Molecular Design of a New Fluorescent Barbiturate Receptor. Sensitive Detection of Barbiturates through Solvent Extraction

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(Received October 19, 1992)

A fluorescent receptor, *N,N'*-bis[6-[4-(1-pyrenyl)butanamido]-2-pyridyl]isophthalamide (**2**), was synthesized to develop a sensitive host–guest-type sensory system for barbiturates. **2** aggregates in cyclohexane and the pyrene fluorescence in **2** almost disappeared because of aggregation-induced concentration quenching. The addition of barbital to the cyclohexane solution of **2**, which induced the deaggregation of **2** through complementary complexation with barbital, increased the fluorescence intensity at 378 nm by a factor of about 70-fold. The barbiturates in water could also be sensitively detected by **2** based on a liquid (water)–liquid (cyclohexane) extraction technique. In this system, **2** was essentially selective for barbiturates and no fluorescence response was observed for guests including a hydantoin skeleton. The analogue of **2**, which has the *N,N'*-di-(2-pyridyl) terephthalamide skeleton, was also investigated as a fluorescent receptor for dicarboxylic acids.

The molecular design of artificial receptors that can precisely recognize guest molecules has been the focus of much recent attentions.[1,2] In the papers reported so far hydrogen-bonding interactions play a central role.[3—11] Although it is undoubted that the hydrogen-bond has several merits which cannot be attained in other interactions, the hydrogen-bonding approach inevitably results in some limitation in the detection of the molecular recognition precess. The examination of the past literatures manifests that ^{1}H NMR spectroscopy is useful but the "sole" effective method to characterize this process. We considered that if the molecular recognition process can be "*read-out*" more conveniently, we can apply the molecular recognition unit as a useful "transducer" from chemical signals to physical signals. One promising method is CD (circular dichroism) spectroscopy which is useful for the detection of chiral polyol compounds.[11—13] We considered that fluorescence spectroscopy might serve as another promising method.[14,15] We previously introduced two pyrenyl groups or a pair of a fluorophore and a quencher onto the lower rim of a calix[4]arene via ester spacers serving as a metal-binding site.[16—19] We found that the fluorescence properties of these functionalized calix[4]-arenes change sensitively in response to metal-binding events.[16,18] The results tempted us to design new artificial receptors in which the molecular recognition process can be "*read-out*" by a fluorescence method. Hamilton et al.[1,5,6] developed an efficient receptor **1a** for barbiturates. We thus synthesized fluorescent receptors **2** with two pyrenyl groups and **3** with one pyrenyl group, expecting that the binding of barbiturates would influence their fluorescence properties.[20] Hamilton et al.[21] also reported that the *N,N'*-di-(2-pyridyl)terephthalamide skeleton serves as a receptor for certain dicarboxylic acids. We thus synthesized compound **4** with two pyrenyl groups and estimated the fluorescence properties in the presence of dicarboxylic acid substrates (Chart 1).

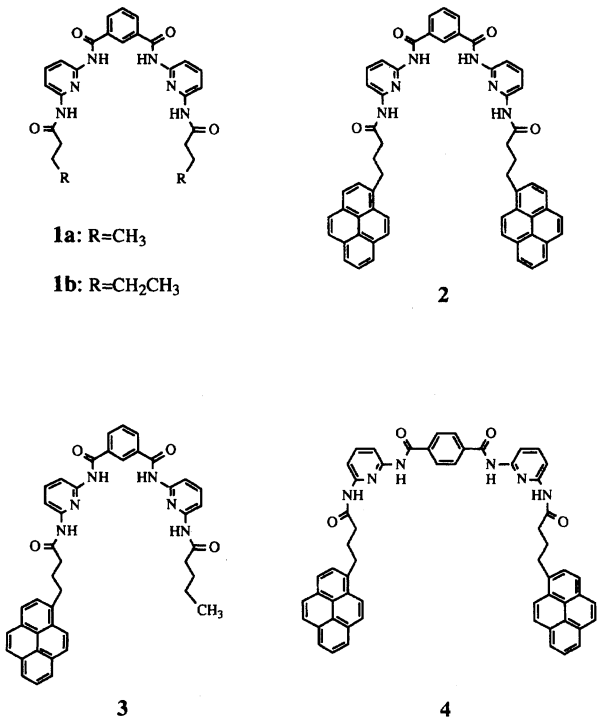


Chart 1.

Experimental

Materials. Compounds **1**—**4** were synthesized in manners similar to those reported by Hamilton et al.[4e]

***N,N'*-Bis(6-pentanamido-2-pyridyl)isophthalamide (1b).** *N,N'*-Bis(6-amino-2-pyridyl)isophthalamide (**5**)[4e] (0.29 g, 0.83 mmol) and triethylamine (0.62 g, 6.1 mmol) were dissolved in dry tetrahydrofuran (THF, 30 cm^3). To this solution was added a THF solution (30 cm^3) containing 1-pentanoyl chloride (0.45 g, 3.73 mmol) dropwise and the reaction was continued for 4 h at room temperature. The precipitate was removed by filtration, the filtrate being concentrated to dryness under reduced pressure. The solid residue was washed successively with aqueous 0.05

M[#] NaOH solution, aqueous 0.05 M HCl solution, and water. The solid product was recrystallized from THF–hexane: White powder, mp 232—234°C, yield 76%; IR (KBr) 3289 (NH), 1651 and 1671 (C=O) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$, 25°C) δ=0.96 (6H, t, J=7 Hz, CH_3), 1.44 (4H, m, $\underline{CH_2}CH_3$), 1.76 (4H, m, $\underline{CH_2}CH_2CO$), 2.42 (4H, t, J=8 Hz, CH_2CO), 7.67 (1H, s, isophth-2H), 7.67 (1H, t, J=8 Hz, isophth-5H), 7.79 (2H, t, J=8 Hz, pyrid-4H), 8.00 and 8.06 (2H each, d each, J=8 Hz each, pyrid-3,5H), 8.12 (2H, dd, J=8 and 2 Hz, isophth-4,6H), 8.41 (2H, s, $CH_2CO\underline{NH}$), 8.46 (2H, s, isophth-CONH). Found: C, 64.95; H, 6.30; N, 16.56%. Calcd for $C_{28}H_{32}N_6O_4$: C, 65.10; H, 6.24; N, 16.27%.

***N,N'*-Bis[6-[4-(1-pyrenyl)butanamido]-2-pyridyl]-isophthalamide (2).** 4-(1-Pyrenyl)butanoic acid (1.00 g, 3.5 mmol) was treated with oxalyl chloride (1.0 ml, 11.4 mmol) in dry dichloromethane (100 cm^3) in the presence of a drop of *N,N'*-dimethylformamide (DMF). The reaction mixture was stirred for 4 h room temperature. The mixture was evaporated to dryness and the solid residue was dried for 4 h under high vacuum. 4-(1-Pyrenyl)butanoyl chroride (**6**) thus obtained was used without further purification for the next reaction.

Compound **5** (0.40 g, 1.1 mmol) and triethylamine (0.35 g, 3.5 mmol) were dissolved in dry THF (50 cm^3). To this solution was added a THF solution (50 cm^3) containing **6** (1.00 g, 3.3 mmol) dropwise and the mixture was stirred for 3 h at room temperature under a nitrogen stream. The precipitate was removed by filtration, the filtrate being concentrated to dryness under reduced pressure. The solid residue was dissolved in DMF (30 cm^3) and the solution was poured into aqueous 0.05 M NaOH solution (300 cm^3). The precipitate was recovered by filtration, washed with water, and dissolved again in DMF (30 cm^3). The solution was poured into a methanol–water (1:1 v/v) mixture (300 cm^3). The precipitate was recovered by filtration, dried in vacuo, and recrystallized from THF–hexane: White powder, mp 265—267°C, yield 71%; IR (KBr) 3289 (NH), 1674 and 1685 (C=O) cm^{-1}; ^1H NMR (250 MHz, THF-d_8, 25°C) δ=2.44 (4H, m, $\underline{CH_2}CH_2CO$), 2.51 (4H, t, J=7 Hz, CH_2CO), 3.45 (4H, t, J=8 Hz, $\underline{CH_2}C_{16}H_9$), 7.61 (1H, t, J=8 Hz, isophth-5H), 7.74 (2H, t, J=8 Hz, pyrid-4H), 7.89—8.43 (24H, m, $C_{16}H_9$ and pyrid-3,5H), 8.45 (1H, s, isophth-2H), 9.16 (2H, s, $CH_2CO\underline{NH}$), 9.47 (2H, s, isophth-CONH). Found: C, 78.29; H, 5.07; N, 9.47%. Calck for $C_{58}H_{44}N_6O_4$: C, 78.36; H, 4.99; N, 9.45%.

***N*-(6-Amino-2-pyridyl)-*N'*-(6-pentanomido-2-pyridyl)isophthalamide (7).** Compound **5** (0.85 g, 2.4 mmol) and triethylamine (0.36 g, 3.6 mmol) were dissolved in dry THF (100 cm^3). To this solution was added a THF solution (100 cm^3) containing 1-pentanoyl chloride (0.44 g, 3.6 mmol) dropwise and the mixture was stirred for 2 h at room temperature under a nitrogen stream. The precipitate was removed by filtration, the filtrate being concentrated to dryness under reduced pressure. The solid residue was washed with aqueous 0.05 M NaOH solution and water. The product was dispersed in aqueous 0.05 M HCl solution (200 cm^3), the insoluble material being removed by filtration. The solution was neutralized with $NaHCO_3$ and then extracted with chloroform (100 cm^3). The chloroform solution was concentrated to dryness and the solid residue was subjected to column purification (silica gel, chloroform–THF): White powder, mp 141—143°C, yield 32%; IR (KBr) 3300 and 3350 (NH), 1670 and 1684 (C=O) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$, 25°C) δ=0.95 (3H, t, J=7 Hz, CH_3), 1.42 (2H, m, $\underline{CH_2}CH_3$), 1.72 (2H, m, $\underline{CH_2}CH_2CO$), 2,44 (2H, t, J=7 Hz, CH_2CO), 4.44 (2H, s, NH_2), 6.28 (1H, d, J=8 Hz, aminopyrid-5H), 7.49 (1H, t, J=8 Hz, aminopyrid-4H), 7.61 (1H, t, J=8 Hz, carbonylaminopyrid-4H), 7.71 (1H, d, J=8 Hz, aminopyrid-3H), 7.73 (1H, t, J=8 Hz, isophth-5H), 7.95 and 8.00 (1H each, d each, J=8 Hz each, carbonylaminopyrid-3,5H), 8.09 (1H, s, isophth-CONH), 8.13 (2H, d, J=8 Hz, isophth-4,6H), 8.45 (1H, s, isophth-CONH), 8.46 (1H, s, isophth-2H), 9.14 (1H, s, $CH_2CO\underline{NH}$). Found: C, 63.80; H, 5.65; N, 19.27%. Calcd for $C_{23}H_{24}N_6O_3$: C, 63.88; H, 5.59; N, 19.45%.

***N*-(6-Pentanamido-2-pyridyl)-*N'*-[6-[4-(1-pyrenyl)-butanamido]-2-pyridyl]isophthalamide (3).** This compound was synthesized from **7** (0.32 g, 0.74 mmol) and **6** (0.32 g, 1.00 mmol) in a manner similar to that described for **2**: White powder, mp 165—167°C, yield 20%; IR (KBr) 3297 (NH), 1651 and 1667 (C=O) cm^{-1}; ^1H NMR (250 MHz, THF-d_8, 25°C) δ=0.90 (3H, t, J=7 Hz, CH_3), 1.33 (2H, m, $\underline{CH_2}CH_3$), 1.66 (2H, m, $\underline{CH_2}CH_2CH_3$), 2.22 (2H, m, $\underline{CH_2}CH_2C_{16}H_9$), 2.35 (2H, t, J=7 Hz, $\underline{CH_2}CH_2CH_2CH_3$), 2.50 (2H, t, J=7 Hz, $\underline{CH_2}CH_2CH_2C_{16}H_9$), 3.41 (2H, t, J=8 Hz, $\underline{CH_2}C_{16}H_9$), 7.53—7.71 (3H, m, pyrid-4H and isophth-5H), 7.84—8.38 (15H, m, $C_{16}H_9$, pyrid-3,5H and isophth-4,6H), 8.47 (1H, s, isophth-2H), 9.11 and 9.21 (1H each, s each, $CH_2CO\underline{NH}$), 9.28 and 9.51 (1H each, s each, isohth-CONH). Found: C, 73.55; H, 5.50; N, 11.87%. Calcd for $C_{43}H_{38}N_6O_4$: C, 73.49; H, 5.45; N, 11.96%.

***N,N'*-Bis[6-[4-(1-pyrenyl)butanamido]-2-pyridyl]-terephthalamide (4).** *N,N'*-Bis(6-amino-2-pyridyl)-terephthalamide[20a] (0.40 g, 1.10 mmol) and compound **6** (0.96 g, 3.10 mmol) were used for the synthesis of **4**. The work-up method is similar to that described for **1**: White powder, mp 303—305°C, yield 67%; IR (KBr) 3306 (NH), 1667 (C=O) cm^{-1}; ^1H NMR (250 MHz, DMF-d_7, 25°C) δ= 2.26 (4H, m, $\underline{CH_2}CH_2CO$), 2.75 (4H, t, J=7 Hz, CH_2CO), 3.51 (4H, t, J=8 Hz, $\underline{CH_2}C_{16}H_9$), 7.90 (2H, t, J=8 Hz, pyrid-4H), 8.01—8.33 (24H, m, $C_{16}H_9$, pyrid-3H and terephth-2,3H), 8.52 (2H, d, J=9 Hz, pyrid-5H), 10.43 (2H, s, $CH_2CO\underline{NH}$), 10.65 (2H, s, tetrphth-CONH). Found: C, 78.54; H, 5.06; N, 9.36%. Calcd for $C_{58}H_{44}N_6O_4$: C, 78.36; H, 4.99; N, 9.45%.

Fluorescence Measurements. (a) Cyclohexane or Chloroform–Cyclohexane System. To a few cm^3 of solvent (cyclohexane or chloroform–cyclohexane mixed solvent) were added 10 μdm^3 of a 1.00×10^{-3} M **2** or **3** solution in THF or a 1.00×10^{-3} M **4** solution in THF–DMF (4:1 v/v) and 10 μdm^3 of a guest in THF or THF–methanol. The mixture was diluted to 5 cm^3 with the solvent. After the solution was allowed to stand for 30 min, the fluorescence spectrum and the fluorescence intensity were measured in a 0.5 cm cell with a Hitach Model F-4500 fluorescence spectrophotometer fitted with a 150 W xenon lamp.

(b) Solid (Guest)–Liquid (Cyclohexane) Extraction System. To 5 cm^3 of cyclohexane were added 10 μdm^3 of a 1.00×10^{-3} M **1** solution in THF and 0.050 mmol of powdered guests. After the mixture was kept in a ultrasonic bath for 10 min, the fluorescence intensity of the so-

[#] 1 M=1 mol dm^{-3}.

lution was measured in the same manner as that described above.

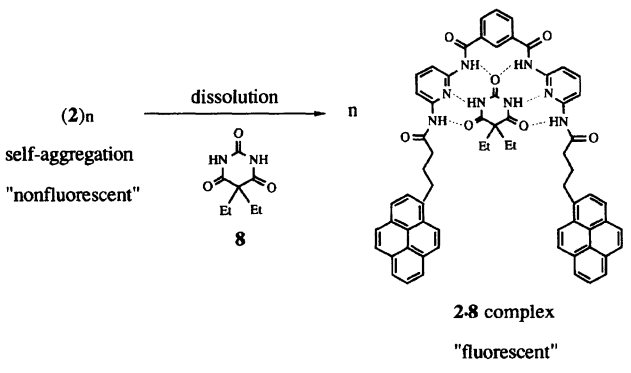
(c) Liquid (Water)–Liquid (Cyclohexane) Extraction System. To 10 cm^3 of cyclohexane were added 20 μdm^3 a 1.00×10^{-3} M **1** solution THF and 10 cm^3 of a guest aqueous solution containing an appropriate pH buffer solution. After the mixture was shaken vigorously for 10 min, the fluorescence of the upper (cyclohexane) phase was measured in the same manner as that described above.

Miscellaneous. The ^{1}H NMR and IR spectra were recorded on a Bruker AC-250P (250 MHz) FT-NMR spectrometer and a Shimadzu FT-IR 8100 spectrometer, respectively. The absorption spectra were measured with a Shimadzu UV-2200 spectrophotometer. A Horiba pH Meter, Model F-8DP, was used for the pH measurements.

Results and Discussion

Spectral Properties of 2, 3, and 4. When compared with **1b** without a pyrenyl group, the solubility of **2**—**4** with one or two pyrenyl group was relatively poor. In chloroform, the absorption spectra satisfied the Lambert–Beer's law and the spectral shapes were scarcely affected by the concentration change. This implies that these compounds are "homogeneously" dispersed in chloroform. On the other hand, the solubility in cyclohexane was very poor and the spectral shapes were quite different from those in chloroform (Fig. 1). The spectral shapes changed with the concentration and did not obey the Lambert–Beer's law. The result indicates that these compounds "aggregate" in cyclohexane because of intermolecular hydrogen-bonding interactions and/or stacking of pyrene rings. Interestingly, we found that in cyclohexane addition of barbital (**8**) changes the spectrum of **2** from the aggregated one to the homogeneous one [Fig. 1A]: that is, **2** is homogeneously dispersed through complexation with **8**. The deaggregation is induced by the substitution of intermolecular hydrogen-bonds among **2**'s in the aggregate with more efficient and more complementary host–guest-type complexation between **2** and **8** (Scheme 1).

The aggregation-deaggregation precess was detected more clearly by fluorescence spectroscopy (Fig. 2). In chloroform monomer emission maxima (378 and 398 nm for **2** and **4**) as well as an excimer emission maximum



Scheme 1.

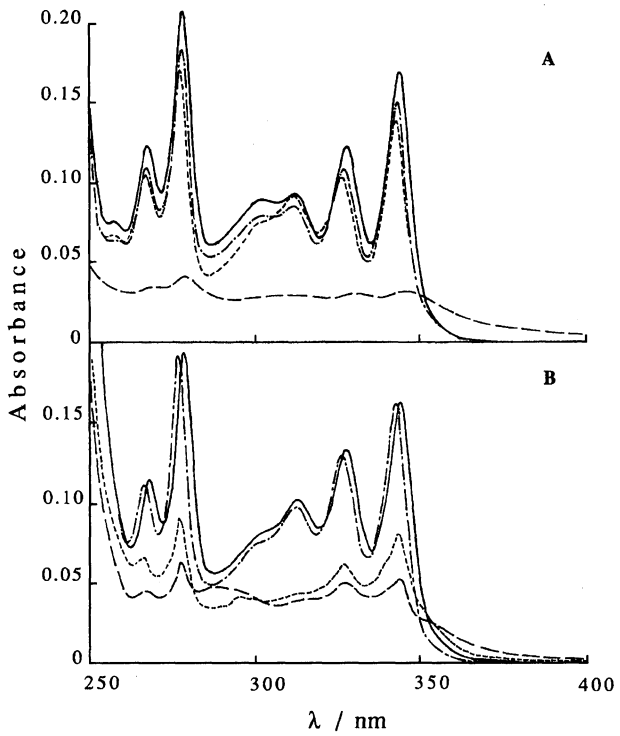


Fig. 1. Absorption spectra of (A) **2** (2.00×10^{-6} M) and (B) **4** (2.00×10^{-6} M): (A) — chloroform, –·– chloroform : cyclohexane = 1 : 4 v/v, --- cyclohexane, ···· cyclohexane in the presence of barbital (5.00×10^{-5} M); (B) — chloroform, –·– chloroform : cyclohexane=2 : 3 v/v, --- chloroform : cyclohexane=1 : 4 v/v, ···· chloroform : cyclohexane=1 : 4 v/v in the presence of adipic acid (1.00×10^{-4} M). Light-path length 1 cm.

(478 nm for **2**; 498 nm for **4**) were observed. In cyclohexane, in contrast, these fluorescence bands almost disappeared because of aggregation-induced concentration quenching. Addition of **8** to the cyclohexane solution of **2**, which induced the deaggregation of **2**, increased both of the monomer emission bands and the excimer emission band. The magnitude of the intensity increase corresponds to about 70-fold.

Similar spectral changes were observed for **4** on the addition of adipic acid [Figs. 1B and 2B]. Although the change in the absorption spectrum induced by added adipic acid was not so significant as the **2**-barbital system, the intensity of the pyrene monomer emission increased sensitively: on the addition of adipic acid the fluorescence intensity increased by 7-fold.

Here, a new idea occurred to us that the dramatic fluorescence change would be useful for the design of a new sensory system for neutral guest molecules.

Fluorescence Sensing of Barbiturates by 2 and 3. We estimated the influence of added barbiturates on the fluorescence intensity of **2** and **3** in cyclohexane. The results are summarized in Table 1. Examination of Table 1 reveals that both monomer emission (378 nm)

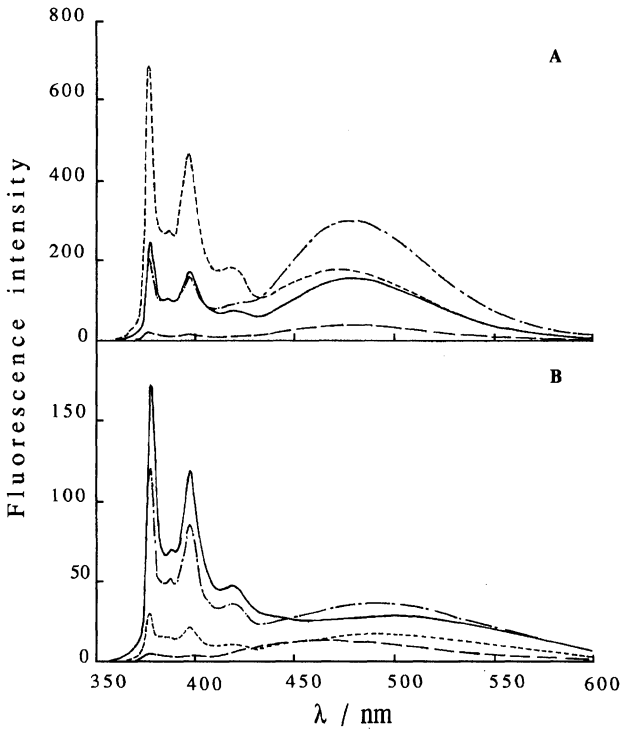


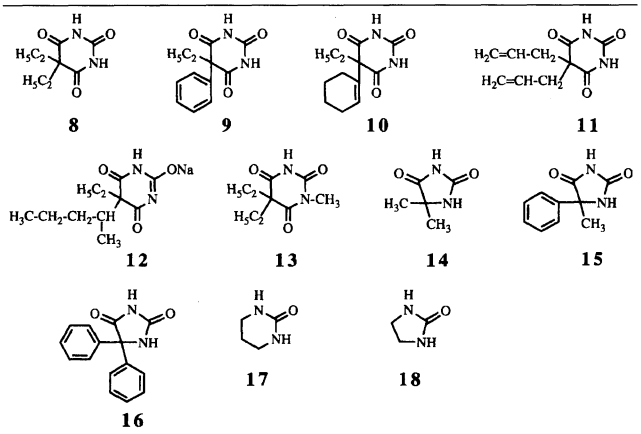
Fig. 2. Fluorescence spectra of (A) **2** (2.00×10^{-6} M) and (B) **4** (2.00×10^{-6} M). (A) — chloroform, –·– chloroform : cyclohexane = 1 : 4 v/v, --- cyclohexane, ···· cyclohexane in the presence of barbital (5.00×10^{-5} M); (B) — chloroform, –·– chloroform : cyclohexane=2 : 3 v/v, --- chloroform : cyclohexane=1 : 4 v/v, ···· chloroform : cyclohexane=1 : 4 v/v in the presence of adipic acid (1.00×10^{-4} M).

and excimer emission (472 nm) of **2** are suppressed in cyclohexane whereas addition of **8** (25-fold of **2**) which can form six complementary hydrogen-bonds with **2** enhances the intensity of monomer emission by a factor of 67 and that of excimer emission by a factor of 7.8. Similarly, the intensity of monomer emission for **3** is enhanced by a factor of 5.8 by the addition of **8**. As supported from spectral evidence, these changes are attributed to dissolution of **2** and **3** into cyclohexane. In fact, **2** and **3** are poorly soluble in cyclohexane whereas they are "very soluble" in the presence of **8**. Since the intensity of monomer emission (I_m) for **2** changes most sensitively, we considered that this band is useful as a marker to detect **8** and its analogs.

In Table 1 we examined 11 guest molecules. The guest molecules that showed the I_m increase by more than 10-fold are **8**, phenobarbital (**9**), cyclobarbital (**10**), allobarbital (**11**), and sodium salt of pentobarbital (**12**). It is worthy to mention that these compounds can form six complementary hydrogen-bonds with **2**, except for **12**: Although **12** can form five hydrogen-bonds (less by one than **8**), the oxide anion would serve as a strong hydrogen-bond acceptor and the pentyl group would be effective to deaggregate **2** in cyclohexane. In

Table 1. Fluorescence Intensities of **2** and **3** in the Presence of Barbital and Its Analogs in Cyclohexane at 25°C[a)]

Guest	Receptor **2**		Receptor **3**
	I_m[b)]	I_e[c)]	I_m[b)]
None	10	21	58
Barbital (**8**)	670	164	339
Phenobarbital (**9**)	272	84	179
Cyclobarbital (**10**)	407	107	330
Allobarbital (**11**)	444	92	214
Sodium salt of pentobarbital (**12**)	760	153	710
N-Methylbarbital (**13**)	36	38	95
5,5-Dimethylhydantoin (**14**)	27	91	207
5-Methyl-5-phenylhydantoin (**15**)	81	76	258
5,5-Diphenylhydantoin (**16**)	65	66	199
Tetrahydro-2(1*H*)-pyrimidinone (**17**)	17	55	78
2-Imidazolidinone (**18**)	15	32	91



a) [**2** or **3**] = 2.00×10^{-6} M, [guest] = 5.00×10^{-5} M, excitation wavelength 345 nm. b) Fluorescence intensity for monomer emission at 378 nm. c) Fluorescence intensity for excimer emission at 472 nm.

N-methylbarbital (**13**) one NH group is converted to the N–CH_3 group. Although this compound can still form five hydrogen-bonds, the I_m value is smaller by 18.6-fold that for **8**. Presumably, the methyl group not only decreases the number of the hydrogen-bond but also disorders the host structure because of the steric bulkiness. In tetrahydro-2(1*H*)-pyrimidinone (**17**) two carbonyl groups are removed from the barbital skeleton and four hydrogen-bonds will be formed with **2**. As shown in Table 1, the I_m increase was only of 1.7-fold. Compounds **14**—**16** all include a hydantoin skeleton and form five hydrogen-bonds with **2**. However, the I_m values were not so enhanced [(2.7—8.1)-fold]. As pointed out by Hamilton et al.,[4e)] the bond angles in **2** are designed so that it can accept the six-membered barbital skeleton. Hence, the relatively small I_m increase is ascribed to the decrease in the number of the hydrogen-bond and the weak hydrogen-bond.

In addition to 11 guest molecules recorded in Table 1, we also examined urea, 1,3-dimethylurea, uracil, 5,6-dihydrouracil, uric acid, molonic acid, glutaric acid,

phthalic acid, pyrogallol, and butylamine hydrochloride. These guest molecules are either less lipophilic or inferior in the number of the hydrogen-bond. We could not detect the perceptible I_m increase for these compounds.

Next, we tried to determine the associations constant (K_{ass}) for the **2·8** complex. In Fig. 3 the I_m was plotted against [**8**]. The plot was reasonably analyzed by using the equilibrium-shift method for the formation of a 1:1 complex to give $K_{ass}=5.90\times10^5$ M^{-1}. This value is larger than that determined in chloroform ($K_{ass}=4.1\times10^4$ M^{-1}) but smaller than that determined in chloroform–cyclohexane (1:4 v/v; $K_{ass}=2.5\times10^6$ M^{-1}).[20] In the present system the driving force for host–guest complexation is the hydrogen-bond, so that the K_{ass} should be increased in nonpolar solvents. Actually, the K_{ass} in chloroform–cyclohexane (1:4 v/v) is greater by 61-fold than that in chloroform. The unexpected decrease in the K_{ass} in cyclohexane is attributed to the aggregation of **2** (Scheme 2): that is, for **8** to form a 1:1 complex with **2**, it must disrupt the hydrogen-bonds formed among **2**'s in the aggregate. This additional energy results in the decrease in the K_{ass} value for the **2·8** complex.

The fluorescence intensity of **4** in the presence of various dicarboxylic acid substrates are listed in Table 2. Among a series of aliphatic dicarboxylic acids, **4** shows a selectivity towards adipic acid and pimeric acid. This size selectivity is reasonable because the two pyridine units in **4** are in an optimal position to complex

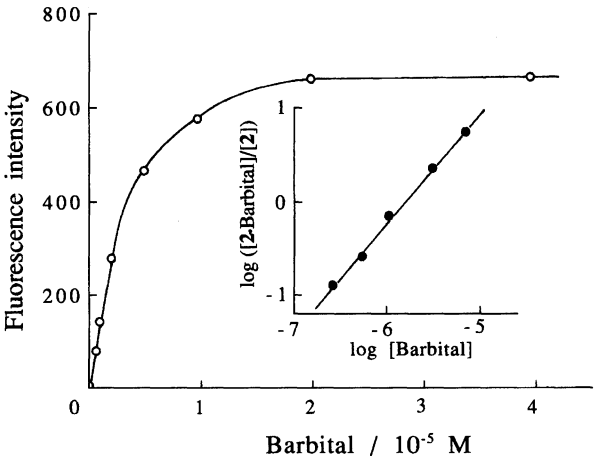
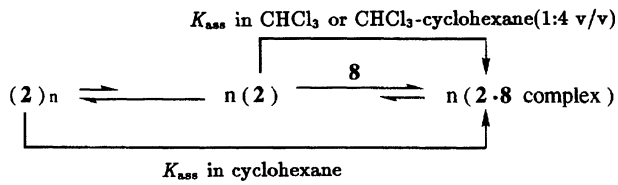


Fig. 3. Plot of the monomer fluorescence intensity (I_m) at 378 nm vs. [**8**]: [**2**]$=2.00\times10^{-6}$ M, cyclohexane, 25°C. The inserted figure shows the equilibrium-shift plot.



Scheme 2.

Table 2. Fluorescence Intensities of **4** in the Presence of Dicarboxylic Acids in a Chloroform–Cyclohexane (1:4 v/v) Mixed Solvent at 25°C[a]

Guest	I_m[b]	Guest	I_m[b]
None	4.2	*o*-Phenylenediacetic acid	13.3
Malonic acid	4.2	*m*-Phenylenediacetic acid	21.5
Succinic acid	5.6	*p*-Phenylenediacetic acid	6.0
Glutaric acid	12.8	Phthalic acid	5.8
Adipic acid	28.8	Isophthalic acid	5.8
Pimeric acid	27.0	Acetic acid[c]	4.2
Suberic acid	15.9	Trifluoroacetic acid[c]	4.6
Sebacic acid	4.2	Decanoic acid [c]	7.1

a) [**4**]$=2.00\times10^{-6}$ M, [guest]$=1.00\times10^{-4}$ M, excitation wavelength 345 nm. b) Pyrene monomer fluorescence, at 377 nm. c) [guest]$=2.00\times10^{-4}$ M.

adipic acid.[21] Monocarboxylic acids and dicarboxylic acids having a too short or too long spacer between two carboxylic groups are scarcely respondent to **4**.

Fluorescence Sensing in Solvent Extraction System.

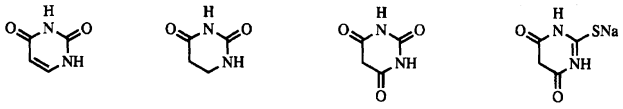
The guest molecules tested herein are well soluble in water and their analysis is usually carried out in an aqueous system. However, the hydrogen-bond, on the basis of which the present molecular recognition system is designed, is almost impotent in an aqueous system. As an attempt to apply compound **2** to the analysis of guest molecules dissolved in water, we adopted the solvent extraction method.

To examine if such solvent extraction really takes place, we first extracted **8** from the solid phase or from the aqueous solution with **1b** or **2** into the organic phase at 25°C. In liquid–liquid extraction, **1b** was used instead of **2** to enhance the host concentration to the ^{1}H NMR measurement level. A D_2O solution (1 cm^3) containing **8** (5.0 mM) was shaken with a $CDCl_3$ solution (0.5 cm^3) containing **1b** (10 mM). The ^{1}H NMR spectra of the organic phase established that in the presence of **1b** almost 100% of **8** was extracted into the $CDCl_3$ phase whereas in the absence of **1b** only 30.5% of **8** was distributed to the $CDCl_3$ phase. In solid–liquid extraction, a cyclohexane solution containing **2** (2.00×10^{-6} M) was mixed with solid guest molecules at 25°C. The supernatant of the cyclohexane solution was subjected to the fluorescence measurement. The results are summarized in Table 3. It is seen from Table 3 that the I_m values thus obtained are roughly correlated with those obtained from the homogeneous system (Table 1). The large I_m value was again observed for **8**. The I_m values for **13** and **17** are somewhat enhanced probably because of the relatively high solubility of these guests into cyclohexane.

To conduct liquid (water)–liquid (cyclohexane) extraction, we first examined the influence of pH in the aqueous phase (Fig. 4). After two-phase solvent extrac-

Table 3. Fluorescence Intensities of **2** in Solvent Extraction Systems at 25°C

Guest	Solid–liquid extraction[a]		Liquid–liquid extraction[b]	
	I_m	I_e	I_m	I_e
None	10	21	5	4
Barbital (**8**)	680	162	310	74
Phenobarbital (**9**)	195	54	276	86
Cyclobarbital (**10**)	435	123	368	106
Allobarbital (**11**)	400	79	247	57
Sodium salt of pentobarbital (**12**)	328	70	591	136
N-Methylbarbital (**13**)	198	61	15	21
5,5-Dimethylhydantoin (**14**)	13	23	8	16
5-Methyl-5-phenylhydantoin (**15**)	21	16	6	10
5,5-Diphenylhydantoin (**16**)	12	15	—	—
Tetrahydro-2(1*H*)-pyrimidinone (**17**)[c]	57	42	6	9
2-Imidazolidinone (**18**)[c]	11	28	6	9
Urea[c]	—	—	6	9
1,3-Dimethylurea[c]	—	—	14	11
Uracil (**19**)	—	—	6	7
5,6-Dihydrouracil (**20**)	—	—	6	7
Barbituric acid (**21**)	—	—	6	8
S-Sodium 2-thiobarbiturate (**22**)	—	—	6	9



19 **20** **21** **22**

a) [**2**] = 2.00×10^{-6} M in cyclohexane. 5000 equivalents of the solid guest were added. b) Organic phase (10 cm^3 cyclohexane): [**2**] = 2.00×10^{-6} M. Aqueous phase (10 cm^3 water buffered with 0.05 M phosphate to pH 6.5): [guest] = 1.00×10^{-3} M. c) [guest] = 1.00×10^{-1} M. This concentration was employed because of the high solbility in water and the weak I_m increase.

tion, the I_m value of **2** (2.00×10^{-6} M) in the cyclohexane phase was determined. In case the aqueous phase did not contain any guest molecule, the I_m was constantly low. When **8** (1.00×10^{-3} M) was added to the aqueous phase, the I_m increased with lowering aqueous pH and reached a constant value at around 7. The pH dependence is related to the dissociation of **8** (pK_a 7.43 at 25°C)[22] as in Scheme 3: that is, neutral **8** is readily extracted by **2** into the cyclohexane phase whereas anionic **8** is scarcely extracted because of the high water solubility. We thus carried out the extraction at pH 6.5.

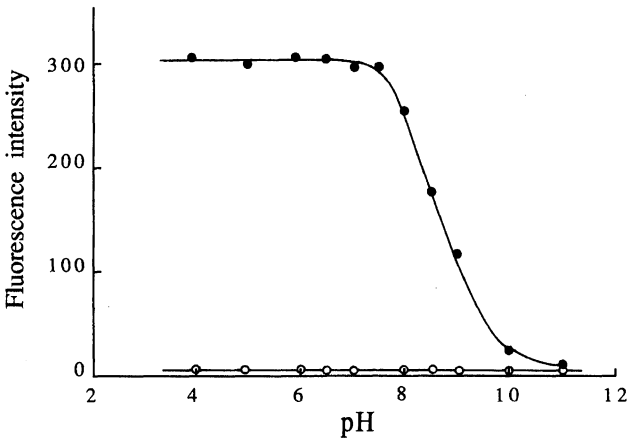
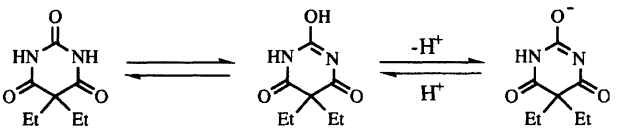


Fig. 4. pH dependence of I_m (at 378 nm) in the liquid (water)–liquid (cyclohexane) extraction system: [**2**] in cyclohexane = 2.00×10^{-6} M, [**8**] in water = 0 M (○) or 1.00×10^{-3} M (●), 25°C. The pH was adjusted with 0.05 M potassium hydrogen phthalate for pH 4—5, 0.05 M phosphate for pH 5.5—7.5, 0.05 M borate for pH 8.0—10.0, and 0.05 M carbonate for above pH 11. The fine pH value was adjusted by using a 0.1 M NaOH solution or a 0.1 M HCl solution.

As shown in Table 3, several guest molecules can be sensitively detected by the solvent extraction method. The guest molecule that gave the largest intensity increase was again **12** (91-fold), however: it was extracted as the neutral species in this system. The other barbiturates **8**—**11** are also detectable sensitively. We measured the I_m as a function of [**8**], [**9**] or [**12**] to know the minimum concentrations detectable by the present method (Fig. 5). If one defines the minimum detection concentration to be signal/noise=2 (this is a reasonable



Scheme 3.

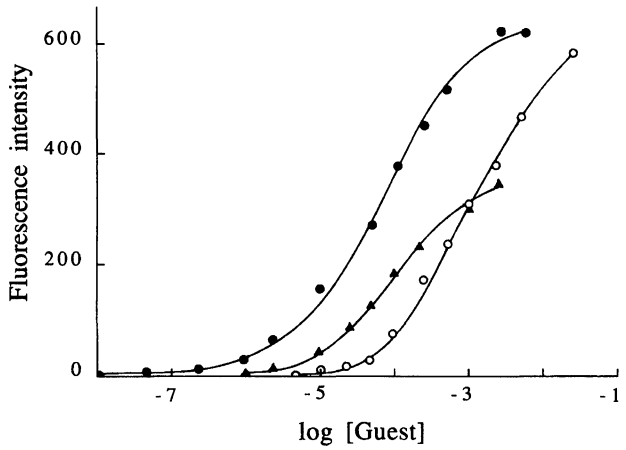


Fig. 5. Concentration dependence of I_m (at 378 nm) in the liquid (water)–liquid (cyclohexane) extraction system: [**2**] in cyclohexane=2.00×10^{-6} M, pH (of the aqueous phase) 6.5 with 0.05 M phosphate buffer, 25°C. Guest: **8** (○), **9** (▲), and **12** (●).

definition because the error for the I_m value is less than 10%), it follows that it is 1×10^{-5} M for **8**, 2×10^{-6} M for **9** and 2×10^{-7} M for **12**. This concentration order corresponds with the hydrophobicity of the guest molecules: the distribution ratio between 1-octanol and water is 4.47 for **8**, 26.3 for **9**, and 89.1 for **12**.[23]

In contrast to the sensitive detection of barbiturates, adipic acid (1.00×10^{-3} M) in water could not be extracted with **4** (2.00×10^{-6} M) into chloroform–cyclohexane (1:4 v/v) over the pH ranges 2—10. This is probably due to the high water solubility of adipic acid.

In conclusion, the present paper demonstrated that the aggregation–deaggregation process of a fluorescent receptor which can recognize guest molecules by hydrogen-bonding interactions is useful for developing a novel sensory system for neutral molecules.

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