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A New Chiral *Rigid Cone* Water Soluble Peptidocalix[4]arene and Its Inclusion Complexes with α-Amino Acids and Aromatic Ammonium Cations

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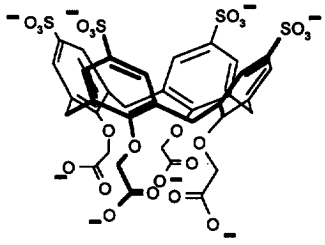
Abstract. A remarkable influence of the rigidity of the calix[4]arene platform in determining the recognition properties of *mobile* **2** and *rigid cone* **6** water soluble peptidocalix[4]arene receptors towards α-amino acids and aromatic quaternary ammonium cations has been found.

Keywords: calixarenes; molecular recognition; α-amino acids; water soluble receptors.

The design of water soluble synthetic receptors is a topic of current interest in supramolecular chemistry since they allow the study of host-guest interactions in a solvent where most biological processes take place.[1] Since 1984[2] we have reported the synthesis of several water soluble calixarenes and studied their inclusion properties towards alkylammonium ions,[3] amino acids,[4] and aliphatic alcohols.[5] Particularly studied was the receptor **1** which is highly soluble in water thanks to the presence of carboxylates at the lower rim and sulfonates at the upper rim of the calix.

More recently we reported the synthesis and structure of several *cone* calix[4]arenes functionalized with L-alanine units at the upper rim.[6] One of these derivatives (**2**) containing four amino acid moieties was water soluble at neutral pH.



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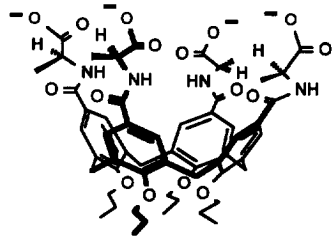

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This prompted us to investigate the inclusion properties of host **2** with several guest molecules and especially chiral amino acids, in order to compare the results with those previously obtained[3,4] with the achiral host **1**. We studied the complexation properties of host **2** in water at pH = 7.3 (phosphate buffer 0.067 M) by ^{1}H NMR titration experiments[7] and surprisingly we did not obtain evidence for the inclusion of L-phenylalanine (L-Phe) or its methyl ester hydrochloride (L-PheMe) into the calixarene cavity. At the same time the association constants obtained with **2** and trimethylanilinium (TMA, K = 220 M^{-1}) and benzyltrimethylammonium (BTMA, K = 80 M^{-1}) cations are lower than those obtained with the tetrasulphonate derivative **1** (K = 2×10^3 M^{-1}).[3]

Suspecting that this behaviour could be partly due to the fact that host **2** possesses a residual conformational mobility between two C_{2v} conformations as observed in other systems,[8] we looked for a more

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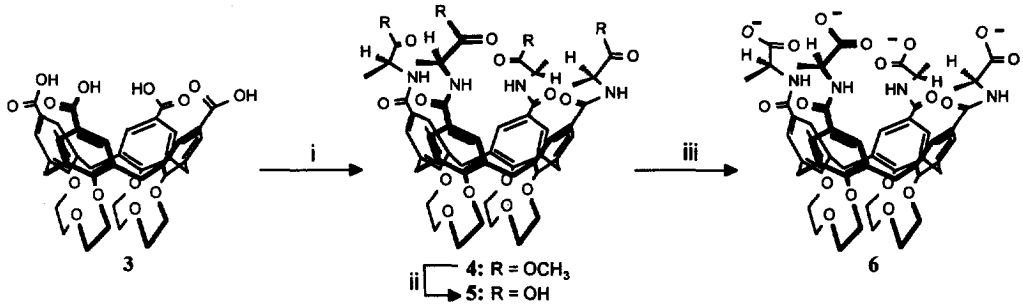
rigid platform to build up our receptors. It has previously been shown that the insertion of two short di(ethylene glycol) units in proximal (1,2) positions at the lower rim of calix[4]arene inhibits its residual conformational freedom, blocking it in a *rigid cone* structure.[8] Thus, by oxidation of the known tetraformyl derivative[8] we obtained the corresponding tetracarboxylic acid **3** which was easily converted into the water soluble *rigid cone* peptidocalix[4]arene **6**.[9]



Reagents and conditions. i) *Method A*: HBTU,[10] L-alanine methyl ester, NEt_3, r.t.; *Method B*: 1) $(COCl)_2$, reflux; 2) L-alanine methyl ester, NEt_3, r.t.. ii) 1) $LiOH{\cdot}H_2O$, THF/H_2O, r.t.; 2) HCl 1N. iii) $LiOH{\cdot}H_2O$.

The complexation properties of the new host **6** towards quaternary ammonium salts, amino acids and their methyl ester hydrochlorides were evaluated in D_2O at pD = 7.3 (phosphate buffer). The binding constants, reported in table 1, were obtained by ^1H NMR titration experiments by varying both the guest and the host concentration from 9.5×10^{-4} to 5.0×10^{-4} M and from 4.3×10^{-4} to 4.4×10^{-3} M respectively. The observed proton upfield shifts of the guests were treated by using a non-linear least squares fitting procedure.[11]

Table 1. Association constants (M^{-1}) for complexes with host **6** (25 °C, pD = 7.3). The values are affected by error ≤10%.

Guest	K	Guest	K	Guest	K	Guest	K	Guest	K
TMA	1440	L-Trp	110	L-TrpMe	620	L-Leu	<20	L-LeuMe	290
BTMA	1120			D-TrpMe	710	L-Val	no inclusion	L-ValMe	220
TEMA	150	L-Phe	70	L-PheMe	400	L-Ala	no inclusion	L-AlaMe	110
				D-PheMe	430	Gly	no inclusion	GlyMe	no inclusion
		L-Tyr	<20	L-TyrMe	180				
				L-PheglyMe	430				

TMA = trimethylanilinium chloride, BTMA = benzyltrimethylammonium chloride, TEMA = tetramethylammonium chloride.

The K values clearly indicate that the *rigid cone* peptidocalixarene **6** is much more efficient than the *mobile cone* analogue **2** in the complexation of all guests, pointing out the importance of the rigidity in the recognition process. Figure 1 shows the observed upfield shifts experienced by the TMA protons in the presence of variable concentrations of host **6**; here we have a clear indication that the aromatic group of TMA is selectively complexed by the calixarene apolar cavity. Interestingly this is also true for the BTMA cation which is unselectively complexed *via* both the aromatic nucleus and the trimethylammonium head group by the tetrasulphonate receptor **1**. Evidently, once the aromatic moiety of BTMA enters the cavity, the positively charged ammonium ion is closer to the carboxylate groups of host **6** than to the sulfonate groups of host **1**.

Also the α-amino acids are complexed by **6** *via* their apolar moiety. All data show that they are better complexed as methyl esters rather than as zwitterions, since a better complementarity with the tetra-anionic host **6** is achieved with substrates terminating in a positive charge only. The same conclusion is reached by examining the pH dependence of the association constant between **6** and L-PheMe. It decreases regularly from pH = 6.0 ($K = 710\ M^{-1}$) to pH = 7.3 ($K = 400\ M^{-1}$) and pH = 8.0 ($K = 220\ M^{-1}$), following the corresponding decrease in the percentage of protonated species in the guest.[12] Aromatic amino acids are more strongly complexed than the aliphatic ones and the highest association constant is observed for tryptophan. Although this is the most hydrophobic amino acid tested, we must not rule out a more specific π-π interaction between the indole nucleus of Trp and the calix[4]arene apolar cavity. This interaction might be stronger for the indole of Trp than for the aromatic (π-π) or aliphatic (CH-π) groups of other amino acids. No inclusion for the very hydrophilic glycine (Gly) and its methyl ester and a very modest chiral discrimination between L and D amino acids are observed.

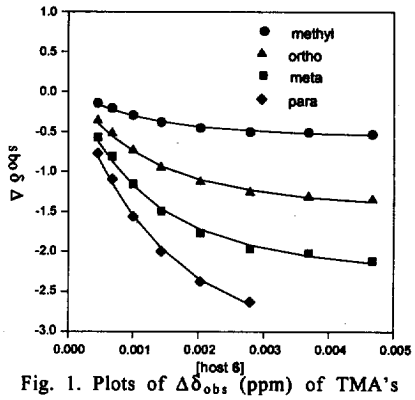


Fig. 1. Plots of $\Delta\delta_{obs}$ (ppm) of TMA's protons versus [**6**] (25 °C, pD = 7.3, phosphate buffer).

In conclusion, this work shows a remarkable influence of subtle conformational changes of the *cone* calix[4]arene platform, which can be tuned by the nature of functionalization at the lower rim, on the recognition properties of peptidocalix[4]arenes towards ammonium cations and amino acids. In these cases the complexation occurs exclusively through the interaction of the calixarene cavity with the apolar groups of the guests and the rigidity of the platform increases the binding. On the other hand, we recently found that where the recognition process involves more directional hydrogen bonding, a better binding with more flexible hosts is observed.[13]

Acknowledgements

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9. **5,11,17,23-Tetracarboxy-calix[4]arene-25,26:27,28-biscrown3 (3).** To a solution of 5,11,17,23-tetraformyl-calix[4]arene-25,26:27,28-biscrown3[7] (1.0 g, 1.47 mmol) in $CHCl_3$/acetone (20 ml, 1/1 v/v) cooled with ice bath are added sulfamic acid (1.72 g, 17.73 mmol) and sodium chlorite (1.34 g, 14.7 mmol) dissolved in water (2 ml). The reaction proceeds overnight then is quenched by removal of the organic solvents. The solid residue is crystallized with CH_3OH to obtain **3** as a white solid (760 mg, 70%). mp > 360 °C (from CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (s, 8 H; Ar), 5.00 (d, 2 H, J = 12.0 Hz; H_{ax} di ArCH_2Ar), 4.45 (d, 2 H, J = 12.3 Hz; H_{ax} di ArCH_2Ar), 4.41–4.20, 3.75–3.65 (2 m, 16 H; OCH_2CH_2O), 3.51 (d, 2 H, J = 12.3 Hz; H_{eq} di ArCH_2Ar), 3.43 (d, 2 H, J = 12.0 Hz; H_{eq} di ArCH_2Ar); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7 (C=O), 159.2 (Ar ipso), 135.5 (Ar orto), 130.5, 129.7 (Ar meta), 126.2 (Ar para), 76.6 (ArOCH_2CH_2O), 73.8 (ArOCH_2CH_2O), 29.5, 28.9 (ArCH_2Ar); IR (KBr) ν_{max} 3400 cm^{-1} (OH), 1694 (C=O); CI-MS *m/z* 740 [18%, M^+], 723 [100%, $(M - OH)^+$]. **5,11,17,23-Tetrakis(carbonyl-*N*-(L)-alanine methyl ester)-calix[4]arene-25,26:27,28-biscrown3 (4).** *Method A.* To a suspension of 5,11,17,23-tetracarboxy-calix[4]arene-25,26:27,28-biscrown3 (0.5 g, 0.68 mmol) and *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU)[10] (1.3 g, 3.38 mmol) in dry CH_2Cl_2 (15 ml) L-alanine methyl ester hydrochloride (755 mg, 5.40 mmol) and NEt_3 (0.75 ml, 5.40 mmol) are added. The mixture is stirred at r. t. 4 h, then HCl 1.0 N (10 ml) is added and the organic layer is separated, washed with H_2O (10 ml), aqueous solution of $NaHCO_3$ (5%, 10 ml), H_2O (10 ml) and finally concentrated. *Method B.* To a suspension of 5,11,17,23-tetracarboxy-calix[4]arene-25,26:27,28-biscrown3 (0.5 g, 0.68 mmol) in dry CH_2Cl_2 (15 ml) oxalyl chloride (4.1 ml, 47.3 mmol) is added and the mixture is refluxed 5 h under nitrogen. The reaction is evaporated to dryness under vacuum then the solid is dissolved in dry CH_2Cl_2 (15 ml) and L-alanine methyl ester hydrochloride (755 mg, 5.40 mmol) and NEt_3 (1.6 ml, 10.81 mmol) are added. After 4 h at r.t. HCl 1 N (10 ml) is added and the organic layer is separated, washed with H_2O (10 ml, 2×), and concentrated. With both methods the pure product is obtained by flash chromatography on silica gel (CH_2Cl_2/acetone 5:1) (yield: 60 %). mp 175 °C (from CH_2Cl_2/acetone); $[\alpha]^{25}_D$ +31.7° (*c* = 1.01, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, 4 H, J = 1.9 Hz; Ar), 7.51 (d, 4 H, J = 1.9 Hz; Ar), 6.65 (d, 4 H, J = 7.2 Hz; NH), 5.08 (d, 2 H, J = 12.3 Hz; H_{ax} di ArCH_2Ar), 4.72–4.60 (m, 4 H; CH-alanine), 4.50 (d, 2 H, J = 12.0 Hz; H_{ax} di ArCH_2Ar), 4.40–4.17 e 3.92–3.77 (2 m, 16 H; OCH_2CH_2O), 3.72 (s, 12 H; OCH_3), 3.39 (d, 2 H, J = 12.0 Hz; H_{eq} di ArCH_2Ar), 3.34 (d, 2 H, J = 12.3 Hz; H_{eq} di ArCH_2Ar), 1.46 (d, 12 H, J = 7.2 Hz; CH_3-alanine); ^{13}C NMR (300 MHz, $CDCl_3$) δ 173.7 (C=O ester), 166.9 (C=O amide), 158.2 (Ar ipso), 135.5, 135.4 (Ar orto), 130.0 (Ar para), 128.6, 128.3, 127.7, 127.4 (Ar meta), 76.6 (ArOCH_2CH_2O), 74.6 (ArOCH_2CH_2O), 52.3 (OCH_3), 48.5 (CH-alanine), 30.6, 29.7 (ArCH_2Ar), 18.1 (CH_3-alanine); IR (KBr) ν_{max} 1738 cm^{-1} (C=O ester), 1650 (C=O amide); CI-MS *m/z* 1081 [100%, M^+]. **5,11,17,23-Tetrakis(carbonyl-*N*-(L)-alanine)-calix[4]arene-25,26:27,28-biscrown3 (5).** To a solution of **4** (0.5 g, 0.46 mmol) in THF (10 ml), cooled at 0°C, a solution of LiOH monohydrate (160 mg, 3.7 mmol) in H_2O (2 ml) is added. The reaction proceeds at r. t. for 6 h, then it is quenched by removal of organic solvent under vacuum and by adding of HCl 4.0 N (10 ml). Compound **5** is obtained as a white solid in quantitative yield by filtration on a Buchner and without further purification. mp 248 °C (from H_2O); $[\alpha]^{25}_D$ +326.8 (*c* = 0.505, EtOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (bs, 4 H; Ar), 7.56 (bs, 4 H; Ar), 5.24 (d, 2 H, J = 12.5 Hz; H_{ax} di ArCH_2Ar), 4.63 (d, 2 H, J = 12.5 Hz; H_{ax} di ArCH_2Ar), 4.50–4.21 (m, 16 H; CH-alanine and OCH_2CH_2O), 3.85 (m, 4 H, OCH_2CH_2O), 3.40 (d, 2 H, J = 12.5 Hz; H_{eq} di ArCH_2Ar), 3.32 (d, 2 H, J = 12.5 Hz; H_{eq} di ArCH_2Ar), 1.43 (d, 12 H, J = 7.4 Hz; CH_3-alanine); ^{13}C NMR (75 MHz, CD_3OD) δ 176.4 (C=O acid), 170.1 (s, C=O amide), 159.9 (s, Ar ipso), 136.7, 136.5 (s, Ar orto), 130.4, 130.0, 129.5, 129.2 (d, Ar meta), 128.8 (s, Ar para), 77.6 (t, ArOCH_2CH_2O), 75.1 (t, ArOCH_2CH_2O), 50.4 (d, CH-alanine), 31.8, 30.9 (t, ArCH_2Ar), 17.4 (q, CH_3-alanine); IR (KBr) ν_{max} 3408 cm^{-1} (OH), 1732 (C=O acid), 1639 (C=O amide); CI-MS *m/z* 1021 [100%, $(M + H)^+$]. **5,11,17,23-Tetrakis(carbonyl-*N*-(L)-alanine)-calix[4]arene-25,26:27,28-biscrown3 tetralithium salt (6).** A solution of compound **5** (0.5 g, 0.49 mmol) in CH_3OH (5 ml) is treated with 4 equiv. of LiOH•H_2O (82 mg, 1.96 mmol), then the solvent is removed under vacuum to dryness to obtain **6**. ^{1}H NMR (300 MHz, D_2O) δ 7.69 (d, 4 H, J = 2.7 Hz, Ar), 7.66 (d, 4 H, J = 2.7 Hz, Ar), 5.19 (d, 2 H, J = 12.4 Hz, H_{ax} di ArCH_2Ar), 4.73 (d, 2 H, J = 12.9 Hz, H_{ax} di ArCH_2Ar), 4.62 (d, 4 H, J = 10.9 Hz, OCH_2), 4.50–4.35 (m, 8 H, OCH_2), 4.35 and 4.34 (q, 4 H, J = 7.3 Hz, CH-alanine), 4.08–3.92 (m, 4 H, OCH_2), 3.61 (d, 2 H, J = 12.9 Hz, H_{eq} di ArCH_2Ar), 3.57 (d, 2 H, J = 12.4 Hz, H_{eq} di ArCH_2Ar), 1.46 (d, 12 H, J = 7.3 Hz, CH_3-alanine).
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