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Recognition of guests bearing donor and acceptor hydrogen bonding groups by heteroditopic calix[4]arene receptors

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Abstract—The synthesis of new hosts specifically designed for the recognition of neutral guests bearing donor–acceptor hydrogen bonding groups is described. These hosts are characterized by the presence of two distinct binding region in close proximity: the rigid π-donor cavity and the H-bond donor *N*-methylene-*N'*-phenylureido group inserted onto the upper rim of the calix[4]arene skeleton. The binding abilities of these receptors were investigated toward a series of neutral ditopic organic molecules in $CDCl_3$ solution by ^1H NMR spectroscopy. The results obtained show that rigidity of the calix[4]arene apolar cavity is the control element in determining efficiency. In fact, compared with the more rigid **2**, host **10**, where the rigidity of the *cone* structure is maintained by hydrogen bonding of the OH of the lower rim, a decrease of efficiency of almost one order of magnitude was observed. The cooperative effect of the two binding region of host **2** was verified with different classes of ditopic guests. Good efficiency in the recognition of urea derivatives and dimethylsulfoxide was achieved.

1. Introduction

In apolar media, the binding abilities of the π-donor cavity of calix[4]arenes[1–3] are strictly related to the conformational flexibility of the macrocycle. In fact, it has been verified that, because of their residual flexibility, simple tetraalkoxy calix[4]arenes in the *cone* conformation are not able to form stable *endo* cavity inclusion complexes with neutral organic molecules.[4] On the contrary, calix[4]arenes held in a 'rigid' *cone* conformation through the linkage of two proximal phenol groups with short diethyleneglycol bridges or hydrogen bonding interactions in partially alkylated calix[4]arenes are able to form inclusion complexes with neutral organic guests.[5–8] Regardless of the strategy adopted to immobilize the *cone* structure, in all binding studies, only guests bearing acid CH_3 or CH_2 groups were found to be suitable guests, recognised with association constants that occasionally exceed $50\ M^{-1}$. From the structure of these complexes, inferred in apolar media and observed in the solid state, it emerges that specific CH-π(aromatic) interactions[9,10] represent the main driving force for their formation.

More recently, attempts to enhance the efficiency of the binding ability of rigidified calix[4]arenes as hosts, have brought us to the synthesis of heteroditopic receptors able to recognise very efficiently amides of formic, acetic and benzoic acids.[11] In particular, it was observed that the introduction of aN *N*-methylene-*N'*-phenylureido group onto the upper rim of the rigid calixarene skeleton of host **1** renders receptor **2** able to interact through its NHs with the carbonyl group of the guest. In addition the NH and/or CH groups of the guest directly linked to the carbonyl interact with the calix[4]arene π-donor cavity through NH-π[12,13] or CH-π[9,10] interactions. As a result of the proper structural matching between the host and the neutral guest, the association constants measured in apolar media approached $700\ M^{-1}$ in some instances. It thus appears that the synthesis of simple heteroditopic receptors able to efficiently bind neutral organic molecules bearing specific functional groups could be a powerful tool for the comprehension not only of the role and magnitude of the host preorganization on the binding efficiency, but also of the nature of the weak intermolecular interactions that are involved in biological and molecular recognition processes.

A possible approach to a deeper understanding of these phenomena could derive from the extension of the study, performed using **2** as reference host, to other classes of guest bearing donor and acceptor hydrogen bonding groups. Moreover, other useful information could be obtained studying the effect of the preorganization and shape of the calix[4]arene aromatic cavity on the recognition process. To address these issues the synthesis of new heteroditopic hosts held in a 'rigid' *cone* conformation through intramolecular H-bonds was undertaken. Moreover, these more versatile synthetic hosts could be easily modified to be employed as

Keywords: calix[4]arenes; heteroditopic receptors; molecular recognition; neutral organic guests.
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active components, bound onto appropriate surfaces, for the preparation of new selective sensors for neutral molecules.

2. Results and discussion

2.1. Design and synthesis of the new hosts

In a previous paper[11] we studied the binding ability of receptors 1, 2, and 3 (Fig. 1) toward simple amides derived from formic, acetic and benzoic acids. We found that the rigid 1, where only the π donor cavity can act as a binding site, experiences quite poor binding efficiency, comparable with that of **3**, where the hydrogen bond donor methylene-phenylureido group is anchored to the flexible tetrapropoxy-calix[4]arene skeleton. On the contrary **2**, where the same additional binding site was inserted onto the rigid platform of **1**, experiences quite remarkable efficiency. To give an insight into the mutual role of both host rigidity and additional binding site, the synthesis of the new receptor **10** was tackled.[14]

Receptor **10** is characterized by the presence of the *N*-methylen-*N′*-phenylureido group inserted at the upper rim of the calix[4]arene skeleton, held in a rigid *cone* conformation by intramolecular hydrogen bonding between the two distal OH groups present at its lower rim.

Attempts to apply the procedure used for the synthesis of host **2**,[11] based on a Tscherniac–Einhorn amidomethylation reaction[15–17] between *N*-hydroxymethyl-*N′*-phenylurea and **1**, using trifluoroacetic acid as catalyst on the 25,27-dipropoxy-calix[4]arene (**4**) were unsuccessful. Therefore compound **10** was synthesized in 9% overall yield through the reaction sequence reported in Scheme 1 that, together with the necessary steps for functional group insertion and transformation, also involves the protection–deprotection steps of the calixarene OH groups, which contribute to a decrease of the overall yield. The low overall yield prompted us to study in more detail the direct amidomethylation reaction. The systematic investigation of the reaction conditions brought us to the selection of $AlCl_3$ as the catalyst of choice for the direct amidomethylation of **4** with *N*-hydroxymethyl-*N′*-phenylurea that gave **10** in 30% (Scheme 1). Using the same synthetic approach, receptor **12** was synthesized (Scheme 2) from 25,26-dihydroxy-27,28-mono(crown-4)-calix[4]arene (**11**) in 55% yield. It is characterized by the presence of two proximal *N*-methylen-

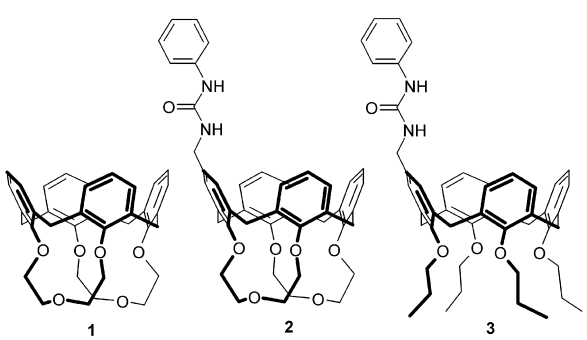
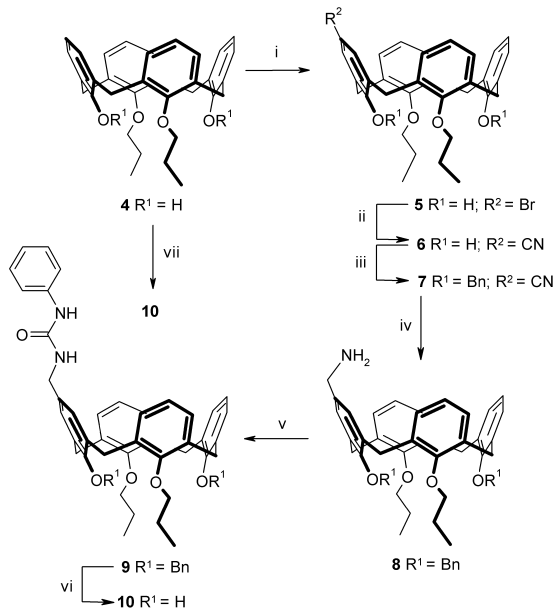


Figure 1.



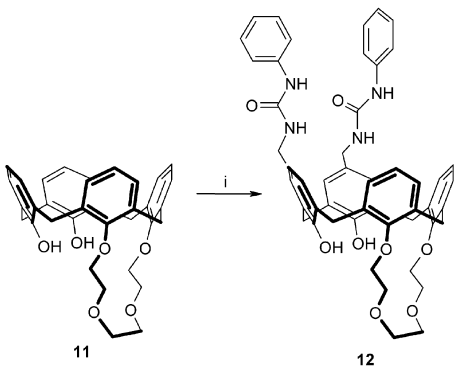
Scheme 1. *Reagents and conditions*: (i) NBS, 2-butanone, η=45%; (ii) CuCN, DMF, T=200°C, η=90%; (iii) NaH, BnBr, DMF, T=70°C, η=75%; (iv) B_2H_6, THF, T=60°C, η=55%; (v) C_6H_5NCO, CH_2Cl_2, rt, η=90%; (vi) H_2, Pd/C (cat.), η=60%; (vii) $C_6H_5NHCONHCH_2OH$, $AlCl_3$, CH_2Cl_2, η=30%.

N′-phenylureido groups onto its upper rim. Moreover a triethyleneglycol chain, that links the two proximal phenol groups, could contribute, together with the formation of intramolecular hydrogen bonding between remaining phenolic groups, to the stiffening of the whole calixarene skeleton.

Both receptors **10** and **12** were fully characterized by NMR and MS spectroscopy (see Section 4). The main features of the ^{1}H NMR spectrum of **10** in $CDCl_3$ are two singlets at δ=8.30 and 8.36 ppm for the phenolic OHs, and two overlapped AX systems at δ=3.35, 3.37, 4.30 and 4.32 ppm for the equatorial and axial methylene protons, respectively. The ureido NH protons resonate at δ=5.1 and 6.5 ppm as two broad singlets. The overall pattern of peaks is typical for a 1,3-dialkoxy-calix[4]arene derivative mono-substituted at the upper rim, in a flattened *cone* conformation on the NMR timescale. The ^{1}H NMR spectrum in $CDCl_3$ of derivative **12** is rather complicated and shows broad peaks especially in the aromatic region. A well resolved spectrum was recorded in DMSO d_6 in which all the characteristic signals of this compound were fully assigned by means of ^{1}H–^{1}H 2D COSY and ^{1}H–^{13}C 2D HETCOR spectroscopy.

2.2. Binding studies

Initially formamide, acetamide and their *N*-methyl derivatives were selected as guests to compare the binding efficiency of the new receptor **10** with that of **2**.[18] Therefore ^{1}H NMR titration experiments were performed adding increasing amounts of a $0.5–1.0\times10^{-1}$ M stock solution of the guest to a $0.5–1.0\times10^{-2}$ M stock solution of the host in $CDCl_3$, by monitoring the host ureido chemical shift variation. All the NMR spectra showed time-averaged signals for the free and complexed species and, having verified a 1:1 stoichiometry for the host–guest association



Scheme 2. *Reagents and conditions*: (i) $C_6H_5NHCONHCH_2OH$, $AlCl_3$, CH_2Cl_2, η=55%.

by means of continuous variation methods,[19] the stability constants (K) for the complex formation were calculated using methods that have been previously described based on the non-linear fitting of the experimental data.[20–22] The results are summarized in Table 1.

From the comparison of the efficiency of **2** and **10**, it emerges that the lower hydrogen bonding driven pre-organization of the calixarene cavity of **10** results in a general decrease of efficiency of almost one order of magnitude. Interestingly, **10** experiences a different selectivity order compared with **2** and a higher stability constant was observed with the bulkiest *N*-methylacetamide. Probably the larger flexibility of **10**, that could be responsible for this lower efficiency, could also, on the other hand, render it more adaptable to the bulkiest guest.

From the variation of the chemical shift experienced by the protons of both the hosts and the different guests during the titration experiments, general information on the structure of the complexes formed can be inferred (see Fig. 2). For example, during the titration of **10** with acetamide, a downfield complexation induced shift of about 0.8 and 0.5 ppm was experienced by the two ureido NH protons of the host, as expected on the basis of their involvement in hydrogen bonding with the oxygen of the guest carbonyl group. On the other hand, the guest NH_2 and CH_3 group signals were upfield shifted up to 0.8 and 0.35 ppm, respectively, as a consequence of the anisotropy shielding effects exerted by the aromatic rings of the cavity, thus accounting for their inclusion into the host cavity. Furthermore a downfield shift of about 0.2 ppm was experienced by hydroxyl protons belonging to the aromatic nucleus functionalized with the phenylureido moiety, indicating that during the titration a partial rearrangement of the calixarene skeleton takes place. Analogous behavior was also observed, although with different magnitude, with all the other guests studied.

Table 1. Association constants (K, M^{-1}) for hosts **2** and **10** with different amide-based guests, determined by ^1H NMR in $CDCl_3$ (T=300 K)

Entry	Guest	**2**[a]	**10**
1	$HCONH_2$	750(160)	30(5)
2	$HCONHCH_3$	204(8)	25(5)
3	CH_3CONH_2	350(25)	40(7)
4	$CH_3CONHCH_3$	260(25)	65(5)

Calculated monitoring the chemical shift variation of the Ar(calix)–CH_2NH–$CONHC_6H_5$ signal. All values result from at least duplicate experiments, standard deviations are in brackets.
[a] See Ref. 11.

Owing to its higher efficiency, the binding properties of host **2** were evaluated toward different series of ditopic guests, selected on the basis of the different extent of their hydrogen bond donor–acceptor ability (see Fig. 3). In fact, the common structural feature of the guests reported in Table 2 is the presence of a methyl group linked to moieties having different electron-withdrawing and H-bond acceptor ability (CH_3–G, where G=C(O)R^1, C(S)R^2, S(O)R^2, S(O_2)R^2 with R^1=Me, NH_2, NHMe, OMe and R^2=Me, NH_2). As a consequence, these CH_3 groups, having different acidity, should be able to interact with the calixarene cavity through 'hydrogen bond like' C–H/π interactions, whose magnitude could be related to their acidity.[7] In addition, the higher upfield shift experienced by the NH protons of acetamide and thioacetamide indicates, that for these guests, NH/π interactions with the host cavity could be the major driving force for complex formation (see Fig. 3).

By looking at the K values reported in Table 2, it emerges that the Brønsted acidity in DMSO[23–28] of the guest CH_3 and/or NH_2 groups, does not account, as the unique parameter, for the efficiency order observed. In fact, methyl acetate (entry 2) that possesses the more acid CH_3 group, is the least efficiently bound, while DMSO (entry 5), that is the weakest acid among the guests, is the most strongly bound. Similarly, the comparison of the NH_2 acidity, for example, of acetamide (entry 3) and methansulfonamide (entry 7) shows that the latter, in spite of its more acid NH_2 is less strongly bound.

A better rationalization of the K values reported in both Tables 1 and 2 could derive from the quantitative evaluation of the mutual and co-operative actions of the two binding sites of the host on the recognition of a ditopic guest that, unfortunately, has been scarcely studied. Nevertheless we attempted to analyze the results obtained in the present study through the solute effective hydrogen bond basicity and acidity scales introduced by Abraham.[29,30] In these scales the H-bond acceptor (A) and H-bond donor (B) parameters have been defined as free energy properties that reflect the tendency of a certain solute to accept and donate H-bonding in aprotic chlorinated solvents, measured toward an acid and a base taken as reference, respectively.

It should however be noted that these scales do not take into account the H-bond acidity of aliphatic protons. Moreover, considering the low H-bond basicity of the aromatic systems (e.g. B for benzene is 0.14), the very weak H-bond

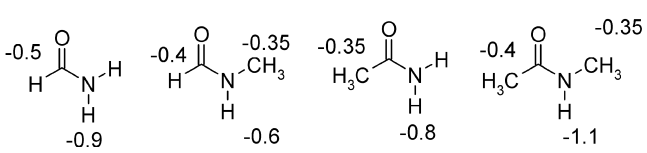


Figure 2. Observed upfield CIS (Complexation Induced Shifts) for amide-based guests during the titration with host **10** in $CDCl_3$ (T=300 K, $[G_0]/[H_0]$=0.3).

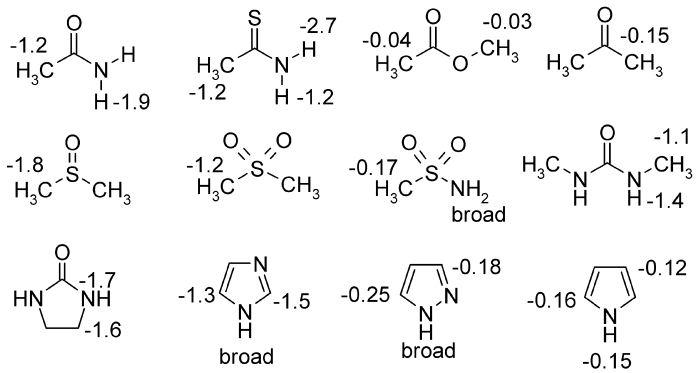


Figure 3. Observed upfield CIS (Complexation Induced Shifts) of the guest proton signals for the titration experiments when receptor **2** is used as host ($CDCl_3$, T=300 K, $[G_0]/[H_0]$=0.3).

interactions, like the CH/π (aromatic), that are often responsible for the formation of several supramolecular complexes, are neglected in such an approach.

As a consequence, for the data analyses, only the H-bond acidity of the NH groups present in the different guests could be utilized. Nevertheless, considering the correlation of the H-bond donor (A) and acceptor (B) ability with the binding efficiency of guests having related functional groups such as acetamide (A=0.55; B=0.68; K=350±25 M^{-1}), N-methylacetamide (A=0.40; B=0.71; K=260±25), acetone (A=0.04; B=0.49; K=16±2) and methyl acetate (A=0.00; B=0.45; K=13±2), it emerges that these parameters account for the trend of the measured binding constants. In fact, it could be reasonable to assume that acetone and methyl acetate because of the lack of H-bond donor ability, can be bound by host **2** only through their carbonyl group and hence less efficiently than acetamide and N-methylacetamide that, because of the H-bond donor ability of their NH, can be recognised more efficiently by a co-operative interactions of their CO and NH groups. However, as expected, any attempt to apply this approach to rationalize the K values for all the different classes of guest studied failed. It is reasonable, in fact, to assume that, besides the H-bond parameters, other structural properties of the guests (e.g. polarity, polarizability, shape and size) can affect the recognition process.

Table 2. Association constants (K, M^{-1}) and limiting chemical shift values (δ_∞, ppm) for hosts **2** with neutral guests, determined by ^1H NMR in $CDCl_3$ (T=300 K)

Entry	Guest	pK_a	K (M^{-1})	$\delta(NH)_\infty$
1	CH_3COCH_3	26.5[a]	16(2)	5.3(0.2)
2	CH_3COOCH_3	22.7[b]	13(2)	5.0(0.1)
3	CH_3CONH_2	35(Me)[c], 25.5(NH_2)[d]	340(25)	5.8(0.2)
4	CH_3CSNH_2	25.7(Me)[e], 18.5(NH_2)[f]	270(30)	5.6(0.2)
5	CH_3SOCH_3	35.1[a]	1250(350)	6.0(0.2)
6	$CH_3SO_2CH_3$	31.1[a]	200(20)	5.5(0.2)
7	$CH_3SO_2NH_2$	17.5(NH_2)[g]	85(25)	5.2(0.2)

Calculated monitoring $ArCH_2NHCONH$ chemical shift variation.
[a] Ref. 23.
[b] Determined as *tert*-butylacetate, Ref. 24.
[c] Determined as *N*,*N*-diethylacetamide, Ref. 24.
[d] Ref. 25.
[e] Determined as *N*,*N*-diethylthioacetamide, Ref. 24.
[f] Ref. 26.
[g] Ref. 27.

A further series of guests examined is represented by the pentaatomic heterocycles; pyrrole, 1-*H*-pyrazole and 1-*H*-imidazole (see Table 3, entries 1–3). These potential guests are all characterized by a relatively acidic NH proton, potentially able to interact with the host cavity of **2**. Pyrazole and imidazole could be recognized as ditopic guests since the second nitrogen atom located in the 2 and 3 positions, respectively, could participate in the overall recognition process by interacting with the host ureido NH. As expected these latter guests are bound more efficiently than the monotopic pyrrole for which negligible binding was observed.

Furthermore the higher efficiency exhibited by **2** toward imidazole suggests that, for this guest, the H-donor and acceptor atoms are in more appropriate relative positions. These latter results, together with those obtained in the case of amide recognition, prompted us to verify whether host **2** could be exploited for the recognition of structurally related guests like ureas. Unfortunately, in spite of their very effective H-bond donor and acceptor ability and the current interest in their recognition and sensing, the study was restricted to *N*,*N*′-dimethylurea and ethylenurea (entries 4 and 5, Table 3) for solubility reasons. Interestingly both ureas are bound to the same extent and with a magnitude almost identical to that of acetamide, suggesting a very similar binding mode.

2.3. Binding mode

Attempts to obtain suitable crystals for X-ray analysis of the complexes formed by **2** with the different guests failed. However, because of the unexpected and unprecedented

Table 3. Association constants (K, M^{-1}), limiting chemical shift values (δ_∞, ppm), for hosts **2** with urea and heterocycles-based guests, determined by ^1H NMR in $CDCl_3$ (T=300 K)

Entry	Guest	K (M^{-1})	$\delta(NH)_\infty$
1	Pyrrole	[a]	–
2	1-*H*-imidazole[b]	330(20)	7.9(0.1)
3	1-*H*-pyrazole[b]	120(15)	7.1(0.1)
4	$CH_3NHCONHCH_3$	330(30)	5.9(0.1)
5	Ethylenurea	340(30)	6.4(0.2)

Calculated monitoring $ArCH_2NHCONH$ chemical shift variation, unless otherwise stated.
[a] Negligible binding.
[b] Calculated monitoring $NHCONHC_6H_5$ signal chemical shift variation.

high association between **2** and DMSO, a detailed NMR investigation on the structure of the complex was carried out.

In the NMR spectra of the 1:1 complex (see Fig. 4(c)), the signals of the host NHs are downfield of about 1 ppm and resonate at δ=6.0 and 7.6 ppm, suggesting their involvement in hydrogen bonding with the guest S–O. On the other hand, the methyl groups of DMSO support an upfield shift of 2.1 ppm and resonate at δ=0.55 ppm. This indicates that they are in close proximity to the host aromatic cavity. These chemical shifts variation suggest a binding mode in which the guest SO moiety is involved in hydrogen bonding with the NH ureido protons and it seems reasonable to assume that this interaction pivots the orientation of at least one methyl group of DMSO toward the π-rich calix[4]arene cavity.

While the complete assignment of all host signals was obtained from ^{1}H–^{1}H 2D NMR DQF-COSY experiments, the structure of the complex was inferred through NOESY spectra.[31] The main feature of these latter spectra are the NOE cross-peaks between the methyl protons of DMSO with the aromatic and NH protons of the host.[32] In particular large cross-peak volumes were observed for the guest protons with H-5, 7, 11, 13 and 15 (see Fig. 5).

These data suggest the hypothesis that, beside the formation of classical H-bonding, a not negligible contribution to the complex stability also derives from the interaction of the methyl group of DMSO with the host cavity.

3. Conclusion

The present study shows an efficient route for the synthesis of new heteroditopic calix[4]arene-based receptors having one and two phenylureido groups at their upper rim, whose degree of preorganization is modulated through the formation of intramolecular hydrogen bonding at their lower rim. The recognition properties of the new hosts was tested toward a series of small amide derivatives having complementary hydrogen bonding properties, and compared to those experienced by a corresponding highly preorganised calix[4]arene-biscrown-3 (**2**). These studies show that the recognition of this type of guests can also be achieved, although less efficiently, with hosts whose degree of rigidity is only determined by intramolecular hydrogen bonding. These findings open new possibilities to employ these latter hosts as active components for the development of new artificial sensors for small organic molecules. In fact, it could be easy to realize new calix[4]arene receptors having 1,3-dialkoxy substituents functionalized with groups that allow the anchoring of the host onto sensor surfaces. On the other hand, the results obtained with **2** show interesting binding ability of this receptor toward DMSO. The stability constant calculated for this derivative (K>10^3 M^{-1}) in apolar solvents is one of the highest among those reported in literature for other synthetic hosts where the complexation of DMSO is driven only by hydrogen bonds[5,33,34] or mediated by metal cations.[35] The NMR studies accomplished on this complex show that its complexation is the result of the co-operation of the two binding sites of host **2** towards DMSO, which behaves as ditopic guest.

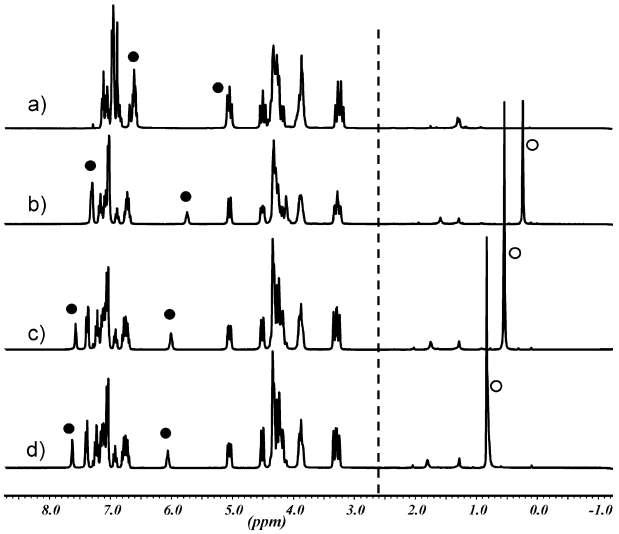


Figure 4. Stack plot of the titration of DMSO with host **2** in $CDCl_3$ (T=300 K): (a) free host; (b) [DMSO]/[**2**]=0.75; (c) [DMSO]/[**2**]=1.0; (d) [DMSO]/[**2**]=1.25; (●) host ureido NH signals; (○) guest *Me* signal; (--) free guest.

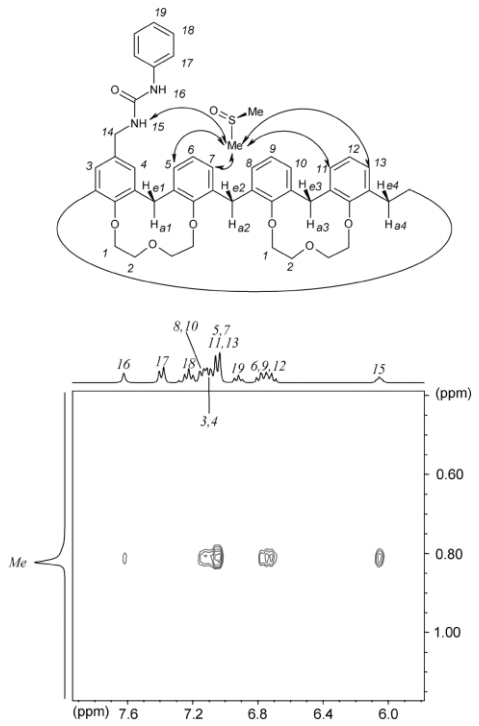


Figure 5. Expanded region of the NOESY spectrum of **2**⊃DMSO complex (1:1.25) showing negative cross-peaks corresponding to intermolecular interactions between the methyl groups of the guest with the aromatic part of the host (300 MHz, T=300 K, mixing time=0.7 ms).

4. Experimental

4.1. General remarks

All reactions were carried out under nitrogen; all solvents were freshly distilled under nitrogen and stored over molecular sieves for at least 3 h prior to use. All other

reagents were of reagent grade quality as obtained from commercial suppliers and were used without further purification. Column chromatography were performed on silica gel 63–200 mesh. NMR spectra were recorded in $CDCl_3$ at 300 K unless otherwise indicated. Mass spectra were determined in the CI mode (CH_4) as appropriate. Melting point are uncorrected. *N*-hydroxymethyl-*N'*-phenylurea,[36] calix[4]arenes **2**,[37] **4**[37] and **11**[38] were synthesized according to literature procedures.

4.1.1. 5-Bromo-25,27-dipropoxycalix[4]arene (5). To a solution of **4** (2.0 g, 3.9 mmol) in methyl ethyl ketone (200 mL), NBS was added (0.6 g, 3.5 mmol). After stirring at rt for 4 h, the solution was diluted with CH_2Cl_2 (100 mL) and then treated with a saturated aqueous solution of Na_2SO_3 (100 mL). The organic layer was separated, washed with brine (2×100 mL), dried (Na_2SO_4), and the solvent was completely evaporated under reduce pressure. Purification of the residue by chromatography (hexane/CH_2Cl_2, 60:40) gave 0.9 g (45%) of **5** as a pale yellow solid: mp 180–181°C (dec.). ^{1}H NMR (300 MHz) δ: 1.31 (t, 6H, J=7.4 Hz); 2.0–2.1 (m, 4H); 3.32, 3.38 (2d, 4H, J=13 Hz); 3.97 (t, 4H, J=6 Hz); 4.28, 4.31 (2d, 4H, J=13 Hz); 6.66 (t, 1H, J=7.5 Hz); 6.76 (t, 2H, J=7.5 Hz); 6.8–6.9 (m, 4H); 7.12 (d, 2H, J=7.5 Hz); 7.17 (s, 2H); 8.4 and 8.2 (2s, 2H). ^{13}C NMR (75 MHz) δ: 10.8, 23.4, 29.6, 31.1, 31.3, 78.4, 110.2, 118.9, 125.3, 127.9, 128.4, 129.2, 130.1, 130.6, 132.5, 133.5, 151.8, 152.5, 153.2. MS-CI(+) *m/z*: 587 [MH^+]; Anal. Calcd for $C_{34}H_{35}BrO_4$: C, 69.50; H, 6.00. Found: C, 69.32; H, 6.05.

4.1.2. 5-Cyano-25,27-dipropoxycalix[4]arene (6). To a solution of **5** (2.0 g, 3.4 mmol) in DMF (100 mL), CuCN (0.90 g, 10.2 mmol) was added. The resulting heterogeneous mixture was poured into a thick wall glass autoclave and then heated at 200°C for 48 h under vigorous stirring. After cooling, the solvent was completely evaporated under reduced pressure. The resulting sticky residue was extracted twice with hot ethyl acetate (2×50 mL). The combined organic phases were then washed twice with brine (2×100 mL), dried (Na_2SO_4), and the solvent was completely evaporated to dryness (the separated water phase was carefully treated with a solution of sodium hypochlorite to destroy the residuals cyanide ions). Purification of the solid residue by chromatography (hexane/CH_2Cl_2, 50:50) afforded 1.8 g (90%) of **6** as a white solid: mp 200–201°C (dec.). ^{1}H NMR (300 MHz) δ: 1.31 (t, 6H, J=7.4 Hz); 2.0–2.1 (m, 4H); 3.37, 3.39 (2d, 4H, J=13.1 Hz); 3.9–4.0 (m, 4H); 4.28, 4.30 (2d, 4H, J=13 Hz); 6.6–6.7, 6.7–6.8, 6.9–7.0, 7.0–7.1, (4m, 9H); 7.37 (s, 2H); 8.19 and 9.19 (2s, 2H). ^{13}C NMR (75 MHz) δ: 10.8, 23.4, 31.1, 31.3, 78.4, 118.9, 119.0, 125.4, 127.7, 128.3, 128.4, 128.7, 128.8, 129.2, 129.5, 131.8, 132.4, 133.6, 151.7, 153.2. MS-CI(+) *m/z*: 534 [MH^+]; Anal. Calcd for $C_{35}H_{35}NO_4$: C, 78.77; H, 6.61; N, 2.62. Found: C, 78.99; H, 6.67; N, 2.53.

4.1.3. 5-Cyano-26,28-dibenzyloxy-25,27-dipropoxycalix[4]arene (7). To a solution of **6** (0.80 g, 1.5 mmol) in DMF (60 mL), NaH (0.22 g, 9.2 mmol) was added. The resulting heterogeneous mixture was stirred for 30 min, then benzyl bromide (0.65 g, 3.8 mmol) was added. The mixture was heated at 65°C for 3 h, cooled and diluted with ethyl acetate (100 mL). The reaction was then quenched by treatment with a 10% HCl solution (50 mL, CAUTION!). The organic layer was separated, washed with a saturated solution of Na_2CO_3 (100 mL), dried (Na_2SO_4), and evaporated to dryness. Purification of the residue by crystallization with hexane, gave 0.6 g (75%) of **7**: mp 146–148°C. ^{1}H NMR (300 MHz) δ: 0.63 (t, 6H, J=7.2 Hz); 1.5–1.7 (m, 4H); 3.05 and 3.15 (2d, 4H, J=13.5 Hz); 3.7–3.9 (m, 4H); 4.41 and 4.44 (2d, 4H, J=13.4 Hz); 4.77 and 4.82 (2s, 4H); 6.21 (d, 2H, J=7.5 Hz); 6.44 (s, 2H); 6.50 (t, 1H, J=7.8 Hz); 6.92 (t, 2H, J=7.3 Hz); 6.98 and 7.07 (2dd, 4H, J_1=7.3 Hz, J_2=1.7 Hz); 7.3–7.5 (m, 10H). ^{13}C NMR (75 MHz) δ: 9.4, 22.8, 30.8, 31.0, 76.5, 105.9, 119.5, 122.3, 122.5, 127.6, 127.8, 128.2, 128.25, 128.3, 128.5, 128.7, 129.5, 130.0, 131.6, 133.5, 135.4, 135.9, 136.6, 136.9, 137.7, 154.6, 157.5. MS-CI(+) *m/z*: 714 [MH^+]; Anal. Calcd for $C_{49}H_{47}NO_4$: C, 82.44; H, 6.64, N, 1.96. Found: C, 82.62; H, 6.70 N, 1.90.

4.1.4. 5-Aminomethyl-26,28-dibenzyloxy-25,27-dipropoxycalix[4]arene (8). To a solution of **7** (0.6 g, 0.84 mmol) in dry THF (30 mL), B_2H_6 (1 M solution in THF, 8.4 mL) was added. The resulting mixture was refluxed for 24 h under argon atmosphere, cooled, treated with methanol (20 mL, CAUTION!), and then refluxed for an additional 30 min. The solvent was then completely evaporated under reduced pressure and the residue taken up with CH_2Cl_2 (50 mL) and a saturated solution of Na_2CO_3 (50 mL). The organic layer was separated, washed with brine (2×100 mL), dried (Na_2SO_4), and the solvent was completely evaporated. Purification of the solid residue by chromatography (CH_2Cl_2/CH_3OH, 95:5) gave 0.33 g (55%) of **8** as a white solid: mp 200–201°C (dec.). ^{1}H NMR (300 MHz) δ: 0.65 (t, 6H, J=7.4 Hz); 1.33 (bs, 2H); 1.7–1.8 (m, 4H); 3.14, 3.15 (2d, 4H, J=13.3 Hz); 3.30 (s, 2H); 3.8–3.9 (m, 4H); 4.47, 4.48 (2d, 4H, J=13.2 Hz); 4.82 (s, 4H); 6.20 (s, 2H); 6.36 (bs, 3H); 6.88 (t, 2H, J=7.5 Hz); 7.0 (d, 4H, J=7.5 Hz); 7.3–7.5 (2m, 10H). ^{13}C NMR (75 MHz) δ: 9.5, 22.8, 31.0, 31.1, 45.8, 76.5, 77.4, 121.9, 126.4, 127.6, 127.8, 127.83, 128.1, 128.6, 128.7, 128.9, 129.0, 134.0, 136.3, 137.6, 137.8, 153.4, 154.9, 157.5. MS-CI(+) *m/z*: 717 [MH^+]; Anal. Calcd for $C_{49}H_{51}NO_4$: C, 81.98; H, 7.16, N, 1.95. Found: C, 82.12; H, 7.20; N, 1.90.

4.1.5. 5-(*N*-Phenylureido)methyl-26,28-dibenzyloxy-25,27-dipropoxycalix[4]arene (9). To a solution of **8** (0.3 g, 0.42 mmol) in dry CH_2Cl_2 (25 mL), phenyl isocyanate (0.05 g, 0.42 mmol) was added. After 3 h under stirring, the mixture was poured into water (50 mL) and diluted with CH_2Cl_2 (50 mL). The organic layer was separated, washed with water (2×100 mL), dried (Na_2SO_4), and the solvent was completely evaporated. Crystallization with diethyl ether afforded 0.27 g (90%) of **9** as pure white solid: mp 116–118°C. ^{1}H NMR (300 MHz) δ: 0.62 (t, 6H, J=7.4 Hz); 1.6–1.7 (m, 4H); 3.07, 3.11 (2d, 4H, J=13 Hz); 3.7–3.8 (m, 4H); 3.89 (d, 2H, J=5.3 Hz); 4.31 (bt var, 1H); 4.40 and 4.43 (2d, 4H, J=13.2 Hz); 4.78, 4.79 (2s, 4H); 6.03 (bs var, 1H); 6.16 (s, 2H); 6.2–6.3 (m, 3H); 6.78 (t, 2H, J=7.4 Hz); 6.9–7.0 (m, 4H); 7.0–7.1, 7.2–7.3 (2m, 5H); 7.3–7.5 (m, 10H). ^{13}C NMR (75 MHz) δ: 10.3, 22.8, 29.5, 31.1, 32.7, 42.4, 44.1, 75.5, 120.3, 121.3, 121.96, 122.65, 124.7, 126.94, 127.3, 127.86, 128.8, 129.0, 129.4, 129.98, 131.8, 134.2, 136.2, 137.6, 154.4, 155.3, 157.4. MS-

CI(+) *m/z*: 836 [MH^+]; Anal. Calcd for $C_{56}H_{56}N_2O_5$: C, 80.35; H, 6.74, N, 3.35. Found: C, 80.51; H, 6.84; N, 3.28.

4.1.6. 5-(*N*-Phenylureido)methyl-25,27-dipropoxycalix[4]arene (10). To a solution of **9** (0.27 g, 0.32 mmol) in dry CH_2Cl_2 (15 mL) and MeOH (5 mL) solvents mixture, a spatula tip of Pd/C catalyst was added (CAUTION!). The resulting dark mixture was stirred for 24 h under atmospheric hydrogen pressure, then the palladium catalyst was filtered off, under nitrogen atmosphere, through a short pad of celite. The filtered solution was evaporated to dryness and the resulting solid residue was triturated with hot cyclohexane to afford 0.16 g (60%) of **10** as white solid: mp 152–154 °C. ^{1}H NMR (300 MHz) δ: 1.31 (t, 6H, J=7.5 Hz); 2.0–2.2 (m, 4H); 3.35, 3.37 (2d, 4H, J=13 Hz); 3.97 (t, 4H, J=6.3 Hz); 4.09 (d, 2H, J=5.3 Hz); 4.30, 4.32 (2d, 4H, J=12.9 Hz); 5.13 (bt var, 1H); 6.48 (bs var, 1H); 6.6–6.7 (m, 3H); 6.8–6.9 (m, 6H); 6.96 (s, 2H); 7.1–7.2 (m, 5H); 8.30 and 8.36 (2s, 2H). ^{13}C NMR (75 MHz) δ: 10.8, 23.4, 31.3, 78.3, 121.0, 127.9, 128.3, 128.9, 129.1. MS-CI(+) *m/z*: 656 [MH^+]; Anal. Calcd for $C_{42}H_{44}N_2O_5$: C, 76.80; H, 6.75, N, 4.26. Found: C, 77.07; H, 6.82; N, 4.20.

4.2. Direct synthesis of 5-(*N*-phenylureido)methyl-25,27-dipropoxycalix[4]arene (10)

To a solution of **4** (1.0 g, 1.97 mmol) and anhydrous $AlCl_3$ (0.52 g, 3.94 mmol) in CH_2Cl_2 (50 mL), kept at 0°C by means of an external ice bath, a solution of *N*-hydroxymethyl-*N′*-phenylurea (0.33 g, 1.97 mmol) in CH_2Cl_2 (20 mL) was added dropwise. After stirring at room temperature for 3 h, the reaction was quenched with a 10% solution of HCl (50 mL). The organic phase was diluted with CH_2Cl_2 (50 mL), separated, washed with water (2×100 mL) up to neutrality, dried (Na_2SO_4), and completely evaporated. Purification of the solid residue by chromatography (hexane/CH_2Cl_2, 5:5) gave 0.38 g (30%) of **10**.

4.2.1. 17,23-Bis-(*N*-phenylureido)methyl-25,26-didroxy-27,28-mono(crown-4)-calix[4]arene (12). As reported for the direct synthesis of **10** using the following reagents and quantities: **11** (0.5 g, 0.9 mmol), $AlCl_3$ (0.5 g, 3.7 mmol), and *N*-hydroxymethyl-*N′*-phenylurea (0.31 g, 1.86 mmol) in dry CH_2Cl_2 (70 mL). Purification of the residue by crystallization from a CH_2Cl_2/ethyl acetate mixture gave 0.43 g (55%) of **12** as white solid: mp 241–242°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.35 (bs, 1H); 3.45 (d, 3H, J=12.9 Hz); 3.7–3.8 (m, 3H); 3.9–4.2 (m, 20H); 4.65 (d, 1H, J=12.3 Hz); 6.2–6.3 (m, 2H); 6.79 (t, 2H, J=7.5); 6.88 (t, 2H, J=7.5); 7.0–7.1 (m, 6H); 7.20 (t, 4H, J=7.8); 7.28 (d, 2H, J=7.5); 7.36 (d, 4H, J=7.8); 8.25 (s, 2H); 8.54 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_d) δ: 28.48, 30.62, 30.93, 42.57, 68.88, 70.39, 75.39, 117.57, 120.95, 124.57, 127.59, 128.05, 128.55, 128.74, 128.91, 129.30, 131.75, 133.99, 135.25, 140.35, 149.79, 153.40, 154.92. MS-CI(+) *m/z*: 835 [MH^+]; Anal. Calcd for $C_{50}H_{50}N_4O_8$: C, 71.92; H, 6.04, N, 6.71. Found: C, 71.63; H, 6.14; N, 6.60.

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