University of Salerno
Department of Chemistry and Biology

PhD in
SCIENCE AND TECHNOLOGY FOR
CHEMICAL, PHARMACEUTICAL AND FOOD INDUSTRY”
XII CYCLE - Chemistry Curriculum
2013/2014

New Chemical Topologies
Based on Calixarene Threading

Roberta Ciao
Ph.D in
SCIENCE AND TECHNOLOGY FOR CHEMICAL, PHARMACEUTICAL,
AND FOOD INDUSTRY”
XII CYCLE - Chemistry Curriculum
2011-2014

Ph.D. Thesis

New Chemical Topologies

Based on Calixarene Threading

Roberta Ciao

Supervisors

Prof. Placido Neri
University of Salerno

Prof. Yoram Cohen
University of Tel Aviv

Counter Supervisor

Prof. Francesco De Riccardis
University of Salerno

Coordinator

Prof. Gaetano Guerra
University of Salerno
CHAPTER I

1. Supramolecular Topology Outline 15
   1.1 Supramolecular Topology. An informal discussion 15
   1.2 DNA topological isomerism 22
   1.3 Supramolecular topologies: from paper to laboratory 24
      1.3.1 Catenanes and Rotaxanes 24
      1.3.2 Molecular knots 34
   1.4 Topological and functional complexity 43
      1.4.1 Rotaxanes and Catenanes as supramolecular devices 43
      1.4.2 Rotaxanes and catenanes in nanotechnology 51

CHAPTER II

2.1 Complex Topologies based on Calixarenes 56
2.2 Supramolecular Properties of Calix[n]arenes 60
2.3 Through-the-Annulus Threading of Large Calixarenes Induced by Very Loose Alkylammonium Ion Pairs 62

CHAPTER III

3.1 Goal of the research 71

CHAPTER IV

4.1 Introduction 76
4.3 Synthesis of double-threaded[2]pseudorotaxane 14,16 82
   4.3.1 Threading of double-calix[6]arene 12 with di-n-pentylammonium axle 2" 83
   4.3.2 Directional threading of double-calix[6]arene 12 with nonsymmetrical n-butylbenzylammonium axle 89
   4.5.1 Threading of double-calix[6]arene 21 with di-n-pentylammonium axle 2" 97
   4.5.2 Directional threading of double-calix[6]arene 21 with nonsymmetrical n-butylbenzylammonium axle 3" 100
   4.5.3 Threading of double-calix[6]arene 21 with dibenzylammonium axle 4" 103
4.6 Conclusion 104
4.7 Experimental section 106
   4.7.1 Synthesis and characterization of double-calixarene 12. 108
4.7.2 Preparation of singly-threaded pseudo[2]rotaxanes 13,15

4.7.3 Preparation of doubly threaded pseudo[3]rotaxanes 14,16 119

4.7.4 Synthesis and characterization of double-calixarene 21 122

4.7.5 Preparation of singly-threaded pseudo[2]rotaxanes 22
24, 26 130


4.7.7 Determination of $K_{\text{ass}}$ values of $(2^*)_2 \subset 12$ and $(3^*)_2 \subset 12$
complexes by quantitative $^1$H NMR analysis 139

CHAPTER V

5.1 Introduction 141

5.2 Stereo-programmed synthesis of (T,T)-handcuff-[2]rotaxane 146

5.2.1 Stereo-programmed formation of an (T,T)-handcuff-
pseudo[2]rotaxane (29$^{2+}$) 146

5.2.2 Stereo-programmed synthesis of an (T,T)-handcuff-
[2]rotaxane (32$^{3+}$) 153

5.3 Stereo-programmed formation of (H,H)-handcuff-[2]rotaxane 158

5.3.1 Stereo-programmed formation of an (H,H)-handcuff-
pseudo[2]rotaxane (34$^{2+}$) 158

5.3.2 Stereo-programmed synthesis of an (H,H)-handcuff-
[2]rotaxane (37$^{2+}$) 161

5.4 Stereo-programmed formation of (H,T)-handcuff-[2]rotaxane 167

5.4.1 Stereo-programmed formation of an (H,T)-handcuff-
pseudo[2]rotaxane (39$^{2+}$) 167

5.4.2 Stereo-programmed synthesis of an (H,T)-handcuff-
[2]rotaxane (42$^{2+}$) 169

5.5 Experimental section 176

5.5.1 Preparation of handcuff pseudo[2]rotaxane (T,T)-29$^{2+}$ 177

5.5.2 Preparation of handcuff pseudo[2]rotaxane (T,T)-31$^{2+}$ 179

5.5.3 Synthesis of handcuff [2]rotaxane (T,T)-32$^{2+}$ 180

5.5.4 Simulated Annealing Experiments on handcuff
pseudo[2]rotaxane (T,T)-31$^{2+}$ 185

5.5.5 Simulated Annealing Experiments on handcuff
[2]rotaxane (T,T)-32$^{2+}$ 185

5.5.6 Preparation of handcuff pseudo[2]rotaxane (H,H)-34$^{2+}$ 186

5.5.7 Preparation of handcuff pseudo[2]rotaxane (H,H)-36$^{2+}$ 188

5.5.8 Synthesis of handcuff [2]rotaxane (H,H)-37$^{2+}$ 189

5.5.9 Preparation of handcuff pseudo[2]rotaxane (H,T)-39$^{2+}$ 193

5.5.10 Synthesis and characterization of thread 40$^{2+}$ and its
precursors 194

5.5.11 Preparation of handcuff pseudo[2]rotaxane (H,T)-41$^{2+}$ 212

5.5.12 Synthesis of handcuff [2]rotaxane (H,T)-42$^{2+}$ 214
## CHAPTER VI

6.1 Introduction
   6.3.1 Threading of triple-calix[6]arene 57 with di-n-pentylammonium axle 2'
   6.3.2 Threading of triple-calix[6]arene 57 with di-benzylammonium axle 4'
   6.3.3 Directional threading of triple-calix[6]arene 57 with nonsymmetrical n-butylbenzylammonium axle 3'
6.4 Experimental section
   6.4.1 Synthesis of triple tert-butylated-calix[6]arene 57
   6.4.2 Preparation of singly-threaded pseudo[2]rotaxanes 58', 61' and 64'
   6.4.3 Preparation of triply-threaded pseudo[4]rotaxanes 603', 633' and 663'
6.5 Conclusion

## CHAPTER VII

7. Conclusion
FIGURE LISTS

Figure 1. Classical topological transformation: a donut that becomes a cup.

Figure 2. Some simple objects for the consideration of Euclidean geometry.

Figure 3. Topologically equivalent constructions.

Figure 4. Two topologically distinct closed curve structures.

Figure 5. Structure and graph of a fungicide.

Figure 6. On the left a planar graph, on the right a non-planar graph.

Figure 7. (a) A right and left handed trefoil knot; (b) an achiral figure-of-eight knot.

Figure 8. Topoisomerase General mechanism.

Figure 9. Micrograph of DNA topological isomers by the action of DNA topoisomerase.

Figure 10. A pseudorotaxane: common precursor to catenane and rotaxane.

Figure 11. Pseudorotaxane Rotaxane.

Figure 12. A catenane structure and its cartoon representation.

Figure 13. Nomenclature pseudorotaxanes rotaxanes and catenanes.

Figure 14. Wasserman [2]catenane.

Figure 15. Schill and Lüttingraus [2]catenane by template synthesis.

Figure 16. Schematic representation of the synthesis of a catenane by the metal template effect.

Figure 17. A supramolecular self-assembly process of [2]-catenane.

Figure 18. Rotaxane obtain by threading e stoppering.

Figure 19. Cartoon representation of the clipping strategy for the synthesis of a rotaxane.

Figure 20. Cartoon representation of a slipping strategy for the synthesis of a rotaxane.

Figure 21. Cartoon representation of (a) a statistical, (b) a directed, (c) a metal templated synthesis and (d) electrostatic interaction or H-bonding templated synthesis for a catenane.

Figure 22. (a) Lactoferrin structure (b) acid ascorbic structure.

Figure 23. A schematic tabulation of simple knots and links, accompanied by their trivial names and descriptors using the Alexander-Briggs notation.

Figure 24. Trefoil knot representation.

Figure 25. A visualization of the inherent topological chirality of Trefoil Knots.

Figure 26. Knot Sauvage Synthesis.

Figure 27. Sauvage Trefoil Knot Synthesized via the metathesis.

Figure 28. Examples of decorative Solomon Link (on the left) on an Italian mosaic tile;
Figure 29. Schematic representation of Borromean rings and molecular equivalents of its constituents.

Figure 30. Stoddart olympiadane

Figure 31. Ball-and-stick representation of the solid-state structure of a suit[2]ane

Figure 32. A molecular clamp by light activated, structure (on the left) and cartoon representation (on the right).

Figure 33. Molecular Shuttle driven by chemical impulses

Figure 34. A four-stroke engine based on rotaxane

Figure 35. Diagram of a pH dependent molecular shuttle.

Figure 36. Redox controlled ring rotation in a catenane containing a non-symmetric ring

Figure 37. The molecular elevator of Stoddart and Balzani in a graphical representation.

Figure 38. Structure of the bistable [2]rotaxane used in the crossbar memory and SEMs of the nanowire crossbar memory

Figure 39. Molecular junction constituted by a layer of bistable [2]catenanes.

Figure 40. Schematic representation of a molecular carrier and bistable [2]rotaxane structure

Figure 41. AFM image on the writing on a rotaxane film

Figure 42. Calix crater and p-terz-butilcalix[4]arene structure

Figure 43. The phenol-derived calixarene family

Figure 44. (a) Crown ethers, cucurbiturils and cyclodextrines; (b) Structure of larger calix[n]arene (n = 6, 7 e 8)

Figure 45. Threading of calix[6]arene derivative.

Figure 46. Threading of calix[6]arene derivative.

Figure 47. Through-the-Annulus Threading of Calixarenes. Induced by Very Loose Alkylammonium Ion Pairs

Figure 48. The endo alkyl preference

Figure 49. [2]rotaxane synthesis and its unprecedented inversion of the wheel orientation

Figure 50. Through-the-annulus threading of nonsymmetrical alkylbenzyl ammonium cations by hexaalkoxylcalix[6]arene

Figure 51. The three stereoisomeric [3]rotaxane structures named a. Tail to Tail, b. Head to head, and c. Head to Tail.

Figure 52. Calix[6]arene-based catenane

Figure 53. Double-calix[6]arene and tris-calix[6]arene structures

Figure 54. Double-calixarene-based handcuff-rotaxane

Figure 55. Three stereoisomeric handcuff-pseudo[3]rotaxane structures

Figure 56. Double-threaded[2]catenane topologies

Figure 57. The variety of topological isomers obtainable from a tris-calixarene host.

Figure 58. The endo-alkyl rule

Figure 59. Schematic representation of the currently known prototypical
examples of handcuff-derived architectures

**Figure 60.** Stereoisomeric oriented handcuff pseudo-[2]rotaxanes

**Figure 61.** $^1$H NMR spectrum of double-calixarene derivative 12

**Figure 62.** Linear mono-ammonium systems, double-calixarene host

**Figure 63.** Schematic representation of directional threading of 12 with the $n$-butylbenzylammonium axle 3$^+$

**Figure 64.** $^1$H NMR of: a) 12 (aa x 10$^{-3}$ M); b) 12 and 0.5 equiv of 2$^+$; c) 12 and 1 equiv of 2$^+$; d) 12 and 2 equiv of 2$^+$; e) 12 and 3 equiv of 2$^+$; f) significant portion of the ESI(+) mass spectrum of a mixture of 12 and 1 equiv of 2$^+$.

**Figure 65.** 2D COSY spectrum of the 1:1 mixture of 12 and 2$^+\cdot$TFPB$^-$. 

**Figure 66.** HSQC spectrum of an equimolar solution of 2$^+$ and 12.

**Figure 67.** DFT calculation.

**Figure 68.** Schematic representation of Directional threading of 12 with the $n$-butylbenzylammonium axle 3$^+$.

**Figure 69.** The “endo-alkyl rule” and related examples of oriented interpenetrated architectures.

**Figure 70.** Possible double-threaded pseudo[2]rotaxane stereoisomers by directional threading of 12 with the $n$-butylbenzylammonium axle 3$^+$.

**Figure 71.** $^1$H NMR of: a) 12 (aa x 10$^{-3}$ M); b) 12 and 0.25 equiv of 3$^+$; c) 12 and 0.5 equiv of 3$^+$; d) 12 and 0.75 equiv of 3$^+$; e) 12 and 1.0 equiv of 3$^+$; f) 12 and 2.0 equiv of 3$^+$; g) ESI(+) mass spectrum of a mixture 1:1 of 12 and 3$^+$.

**Figure 72.** Energy-minimized structures of the (3$^+$)$_2\subset$12 complex (B3LYP DFT calculation)

**Figure 73.** Energy-minimized structures of endo-alkyl/endo-alkyl (3$^+$)$_2\subset$12 (B3LYP DFT calculation using the 6-31G* basis set).

**Figure 74.** $^1$H NMR spectrum of double-calixarene derivative 21

**Figure 75.** Linear mono-ammonium systems, double-calixarene host 21

**Figure 76.** Schematic representation of directional threading of 21 with the $n$-butylbenzylammonium axle 2$^+$.

**Figure 77.** $^1$H NMR (400 MHz, 298 K, CDCl$_3$) of: a) 21 (aa x 10$^{-3}$ M); b) 21 and 1.0 equiv of 2$^+$; c) 21 and 2.0 equiv of 2$^+$; d) 21 and 5.0 equiv of 2$^+$; significant portion of the ESI(+) mass spectrum of a mixture of 21 and 1.0 equiv of 2$^+$.

**Figure 78.** Significant portions of the 2D COSY spectrum of the 1:2 mixture of 21 and 2$^+\cdot$TFPB$^-$. 

**Figure 79.** Schematic representation of Directional threading of 21 with the $n$-butylbenzylammonium axle 3$^+$.

**Figure 80.** Possible double-threaded pseudo[2]rotaxane stereoisomers by directional threading of 21 with the $n$-butylbenzylammonium axle 3$^+$.

**Figure 81.** $^1$H NMR of: a) 21 (aa x 10$^{-3}$ M); b) 21 and 1.0 equiv of 3$^+$; c) 21
and 2.0 equiv of \(3^+\); d) \(21\) and 5.0 equiv of \(3^+\);

**Figure 82.** Significant portions of the 2D COSY spectrum of the 1:2 mixture of \(21\) and \(3^+\)-TFPB<sup>−</sup>.

**Figure 83.** Schematic representation of threading of \(21\) with the dibenzylammonium axle \(4^+\).

**Figure 84.** \(^1\)H NMR of: a) \(21\) (aa x10<sup>−3</sup> M); b) \(21\) and 0.5 equiv of \(4^+\); c) \(21\) and 1.0 equiv of \(4^+\); d) \(21\) and 2.0 equiv of \(4^+\)

**Figure 85.** Significant portions of the 2D COSY spectrum of the 1:2 mixture of \(21\) and \(4^+\)-TFPB<sup>−</sup>.

**Figure 86.** \(^1\)H NMR of derivative \(9\).

**Figure 87.** \(^1\)H NMR spectrum of derivative \(9\).

**Figure 88.** \(^1\)H NMR spectrum of derivative \(10\).

**Figure 89.** \(^1\)H NMR spectrum of derivative \(10\).

**Figure 90.** \(^1\)H NMR spectrum of derivative \(11\).

**Figure 91.** \(^1\)H NMR spectrum of derivative \(11\).

**Figure 92.** \(^1\)H NMR spectrum of derivative \(12\).

**Figure 93.** \(^1\)H NMR spectrum of derivative \(12\).

**Figure 94.** ESI(+)(+) MS spectrum of derivative \(11\).

**Figure 95.** \(^1\)H NMR spectrum of the 1:1 mixture of \(12\) and \(2^+\)-TFPB<sup>−</sup>.

**Figure 96.** 2D COSY spectrum of the 1:1 mixture of \(12\) and \(2^+\)-TFPB<sup>−</sup>.

**Figure 97.** \(^1\)H NMR spectrum of the 1:1 mixture of \(12\) and \(3^+\)-TFPB<sup>−</sup>.

**Figure 98.** \(^1\)H NMR spectrum of the 1:2 mixture of \(12\) and \(2^+\)-TFPB<sup>−</sup>.

**Figure 99.** \(^1\)H NMR spectrum of the 1:2 mixture of \(12\) and \(3^+\)-TFPB<sup>−</sup>.

**Figure 100.** Spectrum \(^1\)H NMR of derivative \(18\).

**Figure 101.** Spectrum \(^1\)H NMR of derivative \(18\).

**Figure 102.** Spectrum \(^1\)H NMR of derivative \(19\).

**Figure 103.** Spectrum \(^1\)H NMR of derivative \(19\).

**Figure 104.** Spectrum \(^1\)H NMR of derivative \(20\).

**Figure 105.** Spectrum \(^1\)H NMR of derivative \(20\).

**Figure 106.** Spectrum \(^1\)H NMR of derivative \(21\).

**Figure 107.** Spectrum \(^1\)H NMR of derivative \(21\).

**Figure 108.** ESI(+)(+) MS spectrum of derivative \(21\).

**Figure 109.** \(^1\)H NMR spectrum of the 1:1 mixture of \(21\) and \(2^+\)-TFPB<sup>−</sup>.

**Figure 110.** \(^1\)H NMR spectrum of the 1:1 mixture of \(21\) and \(3^+\)-TFPB<sup>−</sup>.

**Figure 111.** \(^1\)H NMR spectrum of the 1:2 mixture of \(21\) and \(3^+\)-TFPB<sup>−</sup>.

**Figure 112.** \(^1\)H NMR spectrum of the 1:2 mixture of \(21\) and \(4^+\)-TFPB<sup>−</sup>.

**Figure 113.** Cartoon representations of handcuff-like systems.

**Figure 114.** Stereo-programmed direct synthesis of calixarene-based [2]rotaxanes.

**Figure 115.** Handcuff-rotaxanes.

**Figure 116.** Sequence stereoisomerism in handcuff-[2]rotaxane.

**Figure 117.** \(^1\)H NMR spectra of: (a) \(12\), (b) an equimolar mixture of \(12\) and \(28^{2+}\)-TFPB<sup>−</sup>, (c) an equimolar mixture of \(12\) and \(30^{2+}\)-TFPB<sup>−</sup>.

**Figure 118.** ESI(+)(+) mass spectrum of \((T, T)\cdot29^{2+}\) and its AMBER energy-
minimized structure (inset)

Figure 119. Expansions of 2D COSY-45 spectrum of an equimolar mixture of 12 and 28$^{2+}$·2TFPB$^-$

Figure 120. Lowest energy structure of three possible handcuff-pseudo[2]rotaxane 31$^{2+}$ (from left to right: T,T; H,T and H,H)

Figure 121. (a-c) Snapshot from an MD simulation of (T,T)-handcuff-pseudo[2]rotaxane 31$^{2+}$

Figure 122. ESI(+) mass spectrum of (T,T)-32$^{2+}$ and its AMBER energy-minimized structure (inset)

Figure 123. (a) $^1$H NMR spectrum of handcuff-[2]rotaxane (T,T)-32$^{2+}$

Figure 124. Lowest energy structure of the (T,T)-handcuff-pseudo[2]rotaxane 31$^{2+}$

Figure 125. $^1$H NMR spectra of: (a) 12, (b) an equimolar mixture of 12 and 37$^{2+}$·2TFPB$^-$, (c) an equimolar mixture of 12 and 31$^{2+}$·2TFPB$^-$

Figure 126. 2D COSY-45 spectrum of 12 and 37$^{2+}$·2TFPB$^-$

Figure 127. ESI(+) mass spectrum of doubly-charged (H,H)-handcuff-pseudo[2]rotaxane 39$^{2+}$

Figure 128. ESI(+) mass spectrum of (H,H)-41$^{2+}$ and its AMBER energy-minimized structure (inset)

Figure 129. (a) $^1$H NMR spectrum of handcuff-[2]rotaxane (H,H)-37$^{2+}$·2TFPB$^-$

Figure 130. Expansions of 2D COSY-45 spectrum of the (H,H)-handcuff-[2]rotaxane 37$^{2+}$

Figure 131. Lowest energy structure of the (H,H)-handcuff-[2]rotaxane 37$^{2+}$

Figure 132. $^1$H NMR spectra of: (a) 12, (b) an equimolar mixture of 12 and 40$^{2+}$·2TFPB$^-$ (c) an equimolar mixture of 12 and 40$^{2+}$·2TFPB$^-$

Figure 133. ESI(+) mass spectrum of (H,T)-42$^{2+}$ and its AMBER energy-minimized structure (inset)

Figure 134. $^1$H NMR spectrum of handcuff-[2]rotaxane (H,T)-42$^{2+}$

Figure 135. 2D COSY-45 spectrum of the (H,T)-handcuff-[2]rotaxane 42$^{2+}$

Figure 136. Lowest energy structures of the (H,T)-handcuff-[2]rotaxane 42$^{2+}$

Figure 137. The three possible (H,H)-, (T,T)-, and (H,T)-handcuff-[2]rotaxane

Figure 138. 2D COSY spectrum of handcuff pseudo[2]rotaxane (T,T)-29$^{2+}$·2TFPB$^-$

Figure 139. 2D COSY spectrum of handcuff pseudo[2]rotaxane (T,T)-31$^{2+}$·2TFPB$^-$

Figure 140. $^1$H NMR spectrum of handcuff [2]rotaxane (T,T)-32$^{2+}$·2TFPB$^-$

Figure 141. $^{13}$C NMR spectrum of handcuff [2]rotaxane (T,T)-30$^{2+}$

Figure 142. 2D COSY spectrum of handcuff [2]rotaxane (T,T)-30$^{2+}$·2TFPB$^-$

Figure 143. Dihedral angle values (°) around C(1)-C(2) and C(2)-C(3) bonds measured for the SA structures of (T,T)-36$^{2+}$ in the 4 kcal/mol lowest energy window.
Figure 144. Dihedral angle values (°) around C(1)-C(2) and C(2)-C(3) bonds measured for the SA structures of (T,T)-302+ in the 4 kcal/mol lowest energy window.

Figure 145. 1H NMR spectrum of handcuff pseudo[2]rotaxane (H,H)-382+ 2TFPB−

Figure 146. 2D COSY spectrum of handcuff pseudo[2]rotaxane (T,T)-382+ 2TFPB−

Figure 147.

Figure 148. 1H NMR spectrum of handcuff [2]rotaxane (H,H)-402+

Figure 149. 13C NMR spectrum of handcuff [2]rotaxane (H,H)-402+

Figure 150. 2D COSY-45 spectrum of Handcuff [2]rotaxane (H,H)-402+

Figure 151. 13C NMR spectrum of handcuff [2]rotaxane (H,H)-402+

Figure 152. Spectrum 1H NMR of derivative 51

Figure 153. Spectrum 13C NMR of derivative 51

Figure 154. Spectrum 1H NMR of derivative 53

Figure 155. Spectrum 13C NMR of derivative 53

Figure 156. Spectrum 1H NMR of derivative 54

Figure 157. Spectrum 13C NMR of derivative 53

Figure 158. Spectrum 1H NMR of derivative 56

Figure 159. Spectrum 13C NMR of derivative 56

Figure 160. Spectrum 1H NMR of derivative 57

Figure 161. Spectrum 13C NMR of derivative 57

Figure 162. Spectrum 1H NMR of derivative 58

Figure 163. Spectrum 1H NMR of derivative 60

Figure 164. Spectrum 13C NMR of derivative 60

Figure 165. Spectrum 1H NMR of derivative 61

Figure 166. Spectrum 13C NMR of derivative 61

Figure 167. Spectrum 1H NMR of derivative 34

Figure 168. Spectrum 13C NMR of derivative 342+

Figure 169. 2D COSY spectrum of handcuff pseudo[2]rotaxane (H,T)-622+ 2TFPB−

Figure 170. 1H NMR spectrum of handcuff [2]rotaxane (H,T)-632+ 2TFPB−

Figure 171. 13C NMR spectrum of handcuff [2]rotaxane (H,T)-632+ 2TFPB−

Figure 172. 2D COSY-45 spectrum of Handcuff [2]rotaxane (H,H)-402+

Figure 173. Threading of triple-calixarene hosts with TFPB salts of n-alkylammonium cations

Figure 174. (a) 1H NMR spectrum of tris-calixarene derivative 67; (b) ESI(+) MS spectra of derivative 67

Figure 175. Linear mono-ammonium systems (left), triple–calix[6]arene host (right)

Figure 176. Cartoon representations of new topologies obtained through the threading of triple calix[6]arene with mono-ammonium axles.

Figure 177. 1H NMR of: a) 57 (aa x10⁻³ M); b) 57 and 1 equiv of 2⁺; c) 57 and 2 equiv of 2⁺; d) 57 and 3 equiv of 2⁺; e) 57 and 8 equiv of
Figure 178. $^1$H NMR of: a) 57(aa x 10^{-3} M); b) 57 and 1 equiv of 4$^+$; c) 57 and 2 equiv of 4$^+$; d) 57 and 3 equiv of 4$^+$; e) 57 and 8 equiv of 4$^+$: on the right expansion of shielded benzyl region.

Figure 179. Schematic representation of directional threading of 57 with the $n$-butylbenzylammonium axle 3$^+$. 

Figure 180. Possible triple-threaded pseudo[4]rotaxane stereoisomers by directional threading of 67 with the $n$-butylbenzylammonium axle 3$^+$. 

Figure 181. $^1$H NMR of: a) 57(aa x 10^{-3} M); b) 44 and 1 equiv of 3$^+$; c) 7 and 2 equiv of 3$^+$; d) 57 and 3 equiv of 3$^+$; e) 57 and 8 equiv of 3$^+$: on the right expansion of shielded alkyl region. 

Figure 182. Spectrum $^1$H NMR of derivative 57 

Figure 183. Spectrum $^{13}$C NMR of derivative 57 

Figure 184. $^1$H NMR spectrum of the 1:1 mixture of 57 and 3$^+$·TFPB$^-$. 

Figure 185. $^1$H NMR spectrum of the 1:1 mixture of 57 and 3$^+$·TFPB$^-$. 

Figure 186. $^1$H NMR spectrum of the 1:1 mixture of 57 and 3$^+$·TFPB$^-$. 

Figure 187. $^1$H NMR spectrum of the 1:3 mixture of 57 and 2$^+$·TFPB$^-$. 

Figure 188. $^1$H NMR spectrum of the 1:3 mixture of 57 and 4$^+$·TFPB$^-$. 

Figure 189. $^1$H NMR spectrum of the 1:3 mixture of 57 and 4$^+$·TFPB$^-$. 

12
Abstract

Recently, Neri et al. have introduced an efficient method to obtain endo-cavity complexation and through-the-annulus threading of large calixarenes by exploiting the inducing effect of the weakly coordinating tetrakis[3,5-bis-trifluoromethyl]phenyl]borate (TFPB-) anion. The corresponding calix[6]-arene/dialkylammonium pair can be considered a versatile recognition motif, which can be used for the construction of a large variety of calixarene-threaded architectures.

This Ph. D. thesis deals with the exploration of the stereochemical features of the threading of hosts containing multiple cavities. Therefore, the synthesis of double- and triple-calixarenes is reported, which is followed by the subsequent study of their threading abilities with dialkylammonium axles.

The results confirmed the now well-known endo-alkyl rule of calix[6]arenes that give the inclusion of alkyl chains inside the calix-cavity. On this basis, we were then able to build new attractive chemical topologies. In particular, doubly-threaded pseudo[3]rotaxane structures have been obtained by the threading of double-calixarene hosts with mono-ammonium axles. The subsequent extension to triple-calixarene hosts, in which three macrocycles are covalently linked to one another by means of an appropriate spacer, gave triply-threaded pseudo[4]rotaxane structures.

Because of the three-dimensional nonsymmetrical nature of the calix[6]arene wheels, by threading double-calixarene hosts with bis-ammonium axles three examples of beautiful stereoisomeric calixarene-based handcuff rotaxanes were obtained, which could be termed as head-to-head (H,H), head-to-tail (H,T), and tail-to-tail (T,T).

On the basis of these results, it is conceivable that the extension of this approach could lead to novel mechanically interlocked architectures with high-order topologies.
E il tempo che hai perso per la tua rosa, a rendere importante la tua rosa.
CHAPTER I

Introduction

The main interpretation of supramolecular chemistry as the chemistry that goes beyond the covalent bond is inherent to its original foundation by Lehn.\textsuperscript{1} In particular, anyone who approaches to that branch of chemistry is introduced to the weak interactions between molecular components. These interactions can be recognized within these supramolecular structures and lead to a superior organization of the matter. Supramolecular chemistry has found quite different applications, ranging from chemistry to biological mimicry, including nanotechnology and computing at the molecular level.

Starting from the Lehn's approach\textsuperscript{1} as the basic foundation of supramolecular chemistry, during the 90s of the last century there has been a gradual development of a new field: supramolecular topology. This field of research, will be introduced in the first chapter, and afterward will be used to frame the subject of the experimental work of this thesis, that is, the development of new supramolecular architectures based on calixarene\textsuperscript{2} macrocycles. Such compounds are able to accommodate guest molecules in their cavities and therefore are well suitable to give systems of higher complexity.

1. Supramolecular Topology Outline

1.1 Supramolecular Topology. An informal discussion

The *rubber geometry* metaphor, which we frequently encounter leafing through the classics on the subject, is particularly useful to introduce topology, the mathematic’s branch that classifies its objects by their connectivity. Through the use of transformations such as contraction or expansion of distances and angles in order to establish equivalence relationships, it marks a clear boundary with the Euclidean geometry. The rigidity of the objects is essential for the latter, whilst for the former two objects are equivalent as long as they can convert one into the other by steps that do not involve tearing or gluing, which is a *homeomorphism:* a sewing needle and a pipe, a donut and a cup of coffee, Euclidean objects completely different, are topologically equivalent.

![Figure 1](image)

**Figure. 1** – Classical topological transformation: a donut that becomes a cup. The two objects are equivalent, since both are geometrical toruses.

Euclidean geometry and topology are two complementary approaches to molecular structure which show alternative aspects of the chemical aggregates. However, in Euclidean geometry the key idea is that of *geometrically equivalent or congruent figures.* Two figures are called congruent if an intellectual transformation allows one figure to be “placed on the other” so that the two figures exactly coincide in all the geometrical

---

properties. In Euclidean geometry, squares A and B (Figure 2), which have the same side lengths and areas, would be said to be equivalent (or congruent in mathematical terminology). Square C, which differs in size, is not equivalent but is said to be similar. Clearly, triangle D and circle E are entirely different objects.

![Figure 2. Some simple objects for the consideration of Euclidean geometry.](image)

In topological geometry however, we consider different properties such as connectivity: lengths, angles and size are no longer considered. Topologically, two objects are identical if one can be deformed into the other in a continuous fashion as long as nothing is broken or no holes are punched through during the process. If we now look again at the object in Figure 3, we can see that in terms of topology they are all equivalent, since given complete freedom to distort lengths and angles, any one object can be transformed into any other. Indeed, they are all simply different representations of the same topological object, a closed curve. When considering the transformation of any object into another in topological geometry, one may consider the object to be totally flexible with no restriction on length and angle changes. However, connectivity is a property that must remain unchanged and for this question the circle, square and triangle are termed homeomorphic.\(^5\)

---

If we consider the circle and the knot in Figure 4: both are closed curves, but they are topologically distinct. So we can see that while normal chemical isomerism such as stereoisomerism arises from the consideration of Euclidean properties (bond types, angles), to classify topological isomers, we must consider topological geometry.\(^6\)

\[ \text{Figure 3. Topologically equivalent constructions.} \]

It is clear that, topologically, a molecule is simply a collection of vertices (atoms) connected to each other by edges (bonds), with no regard to angles or lengths, considering only the connectivity.

The above discussion of the topology of one dimensional constructions such as closed curves and lines is directly transferable to molecular structures once the \textit{molecular graph} is defined. A graph is a simply collection of vertices bound together by lines,\(^7\) in correspondence with its atoms and bonds. We define the molecular graph as simply the graph where nuclei define the points and bonds define the edges (Figure. 5).


Thus, the molecular graph is exactly the common structural formula embedded in a 3D-space.

![Figure 5](image.png)

**Figure 5.** Structure and graph of a fungicide. From the most structural to the most essential.

Embedding in 3D-space imparts *extrinsic topological properties* to a construction. Two constructions which are topologically equivalent in a 3D-space are termed *isotopic*. All isotopic constructions are homeomorphic. Not all homeomorphic constructions, however, are isotopic. The distinct character of the mirror image of trefoil knots, or of the linked and separated rings, are extrinsic *topological properties*. These constructions are homeomorphic, but not isotopic. Any topological chirality must necessarily be a property of embedding of an object in some space and is an extrinsic property - all mirror image objects are homeomorphic. Topological stereochemistry involves a collection of extrinsic topological properties deriving from the embedding of molecular graphs in a 3D-space.

The graphological footprint of a supramolecular architectures, whatever discernes it from a molecular entity in the topology of its graph, is the *non-planarity*. In fact, if the three-dimensional structure is projected on a plane, a graph whose lines intersect in an irreversible way is obtained (Figure 6). It is impossible to remove the overlapping by a topological transformation.

---

Going to the insertion of a graph in three-dimensional space, the topoisomerism is evidently a stereochemical property and it is therefore subjected to the same wording as the stereoisomerism (i.e. there will be topological enantiomers and topological diastereoisomers). It becomes clear that, to a higher complexity of the construct corresponds to a higher number of topological stereoisomers that it is possible to find. Although, to date, the math has not yet produced a consolidated and comprehensive survey of the stereotopological tools, in chemistry it has been suggested a conjecture\textsuperscript{9,10} which involves the occurrence of topological enantiomers when at least one of the following elements in the graph is present: an oriented ring, a chiral ring, a chiral knot, a chiral non-planar graph. The condition given for the topological diastereoisomerism is, instead, more elastic, since it requires that the graphs are just not planar (they do not need be topologically chiral).

The topological isomerism of molecules becomes more interesting when chirality is considered. For molecules to be topologically chiral, they must remain non-superimposable with their mirror image under all distortions. In fact, the necessary and sufficient condition for topological chirality is that

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{example_graph.png}
\caption{On the left a planar graph, where you can move the internal bond above the structure and avoid overlapping; on the right a non-planar graph.}
\end{figure}

\textsuperscript{9} Ibid., 3167.
any presentation of the construction must be topologically distinct from its mirror image. This implies that no presentation may be converted into its mirror by continuous deformation in the 3D-space. This property implies that the graph of the molecule is non-planar. Molecular systems with an intrinsically non-planar graph are scarce. If any rigidly achiral presentation is found for a construction, topological chirality is ruled out. Of course, such a presentation must possess an improper axis of symmetry. This constraint readily allows topological chirality to be ruled out for most molecular graphs. Specifically, any planar presentation of a molecular graph is rigidly achiral in the 3D-space. It possesses at least one \( \sigma \) plane (the plane in which it is embedded), and is therefore a rigidly achiral presentation. Most topologically non-planar one-dimensional objects contain either a link, knot, or non-planar graph. The non-planar graphs are intrinsically non-planar, the links and knots are non-planar when embedded in a 3D-space. For example the trefoil knot is chiral, whereas a figure-of-eight knot is achiral (Figure 7).

\[\text{Figure 7. (a) A right and left handed trefoil knot; (b) an achiral figure-of-eight knot.}\]

\[\text{Figure 7. (a) A right and left handed trefoil knot; (b) an achiral figure-of-eight knot.}\]

1.2. **DNA topological isomerism**

*Nature* gives us the most emblematic example of structural complexity through the topological isomerism of DNA. After the discovery in 1953 of the double helix by Watson and Crick,\(^{13}\) the scientific community was puzzled to explain how it was possible that the strand of human DNA, even one meter long, wrapped on itself, could be in the cell’s nucleus, which has a size of about five millionths of a meter. In addition, remained to explain what was the mechanism by which the structure was carried out to expose the nucleotide bases during replication and transcription. It took some time before realizing that the answer lays in topology, in particular in the theory of knots. It became soon clear that it was necessary to think DNA as a twisted band in space, a kind of node (open), whose geometry can be modified by enzymes which were responsible for relax (remove) the tensions created in the double helix DNA afterwards the transcription or other cellular processes and therefore involve a change in DNA topology (Figure 8). Knot theory will be important here, because while the effects of enzymes on DNA can not be seen directly, it is possible to realize the changes induced in its geometry.

In practice, these enzymes, called topoisomerases\(^\text{14}\), resolve the helix supercoiling, producing DNA breakage at the phosphodiester bond level (Figure 8) and allowing the opening of the replication fork without excessive torsional stress.

These results encouraged the scientific research and shortly thereafter followed a successful series of articles in which even more intriguing topologies were characterized, such as knots and catenanes, of both artificial\(^\text{15,16}\) and natural DNA (Figure 9).\(^\text{17,18,19}\) Thus, from simple [2]catenanes up to networks of thousands of interlocked circular DNA were found in mitochondrial of trypanosomes.

---


1.3. **Supramolecular topologies: from paper to laboratory**

Now we will discuss the most important complex architectures that have been obtained from experimental chemistry to date. Leafing through the classics of supramolecular chemistry one easily comes across in topologically complex structures and this shows the fervent interest in this attractive field of research. In particular, we intend to focus our attention to rotaxanes, catenanes, and knots.

1.3.1 **Catenanes and rotaxanes**

Rotaxanes and catenanes are interpenetrated structures topologically more relevant in the literature. Both derive from the same precursor known as pseudorotaxane ([Figure 10](#)).

![Figure 10. A pseudorotaxane: common precursor to catenane and rotaxane](image)

Specifically, a pseudorotaxane is a supramolecular system consisting of a linear molecule, the "axle", stuck in a cyclic molecule, the "wheel", in

---

which the two units are held together only by secondary chemical forces, such as hydrogen bonds, electrostatic interactions, or π-π stacking interactions. The peculiarity of pseudorotaxane systems is that they can "disassemble" by means of the "pulling out" of the two parts. A rotaxane can be seen as a pseudorotaxane in which the ends of the linear component are covalently linked to groups bulky enough to not allow the "parade" of the axle (stoppering) (Figure 11).

![Pseudorotaxane & rotaxane](image)

**Figure 11.** Pseudorotaxane & rotaxane

Starting from the pseudorotaxane we can get a different kind of interlocked structure if a suitably functionalized axis is subjected to a *macrocyclization* reaction rather than a *stoppering* reaction, as previously discussed. The interlocked structure thus obtained is called “catenane” (Figure 12) and it consists of two cyclic interpenetrated molecules that cannot be separated unless that a covalent bond is broken (Figure 12).

![Catenane structure](image)

**Figure 12.** A catenane structure and its cartoon representation.

The nomenclature for interpenetrated systems provides to indicate, in square brackets, the number of constituents that make up the system,
followed by the name of the given system (pseudorotaxane, rotaxane and catenane) (Figure 13).

Figure 13. Nomenclature pseudorotaxanes rotaxanes and catenanes

The first examples date back as far as 1960, when Wasserman,\textsuperscript{21} with the so-called “statistical approach”, began to cyclize an thirty-eight carbon atom ester by acyloin condensation, then reducing it with deuterium chloride by Clemmensen reaction. The acyloin condensation of the same substrate in presence of a macrocycle led to a mixture of topoisomers, including the [2]catenane. Wasserman proved the goodness of the synthesis by purifying the small amount of interlocked compound from the starting materials by column chromatography and by checking carbon-deuterium stretching with infrared spectroscopy (Figure 14).

\textsuperscript{21} E. Wasserman, \textit{J. Am. Chem. Soc.}, 1960, 82, 4433.
Notwithstanding the very low yields (ca. 1%), the most interesting aspect of this work concerned the introduction of the concept of mechanical bond in chemistry and the definition of the field of chemical topology. However, the insertion of a linear bifunctional molecule through a macrocycle is a strategy that is still used today, the so-called threading. The success of Wasserman prompted further scientific researches to develop new synthetic routes with improved yields. Thus, Schill and Lüttingraus published in 1964 the first template synthesis strategy of a [2]catenane.\(^\text{22}\) The rather long synthesis (Figure 15) took advantage of the acetal formed by a substituted cyclic catechol with bis-(12-chlorododecyl) ketone. The introduction of an amino group to give a nucleophilic intramolecular substitution, followed by acetal removal and oxidation phenol, brought the desired architecture. In this methodology the role of the acetal intermediate is crucial, because the intramolecular cyclization on nitrogen is forced by the tetrahedral geometry of the protected carbonyl carbon. This synthetic approach was subsequently used for the construction of the first [3]catenane.\(^\text{23}\)


In the early 80’s, Sauvage developed a very effective template synthesis strategy, taking advantage of the use of metal coordination centers. With this methodology he assembled a polyether bridged [2]catenane. A phenanthroline formed a complex with copper (I) threading a pre-formed macrocycle (Figure 16). A bis-iodide terminal polyether was used to bridge the free phenolic functions under high dilution conditions. Finally, the metal was removed with cyanide.

a)
The template effect can also come into play by exploiting electrostatic interactions. This strategy was widely investigated by the group of F. Stoddart,\(^\text{28,29}\) and is specific for aromatic compounds, as it develops on their packing interactions. In fact, \(\pi\)-donor/\(\pi\)-acceptor interactions between electron-poor paraquat (\(N,N\)-dimethyl-4,4\(^{\prime}\)-bipyridinium) and electron-rich hydroquinone or naphthoquinone moieties were utilized in order to preorganize a pseudorotaxane that can then be functionalized with appropriate groups to give a rotaxane or a catenane.


Quite different is the Hunter method,\textsuperscript{30} that uses H-bonding and $\pi$-$\pi$ interaction. With this method a \textbf{34\%} yield of catenane was obtained from a one-pot double macrocyclization reaction (\textbf{Figure 17}).

After this short background, required to introduce the reader into the world of interlocked structures, it is important to illustrate the procedures allowing their synthesis. Hereinafter, it is shown how scientists have developed, over the years, synthetic strategies able to realize complex interlocked structures with increased efficiency. From the simplest structure, the rotaxane, we will move on to catenanes until we will come to a growing of complexity in knot structures.

As in asymmetric synthesis, where absolute stereochemistry is controlled, control of topological stereochemistry has required the development of new synthetic strategies. The key step in any synthesis is the generation of an intermediate that contains latent topological properties: the latent topology is realised by macrocyclisation of this intermediate. In fact, as mentioned above, the synthesis of the more complex catenane must proceed via the simpler pseudo-rotaxane.

These intermediates are often not isolated and may only be present in the reaction mixture in very small amounts, but it is their properties that define the limits on the yields of catenane. Chemists working in this field soon realised that it was possible to trap the latent topological properties of the pseudo-rotaxane by capping the ends of the linear molecule with two bulky stopper groups to prevent the unthreading (Figure 18).  

![Diagram of rotaxane and pseudorotaxane structures](image)

**Figure 18.** Rotaxane obtained by *threading* and *stoppering*.

One special feature in common to all pseudorotaxane structures is related to their ability to "disassemble" due to the unthreading of the axle with respect to the wheel portion. Therefore, the structure can be defined as "interpenetrated" but not "interlocked", whereas an "interlocked" structure requires the presence of mechanical restriction between the constitutive components as in the case of rotaxanes. Thus, strictly speaking, rotaxanes are not topological isomers of their separate components, because they accept continuous deformations of the graphs until they are split. However, in experimental practice this

---

fracture does not occur if it is not accompanied by the breaking of a covalent bond, making these structures quite peculiar.

In addition to *threading*, a rotaxane can also be assembled by *clipping*, i.e. by closing the wheel around the axle. This method, shown in Figure 19, provides an initial complexation of a preformed dumbbell with a half wheel and subsequent [1 +1] cyclization reaction with an appropriate species.

![Figure 19: Cartoon representation of the clipping strategy for the synthesis of a rotaxane.](image)

In order to achieve high yields, is an essential prerequisite that balance is strongly shifted in the direction of complex formation. In this way, it is possible to minimize the formation of unthreaded cyclic species. A further synthetic method is known as *slipping strategy*, when, by temperature increasing, the macrocycle possesses enough energy to overtake one of the stoppers, slipping it reversibly. This approach takes advantage from rotaxane kinetic stability. In fact, cooling the dynamic complex, it becomes kinetically trapped as rotaxane.
Catenanes also follow the same synthetic strategies used for rotaxanes as summarized in Figure 21.

**Figure 20.** Cartoon representation of a *slipping* strategy for the synthesis of a rotaxane

**Figure 21.** Cartoon representation of (a) a statistical, (b) a directed, (c) a metal templated synthesis and (d) electrostatic interaction or H-bonding templated synthesis for a catenane.
1.3.2 Molecular knots

Knots and links are all around us (Figure 22). Be they functional or decorative, we encounter them constantly, when, for example, tying simple bow knots in our shoelaces or the four-in-hand knot in a necktie. These entangled and interlocked entities have also developed strong spiritual and symbolic meanings as human culture has advanced from prehistory, and now embody many tenets of both religious and secular societies. However, from a scientific point of view, the attention given to these systems is mainly due to their presence in natural systems such as DNA and proteins. In fact, aside from the DNA knottiness, of which has been exhaustively discussed in the previous pages, examples of natural macromolecules that contain knots, such as the protein lactoferrin and the enzyme ascorbic acid oxidase\textsuperscript{32} are known (Figure 22).

Following the mathematical rigorism, a knot is defined as a closed curve in the three-dimensional space that does never not intersect. Its representation on a plane is a “projection”. If knot projection intersects no more than two points, it is isomorphous to a loop, which is equivalent to a trivial knot: a twisted knot admits at least three intersections. A knot is “oriented” when it is possible to choose a traveling direction along it. It is clearly lawful to block together more than one knot to form a chain. From the just given definitions, it is clear the high lushness of this approach with a literally imaginable unlimited number of knot and chains, so that mathematicians have classified them in various tabs. For our purposes the most suitable is the one using the Alexander-Briggs notation (Figure 23). This notation takes advantage of a descriptor $x'y'z$ that represents a knot or link, where $x$ is the minimum number of nodes, or crossing points of any projection of the knot or link, $y$ is the number of components (in a knot, $y = 1$ and is usually not displayed in the Alexander-Briggs notation), and $z$ is the order of the knot among its peers with the same number of nodes and components, which describes, when embedding the knot or link on a sphere, the number of handles that must be added such that no crossings are observed.

---

Figura 23. A schematic tabulation of simple knots and links, accompanied by their trivial names and descriptors using the Alexander-Briggs notation.

However, the above described simplicity of method has not deterred chemists to employ commonly used names. Thus, for example, it is usual to refer to knot $3_1$ with the term *trefoil knot*, for his clear similarity with the plant. The $2_1^2$ knot is called *Hopf ring* (corresponding to catenane), whereas $4_1^2$ one is called *Solomon ring*. The advantage to use similar practical terminology finds its counterpart in an apparent loss of information about architecture topology.

The search for an appropriate nomenclature that allows to draw the supramolecular assembly graph from its name is a problem still open at the present. At the dawn of the millennium, Vögtle\textsuperscript{36} e co-workers have devised a very rigorous systematic, but unfortunately equally abstruse and poorly

manageable. At the moment, it is common to name catenanes or knots that have Alexander-Briggs zenith greater than or equal to 2 and to precede, between brackets, the number of interlocked units making up the architecture. e.g. [3]catenane (3 denoting the number of interlocked rings). The limitations of this approach are obvious; for example, *Hopf knot* and *Solomon Knot* are expressed with the same word of [2]catenane.

The search for aesthetically appealing molecules has been a goal of chemistry since its origins. In fact, nowadays chemists know how to create all types of exotic molecules. For example, Sauvage is a chemical has been primarily interested to *trefoil knot* which is the “simplest example of a non-trivial first knot’ (Figure 24). It is known to mathematicians as $3_1$, because it is formed by three crossings: is a *first knot* because, it is not separable into its components and, as such, it is the cornerstone of the mathematical knots theory.

![Figure 24. Trefoil knot representation](image)

Knots such as *trefoil knot* inspired the scientific community for several years. Apart from their aesthetic beauty, an interesting aspect that surrounds these architectures is related to their properties in relation to chirality. In fact, in the particular case of the trefoil, due to the asymmetric nature of the structure, two form of *trefoil knot* are known, a left-handed and a right-handed. Therefore, the object is chiral and, as a consequence of this peculiarity, it exists as two enantiomers (Figure 25).
Figure 25. A visualization of the inherent topological chirality of Trefoil Knots, wherein the two nonsuperimposable mirror images which comprise the (+) and (-) isomers are displayed side-by-side about a mirror plane, m.

Returning to Sauvage, his interest for this molecular architecture was such that immediately, in collaboration with Buchecker, he realized its synthesis (Figure 26). In fact, just after the development of the template method, he\textsuperscript{37} was able to intertwine a phenanthroline double helix, through intramolecular cyclization.

The yields in the first instance were scarce, but they were optimized by changing the bridging groups between the aromatic units and by exploiting a metathesis reaction in order to close the macrocycle (76%) (Figure 27). 

Figure 26. Knot Sauvage Synthesis.

Figure 27. Sauvage Trefoil Knot Synthesized via the metathesis.

More recently, Vögtle was successful in joining a macrocyclic trefoil by exploiting an appropriate hydrogen-bonding. This discovery led to a series of differently functionalized chiral knots.\(^{39}\) By suitable changes to the modern synthetic protocols, various laboratories have enriched the literature with even more intricate geometrical knots, among which the above mentioned Solomon and Borromean ring (Figure 28).\(^{40}\)

**Figure 28.** Examples of decorative Solomon Link (on the left) on an Italian mosaic tile; A schematic diagram of Solomon ring; Examples of Borromean Rings depicted in art, religion, and science. Schematic diagram showing metal template approach to Borromean Ring synthesis.

The latter, recalling to mind the heraldic family crest of a prestigious Italian Renaissance family from which it is named. It is composed of three macrocycles not concatenated but interlocked, for which the breaking of just one causes the dissociation of the others two. The building of this architecture is not trivial job. For example, the ring by ring assembly requires that they lie on orthogonal planes: a condition that is obtained through weak coordinating interactions (Figure 29).


Figura 29. Schematic representation of Borromean rings and molecular equivalents of its constituents.

At the peak of this crescendo in topological complexity, we come to discuss the most complex systems made in laboratory up to now: olimpiadane and suitanes. The first,\textsuperscript{41} represent the most ambitious outcome at the present obtained in the context of catenane architectures, because they consist of five linearly interlocked macrocycles (Figure 30). Their synthesis, carried out by Stoddart,\textsuperscript{42} provides that the architecture is composed of two crown ethers and three cycle-bis(paraquat-p-phenylene) units.

Suitanes, instead, consist of two units, one of which has a central body which exhibits a number of arms capable of recognizing cyclic molecules. These latter are connected with spacers to give the second unit, as if it was a suit sewn on the central body: an analogy used by its inventor, Stoddart. The number of protruding limbs is inserted between “suit” and “ane” in square brackets according a supramolecular chemistry custom. Thus, the simplest member of the series would be a suit[2]ane, a linear structure in which the suit surrounds the body with limbs protruding outwards in opposite directions (Figure 31), such that there is no easy way by which the suit can be removed from the body. Thus, the suit[2]ane depicted in Figure 32 was synthesized upon recognition of two [24]crown-8 molecules which were linked to a planar molecule bearing two bis-benzilammonium arms.

---

1.4. **Topological and functional complexity**

1.4.1 Rotaxanes and catenanes as supramolecular devices

After the journey through the classifications, now we will show how the topological complexity can imply a functional aspect. This will be done through a number of molecular machines representing the most natural evolution in the synthesis of such interlocked structures.

Artificial molecular machines capable of converting chemical, photochemical and electrochemical energy into mechanical motion represent a high-impact, fast-growing field of interdisciplinary research. These molecular-scale systems utilize a “bottom-up” approach centered on the design and manipulation of molecular assemblies and are potentially capable of delivering efficient actuation at length scales dramatically smaller than traditional microscale actuators. Much of the inspiration to construct such molecular devices and machines comes from the outstanding progress in molecular biology that has begun to reveal the functioning of the natural nanodevices, which are essential for life. Mechanically interlocked molecules, such as rotaxanes, are one of the most suitable candidates for molecular machines because the mechanical bond allows a large variety of

---

mutual arrangements of the molecular components, while conferring stability to the system. The interlocked architecture limits the amplitude of the intercomponent motion in the three dimensions; the stability of a specific arrangement is determined by the strength of the intercomponent interactions; and such interactions can be modulated by external stimulation.

These systems, initially gained interest due to their peculiar topology and the associated synthetic challenge, but recent efforts have showed that they are also attractive as nanoscale switches for molecular electronics and nanoelectromechanical systems because of their electrical properties and bi- or multistable behavior.

In general, these devices consist of at least two structures, one fixed and one sliding, which are mutually interlocked. The fixed part has a pair of sites of the same or different affinity against the complementary sliding one. In the first case, the sliding unit will oscillate between two identical stations. In the second, however, it will have a preferential status, from which the system may be removed by chemical stimulus, usually hydrogen acids or oxidizing agents, electronic or light, thus through reversible transformations.

Let us see some examples.

*Molecular shuttles.*

One of the first examples found in the literature of artificial molecular machine of a single component, is known as *molecular clamp* (Figure 32).

---

The system consists of two cyclic molecules (crown ethers) \( (a_1) \), connected by a central unit \( (b) \) that can give isomerization of the double bond after irradiation with an appropriate wavelength. In the ground state the central double bond has a *trans* stereochemistry; by beaming the system at 360 nm the *cis*-isomer is obtained leading to a closeness of the two units \( a_1 \). This structural change of the central unit enables the system to complex a potassium ion \( (K^+) \). Using light of 440 nm or leaving the system in the dark, the reverse process occurs, with consequent release of potassium ion \( (K^+) \). This mechanical action is comparable to a nanometric clamp which could be a prototype of systems able to "clean up" a body waste.

In a rotaxane, the movement of the ring along the axle corresponds, on a molecular level, to a "shuttle" motion along a track. An example of this type is represented by bistable rotaxane shown in Figure 33. It is comprised of a ring component that encircles a dumbbell-shaped component. The interaction between the two components is such that the ring can choose between one of the two recognition sites along the rod section of the dumbbell component. If two recognition sites with very different binding affinities can be grafted into the rod section of the dumbbell component, a bistable [2]rotaxane with switchable properties can be obtained (*Figure 33*) without damage to its molecular structure.

*Figure 32.* A molecular clamp by light activated, structure (on the left) and cartoon representation (on the right).
In particular, the dumbbell component $f$ bears two separate units, $f_1$ e $f_2$; the first unit is a ammonium site, the second unit is a di-pyridine cationic one which has donors-acceptors properties. These units represent two potential "stations" for the ring because it can interact with both $f_1$, through the formation of hydrogen bonding, and $f_2$, through a donor-acceptor interaction.\textsuperscript{47} Initially the ring lies on the station $f_1$ because the first type of interaction is stronger than the second; however, upon deprotonation of ammonium unit $f_1$ with a base, the interaction becomes weaker, which would lead to a switching to station $f_2$.

An acid addition will be able to restore the ammonium unit $f_1$ which would become free to interact again with ring $e$. The alternate movement of $e$ between $f_1$ and $f_2$ can be repeated several times due to the reversibility of the acid/base reaction. However, keep in mind that, the subsequent additions of acid and base lead to the formation of waste products, which cannot be eliminated, but accumulate, until they can impair the functioning of the system.

A higher level of complexity both in design and in construction of molecular machines is largely demonstrated by the system shown in Figure 34. It is a rotaxane constituted by a ring component $g$, with electron-donor characteristics, and a linear component consisted of several subcomponents: 1) a ruthenium complex $h$ which is able to absorb light and to act as a stopper; 2) two units, $j_1$ and $j_2$, with electron-acceptor properties, which are the two "stations" on which it may stop the ring $g$; 3) a rigid spacer $i$ and a second stopper $k$.

![Figure 34. A four-stroke engine based on rotaxane.](image)

The architecture of the system is such that following a light pulse, through a four-stage process, an alternate movement of the ring along the thread from right to left and back again is triggered without waste products production differently to the previous case. In particular, the initial situation of the system is one in which $g$ ring surrounds $j_1$ unit, that is a better electron-acceptor than $j_2$. Following light excitation of ruthenium complex $h$, occurs in
the system, a series of movements that can be described very schematically in this way:

a) destabilization of initial structure: following the absorption of light (process 1) is obtained an excited state of \( h \), which transfers an electron to \( j_1 \) station (process 2) surrounded by \( g \) ring. Following this electronic transfer \( j_1 \) station loses its electron acceptor characteristics of and no longer interacts with \( g \);

b) ring shift: failing its interaction with \( j_1 \), \( g \) ring moves (process 3) and switches to the station \( j_2 \) with which it is able to interact;

c) electronic reset: at this point a opposite process brings an electron from destabilized \( j_1 \) station, no longer surrounded by \( g \), to ruthenium complex that had initially transferred (process 4), in this way, electron acceptor character of \( j_1 \) station which is thus resumed;

d) nuclear reset: following electronic reset, \( g \) ring comes back to \( j_1 \) station (process 5), restoring structure.

Going on the same level of structural complexity, a very interesting example of molecular device is the Stoddart shuttle shown in Figure 35 as a prototype of a pH-dependent structure. It is a linear shuttle of nanometric size incorporating, in a macrocycle of bis piridyne units, an axle with a benzidine and alkoxy-phenolic site. Electrons poorness of the macrocycle makes its favorable placement near benzidine site; however, lowering the pH with trifluoroacetic acid, the protonation of amino groups leads migration to bis-phenol station.

**Figura 35.** Diagram of a pH dependent molecular shuttle.
This process is also capable of activation by a mono-electron electrochemical oxidation. Sauvage developed and realized a similar device using oxidation by electrolysis of copper from (I) to (II), coordinating a phenanthroline macrocycle along an axle with phenanthroline and tris-pyridine sites. Balzani demonstrated the ability to power photochemically it.

*Rotors.* Rotary movements are accessible with catenane species. In a catenane, structural changes caused by rotation of one ring with respect to the other can be clearly evidenced when one of the two rings contains two non-equivalent units. A bis-pyridinium cyclophane and a crown ether, integrated with a porphyrin ring, make up a motor that, at suitable pH, in acetonitrile rotates at 1500 Hz. The same mobile unit can be disposed on a crown ether integrated with a tetra-tiafulvalene ring, whose driving force can be also of electrochemical nature (Figure 36).

![Figure 36. Redox controlled ring rotation in a catenane containing a non-symmetric ring](image)

---

48 Ibid., 3376.
Artificial myofibrils.\textsuperscript{50} Molecular muscles, that convert chemical electrochemical and photochemical energy into mechanical work have attracted the attention of scientists working in the field of nanotechnology and rotaxanes are turning out very versatile in this regard. An example is the dibenzo[24]crown-[8] ether penetrated by an axle with secondary dialkylammonium sites and N,N'-dialkyl-4,4'-bipyridine. The device is controlled by pH value of environment.

Molecular Elevator.\textsuperscript{51} From the fusion of three crown ethers with an aromatic bridging unit a tritopic receptor (platform) has been obtained, which is able to recognize a tris-benzyl-ammonium salt (\textbf{Figure 37}). Climbs and descents of the platform are regulated by acid. The authors estimated that the force expressed by the device is around 200 pN for a movement of about 0.7 nm.

\textbf{Figure 37.} The molecular elevator of Stoddart and Balzani in a graphical representation.

1.4.2 Rotaxanes and catenanes in nanotechnology.

Over the last years, the scientific and technological world saw a continuous and intensive development of the so-called "nanotechnology", considered by many to be the engine of a new industrial revolution of the twenty-first century.

In current terminology the suffix “nano” is used to indicate materials and structures in which at least one dimension is less than 100 nm, but in reality Nanotechnology is a multidisciplinary field which goes back to theories and techniques developed in various scientific fields ranging from physics, to chemistry, to biology and material sciences, and to mathematics, directed to produce materials, components and devices at the molecular scale and then with dimensions on the order of nanometers.

**Figure 38.** Structure of the bistable [2]rotaxane used in the crossbar memory and SEMs of the nanowire crossbar memory
Particularly interesting in this field is the use of rotaxanes as a data storage elements in an electronic molecular 160 Kbit memory\textsuperscript{52} built with a density of $10^{11}$ bit/cm$^2$ (Figura 38).

Another example of application in molecular electronics involves the use of catenanes to build integrated circuits (Figura 39).\textsuperscript{53} Here, in fact, a layer of bistable [2]catenane, self-assembled on a particular support, acts as the junction between two electrodes.

![Figure 39. Molecular junction constituted by a layer of bistable [2] catenanes.](image)

Particularly symbolic is, however, the use of a rotaxane system as controller in a small molecular carrier.\textsuperscript{54} In particular, a film of silica was used as a solid support to connect a rotaxane system to nanoporous silica materials that can accommodate small organic molecules. In the interlocked system the cyclic unit can assume, as a function of an appropriate stimulus, different positions with respect to the linear unit. Thus, it plays a crucial role in the release and transport of small molecules (Figure 40).

---


A final application is a demonstration of the important connection/transduction of molecular properties to the macroscopic world. In particular, Leigh and coworkers \(^5\) have reported an example of supramolecular writing in which instead of using the usual magnetic support, the writing takes place on a surface covered with a thin film of rotaxanes.

(Figure 41). The authors observed that by applying to the rotaxane film an external perturbation through an atomic force microscope, the interaction between the tip and the thin film causes a "spontaneous" alignment of the molecules at the touched points', thus creating chains of "nano-balls" of 20-40 nanometers in diameter separated by 100 nanometers. The patterns thus obtained are permanently stable. There are three fundamental advantages with respect to the usual magnetic support: 1) the small dimensions of “nano-balls” which allow a higher density of information storage; 2) the absolute precision of pattern reproduction; 3) the unrequired planarity of the support. This latter can open up the way to three-dimensional flexible and complex devices with unlimited possibilities.
CHAPTER II

2.1 Complex Topologies based on Calixarenes

Chemical reaction of molecules in specific and selective ways forms the basis of the living world. Taking clue from this, chemists have shifted their focus from molecular chemistry to supramolecular chemistry. In the majority of cases, the supramolecular architecture is constructed through selective host-guest interactions. During the molecular evolution of biological system, the highly selective complexation process between the host and the guest must have played a central role. This attribute of biological life was mimicked in synthetic chemistry which later came to be known as the host-guest chemistry. A molecular complex is composed of at least one host and one guest components. The host is an organic molecule or ion whose binding sites converge. The guest is an organic molecule or ion or metal ion whose binding sites diverge.

The complexes of the host-guest chemistry are held together in unique structural relationship by forces other than those of covalent nature. Before the accidental discovery of crown ethers by Pedersen, the word “supramolecule” was not commonly used; but along with cyclodextrins the crown ethers were also included as another family of hosts. Later came the discovery of calixarenes, which became the third generation of supramolecules.

---

60 Gutsche CD, Dhawan B, No KH, Muthukrishnan R. Calixarenes. 4. The synthesis, characterization, and properties of the calixarenes from p-tert-butyphenol. *Journal of the American Chemical Society*. 1981;103(13):3782
supramolecules. All coexist under the title of supramolecules as well as host-guest chemistry.

There were and there are a good number of research groups in the field of calixarene chemistry whose work has already generated hundreds of original journal articles, extensive literature reviews and monographs\(^{61}\) in supramolecular chemistry. Although worked upon by many before his arrival in this field, the credit of naming this class of compounds goes to C. D. Gutsche, who perceived a similarity between the shapes of these cyclic tetramers and a type of Greek vase known as calix crater (Figure 42). He suggested the compound to be called “Calixarene.”

\[\text{Figure 42. calix crater and } p\text{-terz-butilcali}[4]\text{arenne structure}\]

This “crater” or “basket” plays a very important role in shaping the entire architecture of calixarene for its function in host-guest chemistry. With their well organized and preformed cavities, the calixarenes are able to act as

host molecules. Over the years, calixarenes have been frequently employed as platforms that permit the design and synthesis of interesting host compounds. The common convention to refer to calixarene derivatives is to report in square bracket the number $n$ of phenolic units involved in the structure: in this way the tetramer will be the calix[4]arene, the pentamer will be the calix[5]arene, etc. (Figure 43).

![Figure 43. The phenol-derived calixarene family.](image)

Two are the methodologies for synthesizing calix[n]arene macrocycles: the one-step synthesis and the multi-step approach.\(^\text{62}\)

**ONE-STEP SYNTHESIS:** this field was explored mainly by Gutsche and coworkers. The very early procedures were not reproducible and led to difficulty separable mixtures. In the early 1980s reliable procedures for $p$-tert-butylcalix[4]arene, $p$-tert-butylcalix[6]arene, and $p$-tert-butylcalix[8]arene were introduced. $p$-tert-butylphenol is the eligible starting material for this

synthetic approach and the choice of the right catalytic reagent for the condensation between \textit{p-tert}-butylphenol itself and formaldehyde (acidic or basic catalysis) is fundamental:

a) the condensation of the \textit{p-terz}-butylphenol with formaldehyde using NaOH as the base, followed by dissolution in diphenyl ether, produces \textit{p-tert}-butylcalix[4]arene;\textsuperscript{63}

b) the condensation of the \textit{p-tert}-butylphenol with formaldehyde using KOH as the base, followed by dissolution in xylene, produces \textit{p-tert}-butylcalix[6]arene;\textsuperscript{64}

c) the condensation of the \textit{p-tert}-butylphenol with formaldehyde in xylene, using NaOH as the base, produces \textit{p-tert}-butylcalix[8]arene.\textsuperscript{62}

The base-induced mechanism of formation of calixarenes has been studied in some details and the most reasonable proposal is that the precursor of any macrocycle is a linear oligomer carrying the appropriate number of aryl moieties.\textsuperscript{65}

Furthermore from the acid-catalyzed reaction a number of large calixarenes (\textit{n} = 9-20) can be isolated. The one-step synthesis is the widest used method to synthesize calixarene compounds.

2. \textit{FRAGMENT CONDENSATION SYNTHESIS}: this approach was studied mainly by Kämmerer and then by Böhmer and co-workers. It is useful when polysubstituted, dissymmetric, asymmetric and bridged structures are desired. The schematic route is to synthesize separately different parts and then to build the calixarene structure using a final reaction.

A large number of procedures to obtain calixarene related compounds are also known (oxa-, aza-, thio-, homocalixarenes).66

2.2 Supramolecular Properties of Calix[n]arenes

The easy functionalization is the major explanation for the rapid and widely differentiated development of calixarene chemistry.

The \( p \)-tert-butylphenol unit, in addition to making calixarenes readily available, allows their easy functionalization: the functionalization chemistry of calixarenes follows, in substance, the functionalization chemistry of generic phenols. The \(-\text{OH}\) at the calixarene lower rim can be converted to a variety of esters and ethers, while the \( p \)-tert-butyl group at the upper rim can be easily removed and replaced by a wide variety of different groups.

These two ways of functionalization can be carried out separately, and this makes calixarenes more advantageous with respect to other hosts, e.g. cyclodextrins.67

FUNCTIONALIZATION AT THE LOWER RIM: an \( O \)-alkylation or an \( O \)-acylation can be easily realized at the calixarene lower rim. The introduction of these groups can block the macrocycle in specific conformations if the substituents are bulky enough. Conformational properties and easy functionalization make calixarenes an interesting family of macrocycles from the supramolecular point of view.


FUNCTIONALIZATION AT THE UPPER RIM: the general way to functionalize the upper (or also called wide or exo) rim of calixarenes is to use an electrophilic substitution approach: the calixarene is able to react as a nucleophile, in virtue of the presence of the activated aromatic rings, with electrophilic agents. For this kind of approach, the para-position must be free, and this is easily accomplished by means of a complete or selective de-tert-butylation reaction, using $\text{AlCl}_3$ as Lewis acid catalyst, in toluene.\(^6\)

Different types of reactions have been carried out using the de-tert-butylated product, with the introduction of several functional groups at the upper rim. For example, halogenated derivatives can be obtained through a complete or selective bromination, using NBS in $\text{CH}_2\text{Cl}_2$, on a substrate bearing ether moieties at the lower rim.

As receptors, calixarenes are able to conjugate the two fundamental characteristics previously discussed: rigidity and flexibility. A series of books and reviews have been published which discuss in length the use of calixarene derivatives for the recognition of cation, anion, and neutral molecules.\(^1\)\(^3\) One of the major factors that have contributed to the proliferation of these research papers is the versatile structure of calixarenes for their use as complexing agents. The almost unlimited possibilities of chemical modification of calixarenes have made them a powerful class of host compounds. Their applications range from selective sensors, for analytical and medical applications, to wastewater decontaminant, to electrodes and membranes. The ability in terms of sensitivity and selectivity of calixarene hosts to discriminate among a group of guests make them a special class in supramolecular chemistry.

The possibility to functionalize calixarenes with alkyl chains of appropriate length or with bridging elements, allows to decrease the conformational mobility of the macrocycles and to increase their preorganization, which is

fundamental for a selective molecular recognition process. On the other hand, the rotational freedom allows the conformational changes necessary to accommodate guests in thermodynamically favored events.

The great development of the calixarenes chemistry allows, nowadays, to design receptors for every supramolecular challenge.\textsuperscript{69,70}

Furthermore, the appropriate introduction of functional groups at both rims allows to modulate their solubility in organic or aqueous solvents. For these reasons calixarenes have been used in every field of supramolecular chemistry: supramolecular recognition,\textsuperscript{71} self-assembly, crystal-engineering,\textsuperscript{72} supramolecular catalysis, etc. They have been used as platforms for the construction of more complex structures or for the realization of supramolecular adducts in solution or solid state.

\section*{2.3 Through-the-Annulus Threading of Large Calixarenes Induced by Very Loose Alkylammonium Ion Pairs}

As seen above, the most common technique for the construction of interlocked structures is the threading. In contrast with other macrocyclic hosts such as cyclodextrins,\textsuperscript{73} crown ethers,\textsuperscript{74} and cucurbiturils,\textsuperscript{75} \textbf{(Figure 44a)}, which readily give endo-complexation with native unsubstituted

\begin{thebibliography}{99}
\end{thebibliography}
derivatives, the threading of larger calix[n]arenes \((n = 6, 7 \text{ and } 8)\) (Figure 9b) is a rare event. In fact, calixarenes often require an extensive chemical modification to give similar results, and more frequently they act as simple scaffolds to construct podand-like receptors where the calix cavity very often remains unexploited.

![Crown Ethers, Cucurbiturils and Cyclodextrins](image)

**Figure 44.** (a) Crown Ethers, Cucurbiturils and Cyclodextrins; (b) Structure of larger calix[n]arene \((n = 6, 7 \text{ e } 8)\).

From another point of view, simple ethers of calixarenes can be seen as modified crown ethers, and in principle they should be able to give *endo*-complexation of organic cations in a similar way. Unfortunately, with the notable exception of some calix[5]arene derivatives, \(^{76}\) simple ethers of calixarenes have not been extensively studied.

---

calixarenes do not give such kind of complexation because they are too small in the case of calix[4]arenes or because they are not well preorganized in the case of larger calix[6-8]arenes.

Thus, for example, it is well-known that hexamethoxy-\(p\)-tert-butylcalix[6]arene (Figure 45) is conformationally mobile and does not complex halide, hexafluorophosphate, or tetraphenylborate salts of organic dialkylammonium cations.

![Figure 45. Threading of calix[6]arene derivative.](image)

On the other hand, the \textit{through-the-annulus} threading of dialkylammonium or bipyridium cations to give pseudorotaxane structures, which is very popular with large crown ethers, such as \([24]\)crown-8 or \([34]\)crown-10, is very rare in the calix[n]arene series. In fact, the very few examples regard the threading of viologen derivatives through the annulus of calix[6]arene hosts in which the anion coordinating ability of their ureido groups is exploited to favor the ion-pair dissociation (Figure 46).
Instead, no examples of threading through conformationally mobile, larger calix[6-7]arene annuli by dialkylammonium cations were known until the 2010 year.

At that time Gaeta et al\textsuperscript{77} introduced an efficient method to obtain \textit{endo}-cavity complexation and \textit{through-the annulus} threading of large calix[6-7]arenes exploiting the inducing effect of a weakly coordinating anion, tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB\textsuperscript{-}), which is known to be noncoordinating (or very weakly coordinating) to metal ions and therefore is referred to as a “superweak” anion (Figure 47).

In particular, they observed that the complexation of a nonsymmetrical alkylbenzylammonium cation (e.g., 3) by hexaalkoxycalix[6]arenes (e.g., 1a) can lead to a preference for the endo-alkyl stereoisomer (3⊂1a) over the endo-benzyl one up to a 30:1 ratio (Figure 48)\(^\text{77}\)

![Figure 47. Through-the-Annulus Threading of Calixarenes Induced by Very Loose Alkylammonium Ion Pairs](image)

This approach has been used for the synthesis of [2]rotaxanes\(^\text{78}\), which showed an unprecedented inversion of the wheel orientation. (Figure 49)

---

Subsequently, on this basis Gaeta and al envisioned that the appropriate covalent linkage of two such alkylbenzylammonium recognition motifs could allow good control of the consequent, it was extended to the synthesis of pseudo[3]rotaxane systems in which two calix[6]arene macrocycles are threaded by a bis(benzylalkylammonium) axle.\textsuperscript{79}

Because of the three-dimensional non-symmetrical nature of the calix[6]arene wheels and on the basis of the above-mentioned preference for the endo-alkyl complexation by calix[6]arene macrocycles three sequence stereoisomers could be obtained, which were termed as head-to-head (H,H), head-to-tail (H,T) and tail-to-tail (T,T) (Figure 51) with rationally designed axles. Taking advantage of these systems, it was possible to obtain the stereoprogrammed synthesis of the first examples of calixarene-based [3]rotaxane architectures. The base-acid treatment demonstrated that these systems act as molecular shuttles, which move on a nanometer scale level.⁸⁰

---

Figure 51. The three stereoisomeric [3]rotaxane structures named a. Tail to Tail, b. Head to head, and c. Head to Tail, d. Calix[6]arene-based catenane.

The directionality of the threading and the observed high stereoselection have enabled the synthesis of directional calix[6]arene-based catenane (Figure 51d.).
3.1 Goal of the research

All the above aspects represent interesting peculiar features of *calixarene threading*, which could be exploited for designing interlocked structures with new properties or functions. The present Ph.D. project was born with the basic logic to exploit the "superweak anion" approach for the synthesis and study of supramolecular interpenetrated and interlocked systems that have bis- and tris-calixarenes as cyclic units.

The above mentioned directionality of the threading and the observed high stereoselection have enabled the synthesis of directional double-calix[6]arene-based rotaxane and tris-calix[6]arene-based rotaxane.

![Diagram of rotaxane structures](image)

**Figure 52.** double-calix[6]arene and tris-calix[6]arene structures

Initially, the interest was directed towards the synthesis of a double-calixarene macrocycle, in which two calix-rings are linked to one another by a short *m*-xylylene spacer (*Figure 52*). Afterwards, the attention was
directed to the study of the *threading* of derivatives bearing two or more ammonium units in order to assess whether it is possible to obtain higher order double-threaded-pseudorotaxane systems (**Figure 53**).

**Figure 53.** Double-Calixarene-Based Handcuff-Rotaxane

Due to the natural asymmetry of calixarene macrocycle, which possess a lower rim different from the upper rim, it may be particularly interesting to evaluate the possibility to control the formation of adducts bearing a different sequence of *threading* of the cyclic systems with respect to the linear unit.

In fact, the marked *endo*-alkyl preference of calixarene derivatives could play a crucial role for the synthesis of a *double-threaded-[2]-pseudorotaxane* in which the two calix[6]arene wheels have a programmed relative orientation obtained through the rational choice of the threading element.
It will be also interesting to try to obtain a double-threaded calix[2]catenane system exploiting a macrocyclization reaction of a suitably functionalized double–threaded-pseudorotaxane. (Figure 55)

Figure 56. Polymeric double-threaded[2]catenane
Another interesting challenge will concern the study of threading conducted on tritopic receptor with a wide variety of ammonium axles.

**Figure 57.** A tritopic receptor

This triple-calixarene receptor could prove incredibly versatile, making accessible several topologies so far never obtained with the cyclophanes, such as triple-threaded [4]rotaxanes and even a triple-threaded [2]catenane.

**Figure 58.** The variety of topological isomers obtainable from a tris-calixarene host.
CHAPTER IV

Threading of a double-calix[6]arene systems

with mono-ammonium axles

4.1 Introduction
Rotaxanes and catenanes\textsuperscript{81} have gained a prominent position in the field of nanotechnology wherein they have been used as mechanical or electronic molecular devices. In addition, novel and fascinating functions are continuously emerging for interpenetrated architectures. At this regard, Leigh and coworkers\textsuperscript{82} have reported a sequence-specific synthesis of a peptide by a prototype of rotaxane-based artificial ribosome. Rotaxane and catenane architectures can be efficiently obtained through a template-directed threading\textsuperscript{83} of a linear axle through a macrocycle to form a pseudorotaxane derivative, which can be considered as the precursor of both rotaxanes and catenanes. At this regards, an early strategy provides the use of dialkylammonium axles and macrocycles such as cyclodextrins,\textsuperscript{84} crown ethers\textsuperscript{86} and cucurbiturils.\textsuperscript{86}

As illustrated in the first section an important contribution to this field of research comes from the work conducted by Neri et al.\textsuperscript{77} They have introduced an efficient method to obtain \textit{endo}-cavity complexation and \textit{through-the-annulus} threading of large calix[6-7]arenes exploiting the inducing effect of a weakly coordinating anion, tetrakis [3,5-bis (trifluoromethyl) phenyl]borate (TFPB).
From their studies they have deduced a sort of “molecular code” which has been used to develop an integrative self-sorting\(^{87}\). This molecular code includes the following stereochemical “\textit{endo-alkyl rule}” (Figure 59): "threading of a directional alkylbenzylammonium axle through a non-tert-butylated hexaalkoxy-calix[6]arene occurs with an endo-alkyl preference".\(^{86a}\)

![Diagram of endo-alkyl rule](image)

**Figure 59.** The endo-alkyl rule.\(^{86a}\) "threading of a directional alkylbenzylammonium axle through a non-tert-butylated hexaalkoxy-calix[6]arene occurs with an endo-alkyl preference."

On the basis of the endo-alkyl rule the first example of oriented calix[2]catenane \(5^+\) has been obtained,\(^{87}\) after macrocyclization, by using a directional alkylbenzylammonium axle. Encoding the endo-alkyl rule on a bis(benzylalkylammonium) axle two calix[6]arene directional wheels can be ordered in the right stereosequence (e.g.: \((H,H)-6^{2+}\) and \((T,T)-7^{2+}\)) to obtain stereoisomeric pseudo[3]rotaxane architectures.\(^{86b}\)

With regard to hosts with multiple cavities or rings, spectacular interpenetrated architectures have been obtained by double-threading of dialkylammonium axles through covalently linked macrocycle derivatives\(^88\) (e.g.: double-crown ethers, double-cucurbiturils or double-cyclodextrins). In particular, non-trivial "handcuff" catenane\(^89\) architectures have been reported in which two flat crown-rings, rigidly linked to one another, were threaded with a bis(ammonium) axle to perform a double-leg elevator (Figure C).

![Figure 60](image)

**Figure 60.** Schematic representation of the currently known prototypical examples of handcuff-derived architectures (A-C)

With respect to the use of flat macrocycles in C (Figure 60), a major synthetic challenge is represented by the use of three-dimensional nonsymmetrical rings (directional wheels), such as double-calixarene 8 because of the inherent difficulty in controlling the stereochemistry of the entire system.\(^90\)

---


fact, their threading abilities with dialkylammonium axles has remained unexplored (Figure 61).

Figure 61. Stereoisomeric oriented handcuff (pseudo)-[2]rotaxanes


Double-calix[6]arene 12 was synthesized exploiting the reaction sequence shown in Scheme 1. In particular, p-H-calix[6]arene 8 was monobenzylated with benzyl bromide in the presence of potassium carbonate as the base in refluxing dry CH$_3$CN to give mono-benzyl ether 9 (83%) after usual work-up. Compound 9 was smoothly hexamethylated by treatment with Mel in acetone in the presence of Cs$_2$CO$_3$ as the base to give 10 in 91% yield. Removal of the benzyl groups of 10 was easily accomplished by hydrogenolysis (H$_2$, Pd/C) to give pentamethoxy-calix[6]arene-mono-ol 11 in 51% yield. Finally, treatment of 2 equiv of 11 with 1 equiv of 1,3-bis(bromomethyl)benzene in dry THF/DMF (at reflux) in the presence of
NaH as the base afforded double-calix[6]arene derivative 12 in 33% yield, after purification by column chromatography.

Compounds 9, 10, 11 and 12 were fully characterized by $^1$H NMR, $^{13}$C NMR, and ESI(+) MS spectra. Regarding double-calix[6]arene 12 its $^1$H and $^{13}$C NMR data, acquired at room temperature, were fully consistent with its molecular symmetry. In particular, three singlets were present at 2.92, 3.11 and 3.17 ppm relative to OMe groups, while three singlets were present at 3.92, 3.97 and 4.00 ppm relative to ArCH$_2$Ar protons (Figure 62). In fact, compounds 12 is conformationally mobile and gives rise to sharp signals for the three symmetry related ArCH$_2$Ar groups. This is due to the small dimension of both the methoxy groups at the lower rim and the $p$-H “substituents” at the upper rim, which allow the through-the-annulus passage of both rims.\(^91\) Finally, a singlet was present at 4.84 ppm in the $^1$H NMR spectrum (Figure 62) of 12 relative to the OCH$_2$ protons of the m-xylylene bridge.

Figure 62. $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of double-calixarene derivative 12

4.3 Threading studies of 8 with mono-ammonium axles: double-threaded pseudo[2]rotaxanes 14 and 18

The synthesized derivative 8 was then used to study the threading with mono-ammonium axles $2^+$, $3^+$ and $4^+$ (Figure 63).

Figure 63. Linear mono-ammonium systems and double–calixarene host
4.3.1 Threading of double-calix[6]arene 12 with di-n-pentylammonium axle 2⁺

![Diagram](image)

**Figure 64.** Schematic representation of Directional threading of 12 with the di-n-pentylammonium axle 2⁺.

Interestingly, the addition of di-n-pentylammonium salt 2⁺·TFPB⁻ to a CDCl₃ solution of double-calix[6]arene 12 caused dramatic changes in its ¹H NMR spectrum (Figure 65). In fact, upon addition of 1 equivalent of 2⁺·TFPB⁻ salt a new set of signals emerged (Figure 65c) due to the formation of the singly threaded pseudo[2]rotaxane ion 2⁺⊂12 (Scheme 2). Under these conditions (1 equiv of axle 2⁺), the formation of a singly threaded pseudo[2]rotaxane ion 2⁺⊂12 (Scheme 2) was ascertained by the ESI(+) mass spectrum that gave a value of 1672.4 m/z as the base peak (Figure 65f), corresponding to a 1:1 host/guest stoichiometry, in which only one dialkylammonium axle was threaded into one of the two macrocycles of 12.

![Scheme 2](image)

**Scheme 2.**
In addition, the appearance of $n$-alkyl resonances in the upfield negative region of the $^1$H NMR spectrum of the 1:1 mixture of $2^+$ and 12 in CDCl$_3$ and the formation of AX systems for ArCH$_2$Ar groups corroborate the formation of the pseudo[2]rotaxane.$^{77}$ The 1:1 stoichiometry was confirmed by spectral integration. A COSY-45 spectrum (CDCl$_3$, 400 MHz, 298 K) of the 1:1 mixture of thread $2^{2+}$ and double-calix[6]arene 12 allowed a complete confident assignment of all shielded alkyl resonances. Thus, $\alpha$ protons at $-0.02$ ppm show a coupling with $\beta$ methylene group at $-1.07$ ppm, which presents a cross-peak with $\gamma$ protons at $-0.17$ ppm, finally
coupled with $\delta$ protons at 0.37 ppm (accidently isochronous with $\varepsilon$ methyl) (Figure 66).

![2D COSY spectrum](image)

Figure 66. Significant portion of the 2D COSY spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:1 mixture of 12 and 2$^+\cdot$TFPB$^-$. Interestingly, a 2D HSQC spectrum (Figure 67) revealed the presence of two cross-peaks between two singlets at 4.99 and 4.78 ppm and two pertinent carbon resonances at 75.4 and 74.5 ppm, which can be assigned to OCH$_2$ groups of the $m$-xylylene bridge. Naturally, these $^1J$ direct correlations were consistent with a singly-threaded pseudo[2]rotaxane structure.
Figure 67. Expansion of the HSQC (400 MHz, 298 K, CDCl\textsubscript{3}) spectrum of an equimolar solution of 2\textsuperscript{+} and 12.

Further addition of 1 equivalent of di-\textit{n}-pentylammonium axle 2\textsuperscript{+} led to a simplification of the \textsuperscript{1}H NMR spectrum of the mixture of 2\textsuperscript{+}-TFPB\textsuperscript{-} and 12 (Figures 65c-d). In particular, the appearance of a new singlet at 4.98 ppm (integrating for 4H) relative to OCH\textsubscript{2} groups of the \textit{m}-xylene bridge, and the presence of three singlets relative to OMe groups were indicative of the formation of a new, higher-symmetry, doubly-threaded pseudo[3]rotaxane (2\textsuperscript{+})\textsubscript{2}⊂12 in which two axles 2\textsuperscript{+} were threaded into the two macrocycles of 12. This was confirmed by \textsuperscript{1}H NMR signal integration and by an ESI(+) mass spectrum of a 2:1 mixture of 2\textsuperscript{+}-TFPB\textsuperscript{-} and 12, which gave a value of 915.5 \textit{m/z} as the base peak, corresponding to a 1:2 host/guest stoichiometry.

DFT calculations at the B3LYP/6-31G\textsuperscript{*} level of theory\textsuperscript{92} evidenced strongly stabilizing N-H···O hydrogen-bonds (Figure 68a) between the

\textsuperscript{92} Gaussian 09, Revision A.1, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.;
ammonium threads and the calix-wheels. Interestingly, the optimized structure of the \((2^+)_2\subset 12\) complex (Figure 68a) revealed an approximate \(C_2\) symmetry axis bisecting the \(m\)-xylol bridge and an \(anti\) conformation around the \(\text{O}^{\text{calix}}\cdot \text{CH}_2 \text{xylyl}\) bonds of with dihedral angles of 168.2° and 167.2° (Figure 68). Consequently, the two calix cavities are diverging with their main axes almost perpendicular. In particular, the two mean planes of the calixarene oxygens form an angle of 60.0°. The preference for the \(anti\) conformation around the \(\text{O}^{\text{calix}}\cdot \text{CH}_2 \text{xylyl}\) bonds was confirmed by molecular dynamics (MD) simulation at 500 K which clearly evidenced that about 48% of the coconformers sampled during the entire MD simulation (20000 ps) showed a dihedral angle in the 150-170° range around the \(\text{O}^{\text{calix}}\cdot \text{CH}_2 \text{xylyl}\) bonds (Figure 68c).
Figure 68. (a) Energy-minimized structures of the $(2^+)_2\subset 12$ complex (B3LYP DFT calculation using the 6-31G* basis set). (b) Detailed view of the predicted staggered conformations around O$_{calix}$-CH$_2$ bonds of the m-xylyl bridge. (c) Variation in the dihedral angle between O$_{calix}$-CH$_2$ observed during the MD simulation at 500 K (time given in ps).

The total binding constant ($K_{tot}$) for $(2^+)_2\subset 12$ complex, determined by quantitative $^1$H NMR analysis of its 2:1 titration mixture in CDCl$_3$ and using tetrachloroethane (TCHE) as internal standard,$^{93}$ gave a value $>10^7$ M$^{-2}$, which was beyond the limit of reliability of the NMR technique. Therefore, in order to have more accurate measurements$^{94}$ we decided to evaluate $K_{tot}$ in the presence of a polar competing solvent such as CD$_3$CN. Thus, in a

---


mixture of CDCl₃/CD₃CN (99/1, v/v), we were able to measure a value of $K_{\text{tot}} = 4.4 \pm 0.3 \times 10^4$ M⁻² for the (2⁺)₂⊂12 complex by quantitative $^1$H NMR analysis and using TCHE as internal standard.

4.3.2 **Directional threading of double-calix[6]arene 12 with nonsymmetrical n-butylbenzylammonium axle 3⁺**

![Figure 69](image)

Figure 69. Schematic representation of Directional threading of 12 with the n-butylbenzylammonium axle 3⁺

On the basis of results we decided to verify what happens in the case of double-calix[6]arene host 12. In fact, the threading of 12 with the n-butylbenzylammonium axle 3⁺ could give rise to three directional double-threaded pseudo[3]rotaxane diastereoisomers with *endo*-alkyl/*endo*-alkyl, *endo*-benzyl/*endo*-alkyl, or *endo*-benzyl/*endo*-benzyl relative orientations (Figure 71).

![Figure 71](image)

Figure 71. Possible double-threaded pseudo[3]rotaxane stereoisomers by directional threading of 12 with the n-butylbenzylammonium axle 3⁺.
Of course, on the basis of the above discussed endo-alkyl rule (Figure 59), we expected that the endo-alkyl/endo-alkyl stereoisomer should be favored. As above, the addition of butylbenzylammonium 3+ to a solution of 12 in CDCl₃ caused significant changes in its ¹H NMR spectrum. In particular, 1 equiv of butylbenzylammonium 3+ led to a new specie corresponding to the singly-threaded complex 3+⊂12, in slow exchange with the free host 12. The 1:1 host/guest stoichiometry was confirmed by means of ESI(+) MS and integration of ¹H NMR signals. In fact, the ESI(+) mass spectrum of a 1:1 mixture of 3+-TFPB− and 12 gave a value of 1680.3 m/z as the base peak (Figure 70g), corresponding to a singly threaded pseudo[2]rotaxane ion 3+⊂12 (Scheme 3).

Scheme 3

In analogy to the above discussed 2+⊂12 pseudorotaxane, also in this case two singlets at 4.96 and 4.77 ppm relative to the OCH₂ protons of the m-xylene bridge corresponding to the singly-threaded pseudo[2]rotaxane were observed in the ¹H NMR spectrum of the 0.75:1 mixture of 3+ and 12 (Figure 71d).

The progressive addition of 3+ led to the disappearance of these two singlets while a new singlet emerged at 4.96 ppm corresponding to the doubly-threaded pseudo[3]rotaxane (3+)₂⊂12, as confirmed by ESI(+) MS and ¹H NMR signal integration.

As concerns the stereochemistry of the threading, the ¹H NMR spectra of the 1:1 and 1:2 mixtures of 12 and 3+ showed typical signatures at highfield negative values (from 1.0 to −1.0 ppm) characteristic of an endo-alkyl complexation.⁷⁷,⁸⁰ This result and the absence of shielded
benzylic resonances in the 4–6 ppm region, typical of an endo-benzyl complexation, were clear-cut proofs that in both singly- and doubly-threaded pseudorotaxanes an endo-alkyl orientation of butylbenzylammonium threads was present. This result demonstrated the validity of the endo-alkyl rule also for double-calixarenes and confirmed the possibility to control the directionality of the double-threading in these systems.

Figure 71. $^1$H NMR (400 MHz, 298 K, CDCl$_3$) of: a) 12 (aa x10$^{-3}$ M); b) 12 and 0.25 equiv of 3$^+$; c) 12 and 0.5 equiv of 3$^+$; d) 8 and 0.75 equiv of 3$^+$; e) 12 and 1.0 equiv of 3$^+$; f) 12 and 2.0 equiv of 3$^+$; g) significant portion of the ESI(+) mass spectrum of a mixture of 12 and 1 equiv of 3$^+$.

---

In analogy to the $(2^+)_2\subset 12$ complex above described, DFT calculations at the B3LYP/6-31G* level of theory also in this case confirmed the presence of stabilizing N-H····O hydrogen-bonds (Figure 72a) between the ammonium cations and the calixarene oxygens. The optimized structure of the $(3^+)_2\subset 12$ complex (Figure 72a) was very similar to that $(2^+)_2\subset 12$, showing again an anti conformation around $O^{\text{calix}}$-CH$_2$xylyl bonds with dihedral angles of 162.7° and 166.1°. This conformational preference was confirmed by MD simulation at 500 K, which clearly evidenced that about 46% of the sampled coconformers showed a dihedral angle in the 150-170° range around the $O^{\text{calix}}$-CH$_2$xylyl bonds (Figure 72b).

![Figure 72](image)

**Figure 72.** (a) Energy-minimized structures of the $(3^+)_2\subset 12$ complex (B3LYP DFT calculation using the 6-31G* basis set). (b) Variation in the dihedral angle between $O^{\text{calix}}$-CH$_2$ observed during the MD simulation at 500 K (time given in ps).
The extension of DFT calculations to the other endo-benzyl/endo-alkyl and endo-benzyl/endo-benzyl orientational isomers of (3*)₂⊂12 (Figure 73) confirmed the higher stability of endo-alkyl/endo-alkyl orientation experimentally evidenced by ¹H NMR. In fact, this latter orientation was 3.9 kcal/mol more stable than the endo-benzyl/endo-alkyl one, which in turn was 4.3 kcal/mol more stable than the endo-benzyl/endo-benzyl orientational isomer.

Figure 73. Energy-minimized structures of endo-alkyl/endo-alkyl (a), endo-benzyl/endo-alkyl (b) endo-benzyl/endo-benzyl (c) orientational isomers of pseudo[3]rotaxane (3*)₂⊂12 (B3LYP DFT calculation using the 6-31G* basis set).

As discussed above, also in this case the total binding constant (Kₜₒₜ) of the (3*)₂⊂12 complex was determined by quantitative ¹H NMR analysis of its 2:1 titration mixture in CDCl₃/CD₃CN (99/1, v/v), to give a value of 8.8±0.2×10⁴ M⁻². This value is only slightly higher with respect to that found (4.4±0.3×10⁴ M⁻²) for dipentylammonium complex (2*)₂⊂12.

In order to further validate the endo-alkyl rule we decided to study the threading of double-calix[6]arene 12 with dibenzylammonium axle 4⁺. In particular, the addition of increasing amounts (0.5-1.5 equivalents) of dibenzylammonium salt 4⁺-TFPB⁻ to a CDCl₃ solution of 12 (Scheme 4) did not caused significant changes in its ¹H NMR spectrum. This results and the absence of shielded benzylic resonances in the 4─6 ppm region typical of an endo-benzyl complexation, were clear-cut proof that both singly-threaded
and doubly-threaded pseudorotaxane structures were not formed. This indicates that the endo-benzyl complexation by double-calix[6]arene 12 is an unfavourable process.

\[ 12 + 4^\prime\cdot\text{TFPB} \xrightarrow{\text{CDCl}_3} \]  
\[ + 2^\prime\cdot\text{TFPB}^- \xrightarrow{\text{CDCl}_3} \]

**Scheme 4**


As a natural complement to derivative 12, we decide to synthesize double-calix[6]arene 21 bearing \( p \)-tert-butyl groups at the upper rim in place of the “\( p \)-H-substituents” of 12. The synthesis of 21 was realized by exploiting the reaction sequence shown in **Scheme 5**, very similar to that already described for derivative 12.

All the compounds 18, 19, 20 and 21 were fully characterized by means of $^1$H NMR, $^{13}$C NMR, and ESI(+) MS spectra. Regarding double-calix[6]arene derivative 16, its $^1$H and $^{13}$C NMR data, acquired at room temperature, were fully consistent with its molecular symmetry. In particular, three singlets were presents at 2.92, 3.11 and 3.17 ppm relative to OMe groups (Figure 73). It is interesting to note that the increased steric hindrance to the conformational interconversion generated by the t-Bu groups at the upper rim gives rise to the fine structure of three AX systems for the symmetry related ArCH$_2$Ar protons [4.50 e 3.58 ppm ($J = 14.7$ Hz), 4.17 e 4.04 ppm ($J$...
= 14.5 Hz), 3.83 e 3.68 ppm (J = 15.1 Hz)]. Finally, a singlet was present at 4.89 ppm in the $^1$H NMR spectrum (Figure 74) of 21 relative to OCH$_2$ protons of the m-xylylene bridge.

**Figure 74.** $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of double-calixarene derivative 21
4.5 Threading studies of 21 with mono-ammonium axles: double-threaded pseudo[2]rotaxanes 22 and 23

Figure 75. Linear mono-ammonium systems (2+, 3+, 4+) and double-calixarene host 21

4.5.1 Threading of double-calix[6]arene 21 with di-n-pentylammonium axle 2+

Figure 76. Schematic representation of Directional threading of 21 with the di-n-pentylammonium axle 2+.
In analogy to the above discussed $2^+ \subset 12$ pseudorotaxane, also in this case two singlets at 4.96 and 4.77 ppm relative to the OCH$_2$ protons of the $m$-xylylene bridge corresponding to the singly-threaded pseudo[2]rotaxane were observed in the $^1$H NMR spectrum of the 0.75:1 mixture of $2^+$ and 21 (Figure 76d). The progressive addition of $2^+$ led to the disappearance of these two singlets while a new singlet emerged at 4.96 ppm corresponding to the doubly-threaded pseudo[3]rotaxane ($2^+$)$_2 \subset 21$, as confirmed by ESI(+) MS and $^1$H NMR signal integration. (Figure 76)

Scheme 6

Figure 77. $^1$H NMR (400 MHz, 298 K, CDCl$_3$) of: a) 21(aa x 10$^{-3}$ M); b) 21 and 1.0 equiv of $2^+$; c) 21 and 2.0 equiv of $2^+$; d) 21 and 5.0 equiv of $2^+$; significant portion of the ESI(+) mass spectrum of a mixture of 16 and 1 equiv of $2^+$. 
In addition, the appearance of \( n \)-alkyl resonances in the upfield negative region of the \( ^1 \)H NMR spectrum of the 1:1 mixture of \( 2^* \) and \( 21 \) in CDCl\(_3\) and the formation of new AX systems for ArCH\(_2\)Ar groups corroborate the formation of the pseudo[2]rotaxane. In addition, the 1:1 stoichiometry was confirmed by spectral integration. A COSY-45 spectrum (CDCl\(_3\), 400 MHz, 298 K) of the 1:1 mixture of thread \( 2^2^+ \) and double-calix[6]arene \( 21 \) allowed a complete confident assignment of all shielded alkyl resonances. Thus, \( \alpha \) protons at −0.02 ppm show a coupling with \( \beta \) methylene group at −1.04 ppm, which presents a cross-peak with \( \gamma \) protons at −0.15 ppm, finally coupled with \( \delta \) protons at 0.37 ppm (accidentally isochronous with \( \varepsilon \) methyl) (Figure 78.).

![Figure 78.](image)

**Figure 78.** Significant portions of the 2D COSY spectrum (400 MHz, 298 K, CDCl\(_3\)) of the 1:2 mixture of \( 21 \) and \( 2^*\)·TFPB\(^-\).
4.5.2 Directional threading of double-calix[6]arene 21 with nonsymmetrical n-butylbenzylammonium axle 3+

As illustrated above the “endo-alkyl rule” (Figure 59) is also valid for calix[6]arenes bearing the tert-butyl groups at the upper rim, consequently it is particularly relevant to verify its validity on double-calix[6]arene host 21. Thus, in analogy to 12, the threading of 21 with n-butylbenzylammonium axle 3+ could give rise to three directional double-threaded pseudo[3]rotaxane diastereoisomers with endo-alkyl/endo-alkyl, endo-benzyl/endo-alkyl, or endo-benzyl/endo-benzyl relative orientations (Figure 81).

**Figure 79.** Schematic representation of Directional threading of 21 with the n-butylbenzylammonium axle 3+.

**Figure 80.** Possible double-threaded pseudo[3]rotaxane stereoisomers by directional threading of 21 with the n-butylbenzylammonium axle 3+.

Of course, on the basis of the above discussed endo-alkyl rule, we expected that the endo-alkyl/endo-alkyl stereoisomer should be favored. As above, the addition of butylbenzylammonium 3+ to a solution of 21 in CDCl₃
caused significant changes in its $^1$H NMR spectrum. In particular, 1 equiv of $3^+$ led to a new species corresponding to the singly-threaded complex $3^+\subset 21$, in slow exchange with the free host $21$. The 1:1 host/guest stoichiometry was confirmed by means of ESI(+) MS and integration of $^1$H NMR signals.

Scheme 7.

In analogy to the above discussed $2^+\subset 12$ pseudorotaxane, also in this case two singlets at 4.96 and 4.77 ppm relative to the OCH$_2$ protons of the $m$-xylylene bridge corresponding to the singly-threaded pseudo[2]rotaxane were observed in the $^1$H NMR spectrum of the 0.75:1 mixture of $3^+$ and $12$ (Figure 70d). The progressive addition of $3^+$ led to the disappearance of these two singlets while a new singlet emerged at 4.96 ppm corresponding to the doubly-threaded pseudo[3]rotaxane $(3^+)_2\subset 12$, as confirmed by ESI(+) MS and $^1$H NMR signal integration.
As concerns the stereochemistry of the threading, the $^1$H NMR spectra of the 1:1 and 1:2 mixtures of 21 and 3$^+$ showed typical signatures at highfield negative values (from 1.0 to $-1.0$ ppm) characteristic of an endo-alkyl complexation.$^{77,80}$ This result and the absence of shielded benzylic resonances in the 4–6 ppm region, typical of an endo-benzyl complexation, were clear-cut proofs that in both singly- and doubly-threaded pseudorotaxanes an endo-alkyl orientation of butylbenzylammonium threads was present.$^{95}$ This result demonstrated the validity of the endo-alkyl rule also for double-calixarenes bearing the t-Bu groups at the upper rim and confirmed the possibility to control the directionality of the double-threading in these systems.
Figure 82. Significant portions of the 2D COSY spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:2 mixture of 21 and 3$^+$·TFPB$^-$.

4.5.3. **Threading of double-calix[6]arene 21 with dibenzylammonium axle 4$^+$**

![Diagram showing threading](image)

Figure 83. Schematic representation of threading of 21 with the dibenzylammonium axle 4$^+$.

Scheme 8
Additional interesting threading results were obtained with dibenzylammonium salt (Bn$_2$NH$_2$*•TFPB$^-$) and tert-butylated double-calix[6]arene 21 in CDCl$_3$ (Scheme 8). In fact, unlike derivative 12, the threading of 21 and 4*•TFPB$^-$ gave rise to endo-benzyl complexation.

In particular, the addition of 0.5 equivalents of di-benzylammonium salt 4*•TFPB$^-$ to a solution of 21 in CDCl$_3$, caused dramatic changes in its $^1$H NMR spectrum (Figure 80). A new set of signals emerged (Figure 80c) due to the formation of the singly threaded pseudo[2]rotaxane. Particularly diagnostic was the presence of shielded benzylic resonances in the 4—6 ppm region, related to benzylic orto, meta e para protons shielded by calix[6]arene cavity (Figure on the left) that were clear-cut proof that a singly-threaded pseudorotaxane structure was formed by endo-benzyl complexation.
A further addition of 1 equivalent of di-benzylammonium axle $4^+$ led to a simplification of the $^1$H NMR spectrum of the mixture of $4^+\cdot$TFPB$^-$ and 21 (Figures c-d). In particular, the appearance of a new singlet at 4.98 ppm (integrating for 4H), relative to OCH$_2$ groups of the m-xylylene bridge, and the upfield shift of the three OMe singlets was indicative of the formation of a new, higher-symmetry, doubly-threaded pseudo[3]rotaxan ($4^+)_2\subset 21$ in which two axles $4^+$ were threaded into the two macrocycles of 21. This was confirmed by $^1$H NMR signal integration and by an ESI(+) mass spectrum of a 2:1 mixture of $4^+\cdot$TFPB$^-$ and 21, which gave a value of 1290.3 m/z as the base peak, corresponding to a 1:2 host/guest stoichiometry. A COSY-45 spectrum (CDCl$_3$, 400 MHz, 298 K) allowed a complete confident assignment of all shielded benzyl resonances. Thus, ortho protons at 4.6 ppm show a coupling with meta protons at 5.3 ppm, which are coupled to para ones at 6.0 ppm.
Figure 85. Significant portions of the 2D COSY spectrum (400 MHz, 298 K, CDCl₃) of the 1:2 mixture of 21 and 4⁺:TFPB⁻.

4.6 Conclusion

In summary, this part of the Ph.D. thesis has been devoted to the threading abilities of double-calix[6]arene hosts 12 and 21 with mono-ammonium axles in the presence of the TFPB superweak anion. ¹H NMR and ESI(+) MS spectra evidenced the stepwise formation of singly- and doubly-threaded pseudorotaxane architectures by changing the host/guest stoichiometry from 1:1 to 1:2. The directional threading of nonsymmetrical n-butylbenzylammonium axle 3⁺ with double-calix[6]arene host 12 and 21 occurs with an endo-alkyl preference in accordance with the known “endo-alkyl rule”.

DFT calculations indicated that the lowest-energy structures of the pseudo[3]rotaxane complexes are characterized by an anti conformation.
around the $O^{calix} \cdot CH_2^{bridge}$ bonds which led to a spatial divergence of the two calix-cavities. Another important aspect concerns the *endo*-benzyl complexation observed only in the case of "*tert*-butylated" double-calix[6]arene 21 to form singly- and doubly-threaded pseudorotaxane architectures.

4.7 Experimental section

ESI(+)-MS measurements were performed on a Micromass Bio-Q triple quadrupole mass spectrometer equipped with electrospray ion source, using CHCl$_3$ as solvent. All chemicals were reagent grade and were used without further purification. When necessary compounds were dried in vacuo over CaCl$_2$. Reaction temperatures were measured externally. Derivative $2^+ \cdot TFPB^{-77}$, $3^+ \cdot TFPB^{-77}$, and $12^{96}$ were synthesized according to literature procedures. NMR spectra were recorded on Bruker Avance-400 spectrometer [400 (1H) and 100 MHz (13C)], Bruker Avance-300 spectrometer [300 (1H) and 75 MHz (13C)], or Bruker Avance-250 spectrometer [250 (1H) and 63 MHz (13C)]; chemical shifts are reported relative to the residual solvent peak (CHCl$_3$: $\delta$ 7.26, CDCl$_3$: $\delta$ 77.23). COSY-45 spectra were taken using a relaxation delay of 2 seconds with 30 scans and 170 increments of 2048 points each. 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. Monte Carlo conformational searches (10000 steps) were performed with MacroModel-9/Maestro-4.1 program using CHCl$_3$ as solvent (GB/SA model). MD simulations were performed at $T = 500$ K, for 20000 ps, using a time step of 1.0 fs.

---

4.7.1 Synthesis and characterization of double-calixarene 12.\textsuperscript{96}

CHART 1
**Synthesis of Double-Calix[6]arene 12**

K$_2$CO$_3$ (0.94 g, 6.80 mmol) was added, under stirring, to a solution of $p$-H-calix[6]arene 8 (4.00 g, 6.30 mmol) in dry CH$_3$CN (300 mL). The mixture was kept at reflux under stirring, and after 1 h benzylbromide (0.82 mL, 1.18 g, 6.80 mmol) was added. The reaction was stirred at reflux for 3 h, 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 (50 mL), brine (50 mL) and dried over Na₂SO₄. The crude product was triturated with methanol, collected by filtration, and dried, to give 9 as a white solid (3.74 g, 5.17 mmol, 76%), which was sufficiently pure for subsequent synthetic manipulations. **ESI(+) MS:** m/z = 727 (MH⁺); **¹H NMR** (400 MHz, CDCl₃, 323 K): δ 9.68 (s, OH, 2H), 9.58 (s, OH, 1H), 8.66 (s, OH, 2H), 7.72 (br d, ArH, 2H), 7.60 (br t, ArH, 2H), 7.40 (br t, ArH, 1H), 7.11−6.77 (overlapped, ArH, 18H), 5.22 (s, OCH₂Ar, 2H), 4.01 (br s, ArCH₂Ar, 4H), 3.92 (s, ArCH₂Ar, 4H), 3.71 (s, ArCH₂Ar, 4H); **¹³C NMR** (63 MHz, CDCl₃, 298 K): δ 152.6, 151.8, 150.8, 149.4, 136.4, 133.5, 129.7, 129.6, 129.4, 129.2, 129.1, 129.0, 128.7, 128.1, 127.7, 125.7, 122.1, 121.4, 120.8, 77.9, 32.2, 32.1, 32.09; Anal. Calcd for C₄₉H₄₂O₆: C, 80.97; H, 5.82. Found: C, 81.05; H, 5.73.

**Figure 86.** ¹H NMR spectrum of derivative 9 (400 MHz, CDCl₃, 323 K).
A solution of 9 (3.74 g, 5.17 mmol) in acetone (300 mL) was added of Cs₂CO₃ (15 g, 46 mmol). The mixture was stirred at the reflux temperature for 2 h and then iodomethane (15 g, 6.6 mL, 106 mmol) was added. The reaction mixture was stirred for 12 h under reflux. The solution was concentrated to dryness and the residue was partitioned between 1 N HCl (50 mL) and CH₂Cl₂ (50 mL). The organic phase was washed with water (3×30 mL) and dried over Na₂SO₄. Solvent evaporation under vacuum afforded a crude product that was triturated with methanol, collected by filtration, and dried, to give 10 as a white solid (3.70 g, 4.64 mmol, 90%), which was sufficiently pure for subsequent synthetic manipulations. ESI(+)
**MS:** $m/z$ 797 ($\text{MH}^+$); $^1\text{H NMR}$ (250 MHz, CDCl$_3$, 298 K) $\delta$: 7.40–6.79 (overlapped, ArH, 23H), 4.76 (s, OCH$_2$Ar, 2H), 3.99, 3.97, 3.94 (s, ArCH$_2$Ar, 4H each one), 3.23 (s, OCH$_3$, 6H), 3.13 (s, OCH$_3$, 3H), 2.97 (s, OCH$_3$, 6H);

$^{13}\text{C NMR}$ (63 MHz, CDCl$_3$, 298 K): $\delta$ 156.2, 156.1, 154.6, 137.8, 134.9, 134.75, 134.71, 134.58, 134.56, 134.4, 129.8, 129.6, 129.3, 128.9, 128.7, 128.5, 128.3, 127.9, 127.8, 123.8, 123.6, 123.5, 74.9, 60.3, 60.2, 30.6, 30.4, 29.8. Anal. Calcd for C$_{54}$H$_{52}$O$_6$: C, 81.38; H, 6.58. Found: C, 81.45; H, 6.49.

**Figure 88.** $^1\text{H NMR}$ spectrum of derivative 10 (250 MHz, CDCl$_3$, 298 K).
A solution of 10 (3.70 g, 4.64 mmol) in CHCl₃ (400 mL) was added of Pd/C (10%) and stirred for 4 h under H₂ at 25 °C. After filtration of the catalyst, the solvent was evaporated to give 11 (1.47 g, 2.07 mmol, 45%). ESI(+) MS: m/z 707 (MH⁺); ¹H NMR (300 MHz, CDCl₃, 298 K): δ 7.38 (s, OH, 1H), 7.12–6.72 (overlapped, ArH, 18H), 3.99 (s, ArCH₂Ar, 8H), 3.85 (s, ArCH₂Ar, 4H), 3.57 (s, OMe, 3H), 3.34 (s, OMe, 6H), 3.23 (s, OMe, 6H); ¹³C NMR (63 MHz, CDCl₃, 298 K): δ 156.8, 156.6, 155.1, 152.3, 134.34, 134.32, 134.1, 134.07, 133.4, 129.7, 129.3, 128.9, 127.2, 124.3, 123.5, 123.4, 119.6, 61.0, 60.5, 60.3, 31.4, 31.1, 30.6. Anal. Calcd for C₄₇H₄₆O₆: C, 79.86; H, 6.56. Found: C, 79.95; H, 6.45.
Figure 90. $^1$H NMR spectrum of derivative 11 (300 MHz, CDCl$_3$, 298 K).

Figure 91. $^{13}$C NMR spectrum of derivative 11 (63 MHz, CDCl$_3$, 298 K).
NaH (1.30 g, 54.2 mmol) was added at 0 °C, under stirring, to a solution of derivative 11 (1.47 g, 2.07 mmol) in dry THF/DMF (75 mL, 7/3 v/v). The mixture was kept at 25 °C under stirring, and after 1 h, 1,3-bis(bromomethyl)benzene (0.26 g, 0.98 mmol) was added. The reaction was stirred at reflux 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 (50 mL), brine (50 mL), and dried over Na₂SO₄. The crude product was purified by column chromatography (SiO₂; Et₂O/CH₂Cl₂ 3/97) to give derivative 12 as a white solid (1.04 g, 0.69 mmol, 33%).

ESI(+) MS: m/z = 1516 (MH⁺); ¹H NMR (400 MHz, CDCl₃, 298 K): δ 7.06-6.78 (overlapped ArH, 40H), 4.80 (s, OCH₂Ar, 4H), 3.96 (s, ArCH₂Ar, 8H), 3.92 (s, ArCH₂Ar, 8H), 3.90 (s, ArCH₂Ar, 8H), 3.16 (s, OCH₃, 12H), 3.11 (s, OMe, 6H), 2.89 (s, OCH₃, 12H); ¹³C NMR (75 MHz, CDCl₃, 298 K): δ 156.7, 156.6, 156.3, 154.5, 137.9, 134.9, 134.8, 134.7, 134.6, 134.5, 130.0, 129.7, 129.3, 128.9, 128.8, 128.7, 128.2, 127.5, 127.4, 123.6, 74.9, 60.4, 60.2, 30.7, 30.4. Anal. Calcd for C₁₀₂H₉₈O₁₂: C, 80.82; H, 6.52. Found: C, 81.80; H, 6.43.
Figure 92. $^1$H NMR spectrum of derivative 12 (400 MHz, CDCl$_3$, 298 K).

Figure 93. $^{13}$C NMR spectrum of derivative 12 (75 MHz, CDCl$_3$, 298 K).
Figure 94. ESI(+) MS spectrum of derivative 12.


Derivative 13

Double-calixarene derivative 12 (1.81·10⁻³ g, 1.2·10⁻³ mmol) and the ialkylammonium derivative 2⁺ (1.2·10⁻³ mmol) were dissolved in 0.4 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. Selected
spectral data for singly threaded pseudo[2]rotaxane ion $2^\ast$$\subset$12. ESI(+) MS: $m/z = 1672.4 \ [2\subset12]^+$.

$^1$H NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$ -1.07 [broad, (CH$_2$)$_{\beta}$, 2H], -0.17 [broad, (CH$_2$)$_{\gamma}$, 2H], -0.02 [broad, (CH$_2$)$_{\alpha}$, 2H], 0.37 [broad, (CH$_2$)$_{\delta}$ + (CH$_3$)$_{\epsilon}$, 5H], 3.53 and 4.29 (broad overlapped, ArCH$_2$Ar, 24H), 2.87, 2.99, 3.23 (br s, OCH$_3$, 6H, 3H, 6H), 3.78, 3.84, 3.90 (br s, OCH$_3$, 3H, 6H, 6H), 4.79 (br s, OCH$_2$, 2H), 4.98 (br s, OCH$_2$, 2H), 6.63—6.99 (overlapped, ArH, 40H).

Figure 95. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:1 mixture of 12 and $2^\ast$-TFPB$^-$. 
Figure 96. Significant portion of the 2D COSY spectrum (400 MHz, 298 K, CDCl₃) of the 1:1 mixture of 12 and 2⁺-TFPB⁻.

Derivative 15.

Double-calixarene derivative 12 (2.0·10⁻³ g, 1.3·10⁻³ mmol) and the dialkylammonium derivative 3⁺ (1.3·10⁻³ mmol) were dissolved in 0.5 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

ESI(+) MS: m/z = 1680.3 [3⁺12⁺]. ¹H NMR (CDCl₃, 400 MHz, 298 K): δ -1.02 [broad, (CH₂)₂, 2H], 0.04 [broad, (CH₂)ᵢ + (CH₃)ᵢ, 5H], 0.22 [broad, (CH₂)ᵢ, 2H], 2.30 [broad, (H₂N⁺CH₃²⁺Ph), 2H], 2.87–3.93 (broad overlapped OCH₃+ ArCH₂Ar, 42H), 4.31–4.41 (broad overlapped, ArCH₂Ar, 12H), 4.78
(br s, OCH₂, 2H), 4.98 (br s, OCH₂, 2H), 5.37 (br s, NH₂, 2H), 6.76–7.40 (overlapped, ArH, 45H).

Figure 97. ¹H NMR spectrum (400 MHz, 298 K, CDCl₃) of the 1:1 mixture of 12 and 3⁺·TFPB⁻.

4.7.3 Preparation of doubly threaded pseudo[3]rotaxanes 14,16

Derivative 14
Double-calixarene derivative 12 (1.81·10⁻³ g, 1.2·10⁻³ mmol) and the dialkylammonium derivative 2⁺ (2.4·10⁻³ mmol) were dissolved in 0.4 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+) MS: \( m/z = 915.5 \ [((\text{2}_{2}\text{2}_{12})^{2+}. \)¹H NMR (CDCl₃, 400 MHz, 298 K): \( \delta \) -1.07 [broad, (CH₂)β, 4H], -0.18 [broad, (CH₂)γ, 4H], -0.01 [broad, (CH₂)α, 4H], 0.39 [broad, (CH₂)δ + (CH₃)ε, 10H], 3.52 and 4.33 (broad, ArCH₂Ar, 24H), 3.79, 3.84, 3.91 (br s, OCH₃, 6H, 12H, 12H), 4.98 (br s, OCH₂, 4H), 6.66–7.61 (overlapped, ArH, 40H).

Figure 98. ¹H NMR spectrum (400 MHz, 298 K, CDCl₃) of the 1:2 mixture of 12 and 2⁺·TFPB⁻.
Double-calixarene derivative 12 (2.0·10^{-3} g, 1.3·10^{-3} mmol) and the dialkylammonium derivative 3^+ (2.6·10^{-3} mmol) were dissolved in 0.5 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

ESI(+) MS: \textit{m/z} = 921.5 [(\textit{3})_2\textit{12}]^{2+}. \textit{¹H NMR (CDCl₃, 400 MHz, 298 K):} δ -1.01 [broad, (CH₂)\text{β}, 4H], 0.03 [broad, (CH₂)\text{γ} + (CH₃)\text{δ}, 10H], 0.23 [broad, (CH₂)\text{α}, 4H], 2.27 [broad, (\textit{⁺H₂NCHF}_{2}\text{Ph}), 4H], 3.28, 3.59, 3.70 (s, OCH₃, 6H, 12H, 12H), 3.50 and 4.44 (broad, ArCH₂Ar, 8H), 3.52 and 4.32 (broad, ArCH₂Ar, 8H), 3.58 and 4.43 (broad, ArCH₂Ar, 8H), 4.97 (br s, OCH₂, 4H), 5.35 (br s, \textit{⁺NH₂}, 4H), 6.64–7.59 (overlapped, ArH, 50H).
Figure 99. \(^1\)H NMR spectrum (400 MHz, 298 K, CDCl\(_3\)) of the 1:2 mixture of 12 and 3\(^+\)-TFPB\(^-\).

4.7.4 Synthesis and characterization of double-calixarene 21

![Diagram of double-calixarene 21]


K\(_2\)CO\(_3\) (8.0 g, 8.21 mmol) was added, under stirring, to a solution of \(p\)-\(t\)-but-calix[6]arene 17 (1.2 g, 9.0 mmol) in dry CH\(_3\)CN (300mL). The mixture was kept at reflux under stirring, and after 1 h benzylbromide (1.1 mL, 9.0 mmol) was added. The reaction was stirred at reflux for 3 h, then the solvent was removed under reduced pressure and the mixture was partitioned between CH\(_2\)Cl\(_2\) and H\(_2\)O. The organic layer was washed with 1N HCl (100 mL), brine (100 mL) and dried over Na\(_2\)SO\(_4\). The crude product was triturated with methanol, collected by filtration, and dried, to give 18 as a white solid (6.7 g, 6.31 mmol, 84%), which was sufficiently pure for subsequent
synthetic manipulations. **ESI(+) MS:** $m/z = 1062$ (MH$^+$); **$^1$H NMR** (250 MHz, CDCl$_3$, 298 K): $\delta$ 1.19 (s, C(CH$_3$)$_3$, 9H) 1.23 (s, C(CH$_3$)$_3$, 9H), 1.29 -1.28 (s, C(CH$_3$)$_3$, 36H), 4.02 e 3.39 (AX, ArCH$_2$Ar, $J=13.9$ Hz, 4H), 4.27 e 3.56 (AX, ArCH$_2$Ar, $J=14.0$ Hz, 4H), 4.45 e 3.54 (AX, ArCH$_2$Ar, $J=13.4$ Hz, 4H), 5.21 (s, CH$_2$Ar$_{Bn}$, 2H), 7.17-7.09 (overlapped, ArH$_{calix}$, 12H), 7.45 (m, ArH$_{Bn}$, 1H), 7.64 (m, ArH$_{Bn}$, 2H), 7.78 (m, ArH$_{Bn}$, 2H), 9.12 (s, OH, 2H), 9.82 (s, OH, 1H), 10.02 (s, OH, 2H). **$^{13}$CNMR** (250 MHz, CDCl$_3$, 298 K): $\delta$ 31.8 (2), 32.9, 33.5, 34.1, 34.2, 34.5, 78.1, 125.6, 125.9, 126.2, 126.4, 126.9, 127.0, 127.2, 127.4, 127.6, 128.6, 129.3, 132.7, 136.6, 143.1, 143.7, 144.6, 146.7, 148.3, 148.4, 149.4, 149.6.

**Figura 100.** Spectrum $^1$H NMR of derivative 18 in CDCl$_3$ (250 MHz, 298 K).
A solution of 18 (6.7 g, 6.31 mmol) in acetone (300 mL) was added of Cs$_2$CO$_3$ (18.5 g, 56.8 mmol). The mixture was stirred at the reflux temperature for 2 h and then iodomethane (17.3 g, 7.6 mL, 126.2 mmol) was added. The reaction mixture was stirred for 12 h under reflux. The solution was concentrated to dryness and the residue was partitioned between 1 N HCl (100 mL) and CH$_2$Cl$_2$ (100 mL). The organic phase was washed with water (3×90 mL) and dried over Na$_2$SO$_4$. Solvent evaporation under vacuum afforded a crude product that was triturated with methanol, collected by filtration, and dried, to give 19 as a white solid (6.03 g, 5.32 mmol, 90%), which was sufficiently pure for subsequent synthetic
manipulations. ESI(+) MS: \text{m/z} 1132 (MH\textsuperscript{+}); \textsuperscript{1}HNMR (250 MHz, CDCl\textsubscript{3}, 298 K): \delta 1.03 - 0.97 (s, C(CH\textsubscript{3})\textsubscript{3}, 18H), 1.25 - 1.24 (s, C(CH\textsubscript{3})\textsubscript{3}, 27H), 2.53 (s, OCH\textsubscript{3}, 6H), 2.78 (s, OCH\textsubscript{2}, 3H), 3.20 (s, OCH\textsubscript{3}, 6H), 4.05 e 3.54 (AX, ArCH\textsubscript{2}Ar, J=14.2 Hz, 4H), 4.18 e 3.82 (AX, ArCH\textsubscript{2}Ar, J=15.3 Hz, 4H), 4.46 e 3.68 (AX, ArCH\textsubscript{2}Ar, J=14.7 Hz, 4H), 4.88 (s, CH\textsubscript{2}Ar\textsubscript{Bn}, 2H), 7.55 - 6.9 (overlapped, ArH\textsubscript{calix} e ArH\textsubscript{Bn}, 16H); \textsuperscript{13}CNMR (250 MHz, CDCl\textsubscript{3}, 298 K): \delta 31.4, 31.5, 31.6, 34.2, 34.3, 60.0, 60.1, 60.2, 74.5, 77.4, 124.7, 125.2, 125.3, 126.9, 127.0, 127.4, 127.9, 128.6, 133.3, 133.4, 133.5, 133.7, 134.0, 138.0, 145.8 (2), 146.0, 152.2, 153.7, 154.4, 154.5.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{spectrum.png}
\caption{Figura 102. Spectrum \textsuperscript{1}H NMR of derivative 19 in CDCl\textsubscript{3} (250 MHz, 298 K).}
\end{figure}
A solution of 19 (6.0 g, 5.32 mmol) in CHCl₃ (400 mL) was added of Pd/C (10%) and stirred for 4 h under H₂ at 25 ºC. After filtration of the catalyst, the solvent was evaporated to give 20 (5.4 g, 5.23 mmol, 90%). **ESI(+) MS:** m/z 1032 (MH⁺); **¹³HNMR** (250 MHz, CDCl₃, 298 K): δ 1.12 (s, C(CH₃)₃, 9H) 1.19 - 1.17 (s, C(CH₃)₃, 45H), 3.07 (s, OCH₃, 12H), 3.52 (s, OCH₃, 3H), 3.82 (broad, ArCH₂Ar, 4H), 3.97 e 3.94 (broad, ArCH₂Ar, 8H), 6.80 (s, ArH_calix, 2H), 6.89 (s, ArH_calix, 2H), 7.04- 7.01 (overlapped, ArH_calix, 6H), 7.12- 7.11 (overlapped, ArH_calix, 2H), 7.38 (s, OH). **¹³CNMR** (250 MHz, CDCl₃, 298 K): δ 31.4, 31.6 (2), 34.1, 34.2, 34.4, 60.8, 61.1, 77.5, 124.9, 125.6, 125.9, 126.3, 126.5, 126.7, 127.3, 132.8, 133.4, 133.6, 133.9, 142.2, 145.3, 146.0, 146.7, 149.7, 153.3, 154.2, 154.6.
Figura 104. Spectrum $^1$H NMR of derivative 20 in CDCl$_3$ (400 MHz, 298 K).
NaH (1.30 g, 54.2 mmol) was added at 0 °C, under stirring, to a solution of derivative 20 (1.47 g, 2.07 mmol) in dry THF/DMF (75 mL, 7/3 v/v). The mixture was kept at 25 °C under stirring, and after 1 h, 1,3-bis(bromomethyl)benzene (0.26 g, 0.98 mmol) was added. The reaction was stirred at reflux for 12 h under a nitrogen atmosphere, then the solvent was removed under reduced pressure and the mixture was partitioned between CH$_2$Cl$_2$ and H$_2$O. The organic layer was washed with 1N HCl (50 mL), brine (50 mL), and dried over Na$_2$SO$_4$. The crude product was purified by column chromatography (SiO$_2$; Et$_2$O/CH$_2$Cl$_2$ 3/97) to give derivative 21 as a white solid (1.04 g, 0.69 mmol, 80%).

ESI(+) MS: m/z 2188 (MH$^+$) (Figure 107); $^1$HNMR (400 MHz, CDCl$_3$, 298 K): $\delta$ 1.07 (s, C(CH$_3$)$_3$, 36H), 1.24 (2) (overlapped C(CH$_3$)$_3$, 54H), 2.58 (s, OCH$_3$, 18H), 2.82 (s, OCH$_3$, 9H), 3.15 (s, OCH$_3$, 18H), 3.83 e 3.68 (AX, ArCH$_2$Ar, $J$=15.1Hz, 6H), 4.17 - 4.04 (AX, ArCH$_2$Ar, $J$=14.5Hz, 6H), 4.50 - 3.58 (AX, ArCH$_2$Ar, $J$=14.7Hz, 6H), 5.06 (s, ArH $^B_{Bn}$, 6H), 6.94 – 6.87 (overlapped, ArH$_{calix}$, 18H), 7.11 - 7.06 (overlapped ArH$_{calix}$, 12H), 7.28 - 7.26 (overlapped ArH$_{calix}$, 6H), 7.78 (s, ArH, 3H); $^{13}$CNMR (250 MHz, CDCl$_3$, 298 K): $\delta$ 14.4, 22.7, 30.6, 30.8, 31.5 (2), 31.7(2), 31.8, 34.3 (2), 60.0, 60.2, 74.8, 75.9, 77.5, 124.8, 125.3, 125.5, 126.9, 127.5, 133.4, 133.6, 133.7, 133.9, 134.1, 138.8, 145.7, 145.9, 152.4, 153.9, 154.4, 154.5.
Figura 106. Spectrum $^1$H NMR of derivative 21 in CDCl$_3$ (250 MHz, 298 K).

Figura 107. Spectrum $^{13}$C NMR of derivative 21 in CDCl$_3$ (250 MHz, 298 K).

Derivative 22

Double-calixarene derivative 21 (2.0·10⁻³ g, 0.91·10⁻³ mmol) and the dialkylammonium derivative 2⁺ (0.91·10⁻³ mmol) were dissolved in 0.4 mL of
CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. Selected spectral data for singly threaded pseudo[2]rotaxane ion 2⁺∙21. ESI(+) MS: m/z = 2344.3 [2⁺∙21]⁺. ¹H NMR (CDCl₃, 400 MHz, 298 K): δ -1.00 [broad, (CH₂)ᵦ, 2H], -0.88 [broad, (CH₂)ᵦ, 2H], -0.52 [broad, (CH₂)ᵦ, 2H], -0.41 [broad, (CH₂)ₓ + (CH₃)ₙ, 5H], 3.18 and 4.24 (broad overlapped, ArCH₂Ar, 24H), 3.20, 3.54, 3.76 (br s, OCH₃, 6H, 3H, 6H), 3.78, 3.84, 3.90 (br s, OCH₃, 3H, 6H, 6H), 4.79 (br s, OCH₂, 2H), 4.85 (br s, OCH₂, 2H), 6.86—7.20 (overlapped, ArH, 40H).

Figure 109. ¹H NMR spectrum (400 MHz, 298 K, CDCl₃) of the 1:1 mixture of 21 and 2⁺∙TFPB⁻.

Derivative 24
Double-calixarene derivative 21 (2.0·10⁻³ g, 0.91·10⁻³ mmol) and the dialkylammonium derivative 3⁺ (0.91·10⁻³ mmol) were dissolved in 0.5 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

ESI(+) MS: \( m/z = 2350.3 [3\mathbf{−21}]^+ \). \(^1\)H NMR (CDCl₃, 400 MHz, 298 K): \( \delta \) -1.02 [broad, (CH₂)₂, 2H], 0.04 [broad, (CH₂)₁ + (CH₃)₅, 5H], 0.22 [broad, (CH₂)₅, 2H], 2.30 [broad, (H₂N⁺CH₂⁺Ph), 2H], 2.87–3.93 (broad overlapped OCH₃ + ArCH₂Ar, 42H), 4.31–4.41 (broad overlapped, ArCH₂Ar, 12H), 4.78 (br s, OCH₂, 2H), 4.98 (br s, OCH₂, 2H), 5.37 (br s, NH₂, 2H), 6.76–7.40 (overlapped, ArH, 45H).
Figure 110. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:1 mixture of 21 and 3$^+$·TFPB$^-$.

Derivative 26

Double-calixarene derivative 21 (2.0·10$^{-3}$ g, 0.91·10$^{-3}$ mmol) and the dibenzylammonium derivative 3$^+$ (1.3·10$^{-3}$ mmol) were dissolved in 0.5 mL of CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

$^1$H NMR (400 MHz, CDCl$_3$, 298 K): δ 1.04 [s, (CH$_3$)$_3$C-, 45H], 1.28 [s, (CH$_3$)$_3$C-, 45H], 1.38 [m, (CH$_2$)$_n$, 2H)], 1.53 [m, (OCH$_2$)$_j$, 2H], 1.61 [m, (OCH$_2$)$_x$, 2H], 3.45 and 4.39 (AX, ArCH$_2$Ar, 12H, J = 12.0 Hz), 3.75 [s, OCH$_3$, 15H], 3.75 [s, OCH$_3$, 15H], 4.01 and 4.51 (d, ArH$_n$, 2H, J = 8.0 Hz), 5.07 [s, (OCH$_2$)$_s$, 4H$_s$), 4.78 (d, ArH$_o$, J = 8.0 Hz, 1H), 5.33 (dd, ArH$_m$, J$_1$=J$_2$=7.6 Hz, 4H), 5.98 (t, ArH$_p$, J = 7.6 Hz, 2H), 6.11 (broad, NH$_2^+$, 2H), 7.14-7.30 (overlapped, ArH, 52H), 7.50 (s, ArH$_{TFPB}$, 4H), 7.71 (t, ArH$_{TFPB}$, 8H, J = 4.0 Hz ), 7.83 (d, ArH$_n$, 2H, J = 8.0 Hz);

Derivative 23

Double-calixarene derivative 21 (2.0·10⁻³ g, 0.91·10⁻³ mmol) and the dialkylammonium derivative 2⁺ (2.4·10⁻³ mmol) were dissolved in 0.4 mL of
CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+) MS: $m/z = 1248.33 [(2)_2\cdot21]^2+$. $^1$H NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$ −1.07 [broad, (CH$_2$)$_\beta$, 4H], −0.18 [broad, (CH$_2$)$_\gamma$, 4H], −0.01 [broad, (CH$_2$)$_\alpha$, 4H], 0.39 [broad, (CH$_2$)$_\delta$ + (CH$_3$)$_\epsilon$, 10H], 1.15 (s, Bu', 90H), 3.52 and 4.33 (broad, ArCH$_2$Ar, 24H), 3.79, 3.84, 3.91 (br s, OCH$_3$, 6H, 12H, 12H), 4.98 (br s, OCH$_2$, 4H), 6.66–7.61 (overlapped, ArH, 40H).

Figure 112. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:2 mixture of 21 and $2^*\cdot$TFPB$^\cdot$.

Derivative 25
Double-calixarene derivative 21 (2.0·10⁻³ g, 0.91·10⁻³ mmol) and the dialkylammonium derivative 2⁺ (2.4·10⁻³ mmol) were dissolved in 0.4 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+) MS: \(m/z = 1257 \ [(3)_2\cdot\text{21}]^{2+}\).

\(^1\text{H NMR (CDCl}_3, 400 \text{ MHz, 298 K})\): \(\delta -1.01 \) [broad, (CH₃)₂β, 4H], 0.03 [broad, (CH₂)_¢+(CH₃)₃δ, 10H], 0.23 [broad, (CH₂)₂α, 4H], 1.67(s, Bu', 90H), 2.27 [broad, (\(^\text{1H}_2\text{NCH}_2\text{Ph}\), 4H], 3.28, 3.59, 3.70 (s, OCH₃, 6H, 12H, 12H), 3.50 and 4.44 (broad, ArCH₂Ar, 8H), 3.52 and 4.32 (broad, ArCH₂Ar, 8H), 3.58 and 4.43 (broad, ArCH₂Ar, 8H), 4.97 (br s, OCH₂, 4H), 5.35 (br s, \(^\text{1NH}_2, 4H), 6.64–7.59 \) (overlapped, ArH, 50H).
Figure 113. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:2 mixture of 21 and $^3^+$-TFPB$^-$. 

Derivative 27

Double-calixarene derivative 21 (2.0·$10^{-3}$ g, 0.91·$10^{-3}$ mmol) and the dibenzylammonium derivative 4$^+$ (2.4·$10^{-3}$ mmol) were dissolved in 0.5 mL of CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+) MS: $m/z$ =1290.3 [(4)$_2$$\subset$21]$^{2+}$. 

$\delta$1.17 (s, Bu', 90H), 1.45 [(CH$_2$)$_d$, 4H], 2.94 [(CH$_2$)$_e$, 4H], 3.51 and 4.40 (AX, ArCH$_2$Ar, $J$=13.6 Hz, 12H each), 3.86 (s, OCH$_3$, 36H), 4.67 (d, ArH$_o$, $J$= 8.0 Hz, 2H), 5.34 (dd, ArH$_m$, $J_1$=$J_2$=7.6 Hz, 4H), 5.40 [s (OCH$_2$)$_s$], 6.01 (t, ArH$_p$, $J_1$ = 7.6 Hz, 4H), 6.09 (broad, NH$_2^+$, 4H), 7.01 (s, ArH$_{calix}$, 24H), 7.48 (s, ArH$_{TFPB^-}$, 8H), 7.71 (ArH$_{TFPB^-}$, 16H).
4.7.7 Determination of $K_{\text{ass}}$ values of $(2^+)_2\subset 12$ and $(3^+)_2\subset 12$ complexes by quantitative $^1\text{H}$ NMR analysis

The samples were prepared by dissolving $8(1.24 \times 10^{-3}\text{ mmol})$ and the appropriate alkylammonium guest $2^+$ or $3^+(2.48 \times 10^{-3}\text{ mmol})$ in CDCl$_3$ (0.4 mL) containing 1 μL of 1,1,2,2-tetrachloroethane ($d = 1.59 \text{ g/mL}$) as internal standard. The complex concentration [complex] was evaluated by integration of the $^1\text{H}$ NMR signal of CHCl$_2$CHCl$_2$ vs the shielded signals at negative values of the guest molecule. The following equation was used to obtain the moles of the complex:

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

\( G_a \) = 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 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)
Stereo-Programmed Synthesis of
Calixarene-Based Handcuff Rotaxanes
5.1 Introduction

In the last decade, macrocyclic hosts with multiple cavities or multiple recognition sites have attracted increasing attention because they are particularly useful for developing non-trivial interlocked architectures.\textsuperscript{20} At this regards, handcuff-like systems in which two interpenetrated rings are linked to one another, represent a significative synthetic challenge.\textsuperscript{97} Based on the template-direct threading\textsuperscript{98,20} of linear axles through double-macrocycles, spectacular handcuff-like architectures have been reported to date, which shows interesting properties and functions.

In 1993 Stoddart\textsuperscript{99} and coworkers reported the first example of handcuff architecture B (Figure 116), which was followed by the similar [2]catenane polymer C.\textsuperscript{100} Successively, Becher\textsuperscript{101} reported the handcuff architecture D, in which two connected macrocycles were threaded through the same large ring to form an handcuff [3]catenane. A similar topology was built later by Sauvage\textsuperscript{102} in 2005 through the template effect of Cu(I) and recently by Beer\textsuperscript{103} through an anion templation. In 2000 Vögtle\textsuperscript{104} and coworkers


reported a molecular “pretzelane” A (Figure 115) in which the two rings of a [2]catenane were bridged with a short spacer.

Regarding the handcuff [3]rotaxane architecture E (Figure 115), recently an example has been reported in which two flat crown-rings, rigidly linked to one another, were threaded with a bis(ammonium) axle to give a double-leg elevator.

With respect to the use of flat macrocycles in E (Figure 115), an increased synthetic challenge is represented by the use of three-dimensional nonsymmetrical rings (directional wheels), such as double-calixarene B (Figure 48), because of the inherent difficulty in controlling the stereochemistry of the entire system. In fact, the synthesis of an handcuff-rotaxane by threading 12 with a bis(ammonium) axle could give rise to three stereoisomeric handcuff pseudo[2]rotaxane structures, in which the calixwheels could show three different relative orientations, head-to-head (H,H), head-to-tail (H,T), and tail-to-tail (T,T), represented by F–H in Figure 115.

Figure 115. Cartoon representations of handcuff-like systems.
The control of the relative orientation of the two directional wheels along bis(alkylbenzylammonium) threads could be obtained on the basis of the previously reported “endo-alkyl rule”. This control was already realized by Gaeta et al. in the stereo-programmed direct synthesis of calixarene-based pseudo[3]rotaxanes (Figure 116).

Figure 116. Stereo-programmed direct synthesis of calixarene-based pseudo[3]rotaxanes.
It is obvious that to obtain handcuff rotaxanes with different orientation of the calix-wheels you must rely on an appropriate design of the threading element (Figure 117).

In fact, the suitable choice of the alkyl-benzyl sequence along bis(benzylalkylammonium) axles in conjunction with the above mentioned endo-alkyl rule could drive the stereochemistry of the rotaxane adducts. Thus, two calix[6]arene directional wheels can be ordered in the right stereo-sequence by their through-the-annulus threading (Figure 117).

Thus, as a first step, we have designed three axles in which the appropriate covalent linkage of two alkylbenzylammonium recognition motifs could have a good control of the consequent sequence stereoisomerism (Figure 118).
Thus, the specific stereosequences of handcuff pseudo[2]rotaxanes ($T,T$)-12⊂28, ($H,H$)-12⊂33 and ($H,T$)-12⊂38 (Figure 118) could be obtained. The introduction of appropriate stoppers would lead to handcuff [2]rotaxane structures with a programmed orientation of the calix-wheels.

**Figure 118.** Predicted sequence stereoisomerism in handcuff pseudo[2]rotaxane by threading with rationally designed bis(benzylalkylammonium) axles (28, 33, 38).

### 5.2 Stereo-programmed synthesis of ($T,T$)-handcuff [2]rotaxane

#### 5.2.1 Stereo-programmed formation of a ($T,T$)-handcuff pseudo[2]rotaxane (29)

In order to prepare a handcuff-pseudo-[2]rotaxane with a programmed tail-to-tail stereosequence, we decided to connect two alkylbenzylammonium moieties by the benzyl ends to give the thread $28^{2+}$ exposing alkyl chains at the terminations [Figure 118 a]). On the basis of the *endo-alkyl rule*, the threading of $28^{2+}$ with 12 should result in a tail-to-tail orientation of the calix-wheels of 12 and thus in a tail-to-tail handcuff pseudo[2]rotaxane.

Therefore, the TFPB\textsuperscript{105} salt of 282+ was equilibrated with double-calix[6]arene 12 (Scheme 9). Then, the formation of handcuff pseudo[3]rotaxane architecture 292+ was confirmed by 1D and 2D NMR spectroscopy and ESI(+) mass spectrum. In particular, the \textsuperscript{1}H NMR spectrum (CDCl\textsubscript{3}, 400 MHz, 298 K) of a 1:1 mixture (Figure 119b) of TFPB salt of dicationic thread 282+ and double-calix[6]arene 12 (Scheme 9) showed a typical signature at highfield negative values (from 1.0 to −1.0 ppm, see Figure 119b) characteristic of an endo-complexation of the alkyl chains shielded by calixarene aromatic rings. This result and the absence of shielded benzylic resonances in the 4−6 ppm region, typical of endo-benzyl complexation,\textsuperscript{77,80} were a clear-cut proof that tail-to-tail (T,T)-handcuff pseudo[3]rotaxane 292+ (Scheme 9) had been stereo-selectively formed. This result indicates that the presence of a short \textit{m}-xylylene spacer between the two calix-wheels of 12 does not generate any abnormal stereo-sequence of the directional wheels with respect to that expected by the endo-alkyl rule.

\textsuperscript{105} For a review on superweak anion TFPB\textsuperscript{−}: Strauss, S. H. Chem Rev. 1993, 93, 927.
The formation of the handcuff pseudo[3]rotaxane $29^{2+}$ was confirmed by a prominent peak at 980.3 $m/z$ in the ESI(+) mass spectrum in Figure 120, corresponding to the doubly-charged molecular ion $29^{2+}$. In fact, as showed in the expansion of ESI mass spectrum (Figure 120 inset), the $\Delta$ spacing of 0.5 $m/z$ between the peaks in the isotopic envelop solely accounts for a doubly-charged species of $29^{2+}$, excluding architectures with higher charges, such as a cyclic or square-type supramolecular architecture A or an undefined supramolecular oligomer or polymer B.

A COSY-45 spectrum (CDCl$_3$, 400 MHz, 298 K) of the 1:1 mixture of thread $28^{2+}$ and double-calix[6]arene 12 (Figure 121) allowed a complete confident assignment of all shielded alkyl resonances. Thus, $\alpha$ protons at 0.17 ppm, shows a coupling with $\beta$ methylene group at −0.96 ppm, which presents a cross-peak with $\gamma$ protons at −0.03 ppm, finally coupled with $\delta$ methyl at 0.39 ppm, which was coupled with $\varepsilon$ protons at 0.40 ppm (Figure 121 right).
Figure 119. $^1$H NMR spectra (400 MHz, CDCl$_3$, 298 K) of: (a) 12, (b) an equimolar mixture (3 mM) of 12 and 28$^{2+}$-2TFPB$^-$, (c) an equimolar mixture (3 mM) of 12 and 31$^{2+}$-2TFPB$^-$.

In addition, the ArCH$_2$Ar region (3–5 ppm, Figure 121 left) of the COSY-45 spectrum revealed the presence of three AX systems at 4.43/3.58, 4.38/3.49 and 4.31/3.49 ppm relative to calixarene ArCH$_2$Ar groups. Furthermore, the corresponding $^1$H NMR spectrum showed three singlets in a 2:1:2 ratio at 3.77, 3.63 and 3.33 ppm, relative to OMe groups, and a singlet a 4.95 ppm relative to oxymethylene groups of the m-xylylene bridge. These data were in accordance with the presence of a symmetry plane bisecting the m-xylylene bridge and the 1,4-diphenoxybutane chain, which is only possible in the case of a handcuff-threading of 12.

The presence of well-defined AX systems for ArCH$_2$Ar groups in the COSY-45 spectrum in Figure 121 (left), is a clear indication that the thread 29$^{2+}$ gave a through-the-annulus-threading with double-calix[6]arene 12 in CDCl$_3$. In fact, the ArCH$_2$Ar protons appear as singlets for the conformationally mobile free host 12 (see $^1$H NMR spectrum of 12 in Figure 120a), whereas they give rise to a couple of doublets (AX system) when the wheel is conformationally blocked by pseudorotaxane formation.
Figure 120. ESI(+) mass spectrum of (\(T,T\))-29\(^{2+}\) and its AMBER energy-minimized structure (inset).

The preference for tail-to-tail stereo-sequence of two wheels of 12 along thread 29\(^{2+}\) was also confirmed by molecular mechanics calculations\(^{106,17}\) (AMBER force field, CHCl\(_3\), GB/SA model solvent), which indicated the (\(T,T\))-29\(^{2+}\) stereoisomer as the lowest in energy with respect to (\(H,T\)) and (\(H,H\)) ones (Figure 123). Molecular mechanics calculations revealed that a folding of the thread 28\(^{2+}\) is required to simultaneously thread the two calix-wheels of 12. Thus, the folded conformation adopted by 29\(^{2+}\) in (\(T,T\))-handcuff pseudo[2]rotaxane 29\(^{2+}\) was characterized by unfavorable gauche conformations around the O(1)-C(1) and C(2)-C(3) bonds of the central 1,4-diphenoxybutane fragment (Figures 123 and 124). The dihedral angles around O(1)-C(1) and C(2)-C(3) bonds are 65° and 78°, respectively (see Figure 125c).

Figure 121. Expansions of 2D COSY-45 spectrum (400 MHz, CDCl$_3$, 298 K) of an equimolar mixture (3 mM) of 12 and 28$^{2+}$·2TFPB$^-$. 

Figure 122. Lowest energy structure of three possible handcuff-pseudo[2]rotaxane 29$^{2+}$ (from left to right: T,T; H,T and H,H) found by Monte Carlo conformational search (10000 steps, MacroModel V. 9.0, AMBER force field).
Figure 123. Dihedral angle values (°) around C(1)-C(2) and C(2)-C(3) bonds measured for the SA structures of (T, T)-29\textsuperscript{2+} in the 4 kcal/mol lowest energy window. The structures are ranked according to their energy difference (ΔE) with respect to the lowest minimum one (0.0 kcal/mol).

Molecular Dynamics (MD) simulation at 500 K clearly showed that about 55% of the coconformers sampled during the entire MD simulation (20000 ps) showed a dihedral angle in the 65-80° range around the O-C bond (Figure 124). Analogous results were obtained upon sampling the dihedral angle between C(2)-C(3) bond (Figure 124). In fact, about 69% of the coconformers sampled during the entire MD simulation (20000 ps) showed a dihedral angle in the 65-90° range.
Figure 124. (a-c) Snapshot from an MD simulation of \((T,T)\)-handcuff-pseudo[2]rotaxane \(29^{2+}\) illustrating the thread \(28^{2+}\) in an folded conformation. (d-e) Variation in the dihedral angle between \(O(1)-C(1), C(2)-C(3)\) and \(C(1)-C(2)\) observed during the MD simulation at 500 K.

This situation is resembling those observed by Rebek,\(^{107}\) in the encapsulation of long alkanes in a coiled form inside a self-assembled capsule, and recently by us,\(^{108,15f}\) in the \(endo\)-complexation of large di-\(n\)-alkylammonium cations inside the narrow cavity of 18-membered dihomooxa[4]arene ring. In analogy to those complexes, in the present


case the energy loss due to the folding of the thread to a less stable form is counterbalanced by the gain of H-bonds due to the threading of the calix[6]-cavities.

The apparent total association constant \( (K_{tot} = K_1 \times K_2 = 1.6 \pm 0.3 \times 10^3 \) percentage of formation 58%) for complex \((T,T)-29^{2+}\) was determined by integration of \(^1\)HNMR spectrum of its 1:1 titration mixture in CDCl\(_3\) which showed slowly exchanging signals for both the free and complexed guest.

5.2.2 Stereo-programmed synthesis of \((T,T)\)-handcuff [2]rotaxane 32\(^{2+}\)

In order to obtain a handcuff [2]rotaxane architecture we attempted the handcuff-threading of derivative 12 with axle 30\(^{2+}\) encoding alkyl chains at the terminations and bearing two terminal OH groups, which are derivatizable with a trityl-bearing isocyanate as the stoppering reagent (Scheme 10).\(^{80}\)

Therefore, the TFPB salt of 30$^{2+}$ was equilibrated with double-calix[6]arene 12 to give (T,T)-handcuff pseudo[2]rotaxane 31$^{2+}$ (Scheme 10).

The $^1$H NMR spectrum (Figure 118c) of a 1:1 mixture of TFPB salt of dicationic 30$^{2+}$ and double-calix[6]arene 12 in CDCl$_3$ showed a typical signature at highfield and negative values (from 1.0 to −1.0 ppm) characteristic of an endo-complexation of the alkyl chains shielded by calixarene aromatic rings.

As above, the absence of shielded benzylic resonances in the 4-6 ppm region, typical of endo-benzyl complexation, were a clear-cut proof that tail-
to-tail handcuff pseudo[2]rotaxane $31^{2+}$ had been stereoselectively formed. Again, the validity of the endo-alkyl rule is confirmed. As above, a COSY-45 spectrum allowed a complete confident assignment of all n-alkyl resonances of the axle of $(T,T)$-handcuff pseudo[2]rotaxane $31^{2+}$. Thus, one strongly shielded chain inside the cavity was observed, with $\zeta$, $\epsilon$, $\delta$, $\gamma$, $\beta$, and $\alpha$ protons resonating at 3.43, 1.00, 0.45, 0.04, −0.92 and −0.23 ppm, respectively while three ArCH$_2$Ar AX systems were observed at 4.44/3.60, 4.42/3.52 and 4.34/3.50 ppm.

![Figure 125. ESI(+) mass spectrum of $(T,T)$-$31^{2+}$ and its AMBER energy-minimized structure (inset).](image)

This pseudorotaxane was then stoppered by reaction with 4-tritylphenyl isocyanate to give the first example of calixarene-based handcuff [2]rotaxane $(T,T)$-$32^{2+}$ in 65% yield (Scheme 9). The formation of $(T,T)$-$32^{2+}$ was confirmed by a prominent peak at 1370.1 m/z in the ESI(+) mass spectrum (Figure 125) corresponding to the doubly-charged supramolecular ion. The tail-to-tail stereosequence of the two calix-wheels in the precursor $(T,T)$-handcuff pseudo[2]rotaxane $29^{2+}$ was retained after the stoppering
reaction as evidenced by the $^1$H NMR spectrum (Figure 126a) of $(T,T)$-32$^{2+}$ in CDCl$_3$, which showed a typical signature at highfield values (from 1.0 to −1.0 ppm) and no resonances in the 4-6 ppm region. As above the COSY-45 spectrum (Figure 127) (CDCl$_3$, 400 MHz, 298 K) of $(T,T)$-32$^{2+}$ in CDCl$_3$ allowed a complete confident assignment of all shielded alkyl resonances.

Thus, $\alpha$ protons at 0.21 ppm, shows a coupling with $\beta$ methylene group at −0.92 ppm, which presents a cross-peak with $\gamma$ protons at 0.04 ppm, finally coupled with $\delta$ methylene at 0.44 ppm which was coupled with $\epsilon$ protons at 1.11 ppm that shows a coupling with $\delta$ methylene at 3.91 ppm.
Figure 127. Expansions of 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of the (T,T)-handcuff-[2]rotaxane 32²⁺.

Analogously to handcuff-architectures (T,T)-29²⁺ and (T,T)-31²⁺ above described, the ArCH₂Ar region (3−5 ppm) of the COSY-45 spectrum (Figure 127c) (CDCl₃, 400 MHz, 298 K) of (T,T)-32²⁺, revealed the presence of three AX systems at 4.45/3.60, 4.41/3.50 and 4.34/3.49 ppm relative to calixarene ArCH₂Ar groups, while its ¹H NMR spectrum showed three singlets in a 2:2:1 ratio at 3.78, 3.67 and 3.34 ppm relative to OMe groups and a singlet a 4.96 ppm relative to oxymethylene groups of the m-xylylene bridge. As above molecular mechanics calculations (AMBER force field, CHCl₃, GB/SA model solvent) indicated a folded conformation of the thread in handcuff rotaxane (T,T)-32²⁺ (Figure 129).
Figure 128. Lowest energy structure of the \((T,T)\)-handcuff-pseudo\([2]\)rotaxane \(32^{2+}\) found by Monte Carlo conformational search (10000 steps, MacroModel V. 9.0, AMBER force field).

5.3 Stereo-programmed synthesis of \((H,H)\)-handcuff \([2]\)rotaxane

5.3.1 Stereo-programmed formation of \((H,H)\)-handcuff pseudo\([2]\)rotaxane \(34^{2+}\)

The logical extension of the above approach was the preparation of a handcuff pseudo-[2]rotaxane with a programmed head-to-head stereosequence. For this purpose, we decided to connect two alkylbenzylammonium moieties by the alkyl ends to give thread \(33^{2+}\) (Figure 119b) exposing benzyl chains at the terminations (Scheme 11).

On the basis of the endo-alkyl rule, we expected that the threading of \(12\) with \(33^{2+}\) should result in a head-to-head orientation of the calix-wheels of \(12\).

Scheme 11. Stereo-programmed handcuff-threading of \(12\) with \(33^{2+}\) to give \((H,H)\)-handcuff-pseudo[2]rotaxane \(34^{2+}\).
Therefore, the TFPB\textsuperscript{109} salt of 33\textsuperscript{2+} was equilibrated with double-calix[6]arene derivative 12 (Scheme 9). Then, the formation of handcuff-pseudo[2]rotaxane architecture 33\textsuperscript{2+} was confirmed by 1D and 2D NMR spectroscopy and ESI(+) mass spectrum.\textsuperscript{3}

In particular, the \textsuperscript{1}H NMR spectrum (CDCl\textsubscript{3}, 400 MHz, 298 K) of a 1:1 mixture (Figure 130b) of 33\textsuperscript{2+} and 12 (Scheme 11) showed a typical signature at highfield negative values (from 1.0 to −1.0 ppm, see Figure 130b) characteristic of an endo-complexation of the alkyl chains shielded by calixarene aromatic rings. This result and the absence of shielded benzylic resonances in the 4−6 ppm region, typical of endo-benzyl complexation,\textsuperscript{77,80}, were a clear-cut proof that head-to-head (H,H)-handcuff pseudo[2]rotaxane 34\textsuperscript{2+} (Scheme 11) had been stereo-selectively formed. This result confirms once more the scarce influence of the short \textit{m}-xylylene spacer with respect to the endo-alkyl rule. The formation of the handcuff pseudo[3]rotaxane 34\textsuperscript{2+} was confirmed by a prominent peak at 935.3 \textit{m}/z in the ESI(+) mass spectrum (Figure 132) corresponding to the doubly-charged ion.

\textsuperscript{109} For a review on superweak anion TFPB\textsuperscript{−}: Strauss, S. H. Chem Rev. 1993, 93, 927.
Figure 129. $^1$H NMR spectra (400 MHz, CDCl$_3$, 298 K) of: (a) 12, (b) an equimolar mixture (3 mM) of 12 and 33$^{2+}$·2TFPB$^-$, (c) an equimolar mixture (3 mM) of 12 and 36$^{2+}$·2TFPB$^-$.

Figure 130. Expansions of 2D COSY-45 spectrum (400 MHz, CDCl$_3$, 298 K) of an equimolar mixture (3 mM) of 12 and 33$^{2+}$·2TFPB$^-$. 
5.3.2 Stereo-programmed synthesis of (H,H)-handcuff-[2]rotaxane 37²⁺

In order to obtain a handcuff [2]rotaxane architecture we attempted the handcuff-threading of derivative 12 with axle 35²⁺ encoding inner alkyl chains and bearing two terminal OH groups, which are derivatizable with a trityl-bearing isocyanate as the stoppering reagent (Scheme 12).

Therefore, the TFPB salt of 35$^{2+}$ was equilibrated with double-calix[6]arene 12 to give (H,H)-handcuff pseudo[2]rotaxane 36$^{2+}$ (Scheme 12). The $^1$H NMR spectrum (Figure 130c) of a 1:1 mixture of 35$^{2+}$ and 12 in CDCl$_3$ showed a typical signature at highfield and negative values (from 1.0 to $-1.0$ ppm) characteristic of an endo-complexation of the alkyl chains shielded by calixarene aromatic rings.

As above, the absence of shielded benzylic resonances in the 4-6 ppm region, typical of endo-benzyl complexation, were a clear-cut proof that tail-to-tail (H,H)-handcuff pseudo[2]rotaxane 36$^{2+}$ had been stereoselectively formed.
Again, the validity of our *endo-alkyl rule* is confirmed. As above, a COSY-45 spectrum allowed a complete confident assignment of all *n*-alkyl resonances of the axle of \((H,H)\)-handcuff-pseudo[2]rotaxane 36\(^{2+}\). Thus, one strongly shielded chain inside the cavity was observed, with ε, δ, γ, β, and α protons resonating at 1.00, 0.45, 0.04, −0.92 and 0.23 ppm, respectively, while three ArCH\(_2\)Ar AX systems was observed at 4.54/3.70, 4.42/3.52 and 4.34/3.50 ppm.

![Figure 132. ESI(+) mass spectrum of doubly-charged \((H,H)\)-handcuff-pseudo[3]rotaxane 36\(^{2+}\)](image)

This pseudorotaxane was then stoppered by reaction with 4-tritylphenyl isocyanate to give handcuff [2]rotaxane \((H,H)\)-37\(^{2+}\) in 35% yield (*Scheme 12*). The formation of \((H,H)\)-37\(^{2+}\) was confirmed by a prominent peak at 1370.1 m/z in the ESI(+) mass spectrum in *Figure 134*, corresponding to the doubly-charged supramolecular ion. The *head-to-head* stereosequence of the two calix-wheels in the precursor \((H,H)\)-handcuff pseudo[2]rotaxane 36\(^{2+}\) was retained after the stoppering reaction as evidenced by \(^1\)H NMR spectrum (*Figure 135a*) of \((H,H)\)-37\(^{2+}\) in CDCl\(_3\), which showed a typical signature at highfield values (from 1.0 to −1.0 ppm) and no resonances in the 4-6 ppm region. As above the COSY-45 spectrum (*Figure 136*) (CDCl\(_3\), 164
400 MHz, 298 K) of \((H,H)-37^{2+}\) in CDCl\(_3\) allowed a complete confident assignment of all shielded alkyl resonances.

Figure 133. ESI(+) mass spectrum of \((H,H)-37^{2+}\) and its AMBER energy-minimized structure (inset).
Thus, $\alpha$ protons at 0.19 ppm, shows a coupling with $\beta$ methylene group at −0.99 ppm, which presents a cross-peak with $\gamma$ protons at 0.04 ppm, finally coupled with $\delta$ methylene at 0.44 ppm which was coupled with $\varepsilon$ protons at 1.11 ppm.
Figure 135. Expansions of 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of the (H,H)-handcuff-[2]rotaxane 37²⁺.

Analogously to handcuff-architectures (H,H)-34²⁺ and (H,H)-36²⁺ above described the ArCH₂Ar region (3–5 ppm) of the COSY-45 spectrum (Figure 135c) (CDCl₃, 400 MHz, 298 K) of (H,H)-37²⁺, revealed the presence of three AX systems at 4.45/3.60, 4.41/3.50 and 4.34/3.49 ppm relative to calixarene ArCH₂Ar groups, while the ¹H NMR spectrum of (H,H)-37²⁺ in CDCl₃, showed three singlets in a 2:2:1 ratio at 3.78, 3.67 and 3.34 ppm relative to OMe groups and a singlet a 4.96 ppm relative to methylene groups of the m-xylylene bridge.

The stereochemical preference was also confirmed by molecular mechanics calculations¹¹⁰ (AMBER force field, CHCl₃, GB/SA model solvent), that

indicates an folded conformation of the thread in handcuff-rotaxane \((H,H)\)-37\(^{2+}\) (Figure 136).

![Figure 136. Lowest energy structure of the \((H,H)\)-handcuff-[2]rotaxane 37\(^{2+}\) found by Monte Carlo conformational search (10000 steps, MacroModel V. 9.0, AMBER force field) on the left and its expansion on the right]

5.4 Stereo-programmed synthesis of a \((H,T)\)-handcuff [2]rotaxane

5.4.1 Stereo-programmed synthesis of \((H,T)\)-handcuff pseudo[2]rotaxane 39\(^{2+}\)

The most natural conclusion of the above approach was the preparation of a handcuff pseudo[2]rotaxane with a programmed head-to-tail stereosequence. For this purpose it was very interesting to design an asymmetric axle in which there was both one inner and one outer alkyl chain to allow the head-to-tail stereosequence.

At this regard we decided to connect an outer alkylbenzylammonium moiety to a inner ones to give thread 38\(^{2+}\) bearing an alkyl and a benzyl unit at the two extremities (Scheme 13).
Scheme 13. Stereo-programmed handcuff-threading of 12 with 38$^{2+}$ to give $(H,T)$-handcuff-pseudo[2rotaxane 39$^{2+}$.  

To verify the predicted stereochemical outcome, the TFPB$^{111}$ salt of 38$^{2+}$ was equilibrated with double-calix[6]arene 12 (Scheme 13). Then, the formation of the handcuff pseudo[2]rotaxane architecture 39$^{2+}$ was confirmed by 1D and 2D NMR spectroscopy and ESI(+) mass spectrum. 

As expected, the $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K), of a 1:1 mixture (Figure 137b) of 38$^{2+}$ and 12 (Scheme 13) showed a typical signature at highfield negative values (from 1.0 to −1.0 ppm, see Figure 137b) characteristic of an endo-complexation. This result and the absence of shielded benzylic resonances were a clear-cut proof that head-to-tail handcuff pseudo[2]rotaxane 39$^{2+}$ (Scheme 13) had been stereo-selectively formed. This formation was confirmed by a prominent peak at 1022 m/z in the ESI(+) mass spectrum corresponding to the doubly-charged 39$^{2+}$ ion.

---

Figure 137. $^1$H NMR spectra (400 MHz, CDCl$_3$, 298 K) of: (a) 12, (b) an equimolar mixture (3 mM) of 8 and 38$^{2+}$·2TFPB$^-$, (c) an equimolar mixture (3 mM) of 12 and 40$^{2+}$·2TFPB$^-$. 

5.4.2 Stereo-programmed synthesis of (H,T)-handcuff [2]rotaxane 42

In order to obtain the corresponding handcuff [2]rotaxane architecture, we decided to study the handcuff-threading of derivative 12 with axle 40$^{2+}$ encoding the same elements of 38$^{2+}$ and bearing two terminal derivatizable OH groups, (Scheme 14).$^{80}$ Since axle 40$^{2+}$ was unknown, we decided to synthesize it with the synthetic route reported in Scheme 14.

Thread 40$^{2+}$ (Scheme 14) was obtained by following a six step sequence with the isolation and characterization of five intermediates. The sequence started with the Gabriel synthesis that involved the alkylation of potassium
phthalimide 43 with the 1,6-dibromohexane 44 in DMF$^{\text{dry}}$ at reflux for 48 hours, to give derivative 45 with a 87.4% yield.

Compound 45 was then involved in a nucleophilic substitution with $p$-cyanophenol 46, which led to the formation of compound 47. The reaction was carried out in acetonitrile in the presence of potassium carbonate to generate the nucleophilic agent, the phenoxide anion. The protected amino function of 47 was then released with hydrazine under an Argon atmosphere to obtain the cyano-ammina 48 with a 90% yield. This derivative is a key intermediate, the central building block for the construction of the bis-ammonium axle.

The coupling of derivative 48 with aldehyde 49, followed by an in-situ imine reduction with sodium borohydride in anhydrous methanol, led to the formation of a secondary amine 50 with a yield higher than 90%.

The next step involved the reduction of the cyano-group of 50 to aldehyde 51 with Adams's catalyst. The subsequent treatment with hydrochloric acid allowed the isolation of chlorohydrate 52. The latter derivative was then involved in another amination reaction through the coupling with 1-ammino-6-hexanol 53, followed by imine reduction, to lead to the formation of the second secondary amine function of 54. This was then treated with 37% HCl to obtain the bis-chloride 55. The last step involved an ion-exchange reaction by treatment of this bis-chloride with the sodium salt of TFPB, to give derivative 40$^{2+}$.

All synthetic intermediates were isolated and fully characterized by mass spectrometry and NMR spectroscopy.
Scheme 14. synthesis of thread $40^{2+}$

The TFPB salt of 40\(^2+\) was equilibrated with double-calix[6]arene 12 to give \((T,H)\)-handcuff pseudo[2]rotaxane 41\(^2+\) (Scheme 15). The \(^1\)H NMR spectrum (Figure 137c) of a 1:1 mixture of 40\(^2+\) and 12 in CDCl\(_3\) showed a typical signature at highfield and negative values (from 1.0 to −1.0 ppm) characteristic of an endo-complexation of the alkyl chains. As in the previous instances, this and the absence of shielded benzylic resonances were a clear evidence that head-to-tail handcuff pseudo[2]rotaxane 41\(^2+\) had been stereoselectively formed. Again, the validity of our endo-alkyl rule is confirmed. As above, a COSY-45 spectrum allowed a complete confident assignment of all \(n\)-alkyl resonances of the axle of 40\(^2+\). Thus, two different, strongly shielded chains inside the cavity were now observed; the
asymmetry of the system was reflected in the presence of six ArCH$_2$Ar AX systems three each at 4.45/3.62, 4.44/3.60, 4.42/3.52, 4.40/3.57, 4.38/3.55, 4.36/3.524, .34/3.50 ppm, respectively. (Figure 137)

*Head-to-tail* handcuff pseudo[2]rotaxane 41 was then stoppered by reaction with 4-tritylphenyl isocyanate to give handcuff [2]rotaxane (H,T)-42 in 60% yield (Scheme 15). The formation of (H,T)-42 was confirmed by a prominent peak at 1370.1 m/z in the ESI(+) mass spectrum (Figure 138) corresponding to the doubly-charged supramolecular ion.

The *head-to-tail* stereosequence of the two calix-wheels was evidenced by a $^1$H NMR spectrum (Figure 139) of (H,T)-42 in CDCl$_3$, which showed a typical signature at highfield values (from 1.0 to −1.0 ppm) and no resonances in the 4-6 ppm region. As above the COSY-45 spectrum (Figure 140) in CDCl$_3$ allowed a complete confident assignment of all shielded alkyl resonances. In particular the asymmetry of the system is evident by the presence of six AX systems, related to ArCH$_2$Ar groups, as it is well documented by the 2D-COSY spectrum (Figure 140b).

![Figure 138. ESI(+) mass spectrum of (H,T)-42$^{2+}$ and its AMBER energy-minimized structure (inset).](image-url)
Figure 139. (a) $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of handcuff-[2]rotaxane $(H,T)$-42$^{2+}$. 2TFPB$. (b) Expansion of the negative region of the $^1$H NMR spectrum of $(H,T)$-42.
Figure 140. (a) 2D COSY-45 spectrum (400 MHz, CDCl$_3$, 298 K) of the (H, T)-handcuff-[2]rotaxane 42; (b) Expansions of 2D COSY-45 spectrum of methylene region of the (H, T)-handcuff-[2]rotaxane 42; (c) Expansions of 2D COSY-45 spectrum of negative region of the (H, T)-handcuff-[2]rotaxane 42.

Figure 141. Lowest energy structures of the (H, T)-handcuff-[2]rotaxane 42 found by Monte Carlo conformational search (10000 steps, MacroModel V. 9.0, AMBER force field).
5.5 EXPERIMENTAL SECTION

GENERAL COMMENTS

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 spraying with H₂SO₄-Ce(SO₄)₂ or phosphomolybdic acid. Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate¹¹², was synthesized according to literature procedures.

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)], or Bruker Avance-250 spectrometer [250 (¹H) and 63 MHz (¹³C)]; chemical shifts are reported relative to the residual solvent peak (CHCl₃: δ 7.26, CDCCl₃: δ 77.23; C₆H₅CH₃: δ 7.09, 7.00, 6.98, 2.09, C₆D₅CD₃: δ 137.9, 129.2, 128.3 125.5, 20.4; CD₃OH: δ 4.87, CD₃OD: δ 49.0; THF-d₈: 3.58, 1.73). Standard pulse programs, provided by the manufacturer, were used for 2D COSY and 2D ROESY experiments. Monte Carlo conformational searches (10000 steps) were performed with MacroModel-

9/Maestro-4.1\textsuperscript{113} program using OPLS force-field and CHCl$_3$ solvent (GB/SA model).

### 5.5.1 Preparation of handcuff pseudo[2]rotaxane ($T,T$)-29\textsuperscript{2+}

![Scheme 16](image)

**Scheme 16.** Formation of handcuff pseudo[2]rotaxane ($T,T$)-29\textsuperscript{2+}∙2TFPB\textsuperscript{−}.

Double-calixarene derivative 12 (1.81·10\textsuperscript{-3} g, 1.2·10\textsuperscript{-3} mmol) and derivative 28\textsuperscript{2+} (2.60·10\textsuperscript{-3} g, 1.2·10\textsuperscript{-3} mmol) were dissolved in 0.4 mL of CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

2D COSY-45 Spectrum of handcuff pseudo[2]rotaxane \((T,T)-29^{2+}\)

**Figure 142.** Relevant portions of the 2D COSY spectrum (400 MHz, CDCl\(_3\), 298 K) of handcuff pseudo[2]rotaxane \((T,T)-29^{2+} \cdot 2\text{TFPB}^-\).
5.5.2. Preparation of handcuff pseudo[2]rotaxane ($T,T$)-$31^{2+}$


Double-calixarene derivative $12$ (1.81·$10^{-3}$ g, 1.2·$10^{-3}$ mmol) and derivative $30^{2+}$ (2.67·$10^{-3}$ g, 1.2·$10^{-3}$ mmol) were dissolved in 0.4 mL of CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.
2D COSY-45 Spectrum of handcuff pseudo[2]rotaxane (T,T)-31^{2+}

Figure 143. Relevant portion of the 2D COSY spectrum (400 MHz, CDCl₃, 298 K) of handcuff pseudo[2]rotaxane (T,T)-31^{2+}·2TFPB⁻.

5.5.3. Synthesis of Handcuff [2]Rotaxane (T,T)-32^{2+}

Di-n-butyltin dilaurate (3 drops) was added to a solution of 30$^{2+}$ (0.18 mmol, 0.41 g) and 12 (0.11 mmol, 0.17 g) in dry CHCl$_3$ (12 mL) at room temperature, and subsequently, 4-triphenylmethylphenylisocyanate (0.083 g, 0.230 mmol) was added. The reaction was kept under stirring at 60 °C for 5 days. The crude product was purified by column chromatography (SiO$_2$; hexane/CH$_2$Cl$_2$ 2/8) to give handcuff rotaxane (T,T)$^{−32^{2+}}$ as a white solid (0.14 g, 0.031 mmol, 28%). ESI(+) MS: $m/z$ = 1370 (M$^{2+}$/2);$^1$H NMR (400 MHz, CDCl$_3$, 298 K): $\delta$ −0.92 (broad, 4H, CH$_2$), 0.04 (broad, 4H, CH$_2$), 0.21 (broad, 4H, CH$_2$), 0.44 (broad, 4H, CH$_2$), 1.11 (broad, 4H, CH$_2$), 1.94 (broad, OCH$_2$CH$_2$CH$_2$CH$_2$O, 4H), 2.23 (broad, 4H, CH$_2$), 3.49 and 4.34 (AX, ArCH$_2$Ar, 8H), 3.50 and 4.41 (AX, ArCH$_2$Ar, 8H), 3.60 and 4.45 (AX, ArCH$_2$Ar, 8H), 3.34, 3.67 and 3.78 (s, OMe, 12, 6 and 12H respectively), 3.91 (broad, 4H, CH$_2$), 4.05 (broad, OCH$_2$CH$_2$CH$_2$CH$_2$O, 4H), 4.96 (s, OCH$_2$-xylenyl, 4H), 5.48 (broad, $^{+}$NH$_2$, 4H), 6.77–7.28 (overlapped, 88H, ArH+NHCO) , 7.52 [s, 8H, (ArH)$_{TFPB}$], 7.72 [s, 16H, (ArH)$_{TFPB}$]; $^{13}$C NMR (100 MHz, CDCl$_3$, 298 K): $\delta$ 24.5, 29.9, 64.7, 117.6, 120.7, 123.4, 126.1, 127.7, 131.3, 134.9, 135.1, 142.5, 146.9, 161.1, 161.6, 162.1. Anal. Calcd for C$_{248}$H$_{210}$B$_2$F$_{48}$N$_4$O$_{18}$: C, 66.67; H, 4.74. Found: C, 66.75; H, 4.67.86.
1D and 2D NMR Spectra of Handcuff [2]Rotaxane $(T,T)-32^{2+}$

Figure 144. (a) $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of handcuff [2]rotaxane $(T,T)-32^{2+}$·2TFPB$^\cdot$. (b) Expansion of the methylene region of the $^1$H NMR spectrum of $(T,T)-32^{2+}$. (c) Expansion of the methylene region of the 2D COSY-45 spectrum of $(T,T)-32^{2+}$.
Figure 145. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 298 K) of handcuff [2]rotaxane ($T,T$)-$^{32}_{32}$\textsuperscript{2+}.
2D COSY-45 Spectrum of handcuff [2]rotaxane \((T,T)-32^{2+}\)

**Figure 146.** Relevant portion of the 2D COSY spectrum (400 MHz, CDCl\(_3\), 298 K) of handcuff-[2]rotaxane \((T,T)-32^{2+}\)∙2TFPB\(^-\).
5.5.4 Simulated Annealing Experiments on Handcuff pseudo[2]rotaxane \((T,T)-31^{2+}\)

Simulated Annealing (SA) experiments performed on pseudo[2]rotaxane \((T,T)-31^{2+}\) evidenced that about 75% of the coconformers in the 4 kcal/mol lowest energy window (200 independent annealing experiments) showed a dihedral angle in the \(42-87^\circ\) range around the C(1)-C(2) bond (Figure S15, black squares). Analogously, about 85% of the same structures gave a C(2)-C(3) dihedral angle in the \(50-75^\circ\) range (Figure 148, red circles).

Figure 147. Dihedral angle values \(^{\circ}\) around C(1)-C(2) and C(2)-C(3) bonds measured for the SA structures of \((T,T)-36^{2+}\) in the 4 kcal/mol lowest energy window. The structures are ranked according to their energy difference \((\Delta E)\) with respect to the lowest minimum one (0.0 kcal/mol).

5.5.5 Simulated Annealing Experiments on handcuff [2]rotaxane \((T,T)-32^{2+}\)

Analogously to pseudorotaxane \((T,T)-31^{2+}\), SA experiments performed on \((T,T)-32^{2+}\) evidenced that about 65% of the coconformers in the 4 kcal/mol lowest energy window (200 independent experiments) showed a dihedral angle in the
45-70° range around the C(1)-C(2) bond (Figure 149, black squares). Analogously, about 50% of the same structures gave a C(2)-C(3) dihedral angle in the 59-84° range (Figure 149, red circles).

**Figure 148.** Dihedral angle values (°) around C(1)-C(2) and C(2)-C(3) bonds measured for the SA structures of \((T,T)\)-30\(^2+\) in the 4 kcal/mol lowest energy window. The structures are ranked according to their energy difference (\(\Delta E\)) with respect to the lowest minimum one (0.0 kcal/mol).

### 5.5.6. Preparation of handcuff pseudo[2]rotaxane \((H,H)\)-34\(^2+\)

![Scheme 19. Formation of handcuff pseudo[2]rotaxane \((H,H)\)-34\(^2+\)-2TFPB\(^-\).](image)
Double-calixarene derivative 12 (1.89·10⁻³ g, 1.2·10⁻³ mmol) and derivative 33²⁺ (2.60·10⁻³ g, 1.25·10⁻³ mmol) were dissolved in 0.4 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

¹H NMR (400 MHz, CDCl₃, 298 K): δ-0.96 [broad, (CH₂)₂, 4H], -0.06 [broad, (CH₂)₇, 4H], 0.22 [broad, (CH₂)₉ + (CH₂)₁₀, 8H], 0.51 [broad, (CH₂)₁₁, 4H], 3.52 and 4.36 (AX, ArCH₂Ar, J = 12.6 Hz, 12H), 3.60 (s, OCH₂, 36H), 4.98 (s, OCH₂₃-xylenyl, 4H), 5.34 [broad, (NH₂)₂, 4H], 6.84-6.95 (overlapped, ArHcalix, 36H), 7.49 (s, ArH₄TPB-, 8H), 7.59 (dd, ArHb, J₁ = J₂ = 7.6 Hz, 4H), 7.70 (s, ArH₄TPB-, 16H).

Figure 149. ¹H NMR spectrum (400 MHz, CDCl₃, 298 K) of handcuff pseudo[2]rotaxane (H,H)-34²⁺·2TFPB⁻.
2D COSY-45 Spectrum of handcuff pseudo[2]rotaxane (H,H)-34^{2+}

**Figure 150.** Relevant portions of the 2D COSY spectrum (400 MHz, CDCl₃, 298 K) of handcuff pseudo[2]rotaxane (H,H)-34^{2+}·2TFPB⁻.

### 5.5.7 Preparation of handcuff pseudo[2]rotaxane (H,H)-36^{2+}.

Double-calixarene derivative 12 (1.89·10^{-3} g, 1.2·10^{-3} mmol) and derivative 31^{2+} (2.67·10^{-3} g, 1.2·10^{-3} mmol) were dissolved in 0.4 mL of CDCl\textsubscript{3} and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

2D COSY-45 Spectrum of handcuff pseudo[2]rotaxane (H,H)-36^{2+}

Figure 151. Relevant portion of the 2D COSY spectrum (400 MHz, CDCl\textsubscript{3}, 298 K) of handcuff pseudo[2]rotaxane (H,H)-36^{2+}∙2TFPB\textsuperscript{−}.


Di-\textit{n}-butyltin dilaurate (3 drops) was added to a solution of 35\textsuperscript{2+} (0.18 mmol, 0.41 g) and 12 (0.11 mmol, 0.17 g) in dry CHCl\textsubscript{3} (12 mL) at room temperature, and subsequently, 4-triphenylmethylphenylisocyanate (0.083 g, 0.230 mmol) was added. The reaction was kept under stirring at 60 °C for 5 days. The crude product was purified by column chromatography (SiO\textsubscript{2}; CH\textsubscript{2}Cl\textsubscript{2} 100%) to give handcuff rotaxane \((H,H)\textsuperscript{−37}\textsuperscript{2+}\) as a white solid (0.050 g, 0.035 mmol, 32%).

**ESI(+) MS:** \(m/z = 1412.82\) (\(M^{2+}/2\));

**\(^{1}H\ NMR\)** (400 MHz, CDCl\textsubscript{3}, 298 K): \(\delta = -0.92\) (broad, 4H, CH\textsubscript{2}\textsubscript{β}), 0.04 (broad, 4H, CH\textsubscript{2}\textsubscript{γ}), 0.21 (broad, 4H, CH\textsubscript{2}\textsubscript{α}), 0.44 (broad, 4H, CH\textsubscript{2}\textsubscript{δ}), 1.11 (broad, 4H, CH\textsubscript{2}\textsubscript{ρ}), 1.94 (broad, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}O, 4H), 2.23 (broad, 4H, CH\textsubscript{2}\textsubscript{ω}), 3.49 and 4.34 (AX, ArCH\textsubscript{2}Ar, 8H), 3.50 and 4.41 (AX, ArCH\textsubscript{2}Ar, 8H), 3.60 and 4.45 (AX, ArCH\textsubscript{2}Ar, 8H), 2.84, 3.25 and 3.69 (s, OMe, 12, 6 and 12H respectively), 3.89 (broad, 4H, CH\textsubscript{2}\textsubscript{ζ}), 4.05 (broad, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}O, 4H), 4.98 (s, OCH\textsubscript{2}m-xylenyl, 4H), 5.48 (broad, \(^{+}\text{NH}_{2},\ 4H\)), 6.77−7.28 (overlapped, 88H, ArH+NHCO), 7.52 [s, 8H, (ArH)\textsubscript{TFPB}], 7.72 [s, 16H, (ArH)\textsubscript{TFPB}];

**\(^{13}C\ NMR\)** (100 MHz, CDCl\textsubscript{3}, 298 K): \(\delta = 24.5, 29.9, 60.6, 65.1, 68.7, 115.6, 118.1, 119.3, 122.9, 124.0, 125.6, 126.4, 127.2, 128.0, 128.4, 129.3, 131.4, 131.6, 132.3, 135.0, 161.6, 162.1.\)
Figure 152. $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of handcuff-[2]rotaxane (H,H)-37$^{2+}$ · 2TFPB$^-$.

Figure 153. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 298 K) of handcuff [2]rotaxane (H,H)-37$^{2+}$. 

Figure 154. (a) 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of Handcuff [2]rotaxane (H,H)-37²⁺; (b) Expansions of 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of methylene region of Handcuff [2]rotaxane (H,H)-37²⁺; (c) Expansions of 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of negative region of Handcuff [2]rotaxane (H,H)-37²⁺.
5.5.9 Preparation of Handcuff Pseudo[2]rotaxane (H,T)-39^{2+}.


Double-calixarene derivative 12 (1.89·10^{-3} g, 1.2·10^{-3} mmol) and derivative 38^{2+} (2.60·10^{-3} g, 1.25·10^{-3} mmol) were dissolved in 0.4 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

^1H NMR (400 MHz, CDCl₃, 298 K): δ-0.96 [broad, (CH₂)₉, 4H], -0.06 [broad, (CH₂)₉, 4H], 0.22 [broad, (CH₂)₉ + (CH₂)₂, 8H], 0.51 [broad, (CH₂)₉, 4H], 3.52 and 4.36 (AX, ArCH₂Ar, J = 12.6 Hz, 12H), 3.60 (s, OCH₃, 36H), 4.89 (s, OCH₂₃₉⁻xylenyl, 4H), 5.34 [broad, (NH₂⁺)e, 4H], 6.84-6.95 (overlapped, ArH₃calix, 36H), 7.49 (s, ArH₁TFPB⁻, 8H), 7.59 (dd, ArH₂b, J₁ = J₂ = 7.6 Hz, 4H), 7.70 (s, ArH₂TFPB⁻, 16H).
Figure 155. $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of handcuff pseudo[2]rotaxane $(H,T)$·39$^{2+}$.

5.5.10 Synthesis and characterization of thread 40$^{2+}$ and its precursors.
Scheme 23. Thread $40^{2+}$ synthesis

**Derivative 45**

To a suspension of potassium phthalimide $43$ (7.5 g, 40.49 mmol) in DMF (300 mL) was added 1,6-dibromohexane $44$ (19.75 g, 80.98 mmol); The mixture was kept under stirring at room temperature for 48h. KBr was filtered and the product was evaporated under reduced pressure; The crude product was purified by column chromatography (SiO$_2$ petroleum ether /CH$_2$Cl$_2$ 50/50) to give derivative $45$ as a white solid (10.98 g, 35.40 mmol 87.4%); $^1$H NMR (400 MHz, CDCl$_3$, 298 K): $\delta$ 7.70 (overlapped, H$_{h,g}$, 4H), 3.66 (t, H$_a$, 2H, J= 7.2 Hz), 3.37 (t, H$_i$, 2H, J= 6.8 Hz), 1.83 (m, H$_b$,
2H), 1.67 (m, H_e, 2H), 1.44 (overlapped m, H_d,c, 4H). \(^{13}\text{C NMR}\) (100 MHz, CDCl\(_3\), 298 K): \(\delta\) 26.2, 27.9, 28.6, 32.8, 33.9, 38.0, 123.4, 132.3, 134.1, 168.6.

**Figura 156.** Spectrum \(^1\text{H NMR}\) of derivative 45 in CDCl\(_3\) (400 MHz, 298K)
Derivative 47

To a solution of cyanophenol 46 (3.84g, 32.2 mmol) in dry CH$_3$CN (400 mL) was added K$_2$CO$_3$ (18.00g, 130.23 mmol). The mixture was refluxed for 1h under N$_2$. The solution was cooled at room temperature and subsequently, derivative 45 (10 g, 32.25 mmol) was added. The reaction was kept under stirring at 80 °C for 12h, then the solvent was concentrated under reduced pressure. The product was extracted with CH$_2$Cl$_2$ (3×80 mL) and the organic layers were collected and dried over MgSO$_4$, filtered and evaporated under reduced pressure to give 47 as a white solid (11.23 g 100%); $^1$H NMR (CDCl$_3$, 65 MHz, 298K).

Figura 157. Spectrum $^{13}$C NMR of derivative 45 in CDCl$_3$ (65 MHz, 298K)
400 MHz, 298 K): δ 7.82 (overlapped, H_ab, 4H), 7.70 (overlapped, H_j+i, 4H), 3.97 (t, H_c, 2H, J= 6.3 Hz), 3.69 (t, H_h, 2H, J= 7.0 Hz), 1.79 (m, H_d, 2H), 1.71 (m, H_e, 2H), 1.51 (H_f, 2H), 1.41 (H_g, 2H).

$^{13}$C NMR (CDCl₃, 250 MHz, 298 K): δ 26.9, 27.87, 29.8, 30.2, 39.2, 60.5, 116.5, 124.6, 135.3.

![Chemical Structure](image)

**Figura 158.** Spectrum $^1$H NMR of derivative 47 in CDCl₃ (400 MHz, 298 K).
A solution of derivative 47 (5.00 g, 14.36 mmol) and hydrazine (73.3 mmol, 4.6 mL, 50-60% v/v solution in H$_2$O) in EtOH (40 mL) was refluxed for 1h under N$_2$. The solution was cooled at room temperature and H$_2$O (100 mL) was added. The product was extracted with AcOEt (3×80 mL) and the organic layers were collected and dried over MgSO$_4$, filtered and evaporated under reduced pressure to give 48 as a white solid (2.81 g, 90%). $^1$H-NMR (CDCl$_3$, 300 MHz, 298 K): $\delta$ 7.58 (H$_h$, 2H), 6.93 (H$_i$, 2H), 3.99 (t, H$_g$, 2H, J= 7.2 Hz), 2.70 (t, H$_b$, 2H, J= 6.8 Hz), 1.83 (m, H$_f$, 2H), 1.47 (overlapped, m, H$_e$+$d$+$c$, 6H). $^{13}$C-NMR (CDCl$_3$, 300 MHz, 298 K): $\delta$ 26.0, 26.8, 33.9, 42.3, 68.5, 103.9, 115.4, 119.5, 134.1, 162.6.
**Figura 160.** Spectrum $^1$H NMR of derivative 48 in CDCl$_3$ (300 MHz, 298 K).

**Figura 161.** Spectrum in CDCl$_3$ of derivative 48 $^{13}$C NMR (65 MHz, 298 K).
Derivative 50

A mixture of benzaldehyde 49 (0.071 mL, 0.86 mmol) and derivate 48 (0.13 g, 0.044 mmol,) in CHCl$_3$ (5 mL) was stirred at room temperature for 30 min. The solvent was evaporated under reduced pressure to give the imine intermediate as a yellow oil in a quantitative yield. The imine intermediate was used for the next step without further purification. The imine was dissolved in dry MeOH (20 mL), and NaBH$_4$ (6.15 g, 162.61 mmol) 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 (50 mL) and an aqueous saturated solution of NaHCO$_3$ (100 mL). The organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure, to give derivative 50 as a white solid (6.63 g, 98%).

$^1$H NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$ 7.61 (d, H$_r$, 2H, J=8.6), 7.26 (H$_s$, 2H, J=8.4), 6.97 (d, H$_h$, 2H, J=8.8), 6.89 (H$_i$, 2H, J=8.5), 4.63 (s, H$_j$, 2H), 4.03 (t, H$_g$, 2H, J=6.5), 3.99 (t, H$_q$, 2H, J=6.54), 3.76 (s, H$_a$, 1H), 3.67 (t, H$_b$, 2H, J=6.45), 2.66 (t, H$_l$, 2H, J=7.3), 2.36 (s, H$_k$, 1H), 1.87-1.8 (overlapped, m, H$_{e+p+c}$, 6H), 1.62 (m, H$_m$, 2H), 1.56-1.43 (overlapped, m, H$_{e+d+o+n}$, 8H).

$^{13}$C NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$ 25.7, 25.9, 25.96, 27.1, 28.9, 29.3, 29.8, 32.8, 49.1, 53.4, 62.6, 64.7, 67.9, 68.3, 103.6, 114.4, 115.2, 119.4, 128.6, 129.4, 131.9, 134.0, 158.2, 162.5.
Figura 162. Spectrum $^1$H NMR of derivative 50 in CDCl$_3$ (400 MHz, 298 K).

Figura 163. Spectrum $^{13}$C NMR of derivative 50 in CDCl$_3$ (100 MHz, 298 K).
Derivative 51

To a solution of derivative 50 (2.57 g, 6.06 mmol) and HCOOH/H₂O (80% w/w 116 ml + 34 ml); was added (0.2728 g, 1.20 mmol) and the reaction was stirred at 60°C for 12h. The solution was cooled at room temperature and the catalyst was filtered. The crude product was extracted with AcOEt and the organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure, to give derivative 51 (2.00 g, 95%) as a white solid. ¹H NMR (CDCl₃, 250 MHz, 298 K): δ 9.85 (s, Hᵣ, 1H), 7.79 (d, Hₛ, 2H, J=8.9), 7.47 (Hₜ, 2H, J=8.2), 6.92 (d, Hᵣ, 2H, J=8.5), 6.86 (Hᵣ, 2H, J=8.1), 4.15 (t, Hᵣ, 2H, J=6.6), 3.93 (s, Hₛ, 1H), 3.99 (t, Hᵣ, 2H, J=6.54), 3.76 (s, Hₛ, 1H), 3.85 (t, Hᵣ, 2H, J=6.4), 2.66 (t, Hᵣ, 2H, J=7.3), 2.74 (s, Hₛ, 1H), 1.87-1.8 (overlapped, m, Hᵣ+p+c, 6H), 1.62 (m, Hₐ, 2H), 1.87-1.42 (overlapped, m, Hₐ+p+c, 16H). ¹³C NMR (CDCl₃, 400 MHz, 298 K): δ 25.5, 25.7, 25.8, 25.8, 26.6, 28.6, 28.9, 29.2, 45.6, 50.1, 64.0, 67.9, 68.1, 114.8, 115.0, 115.3, 121.9, 129.9, 131.9, 132.1, 134.1, 160.0, 161.3, 164.2, 190.9
Figure 164. Spectrum $^1$H NMR of derivative 51 in CDCl$_3$ (250 MHz, 298K)

Figure 165. Spectrum $^{13}$C NMR of derivative 51 in CDCl$_3$ (100 MHz, 298K)
Derivative 52

Derivative 51 (0.015 g, 0.035 mmol) was dissolved in Et₂O (20 mL) at room temperature and an aqueous solution of HCl (37% w/w, 1 mL) was added dropwise. The mixture was kept under stirring for 30 min, until a white precipitate was formed. The solid was collected by filtration, washed with MeOH (15 mL) and CH₃CN (15 mL) and dried under vacuum, to give derivative 58 as a white solid (1.91 g, 4.46 mmol, 73.8%).

Figure 166. Spectrum \(^1\)H NMR of derivative 52 in CDCl₃ (250 MHz, 298K)
A mixture of derivative 52 (1.5 g, 3.50 mmol) and 53 (0.43 g, 3.61 mmol) in CHCl₃ (5 mL) was stirred at room temperature for 30 min. The solvent was evaporated under reduced pressure to give the imine intermediate as a yellow oil in a quantitative yield. The imine intermediate was used for the next step without further purification. The imine was dissolved in dry MeOH (40 mL), and NaBH₄ (1.31 g, 35.34 mmol) 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 (50 mL) and an aqueous saturated solution of NaHCO₃ (100 mL). The organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure, to give derivative 54 as a white solid 54.6 % (1 g, 1.77 mmol); ¹H NMR (CDCl₃, 400 MHz, 298 K): δ 7.23 (overlapped, Hₙ, 4H), 6.87 (overlapped, Hₙ, 4H), 3.98-3.92 (overlapped, Hₙ+q+z', 10H), 3.68-3.61 (overlapped, t, Hₙ+g+q+z', 8H), 2.62 (t, Hₙ, 2H, J=7.0), 2.18 (s, Hₙ, 2H), 2.06 (s, Hₙ, 1H), 1.80-1.26 (overlapped, m, Hₙ+q+p+w+z+e+d+o+m+y+x, 24H). ¹³C NMR (CDCl₃, 250 MHz, 298 K): δ 26.9, 27.3, 28.5, 30.6, 31.3, 34.0, 50.5, 54.8, 64.2, 69.2, 115.8, 130.0, 130.7, 159.6.
**Figura 167.** Spectrum $^1$H NMR of derivative 54 in CDCl$_3$ (250 MHz, 298K)

**Figura 168.** Spectrum $^{13}$C NMR of derivative 54 in CDCl$_3$ (250 MHz, 298K)
To a solution of derivative 54 (1 g, 1.77 mmol) in MeOH (20 mL) was added dropwise an aqueous solution of HCl (37% w/w, 15 mL). The mixture was stirred overnight at 70 °C, then the solvent was concentrated under reduced pressure and the residue partitioned between AcOEt (50 mL) and H₂O (100 mL). The organic layer was dried over MgSO₄, filtered and the solvent was removed under reduced pressure, to give derivative 55 as a yellow oil in a quantitative yield (0.6 g, 56.36 %). ¹H NMR (CDCl₃, 400 MHz, 298 K): δ 7.40 (overlapped, Hₙ+r, 4H), 6.99 (overlapped, Hᵢ+s, 4H), 4.12 (s, Hₜ+a+z'', 2H), 3.99 (overlapped, t, Hₜ+b+g+q+z', H, J=6.3), 3.56 (t, Hₗ+i, 4H, J=6.6), 3.05-2.98 (overlapped, Hₘ+v, 4H) 2.02 (s, Hₖ+u, 4H), 1.80-1.42 (overlapped, m, Hₖ+ₙ+i+n+p+w+z+e+d+o+m+y+x, 24H). ¹³C NMR (MeOD, 400 MHz, 298 K): δ 25.5, 25.8, 26.1, 26.2, 26.5, 29.2, 29.4, 32.3, 32.7, 50.9, 61.7, 61.9, 67.8, 67.9, 68.1, 114.4, 115.1, 115.1, 123.3 123.4, 129.8, 131.6, 131.7, 160.6, 160.7.
**Figura 169.** Spectrum $^1$H NMR of derivative 55 in MeOD (400 MHz, 298K)

**Figura 170.** Spectrum $^{13}$C NMR of derivative 55 in MeOD (400 MHz, 298K)
Derivative 40$^{2+}$

Derivative 55 (0.4 g, 0.67 mmol) was dissolved in warm dry MeOH (25 mL), then a solution of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate$^4$ (1.23 g, 1.39 mmol) in dry MeOH (5 mL) was added. 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 for 48 h to give derivative 40$^{2+}$ as a brown solid (1.23 g, 1.39 mmol, 38%).

$^1$H NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$ 7.59 (s, ArH$_{TFPB}$, 12H, $J=8.6$), 7.26 (H$_g$, 2H, $J=8.4$), 6.97 (d, H$_h$, 2H, $J=8.2$), 6.89 (H$_i$, 2H, $J=8.5$), 4.63 (s, H$_j$, 2H), 4.03 (t, H$_g$, 2H, $J=6.5$), 3.99 (t, H$_q$, 2H, $J=6.5$), 3.76 (s, H$_a$, 1H), 3.67 (t, H$_b$, 2H, $J=6.5$), 2.66 (t, H$_l$, 2H, $J=7.3$), 2.36 (s, H$_k$, 1H), 1.87-1.8 (overlapped, m, H$_{l+p+c}$, 6H), 1.62 (m, H$_m$, 2H), 1.56-1.43 (overlapped, m, H$_{e+d+o+n}$, 8H).

$^{13}$C NMR (MeOD, 400 MHz, 298 K): $\delta$ 26.4, 26.7, 26.9, 27.1, 27.3, 30.1, 30.2, 30.6, 33.1, 33.5, 51.9, 62.5, 62.8, 68.8, 69.0, 115.9, 116.1, 118.5, 119.3, 123.6, 124.1, 124.2, 127.9, 129.7, 130.2, 130.6, 131.2, 132.2, 132.4, 135.8, 161.6, 162.4, 163.2, 164.0.
Figura 171. Spectrum $^1$H NMR of derivative 40 in MeOD(400 MHz, 298K)

Figura 172. Spectrum $^{13}$C NMR of derivative 40* in MeOD(400 MHz, 298K)
5.5.11 Preparation of handcuff pseudo[2]rotaxane (\(H,T\))-41\(^{2+}\)

**Scheme 23.** Formation of handcuff pseudo[2]rotaxane (\(H,T\))-41\(^{2+}\)-2TFPB\(^{-}\).

Double-calixarene derivative 12 (1.81·10\(^{-3}\) g, 1.2·10\(^{-3}\) mmol) and derivative 40\(^{2+}\) (2.67·10\(^{-3}\) g, 1.2·10\(^{-3}\) mmol) were dissolved in 0.4 mL of CDCl\(_3\) and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

**2D COSY-45 Spectrum of handcuff pseudo[2]rotaxane (\(H,T\))-41\(^{2+}\)**

**Figure 173.** Relevant portion of the 2D COSY spectrum (400 MHz, CDCl\(_3\), 298 K) of handcuff pseudo[2]rotaxane (\(H,T\))-41\(^{2+}\)-2TFPB\(^{-}\).
5.5.12 Synthesis of handcuff [2]rotaxane \((H,T)-42^{2+}\)


Di-\(n\)-butyltin dilaurate (3 drops) was added to a solution of \(40^{2+}\) (0.18 mmol, 0.41 g) and \(12\) (0.11 mmol, 0.17 g) in dry CHCl\(_3\) (12 mL) at room temperature, and subsequently, 4-triphenylmethylphenylisocyanate (0.083 g, 0.230 mmol) was added. The reaction was kept under stirring at 60 °C for 5 days. The crude product was purified by column chromatography (SiO\(_2\); CH\(_2\)Cl\(_2\) 100%) to give handcuff rotaxane \((H,T)-42^{2+}\) as a white solid (0.14 g, 0.031 mmol, 27%).

**ESI(+) MS:** \(m/z = 1384 \ (M^{2+}/2)\); \(^1\)H NMR \((400 \text{ MHz}, \text{CDCl}_3, 298 \text{ K}): \delta\)

- 0.67 (broad, 4H, CH\(_2\)\(^\beta\)), 0.04 (broad, 4H, CH\(_2\)\(^\gamma\)), 0.21 (broad, 4H, CH\(_2\)\(^\alpha\)), 0.44 (broad, 4H, CH\(_2\)\(^\delta\)), 1.11 (broad, 4H, CH\(_2\)\(^\epsilon\)), 1.94 (broad, OCH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)O, 4H), 2.23 (broad, 4H, CH\(_2\)\(^\omega\)), 3.49 and 4.34 (AX, ArCH\(_2\)Ar, 8H), 3.50 and 4.41 (AX, ArCH\(_2\)Ar, 8H), 3.60 and 4.45 (AX, ArCH\(_2\)Ar, 8H), 3.34, 3.67 and 3.78 (s, OMe, 12, 6 and 12H respectively), 3.91 (broad, 4H, CH\(_2\)\(^\zeta\)), 4.05 (broad, OCH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)O, 4H), 4.96 (s, OCH\(_2\)\(^m\)-xylenyl, 4H), 5.48 (broad, \(^+\)NH\(_2\), 4H), 6.77–7.28 (overlapped, 88H, ArH+NHCO), 7.52 [s, 8H, (ArH)\(^{TFPB}\)], 7.72 [s, 16H, (ArH)\(^{TFPB}\)]; \(^{13}\)C NMR \((100 \text{ MHz}, \text{CDCl}_3, 298 \text{ K}): \delta\)

- 24.5, 29.9, 64.7, 117.6, 120.7, 123.4, 126.1, 127.7, 131.3, 134.9, 135.1, 142.5, 146.9, 161.1, 161.6, 162.1.
Figure 174. (a) $^1$H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of handcuff [2]rotaxane ($H,T$)-42$^{2+}$·2TFPB$^-$.

Figure 175 $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 298 K) of handcuff [2]rotaxane ($H,T$)-42$^{2+}$.
2D COSY-45 Spectrum of handcuff [2]rotaxane (H,T)-42²⁺

Figure 176 (a) 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of handcuff [2]rotaxane (H,T)-42²⁺; (b) Expansions of 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of methylene region of handcuff [2]rotaxane (H,T)-42²⁺; (c) Expansions of 2D COSY-45 spectrum (400 MHz, CDCl₃, 298 K) of negative region of handcuff [2]rotaxane (H,T)-42²⁺
5.6 Conclusion

In conclusion, we have reported here the first examples of calixarene-based handcuff pseudorotaxanes and rotaxanes architectures obtained by through-the-annulus threading of a double-calix[6]arene system with a bis-ammonium axle. The relative orientation of the two calix-wheels can be predefined by exploiting the "endo-alkyl rule" which controls the directionality of the threading of alkylbenzylammonium axles with calixarene macrocycles. Thus, following this approach were obtained three examples of beautiful calixarene-based handcuff rotaxane stereoisomers which could be termed as head-to-head \((H,H)\), head-to-tail \((H,T)\), and tail-to-tail \((T,T)\).

Therefore, it is conceivable that the extension of this approach could lead to novel mechanically interlocked architectures with high-order topologies.
CHAPTER VI

Threading of a *triple-calixarene* host:

“a wealth of new possible architectures”
6.1 Introduction.

In the last 15 years research in the field of artificial molecular machinery has grown exponentially and it has been stimulated by several major scientific breakthroughs. The long been used top-down approach to miniaturization of components was profitably replaced by a (chemical) bottom-up approach. The latter strategy relies on the control of the self-assembly\textsuperscript{20} of molecular components with the aim to construct nanosized molecular devices able to store, process, and interpret information under appropriate external stimuli.\textsuperscript{114} Among the possible candidates for bottom-up approach, [\textit{n}]rotaxanes are particularly attractive because of their different, easily accessible co-conformations that make them ideal bi- or multistable systems. Molecular machines are mechanically interlocked molecules,\textsuperscript{20} and they can be considered as topological complex systems able to translocate a macrocyclic component over two or more sites (“stations”) under the influence of an external stimulus.\textsuperscript{115} Usually, flat or symmetrical wheels, such as crown ethers,\textsuperscript{116} azacyclopahnes,\textsuperscript{117} cycloamides,\textsuperscript{118} or cucurbiturils,\textsuperscript{119} are used, which lead to a

non-directional shuttling component. Instead the use of three-dimensional nonsymmetrical wheels, such as cyclodextrins\(^{120}\) or calixarenes,\(^{121}\) gives rise to directional shuttles with a determined relative orientation of their components and with new related potential properties. However, such systems still remain largely less exploited probably because of the inherent difficulty in controlling their relative orientation.

As above mentioned, concerning the hosts with multiple cavities or rings, spectacular interpenetrated architectures have been obtained by double-threading through systems in which two macrocycles are covalently linked to one another in a handcuff-like fashion\(^{122}\) [e.g.: double-crown ethers, double-calixarene systems (e.g. 12)]\(^{96}\).

However, it would be very attractive to design interpenetrated architectures obtained by threading a triple-host in which three macrocycles are covalently linked to one another by means of an appropriate spacer. To the best of our

---


\(^{121}\) To the best of our knowledge has been reported only one example of calix[6]arene-based rotaxane system in which calix-wheel encircle a viologen axle. In this case no shuttling or inversion motion of the calix-wheel has been evidenced: A. Arduini, R. Ferdani, A. Pochini, A. Secchi, F. Ugozzoli, *Angew. Chem.* 2000, 112, 3595–3598; *Angew. Chem. Int. Ed.* 2000, 39, 3453–3456.C. D. Gut sche.

knowledge, no-examples of threading studies on triple-calixarene systems (e.g. 57), have been so far reported in the literature. Undoubtedly, the design and synthesis of such a triple-calixarene could represent a new horizon of possible chemical topologies as exemplified by Figure 177.

Figure 177. Threading of triple-calixarene hosts with TFPB salts of \(n\)-alkylammonium cations: horizons of new architectures.


The tritopic host 57 was designed with the intention to obtain three equivalent recognition sites. Therefore, a bridge with \(C_3\) symmetry and a single kind of macrocyclic unit were chosen for the last step. Of course, this decision is reflected in the high symmetry of the final architecture 57.

Triple-calix[6]arene 57 was synthesized exploiting the reaction sequence shown in Scheme 25.\(^{96}\) In particular, in the key step, pentamethoxy-calix[6]arene-mono-ol 20 was reacted with 1,3,5-tris(bromomethyl)benzene 56 in the presence of NaH to give triple-calix[6]arene 57 in 33% yield.
Compound 57 was fully characterized by means of its $^1$H NMR, $^{13}$C NMR, and ESI(+) MS spectra (Figure 178).


The $^1$H and $^{13}$C NMR data of triple-calix[6]arene 57, acquired at room temperature, were fully consistent with its molecular symmetry. In particular, three singlets were present in the $^1$H NMR spectrum (Figure 176) at 0.96, 1.07 and 1.29 ppm relative to the four symmetry related tert-butyl groups, because of an accidental isochrony. More diagnostic were the three singlets at 2.92, 3.11 and 3.17 ppm (2:1:2) relative to OMe groups. The steric hindrance to conformational interconversion, due to the t-Bu groups at the upper-rim, was evident by the three AX systems at 3.58/4.50 ppm (J = 14.7 Hz), 4.17/4.04 ppm (J = 14.5 Hz), and 3.83/3.68 ppm (J = 15.1 Hz). Finally, a singlet was present at 5.05 ppm relative to OCH$_2$ protons of the bridge.
In order to design new topologically complex architectures it is fundamental to know the threading abilities of the new host. Therefore, as a first step we decided to investigate the threading of triple-calix[6]arene derivative 57 with mono-ammonium axles.

It is interesting to note that in this case, three new chemical topologies could be formed. In fact, as showed in Figure 178, the threading of triple-calix[6]arene 57 with mono-ammonium axle could give rise to:

1) a singly threaded pseudo[2]rotaxane (Figure 178a),
2) a doubly threaded pseudo[3]rotaxane (Figure 178b),
3) a triply threaded pseudoro[4]taxane (Figure 178c).
Of course, the stoppering of such pseudo[n]rotaxanes would give rise to the corresponding [n]rotaxanes.

Figure 179. Linear mono-ammonium systems (left), triple–calix[6]arene host (right)

Figure 180. Cartoon representations of the possible topologies obtainable through threading of a triple-calix[6]arene with mono-ammonium axles.
6.3.1 Threading of triple-calix[6]arene 57 with di-n-pentylammonium axle 2^+

Scheme 26. Schematic representation of Directional threading of 57 with the di-n-pentylammonium axle 2^+.

Interestingly, the addition of di-n-pentylammonium salt 2^+·TFPB^- to a CDCl_3 solution of triple-calix[6]arene 57 caused the usual changes in its ¹H NMR spectrum (Figure 179). In fact, upon addition of 1 equivalent of 2^+·TFPB^- salt a new set of signals emerged (Figure 179b) due to the formation of the singly threaded pseudo[2]rotaxane ion 2^+⊂57 (Scheme 27). Under these conditions (1 equiv of axle 2^+), the formation of a singly threaded pseudo[2]rotaxane ion 2^+⊂57 (Scheme 27) was ascertained by the ESI(+) mass spectrum that gave a value of 3402 m/z as the base peak, corresponding to a 1:1 host/guest stoichiometry, in which only one dialkylammonium axle was threaded into one of the three macrocycles of 57.

Scheme 27
Figure 181. $^1$H NMR (400 MHz, 298 K, CDCl$_3$) of: a) 57 (aa x10$^{-3}$ M); b) 57 and 1 equiv of 2$^+$; c) 57 and 2 equiv of 2$^+$; d) 57 and 3 equiv of 2$^+$; e) 57 and 8 equiv of 2$^+$.

In addition, the appearance of n-alkyl resonances in the upfield negative region of the $^1$H NMR spectrum of the 1:1 mixture of 2$^+$ and 57 in CDCl$_3$ corroborates the formation of the pseudo[2]rotaxane. The 1:1 stoichiometry was confirmed by spectral integration. A COSY-45 spectrum of the above 1:1 mixture allowed a complete assignment of all shielded alkyl resonances. Thus, $\alpha$ protons at −0.03 ppm show a coupling with $\beta$ methylene group at −1.06 ppm, which presents a cross-peak with $\gamma$ protons at −0.17 ppm, finally coupled with $\delta$ protons at 0.37 ppm (accidentally isochronous with $\varepsilon$ methyl).

Interestingly, the addition of increasing amounts of di-$n$-pentylammonium axle 2$^+$ led to the appearance of new signals attributable to the intermediate formation of pseudo[3]rotaxane which then evolved to pseudo[4]rotaxane (Figure 179). The addition of 8 equivalent of 2$^+$ led to a
simplification of the $^1$H NMR spectrum of the mixture of $2^+\cdot$TFPB$^-$ and 57 (Figures 179c-e). In particular, the appearance of a new singlet at 4.98 ppm integrating for 6H and relative to OCH$_2$ groups of the bridge, and the presence of three singlets relative to OMe groups were indicative of the prevalence of the higher-symmetry (with respect to singly threaded pseudo[2]rotaxane $2^+\subset57$) triply threaded pseudo[4]rotaxane $(2^+)_3\subset57$ in which three axles $2^+$ were threaded into the three subcavities of 57. Both, $^1$H NMR signal integration and ESI(+) mass spectrum of 8:1 mixture of $2^+\cdot$TFPB$^-$ and 57 gave a value of 1239.6 m/z as the base peak, corresponding to triply threaded pseudo[4]rotaxane ion $(2^+)_3\subset57$ with a 1:3 host/guest stoichiometry.

6.3.2. Threading of triple-calix[6]arene 57 with di-benzylammonium axle 4$	extsuperscript{+}$

Scheme 28. Schematic representation of threading of 57 with the di-$n$-benzylammonium axle 4$	extsuperscript{+}$.

Quite interesting was the through-the-annulus threading of triple-calix[6]arene 57 with dibenzylammonium axle (Bn$_2$NH$_2^+\cdot$TFPB$^-$)(Scheme 28). In analogy to the homologue double-calix[6]arene 21, the tert-butylated triple-calix[6]arene 57 gave also rise to endo-benzyl complexation. In fact, the addition of 1 equivalent of di-benzylammonium salt $4^+\cdot$TFPB$^-$ to a solution of 57 in CDCl$_3$, evidenced the presence of diagnostic shielded benzylic resonances in the 4─6
ppm region of its $^1$H NMR spectrum (Figure 180), typical of ortho, meta and para protons (Figure 182 on the right expansion of shielded benzyl region).

**Figure 182.** $^1$H NMR (400 MHz, 298 K, CDCl$_3$) of: a) 57(aa x10$^{-3}$ M); b) 57 and 1 equiv of 4$^*$; c) 57 and 2 equiv of 4$^*$; d) 57 and 3 equiv of 4$^*$; e) 57 and 8 equiv of 4$^*$: on the right expansion of shielded benzyl region.
Further addition of 3 equivalents of di-benzylammonium axle $4^+$ led to a simplification of the $^1$H NMR spectrum (Figure 180 d-e, see in particular the resonance of para protons). This was indicative of the formation of a new, higher-symmetry, triple-threaded pseudo[4]rotaxane $(4^+)_3\subset 57$ in which three axles $4^+$ were threaded into the three macrocycles of 57. A confirmation was obtained by $^1$H NMR signal integration and by an ESI(+) mass spectrum of a 3:1 mixture of $4^+\cdot$TFPB$^-$ and 57, which gave a value of 1245.3 m/z as the base peak, corresponding to a 1:3 host/guest stoichiometry.

**6.3.3 Directional threading of triple-calix[6]arene 57 with nonsymmetrical n-butylbenzylammonium axle $3^+$**

![Scheme 29](image)

**Figure 183.** Schematic representation of Directional threading of 57 with the n-butylbenzylammonium axle $3^+$. 
As in the case of double calix[6]arene 21 (Figure 82), the threading of 57 with the \(n\)-butylbenzylammonium axle 3\(^+\) could give rise to four directional triply-threaded pseudo[4]rotaxane diastereoisomers with all possible combination of \textit{endo-}alkyl, or \textit{endo-}benzyl relative orientations (Figure 184). Of course, on the basis of the above discussed \textit{endo-}alkyl rule (Figure 79), we expected that the triple-\textit{endo-}alkyl stereoisomer should be favored.

![Figure 184. Possible triply-threaded pseudo[4]rotaxane stereoisomers by directional threading of 57 with the \(n\)-butylbenzylammonium axle 3\(^+\).](image)

As above, the addition of butylbenzylammonium 3\(^+\) to a solution of 57 in CDCl\(_3\) caused the usual changes in its \(^1\)H NMR spectrum. As in the previous instances, 1 equiv of guest led to the singly-threaded complex 3\(^+\)\(\subset\)57 (as confirmed by ESI(+) MS and \(^1\)H NMR signal integration. In analogy to the above discussed 2\(^+\)\(\subset\)57 pseudorotaxane, particularly useful were the singlets
relative to the OCH$_2$ protons of the bridge, which resonate at 5.02-5.07 ppm (Figure 185b). The progressive addition of 3$^+$ led to the emerging of a sole singlet at 5.04 ppm corresponding to the triply-threaded pseudo[4]rotaxane (3$^+$)$_3$::$57$, as confirmed by ESI(+) MS and $^1$H NMR signal integration.

Scheme 30

Figure 185. $^1$H NMR (400 MHz, 298 K, CDCl$_3$) of: a) 57(aa x10$^{-3}$ M); b) 57 and 1 equiv of 3$^+$; c) 57 and 2 equiv of 3$^+$; d) 56 and 3 equiv of 3$^+$; e) 57 and 8 equiv of 3$^+$: on the right expansion of shielded alkyl region.
As concerns the stereochemistry of the threading, the $^1$H NMR spectra of the mixtures of 57 and 3$^+$ showed typical highfield signals characteristic of an endo-alkyl complexation.$^{77,80}$ This result in conjunction with the absence of shielded benzylic resonances indicated an endo-alkyl orientation of butylbenzylammonium threads in the corresponding pseudorotaxanes.$^{95}$ Thus, the validity of the endo-alkyl rule is confirmed once again, which would allow to control the threading directionality in interpenetrad systems based on such triple-calixarene.
6.4 EXPERIMENTAL SECTION

ESI(+)–MS measurements were performed on a Micromass Bio-Q triple quadrupole mass spectrometer equipped with electrospray ion source, using CHCl₃ as solvent. All chemicals were reagent grade and were used without further purification. When necessary compounds were dried in vacuo over CaCl₂. Reaction temperatures were measured externally. Derivative 2⁺·TFPB⁻⁷⁷, 3⁺·TFPB⁻⁷⁷, and 12¹²³ were synthesized according to literature procedures. NMR spectra were recorded on Bruker Avance-400 spectrometer [400 (1H) and 100 MHz (13C)], Bruker Avance-300 spectrometer [300 (1H) and 75 MHz (13C)], or 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). COSY-45 spectra were taken using a relaxation delay of 2 seconds with 30 scans and 170 increments of 2048 points each. 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. Monte Carlo conformational searches (10000 steps) were performed with MacroModel-9/Maestro-4.1 program using CHCl₃ as solvent (GB/SA model). MD simulations were performed at T = 500 K, for 20000 ps, using a time step of 1.0 fs.

6.4.1 Synthesis of triple *tert*-butylated-calix[6]arene 56

NaH (1.05 g, 54.2 mmol) was added at 0 °C, under stirring, to a solution of derivative 20 (3.11, 4.37 mmol) in dry THF/DMF (180 mL, 7/3 *v/v*). The mixture was kept at 25 °C under stirring, and after 1 h, 1,3,5-tris(bromomethyl)benzene 56 (0.36 g, 1.36 mmol) was added. The reaction was stirred at reflux for 24 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 (100 mL), brine (100 mL), and dried over Na₂SO₄. The crude product was purified by column chromatography (SiO₂; Et₂O/CH₂Cl₂ 3/97) to give derivative 57 as a white solid (2.22 g, 0.69 mmol, 69%). 

**ESI(+) MS**: *m/z* 3287.2 (MH⁺). 

**¹HNMR** (400 MHz, CDCl₃, 298 K): δ 7.78 (s, ArH₃, 3H), 7.28 - 7.26 (overlapped ArH₃, 6H), 7.11 - 7.06 (overlapped, ArH₁, 12H), 6.94 - 6.87 (overlapped, ArH₁, 18H), 5.06 (s, ArH₃, 6H), 4.50 e 3.58 (AX, ArCH₂Ar, *J*=14.7Hz, 12H), 4.17 e 4.04 (AX, ArCH₂Ar, *J*=14.5Hz, 12H), 3.83 e 3.68 (AX, ArCH₂Ar, *J*=15.1Hz, 12H), 3.15 (s, OCH₃, 18H), 2.82 (s, OCH₃, 9H), 2.58 (s, OCH₃, 18H), 1.24 (2) (overlapped, C(CH₃)₃, 81H), 1.07 (s, C(CH₃)₃, 54H), 0.97 (s, C(CH₃)₃, 26H). 

**¹³CNMR** (250 MHz, CDCl₃, 298 K): δ 154.5, 154.4, 153.9, 152.4,
145.9, 145.7, 138.8, 134.1, 133.9, 133.7, 133.6, 133.4, 127.5, 126.9, 125.5, 125.3, 124.8, 77.5, 75.9, 74.8, 60.2, 60.0, 34.3 (2), 31.8, 31.7(2), 31.5 (2), 30.8, 30.6, 22.7, 14.4.

Figura 186 Spectrum $^1$H NMR of derivative 57 in CDCl$_3$ (400 MHz, 298 K).
Figura 187. Spectrum $^{13}$C NMR of derivative 57 in CDCl$_3$ (250 MHz, 298 K).

6.4.2 Preparation of singly-threaded pseudo[2]rotaxanes 58$^+$, 61$^+$ and 64$^+$

Derivative 58$^+$
Triple-calixarene derivative 57 (1.81·10^{-3} g, 1.2·10^{-3} mmol) and the di-n-pentylammonium derivative 2^+ (0.6·10^{-3} mmol) were dissolved in 0.4 mL of CDCl\textsubscript{3} and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. Selected spectral data for singly threaded pseudo[2]rotaxane ion 2^+\subset57 ESI(+) MS: m/z = 3423.4 [2\subset57]^+. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz, 298 K): δ −1.07 [broad, (CH\textsubscript{2})\textsubscript{β}, 2H], −0.17 [broad, (CH\textsubscript{2})\textsubscript{γ}, 2H], −0.02 [broad, (CH\textsubscript{2})\textsubscript{α}, 2H], 0.37 [broad, (CH\textsubscript{2})\textsubscript{δ} + (CH\textsubscript{3})\textsubscript{ε}, 5H], 3.53 and 4.29 (broad overlapped, ArCH\textsubscript{2}Ar, 36H), 2.87, 2.99, 3.23 (br s, OCH\textsubscript{3}, 6H, 3H, 6H), 3.78, 3.84, 3.90 (br s, OCH\textsubscript{3}, 18H, 9H, 18H), 4.79 (br s, OCH\textsubscript{2}, 3H), 4.98 (br s, OCH\textsubscript{2}, 3H), 6.63–6.99 (overlapped, ArH, 40H).

Figure 188. \textsuperscript{1}H NMR spectrum (400 MHz, 298 K, CDCl\textsubscript{3}) of the 1:1 mixture of 57 and 3^+\textsuperscript{-}TFPB\textsuperscript{-}.
Triple-calixarene derivative 57 (2.0·10⁻³ g, 0.91·10⁻³ mmol) and the dibenzylammonium derivative 3⁺ (1.3·10⁻³ mmol) were dissolved in 0.5 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.

¹H NMR (400 MHz, CDCl₃, 298 K): δ 1.04 [s, (CH₃)₃C-, 90H], 1.28 [s, (CH₃)₃C-, 90H], 1.38 [m, (CH₂)n, 2H], 1.53 [m, (OCH₂)₃, 2H], 1.61 [m, (OCH₂)₃, 2H], 3.45 and 4.39 (AX, ArCH₂Ar, 12H, J = 12.0 Hz), 3.75 [s, OCH₃, 15H], 3.75 [s, OCH₃, 15H], 4.01 and 4.51 (d, ArHᵣ, 2H, J = 8.0 Hz), 5.07 [s, (OCH₂)₃, 6H,], 4.78 (d, ArHᵣ, J= 8.0 Hz, 1H), 5.33 (dd, ArHₘ, J₁=J₂=7.6 Hz, 4H), 5.98 (t, ArHᵢ, J₁ = 7.6 Hz, 2H), 6.11 (broad, NH₂⁺, 2H), 7.14-7.30 (overlapped, ArH, 52H), 7.50 (s, ArHₜₚ, 4H), 7.71 (t, ArHₜₚ, 8H, J = 4.0 Hz), 7.83 (d, ArHₘ, 2H, J = 8.0 Hz)
Derivative 64⁺

Figure 189. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:1 mixture of 57 and 3⁺-TFPB⁻.

Triple-calixarene derivative 57 (2.0·10⁻³ g, 0.91·10⁻³ mmol) and the dialkylammonium derivative 3⁺ (0.65·10⁻³ mmol) were dissolved in 0.5 mL of CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition.
ESI(+) MS: $m/z = 3456 \ [3\cdot57]^+$. $^1$H NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$

$-1.02$ [broad, (CH$_2$)$_\beta$, 2H], $0.04$ [broad, (CH$_2$)$_\gamma$ + (CH$_3$)$_\delta$, 5H], $0.22$ [broad, (CH$_2$)$_\alpha$, 2H], $2.30$ [broad, (H$_2$N$^+$/CH$_2$)$_\beta$Ph), 2H], $2.87$–$3.93$ (broad overlapped OCH$_3$ + ArCH$_2$Ar, 42H), $4.31$–$4.41$ (broad overlapped, ArCH$_2$Ar, 12H), $4.78$ (br s, OCH$_2$, 2H), $4.98$ (br s, OCH$_2$, 2H), $5.37$ (br s, $^+$NH$_2$, 2H), $6.76$–$7.40$ (overlapped, ArH, 45H).

**Figure 190.** $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:1 mixture of 57 and $^+$TFPB$^-$. 
6.4.3 Preparation of triply-threaded pseudo[4]rotaxanes 60\(^{3+}\), 63\(^{3+}\) and 66\(^{3+}\)

**Derivative 60\(^{3+}\)**

![Diagram of the formation of derivative 60\(^{3+}\)]

Triple-calixarene derivative 57 (2.0 \(\cdot 10^{-3}\) g, 0.91 \(\cdot 10^{-3}\) mmol) and the di-\(n\)-pentylammonium derivative 2\(^+\) (1.94 \(\cdot 10^{-3}\) mmol) were dissolved in 0.5 mL of CDCl\(_3\) and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+) MS: \(m/z = 1239.8\ [(2)_3\subset 57]^{3+}\). \(^1\)H NMR (CDCl\(_3\), 400 MHz, 298 K): \(\delta = -1.07\) [broad, (CH\(_2\))\(_\beta\), 6H], −0.18 [broad, (CH\(_2\))\(_\gamma\), 6H], −0.01 [broad, (CH\(_2\))\(_\alpha\), 6H], 0.39 [broad, (CH\(_2\))\(_\delta\) + (CH\(_3\))\(_\epsilon\), 15H], 3.52 and 4.33 (broad, ArCH\(_2\)Ar, 36H), 3.79, 3.84, 3.91 (br s, OCH\(_3\), 9H, 18H, 18H), 4.98 (br s, OCH\(_2\), 6H), 6.66—7.61 (overlapped, ArH, 60H).
Figure 191. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:3 mixture of 57 and 2$^+\cdot$TFPB$^-$. 

Derivative 63$^{3+}$

Triple-calixarene derivative 57 (2.0·10$^{-3}$ g, 0.91·10$^{-3}$ mmol) and the dibenzylammonium derivative 4$^+$ (1.97 10$^{-3}$ mmol) were dissolved in 0.5 mL of CDCl$_3$ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+ ) MS: $m/z = 1245.6 [(4)_3\subset57]^{3+}$. $^1$H NMR (CDCl$_3$, 400 MHz, 298 K): $\delta$ 1.17 (s, Bu', 135H), 1.45 [(CH$_2$)$_d$, 6H], 2.94 [(CH$_2$)$_f$, 6H], 3.51 and 4.40 (AX, ArCH$_2$Ar, $J = 13.6$ Hz, 18H each), 3.86 (s, OCH$_3$, 54H), 4.67 (d, ArH$_o$, $J = 8.0$ Hz, 6H), 5.34
(dd, ArH<sub>m</sub>, J<sub>1</sub>=J<sub>2</sub>=7.6 Hz, 6H), 5.40 [s (OCH<sub>2</sub>)<sub>bridge</sub>,6H], 6.01 (t, ArH<sub>p</sub>, J<sub>1</sub>= 7.6 Hz, 6H), 6.09 (broad, NH<sub>2</sub><sup>+</sup>, 6H), 7.01 (s, ArH<sub>calix</sub>, 36H), 7.48 (s, ArH<sub>TFPB</sub><sup>−</sup>, 12H), 7.71 (ArH<sub>TFPB</sub><sup>−</sup>, 24H).

Figure 192. <sup>1</sup>H NMR spectrum (400 MHz, 298 K, CDCl<sub>3</sub>) of the 1:3 mixture of 57 and 4<sup>+</sup>·TFPB<sup>−</sup>.

Triple-calixarene derivative 57 (2.0·10^{-3} g, 0.91·10^{-3} mmol) and the alkylbenzylammonium derivative 3+ (2.73·10^{-3} mmol) were dissolved in 0.5 mL of CDCl₃ and the mixture was stirred for 5 min at 25 °C. Then, the solution was transferred in a NMR tube for 1D and 2D NMR spectra acquisition. ESI(+) MS: m/z = 1239.6 [(3)₃·57]^{3+}. 

$^1$H NMR (CDCl₃, 400 MHz, 298 K): δ = 1.01 [broad, (CH₂)₂, 6H], 0.03 [broad, (CH₂)γ + (CH₃)δ, 15H], 0.23 [broad, (CH₂)α, 6H], 2.27 [broad, ($^1$H₂NCH₂Ph), 6H], 3.28, 3.59, 3.70 (s, OCH₃, 9H, 18H, 18H), 3.50 and 4.44 (broad, ArCH₂Ar, 12H), 3.52 and 4.32 (broad, ArCH₂Ar, 12H), 3.58 and 4.43 (broad, ArCH₂Ar, 12H), 4.97 (br s, OCH₂, 6H), 5.35 (br s, $^1$NH₂, 6H), 6.64–7.59 (overlapped, ArH, 75H).
Figure 193. $^1$H NMR spectrum (400 MHz, 298 K, CDCl$_3$) of the 1:3 mixture of 57 and 3·TFPB$\textsuperscript{-}$. 
6.5 Conclusion

In the last part of the Ph.D. thesis has been devoted to the threading abilities of triple-calix[6]arene hosts 57 with mono-ammonium axles in the presence of the TFPB superweak anion.

$^1$H NMR and ESI(+) MS spectra evidenced the stepwise formation of singly-doubly- and tply-threaded pseudorotaxane architectures by changing the host/guest stoichiometry from 1:1 to 1:3. The directional threading of nonsymmetrical $n$-butylbenzylammonium axle 3$^+$ with triple-calix[6]arene host 57 occurs with an endo-alkyl preference in accordance with the known “endo-alkyl rule”.
Figure 194. Possible triply-threaded pseudo[4]rotaxane stereoisomers by directional threading of 57 with the n-butylbenzylammonium axle 3*.
7. Conclusion

In this Ph. D. thesis, we have briefly introduced the main concepts of chemical topology in order to provide a rational perspective to frame what appear to be the most advanced molecular machines that chemistry has been able to assemble up to now.

From the considerable list of available approaches, it was decided to focus our study to the calixarene/dialkylammonium recognition motif, reporting some recently discovered peculiarities, which are related to the possible orientations of the directional calix-wheel.\textsuperscript{77,78,79}

![Image of endo-Cavity Complexation and Through-the-Annulus Threading of Calixarenes Induced by Very Loose Alkylammonium Ion Pairs](image)

**Figure 195.** endo-Cavity Complexation and *Through-the-Annulus* Threading of Calixarenes Induced by Very Loose Alkylammonium Ion Pairs

Then, the Ph. D. thesis deals with the exploration of these stereochemical features in the threading of hosts containing multiple cavities. Therefore, the synthesis of double- and triple-calixarenes is reported, which is followed by the subsequent study of their threading abilities with dialkylammonium axles. The results confirmed the well-known *endo-alkyl* preference of calix[6]arenes to give the inclusion of alkyl chains inside the calix-cavity. On this basis we were then able to build new chemical topologies such as oriented handcuff rotaxanes.
The results were particularly satisfying and can be considered as a prelude to the possible use of multi-calixarenes in the synthesis of topologically more complex architectures.