Semiquantitative Visual Chiral Assay by a Pseudoenantiomeric Fluorescent Sensor Pair

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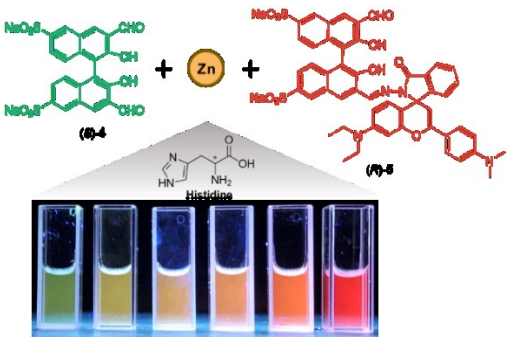
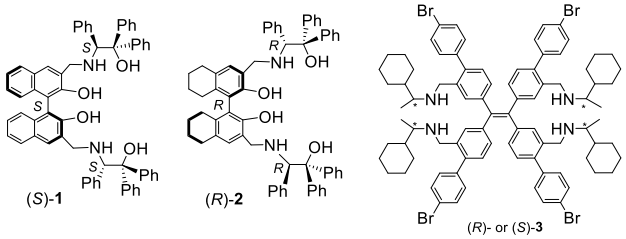
Abstract. A new red-light emitting fluorescent probe (*R*)-**5** was synthesized. In the presence of Zn^{2+}, this compound was found to exhibit good enantioselective fluorescence enhancement at λ = 655 nm when treated with a variety of amino acids in aqueous solution. This probe in combination with a green-light emitting probe (*S*)-**4** that has enantioselective fluorescence enhancement at λ = 505 nm has formed a pseudoenantiomeric sensor pair because of their opposite enantioselectivity. This sensor pair can simultaneously detect both enantiomers of a chiral amino acid at two very different wavelengths (Δ = 150 nm). It was used to visually and semiquantitatively determine the enantiomeric compositions of amino acids. For example, when a 1:1 mixture of (*R*)-**5** and (*S*)-**4** was treated with $Zn(OAc)_2$ and histidine samples of 0 – 100% [D-His], the color of the mixtures changed from green to yellow, orange and red under a UV lamp (365 nm) which allowed a quick quantification of [D-His]%. This is the first example of using fluorescence to visually quantify the enantiomeric composition of chiral compounds.



Introduction

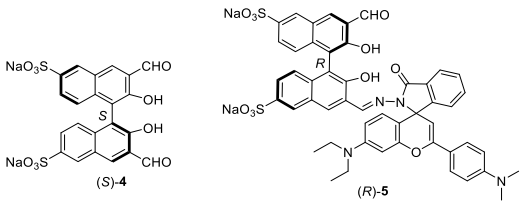
The most common *semiquantitative* visual analysis is the use of pH test papers to determine the acidity and basicity of diverse matters. It provides a very fast and convenient method for routine laboratory, field and even household tests by simply observing the color change of the pH papers. Because of the prevalence of chiral molecules in nature, chemical laboratories and industries of pharmaceutical, agriculture and materials, their rapid analysis has attracted extensive research activities. It would greatly facilitate the study of chiral molecules, such as in the screening of asymmetric reactions, if their enantiomeric composition can be determined by visual observation. In recent years, great progress has been made in the development of optical probes to discriminate enantiomers of chiral molecules.[1-3] Among these studies, we are particularly interested in the use of fluorescent probes to conduct chiral recognition because they use easily available instruments and provide high sensitivity and fast analysis. Although color changes are observed in a few systems when enantioselective fluorescent probes are treated with the enantiomers of a chiral compound,[1-3] the differences in the color change caused by the two enantiomers are often not large enough to allow quantitative visual analysis. Thus, there is no fluorescence-based technique like the pH test papers that can be used to quantitatively determine the enantiomeric composition of chiral compounds by visual observation.

A fluorescent probe needs to meet the following requirements to be useful for quantitative visual analysis: (1) It should show enantioselective fluorescent response in the visible region (400 – 700 nm); (2) It should display very different emitting colors (preferably over 100 - 300 nm difference in wavelength) toward the two enantiomers of a substrate; (3) It should show distinctive color changes as the enantiomeric composition changes. In 2010, we reported the use of a pseudoenantiomeric sensor pair (*S*)-**1** and (*R*)-**2** for the recognition of an α-hydroxy carboxylic acid, mandelic acid (MA).[4] (*S*)-**1** shows greatly enhanced fluorescence by (*R*)-MA at 374 nm but not by (*S*)-MA, and (*R*)-**2** shows greatly enhanced fluorescence by (*S*)-MA at a different emitting wavelength of 330 nm but much less by (*R*)-MA. Thus, using a mixture of (*S*)-**1** and (*R*)-**2**, we can simultaneously detect both (*R*)- and (*S*)-MA at two different wavelengths. However, because of the short emission wavelengths of this sensor pair, they cannot be used for visual recognition. In 2020, Zheng and coworkers reported the use of the fluorescent probe (*R*)- and (*S*)-**3** for enantioselective fluorescent recognition of a series of chiral carboxylic acids.[5] They found that the two enantiomers of a chiral acid can shift the emission of the probe to different wavelengths. Using the difference in emission wavelengths, they were able to determine the enantiomeric composition of a substrate. Although the two enantiomers of a substrate generated different visible colors in the presence of this probe, because the wavelength difference was generally in the range of 22 – 33 nm, it is difficult to use this probe for quantitative visual analysis. Thus, the challenge in quantitative visual analysis is to develop an enantioselective fluorescent sensing system to exhibit large visible emission wavelength differences when interacting with the two enantiomers of a chiral molecule.



In 2020, we reported the use of a water-soluble fluorescent probe (*S*)-**4** and its enantiomer to discriminate the enantiomers of a number of amino acids in aqueous solution.[6] It was found that in the presence of $Zn(OAc)_2$, (*S*)-**4** shows greatly enhanced green-light emission with a L-amino acid like L-Met at λ_{em} = 505 nm but much smaller fluorescence enhancement with the enantiomer D-Met at the same wavelength. We further found that a monoaldehyde compound (*R*)-**5** exhibits enantioselective red-

light emission with amino acids under similar conditions (*vide infra*). The enantioselectivity of (*R*)-**5** was the opposite of that of (*S*)-**4** and it gave a greatly red-shifted emission signal at λ_{em} = 655 nm in the presence of $Zn(OAc)_2$ and an amino acid. That is, a D-amino acid can greatly enhance the red-light emission of (*R*)-**5** but the L-enantiomer shows smaller fluorescence enhancement at the same wavelength.



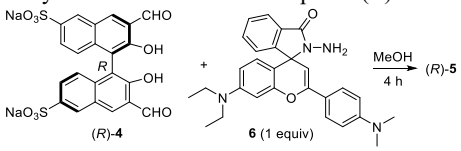
Therefore, we have combined the green-light emitting probe (*S*)-**4** with the red-light emitting probe (*R*)-**5** as a pseudoenantiomeric sensor pair to simultaneously detect both enantiomers of a chiral amino acid at two very different wavelengths. Because of the large emission wavelength difference between (*S*)-**4** and (*R*)-**5** (Δ = 150 nm) when they are treated with the two enantiomers of an amino acid, we have established the first fluorescent sensing system that can carry out semiquantitative visual analysis of chiral compounds. It can be used to quickly determine the enantiomeric composition of chiral amino acids. Herein, these results are reported.

Results and Discussion

1. Synthesis of the Probe (*R*)- and (*S*)-5 and Fluorescent Recognition of Methionine

We synthesized compound (*R*)-**5** from the condensation of (*R*)-**4** with one equiv of a rhodamine derivative **6**[7,8] at room temperature (Scheme 1).[9] In spite of the sulfonate groups of (*R*)-**5**, this compound was purified by flash column chromatograph on silica gel eluted with CH_2Cl_2/MeOH (7:1) to give the product as a green solid in 41.5% yield. The ^{1}H NMR spectrum of (*R*)-**5** in DMSO-d_6 gave two aldehyde singlets at δ 10.30 and 10.29 with 1:1 ratio, indicating the formation of a 1:1 mixture of two diastereomers due to the spiral chiral carbon of **6**. The spiral lactam product (*R*)-**5** displayed very weak fluorescence in aqueous solution.

Scheme 1. Synthesis of the fluorescent probe (*R*)-**5**



Fluorescent response of (*R*)-**5** toward the enantiomers of methionine in the presence of Zn^{2+} was investigated. In HEPES neutral solution (pH = 7.4), (*R*)-**5** (20 μM) gave a weak fluorescence signal at λ = 655 nm which showed little change in the presence of $Zn(OAc)_2$ (2 equiv) (Figure 1). When the (*R*)-**5**+Zn^{2+} solution was treated with D-Met (100 equiv), a large fluorescent enhancement at λ = 655 nm was observed upon excitation at 420 nm. When L-Met was used to interact with the (*R*)-**5**+Zn^{2+} solution, the fluorescence enhancement at 655 nm was much smaller. The enantioselective fluorescent enhancement ratio (*ef*) was found to be 2.5 [$ef = (I_D - I_0)/(I_L - I_0)$, where I_0 is the fluorescence intensity of the probe in the absence of the amino acid]. A smaller emission enhancement at a shorter wavelength (545 nm) was also observed but it had almost no enantioselectivity.

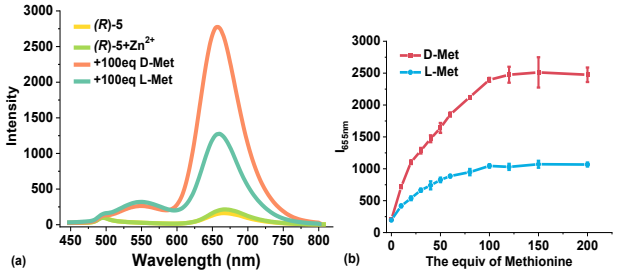


Figure 1. (a) Fluorescence spectra of (*R*)-**5** (2.0×10^{-5} M) with $Zn(OAc)_2$ (2.0 equiv) in the presence of D-and L-Met (100 equiv) in HEPES/1% DMSO (pH = 7.4). (b) Fluorescence intensities at 655 nm versus the equiv of methionine. (λ_{exc} = 420 nm, slit: 5/5 nm)

Compound (*S*)-**5** was prepared as the enantiomer of (*R*)-**5**, and its fluorescence response toward *D*- and *L*-Met was studied under the same conditions. The fluorescence responses of these enantiomeric probes display a mirror image relationship at λ = 655 nm when treated with the enantiomers of the amino acid (Figure S3 and S7). This confirms the inherent recognition process of these two probes. The fluorescence response of both (*R*)- and (*S*)-**5** toward methionine (100 equiv) at various enantiomeric compositions was investigated. Figure 2 plots I_{655} of each enantiomeric probe versus the enantiomeric purity of methionine up excitation at 420 nm. These plots can be used to determine the enantiomeric composition of the amino acid. The average absolute error (AAE) between the measured ee values and the fitted ones was 1.58% *ee*.

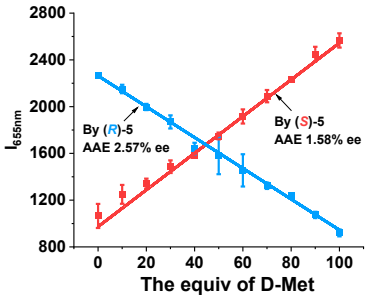


Figure 2. Fluorescence intensity of (*R*)-**5** and (*S*)-**5** (2.0×10^{-5} M in HEPES/1% DMSO) + Zn^{2+} (2 equiv) at 655 nm versus the enantiopurity of methionine (100 equiv). (λ_{exc} = 420 nm, slit: 5/5 nm). The lines were obtained by fitting with OriginPro 2021; Error bars were obtained from three measurements.

Because of the large emitting wavelength difference as well as their opposite enantioselectivity, the probes (*R*)-**5** and (*S*)-**4** were used as a pseudoenantiomeric sensor pair to conduct visual quantification of the enantiomeric composition of the amino acid. We investigated the use of a mixture of (*R*)-**5** and (*S*)-**4** at various ratios in combination with $Zn(OAc)_2$ to interact with methionine samples at varying [D-Met]% (Figures S8a-S8d). Figure 3 shows that when [D-Met]% increased from 0% to 20%, 40%, 60%, 80% and 100%, the color of the solution (under the irradiation of a UV lamp at 365 nm) containing a 1:9 mixture of (*R*)-**5** versus (*S*)-**4** changed gradually from green to red. Thus, on the basis of this color change, the enantiomeric composition of this amino acid can be quickly and semiquantitatively determined.

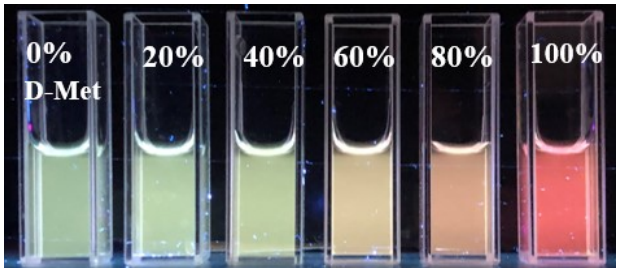


Figure 3. Photographs (under 365 nm light) of (*R*)-**5** (2 μM in DMSO) + (*S*)-**4** (18 μM in MeOH) + Zn^{2+} (40 μM) with 100 equiv methionine at various enantiomeric composition in 50 mM HEPES (pH = 7.4).

2. Enantioselective Fluorescent Recognition of Other Amino Acids

We have studied the fluorescent responses of (*R*)-**5**+Zn^{2+} toward additional common amino acids (18 pairs of enantiomers including methionine). It is generally observed that the D-enantiomers of these amino acids greatly enhance the fluorescence of the probe at λ = 655 nm, while L-enantiomers do not significantly change the fluorescence (Figure 4). In addition to methionine, (*R*)-**5** has showed good enantioselectivity toward leucine (*ef* = 1.9), lysine (*ef* = 2.2), tryptophan (*ef* = 2.5), phenylalanine (*ef* = 3.1), arginine (*ef* = 4.1), valine (*ef* = 2.9), asparagine (*ef* = 2.6), histidine (*ef* = 6.9) and glutamic acid (*ef* = 6.2) at λ = 655 nm but little fluorescence response toward aspartic acid, cysteine, and proline (Figures S9-S17).

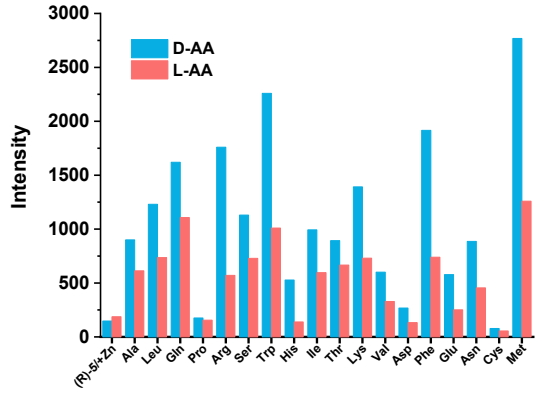
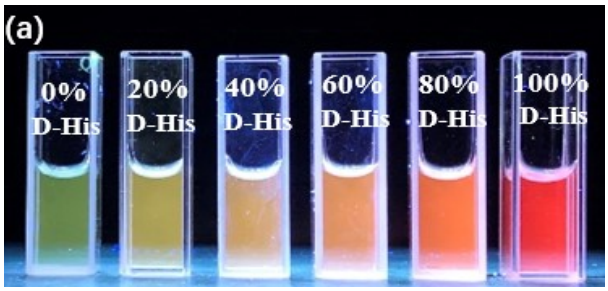


Figure 4. Fluorescence intensity at 655 nm of (*R*)-**5** (2.0×10^{-5} M) + $Zn(OAc)_2$ (2.0 equiv) with 18 chiral amino acids (100 equiv) in pH = 7.4 HEPES/1% DMSO. (λ_{exc} = 420 nm, slit: 5/5 nm.)

We also conducted visual quantifications of the enantiomeric compositions of more amino acids. Through a systematic screening of the ratio of the two probes in the pseudoenantiomeric pair, the desired color change can be obtained (Figure S18-S24). As shown in Figure 5a, when a 1:1 mixture of (*R*)-**5** and (*S*)-**4** in combination with $Zn(OAc)_2$ was used to interact with histidine samples, distinctive color changes from green to yellow, orange and red as [D-His]% increased from 0% to 20%, 40%, 60%, 80% and 100%. Thus, the enantiomeric composition of this amino acid can be visually determined by using the fluorescent sensor system. The fluorescence spectra of these samples are given in Figure 5b. In this 1:1 mixture of (*R*)-**5** and (*S*)-**4**, the L-enantiomer of the amino acid turns on the fluorescence of (*S*)-**4** at ca 500 nm and the D-enantiomer turns on the fluorescence of (*R*)-**5** at ca 650 nm, giving the visual observation of the color change from green to red as the D-component increases.



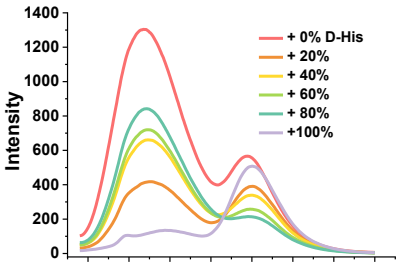


Figure 5. (a) Photographs (under 365 nm light and (b) fluorescence spectra of (*R*)-**5** (10 μM in DMSO) + (*S*)-**4** (10 μM in MeOH) + Zn^{2+} (40 μM) with 50 equiv histidine at various enantiomeric composition in 50 mM HEPES (pH = 7.4).

Figure 6 shows that when 1:4 mixtures of (*R*)-**5** and (*S*)-**4** in combination with $Zn(OAc)_2$ were used to interact with asparagine, arginine and lysine at varying enantiomeric compositions, distinctive color changes were observed. Thus, the enantiomeric compositions of these amino acids can also be semiquantitatively determined by visual analysis.

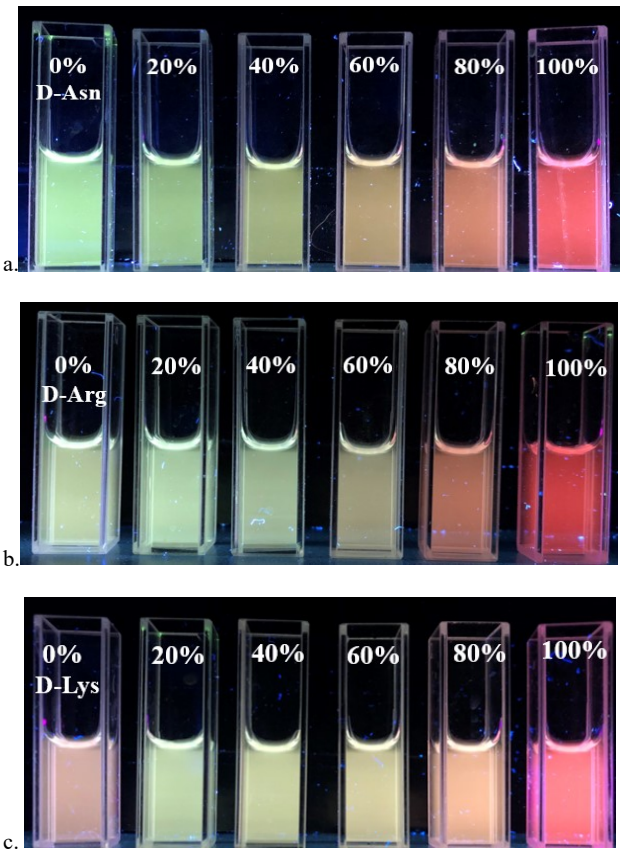
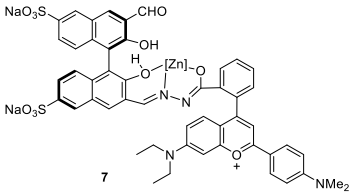


Figure 6. Photographs of (*R*)-**5** (4 μM in DMSO) +(*S*)-**4** (16μM in MeOH) + Zn^{2+} (2 equiv, 40μM) with various enantiomeric

compositions of (a) Asn (100 eq), (b) Arg (100 eq), and (c) Lys (100 eq) in 50 mM HEPES (pH = 7.4), respectively (under 365 nm light).

3. Additional Spectroscopic Studies for the Reaction of (*R*)-5 with Zn(OAc)$_2$ and Methionine

We studied the UV-vis spectroscopic response of (*R*)-**5** toward Zn^{2+} and methionine. As shown in Figure S25, when (*R*)-**5** (2.0×10^{-5} M in HEPES/1% DMSO) was treated with 2 equiv $Zn(OAc)_2$, an increased absorption around 600 nm was observed. This suggests that coordination of Zn^{2+} with (*R*)-**5** might facilitate the opening of its spiral lactam ring to generate a more conjugated structure as represented by **7**.[6,7] Although this ring-opening product gave increased long wavelength absorption, no fluorescence enhancement was observed as shown in Figure 1. When the (*R*)-**5**+$Zn(OAc)_2$ solution was treated with D-Met (2 equiv), further increase in the absorption around 600 nm was observed which was accompanied with large fluorescence enhancement at 655 nm (Figure S26). However, when the (*R*)-**5**+$Zn(OAc)_2$ solution was treated with *L*-Met (2 equiv), the absorption at around 600 nm decreased and the fluorescence enhancement at 655 nm was also much smaller than that with *D*-Met (2 equiv) (Figure S26).



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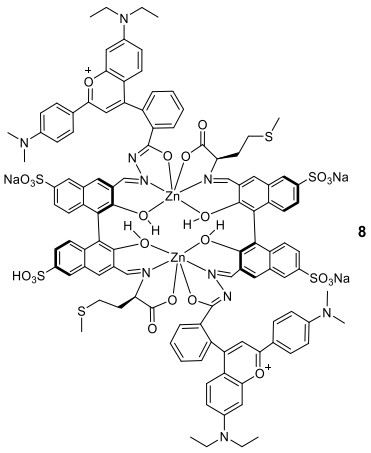
The ^{1}H NMR spectroscopic study (Figure S27) shows that when a (*R*)-**5**+$ZnBr_2$ (2 equiv) solution [DMSO-d_6:HEPES buffer prepared from D_2O, 3:1 v/v) was treated with more than 2 equiv of either *L*-Met or *D*-Met, the aldehyde signal of (*R*)-**5** disappeared, indicating a condensation with the amino acid to form an imine product (In the NMR study, $ZnBr_2$ was used for its better solubility and absence of NMR signal). The above NMR samples containing the product mixture of (*R*)-**5** (4 mM) + $ZnBr_2$ (2 equiv) + *D-/L*-Met (2 equiv) were used for ESI-TOF mass spectroscopic analyses. It was found that the reaction of (*R*)-**5** + Zn^{2+} with *D*-Met gave a dianion signal at m/z (z = 2) = 1174.10 with m = 2348.20 (Figure S28 in SI). This can be attributed to the formation of a [2+2] complex like **8** (calcd for **8**+H_2O: 2348.34). Another peak at *m/z* (*z* = 2) = 1162.61 with *m* = 2325.22 was also observed for **8**+H_2O-Na (calcd: 2325.35). Although previously a z =2 peak was not identified in the mass spectrum obtained for the reaction of an analogue of (*R*)-**5** with *D*-Met and $Zn(OAc)_2$,[9] formation of the [2+2] complex is consistent with our studies on the reaction of (*R*)-**4** and other analogues with the chirality-matched amino acids in the presence of Zn^{2+}.[6,10] In the ESI-TOF mass spectrum of the reaction mixture of (*R*)-**5** + Zn^{2+} with L-Met, there was no z = 2 signal. A peak at *m/z* = 1174.2007 was observed which is consistent with the formation of a monomeric complex like **9** (calcd. for [**9**-H]: 1174.1386). Thus, the enantioselective fluorescence responses of (*R*)-**5**+Zn^{2+} toward the two enantiomers of methionine were attributed to the difference in the stability and structural rigidity of the complexes like **8** and **9**. Thus, the ring-opening of the spiral lactam unit of (*R*)-**5** alone like that in **7** and **9** might not be enough to generate large fluorescence enhancement because of the flexibility of the binaphthyl rings which can relax the excited state energy. However, the dimeric structure like **8** should provide a more rigid structure for greater fluorescence enhancement.



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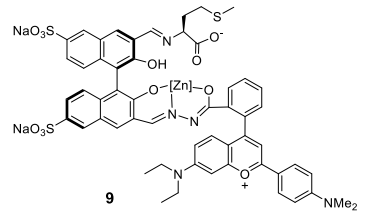

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When a mixture of (*S*)-**4** and (*R*)-**5** was used to interact with the amino acids, it is expected that the fluorescent recognition mechanism is not simply a linear combination of the responses of the individual probes. The formation of the [2+2] complex like **8** suggests that there should also be intermolecular interactions between the two probes to generate a mixture of hetero- and homodimeric complexes upon reaction with Zn^{2+} and the amino acid enantiomers. In spite of the complexity of the sensing mechanism, the observed distinctive color changes in Figure 3, 5 and 6 demonstrate that this sensing system is practically useful for semiquantitative visual chiral analysis.

4. Conclusion

In summary, we have developed a fluorescence method to carry out semiquantitative visual analysis of the enantiomeric composition of chiral compounds. It provides a very convenient way to quickly determine the enantiomeric composition of chiral amino acids. This method employs a new fluorescent probe that exhibits enantioselective recognition of amino acids with red-light emission. The combination of this red-light emitting probe with a previously reported green-light emitting probe forms a pseudoenantiomeric sensor pair because of their opposite enantioselectivity. They can be used to simultaneously detect both enantiomers of a chiral amino acid at two very different wavelengths. The distinctive color change of this sensing system in the presence of the enantiomers of the substrates allows visual quantification of the enantiomeric composition. This study has established a new and potentially general strategy to develop fluorescent probes for quantitative visual analysis of chiral compounds. Absorption of the sensing system on cellulose papers or other absorbing materials to build enantioselective visual testing papers similar to pH papers represents one of the many promising applications of this sensing system. We are currently exploring the diverse applications of these enantioselective fluorescent probes.

Experimental Section

General Information. Unless otherwise noted, reagents were obtained from commercial suppliers and used without further purification. In optical studies, all solvents were HPLC or spectroscopic grade. A Bruker AM400 NMR spectrometer and a Bruker AM800 NMR spectrometer were used for NMR analysis.

HRMS spectra were measured on a Bruker Daltonics Bio TOF mass spectrometer. UV absorption spectra were recorded on a Persee TU-1901 UV-visible spectrophotometer. Fluorescence spectra were collected on a Hitachi F-7000 fluorescence spectrophotometer.

Compound (*R*)-**4** were synthesized by following our previously reported procedures[6] in 29.3% (527.7 mg) yield as a yellow solid. (*S*)-**4** was prepared in the same way as (*R*)-**4** in 26.8% (386.3 mg) yield. Compound **6** was synthesized by following the literature procedure[7a] in 82.1% (0.85 g) yield as a gray solid.

Synthesis and Characterization of (*R*)-5. Compound **6** (421 mg, 0.927 mmol) was added, in batches, into a solution of (*R*)-**4** (505 mg, 0.927 mmol) in anhydrous methanol with stirring. The reaction mixture was continuously stirred at rt for 4 h. The solvent was then evaporated, and the crude product was subjected to flash column chromatography on silica gel eluted with CH_2Cl_2/MeOH (7:1) to afford (*R*)-**5** as a green solid in 41.5% yield (377.8 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 0.5H), 10.87 (s, 0.5H), 10.30 (s, 0.5H), 10.29 (s, 0.5H), 10.04 (s, 1H), 9.31 (s, 0.5H), 9.18 (s, 0.5H), 8.65 (s, 1H), 8.28 (s, 1H), 8.16 (t, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.69 (t, *J* = 7.4 Hz, 1H), 7.65 (m, 1H), 7.63–7.57 (m, 2H), 7.54 (m, 1H), 7.45 (t, *J* = 1.7 Hz, 0.5H), 7.43 (t, *J* = 1.8 Hz, 0.5H), 7.31 (d, *J* = 2.7 Hz, 0.5H), 7.30 (d, *J* = 2.6 Hz, 0.5H), 6.89 (m, 1H), 6.81 (m, 1H), 6.68 (t, *J* = 9.1 Hz, 2H), 6.50 (t, *J* = 1.8 Hz, 1H), 6.43 (m, 1H), 6.39 (m, 1H), 5.51 (s, 0.5H), 5.46 (s, 0.5H), 3.28 (m, 4H), 2.94 (s, 3H), 2.91 (s, 3H), 1.01 (m, 6H). ^{13}C{^{1}H} NMR (201 MHz, DMSO-d_6) δ 197.9, 164.9, 155.3, 154.7, 154.4, 154.2, 154.1, 152.7, 152.5, 152.4, 150.4, 150.3, 145.6, 145.0, 138.3, 135.7, 135.1, 135.0, 130.8, 129.8, 129.7, 129.0, 128.2, 128.0, 127.9, 127.81, 127.76, 126.8, 125.9, 125.2, 125.0, 122.4, 121.5, 121.4, 118.6, 116.7, 113.3, 110.9, 105.8, 105.6, 99.6, 99.5, 95.0, 66.6, 50.3, 45.4, 14.0. HRMS (ESI): m/z calcd for $C_{50}H_{40}N_4O_{11}S_2^{2-}$: 468.1065 [M-2Na]$^{2-}$; found: 468.1066. $[\alpha]^{25}_D$ = +31.6 (*c* = 1 mg/mL, DMSO).

Synthesis and Characterization of (*S*)-5. (*S*)-**5** was prepared in the same way as (*R*)-**5** by using (*S*)-**4** (210 mg) which gave the product in 37.6% (142 mg) as a green solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 0.5H), 10.29 (s, 0.5H), 9.31 (s, 0.5H), 9.18 (s, 0.5H), 8.64 (s, 1H), 8.28 (s, 1H), 8.15 (t, J = 9.0 Hz, 2H), 7.92 (d, *J* = 7.3 Hz, 1H), 7.69 (m, 1H), 7.65 (m, 1H), 7.62–7.57 (m, 2H), 7.53 (m, 1H), 7.45 (t, *J* = 2.1 Hz, 0.5H), 7.43 (t, *J* = 1.8 Hz, 0.5H), 7.32 (d, *J* = 2.7 Hz, 0.5H), 7.30 (d, *J* = 2.9 Hz, 0.5H), 6.88 (m, 1H), 6.80 (m, 1H), 6.68 (t, *J* = 9.0 Hz, 2H), 6.50 (t, *J* = 1.8 Hz, 1H), 6.45–6.41 (m, 1H), 6.38 (m, 1H), 5.51 (s, 0.5H), 5.46 (s, 0.5H), 3.28 (m, 4H), 2.94 (s, 3H), 2.92 (s, 3H), 1.01 (m, 6H). ^{13}C{^{1}H} NMR (201 MHz, DMSO-d_6) δ 197.9, 164.9, 155.4, 154.8, 154.4, 154.2, 154.1, 152.7, 152.5, 152.4, 150.4, 150.3, 145.5, 145.0, 138.3, 135.8, 135.2, 135.0, 130.9, 129.8, 129.7, 129.0, 128.2, 128.0, 127.9, 127.8, 127.77, 126.9, 125.9, 125.2, 125.0, 122.5, 121.5, 121.4, 118.6, 116.7, 113.3, 110.9, 105.8, 105.6, 99.6, 99.5, 94.9, 66.7, 50.3, 45.4, 14.0. HRMS (ESI): m/z calcd for $C_{50}H_{40}N_4O_{11}S_2^{2-}$: 468.1065 [M-2Na]$^{2-}$; found: 468.1045. $[\alpha]^{25}_D$ = -32.4 (*c* = 1 mg/mL, DMSO).

Sample Preparation for Fluorescence Measurement. Stock solutions of (*R*)-**5** (2.0 mM, DMSO, 20 μL), $Zn(OAc)_2$ (4.0 mM, deionized water), amino acids (40.0 mM, 50 mM HEPES, pH = 7.4) were freshly prepared for each measurement. The stock solution of (*R*)-**5** was diluted to the corresponding concentration (20 μM) with HEPES to which the $Zn(OAc)_2$ solution and an appropriate amino acid solution were added. After the mixture was allowed to stand at room temperature for 3 h, it was chilled in an ice bath for 30 min to quench the reaction and the fluorescence spectrum was then recorded at 5 °C (λ_{exc} = 420 nm, slits:5/5 nm).

Sample Preparation for UV-Vis Measurement. Stock solutions of (*R*)-**5** (2.0 mM, DMSO), $Zn(OAc)_2$ (4.0 mM, deionized water), amino acid (2.0 mM, 50 mM HEPES, pH = 7.4) were freshly prepared for each measurement. A solution of (*R*)-**5** + Zn^{2+} (2.0 equiv) was mixed with D/L-methionine (2.0 equiv) in a 5 mL test tube. The resulting solution was allowed to stand at room temperature for 3 h, which was then diluted to 20 μM with HEPES before measurements.

Sample Preparation for Visual Analysis. Stock solutions of (*R*)-**5** (1.0 mM in DMSO), (*S*)-**4** (1.0 mM in MeOH), $Zn(OAc)_2$ (4.0 mM in deionized water), and amino acids (40.0 mM in HEPES) were freshly prepared for each measurement. For visual fluorescent study, the sensor solution was (*R*)-**5**+(*S*)-**4** (1:1-9, total concentration: 20 μM, total volume: 40 μL). The stock solution of (*R*)-**5**+(*S*)-**4** was diluted to 20 μM with HEPES to which the $Zn(OAc)_2$ solution (20 μL) and an amino acid solution (total volume:100 μL, with various enantiomeric compositions) were added. After the mixtures were allowed to stand at rt for 3 h, their photographs were taken under a UV lamp (365 nm, 5W).

Supplementary Materials Available: Additional experimental description and spectroscopic data.

Keywords: fluorescent probe, visual analysis, enantioselective, amino acids

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