Supporting Information:

Synthesis of Novel Fluorescence Probes and Their Application in the Enantioselective Recognition of Amino Acids

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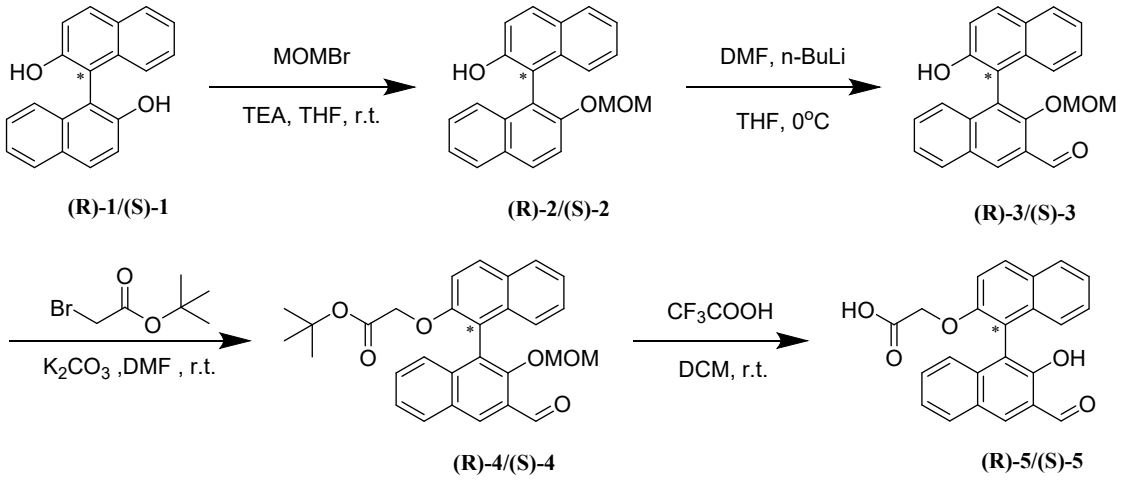
1. General information

The ^{1}H NMR and ^{13}C NMR of the compounds were measured by Bruker AV 300 spectrometer, and the solvents were internally standard using DMSO-d_6, and tetramethylsilane (TMS; δ ppm = 0 ppm）. Mass spectrometry was determined using the Waters Q-TOF mass spectrometer and fluorescence spectra were determined using the SpectraMax iD5 microplate detection system.

Unless otherwise stated, materials were obtained from commercial suppliers and could be used without further purification.

2. Synthesis and characterization of compounds



(1) Synthesis of compound S-2

Under 0°C, 10 g (35 mmol) of S-1 (1,1'-binaphthol) and 10 mL (56 mmol) of DIPEA were dissolved in THF and stirred until fully dissolved. Then, MOMBr (4.3 mL, 52.5 mmol) was added dropwise and stirred at 0°C for 10 minutes and then at room temperature for 30 minutes. After the reaction was complete, the mixture was extracted with water and ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The resulting product was purified by column chromatography with petroleum ether:ethyl acetate (25:1) as eluent. This yielded white oil-like compound S-2, weighing 8.2 g with a yield of 71%. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 9.38 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 7.87 (dd, J = 8.6, 6.8 Hz, 2H), 7.60 (d, J = 9.1 Hz, 1H),

7.36 – 7.30 (m, 2H), 7.24 (ddd, *J* = 8.1, 7.0, 1.4 Hz, 2H), 7.17 (ddd, *J* = 8.2, 6.8, 1.5 Hz, 1H), 7.03 – 6.98 (m, 1H), 6.88 (dd, *J* = 8.2, 1.4 Hz, 1H), 5.08 (q, *J* = 6.7 Hz, 2H), 3.10 (s, 3H).

(2) Synthesis of compound S-3

Under N_2 at 0°C, 4.5 g (13.5 mmol) of S-2 was dissolved in 50 mL of anhydrous THF. Then, n-BuLi (19 mL, 47.25 mmol) was added dropwise and stirred for 30 minutes at 0°C, followed by stirring at room temperature for 1 hour. Next, anhydrous DMF (1.55 mL, 20 mmol) was added dropwise and the mixture was stirred at room temperature for 45 minutes. The reaction was quenched with saturated NH_4Cl solution, and the organic layer was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The resulting product was purified by column chromatography with petroleum ether:ethyl acetate(15:1) as eluent. This yielded a yellow solid compound S-3, weighing 2.7 g with a yield of 56%. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 10.46 (s, 1H), 9.74 (s, 1H), 8.58 (s, 1H), 8.26 – 8.20 (m, 1H), 7.96 (d, *J* = 8.9 Hz, 1H), 7.89 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.53 (ddd, *J* = 8.1, 6.8, 1.3 Hz, 1H), 7.45 (ddd, *J* = 8.3, 6.8, 1.4 Hz, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 7.25 (dddd, *J* = 17.8, 8.2, 6.8, 1.4 Hz, 2H), 7.15 – 7.10 (m, 1H), 6.91 (dd, *J* = 8.2, 1.5 Hz, 1H), 4.82 (d, *J* = 5.8 Hz, 1H), 4.64 (d, *J* = 5.8 Hz, 1H), 2.97 (s, 3H).

(3) Synthesis of compound S-4

Under room temperature, S-3 (300 mg, 0.84 mmol) and K_2CO_3 (232 mg, 1.68 mmol) were dissolved in 3 mL of DMF. tert-butyl bromoacetate (82 mg, 1.26 mmol) was slowly added and stirred at room temperature for 3 hours. After the reaction was complete, the mixture was extracted with water and ethyl acetate, the organic phase was dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified with column chromatography with petroleum ether:ethyl acetate(25:1) as eluent to obtain a yellow solid compound S-4, weighing 390 mg with a yield of 99%. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 10.45 (s, 1H), 8.59 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.13 (d, *J* = 9.1 Hz, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.53 (ddd, *J* = 8.1, 6.8, 1.2 Hz, 1H), 7.46 – 7.35 (m, 3H), 7.29 (ddd, *J* = 8.2,

6.7, 1.4 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 4.86 (d, *J* = 5.8 Hz, 1H), 4.70 (s, 2H), 4.64 (d, *J* = 5.8 Hz, 1H), 2.92 (s, 3H), 1.34 (s, 9H).

(4) Synthesis of compound S-5

Under room temperature, 390 mg (0.84 mmol) of S-4 was dissolved in 5 mL of DCM. Then, 3 mL of CF_3COOH was added dropwise and the reaction was carried out at room temperature for 20 hours. After the reaction was complete, the solvent and CF_3COOH were removed under reduced pressure. The remaining residue was dissolved in DCM and the solvent and CF_3COOH were removed under reduced pressure three times. The crude product was purified with column chromatography with petroleum ether: ethyl acetate: acetic acid (100:25:1) to obtain a yellow solid compound S-5, weighing 290 mg with a yield of 94%. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 10.33 (s, 1H), 10.11 (s, 1H), 8.63 (s, 1H), 8.15 – 8.03 (m, 2H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 9.1 Hz, 1H), 7.41 – 7.32 (m, 3H), 7.26 (ddd, *J* = 8.3, 6.7, 1.4 Hz, 1H), 7.10 – 7.02 (m, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.76 – 4.61 (m, 2H). The ^{13}C NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 196.73, 170.45, 153.81, 152.92, 136.96, 136.45, 133.27, 130.07, 129.90, 129.79, 129.07, 128.12, 127.29, 126.70, 124.83, 124.37, 124.14, 123.75, 122.86, 117.61, 117.02, 114.81, 65.39. HRMS: m/z calcd. for $C_{23}H_{16}O_5$ $[M+H]^+$: 373.09977, found 373.10686.

(5) Synthesis of compound R-5

The experimental procedure for the synthesis of compound R-5 from compound R-1 was the same as described above. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 10.33 (s, 1H), 10.11 (s, 1H), 8.63 (s, 1H), 8.15 – 8.04 (m, 2H), 7.96 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 9.1 Hz, 1H), 7.43 – 7.31 (m, 3H), 7.26 (ddd, J = 8.2, 6.7, 1.4 Hz, 1H), 7.09 – 6.95 (m, 2H), 4.78 – 4.60 (m, 2H). The ^{13}C NMR spectrum (300 MHz, DMSO-d_6) showed peaks at δ 196.72, 170.44, 153.80, 152.91, 136.95, 136.44, 133.26, 130.07, 129.89, 129.78, 129.06, 128.12, 127.28, 126.69, 124.82, 124.37, 124.14, 123.74, 122.86, 117.60, 117.00, 114.80, 65.38. HRMS: m/z calcd. for $C_{23}H_{16}O_5$ $[M+H]^+$: 373.09977, found 373.10724.

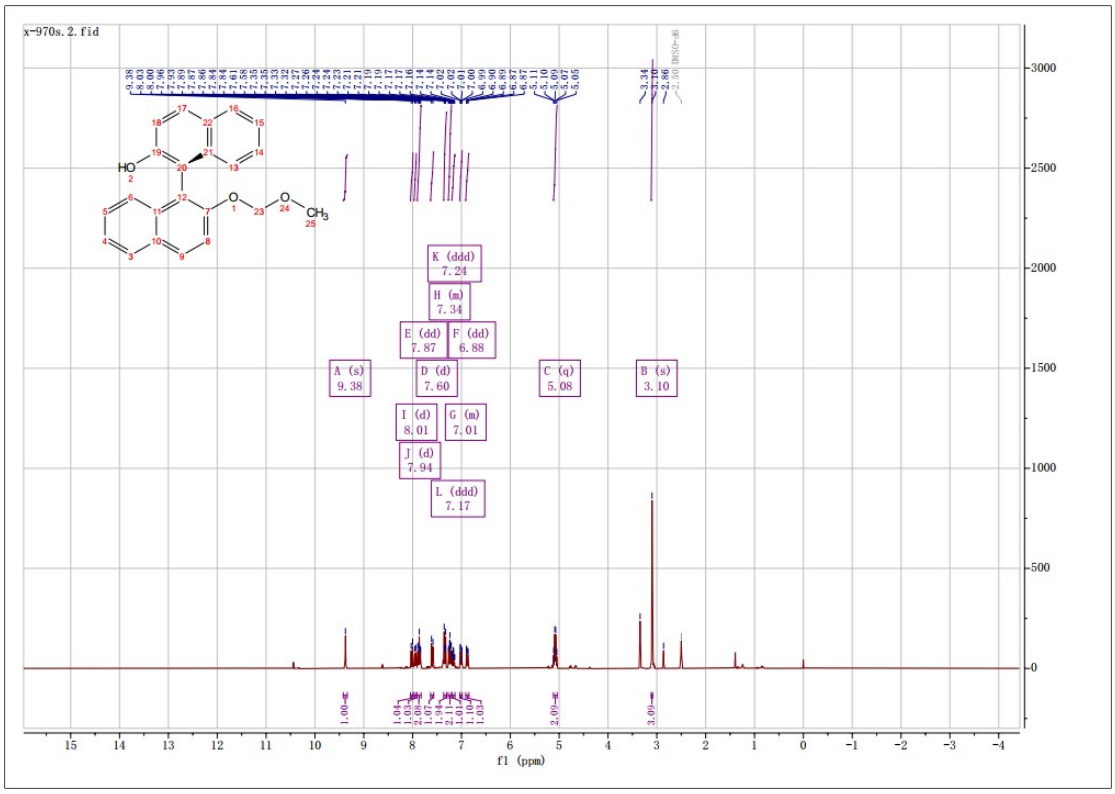


Figure S1. ^{1}H NMR spectra of (S)-2 in DMSO-d_6 (300 MHz)

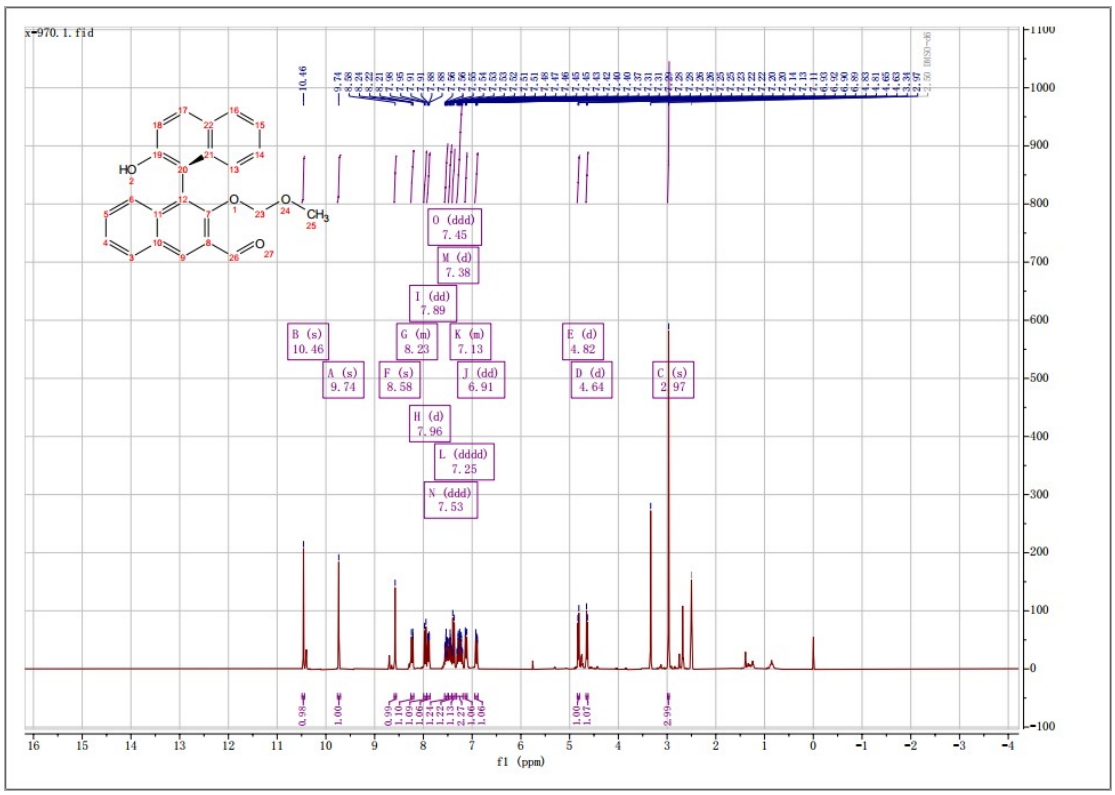


Figure S2. ^{1}H NMR spectra of (S)-3 in DMSO-d_6 (300 MHz)

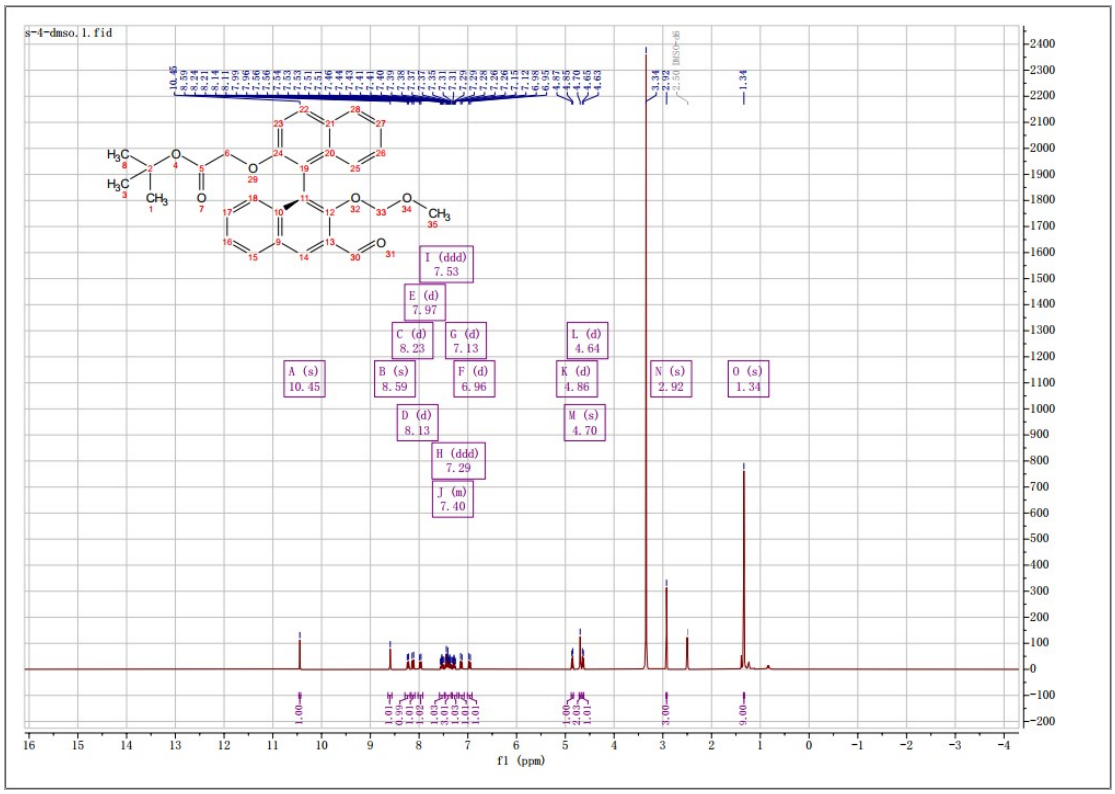


Figure S3. ^{1}H NMR spectra of (S)-4 in DMSO-d_6 (300 MHz)

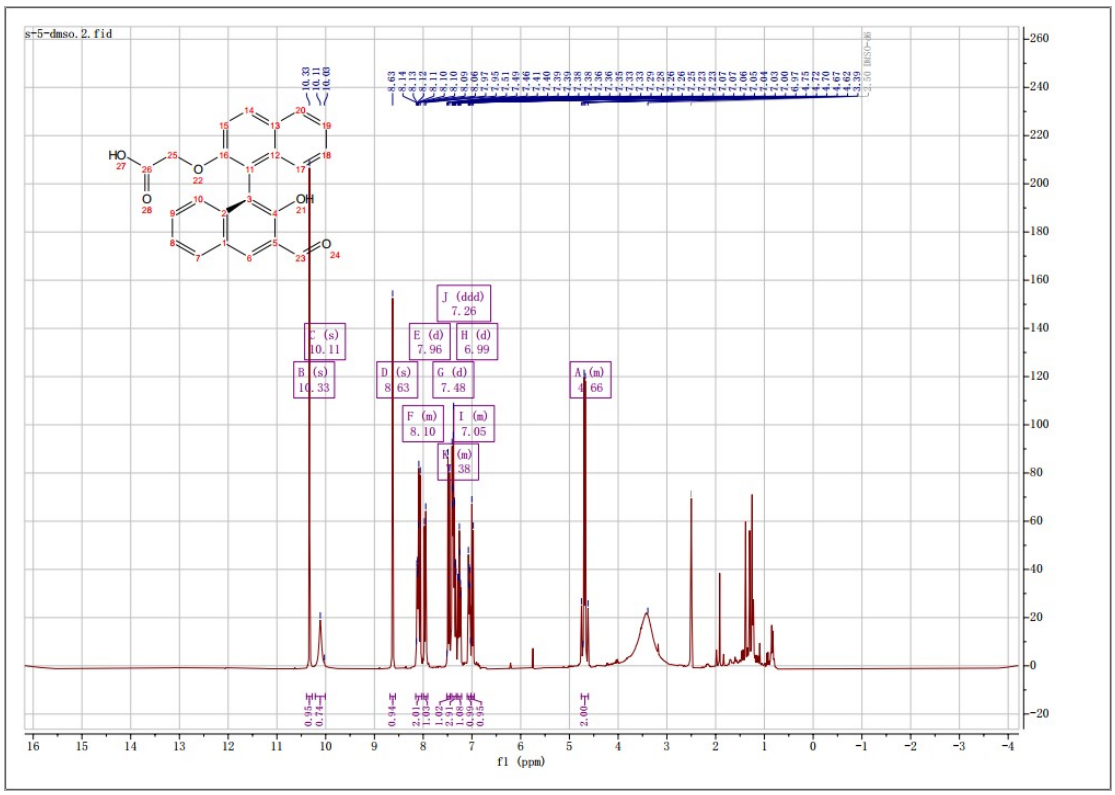


Figure S4. ^{1}H NMR spectra of (S)-5 in DMSO-d_6 (300 MHz)

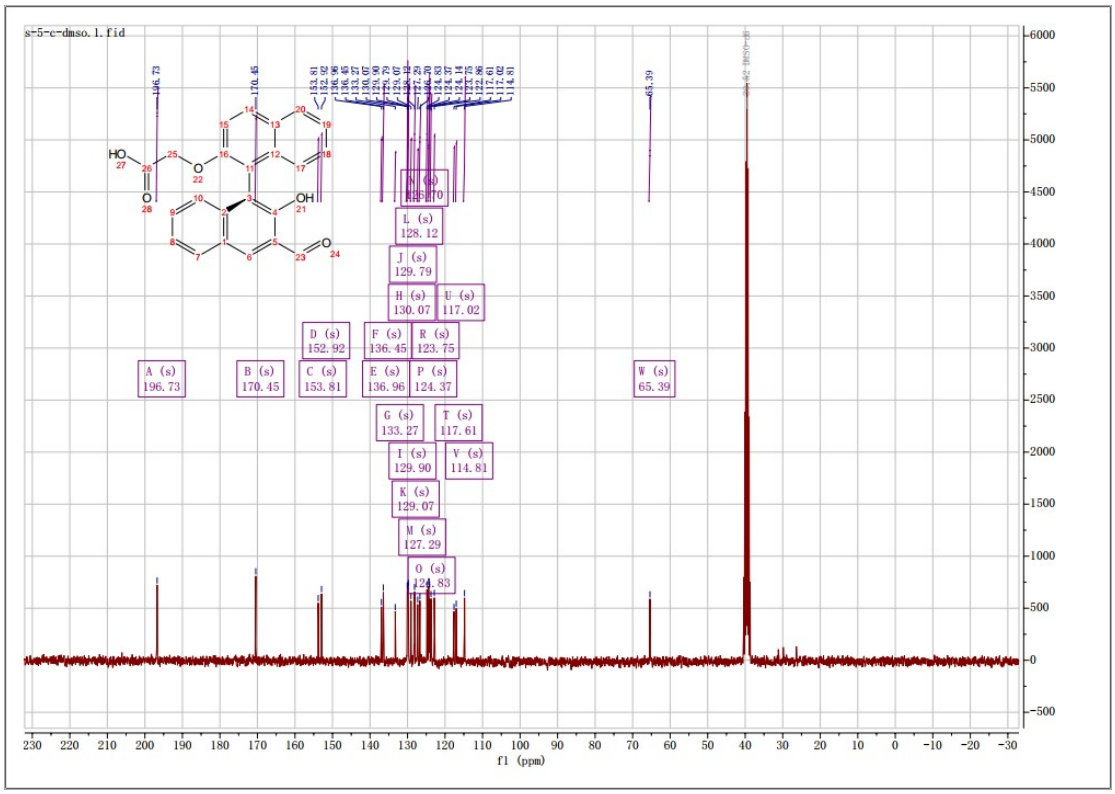


Figure S5. ^{13}C NMR spectra of (S)-5 in DMSO-d_6 (300 MHz)

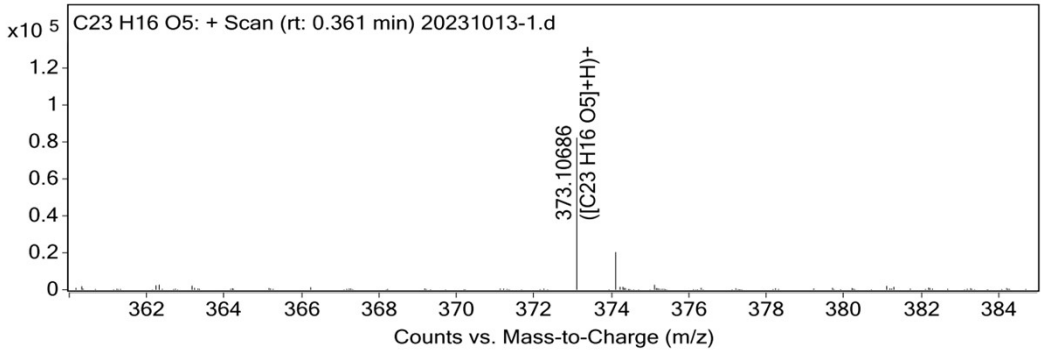


Figure S6. HRMS: m/z calcd. for (S)-5 $[M+H]^+$: 373.09977, found 373.10686.

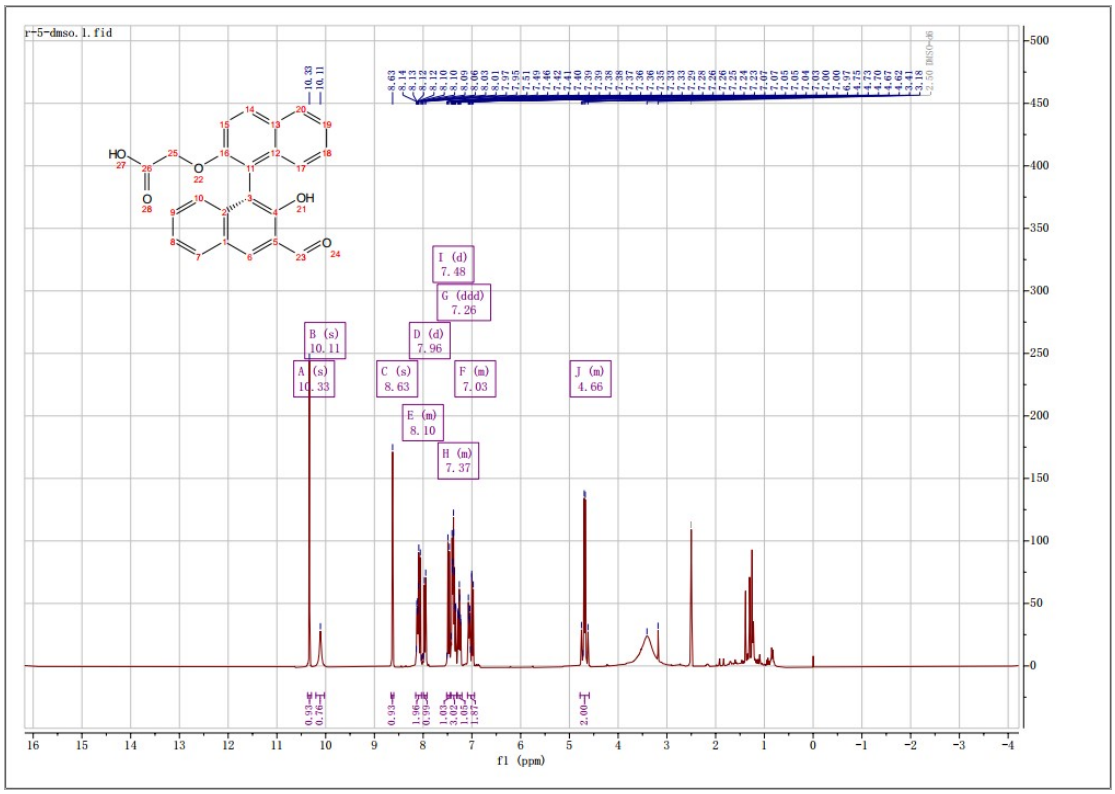


Figure S7. ^{1}H NMR spectra of (R)-5 in DMSO-d_6 (300 MHz)

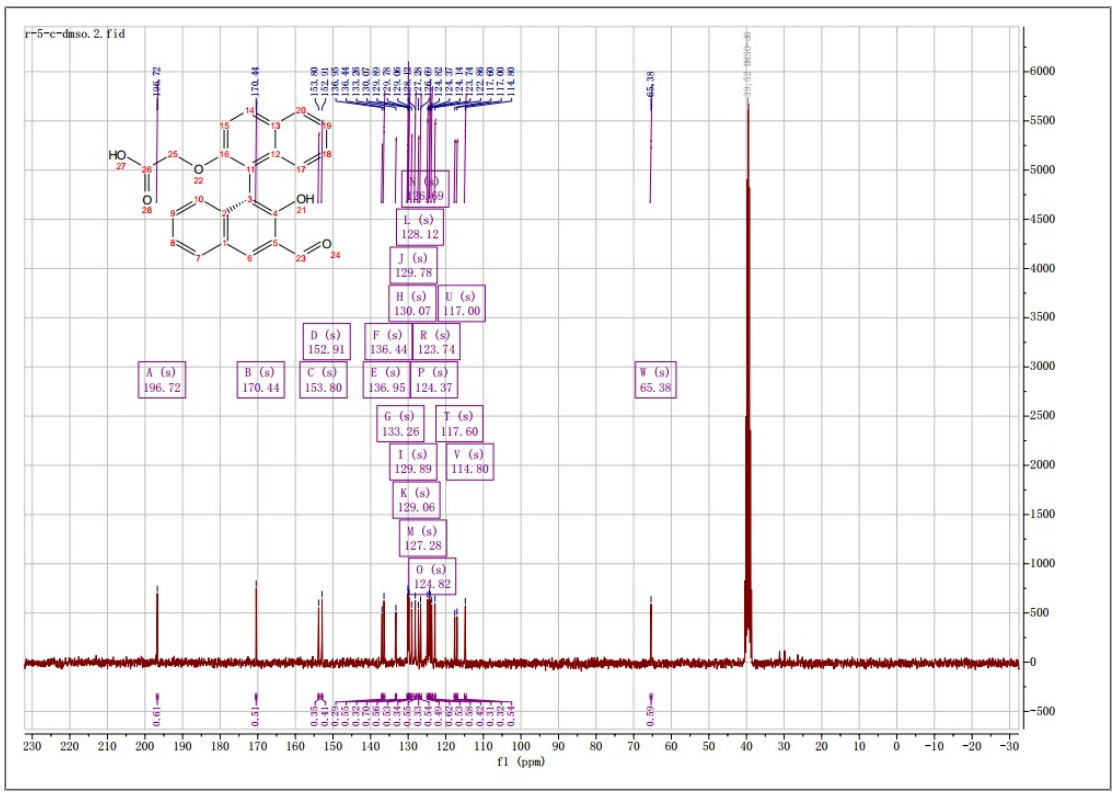


Figure S8. ^{13}C NMR spectra of (R)-5 in DMSO-d_6 (300 MHz)

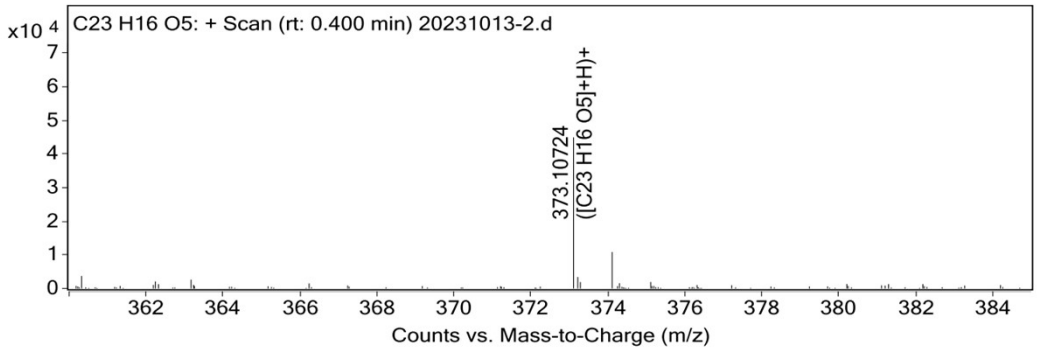


Figure S9. HRMS: m/z calcd. for (R)-5 $[M+H]^+$: 373.09977, found 373.10724.

3. Fluorescence spectra of (R)-5 /(S)-5 with amino acids & fluorescence lifetime

First, a 1.0 mM probe stock solution was prepared by dissolving (R)-5 in chromatography-grade methanol. Subsequently, $Zn(AcO)_2$ and amino acids were dissolved in ultrapure water, and 1.0 mM zinc ion stock solution and amino acid stock solution were prepared. 10 μL of probe solution, 10 μL of Zn^{2+}, and 50 μL of amino acids were added to the centrifuge tube and diluted to 1 mL with 930 μL of methanol to make the final concentration of the probe in solution 10 μM. The fluorescence determination was performed after 1.0 h of reaction at room temperature.

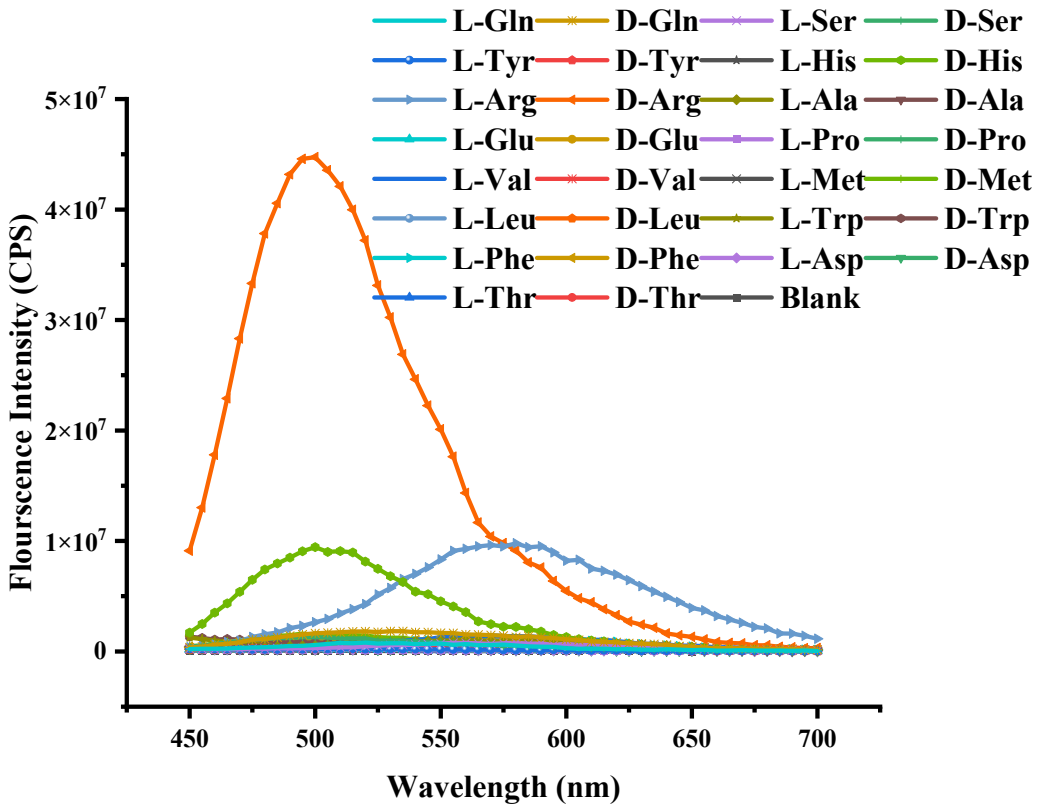


Figure S10. (R)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 5.0 eq of D-/L-amino acids, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

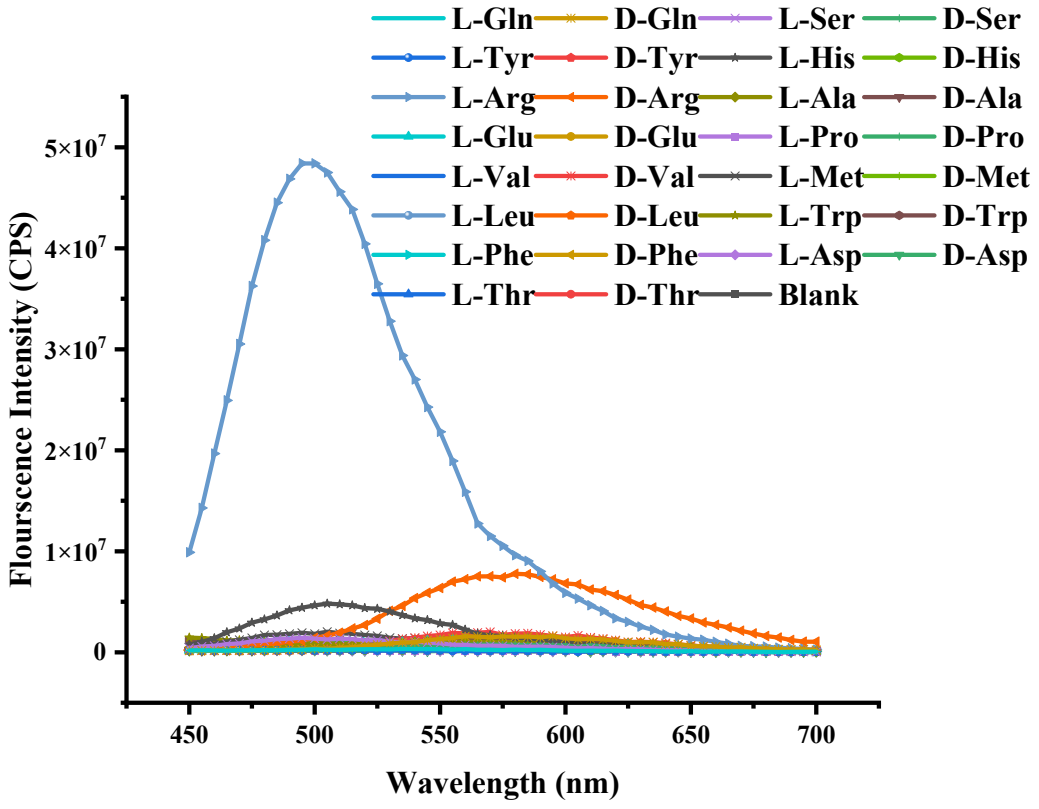


Figure S11. (S)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 5.0 eq of D-/L-amino acids, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

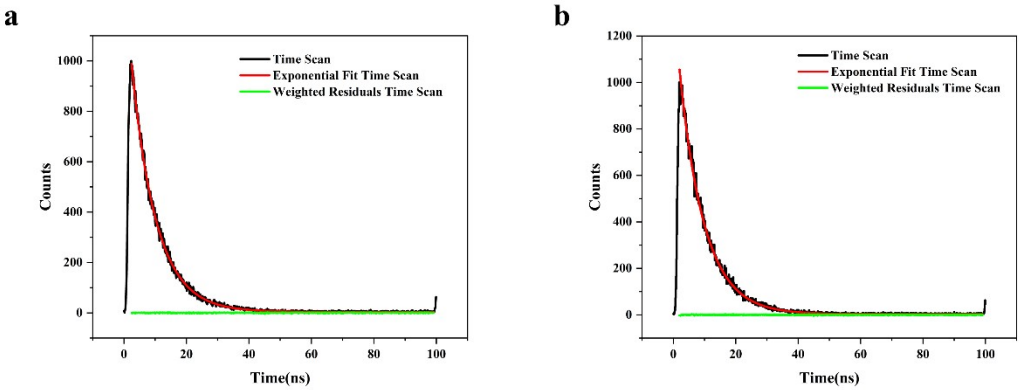


Figure S12. (a) Fluorescence lifetime curves and its fitting curve of (R)-5 (10 μM) with the addition of 1.0 eq of Zn^{2+} and 5.0 eq of D-Arg, MeOH:H_2O = 94:6 (λ_{ex} = 265 nm, λ_{em} = 470 nm, slit width = 5.0/5.0 nm, t = 1.0 h). (b)Fluorescence lifetime curves and its fitting curve of (S)-5 (10 μM) with the addition of 1.0 eq of Zn^{2+} and 5.0 eq of L-Arg, MeOH:H_2O = 94:6 (λ_{ex} = 265 nm, λ_{em} = 470 nm, slit width = 5.0/5.0 nm, t = 1.0 h).

4. Fluorescence spectra of (R)-5 /(S)-5 with D-Arg, L-Arg

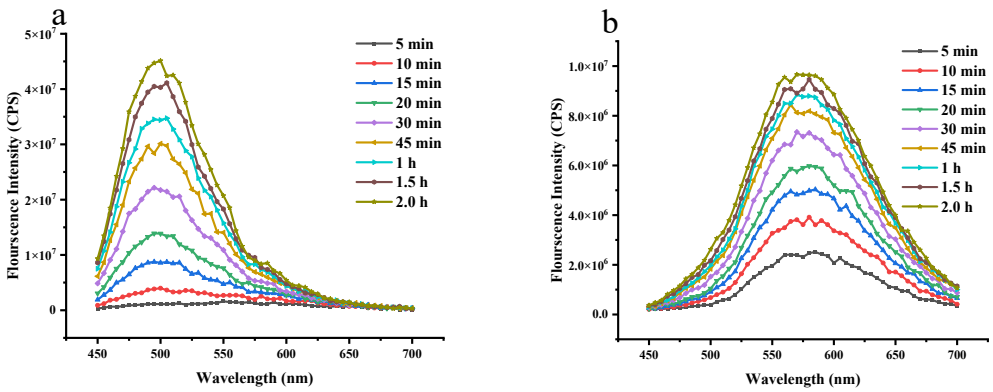


Figure S13. (R)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 5.0 eq of D- Arg（a）/L-Arg（b）, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t =5 min-2.0 h).

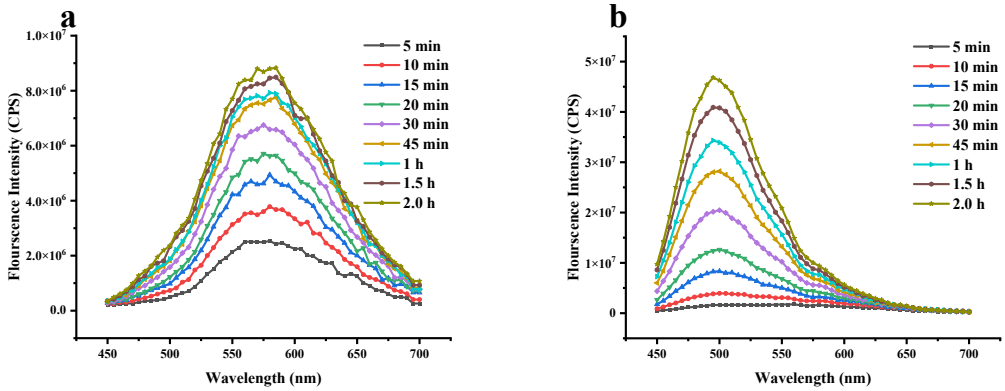


Figure S14. (S)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 5.0 eq of D- Arg（a）/L-Arg（b）, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t =5 min-2.0 h).

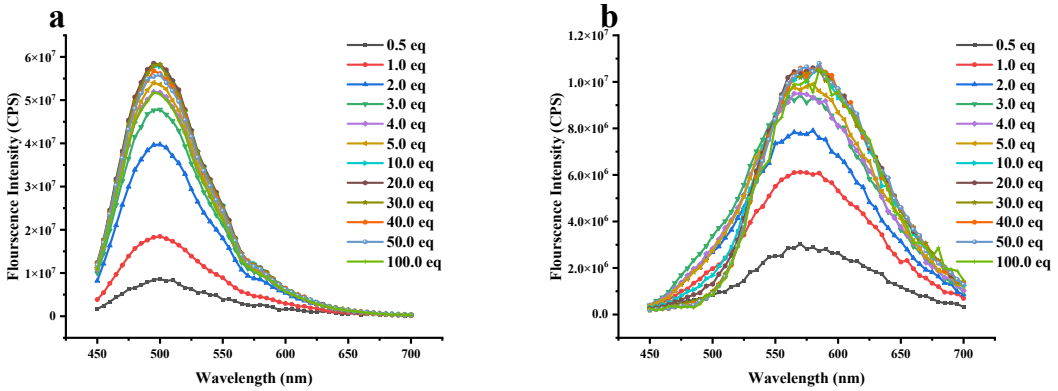


Figure S15. (R)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 0.5 -100 eq of D- Arg（a）/L-Arg（b）, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

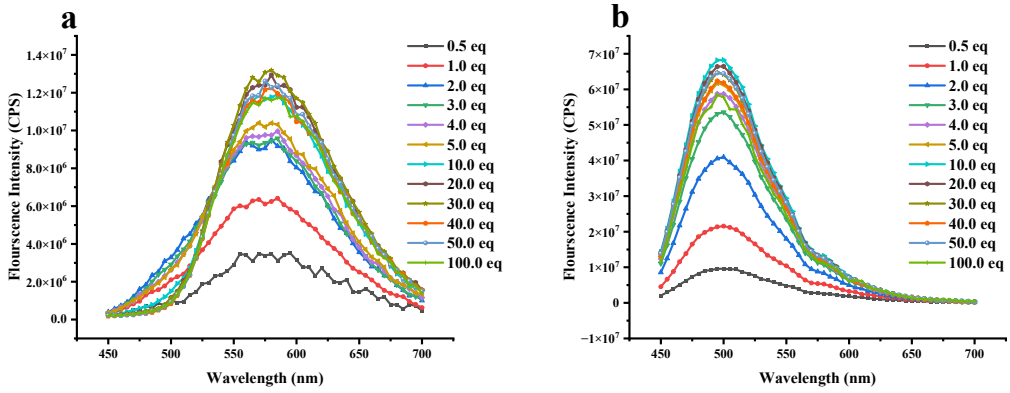


Figure S16. (S)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 0.5 -100 eq of D- Arg（a）/L-Arg（b）, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

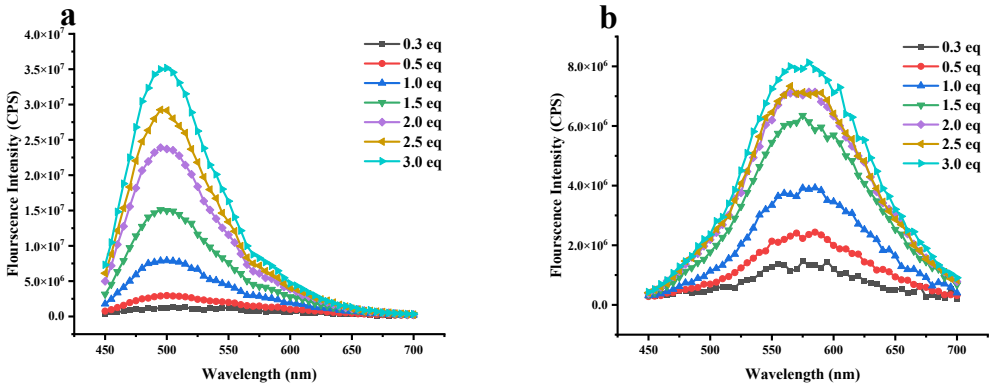


Figure S17. (R)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 0.3 -3.0 eq of D- Arg（a）/L-Arg（b）, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

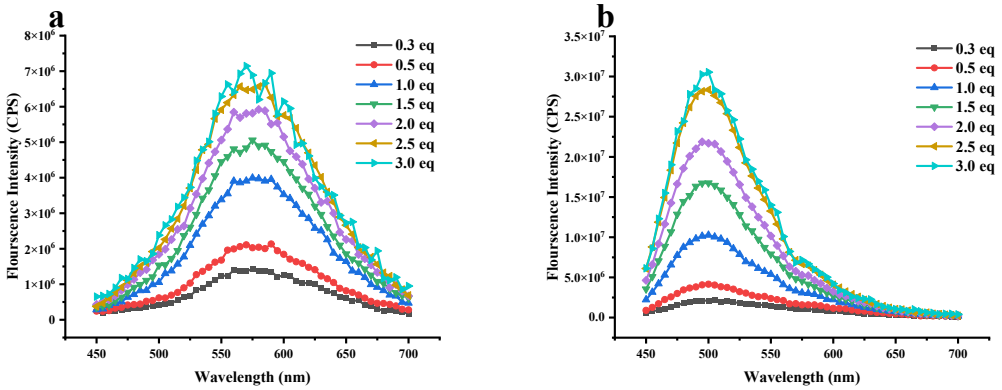


Figure S18. (S)-5 (10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 0.3 -3.0 eq of D- Arg（a）/L-Arg（b）, MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

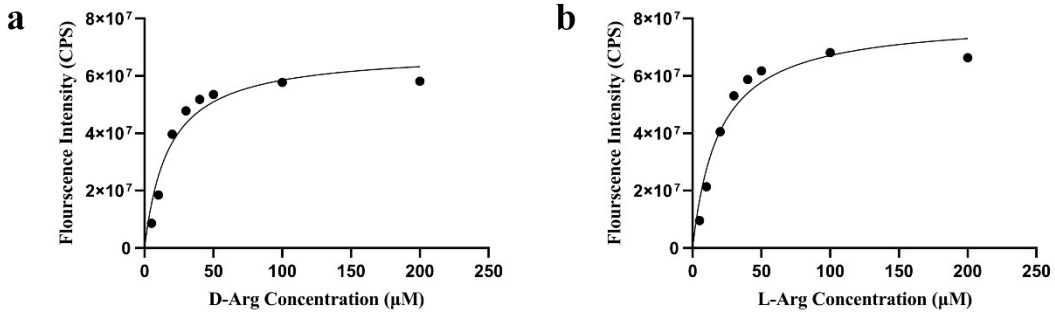


Figure S19. (a) Nonlinear fitting of One-site--Specific binding fluorescence intensity curve of (R)-5 (10 μM) with the addition of 1.0 eq of Zn^{2+} and varying eq of D-Arg, MeOH:H_2O = 94:6 (λ_{ex} = 265 nm, λ_{em} = 470 nm, slit width = 5.0/5.0 nm, t = 1.0 h). (b) Nonlinear fitting of One-site--Specific binding fluorescence intensity curve of (S)-5 (10 μM) with the addition of 1.0 eq of Zn^{2+} and varying eq of L-Arg, MeOH:H2O = 94:6 (λ_{ex} = 265 nm, λ_{em} = 470 nm, slit width = 5.0/5.0 nm, t = 1.0 h).

5. Fluorescence spectra of ee studies of Arg

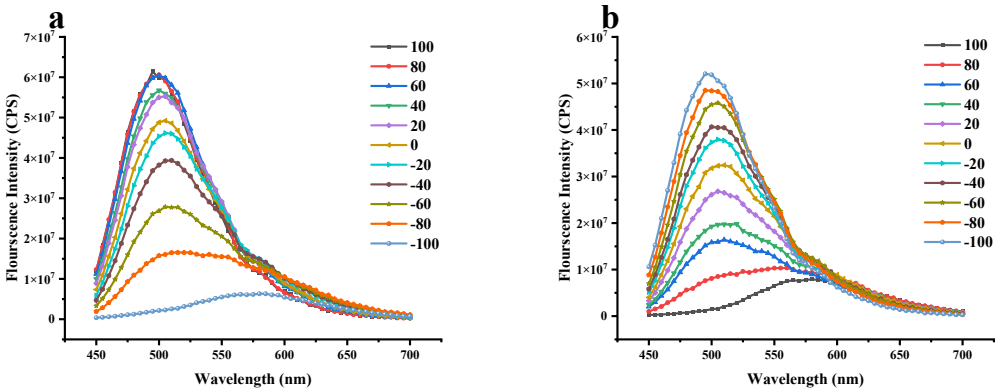


Figure S20. (R)-5（a）/(S)-5（b）(10 μM) in fluorescence emission spectra with 1.0 eq of Zn^{2+} and 5.0 eq of Arg , MeOH:H_2O = 94:6. (λ_{ex} = 265 nm, λ_{em} = 450 nm – 700 nm, slit = 5.0/5.0 nm, t = 1.0 h).

6. Mass studies

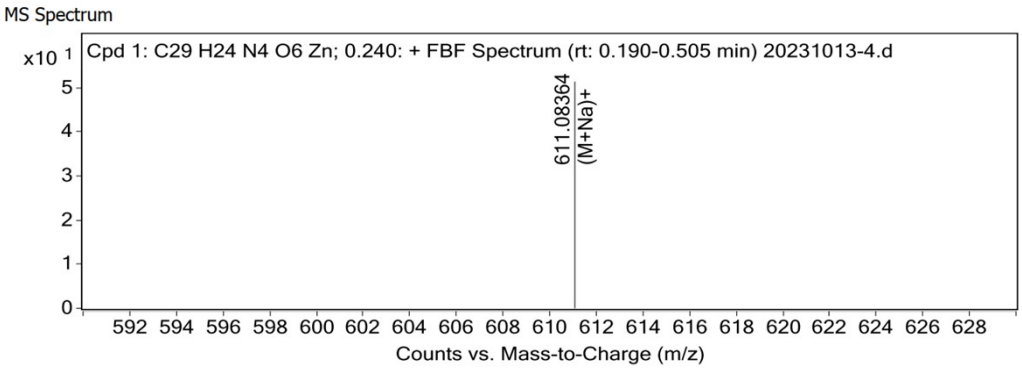


Figure S21. HRMS: m/z calcd. for Complex (S)-5+L-Arg+Zn^{2+} $[M+Na]^+$: 611.07646, found 611.08364.

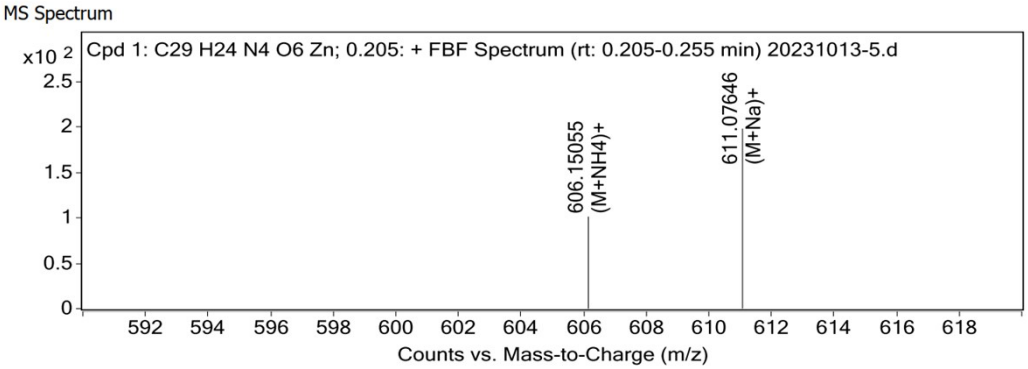


Figure S22. HRMS: m/z calcd. for Complex (R)-5+D-Arg+Zn^{2+} $[M+Na]^+$: 611.07646, found 611.07646.