Supporting Information For

Highly Selective Fluorescent Recognition of Histidine by a Crown Ether-Terpyridine-Zn(II) Sensor

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Contents

1. General data

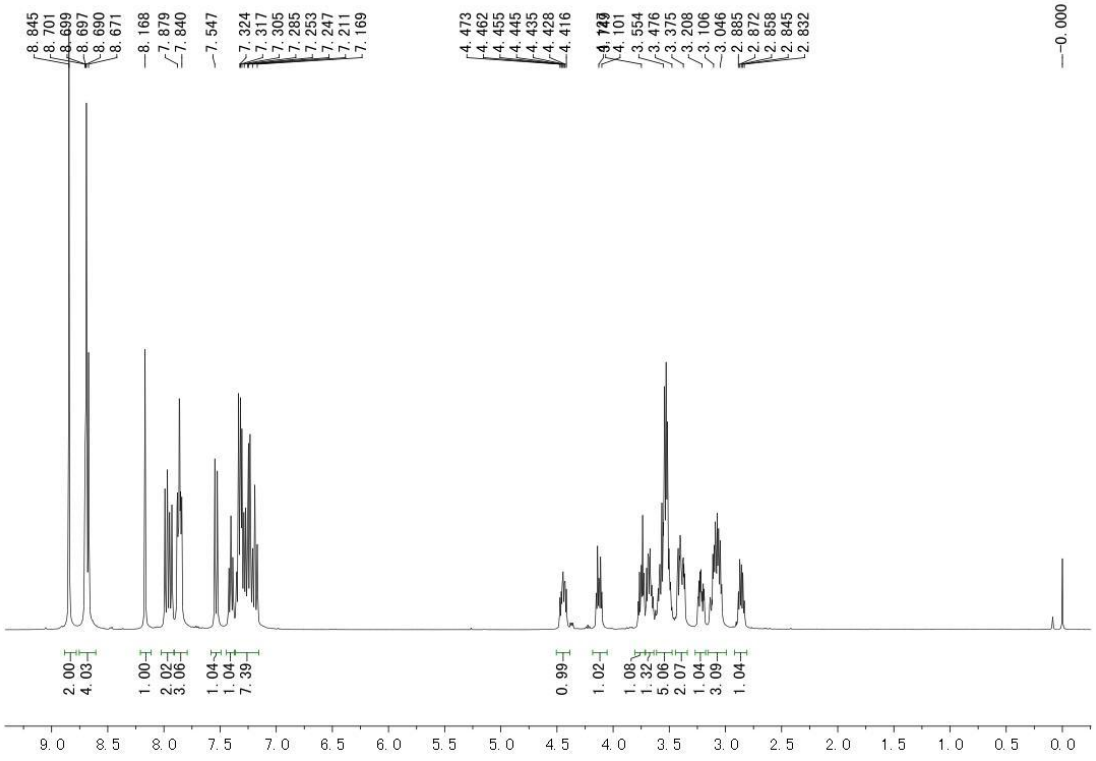
^{1}H and ^{13}C NMR spectra were measured on a Bruker AM400 NMR spectrometer. Proton chemical shifts of NMR spectra were given in ppm relative to internal reference TMS (1H, 0.00 ppm). ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluorophotometer (HORIBA Jobin Yvon) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

2. Preparation and Spectroscopic Characterization of New Compounds

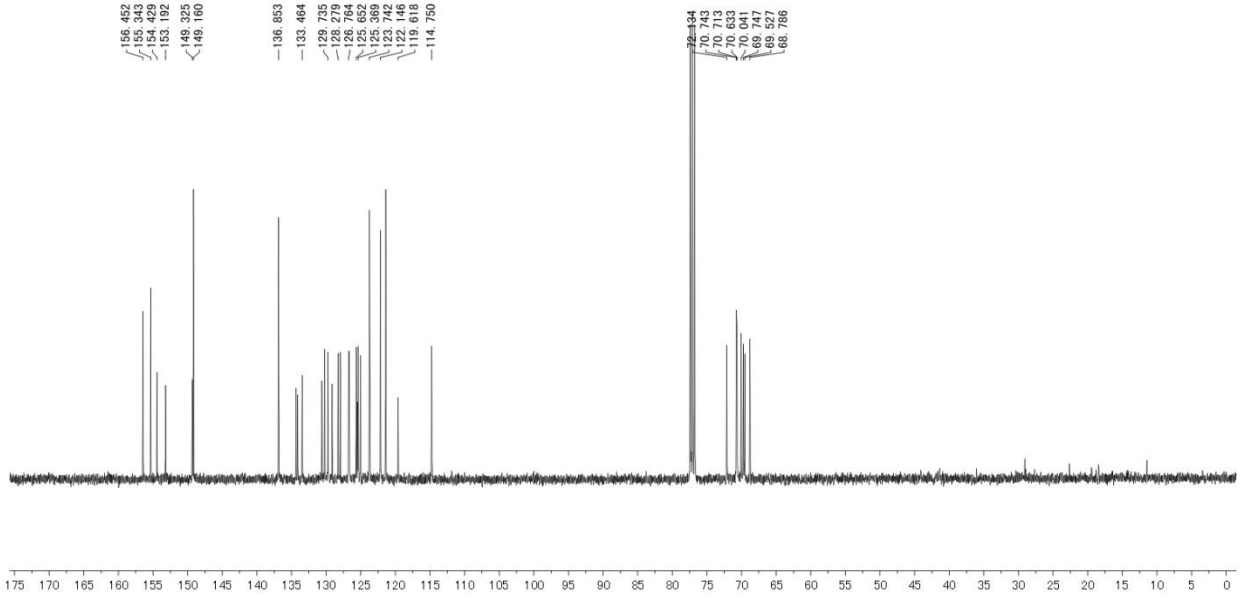
Preparation and characterization of 4'-(12,13,15,16,18,19,21,22-octahydrodinaphtho[2,1-n:1',2'-p][1,4,7,10,13]pentaoxacycloheptadecin-10-yl)-2,2':6',2''-terpyridine 8. 3-([2,2':6',2''-terpyridin]-4'-yl)-[1,1'-binaphthalene]-2,2'-diol **7** (0.98 g, 1.89 mmol) was stirred with dry K_2CO_3 (0.68 g, 4.92 mmol) in DMF at 90 ^{o}C. After 1 h, ((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl)bis(4-methylbenzenesulfonate) (1.14 g, 2.27 mmol) was added to the reaction mixture. The solution was heated at 90 ^{o}C for 24 h. Then water (30 mL) and dichloromethane (50 mL) were added. The organic layer was separated and the water layer was extracted by dichloromethane (50 mL× 3). The organic layers were combined and washed with saturated NaCl (30 mL × 3). After dried over Na_2SO_4, the solvent was removed and the residue was subjected to column chromatography on silica gel eluted with petroleum ether/ethyl acetate (2:1) to afford the product **8** as a white solid in 46% yield (0.57 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.85 (s, 2H), 8.79- 8.67 (m, 4H), 8.17 (s, 1H), 7.98 (d, 1H, *J* = 9.2 Hz), 7.94 (d, 1H, *J* = 8.00 Hz), 7.88- 8.84 (m, 3H), 7.54 (d, 1H, *J* = 9.2 Hz), 7.41 (t, 1H, *J* = 16.0 Hz), 7.35- 7.17 (m, 7H),

4.47- 4.42 (m, 1H), 4.15- 4.10 (m, 1H), 3.78- 3.72 (m, 1H), 3.70- 3.65 (m, 1H), 3.60- 3.48 (m, 5H), 3.43- 3.36 (m, 2H), 3.24- 3.19 (m, 1H), 3.14- 3.03(m, 3H), 2.89- 2.83 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4, 155.3, 154.4, 153.2, 149.3, 149.2, 136.9, 134.3, 134.1, 133.5, 130.6, 130.2, 129.7, 129.1, 128.3, 128.0, 126.8, 126.7, 125.7, 125.5, 123.7, 123.7, 122.1, 121.4, 119.6, 114.8. HR-MS (ES+) calcd for $C_{43}H_{37}N_3O_5$ (M+Na) 698.2625 and (M+H) 676.2806, found 698.2653 and 676.2802.

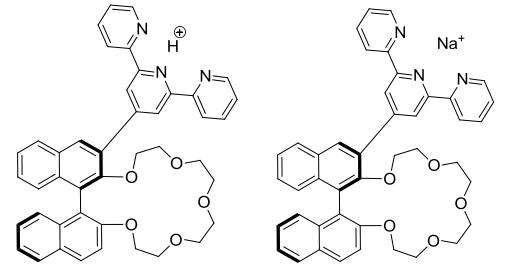
^{1}H-NMR of 8 (400MHz in $CDCl_3$)

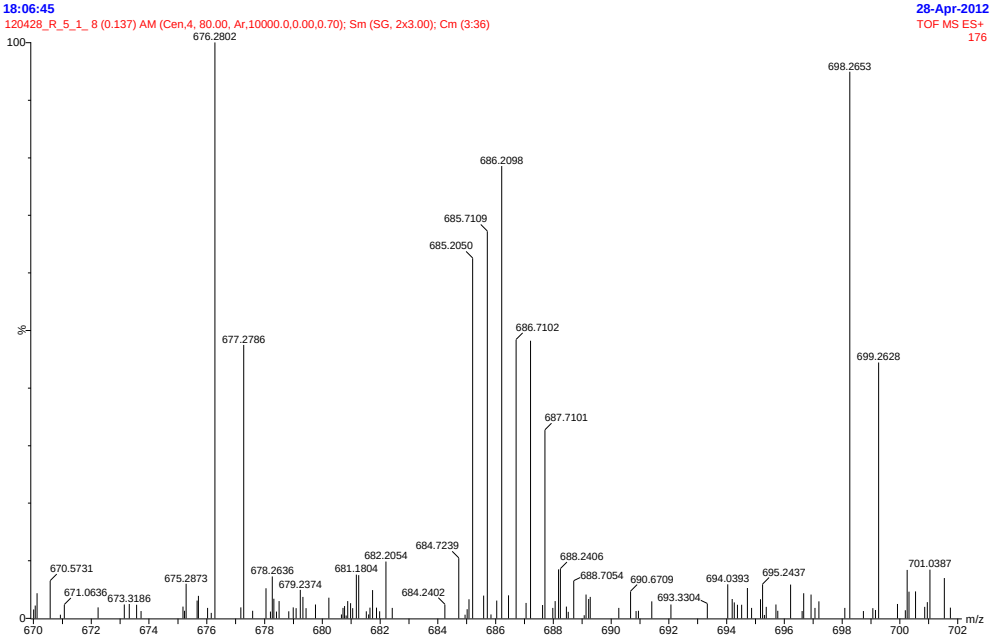


^{13}C-NMR of 8 (100 MHz in $CDCl_3$)



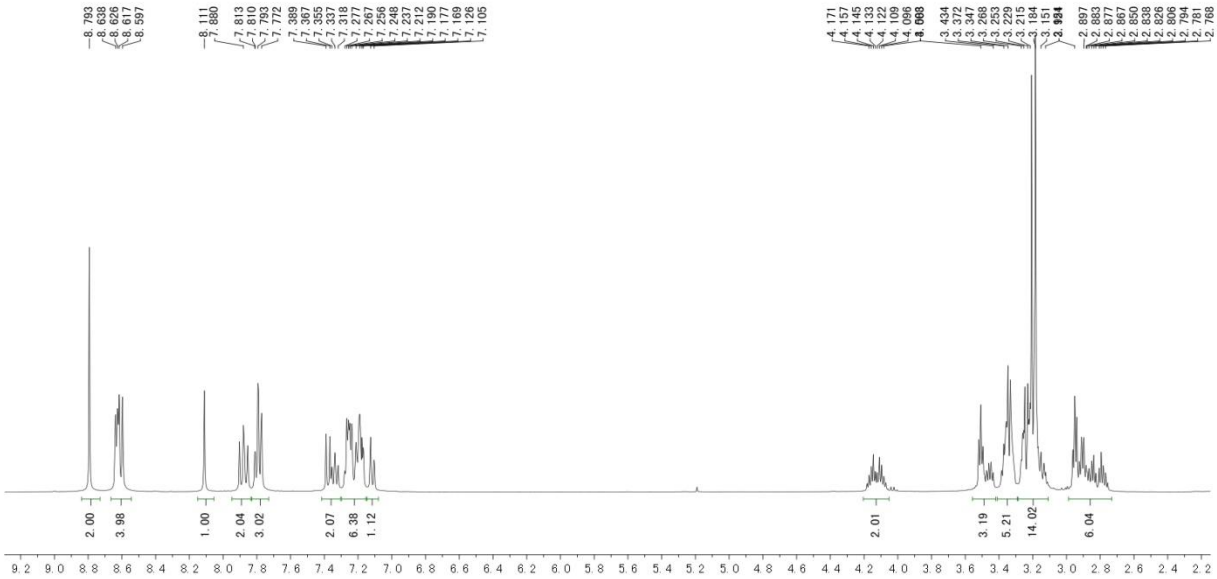
HRMS (TOF MS ES+) of 8



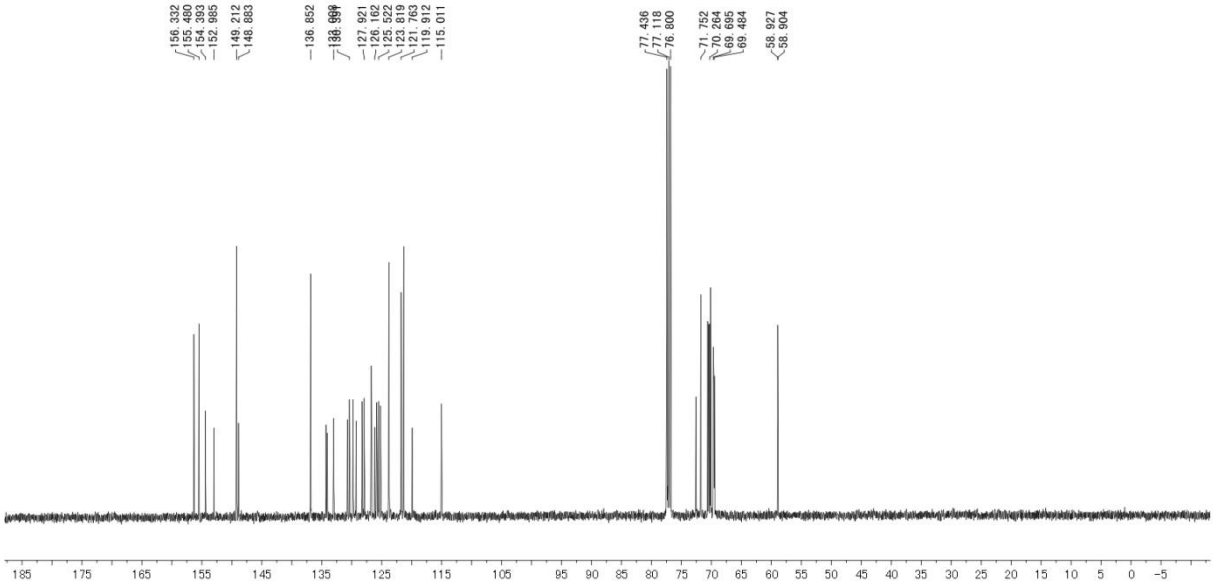


Preparation and characterization of 4'-(2,2'-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-[1,1'-binaphthalen]-3-yl)-2,2':6',2''-terpyridine 9. 3-([2,2':6',2"-terpyridin]-4'-yl)-[1,1'-binaphthalene]-2,2'-diol **7** (0.50 g, 0.97 mmol) was stirred with dry K_2CO_3 (0.35 g, 2.53 mmol) in DMF at 90 ^oC. After 1 h, ((oxybis (ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl)bis(4-methylbenzenesulfonate) (0.92 g, 2.89 mmol) was added to the reaction mixture. The solution was heated at 90 ^oC for 24 h. Then water (30 mL) and dichloromethane (50 mL) were added. The organic layer was separated and the water layer was extracted by dichloromethane (50 mL× 3). The organic layers were combined and washed with saturated NaCl (20 mL × 3). After dried over Na_2SO_4, the solvent was removed and the residue was subjected to column chromatography on basic Al_2O_3 gel eluted with petroleum ether/ethyl acetate (1:1) to afford the product **9** as a light yellow oily solid in 54.7% yield (0.43 g). ^1H NMR (400 MHz , $CDCl_3$) δ 8.79 (s, 2H), 8.64- 8.60 (m, 4H), 8.17 (s, 1H), 7.88 (t, 2H, J = 10.4 Hz), 7.79 (t, 3H, J = 8.4 Hz), 7.39- 7.32 (m, 2H), 7.28- 7.24 (m, 3H), 7.21- 7.17 (m, 3H), 7.11 (d, 1H, J = 8.4 Hz), 4.18- 4.07 (m, 2H), 3.51 (t, 2H, J = 4.8 Hz), 3.47- 3.43 (m, 1H), 3.85- 3.33 (m, 5H), 3.27- 3.10 (m, 14H), 2.96- 2.76 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 155.5, 154.4, 153.0, 149.2, 148.9, 136.9, 134.3, 134.1, 133.0, 130.7, 130.4, 129.8, 129.3, 128.3, 127.9, 126.7, 126.7, 126.2, 125.8, 125.5, 125.2, 123.8, 123.8, 121.8, 121.3, 119.9, 115.0, 72.6, 71.8, 71.8, 70.6, 70.4, 70.3, 70.1, 70.1, 69.7, 69.6, 69.6, 69.5, 58.9, 58.9. HR-MS (ES+) calcud for $C_{49}H_{52}N_3O_8$ (M+H) 810.3746, found 810.3759.

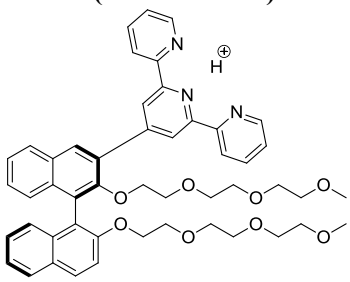
^{1}H-NMR of 9 (400 MHz in $CDCl_3$)



^{13}C-NMR of 9 (100 MHz in $CDCl_3$)

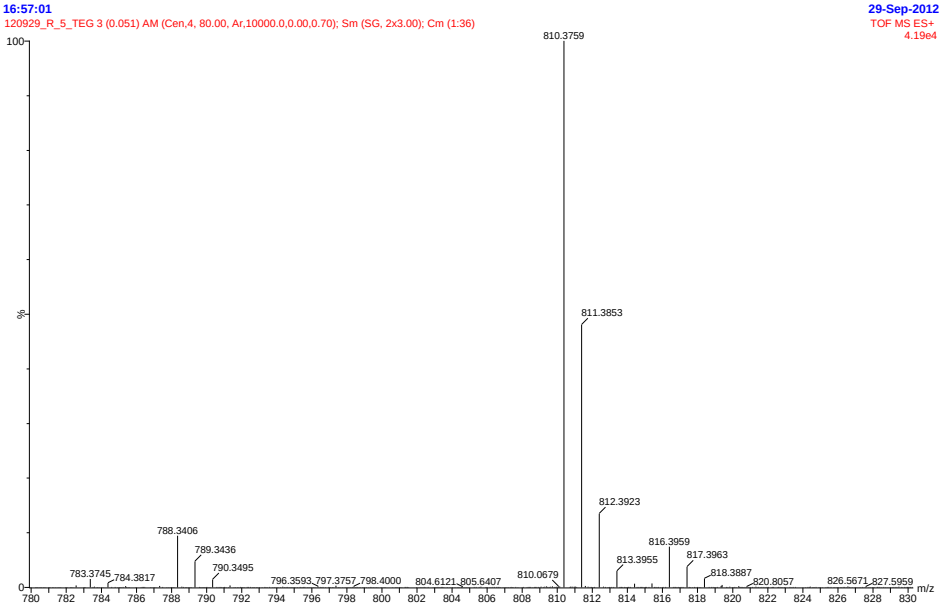


HRMS (TOF MS ES+) of 9



Chemical Formula: $C_{49}H_{52}N_3O_8^+$

Exact Mass: 810.3749



3. Fluorescence Experiments and Spectra

Figure S1. Fluorescence Responses of Tpy+Zn^{2+}(0.6 equiv) (2.0×10^{-5} M in H_2O with 1% THF) at λ_{emi} = 353 nm in the Presence of 10 equiv Amino Acids (λ_{exc} = 298 nm, slits: 2 nm/ 2 nm).

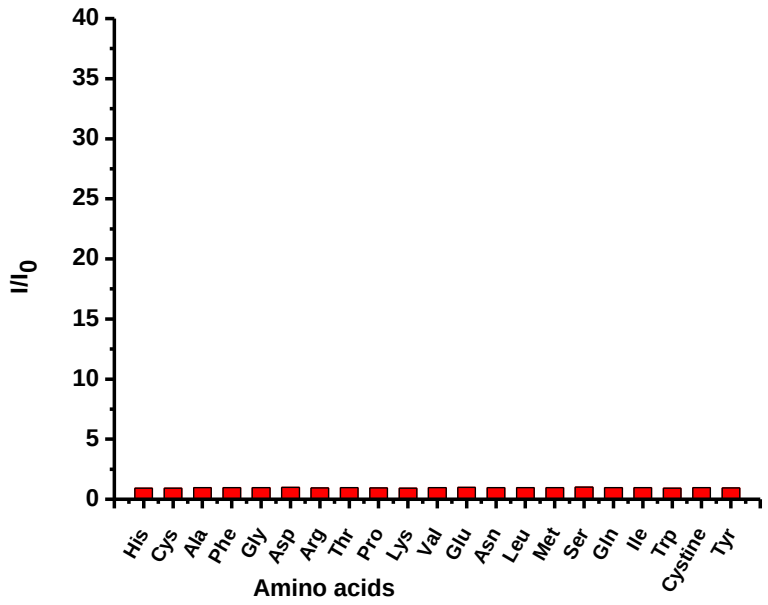


Figure S2. Fluorescence Titration of **7** (2.0×10^{-5} M in THF: H_2O = 1:1) with $Zn(NO_3)_2.6H_2O$ (λ_{exc}= 321 nm, slits: 5nm/5nm).

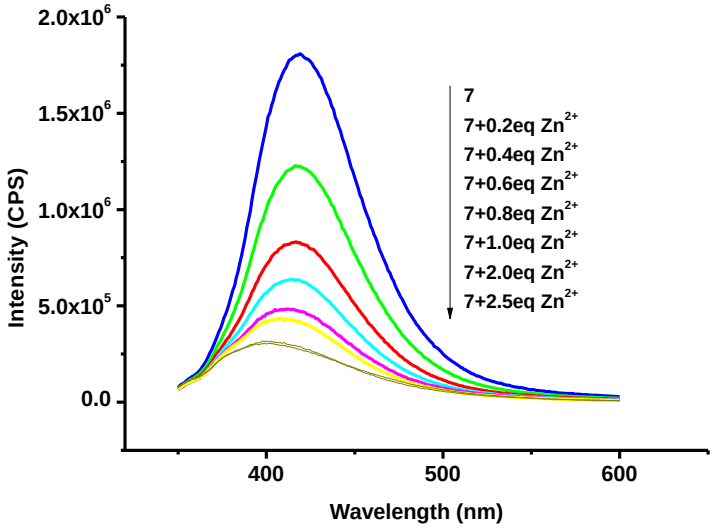


Figure S3. Fluorescence Titration of **8** (2.0×10^{-5}M in THF: H_2O = 1:1) with $Zn(NO_3)_2.6H_2O$ (λ_{exc}= 300 nm, slits: 5nm/5nm).

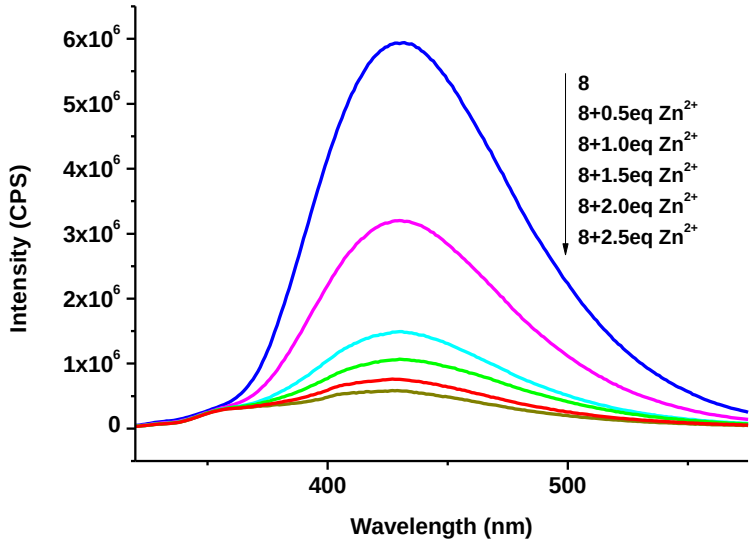


Figure S4. Fluorescence enhancement of **7** +2.5eqZn^{2+} (2.0 × 10-5 M in THF: HEPES= 1: 9) at 439 nm when treated with10eq amino acids .（λexc = 321 nm, slits: 5 nm/ 5 nm).

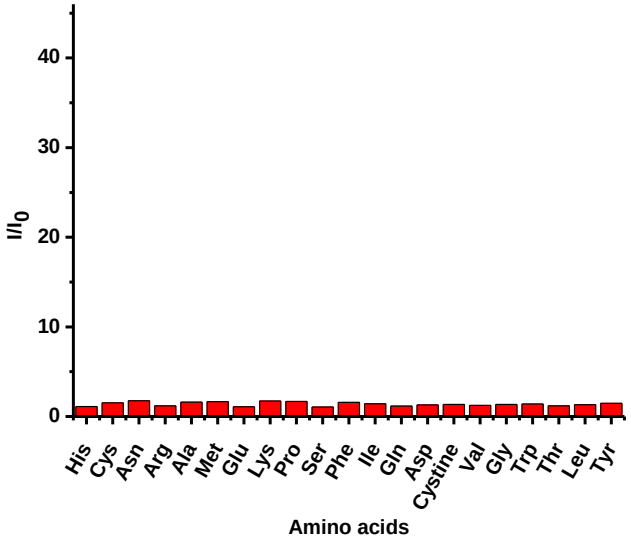


Figure S5. Fluorescence spectra of **8**+Zn^{2+}(2.5 equiv) (2.0×10^{-5} M in 25 mM pH = 7.35 hepes buffer with 1% THF) with histidine (10 equiv) and other amino acids (10 equiv) (λ_{exc} = 343 nm, slits: 5 nm/ 5 nm).

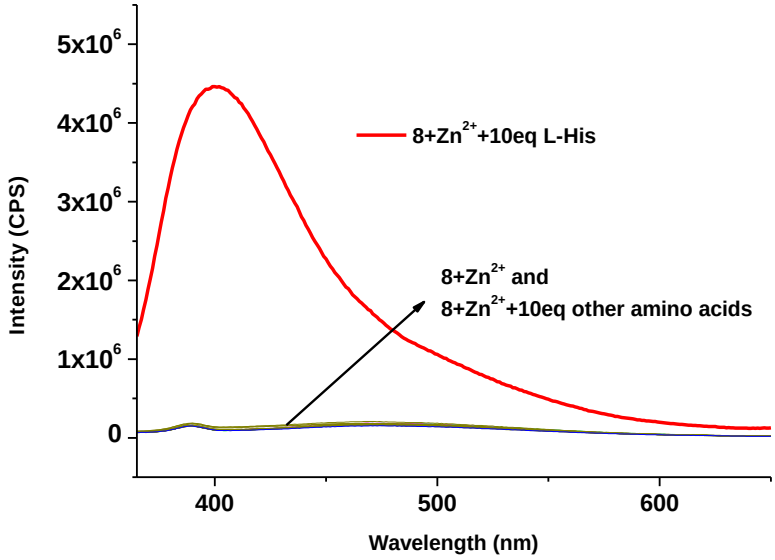


Figure S6. Photos of **8**+Zn^{2+}(2.5 equiv) ($1x10^{-4}$ M in H_2O with 1% THF) with (left) and without (right) histidine (10 equiv).

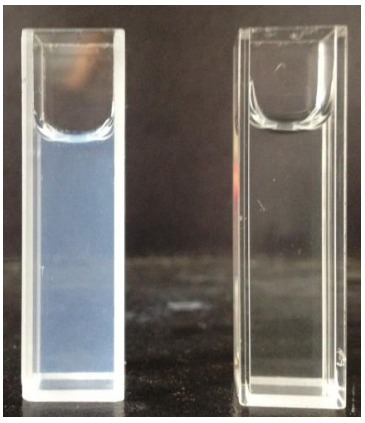


Figure S7. Fluorescence Spectra of **8**+Zn^{2+} (2.5 equiv) (1 x 10^{-4} M H_2O with 1% THF) with histidine (10 equiv) before and after centrifugation (λ_{exc} = 343 nm, slits: 5 nm/ 5 nm).

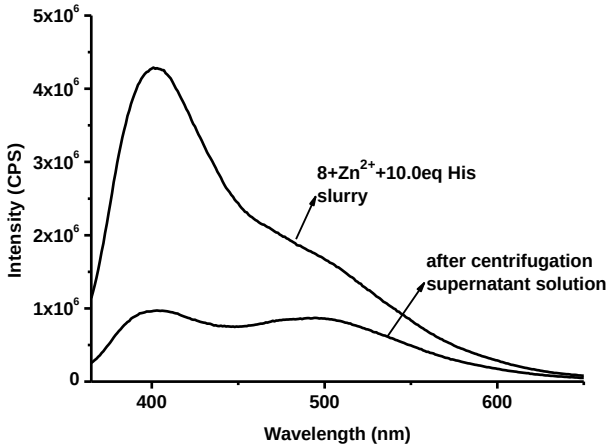


Figure S8. Fluorescence response of **8**+Zn^{2+}(2.5 equiv) (2.0 × 10^{-5} M in 25 mM pH = 7.35 hepes buffer with 1% THF) toward histidine and the derivatives of histidine and imidazole at λ_{emi} = 400 nm (λ_{exc} = 343 nm, slits: 5 nm/ 5 nm).

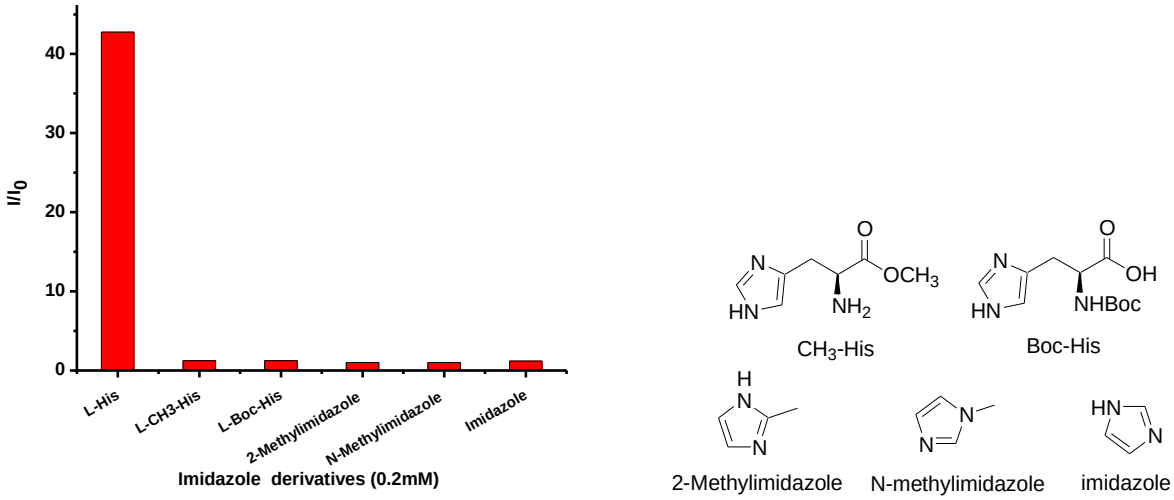
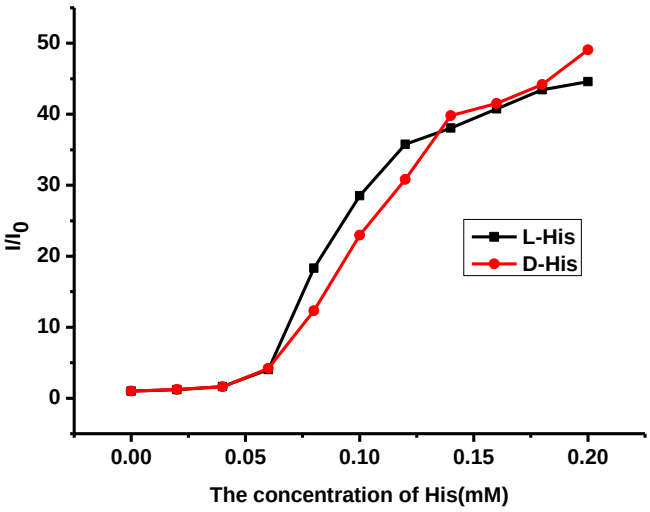


Figure S9. Fluorescence enhancement of (*R*)-**8**+Zn^{2+}(2.5 equiv) (2.0×10^{-5} M in 25mM pH=7.35 hepes buffer with 1% THF) at λ_{emi} = 400 nm when treated with L- and D- histidine.（λ_{exc} = 343 nm, slits: 5 nm/ 5 nm).



4. Study of Compound 9

We have prepared compound **9** as an acyclic polyether analogue of **8** without the crown ether ring. Similar to **7** and **8**, when **9** was treated with Zn^{2+}, its fluorescence was significantly quenched upon coordination of Zn^{2+} with the Tpy unit (Fig. S10). However, when the **9**+Zn^{2+}(2.5 equiv) complex was treated with histidine as well as other amino acids, almost no fluorescence enhancement was observed (Fig. S11) and there was also no precipitate formation in these interactions. These results demonstrate that the crown ether ring of **8** is very important for the highly selective fluorescent recognition of histidine in the presence of Zn^{2+}. Coordination of the crown ether ring with Zn^{2+} and its subsequent interaction with histidine is very likely. (For the coordination of 15-crown-5 with Zn^{2+}, see: Cooper, T. E.; Carl, D. R.; Oomens, J.; Steill, J. D.; Armentrout, P. B.. *J. Phys. Chem. A,* **2011**, *115*, 5408–5422.)

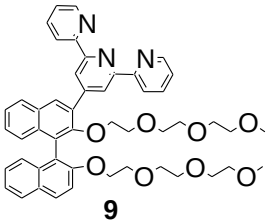


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Figure S10. Fluorescence titration of **9** (2.0×10^{-5}M in THF: H_2O=1:1) with $Zn(NO_3)_2.6H_2O$ (λ_{exc}=322nm, slits: 5nm/5nm).

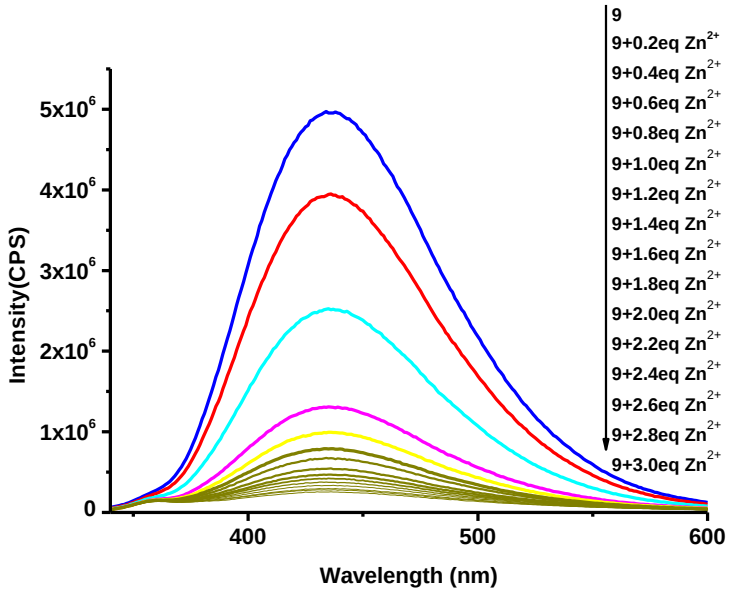
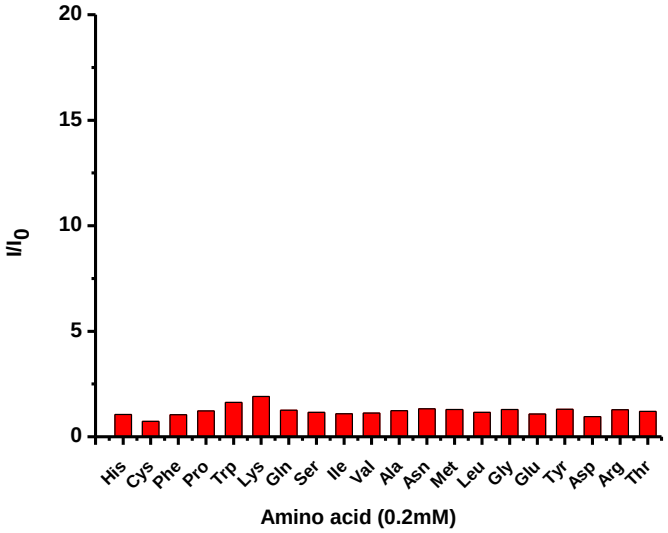


Figure S11. Fluorescence response of **9**+Zn^{2+}(2.5 equiv) (2.0×10^{-5} M in 20mM pH=7.4 hepes buffer with 0.02% THF) at λ_{emi} = 414 nm when treated with Amino Acids (10 equiv) (λ_{exc} = 322 nm, slits: 5 nm/ 5 nm).



5. Job Plots

The Job plot for the interaction of **8** with Zn^{2+} was obtained by measuring the fluorescence response of **8** with varying ratio of Zn^{2+} versus **8** while the total concentration of **8**+Zn^{2+} was maintained. As Figure S12 shows, the major fluorescence quenching of **8** by Zn^{2+} occurs at 1:1 ratio. On the basis of the fluorescence responses of compounds **7**, **8** and **9** to Zn^{2+}, we propose that when **8** was treated with Zn^{2+}, the binding of its Tpy unit with Zn^{2+} led to the major fluorescence quenching. Previously, we observed that Tpy forms strong complex with Zn^{2+} in water with an association constant of 3.35×10^7 (Huang, Z.; Du J.; Zhang, J.; Yu, X. Q.; Pu, L. *Anal. Methods*, **2012**, *4*, 1909-1912). Figure S12 also indicates that there are additional bindings between **8** and Zn^{2+} which led to smaller fluorescence quenching. This could be attributed to the interaction of the crow ether ring of **8** with Zn^{2+}. We then obtained the Job plot for the interaction of the complex **8**+Zn^{2+}(2.5 equiv) with varying ratio of histidine by measuring the fluorescence response while the total concentration was maintained (Figure S12'). It shows that the fluorescence enhancement of complex **8**+Zn^{2+} started at the complex versus histidine ratio greater than 1:1 and peaked at 1:4. Because of the precipitate formation, the accurate association constant could not be obtained.

Figure S12. The Fluorescence Intensity of **8** at 439 nm with varying ratio of Zn^{2+} (The total concentration of **8**+[Zn^{2+}] = 2 x 10^{-5} M in THF:H_2O = 1:4)

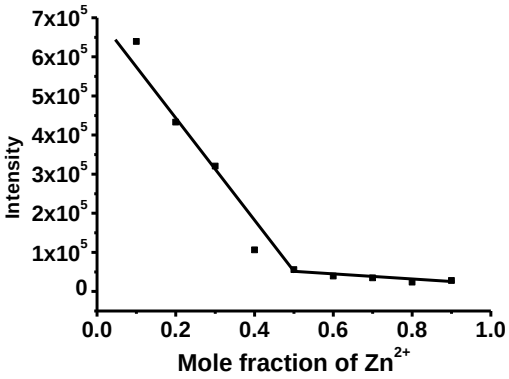
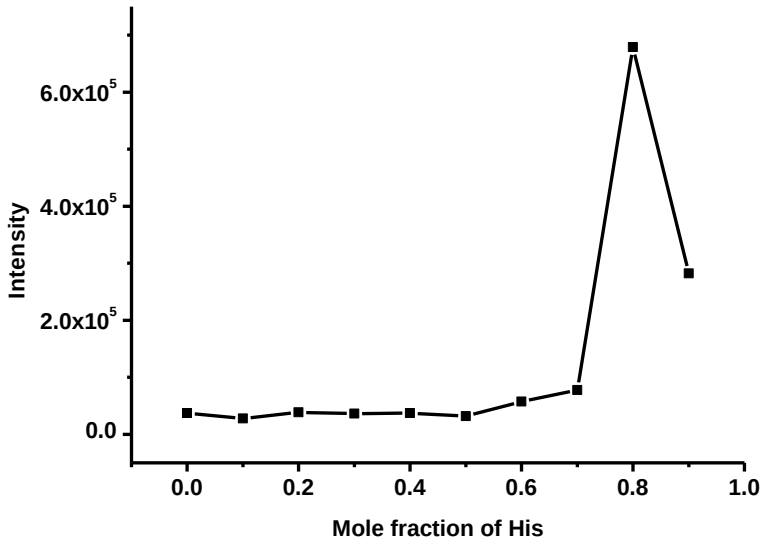
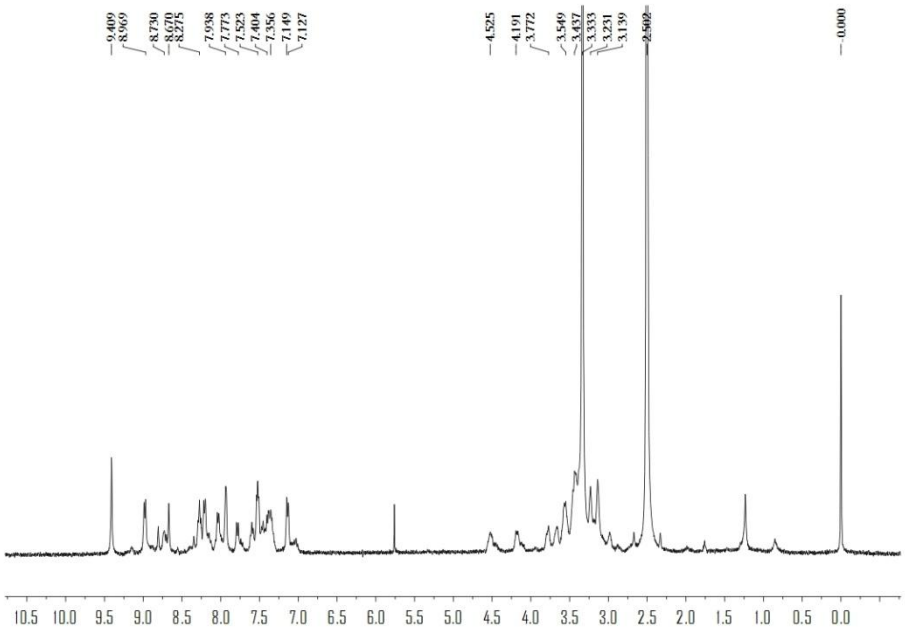


Figure S12'. Fluorescence intensity of **8**+Zn^{2+}(2.5 equiv) complex at 400 nm in the presence of varying amount of histidine (The total concentration of [**8**+Zn^{2+}]+[His] = 1 x 10^{-4} M in 25mM pH=7.35 hepes buffer solution at 37 ^{o}C).



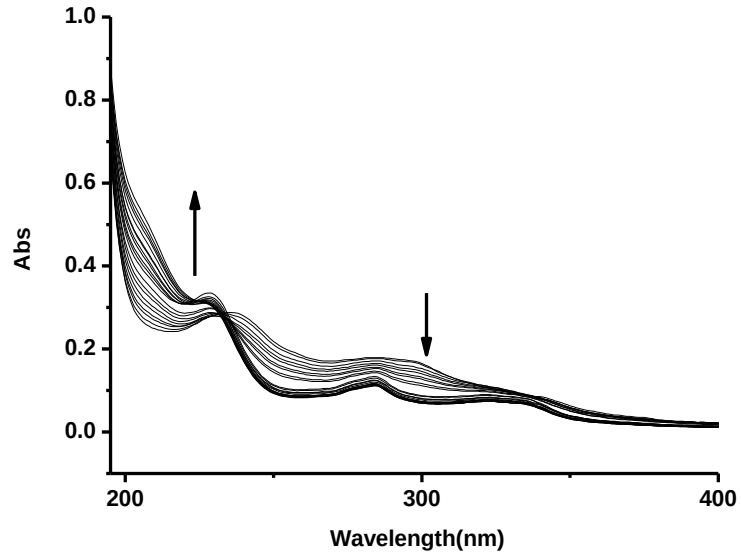
6. ^{1}H-NMR Spectrum of the Isolated Precipitate

Figure S13. ^{1}H-NMR spectrum of the precipitate isolated from the reaction of **8**+Zn^{2+}(2.5 equiv) with histidine in H_2O solution (400 MHz in DMSO-d_6)

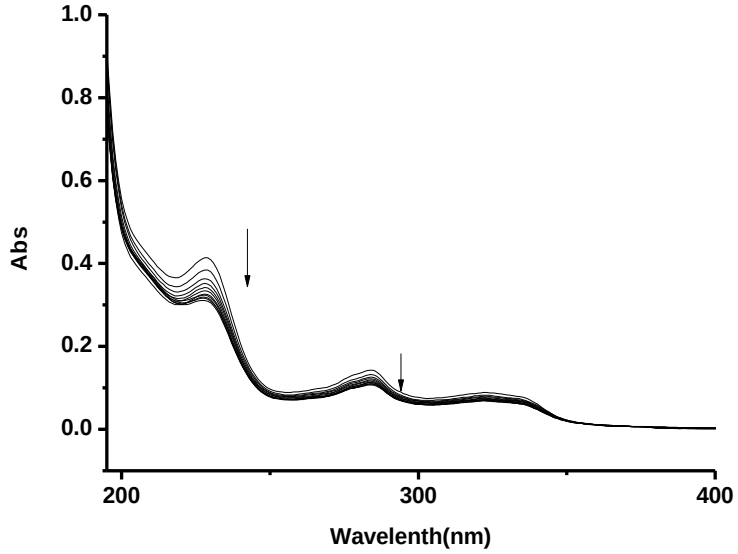


7. UV Absorption Spectra

Figure S14. (a) Absorption spectra of **8** (4.0×10^{-6} M in water with 0.2% THF) when treated with Zn^{2+} (from 0.2 to 4.0 equiv). (b) Absorption spectra of **8**+Zn^{2+}(2.5 equiv) (4.0×10^{-6} M in water with 0.2% THF) when treated with 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 equiv histidine. (The solution remained homogenous because of the low concentration of **8**)



(`a)



(b)

8. Fluorescence Recovery of 8+Zn^{2+}(2.5 equiv) Complex in the Presence of the Mixtures of Histidine with Other Species

We tested the fluorescence recovery of the **8**+Zn^{2+}(2.5 equiv) complex in the presence of the mixtures of histidine with other species including natural amino acids and more. As summarized in Table S1, all the other natural amino acids had little effect on the fluorescent recognition of histidine by the Zn^{2+} complex. Alkaline metal and alkaline earth metal salts also did not interfere with the histidine recognition. Among the compounds examined, $FeCl_3$ at concentration greater than 20 μM was found to interfere with the measurement probably due to the competitive binding of the Tpy unit of **8** with Fe^{3+}.

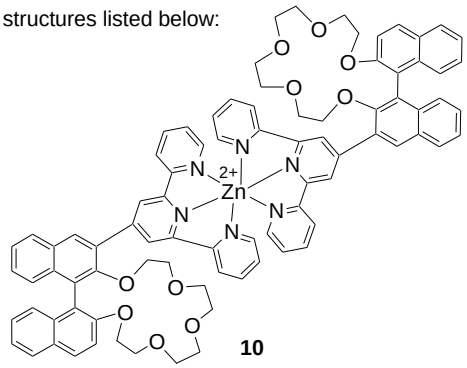
Table S1. Fluorescence Response of **8**+Zn^{2+}(2.5 equiv) (2 x 10^{-5} M) to the Mixtures of Histidine (200 μM) with Other Substances (All solutions were prepared in 25 mM hepes buffer at pH = 7.35. λ_{exc}=343nm, slits: 5nm/5nm)

Added species	Conc. (μM)	Recovery (%)	Added species	Conc. (μm)	Recovery (%)
L-Ala	200	99.7	L-Thr	200	104.5
L-Glu	200	99.8	L-Asp	200	98.4
L-Phe	200	99.4	L-Cys	200	93.6
L-Lys	200	99.2	L-Cystine	200	95.6
L-Trp	200	95.7	NaCl	6000	94.9
L-Ser	200	95.0	$Ca(NO_3)_2$	4000	96.0
L-Arg	200	94.5	$MgCl_2$	8000	96.2
Gly	200	97.5	KNO_3	8000	95.1
L-Val	200	93.8	$NaHCO_3$	4000	96.1
L-Met	200	97.0	Na_2SO_4	800	96.5
L-Pro	200	92.5	Na_3PO_4	1000	100.3
L-Tyr	200	95.6	$FeCl_3$	20	89.6
L-Ile	200	99.4	Ascobic acid	600	95.6
L-Gln	200	99.9	Glucose	800	99.8
L-Leu	200	99.2	Acetylcholine chloride	400	95.6
L-Asn	200	94.8	L-Phenylalaninol	800	95.1

9. Mass Analyses for the 8+Zn^{2+} Complex and Its Interaction with Histidine

MS spectra (TOF MS ES+) of **8** with the addition of $Zn(NO_3)_2$ (2.5 equiv) and Histidine in water with 1% THF

Possible structures listed below:



10

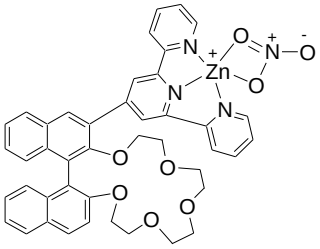
$C_{86}H_{74}N_6O_{10}Zn$

Exact Mass ($^{64}Zn_{\text{natural aboundance 48.6\%}}$): 1414.48

1416.48/2 = 707.24

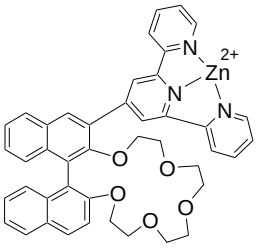
Exact Mass ($^{66}Zn_{\text{natural aboundance 27.9\%}}$): 1416.48

1416.48/2 = 708.24



11 $C_{43}H_{37}N_4O_8Zn$

Exact mass = 801.19

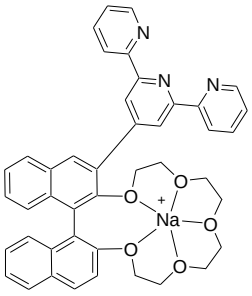


11-NO_3^-

$C_{43}H_{37}N_3O_5Zn$

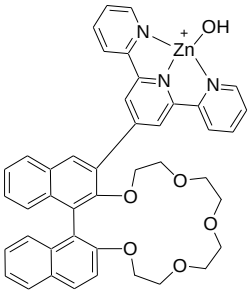
Exact mass: 739.20

739.20/2 = 369.60



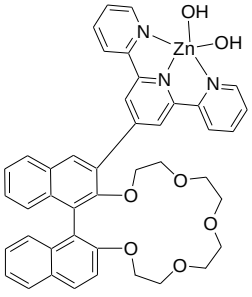
$C_{43}H_{37}N_3NaO_5$

Exact Mass: 698.2625



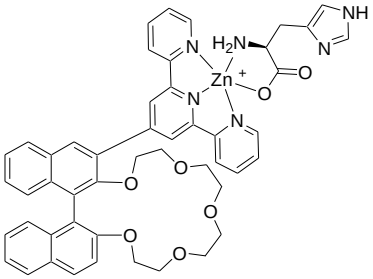
$C_{43}H_{38}N_3O_6Zn$

Exact Mass: 756.21



$C_{43}H_{40}N_3O_7Zn$

Exact Mass of $M+H^+$: 774.22

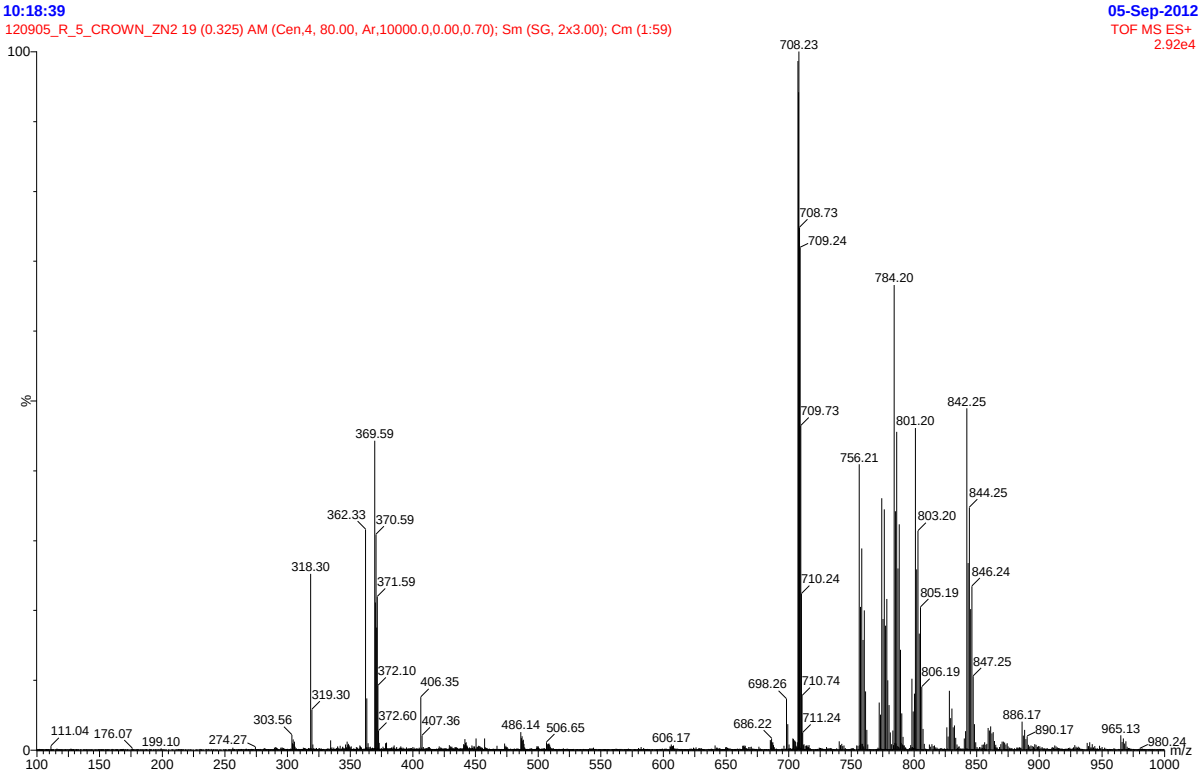


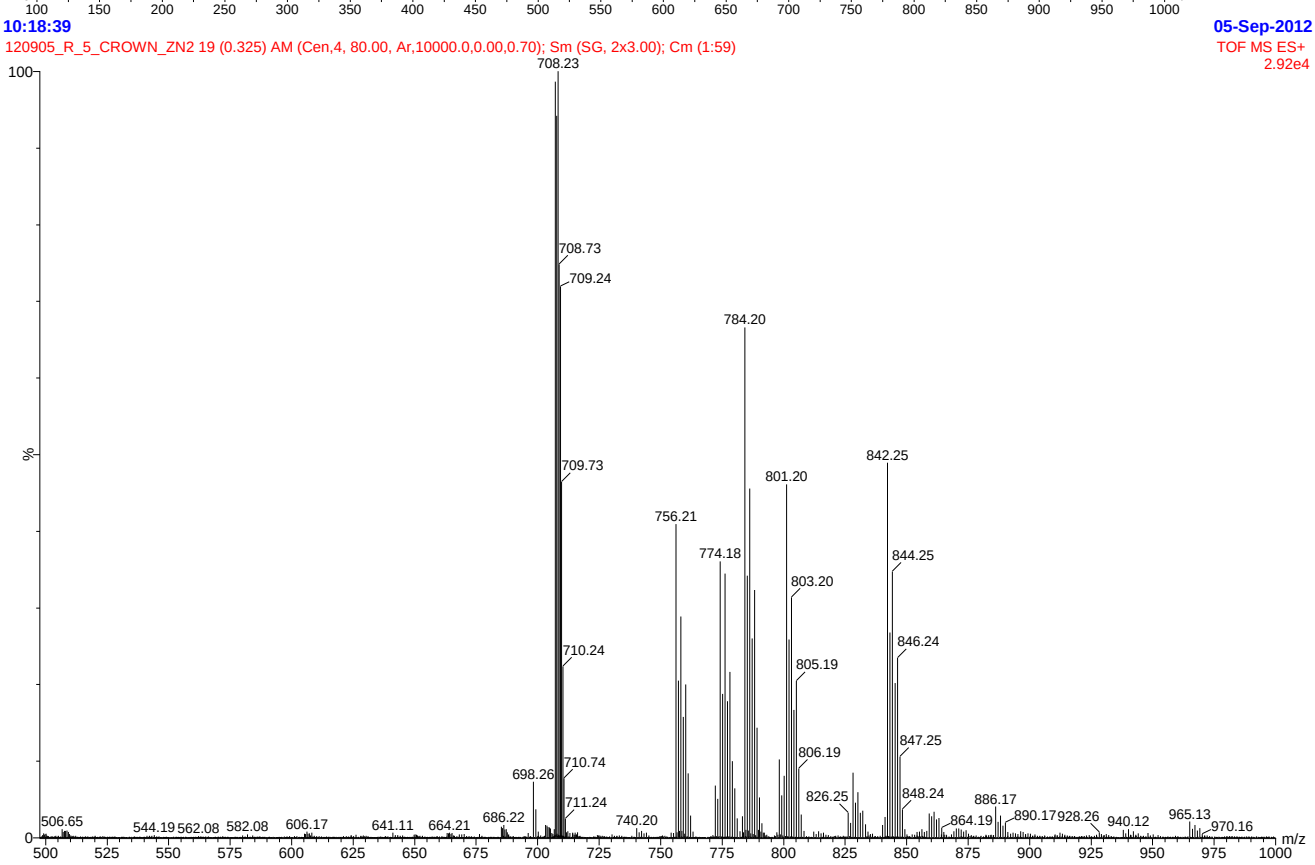
15

$C_{49}H_{45}N_6O_7Zn$

Exact Mass: 893.26

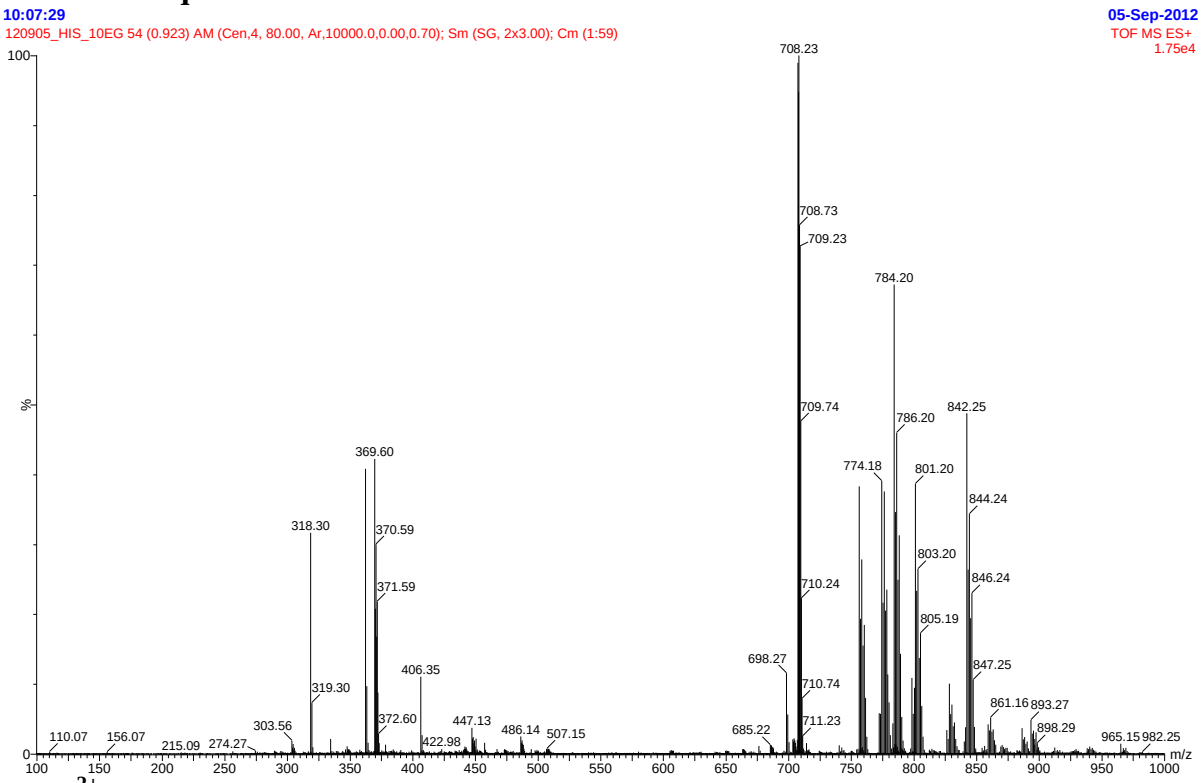
8+Zn^{2+}



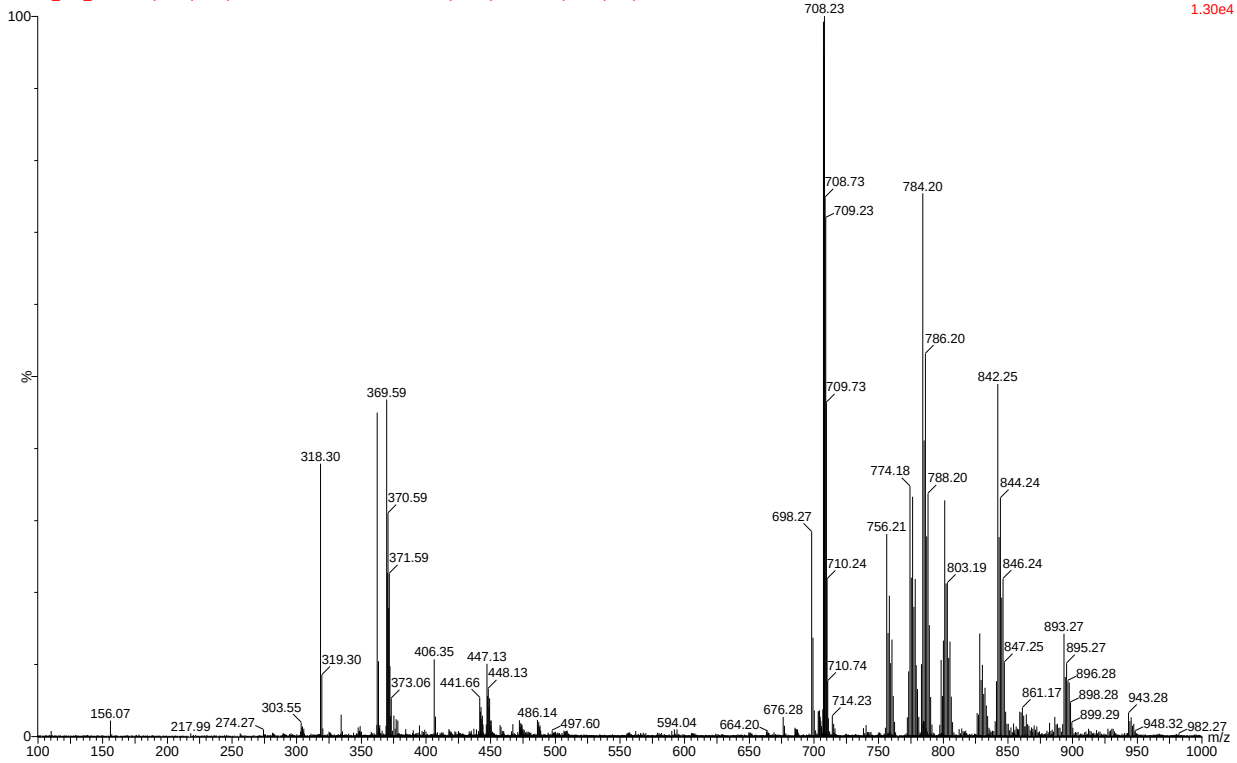


a peak is also observed at 707.23.

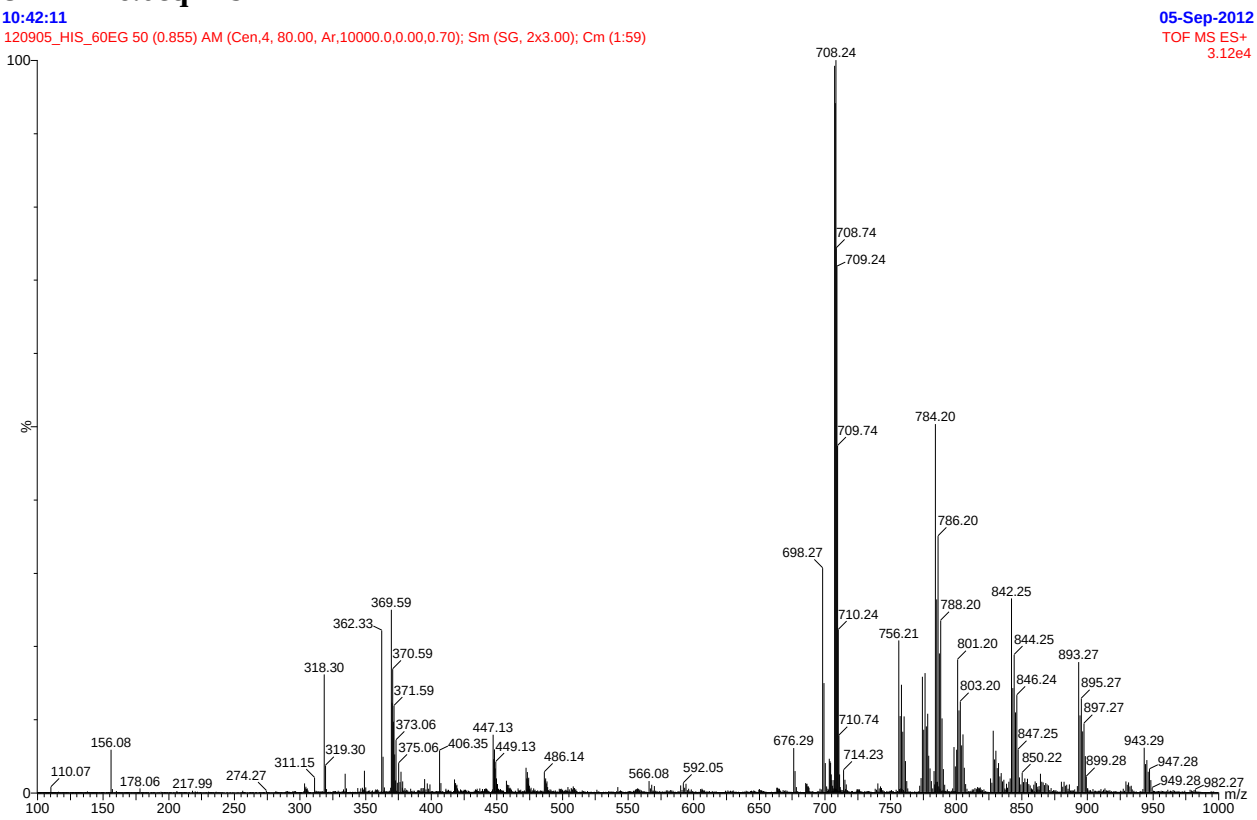
8+Zn^{2+}+1.0eq His



8+Zn^{2+}+3.0eq His



8+Zn^{2+}+6.0eq His



8+Zn^{2+}+10.0 eq His