*Journal of Inclusion Phenomena and Molecular Recognition in Chemistry* **15:** 273–279, 1993.



# Dansyl-Modified γ-Cyclodextrin as a Fluorescent Sensor for Molecular Recognition

FUMIO HAMADA,* YOSHIHIKO KONDO and RIKO ITO
*Department of Material Engineering and Applied Chemistry, Mining College, Akita University, Tegata, Akita 010, Japan.*

IWAO SUZUKI and TETSUO OSA*
*Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan.*

and

AKIHIKO UENO*
*Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 227, Japan.*

(Received: 30 December 1992; accepted: 14 May 1993)

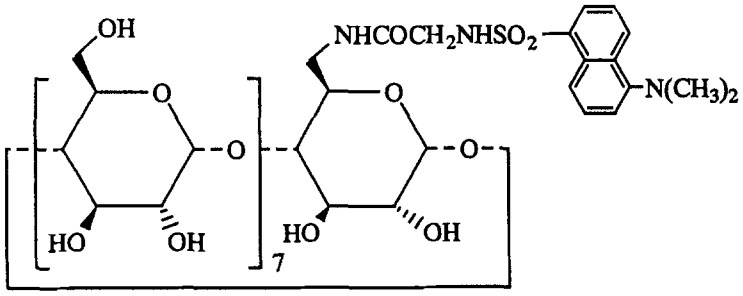
**Abstract.** Dansyl-Modified γ-Cyclodextrin (**1**) has been prepared as a sensor for detecting organic compounds. **1** shows pure monomer fluorescence whose intensity is decreased or enhanced upon addition of guest species. The value $\Delta I/I^0$, where $I$ and $I^0$ are fluorescence intensities in the presence and absence of a guest and $\Delta I$ is $I^0 - I$, was used as a parameter of sensitivity. **1** exhibits highly sensitive and selective molecular recognition ability, particularly, for ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid.

**Key words:** Dansyl-modified cyclodextrin, fluorescent sensor system, chenodeoxycholic acid, ursodeoxycholic acid.

## 1. Introduction

Cyclodextrins, which are torus-shaped cyclic oligomers of D-glucopyranose, named α-, β-, and γ- for the hexamer, heptamer and octamer, respectively, can include a variety of organic compounds in their cavities in aqueous solution [1]. When the inclusion phenomena of cyclodextrins are studied, spectroscopically active guests should be used because cyclodextrins are spectroscopically inert. Cyclodextrins, however, can be converted into spectroscopically active compounds by modification with chromophores, and spectroscopically inert guests may be detected by spectral changes of the modified cyclodextrins. Recently we reported dansylglycine-modified β-cyclodextrin as a sensor which exhibited a decrease in its fluorescence intensity upon guest binding [2]. Here we report on the system and molecular recognition ability of γ-cyclodextrin modified with dansylglycine (**1**) which shows higher recognition ability in comparison to the β-cyclodextrin analogue.

* Authors for correspondence.




Scheme 1.

## 2. Experimental

### 2.1 Preparation of Dansyl-Modified γ-Cyclodextrin (1)

To a cooled solution (−5°C) of dansylglycine free acid (0.56 g, 1.82 mmol) in 45 mL of *N*, *N'*-dimethylformamide (DMF) was added dicychlohexyl carbodiimide (0.42 g, 2.04 mmol). The reaction mixture was stirred at −5°C for 30 min. To the stirred solution was added portionwise 6-deoxy-6 amino γ-cyclodextrin (0.70 g, 0.54 mmol) [3], and the solution was stirred at −5°C for another 30 min, and then the reaction mixture was stirred at room temperature for 48 h. It was then concentrated under reduced pressure. The residue was diluted with water and extracted with $CHCl_3$. The water-soluble fraction was concentrated and poured into 300 mL of acetone. The resultant precipitates were collected by filtration. The crude product was chromatographed on a column of CM-Sephadex C-50 (4 × 25 cm) using water as the eluent. After 200 mL of water eluted, the fractions (100 mL) which eluted next were collected and evaporated to yield pure **1** (191.5 mg, 22.2% isolated yield). $R_f$ 0.39 (1-butanol : ethanol : water 5 : 4 : 3 by volume). $^1$H-NMR(DMSO-$d_6$) = 3.25 (6*H*, *s*, $NMe_2$), 3.4–4.2 (50H, *m*, $CH_2$ and $C_2H$-$C_6H$ of cyclodextrin), 4.4–4.5 (23H, *br*, $O_2H$, $O_3H$, and $O_6H$), 5.2–5.35 (8H, *br*, $C_1H$), 7.65 (1H, *d*, Ar-H), 7.98 (2H, *q*, Ar-H), 8.45 (1H, *d*, Ar-H), 8.62 (1H, *d*, Ar-H), 8.86 (1H, *d*, Ar-H), $C_{62}H_{95}O_{42}N_3S$ *Calc.* for C, 46.94 H, 6.04 N, 2.65 S, 2.02. *Found* C, 46.86 H, 6.10 N, 2.60 S, 2.35 MS(FAB) : 1586($[M + H^+]$).

### 2.2 Measurement

Fluorescence spectra were measured at 25°C with a Shimadzu RF-500 spectrofluorophotometer. Dimethyl sulfoxide aqueous solution (10 vol.-%) was used as the solvent for the fluorescence measurements because the solubility of **1** in pure water is poor. The fluorescence measurements were performed by using a cell holder whose temperature was kept at 25°C by circulating water. The excitation wavelength was 370 nm.

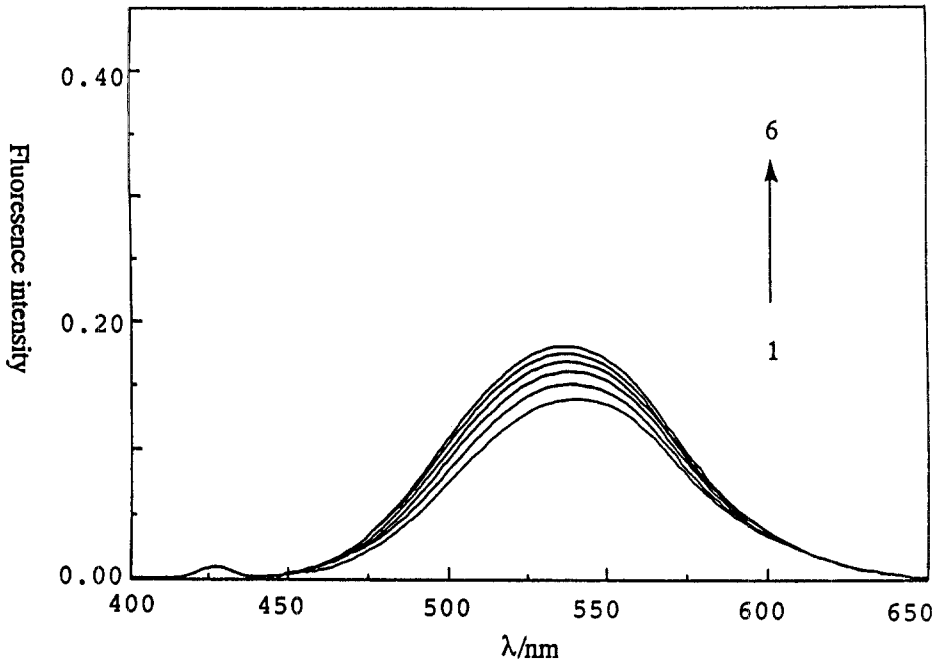



Fig. 1. Fluorescence spectra of **1** ($2.66 \times 10^{-6}$ M) in a 10 vol.-% dimethyl sulfoxide aqueous solution at various cyclohexanol concentrations (1, 0; 2, 20; 3, 40; 4, 60; 5, 80; 6, 100 mM). Excitation wavelength was 370 nm.

## 3. Results and Discussion

γ-Cyclodextrin is larger than β-cyclodextrin in molecular size and shows different inclusion behavior in many cases in comparison to β-cyclodextrin [4]. In the previous report we have shown that pyrene-appended γ-cyclodextrin, which forms an association dimer that is converted into 1 : 1 host-guest complexes upon addition of a guest, is capable of detecting various guests by changing the monomer and excimer emission intensities [5]. In the case of dansyl-modified β-cyclodextrin we showed that it decreases its fluorescence intensity upon guest binding and acts as a sensor which has remarkable molecular recognition ability, as shown by particularly high sensitivities for ursodeoxycholic acid and chenodeoxycholic acid. Figure 1 shows fluorescence spectra of **1** in a 10 vol.-% dimethyl sulfoxide aqueous solution in the presence and absence of cyclohexanol. The spectrum of **1** alone exhibits a fluorescence peak at 540 nm, and the fluorescence intensity increases with increasing cyclohexanol concentration. Ueno *et al.* have reported that another kind of dansyl-modified γ-cyclodextrin also shows guest-induced fluorescence enhancement [6].

Warner and coworkers have reported that fluorescence intensity is significantly enhanced for the pyrene-β-cyclodextrin complex in the presence of certain alcohols

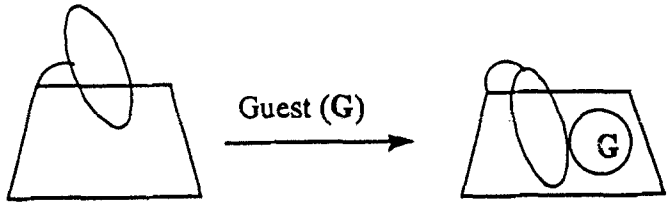
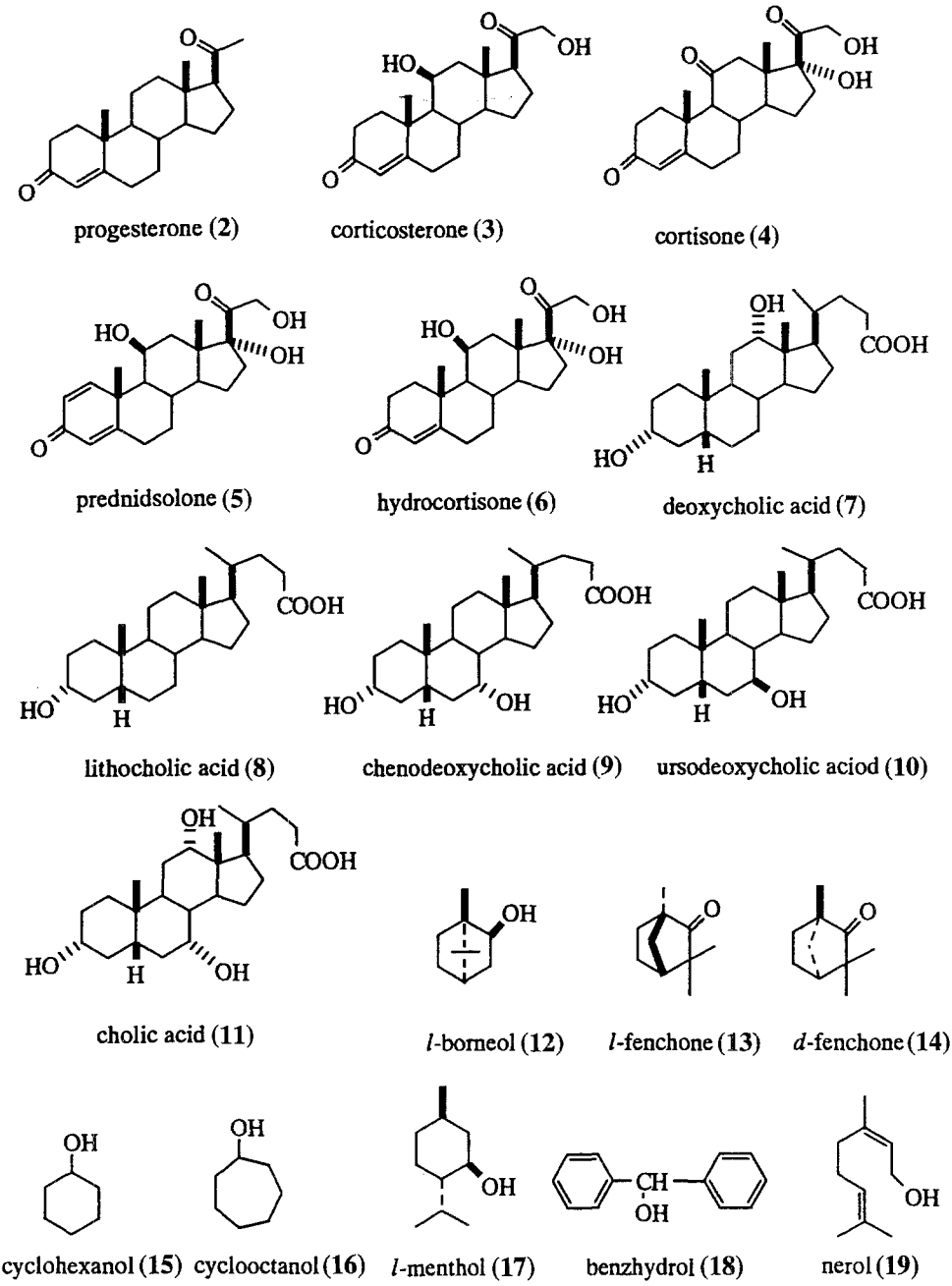



Fig. 2. Induced-fit type of space regulation by the appended moiety for inclusion of a guest molecule (G) in the cyclodextrin cavity.

[7]; however, no guest-induced fluorescence enhancement has ever been observed in the case of dansyl-modified $\beta$-cyclodextrin. This guest-induced variation in the fluorescence intensity suggests that the dansylglycine moiety acts as a spacer which enables the cyclodextrin to form 1 : 1 host-guest complexes by narrowing the large $\gamma$-cyclodextrin cavity (Figure 2) [8].

This argument is consistent with the fact that the fluorescence of the dansyl unit is enhanced in the hydrophobic microenvironment of enzymes or micelles [9]. When cyclooctanol, *l*-menthol and benzhydrol were used as guests the fluorescence intensities of **1** increased. When the other guests (Scheme 2) were used the fluorescence intensity of **1** decreased, as shown in the case of dansylglycine-modified $\beta$-cyclodextrin, in which the dansylglycine moiety was excluded from the cyclodextrin cavity upon guest binding and acts as a hydrophobic cap (Figure 3). When the fluorescence intensity at 540 nm is expressed as $I^0$ for **1** alone, and $I$ for a mixture of **1** and a guest, the $\Delta I/I^0$ value, where $\Delta I$ is $I^0 - I$, can be used as a factor reflecting the sensitivity of the system to the guest. Figure 4 (A) shows the $\Delta I/I^0$ values of **1** obtained with steroids at 0.1 mM (M = mol dm$^{-3}$), except for lithocholic acid (**8**), which was examined at 0.01 mM because 0.1 mM of lithocholic acid is not soluble in 10 vol.-% dimethyl sulfoxide aqueous solution. It is obvious that chenodeoxycholic acid (**9**) and ursodeoxycholic acid (**10**) are detected with remarkably high sensitivities, exhibiting values of 0.21 and 0.15 for $\Delta I/I^0$, respectively. Lithocholic acid (**8**) was detected with high sensitivity, even at one tenth concentration (0.01 mM), exhibiting a value of 0.194 for $\Delta I/I^0$. This value is higher than that of the $\beta$-cyclodextrin analogue [10]. Deoxycholic acid (**7**), which is different from the other steroids only in the position of one hydroxy group, was detected with lower sensitivity. Cholic acid (**11**), which bears one more hydroxy group than **9** and **10**, was hardly detected, probably due to its increased polarity. Compound **1** shows only little sensitivity for ketosteroids which have two (corticosterone and cortisone) and three (prednisolone and hydrocortisone) hydroxyl groups. Progesterone (**2**), which carries no hydroxyl group and is more hydrophobic than the other ketosteroids, is detected with a value of 0.067 for $\Delta I/I^0$, which is higher than the value exhibited by the analogous $\beta$-cyclodextrin. Figure 4 (B) shows the sensitivity data of **1** for smaller molecular size guests measured at 1.0




Scheme 2.

mM. The complexation behavior of **1** was affected by the molecular structure and size because *l*-borneol, *l*-fenchone and *d*-fenchone, which are bicyclic derivatives, were detected with negative $\Delta I/I^0$ values, while monocyclic derivatives,

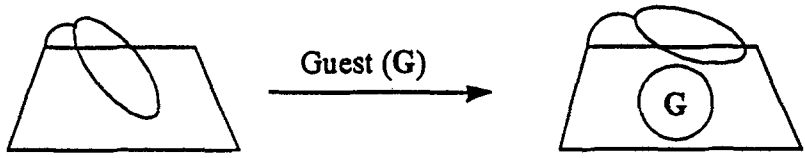



Fig. 3. Induced-fit type of complexation of modified cyclodextrin with a pendant moiety acting as a hydrophobic cap for inclusion of a guest molecule (G) in the cyclodextrin cavity.

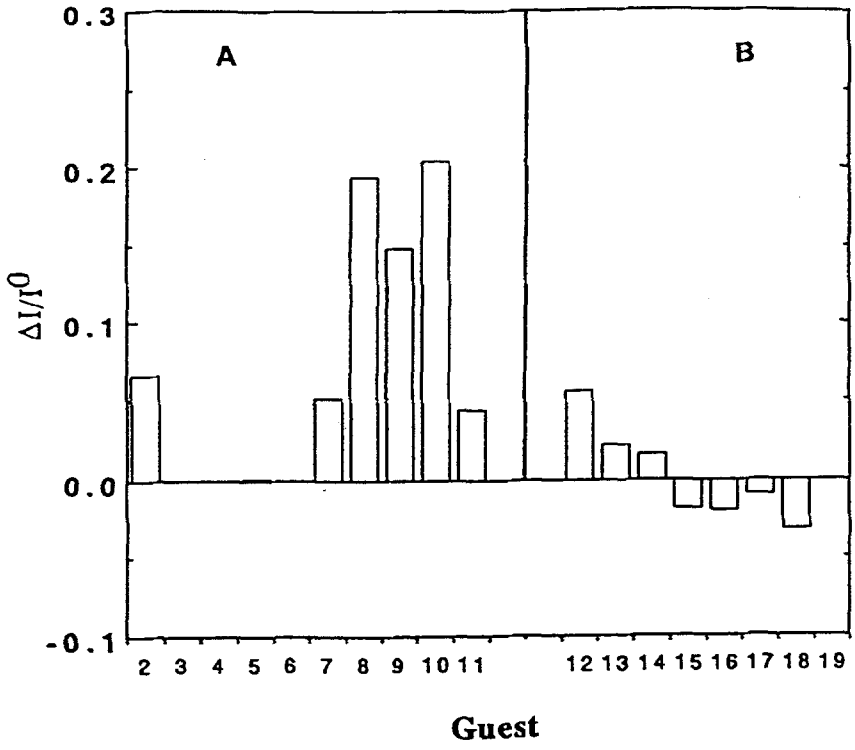



Fig. 4. The sensitivity factor $\Delta I/I^0$ of **1** ($2.26 \times 10^{-6}$ M) for various guests.

such as cyclooctanol, cyclohexanol, and *l*-menthol, were detected with positive $\Delta I/I^0$ values. Bicyclic and monocyclic derivatives show $\Delta I/I^0$ values ranging from 0.05580 to 0.01569 for bicyclic compounds, and from $-0.02$ to $-0.0089$ for monocyclic compounds. Nerol, which is a noncyclic compound, was not detected with this system.

## 4. Conclusion

The sensory system **1** exhibits a high selective molecular recognition ability in detecting organic compounds by its fluorescence. It seems to be very important

that ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid, which are biologically significant substances, were detected by this system with high sensitivities. The concentration at which these bile acids can be detected by the system is very low, ranging from 0.01 to 0.1 mmol unit. This system should also be applicable for many organic substances, which can be inserted partially or fully into the γ-cyclodextrin cavity, and is very different from enzymatic sensors, which usually detect only one species. In this system the attached chromophore acts either as an effective spacer, which narrows the large cavity of γ-cyclodextrin, or as a hydrophobic cap, resulting in an enlarged range in its molecular recognition ability.

Further work with many other guests is needed to clarify the relationship between guest structure and the sensitivity of this system.

## Acknowledgements

This research was supported by Grants from the Ministry of Education, Culture and Science of Japan.

## References

1. M.L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer Verlag (1978).
2. A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Okhubo, T. Osa, F. Hamada and K. Murai: *Chem. Lett.* 605 (1990).
3. F. Hamada, K. Murai, A. Ueno, I. Suzuki and T. Osa: *Bull. Chem. Soc. Jpn.* **61**, 3758 (1988).
4. A. Ueno, F. Moriwaki, T. Osa, F. Hamada and K. Murai: *J. Am. Chem. Soc.* **110**, 4323 (1988) and references therein.
5. A. Ueno, I. Suzuki and T. Osa: *J. Am. Chem. Soc.* **111**, 6391 (1989); I. Suzuki, A. Ueno and T. Osa: *Chem. Lett.* 2013 (1989).
6. Y. Wang, T. Ikeda, A. Ueno and F. Toda: *Chem. Lett.* 863 (1992).
7. G. Nerson, G. Patonay and I.M. Warner: *J. Incl. Phenom.* **6**, 277 (1988).
8. A. Ueno, Y. Tomita and T. Osa: *J. Chem. Soc., Chem. Commun.* 976 (1983).
9. W. Rettig: *Angew. Chem., Int. Ed. Engl.* **25**, 971 (1986).
10. Unpublished data. In the case of β-cyclodextrin analogue, $\Delta I/I^0$ for lithocholic acid is 0.148.