Supporting Information

In vivo fluorescence imaging of β-amyloid plaques with push-pull dimethylaminothiophene derivatives

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Chemistry

General

All reagents were obtained commercially and used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained on a JEOL JNM-LM400 (JEOL Ltd., Tokyo, Japan) spectrometer with TMS as an internal standard. Coupling constants are reported in hertz. Multiplicity was defined by s (singlet), d (doublet), and m (multiplet). Mass spectra were obtained on a JEOL JMS-GC-mate mass spectrometer (JEOL Ltd.).

5-(Dimethylamino)thiophene-2-carbaldehyde (1)

5-Bromo-2-thiophenecarboxaldehyde (1.0 g, 5.23 mmol) was dissolved in H_2O (3.0 mL) with dimethylamine (1.64 mL, 15.69 mmol). The mixture was stirred under reflux for 6 h. After the mixture cooled to room temperature, it was extracted with chloroform. The organic phase was dried over Na_2SO_4 and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (ethylacetate : hexane = 1:1) to give 521 mg of **1** (64.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.08 (s, 6H), 5.92 (d, J = 4.6 Hz, 1H), 7.46 (d, J = 4.1 Hz, 1H), 9.49 (s, 1H).

2-((5-(Dimethylamino)thiophen-2-yl)methylene)malononitrile (2, DTM-0)

A solution of **1** (118 mg, 0.76 mmol), malononitrile (50 mg, 0.76 mmol) and pyridine (91 μL) in 2-propanol (5.0 mL) was stirred and refluxed for 4 h. The precipitate was collected by filtration and washed with 2-propanol to give 28 mg of **2** (18.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20 (s, 6H), 6.04 (d, J = 4.6 Hz, 1H), 7.30-7.42 (m, 2H). HRMS (EI): m/z calcd for $C_{10}H_9N_3S$ (M^+) 203.0517, found 203.0514.

(*E*)-3-(5-(Dimethylamino)thiophen-2-yl)acrylaldehyde (3)

The solution of **1** (360 mg, 2.32 mmol), 1,3-dioxan-2-yl- methyl)triphenyl-phosphoniumbromide (1919 mg, 4.47 mmol) and 18-crown-6 (5 mg) in THF (10 mL), NaH (230 mg, 9.6 mmol) was added in one portion. After 12 h stirring at room temperature, a few drops of 1 M HCl were added. The mixture was alkalized with saturated $NaHCO_3$ and extracted with ethylacetate. The organic phase was dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (ethylacetate : hexane = 1 : 1) to give 315 mg of **3** (74.9 %). ^{1}H NMR

(400 MHz, $CDCl_3$) δ3.06 (s, 6H), 5.86 (d, J = 4.0 Hz, 1H), 6.47 (dd, J = 8.0, 15.0 Hz, 1H), 7.11 (d, J = 4.0 Hz, 1H), 7.40 (d, J = 15.2 Hz, 1H), 9.45 (d, J = 8.0 Hz, 1H).

2-((*E*)-3-(5-(Dimethylamino)thiophen-2-yl)allylidene)malononitrile (4, DTM-1)

The same reaction as described above to prepare **2** was used, and 111 mg of **4** was obtained from **3** in a yield of 66.2 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ3.15 (s, 6H), 5.96 (d, J = 4.6 Hz, 1H), 6.08 (dd, J = 12.4, 14.0 Hz, 1H), 7.18-7.21 (m, 2H), 7.33 (d, J = 11.9 Hz, 1H). HRMS (EI): m/z calcd for $C_{12}H_{11}N_3S$ (M^+) 229.0674, found 229.0670.

(2*E*,4*E*)-5-(5-(Dimethylamino)thiophen-2-yl)penta-2,4-dienal (5)

The same reaction as described above to prepare **3** was used, and 335 mg of **5** was obtained from **3** in a yield of 92.9 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ3.02 (s, 6H), 5.78 (d, J = 4.0 Hz, 1H), 6.08 (dd, J = 8.4, 14.6 Hz, 1H), 6.36 (dd, J = 10.8, 15.0 Hz, 1H), 6.92 (d, J = 4.0 Hz, 1H), 7.01 (d, J = 14.8 Hz, 1H), 7.16 (dd, J = 11.2, 15.0 Hz, 1H), 9.49 (d, J = 8.4 Hz, 1H).

2-((2*E*,4*E*)-5-(5-(Dimethylamino)thiophen-2-yl)penta-2,4-dienylidene)malononitrile (6, DTM-2)

The same reaction as described above to prepare **2** was used, and 62 mg of **6** was obtained from **5** in a yield of 60.6 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ3.09 (s, 6H), 5.87 (d, J = 4.4 Hz, 1H), 6.32 (dd, J = 11.6, 14.6 Hz, 1H), 6.57 (dd, J = 12.4, 13.7 Hz, 1H), 6.94-7.05 (m, 3H), 7.35 (d, J = 12.0 Hz, 1H). HRMS (EI): m/z calcd for $C_{14}H_{13}N_3S$ (M^+) 255.0830, found 255.0835.

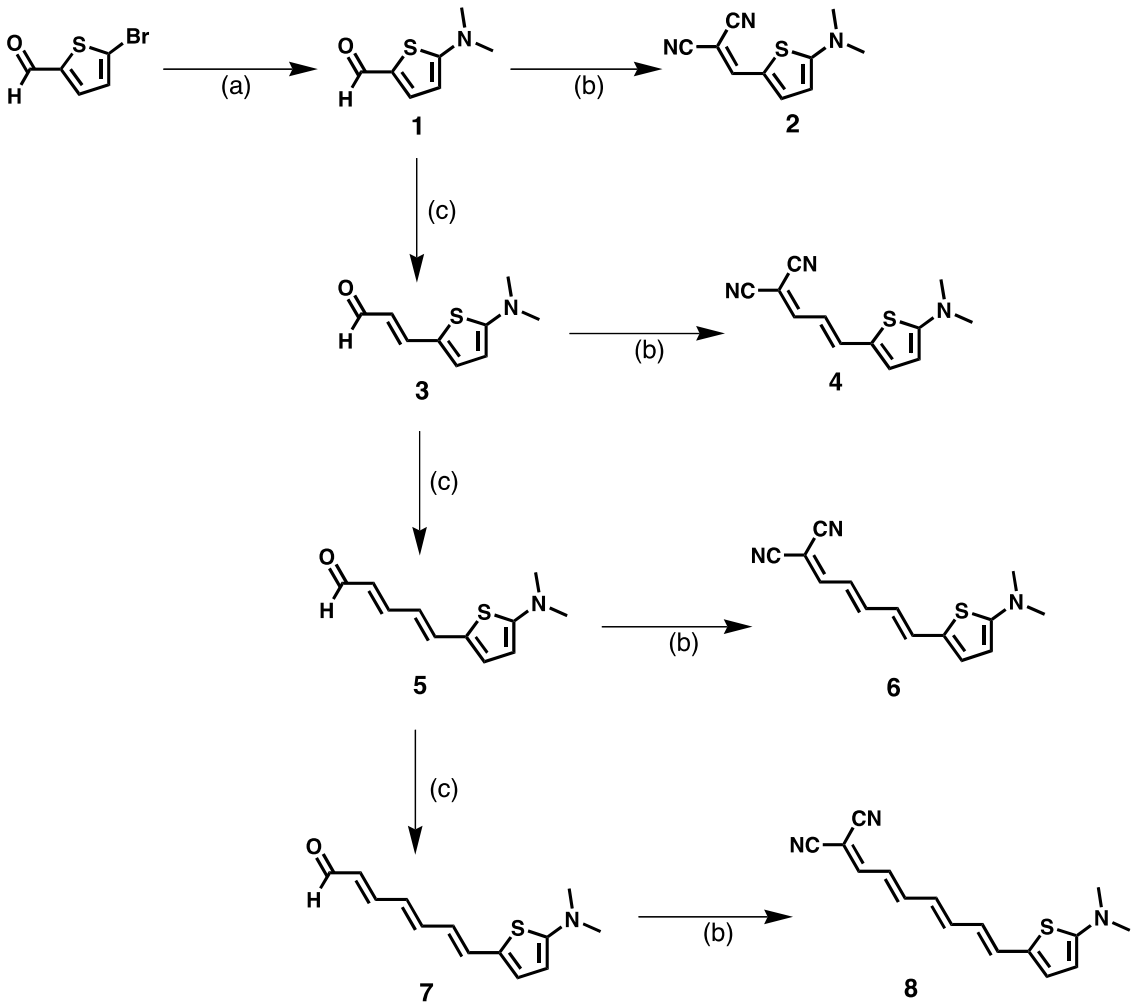
(2*E*,4*E*,6*E*)-7-(5-(Dimethylamino)thiophen-2-yl)hepta-2,4,6-trienal (7)

The same reaction as described above to prepare **3** was used, and 93 mg of **7** was obtained from **5** in a yield of 24.7 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ3.01 (s, 6H), 5.76 (d, J = 3.7 Hz, 1H), 6.09 (dd, J = 8.2, 15.1 Hz, 1H), 6.30 (dd, J = 11.0, 14.9 Hz, 1H), 6.38 (dd, J = 11.5, 14.7 Hz, 1H), 6.71-6.82 (m, 3H), 7.15 (dd, J = 11.5, 15.1 Hz, 1H), 9.52 (d, J = 7.8 Hz, 1H).

2-((2*E*,4*E*,6*E*)-7-(5-(Dimethylamino)thiophen-2-yl)hepta-2,4,6-trienylidene)malononitrile (8, DTM-3)

The same reaction as described above to prepare **2** was used, and 66 mg of **8** was obtained from **7** in a yield of 53.5 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 6H), 5.81 (d, J = 4.1 Hz, 1H), 6.30-6.37 (m, 1H), 6.60 (dd, J = 11.9, 14.0 Hz, 1H), 6.80 (dd, J = 11.5, 14.2 Hz, 1H), 6.87 (d, J = 15.1 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.96 (dd, J = 11.9, 14.2 Hz, 1H), 7.37 (d, J = 11.9 Hz, 1H). HRMS (EI): m/z calcd for $C_{16}H_{15}N_3S$ (M^+) 281.0987, found 281.0990.

Scheme S1. Synthetic route of DTM derivatives



Reagents: (a) dimethylamine, H_2O; (b) malononitrile, pyridine, 2-propanol; (c) (1,3-dioxan-2-yl-methyl)triphenyl-phosphoniumbromide, 18-crown-6, NaH, THF.

Fluorescence Characterization

Absorption wavelength, fluorescence excitation and emission wavelength, and quantum yields were determined with 10 μM of the compounds in $CHCl_3$ (UV-1800, SHIMADZU Corp, Kyoto, Japan or Fluorolog-3, HORIBA Jobin Yvon Inc., Kyoto, Japan).

Measurement of the Constant for Binding of Aβ Aggregates

A solid form of Aβ(1-42) was purchased from Peptide Institute (Osaka, Japan). Aggregation was carried out by gently dissolving the peptide (0.25 mg/mL) in phosphate buffered saline (PBS) (pH 7.4). The solution was incubated at 37 ˚C for 42 h with gentle and constant shaking. A mixture (100 μL of 10% EtOH) containing DTM-0, DTM-1, DTM-2, and DTM-3 (final concentration 0–3.75 μM) and Aβ(1-42) aggregates (final concentration 2.2 μM) was incubated at room temperature for 30 min. Fluorescence intensity at 600 nm for DTM-1, 692 nm for DTM-2, and 834 nm for DTM-3 was recorded (excitation: 572, 656, and 694 nm, respectively, Infinite M200PRO, TECAN, Männedorf, Switzerland). The K_d binding curve was generated by Prism 4.0 software (GraphPad Software, San Diego, CA).

In Vitro Fluorescent Staining of Mouse Brain Sections

The experiments with animals were conducted in accordance with our institutional guidelines and approved by the Kyoto University Animal Care Committee. A PS2APP transgenic mouse (female, 9 months old) was used as the AD model. After the animal was sacrificed by decapitation, the brain was removed and sliced into serial sections (10 μm thick). Each slide was incubated with a 50% EtOH solution (100 μM) of DTM-1, DTM-2, and DTM-3 for 10 min. The sections were washed in 50% EtOH for 1 min two times and examined using a microscope (BIOREVO BZ-9000, Keyence Corp., Osaka, Japan) equipped with a Tex Red for DTM-1, a Cy5 for DTM-2, and a Cy7 for DTM-3 filter set. Thereafter, the serial sections were also stained with thioflavin S, a pathologic dye commonly used for staining Aβ plaques in the brain, and examined using a microscope equipped with a GFP filter set.

In vivo and Ex Vivo Imaging of PS2APP and Wild-type Mouse Brain

PS2APP mice (Female, 21-22 months old) and wild-type mice (22-25 months old) were shaved before background imaging and intravenously injected with 100 μL of DTM-2 (400 μM, 20% DMSO and 80% propylene glycol). Fluorescence images of the brain were acquired with an IVIS Spectrum imaging system (Caliper Life Sciences Inc., Hopkinton, MA) with a 0.1-second exposure (f-stop = 2) and a customized filter set (excitation, 640 nm; emission, 700 nm). After 1 h, the mice were sacrificed and the brain was removed. Thereafter, fluorescence images of the brain were acquired with an IVIS Spectrum imaging system. Then, the brain was frozen in powdered dry ice. The frozen blocks were sliced into serial sections (20 μm thick) and examined using a microscope (BIOREVO BZ-9000) equipped with a Cy5 filter set. After being immersed in 50% EtOH for 15 minutes, the same sections were stained with thioflavin S and observed with a GFP filter set.

Spectra Analysis in Mouse Brain Section

The sections were examined using a microscope (Eclipse 80i, Nikon, Tokyo, Japan) equipped with a Cy5 filter set. Fluorescence spectra were recorded with a multispectral imaging system (Nuance FX, Caliper Life Sciences Inc, Hopkinton, MA) and analyzed with the Nuance software.

Fluorescence Measurement Using Aβ(1-42) and Bovine Serum Albumin

A mixture (10% EtOH) containing DTA derivatives (10 μM) and Aβ(1-42) aggregates (0, 5, and 10 μM) or bovine serum albumin (BSA) (45 mg/mL) was incubated at room temperature for 30 min. After incubation, fluorescence emission spectra of DTM-0, DTM-1, DTM-2, and DTM-3 were collected with excitation at 430, 525, 600, and 650 nm (Infinite M200PRO, TECAN).

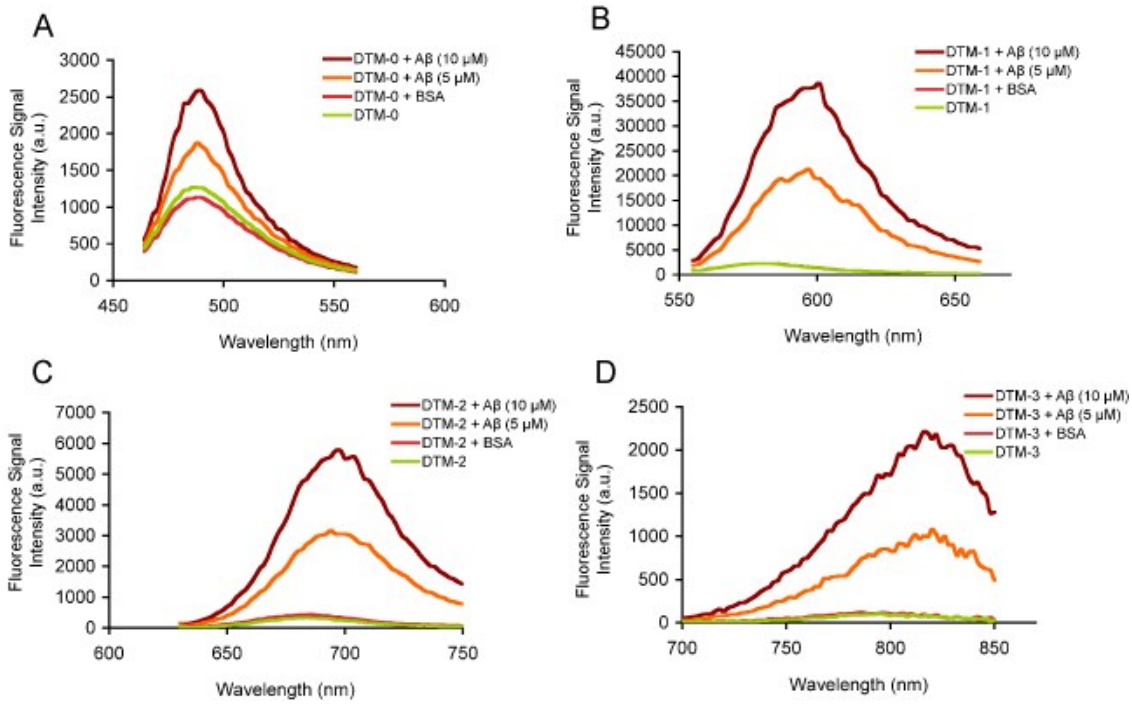


Figure S1. The fluorescence intensity of the DTM derivatives on interaction with Aβ(1–42) aggregates and BSA. A, B, C, and D show the fluorescent spectra of DTM-0, DTM-1, DTM-2, and DTM-3, respectively.

Effect of pH on the fluorescence properties

Fluorescence emission spectra of DTM-1, DTM-2, and DTM-3 (100 μM in 30% EtOH) were collected at a different pH (pH = 3, 7.4, and 12, Infinite M200PRO, TECAN).

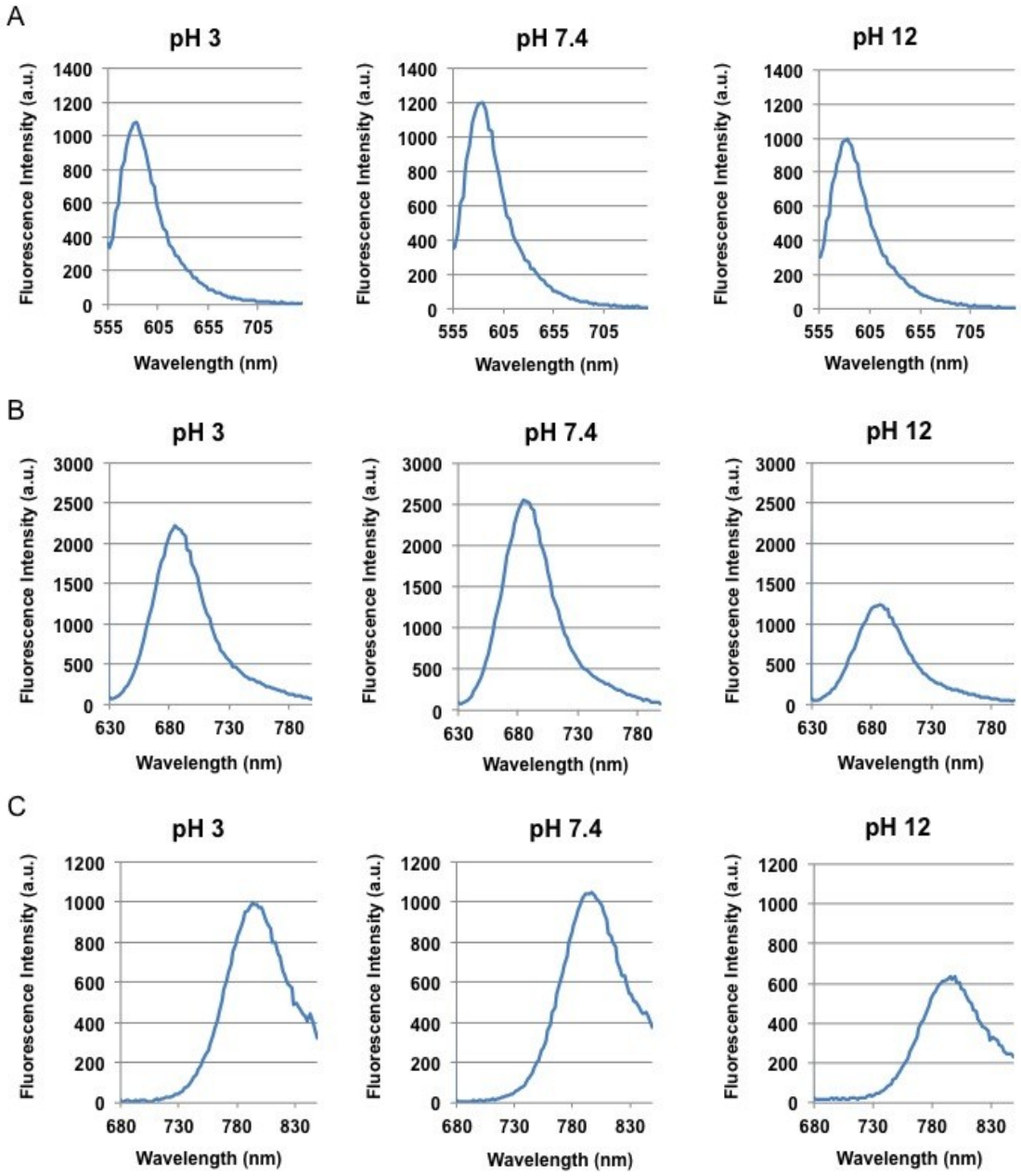


Figure S2. Effect of pH on the fluorescence properties of DTM-1 (A), DTM-2 (B), and DTM-3 (C) in the 30% EtOH solution.

Effect of metal ions on the fluorescence properties

Fluorescence emission spectra of DTM-1, DTM-2, and DTM-3 (10 μM in 20% EtOH) were collected in the presence of a metal (100 nM of $ZnCl_2$, $CuCl_2$, and $FeCl_3 \cdot 6H_2O$, Infinite M200PRO, TECAN).

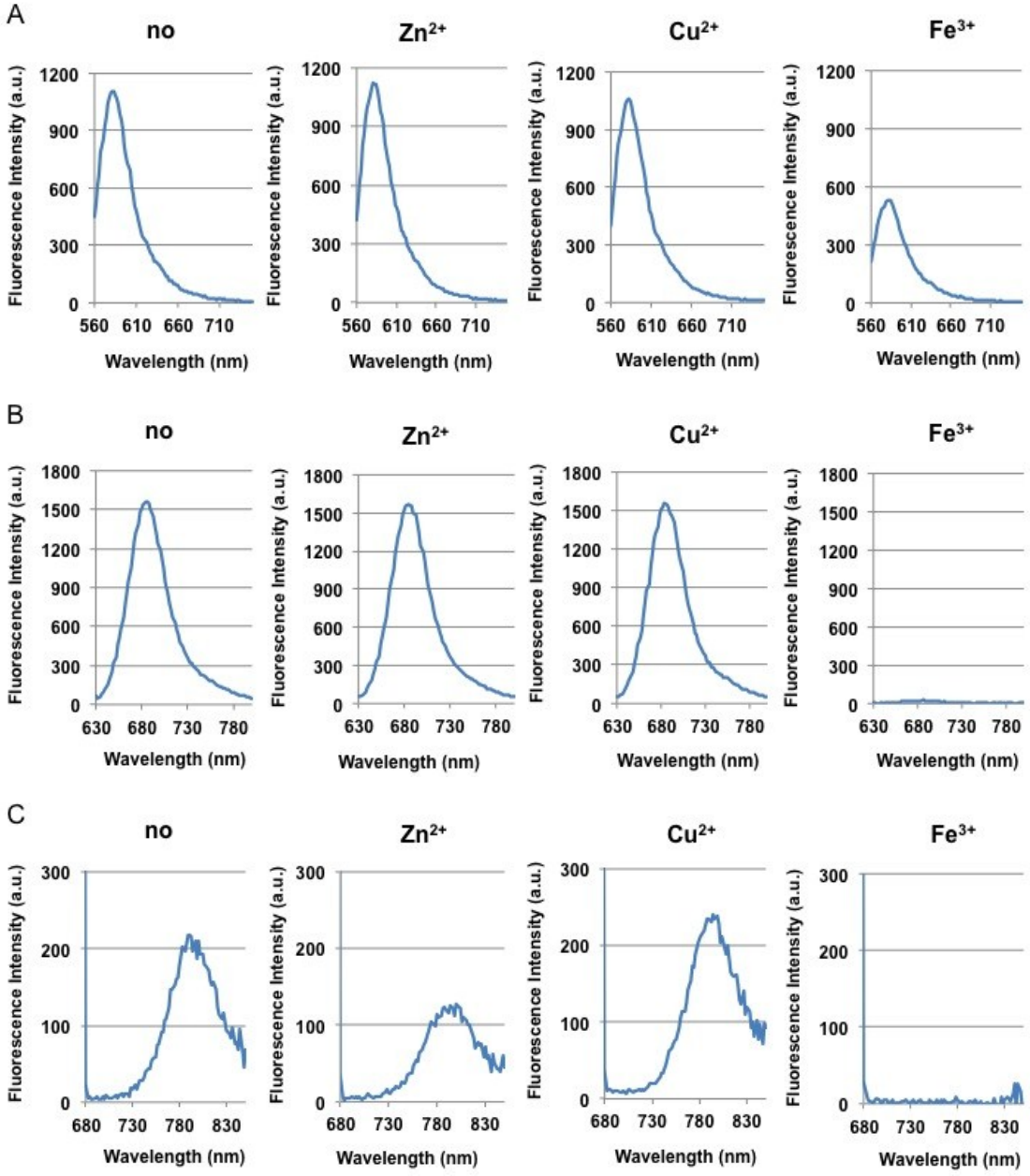


Figure S3. Fluorescence properties of DTM-1 (A), DTM-2 (B), and DTM-3 (C) in the 20% EtOH solution of in the presence of a metal.

Ex Vivo Imaging of Brains from Normal Mice

A mixed solution consisting of 20% DMSO and 80% propylene glycol (100 μL) of DTM-2 and DTM-3 (400 μM) was injected intravenously directly into the tail of ddY mice (5 weeks old). The mice (n = 4-5 for each time point) were sacrificed at 2, 10, 30, and 60 minutes postinjection. The brain was removed and fluorescence images of brains were acquired with an IVIS Spectrum imaging system with a 0.1-second exposure (f-stop = 2) and a customized filter set (excitation, 640 nm; emission, 700 nm). The fluorescence intensity in each region of interest covering an entire tissue was expressed as p/s/cm2/sr after the subtraction of background signals obtained in a region of interest set over an area without any tissue.

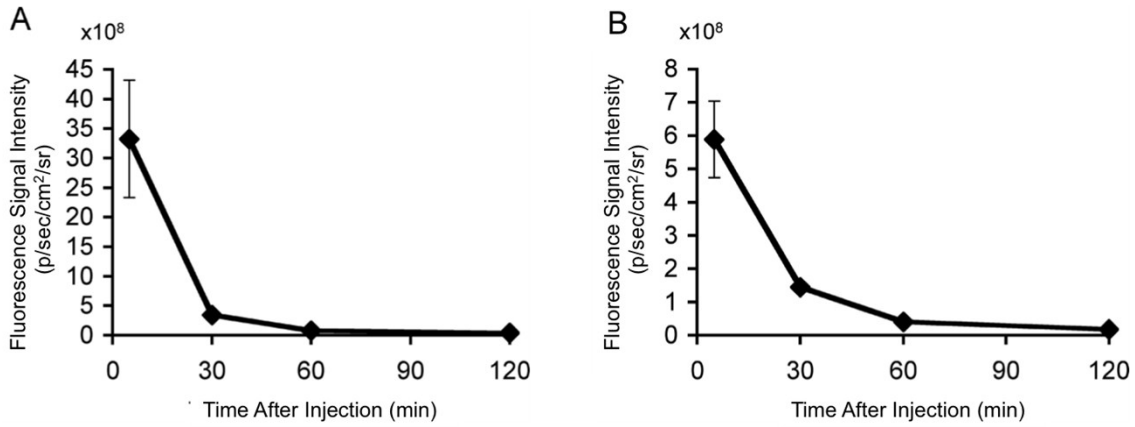


Figure S4. The fluorescence intensity in the brain after injection of DTM-2 and DTM-3 in ddY mice.