Electronic Supplementary Information for

Intracellular Delivery of Chemical Probes Using a Glutathione-Responsive Traceless Tag

Eriko Aoyama, Kazuhiro Fuchida, Yuji Oshikawa, Shohei Uchinomiya, Akio Ojida*

Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan.

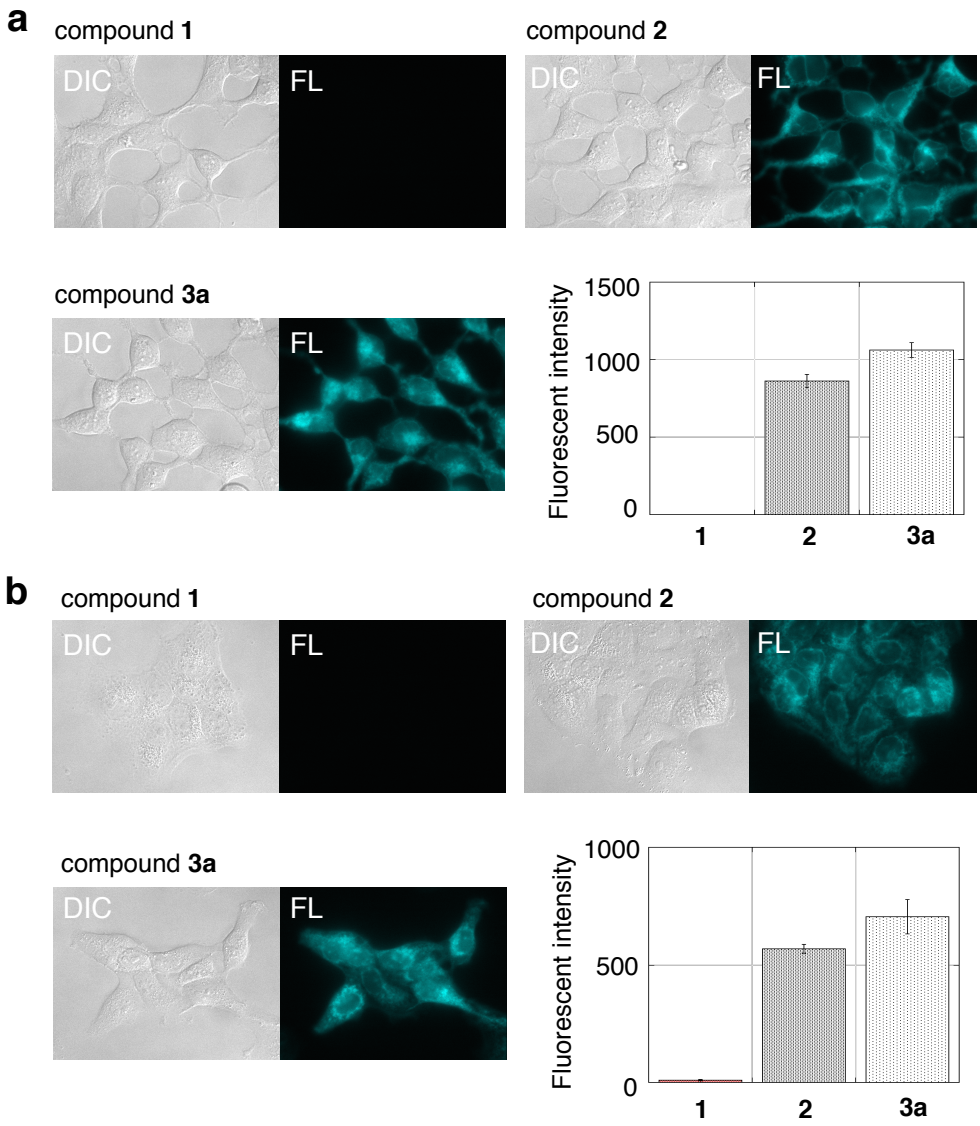


Fig S1. Fluorescence imaging of (a) HEK293 (b) A431 cells treated with **1**, **2** and **3a**. The bar graph shows the average fluorescence intensity of the cells (n = 5). The cells were incubated with 10 uM of **1**, **2** or **3a** in HBS for 30 min at 37 °C and subjected to fluorescence imaging.

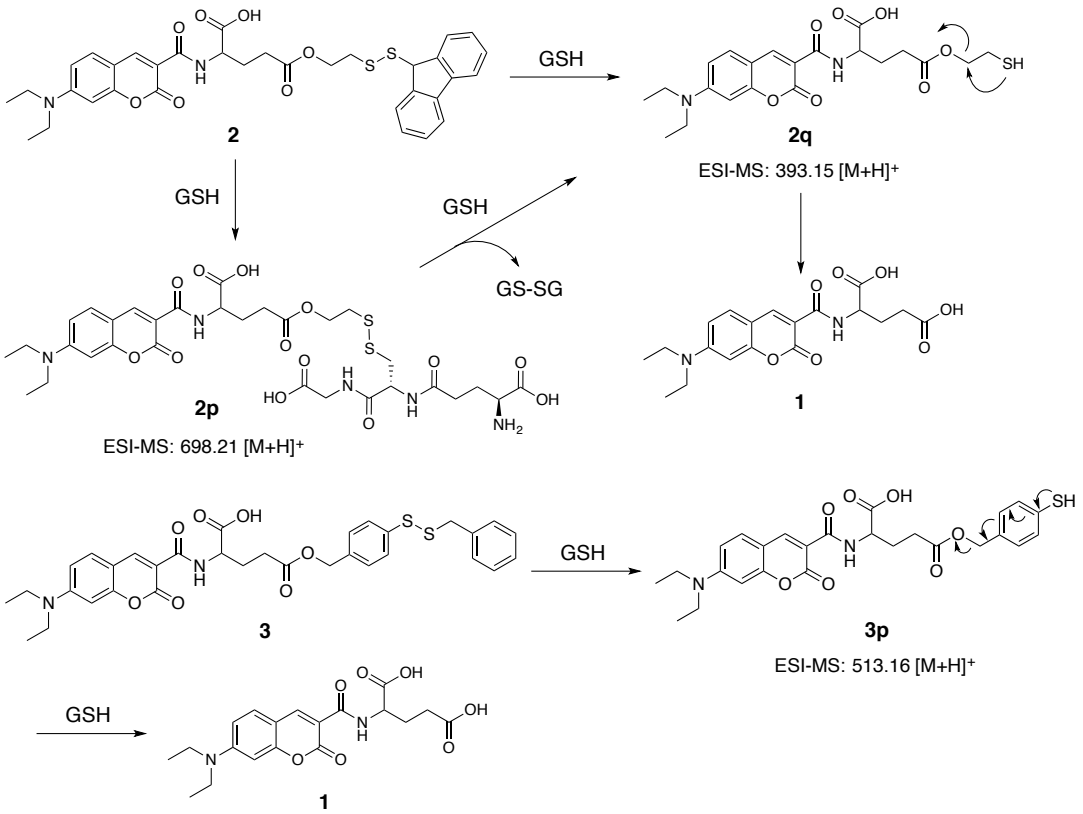


Fig S2. GSH-responsive tag cleavage reactions of **2** and **3**. The reactions were traced by HPLC analysis (see **Fig. 3**). The structures of the reaction intermediates **2p**, **2q**, and **3p** were confirmed by ESI mass analyses.

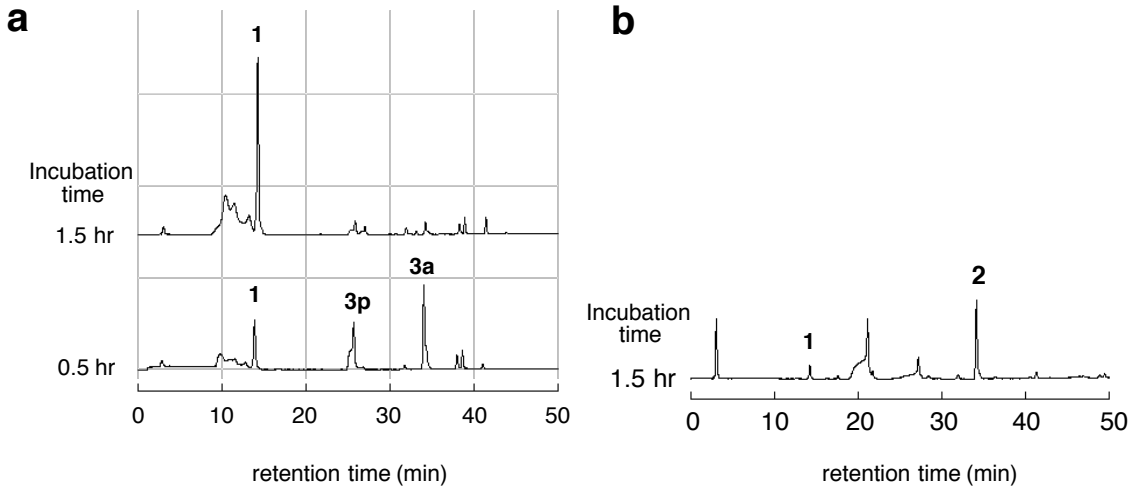


Fig S3. HPLC analysis of the tag-cleavage reaction of **3a** (a) and **2** (b) in HeLa cells. The detail experimental conditions were described below.

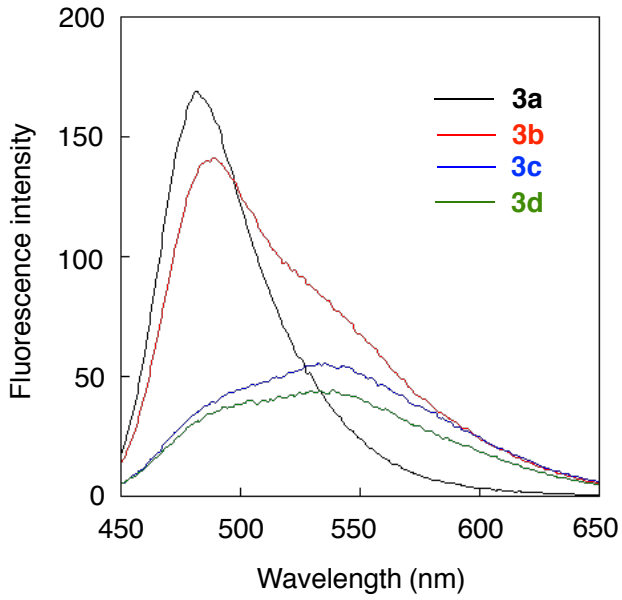


Fig S4. Fluorescence spectra of **3a-d** (5 uM) in HBS. λ_{ex} = 430 nm.

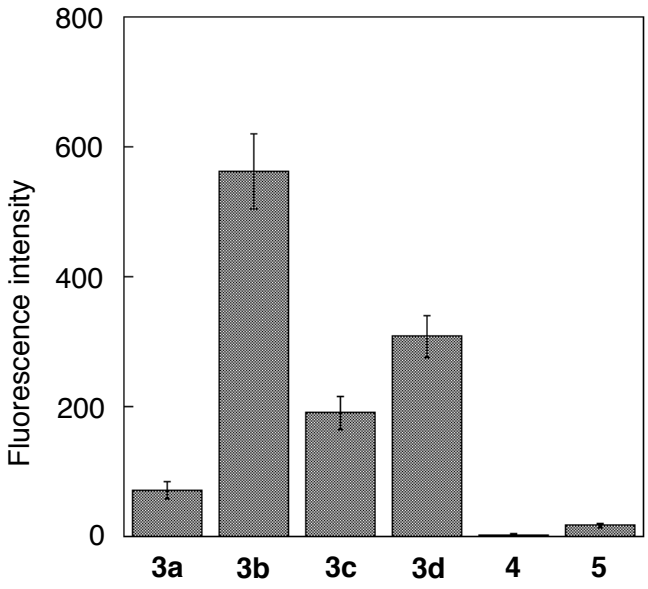


Fig. S5 Comparison of the average fluorescence intensity of HeLa cells (n = 5) after the treatment with **3a-d**, **4** or **5**. The cells were incubated with 1 uM of each coumarin derivative in HBS for 30 min at 37°C, washed with HBS (x2), further incubated in HBS for 1hr at 37°C and subjected to fluorescence imaging.

Cell culture

HeLa cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) under a humidified atmosphere of 5% CO_2 in air. For all experiments, cells were harvested from subconfluent (<80%) cultures using a trypsin-EDTA solution and then resuspended in fresh medium. A subculture was performed every 2–3 days.

Fluorescence imaging of the coumarin probe in living cell

Cells (HeLa, HEK293, or A431) cultured in a glass-based dish (35 mm, Iwaki) were washed twice with HEPES-buffered saline (HBS). The cells were incubated with the coumarin probe (1, 5, 10 μM) in HBS cells for 30 min at 37 °C, washed with HBS, and further incubated for 1 hr at 37 °C under a humidified atmosphere of 5% CO_2 in air. After washing twice with HBS, the cells were subjected to imaging analysis. The fluorescence images were collected with a fluorescence microscope (LX71, Olympus) with a 63x oil-immersion objective lens (excitation: 387/11 nm, dichroic mirror: 490 nm, emission: 520/35 nm) and analyzed using Aquacosmos software (Hamamatsu Photonics).

HPLC analysis of the tag-cleavage reaction

(a) In vitro experiment

A solution of the coumarin probe (10 μM) in 50 mM HEPES buffer (pH 7.4) was incubated at 37 °C in the presence or absence of glutathione (GSH, 10 mM). The solution was sampled at the appropriated time and subjected to HPLC analysis (Hitachi, L-2000 series) using *o*-nitroaniline as an internal standard.

(b) In cell experiment

HeLa cells (2×10^5) cultured in a 60 mm dish (Falcon) for 2 days were washed twice with HEPES-buffered saline (HBS) and incubated with 10 μM of **3a** or **2** for 30 min or 1.5 hr in HBS (1.5 mL) at 37 °C in CO_2 incubator. The cells were collected with a cell scraper and the cell suspension was treated with the same volume of RIPA (containing 1mM EDTA as a protease inhibitor) for 15 min on ice. After centrifugation (13,000 rpm, 1min), the supernatant was mixed with the same volume of CH_3CN containing 0.1% TFA and subjected for HPLC analysis.

HPLC conditions:

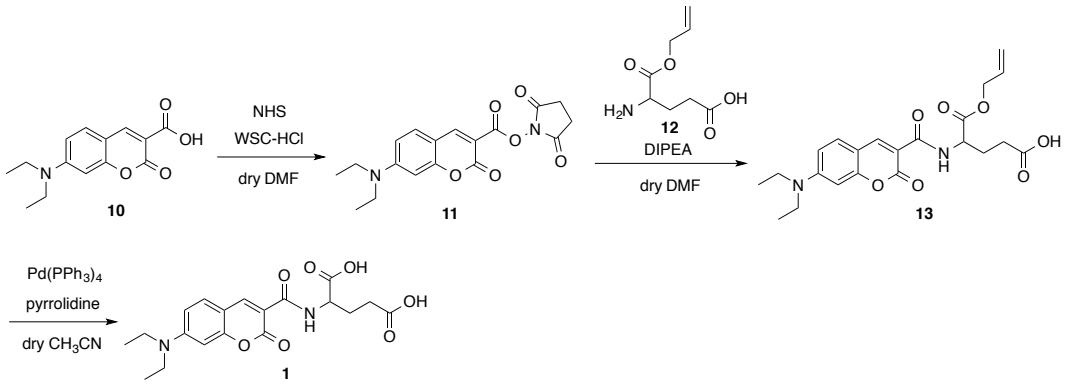
column; YMC-Actus Triart C18, 4.6 mm x 250 mm, flow rate; 1.0 mL/min, detection; UV (430 nm), gradient; A: MeCN (0.1% TFA), B: H_2O (0.1% TFA), A / B = 10 / 90 (0 min) → 80 / 20 (10 min) → 100 / 0 (40 min).

Viability assay of the HeLa cells treated with Pt complexes

HeLa cells ($9x10^3$ cells) were seeded on 96-well plate (Iwaki) and cultured for 24h in DMEM containing 10% FBS. The cells were washed with PBS twice and incubated with a platinum complex in serum-free DMEM for 6 hr at 37 °C. After changing the DMEM, the cells were further incubated in DMEM containing 10% FBS for 18h at 37 °C. The cell viability was evaluated with Cell Count Reagent (Nacalai tesque) by measurement of OD_{450}.

General materials and methods for organic synthesis

Unless otherwise noted, chemical reagents were purchased from commercial suppliers (Sigma-Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical Industries) and used without further purification. ^{1}H NMR spectra were recorded using a Varian UNITY-400 (400 MHz) spectrometer (Varian, USA), and chemical shifts (δ, ppm) were referenced to residual solvent peak. ESI mass spectrometry was recorded using a Bruker microTOF II (Bruker Daltonics, USA) spectrometer. MALDI-TOF mass spectrometry was recorded using a Bruker autoflex III (Bruker Daltonics, USA) spectrometer. HPLC purification was conducted with a HITACHI L-7000 series (Hitachi, Japan).



Scheme S1. Synthesis of **1**

Synthesis of 11

WSC•HCl (462 mg, 2.4 mmol) was added to an ice-cooled solution of **10**[S1] (524 mg, 2.0 mmol) and N-hydroxysuccinimide (277 mg, 2.4 mmol) in dry DMF (7 mL), and the mixture was stirred for 4 hr at rt. After dilution with water, the mixture was extracted with AcOEt (x2). The combined organic layers were washed with sat. $NaHCO_3$ and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was filtered and washed with hexane to give **11** (543 mg, 76%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.25-1.28 (6H, t, J = 7.0 Hz), 2.88 (4H, s), 3.46-3.50 (4H, q, J = 7.0 Hz), 6.46-6.47 (1H, d, J = 2.5 Hz), 6.62-6.45 (1H, dd, J = 2.5, 9.0 Hz), 7.37-7.38 (1H, d, J = 9.0 Hz), 8.58 (1H, s).

Synthesis of 13

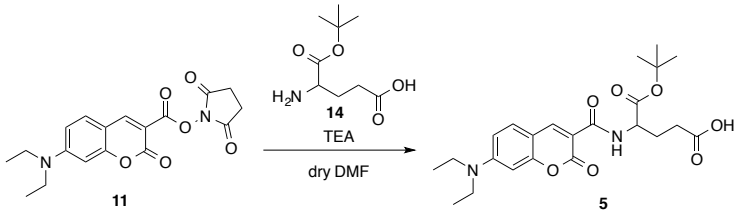
A solution of **11** (214 mg, 0.6 mmol), **12** (289 mg, 0.7 mmol) and DIPEA (0.32 mL, 1.8 mmol) in dry DMF (2 mL) was stirred overnight at rt. After dilution with water, the solution was acidified with conc. HCl to pH 1and extracted with AcOEt (x3). The combined organic layers were dried over $MgSO_4$ and concentrated by evaporation. The residue was purified by flash column chromatography on SiO_2 (hexane : AcOEt : AcOH = 150 : 50 : 1 → 50 : 50 : 1 → 0 : 100 : 1) to give **13** (238 mg, 92%) as a yellow solid.

^{1}H NMR (500 MHz, CD_3OD) : δ 1.23-1.25 (6H, t, *J* = 7.0 Hz), 2.07-2.14 (1H, m), 2.25-2.32 (1H, m), 2.38-2.49 (2H, m), 3.52-3.56 (4H, q, *J* = 7.0 Hz), 4.67-4.69 (2H, m), 4.73-7.76 (1H, m), 5.24-5.27 (1H, m), 5.35-5.40 (1H, m), 5.94-6.02 (1H, m), 6.58-6.59 (1H, d, *J* = 2.5 Hz), 6.82-6.84 (1H, dd, *J* = 2.5, 9.0 Hz), 7.54-7.56 (1H, d, *J* = 9.0 Hz), 8.61 (1H, s), 9.39-9.41 (1H, d, *J* = 7.5 Hz). ESI-MS (negative mode): 429.1698 $[M-H]^-$.

Synthesis of 1

A solution of **13** (59 mg, 0.137 mmol), $Pd(PPh_3)_4$ (4.8 mg, 0.004 mmol), and pyrrolidine (39 μL, 0.48 mmol) in dry CH_3CN (2 mL) was stirred for 4 hr at rt. After removal of the solvent by evaporation, the residue was purified by flash column chromatography on SiO_2 (CH_3Cl : MeOH : AcOH = 100 : 1 : 1 → 100 : 10 : 1) to give **1** (49 mg, 92%) as a yellow solid.

^{1}H NMR (500 MHz, CD_3OD) : δ 1.23-1.25 (6H, t, *J* = 7.0 Hz), 2.10-2.16 (1H, m), 2.27-2.34 (1H, m), 2.37-2.48 (2H, m), 3.52-3.56 (4H, q, *J* = 7.0 Hz), 4.68-4.70 (1H, m), 6.58-6.59 (1H, d, *J* = 2.0 Hz), 6.82-6.84 (1H, dd, *J* = 2.5, 9.0 Hz), 7.55-7.56 (1H, d), 8.62 (1H, s). ESI-MS (positive mode): 391.1499 $[M+H]^+$.



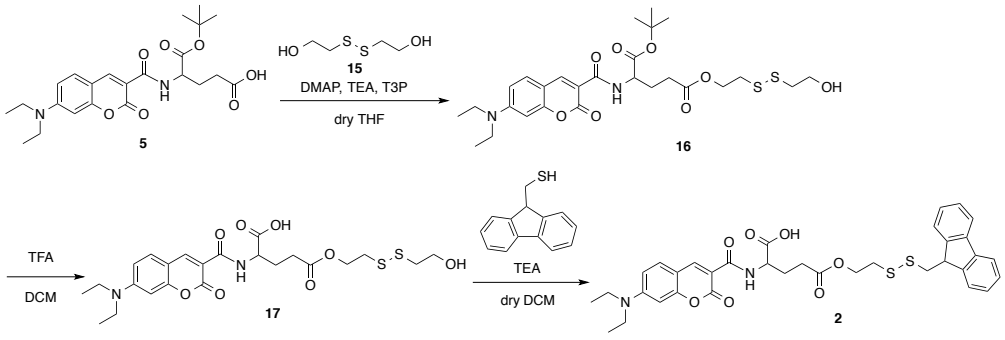
Scheme S2. Synthesis of **5**

Synthesis of 5

A solution of **11** (71 mg, 0.20 mmol), **14** (60 mg, 0.30 mmol) and NEt_3 (0.11 mL, 0.60 mmol) in dry CH_2Cl_2 (2 mL) was stirred overnight at rt. After dilution with water, the solution was acidified with conc. HCl to pH 1and extracted with AcOEt (x3). The combined organic

layers were dried over $MgSO_4$ and concentrated by evaporation. The residue was filtered and washed with hexane to give **5** (88 mg, 98%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.23-1.26 (6H, t, *J* = 7.5 Hz), 1.50 (9H, s), 2.00-2.09 (1H, m), 2.31-2.38 (1H, m), 2.47-2.50 (2H, t, *J* = 6.5 Hz), 3.44-3.49 (4H, q, *J* = 7.0 Hz), 4.69-4.74 (1H, m), 6.51 (1H, d, *J* = 2.5 Hz), 6.64-6.66 (1H, dd, *J* = 2.5, 9.0 Hz), 7.42-7.43 (1H, d, *J* = 9.0 Hz), 8.68 (1H, s), 9.54-9.55 (1H, d, *J* = 7.5 Hz). ESI-MS (negative mode): 445.1987 $[M-H]^-$.



Scheme S3. Synthesis of **2**

Synthesis of 16

Propylphosphonic anhydride (T3P®, 50 wt% in AcOEt) (0.34 mL, 0.57 mmol) was slowly added to a solution of **5** (84 mg, 0.19 mmol), **15** (26 μL, 0.19 mmol), DMAP (3.6 mg, 0.025 mmol) and NEt_3 (0.24 mL, 1.71 mmol) in dry THF (2 mL). The solution was stirred overnight at rt. After dilution with water, the mixture was extracted with AcOEt(x3). The combined organic layers were dried over Na_2SO_4 and concentrated by evaporation. The residue was purified by flash column chromatography on SiO_2 (CH_3Cl) to give **16** (39 mg, 35%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.22-1.25 (6H, t, *J* = 7.0 Hz), 1.49 (9H, s), 2.05-2.13 (1H, m), 2.29-2.36 (1H, m), 2.41-2.54 (2H, m), 2.87-2.89 (2H, t, *J* = 6.0 Hz), 2.92-2.95 (2H, t, *J* = 6.5 Hz), 3.43-3.47 (4H, q, *J* = 7.0 Hz), 3.86-3.89 (2H, t, *J* = 6.0 Hz), 4.33-4.36 (2H, m), 4.71-4.75 (1H, m), 6.50 (1H, d, *J* = 2.0 Hz), 6.63-6.65 (1H, dd, *J* = 2.0, 9.0 Hz), 7.41-7.42 (1H, d, *J* = 9.0 Hz), 8.65 (1H, s), 9.26-9.28 (1H, d, *J* = 7.5 Hz). ESI-MS (positive mode): 605.1931 $[M+Na]^+$.

Synthesis of 17

TFA (1.5 mL) was added dropwise to an ice-cooled solution of **16** (39 mg, 0.07 mmol) in dry CH2Cl2 (1.5 mL). The solution was stirred for 2 hr at rt. TFA was removed in vacuo to give

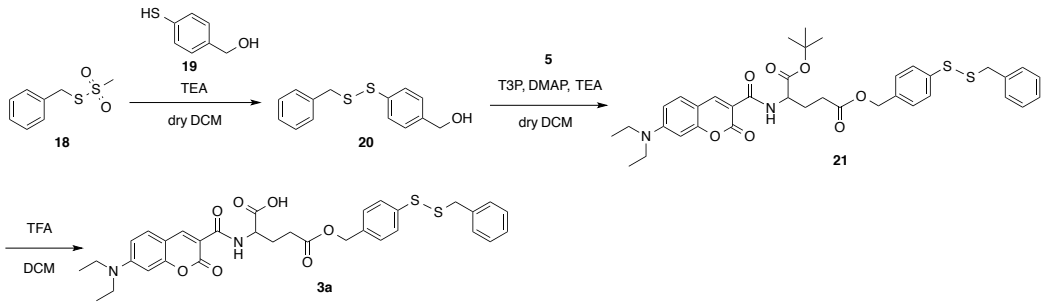
17 (41 mg, quant) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.23-1.26 (6H, t, *J* = 7.5 Hz), 2.18-2.24 (1H, m), 2.37-2.44 (1H, m), 2.51-2.58 (2H, m), 2.89-2.95 (3H, m), 2.98-3.01 (1H, t, *J* = 7.0 Hz), 3.45-3.49 (4H, q, *J* = 7.0 Hz), 3.92-3.95 (1H, t, *J* = 6.0 Hz), 4.33-4.37 (2H, m), 4.58-4.61 (1H, t, *J* = 7.0 Hz), 4.80-4.83 (1H, m), 6.51 (1H, d, *J* = 2.0 Hz), 6.67-6.69 (1H, dd, *J* = 2.0, 9.0 Hz), 7.45-7.47 (1H, dd, *J* = 2.5, 9.0 Hz), 8.70 (1H, s), 9.50-9.53 (1H, t, *J* = 7.5 Hz).

Synthesis of 2

A solution of **17** (53 mg, 0.10 mmol), 9-fluorenylmethlthiol (28 mg, 0.13 mmol), and NEt_3 (82 μL, 0.59 mmol) in dry CH_2CH_2 (2 mL) was stirred overnight at rt. After concentration by evaporation, the residue was purified by flash column chromatography on SiO_2 ($CHCl_3$: MeOH : AcOH = 200 : 1 : 2) to give **2** (21 mg, 31%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.21-1.24 (6H, t, *J* = 7.0 Hz), 2.15-2.22 (1H, m), 2.37-2.44 (1H, m), 2.48-2.59 (2H, m), 2.82-2.85 (2H, t, *J* = 7.0 Hz), 3.25-3.26 (2H, d, *J* = 6.5 Hz), 3.41-3.46 (4H, q, *J* = 7.0 Hz), 4.24-4.26 (1H, t, *J* = 6.5 Hz), 4.28-4.31 (2H, t, *J* = 6.5 Hz), 4.74-4.78 (1H, q, *J* = 7.5 Hz), 6.46 (1H, d, *J* = 2.0 Hz), 6.61-6.63 (1H, dd, *J* = 2.5, 9.0 Hz), 7.29-7.32 (2H, m), 7.36-7.40 (3H, m), 7.65-7.67 (2H, d, *J* = 2.5 Hz), 7.72-7.73 (2H, d, *J* = 2.5 Hz), 8.65 (1H, s), 9.31-9.32 (1H, d, *J* = 7.5 Hz). ESI-MS (negative mode) 659.1890: $[M-H]^-$.



Scheme S4. Synthesis of **3a**

Synthesis of 20

A solution of **18**[S2] (244 mg, 1.2 mmol), **19**[S3] (211 mg, 1.5 mmol), and NEt_3 (0.50 mL, 3.6 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 2.5 hr at rt. After dilution with water, the mixture was extracted with AcOEt (x3). The combined organic layers were dried over N2SO4 and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 (hexane : AcOEt = 3 : 1 → 2 : 1) to give **20** (147 mg, 47%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 3.94 (2H, s), 4.68 (2H, s), 7.28-7.30 (7H, m), 7.43-7.45 (2H, d, *J* = 8.0 Hz). ESI-MS (positive mode): 285.0373 $[M+Na]^+$.

Synthesis of 21

Propylphosphonic anhydride (T3P®, 50 wt% in AcOEt) (0.30 mL, 0.51 mmol) was slowly added to a solution of **5** (76 mg, 0.17 mmol), **20** (53 mg, 0.20 mmol), DMAP (3.4 mg, 0.028 mmol) and NEt_3 (0.19 mL, 1.36 mmol) in dry CH_2Cl_2 (2 mL). The solution was stirred overnight at rt. After dilution with water, the mixture was extracted with AcOEt (x3). The combined organic layers were dried over Na2SO4 and concentrated by evaporation. The residue was purified by flash column chromatography on SiO_2 (hexane : AcOEt = 3 : 1 → 2 : 1) to give **21** (89 mg, 76%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.22-1.24 (6H, t, *J* = 7.0 Hz), 1.49 (9H, s), 2.10-2.17 (1H, m), 2.31-2.34 (1H, m), 2.44-2.54 (2H, m), 3.42-3.46 (4H, q, *J* = 7.0 Hz), 3.92 (2H, s), 4.71-4.76 (1H, m), 5.07 (2H, s), 6.49 (1H, d, *J* = 2.0 Hz), 6.61-6.64 (1H, dd, *J* = 2.5, 9.0 Hz), 7.23-7.27 (7H, m), 7.38-7.41 (3H, m), 8.64 (1H, s), 9.25-9.27 (1H, d, *J* = 8.0 Hz). ESI-MS (positive mode): 713.2299 $[M+Na]^+$.

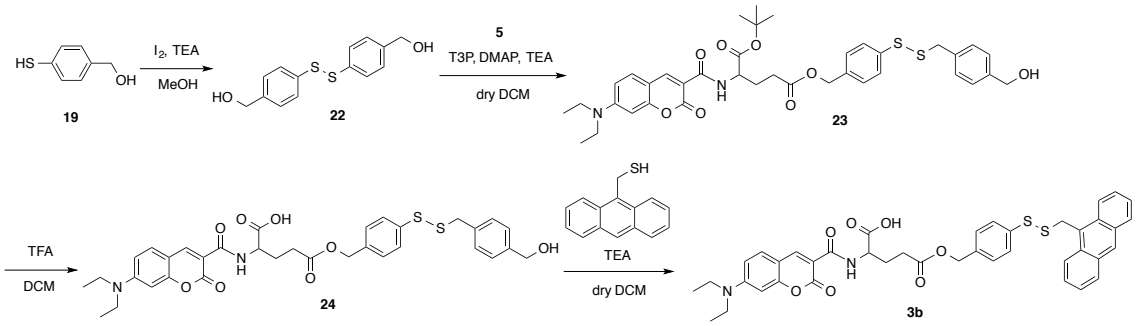
Synthesis of 3a

TFA (1.0 mL) was added dropwise to an ice-cooled solution of **21** (34.5 mg, 0.05 mmol) in dry CH_2Cl_2 (2 mL). The solution was stirred for 40 min at rt. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on SiO_2 ($CHCl_3$: AcOH = 100 : 1) and HPLC to give **3a** (3.9 mg, 12%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.24-1.26 (6H, t, *J* = 7.0 Hz), 2.10-2.23 (1H, m), 2.43-2.47 (1H, m), 2.58-2.62 (2H, m), 3.44-3.49 (4H, q, *J* = 7.0 Hz), 3.93 (2H, s), 4.67-4.71 (1H, q, *J* = 7.0 Hz), 5.09 (2H, s), 6.50 (1H, d, *J* = 2.5 Hz), 6.65-6.67 (1H, dd, *J* = 2.0, 9.0 Hz), 7.24-7.28 (7H, m), 7.40-7.43 (3H, m), 8.66 (1H, s), 9.34-9.36 (1H, d, *J* = 7.0 Hz). ESI-MS (positive mode): 635.1891 $[M+H]^+$.

HPLC conditions:

column; YMC-Actus Triart C18, 20 mm x 250 mm, flow rate; 9.9 mL/min, detection; UV (220 nm), gradiant; A: MeCN (0.1% TFA), B: H_2O (0.1% TFA), A / B = 50 / 50 (0 min) → 80 / 20 (10 min) → 100 / 0 (30 min).



Scheme S5. Synthesis of **3b**

Synthesis of 22

A solution of I_2 (198 mg, 1.56 mmol) in MeOH (5 mL) was added dropwise to the solution of **19**[S3] (168 mg, 1.2 mmol) in MeOH (5 mL) over 10 min. The solution was stirred for 1 hr at rt. After dilution with water, the solution was extracted with AcOEt (x3). The combined organic layers were washed with brine and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by flash column chromatography on SiO_2 ($CHCl_3$: MeOH = 200 : 1 → 20 : 1) to give **22** (110 mg, 66%) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 4.67 (4H, s), 7.30-7.32 (4H, d, *J* = 8.0 Hz), 7.48-7.50 (4H, d, *J* = 8.5 Hz).

Synthesis of 23

Propylphosphonic anhydride (T3P®, 50 wt% in AcOEt) (72 μL, 0.12 mmol) was slowly added to a solution of **5** (22 mg, 0.05 mmol), **22** (27 mg, 0.10 mmol), DMAP (4.0 mg, 0.033 mmol) and NEt_3 (56 μL, 0.40 mmol) in dry CH_2Cl_2 (4 mL). The solution was stirred overnight at rt. After dilution with water, the mixture was extracted with AcOEt (x3). The combined organic layers were dried over Na_2SO_4 and concentrated by evaporation. The residue was purified by flash column chromatography on SiO_2 (hexane : AcOEt = 3 : 2 → 1 : 2) to give **23** (11 mg, 32%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.22-1.25 (6H, t, *J* = 7.0 Hz), 1.48 (9H, s), 2.07-2.12 (1H, m), 2.29-2.33 (1H, m), 2.41-2.53 (2H, m), 4.66 (2H, s), 4.69-4.74 (1H, m), 5.06 (2H, s), 6.49-6.50 (1H, d, *J* = 2.5 Hz), 6.62-6.64 (1H, dd, *J* = 2.5, 9.0 Hz), 7.28-7.30 (3H, m), 7.38-7.40 (1H, d, *J* = 9.0 Hz), 7.44-7.47 (4H, t, *J* = 8.0 Hz), 8.63 (1H, s), 9.24-9.25 (1H, d, *J* = 7.5 Hz). ESI-MS (positive mode): 729.2102 $[M+Na]^+$.

Synthesis of 24

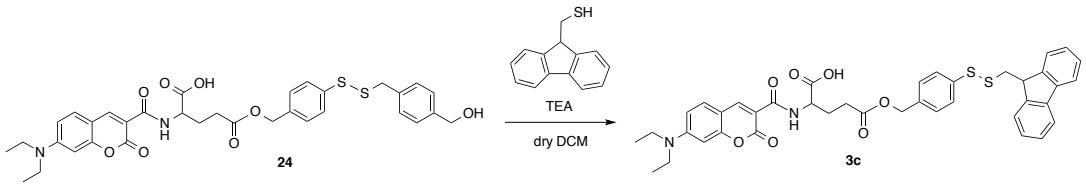
TFA (1.5 mL) was added dropwise to an ice-cooled solution of **23** (11.3 mg, 0.016 mmol) in dry CH_2Cl_2 (1.5 mL). The solution was stirred for 40 min at rt. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on SiO_2 ($CHCl_3$: MeOH : AcOEt = 100 : 0 : 1 → 100 : 1 : 1) to give **24** (4.5 mg, 43%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.24-1.27 (6H, t, *J* = 7.0 Hz), 2.15-2.22 (1H, m), 2.41-2.48 (1H, m), 2.56-2.59 (2H, m), 3.45-3.49 (4H, q, *J* = 7.0 Hz), 4.64-4.68 (1H, q, *J* = 7.0 Hz), 5.08 (2H, s), 5.31 (2H, s), 6.50-6.51 (1H, d, *J* = 4.0 Hz), 6.65-6.67 (1H, dd, *J* = 2.0, 9.0 Hz), 7.28-7.33 (4H, m), 7.42-7.47 (3H, m), 7.50-7.52 (2H, d, *J* = 8.5 Hz), 8.66 (1H, s), 9.31-9.32 (1H, d, *J* = 6.5 Hz). ESI-MS (negative mode): 651.1657 $[M-H]^-$.

Synthesis of 3b

A solution of **23** (11 mg, 0.017 mmol), 9-fluorenylmethylthiol (3.8 mg, 0.018 mmol), and NEt_3 (10 μL, 0.072 mmol) in dry CH_2Cl_2 (3 mL) was stirred overnight at rt. After concentration by evaporation, the residue was purified by flash column chromatography on SiO_2 ($CHCl_3$: AcOH = 100 : 1) to give **3b** (4.2 mg, 34%) as a yellow solid.

^{1}H NMR(500 MHz, $CDCl_3$) : δ 1.21-1.24 (6H, t, *J* = 7.0 Hz), 2.17-2.24 (1H, m), 2.40-2.49 (1H, m), 2.50-2.64 (2H, m), 3.23-3.25 (2H, d, *J* = 6.0 Hz), 3.41-3.46 (4H, q, *J* = 7.5 Hz), 4.26-4.29 (1H, t, *J* = 6.5 Hz), 4.69-4.73 (1H, q, *J* = 6.5 Hz), 5.09 (2H, s), 6.47 (1H, d, *J* = 1.5 Hz), 6.61-6.63 (1H, d, *J* = 7.5 Hz), 7.28-7.31 (4H, t, *J* = 6.5 Hz), 7.36-7.40 (3H, m), 7.46-7.48 (2H, d, *J* = 8.0 Hz), 7.61-7.62 (2H, d, *J* = 7.5 Hz), 7.72-7.74 (2H, d, *J* = 7.5 Hz), 8.64 (1H, s), 9.31-9.32 (1H, d, *J* = 6.5 Hz). ESI-MS (negative mode): 721.2030 $[M-H]^-$.



Scheme S6. Synthesis of **3c**

Synthesis of 3c

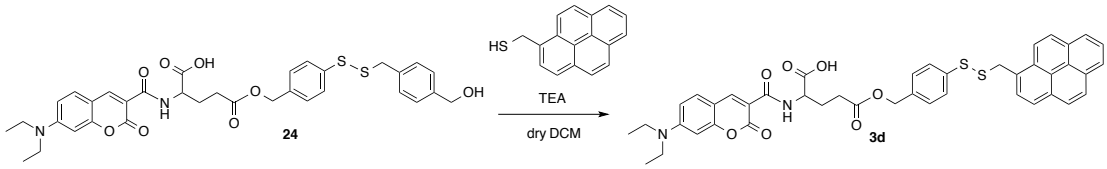
A solution of **24** (15.5 mg, 0.024 mmol), 9-anthracenemethylthiol[S4] (6.5 mg, 0.029 mmol), and NEt_3 (13 μL, 0.076 mmol) in dry CH_2Cl_2 (3 mL) was stirred overnight at rt. After concentration by evaporation, the residue was purified by flash column chromatography on SiO_2

($CHCl_3$: AcOH = 100 : 1) and HPLC to give **3c** (3.5 mg, 23%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.20-1.23 (6H, t, *J* = 7.0 Hz), 2.22-2.26 (1H, m), 2.46-2.50 (1H, m), 2.61-2.65 (2H, m), 3.40-3.44 (4H, q, *J* = 7.0 Hz), 4.71-4.75 (1H, q, *J* = 6.5 Hz), 5.00 (2H, s), 5.12 (2H, s), 6.45 (1H, d, *J* = 2.5 Hz), 6.58-6.60 (1H, dd, *J* = 2.0, 9.0 Hz), 7.25-7.26 (2H, d, *J* = 7.0 Hz), 7.34-7.56 (1H, d, *J* = 9.0 Hz), 7.44-7.51 (6H, m), 7.95-7.97 (2H, d, *J* = 9.0 Hz), 8.15-8.17 (2H, d, *J* = 9.0 Hz), 8.36 (1H, s), 8.62 (1H, s), 9.36-9.37 (1H, d, *J* = 6.5 Hz). ESI-MS (negative mode): 733.2064 $[M-H]^-$.

HPLC conditions:

column; YMC-Actus Triart C18, 20 mm x 250 mm, flow rate; 9.9 mL/min, detection; UV (220 nm), gradient; A: MeCN (0.1% TFA), B: H_2O (0.1% TFA), A / B = 50 / 50 (0 min) → 80 / 20 (10 min) → 100 / 0 (30 min).



Scheme S7. Synthesis of **3d**

Synthesis of 3d

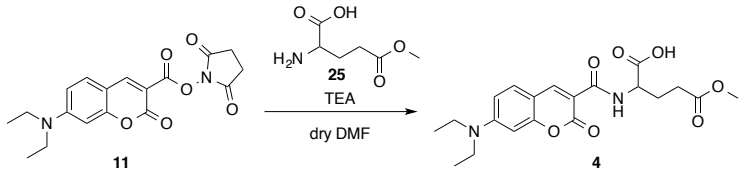
A solution of **24** (15.6 mg, 0.024 mmol), 1-pyrenylmethythiol[S5] (4.6 mg, 0.019 mmol), and NEt_3 (13 μL, 0.076 mmol) in dry CH_2Cl_2 (3 mL) was stirred 8.5 hr at rt. After concentration by evaporation, the residue was purified HPLC to give **3d** (2.7 mg, 15%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.20-1.23 (6H, t, *J* = 7.0 Hz), 2.16-2.23 (1H, m), 2.41-2.46 (1H, m), 2.54-2.58 (2H, m), 3.40-3.44 (4H, q, *J* = 7.0 Hz), 4.67 (2H, s), 4.68-4.72 (1H, q, *J* = 7.0 Hz), 4.81 (2H, s), 6.45 (1H, d, *J* = 2.0 Hz), 6.58-6.61 (1H, dd, *J* = 2.5, 9.0 Hz), 6.95-6.97 (2H, d, *J* = 8.5 Hz), 7.25-7.26 (2H, d, *J* = 7.5 Hz), 7.35-7.37 (1H, d, *J* = 9.0 Hz), 7.85-7.86 (1H, d, *J* = 8.0 Hz), 7.97-8.04 (4H, m), 8.09-8.11 (1H, d, *J* = 9.5 Hz), 8.17-8.20 (2H, t, *J* = 7.0 Hz), 8.23-8.25 (1H, d, *J* = 9.0 Hz), 8.63 (1H, s), 9.34-9.36 (1H, d, *J* = 6.5 Hz). ESI-MS (negative mode): 757.2041 $[M-H]^-$.

HPLC conditions:

column; YMC-Actus Triart C18, 20 mm x 250 mm, flow rate; 9.9 mL/min, detection UV (220

nm), gradient; A: MeCN (0.1% TFA), B: H_2O (0.1% TFA), A / B = 50 / 50 (0 min) → 80 / 20 (10 min) → 100 / 0 (30 min).

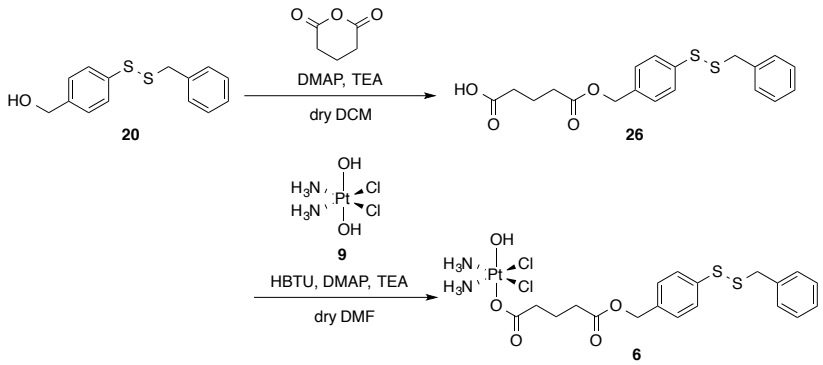


Scheme S8. Synthesis of **4**

Synthesis of 4

A solution of **11** (36 mg, 0.10 mmol), **25** (19 mg, 0.12 mmol) and NEt_3 (0.42 mL, 0.30 mmol) in dry CH_2Cl_2 (4 mL) was stirred overnight at rt. After dilution with water, the solution was acidified with conc. HCl to pH 1and extracted with AcOEt (x3). The combined organic layers were dried over $MgSO_4$ and concentrated by evaporation. The residue was filtered and washed with hexane to give **4** (42 mg, quant) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.23-1.25 (6H, t, *J* = 7.5 Hz), 2.15-2.22 (1H, m), 2.36-2.44 (1H, m), 2.48-2.58 (2H, m), 3.43-3.48 (4H, q, *J* = 7.0 Hz), 4.72-4.76 (1H, q, *J* = 7.5 Hz), 6.49 (1H, d, *J* = 2.5 Hz), 6.34-6.66 (1H, dd, *J* = 9.0 Hz), 8.68 (1H, s), 9.31-9.33 (1H, d, *J* = 7.5 Hz). ESI-MS (negative mode): 403.15 $[M-H]^-$.



Scheme S9. Synthesis of **6**

Synthesis of 26

A solution of **20** (66 mg, 0.25 mmol), glutaric anhydride (34 mg, 0.30 mmol), DMAP (3.0 mg, 0.025 mmol) and NEt_3 (42 μL, 0.30 mmol) in dry CH_2Cl_2 (4 mL) was stirred for 7.5 hr at rt. After dilution with sat. $NaHCO_3$ aq., the mixture was extracted with AcOEt (x2). The combined organic layers were dried over $MgSO_4$. After concentration by evaporation, the residue was

purified by flash column chromatography on SiO_2 (Hexane : AcOEt : CH_3COOH= 4 : 1 : 0.05 → 3 : 1 : 0.04) to give **26** (59 mg, 63%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.94-2.00 (2H, m), 2.41-2.46 (4H, t, *J* = 7.5 Hz), 3.93 (2H, s), 5.07 (2H, s), 7.22-7.28 (7H, m), 7.41-7.43 (2H, t, *J* = 8.5 Hz). ESI-MS (negative mode): 375.0767 $[M-H]^-$.

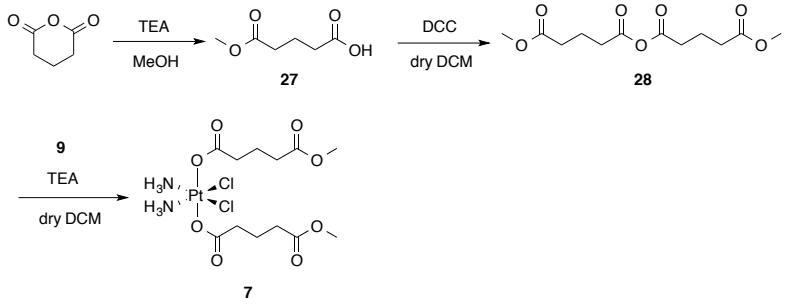
Synthesis of 6

A solution of **9**[S6] (17 mg, 0.05 mmol), 20 (38 mg, 0.10 mmol), DMAP (3.0 mg, 0.025 mmol), HBTU (38 mog, 0.10 mmol) and NEt_3 (14 μL, 0.10 mmol) in dry DMF (1 mL) was stirred for 1 day at rt. After concentration by evaporation, the residue was purified HPLC to give **6** (7.2 mg, 20%) as a yellow solid.

^{1}H NMR (500 MHz, CD_3OD) : δ 1.87-1.93 (2H, m), 2.38-2.41 (2H, t, *J* = 7.5 Hz), 2.46-2.49 (2H, t, *J* = 7.5 Hz), 3.97 (2H, s), 5.08 (2H, s), 7.25-7.29 (7H, m), 7.40-7.41 (2H, d, *J* = 8.5 Hz). ESI-MS (positive mode): 715.0196 $[M+Na]^+$.

HPLC conditions

column; YMC-Actus Triart C18, 20 mm x 250 mm, flow rate; 9.9 mL/min, detection; UV (220 nm), gradient; A: MeCN, B: H_2O, A / B = 20 / 80 (0 min) → 100 / 0 (40 min) → 100 / 0 (50 min) → 20 / 80 (60 min)



Scheme S10. Synthesis of **7**

Synthesis of 27 [S7]

A solution of **181** (507 mg, 5.0 mmol) and NEt_3 (1.14 μL, 10 mmol) in MeOH (5 mL) was stirred overnight at rt. After dilution with water, the solution was acidified with conc. HCl to pH 1 and extracted with AcOEt (x3). The combined organic layers were dried over $MgSO_4$ and concentrated by evaporation to give **27** (583 mg, 80%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.97-1.99 (2H, m), 2.40-2.46 (4H, m), 3.68 (3H, s).

Synthesis of 28 [S7]

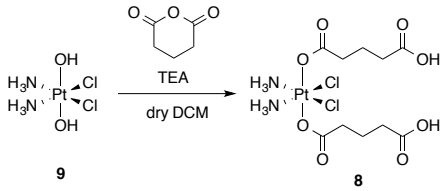
A solution of **27** (292 mg, 2.0 mmol) and DCC (206 mg, 1.0 mmol) in dry CH_2Cl_2 (2 mL) was stirred for 1 hr at 0 °C. After removal of a precipitation by filtration, the solution was evaporated to give **28** (273 mg, 99%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 1.97-2.00 (4H, m), 2.41-2.44 (4H, t, J = 7.5 Hz), 2.53-2.56 (4H, t, J = 7.5 Hz), 3.69 (6H, s). ESI-MS (positive mode): 297.0972 $[M+Na]^+$.

Synthesis of 7[S7]

A solution of **9** (33 mg, 0.10 mmol), **28** (69 mg, 0.25 mmol) in dry DMF (5 mL) was stirred for 1 day at rt. After additional of water (1 mL), the solution was kept at 2 °C overnight. After concentration by evaporation and dilution with sat. $NaHCO_3$ aq., the mixture was extracted with AcOEt (x2). The combined organic layers were dried over $MgSO_4$. After concentration by evaporation, the residue was washed with CH_3CN : ether = 1 : 1 to give **7** (1.9 mg, 3.2%) as a white solid.

^{1}H NMR (500 MHz, CD_3OD) : δ 1.84-1.90 (4H, m), 2.40-2.43 (8H, m), 3.66 (6H, s). ESI-MS (positive mode): 613.0419 $[M+Na]^+$.



Scheme S11. Synthesis of **8**

Synthesis of 8[S8]

A solution of **9** (80 mg, 0.24 mmol), glutaric anhydride (118 mg, 1.03 mmol) and Et_3N (4.2 μL, 0.030 mmol) in dry DMF (6 ml) was stirred for 1 day at rt. After additional of water (1 ml), the solution was kept at 2 °C overnight. After removal of the solvent by evaporation, the residue was carefully washed with EtOH/ether several times to give **8** (2.6 mg, 2.2%) as a yellow solid.

^{1}H NMR (500 MHz, CD_3OD) : δ 1.84-1.90 (4H, m), 2.33-2.44 (8H, m). ESI-MS (negative mode): 561.0189 $[M-H]^-$.

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