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Zn(II) coordinated organic small molecules as chiral fluorescent probe for discrimination and detection of tyrosine enantiomers

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Contents

Experimental section

Reagents and apparatus. Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), ferric chloride ($FeCl_3$), cobaltous nitrate hexahydrate ($Co(NO_3)_2{\cdot}6H_2O$), cadmium sulfate 8/3hydrate ($CdSO_4{\cdot}8/3H_2O$), cupric sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), nickel sulfate hexahydrate ($NiSO_4{\cdot}6H_2O$), ferrous sulfate heptahydrate **(**$FeSO_4{\cdot}7H_2O$), petroleum ether (PE), dichloromethane (DCM), ethanol, methanol, hexamethylenetetramine (HMTA), ethyl acetate, N,N-diisopropylethylamine (DIPEA), anhydrous sodium sulfate (Na_2SO_4), potassium chloride (KCl) and hydrochloric acid (HCl) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). D-tyrosine (D-Tyr), L-tyrosine (L-Tyr), (1R,2R)-(+)-1,2-diphenyl-1,2-ethylenediamine, trifluoroacetic acid (TFA), tetrahydrofuran (THF), 2–(7-azabenzotriazole)-N,N,N’,N’-tetramethylurea hexafluorophosphate (HATU), N,N-dimethylformamide (DMF), acetonitrile, 5-methylsalicylic acid and 4-(2-hydroxyerhyl) piperazine-1-erhaesulfonic acid (HEPES) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Sodium bicarbonate ($NaHCO_3$) was received from Rhawn Chemical Technology Co., Ltd. (Shanghai, China). Ultrapure water (18.2 MΩ·cm) was used throughout.

The proton nuclear magnetic resonance (^{1}H NMR) spectra of the intermediate P1, COSM and COSM/Zn(II) were acquired with an Avance III 400MHz NMR spectrometer (Bruker, Switzerland) by using DMSO-d_6 as the solvent. The High-resolution mass spectra (HRMS) were acquired with a 6540 Q-TOF Mass spectrometer (Agilent Technologies Inc., United States). The optical rotation and specific rotation values were acquired with a Rudolph AUTOPOL VI polarimeter (Rudolph Research Analytical, United States). The Fourier transform infrared (FTIR) spectra of different samples were acquired with a FTIR-8400S FT-IR spectrometer (Shimadzu, Japan). The fluorescence spectra of different systems were recorded by using a G9800A fluorescence spectrometer (Agilent Technologies Inc., United States). The ultraviolet-visible (UV-vis) spectra of COSM and COSM/Zn(II) complex were acquired with a L5S UV-vis spectrophotometer (Shanghai Yidian Analytical Instrument Co., Ltd., China), and the CD spectra of COSM and COSM/Zn(II) were acquired with a J-1500 CD spectrometer (JASCO, Japan). The electrochemical measurements were carried out on a CHI 660E electrochemical workstation (Shanghai

Chenhua Instruments Co. Ltd., China). Density functional theory (DFT) was accomplished with the GaussView 6.0 software package.

Synthesis of intermediate P1. The intermediate P1 was synthesized by the procedure previously reported with slight modifications.[S1] Briefly, 0.78 g of 5-methylsalicylic acid, 2.14 g of HATU and 1.64 mL of DIPEA were successively added into 50 mL of DCM and magnetically stirred for 1 h, and then 0.5 g of (1R,2R)-(+)-1,2-diphenyl-1,2-ethylenediamine was added into the mixture. The reaction was maintained at room temperature overnight with continuous stirring until the starting material cannot be observed by thin-layer chromatography (TLC). Next, the mixture was transferred to a separatory funnel and washed with 50 mL of 1 M HCl, and then the organic phase was collected and washed with 100 mL of saturated $NaHCO_3$ solution. The organic phase was separated with the separatory funnel, and the residual moisture was removed with anhydrous Na_2SO_4. After that, the organic phase was evaporated in vacuum to yield the crude product, which was purified by column chromatography using DCM/PE (4/1, v/v) as the eluent. Finally, the pure product was evaporated in vacuum to obtain the intermediate P1 (white solid, 0.43g, 0.895 mmol, 38% yield).

Synthesis of COSM and COSM/Zn(II). The intermediate P1 (0.43 g) and 2.7 g of HMTA were dissolved in 30 mL of TFA, which was refluxed at 85 °C overnight. Next, 50 mL of 1 M HCl was added into the mixture and continuously stirred for 24 h, and then the pH of the mixture was adjusted to 7.0 with saturated $NaHCO_3$ solution. After that, the mixture was extracted with ethyl acetate (3 × 150 mL), and the organic phase was separated with the separatory funnel. After the removal of the residual moisture with anhydrous Na_2SO_4, the organic phase was evaporated in vacuum to yield the crude product, which was purified by column chromatography using DCM/methanol (95/5, v/v) as the eluent. Finally, the pure product was evaporated in vacuum to obtain the COSM (brownish-yellow solid, 0.13 g, 0.241 mmol, 27% yield).

To synthesize the COSM/Zn(II) complex, 250 μL of acetonitrile, 50 μL of 1 mM COSM (solvent, acetonitrile), 100 μL of 0.1 M HEPES buffer solution (solvent, acetonitrile/H_2O = 9/1, v/v; pH = 8.5) and

50 μL of 2 mM $Zn(NO_3)_2$ were successively added into a 2 mL centrifuge tube, which was allowed to stand for 10 min at room temperature.

Electrochemical measurements. First, 150 μL of 1 mM L-Tyr and D-Tyr was respectively injected into the COSM/Zn(II) solution in a 2 mL centrifuge tube, which was allowed to stand for 90 min at 38 °C. Next, 5 μL of COSM/Zn(II) + D-Tyr and COSM/Zn(II) + L-Tyr solution was respectively cast onto the surface of a clean glassy carbon electrode (GCE), and the solvent was evaporated under an infrared lamp. After that, the differential pulse voltammograms (DPVs) of COSM/Zn(II) + L-Tyr/GCE and COSM/Zn(II) + D-Tyr/GCE in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$ were recorded in a three-electrode cell consisting of COSM/Zn(II) + L-Tyr/GCE or COSM/Zn(II) + D-Tyr/GCE as the working electrode, a platinum plate as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode.

Fluorescent discrimination and detection of Tyr enantiomers. For fluorescent discrimination of the Tyr enantiomers, 50 μL of 1 mM L-Tyr and D-Tyr was respectively injected into the COSM/Zn(II) solution in a 2 mL centrifuge tube, which was allowed to stand for 90 min at 38 °C. After that, the fluorescence emission spectra of COSM/Zn(II) + L-Tyr and COSM/Zn(II) + D-Tyr were respectively recorded at an excitation wavelength (λ_{ex}) of 390 nm, and the Tyr enantiomers were discriminated according to the difference in the fluorescence intensity at the maximum emission wavelength.

For fluorescent detection of the Tyr enantiomers, 50 μL of D-Tyr and L-Tyr of different concentrations was respectively injected into the COSM/Zn(II) solution in a 2 mL centrifuge tube and allowed to stand for 90 min at 38 °C, and then the fluorescence emission spectra of COSM/Zn(II) + L-Tyr and COSM/Zn(II) + D-Tyr were recorded (λ_{ex} = 390 nm). D-Tyr was detected according to the linear relationship between the concentration of D-Tyr and the fluorescence intensity, and L-Tyr was detected according to the linear relationship between the concentration of L-Tyr and the maximum emission wavelength.

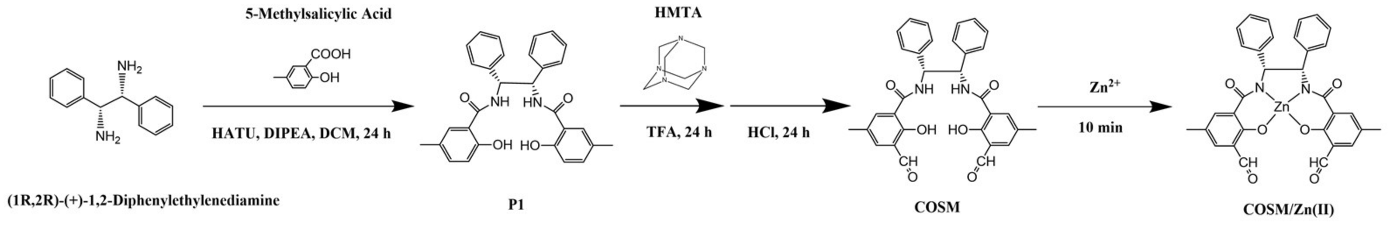
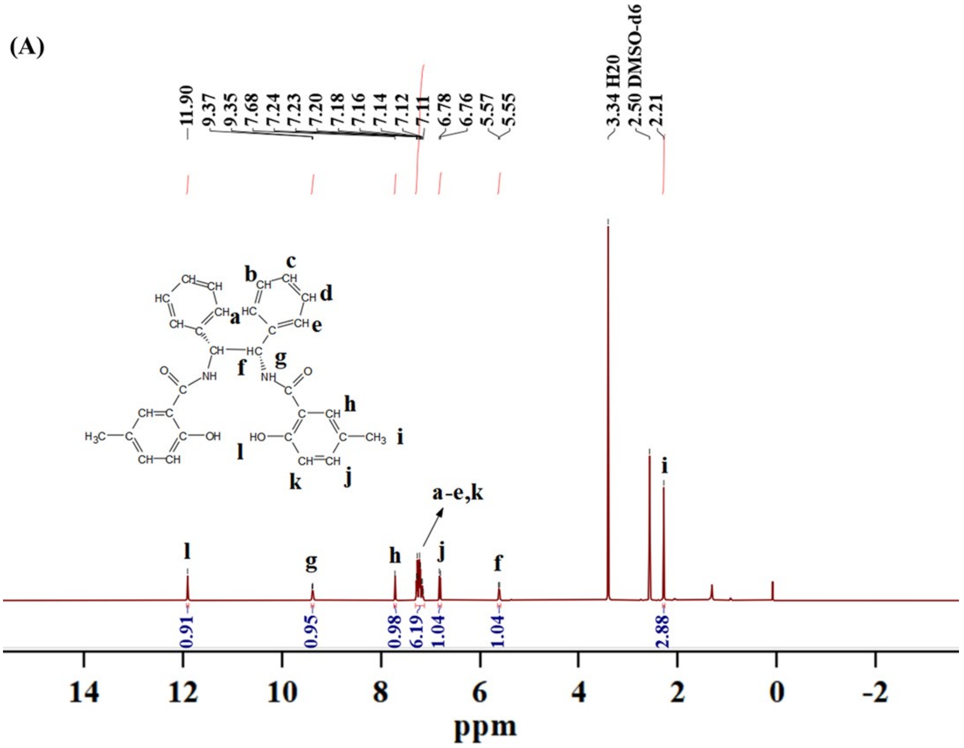


Fig. S1 Schematic illustrating the synthesis of intermediate P1, COSM and COSM/Zn(II).

P1 ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1H), 9.36 (d, *J* = 5.4 Hz, 1H), 7.68 (s, 1H), 7.27–7.08 (6H), 6.77 (d, *J* = 8.3 Hz, 1H), 5.56 (d, *J* = 7.7 Hz, 1H), 2.21 (s, 3H).



COSM ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.13 (d, J = 9.5 Hz, 1H), 10.28 (s, 1H), 9.48 (s, 1H), 7.89 (s, 1H), 7.51 (s, 1H), 7.34–7.07 (5H), 5.65 (d, J = 9.1 Hz, 1H), 2.19 (s, 3H).

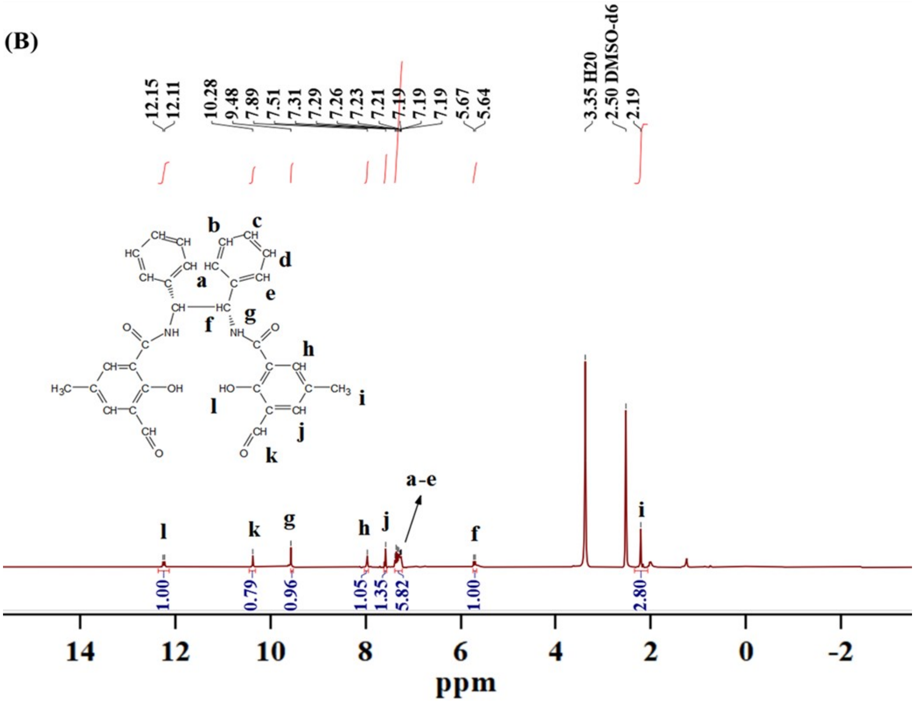
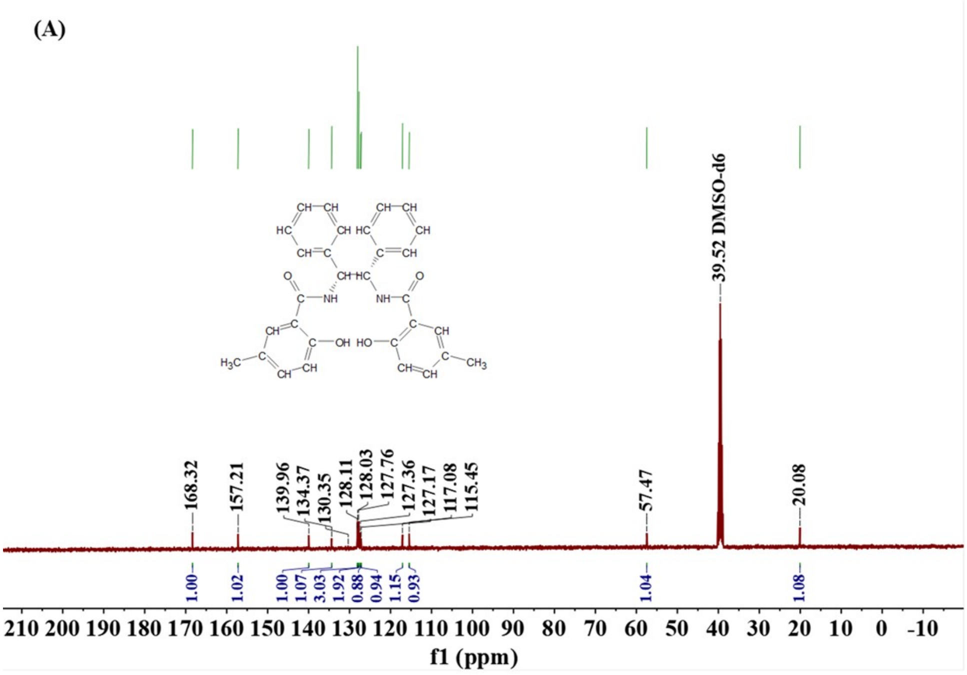


Fig. S2 ^{1}H NMR spectra of intermediate P1 (A) and COSM (B).

P1 ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.32, 157.21, 139.96, 134.37, 130.35, 128.11, 128.03, 127.76, 127.36, 127.17, 117.08, 115.45, 57.47, 20.08.



COSM ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 195.39, 168.78, 165.27, 142.31, 134.54, 128.06, 127.99, 127.77, 126.68, 124.45, 120.96, 57.46, 19.58.

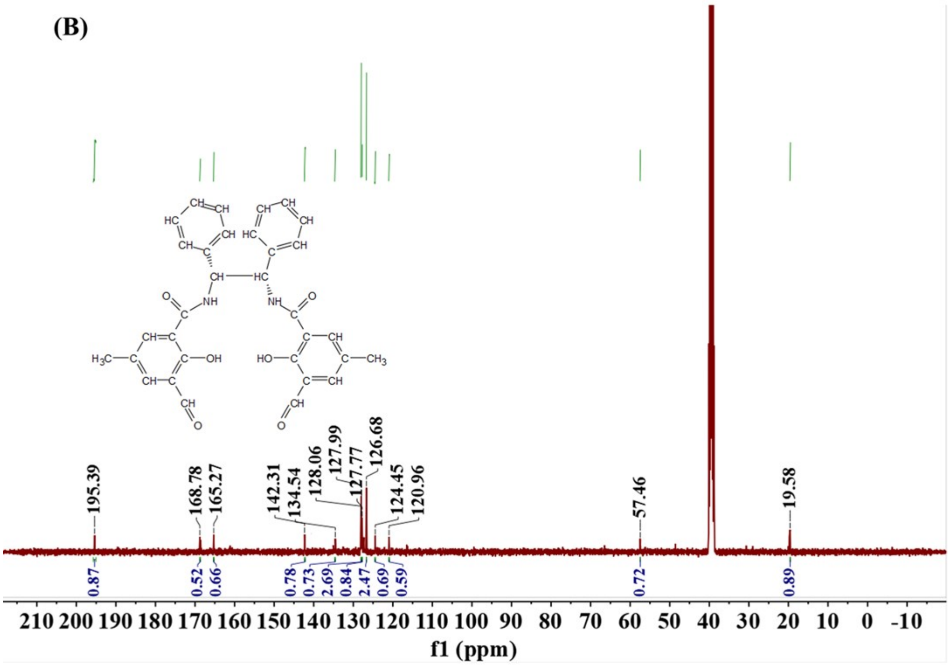
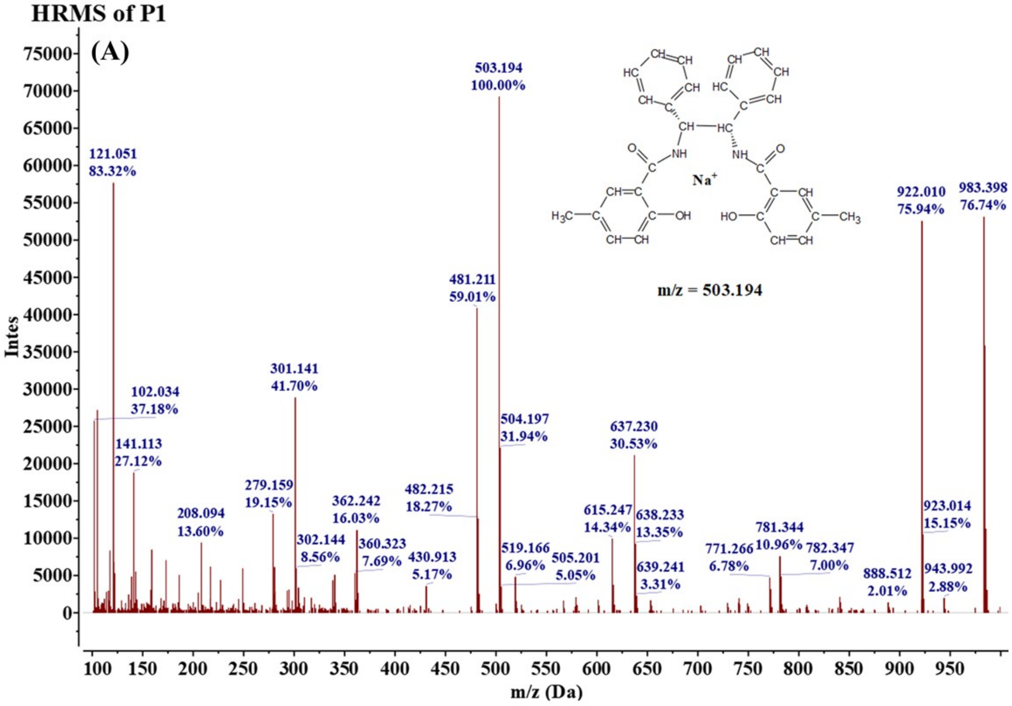


Fig. S3 ^{13}C NMR spectra of intermediate P1 (A) and COSM (B).



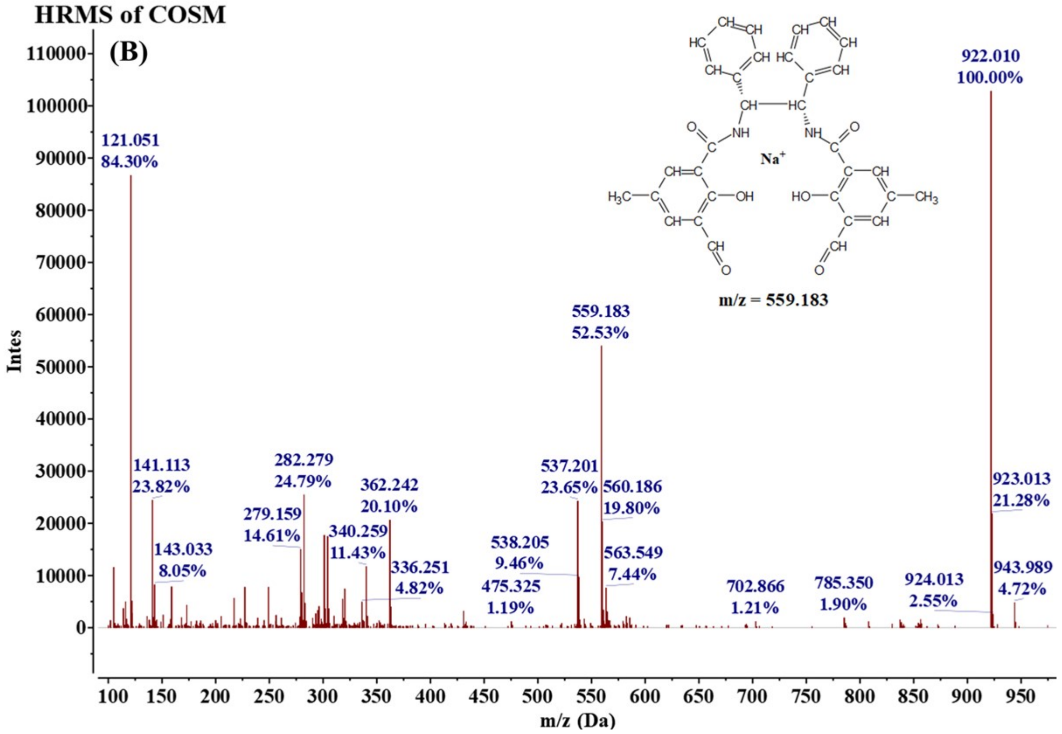


Fig. S4 HRMS of P1 (A) and COSM (B).

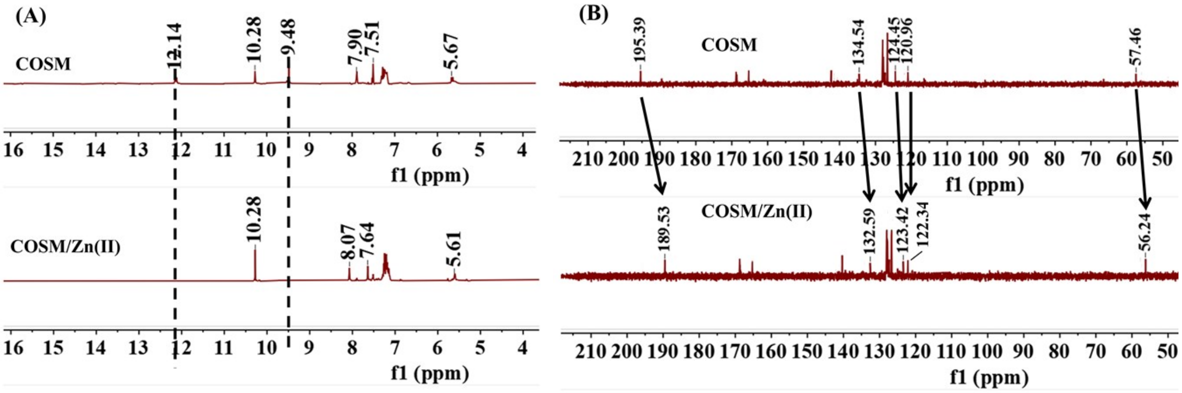


Fig. S5 ^{1}H NMR (A) and ^{13}C NMR (B) spectra of COSM and COSM/Zn(II)**.**

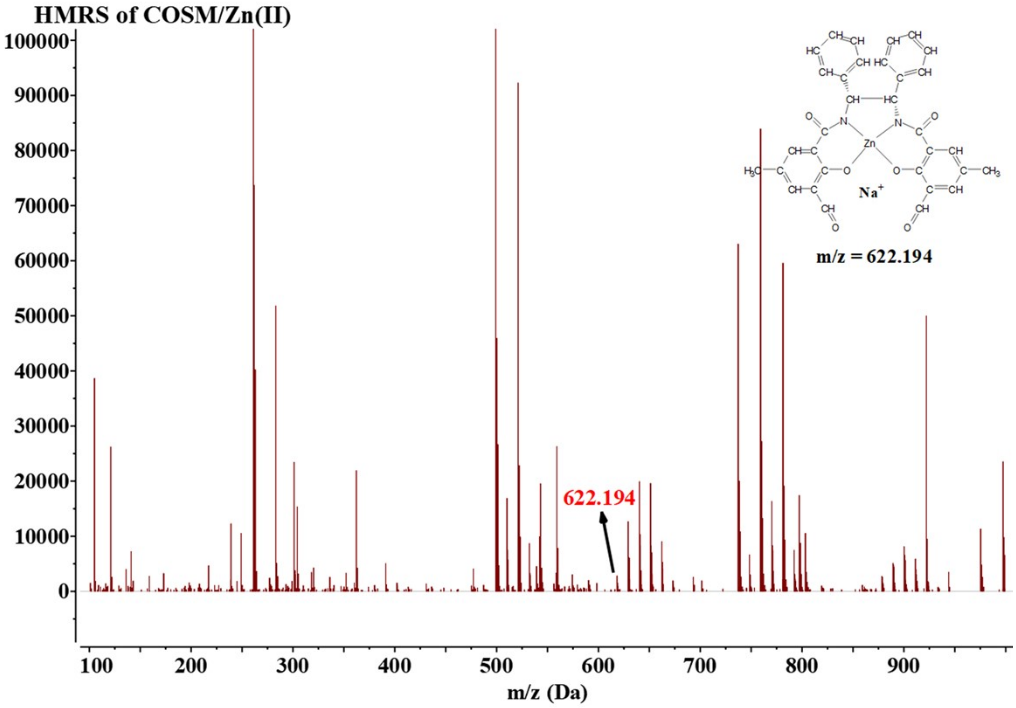


Fig. S6 HRMS of COSM/Zn(II).

Table. S1 Specific optical rotation values of COSM and COSM/Zn(II).

Sample	Wavelength (nm)	Pathlength (dm)	Concentration (g 100mL^{-1})	Solvent	Specific optical rotation value
COSM	589	1	0.00785	acetonitrile	105.73° mL g^{-1} dm^{-1}
COSM/Zn(II)	589	1	0.00785	acetonitrile	280.54° mL g^{-1} dm^{-1}

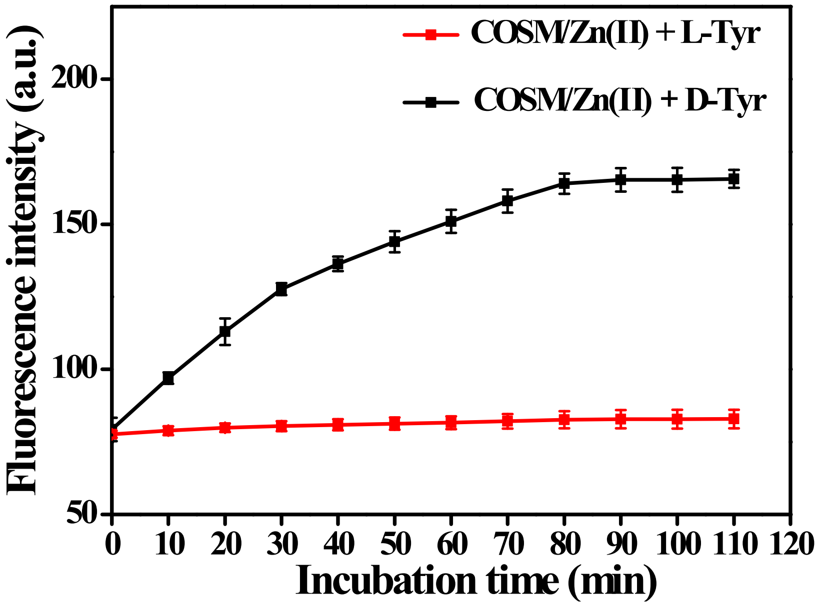
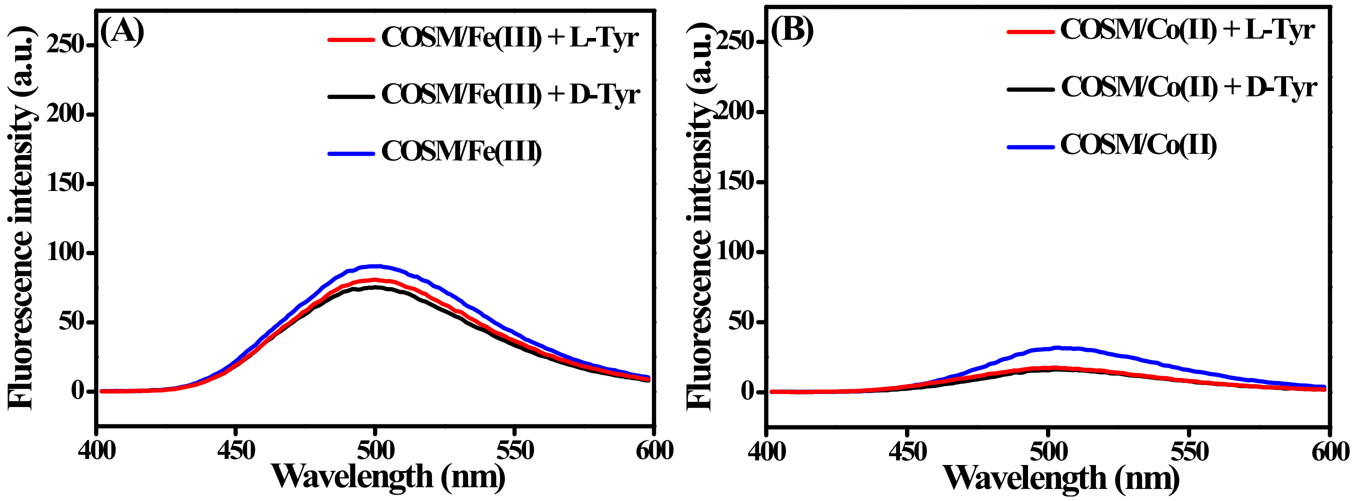


Fig. S7 Fluorescence intensity of COSM/Zn(II) + L-Tyr and COSM/Zn(II) + D-Tyr at different incubation times.



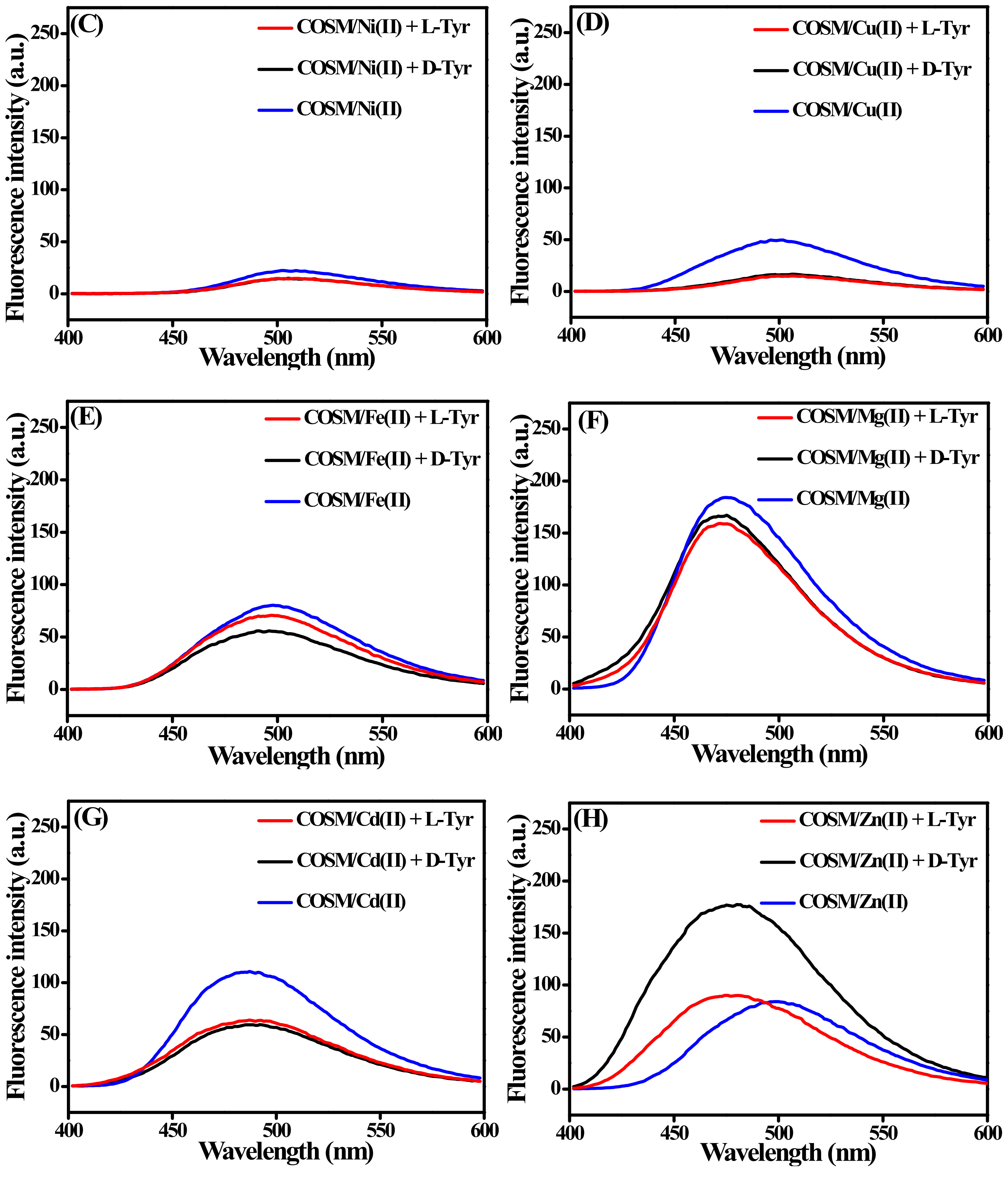


Fig. S8 Fluorescence spectra of COSM/metal ions and COSM/metal ions + Tyr enantiomers (1 mM). (A) Fe^{3+}, (B) Co^{2+}, (C) Ni^{2+}, (D) Cu^{2+}, (E) Fe^{2+}, (F) Mg^{2+}, (G) Cd^{2+}, and (H) Zn^{2+}.

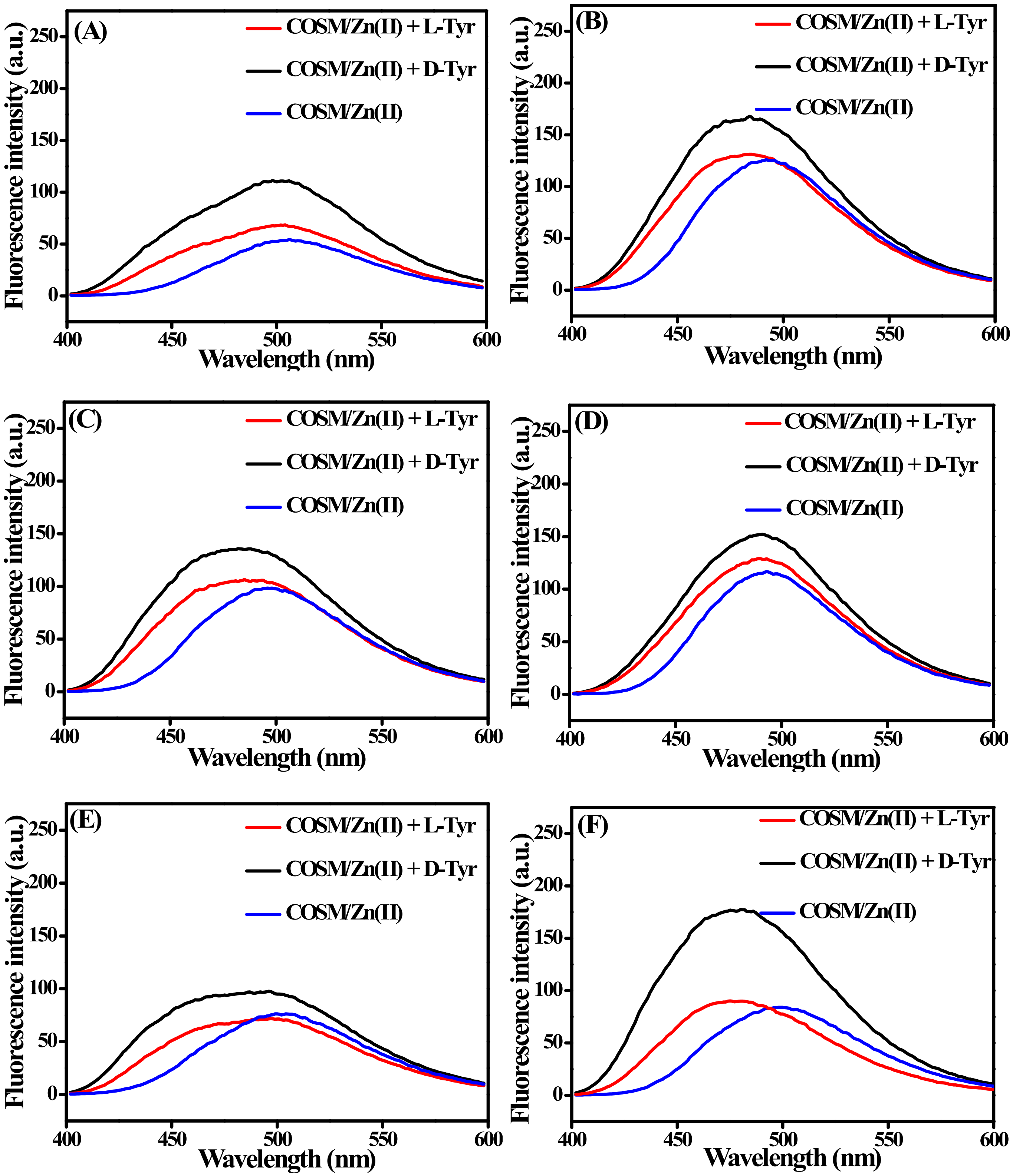


Fig. S9 Fluorescence spectra of COSM/Zn(II) and COSM/Zn(II) + Tyr enantiomers (1 mM) by using H_2O (A), THF (B), ethanol (C), DMF (D), methanol (E) and acetonitrile (F) as the solvent.

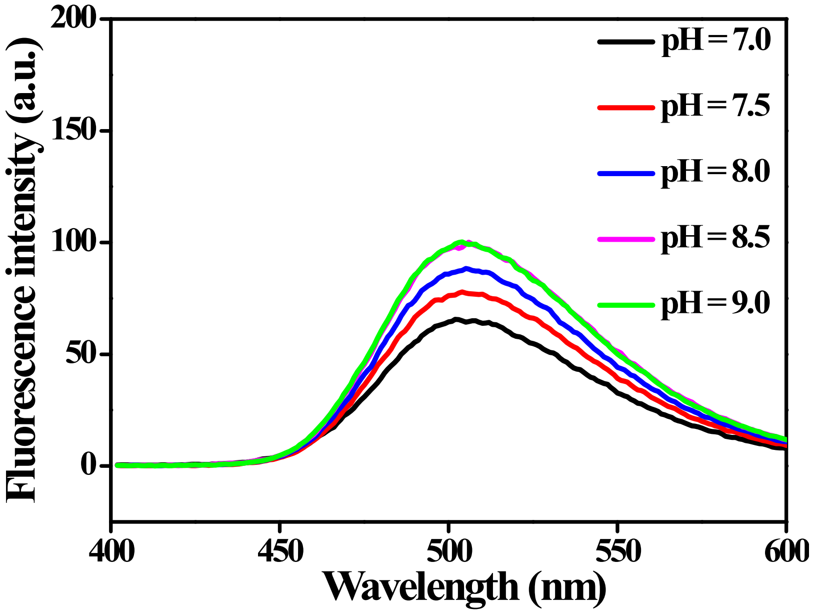


Fig. S10 Fluorescence spectra of COSM/Zn(II) prepared at different pH values.

Table. S2 Comparison with the results obtained with previous work.

Sensor	Method	Linear range	LOD (μM)	Ref.
Cdots@Van	Fluorescence	10–120 μM	7.22 (D-Tyr) 8.12 (L-Tyr)	41
	CD	10–80 μM (D-Tyr)	6.38 (D-Tyr)	
Adonitol/GCE	SWV	10–1000 μM	6.85 (D-Tyr) 4.26 (L-Tyr)	42
Mal-βCD/BPNSs/GCE	SWV	10–1000 μM	6.89 (D-Tyr) 4.81 (L-Tyr)	43
L-NAC-CdSe/CdS QDs	Fluorescence	40–120 μM	–	44
AuNPs/D-sorbitol/GCE	SWV	10–1000 μM	8.07 (D-Tyr) 4.12 (L-Tyr)	45
COSM/Zn(II)	Fluorescence	10–1820 μM (D-Tyr) 10–1500 μM (L-Tyr)	2.77 (D-Tyr) 3.35 (L-Tyr)	this work

References

S1 Z. Wang, L. Zhao, B. Zhang, J. H. Feng, Y. L. Wang, B. Zhang, H. X. Jin, L. J. Ding, N. Wang and S. He, *J. Enzyme Inhib. Med. Chem.*, 2023, **38**, 2206581.