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Determine Concentration and Enantiomeric Composition of Histidine by One Fluorescent Probe

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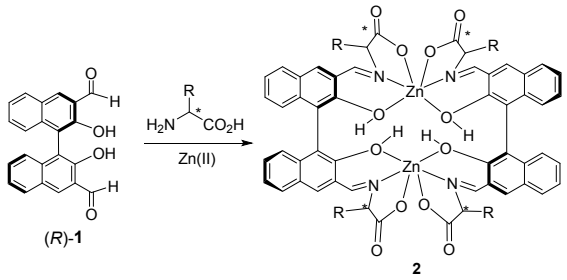
A chemoselective as well as enantioselective fluorescent probe has been developed to determine both the concentration and enantiomeric composition of the biologically important amino acid histidine by measuring fluorescent responses when excited at two different wavelengths.

Fluorescent probes to detect amino acids have been actively investigated in the past two decades because of the easily available instruments, fast analysis, multiple sensing modes and capability of high throughput assay.[1-3] L-Amino acids are the structural units of proteins and play diverse biological functions.[4,5] In recent years, more and more research has revealed that D-amino acids also exist in biosystems including human.[6-8] For example, they are found in central never systems as important neural transmitters. The enantiomerically pure L- and D-amino acids are extensively used in the asymmetric synthesis of functional organic compounds including many pharmaceutical products as either the starting materials or the chirality sources.[9,10] However, fluorescent recognition of the biologically important amino acids with both chemoselectivity and enantioselectivity still presents significant challenges in this area.[3]

Histidine is an essential amino acid for human growth.[11] It is a precursor for histamine, an amine produced in the body necessary for inflammation.[12a] Proteins enriched with histidine play many important roles in human body.[12] It is also useful in asymmetric synthesis and has been applied to catalyze the cross-aldol reactions of enolizable aldehydes.[13] Although several fluorescent probes were developed for the detection of histidine, they cannot be used to distinguish the L- and D-enantiomers.[14] For example, a metal organic framework-based fluorescence probe was found to show selective recognition of histidine but its enantioselectivity was not high.[14g]

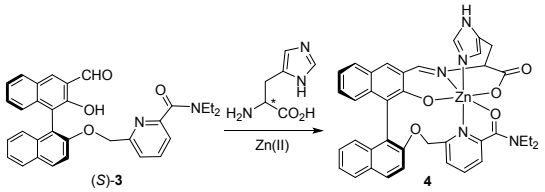
Previously, we discovered that the 1,1'-bi-2-naphthol (BINOL)-based dialdehyde (*R*)-**1** in combination with Zn(II) exhibits enantioselective fluorescent enhancement with amino acids via an amine-aldehyde condensation followed by Zn(II) coordination and intermolecular association to generate dimeric complexes like **2**.[15] A two-stage fluorescence enhancement mechanism is proposed for this process: (1) The Zn(II) coordination with the imine restricts the excited state C=N bond isomerization; (2) The formation of the macrocyclic dimeric product **2** rigidifies the structure and restricts the rotation of the BINOL units around its 1,1'-bonds.[15,16] Although (*R*)-**1** exhibits enantioselective fluorescent responses toward several amino acids, it is not chemoselective for a specific substrate. In order to develop fluorescent probes that are both chemoselective and enantioselective for specific amino acids such as histidine, we have conducted a systematic modification of the structure of the BINOL-aldehyde-based probe.

Scheme 1. The reaction of the fluorescent probe (*R*)-**1** with an amino acid and Zn(II).



We have designed the monoaldehyde probe (*S*)-**3** for the selective recognition of histidine in the presence of Zn(II). As shown in Scheme 2, when (*S*)-**3** is treated with histidine and Zn^{2+}, a six-coordinated Zn(II) complex like **4** could be generated to restrict the rotation of the BINOL unit to show fluorescence enhancement. The L- and D-enantiomers of histidine may lead to different stability and/or structural rigidity of **4**, giving enantioselective response. Other amino acids without the imidazole substituent of histidine cannot generate the structure of **4** which would allow histidine to be selectively detected by (*S*)-**3**. We have discovered that (*S*)-**3** in combination with Zn(II) is an unprecedented highly chemoselective as well as highly enantioselective fluorescent probe for histidine. Herein, this result is reported.

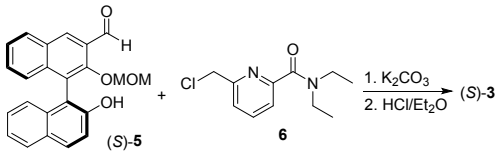
Scheme 2. A proposed reaction of (*S*)-**3** with histidine and Zn(II) to form **4**.



We prepared the proposed fluorescent probe (*S*)-**3** according to Scheme 3. Reaction of the monoBINOL aldehyde (*S*)-**5**[17] with **6**[18] in the presence of a base at 80 °C gave a MOM protected intermediate. Removal of the MOM

protecting group of this intermediate with HCl (anhydrous Et_2O) led to the formation of the desired product (*S*)-**3** in 91% yield. In the ^1H NMR spectrum of (*S*)-**3** in $CDCl_3$, the singlet at δ 10.21 is assigned to the hydroxyl proton as it disappeared with the addition of D_2O. This low field signal of the hydroxyl proton is consistent with a strong intramolecular hydrogen bond similar to that observed in the dialdehyde compound (*R*)-**1**.

Scheme 3. Synthesis of the proposed fluorescent probe (*S*)-**3**



Compound (*S*)-**3** displays an emission at 470 nm upon excitation at 378 nm (Figure 1a). Addition of Zn(II) has no significant influence on the fluorescence (*S*)-**3**. It was found that both D- and L-histidine reduced the fluorescence intensity of the (*S*)-**3**+Zn(II) solution at λ_1 = 470 nm equally (Figure 1a-d). That is, the fluorescence quenching at this wavelength is independent of the amino acid configuration.

We further found that a new emission emerged at λ_2 = 560 nm (λ_{exc} = 450 nm) when (*S*)-**3**+Zn^{2+} was treated with histidine. As shown in Figure 2, D-histidine greatly enhanced the fluorescence at this wavelength but L-histidine generated much weaker response. Thus, the fluorescent probe (*S*)-**3**+Zn(II) exhibits high enantioselectivity for histidine at λ_2. At 10 equiv histidine, the enantioselective fluorescent enhancement ratio [*ef* = $(I_D–I_0)/(I_L–I_0)$. I_0: the fluorescence intensity in the absence of D- and L-histidine] was found to be at 24.3 (Figure 2a).

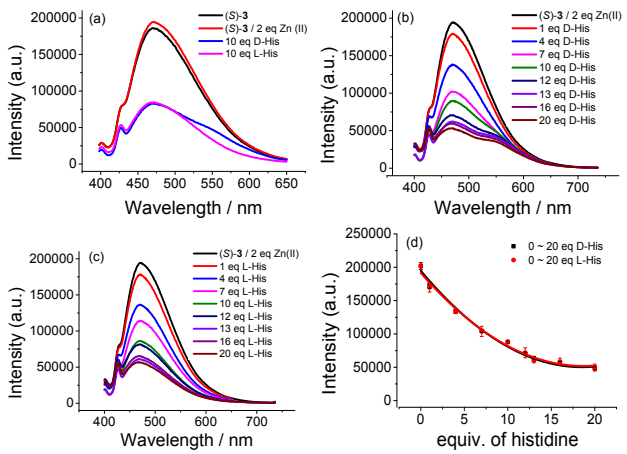


Figure 1. Fluorescence spectra of (*S*)-**3** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) with (a) D- and L-histidine (10.0 equiv), (b) D-histidine, and (c) L-histidine. (d) Fluorescence intensity at 470 nm versus the equivalence of D- and L-histidine (Error bars from three independent experiments. λ_{exc} = 378 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer)

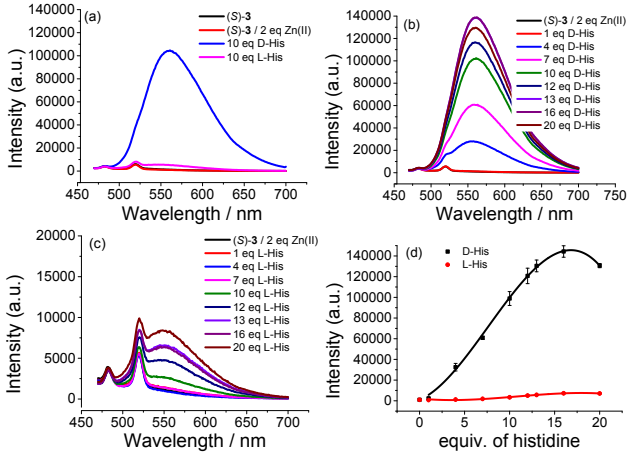


Figure 2. Fluorescence spectra of (*S*)-**3** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) with (a) D- and L-histidine (10.0 equiv), (b) D-histidine, and (c) L-histidine. (d) Fluorescence intensity at 560 nm versus the equivalence of D- and L-histidine (Error bars from three independent experiments. λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer)

The enantiomer of (*S*)-**3**, compound (*R*)-**3**, was also prepared from (*R*)-BINOL, and its fluorescence response toward D- and L-histidine was studied under the same conditions. As shown in Figure S2, a mirror-image relation was observed between the fluorescence responses of (*R*)-**3** and (*S*)-**3** toward the enantiomers of the histidine at λ_2, which confirms the inherent chiral recognition process.

We studied the interaction of both (*S*)- and (*R*)-**3** with histidine at various enantiomeric composition and plotted the fluorescence response of each enantiomeric probe at λ_2 = 560 nm versus the enantiomeric excess [ee = ([D]-[L])/([D]+[L])%] of histidine in Figure 3. The mirror-image relation was observed between the fluorescence responses of this enantiomeric probe pair. These plots can be used to determine the enantiomeric composition of histidine.

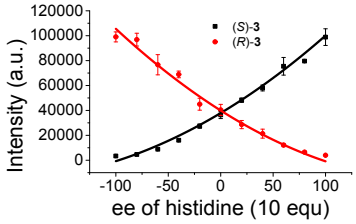


Figure 3. Fluorescence intensity of (*S*)-**3** or (*R*)-**3** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) at 560 nm versus the ee of histidine (10.0 equiv) (Error bars from three independent experiments. λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer)

We investigated the fluorescence responses of (*S*)-**3**+Zn(II) toward 17 enantiomeric pairs of common amino acids (including histidine). Under the same conditions, all 17 amino acids reduced the fluorescence of the probe at λ_1 = 470 nm (λ_{exc} = 378 nm) with no enantioselectivity (Figure S22 in SI). However, at λ_2 = 560 nm (λ_{exc} = 450 nm), only D-histidine showed greatly enhanced fluorescence (Figure 4). Thus, the probe (*S*)-**3**+Zn(II) is highly chemoselective and enantioselective for fluorescent recognition of histidine.

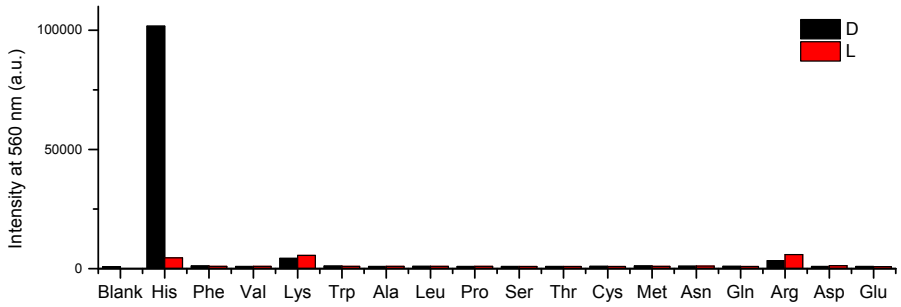


Figure 4. Fluorescence intensity at 560 nm of (*S*)-**3** (0.01 mM) + $Zn(OAc)_2$ (2.0 equiv) with 17 amino acids (10 equiv) (λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer).

As described above, when excited at 378 nm (Figure 1), the two enantiomers of histidine produced similar fluorescence quenching on (*S*)-**3**+Zn(II) at λ_1; however, when excited at 450 nm (Figure 2), one enantiomer of histidine greatly enhanced fluorescence of the probe at λ_2 while the other enantiomer did not. This indicates that the excitation at 378 nm should allow the determination of the histidine concentration, and the excitation at 450 nm should allow the determination of the histidine enantiomeric composition.[19] We thus measured the fluorescence intensities of (*S*)-**3**+Zn^{2+}, that is, I_{470} (λ_{exc} = 378 nm) and I_{560} (λ_{exc} = 450 nm), with D- and L-histidine at varying concentrations and ee's (Figure 5). As shown in Figure 5a, I_{470} is only correlated

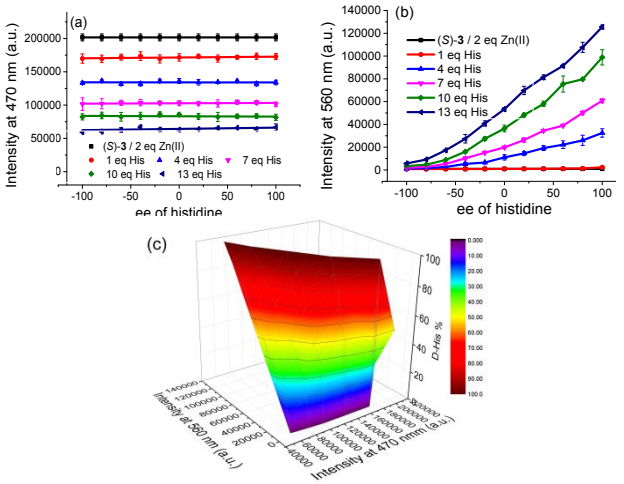


Figure 5. Fluorescence response of (*S*)-**3** (0.01 Mm) + $Zn(OAc)_2$ (2.0 equiv) toward histidine (0 - 13 equiv). (a) I_{470} (λ_{exc} = 378 nm) versus ee of histidine at varying concentration. (b) I_{560} (λ_{exc} = 450 nm) versus ee of histidine at varying concentration. (c) I_{470} versus I_{560} at varying [D-His]%. (Solvent: DMF/1% pH 6.35 phosphate buffer. All the data were obtained from three independent experiments. Slit: 5/5 nm)

with the histidine concentration but independent of its ee's which can be used to determine the total concentration of a histidine sample containing both enantiomers. Figure 5b shows that I_{560} is strongly influenced by both the concentration and ee's of histidine. Combining the data of Figure 5a,b, we obtained the 3D graph Figure 5c by plotting the [D-His]% versus the fluorescence intensities I_{470} and I_{560}. Therefore, we can determine the enantiomeric composition of a given histidine sample by using the fluorescence responses at the two wavelengths and this 3D plot.

We have applied the 3D plot Figure 5c to analyze the concentration and enantiomeric composition of 7 histidine samples. As the results summarized in Table 1 (see more details in Table S1 in SI) show, the values of [D-His]% and histidine concentration obtained from the fluorescent measurements had good agreement with the actual data.

Table 1. Using fluorescence measurements to determine the concentration and enantiomeric compositions of histidine samples.[a]

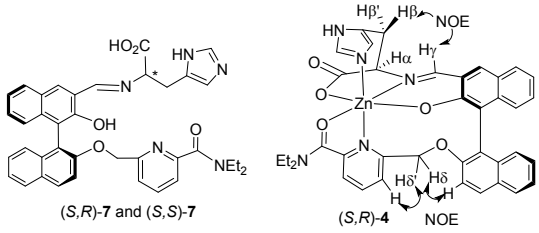
entry	[D-His]%		[His]/*10^{-5}M	
	actual	found	actual	found
1	80.0	75.1	3.50	3.69
2	45.0	46.2	4.50	4.92
3	70.0	71.9	5.50	6.05
4	30.0	28.9	7.50	7.54
5	65.0	69.3	8.50	8.32
6	25.0	25.3	9.50	9.67
7	55.0	56.3	11.50	11.08

a. The plots and conditions in Figure 5 were used. All the data were obtained by averaging three independent experiments.

We have synthesized and characterized the imine compounds (*S,R*)- and (*S,S*)-**7** from the condensation of (*S*)-**3** with L- and D-histidine respectively (see the synthesis in SI). Both of these diastereomeric imine compounds are found to form 1:1 complex with Zn(II) in the absence of a buffer (see Figure S3 in SI) with similar fluorescence enhancement at 550 nm. However, when these complexes were exposed to the pH 6.35 phosphate buffer used in the fluorescence measurement, the emission of (*S,S*)-**7** greatly diminished, but that of (*S,R*)-**7** was observed at much higher intensity though still reduced from that in the absence of a buffer (Figure S4). Thus, it is proposed that under the fluorescence measurement conditions, the Zn(II) complex of (*S,R*)-**7** should be more stable than that of (*S,S*)-**7**, giving the observed enantioselective fluorescent response at λ_2. The fluorescent quenching observed at λ_1 for the interaction of (*S*)-**3** with both D- and L-histidine should be due to the

consumption of (*S*)-**3** as it was converted to the corresponding imine-Zn(II) complexes.

We conducted COSY and NOESY NMR spectroscopic analyses on the Zn(II) complex of (*S,R*)-**7** in DMSO-d_6 solution (Figure S12-S15 in SI). It was found that the imine proton Hγ as shown in the proposed structure (*S,R*)-**4** exhibited stronger NOE effect with one of the diastereotopic hydrogens of Hβ and Hβ' than with the other one. This NOE effect was also stronger than that observed in the imine compound (*S,R*)-**7** in the absence of Zn(II). The NOE effects of Hδ/Hδ' in (*S,R*)-**4** with more aromatic hydrogens than those in (*S,R*)-**7** were also observed. These observations support the proposed multidented macrocyclic coordination of the histidine unit with the Zn(II) center as depicted in (*S,R*)-**4**. The mass spectrum (MALDI-TOF) of (*S,R*)-**4** was obtained (Figure S5) which gave a predominate signal at *m*/*z* = 704.1 consistent with the structure [calcd for (*S,R*)-**4**+H: 704.2). We were not able to obtain the single crystal of this complex for X-ray analysis yet at this stage, but are still working on getting more structural information about this compound. Molecular modeling study will be conducted in order to understand the stability difference of the complexes generated from the reaction of (*S*)-**3** with the enantiomers of histidine as well as other amino acids.



In summary, we have discovered a highly chemoselective and enantioselective fluorescent probe for histidine. Upon excitation at two different wavelengths, this probe exhibits very different fluorescent responses toward the enantiomers of histidine which allows the determination of both the concentration and enantiomeric composition of amino acids.

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Supplementary Information Available: Experimental procedures and additional spectroscopic data are provided.

Keywords: fluorescent probe, histidine, enantioselective, chemoselective, amino acids

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