

Supplementary Information

for

Water-soluble doubly N-confused hexaphyrin: A near-IR fluorescent Zn(II) ion sensor in water

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General

Commercially available reagents and solvents were used without further purification unless otherwise mentioned. Tetrahydrofuran (THF) was distilled over benzophenone and sodium under Ar atmosphere. Silica gel column chromatography was performed on KANTO Silica Gel 60 N (spherical, neutral, particle size 40–50 μm). Fmoc-Arg(Pbf)-OH, *N,N*-diisopropylethylamine (DIPEA), *O*-benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), hydroxybenzotriazole (HOBT), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), piperidine were purchased from Watanabe Chemical (Hiroshima, Japan), and Fmoc-Sieber Amide resin was purchased from Novabiochem (Laufelfingen, Switzerland). ^1H NMR spectra were recorded on a JEOL JNM-AL300 spectrometer (operating at 300.40 MHz for ^1H). Chemical shifts were expressed in parts per million (ppm) from a residual portion of deuterated solvent, CHCl_3 ($\delta = 7.26$). MALDI-TOF-mass spectra were recorded on a Bruker Daltonics Autoflex with linear positive ion mode. UV-vis-NIR spectra were recorded on a Shimadzu UV-3150PC spectrometer. Fluorescence spectra were recorded on a Horiba Fluorolog-3-NIR luminescence spectrophotometer. For absorption/emission measurements, spectroscopic grade DMF (purchased from Nacalai Tesque, Kyoto, Japan) and ultrapure water (prepared by Organo Puric-Z, Tokyo, Japan) were employed. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-20AB with an SPD-M10A photodiode array detector.

Synthesis

5,20-Bis(2',3',5',6'-tetrafluoro-4'-iodophenyl)-10,15,25,30-tetrakis(pentafluorophenyl) doubly N-confused dioxohexaphyrin (N_2CH , 1):

5,10-Bis(pentafluorophenyl) N-confused tripyrrane^[1] (100 mg, 0.18 mmol) and 2,3,5,6-tetrafluoro-4-iodo-benzaldehyde^[2] (55 mg, 0.18 mmol) in CH_2Cl_2 (30 mL) were stirred under N_2 for 15 min at room temperature. *p*-TsOH $\cdot\text{H}_2\text{O}$ (3.4 mg, 0.1 equiv) was added to the mixture. The solution was stirred for 4 h under dark conditions with an aluminum foil to avoid the light. The solution was opened to the air and DDQ (0.5 mmol, 125 mg, 3.0 equiv) was added. The resulting mixture was stirred for 12 h at room temperature. Then, the crude product was separated roughly by an alumina column with CH_2Cl_2 and a purple color fraction was collected. After the solvent was removed, the residue was dissolved in CH_2Cl_2 (60 mL) and $\text{Cu}(\text{OAc})_2\cdot\text{H}_2\text{O}$ (100 mg) in methanol was added. After stirring for 5 h, the solvent was removed and the residue was purified by a silica gel column with CH_2Cl_2 /Hexane (v/v = 1/1). Green color fraction was collected and the solvent was removed. The residue was dissolved in CH_2Cl_2 (20 mL) and trifluoroacetic acid (TFA) (1 mL) was added. After 12 h, an aqueous NaOH (1 M) solution was added and the organic phase was separated, washed with water and brine, dried over Na_2SO_4 , and evaporated. The product was recrystallized from CH_2Cl_2 /hexane. Gold solid was obtained in 4.9% (7.5 mg, 4.4 μmol).

^1H NMR (CDCl_3 , 300 MHz, ppm): $\delta = 10.75$ (s, 2H), 9.50 (d, $J = 4.8$ Hz, 2H), 9.47 (d, $J = 4.8$ Hz, 2H), 9.22 (s, 4H), -0.28 (s, 2H), -0.76 (s, 2H); MALDI-TOF-MS: calcd: 1708.62 for $\text{C}_{66}\text{H}_{14}\text{F}_{28}\text{I}_2\text{N}_6\text{O}_2$, found: 1710.02 ($[\text{M}+\text{H}]^+$); UV/vis/NIR (CH_2Cl_2): $\lambda_{\text{max}}[\text{nm}] = 1049, 908, 794, 721, 662, 568, 383$.

Bis-Cu(II) complex of 5,20-bis{2',3',5',6'-tetrafluoro-4'-[(triisopropylsilyl)ethynyl]-phenyl}-10,15,25,30-tetrakis(pentafluorophenyl) doubly N-confused Dioxohexaphyrin:

5,20-Bis(2',3',5',6'-tetrafluoro-4'-iodophenyl)-10,15,25,30-tetrakis(pentafluorophenyl)-doubly N-confused dioxohexaphyrin was treated with Cu(OAc)₂·H₂O, affording the corresponding bis-Cu(II) complex, quantitatively. To a dried Schlenk tube degassed by purging with Ar gas, bis-Cu(II) complex of 5,20-bis-(2',3',5',6'-tetrafluoro-4'-iodophenyl)-10, 15, 25, 30-tetrakis(pentafluorophenyl) doubly N-confused dioxohexaphyrin (40.4 mg, 22.1 μmol), Pd(PPh₃)₂Cl₂ (31.0 mg, 2.0 equiv), THF (3 mL) and (triisopropylsilyl)acetylene (49 μL, 10 equiv) were added and the solution was stirred for 5 min. Then, CuI (8.4 mg, 2.0 equiv) and triethylamine (1.5 mL) were added. Then, the reaction was conducted further for 24 h at room temperature. The reaction mixture was filtered and diluted with CH₂Cl₂. The reaction mixture was washed with an aqueous NH₄Cl solution. The organic phase was separated, dried over Na₂SO₄, and evaporated. The residue was purified by a silica gel column with CH₂Cl₂/Hexane (v/v = 1/1), and the product was recrystallized from CH₂Cl₂/hexane. Gold solid was obtained in 49% (20.8 mg, 10.7 μmol).

MALDI-TOF-MS: calcd: 1940.61 for C₈₈H₅₂Cu₂F₂₈N₆O₂Si₂, found: 1941.88 ([M+H]⁺); UV/vis/NIR (CH₂Cl₂): λ_{max}[nm] = 1050, 912, 617, 465, 402.

Freebase of 5,20-bis{2',3',5',6'-tetrafluoro-4'-[(triisopropylsilyl)ethynyl]phenyl}-10,15,25,30-tetrakis(pentafluorophenyl) doubly N-confused dioxohexaphyrin:

¹H NMR (CDCl₃, 300 MHz, ppm): δ = 10.74 (s, 2H), 9.49 (d, J = 4.8 Hz, 4H), 9.24 (d, J = 5.1 Hz, 2H), 9.20 (d, J = 4.8 Hz, 2H), 1.32 (m, 42H), -0.29 (s, 2H), -0.77 (s, 2H); MALDI-TOF-MS: calcd: 1817.55 for C₈₈H₅₆F₂₈N₆O₂Si₂, found: 1818.528 ([M+H]⁺); UV/vis/NIR (CH₂Cl₂): λ_{max}[nm] = 1047, 908, 794, 720, 569, 383, 334.

Bis-Cu(II) complex of 5, 20-bis(2',3',5',6'-tetrafluoro-4'-[(ethynyl)phenyl]-10,15,25,30-tetrakis(pentafluorophenyl) doubly N-confused dioxohexaphyrin (3):

To a dried Schlenk tube degassed by purging with Ar gas, the THF solution of bis-Cu(II) complex of 5,20-bis{2',3',5',6'-tetrafluoro-4'-[(triisopropylsilyl)ethynyl]-phenyl}-10,15,25,30-tetrakis(pentafluorophenyl) doubly N-confused dioxohexaphyrin (13.0 mg, 6.7 μmol) was added. Then, tetra-*n*-butylammonium fluoride (TBAF) (20 μL of 1.0 M THF solution, 3.0 equiv) and acetic acid (20 μL of 1.0 M THF solution, 3.0 equiv) were added. The solution was stirred for 8 h at room temperature under Ar. Then the reaction was quenched by an excess amount of acetic acid and the solvent was removed. The product was washed with water and the organic phase was separated, dried over Na₂SO₄ and the solvent was removed. Residue was used for the next reaction without further purification.

Synthesis of side-chain-protected octa-arginine peptide having an azide moiety (5).

Fmoc-Sieber Amide Resin (156 mg, 0.1 mmol of Fmoc-protected amino group) was washed with CH₂Cl₂ and then *N,N*-dimethylformamide (DMF) three times. To remove Fmoc groups, the resin was treated with 20% piperidine in DMF (2 mL) for 20 min and then washed with DMF five times. A DMF solution containing Fmoc-Arg(Pbf)-OH (210 mg, 0.3 mmol), HOBt (46 mg, 0.3 mmol) and HBTU (114 mg, 0.3 mmol) and then DIPEA (104 μL, 0.6 mmol) were added to the resin. The resin mixture was shaken

gently for 20 min. The resin was washed with DMF five times. These procedures were repeated eight times. Additionally, the resin was treated with 20% piperidine in DMF for 20 min and then washed with DMF five times. Then DMF solution containing $\text{N}_3\text{CH}_2\text{CO}_2\text{H}$ (10 equiv, 1.0 mmol), HOBt (5 equiv, 76 mg, 0.5 mmol) and PyBOP (260 mg, 0.5 mmol) and DIPEA (20 equiv, 2.0 mmol) were added. The resin mixture was shaken gently for 2 h. The resin was washed with DMF five times. After each coupling reaction, a little portion of the resin was transferred to a sample tube and subjected to Kaiser test. The coupling reaction was repeated until the test showed no blue color change. The resin was washed with CH_2Cl_2 five times. To cleave the side-chain-protected peptide from the resin, 1% TFA in CH_2Cl_2 was added to the resin and the mixture was shaken for 2 min and then filtrated. The filtrated solution was dropped into the 10% pyridine in methanol. The resin was washed with CH_2Cl_2 and methanol three times, alternately. Solvents were removed by evaporation. The residue was diluted with CH_2Cl_2 , washed with an aqueous NaHCO_3 solution, then dried over Na_2SO_4 , and the solvent was evaporated to afford pale yellow color solid **5**. MALDI-TOF-MS: calcd: 3368.20 for $\text{C}_{154}\text{H}_{228}\text{N}_{36}\text{O}_{33}\text{S}_8$, found: 3366.278 ($[\text{M}]^+$).

Synthesis of $\text{N}_2\text{CH-R}_8$ by the click reaction between **3** and azido peptide **5**.

To a 50 mL round flask, compound **3** (ca. 6.7 μmol), protected azido peptide **5** (20 μmol , 3.0 equiv), CuI (2.6 mg, 13.4 μmol , 2 equiv), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1 mg, 1.88 μmol , 0.3 equiv), and CH_2Cl_2 (20 mL) were added and the solution was stirred for 12 h at ambient temperature under N_2 atmosphere. The reaction mixture was washed with aqueous NaHCO_3 and the organic phase was separated, dried over Na_2SO_4 and the solvent was removed by evaporation. The residue was transferred into a plastic tube and a cocktail of TFA/thioanisole/*m*-cresol ($v/v/v = 85/7.5/7.5$) was added. The mixture was shaken for 5 h at ambient temperature. Diethyl ether (10 mL) was added to the solution and a precipitated crude target compound was collected by centrifugation. The residue was dissolved into a ultra-pure water and purified by HPLC with YMC R-ODS-5 column (4.6 \times 250 mm) using a mixed solvent $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ($v/v = 40/60$) containing 0.1% TFA. Flow rate was 1 mL/min and the absorptions at 210 nm and 566 nm were employed for detection of peptide and N_2CH moiety, respectively. The target compound was collected and lyophilized to afford $\text{N}_2\text{CH-R}_8$ as TFA salts in 46% yield (13 mg, 3.1 μmol).

Spectroscopic measurements

Effects of metal cations on absorption and fluorescence spectra of $\text{N}_2\text{CH-R}_8$.

To an aqueous solution of $\text{N}_2\text{CH-R}_8$, an acetate salt of metal cation of interest was added to prepare the solution containing 0.53 μM of $\text{N}_2\text{CH-R}_8$ and 530 μM (1000 equiv) of metal cation. The mixture was stirred at ambient temperature for 1 h and the absorption and fluorescence spectra were recorded.

Effects of second metal ions on fluorescence spectra of $\text{N}_2\text{CH-R}_8$ in the presence of Zn^{2+} .

To an aqueous solution of $\text{N}_2\text{CH-R}_8$, $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ and water were added to prepare the solution containing $\text{N}_2\text{CH-R}_8$ and 1000-fold excess Zn^{2+} . After stirring at ambient temperature for 1 h, an aqueous solution of acetate or chloride salt of metal cation of interest was added. Four sets of solutions containing 0.53 μM of $\text{N}_2\text{CH-R}_8$, 530 μM (1000 equiv) of Zn^{2+} , and different concentration of metal cation of interest, 0.53 μM

(1.0 equiv), 1.06 μM (2.0 equiv), 10.6 μM (20 equiv), or 530 μM (1000 equiv), respectively, were prepared. After additional stirring for 1 h (or different times in the experiment for Figure S13) at ambient temperature, fluorescence spectra were recorded.

References

- [1] A. Srinivasan, T. Ishizuka, A. Osuka and H. Furuta, *J. Am. Chem. Soc.*, 2003, **125**, 878.
- [2] J. Leroy, B. Schoellhorn, J.-L. Syssa-Magale, K. Boubekeur and P. Palvadeau, *J. Fluorine Chem.* 2004, **125**, 1379.

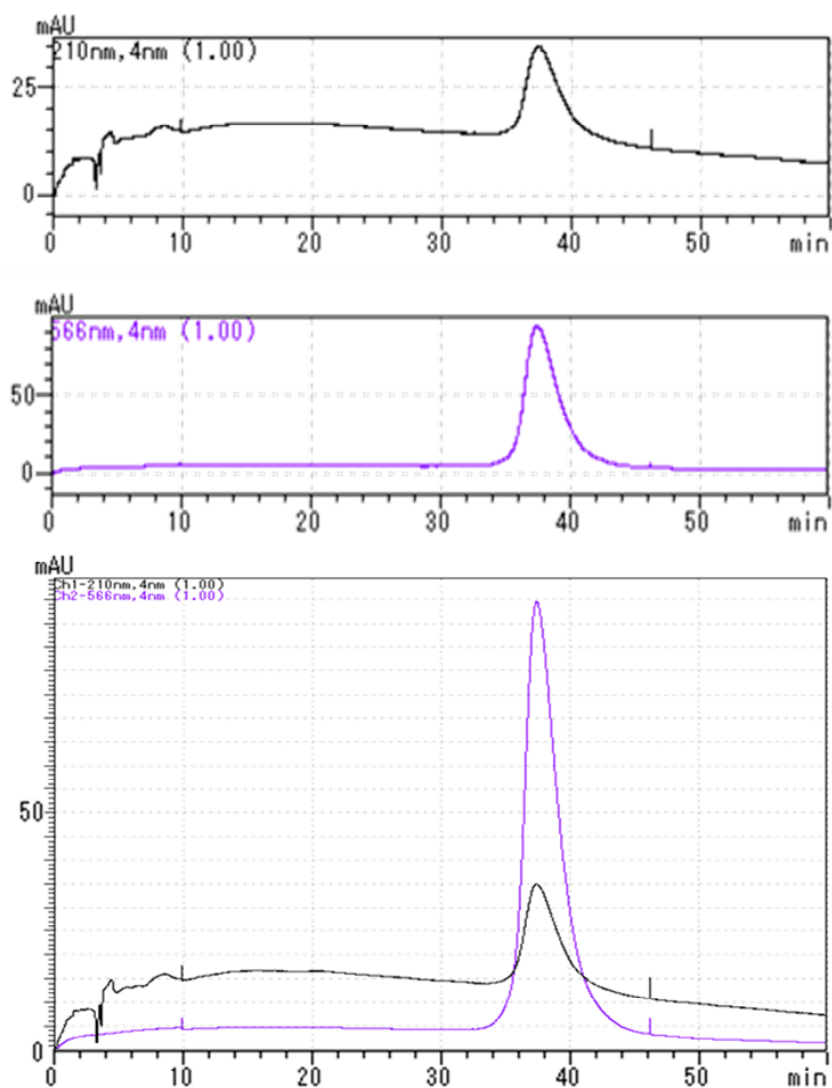
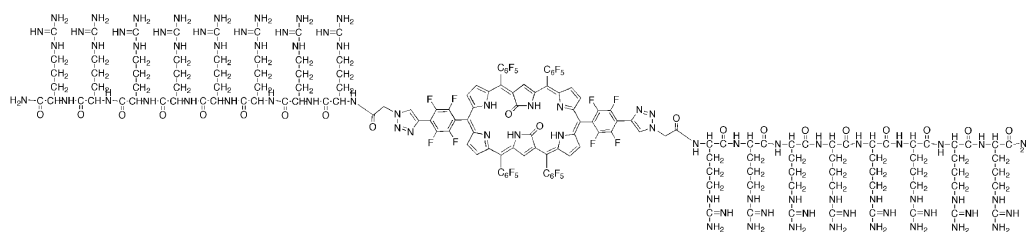


Fig. S1. HPLC chromatograms of N₂CH-R8. Absorption at 210 nm (top) or 566 nm (middle) was employed for detection of the peptide or N₂CH moiety, respectively. Absorption was monitored at two wavelengths, simultaneously, by using a photodiode array detector.



Comment 1

Comment 2

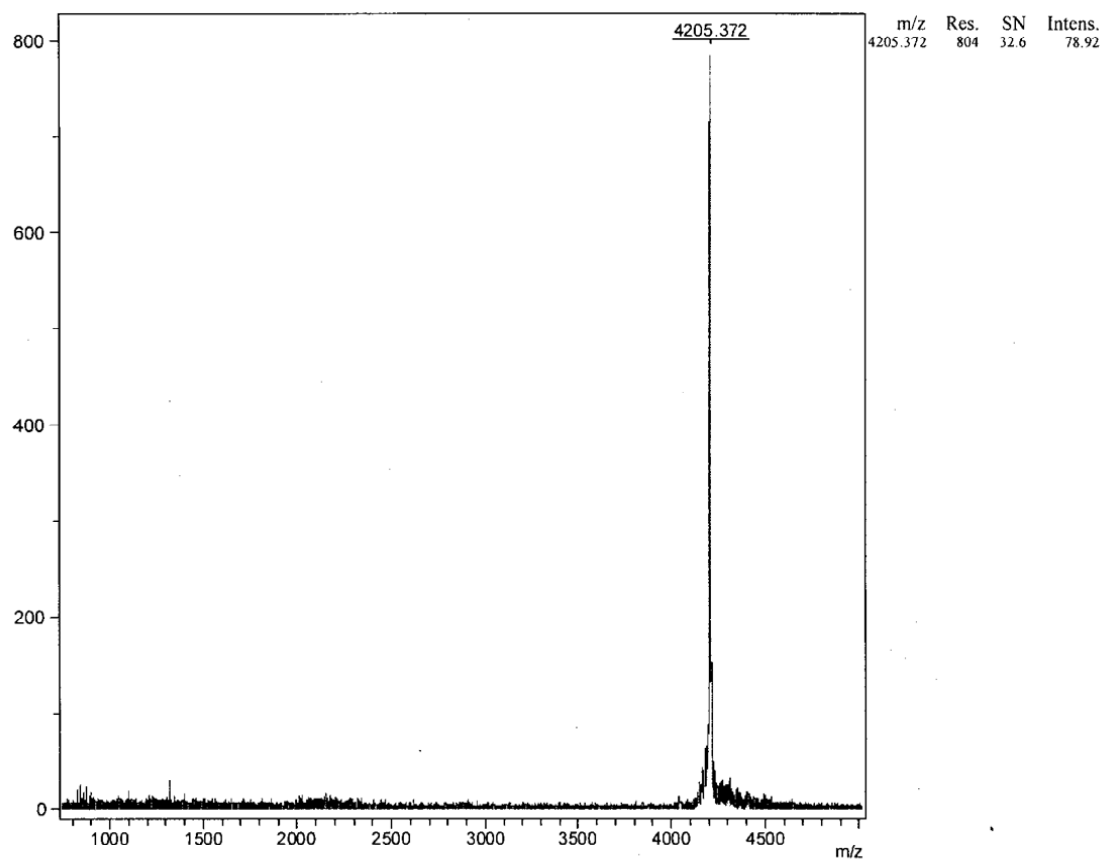


Fig. S2. MALDI-TOF-MS spectrum of **N₂CH-R8**.

MALDI-TOF-MS: calcd: 4204.00 for C₁₇₀H₂₁₆ F₂₈N₇₈O₂₀, found: 4205.372 ([M+1]⁺).

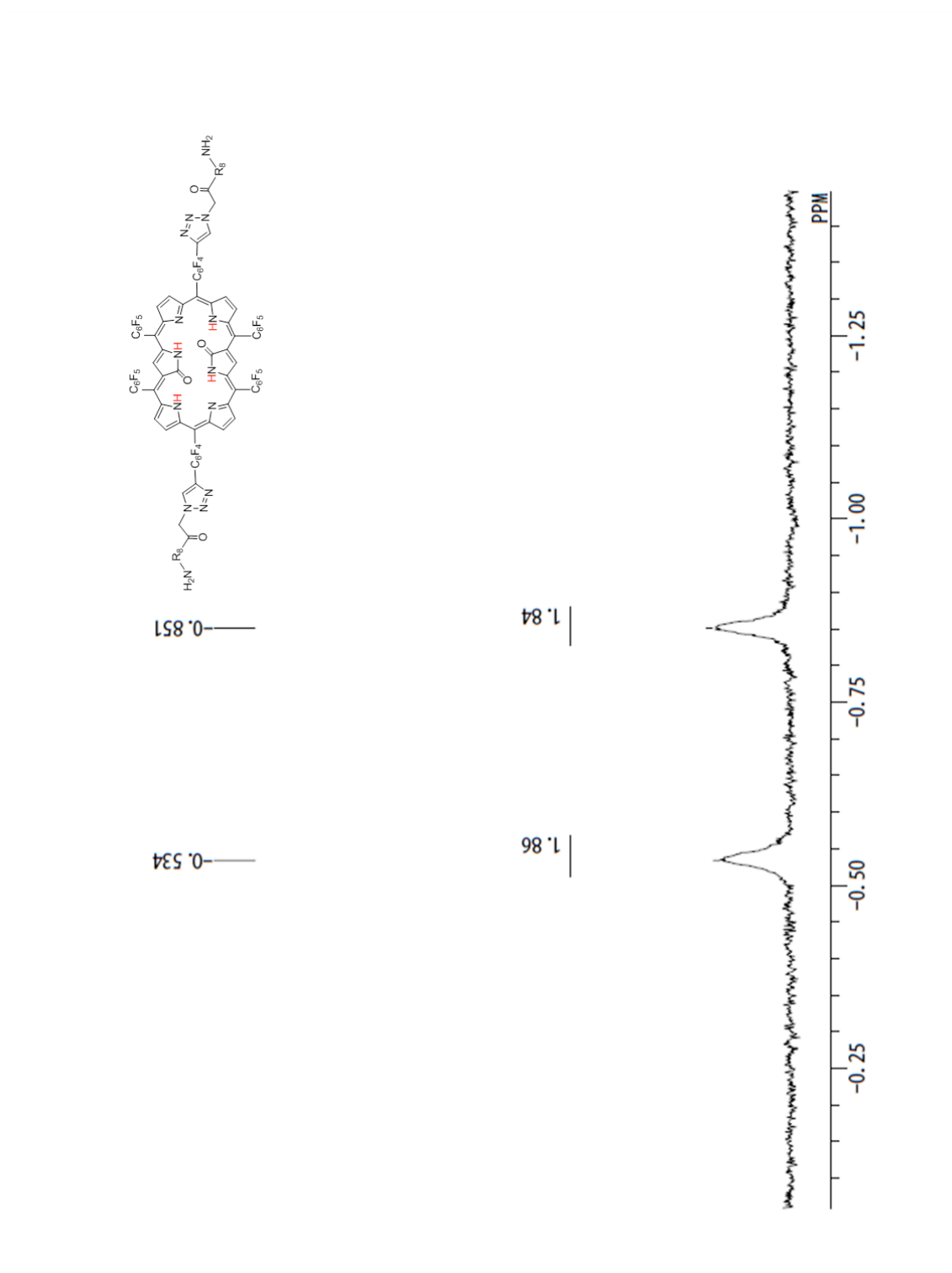


Fig. S3. Expanded 1H NMR spectrum of N_2CH-R8 in $DMF-d_7$. In the structure of N_2CH-R8 , protons observed in this region are indicated in red.

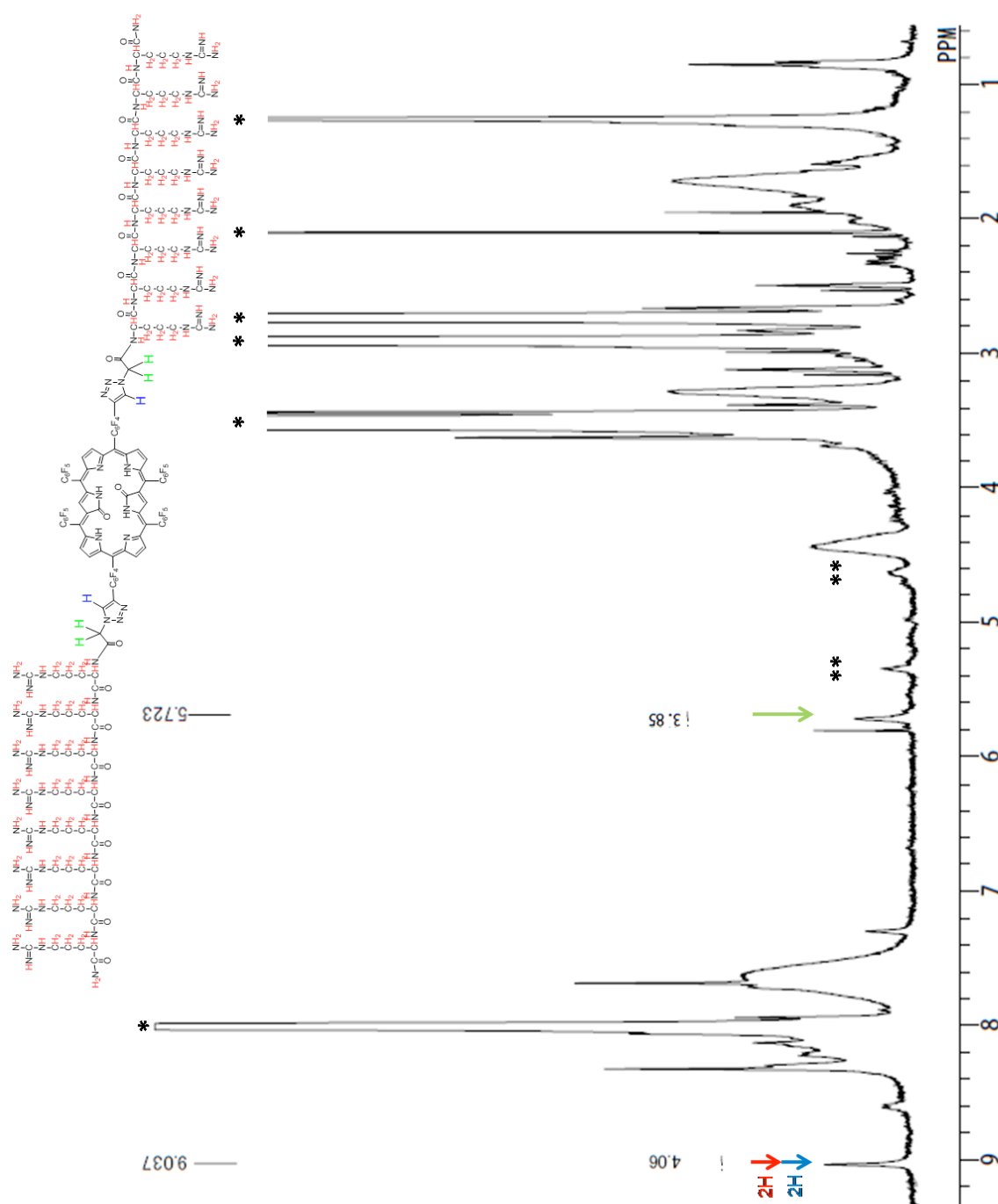


Fig. S4. Expanded ^1H NMR spectrum of $\text{N}_2\text{CH-R8}$ in DMF-d_7 . In the structure of $\text{N}_2\text{CH-R8}$, protons observed in this region are indicated in red (two octa-arginine peptide units), green (two methylene units between the peptide and triazole units), or blue (two triazole units). Signals of octa-arginine units are closely similar with those of octa-arginine amide prepared separately. Signals of triazole units and two methylenes are also similar to those of an N_2CH derivative prepared by a click reaction between compound **3** and ethyl azidoacetate.

*: solvent, **: impurity.

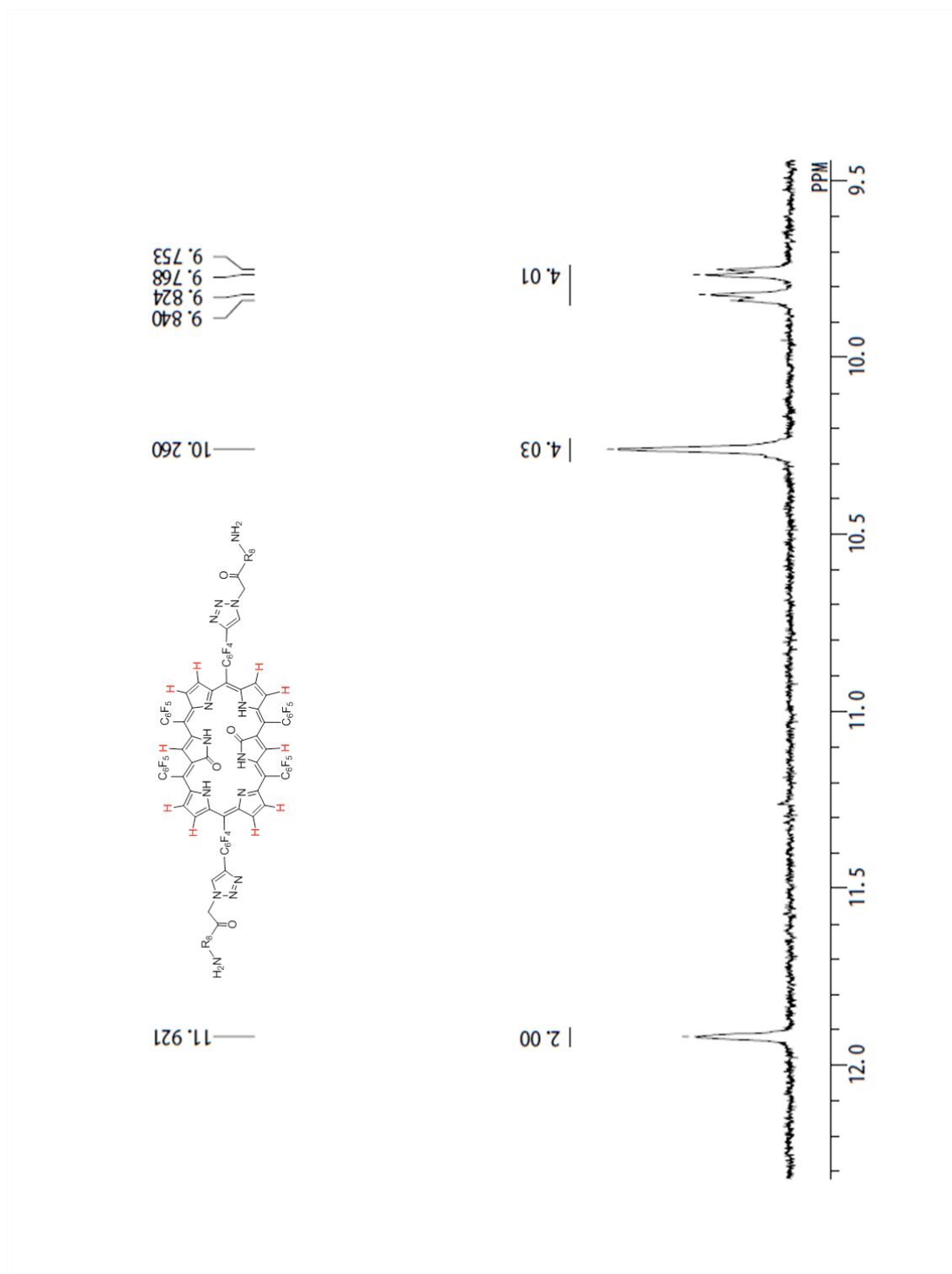


Fig. S5. Expanded 1H NMR spectrum of N_2CH-R8 in $DMF-d_7$. In the structure of N_2CH-R8 , protons observed in this region are indicated in red.

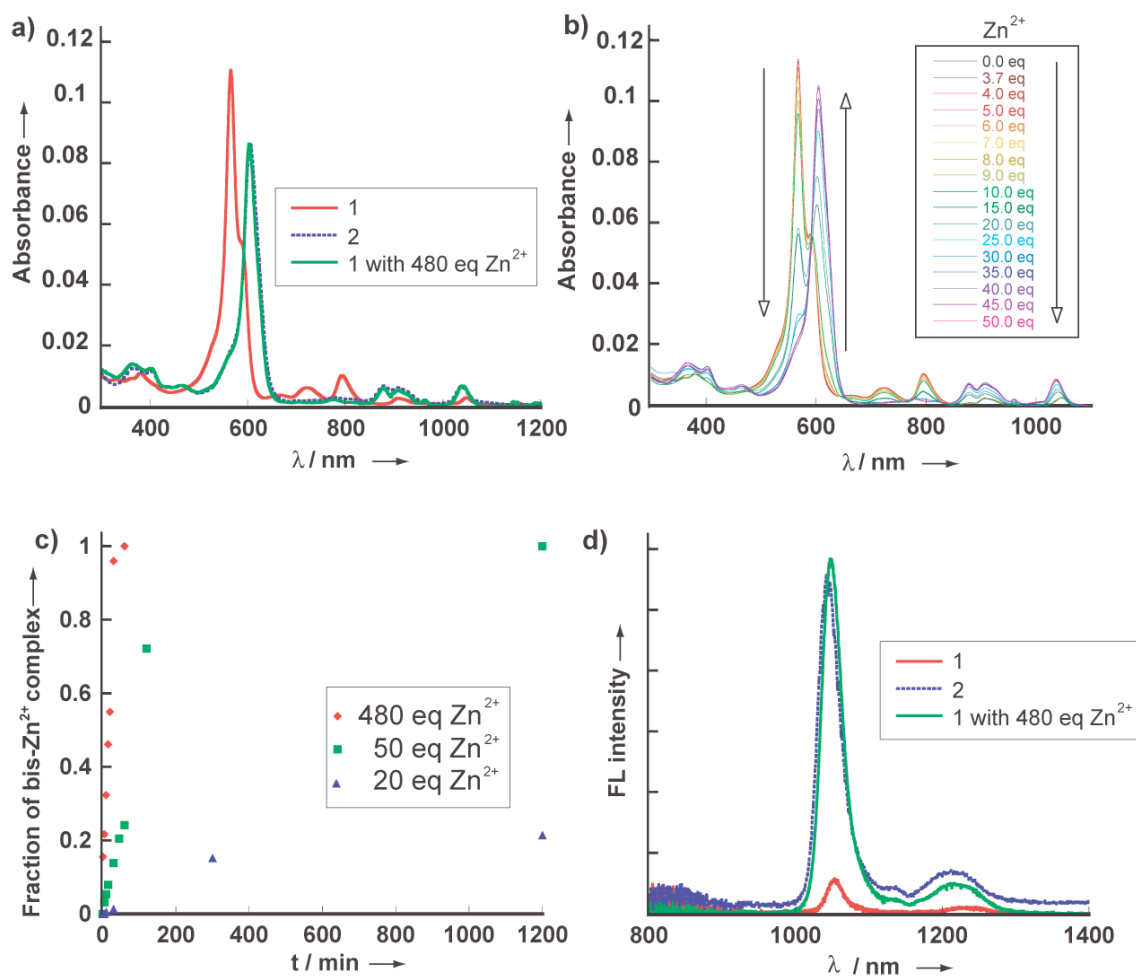


Fig. S6. Effects of Zn²⁺ on the absorption and fluorescence spectra of N₂CH in CH₂Cl₂. (a) Absorption spectra of freebase N₂CH (**1**), bis-Zn²⁺ complex of N₂CH (**2**), and **1** treated *in situ* with 480 equiv of Zn(OAc)₂. [**1**] = [**2**] = 0.27 μM. (b) Absorption spectra of **1** (0.27 μM) treated with different amount of Zn(OAc)₂. (c) Time dependent Zn²⁺ coordination of **1** (4.0 μM). (d) Fluorescence spectra of freebase N₂CH (**1**, λ_{ex} = 566 nm), bis-Zn²⁺ complex of N₂CH (**2**, λ_{ex} = 600 nm), and **1** treated *in situ* with 480 equiv of Zn(OAc)₂ (λ_{ex} = 600 nm). [**1**] = [**2**] = 0.53 μM.

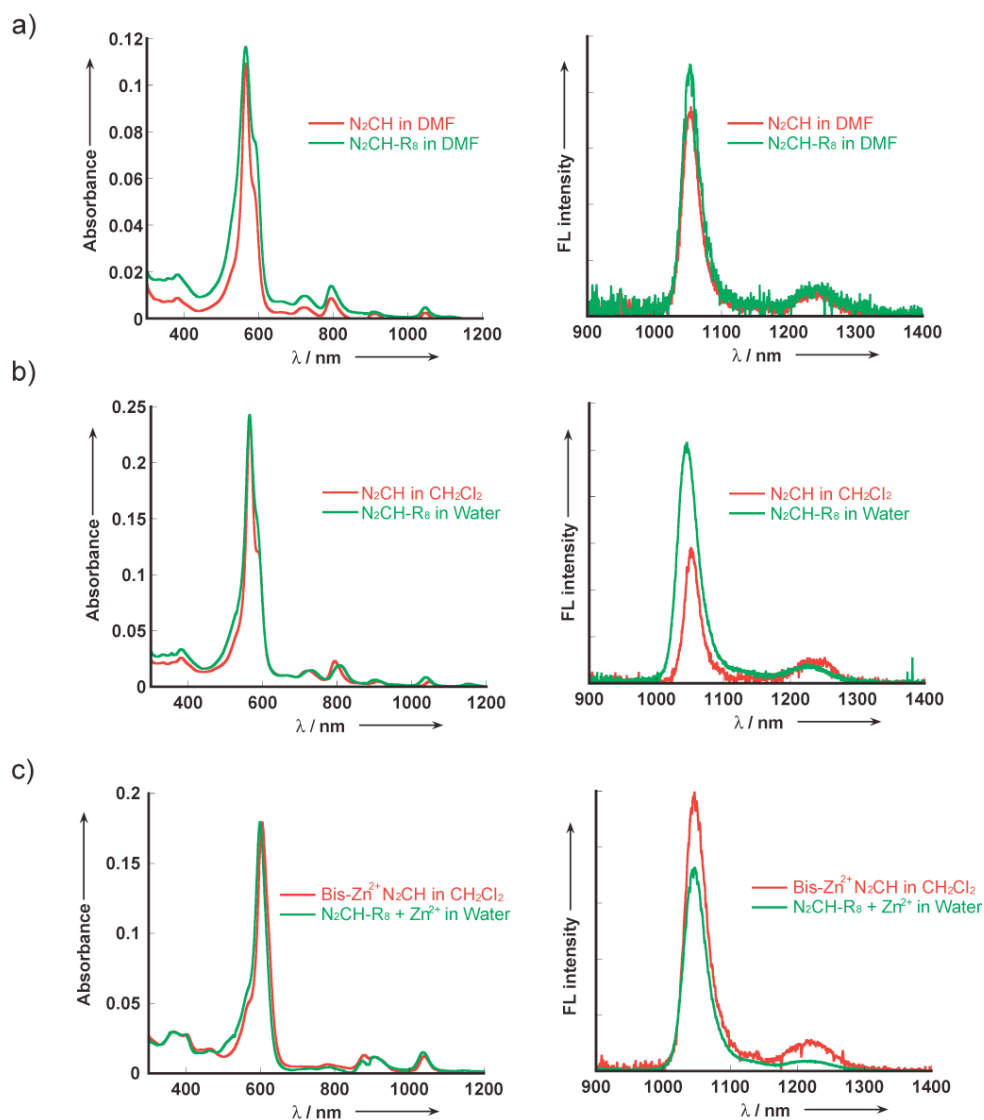


Fig. S7. Comparison of absorption and fluorescence spectra of **N₂CH (1)** and **N₂CH-R₈**. (a) Absorption (left) and fluorescence (right, $\lambda_{\text{ex}} = 566$ nm) spectra of **1** in DMF (absorption $\lambda_{\text{max}} = 566$ nm) and **N₂CH-R₈** in DMF (absorption $\lambda_{\text{max}} = 566$ nm). (b) Absorption (left) and fluorescence (right, $\lambda_{\text{ex}} = 566$ nm) spectra of **1** in CH_2Cl_2 (absorption $\lambda_{\text{max}} = 566$ nm) and **N₂CH-R₈** in H_2O (absorption $\lambda_{\text{max}} = 566$ nm). (c) Absorption (left) and fluorescence (right, $\lambda_{\text{ex}} = 600$ nm) spectra of bis-Zn²⁺ complex of **1 (2)** in CH_2Cl_2 (absorption $\lambda_{\text{max}} = 600$ nm) and **N₂CH-R₈** with 1000 equiv of $\text{Zn}(\text{OAc})_2$ in H_2O (absorption $\lambda_{\text{max}} = 600$ nm).

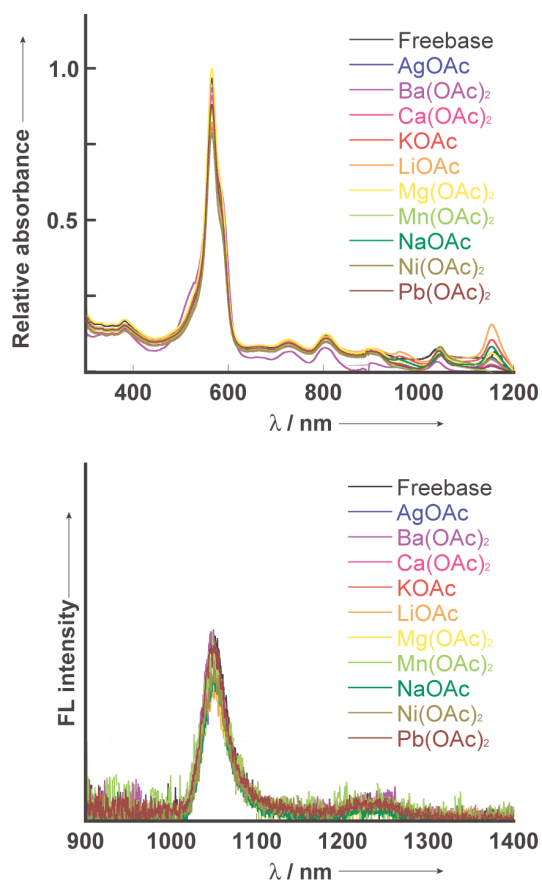


Fig. S8. Absorption (top) and fluorescence (bottom) spectra of **N₂CH-R8** in H₂O in the presence of metal ions that cause no significant spectral change. Absorption and fluorescence ($\lambda_{\text{ex}} = 600 \text{ nm}$) spectra were measured in the presence of $0.27 \mu\text{M}$ and $0.53 \mu\text{M}$ of **N₂CH-R8** with 1000 equiv of cations of interest, respectively.

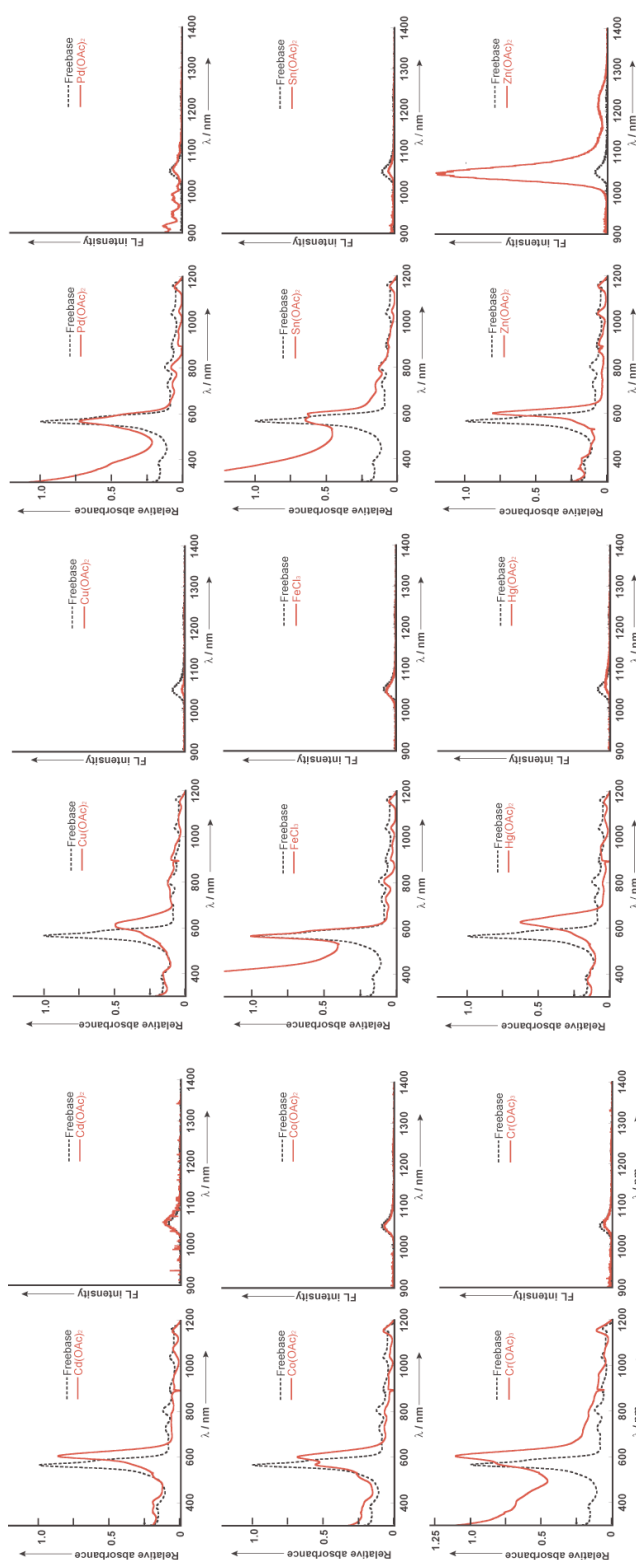


Fig. S9. Effects of metal ions that cause spectral changes on absorption and fluorescence of N_2CH-R8 in H_2O . Absorption and fluorescence ($\lambda_{ex} = 600$ nm) spectra were measured in the presence of $0.27 \mu M$ and $0.53 \mu M$ of N_2CH-R8 , respectively, with 1000 equiv of cations of interest.

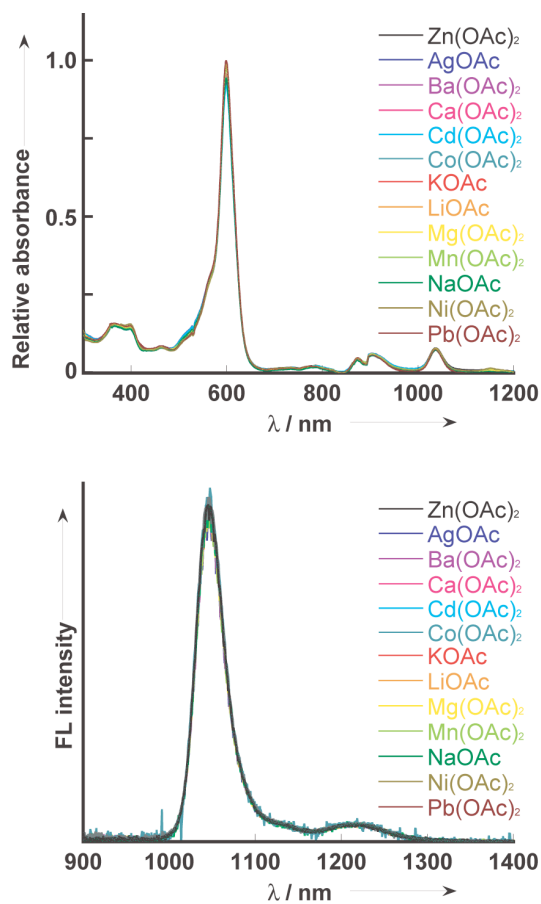


Fig. S10. Absorption (top) and fluorescence (bottom) spectra of bis-Zn²⁺ complex **N**₂**CH-R8** in H₂O in the presence of metal ions that cause no significant spectral change. Absorption and fluorescence ($\lambda_{\text{ex}} = 600$ nm) spectra were measured in the presence of 0.27 μM and 0.53 μM of **N**₂**CH-R8** with 1000 equiv of Zn²⁺ and 1000 equiv of cations of interest, respectively.

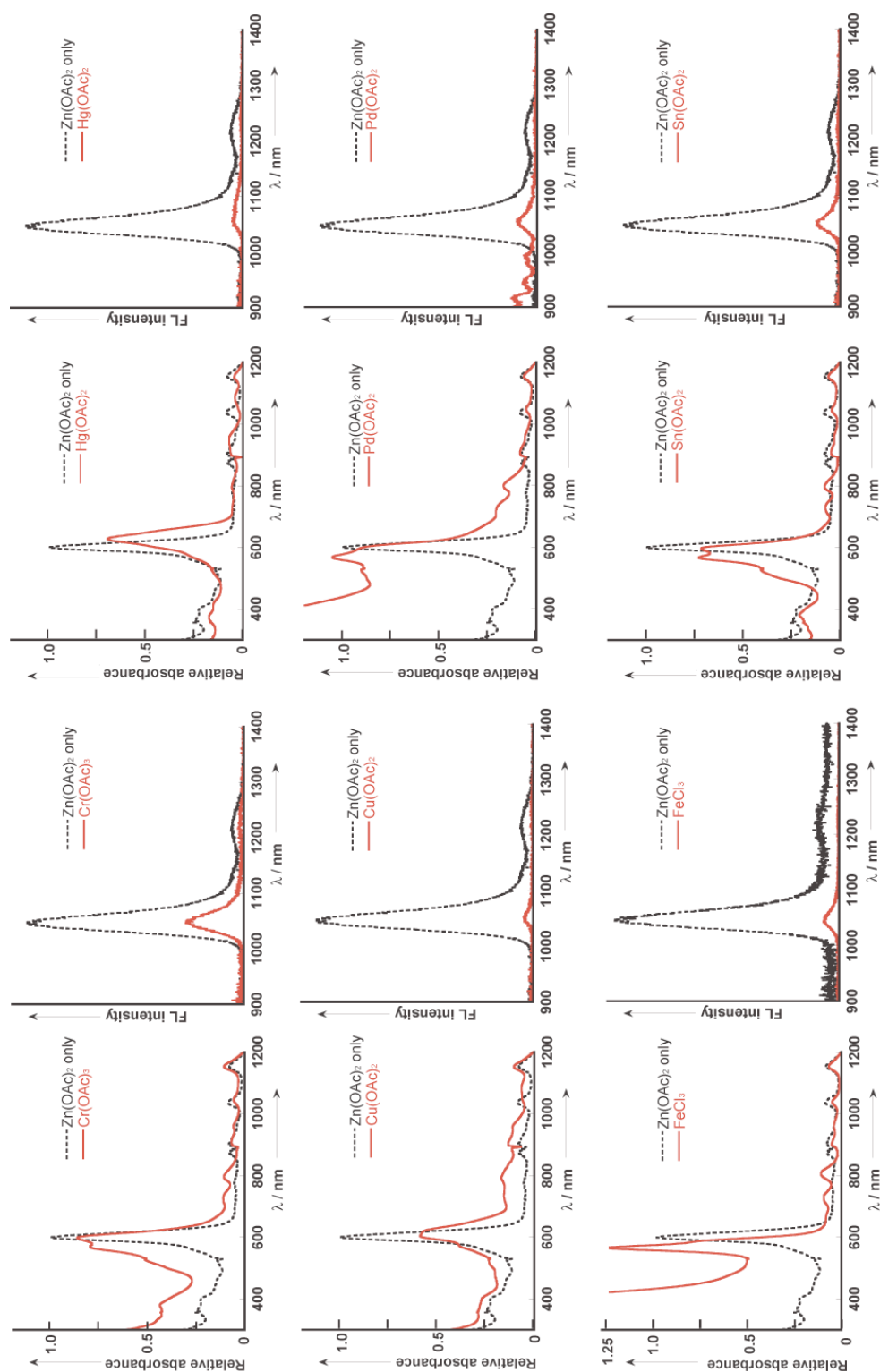


Fig. S11. Effects of metal ions that cause spectral changes on absorption and fluorescence of bis-Zn²⁺ complex of **N₂CH-R8** in H₂O. Absorption and fluorescence ($\lambda_{\text{ex}} = 600 \text{ nm}$) spectra were measured in the presence of $0.27 \mu\text{M}$ and $0.53 \mu\text{M}$ of **N₂CH-R8**, respectively, with 1000 equiv of Zn²⁺ and 1000 equiv of cations of interest.

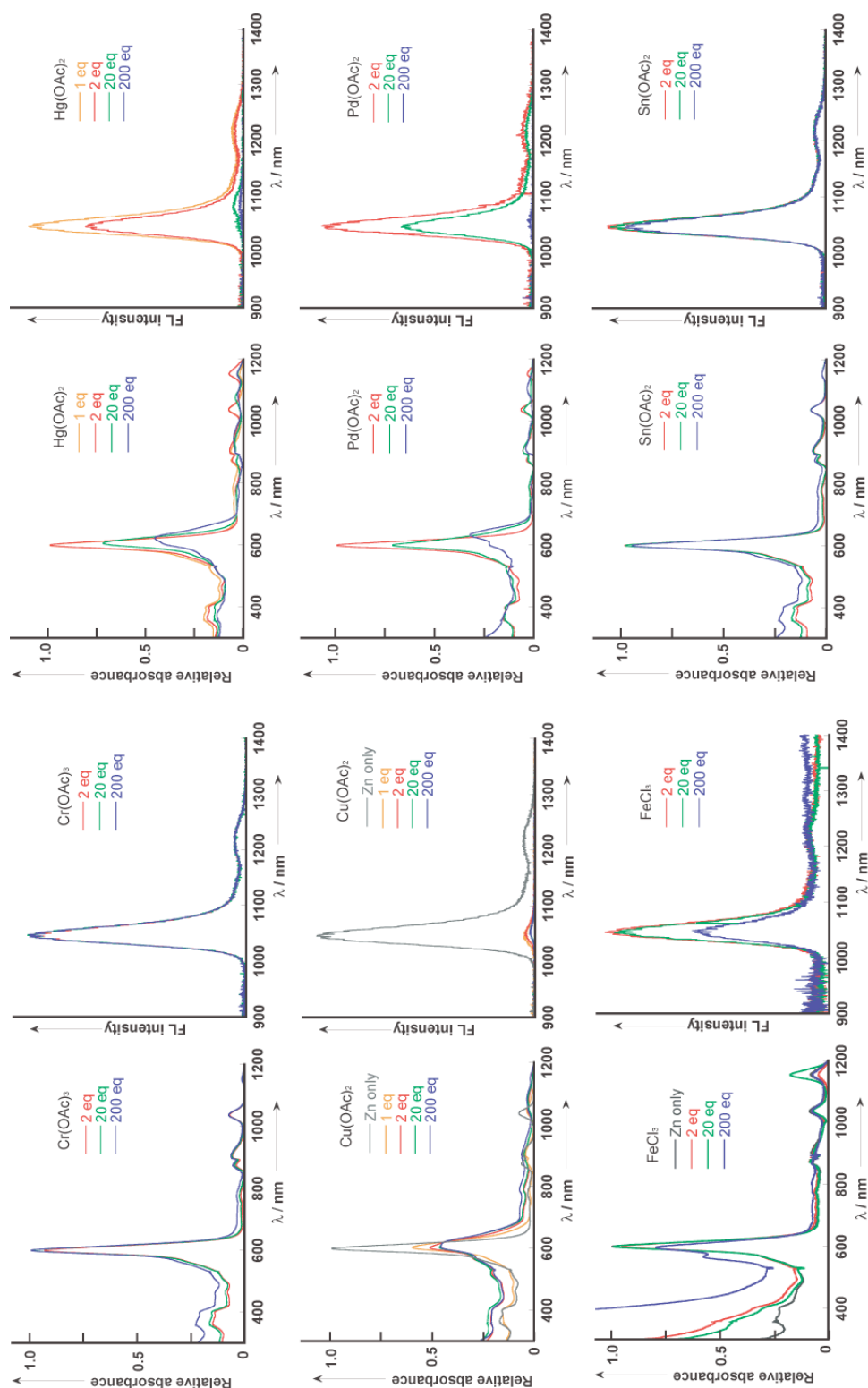


Fig. S12. Effects of different amount of metal ions that cause spectral changes on fluorescence of bis-Zn²⁺ complex of **N₂CH-R8** in H₂O. Fluorescence spectra ($\lambda_{\text{ex}} = 600$ nm) were measured in the presence of 0.53 μM of **N₂CH-R8** with 1000 equiv of Zn²⁺ and different molar equivalent of cations of interest.

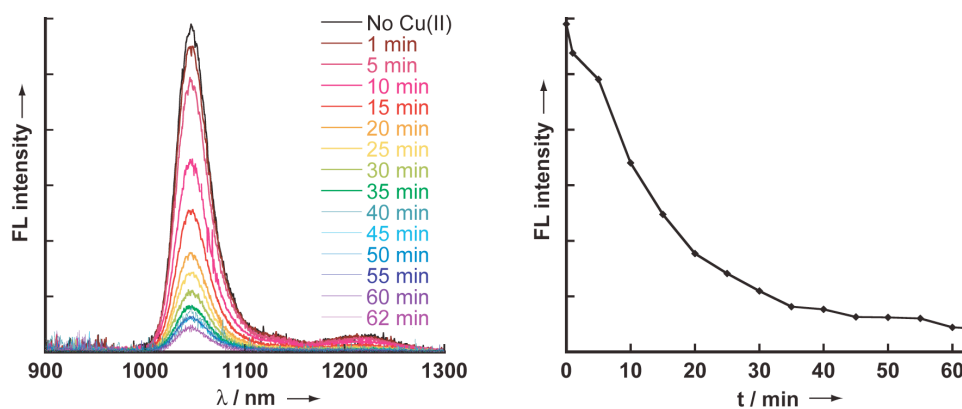


Fig. S13. The time dependence of fluorescence quenching of bis-Zn²⁺ complex **N₂CH-R8** by equivalent amount of Cu²⁺ in H₂O. Fluorescence spectra ($\lambda_{\text{ex}} = 600 \text{ nm}$) were measured in the presence of $0.53 \mu\text{M}$ of **N₂CH-R8**, $530 \mu\text{M}$ of Zn(OAc)₂, and $0.27 \mu\text{M}$ of Cu(OAc)₂.

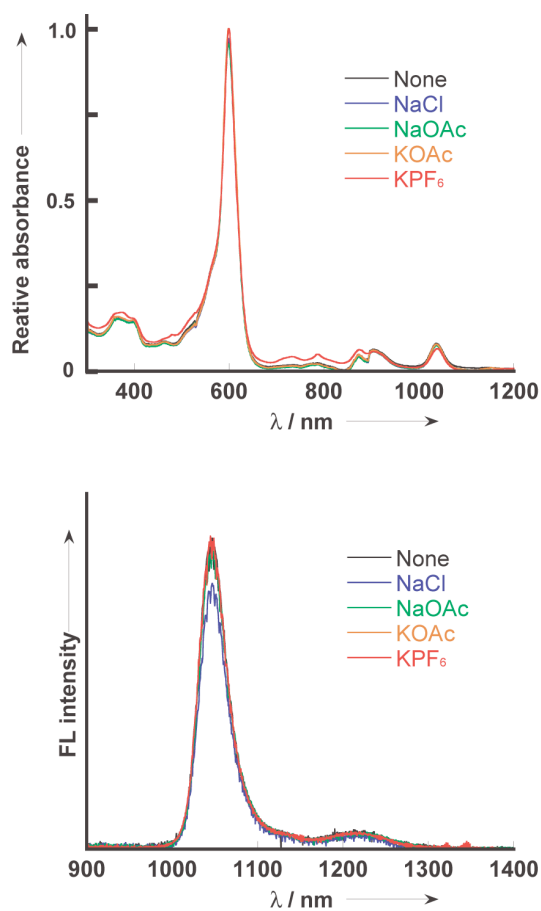


Fig. S14. Effects of monovalent salt on absorption (top) and fluorescence (bottom) spectra of bis-Zn²⁺ complex of **N₂CH-R8** in H₂O. Absorption and fluorescence ($\lambda_{\text{ex}} = 600 \text{ nm}$) spectra were measured in the presence of 0.27 μM and 0.53 μM of **N₂CH-R8**, respectively, with 1000 equiv of Zn(OAc)₂ and 1000 equiv of salts of interest.