Journal of Inclusion Phenomena and Macrocyclic Chemistry **44:** 335–339, 2002.

Synthesis of γ-Cyclodextrin Pyrene-Labeled at the Hetero Rim and Its Use in Fluorescent Molecular Sensing

MIYUKI NARITA and FUMIO HAMADA
Department of Materials-process Engineering and Applied Chemistry for Environments, Faculty of Engineering and Resource Science, Akita University, Tegata, Akita 010-8502, Japan

(Received: 7 May 2002; in final form: 1 October 2002)

Key words: excimer formation, fluorescent molecular sensing, γ-cyclodextrin, hetero rim

Abstract

Fluorogenic active host labeled at the upper and lower rims of γ-cyclodextrin, namely, mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-6^{X}-*O*-mono-pyrenebutylate-mono-altro-γ-cyclodet rin (**mixture γ-1**, X = A, B, C, D, E, F, G, or H), has been synthesized in order to investigate their host-guest complexation with steroidal compounds using fluorescence spectra. Monomer and excimer fluorescence was observed for mixture host. Inclusion of a guest molecule in the cyclodextrin cavity resulted in increased monomer fluorescence and decreased excimer fluorescence. The extent of monomer and excimer fluorescence variations of **mixture γ-1** with the guest was used as an indication for the sensing ability. The guest induced fluorescence changes were measured for 10^{-7} M solutions of **mixture γ-1**. The values $\Delta I/I^0$, where I^0 and I are fluorescence intensities in the absence and presence of a guest, respectively, and ΔI is $I^0 - I$, were then used to describe the sensing ability.

Introduction

Cyclodextrins (CDs) are spectroscopically inert, however, CDs can be converted into spectroscopically active host by modification with fluorescent active units. Fluorogenic CDs exhibit fluorescence spectral changes for guest-inclusion and this change is used for molecular recognition of a guest compounds at a low concentration of these CDs [1–19]. Recently, we have studied synthesis of a selective fluorescent sensing system based on γ-CD labeled with pyrene and tosyl on the hetero rim, in which the appended units were able to function to distinguish the steroidal compounds with *trans*- or *cis*-AB-fursion [20]. This report has clarified that the co-operation of the hetero unit on the CD hetero rim produces higher selective molecular recognition than that of the hetero units on the CD upper rim reported previously [9, 14, 19]. In a series of the fluorogenic CDs labeled at the hetero rim, we synthesized homo fluorogenic γ-CD labeled at the hetero rim, which is mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-6^{X}-*O*-mono-pyrenebutylate-mono-altro-γ-CD (**mixture γ-1**, X = A, B, C, D, E, F, G, and H), as a new chemo-sensing system based on intermolecular excimer formation of this compound.

Experimental

Preparation of mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-6^{X}-O-mono-pyrenebutylate mono-altro-γ-CD (mixture γ-1, X = A, B, C, D, E, F, G, or H)

A mixture of mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-6^{X}-*O*-(*p*-toluenesulfonyl)mono-altro γ-CD (**I**, X = A, B, C, D, E, F, G, and H) [20] (40 mg, 0.023 mmol) and sodium 1-pyrenebutylate (14 mg, 0.046 mmol) in 5 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 200 mL of acetone. The resulting precipitate was filtered off and dissolved in 1 mL of DMF. The DMF soluble fraction was applied to a reversed-phase column (Lobar column Lichroprep RP-18, 240 × 10 mm). Stepwise elution using 100 mL of 10 vol.%. 100 mL of 30 vol.%, and 100 mL of 50 vol.% aqueous MeOH, and 100 mL of 60 vol.% aqueous MeOH was used to obtain **mixture γ-1** (18 mg, 1.6%, isolated yield).

R_f 0.62 (butanol-ethanol-water 5:4:3 by volume, TLC; silica gel 60F254; Merck Ltd.) and 0.35 (methanol-water 2:1 by volume, TLC; RP-18F$_{254}$S; Merck Ltd.) ^{1}H-NMR (DMSO-d$_6$): δ = 3.2–3.8 (46H, m, C^{2}H and C^{3}H of glucose unit without pyrene and C^{4}H, C^{5}H and C^{6}H of CD), 3.8–3.95 (1H, br., C^{3}H of glucose unit with pyrene), 4.1–4.2 (H, br., C^{2}H of glucose unit with pyrene), 4.4–4.65 (7H, br., O^{6}H of CD), 4.7–5.1 (7H, br., C^{1}H of glucose units without pyrene), 5.4–5.5 (1H, m, C^{1}H of glucose unit with pyrene), 5.6–6.0 (15H, m, O^{2}H and O^{3}H of CD), 7.96 (2H, d, J = 7.8 Hz, aromatic-H of pyrene), 8.06 (2H, t, J = 7.8 Hz, aromatic-H of pyrene), 8.13 (4H, s, aromatic-H of pyrene), 8.20–8.35

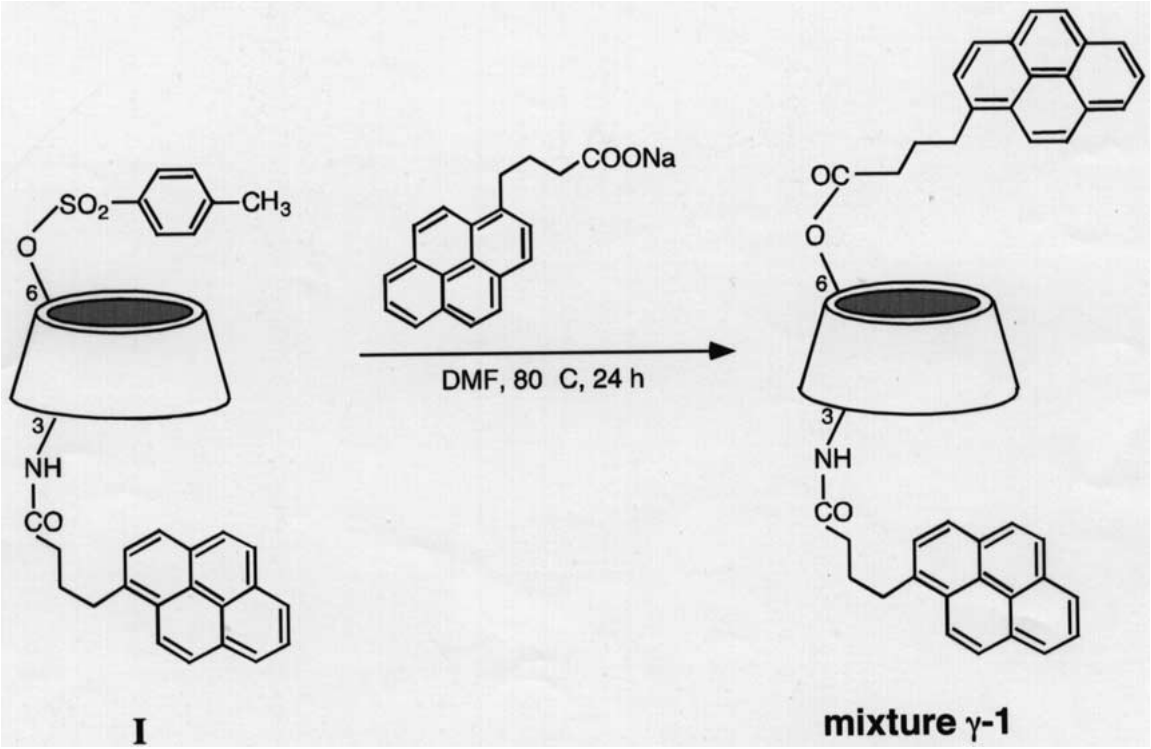


Figure 1. Preparation of **mixture γ-1**.

(8H, m, aromatic-H of pyrene), 8.42 (2H, d, J = 9.3 Hz, aromatic-H of pyrene). Calcd. for $C_{88}G_{109}O_{41}N{\cdot}2H_2O$: C, 56.44; H, 6.08; N, 0.75%. Found: C, 56.22; H, 6.19; N, 0.70%. MS(FAB): 1836 ($[M]^+$).

Measurements

Fluorescence and circular dichroism spectra were measured at 25 °C using a Perkin-Elmer LS 40B fluorescence spectrophotometer and a JASCO J-700 spectropolarimeter, respectively. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 345 nm and the excitation and emission slits were 4 nm wide. Ethylene glycol aqueous solution (10 vol.%) was used as a solvent for the spectroscopic measurements because the solubility of the host in pure water is poor. Five μL of guest species (0.05 and 0.005 M) in dimethyl sulofoxide (DMSO) or MeOH were injected into a 10 vol.% ethylene glycol aqueous solution of the host (2.5 mL) to make a sample solution with a host concentration of 1.0×10^{-7} M and guest concentration of 0.1 and 0.01 mM.

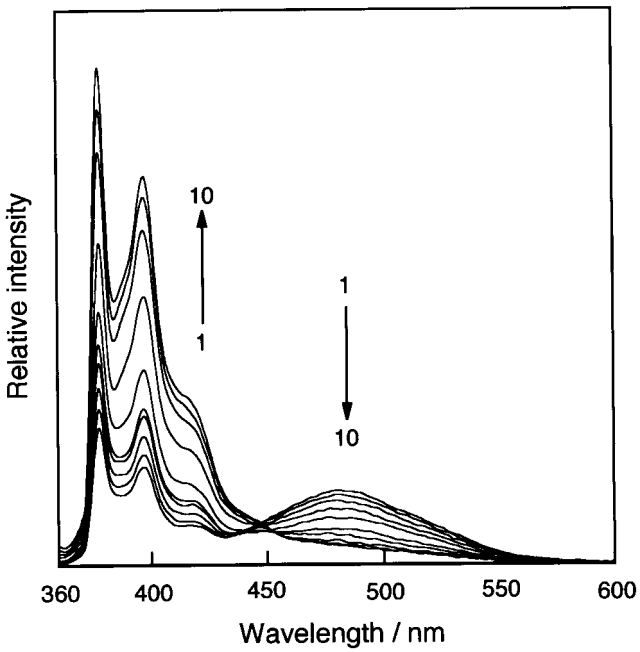


Figure 2. Fluorescence spectra of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-7} M, 25 °C) at various concentrations of lithocholic acid (1: 0, 2: 4×10^{-7}, 3: 1.2×10^{-6}, 4: 2.4×10^{-6}, 5: 4.0×10^{-6}, 6: 6.0×10^{-6}, 7: 8.3×10^{-6}; 8: 1.1×10^{-5}, 9: 1.4×10^{-5}, 10: 1.8×10^{-5} M).

Results and discussion

The preparation of mono-3^A-deoxy-3^A-pyrenebutylamido-6^X-O-mono-pyrenebutylate mono-altro-γ-CD (mixture γ-1, X = A, B, C, D, E, F, G, or H)

As previous report [20], compound **I** would be mixture of regioisomer bearing the pyrene unit at 3A-position of glucose units and the tosyl unit at 6A to 6H-positions of glucose units. Therefore, we attempted to determine the positions of the tosyl unit of compound **I** by ^{1}H-NMR analysis, X-ray analysis of single crystals obtained from recrystallization and charge density of energy-minimized structures. Unfortunately, our attempts of ^{1}H-NMR analysis, X-ray analysis were not successful. The charge density of energy-minimized structure of mono-3-deoxy-3-

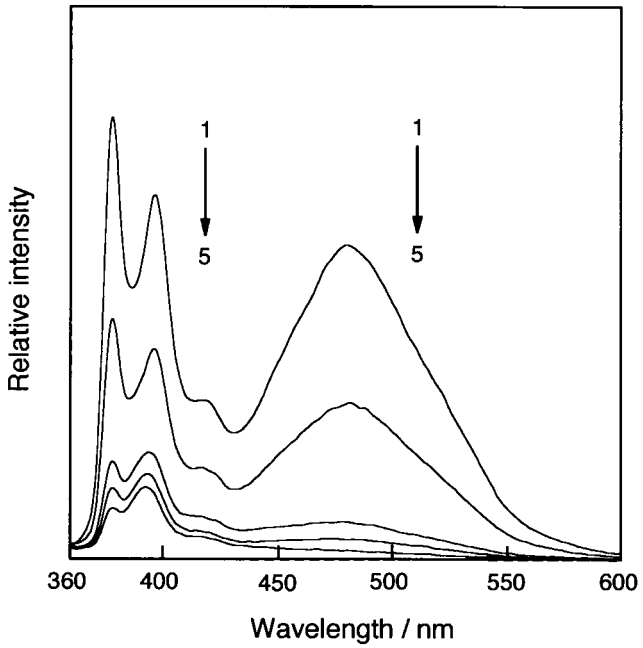


Figure 3a. Monomer and excimer emissions depending on concentration of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1: 1.0×10^{-7}, 2: 5.0×10^{-8}, 3: 1.0×10^{-8}, 4: 5.0×10^{-9}, 5: 1.0×10^{-9} M).

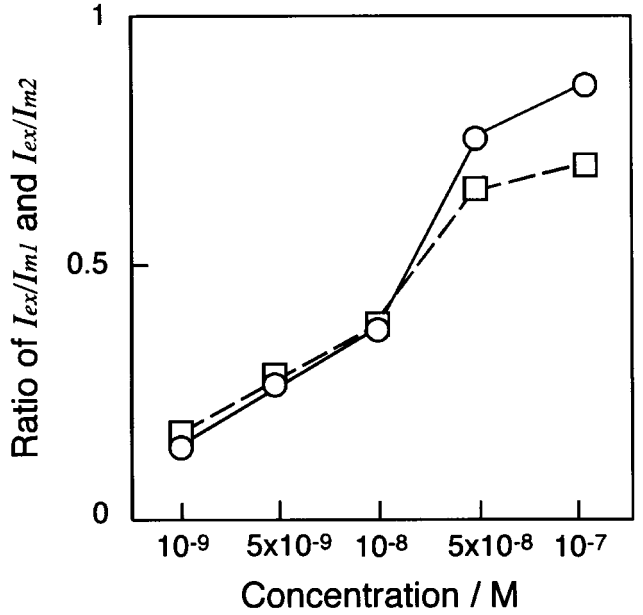


Figure 3b. Parameters of I_{ex} versus I_{m1} (○) and I_{ex} versus I_{m2} (□) in various concentrations of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution.

pyrenebutylamido-mono-altro-γ-CD [20] exhibited higher charge density at the H-position of C-6 primary hydroxyl groups than those at other positions. These results suggest that the tosyl unit of compound **I** will be located at the H-position in C-6. However, these results are not sufficient to determine the structure. Therefore, in this study, **mixture γ-1** was assumed to exist as mixture of diastereomers which is mono-3^A-deoxy-3^A-pyrenebutylamido-6^X-*O*-mono-pyrenebutylate mono-altro-γ-CD (X = A, B, C, D, E, F, G, or H).

Fluorescence spectra

The fluorescence spectra of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution in the absence and the presence of lithocholic acid are shown in Figure 2. The concentration of **mixture γ-1** in the fluorescence measurement is 1.0×10^{-7} M. In our studies reported previously, the host concentrations in the fluorescence measurements were 1.0×10^{-6} M [5–9, 14, 18, 19, 20], and other reports of bis pyrene-labeled (upper rim) CDs described that those concentrations were larger than 1.0×10^{-6} M [21, 22–24]. Thus the introduction of two pyrenes to the hetero rim of γ-CD will contribute to increased fluorescence intensity, which can be measured at 1.0×10^{-7} M concentration, whereas γ-CD, modified with a pyrene and a tosyl group, cannot be measured at this concentration [20]. Probably, the modification with two pyrenes at the hetero rim will be make hydrophobicity around the CD cavity larger, therefore, the fluorescence intensity of the pyrene units at the hetero rim of **mixture γ-1** is larger than that of other pyrene-labeled (upper rim) CDs. The spectra of **mixture γ-1** are composed of monomer and excimer fluorescence with the peaks at around 377, 397 and 480 nm, respectively, indicating that the pyrene excimer of **mixture γ-1** is derived from intermolecular or intramolecular interaction, because the excimer fluorescence are caused by the face-to-face formation of two pyrene units of **mixture γ-1**. In order to decide whether the pyrene excimer of **mixture γ-1** produced in the intermolecular or intramolecular interaction, a ratio of monomer versus excimer fluorescence intensities depending on the concentration of **mixture γ-1** is figured out, as shown in Figure 3a and 3b. The values I_{ex}/I_{m1} and I_{ex}/I_{m2} were used as parameters, where I_{m1} and I_{m2}, and I_{ex} are intensities of monomer fluorescence at 377 and 397 nm and intensity of excimer fluorescence at 480 nm, respectively. The values I_{ex}/I_{m1} and I_{ex}/I_{m2} of **mixture γ-1** were changed in 1.0×10^{-9} to 1.0×10^{-7} M. The linear equations of the range 1.0×10^{-9}–1.0×10^{-8} and 5.0×10^{-8}–1.0×10^{-7} M of **mixture γ-1** is not connected. This indicates that the pyrene excimer formation between the range 1.0×10^{-9}–1.0×10^{-8} and over 5.0×10^{-8} M of **mixture γ-1** are different. It was reported that large concentration of pyrene produced excimer fluorescence by the intermolecular interaction of pyrenes and small concentration of pyrene caused excimer fluorescence by the intramolecular interaction of pyrenes [25]. It is estimated that the pyrene excimer of **mixture γ-1** is derived from the intermolecular interaction in the range of 5.0×10^{-8}–1.0×10^{-7} M of **mixture γ-1**, on the other hand, the pyrene excimer of **mixture γ-1** is speculated to be produced by the intramolecular interaction in the range of under 5.0×10^{-8} M of **mixture γ-1**. These suggestions mean that the pyrene excimer of **mixture γ-1** at 1.0×10^{-7} M might be caused by the intermolecular interaction. Therefore, **mixture γ-1** is seemed to form the association dimer at 1.0×10^{-7} M, in which the pyrene unit at the upper rim might make an excimer formation with the pyrene unit at the upper rim of another CD.

The monomer and excimer fluorescence spectra of **mixture γ-1** increase and decrease, respectively, with increasing

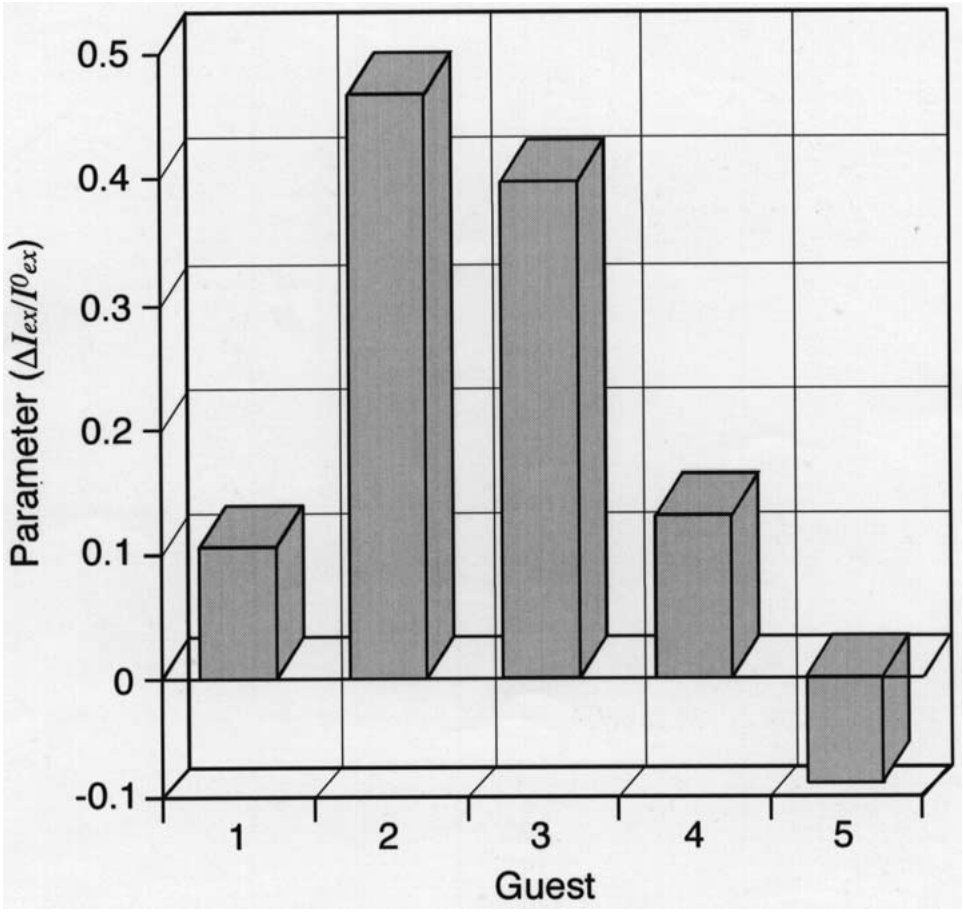
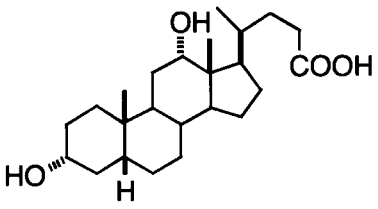
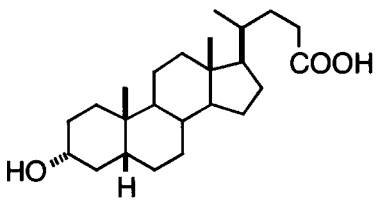


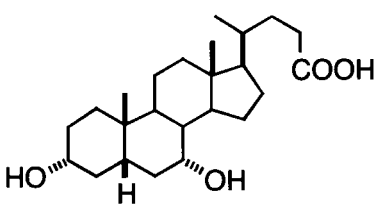
Figure 4. Sensitivity factors of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-7} M, 25 °C) at excimer emission for steroidal guests examined.



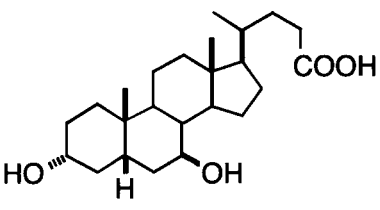
deoxycholic acid (**1**)



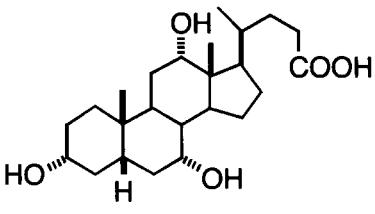
lithocholic acid (**2**)



chenodeoxycholic acid (**3**)



ursodeoxycholic acid (**4**)



cholic acid (**5**)

Scheme 1. Guest molecules.

lithocholic acid concentration. The results obtained as the guest-induced fluorescence spectral changes suggest that the pyrene excimer formation of **mixture γ-1** will be cancelled, when a guest is included into the CD cavity.

Sensing ability of **mixture γ-1** *for bile acids*

In order to evaluate the sensing ability of **mixture γ-1**, the $\Delta I_{ex}/I_{ex}^0$, value was used as sensitivity parameters. Here, ΔI_{ex} is $I_{ex}^0 - I_{ex}$, where I_{ex}^0 is the intensity of excimer at 480 nm for the host, and I_{ex} is the intensity of excimer in the presence of a guest. Figure 4 shows the parameter values of **mixture γ-1** obtained using bile acid at 0.1 mM except for lithocholic acid (2), which was examined at 0.01 mM because 0.1 mM of lithocholic acid is not soluble to a 10 vol.% ethylene glycol aqueous solution. Lithocholic acid (**2**) is detected by **mixture γ-1** with the highest value $\Delta I_{ex}/I_{ex}^0$ of 0.469. Chenodeoxycholic acid (**3**) is detected by **mixture γ-1** with the next highest value $\Delta I_{ex}/I_{ex}^0$ of 0.399. Deoxycholic acid (**1**) is detected by **mixture γ-1** with the low value $\Delta I_{ex}/I_{ex}^0$ of 0.104. Ursodeoxycholic acid (**4**), which is diastereoisomer of guest **3**, is also detected by **mixture γ-1** with the next highest value $\Delta I_{ex}/I_{ex}^0$ of 0.130. Cholic acid (**5**), which bears three hydroxyl groups in a steroidal framework, is hardy detected by **mixture γ-1**. The sensing parameters of **mixture γ-1** for guests **2** and **3** at the excimer emission are smaller than those of γ-CD derivatives at excimer or exciplex emissions reported previously [26, 14], however, concentration of **mixture γ-1** in the fluorescence measurements is smallest of these CD derivatives. These results suggest that **mixture γ-1** can selectively detect guests **2** and **3**, whereas guest **4** can be hardly detected by **mixture γ-1**. It means that **mixture γ-1** can distinguish guest structure which is equatorial or axial bonding of hydroxyl group at C-7 in the steroidal framework of guests **3** and **4**. As compared with guests **1** and **2**, guest **2** is detected by **mixture γ-1** with higher sensitivity than guest **1**. Additionally, the sensing ability of **mixture γ-1** for guest **5** is negligible. These indicated that the position of hydroxyl groups in the guests affect the sensing ability of **mixture γ-1**. Thus it can be concluded that the introduction of two pyrene units on the hetero rim of γ-CD can improve the selectivity and sensitivity of fluorescent molecular sensing of guest molecules.

Acknowledgements

This study was supported by a Grant-in-Aid for Specially Promoted Research (No. 404: Molecular Synchronization for Design of New Materials System) from the Ministry of Education Science, Sports and Culture of Japan.

References

1. M. Eddaoudi, H.-P. Lopez, S.F. de Iamotte, D. Ficheux, P. Probnon, and A.W. Coleman: *J. Chem. Soc., Perkin Trans. 2*, 1711 (1996).
2. B.K. Hubbard, L.A. Beilstein, C.E. Heath, and C.J. Abelt: *J. Chem. Soc., Perkin Trans. 2*, 2751 (1996).
3. S.R. McAlpine and M.A.G. Garibay: *J. Am. Chem. Soc.* **118**, 2750 (1996).
4. R. Corradini, A. Dossena, G. Galaverna, R. Marchelli, A. Panagia, and G. Sartor: *J. Org. Chem.* **62**, 6283 (1997).
5. M. Narita, F. Hamada, I. Suzuki, and T. Osa: *J. Chem. Soc., Perkin Trans. 2*, 2751 (1998).
6. M. Narita, F. Hamada, M. Sato, I. Suzuki, and T. Osa: *J. Incl. Phenom. Macrocyclic Chem.* **34**, 421 (1999).
7. M. Sato, M. Narita, N. Ogawa, and F. Hamada: *Anal. Sci.* **15**, 1199 (1999).
8. M. Narita, S. Koshizaka, and F. Hamada: *J. Incl. Phenom. Macrocyclic Chem.* **35**, 605 (1999).
9. M. Narita and F. Hamada: *J. Chem. Soc., Perkin Trans. 2*, 823 (2000).
10. M. Narita, N. Ogawa, and F. Hamada: *Anal. Sci.* **16**, 37 (2000).
11. M. Narita, S. Mima, N. Ogawa, and F. Hamada: *Anal. Sci.* **16**, 865 (2000).
12. M. Narita, N. Ogawa, and F. Hamada: *Anal. Sci.* **16**, 701 (2000).
13. F. Hamada, M. Narita, A. Makabe, and H. Itoh: *J. Incl. Phenom. Macrocyclic Chem.* **40**, 83 (2001).
14. F. Hamada, M. Narita, K. Kinoshita, A. Makabe, and T. Osa, *J. Chem. Soc., Perkin Trans. 2*, 388 (2001).
15. M. Narita, S. Mima, N. Ogawa, and F. Hamada: *Anal. Sci.* **17**, 379 (2001).
16. M. Narita, J. Itoh, and F. Hamada: *J. Incl. Phenom. Macrocyclic Chem.* **42**, 107 (2002).
17. H. Nakashima, Y. Takenaka, M. Highshi, and N. Yishida: *J. Chem. Soc., Perkin Trans. 2*, 2096 (2001).
18. M. Narita, E. Tshiro, and F. Hamada: *Anal. Sci.* **17**, 1453 (2001).
19. A. Makabe, K. Kinoshita, M. Narita, and F. Hamada: *Anal. Sci.* **18**, 119 (2002).
20. M. Narita, E. Tashiro, and F. Hamada: *J. Incl. Phenom. Macrocyclic Chem.* **42**, 13 (2002).
21. A. Ueno, I. Suzuki, and T. Osa: *J. Am. Chem. Soc.* **111**, 6391 (1989).
22. I. Suzuki, A. Ueno, and T. Osa: *Chem. Lett.*, 2013 (1989).
23. A. Ueno, I. Suzuki and T. Osa: *Anal. Chem.* **62**, 2461 (1990).
24. I. Suzuki, M. Ohkubo, A. Ueno, and T. Osa: *Chem. Lett.*, 269 (1992).
25. N. Kobayashi, R. Saito, H. Hino, Y. Hino, A. Ueno, and T. Osa: *J. Chem. Soc., Perkin Trans. 2*, 1031 (1983).
26. F. Hamada, S. Minato, T. Osa, and A. Ueno: *Bull. Chem. Soc. Jpn.* **70**, 1339 (1997).