SYNTHESIS OF FLUORESCENT MOLECULAR SENSORY SYSTEM FOR ENDOCRINE DISRUPTORS BASED ON DANSYLTHIACALIX[6]ARENES

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Dedicated to Professor Ivan Stibor on the occasion of his 60th birthday.

Flexible fluorescent hosts, mono-, di- and tridansyl-modified *tert*-butylthiacalix[6]arenes (**TC[6]A-1**, **TC[6]A-2** and **TC[6]A-3**, respectively) and didansyl-*tert*-butylcalix[6]arene (**C[6]A-2**) have been synthesized in order to investigate their fluorescent chemosensor functionality, which detects endocrine disruptors. The hosts, **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** exhibit pure monomer fluorescence spectra, of which the spectra shows a decrease fluorescence intensity in the presence of guests. The extent of fluorescence variation of **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** upon a guest addition was recognized as the manifestation of sensing ability of the hosts. A sensing parameter ($\Delta I/I^0$) was used to describe the sensing ability of four hosts. Host **TC[6]A-2** was able to detect 2,4-dichlorophenoxyacetic acid with high sensitivity. The guest-induced variations in the fluorescence intensities and MM2-minimized structures of the hosts suggest that the appended moieties of the hosts act as a hydrophobic cap and a probe showing the host to form a 1:1 host–guest complex.

Keywords: Calixarenes; Thiacalixarenes; Fluorescent sensors; Receptors; Dioxins; Endocrine disruptors; Chlorinated phenols; 2,4-Dichlorophenoxyacetic acid.

Fluorescent macrocyclic host molecules, such as calixarenes, cavitands and cyclodextrins[1–4], have attracted attention as supramolecular precursors, because these hosts can accommodate a variety of guests including organic molecules and metal cations in their cavities and their guest-responsive fluorescence spectra are used as a probe to show the activity of molecular sensors[5–13]. Calixarenes (CAs) are macrocycles comprising phenolic and

methylene units, which can readily be assembled by the base-catalyzed condensation of alkylphenols with formaldehyde. Therefore, CAs have phenolic hydroxy and alkyl groups at lower and upper rims, respectively, for each benzene unit, in which CAs themselves can hardly make a host–guest complexation with metal cations[2,14]. On the other hand, in thiacalix[4]arenes (TC[4]A), which are analogs of CAs composed of benzene rings linked via sulfide bridges, host–guest complexation proceeds with metal cations because the sulfide functional groups have affinity to metal cations[15–18]. Previously, we have reported that fluorescent TC[4]A recognizes metal cations such as Cd^{2+}, and the host–guest binding mechanism operates in aqueous solution, in which the dansyl units function as a hydrophobic cap and a fluorescent active probe[5]. Furthermore, we have been successful in the synthesis of regioselective de-*tert*-butylation of *tert*-butyl-TC[4]A and we have constructed their fluorescent metal sensory system, in which the appended units such as dansyl moieties also act as a hydrophobic cap to elevate binding ability for the metal cations[6]. Recently, Miyano and Iki and their co-workers have reported the synthesis of *tert*-butylthiacalix[6]arene (**TC[6]A**) and its crystal structure[19]. Recently, we have also reported porous crystals with a large cavity based on a metal complex of **TC[6]A** [20]. Anticipating complexation with big molecules, we have synthesized new fluorescent thiacalixarene (TCA) derivatives such as mono-, di- and tridansyl-*tert*-butylthiacalix[6]arenes (**TC[6]A-1**, **TC[6]A-2** and **TC[6]A-3**, respectively), which have larger cavities than those of TC[4]A derivatives. In the present study, we would like to report on a fluorescent molecular sensing system for endocrine disruptors based on **TC[6]A-1**, **TC[6]A-2** and **TC[6]A-3** compared with didansyl-*tert*-butylcalix[6]arene (**C[6]A-2**). Out of these hosts, **TC[6]A-2** can detect 2,4-dichlorophenoxyacetic acid with the highest sensitivity.

EXPERIMENTAL

Measurements

Fluorescence spectra were measured with a Perkin–Elmer LS 40B fluorescence spectrometer at 25 °C, the excitation wavelength was 340 nm, and the excitation and emission slits were 7 nm. 10 vol.% DMF aqueous solution was used as a solvent for the hosts because their solubility in pure water is poor. Five μl of guest species (0.5, 0.05 and 0.005 mol/l) in DMSO or MeOH were injected into a 10 vol.% DMF aqueous solution of the hosts (2.5 ml) to make a sample solution with a host concentration of 1.0×10^{-6} mol/l and a guest concentration of 1.0, 0.1 or 0.001 mmol/l. In NMR spectra, chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz.

Preparation of Mono-, Di- and Tridansylthiacalix[6]arenes (**TC[6]A-1**, **TC[6]A-2** and **TC[6]A-3**, Respectively)

To a suspension of *tert*-butylthiacalix[6]arene (**TC[6]A**; 0.2 g, 0.2 mmol) and NaH (27 mg, 1.3 mmol) in THF (20 ml), dansyl chloride (94 mg, 0.4 mmol) was added. The reaction mixture was stirred at room temperature for 24 h and then evaporated. The residue was extracted with chloroform, the organic phase was washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography on silica gel (Kiesel gel 60, 230–400 mesh, Merck) with hexane/acetone mixtures as eluents. Fractions eluted with 10 vol.% acetone/hexane were collected and evaporated to afford monodansyl-TC[6]A (**TC[6]A-1**; 32 mg, isolated yield 12%) as a yellow powder. 15 vol.% Acetone/hexane eluted fractions were collected and evaporated to yield crude of didansyl-TC[6]A. 20 vol.% Acetone/hexane eluted fractions were collected and evaporated to give tridansyl-modified TC6A (**TC[6]A-3**; 71 mg, isolated yield 20%) as a yellow powder. The resulting crude product of didansyl-TC[6]A was dissolved in DMF (1 ml). The DMF-soluble fraction was applied to a reverse-phase column (Lobar column Lichrorep RP-18). After stepwise elution with 100 ml of 10 vol.% and 100 ml of 50 vol.% acetone aqueous solution and with 500 ml of 80 vol.% acetone aqueous solution, didansyl-TC[6]A (**TC[6]A-2**; 15 mg, isolated yield 4.8%) was given as a yellow powder.

TC[6]A-1: R_F 0.49 (TLC: silica gel $60F_{254}$; hexane/acetone 2:1 by volume). ^{1}H NMR: 0.94 (9 H, s, $C(CH_3)_3$ of TC6A); 1.14 (18 H, s, $C(CH_3)_3$ of TC6A); 1.22 (18 H, s, $C(CH_3)_3$ of TC6A); 1.26 (9 H, s, $C(CH_3)_3$ of TC6A); 2.88 (6 H, s, $N(CH_3)_2$ of dansyl); 6.75 (2 H, s, arom. H of TC6A); 7.03 (2 H, d, J = 2.7, arom. H of dansyl); 7.33 (2 H, d, J = 2.4, arom. H of TC6A); 7.45 (2 H, d, J = 2.4, arom. H of TC6A); 7.51 (2 H, d, J = 2.4, arom. H of TC6A); 7.59 (4 H, s, arom. H of TC6A); 7.70–7.78 (3 H, br, OH of TC6A); 7.89 (1 H, t, J = 8.1, arom. H of dansyl); 8.10–8.24 (2 H, br, OH of TC6A); 8.39 (1 H, d, J = 6.6, arom. H of dansyl); 8.64 (1 H, d, J = 5.7, arom. H of dansyl); 8.67 (1 H, d, J = 7.2, arom. H of dansyl). For $C_{72}H_{83}NO_8S_7 \cdot 3H_2O$ (1369.0) calculated: 63.17% C, 6.55% H, 1.02% N; found: 63.41% C, 6.39% H, 1.28% N. MS (FAB), m/z: 1314 ($[M + 1]^+$).

TC[6]A-2: R_F 0.43 (TLC: silica gel $60F_{254}$; hexane/acetone 2:1 by volume) and 0.26 (TLC: RP-$18F_{254S}$, Merck; acetone/water 8:1 by volume). ^{1}H NMR: 0.79 (18 H, s, $C(CH_3)_3$ of TC6A); 1.19 (18 H, s, $C(CH_3)_3$ of TC6A); 1.23 (9 H. s, $C(CH_3)_3$ of TC6A); 1.26 (9 H, s, $C(CH_3)_3$ of TC6A); 2.88 (12 H, s, $N(CH_3)_2$ of dansyl); 6.47 (2 H, d, J = 2.1, arom. H of TC6A); 6.54 (1 H, s, arom. H of TC6A); 6.70 (2 H, d, J = 2.1, arom. TC6A); 7.11–7.19 (2 H, m, arom. H of dansyl); 7.22 (2 H, s, arom. H of dansyl); 7.37 (2 H, d, J = 2.7, arom. H of TC6A); 7.41 (2 H, d, J = 2.1, arom. H of TC6A); 7.43 (2 H, s, arom. H of TC6A); 7.54 (2 H, s, arom. H of dansyl); 7.50–7.62 (4 H, m, 1 H of arom. H and 3 H of OH of TC6A); 7.69 (1 H, t, J = 7.8, OH of TC6A); 8.36 (2 H, d, J = 7.2, arom. H of dansyl); 8.63 (2 H, d, J = 8.7, arom. H of dansyl); 8.67 (2 H, d, J = 8.7, arom. H of dansyl). For $C_{84}H_{94}N_2O_{10}S_8 \cdot 3H_2O$ (1601.0) calculated: 63.69% C, 6.24% H, 1.77% N; found: 63.42% C, 6.39% H, 1.28% N. MS (FAB), m/z: 1547 ($[M + 1]^+$).

TC[6]A-3: R_F 0.39 (TLC: silica gel $60F_{254}$; hexane/acetone 4:1 by volume). ^{1}H NMR: 0.78 (27 H, s, $C(CH_3)_3$ of TC6A); 1.23 (27 H, s, $C(CH_3)_3$ of TC6A); 2.87 (18 H, s, $N(CH_3)_2$ of dansyl); 6.39 (3 H, s, arom. H of TC6A); 6.73 (6 H, s, arom. H of TC6A); 7.20 (3 H, d, J = 7.2, arom. H of dansyl); 7.47 (5 H, s, 3 H of arom. H of TC6A and 2 H of arom. H of dansyl); 7.51–7.63 (7 H, m, 4 H of arom. H of dansyl and 3 H of OH of TC6A); 8.29 (3 H, d, J = 7.2, arom. H of dansyl); 8.55 (3 H, d, J = 9.0, arom. H of dansyl); 8.62 (3 H, d, J = 8.7, arom. H

of dansyl). For $C_{96}H_{105}N_3O_{12}S_9 \cdot 2H_2O$ (1817.5) calculated: 62.82% C, 6.10% H, 2.29% N; found: 62.95% C, 6.08% H, 2.32% N. MS (FAB), *m/z*: 1780 ($[M + 1]^+$).

Preparation of Didansylcalix[6]arene (**C[6]A-2**)

To a suspension of *tert*-butylcalix[6]arene (**C[6]A**; 0.5 g, 0.52 mmol) and NaH (72 mg, 3.09 mmol) in THF (50 ml), dansyl chloride (260 mg, 1.13 mmol) was added. The reaction mixture was stirred at room temperature for 24 h and then evaporated. The residue was extracted with chloroform, the organic phase was washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography on silica gel (Kiesel gel 60, 230–400 mesh, Merck) with hexane/acetone mixtures as eluents. Fractions eluted with 20 vol.% acetone/hexane were collected and evaporated to afford a crude of didansyl-C[6]A, which was purified with a reverse-phase column (Lobar column Lichrorep RP-18). After stepwise elution with 100 ml of 10 vol.% and 100 ml of 80 vol.% acetone aqueous solution and with 500 ml of acetone solution, didansyl-C[6]A (**C[6]A-2**; 22 mg, isolated yield 4.8%) was given as a yellow powder.

C[6]A-2: R_F 0.45 (TLC: silica gel $60F_{254}$; hexane/acetone 2:1 by volume). ^{1}H NMR: 0.86 (9 H, s, $C(CH_3)_3$ of C6A); 0.94 (9 H, s, $C(CH_3)_3$ of C6A); 1.22 (18 H, s, $C(CH_3)_3$ of C6A); 1.25 (18 H, s, $C(CH_3)_3$ of C6A); 2.87 (12 H, s, $N(CH_3)_2$ of dansyl); 3.12 (2 H, d, J = 15.3, CH_2 of C6A); 3.40–3.52 (1 H, m, CH_2 of C6A); 3.57 (2 H, s, CH_2 of C6A); 3.71 (2 H, s, CH_2 of C6A); 3.74–3.79 (1 H, s, CH_2 of C6A); 3.94 (2 H, d, J = 16.8, CH_2 of C6A); 4.11 (2 H, d, J = 15.0, CH_2 of C6A); 5.63 (2 H, s, arom. H of C6A); 6.63 (2 H, s, arom. H of C6A); 6.74 (2 H, s, arom. H of C6A); 6.78 (2 H, s, arom. H of C6A); 6.94 (2 H, d, J = 2.7, arom. H of C6A); 7.00 (2 H, d, J = 2.4, arom. H of dansyl); 7.13 (2 H, d, J = 2.4, arom. H of C6A); 7.21 (2 H, d, J = 7.2, arom. H of dansyl); 7.50–7.65 (5 H, m, 2 H of arom. H of dansyl and 3 H of OH of C6A); 7.83 (1 H, t, J = 7.8, OH of C6A); 8.26 (1 H, d, J = 7.5, arom. H of dansyl); 8.33 (1 H, d, J = 8.1, arom. H of dansyl); 8.56 (1 H, d, J = 9.0, arom. H of dansyl); 8.62 (3 H, d, J = 8.7, arom. H of dansyl). For $C_{90}H_{106}N_2O_{10}S_2 \cdot H_2O$ (1457.0) calculated: 74.14% C, 7.47% H, 1.92% N; found: 73.94% C, 7.84% H, 1.99% N. MS (FAB), *m/z*: 1439 ($[M]^+$).

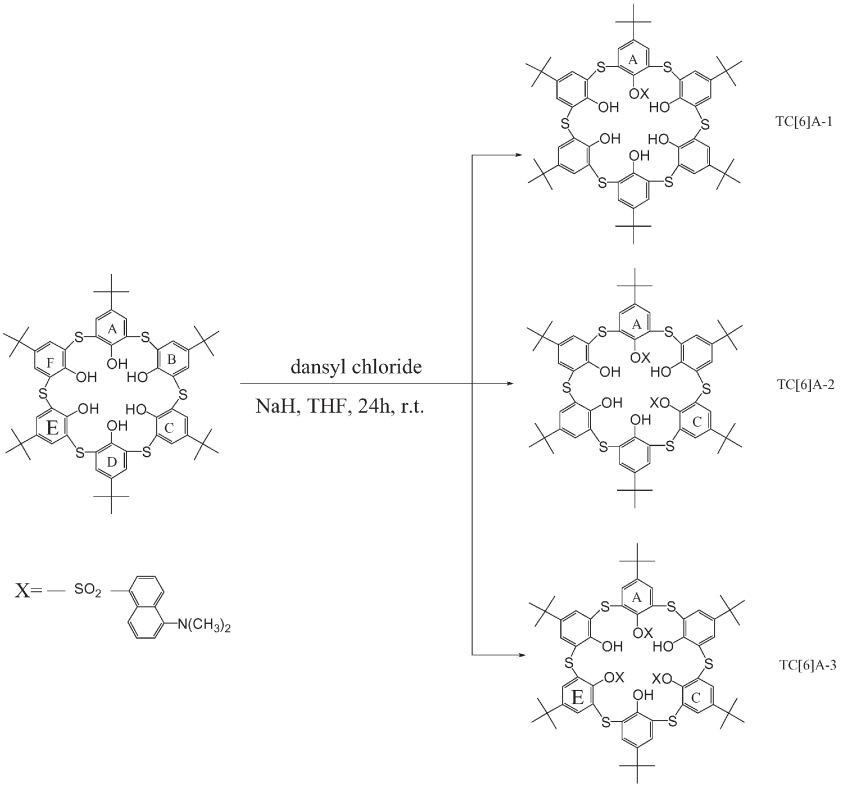
Energy-Minimized Structures

Energy-minimized structures were calculated by molecular mechanics using MM2 in CS Chem 3D. The parameters of MM2 are improved, obtained from studies by Allinger[20] based on TINKER system researched by Ponder[21].

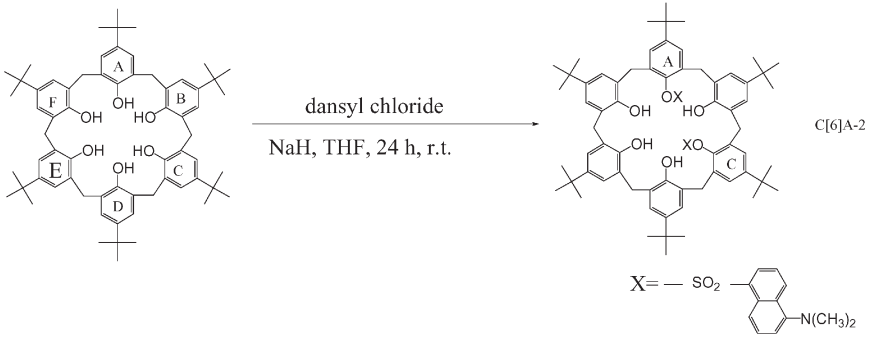
RESULTS AND DISCUSSION

*Preparations of Mono-, Di- and Tridansylthiacalix[6]arenes (**TC[6]A-1**, **TC[6]A-2** and **TC[6]A-3**, Respectively) and Didansylcalix[6]arene (**C[6]A-2**)*

Hosts **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** were directly prepared from **TC[6]A** and **C[6]A**, respectively, with excess of dansyl chloride in THF at room temperature, as shown in Schemes 1 and 2. Hosts **TC[6]A-1**, **TC[6]A-3** and **C[6]A-2** were purified by silica gel column chromatography.



SCHEME 1
Preparations of dansyl-TC[6]A analogs



SCHEME 2
Preparation of dansyl-C[6]A-2

In contrast, host **TC[6]A-2** was purified by reverse-phase column chromatography after separation by silica gel column chromatography. Figure 1 shows *tert*-butyl parts of the ^{1}H NMR spectra of **TC[6]A-1**, **TC[6]A-2**,

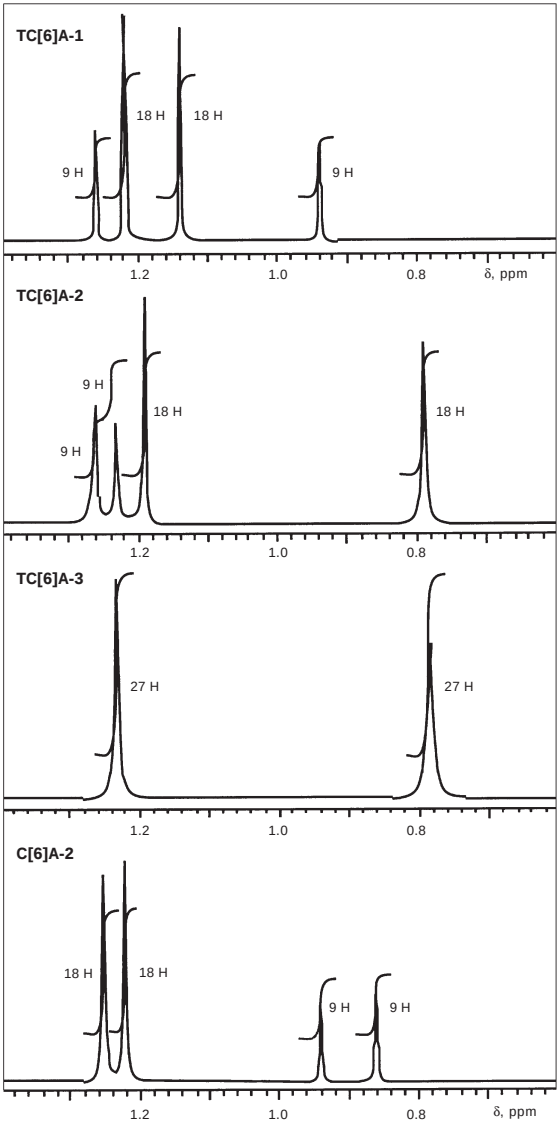


FIG. 1
^{1}H NMR spectra of **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** (in $CDCl_3$, 20 °C)

TC[6]A-3 and **C[6]A-2**. The ^{1}H NMR spectra of **TC[6]A-1** show 0.95, 1.14, 1.22 and 1.26 ppm signals as singlets, exhibiting integral values of 9 H, 18 H, 18 H and 9 H, respectively. In didansyl analogs, such as **TC[6]A-2** and **C[6]A-2**, the signals of ^{1}H NMR spectra are 0.79, 1.19, 1.23 and 1.26 ppm as singlets, exhibiting integral values of 18 H, 18 H, 9 H and 9 H, respectively, for **TC[6]A-2**, and at 0.86, 0.94, 1.22 and 1.25 ppm as singlets, exhibiting integral values of 9 H, 9 H, 18 H and 18 H, respectively, for **C[6]A-2**. Further, the ^{1}H NMR spectra of **TC[6]A-3** show 0.78 and 1.23 ppm signals as singlets, exhibiting integral values of 27 H and 27 H, respectively. These results suggest that four types of proton groups exist in **TC[6]A-2** and **C[6]A-2**, and two types of proton groups exist in **TC[6]A-3**. This means that the positions of dansyl units are A and C in **TC[6]A-2** and **C[6]A-2**, and A, C and E in **TC[6]A-3** for each structure as shown in Scheme 1.

Fluorescence Spectra

The fluorescence spectra of **TC[6]A-2** in a 10 vol.% DMF aqueous solution in the absence and presence of 3,4-dichlorobenzoic acid are shown in Fig. 2. The spectra of **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** are composed of monomer fluorescence with peaks around 490, 495, 505 and 500 nm, respectively. The intensity decreased with increasing guest addi-

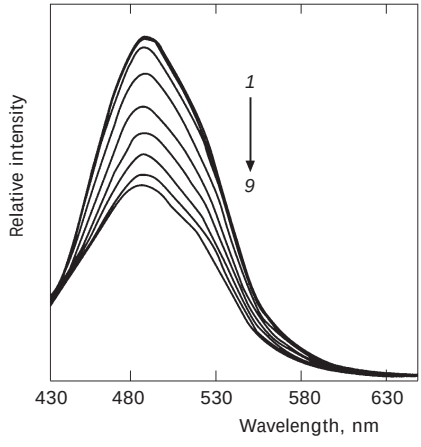
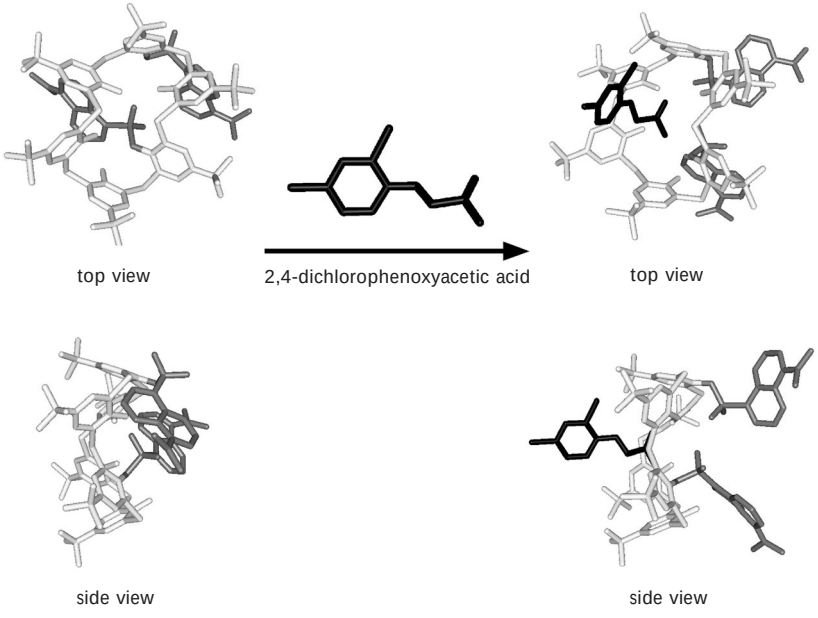


FIG. 2
Fluorescence spectra of **TC[6]A-2** in a 10 vol.% DMF aqueous solution (1.0×10^{-6} mol/l, 25 °C) at various concentrations of 3,4-dichlorobenzoic acid (**4**) (*1* 0, *2* 4.0×10^{-6}, *3* 1.2×10^{-5}, *4* 2.4×10^{-5}, *5* 4.0×10^{-5}, *6* 6.0×10^{-5}, *7* 8.3×10^{-5}, *8* 1.1×10^{-4}, *9* 1.4×10^{-4} mol/l)

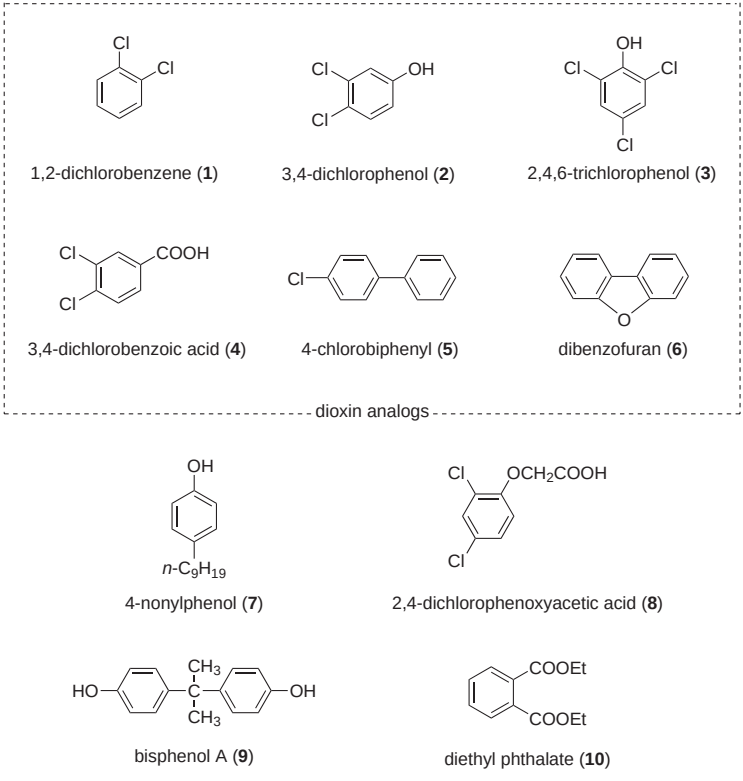
tion. It is reported that a decrease in the guest-induced fluorescence spectra means that appended unit moves towards the bulk water from the hydrophobic host cavity[9,10], whereas an increase means that the appended unit moves deep in the hydrophobic host cavity[8]. The fluorescence spectra changes suggest that the dansyl units of the four hosts move far away from the hydrophobic environment of TC[6]A cavity upon guest binding and play a role of a hydrophobic cap. The MM2-energy-minimized structure, which is **TC[6]A-2** alone and with 2,4-dichloroacetic acid as a guest, supports this mechanism, as illustrated in Scheme 3. The CPK models obtained for MM2-energy-minimized structures indicate that the cavities of four hosts, **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2**, are not large enough to include two guests. It means that four hosts make 1:1 host–guest complexes. It is probably suggested that the structures obtained from MM2 in CS Chem 3D should be local minimized structure, because the effect of solvent for the formation of the complex was neglected and stereo hindrance of resulting the complex was only considered.



SCHEME 3
Energy-minimized structure of **TC[6]A-2** including a guest obtained using MM2 in CS Chem 3D

*Detection of Endocrine Disruptors by **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2***

The detection of endocrine disruptors is still a big problem because complex and costly procedures and high proficiency are needed to obtain analytical results[22–24]. Therefore, it is urgent to construct a new direct and low-cost system to detect endocrine disruptors. A couple of years ago, we have reported a detection of endocrine disruptors based on fluorescent active cyclodextrins[25–29]. This makes us develop a new system, which is a fluorescent molecular sensing system based on fluorescent-active thiacalixarenes, because these hosts are soluble in a 10 vol.% DMF aqueous solution at fluorescence spectra concentration levels. Unfortunately, at ^{1}H NMR and UV spectra concentration levels, these hosts are not soluble in a 10 vol.% DMF aqueous solution. When we increase the DMF content in solution, the



SCHEME 4
Guest molecules

host–guest complexation phenomena was not observed. So we could not use ^{1}H NMR and UV analysis. Probably, the mechanism of the host–guest complexation is based on hydrophobic interactions. Accordingly, we have tried to investigate fluorescence molecular sensing ability of **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** for endocrine disruptors as shown in Scheme 4. It has been reported[5,6] that the extent of the variation of the fluorescence intensity of the host such as **TC[4]A** depended on the nature of a guest, even at low concentrations, therefore, those hosts also can probably be used as molecular sensors as reported previously. To evaluate the sensing ability of the fluorescent TC[6]As and their analogs, the $\Delta I/I^0$ value was used as a sensitivity parameter. Here, ΔI is $I^0 \cdot I$, where I^0 is the fluorescence intensity for the host alone and I that for complex. Figure 3 shows the parameter values of **TC[6]A-1**, **TC[6]A-2**, **TC[6]A-3** and **C[6]A-2** with 1,2-dichlorobenzene (**1**), 3,4-dichlorophenol (**2**), 2,4,6-trichlorophenol (**3**), 4-nonylphenol (**7**), 2,4-dichlorophenoxyacetic acid (**8**), bisphenol A (**9**) and diethyl phthalate (**10**) at 1.0 mmol/l, 3,4-dichlorobenzoic acid (**4**) at 0.1 mmol/l, and 4-chlorobiphenyl (**5**) and dibenzofuran (**6**) at 0.01 mmol/l; 0.5 M solution of **4**, and 0.05 M solutions of **5** and **6** are not soluble in the host solution. Of dioxin analogs, which are precursors of dioxin, guest **1**, which has two chlorine atoms in ortho positions of the benzene ring, was detected by **TC[6]A-1** with high sensitivity, exhibiting $\Delta I/I^0$ value of 0.252, whereas the sensing parameters of other hosts for guest **1** are below ca. 0.1. Similarly, guest **5** was detected by **TC[6]A-1** with high sensitivity, exhibit-

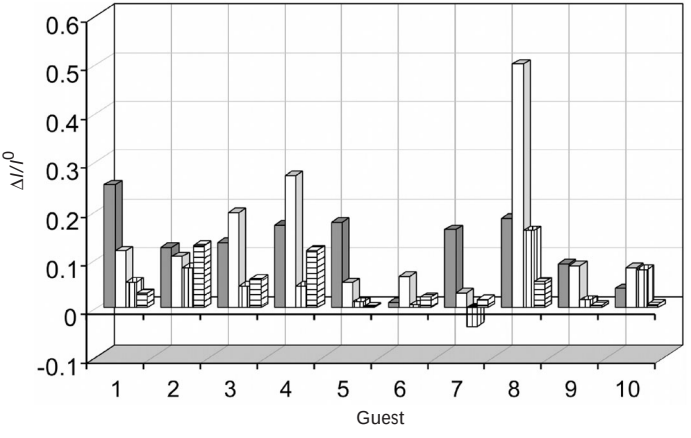


FIG. 3
Sensitivity factors of **TC[6]A-1** (■), **TC[6]A-2** (□), **TC[6]A-3** (▥) and **C[6]A-2** (▤) in a 10 vol.% DMF aqueous solution (1.0×10^{-6} mol/l, 25 °C) for endocrine disruptors

ing $\Delta I/I^0$ value of 0.176 even at a guest **5** concentration of 0.01 mmol/l, whereas the sensing parameters of other hosts for guest **5** are negligible. Guest **3**, which bears three chlorine atoms and one hydroxy group, and guest **4**, which has two chlorine atoms and one carboxylic group, were detected with **TC[6]A-2** with higher sensitivity than with other hosts, exhibiting $\Delta I/I^0$ values of 0.195 and 0.271, respectively, even at 0.1 mM concentration of guest **4**. Four hosts recognized guest **2**, which bears two chlorine atoms and one hydroxy group, and guest **6**, which has furan structure, with low sensitivity below ca. 0.1. Host **TC[6]A-2** detected peculiarly guest **8**, which bears two chlorine atoms and one carboxylic group with the highest sensitivity, exhibiting $\Delta I/I^0$ value of 0.500. Host **TC[6]A-1** detected guest **7**, which bears a long alkyl chain with high sensitivity, exhibiting $\Delta I/I^0$ value of 0.161, whereas the sensing parameters of other hosts for guest **7** are negligible. However, the sensing parameters of four the hosts for guest **9**, which has two benzene rings and hydroxy groups, and **10**, which bears two ethoxycarbonyl groups in ortho position in the benzene ring, are below 0.1. These results suggest that **TC[6]A-1** and **TC[6]A-2** are favorable as fluorescent molecular sensors, because one and two dansyl units of **TC[6]A-1** and **TC[6]A-2**, respectively, can easily move around the **TC[6]A** lower rim. Peculiarly, **TC[6]A-2** can recognize 2,4-dichlorophenoxyacetic acid (**8**) with remarkable sensitivity. On the other hand, because three dansyl units of **TC[6]A-3** are much crowded at the **TC[6]A** lower rim in comparison with **TC[6]A-1** and **TC[6]A-2**, three dansyl units enhance the host–guest binding ability less than one and two appended moieties. It is shown that **C[6]A-2** can hardly recognize endocrine disruptors. It was confirmed that sulfide, bridging benzene rings of **TC[6]A**, is effective in the fluorescent molecular sensing in aqueous solutions. It is probable that the high electron density from lone pairs of sulfide groups effectively binds dioxin analogs, which have a low electron density due to existing of electron-withdrawing groups such as chlorine. The guest-induced monomer fluorescence variations at 495 nm was employed to obtain the binding constants of **TC[6]A-2**. The binding constants are calculated by using a Benesi–Hildebrand equation (*1*) as reported previously[5,6].

$$\frac{1}{I_f - I_f^0} = \frac{1}{a[\mathrm{TC[6]A}]} + \frac{1}{a[\mathrm{TC[6]A}]K} + \frac{1}{[\mathrm{G}]} \qquad (1)$$

Here, I is the fluorescence intensity at 495 nm (I_f for complex, I_f^0 for the host alone), [TC[6]A] is the total host concentration, [G] is the total guest

concentration, *a* is a constant. A computer simulation using fluorescence intensity at 495 nm as a function of guest concentration proved that experimental data could fit to liner equations, indicating a Benesi–Hildebrand equation for a 1:1 complex formation, as shown in Fig. 4. The binding constants of **TC[6]A-2** for guests **4** and **8** are 8710 ± 670 and 11800 ± 430, respectively, roughly parallel with the sensing parameters. Host **TC[6]A-2** detects 2,4-dichlorophenoxyacetic acid (**8**) at $10^{-5.3}$–10^{-3} mol/l, as shown in Fig. 5. This suggests that **TC[6]A-2** gives clear concentration dependence for **8**, reflecting the sensitivities of the system to the guest.

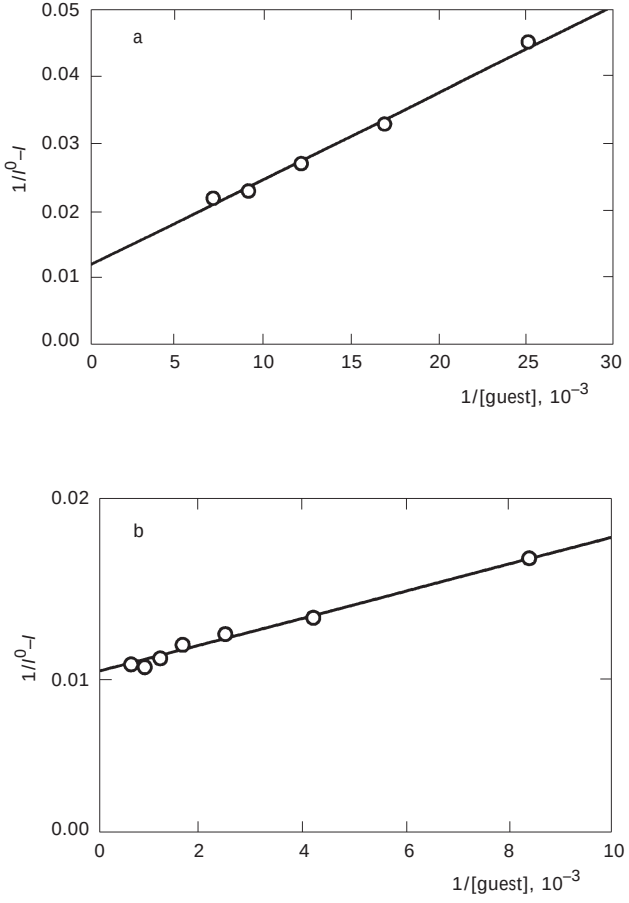


FIG. 4

Binding curves of **TC[6]A-2** (1×10^{-6} mol/l, 25 °C) in a 10 vol.% DMF aqueous solution for 3,4-dichlorobenzoic acid (**4**) (a) and 2,4-dichlorophenoxyacetic acid (**8**) (b)

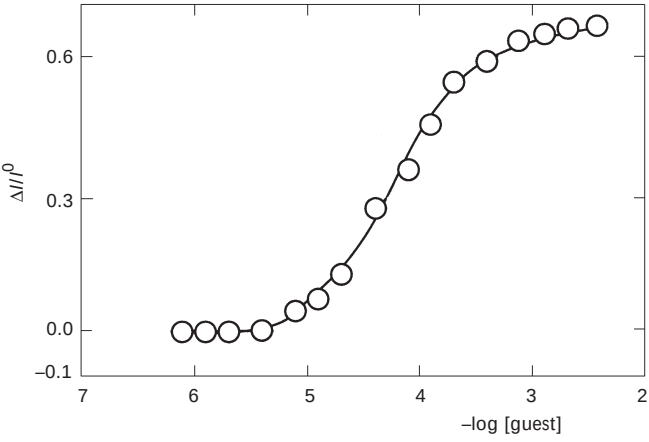


FIG. 5
Fluorescence variations of **TC[6]A-2** (1.0×10^{-6} mol/l, 25 °C) for 2,4-dichlorophenoxyacetic acid (**8**) as a function of guest concentration (in mol/l)

CONCLUSION

Three new analogs of *tert*-butylthiacalix[6]arenes selectively modified with dansyl units together with a C[6]A analog, which are soluble in 10 vol.% DMF aqueous solution, have been synthesized to investigate their molecular sensory system by guest-responsive fluorescence spectra. It is shown that didansyl-TC[6]A detects endocrine disruptors such as 2,4-dichlorophenoxyacetic acid (**8**) with peculiarly high sensitivity, forming a 1:1 host–guest complex. Furthermore, cooperation of two dansyl units in TC[6]A enhances the binding of endocrine disruptors compared with that of one or three dansyl units in TC[6]A.

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