Chemoselective and Enantioselective Fluorescent Identification of Specific Amino Acid Enantiomers

Lin Pu

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904, USA. E-mail: lp6n@virginia.edu

Abstract

The enantiomers of chiral amino acids play versatile roles in biological systems including humans. They are also very useful in the asymmetric synthesis of diverse chiral organic compounds. Therefore, identification of a specific amino acid and distinguishing it from its enantiomer are of great importance. Although significant progress has been made in the development of fluorescent probes for amino acids, most of them are not capable of conducting simultaneous chemoselective and enantioselective detection of a specific amino acid enantiomer. In this article, several fluorescent probes have been designed and synthesized for chemoselective as well as enantioselective recognition of certain amino acid enantiomers. (*S*)-**1** shows greatly enhanced fluorescence in the presence of L-glutamic acid and L-aspartic acid, but produces no or little fluorescent response toward their opposite enantiomers and other amino acids. (*R*)-**4** in combination with Zn^{2+} shows greatly enhanced fluorescence in the presence of L-serine. (*S*)-**6** is designed for the selective recognition of histidine. Micelles made of an amphiphilic diblock copolymer are used to encapsulate the water-insoluble compound (*S*)-**8** which shows chemoselective as well as enantioselective fluorescence enhancement with L-lysine in the presence of Zn^{2+} in aqueous solution. The same micelles are also used to encapsulate several (*S*)-1,1'-binaphthyl-based monoaldehydes (*S*)-**10** for the chemoselective and enantioselective fluorescent recognition of L-tryptophan in the presence of Zn^{2+} in aqueous solution. These findings have demonstrated that highly selective fluorescent identification of a specific amino acid enantiomer can be achieved by incorporating certain functional groups at the designated locations of the 1,1'-binaphthyls. The binaphthyl core structure of these probes provides both chirality source and highly tunable fluorescence property. Matching the structure and chirality of these probes with those of the specific amino acid enantiomers can generate structurally rigid reaction products and give rise to greatly enhanced fluorescence. The strategies of this work can be further expanded to develop fluorescent probes for the specific identification of many amino acids of interests. It should facilitate the analysis of chiral amino acids in various applications. The outlook of this research and its comparison with other methods are also discussed.

1. Introduction

Amino acids, specifically the L-enantiomers of α-amino acids, are the building blocks of life.[1] They link together through amid bonds to form proteins that are essential for all the life systems including humans. Among 20 canonical amino acids used in the protein syntheses of human body, only half of them can be produced by humans and the other half are named essential amino acids which need to be provided in daily diet. Besides L-amino acids, their opposite enantiomers, D-amino acids, are also found in humans, other animals, plants and microbes and exhibit various biological functions.[2-6] For example, D-aspartic acid and D-serine in the β-amyloid protein of brains were obtained from patients with Alzheimer's disease.[2] The level of D-aspartic acid in human lenses was found to increase with aging.[3] Other D-amino acids such as D-alanine, D-glutamate, N-methyl-D-asparate, and D-glutamine were found in brain to play important roles as neurotransmitters and messenger molecules.[4] Detection of D-amino acids and understanding the mechanism of their functions are important for the development of treatment for neurological diseases such as amyotrophic lateral sclerosis, Alzheimer's disease and schizophrenia.[4] The ratio of the enantiomers of amino acids in foods can be used to estimate the sample age and provide quality control since D-amino acids can be generated under certain food processing conditions or from the contamination of microbes.[5] Both enantiomers of amino acids are also extensively used in organic synthesis including pharmaceutical production.[7]

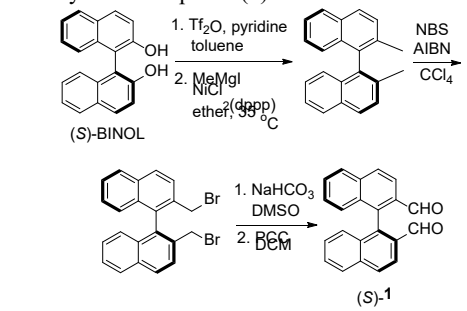
In order to analyze the enantiomeric composition of chiral amino acids, a number of methods have been used including HPLC, GC and electrophoresis.[8-10] Optical methods such as UV-vis absorption, circular dichroism and fluorescence spectrometry have also been developed to distinguish the enantiomers of amino acids.[11-13] In our laboratory, we have conducted projects on the design and synthesis of chiral molecular hosts for the enantioselective fluorescent recognition of chiral organic compounds including α-hydroxy carboxylic acids, amines, amino alcohols and amino acids because using fluorescence spectroscopy has advantages of easily available instrument, fast and on-site analysis, high throughput assay, remote sensing, etc.[14-19] In spite of the progress in the development of fluorescent probes for amino acid analysis,[13] simultaneous chemoselective as well as enantioselective identification of a specific amino acid enantiomer by using a fluorescent probe was rare.[20] Recently, we have designed and synthesized several fluorescent probes that have exhibited both high chemoselectivity and high enantioselectivity in the recognition of amino acids. These probes give greatly enhanced fluorescence in the presence of a specific amino acid enantiomer and they are potentially useful in various applications to distinguish one amino acid enantiomer from other amino acids and chemicals. Herein, our study on the development of fluorescent probes for chemoselective as well as enantioselective recognition of free amino acids is discussed.

II. Selective Recognition of Glutamic Acid and Aspartic Acid.

We synthesized 2,2'-diformyl-1,1'-binaphthyl (*S*)-**1** from (*S*)-1,1'-bi-2-naphthol [(S)-BINOL] according to Scheme 1.[21] When (*S*)-**1** was excited at 280 nm, it gave weak emissions at λ = 340 nm and 410 nm. It was interacted with the tetrabutylammonium (TBA) salts of 18 enantiomeric pairs of common amino acids, and only L-Glu-TBA and L-Asp-TBA generated large fluorescence enhancement at 365 nm (Figure 1). The TBA salts of these amino acids were used for better solubility in methanol. All other amino acid enantiomers including the opposite enantiomers D-Glu-TBA and D-Asp-TBA produced no or little fluorescence response. Figure 2 shows the fluorescence responses of (*S*)-**1** toward L- and D-Glu-TBA. In Figure 2a, (*S*)-**1** (2.0 mM) in CH_2Cl_2 was first mixed with 5.0 equiv of L- or D-Glu-TBA at 300 K for 2 h and then diluted to 2.0 x 10^{-5} M with methanol for fluorescence measurement. Figure 2b shows that when the concentration of L-

Glu-TBA was increased from 0.5 to 5.0 equiv, the fluorescence enhancement reached maximum (81 folds of the original intensity), after which the fluorescence intensity started decreasing while the concentration of L-Glu-TBA was increasing. However, little fluorescence response was observed when the concentration of D-Glu-TBA was increased from 0 to 8.0 equiv. The maximum enantioselective fluorescence enhancement ratio [$ef = (I_L - I_0)/(I_D - I_0)$] was 25 at 5.0 equiv of the amino acid. The limit of detection (LOD) for L-Glu was 4.76×10^{-8} M.

Scheme 1. Synthesis of probe (*S*)-**1**.



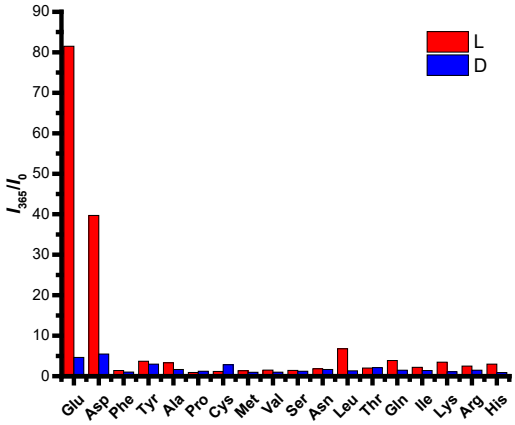
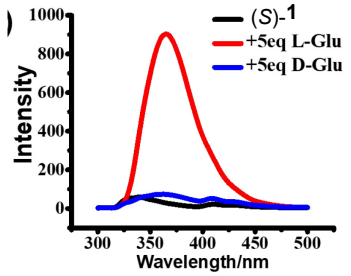
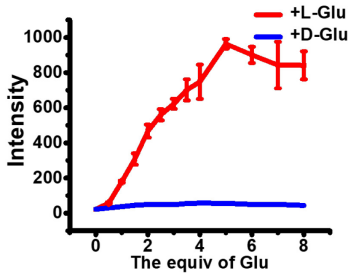


Figure 1. Fluorescent response at 365 nm, I_{365}/I_0, for the interaction of (*S*)-**1** (2.0×10^{-5} M) with 18 pairs of D-/ L-amino acids (5 equiv) (Solvent: MeOH/CH_2Cl_2 = 99/1, v/v. λ_{exc} = 280 nm, slits = 5/5 nm. I_0: Fluorescence intensity of (*S*)-**1** at 365 nm in the absence of amino acids).



(a)



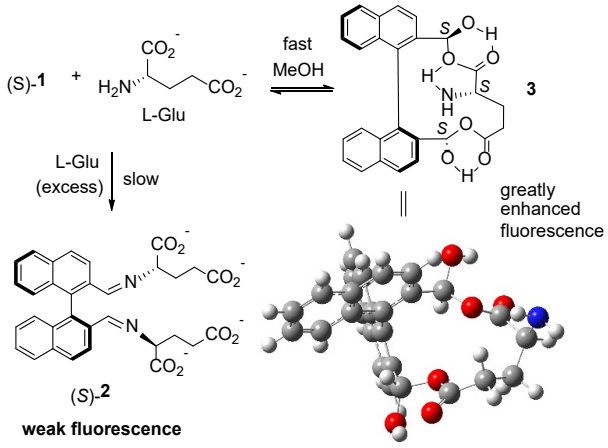
(b)

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

The fluorescence responses of probe (*S*)-**1** and its enantiomer (*R*)-**1** toward L- and D-Glu-TBA at various enantiomeric composition show a mirror image relationship which confirms the chiral recognition process. The enantiomeric composition of the amino acid can thus be determined by the fluorescence measurement.

No fluorescence response was observed when (*S*)-**1** was treated with 4-amino butyric acid. Reaction of (*S*)-**1**–with excess amount of L-Glu-TBA over extended reaction time gave the diimine product (*S*)-**2** which gave only weak fluorescence (Scheme 2). Thus, the stereoselective formation of the dihemiacetal adduct **3** was proposed to account for the chemoselective and enantioselective recognition of the amino acid. The greatly increased structural rigidity of **3** over (*S*)-**1** should have contributed to the greatly enhanced fluorescence. (*S*)-**1** might not be able to form a compound like **3** with D-Glu-TBA and other amino acid enantiomers and thus cannot produce significant fluorescence enhancement.

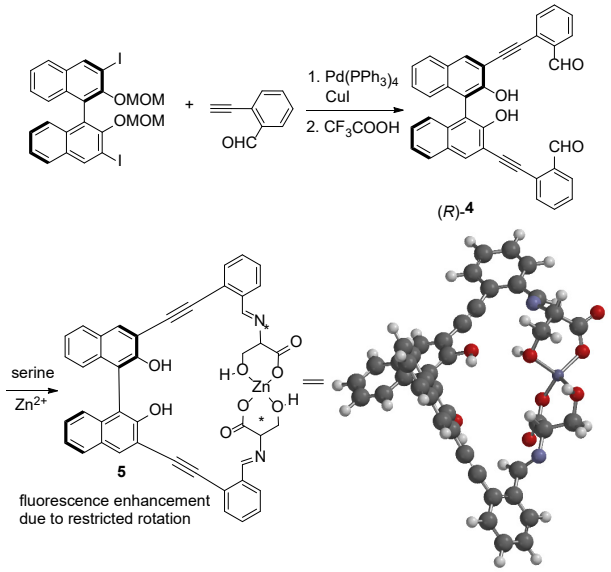
Scheme 2. A proposed mechanism for the reaction of (*S*)-**1** with L-Glu.



III. Selective Recognition of Serine.

We designed and synthesized compound (*R*)-**4** for the chemoselective and enantioselective detection of serine as shown in Scheme 3.[22] This compound contains a chiral 1,1'-binaphthyl unit with two freely rotating arylethynyl groups. The two aldehyde groups of (*R*)-**4** can condense with the amine group of serine to form an imine product which upon coordination with a Zn^{2+} ion could give a macrocyclic Zn^{2+} complex **5**. This should restrict the rotation of the arylethynyl units of (*R*)-**4** as well as that of its binaphthyl unit to give enhanced fluorescence. It was proposed that when the chirality of the serine unit in **5** matches that of the binaphthyl unit, more of this macrocycle could be generated to give greater fluorescence enhancement than the mismatched one. Other amino acids that cannot form a stable Zn^{2+} coordination like that in **5** will not be able to generate the same fluorescence enhancement.

Scheme 3. Design of a fluorescent probe (*R*)-**4** for selective recognition of serine.



The fluorescent response of (*R*)-**4** toward the TBA slats of the enantiomeric pairs of 18 common amino acids in the presence of $Zn(OAc)_2$ was investigated. As shown in Figure 3a, treatment of (*R*)-**1** with $Zn(OAc)_2$ (4.0 equiv) caused little change in its fluorescence at 402 nm (λ_1). When L-Ser-TBA (10.0 equiv) was added to the (*R*)-**4** (2.0×10^{-5} M) + $Zn(OAc)_2$ (4.0 equiv) solution, there was a large fluorescence enhancement at 471 nm (λ_2) (Figure 3a), however, D-Ser-TBA (Figure 3a) and 17 pairs of other amino acid enantiomers cannot produce significant fluorescence response under the same conditions (Figure 3b). In Figure 3a, ef ($[I_L\text{-}I_0]/[I_D\text{-}I_0]$) was found to be 15 for the fluorescence response of (*R*)-**4** toward serine at 471 nm.

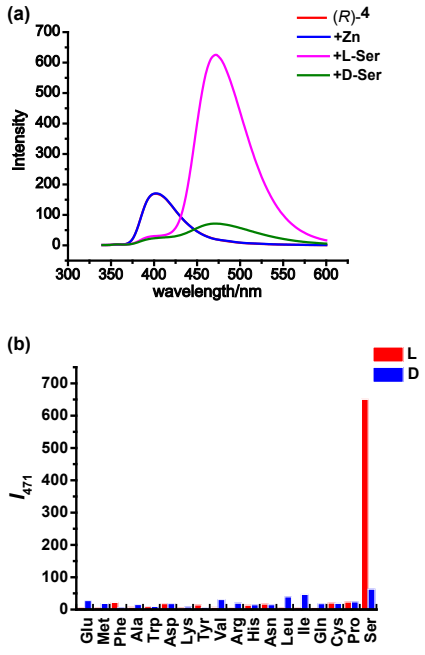


Figure 3. (a) Fluorescence spectra of (*R*)-**4**, (*R*)-**4** + $Zn(OAc)_2$, (*R*)-**4** + $Zn(OAc)_2$ + L-Ser-TBA, and (*R*)-**4** + $Zn(OAc)_2$ + D-Ser-TBA. (b) Fluorescence intensity of (*R*)-**4** with $Zn(OAc)_2$ and 18 D-/L-amino acid-TBAs. [(*R*)-**4**: 2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2. $Zn(OAc)_2$: 4.0 equiv. L-/D-amino acids: 10.0 equiv. Reaction time: 5 h at rt. λ_{exc} = 320 nm, slit 5/5 nm]. Adapted from *Tetrahedron Lett*. **2021**, *66*, 152803. Wang, Y.; Tian, J.; Zhao, F.; Chen, Y.; Huo, B.; Yu, S.; Yu, X.; Pu, L. Highly Chemoselective and Enantioselective Recognition of Serine by a Fluorescent Probe. Copyright (2021), with permission from Elsevier.

Figure 4 shows the effect of the concentration of serine on the fluorescent response of (*R*)-**4**. When the concentration of L-Ser-TBA increased from 0 – 10 equiv, the fluorescence intensity I_{471} of (*R*)-**4**+Zn^{2+} increased greatly. Then, the fluorescence decreased slowly when the concentration of L-Ser-TBA was over 10 equiv. Over the entire concentration range (0 – 20 equiv), D-Ser-TBA caused only small change in fluorescence at 471 nm. Thus, this fluorescent probe can be used to determine the enantiomeric composition of serine.

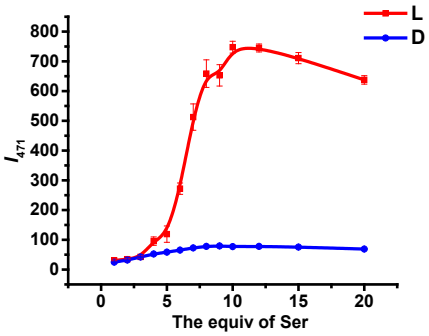


Figure 4. Plots of I_{471} for (*R*)-**4** (2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2) + Zn^{2+} (4 equiv) in the presence of varying concentrations of D- and L-Ser-TBA (0 - 40×10^{-5} M). (Reaction time: 5 h at rt. λ_{exc} = 320 nm, slit 5/5 nm). Reprinted from *Tetrahedron Lett*. **2021**, *66*, 152803. Wang, Y.; Tian, J.; Zhao, F.; Chen, Y.; Huo, B.; Yu, S.; Yu, X.; Pu, L. Highly Chemoselective and Enantioselective Recognition of Serine by a Fluorescent Probe. Copyright (2021), with permission from Elsevier.

IV. Selective Recognition of Histidine.

Compound (*S*)-**6** was synthesized from (*S*)-BINOL as shown in Scheme 4 for the recognition of histidine.[23] It was proposed that treatment of (*S*)-**6** with the chirality matched histidine in the presence of Zn^{2+} might generate the structurally rigid complex **7** to give enhanced fluorescence.

Scheme 4. Preparation of probe (*S*)-**6** and its proposed reaction with histidine and Zn(II) to form **7**.

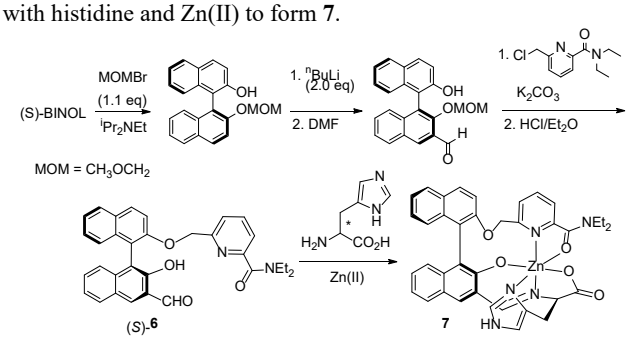
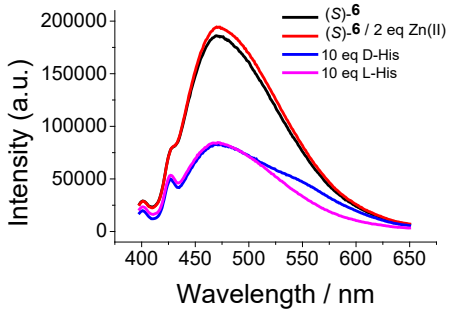
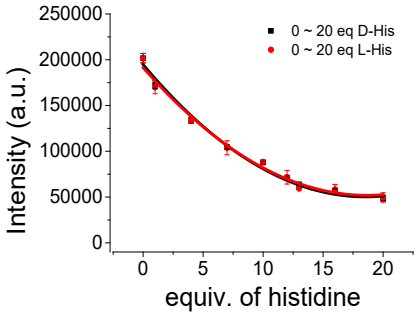


Figure 5a shows that upon excitation at 378 nm, the emission of compound (*S*)-**6** was observed at 470 nm. When it was treated with Zn^{2+}, little change in fluorescence was observed. Addition of D- or L-His decreased the fluorescence of (*S*)-**6**+Zn^{2+} (2.0 equiv) at λ_1 = 470 nm with no enantioselectivity (Figure 5a-d). However, when excited at 450 nm, D-His greatly enhanced the fluorescence (*S*)-**6**+Zn^{2+} (2.0 equiv) at λ_2 = 560 nm and L-His caused only very small change for fluorescence at this wavelength (Figure 6a-d). The ef $[(I_D–I_0)/(I_L–I_0)]$ was found to be 24.3 at 10 equiv D-His.

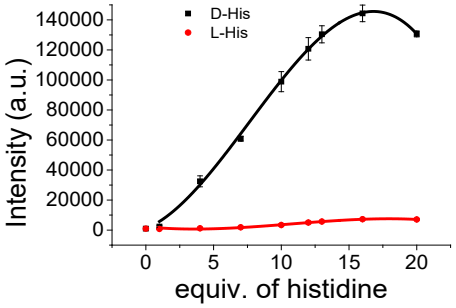


(a)



(b)

Figure 5. (a) Fluorescence spectra of (*S*)-**6** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) with (a) D- and L-His (10.0 equiv). (b) Fluorescence intensity at 470 nm versus the equivalence of D- and L-His [Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer (0.25 mM)]

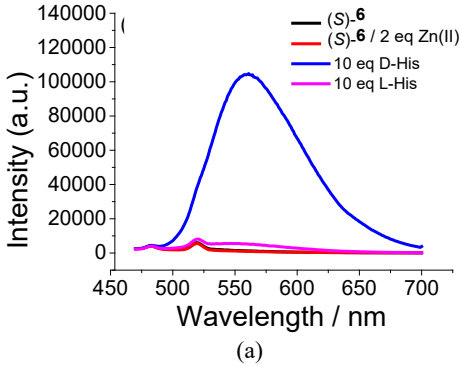


(b)

Figure 6. (a) Fluorescence spectra of (*S*)-**6** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) with D- and L-histidine (10.0 equiv). (b) Fluorescence intensity at 560 nm versus the equivalence of D- and L-histidine [(λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer (0.25 mM)]

Both enantiomeric probes (*S*)- and (*R*)-**6** were used to interact with histidine at various enantiomeric composition. The fluorescence responses of (*S*)- and (*R*)-**6** at λ_2 give the expected mirror image relationship.

The fluorescence responses of (*S*)-**6**+Zn^{2+} toward 17 pairs of common amino acid enantiomers (including D- and L-His) were investigated. It showed that these amino acids caused fluorescence quenching at λ_1 = 470 nm (λ_{exc} = 378 nm) with no enantioselectivity, and only D-histidine greatly enhanced the fluorescence at λ_2 = 560 nm (λ_{exc} = 450 nm) but not L-His and other amino acid enantiomeric pairs (Figure 7). That is, (*S*)-**6**+Zn^{2+} has exhibited highly chemoselective and enantioselective fluorescent recognition of histidine.



(a)

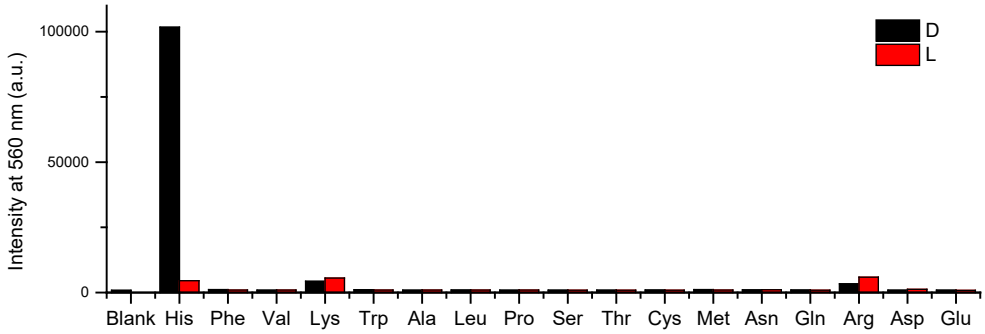


Figure 7. Fluorescence intensity at 560 nm of (*S*)-**6** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) with 17 amino acids (10 equiv) [λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer (0.25 mM)].

Since nonenantioselective fluorescent quenching at 470 nm while excited at 378 nm was observed for (*S*)-**6**+Zn^{2+} in the presence of histidine, these fluorescence responses are only dependent on the concentration of this amino acid (Figure 8a) which can be used to determine the substrate concentration. The fluorescent responses at 560 nm while excited at 450 nm are highly enantioselective and are dependent on both the enantiomeric composition of the amino acid and the total concentration of the two enantiomers (Figure 8b). Combining Figure 8a and 8b led to the 3D plot Figure 8c which can be used to determine the enantiomeric composition of histidine by

measuring the fluorescence responses at the two emission wavelengths.

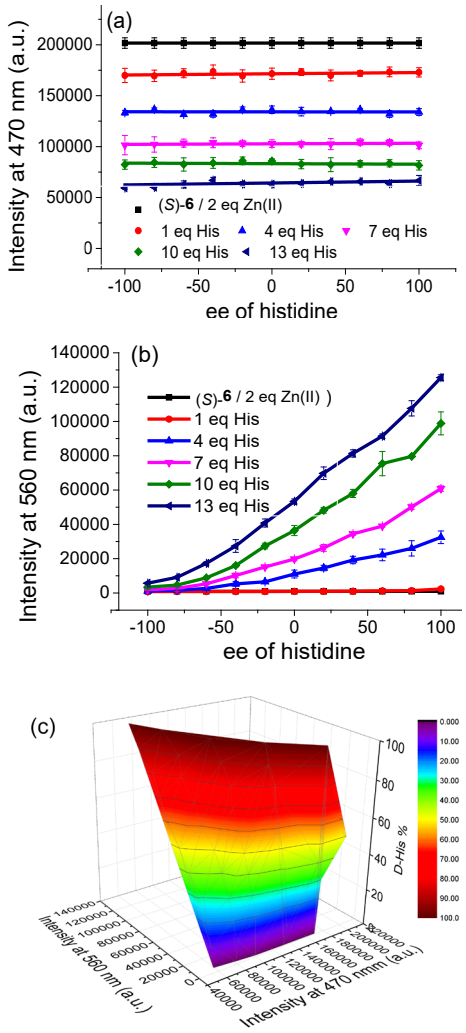
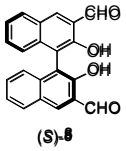


Figure 8. Fluorescence response of (*S*)-**6** (0.01 Mm) + $Zn(OAc)_2$ (2.0 equiv) toward histidine (0 - 13 equiv). (a) I_{470} (λ_{exc} = 378 nm) versus ee of histidine at varying concentration. (b) I_{560} (λ_{exc} = 450 nm) versus ee of histidine at varying concentration. (c) I_{470} versus I_{560} at varying [D-His]%. [Solvent: DMF/1% pH 6.35 phosphate buffer (0.25 mM). Slit: 5/5 nm]

It was proposed that the fluorescence quenching at λ_1 should be due to the consumption of (*S*)-**6** upon reaction with D- and L-His. The highly enantioselective fluorescent response at λ_2 can be attributed to the different stability of the Zn^{2+} complex **7** formed from the reaction of (*S*)-**6** with the histidine enantiomers. The more stable complex formed from the reaction of (*S*)-**6**+Zn^{2+} with D-His led to the observed large fluorescence enhancement at λ_2.

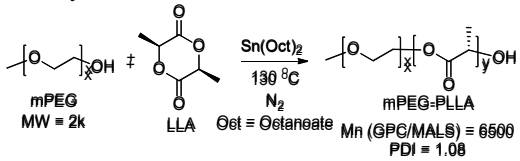
V. Selective Recognition of Lysine.

In order to use the water-insoluble probe (*S*)-**8** to detect amino acids in aqueous solution, we have encapsulated it into the micelles of an amphiphilic diblock copolymer.[24] The diblock copolymer polyethylene glycol–poly L-lactic acid (PEG–PLLA)



containing a hydrophilic PEG chain and a hydrophobic PLLA chain was prepared according to Scheme 5 which can form micelles in aqueous solution. The micelle solution of (*S*)-**8** (2.0 x 10^{-5} M) with PEG-PLLA was prepared by addition of a DMF solution of (*S*)-**8**+PEG-PLLA to water followed by dialysis in water. This micelle solution, named ML-S**8**, was used to interact with various L- and D-amino acids in the presence of $Zn(OAc)_2$ in carbonate buffer solution (CBS, 25 mM, pH = 10.1). As shown in Figure 9, D-Lys greatly enhanced the fluorescence of ML-S**8** at 528 nm but L-Lys did not. Most of the other amino acids cannot generate significant fluorescence response and only smaller fluorescence enhancements were observed with tryptophan and arginine. Thus, the micelle-based probe ML-S**8** has exhibited chemoselective as well as enantioselective fluorescent recognition of L-Lys.

Scheme 5. Synthesis of mPEG-PLLA.



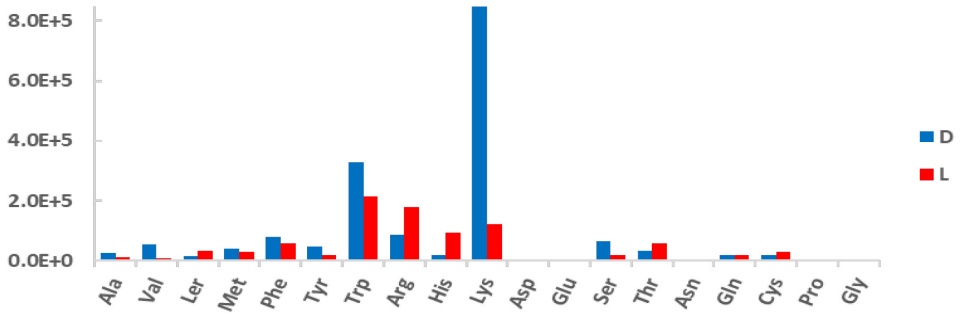
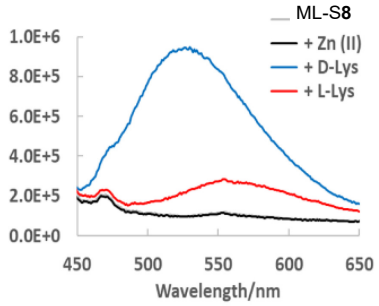


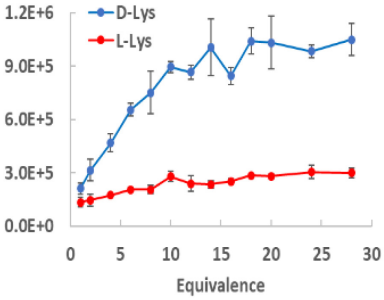
Figure 9. Fluorescence intensity of micelle M*L*-S**8** [2.0 x 10^{-5} M (*S*)-**8**] with $Zn(OAc)_2$ (2.0 equiv) and D-/L-amino acids (10 equiv) in carbonate buffer solutions (25 mM, pH = 10.1). Peak intensities at 528 nm were recorded at 5 °C after mixed at rt for 3 h and quenched with ice-water bath (λ_{exc}= 407 nm, slit 3/3 nm, int time 0.3 s. Y axis: fluorescence intensity minus that of M*L*-S**8**+Zn(II)/arbitrary unit.). Reprinted with permission from *Org. Lett.* **2019**, *21*, 4777. Copyright {2019} American Chemical Society.

The fluorescence spectra of ML-S**8**+$Zn(OAc)_2$ with D-/L-Lys (10.0 equiv) are given in Figure 10a in which D-Lys greatly enhanced the fluorescence at 528 nm but L-Lys enhanced much less. The enantioselective fluorescence enhancement ratio [ef =

(I_D-I_0)/(I_D-I_0). I_D, I_L and I_0: fluorescence intensity at 528 nm with and without D-/L-Lys respectively.] was 6.6. Figure 10b plots the concentration effect of D- and L-Lys on the fluorescence of the micelle probe at 528 nm. It shows that as the concentration of D-Lys increased from 0 – 10 equiv, there was large fluorescence enhancement. Then, the fluorescence enhancement became smaller. L-Lys did not give significant fluorescence enhancement in the entire concentration range.



(a)

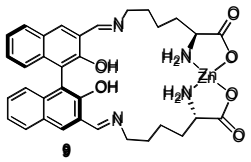


(b)

Figure 10. (a) Fluorescence spectra of M*L*-*S***8** (2.0 x 10^{-5} M of (*S*)-**8**) with $Zn(OAc)_2$ (2.0 equiv), Lys (10.0 equiv) in carbonate buffer solutions (25 mM) (using 20 mM Lys stock solution). Spectra were recorded at 5 °C after mixing at rt for 3 h. (b) Fluorescence intensity at λ = 528 nm versus equivalency of D- and L-Lys. (λ_{exc}= 407 nm, slit 3/3 nm). Adapted with permission from *Org. Lett.* **2019**, *21*, 4777. Copyright {2019} American Chemical Society.

The diblock copolymer mPEG-PDLA was made from D-lactic acid (DLA) following the same procedure as in Scheme 5. The micelles MD-R**8** were prepared by using mPEG-PDLA and (*R*)-**8**. Although the micromolecular structure of MD-R**8** is not the exact mirror image of ML-S**8** due to the random conformations of the block copolymers in the micelles and the distributions of the micelle sizes, their fluorescence responses were close to a mirror image relationship.

It was found that when (*S*)-**8** was treated with L- or D-Lys in DMSO-d_6/D_2O solution, the terminal amine group of the amino acid reacted selectively with the aldehyde groups of the probe to give a diimine which could coordinate with Zn^{2+} to form a macrocyclic product **9**. It was proposed that the imine compound made of D-Lys might form a more stable Zn^{2+} complex than that made of L-Lys, leading to the greatly enhanced fluorescence and the chemoselective and enantioselective fluorescent recognition of lysine under the micelle conditions in aqueous solution. In the absence of the diblock copolymer-based micelles, (*S*)-**8** cannot be used in aqueous solution for the fluorescent recognition of amino acids.

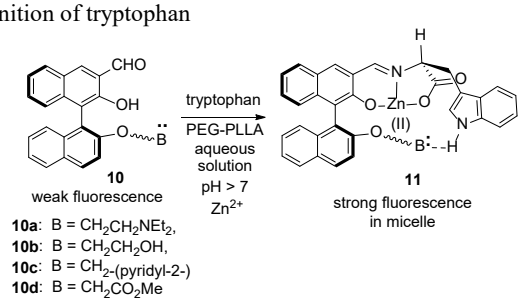


9

VI. Selective Recognition of Tryptophan.

The micelle-based strategy in Section V was also used to design probe for the recognition of tryptophan in aqueous solution. Scheme 6 shows the use of compounds **10** in combination with Zn^{2+} for the fluorescent recognition of tryptophan.[25] Compounds **10a-d** containing a Lewis basic site were prepared which upon reaction with tryptophan and Zn^{2+} inside the hydrophobic environment of the diblock copolymer mPEG-PDLA could generate complex **11** with an intramolecular hydrogen bonding interaction to give enhanced fluorescence in aqueous solution.

Scheme 6. Design of the fluorescent probes **10** for the selective recognition of tryptophan

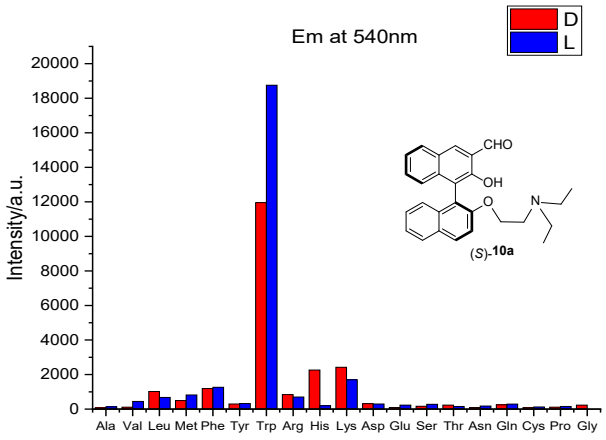


10
weak fluorescence

11
strong fluorescence
in micelle

10a: B = $CH_2CH_2NEt_2$,
10b: B = CH_2CH_2OH,
10c: B = CH_2-(pyridyl-2-)
10d: B = CH_2CO_2Me

Compounds (*S*)-**10a-d** were encapsulated into mPEG-PLLA which were used to interact with the two enantiomers of 19 common amino acids in the presence of $Zn(OAc)_2$ in carbonate buffer solution (25 mM, pH = 10.1). It was found that compounds (*S*)-**10a** and (*S*)-**10c** showed good chemoselective as well as enantioselective fluorescent response toward tryptophan. As shown in Figure 11, L-Trp enhanced the fluorescence of the micelles (*S*)-**10a,c**@PEG-PLLA at λ = 540 or 545 nm to a greater extent than D-Trp. Other amino acids caused little fluorescence response on the micelle probes.



(a)

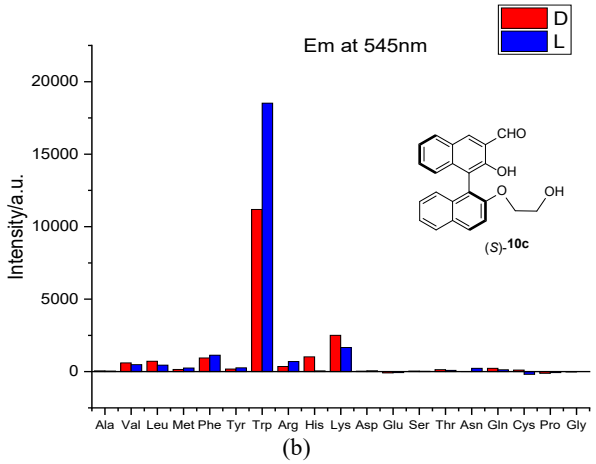


Figure 11. Fluorescence responses of the micelle probes (a) (*S*)-**10a**@PEG-PLLA and (b) (*S*)-**10c**@PEG-PLLA towards various amino acids [Conditions: 10 μM micelle probe, 2 equiv $Zn(OAc)_2$, and 10 equiv amino acids in carbonate buffer solution (25 mM). Peak intensities were used. Spectra were taken after 3 h of reaction at rt. λ_{ex} = 430 nm. slit = 3/3 nm.]

Figure 12 shows the effect of the concentration of L- and D-Trp on the fluorescence response of (*S*)-**10c**@PEG-PLLA. L-Trp greatly enhanced the fluorescence of the micelle probe from 0 – 20 equiv after which the fluorescence enhancement was saturated.

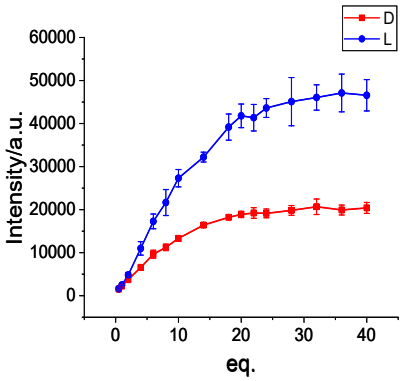


Figure 12. Fluorescence intensity of (*S*)-**10c**@PEG-PLLA (10 μM) and $Zn(OAc)_2$ (2 equiv) at λ = 545 nm versus the stoichiometry of tryptophan. (λ_{ex} = 430 nm, slit = 3/3 nm, integration time = 0.1 s).

The enantiomeric probe (*R*)-**10c** was encapsulated in PEG-PDLA for the fluorescent recognition of L- and D-Trp. The fluorescence responses of (*S*)-**10c**@PEG-PLLA and (*R*)-**10c**@PEG-PDLA toward L- and D-Trp at various enantiomeric compositions give mirror image like response at λ = 454 nm.

6. Summary and Outlook

In this article, several fluorescent probes that can conduct chemoselective as well as enantioselective recognition of certain amino acid enantiomers are discussed. All of these probes exhibit large fluorescence enhancement in the presence of a specific amino acid enantiomer but little or non-fluorescence enhancement with the opposite enantiomer or other amino acids. The probe (*S*)-**1** contains two aldehyde groups at the 2.2'-position of its 1.1'-binaphthyl structure. It can be used to detect the L-enantiomers of glutamic acid and aspartic acid. The chemoselectivity and enantioselectivity of this probe are attributed to a stereoselective double nucleophilic addition of the two carboxylate groups of these acidic amino acids to the two aldehyde groups of the probe. This reaction can generate a rigid macrocyclic structure to give greatly enhanced fluorescence. The probe (*R*)-**4** contains two arylethynyl units at the 3,3'-position of its 1,1'-binaphthyl core. It shows greatly enhanced fluorescence upon interaction with L-Ser and Zn^{2+}. Condensation of the two aldehyde groups of (*R*)-**4** with L-Lys to form a diimine product and the subsequent stereoselective chelate coordination with a Zn^{2+} center is proposed for the observed selective recognition of L-Lys. The monoaldehyde compound (*S*)-**6** shows greatly enhanced fluorescence upon reaction with D-His and Zn^{2+}. Stereoselective formation of a structurally rigid Zn^{2+} complex is proposed to explain the observed fluorescence enhancement. The water-insoluble dialdehyde probe (*S*)-**8** can be used to detect amino acids in aqueous solution by using an amphiphilic diblock copolymer to encapsulate it into micelles. In micelles, this probe shows chemoselective as well as enantioselective fluorescence enhancement with D-Lys in the presence of Zn^{2+}. In the presence of the same micelles, the monoaldehyde probes (*S*)-**10a,c** are capable of chemoselective and enantioselective recognition of L-Trp in water with significant fluorescence enhancement.

All of these probes contain a 1,1'-binaphthyl core which provides the source of chirality as well as highly tunable fluorescence property. In these probes, one or two electrophilic aldehyde groups are incorporated which can selectively react with the functional groups of a specific amino acid enantiomer. In most of the cases, when the structure and chirality of a substrate match those of a probe, their reaction can generate a macrocyclic product with restricted rotation of the binaphthyl unit and other structural components. Formation of such a structurally rigid product should have contributed to the observed chemoselective and enantioselective fluorescence enhancement. No or very small fluorescence response was observed for the interaction of the structurally mismatched probes and substrates. The strategies described in this work can be expanded to design fluorescent probes for selective recognition of other amino acids of interest. Further advancement of this research includes the development of amino acid probes with chemoselective as well as enantioselective fluorescent response at near IR excitation and emission wavelengths in aqueous solutions. Such probes will be useful in fluorescent imaging of specific amino acid enantiomers in biological systems including humans, and contributing to disease diagnosis and treatment.

Currently, in the chiral analysis of amino acids, the most popular methods involve the use of chromatographic and electrophoretic techniques equipped with chiral stationary or mobile phases and coupled with mass spectrometry. These methods have allowed precise detection and quantification of amino acid enantiomers in samples of diverse sources.[8-10] Using molecular probes to conduct fluorescent chiral analysis of amino acids, however, has other advantages such as more readily available instrumentation, capability for high throughput parallel analyses, potential for *in vivo/in vitro* imaging, etc. In recent years, the use of molecular probes in the circular dichroism (CD)-based detection of amino acid enantiomers have also been developed.[11] This technique involves the conversion of amino acid enantiomers to derivatives that have enhanced CD signals in the UV-vis absorption range. Using this method will be less interfered by the presence of CD-silent achiral components in analytical samples. Since fluorescence signals can often appear at much longer wavelengths than absorptions, the fluorescence-based technique would have broader application in biological imaging such as in monitoring amino acids in humans and other animals. The high sensitivity of fluorescence is also another advantage of the fluorescence-based chiral analysis.

Although the studies described in this article are limited to the analysis of individual amino acid samples at this stage, they have provided important information on the molecular interaction of the probes with various amino acid enantiomers which lays the foundation for further development of this technique. With

continuous research effort in this area, practically useful methods for chemoselective as well as enantioselective fluorescent detection of specific amino acid enantiomers in various applications would be expected.

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BIOGRAPHICAL INFORMATION

Lin Pu was born in 1965 in Xuyong, Sichuan Province, China. He received his B.S. degree in chemistry from Sichuan University in 1984. He then obtained the Doering Fellowship (CGP) to undertake graduate study in the department of chemistry at University of California San Diego in 1985. Under the supervision of Professor Joseph M. O'Connor, he obtained his Ph.D. degree in 1990. As a postdoctoral fellow, he worked with Professor Henry Taube at Stanford University from January 1991 to November 1992, and with Professor Robert Grubbs at California Institute of Technology from November 1992 to August 1994. In the fall of 1994, he was appointed as an assistant professor at North Dakota State University. He then moved to University of Virginia as an associate professor in the department of chemistry in 1997 and as a professor in 2003. The research projects in his laboratory focus on the design and synthesis of novel chiral molecules and macromolecules for applications in areas such as enantioselective fluorescent sensors, asymmetric catalysis, and materials.

Dedication

In memorial of Professor Robert Grubbs (1942-2021), a great chemist and a wonderful human being

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