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PAPER

Allosterically driven self-assemblies of interlocked calix[6]arene receptors†

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The construction of self-assembled receptors based on flexible concave subunits is a challenging task and constitutes an interesting approach to mimic binding processes occurring in biological systems. The receptors studied herein are based on flexible calix[6]arene skeletons bearing three (or more) acid–base functionalities at their narrow rim. When complementary, they self-assemble in a tail-to-tail manner to give a diabolo-like complex, provided that each calixarene subunit hosts a guest. The allosterically-driven multi-recognition pattern is highly selective and leads to stable quaternary adducts. In order to evaluate the scope of this system, various polyamino and polyacidic calix[6]arenes have been studied. It is shown that modifications of the nature of the wide rim substituents do not alter the efficiency of the quaternary self-assembling process, even with the more flexible macrocycles that lack *t*Bu substituents. On the contrary, the replacement of the latter by smaller groups led to receptors with broader scope, as larger guests such as tryptamine and dopamine derivatives were stabilized in the cavities. Implementation of extra-functionalities at the narrow rim were revealed also to be of high interest. Indeed, it is shown that secondary interactions take place between the two calix-subunits when they present additional and complementary functions such as carboxylate and ureido moieties. The ureido arms are also capable of binding the counter anion Cl^- of the ammonium guest, thus leading to a quinternary neutral complex. Such remarkable behavior is due to the versatility of the calix[6]arene platform, which allows the implementation of a high number of functions, leading to multiple non-covalent attractive interactions, whereas the macrocycle remains flexible, thus allowing induced-fit processes to occur.

Introduction

Synthetic self-assembled receptors are a particular class of host molecules that are built through the non-covalent assembly of several (identical or not) subunits.[1] Over the last two decades, particular attention has been directed toward the design of self-assembled receptors that display capsule-like, tubular-like or cage-like shapes. The confined, generally hydrophobic, nano-environment defined by these three-dimensional structures allows selection of guest molecules and their separation from the bulk. Such isolated molecules can display unique physical properties and chemical reactivities.[2] This field of research is now at a stage where applications such as molecular flasks are becoming realistic.[3]

Self-assembly[4] of molecular receptors present clear advantages over covalent synthesis: (i) it avoids tedious multistep synthesis thanks to error correcting processes; (ii) it enhances rates of guest exchange through partial dissociation of the host.[5] The use of rigid skeletons that can provide reversible interactions with a high directionality has become a widespread strategy to drive and direct the assembly process into discrete structures with a precise positioning of the subunits. Planar poly-aryl or aromatic molecules (*e.g.* tris(4-pyridyl)triazines or porphyrins), curved shape molecules (*e.g.* glycoluril derivatives) or bowl shape molecules (*e.g.* calix[4]arenes or resorcinarene cavitands) are among the most popular subunits and their assembly has been achieved mainly *via* H-bonding interactions[6] or coordinative bonds.[7] Such a strategy for elaborating self-assembled receptors strongly differs from what is encountered in natural systems. Indeed, most of the biological receptors are based on rather flexible structures (*e.g.*, protein scaffolds), and nanomolar affinity is commonly achieved in aqueous media, several orders of magnitude better than what synthetic-rigid-receptors can do.[8] Recent insights into molecular recognition by proteins have highlighted that the binding of the ligand can induce a reinforcement of intra-protein interactions through a reduction of the receptor dynamics.[9] This reinforced molecular recognition strategy, that is the use of secondary intra-receptor interactions to strengthen ligand binding, can likely explain the

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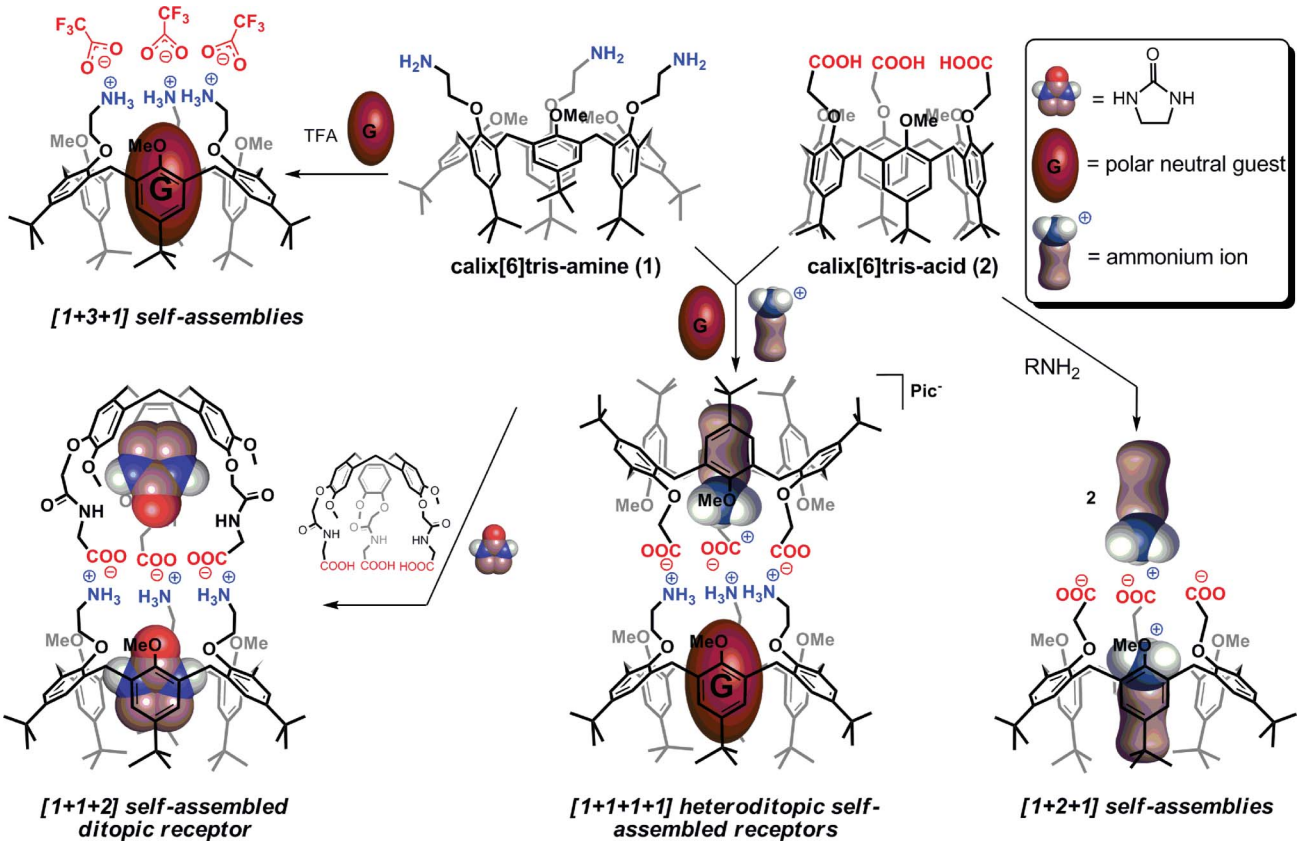


Fig. 1 The use of calix[6]tris-amine **1** and calix[6]tris-acid **2** for the elaboration of self-assembled receptors.

high affinities observed with natural systems. Transposed into synthetic systems, such a strategy represents an emergent approach contrasting with the design of rigid receptors focused exclusively on the direct host–guest interactions.[10] Thus, even if synthetic self-assembled receptors based on flexible macrocyclic scaffolds are still scarcely studied,[11] there is a considerable interest in developing such receptors since secondary intra-receptor interactions could arise from conformational rearrangement triggered by guest binding.

In the field of molecular recognition, calix[6]arenes are less studied than the smaller tetramers.[12] This is mainly due to the difficulty of constraining the calix[6]arene structure into a particular conformation since, in contrast to calix[4]arenes, the narrow rim is wide enough to allow rotation of the bulky *p*-tBu groups of the phenolic units through the annulus. Thus, to behave as a molecular host, the flexible calix[6]arene skeleton needs to be rigidified into the cone conformation, this conformation providing a well defined cavity ideally suited for guest inclusion. For that purpose, several strategies involving covalent bridging,[13] metal ion coordination[14] or anion binding[15] have been developed. The resulting conformationally constrained but still flexible calix[6]arene hosts have exhibited unique host–guest properties and, in many cases, the flexibility of the host was turned into an advantage since induced fit processes were observed.[16] However, despite an appealing potential in molecular recognition, the design of self-assembled calix[6]arene edifices remains a challenging task. Indeed, only a few examples of such supramolecular hosts have been described, most of them being homo-dimers connected through the wide rim *via* H-bonding interactions.[17]

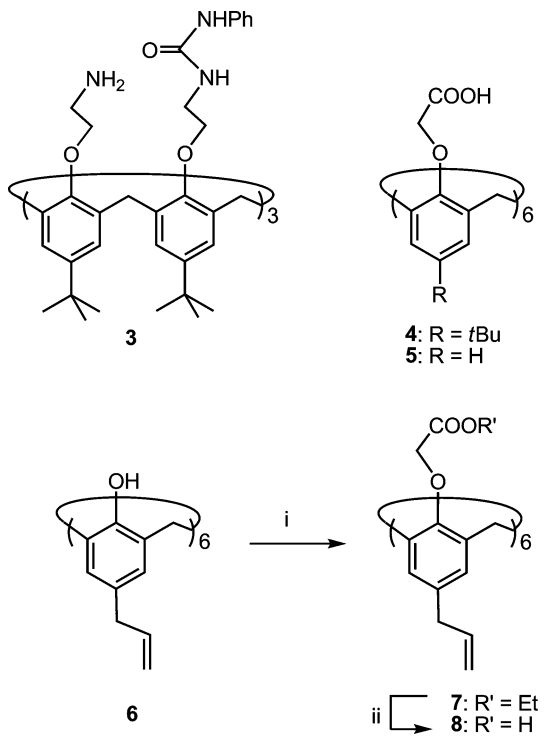
As part of our research on biomimetic host–guest systems, we were interested in the design of multi-cavities self-assembled receptors, as such receptors could provide a means to achieve allosteric control of guest binding.[18,19] Thus, we recently turned our attention to the narrow rim self-assembly of calix[6]arenes through ionic interactions (Fig. 1). We first found that the flexible calix[6]tris-amine **1** can be frozen into its cone conformation upon protonation, the rigidification arising from the formation of an ion paired cap and a network of H-bonding interactions between the ammonium arms and their counter anions.[20] As a result, the positively charged calixarene presents a polarized cavity that hosts efficiently polar neutral molecules through charge–dipole, CH-π and H-bonding interactions. The supramolecular capping strategy has then been used to associate a rigid concave cavity such as a cyclotriveratrylene bearing three carboxylic groups to the tris-amino calix[6]arene (Fig. 1).[21] The resulting self-assembled heteroditopic receptor was shown to encapsulate a polar neutral guest into each cavity with, however, a different recognition mode and thus different affinities.

Conversely, the calix[6]tris-acid **2** was shown to be efficiently constrained into a cone through an acid–base reaction with three equivalents of amine. The resulting self-assembled structure is shaped by two ammonium ions capping the narrow rim of the calixarene in an *exo* position through ion pairing with the carboxylate arms, and by a third one in an *endo* position (Fig. 1).[22]

Indeed, the resulting tris-carboxylate polarized host was found to display a high affinity for ammonium ions as guests thanks to a combination of weak interactions (charge–dipole, H-bonding and CH-π) in a way that is very similar to the above described case of calix[6]tris-amine **1**.

Finally, the complementary calix[6]tris-amine **1** and calix[6]tris-acid **2** subunits were self-assembled into a diabolo-like heterodimer displaying divergent cavities,[23] at the very condition however that the cavities were shaped by their respective guest molecules.[24] Hence, the selective formation of a rare [1+1+1+1] quaternary complex through an allosterically coupled, double induced fit process was demonstrated. Such a system is characterized by the triple ammonium-carboxylate ion-pairing that drives the assembly process and polarizes the cavities, while the specific host–guest interactions direct the process into a discrete and highly ordered structure. This last result clearly highlighted that a structure with controlled flexibility can be a valuable alternative to rigid subunits for the construction of sophisticated self-assembled receptors.

We thus became interested in exploring this further and possibly expanding the scope of these self-assembled bis-calix[6]arene-based receptors. On the one hand, in view of practical applications, more accessible calix[6]arene subunits that can *endo*-complex large guests were desirable. On the other hand, we thought of implementing additional recognition motifs on the calixarene subunits. Here, we present the results obtained with calix[6]arenes bearing various functionalities at the narrow rim and various substituents at the wide rim (Scheme 1).



Scheme 1 Structures of the calix[6]arene subunits **3**, **4**, **5** and **8**. i) $BrCH_2COOEt$, K_2CO_3, acetone, reflux, 62%; ii) NMe_4OH, THF, reflux, 97%.

Results

• Synthesis of the calixarene subunits 3, 4, 5 and 8

The calixarene subunits **3**, **4**, **5** and **8** were chosen for the elaboration of the new self-assembled receptors (Scheme 1). The synthesis of calix[6]-1,3,5-trisamine-2,4,6-trisurea **3** was previously reported[25] *via* a strategy involving a selective tris-protection reaction.[26] For the poly-acidic subunits, we focused on calix[6]hexa-acid derivatives for the following reasons: (i) they are more accessible than the corresponding 1,3,5-trisacids (such as **2**); (ii) they provide additional carboxylic groups that could participate in the ion-pairing assembly; (iii) they can be readily derivatized at the wide rim. Hence, calix[6]hexa-acid **4** was prepared as described in the literature.[27] This compound however, suffers from the presence of the bulky *t*Bu groups on the wide rim that restrict the size of the cavity. The related calix[6]hexa-acids **5** and **8**, which present an open cavity in the cone conformation, were thus also synthesized. In contrast to **5**, compound **8** was expected to be highly soluble in organic media. Calix[6]hexa-acid **5** was prepared by following a literature procedure[28] while **8** was readily obtained in two steps from the known calix[6]arene **6** (Scheme 1).[29]

• NMR studies of the host–guest properties of the calixarene subunits 3, 4, 5 and 8

In a first set of experiments, the host–guest properties of the calixarene subunits **3**, **4**, **5** and **8** were evaluated by ^{1}H NMR spectroscopy and compared to those of the parent tris-functionalized receptors (*i.e.* **3** *vs.* **1** and **4**, **5**, **8** *vs.* **2**). Hence, upon protonation by trifluoroacetic acid (TFA), **3** was found to *endo*-complex quantitatively one equivalent of the neutral 2-imidazolidinone (IMI) (Scheme 2, top left). The resulting host–guest complex $\mathbf{3}_{\mathbf{IMI}}^{\mathbf{3H+,3TFA-}}$ displays a C_{3v} symmetrical NMR pattern with a high-field singlet corresponding to the included IMI ($\delta_{CH2}IMI_{in} = 0.36$ ppm),[30] the *in* and *out* guest exchange being slow on the NMR time scale. It is noteworthy that the complexation induced shift (CIS) of the included IMI is similar to the one observed with $\mathbf{1}_{\mathbf{IMI}}^{\mathbf{3H+,3TFA-}}$ ($\Delta\delta_{CH2} \approx -3.2$ ppm). The addition of an excess of $PrNH_2$ to a $CDCl_3$ solution of calix[6]hexa-acid **4** led to the formation of the host–guest complex $\mathbf{4}_{\mathbf{PrNH3+}}^{\mathbf{-6H+,5PrNH3+}}$ (Scheme 2, top right).[31] A ^{1}H NMR titration experiment revealed that, similarly to the parent receptor **2**, only two *exo*-complexed ammonium ions are necessary to achieve the supramolecular capping of the host.[30] Complex $\mathbf{4}_{\mathbf{PrNH3+}}^{\mathbf{-6H+,5PrNH3+}}$ displays a C_{6v} symmetrical ^{1}H NMR pattern at room temperature that likely corresponds to an average spectrum of the two identical C_{3v} symmetrical flattened cone conformations in fast inter-conversion on the NMR time scale.[30,32] Again, a slow host–guest exchange occurs at the NMR time scale and the CISs of the propylammonium guest are similar to those observed with the parent *endo*-complex $\mathbf{2}_{\mathbf{PrNH3+}}^{\mathbf{-3H+,2PrNH3+}}$ ($\Delta\delta_{CH3} \approx -2.6$ ppm).[22] Despite an extremely low solubility in $CDCl_3$, the calix[6]hexa-acid **5** was fully extracted upon addition of an excess of octylamine (> 6 equiv) and the quantitative *endo*-complexation of the octylammonium ion was observed. The CISs indicate that the protons in the β position relative to the charged nitrogen atom are located in the heart of the cavity, while the chain extremity is protruding outside of the cavity (Scheme 2, bottom, and Fig. 2a). Calix[6]arene **8** was fully soluble in organic media thanks to the hexa-allylic groups, and the ammonium salts of the biologically relevant

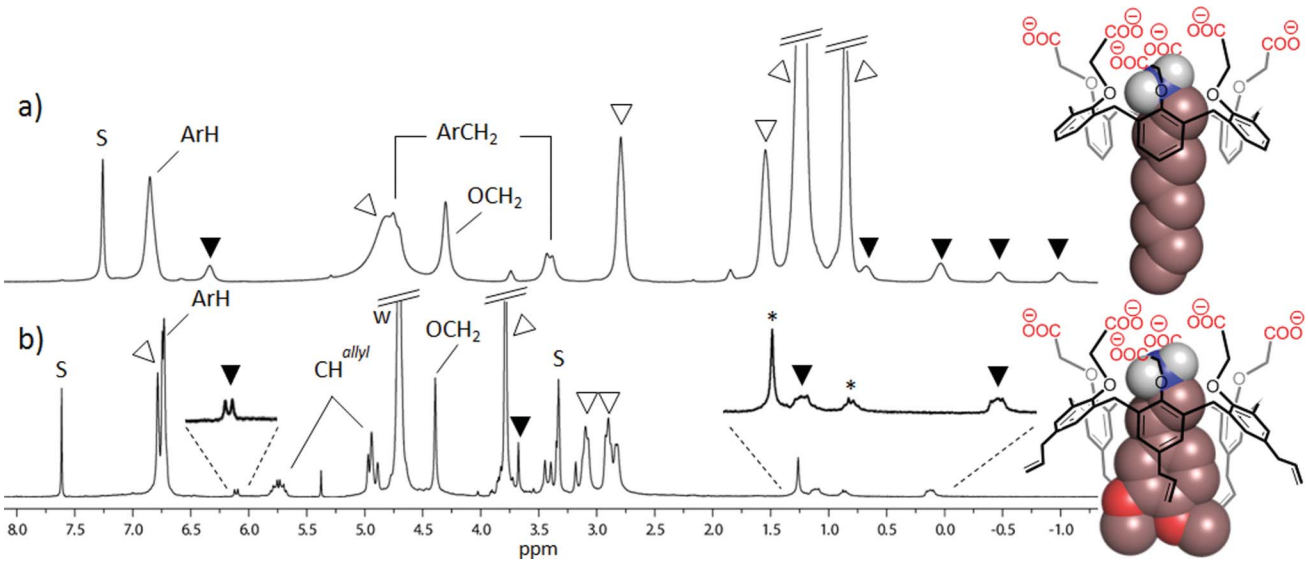


Fig. 2 ^{1}H NMR spectra at 298 K of host–guest complexes (a) $\mathbf{5}^{-6H+,5OctNH3+}_{OctNH3+}$ ($CDCl_3$) and (b) $\mathbf{8}^{-6H+,5DopaMe2NH3+}_{DopaMe2NH3+}$ (2 : 1 CD_3OD : $CDCl_3$ solution). ▼: $OctNH_3^+$ *in* or $DopaMe_2$ *in*; Δ: $OctNH_2$ free or $DopaMe_2$ free; S: solvent; w: water; *: residual grease.

O-methylated derivatives of 6-hydroxytryptamine and dopamine were fully *endo*-complexed, even in a polar protic media (*i.e.* in a 2 : 1 CD_3OD : $CDCl_3$ solution) (Fig. 2b).[30] Again, the CISs attest to the presence of these large guests in the heart of the hydrophobic cavity (Scheme 2, bottom). This result highlights the efficiency of ionic interactions combined to a polarized hydrophobic cavity for the building of stable host–guest adducts in polar protic media.

All these NMR data show that the hexa-functionalized calix[6]arenes (*i.e.* **3**, **4**, **5** and **8**) display similar host–guest properties compared with the parent tris-functionalized compounds (*i.e.* **1** and **2**):

- they are rigidified in the same flattened cone conformation upon ionization (protonation to yield poly-ammonium hosts or deprotonation to yield poly-carboxylate hosts) thanks to supramolecular capping by the counter ions;[33]
- their polarized cavity readily accommodates polar guests (neutral, dipolar in the case of ammonium hosts and ammonium ions in the case of carboxylic acid hosts);
- the host–guest complexes are remarkably resistant in H-bonding competitive media.

Finally, the replacement of the *t*Bu groups by smaller substituents (H or allyl) led to the "opening" of the calixarene cavity, thus allowing the inclusion of large ammonium guests in spite of the high flexibility of the calix[6]arene skeleton.

• NMR studies of the self-assembly process between the calixarene subunits 3, 4, 5 and 8 into [1+1+1+1] complexes

The receptor ability of the calixarene subunits **3**, **4**, **5** and **8** being checked, their self-assembly in tail-to-tail bis-calix[6]arenes was investigated by ^{1}H NMR spectroscopy in $CDCl_3$ (Scheme 3, entries 2 to 9). Common features were observed for the self-assembly processes:

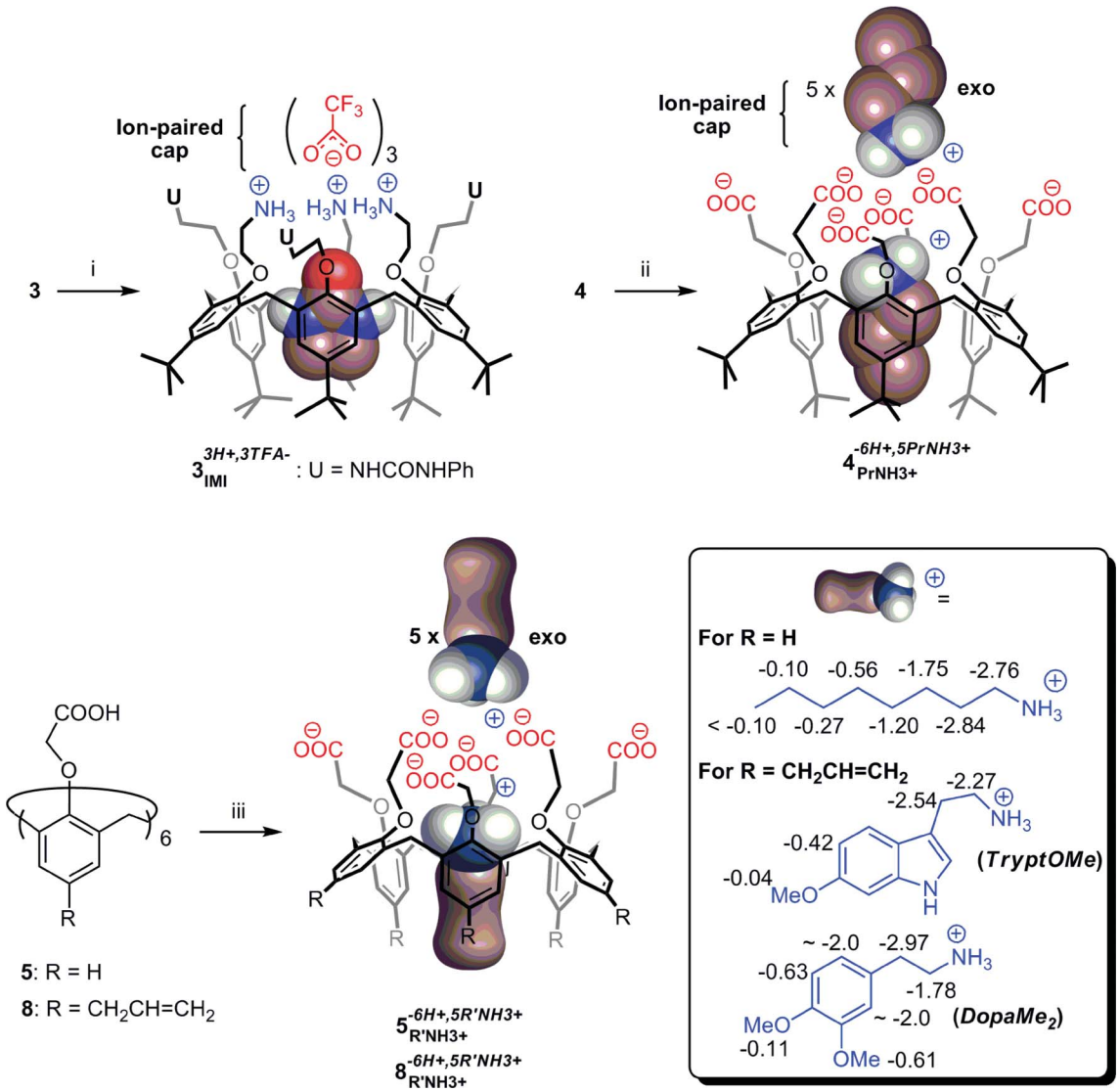
i) when calix[6]tris-amines **1** or **3** were combined with the complementary calix[6]hexa-acids **4**, **5** or **8** (1 : 1 ratio), broad ^{1}H NMR spectra that differ from the NMR signatures of the separated compounds were observed (see Fig. 3a). These ill-defined NMR spectra were found to be insensitive to the temperature and, thus, should correspond to mixtures of non-discrete assemblies. This absence of selectivity in the recognition processes is attributable to the high conformational flexibility of the calix[6]arene partners;

ii) however, upon addition of a polar neutral molecule (*e.g.* 3 to 5 equiv. of IMI) and of an amine or an ammonium ion (1 equiv.),[34] a spectacular sharpening of the spectra together with high-field signals attributed to the included guest molecules were observed (Fig. 3b–d).[35] These well resolved NMR spectra are characteristic of the [1+1+1+1][36] self-assembly of the calixarene subunits into tail-to-tail bis-calix[6]arenes, each calixarene including its specific guest in the heart of the cavity. Two sets of doublets were observed for the $ArCH_2$ protons of the calixarene cores, showing that both partners are rigidified in the cone conformation upon guest inclusion. A broad signal around 8.5–9 ppm, corresponding to the NH_3^+ protons, attested to the proton exchange between the two calixarene partners;

iii) the *in* and *out* guest exchanges were slow on the NMR time scale and the CISs are similar to those observed for the related monomeric *endo*-complexes, which shows an analogous mode of recognition. The small differences, however, reveal a close proximity between the complementary self-assembled hosts;

iv) overall association constants for the whole assembly process were estimated to be $> 10^{11}$ M^{-3} (Scheme 3 entries 2, 3, 6–9).

In each case, the formation of such self-assembled quaternary complexes relies on a highly selective process since no other species were observed. This selectivity is remarkable taking into account the high flexibility of the hosts and the chemical diversity of the four partners (*i.e.* a tris-amine or trisureido-trisamine, a tris- or hexa-carboxylic acid, a cyclic urea and a primary alkyl ammonium ion). The assembly processes proceed through induced-fit processes that are allosterically coupled, the key step being the shaping of both calixarene cores by their respective guests. In other words, the success of this process is due to the high degree of complementarity between the four partners.



Scheme 2 Host–guest properties of the calix[6]arene subunits **3**, **4**, **5** and **8**. i): TFA, IMI (3 equiv.), $CDCl_3$; ii): $PrNH_2$ (> 6 equiv.), $CDCl_3$; iii): $R'NH_2$, (> 6 equiv.), $CDCl_3$ or 2 : 1 CD_3OD : $CDCl_3$.

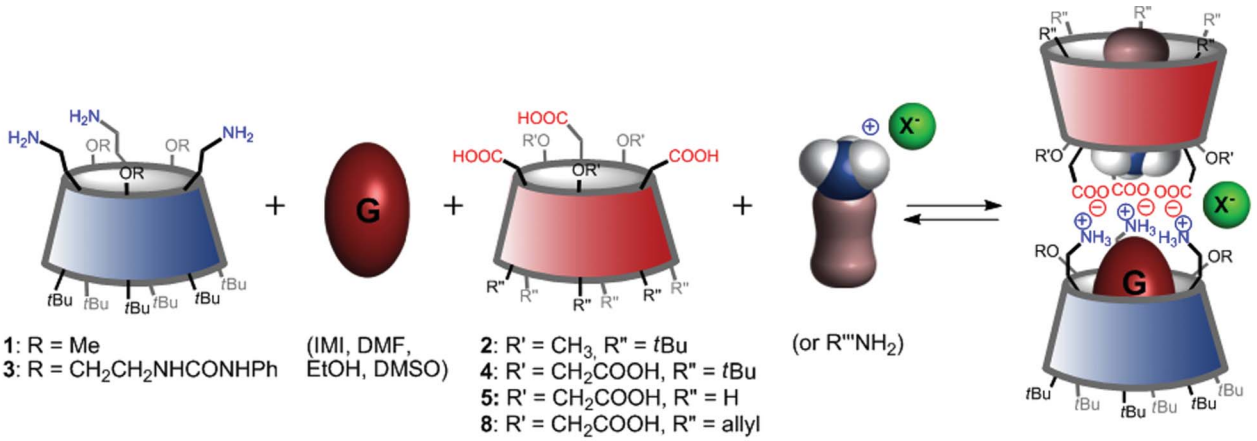
All these NMR considerations are similar to what was observed in the case of the [1+1+1+1] self-assembly of the parent receptors (Scheme 3, entry 1) and thus show that the strategy can be extended to more functionalized and/or flexible calix[6]arene subunits.[24] However, in comparison to the parent receptors and as shown below, the self-assemblies made of hexa-functionalized calix[6]arene subunits can display additional features and, notably, lead to secondary intra-assembly interactions.

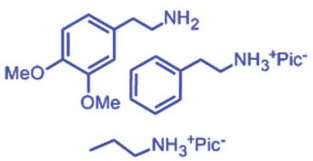
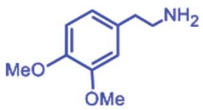
• Assemblies with calix[6]hexa-acid subunits: evidence for versatility

Whereas with calix[6]tris-acid **2**, formation of the quaternary adduct requires the addition of its guest in the form of an ammonium ion, with the calix[6]hexa-acids, the guest could be introduced in the form of its free base as well (Scheme 3, entries 3–5 and 9). Indeed, upon the triple proton exchange between the calix[6]tris-amine and the calix[6]hexa-acid, three carboxylic acid groups remain available for the *in situ* generation of the ammonium guest. For instance, in the case of the subunits **1** and **4** (entry 3), the addition of 1 equiv. of $PrNH_2$ to a mixture of **1**, **4** and IMI (1 : 1 : 3 ratio) led to the tetra-deprotonated species of **4** (*i.e.* $\mathbf{4}^{-4H+}$) and to the selective formation of the assembly $\mathbf{1}_{IMI}^{3H+}\cdot\mathbf{4}_{PrNH3+}^{-4H+}$ that is now a neutral entity (Fig. 3c).[37,38] Thus, the hexa-carboxylic acid **4** plays a triple role: it behaves as a cap for $\mathbf{1}^{3H+}$, as a receptor for the ammonium ion, but also as a proton source for the *in situ* generation of its own guest.

• Assemblies with subunits possessing an enlarged cavity: encapsulation of large bio-relevant guests

With calix[6]hexa-acids **5** and **8**, it was possible to obtain the [1+1+1+1] self-assemblies with larger ammonium guests (Scheme 3, entries 4 and 5).[39] For instance, **5** could be self-assembled with **1** in the presence of IMI and ammonium ions derived either from propylamine, phenylethylamine or *O*-methylated dopamine.[30] The assembly of **8** with **3** in the presence of IMI and the *O*-methylated dopamine was also successful (Fig. 3d). In all



Entry	Calix[6]tris-amine	Neutral guest	Calix[6]tris- or hexa-acid	Ammonium ion or amine	Resulting assembly
1 (*Lit.*)	**1**	IMI, DMF, EtOH	**2**	$NH_3^+Pic^-$	$\mathbf{1}_G^{3H+} \bullet \mathbf{2}_{PrNH3+}^{-3H+}$, Pic^-
2	**1**	IMI, DMSO	**4**	$NH_3^+Pic^-$	$\mathbf{1}_G^{3H+} \bullet \mathbf{4}_{PrNH3+}^{-3H+}$, Pic^-
3	**1**	IMI	**4**	NH_2	$\mathbf{1}_{IMI}^{3H+} \bullet \mathbf{4}_{PrNH3+}^{-4H+}$
4	**1**	IMI	**5**		$\mathbf{1}_{IMI}^{3H+} \bullet \mathbf{5}_{R'''NH3+}^{-3H+}$, Pic^- or $\mathbf{1}_{IMI}^{3H+} \bullet \mathbf{5}_{R'''NH3+}^{-4H+}$
5	**3**	IMI	**8**		$\mathbf{3}_{IMI}^{3H+} \bullet \mathbf{8}_{DopaMe2NH3+}^{-4H+}$
6	**3**	IMI, DMSO	**2**	$NH_3^+Pic^-$	$\mathbf{3}_G^{3H+} \bullet \mathbf{2}_{PrNH3+}^{-3H+}$, Pic^-
7	**3**	IMI, DMSO	**2**	$NH_3^+Cl^-$	$\mathbf{3}_G^{3H+} \bullet Cl^- \bullet \mathbf{2}_{PrNH3+}^{-3H+}$
8	**3**	IMI, DMSO	**4**	$NH_3^+Pic^-$	$\mathbf{3}_G^{3H+} \bullet \mathbf{4}_{PrNH3+}^{-3H+}$, Pic^-
9	**3**	IMI	**4**	NH_2	$\mathbf{3}_{IMI}^{3H+} \bullet \mathbf{4}_{PrNH3+}^{-4H+}$

Scheme 3 Formation of tail-to-tail self-assemblies triggered by guest inclusion.

cases, the CISs values attest to the deep inclusion of the aromatic ring of the guests into the cavities, with a slow *in* and *out* guest exchange process on the NMR time scale, even at high temperature (330 K).[30]

• Assemblies with the calix[6]trisamine-trisurea 3: evidence for secondary interactions

The calix[6]tris-amine-trisurea **3** was well adapted for the establishment of secondary interactions within the assemblies thanks to the presence of the three anion binding ureido groups in alternate positions at the narrow rim. Hence, the NMR spectra of the quaternary assemblies obtained by mixing calix[6]tris-amine **3**, calix[6]hexa-acid **4** and IMI (1 : 1 : 3 ratio) with 1 equiv. of $PrNH_3^+Pic^-$ or 1 equiv. of $PrNH_2$ (Scheme 3, entries 8 and 9) differ by the chemical shift of the PhN*H*CO protons. Indeed, the PhN*H*CO protons of $\mathbf{3}_{IMI}^{3H+}$ experienced a significant downfield shift ($\Delta\delta_{PhNHCO}$ = 0.37 ppm) when assembled with the tetra-deprotonated subunit $\mathbf{4}_{PrNH3+}^{-4H+}$ *vs.* the tris-deprotonated one $\mathbf{4}_{PrNH3+}^{-3H+}$ (Scheme 4, left).[30] This downfield shift is in agreement with the

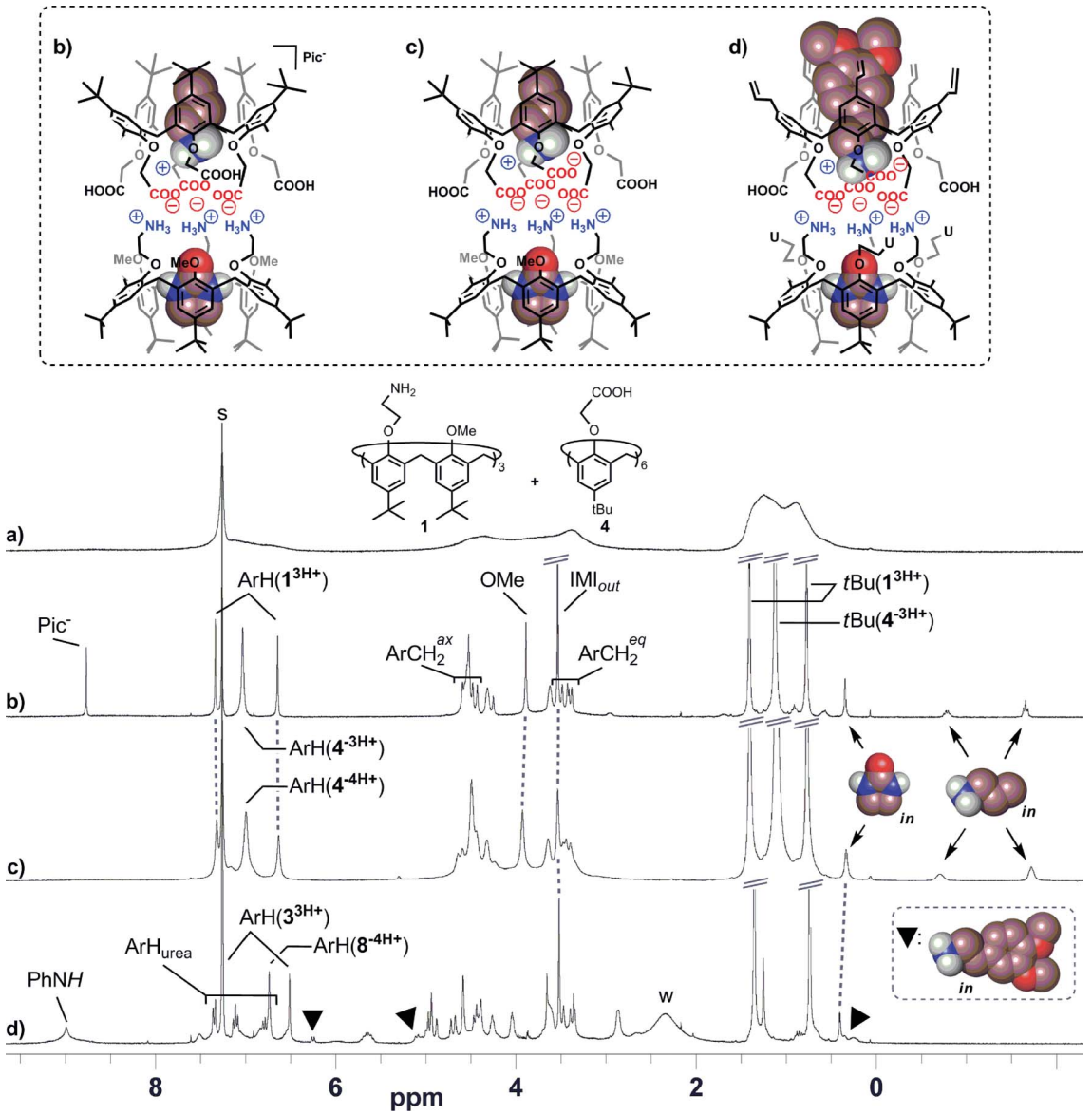
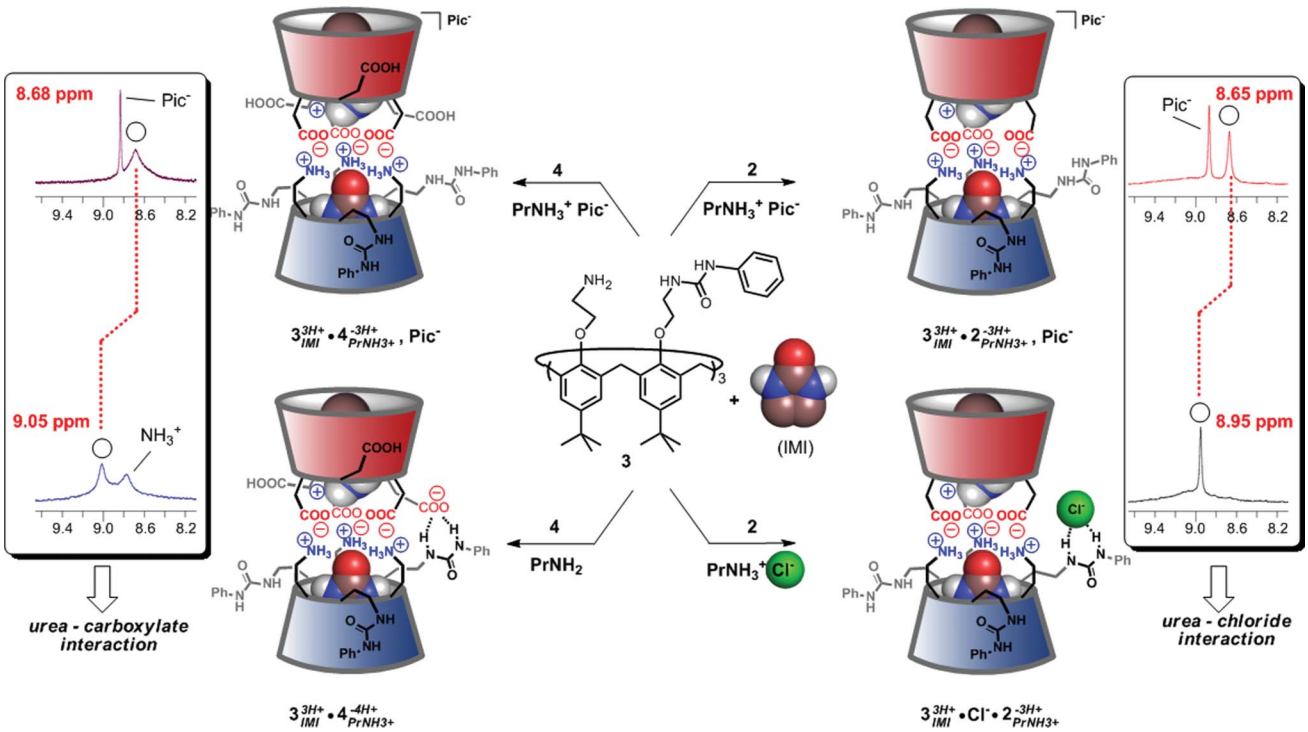


Fig. 3 ^{1}H NMR spectra ($CDCl_3$, 298 K) of a 1 : 1 mixture of **1** and **4** before (a) and after (b) the addition of IMI and $PrNH_3^+Pic^-$ (1 equiv.), leading to the discrete assembly of Pic^-; (c) related assembly obtained upon addition of IMI and $PrNH_2$ (1 equiv.) to a 1 : 1 mixture of **1** and **4**; (d) assembly. S = solvent, w = water.

implication of the ureido groups in H-bonding interactions and thus indicates the presence of intra-assembly interactions between the ureido groups of $\mathbf{3}_{IMI}^{3H+}$ and the fourth carboxylate group of $\mathbf{4}^{-4H+}$ (see the structure displayed in Scheme 4). The comparison of the NMR spectra corresponding to the self-assemblies $\mathbf{3}_{IMI}^{3H+}\cdot\mathbf{2}_{PrNH3+}^{-3H+},\mathbf{X}^-$ (with X^- = Pic^- or Cl^-) was also instructive (Scheme 3, entries 6 and 7) (Scheme 4, right). Again, a significant downfield shift of the signal of the PhN*H*CO protons was observed when a chloride counter ion was used instead of a picrate one ($\Delta\delta_{PhNHCO}$ = 0.30 ppm, Scheme 4 inset). The chemical shift of the CH_2 protons of the included IMI was also influenced by the nature of the counter anion (0.34 ppm in the case of the chloride *vs.* 0.44 ppm in the case of the picrate). These data indicate the binding of the chloride anion by the ureido groups of $\mathbf{3}_{IMI}^{3H+}$. In this case, the binding of the ammonium salt $PrNH_3^+Cl^-$ corresponds to an ion-pair recognition process,[40] where the ammonium ion and the chloride anion are simultaneously recognized by separated binding sites. In other words, the resulting supramolecular structure corresponds to a quinternary [1+1+1+1+1] self-assembly, namely $\mathbf{3}_{IMI}^{3H+}\cdot\mathbf{Cl}^-\cdot\mathbf{2}_{PrNH3+}^{-3H+}$ (see the structure depicted in Scheme 4). This corresponds to a very rare case of a discrete assembly involving five different partners, where a neutral molecule, an ammonium ion and an anion are simultaneously bound to a receptor. All in all, the alternate trisamino-trisureido functionalization at the narrow rim of **3** can be viewed as a dual recognition site since both ammonium and ureido arms are involved in the recognition process with the second calixarene partner.



Scheme 4 Self assembled complexes obtained with calix[6]trisamine-trisurea **3** and calix[6]hexa-acid **4** or calix[6]tris-acid **2**. Inset: ^{1}H NMR spectra ($CDCl_3$, 298 K), selected areas; O: CON*H*Ph protons.

Conclusion

The introduction of three or more acid–base functionalities at the narrow rim of calix[6]arenes was revealed to be a successful strategy for obtaining a supramolecular system that responds to a variety of stimuli in a very specific way. Upon ionization of three acidic functions or three amino-functions, the calix[6]arene core becomes self-assembled into a cone, provided a guest molecule shapes its cavity. The efficiency of the induced-fit process relies on the polarization of the hydrophobic cavity that becomes a good host, providing there is at one extremity, a charged site with hydrogen-bond acceptors and/or donors, and at the other extremity, aromatic walls that can widely open to allow guest inclusion. Combining two complementary calixarenes, one acidic, the other basic, led to a remarkable self-assembled heteroditopic receptor that responds specifically to two different guests. In that case, formation of the quaternary [1+1+1+1] adducts is under double allosteric control.

The goal of this study was to evaluate the scope of these remarkable spontaneous recognition processes, and see whether changes or introduction of additional functions at the calixarene core can lead to new features, thus opening the route to more sophisticated but also more versatile systems. Therefore, the study of the self-assembly process *via* triple ion pairing of calix[6]arenes bearing acid–base functionalities at the narrow rim has been extended to subunits displaying various substitution patterns at the narrow rim and at the wide rim. The formation of host–guest adducts through ionization and capping with counter anions, as well as of the bis-calixarene self-assemblies, has been explored and has shown interesting new features.

• Replacement of the bulky *t*Bu groups at the wide rim by smaller substituents (a hydrogen atom or an allyl group) does not alter the formation of the self-assemblies in spite of the increased flexibility of the macrocycles. On the contrary, this allowed the inclusion of larger guests such as the bio-relevant tryptamine and dopamine derivatives. Indeed, these calix-cavities can largely open to include the aromatic moieties of these guests.

• Introduction of extra functionalities at the narrow rim does not disrupt the self-assembly processes. Indeed, it is shown that extra acid functions can participate in the host–guest process by providing the necessary equivalent of acid to protonate the amine into the guest ammonium, thus expanding the versatility of the system. With ureido groups, secondary interactions between both calixarene units have been demonstrated in the quaternary [1+1+1+1] self-assemblies. This shows a possible strategy to reinforce the stability of such adducts.

• Finally, the same ureido groups have been shown to be capable of interacting with an anion such as a chloride, within the self-assembly, thus leading to a formally neutral [1+1+1+1+1] complex.

In conclusion, this work shows that a sophisticated molecular recognition pattern can be constructed to create additional host–host (urea–carboxylate) and host–guest (urea–counteranion) interactions. These intra-receptor interactions may be viewed as reminiscent of protein–protein recognition and enzyme–substrate interaction, both being interlocked, and under allosteric control. Their achievement is possible because of the versatility of the calix[6]arene functionalization together with the flexibility of its skeleton. Further research in our laboratory will be directed toward the study of the self-assembly

processes in water with calixarene subunits bearing hydrophilic groups.

Experimental

General procedures

All reactions were performed under an inert atmosphere. Silica gel (230–400 mesh) was used for flash chromatography purifications. ^{1}H NMR spectra were recorded at 300 MHz or 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts are expressed in ppm. Traces of residual solvent were used as internal standard in the case of ^{1}H and ^{13}C NMR spectra. In all cases, $CDCl_3$ was filtered over a short column of basic alumina in order to remove traces of HCl. Most of the ^{1}H NMR spectra signals were attributed through 2D NMR analyses (COSY, HSQC, HMQC, HMBC).

Calix[6]hexaallyl-hexaester 7

In a sealed reactor, calix[6]arene **6** (500 mg, 0.57 mmol) was dissolved in acetone (8 mL) and K_2CO_3 (1.42 g, 10.3 mmol) and a solution of ethyl bromoacetate (1.9 mL, 17.0 mmol) in acetone (7 mL) were successively added. The mixture was refluxed for 5 days and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and washed with water (25 mL). The aqueous layer was extracted with dichloromethane (2 × 15 mL). The combined organic layers were concentrated under reduced pressure and the residue was subjected to flash chromatography (CH_2Cl_2–AcOEt; 98 : 2), yielding compound **7** as a white solid (490 mg, 62%). m.p.: 138–141 °C (dec); IR (KBr): ν = 2979, 1756, 1637, 1559, 1472 cm^{-1}; ^{1}H NMR ($CDCl_3$, 328 K, 400 MHz) δ 1.28 (t, 18H, J = 7.0 Hz, $COOCH_2CH_3$), 2.68 (s, 12H, $ArCH_2CHCH_2$), 4.07 (s, 12H, $ArCH_2$), 4.23 (q, 12H, J = 7.0 Hz, $COOCH_2CH_3$), 4.55 (s, 12H, OCH_2COO), 4.83 (m, 12H, $ArCH_2CHCH_2$), 5.66 (d, 6H, J = 6.1 Hz, $ArCH_2CHCH_2$), 6.55 (s, 12H, ArH); ^{13}C NMR ($CDCl_3$, 328 K, 100 MHz) δ 14.4, 31.1, 39.1, 61.0, 71.1, 114.9, 129.5, 133.5, 136.5, 138.3, 153.4, 169.5; HRMS (ESI-TOF) calcd for $C_{84}H_{97}O_{18}$ (M+H$^+$) 1393.6675, found 1393.6650.

Calix[6]hexaallyl-hexaacid 8

In a sealed reactor, calix[6]arene **7** (120 mg, 0.086 mmol) was dissolved in THF (4 mL) and a 10% (v/v) aqueous solution of NMe_4OH (6 mL, 6.25 mmol) was added. The mixture was refluxed for 22 h and, upon cooling to 0 °C, a concentrated aqueous solution of HCl was added dropwise under stirring until acidic pH (~1). The THF was then removed under reduced pressure, leading to the apparition of a white precipitate in the aqueous phase. The solid was isolated by suction filtration on a glass filter and extensive washing with water (pH ~ 5). Compound **8** was obtained as a white solid without further purification (102 mg, 97%). m.p.: 160–165 °C (dec); IR (KBr): ν 3550 to 2700, 1745, 1639 cm^{-1}; ^{1}H NMR (DMSO-d_6, 373 K, 400 MHz) δ 2.66 (s, 12H, $ArCH_2CHCH_2$), 4.02 (s_b, 12H, $ArCH_2$), 4.44 (s, 12H, OCH_2COOH), 4.82 (m, 12H, $ArCH_2CHCH_2$), 5.65 (m, 6H, $ArCH_2CHCH_2$), 6.54 (s, 12H, ArH); ^{13}C NMR (DMSO-d_6, 373 K, 100 MHz) δ 30.1, 37.8, 69.9, 114.0, 128.3, 132.6, 134.8, 137.5, 152.5, 169.6; HRMS (ESI-TOF) calcd for $C_{72}H_{72}NaO_{18}$ (M+Na$^+$) 1247.4616, found 1247.4589.

Representative procedure of a self-assembly: $\mathbf{1}_{IMI}^{3H+}\cdot\mathbf{4}_{PrNH3}^{-3H+}$,$\mathbf{Pic}^-$

The spectrum displayed in Fig. 3a was recorded at 298 K after dissolving **1** (3.0 mg, 2.6 mmol) and **4** (3.5 mg, 2.6 mmol) in $CDCl_3$ (0.6 mL, neutralized with basic alumina). The subsequent addition of IMI (4 equiv.) and $PrNH_3^+Pic^-$ (1 equiv.) led to the spectrum displayed in Fig. 3b, which corresponds to the formation of $\mathbf{1}_{IMI}^{3H+}\cdot\mathbf{4}_{PrNH3+}^{-3H+}$, $\mathbf{Pic}^-$ as a unique species. ^{1}H NMR ($CDCl_3$, 300 MHz) δ –1.66 (t, 3H, J = 7.5 Hz, $H_3N^+CH_2CH_2CH_3$*in*), –0.79 (m, 2H, J = 7.8 Hz, $H_3N^+CH_2CH_2CH_3$*in*), 0.34 (s, 4H, CH_2 IM*in*), 0.58 (m, 2H, J = 7.8 Hz, $H_3N^+CH_2CH_2CH_3$*in*), 0.77 (s, 27 H, *t*Bu), 1.21 (s, 54 H, *t*Bu), 1.41 (s, 27H, *t*Bu), 3.40 (d, 6H, J = 15 Hz, $ArCH_2$), 3.50 (d, 6H, J = 15 Hz, $ArCH_2$), 3.62 (s_b, 6H, NCH_2CH_2O), 3.89 (s, 9H, OMe), 4.25 (s, 2H, NH IM*in*) 4.31(s_b, 6H, NCH_2CH_2O), 4.45 (d, 6H, J = 15 Hz, $ArCH_2$), 4.52 (s, 12H, OCH_2COO), 4.56 (d, 6H, J = 15 Hz, $ArCH_2$), 6.64 (s, 6H, ArH), 7.03 (s, 12H, ArH), 7.33 (s, 6H, ArH), 8.77 (s, 2H, ArH Pic$^-$).

Estimation of the overall association constants for the whole assembly process in $CDCl_3$

Accurate determination of the overall association constants for the formation of the quaternary complexes is not possible as "free" calixarenes are aggregated. However, the values were estimated based on the NMR spectra ($CDCl_3$) obtained with $PrNH_3^+Pic^-$ and IMI according to the following procedure: suitable aliquots of a solution of IMI were added to a 1 : 1 : 1 solution of both calix subunits and $PrNH_3^+Pic^-$ (2.5 10^{-3} M) in such a way that the corresponding ^{1}H NMR spectra recorded at 298 K revealed the quantitative formation of the [1+1+1+1] self-assembly. The concentration of the undetectable species (*i.e.* both free calix subunits and free $PrNH_3^+$) and the concentration of the [1+1+1+1] complex were estimated to be 5% and 95%, respectively, of the starting host concentration. The overall association constant was estimated according to the following equation: K > [complex]/{[first calix subunit]x[second calix subunit]x[$PrNH_3^+Pic^-$]x[IMI]}.

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30 See the Supporting Information†.
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36 This ratio is deduced from the integration of the high-field signals of the guests.
37 The average chemical shift of the OCH_2 protons of **4** was high-field shifted in the case of $\mathbf{1}^{3H+}_{IMI}\cdot\mathbf{4}^{4H+}_{PrNH3+}$ compared to the assembly $\mathbf{1}^{3H+}_{IMI}\cdot\mathbf{4}^{-3H+}_{PrNH3+},\mathbf{Pic}^-$ (δ_{OCH2} = 4.49 *vs.* 4.52 ppm, respectively). This is in agreement with the deprotonation of an additional carboxylic acid group.
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