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Supporting information for

Determine Concentration and Enantiomeric Composition of Histidine by One Fluorescent Probe

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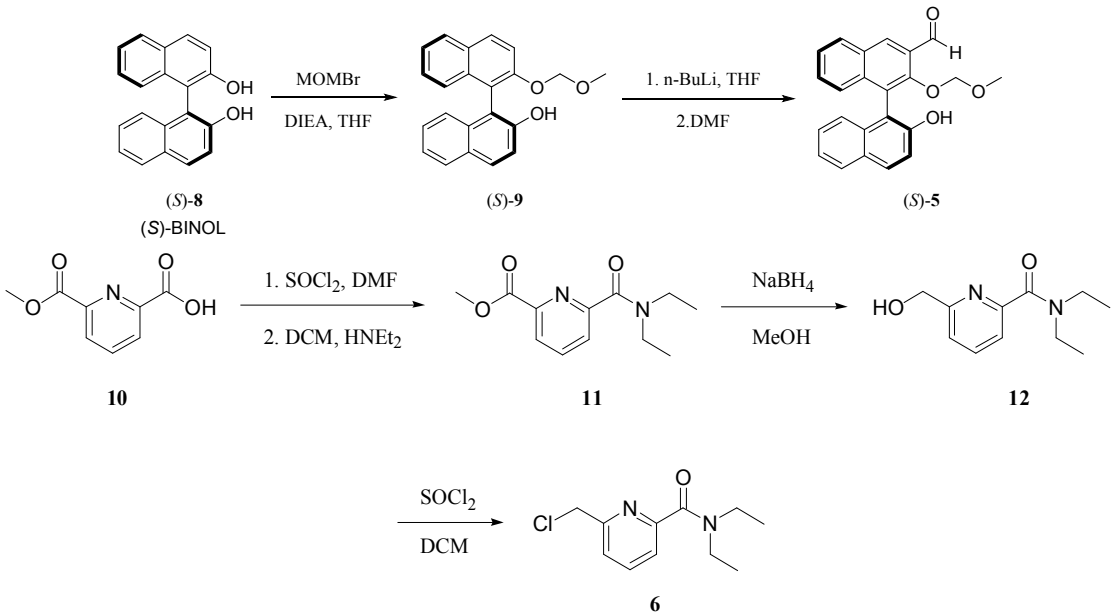
1. General Procedure of the Sample Preparation for Fluorescence Measurement

The following stock solutions were freshly prepared for each measurement: (*S*)-**3** (1.0 mM) in DMF, an amino acid (20 mM) in phosphate bu□er (50 mM, pH = 6.35), and $Zn(OAc)_2$ (4.0 mM) in deionized water. In an measurement, 15 μL $Zn(OAc)_2$ (2 equiv) and 15 μL amino acid (10 equiv or other quantity) were mixed and 30 μL (*S*)-**3** was then added. The resulting solution was allowed to stand at room temperature for 2 h which was then transferred to an ice bath to chill for 30 min. This was done to avoid the potential decomposition of the reaction product during the dilution since the subsequent mixing of an aqueous solution with an organic solvent was found to be exothermic. To the above solution, DMF (prechilled at 0 ^{o}C) was added to dilute it to 10 μM of (*S*)-**3** and the solution was maintained in ice bath for additional 30 min. Then the solution was taken out from the ice bath and was allowed to warm up to room temperature in 30 min, and the fluorescence spectra were recorded at room temperature.

2. Synthesis and characterization of compounds

2.1. General data and synthesis of compounds (*S*)-**5**[S1] and **6**[S2].

General Data. The commercially available compounds were from Sigma Aldrich Chemical Co. or Alfa Aesar. All solvents used in the fluorescence measurement were HPLC or spectroscopic grades. NMR spectra were recorded on a Varian-600 MHz spectrometer, a Bruker-600 MHz spectrometer, and a Bruker-800 MHz spectrometer. Optical rotation measurements were conducted on a Jasco P-2000 digital polarimeter. Chemical shifts for ^{1}H NMR spectra were recorded in parts per million relative to solvent signals at 7.26 ppm for $CDCl_3$, and 2.50 ppm for DMSO-d_6. Chemical shifts for ^{13}C NMR spectra were recorded relative to the centerline of a triplet at 77.16 ppm for $CDCl_3$. Mass spectroscopic analyses were conducted by the University of Illinois at Urbana-Champaign Mass Spectrometry Facility. Steady-state fluorescence spectra were recorded with a Horiba FluoroMax-4 spectrofluorometer. UV-Vis spectra were measured by Shimadzu UV-2600 UV-Vis spectrometer.



Scheme S1.

Synthesis and characterization of compound (*S*)-9. Under nitrogen, (*S*)-**8** (4.29 g, 15 mmol), N,N-diisopropylethylamine (DIEA, 1.4 mL, 1.1 equiv) were dissolved in THF (dry, 30 mL). Then the solution was cooled to 0 ^{o}C and bromomethyl methyl ether (2.06 g in 10 mL THF) was added dropwise in 40 min at 0 ^{o}C. Then the solution was warmed up to rt and was stirred overnight. After being quenched by a.q. $NaHCO_3$ (saturated, 20 mL), THF was removed by rotary-evaporation. CH_2Cl_2 (100 mL) was added to dissolve the residue, and the solution was washed with water (2 x 100 mL) and dried with Na_2SO_4. CH_2Cl_2 was then removed by roto-evaporation. The residue was purified by column chromatography on silica gel, gradient eluted with hexane/ethyl acetate (1/10, v) to give (*S*)-**9** as a white solid in 71% yield (3.61 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, 1H), 7.91 (d, 2H), 7.85 (d, 1H), 7.59 (d, 1H), 7.39 (t, 1H), 7.35 (d, 1H), 7.30-7.27 (m, 2H), 7.23-7.18 (m, 2H), 7.07 (d, 1H), 5.07 (dd, 2H), 4.95 (OH, s, 1H), 3.17 (s, 3H)

Synthesis and characterization of compound (*S*)-5. Under nitrogen, (*S*)-**9** (3.53 g, 10.7 mmol) was dissolved in 30 mL dried THF and cooled to -78 ^{o}C. n-Butyllithium solution (8.5 mL of 2.5 M hexane solution, mixed with 10 mL THF, 2 equiv) was added dropwise and the solution was warmed up to rt and stirred for 4 h. The solution was cooled to -78 ^{o}C again and DMF (2.1 mL, 2.1 equiv) was added dropwise which was then warmed up to rt. The solution was stirred overnight at rt. Aqueous NH_4Cl (saturated, 20 mL) was added to quench the reaction and THF was removed by rotary-evaporation. The residue was dissolved in CH_2Cl_2 (200 mL) which was washed two times with water (300 mL) and dried with Na_2SO_4. CH_2Cl_2 was then removed by rotary-evaporation. The residue was purified by column chromatography on silica gel, gradient eluted with CH_2Cl_2/ethyl acetate (40/1, v) to give (*S*)-**5** as a light-yellow solid. The product was further purified by recrystallization in CH_2Cl_2/hexanes to give (*S*)-**5** as light-yellow crystals in 45% yield

(1.45 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.55 (s, 1H), 8.61 (s, 1H), 8.07 (d, 1H), 7.94 (d, 1H), 7.87 (d, 1H), 7.51 (t, 1H), 7.41 (t, 1H), 7.36-7.30 (m, 2H), 7.30-7.20 (m, 2H), 7.06 (d, 1H), 5.07 (OH, s, 1H), 4.72 (dd, 2H), 3.01 (s, 3H)

Synthesis and characterization of compound 11. Under nitrogen, compound **10** (1.00 g, 5.52 mmol) was cooled to 0 °C. $SOCl_2$ (10 mL) was slowly added and one drop dried DMF was also added. Then the solution was heated to 60 °C and stirred for 1 h until all solid was dissolved. $SOCl_2$ was removed under reduced pressure and the residue was dissolved in dry CH_2Cl_2 (15 mL). The solution was cooled to 0 °C and diethylamine (2.1 mL, 20.8 mmol) was added. After the solution was heated at 40 °C with stirring for 2 h, it was quenched with a.q. NH_4Cl (saturated, 15 mL) and washed two times with water (50 mL). CH_2Cl_2 was then removed by rotary-evaporation to give compound **11** as a brown oil in ~100% yield (1.33 g). Product was pure enough for the next step reaction without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (d, 1H), 7.90 (t, 1H), 7.80 (d, 1H), 3.96 (s, 3H), 3.53 (q, 2H), 3.38 (q, 2H), 1.24 (t, 3H), 1.22 (t, 3H). ^{13}C{^{1}H} NMR (151MHz, $CDCl_3$) δ 167.35, 165.30, 154.92, 146.32, 137.92, 126.76, 125.48, 52.82, 43.57, 40.68, 14.20, 12.77.

Synthesis and characterization of compound 12. Under nitrogen, compound **11** (1.96 g, 8.28 mmol) was dissolved in dry MeOH (36 mL). $NaBH_4$ (2.63 g, 8.4 equiv) was added slowly and the mixture was heated at reflux for overnight. Then a.q. $NaHCO_3$ (saturated, 20 mL) was added to quench the reaction, and MeOH was removed by rotary-evaporation. CH_2Cl_2 (200 mL) was added to dissolve the mixture and washed two times with water (200 mL). Removal of CH_2Cl_2 by rotary-evaporation gave compound **12** as a light-brown solid in 61% (1.059 g). ^{1}H NMR (600 MHz,

$CDCl_3$) δ 7.75 (t, 1H), 7.44 (d, 1H), 7.30(d, 1H), 4.74 (s, 2H), 4.0-3.0 (broad, OH, 1H), 3.56 (q, 2H), 3.31 (q, 2H), 1.26 (t, 3H), 1.16 (t, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 168.45, 158.48, 154.02, 137.73, 121.54, 120.91, 64.22, 43.30, 40.21, 14.48, 12.95.

Synthesis and characterization of compound 6. Under nitrogen, compound **12** (660 mg, 3.17 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and cooled to 0 °C. $SOCl_2$ (0.58 mL, dissolved in 50 mL CH_2Cl_2, 2.5 equiv) was added. The solution was warmed up to rt and stirred overnight. Then 10 mL ice water was added and the solution was washed with water (50 mL) and sat. $NaHCO_3$ (50 mL). The organic phase was separated and dried with Na_2SO_4. After filtration, CH_2Cl_2 was removed by rotary-evaporation to give compound **6** as a light-yellow oil in ~100% yield (729 mg). The product was pure enough for the next step reaction without further purification. ^1H NMR (600 MHz, $CDCl_3$) δ 7.80 (t, 1H), 7.54 (d, 1H), 7.50 (d, 1H), 4.66 (s, 2H), 3.56 (q, 2H), 3.36 (q, 2H), 1.27 (t, 3H), 1.20 (t, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 168.12, 155.44, 154.81, 138.06, 123.23, 122.52, 46.57, 43.51, 40.49, 14.43, 12.97.

2.2. Synthesis and characterization of (*S*)-**3** and (*R*)-**3**

Synthesis and characterization of (*S*)-3. (*S*)-**5** (500 mg, 1.4 mmol), compound **6** (333 mg, 1.05 equiv) and K_2CO_3 (1.54 g, 8 equiv) were dissolved in CH_3CN (25 mL) and the solution was bubbled with N_2 for 15 min. After the solution was heated under nitrogen to reflux with stirring for overnight, it was cooled to rt and filtered to remove the solid. The filtrate was dried with Na_2SO_4 and the solvent was removed to give the intermediate. Under nitrogen, the intermediate was dissolved in dry MeOH (10 mL). HCl (4.1 mL, 2.0 M Et_2O solution, 6 equiv) was added. The solution was stirred for overnight and then quenched with sat. $NaHCO_3$ (10 mL). After MeOH

was removed by rotary-evaporation, CH_2Cl_2 (50 mL) was added and washed with sat. $NaHCO_3$ (50 mL) and dried with Na_2SO_4. CH_2Cl_2 was then removed by rotary-evaporation to yield a yellow solid which was purified by column chromatography on silica gel, gradient eluted with CH_2Cl_2/MeOH to give (*S*)-**3** as a yellow solid in 91% yield (642 mg). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.49 (s, 1H), 10.21 (s, OH, 1H), 8.33 (s, 1H), 8.00 (m, 1H), 7.95 (d, 1H), 7.88 (d, 2H), 7.47 (t, 1H), 7.44-7.31 (m, 5H), 7.28 (m, 1H), 7.23-7.18 (m, 2H), 6.91 (d, 1H), 5.28 (d, 1H), 5.22 (d, 1H), 3.54 (q, 2H), 3.26 (q, 2H), 1.26 (t, 3H), 1.12 (t, 3H). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 196.88, 168.45, 156.61, 154.21, 153.75, 153.59, 137.97, 137.50, 133.77, 130.50, 130.29, 129.89, 129.61, 128.32, 127.65, 126.95, 125.45, 124.97, 124.45, 124.17, 122.21, 121.52, 121.27, 118.60, 118.16, 115.01, 71.44, 43.31, 40.29, 14.36, 12.96. HRMS: m/z calcd. for $C_{32}H_{29}N_2O_4$ $[M + H]^+$: 505.2127, found 505.2119. $[\alpha]_D^{23} = -88.1$ (c = 0.2, $CHCl_3$).

Synthesis and characterization of (*R*)-3. (*R*)-**3** was synthesized starting from (*R*)-BINOL by using the same procedures as the preparation of (*S*)-**3**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.49 (s, 1H), 10.21 (s, OH, 1H), 8.34 (s, 1H), 8.00 (m, 1H), 7.95 (d, 1H), 7.88 (d, 2H), 7.48 (t, 1H), 7.44-7.31 (m, 5H), 7.28 (m, 1H), 7.23-7.18 (m, 2H), 6.91 (d, 1H), 5.28 (d, 1H), 5.22 (d, 1H), 3.54 (q, 2H), 3.26 (q, 2H), 1.26 (t, 3H), 1.12 (t, 3H). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 196.90, 168.36, 156.61, 154.14, 153.75, 153.63, 138.00, 137.66, 133.79, 130.55, 130.34, 129.91, 129.65, 128.36, 127.69, 126.99, 125.50, 125.00, 124.50, 124.21, 122.25, 121.59, 121.36, 118.65, 118.17, 115.02, 71.37, 43.35, 40.33, 14.39, 12.99. HRMS: m/z calcd. for $C_{32}H_{29}N_2O_4$ $[M + H]^+$: 505.2127, found 505.2125. $[\alpha]_D^{23} = 87.5$ (c = 0.2, $CHCl_3$)

2.3. Synthesis and characterization of (*S*,*R*)-**7** and (*S*,*S*)-**7**

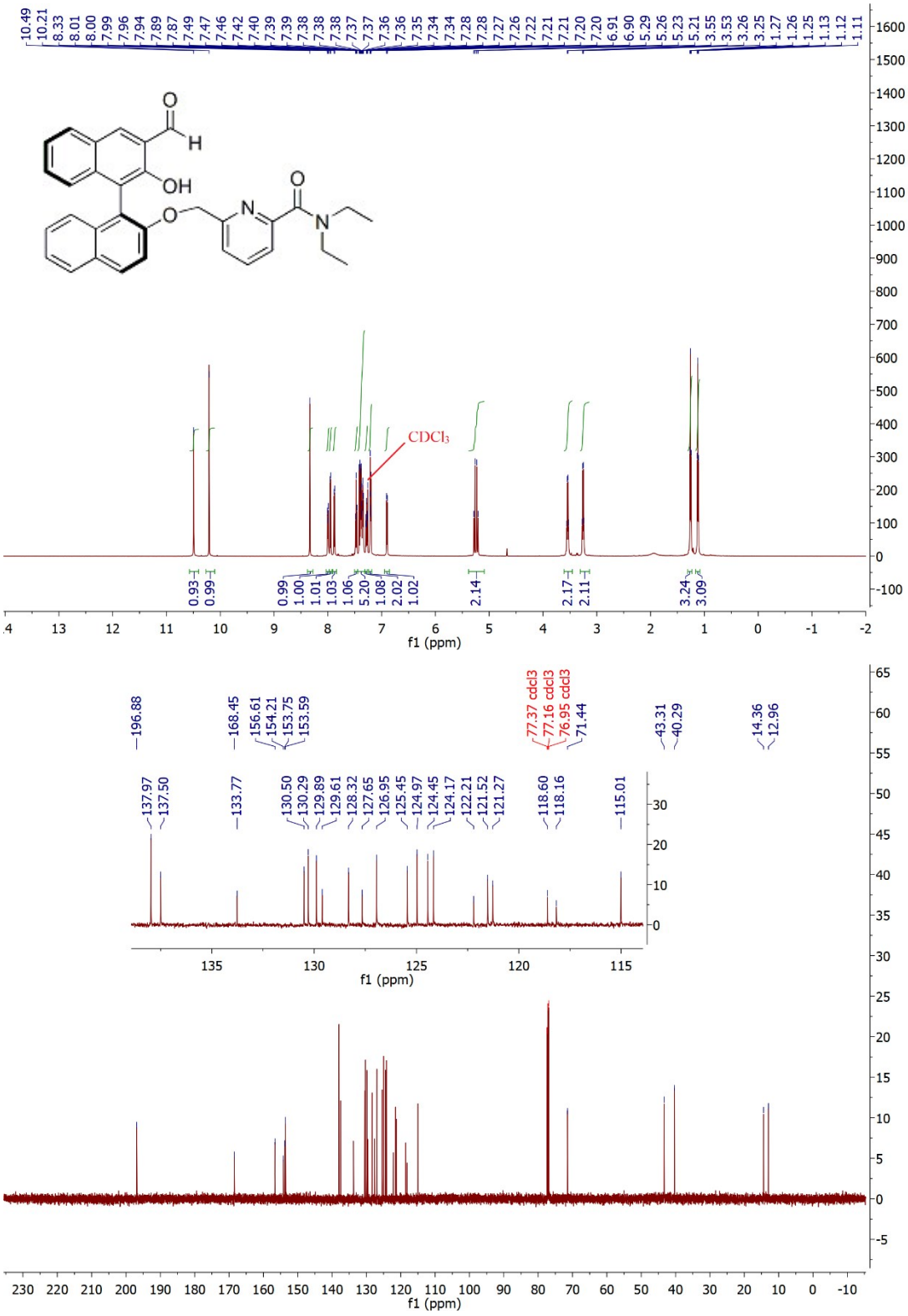
Synthesis and characterization of (*S*,*R*)-7. Under nitrogen, (*S*)-**3** (200 mg, 0.40 mmol) and D-histidine (68 mg, 1.1 equiv) was added into dry MeOH (10 mL). After the reaction mixture was stirred for 2 d, it was filtered to remove the solid. The solvent was then removed by rotary-evaporation and the residue was dissolved in a minimum amount of CH_2Cl_2. The solution was added to Et_2O (20 mL) to generate a pink solid which was collected by filtration and dried to give (*S*,*R*)-**7** as a pink powder in 92% yield (172 mg) [52.8 mg (*S*)-**3** was recovered]. ^{1}H NMR (600 MHz, DMSO-D^6) δ 13.20 (s, COOH, 1H), 8.66 (s, 1H), 8.19 (s, 1H), 8.03 (d, 1H), 8.00-7.92 (m, 2H), 7.62-7.55 (m, 3H), 7.35 (t, 1H), 7.33-7.24 (m, 4H), 7.04 (d, 1H), 6.98-6.92 (m, 2H), 6.76 (s, 1H), 5.27 (d, 1H), 5.20 (d, 1H), 4.45 (dd, 1H), 3.39 (q, 2H), 3.20 (dd, 1H), 3.10 (q, 2H), 3.01 (dd, 1H), 1.12 (t, 3H), 0.98 (t, 3H). ^{13}C{^{1}H} NMR (200 MHz, DMSO-d_6) δ 172.24, 167.56, 167.46, 156.09, 154.25, 154.01, 153.38, 137.62, 134.81, 133.73, 133.37, 129.39, 129.07, 128.98, 128.36, 128.06, 126.94, 126.55, 124.66, 124.11, 123.64, 123.21, 121.09, 121.03, 120.53, 118.74, 116.91, 116.31, 115.13, 70.48, 70.14, 54.92, 42.52, 31.09, 14.08, 12.80. HRMS: m/z calcd. for $C_{38}H_{36}N_5O_5$ $[M + H]^+$: 642.2716, found 642.2715.

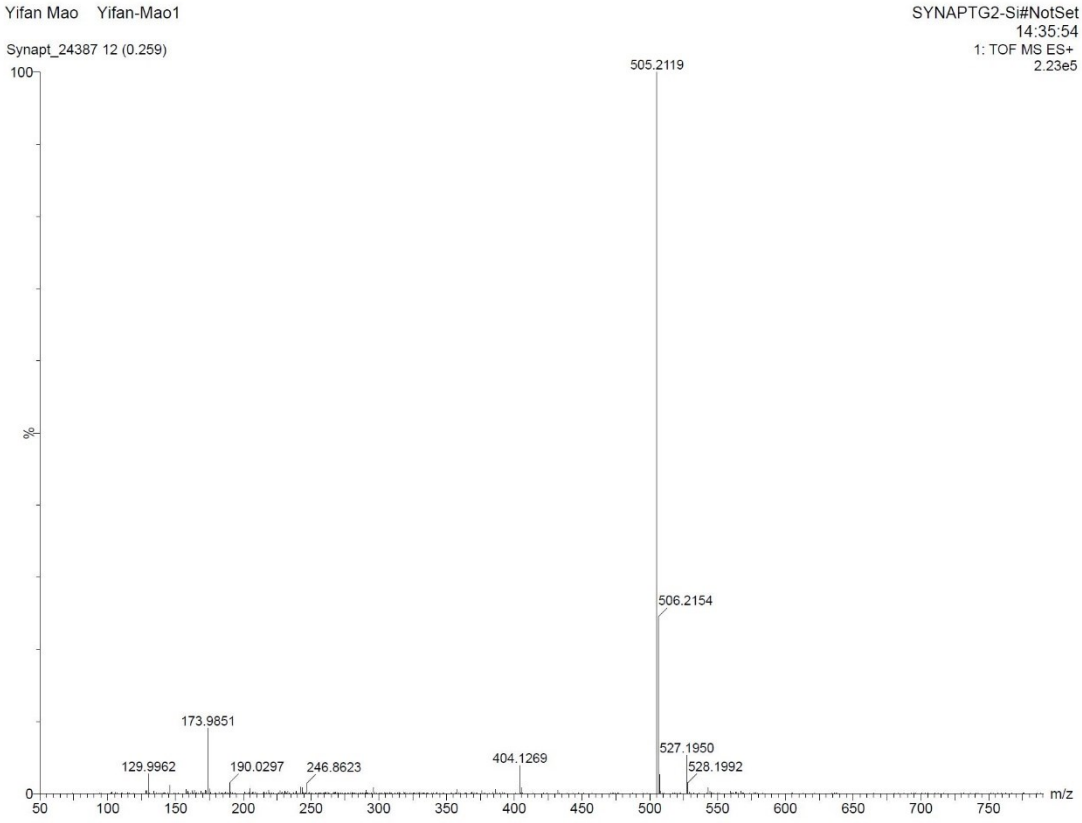
Synthesis and characterization of (*S*,*S*)-7. (*S*,*S*)-**7** was obtained as a light-yellow powder from the reaction of (*S*)-**3** with L-histidine by using the same procedure as the preparation of (*S*,*R*)-**7**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.11 (s, COOH, 1H), 8.66 (s, 1H), 8.20 (s, 1H), 8.03 (d, 1H), 7.99 (d, 1H), 7.95 (d, 1H), 7.62-7.54 (m, 3H), 7.38-7.28 (m, 4H). 7.26 (t, 1H), 7.04 (d, 1H), 6.96 (d, 1H), 6.92 (d, 1H), 6.73 (s, 1H), 5.28 (d, 1H), 5.22 (d, 1H), 4.47 (dd, 1H), 3.41 (q, 2H), 3.19 (dd, 1H), 3.13 (q, 2H), 3.01 (dd, 1H), 1.10 (t, 3H), 1.00 (t, 3H). ^{13}C{^{1}H} NMR (200 MHz, DMSO-d_6) δ 172.17, 167.65, 167.46, 156.18, 154.17, 154.06, 153.34, 137.61 134.83, 133.77, 133.35, 129.38,

129.07, 129.00, 128.38, 128.02, 126.94, 126.57, 124.62, 124.13, 123.68, 123.24, 121.02, 120.90, 120.51, 118.79, 116.71, 116.33, 115.20, 70.48, 69.99, 48.60, 42.52, 31.17, 14.08, 12.79. HRMS: m/z calcd. for $C_{38}H_{36}N_5O_5$ $[M + H]^+$: 642.2716, found 642.2720.

2.4. ^{1}H NMR, ^{13}C NMR and HRMS spectra of (*S*)-**3**, (*R*)-**3**, (*S*,*R*)-**7** and (*S*,*S*)-**7**

(*S*)-**3**

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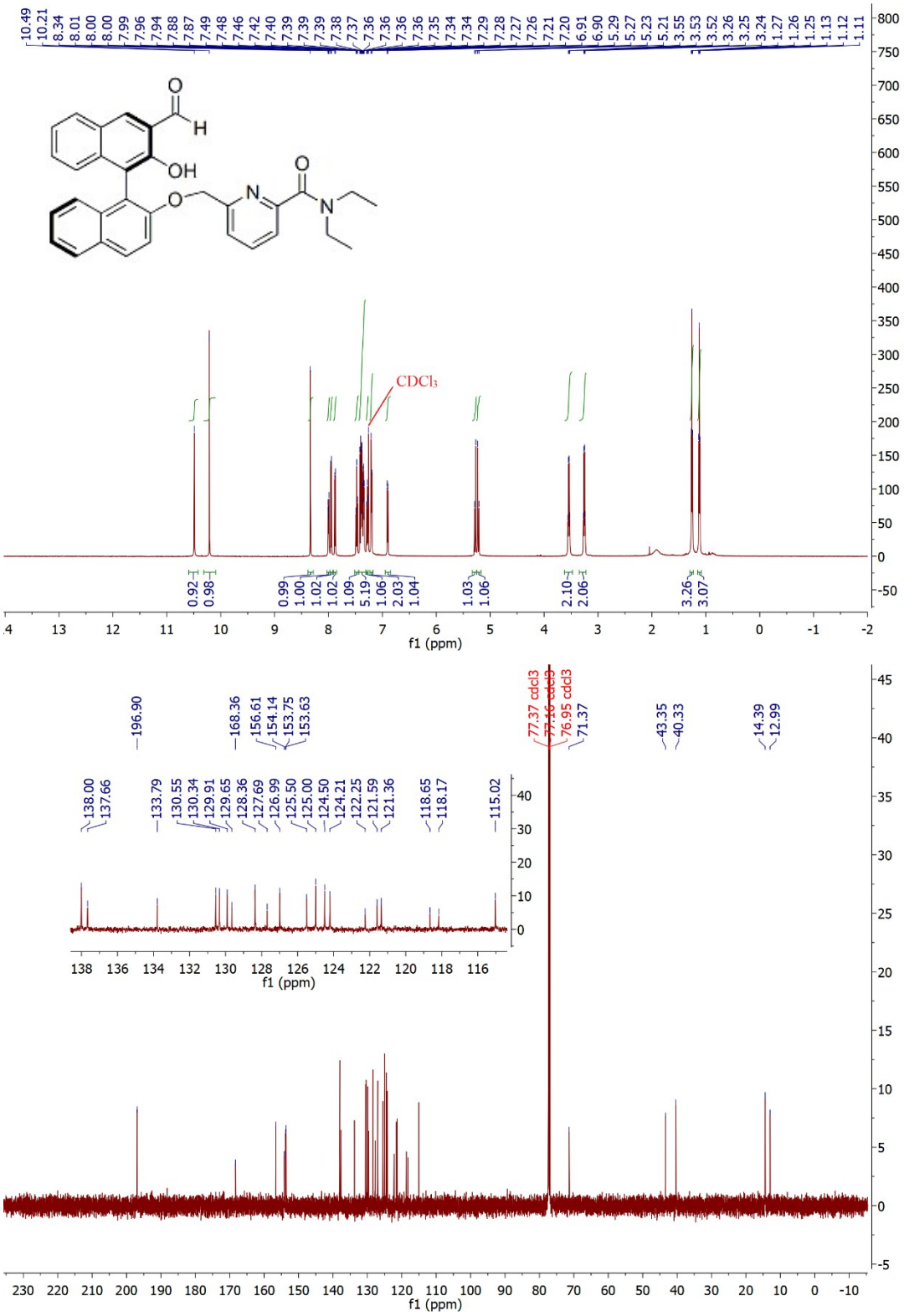
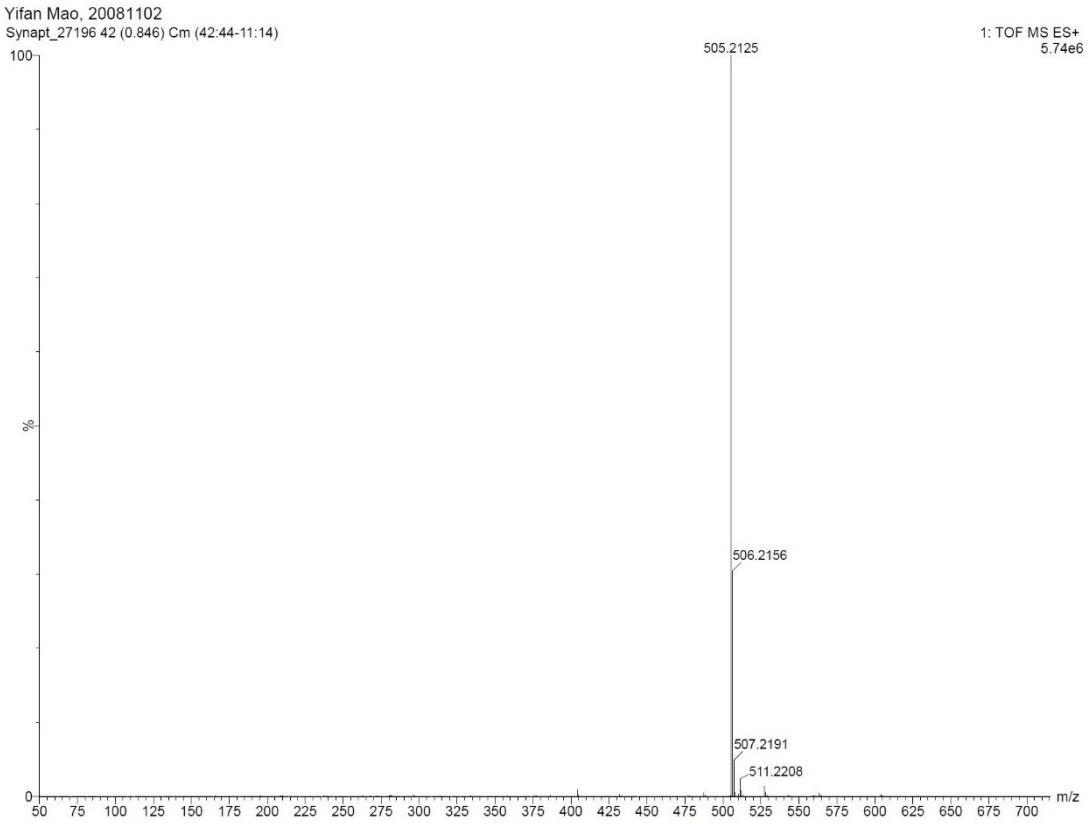
(*R*)-**3**

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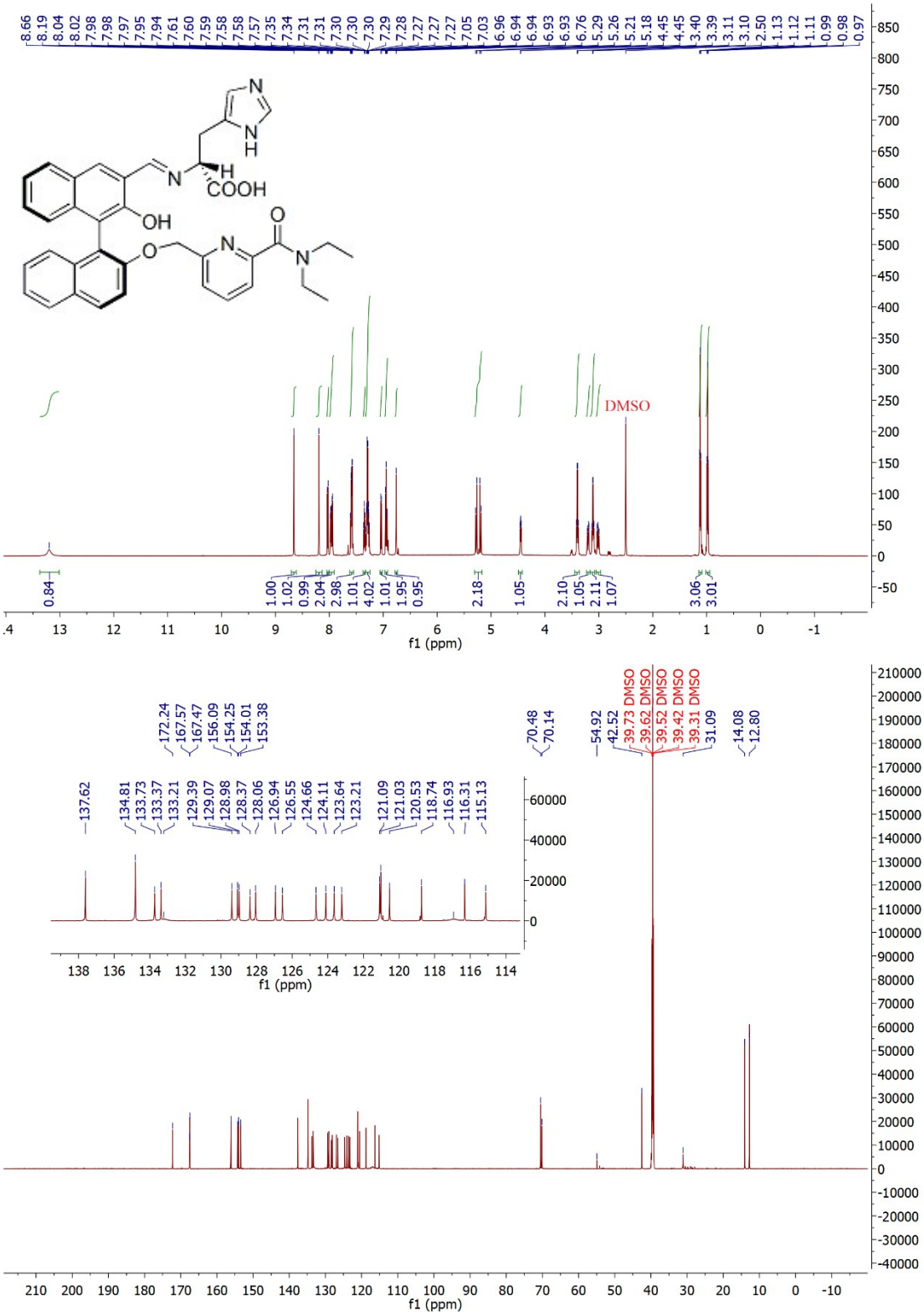
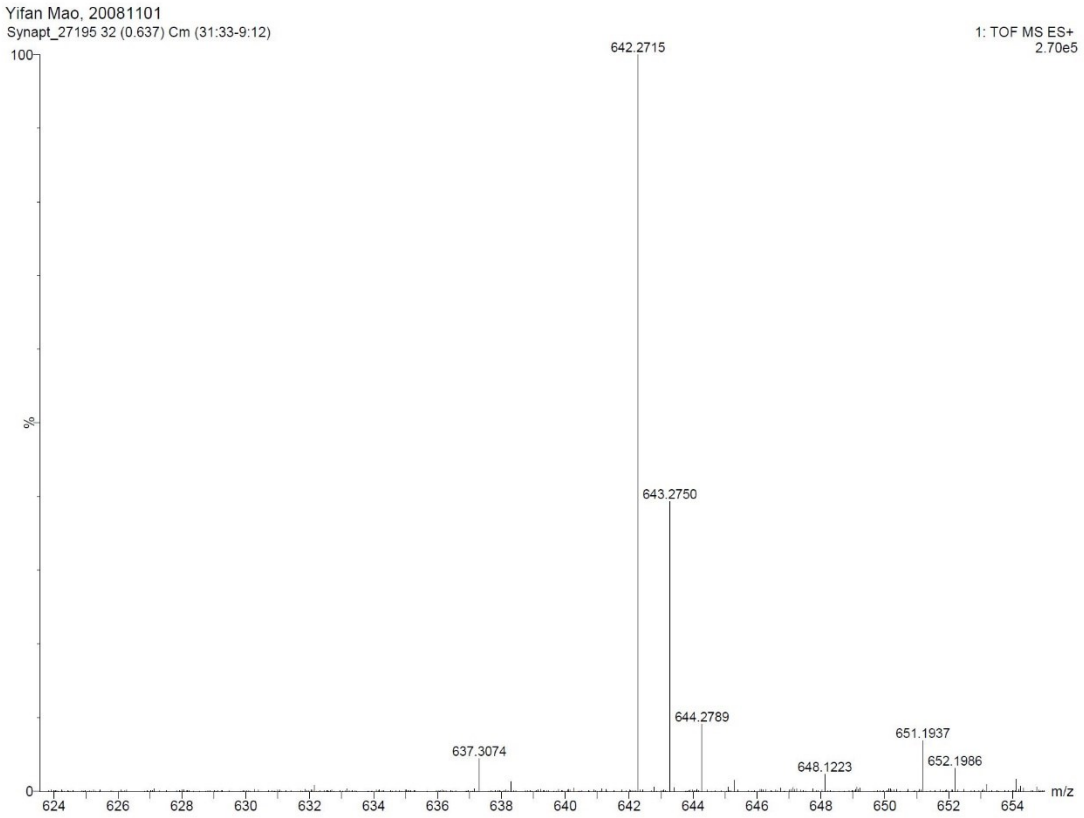
(*S*,*R*)-**7**

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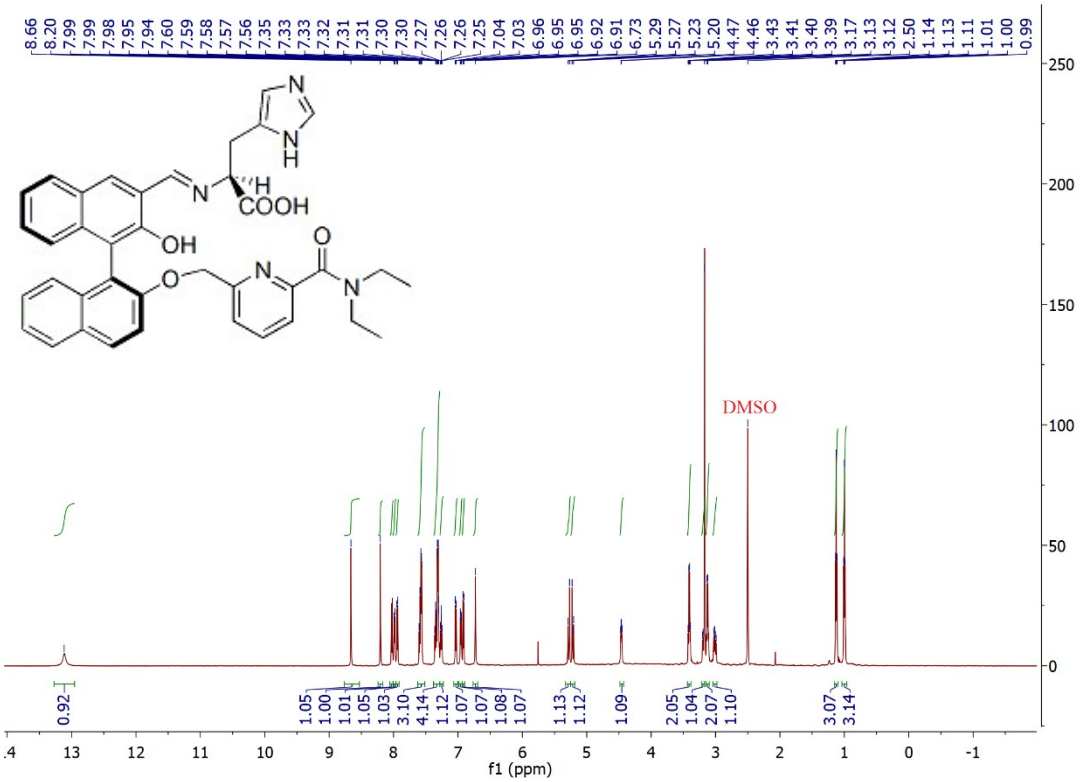
(*S*,*S*)-**7**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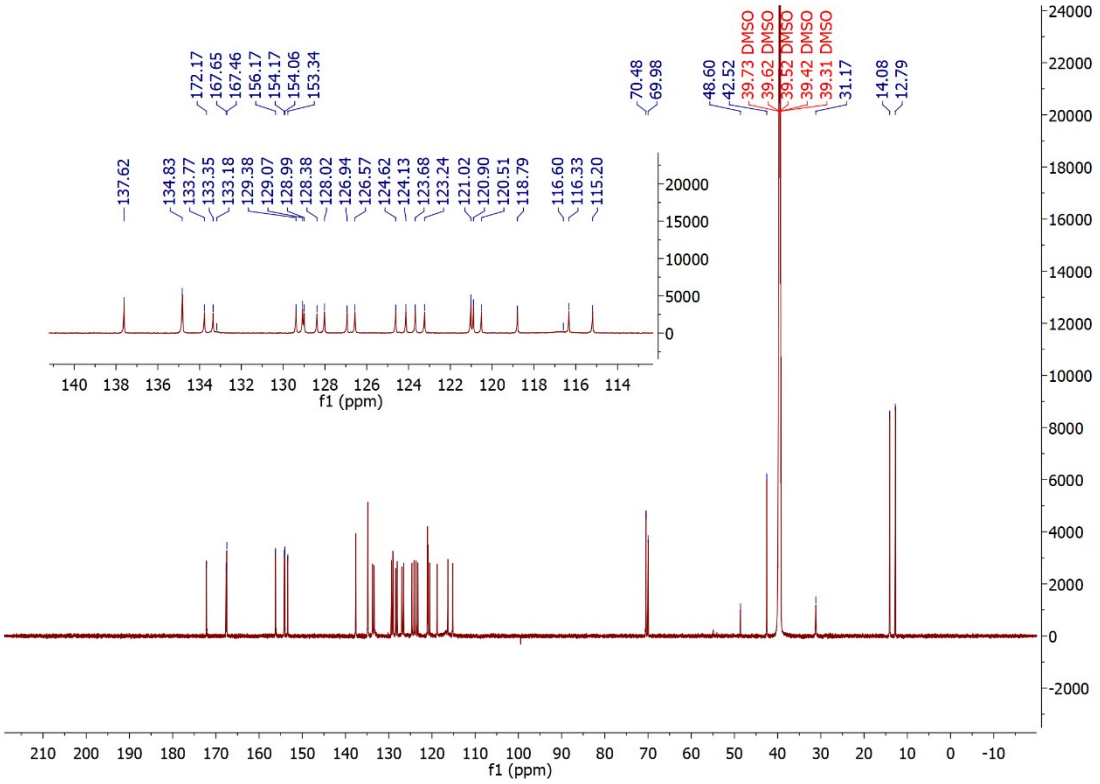
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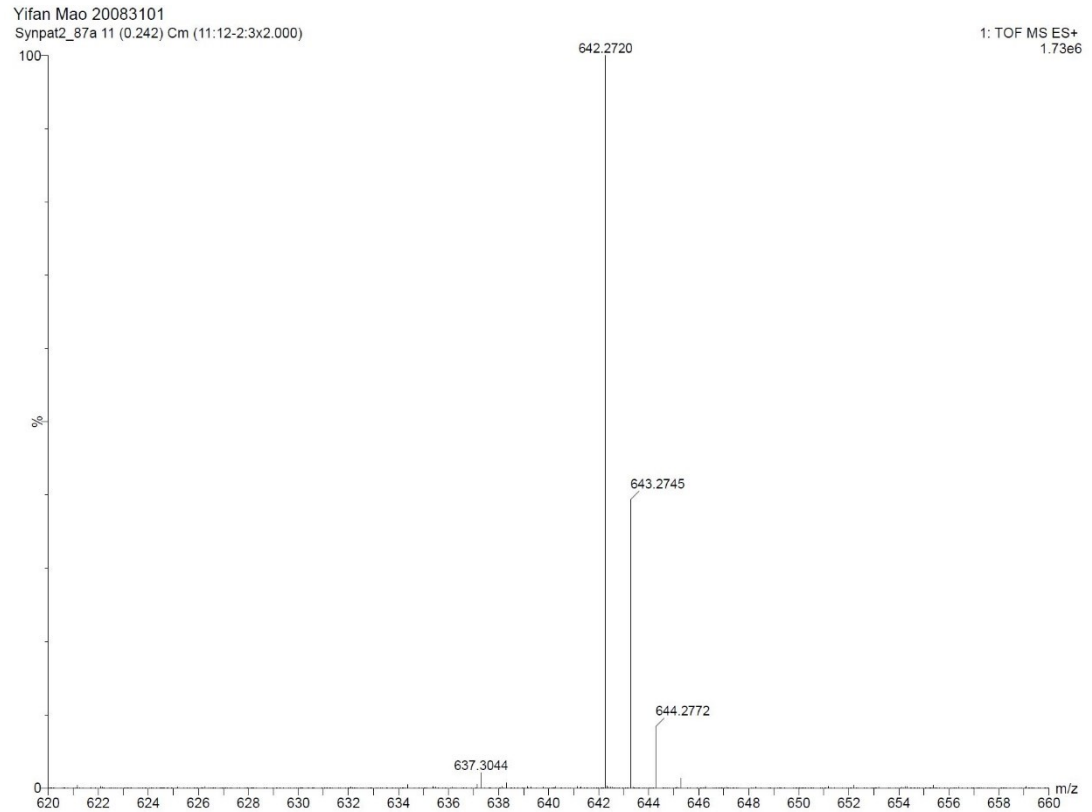
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3. Fluorescence Response of (*S*)-3 Toward Histidine versus Reaction Time

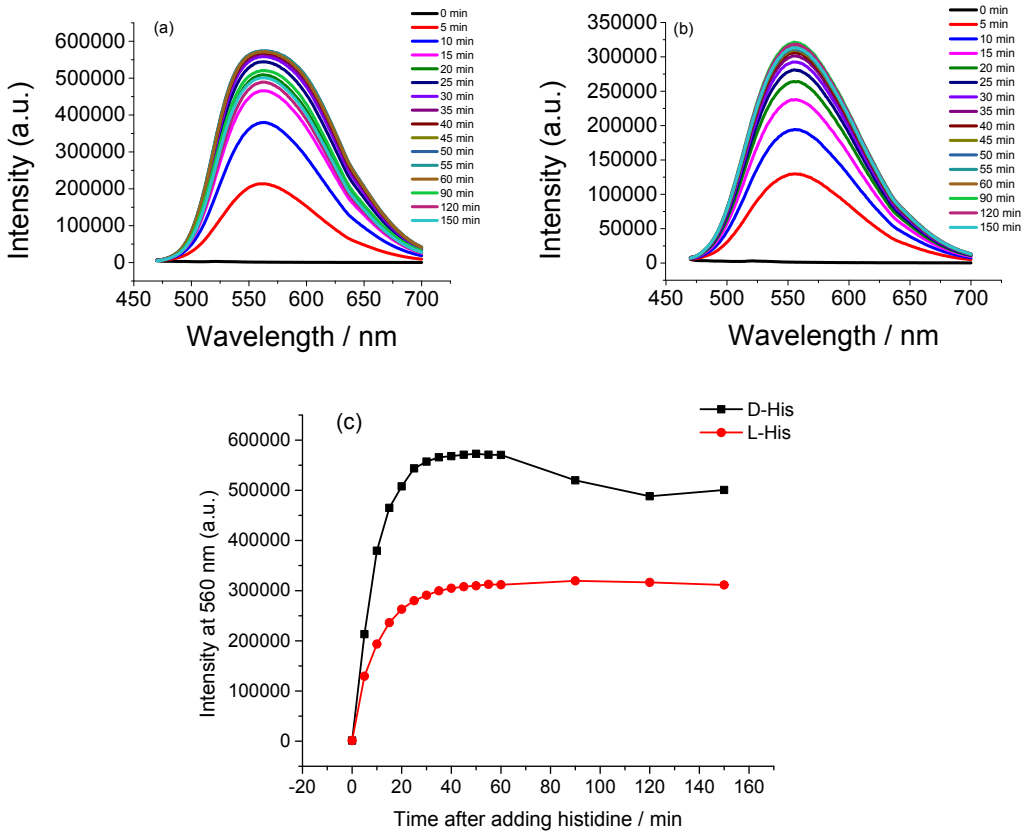

Figure S1. Fluorescence spectra of (*S*)-**3** (0.5 mM in DMF) with (a) D-His or (b) L-His (in pH 6.35 phosphate bu□er, 10 equiv) in the presence of $Zn(OAc)_2$ (in H_2O, 2.0 equiv.) at various reaction time (0 ~ 150 min) (Solvent: 1:1 DMF/pH 6.35 phosphate bu□er). (c) Fluorescence intensity at 560 nm versus the reaction time (λ_{exc} = 450 nm. Slit: 5/5 nm).

The fluorescence spectra were taken without dilution with DMF.

The fluorescence became stable after 2 h reaction. Thus, 2 h reaction time before dilution was chosen for fluorescence measurement.

4. Fluorescence Response of (*S*)-3 and (*R*)-3 Toward Histidine

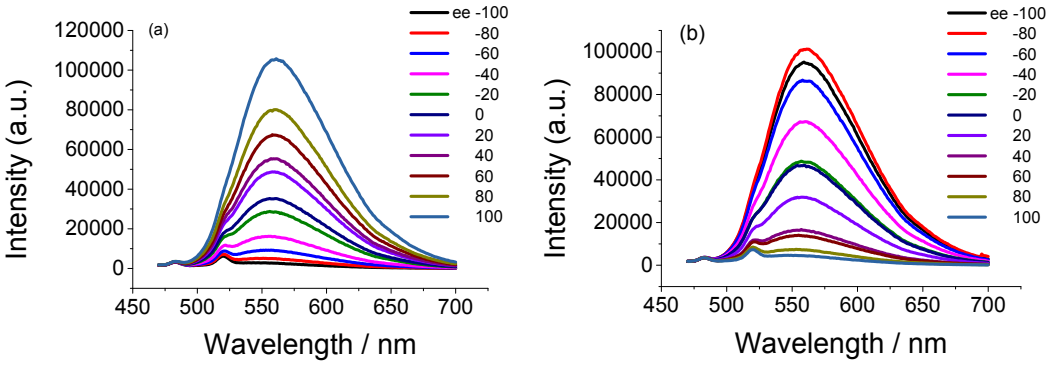

Figure S2. Fluorescence spectra of histidine (in pH 6.35 phosphate bu□er, ee of histidine (ee > 0 when D-His in excess): -100 ~ 100, 10 equiv) and $Zn(OAc)_2$ (in H_2O, 2.0 equiv) with (a) (*S*)-**3** (0.01 mM in DMF) and (b) (*R*)-**3** (0.01 mM in DMF) (λ_{exc} = 450 nm. Slit: 5/5 nm). (Solvent: DMF/1% pH 6.35 phosphate bu□er)

5. Fluorescent Titration of (*S*,*R*)-7 and (*S*,*S*)-7 with Zn(II) in the Absence of a Buffer

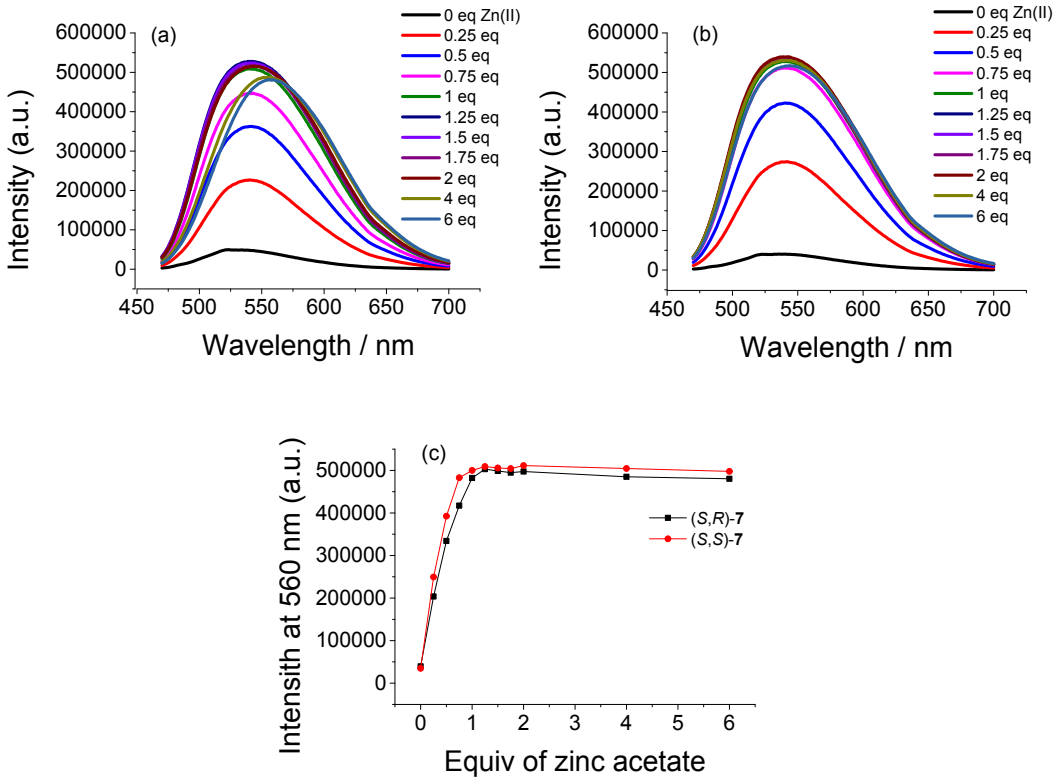

Figure S3. Fluorescence spectra of (a) (*S*,*R*)-**7** (0.01 mM) and (b) (*S*,*S*)-**7** (0.01 mM) in the presence of $Zn(OAc)_2$ (0 ~ 6.0 equiv). (c) Fluorescence intensity at 560 nm versus the equivalent of $Zn(OAc)_2$. (λ_{exc} = 450 nm. Slit: 5/5 nm). (Solvent: DMF/1% water)

6. Stability of the Imine-Zinc Complexes of (*S*,*R*)-7 and (*S*,*S*)-7 in the Presence of Buffer

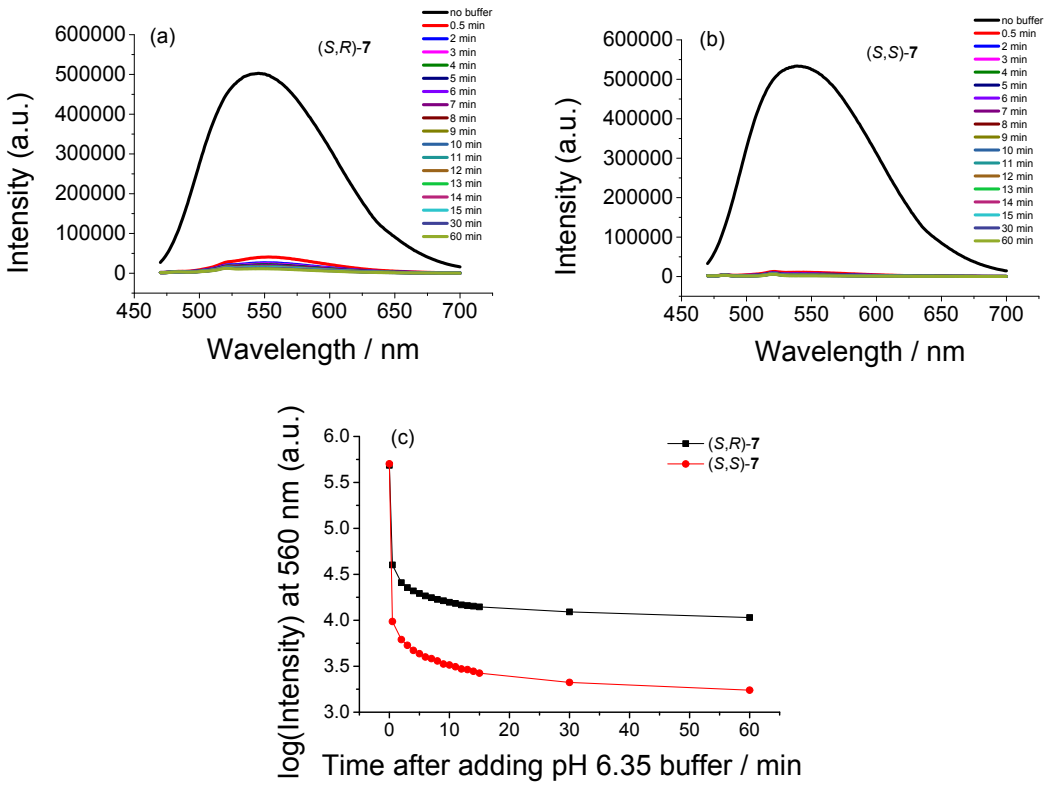

Figure S4. Fluorescence spectra of (a) (*S*,*R*)-**7** (0.01 mM) and (b) (*S*,*S*)-**7** (0.01 mM) in the presence of $Zn(OAc)_2$ (2.0 equiv) at 0 ~ 60 min after adding pH 6.35 phosphate bu□er (50 mM). (c) Fluorescence intensity (1og value) at 560 nm versus the time after adding the buffer. (λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% water or 1% 6.35 phosphate buffer)

7. Mass Spectrum (MALDI-TOF) of (*S*,*R*)-7 + 2 eq $Zn(OAc)_2$ (in DMSO)

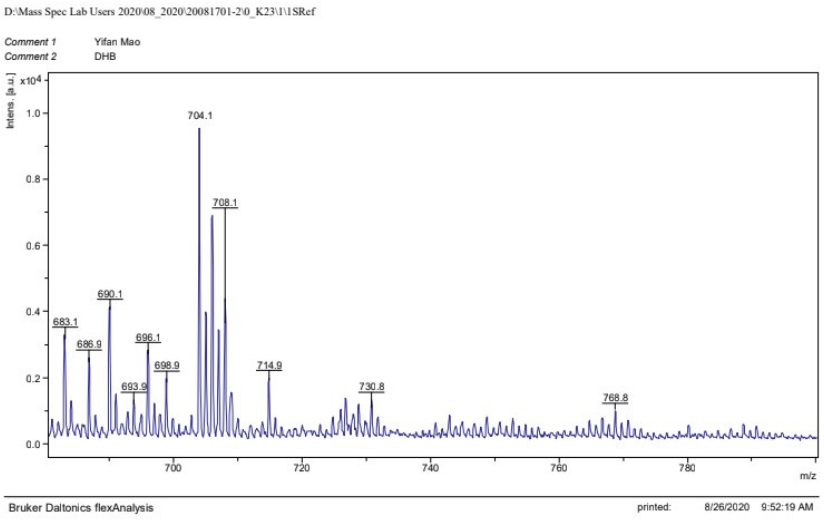

Figure S5. Mass spectrum (MALDI-TOF) of (*S*,*R*)-**7** + 2 eq $Zn(OAc)_2$ (in DMSO)

8. Mathematic Description of the 3D Plot (Figure 5c)

$$I_{470,\ 1\ equ}(n) = \left(0.0122n^4 - 1.8497n^3 + 89.969n^2 - 1154.3n + 79245\right)/100$$
$$R^2 = 0.9758$$

$$I_{470,\ 4\ equ}(n) = \left(0.0353n^4 - 8.2967n^3 + 854.24n^2 - 6904.1n + 120370\right)/100$$
$$R^2 = 0.9969$$

$$I_{470,\ 7\ equ}(n) = \left(0.0979n^4 - 18.968n^3 + 854.24n^2 - 1568.8n + 184613\right)/100$$
$$R^2 = 0.9986$$

$$I_{470,\ 10\ equ}(n) = \left(0.1387n^4 - 33.564n^3 + 3145.6n^2 - 23257n + 339510\right)/100$$
$$R^2 = 0.9963$$

$$I_{470,\ 13\ equ}(n) = \left(0.2501n^4 - 55.048n^3 + 4314.9n^2 - 11502n + 569993\right)/100$$
$$R^2 = 0.9993$$

$$n = D\%\ histidine\ of\ the\ sample$$

Calculation of D% (n) from unknown samples:

$$If\ 1 < C(m) \leq 4,\ I_{470} = \left[I_{470,\ 4\ equ}(n) - I_{470,\ 1\ equ}(n)\right] \times \frac{C(m) - 1}{3} + I_{470,\ 1\ equ}(n)$$

$$If\ 4 < C(m) \leq 7,\ I_{470} = \left[I_{470,\ 7\ equ}(n) - I_{470,\ 4\ equ}(n)\right] \times \frac{C(m) - 4}{3} + I_{470,\ 4\ equ}(n)$$

$$If\ 7 < C(m) \leq 10,\ I_{470} = \left[I_{470,10\ equ}(n) - I_{470,\ 7\ equ}(n)\right] \times \frac{C(m) - 7}{3} + I_{470,\ 7\ equ}(n)$$

$$If\ 10 < C(m) \leq 13,\ I_{470} = \left[I_{470,\ 13\ equ}(n) - I_{470,\ 10\ equ}(n)\right] \times \frac{C(m) - 10}{3} + I_{470,\ 10\ equ}(n)$$

9. Mathematic Description of the Relation between Concentration and I_{470} (λ_{exc} = 378 nm)

$C(m) = 0.0535m^2 - 2.3629m + 25.888$
$R^2 = 0.9991$
$m = FL\ intensity\ at\ 470\ nm/10000$

As shown above (Sections 8 and 9 in SI), the 3D figure (Figure 5c) used to determine the enantiomeric composition and total concentration can be described as mathematic forms. And the mathematic description had good agreement with the actual data (as shown in Table **S1**, 'Found/% versus Fitting').

10. Fluorescence Spectra of (*S*)-3 with 17 Pairs of Amino Acid Enantiomers at λ_{exc} = 378 nm and λ_{exc} = 450 nm)

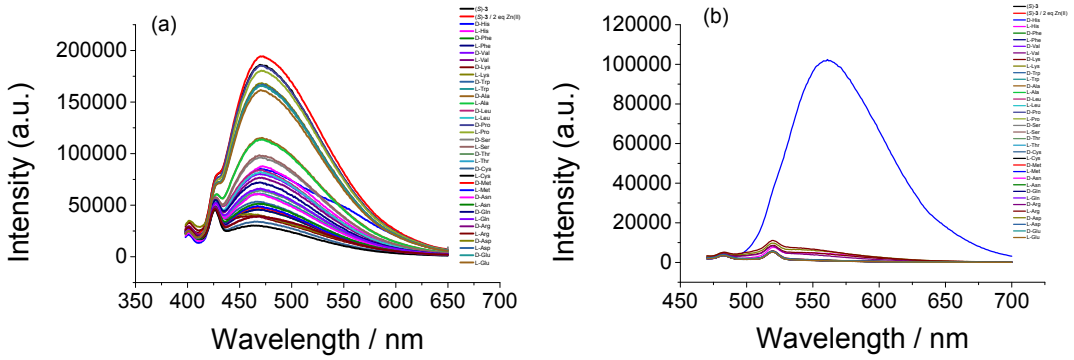

Figure S6. Fluorescence spectra of (*S*)-**3** (0.01 mM in DMF, 1.0 equiv) with 17 pairs of D- and L-amino acid enantiomers (in pH 6.35 phosphate bu□er, 10 equiv) in the presence of $Zn(OAc)_2$ (in H_2O, 2.0 equiv): (a) λ_{exc} = 378 nm; (b) λ_{exc} = 450 nm. (Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate bu□er)

11. Fluorescence Spectra of (S)-3 + Histidine (0 ~ 13 equiv and -100 ~ 100 ee of D-His) (λ_{exc} = 378 nm)

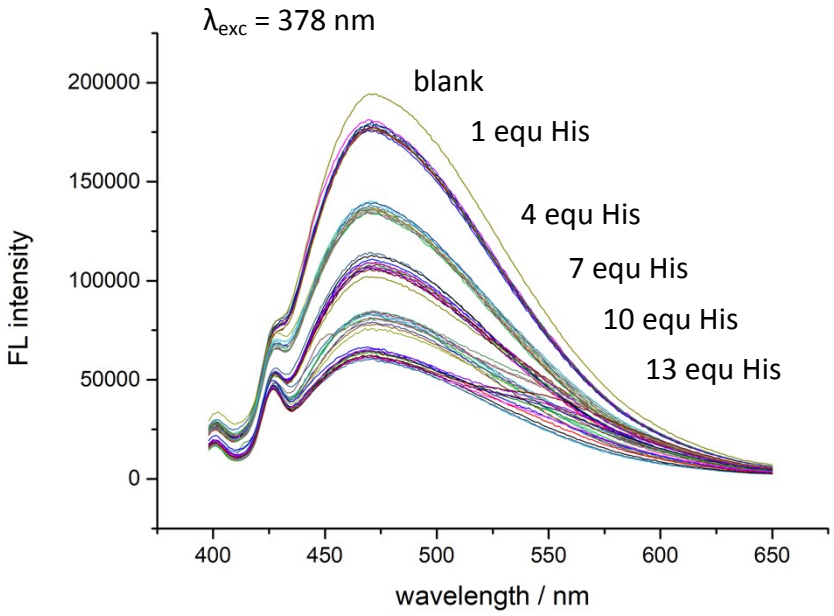

Figure S7. Fluorescence spectra of (*S*)-**3** (0.01 mM in DMF) with $Zn(OAc)_2$ (2.0 equiv) and histidine (0 ~ 13 equiv and -100 ~ 100 ee in phosphate bu□er, pH = 6.35). (λ_{exc} = 378 nm. Slit 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate bu□er)

12. Fluorescence Spectra of (S)-3 + Histidine (0 ~ 13 equiv and -100 ~ 100 ee of D-His) (λ_{exc} = 450 nm)

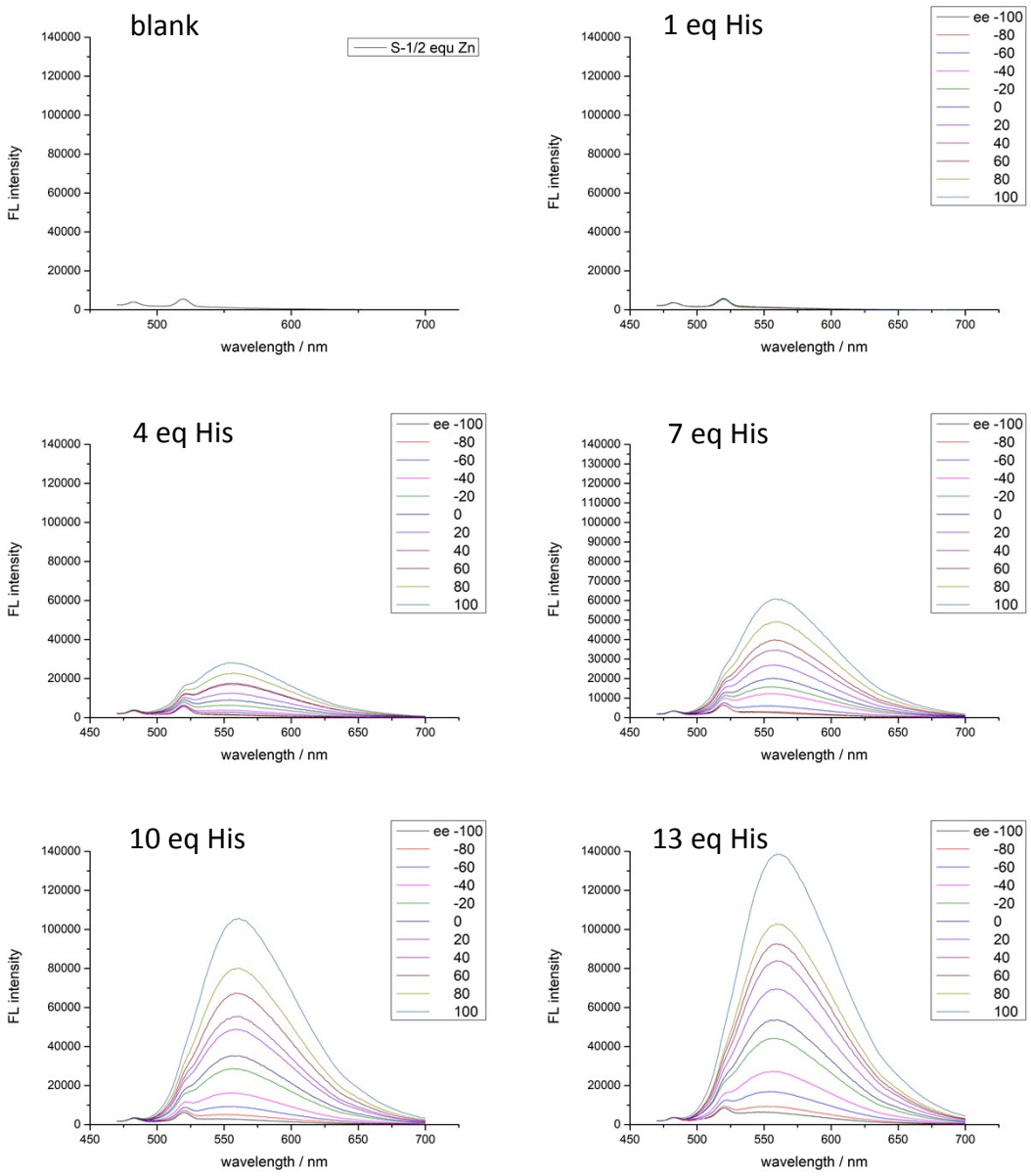

Figure S8. Fluorescence spectra of (*S*)-**3** (0.01 mM in DMF) with $Zn(OAc)_2$ (2.0 equiv) and histidine (0 ~ 13 equiv and -100 ~ 100 ee in phosphate bu□er, pH = 6.35). (λ_{exc} = 450 nm. Slit 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate bu□er)

13. Fluorescent Titration of (*S*)-3 + 10 equiv. D- and L-Histidine with Zn(II)

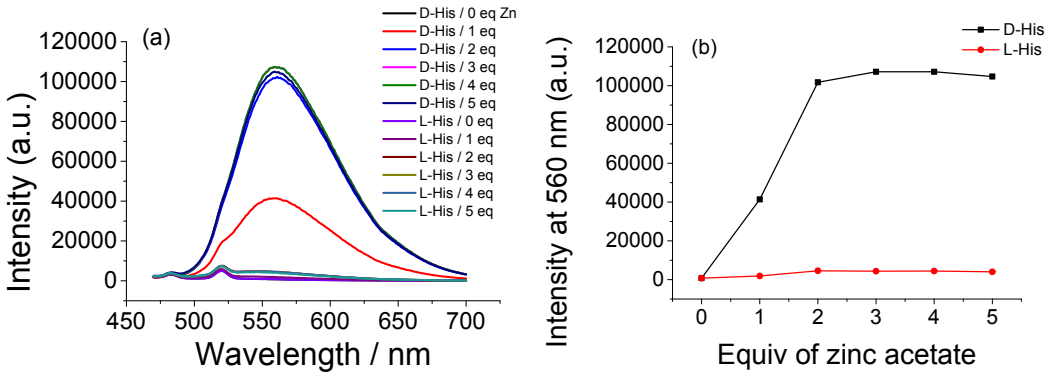

Figure S9. (a) Fluorescence spectra of (*S*)-**3** (0.01 mM in DMF) and D- and L-histidine (in pH 6.35 phosphate bu□er, 10 equiv) in the presence of $Zn(OAc)_2$ (in H_2O, 0 ~ 5.0 equiv) and (b) fluorescence intensity at 560 nm versus the equivalent of $Zn(OAc)_2$. (λ_{exc} = 450 nm. Slit: 5/5 nm. Solvent: DMF/1% 6.35 phosphate buffer)

The fluorescence of (*S*)-**3** reached maximum when 2 equiv. Zn(II) was added. Thus, 2 equiv. Zn(II) was chosen in all fluorescence measurements.

14. Excitation Spectra of the Emissions of (*S*)-3 + D- and L-Histidine at 470 and 560 nm

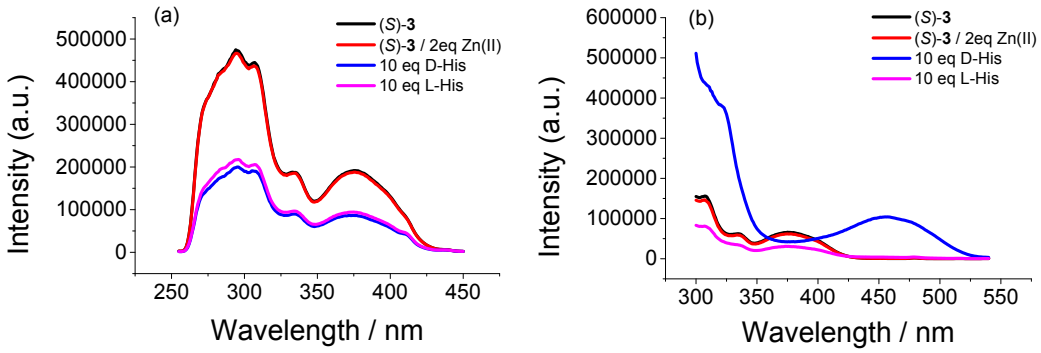

Figure S10. Excitation Spectra of (*S*)-**3** (0.01 mM in DMF) and D- and L-histidine (in pH 6.35 phosphate bu□er, 10 equiv.) in the presence of $Zn(OAc)_2$ (in H_2O, 2.0 equiv.) for emissions at (a) 470 nm and (b) 560 nm. (Solvent: DMF/1% 6.35 buffer)

The excitation spectra showed intense signals at 378 nm (Figure S10a) and 450 nm (Figure S10b). Thus, 378 and 450 nm were chosen as the excitation wavelengths in all fluorescence measurements.

15. Stability of the Fluorescence Responses at 470 and 560 nm after Dilution

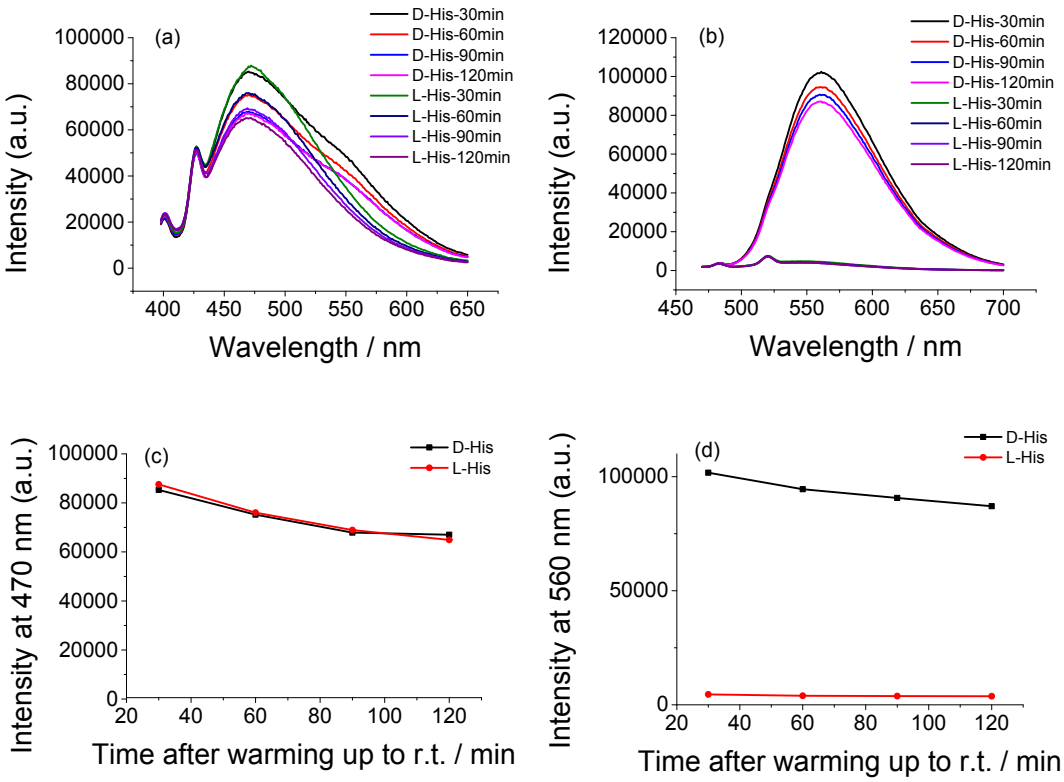

Figure S11. Fluorescence spectra of (*S*)-**3** (0.01 mM in DMF) and D- and L-histidine (in pH 6.35 phosphate bu□er, 10 equiv) in the presence of $Zn(OAc)_2$ (in H_2O, 2.0 equiv): (a) λ_{exc} = 378 nm, slit: 5/5 nm and (b) λ_{exc} = 450 nm, slit: 5/5 nm. Fluorescence intensity at (c) 470 nm (λ_{exc} = 378 nm. Slit: 5/5 nm) and (d) 560 nm (λ_{exc} = 450 nm. Slit: 5/5 nm) versus the time when the solutions were warmed up to room temperature after diluted at 0 °C. (Solvent: DMF/1% 6.35 buffer)

As shown in Figure S11, the fluorescence intensities at 470 and 560 nm were only slightly decreased in 2 h after warmed up to room temperature from their 0 °C dilution. Thus, all the fluorescence measurements were conducted at 30 min after the samples were warmed up to room temperature from their dilutions.

16. COSY and NOESY NMR of (*S*,*R*)-7 and (*S*,*R*)-7 with or without 1 equiv. Zinc Acetate.

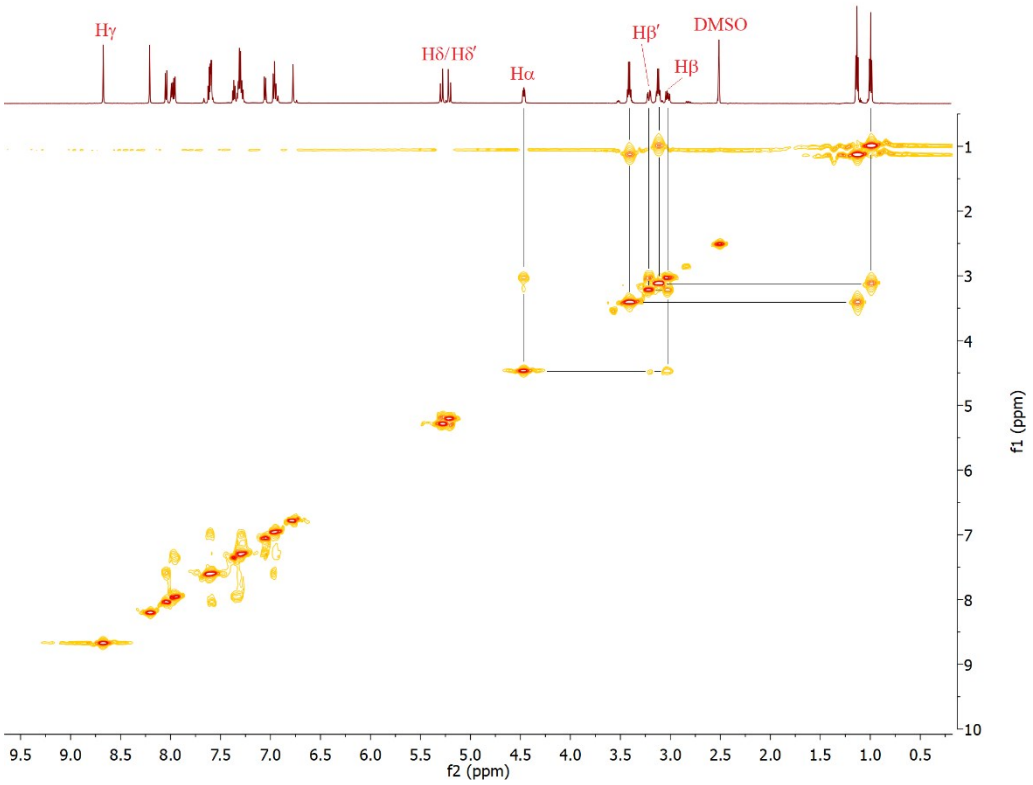

Figure S12. COSY NMR of (*S*,*R*)-**7** in DMSO-d_6

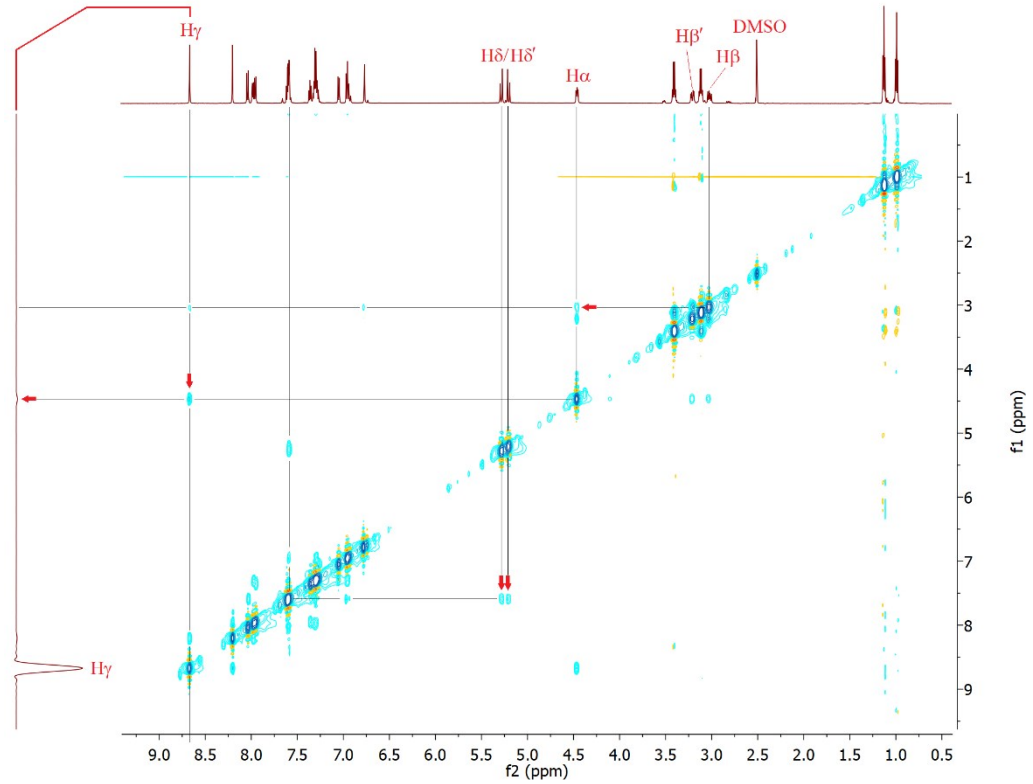

Figure **S13**. NOESY NMR of (*S*,*R*)-**7** in DMSO-d_6

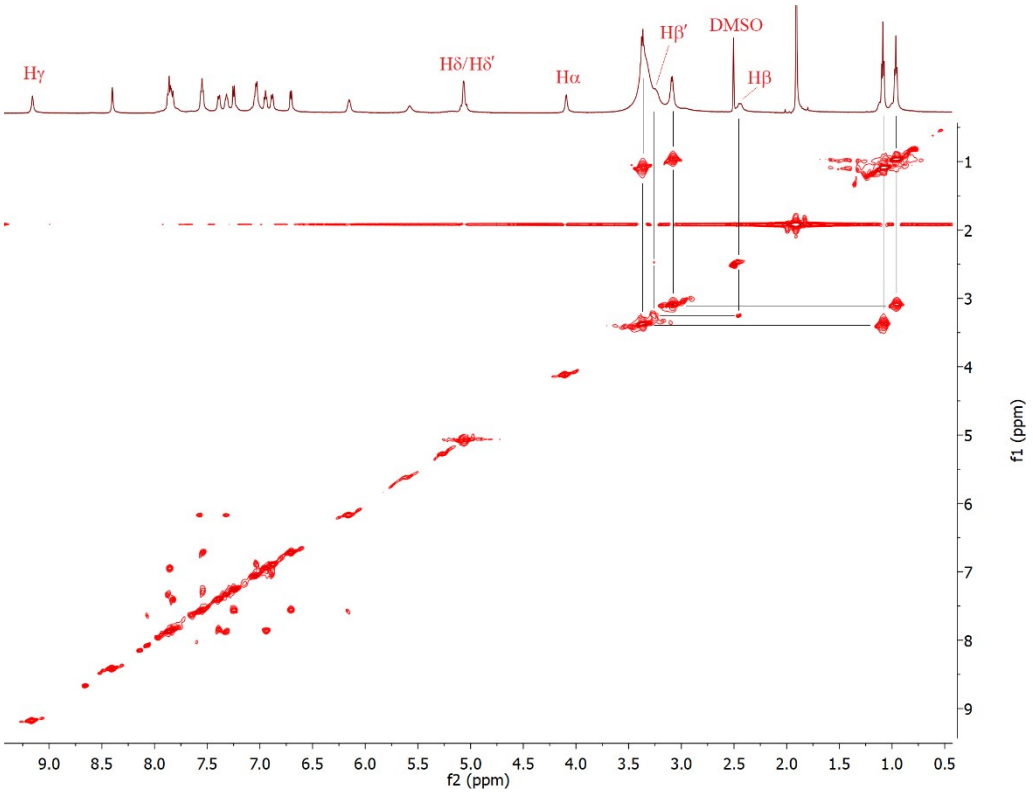

Figure S14. COSY NMR of (*S*,*R*)-**7** with 1 eq $Zn(OAc)_2$ in DMSO- d_6

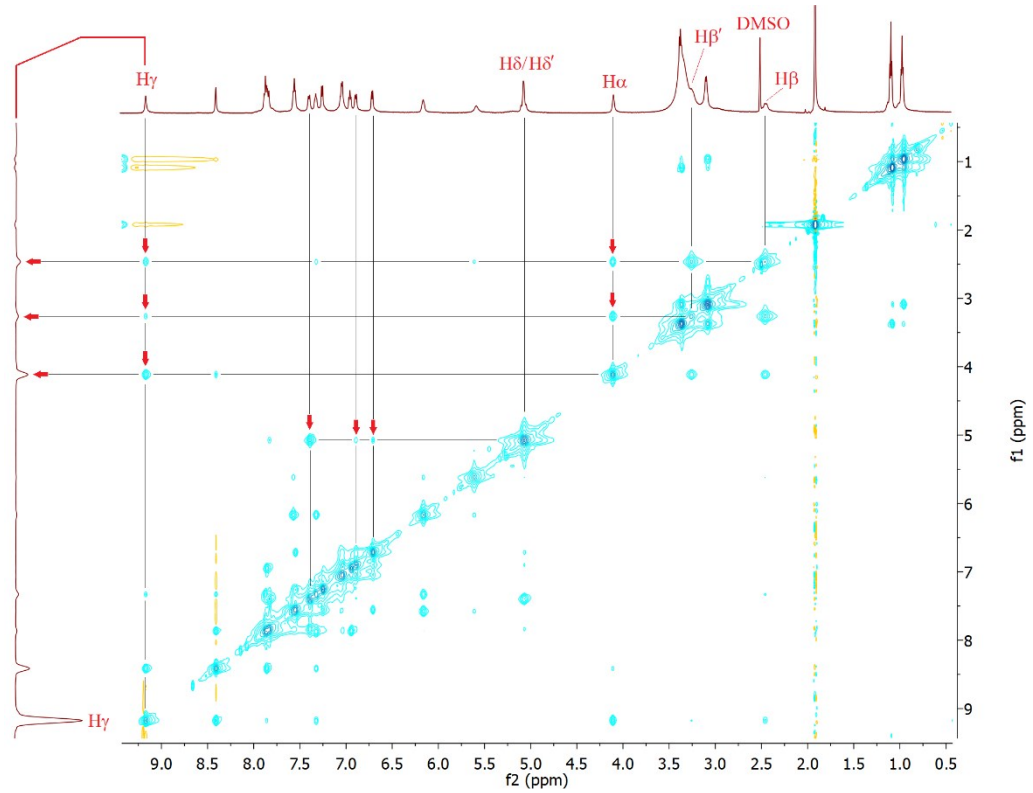

Figure **S15**. NOESY NMR of (*S*,*R*)-**7** with 1 eq $Zn(OAc)_2$ in DMSO-d_6

17. ^{1}H NMR Titration of (*S*,*R*)-7 with Zn(II).

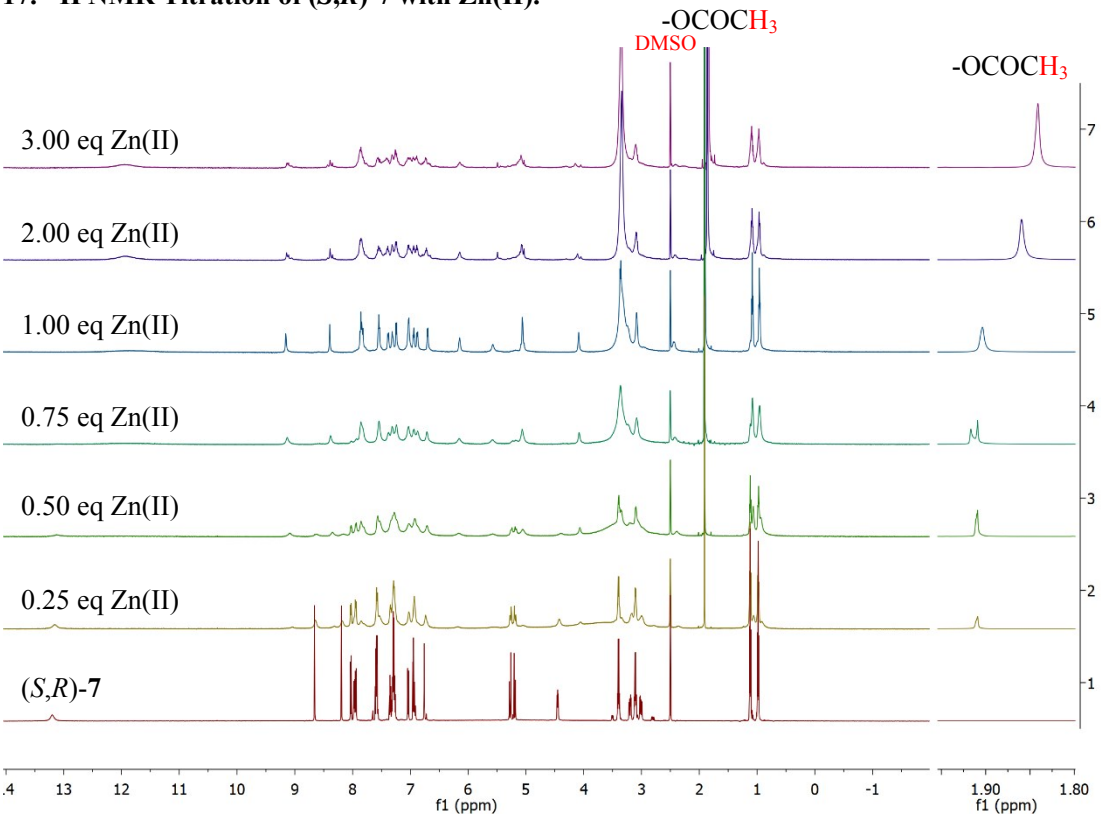

Figure S16. ^{1}H NMR of (*S*,*R*)-**7** with 0 ~ 3 equiv $Zn(OAc)_2$ in DMSO-d_6

As shown in Figure S16, (*S*,*R*)-**7** was completely converted to a new compound after 1 equiv Zn(II) was added. This also suggests that (*S*,*R*)-**7** and Zn(II) form a 1:1 complex. The zoomed region shows the shift of the acetate signal as the reaction proceeded.

18. Fluorescence Spectra of (*S*)-3 + Zn(II)

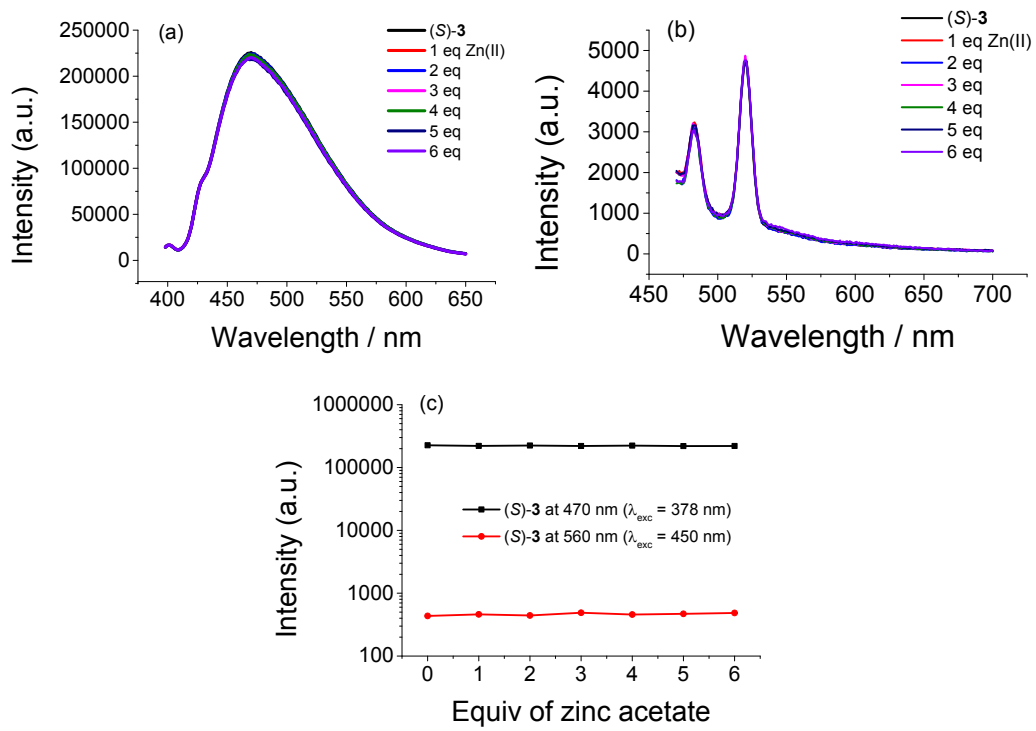

Figure S17. Fluorescence spectra of (*S*)-**3** (0.01 mM) in the presence of $Zn(OAc)_2$ (in H_2O, 0 ~ 6.0 equiv) when (a) λ_{exc} = 378 nm and (b) λ_{exc} = 450 nm. (c) Fluorescence intensity at 470 and 560 nm versus the equivalence of $Zn(OAc)_2$. (λ_{exc} = 378 and 450 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer)

As shown in Figure S17, the fluorescence properties of (*S*)-**3** were not affected by adding Zn(II).

19. Fluorescence Spectra of (*S*)-3 + Histidine without Zn(II)

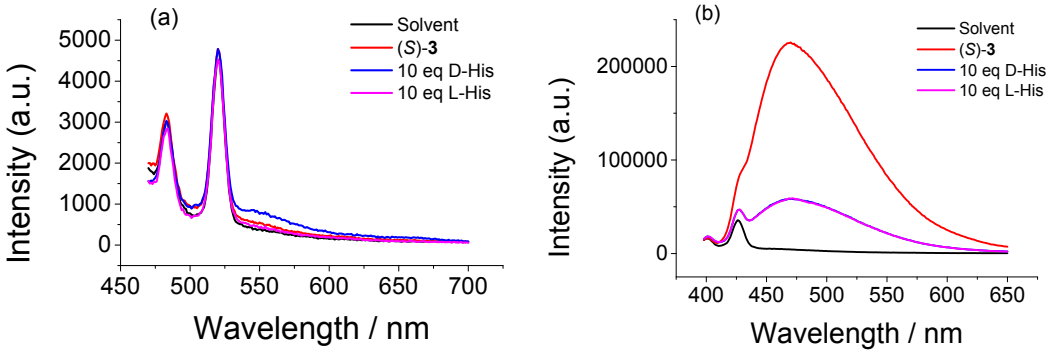

Figure S18. Fluorescence spectra of (*S*)-**3** (0.01 mM) with D- and L-histidine (in pH 6.35 phosphate bu□er, 10 equiv) when (a) λ_{exc} = 450 nm, and (b) λ_{exc} = 378 nm. (Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate buffer)

As shown in Figure S18a, without Zn(II), (*S*)-**3** did not have emission at 560 nm (only the light scattering signals of the solvent were observed). Figure S18b suggests that the fluorescence decreasing at the short wavelength should be due to the consumption of (*S*)-**3** from the reaction with histidine with or without Zn^{2+}.

20. UV-vis Spectrum of (*S*)-3

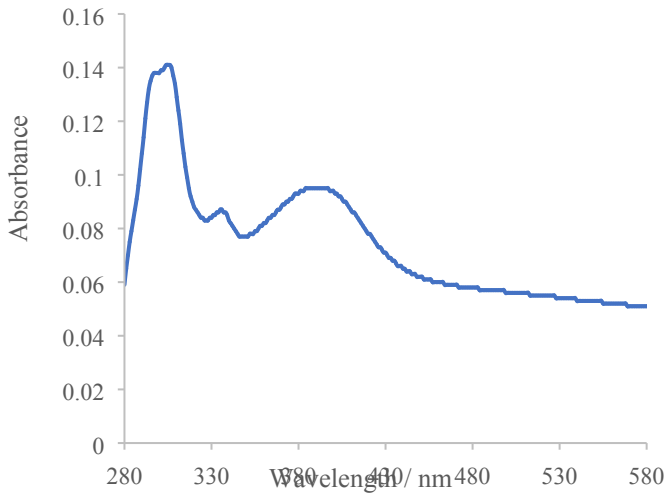

Figure S19. UV-vis spectrum of (*S*)-**3** (0.01 mM, DMF/1% pH 6.35 phosphate buffer).

21. ^{1}H NMR Spectra of (*S*)-3 with and without D_2O in DMSO-d_6

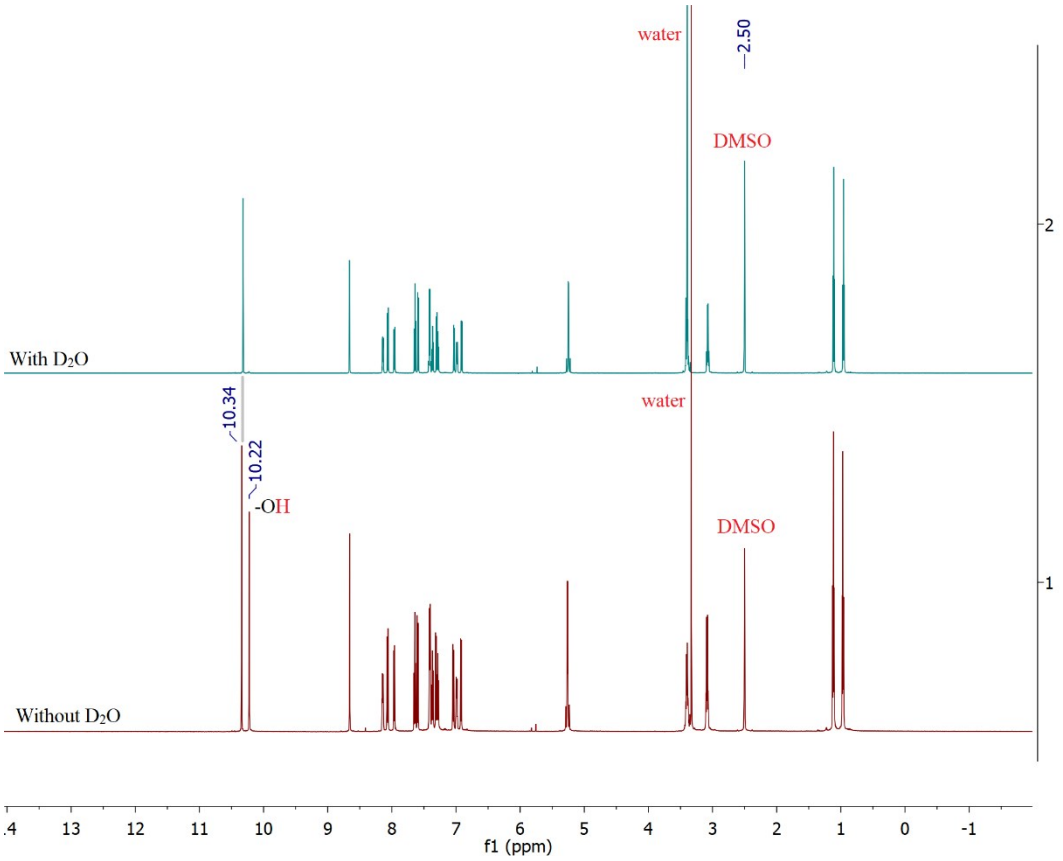

Figure S20. ^{1}H NMR spectra of (*S*)-**3** with and without D_2O in DMSO-d_6

22. Analysis of Total Concentration and Enantiomeric Composition of 7 Histidine Samples

Table S1. Determine the Concentration and Enantiomeric Compositions of the Histidine Samples with (*S*)-**3** by Fluorescence Measurement[a]

entry	actual D/%	Found/% by 3D plot	error	Found/% Fitting	error	actual [His]/*10^{-5}M	found	error
1	80.0	75.1	+6.9 -5.9	76.2	+5.1 -4.8	3.50	3.69	+0.23 -0.23
2	45.0	46.2	+2.2 -2.1	45.4	+2.5 -2.4	4.50	4.92	+0.08 -0.09
3	70.0	71.9	+7.7 -5.0	73.4	+4.8 -4.9	5.50	6.05	+0.18 -0.17
4	30.0	28.9	+2.2 -2.1	29.2	+2.4 -2.3	7.50	7.54	+0.21 -0.21
5	65.0	69.3	+3.4 -3.5	69.6	+3.8 -3.7	8.50	8.32	+0.24 -0.23
6	25.0	25.3	+2.2 -2.1	25.2	+2.0 -2.0	9.50	9.67	+0.20 -0.20
7	55.0	56.3	+3.6 -3.6	56.4	+4.1 -4.0	11.50	11.08	+0.22 -0.20

[a] Figure 5 and its conditions were applied. λ_{exc} = 378 nm, λ_{em} = 450 nm; λ_{exc} = 450 nm, λ_{exc} = 560 nm. Slit: 5/5 nm. All the data were obtained from three independent experiments.

23. Fluorescence Responses of (*S*)-3 toward 17 Pairs of Amino acid Enantiomers at λ_1 = 470 nm (λ_{exc} = 378 nm)

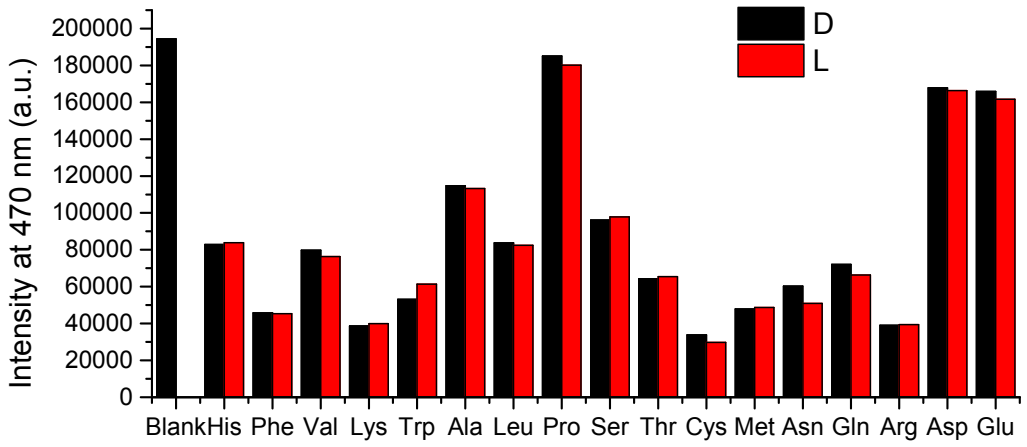

Figure S22. Fluorescence intensity at 470 nm of (*S*)-**3** (0.01 mM in DMF) + $Zn(OAc)_2$ (2.0 equiv in water) with 17 pairs of amino acid enantiomers (10 equiv, pH 6.35 phosphate bu□er) (λ_{exc} = 378 nm. Slit: 5/5 nm. Solvent: DMF/1% pH 6.35 phosphate bu□er).

24. References

S1. Zhu, Y.; Wu, X.; Gu, S.; Pu, L. Free Amino Acid Recognition: A bisbinaphthyl-based fluorescent probe with high enantioselectivity. *J. Am. Chem. Soc*. **2019**, *141*, 175–181.

S2. Bravard, F.; Bretonniere, Y.; Wietzke, R.; Gateau, C.; Mazzanti, M.; Delangle, P.; Pecaut, J. Solid-State and Solution Properties of Cationic Lanthanide Complexes of a New Neutral Heptadentate N4O3 Tripodal Ligand. *Inorg. Chem*. **2003**, *42* (24), 7978–7989.