# Complexation of the basic amino acids lysine and arginine by three sulfonatocalix[*n*]arenes (*n* = 4, 6 and 8) in water: microcalorimetric determination of the Gibbs energies, enthalpies and entropies of complexation

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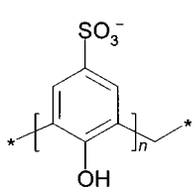
The complexes formed between three *p*-sulfonatocalix[*n*]arenes (*n* = 4, 6 and 8) and the amino acids lysine and arginine in water have been studied by microcalorimetry, at 298.15 K. For each system, both the apparent association constant and enthalpy of reaction have been extracted from the calorimetric data. The Gibbs energies, enthalpies and entropies of complexation have been determined both in acidic medium (pH 1) and in slightly basic medium (pH 8). The thermodynamic parameters for the complexation of arginine markedly differ from those for the complexation of lysine. The three hosts show very different thermodynamic behaviours. Our results are consistent with the formation of 1 : 1 complexes with the calix[4]arenesulfonate and the calix[6]arenesulfonate and with the formation of 1 : 1 and 1 : 2 complexes with the calix[8]arenesulfonate. Whereas the calix[4]arenesulfonate forms relatively strong complexes, the calix[6]arenesulfonate and the calix[8]arenesulfonate form only weak complexes. In all cases, the complexation is driven by a favourable enthalpy change. The enthalpies and entropies of complexing of arginine by the calix[6]arenesulfonate are remarkably negative. The enthalpies and entropies of complexation of the two amino acids by the cyclic tetramer and by the cyclic hexamer become more negative when the pH is changed from 8 to 1; the same effect is observed upon binding of the cyclic octamer with the first guest whereas the opposite effect is observed upon addition of the second guest.

## Introduction

Calixarenes[1] are the third major class of supramolecular host systems along with crown ethers[2] and cyclodextrins.[3] They are obtained from the condensation of phenols with formaldehyde and possess an hydrophobic cavity capable of including molecular guests in solution. A characteristic feature of the calixarenes is their insolubility in water and their low solubility in organic solvents. So it has been of particular interest to confer water solubility on the calixarenes.[4] The *p*-sulfonatocalix[*n*]arenes (*n* = 4, 6 and 8), **1**$_4$, **1**$_6$ and **1**$_8$, have been found to




**1**$_4$, **1**$_6$, **1**$_8$
(*n* = 4, 6, 8)

have solubilities at least as great as 0.1 mol dm$^{-3}$.[5] With the advent of this water solubility, potentiometric and calorimetric measurements of p$K_a$ values have yielded data for several OH groups of **1**$_4$, **1**$_6$ and **1**$_8$.[6–9]

The *p*-sulfonatocalix[*n*]arenes are able to complex a variety of organic compounds as well as inorganic ions in water.[10] Many complexation studies have already been performed with water-soluble calixarenes and analogues. Schneider *et al.*[11] have studied the electrostatic attraction between a water soluble anionic host molecule with a singly positively charged organic cation by $^1$H and $^{13}$C NMR. Shinkai and co-workers[12–16] studied the association of water soluble *p*-sulfonatocalix[*n*]-arenes with neutral and charged guests by spectrophotometry, $^1$H NMR or induced circular dichroism. In particular, they estimated the association properties of **1**$_4$, **1**$_6$ and **1**$_8$ with trimethylanilinium and 1-adamantyltrimethylammonium ions by using NMR methods.[13] Their results showed that **1**$_8$ can form 1 : 2 complexes with both guests. From their study of the influence of pH on the complexation between **1**$_4$ and these guests they concluded that, in the acidic pH region, the phenyl moiety of the guest resides in the cavity whereas, in the neutral pH region, both the trimethylammonium ion and the phenyl moiety are included non-specifically in the cavity.[14] Studies involving **1**$_6$ have also been performed with Auramine O dye[17] using fluorescence intensities and with dimeric bipyridinium guests[18] using $^1$H NMR. Kaifer *et al.*[18] have proposed two possible mechanisms for surface binding of long organic cations to **1**$_6$.

Very recently, a series of calix[4]arene based α-aminophosphonates were synthesized and exhibited remarkable selectivity as carriers for the membrane transport of the zwitterionic form of aromatic amino acids.[19] The synthesis of an antibody mimic based on calix[4]arene linked to four constrained peptide loops, used for recognition of protein surfaces, was also reported.[20] So the biomedical potential of calixarenes becomes of very great interest and recent studies have shown that **1**$_4$, **1**$_6$ and **1**$_8$ could be

excellent candidates as heparin mimics to interact with heparin receptor peptides,[21] which possess many lysine and arginine residues. To comprehend the binding of the *p*-sulfonatocalix[*n*]arenes with the heparin receptor peptides, it is necessary to understand first the nature of the interactions between $\mathbf{1}_4$, $\mathbf{1}_6$ or $\mathbf{1}_8$ and the basic amino acids lysine and arginine. In order to do so, we have first carried out some $^1$H NMR experiments at pH 1, 5 and 13: it has been shown that, at pH 1 and 5, $\mathbf{1}_4$ forms 1 : 1 complexes with lysine and arginine. [22]

In the present paper we apply microcalorimetry to the thermodynamic characterization of the complexation of lysine and arginine by $\mathbf{1}_4$, $\mathbf{1}_6$ and $\mathbf{1}_8$ in water at 298.15 K. The method we use allows the simultaneous determination of the association constant and enthalpy change. We report the Gibbs energies, enthalpies and entropies of complexation at pH 1 and 8.

## Experimental

### Materials

$\mathbf{1}_4$, $\mathbf{1}_6$ and $\mathbf{1}_8$ were synthesized using the method described by Arena *et al*.[7] For each calixarene, the final neutralization before recrystallization was performed at pH 6. $^1$H NMR ($D_2O$ at 20 °C), atomic absorption spectroscopy (Na) and water analysis showed that the products have the following formulas at pH 6: $\mathbf{1}_4$, $C_{28}H_{19}O_{16}S_4Na_5$, 11.7% $H_2O$; $\mathbf{1}_6$, $C_{42}H_{28}O_{24}S_6Na_8$, 23.2% $H_2O$; $\mathbf{1}_8$, $C_{56}H_{38}O_{32}S_8Na_{10}$, 20.4% $H_2O$.

Sodium dihydrogen phosphate dihydrate (Fluka, *pro analysi*), anhydrous disodium hydrogen phosphate (Merck, BioChemika), arginine and lysine (Neosystem) were used without further purification.

The phosphate buffer pH 8 was prepared by mixing 96.6 mL of a 0.01 mol kg$^{-1}$ $Na_2HPO_4$ solution and 3.4 mL of a 0.01 mol kg$^{-1}$ $NaH_2PO_4$ solution. The pH was verified on a pH-meter (U-ISIS 20.000-1 SOLEA-Tacussel) calibrated with two different buffer solutions. Other biological buffers have been tested by NMR [22] but since they interacted strongly with the calixarenes their use for our study was excluded. In fact, the phosphate buffer has no influence on complexation and can be used on a relatively large scale of pH (5 to 8).

All the solutions were prepared by weight from triply distilled water. The pH of the calixarene and amino acid solutions was set at 8 with the phosphate buffer and at about 1 with 0.1 mol kg$^{-1}$ HCl.

## Microcalorimetry

All measurements were performed using a multichannel microcalorimeter (LKB-Thermometric 2277 Thermal Activity Monitor) equipped with a titration–perfusion vessel. Suurkuusk and Wadsö [23] have thoroughly described this twin thermopile heat-conduction calorimeter and analyzed its performance.

For the measurements at pH 8, a 1 mL stainless steel titration vessel was used. At pH 1, it was replaced by a 1 mL glass vessel. Both were fitted with a gold stirrer. The vessel was charged with 0.8 mL of calixarene solution and 14 μL of amino acid solution was injected in each step using a Lund syringe pump (Thermometric) equipped with a 250 μL Hamilton syringe fitted either with a stainless steel (at pH 8) or a gold (at pH 1) cannula. Fifteen injections were made for each titration experiment. The solution molalities were, prior to titration, in the range 0.005–0.01 mol kg$^{-1}$ for calixarenes and 0.05–0.3 mol kg$^{-1}$ for amino acids. Static and dynamic calibrations were used; the power values observed upon titration ranged from 30 to 300 μW.

Separate dilution experiments were performed. Since the heats of dilution of calixarenes were found to be negligible, the heat effects observed upon titration were simply corrected for the heats of dilution of the amino acids. Each experiment was repeated three times for reproducibility.

Values for the apparent association constant $K'$ and apparent standard enthalpy of reaction $\Delta_rH'^{\ominus}$ in a given medium were calculated by use of the Digitam 4.1 minimization program (Thermometric). The three series of data obtained for each system were treated simultaneously in the regression analysis.

## Results and discussion

The thermodynamic property that characterizes a binding reaction of the type given in eqn. (1) reflects the modifications of all

$$\text{host} + \text{guest} = \text{complex} \qquad (1)$$

the species upon complexation. It corresponds in fact, for a given property $X$, to eqn. (2) and reflects the balance of several

$$\Delta_rX'^{\ominus} = X(\text{complex}) - X(\text{host}) - X(\text{guest}) \qquad (2)$$

contributions among which are those due to the modification of the solvation of the host and/or guest, the modification of the degrees of freedom of the host and/or guest, the electrostatic interactions, the hydrophobic interactions, the hydrogen bonding, the π–π interactions, *etc*. It is not possible to evaluate separately the individual contributions for the systems studied here. We know, however, the sign of the major contributions in water: for instance, the partial dehydration of $NH_3^+$ or $SO_3^-$ gives a positive contribution to $\Delta_rH'^{\ominus}$ and to $T\Delta_rS'^{\ominus}$, the loss by the guest or the host of conformational degrees of freedom gives a negative contribution to $T\Delta_rS'^{\ominus}$, the π–π interactions give a negative contribution to $\Delta_rH'^{\ominus}$, the hydrophobic interaction gives a positive contribution to $T\Delta_rS'^{\ominus}$. The contributions due to the electrostatic interactions between the charged sites of the hosts and guests studied here are probably low.

The $K'$ and $\Delta_rH'^{\ominus}$ values characterizing the complexation of arginine and lysine by $\mathbf{1}_4$, $\mathbf{1}_6$ and $\mathbf{1}_8$ in water, deduced from the non-linear regression fit of the microcalorimetric data, are reported with the estimated standard errors in Table 1. The corresponding $\Delta_rG'^{\ominus}$ and $T\Delta_rS'^{\ominus}$ values are also given.

One sees that calix[4]arenesulfonate forms relatively strong complexes with the two amino acids studied here. As expected, our data are consistent with a 1 : 1 binding model. Arginine binds more strongly than lysine; whatever the pH, $K'$($\mathbf{1}_4$–arginine) is twice as large as $K'$($\mathbf{1}_4$–lysine). $T\Delta_rS'^{\ominus}$(arginine) is slightly unfavourable, whereas $T\Delta_rS'^{\ominus}$(lysine) is slightly favourable. With both guests, $\Delta_rH'^{\ominus}$ is negative and the complexation process is enthalpy-driven. A literature survey shows that many complexation processes involving cyclic ligands (crown ethers, cryptands, cyclodextrins, cyclophanes, calixarenes, *etc.*) or acyclic flexible ligands (glymes, podands, enzymes, antibiotics, *etc.*) show similar thermodynamic behaviour whatever the guest (small cations, charged or neutral molecules, apolar aromatic substrates, *etc.*) and whatever the solvent.[24–31] This seems to indicate that the complexation process is governed by the inclusion of the guest itself. According to Smithrud *et al.*,[26] a large part of the favourable enthalpy change results from solvent-specific contributions. Their calorimetric study in 12 solvents of different polarities shows that water is not special in providing an enthalpic driving force for apolar complexation. Their results suggest that the enthalpic driving force for tight apolar inclusion increases with increasing polarity, becoming strongest in polar protic solvents, and ultimately in water. Upon inclusion, the degrees of freedom of both guest and host are reduced, which results in a negative change in $T\Delta_rS'^{\ominus}$. Obviously the hydrophobic interactions, which positively contribute to $T\Delta_rS'^{\ominus}$, do not play a major role in the complexation processes studied here. At pH 8, $\Delta_rH'^{\ominus}$(arginine) is more favourable than $\Delta_rH'^{\ominus}$(lysine): this is probably due to the existence of π–π interactions between the guanidinium group of arginine and the aromatic rings of the calixarene. Changing the pH from 8 to 1 yields negative contributions to both $\Delta_rH'^{\ominus}$ and $T\Delta_rS'^{\ominus}$. The repulsion that exists

**Table 1** Thermodynamic parameters characterizing the complexation of arginine and lysine by the *p*-sulfonatocalix[*n*]arenes ($n = 4, 6, 8$) in water at 298.15 K and at pH 8 and 1 [a]

| | | $K'$ | $\Delta_r G'^{\ominus}$/kJ mol$^{-1}$ | $\Delta_r H'^{\ominus}$/kJ mol$^{-1}$ | $T\Delta_r S'^{\ominus}$/kJ mol$^{-1}$ |
|---|---|---|---|---|---|
| pH 8 | $\mathbf{1}_4$–Arg | 1520 ± 90 | −18.2 ± 0.1 | −20.3 ± 0.3 | −2.1 ± 0.4 |
| | $\mathbf{1}_4$–Lys | 735 ± 10 | −16.4 ± 0.1 | −14.4 ± 0.1 | 2.0 ± 0.2 |
| pH 1 | $\mathbf{1}_4$–Arg | 2830 ± 110 | −19.7 ± 0.1 | −25.9 ± 0.1 | −6.2 ± 0.2 |
| | $\mathbf{1}_4$–Lys | 1400 ± 100 | −18.0 ± 0.1 | −19.4 ± 0.3 | −1.4 ± 0.4 |
| pH 8 | $\mathbf{1}_6$–Arg | 186 ± 7 | −13.0 ± 0.1 | −41.2 ± 0.5 | −28.2 ± 0.6 |
| | $\mathbf{1}_6$–Lys | 94 ± 4 | −11.3 ± 0.1 | −21.8 ± 0.3 | −10.5 ± 0.4 |
| pH 1 | $\mathbf{1}_6$–Arg | 45 ± 1 | −9.4 ± 0.1 | −48.2 ± 0.3 | −38.8 ± 0.4 |
| | $\mathbf{1}_6$–Lys | 18 ± 1 | −7.2 ± 0.1 | −27.1 ± 0.8 | −19.9 ± 0.9 |
| pH 8 | $\mathbf{1}_8$–Arg [b] | 350 ± 50 | −14.5 ± 0.4 | −14.9 ± 0.7 | −0.4 ± 1 |
| | $\mathbf{1}_8$–(Arg)$_2$ [c] | 41 ± 1 | −9.2 ± 0.1 | −43 ± 1 | −34 ± 1 |
| | $\mathbf{1}_8$–Lys [b] | 400 ± 140 | −14.9 ± 0.6 | −6.2 ± 0.6 | 9 ± 1 |
| | $\mathbf{1}_8$–(Lys)$_2$ [c] | 23 ± 1 | −7.8 ± 0.1 | −24 ± 2 | −16 ± 2 |
| pH 1 | $\mathbf{1}_8$–Arg [b] | 73 ± 16 | −10.6 ± 0.6 | −38 ± 6 | −27 ± 7 |
| | $\mathbf{1}_8$–(Arg)$_2$ [c] | 49 ± 1 | −9.6 ± 0.1 | −29 ± 8 | −19 ± 8 |
| | $\mathbf{1}_8$–Lys [b] | 143 ± 27 | −12.3 ± 0.4 | −14.5 ± 0.7 | −2 ± 1 |
| | $\mathbf{1}_8$–(Lys)$_2$ [c] | 27 ± 1 | −8.2 ± 0.1 | −23 ± 2 | −15 ± 2 |

[a] Molar scale. [b] Characterized by $K'_1$, $\Delta_r G'^{\ominus}_1$, $\Delta_r H'^{\ominus}_1$, and $T\Delta_r S'^{\ominus}_1$ [eqn. (3)]. [c] Characterized by $K'_2$, $\Delta_r G'^{\ominus}_2$, $\Delta_r H'^{\ominus}_2$, and $T\Delta_r S'^{\ominus}_2$ [eqn. (4)].

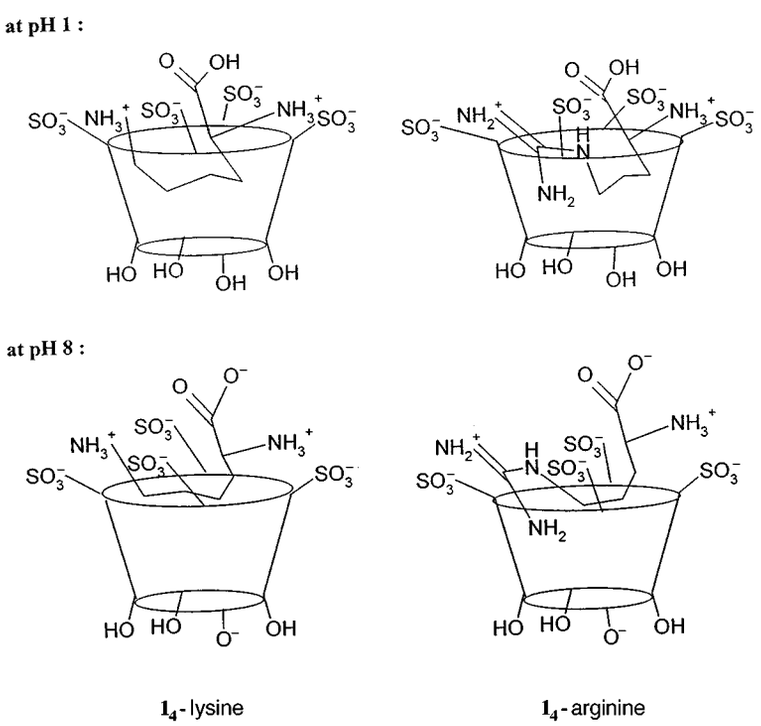



**Fig. 1** Schematic representations of the $\mathbf{1}_4$–arginine and $\mathbf{1}_4$–lysine complexes in aqueous solution at pH 1 and 8.

at pH 8 between the sulfonate groups of the calixarene and the carboxy group of the amino acid disappears when the medium becomes acidic. In the absence of this particular repulsion the guest can penetrate more deeply into the calixarene cone. As expected, this gives a favourable contribution to $\Delta_r H'^{\ominus}$ and an unfavourable contribution to $T\Delta_r S'^{\ominus}$. Schematic representations of the $\mathbf{1}_4$–arginine and $\mathbf{1}_4$–lysine complexes in aqueous solution at pH 8 and 1 are given in Fig. 1. The lysine model is based on the X-ray structure of a $\mathbf{1}_4$–lysine complex.[32] The arginine model may resemble this in some ways but the guanidinium binding to the cavity suggests that deeper insertion will be preferred, as evidenced by the NMR spectra.[22]

It is interesting to compare the results we have found for lysine with those deduced from the microcalorimetric titration of $\mathbf{1}_4$ with alkylammonium ions in dilute aqueous solution at pH 7.1 and 298.15 K:[33] for instance, with $CH_3(CH_2)_4NH_3^+$ $K = 6400$, $\Delta_r G^{\ominus} = -21.72$ kJ mol$^{-1}$, $\Delta_r H^{\ominus} = -20.24$ kJ mol$^{-1}$ and $T\Delta_r S^{\ominus} = 1.48$ kJ mol$^{-1}$, whereas with $CH_3(CH_2)_5NH_3^+$ $K = 4000$, $\Delta_r G^{\ominus} = -20.57$ kJ mol$^{-1}$, $\Delta_r H^{\ominus} = -20.42$ kJ mol$^{-1}$ and $T\Delta_r S^{\ominus} = 0.15$ kJ mol$^{-1}$. The enthalpy of complexation

of lysine at pH 1, which then has the structure HOOC-$CH(NH_3^+)(CH_2)_4NH_3^+$, is quite close ($-19.4$ kJ mol$^{-1}$) to the enthalpy of complexation of an alkylammonium ion of comparable size. This probably shows that for both types of guests, the major contribution to the enthalpy of reaction comes from the inclusion of the alkyl chain within the host cavity. The entropy of complexation we have obtained with lysine is, as observed with the alkylammonium ions, quite small: this could be an indication that the hydrophobic interactions do not play a major role in associations of this type.

It is also interesting to compare the results we have obtained for arginine at pH 1 ($K = 2830$, $\Delta_rG^\ominus = -19.7$ kJ mol$^{-1}$, $\Delta_rH^\ominus = -25.9$ kJ mol$^{-1}$ and $T\Delta_rS^\ominus = -6.2$ kJ mol$^{-1}$) with those determined by $^1$H NMR for the complexation of the trimethylanilinium ion with **1**$_4$ in $D_2O$ ($K = 5600$, $\Delta_rG^\ominus = -21.3$ kJ mol$^{-1}$, $\Delta_rH^\ominus = -25.9$ kJ mol$^{-1}$ and $T\Delta_rS^\ominus = -4.6$ kJ mol$^{-1}$).[13] Note that the enthalpies of complexation are equal and that the entropies of complexation are not very different. This is an indication that the factors governing the complexation of both guests are the same: obviously, the predominant factors are the desolvation and the modification of the degrees of freedom of both guest and host upon inclusion on the one hand, and the interactions between the π electrons of the guanidinium group of arginine or of the phenyl group of the trimethylanilinium ion and the π electrons of the phenyl groups of **1**$_4$, on the other hand. Upon complexation arginine loses more degrees of freedom than the trimethylanilinium ion and, as a result, $T\Delta_rS^\ominus$ is slightly more negative for the former guest than for the latter one.

Our results (Table 1) show that the calix[6]arenesulfonate, which is a less rigid host than the calix[4]arenesulfonate, forms with arginine and lysine 1:1 complexes that are weaker than those formed by the cyclic tetramer. Again, arginine binds more strongly than lysine, $K'$(**1**$_6$–arginine) being twice as large as $K'$(**1**$_6$–lysine) at both pH 1 and 8. Here again, complexation is driven by a favourable enthalpy change. It must be underlined, however, that $\Delta_rH'^\ominus$ and $T\Delta_rS'^\ominus$ are much more negative with **1**$_6$ than with **1**$_4$. At pH 8, $\Delta_rH'^\ominus$(arginine) and $T\Delta_rS'^\ominus$(arginine) are much more negative than $\Delta_rH'^\ominus$(lysine) and $T\Delta_rS'^\ominus$(lysine), respectively. When the pH is changed from 8 to 1, $\Delta_rH'^\ominus$ and $T\Delta_rS'^\ominus$ become more negative for both guests, as observed with the calix[4]arenesulfonate. In solution, **1**$_6$ either retains the double partial cone conformation (double cone with two inverted faces) it has in the solid state[34] or adopts an ellipsoidal cone conformation. Our thermodynamic results cannot totally discriminate between these two conformations but seem to be more consistent with the former one. In fact, CPK models show that the elongated amino acid can fit quite tightly within the double cone cavity of the host, the guest being then totally immobilized and each of its terminal cations being surrounded by the three or four (at pH 8) anions borne by the host partial cone. The formation of such a constrained structure may explain the very important decrease observed for both $\Delta_rH'^\ominus$ and $T\Delta_rS'^\ominus$, the major contributions being probably those associated with the desolvation of the species and with the very important loss of degrees of freedom. The fact that this inclusion structure is particularly favourable to π–π stacking may also explain why the decrease of both $\Delta_rH'^\ominus$ and $T\Delta_rS'^\ominus$ is particularly pronounced for arginine. Thus, although the enthalpy of complexation is extremely favourable, the complex formed happens to be relatively weak because of the highly unfavourable entropy of complexation arising mainly from the important loss of degrees of freedom upon inclusion of the guest. Contributions of that type may also be observed with a host in the ellipsoidal cone conformation but they are not expected to be as pronounced as those associated with the double partial cone structure. It may be noted that Kaifer and co-workers,[18] who studied the association of **1**$_6$ with cationic viologen guests by NMR, also suggested the formation of double partial cone inclusion complexes.

The thermodynamic parameters for the complexation of the trimethylanilinium ion by **1**$_6$ were also determined by $^1$H NMR in $D_2O$ ($K = 550$, $\Delta_rG^\ominus = -15.5$ kJ mol$^{-1}$, $\Delta_rH^\ominus = -1.1$ kJ mol$^{-1}$ and $T\Delta_rS^\ominus = 14.4$ kJ mol$^{-1}$).[13] These enthalpy and entropy of complexation values are very different from the values we found for arginine at pH 1 ($K = 45$, $\Delta_rG^\ominus = -9.4$ kJ mol$^{-1}$, $\Delta_rH^\ominus = -48.2$ kJ mol$^{-1}$ and $T\Delta_rS^\ominus = -38.8$ kJ mol$^{-1}$), whereas the agreement was excellent for the complexation by **1**$_4$. This discrepancy is probably due to the fact that the enthalpy of complexation of the trimethylanilinium ion was deduced from the temperature dependence of the chemical shifts (measured at 0, 40, 60 and 80 °C). The association constant with **1**$_6$ being much smaller than with **1**$_4$, the determination of the enthalpy of reaction from the van't Hoff plot is inevitably much less reliable and hardly comparable with the value determined by microcalorimetry.

We must consider a 1:2 binding model in order to fit the microcalorimetric data we have obtained for the **1**$_8$–arginine and **1**$_8$–lysine systems. This model involves the following step equilibria [eqns. (3) and (4)] where AA stands for the amino acid.

$$\mathbf{1}_8 + \mathrm{AA} = \mathbf{1}_8\text{–AA} \quad (\text{characterized by } K'_1 \text{ and } \Delta_rH'^\ominus_1) \qquad (3)$$

$$\mathbf{1}_8\text{–AA} + \mathrm{AA} = \mathbf{1}_8\text{–(AA)}_2 \quad (\text{characterized by } K'_2 \text{ and } \Delta_rH'^\ominus_2) \qquad (4)$$

The stepwise apparent association constants, $K'_1$ and $K'_2$, and the apparent standard enthalpy changes, $\Delta_rH'^\ominus_1$ and $\Delta_rH'^\ominus_2$, thus deduced from the non-linear regression fit of the microcalorimetric data are reported in Table 1. The fact that $K'_1$ is larger than $K'_2$ indicates that the calix[8]arenesulfonate binds two arginine or lysine molecules in a non-cooperative manner. This may be an indication that **1**$_8$ adopts, in solution, a double partial cone conformation although it does not totally preclude a conformation of the ellipsoidal cone type. At both values of pH, $K'_1$(arginine) is smaller than $K'_1$(lysine) whereas $K'_2$(arginine) is larger than $K'_2$(lysine). At pH 8, the overall constant ($\beta = K'_1\,K'_2$) is larger for arginine than for lysine whereas at pH 1 it is almost the same for both amino acids. Complexation is driven by a favourable enthalpy change: for both guests at pH 8 and for lysine at pH 1 $\Delta_rH'^\ominus_2 \ll \Delta_rH'^\ominus_1$ and $T\Delta_rS'^\ominus_2 \ll T\Delta_rS'^\ominus_1$, whereas for arginine at pH 1 $\Delta_rH'^\ominus_2 > \Delta_rH'^\ominus_1$ and $T\Delta_rS'^\ominus_2 > T\Delta_rS'^\ominus_1$. For both steps [eqns. (3) and (4)], the apparent standard enthalpy of complexation of arginine is more favourable than that of lysine whereas it is the opposite for the entropy.

The complexation of the trimethylanilinium ion by **1**$_8$ was also studied by $^1$H NMR.[13] A break point was observed in the plots of the chemical shifts *versus* the host-to-guest concentration ratio, supporting the formation of a 1:2 complex. This is in good agreement with what is observed here for the complexation of both arginine and lysine. The thermodynamic parameters that were determined from the temperature dependence of the NMR spectra cannot, however, be reasonably compared with the values given here: the fact that the $\Delta_rH'^\ominus_1$ and $\Delta_rH'^\ominus_2$ values deduced from the spectra are both equal to zero is a clear indication that the spectroscopic method is not sufficiently sensitive for this type of determination.

When we plot $\Delta_rH'^\ominus$ against $T\Delta_rS'^\ominus$ for all the complexations studied here, two linear relationships are observed (Fig. 2): one for the complexations by **1**$_4$ and one for the complexations by **1**$_6$ and **1**$_8$ that includes, for the latter host, both the 1:1 and 1:2 step reactions. Inoue and co-workers[24,25,27,28] have shown that the enthalpy–entropy compensation effect holds for complexation of different guests (cations, neutral or charged molecules) by various cyclic (crown ethers, cryptands, cyclodextrins, cyclophanes, calixarenes, *etc.*) or acyclic flexible (glymes, podands, enzymes, antibiotics, *etc.*) hosts. As correlations of this type are usually observed within homologous

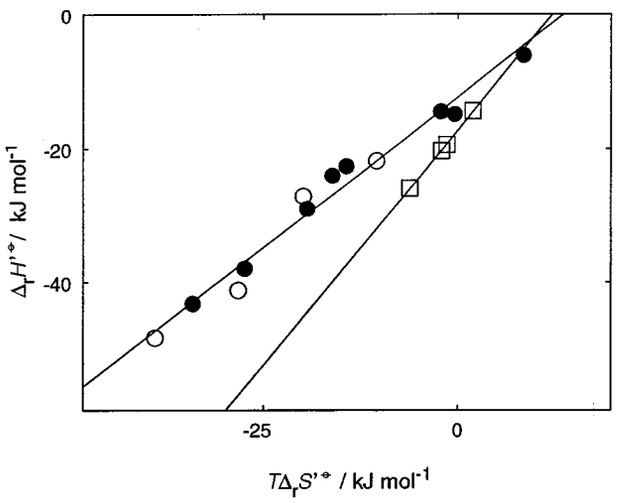



**Fig. 2** Variation of $\Delta_r H'^{\ominus}$ with $T\Delta_r S'^{\ominus}$ for the complexation of lysine and arginine by $\mathbf{1}_4$ (□), $\mathbf{1}_6$ (○) and $\mathbf{1}_8$ (●) in water at both pH 1 and 8. For $\mathbf{1}_8$, the data characterizing the formation of both the 1:1 and 1:2 complexes have been plotted.

series, we can reasonably consider that the data points that fall on the same correlation line are governed by predominant factors of the same type. In fact, the partial dehydration of the species and the modification of the conformational degrees of freedom of the guest and host probably give the most important contributions to the enthalpy and entropy of complexation, whatever the size of the *p*-sulfonatocalixarene. In the present case, it is obvious that the conformational degrees of freedom of $\mathbf{1}_4$ are much less modified than those of $\mathbf{1}_6$ and $\mathbf{1}_8$ upon complexation and that, accordingly, the $\Delta_r H'^{\ominus}$ and $T\Delta_r S'^{\ominus}$ contributions due to this factor are less negative for $\mathbf{1}_4$ than for the other hosts. This may explain why the slope $b$ of the correlation line ($\Delta_r H'^{\ominus} = \Delta_r H'^{\ominus}_o + bT\Delta_r S'^{\ominus}$) is steeper for the $\mathbf{1}_4$ series than for the $\mathbf{1}_6 + \mathbf{1}_8$ series. This is consistent with the observations made by Inoue *et al.*[24, 25, 27, 28] who noticed that the slope $a$, deduced from the $T\Delta_r S - \Delta_r H$ plot ($T\Delta_r S = T\Delta_r S_o + a\Delta_r H$, which means that $a = 1/b$), is smaller for more rigid ligands. Very recently, Tao and Barra[31] observed a good linear relationship between $T\Delta_r S$ and $\Delta_r H$ for complexation between *N*,*N*-dimethylindoaniline and *p*-sulfonated calix[*n*]arenes in aqueous solution. The values they determined by means of UV–Vis spectroscopy for complexation with $\mathbf{1}_4$, $\mathbf{1}_6$ and $\mathbf{1}_8$ fall on a single enthalpy–entropy compensation plot with a resulting slope $a$ of 1.1 ± 0.1 and an intercept $T\Delta_r S_o$ of 17 ± 3 kJ mol$^{-1}$. Tao and Barra have also compiled their values with those reported by Shinkai *et al.*[13] for the complexation of trimethylanilinium chloride and 1-adamantyltrimethylammonium chloride (studied by NMR): the resulting slope and intercept were quite comparable ($a = 1.1 \pm 0.1$ and $T\Delta_r S_o = 19 \pm 2$ kJ mol$^{-1}$). The values we can deduce from our data for the complexation of lysine and arginine are the following: $a = 0.71$ and $T\Delta_r S_o = 12.0$ kJ mol$^{-1}$ for $\mathbf{1}_4$ and $a = 1.12$ and $T\Delta_r S_o = 13.5$ kJ mol$^{-1}$ for $\mathbf{1}_6$ and $\mathbf{1}_8$. Although the thermodynamic properties deduced from spectroscopic data are not as reliable as those obtained by microcalorimetry, the agreement appears to be quite good for $\mathbf{1}_6$ and $\mathbf{1}_8$. But the most interesting point is that microcalorimetry is sufficiently sensitive to discriminate between the rigid tetramer and the flexible hexamer and octamer.

The formation of 1:2 complexes for $\mathbf{1}_8$ is of particular interest: as the heparin binding peptide sequences contain multiple blocks of positive charge, it may be expected that cooperative binding may become an important factor in the natural systems. We are currently extending the studies to include dipeptides and the much larger polypeptide heparin binding sites.

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