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# Fluorescent β-Cyclodextrins Modified by Isomeric Aminobenzamides: Synthesis, Conformational Analysis, and Fluorescent Behaviors

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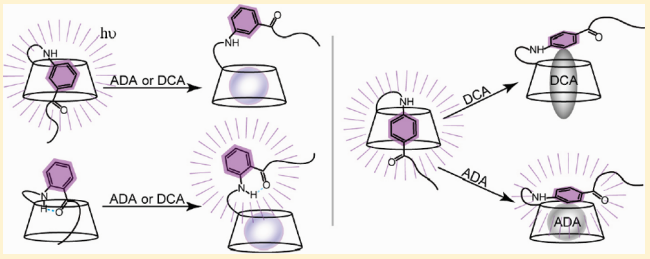
Supporting Information

**ABSTRACT:** Three isomeric fluorescent β-cyclodextrins bearing 2-, 3-, and 4-(2-aminoethyl)amino-*N*-butylbenzamide, respectively (**1**−**3**) have been synthesized. The conformations of these fluorescent CDs have been investigated by 2D NMR and induced circular dichromism. It is confirmed that the *ortho* isomer **1** takes a butyl-included conformation, while the other two isomers **2** and **3** display a phenyl-included conformation, respectively. The three fluorescent CDs **1**−**3** exhibited totally different self- and guest-inclusion fluorescence behavior. In the presence of adamantane carboxylate sodium (ADA) or deoxycholate sodium (DCA), the fluorescence intensity of **1** showed an enhancement over 1-fold, while **2** exhibited dramatic fluorescence quenching. Interestingly, the fluorescent responses of **3** toward two guests respectively were highly distinguishable. The fluorescence intensity of **3** only showed a slight increase upon the addition of ADA, but the addition of DCA led to a large decrease in fluorescence intensity. The investigations have been further carried out by 2D NMR, induced circular dichromism, fluorescence spectroscopy and molecular modeling to explore the relationships between the conformations and the fluorescence characteristics of CDs **1**−**3** in the absence and presence of guest molecules. On the basis of the above investigations, the origins for the different fluorescence behaviors have been proposed.




## INTRODUCTION

Fluorescence-based sensing has been a growing research area because of its high sensitivity, simplicity, and diversity.[1] However, up to now, only very limited cases have been achieved in aqueous media for biomedical analysis.[2]

Cyclodextrins (CDs) are the most important and promising natural macrocyclic hosts. They are water-soluble, nontoxic, commercially available, and readily functionalized. The most important property of CDs is the inclusion of guest molecules into their cavity. These properties made them applicable in various fields, such as enzyme models, drug delivery, chemical sensors, and enantiomeric separations.[3] Among them, the fluorescent sensors based on CD derivatives have attracted much attention due to their potential for biosensing.[4] Ueno and coworkers[5] have pioneered the field of fluorophore-appended CD chemical sensors, which usually adopt a self-inclusion conformation in aqueous solution. The fluorescent CD exhibited strong emission in the self-inclusion state because of the hydrophobic environment of the CD cavity, and the exclusion of fluorophore from CD cavity to bulk aqueous media must weaken its fluorescence intensity. But some exceptions have also been reported,[6] and the fluorescence intensity of these fluorescent CDs was increased by formation of host−guest inclusion complexes. These fluorescent CDs, with a rigid spacer between CD and fluorophore, could not form self-inclusion complexes. The fluorophore just covers the upper rim of the CD cavity, surrounded by a hydrophilic environment. The inclusion of a hydrophobic guest molecule into the cavity of the fluorescent CD makes the fluorophore locate in a more hydrophobic environment; thus, the fluorescence intensity increases.

Recently, we have reported a new fluorescent cyclodextrin with an *N*-butyl-4-(2-aminoethyl)amino-1,8-naphthalimide group connecting to the primary side of β-CD.[7] Upon addition of guest molecules, an unexpected 8-fold enhancement of the fluorescence intensity and a 5 nm blue shift of the fluorescence maximum were observed. On the basis of the combination of the fluorescence data and conformation, it is believed that fluorescence of this fluorescent CD is quenched by the formation of a self-inclusion conformation. On the other hand, Campos and Brochsztain[8] reported that when β-CD was added to an aqueous solution of 4-amino-1, 8-naphthalimide, a blue shift of the fluorescence maximum and an increase in the fluorescence intensity were observed. In other words, the fluorescence behavior of our fluorophore-appended CD in aqueous solution was opposite to that of the inclusion complexes between 4-amino-1,8-naphthalimide and β-CD (namely, in Campos's case). Detailed research has revealed that the orientations of the two fluorophore are opposite to each other, though in both systems the 4-amino-1, 8-naphthalimide moiety was included in the CD cavity.

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Another unusual fluorescence phenomenon for two diastereomeric L/D-Trp-$\beta$-CD (where Trp is tryptophan) was also reported by Liu.[9] It is interesting to note that the L-Trp-$\beta$-CD exhibits usual self- and guest-inclusion fluorescence behavior, as in the case of Ueno's "turn-off" fluorophore-appended CD, while the D-Trp-$\beta$-CD shows entirely opposite behavior, similar to our case above. Moreover, two position-isomeric naphthalene-appended amino-$\beta$-cyclodextrins have also been found to exhibit totally different fluorescent behavior.[10]

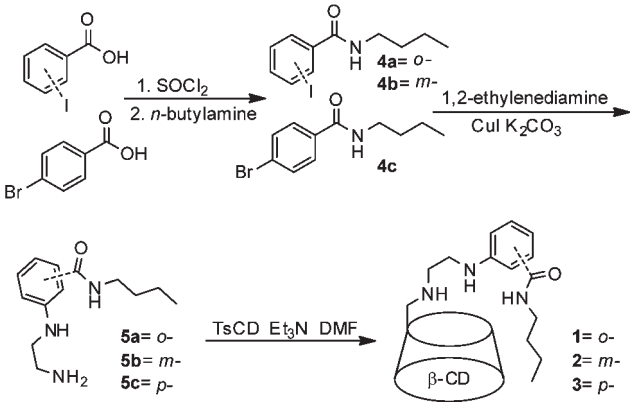
The interesting phenomena described above demonstrated that the nature of the microenvironment around the fluorophore (usually expressed in terms of polarity, hydrogen-bonding ability, hydrophobicity, etc.) is anisotropic, although the interior of the CD cavity is generally considered as a hydrophobic hole. Thus, when we investigate the microenvironment effect of CD on fluorescence, the position and orientation of the fluorophore moiety relative to the CD cavity should be considered as a key factor. Therefore, in order to create new and better fluorophore-appended CD chemical sensors, the relative position and orientation of the fluorophore to the CD cavity should be designed or controlled by changing the molecular structure of the fluorescent CDs, such as by changing the spacer or substitute groups on the fluorophore or by changing the isomeric connecting sites on the fluorophore and so on. The investigations on the relationship between conformation and spectroscopic properties are significant for explaining different fluorescence behaviors and gathering more information of the microenvironment provided by CD. Fluorescent molecules are currently used as probes for the investigation of physicochemical, biochemical, and biological processes, so the study of microenvironment effects on fluorescence behavior should be of great importance for sensing, monitoring, and elucidating various processes in biological systems.

In this paper, we report three isomeric fluorescent CDs **1**−**3**, in which 2-, 3-, and 4-(2-aminoethyl)amino-*N*-butylbenzamide are connected to the primary side of $\beta$-CD, respectively. The aminobenzoyl moiety is an intramolecular charge-transfer (ICT) group, which is sensitive to polarity.[11,12] This ICT characteristic is propitious to offer the information about the microenvironment around the fluorophore moiety.[13] All these compounds have a flexible diaminoethyl group as spacer and a butyl group as terminal on the aminobenzamide moiety to induce the inclusion into the CD cavity. The three position-isomeric aminobenzoyl moieties of fluorescent CDs, **1**−**3**, should take different steric hindrance effects and control the position and orientation of fluorophore moiety relative to the CD cavity. 2D NMR and induced circular dichromism experiments were carried out to investigate the conformations of the fluorescent CDs **1**−**3**, and their spectroscopic properties were also determined. It is interesting that the three fluorescent CDs, **1**−**3**, exhibit totally different self- and guest-inclusion fluorescence behavior. The relationships between their conformations and spectroscopic properties are discussed.

## RESULTS AND DISCUSSION

**Synthesis.** The synthetic route is showed in Scheme 1. Halogen-substituted benzoic acid reacted with thionyl chloride followed by *n*-butylamine to afford **4**. Then under cuprous catalytic conditions, **4** and 1,2-ethylenediamine coupled to generate **5**. Finally, through the classic mono-6-tosyl-$\beta$-CD derivative method, the tosyl group was readily displaced by the amine group of **5** to gain the corresponding fluorescent CDs **1**−**3**.

**Scheme 1. Synthetic Route for Fluorescent CDs 1−3**




**Conformations of Fluorescent CDs 1−3.** NOESY NMR experiments in $D_2O$ were carried out to determine the conformation of **1**−**3**. Figure 1 shows the 2D NOESY NMR spectra of **1**−**3**. In the spectrum of **1**, correlation signals between the protons of CD and the protons of *n*-butyl group can be found. Somewhat unexpectedly, no NOE signal can be observed between the protons of the CD and phenyl group. If the phenyl moiety is included in the CD cavity, the NOE correlations would be observed; therefore, it is possible to estimate that the benzene ring of CD **1** is staying outside the CD cavity. Different from the *ortho* isomer **1**, *meta* and *para* isomers **2** and **3** display clear NOE correlations between protons of CD and the aromatic protons of aminobenzamide other than that between CD and butyl. This result suggests that the benzene ring is included into the CD cavity and the butyl group has passed through the cavity. The 2D NOESY NMR experiment confirms that the three compounds all take self-inclusion conformation in water and the depth of side chains included in CD cavity are different.

Induced circular dichromism (ICD) spectroscopy was used to further provide information about the orientation and position of chromophore relative to the CD cavity. The ICD spectra of **1**−**3** are shown in Figure 2, and a broad band can be observed, respectively, for all three compounds and assigned to the ICT $\pi-\pi^*$ transition relative to about 320, 310, and 290 nm absorption for **1**−**3**, respectively, in UV spectra (Figure4). In the absence of the guests, **1** displayed a negative band and fluorescent CDs **2** and **3** showed a positive band, respectively. According to the rule proposed by Kajtar, Harata, and Kodaka et al.,[14] we can conclude that the phenyl group, the main body of the fluorophore in **1**, is outside the CD cavity, with the benzene plane parallel to the axis of CD cavity and tightly perching on the upper rim of the hydrophobic cavity, and the phenyl group in **2** and **3** is certainly included in the CD cavity. This conclusion is consistent with the results of 2D NOESY NMR experiments.

On the basis of the above experimental information, the molecular modeling of the three fluorescent CDs have been performed by Gaussian 09 program with the density functional theory (DFT) B97D/SVP/def2SV level. The resulting conformations are shown in Figure 3.

The only difference in the three fluorescent CDs is the relative positions of the substituted groups in aminobenzamide moiety, so the position isomerism of side chain is the key factor to yield the differences in self-inclusion conformations. From Figure 3, we can see that because free rotation is strictly limited by two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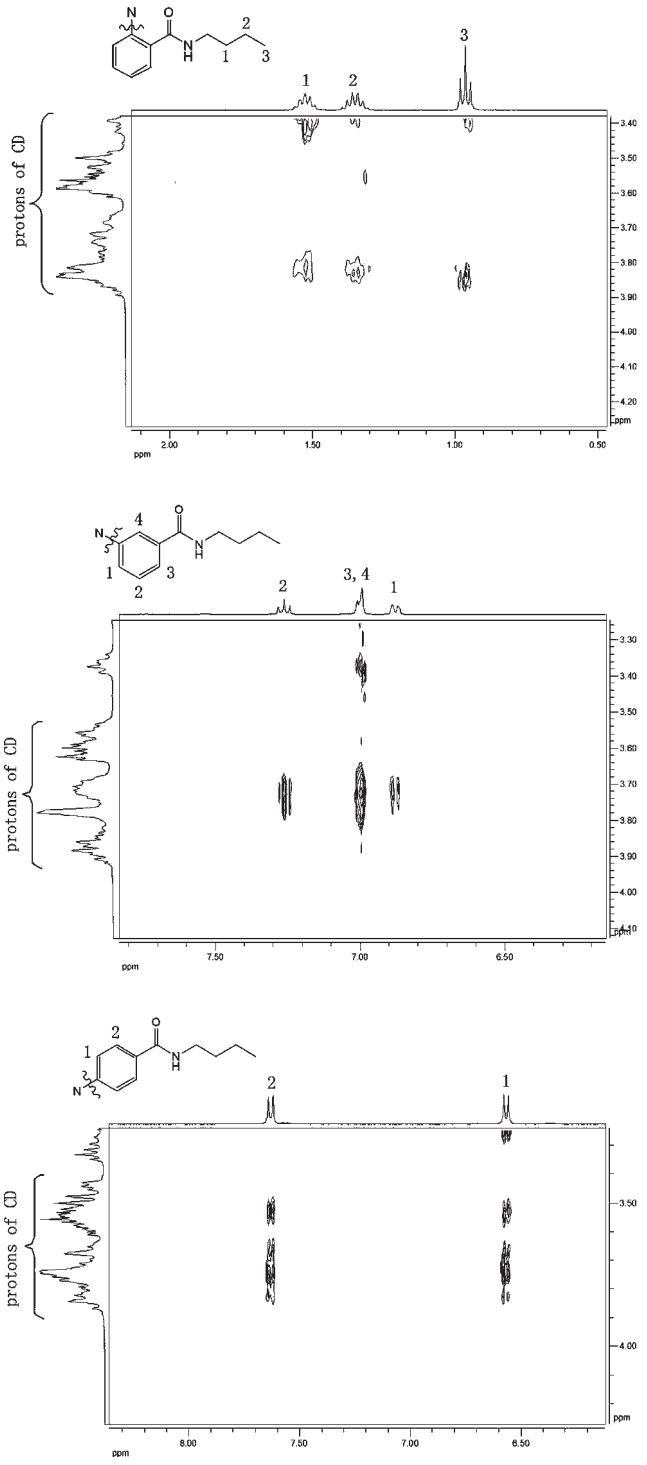


**Figure 1.** 2D NOESY NMR spectra of **1**−**3** with water suppression in $D_2O$ (mixing time = 600 ms).

adjacent substituent groups, the side chain of **1** can only take a folding shape, which holds the benzene ring on the upper rim of the CD cavity, resulting in a butyl-included conformation. In the case of **2**, the *meta*-substituted side chain provides relatively better flexibility than the side chain in **1**, consequently the aminobenzamide moiety can be induced deeply into the CD cavity by butyl group and give a phenyl-included conformation. As for **3**, the linear side chain makes it easier to inlcude the phenyl

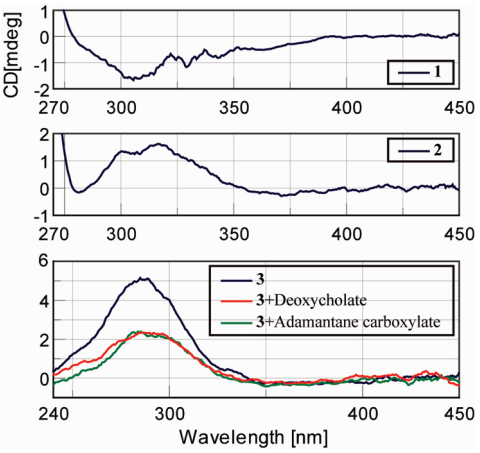


**Figure 2.** Induced circular dichromism spectra of **1**, **2** in the absence, and **3** in the absence and presence of guest molecules in tris-HCl buffer (10 mM, pH 7.5), concentration: **1** and **2**, 0.5 mM; **3**: 0.05 mM; adamantane carboxylate sodium, 2 mM; deoxycholate sodium, 0.4 mM. (The ICD spectra of **1** and **2** with guests are absent because the concentration of two guests cannot satisfy the need for the ICD experiment.)

group in the CD cavity, so it adopts a conformation similar to that of **2**. In both cases, hydrophobicity and the shape-matching effect make the phenyl group stay in the CD cavity and allow the butyl group to pass through the bottom rim of CD into the aqueous solution.

**Spectroscopic Properties.** The emission spectra (in Figure 6) of all three compounds are characterized by a typical ICT band corresponding to the longest wavelength absorption band in absorption spectra, about 320, 310, and 290 nm for **1**−**3**, respectively (Figure 4), which originates mainly from the amino group to the carbonyl group transfer. The excitation wavelengths ($\lambda_{ex}$) for **1**, **2**, and **3** are 326, 315, and 292 nm, respectively, and the maximum emission wavelengths ($\lambda_{max}$) of **1**, **2**, and **3** are 428, 418, and 357 nm, respectively.

Since fluorescent chromophores based on the electron donor/acceptor system are very sensitive to the pH value, the relationship of emission intensity and pH value has been tested. From Figure 5, we can observe that with the increased solution acidity the fluorescence intensity of **2** and **3** enhanced gradually, while in the case of **1**, the change of fluorescence intensity with the increased solution acidity exhibits an opposite tendency. The fluorescence−pH relationships of **2** and **3** show a typical photo-induced electron transfer (PET) feature; namely, with the increased protonation of the amino group directly connected to CD, the PET process was blocked resulting emission enhancement. For the case of **1**, at low pH value, the increased protonation of arylamine quenches the fluorescence emission.

The fluorescence titration of compounds **1**−**3** was performed in 0.01 M pH 7.5 Tris-HCl (tris-(hydroxymethyl)aminomethane) buffer. Well known as having highly affinity to $\beta$-CD, adamantane analogues[15] and bile salts[16] could be tightly bound by $\beta$-cyclodextrin and displace the chromophore in the CD cavity. Thus, adamantane carboxylate sodium (ADA) and deoxycholate sodium (DCA) were chosen as the guests to investigate the fluorescent behavior, while the fluorophore of CDs **1**−**3** stayed outside the CD cavity. The results of the fluorescence titration of compounds **1**−**3** toward these two guests are shown in Figure 6. It was found that the sensing behaviors of the three fluorescent CDs


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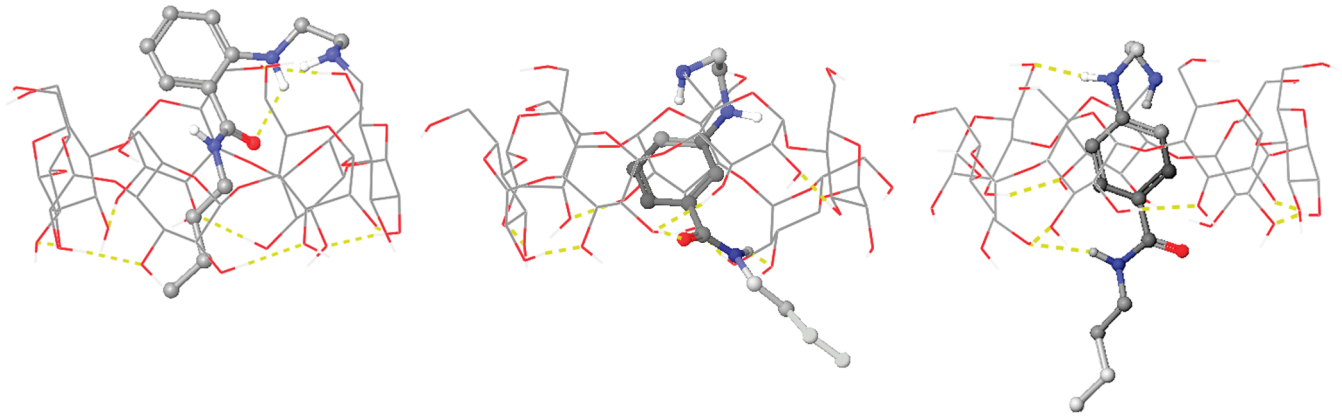

**Figure 3.** Possible geometry structures of **1**−**3**.

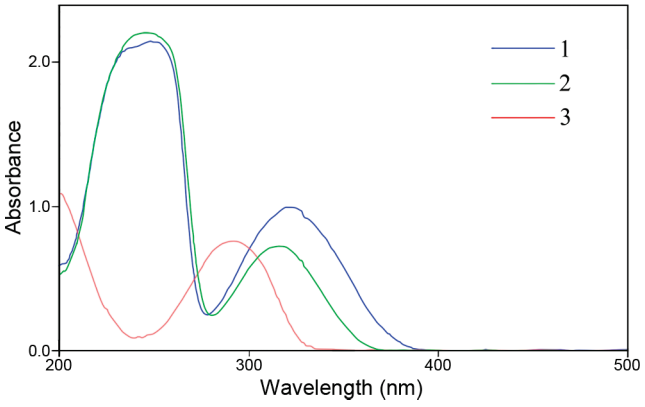


**Figure 4.** Absorption spectra of **1**−**3** in water, concentration (mM): **1** and **2**, 0.5; **3**, 0.05.

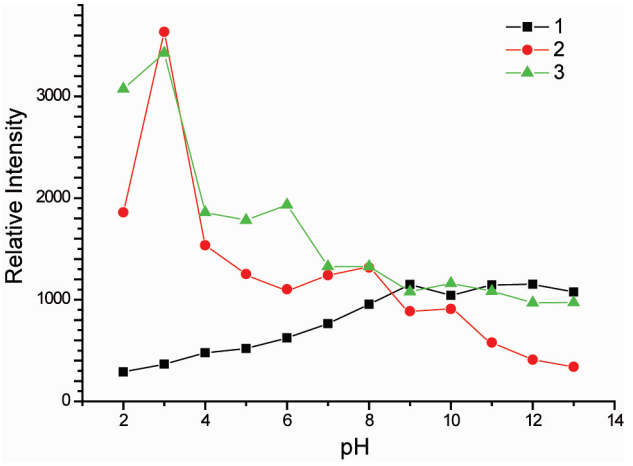


**Figure 5.** Plots of fluorescence intensity of **1**−**3** versus pH value in aqueous solution. Measured wavelengths for **1**−**3** are 428, 418, and 357 nm respectively.

toward these two guests are highly inconsistent. With the gradual addition of ADA or DCA, the fluorescence intensity of **1** showed continuous enhancement. An over 1-fold enhancement of the fluorescence intensity and about 2 nm red shift of the fluorescence maximum were observed when the equilibrium was reached. In the case of compound **2**, a large decrease of the fluorescence intensity and about 18 nm red shift of the fluorescence maximum were observed with the addition of the two guests, respectively. Compared with compounds **1** and **2**, the fluorescent respondses of **3** toward two guests are highly distinguishable. The fluorescence intensity of **3** only showed a slight increase upon the addition of ADA, but the addition of DCA led to a large decrease in fluorescence intensity, and in both cases the fluorescence maximum was red-shifted 5 and 7 nm, respectively. Furthermore, the fluorescence titrations of **3** with 1-adamantanol and taurocholate as guest molecules, respectively, have been carried out, and the same distinguishable emission spectra were obtained in the two situations (see Figures S2 and S3, Supporting Information). This guest-responsive variation of the fluorescence intensity allowed **3** to be used as an effective fluorescent sensor for molecule discrimination.

The stoichiometry of host−guest complexes was determined by the Job's method of continuous variations (Supporting Information). It was confirmed that compounds **1**−**3** all formed a 1:1 complex with the guest. On the basis of the stoichiometry and the fluorescence titration data, the binding constants ($K_S$) between fluorescent CDs **1**−**3** and two guests were calculated by using a nonlinear least-squares curve-fitting method[17] as shown in Table 1. The order of binding constants of fluorescent CDs **1**−**3** with ADA is **3** < **2** < **1**, which reflects the order of steric hindrance caused by the relative substitutions on the benzene ring in aminobenzamide moiety. It indicates the strength of the isomeric side chains being self-included by CD cavity of these modified CDs is **3** > **2** > **1**. Very large and almost equal $K_S$ values for **1**−**3** with DCA show that the combining capacity between DCA and CD cavity are much larger than that of the self-included side chain.

In order to analyze the relationships between the conformation and fluorescent behavior of fluorescent CDs **1**−**3** in detail, the fluorescent spectra of **5a**, **5b**, and **5c** (the fluorophore moiety of **1**−**3**) at the same test conditions as that for **1**−**3** and as well in different solvents were recorded for comparison (as shown in Figure 7 and 8). It should be noted that the effect of the polarity (the polarizability effects and hydrogen bonding ability) of solvents on the fluorescent property of fluorophore moiety with more than one functional group is not easy to predict.[19] The complicacy could be demonstrated by the spectra of **5a**, **5b** ,and **5c** in different solvents (Figure8).

In Figure 7, the spectrum of **1** alone exhibits a weak fluorescent peak at 428 nm, while an over one fold enhancement of emission


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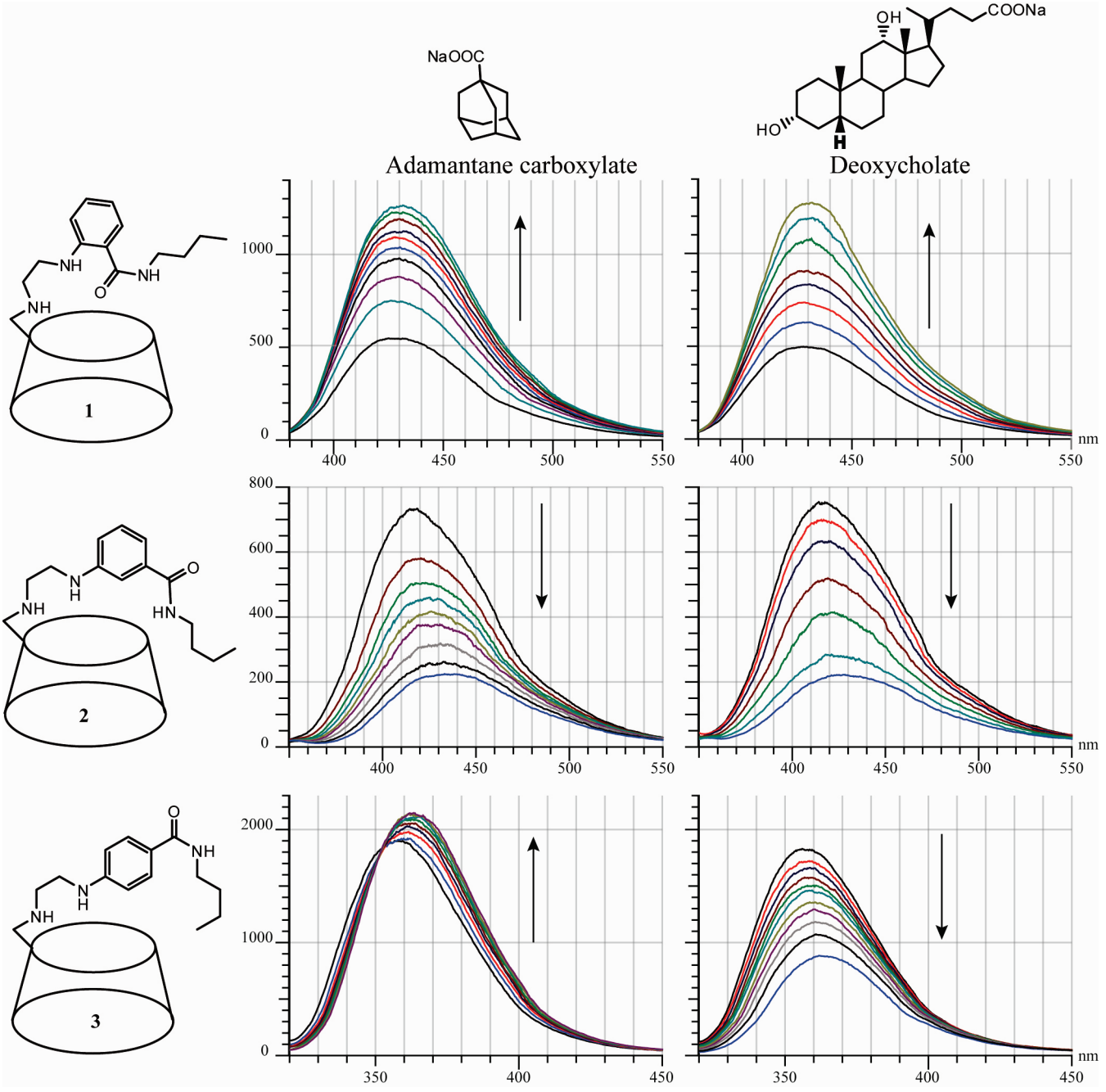


**Figure 6.** Emission spectra of 10 $\mu$M **1**−**3** in the presence of various concentrations of guests in pH 7.5 tris-HCl buffer. Adamantane carboxylate sodium ($\mu$M): (**1**) 0, 20, 40, 60, 79, 98, 116, 151, 186, 235; (**2**) 0, 20, 40, 60, 88, 116, 251, 327; (**3**) 10, 20, 40, 60, 79, 98, 134. Deoxycholate sodium ($\mu$M): (**1**) 0, 0.5, 2, 3.8, 5.6, 10, 14, 17; (**2**) 0, 0.5, 1.5, 3.8, 6.5, 11, 15; (**3**) 0, 1, 2, 3, 4, 4.7, 6.5, 8.2, 10, 14, 20. The excitation wavelength of **1**−**3** are 326, 315, and 292 nm, respectively.

is noticed for **1**+ADA and **1**+DCA, respectively, the intensity is nearly the same as that of **5a**, besides the enhancement of emission, an about 2 nm red shift of fluorescence maximum is also observed in both situations. Combining above spectrum data and the initial conformation of **1**, it is clear that in the cases of **1**+ADA and **1**+DCA, the butyl group, which is self-included in the CD cavity of fluorescent **1**, has been already expelled by guest molecule leading whole fluorophore moiety in **1** exposed to bulk aqueous solution, resulting a concomitant enhancement of fluorescence emission. Namely, the fluorescence of **1** is quenched when it keeps initial conformation, which is the reason for the fluorescence enhancement respond to the guest molecules.

The fluorescence enhancement response is an unusual phenomenon for fluorophore-modified CDs. This phenomenon is often explained by a mechanism which was mentioned previously. However, this mechanism is not suitable for **1**. According to the initial conformation, the fluorophore of **1** should be partially included by the upper rim of the CD cavity, and the exclusion of the fluorophore from the relative hydrophobic environment to bulk aqueous media should weaken its fluorescence intensity.


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**Table 1. Binding Constants of 1−3 with Guests**

| | $K_S$ ($10^4$ $M^{-1}$) | |
|---|---|---|
| host | ADA | DCA |
| **1** | 2.47 ± 0.03 | 11.67 ± 1.6 |
| **2** | 1.49 ± 0.03 | 13.07 ± 1.9 |
| **3** | 0.46 ± 0.06 | 12.03 ± 1.7 |

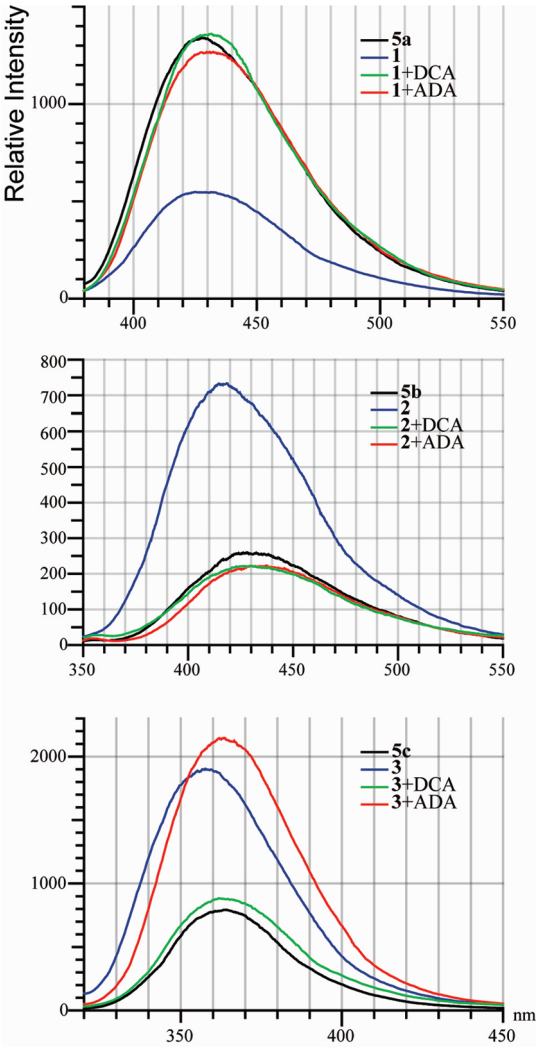


**Figure 7.** Fluorescent spectra of 10 $\mu$M **5a**, **5b**, and **5c** compared with the corresponding CD compounds in the absence and presence of the guest molecules in pH 7.5 tris-HCl buffer.

It should be noted that only fluorescent CD **1** exhibits significant fluorescence enhancement to the guest molecule among these three position isomers. Therefore, the *ortho* substitution on the benzene ring in the fluorophore moiety of **1** should play an important role in the fluorescent behavior. It is expected from this structure that the adjacent amino group and carbonyl group on the benzene ring suitably form a six-member intramolecular hydrogen bond (IHB).[18] From the conformation of **1**, we can see that the butyl group is enfolded in the CD cavity and the benzene ring is located on the upper rim of the cavity. The special conformation makes the IHB stay in a relative

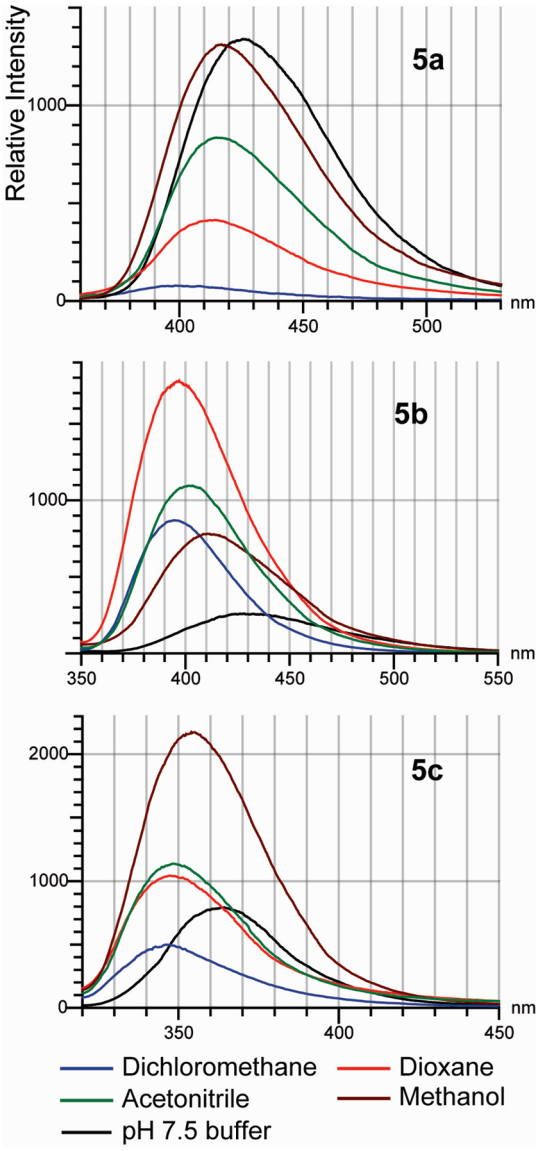


**Figure 8.** Fluorescent spectra of **5a**, **5b**, and **5c** in different solvents.

hydrophobic and close environment avoiding the influence of water molecule, which strongly increases the strength of IHB. On the basis of the above deduction, we postulate that a possible photoinduced proton transfer process via the IHB may be responsible for the fluorescence quenching of **1**. In the presence of the guest molecule, the IHB, which has been exposed to bulk aqueous solution, is broken due to the competition of water molecules; thus, the photoinduced proton transfer was stopped, so as to restore the emission of fluorescence. From the spectra of **5a** in different solvents (In Figure 8), it is clear that the effect of solvent polarity and hydrogen banding ability on the emission intensity and fluorescence maximum of **5a** is consistent with that on the fluorescent behavior of **1**. This comparison further supports the above hypothesis. Moreover, Stalin and Mataga[18] also confirm that 2-aminobenzoic acid exhibits much weaker fluorescence intensity in nonpolar or hydrophobic solvents than in polar solvents.

In the same way, we explore the relationship between the conformation and fluorescent behavior of **2**. From Figure 7, we


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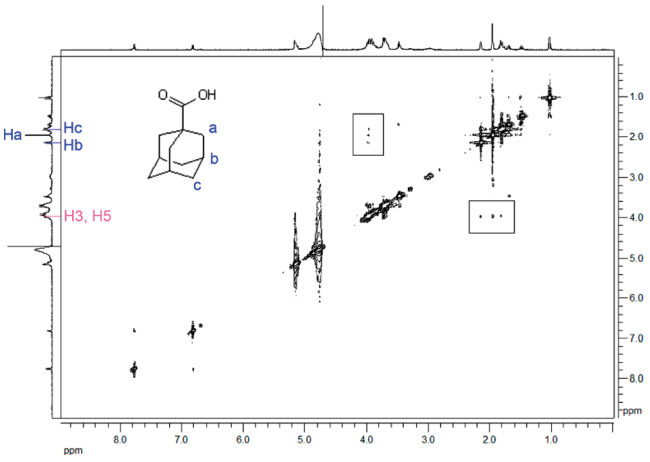


**Figure 9.** NOESY spectra of **3**+ADA with water suppression in $D_2O$ (mixing time = 600 ms).

can find that **2** alone exhibits a relative strong fluorescent emission at 418 nm, while in the case of **2**+ADA and **2**+DCA, a large decrease of the fluorescence emission and about 18 nm red shift has been noticed. The final intensity and florescence maximum of both are nearly the same as those of **5b**. This comparison further confirms the phenyl-included conformation of **2** and concludes that the decrease of the emission intensity in the presence of guest should be attributed to the change of polarity experienced by the fluorophore through the process of being expelled from the cavity. The red shift of florescence maximum in the case of **2** with the addition of the guest is much larger than that of the *ortho* and *para* isomers. This property is in accordance with the performance of **5b** compared to **5a** and **5c** with the increasing polarity (the polarizability effects and hydrogen bonding ability) from dichloromethane to water (Figure 8).

Different from **1** and **2**, compound **3** has exhibited different fluorescent respondses, respectively, toward two guests (Figure 6). It is interesting to explore the origin of this difference. In Figure 7, we find that **3** alone exhibits a strong fluorescent emission at 357 nm, and the emission intensity is much stronger than that of **5c**. This comparison confirms that **3** also takes a phenyl-included conformation. In the situation of **3**+ADA, a slight increase of emission intensity and about 5 nm red shift of florescence maximum are observed, however in the situation of **3**+DCA, a large decrease in fluorescence intensity and a 7 nm red shift of fluorescence maximum occur, and the final intensity and florescence maximum are nearly the same as that of **5c**. These results suggest that the fluorophore moiety in the complex of **3**+ADA is located in a more hydrophobic environment than that of **3** alone, while in the situation of **3**+DCA, the environment polarity around the fluorophore is similar to bulk aqueous media.

The 2D NOESY NMR experiments of **3** in the presence of the two guests have been carried out, respectively, to obtain some structural information. No correlation signal between the protons of CD and fluorophore moiety of **3** is found in Figures 9 and 10, respectively, which suggests that the fluorophore moiety in both situations has been expelled from the CD cavity. The clear correlation signals between all protons of ADA and H3, H5 of CD in Figure 9 indicate the deep immersion of ADA into CD cavity. Because the size and shape of ADA is almost exactly fit for the cavity of $\beta$-CD, the hydrophobic main body of ADA should be tightly accommodated by the cavity, with the charged carboxylic

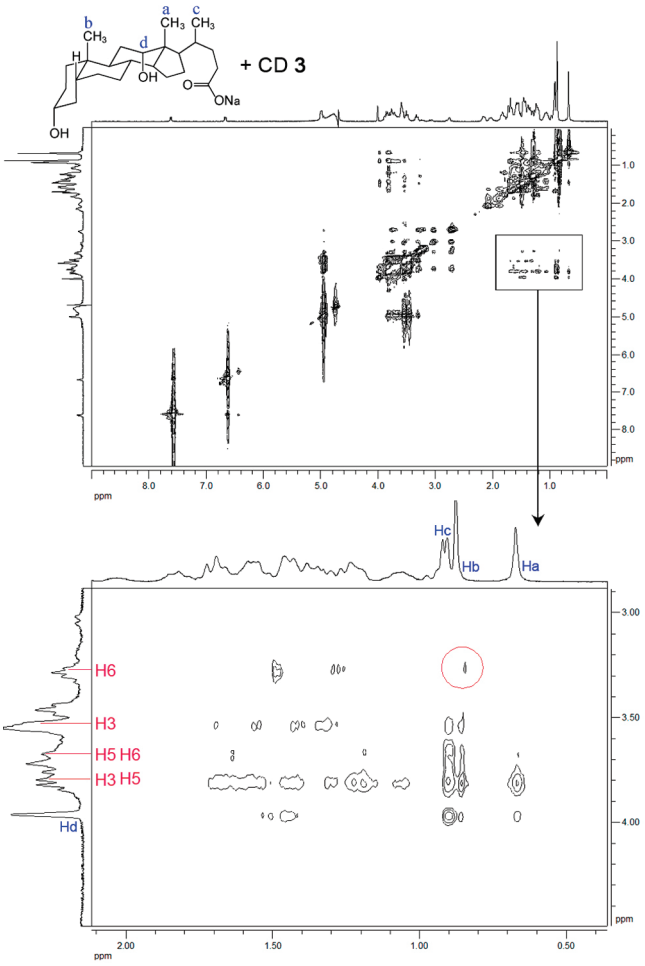


**Figure 10.** NOESY spectra of **3**+DCA with water suppression in $D_2O$ (mixing time = 600 ms).

group exposed to the bulk aqueous solution at the wider opening of the host CD.[20] In the case of **3**+DCA, the clear correlation signals between H3, H5, H6 of CD and the protons of the DCA main body can also be found in Figure 10. In comparison with ADA, DCA is a larger molecule with an elongated shape, and it is clear that there is no enough room for the whole hydrophobic main body of DCA inside the CD cavity. The above correlation signals should be the averages of the spectra of the species in dynamic equilibrium, in which different parts of DCA could be alternately bounded in the CD cavity. The correlation signal between Hb of DCA and H6 of CD is also observed, which indicates that the carboxyl group in DCA also faces to wider side of CD cavity.

The ICD spectra of **3** in the presence of two guests have also been measured, respectively (Figure 2), to determine the position and orientation of the fluorophore moiety relative to the CD cavity in both situations (**3**+ADA and **3**+DCA). Compared with the ICD spectra of **3**, a weaker positive band, at the same position as the original, is observed, respectively, in both cases. It can be assigned to the ICT $\pi-\pi^*$ transition of the fluorophore moiety outside CD cavity with perpendicular orientation. This further confirms that the fluorophore moiety of **3** has been expulsed from the CD cavity by guest molecule and suggests that the fluorophore moiety of **3** in two complexes might cover on the upper rim of CD.


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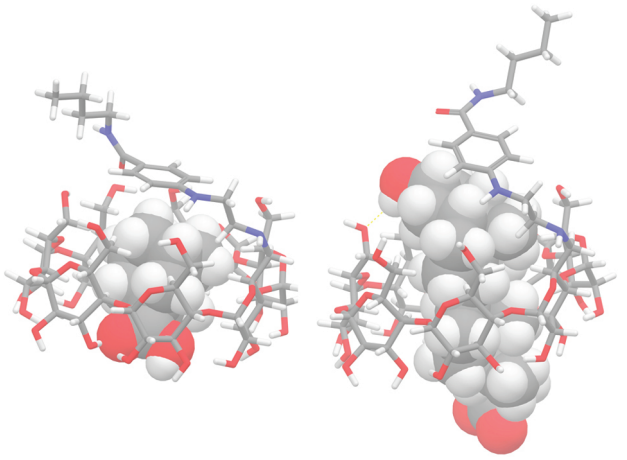

**Figure 11.** Possible geometry structures of host−guest complexes **3**+ADA and **3**+DCA.

The most stable conformations of **3**+ADA and **3**+DCA have been obtained by molecular dynamics simulation of host−guest complexes in water and shown in Figure 11. They are consistent with the experimental data we have obtained so far. As expected, in the complex of **3**+ADA, the size and shape of ADA suitably match the cavity of CD, and the fluorophore moiety closely covers on the upper rim of CD. While in the complex between **3** and DCA, obviously, the hydrophobic main body of DCA has penetrated the CD cavity thoroughly, and a small part of DCA is out of the narrow side of the cavity. Thus, the fluorophore moiety could not closely cover the CD cavity.

Combining the structures of two complexes with the results of the fluorescence titrations of **3** with different guest molecules, it could be inferred the interesting fluorescent phenomenon of **3**, respectively, toward to two guests do not be caused by the interaction between the fluorophore moiety of host molecule and the special functional groups in the guests (see Figures S2 and S3, Supporting Information). All results let us realize that the distinguishable fluorescent responds toward to two guests should be attributed to the tiny difference between two guest-included conformations of **3**. On this basis, possible mechanisms have been provided here to explain the specific fluorescent behavior of **3**. In aqueous solution, **3** alone has adopted a self-included conformation, and the fluorophore moiety is located in a relative hydrophobic microenvironment. Upon the addition of ADA, guest−host association must be accompanied by release of surface/cavity neighboring waters.[6,9,21] The suitable size and shape of ADA must lead to the tight combination of **3** and ADA. The formed complex of **3**+ADA has a guest full-included structure in which the fluorophore moiety could closely cover the upper rim of CD.[20] In this complex, the fluorophore moiety's location is relatively complicated; the microenvironment below the fluorophore moiety must become more hydrophobic because of the deep immersion of the hydrophobic main body of ADA and the accompanied release of surface/cavity neighboring waters, but the upper side of the fluorophore moiety is exposed in bulk aqueous solution. The effect on fluorescent property should come from the changes of the microenvironment polarity. Because of the anisotropy of the microenvironment, its effect on fluorescent property should be much more complicated than that in a homogeneous solvent system. Therefore, the slight increase of emission intensity and about 5 nm red shift of florescence maximum of **3**+ADA could be attributed to the changes of the microenvironment polarity around the fluorophore moiety. In comparison, the large size and elongated shape of DCA leads the complex of **3**+DCA to adopt a guest partly included structure in which the fluorophore moiety has been raised by DCA and covers the top of the guest molecule. From the final intensity and florescence maximum of **3**+DCA, which are nearly the same as those of **5c**, it could be inferred that the gap between the fluorophore moiety and the upper rim of CD should allow the accommodation of water molecules. Therefore, the real polarity experienced by the fluorophore moiety might be like the situation of the moiety entirely exposed in water, which might be the reason for the dramatic quenching of fluorescence emission by DCA.

## CONCLUSIONS

In summary, we have synthesized three isomeric fluorescent-$\beta$-CDs bearing 2-, 3-, and 4-(2-aminoethyl)amino-*N*-butylbenzamide, respectively (**1**, **2**, and **3**). By comparative study of their structures, conformations, and fluorescence characteristics, it can be deduced that the relative position of the substituted groups on the benzene ring in the fluorophore moiety is the key factor in yielding different conformations and fluorescence behaviors of **1**−**3**. In the situation of **1**, the two adjacent substituted groups on benzene ring can offer not only the ability to form an intramolecular hydrogen bond (IHB) but also the butyl-included conformation. The suitable location of benzene ring makes the aminobenzoyl moiety of **1** form a strong IHB, therefore undergoing a photoinduced proton transfer via the IHB results in a concomitant fluorescence quenching. Inclusion of the guest is accompanied by an increase in the intensity of emission due to changing the environment around the fluorophore from the hydrophobic interior to the polar water environment.

In contrast, compound **2**, in which the side chain has better flexibility, takes a phenyl-included conformation as a fluorescent CD bearing an intramolecular charge-transfer group and exhibits a usual fluorescence behavior. The decrease in the intensity of emission in the presence of guest should be attributed to the change of polarity experienced by the fluorophore through the process of being expelled from the cavity.

Compound **3** with a linear side chain has a phenyl-included conformation similar to that of **2** and the strongest self-inclusion ability among three isomers. The distinguishable fluorescent response to two guests should be attributed to the tiny difference between two guest-included conformations of **3**. The differences in the size and shape of two guests (ADA or DCA) let the fluorophore suffer from different changes in environment, yielding an entirely different fluorescence response. This guest-responsive variation of the fluorescence intensity allows **3** to be used as effective fluorescent sensor for molecule discrimination.

This investigation has demonstrated that the different relative position and orientation of the fluorophore to the CD cavity can be obtained by changing the molecular structure of fluorescent CDs. This approach allows us to go deep into the relationship between conformation and spectroscopic properties of fluorescent CDs and also to gather more information about the microenvironment effect of the CD cavity on fluorescence. This study provided some significant information for sensing, monitoring, and elucidating various processes in biological systems.


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## EXPERIMENTAL SECTION

**General Procedures.** $^1$H NMR and $^{13}$C NMR spectra were recorded on a 300 MHz NMR spectrometer, and 2D NOESY NMR was carried out at room temperature on a 400 MHz instrument. Chemical shifts were referenced to the solvent values, and *J* values are reported in hertz. Fluorescence spectra were recorded in a conventional quartz cell (10 mm × 10 mm × 45 mm) on an F-4500 spectrofluorometer. Absorption spectra were carried out on a UV 2550 spectrophotometer. Circular dichroism (CD) spectra were recorded on a J810 spectropolarimeter. Elemental analyses were performed on an EL III instrument.

**Calculations.** The calculations reported here utilized Gromacs 4.5.2 and Gaussian 09 programs.[22] The optimizations of **1**−**3** utilized Grimme's B97D functional, which includes dispersion correction and Aldrich SVP as basis set. Molecular dynamics simulation of host−guest complexes in water used Gromacs program with parameters as follows: opls-aa force field, 1 fs time step, and running in the NVT ensemble for 20 ns.

**Materials.** DMF was dry by $CaH_2$, and other regents were used as reagent grade without further purification. 6-*O*-*p*-Toluenesulfonyl-*β*-cyclodextrin was prepared according to reported methods.[23] Tris(hydroxymethyl)-aminomethane was dissolved in distilled, deionized water and adjusted by HCl to make a 0.01 M pH 7.5 Tris-HCl buffer for fluorescence spectral titration.

*Synthesis of* ***4***. The corresponding halogen-substituted benzoic acid (20 mmol), 25 mL of thionyl chloride, and 1 drop of DMF reacted under reflux until the solid disappeared. Excessive thionyl chloride was removed by vacuum distillation. The residue was dissolved in 30 mL of chloroform and added dropwise to a mixture of *n*-butylamine (20 mmol) and triethylamine (60 mmol) in 50 mL of chloroform under 0 °C. The mixture was stirred for 4 h. Dilute hydrochloric acid (80 mL) was added, the mixture shaken, and the organic layer was extracted with water and saturated brines. The chloroform layer was dried with anhydrous sodium sulfate and concentrated under vacuum.

*2-Iodo-N-butylbenzamide (**4a**).* $^1$H NMR ($CDCl_3$): $\delta_H$ 0.99 (3H, t, *J* = 7.2), 1.32−1.7 (m, 4H), 3.48 (2H, m), 5.84 (NH, s, br), 7.12 (1H, m), 7.37 (2H, m), 7.88 (1H, d, *J* = 8.1).

*3-Iodo-N-butylbenzamide (**4b**).* $^1$H NMR ($CDCl_3$): $\delta_H$ 0.96 (3H, t, *J* = 7.1), 1.3−1.66 (m, 4H), 3.45 (2H, m), 6.11 (NH, s, br), 7.16 (1H, m), 7.11−7.22 (2H, m), 8.10 (1H, d, *J* = 8.1).

*4-Bromo-N-butylbenzamide (**4c**).* $^1$H NMR ($CDCl_3$): $\delta_H$ 0.95 (3H, t, *J* = 6.79), 1.39 (2H, m), 1.54 (2H, m), 3.31 (2H, m), 7.52 (2H, d, *J* = 8.8), 7.75 (2H, d, *J* = 7.6).

*Synthesis of* ***5***. A mixture of **4** (0.012 mol), anhydrous $K_2CO_3$ (0.024 mol), CuI (0.0006 mol), and 1,2-ethylenediamine (50 mL) was stirred and heated to reflux under argon atmosphere for 24 h. After being cooled to room temperature, the resulting solution was collected by filtration and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ and purified by preparative thin layer plates (PLC). The PLC developing solvent was $CHCl_3/CH_3OH/Et_3N$ = 50:5:1 by volume.

*2-(2-Aminoethyl)amino-N-butylbenzamide (**5a**).* MS (ESI): *m*/*z* 235 (M). $^1$H NMR ($CDCl_3$): $\delta_H$ 0.95 (3H, t, *J* = 6), 1.40 (2H, m), 1.57 (2H, m), 2.95 (2H, t, *J* = 5.4), 3.22 (2H, t, *J* = 5.7), 3.38 (2H, t, *J* = 6.3), 6.22 (NH, s, br), 6.58 (1H, t, *J* = 7.2), 6.70 (1H, d, *J* = 8.1), 7.31 (2H, m), 7.63 (NH, s, br).

*3-(2-Aminoethyl)amino-N-butylbenzamide (**5b**).* MS (ESI): *m*/*z* 235 (M). $^1$H NMR ($CDCl_3$): $\delta_H$ 0.94 (3H, t, *J* = 7.2), 1.37 (2H, m), 1.58 (2H, m), 2.94 (2H, t, *J* = 5.7), 3.21 (2H, s), 3.43 (2H, m), 4.30 (NH, s, br), 6.29 (NH, s, br), 6.74 (1H, d, *J* = 6.9), 6.98 (1H,), 7.08 (1H, s), 7.18 (1H, t, *J* = 7.5).

*4-(2-Aminoethyl)amino-N-butylbenzamide (**5c**).* MS (ESI): *m*/*z* 236.2 (M + 1). $^1$H NMR ($CDCl_3$): $\delta_H$ 0.97 (3H, t, *J* = 7.2), 1.41 (2H, m), 1.60 (2H, m), 2.99 (2H, s) 3.23 (2H, s), 3.44 (2H, m), 4.77 (NH, s, br), 5.97 (NH, s, br) 6.62 (2H, d, *J* = 6), 7.63 (2H, d, *J* = 8.1).

*Synthesis of* ***1***−***3***. Mono-6-*O*-(*p*-tolylsulfonyl)-*β*-cyclodextrin (4.2 mmol), **5** (4.2 mmol), and $Et_3N$ (0.5 mL) were added to 10 mL of dry DMF. The resulting solution was stirred at 80 °C under argon atmosphere for 20 h and cooled to room temperature. Afterward, the solution was added to 200 mL of acetone dropwise to form precipitates. The precipitates were collected, washed with acetone followed by ether, and dried in vacuum. The product was dissolved in water, and the solution was layered on a column packed with YMC GEL ODS-A (2.5 × 10 cm, pre-equilibrated with water). A step gradient elution was applied. The fractions containing title compounds were combined, and EtOH was removed under reduced pressure.

*Mono-6-deoxy-6-(2-(2-aminoethyl)amino-N-butylbenzamide)-β-CD (**1**).* 20% yield based on **5a**. MS (ESI): *m*/*z* 1352 (M). $^1$H NMR ($D_2O$): $\delta_H$ 0.96 (3H, t, *J* = 7.2), 1.34 (2H, m), 1.53 (2H, m), 2.67 (2H, m), 2.84 (1H, m), 2.95 (1H, s), 2.98(1H, s), 3.2−3.9 (43H, m), 4.96−5.03 (7H, m), 6.76 (1H, t, *J* = 7.6), 6.92 (1H, d, *J* = 8.4), 7.22 (1H, d, *J* = 8), 7.38 (1H, t, *J* = 8). $^{13}$C NMR (DMSO-$d_6$): $\delta_C$ 11.0, 16.9, 28.5, 39.2, 45.3, 57.0, 67.3, 70.2, 78.8, 80.2, 99.2, 108.5, 111.6, 112.9, 125.4, 129.5, 146.2, 166.4. Anal. Calcd for $C_{55}H_{89}N_3O_{35}\cdot 8H_2O$: C, 44.14; H, 7.07; N, 2.81. Found: C, 44.29; H, 7.15; N, 2.99.

*Mono-6-deoxy-6-(3-(2-aminoethyl)amino-N-butylbenzamide)-β-CD (**2**).* 17% yield based on **5b**. MS (ESI): *m*/*z* 1352 (M). $^1$H NMR ($D_2O$): $\delta_H$ 0.96 (3H, t, *J* = 7.6), 1.39 (2H, m), 1.6 (2H, m), 2.71 (1H, m), 2.77 (2H, m), 3.01 (1H, s), 3.05 (1H, s), 3.19 (2H, m), 3.37 (3H, m), 3.53−3.92 (38H, m), 5.02−5.04 (7H, m), 6.87 (1H, d, *J* = 9.2), 6.99 (1H, s), 7.01 (1H, s), 7.26 (1H, t, *J* = 8). $^{13}$C NMR (DMSO-$d_6$): $\delta_C$ 10.7, 16.6, 28.2, 39.7, 45.1, 45.8, 56.9, 69.0, 69.3, 70.0, 78.5, 80.1, 98.9, 107.5, 111.3, 111.7, 125.6, 132.6, 145.8, 163.9. Anal. Calcd. for $C_{55}H_{89}N_3O_{35}\cdot 5H_2O$: C, 45.80; H, 6.92; N, 2.91. Found: C, 45.65; H, 7.02; N, 2.79.

*Mono-6-deoxy-6-(4-(2-aminoethyl)amino-N-butylbenzamide)-β-CD (**3**).* 27% yield based on **5c**. MS (ESI): *m*/*z* 1352 (M). $^1$H NMR ($D_2O$): $\delta_H$ 0.86 (3H, t, *J* = 7.6), 1.31 (2H, m), 1.53 (2H, m), 2.5−2.8 (3H, m), 3.0 (1H, d), 3.1 (1H, m), 3.2−3.9 (43H, m), 4.8−5.0 (7H, m), 6.6 (2H, d, *J* = 8.8), 7.6 (2H, d, *J* = 8.4). $^{13}$C NMR ($D_2O$/DMSO-$d_6$): $\delta_C$ 11.6, 17.9, 29.3, 37.7, 58.4, 68.1, 70.3, 71.5, 79.0, 79.5, 82.0, 99.7, 100.3, 110.2, 119.6, 127.1, 149.9, 167.3. Anal. Calcd for $C_{55}H_{89}N_3O_{35}\cdot 4H_2O$: C, 46.38; H, 6.86; N, 2.95. Found: C, 46.45; H, 6.99; N, 2.90.

## ASSOCIATED CONTENT

**Supporting Information.** Job's method of continuous variations, 2D NOESY NMR spectrum of **3** and ADA, copy of **1**−**3** NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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