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Synthesis of Novel Benzo-15-Crown-5-Tethered β-Cyclodextrins and Their Enhanced Molecular Binding Abilities by Alkali Metal Cation Coordination

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Abstract

Two novel benzo-15-crown-5 tethered β-cyclodextrins **1** and **2** have been synthesized by coupling substituted benzo-15-crown-5 with corresponding β-cyclodextrin derivatives. Their inclusion complexation behavior with representative guests, such as cyclohexanol, cyclohexane carboxylic acid, cyclohexane acetic acid, sodium cyclohexane carboxylate, and potassium cyclohexane carboxylate, was investigated in aqueous solution by means of fluorescence spectrometry. As compared with parent β-cyclodextrin, benzo-15-crown-5 tethered β-cyclodextrins **1–2** display significantly enhanced molecular binding abilities and selectivities towards model substrates, especially towards substrates containing alkali-metal cations. These results indicate that, bearing two recognition sites in a single molecule, these supramolecular architectures can strongly enhance the molecular binding ability of parent β-cyclodextrin by the cooperative binding of the β-cyclodextrin cavity and the crown ether moiety. Possessing a shorter linker, crown ether-β-cyclodextrin **2** shows much higher binding affinity with guest molecules than crown ether-β-cyclodextrin **1**, which may be attributed to the binding size and molecular multiple recognition behavior between host and guest.

Introduction

Among the diverse molecular templates available for supramolecular systems, crown ethers and cyclodextrins can be taken as molecular receptors to selectively binding cations and molecules respectively forming host–guest complexes or supramolecular species. Therefore, a lot of effort has been contributed to the design and syntheses of functional crown ether and cyclodextrin derivatives in order to enhance their original ionic/molecular affinities and selectivities [1]. Recently, many approaches to enhance the binding abilities and molecular selectivities of cyclodextrins by appropriately appending additional recognition sites to cyclodextrins have been reported, including dimeric cyclodextrins, calix[4]arene tethered cyclodextrins and crown ether tethered cyclodextrins [2–7]. As a new-typed supramolecular architectures bearing two different recognition sites, i.e., a hydrophobic cavity of cyclodextrin and a crown ether moiety as a cation receptor site, in a single molecule, crown ether-cyclodextrins can significantly enhance the original binding ability of parent β-cyclodextrin through the cooperative binding of the crown ether and cyclodextrin moieties, while the difference in the length and flexibility of tether group allows them displaying different binding abilities and molecular selectivities upon cooperative complexation with model substrates. In the present work, we wish to report the synthesis of two crown ether-β-cyclodextrins, i.e. 4′,5′-dimethylene-benzo-15-crown-5 tethered 6-diethylene triamino-6-deoxy-β-cyclodextrin (**1**) and 4′,5′-dimercaptomethylene-benzo-15-crown-5 tethered 6-deoxy-β-cyclodextrin (**2**), (scheme 1), which possess the same supramolecular moieties, such as cyclodextrin and benzo-15-crown-5, and different linker groups, and their enhanced molecular binding abilities towards alkali-metal cyclohexane carboxylates through the coordination of alkali-metal cation with appended crown ether moiety and the hydrophobic interaction between cyclodextrin cavity with accommodated cyclohexane carboxylate anion. It is of our particular interest to explore the contribution of the cooperative effect of different recognition receptor sites and binding modes in supramolecular system.

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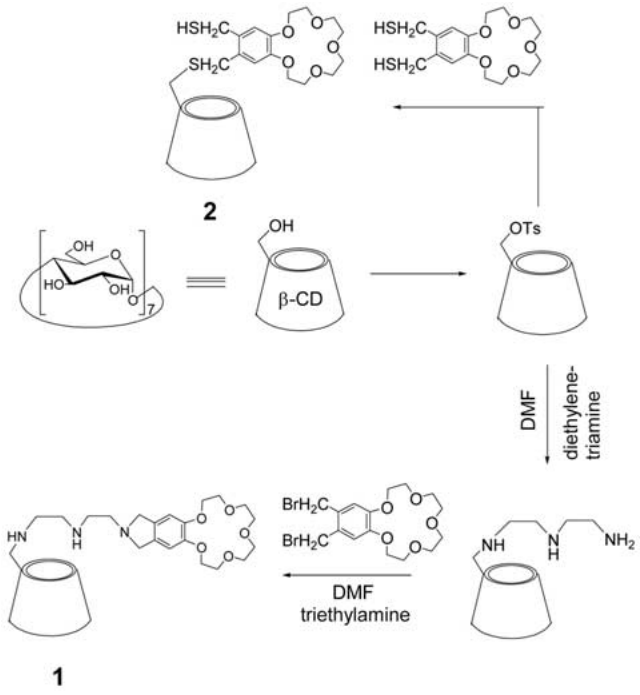
Experimental section

General

Cyclohexanol, cyclohexane carboxylic acid, cyclohexane acetic acid, potassium cyclohexane carboxylate and sodium cyclohexane carboxylate were purchased from Wako. All chemicals were reagent grade and used without further purification unless noted otherwise. β-Cyclodextrin of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried in vacuo for 12 h at 100 °C. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and distilled under reduced pressure prior



Scheme 1.

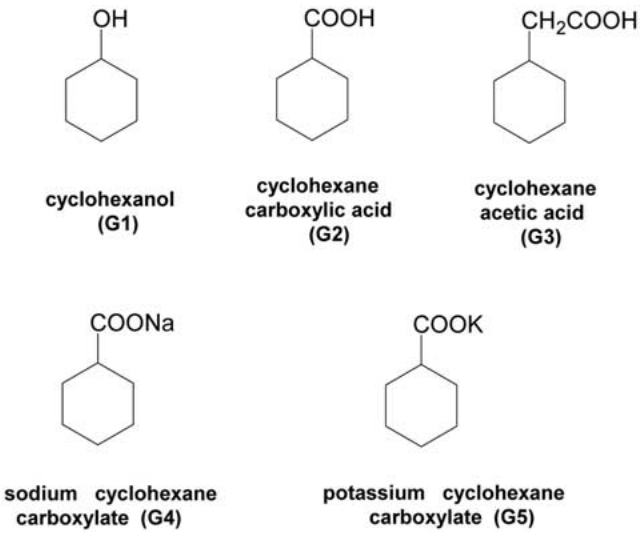


Chart 1.

to use. 6-Diethylenetriamino-6-deoxy-β-cyclodextrin and 2,3-[4′,5′-bis-(bromomethyl)benzo]-1,4,7,10,13-pentaoxa-2-cyclopentadecene (4′,5′-dibromomethene-benzo-15-crown-5) was prepared according to the procedures reported by Shen [8] and Luboch [9], respectively. Mass spectrum was obtained on a JEOL JMS-DX-303 instrument. ^{1}H-NMR spectra was recorded on a Mercury Vx300 instrument at 300 MHz using tetramethylsilane as an internal reference. Elemental analysis was performed on a Perkin-Elmer 2400C instrument. Circular dichroism and UV-vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JSACO J-720S or a Shimadzu UV-2401/PC instrument equipped with a PTC-348WI temperature controller to keep the temperature at 25°C. Electronic conductivity was measured on a DDS-12A (Zhejiang) digital conductive instrument. Fluorescence spectra were measured in a conventional quartz cell (10 × 10 × 45 mm) at 25 °C on a JASCO FP-750 spectrometer equipped with a temperature controller, with the excitation and emission slits of 5 nm width. Deionized, distilled water was used as solvent in all measurements.

Synthesis

6-diethylenetriamino-6-deoxy-β-cyclodextrin were synthesized by mono-[6-O-(p-toluenesulfonyl)]-β-cyclodextrin and diethylenetriamino according to the reference[8]. 4′,5′-dibromomethene-benzo-15-crown-5[9]: benzo-15-crown-5 (0.1 mmol) and paraformaldehyde (14.5 g, 95%) were dissolved in hydrogen bromide (160 ml, 30%) and acetic acid. The reaction mixture was allowed to stand for 1 h at room temperature and then for 48 h under 4 °C. The solvent was removed under reduced pressure at a temperature not exceeding 50 °C and then the residue was recrystallized by tetrahydrofuran.

4′,5′-Dimethylene-benzo-15-crown-5 tethered 6-diethylenetriamino-6-deoxy-β-cyclodextrin (**1**). 6-Diethylenetriamino-6-deoxy-β-cyclodextrin (1.22g, 1mmol) and 4′,5′-dibromomethene-benzo-15-crown-5 (0.45 g, 1mmol) were dissolved in dry DMF (40 mL) containing triethylamine (4 mL). The resultant mixture was stirred at 70–80 °C for 4 days under nitrogen atmosphere. Then the solvent was evaporated under a reduced pressure to dryness. The residue was dissolved in a minimum amount of hot water, and then acetone was poured to the solution to give the crude product as a reddish precipitate. After drying, the crude product was purified on a column of Sephadex G-25 to give **1** (0.5 g, 0.3 mmol) in 30% yield as a reddish solid: FAB-MS (*m/z*): 1536 (M + Na^+); ^{1}H-NMR (300Mz, D_2O, TMS) δ 2.71–3.99 (m, 66H, C_{2-6}–H of CD; $NHCH_2$; OCH_2), 4.12 (m, 4H, $ArCH_2N$), 4.87 (m, 7H, C_1–H of CD), 6.84 (s, 2H, ArH). ^{13}C-NMR (300Mz, D_2O): 149.03, 148.30, 124.84, 116.41, 108.11, 101.99, 83.31, 81.23, 73.17, 72.07, 71.83, 69.87, 69.38, 68.77, 68.28, 60.34, 50.33, 48.49. FT-IR (KBr), ν = 3337, 2930, 1652, 1508, 1456, 1301, 1154, 1081, 1032, 941, 852, 755, 577 cm^{-1}. Anal. Calcd. for $C_{62}H_{101}O_{39}N_3 \cdot 7H_2O$: C, 45.45; H, 7.07; N, 2.56. Found: C, 45.49; H, 7.00; N, 2.55. UV-vis (H_2O) λmax/nm (ϵ/M^{-1} cm^{-1}) 282.5 (4790), 206.5 (28300).

4′,5′-Dimercaptomethylene-benzo-15-crown-5. 4′,5′-Dibromomethylene-benzo-15-crown-5 (2.27 g, 5 mmol) and thiourea (0.76 g, 10 mmol) were dissolved in ethanol, and the resultant mixture was stirred at 50 °C for 24 h. Then the reaction mixture was hydrolyzed with aqueous NaOH (10%). After acidification by ice and concentrated hydrochloric acid, it is extracted with ether. The extract was washed with deionized water and dried over anhydrous $MgSO_4$, then the solvent was removed to leave a white powder, which was recrystallized from cyclohexane to give the pure sample (0.4 g, 1mmol) in 20% yield as a white solid: m.p. 108–110 °C; ^{1}H-NMR (300Mz, $CDCl_3$, TMS)

δ 1.83 (t, 2H, SH), 3.75 (m, 12H, CH_2OCH_2), 3.88 (t, 4H, $ArOCH_2$), 4.12 (t, 4H, $SHCH_2$).

4′,5′-Dimercaptomethylene-benzo-15-crown-5 tethered 6-deoxy-β-cyclodextrin (**2**). Mono-[6-O-(p-toluenesulfonyl)]-β-cyclodextrin (1.0 g, 0.76 mmol) and 4′,5′-dimercaptomethylene-benzo-15-crown-5 (0.5 g, 1.4 mmol) were dissolved in dry DMF (30mL), and the resultant mixture was stirred at 70 °C for 10 days under nitrogen atmosphere. Then the solvent was evaporated under a reduced pressure to dryness. The residue was dissolved in water, and then acetone was added to the solution to give a brown precipitate. After drying, the precipitate was purified on a column of Sephadex G-25 to give 0.1 g (9.4% yield) of **2** as a brown solid: FAB-MS (*m/z*): 1477 (M^+); ^{1}H-NMR (300Mz, D_2O, TMS) δ 2.7–3.9 (m, 58H, C_{2-6}–H of CD; OCH_2), 4.12 (m, 4H, $ArCH_2$), 4.83 (m, 7H, C_1–H of CD), 6.5–7.2 (m, 2H, ArH). FT-IR (KBr), ν = 3297, 2928, 2879, 2504, 1599, 1504, 1453, 1405, 1360, 1291, 1250, 1152, 1078, 1032, 940, 853 cm^{-1}. Anal. Calcd. for $C_{58}H_{92}O_{39}S_2{\cdot}2H_2O$: C, 46.03; H, 6.39; S, 4.24. Found: C, 45.88; H, 6.40; S, 4.48. UV-vis (H_2O) λmax/nm (ϵ/M^{-1} cm^{-1}) 290.5 (11810).

Results and discussion

Circular dichroism spectra

In order to obtain information about the original conformation of crown ether appended β-cyclodextrins in diluted solution, the circular dichroism spectra of hosts **1–2** were taken at a concentration of 1.0×10^{-4} mol dm^{-3} in aqueous solution. As can be seen from Figure 1, host **1** displays very weak induced circular dichroism (ICD) signals for the transitions of the benzo-15-crown-5 chromophore. However, host **2** shows distinctly different ICD spectrum; weak positive ICD signals around 270 nm for the ^1L_b transition and weak negative ICD signals around 245 nm for the ^1L_a transition of benzo-15-crown-5 moiety. According to the empirical rule that interprets the ICD observed for a chromophore inside or outside of the cyclodextrin cavity proposed by Kajtar [10], Harata [11], and Kodaka [12], we can deduce that the aromatic ring of host **1** locates distant from the cyclodextrin cavity, while the benzo-15-crown-5 unit of host **2** shallowly penetrates partly into the hydrophobic cavity of cyclodextrin. This conformational difference between hosts **1** and **2** will subsequently result in the dramatic difference in the complex stabilities upon inclusion complexation with guest molecules to some extent.

Complex stoichiometry

As the conductivity of the system reduces with the complex formation, conductivity measurements can be applied to explore the complex stoichiometry for the host–guest association. Figure 2 illustrates the continuous variation plot for the crown ether-β-cyclodextrin **1** with sodium cyclohexane carboxylate system in aqueous solution. In the concentration range used, the plot shows a maximum at a molar fraction of 0.5, indicating 1:1 inclusion complexation between host and guest. The same results are obtained in the other cases of the inclusion complexation of host–guest association.

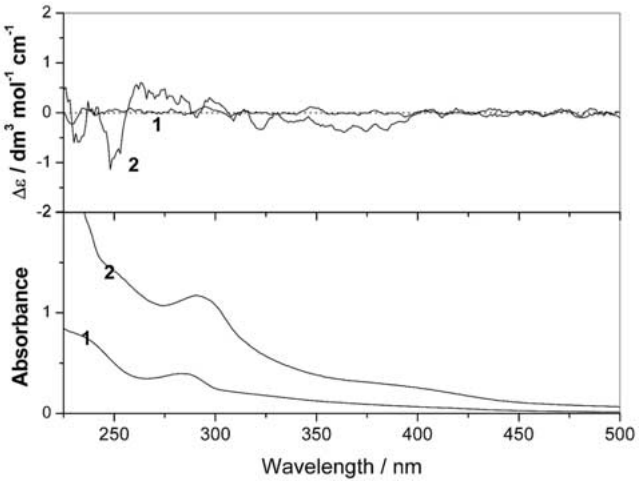


Figure 1. UV and Circular dichroism spectrum of hosts **1–2** (1.0×10^{-4} mol dm^{-3}) in aqueous solution.

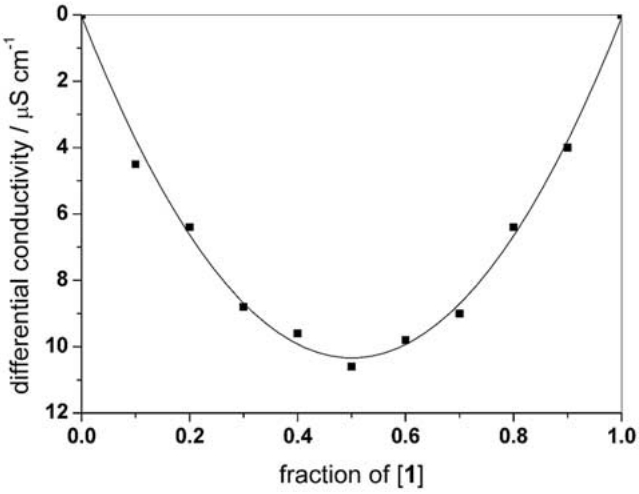


Figure 2. A Job plot of the complexation of crown ether-cyclodextrin **1** with sodium cyclohexane carboxylate (G1) in aqueous solution. ([**1**] + [G1]= 1.0×10^{-4} mol dm^{-3}).

Spectral titration

Complex formation between host and guest usually alters the original spectrum of the host or guest molecule. Herewith, in order to study quantitatively the binding ability of crown ether-β-cyclodextrins, the inclusion complexation behavior of hosts **1–2** was investigated in aqueous solution at 25 °C by the fluorometric titration method, employing cyclohexanol (G1), cyclohexane carboxylic acid (G2), cyclohexane acetic acid (G3), sodium cyclohexane carboxylate (G4) and potassium cyclohexane carboxylate (G5) as representative guest molecules (Chart 1). Figure 3 illustrates the typical fluorescence spectral changes of host **1** upon gradual addition of cyclohexanol. As can be seen in Figure 3, the relative fluorescence intensity of host **1** distinctly enhanced upon gradual addition of guest molecule, accompanying slightly hypsochromic shifts of the fluorescence peaks, which jointly

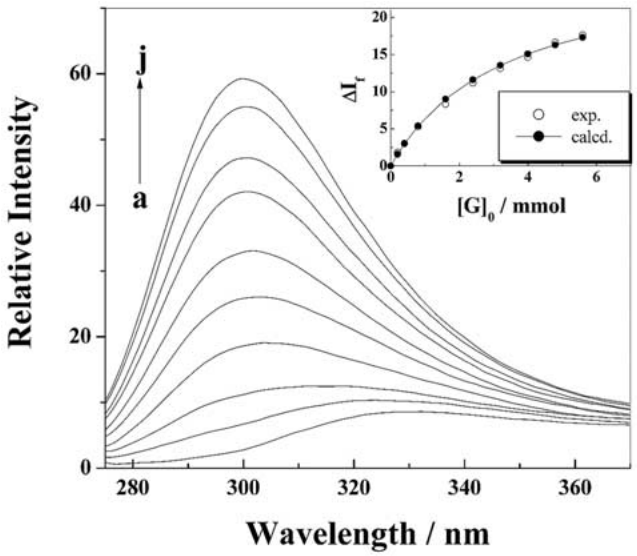


Figure 3. Fluorescence spectra changes of host **1** (0.96×10^{-5} mol dm^{-3})and the non-linear least-squares analysis (inset) of the relative fluorescence intensity (ΔI_f) to calculate the complex stability constant (K_S) upon addition of cyclohexanol ($0 - 550 \times 10^{-5}$ mol dm^{-3} from a to j) at 25 °C in deionized water. (Ex = 260 nm, Ex and Em slit 5 nm, sensitivity medium).

indicates the formation of the host-guest inclusion complexes. In the control experiment, the fluorescence of host **1** or **2** shows no significant changes upon adding sodium or potassium nitrate under the same conditions. Validating the 1:1 complex stoichiometry, the inclusion complexation of guest (G) with host (H) is expressed by Equation (1).

$$\mathrm{H} + \mathrm{G} \overset{K_S}{\rightleftharpoons} \mathrm{G} \cdot \mathrm{H}. \qquad (1)$$

The complex stability constant (K_S) can be calculated from the analysis of the sequential changes in fluorescence intensity (ΔI_f) of host molecule at various guest concentration, using a non-linear least squares method according to the curve fitting Equation (2) [13, 14].

$$\Delta I_f = \{\alpha([\mathrm{H}]_0 + [\mathrm{G}]_0 + 1/K_S) \pm \sqrt{\alpha^2([\mathrm{H}]_0 + [\mathrm{G}]_0 + 1/K_S)^2 - 4\alpha^2[\mathrm{H}]_0[\mathrm{G}]_0}\}/2, \qquad (2)$$

where $[\mathrm{G}]_0$ and $[\mathrm{H}]_0$ refer to the initial concentrations of guest and host molecule, respectively, and α is the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence change upon complexation. For each guest examined, the ΔI_f values were plotted as a function of $[\mathrm{H}]_0$ to give an excellent fit. The experimental data do not show any significant deviations from the theoretical curve in each case. In the repeated measurements, the K_S values were reproducible within an error of ±5%. The K_S values obtained are listed in Table 1, along with the free energy changes of complex formation ($-\Delta G°$). In order to visualize the inclusion complexation behavior between host and guest, the K_S values are also plotted against the guests in Figure 4.

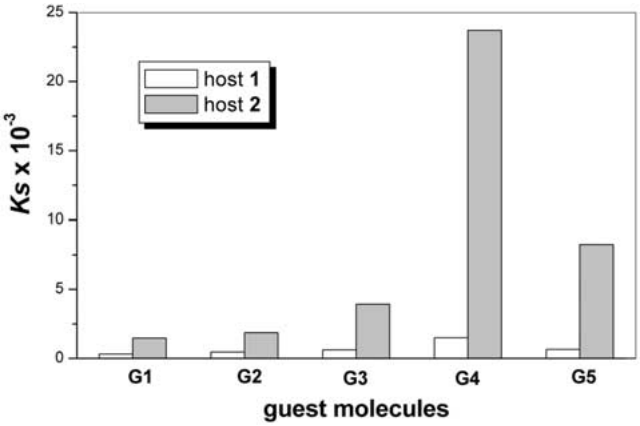


Figure 4. Complex stability constants K_S upon inclusion complexation of hosts **1–2** with various guest molecules at 25 °C in aqueous solution. G1, cyclohexanol; G2, cyclohexane carboxylic acid; G3, cyclohexane acetic acid; G4, sodium cyclohexane carboxylate; G5, potassium cyclohexane carboxylate.

Molecular binding ability and selectivity

Among the weak interactions involved in the inclusion complexation of cyclodextrins, van der Waals and hydrophobic interaction are found to play important roles to determine the complex stability. According to our previous report, simply modified β-cyclodextrin showed weaker binding ability towards cyclohexane carboxylic acid (G2) than towards cyclohexanol (G1), attributing to the poor hydrophobicity of cyclohexane carboxylic acid as compared with cyclohexanol [15]. However, in the present case, hosts **1** and **2** display stronger affinity towards cyclohexane carboxylic acid and cyclohexane acetic acid than towards cyclohexanol. This phenomenon is reasonably accounted for that the relatively strong interaction between the carboxyl group of guest molecule and the alkali crown ether sidearm in hosts **1–2** supplies an additional association. As a cooperative effect of these two factors, the complex stability constants of sodium and potassium cyclohexane carboxylates with hosts **1–2** are dramatically enhanced up to 2.5–90 times as compared with parent β-cyclodextrin. In the best case, crown ether-β-cyclodextrin **2** extends the original binding ability of parent β-cyclodextrin towards sodium cyclohexane carboxylate (G4) by a factor of 90, and shows a significantly high molecular selectivity towards G4/G1 pair up to 16.2. On the other hand, hosts **1** and **2** give also high molecular selectivity up to 2.3 and 2.9 towards sodium cyclohexane carboxylate (G4)/potassium cyclohexane carboxylate (G5) pair respectively, attributing to the crown ether moiety appended to the β-cyclodextrin cavity upon selectively binding with alkali-metal cations. Additionally, crown ether-β-cyclodextrin **2** forms more stable complex with guest molecules G1–G5 than host **1**. One possible explanation should be that the crown ether and cyclodextrin moieties of host **2** are located in close vicinity, which will be advantageous to the inclusion complexation between host and guest.

Although the results described above are deduced from limited data, we still can conclude that the crown ether tethered β-cyclodextrins can remarkably enhance the ori-

Table 1. Complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G^\circ$) for 1:1 inclusion complexation of various guests with β-cyclodextrin and hosts **1–2** in aqueous solution at 25 °C

Host	Guest	K_S	log K_S	$-\Delta G^0$ (kJ mol^{-1})	Method[a]	Ref.
β-CD	Cyclohexanol	688	2.838	16.19	cal	b
		500	2.70	15.3	uv	c
		704	2.85	16.3	cal	d
	Cyclohexane carboxylate	263	2.42	13.8	pot	e
1	Cyclohexanol	312 ± 10	2.50	14.24	fl	f
	Cyclohexanol	315 ± 10	2.50	14.24	uv	f
	Cyclohexane carboxylic acid	465 ± 20	2.67	15.25	fl	f
	Cyclohexane acetic acid	620 ± 20	2.79	15.94	fl	f
	Sodium cyclohexane carboxylate	1500 ± 50	3.18	18.13	fl	f
	Potassium cyclohexane carboxylate	647 ± 20	2.87	16.04	fl	f
2	Cyclohexanol	1460	3.16	18.06	fl	f
	Cyclohexane carboxylic acid	1860	3.27	18.66	fl	f
	Cyclohexane acetic acid	3920	3.59	20.51	fl	f
	Sodium cyclohexane carboxylate	23700	4.37	24.97	fl	f
	Potassium cyclohexane carboxylate	8240	3.92	22.35	fl	f

[a]Method employed: cal, calorimetry; fl, fluorometry; uv, spectrophotometry; pot, potentiometry.
[b]Reference [16], in H_2O at pH 6.90.
[c]Reference [17], in H_2O.
[d]Reference [18], in H_2O at pH 6.90.
[e]Reference [19], in H_2O.
[f]This work.

ginal binding ability of parent β-cyclodextrin by the cooperative binding of one guest molecule by two closely located binding sites (crown ether and cyclodextrin), especially for model substrates with metal cations, giving the highest binding ability towards sodium cyclohexane carboxylate up to 90 times higher than native β-cyclodextrin and the enhancement of molecular selectivity for sodium cyclohexane carboxylate/cyclohexanol pair by 16.2 times as compared with parent β-cyclodextrin for host **2**. So we can deduce that crown ether-cyclodextrin couples possess the inherent advantage of binding specific substrates containing metal cation and anionic hydrophobic skeleton. Further studies are currently in progress concerning the design and synthesis of new-typed supramolecular hosts bearing multiple binding sites along with the elucidation of the detailed cooperative binding mechanism.

Acknowledgements

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