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Multipoint Molecular Recognition of Amino Acids and Biogenic Amines by Ureidocalix[5]arene Receptors

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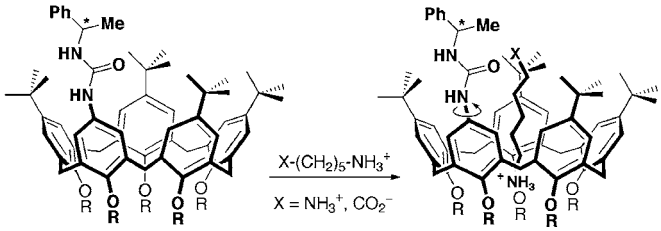
ABSTRACT



***p-tert*-Butylcalix[5]arenes in a fixed cone conformation, endowed with a urea functionality at the upper rim, behave as remarkably efficient abiotic receptors of ω-amino acids and biogenic amines, which are bound with one end of the chain within the π-basic cavity (primary recognition site) and the other grasped by the secondary hydrogen bonding donor/acceptor binding site.**

Basic α-amino acids, aliphatic biogenic di- and polyamines, and ω-amino acids are all interrelated bioactive species that share biosynthetic and metabolic pathways. Decarboxylation of lysine and ornithine produces cadaverine and putresceine, respectively.[1] Putresceine generates spermine and spermidine in the presence of methionine and is also a precursor[2] of γ-aminobutyric acid (GABA). Besides the paramount importance of α-amino acids in living organisms and the well-known inhibitory activity of GABA as a neurotransmitter in mammalian central nervous systems,[3] several studies have demonstrated the crucial role played by aliphatic biogenic amines in the biochemical clinical field. The latter have been used as cancer markers[4] and, in relation to food quality and toxicity for human health,[5] are currently being monitored in several foodstuffs (fish, meat, cheese, wine) susceptible to microbial degradation during aging and storage.[6]

Analytical methods for GABA and aliphatic biogenic amine determination mainly rely on chromatographic (LC, HPLC, GC, ion-exchange) and electrophoretic techniques. However, because of their UV- and fluorescence-transparency, pre- or postcolumn derivatization is generally required for detection.[7] A more recent approach to direct/indirect biogenic amine determination involves the use of specific synthetic macrocycles. Calixarenes and crown ethers have successfully been employed as selectivity modifiers in

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(1) Tabor, W. C.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749−790.
(2) Demello, M. C. F.; Guerrapeixe, R.; Demello, F. G. *Neurochem. Int.* **1993**, *22*, 249−253.
(3) Perry, T. L.; Berry, K.; Diamond, S.; MoK, J. *J. Neurochem.* **1971**, *18*, 513−519. Fahn, S.; Côte, L. J. *J. Neurochem.* **1968**, *15*, 209−213.
(4) Moulinoux, J.-P.; Quemener, V.; Delcros, J.-G.; Cipolla, B. In *Polyamines in cancer: Basic Mechanisms and Clinical Approaches*; Nishioka, K., Ed.; Springer: New York, 1996; Chapter 10, pp 233−249.
(5) Bartholomew, B. A.; Berry, P. R.; Rodhouse, J. C. Gilbert, R. J. *Epidemiol. Infect.* **1987**, *99*, 775−782.
(6) Shalaby, A. R. *Food Res. Int.* **1996**, *29*, 675−690.
(7) Khuhawar, M. Y.; Qureshi, G. A. *J. Chromatogr. B* **2001**, *764*, 385−407.

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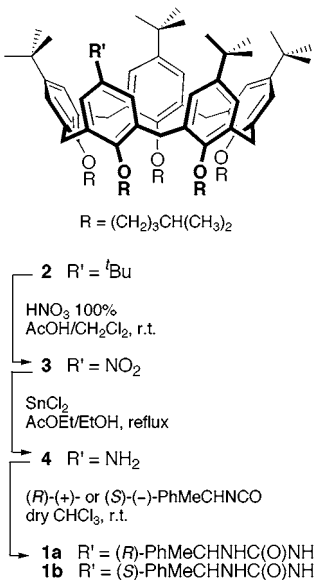
capillary electrophoresis[8] or as sensing agents in potentiometric detection with liquid-membrane electrodes.[9]

In connection with the increasing interest in calixarenes as biomimetic receptors[10] and their use as sensing agents,[11] we have recently reported that calix[5]arenes locked in a C_{5v}-symmetric cone conformation provide highly preorganized cavities for the formation of strong 1:1 inclusion (endocavity) complexes with linear alkylammonium ions in organic media.[12] We have also developed calix[5]arene-based *n*-butylammonium selective liquid-membrane electrodes[13] (insensitive to commonly interfering inorganic cations) and disclosed preliminary data on the potential of these macrocycles as receptors for carboxyl-protected bioactive substrates.[12a,14] Structural modifications to improve such properties were then concentrated on the replacement of a *p-tert*-butyl with a ureido group. The latter has been successfully used for the complexation of carboxylates[15] and amides[16] in the calix[4]arene series.[17] We now report that calix[5]arenes **1** in a fixed cone conformation bearing a urea unit at the upper rim show a strong affinity for biogenic amines, ω-amino acids, and lysine derivatives.

Selective ipso-nitration of penta-*O*-isohexyl ether **2**[12a] with 100% nitric acid in a AcOH−CH_2Cl_2 mixture at room temperature produced 5-nitro derivative **3** (30%), which was converted into 5-aminocalix[5]arene **4** (68%) by reduction with tin(II) chloride[18] in refluxing AcOEt/EtOH. Subsequent reaction of **4** with enantiopure (*R*)-(+)- and (*S*)-(−)-α-methylbenzyl isocyanate in dry chloroform afforded receptors **1** (80−83%), Scheme 1.[19] Distinctive ^{1}H and ^{13}C NMR patterns and chemical shifts of the bridging methylene and *tert*-butyl groups fully support the formation of ureido

Scheme 1



derivatives **1** and their C_s-symmetric precursors **3** and **4** as rigid cone conformers.[20]

A preliminary NMR screening of the binding affinities of **2** and the new derivatives **3**, **4**, and **1a** toward N^{α}-Ac-L-Lys-OMe·HCl (in $CDCl_3/CD_3OD$, 9:1) showed a pronounced dependence of the association constant (K_a) on the nature of the upper rim substituents. With respect to the parent receptor **2** ($K_a = 89 \pm 10\ M^{-1}$), the introduction of a strong electron-withdrawing *p*-NO_2 group totally deactivates the binding properties of **3**.[12b] On the other hand, in the case of **4** and **1a** the presence of a *p*-NH_2 or *p*-ureido functionality, respectively, produces either a marginal ($K_a = 101 \pm 12\ M^{-1}$) or a drastic enhancement of the association constants ($K_a = 1690 \pm 185\ M^{-1}$).

The full potential of ureido derivatives **1** as molecular receptors for bioactive ammonium-containing substrates was later investigated by ^{1}H NMR spectroscopy, in $C_2D_2Cl_4/CD_3OD$ (2:1) solution.[21] Cadaverine dihydrochloride (Cad·2HCl), 6-aminohexanoic acid (ϵ-Ahx),[22] and L-lysine methyl ester dihydrochloride (Lys-OMe·2HCl) were chosen as prototype ammonium substrates bearing an additional recognizable moiety (i.e., ammonium, carboxylate, and alkoxycarbonyl, respectively), located five methylenes away from the primary ammonium group (Figure 1).

In all instances, the spectra of equimolar amounts of host and guest ([H] = [G] = 10^{-3} M) showed the formation of inclusion complexes (vide infra). The complexation process is slow on the NMR time scale, and the 1:1 stoichiometry

(8) Arce, L.; Carretero, A. S.; Rios, A.; Cruces, C.; Fernandez, A.; Valcarcel, M. *J. Chromatogr. A* **1998**, *816*, 243−249.

(9) Poels, I.; Nagels, L. J. *Anal. Chim. Acta* **2001**, *440*, 89−98.

(10) Sansone, S.; Segura, M.; Ungaro, R. In *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer Academic: Dordrecht, 2001; Chapter 27, pp 496−512.

(11) Cadogan, F.; Nolan, K.; Diamond, D. In *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer Academic: Dordrecht, 2001; Chapter 34, pp 627−641.

(12) (a) Arnaud-Neu, F.; Fuangswasdi, S.; Notti, A.; Pappalardo, S.; Parisi, M. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 112−114. (b) Ferguson, G.; Notti, A.; Pappalardo, S.; Parisi, M. F.; Spek, A. L. *Tetrahedron Lett.* **1998**, *39*, 1965−1968. (c) Cafeo, G.; Gattuso, G.; Kohnke, F. H.; Notti, A.; Occhipinti, S.; Pappalardo, S.; Parisi, M. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 2122−2126.

(13) Giannetto, M.; Mori, G.; Notti, A.; Pappalardo, S.; Parisi, M. F. *Anal. Chem.* **1998**, *70*, 4631−4635.

(14) For the molecular recognition of *N*-α-protected amino acid esters by a self-assembled cylindrical capsule, see: Hayashida, O.; Sebo, L.; Rebek, J., Jr. *J. Org. Chem*. **2002**, *67*, 8291−8298.

(15) (a) Pelizzi, N.; Casnati, A.; Friggeri, A.; Ungaro, R. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1307−1311. (b) Budka, J.; Lhoták, P.; Michlová, V.; Stibor, I. *Tetrahedron Lett*. **2001**, *42*, 1583−1586.

(16) Arduini, A.; Secchi, A.; Pochini, A. *J. Org. Chem*. **2000**, *65*, 9085−9091.

(17) Calix[4]-, calix[5]-, and calix[6]arenes with ureido functionalities at the upper rim have also been employed for the self-assembling of molecular capsules; see: Rebek, J., Jr. *Chem. Commun*. **2000**, 637−643; Vysotsky, M. O.; Pop, A.; Broda, F.; Thondorf, I.; Böhmer, V. *Chem. Eur. J*. **2001**, *7*, 4403−4410. Yanase, M.; Haino, T.; Fukazawa, Y. *Tetrahedron Lett*. **1999**, *40*, 2781−2784. Gonzáles, J. J.; Ferdani, R.; Albertini, E.; Blasco, J. M.; Arduini, A.; Pochini, A.; Prados, P.; de Mendoza, J. *Chem. Eur. J*. **2000**, *6*, 73−80.

(18) Bellamy, F. D.; Ou, K. *Tetrahedron Lett*. **1984**, *25*, 839−842.

(19) All new compounds were characterized by elemental analysis and NMR and MS spectroscopy.

(20) Stewart, D. R.; Krawiec, M.; Kashyap, R. P.; Watson, W. H.; Gutsche, C. D. *J. Am. Chem. Soc.* **1995**, *117*, 586−601. De Salvo, G.; Gattuso, G.; Notti, A.; Parisi, M. F.; Pappalardo, S. *J. Org. Chem*. **2002**, *67*, 684−692.

(21) This solvent mixture is more suitable than $CDCl_3/CD_3OD$ to solubilize the dication and amino acid guests under study.

(22) ϵ-Ahx is a lysine analogue employed as plasminogen inhibitor, see: Markus, G. *Fibrinolysis* **1996**, *10*, 75−85.

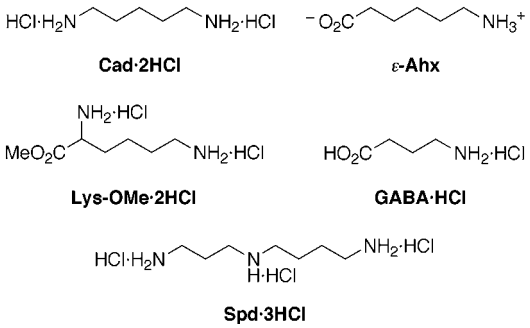


Figure 1. Structures of the ammonium-containing guests under investigation.

and association constants were readily determined by direct analysis of the peak intensity ratio of complexed and uncomplexed species (Table 1).

Table 1. Association Constants (K_a, M^{-1}) of Selected Ammonium-Containing Guests with Hosts **1a,b** and **2**[a]

host	Cad·2HCl	ϵ-Ahx	Lys-OMe·2HCl
1a	12820	16140	2240
1b	11860	16850	2190
2	300	1070	43

[a] Determined by ^{1}H NMR at 22 ± 1 °C in $C_2D_2Cl_4$/CD_3OD (2:1), using equimolar (10^{-3} M) host−guest mixtures. Values derived from the average of at least three independent measurements (standard error ≤15%). No intermolecular self-association of hosts **1** was detected in the (5×10^{-4})−(5×10^{-3}) M range.

5-Ureidocalix[5]arene receptors **1** display much higher K_a values than their progenitor **2**. These results imply that, besides the known proclivity of alkylammonium chains to fill the cavity of preorganized calix[5]arenes,[12] complexation is strongly enhanced by the synergic action of the additional binding site introduced at the upper rim. Notably, the ureido "secondary binding site" of receptors **1a**,**b**—by simply rotating around the Ar—NH single bond—acts as a powerful hydrogen bond acceptor or donor and efficiently interacts with both the α-ammonium[23] and α-carboxylate moieties of Cad·2HCl and zwitterionic ϵ-Ahx, respectively. Spectroscopic evidence for this dual mode of interaction of the ureido moiety was provided by the ^{1}H NMR measurements in neat $C_2D_2Cl_4$, which allowed us to follow the complexation induced shifts (CISs) of the guest NH_3^+ and host NH resonances upon binding (Table 2). Formation of 1:1 endocavity complexes between **1a** and Cad·2HCl or ϵ-Ahx is unambiguously supported by the four resonances of the β-CH_2−ϵ-CH_2 groups in the 0.5 to −2.0 ppm region of the ^{1}H NMR spectra (Figure 2, traces b and c). In both cases,

(23) For the sake of consistency with the ϵ-ammonium group of ϵ-Ahx, in the case of Cad·2HCl we arbitrarily designate the ammonium groups located inside the calixarene cavity and the one interacting with the ureido moiety as ϵ-NH_3^+ and α-NH_3^+, respectively.

Table 2. Selected Resonances of the ^{1}H NMR (300 MHz, in $C_2D_2Cl_4$) Spectra of Model Ammonium Substrates (*n*-$BuNH_2$·HCl and ϵ-Ahx-OMe·HCl),[24] Host **1a**, and Its Inclusion Complexes with Cad·2HCl and ϵ-Ahx

	α-NH_3^+	ϵ-NH_3^+	Ar*N*H	CH*N*H
n-$BuNH_2$·HCl		8.23 (br s)		
ϵ-Ahx-OMe·HCl		8.22 (br s)		
1a			4.75 (s)	5.56 (d)
Cad·2H$^+$⊂**1a**[a]	7.81 (br s)	5.37 (br s)	9.34 (s)	6.95 (d)
ϵ-Ahx⊂**1a**[a]		5.44 (br s)	8.65 (s)	7.02 (d)

[a] Solutions previously used for K_a determination were first concentrated to dryness and then redissolved in $C_2D_2Cl_4$.

binding of the common pentylenammonium chain inside the calix[5]arene cavity takes advantage of a combination of weak noncovalent interactions including cation−π, CH−π, and hydrogen bonding(s) with the phenolic oxygens. Inclusion is further confirmed by the upfield shifts of the broad singlets (δ 5.37 and 5.44 ppm for Cad·2HCl and ϵ-Ahx, respectively, Table 2)[24] of the ϵ-NH_3^+ groups,[23] which enjoy the shielding of five aryl rings.

In the Cad·2H$^+$⊂**1a** complex the second ammonium group of cadaverine (α-NH_3^+)[23] is engaged in hydrogen bonding(s) with the carbonyl group of the ureido moiety of **1a** and resonates at 7.81 ppm. This interaction causes the concomitant downfield shift of the ureido NH resonances of **1a** (Table 2).

On the other hand, in the ϵ-Ahx⊂**1a** complex, formation of hydrogen bonding(s) between the carboxylate group of the guest and the NH hydrogen(s) of **1a** (CO_2^-···H—N), produces a similar downfield shift on the resonances of the latter.[15a,16]

A scrutiny of the K_a values in Table 1 reveals that the ureido secondary binding site of **1** displays a higher affinity for carboxylate rather than ammonium moieties, and that the presence of a bulky α-amino ester moiety (Lys-OMe·2HCl) lowers the association constant by about 1 order of magnitude. Remarkably, in the absence of CD_3OD complexation of ϵ-Ahx by **1a** increases from 82 to nearly 100% (see Figure 2, trace c). Data in Table 1 also show that no enantioselective recognition of L-Lys-OMe·2HCl by (*R*)- and (*S*)-α-methylbenzylureido derivatives **1a**,**b** is taking place.

Complexation studies were also extended to spermidine trihydrochloride (Spd·3HCl) and GABA. Noteworthy, the former was toposelectively included inside the calixarene cavity of **1a** with its butylenammonium moiety (K_a = 1010 ± 89 M^{-1}).[25] On the other hand, when zwitterionic GABA was tested with the same receptor only a slight broadening of signals was detected, indicating no inclusion and a likely single-point interaction (docking of the α-carboxylate group

(24) Because of the insolubility of Cad·2HCl and ϵ-Ahx in $C_2D_2Cl_4$, *n*-butylammonium hydrochloride (*n*-$BuNH_2$·HCl) and 6-aminohexanoic acid methyl ester hydrochloride (ϵ-Ahx-OMe·HCl) were used as model substrates to evaluate the CISs of the ϵ-NH_3^+ (and α-NH_3^+) groups in the Cad·2H$^+$⊂**1a** and ϵ-Ahx⊂**1a** complexes.

(25) Determined by ^{1}H NMR, under the experimental conditions reported in Table 1.

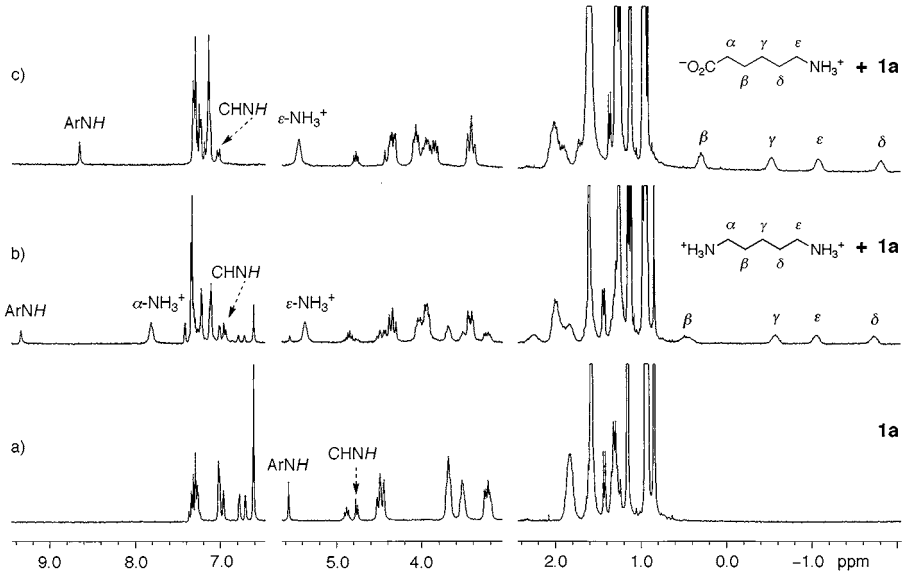


Figure 2. Endo-cavity complexation (slow exchange regime) of Cad·2HCl and ϵ-Ahx·HCl by **1a**. Selected regions of the ^{1}H NMR (300 MHz; $C_2D_2Cl_4$; 22 ± 1 °C) spectra of (a) free host, (b) equimolar mixture of **1a** and Cad·2HCl (10^{-3} M), and (c) equimolar mixture of **1a** and ϵ-Ahx·HCl (10^{-3} M).

to the ureido moiety). However, a single set of broad but diagnostic high field signals of the included guest readily appeared in the ^{1}H NMR spectrum of GABA·HCl. Sharpening of both host and guest resonances at −20 °C seems to indicate that at room temperature the short GABA·H$^+$ ion is rapidly hopping between the two recognition sites, while the ureido-containing aryl ring is swinging in and out of the calixarene cavity. At a lower temperature this motion slows down and complexation can readily be quantified (K_a = 12230 ± 1071 M^{-1}).[26]

In conclusion, the selective introduction of one ureido functionality at the upper rim of preorganized calix[5]arenes turns simple alkylammonium ion hosts into powerful abiotic receptors of biologically relevant substrates (biogenic amines, ω-amino acids, and basic α-amino acids). The effectiveness of these receptors derives from the versatility of the ureido moiety, which plays a dual role as hydrogen bonding acceptor (Cad·2HCl) and donor (ϵ-Ahx), synergically assisting the calix[5]arene cavity in the recognition and binding of the ϵ-alkylammonium moiety of the targeted substrates.

Future structural refinement of the calixarene hosts described above will be directed toward the development of sensors (e.g., optical) and chromatographic media.

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Supporting Information Available: Experimental procedures and characterization data for compounds **1**, **3**, and **4**. ^{1}H NMR spectra of the complexation between GABA, GABA·HCl, and **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) Determined by ^{1}H NMR at −20 ± 1 °C in $C_2D_2Cl_4$/CD_3OD (2:1), using equimolar (10^{-3} M) host−guest mixtures.