Highly constrained guests in complexes of *p-tert*-butylcalix[6]arene dianion: Pentane-1,5-diammonium and choline

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GRAPHICAL ABSTRACT

Two choline units (yellow and pind) deep immersed in the cavity of *p-t*-bu-calix[6]arene dianion (light green).
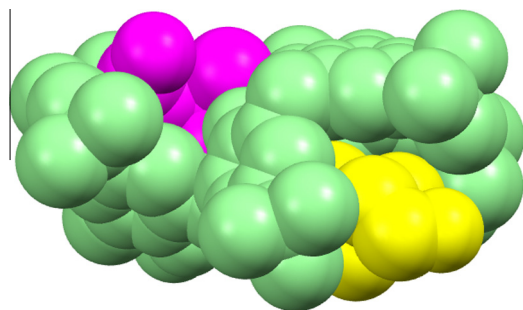
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ABSTRACT

The structures of the complexes of choline and pentane-1,5-diammonium with *p-t*-bu-calix[6]arene dianion were determined. Both salts display the calixarene moiety at 1,2,3-alternate conformation, with two concave surfaces formed by three aromatic rings, and the phenolate units are at distal positions, interacting with two phenol units by hydrogen bonds.

The salt of pentane-1,5-diammonium shows the diammonium connecting both calixarene dianion units, and one NH_3^+ is located *endo*-calix position and the other occupies an *exo*-calix position. The *t*-butyl groups and the calix cavity constrains the pentane-1,5-diammonium chain to near *syn-eclipsed* and *gauche* conformations. The other semi-calix accomodates a THF solvent molecule. The salt of choline shows the trimethylammonium groups of choline units immersed in these concave surfaces, with several interactions N^+–C–H–π interactions with the aromatic semi-cavities.

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Introduction

Calixarene based receptors are able to interact with organic and inorganic molecules and ions [1], and the interactions with biomolecules [2–4] have special interest for sensors and carriers, which increase the solubility of bioactive molecules [5] and allow the controlled delivery of pharmaceuticals.

The aromatic cavity of calixarenes **1** (Fig. 1) is able to interact by π stacking and C–H–π from one side [6] and the phenolic hydroxyl groups interact by hydrogen bonds or electrostatic by the other side [7]. The complexes of calix[6]arene with ammonium cations
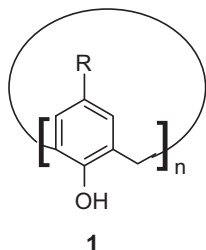
Fig. 1. General structure of a calix[n]arene.

have been described with 2:1 ammonium: calix stoichiometry. The relative position of ammonium complexes of calix[6]arene salts is related with the size complementarity of the cation and the cavity. Tetramethylammonium cation complexes have both cations placed *endo*-calix [8], whereas the bis-hexylammonium [9] salt have one ammonium in the *endo*-calix and the other *exo*-calix positions. Bulkier ammonium cations like triethylammonium have both ammonium units placed out of the cavity of calix[6]arene [10].

Choline and acetylcholine are neurotransmitters acting in the nervous central systems, which can be considered important targets for molecular recognition by their role on the enhancing of cation transport in cation channels and their esters are in the surface of celular membranes. Signal deactivation is mediated by acetylcholinesterase (AChE) enzymes, which are targets for insecticides, and their irreversible inactivation kills by neuromuscular paralysis [11]. The recognition of the trimethylcation moiety in acetylcholinesterase *from torpedo californica* [12] and choline oxidase from *sinorhizobium meliloti* [13] is provided by N$^+$–CH$_3$–π interaction with residues of Tyr, Phe and Trp, and the affinity of the synthetic receptor sulphonate-calixarenes by choline was reported by Lehn et al. [14] as comparable with biological receptors, with one choline unit inside of the calix cavity. The binding of calixarene in the outer surface of membranes has been pointed as the possible reason for antibacterial properties of calix-guanidine derivatives [15]. This work describes the salts of *p-t*-bu-calix[6]arene dianion with two ammonium cations: pentane-1,5-diammonium (cadaverine diammonium) and choline in order to evaluate the main forces that control the complexation.

Experimental

The complex of pentane-1,5-diammonium and *p-t*-bu-calix[6]-arene dianion was obtained from a solution of 1:1 mol ratio of *p-t*-bu-calixarene and pentane-1,5-diamine (cadaverine) from in

CH$_3$CN/THF. The suspension was heated and filtered, and the slow evaporation of the solvent mixture yielded colorless crystals. Crystals of calixarene dianion with choline were obtained by previous reaction of *p-t*-bu-calix[6]arene with KOH with a 1:2 mol ratio in ethanol. Subsequent addition of choline chloride until 10:1 choline: calix ratio, yielded colorless crystals after two days.

Data were collected on an Enraf–Nonius Kappa-CCD diffractometer with graphite monochromated Mo K radiation (λ = 0.71073 Å), up to 50° in 2 and final unit cell parameters were based on all reflections. The temperature was controlled using an Oxford Cryosystem low temperature device operating at 120 K. Data collection was carried out using the COLLECT program [16]. Integration and scaling of the reflections were performed with the HKL Denzo–Scalepack system of programs [17,18], and numerical absorption corrections were performed. The structure was solved using direct methods with SHELXS-97 [19]. The model was refined by full-matrix least-squares on F2 by with SHELXL-97. Hydrogen atoms were located on stereochemical grounds and refined with the riding model; for methyl and phenolic hydrogens, the rotating model was used. Hydrogen atoms were set isotropic with a thermal parameter 20% greater than the equivalent isotropic displacement parameter of the carbon to which each one is bonded; this percentage was set to 50% for the hydrogen atoms of methyl groups. The infrared analysis of the salts were collected using a Varian 640-IR as KBr pellets.

Results and discussion

This work is part of our efforts to evaluate the forces involved in the complexation of ammonium cations with calixarenes, focusing in biomolecules. We also sought organized structures, where an diammonium cation is able to connect two calixarene units, so we tried to obtain crystals with 1,8 – 1,6 – 1,5 and 1,4 diammonium linear cations, and only the reaction with pentane-1,5-diammonium (cadaverine) shown in Fig. 2, yielded good crystals from a solution with 1:1 diamine: calix. We tried other calix: diamine molar ratios to grow crystals: 1:1, 1:2, 1:4 and 1:10, but only the first resulted crystals with good quality for X-ray analysis. The other ratios gave the 2:1 ammonium: calix, although none formed monocrystal with quality to X-ray analysis.

The synthesis of the choline came from our observations about the competition between quaternary ammonium cations and alkaline cations. In a first attempt, alkaline metal hydroxides were added to a suspension of *p-t*-bu-calix[6]arene, followed by the addition of tetramethylammonium chloride. The tetramethylammonium salt with the calixarene crystallized in all experiments performed, instead of the metal salt, as seen as seen by infrared analysis of the solids obtained. So, we used choline and
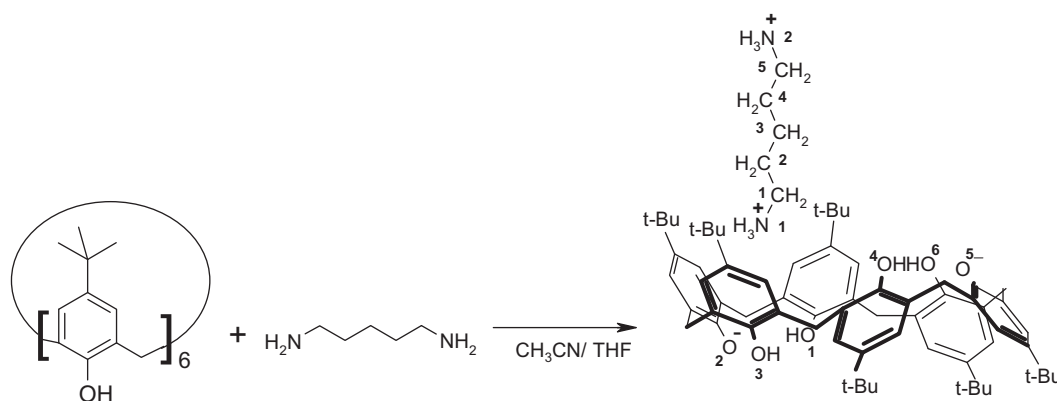

Fig. 2. Synthesis of the complex of *p-t*-bu-calix[6]arene dianion with pentane-1,5-diammonium.
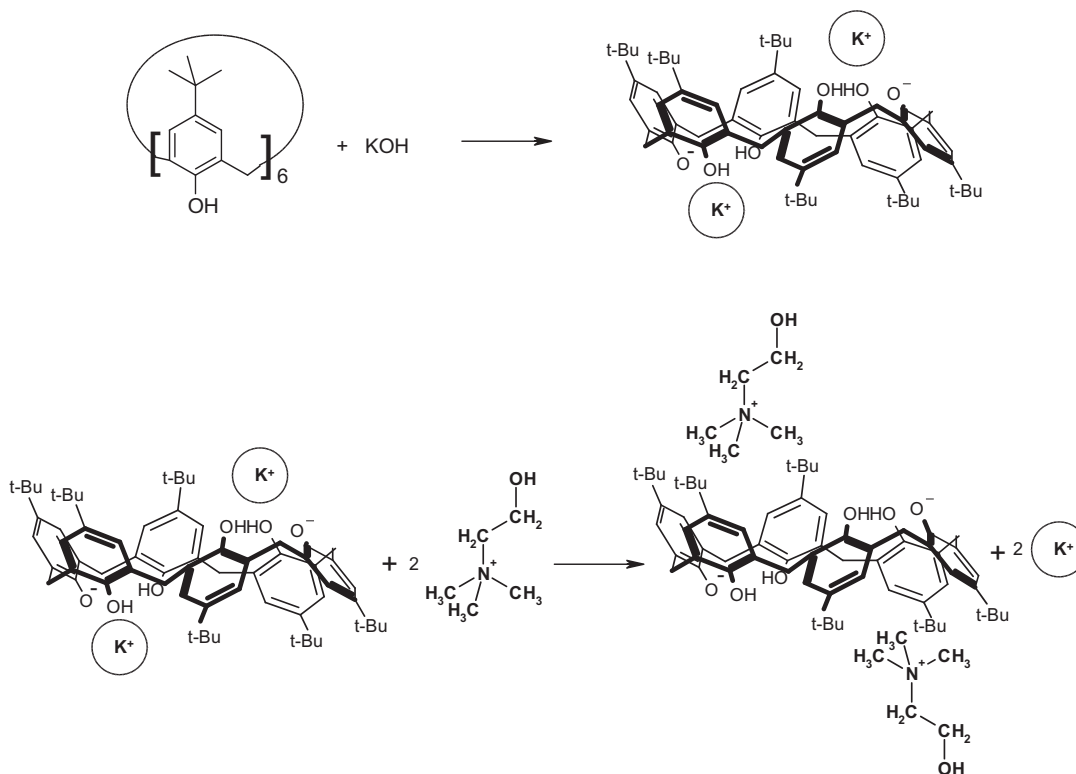
Fig. 3. Synthesis of the complex of *p-t*-bu-calix[6]arene dianion with choline.

acetylcholine to exchange with potassium cation, as seen in Fig. 3, which have the trimethylammonium group to achieve crystals of molecules of biological relevance. Both gave monocrystals, but only choline had sufficient quality for X-ray analysis. Crystal data obtained for the salts are summarized in Table 1.

The CIF file of 1,5-pentanediammonium-*p-t*-bu-calix[6]arene dianion has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number 288968. The CIF file of bis-choline-*p-t*-bu-calix[6]arene dianion has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number 288969.

Pentane-1,5-diammonium-p-t-bu-calix[6]arene dianion complex

The asymmetric unit of pentane-1,5-diammonium complex is composed by one *p-t*-bu-calix[6]arene dianion, one pentane-1,5-diammonium cation, one acetonitrile and one THF molecule by asymmetric unit. The pentanediammonium cation and calixarene are disposed as a one-dimensional array with an infinite hydrogen bond network alternating calixarene and ammonium cation, as seen in Fig. 4, which can be considered as a regular supramolecular copolymer linked by hydrogen bonds between the singular constitutional units.

Table 1
Crystallographic parameters for 1,5-pentanodiammonium and choline salts with *p-t*-bu-calix[6]arene dianion.

|	1,5-pentanediammonium	Choline
Formula	$C_{77}H_{109}N_3O_7$	$C_{154}H_{226}N_4O_{17}$
Formula weight	1188.67	2405.39
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/n$	$P-1$
a (Å)	12.8220(5)	16.9010(10)
b (Å)	27.0070(13)	18.4450(10)
c (Å)	21.4270(10)	27.510
$\alpha=$		98.560(10)
β	96.374(3)	98.850(10)
γ		110.540(10)
Z	4	2
V	7374.0(6) Å^3	7742.15
Crystal size (mm)	0.05 × 0.05 × 0.10	0.05 × 0.05 × 0.10
Crystal color	Colorless	Colorless
m	0.067 mm^{-1}	0.066 mm^{-1}
Reflections	37,638	69,324
Observed reflections	5833	12,445
R; $wR2$	0.087; 0.3075	10.44; 0.3118
GOF; Npar	1.083; 810	1.019;1495
Min and max res dens (e/Å^3)	−0.393; 0.904	−0.525; 1.201
W where $P = (Fo2 + 2Fc2)/3$	$1/[\sigma^2(Fo^2) + (0.1378P)^2 + 23.7508P]$	$1/[\sigma_2(Fo^2) + (0.1699P)^2 + 14.289P]$
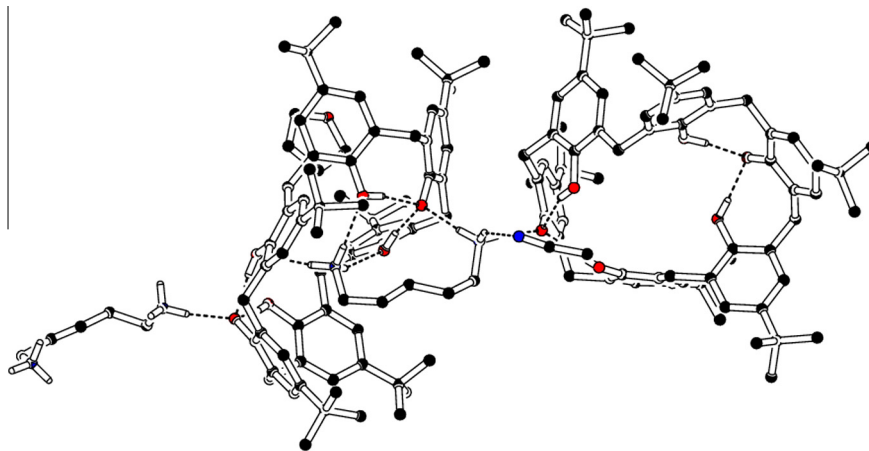
Fig. 4. View of the hydrogen bond network of the complex of pentane-1,5-diammonium with *p-t*-bu-calix[6]arene dianion. Acetonitrile, THF and hydrogen atoms were omitted for clarity.

The pentane-1,5-diammonium moiety is deeply immersed (Fig. 5) in the calix cavity and the chain suffers high distortion (Table 2) from the *all*-antiperiplanar conformation reported in early structures [20] of pentanediammonium salts. The almost *eclipsed* conformation seen by the dihedral angle formed by N1–C1–C2–C3 = 5.72°, reflects the constraint imposed on the host by van der Waals repulsion with the *t*-butyl groups, and the needing of the optimal geometry in order to fit the requirements for the other interactions: electrostatic attraction of N1 with the phenolic oxygen atoms and N^+–CH_3—π between the ammonium cation and the aromatic rings.
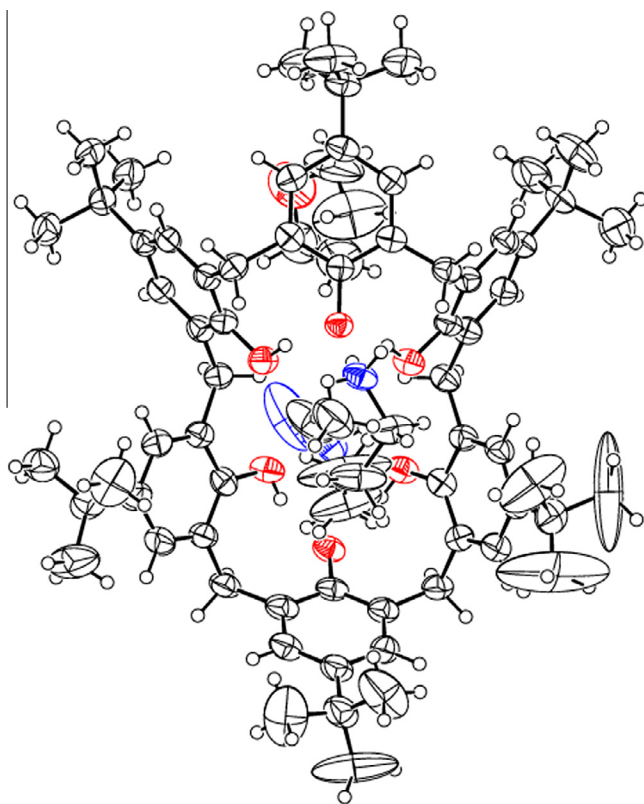

Fig. 5. ORTEP diagram of the pentane-1,5-diammonium along the axis in the cavity showing the thermal ellipsoids. The conformation of ammonium suffers severe restriction by the cavity.

Similar examples of chain distortions promoted by complexation can be found in the complexes of *p-t*-bu-calix[6]arene dianion with hexylammonium [9] and diethylammonium [21], and calix[4]arene derivatives with *L*-lysine [22], however any eclipsed conformation was reported in these structures. For example, the dihedral angles formed by N^+–C_1–C_2–C_3 and C_1–C_2–C_3–C_4 for the structure of hexylammonium are 60.9° and 56.3°, with a *gauche* conformation, but *gauche* is a local minimum for the plot of energy vs. dihedral angle, and *eclipsed* is the absolute maximum, so the constraint imposed for pentanediammonium cation is higher than for the previously reported structures.

Both nitrogen atoms of the pentanediammonium moiety are located at non-equivalent positions with respect to the calix dianion, and the interactions have distinct environments: N1 occupies the inner space of one of the *semi*-calixes, surrounded by three aromatic rings with twelve short contacts with the NH_3^+ group. The short contact is pointed when the atoms are located at positions smaller than the sum of the van der Walls radius. In addition, this NH_3^+ is anchored by hydrogen bonds with three phenolic hydroxyl groups. The other NH_3^+ group is placed out of the cavity and displays two hydrogen bonds with two phenolates, as seen in Fig. 4, each one from distinct calix macrocycle units, besides one hydrogen bond with an ethanol molecule. Such kind of different binding sites has been modeled by Kim and Choe [23] The distances of hydrogen bonds between *exo*-cavity NH_3^+ N2 and the receptor atoms are considerably shorter (*circa* 2.7 Å) than the distances involving the *endo*-cavity NH_3^+ (N1) and the phenol groups (*circa* 2.9 Å), as seen in Table 3. This is a consequence of the strength of the ionic hydrogen bonds (N^+–H—O^-) and the crowding near N1 that does not allow an approximation for hydrogen bonds.

Another important contribution for the inclusion of the diammonium cation are the C–H—π interactions between both hydrogen atoms from C1 and two aromatic rings, when the C–H bonds point to the face of the aromatic rings, with distances of 3.53 Å from C1 to the centroid formed by C51–C52–C53–C54–C55–C56

Table 2
Dihedral angles of 1,5-pentanediammonium included in the calix cavity.

Atoms	Dihedral Angle (°)A
N1–C1–C2–C3	5.70
C1–C2–C3–C4	173.40
C2–C3–C4–C5	135.85
C3–C4–C5–N2	72.63

Table 3
Distances of hydrogen bonds of pentane-1,5-diammonium p-t-bu-calix[6]arene dianion.

D–H	A	d (D···A), Å
O1–H1	O2	2.56
N1–H1A	O3	2.86
N1–H1B	O4	2.99
N1–H1C	O1	2.90
N2–H2A	O2	2.70
N2–H2B	O5	2.70
N2–H2C	N4	2.92
O3–H3	O2	2.56
O4–H4	O5	2.55
O6–H6	O5	2.60

and 4.02 Å from C-1 to the centroid formed by C41–C42–C43–C44–C45–C46.

The infrared showed a shift for $\nu_{O–H}$ of the calix, which is result of the break of the hydrogen bond belt of neutral p-t-bu-calix[6]arene, and the displacement and splitting of $\nu_{N–H}$ bonds, which occurs at 3190 and 3140 cm^{-1}, whereas for the hydrogen sulfate [24] salt appears at 3026 cm^{-1}. The absorption corresponding to the $\nu_{O–H}$ of the calix appears at 3560 and 3400 cm^{-1}, also displaced when compared with the respective sodium salt prepared, which shows a broad band between 3150 to 3640 cm^{-1}.

Choline-p-t-bu-calix[6]arene dianion complex

The structure of the complex of choline and p-t-bu-calix[6]arene dianion has been resolved and shows two units of calix dianion, four choline molecules and one ethanol molecule by asymmetric unit, as seen in Fig. 6. The calix moiety also displays the 1,2,3-alternate, with structural features similar to the pentane-diammonium cation, which shows the robustness of this conformation, sustained by ionic hydrogen bonds and the minimization of sterical crowding between the *tert*-butyl groups.

The most interesting structural features come from the included choline cations. The methyl radicals of the choline molecules are deeply immersed in the cavity of the semi-calix, with 22 contacts between the N$^+$–C–H of choline and the aromatic rings. The main driving force that directs the choline to the inner space of the cavity is the attraction between the polarized C–H bonds and the π electrons of the aromatic rings, instead hydrogen bonds of OH of choline and phenolic groups. This mode of binding is alike to the binding site for NMe$_3^+$ in acetylcholinesterase from *Torpedo californica* [12], where the acetylcholine in the binding site interacts with 14 aromatic residues (Trp, Phe and Tyr) of aminoacids rather than anionic residues.

The O–H—O$^-$ hydrogen bonds between phenolate and neighbor hydroxyl groups of the calixarene are considerably shorter than the hydrogen bonds involving calixarene and choline or ethanol molecules (Table 4). For phenolic–phenolate hydrogen bonds, the range falls between 2.54 and 2.59 Å, whereas for phenolate-hydroxyl hydrogen bonds, the range falls between 2.62 and 2.66 Å, and this shows that the phenolic–phenolate are stronger and that intramolecular hydrogen bonding have the main role for the conformation of the calixarene. The infrared of the solid shows an almost flat band between 3100 and 3600 cm^{-1}, result of the high dispersion of $\nu_{O–H}$ bands in the solid.

Each semi-calix has a NMe$_3^+$ inside (Fig. 7). Choline OH and NMe$_3^+$ groups connect the cavities by hydrogen bonding and C–H—π interactions, respectively, and build a one-dimensional supramolecule in a columnar array. Choline molecules also suffer severe sterical restraints by the calix cavity, as seen by the dihedral angles around C–C bonds: O1–C11–C12–N1: 77.89° for choline 1; O2–C21–C22–N2: 72.55° for choline 2; O3–C31–C32–N3: 0.04° for choline 3 and O4–C41–C42–N4: 54.52° for choline 4. This means that neither choline is able to adopt the *anti* conformation, being three as *gauche* and one as *eclipsing* conformation.

When the conformation of p-t-bu-calix[6]arene dianion with several counter-ions are compared, it is possible to state that is almost the same, and only minor influence of guest size and shape are detected. Thus, this can be considered a robust conformation
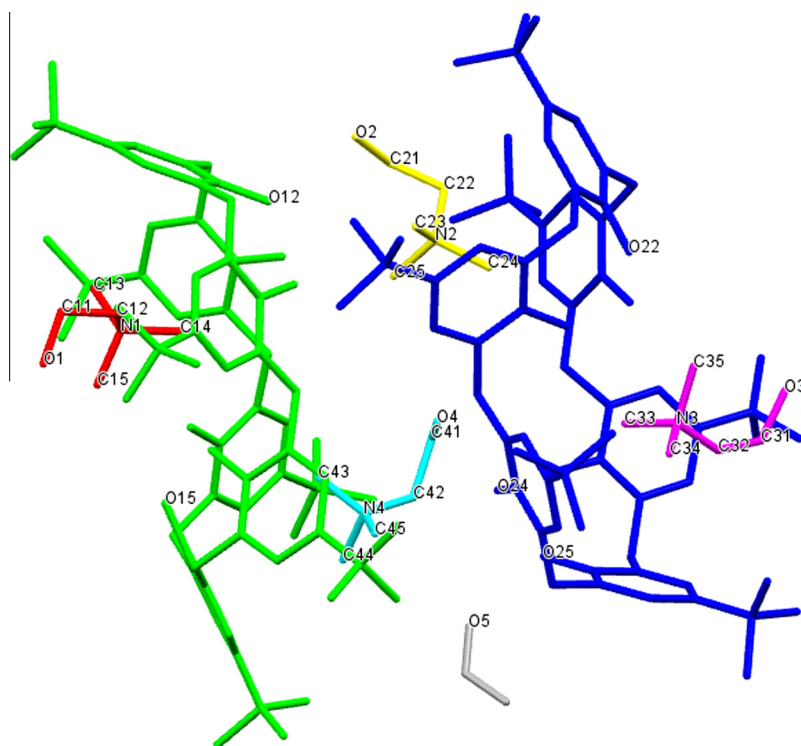

Fig. 6. Structure of the p-t-bu-calix[6]arene dianion with both calix, the four choline and one ethanol; hydrogen atoms were omitted for clarity.

Table 4
Distances of hydrogen bonds of bis-choline *p-t*-bu-calix[6]arene dianion.

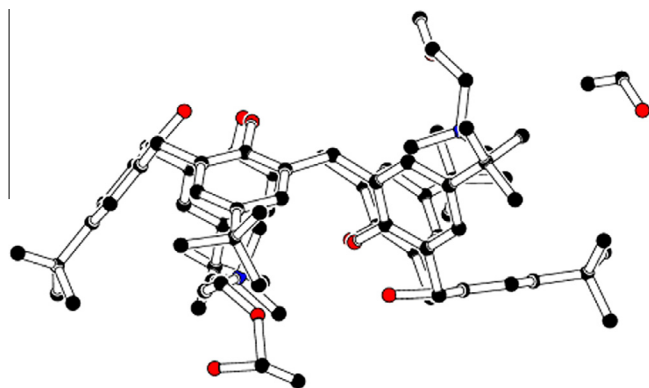| D–H	A	d (D···A), Å
O1–H1	O15	2.63
O3–H3	O22	2.66
O4–H4	O24	2.93
O5–H5	O25	2.66
O11–H11	O12	2.56
O13–H13	O12	2.55
O14–H14	O15	2.55
O16–H16	O15	2.56
O21–H21	O22	2.56
O23–H23	O22	2.59
O24–H24	O25	2.55
O26–H26	O25	2.55

Fig. 7. One calix unit from the complex of choline with *p*-t-bu-calix[6]arene dianion showing that both semi-calixes are filled with one choline moiety; hydrogen atoms were omitted for clarity.

with binding sites turned to opposite directions that makes calix[6]arene dianions useful as supramolecular synthons for crystal engineering. The stability of the 1,2,3-conformation can be understood by the restriction imposed on the guests.

Hydrogen bonding between the phenolate oxygen with two vicinal phenolic oxygen atoms and repulsion between the *t*-butyl groups are the structural features that control the conformation and opening of the calix cavity. The guest lies on the cavity and its conformation should adapt to the inner space. If it is not possible the guest goes out of the cavity, like triethylammonium cation.

Conclusion

This work aimed to obtain inclusion ammonium salts with calixarene macrocycles, and in both cases the dianion of *t*-bu-calix[6]arene was achieved from the neutral macrocyle. In the case of the complex with pentanediammonium cation, the salt was

obtained by proton transfer between the calixarene and the respective amine, whereas for choline was obtained from the previously prepared potassium cation.

The pentanediammonium cation acts like a bridge between the calix moieties, and the C–H−π interactions and sterical crowding with *t*-butyl groups enforces high constraints, seen by the almost eclipsed groups. The choline salt shows the trimethylammonium groups deeply immersed in the calix cavity, also with several sterical constrained groups, and the calix[6] has a great complementarity with choline and other molecules carrying the trimethylammonium group.

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