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ORIGINAL ARTICLE

Inclusion complexation of octaethyl-*p*-*tert*-butylcalix[8]arene octaacetate with methylene blue and control of its inclusion ability by alkali metal cations

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Abstract The equilibrium constants for the inclusion complexation of octaethyl-*p*-*tert*-butylcalix[8]arene octaacetate (Calix-B8-EA) with methylene blue (MB) were determined spectrophotometrically. Calix-B8-EA, which has a flexible hydrophilic pseudo-cavity formed by polyfunctional esters on the lower rim, is an effective receptor for alkali and alkaline-earth cations, and its ester carbonyl group forms the complex with metal cations. We have examined the ability to include the organic molecule (methylene blue (MB)) into the upper main cavity of Calix-B8-EA formed the complex of alkali metal cations with ester carbonyl groups on the lower rim. It was found that Calix-B8-EA forms a 1:1 inclusion complex of MB with the upper main cavity and, in the presence of excess alkali metal cations, the association constants increase with an increase in the size of the metal cations complexed with the polyfunctional groups on the lower rim. Further, the structure of the inclusion complex of MB with cation-complexed Calix-B8-EA is characterized by 2D ROESY-NMR measurements. Based on the results, we have demonstrated the control of the inclusion ability by changing the portal size of the calixarene cavity.

Keywords Calixarene · Inclusion complex · Inclusion ability · ROESY-NMR · Methylene blue

Introduction

Calixarenes are macrocycle oligomers of *p*-substituted phenolic residues bridged by methylene groups. They are useful host molecules; organic molecules of appropriate sizes and shapes can be incorporated into their hydrophobic cavities through hydrophobic, van der Waals, π–π, and cation–π interactions [1–3]. The ability of calixarenes to form inclusion complexes with organic molecules has found applications in many areas. Some efforts to control inclusion ability have been made by changing the size of the calixarene cavity and substituents of phenolic oxygens [2, 4–6], while further investigations seem to be necessary to obtain better results.

Chemically modified calixarenes are reported to have significant ionophoric activity [7, 8]. In particular, octopus-type calixarenes containing polyfunctional esters and ketones serve as complexing agents for alkali metal cations [9–12]. In the inclusion of *p*-*tert*-butylmethoxycalix[4]arene, Bott et al. [13] suggested that methoxycalixarene includes both Na^+ cations and organic molecule such as toluene. In brief, sodium cation chelates with methoxy groups on the lower rim and the organic molecule are encapsulated into the upper main cavity of calixarene. However, the inclusion ability of the main cavity (upper-rim side) of calixarene derivatives complexed with metal cations at the lower-rim side has not been well established.

In this study, to obtain information concerning the inclusion complexation of the upper main cavity of calixarene containing ester carbonyl groups on the lower rim, we have established the inclusion behavior of octaethyl-*p*-*tert*-butylcalix[8]arene octaacetate (Calix-B8-EA) by means of UV–Vis spectroscopic and 2D ROESY-NMR experiments. As a guest organic molecule, methylene blue (MB) dye, which is spectroscopically active in the visible

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region, has been employed. In Calix-B8-EA, the eight ester groups on the lower rim are good receptors for alkali metal cations [9, 11]. We have examined the ability to include MB into the upper main cavity of the *tert*-butyl group side of Calix-B8-EA complexed with alkali metal cations at the lower-rim side, and showed the possibility of controlling inclusion ability for calixarene by metal–ion complexation.

Experimental

Materials

Calix-B8-EA (Fig. 1) was prepared by the method reported previously [9] and recrystallized from ethanol: mp 501 K (lit. 501–503 K [9]). Methylene blue (MB) was purchased from Chroma Gesellschaft Schmidt and Co. The phenothiazine dye MB was recrystallized from ethanol and dried under vacuum before use. Reagent-grade acetonitrile was purified by distillation and was used as a solvent.

Spectral measurements

The spectral change of MB in the presence of Calix-B8-EA was monitored by using a Hitachi U-3200 spectrometer (Tokyo, Japan); the reaction temperature was controlled at 298 ± 0.1 K by circulating thermostated water. The typical solution concentrations were [MB] = 2.0×10^{-5} mol dm^{-3} and [Calix-B8-EA] = $(0–4.3) \times 10^{-4}$ mol dm^{-3}.

In the Calix-B8-EA complexation, Meier and Detellier [11] suggested that the ester carbonyl groups on the lower rim of Calix-B8-EA form a 1:3 complex with Na^+ cations under the condition of [Calix-B8-EA]/[Na^+ cation] < 0.3. Based on the results and maintaining ionic strength $I = 0.01$, we have determined the association constants for the inclusion complexation of cation-complexed Calix-B8-EA with MB under the condition of [Calix-B8-EA]/[metal cation] < 0.2. In the present study, the cation-concentration dependence of UV–Vis absorption spectra of MB was not observed, suggesting that the interaction of the alkali metal cations with the dimethylamino groups of MB is not operative efficiently.

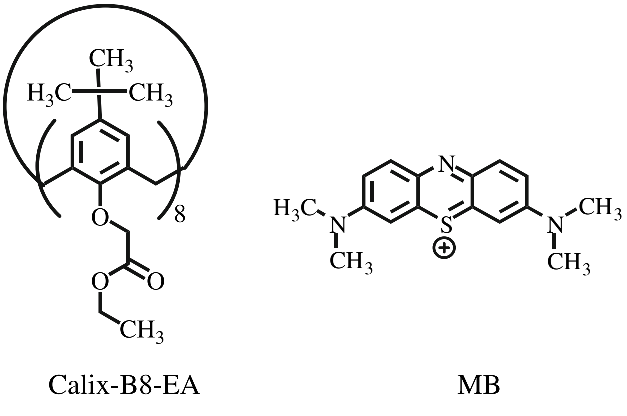


Fig. 1 Structures of Calix-B8-EA and MB

2D ROESY-NMR experiments were recorded at 600 MHz in CD_3CN on a Varian Inova AS600 NMR spectrometer at 303 K. The mixing time for each ROESY experiment was 100 ms.

Results and discussion

Inclusion complexation of Calix-B8-EA

The typical absorption spectra of MB upon consecutive addition of Calix-B8-EA are shown in Fig. 2. The peak in the vicinity of 660 nm due to MB decreased with an increase in the concentration of Calix-B8-EA. Upon the addition of 4-*tert*-butylphenol to the MB solution, any changes in the UV–Vis spectrum of MB were not observed, suggesting that the spectral changes in the absorption of MB appear by the inclusion complex formation with Calix-B8-EA. In previous UV–Vis and NMR studies, we suggested that MB forms a 1:1 inclusion complex with *p*-sulfonatocalix[8]arene (Calix-S8); the inclusion constants have been determined according to Benesi–Hildebrand (B–H) treatments [14]. However, analysis of the spectral data using a B–H type equation is not suitable for the inclusion complexation of Calix-B8-EA. The Calix-B8-EA concentrations used for the determination of the inclusion association constants are comparable to the MB concentration because of the high stability of the inclusion complexes. Therefore, we have employed the optimization method of Lang's treatments for determining the association constants (K) of the 1:1 inclusion complex [15].

$$\text{Calix - B8 - EA} + \text{MB} \overset{K}{\rightleftharpoons} \text{Calix - MB}$$
$$K = \frac{[\text{Calix} - \text{MB}]}{[\text{Calix - B8 - EA}][\text{MB}]} \tag{1}$$

$$\frac{[\text{Calix} - \text{B8} - \text{EA}]_0[\text{MB}]_0}{\Delta E} = \left([\text{Calix - B8 - EA}]_0 + [\text{MB}]_0 - \frac{\Delta E}{\Delta\varepsilon}\right)\frac{1}{\Delta\varepsilon} + \frac{1}{\Delta\varepsilon K} \tag{2}$$

where $[\text{Calix-B8-EA}]_0$ and $[\text{MB}]_0$ are the total concentrations of Calix-B8-EA and MB, respectively. $\Delta\varepsilon$ is the difference between the molar extinction coefficients for bound and unbound MB with Calix-B8-EA, and ΔE is the change in the absorption intensity of MB. Tentative values of $\Delta\varepsilon$

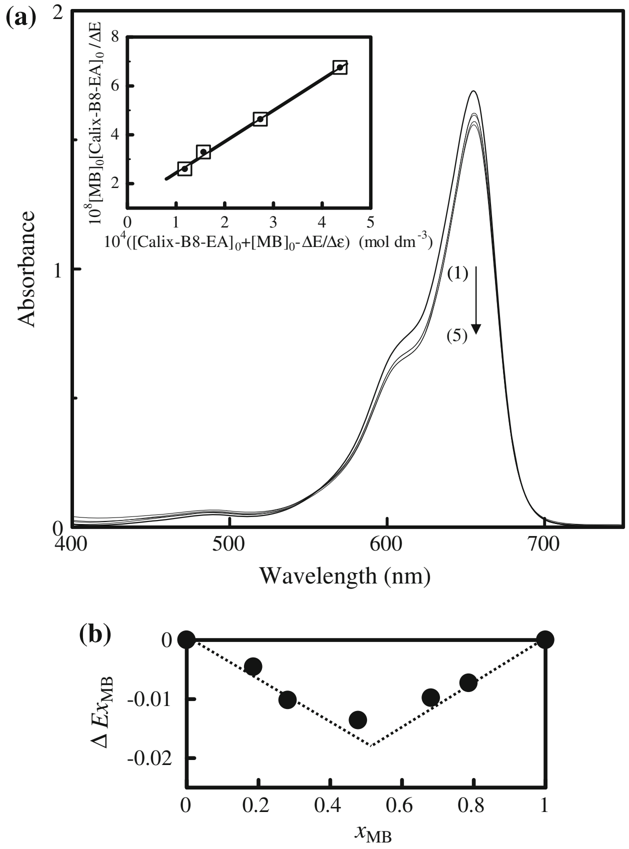


Fig. 2 **a** Absorption spectra of MB (2.01×10^{-5} mol dm^{-3}) containing different concentrations of Calix-B8-EA in acetonitrile at 298 K: [Calix-B8-EA] = (1) 0, (2) 1.09×10^{-4}, (3) 1.48×10^{-4}, (4) 2.68×10^{-4}, and (5) 4.33×10^{-4} mol dm^{-3}. *Inset*: determination of the 1:1 inclusion equilibrium constants for the Calix-B8-EA/MB complexation according to Eq. 2: (*open square*) plot using the second tentative value of Δε, and (*closed circle*) plot using the third tentative value of Δε. The variation in magnitude of the slope of this plot converges to within 5% relative to that of a line drawn immediately before. **b** Continuous variation plots (Job's plot)

and K for the inclusion complexation were established according to Eq. 2. Using this value of Δε, a plot of [Calix-B8-EA]$_0$[MB]$_0$/ΔE against {[Calix-B8-EA]$_0$ + [MB]$_0$ − (ΔE/Δε)} was made according to Eq. 2, producing a linear relationship between the two. A new value for Δε can be determined from a new value for K. This procedure is repeated until the variation in the magnitude of the slope of a new plot for Eq. 2 converges to within 5% relative to the slope of the previous plot for Eq. 2. Figure 2 shows the plots of Eq. 2, indicating the convergence of the slope relative to a previously drawn line. The linearity for the plots of Eq. 2 suggests the 1:1 inclusion complex formation of MB with Calix-B8-EA. The association constant ($K = 1.09 \times 10^4$ mol^{-1} dm^3) obtained for Calix-B8-EA complexation is listed in Table 1, which is comparable to that for Calix-S8 complexation with MB ($K = 7.53 \times 10^3$ mol^{-1} dm^3) [14]. The large association constant reflects the high degree of stability of the inclusion complex.

Table 1 The equilibrium constants (K) for inclusion complexation of MB with Calix-B8-EA in acetonitrile in excess alkaline salts at 298 K

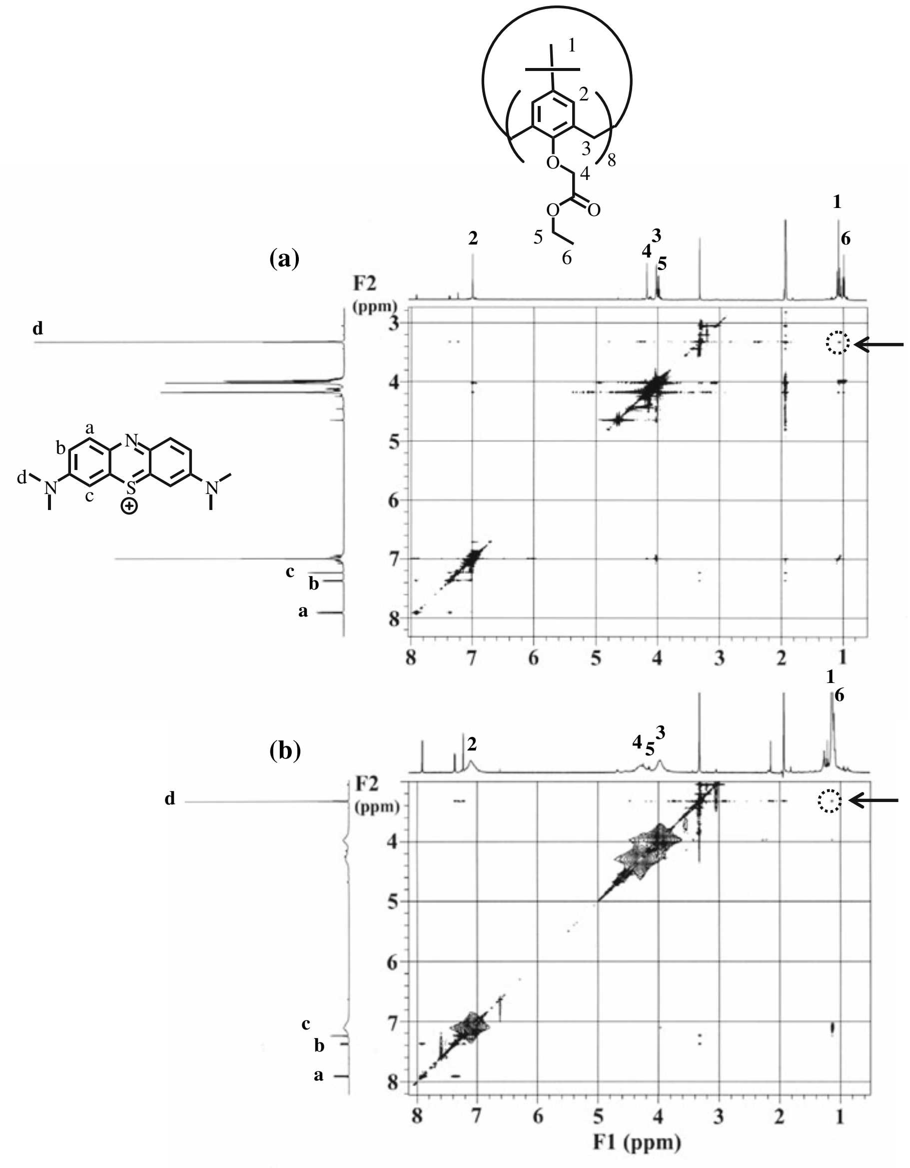
Salts[a]	Ionic radius (nm)[b]	$10^{-3}K$ (mol^{-1} dm^3)	$-\Delta G^o$ (kJ mol^{-1})
Non-salts	–	10.9 ± 0.3	23.0
$LiClO_4$	0.059	–	–
$NaClO_4$	0.099	1.11 ± 0.02	17.4
$KClO_4$	0.137	5.69 ± 0.27	21.4
$RbClO_4$	0.152	7.70 ± 0.30	22.2

[a] [$MClO_4$] = 0.01 mol dm^{-3}

[b] Cited from [18]

The 2D ROESY-NMR experiments are instructive for elucidating the position of the guest molecules in the calixarene cavity. The assignments of Calix-B8-EA and MB protons were made according to the previous reports [9, 14]. Figure 3a shows the ROESY-NMR spectrum of the inclusion complex of MB with Calix-B8-EA. As indicated by the arrow in Fig. 3a, the cross peak arising from the C(d)–H protons of MB and C(1)–H protons of the *tert*-butyl-groups in Calix-B8-EA was observed. However, the interaction between the C(a, b, c)–H protons of MB and C(2, 3, 4, 5, 6)–H protons of Calix-B8-EA can not be seen, indicating that the dimethylamino group of MB is encapsulated in the main cavity of Calix-B8-EA from the upper rim of the *tert*-butyl group side. In calixarene complexation, a cone conformation is more suitable to form inclusion complexes than is an alternative conformation [1, 2, 14]. Based on the results, a plausible structure of the inclusion complex of MB with Calix-B8-EA is depicted in Fig. 4a. In a previous NMR study for Calix-S8 complexation, we suggested that the N–$(CH_3)_2$ and C(c)–H moieties of both sides of MB are included inside the calixarene cavity, which is different from the inclusion aspect for Calix-B8-EA. In Calix-S8, eight sulfonato anions are situated on the upper rim, and the repulsion between them may result in an increase in the portal size of the Calix-S8 cavity. In contrast, the steric repulsion between the eight polyfunctional ester carbonyl groups on the lower rim of Calix-B8-EA decreases the portal size of the upper main cavity of the *tert*-butyl group side, which is responsible for the difference in the inclusion aspects. In MB/Calix-B8-EA inclusion, judging from the hydrophobic nature of the *tert*-butyl groups, we believe that the van der Waals interactions between the eight *tert*-butyl groups of calixarene and the dimethylamino group of MB is an important factor in forming a stable inclusion complex in acetonitrile.

Fig. 3 2D ROESY-NMR spectra for the inclusion complexes of MB (1.47×10^{-3} mol dm^{-3}) at 303 K in CD_3CN: **a** [Calix-B8-EA] = 1.57×10^{-3} mol dm^{-3} in the absence of alkali metal cations. **b** [Calix-B8-EA] = 1.26×10^{-3} mol dm^{-3} under the condition of [Calix-B8-EA]/[$NaClO_4$] = 0.14



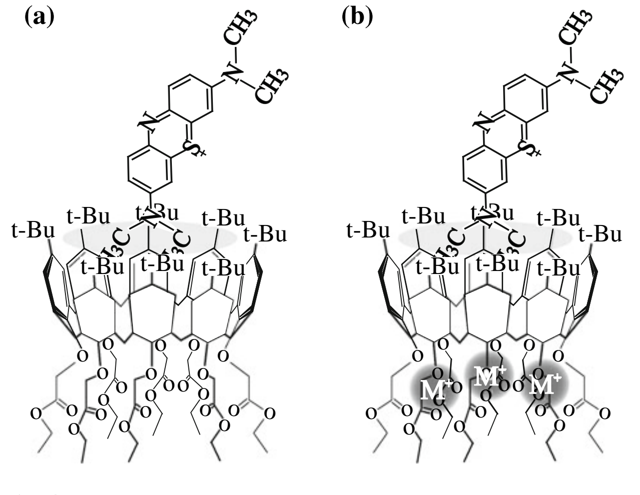


Fig. 4 Plausible structures of inclusion complexes of MB with Calix-B8-EA in the absence (**a**) and presence (**b**) of alkali metal cations

Inclusion by Calix-B8-EA complexed with metal cations

Many complexation studies on alkali metal cations have been performed by using pendant ester groups on the calixarene rim [9, 12]. Meier and Detellier [11] suggested that alkali metal cations are encapsulated into the hydrophilic pseudo-cavity (lower side) defined by the ester groups of Calix-B8-EA, resulting in a 1:3 complex formation with Na^+ cations under the condition of [Calix-B8-EA]/[Na^+] < 0.3. The ester carbonyl groups of Calix-B8-EA are good receptors for metal cations, and we assumed that Calix-B8-EA, which has complexed with metal cations at the lower-rim side, exists in the cone conformation as depicted for *p-tert*-butylcalix[6]arene *syn*-1,3,5-trimethoxy-2,4,6-triamide complexed with alkali metal cations

[16]. This is also supported from the visual inspection of the Corey–Pauling–Koltun (CPK) space-filling model (this figure is not shown). To obtain the information on the conformation of Calix-B8-EA in excess alkali metal cations, the NMR measurements were carried out in the range of 273–328 K. The change in the NMR spectral shape was not observed at various temperatures, suggesting the fixed conformation of Calix-B8-EA in excess alkali metal cations.

It is instructive to examine the inclusion ability for the upper main cavity of Calix-B8-EA complexed with metal cations at the lower-rim side. In the presence of excess $NaClO_4$, $KClO_4$, or $RbClO_4$, UV–Vis spectral changes similar to those in the absence of alkali metal cations were obtained. However in the presence of excess of $LiClO_4$, the addition of Calix-B8-EA did not cause any changes in the UV–Vis spectrum of MB, which suggests the stable complex formation of MB with Calix-B8-EA in the presence of alkali salts other than $LiClO_4$.

Using 2D ROESY-NMR methods, we have discussed the structure of the inclusion complex of organic compounds with Calix-B8-EA complexed with metal cations at the lower-rim side. Figure 3b shows the 2D ROESY-NMR spectrum for the inclusion complex of MB with Calix-B8-EA in the presence of excess $NaClO_4$ ([Calix-B8-EA]/$[NaClO_4] = 0.14$). Analogous with the ROESY-NMR spectrum for the MB/Calix-B8-EA complex in the absence of alkali salts, cross peak between the *tert*-butyl C(1)–H protons of Calix-B8-EA and dimethylamino C(d)–H protons of MB was detected, while those between the C(2,4,5,6)–H protons of calixarene and C(a,b,c)–H protons of MB did not appear. These results indicate that the dimethylamino moiety of MB is included in the upper main cavity (Fig. 4b), which is similar to the structure of the inclusion complex with Calix-B8-EA in the absence of alkali salts. The ability of Calix-B8-EA to encapsulate organic molecules into the main cavity is not lost by complexation with alkali metal cations.

As suggested above, in large excess of alkali salts, Calix-B8-EA forms the complex of three metal cations with the eight ester carbonyl groups on the lower rim [11]. To confirm the stoichiometry of inclusion complex of MB with cation-complexed Calix-B8-EA, we conducted absorption measurements by varying the mole fraction of MB and Calix-B8-EA in excess alkali metal cations: the continuous variation method (Job's method) [17]. Job's plots for the changes in the absorption of MB are shown in Fig. 2b. From the plots, it is clear that the complex of MB is formed with 1:1 stoichiometry. From analyses of UV–Vis spectral changes in the presence of excess alkali salts, we have determined the association constants of MB with cation-complexed Calix-B8-EA according to Eq. 2. The inclusion equilibrium constants obtained in the presence of excess $NaClO_4$, $KClO_4$, or $RbClO_4$ are given in Table 1. The K values in the excess alkali metal cations are small compared with that in the absence of metal cations. We believe that the complex formation between cations and pendant ester carbonyl groups decreases steric interference between the eight polyfunctional ester groups. This conformational change of Calix-B8-EA is related to the enlargement of the upper main cavity of the *tert*-butyl group side, resulting in a decrease in the inclusion equilibrium constants, compared with that in the absence of alkali salts. Table 1 reports an interesting observation. The inclusion equilibrium constants increase with an increase in cation size. Further, we have examined the inclusion complexation of hexaethyl-*p*-*tert*-butylcalix[6]arene hexaacetate (Calix-B6-EA) of six benzene-ring units. In the inclusion complex of Calix-B6-EA with MB, the changes in the UV–Vis absorption spectra due to inclusion of MB were obtained in the absence of alkali metal cations; however, any spectral changes of MB were not observed in the excess alkali metal cations. In Calix-B6-EA having a smaller cavity, we believe that the lower-rim complexation with metal cations decreases the portal size of the upper cavity. The above results indicate that the lower-rim complexation of alkali metal cations causes the change in the portal size of the upper rim. One can effectively control inclusion ability by changing the portal size of the calixarene cavity.

The free energy change of the inclusion complexation can be given by:

$$\Delta G^\circ = -RT\ln K \tag{3}$$

The ΔG^o values express the stability of the inclusion complexes and are listed on the right of Table 1. The $-\Delta G^o$ values for the complexation in the absence of alkali salts are large compared with that for the inclusion of the dimethylamino group of MB by *p*-sulfonatocalix[6]arene (Calix-S6): $K = 1.9 \times 10^3\ \mathrm{mol^{-1}\ dm^3}$ and $-\Delta G^o = 18.7\ \mathrm{kJ\ mol^{-1}}$ for Calix-S6 [14], which is comparable to that for the Na^+-complexed Calix-B8-EA. The large $-\Delta G^o$ values for Calix-B8-EA are attributed to the van der Waals interaction enhanced by the *tert*-butyl groups on the calixarene rim.

In summary, we have shown that the upper main cavity of Calix-B8-EA that formed the complex of alkali metal cations with polyfunctional ester carbonyl groups on the lower rim does not lose its inclusion ability for organic molecules. The complex formation of metal cations with pendant ester carbonyl groups serves to fix the cone conformation of Calix-B8-EA. In the presence of excess alkali metal cations, the association constants for the inclusion complex formation of Calix-B8-EA with MB increase with an increase in the sizes of metal cations encapsulated into the pseudo-cavity of the ester carbonyl groups on the lower rim. The above results suggest that the inclusion ability for the upper main cavity (the *tert*-butyl group side) of Calix-B8-EA is effectively controlled by inserting cations into the ester carbonyl groups on the lower rim.

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