1 Supplementary Information for:

2

3 **Design of Multinuclear Zn(II) Complex as New Molecular Probe for**

4 **Fluorescence Imaging of His-tag Fused Proteins**

5

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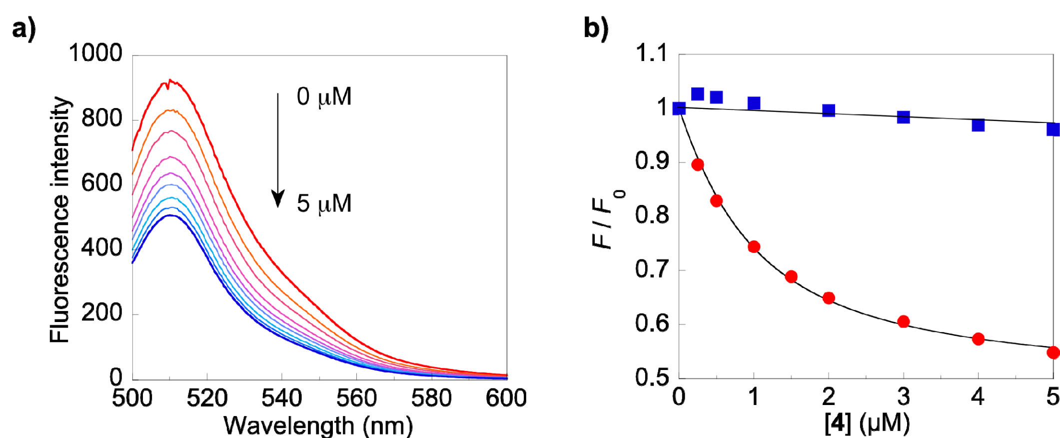
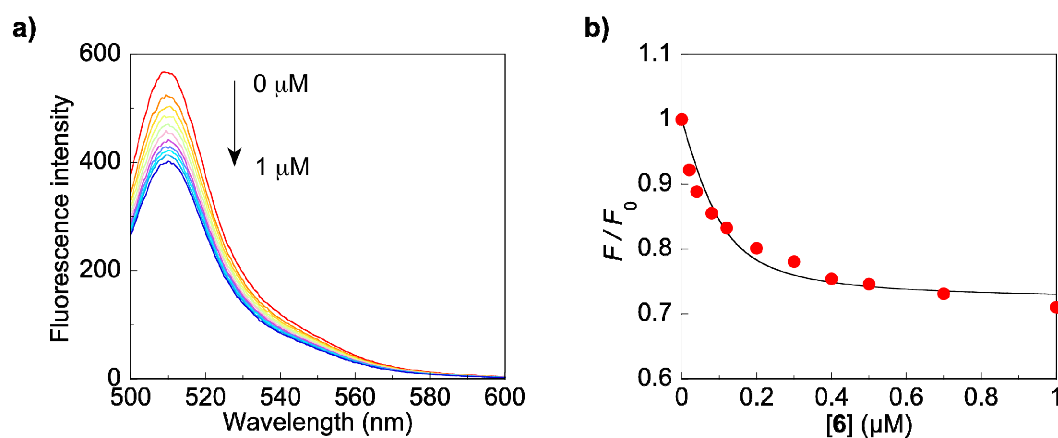
1 # Supplementary Figures



2

3 **Fig. S1** (a) Fluorescence spectral change of the EGFP tetherd with a His10 tag (His10-EGFP,
4 0.5 μM) upon addition of **4** (0-5 μM). (b) Plots of the fluorescence emission change at 510 nm
5 observed in the titration of His10-EGFP (●) or EGFP lacking a His10 tag (■) with **4**.
6 Fluorescence titration curve was analyzed using nonlinear least-square curve-fitting assuming
7 1:1 binding to evaluate the apparent binding constant. Measurement conditions: 50 mM HEPES
8 (pH 7.2), 100 mM NaCl, 25 ˚C.

9



1

2 **Fig. S2** (a) Fluorescence spectral change of the EGFP tethered with a His10 tag (His10-EGFP,

3 0.1 μM) upon addition of **6** (0-1 μM) in the presence of 10 μM of Zn(II)-Ida dimer **28** as a

4 competitive binder (see **Supplementary Methods**). (b) Curve-fitting analysis of the

5 fluorescence emission change at 510 nm.[S1] Measurement conditions: 50 mM HEPES (pH

6 7.2), 100 mM NaCl, 25 ˚C.

7

8

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Supplementary Methods

General materials and methods for organic synthesis

All chemical reagents and solvents were obtained from commercial suppliers (Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical Industries, Acros Organics, Sasaki Chemical, or Watanabe Chemical Industries) and used without further purification.

Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated aluminium sheets (Merck) and visualized by fluorescence quenching or ninhydrin staining. Chromatographic purification was conducted by flash column chromatography on silica gel 60 N (neutral, 40–50 μm, Kanto Chemical). ^{1}H NMR spectra were recorded in deuterated solvents on a Varian Mercury 400 (400 MHz) spectrometer and calibrated to the residual solvent peak or tetramethylsilane (= 0 ppm). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, dd = double doublet. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) was measured by an Autoflex II instrument (Bruker Daltonics) using α-cyano-4-hydroxycinnamic acid (CHCA) or sinapinic acid as the matrix. High-resolution electrospray ionization quadrupole fourier transform mass spectroscopy (HR-ESI Qq-LTMS) was measured by a Bruker apex-ultra (7T) mass spectrometer. We acknowledge Dr. Keiko Kuwata (Graduate School of Engineeing, Kyoto University) for the measurements of the high-resolution mass spectroscopy.

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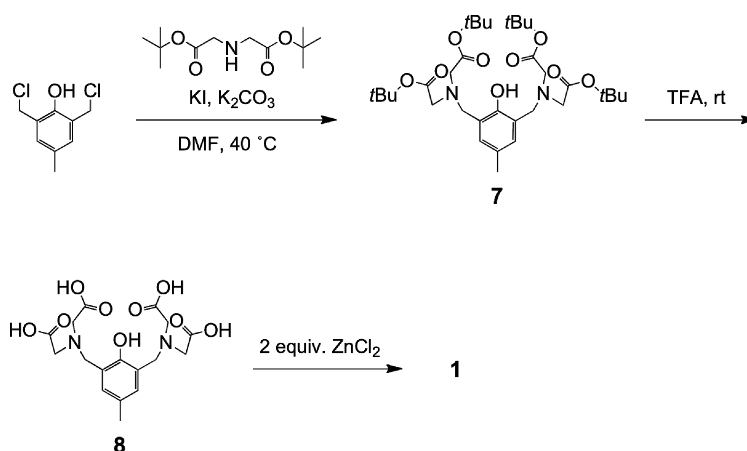
Synthesis of 1



Compound **7**

A solution of 2,6-bis(chloromethyl)-*p*-cresol (50 mg, 241 μmol), di-*tert*-butyl iminodiacetate (130 mg, 530 μmol), potassium carbonate (74 mg, 530 μmol) and potassium iodide (16 mg, 96 μmol) in dry DMF (5 mL) was stirred for 3 h at 40 ˚C. After removal of the solvent by evaporation, the residue was diluted with $CHCl_3$. The organic layer was washed with *sat*. $NaHCO_3$ and brine followed by drying over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by flash column chromatography on SiO_2 (Hexane : AcOEt = 8 : 1) to give **7** (53 mg, 35%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1H), 6.95 (s, 2H), 3.91 (s, 4H), 3.42 (s, 8H), 2.22 (s, 3H), 1.47 (s, 36H).

Compound **8**

A solution of **7** (53 mg, 85 μmol) in TFA (3 mL) was stirred for 4 h at rt. The solvent was removed to give **8** (59 mg, quant.) as a white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.08 (s, 2H), 4.27 (s, 4H), 3.83 (s, 8H), 2.26 (s, 3H). HR-ESI MS *m/e* calcd for $[M+H]^+$ 399.1398, found 399.1393.

Compound **1**

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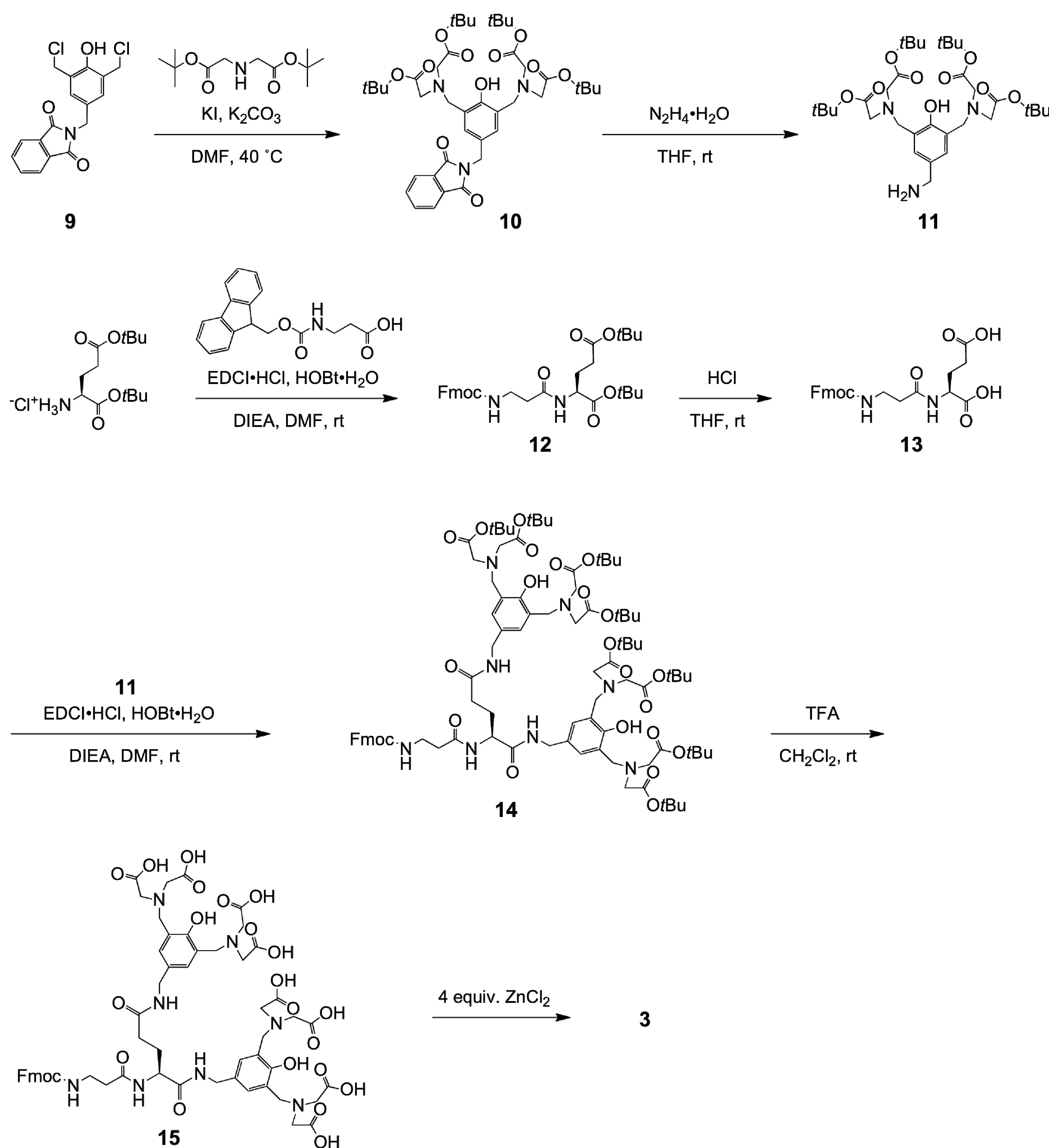
1 A solution of **8** (28 mg, 70 μmol), $ZnCl_2$ (140 μmol) in H_2O (1 mL) was stirred for 1 h at
2 rt. The solution was filtered through a cellulose acetate filter and then lyophilized. The
3 solid was filtered and washed with AcOEt followed by drying in vacuo at 40 ˚C to give **1** (33
4 mg, 90%) as a white powder.
5

1 **Synthesis of 3**



2

3 Compound **10**

4 A solution of **9**[S2] (100 mg, 286 µmol), di-*tert*-butyl iminodiacetate (154 mg, 628 µmol),
5 potassium carbonate (87 mg, 628 µmol) and potassium iodide (19 mg, 114 µmol) in dry DMF
6 (5 mL) was stirred for 3 h at 40 ˚C. The solution was diluted with *sat*. $NaHCO_3$ and extracted
7 with $CHCl_3$. The organic layer was washed with brine followed by drying over Na_2SO_4.
8 After removal of the solvent by evaporation, the residue was purified by flash column

1 chromatography on SiO_2 (Hexane : AcOEt = 4 : 1) to give **10** (124 mg, 57%) as a colorless oil.
2 ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.82 (d, *J* = 5.2 Hz, 2H), 7.68 (d, *J* = 5.2 Hz, 2H),
3 7.19 (s, 2H), 4.73 (s, 2H), 3.91 (s, 4H), 3.41 (s, 8H), 1.45 (s, 36H). HR-ESI MS *m/e* calcd for
4 $[M+H]^+$ 768.4066, found 768.4058.
5
6 Compound **11**
7 A solution of **10** (81 mg, 106 μmol) in THF (5 mL) / hydrazine monohydrate (0.4 mL)
8 was stirred for 21 h at rt. After addition of 2 N aqueous NaOH (10 mL), the reaction mixture
9 was further stirred for 1.5 h at rt. The resultant mixture was extracted with $CHCl_3$ and the
10 organic layer was washed with brine followed by drying over Na_2SO_4. After removal of the
11 solvent by evaporation, the residue was purified by flash column chromatography on SiO_2
12 ($CHCl_3$: MeOH : 25% aqueous NH_3 = 400 : 10 : 1) to give **11** (55 mg, 82%) as a pale-yellow
13 oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 7.09 (s, 2H), 3.94 (s, 4H), 3.73 (s, 2H), 3.42
14 (s, 8H), 1.47 (s, 36H). HR-ESI MS *m/e* calcd for $[M+H]^+$ 638.4011, found 638.4006.
15
16 Compound **12**
17 A solution of H-Glu(O*t*Bu)-O*t*Bu•HCl (210 mg, 710 μmol), Fmoc-βAla-OH (265 mg,
18 852 μmol), EDCI•HCl (191 mg, 995 μmol), HOBt•H_2O (152 mg, 995 μmol) and DIEA (238 μL,
19 1.42 mmol) in dry DMF (5 mL) was stirred for 15 h at rt. The reaction mixture was diluted
20 with *sat*. $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with water and
21 brine followed by drying over Na_2SO_4. After removal of the solvent by evaporation, the
22 residue was purified by flash column chromatography on SiO_2 (Hexane : AcOEt = 2 : 1) to give
23 **12** (400 mg, quant.) as a white amorphous powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J*
24 = 7.6 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.30 (t, *J* = 7.6 Hz, 2H), 6.29 (d,
25 *J* = 8.0 Hz, 1H), 5.67 (t, *J* = 5.6 Hz, 1H), 4.45-4.51 (m, 1H), 4.36 (d, *J* = 7.2 Hz, 2H), 4.21 (t, *J*
26 = 6.8 Hz, 1H), 3.52 (t, *J* = 6.0 Hz, 2H), 2.46 (t, *J* = 5.6 Hz, 2H), 2.22-2.38 (m, 2H), 2.09-2.17

1 (m, 1H), 1.88-1.97 (m, 1H), 1.48 (s, 9H), 1.44 (s, 9H). HR-ESI MS *m/e* calcd for $[M+H]^+$
2 553.2908, found 553.2909.
3
4 Compound **13**
5 A solution of **12** (400 mg, 724 μmol) in THF (5 mL) / 4 N aqueous HCl (5 mL) was
6 stirred for 19 h at rt. After removal of the solvent by evaporation, the residue was dissolved
7 in *sat.* $NaHCO_3$. The aqueous layer was washed with AcOEt, acidified to pH 2 with 1 N
8 aqueous HCl, and then extracted with AcOEt. The organic layer was dried over Na_2SO_4 and
9 concentrated by evaporation to give **13** (327 mg, quant.) as a white amorphous powder.
10 ^{1}H-NMR (400 MHz, CD_3OD) δ 7.78 (d, *J* = 7.2 Hz, 2H), 7.64 (d, *J* = 7.6 Hz, 2H), 7.38 (t, *J* =
11 7.2 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 2H), 4.43 (q, *J* = 4.4 Hz, 1H), 4.31 (d, *J* = 7.2 Hz, 2H), 4.19 (t,
12 *J* = 6.8 Hz, 1H), 3.39 (t, *J* = 5.6 Hz, 2H), 2.45 (t, *J* = 6.8 Hz, 2H), 2.41 (t, *J* = 7.6 Hz, 2H),
13 2.13-2.22 (m, 1H), 1.90-1.98 (m, 1H). HR-ESI MS *m/e* calcd for $[M+Na]^+$ 463.1476, found
14 463.1476.
15
16 Compound **14**
17 A solution of **13** (80 mg, 182 μmol), **11** (244 mg, 382 μmol), EDCI•HCl (98 mg, 510
18 μmol), HOBt•H_2O (78 mg, 510 μmol) and DIEA (123 μL, 728 μmol) in dry DMF (5 mL) was
19 stirred for 20 h at rt. The reaction mixture was diluted with *sat.* $NaHCO_3$ and extracted with
20 $CHCl_3$. The organic layer was washed with *sat.* $NaHCO_3$, brine and dried over Na_2SO_4
21 followed by concentration in vacuo. The residue was purified by flash column
22 chromatography on SiO_2 ($CHCl_3$: MeOH = 30 : 1) to give **14** (116 mg, 38%) as a pale-yellow
23 oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.59 (s, 1H), 9.56 (s, 1H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.59
24 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.07 (s, 2H), 7.05 (s, 2H),
25 6.18 (br s, 1H), 5.67 (br s, 1H), 4.19-4.46 (m, 8H), 3.90 (s, 4H), 3.89 (s, 4H), 3.49 (br s, 2H),
26 3.40 (s, 8H), 3.39 (s, 8H), 2.48 (br s, 2H), 2.29-2.43 (m, 2H), 2.02-2.17 (m, 2H), 1.47 (s, 36H),

1 1.45 (s, 36H). HR-ESI MS *m/e* calcd for $[M+H]^+$ 1679.9322, found 1679.9328.

2

3 Compound **15**

4 A solution of **14** (25 mg, 14.9 μmol) in CH_2Cl_2 (1 mL) / TFA (1 mL) was stirred for 1 h at

5 rt. After removal of the solvent by evaporation, the residue was purified by RP-HPLC

6 (column: YMC-pack ODS-A, 250 x 25 mm, mobile phase; CH_3CN (containing 0.1% TFA) :

7 H_2O (containing 0.1% TFA) = 20 : 80 (0 min) → 40 : 60 (40 min), flow rate; 10 mL/min,

8 detection; UV (220 nm)) to give **15** (11 mg, 60%) as a white powder. ^1H-NMR (400 MHz,

9 CD_3OD) δ 7.78 (d, *J* = 7.2 Hz, 2H), 7.59 (d, *J* = 6.4 Hz, 2H), 7.37 (t, *J* = 7.2 Hz, 2H), 7.28 (t, *J*

10 = 7.2 Hz, 2H), 7.14 (s, 4H), 4.10-4.36 (m, 16H), 3.79 (s, 8H), 3.78 (s, 8H), 3.41 (t, *J* = 6.8 Hz,

11 2H), 2.45 (t, *J* = 6.4 Hz, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 2.17 (m, 1H), 1.93 (m, 1H). HR-ESI

12 MS *m/e* calcd for $[M+H]^+$ 1231.4314, found 1231.4279.

13

14 Compound **3**

15 A solution of **15** (200 μM, determined by $\varepsilon(\mathbf{15})_{299\ nm} = 7.63 \times 10^3\ M^{-1}cm^{-1}$) in 50 mM

16 HEPES buffer (2 mL, pH 7.2, 100 mM NaCl) was mixed with $ZnCl_2$ (4 equiv.) to give a stock

17 solution of **3** (200 μM), which was used for ITC titration experiments.

18

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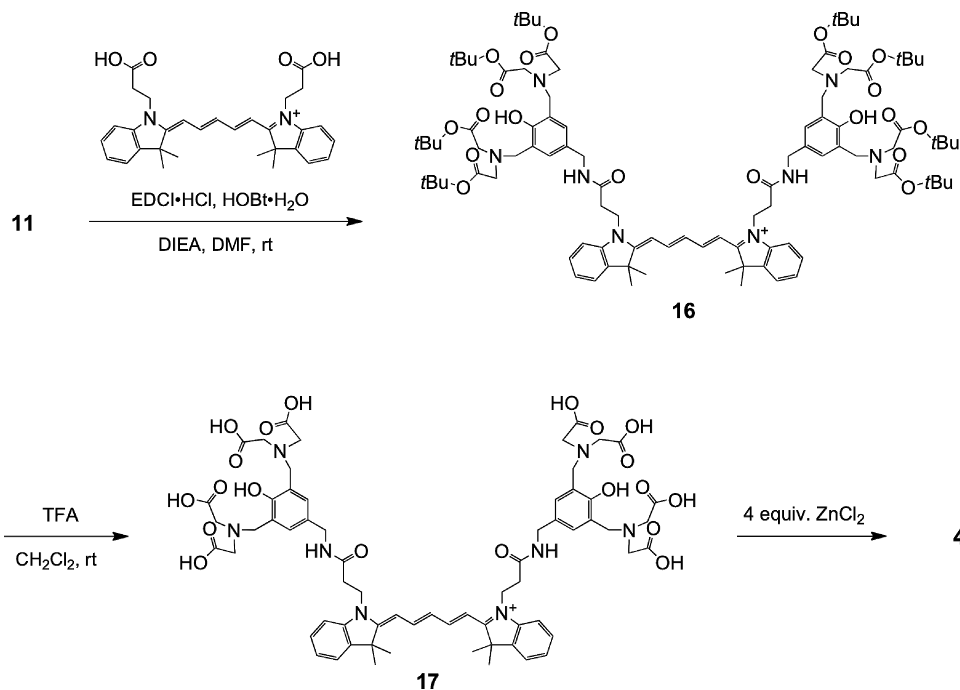
Synthesis of 4



Compound **16**

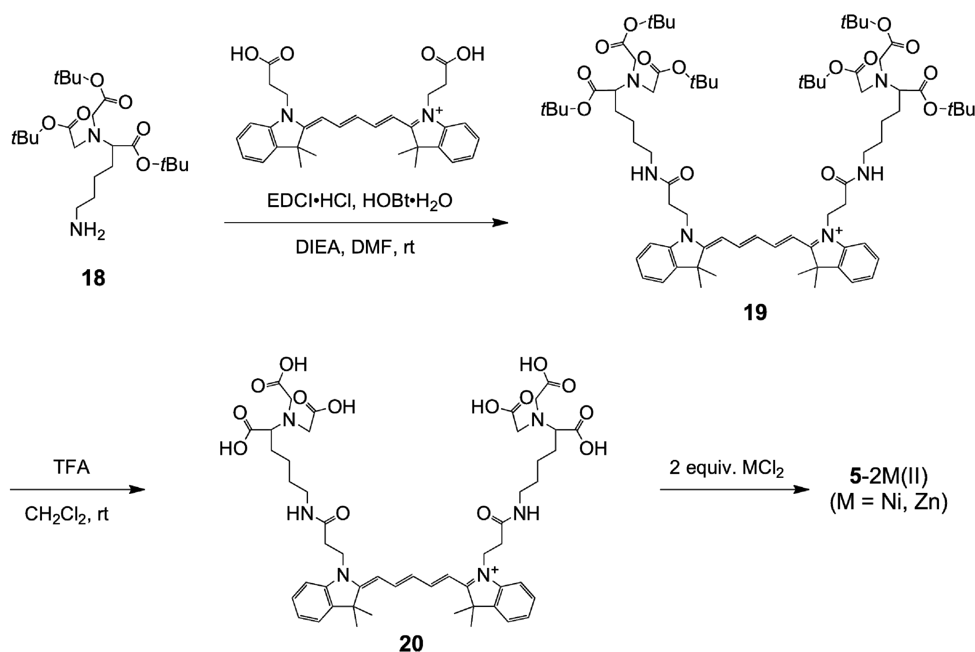
A solution of Cy5-bisCO_2H[S3] (21 mg, 39 µmol), **11** (55 mg, 86 µmol), EDCI•HCl (11 mg, 55 µmol), HOBt•H_2O (8 mg, 55 µmol) and DIEA (27 µL, 155 µmol) in dry DMF (3 mL) was stirred for 15 h at rt. After removal of the solvent by evaporation, the residue was purified by RP-HPLC (column: YMC-pack ODS-A, 250 x 25 mm, mobile phase; CH_3CN (containing 0.1% TFA) : H_2O (containing 0.1% TFA) = 50 : 50 (0 min) → 90 : 10 (40 min), flow rate; 10 mL/min, detection; UV (220 nm)) to give **16** (27 mg, 37%) as a deep blue powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 2H), 7.87 (t, J = 12.8 Hz, 2H), 7.29-7.38 (m, 8H), 7.19 (s, 4H), 6.61 (t, J = 12.4 Hz, 1H), 6.26 (t, J = 13.2 Hz, 2H), 4.37 (t, J = 7.2 Hz, 4H), 4.29 (d, J = 5.6 Hz, 4H), 4.25 (s, 8H), 3.72 (s, 16H), 2.78 (t, J = 7.6 Hz, 4H), 1.65 (s, 12H), 1.46 (s, 72H). HR-ESI MS *m/e* calcd for $[M]^+$ 1738.0257, found 1738.0252.

Compound **17**

A solution of **16** (20 mg, 11 µmol) in CH_2Cl_2 (1 mL) / TFA (1 mL) was stirred for 6 h at rt.

1 After removal of the solvent by evaporation, the residue was purified by RP-HPLC (column:
2 YMC-pack ODS-A, 250 x 25 mm, mobile phase; CH_3CN (containing 0.1% TFA) : H_2O
3 (containing 0.1% TFA) = 20 : 80 (0 min) → 50 : 50 (40 min), flow rate; 10 mL/min, detection;
4 UV (220 nm)) to give **17** (11 mg, 73%) as a deep blue powder. ^{1}H-NMR (400 MHz, CD_3OD)
5 δ 8.21 (t, *J* = 12.8 Hz, 2H), 7.47 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.31 (d, *J* = 7.6 Hz,
6 2H), 7.26 (t, *J* = 7.2 Hz, 2H), 7.10 (s, 4H), 6.54 (t, *J* = 12.0 Hz, 1H), 6.32 (d, *J* = 13.6 Hz, 2H),
7 4.41 (t, *J* = 7.2 Hz, 4H), 4.22 (s, 8H), 4.19 (s, 4H), 3.76 (s, 16H), 2.72 (t, *J* = 6.8 Hz, 4H), 1.67
8 (s, 12H). HR-ESI MS *m/e* calcd for $[M]^+$ 1289.5249, found 1289.5182.
9
10 Compound **4**
11 Compound **4** was dissolved in DMSO. The concentration of **4** was determined based
12 on the UV absorbance at 647 nm using the extinction coefficient of Cy5 ($\varepsilon_{647\ nm}$ = 250,000
13 $M^{-1}cm^{-1}$).[S3] The solution was mixed with 4 equiv of $ZnCl_2$ (100 mM in H_2O) to give a stock
14 solution of **4** (1.0 mM), which was stored in a refrigerator (- 30 ˚C) and thawed before use.

1 **Synthesis of 5-2M(II) (M = Zn, Ni)**



2

3 Compound **19**

4 A solution of **18**[S4] (60 mg, 140 µmol), Cy5-bisCO_2H (30 mg, 56 µmol), EDCI•HCl (30
5 mg, 157 µmol), HOBt•H_2O (24 mg, 157 µmol) and DIEA (38 µL, 218 µmol) in dry DMF (2
6 mL) was stirred for 13 h at rt. After removal of the solvent by evaporation, the residue was
7 diluted with $CHCl_3$. The organic layer was washed with brine followed by drying over
8 Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by flash
9 column chromatography on SiO_2 ($CHCl_3$: MeOH : 25% aqueous NH_3 = 400 : 40 : 1) to give **19**
10 (77 mg, quant.) as a deep blue oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96 (t, J = 13.2 Hz, 2H),
11 7.31-7.40 (m, 6H), 7.19-7.23 (m, 2H), 6.95 (t, J = 12.4 Hz, 1H), 6.52 (d, J = 13.2 Hz, 2H), 4.42
12 (t, J = 7.6 Hz, 4H), 3.72 (d, J = 6.8 Hz, 2H), 3.43-3.49 (m, 8H), 3.18-3.29 (m, 4H), 2.83 (t, J =
13 7.6 Hz, 4H), 1.68 (s, 12H), 1.54-1.73 (m, 12H), 1.45 (s, 18H), 1.42 (s, 36H). HR-ESI MS
14 *m/e* calcd for $[M]^+$ 1323.8466, found 1323.8460.

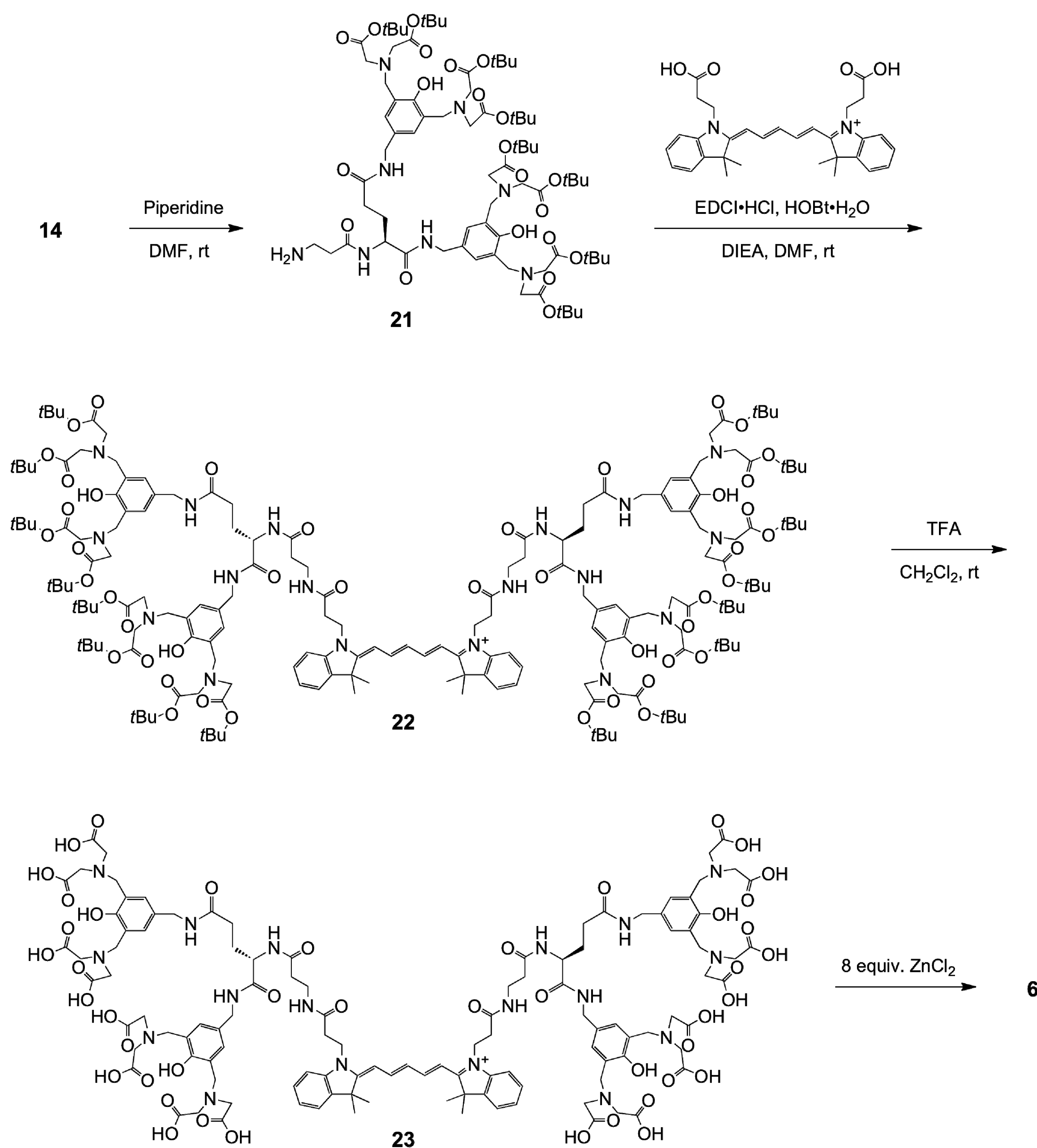
15

16 Compound **20**

17 A solution of **19** (42 mg, 31 µmol) in CH_2Cl_2 (1 mL) / TFA (1 mL) was stirred for 12 h at

1 rt. After removal of the solvent by evaporation, the residue was purified by RP-HPLC
2 (column: YMC-pack ODS-A, 250 x 25 mm, mobile phase; CH_3CN (containing 0.1% TFA) :
3 H_2O (containing 0.1% TFA) = 20 : 80 (0 min) → 50 : 50 (40 min), flow rate; 10 mL/min,
4 detection; UV (220 nm)) to give **20** (19 mg, 56%) as a deep blue powder. ^{1}H-NMR (400
5 MHz, CD_3OD) δ 8.24 (t, *J* = 12.8 Hz, 2H), 7.48 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H),
6 7.32 (d, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.6 Hz, 2H), 6.63 (t, *J* = 12.4 Hz, 1H), 6.35 (d, *J* = 13.6 Hz,
7 2H), 4.40 (t, *J* = 6.0 Hz, 4H), 3.62 (d, *J* = 8.8 Hz, 8H), 3.40 (t, *J* = 7.2 Hz, 2H), 3.10 (br s, 4H),
8 2.67 (t, *J* = 6.0 Hz, 4H), 1.72 (s, 12H), 1.28-1.39 (m, 12H). HR-ESI MS *m/e* calcd for $[M]^+$
9 987.4710, found 987.4734.
10
11 Compound **5**-2M(II)
12 Compound **20** was dissolved in DMSO. The concentration of **20** was determined based
13 on the UV absorbance at 647 nm. The solution was mixed with 2 equiv of $NiCl_2$ and $ZnCl_2$
14 (100 mM in H_2O) to give a stock solution of **5**-2Ni(II) and **5**-2Zn(II) (1.0 mM), respectively.
15 The stock solution was stored in a refrigerator (- 30 ˚C) and thawed before use.
16

Synthesis of 6



Compound **21**

A solution of **14** (88 mg, 52 μmol) in DMF (2 mL) / piperidine (0.2 mL) was stirred for 30 min at rt. After removal of the solvent by evaporation, the residue was dissolved in *n*-hexane. The precipitate was collected and dried in vacuo to yield **21** (45 mg, 59%) as a yellow amorphous powder. ^{1}H-NMR (400 MHz, CD_3OD) δ 9.56 (br s, 2H), 7.86 (s, 1H),

1 7.84 (s, 1H), 7.08 (s, 2H), 7.06 (s, 2H), 6.40 (br s, 1H), 4.18-4.52 (m, 5H), 3.91 (s, 4H), 3.89 (s,
2 4H), 3.41 (s, 8H), 3.40 (s, 8H), 2.91-3.03 (m, 2H), 2.30-2.41 (m, 4H), 2.07-2.26 (m, 2H), 1.46 (s,
3 72H). HR-ESI MS *m/e* calcd for $[M+H]^+$ 1457.8641, found 1457.8622.
4
5 Compound **23**
6 A solution of **21** (31 mg, 21 μmol), Cy5-bisCO_2H (5 mg, 9.3 μmol), EDCI•HCl (5 mg, 26
7 μmol), HOBt•H_2O (4 mg, 26 μmol) and DIEA (6 μL, 37 μmol) in dry DMF (2 mL) was stirred
8 for 22 h at rt. After removal of the solvent by evaporation, the residue was diluted with
9 AcOEt. The organic layer was washed with 2.5% NH_3aq and brine followed by drying over
10 Na_2SO_4, and concentrated by evaporation. The residue was purified by RP-HPLC (column:
11 YMC-pack ODS-A, 250 x 25 mm, mobile phase; CH_3CN (containing 0.1% TFA) : H_2O
12 (containing 0.1% TFA) = 50 : 50 (0 min) → 90 : 10 (40 min), flow rate; 10 mL/min, detection;
13 UV (220 nm)) to give **22** (20 mg) as a deep blue powder.
14 A solution of **22** (20 mg, 5.7 μmol) in CH_2Cl_2 (1 mL) / TFA (1 mL) was stirred for 6 h at
15 rt. After removal of the solvent in vacuo, the crude residue was purified by RP-HPLC
16 (column: YMC-pack ODS-A, 250 x 25 mm, mobile phase; CH_3CN (containing 0.1% TFA) :
17 H_2O (containing 0.1% TFA) = 20 : 80 (0 min) → 40 : 60 (40 min), flow rate; 10 mL/min,
18 detection; UV (220 nm)) to give **23** (5.1 mg, 21% in 2 steps) as a deep blue powder.
19 ^1H-NMR (400 MHz, CD_3OD) δ 8.22 (t, J = 13.2 Hz, 2H), 7.47 (d, J = 7.6 Hz, 2H), 7.38 (t, J =
20 7.6 Hz, 2H), 7.30 (d, J = 7.6 Hz, 2H), 7.25 (t, J = 7.6 Hz, 2H), 7.18 (s, 4H), 7.16 (s, 4H), 6.58 (t,
21 J = 12.0 Hz, 1H), 6.31 (d, J = 13.2 Hz, 2H), 4.31-4.34 (m, 10H), 4.20-4.25 (m, 20H), 3.79 (s,
22 32H), 3.41 (q, J = 3.6 Hz, 4H), 2.65 (t, J = 6.8 Hz, 4H), 2.42 (q, J = 4.4 Hz, 4H), 2.35 (t, J =
23 6.8 Hz, 4H), 2.12-2.17 (m, 2H), 1.92-1.98 (m, 2H), 1.69 (s, 12H). HR-MALDI-TOF MS *m/e*
24 calcd for $[M]^+$ 2479.9500, found 2479.9553.
25
26 Compound **6**

Electronic Supplementary Material (ESI) for Chemical Communications
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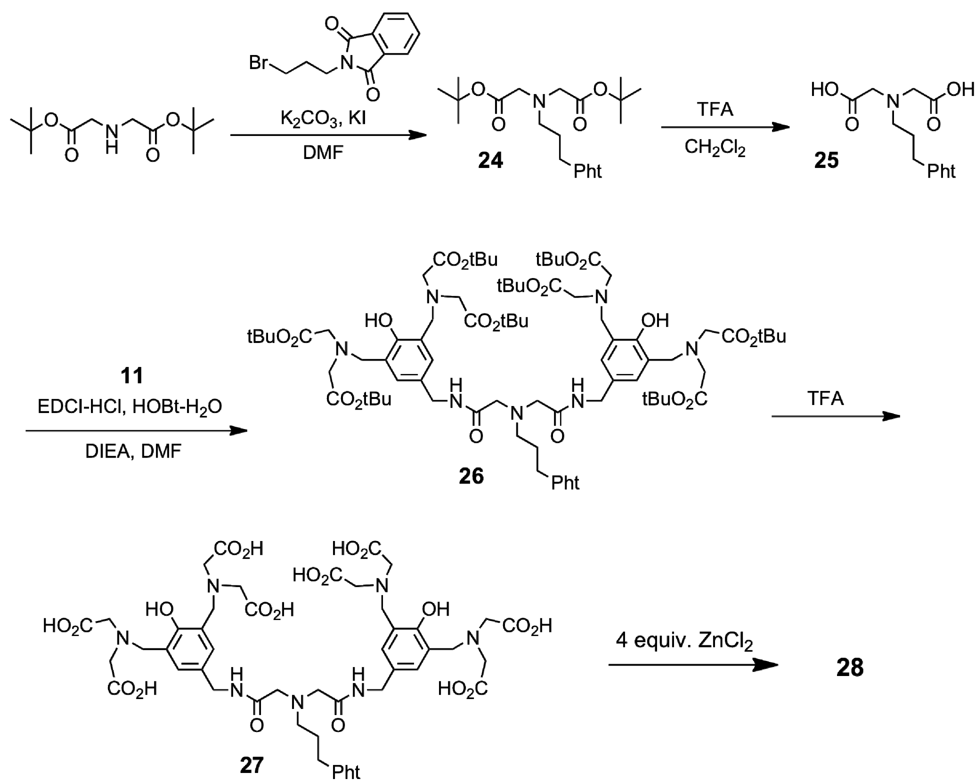
1 Compound **23** was dissolved in DMSO. The concentration of **23** was determined based
2 on the UV absorbance at 647 nm. The solution was mixed with 8 equiv of $ZnCl_2$ (100 mM in
3 H_2O) to give a stock solution of **6** (1.0 mM), which was stored in a refrigerator (- 30 ˚C) and
4 thawed before use.
5

1 **Synthesis of 28**



2

3 Compound **24**

4 A solution of di-*tert*-butyl iminodiacetate (587 mg, 2.39 mmol), 3-bromopropyl
5 phthalimide (706 mg, 2.63 mmol), potassium carbonate (363 mg, 2.63 mmol) and potassium
6 iodide (159 mg, 0.956 mmol) in dry DMF (10 mL) was stirred overnight at 50 ˚C. After
7 removal of the solvent by evaporation, the residue was diluted with $CHCl_3$. The organic layer
8 was washed with *sat*. $NaHCO_3$ and brine followed by drying over Na_2SO_4. After removal of
9 the solvent by evaporation, the residue was purified by flash column chromatography on SiO_2
10 (Hexane : AcOEt = 4 : 1) to give **24** (347 mg, 34%) as a yellow oil. ^{1}H-NMR (400 MHz,
11 $CDCl_3$) δ 7.83 (dd, J = 5.6, 3.2 Hz, 2H), 7.70 (dd, J = 5.6, 2.8 Hz, 2H), 3.77 (t, J = 7.2 Hz, 2H),
12 3.44 (s, 4H), 2.79 (t, J = 7.2 Hz, 2H), 1.85 (q, J = 7.2 Hz, 2H), 1.44 (s, 18H).

13

14 Compound **25**

1 A solution of **24** (347 mg, 0.802 mmol) in CH_2Cl_2 (2 mL) / TFA (2 mL) was stirred for 13
2 h at rt. After removal of the solvent by evaporation, the residue was dissolved in H_2O. The
3 aqueous solution was neutralized with 25% aqueous NH_3 and washed with AcOEt, then
4 acidified with 1 N aqueous HCl to precipitate the white solid. The solid was filtered and
5 dried in vacuo to give **25** (142 mg, 50%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.80-7.85 (m,
6 4H), 3.59 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 1.69 (q, J = 7.2 Hz, 2H).
7
8 Compound **26**
9 A solution of **25** (100 mg, 280 μmol), **11** (375 mg, 589 μmol), EDCI•HCl (152 mg, 793
10 μmol), HOBt•H_2O (121 mg, 793 μmol) and DIEA (189 μL, 1.12 mmol) in dry DMF (5 mL) was
11 stirred for 3 h at 50 ˚C. After removal of the solvent by evaporation, the residue was
12 dissolved in $CHCl_3$. The organic layer was washed with *sat*. $NaHCO_3$ and brine followed by
13 drying over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by
14 flash column chromatography on SiO_2 (Hexane : AcOEt = 1 : 1 → 1 : 2) to give **26** (250 mg,
15 57%) as a white amorphous powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 2H), 7.79
16 (dd, J = 5.6, 2.8 Hz, 2H), 7.69 (dd, J = 5.2, 3.2 Hz, 2H), 7.11 (t, J = 5.2 Hz, 2H), 7.07 (s, 4H),
17 4.32 (d, J = 5.6 Hz, 4H), 3.89 (s, 8H), 3.65 (t, J = 6.8 Hz, 2H), 3.39 (s, 16H), 3.28 (s, 4H), 2.73
18 (t, J = 6.8 Hz, 2H), 1.83 (q, J = 7.2 Hz, 2H), 1.45 (s, 72H).
19
20 Compound **27**
21 A solution of **26** (58 mg, 37 μmol) in TFA (3 mL) was stirred for 13 h at rt. After
22 removal of the solvent by evaporation, the residue was washed with di-isopropylether, then
23 filtered and dried in vacuo to give **28** (35 mg, 55%) as a pale-purple solid. ^{1}H-NMR (400
24 MHz, CD_3OD) δ 7.79-7.87 (m, 4H), 7.22 (s, 4H), 4.31 (s, 4H), 4.29 (s, 8H), 3.84 (s, 4H), 3.82 (s,
25 16H), 3.70 (t, J = 6.8 Hz, 2H), 3.16 (t, J = 7.6 Hz, 2H), 2.00 (q, J = 7.2 Hz, 2H).
26

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1 Compound **28**

2 A solution of **27** (1 mM, determined by ε(**27**)$_{299\ nm}$ = 5.00 x 10^3 $M^{-1}cm^{-1}$) in 50 mM

3 HEPES buffer (pH 7.2, 100 mM NaCl) was mixed with $ZnCl_2$ (4 equiv.) to prepare a stock

4 solution of **28** (1 mM) and evaluate the binding affinity for His10-peptide by ITC measurement

5 to afford 2.8 x 10^6 M^{-1} (data not shown).

6

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1 **Syntheses of Ac-WA(H)$_n$-NH$_2$ (n = 6 (His6) or 10 (His10)) peptide**

2 For the synthesis of His6 peptide, the solid-phase synthesis was carried out using Rink
3 Amide resin (Novabiochem). The coupling reactions (0.1 mmol scale) were performed with a
4 mixture of the corresponding carboxylic acid (3 eq.), HBTU (3 eq.), HOBt•H_2O (3 eq.), and
5 DIEA (6 eq.) in *N*-methylmorpholine (NMP). Fmoc deprotection was performed with 20%
6 piperidine in NMP. All coupling and deprotection steps were monitored by Kaiser test. The
7 cleavage from the resin was performed with TFA containing 2.5% TIS and 2.5% H_2O. The
8 crude peptide product was purified by RP-HPLC to give His6 peptide (9 mg, 8%) as
9 hygroscopic viscous oil. MALDI-TOF MS *m/e* calcd for $[M+H]^+$ 1139.51, found 1140.59.

10 His10 peptide was synthesized by the same procedure described above. The crude
11 peptide was purified by RP-HPLC to give His10 peptide (5 mg, 3%) as hygroscopic viscous oil.
12 MALDI-TOF MS *m/e* calcd for $[M+H]^+$ 1687.75, found 1687.31.

13

14

15

16

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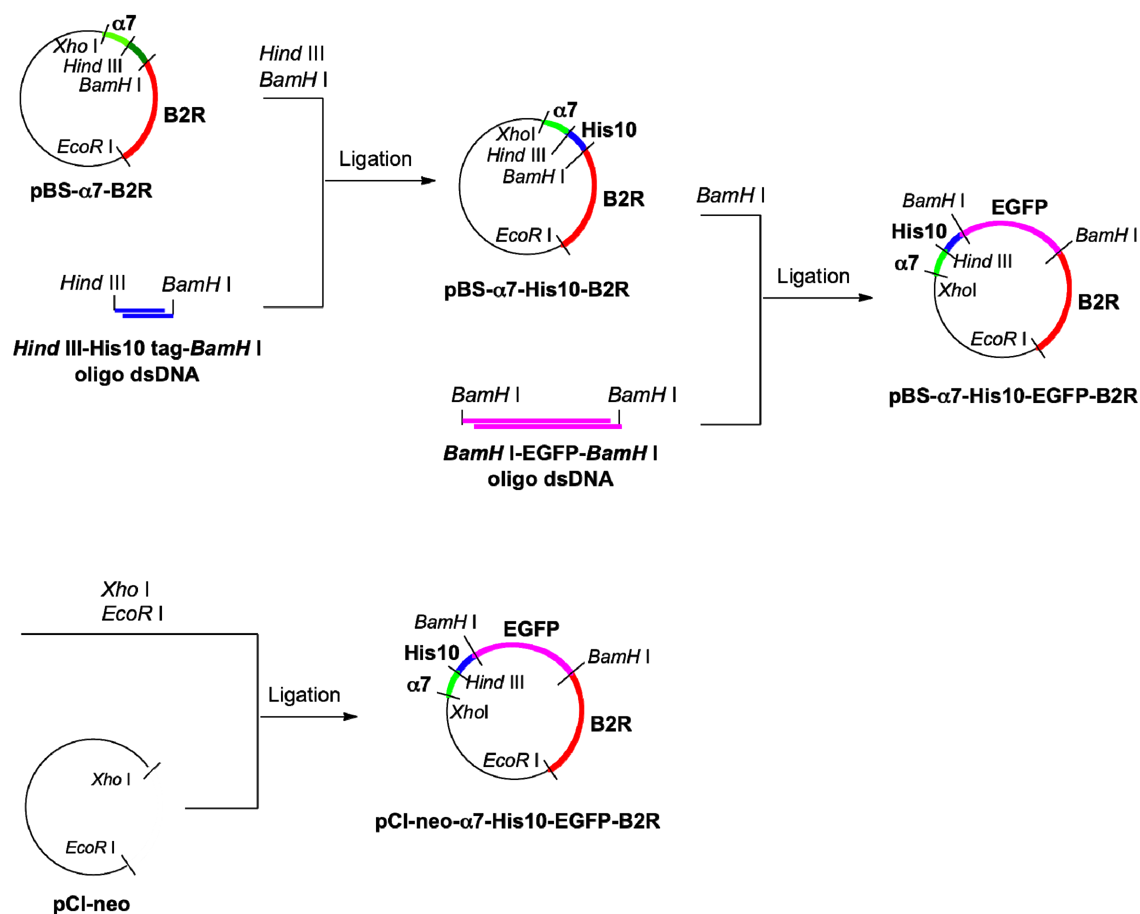
1 **General materials and methods for biochemical/biological experiments**

2 Unless otherwise noted, all proteins/enzymes and biochemical reagents were obtained from
3 commercial suppliers (Sigma, Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical
4 Industries, Pierce Biotechnology, or Calbiochem) and used without further purification.
5 UV-visible spectra and fluorescence spectra were recorded on a UV-2550 spectrophotometer
6 (Shimadzu) and LS55 (Perkin Elmer), respectively. SDS-polyacrylamide gel electrophoresis
7 (SDS-PAGE) was carried out using a Bio-Rad Mini-Protean III electrophoresis apparatus. Cell
8 imaging was performed using a confocal laser scanning microscope (CLSM, Olympus, FV1000,
9 IX81) equipped with a 60× objective lens. Fluorescence images were acquired using the 488 nm
10 line of an argon laser for excitation of EGFP (emission, 500-530 nm) and the 633 nm line of a
11 HeNe Red laser for excitation of Cy5 (emission, 645-745 nm).

12

Construction of GPCR expression plasmids

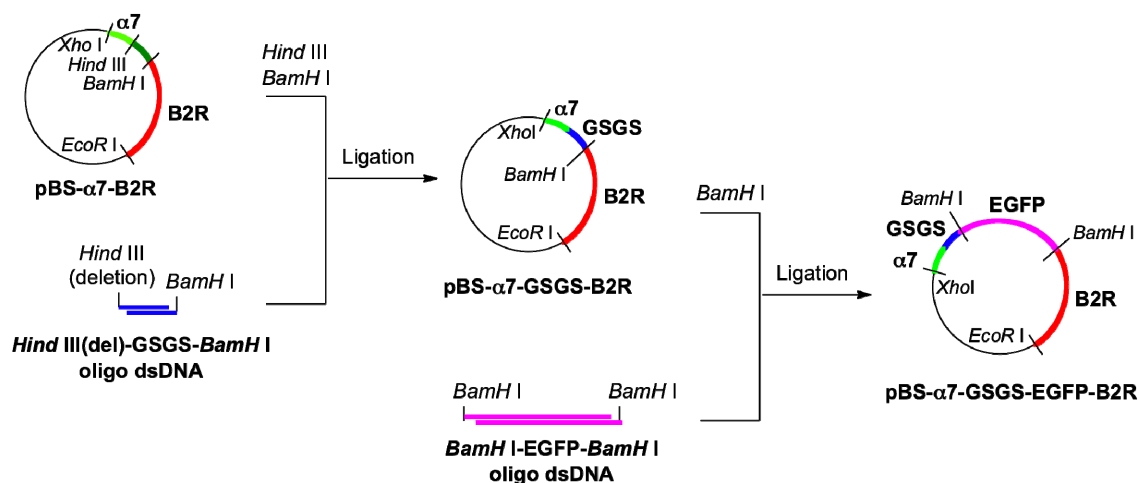
His10-EGFP-B2R plasmid

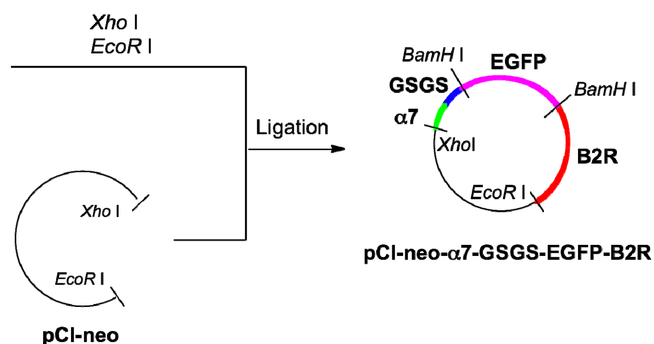


The oligo DNA fragments coding His10 tag were inserted into *Hind* III-*BamH* I site of pBluescript II SK(-) (pBS, Stratagene) coding *N*-terminal of α7 nicotinic acetylcholine receptor(α7)-B2R to yield pBS-α7-His10-B2R. The sequences of the 5'-phosphorylated DNA fragments were as follows: 5'-AGC TTG CAT CAC CAT CAC CAT CAC CAT CAC CAT CAC GCT AGG GGC TCT GGC TCG-3'(forward) and 5'-GAT CCG AGC CAG AGC CCC TAG CGT GAT GGT GAT GGT GAT GGT GAT GGT GAT GCA-3'(backward).

The dsDNA fragment coding *BamH* I-EGFP-*BamH* I was inserted into *BamH* I site of pBS-α7-His10-B2R to yield pBS-α7-His10-EGFP-B2R.

1 A *Xho* I-*EcoR* I fragment of pBS-α7-His10-EGFP-B2R was inserted into the *Xho* I-*EcoR*
2 I digestion site of pCI-neo plasmid (Promega). The plasmid purification using Qiagen
3 Plasmid Maxi kit (Qiagen) yielded pCI-neo-α7-His10-EGFP-B2R, which was used for protein
4 expression in mammalian cells.
5
6 **GSGS-EGFP-B2R plasmid**





7
8
9 An oligo DNA fragments coding a mock tag (GSGS) were inserted into *Hind* III-*BamH* I
10 site to yield pBS-α7-GSGS-B2R. The sequences of the 5'-phosphorylated DNA fragments
11 were as follows: 5'-AGC TCA GGC TCT GGC TCG-3'(forward) and 5'-GAT CCG AGC CAG
12 AGC CTG-3'(backward).

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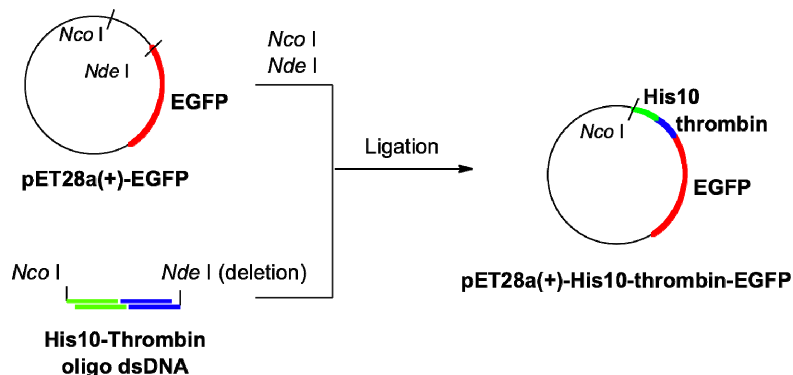
1 The dsDNA fragment coding *BamH* I-EGFP-*BamH* I was inserted into *BamH* I site of
2 pBS-α7-GSGS-B2R to yield pBS-α7-GSGS-EGFP-B2R. This plasmid was placed into
3 pCI-neo and purified by Qiagen Plasmid Maxi kit (Qiagen) to give
4 pCI-neo-α7-GSGS-EGFP-B2R.
5
6 **His10-EGFP-m1AchR plasmid**
7 The dsDNA fragment coding *BamH* I-m1AchR-*EcoR* I obtained from
8 pCI-neo-D4x3-m1AchR[S5] was inserted into *BamH* I-*EcoR* I site of pBS-α7-His10-B2R to yield
9 pBS-α7-His10-m1AchR.
10 The dsDNA fragment coding *BamH* I-EGFP-*BamH* I was inserted into *BamH* I site of
11 pBS-α7-His10-m1AchR to yield pBS-α7-His10-EGFP-m1AchR. This plasmid was placed
12 into pCI-neo and purified by by Qiagen Plasmid Maxi kit (Qiagen) to give
13 pCI-neo-α7-His10-EGFP-m1AchR.
14
15 **GSGS-EGFP-m1AchR plasmid**
16 The oligo DNA fragments coding a mock tag (GSGS) were inserted into *Hind* III-*BamH* I
17 site of pBS-α7-His10-m1AchR to yield pBS-α7-GSGS-m1AchR.
18 The dsDNA fragment coding *BamH* I-EGFP-*BamH* I was inserted into *BamH* I site to
19 yield pBS-α7-GSGS-EGFP-m1AchR. This plasmid was placed into pCI-neo and purified by
20 the same procedure to give pCI-neo-α7-GSGS-EGFP-m1AchR.
21

1 **Plasmid construction and protein expression of EGFP**

2

3 **Construction of His10-thrombin-EGFP plasmid**



4

5

6 The oligo DNA fragments coding His10-thrombin (-HHHHHHHHHHSSGLVPRGS-)
7 were inserted into *Nco* I-*Nde* I site of pET28a(+) vector (Novagen) subcloned with EGFP to
8 yield pET28a(+)-His10-thrombin-EGFP. The sequences of the 5'-phosphorylated DNA
9 fragments were as follows: 5'-CAT GGG CAG CAG CCA TCA TCA TCA TCA TCA TCA
10 TCA TCA TCA TAG CAG CGG CCT GGT GCC GCG CGG CAG CGG-3' (forward) and
11 5'-TAC CGC TGC CGC GCG GCA CCA GGC CGC TGC TAT GAT GAT GAT GAT GAT
12 GAT GAT GAT GAT GGC TGC TGC C-3' (backward).

13

14 **Expression of His10-EGFP and EGFP lacking His-tag**

15 pET28a(+)-His10-thrombin-EGFP vector was transformed into *E. coli* BL21(DE3) pLysS.
16 The cells were grown in 500 mL of LB medium at 37 °C until an optical density (OD) at 600
17 nm increased to 0.6, and further grown at 16 °C overnight with IPTG induction (0.3 mM).
18 The cells were spun down for 10 min at 3500 rpm, and re-suspended in 25 mL HEPES buffer
19 (50 mM, pH 7.2). The collected cells were re-suspended in 25 mL HEPES buffer and lysed
20 by sonication (10 shots x 20 times). Insoluble materials were removed by centrifugation
21 (12,000 rpm, 10 min x 2) to collect the soluble fraction containing the EGFP fused with

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1 His10-tag. The soluble fraction was passed through a plastic column filled with 2 mL of
2 TALON resin (1 mL, Clontech). After washing with the HEPES buffer, the resin-bound
3 protein was eluted with HEPES buffer (50 mM, pH 7.2) containing 150 mM imidazole. The
4 fractions containing His10-EGFP (confirmed by SDS-PAGE) was dialyzed twice against
5 HEPES buffer (50 mM, pH 7.2, 100 mM NaCl) to remove the excess imidazole to give
6 His10-EGFP solution (32 μM, 3 mL).

7 A solution of His10-EGFP in HEPES buffer (15 μM, 1 mL) was mixed with thrombin (5
8 U), and the mixture was incubated for 16 h at 22 °C. After removal of the thrombin by
9 incubation with benzamidine sepharose 6B, elute was dialyzed twice against HEPES buffer (50
10 mM, pH 7.2, 100 mM NaCl) to remove His10 peptide fragment to give EGFP lacking His-tag
11 (12 μM, 1 mL). The cleavage of the His-tag site by thrombin was confirmed by SDS-PAGE.
12

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1 **Isothermal titration calorimetry (ITC) measurement**

2 ITC titration was performed with Isothermal Titration Calorimeter (MicroCal Inc). All
3 measurements were conducted at 298 K. A solution of metal complex in a buffer solution (50
4 mM HEPES, 100 mM NaCl, pH 7.2) was injected stepwise (10 μL x 24 times) to a solution of
5 the His6 or His10 peptide in the same solvent system. The measured heat flow was recorded
6 as function of time and converted into enthalpies (ΔH) by integration of the appropriate reaction
7 peaks. Dilution effects were corrected by subtracting the result of a control experiment with
8 an injection of the metal complexes into the blank HEPES buffer under identical experimental
9 conditions. The binding parameters (K_{app}, ΔH, ΔS, n) were evaluated by applying one site
10 model using the software Origin (MicroCal Inc).
11

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1 # References

2 S1. The apparent binding constant obtained by the competitive titration was 2.8 x 10^7 M^{-1}.
3 The value was further analyzed based on the competitive binding equation described in our
4 previous work; A. Ojida, S. Fujishima, K. Honda, H. Nonaka, S. Uchinomiya and I.
5 Hamachi, *Chem. Asian J.*, 2010, **5**, 877-886.

6 S2. A. Johansson, M. Abrahamsson, A. Magnuson, P. Huang, J. Mårtensson, S. Styring, L.
7 Hammarström, L. Sun and B. Åkermark, *Inorg. Chem.,* 2003, **42**, 7502-7511.

8 S3. M. A. Brun, K.-T. Tan, E. Nakata, M. J. Hinner and K. Johnsson, *J. Am. Chem. Soc.,* 2009,
9 **131**, 5873-5884.

10 S4. W. M. Hussein, B. P. Ross, M. J. Landsberg, D. Lévy, B. Hankamer and R. P. McGeary, *J.*
11 *Org. Chem.,* 2009, **74**, 1473-1479.

12 S5. A. Ojida, K. Honda, D. Shinmi, S. Kiyonaka, Y. Mori and I. Hamachi, *J. Am. Chem. Soc.,*
13 2006, **128**, 10452-10459