Ion Template Effects on the Conformation of Water-Soluble Calixarenes

Seiji Shinkai,* Koji Araki, Michio Kubota, Takashi Arimura, and Tsutomu Matsuda

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

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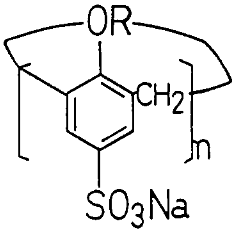
The template effects of alkali metal ions and organic ammonium ions on the rate of calixarene ring inversion were investigated. In the temperature-dependent ^{1}H NMR spectra in D_2O, the coalescence temperature (T_c) of *p*-sulfonatocalix[4]arene (1_4H) was enhanced up to 17–26 °C in the presence of alkali metal ions and up to 50–65 °C in the presence of ammonium ions from 9 °C in the absence of these ions. The temperature rise, which means the suppression of the ring-inversion rate, was explained as such that these ions have the template effects on the calixarene ring inversion. Organic ammonium ions, in comparison to alkali metal ions, exerted large template effects. In particular, di- and tricationic ammonium ions (e.g., *m*- and *p*-*N*,*N'*-hexamethylphenylenediammonium ions) were very effective. This is rationalized in terms of the electrostatic bridge occurring on the cavity edge. From plots of ^{1}H NMR chemical shift versus $[1_n\text{H}]/[\text{guest}]$, the association constants for these template molecules were estimated. The possible relations between the binding mode and the association ability and the mechanisms of ring inversion were discussed.

Calixarenes are cyclic oligomers made up of benzene units just as cyclodextrins are made up of glucose units. Although these two macrocycles have similar cylindrical architecture, there exists an essential difference that leads to an important difference in their molecular recognition abilities: the cyclodextrin cavity has been fixed whereas the calixarene cavity is still flexible because of the rotational freedom remaining in each benzene unit.[1,2] Thus, calixarenes have conformational isomers called "cone" and "alternate".[1,2] The cone conformation in calix[4]arene and its derivatives can be conveniently discriminated from others by ^{1}H NMR spectroscopy: observing the resonances arising from the $ArCH_2Ar$ methylene protons, one can detect a pair of doublets for cone calixarenes under slow-exchange conditions whereas it becomes a sharp singlet at high temperature.[1-3] From the temperature dependence one can estimate the coalescence temperature (T_c) which reflects the rate of ring invension between mirror-image cone conformations. Gutsche and Bauer[4,5] determined the T_c values of calixarenes in various solvents and reached a conclusion that the high T_c is attained in aprotic solvents such as chloroform and toluene whereas the T_c is lowered in basic solvents such as acetone and pyridine. The results indicate that the cone conformation is stabilized by intramolecular hydrogen-bonding interactions among OH groups, which compete with intermolecular hydrogen-bonding interactions with solvent molecules. Thus, the intramolecular hydrogen-bonding interactions can reduce the conformational freedom in calixarenes. Similar template effects may be expected for calixarene–guest interactions if guest molecules can cross-link each phenol unit as do hydrogen bonds. However, the study of such a guest template effect has been very limited: the difficulty is related to the absence of the calixarenes suitably functionalized for this purpose.[6] We recently synthesized water-soluble *p*-sulfonatocalix[*n*]arene derivatives (1_nR: $n = 4$, 6, and 8), host-guest interactions of which have been well characterized.[7-10] Thus, this system is undoubtedly suitable to examination of the guest template effect. In this paper we report a new finding that alkali metal cations and organic ammonium cations serve as efficient template ions to suppress the rate of ring inversion.

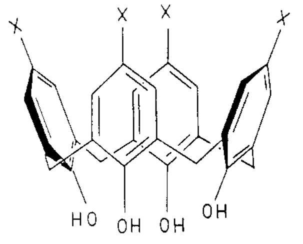
Table I. pD Dependence of T_c (°C) for 1_4H[a]

guest (concn, M)	pD 1.0	pD 8.9	pD 12
none[b]	<0	9	10
NaCl (2.2)	ppt[c]	17	25
2 (2.0)	30	65	65

[a] $[1_4] = 1.10 \times 10^{-2}$ M. The pD was adjusted with DCl (pD 1.0), 0.5 M borate (pD 8.9), and NaOD (pD 12). [b] The same solution contains Na^+ (0.077 M) as counterions and buffer species. [c] The precipitation of 1_4H was observed.



1_nR (R=H,CH$_3$)



"Cone" structure of 1_nR

Results and Discussion

pD Dependence. Izatt et al.[11,12] found that *p*-*tert*-butylcalix[*n*]arenes ($n = 4$, 6, and 8) can extract alkali metal cations from basic aqueous media to organic media. The finding supports the view that alkali metal cations strongly

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Table II. Influence of Bound Alkali Metal Cations on T_c of 1_4H[a]

guest salt (concn, M)	T_c, °C	$\Delta G^{\ddagger}$ at T_c,[c] kcal mol^{-1}	$\Delta G^{\ddagger}$ at 25 °C,[d] kcal mol^{-1}	$\Delta H^{\ddagger}$,[d] kcal mol^{-1}	$\Delta S^{\ddagger}$,[d] cal mol^{-1} deg^{-1}
none[b]	9	12.8	14.1[e]	10.4[e]	-12.0[e]
LiCl (0.17)	9	12.8			
LiCl (4.60)	26	13.6			
NaCl (0.25)	9	12.8			
NaCl (2.20)	17	13.2	14.6	12.0	-8.8
NaCl (4.70)	25	13.5			
KCl (0.17)	11	12.9			
KCl (2.30)	20	13.3			
CsCl (0.17)	17	13.2			
CsCl (3.80)	17	13.2			
$MgCl_2$[f] (0.17)	18	13.2			
$MgCl_2$[f] (0.80)	20	13.3			
$CaCl_2$[f] (0.17)	18	13.2			
$CaCl_2$[f] (0.80)	23	13.5			

[a] pD 8.9 with 0.5 M borate buffer, $[1_4H] = 1.0 \times 10^{-2}$ M. [b] The sample solution contains Na^+ (0.077 M) as counterions and buffer species. [c] $\Delta G^{\ddagger}$ values at T_c were calculated by the method described in ref 19. [d] $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ values were determined by the computer-assisted complete line-shape analysis method. The $\Delta G^{\ddagger}$ values determined from the complete line-shape analysis method are greater by 0.4–1.0 kcal mol^{-1} than those calculated from T_c. [e] Cited from ref 29. [f] pD 6.77 with 0.5 M borate buffer.

interact with oxyanions on the lower rim of calixarenes, but it is still unknown if the bound metal cations exert the template effect on the calixarene conformation in solution. We selected water-soluble calixarenes 1_nR as host molecules because the aqueous system is most convenient to assess metal–calixarene interactions quantitatively. Since the basicity of *p*-sulfonate groups is so weak that the template effects (if any) should be caused by the metal–oxide interactions on the lower rim. This view is supported by the fact that in every case, added metal cations show no effect on T_c of 1_nMe ($n = 4, 6$), which have no oxide anions on the lower rim.

It is known that four pK_a values of 1_4H split because of strong intramolecular hydrogen bonding: $pK_{a1} < 1$, $pK_{a2} = 3.0$, $pK_{a4} = 4.0$, and $pK_{a4} > 11$.[13] We thus determined the T_c as a function of medium pD in D_2O. In neutral and basic media ^1H NMR spectra of 1_4H show a single peak for the $ArCH_2Ar$ protons at 3.93 ppm at high temperature and split peaks at low temperature (Figure 1). The T_c values were estimated to be 9 °C (Table I). In acidic media, on the other hand, the peak did not split even at 0 °C. This suggests that in an aqueous system, the oxide anions act as stronger acceptors for intramolecular hydrogen bonds than the hydroxyl groups. This difference should lead to the stabilization of the cone conformation in neutral and basic pH region. We found that addition of NaCl or trimethylanilinium chloride (2) significantly enhances the T_c at pH 1–12. We thus carried out more quantitative experiments at intermediary pD (mainly at pD 8.9) because three OH groups are ionized at pD 8.9 and the dissociation of the fourth OH group does not take place at this pD region.

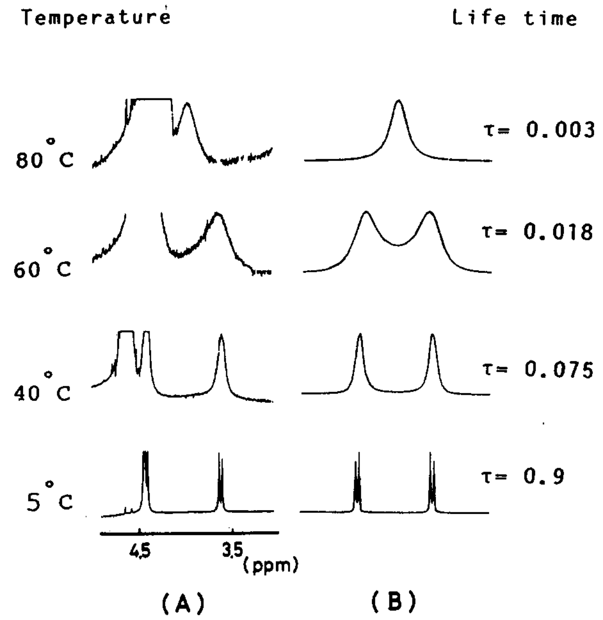

Figure 1. (A) Partial ^1H NMR for $ArCH_2Ar$ protons in 1_4H (1.00×10^{-2} M) in the presence of 5 (2.00×10^{-2} M): D_2O, pD 8.9 with 0.5 M borate buffer. In the absence of 5, the peaks coalesced at 9 °C. The signal which overlaps with the low-field protons is HDO. This signal moves to higher magnetic field with increasing temperature. (B) Simulated spectra assuming life times.

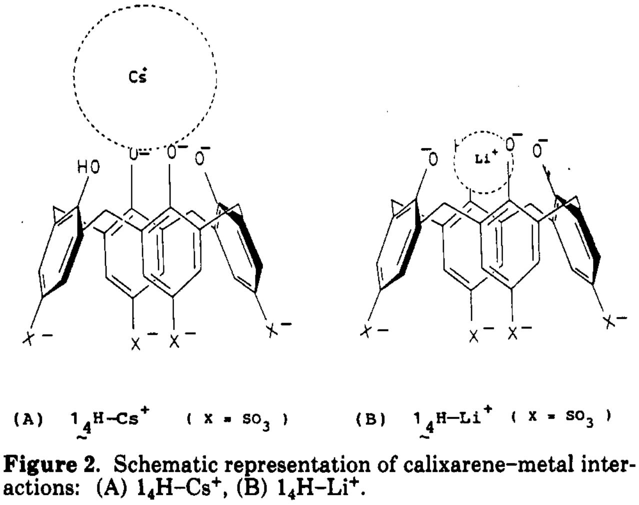

Figure 2. Schematic representation of calixarene–metal interactions: (A) 1_4H-Cs^+, (B) 1_4H-Li^+.

Metal Template Effects. The T_c values for 1_4H determined by ^1H NMR analysis are summarized in Table II. It is seen from Table II that the T_c increases with increasing alkali metal concentrations. The large metal cations (e.g., Cs^+) are more effective at low concentration whereas the small metal cations (e.g., Li^+) are more effective at high concentration. The highest T_c (26 °C) was attained in the presence of 4.60 M Li^+, which was higher by 17 °C than that in the absence of alkali metal cations (9 °C). It is known that the cavity composed of four oxygens in calix[4]arenes is relatively small and possibly fits the size of Li^+ or Na^+.[6,14–18] Thus, the template effects observed for alkali metal cations suggest that the T_c in aqueous solution is affected not only by the "hole-size

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Table III. Influence of Bound Ammonium Cations on T_c[a]

guest (concn, M)	T_c, °C		for 1_4H			
	1_4H	1_6H	$\Delta G^\ddagger$ at T_c,[b] kcal mol^{-1}	$\Delta G^\ddagger$ at 25 °C,[c] kcal mol^{-1}	$\Delta H^\ddagger$,[c] kcal mol^{-1}	$\Delta S^\ddagger$,[c] cal mol^{-1} deg^{-1}
none	9	<0	12.8	14.1[d]	10.4[d]	-12.0[d]
2 (0.005)	20		13.3	14.5[e]	10.6[e]	-13.0[e]
2 (0.010)	43		14.4	15.6[e]	14.5[e]	-3.1[e]
2 (0.020)	50		14.7	15.7[d]	14.6[d]	-1.8[d]
2 (2.00)	65	30	15.4	16.4	17.0	2.0
3 (0.010)	30	<0	~13.8			
3 (2.00)	65	50	15.4			
4 (0.010)	50	25	14.7			
5 (0.010)	50	<0	14.7			
5 (0.020)	65		15.4	16.6	17.2	2.0
6 (0.010)	50	<0	14.7			
7 (0.005)	25		13.5			
7 (0.010)	50		14.7			
7 (0.020)	57		15.0	16.7[d]	18.7[d]	6.7[d]

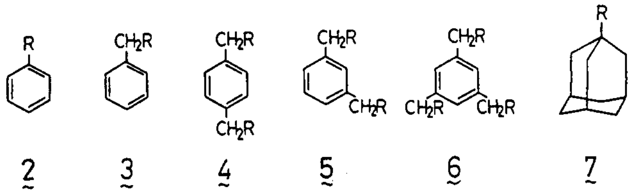
[a] pD 8.9 with 0.5 M borate buffer, $[1_nH] = 1.00 \times 10^{-2}$ M. [b] $\Delta G^\ddagger$ values at T_c were calculated by the method described in ref 19. [c] $\Delta G^\ddagger$, $\Delta H^\ddagger$, and $\Delta S^\ddagger$ values were determined by the computer-assisted complete line-shape analysis method. [d] Cited from ref 29. At [**2**] = 0.020 M 98.3% of 1_4H include **2**. [e] These data should be used only for qualitative discussion because at [**2**] = 0.005 M the temperature change in the NMR spectra is affected not only by the change in the inversion rate but also by the change in the association degree.

fitness" but also by the metal solvation effect. Cs^+ is less hydrated in aqueous media, so that it can interact with 1_4H even at the low concentration. However, the T_c is not enhanced appreciably because of the mismatch between the ion size and the cavity size (Figure 2, A). In contrast, Li^+ is strongly hydrated in aqueous media, so that it exerts little effect on T_c at the low concentration. However, once it is bound to 1_4H, it exerts a strong template effect on T_c (Figure 2, B). We found that alkaline earth metal cations are also effective as template ions (Table II), but they precipitated at the higher concentrations.

We calculated the $\Delta G^\ddagger$ at T_c according to the method described by Kurland et al.[19] The $\Delta G^\ddagger$ value in the absence of guest ions (12.8 kcal mol^{-1}) is somewhat smaller than those determined for calix[4]arene derivatives in organic media (13–16 kcal mol^{-1}).[3,5] This suggests that intramolecular hydrogen bonding interactions, a driving force for suppressing the rate of ring inversion in organic media, are weakened in aqueous media. The T_c rise from 9 to 26 °C corresponds to the increase in $\Delta G^\ddagger$ by 0.8 kcal mol^{-1}. This is attributed to the template effect on Li^+. According to computer-assisted complete line shape analysis,[3] we also determined the activation parameters for the rate of ring inversion in the presence of Na^+ (the method will be described in detail later for ammonium cations). The data in Table II indicate that the increase in $\Delta G^\ddagger$ is mainly caused by the increase in $\Delta H^\ddagger$.

The foregoing results indicate that the T_c rise should be ascribed to the "template effect" of metal cations which are entrapped in the cavity composed of oxide and/or hydroxyl groups. Under the similar conditions, the ^1H NMR absorption of the $ArCh_2Ar$ methylene protons in 1_6H appeared as a sharp singlet even at 0 °C (also in the presence of these metal cations). The extraction data by Izatt et al.[11,12] indicate that metal–calixarene interactions can also occur in calix[6]arenes. Thus, this result indicates that even though metal cations are bound to 1_6H, the interaction is not strong enough to suppress the molecular motion of 1_6H (at least, not strong enough to be detected by the NMR method). Probably, the difference between 1_4H and 1_6H would be accounted for by the rigid ring structure of calix[4]arenes.[1,2,6]

Templete Effects of Ammonium Cations. In order to study if organic ammonium cations raise the T_c through host–guest interactions, we selected six ammonium cations as guest molecules; monocationic **2**, **3**, and **7**, dicationic **4** and **5**, and tricationic **6**.



Guest molecules [R = $N^+(CH_3)_3Cl^-$]

As shown in Table III, the T_c was efficiently anhanced in the presence of these ammonium cations. In particular, dicationic **4** and **5**, tricationic **6**, and hydrophobic **7** were very effective, the T_c values being increased up to 50–65 °C even in the presence of small amounts of these cations (~0.010 M). Furthermore, they could elevate the T_c of 1_6H, for which alkali metal cations were totally ineffective. The results suggest that guest inclusion in the calixarene cavity plays an important role. This view is supported by the following findings: (i) although the T_c for 1_6Me is still lower than 0 °C (even in the presence of excess ammonium cations), the ^1H NMR peaks are significantly broadened (e.g., the half-height line width for the $ArCH_2Ar$ methylene protons in 1_6Me changes from 3.4 Hz in the absence of guests to 17.0 Hz in the presence of 2.0 M of **4** at 25 °C) and (ii) the chemical shifts of these guest molecules move to higher magnetic field in the presence of 1_4H and 1_6H (vide ante). Since 1_6Me has no hydroxyl group, finding i indicates that these ammonium cations interact with the cavity of 1_6Me to enhance the T_c. Finding ii indicates that the protons in these guest molecules are affected by the ring current of the benzene components in host calixarenes. These findings suggest that also in 1_nH, ammonium guests interact not only with the ionophoric OH (or O^-) groups but also with the hydrophobic calixarene catity. The CPK model building and the X-ray crystallographic studies both reveal that cone calixarenes (or "winged" for $1_6R^{1,2,5}$)

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[15] Bott, S. G.; Coleman, A. W.; Atwood, J. L. *J. Am. Chem. Soc.* **1986**, *108*, 1709.

[16] Bott, S. G.; Coleman, A. W.; Atwood, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 610.

[17] Böhmer, V.; Schade, E.; Vogt, W. *Makromol. Chem., Rapid Commun.* **1984**, *5*, 221.

[18] We found that the THF solution of *p*-(4-nitrophenyl)azocalix[4]arene colors only in the presence of $LiClO_4$ whereas other alkali metal cations greater than Li^+ are totally ineffective. This shows that the ionophoric cavity of this calix[4]arene strictly fits the size of Li^+.

[19] Kurland, R. S.; Rubin, N. B.; Wise, W. B. *J. Chem. Phys.* **1964**, *40*, 2426.

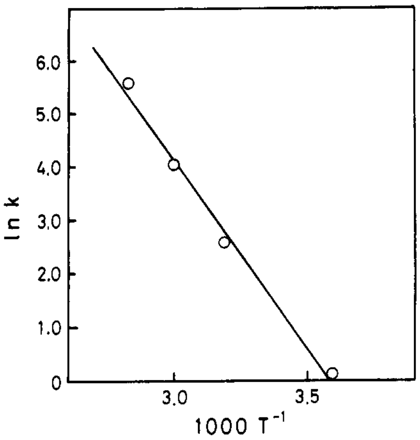


Figure 3. Arrhenius plot for the rate of 1_4H ring inversion: [1_4H] = 1.00 × 10^{-2} M, [5] = 2.00 × 10^{-2} M.

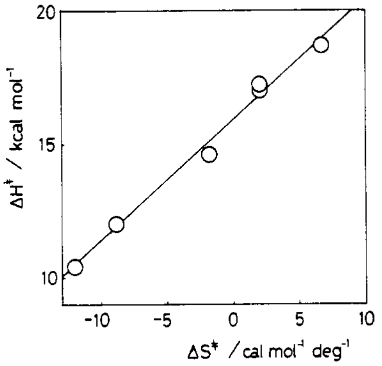
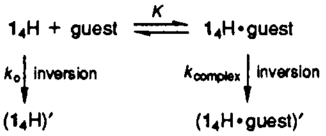


Figure 4. ΔH^*–ΔS^* Compensation relationship.

provide a cavity suitable for guest inclusion.[14-16] Recent circular dichroism studies using chiral calixarenes also support this: the conformation of water-soluble calixarenes tends to adopt the cone conformation upon inclusion of guest molecules.[20-22] Based on these reasons, we consider that both electrostatic and hydrophobic interactions operate for the efficient template effect by the ammonium guest moelcules.

Here, we calculated the ΔG^* at T_c according to the method of Kurland et al.[19] and ΔH^* and ΔS^* according to computer-assisted complete line shape analysis.[3] As shown in Figure 1, the temperature-dependent ^{1}H NMR spectral change was simulated assuming a life time (τ) at each temperature. Excellent agreement is seen between the observed (A) and the simulated spectra (B). An Arrhenius plot of log k (rate constant for the spin exchange: τ^{-1}) vs T^{-1} afforded an excellent linear relationship ($r > 0.99$; Figure 3). By least-squares computation we obtained log A and E_a, from which we calculated ΔH^* and ΔS^*. It is seen from Table III that the T_c rise from 9 to 65 °C corresponds to the increase in ΔG^* by 2.6 kcal mol^{-1}. Careful examination of Table III reveals that both ΔH^* and ΔS^* increase with increasing ΔG^*. This implies that the ΔG^* increase (i.e., suppression of the ring inversion rate) is caused by the ΔH^* increase. As shown in Figure 4, ΔH^* and ΔS^* compensate each other. The isokinetic temperature, β = 450 K (r = 0.995), supports the view that the process is primarily governed by the enthalpy term.

Scheme I



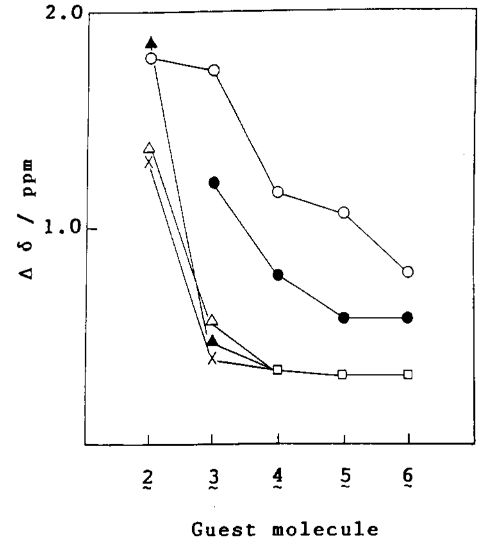


Figure 5. Upfield shifts of ^{1}H NMR peaks of guest molecules in the presence of excess 1_4H (10·[guest]): D$_2$O, pD 7.3, 25 °C, (○) N$^+$-CH$_3$, (●) CH$_2$, (△) o-H, (×) m-H, (▲) p-H, (□) Ar-H.

Here, we discuss how the guest molecule suppresses the rate of ring inversion. There exist two possible mechanisms for ring inversion (Scheme I); that is, (i) the 1_4H·guest complex cannot be inverted by itself and inversion proceeds only from "free" 1_4H (i.e., $k_{complex} = 0$) or (ii) ring inversion occurs both from "free" 1_4H and complexed 1_4H (i.e., $k_o > k_{complex} > 0$), where (1_4H)′ and (1_4H·guest)′ denote the mirror-image conformers of 1_4H and 1_4H·guest, respectively.

As shown in Table III, the ΔG^* value in the absence of guest is 14.1 kcal mol^{-1} (k = 282 s^{-1}). Compound 1_4H can be inverted even in the presence of excess 2 (0.50–2.00 M) where 1_4H totally exists as a 1_4H·2 complex. The ΔG^* values determined at several 2 concentrations were almost constant (16.4 ± 0.1 kcal mol^{-1}; k = 5.76 s^{-1}). At [2] = 0.005 M, 48.3% of the 1_4H form the complex (this can be calculated from $K = 2.10 \times 10^4$ M^{-1}).[29] If the rate of ring inversion is given by the sum of these two processes, the k value at [2] = 0.005 M is expected to be 149 s^{-1}

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(25) Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6085.

(26) The upfield shifts (Δδ) observed for 2 in the presence of excess 1_4H at pD 1.0 are 0.25 ppm for N$^+$-CH$_3$, 0.82 ppm for o-H, 1.26 ppm for m-H, and 2.42 ppm for p-H.

(27) The upfield shifts (Δδ) observed for 3 in the presence of excess 1_4H at pD 1.0 are 0.34 ppm for N$^+$-CH$_3$, 0.30 ppm for CH$_2$, 0.38 ppm for o-H, 0.60 ppm for m-H, 0.79 ppm for p-H.

(28) It is not clear yet why the phenyl group in 2 is included in the cavity more favorably than that in 3. We recently obtained the X-ray crystal data of the 1_4H·2 complex: it shows that the phenyl moiety in 2 is included in the cone-shaped cavity of 1_4H and the ammonium group can exactly interact with SO$_3^-$ on the cavity edge. Supposedly, when the phenyl moiety in 3 is included in the cavity, the ammonium group is too far to interact with SO$_3^-$. The difference may rationalize the fact that the phenyl group in 2 is included in the cavity more favorably.

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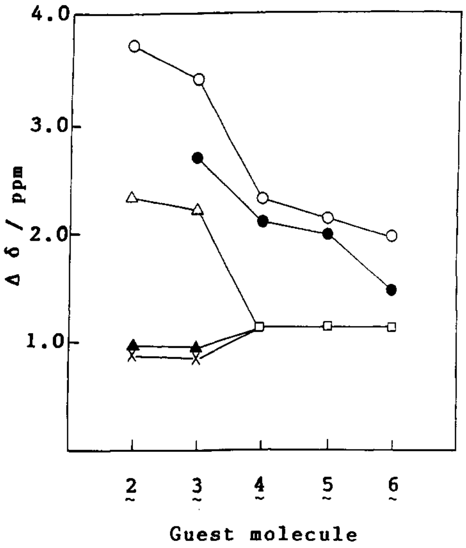

Figure 6. Upfield shift of ^1H NMR peaks of guest molecules in the presence of excess 1_6H (= 10·[guest]): D_2O, pD 7.3, 25 °C. For assignment of plots see caption to Figure 5.

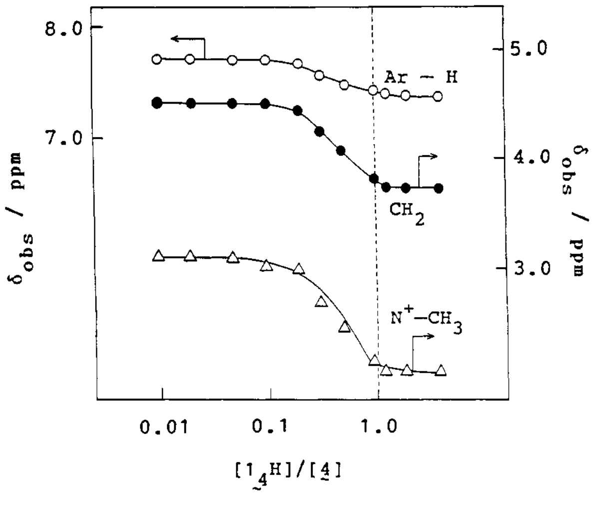

Figure 7. Plots of δ_{obs} versus $[1_4H]/[4]$. The concentration of 1_4H was maintained constant (1.00 mM) while that of 4 was varied (0.25–100 mM): D_2O, 25 °C, pD 7.3 with 0.1 M phosphate buffer, external standard DSS. The dotted line indicates a break point which corresponds to the stoichiometry of this complex.

Table IV. Stoichiometry and Association Constants (K)

	stoichiometry		$10^{-3}K$, M^{-1}	
guest	1_4H	1_6H	1_4H	1_6H
2[a]	1:1	1:1	5.6 ± 0.4	0.55 ± 0.04
3	1:1	1:1	*b*	*b*
4	1:1	1:1	31 ± 2	0.63 ± 0.03
5	1:1	1:3	30 ± 2	*c*
6	1:2	1:3	$K_1 = 5.0 \pm 0.5$ $K_2 = 4.6 \pm 0.5$	*c*
7[a]	1:1	1:1	21 ± 2	1.0 ± 0.1

[a] Cited from refs 9 and 29. [b] Accurate determination of K was difficult because of the overlap of calixarene and guest NMR peaks. [c] It was difficult to determine three K values for the three-step complexation, 1_6H + guest ⇌ 1_6H·guest ⇌ 1_6H·(guest)$_2$ ⇌ 1_6H·(guest)$_3$, by the computer-assisted nonlinear least-squares procedure.

[(282)0.517 + (5.76)0.483], i.e., $\Delta G^{\ddagger}$ = 14.5 kcal mol^{-1}). This value is in agreement with the $\Delta G^{\ddagger}$ value (14.5 kcal mol^{-1}) determined by the complete line-shape analysis for the NMR spectra at [2] = 0.005 M (however, see footnote e in Table III). The result indicates that mechanism ii is more likely. This conclusion is also acceptable from a dynamic viewpoint. It is known that the rates for association–dissociation processes are close to the diffusion rate (about 10^9 s^{-1}). On the other hand, the rates for ring inversion are about 10^2 s^{-1}. Thus, even though a trace amount of free 1_4H is produced through decomplexation of a 1_4H·2 complex, recombination with 2 occurs 10^7 times faster than ring inversion. This suggests that ring inversion from free 1_4H is almost meaningless in the presence of excess guest.

Inclusion of Ammonium Cations in the Calixarene Cavity. It is now clear that inclusion of ammonium cations in the calixarene cavity is the origin of the T_c rise. This view is also supported by the change in the chemical shifts of 2–7 in the presence of 1_4H and 1_6H (Figures 5 and 6). In the presence of excess calixarenes (where more than 98% of guests are included in 1_4H, which can be estimated by the association constants (vide ante)), ^1H NMR peaks of monocationic 2 and 3 show large upfield shifts. In 2, not only the aromatic protons but also the protons in the ammonium group ($CH_3N^+CH_2$) move to higher magnetic field. In 3, on the other hand, the protons in the ammonium group specifically move to higher magnetic field. The shift of the aromatic protons is readily rationalized in terms of hydrophobic interactions between the phenyl group and the calixarene cavity. Then, what is the driving force for the inclusion of the ammonium group? At pD 7.3, three of four OH groups are dissociated, so that the benzene rings in 1_4H should be considerably electron-rich. According to Dougherty and co-workers,[23-25] ammonium cations are easily bound to the cavity composed of electron-rich benzene π-systems. The concept well explains the inclusion of the ammonium group. If this is the case, the binding mode should be changed at low pD where the OH groups are scarcely dissociated. We measured ^1H NMR spectra of 2 and 3 at pD 1.0 (adjusted with DCl) where none or one of four OH groups is dissociated. In 2, the aromatic protons specifically moved to higher magnetic field.[26] In 3, on the other hand, both the aromatic protons and the protons in the ammonium group moved to higher magnetic field.[27] As the calixarene π-system is less electron-rich at pD 1.0, the results support the view that inclusion of the ammonium group at pD 7.3 should be attributed to the interaction between N^+ and the electron-rich calixarene π-systems.[28]

The high T_c values attained in the presence of 4–6 are accounted for by the bridge effect of these guest cations with two (or three) *p*-sulfonate anions. This view is supported by the chemical shifts of these polycationic guest molecules which are fairly different from those of 2 and 3: ^1H NMR peaks of 4, 5, and 6 shift to higher magnetic field only to a smaller extent (Figure 5). This indicates that 2 and 3 are bound deeply in the cavity[28] whereas 4, 5, and 6 rather perch on the cavity edge.

Stoichiometry and Association Constants. We previously studied the shift of the ^1H NMR peaks of guest molecules (2 and 7) in the presence of 1_nH.[9,29] From plots of δ_{obs} (chemical shift change) vs $[1_nH]/[\text{guest}]$ one can estimate the stoichiometry and the association constants (K) for calixarene complexes.[9,29] It was found that 1_4H and 1_6H form 1:1 complexes whereas 1_8H forms a 1:2 1_8H/guest complex.[9] The result establishes that the "hole-size" concept is also operative in molecular recognition with calixarene complexes. We applied the ^1N NMR method to evaluate complex formation with 4–6. Typical plots are shown in Figure 7.[30] The results obtained from these plots

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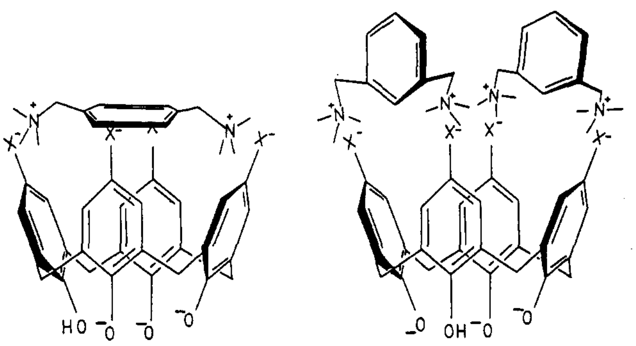
[30] Plots of δ_{obs} against $[1_6H]/[4]$, $[1_4H]/[6]$, and $[1_6H]/[6]$, are available as supplementary material.



(A) 1_nH-4 (X = SO_3^-) (B) 1_nH-5(or 6) (X = SO_3^-)

Figure 8. Possible binding modes for calixarene–ammonium complexes: (A) 1_4H–4 (having ammonium cations at the para position), (B) 1_4H–5(or 6) (having ammonium cations at the meta position).

are summarized in Table IV.

It is seen from Table IV that monocationic guests (2, 3, and 7) form 1:1 complexes with 1_4H and 1_6H. Probably, this is related to the fact that these guests are included in the calixarene cavity. On the other hand, the stoichiometry for 4–6 is fairly complex. Although 4 forms 1:1 complexes, 5 and 6 tend to form 1:2 or 1:3 complexes. As mentioned above, these di- and tricationic guests are bound onto the calixarene cavity mainly by the electrostatic force. Examination of CPK molecular models assuming an electrostatic interaction between ammonium cations and sulfonate anions suggests that 4, having ammonium cations at *p*-position, acts as a "lid" of the cavity and interferes with the approach of the second guest molecule (Figure 8, A). In contrast, 5 and 6, having ammonium cations at the meta position, stand vertically on the cavity edge (Figure 8, B). This binding mode allows the second guest to approach the cavity edge. The fact that in ^1H NMR the $\Delta\delta$ values for 5 and 6 are less affected by the ring current of 1_nH than that for 4 is compatible with this binding mode. Probably, this is why 5 and 6 can form 1:2 or 1:3 complexes.

Examination of K values for 1_4H in Table IV reveals that the K values for 4 and 5 are greater by a factor of 5 than that for 2. This increase is attributed to the multipoint interaction expected for these guest molecules. In contrast, the K values for tricationic 6 are smaller than those for 4 and 5 and rather comparable with those for 2. This suggests that the third cationic charge in 6 scarcely contributes to the complexation. This view is compatible with the association mode proposed for 6 (i.e., vertical to the calixarene edge: Figure 8, B). The K_1 and K_2 values were determined by the computer-assisted nonlinear least-squares method assuming the two-step association in Scheme II.[29] The result shows that the K_1 for 1_4H + 6 is comparable with the K_2 for 1_4H·6 + 6: that is, the association of the first 6 does not influence the association of the second 6. We consider that if the first 6 is bound vertically onto the calixarene edge, enough room for binding of the second 6 remains.

Scheme II

$$1_4H + 6 \underset{}{\overset{K_1}{\rightleftharpoons}} 1_4H\cdot 6$$

$$1_4H\cdot 6 + 6 \underset{}{\overset{K_2}{\rightleftharpoons}} 1_4H\cdot(6)_2$$

It is also seen from Table IV that the K values for 1_6H are generally smaller than those for 1_4H. We previously determined the thermodynamic parameters for 1_nH + 2.[9,29] Interestingly, the binding to 1_4H is mainly due to a negative ΔH, whereas that to 1_8H is due to a positive ΔS.[9,29] The results suggest that the association with 1_4H is based mainly on electrostatic interactions whereas that with 1_8H is based on hydrophobic interactions. Conceivably, the strong electrostatic interaction in 1_4H stems from concentrated anionic charges on the narrow calix[4]arene ring. For example, the benzene π-system may become more electron-rich because of electrostatic repulsion among the oxyanions. In 1_8H, on the other hand, the hydrophobic interaction operates efficiently because of the flexible nature of the calix[8]arene ring. The thermodynamic parameters for 1_6H are intermediary: ΔH is not so negative as 1_4H and ΔS is not so positive as 1_8H. Thus, neither the electrostatic force nor the hydrophobic force operates efficiently in 1_6H. This is the possible reason why the K values for 1_6H are generally smaller than those for 1_4H (Table IV).

In conclusion, the present study established for the first time that the calixarene conformation is subject to the template effects. Organic ammonium ions which are bound into or onto the calixarene cavity are particularly effective. The results consistently support the view that host–guest interactions in calixarenes are profoundly associated with the conformational change: that is, guest inclusion in calixarene hosts occurs basically in an induced-fit manner.

Experimental Section

The synthesis of 1_nH was reported previously.[7,31] ^1H NMR spectra were measured at 400 MHz. Association constants were evaluated from plots of δ_{obs} vs $[1_nH]/[guest]$ by using a computer-assisted nonlinear least-squares procedure.[9,29]

Supplementary Material Available: Plots of δ_{obs} vs $[1_6H]/[4]$, $[1_4H]/[6]$, and $[1_6H]/[6]$ (3 pages). Ordering information is given on any current masthead page.

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(31) Shinkai, S.; Araki, K.; Tsubaki, T.; Arimura, T.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* 1987, 2297.