Qualitatively Fluorescent Guest-Response of 2^A,2^X-Disulfonyl Dibenzosulfolane-Diphenyl-Capped β - and γ -Cyclodextrins for Endocrine Disruptors

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Fluorogenic host capped regioselectively at the lower rim, 2^A,2^X-disulfonyl dibenzosulfolane-diphenyl capped β -cyclodextrin (X=D for β -1) and γ -cyclodextrins (X=C, D and E for γ -1, γ -2 and γ -3), respectively, have been investigated their fluorescent molecular sensing properties for endocrine disruptors in an aqueous solution. These capping cyclodextrins exhibited monomer fluorescence, in which these hosts fluorescence decreased in intensity on accommodation of endocrine disruptors. The extent of fluorescence variation with a guest was employed to evaluate the sensing abilities of the hosts, using the parameter ($\Delta I/I^0$) as a sensing factor of the hosts for the guests. Hosts β -1, γ -2 and γ -3 detected phenolic guests such as 3,4-dichlorophenol, 2,4,6-trichlorophenol, *p*-nonylphenol, and bisphenol A with high sensitivities, and especially, these three hosts exhibited the highest sensitivities for bisphenol A. The sequences of binding of the hosts were γ -3 > β -1 > γ -2 > γ -1 for 3,4-dichlorophenol and 2,4,6-trichlorophenol and β -1 > γ -3 > γ -2 > γ -1 for *p*-nonylphenol and bisphenol A. The behaviors of the capped units of the hosts during a host-guest complexation were studied by fluorescence spectra and MM2-energy minimized structures. These studies suggest that the capping linker units, which are disulfonyl dibenzosulfolane-diphenyl, work as qualitatively fluorescent probe detecting endocrine disruptors.

Key Words : cyclodextrins, fluorescent sensor, molecular sensing, bisphenol A.

1. Introduction

Cyclodextrins (CyD), which are torus-shaped cyclic oligomers of composed of D-glycopyranose, are named α -, β - and γ - for the hexamer, heptamer and octamer, respectively. CyDs form inclusion complexes with various organic guests in an aqueous solution.¹⁻³⁾ In investigation of the resultant phenomena, we should use spectroscopically-active guests because CyDs are basically inert with respect to optical spectroscopy. However, CyDs can be transformed into spectroscopically-active hosts by modification of the chromophore units. Recently, chromophore modified CyDs have shown to exhibit remarkably variations in ICD and fluorescence spectra associated with the formation of inclusion complexes.⁴⁻⁷⁾ In these reports, these spectral variations of chromophore-modified CyDs were depending on the formation of the host-guest complexation; therefore, appended chromophores can be used as a probe to sense guest molecules. Previously, we have discussed the fluorescence sensing systems of CyDs modified with fluorescent units at upper rim.⁸⁻¹⁷⁾ In these systems, it was found that the fluorescence spectra are most sensitive observed in

spectrophotometry, in which the variations of guest-induced fluorescence intensities of the modified CyDs effectively work as a sensing probe showing much more complete and accurate sensing patterns. Furthermore, the position of the modification of the appended moieties on the upper rim of the CyD cavity was found to affect the sensing ability of these modified CyDs. In contrast, these are several papers describe the fluorescent binding property of CyD derivatives substituted at the C-2 or C-3 positions. However, the modification of the secondary hydroxyl group of CyD are very complicated.¹⁸⁻²⁷⁾ Fortunately, Teranishi has succeeded to prepare mono and regioselectively disulfonyl β - and γ -CyDs at the C-2 position from sulfonyl imidazole treated with molecular sieves 4A under mild condition.²⁸⁻²⁹⁾ A couple years ago, we discussed with the selective fluorescent sensing system of CyD modified with pyrene and tosyl at the lower and upper rims, respectively.³⁰⁾ In this system, pyrene moiety at the lower rim of CyD worked as more sensitive fluorescent probe to distinguish the structure of steroidal compounds than those of fluorescent CyD derivatives modified at the upper rim.

For further extension of our work, we tried to study the

fluorescent molecular sensing abilities for endocrine disruptor analogues based on β -CyD capping at the lower rim, which is 2^A, 2^D-disulfonyl dibenzosulfolane-diphenyl capped β -CyD (β -1).³¹⁻³² It was the first example to investigate molecular indicator system based on a fluorescent capped β -CyD at the lower rim for endocrine disruptors. In this contribution, we would like to report the sensor system of γ -CyD analogues of fluorescent capped CyD at the lower rim, which are 2^A, 2^C-, 2^A, 2^D- and 2^A, 2^E-disulfonyl dibenzosulfolane-diphenyl capped γ -CyDs (γ -1, γ -2 and γ -3, respectively)³³ as shown in Scheme 1. A couple of fluorescent capped CyDs at the upper rim have been reported to show low molecular sensing ability due to difficulty of movement of these fluorescent linker.³⁴ Although, hosts β -1, γ -2 and γ -3 can peculiarly recognize phenolic guests, such as *p*-nonylphenol and bisphenol A.

2. Experimental

Material

Host β -1, γ -1, γ -2 and γ -3 were prepared according to previous reported procedure.³¹⁻³³

Measurements

Fluorescence spectra were measured at 25°C using a Perkin-Elmer LS 40B fluorescence spectrometer.

For the fluorescence measurements, the excitation wavelength was 330 nm and excitation and emission slits were 4 nm. Five μ L of guest species (0.5 M) in MeOH or dimethyl sulfoxide (DMSO) were injected into a 10 vol.% ethylene glycol aqueous solution of a host (2.5 mL, 1.0×10^{-6} M). This procedure gave a guest concentration of 1.0 mM in the solution. For measurement, aqueous ethylene glycol (10%) was used as a solvent for the host because the solubility of a host in pure water is poor.

Energy-minimized structures

Energy-minimized structures were calculated by molecular mechanics using MM2 in CS Chem 3D. The parameters of MM2 were improved over those obtained from studies by N. L. Allinger³⁵ based on the TINKER system researched by J. W. Ponder.³⁶

Determination of binding constants

The binding constants of β -1, γ -1, γ -2 and γ -3 for several guests were obtained from guest-induced fluorescence variations at 395 nm by employing a Benesi-Hildbrand type equation (1) as reported previously.^{11, 14-15}

$$\frac{1}{I_f - I_f^0} = \frac{1}{a[CD]} + \frac{1}{a[CD]K} \times \frac{1}{[G]} \quad (1)$$

Here, I is the fluorescence intensity at 395 nm (I_f for complex, I_f^0 for the host alone), $[CD]$ is the total host concentration, $[G]$ is the guest concentration, a is a constant.

3. Results and discussion

Fluorescence spectra

Figure 1a shows the fluorescence spectra of γ -3 alone and in the presence of bisphenol A in a 10 vol.% ethylene glycol aqueous solution. The spectra of β -1, γ -1, γ -2 and γ -3 are composed of monomer fluorescence. Increasing bisphenol A concentration resulted in large decrease of the intensities of β -1, γ -2 and γ -3, whereas γ -1 exhibits smaller guest-induced fluorescence spectral changes than other hosts, as shown in Figure 1b.

As reported previously, the extent of the variation of the fluorescence intensity of the host depended on the nature of a guest, such as endocrine disruptor³⁷⁻⁴¹; therefore, the host can be used as an indicator of molecular recognition for endocrine disruptors. In order to evaluate the sensing ability of β -1, γ -1, γ -2 and γ -3, the $\Delta I/I^0$ was used as a sensing parameter. Here, ΔI is $I^0 - I$, where I^0 and I are the fluorescence intensities at 395 nm for the host alone and a complex, respectively. Figure 2 shows the parameter values of β -1, γ -1, γ -2 and γ -3 with 1,2-dichlorobenzene (1), 3,4-dichlorophenol (2), 2,4,6-trichlorophenol (3), *p*-nonylphenol (4), 2,4-dichlorophenoxyacetic acid (5), bisphenol A (6), and diethylphthalate (7) at 1.0 mM. The structures of guest molecules are shown in scheme 2. Hosts β -1, γ -2 and γ -3 detect with 6, which has two hydroxyl groups and two benzene rings with the greatest sensitivity, exhibiting the values of 0.946, 0.876 and 0.893, respectively. Guest 4, which are phenol having alkyl chain, is detected by β -1, γ -2, γ -3 with the next highest

Scheme 1 Structures of β -1, γ -1, γ -2 and γ -3.

sensitivity, exhibiting the values of 0.712, 0.632, and 0.617, respectively. These sensing parameters of β-1, γ-2 and γ-3 for 4 and 6 are higher than those of fluorescent CyDs reported previously. Guest 2, which is phenol having two chlorines is detected by β-1 and γ-3 with high sensitivity, exhibiting the values of 0.503 and 0.604, respectively, whereas γ-1 and γ-2 detected 2 with low sensitivities, exhibiting the values of 0.145 and 0.317, respectively. Guest 3, which is phenol bearing three chlorines, is also detected by γ-3 with high sensitivity, exhibiting a value of 0.661, whereas β-1, γ-1, and γ-2 detected 3 with low

sensitivities, exhibiting the values of 0.361, 0.160 and 0.323, respectively. These sensing parameters of β-1 and γ-3 for 2 and 3 are also higher than those of fluorescent CyDs reported previously. Guest 5, which has two chlorines and one carboxylic acid, is detected by all the hosts examined with low sensitivity, exhibiting the value ranging from 0.355 to 0.085. The sensing parameters for guests 1 and 7 are negligible by all the hosts examined. These results suggest that β-1, γ-2 and γ-3 can recognize phenolic molecules such as 4 and 6, which have longer molecular size than other guests, with high sensitivity. However, for guests 2 and 3, which have roughly spherical structure, β-1 and γ-3 can exhibit high sensitivities whereas γ-2 indicates low sensitivity. The sequence of the sensing abilities for guests 4 and 6 is β-1 > γ-2 ≥ γ-3 > γ-1, and for guests 2 and 3 is γ-3 > β-1 > γ-2 > γ-1. It suggests that the capping linker at A- and E-positions of eight glucose units of γ-CyD can contribute to elevate sensing ability for phenolic guests, which are spherical or longer structure. Additionally the capping linker at A and D-positions of eight glucose units of β- and γ-CyD can contribute to elevate sensing ability for phenolic guests which are longer structure. It is caused that the capping linker works to extent the hydrophobic domain of CyD cavity in an aqueous solution, therefore hosts β-1, γ-2 and γ-3 seem to peculiarly recognize phenolic guests which have long molecular size, such as *p*-nonylphenol and bisphenol A.

Binding constant

In order to examine the complexations of those four hosts examined here for the guests, the binding constants obtained as the guest-induced fluorescent spectral variation were investigated. The results are shown in Table 1. The host-guest complexation is 1:1 type, because a computer simulation using fluorescence intensity at 395 nm as a function of the guest concentration proved that experimental data could be fitted to the linear equations very well, indicating a Benesi-Hildebrand type equation for 1:1 complex formation, as shown in Figure 3. Moreover, the Corey-Pauling-Koitun (CPK) models calculated by MM2-energy minimized structures indicate that the cavity spaces of β-1, γ-1, γ-2 and

Figure 1a Fluorescent spectra of γ-3 in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) at various concentrations of bisphenol A (1: 0; 2: 1.0×10^{-5} ; 3: 2.0×10^{-5} ; 4: 6.0×10^{-5} ; 5: 1.2×10^{-4} ; 6: 2.0×10^{-4} ; 7: 3.0×10^{-4} ; 8: 4.2×10^{-4} ; 9: 5.5×10^{-4} ; 10: 7.1×10^{-4} M).

Figure 1b Fluorescent spectra of γ-1 in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) at various concentrations of bisphenol A (1: 0; 2: 1.0×10^{-5} ; 3: 2.0×10^{-5} ; 4: 6.0×10^{-5} ; 5: 1.2×10^{-4} ; 6: 2.0×10^{-4} ; 7: 3.0×10^{-4} ; 8: 4.2×10^{-4} ; 9: 5.5×10^{-4} ; 10: 7.1×10^{-4} M).

Figure 2 Sensitivity factors of β-1 (□), γ-1 (▨), γ-2 (▤), and γ-3 (■) in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for endocrine disruptors examined.

Table 1 Binding constants ($K/\text{mol}^{-1}\text{dm}^3$) of β -1, γ -1, γ -2 and γ -3 in a 10 vol.% ethylene glycol aqueous solution ($1.0 \times 10^{-6} \text{ M}$, 25°C).^{a)}

Guest	β -1	γ -1	γ -2	γ -3
3,4-Dichlorophenol (2)	640 ± 35 ^{b)}	300 ± 30	100 ± 5	730 ± 60
2,4,6-trichlorophenol (3)	480 ± 10	310 ± 30	120 ± 25	940 ± 70
<i>p</i> -Nonylphenol (4)	$532,000 \pm 34,600$	$53,200 \pm 9,130$	$65,500 \pm 4,540$	$196,000 \pm 5,530$
Bis-phenol A (6)	$141,000 \pm 2,370$	$1,170 \pm 80$	$6,070 \pm 80$	$11,200 \pm 310$

a) The K values were obtained from guest-induced fluorescence variations.

b) The statistical errors were values of standard deviation assessed by guest-induced fluorescence variations.

γ -3 are not large enough to include two guests. The binding constant is roughly in the order $4 > 6 > 3 > 2$ for the four hosts, which is not parallel with the sensing factors meaning that the sensitivity values gives relative but not absolute sensing ability.

Response ranges

It is very important to investigate the lower detection limits for endocrine disruptors by those host molecules. The response curves of four hosts examined here for some guests, which are *p*-nonylphenol (**4**) and bisphenol A (**6**) are shown in Figure 4. Guest 4 is detected with response ranges of $10^{-7.5}$ - $10^{-2.5}$, $10^{-6.5}$ - $10^{-2.5}$, $10^{-7.5}$ - $10^{-2.5}$ M by β -1, γ -1, γ -2 and γ -3 respectively. Guest 6 is detected with response ranges of 10^{-7} - $10^{-2.5}$, $10^{-4.5}$ - $10^{-2.5}$, 10^{-5} - $10^{-2.5}$, $10^{-5.5}$ - $10^{-2.5}$ M by β -1, γ -1, γ -2 and γ -3, respectively. The lower detection limits of β -1 and γ -3 for 4 is similar to the smallest values of the lower detection limits in the fluorescent CyDs reported previously,³⁹⁾ however, that of β -1 for 6 is one tenth lower than those in the fluorescent CyDs reported. The results obtained as the binding constants and the

response ranges suggest that the hosts such as β -1 and γ -3 can recognize 4 and 6 with the most qualitative sensitivity.

4. Conclusion

Fluorescent capped β - and γ -CyDs at the lower rim were investigated as a new molecular indicator for endocrine disruptors. These hosts showed pure monomer fluorescence, and the variations were used as a parameter to describe the sensing ability. Modification at the lower rim of the γ -CyD as a cap can contribute more qualitative sensing ability of the CyD than the fluorescent CyD modified at upper rim. It is recognized that the capping at the lower rim by the fluorescent active unit works to extent hydrophobic domain of the CyD; the capping CyD can peculiarly detect phenolic guests, such as *p*-nonylphenol and bisphenol A.

Acknowledgements

This study was supported by a Grant-in-Aid for Specially Promoted Research (No.404: Molecular Synchronization for

Scheme 2 Guest molecules.

Figure 3 Binding curves of β -1 and γ -3 in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for p -nonylphenol (○; β -1: a, γ -3: c) and bisphenol A (△; β -1: b, γ -3: d).

Figure 4 Fluorescence variations of β -1, γ -1, γ -2, and γ -3 in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for p -nonylphenol (○) and bisphenol A (□) as a function of guest concentration.

Design of New Materials System) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Notes and References

1. M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry", 1978, Spring-verlag, New York.
2. J. Szejtli, "Cyclodextrin Technology", 1988, Kluwer, Dordrecht.
3. S. Dumitriu, "Polysaccharides: Structural Diversity and Functional Versatility", 1998, Marcel Dekker, New York.
4. M. Eddaoudi, H. -P. Lopez, S. F. de lamotte, D. Ficheux, P. Probnon and A. W. Coleman, "Fluorescent amino acids as reporter systems in peptido-cyclodextrin inclusion compounds", *J. Chem. Soc., Perkin Trans. 2*, 1996, 1711.
5. S. R. Mcalpine and M. A. G. Garibay, "Inside-outside isomerism of β -cyclodextrin covalently linked with a naphthyl group", *J. Am. Chem. Soc.*, 1996, 118, 2750.
6. R. Corradini, A. Dossena, G. Galaverna, R. Marchelli, A. Panagia and G. Sartor, "Fluorescent chemosensor for organic guests and copper (II) ion based on dansyldiethylenetriamine-modified β -cyclodextrin", *J. Org. Chem.*, 1997, 62, 6288.
7. H. Nakashima, Y. Takenaka, M. Higashi and N. Yoshida, "Fluorescent behaviour in host-guest interactions. Part 2. Thermal and pH-dependent sensing properties of two geometric isomers of fluorescent amino- β -cyclodextrin derivatives", *J. Chem. Soc., Perkin Trans. 2*, 2001, 2096.
8. M. Narita, F. Hamada, I. Suzuki and T. Osa, "Variations of fluorescent molecular sensing for organic guests by regioselective anthranilate modified β - and γ -cyclodextrins", *J. Chem. Soc., Perkin Trans. 2*, 1998, 2751.
9. M. Narita, F. Hamada and M. Sato, I. Suzuki and T. Osa, "Fluorescent molecular recognition and sensing system of bis-dansyl modified γ -cyclodextrins", *J. Inclusion. Phenom. Macrocycl. Chem.*, 1999, 34, 421.
10. M. Sato, M. Narita, N. Ogawa and F. Hamada, "Fluorescent chemo-sensor for organic guests based on regioselectively modified 6^A,6^B-, 6^A,6^C-, and 6^A,6^D-bis dansylglycine-modified β -cyclodextrin", *Anal. Sci.*, 1999, 15, 1199.
11. M. Narita, S. Koshizaka and F. Hamada, "Fluorescent pyrrolinone-modified cyclodextrins as a chemo-sensor for organic Guests", *J. Inclusion. Phenom. Macrocycl. Chem.*, 1999, 35, 605.
12. S. Ito, M. Narita and F. Hamada, "Fluorescent molecular recognition systems of terphenyl modified β - and γ -cyclodextrins", *Int. J. Soc. Mater. Eng. Resour.*, 1999, 7, 156.
13. M. Narita and F. Hamada, "The synthesis of a fluorescent chemo-sensor system based on regioselectively dansyl-tosyl-modified β - and γ -cyclodextrins", *J. Chem. Soc., Perkin Trans. 2*, 2000, 823.
14. Y. Higuchi, M. Narita, S. Sugawara, N. Ogawa, F. Hamada and H. Suzuki, "Fluorescent chiral molecular sensing based on β -cyclodextrins modified with mono and bis (R)- or (S)-3-hydroxy-3-phenylpropanoic Acid", *Int. J. Soc. Mater. Eng. Resour.*, 2000, 8, 19.
15. F. Hamada, M. Narita, A. Makabe and H. Itoh, "The effect of dansyl-modified β -cyclodextrin on the chaperone activity of heat shock proteins", *J. Inclusion. Phenom. Macrocycl. Chem.*, 2001, 40, 8.
16. F. Hamada, M. Narita, K. Kinoshita, A. Makabe and T. Osa, "Synthesis and fluorescent molecular sensing at exciplex emission of pyrene- and cyanobenzene-modified γ -cyclodextrins", *J. Chem. Soc., Perkin Trans. 2*, 2001, 388.
17. A. Makabe, K. Kinoshita, M. Narita and F. Hamada, "Guest-responsive fluorescence variations of γ -cyclodextrins labeled with hetero-functionalized pyrene and tosyl moieties", *Anal. Sci.*, 2002, 18, 119.
18. Y. Wang, T. Ikeda, A. Ueno, and F. Toda, "Syntheses and molecular recognition abilities of 6-O-, 2-O- and 3-O-dansyl- γ -cyclodextrin", *Chem. Lett.*, 1992, 863.
19. Y. Wang, T. Ikeda, H. Ikeda, A. Ueno and F. Toda, "Dansyl- β -cyclodextrin as fluorescent sensors responsive to organic compounds", *Bull. Chem. Soc. Jpn.*, 1994, 67, 1598.
20. J. Bgler, J. F. J. Engbersen and D. N. Reinhoudt, "Novel water-soluble β -cyclodextrin-calix[4]arene couples as fluorescent sensor molecules for the detection of neutral analytes", *J. Org. Chem.*, 1998, 63, 5339.
21. J. Bgler, N. A. J. M. Sommerdijk, A. J. W. G. Visser, A. van Hoek, R. J. M. Nolte, J. F. J. Engbersen and D. N. Reinhoudt, "Interconnective host-guest complexation of β -cyclodextrin-calix[4]arene couples", *J. Am. Chem. Soc.*, 1999, 121, 28.
22. H. F. M. Nelissen, A. F. J. Schut, F. Venema, M. C. Feiters and R. J. M. Nolte, "Switch-on luminescence detection of steroids by tris(bipyridyl)ruthenium(II) complexes containing multiple cyclodextrin binding sites", *J. Chem. Soc., Chem. Commun.*, 2000, 577.
23. A. Ueno and R. Breslow, "Selective sulfonation of a secondary hydroxyl group of β -cyclodextrin", *Tetrahedron Lett.*, 1982, 23, 3451.
24. K. Fujita, S. Nagamura and T. Imoto., "Convenient preparation and effective separation of the C-2 and C-3 tosylates of β -cyclodextrin", *Tetrahedron Lett.*, 1984, 25, 5673.
25. T. Murakami, K. Harata and S. Morimoto, "Regioselective sulfonation of a secondary hydroxyl group of cyclodextrins", *Tetrahedron Lett.*, 1987, 28, 321.
26. D. Pong and V. T. D'Souza, "A convenient method for functionalization of the 2-position of cyclodextrins", *Tetrahedron Lett.*, 1990, 31, 4275.
27. F. Venema, C. M. Baselier, E. van Dienst, B. H. M. Ruel, M. C. Feiters, J. F. J. Engbersen, D. N. Reinhoudt and R. J. M. Nolte, "Synthesis and binding properties of novel cyclodextrin dimers", *Tetrahedron Lett.*, 1994, 35, 1773.
28. K. Teranishi, "Practicable regiospecific bifunctionalization on the secondary face of α - and β -cyclodextrins", *J. Chem. Soc., Chem. Commun.*, 2000, 1255.
29. K. Teranishi, M. Hisamatsu and T. Yamada, "Regiospecific synthesis of 2^A,2^B-disulfonated γ -cyclodextrin", *Tetrahedron Lett.*, 2000, 41, 933.
30. M. Narita, E. Tashiro and F. Hamada, "Synthesis of a selective fluorescent sensing system based on γ -cyclodextrin modified with pyrene and tosyl on the hetero Rims", *J. Inclusion. Phenom. Macrocycl. Chem.*, 2002, 42, 137.
31. K. Teranishi, "Regioselective 2^A,2^D-disulfonyl capping of β -cyclodextrin for practical bifunctionalization on the secondary hydroxyl face", *Tetrahedron Lett.*, 2001, 42, 5477.
32. M. Narita, N. Dorjpalam, K. Teranishi and F. Hamada, "Selective fluorescent molecular sensing based on 2^A,2^D-disulfonyl dibenzosulfolane-diphenyl-capped β -cyclodextrin for phenolic guests", *Anal. Sci.*, 2002, 18, 711.
33. Hosts γ -1, γ -2 and γ -3 have been synthesized by Teranishi.
34. F. Hamada, K. Ishikawa, I. Tamura, K. Murai, Y. Akagami

- and A. Ueno, "Molecular sensing for organic guests by cyclodextrins modified with anthranilate moieties", *Int. J. Soc. Mater. Eng. Resour.*, 1997, 1, 69.
35. U. Burkert, N. L. Allinger, "*Molecular Mechanics*", 1982, ACS Monograph 177, American Chemical Society, Washington, D. C.
 36. M. J. Dudek and J. W. Ponder, "Accurate modeling of the intramolecular electrostatic energy of proteins", *J. Comput. Chem.*, 1995, 16, 791.
 37. M. Narita, N. Ogawa and F. Hamada, "Fluorescence molecular sensing for endocrine-disrupting chemicals and their analogues based on dansyl or anthranilate modified β - and γ -cyclodextrins" *Anal. Sci.*, 2000, 16, 37.
 38. M. Narita, N. Ogawa and F. Hamada, "Fluorescent molecular recognition for endocrine-disrupting chemicals and their analogs by fluorescent hetero-modified cyclodextrins", *Anal. Sci.*, 2000, 16, 701.
 39. M. Narita, A. Makabe, K. Kinoshita, K. Endo and F. Hamada, "High sensitive molecular recognition by monomer and exciplex emissions for endocrine-disrupting chemicals and their analogs based on fluorescent hetero-modified cyclodextrins", *Int. J. Soc. Mater. Eng. Resour.*, 2001, 9, 6.
 40. M. Narita, E. Tashiro and F. Hamada, "Selective recognition of endocrine disruptors by fluoregenic γ -cyclodextrin having pyrene-tosyl substituents on hetero rims", *Anal. Sci.*, 2001, 17, 1453.
 41. M. Narita, S. Mima, N. Ogawa and F. Hamada, "Fluorescent molecular sensory system based on bis pyrene-modified γ -cyclodextrin dimer for steroids and endocrine disruptors", *Anal. Sci.*, 2001, 17, 379.