Electronic Supplementary Information

Design Strategy for Small Molecule-based Targeted MRI Contrast Agents: Application for Detection of Atherosclerotic Plaques

Shimpei Iwaki,[a] Kazuya Hokamura,[e] Mikako Ogawa,[f] Yasuo Takehara,[g] Yasuaki Muramatsu,[a] Takehiro Yamane,[a] Kazuhisa Hirabayashi,[a] Yuji Morimoto,[h] Kohsuke Hagisawa,[i] Kazuhide Nakahara,[j] Tomoko Mineno,[j] Takuya Terai,[a] Toru Komatsu,[a,k] Tasuku Ueno,[a] Keita Tamura,[b] Yusuke Adachi,[b] Yasunobu Hirata,[c] Makoto Arita,[a] Hiroyuki Arai,[a] Kazuo Umemura,[e] Tetsuo Nagano,[d] and Kenjiro Hanaoka[*a],

[a]*Graduate School of Pharmaceutical Sciences,* [b]*Department of Physiology, Graduate School of Medicine,* [c]*Department of Cardiovascular Medicine, Graduate School of Medicine and* [d]*Open Innovation Center for Drug Discovery, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.*
E-mail: khanaoka@mol.f.u-tokyo.ac.jp; Fax: +81 3 5841 4855 ;Tel: +81 3 5841 4852
[e]*Department of Pharmacology,* [f]*Photon Medical Research Center and* [g]*Department of Radiology Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, Shizuoka 431-3192, Japan.*
[h]*Department of Integrative Physiology and Bio-Nano Medicine and* [i]*Department of Physiology, National Defense Medical College, Namiki 3-2, Tokorozawa, Saitama 359-8513, Japan.*
[j]*Faculty of Pharmacy, Takasaki University of Health and Welfare, 60* Nakaorui, Takasaki-shi, Gunma 370-0033, Japan.
[k]*JST, PRESTO, 5 Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan*

*Correspondence should be addressed to K.H. (email): khanaoka@mol.f.u-tokyo.ac.jp; (tel) +81-3-5841-4852; (fax) +81-3-5841-4855

Supplementary methods

General procedures and materials

All reagents and solvents were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan), Dojindo Laboratories (Kumamoto, Japan), Invitrogen (Carlsbad, CA), Macrocyclics (Dallas, Texas, USA) or Aldrich Chemical Co. (St. Louis, MO) and were used without further purification. Silica gel column chromatography was performed with Chromatorex-NH (Fuji Silysia Chemical, Kasugai, Japan) or Silica Gel 60 (Spherical) (Kanto Chemical Co., Tokyo, Japan).

Instruments

^{1}H and ^{13}C NMR spectra were recorded by using a JEOL JNM-LA300 or JNM-LA400 spectrometer. Mass spectra (ESI) were measured by using a JEOL JMS-T100LC AccuTOF spectrometer. UV-visible spectra were obtained on a Shimadzu UV-1650 spectrophotometer. Fluorescence spectroscopic studies were performed on a Hitachi F-4500 spectrofluorometer. Longitudinal water proton relaxation time (T_1) was measured by using an NMR instrument operating at 20 MHz, 0.47 T (Minispec mq20, Bruker, Germany). HPLC analyses were performed on an Inertsil ODS-3 column (GL Sciences Inc.; 4.6 mm × 250 mm) using an HPLC system composed of a pump (PU-2080, JASCO) and a detector (MD-2015 or FP-2025, JASCO). HPLC purifications were performed on an Inertsil ODS-3 column (GL Sciences Inc.; 30 × 250 mm) using an HPLC system composed of a pump (PU-1587, JASCO) and a detector (UV-1570, JASCO).

Animals

ApoE-knock out (*ApoE*$^{-/-}$) mice were kindly supplied by the Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo. C57BL/6JJcl mice (male, 8-week-old) were purchased from CLEA, Japan.

Relaxivity

The longitudinal water proton relaxation time (T_1) of aqueous solutions of Gd^{3+} complexes was measured in phosphate-buffered saline (PBS; Dulbecco's phosphate-buffered saline, pH 7.4) at 20 MHz, 0.47 T (37°C) (Minispec mq20, Bruker). The values of T_1 were calculated from 30 points generated by using the standard inversion-recovery procedure. The r_1 relaxivities ($mM^{-1}s^{-1}$) of Gd^{3+} complexes were determined from the slope of the plot of $1/T_1$ versus the concentration of Gd^{3+} complex (0.50, 0.20, 0.10, 0.05 and 0 mM).

Fluorometric analysis

The slit width was 2.5 nm for both excitation and emission. The photon multiplier voltage was 700 V. For determination of the relative quantum efficiency of fluorescence (Φ_{fl}), a solution of fluorescein in 0.1 M NaOH aq. ($\Phi_{fl} = 0.85$) was used as a standard. Values were calculated according to the following equation.

$\Phi_x/\Phi_{st} = [A_{st}/A_x][n_x^2/n_{st}^2][D_x/D_{st}]$

st: standard

x: sample

A: absorbance at the excitation wavelength

n: refractive index

D: area under the fluorescence spectra on an energy scale

Dialysis assay of MRI contrast agents

200 μL of 50 μM probe solution in PBS (pH 7.4) was dialyzed (dialysis membrane; Spectra/Por® Dialysis Membrane, MWCO: 25,000) for 24 hr in PBS. The sample solution was collected and 100 μL of the sample was added to 2.4 mL of H_2O containing H_2O_2 and HNO_3 (final concentration; H_2O_2, 4.8% w/w; HNO_3, 8 M). Then, the sample was thoroughly decomposed by microwave irradiation. The concentration of Gd^{3+} was measured by ICP-AES (iCAP DUO-6300, Thermo).

Cell culture

Preadipocytes (Takara Bio Inc.) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), ascorbic acid (100 μM), $_D$-biotin (33 μM), $_D$-pantothenic acid (17 μM), triiodothyronine (50 nM) and octanoic acid (1 μM) in a humidified incubator under an atmosphere of 5% CO_2 in air. For differentiation to adipose cells, confluent cells were treated for 24 hr with a hormone cocktail containing dexamethasone (2.5 μM), 3-isobutyl-1-methylxanthine (0.5 mM) and insulin (10 μg/mL) in DMEM supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), ascorbic acid (100 μM), $_D$-biotin (33 μM), $_D$-pantothenic acid (17 μM), triiodothyronine (50 nM) and octanoic acid (1 μM). The cocktail was then removed, and the cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), ascorbic acid (100 μM), $_D$-biotin (33 μM), $_D$-pantothenic acid (17 μM), triiodothyronine (50 nM), octanoic acid (1 μM) and insulin (10 μg/mL).

Fluorescence microscopic imaging of adipocytes

Preadipocytes were plated onto 35-mm poly-(*l*)-lysine-coated glass-bottomed dishes (MatTek

Corporation) and differentiated to adipocytes. Adipocytes were cultured in DMEM supplemented with insulin (10 μg/mL) for 2 days. A solution of the probe in DMEM containing 0.1% DMSO as a cosolvent was added to give 10 μM final concentration and incubation was continued for 20 min. The stained cells were washed twice with PBS, and confocal fluorescence images were captured in PBS. We used a confocal imaging system (TCS-SP5, Leica) equipped with a white-light laser, Leica Application Suite Advanced Fluorescence (LAS-AF) and a 63x objective lens (HSXPLAPO 63x/1.40-0.60 oil CS, Leica). The excitation wavelength and fluorescence emission wavelengths were 495 nm and 505-540 nm, respectively.

***In vivo* fluorescence imaging of LDLR$^{-/-}$ mice**

Animal experiments were performed according to the protocol approved by the Ethics Committee for Laboratory Animals of the National Defense Medical College. Male homozygous low-density-lipoprotein receptor-negative mice (LDLR$^{-/-}$ mice) (35 g) that had been fed 1.25% cholesterol diet for > 3 months were used. **2BDP3Gd** was intravenously administered via the tail vein (7.4 μmol/kg) 24-72 hr prior imaging. Mice were anaesthetized with ketamine (50 mg/kg intraperitoneally) and xylazine (10 mg/kg intraperitoneally) and the right carotid artery was exposed after skin incision. Animals were placed into the light-tight chamber of the CCD camera cooled to −90°C (IVIS Lumina XR, PerkinElmer). Fluorescence images were obtained using one filter set (excitation: 480 nm, emission: 520 nm). Acquisition parameters (illumination intensity, binning, sensitivity and exposure time) were kept constant so that fluorescence intensities (dependent on incubation time) could be compared.

Ultrathin endoscopic fluorescence imaging

Intraluminal imaging of the abdominal aorta of mice was conducted with an ultrathin endoscope fluorescence imaging system composed of an ultrathin endoscope, an imaging unit and a light source (FiberTech, Tokyo, Japan). The image fiber bundle of the ultrathin endoscope consists of 15,000 microfibers and the tip of the fiber bundle is coupled with two microlenses having a diameter of 0.35 mm each (http://www.fibertech.jp/eng/m4.html), resulting in a viewing angle of 65°. As the depth of focus is 7.5 mm, spatial resolution is < 50 μm even though the outer diameter of the endoscope is 0.8 mm. The imaging unit consisted of a 3CCD camera (HV-D30, Hitachi Kokusai Electric, Japan) and a filter wheel for attaching fluorescence transmission (band-pass) filters. The light source consisted of a 180 W xenon lamp and a filter wheel for attaching fluorescence excitation filters. For the observation of **2BDP3Gd**, 520/35 nm (em.) and 472/30 nm (ex.) filters was used. Imaging could be easily and promptly switched by means of a foot-operated switch, so that the two types of image (bright field ↔ green fluorescence) could be monitored seamlessly.

Anesthetized mice were placed on a heating pad. After skin incision, the abdominal aorta was exposed

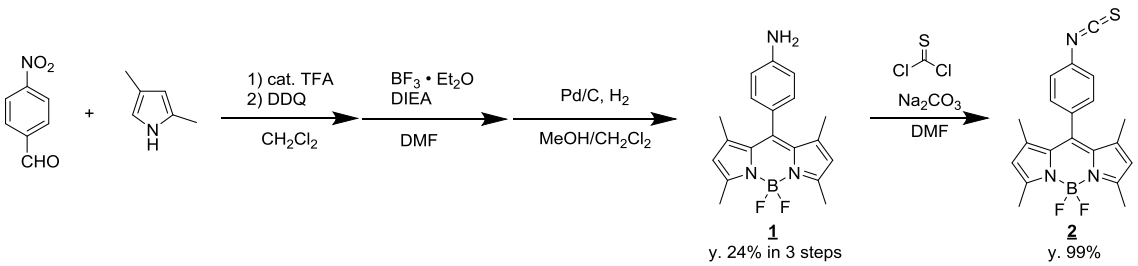
and an 18G catheter was inserted. A Y-shaped connector (AP-YC15S, Terumo) was joined to the catheter. The ultrathin endoscope was introduced into the catheter via the main line of the Y-shaped connector. Image acquisition was performed by flushing blood with heparinized saline (100 u/mL) at a speed of 1.0-1.5 mL/min (total 1 mL injected) via the bypass line of the Y-shaped connector. (Supplementary video 1)

Fluorescence imaging and Oil red O staining of macrophages

Macrophages were harvested from the intraperitoneal cavity of JCL:ICR mice. The mice were sacrificed by cervical dislocation, and 5 mL of PBS was injected intraperitoneally. Peritoneal fluid enriched with macrophages was collected and centrifuged at 1000 rpm for 5 min. Then, the supernatant was removed and the pellet was washed with PBS. The macrophages were resuspended in PBS, plated onto 35-mm poly-(*l*)-lysine-coated glass-bottomed dishes (MatTek Corporation) and incubated for 2 hr. After the removal of PBS, the cells were washed with PBS three times, then DMEM was added. For the "AcLDL (+)" sample, 200 μg/mL of acetylated LDL (Biomedical Technologies Inc.) was added, and then incubation was continued for 24 hr. After removal of the medium, **2BDP3Gd** in DMEM (10 μM; containing 0.1% DMSO as a cosolvent) was added and incubation was continued for 2 hr. The stained cells were washed with PBS three times, and the fluorescence images were obtained in PBS. After fluorescence imaging, the medium was removed, and the cells were fixed with 4% formaldehyde in PBS. The cells were washed with PBS three times, and then saturated Oil red O in 60% isopropanol/water was added. The cells were incubated for 15 min and then washed with PBS three times, and microscopic images (color) were obtained in PBS. The imaging system consisted of an inverted microscope (IX 71, Olympus) and cooled CCD color camera (C7780-20-YT; Hamamatsu Photonics K.K.). Bright field images were captured using MetaMorph 7.7 software (Universal Imaging, Media, PA) with a 60x objective lens (UPlanFLN 60x/1.25 oil, Olympus). The excitation wavelength and fluorescence emission wavelength were 470-490 nm and 510-550 nm, respectively.

Synthesis

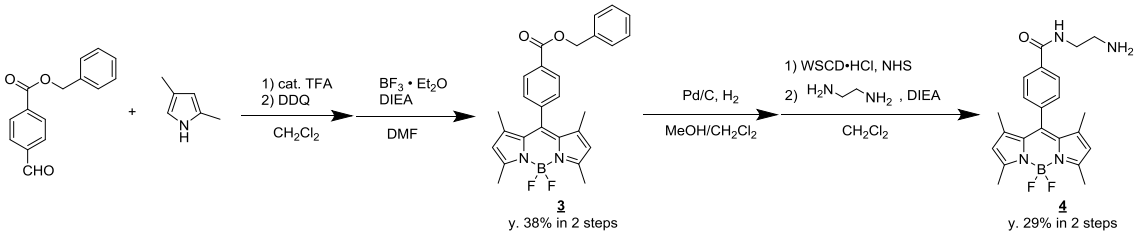
BDP-Gd was synthesized according to reference 1.



Compound 1 was synthesized according to reference 2.

Compound 2:

Compound 1 (101 mg, 0.30 mmol, 1.0 eq.) and Na_2CO_3 (151 mg, 1.4 mmol, 4.7 eq.) were dissolved in 25 mL of DMF. The resulting mixture was stirred at 0°C under an argon atmosphere for 15 min, and thiophosgene (100 μL, 1.3 mmol, 4.3 eq.) was added dropwise to it. The reaction mixture was warmed to room temperature and stirred for 2 hr. The solvent was removed by evaporation. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/hexane = 1/2), affording **2** as an orange solid (113 mg, 0.30 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (s, 6H), 2.56 (s, 6H), 6.00 (s, 2H), 7.30 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.6, 14.7, 121.5, 126.5, 129.6, 131.1, 132.3, 134.0, 137.1, 139.8, 142.8, 156.0; HRMS (ESI$^+$): $[M+H]^+$ calcd. for $C_{20}H_{19}BF_2N_3S$, 382.1361; found, 382.1326 (–3.5 mmu).



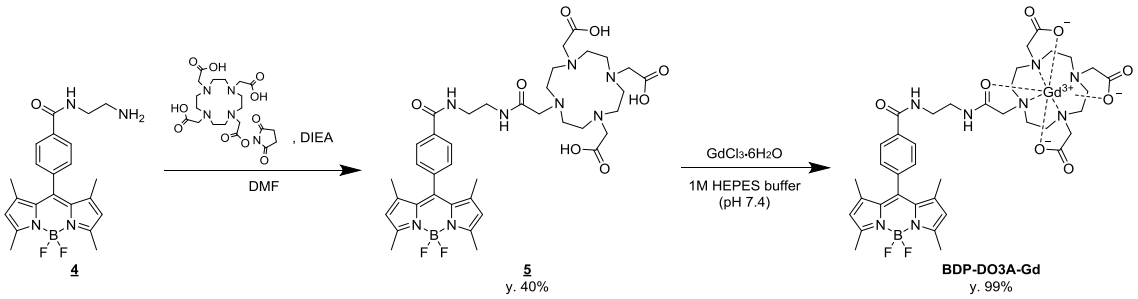
Compound 3:

4-Formylbenzoic acid phenylmethyl ester (5.86 g, 24.4 mmol, 1.0 eq.) and 2,4-dimethylpyrrole (5.00 g, 52.6 mmol, 2.2 eq.) were dissolved in 800 mL of CH_2Cl_2. One drop of TFA was added to the solution under Ar, and the reaction mixture was stirred at room temperature for 9 hr. Then DDQ (5.30 g, 23.3 mmol, 0.9 eq.) was added to the solution. The reaction mixture was stirred at room temperature for 3 hr, washed with H_2O, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The crude compound was purified by column chromatography (alumina, CH_2Cl_2, 1% triethylamine), affording a brown solid. This solid was dissolved in 500 mL of toluene, and DIEA (17 mL, 97.8 mmol, 4.0 eq.) was added. Then BF_3-Et_2O (18 mL, 146 mmol, 6.0 eq.) was added dropwise to the solution under Ar. The reaction mixture was stirred at room temperature for 4 hr, washed with H_2O, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/hexane = 1/1), affording **3** as a orange solid (4.23 g, 9.2 mmol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 6H), 2.56 (s, 6H), 5.40 (s, 2H), 5.98 (s, 2H), 7.37–7.44 (m, 5H), 7.49–7.50 (m, 2H), 8.21 (d, J = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5, 14.6, 67.2, 121.5, 128.4, 128.5, 128.7, 130.5, 130.8, 130.9, 135.7, 140.0, 140.2, 142.9, 156.0, 165.8;

HRMS (ESI$^+$): $[M+H]^+$ calcd. for $C_{27}H_{26}BF_2N_2O_2$, 459.2055; found, 459.2068 (+1.3 mmu).

Compound 4:

Compound 3 (210 mg, 0.46 mmol, 1.0 eq.) was dissolved in 15 mL of CH_2Cl_2, and MeOH 15 mL was added. Then 10% palladium-carbon was added and the mixture was stirred at room temperature under H_2 for 3 hr. The reaction mixture was filtered and the filtrate was evaporated to dryness. The crude product, *N*-hydroxysuccinimide (395 mg, 3.4 mmol, 7.4 eq.) and WSCD-HCl (496 mg, 3.2 mmol, 7.0 eq.) were dissolved in 30 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 24 hr. DIEA (0.24 mL, 1.4 mmol, 3.0 eq.) and ethylenediamine (0.30 mL, 4.5 mmol, 9.8 eq.) were added, and stirring was continued at room temperature for 5 hr. The mixture was washed with H_2O, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The crude compound was purified by column chromatography (NH silica gel, CH_2Cl_2/MeOH = 95/5), affording **4** as an orange solid (55 mg, 0.13 mmol, 29%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, 6H), 2.56 (s, 6H), 3.00 (t, J = 5.9 Hz, 2H), 3.52–3.54 (m, 2H), 5.99 (s, 2H), 6.94 (br, 1H), 7.39 (d, J = 8.1 Hz, 2H), 7.96 (d, J = 8.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 41.1, 42.3, 121.4, 127.8, 128.4, 131.0, 135.0, 138.3, 140.3, 142.9, 155.9, 166.7; HRMS (ESI$^+$): $[M+H]^+$ calcd. for $C_{22}H_{26}BF_2N_4O$, 411.2168; found, 411.2213 (+4.5 mmu).



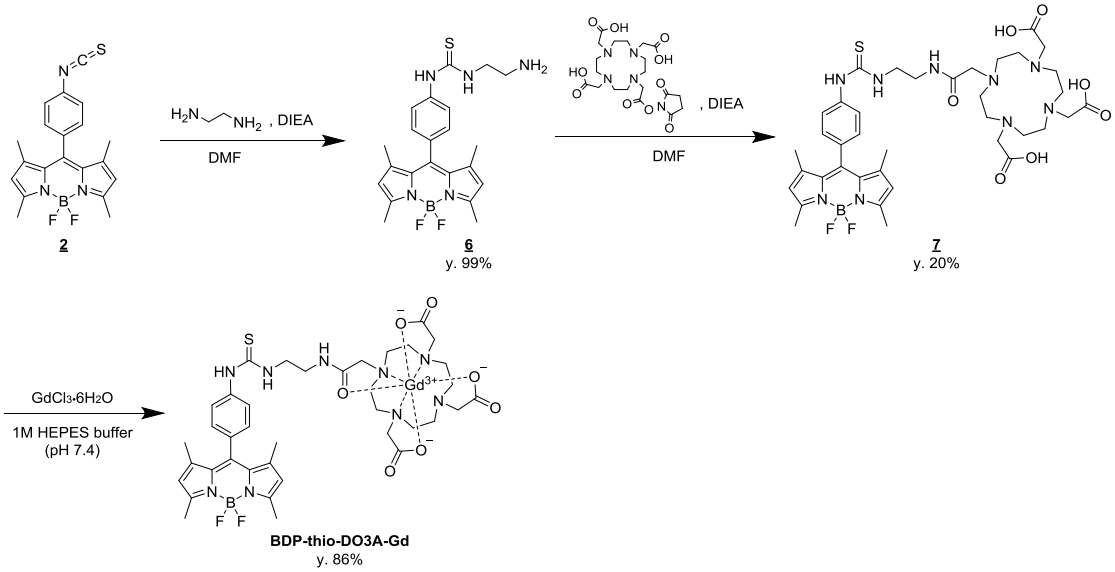
Compound 5:

Compound 4 (50 mg, 0.12 mmol, 1.0 eq.) and DOTA-NHS-ester (60 mg, 0.12 mmol, 1.0 eq.) were dissolved in 10 mL of DMF. DIEA (42 μL, 0.24 mmol, 2.0 eq.) was added, and the reaction mixture was stirred at room temperature for 17 hr. The solvent was removed by evaporation. The crude product was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **5** was obtained as an orange solid (38 mg, 0.048 mmol, 40%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.40 (s, 6H), 2.49 (s, 6H), 3.22 (br, 8H), 3.39 (br, 10H), 3.59 (t, J = 5.9 Hz, 2H), 3.64 (br, 2H), 3.77 (s, 2H), 3.90 (br, 4H), 6.08 (s, 2H), 7.46 (d, J = 8.3 Hz, 2H), 8.09 (d, J = 8.3 Hz, 2H); ^{13}C NMR (100 MHz,

CD_3OD): δ 14.6, 15.8, 40.2, 40.8, 51.0, 51.1, 51.4, 51.6, 54.7, 55.8, 116.5, 119.4, 122.5, 129.5, 129.8, 132.3, 136.5, 139.7, 142.3, 144.3, 157.2, 162.2, 162.6, 169.8; HRMS (ESI^+): $[M+H]^+$ calcd. for $C_{38}H_{52}BF_2N_8O_8$, 797.3969; found, 797.3982 (+1.3 mmu).

BDP-DO3A-Gd:

Compound 5 (20 mg, 0.025 mmol, 1.0 eq.) was dissolved in 3 mL of 1 M HEPES buffer (pH 7.4) and $GdCl_3 \cdot 6H_2O$ (38 mg, 0.10 mmol, 4.0 eq.) was added. The reaction mixture was stirred at room temperature for 21 hr, and then purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **BDP-DO3A-Gd** was obtained as an orange solid (20 mg, 0.025 mmol, 99%). HRMS (ESI^+): $[M–F]^+$ calcd. for $C_{38}H_{48}BFGdN_8O_8$, 932.2913; found, 932.2954 (+4.1 mmu). HPLC analysis: retention time, 12.5 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm, purity, 98.7% integrated intensity.



Compound 6:

Compound 2 (113 mg, 0.30 mmol, 1.0 eq.) and DIEA (0.1 mL, 0.60 mmol, 2.0 eq.) were dissolved in 25 mL of DMF. Ethylenediamine (0.2 mL, 3.0 mmol, 10 eq.) was added and the reaction mixture was stirred at room temperature for 15 hr. The solvent was removed by evaporation. The crude compound was purified by column chromatography (NH silica gel, CH_2Cl_2/MeOH = 95/5), affording **6** as an orange solid (136 mg, 0.30 mmol, 99%). ^1H NMR (300 MHz, $(CD_3)_2SO$): δ 1.43 (s, 6H), 2.45 (s, 6H), 2.74 (t, J = 5.9 Hz, 2H), 3.46 (br, 2H), 6.18 (s, 2H), 7.26 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 40.5, 47.2, 53.5, 121.4, 124.5, 129.5, 131.4, 132.8, 137.8, 140.5, 142.9, 155.7, 180.2; HRMS (ESI^+): $[M+H]^+$ calcd. for $C_{22}H_{27}BF_2N_5S$, 442.2048; found, 442.2098

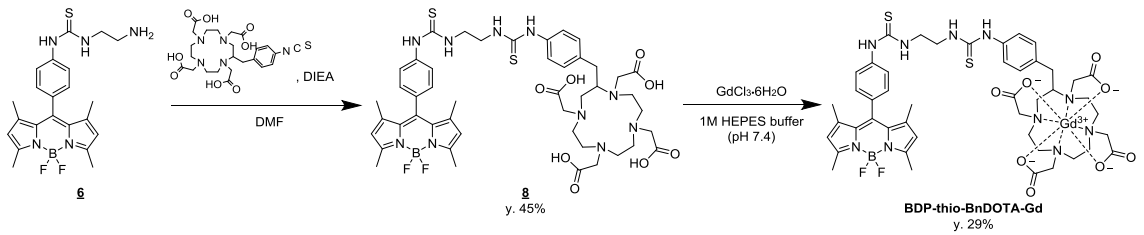
(+5.0 mmu).

Compound 7:

Compound 6 (63 mg, 0.14 mmol, 1.0 eq.) and DOTA-NHS-ester (73 mg, 0.15 mmol, 1.1 eq.) were dissolved in 10 mL of DMF. DIEA (50 μL, 0.29 mmol, 2.1 eq.) was added and the reaction mixture was stirred at room temperature for 18 hr. The solvent was removed by evaporation. The crude compound was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **7** was obtained as an orange solid (23 mg, 0.028 mmol, 20%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.49 (s, 6H), 2.48 (s, 6H), 2.99–3.38 (m, 18H), 3.84 (br, 6H), 3.95–4.07 (m, 4H), 6.06 (s, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 7.83 (br, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 14.6, 14.9, 40.3, 44.0, 50.0, 51.2, 54.5, 55.4, 115.8, 119.6, 122.2, 124.8, 129.3, 132.8, 142.2, 143.5, 144.7, 156.5, 161.8, 162.3, 182.6; HRMS (ESI$^-$): $[M–H]^-$ calcd. for $C_{38}H_{51}BF_2N_9O_7S$, 826.3693; found, 826.3659 (–3.4 mmu).

BDP-thio-DO3A-Gd:

Compound 7 (14 mg, 0.017 mmol, 1.0 eq.) was dissolved in 3 mL of 1 M HEPES buffer (pH 7.4) and $GdCl_3 \cdot 6H_2O$ (16 mg, 0.044 mmol, 2.6 eq.) was added. The reaction mixture was stirred at room temperature for 16 hr. Then, the mixture was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **BDP-thio-DO3A-Gd** was obtained as an orange solid (14 mg, 0.015 mmol, 86%). HRMS (ESI$^+$): $[M–F]^+$ calcd. for $C_{38}H_{49}BFGdN_9O_7S$, 963.2794; found, 963.2800 (+0.6 mmu). HPLC analysis: retention time, 14.0 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm, purity, 96.6% integrated intensity.



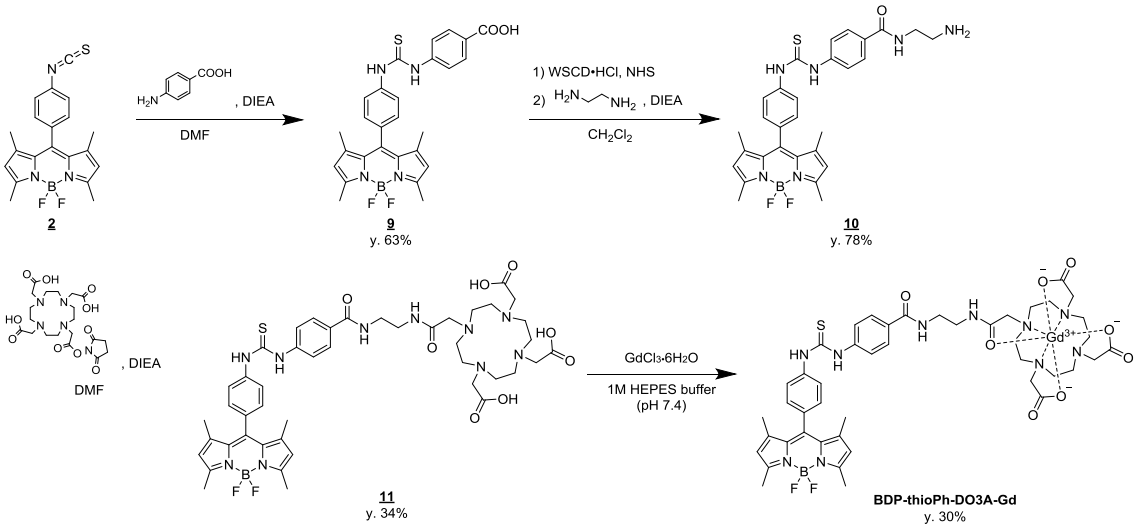
Compound 8:

Compound 6 (54 mg, 0.12 mmol, 1.0 eq.) and *p*-SCN-Bn-DOTA (83 mg, 0.15 mmol, 1.3 eq.) were dissolved in 10 mL of DMF. DIEA (42 μL, 0.24 mmol, 2.0 eq.) was added and the reaction mixture

was stirred at room temperature for 15 hr. The solvent was removed by evaporation. The crude compound was purified by preparative HPLC under the following conditions: A/B = 50/50 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **8** was obtained as an orange solid (54 mg, 0.055 mmol, 45%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.49 (s, 6H), 2.49 (s, 6H), 2.57–3.26 (m, 10H), 3.34–4.10 (m, 17H), 4.26–4.35 (m, 2H), 6.07 (s, 2H), 7.25–7.39 (m, 6H), 7.57–7.60 (m, 2H); HRMS (ESI$^-$): $[M–H]^-$ calcd. for $C_{46}H_{58}BF_2N_{10}O_8S_2$, 991.3942; found, 991.3893 (–4.9 mmu); HPLC analysis: retention time, 14.1 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 495 nm, purity, 99.5% integrated intensity.

BDP-thio-BnDOTA-Gd:

Compound 8 (15 mg, 0.015 mmol, 1.0 eq.) was dissolved in 3 mL of 1 M HEPES buffer (pH 7.4) and $GdCl_3 \cdot 6H_2O$ (28 mg, 0.075 mmol, 5.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 hr, and then purified by preparative HPLC under the following conditions: A/B = 50/50 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **BDP-thio-BnDOTA-Gd** was obtained as an orange solid (4.9 mg, 0.0043 mmol, 29%). HRMS (ESI$^-$): $[M]^-$ calcd. for $C_{46}H_{55}BF_2GdN_{10}O_8S_2$, 1146.2948; found, 1146.2926 (–2.2 mmu). HPLC analysis: retention time, 15.7 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm, purity, 98.5% integrated intensity.



Compound 9:

Compound 2 (80 mg, 0.21 mmol, 1.0 eq.) was suspended in 20 mL of EtOH. *p*-Aminobenzoic acid (58 mg, 0.42 mmol, 2.0 eq.) and DIEA (73 μL, 0.42 mmol, 2.0 eq.) were added and the reaction mixture was stirred at 50 °C for 24 hr. The mixture was washed with H_2O, dried over anhydrous

sodium sulfate, filtered and evaporated to dryness. The crude compound was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 90/10), affording **9** as an orange solid (68 mg, 0.13 mmol, 63%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.44 (s, 6H), 2.47 (s, 6H), 6.03 (s, 2H), 7.23 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.97 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 14.5, 14.9, 122.2, 123.2, 125.6, 127.8, 129.6, 131.6, 132.6, 132.7, 141.7, 143.2, 144.7, 144.9, 156.6, 169.5, 181.9; HRMS (ESI$^+$): $[M+H]^+$ calcd. for $C_{27}H_{26}BF_2N_4O_2S$, 517.1681; found, 517.1716 (+3.5 mmu).

Compound 10:

Compound 9 (59 mg, 0.11 mmol, 1.0 eq.), *N*-hydroxysuccinimide (78 mg, 0.68 mmol, 6.2 eq.) and WSCD-HCl (105 mg, 0.68 mmol. 6.2 eq.) were dissolved in 30 mL of DMF. The reaction mixture was stirred at room temperature for 19 hr. DIEA (38 μL, 0.22 mmol, 2.0 eq.) and ethylenediamine (73 μL, 1.1 mmol, 10.0 eq.) were added to the mixture. Stirring was continued at room temperature for 3 hr, and then the solvent was removed by evaporation. The crude compound was purified by column chromatography (NH silica gel, CH_2Cl_2/MeOH = 95/5), affording **10** as an orange solid (49 mg, crude).

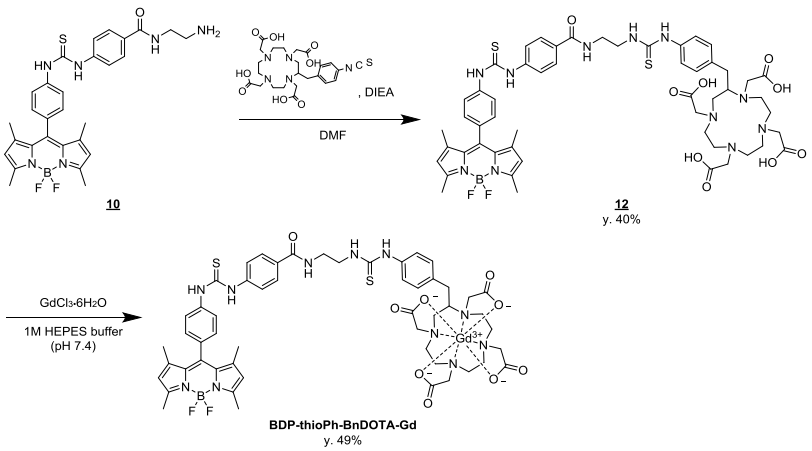
Compound 11:

Crude **10** (18 mg, 0.032 mmol, 1.0 eq.) and DOTA-NHS-ester (28 mg, 0.055 mmol, 1.7 eq.) were dissolved in 10 mL of DMF. DIEA (11 μL, 0.063 mmol, 1.9 eq.) was added and the reaction mixture was stirred at room temperature for 18 hr. The solvent was removed by evaporation. The crude compound was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **11** was obtained as an orange solid (9.9 mg, 0.011 mmol, 27% in 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 1.48 (s, 6H), 2.48 (s, 6H), 3.27–3.34 (m, 16H), 3.40 (t, *J* = 6.6 Hz, 2H), 3.56 (t, *J* = 6.6 Hz, 2H), 3.63–3.98 (m, 8H), 6.06 (s, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 14.6, 14.9, 40.3, 40.6, 50.8, 51.1, 51.6, 54.3, 54.5, 54.6, 55.8, 122.2, 124.2, 125.6, 129.0, 129.6, 131.3, 132.7, 141.7, 143.2, 144.0, 144.7, 156.7, 169.8, 181.9; HRMS (ESI$^-$): $[M–H]^-$ calcd. for $C_{45}H_{56}BF_2N_{10}O_8S$, 945.4064; found, 945.4016 (–4.8 mmu).

BDP-thioPh-DO3A-Gd:

Compound 11 (9.9 mg, 0.011 mmol, 1.0 eq.) was dissolved in 3 mL of 1 M HEPES buffer (pH 7.4) and $GdCl_3 \cdot 6H_2O$ (14 mg, 0.037 mmol, 3.4 eq.) was added. The reaction mixture was stirred at room temperature for 16 hr, and then purified by preparative HPLC under the following conditions: A/B = 50/50 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **BDP-thioPh-DO3A-Gd** was obtained as an orange solid (6.8

mg, 0.0060 mmol, 59%). HRMS (ESI^+): $[M–F]^+$ calcd. for $C_{45}H_{54}BFGdN_{10}O_8S$, 1082.3165; found, 1082.3210 (+4.5 mmu). HPLC analysis: retention time, 8.1 min (eluent: A/B = 50/50 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm, purity, 99.4% integrated intensity.



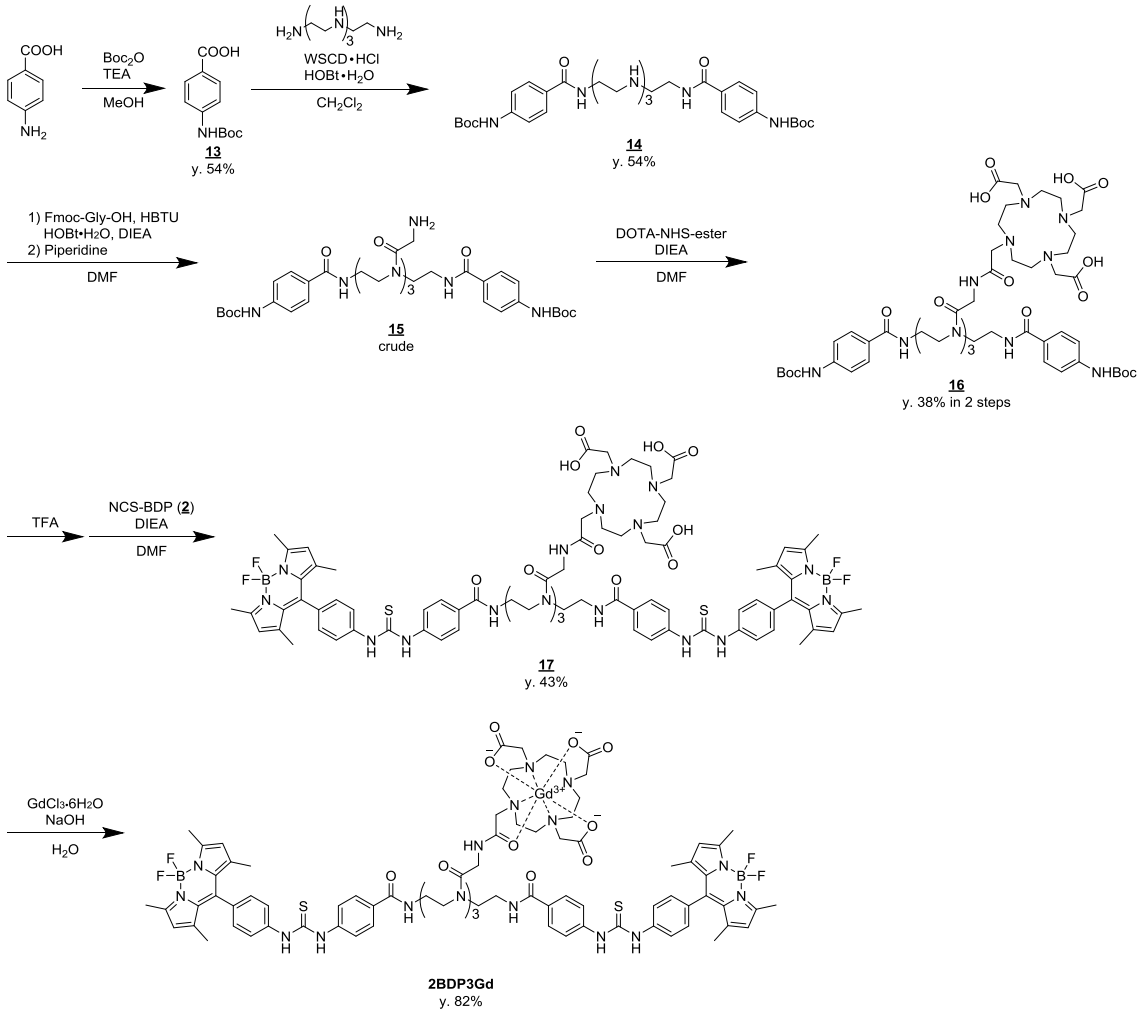
Compound <u>12</u>:

Crude **<u>10</u>** (14 mg, 0.025 mmol, 1.0 eq.) and *p*-SCN-Bn-DOTA (17 mg, 0.031 mmol, 1.2 eq.) were dissolved in 5 mL of DMF. DIEA (9 μL, 0.05 mmol, 2.0 eq.) was added and the reaction mixture was stirred at room temperature for 15 hr. The solvent was removed by evaporation. The crude compound was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **<u>12</u>** was obtained as an orange solid (11 mg, 0.010 mmol, 31% in 2 steps). ^1H NMR (300 MHz, CD_3OD): δ 1.45 (s, 6H), 2.48 (s, 6H), 2.61–3.29 (m, 9H), 3.32–4.33 (m, 20H), 6.06 (s, 2H), 7.26–7.29 (m, 6H), 7.62 (d, 2H, J = 8.8 Hz), 7.69 (d, 2H, J = 8.8 Hz), 7.80 (d, 2H, J = 8.1 Hz); HRMS (ESI^+): $[M+H]^+$ calcd. for $C_{53}H_{65}BF_2N_{11}O_9S_2$, 1112.4469; found, 1112.4471 (+0.2 mmu); HPLC analysis: retention time, 18.7 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 480 nm, purity, 99.7% integrated intensity.

BDP-thioPh-BnDOTA-Gd:

Compound <u>12</u> (19 mg, 0.019 mmol, 1.0 eq.) was dissolved in 3 mL of 1 M HEPES buffer (pH 7.4) and $GdCl_3 \cdot 6H_2O$ (26 mg, 0.070 mmol, 3.7 eq.) was added. The reaction mixture was stirred at room temperature for 16 hr, and then purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **BDP-thioPh-BnDOTA-Gd** was obtained as an orange solid (11 mg, 0.0092 mmol, 49 %). HRMS ($ESI^–$): $[M]^–$ calcd. for $C_{53}H_{60}BF_2GdN_{11}O_9S_2$, 1265.3319; found,

1265.3348 (+2.9 mmu). HPLC analysis: retention time, 16.6 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm, purity, 95.7% integrated intensity.



Compound <u>13</u>:

p-Aminobenzoic acid (6.06 g, 44.2 mmol, 1.0 eq.) and triethylamine (20 mL, 144 mmol, 3.3 eq.) were dissolved in 70 mL of MeOH. Boc_2O (20 mL, 94 mmol, 2.1 eq.) was added to the solution at 0°C. The reaction mixture was warmed to room temperature and stirred for 16 hr. The solvent was removed by evaporation. The residue was dissolved in CH_2Cl_2 and the solution was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 95/5), affording **<u>13</u>** as a white solid (5.65 g, 23.8 mmol, 54%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.53 (s, 9H), 7.50 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 28.0, 79.6, 117.2, 123.9, 130.3, 143.8, 152.5, 167.0; HRMS (ESI$^-$): $[M–H]^-$ calcd. for $C_{12}H_{14}NO_4$, 236.0923; found, 236.0881 (–4.2 mmu).

Compound 14:

Compound 13 (1.00 g, 4.2 mmol, 1.0 eq.), HOBt·H_2O (645 mg, 4.2 mmol, 1.0 eq.) and WSCD·HCl (968 mg, 5.1 mmol, 1.2 eq.) were dissolved in 50 mL of CH_2Cl_2. The resulting solution was stirred at 0°C under an argon atmosphere for 1 hr. Then, tetraethylenepentamine (399 mg, 2.1 mmol. 0.5 eq.) in 5 mL of CH_2Cl_2 was added to it at 0°C. The reaction mixture was warmed to room temperature and stirred overnight under an argon atmosphere. The supernatant was removed by decantation and the remaining solvent was removed by evaporation. The crude product was recrystallized twice from MeOH, affording pure **14** as a white solid (713 mg, 1.1 mmol, 54%). ^{1}H NMR (300 MHz, D_2O): δ 1.33 (s, 18H), 3.00 (br, 4H), 3.17 (br, 8H), 3.49 (br, 4H), 7.17 (d, J = 8.8 Hz, 4H), 7.45 (d, J = 8.8 Hz, 4H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 28.1, 35.7, 44.1, 46.2, 46.4, 79.5, 117.0, 127.1, 128.3, 142.5, 152.6, 166.2; HRMS (ESI$^+$): $[M+H]^+$ calcd. for $C_{32}H_{50}N_7O_6$, 628.3823; found, 628.3803 (–2.0 mmu).

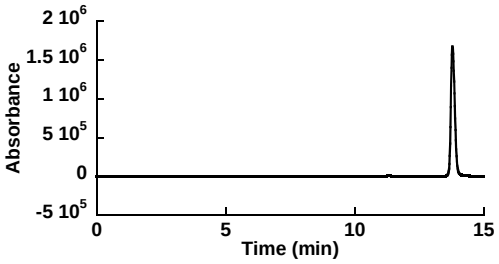
Compound 15:

Compound 14 (69 mg, 0.11 mmol, 1.0 eq.), Fmoc-Gly-OH (329 mg, 1.1 mmol, 10.0 eq.), HOBt·H_2O (174 mg, 1.1 mmol, 10.0 eq.) and HBTU (429 mg, 1.1 mmol, 10.0 eq.) were dissolved in 200 mL of DMF. DIEA (380 μL, 2.1 mmol, 20.0 eq.) was added, and the reaction mixture was stirred for 10 hr at room temperature under an argon atmosphere. The solvent was removed by evaporation. The residue was dissolved in CH_2Cl_2. The solution was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography (NH silica gel, CH_2Cl_2/MeOH = 100/0 to 90/10). The resulting product was dissolved in 20% piperidine in DMF. The reaction mixture was stirred at room temperature for 2 hr, and the solvent was removed by evaporation. The residue was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA), affording **15** as a white solid (62 mg, crude).

Compound 16:

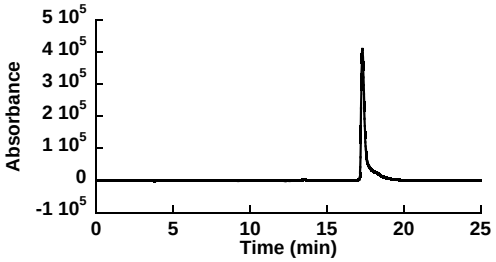
Crude **15** (58 mg, 0.73 mmol, 1.0 eq.) and DOTA-NHS-ester (121 mg, 0.241 mmol, 3.3 eq.**)** were dissolved in 10 mL of DMF. DIEA (76 μL, 0.44 mmol, 6.0 eq.) was added, and the reaction mixture was stirred at 50°C under an argon atmosphere for 24 hr. The solvent was removed by evaporation and the crude product was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA), affording **6** as a white solid (78 mg, 0.040 mmol, 38% in 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 1.52 (s, 18H), 3.16–4.20 (m, 94H), 7.48–7.51 (m, 4H), 7.71–7.80 (m, 4H); ^{13}C NMR (75 MHz, D_2O/$(CD_3)_2SO$ = 1/1): δ 28.9, 48.8, 49.1, 49.5, 51.3, 51.9, 54.2, 56.1, 57.6, 81.3, 118.5, 128.2, 129.2, 143.0, 154.0, 168.2, 168.3, 168.7, 168.7, 170.5, 171.5, 173.9; HRMS (ESI$^-$): $[M–H]^-$

calcd. for $C_{86}H_{135}N_{22}O_{30}$, 1955.9714; found, 1955.9754 (+4.0 mmu); HPLC analysis: retention time, 13.8 min (eluent: A/B = 95/05 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 254 nm, purity, 99.5% integrated intensity.



Compound <u>17</u>:

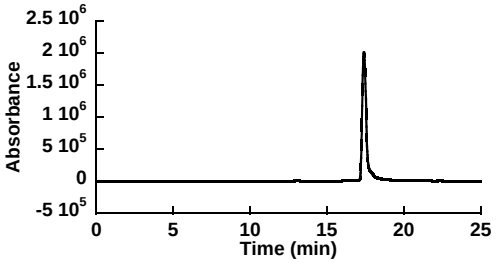
Compound <u>16</u> (561 mg, 0.29 mmol, 1.0 eq.) was dissolved in 15 mL of TFA. The reaction mixture was stirred at room temperature for 30 min. TFA was removed by evaporation, and the residue was dissolved in water and lyophilized. The crude product and **<u>2</u>** (273 mg, 0.72 mmol, 2.5 eq.) were dissolved in 20 mL of DMF. DIEA (199 μL, 1.1 mmol, 4.0 eq.) was added, and the reaction mixture was stirred at 50°C under an argon atmosphere for 10 hr. The solvent was removed by evaporation, and the residue was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (30 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA), affording **<u>17</u>** as an orange solid (283 mg, 0.11 mmol, 43%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.44 (s, 12H), 2.46 (s, 12H), 3.13–4.11 (m, 94H), 6.03 (s, 4H), 7.21 (d, J = 7.8 Hz, 4H), 7.69–7.87 (m, 12H); HRMS (ESI$^+$): $[M+2H]^{2+}$ calcd. for $C_{116}H_{158}B_2F_4N_{28}O_{26}S_2$, 2521.1466; found, 2521.1428 (–3.8 mmu). HPLC analysis: retention time, 17.3 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm.

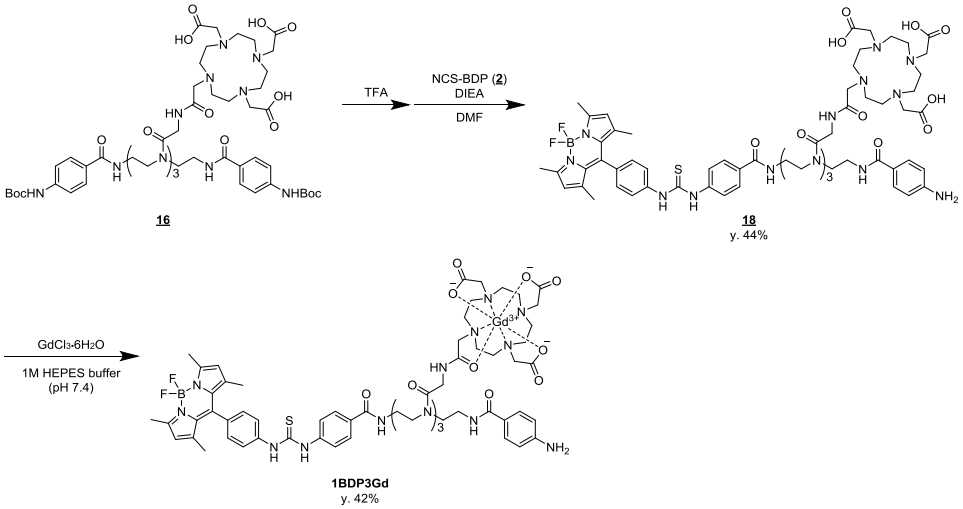


2BDP3Gd:

Compound <u>17</u> (283 mg, 0.112 mmol, 1.0 eq.) was dissolved in 20 mL of H_2O and $GdCl_3 \cdot 6H_2O$ (375 mg, 1.0 mmol, 9.0 eq.) was added to it. The reaction mixture was adjusted to pH 4-5 by addition of 1 N NaOH aq., stirred at room temperature for 48 hr, and then purified by preparative HPLC under the

following conditions: A/B = 80/20 (0 min) to 0/100 (30 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **2BDP3Gd** was obtained as an orange solid (275 mg, 0.092 mmol, 82%). HRMS (ESI^+): $[M+2H]^{2+}$ calcd. for $C_{116}H_{149}B_2F_4Gd_3N_{28}O_{26}S_2$, 2985.8484; found, 2985.8524 (+4.0 mmu). HPLC analysis: retention time, 17.4 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 500 nm.

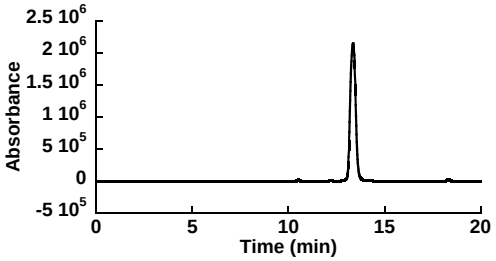




Compound 18:

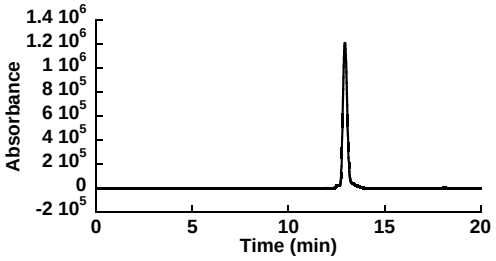
Compound 16 (256 mg, 0.13 mmol, 1.0 eq.) was dissolved in 10 mL of TFA, and the solution was stirred at room temperature for 2 hr. After removal of TFA by evaporation, the residue was dissolved in water and lyophilized. The resulting solid was dissolved in 10 mL of DMF, and then **2** (50 mg, 0.13 mmol, 1.0 eq.) and DIEA (91.3 μL, 0.52 mmol, 4.0 eq.) were added to it. The mixture was stirred overnight at 50°C under an argon atmosphere. After removal of the solvent by evaporation, the residue was purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (30 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). Compound **18** was obtained as an orange solid (123 mg, 0.058 mmol, 44%). ^1H NMR (400 MHz, CD_3OD): δ 1.48 (s, 6H), 2.48 (s, 6H), 3.13–4.19 (m, 94H), 6.07 (s, 2H), 6.77–6.80 (m, 2H), 7.27–7.30 (m, 2H), 7.62–7.88 (m, 8H); HRMS (ESI^+): $[M+2H]^{2+}$ calcd for $C_{96}H_{140}BF_2N_{25}O_{26}S$, 2140.0183; found, 2140.0212 (+2.9 mmu). HPLC analysis: retention time, 14.0 min (eluent: A/B = 80/20 to 0/100,

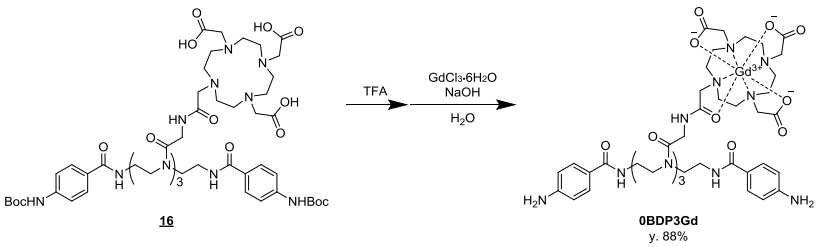
20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 490 nm.



1BDP3Gd:

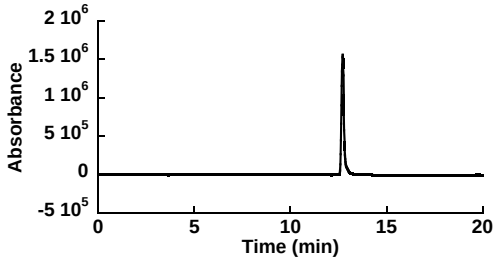
Compound 18 (75 mg, 0.035 mmol, 1.0 eq.) was dissolved in 8 mL of 1 M HEPES buffer (pH 7.4), then $GdCl_3 \cdot 6H_2O$ (111 mg, 0.30 mmol, 8.5 eq.) was added to it. The mixture was stirred overnight at room temperature, and then purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (30 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). **1BDP3Gd** was obtained as an orange solid (29 mg, 0.011 mmol, 42%). HRMS (ESI^+): $[M+2H]^{2+}$ calcd. for $C_{96}H_{129}BF_2Gd_3N_{25}O_{26}S$, 2604.7202; found, 2604.7160 (–4.2 mmu). HPLC analysis: retention time, 13.8 min (eluent: A/B = 80/20 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 490 nm.





0BDP3Gd:

Compound 16 (45.7 mg, 0.023 mmol, 1.0 eq.) was dissolved in 1 mL of TFA, and the solution was stirred for 2 hr at room temperature. After removal of TFA by evaporation, the residue was dissolved in water and lyophilized. The resulting solid was dissolved in 2 mL of H_2O, and $GdCl_3 \cdot 6H_2O$ (82 mg, 0.22 mmol, 9.5 eq.) was added to the solution. The reaction mixture was adjusted to pH 4-5 by addition of 1 N NaOH aq., stirred overnight at room temperature, and then purified by preparative HPLC under the following conditions: A/B = 80/20 (0 min) to 0/100 (20 min) linear gradient, (solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA). After removal of excess Gd^{3+} ions using Chelex®100 resin (Bio-Rad), **0BDP3Gd** was obtained as a white solid (45.7 mg, 0.021 mmol, 88%). HRMS (ESI^+): $[M+2Na]^{2+}$ calcd. for $C_{76}H_{111}Gd_3N_{22}Na_2O_{26}$, 2267.5558; found, 2267.5519 (–4.0 mmu). HPLC analysis: retention time, 12.7 min (eluent: A/B = 99/01 for 5 min, then 99/01 to 0/100, 20 min, linear gradient; solvent A: H_2O, 0.1% TFA; solvent B: acetonitrile/H_2O = 80/20, 0.1% TFA); flow rate, 1.0 mL/min; detection wavelength, 254 nm.



Supplementary Figures

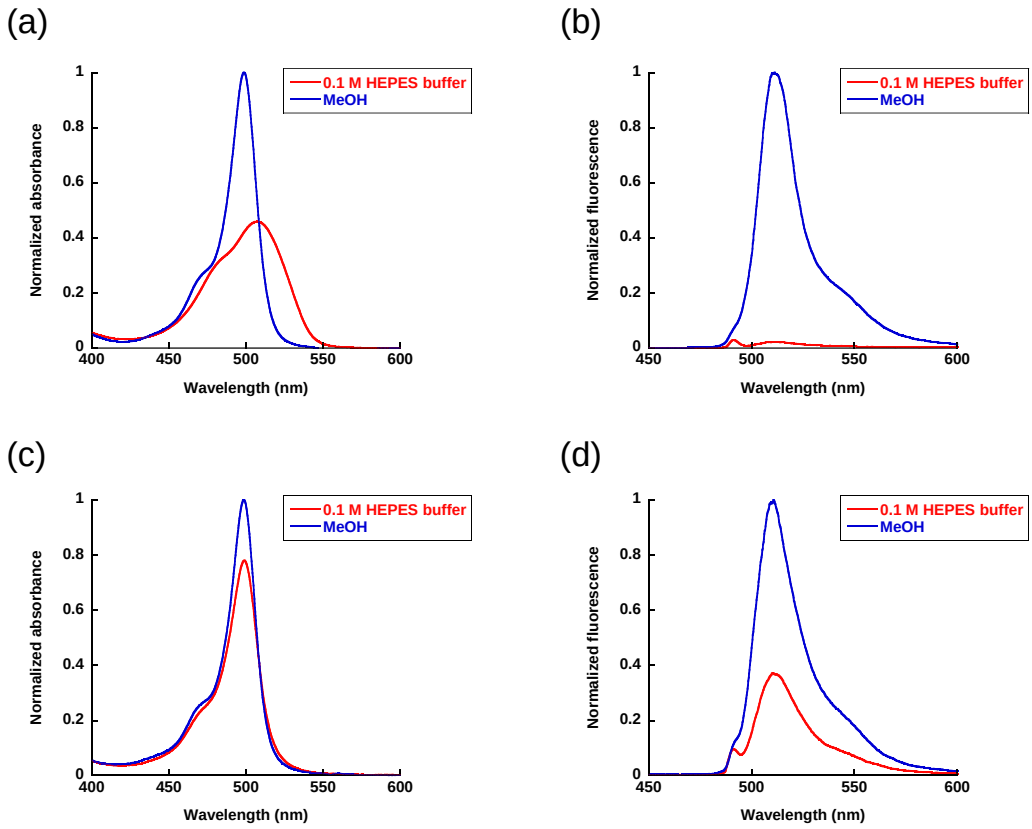


Fig. S1. Absorption and fluorescence spectra of **2BDP3Gd** and **1BDP3Gd**. (a) Absorption spectra of **2BDP3Gd** in 0.1 M HEPES buffer (red) and MeOH (blue) containing 0.3% DMSO as a cosolvent. (b) Fluorescence spectra of **2BDP3Gd** in 0.1 M HEPES buffer (red) and MeOH (blue) containing 0.3% DMSO as a cosolvent. Excitation wavelength was 490 nm. (c) Absorption spectra of **1BDP3Gd** in 0.1 M HEPES buffer (red) and MeOH (blue) containing 0.3% DMSO as a cosolvent. (d) Fluorescence spectra of **1BDP3Gd** in 0.1 M HEPES buffer (red) and MeOH (blue) containing 0.3% DMSO as a cosolvent. Excitation wavelength was 490 nm.

Table S1. Fluorescence quantum yields of **2BDP3Gd** and **1BDP3Gd** in aqueous buffer or MeOH

probe	$\Phi_{fl\ buffer}$[a]	$\Phi_{fl\ MeOH}$[b]
2BDP3Gd	0.010	0.24
1BDP3Gd	0.11	0.31

Φ_{fl} is the relative fluorescence quantum yield determined by using fluorescein in 0.1 M NaOH aq. (0.85) as a fluorescence standard. [a]All data were measured in 100 mM HEPES buffer (pH 7.4). [b]All data were measured in MeOH.

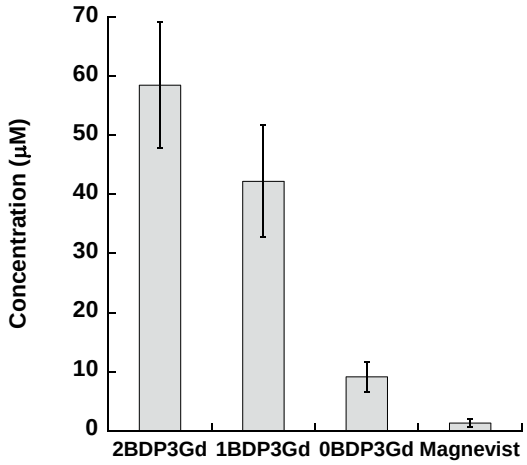


Fig. S2. The concentration of Gd^{3+} complexes in the dialysis membrane (initial concentration: 50 μM) after the dialysis in PBS (pH 7,4) for 24 hr. The error bar shows standard deviation (n = 3). **2BDP3Gd** and **1BDP3Gd** were highly retained in the dialysis membrane.

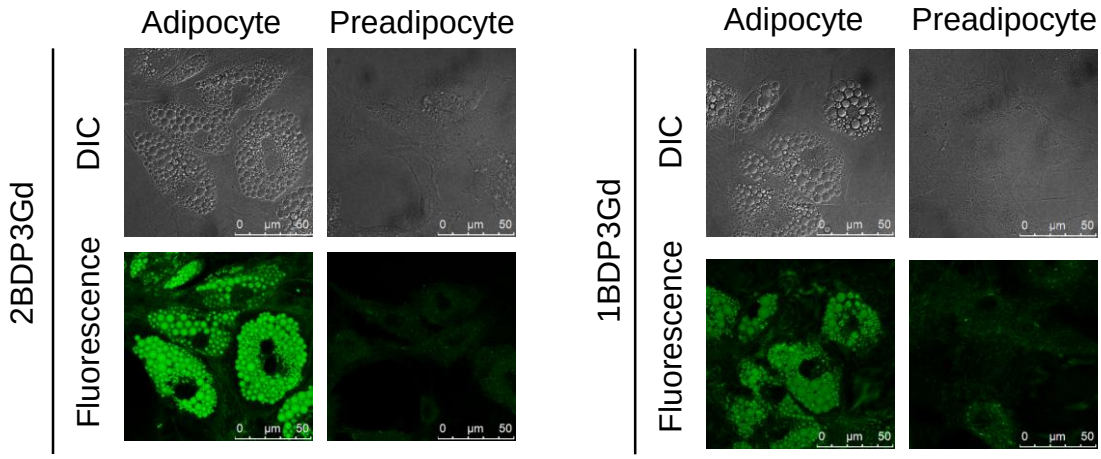


Fig. S3. Confocal microscopic images of adipocytes (left) and preadipocytes (right) loaded with **2BDP3Gd** or **1BDP3Gd** (10 μM in DMEM containing 0.1% DMSO as a cosolvent). Upper and lower panels show DIC and fluorescence images, respectively.

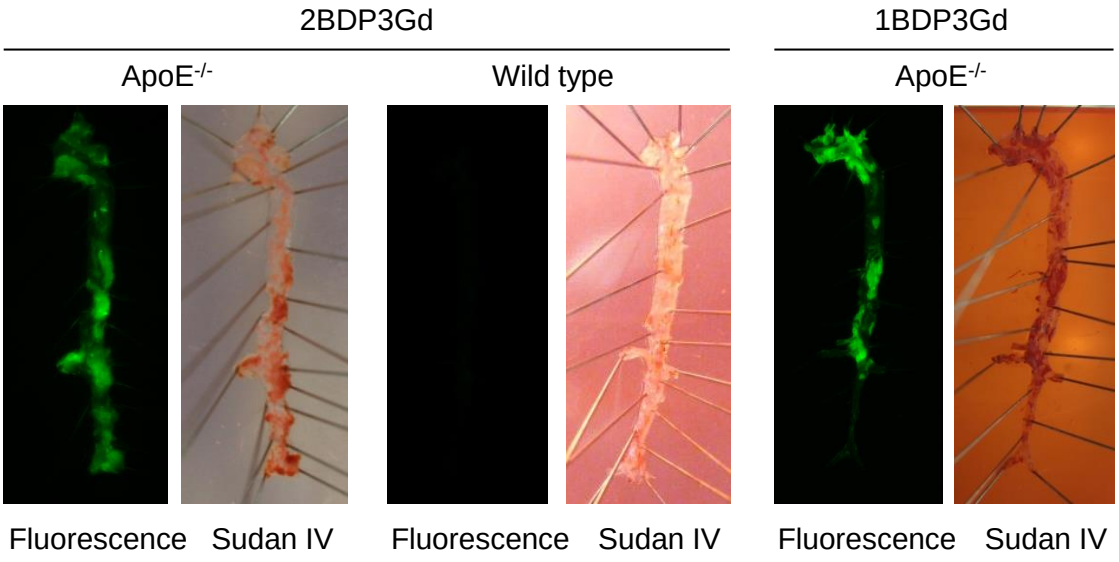


Fig. S4. Fluorescence (left) and Sudan IV staining (right) images of aorta isolated from *ApoE*$^{-/-}$ mouse injected with **2BDP3Gd** (1st injection; 5 mM, 100 μL. 2nd injection; 5 mM, 150 μL, 2 hr after 1st injection) or **1BDP3Gd** (1st injection; 5 mM, 100 μL. 2nd injection; 20 mM, 100 μL, 2 hr after 1st injection). Fluorescence images were captured 1 hr after the 2nd injection.

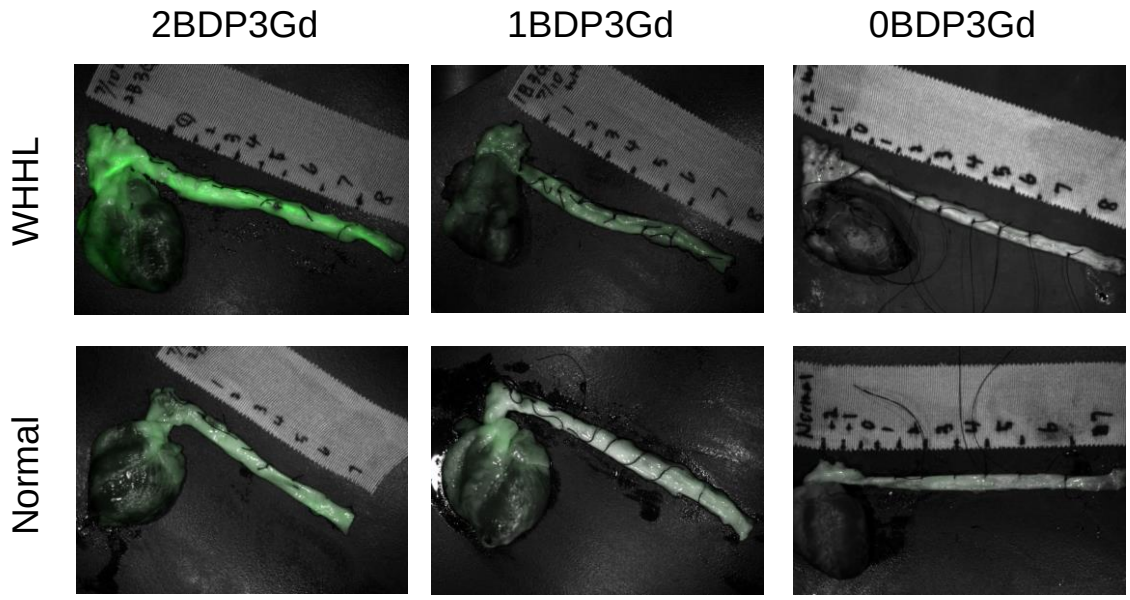


Fig. S5. Fluorescence images of aorta isolated from WHHL or normal rabbit injected with **2BDP3Gd**, **1BDP3Gd** or **0BDP3Gd** (7.4 μmol/kg).

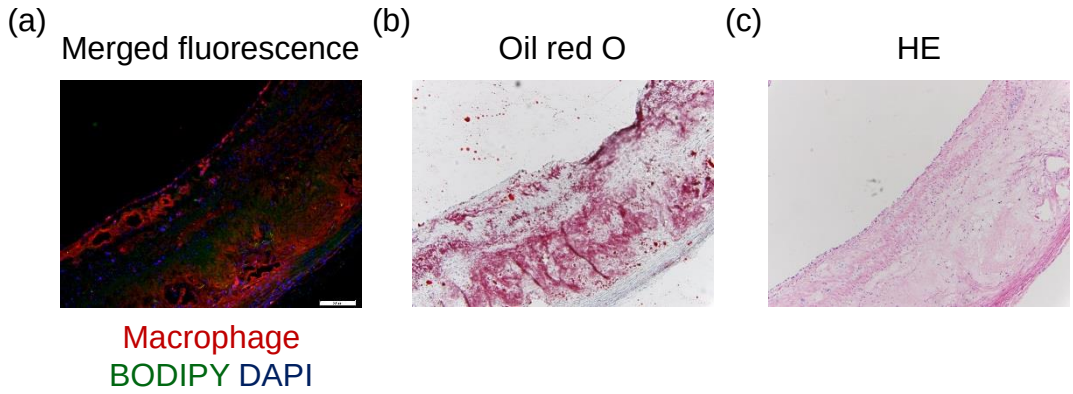


Fig. S6. Frozen section of the aorta of WHHL rabbit after administration of **1BDP3Gd**. Frozen sections were histologically examined by (a) fluorescence imaging (red: anti-RAM antibody, green: BODIPY, blue: DAPI), (b) Oil red O staining, and (c) haematoxylin and eosin (HE) staining. Scale bar: 50 μm. Thickening of aorta, infiltration of leucocytes and accumulation of lipids were observed, i.e., formation of atherosclerotic plaques was confirmed. On the other hand, the fluorescence of **1BDP3Gd** was very weak.

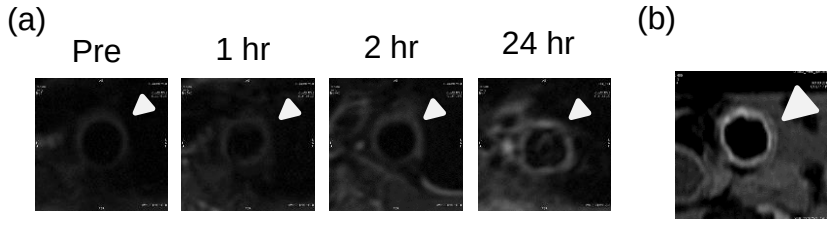


Fig. S7. Reproducibility of MR imaging of WHHL rabbit with **2BDP3Gd**. White arrowheads indicate the aorta. (a) T_1-weighted MR images (*in vivo* imaging) of the aorta of WHHL rabbit before and at 1 hr, 2 hr and 24 hr after the administration of **2BDP3Gd** (7.4 μmol/kg). (b) T_1-weighted MR image (*ex vivo* imaging) of the isolated aorta of WHHL rabbit injected with **2BDP3Gd**. Atherosclerotic plaque was successfully visualized with **2BDP3Gd**. Reproducibility was confirmed by additional imaging (data not shown).

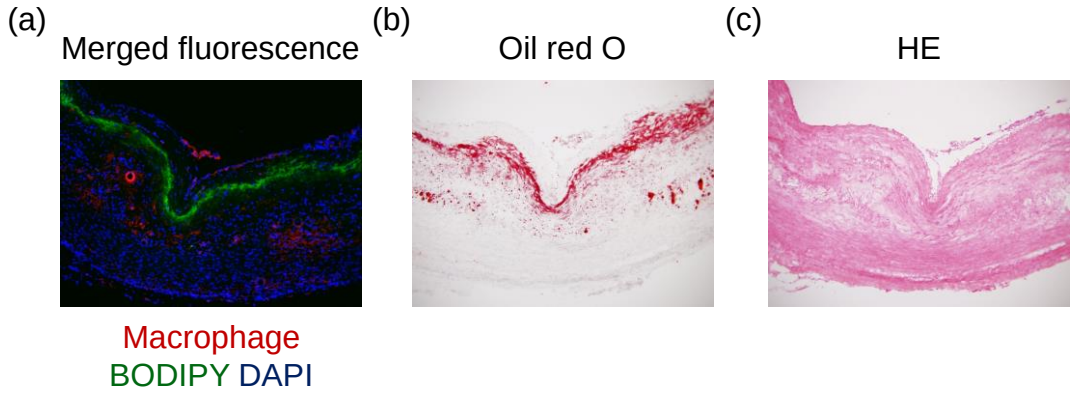


Fig. S8. Reproducibility of histological staining of frozen sections of the aorta of WHHL rabbit after administration of **2BDP3Gd** (*in vivo* MR imaging). (a) Fluorescence image (red: anti-RAM antibody, green: BODIPY, blue: DAPI). (b) Oil red O staining. (c) Haematoxylin and eosin (HE) staining. Scale bar: 50 μm. Thickening of aorta, infiltration of leucocytes and accumulation of lipids were observed, i.e., formation of atherosclerotic plaques was confirmed. Strong fluorescence of **2BDP3Gd** was observed and its location was well matched with that of Oil red O staining.

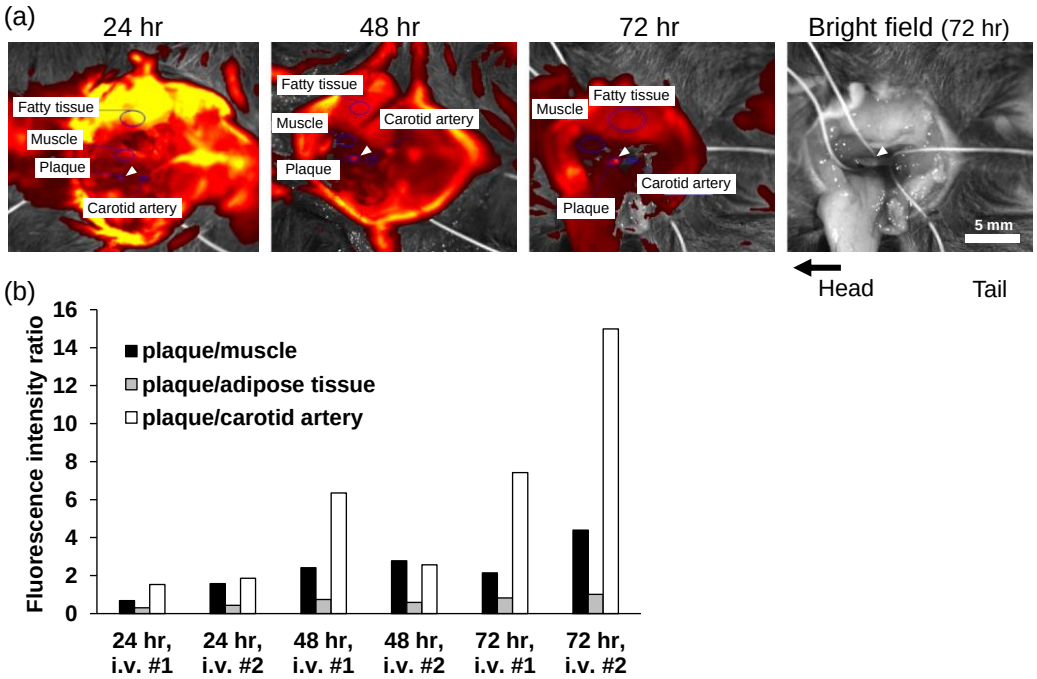


Fig. S9. In *vivo* fluorescence imaging of atherosclerotic plaques in carotid artery in $LDLR^{-/-}$ mice. **2BDP3Gd** (7.4 μmol/kg) was intravenously administered via the tail vein at 24 hr, 48 hr or 72 hr before imaging. (a) Fluorescence images of atherosclerotic plaques. White arrowhead indicates atherosclerotic plaques. (b) Fluorescence intensity ratio at each time point. n = 2.

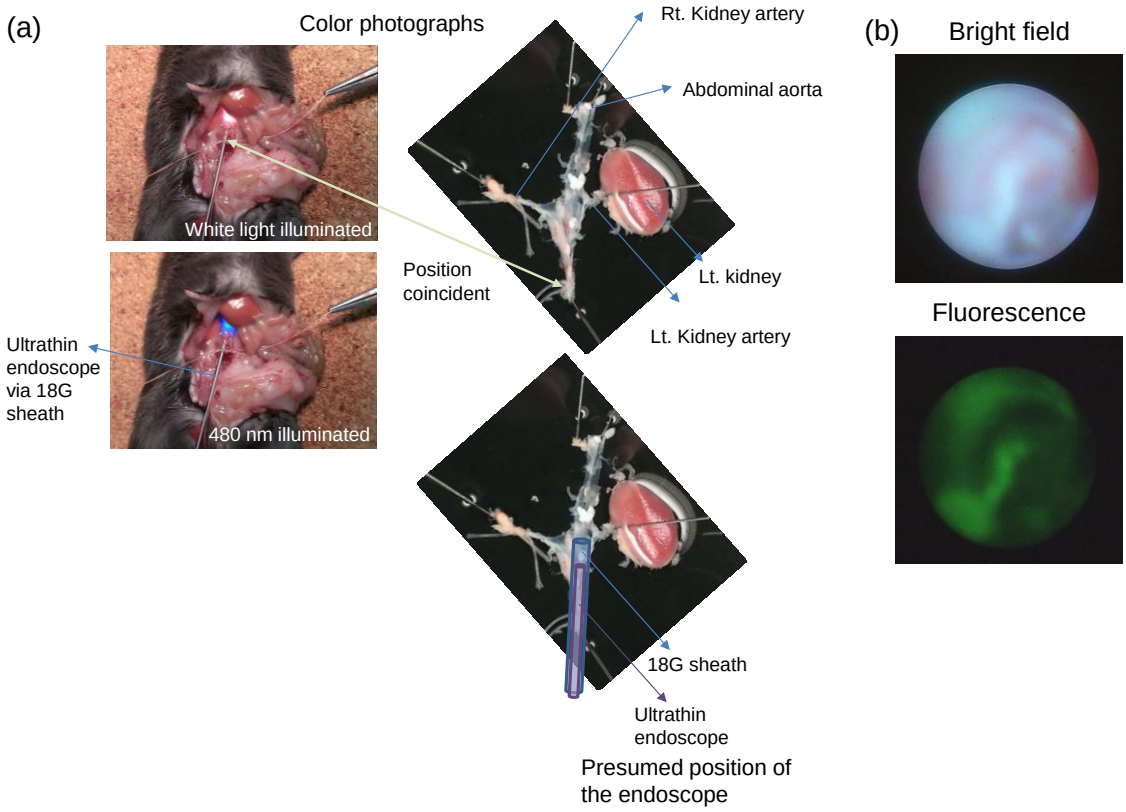


Fig. S10. Fluorescence endoscopic images of atherosclerotic plaques in LDLR$^{-/-}$ mice. **2BDP3Gd** (7.4 μmol/kg) was intravenously administered via the tail vein 48 hr before imaging. (a) Overview of the endoscopic imaging of atherosclerotic plaques. (b) Fluorescence images obtained from lumen of aorta. Elastica van Gieson (EVG) staining of another artery stained with **2BDP3Gd** was performed, and formation of foam cell-rich plaques was confirmed (data not shown).

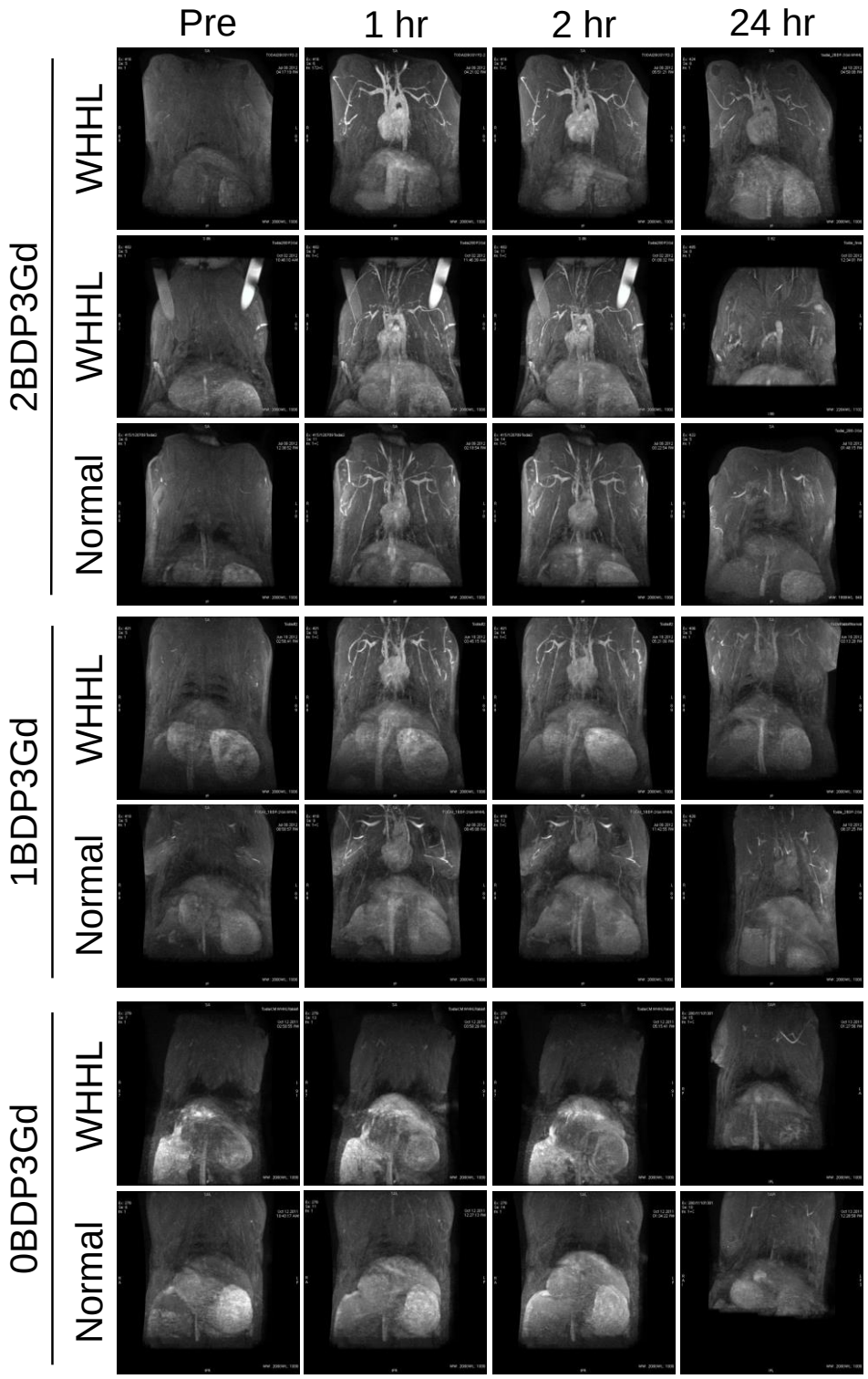


Fig. S11. T_1-weighted MR angiography of WHHL or normal rabbit before and at 1 hr, 2 hr and 24 hr after administration of **2BDP3Gd**, **1BDP3Gd**, or **0BDP3Gd** (7.4 μmol/kg). **0BDP3Gd** was excreted within 1 hr after its injection. In contrast, **2BDP3Gd** and **1BDP3Gd** showed prolonged circulation times (~24 hr).

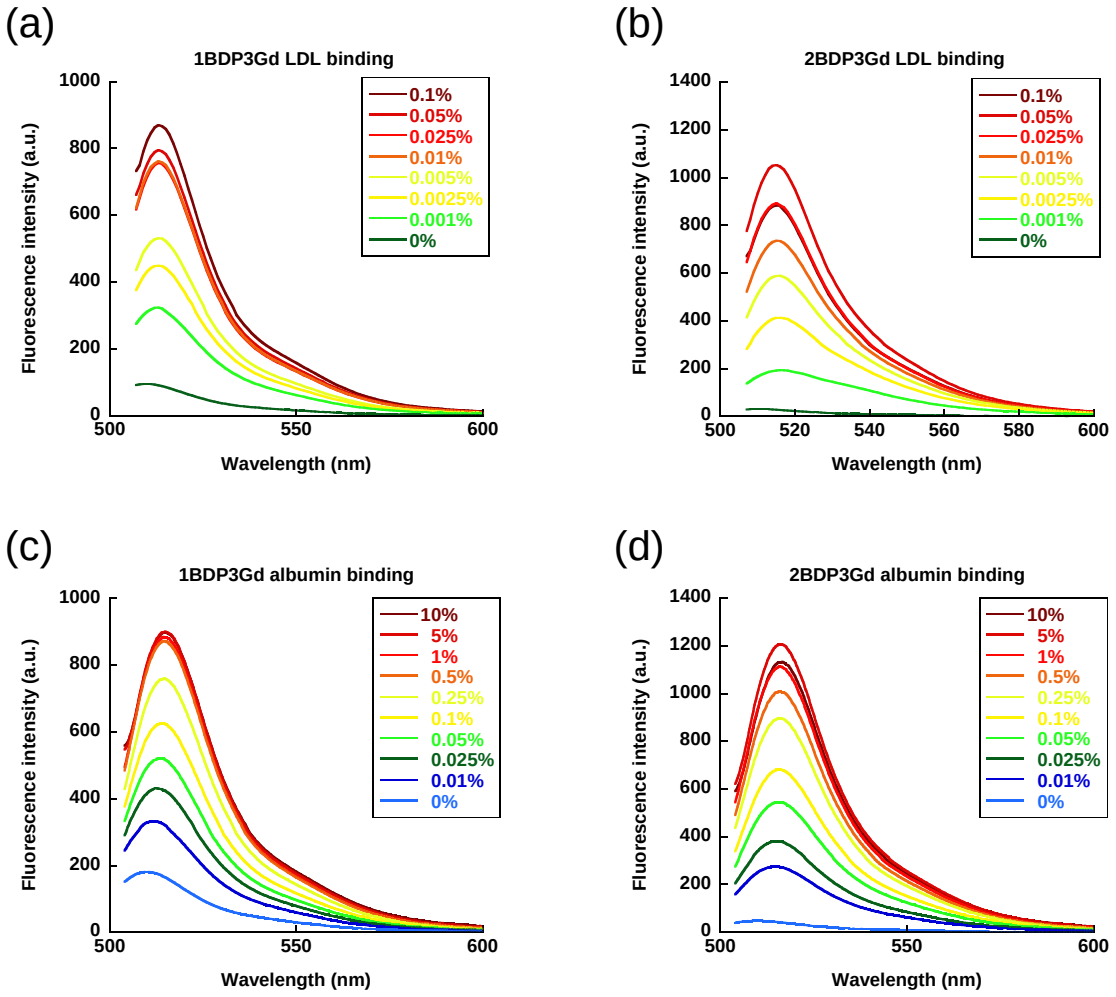


Fig. S12. Fluorescence spectra of **1BDP3Gd** or **2BDP3Gd** (1 μM) in the presence of various concentrations (w/v %) of HSA or LDL in PBS (pH 7.4). Excitation wavelength was 490 nm. (a) **1BDP3Gd** in the presence of LDL. (b) **2BDP3Gd** in the presence of LDL. (c) **1BDP3Gd** in the presence of HSA. (d) **2BDP3Gd** in the presence of HSA. **1BDP3Gd** and **2BDP3Gd** have low fluorescence quantum yields in aqueous solutions, but their fluorescence intensity increased when LDL or HSA was added to the solution. This is because **2BDP3Gd** and **1BDP3Gd** bind to LDL or HSA via hydrophobic interaction. The physiological concentration of LDL is 0.4-0.01% w/v,[3] and that of HSA is 4.7-3.6% w/v.[4]

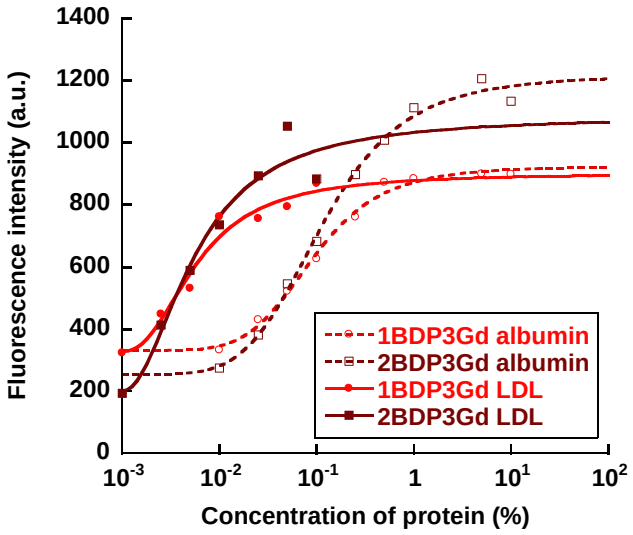


Fig. S13. Fluorescence intensity of **2BDP3Gd** or **1BDP3Gd** (1 μM) in the presence of various concentrations (w/v %) of HSA or LDL in PBS (pH 7.4). Ex/Em = 490 nm/510 nm.

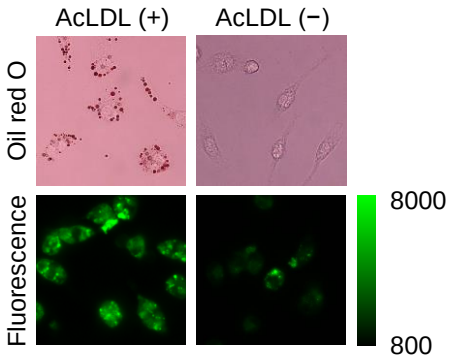


Fig. S14. Fluorescence and Oil red O staining images of foam cells loaded with **2BDP3Gd** (1 μM in DMEM containing 0.1% DMSO as a cosolvent). Upper and lower panels show Oil red O staining and fluorescence images, respectively. **2BDP3Gd** showed the accumulation to LDL in cellular level.

Supplementary References

1. T. Yamane, K. Hanaoka, Y. Muramatsu, K. Tamura, Y. Adachi, Y. Miyashita, Y. Hirata, and T. Nagano, *Bioconjugate Chem.*, 2011, **22**, 2227–2236.
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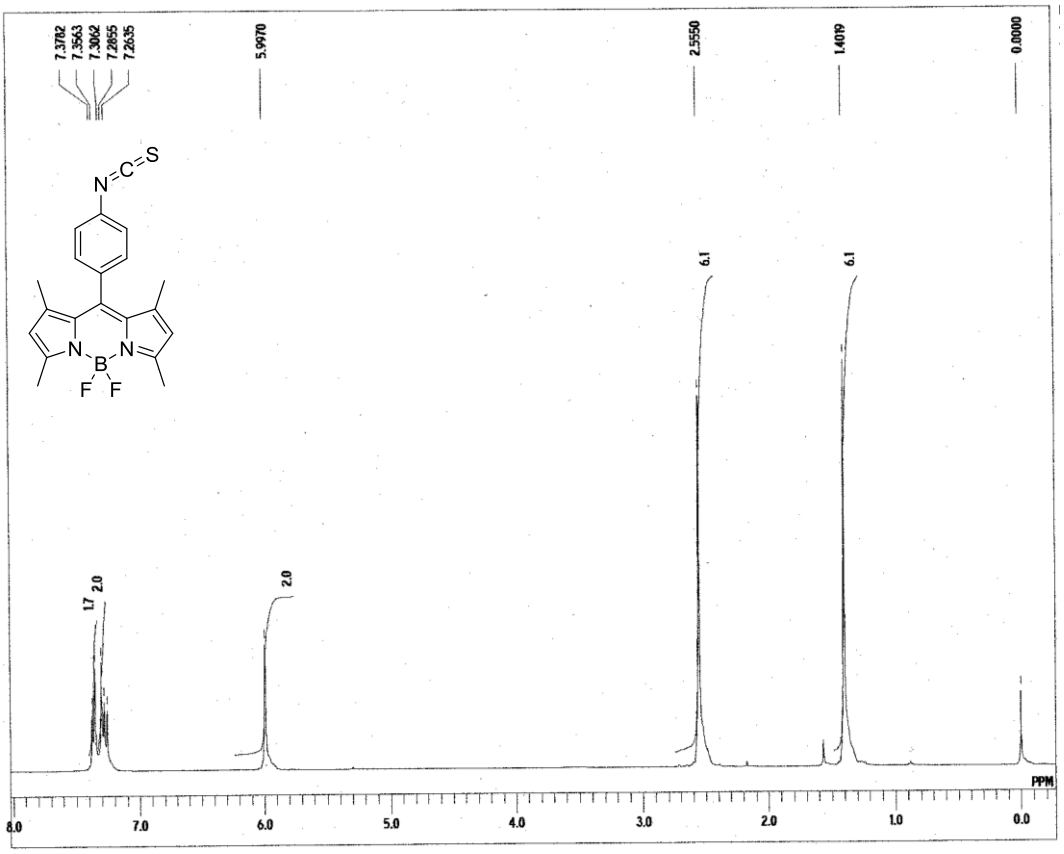
3. R. J. Havel, T. Kita, L. Kotite, J. P. Kane, R. L. Hamilton, J. L. Goldstein, and M. S. Brown, *Arter. Thromb. Vasc. Biol.*, 1982, **2**, 467–474.
4. K. C. Fearon, J. S. Falconer, C. Slater, D. C. McMillan, J. A. Ross, and T. Preston, *Ann. Surg.*, 1998, **227**, 249–54.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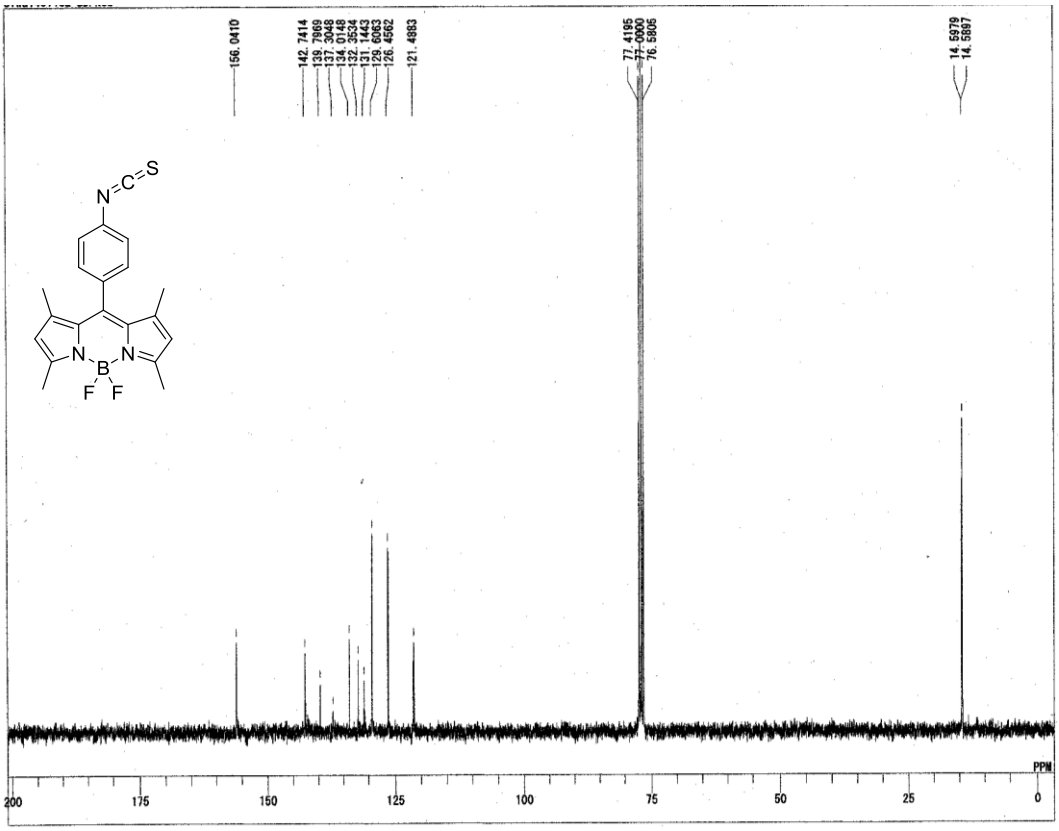
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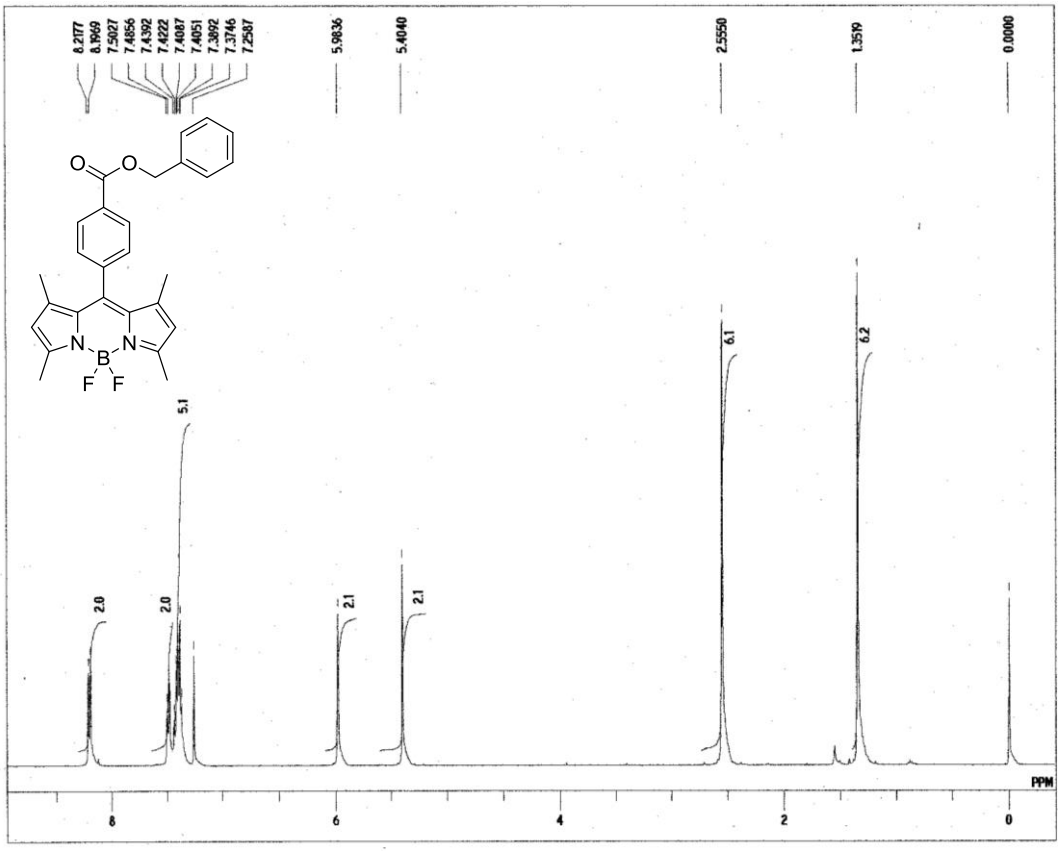
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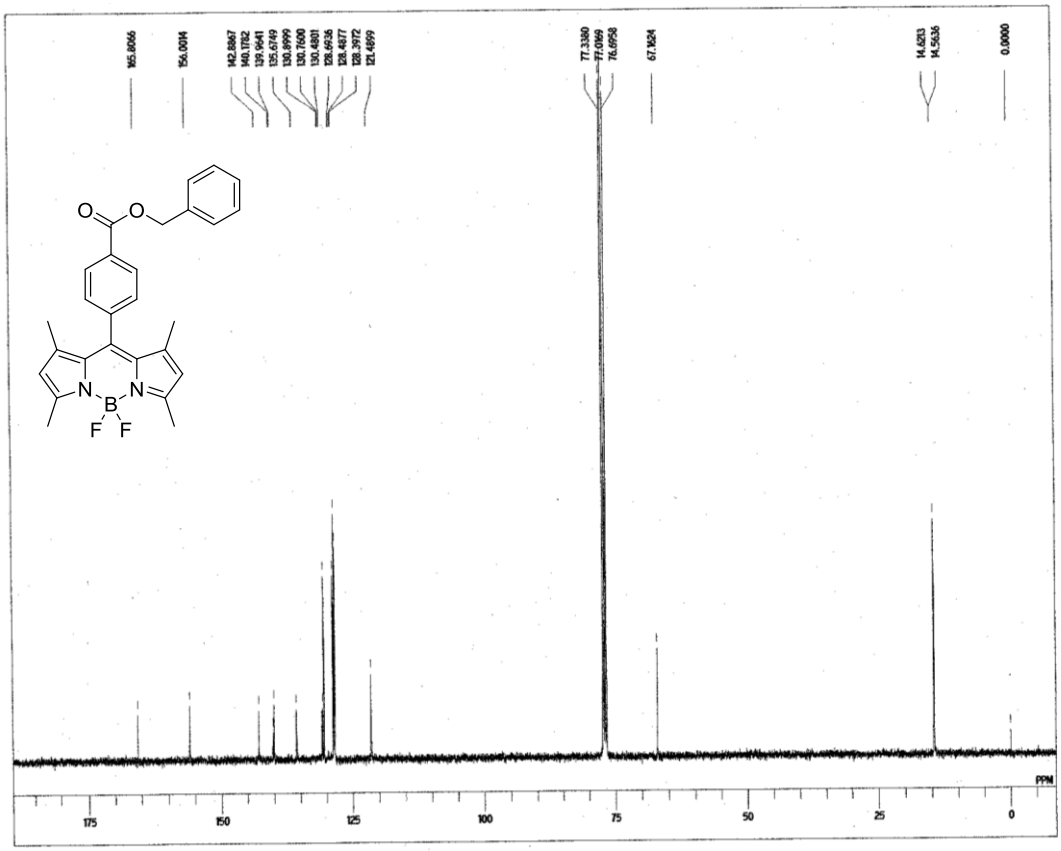
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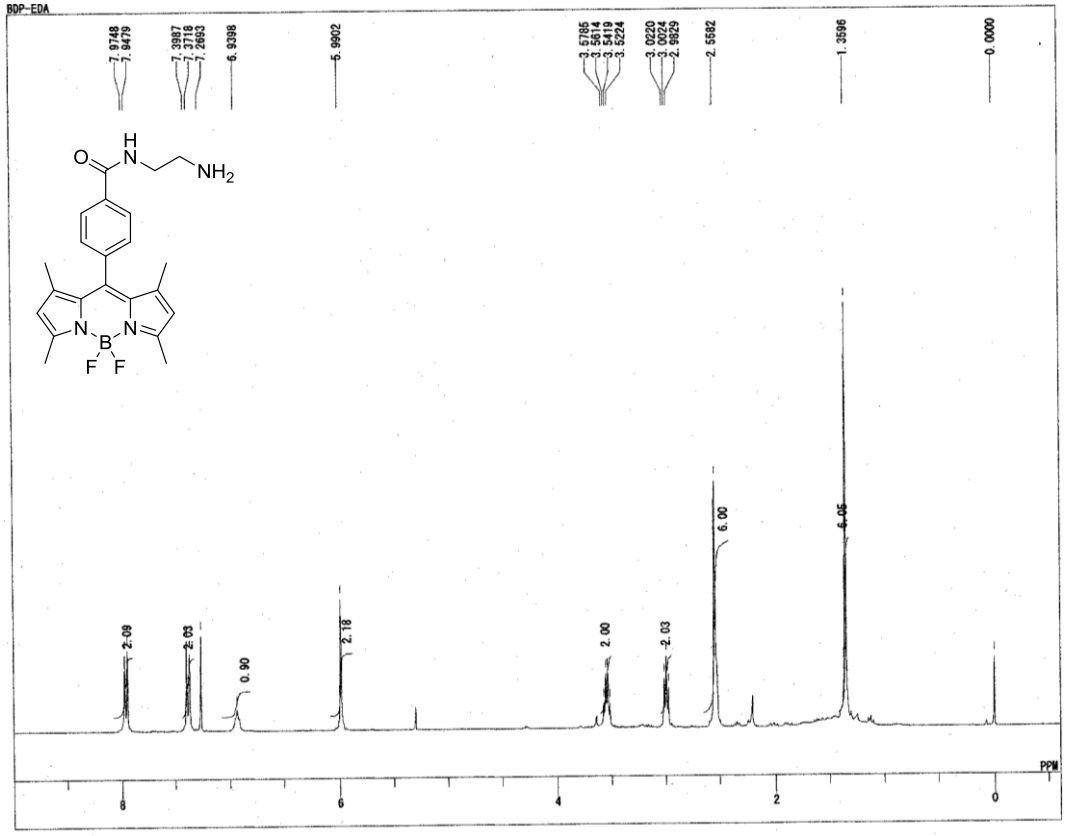
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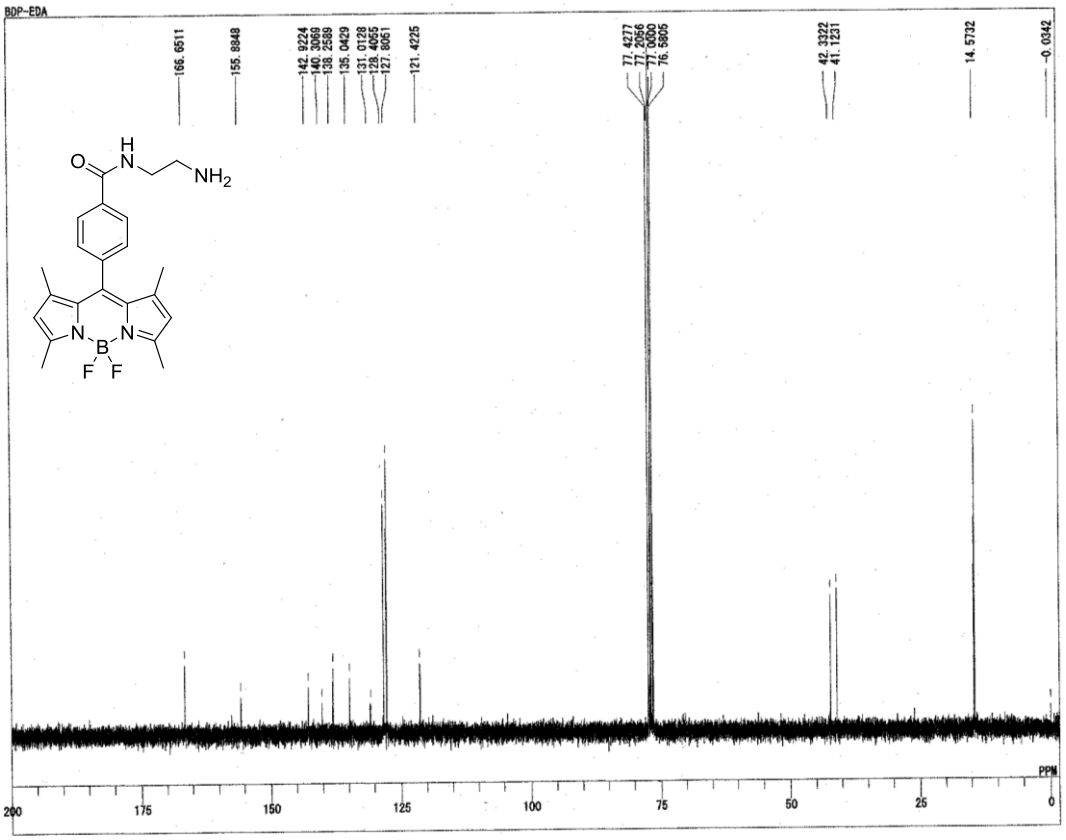
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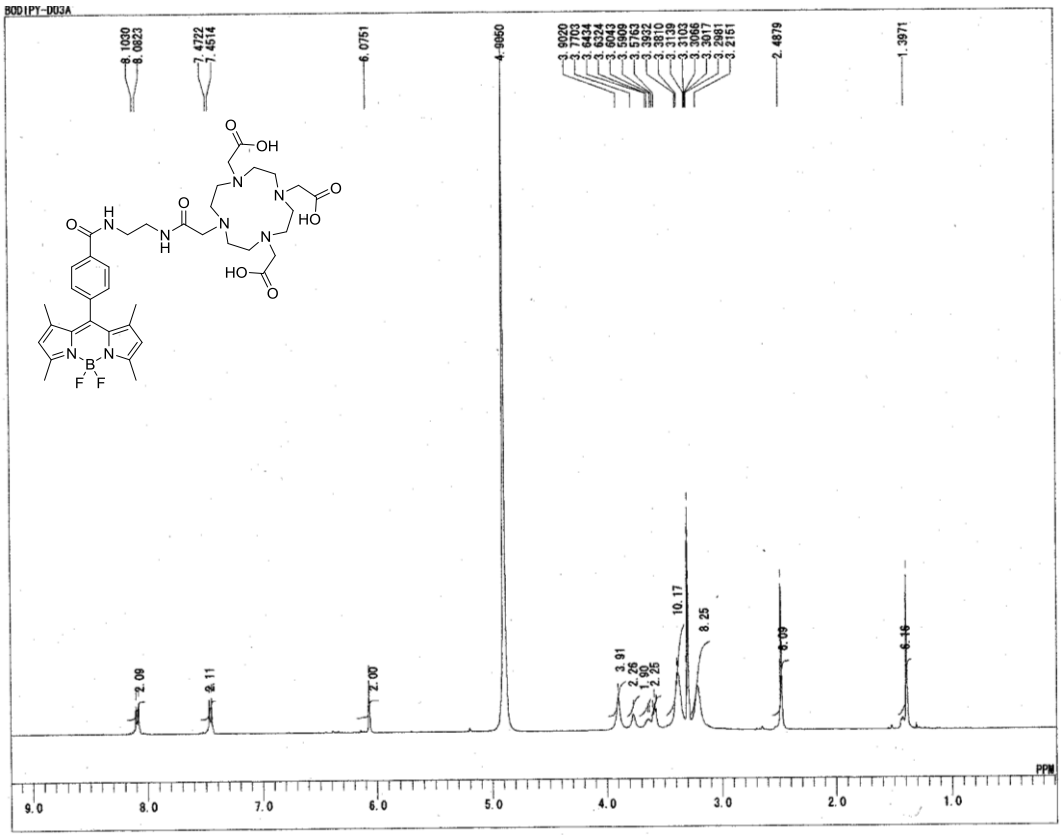
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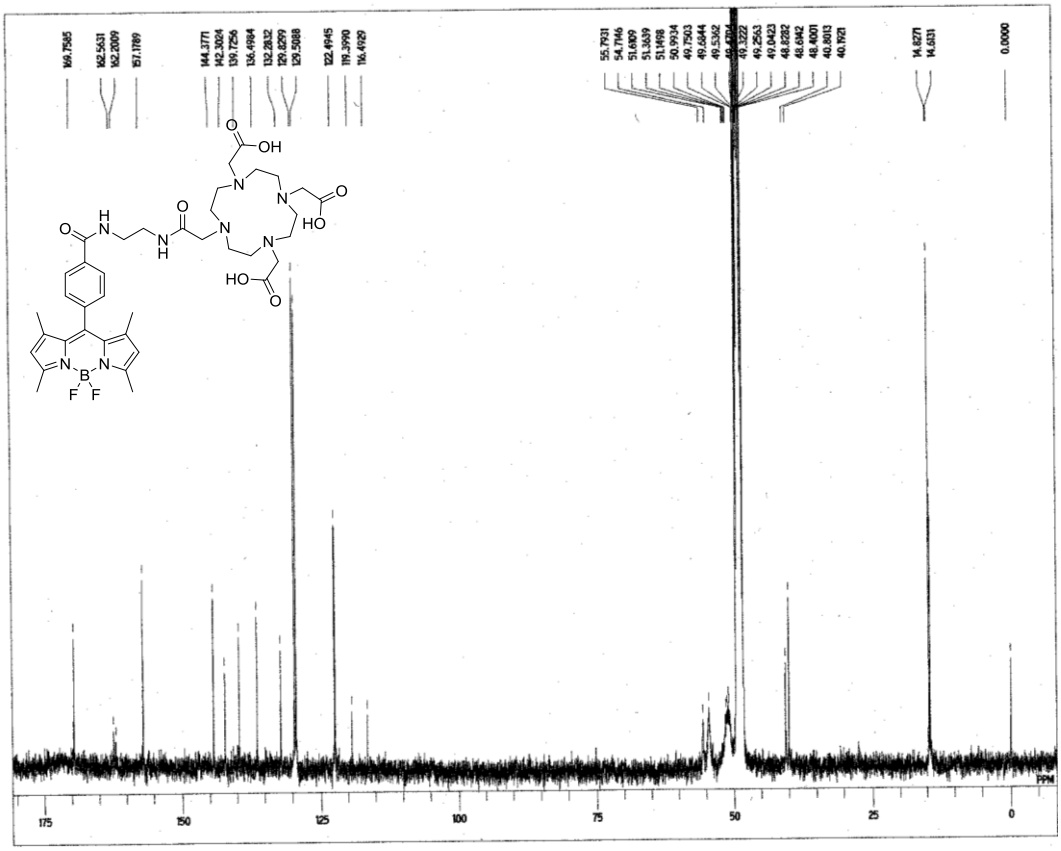
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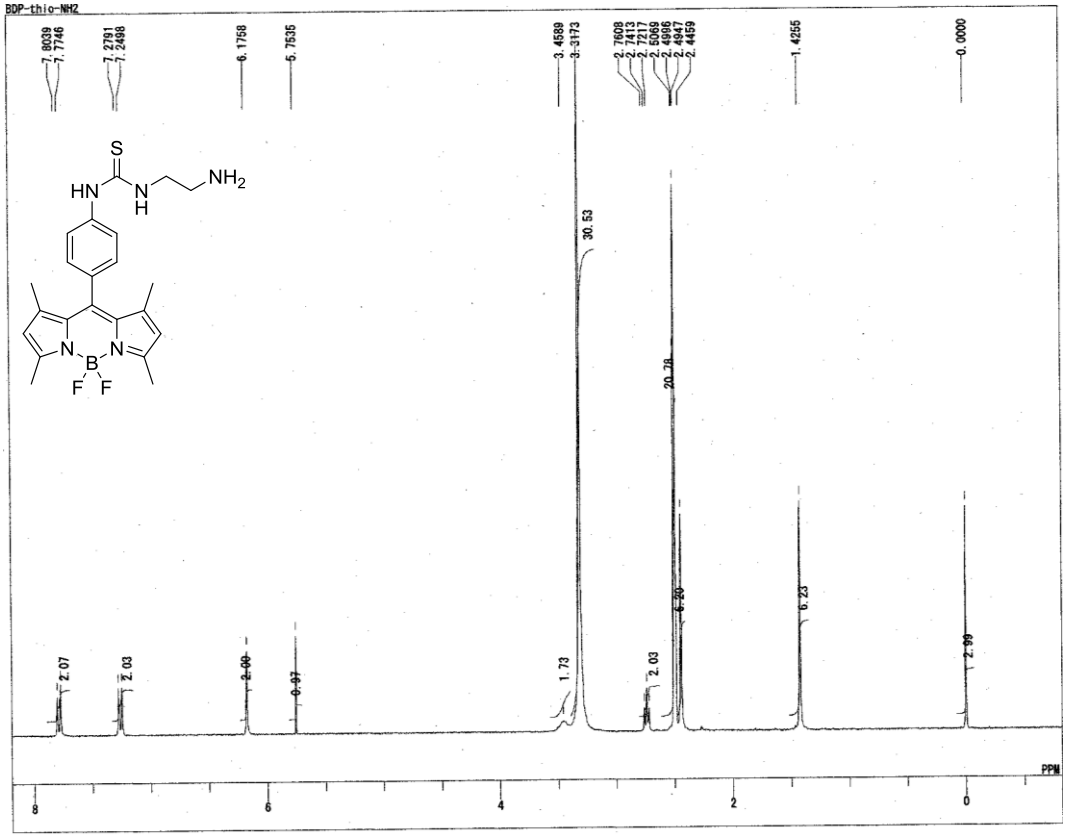
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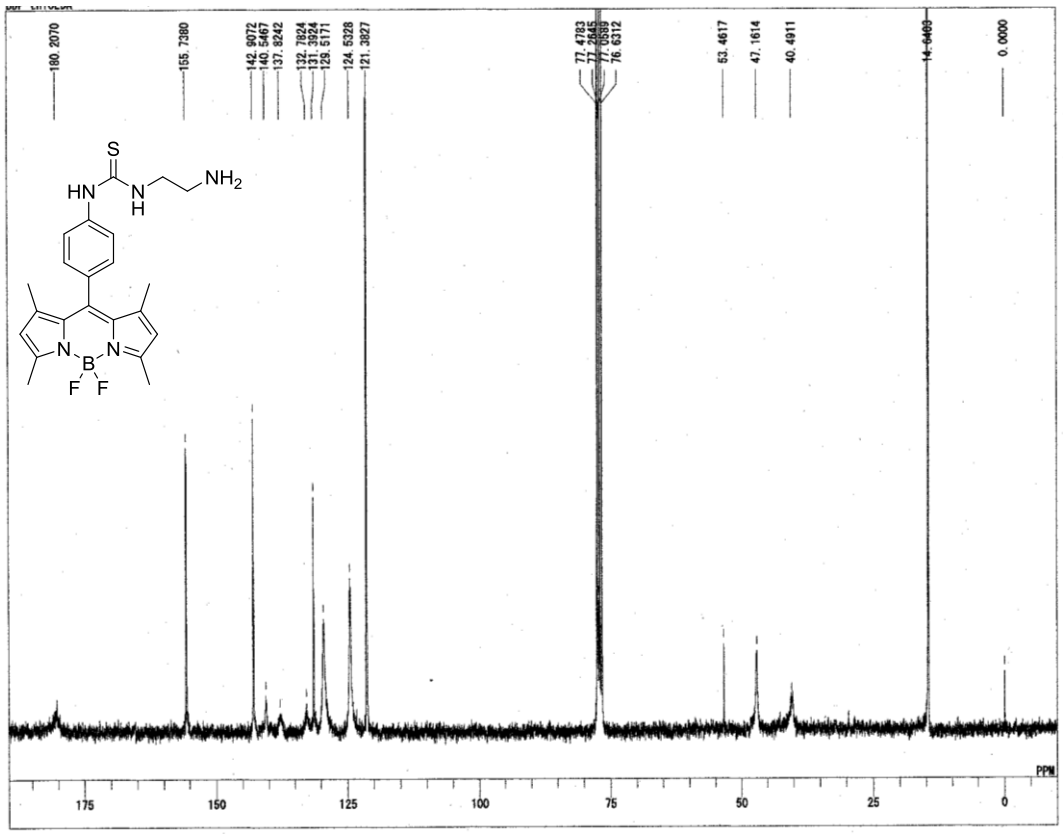
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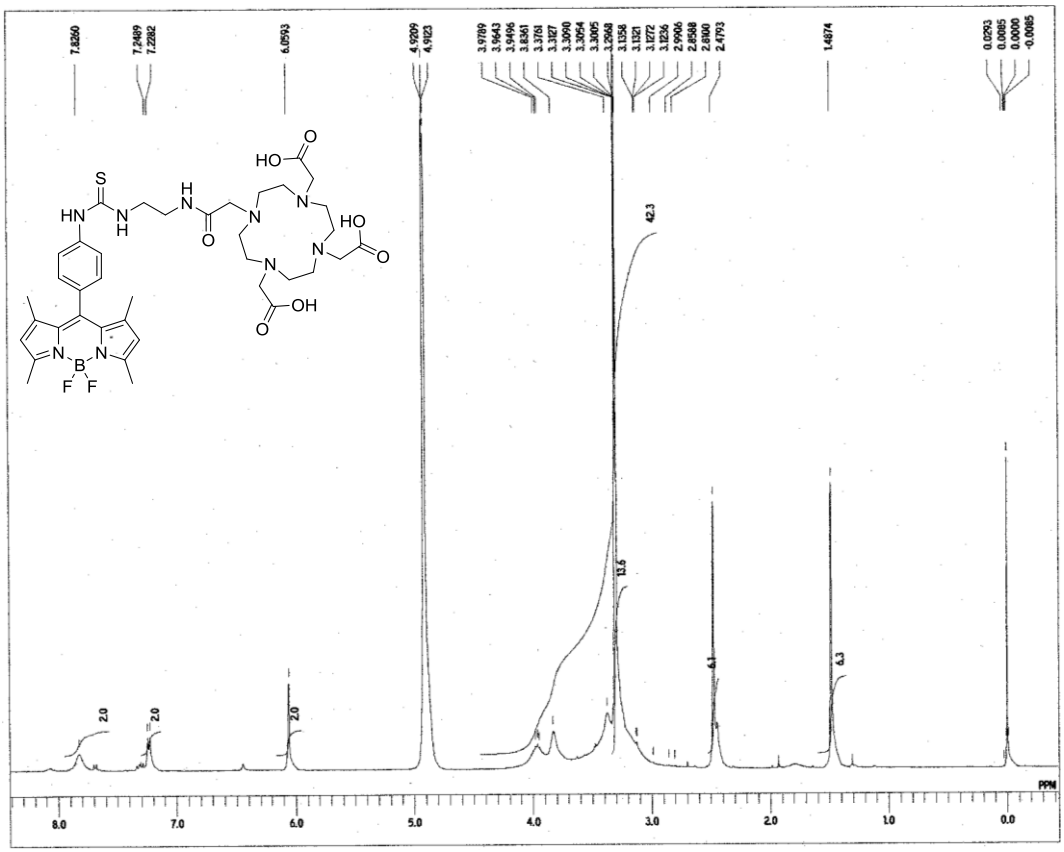

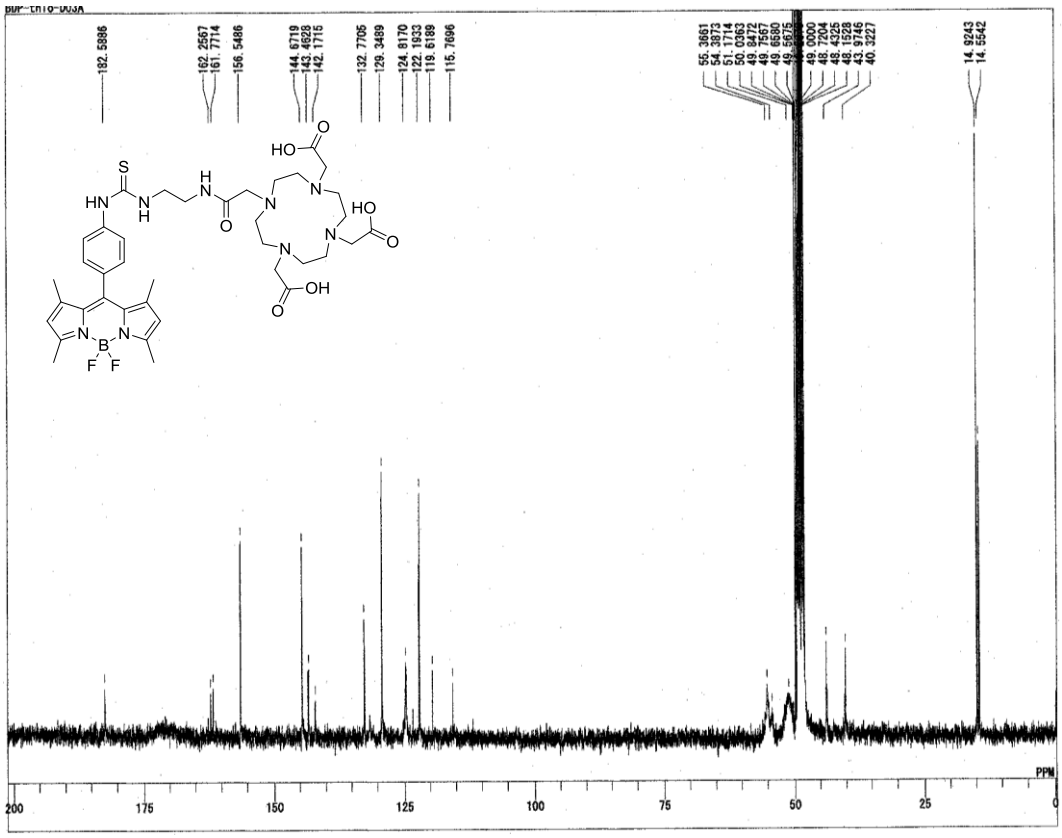

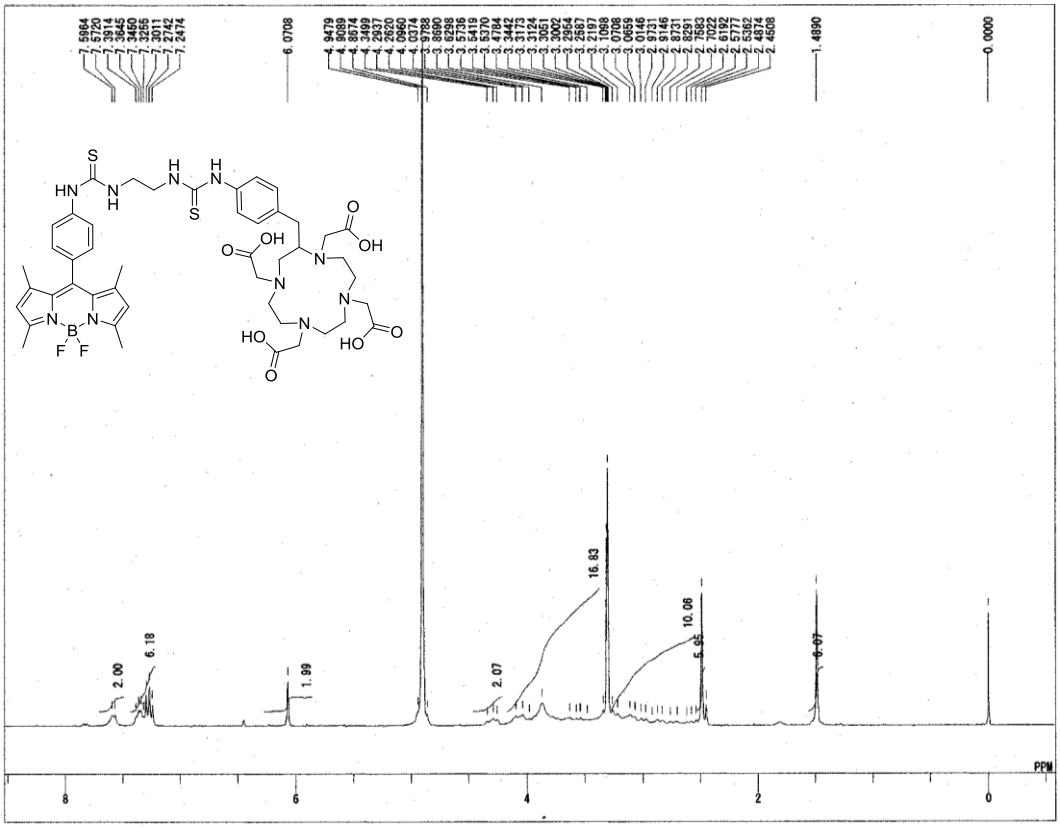

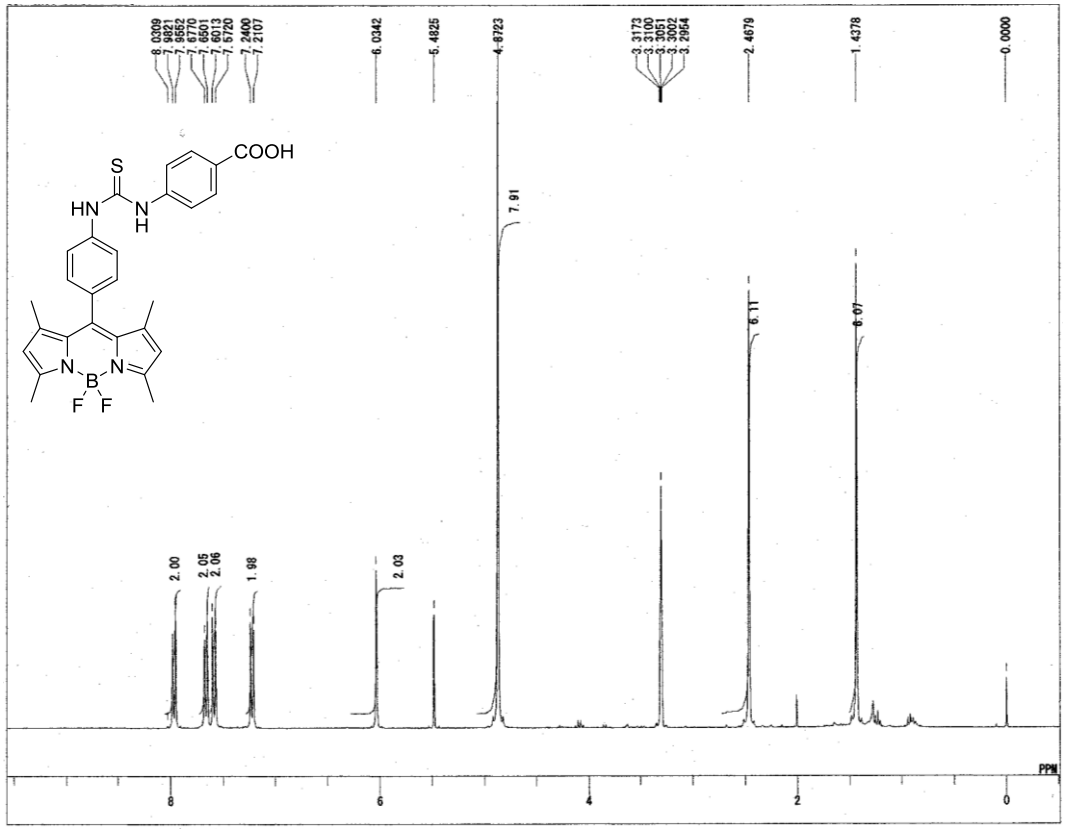

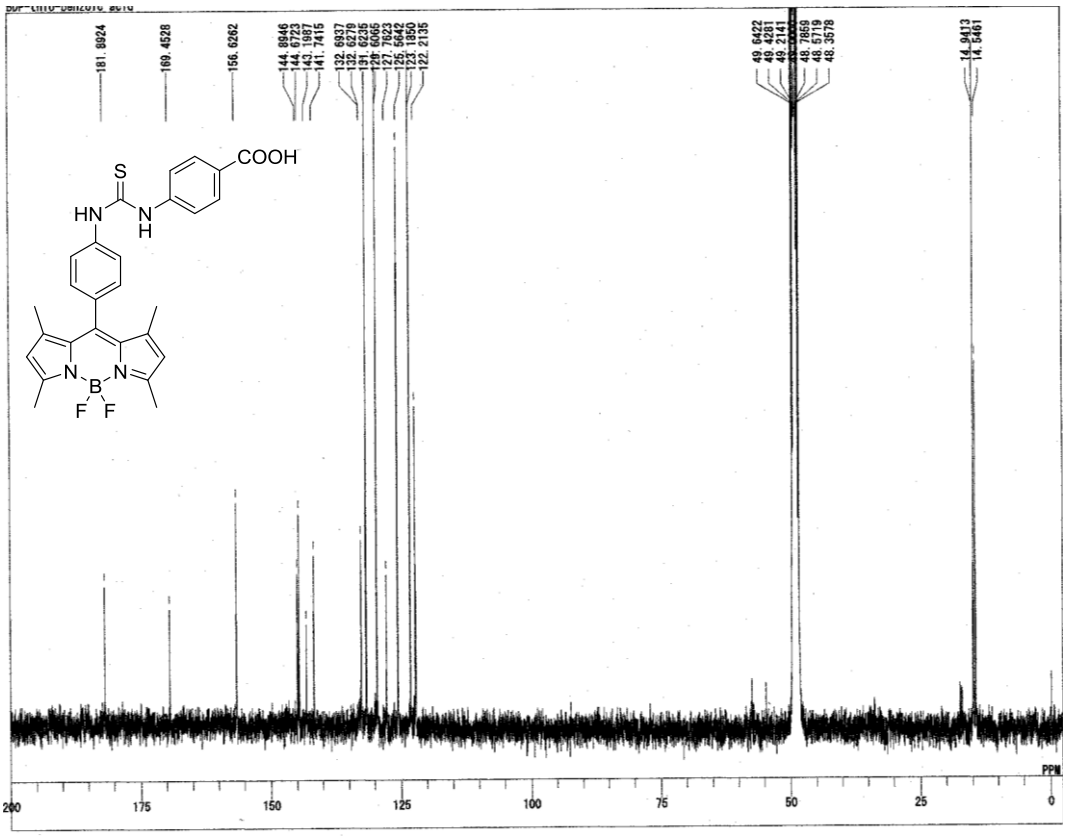
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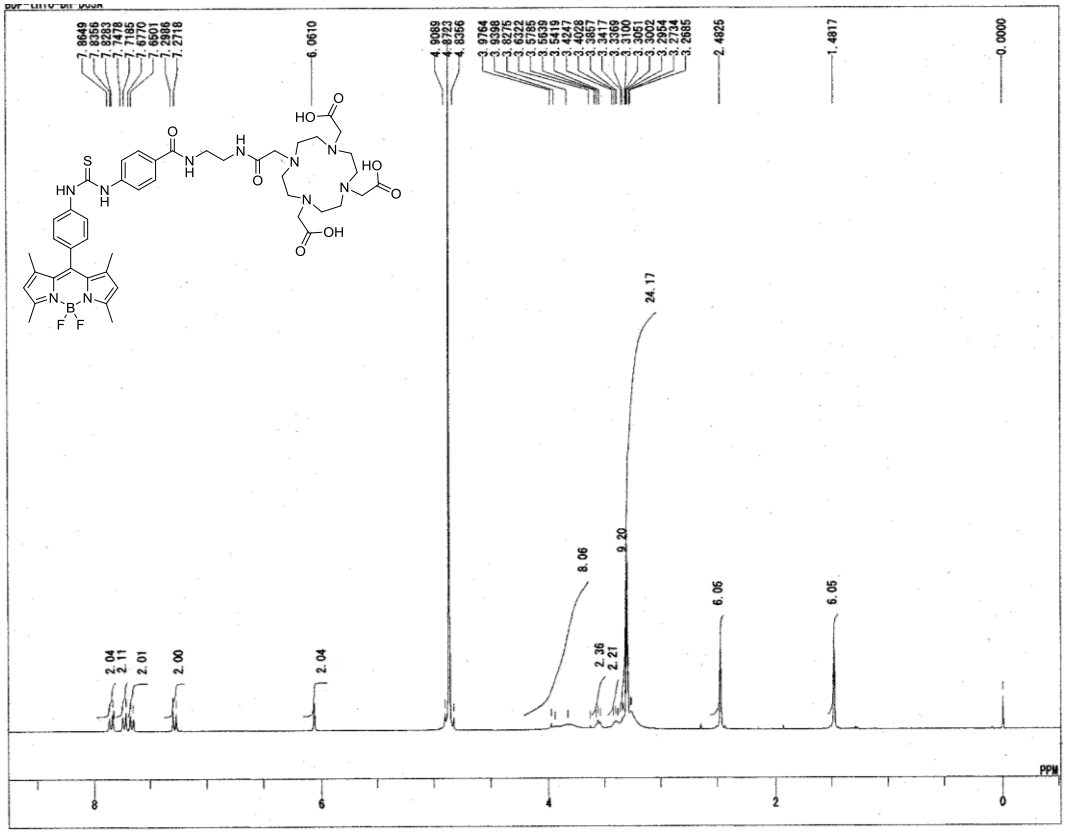
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2.04
2.11
2.01
2.00
2.04
2.36
2.21
HO
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OH
HN
S
N
H
O
B
F
F
PPM
8
6
4
2
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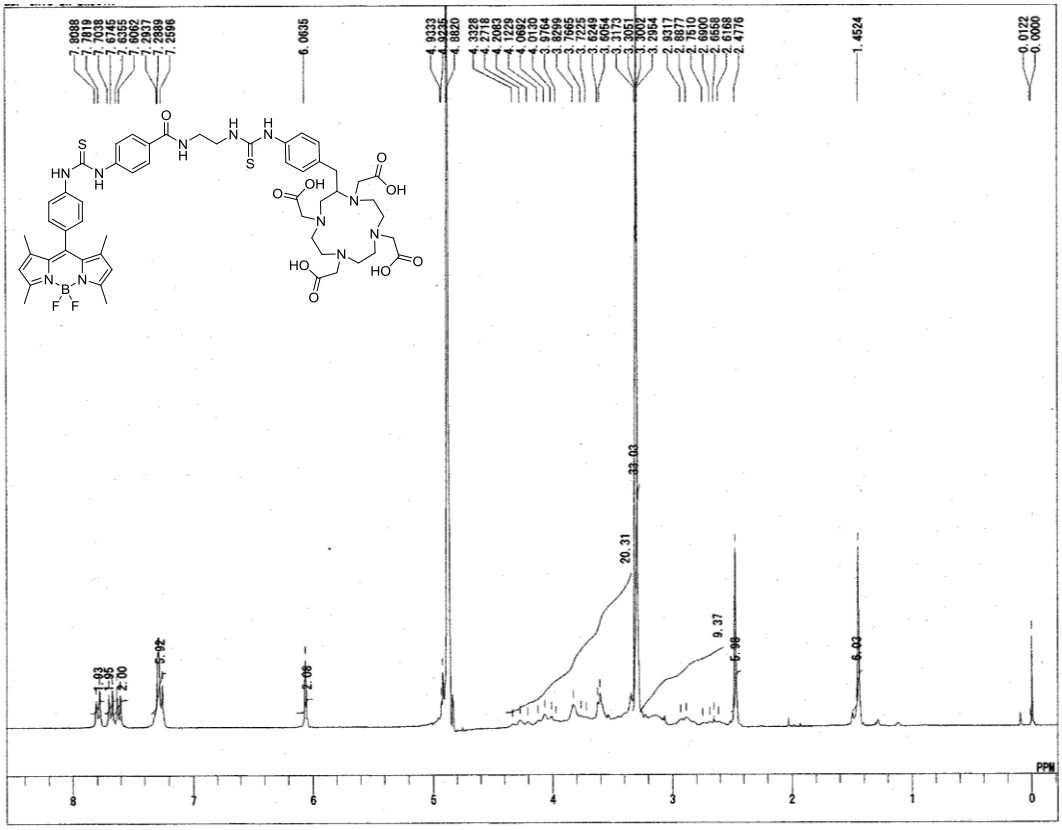

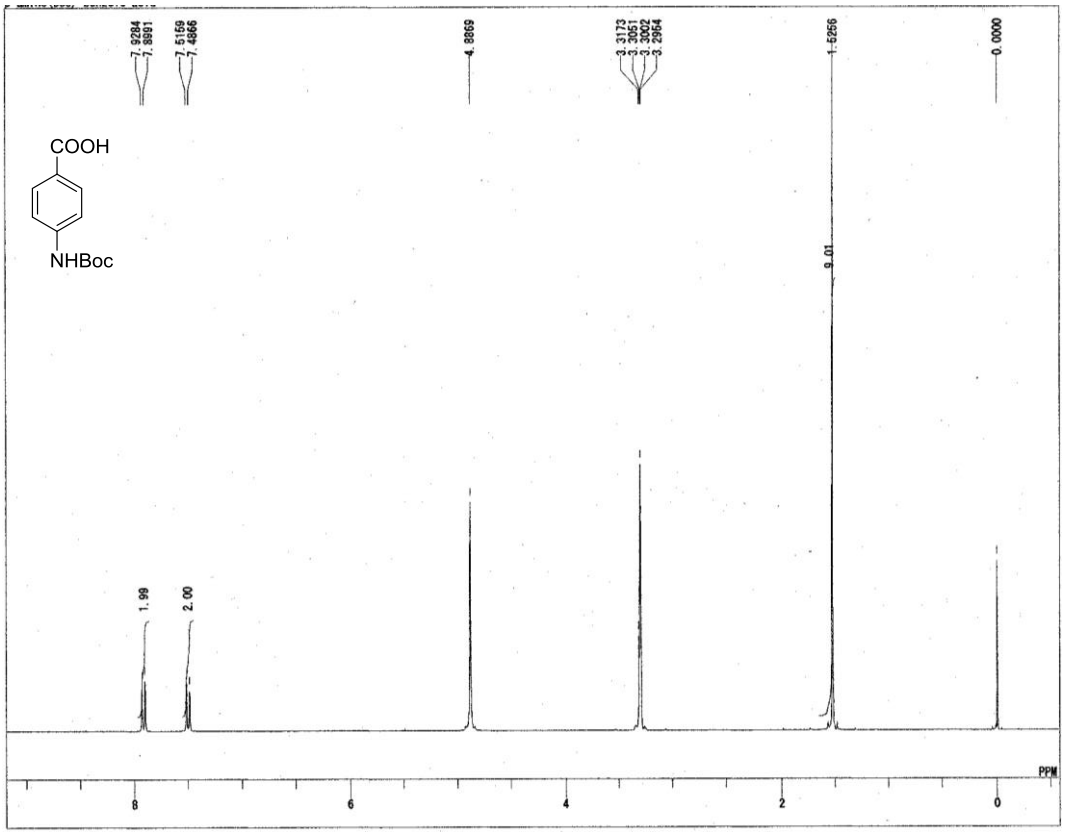

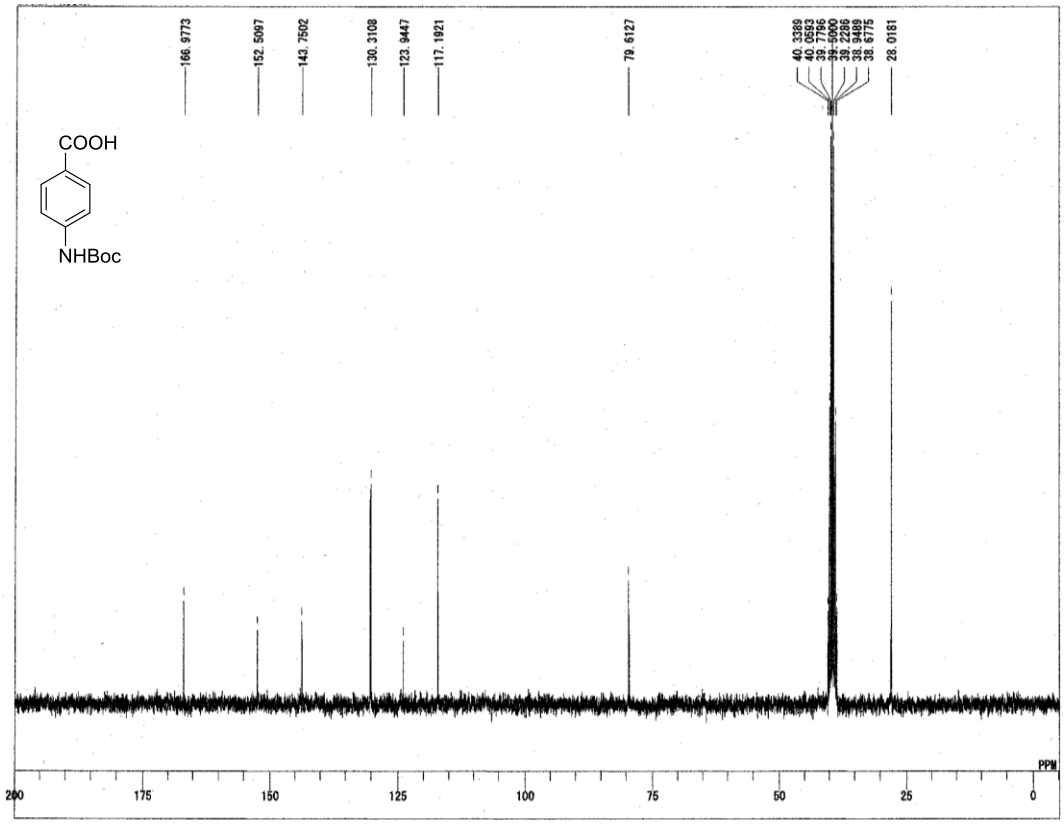
COOH
NHBoc
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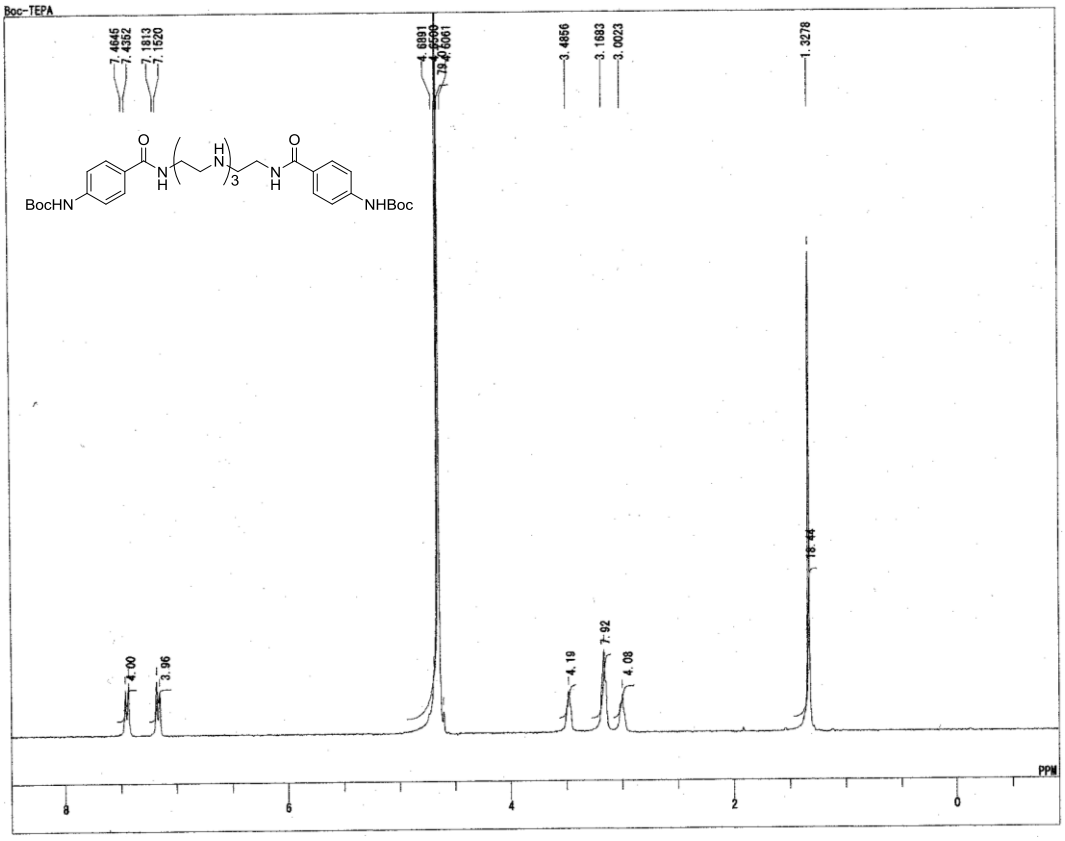
143.7502
130.3108
123.9447
117.1921
79.6127
40.3389
40.0583
39.7796
39.5000
39.2206
38.9419
38.6775
28.0161
PPM
200
175
150
125
100
75
50
25
0


Boc-TEPA
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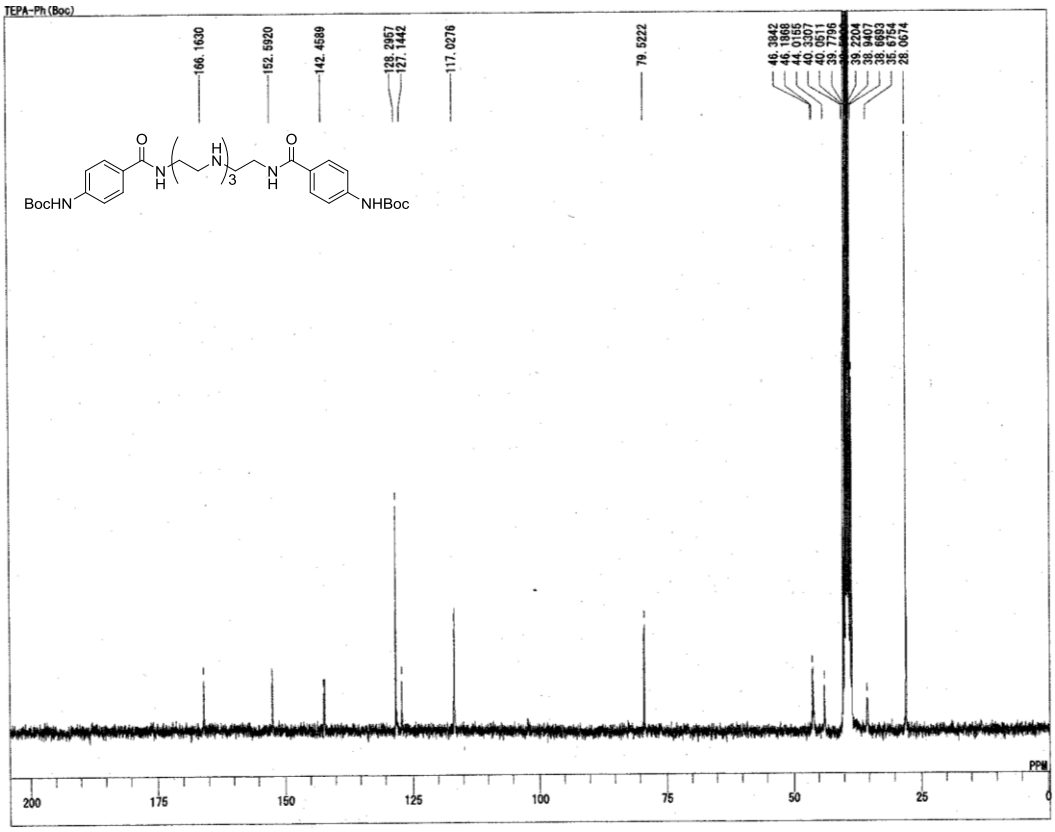
7.4352
7.1813
7.1520
4.6801
3.4866
3.1683
3.0023
1.3278
BocHN
NHBoc
18.44
7.92
4.19
4.08
4.00
3.96
PPM
8
6
4
2
0


TEPA-Ph(Boc)
166.1630
152.5920
142.4589
128.2957
127.1442
117.0276
79.5222
46.3842
46.1868
44.0165
40.3077
40.0911
39.7796
39.2204
38.9407
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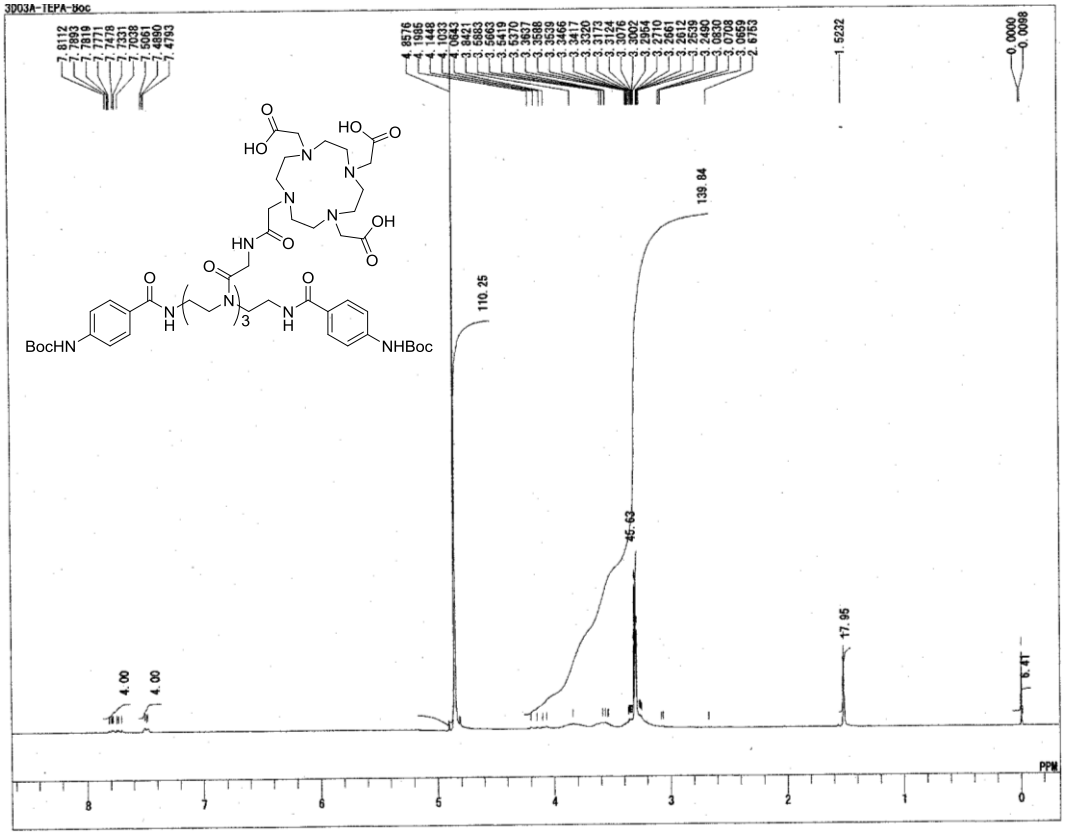
28.0674
BocHN
NHBoc
PPM
200
175
150
125
100
75
50
25
0


3D03A-TEPA-Boc
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7.7519
7.7371
7.7478
7.7331
7.7038
7.5061
7.4850
7.4793
4.8576
4.1986
4.1448
4.1033
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3.8421
3.6663
3.6465
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3.5370
3.3637
3.3588
3.3539
3.3446
3.3417
3.3320
3.3173
3.3124
3.3076
3.3027
3.2954
3.2710
3.2661
3.2612
3.2539
3.2490
3.2030
3.1708
3.0659
2.6753
1.5232
0.0000
0.0088
139.84
110.25
45.63
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6.47
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4.00
BocHN
NHBoc
PPM
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7
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5
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1
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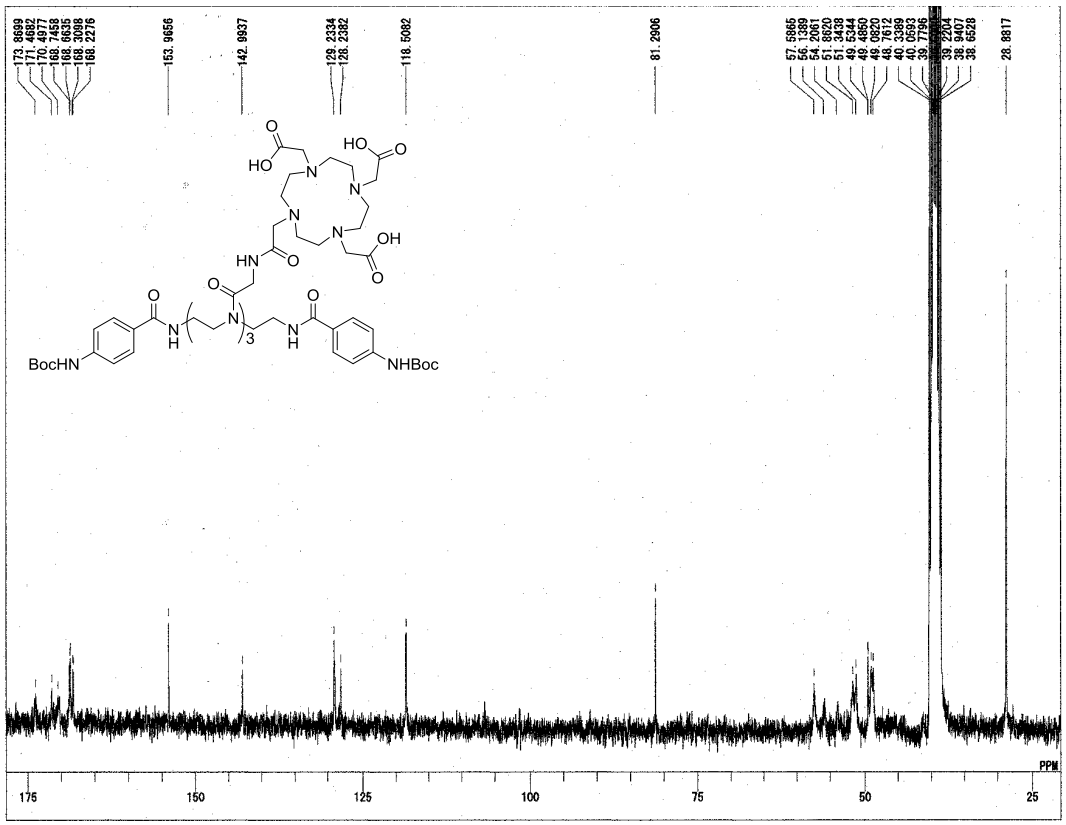
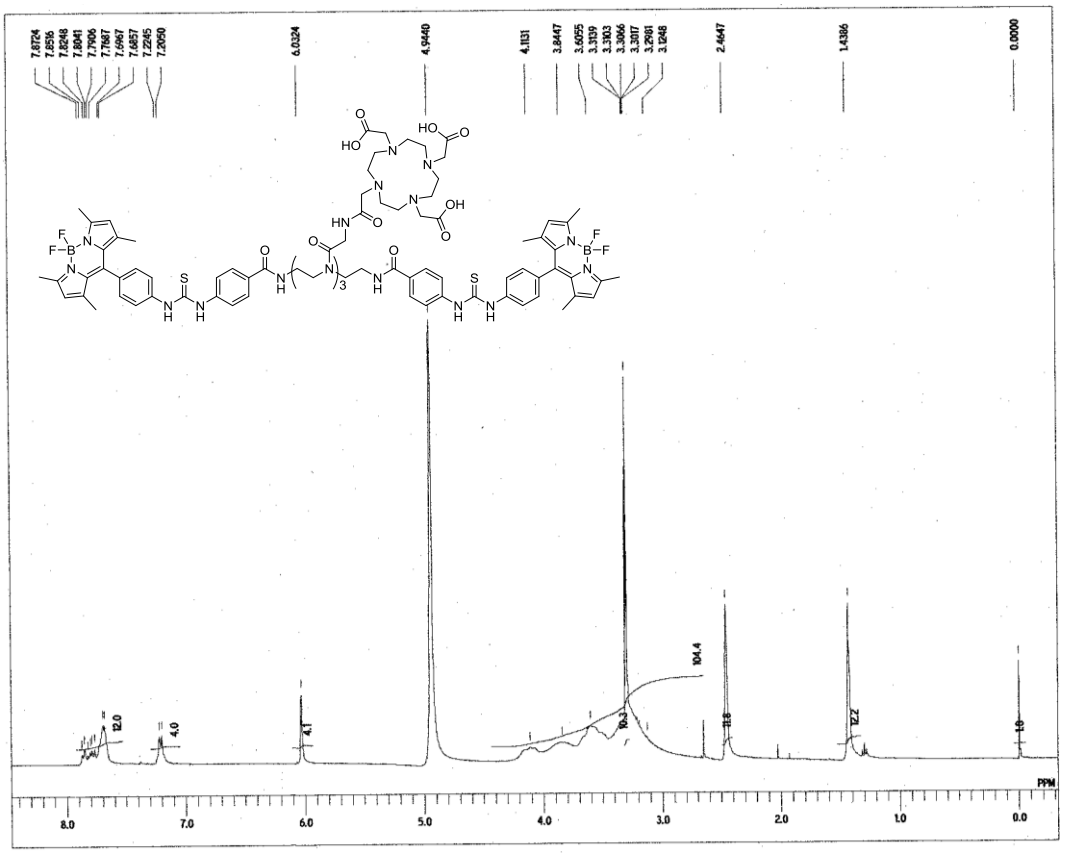

BocHN
NHBoc
PPM


PPM