Supporting Information

Towards A Chemical Marker for Inflammatory Disease: A Fluorescent Probe for Membrane-Localized Thioredoxin

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**Synthetic materials and methods.** All reactions were carried out under a nitrogen atmosphere. Silica gel 60 (Merck, 0.063–0.2 mm) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F254 silica gel (precoated sheets, 0.25 mm thick). $^1$H and $^{13}$C NMR spectra were recorded in CDCl$_3$, CD$_3$OD (Cambridge Isotope Laboratories, Cambridge, MA) and collected on either Varian 300 or Varian 400 MHz spectrometers. All chemical shifts are reported in ppm using either residual proton signals of the deuterated solvent or TMS as an internal reference. ESI mass spectral analyses were carried out using a LC/MS-2020 Series (Shimadzu) instrument. MALDI-TOF mass spectral analyses were carried out at the Seoul National University National Center for Inter-university Research analytical facilities.

**Spectroscopic materials and methods.** Stock solutions of biologically relevant metals [GSH, Hcy, Cys, H$_2$O$_2$, Na(I), K(I), Zn(II), Mg(II), Fe(II), Fe(III), Cu(II), Mn(II) and Ca(II)] were prepared in triple distilled water. Stock solutions of compounds were prepared in DMSO. All spectroscopic measurements were acquired 30 min after addition of the analytes in question and were recorded in PBS solution (pH 7.4) containing 5% (v/v) DMSO at 37 °C. Absorption spectra were recorded on an S-3100 (Scinco) spectrophotometer, and fluorescence spectra were recorded using an RF-5301 PC spectrofluorometer (Shimadzu) equipped with a xenon lamp. Samples for absorption and emission measurements were contained in quartz cuvettes (3 mL volume). Excitation was effected at 430 nm using excitation and emission slit widths that were both 5 nm.

**Liposome preparation.** Liposomes were prepared by the solvent evaporation method$^1$. Briefly, 36 μL of (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) DPPC (0.1 M in chloroform) and 24 μL of cholesterol (0.1 M in chloroform) were added to a 50 mL round bottom flask containing 940 μL of chloroform and 200 μL of methanol. The aqueous phase (7 mL of HEPES buffer, 10 mM, pH = 7.4) was then carefully added along the flask walls. The organic solvents were removed in a rotary evaporator under reduced pressure at 40 °C and 40 rpm. Subsequently, the resulting aqueous solution was subjected to sonication for 30 min. The liposomes were characterized by transmission electron microscopy (TEM) (Fig. S4). To stain the liposomes, a small amount of 1 in DMSO was added with measurements being made after 1 h at 37 °C. The [1]:[liposome] ratio was around 1:170.
**Cell culture and imaging.** A human cervical cancer cell line (HeLa) and a human hepatoma cell line (HepG2) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and in RPMI 1640, respectively, each supplemented with 10% FBS (WelGene), penicillin (100 units/mL), and streptomycin (100 μg/mL). Two days before imaging, the cells were placed on glass-bottomed dishes (MatTek) which were incubated in a humidified atmosphere containing 5% (v/v) CO₂ at 37 °C. Cell images were obtained using confocal microscopes from Leica (Leica TCS SP2 model) and Zeiss (Zeiss LSM 510 model). All fluorescence images of probe 1 were obtained using an excitation wavelength of 458 nm and a long path (> 505 nm) emission filter. Other information is in the Figure captions.

**Treatment of HeLa cells with cycloheximide and dinitrophenol.** For the tests of Trx secretion from HeLa cells, the cells were plated at 2 × 10⁵/well in glass-bottom dish. After incubation overnight, the cells were treated with Dulbecco's Modified Eagle’s Medium (DMEM) containing 0.5 mM dinitrophenol or 0.1 mM cycloheximide for 3 h at 37 °C. The media were exchanged for PBS containing 1 (5.0 μM) prior to confocal microscopic imaging.

**Treatment of HepG2 cells with fatty acids.** HepG2 cells were plated 2 × 10⁵/well in glass-bottom dishes and incubated for 24 h. The media were changed to ones containing 0.7 mM oleic acid or 0.7 mM palmitic acid with BSA for 24 h at 37 °C. The media were then replaced with RPMI (Roswell Park Memorial Institute) medium without FBS for 4 h. The cells were incubated with PBS containing 1 (5.0 μM) prior to confocal microscopic imaging.

**Western blot experiments.** To collect Trx protein that was presumably secreted into the extracellular medium, media from each well were treated with 20% trichloroacetic acid for 30 min in ice and centrifuged at 14,000 rpm for 15 min at 4 °C. The resulting pellet was washed with 200 μL cold acetone and centrifuged two more times before removing the acetone by exposure to the laboratory atmosphere for 10 min. After being air-dried in this way, the pellet was resuspended in a SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) sample buffer. The secreted Trx was separated by SDS-PAGE (15% polyacrylamide) and blotted onto a PVDR membrane (pore size 0.2 μm) using a Bio-Rad Transblot (Trans-Blot SD Semi-Dry transfer Cell) kit (Bio-Rad, Inc., CA). The membrane
was treated with a blocking solution (5% (w/v) non-fat dry milk in TBS-T) for 1 h at room temperature, before being subjected to primary antibody binding using goat anti-Trx antibody (ab16965, Abcam, Inc., MA, USA); this was done using a blocking solution and overnight treatment at 4 °C. The excess antibody was removed by rinsing 3 times with TBS-T. For the secondary antibody binding studies, the membrane was incubated with a donkey anti-goat IgG-HRP antibody (sc-2005, Santa Cruz, Inc., Texas) in a blocking solution for 1 h at room temperature. After the membrane was rinsed with TBS-T buffer 3 times, the immunoreactive bands were detected via treatment with an ECL substrate solution (Western Blot Detection System, iNtRON, Inc., Kyunggi-do, Korea), followed by visualization on X-ray film AGFA. The density ratio was normalized to that of the control. The values are expressed as mean ± S.D, with an n = 4 (i.e., four independent samples from different cells). Data were analyzed statistically by the student’s t-test and a value of p < 0.05 was considered to be statistical significant.

**Immunohistochemistry-based colocalization of Trx with TXNIP or NLRP3.** Cells in PBS solution were fixed with 4% paraformaldehyde in PBS for 30 min at room temperature and washed with a buffer (0.1% BSA and 0.1% Triton X-100). The fixed cells were blocked with a blocking buffer (1% BSA). After the blocking buffer was discarded, the cells were incubated with rabbit anti-Trx antibody (1:200, diluted with 0.1% BSA) overnight at 4 °C. After removing the unbound antibody, the cells were incubated with goat anti-TXNIP or mouse anti-NLRP3 antibodies (1:200, diluted with 0.1% BSA) for 1 h at room temperature, followed by washing with the above buffer 3 times. The cells were incubated with the corresponding fluorescent secondary antibodies (1:1000, diluted with 0.1% BSA) for 1 h at room temperature. Finally, after washing, the fluorescence images of the cells were obtained based on a Z-stack (3D image stack) using a confocal microscope (Zeiss LSM 510) where the Z-stack images were collected at 1 μm intervals over a 0 to 9 μm range. Merged Z-stack and orthogonal images were then obtained. Trx (ab26320, Abcam, Inc., MA), TXNIP (SC-33099, Santa Cruz, Inc., Texas), and NLRP3 (Cryo-2, Adipogen, Inc., Incheon, Korea) were detected using 488 donkey anti-rabbit IgG, 546 rabbit anti-goat IgG, and 633 goat anti-mouse IgG (A-21206, A-21085, and A-21050 from Molecular Probes, Inc., OR) antibodies. The images corresponding to Trx (green), TXNIP (red), and NLRP3 (red) were obtained using excitation wavelengths of 488 nm, 543 nm, 633 nm and 505-530 nm band-path, 560 nm and 650 nm long-path filters, respectively.
Supplementary data

Figure S1. (a) UV/Vis absorption and (b) fluorescence spectra of 1 (5.0 μM) recorded in the presence of Trx (5.0 μM), other metabolic thiols (5.0 mM of GSH, Cys, or Hcy), various metal cations (100 μM each), and H₂O₂ (100 μM). Spectra were acquired 30 min after addition of the analytes in question and were recorded in PBS solution (pH 7.4) containing 5% (v/v) of DMSO at 37 °C. Excitation was effected at 430 nm.
Figure S2. MALDI-TOF MS spectrum of 1 recorded upon the addition of Trx.
Figure S3. (a) Normalized absorption and (b) fluorescence spectra of 1 recorded in the absence (black line) and presence (red line) of Trx, and of 2 (blue line) in PBS solution (pH 7.4) containing 5% (v/v) of DMSO at 37 °C. Excitation was effected at 430 nm.

Figure S4. (a) TEM image of liposomes and (b) enlarged image of the red boxed area in panel (a).
Figure S5. (a) Fluorescence changes of 1 (1.0 μM) upon treatment with increasing concentrations of Trx (0-5.0 μM). (b) Change in fluorescence intensity (FI) at 530 nm as a function of Trx concentration. All data were acquired 30 min after Trx addition in the mixture of 1 and liposome (DPPC/40 mol% cholesterol) at 37 °C. Excitation was effected at 430 nm.

Figure S6. Fluorescence spectra of 1 (5.0 μM) recorded in the presence of Trx (5.0 μM), metabolic thiols (5.0 mM of GSH, Cys, Hcy, respectively), H₂O₂ (100 μM) and various metal cations (100 μM of Na⁺, K⁺, Zn²⁺, Cu²⁺, Fe²⁺, Mg²⁺, Ca²⁺, Fe³⁺, respectively). All data were acquired 30 min after analytes addition in the mixture of 1 and liposome (DPPC/40 mol % cholesterol) at 37 °C. Excitation was effected at 430 nm.
Figure S7. Confocal microscopy (top) images and the overlay with the corresponding differential interference contrast images (bottom) of HeLa cells treated with 1. The cells were incubated with PBS containing 1 (5 μM) at 37 °C. The images were obtained at time points consisting of 1, 3, 5, 10, 20, 30, and 60 min after the addition of 1.

Figure S8. Confocal microscopy (top) images and the overlay with the corresponding differential interference contrast images (bottom) of HeLa cells treated with 2. The cells were incubated with PBS containing 2 (1 μM) at 37 °C. The images were obtained at time points consisting of 1, 3, 5, 10, 20, 30, and 60 min after the addition of 2.
Figure S9. Confocal microscopy (top) images and the overlay with the corresponding differential interference contrast images (bottom) of HeLa cells treated with 12. The cells were incubated with PBS containing 12 (1 μM) at 37 °C. The images were obtained at time points consisting of 1, 2, 3, 5, 10, 20, 30, and 60 min after the addition of 12.
**Figure S10.** Colocalization experiments using probe 1, fluorescent membrane tracker, and early endosome tracker in HeLa cells. The cells were incubated with 5.0 µM of 1 for 10 min at 37 °C and the media were replaced with fresh media containing 1.0 µM of membrane tracker (DiIC12) (a-d) or 5.0 µM of early endosome tracker (Early Endosomes-RFP, BacMam 2.0) (e-h), respectively, and incubated for 10 min at 37 °C. The fluorescence images were obtained using excitation wavelengths of 458 nm (green channel, panels a and e) and 543 nm (red channel, panels b and f) and 475–525 nm band-path (green channel, panels a and e) and 560 nm (red channel, panels b and f) long-path emission filters, respectively. The corresponding differential interference contrast images (c and g) and the overlay images of probe 1 with DiIC12 (d) or with Early Endosomes-RFP (h).
Figure S11. (a) Confocal microscopy (top) images and the overlay with the corresponding differential interference contrast images (bottom) of HeLa cells treated with 1 in the presence of bacitracin. The cells were separately pretreated with media containing bacitracin (0, 100, 250, and 500 μM) for 15 min at 37 °C. The media were replaced with PBS containing 1 (5.0 μM). After incubation for 20 min at 37 °C, the fluorescence images were recorded. (b) Effect of PX-12 or E-64 on the fluorogenic responses of 1 in HeLa cytosolic proteins (1.0 mg/mL). The cell extracts were separately pretreated with media containing PX-12 (10 μM) and E-64 (10 μM) for 30 min at 37 °C. After incubation with 1 (5.0 μM), the fluorescence changes were recorded. Changes in the fluorescence intensity (FI) of each cell in the images were averaged and plotted. The histograms are based on the average and show the standard deviation in the form of error bars. The excitation and emission wavelengths were 485 and 535 nm, respectively.
Figure S12. Studies designed to assess the extent of colocalization of Trx with TXNIP or NLRP3. Colocalization of Trx with TXNIP or NLRP3 in fixed HepG2 cells. Confocal immunofluorescence images of fixed HepG2 cells with antibodies for Trx (green, a and f), TXNIP (red, b), and NLRP3 (red, g), the corresponding differential interference contrast (DIC) images (c and h), and the overlay images of Trx with TXNIP (d), and with NLRP3 (i), respectively. The corresponding Z-stack orthogonal images (e and j) were collected at 1 μm intervals ranging from 0 to 9 μm along a Z-optical axis.
Figure S13. Confocal microscopy images of HeLa cells treated with 1. The cells were separately pretreated with media containing dinitrophenol (0.5 mM) and cycloheximide (0.1 mM) for 3 h at 37 °C. The cells were incubated with PBS containing 1 (5 μM) at 37 °C. Fluorescence images were obtained at time points consisting of 1, 2, 3, 5, 10, 20, 30, 60 min after the addition of 1.

Figure S14. Confocal microscopy images of HeLa cells treated with 12. The cells were separately pretreated with media containing dinitrophenol (0.5 mM) and cycloheximide (0.1 mM) for 3 h at 37 °C. The cells were incubated with PBS containing 12 (1 μM) at 37 °C. Fluorescence images were obtained at time points consisting of 1, 2, 3, 5, 10, 20, 30 min after the addition of 12.
Figure S15. Fluorescence changes observed for 1 (a) and mitochondrial Trx responding probe (b) in HeLa cells treated with the Trx secretion activators cycloheximide and dinitrophenol. Cells were separately pretreated with media containing cycloheximide (0.1 mM) and dinitrophenol (0.5 mM) for 3 h at 37 °C. The media were replaced with PBS containing 1 or mitochondrial Trx responding probe (5.0 μM, respectively). Fluorescence images were recorded at time points consisting of 1, 2 and 3 min. Changes in the fluorescence intensity (FI) of each cell in the images were averaged and plotted. The histograms are based on the average and show the standard deviation in the form of error bars.
Figure S16. Confocal immunofluorescence images of fixed HepG2 cells (untreated-, PA-, OA-treated cells) with antibodies for Trx (green) and NLRP3 (red). The corresponding differential interference contrast (DIC) images, and the overlay images of Trx with NLRP3, respectively. The corresponding Z-stack orthogonal images were collected at 1 μm intervals ranging from 0 to 9 μm along a Z-optical axis.
**Figure S17.** Confocal microscopy images (top) and the overlay with the corresponding differential interference contrast (bottom) of various cell lines, including HT-29, HepG2, and HeLa cells, treated with probe 1, respectively.
Synthesis

Synthesis of 1. A solution of TFA/CH₂Cl₂ (v/v, 2:1) was added to compound 11 (38.0 mg, 0.0344 mmol). After 12 h of stirring, the volatiles were removed under reduced pressure. The residue was dried in vacuo to yield 1 as yellow oil (28.0 mg, 93%). MALDI-TOF-MS m/z (M⁺) calcd 877.36, found 878.2319 (M⁺H⁺). ¹H NMR (CD₃OD, 400 MHz): δ 8.63–8.32 (br s, 3 H); 8.26–8.07 (br s, 1 H); 7.84–7.62 (br s, 1 H); 4.64–4.32 (br s, 4 H); 4.04–3.75 (br s, 2 H); 3.66–3.15 (m, 12 H); 2.14–2.85 (br s, 6 H); 2.62 (br s, 2 H); 1.86–1.64 (br s, 2 H); 1.52–1.00 (m, 18 H); 0.69 (br s, 3 H). ¹³C NMR (CD₃OD, 100 MHz): 176.8, 164.6, 161.8, 141.4, 132.3, 131.5, 129.1, 126.3, 118.3, 115.3, 63.2, 55.0, 54.0, 53.3, 50.2, 49.5, 44.4, 37.0, 34.6, 31.9, 29.5, 29.6, 29.3, 29.1, 26.4, 23.5, 22.6, 13.3 ppm.

Synthesis of 2. A solution of TFA/CH₂Cl₂ (v/v, 1:1) was added to compound 7 (45.0 mg, 0.0064 mmol). After 1 h of stirring, the volatiles were removed under reduced pressure. The residue was dried in vacuo to yield 2 as yellow oil (30.0 mg, 80%). ESI-MS m/z (M⁺) calcd 582.34, found 583.4 (M⁺H⁺). ¹H NMR (CD₃OD, 400 MHz): δ 8.57–8.37 (m, 2 H); 8.25–8.12 (br d, 1 H); 7.64–7.48 (br d, 1 H); 6.84–6.69 (br d, 1 H); 4.54–4.39 (br s, 2 H); 3.62–3.40 (m, 4 H); 3.40–3.23 (m, 6 H); 3.11–2.97 (br s, 2 H); 1.81–1.62 (br s, 2 H); 1.49–1.07 (m, 18 H); 0.80 (t, 3 H, J = 8.0 Hz). ¹³C NMR (CD₃OD, 100 MHz): 174.9, 165.2, 164.4, 153.8, 134.8, 131.8, 130.6, 129.6, 124.1, 121.8, 120.0, 108.6, 107.7, 55.1, 54.0, 53.3, 50.3, 49.5, 34.4, 31.9, 29.6, 29.4, 29.3, 29.0, 26.4, 23.5, 22.6, 13.3 ppm.

Synthesis of 3. 4-dodecyldiethylenetriamine (1.0 g, 3.6 mmol) and TEA (414.0 μL, 3.6 mmol) were dissolved in anhydrous CH₂Cl₂ (400 mL) and the solution was cooled to 0 °C under nitrogen atmosphere. Benzyl chloroformate (519.0 μL, 3.6 mmol) in anhydrous CH₂Cl₂ (100 mL) was then added dropwise to the solution. The mixture was warmed to room temperature and stirred for overnight. Water (200 mL) was added to it, and the
mixture was extracted with CH₂Cl₂. The CH₂Cl₂ layer was collected and dried with anhydrous MgSO₄. After removal of the solvents, the crude product was purified by silica gel column chromatography using CH₂Cl₂/MeOH (v/v, 22:3) as the eluent to yield 3 as a colorless oil (0.5 g, 34%). ESI-MS m/z (M⁺) calcd 405.62, found 406.35 (M+H⁺). ¹H NMR (CDCl₃, 400 MHz): δ 7.45–7.15 (m, 5 H); 6.48 (s, 2 H); 6.32 (s, 1 H); 5.08 (s, 1 H); 3.33–3.06 (s, 2 H); 2.96–2.74 (s, 2 H); 2.67–2.24 (m, 6 H); 1.59–1.03 (m, 20 H); 0.92–0.83 (t, 3 H, J = 6.0 Hz). ¹³C NMR (CDCl₃, 100 MHz): 157.3, 137.1, 128.4, 128.0, 127.9, 66.8, 66.4, 54.3, 53.9, 53.1, 51.7, 39.6, 38.2, 37.5, 32.1, 29.4, 27.5, 26.6, 25.0, 22.9, 14.4 ppm.

**Synthesis of 4.** Compound 3 (0.3 g, 0.7 mmol) and K₂CO₃ (0.1 g, 0.7 mmol) were dissolved in anhydrous DMF (50 mL) and the solution was cooled to 0 ºC under nitrogen atmosphere. tert-butyl bromoacetate (216.0 µL, 1.5 mmol) was then added dropwise to the solution. The mixture was warmed to room temperature and stirred for overnight. The solvent was evaporated off, at which point CH₂Cl₂ (100 mL) and water (100 mL) were added, and the organic layer collected. The CH₂Cl₂ layer was dried over anhydrous MgSO₄. After removal of the solvents, the crude product was purified by silica gel column chromatography using ethyl acetate/hexanes (v/v, 2:1) as the eluent to yield 4 as a colorless oil (0.1 g, 22%). ESI-MS m/z (M⁺) calcd 633.90, found 634.55 (M+H⁺). ¹H NMR (CDCl₃, 300 MHz): δ 7.38–7.28 (m, 5 H); 5.86 (s, 1 H); 5.08 (s, 2 H); 3.42 (s, 4 H); 3.23 (s, 2 H); 2.78 (t, 2 H, J = 6.0 Hz); 2.59–2.50 (s, 4 H); 2.45–2.35 (br t, 2 H); 1.44 (s, 18 H); 1.35–1.15 (s, 20 H); 0.91–0.83 (t, 3 H, J = 6.0 Hz). ¹³C NMR (CDCl₃, 100 MHz): 171.0, 170.9, 156.9, 137.1, 128.6, 128.1, 128.1, 81.4, 66.6, 56.3, 55.0, 53.0, 52.4, 51.4, 39.3, 32.0, 29.7, 29.4, 28.2, 27.5, 27.0, 22.7, 14.2 ppm.

**Synthesis of 5.** Compound 4 (0.5 g, 0.8 mmol) and Pd/C (0.2 g) was dissolved in degassed EtOH (50 mL), and stirred under H₂ (g) atmosphere at room temperature. After 5 h, the Pd/C was filtered off and the residue was dried in vacuo to yield 5 as brownish oil (0.39 g, 100%). ESI-MS m/z (M⁺) calcd 499.77, found 500.45 (M+H⁺). ¹H NMR (CDCl₃, 400 MHz): δ 5.74 (s, 2 H); 3.29 (s, 4 H); 2.77–2.70 (br t, 2 H); 2.68–2.61 (t, 2 H, J = 6.0 Hz); 2.53–2.45 (br t, 2 H); 2.44–2.36 (t, 2 H, J = 6.0 Hz); 2.32–2.25 (t, 2 H, J = 6.0 Hz); 1.29 (s, 18 H); 1.19–1.00 (s, 20 H); 0.72–0.69 (t, 3 H, J = 6.0 Hz). ¹³C NMR (CDCl₃, 100 MHz): 170.8, 81.3, 55.8, 55.2, 52.4, 51.4, 39.3, 32.0, 29.7, 29.4, 28.2, 27.5, 27.0, 22.7, 14.2 ppm.
Synthesis of 6. Compound 5 (0.39 g, 0.78 mmol) and 4-nitro-1,8-naphthalic anhydride (0.19 g, 0.78 mmol) were dissolved in ethanol (50 mL) and the solution was refluxed at 80 °C for 6 h under nitrogen atmosphere. The solvent was evaporated off, and the crude product was purified by silica gel column chromatography using CH$_2$Cl$_2$/MeOH (v/v, 30:1) as the eluent to yield 6 as yellowish oil (0.39 g, 70%). ESI-MS $m/z$ (M$^+$) calcd 724.93, found 725.55 (M$+$H$^+$). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 8.81 (d, 1 H, $J = 8.8$ Hz); 8.70–8.65 (m, 2 H); 8.40 (d, 1 H, $J = 8.0$ Hz); 7.97 (t, 1 H, $J = 8.0$ Hz); 4.29 (t, 2 H, $J = 4.0$ Hz); 3.48 (s, 4 H); 2.90–2.65 (m, 6 H); 2.52 (t, 2 H, $J = 4.0$ Hz); 1.52–1.41 (s, 18 H); 1.40–1.05 (m, 20 H); 0.88 (t, 3 H, $J = 8.0$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz): 170.8, 163.1, 162.3, 149.4, 132.3, 129.9, 129.7, 129.1, 129.0, 127.0, 123.9, 123.6, 123.0, 80.8, 56.2, 54.6, 52.8, 52.2, 51.3, 38.5, 32.0, 29.8, 29.7, 29.5, 28.3, 27.6, 27.5, 22.9, 14.3 ppm.

Synthesis of 7. Compound 6 (0.4 g, 55.1 mmol) and Pd/C (0.2 g) was dissolved in degassed EtOH (50 mL), and stirred under H$_2$ (g) atmosphere at room temperature. After 5 h, the Pd/C was filtered off and the solvent was removed. The crude product was purified by silica gel column chromatography using CH$_2$Cl$_2$/MeOH (v/v, 30:1) as the eluent to yield 7 as yellow oil (0.27 g, 71%). ESI-MS $m/z$ (M$^+$) calcd 694.94, found 695.60 (M$+$H$^+$). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 8.44 (d, 1 H, $J = 4.0$ Hz); 8.34 (d, 1 H, $J = 8.0$ Hz); 8.26 (d, 1 H, $J = 8.0$ Hz); 7.50 (t, 1 H, $J = 8.0$ Hz); 6.86 (d, 1 H, $J = 8.0$ Hz); 6.09 (s, 2 H); 4.26 (br t, 2 H); 3.51 (s, 4 H); 3.03–2.81 (m, 6 H); 2.76–2.63 (s, 2 H); 1.47 (s, 18 H); 1.35–1.07 (m, 20 H); 0.88 (t, 3 H, $J = 8.0$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz): 171.2, 164.8, 164.0, 151.1, 134.2, 131.4, 130.0, 128.3, 124.5, 122.5, 120.0, 110.3, 109.3, 81.3, 56.6, 55.0, 53.0, 52.2, 51.6, 37.3, 32.1, 29.9, 29.5, 28.3, 27.6, 26.9, 22.9, 14.3 ppm.
Synthesis of 9. Compound 8$^2$ (1.5 g, 4.3 mmol) and thiourea (1.3 g, 17.0 mmol) were dissolved in ethanol (200 mL) and the solution was heated at reflux for 5 h under a nitrogen atmosphere. The solvent was evaporated off, and the residue was dissolved in CHCl$_3$ (150 mL). Subsequently, NaOH (0.34 g, 8.5 mmol) dissolved in water (10 mL) was added and the reaction mixture heated at reflux for 5 h. The mixture was diluted with CH$_2$Cl$_2$ and washed with H$_2$O (200 mL) twice. The organic layer was collected and dried with anhydrous MgSO$_4$. After removal of the solvents, the crude product was purified by silica gel column chromatography using ethyl acetate/hexanes (v/v, 1:6) as the eluent to yield 9 as a colorless oil (0.96 g, 74%). ESI-MS $m/z$ (M$^+$) calcd 305.43, found 306.20 (M$^+$H$^+$) and 328.15 (M$^+$Na$^+$). $^1$H NMR (CDCl$_3$, 400 MHz): δ 3.45 (s, 4 H); 2.94 (t, 2 H, $J$ = 6.0 Hz); 2.65–2.56 (m, 2 H); 1.86 (t, 1 H, $J$ = 8.0 Hz); 1.47 (s, 18 H). $^{13}$C NMR (CDCl$_3$, 100 MHz): 170.6, 81.1, 57.7, 56.1, 28.1, 23.5 ppm.

Synthesis of 10. Compound 9 (0.4 g, 1.3 mmol) and 2,2'-dithiodiethanol (0.7 g, 3.9 mmol) were dissolved in CH$_2$Cl$_2$/methanol (v/v, 1:1) under nitrogen atmosphere and the solution was stirred at room temperature in dark for 48 h. Subsequently, the solution of iodine (0.5 g) dissolved in methanol (10 mL) was added until the mixture solution showed slightly yellow. The solvents were removed and the residue was dissolved in ethyl acetate (200 mL). The solution was washed with a saturated sodium bicarbonate solution twice, and the organic layer was dried over MgSO$_4$. The solvent was evaporated off, and the crude product was purified by silica gel column chromatography using ethyl acetate/hexanes (v/v, 1:4) as the eluent to yield 10 as a colorless oil (0.35 g, 70%). ESI-MS $m/z$ (M$^+$) calcd 381.55, found 382.15 (M$^+$H$^+$). $^1$H NMR (CDCl$_3$, 400 MHz): δ 3.88 (t, 2 H, $J$ = 6.0 Hz); 3.55 (s, 4 H); 3.13 (t, 2 H, $J$ = 6.0 Hz); 2.89 (t, 4 H, $J$ = 8.0 Hz); 1.48 (s, 18 H) ppm. $^{13}$C NMR (CDCl$_3$, 100 MHz): 170.1, 81.8, 60.3, 56.0, 54.0, 41.8, 36.6, 28.2 ppm.
Synthesis of 11. To a mixture of 7 (135.0 mg, 0.2 mmol) and 20% phosgene (CAUTION: TOXIC) dissolved in toluene (865 μL, 1.7 mmol) in 20 ml anhydrous CH₂Cl₂, N,N-diisopropylethylamine (DIPEA) (305 μL, 1.7 mmol) was added dropwise. The resulting solution was stirred at room temperature for 3 h. The reaction mixture was flushed with nitrogen gas. After removal of unreacted phosgene gas (CAUTION: TOXIC) and neutralizing in an NaOH bath, a solution of 10 (150.0 mg, 0.4 mmol) in anhydrous CH₂Cl₂ was added to the mixture. The reaction mixture was stirred overnight. The solvent was evaporated off, at which point CH₂Cl₂ (100 mL) and water (100 mL) were added, and the organic layer collected. The CH₂Cl₂ layer was dried over anhydrous MgSO₄. After removal of the solvent, the crude product was purified by silica gel column chromatography using ethyl acetate/hexane (v/v, 1:1) as the eluent to yield 11 as yellowish oil (43.0 mg, 20%).

ESI-MS m/z (M⁺) calcd 1102.49, found 1100.90 (M-H⁺). ¹H NMR (CDCl₃, 300 MHz): δ 8.60-8.56 (m, 2 H); 8.36-8.31 (m, 2 H); 8.12 (s, 1 H); 7.73 (t, 1 H, J = 9.0 Hz); 4.52 (t, 2 H, J = 6.0 Hz); 4.26 (t, 2 H, J = 6.0 Hz); 3.48 (s, 4 H); 3.46 (s, 4 H); 3.11-3.01 (m, 4 H); 2.92-2.67 (m, 8 Hz); 2.53 (t, 2 H, J = 9.0 Hz); 1.28-1.14 (m, 20 H); 0.87 (t, 3 H, J = 6.0 Hz). ¹³C NMR (CDCl₃, 100 MHz): 170.9, 168.9, 167.4, 163.6, 156.1, 153.5, 133.7, 132.4, 131.9, 130.9, 129.0, 128.5, 127.9, 126.5, 123.3, 117.5, 83.6, 81.5, 81.1, 63.5, 56.6, 54.9, 54.0, 53.1, 52.4, 51.5, 50.9, 45.0, 37.9, 37.0, 32.1, 29.8, 29.6, 28.4, 27.6, 22.9, 14.4 ppm.
\(^1\)H- and \(^{13}\)C NMR spectroscopic, ESI-MS, and MALDI-TOF-MS analyses

**Figure S18.** \(^1\)H-NMR spectrum of 1 in CD\(_3\)OD.

**Figure S19.** \(^{13}\)C NMR spectrum of 1 in CD\(_3\)OD.
Figure S20. MALDI-TOF-MS spectrum of 1.

Chemical Formula: C_{41}H_{59}N_{5}O_{12}S_{2}
Exact Mass: 877.36

Figure S21. $^1$H NMR spectrum of 2 in CD$_3$OD.
Figure S22. $^{13}$C NMR spectrum of 2 in CD$_3$OD.

Figure S23. ESI-MS spectrum of 2.
Figure S24. $^1$H NMR spectrum of 3 in CDCl$_3$.

Figure S25. $^{13}$C NMR spectrum of 3 in CDCl$_3$. 
Figure S26. ESI-MS spectrum of 3.

Figure S27. $^1$H NMR spectrum of 4 in CDCl$_3$. 

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Figure S28. $^{13}$C NMR spectrum of 4 in CDCl$_3$.

Figure S29. ESI-MS spectrum of 4.
**Figure S30.** $^1$H NMR spectrum of 5 in CDCl$_3$.

**Figure S31.** $^{13}$C NMR spectrum of 5 in CDCl$_3$. 
**Figure S32.** ESI-MS spectrum of 5.

**Figure S33.** $^1$H NMR spectrum of 6 in CDCl$_3$. 
Figure S34. $^{13}$C NMR spectrum of 6 in CDCl$_3$.

Figure S35. ESI-MS spectrum of 6.
Figure S36. $^1$H NMR spectrum of 7 in CDCl$_3$.

Figure S37. $^{13}$C NMR spectrum of 7 in CDCl$_3$. 
Figure S38. ESI-MS spectrum of 7.

Figure S39. $^1$H NMR spectrum of 9 in CDCl$_3$. 
Figure S40. $^{13}$C NMR spectrum of 9 in CDCl$_3$.

Figure S41. ESI-MS spectrum of 9.
Figure S42. $^1$H NMR spectrum of 10 in CDCl$_3$.

Figure S43. $^{13}$C NMR spectrum of 10 in CDCl$_3$. 
Figure S44. ESI MS spectrum of 10.

Figure S45. $^1$H NMR spectrum of 11 in CDCl$_3$. 
Figure S46. $^{13}$C NMR spectrum of 11 in CDCl$_3$.

Figure S47. ESI-MS spectrum of 11.
References

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