Chemoselective and Enantioselective Fluorescent Recognition of Glutamic and Aspartic Acids

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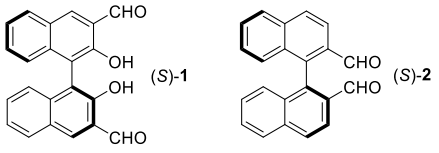
A highly chemoselective as well as enantioselective fluorescent probe has been discovered for the recognition of the acidic amino acids including glutamic acid and aspartic acid. This study has established a novel amino acid recognition mechanism by an aldehyde-based fluorescent probe.

Amino acids are important molecules in nature as well as in laboratories and industry. They play essential roles in many biological systems and serve as starting materials to diverse organic compounds including a number of pharmaceutical products. For example, the acidic amino acids L-glutamic acid (L-Glu) and L-aspartic acid (L-Asp) are major excitatory neurotransmitters in the mammalian central nervous system and are found to be vital in learning, memory, movement and brain functions.[1-5] Their deficiency may cause several neurological or psychiatric disorders.[6,7] Besides L-amino acids, the existence of the enantiomeric D-amino acids in biosystems and their diverse functions have also been recognized.[8-11] For example, a recent study shows a significant level of D-glutamate in the hearts of certain mice as well as the presence of a mammalian D-glutamate cyclase gene which indicate possible physiological roles of D-Glu in mammals.[12] In central nervous, neuroendocrine, and endocrine systems, D-Asp has been found to regulate hormone secretion and steroidogenesis.[13,14] It is identified as a neuro transmitter and is found to be associated with Alzheimer's disease[15].

Because of the importance of amino acids, many analytical methods have been developed.[16,17] Among these methods, the use of fluorescence has attracted significant attention because of the easily available instrument, high sensitivity, multiple sensing modes, rapid analysis and potential for noninvasive bioimaging.[18,19] Although significant work has been conducted on the fluorescent detection of amino acids, no fluorescent probe has been obtained for the chemoselective as well as enantioselective recognition of glutamic acid and aspartic acid.[20-22]

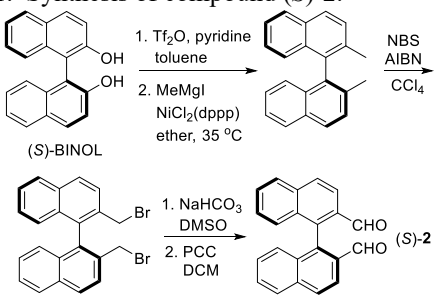
In 2014, we reported the use of the 1,1′-bi-2-naphthol (BINOL)-based dialdehyde (*S*)-**1** in combination with Zn^{2+} as an enantioselective fluorescent sensor for amino acids.[23] It was found that the aldehyde groups of (*S*)-**1** provided the covalent binding sites for the amino acid substrates to generate the observed fluorescent responses. In order to further improve the selectivity of (*S*)-**1** for the fluorescent recognition of amino acids, we have proposed to move its 3,3'-diformyl groups to the 2,2'-positions to prepare the optically active 2,2'-diformyl-1,1'-binaphthyl (*S*)-**2**.[24] In (*S*)-**2**, the two aldehyde groups are located much closer to the chiral cavity of the 1,1'-binaphthyl structure which has potential to enhance the chiral bias of the probe. In addition, the proximity of the two aldehyde groups of (*S*)-**2** may allow them to cooperate with each other to react with specific amino acids with potentially more selective response. Recently, we have discovered that this compound and its enantiomer have exhibited unprecedented chemoselective and enantioselective fluorescent recognition of glutamic acid and aspartic acid without the addition of a metal ion. Herein, these results are reported.



We prepared (*S*)-**2** from (*S*)-BINOL by combining and modifying the literature procedures as shown in Scheme 1.[24] The enantiomer (*R*)-**2** was also prepared by starting with (*R*)-BINOL. Figure 1 gives the fluorescence and UV-vis absorption spectra of (*S*)-**2** (2.0×10^{-5} M in $MeOH/CH_2Cl_2$ = 99:1, v). It shows that when (*S*)-**2** was excited at 280 nm, there were weak emissions at 340 nm and 410 nm. The UV-vis spectrum displays absorptions at λ (ε) = 250 (4.6×10^{-4}), 285 (1.5×10^{-4}), and 352 (3.6×10^{-5}) nm.

We investigated the fluorescence response of (*S*)-**2** toward the tetrabutylammonium (TBA) salts of the enantiomeric pairs of 18 common amino acids in the absence of a metal ion. In these experiments, a CH_2Cl_2 solution of (*S*)-**2** (2.0×10^{-3} M, 25 μL) was mixed with each enantiomer (5.0 equiv) of the amino acid salts in methanol at 27.0 °C for 2.0 h which was then diluted with methanol to a final concentration of (*S*)-**2** at 2.0 x 10^{-5} M (2.5 mL, $MeOH/CH_2Cl_2$ = 99:1, v/v). As the results summarized in Figure 2 show, L-Glu and L-Asp greatly enhanced the fluorescence of (*S*)-**2** at λ = 365 nm with I/I_0

Scheme 1. Synthesis of compound (*S*)-**2**.



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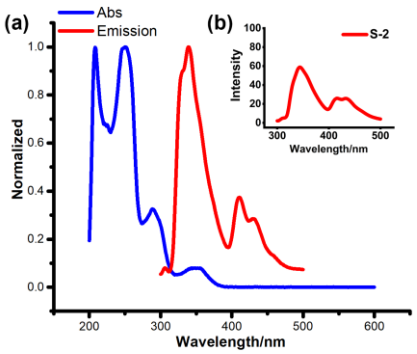


Figure 1. (a) Normalized UV-vis and fluorescence spectra of (*S*)-**2.** (b) Fluorescence spectrum of (*S*)-**2** (2.0×10^{-5} M in MeOH/CH_2Cl_2 = 99:1, v/v) (λ_{exc} = 280 nm).

of 81.5 and 39.7 respectively. However, the enantiomers D-Glu, D-Asp as well as all the other amino acids generated little fluorescence response with the probe (See Figure S1 for more details). Thus, (*S*)-**2** exhibits highly chemoselective and enantioselective fluorescent responses toward glutamic acid and aspartic acid.[25]

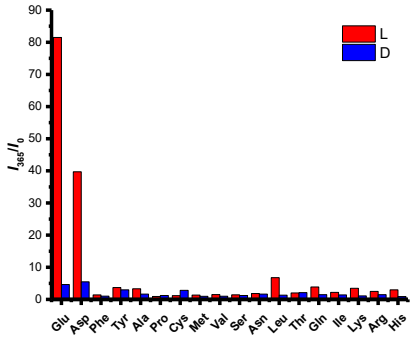


Figure 2. Fluorescent response at 365 nm, I_{365}/I_0, for the interaction of (*S*)-**2** (2.0×10^{-5} M) with 18 pairs of D-/ L-amino acids (5 equiv) (Solvent: MeOH/CH_2Cl_2 = 99/1, v/v. λ_{exc} = 280 nm, slits = 5/5 nm. I_0: Fluorescence intensity of (*S*)-**2** at 365 nm in the absence of amino acids).

A detailed investigation on the fluorescent response of (*S*)-**2** toward L- and D-Glu was then conducted. Figure 3a gives the fluorescence spectra of (*S*)-**2** after treated with 5.0 equiv of L- and D-Glu. In these measurements, (*S*)-**2** (2.0 mM) in CH_2Cl_2 was mixed with L- or D-Glu (5 equiv) at 300 K for 2 h and then diluted with methanol to 2.0×10^{-5} M. Figure 3b,c give the fluorescence spectra of (*S*)-**2** in the presence of 0.5 – 8.0 equiv of L- and D-Glu. We studied the influence of the reaction time before dilution on the fluorescence response. As shown in Figure S2 in SI, the fluorescence enhancement of (*S*)-**2** by L-Glu (5 equiv) became stable after 60 min, and little fluorescence enhancement was observed with the addition of D-Glu over 120 min. Therefore, all the following fluorescence measurements were conducted after 90 min of reaction. As shown in Figure 3d, when the concentration of L-Glu increased to 5 equiv, the fluorescence intensity of (*S*)-**2** at 365 nm increased to 81 folds of its original value. When more than 5 equiv of L-Glu was added, the fluorescence started to decrease. When D-Glu was used, however, the fluorescence of (*S*)-**2** did not change significantly over the entire concentration range (Figure 3c,d). At 5.0 equiv of the amino acid, the maximum enantioselective fluorescence enhancement ratio [$ef = (I_L - I_0)/(I_D - I_0)$ where I_0 is the fluorescence of the sensor without the amino acid][21a] is 25. The limit of detection (LOD) for L-Glu by using (*S*)-**2** was determined to be 4.76×10^{-8} M (Figure S3).

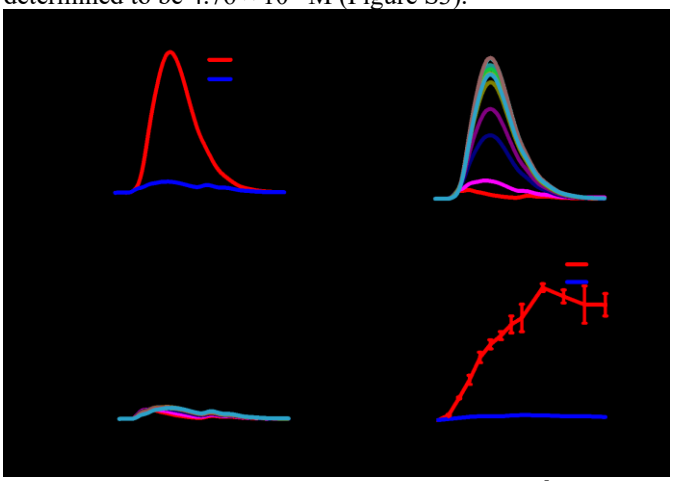


Figure 3. Fluorescence spectra of (*S*)-**2** (2.0×10^{-5} M) with (a) L- and D-Glu (5.0 equiv), (b) L-Glu, and (c) D-Glu. (d) Fluorescence intensity at 365 nm versus the equivalent of L- and D-Glu. (Solvent: MeOH/CH_2Cl_2 = 99/1, v/v. Error bars from three independent experiments. λ_{exc} = 280 nm. Slit: 5/5 nm)

The fluorescence response of (*R*)-**2**, the enantiomer of (*S*)-**2**, toward D- and L-Glu was also studied under the same conditions. As shown in Figure S4 in SI, a mirror image relation was observed between the fluorescence responses of (*R*)- and (*S*)-**2** toward the enantiomers of glutamic acid, which confirms the inherent chiral recognition process. We also investigated the fluorescence responses of (*S*)-**2** toward L-Glu (4.0 equiv) in the presence of other amino acids (1.0 equiv). As shown by Figure S5, no significant interference on the fluorescence measurement was observed except with Lys or Ile.

We examined the interaction of (*S*)- and (*R*)-**2** with glutamic acid at various enantiomeric compositions and plotted the fluorescence intensities of each enantiomeric probe versus the glutamic acid enantiomeric excess (Figure 4). A mirror image relationship was observed between the fluorescence responses of this pair of enantiomeric sensors. These figures can be used to determine the enantiomeric composition of amino acids. The highly enantioselective

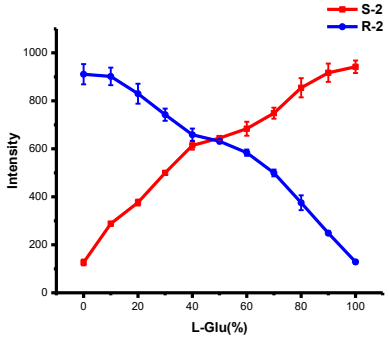
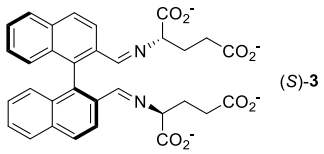


Figure 4. Fluorescence intensity of (*S*)- and (*R*)-**2** (2.0×10^{-5} M) at 365 nm versus the [L-Glu] % (5 equiv) (Solvent: MeOH/CH_2Cl_2 = 99/1, v/v. Error bars from three independent experiments. λ_{exc} = 280 nm. Slit: 5/5 nm).

fluorescence response of (*S*)-**2** toward aspartic acid was also investigated which is shown in Figures S6 in SI.

In order to understand the observed chemoselective and enantioselective recognition of glutamic acid, we monitored the reaction of (*S*)-**2** with L- and D-Glu by ^{1}H NMR analysis (Figure S7 in SI). It was found that when (*S*)-**2** was treated with excess L-Glu over 12 h, it was completely converted to the diimine product (*S*)-**3**. The 2D NMR HSQC spectrum of (*S*)-**3** (Figure S8 in SI) shows a cross peak between the imine proton signal of (*S*)-**3** at δ 7.73 and the imine carbon signal at δ 159.9 which confirms the imine structure. We also isolated the diimine product (*S*)-**3** from the reaction of (*S*)-**2** with L-Glu and obtained its fluorescence and UV-vis absorption spectra (Figure S9 in SI).

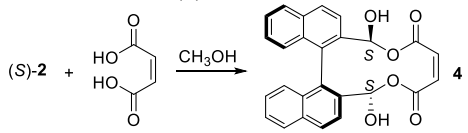


(*S*)-**3**

The fluorescence of the diimine product (*S*)-**3** was found to be very weak which cannot explain the greatly enhanced fluorescence of (*S*)-**2** with L-Glu. Thus, formation of a strongly fluorescent intermediate is proposed for the reaction of (*S*)-**2** with L-Glu. As shown in Figure 1, only the TBA salts of L-Glu and L-Asp that contain two carboxylic acid groups can generate large fluorescence enhancement. This suggests a possible cooperative effect of the two carboxylate groups. We found, however, that not all organic dicarboxylic acids or their dicarboxylate salts can increase the fluorescence of (*S*)-**2** (see Figure S10 in SI). For example, D-Glu, D-Asp, succicinic acid, glutaric acid and its TBA salt gave little or no fluorescence response. That is, the fluorescence enhancement requires a specific structural match between a dicarboxylic acid and (*S*)-**2**.

When (*S*)-**2** was treated with maleic acid, a large fluorescence enhancement was observed (see Figure S11 in SI). The ^{1}H NMR spectrum in CD_3OD and mass analyses (see Figure S12 in SI) demonstrate that (*S*)-**2** reacts with maleic acid to form the dicarboxylate adduct **4** (calcd. for **4**-H_2O+Na^+: 431.1, found 431.2) (Scheme 2). The ^{1}H NMR spectrum of **4** suggests a symmetric structure and the proton signal on the hemiacetal carbon appears at δ 4.75. On the basis of the CD spectrum of **4** and the CD calculation (see Figure S15 in SI), it is proposed that the two newly formed hemiacetal chiral carbon centers of **4** should have two S configurations. We conducted a density functional theory calculation by using the RB3LYP, 6-311G** program on the stability of the 3 stereoisomers of **4** (see Figure S16-S18). It was found that the proposed structure **4** with *SS* configurations at the two hemiacetal centers is the most stable one. The rigid macrocyclic structure of **4** should have restricted the rotation of the naphthyl units and contributed to the greatly enhanced fluorescence. In addition, conversion of the aldehyde groups of (*S*)-**2** to the two hemiacetal groups of **4** also removes the charge transfer between the naphthyl groups and the aldehyde groups, contributing to the greatly enhanced fluorescence at the short emission wavelength (λ = 340 nm).

Scheme 2. Reaction of (*S*)-**2** with maleic acid.

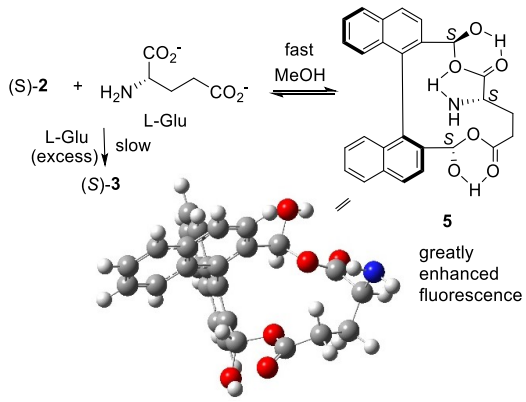


When compound (*S*)-**2** interacts with maleic acid, the two carboxylic acid groups have a synergistic effect to react with both of the aldehyde groups of (*S*)-**2** to form the macrocyclic bis(hemiacetal) product **4**, and the structural rigidity of this macrocyclic hemiacetal product contributes to the increased fluorescence. Among the diacids used to interact with (*S*)-**2** in Figure S10b,c, oxalic acid was another substrate that can enhance the fluorescence of (*S*)-**2**, but all the other diacids gave little fluorescence enhancement. As shown in the ^{1}H NMR spectra in Figure S12c, only maleic acid and oxalic acid can completely convert (*S*)-**2** to the corresponding bis(hemiacetal) products with no aldehyde signal of the starting material left. All other diacids showed only partial reaction with significant aldehyde signals remained around 9.5 ppm. Therefore, the structures of maleic acid and oxalic acid match that of (*S*)-**2** to give complete reaction and fluorescence enhancement.

In the ^{1}H NMR spectra obtained for the reaction of (*S*)-**2** with L-Glu, it was found that at lower concentration of L-Glu, several small peaks appeared at δ 4.7 ~ 5.2 (see Figure S7 in SI), which disappeared when excess L-Glu was added for an extended reaction time to form the final diimine product (*S*)-**3**. This together with the above study for the reaction of (*S*)-**2** with maleic acid indicate that a dicarboxylate adduct intermediate like **5**, similar to the bis(hemiacetal) compound **4**, might be produced to give the observed greatly enhanced fluorescence (Scheme 3). In **5**, an intramolecular hydrogen bonding between a hemiacetal group and the amine might facilitate its formation. Similar to **4**, the rigid macrocyclic structure of **5** should contribute to the greatly enhanced fluorescence.

We calculated the energies of all 8 stereoisomers of **5** with a (*S*)-BINOL unit, and found that the *SSS* structure as depicted for **5** in Scheme 2 is the most stable one (see Figure S20-S28 in SI) probably due to the extra the hydrogen bonding interaction between the amine group and one of the hemiacetal groups. This stability difference might have contributed to the observed chemoselective as well as enantioselective recognition of L-Glu by (*S*)-**2**. When D-Glu was used, the intramolecular hydrogen bond like that in the intermediate **5** might be too weak to allow the formation of significant amount of such a dicarboyxlate adduct, giving the observed much lower fluorescence. When the N-Boc protected L-Glu or glutaric acid (see Figure S19 in SI) was used, much smaller fluorescence enhancement was observed probably because their hydrogen bonding interactions were much weaker than those in **5**. As shown in Scheme 3, when (*S*)-**2** was treated with excess L-Glu over the extended reaction time, it was converted to the weakly fluorescent final product, the diimine (*S*)-**3**.

Scheme 3. A proposed mechanism for the reaction of (*S*)-**2** with L-Glu.



We also studied the interaction of (*S*)-**2** with 4-amino butyric acid and its TBA salt in methanol, which did not show fluorescence enhancement (See Figure S19 in SI). When (*S*)-**2** was treated with the dimethyl ester of L-Glu, there was very weak fluorescence enhancement (see Figure S19 in SI). These results further support the proposed dicarboxylate adduct intermediate **5** for the chemoselective and enantioselective recognition of L-Glu by (*S*)-**2**.

In summary, we have discovered that the 1,1'-binaphthyl-based dialdehyde (*S*)-**2** is a highly chemoselective as well as enantioselective fluorescent probe for glutamic acid and aspartic acid. Unlike the previously reported BINOL-based probes such as (*S*)-**1**, no metal ion is required for the use of (*S*)-**2** in fluorescent sensing. On the basis of a series of spectroscopic and molecular modeling study, it is proposed that when the chirality of the probe matches that of the substrate, the two carboxylate groups of the acidic amino acids add to the two aldehyde groups of (*S*)-**2** to generate an intermediate with greatly enhanced fluorescence. That is, the two aldehyde groups of the probe cooperate with each other to exhibit the unprecedented selectivity in the fluorescent recognition of amino acids. This represents a new fluorescent recognition mechanism provided by an aldehyde-based probe.

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