A Near IR Fluorescent Probe for Enantioselective Recognition of Amino Acids in Aqueous Solution

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Abstract

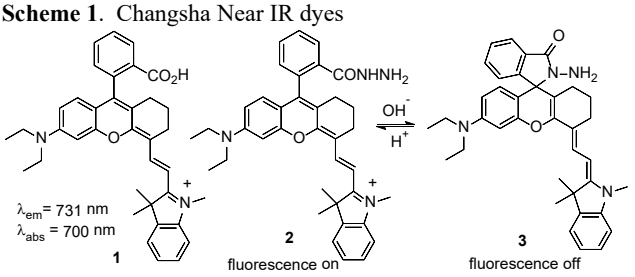
A novel BINOL-based near IR fluorescent probe by incorporating a rhodamine-like dye has been designed and synthesized. In the presence of Zn(II), this compound was found to exhibit highly enantioselective fluorescent enhancement at λ_{emi} > 730 nm and λ_{exc} = 690 nm when treated with 14 common amino acids in aqueous solution. The enantioselective fluorescent enhancement ratio up to 163 was observed. The mechanism of the fluorescent response of this probe was investigated by various spectroscopic methods.

Introduction

In recent years, there has been growing research attention on the enantioselective fluorescent recognition of chiral amino acids[1,2] because of the importance of these molecules in biological systems[3] as well as in asymmetric synthesis.[4] L-Amino acids are used by nature to construct proteins and D-amino acids are also found to play various biological functions such as neurotransmitters.[3,5] Up to date, structurally diverse chiral molecular probes have been reported and various degrees of enantioselectivity and sensitivity of chiral amino acids have been achieved.[1] Most of these probes show emissions and absorptions at wavelengths less than 600 nm. It is highly desirable to develop fluorescent probes that can conduct the substrate detection in red to near infrared (IR) region (650 – 900 nm) since it will provide less damage to cells, better tissue penetration, and minimum interference from the background autofluorescence of biomolecules in living systems.[6] However, no molecular probe was obtained to carry out enantioselective fluorescent recognition with absorption and emission in the near IR region.[7]

In 2012, Lin and coworkers reported a class of rhodamine-like near IR dyes (Changsha dyes) such as **1** and **2** with absorptions and emissions at wavelengths greater than 700 nm (Scheme 1).[8] These dyes exhibited high fluorescence intensity and good photo and chemical stability and were shown to be useful for fluorescent imaging of the endogenously produced HClO in living animals. These dyes can undergo interconversion from the fluorescence "off" state of the spirolactone or spirolactame form to the ring-opened fluorescence "on" state under various conditions depending on the specific functional groups incorporated. For example, under neutral or basic conditions, the strongly fluorescent probe **2** can be converted to the nonfluorescent spirolactame **3** whose fluorescence can then be turned on upon treatment with acid to give the ring-opened form.

Scheme 1. Changsha Near IR dyes



In our laboratory, we have discovered that 1,1'-bi-2-naphthol (BINOL)-based chiral aldehydes can be used to conduct the enantioselective fluorescent recognition of amino acids in the presence of Zn^{2+}.[9,10] We thus propose to combine the BINOL-based aldehydes with the Changsha near IR dyes to build molecular probes for the enantioselective fluorescent recognition of amino acids in the near IR region. Herein, we report our discovery of a novel near IR probe that shows highly enantioselective fluorescent enhancement with amino acids in aqueous solution. In addition, this probe also allows the visual discrimination of the enantiomers of amino acids.

Results and Discussion

1. Design and Synthesis of a Near IR Fluorescent Probe

We have designed the BINOL-based compound (*R*)-**4** for the recognition of amino acids in the near IR region. In (*R*)-**4**, the ionic sulfonate groups are introduced to the BINOL unit to make it soluble in aqueous solution. The aldehyde group will allow it to react with amino acids to form the covalent imine bonds. Since the near IR dye **3** exists in the spirolactam form under neutral conditions, we expect that it should also be in such a nonfluorescent spirolactam form in (*R*)-**4**. It is proposed that when (*R*)-**4** is treated with an amino acid and Zn^{2+}, formation of the imine product from the condensation and the subsequent coordination with the Lewis acidic Zn^{2+} may induce the

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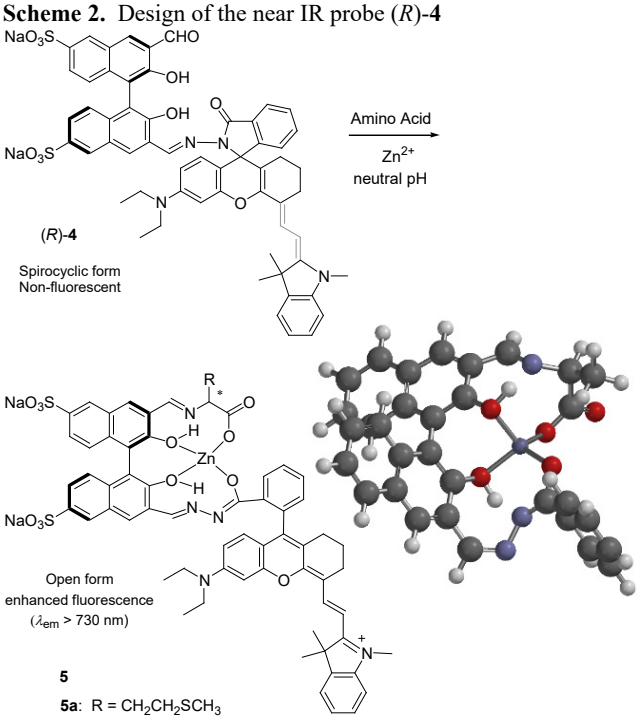
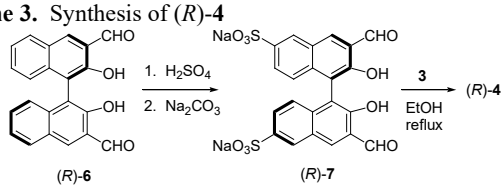
ring-opening of the lactam to generate a Zn(II) complex like **5** to turn on the fluorescence. A molecular modeling structure of a simplified analog of **5** obtained by using the semiemperical method (Spartan, PM3), is given in Scheme 2 which shows a possible tetrahedral Zn(II) coordination. Different reactivity, stability and structural rigidity might be observed for the reactions of the chirality matched and mismatched BINOL and amino acid units, which can produce the desired enantioselective fluorescent response.

Scheme 2. Design of the near IR probe (*R*)-**4**



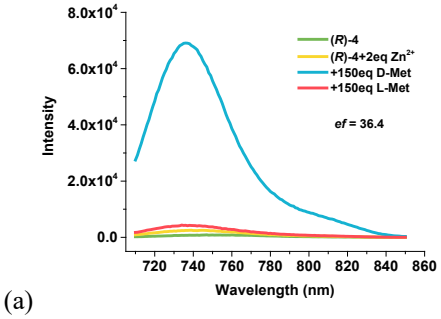
In order to make a water soluble fluorescent probe, we first carried out the 6,6'-disulfonation of the BINOL dialdehyde (*R*)-**6** to give (*R*)-**7** (Scheme 3).[11] Then, condensation of (*R*)-**7** with the near IR dye **3** directly generated the desired Schiff base product (*R*)-**4**. The ^{1}H NMR spectrum of (*R*)-**4** in DMSO-d_6 gives six peaks of similar intensities at $\delta > 10$ for the aldehyde and hydroxyl proton signals. When in a DMSO-d_6-D_2O buffer solution, only two aldehyde singlets at δ 10.16 and 10.12 were observed. This is consistent with the retention of the spirolatam structure of **3** in (*R*)-**4** since the chiral carbon center of the racemic compound **3** is expected to generate a 1:1 mixture of two diastereomeric products of (*R*)-**4**. The other four peaks at $\delta > 10$ can be assigned to the two intramolecularly bonded hydroxyl protons of the two diastereomers. This spirolactam product shows very weak fluorescence as expected.

Scheme 3. Synthesis of (*R*)-**4**

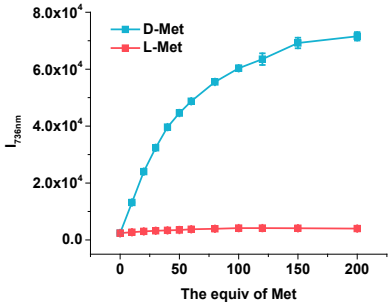


2. Fluorescent Response of (*R*)-4 toward Methionine in the Presence of Zn(II)

We studied the fluorescence response of (*R*)-**4** toward the enantiomers of an amino acid methionine in the presence of Zn^{2+}. When (*R*)-**4** (10 μM in 50 mM HEPES buffer/1% DMSO, pH = 7.4) was combined with $Zn(OAc)_2$ (2 equiv), only a slightly increased emission was observed at λ = 736 nm (Figure 1a). When the (*R*)-**4**+Zn^{2+} solution was treated with D-Met, there was a large fluorescence enhancement at λ = 736 nm while excited at 690 nm. When L-Met was used to interact with the (*R*)-**4**+Zn^{2+} solution, the fluorescent enhancement at λ = 736 nm was much smaller. That is, the fluorescent probe (*R*)-**4**+Zn^{2+} exhibits highly enantioselective response. The fluorescence intensity at λ = 736 nm is plotted against the concentration of D- and L-Met in Figure 1b. It shows that as the concentration of D-Met increased, there was large fluorescence enhancement, but only small fluorescence enhancement was observed as the concentration of L-Met increased. In the presence of 150 equiv methionine, the enantioselective fluorescence enhancement ratio *ef* [*ef* = $(I_D-I_0)/(I_L-I_0)$, where I_0 is the fluorescence intensity of the probe in the absence of the amino acid] is 36.4. The fluorescence measurement was conducted after the reaction of (*R*)-**4**+Zn^{2+} with the amino acid at 37 °C for 4 h. Varying the counter anions of the Zn^{2+} salt did not significantly influence the enantioselective fluorescent enhancement (See Figure S24 in SI).



(a)



(b)

Figure 1. Fluorescence spectra of (*R*)-**4**+Zn^{2+} (2 equiv.) (10 μM) toward D- and L-Met (150.0 equiv) in 50 mM HEPES (pH = 7.4)/ 1% DMSO (a). Fluorescence intensity at 736 nm versus the equiv of methionine (b). Spectra were recorded at rt after mixing at 37 °C for 4 h. (λ_{exc} = 690 nm, slits = 5/5 nm)

Compound (*S*)-**4** was prepared as the enantiomer of (*R*)-**4** from (*S*)-BINOL. We found its fluorescence responses toward D- and L-Met under the same conditions were mirror-images of those observed for (*R*)- (Figure S3). This confirms the inherent chiral recognition process.

We studied the fluorescent response of both (*R*)- and (*S*)-**4** toward methionine of various enantiopurity . The fluorescence intensity I_{736} versus the enantiomeric composition of the amino acid is plotted in Figure 2 (also see Figure S4 in SI). A mirror image relationship is shown between the fluorescence responses of(*R*)- and (*S*)-**4**. The relationship between the fluorescence intensity and D-Met% was linear, which could be utilized to determine the enantiopurity of the amino acid.

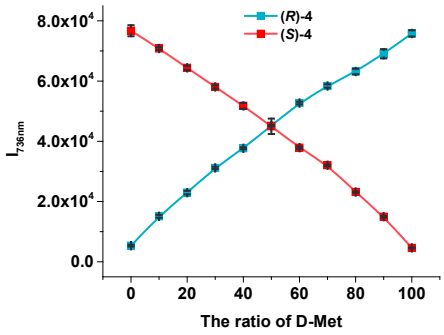


Figure 2. Fluorescence intensity of (*R*)-**4** and (*S*)-**4** (10 μM) + Zn^{2+} (2 equiv) at 736 nm versus the enantiopurity of methionine (150 equiv) in 50 mM HEPES (pH = 7.4)/ 1% DMSO. Spectra were recorded at rt after mixing at 37 ℃ for 4 h. (λ_{exc} = 690 nm, slits = 5/5 nm)

3. Fluorescent Responses toward Additional Amino Acids.

We further investigated the fluorescent responses of (*R*)-**4**+Zn^{2+} toward additional 18 common amino acids in aqueous solution. In HEPES buffer solution (pH = 7.4), the (*R*)-**4**+Zn^{2+} (2 equiv) probe has exhibited enantioselective fluorescence enhancements with the following 14 amino acids: alanine, arginine, asparagine, glutamine, histidine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine. It was generally observed that the D-enantiomers of these amino acids greatly enhance the fluorescence of the probe at 736 nm while the L-enantiomers do not significantly change the fluorescence. Figure 3 gives several examples of the observed highly enantioselective fluorescent responses with the following high *ef* values: 77.7 for arginine, 55.5 for asparagine, 32.8 for glutamine, 47.9 for histidine, 56.5 for leucine, 50 for serine, 162.6 for threonine and 46 for valine. (*R*)-**4** also showed good enantioselectivity toward alanine (27.2), lysine (27.2), tryptophan (19.9), phenylalanine (15.6) and tyrosine (11.2), but little fluorescence response toward aspartic acid, cysteine, glutamic acid and proline (see Figure S7-S19 in SI).

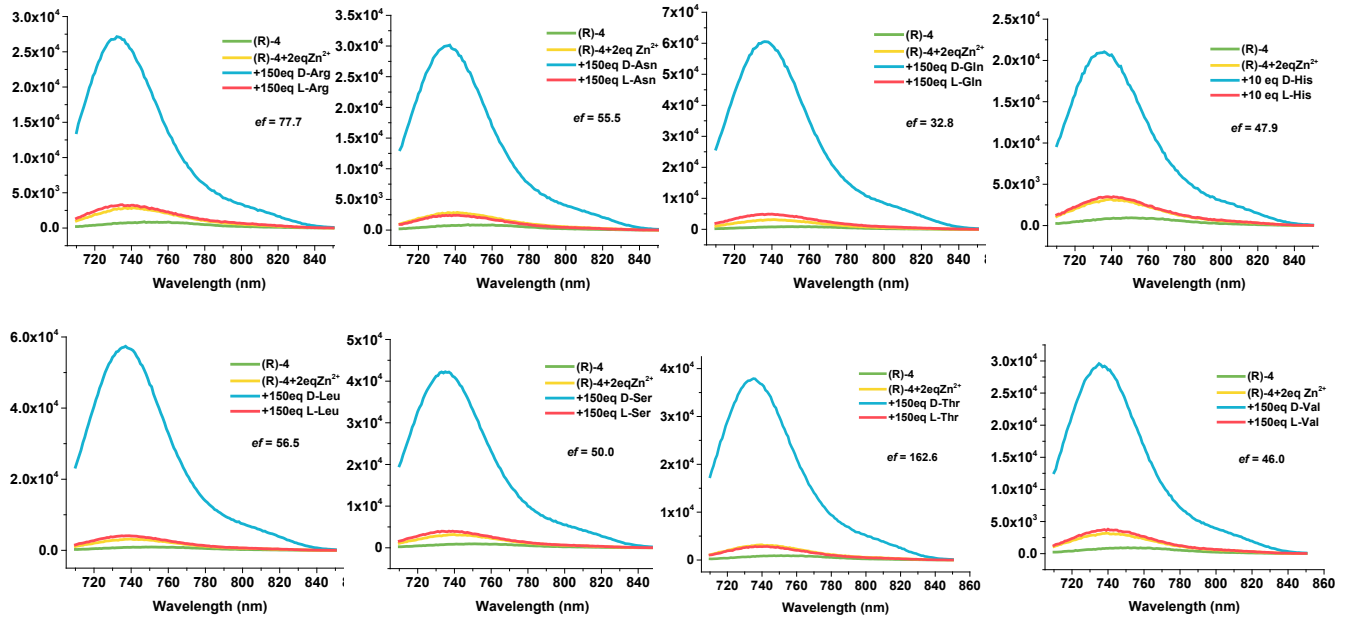


Figure 3. Selected fluorescence spectra of (*R*)-**4** (10 μM) + Zn^{2+} (2 equiv) with D-/ L- amino acids in 50 mM HEPES (pH = 7.4)/1% DMSO. Spectra were recorded at rt after mixing at 37 ℃ for 4 h. (λ_{exc} = 690 nm, slits = 5/5 nm)

4. Study of the Interaction of (*R*)-4+Zn^{2+} with Methionine by Additional Spectroscopic Methods

In order to gain further understanding on the fluorescent responses of (*R*)-**4** toward the amino acids, we conducted additional spectroscopic studies on the reaction of (*R*)-**4** with methionine in the presence of Zn^{2+}.

We found that at low concentration of (*R*)-**4** (1.0 x 10^{-5} M), when it was treated with Zn^{2+} and L-Met or D-Met, the changes in the UV-vis absorption spectra were very small. This indicates that there was only a small degree of reaction between these sensing components which however has led to the observed large differences in the fluorescence responses. When the concentration of (*R*)-**4** was greatly increased to 1 mM, its absorption at over 700 nm showed much bigger differences upon treatment with Zn^{2+} and D- or L-Met. As shown in Figure 4a, the (*R*)-**4** solution (1 mM) in DMSO:HEPES buffer (3:1) gave a long wavelength absorption at λ = 723 nm with a yellow-orange color. When it was treated with Zn^{2+} (1 equiv, $ZnBr_2$ was

used because of its better solubility at the high concentration), the absorption at λ = 723 nm increased significantly with a shoulder at λ = 660 nm. The color of the solution also turned brown. When D-Met was added to the above (*R*)-**4**+Zn^{2+} solution, the absorption intensity further increased with λ_{max} = 718. The color of the solution turned green. When the (*R*)-**4**+Zn^{2+} solution was interacted with L-Met, however, the long wavelength absorption decreased with the brown color of the solution turning toward yellow. This distinct color change allows the two enantiomers of the amino acids to be visually discriminated. The significant changes of the solution color of (*R*)-**4**+Zn^{2+} with methionine could be used to determine the enantiomeric purity of the chiral amino acids. While the molar ratio of (*R*)-**4** to the mixture of D-Met and L-Met was maintained at 1:1, it was found that the absorption intensity of (*R*)-**4** at λ = 718 nm and 660 nm gradually increased with *ee*% of D-Met in the range of -100% to 100% (Figure 4b). A progressive changing of the solution color from yellow-orange to green was clearly observed with the changing *ee*. Thus, the probe can be used as a colorimetric probe for visual recognition of methionine enantiomers (See Figure S23 in SI for additional photos with other amino acids).

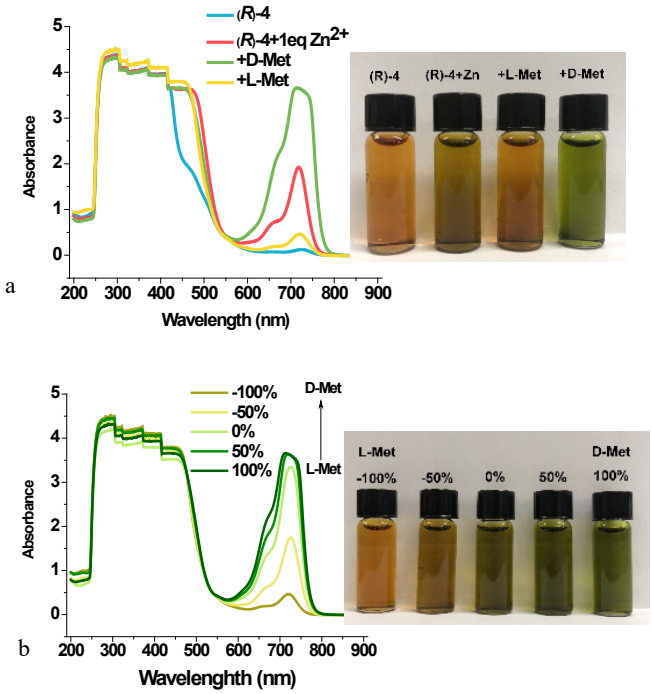


Figure 4. UV-vis spectra and photographs (under room light) of (*R*)-**4** (1 mM) + $ZnBr_2$ (1 equiv) with 1 equiv of D- or L-Met (a) and (*R*)-**4** (1 mM) + $ZnBr_2$ (1 equiv) with 1 equiv of methionine at various *ee*'s [*ee* = (D-L)/(D+L)] in DMSO:HEPES buffer (3:1) (b).

A ^{1}H NMR spectroscopic investigation was conducted on the reaction of (*R*)-**4** with methionine and Zn^{2+}. As shown in Figure 5a, (*R*)-**4** gave two aldehyde singlets as a 1:1 mixture of two diastereomers. When 1 equiv $ZnBr_2$ was added, the two singlets were changed to one new singlet at δ 10.20. This indicates that Zn^{2+} should have induced the spirolactam ring-opening to remove the chiral spiro carbon center. Addition of L- or D-Met led to the disappearance of the aldehyde signal at δ 10.20 with the appearance of a few new singlets at δ 8 – 9 for the aromatic protons in the BINOL unit and the newly formed imine protons from the reaction of the amine group with the aldehyde group. The very different chemical shifts of the ^{1}H NMR signals formed from the reactions of L – and D-Met demonstrate very different structures of the products which should contribute to the highly enantioselective fluorescent responses. The ^{1}H NMR study also suggests that even though Zn^{2+} might have induced the ring-opening of the spirolactam, large fluorescence enhancement did not occur until it reacted with the chirality-matched amino acid. It is proposed that the structural rigidity of the chirality-matched adduct, as shown in the molecular modeling structure in Scheme 1, should be important for the observed enhanced fluorescence.

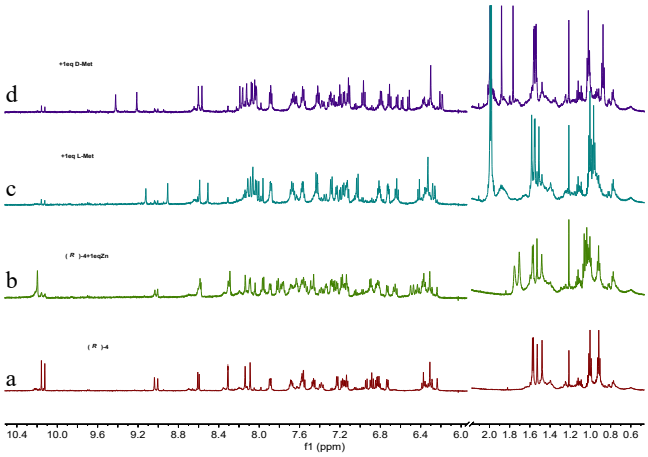


Figure 5. ^{1}H NMR spectra for the reaction of (*R*)-**4** (4 mM) with Zn^{2+} (1 equiv) and D-/ L-Met (1 equiv) in DMSO-d_6: HEPES buffer (3:1) (50 mM at pD = 7.4, prepared with D_2O). Other signals at δ 2.1-6.0 are removed for clarity.

We further conducted a time-of-flight mass spectroscopic (ES-) study on the reaction mixture of (*R*)-**4** (20 μM) + $Zn(OAc)_2$ (2 equiv) with D- Met and L- Met (150 equiv) in 50 mM HEPES/1% DMSO (pH = 7.4) (reaction time: 1 d at room temperature) (Figure S21 and S22 in SI). The reaction of (*R*)-**4**+Zn^{2+} with D-Met produced a peak at m/z = 1346.4 for the proposed complex **5a** (calcd. for **5a**+H_2O+2OH: 1346.2). Signals at m/z = 1277.4 (calcd. for **5a**-OH: 1277.2) and 1537.5 (calcd. for **5a**+$Zn(OAc)_2$+AcO+2H: 1537.2) were also observed. However, the mass spectrum for the reaction of (*R*)-**4**+Zn^{2+} with L-Met did not give evidence for the formation of a complex like **5a**. Thus, the ^{1}H NMR and mass spectroscopic data support the proposed reaction of (*R*)-**4** with the chirality-matched amino acids to give the corresponding imine product with the ring-opened near IR dye unit to give the greatly enhanced fluorescence.

Conclusion

In conclusion, using the BINOL-based aldehyde and a Changsha near IR dye, we have constructed the first molecular probe that can carry out the enantioselective

fluorescent recognition of amino acids in the near IR region. It was found that in the presence of Zn^{2+}, the probe (*R*)-**4** shows highly enantioselective fluorescent enhancement when treated with the enantiomers of the structurally diverse amino acids with *ef*'s up to 163 in aqueous solution. In addition, this probe can also allow visual discrimination of the two enantiomers of an amino acid. Spectroscopic analysis indicates that the ring-opening of the spirolactam structure of the probe as well as the formation of the chirality-matched complex with the amino acid enantiomer should be responsible for the observed enantioselective fluorescent enhancement. The excellent near IR fluorescent response of this probe makes it potentially useful for application in the enantioselective fluorescent imaging of amino acids in biological systems. We are currently conducting the investigation of this probe along this direction.

Experimental Section

General data. A Varian-600 MHz spectrometer and a Bruker-800 MHz spectrometer were used for NMR analyses. A Horiba FluoroMax-4 spectrofluorometer was used to acquire the steady-state fluorescence spectra. The UV-Vis spectra were measured by Shimadzu UV-2600 UV-Vis spectrometer. A Jasco P-2000 digital polarimeter was used for optical rotation measurements. The University of Illinois at Urbana-Champaign (UIUC) Mass Spectrometry Facility conducted mass spectroscopic analyses. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All solvents used in the fluorescence measurement were HPLC or spectroscopic grades. Water used for all the experiments was deionized.

Synthesis and Characterization of (*R*)-4. Compound **3** (119 mg, 0.208 mmol) and (*R*)-**7** (170 mg, 0.312 mmol) were dissolved in anhydrous EtOH (25.0 mL). The reaction mixture was heated at reflux in an oil bath for 6 h. The solvent was then evaporated, and the crude product was purified by flash column chromatography on silica gel (eluted with CH_2Cl_2/MeOH = 5:1) to give (*R*)-**4** as a green solid in 34.9% yield (80 mg). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 0.5H), 10.97 (s, 0.5H), 10.29 (s, 0.5H), 10.27 (s, 0.5H), 10.13 (s, 0.5H), 10.10 (s, 0.5H), 9.07 (s, 0.5H), 9.05 (s, 0.5H), 8.64 (s, 1H), 8.29 (s, 1H), 8.14 (d, *J* = 11.0 Hz, 2H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.70 – 7.66 (m, 1H), 7.57 (m, 2H), 7.47 (m, 2H), 7.30 (d, *J* = 5.8 Hz, 1H), 7.28–7.24 (m, 1H), 7.15 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 1H), 6.84–6.81 (m, 2H), 6.79 (m, 1H), 6.41–6.33 (m, 3H), 5.40 (d, *J* = 12.7 Hz, 0.5H), 5.36 (d, *J* = 12.7 Hz, 0.5H), 3.28 (m, 4H), 3.14 (d, *J* = 8.8 Hz, 3H), 2.48–2.39 (m, 2H), 1.79–1.72 (m, 2H), 1.64 (d, *J* = 12.3 Hz, 6H), 1.47 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.0 Hz, 3H). ^{13}C{^{1}H} NMR (201 MHz, DMSO-d_6) δ 196.2, 163.8, 157.7, 153.7, 153.0, 151.9, 150.8, 149.6, 148.6, 147.7, 145.0, 143.9, 143.5, 138.3, 136.8, 136.5, 134.1, 133.4, 129.1, 128.3, 128.1, 127.7, 127.5, 126.5, 126.2, 125.1, 124.1, 123.6, 123.2, 122.3, 121.7, 120.7, 120.0, 119.3, 118.9, 116.8, 115.1, 108.8, 108.4, 106.2, 104.0, 102.7, 97.2, 92.0, 67.3, 45.0, 43.6, 35.8, 28.9, 28.1, 28.0, 25.9, 24.6, 24.1, 22.6, 21.7, 12.4. HRMS (ESI) m/z: $[M-2Na+3H]^+$ Calcd for $C_{59}H_{53}N_4O_{11}S_2$ 1057.3152; Found 1057.3131. $[\alpha]_D^{22}$ = 18.5 (*c* = 0.1, DMSO).

Synthesis and Characterization of (*S*)-4. (*S*)-**4** was prepared in the same way as (*R*)-**4** by starting with (*S*)-**7**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 0.5H), 10.97 (s, 0.5H), 10.30 (s, 0.5H), 10.28 (s, 0.5H), 10.13 (s, 0.5H), 10.10 (s, 0.5H), 9.06 (s, 0.5H), 9.04 (s, 0.5H), 8.64 (s, 1H), 8.29 (s, 1H), 8.16-8.12 (m, 2H), 7.91 (d, *J* = 7.1 Hz, 1H), 7.71-7.66 (m, 1H), 7.58 (m, 2H), 7.50-7.45 (m, 2H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.27-7.23 (m, 1H), 7.15 (t, *J* = 7.0 Hz, 1H), 6.94 (m, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 6.80 (m, 3H), 6.40-6.31 (m, 3H), 5.39 (d, *J* = 12.7 Hz, 0.5H), 5.35 (d, *J* = 12.7 Hz, 0.5H), 3.29-3.25 (m, 4H), 3.14 (d, *J* = 9.4 Hz, 3H), 2.48-2.40 (m, 2H), 1.76 (m, 2H), 1.63 (m, 6H), 1.49-1.43 (m, 2H), 1.09-1.06 (m, 3H), 1.03-1.00 (m, 3H). ^{13}C{^{1}H} NMR (201 MHz, DMSO-d_6) δ 196.2, 163.8, 157.7, 153.7, 153.0, 151.9, 149.6, 148.6, 147.7, 145.0, 143.8, 143.4, 138.3, 136.6, 135.2, 134.3, 133.3, 129.1, 128.3, 128.1, 127.7, 127.5, 126.3, 125.1, 124.1, 123.5, 123.3, 122.3, 121.7, 120.7, 120.0, 119.3, 118.9, 116.8, 115.2, 108.8, 108.4, 106.2, 104.0, 102.7, 97.2, 92.0, 67.3, 45.0, 43.6, 35.8, 28.9, 28.1, 25.9, 24.6, 24.1, 22.6, 21.7, 12.4. HRMS (ESI) m/z: $[M-2Na+3H]^+$ Calcd for $C_{59}H_{53}N_4O_{11}S_2$: 1057.3152; Found 1057.3137. $[\alpha]_D^{22}$ = -16.2 (*c* = 0.1, DMSO).

Sample preparation for fluorescence measurement. The following stock solutions were freshly prepared for each measurement: an amino acid (20.0 mM) in HEPES (50 mM, pH = 7.4), (*R*)-**4** (1.0 mM) in DMSO, and $Zn(OAc)_2$ (2.0 mM) in deionized water. The stock solution of (*R*)-**4** was diluted to the corresponding concentration (10 μM) with HEPES, to which the $Zn(OAc)_2$ solution and the proper amino acid solution were added. The resulting solutions were allowed to stand at 37 ℃ for 4 h and the fluorescence spectra were recorded at room temperature.

Preparation of samples for visual recognition. 1 mL of (*R*)-**4** (2 mM, DMSO), 0.5 mL of $ZnBr_2$ (4 mM, DMSO) and 0.5 mL of Met (4 mM, HEPES buffer) were sequentially added into one 2 mL vial. The above mixture was incubated at room temperature (about 25 °C) for 2 d. The photos of the solutions and their UV-vis spectra were then recorded.

Supporting Information: ^{1}H/^{13}C{^{1}H} NMR and HRMS spectra of **4**, fluorescence spectra of (*R*)-**4** with amino acids, NMR and mass spectroscopic studies, photographs of (*R*)-**4** and $ZnBr_2$ with D- or L-amino acids, and fluorescence spectra of (*R*)-**4** with D-Met or L-Met in the presence of Zn^{2+} salts of various counter anions.

Keywords: near infrared, fluorescence probe, amino acid, enantioselective, chiral recognition

Acknowledgement: We thank the partial support of this project by the US National Science Foundation (CHE-

1565627 and 1855443). F.Z. is grateful for the support by China Scholarship Council (CSC, 201806240193).

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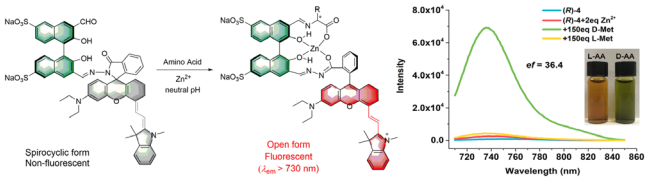
TOC Graph


Amino Acid
Zn^{2+}
neutral pH
Spirocyclic form
Non-fluorescent
Open form
Fluorescent
(λ_{em} > 730 nm)
(R)-4
(R)-4+2eq Zn^{2+}
+150eq D-Met
+150eq L-Met
ef = 36.4
L-AA
D-AA
Intensity
Wavelength (nm)