One Molecular Probe with Opposite Enantioselective Fluorescence Enhancement at Two Distinct Emissions

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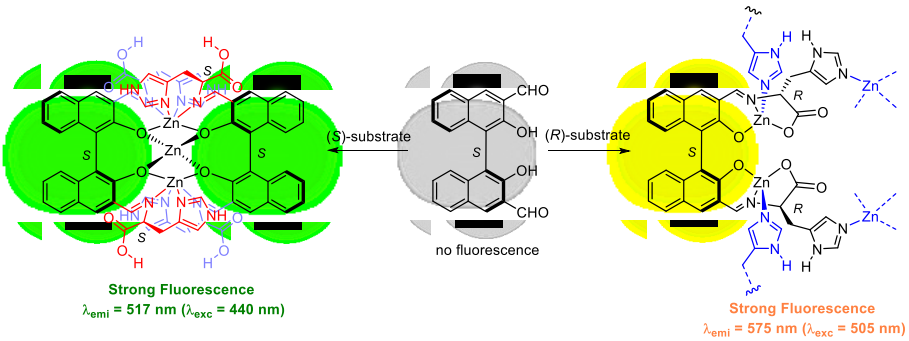
Received Date (will be automatically inserted after manuscript is accepted)

It is discovered that one enantiomer of a chiral substrate can greatly enhance the fluorescence of one molecular probe at one emitting signal (λ_1 = 517 nm), while the opposite enantiomer of the substrate greatly enhances the fluorescence of the same probe at a distinctively different emission (λ_2 = 575 nm). This probe is made of a 1,1'-binaphthyl-based chiral dialdehyde which in combination with Zn^{2+} under slightly acidic conditions shows chemoselective as well as enantioselective fluorescent response to histidine. The opposite enantioselective fluorescent response of the probe at two emissions allows it to be used to determine both the concentration and enantiomeric composition of the substrate by a single probe. The mechanistic study has revealed two very different reaction pathways when the two enantiomers of the substrate are treated with the probe. These reaction pathways generate two different products, one dimeric and another polymeric, with very different emissions.

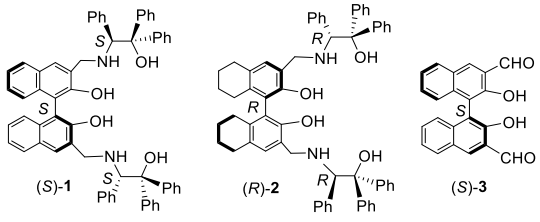


Enantioselective fluorescent recognition of chiral organic compounds by using molecular probes has been actively investigated in the past several decades.[1] These studies not only can provide fundamental information on the interaction of chiral molecules at both the ground and excited states, but also can allow the development of a rapid analytical tool for chiral analysis. Generally, a conventional fluorescent probe reports the concentration of an analyte by showing fluorescence enhancement or quenching at its emission signal after being treated with the substrate. The analysis of a chiral molecule, however, requires the determination of both the concentration and enantiomeric composition of the substrate which cannot be accomplished by observing fluorescence enhancement or quenching at only one emission signal of the fluorescent probe.

In order to achieve the above goal, we previously 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).[2] (*S*)-**1** shows greatly enhanced fluorescence by (*R*)-MA at λ_{emi} = 374 nm but not by (*S*)-MA. Whereas, (*R*)-**2** shows greatly enhanced fluorescence by (*S*)-MA at a different emitting wavelength with λ_{emi} = 330 nm but much less by (*R*)-MA. In the presence of a mixture of (*S*)-**1** and (*R*)-**2**, both (*R*)- and (*S*)-MA enhance the fluorescence of the pseudoenantiomeric sensor pair but at two distinctively different emitting signals. The sum of the fluorescence intensities at the two signals can be correlated with the total concentration of the two enantiomers, and the difference of the fluorescence intensities at the two signals can be correlated with the enantiomeric composition. Thus, using this pseudoenantiomeric sensor pair, we can determine both of the parameters of the chiral substrate.

Although additional strategies have also been developed to measure the concentration and enantiomeric composition of a chiral molecule by using fluorescent probes,[3] there was no report on using a *single* fluorescent probe that can behave like the pseudoenantiomeric sensor pair (*S*)-**1** and (*R*)-**2** with the *opposite* enantioselective fluorescence responses at two distinctively different emitting signals.

The chiral dialdehyde (*S*)-**3** in combination with $Zn(OAc)_2$ was previously reported to show enantioselective fluorescent responses toward a number of chiral amino acids in the presence of an excess amount of Bu_4NOH in methanol solution.[4] Our further study of this probe leads to the discovery that in DMF solution under slightly acidic conditions, (*S*)-**3** in combination with $Zn(OAc)_2$ not only exhibits chemoselective and enantioselective fluorescence response toward an essential amino acid histidine,[5-12] but also shows opposite enantioselective fluorescence enhancement at two distinctively different emitting signals. That is, while L-His turns on the fluorescence of (*S*)-**3** at one emitting signal, D-His turns on the fluorescence at a distinctively different emitting signal. Therefore, (*S*)-**3** as a single molecular probe can be used to detect both enantiomers of the chiral substrate individually by observing fluorescence enhancement at two separate signals. This allows the determination of the concentration and enantiomeric composition of the substrate. The mechanism of these fluorescence responses has been investigated. Herein, these results are reported.



Compound (*S*)-**3** is synthesized from (*S*)-1,1'-bi-2-naphthol (BINOL) by modifying the literature procedure[13], and it shows no fluorescence with or without $Zn(OAc)_2$. When (*S*)-**3** is treated with 17 common amino acid enantiomers in DMF/H_2O (1:1) (phosphate buffer, pH = 6.4) in the presence of $Zn(OAc)_2$ (2.0 equiv), only L-

His greatly enhances the fluorescence of (*S*)-**3** at λ_{emi} = 517 nm (λ_{exc} = 440 nm), but D-His and all the other amino acid enantiomers cause little or no fluorescence response (Figure 1 and S1a). This is in sharp contrast to the previously reported use of the Bu_4N^+ salts of the amino acids, where a number of the L-amino acid salts can turn on the fluorescence of (*S*)-**3**.[4] Thus, by using the slightly acidic conditions, (*S*)-**3** is converted to a highly chemoselective as well as enantioselective fluorescent probe for histidine.

Histidine is an essential amino acid for human growth and development.[5] Its imidazole side chain is an important active site of enzymes and metalloproteins.[6,7] Histidine is a precursor of histamine, which serves as a neurotransmitter and a potent inflammation mediator.[8,9] Histidine is also found to be useful in asymmetric catalysis, such as asymmetric aldol reactions, for the synthesis of chiral organic compounds.[10,11] Fluorescent probes have been developed for the detection of histidine,[12] but only one of them shows good enantioselective fluorescence response.[12j] Figure 1 demonstrates that (*S*)-**3** is a readily available and highly chemoselective and enantioselective fluorescent probe for histidine.

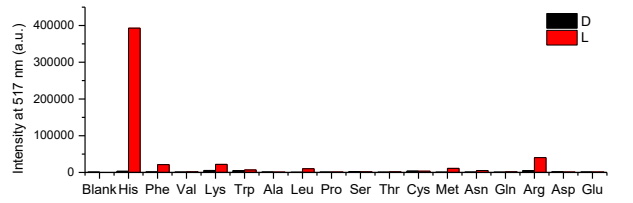


Figure 1. Fluorescence intensity at λ = 517 nm for (*S*)-**3** (0.5 mM) + $Zn(OAc)_2$ (2.0 equiv) with 17 pairs of amino acid enantiomers (10 equiv) (λ_{exc} = 440 nm, slit: 3/3 nm for. Solvent: DMF/H_2O = 1/1 with 12.5 mM phosphate pH = 6.4 buffer).

The fluorescence response of (*S*)-**3** toward histidine is studied in detail. Figure 2a gives the fluorescence spectra of (*S*)-**3** with D- and L-His in the presence of $Zn(OAc)_2$. It shows that L-His (10 equiv) greatly enhances the fluorescence of (*S*)-**3** at λ_1 = 517 nm (λ_{exc} = 440 nm) (for excitation spectrum see Figure S2a), but D-His produces much weaker emission. Under these conditions, the enantioselective fluorescence enhancement ratio [$ef_1 = (I_L-I_0)/(I_D-I_0)$. I_0: the fluorescence intensity in the absence of D- and L-histidine] is 168.3 at λ_1 (Figure 2a). Figure 2b shows the fluorescence response at λ_1 versus the equivalence of L- and D-His. When the concentration of L-His increases from 0 – 10 equiv, the fluorescence intensity greatly increases. Whereas, the fluorescence intensity remains low in the presence of D-His.

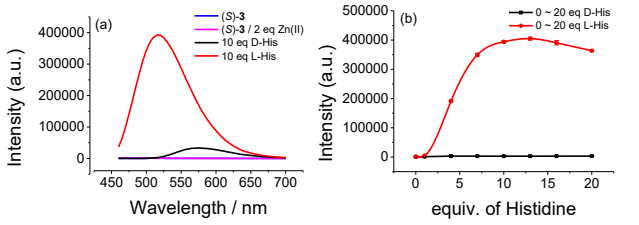


Figure 2. (a) Fluorescence spectra of (*S*)-**3** (0.5 mM) + $Zn(OAc)_2$ (2.0 equiv) with D- and L-His (10.0 equiv); (b) Fluorescence intensity at λ_1 = 517 nm *versus* the equivalence of D- and L-His (error bars from three independent experiments. λ_{exc} = 440 nm. Slit: 3/3 nm. Solvent: DMF/H_2O = 1/1 with 12.5 mM pH = 6.4 phosphate buffer).

In contrast to the above observations, D-His greatly enhances the fluorescence of (*S*)-**3** at λ_2 = 575 nm while excited at 505 nm (for excitation spectrum see Figure S2b), but L-His gives only a weak signal (Figure 3a). Figure 3b plots the fluorescence intensity at λ_2 versus the concentration of L- and D-His. From 0 – 10 equiv, D-His greatly enhances the fluorescence of (*S*)-**3** at λ_2, but L-His does not. At 10 equiv histidine, the enantioselective fluorescence enhancement ratio [$ef_2 = (I_D-I_0)/(I_L-I_0)$. I_0: the fluorescence intensity in the absence of D- and L-His] is 13.9 at λ_2.

As shown in Figure 2 and 3, (*S*)-**3** exhibits opposite enantioselective fluorescent responses at two emission signals toward the enantiomers of histidine. While L-His greatly enhances the fluorescence of (*S*)-**3** at λ_1 = 517 nm, D-His greatly enhances the fluorescence of (*S*)-**3** at λ_2 = 575 nm. This represents the first example that a single fluorescence probe displays opposite enantioselective enhancement at two distinctively different emission signals toward a chiral substrate. It can be used to directly detect both of the enantiomers of the substrate.

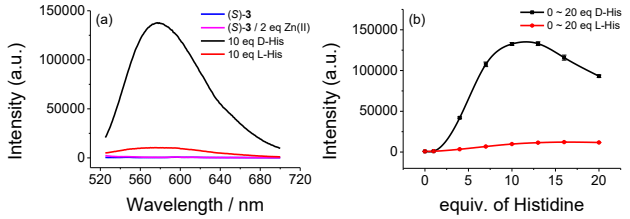


Figure 3. (a) Fluorescence spectra of (*S*)-**3** (0.5 mM) + $Zn(OAc)_2$ (2.0 equiv) with D- and L-His (10.0 equiv). (b) Fluorescence intensity at λ_2 = 575 nm *versus* the equivalence of D- and L-His (error bars from three independent experiments. λ_{exc} = 505 nm. Slit: 5/5 nm. Solvent: DMF/H_2O = 1/1 with 12.5 mM phosphate buffer pH = 6.4).

The presence of Zn(II) is found to be necessary for the observed enantioselective fluorescence enhancement of (*S*)-**3** with histidine. When Zn(II) is replaced with other metal cations, such as Mg(II), Ni(II), Cu(II), Fe(III), and Mn(II), no fluorescence enhancement can be observed (see Figure S3 and S4. for screening of the reaction conditions see Figure S5-S9).

We have further found that at λ_2 = 575 nm, (*S*)-**3** is also highly chemoselective and enantioselective toward histidine. As shown in Figure 4 and S1b, among 17 pairs of common amino acid enantiomers, only D-His can greatly enhance the fluorescence of (*S*)-**3** at λ_2 but not L-His and other amino acids. Thus, (*S*)-**3** can be used to conduct chemoselective and enantioselective recognition of histidine at two different emissions.

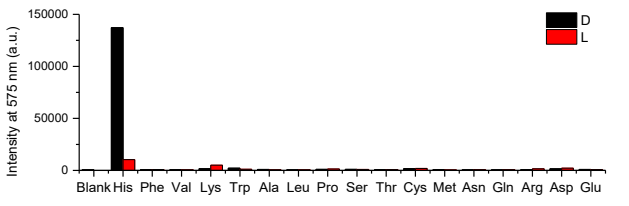


Figure 4. Fluorescence intensity at λ_2 = 575 nm for (*S*)-**3** (0.5 mM) + $Zn(OAc)_2$ (2.0 equiv) with 17 pairs of amino acid enantiomers (10 equiv) (λ_{exc} = 505 nm, slit: 5/5 nm. Solvent: DMF/H_2O = 1/1 with 12.5 mM pH = 6.4 phosphate buffer).

The effect of pH on the fluorescence response of (*S*)-**3** toward histidine is investigated. It is found that at 6.4 < pH < 9.4, (*S*)-**3** maintains the opposite enantioselective fluorescence responses toward histidine at λ_1 and λ_2. However, as the basicity increases, (*S*)-**3** starts to show fluorescence enhancement with many of the other 16 pairs of amino acid enantiomers studied (for the response at pH = 9.4 see Figure S10 - S13). At pH < 6.4, the fluorescence enhancement at λ_1 and λ_2 decreases (Figure S14). Thus, the optimum conditions for the chemoselective as well as enantioselective fluorescent recognition of histidine is at pH = 6.4.

Compound (*R*)-**3**, the enantiomer of (*S*)-**3**, is synthesized from (*R*)-BINOL, and its fluorescence response toward D- and L-His has been studied under the same conditions. As shown in Figure S15 - S17, a mirror-image relationship is observed between the fluorescence responses of (*R*)-**3** and (*S*)-**3** toward the enantiomers of histidine at both λ_1 and λ_2, which confirms the inherent chiral recognition process of the molecular probe.

Because of the opposite enantioselective fluorescence responses of (*S*)-**3** at two emitting signals, both the concentrations of the two enantiomers of histidine can be determined by using this probe. That is, the total concentration of [L-His]+[D-His] and the enantiomeric composition of a given sample of this amino acid can be determined. The fluorescence responses of (*S*)-**3** toward D- and L-His at varying concentrations and *ee*'s {*ee* = ([D]-[L])/([D]+[L])} in the presence of $Zn(OAc)_2$ are investigated (Figure 5, S18 and S19). As shown in Figure 5a, I_{517} increases as the concentration of L-His increases and the -*ee* increases. Figure 5b shows that I_{575} increases as the concentration of D-His increases and the *ee* increases. On the basis of the data in Figure 5a,b, two 3D plots can be obtained. Figure 5c is the 3D plot of [D-His]% *versus* I_{517} and I_{575}. Using this plot, [D-His]% and [L-His]% ([D-His]%+[L-His]% = 100%) can be determined from I_{517} and I_{575}. Figure 5d is the 3D plot of [His] ([His] = [D-His]+[L-His]) (0 – 3.5 mM) *versus* I_{517} and I_{575}. Using this plot, [His] can be determined from I_{517} and I_{575}.

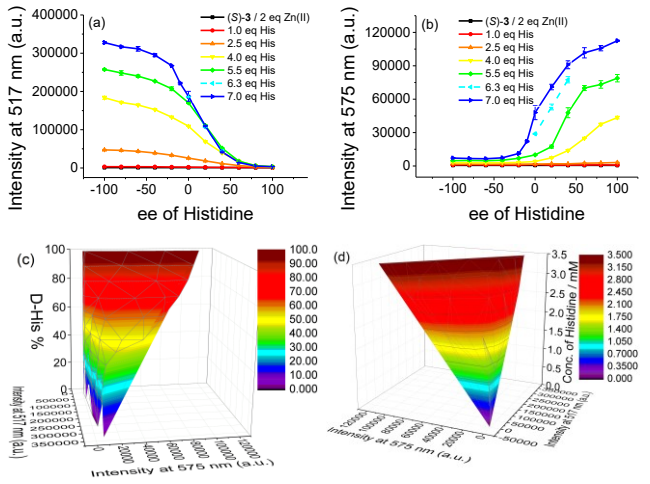


Figure 5. Fluorescence response of (*S*)-**3** (0.5 mM) + $Zn(OAc)_2$ (2.0 equiv) toward histidine (0 ~ 3.5 mM). (a) I_{517} (λ_{exc} = 440 nm) *versus ee* of histidine at varying concentrations. (b) I_{575} (λ_{exc} = 505 nm) *versus ee* of histidine at varying concentrations. (c) I_{517} *versus* I_{575} at varying [D-His]%. (d) I_{517} *versus* I_{575} at varying [His]. (Solvent: DMF/H_2O = 1/1 with 12.5 mM pH 6.4 buffer. All the data were obtained from three independent experiments. Slit: 3/3 nm for I_{517}, and slit: 5/5 nm for I_{575})

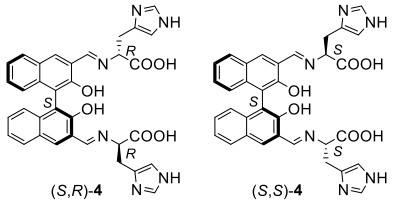
We have used the 3D plots Figure 5c and 5d and their reaction conditions to determine the enantiomeric composition and concentration of 7 histidine samples in the presence of (*S*)-**3**+$Zn(OAc)_2$. As shown in Table 1, the fluorescence-determined data of [D-His]% and [His] on the basis of Figure 5c and 5d match well with the actual data.

Table 1. Using the fluorescence response of (*S*)-**3** + $Zn(OAc)_2$ to determine the concentration and enantiomeric compositions of histidine samples.[a]

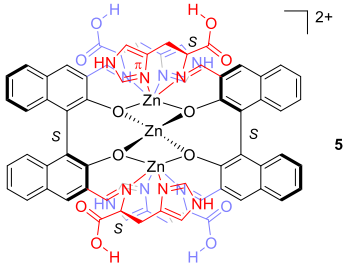
Entry	[D-His]%		[His]/mM	
	Actual	Found	Actual	Found
1	90.0	91.3	1.00	0.95
2	45.0	42.2	1.50	1.47
3	80.0	79.3	1.75	1.70
4	35.0	33.0	2.25	2.40
5	70.0	71.8	2.50	2.56
6	25.0	29.0	3.00	3.10
7	60.0	60.4	3.25	3.48

a. The plots and conditions of Figure 5 were used. All the data were obtained from three independent experiments.

In order to understand the origin of the selective fluorescence responses of (*S*)-**3** toward D- and L-His in the presence of Zn^{2+}, we have synthesized the two diastereomeric imine compounds (*S*,*R*)-**4** and (*S*,*S*)-**4** from the condensation of (*S*)-**3** with D- and L-His. We have conducted ^{1}H NMR spectroscopic titrations of (*S*,*R*)-**4** and (*S*,*S*)-**4** with $Zn(OAc)_2$ in DMSO-d_6 solution at room temperature. As shown in Figure S20a, the addition of $Zn(OAc)_2$ to (*S*,*R*)-**4** leads to the disappearance of the ^{1}H NMR signals (slurry formed later over an extended period of time). One explanation for this is the

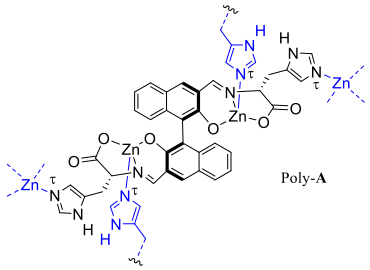


formation of a polymeric network to give diminished NMR signals. However, when (*S*,*S*)-**4** is treated with 1.5 equiv $Zn(OAc)_2$, a new complex is generated whose clear and sharp ^{1}H NMR signals indicate the formation of a symmetric complex (Figure S20b). The 2D NMR spectroscopic analyses, including COSY, NOESY, HSQC, HMBC and gated decoupling ^{13}C-NMR spectroscopic methods, support the formation of a [2+3] complex **5** from the reaction of (*S*,*S*)-**4** with $Zn(OAc)_2$ (Figures S21 – S36). This is also supported by the high-resolution mass spectrum (TOF ES+) which gives a prominent peak at m/z = 710.0862 (z = 2) (Calcd for **5**, M/2: 710.0851) (Figure S37 - S39).



Our study demonstrates that in **5** there is a chelate coordination of an imine N and the π-N of an imidazole ring to a Zn(II) center formed from either the reaction of (*S*,*S*)-**4** with $Zn(OAc)_2$ or the reaction of (*S*)-**3** with L-His and $Zn(OAc)_2$ (see Section 19 and Figure S40-S62 in SI). When (*S*,*R*)-**4** is treated with $Zn(OAc)_2$ or (*S*)-**3** is treated with D-His and $Zn(OAc)_2$, the dimeric complex like **5** cannot form but a polymeric network such as Poly-**A** may be produced through the intermolecular coordination of the τ-N of the

imidazole units with the Zn(II) centers (see Section 19 and Figure S40 - S62 in SI). The imine units in this polymer might be able to achieve a better conjugation with the naphthalene rings to give the observed significantly longer wavelength emission than the [2+3] complex **5** that might have more intramolecular steric interaction to disturb the conjugation. The Zn(II) coordination-generated fluorescence enhancement in the formation of the polymer can be attributed to the inhibition of both the excited state isomerization of the imine bonds and the excited state intramolecular proton transfer, similar to those involved in the formation of **5**. In addition, formation of the polymeric network and its intermolecular aggregation might also reduce the bond rotations and produce aggregation-induced emission.[14]



In conclusion, we have discovered that under slightly acidic conditions, compound (*S*)-**3** exhibits chemoselective as well as enantioselective fluorescence responses toward an essential amino acid, histidine. It is further found that this molecular probe shows opposite enantioselective fluorescence enhancement at two distinctively different emitting signals upon interaction with the chiral substrate. This is the first example of a molecular probe that allows the detection of both enantiomers of a chiral substrate individually by observing fluorescence enhancement at two signals. It can be used to determine the concentration and the enantiomeric composition of the chiral substrate. Our mechanistic study has revealed that (*S*)-**3** reacts with the two enantiomers of the chiral substrate in the presence of Zn(II) to form two structurally very different products, one dimeric and one polymeric. These two different types of products give greatly enhanced fluorescence with two completely different signals. This study should contribute to developing more convenient and efficient enantioselective fluorescent probes for chiral analysis.

ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supplementary Information Available

Additional experimental procedures and spectroscopic data are provided.

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Notes

The authors declare no competing financial interest.

Acknowledgement

We thank the support of the US National Science Foundation (CHE-1855443 and CHE-2153466) for this project.

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