Orientational preferences of aromatic guests in dimeric capsules of tetraurea calix[4]arenes—MD and NMR studies

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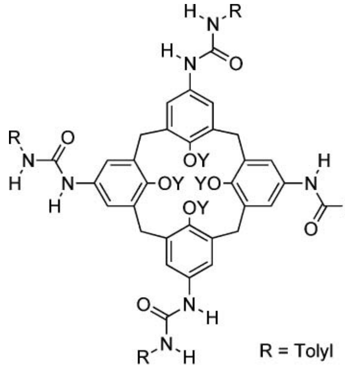
Molecular dynamics (MD) simulations have been performed for complexes of a dimeric capsule of a tetraurea calixarene with a series of twelve aromatic guests. A distinct orientational preference and a restriction of the internal mobility was found which depend on the size and electronic properties of the guests. The results are in agreement with the CIS values obtained from ^{1}H NMR spectroscopic measurements and with complexation selectivities obtained by competition experiments.

Introduction

The reversible encapsulation of guests in self-assembled molecular containers has attracted considerable interest. Potential applications as storage, transport and delivery systems for bioactive substances, vessels for chemical reactions or even for the stabilization of reactive species may be envisioned.[1] Hydrogen-bonded capsules, which have been constructed from a variety of building blocks, are promising candidates due to their easy availability and their often considerable kinetic stability, even in polar environments.[2,3] These capsules can enclose a wide variety of guests ranging from the small, neutral methane (in the "tennis ball"[4]) to long-chain alkylammonium cations (in a hexameric capsule formed by resorcarenes[5]). In addition, even more than a single guest molecule can occupy the interior of a capsule and if the size and shape of the capsule limits the mobility of these guests, this can result in the occurrence of different stereoisomers.[6]

Despite the increasing number of such encapsulation studies, our understanding of the behaviour of the included guest molecules still remains limited. Only a few X-ray analyses are known in which the guest position has been definitely found.[7] We have recently reported a detailed NMR, X-ray and theoretical investigation of the structure and dynamics of a self-assembled capsule formed by two tetraurea calix[4]arenes **1** (Fig. 1) with a tetraethylammonium cation as guest.[8,9] The inclusion of this, in comparison to the capsule volume ($V = 190$–200 Å^3), voluminous cation ($V = 154$ Å^3) has several dramatic consequences for the structure and dynamics of the capsule: NMR studies showed that the hydrogen bonded belt changes its direction rapidly on the NMR timescale while the rotation of the guest around the pseudo-C_2 axis† in the equatorial plane of the capsule is restricted.‡ Molecular dynamics studies of the complex provided the explanation that the two hemispheres of the capsule are pushed apart by the guest thus leading to an appreciable weakening of the hydrogen bonding system which is, however, compensated by favourable cation $\cdots$ π interactions.[8] This prediction was subsequently corroborated by the X-ray analysis of the **1b**·NEt$_4^+$·**1b** complex.[9] The distorted shape of the crystal structure in comparison to the regular structure of similar dimers is reflected by the MD simulations over a short time while longer simulation times provided the high symmetry found by ^{1}H NMR spectroscopy in solution. In order to evaluate further the possibilities and limits of computational methods for the description of the structure and dynamics of hydrogen bonded capsular assemblies we have extended our studies to a series of



1a: Y = C_2H_5 **1b:** Y = C_5H_{11}

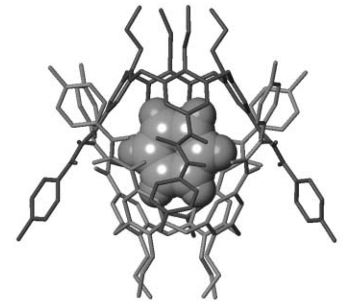


Fig. 1 General formula of a tetraurea calix[4]arene and stick representation of a dimeric capsule with benzene (spacefilling) as guest.

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† With respect to the molecular skeleton, neglecting the directionality of the hydrogen-bonded belt, this would be a C_2 axis.

‡ The rotation around the S_8 axis of the capsule is rapid under all experimental conditions.

aromatic guests. We were interested how the rather limited space in the interior of the capsule influences the mobility of a particular guest and whether the internal properties of the walls force the guest into a certain orientation. Herein we describe the outcome of the MD simulations and we compare the results with those obtained from ^{1}H NMR measurements.

Experimental

Computational methods

All molecular dynamics simulations were performed using the AMBER 7 software package and the *gaff* parameter set.[10] The initial geometry of all models was obtained from the crystal structure of a tetraurea calixarene dimer by converting the ethoxycarbonyl methyl groups into ethyl residues and by placing the guests listed in Table 1 in the interior of the capsule.[11,12] Charges were derived following the standard RESP procedure from a 6–31G* electrostatic potential calculated with the GAMESS program and the assemblies were transferred into the LEaP format.[13,14] Subsequently, a rectangular box of chloroform molecules (approximately 14 Å solvent layer thickness on each side) was added. The solvated structures were subjected to 5000 steps of minimization followed by a 30 ps belly dynamics (300 K, 1 bar, 1 fs timestep) for solvent relaxation and by a 100 ps equilibration period. Subsequently, MD simulations were performed in a NTP (300 K, 1 bar) ensemble for 9–18 ns using a 1 fs time step. Constant temperature and pressure conditions were achieved by the weak coupling algorithm and isotropic position scaling. Temperature and pressure coupling times of 0.5 and 1.0 ps, respectively, and the experimental compressibility value of 100×10^{-6} bar^{-1} for chloroform were used. Bonds containing hydrogen atoms were constrained to their equilibrium length using the SHAKE algorithm. Snapshots were recorded every 2 ps. The isolated guest molecules and the tetraurea calixarene **1a** were subjected to a MD simulation of 3 ns using the conditions as described above.

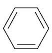
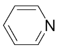
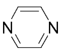
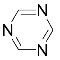
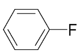
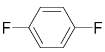
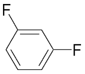
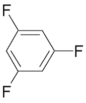
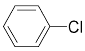
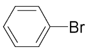
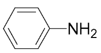
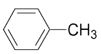
For analysis purposes, the following axes were defined (see also Fig. 4): a pole–pole axis connecting the centroids of the methylene carbon atoms of the two calixarenes (S_8-axis), two pseudo-C_2 axes perpendicular to the S_8 axis, an axis connecting the 1,4-carbon atoms of the aromatic guest and the normal of the plane passing through the aromatic carbon atoms of the guest. Angles between the guest and capsule axes were determined and used to classify snapshots according to guest orientation.

Molecular surfaces and volumes were calculated with the MOLCAD module of the SYBYL program package.[15]

^{1}H NMR measurements

^{1}H NMR spectra were recorded in cyclohexane-d$_{12}$ as solvent on a Bruker DRX400 Avance instrument at 400 MHz frequency. The chemical shifts were calibrated towards the residual signal of this solvent. In a typical experiment, the tetraurea calix[4]arene **1b** (8–12 mg) is dissolved using the desired guest as solvent (100 μL) with an ultrasonic bath. The solution is evaporated under reduced pressure and the complex thus obtained as a dry residue is dissolved in cyclohexane-d$_{12}$ and the ^{1}H NMR spectrum is measured immediately.[3]

Table 1 Complexation-induced shifts[a] (CIS, ^{1}H NMR) of the aromatic guest in the **1b**·G·**1b** complex and preferred orientations of the guest within the capsule (**1a**·G·**1a**) derived from the MD trajectories

Guest	*ortho*	*meta*	*para*	R	Orientation[b]
	−3.34				π_{eq}
	−3.86	−3.99	−2.15		π_{eq}
	−4.04				π_{eq}
	[c]				[d]
	−4.08	−4.03	−1.95		π_{eq}
	−4.05				π_{eq}
	−3.69 (1H) −3.17 (2H)	−4.05			π_{eq}
	−3.31				π_{eq}
	−4.00	−3.84	−1.77		π_{eq}
	−3.95	−3.78	−1.63		[e]
	−3.45	−3.50	−2.67	[f]	π_{eq} (90%) π_{ax} (10%)
	−3.03	−3.29	−3.17	−3.12	π_{eq} (10%) π_{ax} (90%)

[a] CIS = $\delta_{encapsulated} - \delta_{free}$ (ppm). Spectra were recorded in cyclohexane-d$_{12}$ solutions. [b] For the definition of the guest orientation see Fig. 2. [c] No complex formation observed. [d] No preferred orientation. [e] See text. [f] Not detected.

Competition experiments

All competition experiments were performed in mixtures of benzene and a second guest. The relative binding constants (K_{rel}) were determined from the integral ratios of the signals (encapsulated guest or calixarene) belonging to different complexes, taking into account the guest ratio which was chosen allowing to observe the two different species in a ratio smaller than 10.

In a typical experiment, the tetraurea calix[4]arene **1b** (8–12 mg) is dissolved at 22–25 °C in a mixture of benzene and fluorobenzene (10 : 1 molar ratio, 100 μL) using an ultrasonic bath. After a clear solution was formed, the mixture was evaporated using an oil pump (0.02–0.1 mbar). Due to the small amount of solvent, this evaporation takes place "immediately" and does not change the equilibrium in a measurable way. The residue obtained was dissolved in 0.7–0.8 mL of cyclohexane-d$_{12}$ (ultrasonic bath) and the ^{1}H NMR spectrum of the solution was recorded immediately. Usually the kinetic stability of the original capsules is sufficient to determine their ratio by the integration of suitable signals (*e.g.* from the included guest). Only the exchange of aniline against cyclohexane was too fast and the NMR spectrum contained signals of three capsules. Consequently the sum of capsules filled with aniline and cyclohexane was taken for the amount of capsules originally filled with aniline.

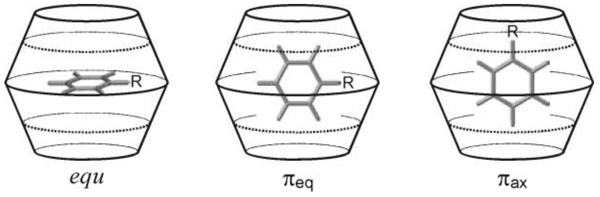


Fig. 3 Idealized orientations of aromatic guests within the tetraurea calixarene capsules. Dashed lines denote the border of the region of the calixarene aromatic rings. The middle circle represents the equatorial plane defined by the carbonyl carbon atoms of the urea functions.

Results and discussion

General considerations

Our present knowledge about the geometry of tetraurea calixarene capsules stems mainly from the four published crystal structures.[9,11,16,17] In the presence of a neutral guest the dimer is held together by sixteen hydrogen bonds, eight of them being characterized as "strong" on the basis of the N · · · O distances (average distance = 2.85 Å) and eight as "weak" (average distance = 3.18 Å).[18] The cavity has a volume of about 190–200 Å^{3} and the shape of the interior has been described as two square pyramids rotated by 45° with respect to each other.[11,19] The extension along the pole · · · pole axis (defined by the centroids of the methylene carbon atoms) is larger than the equatorial diameter (the region of the hydrogen bonded belt). From the Connolly surface calculated for the crystal structure of $\mathbf{1}\cdot C_6H_6\cdot\mathbf{1}$, which is shown in Fig. 2, upper limits of 10.2 and 7.6 Å can be estimated for these two characteristic distances.[11] The wall of the cavity is composed of a polar, hydrophilic equatorial zone and two lipophilic, π-electron rich hemispheres, delineated by the aromatic rings. The comparison of the shape and size of the cavity with the dimensions of various aromatic compounds (Fig. 2) suggests a tight fit even if 53–68% of the cavity remains unoccupied due to the discoidal shape of the guests (*cf.*, Table 3).[19–21]

In principle, an aromatic guest inside the tetraurea capsule can adopt three extreme orientations (Fig. 3). In the first case, the plane of the aromatic ring coincides with the equatorial plane and all substituents point to the seam of the hydrogen bonds. This orientation we will refer to in the following as the *equ* position. In the other two cases, the S_8 axis of the capsule (the long axis connecting the poles) bisects the plane of the aromatic ring. Since the aromatic plane is perpendicular to the equatorial plane, there is one (idealized) situation where two opposing substituents lie in the equatorial plane (π_{eq} position) while in the second orientation the ring is rotated by 30° and two substituents point to the poles (π_{ax} position).§ In each of the three orientations the shielding of the ring protons is different and provided that the guest motion inside the capsule is restricted and/or the guest prefers a single orientation it should be possible to correlate the complexation-induced shifts obtained by ^{1}H NMR spectroscopy with the guest orientation found by MD. In order to identify those orientations 9–18 ns of molecular dynamics simulations have been performed for each of the complexes listed in Table 1.

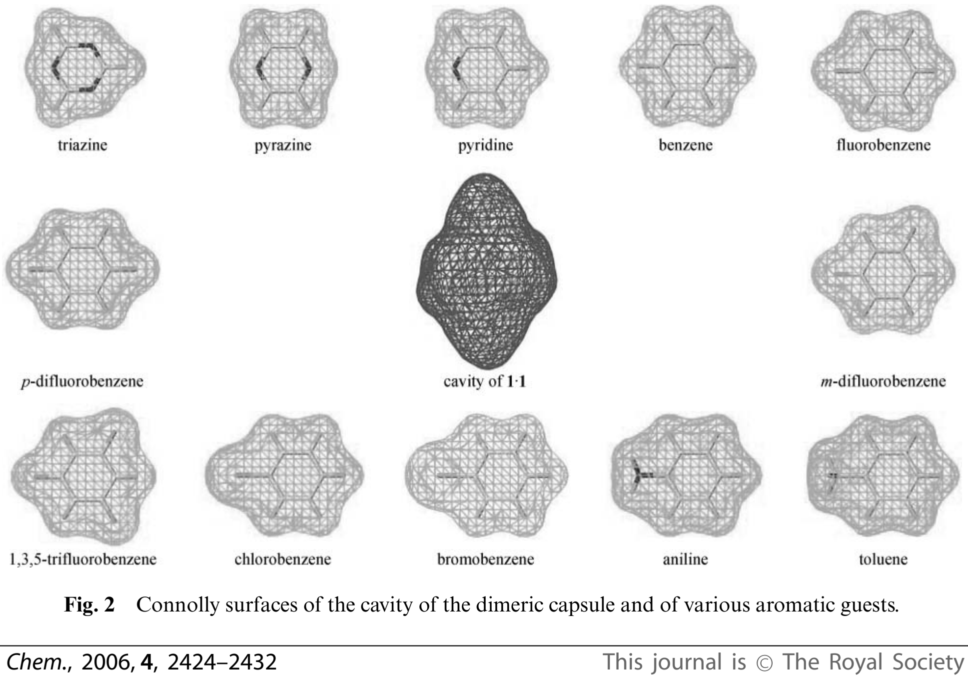


Fig. 2 Connolly surfaces of the cavity of the dimeric capsule and of various aromatic guests.

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§ Although the difference in the two positions (π_{eq} and π_{ax}) seems to be small, as judged by the angle of 30°, it was possible to distinguish clearly between the two positions in the MD trajectories, see, *e.g.*, Fig. 4a.

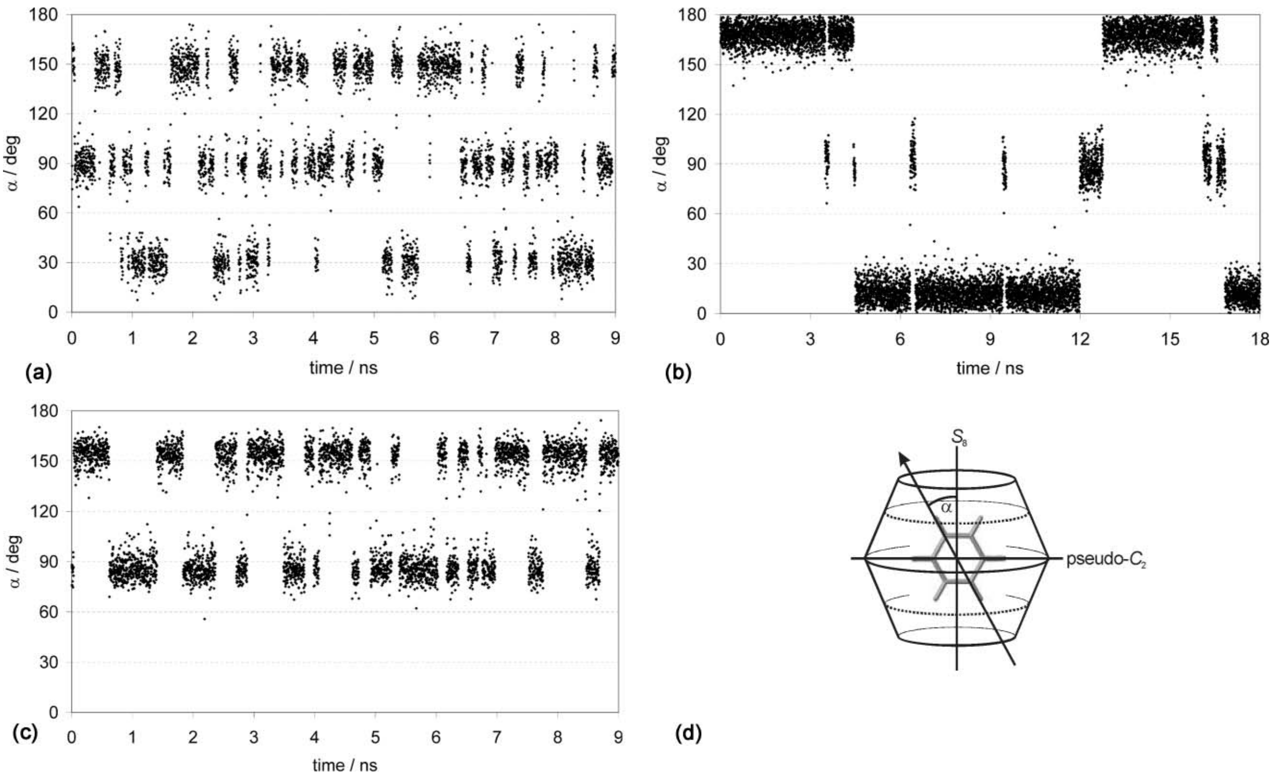

Fig. 4 Time-dependent guest orientation of the benzene (a), toluene (b) and *m*-difluorobenzene (c) complexes. The definition of the angle α is depicted in the sketch (d).

Benzene complex

As illustrated in Fig. 4a, in the MD simulations benzene as the simplest aromatic guest molecule showed an preference for the π_{eq} orientation. Since neither a π_{ax} nor an *equ* orientation was observed in the trajectories a comparison of the energetical and geometrical parameters for the three orientations was not possible. In the π_{eq} form favorable edge-to-face aromatic interactions of the tilted-T type with a mean ring center separation of 4.93 Å are possible which may be the reason for the stabilization of this orientation.[22] The rotation of the benzene molecule around the S_8 axis of the capsule and about its 6-fold axis of symmetry is fast on the MD timescale (*cf.*, Fig. 4a). The latter motion exchanges the individual positions of all hydrogen atoms resulting in an equal average environment which is commensurate with the single CIS value of −3.34 ppm measured by ^{1}H NMR spectroscopy.

Pyridine, pyrazine, fluorobenzene, chlorobenzene and *p*-difluorobenzene complexes

A pronounced preference for the π_{eq} position was also observed in the MD simulations of the complexes with pyridine, pyrazine, fluorobenzene, chlorobenzene and *p*-difluorobenzene.[23] Since the guests must edge through the narrow cavity to reach the π_{ax} orientation a high barrier for the interconversion between the π_{eq} and π_{ax} positions, which cannot be surmounted during the relatively short simulation time, might be responsible for the predominance of the π_{eq} orientation.¶ Therefore, the simulations were also started from the corresponding π_{ax} oriented guests. In all cases a reorientation to the π_{eq} orientation was observed and, as expected, dependent on the guest size, the time until the interconversion occurred ranged from the equilibration period (pyrazine, pyridine), over a few steps of sampling (fluorobenzene) to 2.6 and 6 ns of simulation time (chlorobenzene, *p*-difluorobenzene). Finally, the π_{eq} orientation was retained by all guests over the remaining simulation period (which was extended from 9 to 18 ns in the case of *p*-difluorobenzene and chlorobenzene). This does not necessarily mean that the guests adopt exclusively the π_{eq} position but it suggests that the π_{eq} position is energetically preferred over the π_{ax} position. Obviously, the halogen substituents as well as the lone electron pairs of the nitrogen-containing heterocycles tend to avoid the contact with the π-electron rich aromatic walls of the capsule and orient towards the equator with less electron density. This assumption is supported by the energy component analysis of the complexes with *p*-difluorobenzene and chlorobenzene in π_{eq} and π_{ax} orientation (Table 2) showing that the host–guest interaction is more favourable by about 8 kcal mol^{-1} when the halogen substituents point to the equator. In the chlorobenzene complex the energy gain in the π_{eq} position is in part compensated by the deformation of the single calixarenes ($\Delta\Delta E$ = 0.9 kcal mol^{-1} per calixarene) and less favourable interactions

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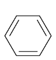
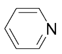
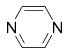
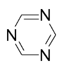
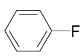
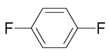
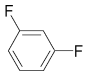
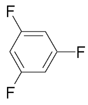
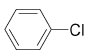
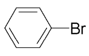
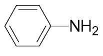
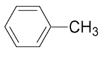
¶ By using the equation $\tau = \tau_0 \cdot \exp(\Delta G^{\neq}/RT)$ where τ_0 is the timestep of the molecular dynamics simulation (1 fs = 10^{-15} s) the time till the first occurrence of an interconversion can be estimated. For a barrier of $\Delta G^{\neq}$ = 10 kcal mol^{-1} τ would be about 19 ns.

Table 2 Average energy components[a] (kcal mol^{-1}) for the complexes **1a**·G·**1a**

G		E_{h1}	E_{h2}	$\Delta E_{interact}$	ΔE_{hg1}	ΔE_{hg2}	ΔE_{steric}	$\Delta E_{complex}$
		−102.1	−102.7	−116.6	−11.1	−11.1	−329.5	−62.1
		−101.5	−101.5	−116.2	−12.2	−12.2	−336.1	−62.3
		−101.6	−102.1	−116.4	−13.1	−13.1	−301.6	−63.3
		−101.7	−100.8	−117.2	−11.2	−11.2	−593.2	−61.4
		−101.8	−101.6	−116.4	−12.5	−12.5	−328.3	−62.7
	π_{eq} π_{ax}	−101.8 −101.9	−101.5 −101.1	−116.3 −116.2	−13.7 −9.6	−13.9 −9.7	−334.6 −325.3	−63.9 −59.3
		−101.2	−101.8	−115.9	−9.3	−13.9	−328.7	−61.1
		−101.2	−101.5	−115.3	−10.5	−10.6	−338.2	−59.8
	π_{eq} π_{ax}	−101.3 −102.2	−101.5 −102.4	−114.8 −115.4	−13.2 −13.3	−13.2 −9.1	−329.9 −328.4	−62.0 −61.7
	π_{eq} π_{ax}	−99.1 −102.3	−100.9 −101.6	−109.6 −113.9	−13.8 −8.6	−14.3 −13.9	−322.9 −325.7	−51.7 −53.1
	π_{eq} π_{ax}	−101.2 −102.5	−101.6 −102.2	−115.2 −115.6	−14.0 −12.8	−14.2 −12.2	−361.5 −360.5	−63.2 −62.7
	π_{eq} π_{ax}	−100.1 −102.1	−102.0 −102.3	−114.5 −114.7	−12.2 −11.7	−12.3 −11.8	−326.5 −328.3	−60.7 −61.6

[a] E_{h1}, E_{h2}: energies of the two tetraurea calixarenes in the capsule; $\Delta E_{interact}$: interaction energy between the two tetraurea calixarenes in the capsule; ΔE_{hg1}, ΔE_{hg2}: interaction energies between the guest and each of the two tetraurea calixarene units; ΔE_{steric}: steric energy of the capsule = $E_{h1} + E_{h2} + E_{guest} + \Delta E_{interact} + \Delta E_{hg1} + \Delta E_{hg2}$; $\Delta E_{complex}$: complexation energy per calixarene = $0.5 \times \Delta E_{steric} - 0.5 \times E_{guest,free} - E_{calixarene,free}$.

between them ($\Delta\Delta E = 0.6$ kcal mol^{-1}). Thus, the complexation energies (see Table 2) are −9.3 and −1.6 kcal mol^{-1} in favour of the π_{eq} orientation for the *p*-difluorobenzene and chlorobenzene complexes, respectively.* Interestingly, a ^{19}F-NMR study and a X-ray diffraction analysis of the *t*-butylcalix[4]arene–fluorobenzene complex showed that also in the "open" host–guest system the fluorine substituent avoids contact with the aromatic rings.[24]

On the MD timescale, the included guests pyridine, pyrazine, fluorobenzene, chlorobenzene and *p*-difluorobenzene in the π_{eq} orientation tumble more or less fast, depending on the size of the guest, around the S_8 axis of the capsule. On average, all protons of pyrazine and *p*-difluorobenzene as well as the *ortho*- and *meta*-protons of pyridine, fluorobenzene and chlorobenzene experience the strongly shielding environment of the π-electron clouds of the calixarene hemispheres which correlates well with their large CIS values of −3.84 to −4.08 ppm measured by ^{1}H NMR spectroscopy (Table 1). In contrast, the *para*-protons of pyridine, fluorobenzene and chlorobenzene point exclusively to the less shielding hydrogen-bonded belt which results in CIS values which are about 1.7–2.2 ppm lower than for the *ortho*- and *meta*-protons.

In principle, all guests discussed in this chapter can act as hydrogen bond acceptors for the urea protons of the hydrogen bonded belt. This would lead to a weakening of the interactions between the two hemispheres. However, taking **1a**·C_6H_6·**1a** as the reference, there is no significant loss in the interaction energies ($\Delta E_{interact}$ in Table 2) nor a reduction of the number of hydrogen bonds (Table 3), with the exception of the chlorobenzene complex. In this complex the number of hydrogen bonds is diminished by one as compared to **1a**·C_6H_6·**1a** and the difference in the interaction energies $\Delta\Delta E_{interact}$ is 1.8 kcal mol^{-1}. Indeed, in approximately 50%

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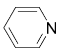
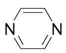
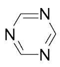
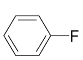
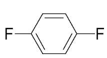
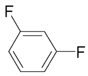
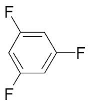
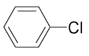
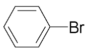
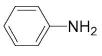
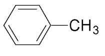
* According to the Boltzmann equation this energy difference would correspond to 100% for the π_{eq} form of the *p*-difluorobenzene complex and to a ratio of 95 : 5 for the π_{eq} and π_{ax} forms of the chlorobenzene complex.

Table 3 Average geometric parameters of **1a**·G·**1a** complexes

G	Orientation	r_{gyr}[a]/Å	$d_{pole-pole}$[b]/Å	V_{cavity}[c]/Å^3	V_{guest}[d]/Å^3	Packing (%)	d_{min}[e]/Å	d_{max}[e]/Å	$n_{H\text{-}bonds}$[f]
X-Ray[11]		5.78	9.78	202	78	39	7.6[c]	10.2[c]	16.0
	π_{eq}	5.94	9.34	206	78	38	6.2	6.9	15.1
	π_{eq}	5.95	9.35	202	71	35	6.2	6.2	15.1
	π_{eq}	5.96	9.33	204	65	32	5.5	6.2	15.4
		5.96	9.26	201	61	30	6.1	6.2	15.4
	π_{eq}	5.94	9.37	199	79	40	6.2	7.4	14.9
	π_{eq}	5.95	9.40	205	81	40	6.2	7.9	14.8
	π_{eq}	5.92	9.48	208	75	36	6.4	7.4	14.6
	π_{eq}	5.89	9.60	214	81	38	6.4	7.4	14.0
	π_{eq}	5.92	9.49	209	86	41	6.2	8.3	13.9
	π_{ax}	5.87	9.60	211		41			14.1
	π_{eq}	5.91	9.79	238	91	38	6.2	8.4	12.7
	π_{ax}	5.81	9.84	238		38			13.3
	π_{eq}	5.94	9.42	200	88	44	6.2	7.6	14.5
	π_{ax}	5.91	9.48	203		43			14.5
	π_{eq}	5.90	9.51	217	86	40	6.2	7.8	13.5
	π_{ax}	5.86	9.71	217		40			13.9

[a] Radius of gyration of the carbonyl oxygen atoms in the plane of the equator. [b] Distance of the centroids of the methylene carbon atoms. [c] Cavity volume. [d] Volume of the guest. [e] d_{min} and d_{max} denote the smallest and biggest diameter of the guest. [f] Number of hydrogen bonds between the urea residues.

of all snapshots the chlorine substituent is in close proximity to the urea proton attached to the calixarene skeleton (N···Cl distance less than 3.50 Å), but also a deformation of the capsule by this guest (whose long axis exceeds the equatorial diameter, see Table 3) must be considered as explanation for the deviation from the "normal" behaviour of the other guests discussed so far.

Bromobenzene

The measured CIS values for the protons of bromobenzene in the capsule **1b**·C_6H_5Br·**1b** are somewhat smaller, but roughly comparable, to those obtained for the pyrazine, pyridine, fluorobenzene, chlorobenzene and *p*-difluorobenzene complexes which give rise to the assumption that bromobenzene also adopts the π_{eq} orientation within the capsule. Especially indicative in this case is the small CIS value of −1.63 ppm for the *para*-proton. In the MD simulations starting from either the π_{eq} or π_{ax} position no reorientation of the guest was observed suggesting that the barrier for the interconversion between both positions is too high to be passed on the chosen MD timescale. The results indicate that the π_{ax} orientation is energetically favoured by 2.8 kcal mol^{-1}. As shown in Table 2, this energy difference mainly results from the strong, asymmetric deformation of the capsule (E_{h1}, E_{h2}, $\Delta E_{interact}$) in the π_{eq} orientation due to the bulky bromine substituent while the host–guest interactions are again more favourable in this orientation. The contradiction between experiment and theory might be explained by electronic factors. Bromine is a big and polarizable substituent which may adapt its electron cloud to the interior of the capsule while in a force field calculation it is handled as an elastic sphere with a predefined van der Waals radius.

Aniline

Inspection of the trajectories recorded for the complex with aniline again revealed a predominance of the π_{eq} orientation and a fast rotation of the guest about the S_8-axis of the capsule. In contrast to the complexes discussed so far, a single $\pi_{eq} \rightarrow \pi_{ax} \rightarrow \pi_{eq}$ interconversion was observed, the time in the π_{ax} position being roughly 1 ns. Extension of the simulation time from 9 to 18 ns did not result in the occurrence of any other interconversion. When the guest spends its time inside the capsule in both the π_{eq} and π_{ax} orientations, the experimentally measured CIS value should correspond to the Boltzmann-weighted average of the two orientations. Judging from a single interconversion during 18 ns, the simulation time to obtain a Boltzmann-weighted ensemble would be prohibitively long on the human timescale. Therefore, the discussion concerning the correlation of CIS values and orientations of the guest in the MD simulations will be restricted to qualitative aspects.

As already discussed above, in the π_{eq} orientation the *ortho*- and *meta*-protons are considerably more shielded from the environment than the *para*-proton (average distances to the center of the nearest aromatic ring 2.84, 2.73 and 3.59 Å, respectively). In the π_{ax} position the *ortho*-protons are shifted toward the equator due to the presence of the amino substituent while the *meta*- and *para*-protons are in the vicinity of the aromatic rings (average distances of 3.16, 2.99 and 2.88 Å, respectively). Therefore, in the case of aniline the CIS values should be smaller for the *ortho*- and *meta*-protons but bigger for the *para*-proton than in the complexes of **1** with pyrazine, fluorobenzene, *p*-difluorobenzene and chlorobenzene. This qualitative consideration agrees well with the experimental data shown in Table 1. The π_{eq} and π_{ax} forms differ in their total energies by 1.0 kcal mol^{-1}, which would correspond to a calculated ratio of 85 : 15. Favourable host–guest interactions are responsible for the preference of the π_{eq} orientation which are, however, in part counterbalanced by less favourable energies for the calixarenes and their interaction. In the π_{eq} orientation the aniline protons are actively involved in the hydrogen bonded belt. Careful analysis of the interactions revealed that, on average, in each second snapshot of the MD simulations a hydrogen bond is formed between an aniline proton and a carbonyl function of the urea groups.

Toluene

Toluene is the only guest in the present study that preferred the π_{ax} position which means that the methyl substituent is deeply embedded in the aromatic basket of the calixarenes. As shown in Fig. 4b the guest slowly (on the MD timescale) toggles between the π_{eq} and π_{ax} orientations with a relative occupancy of about 10 : 90. In both forms the guest rotates fast around the S_8 axis of the capsule. When the positions of the toluene ring protons are averaged over the whole MD run, $Ar_{center} \cdots H$ distances of 3.37, 2.88 and 2.95 Å for the *ortho*-, *meta*- and *para*-protons are obtained indicating a moderate shielding by the aromatic walls which is commensurate with the moderate CIS values of −3.03, −3.29 and −3.17 ppm.

The analysis of the individual energy components of the **1a**·$C_6H_5CH_3$·**1a** complex (Table 2) reveals that the host–guest interaction energies are in favour of the π_{eq} form although the energy differences ($\Delta E = -1.0$ kcal mol^{-1}) are smaller than for the *p*-difluorobenzene, chlorobenzene and aniline complexes, most probably due to favourable $Ar \cdots CH_3$ contacts in the π_{ax} position. This energy gain is, however, more than compensated by the unfavourable steric energies of the calixarenes and their interaction in the π_{eq} form ($\Delta E = 2.6$ kcal mol^{-1}) which explains the predominance of the π_{ax} orientation in the simulations ($\Delta\Delta E_{steric} =$ 1.8 kcal mol^{-1} corresponding to a ratio of 5 : 95 for $\pi_{eq} : \pi_{ax}$ which agrees well with the occupancy of about 10 : 90 observed in the simulations).

Triazine, *m*-difluorobenzene and 1,3,5-trifluorobenzene complexes

Since we have observed that electron-rich substituents orient toward the seam of hydrogen bonds, we were interested to study the orientations of triazine, *m*-difluorobenzene and 1,3,5-trifluorobenzene. In the equatorial position the CIS values for all protons of these guests should be similar to those for the *para*-protons of fluorobenzene, chlorobenzene and *p*-difluorobenzene (Table 1) which are in the range of −1.63 to −1.95 ppm.

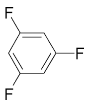
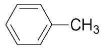
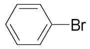
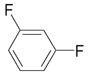
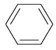
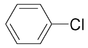
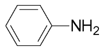
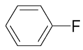
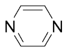
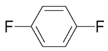
While no complex formation could be detected with triazine, the CIS values measured for the protons of *m*-difluorobenzene and 1,3,5-trifluorobenzene are in the range of −3.17 to −4.05 ppm indicating that the protons most probably experience the shielding of the aromatic walls. In the MD simulations, which were started from the *equ* orientation, the two fluorine substituted guests rearranged immediately to the π_{eq} position while no definite orientation was observed for the triazine guest due to its very fast rotation inside the capsule.

In the complexes with *m*-difluorobenzene and 1,3,5-trifluorobenzene the guests rotate fast around the S_8-axis but slower around the axis passing through the center of their aromatic rings. For *m*-difluorobenzene, the latter rotation resembled a seesaw motion by 60° forth and back which exchanges the positions of the fluorine substituents with respect to the walls of the capsule (Fig. 4c). No complete rotation of 360° was observed. In both (equivalent) π_{eq} positions the protons in 2- and 5-position have close contacts to the aromatic walls (average $Ar_{center} \cdots H$ distances of 2.73 and 2.76 Å) while the protons in 4-position spend equal time in the polar and equatorial regions of the capsule (average $Ar_{center} \cdots H$ distance 3.18 Å). This observation agrees well with the CIS values of −3.69, −3.17 and −4.05 ppm measured for the protons in 2-, 4- and 5-position, respectively.

Competition experiments

In order to evaluate the relative stabilities of the complex **1b**·G·**1b**, we have performed competition experiments with benzene as standard. For this purpose, appropriate amounts of a second guest were mixed with benzene, **1b** was dissolved in this mixture and the solvent was rapidly evaporated after equilibration. The dry residue consisting of the two capsules was dissolved in cyclohexane-d_{12} and their ratio was determined by ^1H NMR. Table 4 contains the relative stability constants $K_{rel} = K_G/K_{benzene}$ determined from the ratio of the two complexes regarding the respective ratio of the guests during complex formation. The differences in the complexation energies $\Delta\Delta E_{comp}$ calculated from the MD trajectories agree reasonably with the free enthalpies ΔG calculated from the thermodynamic stability constants K_{rel}.

Table 4 Relative thermodynamic stability constants K_{rel} and free enthalpy differences (kcal mol^{-1}) from competition experiments compared to the differences in complexation energy (kcal mol^{-1}) from MD simulations

G	K_{rel}[a]	$\Delta\Delta G$[b]	$\Delta\Delta E_{comp}$
	0.05	1.8	2.3
	0.14	1.2	0.8
	0.53	0.4	9
	0.57	0.4	0.9
	1.0	0	0
	2.0	−0.4	−0.4
	2.8	−0.6	−1.1
	7.0	−1.1	−0.6
	7.9	−1.2	−1.2
	150	−2.9	−1.8

[a] All competition experiments have been carried out in presence of a given guest with benzene. Relative errors are ±50% for aniline (less accurate due to the kinetic instability of the capsule) and ±10% for the other compounds. [b] Calculated from the K_{rel} values.

Conclusions

With the exception of triazine, which is tumbling fast in all directions, the MD studies revealed that all aromatic compounds adopt a preferred orientation perpendicular to the equatorial plane of the capsule. An electron rich substituent or a nitrogen lone electron pair (in the case of azaheterocycles) lies in the equatorial plane with the necessary exception of *meta*-substituted compounds. A different orientation with the methyl group pointing to the pole was found only for toluene. On the MD timescale, rotations of the guests around the S_8-axis of the capsule are fast, while rotations about the pseudo-C_2 axes are much slower. The CIS values for the aromatic protons measured by ^{1}H NMR spectroscopy correlate well with the different shielding expected for the preferred orientations of the guests in the MD simulations. Competition experiments for guest inclusion also agree reasonably with the calculated energy differences for the different host–guest inclusion complexes. These competition experiments show a selectivity ratio of about 3000 between the best (*p*-difluorobenzene) and the worst guest (1,3,5-trifluorobenzene).

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