 7.48 (overlapped, 5H, \( H_{e-g} \)), 7.59 (overlapped, 12H, \( \text{ArH}_{TFPB} \)); \(^{13}\)C NMR (65 MHz, CD₃OD, 298 K): \( \delta \) 8.6, 22.8, 50.8, 63.1, 117.7, 118.2, 122.5, 126.8, 128.2, 128.8, 128.9, 129.3, 129.8, 130.6, 131.2, 131.8, 134.9, 160.6, 161.4, 162.2, 163.0.
General procedure for the preparation of [2]Pseudorotaxane 2.4⁺·TFPB⁻

Calixarene derivative 2.1a (1.9·10⁻³ mmol) was dissolved in 0.5 mL of CDCl3 (3.8·10⁻³ M solution). Then, the appropriate TFPB⁻ salt 2.3⁺·TFPB⁻ was added (1.9·10⁻³ mmol, 3.8·10⁻³ M) and the mixture was stirred for 15 min. Then, the solution was transferred in a NMR tube for 1D NMR spectra acquisition.
\(^{1}\)H NMR determination of \(K_{\text{ass}}\) values\(^{44}\) for the endo-alkyl pseudorotaxanes \(2.4f\cdot\text{TFPB}^-,\ 2.4j\cdot\text{TFPB}^-,\ 2.4k\cdot\text{TFPB}^-,\ 2.4l\cdot\text{TFPB}^-,\ 2.4m\cdot\text{TFPB}^-,\ 2.4n\cdot\text{TFPB}^-\).

The association constant values were calculated by integration of free and complexed \(^{1}\)H NMR peaks of host or guest. The calixarene derivative \(2.1a\) \((1.9\cdot10^{-3}\text{ mmol})\) was dissolved in 0.5 mL of CDCl\(_3\) \((3.8\cdot10^{-3}\text{ M solution})\). Then, the appropriate TFPB\(^-\) salt \(2.4^+\) was added \((1.9\cdot10^{-3}\text{ mmol, 3.8}\cdot10^{-3}\text{ M})\) and the mixture was stirred for 15 min. Then, the solution was transferred in a NMR tube for spectra acquisition.

An representative example of \(K_{\text{ass}}\) calculation is reported in Figure 2.17.

Figure 2.17 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.8·$10^{-3}$ M) of 2.1a and 2.3m$^+$·TFPB$^-$. The association constant $K_{ass}$ value was calculated by integration of complexed (▲) and free (●) derivative 2.1a: $[(6.00 \times 3 / 21.89) \times 3.8 \times 10^{-3}] / [(3.89/21.89) \times 3.8 \times 10^{-3}]^2 = 6.9 \times 10^3$ M$^{-1}$. 
Figure 2.18 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution ($3.8 \cdot 10^{-3}$ M) of $2.1a$ and $2.3j^+$·TPFB$^-$. The association constant $K_{ass}$ value was calculated by integration of complexed ($\Delta$) and free derivative $2.1a$ ($\bullet$).
Figure 2.19 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.8·10$^{-3}$ M) of 2.1a and 2.3k$^+$·TFPB$^-$. The association constant $K_{ass}$ value was calculated by integration of complexed (▲) and free (●) derivative 2.1a.
Figure 2.20 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.8·10$^{-3}$ M) of 2.1a and 2.3f·TFPB$^-$. The association constant $K_{ass}$ value was calculated by integration of endo-alkyl complexed (▲), endo-benzyl complexed (▲) and free derivative 2.1a (●).
Figure 2.21 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.8·10$^{-3}$ M) of 2.1a and 2.3n$^+$·TPPB$^-$. The association constant $K_{ass}$ value was calculated by integration of complexed (▲) and free (●) derivative 2.1a.
Figure 2.22 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.8·10$^{-3}$ M) of 2.1a and 2.3l·TFPB$^-$. The association constant $K_{ass}$ value was calculated by integration of complexed (▲) and free (●) derivative 2.1a.
$^1$H NMR determination of the $K_{ass}$ value for the $2.4o^+\cdot\text{TFPB}^-$ pseudorotaxane by quantitative NMR Analysis.

The sample was prepared by dissolving 2.1a ($1.9 \times 10^{-3}$ mmol) and the 2.3o$^+\cdot\text{TFPB}^-$ benzylalkylammonium guest ($1.9 \times 10^{-3}$ mmol) in CDCl$_3$ (0.5 mL) containing 1 μL of 1,1,2,2-tetrachloroethane ($d = 1.59$ g/mL) as internal standard. The complex concentration was evaluated by integration of the $^1$H NMR signal of CHCl$_2$CHCl$_2$ $Vs$ the signal of the complex. The following equation was used to obtain the moles of the complex:

$$\frac{G_a}{G_b} = \frac{F_a}{F_b} \times \frac{N_a}{N_b} \times \frac{M_a}{M_b}$$

$G_a$ = grams of 1,1,2,2-tetrachloroethane; 
$G_b$ = grams of complex 
$F_a$ and $F_b$ = areas of the signals of 1,1,2,2-tetrachloroethane and the shielded signal of the guest 
$N_a$ and $N_b$ = numbers of nuclei which cause the signals ($N_a$ for 1,1,2,2-tetrachloroethane; $N_b$ for guest) 
$M_a$ and $M_b$ = molecular masses of 1,1,2,2-tetrachloroethane (a) and complex (b)
Figure 2.23. $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.8·10$^{-3}$ M) of 2.1a and 2.3o$^+$·TFPB$^-$. The association constant $K_{ass}$ value was calculated by integration of the complexed derivative 2.1a (▲) and the signal of CHCl$_2$CHCl$_2$ (●).
$^1$H NMR determination of the $K_{ass}$ values for endo-alkyl 2.4d$^+\cdot$TFPB$^-$, 2.4i$^+\cdot$TFPB$^-$, 2.4h$^+\cdot$TFPB$^-$ and 2.4g$^+\cdot$TFPB$^-$ pseudorotaxanes by competition experiments.

$^1$H NMR competition experiments were performed by analysis of a 1:1:1 mixture of host H 1, guest GA and guest GB in a NMR tube using CDCl$_3$ as solvent. Calixarene derivative 2.1a (1.9·10$^{-3}$ mmol) was dissolved in 0.5 mL of CDCl$_3$ (3.8·10$^{-3}$ M solution). Then, the appropriate guests GA (1.9·10$^{-3}$ mmol, 3.8·10-3 M) and GB (1.9·10$^{-3}$ mmol, 3.8·10$^{-3}$ M) were added. Then, the solution was transferred in a NMR tube for 1D NMR spectra acquisition.

\[
K_{A\subset H} = \frac{[HG_A]}{[H][G_A]} \quad \text{and} \quad K_{B\subset H} = \frac{[HG_B]}{[H][G_B]} \quad \Rightarrow \quad K_{rel} = \frac{K_{A\subset H}}{K_{B\subset H}} = \frac{[HG_A][H][G_B]}{[HG_B][H][G_A]}
\]

\[
K_r = \frac{K_{A\subset H}}{K_{B\subset H}} = \frac{[HG_A]^2}{[HG_B]^2}
\]
Figure 2.24 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of derivative 2.1a in presence of 1 equivalent of 2.3d$^+$·TFPB$^-$ and 1 equivalent of 2.3j$^+$·TFPB$^-$ (3.8·10$^{-3}$ M each one). (●) Resonance relative to the endo-alkyl-2.4d$^+$·TFPB$^-$ complex, (▲) resonance relative to the endo-alkyl-2.4j$^+$·TFPB$^-$.
Figure 2.25 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of derivative 2.1a in presence of 1 equivalent of 2.3i$^+$·TFPB$^-$ and 1 equivalent of 2.3c$^+$·TFPB$^-$ (3.8·10$^{-3}$ M each one). (●) Resonance relative to the endo-alkyl-2.4i$^+$·TFPB$^-$ complex, (▲) resonance relative to 2.4c$^+$·TFPB$^-$ complex.
Figure 2.26 ¹H NMR spectrum (CDCl₃, 400 MHz, 298 K) of derivative 2.1a in presence of 1 equivalent of 2.3h⁺·TFPB⁻ and 1 equivalent of 2.3c⁺·TFPB⁻ (3.8·10⁻³ M each one). (●) Resonance relative to the endo-alkyl-2.4h⁺·TFPB⁻ complex, (▲) resonance relative to the 2.4c⁺·TFPB⁻ complex.
Figure 2.27 $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of derivative 2.1a in presence of 1 equivalent of 2.3$i^+\cdot$TFPB$^-$ and 1 equivalent of 2.3$g^+\cdot$TFPB$^-$ (3.8·$10^{-3}$ M each one). (●) resonance relative to 2.4$i^+\cdot$TFPB$^-$ complex, (▲) resonance relative to 2.4$g^+\cdot$TFPB$^-$ complex.
Packing Coefficient and CCs calculation.

A. Pseudorotaxane structures \textit{endo-alkyl-2.4^+\cdot TFPB^-} were minimized using the force field OPLS-2005 in the MacroModel program. The structures obtained were subsequently defined by DFT calculations using B3LYP/6 31G level of the theory and Grimme's dispersion corrections (IOp(3/124 = 3).

B. The Swiss-pdb program was used to calculate the volume of the internal calixarene cavity and the guest volume. In detail, the guest was removed from each pseudorotaxane, the resulting calixarene was capped with a meta xylene unit and the inner volume was calculated with a probe of 1.4 Å as diameter. The guest volume was obtained considering only the portion hosted in the cavity by cutting the guest at the ammonium site level

C. \textbf{Packing Coefficients.} PCs were calculated dividing the guest volume by the calixarene volume, both calculated as described in section B.

D. \textbf{Contacting Coefficients.} Using the Swiss-pdb program each pseudorotaxane was divided in two portions: calixarene and guest and their surfaces were calculated (for the guest surface, \(S_{\text{guest}}\), it was considered only the portion hosted in the cavity by
cutting the guest at the ammonium site level). Again with the Swiss-pdb program, these two surface values were used to define the contact surface between host and guest ($S_{\text{host-guest contact}}$). The CC values, for each pseudorotaxane, were obtained dividing the contact surface $S_{\text{host-guest contact}}$ by the guest surface $S_{\text{guest}}$. 
CHAPTER III
CHIRALITY AND CHIRAL MOLECULAR RECOGNITION IN THE CALIXARENE THREADING

3.1 CHIRAL RECOGNITION IN SUPRAMOLECULAR STRUCTURES

One of the most important aims of the Supramolecular Chemistry is the recognition of chiral guests. In fact, differently by the natural receptors the artificial chiral discrimination is quite difficult to achieve with synthetic hosts. Usually, the use of a chiral host can be a good way to distinguish between two chiral guests.

For example when an enantiopure host is added to a mixture of two enantiomers, the two diastereoisomeric complexes formed are characterized by different properties and so their separation becomes easier to achieve.

An interesting example of chiral recognition is represented by the Cram’s machine (Figure 3.1).

---

The machine operated in the way that from the central tank of the W-tube containing an aqueous solution of racemic salt 3.2 (Figure 3.2), the (R)-enantiomer 3.2 was delivered to the left hand aqueous layer by (S,S)-3.1, while the (R)-enantiomer was transported by (R,R)-3.1 and delivered to the right hand aqueous layer. Fresh racemic guest was continuously added to the central tank and (S)- and (R)-C₆H₅H(CO₂CH₃)NH₃PF₆ of 86-90 % enantiomeric excess were continuously removed from the left and right hand aqueous tanks, respectively.
There are several spectroscopic techniques that have been used to study the chiral recognition, naturally the most common are those who study chiroptical properties such as electron circular dichroism (ECD), vibrational circular dichroism (VCD) and optical rotary dispersion (ORD). All these techniques represent also an useful tool for the assignment of the absolute configuration.

Interestingly, Habata and co-workers showed that an achiral crown-ether macrocycle was able to recognize chiral sec-ammonium cations and that an amplification of the chirality was observed due to the formation of a chiral pseudo[2]rotaxane (Figure 3.3).

In detail with the intent to investigate the chirality...
transcription and amplification by the [2]pseudorotaxanes, the UV-vis and CD spectra of the crown ether 3.3, ammonium salts (R)/(S)-3.4a-b, and [2]pseudorotaxanes were measured (Figure 3.3).

![Diagram of the formation of [2]pseudorotaxanes.](image)

**Figure 3.3** Diagram of the formation of [2]pseudorotaxanes.

The host 3.3 was achiral and no Cotton effect was observed. The chiral ammonium salt (R)-3.4a-H·PF₆ showed CD Cotton effects around 260 nm (aromatic ¹L₅ band) but the amplitude was very low. On the other hand, the [2]pseudorotaxane [3.3·(R)-3.4a-H][PF₆] displayed significant CD Cotton effects due to the exciton coupling between the two biphenyl chromophores in the 2’,2’’-quaterphenyl group.
3.2.2 Chiral recognition in gas phase.

Supramolecular species are sometimes quite big, with high molecular masses and their subunits are weakly bound and easy to destroy during the ionization process in mass spectrometry. For this reason the conventional mass spectrometry methods, in particular the harsh ionization methods such as EI and CI have delayed the development of mass spectrometry as tool to characterize supramolecular aggregates.

However since the introduction of the soft ionization methods in FAB, MALDI and ESI, mass spectrometry has gained a position among the analytical tools used to characterize noncovalent species.

As it is well-known, MS is insensitive to chirality differences; thus, MS cannot provide enantiomer differentiation but there
are several methods to use this technique to differentiate diastereomers. The first method is the “Enantiomer Labelled” guest method (EL). In this case the enantiopure host is mixed with the guest in racemic form. One of the two enantiomers is isotopically labelled, and the other not, so the signals for the two diastereomeric host-guest complexes appear at different m/z values in a MS spectra.

Directly from the intensity ratio of the two complexes, it is possible to determine the stereochemical effect in absence of the isotopic effect.

This method was successfully applied to some crown ethers by Sawada et al. using FAB\textsuperscript{47} and ESI-MS\textsuperscript{48} ionization techniques and it was extensively reviewed by Schalley in 2001.\textsuperscript{49}


Figure 3.5 Chiral crown ethers and ammonium guest ions studied by Sawada with EL method.

This study revealed that in the FAB experiments the crown (R,R,R,R)-3.5 recognized the guest (R)-3.7 (Figure 3.5) with a large excess compared to the S-isomer, but in ESI experiment this difference was less evident.

This trend was found to be common to all host-guest pairs.
investigated and was ascribed to the electrospray process, although no valid explanation was given about how the spray procedure affected the result.

An alternative method to the EL needs two independent measurements of each host enantiomer with a given guest used as a reference. It has been used in chiral recognition experiments between some crown ethers and ammonium ion guests by Pòcsfalvi\textsuperscript{50} and a similar work was made always by Sawada (Figure 3.6).\textsuperscript{51}

![Figure 3.6 Chiral crown ether and ammonium guest ions studied by Sawada with the reference method](image)


Also in this case a stero-preference was observed and was possible to enhance the crown’s enantiodiscrimination ability upon replacement of the two methyl groups with two phenyl substituents.

3.3 AIMS

In the previous chapter we have showed that the calix[6]arene macrocycle was able to thread branched dialkylammonium cations. On these basis and considering the importance of the chiral recognition, another aim of this PhD project has been the study of the chiral recognition abilities of calix[6]arene macrocycles toward chiral dialkylammonium guests. Therefore the goal was to explore the changes in the chiroptical properties of the single components calix-wheel/dialkylammonium axle of the calixarene-based pseudorotaxane after the threading process and finally to investigate the possibility to obtain a chiral recognition between chiral hosts and guests.

3.4 RESULTS AND DISCUSSIONS

In the first instance we decided to study what happens to the chirooptical properties of the achiral calixarene host 3.13 after threading with a chiral enantiopure dibenzylammonium guest 3.14⁺·TFPB⁻.
The complexation experiment was conducted mixing the calixarene 3.13 and 1 equivalent of 3.14\(^+\)-TFPB\(^-\) in CDCl\(_3\) and the mixture analysed by \(^1\)H NMR.

The first surprising results was the formation of the pseudorotaxane endo-alkyl-3.15\(^+\)-TFPB\(^-\) in Figure 3.8 in which the α-methylbenzyl moiety was inside the calixarene cavity.

Figure 3.7 Host and guest studied in complexation experiments
Figure 3.8. Complexation experiments between the derivative 3.13 and the derivative $3.14^+\cdot$TFPB$^-$. Indeed the signal at -0.9 ppm was related to the methyl group of the guest $3.14^+\cdot$TFPB$^-$ shielded inside the calixarene cavity.
Figure 3.9 Significant portion of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of 1:1 mixture of $3.13$ (3.8·$10^{-3}$M) and $3.14^+\cdot$TFPB$^-$ (3.8·$10^{-3}$M)

However the most important aspect in the formation of the [2]pseudorotaxane $3.15^+\cdot$TFPB$^-$ was that mixing the chiral guest $3.14^+\cdot$TFPB$^-$ with the achiral calixarene-host 3.13 it was observed a chirality induction and amplification from the guest to the entire complex. The ORD experiment reported below was made in collaboration with the Prof. Superchi from “Università degli Studi della Basilicata”.

As it can be seen from the graphic (Figure 3.10), moving from the axle to the complex there is a shift in the ORD spectra that indicates an induced optical rotary dispersion demonstrating an induced and amplification of chirality in the complex formation.
3.4.1 Synthesis of chiral hosts and guest

Once having experimented a change in the chiroptical properties of the complex the study moved towards more complex chiral systems. At this point the through the annulus threading was explored using the chiral host (±)-3.16 showed in Figure 3.11 and different guests. The synthesis of the chiral calixarene (±)-3.16 was outlined in the Scheme 3.1.
Scheme 3.1 Synthesis of derivative 28

*p*-terz-butyl-calix[6]arene 3.17 was monobenzylated with benzyl bromide in the presence of potassium carbonate as base in dry CH₃CN at reflux to give mono-benzyl ether 3.18 (45%) after usual work-up. Compound 3.18 was exhaustively methylated by treatment with MeI in acetone in presence of Cs₂CO₃.

The removal of the benzyl groups was easily accomplished by hydrogenolysis to give pentamethoxy-calix[6]arene-mono-ol.
3.20 in 95% yield.
The $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of 3.20 showed three sharp singlets due to ArCH$_2$Ar groups indicating a fast conformational interconversion, which is due to the small dimension of the methoxy groups at the lower rim of the macrocycle.

Figure 3. 12 $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of the derivative 3.20

Finally, the treatment of 3.20 with $\alpha$-methylbenzylbromide in dry DMF and NaH as base, gave the racemic compound 3.16.
ESI(+)-MS spectrum confirmed the molecular formula of (±)-3.16, in addition ¹H and ¹³C NMR spectra confirmed the structure (Figura 3.13) in fact, two signals were present at 5.05 and 1.73 ppm related, respectively to the benzylic CH and methyl CH₃ both from the chiral substituent.

The aromatic region showed a complex signal pattern (6.75-7.53) related to the benzylic protons of both calixarene scaffold and substituent. Again there were 6 AX systems due to ArCH₂Ar groups belonging to the calixarene scaffold at 4.64 and 3.63 ppm (J = 13.4 Hz), 4.27 and 3.69 ppm (J = 14.0 Hz), 4.25 and 3.59 ppm (J = 14.0 Hz), 4.16 and 3.00 ppm (J = 13.4 Hz), 4.12 and 3.84 ppm (J = 13.9 Hz); 4.12 and 3.77 ppm (J = 13.9 Hz).
The compound \((\pm)3.16\) was studied in complexation experiments with the chiral guest \(3.14^+\cdot\text{TFPB}^-\).

![TFPB-](image)

\[
3.14^+\text{TFPB}^-
\]

**Figure 3.14.** Chiral derivative \(3.14^+\cdot\text{TFPB}^-\).

In this case the formation of \([2]\)pseudorotaxane could lead at four different stereoisomers. The dibenzylammonium cation \(3.14^+\cdot\text{TFPB}^-\) was constitutionally asymmetric so two stereoadducts were possible with the stereogenic center into the calixarene cavity for both calixarene enantiomers (being the calixarene in racemic form) and other two having the stereogenic center outside the cavity for the two calixarene enantiomers as well.
Figure 3.15 Stereoisomeric [2]pseudorotaxane obtainable.

The $^1$H NMR spectrum of the complexation experiment showed the formation of the only “endo-chiral” stereoisomers.

Indeed there was the signal, at negative chemical shift, related to the methyl group of the $\alpha$-methylbenzyl moiety into the calixarene cavity and shielded by the aromatic wall.
In addition in the range 4.8 - 6.5 ppm there was the classic pattern, already seen before, related to the benzylic hydrogens shielded by the calixarene cavity.

![Figure 3.16 Significant portion of the $^1$H NMR spectra (400 MHz, 298 K CDCl$_3$) of a) 1:1 mixture of 3.16 (3.8\cdot10^{-3}\text{M}) and 3.14\cdot\text{TFPB}$^-$ (3.8\cdot10^{-3}\text{M}); b) 3.16 (3.8\cdot10^{-3}\text{M}).](image)

Furthermore, focussing the attention on the methoxy and terz-butyl $^1$H NMR region, a qualitative valuation about the number and the intensity of these signals, suggested that the two diastereoisomers were formed in equal ratio.

This meant that no enantiodiscrimination was observed in the formation of the [2]pseudorotaxane probably because the two chiral centres were too distant, in the space, to act as a discriminating agent.
With the intent to observe a discrimination effect in the formation of [2]pseudorotaxane it was designed a new derivative that would have brought the two chiral centres closer to each other after the threading. This was possible, again, assuming the validity of the *endo*-alkyl rule for the new axle 3.22⁺·TFPB⁻ reported.

The proposed guest was synthesised from α-methyl-benzylamine and acetone at reflux for 18 h and reduced with NaBH₄. HCl (37%) was then used to obtain the chloride that was exchange with NaTFPB (Scheme 3.2).
Scheme 3.2 Synthesis of the chiral axle $3.22^+\cdot\text{TFPB}^-$.  

The threading between $3.22^+\cdot\text{TFPB}^-$ and the derivative $(\pm)3.16$ could lead at four different stereoisomers as in the previous case: two stereoadducts $(R,R$ and $R,S)$ with the isopropyl moiety into the calixarene cavity and other two with the benzyl moiety into the cavity for the two calixarene enantiomers, $R$ and $S$ as well.
Figure 3. 18 Stereoisomeric [2]pseudorotaxane obtainable.

The $^1$H NMR spectrum of the complexation experiment showed the characteristic isopropyl signal at negative chemical shift and the absence of the classic pattern related to the benzylic hydrogen shielded by the cavity. Therefore it was possible to claim that, the only stereoisomers formed were the ones with the isopropyl group into the calixarene cavity.
The two stereo-adducts had the two chiral centres closer to each other this time but looking at the metoxyl group region it was difficult to establish if there was enantiodiscrimination or not. The only 1D and 2D NMR techniques were not sufficient to discriminate and fully understand the complexation process.

For this reason it was decided to explore the enantiomeric discrimination in the calixarene threading by mass spectroscopy through the "enantiomer labelled guest method (EL).

3.4.2 Gas-phase study

In this study it was applied the "enantiomer labelled guest method (EL) as described in the introduction section.
An enantiopure host is mixed with an excess of a racemic mixture of the guest. One of the enantiomeric guests is isotopically labelled, and the other is not so the signals for the two diastereomeric host-guest pairs appear at different $m/z$ ratios.

3.4.3 Synthesis of enantiopure hosts

As well know, a good and easy way to obtain chiral calixarenes in enantiopure form is the functionalization of the calixarene lower rim with a chiral substituent.

**Figure 3.20** Chiral hosts synthesised

The hosts 3.27, 3.28 and 3.29 in the **Figure 3.18** are monofunctionalised and were obtained by the functionalization of the derivative pentamethoxy-calix[6]arene-mono-ol 3.20, in
particular, the synthesis of chiral derivatives 3.27 and 3.28 were outlined in **Scheme 3.3**.

**Scheme 3.3** Synthesis of derivatives 3.27 and 3.28.

Compound 3.29 was obtained after the esterification of the hydroxyl group of the derivative 3.20 with 3.34.

**Scheme 3.4** Synthesis of derivative 3.29

The fourth compound was obtained exploiting the reaction sequence shown in the **Scheme 3.5**.
Scheme 3.5 Synthesis of derivative 3.30

The \( p \)-terz-butyl-calix[6]arene was mixed with \( \text{K}_2\text{CO}_3 \) as base and \( \text{MeI} \) in dry acetone in autoclave. Compound 3.35 was obtained after purification by recrystallization. Compounds 3.30 was obtained through a \( \text{S}_2 \text{N}_2 \) reaction in which the derivative 3.35 was deprotonated with \( \text{NaH} \) and \((S)\)-1-iodo-2-methylbutane was added in DMF. The four compounds were fully characterized by the use of NMR and MS spectroscopy that was particularly helpful to assume the obtaining of the desired products.
Figure 3.21 Mass spectra of compounds 3.27, 3.28, 3.29 and 3.30.

3.4.4 Synthesis of labelled enantiopure guest

As already mentioned before the EL method requires an
isotopically labelled enantiomer of the selected guest.

On the basis of the results obtained by threading studies with the chiral ammonium cation (S)-3.22$^+_\cdot$TFPB$^-$ (see Figure 3.16 at pag. 94) it was synthesized the labelled enantiomer (R)-3.22-d$_6^+$·TFPB$^-$

![Figure 3.22 Deuteration strategy explored for the synthesis of (R)-3.22-d$_6^+$·TFPB$^-$.](image)

The synthesis of (R)-3.22-d$_6^+$·TFPB$^-$ was outlined in Scheme 3.6. The amine-acetone coupling was made by using Ti(IV)OiPr$_4$ as catalyst. The acidification with HCl (37%) gave the ammonium chloride and the usual salt exchange gave the axle required
Scheme 3.6 Synthesis of deuterated axle (R)-3.22-d₆⁺·TFPB⁻.

The guest (R)-3.22-d₆⁺·TFPB⁻ was characterized by NMR and mass spectrometry. Due to the inevitable H/D exchange in the first reaction step the resulting axle was not fully deuterated but a mixture of partially deuterated compounds (d₄, d₅ and d₆). This was evident in the ¹H NMR (Figure 3.23) by the presence of the low signal at 1.3 ppm and from the MS spectra (Figure 3.22).
3.4.5 MS experiments

A fundamental feature of this method is the use of an isotopically labelled enantiomer of a selected guest to
distinguish two diastereomeric host-guest complexes in a mass spectrum. A 1/1 mixture of labelled and unlabelled guest enantiomers was mixed with 0.5 equivalent of the target chiral host.

It was considered the competitive equilibrium system through eqs. 3.1 and 3.2

\[ \text{H} + \text{G}_S^+ \rightleftharpoons K_S (\text{HG}_S)^+ \] (3.1)

\[ \text{H} + \text{G}_{R-d_6}^+ \rightleftharpoons K_R (\text{HG}_{R-d_6})^+ \] (3.2)

Therefore, the peak intensity ratio, \( I[(\text{HG}_S)^+] / I[(\text{HG}_{R-d_6})^+] \), of the diastereomeric host-guest complex ions, was expected to become a measure of the enantio-discrimination ability of the host toward the two enantiomers of the chiral guest.

- If \( I_S / I_{R-d_6} > 1 \) means that a given chiral host binds more strongly the (S)-enantiomer. The larger the \( I_S / I_{R-d_6} \) ratio value and higher the degree of chiral recognition of the host.
- \( I_S / I_{R-d_6} < 1 \) means that a given chiral host binds more strongly the R-labelled guest.
• $I_S/I_{R-d_6} = 1.0 \pm 0.1$ means that a given chiral host cannot differentiate the chirality of a given guest.

3.4.6 Concentration Effect

The concentration solution can affect the ionization process and therefore the intensity ratio $I[(H + G_S)^+] / I[(H + G_{R-d_6})^+]$. Therefore the first step was to choose the best concentration value to use.

It was seen that the peak intensities were quite low up to a 300 μM solution and the intensity ratio did not change increasing the concentration over 300 μM. Therefore 300 μM solutions were used in determining isotopic and chiral recognition effects.

3.4.7 Isotopic Effects

In order to determine certain effects of deuterium labelling on the mass spectral intensities of the corresponding host-guest complex ions, it was used here a 1:1 mixture of a pair of labelled (R)-and unlabelled (R)-enantiomer guests and we evaluated the $[I_R/I_{R-d_6}]$ values with the corresponding host.

The unlabelled (R)-enantiomer, (R)-3.22$^+\cdot$TFPB$^-$ was
synthesised using the same procedure used for the synthesis of (S)-3.22\textsuperscript{+}·TFPB\textsuperscript{−} described before in Figure 3.16 and Scheme 3.2.

The resulting MS spectra for the investigation of the isotopic effect are reported below.

![Mass spectrum diagram](image.png)

**Figure 3.25** Significant portion of the mass spectrum of a 1:2:2 mixture (CH\textsubscript{2}Cl\textsubscript{2}, 300 μM) of the derivatives 3.27, (R)-3.22\textsuperscript{+}·TFPB\textsuperscript{−} and (R)-3.22\textsubscript{d6}\textsuperscript{+}·TFPB\textsuperscript{−} respectively (sample cone 25V, HV 2500 V).
Figure 3. 26 Significant portion of the mass spectrum of a 1:2:2 mixture (CH$_2$Cl$_2$, 300 μM) of the derivatives 3.28, (R)-3.22$^+$·TFPB$^-$ and (R)-3.22-d$_6$$^+$·TFPB$^-$ respectively (sample cone 25V, HV 2500 V).

Figure 3. 27 Significant portion of the mass spectrum of a 1:2:2 mixture (CH$_2$Cl$_2$, 300 μM) of the derivatives 3.29, (R)-3.22$^+$·TFPB$^-$ and (R)-3.22-d$_6$$^+$·TFPB$^-$ respectively (sample cone 25V, HV 2500 V).
Figure 3. 28 Significant portion of the mass spectrum of a 1:2:2 mixture (CH$_2$Cl$_2$, 300 μM) of the derivatives 3.30, (R)-3.22$^\ddagger$·TFPB$^-$ and (R)-3.22-d$_6$$^\ddagger$·TFPB$^-$ respectively (sample cone 25V, HV 2500 V).

The [$I_R/I_{R-d_6}$] values for the guest 3.30 was close to the unit indicating that no isotopic effect was observed, however the MS spectra for the compound 3.27, 3.28 and 3.29 showed a slight isotopic effect (see experimental section for the exact values and the calculation procedures).

Accordingly to the literature, the directionality of isotope effects is difficult to predict from system to system.

The literature supports observation of deuterium IEs from both solution and gas phase.$^{52}$

Therefore, the observed effects in our system might be due to a decreased van der Waals interactions between the guest’s

---

deuterated moiety and the host; or a preferential ionization of one diastereomeric complex over the other one; or a different gas-phase behaviour between the deuterated and non-deuterated guests.

3.4.8 Chiral Recognition

In order to determine a chiral recognition effect of a 1:1 mixture of a pair of labelled (R)-enantiomer, (R)-3.22-d₆⁺·TFPB⁻ and unlabelled (S)-enantiomer guest (S)-3.22⁺·TFPB⁻ was used with 0.5 equivalent of the corresponding host and the Iₛ/Iᵣ₆ ratio value was measured by inspection of the mass spectrum. The resulting MS spectra for the investigation of the isotopic effect are reported below.
Figure 3.29 Significant portion of the mass spectrum of a 1:2:2 mixture (CH₂Cl₂, 300 μM) of the derivatives 3.27, (S)-3.22⁺·TFPB⁻ and (R)-3.22-d₆⁺·TFPB⁻ respectively (sample cone 25V, HV 2500 V).

Figure 3.30 Significant portion of the mass spectrum of a 1:2:2 mixture
(CH₂Cl₂, 300 μM) of the derivatives 3.28, (S)-3.22⁺·TFPB⁻ and (R)-3.22-d₆⁺·TFPB⁻ respectively (sample cone 25V, HV 2500 V).

Figure 3. 31 Significant portion of the mass spectrum of a 1:2:2 mixture (CH₂Cl₂, 300 μM) of the derivatives 3.29, (S)-3.22⁺·TFPB⁻ and (R)-3.22-d₆⁺·TFPB⁻ respectively (sample cone 25V, HV 2500 V).

Figure 3. 32 Significant portion of the mass spectrum of a 1:2:2 mixture (CH₂Cl₂, 300 μM) of the derivatives 3.30, (S)-3.22⁺·TFPB⁻ and (R)-3.22-d₆⁺·TFPB⁻ respectively (sample cone 25V, HV 2500 V).
For the hosts 3.30 the $I_S/I_{R-d_6}$ was, again close to the unit so no enantiodiscrimination was observed.

For the hosts 3.27, 3.28 and 3.29 the $I_S/I_{R-d_3}$ were slightly different from the unit however their values were identical to those regarding the isotopic effect and therefore there was not a chiral enantiodiscrimination recognition with the hosts studied (see experimental section for the exact values and the calculation procedures).
3.5 Conclusion

In this chapter it was studied the calixarene threading with chiral systems. In particular it was demonstrated that in the formation of a [2]pseudorotaxane, starting form a chiral axle and an achiral calix, there was a chiral induction and transfer from the axle to the pseudorotaxane.

Soon later, the attention moved toward the possibility to obtain chiral recognition during the calixarene threading and the formation of the [2]pseudorotaxane.

In the first instance the NMR spectroscopy was selected as the technique for exploring the enantiodiscrimination formation of the [2]pseudorotaxanes. However the complexity and the difficulty to interpret the results obtained, suggested investigating the enantiodiscrimination process differently.

For this reason it was decided to explore the enantiomeric discrimination in the calixarene threading.

The enantiodiscrimination process was then studied in gas-phase by means of the MS spectrometry.

This required the synthesis of some enantiopure hosts and a pair of guests in which one of them was deuterated. Unfortunately the study did not provide the awaited results for the hosts objected of the study but it provided another way to study processes that occur in the calixarene threading.
3.6 EXPERIMENTAL SECTION

Absorption and ORD spectra were recorded on a JASCO J600 spectropolarimeter at room temperature, in acetonitrile, using 0.1 mm cells and concentrations of about $1 \times 10^{-3}$ M. During the measurement, the instrument was thoroughly purged with nitrogen.

Mass spectra were recorded with a Finnigan Mat 711 (EI, 80 eV, 8 kV), an Agilent 6210 ESI-TOF, and an Agilent QFT-7 FTICR mass spectrometer with Micromass Z-Spray ESI source. Flash chromatography was performed on Merck silica gel (60, 40-63 μm). All chemicals were reagent grade and were used without further purification. Anhydrous solvents were purchased from Aldrich. When necessary compounds were dried in vacuo over CaCl$_2$. Reaction temperatures were measured externally. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light, or by spraying with H$_2$SO$_4$-Ce(SO$_4$)$_2$ or phosphomolybdic acid.

1D NMR spectra were recorded on a Bruker Avance-400 spectrometer [400 (1H) and 100 MHz (13C)], Bruker Avance-300 spectrometer [300 (1H) and 75 MHz (13C)] and Bruker Avance-250 spectrometer [250 (1H) and 63 MHz (13C)]; chemical shifts are reported relative to the residual solvent peak (CHCl$_3$: δ 7.26, CDCl$_3$: δ 77.23; CD$_3$OH: δ 3.31,
CD$_3$OD: δ 49.0). Derivatives 3.17, 3.18, 3.19 and 3.35 were synthesized according to literature procedures.\textsuperscript{53-54}


Synthesis of derivative 35^+

(S)-α-Methylbenzylamine (0.010 mol) was added to benzaldehyde in CHCl₃ dry (2 mL) and the reaction mixture was stirred at room temperature for 2 h to give the imine intermediate in a quantitative yield.

The resulting imine (0.010 mol) was dissolved in dry MeOH (20 mL) under a nitrogen atmosphere and NaBH₄ (0.10 mol) was added at 0 °C and then the mixture was allowed to warm at room temperature. The solution was kept under stirring for 3 h. The solvent was removed under reduced pressure and the residue partitioned between AcOEt (30 mL) and an aqueous saturated solution of NaHCO₃ (30 mL). The organic layer was dried over MgSO₄ and the solvent was removed under
reduced pressure, to give derivative 3.38 as a yellow viscous liquid. The compound was used for the next step without further purification. The crude product (0.010 mol) was dissolved in Et₂O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 0.011 mol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with acetonitrile and dried under vacuum, to give derivative 3.39⁺ as a white solid.

Derivative 3.39⁺ was dissolved in dry MeOH (C= 0.2 M), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (1.1 eq) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives 3.14⁺TFPB⁻.

Derivative 3.14⁺TFPB⁻: (0.090 g, 0.22 mmol, 95%). ESI(+) MS: m/z = 212.15 (M+). ¹H NMR (400 MHz, CD₃OD, 298 K): δ 1.65 (d, J = 7, 3H, Hf), 3.85 and 4.05 (d, J = 13, 2H,
Hd), 4.36 (q, J = 6, 1H, He), 7.32-7.42 (overlapped, 6H, ArH, + 4H, ArH^TPFb), 7.56-7.59 (overlapped, 4H, ArH + 12 H, ArH^TPFb); ^13C NMR (100 MHz, CD$_3$OD, 298 K) δ 18.3, 49.3, 58.3, 117.1, 120.3, 123.0, 125.7, 127.2, 128.4, 128.6, 128.9, 129.2, 129.3, 129.4, 129.5, 130.9, 134.4, 136.0, 160.8, 161.2, 161.7, 162.2.
General Procedure for the Preparation of [2]Pseudorotaxane 3.15$^{+}$TFPB$^{-}$

Calixarene derivative 3.13 (1.9·10$^{-3}$ mmol) was dissolved in 0.5 mL of CDCl$_3$ (3.8·10$^{-3}$ M solution). Then, the TFPB- salt 3.14$^{+}$TFPB$^{-}$ was added (1.9·10$^{-3}$ mmol, 3.8·10$^{-3}$ M) and the mixture was stirred for 15 min. Then, the solution was transferred in a NMR tube for 1D NMR spectra acquisition.
$^1$H NMR determination of $K_{ass}$ values.

The association constant value was calculated by integration of free and complexed $^1$H NMR peaks of host and guest. Calixarene derivative 3.13 (1.9·$10^{-3}$ mmol) was dissolved in 0.5 mL of CDCl$_3$ (3.8·$10^{-3}$ M solution). Then, the TFPB- salt 3.14$^{+}$TFPB$^{-}$ was added (1.9·$10^{-3}$ mmol, 3.8·$10^{-3}$ M) and the mixture was stirred for 15 min. Then, the solution was transferred in a NMR tube for spectra acquisition.
Figure 3. $^{33}$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of equimolar solution (3.9 $10^{-3}$ M) of 3.13 and 3.14$^+$.TFPB$^-$. The association constant $K_{ass}$ value was calculated by integration of the complexed (▲) and free derivative 3.13 (●).
Procedure for ORD calculation

7.13 mg of derivative \(3.14^+\text{TFPB}^-\) were dissolved in 2mL of CHCl₃ and the ORD was measured for the guest. Then an equimolar solution of the derivative \(3.14^+\text{TFPB}^-\) (6.96 mg) and \(3.13\) (6.32 mg) in 2 mL of CHCl₃ was used for the measurement of the ORD for the complex.

\[
\alpha_{\text{tot}} = \alpha_{\text{comp}} + \alpha_{\text{guest}} \\
\alpha_{\text{comp}} = \alpha_{\text{tot}} - \alpha_{\text{guest}} \\
\alpha_{\text{guest}} = [\alpha]_{\text{guest}} \times C'_{\text{guest}} / 100 \quad (C' \text{ expressed in g/100mL})
\]

guest = 6.69 mg \quad C°_{\text{guest}} = 3.11 \times 10^{-3} \text{ M}
host = 6.32 mg \quad C°_{\text{host}} = 2.99 \times 10^{-3} \text{ M}

From the NMR spectrum the percentage of complexation is 42% at equilibrium, then:

\[
C'_{\text{comp}} = C°_{\text{host}} \times 0.42 = 1.256 \times 10^{-3} \text{ M} \\
C'_{\text{guest}} = C°_{\text{guest}} - C'_{\text{comp}} = 1.854 \times 10^{-3} \text{ M}
\]

In g/100mL:

\[
C'_{\text{comp}} = 1.256 \times 10^{-3} \text{ M} \times MW_{\text{comp}} / 10 = 0.268 \text{ g/100mL} \\
C'_{\text{guest}} = 1.854 \times 10^{-3} \text{ M} \times MW_{\text{guest}} / 10 = 0.199 \text{ g/100mL}
\]
<table>
<thead>
<tr>
<th>$\lambda$</th>
<th>$\alpha_{\text{tot}}$</th>
<th>$[\alpha]_{\text{guest}}$</th>
<th>$\alpha_{\text{guest}}$</th>
<th>$\alpha_{\text{comp}}$</th>
<th>$[\alpha]_{\text{comp}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>589</td>
<td>-0.030</td>
<td>0.8</td>
<td>0.0022</td>
<td>-0.0322</td>
<td>-2.0</td>
</tr>
<tr>
<td>546</td>
<td>-0.0686</td>
<td>-4.8</td>
<td>-0.0128</td>
<td>-0.0588</td>
<td>-20.8</td>
</tr>
<tr>
<td>435</td>
<td>-0.153</td>
<td>-14.3</td>
<td>-0.0384</td>
<td>-0.1146</td>
<td>-42.8</td>
</tr>
<tr>
<td>405</td>
<td>-0.195</td>
<td>-19.6</td>
<td>-0.0527</td>
<td>-0.1423</td>
<td>-53.1</td>
</tr>
</tbody>
</table>
Synthesis of derivatives (R)/(S)-3.22\textsuperscript{+}TFPB\textsuperscript{-}

(R)/(S)-\(\alpha\)-Methylbenzylamine (0.010 mol) was dissolved into acetone (40 mL) and the reaction mixture was stirred at reflux for 18 h. The reaction mixture was then cooled to room temperature and the excess of ketone was removed under reduced pressure.

The resulting imine (0.010 mol) was dissolved in dry MeOH (20 mL) under a nitrogen atmosphere and NaBH\(_4\) (0.10 mol) was added at 0 °C and then the mixture was allowed to warm at room temperature. The solution was kept under stirring for 3 h. The solvent was removed under reduced pressure and the residue partitioned between AcOEt (30 mL) and an aqueous saturated solution of NaHCO\(_3\) (30 mL). The organic layer was dried over MgSO\(_4\) and the solvent was removed under

124
reduced pressure, to give derivative \((R)/(S)-3.25\) as a yellow viscous liquid. The compound was used for the next step without further purification. The crude product (0.010 mol) was dissolved in Et\(_2\)O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 0.011 mol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with Exane/MeOH and dried under vacuum, to give derivative \((R)/(S)-3.26^+\text{Cl}^-\) as a white solid.

Derivative \((R)/(S)-3.26^+\text{Cl}^-\) was dissolved in dry MeOH (C= 0.2 M), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (1.1 eq) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives \((R)/(S)-3.22^+\text{TFPB}^-\)

Derivative \((S)-3.22^+\text{TFPB}^-\): (0.090 g, 0.22 mmol, 95%).

ESI(+) MS: m/z = 164.14 (M+). \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\),
298 K): δ 1.24 and 1.29 (d, J = 6, 6H, Ha-a’), 1.66 (d, J = 7, 3H, Hd), 4.32 (m, J = 6, 1H, Hb), 4.41 (q, J = 6, 1H, Hc), 7.22 (d, J = 7, 2H, He-e’), 7.46-7.53 (overlapped 3H, Hf-f’-g + 4H, ArH\textsuperscript{TFPB}), 7.69 (s, 8H, ArH\textsuperscript{TFPB}); \textsuperscript{13}C \textsuperscript{NMR} (100 MHz, CD\textsubscript{3}OD, 298 K) δ 19.1, 19.4, 20.0, 50.4, 57.8, 117.8, 120.7, 123.4, 126.1, 126.5, 128.6, 128.8, 129.0, 129.3, 129.6, 130.6, 131.4, 135.0, 161.1, 161.6, 162.1, 162.6.

**Derivative (R)-3.22\textsuperscript{+}TFPB\textsuperscript{-}:** (0.090 g, 0.22 mmol, 95%)

Synthesis of derivative (R)-3.22-d\textsubscript{6}\textsuperscript{+}TFPB\textsuperscript{-}

(R)-α-Methylbenzylamine (1.2g, 0.010 mol), Ti(IV)i-OPr\textsubscript{4} (8.5g, 0.030 mol) and deuterated acetone (0.030 mol) were mixed together and stirred for 2h.

126
The resulting mixture was diluted with MeOD (10 mL) under a nitrogen atmosphere and NaBH₄ (0.020 mol) was added at 0 °C and then the mixture was allowed to warm at room temperature. The solution was kept under stirring for 3 h.

The solvent was removed under reduced pressure and the residue partitioned between AcOEt (30 mL) and an aqueous saturated solution of NaHCO₃ (30 mL). The organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure, to give derivative (R)-3.25-d₆ as a yellow viscous liquid. The compound was used for the next step without further purification. The crude product (0.010 mol) was dissolved in Et₂O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 0.011 mol) was added dropwise. The mixture was kept under stirring for 1 h, until the formation of a white precipitate. The solid was collected by filtration, purified by crystallization with Exane/MeOH and dried under vacuum, to give derivative (R)-3.25-d₆⁺Cl⁻ as a white solid.

Derivative (R)-3.25-d₆⁺Cl⁻ was dissolved in dry MeOH (C= 0.2 M), then sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (1.1 eq) was added and the mixture was kept under stirring overnight in the dark. The solvent was removed and deionized water was added, obtaining a brown precipitate that was filtered off and dried under vacuum to give derivatives (R)-3.22-d₆⁺TFPB⁻.
Derivative \((\text{R})-3.22-\text{d}_6^+\text{TFPB}^-\): (0.090 g, 0.22 mmol, 95%).

ESI(+) MS: \(m/z = 170.18\) (M+).

\(^1\text{H NMR}\) (400 MHz, CD\(_3\)OD, 298 K): \(\delta 1.64\) (d, \(J = 7\), 3H, H\(_c\), ), 3.09 (s, 1H, H\(_a\)), 4.49 (q, \(J = 7\), 1H, H\(_b\)), 7.47-7.59 (overlapped, 5H, ArH, + 12H, ArH\(_{\text{TFPB}}\)).

\(^{13}\text{C NMR}\) (100 MHz, CD\(_3\)OD, 298 K) \(\delta 19.8, 51.0, 58.5, 118.2, 121.1, 123.8, 126.5, 126.9, 129.1, 129.3, 129.4, 129.7, 130.0, 131.1, 131.9, 134.0, 135.4, 161.5, 162.0, 162.5, 163.0\).

Synthesis of derivative 31

To a suspension of 20 g (21 mmol) of \(p\text{-}\text{tert-}\)butylcalix[6]arene in 1.1 L of dry CH\(_3\)CN were added 2.8 g (1 eq.) di K\(_2\)CO\(_3\).

The mixture was kept under stirring at reflux for 3h and
benzyl bromide (2.4 mL, 1eq) was added. The mixture was kept under stirring at reflux for 3 h again and then the crude product was treated with NH₃ (30 %) and HCl (1 N). The residue was extracted with CH₂Cl₂ and purified by silica gel chromatography (petroleum ether/ CH₂Cl₂). 10 g of derivative 3.18 were recovered with a yield of 45%.

**ESI(+) MS:** m/z =1063.70 (MH⁺). **¹HNMR** (250 MHz, CDCl₃, 298 K): δ 10.02 (s, OH, 2H), 9.82 (s, OH, 1H), 9.12 (s, OH, 2H), 7.78 (m, ArH₆Bn, 2H), 7.64 (m, ArH₆Bn, 2H), 7.45 (m, ArH₆Bn, 1H), 7.17-7.09 (overlapped, ArH₆calix, 12H), 5.21 (s, CH₂ArBn, 2H), 4.45 e 3.54 (AX, ArCH₂Ar, J=13.4 Hz, 4H), 4.27 e 3.56 (AX, ArCH₂Ar, J=14.0 Hz, 4H), 4.02 e 3.39 (AX, ArCH₂Ar, J=13.9 Hz, 4H), 1.29 -1.28 (s, C(CH₃)₃, 36H), 1.23 (s, C(CH₃)₃, 9H), 1.19 (s, C(CH₃)₃, 9H).

**¹³CNMR** (63 MHz, CDCl₃, 298 K): δ 149.6, 149.4, 148.4, 148.3, 146.7, 144.6, 143.7, 143.1, 136.6, 132.7, 129.3, 128.6, 127.6, 127.4, 127.2, 127.0, 126.9, 126.4, 126.2, 125.9, 125.6, 78.1, 34.5, 34.2, 34.1, 33.5, 32.9, 31.8 (2).

Synthesis of derivative 3.19
5.7 g of derivative 3.18 were mixed with Cs$_2$CO$_3$ (45 g, 30 eq.) in 0.30 L of acetone and kept under stirring at reflux for 1.5 h. The reaction mixture was cooled at room temperature and MeI 29 ml (100 eq) was added and the mixture kept at reflux for 12 h. The solvent was removed under reduced pressure. After usual work-up 5.2 g of derivative 3.19 were recovered (90% yield).

**ESI(+) MS**: m/z =1133.80 (MH$^+$).

**$^1$HNMR** (250 MHz, CDCl$_3$, 298 K): δ 7.55- 6.9 (overlapped, ArH$_{calix}$ e ArH$_{Bn}$, 16H), 4.88 (s, CH$_2$Ar$_{Bn}$, 2H), 4.46 e 3.68 (AX, ArCH$_2$Ar, J=14.7 Hz, 4H), 4.18 e 3.82 (AX, ArCH$_2$Ar, J=15.3 Hz, 4H), 4.05 e 3.54 (AX, ArCH$_2$Ar, J=14.2Hz, 4H), 3.20 (s, OCH$_3$, 6H), 2.78 (s, OCH$_3$, 3H), 2.53 (s, OCH$_3$, 6H), 1.25 -1.24 (s, C(CH$_3$)$_3$, 27H), 1.03 -0.97 (s, C(CH$_3$)$_3$, 18H).

**$^{13}$CNMR** (63 MHz, CDCl$_3$, 298 K): δ 154.5, 154.4, 153.7, 152.2, 146.0, 145.8 (2), 138.0, 134.0, 133.7, 133.5, 133.4, 133.3, 128.6, 127.9, 127.4, 127.0, 126.9, 125.3, 125.2, 124.7, 77.4, 74.5, 60.2, 60.1, 60.0, 34.3, 34.2, 31.6, 31.5, 31.4.

Synthesis of derivative 3.20
To a solution of 5.22 g of 3.19 in 0.10 L of CH₂Cl₂ Pd/C was added and the mixture kept under stirring at RT for 12 h. The crude product was filtered with celite and the solvent removed under reduced pressure. The derivative 3.20 was recovered with a 95% yield (5.00 g).

**ESI(+) MS:** m/z = 1043.71 (MH⁺).

**¹HNMR** (250 MHz, CDCl₃, 298 K): δ 7.38 (s, OH), 7.12-7.11 (overlapped, ArH_calix, 2H), 7.04-7.01 (overlapped, ArH_calix, 6H), 6.89 (s, ArH_calix, 2H), 6.80 (s, ArH_calix, 2H), 3.97 e 3.94 (broad, ArCH₂Ar, 8H), 3.82 (broad, ArCH₂Ar, 4H), 3.52 (s, OCH₃, 3H), 3.07 (s, OCH₃, 12H), 1.19 -1.17 (s, C(CH₃)₃, 45H), 1.12 (s, C(CH₃)₃, 9H).

**¹³CNMR** (63 MHz, CDCl₃, 298 K): δ 154.6, 154.2, 153.3, 149.7, 146.7, 146.0, 145.3, 142.2, 133.9, 133.6, 133.4, 132.8, 127.3, 126.7, 126.5, 126.3, 125.9, 125.6, 124.9, 77.5, 61.1, 60.8, 34.4, 34.2, 34.1, 31.6 (2), 31.4.

Synthesis of derivative (±)-3.16
NaH (0.072g, 3.0 mmol) was added to a solution of derivative 3.20 (0.31g, 0.30 mmol) in dry DMF (15 mL) and stirred for 1h. The mixture was allowed to cool at room and benzylbromide (0.17g, 1.00 mmol) was added. The resulting mixture was kept at 80°C for 12h under a nitrogen atmosphere, then the solvent was removed under reduced pressure and the mixture was partitioned between CH₂Cl₂ and H₂O. The organic layer was washed with 1N HCl (30 mL), brine (30 mL), and dried over Na₂SO₄. The crude product was purified by column chromatography (SiO₂; CH₂Cl₂) to give (0.16g, 0.14 mmol) of derivative (±)-3.16 as a white solid.

**ESI(+) MS:** m/z =1147.81 (MH⁺), **¹H NMR** (400 MHz, CDCl₃, 298 K): δ 7.53-6.75 (overlapped, 17H, ArH_calix + ArH_Bn), 5.06 (q, J = 8 , 1H, CH_Bn), 4.64 and 3.63 (AX, J = 13.4 Hz, 2H, ArCH₂Ar), 4.27 and 3.69 (AX, J = 14 Hz, ArCH₂Ar, 2H), 4.25 and 3.59 (AX, J = 14 Hz, ArCH₂Ar, 2H), 4.16 and 3.00 (AX, J = 13.6 Hz, ArCH₂Ar, 2H), 4.12 and 3.84 (AX, J = 13.9 Hz, ArCH₂Ar, 2H), 4.12 and 3.77 (AX, J = 13.9 Hz, ArCH₂Ar, 2H), 3.36 (s, overlapped, OCH₃, 6H), 2.75 (s, OCH₃, 3H), 2.52 (s, OCH₃, 3H), 2.35 (s, OCH₃, 3H), 1.68
(d, J = 8, 3H, CH₃Br), 1.34 (s, C(CH₃)₃, 9H), 1.29 and 1.28 (s, overlapped, C(CH₃)₃, 18H), 1.00 and 0.98 (s, overlapped C(CH₃)₃, 18H), 0.92 (s, C(CH₃)₃, 9H)

$^{13}$C NMR (100 MHz, CDCl₃, 298 K): δ 154.5, 154.4, 154.4, 153.6, 151.3, 145.8, 145.7, 145.4, 143.3, 134.2, 134.0, 133.8, 133.6, 133.6, 133.5, 133.4, 133.4, 128.4, 127.8, 127.1, 126.7, 124.9, 124.3, 124.1, 81.0, 77.4, 60.3, 60.2, 60.0, 59.9, 34.3, 34.2, 34.2.

Synthesis of derivative 3.27

NaH (0.024g, 1.0 mmol) was added to a solution of derivative 3.20 (0.10g, 0.10 mmol) in dry DMF (15 mL) and stirred for 1h.

The mixture was allowed to cool at room and (S)-(+)–1-iodo-2-methylbutane (0.99g 0.5 mmol) was added. The resulting mixture was kept at 80°C for 12h under a nitrogen atmosphere, then the solvent was removed under reduced pressure and the mixture was partitioned between CH₂Cl₂ and H₂O.
The organic layer was washed with 1N HCl (30 mL), brine (30 mL), and dried over Na₂SO₄. The crude product was purified by column chromatography (SiO₂; CH₂Cl₂) to give derivative 3.27 as a white solid (0.87g, 0.078 mmol, 78%)

**ESI(+) MS:** m/z =1130.77 [M+NH₄]+, **¹H NMR** (300 MHz, CDCl₃, 298 K): δ 7.27-7.26 (overlapped, ArH, 2H), 7.13-7.11 (overlapped, ArH, 4H), 6.86 (br s, ArH, 2H), 6.84 (br s, ArH, 2H), 6.76 (br s, ArH, 2H), 6.76 (br s, ArH, 2H), 3.94-3.70 (br, ArCH₂Ar + OCH₂CH(CH₃)CH₂CH₂, 14H), 3.28 (s, OCH₃, 6H), 2.71 (s, OCH₃, 3H) 2.47 (s, OCH₃, 6H), 1.95 (m, OCH₂CH(CH₃)CH₂CH₂, 1H), 1.69 (m, OCH₂CH(CH₃)CH₂CH₂, 2H), 1.30 (s, overlapped, C(CH₃)₃, 18H), 1.27 (s, overlapped, C(CH₃)₃, 9H), 1.11 (br, d, J = 9, OCH₂CH(CH₃)CH₂CH₂, 1H), 1.00 (s, overlapped, C(CH₃)₃, 18H), 0.98 (br, m, OCH₂CH(CH₃)CH₂CH₂, 3H), 0.91 (s, C(CH₃)₃, 9H); **¹³C NMR** (75 MHz, CDCl₃, 298 K): δ 154.4 (2), 153.6, 152.2, 145.8, 145.6, 133.9, 133.7, 133.6 (2), 133.5, 133.3, 127.6, 127.0, 125.0, 124.2, 60.2, 60.1, 60.0, 36.2, 34.3, 34.2, 32.1, 31.7, 31.4(2), 30.6, 30.3, 29.9, 29.5, 26.4, 22.9, 16.9, 14.3, 11.7.
Synthesis of derivative 3.33

(R)-Myrtenol (3.0 g, 0.020 mol) was dissolved in DMF (80 mL) and I₂ (22g, 0.087 mol) and PPh₃ (20g, 0.079 mol) were added and the mixture was kept at 40°C for 2h. DMF was removed under reduced pressure and the mixture partitioned between CH₂Cl₂ and H₂O. the organic layer was washed with brine and dried over Na₂SO₄. The crud product was used directly for the next step without any purification (0.52g, 2.0 mmol 10%)

Synthesis of derivative 41
NaH (0.05 g, 2.0 mmol) was added to a solution of derivative 3.20 (0.20 g, 0.20 mmol) in dry DMF (10 mL) and stirred for 1 h.
The mixture was allowed to cool at room and derivative 3.33 (0.27 g, 1.0 mmol) was added. The resulting mixture was kept at 80°C for 12 h under a nitrogen atmosphere, then the solvent was removed under reduced pressure and the mixture was partitioned between CH₂Cl₂ and H₂O.
The organic layer was washed with 1 N HCl (30 mL), brine (30 mL), and dried over Na₂SO₄. The crude product was purified by column chromatography (SiO₂; CH₂Cl₂) to give derivative 3.28 as a white solid (0.16 g, 0.14 mmol, 75%).

**ESI(+) MS:** m/z = 1194.75 [M+NH₄]⁺, ¹H NMR (300 MHz, TCDE, 353 K): δ 7.39 (br s, ArH, 2H), 7.28-7.26 (overlapped, ArH, 4H), 7.02 (s, ArH, 2H), 6.98 (s, ArH, 2H), 6.94 (s, ArH, 2H), 5.90 (br s, Hₐ, 1H), 4.59 and 3.61 (AX, J = 14 Hz, ArCH₂Ar, 2H), 4.40 (s OCH₂, 2H), 4.39 and 3.77 (AX, J = 15 Hz, ArCH₂Ar, 2H), 4.27 and 3.87 (AX, J = 15 Hz, ArCH₂Ar, 2H), 3.49 (s, OCH₃, 3H), 3.47 (s, OCH₃, 3H), 2.87 (s, OCH₃, 3H), 2.73 (s, OCH₃, 3H), 2.68 (s, OCH₃, 3H), 2.61 (br m, Hₐ, 2H), 1.98 (br s, H₂O)
1H), 2.52 (overlapped Hc+He, 3H), 2.30 (br s, Hf, 1H), 1.47 (s, C(CH₃)₃, 9H), 1.46 (s, C(CH₃)₃, 9H), 1.42 (s, C(CH₃)₃, 9H), 1.16 (s, overlapped, C(CH₃)₃, 18H), 1.08 (s, C(CH₃)₃, 9H), 1.01 (br s Hg, 6H). ¹H NMR (75 MHz, CDCl₃, 298 K) 154.9, 154.2, 152.8, 146.3, 146.2, 145.4, 134.5, 134.4, 134.2, 134.1, 133.8 (2), 127.5, 125.6, 124.8, 120.0, 77.9, 75.9, 60.5, 44.3, 41.6, 38.9, 35.3, 34.9, 34.7, 32.4, 32.2, 31.2, 32.0, 31.9, 31.1, 30.9, 27.0, 25.9, 21.9.

Synthesis of derivative 3.29

An oven-dried flask was charged with derivative 3.20 (0.10g, 0.10 mmol), DMAP (3.7mg, 0.030 mmol), and triethylamine (1.0 mL) in DMF (5.0 mL). Finally, (S)-Mosher’s acid chloride (47 mg, 0.20 mmol) was added and the reaction mixture was stirred at 70 °C for 12 h. The reaction mixture was cooled to room temperature followed by standard workup procedure using dichloromethane for extraction. The crude product was purified by silica gel column chromatography (CH₂Cl₂: CH₃OH, 98:2, v/v) to yield 0.12 g (0.12g, 0.093
mmol, yield: 93%) of derivative $3.29$ as white solids.

**ESI(+) MS:** m/z =1259.74 [M+H]$^+$, $^1$H NMR (300 MHz, CDCl$_3$, 353 K): \(\delta\) 7.93 (br m, ArH$_{Mosher(\text{para})}$, 1H), 7.58 (overlapped ArH$_{Mosher(\text{meta})}$ + ArH$_{Mosher(\text{orto})}$, 4H), 7.35 (br m, ArH$_{\text{calix}}$, 2H), 7.30-7.28 (overlapped, ArH$_{\text{calix}}$, 6H), 6.99-6.94 (overlapped, ArH$_{\text{calix}}$, 6H), 4.44 and 3.73 (AX, \(J=10\) Hz, ArCH$_2$Ar, 2H), 4.36 and 3.75 (AX, \(J=12\) Hz, ArCH$_2$Ar, 2H), 4.06 and 3.46 (AX, \(J=15\) Hz, ArCH$_2$Ar, 2H), 4.33-4.22 and 3.82-3.49 (overlapped ArCH$_2$Ar, 6H), 3.89 (s, OCH$_3$-Mosher, 3H), 3.63 (s, OCH$_3$, 3H) 3.51 (s, OCH$_3$, 3H), 2.80 (s, OCH$_3$, 3H), 2.67 (s, OCH$_3$, 3H), 2.57 (s, OCH$_3$, 3H), 1.50 (s, C(CH$_3$)$_3$, 9H), 1.46 (s, C(CH$_3$)$_3$, 9H), 1.44 (s, C(CH$_3$)$_3$, 9H), 1.41 (s, C(CH$_3$)$_3$, 9H), 1.13 (s, C(CH$_3$)$_3$, 9H), 1.04 (s, C(CH$_3$)$_3$, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$, 353 K): \(\delta\) 165.9, 155.3, 155.1, 154.2, 149.5, 146.6, 146.4, 144.1, 134.2, 134.0, 133.9, 133.6, 133.5, 132.8, 132.3, 130.6, 129.3, 128.7, 128.3, 128.2, 128.0, 127.8, 127.7, 125.6, 125.3, 125.2, 125.1, 60.7, 60.6, 56.5, 34.8, 34.7, 32.2, 32.0, 31.8, 31.3, 31.1.

Synthesis of derivative $3.35$
A mixture of \( p \)-\textit{tert}-butylcalix[6]arene (1.0 g, 1.0 mmol), K\textsubscript{2}CO\textsubscript{3} (4.1 mmol) and MeI (320 \( \mu \)L, 5.1 mmol) in dry acetone (70 mL) was heated at 70\(^\circ\)C in an autoclave for 20 h. The mixture was poured into 10% HCl (100 mL), and the aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (2 x 50 mL), washed with brine (2 x 50 mL), dried over Na\textsubscript{2}SO\textsubscript{4} and evaporated to dryness.

The crude product was purified by column chromatography (SiO\textsubscript{2}; Hexane/THF, 9:1) to give derivative \textbf{3.35} as a white solid (0.39 g, 35%).

\textbf{ESI(+) MS:} m/z = 1028.41 (M\textsuperscript{+}). \textbf{\( ^1\)HNMR (300 MHz, CDCl\textsubscript{3}, 298 K):} \( \delta \) 8.03 (s, ArH, 4H), 7.06 (m, ArH, 8H), 6.70 (s, ArH, 4H), 3.97 (br s, ArCH\textsubscript{2}Ar, 4H), 3.87 (br s, ArCH\textsubscript{2}Ar, 8H), 3.14 (s, OCH\textsubscript{3}, 6H), 1.18 (s, C(CH\textsubscript{3})\textsubscript{3}, 36H), 0.94 (s, C(CH\textsubscript{3})\textsubscript{3}, 18H);

\textbf{\( ^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}, 298 K)} \( \delta \) 153.4, 149.6, 146.8, 142.0, 134.2, 132.4, 127.5, 126.7, 126.3, 124.1, 61.7, 34.4, 34.0, 31.6, 31.5, 31.2, 31.0.

\textbf{Synthesis of derivative 3.30}

![Chemical diagram of 3.30]
NaH (0.024 g, 1.0 mmol) was added to a solution of derivative 3.35 (0.10 g, 0.10 mmol) in dry DMF (15 mL) and stirred for 1h.

The mixture was allowed to cool at room and (S)-(+)−1-iodo-2-methylbutane (0.20 g, 1.0 mmol) was added. The resulting mixture was kept at 80°C for 12 h under a nitrogen atmosphere, then the solvent was removed under reduced pressure and the mixture was partitioned between CH₂Cl₂ and H₂O. The organic layer was washed with 1N HCl (30 mL), brine (30 mL), and dried over Na₂SO₄. The crude product was purified by column chromatography (SiO₂; CH₂Cl₂) to give derivative 3.30 as a white solid (0.11g, 0.096 mmol, 96%).

**ESI(+) MS**: m/z =1286.88 [M+NH₄]⁺. ¹H NMR (400 MHz, CDCl₃, 298 K): δ 7.24 (br s, ArH, 4H), 6.92 (br s, ArH, 8H), 4.23 and 3.60 (br AX, ArCH₂Ar, 6H), 3.87 (br AX, ArCH₂Ar, 6H), 3.67 (br m, OCH₂CH(CH₃)CH₂CH₃, 4H), 2.72 (br s, OCH₃, 12H), 1.97 and 1.71 (m, OCH₂CH(CH₃)CH₂CH₃, 2H), 1.21 (s, C(CH₃)₃, 36H), 1.11 (d, J = 7, OCH₂CH(CH₃)CH₂CH₃, 6H), 1.02 (s, C(CH₃)₃, 18H), 0.98 (t, J = 7, OCH₂CH(CH₃)CH₂CH₃, 6H); ¹³C NMR (75 MHz, CDCl₃, 298 K) δ 155.0, 152.9, 146.4, 146.3, 134.8, 134.4, 134.3, 127.6, 126.6, 125.4, 78.7, 60.5, 36.9, 35.0, 34.9, 32.3, 32.1, 31.5, 31.3, 27.2, 17.6, 12.4.
General procedure for MS experiments (isotopic effect)

Sample preparation
Calixarene derivatives (1.9·10⁻³ mmol) were dissolved in 0.5 mL of CHCl₃ (3.8·10⁻³ M solution). Then, the appropriate TFPB⁻ salts (R)-3.22⁺TFPB⁻ (3.8·10⁻³ mmol, 7.6·10⁻³ M) and (R)-3.22-d₆⁺TFPB⁻ (3.8·10⁻³ mmol, 7.6·10⁻³ M) were added and the mixture was stirred for 15 min. Then, the solution was diluted at a concentration of 300 μM with CH₂Cl₂ before the MS injection.

MS conditions
Sample concentration 300 μM; flow rate 2-4 μL/min; sample cone: 25 V; HV 2500 V; source temperature and temperature of desolvation gas were kept constant at 40 °C, no nebulizer gas was used for the experiments.

Iᵣ/Iᵣ-dₙ evaluation
The deuterated axle is a mixture of partially deuterated compounds so Iᵣ-dₙ can be evaluated as the sum of the intensity of each peak related to the deuterated compounds. This operation is valid assuming that no significant differences and isotopic effect occur between the partially deuterated compounds (d₄ and d₅) and the fully deuterated one (d₆). The intensity of the non-deuterated compounds, is than evaluated as the sum of each peak related to its distribution. The corresponding peak intensities are pasted from the
Omega software controlling the mass spectrometer to the spreadsheet, which calculates the intensity of each peak.

a) 1:2:2 mixture of 3.27, (R)-3.22\textsuperscript{+}TFPB\textsuperscript{-} and (R)-3.22-d\textsubscript{6}\textsuperscript{+}TFPB\textsuperscript{-}
b) 1:2:2 mixture of 3.28, (R)-3.22\textsuperscript{+}TFPB\textsuperscript{-} and (R)-3.22-d\textsubscript{6}\textsuperscript{+}TFPB\textsuperscript{-}

<table>
<thead>
<tr>
<th>Peak</th>
<th>m/z</th>
<th>Rel. Abund.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1340,9037</td>
<td>100,00</td>
</tr>
<tr>
<td>A+1</td>
<td>1341,9070</td>
<td>94,80</td>
</tr>
<tr>
<td>A+2</td>
<td>1342,9116</td>
<td>40,48</td>
</tr>
<tr>
<td>A+3</td>
<td>1343,9166</td>
<td>9,94</td>
</tr>
<tr>
<td>A+4</td>
<td>1344,9262</td>
<td>3,17</td>
</tr>
</tbody>
</table>

\[ I_\text{R} = 248,39 \]

<table>
<thead>
<tr>
<th>Peak</th>
<th>m/z</th>
<th>Rel. Abund.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(D5)</td>
<td>1345,9369</td>
<td>27,75</td>
</tr>
<tr>
<td>A(D5)+1 + A(D6)</td>
<td>1346,9401</td>
<td>87,92</td>
</tr>
<tr>
<td>A(D5)+2 + A(D6)+1</td>
<td>1347,9440</td>
<td>66,63</td>
</tr>
<tr>
<td>A(D5)+3 + A(D6)+2</td>
<td>1348,9493</td>
<td>25,81</td>
</tr>
<tr>
<td>A(D5)+4 + A(D6)+3</td>
<td>1349,9548</td>
<td>6,48</td>
</tr>
<tr>
<td>A(D6)+4</td>
<td>1350,9563</td>
<td>0,00</td>
</tr>
</tbody>
</table>

\[ I_{\text{Rdn}} = 214,59 \]

\[ I_\text{R}/I_{\text{Rdn}} = 1,16 \]
c) 1:2:2 mixture of 3.29, (R)-3.22⁺TFPB⁻ and (R)-3.22⁻d₆⁺TFPB⁻
(R)-3.22′TFPB−

(R)-3.22-d6'TFPB−
d) 1:2:2 mixture of \(3.30\), (R)-\(3.22^{+}\text{TFPB}^-\) and (R)-\(3.22-\text{d}_6^{+}\text{TFPB}^-\)

<table>
<thead>
<tr>
<th>Peak</th>
<th>(m/z)</th>
<th>Rel. Abund.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1340,9037</td>
<td>98,35</td>
</tr>
<tr>
<td>A+1</td>
<td>1341,9070</td>
<td>100,00</td>
</tr>
<tr>
<td>A+2</td>
<td>1342,9116</td>
<td>46,13</td>
</tr>
<tr>
<td>A+3</td>
<td>1343,9166</td>
<td>14,17</td>
</tr>
<tr>
<td>A+4</td>
<td>1344,9262</td>
<td>4,87</td>
</tr>
<tr>
<td>(I_\text{R})</td>
<td></td>
<td>263,52</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak</th>
<th>(m/z)</th>
<th>Rel. Abund.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(D5)</td>
<td>1345,9369</td>
<td>30,36</td>
</tr>
<tr>
<td>A (D5)+1 + A (D6)</td>
<td>1346,9401</td>
<td>93,33</td>
</tr>
<tr>
<td>A (D5)+2 + A (D6)+1</td>
<td>1347,9440</td>
<td>75,67</td>
</tr>
<tr>
<td>A (D5)+3 + A (D6)+2</td>
<td>1348,9493</td>
<td>33,01</td>
</tr>
<tr>
<td>A (D5)+4 + A (D6)+3</td>
<td>1349,9548</td>
<td>9,60</td>
</tr>
<tr>
<td>A (D6)+4</td>
<td>1350,9563</td>
<td>2,14</td>
</tr>
<tr>
<td>(I_\text{Rdn})</td>
<td></td>
<td>244,11</td>
</tr>
<tr>
<td>(I_\text{R}/I_\text{Rdn})</td>
<td></td>
<td>1,08</td>
</tr>
</tbody>
</table>

(S,S) 3.30  
(R)-3.22^{+}\text{TFPB}^-  
(R)-3.22-\text{d}_6^{+}\text{TFPB}^-
General procedure for MS experiments (chiral recognition effect)

Calixarene derivatives ($1.9 \cdot 10^{-3}$ mmol) were dissolved in 0.5 mL of CHCl$_3$ ($3.8 \cdot 10^{-3}$ M solution). Then, the appropriate TFPB$^-$ salts (S)-35$^+$ ($3.8 \cdot 10^{-3}$ mmol, $7.6 \cdot 10^{-3}$ M) and (R)-35-$d_6^+$ ($3.8 \cdot 10^{-3}$ mmol, $7.6 \cdot 10^{-3}$ M) were added and the mixture was stirred for 15 min. Then, the solution was diluted at the desired concentration with CH$_2$Cl$_2$ just before the MS analisis. **MS condition**: sample concentration 300 $\mu$M; flow rate 2-4 $\mu$L/min; sample cone: 25 V; HV 2500 V; source temperature and temperature of desolvation gas were kept constant at 40 $^\circ$C, no nebulizer gas was used for the experiments.

$I_S/I_{R-d_n}$ evaluation

The deuterated axle is a mixture of partially deuterated compounds so $I_{Sd_n}$ can be evaluated as the sum of the intensity of each peak related to the deuterated compounds.

This operation is valid assuming that no significant differences and isotopoic effect occur between the partially deuterated compounds ($d_4$ and $d_5$) and the fully deuterated one ($d_6$). The intensity of the non-deuterated compounds, is than evaluated as the sum of each peak related to its distribution.

The corresponding peak intensities are pasted from the Omega software controlling the mass spectrometer to the spreadsheet, which calculates the intensity of each peak and
the $I_S/I_{R-d_n}$.

a) 1:2:2 mixture of 3.27, (S)-3.22$^{+}$TFPB$^-$ and (R)-3.22-d$_6$$^{+}$TFPB$^-$

![Diagram of molecules]
b) 1:2:2 mixture of 3.28, (S)-3.22\textsuperscript{+}TFPB\textsuperscript{−} and (R)-3.22-d\textsubscript{6}\textsuperscript{+}TFPB\textsuperscript{−}

<table>
<thead>
<tr>
<th>Non-Deuterated Complex</th>
<th>Peak</th>
<th>m/z</th>
<th>Rel. Abund.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1340,9037</td>
<td>100,00</td>
<td></td>
</tr>
<tr>
<td>A+1</td>
<td>1341,9070</td>
<td>95,79</td>
<td></td>
</tr>
<tr>
<td>A+2</td>
<td>1342,9116</td>
<td>41,84</td>
<td></td>
</tr>
<tr>
<td>A+3</td>
<td>1343,9166</td>
<td>12,09</td>
<td></td>
</tr>
<tr>
<td>A+4</td>
<td>1344,9262</td>
<td>4,08</td>
<td></td>
</tr>
</tbody>
</table>

\[ I_{R} = 253,80 \]

<table>
<thead>
<tr>
<th>Deuterated Complex</th>
<th>Peak</th>
<th>m/z</th>
<th>Rel. Abund.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(D5)</td>
<td>1345,9369</td>
<td>29,87</td>
<td></td>
</tr>
<tr>
<td>A (D5)+1 + A (D6)</td>
<td>1346,9401</td>
<td>89,38</td>
<td></td>
</tr>
<tr>
<td>A (D5)+2 + A (D6)+1</td>
<td>1347,9440</td>
<td>68,91</td>
<td></td>
</tr>
<tr>
<td>A (D5)+3 + A (D6)+2</td>
<td>1348,9493</td>
<td>28,88</td>
<td></td>
</tr>
<tr>
<td>A (D5)+4 + A (D6)+3</td>
<td>1349,9548</td>
<td>6,99</td>
<td></td>
</tr>
<tr>
<td>A (D6)+4</td>
<td>1350,9563</td>
<td>1,30</td>
<td></td>
</tr>
</tbody>
</table>

\[ I_{R_{Dn}} = 225,33 \]

\[ I_{R_{Dn}/R_{Dn}} = 1,13 \]
c) 1:2:2 mixture of $\text{3.29}$, (S)-$\text{3.22}^+\text{TFPB}^-$ and (R)-$\text{3.22}-d_6^+\text{TFPB}^-$
d) Derivative 42 mixed with (S)-35\(^+\) and (R)-35-\(d_6\)^+.

\[
\begin{array}{|c|c|c|}
\hline
\text{Peak} & m/z & \text{Rel. Abund.} \\
\hline
A & 1340,9037 & 96,36 \\
A+1 & 1341,9070 & 96,98 \\
A+2 & 1342,9116 & 45,11 \\
A+3 & 1343,9166 & 14,18 \\
A+4 & 1344,9262 & 5,38 \\
\hline
I_R = 258,01
\end{array}
\]

\[
\begin{array}{|c|c|c|}
\hline
\text{Peak} & m/z & \text{Rel. Abund.} \\
\hline
A(D5) & 1345,9369 & 31,98 \\
A (D5)+1 + A (D6) & 1346,9401 & 100,00 \\
A (D5)+2 + A (D6)+1 & 1347,9440 & 81,21 \\
A (D5)+3 + A (D6)+2 & 1348,9493 & 35,00 \\
A (D5)+4 + A (D6)+3 & 1349,9548 & 10,44 \\
A (D6)+4 & 1350,9563 & 2,53 \\
\hline
I_{rdn} = 261,16
\end{array}
\]

\[
I_R/I_{rdn} = 0,99
\]
CHAPTER IV
SYNTHESIS OF NEW CALIXARENES AND RESORCINARENES BASED CHIRAL HOSTS

4.1 CHIRALITY AND INHERENT CHIRALITY IN CALIXARENES AND RESORCINARENES

In the previous chapter we showed the importance of the chiral recognition and the use of some chiral calix[6]arenes hosts towards the ammonium guests recognition. These hosts were synthesised by the functionalization of the scaffold using chiral substituent.

Contrary, this chapter reports the pursuit of new potential chiral hosts by means of the study and the synthesis of some chiral macrocycles using achiral substituents.

The structure and properties of calixarenes and resorcinarenes can be easily varied functionalizing the hydroxyl groups at the lower rim or introducing substituents on the aromatic scaffold. This property makes them a versatile building block with regard to structure and function.

The addition of chirality to calixarenes and resorcinarenes is an exciting enhancement of their potential as ligands for chiral catalysis and enantioselective processes.
4.1.1 Inherently chiral calixarenes

The introduction of “inherent chirality” into calixarene scaffolds by asymmetric functionalization with achiral substituents is more challenging and interesting. The term “inherent chirality” was defined for the first time by Böhmer\(^{55}\) for chiral calixarenes and then it was extended to all that compounds that do not match with other type of chirality. The inherent chirality definition was reformulated by Mandolini and Schiaffino and subsequently revised by Szumna that now states: “inherent chirality arises from the introduction of a curvature in an ideal planar structure that is devoid of perpendicular symmetry planes in its bidimensional representation”.

![Inherent chirality in calix[4]arene.](image)

**Figure 4.1** Inherent chirality in calix[4]arene.

To designate inherent chirality two type of notations, (cR)/(cS) and (P)/(M), were suggested.

In the case of calix[4]arenes, once the carbons of the bridges

are labelled as $a$, $b$, $c$, and $d$ according to standard stereochemistry rules, an ideal observer standing on the concave side of the calixarene will see the three highest priority atoms $a$, $b$, and $c$.

![Diagram of calixarene structure]

**Figure 4.2** Mandolini’s assignment rules.

The $(cR)$ and $(cS)$ description indicates respectively a clockwise and counterclockwise priority of their sequence while $c$ stands for curvature.

Similarly, the $(P)/(M)$ one does it as $P$ or $M$, respectively. The latter notation was promoted by Szumna especially for concave molecules like calixarenes and resorcinarenes.

There are mainly two methods to synthesise inherently chiral calixarenes: the condensation of different phenolic units\(^{56}\) (**Figure 4.3**) and the asymmetric functionalization of the calixarene skeleton such as $O$-alkylation or esterification of

phenolic OH on the lower rim and and para- and meta-substitution of phenolic units on the upper rim.\textsuperscript{57}

The condensation method was used only for calix[4]arene because the process is tedious and yields are low.

\textbf{Figure 4.3} Calix[4]arenes obtained by condensation of different phenolic units

On the other hand Reinhoudt synthesized \textit{meta}-substituted inherently chiral calix[4]arenes from \textit{para}-acetamido substituted calix[4]arenes (\textbf{Figure 4.4})

In particular there is a preference of the *meta*-substitution over the *para*-substitution because of the activation of the *meta*-position by the *para*-acetamido group.\textsuperscript{58}

A series of inherently chiral calix[4]arenes, asymmetrically functionalized on the phenolic *meta*-position, were prepared by Neri et al. through the so called “*p*-bromodienone route”.\textsuperscript{59}

4.1.2 *Meta*-functionalization of Calixarenes: the “*p*-bromodienone route”

The first example of calixarene *p*-bromodienone derivatives \textbf{4.4a} and \textbf{4.4b} was reported by Neri et al. by treatment of the tripropoxy-\textit{p-tert}-butylcalix[4]arene \textbf{4.3} with trimethylphenylammonium tribromide and a saturated

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure4.png}
\caption{\textbf{4.2} *meta*-substituted inherently chiral calix[4]arenes}
\end{figure}


solution of NaHCO₃ (Scheme 1).⁶⁰

![Scheme 4.1 Synthesis of p-bromoderivatives 4.4a-b](image)

Successively the same authors reported the Ag⁺-mediated nucleophilic substitution of bromine in 4.4a and 4.4b with alcohols, the successive re-aromatization gives p-alkoxy-calixarene derivatives (Scheme 4.2).

In this procedure, the mixture of p-bromodienone-tripropoxycalix[4]arene 4.4a and 4.4b was treated with an alcoholic solution of AgClO₄ for 2 h at 0 °C. Soon after the addition, a yellow precipitate of AgBr was formed⁶¹, and p-alkoxycalix[4]arene derivatives 4.5a-k were obtained in good yields.⁶²

The formation of the p-substitued adduct suggested the initial formation of the aryloxenium carbocation I, which reacted with methanol to give the intermediate II (Scheme 4.2). The subsequent de-tert-butylation and rearomatization yield to p-

---

alkoxycalix[4]arene 4.5a-k.\textsuperscript{63}

\begin{align*}
\text{Scheme 4.2 } p\text{-bromodienone route with different nucleophiles}
\end{align*}

An important aspect in the \( p\)-bromodienone route was observed using activated aromatic rings as nucleophiles.\textsuperscript{59}
The reaction with activated aromatic nucleophiles such as resorcinol, pyrrole and 2,6-dimethylphenol led mainly to asymmetrical C–C \textit{meta}-substituted derivatives 4.9a-e through a dienone–phenol rearrangement of the intermediate

dienone derivative (Scheme 4.3)\textsuperscript{64}.

The meta-substituted derivatives represented typical examples of inherently chiral calixarenes, as they had a three-dimensional asymmetrical structure.

Thus the p-bromodienone route provided a good way to obtain inherently chiral calix[4]arenes.

4.1.3 Chiral and inherently chiral resorcinarenes

The asymmetric O-alkylation strategy that is used to synthesise inherently chiral calix[4]arene can be also applied to resorcin[4]arenes. The inherent chirality can arise from a $C_1$, $C_2$ or $C_4$ symmetry. $C_4$-symmetric adducts result as preferential product, due to the stabilisation of their structures by the maximum number of intramolecular hydrogen bonds.

Resorcinarene A (Figure 4.5), with four methoxy groups, was obtained exploiting an acid catalysed condensation between 3-methoxyphenol and aldehydes (80% yield).\textsuperscript{65}

The Mannich reaction of a resorcinarene with primary amines and formaldehyde was used for preparing tetrabenzoxazines B\textsuperscript{66} and then a Mannich reaction modification was used for the synthesis of the inherently chiral resorcinarenes C and D.\textsuperscript{67}


4.2 AIMS

Considering the importance of the chiral recognition in supramolecular chemistry, another aim of this PhD project has been the study and the synthesis of new macrocycles as powerful host toward ammonium guests recognition. Therefore the goal was to explore the p-bromodienone route on the calix[6]arene scaffold to synthesize chiral and not chiral hosts.

The exploration of new hosts had also the goal of studying inherently chiral resorcinarenes by means of their synthesis separation and chirality identification.
4.2 RESULTS AND DISCUSSION

4.2.1 Synthesis of calixarene hosts via $p$-bromodienone route

With the intent in mind to synthesise new inherently chiral calix[6]arene hosts it was decided to verify the feasibility of the $p$-bromodienone route on partially $O$-alkylated calix[6]arene derivatives. It was decided to perform the oxidation of the phenol ring of the mono-ol derivative, synthesised as described in the previous section. Thus the mono-ol was treated (in $\text{CH}_2\text{Cl}_2$ at 25 °C) with trimethylphenylammonium tribromide and a saturated solution of $\text{NaHCO}_3$ producing in quantitative yield the first example of calix[6]arene $p$-bromodienone derivative.\(^{68}\)

![Scheme 4. 4. Synthesis of derivative 4.11](image)

The structure of 4.11 was assigned by means of spectral


165
The $^1$H NMR spectrum of $p$-bromodienone derivative 4.11 in CDCl$_3$ at 298 K (600 MHz) showed signals in agreement with the $p$-bromodienone derivative’s structure. There was the presence of 3 singlets in a 2:2:1 ratio at 1.23, 1.16, and 1.11 ppm, respectively, due to $t$-butyl groups on anisole rings; a broad singlet at 0.86 ppm due to $t$-butyl group on the oxidized $p$-bromodienone ring and a broad singlet at 6.60 ppm due to the dienone H atoms.

![Figure 4.6. $^1$H NMR spectrum of derivative 4.11 (600 MHz, CDCl$_3$, 298 K).](image)

While the $^{13}$C NMR spectrum revealed the C=O and C–Br signals at 183.7 and 71.3 ppm, respectively.
In the synthesis of calix[4]arene $p$-bromodienone they obtained two stereoisomers, namely, the exo and endo ones, referring to the relative orientation of the Br atom with respect to the calix[4]arene cavity. An analogous stereoisomerism should be expected for calix[6]arene $p$-bromodienone, but its rapid cone-to-cone inversion (even with respect to the NMR timescale) led to the mutual interconversion between exo- and endo-4.11 stereoisomers.
Figure 4. 8. Cone-to-cone inversion of derivative 4.11

The $^1$H NMR showed a temperature dependent behaviour due to the through-the-annulus rotation of the anisole and $p$-bromodienone rings.

The lowering of the temperature at 233 K gave a very complicated $^1$H NMR spectrum corresponding to the presence of the two exo-/endo stereoisomers in different conformations.

At this point the $p$-bromodienone route was preliminarily tested with small and simple $O$-nucleophiles such as methanol and benzyl alcohol.
The derivative 4.11 was treated with a methanolic solution of AgClO₄ at 0°C to give \( p \)-methoxycalix[6]arene 4.12a in 20% yield, after usual workup.

The ESI(+) mass spectrum confirmed the molecular formula, while the \( C_5 \) molecular symmetry was assigned by pertinent signals in the \( ^1H \) and \( ^{13}C \) NMR spectra.

In particular, from the \( ^1H \) NMR spectrum there was a clear displacement of a \( t \)-Bu group by a methoxyl one, which was corroborated by the presence of four singlets due to OMe groups.
The reaction of the derivative with benzylic alcohol was conducted by treating the $p$-bromodienone derivative 4.11 with BnOH in the presence of AgClO4 in DME as solvent. The obtaining of the desired product was confirmed by spectral analysis, in particular the $^1$H NMR spectrum of 4.12b showed the singlet at 4.73 ppm due to OCH$_2$Ph group, which was indicative of the displacement of the t-Bu group.

Figure 4.9. $^1$H NMR spectrum of derivative 4.12a (400 MHz, CDCl$_3$, 298 K).
The $^{13}$C NMR spectrum of 4.12b confirmed the $C_5$ molecular symmetry by the presence of three signals due to ArCH$_2$Ar groups at 29.7, 30.3, and 31.2 ppm, three signals due to $-C(CH_3)_3$ C atoms at 31.3, 31.4, and 31.6 ppm, and one resonance at 70.2 ppm due to OCH$_2$Ph group.

At this point, to extend the generality of the $p$-bromodienone route on calix[6]arene macrocycle, it was decided to test another calixarene scaffold.

Thus the derivative 4.13 was exhaustively alkylated by treatment with Cs$_2$CO$_3$ and 1-iodohexane in acetone as solvent, to give derivative 4.14 in 80% yield. Successively, the benzyl group at the endo rim of 4.14 was removed by hydrogenolysis (H$_2$ and Pd/C) to give pentahexyloxy-mono-ol...
4.15 in 91% yield.

Scheme 4.5 Synthesis of derivative 4.15

Treatment of pentahexyloxyxalix[6]arene-mono-ol 4.15 under conditions analogous to the synthesis of 4.11 led to the formation of derivative 4.16 in 96% yield.

Scheme 4.6 Synthesis of derivative 4.16

The ESI(+) mass spectrum confirmed the molecular formula of 4.16. The $^1$H NMR spectrum in TCDE at 298 K showed 4
broad singlets due to t-butyl groups at 0.71 (9H), 0.99 (18H), 1.13 (9H), and 1.31 (18H) ppm, while broad signals were present in the methylene region indicating a slow conformational interconversion on the NMR time scale. Analogously to \( p \)-bromodienone derivative 4.11, the treatment of 4.16 with a methanolic solution of AgClO\(_4\) afforded \( p \)-methoxycalix[6]arene 4.17a in 15% yield, while its treatment with benzylic alcohol afforded derivative 4.17b in 17% yield (Scheme 4.7).

\[ \text{Scheme 4.7 Synthesis of derivative 4.17a-b} \]

As already reported in the introduction section, the \( p \)-bromodienone route with active aromatic substrates (e.g: resorcinol) gives a \textit{meta} substitution after a dienone-phenol rearrangement.

At this point the \( p \)-bromodienone route was tested with resorcinol with the intent to verify, also in this case, the \textit{meta} substitution, obtaining an inherently chiral host.
Thus, the treatment of 4.11 with resorcinol and a cold solution of AgClO$_4$ afforded meta-substituted calix[6]arene 4.18 in 30% yield.

Scheme 4.8. Synthesis of derivative 4.18

1D and 2D NMR spectra were in agreement with the asymmetrical structure of 4.18, in which the resorcinol and t-Bu groups were, respectively, meta- and para-linked to the calixarene phenol ring.

In fact, five of the expected six t-Bu singlets (two accidentally isochronous) were present in the $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K) of 4.18 at 1.12, 1.15(18H), 1.19, 1.21, and 1.33 ppm, while five singlets due to ArCH$_2$Ar groups were present at 3.83, 3.97, 4.07, 4.10 (4H), and 4.12 ppm, which correlated in the HSQC spectrum with carbon resonances at 31.8, 32.6, 30.2 (2C), 30.3, and 29.9 ppm.
In addition, the $^{13}$C NMR spectrum evidenced five signals due to $-\text{C(CH}_3\text{)}_3$ atoms at 31.50, 31.51 (2C), 31.54, 31.56, and 31.77 ppm, and four signals due to OMe groups at 60.93, 60.98(2C), 62.27, and 62.34 ppm, which correlated in the HSQC spectrum with singlets at 3.60 (9H), 3.87, and 3.88 ppm.

The asymmetric structure of 4.18 coupled with its three-dimensional nature makes it inherently chiral, and consequently, it should be formed as a racemic mixture. A rapid cone-to-cone inversion of the calix[6]arene skeleton of 4.18 leads to the interconversion between the two enantiomers.
The inherently chiral derivative 4.18 was also tested in complexation experiments with some alkylbenzylamonium·TFPB⁻ guests but unfortunately it did not give the formation of the [2]pseudorotaxane desired. This was probably due to the encumbrance of the resorcinol group that did not allow the entrance of the guest into the calixarene cavity.

4.2.2 Synthesis of inherently chiral resorcin[4]arene

The research of new potential chiral hosts went on with the synthesis of some inherently chiral resorcin[4]arenes. With this intent in mind it was used the regioselective $O$-
substitution procedure already experimented by Neri et al.\textsuperscript{69} However this synthetic procedure gives chiral resorcinarenes in racemic form. Thus the use of these compounds as supramolecular chiral hosts or building blocks requires their separation and identification of their chirality.

Therefore the synthesis of inherently chiral resorcin[4]arenes was followed by HPLC separation and EDC analyses supported by computational techniques.\textsuperscript{70}

The regioselective $O$-substitution procedure started with resorcin[4]arene octol $4.19$. It was subjected to alkylation with an excess of benzylbromide (10 equiv) in the presence of $K_2CO_3$ (4 equiv) as the weak base in acetone at reflux.

Column chromatography of the crude reaction mixtures afforded four tetra-$O$-benzylated derivatives, namely, $4.20a$ (8\%), $4.21a$ (26\%), $4.22a$ (9\%), and $4.23a$ (23\%) and four tetra-$O$-methylated derivatives (Scheme 16).

In the same way resorcin[4]arene octol $4.19$ was subjected to alkylation with methyliodide (50 equiv) in the presence of $K_2CO_3$ (4 equiv) at reflux.

Column chromatography of the crude reaction mixtures afforded four tetra-$O$-methylated derivatives, namely, $4.20b$ (9\%), $4.21b$ (25\%), $4.22b$ (11\%), and $4.23b$ (30\%) (Scheme 16).


Scheme 4.9 Synthesis of the inherently chiral derivative 4.20a-b, 4.20a-b, 4.20a-b and 4.20a-b

Structure assignment for the O-substituted resorcin[4]arenes counted essentially on spectral analysis. The tetra-substitution was confirmed by ESI(+) MS spectra, while the assignment of the substitution pattern was based on a careful analysis of $^1$H and $^{13}$C NMR data assisted by 2D NMR experiments.
The chirality of the derivative 4.20a in particular its C$_4$ symmetry was proved by the presence in the $^1$H NMR spectrum of one signal for the bridging methine groups, two singlets for the upper and lower ArH protons, and one resonance for benzylic OCH$_2$ groups.

![Figure 4.13 $^1$H NMR spectrum of derivative 4.20a (300 MHz, CDCl$_3$, 298 K).](image)

The unsymmetrical 1,2,4,6-substitution pattern of 4.23a, evidenced by eight ArH singlets, was also assigned by considering the presence of four isolated OH signals (7.08, 6.98, 6.86, and 6.82 ppm) only compatible with the 1,2,4,6-tetrasubstitution (Figure 4.14).
The epta-O-substituted resoricinarene is another type of inherent chiral derivative and it was synthesised with the aroylation of the resorcin[4]arene octol. Thus, the aroylation of resorcin[4]arene 4.19 with benzoyl chloride (10 equivalent) in pyridine was then used for the synthesis of the inherently chiral derivative 4.24 (Scheme 17).

Scheme 4. 10. Synthesis of the inherently chiral derivative 4.24

The derivative 4.24 was fully characterised, by MS and NMR.
spectroscopy as in the previous cases for the other derivatives.

4.2.3 HPLC-EDC analysis and Computational Spectroscopy

According to the “racemic approach”\textsuperscript{71}, the semi-preparative resolution of the enantiomers of compounds \textbf{4.20a}, \textbf{4.20b}, and \textbf{4.23b} was performed by enantioselective HPLC using a Chiralpak AD column in normal-phase conditions (\textit{n}-hexane/2-propanol mixtures, proportions ranging from 97:3 to 99:1 v/v, flow rates 1 mL min\textsuperscript{-1}).

The resolution of derivatives \textbf{4.23a} was performed using a Lux Amylose-2 column in normal-phase conditions (\textit{n}-hexane/methanol 96:4 v/v, flow rates 1 mL min\textsuperscript{-1}), while the resolution of derivatives \textbf{4.24} was performed using a Lux Cellulose-1 column in normal-phase conditions (\textit{n}-hexane/2-propanol 97:3 v/v, flow rates 1 mL min\textsuperscript{-1}).

The ECD analysis were performed investigating the hyphenation of enantioselective HPLC with a ECD spectropolarimeter with the possibility of on-line measurements.

The so called “stopped-flow” technique allows a reliable on-line ECD analysis with limited loss in spectral resolution and the additional advantage of reduced amounts of racemate.

necessary for the analysis: under optimized conditions, in general, sub-milligram quantities of racemate may be sufficient depending on the spectroscopic properties of the analyte.

Figure 4.15 Schematic representation of stopped-flow HPLC-ECD system.

Moreover, on-line ECD analysis allowed avoiding fraction collection/solvent evaporation cycles, where impurities in the mobile phase components can be accumulated and degradation can be promoted, affecting the reliability of the subsequent off-line ECD analysis.

The HPLC-ECD analysis were a result of a collaboration with the prof. Bertucci’s group from Università di Bologna; the interpretation of the ECD analysis is summarized in the graphic below.
Figure 4. 16 ECD spectra of 4.20a and 4.20b (1,3,5,7-O-substitution pattern).

Figure 4. 17 ECD spectra of 4.23a and 4.23b (1,2,4,6-O-substitution pattern).
The recorded spectra displayed an excellent signal-to-noise ratio and limited baseline drift even at shorter wavelengths. The enantiomers of tetra-$O$-substituted calix[4]resorcinarenes show a CD exciton couplet centred at the absorption maximum of the aromatic $^{1}L_{b}$ transitions, due to the coupling between resorcinol rings in the calix[4]resorcinarene skeleton. Methyl substituents did not contribute to the CD profile of calix[4]resorcinarenes, whose CD intensity is only affected by the $O$-substitution pattern. Benzyl groups contribute to the CD profile of calix[4]resorcinarenes in the short-wavelength spectral region. The ECD spectra have then been computed by TDDFT at the B3LYP/6-31++G* and PBE0/6-31++G* levels and were a result of a collaboration with the prof. Zanasi’s group from Università degli Studi di Salerno.
The spectrum below is only an example of the good agreement between the experimental CD curve and the theoretical one calculated for the derivative R (M) 4.20a.

**Figure 4.19** Experimental and theoretical CD curve calculated for the derivative R (M) 4.20a
4.3 CONCLUSION

In this part it was studied the p-bromodienone route for the first time on the calix[6]arene scaffold demonstrating that this strategy of functionalization is also effective for the functionalization of the calix[6]arene macrocycle. Therefore, through this route it was possible to introduce alcoholic O-nucleophiles at the calix[6]arene exo rim. In addition, the p-bromodienone route with activated aromatic substrates allowed the first example of meta functionalization of a calix[6]arene macrocycle giving rise to an unprecedented meta-substituted inherently chiral calix[6]arene derivative. Some other inherently chiral hosts resorcinarene based were synthesised using a regioselective O-substitution procedure. This synthetic strategy gave the chiral hosts in racemic mixture and for this reason any application of them required the separation and identification of their chirality. The HPLC separation as well as the ECD analysis and interpretation was realised in collaboration with the prof. Bertucci’s group from University of Bologna. The ECD spectra have then been studied by computational calculations in collaboration with the prof. Zanasi’s group from University of Salerno.
4.4 EXPERIMENTAL SECTION

ESI(+) −MS measurements were performed on a quadrupole mass spectrometer equipped with electrospray ion source, using a mixture of H₂O/CH₃CN (1:1) and 5% HCOOH as solvent. Flash chromatography was performed on Merck silica gel (60, 40-63 μm). All chemicals were reagent grade and were used without further purification. Anhydrous solvents were purchased from Aldrich. When necessary compounds were dried in vacuum over CaCl₂. Reaction temperatures were measured externally. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light, or by spraying with H₂SO₄-Ce(SO₄)₂ or phosphomolybdic acid.

1D and 2D NMR spectra were recorded on a Bruker Avance-600 spectrometer [600 (1H) and 150 MHz (13C)], Bruker Avance-400 spectrometer [400 (1H) and 100 MHz (13C)] and Bruker Avance-300 spectrometer [300 (1H) and 75 MHz (13C)].; chemical shifts are reported relative to the residual solvent peak (CHCl₃: δ 7.26, CDCl₃: δ 77.23; CD₃OH: δ 4.87, CD₃OD: δ 49.0). HSQC spectra were performed with gradient selection, sensitivity enhancement, and phase-sensitive mode using Echo/Antiecho-TPPI procedure. A typical experiment comprised 20 scans with 113 increments of 2048 points each.

Derivatives 4.19, 4.20a-b, 4.21a-b, 4.22a-b, 4.23a-b and
4.24 were synthesized according to a literature procedure\textsuperscript{69}

The spectroscopic analysis was performed using the stopped-flow HPLC-CD technique on an HPLC system consisting of a Jasco PU-980 pump, a LG-2080-02 ternary gradient unit, a DG-2080-53 degasser a Jones (Lakewood, CO) model 7955 column chiller, a Rheodyne (Cotati, CA) 7725i syringe loading injector, and a 20 \( \mu \)L sample loop; this system was connected to the Jasco J-810 spectropolarimeter, equipped with a 10 mm path length HPLC flow cell and a Rheodyne 7010 injector setup as a three-way valve for stopped-flow measurements.
General Procedure for the Synthesis of $p$-bromodienone derivatives 4.11 and 4.16

A solution of phenyltrimethylammonium tribromide (0.13 g, 0.36 mmol) in CH$_2$Cl$_2$ (5.0 mL) was added dropwise over 15 min to a stirred solution at 0 °C of the appropriate pentaalkoxy-calix[6]arene-mono-ol 4.10 or 4.15 (0.24 mmol) in CH$_2$Cl$_2$ (24 mL). Then, 25 mL of a saturated aqueous solution of NaHCO$_3$ was added and the resulting mixture was stirred for 15 min at room temperature. The organic phase was separated and washed with an aqueous solution of Na$_2$SO$_3$ (10% wt) and H$_2$O. The organic phase was dried over Na$_2$SO$_4$ and filtered, and the solvent was removed under reduced pressure, to give the corresponding calix[6]arene $p$-bromodienone derivative 4.11 or 4.16 in quantitative yield.

Derivative 4.11 (0.26 g, 99%). ESI(+) MS: m/z = 1143 (MNa$^+$), 1159 (MK+). $^1$H NMR (600 MHz, CDCl$_3$, 298 K): $\delta$ 0.86 [s, $-C(CH_3)_3$, 9H], 1.11 [s, C(CH$_3$) 3, 9H], 1.16 [s, $-C(CH_3)_3$, 18H], 1.23 [s, C(CH$_3$)$_3$, 18H], 2.90 (br s, OCH$_3$, 18H).
6H), 3.11 (br s, OCH₃, 6H), 3.25 (br s, OCH₃, 3H), 3.51 and 3.60 (AB, ArCH₂Ar, J =15.0 Hz, 4H), 3.71 and 4.09 (AB, ArCH₂Ar, J = 14.8 Hz, 4H), 3.74 and 4.20 (AX, ArCH₂Ar, J = 14.6 Hz, 4H), 6.60 (s, C=CH, 2H), 6.91(br s, ArH, 2H), 7.03 (br s, ArH, 2H), 7.07 (br s, ArH, 4H), 7.11 (br s,ArH, 2H).

¹³C NMR (150 MHz, CDCl₃, 298 K): δ 26.3, 30.0, 30.2,30.8, 31.59, 31.6, 31.7, 34.3, 34.4, 39.5, 60.1, 60.4, 60.6, 71.3, 125.5,125.9, 126.4, 126.8, 127.0, 130.0, 131.3, 133.4, 133.7, 133.8, 134.0,137.0, 143.5, 145.8, 145.9, 146.2, 146.2, 154.0, 154.3, 183.7.

Derivative 4.16 (0.34 g, 96%). ESI(+) MS: m/z =1494 (MNa⁺).

¹H NMR (300 MHz, TCDE, 298 K): δ 0.71 [broad,−C(CH₃)₃, 9H], 0.89 [br s, O(CH₂)₅CH₃, 15H], 0.99 [s, −C(CH₃)₃,18H], 1.13 [s, −C(CH₃)₃, 9H], 1.16 −1.19 (overlapped, OCH₂CH₂CH₂CH₂CH₂CH₃, 30H), 1.31 [s, −C(CH₃)₃, 18H], 1.52 −1.98 (overlapped, OCH₂CH₂CH₂CH₂CH₂CH₃, 10H), 2.92 −2.95 (broad, OCH₂CH₂CH₂CH₂CH₂CH₃, 2H), 2.98 −3.46 (overlapped, ArCH₂Ar + OCH₂CH₂CH₂CH₂CH₂CH₃, 14H), 4.28−4.34 (overlapped, ArCH₂Ar, 6H), 6.61 −7.07 (overlapped, ArH+ C=CH, 12H).

¹³C NMR (150 MHz, CDCl₃, 298 K): δ 14.4, 14.5, 22.8, 22.9,26.0, 26.2, 29.7, 29.9, 30.5, 30.7, 31.7, 31.8, 32.1, 32.3, 34.3, 38.9, 83.9,74.2, 126.7, 126.2, 126.5, 127.0, 127.6, 131.1, 132.8, 133.2, 134.1,136.6, 144.3, 145.1, 145.5, 153.5, 154.1, 183.7.
General Procedure for the Synthesis of Derivatives 56a–b.

A solution of AgClO$_4$ (0.048 g, 0.23 mmol) in the appropriate alcohol (1.6 mL of methanol or benzylic alcohol) was cooled at 0 °C and added to solid 4.11 (0.13 g, 0.12 mmol). The reaction mixture was allowed to warm at room temperature and stirred in the dark overnight. The solvent was removed under reduced pressure, and the residue was solubilized in CH$_2$Cl$_2$ (10 mL). The organic phase was washed 3 times with water, dried on Na$_2$SO$_4$, and filtered, and the solvent was removed under reduced pressure.

**Derivative 4.12a.** The crude product was purified by preparative thin layer chromatography, eluent n-hexane/diethyl ether/methanol 80/20/1 (v/v) to give 4.12a as a white solid, 0.025 g, yield 20%. ESI(+) MS: m/z = 1017 (MH$^+$). $^1$H NMR (400 MHz, CDCl$_3$, 298 K): $\delta$ 0.98 [s, $-C(CH_3)_3$, 9H], 1.13 [s, $-C(CH_3)_3$, 18H], 1.17 [s, $-C(CH_3)_3$, 18H], 3.02 (s, OCH$_3$, 6H), 3.15 (s, OCH$_3$, 6H), 3.49 (s, OCH$_3$, 3H), 3.57 (s, OCH$_3$, 3H), 3.81 (s, ArCH$_2$Ar, 4H), 3.93 (br
s, ArCH₂Ar, 8H), 6.44 (s, ArH, 2H), 6.87 (s, ArH, 2H), 6.92 (s, ArH, 2H), 7.00 (s, ArH, 2H), 7.06 (s, ArH, 4H), 7.27 (s, OH, 1H). ¹³C NMR (CDCl₃, 100 MHz, 298 K): δ 30.5, 31.4, 31.5, 31.7, 34.2, 34.3, 55.3, 60.6, 60.9, 113.2, 125.6, 126.3, 126.5, 129.0, 132.5, 133.2, 133.4, 133.6, 133.8, 145.4, 145.8, 145.9, 146.7, 152.6, 153.2, 154.1, 154.4.

**Derivative 4.12b**. The crude product was purified by column chromatography on silica gel using CHCl₃/n-hexane (96/4, v/v) as eluent to give 4.12b as a colourless solid, 0.040 g, 30% yield. **ESI(+) MS**: m/z = 1093 (MH⁺).

¹¹H NMR (600 MHz, CDCl₃, 298 K): δ 0.93 [s, −C(CH₃)₃, 9H], 1.08 [s, −C(CH₃)₃, 18H], 1.10 [s, C(CH₃)₃, 18H], 2.95 (s, OCH₃, 6H), 3.09 (s, OCH₃, 6H), 3.41 (s, OCH₃, 3H), 3.73 (s, ArCH₂Ar, 4H), 3.85 (bs, ArCH₂Ar, 8H), 4.73 (s, OCH₂Ph, 2H), 6.47 (s, ArH, 2H), 6.81 (s, ArH, 2H), 6.87 and 7.01 (AB, ArH, J = 2.04 Hz, 4H), 6.93 and 6.96 (AB, ArH, J = 2.04 Hz, 4H), 7.18 −7.21 (overlapped, OCH₂C₆H₅ + OH, 6H). ¹³C NMR (150MHz, CDCl₃, 298 K): δ 29.7, 30.3, 31.2, 31.3, 31.4, 31.6, 31.9, 34.05, 34.1, 60.3, 60.7, 70.2, 114.2, 125.4, 125.7, 125.9, 126.1, 126.4, 127.5, 127.7, 128.4, 128.6, 128.7, 132.2, 133.0, 133.2, 133.4, 133.6, 133.7, 137.4, 145.2, 145.6, 146.0, 146.5, 151.8, 153.0, 154.0, 154.3.

Synthesis of Derivative 37
Cs$_2$CO$_3$ (9.8 g, 30 mmol) was added, under stirring, to a solution of compound 4.13 (1.2 g, 1.1 mmol) in dry acetone (60 mL), and the mixture was heated at reflux. After 30 min, 1-iodohexane (15 g, 10 mL, 70 mmol) was added and the resulting mixture was kept at reflux under stirring for 48 h. The reaction was allowed to cool at room temperature and the solvent removed under reduced pressure. The crude product was solubilized in CH$_2$Cl$_2$, washed with aqueous 1N HCl and brine, and then dried over Na$_2$SO$_4$. The solvent was evaporated to dryness, and the product was crystallized from MeOH/CH$_2$Cl$_2$ to give 4.14 as a pale yellow solid (1.32 g, 80% yield). ESI(+) MS: m/z = 1485 (MH$^+$), 1507 (MNa$^+$), 1522 (MK$^+$). $^1$H NMR (300 MHz, TCDE, 383 K): $\delta$ 0.77 [broad, O(CH$_2$)$_5$CH$_3$, 15H], 0.94 [s, –C(CH$_3$), 9H], 0.95 [s, –C(CH$_3$), 18H], 1.04 [s, –C(CH$_3$), 27H], 1.10 −1.25 (overlapped, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, 30H), 1.35 −1.60 (overlapped, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, 10H), 3.28 (broad, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, 4H), 3.39 (broad, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, 4H), 3.48 (t, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, $J = 6.5$ Hz, 2H), 3.79 (broad s,
ArCH₂Ar, 12H), 4.67 (s, OCH₂Ph, 2H), 6.77 (s, ArH, 2H), 6.83 (s, ArH, 4H), 6.90 (s, ArH, 4H), 6.94 (s, ArH, 2H), 7.16 – 7.21 (overlapped, OCH₂C₆H₅, 3H), 7.30 – 7.35 (m, OCH₂C₆H₅, 2H). **13C NMR** (75 MHz, TCDE, 383 K): δ 12.2, 20.8, 24.2, 28.1, 28.3, 28.4, 28.9, 29.7, 30.0, 32.1, 71.7, 73.0, 123.7, 123.9, 124.3, 124.6, 124.8, 125.6, 126.4, 131.1, 131.2, 136.5, 143.1, 143.6, 150.9, 151.6, 152.0.

**Synthesis of Derivative 4.15**

A solution of 4.14 (1.3 g, 0.89 mmol) in CHCl₃ (80 mL) was added of Pd/C, and the mixture stirred for 12h under H₂ at 25 °C. The catalyst was filtered on a Celite pad, and the filtrate was evaporated under vacuum. Precipitation of the residue from methanol gave pure 4.15 as a yellow solid (1.13 g, 91% yield). **ESI(+) MS**: m/z = 1417 (MNa⁺), 1434 (MK⁺).

**1H NMR** (300 MHz, TCDE, 383 K): δ 0.70 [s, −C(CH₃), 9H], 0.77 [broad, O(CH₂)₅CH₃, 15H], 0.94 [s, −C(CH₃), 9H], 1.12 [s, −C(CH₃), 18H], 1.15 [s, −C(CH₃), 18H], 1.06 – 1.40 (overlapped, OCH₂CH₂CH₂CH₂CH₂CH₃, 30H), 1.59 – 1.70 (overlapped, OCH₂CH₂CH₂CH₂CH₂CH₃, 10H ), 3.09 (broad...
t, OCH₂CH₂CH₂CH₂CH₂CH₃, 4H), 3.60 (t, OCH₂CH₂CH₂CH₂CH₂CH₃, J = 7.2 Hz, 2H), 3.71 −3.76 (overlapped, ArCH₂Ar + OCH₂CH₂CH₂CH₂CH₂CH₃, 16H), 6.41, (br s, ArH, 2H), 6.54 (br s, ArH, 2H), 6.57 (br s, OH, 1H), 6.77 (s, ArH, 2H), 6.91 (s, ArH, 2H), 6.96 (br s, ArH, 2H), 7.00 (br s, ArH, 2H). ¹³CNMR (75 MHz, TCDE, 383 K): δ 11.9, 12.0, 20.6, 20.9, 24.0, 27.9, 28.4, 28.6, 29.5, 29.6, 29.8, 30.0, 32.0, 32.1, 71.7, 122.7, 123.0, 124.4, 124.5, 125.3, 130.2, 131.2, 131.8, 140.1, 142.6, 143.0, 143.1, 144.0, 149.3, 149.9, 151.7, 152.4.

General Procedure for the Synthesis of Derivatives 4.17a-b.

As a solution of AgClO₄ (0.048 g, 0.23 mmol) in the appropriate alcohol (1.6 mL) at 0 °C was added to the solid p-bromodienenone derivative 4.16 (0.18 g, 0.12 mmol). The reaction mixture was allowed to warm at room temperature and stirred in the dark overnight. The solvent was removed under reduced pressure, and the residue was solubilized in
CH₂Cl₂ (10 mL). The organic phase was washed 3 times with water, dried on Na₂SO₄, and filtered, and the solvent was removed under reduced pressure.

**Derivative 4.17a.** The crude product was purified by column chromatography on silica gel using CHCl₃/n-hexane 96/4 as eluent to give 4.17a as a white solid, 0.025 g, 15% yield.

**ESI(+) MS:** m/z = 1369 (MH⁺), 1391 (MNa⁺). **¹H NMR** (300 MHz, TCDE, 393 K): δ 0.79 [broad, O(CH₂)₅CH₃, 15H], 0.87 [s, −C(CH₃)₃, 18H], 0.99 [s, −C(CH₃)₃, 9H], 1.14 [s, −C(CH₃)₃, 18H], 1.14 −1.73 (overlapped, OCH₂CH₂CH₂CH₂CH₂CH₃, 40H), 3.17 (br t, OCH₂CH₂CH₂CH₂CH₂CH₃, 2H), 3.60 −3.80 (overlapped, ArCH₂Ar+ OCH₂CH₂CH₂CH₂CH₂CH₃, 20H), 3.70 (br s, OCH₃, 3H), 6.63 (b s, ArH, 2H), 6.70 (b s, ArH, 2H), 6.82 (s, ArH, 2H), 6.94 (b s, ArH, 2H), 7.03 (b s, ArH, 4H). **¹³C NMR** (75 MHz, TCDE, 393 K): δ 11.9, 20.5, 20.8, 21.4, 23.9, 24.1, 27.8, 27.9, 29.0, 29.5, 29.7, 32.1, 71.4, 109.4, 127.7, 123.1, 123.3, 123.8, 125.1, 125.2, 127.9, 129.6, 130.2, 131.2, 131.6, 143.0, 143.2, 144.0, 144.4, 150.2, 150.7, 151.6, 152.3.

Derivative 4.17b. The crude product was purified by column chromatography on silica gel using CHCl₃/n-hexane 40/60 as eluent to give 4.17b as a pale yellow solid, 0.031 g, 17% yield. **ESI(+) MS:** m/z = 1466 (MNa⁺), 1483 (MK⁺). **¹H NMR** (300 MHz, TCDE, 393 K): δ 0.77 −0.80 [overlapped, O(CH₂)₅CH₃ +C(CH₃)₃, 33H], 0.98 [s, −C(CH₃)₃, 9H], 1.12
[s, −C(CH₃)₃, 18H], 1.08 −1.62 (overlapped, OCH₂CH₂CH₂CH₂CH₂CH₃, 40H), 3.16 (br t, OCH₂CH₂CH₂CH₂CH₂CH₃, 2H), 3.49− 3.79 (overlapped, ArCH₂Ar + OCH₂CH₂CH₂CH₂CH₂CH₃, 20H), 4.53 (br s, OH, 1H), 4.82 (s, OCH₂Ph, 2H), 6.50 −6.64 (overlapped, ArH, 6H), 6.80 (br s,ArH, 2H), 6.93 (s, ArH, 2H), 6.99 (s, ArH, 2H), 7.16 −7.23(overlapped, OCH₂C₆H₅, 5H). ¹³C NMR (75 MHz, TCDE, 393 K): δ 17.0, 25.8, 29.1, 33.0, 33.3, 33.7, 34.0, 34.6, 35.0, 37.1, 74.5, 119.6, 127.9, 128.4, 130.1, 130.5, 131.5, 132.8, 134.0, 134.9, 135.4, 136.7, 148.2, 149.1, 155.9, 156.8, 157.4.

Synthesis of derivative 4.18

To a solution of p-bromodienone 4.11 (0.52 g, 0.47 mmol) in DME (3.0 mL) at 0 °C was added a solution of AgClO₄ (0.19 g, 0.93 mmol) and resorcinol (0.52 g, 4.7 mmol) in DME (4 mL). The reaction mixture was allowed to warm at room
temperature and stirred in the dark overnight. The solvent was
removed under reduced pressure, and the residue was
solubilized in CH₂Cl₂ (15 mL) and washed with aqueous 1 N
HCl and successively with water, dried on Na₂SO₄, and
filtered, and the solvent was removed under reduced pressure.
The crude product was purified by column chromatography on
silica gel (CH₂Cl₂) to give derivative 4.18 (0.16 g, 30% yield)
as a white solid.

**ESI(+) MS:** m/z = 1152 (MH+).

**¹H NMR** (600 MHz, CDCl₃, 298 K): δ 1.12 [s, −C(CH₃)₃, 9H], 1.15 [bs, −C(CH₃)₃, 18H],
1.19 [s, −C(CH₃)₃, 9H], 1.21 [s, −C(CH₃)₃, 9H], 1.33 [s, −C(CH₃)₃, 9H], 3.60 (s, OCH₃, 9H), 3.83 (s, ArCH₂Ar, 2H),
3.88 (bs, OCH₃, 6H), 3.97 (s, ArCH₂Ar, 2H), 4.07 (s, ArCH₂Ar, 2H), 4.10 (bs, ArCH₂Ar, 4H), 4.12 (s, ArCH₂Ar,
2H), 4.76 (s, OH, 1H), 5.03 (s, OH, 1H), 6.34 (m, ArH, 1H),
6.36 (bs, ArH, 1H), 6.52 (m, ArH, 1H), 6.55 (m, ArH,
1H), 6.88 −6.97 (overlapped, ArH, 3H), 7.10 −7.14 (overlapped, ArH, 3H), 7.24−7.32 (overlapped, ArH, 3H),
7.81 (s, ArH, 1H), 8.55 (s, OH, 1H).

**¹³C NMR** (150 MHz, CDCl₃, 298 K): δ 29.9, 30.2, 30.3, 31.50, 31.51, 31.54, 31.56,
31.8, 32.6, 34.37, 34.41, 34.51, 34.54, 60.93, 60.98, 62.27,
62.34, 104.1, 104.8, 107.5, 108.6, 119.9, 120.1, 125.3,
125.6, 125.7, 125.9, 126.0, 126.1, 126.4, 126.5, 127.1, 127.2,
127.3, 127.7, 131.0, 132.0, 132.3, 132.4, 132.5, 133.2, 133.3,
133.4, 133.5, 133.6, 144.2, 146.3, 146.6, 148.1, 148.3, 152.0,
General procedure for the synthesis of derivatives 4.20a - 4.23a

A suspension of resorcinarene 4.19 (0.50 g, 0.45 mmol) and K$_2$CO$_3$ (0.25 g, 1.8 mmol) was stirred in acetone (30 mL) under reflux. After 1 h BrCH$_2$Ph (0.54 mL, 4.5 mmol) was
added at room temperature and the mixture was stirred under reflux overnight. The solvent was removed under reduced pressure and the product was extracted in CH₂Cl₂ (50 mL), and washed with 0.1 M HCl (2×30 mL). The organic phase was washed with H₂O (3×20 mL) dried over Na₂SO₄ filtered and evaporated. The crude product was subjected to flash chromatography on deactivated (7.5%) silica gel (CH₂Cl₂/AcOEt, 98/2) to give pure 4.20a and 4.21a and a mixture of derivatives 4.22a and 4.23a, which was separated by preparative TLC (hexane/diethyl ether, 4/6).

**Derivative 4.20a.**
White solid, 0.036 mmol, 54 mg, yield: 8% .

**¹H NMR (300 MHz, CDCl₃, 298 K):** δ 7.38 (m, 20H, CH₂Ph), 7.22 (s, 4H, ArH), 7.12 (s, 4H, OH), 6.38 (s, 4H, ArH), 5.03 and 5.02 (AB, 8H, J = 11.4 Hz, OCH₂Ph), 4.26 (t, 4H, J = 7.6 Hz, ArCHAr), 2.15 (m, 8H, CHCH₂), 1.27 (m, 72H, CH₂(CH₂)₉CH₃), 0.88 (m, 12H, CH₂(CH₂)₉CH₃).

**¹³C NMR (75 MHz, CDCl₃, 298 K):** δ 153.1, 152.9, 135.7, 128.8, 128.6, 128.1, 125.2, 124.9, 123.9, 101.8, 71.7, 34.3, 33.3, 32.0, 29.8, 29.5, 28.1, 22.8, 14.2.

**Derivative 4.21a.** White solid, 0.120 mmol, 174 mg, yield: 26% .

**¹H NMR (300 MHz, CDCl₃, 298 K):** δ 7.39 (m, 20H, CH₂Ph), 7.17 (s, 2H, ArH), 7.11 (s, 2H, ArH), 6.78 (s, 4H, OH), 6.49 (s, 2H, ArH), 6.20 (s, 2H, ArH), 5.10 and 5.06 (AB, 8H, J = 11.4 Hz, OCH₂Ph), 4.28 (t, 4H, J = 6.9 Hz,
ArCHAr), 2.11 (m, 8H, CHCH₂), 1.26 (m, 72H, CH₂(CH₂)₉CH₃), 0.89 (t, 12H, J = 6.3 Hz, CH₂(CH₂)₉CH₃).

¹³C NMR (75 MHz, CDCl₃, 298 K): δ 153.4, 152.6, 135.7, 128.9, 128.6, 127.8, 127.3, 126.3, 122.1, 121.9, 103.9, 98.9, 71.9, 34.5, 33.1, 32.0, 29.8, 29.5, 28.0, 22.8, 14.2.

**Derivative 4.22a.** White solid, 0.041 mmol, 60 mg, yield: 9%.

¹H NMR (400 MHz, CDCl₃, 298 K): δ 7.37 (overlapped, 21H, CH₂Ph + ArH), 7.18 (s, 2H, ArH), 7.10 (s, 1H, ArH), 6.92 (s, 2H, OH), 6.86 (s, 2H, OH), 6.46 (s, 1H, ArH), 6.39 (s, 2H, ArH), 6.21 (s, 1H, ArH), 5.04 (m, 8H, OCH₂Ph), 4.26 (t, 4H, J = 7.7 Hz, ArCHAr), 2.14 (m, 8H, CHCH₂), 1.27 (m, 72H, CH₂(CH₂)₉CH₃), 0.89 (m, 12H, CH₂(CH₂)₉CH₃).

¹³C NMR (100 MHz, CDCl₃, 298 K): δ 153.2, 153.1, 152.8, 152.5, 135.7, 135.3, 128.9, 128.7, 127.9, 126.8, 125.8, 124.6, 124.3, 124.2, 123.4, 122.6, 104.6, 101.8, 98.9, 72.0, 71.5, 34.4, 34.1, 33.4, 33.1, 32.0, 29.8, 29.4, 28.0, 22.7, 14.1.

**Derivative 4.23a.** White solid, 0.104 mmol, 154 mg, yield: 23%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ 7.39 (overlapped, 20H, CH₂Ph), 7.28 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.15 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.05 (s, 1H, OH), 6.96 (s, 1H, OH), 6.84 (s, 1H, OH), 6.79 (s, 1H, OH), 6.49 (s, 1H, ArH), 6.39 (s, 1H, ArH), 6.37 (s, 1H, ArH), 6.21 (s, 1H, ArH), 5.06 (overlapped, 8H, OCH₂Ph), 4.26 (overlapped, 4H, ArCHAr), 2.12 (overlapped, 8H, CHCH₂), 1.27 (overlapped, 72H, CH₂(CH₂)₉CH₃), 0.89 (overlapped, 12H,
CH$_2$(CH$_2$)$_9$CH$_3$). $^{13}$C NMR (62.5 MHz, CDCl$_3$, 298 K): δ 153.6, 153.2, 153.1, 152.9, 152.8, 152.6, 152.4, 153.9, 153.5, 135.9, 135.5, 135.4, 128.9, 128.8, 128.7, 128.6, 128.4, 128.2, 127.9, 127.7, 127.6, 126.7, 125.7, 125.5, 125.4, 124.8, 124.6, 123.6, 123.3, 123.1, 122.2, 104.2, 101.9, 101.3, 99.0, 72.0, 71.96, 71.5, 34.3, 34.1, 33.4, 33.2, 33.1, 33.0, 32.0, 29.8, 29.4, 28.0, 22.7, 14.2.

General procedure for the synthesis of derivatives 4.20b - 4.23b
A mixture of resorcinarene 4.19 (0.50 g, 0.45 mmol) and K₂CO₃ (0.25 g, 1.8 mmol) was stirred in acetone (30 mL) under reflux. After 1 h CH₃I (2.8 mL, 45 mmol) was added at room temperature and the mixture was stirred under reflux for 5 h. The solvent was removed under reduced pressure and the residue partitioned between CH₂Cl₂ (20 mL) and 0.1 M HCl (20 mL). The organic phase was washed with H₂O (3×20 mL) and dried over Na₂SO₄ filtered and evaporated. The crude product was subjected to flash chromatography on deactivated (10%) silica gel (CH₂Cl₂/MeOEt, 98/2) to give pure 4.20b and 4.21b and a mixture of derivatives 4.22b and 4.23b which was separated by preparative TLC (hexane/diethyl ether, 4/6).

**Derivative 4.20b.** White solid, 0.041 mmol, 47 mg, yield: 9%. ¹H NMR (300 MHz, CDCl₃, 298 K): δ 7.54 (s, 4H, OH), 7.22 (s, 4H, ArH), 6.35 (s, 4H, ArH), 4.27 (t, 4H, J = 7.6 Hz, ArCHAr), 3.84 (s, 12H, OCH₃), 2.19 (m, 8H, CH₂CH₂CH₂), 1.27 (m, 72H, CH₂(CH₂)₉CH₃), 0.89 (t, 12H, J = 6.3 Hz, C₁₀H₂₀CH₃). ¹³C NMR (75 MHz, CDCl₃, 298 K): δ 153.6, 152.9, 124.7, 124.6, 123.7, 99.9, 55.8, 33.9, 33.0, 31.9, 29.7, 29.4, 28.1, 22.7, 14.1.

**Derivative 4.21b.** White solid, 0.113 mmol, 131 mg, yield: 25%. ¹H NMR (300 MHz, CDCl₃, 298 K): δ 7.19 (s, 4H, ArH), 7.07 (s, 4H, OH), 6.38 (s, 2H, ArH), 6.26 (s, 2H, ArH), 4.28 (t, 4H, J = 7.5 Hz, ArCHAr), 3.92 (s, 12H, OCH₃), 2.17 (m, 8H, CHCH₂CH₂), 1.26 (m, 72H, CH₂(CH₂)₉CH₃), 0.89 (t,
12H, $J = 6.3$ Hz, $C_{10}H_{20}CH_3$). \textsuperscript{13}C NMR (62.5 MHz, CDCl\textsubscript{3}, 298 K): $\delta$ 153.4, 153.2, 126.4, 125.6, 122.6, 122.2, 104.2, 94.9, 56.2, 34.2, 32.9, 32.0, 29.7, 29.4, 28.0, 22.7, 14.1.

**Derivative 4.22b.** White solid, 0.049 mmol, 59 mg, yield: 11%. \textsuperscript{1}H NMR: (250 MHz, CDCl\textsubscript{3}, 298 K): $\delta$ 7.36 (s, 1H, ArH), 7.30 (s, 2H, OH), 7.21 (s, 2H, OH), 7.19 (s, 2H, ArH), 7.12 (s, 1H, ArH), 6.37 (s, 1H, ArH), 6.35 (s, 2H, ArH), 6.28 (s, 1H, ArH), 4.27 (br t, 4H, ArCHAr), 3.91 (s, 6H, OCH\textsubscript{3}), 3.83 (s, 6H, OCH\textsubscript{3}), 2.18 (m, 8H, CHCH\textsubscript{2}CH\textsubscript{2}), 1.27 (m, 72H, CH\textsubscript{2}(CH\textsubscript{2})\textsubscript{9}CH\textsubscript{3}), 0.88 (m, 12H, $C_{10}H_{20}CH_3$). \textsuperscript{13}C NMR (62.5 MHz, CDCl\textsubscript{3}, 298 K): $\delta$ 153.9, 153.8, 152.8, 152.0, 126.1, 125.5, 124.0, 123.9, 123.9, 123.7, 122.8, 104.7, 99.9, 95.1, 56.2, 55.9, 34.2, 34.1, 33.2, 32.0, 29.8, 29.5, 28.1, 22.8, 14.2.

**Derivative 4.23b.** White solid, 0.135 mmol, 157 mg, yield: 30%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, 298 K): $\delta$ 7.41 (s, 1H, OH), 7.35 (s, 1H, OH), 7.28 (overlapped, 3H, OH + ArH), 7.16 (s, 1H, ArH), 7.15 (s, 1H, ArH), 7.09 (s, 1H, OH), 6.39 (s, 1H, ArH), 6.36 (s, 1H, ArH), 6.34 (s, 1H, ArH), 6.28 (s, 1H, ArH), 4.31-4.26 (overlapped, 4H, ArCHAr), 3.93 and 3.92 (d, 6H, OCH\textsubscript{3}), 3.84 and 3.82 (s, 6H, OCH\textsubscript{3}), 2.19 (m, 8H, CHCH\textsubscript{2}CH\textsubscript{2}), 1.27 (m, 72H, CH\textsubscript{2}(CH\textsubscript{2})\textsubscript{9}CH\textsubscript{3}), 0.90 (overlapped, 12H, $C_{10}H_{20}CH_3$). \textsuperscript{13}C NMR (62.5 MHz, CDCl\textsubscript{3}, 298 K): $\delta$ 153.9, 153.6, 153.5, 153.1, 153.0, 152.8, 152.6, 126.7, 125.9, 125.1, 124.7, 124.2, 123.7, 123.6, 123.2, 122.8, 122.5, 104.5, 99.96, 99.8, 95.0, 56.2, 55.8, 34.0, 33.1.
33.0, 32.0, 29.7, 29.4, 28.1, 22.7, 14.1.

General procedure for the synthesis of derivative 4.24

A suspension of resorcinarene 4.19 (0.25 g, 0.23 mmol) and benzoyl chloride (0.32 g, 2.3 mmol), in 5.0 mL of dry pyridine was stirred at room temperature for 10 min. The mixture was dried under vacuum and partitioned between CH$_2$Cl$_2$ (30 mL) and 0.1 M HCl (10 mL). The organic phase was washed with H$_2$O (3×10 mL) and dried. The crude product was purified by flash chromatography on deactivated (10%) silica gel (petroleum ether/CH$_2$Cl$_2$, 60/40).

**Derivative 4.24:** white solid, 0.13 mmol, 0.23 g, yield 55%.

$^{1}$H NMR (300 MHz, CDCl$_3$, 298 K): $\delta$ 8.41 (s, 1H, ArH), 8.38 (s, 1H, ArH), 7.99-7.45 (overlapped, 36H, ArH), 7.10 (s, 1H, ArH), 7.08 (s, 1H, ArH), 6.80 (s, 1H, ArH), 6.61 (s, 1H, ArH), 6.46 (s, 1H, ArH), 6.02 (s, 1H, OH), 4.51 (m, 2H, ArCHAr), 4.36 (br t, 1H, ArCHAr), 4.21 (br t, 1H, ArCHAr), 2.05
2.07 (m, 4H, CHCH₂CH₂), 1.88 (m, 2H, CHCH₂CH₂), 1.77 (m, 2H, CHCH₂CH₂), 1.19 (m, 72H, CH₂(CH₂)₉CH₃), 0.87 (t, J = 6.5 Hz, 12H, C₁₀H₂₀CH₃). ¹³C NMR (62.5 MHz, CDCl₃, 298 K): δ 14.7, 23.2, 28.5, 29.9, 30.2, 30.4, 32.4, 35.1, 35.7, 37.8, 38.0, 90.0, 92.1, 94.2, 112.2, 113.6, 116.6, 118.6, 125.8, 126.1, 128.9, 129.3, 129.6, 129.8, 130.4, 130.5, 130.6, 130.9, 131.5, 131.8, 133.7, 134.0, 134.1, 134.4, 134.7, 135.8, 136.1, 136.6, 137.0, 146.2, 146.3, 146.8, 147.1, 148.7, 148.0, 149.5, 154.6, 163.2, 164.7, 165.0, 166.7, 178.9.
HPLC analysis

**Chromatographic conditions employed for stopped-flow measurements**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Column</th>
<th>Mobile phase</th>
<th>Flow (mL min⁻¹)</th>
<th>Inj. volume (µL)</th>
<th>t₁ (min)</th>
<th>ki</th>
<th>k₂</th>
<th>σ</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>rac-4.20a Chirpak AD</td>
<td>n-hexane/2-propanol</td>
<td>99:1</td>
<td>1.0</td>
<td>20</td>
<td>100</td>
<td>2.780</td>
<td>4.952</td>
<td>6.631</td>
<td>1.339</td>
</tr>
<tr>
<td>rac-4.20b Chirpak AD</td>
<td>n-hexane/2-propanol</td>
<td>99:1</td>
<td>1.0</td>
<td>20</td>
<td>100</td>
<td>3.040</td>
<td>4.383</td>
<td>6.071</td>
<td>1.385</td>
</tr>
<tr>
<td>rac-4.25a Lux Amylose-2</td>
<td>n-hexane/methanol</td>
<td>98:2</td>
<td>1.0</td>
<td>20</td>
<td>100</td>
<td>2.047</td>
<td>1.071</td>
<td>1.505</td>
<td>1.406</td>
</tr>
<tr>
<td>rac-4.26a Chirpak AD</td>
<td>n-hexane/2-propanol</td>
<td>97:3</td>
<td>1.0</td>
<td>20</td>
<td>100</td>
<td>2.940</td>
<td>1.746</td>
<td>3.079</td>
<td>1.764</td>
</tr>
<tr>
<td>rac-4.24 Lux Cellulose-1</td>
<td>n-hexane/2-propanol</td>
<td>97:3</td>
<td>1.0</td>
<td>20</td>
<td>100</td>
<td>2.800</td>
<td>2.724</td>
<td>3.821</td>
<td>1.403</td>
</tr>
</tbody>
</table>

All separations were carried out at controlled temperature (15 °C). The 20 µL injection loop was used during the optimization of chromatographic conditions, while the 100 µL injection loop was used for stopped-flow measurements.

ECD analysis (in collaboration with Prof. Bertucci and Dott. Tedesco from Università di Bologna) and Theoretical Calculations (in collaboration with Prof. Zanasi and Dott. Monaco from Università degli Studi di Salerno)

The enantiomeric fractions were stopped inside the HPLC flow cell during their elution and analysed by full-spectrum ECD spectroscopy in the 350–215 nm spectral range, using a 4 nm spectral bandwidth, a 50 nm min⁻¹ scanning speed, a 1 s data integration time, a 0.2 nm data pitch and three accumulation cycles.

TD-DFT calculations were performed using combinations of the PBE0 and B3LYP functionals with the 6/31++G* basis sets using Gaussian 03.⁷²

---


*Gaussian 03, Revision B.05*; Gaussian, Inc.: Pittsburgh, PA, 2003.