Opposite Enantioselectivity of Mg(II) versus Zn(II) in the Fluorescent Recognition of Amino Acids

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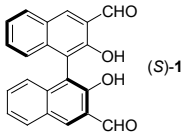
Abstract Addition of Mg^{2+} is found to turn on the fluorescent response of a molecular probe 3,3'-diformyl-1,1'-bi-2-naphthol toward chiral amino acids with high enantioselectivity. It is further found that the enantioselective fluorescence responses of the molecular probe in the presence of Mg^{2+} toward certain amino acids are the opposite of those in the presence of Zn^{2+}, that is, using Mg^{2+} with a L-amino acid generates much greater fluorescence enhancement than with the corresponding D-amino acid but using Zn^{2+} with the D-amino acid gives much greater fluorescence than with the L-enantiomer. Thus, simply changing the metal cation additive allows the chirality sense of the fluorescence-based molecular recognition to be easily regulated.

Introduction

Amino acids are essential components of life. Although naturally occurring amino acids mostly exist in their L-enantiomeric forms,[1] the past decade has witnessed significant growth in the discovery of the biological functions of D-amino acids in humans and other mammals.[2,3] Both enantiomers of chiral amino acids are useful starting materials or chirality sources for the asymmetric synthesis of structurally diverse organic compounds.[4] In our laboratory, we are interested in developing fluorescent sensors for the enantioselective recognition of chiral amino acids aiming at developing fast methods for the analysis of chiral amino acid products in the high throughput screening of asymmetric reactions as well as new imaging tools to monitor amino acid enantiomers in biological systems.[5-7]

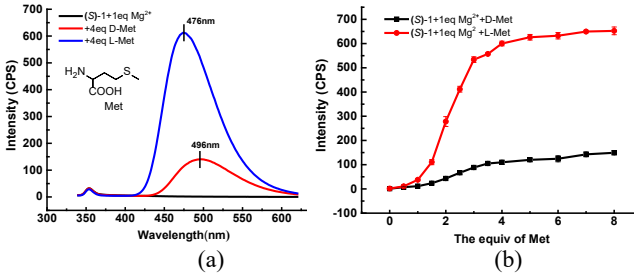
Previously, we reported that Zn^{2+} ions can be used to promote the enantioselective fluorescence enhancement of a 1,1'-bi-2-naphthol (BINOL)-based aldehyde (*S*)-**1** in the presence of functional chiral amines including amino acids.[5a] In the present study, we further found that Mg^{2+} ions can also enhance the fluorescence of (*S*)-**1** in the presence of amino acids with high enantioselectivity. For certain amino acids, (*S*)-**1** in combination with Mg^{2+} exhibits the opposite enantioselective fluorescent responses toward amino acids in comparison with the use of Zn^{2+}. This provides a new method to regulate the sense of chiral recognition in fluorescent sensing. Herein, these results are reported.



(*S*)-**1**

Results and Discussion

Compound (*S*)-**1** was synthesized in three steps from (*S*)-BINOL according to the literature.[8] In methanol solution (1.0×10^{-5} M, MeOH/1% CH_2Cl_2), (*S*)-**1** was not fluorescent. Addition of $MgCl_2$ (1.0 equiv) also caused no fluorescence response (Figure 1). However, when the tetrabutylammonium (TBA) salt of an amino acid L-Met (4.0 equiv, prepared by mixing with 2 equiv TBAOH) in MeOH was added to the (*S*)-**1**+Mg^{2+} solution, a large fluorescence enhancement (413 fold) at λ = 476 nm was observed (Figure 1a and S3a). We then treated the (*S*)-**1**+Mg^{2+} solution with the enantiomer D-Met under the same conditions, a smaller fluorescence (135 fold) enhancement at a different wavelength (λ = 496 nm) was observed (Fig. 1a and S3b). The enantiomeric fluorescence enhancement ratio [*ef* = (I_L-I_0)/(I_D-I_0). I_0, I_L, I_D: fluorescence intensity of (*S*)-**1**+Mg^{2+} without or with L- and D-Met respectively] for the amino acid was found to be 5.3. Figure 1b plots the fluorescence intensity at λ = 476 nm, I_{476}, versus the concentration of the amino acid. It shows that the enantioselectivity in the fluorescence response of the probe toward the amino acids reached maximum when 4.0 equiv of Met was used and this enantioselectivity was maintained as the concentration of the amino acid further increased.



(a) (b)

Figure 1. (a) Fluorescence spectra of (*S*)-**1**+Mg^{2+} (1 equiv) (1.0×10^{-5} M in methanol/1% CH_2Cl_2) in the presence of L- and D-Met-TBA (4.0 equiv). (b) Fluorescence intensity at λ = 476 nm versus the concentration of L- and D-Met-TBA (λ_{exc} = 320 nm, slit = 5/5 nm).

We also prepared (*R*)-**1**, the enantiomer of (*S*)-**1**, and studied the fluorescent responses of its solution with 1 equiv Mg^{2+} in

methanol toward L- and D-Met. The expected mirror image relationship with Fig. 1 was observed which confirmed the observed enantioselective fluorescent recognition (Fig. S4).

The fluorescence intensity ratio I_{476}/I_{496} versus the enantiomeric composition of the chiral amino acid was plotted. Figure 2 shows a linear correlation (R^2 = 0.9956) between the I_{476}/I_{496} and the percent of L-Met in the enantiomeric mixture. Thus, the fluorescent probe (*S*)-**1**+Mg^{2+} can be used to determine the enantiomeric composition of this amino acid.

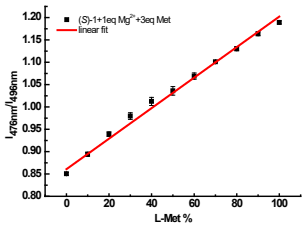
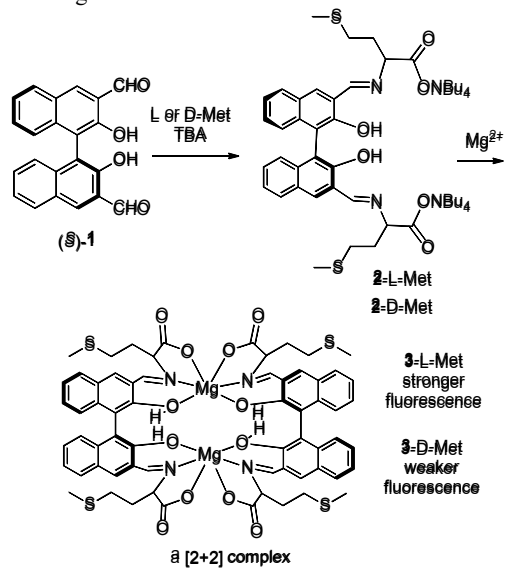


Figure 2. The fluorescence intensity ratio I_{476}/I_{496} of (*S*)-**1**+Mg^{2+} (1 equiv.) (1.0×10^{-5} M in methanol/1% CH_2Cl_2) versus the enantiomeric purity of Met (3 equiv)

A proposed reaction of (*S*)-**1** with the amino acid in the presence of Mg^{2+} based on our NMR and mass spectroscopic studies (*vide infra*) is depicted in Scheme 1. When (*S*)-**1** was treated with an excess amount of L- or D-Met-TBA, condensation of the aldehyde groups of (*S*)-**1** with the amine groups of the amino acid can give the corresponding imine products **2**-L-Met and **2**-D-Met with the characteristic imine proton NMR signal observed at 8.75 ppm in the ^{1}H NMR spectra in Figure 3b,d. These two diastereomers gave almost identical ^{1}H NMR signals in Figure 3b,d. When **2**-L-Met (Figure 3b) was treated with one equiv Mg^{2+}, ~90% of it was converted to a new product **3**-L-Met whose ^{1}H NMR spectrum in Figure 3c shows a symmetric structure with the imine proton signal shifted to 8.44 ppm. Mass spectrum (MALDI-TOF) of this product mixture gave an intense peak at m/z = 2010.18 which can be assigned to the [2+2] complex **3**-L-Met (calcd for **3**+3NBu_4^++CH_3OH-2H^+: 2010.15) (Figure S6a in SI). When **2**-D-Met (Figure 3d) was treated with one equiv Mg^{2+}, there was only ~50% conversion to a new product **3**-D-Met

Scheme 1. Proposed reactions of (*S*)-**1** with L- and D-Met in the presence of Mg^{2+}.



(Figure 3e). The ^{1}H NMR spectrum in Figure 3e also shows a symmetric structure with the imine proton signal shifted to 8.41 ppm. Mass spectrum (MALDI-TOF) of this product mixture gave a significant peak at m/z = 1328.26 which can be assigned to the [2+2] complex **3**-D-Met (calcd for **3**+3H_2O+Na^+-H: 1328.29) (Figure S6b in SI).

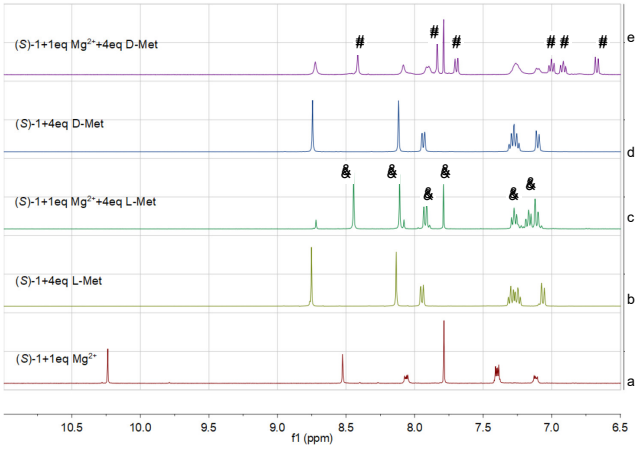
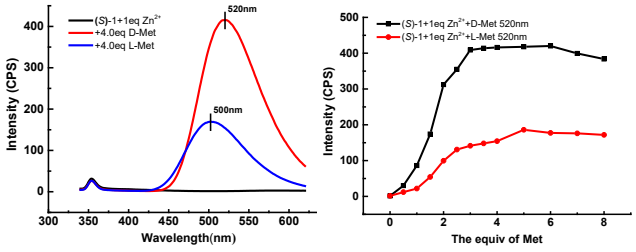


Figure 3. ^{1}H NMR (400 MHz) spectra of (*S*)-**1** (5.0 mM) with 4 equiv of L-Met-TBA or D-Met-TBA in the absence of Mg^{2+} in CD_3OD and in the presence of Mg^{2+} in $CD_3OD/CDCl_3$ (4:1). (&: **3**-L-Met. #: **3**-D-Met.)

Formation of both **3**-L-Met and **3**-D-Met greatly enhanced the fluorescence of (*S*)-**1** from the reaction with the amino acid in the presence of Mg^{2+}. The observed greater fluorescence enhancement with L-Met than with D-Met may be due to the following two factors: (1) More of **3**-L-Met was generated in the reaction than **3**-D-Met under the same conditions as shown by the ^{1}H NMR study; (2) **3**-L-Met could have more rigid structure with inherently stronger fluorescence than **3**-D-Met.

We have compared the fluorescence responses of (*S*)-**1**+Mg^{2+} toward L- and D-Met with those of (*S*)-**1**+Zn^{2+}. As shown in Figure 4, when the methanol solution of (*S*)-**1**+Zn^{2+} was treated with the amino acid, D-Met-TBA greatly enhanced the fluorescence at λ = 520 nm but L-Met-TBA gave much smaller enhancement at λ = 500 nm. Thus, (*S*)-**1**+Zn^{2+} displayed an opposite chirality sense in its fluorescence response toward the enantiomers of the amino acid in comparison with (*S*)-**1**+Mg^{2+}.

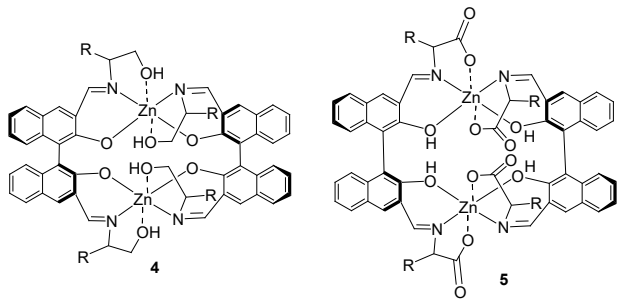


(a) (b)

Figure 4. (a) Fluorescence spectra of (*S*)-**1**+Zn^{2+} (1.0 equiv) (1.0×10^{-5} M in methanol/1% CH_2Cl_2) in the presence of L- and D-Met-TBA (4.0 equiv) [prepared by mixing Met and TBAOH (1:2) in MeOH]. (b) Fluorescence intensity at λ = 520 nm versus the concentration of Met (λ_{exc} = 320 nm, slit = 5/5 nm.).

Previously, we have demonstrated that the condensation product of (*S*)-**1** with an amino alcohol or an amino acid reacts with 1 equiv Zn^{2+} to give the [2+2] complexes like **4**[9] and **5**.[10] The mass spectra of the mixtures of (*S*)-**1**+Zn^{2+} with L- and D-Met also indicate the formation of [2+2] complex (Figure S7 in SI).

The enantioselective fluorescent responses of (*S*)-**1**+Zn^{2+} toward these substrates were attributed to the difference in the stability and structural rigidity of the diastereomeric complexes of **4** and **5**.



We have further compared the fluorescence responses of (*S*)-**1**+Mg^{2+} with those of (*S*)-**1**+Zn^{2+} toward other amino acids. As shown in Figure 5, these two metal ions lead to opposite enantioselective fluorescent enhancement with amino acids such as Phe, Trp, Tyr, Cystine, Lys, Arg, and Ala. Figure S5 shows that only Mg^{2+} or Zn^{2+} can greatly enhance the fluorescence of (*S*)-**1** with an amino acid but not other common metal ions.

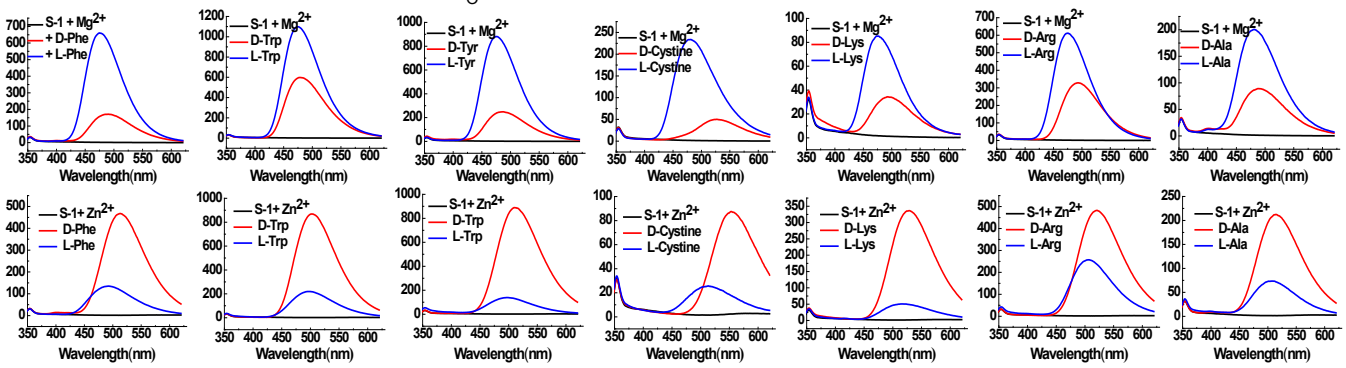


Figure 5. Fluorescence spectra of (*S*)-**1**+Mg^{2+} (1.0 equiv) (first line) or (*S*)-**1**+Zn^{2+} (1.0 equiv) (second line) (1.0×10^{-5} M in methanol/1% CH_2Cl_2) in the presence of various amino acid enantiomers (5.0 equiv, TBA salt prepared by mixing an amino acid and TBAOH (1:2) in MeOH].

Conclusion

We have discovered that in the presence of Mg^{2+}, the BINOL-based dialdehyde (*S*)-**1** exhibits highly enantioselective fluorescent response toward amino acids. The effect of Mg^{2+} is found to be the opposite of Zn^{2+} in the fluorescence response toward the enantiomers of eight amino acids. That is, (*S*)-**1**+Mg^{2+} shows much greater fluorescence enhancement with a L-amino acid than with its D-enantiomer, but (*S*)-**1**+Zn^{2+} shows much greater fluorescence enhancement with the D-enantiomer than with the L-enantiomer. This discovery demonstrates that simply changing the metal ion can inverse the chirality sense in the fluorescence response of the probe toward the substrates. It provides a new method to regulate the BINOL-based fluorescent probe in chiral discrimination.

Experimental Section

General Information: Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All solvents for the optical spectroscopic studies were either HPLC- or spectroscopic-grade.

Preparation of samples for fluorescence measurement. Stock solutions of (*S*)-**1** (1.0×10^{-3} M, CH_2Cl_2), $MgCl_2$ (1.0×10^{-3} M, CH_3OH), $Zn(OAc)_2$ (1.0×10^{-3} M, CH_3OH) and various amino acids (5.0×10^{-3} M, with 2 equiv TBAOH, CH_3OH) were freshly prepared for each measurement. For fluorescence measurement, a portion of the (*S*)-**1** (25 μL) solution was mixed with various equiv of the amino acid solution in a 10 mL test tube. The resulting solution was allowed to stand at rt for 2 h before the addition of the $MgCl_2$ (25 μL) or $Zn(OAc)_2$ (25 μL) solution. The mixture was set at rt for 1.5 h before being diluted to the desired concentration (1.0×10^{-5} M) with the addition of methanol. All the fluorescence spectra were taken within 2 h.

NMR Experiments. (*S*)-**1** + Mg^{2+} + 4 equiv L-/D-Met in $CDCl_3/CD_3OD$ (4:1): To a NMR tube containing (*S*)-**1** (100 μL, 25 mM in $CDCl_3$) was added 0 or 100 μL L-/D-Met-TBA (0.1 M in CD_3OD). The total volume was made up to 0.45 mL with CD_3OD. The resulting solution was allowed to stand at rt for 2 h before addition of $MgCl_2$ (50 μL, 50 mM in CD_3OD). The final concentration of the probe was 5 mM in $CDCl_3/CD_3OD$ (1:4). The resulting solution was allowed to stand at rt for 1.5 h before measurement. (*S*)-**1** + 4 equiv L-/D-Met in CD_3OD: To a NMR tube containing (*S*)-**1** (1.7 mg, 5 μmol) was added 200 μL L-/D-Met-TBA (0.1 M in CD_3OD). The total volume was made up to 1 mL. The final concentration of the probe was 5 mM in CD_3OD. The resulting solution was allowed to stand at rt for 2 h before measurement.

Supplementary Materials Available: Additional fluorescence spectra, NMR and MS spectra. This material is available free of charge via the internet at http://pubs.acs.org.

Keywords: fluorescent sensing amino acids magnesium(II) zinc(II) enantioselective

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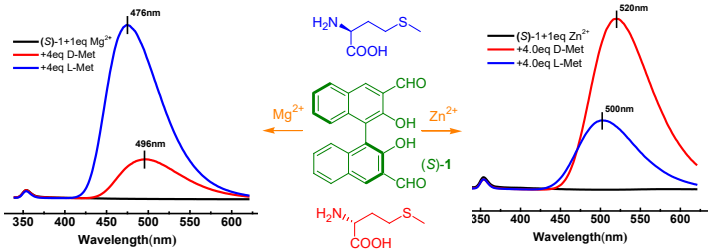
TOC Graph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Wavelength(nm)
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COOH
CHO
OH
Mg2+
Zn2+
OH
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