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Highly enantioselective fluorescent recognition of α-amino acid derivatives

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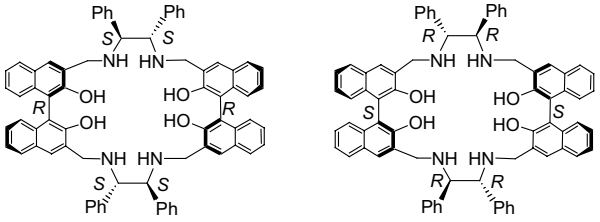
Abstract—Bisbinaphthyl-based macrocycles are found to carry out enantioselective fluorescent recognition of α-amino acid derivatives. It is observed that one enantiomer of a *N*-protected phenyl glycine can increase the fluorescence intensity of the binaphthyl fluorophores by over 4-fold but the other enantiomer does not cause much fluorescence enhancement. This highly enantioselective fluorescent response makes the binaphthyl macrocycles practically useful for the enantioselective fluorescent recognition of the amino acid substrate.

Development of molecule-based enantioselective fluorescent sensors is receiving growing research attention because such sensors can potentially provide a real time technique to determine the enantiomeric composition of chiral molecules.[1–6] Application of this method will be of great significance in the combinatorial high through-put assay of chiral drugs and catalysts. Among the work on the fluorescence-based chiral recognition, only limited reports on amino acids have appeared,[6] despite the extensive studies on the use of other analytical methods such as NMR, UV absorption and various separation techniques in the molecular recognition of amino acids and derivatives.[7–9]

Recently, we have found that the bisbinaphthyl macrocycles (+)- and (−)-**1**[10] can carry out highly enantioselective fluorescent recognition of α-hydroxycarboxylic acids.[1c] We have also studied the use of these chiral macrocycles for the fluorescent recognition of amino acid derivatives. Herein, we report that these compounds can be used as fluorescent sensors to recognize α-amino acid derivatives with high enantioselective responses.

Compound (+)- and (−)-**1** contain secondary nitrogen atoms adjacent to the naphthyl rings. As we have shown earlier,[1a–c] interaction of these nitrogen atoms with acids can turn on the fluorescence of the naphthalenes by inhibiting the fluorescence quenching of the photo-induced-electron-transfer (PET)[11] of the nitrogen lone pair electrons. The multiple chiral functional groups of the macrocycles also provide efficient binding sites for functional organic molecules.



However, our initial study on using these macrocycles for the fluorescent recognition of amino acids met two major obstacles. In order to increase the hydrogen bonding interaction between the macrocycles and amino acids, it is necessary to use less polar organic solvents, but most of the natural amino acids are highly polar and insoluble in organic solvents such as benzene and methylene chloride. In addition, although an amino acid contains a carboxylic acid group that could enhance the fluorescence of the macrocycles by interacting with their nitrogen atoms, the primary amine group in an amino acid could also quench the fluorescence of the fluorophores. This makes the enantioselective fluorescent

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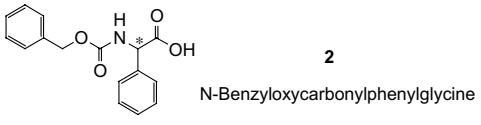
recognition of amino acids more challenging than the recognition of α-hydroxycarboxylic acids or amines.

In order to overcome these problems, we decided to introduce a *N*-carbonyl group into an amino acid substrate. Introduction of an electron-withdrawing *N*-carbonyl group to an amino acid molecule could serve two purposes: (1) It delocalizes the lone pair electrons of the amine group and makes them unavailable for the fluorescence quenching; (2) It also converts the often Zwitterionic amino acid to a neutral molecule, which should increase its solubility in organic solvents.

We first studied the interaction of (+)-**1** with D- and L-*N*-benzyloxycarbonylphenyl glycine (**2**). Figure 1 gives the fluorescence spectra of (+)-**1** in the presence of D- and L-**2**. The fluorescence measurements were conducted in benzene solutions with the addition of a small amount of dimethoxyethylene (DME) in order to increase the solubility of **2**. As shown in the spectra, when (+)-**1** was treated with D- or L-**2**, significant fluorescence enhancement especially at the long wavelength was observed ($\lambda_{long} = 430$ nm when excited 340 nm). The enantiomer D-**2** enhanced the long wavelength emission 5.7 times more than L-**2** did, that is, ef = 5.7 [ef: enantiomeric fluorescence difference ratio $= (I_D - I_0)/(I_L - I_0)$. I_0 is the fluorescence intensity of the receptor. I_D and I_L are the fluorescence intensity of the receptor in the presence of D- and L-**2**, respectively.]. The influence of the concentration of the *N*-protected amino acid on the fluorescence of (+)-**1** at the long wavelength was studied (Fig. 2). In Figure 2, the error bars were obtained by four independent measurements. These experiments demonstrate that while D-**2** led to 4-fold increase for the fluorescence intensity of (+)-**1**, L-**2** caused little change for the fluorescence. Thus, the fluorescence response of the receptor is highly enantioselective towards the amino acid.



2

N-Benzyloxycarbonylphenylglycine

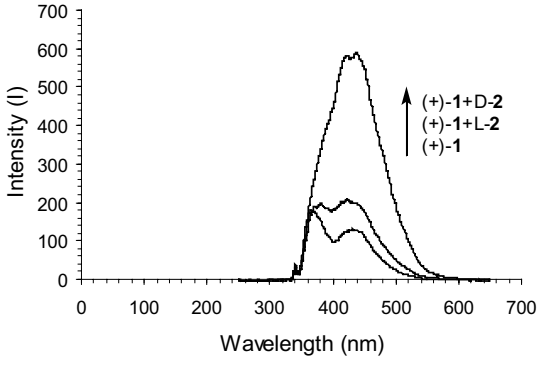


Figure 1. Fluorescence spectra of (+)-**1** (1.0×10^{-4} M in benzene containing 2% DME) with/without D- and L-**2** (4.0×10^{-3} M) ($\lambda_{exc} = 340$ nm).

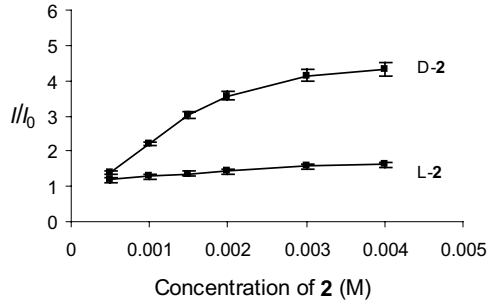


Figure 2. Fluorescence enhancement (I/I_0) at λ_{long} of (+)-**1** (1.0×10^{-4} M in benzene/2% DME) versus concentration of D- and L-**2**.

The general procedure for the fluorescent measurement is described below: A benzene stock solution of the chiral macrocycle (2.0×10^{-4} M) was freshly prepared before each measurement. A 0.01 M stock solution of the chiral amino acid in benzene containing 5% (V) DME was freshly prepared. Then, the sensor solution was mixed with the acid solution at room temperature in a 5 mL volumetric flask and diluted to the desired concentration. Additional DME was added to keep its concentration constant. The resulting solution was allowed to stand at room temperature for 4 h and the fluorescence was measured on a Perkin–Elmer LS-50 B luminescence spectrometer.

The interaction of (−)-**1**, the enantiomer of (+)-**1**, with D- and L-**2** was also studied (Fig. 3). As shown in Figure 3, L-**2** caused much greater fluorescence enhancement for the emission of (−)-**1** at the long wavelength than D-**2**. Figures 2 and 3 are of a mirror image relationship. This confirms the enantioselective response of (+)- or (−)-**1** towards the enantiomers of the amino acid **2**.

The ^{1}H NMR spectra of (−)-**1** and L-**2** in a variety of ratios in benezene-d_6 at a constant total concentration of 4.0×10^{-3} M were taken. It was found that the methine proton signal of L-**2** at δ 5.440 underwent a downfield shift when treated with (−)-**1**. When (−)-**1** versus L-**2** was 8:2, the methine proton signal appeared at δ 5.991. The Job plot of $\Delta\delta X$ versus the mole fraction (X) of L-**2** in the mixture was obtained,[12] which showed a maximum at $X = 0.8$ (Fig. 4). This indicates that (−)-**1** forms a 1:4 complex with the acid under the conditions. Probably all the four nitrogen atoms of (−)-**1** are interacting with the

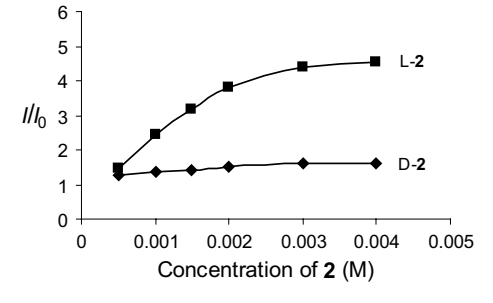


Figure 3. Fluorescence enhancement (I/I_0) at λ_{long} of (+)-**1** (1.0×10^{-4} M in benzene/2% DME) versus concentration of D- and L-**2**.

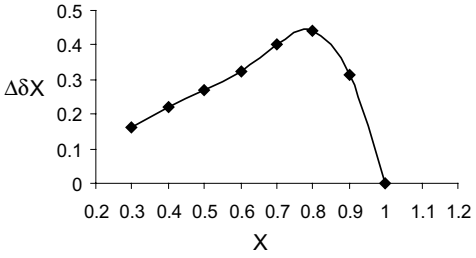


Figure 4. Job plot of (−)-**1** with L-**2** (X = mole fraction of L-**2**, $\Delta\delta$ = the chemical shift change of L-**2**).

acidic protons of four L-**2**. Other binding modes may also exist as there is a shoulder in the Job plot. In the bisbinaphthyl macrocycle, its nitrogen atoms are expected to quench the fluorescence of the naphthyl fluorophores by the PET process.[11] Interaction of these nitrogen atoms with the acidic proton of L-**2** makes their lone pair electrons no longer available for PET, leading to the observed large fluorescence enhancement. The fluorescence experiments demonstrate that L-**2** should bind (−)-**1** much stronger than D-**2**. In the same way, D-**2** should also bind (+)-**1** much stronger than L-**2**, leading to the much greater fluorescence enhancement.

The fluorescence intensity change of (+)-**1** with respect to the enantiomeric composition of **2** was studied. As shown in Figure 5, the fluorescence intensity of (+)-**1** increases linearly with the increasing D-component of the amino acid. Thus, the chiral bisbinaphthyl macrocycle can be used as a fluorescent sensor to readily determine the enantiomeric composition of this amino acid.

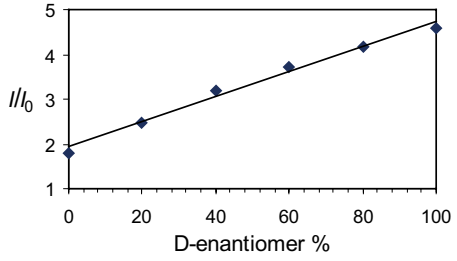


Figure 5. Fluorescence enhancement (I/I_0) at λ_{long} of (+)-**1** (1.0×10^{-4} M in benzene containing 2% DME) versus enantiomeric composition of **2** (3.0×10^{-3} M).

We also studied the interaction of (+)- and (−)-**1** with other *N*-protected amino acids **3**–**8** (Fig. 6). Table 1 summarizes the highest ef's observed for the interaction of (+)- or (−)-**1** with the two enantiomers of these amino acid derivatives. All of these compounds enhanced the fluorescence of the binaphthyl fluorophore. Various degree of enantioselectivity was observed. As shown in Table 1, when the benzyloxycarbonyl protecting group of **3** was replaced with the acetyl group of **4**, the enantioselectivity was inverted. Amino acids **5**, **6** and **8** also showed the opposite enantioselectivity to that of **2**. The additional hydroxyl or carboxylic acid group of **7** and **8** gave a greatly reduced enantioselective fluorescent response.

In Table 1, different amounts of DME were used because of the different solubility of the substrates. The amount of DME in the solution was found to affect the fluorescence spectrum of **1**. In the absence of a substrate, the ratio of the long wavelength emission (λ_{emi} = 435 nm) of (−)-**1** (1.0×10^{-4} M in benzene) versus its short wavelength emission (λ_{emi} = 368 nm) increased from 0.61 to 1.7 as the amount of DME increases from 0% to 20%. This ratio was also strongly influenced by the binding of (+)-**1** with the highly polar amino acids. As shown in Figure 1, the stronger binding of (+)-**1** with D-**2** led to much more intense long wavelength emission than the short wavelength one.

Table 1. Results for the enantioselective fluorescent responses of (+)- or (−)-**1** (1.0×10^{-4} M) to the amino acids **2**–**8**

Amino acid	Receptor	Solvent	Concentration (M)	Ef
2	(+)-**1**	C_6H_6/2% DME	4×10^{-3}	5.7[a]
3	(+)-**1**	C_6H_6/2% DME	2×10^{-3}	1.9[a]
4	(−)-**1**	C_6H_6/20% DME	2×10^{-2}	1.7[a]
5	(+)-**1**	C_6H_6/12% DME	4×10^{-3}	2.2[b]
6	(+)-**1**	C_6H_6/0.1% DME	2×10^{-3}	1.7[b]
7	(+)-**1**	C_6H_6/8% DME	2×10^{-2}	1.2[a]
8	(+)-**1**	C_6H_6/4% DME	5×10^{-3}	1.1[b]

[a] D/L.
[b] L/D.

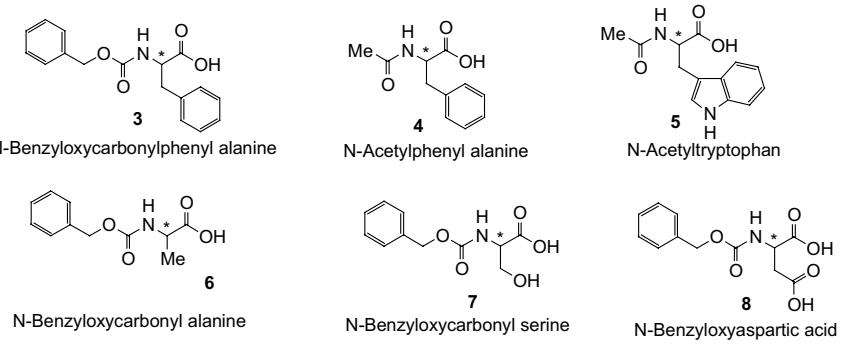


Figure 6. Structures of the *N*-protected amino acids **3**–**8**.

In summary, we have found that the bisbinaphthyl-based macrocycles (+)- and (−)-**1** can carry out enantioselective fluorescent recognition of α-amino acid derivatives. It is observed that one enantiomer of a *N*-protected phenyl glycine can increase the fluorescence intensity of the binaphthyl fluorophores by over 4-fold but the other enantiomer does not cause much fluorescence enhancement. This highly enantioselective fluorescent response makes the binaphthyl macrocycles practically useful for the enantioselective fluorescent recognition of the amino acid substrate.

Acknowledgements

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