Molecular Selective Binding of Pyridinium Guest Ions by Water-Soluble Calix[4]arenes

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The complex stability constants (K_S) and thermodynamic parameters ($\Delta G°$, $\Delta H°$, and $T\Delta S°$) for 1:1 intermolecular complexation of water-soluble calix[4]arene tetrasulfonate (**CAS**) and thiacalix[4]arene tetrasulfonate (**TCAS**) with pyridinium guest ions **1–10** have been determined by means of titration calorimetry in an acidic buffer solution (pH = 2.0) at 298.15 K, and their binding modes have been investigated by NMR spectroscopy. Possessing a smaller cavity and a higher π electron density than **TCAS**, **CAS** affords the stronger binding abilities for all guests examined, giving an exciting molecular selectivity of up to 354 for the 2,6-dimethylpyridine/2,6-pyridinedicarboxylic acid pair. The high binding affinities, attributed to the favorable enthalpic gains, are comprehensively discussed from the viewpoint of π–π stacking, electronic effect and size-fit relationship between host and guest. The thermodynamic parameters that have been obtained, together with the NMR results, jointly demonstrate that the position, number, and type of substituent groups introduced onto the guest molecule are the key factors controlling the structural-energetics correlation for the molecular selective binding of water-soluble calixarenes.

Introduction

Calix[4]arenes,[1] composed of four phenolic units linked via methylene groups, are able to selectively recognize various neutral and charged inorganic/organic species in solution,[2] the solid state[3] and the gas phase[4] through intermolecular complexation. Among these recognition processes, the molecular recognition of calixarenes in aqueous solution seems to be more important because most biological processes occur in aqueous solution. Arena,[5] Stödeman[6,7] and Morel-Desrosiers[8,9] et al. have successively investigated the binding behaviors of some *p*-sulfonatocalix[4]arenes with organic ammonium cations by NMR spectroscopy and microcalorimetry, finding that the sulfonate groups of hosts appear to serve as anchoring points for positively charged guests to give a more stable inclusion complex. Coleman et al.[10] examined the binding thermodynamics of *p*-sulfonatocalix[*n*]arenes (*n* = 4, 6 and 8) with amino acids and polypeptides to understand the nature and manner of interactions between the synthetic receptors and glycosylaminoglycan (GAG) receptor sequences.[11–12] To efficiently remove the environmentally hazardous substances from water, Miyano and co-workers[13] studied the complexation behaviors and mechanism of thiacalix- and calix[4]arene tetrasulfonates with mono-substituted benzenes and halomethanes in neutral aqueous solution. Recently, we have also performed the comparative studies on the fluorescent behaviors and complexation thermodynamics of some representative dye guests with cyclodextrins and calixarenesulfonates.[14] However, studies on the recognition mechanism and thermodynamic behaviors of water-soluble calixarenes are still incomplete,[11,15] although these investigations are very important in understanding the cooperative contributions of several intermolecular interactions working between host (receptor) and guest (substrate) as well as the structure-energetics correlation in the molecular recognition. Therefore, we wish to report herein our investigative results on the intermolecular complexation of water-soluble *p*-sulfonatocalix[4]arenes with some pyridinium guest ions (Scheme 1) in an aqueous phosphate buffer solution (pH, 2.0) by titration microcalorimetry and NMR spectroscopy. A simple reason for choosing these pyridinium guest ions as guest molecules is that these molecules possess good structural comparability, which is favorable to examining the influence of the position, number and electronic effect of substituent groups of guest. Based on the microcalorimetric titration experiments and NMR spectra, we can establish the correlation between the thermodynamic parameters and the complex conformation, which will serve our understanding of the factors governing the molecular binding ability and selectivity of model substrates by water-soluble calixarenes.

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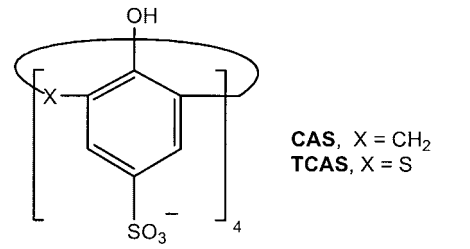

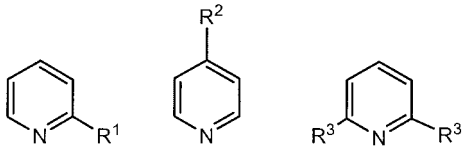

1: R^1 = H
2: R^1 = CH_3
4: R^1 = NH_2
6: R^1 = Cl
3: R^2 = CH_3
5: R^2 = NH_2
10: R^2 = CHO
7: R^3 = CH_3
8: R^3 = Cl
9: R^3 = COOH

Scheme 1. Structures of the host calix[4]arenes and pyridinium guest ions.

Results and Discussion

Binding Stoichiometry

It is widely reported that calix[4]arenes can form typical 1:1 complexes with model substrates. In our experiments, the titration data give the 1:1 binding stoichiometry between host and guest with "*N*" values in the curve fitting results varying from 0.90 to 1.11. Moreover, examinations with the Corey–Pauling–Koltun (CPK) molecular model clearly demonstrate that **CAS** or **TCAS** can only accommodate one pyridinium guest ion in its hydrophobic cavity (Figure 1), which subsequently rationalizes the 1:1 binding stoichiometry between water-soluble calix[4]arenes and pyridinium guest ions. Therefore, a fixed 1:1 binding stoichiometry is used in the curve-fitting analysis of calorimetric titration.

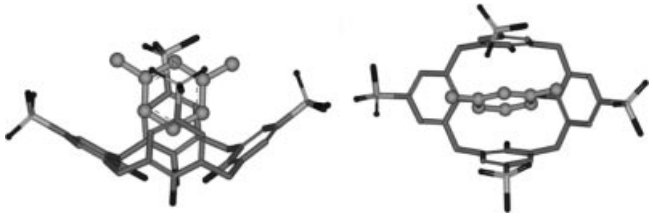

Figure 1. MM2-optimized binding mode of the **CAS-7** complex. (Hydrogen atoms were omitted for clarity, left for side view and right for top view).

Binding Mode

To explore the possible binding mode between water-soluble calix[4]arenes and pyridinium guest ions, ^{1}H NMR spectra were recorded in a pD 2.0 buffer solution and some representative results have been listed in Table 1. In all cases the signals of guest protons are observed as averaged single resonances because of the fast exchange on the NMR timescale between the free and complexed guest.[16] As can be seen in Table 1, the δ values of guest protons shift appreciably to higher fields after complexation with calix[4]arenes as compared with the uncomplexed guest. This indicates that the guest molecule is encapsulated into the calixarene cavity to form the intermolecular complex, which thus leads to an efficient shield toward guest protons. A close comparison of the $\Delta\delta$ values of guest protons after complexation with calix[4]arenes shows that the aromatic proton (or methyl protons) at the 4-position of pyridinium guest ions gives the highest $\Delta\delta$ value ($|\Delta\delta|$ = 0.33–0.86 ppm), followed by the aromatic protons (or methyl protons) at the 3- and 5-positions ($|\Delta\delta|$ = 0.20–0.71 ppm) as well as the aromatic protons (or methyl protons) at the 2- and 6-positions ($|\Delta\delta|$ = 0.15–0.30 ppm), which indicates that the pyridinium guest ions may penetrate into the calix[4]arene cavity from the *p*-position of the N atom, as illustrated in Figure 2a. According to this binding mode, the protonated N atom of the pyridinium guest ions is located close to the anionic sulfonate tails of the calix[4]arene, giving significant electrostatic interactions between host and guest. Interestingly, for

Table 1. ^{1}H NMR spectroscopic data of pyridinium guest ions in a pD 2.0 buffer solution in the presence of host calix[4]arenes.

Sample	δ [ppm] of **2**				
	2-CH_3	3-H	4-H	5-H	6-H
2	2.81	7.91	8.51	7.87	8.63
2 + **CAS**	2.61	7.40	7.79	7.32	8.33
2 + **TCAS**	2.61	7.48	7.96	7.43	8.41
	$\Delta\delta$ [ppm] of **2** induced by complexations				
	2-CH_3	3-H	4-H	5-H	6-H
with **CAS**	0.20	0.51	0.72	0.55	0.30
with **TCAS**	0.20	0.43	0.55	0.44	0.22
	δ [ppm] of **3**				
	2-H	3-H	4-CH_3	5-H	6-H
3	8.57	7.81	2.63	7.81	8.57
3 + **CAS**	8.42	7.61	2.14	7.61	8.42
3 + **TCAS**	8.39	7.53	2.30	7.53	8.39
	$\Delta\delta$ [ppm] of **3** induced by complexations				
	2-H	3-H	4-CH_3	5-H	6-H
with **CAS**	0.15	0.20	0.49	0.20	0.15
with **TCAS**	0.18	0.28	0.33	0.28	0.18
	δ [ppm] of **7**				
	2-CH_3	3-H	4-H	5-H	6-CH3
7	2.72	7.64	8.28	7.64	2.72
7 + **CAS**	2.45	6.93	7.42	6.93	2.45
7 + **TCAS**	2.48	7.08	7.61	7.08	2.48
	$\Delta\delta$ [ppm] of **7** induced by complexations				
	2-CH_3	3-H	4-H	5-H	6-CH3
with **CAS**	0.27	0.71	0.86	0.71	0.27
with **TCAS**	0.24	0.56	0.67	0.56	0.24
	δ [ppm] of **5**				
	2-H	3-H	4-H	5-H	6-H
5	7.99	6.85	–	6.85	7.99
5 + **CAS**	7.54	6.39	–	6.39	7.54
5 + **TCAS**	7.87	6.72	–	6.72	7.87
	$\Delta\delta$ [ppm] of **5** induced by complexations				
with **CAS**	0.45	0.46	–	0.46	0.45
with **TCAS**	0.12	0.13	–	0.13	0.12

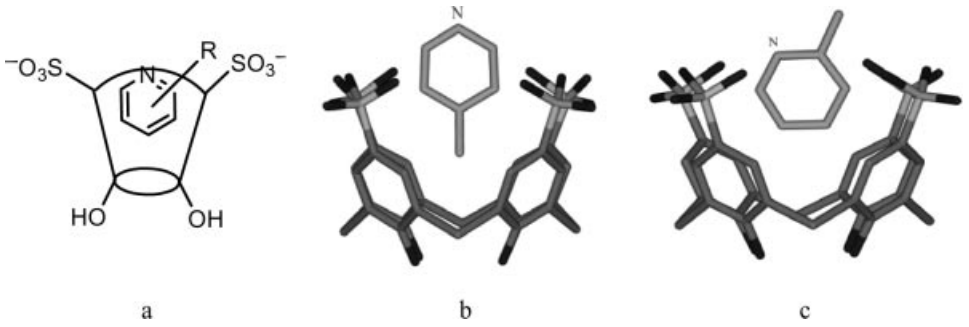

Figure 2. Possible binding modes of the guest with the host calix[4]arene.

symmetrical guests 4-methylpyridinium, 2,6-dimethylpyridinium and 4-aminopyridine, the aromatic protons (or methyl protons) at the 2- and 6-positions of the pyridinium ion give the same $\Delta\delta$ values upon complexation with calix[4]arene, and the same is also observed for the aromatic protons (or methyl protons) at the 3- and 5-positions. This indicates that these guests may penetrate perpendicularly into the calix[4]arene cavity, even in the case of the guest biscation (Figure 2b). However, for asymmetrical guest 2-methylpyridinium, the H-3 proton gives a smaller $|\Delta\delta|$ value than the H-5 proton upon complexation with calix[4]arene. This phenomenon indicates that the 2-methylpyridinium ion may penetrate into the calix[4]arene cavity with a tilt-in conformation as shown in Figure 2c. More interestingly, the calix[4]arene host is found to be able to adjust its conformation to fit the size of the guest molecule. ^{1}H NMR spectroscopic data show that, without pyridinium guest ions, the methylene protons of **CAS** give a single NMR peak assigned to a rapid cone-to-cone interconversion of the uncomplexed host, indicating that **CAS** exists in a flexible conformation. However, upon complexation with guests, this single peak splits into two sets of double peaks with the same integration intensities, which means that **CAS** shows a fixed C_{2v} cone conformation, because the conformation changes of the host become slow on the NMR time-scale following the inclusion of the guest (Figure 1). From the above ^{1}H NMR results, we can draw the conclusion that calix[4]arenes tends to adopt different conformations upon complexation with structure-related guests, and the symmetry of the guest as well as the induced-fit relationship between host and guest may be the main factors controlling these conformational adjustments.

Binding Ability and Thermodynamic Parameter

In order to investigate quantitatively the thermodynamic origin of the inclusion of **TCAS/CAS** with guests **1–10** and establish the structure-energetics correlation, the isothermal titration calorimetry (ITC) titrations were performed at 298.15 K in aqueous phosphate buffer (pH = 2.0), because the calorimetry measurement is the only method that directly measures the heat changes associated with intermolecular interactions. A representative titration curve is shown in Figures 3 and 4, and the thermodynamic parameters obtained are listed in Table 2. As can be seen from Figure 3, each titration of **TCAS** into the sample cell gave an apparent reaction heat, caused by the formation of an inclusion complex between guest **4** and **TCAS** (Figure 4). The reaction heat decreases after each injection of **TCAS** because less and less guest molecules are available to form inclusion complexes.

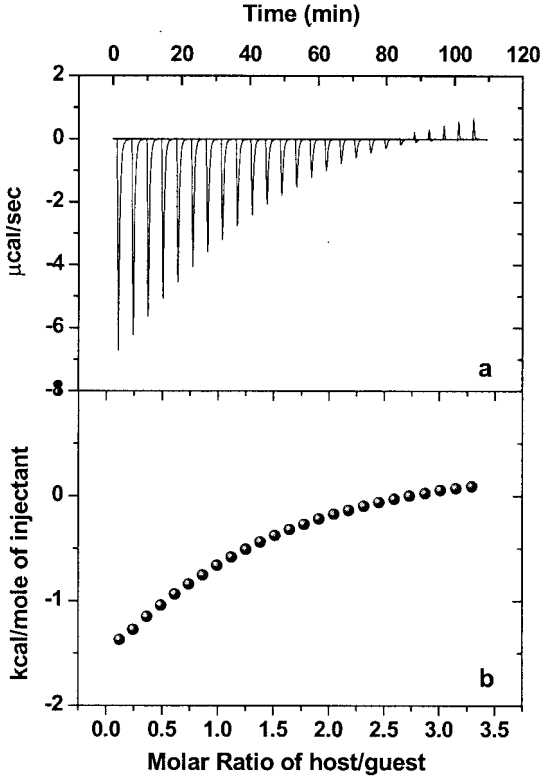

Figure 3. Microcalorimetric titration of **TCAS** with **4**. (a) Raw data for sequential injections of **TCAS** (19.82 mM) into **4** (1.15 mM). (b) Apparent reaction heat obtained from the integration of calorimetric traces.

It is well documented that, among several weak noncovalent interactions between host and guest, the electrostatic, hydrogen bond, π–π, C–H···π and van der Waals interactions mainly contribute to the enthalpic changes, while the conformation change and the desolvation effect contribute to the entropic changes. As can be seen from Table 2, all

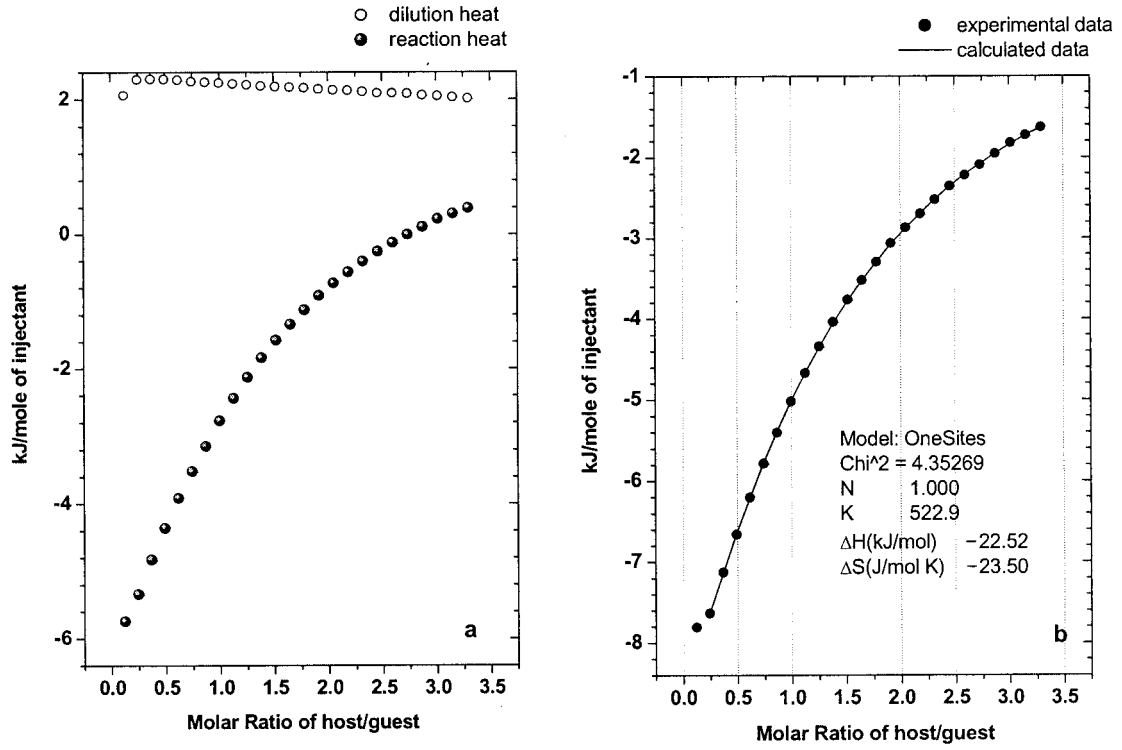

Figure 4. (a) Heat effects of the dilution and of the complexation reaction of **TCAS** with **4**. (b) "Net" heat effects of complexation of **TCAS** with guest **4**.

Table 2. Complex stability constants (K_s) and thermodynamic parameters for 1:1 intermolecular complexation of guests **1–10** with **CAS** and **TCAS** in a phosphate buffer solution (pH = 2.0) at 298.15 K.

Host	Guest	K_s	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]
CAS	**1**	8308 ± 20	–22.38 ± 0.01	–29.71 ± 0.01	–7.34 ± 0.01
	2	13265 ± 35	–23.53 ± 0.01	–34.70 ± 0.02	–11.17 ± 0.03
	3	4450 ± 15	–20.83 ± 0.01	–28.90 ± 0.01	–8.08 ± 0.00
	4	5219 ± 1	–21.25 ± 0.02	–30.97 ± 0.12	–9.73 ± 0.14
	5	858 ± 7	–16.74 ± 0.02	–22.99 ± 0.19	–6.25 ± 0.21
	6	1698 ± 38	–18.44 ± 0.05	–33.71 ± 0.05	–15.27 ± 0.01
	7	24050 ± 410	–25.01 ± 0.03	–38.57 ± 0.22	–13.56 ± 0.26
	8	343 ± 7	–14.47 ± 0.05	–33.50 ± 0.02	–19.03 ± 0.07
	9	68 ± 2	–10.48 ± 0.04	–27.97 ± 0.29	–17.50 ± 0.25
	10	73 ± 1	–10.63 ± 0.01	–26.47 ± 0.01	–15.85 ± 0.02
TCAS	**1**	448 ± 2	–15.13 ± 0.01	–19.95 ± 0.15	–4.83 ± 0.15
	2	1156 ± 10	–17.48 ± 0.02	–28.22 ± 0.44	–10.74 ± 0.45
	3	609 ± 2	–15.89 ± 0.01	–17.62 ± 0.06	–1.73 ± 0.07
	4	523 ± 1	–15.52 ± 0.01	–22.56 ± 0.04	–7.05 ± 0.04
	5	292 ± 6	–14.31 ± 0.31	–6.98 ± 0.18	7.33 ± 0.12
	6	142 ± 2	–12.29 ± 0.04	–26.56 ± 0.10	–14.27 ± 0.06
	7	3054 ± 2	–19.89 ± 0.00	–28.60 ± 0.02	–8.71 ± 0.02
	8	232 ± 1	–13.50 ± 0.00	–12.02 ± 0.04	1.48 ± 0.05
	9	67 ± 1	–10.43 ± 0.03	–12.52 ± 0.16	–2.24 ± 0.07
	10	–[a]			

[a] K_s or $\Delta H°$ was too small to be determined by titration microcalorimetry.

the intermolecular complexation between calix[4]arenes and guests are mainly driven by the favorable enthalpic changes ($\Delta H° < 0$), accompanied by either negative or slightly positive entropic changes ($T\Delta S° > 0$ or $T\Delta S° < 0$), which indicates that the electrostatic, hydrogen bond, π–π, C–H···π and van der Waals interactions may play a crucial role in the host-guest complexation. Since these interactions are closely related to the distance and contacting surface area between host and guest, a good host-guest induced-fit may dominate the stability of the complex formed between calix[4]arene and the model substrate. According to this concept, **CAS**, which possesses a smaller cavity with relatively higher π electron density, should give stronger binding abilities toward pyridinium guest ions than **TCAS**. Compared with **TCAS**, **CAS** shows enhanced binding ability towards the guests examined here. Especially for the complexation

with the pyridinium ion, **CAS** gives a K_s value of up to 8308 ± 20 M^{-1}; that is, 19 times higher than that for **TCAS**. We have demonstrated that the pyridinium guest ion penetrates into the calix[4]arene cavity from the 4-position of the N atom. A further comparison with ^{1}H NMR spectroscopic data indicates that these protons (methyl or H protons located at the 4-position of the N atom) give larger $|\Delta\delta|$ values upon complexation with **CAS** than with **TCAS** (Table 1), which indicates that these protons are more efficiently shielded upon complexation with **CAS**. Therefore, we can deduce that there should be a tighter complexation between **CAS** and guests than for the case of **TCAS**, which will consequently lead to the stronger binding ability of **CAS** to some extent. The significantly high binding ability of **CAS** arising from the host-guest size-fit can also be rationalized from the thermodynamic behaviors. By comparing the thermodynamic parameters for the complexations of calix[4]arenes with pyridinium guest ions, we can see that the stronger binding ability of **CAS** than **TCAS** mainly comes from the enthalpic contribution. As can be seen in Table 2, all of the pyridinium guest ions give more negative enthalpic changes upon complexation with **CAS** than with **TCAS**, which clearly indicates the stronger intermolecular interactions between **CAS** and guests. It is noteworthy that the host-guest complexation process, which leads to the loss of conformational freedom, is inherently accompanied by the entropic loss. Therefore, a good size-fit between **CAS** and pyridinium guest ions should also result in more unfavorable entropic changes than for the case of **TCAS**. However, a comparison of the differential reaction enthalpy (ΔH° = $\Delta H^\circ_{\mathbf{CAS}\text{–guest}}$ – $\Delta H^\circ_{\mathbf{TCAS}\text{–guest}}$ = –6.48 to –21.48 kJ mol^{-1}) and differential reaction entropy ($T\Delta S^\circ$ = $T\Delta S^\circ_{\mathbf{CAS}\text{–guest}}$ – $T\Delta S^\circ_{\mathbf{TCAS}\text{–guest}}$ = –0.43 to –20.51 kJ mol^{-1}) indicates that these additional entropic losses arising from the size-fit between the **CAS** and pyridinium guest ions are over-compensated by the larger enthalpic gain. These results demonstrate that the stronger binding of **CAS** toward the guest molecule is accomplished not by a reduction of the entropic loss, for which the desolvation of both host and guest upon complexation will be responsible, but by an increase of the originally favorable enthalpic gain, that is to say, by a strengthening of the intermolecular interactions.

It is also interesting to discuss the induced-fit effect of the guest molecule upon complexation. As can be seen in Table 2, 2-substituted guests (**2** and **4**) always give stronger binding with the host calix[4]arenes than their 4-substituted analogs (**3** and **5**). For example, the K_s value for the **CAS**/4-aminopyridine complexation is only 858 ± 7 M^{-1}, but significantly enhances to 5219 ± 1 M^{-1} for the **CAS**/2-aminopyridine complexation. ^{1}H NMR experiments demonstrate that the 2-substituted guest adopts a tilt-in conformation when penetrating the calix[4]arene cavity, which enables a close contact of both pyridine ring and methyl (or amino) substituent of guest molecule with aromatic rings of host calix[4]arene and thus leads to strong π–π and C–H···π (or N–H···π)[17] interactions between host and guest. Moreover, CPK molecular model studies also demonstrate that the tilt-in conformation of the 2-substituted guest fits into the calix[4]arene cavity better than the perpendicular-in conformation of the 4-substituted guest, which consequently leads to strong van der Waals interactions between host and guest. As a joint result of these two factors, 2-substituted guests exhibit higher K_s values upon complexation with host calix[4]arenes. This conclusion can be further verified by thermodynamic parameters. That is, the differential reaction enthalpy (ΔH° = ΔH°_{ortho} – ΔH°_{para} = –5.80 to –15.58 kJ mol^{-1}), which responds to the strengthened intermolecular interactions between host and guest, is found to be larger than the corresponding differential reaction entropy ($T\Delta S^\circ$ = $T\Delta S^\circ_{ortho}$ – $T\Delta S^\circ_{para}$ = –3.09 to –14.38 kJ mol^{-1}).

We are especially interested in the effect of the number of substituents of guests in the molecular recognition of calix[4]arenes. By comparing the thermodynamic parameters in Table 2, we find that the Gibbs free energy changes ($-\Delta G^\circ$) for the complexation of 2/6-methylated pyridinium guest ions (guests **1**, **2** and **7**) with calix[4]arenes linearly enhances with an increasing number (N_m) of the methyl groups of the guest (Figure 5), and the unit increments of $-\Delta G^\circ$ per methyl group are 0.23 kJ mol^{-1} for **CAS** and 0.42 kJ mol^{-1} for **TCAS**, respectively. A further examination of the thermodynamic parameters demonstrates that, although both **CAS** and **TCAS** give good linearity between $-\Delta G^\circ$ and N_m, its thermodynamic origin is somewhat different. With the increasing N_m, **TCAS** gives the similar differential exothermic enthalpy ($\Delta H^\circ_{\mathbf{1/2}}$ = $\Delta H^\circ_{\mathbf{TCAS}\text{–}2}$ – $\Delta H^\circ_{\mathbf{TCAS}\text{–}1}$ = –8.27 kJ mol^{-1}, $\Delta H^\circ_{\mathbf{1/7}}$ = $\Delta H^\circ_{\mathbf{TCAS}\text{–}7}$ – $\Delta H^\circ_{\mathbf{TCAS}\text{–}1}$ = –8.65 kJ mol^{-1}) and decremental unfavorable differential entropy ($T\Delta S^\circ_{\mathbf{1/2}}$ = $T\Delta S^\circ_{\mathbf{TCAS}\text{–}2}$ – $T\Delta S^\circ_{\mathbf{TCAS}\text{–}1}$ = –5.91 kJ mol^{-1}, $T\Delta S^\circ_{\mathbf{1/7}}$ = $T\Delta S^\circ_{\mathbf{TCAS}\text{–}7}$ – $T\Delta S^\circ_{\mathbf{TCAS}\text{–}1}$ = –3.88 kJ mol^{-1}), which indicates that the entropic gain from the desolvation effect of the guest molecule should be responsible for the increasing $-\Delta G^\circ$. However, upon complexation with guests **1**, **2** and **7**, **CAS** gives both the incremental exothermic differential enthalpy ($\Delta H^\circ_{\mathbf{1/2}}$ = $\Delta H^\circ_{\mathbf{CAS}\text{–}2}$ –

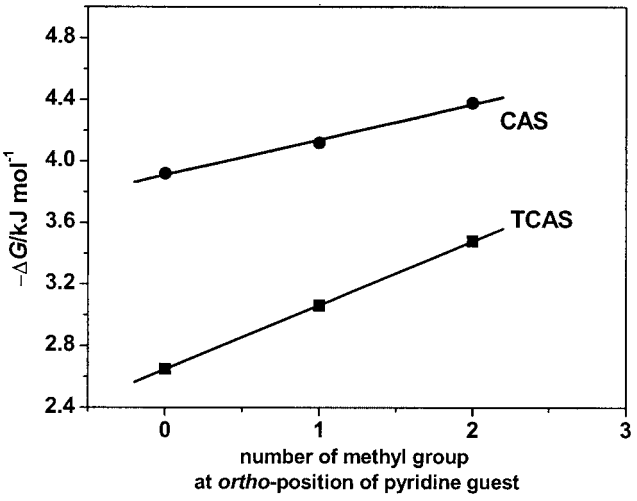

Figure 5. Plots of Gibbs free energy changes ($-\Delta G^\circ$) vs. the number of methyl groups at the 2/6-position of the pyridinium guest ions for the inclusion complexation of calix[4]arenes with 2/6-substituted guests.

$\Delta H°_{CAS-1}$ = –4.99 kJ mol^{-1}, $\Delta H°_{1/7} = \Delta H°_{CAS-7} - \Delta H°_{CAS-1}$ = –8.86 kJ mol^{-1}) and the incremental unfavorable differential entropy ($T\Delta S°_{1/2} = T\Delta S°_{CAS-2} - T\Delta S°_{CAS-1}$ = –3.83 kJ mol^{-1}, $T\Delta S°_{1/7} = T\Delta S°_{CAS-7} - T\Delta S°_{CAS-1}$ = –6.22 kJ mol^{-1}), and the differential enthalpy ($\Delta H°$) is always more negative than the corresponding differential entropy ($T\Delta S°$). This indicates that the enthalpic gain from the strengthened C–H···π interactions mainly contributes to the increasing $-\Delta G°$ for the complexation of **CAS** with guests **1**, **2** and **7**, which over compensate the unfavorable entropic loss.

It is well known that the intensity of π–π interactions, one of the most important intermolecular interactions between calixarene and aromatic guests, depends on the π electron densities of the host and guest to a large degree. The introduction of an electron-donating or electron-withdrawing substituent to the guest's aromatic ring will inevitably change the π electron density of the guest and thus affect the strength of the π–π interaction between the host and guest. Subsequently, this influence will lead to the appreciable changes in the host-guest binding abilities and the thermodynamic parameters. By comparing the structural features of guest molecules, we can divide the examined pyridinium guest ions into three families, that is, 2-substituted guests (**2** and **4**), 4-substituted guests (**3**, **5** and **10**) and 2,6-disubstituted guests (**7** and **9**). In each family, **CAS** and **TCAS** give the same sequence of binding ability towards the guest molecules, which is **2** > **4**, **3** > **5** > **10**, **7** > **9**. That is, the guest molecules possessing an electron-donating substituent (guests **2**, **4**, **3**, **5**, **7**) always give a higher K_s value upon complexation with host calix[4]arenes than the guest molecules bearing an electron-withdrawing substituent (guests **9**, **10**), and the methylated guests (guests **2**, **3**, **7**) always give the highest K_s value upon complexation with calix[4]arenes. These phenomena consequently lead to some exciting molecular selectivity among these three families of guests. For 2-substituted guests, **TCAS** gives the highest molecular selectivity for the **2/6** pair of up to 8.1 (K_{s2}/K_{s6}). For 4-substituted guests, the highest molecular selectivity significantly enhances to 61 for the **3/10** pair by **CAS**. More significantly, for 2,6-disubstituted guests, the highest molecular selectivity even enhances to 354 for the **7/9** pair by **CAS**. The significantly high binding abilities of the methylated guest may mainly result from the C–H···π interaction between the methyl groups of the guest and the aromatic rings of the host calix[4]arenes. In addition, the existence of an electron-donating substituent enhances the π electron density of the guest and thus strengthens the π–π interactions between hosts and guests. This conclusion can also be verified by the thermodynamic parameters, because the methylated pyridinium guest ions always give more negative enthalpic changes upon complexation with calix[4]arene than its non-methylated analogs. On the other hand, although possessing an electron-donating substituent, aminopyridinium ions (**4** or **5**) give smaller K_s values upon complexation with calix[4]arenes than methylpyridinium ions (**2** or **3**). This may be attributed to the relatively high hydrophilicity of the amino substituent, which weakens the hydrophobic interactions between host and guest to some extent and thus leads to the weaker binding abilities. For guests **6**, **8**, **9** and **10**, the existence of an electron-withdrawing substituent reduces the π electron density of the pyridine ring, which consequently leads to a relatively weak π–π interaction between host and guest. This unfavorable π–π interaction, together with the absence of C–H···π (or N–H···π) interactions, jointly result in the weakest binding of these guests.

Enthalpy-Entropy Compensation

The enthalpy-entropy compensation effect is well known for a large amount of data including supramolecular associations,[18–19,20] ligand binding studies,[21,22] calorimetric measurements of protein unfolding,[23] optical detection of thermal denaturation of diverse nucleic acid systems,[24] and solvent transfer,[25] enzyme kinetics,[26,27] etc. The slope (a) and intercept ($T\Delta S°$) of the $\Delta H° - T\Delta S°$ plot can be used as the statistic representation for the degree of conformational change and the extent of desolvation of host and/or guest upon complexation. In the present study, the $T\Delta S°$ values are also plotted against the $\Delta H°$ values by using the thermodynamic parameters obtained in this work and the published data for water-soluble calix[4]arenes.[5,7,9,10,12,19,28,29] As can be seen from Figure 6, the plot of $\Delta H°$ against $T\Delta S°$ gives an excellent straight line with a correlation coefficient of 0.95. The obtained slope (a) and the intercept ($T\Delta S°$) values for water-soluble calix[4]arenes as well as the corresponding values for some natural and synthetic receptors are summarized in Table 3. As can be seen from Table 3, water-soluble calix[4]arenes exhibited the largest a value (a = 1.00) and the second largest $T\Delta S°$ value ($T\Delta S°$ = 18.34 kJ mol^{-1}) among these molecular/ionic receptors. The largest a value suggests that water-soluble calix[4]arenes experience great conformational changes upon complexation with various model substrates, which is in good agreement with the report that calixarenes possess certain conformational flexibility and are easily able to alter their conformations upon complexation. On the other hand, the second largest $T\Delta S°$ value further confirmed the key role of the fairly extensive desolvation of the host and guest upon the complexation. Before the complex formation, both calixarene and the guest molecules are solvated, and the solvent molecules around the solutes are highly ordered. During the complexation, before the guest molecule enters into the calixarene cavity, it has to lose its solvation shell and also, the solvent molecules have to leave the calixarene cavity to jointly cause the disorder to increase. Therefore the entropy of the system considerably increases during this process. Then, the guest molecules enter into the calixarene cavity to form host-guest complexes with a higher ordered conformation. The latest process would slightly decrease the entropy. Consequently, the entropy changes of the calix[4]arene systems would be positive and reasonable up to 18.34 kJ mol^{-1}.

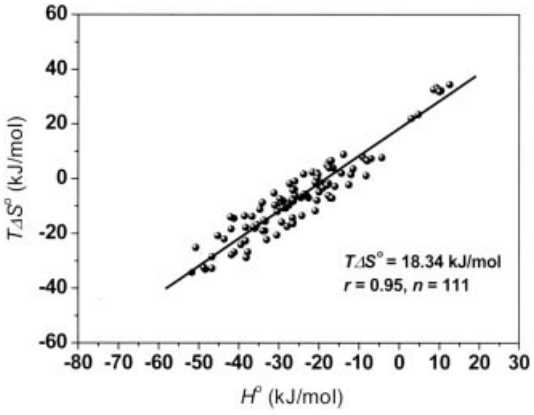

Figure 6. Enthalpy–entropy compensation plot for the inclusion complexation of different guests by water-soluble calix[4]arenes in water at 298.15 K.

Table 3. Slope (a) and intercept ($T\Delta S°$) values of $\Delta H° - T\Delta S°$ plots for complexation of some molecular/ionic receptors in homogeneous solution.

Host	a	$T\Delta S°$ [kJ mol^{-1}]
Antibiotic[30]	0.95	23.43
α-Cyclodextrin[31]	0.79	8
β-Cyclodextrin[31]	0.80	11
γ-Cyclodextrin[31]	0.97	15
Crown ethers[18b]	0.76	10.04
Cryptand[18b]	0.51	16.74
Proteins[32]	0.92	13.82
Nucleic acids[32]	0.87	5.80
Cyclophane[18]	0.78	14.23
Water-soluble calix[4]arene	1.00	18.34

Experimental Section

Materials: Two *p*-sulfonatocalix[4]arenes, i.e. calix[4]arene-tetrasulfonate (**CAS**) and thiacalix[4]arene-tetrasulfonate (**TCAS**), were synthesized and purified according to the literature reports.[13b,33] Guest molecules, pyridine (**1**), 2-picoline (**2**), 4-picoline (**3**), 2-aminopyridine (**4**), 4-aminopyridine (**5**), 2-chloropyridine (**6**), 2,6-dimethylpyridine (**7**), 2,6-dichloropyridine (**8**), 2,6-pyridinedicarboxylic acid (**9**) and 4-pyridinecarbaldehyde (**10**), were purchased from Acros and used without further purification. The phosphate buffer solution (pH, 2.0) was prepared by dissolving sodium dihydrogen phosphate in distilled, deionized water to make a 0.1 mol dm^{-3} solution, which was then adjusted to pH 2.0 by phosphoric acid. The pH value of the buffer solution was verified on a Sartorius pp-20 pH-meter calibrated with two standard buffer solutions. The reason for choosing the phosphate buffer is that it has no apparent influence on the complexation, but some other biological buffers are tested to interact with calixarene sulfonates to some extent.[8] At such a pH value, every sulfonate group of **CAS** or **TCAS** is in the anionic form, while most of the guests except **6** and **8** should exist as pyridinium cations according to the reported pK_a values of *p*-sulfonatocalix[4]arenas[34] and guest pyridines.[35]

Measurement: ^{1}H NMR spectra were recorded at pD 2.0 on a Varian Mercury VX300 spectrometer using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external reference. The observed changes ($\Delta\delta_{obs} = \delta_{obs} - \delta_{free\ guest}$) in chemical shifts induced by the complexation of hosts (1 mM) with representative guests **2**, **3**, **5** and **7** ([**2**] = 5 mM, [**3**] = 6 mM, [**5**] = 8 mM, [**7**] = 8 mM) were calculated. A thermostatted and fully computer-operated isothermal calorimetry (VP-ITC) instrument, purchased from Microcal Inc., Northampton, MA., was used for all microcalorimetric experiments. The VP-ITC instrument was calibrated chemically by measurement of the complexation reaction of β-cyclodextrin with cyclohexanol and the obtained thermodynamic data were shown to be in good agreement (error <2%) with the literature data.[31] All microcalorimetric titrations between water-soluble calix[4]arenes and guest pyridines were performed in aqueous phosphate buffer solution (pH, 2.0) at atmospheric pressure and 298.15 K. Each solution was degassed and thermostatted by a ThermoVac accessory before the titration experiment. Twenty-five successive injections were made for each titration experiment. A constant volume (5 μL/injection or 10 μL/injection) of guest (or host) solution (10.0–20.0 mM) in a 0.250 mL syringe was injected into the reaction cell (1.4227 mL) charged with host (or guest) molecules solution (0.5–1.0 mM) in the same buffer solution. A control experiment was carried out with each run to determine the dilution heat by injecting a guest (or host) buffer solution into a pure buffer solution containing no host (or guest) molecules. The dilution heat determined in these control experiments was subtracted from the apparent reaction heat measured in the titration experiments to give the net reaction heat. The net reaction heat in each run was analyzed by using "one set of binding sites" model (ORIGIN software, Microcal Inc.) to simultaneously compute the binding stoichiometry (N), complex stability constant (K_s), standard molar reaction enthalpy ($\Delta H°$) and standard deviation from the titration curve. Generally, the first point of the titration in a series of injections showed a smaller heat effect than it should have, which could result from bending the syringe needle a little when seating the injector into the barrel, or leakage resulting from having the syringe in the cell a long time before the first injection is made (particularly if it is stirring all the time). Therefore, the first data point of the titration was removed manually before doing the curve-fit. Knowledge of the complex stability constant (K_s) and molar reaction enthalpy ($\Delta H°$) enabled the calculations of the standard free energy ($\Delta G°$) and entropy changes ($\Delta S°$), according to $\Delta G° = -RT\ln K_s = \Delta H° - T\Delta S°$, where R is the gas constant and T is the absolute temperature.

To check the reproducibility of the observed thermodynamic parameters, two independent titration experiments were carried out. The average values and standard deviations of the two independent titration experiments were listed in Table 2.

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