Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 7357–7364

TETRAHEDRON

Synthesis and complexation properties towards amino acids of mono-substituted *p*-sulphonato-calix-[*n*]-arenes

Eric Da Silva and Anthony W. Coleman*

Institut de Biologie et Chimie des Proteines, CNRS UMR5086, 7 Passage du Vercors, Lyon F69367, France

Received 18 April 2003; revised 22 July 2003; accepted 22 July 2003

Abstract—A series of mono-hydroxy functionalised calix-[*n*]-arenes, and *p*-sulphonato-calix-[*n*]-arenes where *n*=4, 6 and 8, have been synthesised, with the pendant functions being ethoxycarbonyl methoxy group, 2-carboxy methoxy group, 2-amido methoxy group or 2-amino ethoxy group. With calix-[4]-arene and calix-[6]-arenes the compounds are obtained in good yield by treatment of the relevant *p*-H-calix-[*n*]-arene with a suitable metal carbonate, as a weak base, in the presence of one equivalent of the corresponding alkyl bromide. However in the case of calix-[8]-arene, the extremely low solubility of *p*-H-calix-[8]-arene prevented its use and *p*-*t*Bu-calix-[8]-arene was used in the monosubstitution reactions. The corresponding sulphonate derivatives were prepared in the case of the 2-carboxy methoxy group, 2-amido methoxy group and 2-amino ethoxy group systems, either by sulphonation of the *para*-H derivatives or by *ipso*-sulphonation of the *tert*-butyl derivatives. The complexation properties of the water-soluble *p*-sulphonato-derivatives with regard to 11 amino acids have been studied by ^{1}H NMR titration experiments. The obtained association constants show a 1:1 stoichiometry. The presence of a pendant group at the lower rim of calix strongly modifies the observed association constant as compared to the parent *p*-sulphonato-calix-[*n*]-arenes. While generally, the cationic amino acids lysine and arginine bind strongly to all the derivatives, the binding of other amino acids is dependant on the nature of the pendant functions, in particular pendant arm-lateral chain function leads to strong binding with aspartic acid, serine and tryptophan.

1. Introduction

There is increasing interest in the biological[1] and material science[2] applications of calix-[*n*]-arenes. In view of this, it is of useful to develop simple routes to derivatives carrying functionalities suitable for grafting onto macromolecules,[3] polymers[4] or into organo-mineral matrices.[5] In a recent communication,[6] we applied the term 'greffons' from the French word 'greffage' for grafting, to such calix-[*n*]-arene derivatives. In order to retain, both the desirable conformational properties of the calix-[*n*]-arenes to act as host molecules, and the mobility for the whole grafted molecule about or within the macromolecular framework, mono-functionalisation, preferably at the hydroxyl face seems a target suitable. While a range of suitable calix-[4]-arene greffons are available,[7–9] the mono functionalisation of calix-[6]-arene[10,11] and calix-[8]-arene[12] has received less attention. A wide range alkyl functions have been coupled at the lower rim of *p*-*t*Bu-calix-[4]-arene in presence of different bases and solvents[13] or by deprotecting a dialkylated calix-[4]-arene derivative in the presence of an equivalent amount of iodotrimethylsilane in chloroform.[14]

The mono-substituted calix-arenes have been used as sensors by coupling onto polymers such as poly-styrene.[15] With the regard to calix-[6]-arene selective functionalisation of the calix-[6]-arene has only recently come to the fore. A recent report by Santoyo-Gonzalez et al. has shown that regioselective mono-alkylation by halo-alkyl esters and halo-alkyl-nitriles can be accomplished using $(Bu_3Sn)_2O$.[16] No mono-substituted *p*-H-calix-[8]-arene derivatives have been reported in the literature, probably due to its extremely low solubility in most organic solvents. Mono-substitution has been reported for *p*-*t*Bu-calix-[8]-arene using activated [*n*]ethylene glycol chains.[17,18] Neri et al. demonstrated the alkylation of *p*-*t*Bu-calix-[8]-arene using *p*-methylbenzyl bromide as an electrophile and CsF as a weak base in THF/DMF.[12]

In terms of actual and potential biological and medical applications, the *p*-sulphonato-calix-[*n*]-arenes have been the focus of considerable interest. Atwood et al. have shown the activity of theses compounds as blockers of calcium dependent chloride ion channels.[19] Their activity as anti-thrombotic agents was demonstrated by Hwang et al.,[20] and their application as anti-viral agents has been patented.[21,22] We have previously shown that *p*-sulphonato-calix-[6]-arene is capable of inhibiting the enzyme activity of Lysyl Oxidase.[23] Their interactions with various amino acids have been studied by ^{1}H NMR,[24] microcalorimetry,[25] and

Keywords: p-sulphonato-calix-arenes derivatives; NMR; amino acids complexation; mono-substitution.

* Corresponding author. Tel.: +33-472-722640; fax: +33-472-722690; e-mail: aw.coleman@ibcp.fr

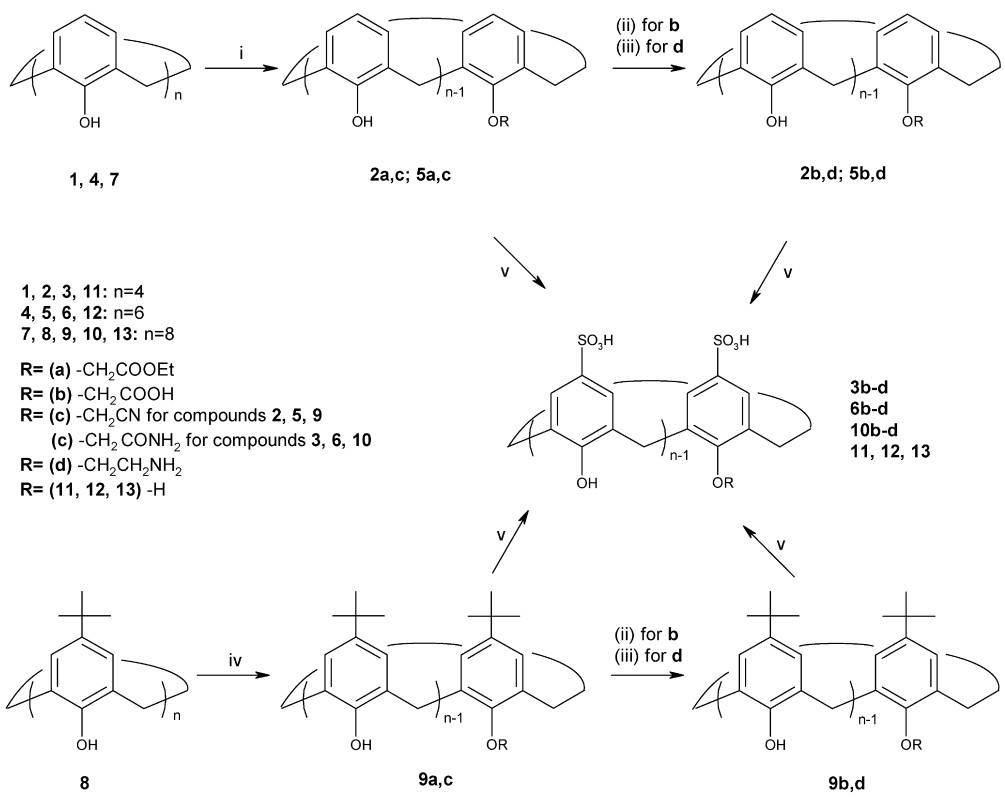
0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0040-4020(03)01137-2

RP-HPLC,[26] and the solid state structures of *p*-sulphonato-calix-[4]-arene with lysine,[27] racemic histidine, racemic alanine, racemic phenylalanine and tyrosine[28] have been determined. Recently we have extended the studies of the interactions to include di- and tri-peptides based on lysine and arginine.[29] The first example of protein/*p*-sulphonato-calix-[*n*]-arene complexes were observed recently by electrospray-mass spectrometry (ES/MS).[30] Complexation between a diamide tetracid calix-[4]-arene and bovine serum albumin has been demonstrated in presence of Gd(III).[31,32] We have recently reported the use of ES/MS in the study of steroid complexation by *p*-sulphonato-calix-[*n*]-arenes.[33] While the mono-functionalisation of the calix-[*n*]-arenes presents considerable interest for subsequent attachment to larger molecular or polymeric skeletons and surfaces, the host–guest properties may also be modified. A recent RP HPLC study on the interaction between a series of amino acids and two mono-functionalised calix-[6]-arene derivatives, *p*-H-37-(2-carboxy-methyloxy)-calix-[6]-arene and *p*-sulphonato-37-(2-carboxy-methyloxy)-calix-[6]-arene, showed considerable differences in the complexation ability between *p*-sulphonato-37-(2-carboxy-methyloxy)-calix-[6]-arene and the parent *para*-sulphonato-calix-[6]-arene.[34] In particular, the observed association constants with Lys and Arg were much higher when a carboxylic acid function was present at the lower rim (2310 M^{-1} for Lys and 3601 M^{-1} for Arg). Of more interest was the very large increase in the association with regard to aspartic acid.

In this paper, we describe a simple and reasonably efficient route to water soluble calix-[4]-arene, calix-[6]-arene and calix-[8]-arene greffons bearing either 2-carboxy methoxy group, 2-cyano methoxy group, 2-amido methoxy group and 2-amino ethoxy group for coupling to macromolecular systems. The synthetic route involves mono-functionalisation of calix-[4]-arene and calix-[6]-arene by reaction with 1 equiv. of bromo-ethyl acetate or bromo-acetonitrile in acetonitrile in the presence of Li_2CO_3, Na_2CO_3, K_2CO_3 or Cs_2CO_3 which, as a weak base, minimises multiple deprotonation of the phenolic hydroxyl functions. In the case of *p*-*t*Bu-calix-[8]-arene, the use of the strong base, CsOH allowed mono-substitution in useful yields. Sulphonation at the upper rim of calix is via treatment with concentrated sulfuric acid for *p*-H-calix-[4]-arene and *p*-H-calix-[6]-arene derivatives or by *ipso*-sulphonation of the *p*-*t*Bu-calix-[8]-arene derivatives with 96% sulphuric acid. We report, also, on the host–guest interaction of *p*-sulphonato-calix-[*n*]-arenes derivatives with 11 amino acids using ^{1}H NMR at pH 8, in the presence of NaOH.

2. Results and discussion

The synthetic route to the *p*-sulphonato-calix-[4]-arene, *p*-sulphonato-calix-[6]-arene and *p*-sulphonato-calix-[8]-arene derivatives is given in Scheme 1. Mono-substitution of the phenolic function was achieved by treatment of **1** and **4** with the corresponding bromo-alkyl derivative, at a molar ratio 1:1, in acetonitrile under reflux for 24 h in the presence of the 1 equiv. of the weak bases, Li_2CO_3, Na_2CO_3, K_2CO_3 or Cs_2CO_3 and **8** with the corresponding bromo-alkyl derivative, at a molar ratio 1:1, in THF under reflux during 1 h in presence of cesium hydroxide. Purification by silica gel chromatography using as eluant chloroform/hexane 4/1 allowed isolation of the mono-functionalised derivatives. The yields are summarised in Table 1.



Scheme 1. *Reagents and conditions*: (i) 1 equiv. alkyl bromide, base, acetonitrile, reflux, 24 h; (ii) KOH 10%, EtOH/H_2O 70:30, 90%; (iii) THF, BH_3/THF complex, reflux, 4 h; (iv) 1 equiv. alkyl bromide, CsOH, THF, reflux, 1 h; (v) H_2SO_4 96%, 50°C, 24 h.

Table 1. Yields obtained for mono substitution of calix in presence of different bases; the yield obtained for the compounds **5a** and **5c** have been previously reported[6]

Compound	Base (%)				
	Li_2CO_3	Na_2CO_3	K_2CO_3	Cs_2CO_3	CsOH
2a	5	28	20	15	–
5a[6]	2	19	46	15	–
9a	<1	<1	<1	<1	6
2c	15	21	23	18	–
5c[6]	<5	23	44	20	–
9c	<1	<1	<1	<1	5

For the calix-[4]-arene derivative **2a** the highest yield is obtained with Na_2CO_3 as base, although at 28% the yield is relatively low. With **2c**, unexpectedly the yield with Na_2CO_3 (21%) is slightly lower than that obtained with K_2CO_3 (23%). In both cases, the yields are significantly lower with bases such Li_2CO_3 and Cs_2CO_3 and no-monosubstitution is obtained using $BaCO_3$ as the base. Given that calix-[4]-arene is generally more selective for Na^+ than K^+[35] the higher yield for **2a** with regard to Na^+ is expected, however the switch to K^+ rather than Na^+ for **2c** suggests that direct cation complexation is not governing the reaction. Recent work on anion co-complexation by ourselves[36] suggests that a combination of cation and anion effects is of importance in salt interactions with calix-[4]-arene derivatives.

In the case of the calix-[6]-arene derivatives **5a** and **5c**, the situation is much clearer, the yields of 46 and 44% obtained for use of K_2CO_3 as the weak base are at least two times greater than those observed with any other base. Apparently in the case of calix-[6]-arene, the mechanism of mono-alkylation is only dependant on the cation.

In the case of *p*-H-calix-[8]-arene, treatment with any of the weak bases led to effectively zero isolated yields of the desired mono-substituted derivative. Tests with various strong bases NaOH, KOH and CsOH showed, again, no substitution. Evidently the almost total insolubility of *p*-H-calix-[8]-arene under the reactions conditions using acetone or THF as solvents prevents the use of this compound. However, the use of direct *ipso*-sulphonation on *p*-*t*Bu-calix-[8]-arene presents an alternative route to the desired compounds.[37] The use of weak bases, led only to the starting material and di or higher substituted derivatives. The use of strong bases such as NaOH, KOH and CsOH showed that only CsOH led to mono-substitution and only in THF under kinetic conditions. In the case of **8**, the yield obtained (6 and 5% for **9a** and **9b**, respectively) with CsOH is low but usable. The formation of the mono-substituted-calix-[8]-arene derivatives is rapid (1–2 h) as compared to the reactions using weak bases to yield the calix-[4]-arene and calix[6]-arene derivatives. For reaction times above 3 h, the formation of di-functionalised calix-[8]-arene derivatives dominates. Little information is available concerning the exact mode of calix/cation interaction and here the kinetics of the reaction appear dominant in the obtained yields (Table 2).[38]

The mono-substitution of both calix-[4]-arene, calix-[6]-arene and calix-[8]-arene reduces the cavity symmetry from four-, six- and eight-fold respectively to two-fold, in all cases. This is reflected in the ^{1}H NMR spectra of the compounds. For **2a** and **2c** the AB signals of methylene groups are observed at 4.45, 4.38; 3.49, 3.35 and 4.32, 4.24; 3.58, 3.48 ppm, respectively. In **2a** and **2c** the two sets of axial and equatorial protons are separated by 1.0 ppm, this value is greater than that of 0.8 ppm accepted as defining the lower limit of a cone eq–ax separation in conformation[39] and both **2a** and **2c** exist in the cone conformation in solution. The ^{1}H NMR spectra of **5b**, **5c** and **5d** is characterised by the presence of three singlets at 3.92, 3.82, 3.75; 4.14, 3.93, 3.74 and 4.10, 3.91, 3.72 ppm corresponding to the three types of methylene protons of calixarene. For **5a**, a broad signal is observed at 3.8 ppm. In the case of **9c**, four singlets are observed at 4.05, 3.99, 3.91 and 3.86 ppm and for **9a**, **9b** and **9d**, broads signals are observed respectively at 3.87, 3.89 and 3.89 ppm.

Table 2. ^{1}H NMR chemical shift displacement of significant protons in the calixarene derivatives (^{1}H); the values of compounds **5a**–**5d** have been previously reported.[6] and the value marked (*) represent a broad signal

Compound	Ar–CH_2–Ar				O–CH_2
2a	4.45	4.38	3.49	3.35	3.87
2b	4.23	4.16	3.42	3.39	4.96
2c	4.32	4.24	3.58	3.48	5.04
2d	4.21	4.12	3.59	3.45	4.52
3b	4.21	4.13	3.61	3.58	4.52
3c	3.99	3.96	3.71	3.69	4.06
3d	3.97	3.92	3.57	3.54	3.65
5a[6]		3.80 (Broad signal)*			4.79
5b[6]	3.92	3.82	3.75	–	4.83
5c[6]	4.14	3.93	3.74	–	4.95
5d[6]	4.1	3.91	3.72	–	4.83
6b	3.85	3.81	3.78	–	4.00
6c	3.77	3.76	3.71	–	3.46
6d	3.80	3.77	3.70	–	3.07
9a		3.87 (Broad signal)*			4.71
9b		3.89 (Broad signal)*			4.46
9c	4.05	3.99	3.91	3.86	4.79
9d		3.89 (Broad signal)*			4.46
10b	3.91	3.86	3.85	3.84	4.01
10c	3.87	3.86	3.78	3.71	4.49
10d		3.77 (Broad signal)*			4.72

Sulphonation of **2b**–**2d**, **5b**–**5d** and *ipso*-sulphonation **9b**–**9d** was achieved by treatment with concentrated sulphuric acid (96%) at 50°C during 24 h. The products were obtained as crystalline solids by precipitation with ether. The nitrile derivatives **2c**, **5c** and **9c** are all hydrolysed to the corresponding amides under the reaction conditions used here. All the ES/MS spectra show a gain of 18 m.u. the CN stretching band at 2160 cm^{-1} is absent and replaced by the CO band at 1660 cm^{-1}. RP HPLC analysis of the products shows them to be pure (>90%). Further hydrolysis to the carboxylic acid does not occur significantly under the current reaction conditions, although at higher temperatures with longer reaction times significant amounts of the carboxylic acid are produced (>30%).

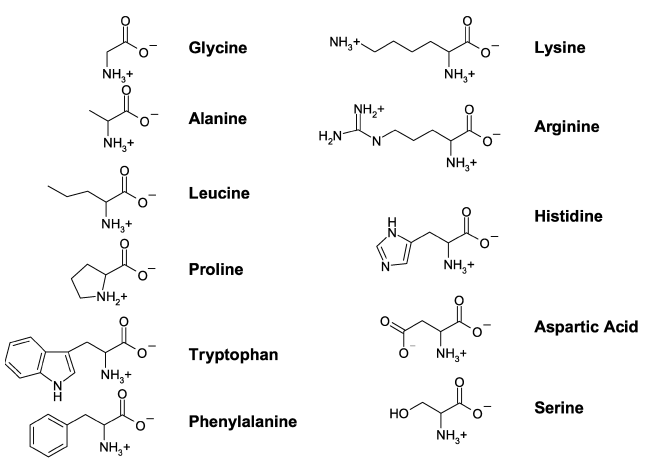
The ^{1}H NMR of **3b**, **3c** and **3d** shows the same pattern as for **2b**, **2c** and **2d** confirming that the *para*-sulphonato-calix-[4]-arene derivatives are present in the cone conformation. In the case of the calix-[4]-arene derivatives, the ^{13}C NMR, signals for the methylene groups investigated in $CDCl_3$ and

D_2O appear between 30 and 32 ppm, as expected for a *syn* arrangment of the neighbouring aromatic rings are in agreement with the proposed cone conformation.

The ^1H NMR spectra of **6b**, **6c** and **6d** are characterized by the presence of three fairly sharp singlets at 3.85, 3.81, 3.78; 3.77, 3.76, 3.71 and 3.80, 3.77, 3.7 ppm, respectively, corresponding to the three types of methylene group and **10b**, **10c** and **10d** are characterized by the presence of four singlets at 3.90, 3.86, 3.85, 3.84; 3.87, 3.85, 3.77, 3.71 and 3.77 ppm as a broad signal. Thus, it may be concluded that both *para*-H and *para*-sulphonato compounds are present in the cone conformation.

The reported solid structure of **3b** shows a pleated cone structure of the macrocycle.[6] The ^1H and ^{13}C NMR values above are in agreement with the retention of such a structure for all the calix-[6]-arene derivatives reported here. Little is known concerning the solid-state structure of substituted calix-[8]-arene derivatives, however, the presence of four signals for the methylene protons in **9c**, **10b** and **10c** can be considered to be consistent with the same folded structure as the calix-[6]-arene derivatives.

^1H NMR titration experiments were carried out with a series of 11 amino acids; glycine (no lateral chain), alanine and leucine (short lateral chain), phenylalanine and tryptophan (aromatic lateral chain), proline (cyclic structure), aspartic acid (carboxylic acid lateral chain), serine (alcohol lateral chain) lysine, arginine and histidine (cationic lateral chain). The structures are given in Scheme 2. The titration experiment were carried out in 95% H_2O/5% D_2O at pH 8 using NaOH to adjust the pH. The use of NaOH and not phosphate buffer to set the pD was chosen to allow comparison with RP HPLC studies.[30] For illustration in Figure 1, the variation of the chemical shifts of the protons is given as a function of molar ratio between *p*-sulphonato-calix-[4]-arene derivatives **3b**–**3d** and lysine. Throughout the titration experiments, no variations in the chemical shifts of the protons of the host calix-arene molecules were observed. With regard to all the determinated association constants, it should be noted that the K_{ass} values differ considerably from those reported by Arena et al.,[40] however in their case a PBS buffer was used, undoubtedly the difference in ionic strength strongly influences the associ-



Scheme 2. List of amino acids and ionisation states at pH **8**.

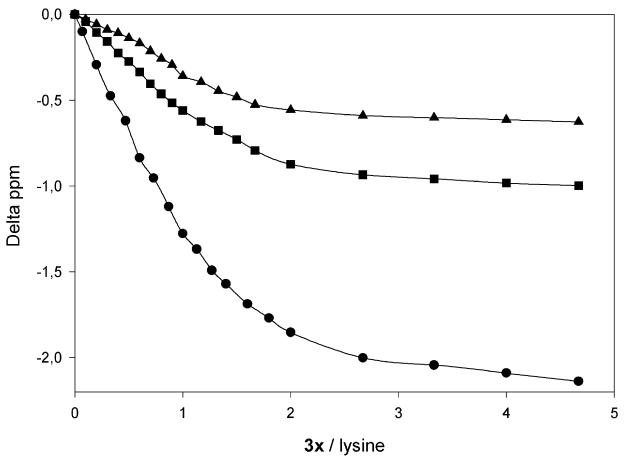


Figure 1. An example of plot of Δ ppm of γ-lysine proton vs. **3b**/lysine (●), **3c**/lysine (■) and **3d**/lysine (▲) in 95:5H_2O/D_2O in 25°C.

ation constant. For example with **11**, Arena reported a K_{ass} of 20 M^{-1} whereas here we find a K_{ass} value of 502 M^{-1} for His. However the use of PBS as a buffer is not totally neutral, as a K_{ass} value of 40 M^{-1} is found in a 0.15 M NaCl solution. We have also noted that many organic biological buffers complex to **11** thus interfering with the complexation. In view of the above we prefer to use NaOH to bring the pD to 8, interestingly under these conditions there exists a much better correlation with results obtained from RP HPLC experiments.[34]

The values of the observed K_{ass} values for the *para*-sulphonato-calix-[4]-arene derivatives, **3b**–**3d** and the parent compound **11** with regard to the various amino acids are given in Table 3. The correlation coefficients observed are consistent in all cases with a 1:1 stoichiometry, Table 3. With respect to glycine, the K_{ass} values vary between 30 (**3c**) and 52 M^{-1} (**3b**) showing that there is essentially zero complexation with any of the calix-[4]-arene derivatives. The observed values for alanine are slightly higher with a maximum of 102 M^{-1} (**3c**). For these two amino acids there is little difference between the observed K_{ass} values for the greffons and the parent compound **11**. However, the situation is quite different for the complexation with leucine, as expected with **11**, there is a relatively strong interaction K_{ass} 782 M^{-1}, arising from inclusion of the hydrophobic lateral chain in the calix

Table 3. Association constants (M^{-1}) for complexes of *p*-sulphonato-calix-[4]-arene and its derivatives with 11 amino acids (25°C)

AA	11	3b	3c	3d
Gly	32 (8)	52 (18)	30 (15)	43 (12)
Ala	78 (13)	83 (18)	102 (15)	99 (16)
Leu	782 (218)	488 (73)	274 (48)	308 (12)
Pro	1272 (25)	479 (49)	367 (41)	208 (35)
Phe	819 (67)	392 (43)	493 (39)	284 (51)
Trp	263 (30)	178 (13)	211 (42)	205 (30)
Asp	512 (24)	2852 (1137)	2250 (1400)	5620 (2618)
Ser	120 (14)	3555 (1859)	540 (232)	420 (20)
Lys	1356 (349)	887 (74)	578 (52)	202 (30)
Arg	1546 (721)	800 (60)	840 (137)	447 (115)
His	502 (135)	306 (62)	208 (13)	206 (32)

Only data for which the correlation coefficient are higher than 0.99 are included.

cavity. For all the mono-substituted systems the K_{ass} values are sharply diminished, between 270 and 490 M^{-1}, this may be explained if the coupled side chain inserts into and partially occupies the hydrophobic calix-[4]-arene cavity, both inter and intra-molecular self-inclusion cannot be ruled out.

For the amino acids, Phe and Trp having aromatic functions on the lateral chains, again the K_{ass} values are lower for **3b**, **3c** and **3d** than **11**. Comparing between the mono-substituted derivatives, **3c** for which a hydrophobic nitrile terminal function is present shows slightly stronger K_{ass} values, this is consistent with the pendant arm interacting within the cavity with the guest. However, the strongest argument for the intervention of the substituent interacting with the amino acid guest arises for Asp and Ser. Both amino acids have polar, hydrogen bonding functions on the lateral chain, and only for these systems are the K_{ass} values for **3b** (2850, 3560 M^{-1}), **3c** (2250, 540 M^{-1}) and **3d** (5620, 420 M^{-1}) higher than those observed with the parent system **11** (512, 110 M^{-1}). Both amino acids show particularly strong interactions with the carboxylic acid derivative **3b**, and Asp shows even stronger binding to the amino functionalized **3d**. These results correlate with those reported on the RP HPLC studies involving **6b** and Asp. The above implies that the pendant arm is capable by an intramolecular mechanism of interacting with the guest amino acid even in a calix-[4]-arene derivatives.

With regard to the calix-[6]-arene derivatives, the overall effects are similar, Gly, Ala, Pro and Leu bind weakly to **6b**, **6c** and **6d**. As expected from electrostatic interactions and previous RP HPLC studies, Lys, His and Arg bind strongly. Again the negatively charged amino acid Asp shows very strongly increased binding to the monosubstituted derivatives as compared to the parent compound **12**, 3201 M^{-1} (**6b**), 2520 M^{-1} (**6c**) and 5423 M^{-1} (**6d**) compared to 354 M^{-1} (**12**). Again the amino derivative **6d** shows the expected strongest bonding. It is interest to note that now Ser, binds only slightly more strongly to **6b** (168 M^{-1}), **6c** (235 M^{-1}) and **6d** (178 M^{-1}) than to **12** (112 M^{-1}), this would suggest that the stereochemistry of the inclusion is no longer optimised for this amino acid, and that pendant group-lateral chain hydroxyl hydrogen bonding does not occur. A particular effect is observed between Trp and **6d** where high selectivity in the K_{ass} is observed (2975 M^{-1}) as compared to **12** (1450 M^{-1}), **6b** (433 M^{-1}) and **6c** (505 M^{-1}), Table 4.

Table 4. Association constants (M^{-1}) for complexes of *p*-sulphonato-calix-[6]-arene and its derivatives with 11 amino acids (25°C)

AA	**12**	**6b**	**6c**	**6d**
Gly	17 (10)	38 (15)	46 (25)	48 (15)
Ala	65 (16)	69 (12)	92 (32)	126 (29)
Leu	346 (58)	109 (16)	140 (32)	195 (20)
Pro	170 (46)	66 (11)	125 (20)	357 (54)
Phe	382 (35)	94 (13)	368 (138)	204 (31)
Trp	1448 (293)	433 (86)	505 (31)	2975 (464)
Asp	354 (38)	3201 (1306)	2516 (1026)	5423 (2291)
Ser	112 (38)	168 (14)	235 (21)	178 (18)
Lys	2200 (276)	1714 (176)	1254 (112)	980 (160)
Arg	3090 (1024)	1475 (108)	2018 (153)	673 (31)
His	1930 (149)	1041 (82)	616 (38)	574 (41)

Only data for which the correlation coefficient are higher than 0.99 are included.

Table 5. Association constants (M^{-1}) for complexes of *p*-sulphonato-calix-[8]-arene and its derivatives with 11 amino acids (25°C)

AA	**13**	**9b**	**9c**	**9d**
Gly	20 (7)	45 (12)	32 (17)	28 (12)
Ala	65 (13)	75 (8)	64 (32)	56 (11)
Leu	1111 (212)	109 (41)	89 (36)	68 (21)
Pro	340 (39)	50 (12)	30 (10)	68 (15)
Phe	2992 (568)	258 (102)	179 (17)	208 (21)
Trp	3465 (1156)	1984 (564)	200 (24)	423 (45)
Asp	614 (68)	ND[a]	ND[a]	ND[a]
Ser	320 (32)	ND[a]	ND[a]	ND[a]
Lys	4288 (1543)	2097 (730)	669 (69)	967 (102)
Arg	10083 (6705)	3879 (1914)	425 (200)	812 (134)
His	2830 (385)	1625 (846)	660 (66)	741 (89)

Only data for which the correlation coefficients are higher than 0.99 are included.

[a] ND: data not calculated. The signals arising from the CH_2 group of the amino acids (Asp, Ser) are masked by the signals arising from the CH_2 groups of the host molecules.

For the calix-[8]-arene derivatives, unfortunately the overlap between host and guest signals prevent determination of the K_{ass} values with Asp and Ser. However, here electrostatic interactions would seem to be totally dominant, with Lys, His and Arg binding far more strongly than the other amino acids, this effect is coupled to a carboxylic acid function-amino lateral chain group hydrogen bonding interaction that reinforces the binding for **9b**, including now the interaction with Trp, Table 5.

3. Conclusion

The synthesis of a range of water soluble calix-[*n*]-arene monosubstituted derivatives has been achieved in usable to good yields by the use of suitable weak or strong bases. ^{1}H NMR studies suggest that the monosubstitution leads to a cone conformation for calix-[4]-arene derivatives and a fan or 'taco' conformation for the calix-[6]-arene and calix-[8]-arene derivatives. A ^{1}H NMR study of the interactions of these derivatives with a series of amino acids shows that the pendant functions on the calix-[*n*]-arene derivatives may selectively interact with certain amino acids.

4. Experimental

4.1. Titration experiments

The ^{1}H NMR spectra of solutions containing the *p*-sulphonato-calix-[*n*]-arene derivatives and amino acids were carried out in 95% H_2O/5% D_2O using aqueous sodium hydroxide to adjust the pH to 8. In the titration experiments, the final concentration of the amino acids was retained constant 2.7 mM. To 300 μL of the amino acid solution at 10 mM was added increasing volumes of a solution of the relevant calix-arene derivative at 18 mM, the final volume was adjusted to 1 mL and the pH of the solution adjusted to pD 8. An NMR spectrum of each tube was recorded. Association constants of the different amino acids complexes were determined by non-linear square regression for

a stoichiometry 1:1.[41–43] A minimum of 18 points was required to have a best fit for calculated the association constant value.

4.2. RP HPLC analysis

All the *p*-sulphonato-calix-[*n*]-arene derivatives were checked for purity by RP HPLC. The LC system consisted of a high pressure pump HP series 1100. The column was packed with Vydac C18 10×250 mm. Both solvents are used as the mobile phase: solvent A (water/TFA 0.1%) and solvent B (acetonitrile/water/TFA 70/30/0.09%). The compounds were purified by increasing the gradient of solvent B from 0 to 40% during 90 min. All chromatograms were obtained at room temperature. The flow rate was 1.5 mL/min, and the UV detector operated at a wavelength of 254 nm.

4.3. Synthesis

All chemicals were purchased from Acros Organics and used without further purification. ^{1}H NMR and ^{13}C NMR was carried out in $CDCl_3$ (TMS for internal standard) or D_2O on a Varian Spectrometer at 500 MHz (^{1}H) and at 125 MHz (^{13}C). Electrospray mass spectrometry (ES-MS) was carried out on a Perkin Elmer Aciex API 165, the solvent was $CHCl_3$/MeOH 2:3 in presence of HCOOH (0.1%) or MeOH/H_2O 1:1 in presence of HCOOH (1%). Column chromatography was performed on silica gel. Melting points were determined using a Stuart Scientific melting point SMP1. Infrared spectra were recorded on a Perkin Elmer FTIR instrument.

4.3.1. 25-Mono-(ethoxycarbonylmethoxy)-calix-[4]-arene (2a). A suspension of **1** (5 g, 11.8 mmol), and an appropriate base (1 equiv.) in acetonitrile (300 mL) was stirred at reflux for 0.5 h. Bromo-ethylacetate (1.31 mL, 1 equiv.) was added and the reaction mixture was stirred under reflux during 24 h. After cooling, the solvent was removed under reduced pressure. The remaining solid was taken up in CH_2Cl_2 (200 mL) and washed with 1 M HCl (2×200 mL) and water (200 mL). The organic layer was dried over $MgSO_4$ and evaporated to give a white powder. The product was purified by column chromatography. R_f: 0.63 (CH_2Cl_2/hexane, 4/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.41 (s, 3H, –CH3), 4.45, 4.38 (d, 4H, Ar–CH_{2ax}–Ar), 3.49, 3.35 (d, 4H, Ar–CH_{2eq}–Ar), 4.53 (s, 2H, –COO–CH_2), 3.87 (s, 2H, –O–CH_2) and 7.30, 7.15 (br, 6H, H–Ar)); ^{13}C NMR (125 MHz, $CDCl_3$): δ170 (–*C*OO), 152.5 (–*C*–OH), 150.8 (*C*–O–CH_2), 134.6, 130, 129.2, 129, 126.7, 122, 121.5 (*C*–Ar), 72.4 (O–*C*H_2–CO), 62.5 (COO–*C*H_2), 32.3 and 32.1 (Ar–*C*H_2–Ar), 14.6 (–*C*H_3); ES/MS, *m/z*: 533.1 (M^++Na), mp=243–247°C.

4.3.2. 25-Mono-(2-carboxymethoxy)-calix-[4]-arene (2b). Compound **2a** (1.9 g, 3.94 mmol) was added to a 10% KOH solution in aqueous ethanol 70/30 (50 mL). The reaction mixture was stirred at room temperature during 24 h. The precipitate obtained was filtered and washed with 1 M HCl (50 mL) and water (50 mL). After column chromatography $CHCl_3$/MeOH/acetic acid 95:5:0.1%. 1.74 g of **2c** (95%) was obtained as a white crystalline solid. ^{1}H NMR (125 MHz, $CDCl_3$): δ 4.23, 4.16 (d, 4H, Ar–CH_{2ax}–Ar), 3.42, 3.39 (d, 4H, Ar–CH_{2eq}–Ar), 4.96 (s, 2H, –O–CH_2), 6.792, 7.08, 7.14 (br, 18H, H–Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 151.5 and 150.4 (*C*–OH), 149.2 (*C*–OCH_2), 134, 130, 129.6, 129.3, 128.8, 128, 127.5, 122.6 (*C*H-Aromatic) 68.5 (O–*C*H_2), 32.2 and 32.1 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 505.1 (M^++Na) mp=>250°C

4.3.3. 25-Mono-(2-cyanomethoxy)-calix-[4]-arene (2c). A suspension of **1** (5 g, 11.8 mmol), and an appropriate base (1 equiv.) in acetonitrile (300 mL) was stirred at reflux for 0.5 h. Bromo-acetonitrile (0.82 mL, 1 equiv.) was added and the reaction mixture was stirred under reflux during 24 h. After cooling, the solvent was removed under reduced pressure. The remaining solid was taken up in CH_2Cl_2 (200 mL) and washed with 1 M HCl (2×200 mL) and water (200 mL). The organic layer was dried over $MgSO_4$ and evaporated to give a white powder. The product was purified by column chromatography. R_f: 0.32 (CH_2Cl_2/hexane, 4/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.35, 4.32, 4.24, 4.21 (2d, 4H, Ar–CH_{2ax}–Ar), 3.59, 3.57, 3.5, 3.47 (2d, 4H, Ar–CH_{2eq}–Ar), 5.04 (s, 2H, –O–CH_2-CN) and 7.09, 6.99, 6.94 (br, 12H, H-Aromatic) and 8.49 (s, 3H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.9 (C–OH), 133.7, 130.3, 129.4, 129.1, 128.7, 128.6, 128.3, 127.8, 122.4 (CH-Aromatic), 121.3 (–CN), 60.7 (O–CH_2), 31.9, and 31.8 (Ar–CH_2–Ar); ES/MS, *m/z*: 486.0 (M^++Na); νCN=2160 cm^{-1}; mp=>250°C

4.3.4. 25-Mono-(2-aminoethoxy)-calix-[4]-arene (2d). Compound **2d** has been synthesised in the same conditions described by Smirnov et al.[44] **2c** (1 g, 2.1 mmol) in presence of 40 mL of THF and 10 mL of a solution of BH_3/THF (1 M) was reacted at room temperature during 2 h under vacuum. The reaction mixture was stirred at reflux during 6 h. After cooling, the solvent was removed under reduced pressure. The remaining solid was taken up in CH_2Cl_2 (200 mL) and washed with 1 M HCl (2×200 mL) and water (200 mL). The organic layer was dried over $MgSO_4$ and evaporated to give a white powder (yield=90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.23, 4.2, 4.13, 4.11 (2d, 4H, Ar–CH_{2ax}–Ar), 3.6, 3.58, 3.47, 3.44 (2d, 4H, Ar–CH_{2eq}–Ar), 4.52 (s, 2H, –O–CH_2), 3.84 (s, 2H, –CH_2NH_2) and 7.04, 6.98, 6.92, 6.07 (br, 12H, H–Ar) and 7.08 (s, 3H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ150.3 (*C*–OH), 148.9 (*C*–O–CH_2), 134, 130, 129.4, 129.2, 128.9, 128.1, 127.3, 122.4, 121.9 (*C*H–Ar), 72.5 (O–*C*H_2), 41.1 (*C*H_2–NH_2), 32.1 and 32 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 468.2 (M+H^+) mp=>250°C

4.3.5. 25-Mono-(2-carboxymethoxy)-5,11,17,23-tetra-sulphonato-calix-[4]-arene (3b). Compound **2b** (1 g, 1.27 mmol) in 3 mL of sulphuric acid (96%) was stirred at 50°C for 24 h. The product is precipitated by diethyl ether. The product is dried by high vacuum overnight. Yield 86% (1.35 g). ^{1}H NMR (500 MHz, D_2O): δ 4.22, 4.2, 4.15, 4.12 (2d, 4H, Ar–CH_{2ax}–Ar), 3.63, 3.6, 3.59, 3.56 (2d, 4H, Ar–CH_{2eq}–Ar), 4.52 (s, 2H, –O–CH_2) and 7.38, 7.37 (br, 12H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 172.5 (*C*OOH), 153.9, 153.3 and 151.9 (*C*–SO_3H), 140, 135.7, 135.1, 134.2, 128.8, 128.6, 128.1, 127, 126.7, 126.5, 126.3 (*C*H–Ar), 71 (O–*C*H_2), 31.6 and 31.2 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 803.0 (M+H^+); νCO=1734 cm^{-1}; mp=210–225°C.

The synthesis of all *p*-sulphonato-calix-[*n*]-arene derivatives is identical to that described for **3b**.

4.3.6. 25-Mono-(2-amidomethoxy)-5,11,17,23-tetra-sulphonato-calix-[4]-arene (3c). ^{1}H NMR (500 MHz, D_2O): δ 3.99, 3.96 (2d, 4H, Ar–CH2ax–Ar), 3.714, 3.69 (2d, 4H, Ar–CH2eq–Ar), 4.06 (s, 2H, –O–CH2) and 7.35, 7.23 (br, 12H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 172.5, 154.1, 153.5, 152.1, 140, 135.7, 135.1, 134.2, 128.9, 128.7, 128.2, 127.1, 126.78, 126.6, 126.4 (CH–Ar), 70.9 (O–CH_2), 31.9 and 31.4 (Ar–CH_2–Ar); ES/MS, *m/z*: 802.0 ($M+H^+$); νCO=1640 cm^{-1}; mp=207–218°C.

4.3.7. 25-Mono-(2-aminoethoxy)-5,11,17,23-tetra-sulphonato-calix-[4]-arene (3d). ^{1}H NMR (500 MHz, D_2O): δ 3.97, 3.92 (2d, 4H, Ar–CH_{2ax}–Ar), 3.57, 3.54 (2d, 4H, Ar–CH_{2eq}–Ar), 3.65 (s, 2H, –O–CH_2), 2.34 (s, 2H, –CH_2NH_2) and 7.52, 7.35 (br, 12H, H–Ar); ^{13}C NMR (125 MHz, D_2O): 153.8, 153.5, 152.0, 142.6, 138.6, 135.4, 134.2, 129.0, 128.9, 128.6, 127.8, 126.9, 126.3, 125.9 (CH–Ar), 68.5 (O–CH_2), 43.6 (CH_2–NH_2), 31.4 and 31.2 (Ar–CH_2–Ar); δ ES/MS, *m/z*: 810.1 (M^++Na); mp=>250°C

The synthesis of compounds **5a**–**5d** have been previously reported.[6]

4.3.8. 37-Mono-(2-carboxymethoxy)-5,11,17,23,29,35-hexa-sulphonato-calix-[6]-arene (6b). ^{1}H NMR (500 MHz, D_2O): δ 3.85, 3.81, 3.78 (3s, 12H, Ar–CH_2–Ar), 4.0 (s, 2H, –O–CH_2) and 7.04, 7.38, 7.41, 7.49 (br, 18H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 172.7 (COOH), 156.3, 153.5 (*C*–SO_3H), 139.2, 135.0, 134.4, 128.5, 128.2, 127.1, 126.3, 125.9, 125.6 (*C*H–Ar), 69.1 (O–*C*H_2), 30.7 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 1175.1 ($M+H^+$); νCO= 1719 cm^{-1}; mp=>250°C.

4.3.9. 37-Mono-(2-amidomethoxy)-5,11,17,23,29,35-hexa-sulphonato-calix-[6]-arene (6c). ^{1}H NMR (500 MHz, D_2O): δ 3.77, 3.76, 3.71 (3s, 12H, Ar–CH_2–Ar), 3.46 (s, 2H, –O–CH_2) and 6.91, 7.0, 7.35, 7.46 (br, 18H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 156.3, 153.3, 135, 134.4, 134.2, 134.1, 129.1, 128.5, 127.8, 127.5, 127.3, 127.1, 126.3, 125.3 (*C*H–Ar), 68.2 (O–*C*H_2), 30.7 and 30.2 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 1174.1 ($M+H^+$); νCO=1660 cm^{-1}; mp=>250°C.

4.3.10. 37-Mono-(2-aminoethoxy)-5,11,17,23,29,35-hexa-sulphonato-calix-[6]-arene (6d). ^{1}H NMR (500 MHz, D_2O): δ 3.80, 3.77, 3.7 (3s, 12H, Ar–CH_2–Ar), 3.07 (s, 2H, –O–CH_2) 1.87 (s, 2H, CH_2–NH_2) and 7.06, 7.08, 7.38, 7.42 (br, 18H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 155.8, 153.8, 153.6, 153.1, 138.7, 135.3, 135, 134.8, 128.9, 128.6, 128.4, 128.2, 126.7, 126.3, 126.1, 125.8 (*C*H–Ar), 67.8 (O–*C*H_2), 39.1 (*C*H_2–NH_2), 31.2 and 30.6 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 1158.3 ($M-H^+$); mp=>250°C.

4.3.11. 49-Mono-(ethoxycarbonylmethoxy)-calix-[8]-arene (9a). A suspension of **8** (5 g, 3.9 mmol), and a base CsOH (1 equiv.) in THF (300 mL) was stirred at reflux until **7** is dissolved. Bromo-ethylacetate (0.49 mL, 1 equiv.) was added and the reaction mixture was stirred under reflux during 1 h. After cooling, the solvent was removed under reduced pressure. The remaining solid was taken up in CH_2Cl_2 (200 mL) and washed with 1 M HCl (2×200 mL) and water (200 mL). The organic layer was dried over $MgSO_4$ and evaporated to give a white powder. The product was purified by column chromatography. R_f: 0.7 (CH_2Cl_2/hexane, 4/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.069 (s, 3H, –CH3), 1.28, 1.344 (br, 72H, *t*Bu), 3.873 (br, 16H, Ar–CH_2–Ar), 4.52 (s, 2H, –COO–CH_2), 4.71 (s, 2H, –O–CH_2) and 7.36 (br, 16H, H–Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.27, 147.41, 146.9, 144.8 (–*C*–OH), 144.42 (*C*–O–CH_2), 128.76, 126.9, 126.1, 125.9, 125.8, 125.6 (*C*–Ar), 61.9 (O–*C*H_2–CO), 34.6, 34.2 (*C*–$(CH_3)_3$), 32.4 ((*C*H_3)$_3$), 31.7 (Ar–*C*H_2–Ar), 14.9 (–*C*H_3); ES/MS, *m/z*: 1384.4 ($M+H^+$), mp=>250°C.

4.3.12. 49-Mono-(2-carboxymethoxy)-calix-[8]-arene (9b). Compound **8b** is obtained in the same condition of **2b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28, 1.253 (br, 72H, *t*Bu), 3.892 (br, 16H, Ar–CH_2–Ar), 4.46 (s, 2H, –O–CH_2) and 7.16 (br, 16H, H–Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.27, 147.41, 146.9, 144.8 (–*C*–OH), 142.4 (*C*–O–CH_2), 128.1, 127.8, 126.9, 126.4, 125.9, 125.7 (*C*–Ar), 56.2 (O–*C*H_2–CO), 34.2 (*C*–$(CH_3)_3$), 32.5 ((*C*H_3)$_3$), 31.72 (Ar–*C*H_2–Ar), ES/MS, *m/z*: 1355.7 (M^++H^+), mp=>250°C.

4.3.13. 49-Mono-(2-cyanomethoxy)-calix-[8]-arene (9c). A suspension of **8** (5 g, 3.9 mmol), and a base CsOH (1 equiv.) in THF (300 mL) was stirred at reflux for 2 h. Bromo-acetonitrile (0.27 mL, 1 equiv.) was added and the reaction mixture was stirred under reflux during 1 h. After cooling, the solvent was removed under reduced pressure. The remaining solid was taken up in CH_2Cl_2 (200 mL) and washed with 1 M HCl (2×200 mL) and water (200 mL). The organic layer was dried over $MgSO_4$ and evaporated to give a white powder. The product was purified by column chromatography. R_f: 0.41 (CH_2Cl_2/hexane, 4/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.326, 1.29 (br, 72H, *t*Bu), 4.05, 3.992, 3.91 (br, 16H, Ar–CH_2–Ar), 4.788 (s, 2H, –O–CH_2–CN) and 7.322, 7.22, 7.163 (br, 16H, H–Ar) and 8.49 (s, 3H, –OH); ^{13}C NMR (125 MHz, $CDCl_3$): ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.3, 147.3, 145.4, 144.8 (–*C*–OH), 144.0 (*C*–O–CH_2), 128.7, 128.5, 128.2, 127.9, 126.5, 126.1 (*C*–Ar), 59.9 (O–*C*H_2–CO), 34.7, 34.2 (*C*–$(CH_3)_3$), 32.9, 32.4 ((*C*H_3)$_3$), 31.8 (Ar–*C*H_2–Ar), ES/MS, *m/z*: 1359.1 (M^++Na); νCN=2179 cm^{-1}; mp=>250°C.

4.3.14. 49-Mono-(2-aminoethoxy)-calix-[8]-arene (9d). Compound **9d** is obtained in the same condition of **2d**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.253 (br, 72H, *t*Bu), 3.892 (br, 16H, Ar–CH_2–Ar), 4.465 (s, 2H, –O–CH_2), 3.526 (s, 2H, –CH_2–NH_2) and 7.263, 7.16 (br, 16H, H–Ar) and 9.448 (s, 2H, NH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.7, 148.5, 147.9, 147.1 (–*C*–OH), 143.8 (*C*–O–CH_2), 127.4, 127.2, 16.6, 126.3, 125.9 (*C*–Ar), 58.8 (O–*C*H_2–CO), 44.2 (*C*H_2–NH_2), 34.2 (*C*–$(CH_3)_3$), 32.1 ((*C*H_3)$_3$), 31.7 (Ar–*C*H_2–Ar), ES/MS, *m/z*: 1340.6 (M^++H^+), mp=>250°C.

4.3.15. 49-Mono-(2-carboxymethoxy)-5,11,17,23,29,35,41,47-octa-sulphonato-calix-[8]-arene (10b). ^{1}H NMR (500 MHz, D_2O): δ 3.906, 3.864, 3.851, 3.841 (s, 16H, Ar–CH_2–Ar), 4.012 (s, 2H, –O–CH_2) and 7.331 (br, 16H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 169.3 (*C*OOH), 158.3, 157.9 (*C*–SO_3H), 148.5, 147.9, 147.1 (–*C*–OH), 143.8 (*C*–O–CH_2), 127.6, 127.2, 126.8, 126.3, 125.6 (*CH*–Ar), 60.2 (O–*C*H_2–CO), 31.2 and 31.5 (Ar–*C*H_2–Ar) ES/

MS, *m/z*: 1547.1 ($M+H^+$), νCO=1735 cm^{-1}; mp=>250°C.

4.3.16. 49-Mono-(2-amidomethoxy)-5,11,17,23,29,35, 41, 47-octa-sulphonato-calix-[8]-arene (10c). ^1H NMR (500 MHz, D_2O): δ 3.874, 3.856, 3.775 (br, 16H, Ar–CH_2–Ar), 4.492 (s, 2H, –O–CH_2) and 7.378, 7.328 (br, 16H, H–Ar) and 9.448 (s, 2H, NH_2); ^{13}C NMR (125 MHz, D_2O): δ 158.3, 156.5 (C–SO_3H); 135.9, 135.4, 154.2, 134.9, 129.9, 128.9, 128.1, 127.5, 127.4, 127.1, 126.8, (*C*H–Ar), 70.2 (O–*C*H_2), 30.9 and 30.6 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 1546.1 (M^++H^+); νCO=1687 cm^{-1}; mp=>250°C.

4.3.17. 49-Mono-(2-aminoethoxy)-5,11,17,23,29,35, 41,47-octa-sulphonato-calix-[8]-arene (10d). ^1H NMR (500 MHz, D_2O): δ 3.77 (br, 16H, Ar–CH_2–Ar), 4.72 (s, 2H, –O–CH_2), 3.4 (s, 2H, –CH_2–NH_2) and 7.36, 7.25, 7.20 (br, 16H, H–Ar); ^{13}C NMR (125 MHz, D_2O): δ 157.2, 156.2, 154.6, 153.9, 139.3, 137.3, 136.3, 135.8, 129.6, 129.2, 128.7, 128.0, 127.3, 126.8, 126.5 (*C*H–Ar), 60.8 (O–*C*H_2), 38.5 (*C*H_2–NH_2), 31.1 and 30.8 (Ar–*C*H_2–Ar); ES/MS, *m/z*: 1547.2 ($M+H^+$), mp=>250°C.

References

1. Hart, P. D.; Armstrong, J. A.; Brodaty, E. *Infect. Immun.* **1996**, *64*, 1491.
2. Goreloff, P.; Lamartine, L.; Coleman, A. W. *Cryst. Engng* **1998**, *1*, 191.
3. Malinowska, El. B.; Gawart, L.; Parzuchowski, P.; Rokicki, G.; Brzózka, Z. *Anal. Chim. Acta* **2000**, *421*, 93.
4. Uysal, G.; Memon, S.; Yilmaz, M. *React. Funct. Polym.* **2001**, *50*, 77.
5. Brindle, R.; Albert, K.; Harris, S. J.; Tröltzsch, C.; Horne, E.; Glennon, J. D. *J. Chromatogr. A* **1996**, *731*, 41.
6. Da Silva, E.; Memmi, L.; Coleman, A. W.; Rather, B.; Zaworotko, *J. Supramol. Chem.* **2001**, *1*, 135.
7. Groenen, L. C.; Ruël, B. H. M.; Casnati, A.; Verboom, W.; Pochini, A.; Ungaro, R.; Reinhoudt, D. N. *Tetrahedron* **1991**, *47*, 8379.
8. Groenen, L. C.; Rël, B. H. M.; Casnati, A.; Timmerman, P.; Verboom, W.; Harkema, S.; Pochini, A.; Ungaro, R.; Reinhoudt, D. N. *Tetrahedron Lett.* **1991**, *32*, 2675.
9. McKervey, M. A.; Owens, M.; Schulten, H.-R.; Vogt, W.; Böhmer, V. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 280.
10. Semwal, A.; Bhattacharya, A.; Nayak, S. K. *Tetrahedron* **2002**, *58*, 5287.
11. Oriol Magrans, J.; Rincon, A. M.; Cuevas, F.; Lopez-prados, J.; Nieto, P. M.; Pons, M.; Prados, P.; de Mendoza, J. *J. Org. Chem.* **1998**, *63*, 1079.
12. Neri, P.; Geraci, C.; Piattelli, M. *J. Org. Chem.* **1995**, *60*, 4126.
13. Mc Mahon, G.; Wall, R.; Nolan, K.; Diamond, D. *Talanta* **2002**, *57*, 1119.
14. Casnati, A.; Arduini, A.; Ghidini, E.; Pochini, A.; Ungaro, R. *Tetrahedron* **1991**, *47*, 2221.
15. Uysal, G.; Memon, S.; Yilmaz, M. *React. Funct. Polym.* **2001**, *50*, 77.
16. Santoyo-Gonzalez, F.; Torres-Pinedo, A.; Sanchez-Ortega, A. *J. Org. Chem.* **2000**, *65*, 4409.
17. Takeshita, M.; Suzuki, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 2587.
18. Hervé, G.; Hahn, D. U.; Hervé, A.-C.; Goodworth, K. J.; Hill, A. M.; Hailes, H. C. *Org. Biomol. Chem.* **2003**, *1*, 427.
19. Atwood, J. L.; Bridges, R. J.; Juneja, R. K.; Singh, A. K. US Patent 5 489 612, 1996.
20. Hwang, K. M.; Qi, Y. M.; Liu, S. Y.; Lee, T. C.; Choy, W.; Chen, J. US Patent 5 409 959, 1995.
21. Hwang, K. M.; Qi, Y. M.; Liu, S. Y.; Lee, T. C.; Choy, W.; Chen, J. US Patent 5 196 452, 1991.
22. Harris, S. J. PCT Int. Appl. WO 0244121, 2002.
23. Hulmes, D. J. S.; Aubert-Foucher, E.; Coleman, A. W. French Patent 98.10074, 1998.
24. Douteau-Guével, N.; Coleman, A. W.; Morel, J.-P.; Morel-Desrosiers, N. *J. Phys. Org. Chem.* **1998**, *11*, 693.
25. Douteau-Guével, N.; Coleman, A. W.; Morel, J.-P.; Morel-Desrosiers, N. *J. Chem. Soc., Perkin Trans. 2* **1999**, 629.
26. Kalchenko, O. I.; Perret, F.; Morel-Desrosiers, N.; Coleman, A. W. *J. Chem. Soc., Perkin Trans. 2* **2001**, 258.
27. Selkti, M.; Tomas, A.; Coleman, A. W.; Douteau-Guevel, N.; Nicolis, I.; Villain, F.; de Rango, C. *J. Chem. Soc., Chem. Commun.* **2000**, *2*, 161.
28. Atwood, J. L.; Ness, T.; Nichols, P. J.; Raston, C. L. *Cryst. Growth Des.* **2002**, *2*, 171.
29. Douteau-Guével, N.; Perret, F.; Coleman, A. W.; Morel, J.-P.; Morel-Desrosiers, N. *J. Chem. Soc., Perkin Trans. 2* **2002**, 524.
30. Memmi, L.; Lazar, A.; Brioude, A.; Ball, V.; Coleman, A. W. *J. Chem. Soc., Chem. Commun.* **2001**, 2474.
31. Bryant, L. H., Jr.; Yordanov, A. T.; Linnoila, J. J.; Brechbiel, M. W.; Frank, J. A. *Angew. Chem. Int. Ed.* **2000**, *39*, 1641.
32. Aime, S.; Barge, A.; Botta, M.; Casnati, A.; Fragai, M.; Luchinat, C.; Ungaro, R. *Angew. Chem. Int. Ed.* **2001**, *40*, 4737.
33. DaSilva, E.; Valmalle, C.; Becchi, M.; Cuilleron, C.-Y.; Coleman, A. W. *J. Incl. Phenom.* **2003**. In press.
34. Kalchenko, O. I.; Da Silva, E.; Coleman, A. W. *J. Incl. Phenom.* **2002**, *43*, 305.
35. Goolsby, B. J.; Brodbelt, J. S.; Adou, E.; Bland, M. *Int. J. Mass Spectrom.* **1999**, *193*, 197–204.
36. Shahgaldian, P.; Coleman, A. W. *Langmuir* **2001**, *17*, 6851.
37. Lamartine, R.; Regnouf De Vains, J.-B.; Choquard, P.; Marcillac, A. PCT Int. Appl. WO 9749677, 1997.
38. Zanotti-Gerosa, A.; Solari, E.; Giannini, L.; Floriani, C.; Chiesi-Villa, A.; Rizzoli, C. *J. Chem. Soc., Chem. Commun.* **1997**, 183.
39. Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. M.; Sanchez, C. *J. Org. Chem.* **1991**, *56*, 3372.
40. Arena, G.; Contino, A.; Gulino, F. G.; Magri, A.; Sansone, F.; Sciotto, D.; Ungaro, R. *Tetrahedron* **1999**, *40*, 1597.
41. Legget, D. J. *J. Chem. Educ.* **1983**, *60*, 707.
42. Shinkai, S.; Araki, K.; Matsuda, T.; Nishiyama, N.; Ikeda, H.; Takasu, I.; Iwamoto, M. *J. Am. Chem. Soc.* **1990**, *112*, 9053.
43. Fielding, L. *Tetrahedron* **2000**, *56*, 6151.
44. Smirnov, S.; Sidorov, V.; Pinkhassik, E.; Havlicek, J.; Stibor, I. *Supramol. Chem.* **1997**, *8*, 187.