Supplementary information

A fluorogenic probe using a catalytic reaction for the detection of trace intracellular zinc

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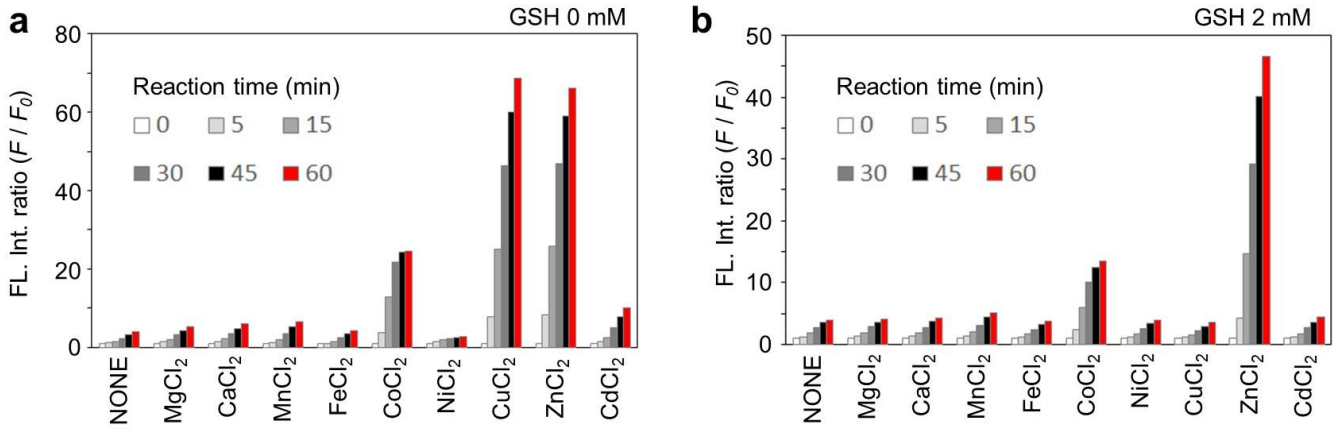


Figure S1. Fluorescence response of 10 μM Dpa-SoxLC towards different metal ions in the absence (a) or presence (b) of 2 mM glutathione (GSH), λ_{ex} = 390 nm, λ_{em} = 470 nm, the fluorescence was measured at the reaction times, 0, 5, 15, 30, 45, or 60 min, at 37 °C after the addition of metal ions. 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO. [Metal ion] = 10 μM except for 500 μM $MgCl_2$ and 2 mM $CaCl_2$. F_0 is the fluorescence intensity before the addition of metal ions.

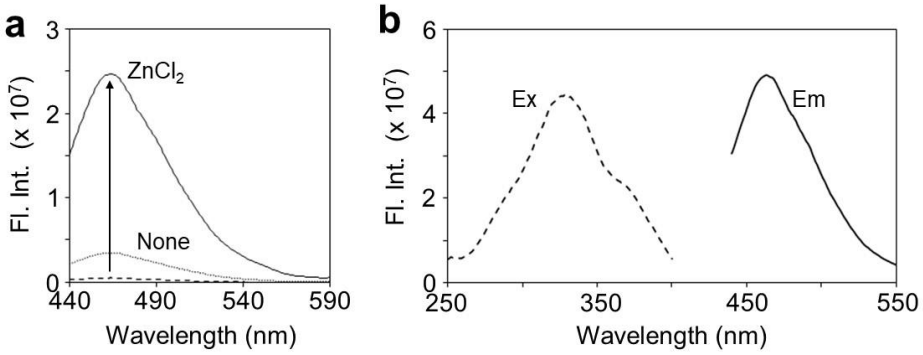


Figure S2. a) Fluorescence spectra (λ_{ex} = 400 nm) of 10 μM Dpa-SoxLC after a 60 min reaction with 10 μM $ZnCl_2$, Solid line: with $ZnCl_2$, dotted line: no addition (None), bold dotted line: before the reaction. b) Fluorescence spectra of 10 μM umbelliferone. 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, 2 mM GSH, at 37 °C. Emission (solid line): λ_{ex} = 400 nm, Excitation (dotted line): λ_{em} = 545 nm.

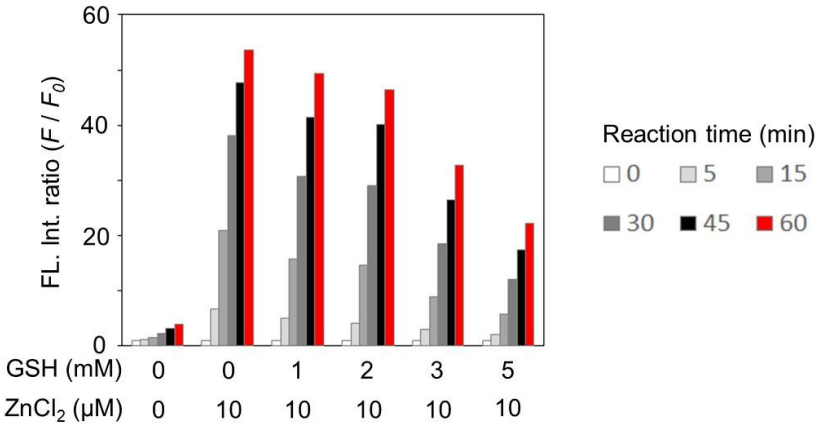


Figure S3. Time-dependent fluorescence response of 10 μM Dpa-SoxLC for detecting 10 μM $ZnCl_2$ in the presence of GSH (0–5 mM). 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, at 37 °C. F_0 is the fluorescence of each probe before the addition of zinc.

Table S1. Summary of the K_m and V_{max} parameters of β-lactamase for each substrate. The parameters calculated by absorbance (A_{486} for Nitrocefin) or fluorescence intensity (λ_{ex} = 390 nm, λ_{em} = 520 nm for Bn-SoxLC and Dpa-SoxLC). The concentrations were calculated using standard samples (reacted nitrocefin or umbelliferone solution). PBS buffer (pH 7.4), 1% (v/v) DMSO, 1% (v/v) DMSO, at 37 °C.

Substrate	K_m (μM)	V_{max} (μMsec^{-1})
Nitrocefin	57.3	0.936
ref [S1]	303	2.46
Bn-SoxLC	6.19	0.161
Dpa-SoxLC	154	0.472

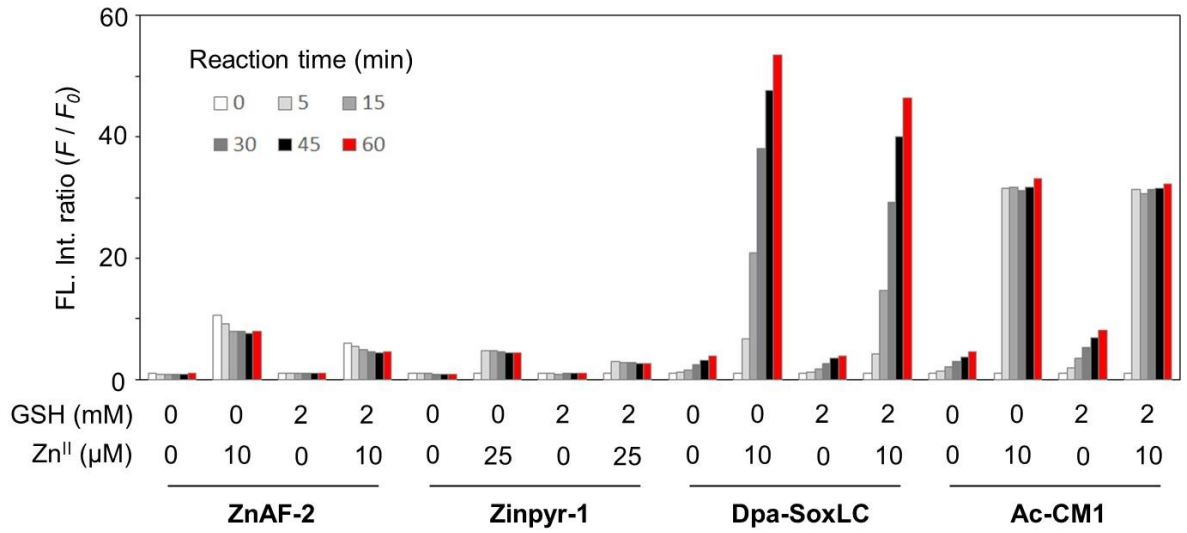


Figure S4. Time-dependent fluorescence response of 10 μM ZnAF-2, Zinpyr-1, Dpa-SoxLC, or Ac-CM1 for detecting 10 μM or 25 μM $ZnCl_2$ in the absence or presence of 2 mM GSH, 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, at 37 °C. F_0 is the fluorescence of each probe before the addition of zinc.

Table S2. Summary of detection limits of 10 μM ZnAF-2, Zinpyr-1, Dpa-SoxLC, and Ac-CM1 for detecting zinc.

Reaction time (min)	Detection limit (nM)							
	ZnAF-2		Zinpyr-1		Dpa-SoxLC		Ac-CM1	
	GSH 0 mM	GSH 2 mM	GSH 0 mM	GSH 2 mM	GSH 0 mM	GSH 2 mM	GSH 0 mM	GSH 2 mM
5	16	191	135	585	84	424	14	25
15	-	-	-	-	23	93	15	27
30	-	-	-	-	11	49	15	27
45	-	-	-	-	8.5	36	15	28
60	-	-	-	-	8.0	30	15	30

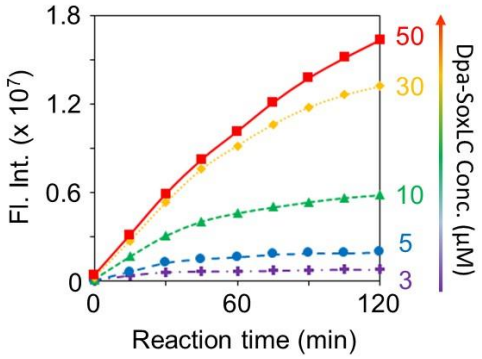


Figure S5. Time-dependent fluorescence change (λ_{ex} = 390 nm, λ_{em} = 535 nm) of Dpa-SoxLC at different concentrations (3–50 μM) upon the addition of 5 μM $ZnCl_2$ (Raw data of Figure 3a). 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, 2 mM GSH, at 37 °C.

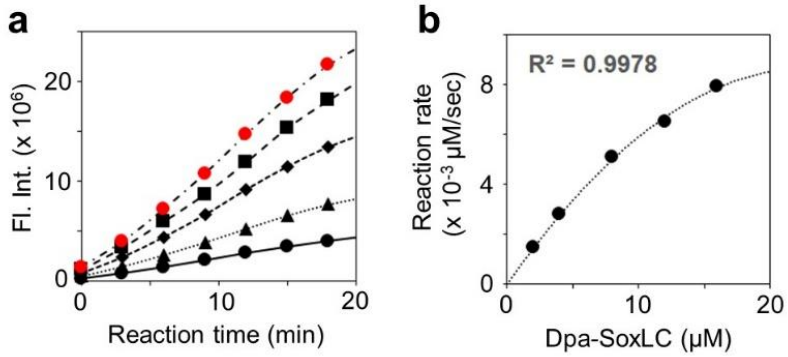


Figure S6. Raw data for calculating K_m and V_{max} of zinc-catalyzed reaction in 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, at 37 °C, a) fluorescence intensities of Dpa-SoxLC, [$ZnCl_2$] = 10 μM, [Dpa-SoxLC] = 2 μM (●), 4 μM (▲), 8 μM (◆), 12 μM (■), 16 μM (●), b) plots of initial reaction rate calculated by the plots of (a) and Dpa-SoxLC concentrations.

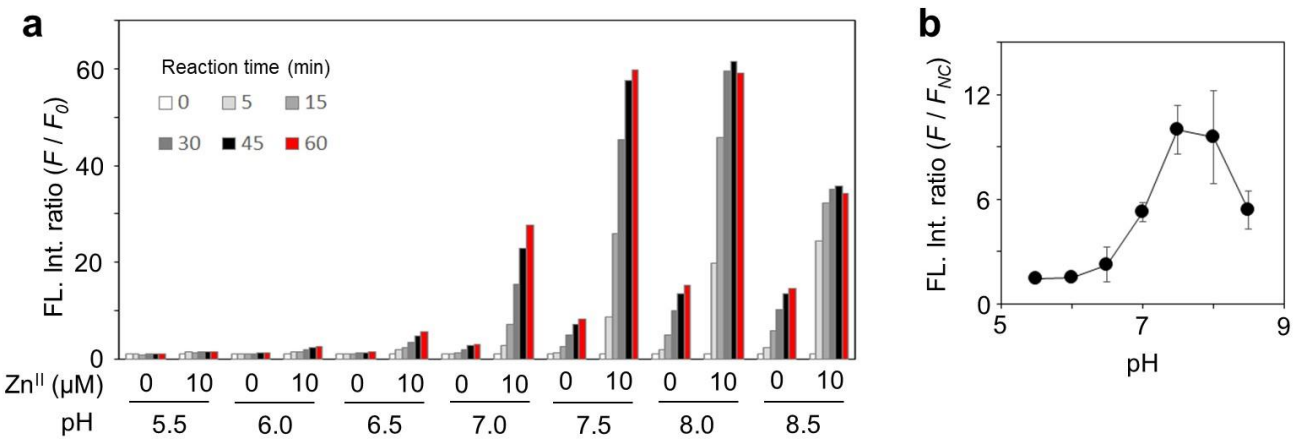


Figure S7. Fluorescence response of Dpa-SoxLC under different pH conditions in 50 mM HEPES buffer, 1% (v/v) DMSO, at 37 °C. a) Fluorescent intensities at different reaction times, b) fluorescence intensity ratio of each pH condition (reaction time 15 min) against negative control (F_{NC}: fluorescence intensity of Dpa-SoxLC on the addition of DMSO).

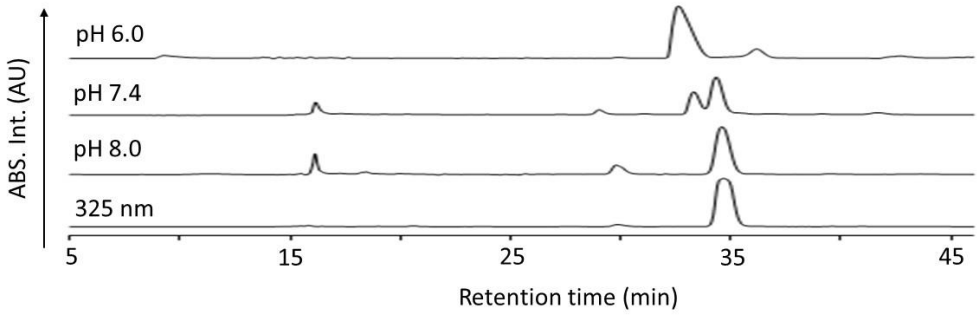


Figure S8. HPLC analyses of zinc-catalyzed reaction of equimolar Dpa-SoxLC in 50 mM HEPES buffer (2.5% CH_3CN, pH 7.4), (reaction time: 1 hour at 36 °C). The absorbance was obtained at 220 nm except for HPLC spectrum at 325 nm. The HPLC analysis at 325 nm was performed to identify the peaks of umbelliferone or umbelliferone conjugates after the reaction at pH 8.0. Retentione time of Dpa-SoxLC: 34 min, umbelliferone: 35 min.

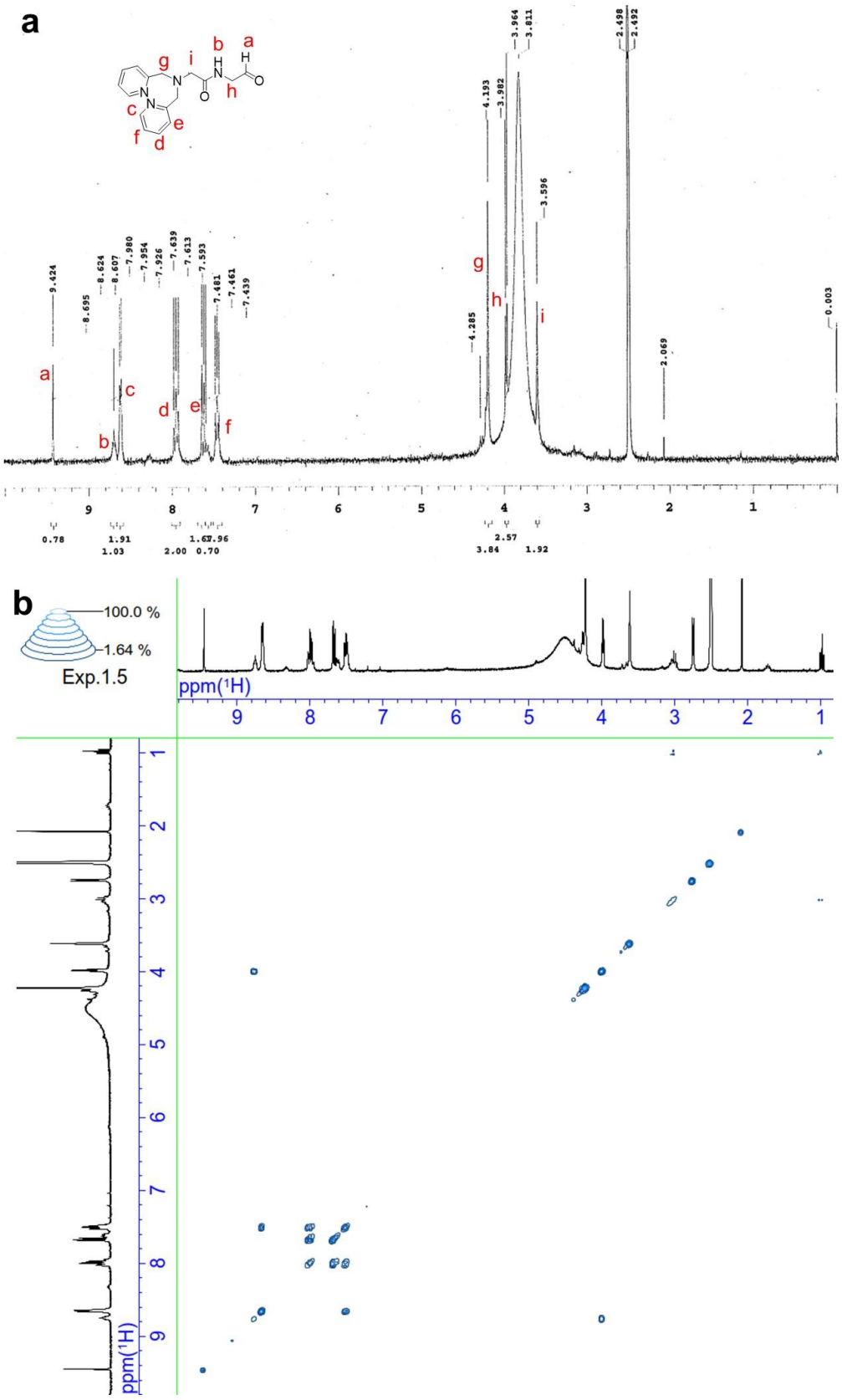


Figure S9. NMR analyses of fraction at retention time16 min (f16) in HPLC analyses (Figure 3c), a) ^{1}H-NMR spectrum (300 MHz, DMSO-*d6*) of f16. Inset structure is the proposed structure of f16 and identification of NMR-peaks, b) HH-COSY spectrum of f16.

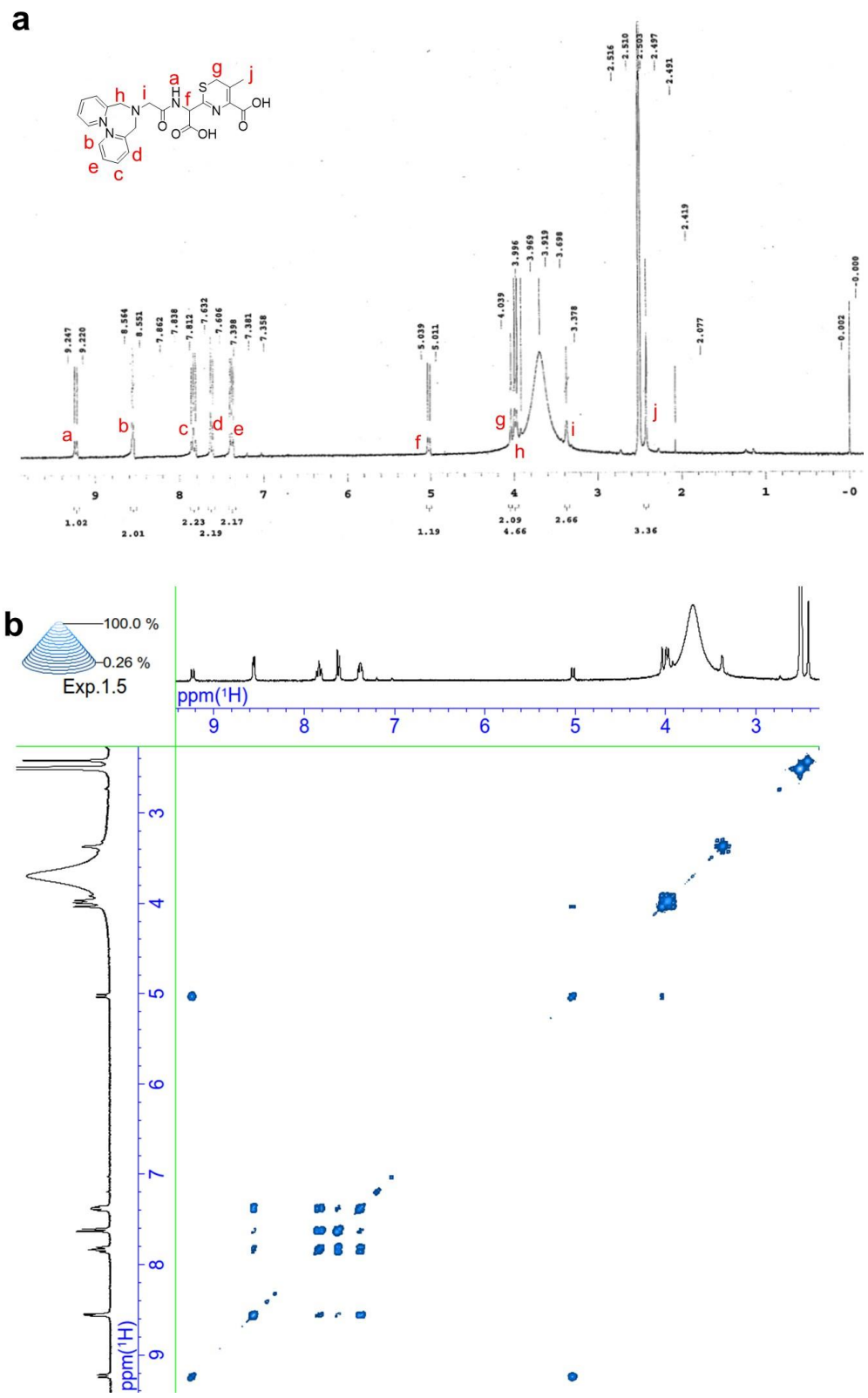


Figure S10. NMR analyses of fraction at retention time 29 min (f29) in HPLC analyses (Figure 3c), a) ^{1}H-NMR spectrum (300 MHz, DMSO-*d6*) of f29. Inset structure is the proposed structure of f29 and identification of NMR-peaks, b) HH-COSY spectrum of f29.

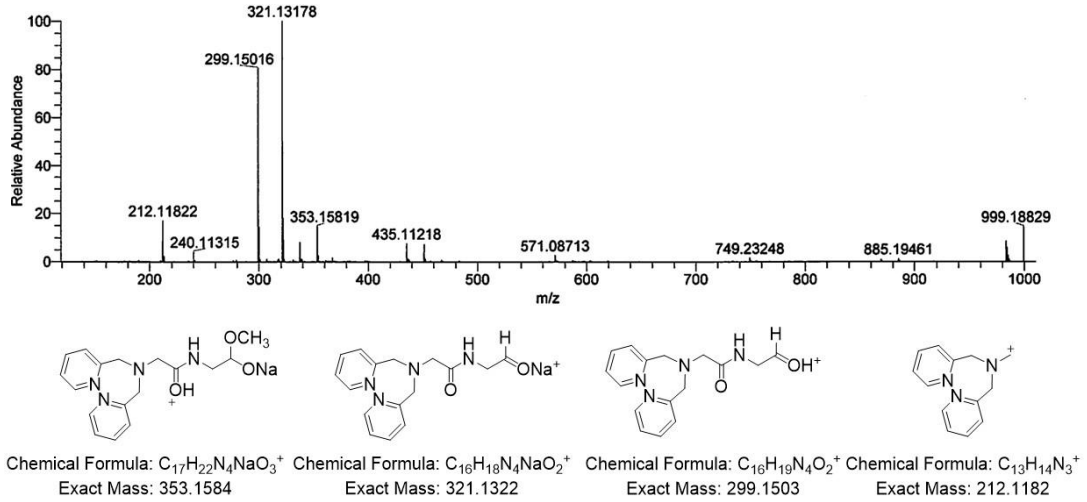


Figure S11. ESI-HRMS spectrum of f16. Structures below the spectrum are the proposed compounds observed for f16 decomposed from Dpa-SoxLC.

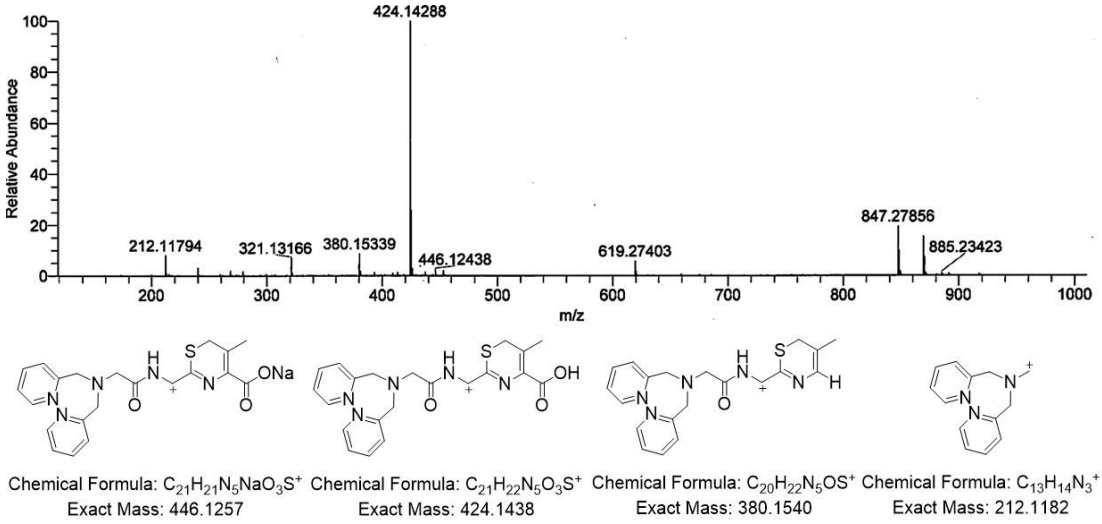


Figure S12. ESI-HRMS spectrum of f29. Structures below the spectrum are the proposed compounds observed for f29 decomposed from Dpa-SoxLC.

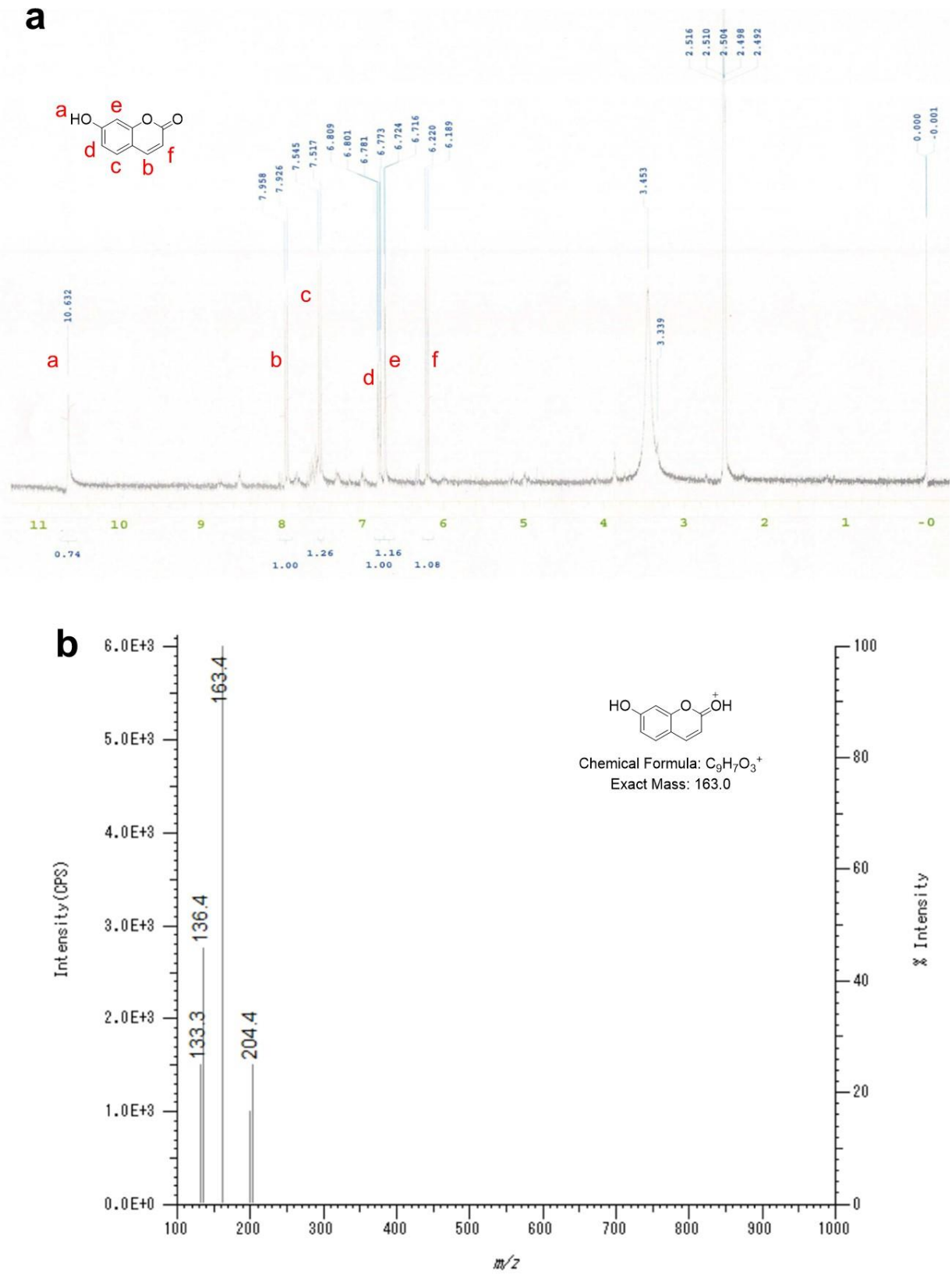


Figure S13. Characterization of fraction at retention time 35 min (f35) in HPLC analyses (Figure 3c), a) ^{1}H-NMR spectrum (300 MHz, DMSO-*d6*) of f35. b) ESI-LRMS spectrum of f35 shows m/z 163.4 identified as [umbelliferrone + H]$^{+}$ (Calcd m/z 163.0).

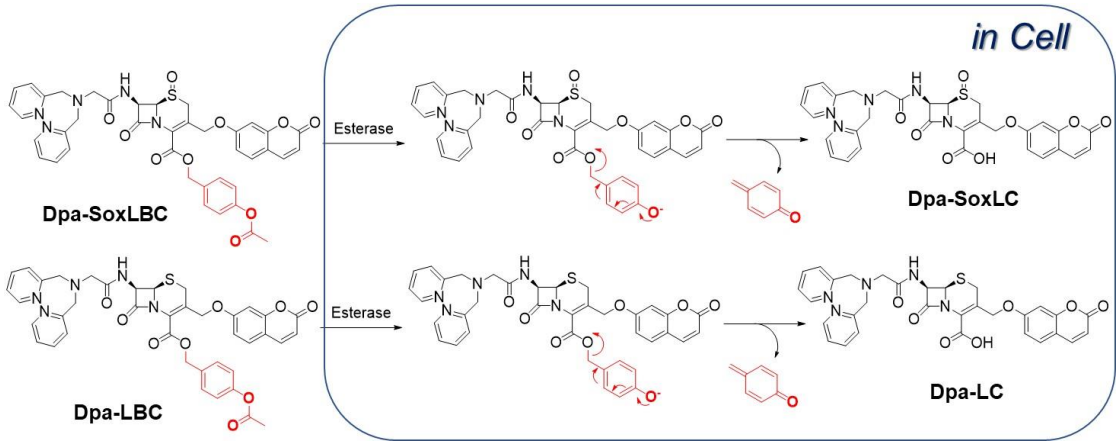


Figure S14. Intracellular transfer concepts of Dpa-SoxLBC and Dpa-LBC. The Dpa-SoxLC and Dpa-LC conjugated with *p*-acetoxybenzyl moieties (Dpa-SoxLBC and Dpa-LBC) are transported into intracellular region and deacetylated by the intracellular esterase with leads to cleavage of quinone methide.

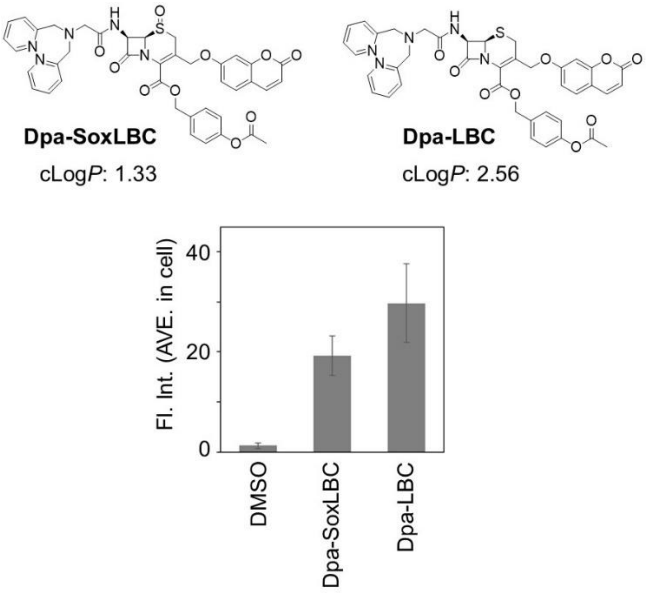


Figure S15. Intracellular detection ability of Dpa-SoxLBC and Dpa-LBC. HeLa cells were treated with 3 μM Dpa-SoxLBC or Dpa-LBC and the intracellular fluorescence was measured at an incubation time for 30 min after the addition of 50 μM zinc pyrithione (ZnPT). Fluorescence channel: λ_{ex} = 405 nm, λ_{em} = 420–530 nm. The parameters, cLog*P* (log$P_{octanol\ /\ water}$ calculated from structure), were calculated by “CLogP” program of ChemDraw professional 16.0 (PerkinElmer Inc.).

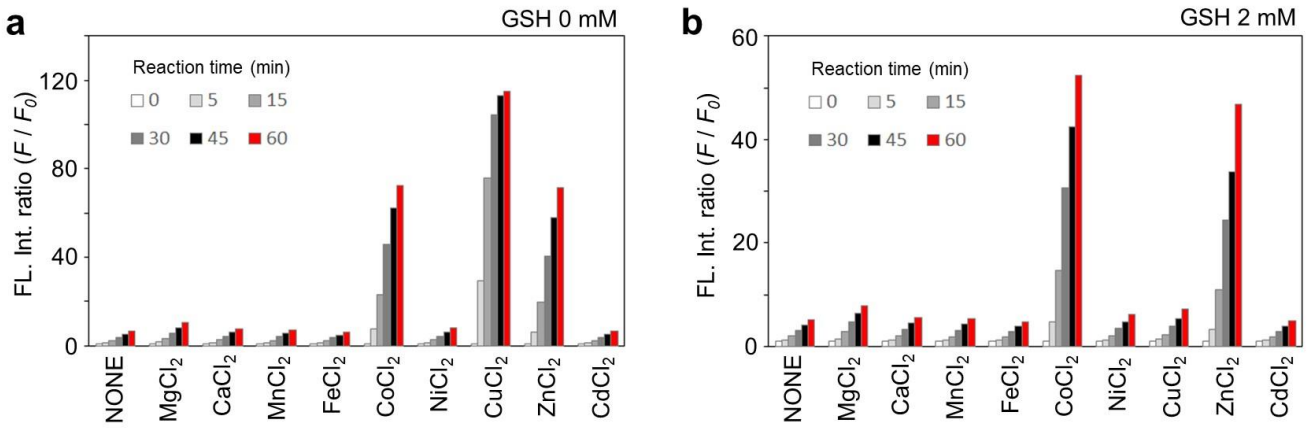


Figure S16. Fluorescence response of 10 μM Dpa-LC towards different metal ions (a) in the absence of GSH or (b) in the presence of 2 mM GSH, λ_{ex} = 390 nm, λ_{em} = 470 nm, the fluorescence was measured at the reaction times, 0, 5, 15, 30, 45, or 60 min after the addition of metal ions. 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO. [Metal ion] = 10 μM except for 500 μM $MgCl_2$ and 2 mM $CaCl_2$. F_0 is the fluorescence intensity before the addition of metal ions.

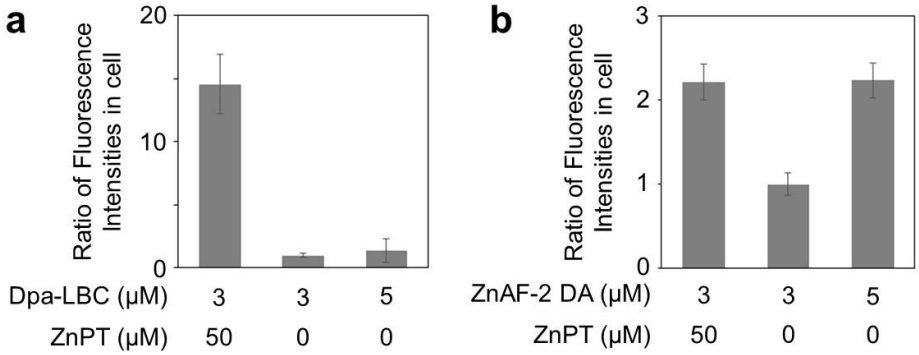


Figure S17. Fluorescence intensities of intracellular region in HeLa cells (n=5), a) fluorescence intensities of HeLa cells treated with 3 μM or 5 μM Dpa-LBC, b) fluorescence intensities of HeLa cells treated with 3 μM or 5 μM ZnAF-2 DA in HEPES buffer saline (HBS) medium with or without 50 μM ZnPT for 30 min at 37 ºC.

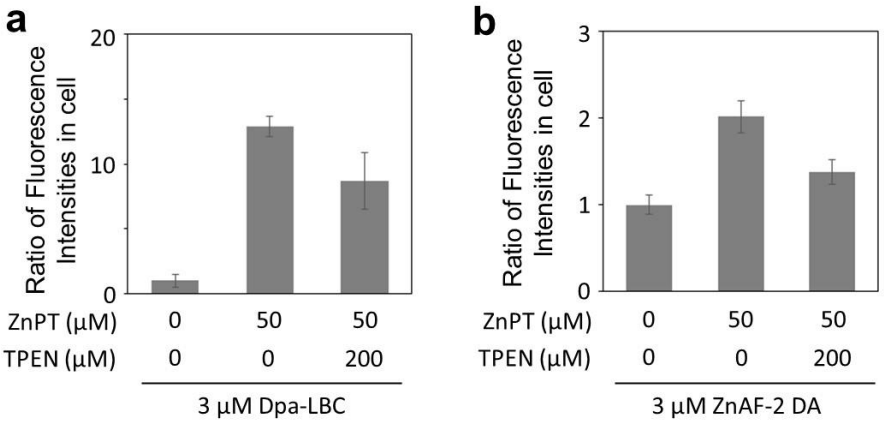


Figure S18. Inhibition effect of *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a) fluorescence intensities of HeLa cells treated with 3 μM Dpa-LBC, b) fluorescence intensities of HeLa cells treated with 3 μM ZnAF-2 DA in HBS medium with or without 50 μM ZnPT, with or without 200 μM TPEN at 37 °C for 30 min.

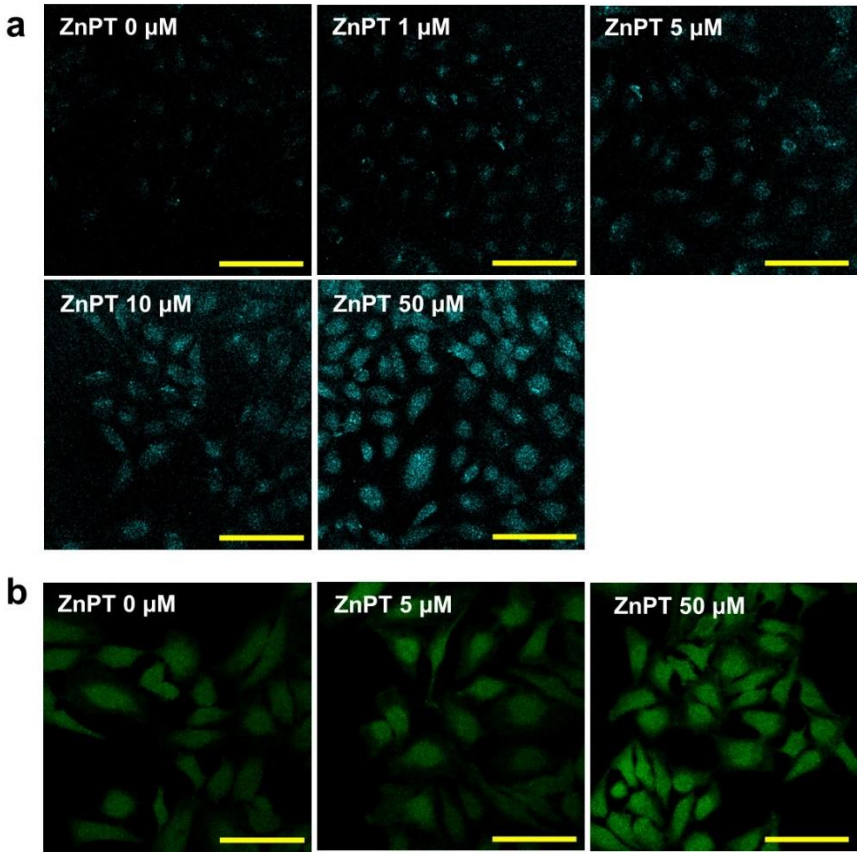


Figure S19. Fluorescent confocal imaging of HeLa cells treated with 3 μM Dpa-LBC (a) and 3 μM ZnAF-2 DA (b). The cells were subsequently treated with 0–50 μM ZnPT for 30 min (a) or 5 min (b) and observed by confocal microscopy. Scale bar: 100 μm.

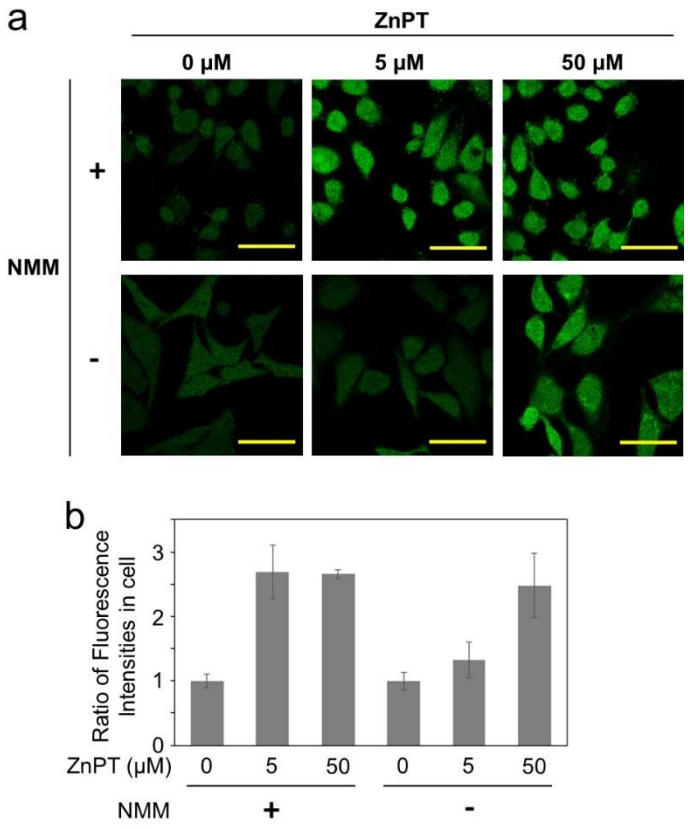


Figure S20. a) Fluorescence confocal imaging of HeLa cells treated with (+) or without (-) 100 μM *N*-methylmaleimide (NMM) and 3 μM ZnAF-2 DA. The cells were subsequently treated with 0, 5, or 50 μM ZnPT for 30 min and observed by confocal microscopy. Scale bar: 100 μm. b) Fluorescence intensities of intracellular region in HeLa cells (n=5) treated with (+) or without (-) 100 μM NMM.

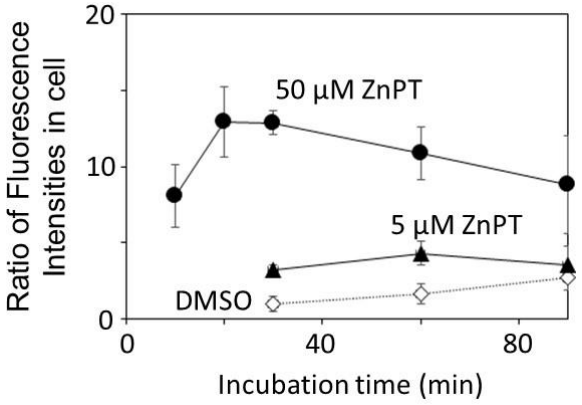


Figure S21. Time-dependent fluorescence of HeLa cells treated with 3 μM Dpa-LBC. After the addition of 0, 5, or 50 μM ZnPT, the cells incubated in HBS medium at 37 °C-5% CO_2 were monitored by confocal microscopy.

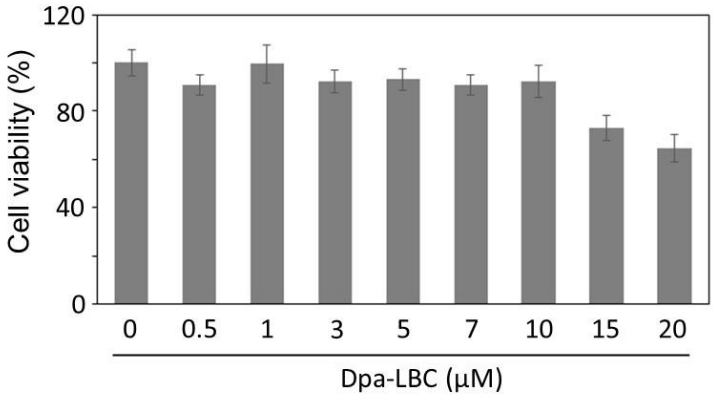
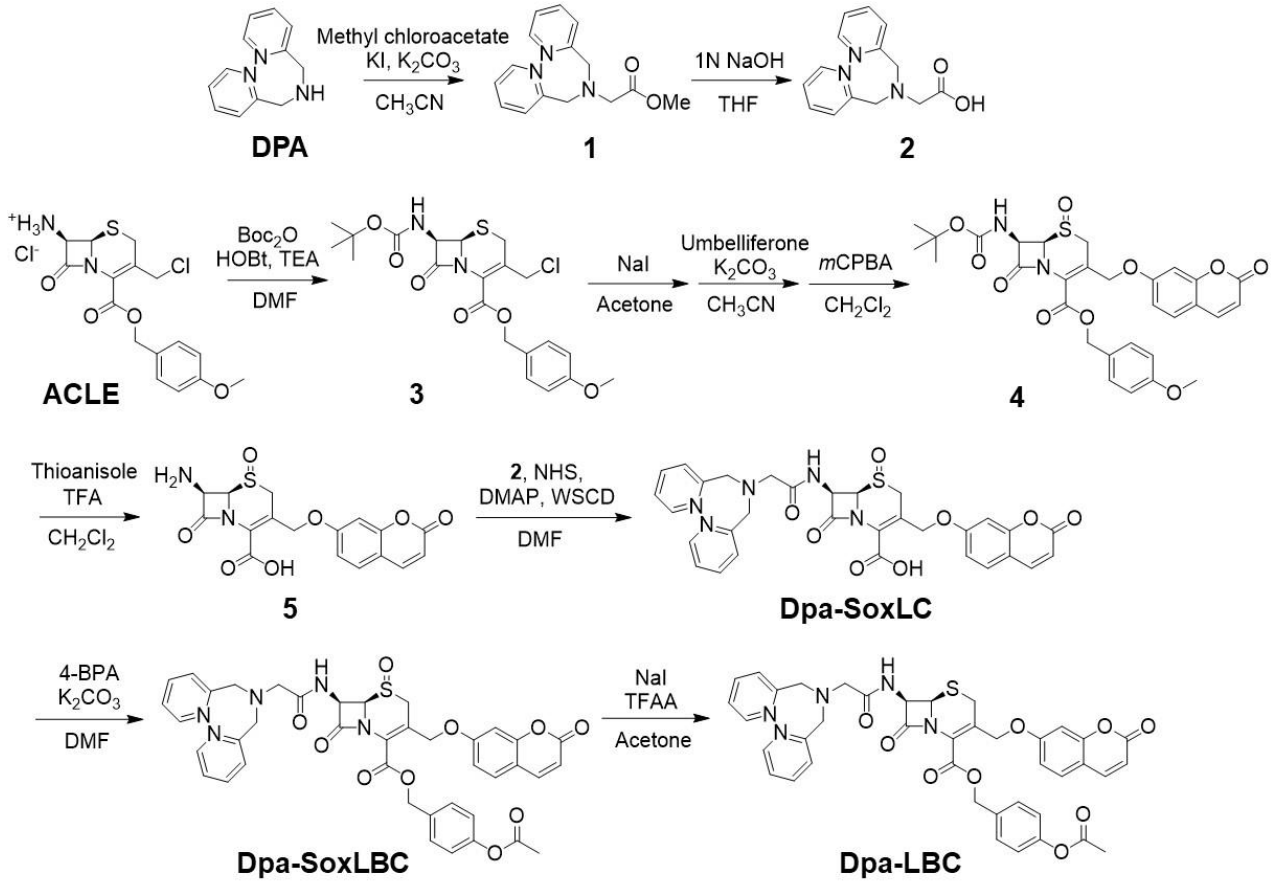


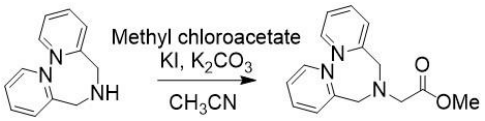
Figure S22. Cell viabilities of HeLa cells after treatment of 0–20 μM Dpa-LBC for 1 hour in incubator at 37 °C-5% CO_2. The absorbance at 450 nm was obtained by the treatment of WST-8 assay reagent for 2 hours.

Synthesis information

The solvents and chemicals for chemical synthesis were used as purchased, with no further purification. ACLE·HCl was purchased from Otsuka Chemical Co. Ltd. All other solvents and chemicals for chemical syntheses were purchased from Aldrich Co., Fujifilm Wako Pure Chemical Co., Kanto Chemical Co., Inc., Nacalai Tesque, Inc., or Tokyo Chemical Industry Co., Ltd. NMR spectra were recorded at 300 MHz / 75 MHz (^{1}H NMR / ^{13}C NMR), HH-COSY using Varian MERCURY plus 300 (300 MHz) spectrometers. Chemical shifts are reported in ppm with the solvent resonance or TMS as the internal standard. Multiplicities are indicated by (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, br = broad). The low-resolution mass spectra (LRMS) were obtained using HITACHI CM5610 MS detector by ESI technique. The high-resolution mass spectra (HRMS) were obtained using Thermo Fisher Scientific Exactive Orbitrap mass spectrometer by ESI technique.

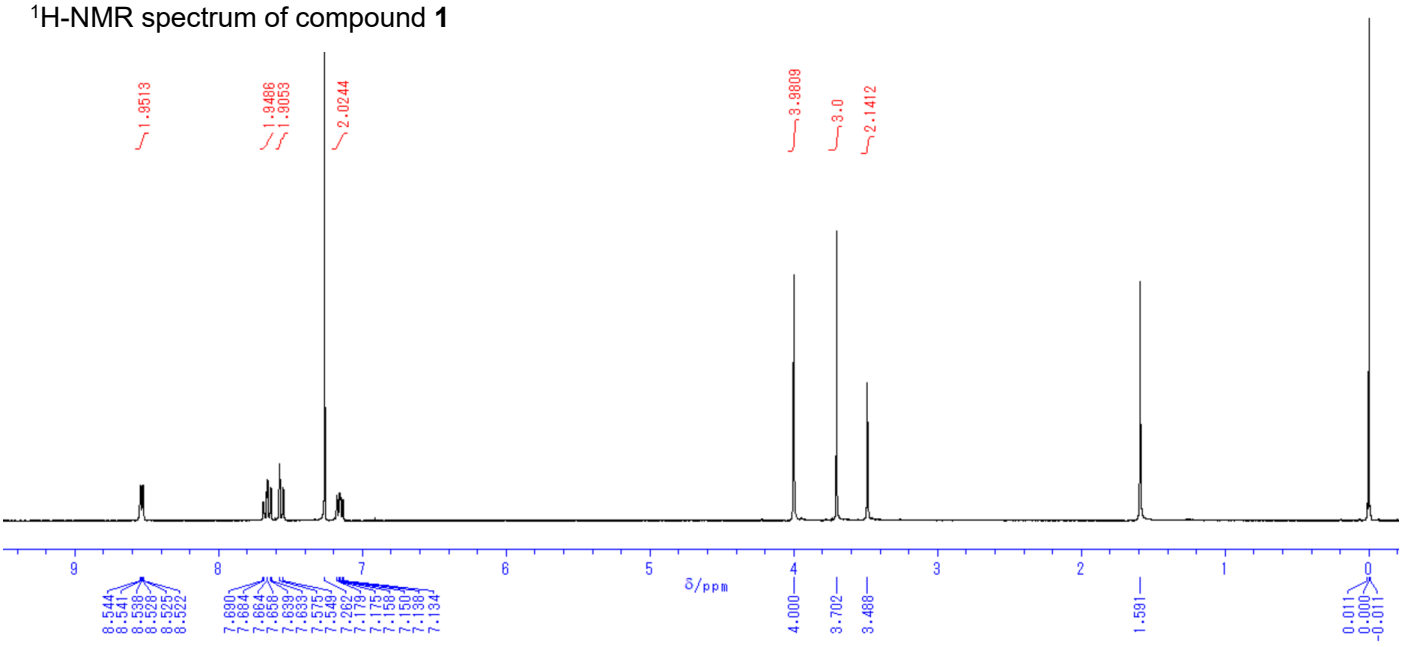


Synthesis of compound 1

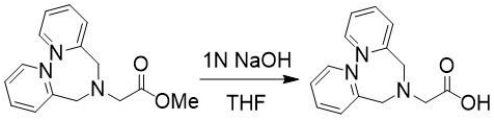


KI (208 mg, 1.35 mmol) and K_2CO_3 (600 mg, 4.35 mmol) were added to a solution of bis(2-pyridylmethyl)amine (355 µL, 394 mg, 1.98 mmol) in CH_3CN (15 mL). After addition of methyl chloroacetate (200 µL, 247 mg, 2.27 mmol), the solution was stirred at 50 °C for 3 hours. After dilution with $CHCl_3$ (150 mL), the suspension was filtered with paper filter. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol / 28% NH_3 aq. = 200 / 10 / 1) to give a brown oil **1** (540 mg, quant). ESI-LRMS m/z found 294.5, calcd 294.1 ($[C_{15}H_{17}N_3O_2+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.49 (2H, s), 3.70 (3H, s), 4.00 (4H, s), 7.16 (2H, td, *J* = 4.8, 1.5 Hz), 7.56 (2H, d, *J* = 7.5 Hz), 7.66 (2H, td, *J* = 7.5, 2.4 Hz), 8.53 (2H, dt, *J* = 5.7, 0.9 Hz). ^{1}H-NMR (300 MHz, DMSO-*d6*). δ 3.45 (2H, s), 3.60 (3H, s), 3.91 (4H, s), 7.25 (2H, td, *J* = 6.0, 1.5 Hz), 7.51 (2H, d, *J* = 7.8 Hz), 7.71 (2H, td, *J* = 7.5, 1.8 Hz), 8.48 (2H, dt, *J* = 4.8, 0.9 Hz).

^{1}H-NMR spectrum of compound **1**

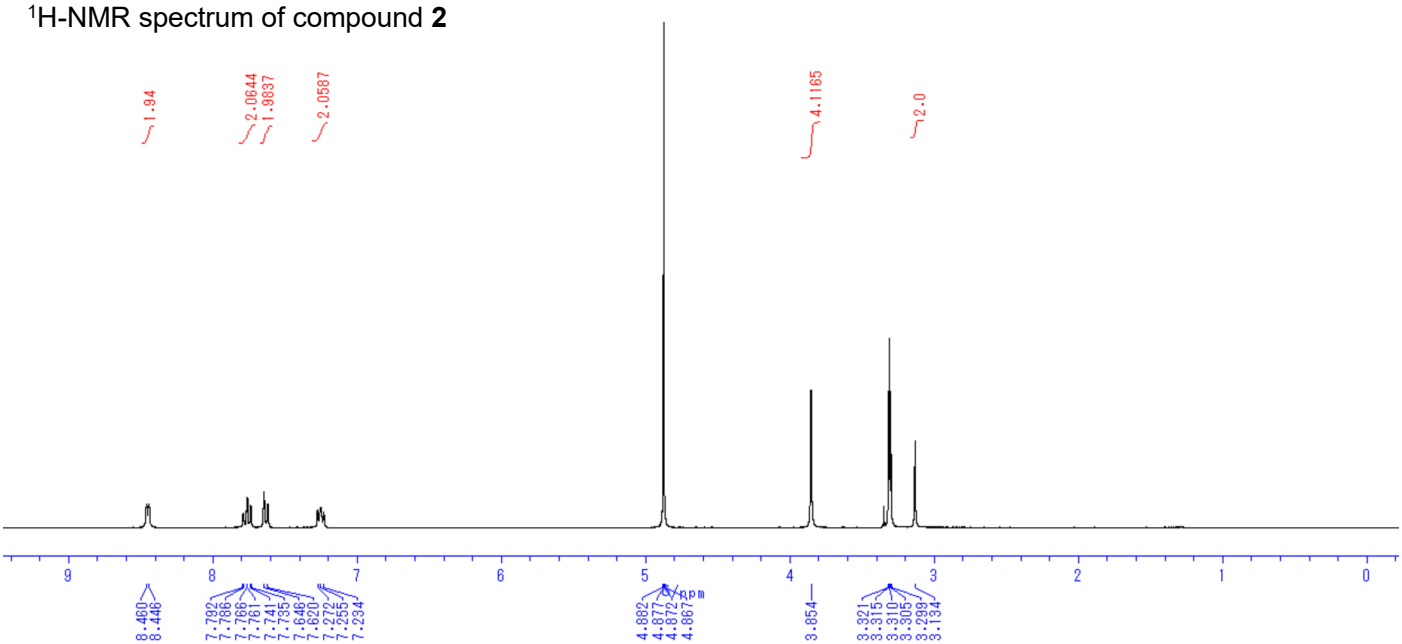


Synthesis of compound 2

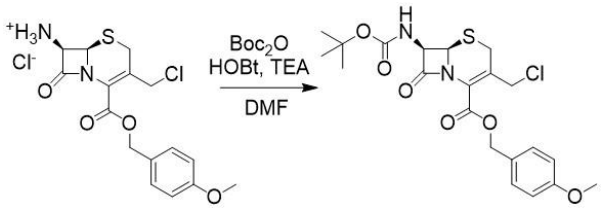


Compound **1** (540 mg, 1.99 mmol) was dissolved in a mixture of THF (tetrahydrofuran, 5 mL) and 1N NaOH (2 mL). The solution was stirred at rt for 6 hours. After removal of THF *in vacuo*, the solution was diluted with water (3 mL). After acidified to pH 5–6 with 1N HCl, the aqueous solution was lyophilized to give a brown amorphous **2** (529 mg, quant). ESI-LRMS m/z found 280.5, calcd 280.1 ($[C_{14}H_{15}N_3O_2+Na]^+$). ^{1}H-NMR (300 MHz, CD_3OD) δ 3.13 (2H, s), 3.85 (4H, s), 7.26 (2H, t, *J* = 5.7 Hz), 7.63 (2H, d, *J* = 8.4 Hz), 7.76 (2H, td, *J* = 8.4, 1.8 Hz), 8.45 (2H, d, *J* = 4.2 Hz).

^{1}H-NMR spectrum of compound **2**

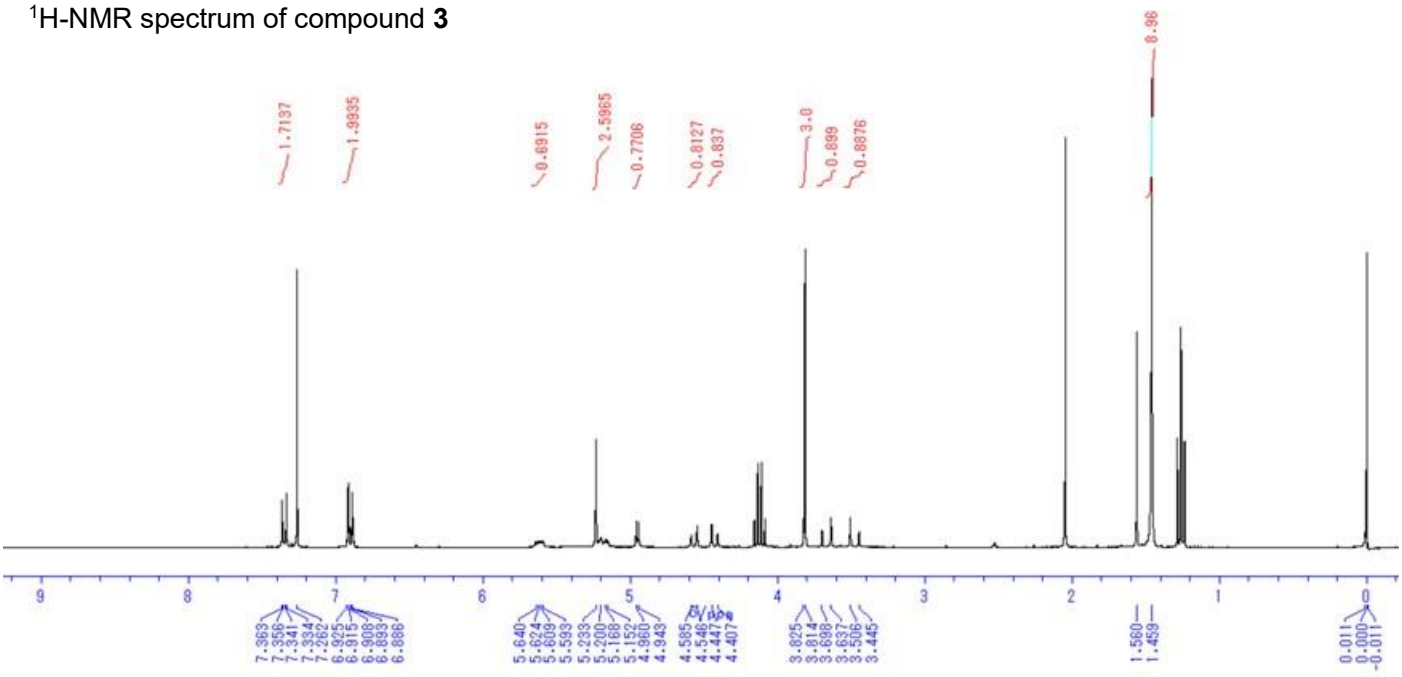


Synthesis of compound 3

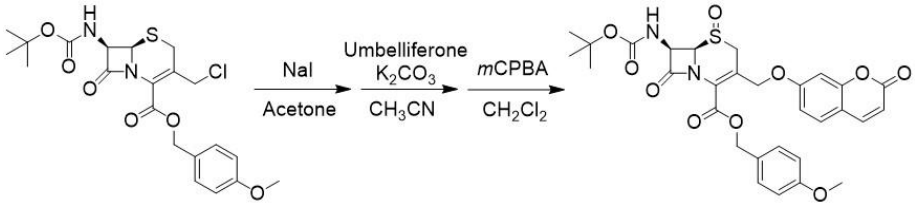


Triethylamine (TEA, 204 µL, 148 mg, 1.46 mmol) was added to a solution of ACLE·HCl (7-amino-3-chloromethyl-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester monohydrochloride) (600 mg, 1.48 mmol), Boc_2O (di-*tert*-butyl dicarbonate, 520 mg, 2.38 mmol), and HOBt·H_2O (1-hydroxybenzotriazole monohydrate, 390 mg, 2.82 mmol). The solution was stirred at 0 °C for 20 min before warming to rt and stirring for 23 hours. After dilution with ethyl acetate (150 mL), the organic layer was washed with water (200 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, hexane / ethyl acetate = 5 / 1 → 3 / 1) to give a clear oil **3** (602 mg, 1.28 mmol, 87%). ESI-HRMS m/z found 491.1007, calcd 491.1014 ($[C_{21}H_{25}ClN_2O_6S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ1.46 (9H, s), 3.48 (1H, d, *J* = 18.3 Hz), 3.67 (1H, d, *J* = 18.3 Hz), 3.81 (3H, s), 4.43 (1H, d, *J* = 11.7 Hz), 4.57 (1H, d, *J* = 12.0 Hz), 4.95 (1H, d, *J* = 4.8 Hz), 5.15–5.21 (1H, m), 5.23 (2H, s), 5.62 (1H, dd, *J* = 9.3, 4.8 Hz), 6.90 (2H, dd, *J* = 6.6, 2.1 Hz), 7.35 (2H, dd, *J* = 6.6, 2.1 Hz).

^{1}H-NMR spectrum of compound **3**

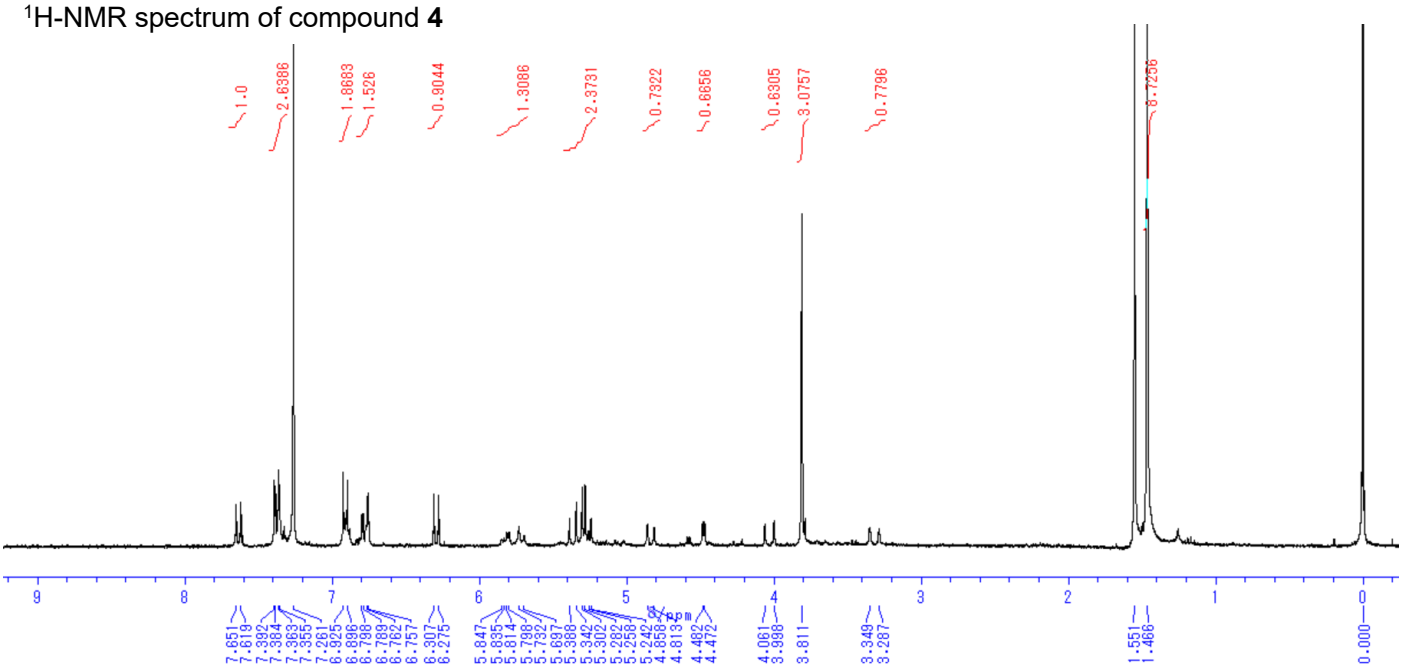


Synthesis of compound 4

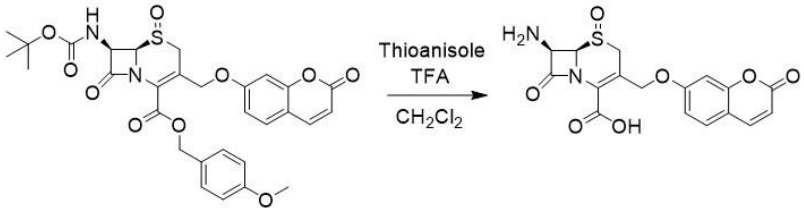


Sodium iodide (1.80 g, 12.0 mmol) was added to a solution of compound **3** (606 mg, 1.29 mmol) in acetone (40 mL). The mixture was stirred at rt for an hour, followed by removal of the solvent *in vacuo*. The residue was dissolved with ethyl acetate (200 mL) and the organic layer was washed with water (200 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. After dried *in vacuo* at rt for 2 hours, the residue was used for the next reaction without further purification. Umbelliferone (300 mg, 1.85 mmol) and K_2CO_3 (370 mg, 2.68 mmol) were added to a solution of the fore-mentioned crude in CH_3CN (20 mL). The mixture was stirred at rt for 3 hours. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 50 / 1) to give a crude of the umbellieferone conjugate (580 mg) as a yellow oil. This oil was dissolved in CH_2Cl_2 (30 mL). After cooling to 0 °C, *m*-chloroperoxybenzoic acid (*m*CPBA, 220 mg, 831 μmol) was added to the solution and after stirring at 0 °C for 30 min. The solution was diluted with $CHCl_3$ (150 mL) and the organic layer was washed with water (150 mL). After dried over Na_2SO_4, the organic solvent was removed *in vacuo*. The residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 50 / 1) to give a pale orange solid **4** (380 mg, 622 μmol, 48%). ESI-LRMS m/z found 633.2, calcd 633.2 ($[C_{30}H_{30}N_2O_{10}S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 3.32 (1H, d, *J* = 18.6 Hz), 3.81 (3H, s), 4.03 (1H, d, *J* = 18.9 Hz), 4.48 (1H, d, *J* = 3.0 Hz), 4.84 (1H, d, *J* = 13.5 Hz), 5.26 (1H, d, *J* = 12.0 Hz), 5.28 (1H, d, *J* = 13.2 Hz), 5.37 (1H, d, *J* = 13.5 Hz), 5.71 (1H, d, *J* = 10.8 Hz), 5.82 (1H, dd, *J* = 10.8, 3.6 Hz), 6.29 (1H, d, *J* = 9.3 Hz), 6.76–6.80 (2H, m), 6.91 (2H, d, *J* = 8.7 Hz), 7.37 (1H, d, *J* = 8.4 Hz), 7.38 (2H, d, *J* = 8.4 Hz), 7.64 (1H, d, *J* = 9.3 Hz).

^{1}H-NMR spectrum of compound **4**

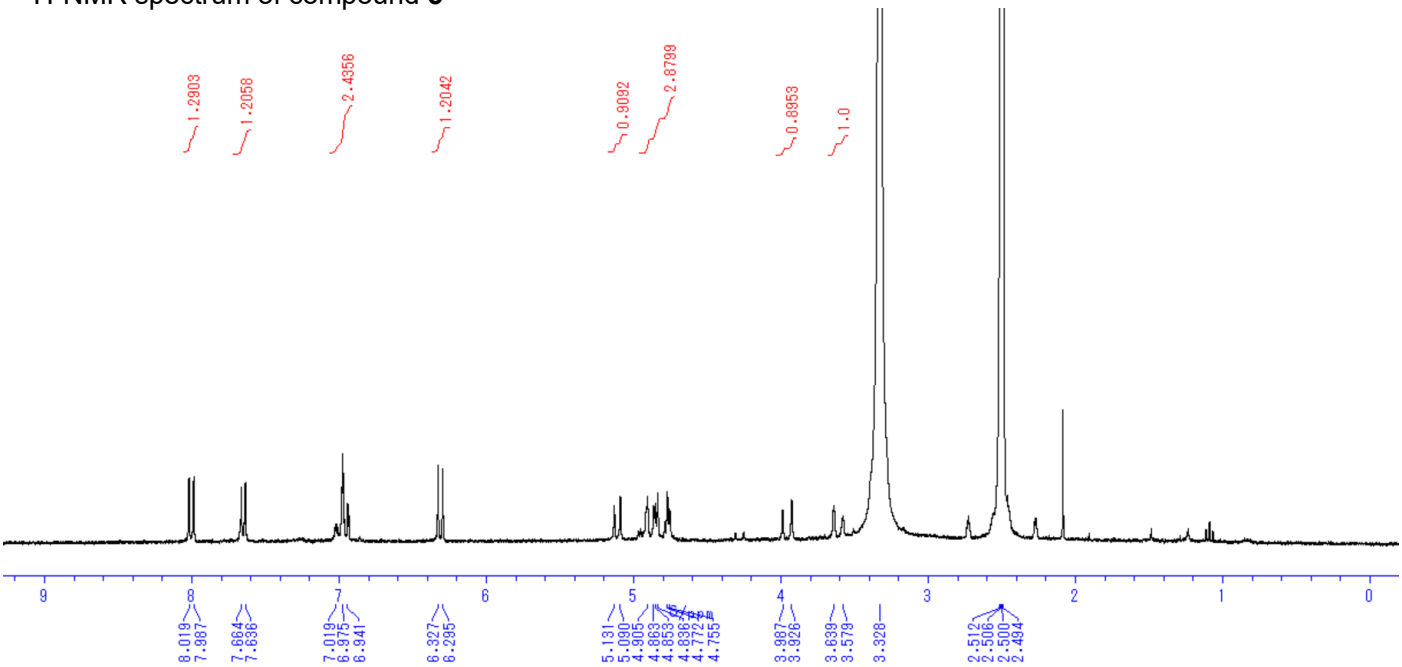


Synthesis of compound 5

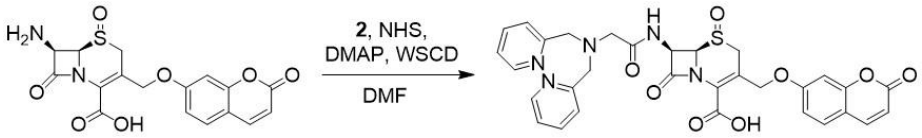


Compound **4** (70.4 mg, 115 μmol) was dissolved in CH_2Cl_2 (2.4 mL). Thioanisole (500 μL) and TFA (trifluoroacetic acid, 1.8 mL) were added to the solution at 0 °C. The solution was stirred at 0 °C for 4 hours. After addition of cold diethyl ether (12 mL), the precipitate was collected by the centrifugation (10000 g × 1 min) and the supernatant was removed. Addition of cold diethyl ether (6 mL) followed by the centrifugation step was repeated 3 times to wash the precipitate. The residue was dried *in vacuo* to give a white solid **5** (35.0 mg, 89.7 μmol, 78%). ESI-HRMS m/z found 391.0592, calcd 391.0594 ($[C_{17}H_{14}N_2O_7S+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.61 (1H, d, *J* = 19.5 Hz), 3.96 (1H, d, *J* = 18.3 Hz), 4.76 (1H, d, *J* = 5.1 Hz), 4.84 (1H, d, *J* = 4.8 Hz), 4.88 (1H, d, *J* = 12.6 Hz), 5.11 (1H, d, *J* = 12.3 Hz), 6.31 (1H, d, *J* = 9.6 Hz), 6.94–7.02 (2H, m), 7.65 (1H, d, *J* = 8.4 Hz), 8.00 (1H, d, *J* = 9.6 Hz).

^{1}H-NMR spectrum of compound **5**

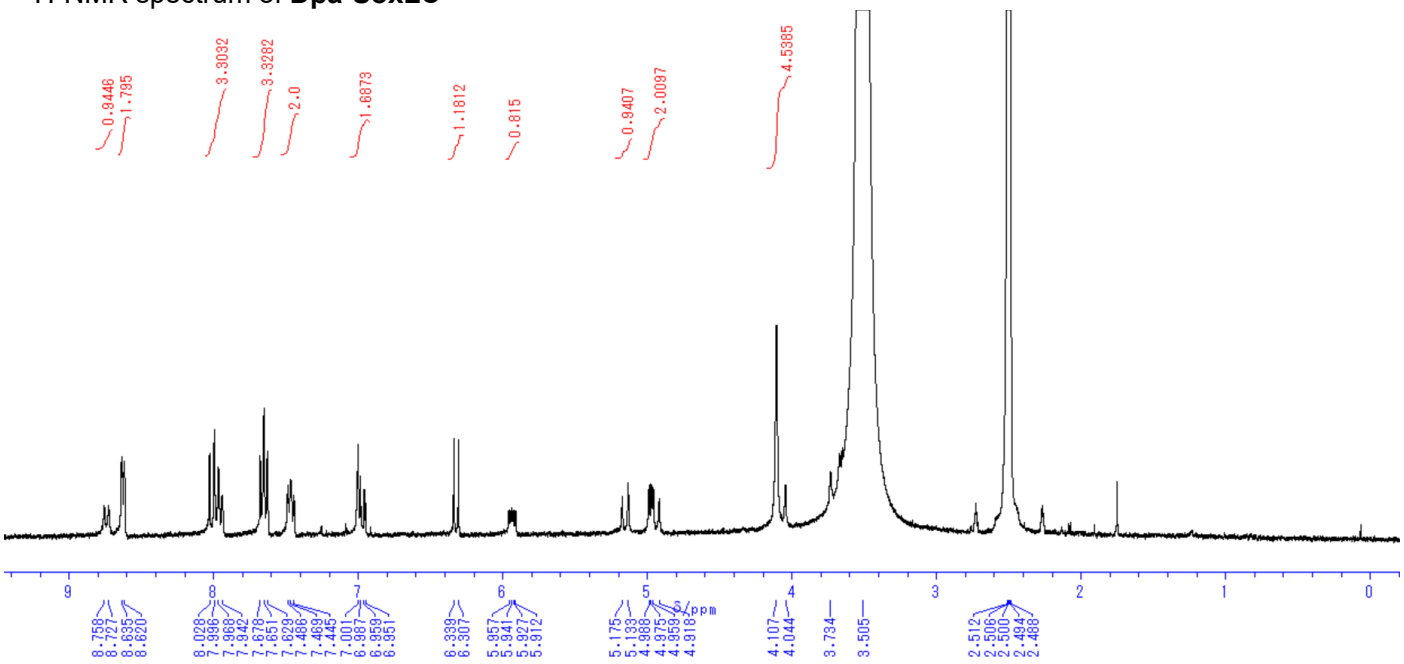


Synthesis of Dpa-SoxLC

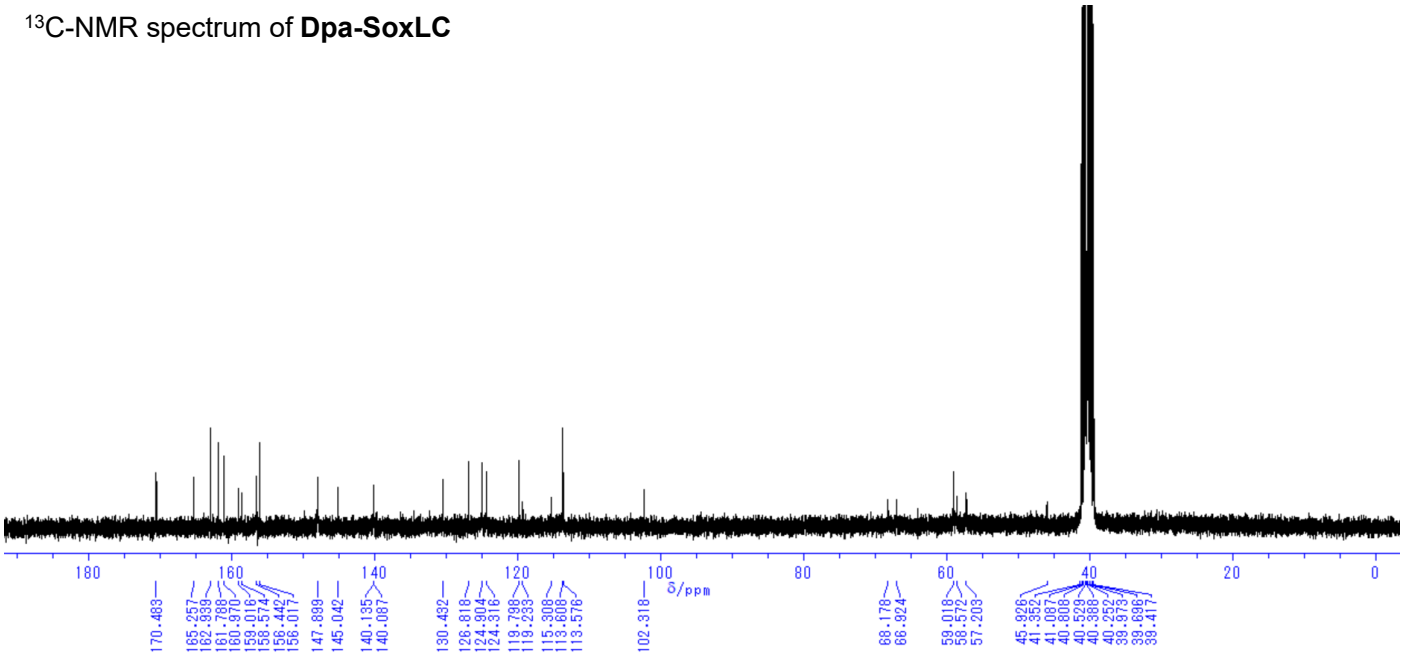


WSCD·HCl (Water-Soluble CarboDiimide, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride, 32.6 mg, 170 μmol) was added into a solution of compound **2** (75.1 mg, 268 μmol), NHS (*N*-hydroxysuccinimide, 26.3 mg, 229 μmol), and DMAP (4-dimethylaminopyridine, 26.0 mg, 213 μmol) in DMF (3 mL) at 0 °C. The solution was stirred at 0 °C for 2 hours, followed by the addition of compound **5** (20.0 mg, 51.2 μmol). After stirring at 0 °C for 1.5 hours, the solution was diluted with a mixture of water / CH_3CN = 2 / 1 (4.5 mL) and purified by HPLC (ODS-A, YMC-Pack, 150 × 30 mm, Flow rate: 9.9 mL/min, A: CH_3CN (0.1% TFA) B: water (0.1% TFA), gradient: (Time (min), B (%)) = (0, 95) (10, 95) (50, 50) (55, 5), retention time 37 min). The obtained solution was lyophilized to give a yellowish white solid **Dpa-SoxLC** (20.5 mg, 32.5 μmol, 64%). ESI-HRMS m/z found 630.1682, calcd 630.1653 ($[C_{31}H_{27}N_5O_8S+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.50 (2H, s), 3.70 (1H, d, *J* = 18.0 Hz), 4.07 (1H, d, *J* = 19.2 Hz), 4.11 (4H, s), 4.94 (1H, d, *J* = 12.3 Hz), 4.98 (1H, d, *J* = 3.9 Hz), 5.15 (1H, d, *J* = 12.9 Hz), 5.93 (1H, dd, *J* = 8.7, 4.5 Hz), 6.32 (1H, d, *J* = 9.6 Hz), 6.95–7.00 (2H, m), 7.47 (2H, t, *J* = 6.3 Hz), 7.64 (2H, d, *J* = 6.6 Hz), 7.65 (1H, d, *J* = 6.3 Hz), 7.96 (2H, t, *J* = 6.3 Hz), 8.01 (1H, d, *J* = 9.6 Hz), 8.63 (2H, d, *J* = 4.5 Hz), 8.74 (1H, d, *J* = 9.3 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 45.93, 57.20, 58.57, 59.02, 66.92, 68.18, 102.32, 113.61, 115.31, 119.23, 119.80, 124.32, 124.90, 126.82, 130.43, 140.09, 145.04, 147.90, 156.02, 156.44, 158.57, 159.02, 160.97, 161.79, 162.94, 165.26, 170.48.

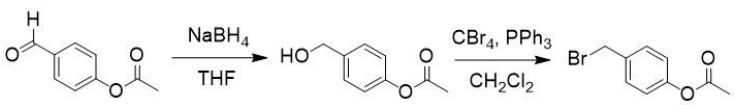
^{1}H-NMR spectrum of **Dpa-SoxLC**



^{13}C-NMR spectrum of **Dpa-SoxLC**



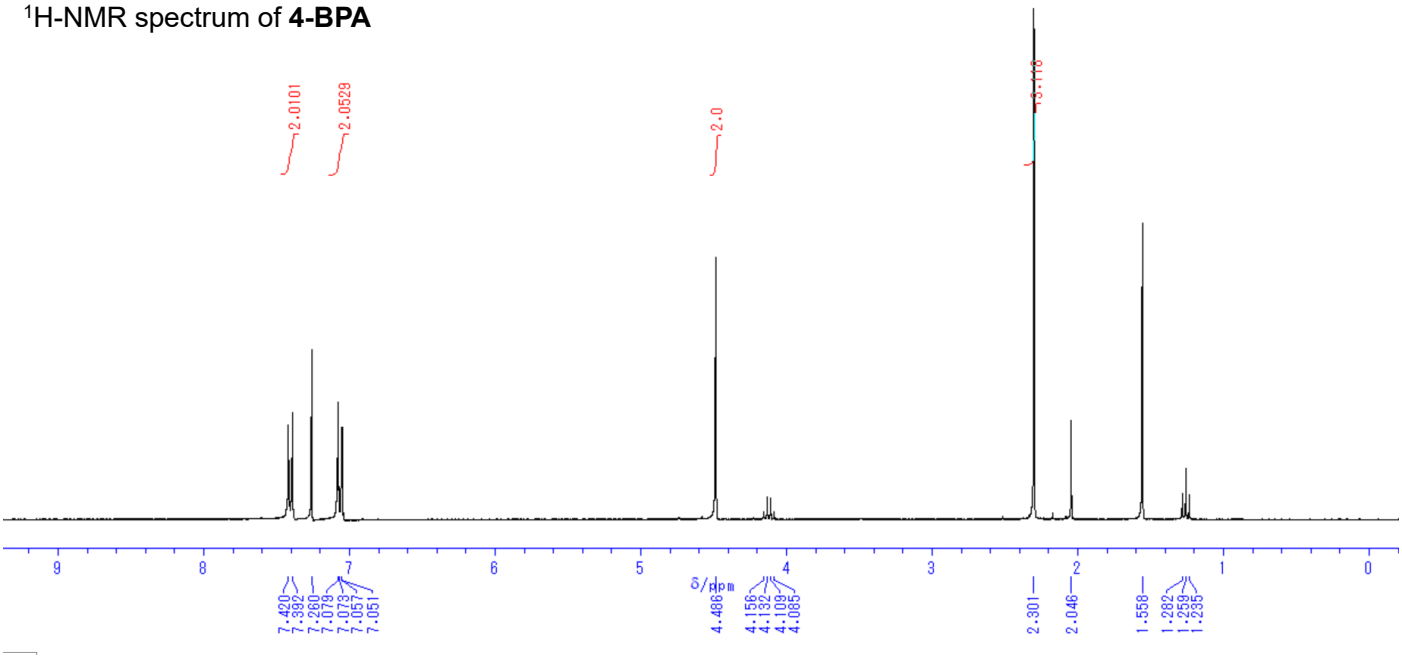
Synthesis of 4-bromomethylphenyl acetate (4-BPA)



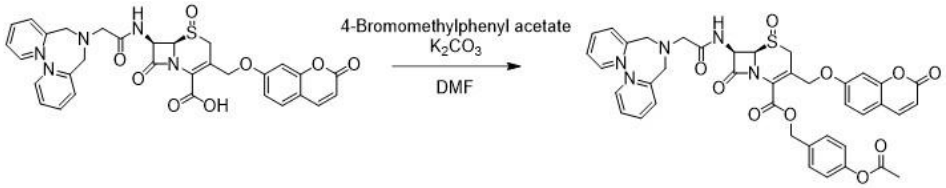
This synthesis was conducted according to the reported method[S3]. $NaBH_4$ (56.0 mg, 1.48 mmol) was added to a solution of 4-acetoxybenzaldehyde (220 mg, 1.34 mmol) in THF (4 mL) at 0 °C. After stirring at 0 °C for 1.5 hours,

the solution was diluted with ethyl acetate (50 mL) and washed with sat. NH_4Cl aq. (20 mL × 2). The organic layer was dried over Na_2SO_4 and evaporated. After drying *in vacuo* at rt for 1.5 hours, the residue was dissolved in CH_2Cl_2 (4 mL) and cooled to 0 °C, then CBr_4 (584 mg, 1.76 mmol) and triphenylphosphine (416 mg, 1.58 mmol) were added to the solution. The solution was stirred at 0 °C for 10 min and at rt for 24 hours. After removal of the solvent, the residue was purified by column chromatography (SiO_2, hexane / ethyl acetate = 12 / 1 → 10 / 1) to give a colorless crystal **4-BPA** (226 mg, 986 μmol, 74%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 4.49 (2H, s), 7.06 (2H, dd, *J* = 6.6, 1.8 Hz), 7.41 (2H, dd, *J* = 6.6, 1.8 Hz). The physical properties are consistent with the reported data.[S3]

^{1}H-NMR spectrum of **4-BPA**



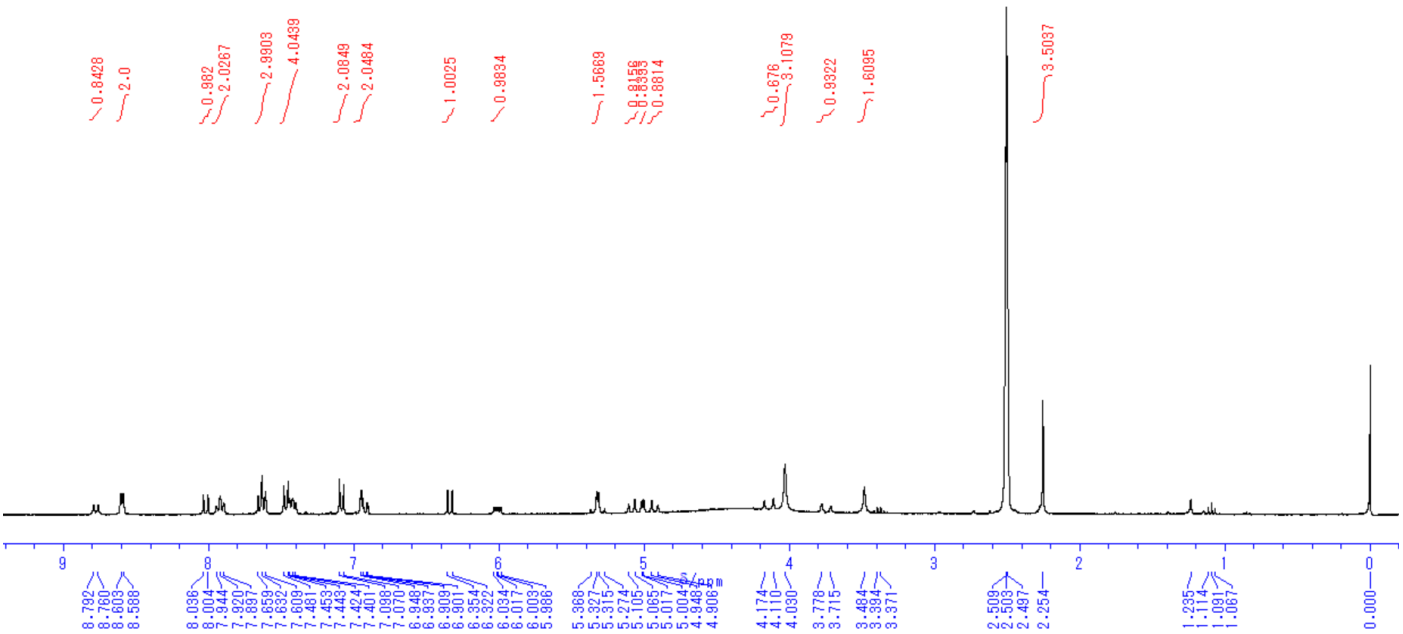
Synthesis of Dpa-SoxLBC



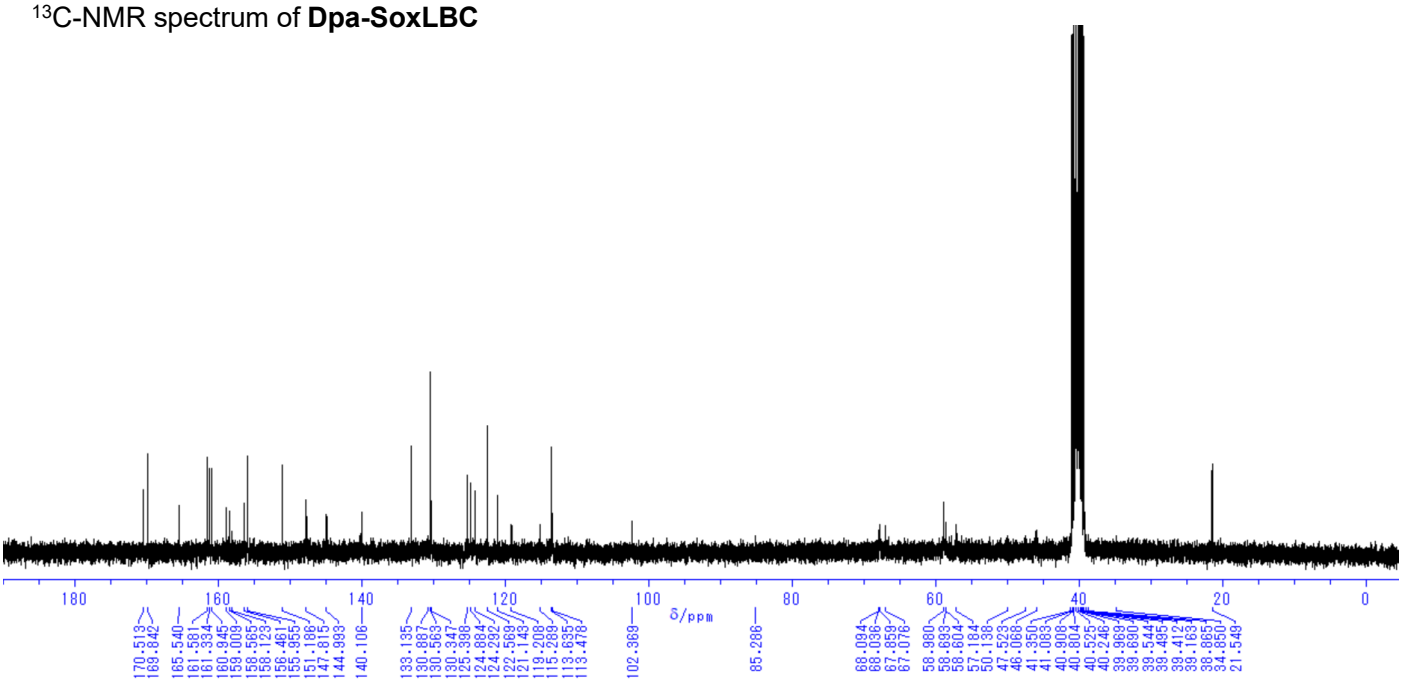
Dpa-SoxLC (17.5 mg, 27.8 μmol) was dissolved in DMF (3 mL), and then K_2CO_3 (10.1 mg, 72.5 μmol) and 4-BPA (26.6 mg, 116 μmol) were added to the solution. After stirring at 0 °C for 6 hours, the solution was diluted with 9 mL of CH_3CN / water = 1 / 2 (0.1% TFA) and filtered with a PVDF filter (Millex-HV, Non-sterile, 13mmφ, 0.45 μm). The filtrate was purified by HPLC (ODS-A, YMC-Pack, 150 × 30 mm, Flow rate: 9.9 mL/min, A: CH_3CN (0.1% trifluoroacetic acid) B: water (0.1% TFA), gradient: (Time (min), B (%)) = (0, 95) (40, 55) (45, 5) (50, 5), retention time 43 min). The obtained solution was lyophilized to give a white solid **Dpa-SoxLBC** (10.2 mg, 13.1 μmol, 47%). ESI-HRMS m/z found 778.2184, calcd 778.2177 ($[C_{40}H_{35}N_5O_{10}S+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 2.25 (3H, s), 3.48 (2H, s), 3.75 (1H, d, *J* = 18.9 Hz), 4.03 (4H, s), 4.14 (2H, d, *J* = 19.2 Hz), 4.93 (1H, d, *J* = 12.6 Hz), 5.01 (1H, d, *J* = 3.9 Hz), 5.09 (1H, d, *J* = 12.0 Hz), 5.32 (1H, d, *J* = 3.6 Hz), 6.01 (1H, dd, *J* = 9.3, 5.1 Hz), 6.34 (1H, d, *J* = 9.6 Hz), 6.90–6.95 (2H, m), 7.08 (2H, d, *J* = 8.4 Hz), 7.40–7.48 (4H, m), 7.63 (2H+1H, t, *J* = 7.5 Hz), 7.92 (2H, t, *J* = 7.2 Hz), 8.02 (1H, d, *J* = 9.6 Hz), 8.60 (2H, d, *J* = 4.5 Hz), 8.78 (1H, d, *J* = 9.6 Hz). ^{13}C-NMR (75 MHz, DMSO-

d6) δ 21.55, 46.07, 57.18, 58.69, 58.98, 67.07, 67.86, 68.04, 68.09, 102.37, 113.48, 113.64, 115.29, 121.14, 122.57, 124.29, 124.88, 125.40, 130.35, 130.56, 133.14, 140.11, 144.99, 147.82, 151.19, 155.96, 156.46, 160.95, 161.34, 161.59, 165.54, 169.84, 170.52.

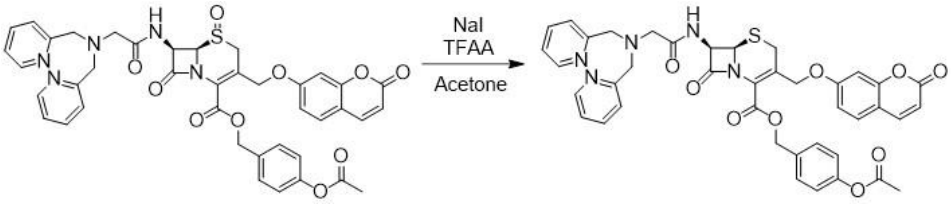
^{1}H-NMR spectrum of **Dpa-SoxLBC**



^{13}C-NMR spectrum of **Dpa-SoxLBC**

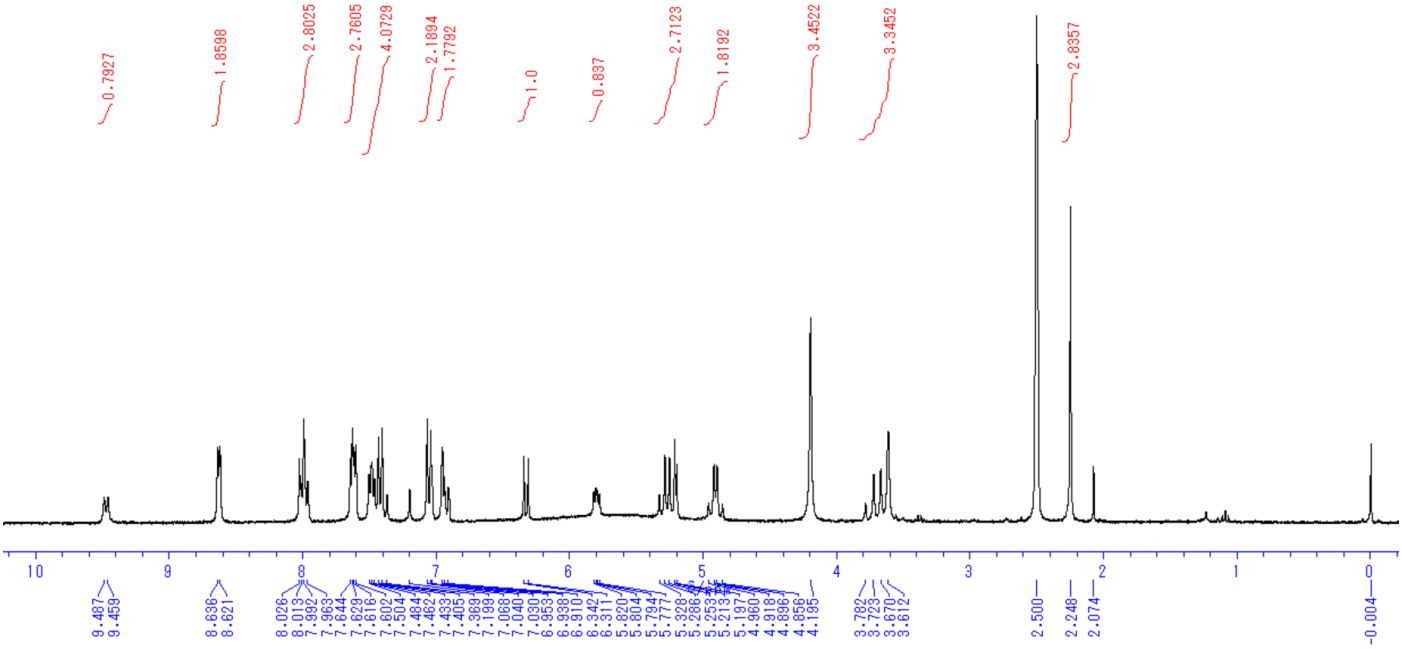


Synthesis of Dpa-LBC

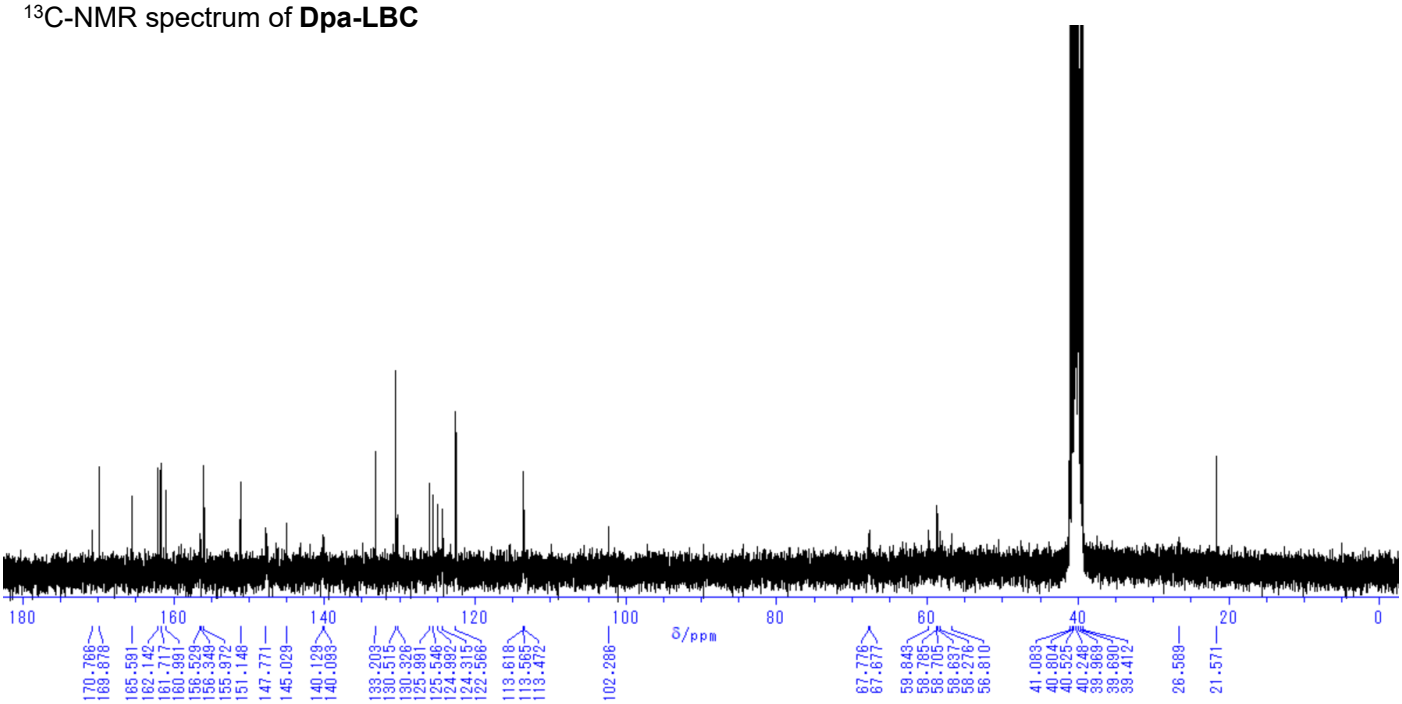


Dpa-SoxLBC (16.7 mg, 21.5 μmol) was dissolved in acetone (2 mL), and then NaI (16.1 mg, 107 μmol) and trifluoroacetic anhydride (16.3 μL, 24.3 mg, 118 μmol) were added to the solution. After stirring at 0 °C for 30 min, the solution was diluted with 5.4 mL of CH_3CN / water = 1 / 2 (0.1% TFA) and filtered with a PVDF filter (Millex-HV, Non-sterile, 13mmφ, 0,45 μm). The filtrate was purified by HPLC (ODS-A, YMC-Pack, 150 × 30 mm, Flow rate: 9.9 mL/min, A: CH_3CN (0.1% TFA) B: water (0.1% TFA), gradient: (Time (min), B (%)) = (0, 80) (30, 32) (31, 0) (36, 0), retention time 24 min). The obtained solution was lyophilized to give a yellow crystalline solid **Dpa-LBC** (11.3 mg, 14.8 μmol, 69%). ESI-HRMS m/z found 762.2221, calcd 762.2228 ($[C_{40}H_{35}N_5O_9S+H]^+$). ^{1}H-NMR(300 MHz, DMSO-*d6*) δ 2.25 (3H, s), 3.61 (2H, s), 3.64 (1H, d, *J* = 17.4 Hz), 3.75 (1H, d, *J* = 17.7 Hz), 4.20 (4H, s), 4.88 (1H, d, *J* = 12.0 Hz), 4.94 (1H, d, *J* = 12.6 Hz), 5.21 (1H, d, *J* = 4.2 Hz), 5.24 (1H, d, *J* = 12.3 Hz), 5.31 (1H, d, *J* = 12.3 Hz), 5.81 (1H, dd, *J* = 8.1, 5.1 Hz), 6.33 (1H, d, *J* = 9.3 Hz), 6.91–6.95 (2H, m), 7.05 (2H, d, *J* = 8.4 Hz), 7.42 (2H, d, *J* = 8.4 Hz), 7.48 (2H, t, *J* = 6.3 Hz), 7.60 (2H, d, *J* = 8.1 Hz), 7.63 (1H, d, *J* = 8.7 Hz), 7.98 (2H, t, *J* = 8.7 Hz), 8.02 (1H, d, *J* = 3.9 Hz), 8.63 (2H, d, *J* = 4.5 Hz), 9.47 (1H, d, *J* = 8.4 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 21.57, 26.59, 56.81, 58.28, 58.71, 59.84, 67.68, 67.78, 102.29, 113.47, 113.57, 122.57, 124.32, 124.99, 125.55, 125.99, 130.33, 130.52, 133.20, 140.13, 145.03, 147.77, 151.15, 155.97, 156.53, 158.94, 160.99, 161.72, 162.14, 165.59, 169.88, 170.77.

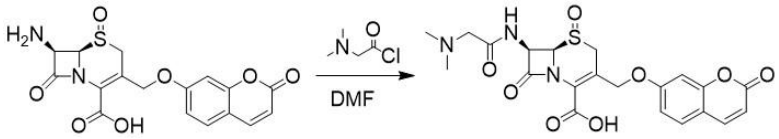
^{1}H-NMR spectrum of **Dpa-LBC**



^{13}C-NMR spectrum of **Dpa-LBC**

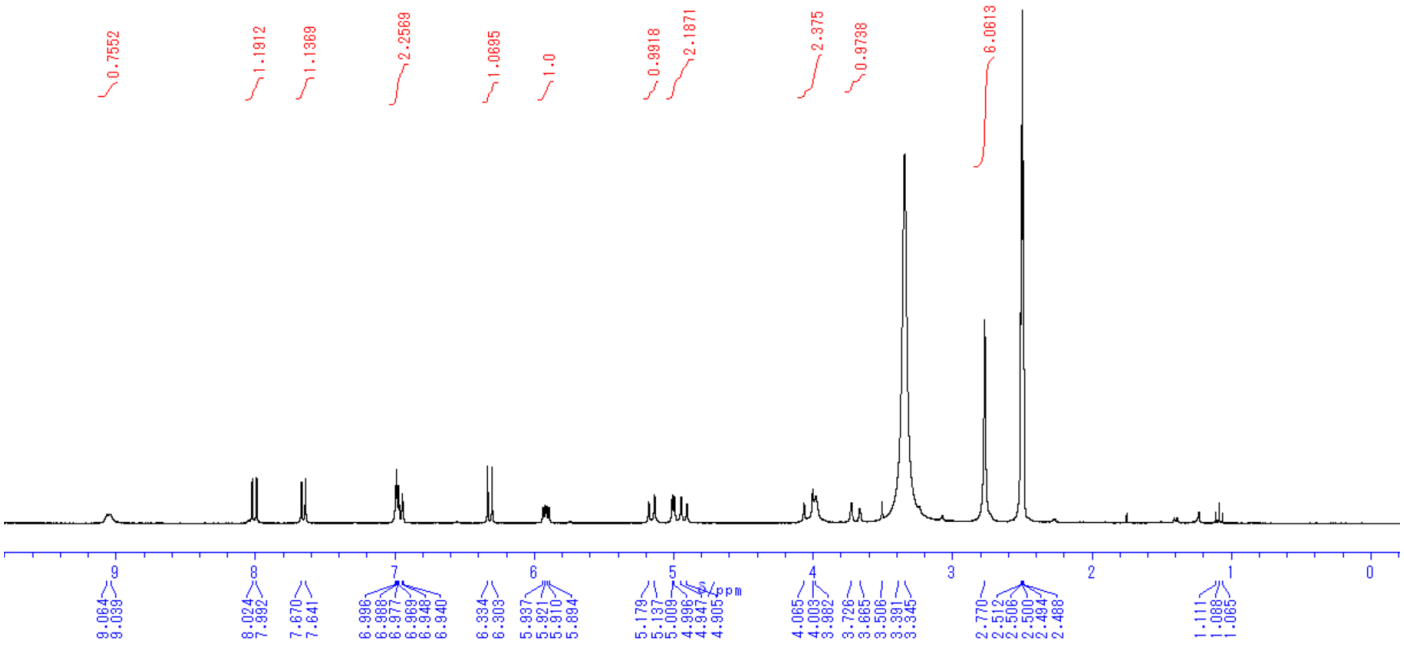


Synthesis of DM-SoxLC

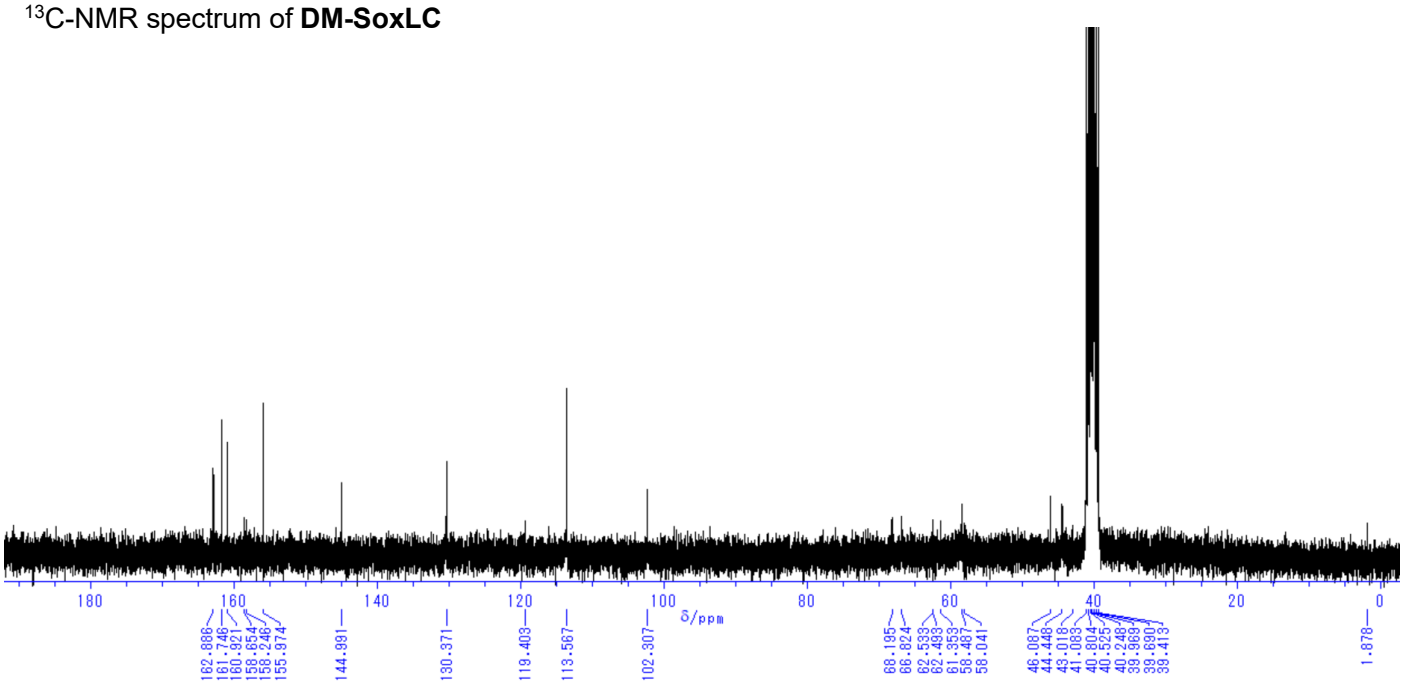


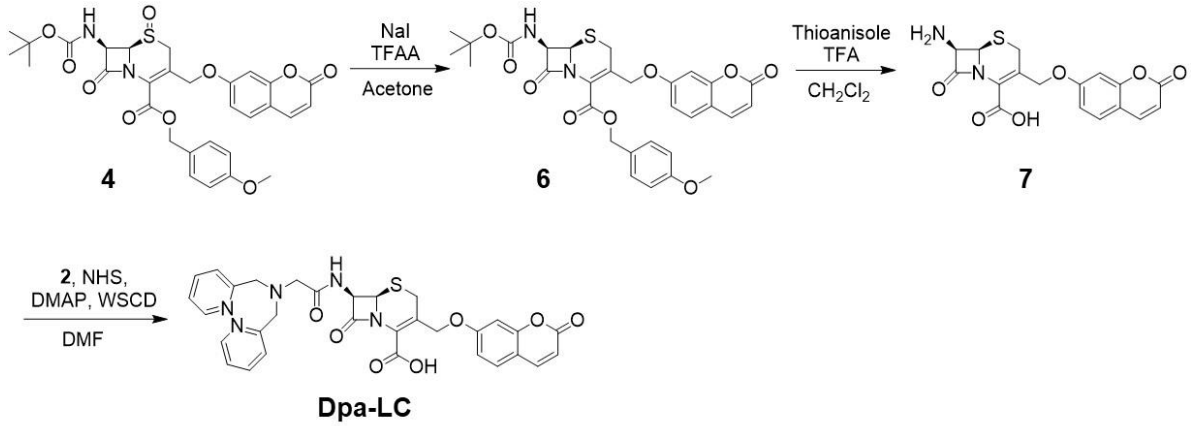
Thionyl chloride (30.0 μL, 49.2 mg, 413 μmol) was added to a solution of *N*,*N*-dimethylglycine (21.0 mg, 204 μmol) in CH_3CN (3 mL) and the solution was stirred at rt for 2 hours. After removal of the solvent *in vacuo*, the residue was dissolved in DMF (2 mL) at 0 °C. The solution was added dropwise to a solution of compound **5** (35.0 mg, 89.6 μmol) in DMF (1.5 mL) at 0 °C. The mixture was stirred at 0 °C for 2 hours. After dilution with a mixture of CH_3CN / water = 1 / 2 (10 mL, 0.1% (v/v) TFA), the solution was filtered with a PVDF filter (Millex-HV, Non-sterile, 13 mmφ, 0.45 μm) and purified by HPLC (ODS-A, YMC-Pack, 150 × 30 mm, Flow rate: 9.9 mL/min, A: CH_3CN (0.1% TFA) B: water (0.1% TFA), gradient: (Time (min), B (%)) = (0, 95) (10, 95) (50, 50) (55, 5), retention time 35 min). The obtained fraction was lyophilized to give a white solid **DM-SoxLC** (17.0 mg, 35.7 μmol, 40%). ESI-HRMS m/z found 476.1122, calcd 476.1122 ($[C_{21}H_{21}N_3O_8S+H]^+$). ^{1}H-NMR(300 MHz, DMSO-*d6*) δ 2.77 (6H, s), 3.70 (1H, d, *J* = 18.3 Hz), 3.98 (2H, br), 4.03 (1H, d, *J* = 18.6 Hz), 4.93 (1H, d, *J* = 12.6 Hz), 5.00 (1H, d, *J* = 3.9 Hz), 5.16 (1H, d, *J* = 12.6 Hz), 5.92 (1H, dd, *J* = 8.1, 4.8 Hz), 6.32 (1H, d, *J* = 9.3 Hz), 6.96 (1H, dd, *J* = 8.7, 2.4 Hz), 6.99 (1H, d, *J* = 2.4 Hz), 7.66 (1H, d, *J* = 8.7 Hz), 8.01 (1H, d, *J* = 9.6 Hz), 9.05 (1H, d, *J* = 7.5 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 44.45, 46.09, 58.49, 61.35, 62.49, 62.53, 66.82, 68.20, 102.31, 113.57, 119.40, 130.07, 130.37, 144.99, 155.97, 158.25, 158.65, 160.92, 161.75, 162.89.

^{1}H-NMR spectrum of **DM-SoxLC**



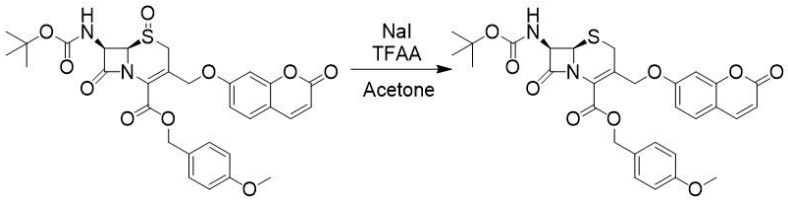
^{13}C-NMR spectrum of **DM-SoxLC**





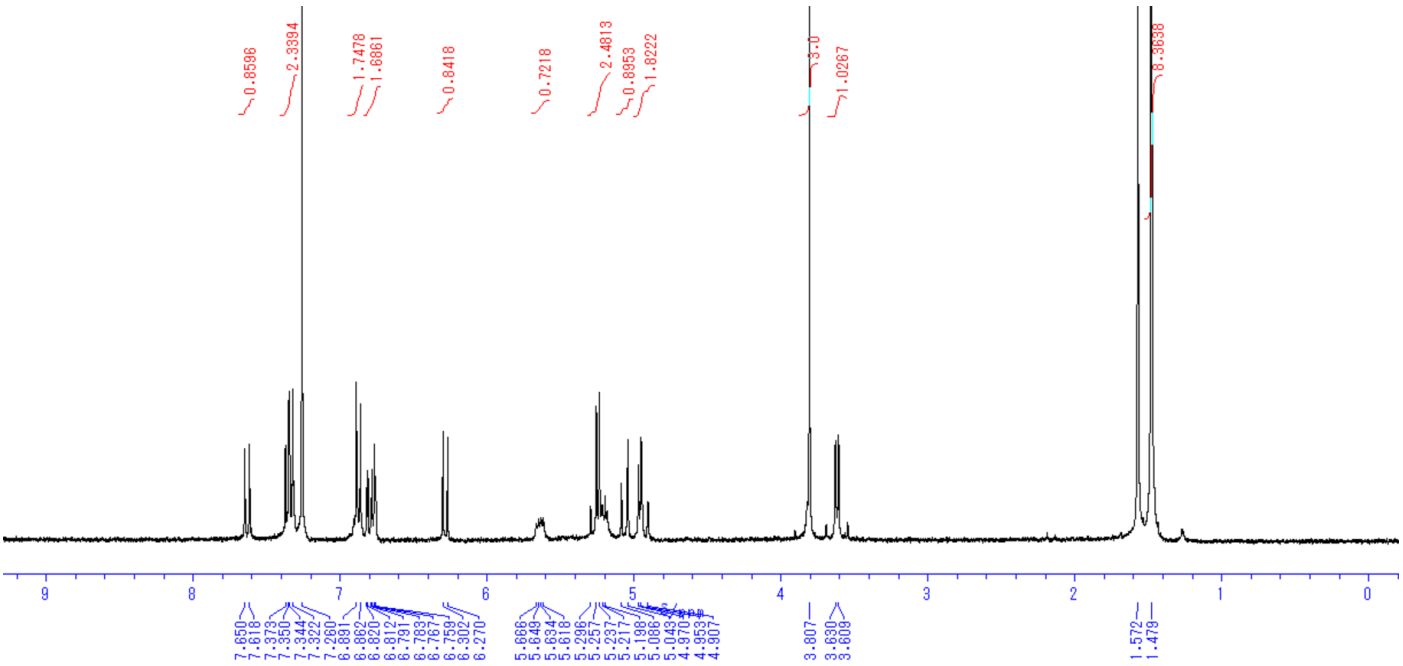
Dpa-LC

Synthesis of compound 6

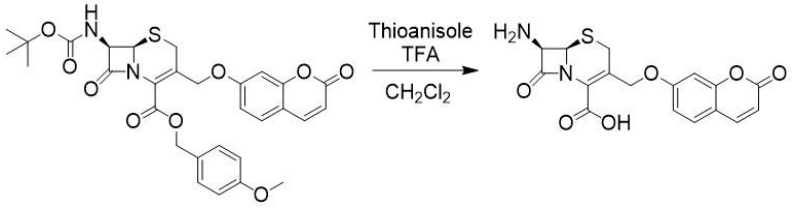


Sodium iodide (236 mg, 1.57 mmol) was added to a solution of compound **4** (195 mg, 319 μmol) in acetone (7 mL). The solution was stirred at 0 ºC for 5 min, followed by the dropwise addition of trifluoroacetic anhydride (252 μL, 378 mg, 1.80 mmol). The mixture was stirred at 0 ºC for 20 min. After dilution with saturated $NaHCO_3$ (30 mL), the solution was extracted with $CHCl_3$ (30 mL × 3). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 100 / 1) to give an orange oil **6** (118 mg, 198 μmol, 62%). ESI-LRMS m/z found 617.7, calcd 617.2 ($[C_{30}H_{30}N_2O_9S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.48 (9H, s), 3.62 (1H, d, *J* = 6.3 Hz), 3.81 (3H, s), 4.93 (1H, d, *J* = 13.2 Hz), 4.96 (1H, d, *J* = 5.1 Hz), 5.06 (1H, d, *J* = 12.9 Hz), 5.20–5.30 (3H, m), 5.64 (1H, dd, *J* = 9.3, 4.8 Hz), 6.29 (1H, d, *J* = 9.6 Hz), 6.76 (1H, d, *J* = 2.4 Hz), 6.80 (1H, dd, *J* = 8.7, 2.4 Hz), 6.88 (2H, d, *J* = 8.7 Hz), 7.33 (2H, d, *J* = 6.6 Hz), 7.36 (1H, d, *J* = 6.9 Hz), 7.63 (1H, d, *J* = 9.6 Hz).

^{1}H-NMR spectrum of compound **6**

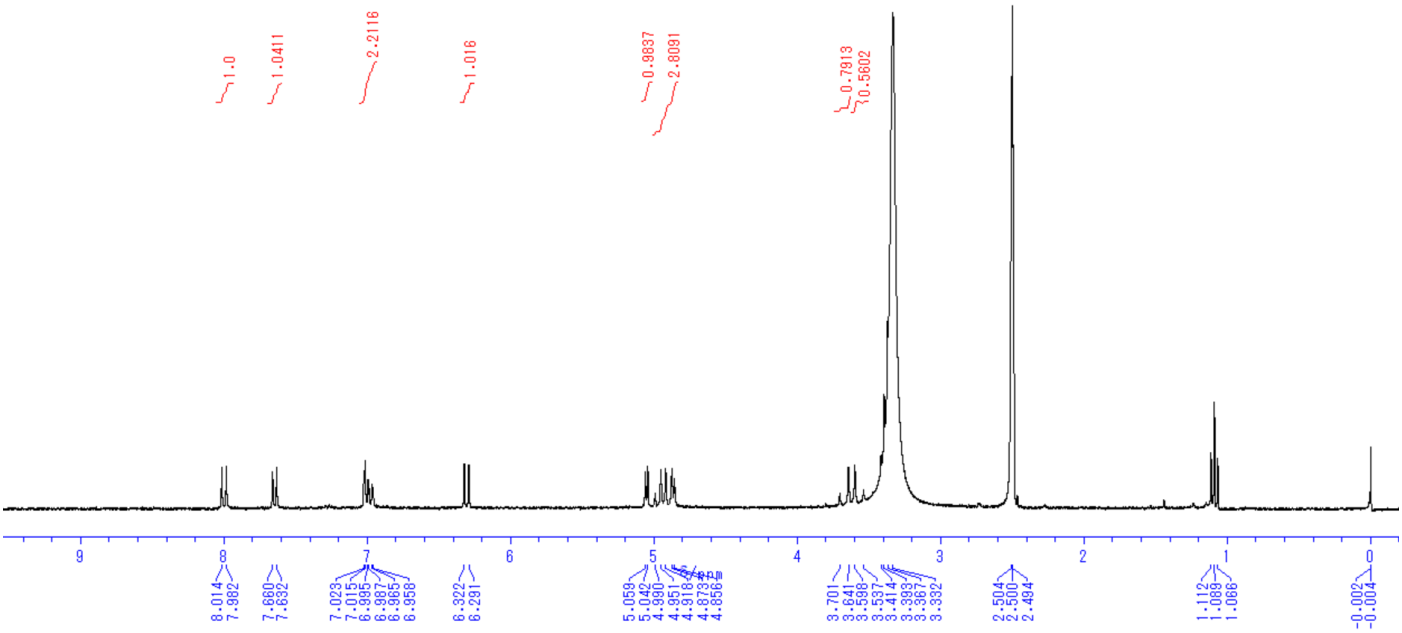


Synthesis of compound 7

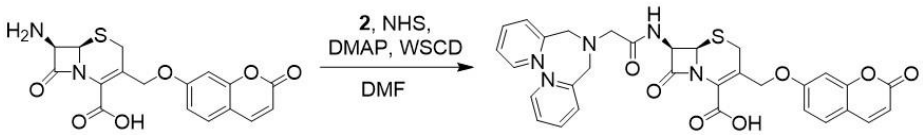


Compound **6** (118 mg, 198 μmol) was dissolved in CH_2Cl_2 (2.0 mL). Thioanisole (500 μL) and TFA (1.5 mL) were added to the solution at 0 °C. The solution was stirred at 0 °C for 4 hours. After addition of cold diethyl ether (13 mL), the precipitate was collected by the centrifugation (10000 g × 1 min) and the supernatant was removed. For washing the precipitate, the addition of diethyl ether (6 mL) and the following centrifugation were repeated 3 times. The residue was dried *in vacuo* to give a white solid **7** (50.5 mg, 135 μmol, 68%). ESI-LRMS m/z found 375.6, calcd 375.1 ($[C_{17}H_{14}N_2O_6S+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.39 (2H, td, *J* = 6.9, 0.6 Hz), 3.57 (1H, d, *J* = 18.0 Hz), 3.68 (1H, d, *J* = 18.0 Hz), 4.87 (1H, d, *J* = 5.7 Hz), 4.90 (1H, d, *J* = 11.4 Hz), 4.98 (1H, d, *J* = 11.4 Hz), 5.05 (1H, d, *J* = 5.1 Hz), 6.21 (1H, d, *J* = 9.6 Hz), 6.96–7.03 (2H, m), 7.65 (1H, d, *J* = 8.7 Hz), 8.00 (1H, d, *J* = 9.6 Hz).

^{1}H-NMR spectrum of compound **7**

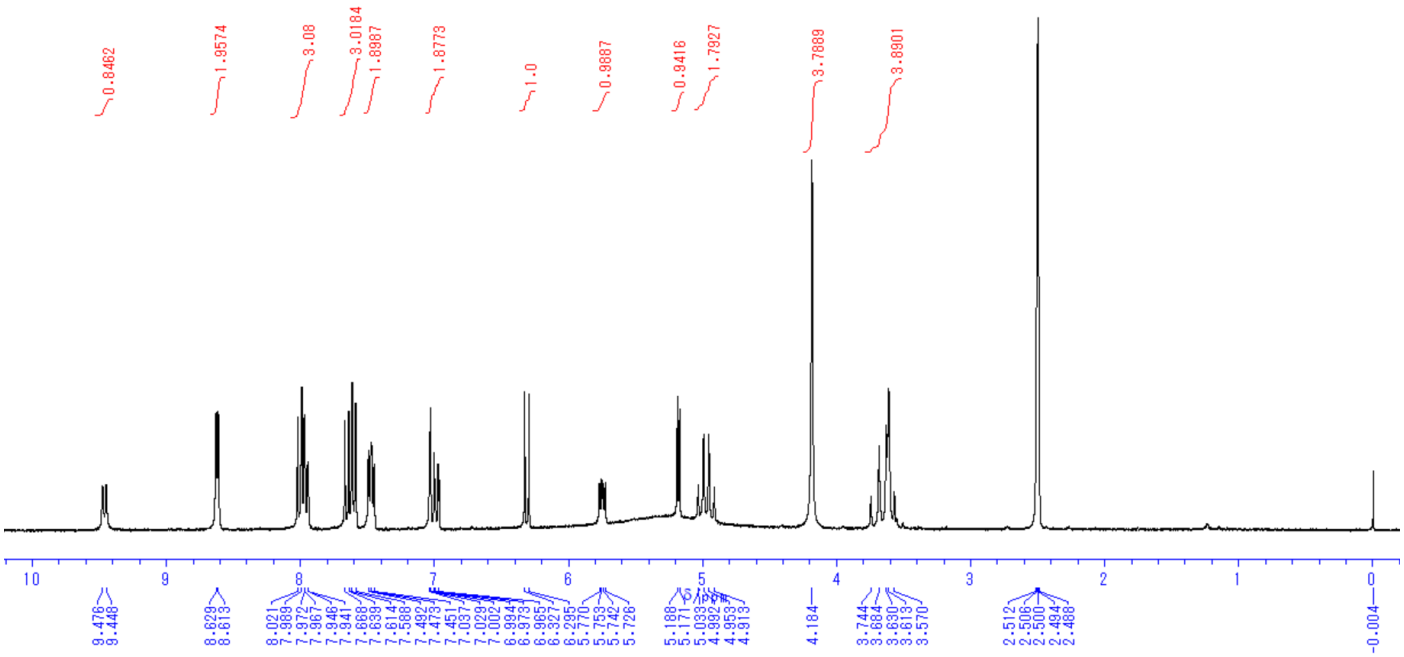


Synthesis of Dpa-LC

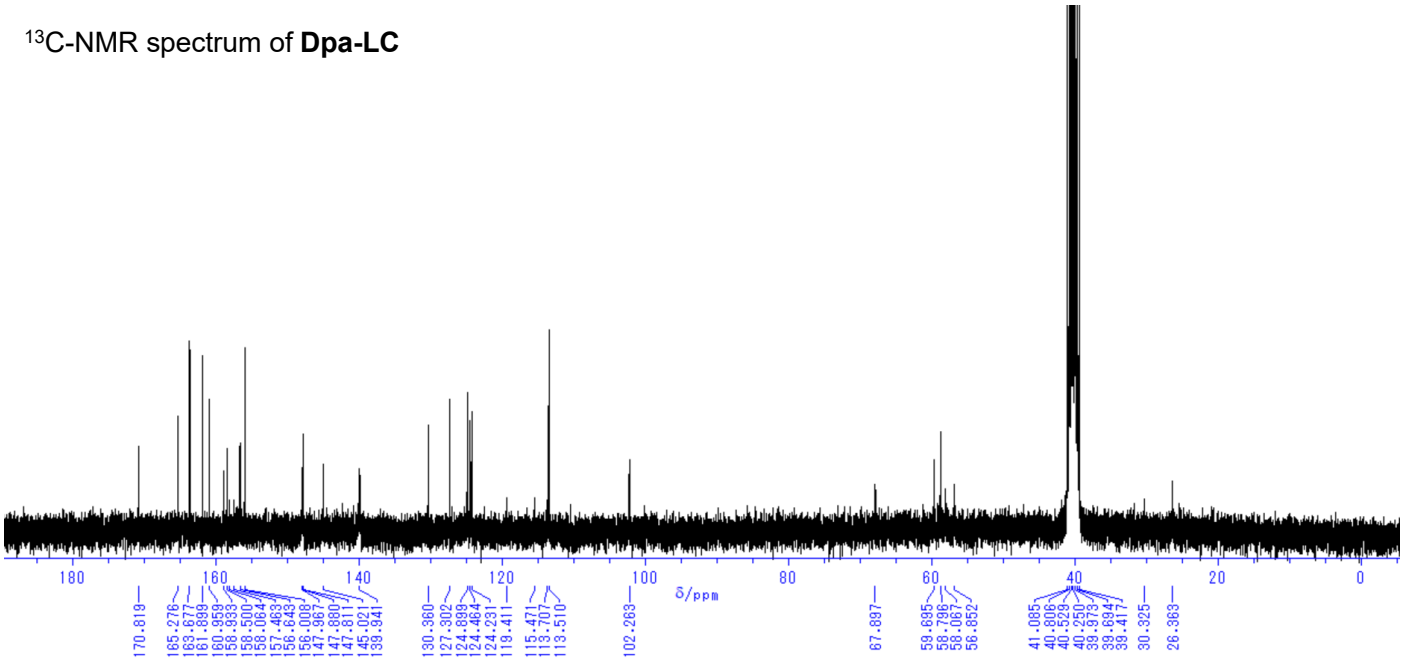


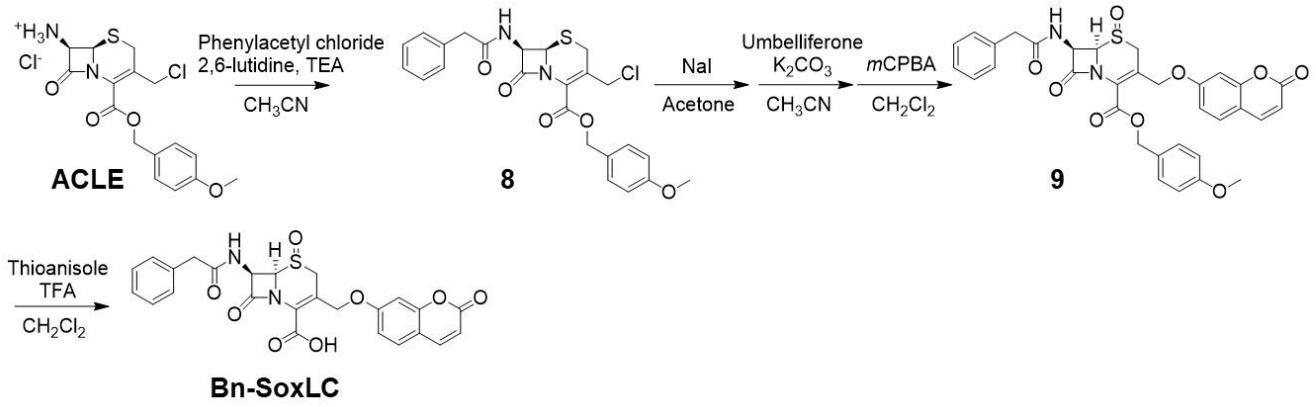
WSCD·HCl (16.5 mg, 84.7 μmol) was added into a solution of compound **2** (38.2 mg, 136 μmol), NHS (13.3 mg, 115 μmol), and DMAP (17.4 mg, 143 μmol) in DMF (2.4 mL) at 0 °C. The solution was stirred at 0 °C for 2 hours, followed by the addition of compound **7** (11.1 mg, 29.7 μmol). After the reaction at 0 °C for 1.5 hours, the solution was diluted with 4.8 mL water (0.1% TFA) and purified by HPLC (ODS-A, YMC-Pack, 150 × 30 mm, Flow rate: 9.9 mL/min, A: CH_3CN (0.1% TFA) B: water (0.1% TFA), gradient: (Time (min), B (%)) = (0, 95) (5, 95) (40, 60) (42, 5), retention time 37 min). The obtained solution was lyophilized to give a yellowish white solid **Dpa-LC** (10.0 mg,16.4 μmol, 55%). ESI-HRMS m/z found 614.1698, calcd 614.1704 ($[C_{31}H_{27}N_5O_7S+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.60 (1H, d, *J* = 18.0 Hz), 3.61 (2H, s), 3.71 (1H, d, *J* = 18.0 Hz), 4.18 (4H, s), 4.93 (1H, d, *J* = 12.0 Hz), 5.01 (1H, d, *J* = 12.3 Hz), 5.18 (1H, d, *J* = 4.8 Hz), 5.75 (1H, dd, *J* = 8.1, 4.8 Hz), 6.31 (1H, d, *J* = 9.6 Hz), 6.98 (1H, dd, *J* = 8.7, 2.4 Hz), 7.03 (1H, d, *J* = 2.4 Hz), 7.47 (2H, t, *J* = 6.3 Hz), 7.60 (2H, d, *J* = 7.8 Hz), 7.65 (1H, d, *J* = 8.7 Hz), 7.97 (2H, td, *J* = 8.4, 1.5 Hz), 8.01 (1H, d, *J* = 9.6 Hz), 8.62 (2H, d, *J* = 4.8 Hz), 9.46 (1H, d, *J* = 8.4 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 26.36, 56.85, 58.07, 58.80, 59.70, 67.90, 102.26, 113.51, 115.47, 119.41, 124.23, 124.46, 124.90, 127.30, 130.36, 139.94, 145.02, 147.88, 156.01, 156.64, 160.96, 161.90, 163.68, 165.28, 170.82.

^{1}H-NMR spectrum of **Dpa-LC**

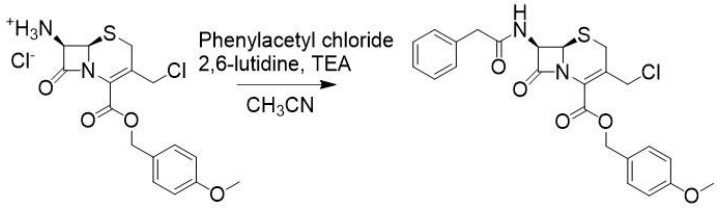


^{13}C-NMR spectrum of **Dpa-LC**



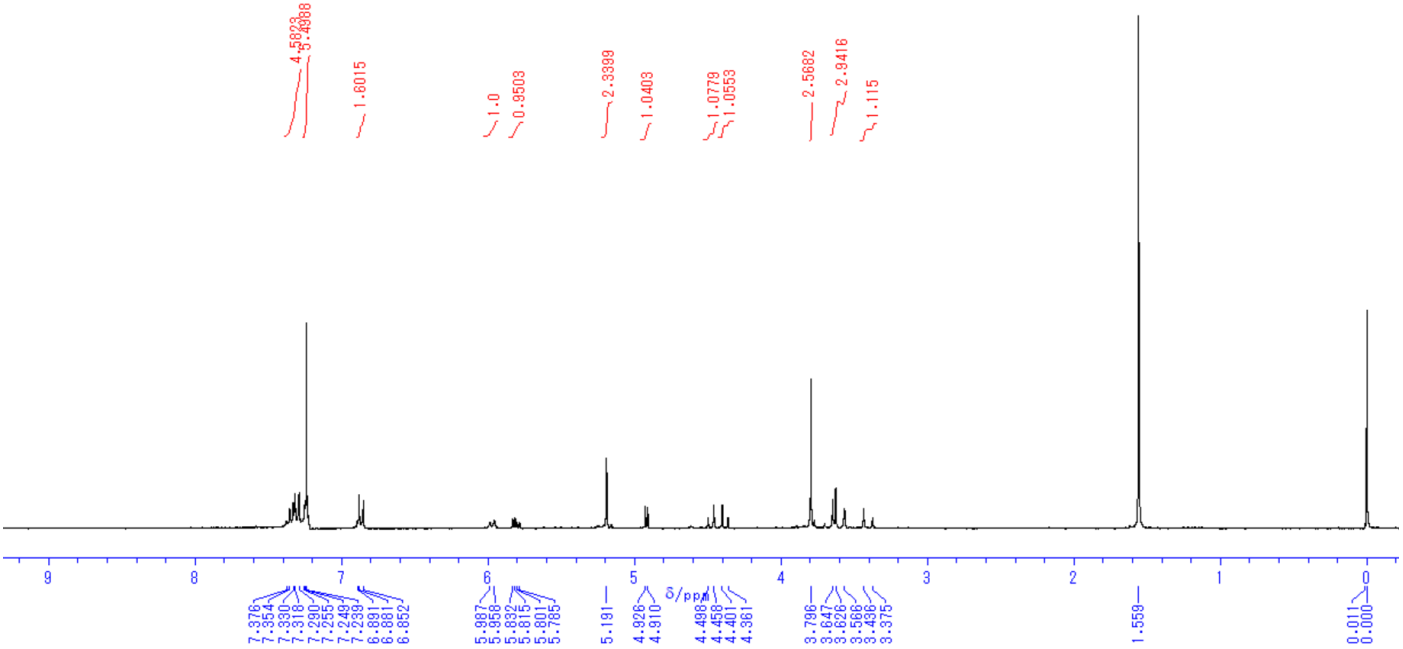


Synthesis of compound 8

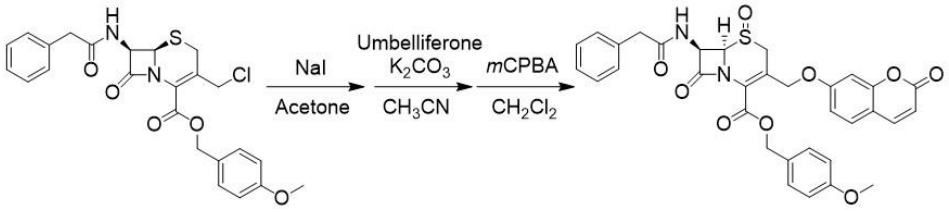


Phenylacetyl chloride (92.8 μL, 108 mg, 702 μmol) was added into a solution of ACLE·HCl (208 mg, 513 μmol) and 2,6-lutidine (115 μL, 106 mg, 986 μmol) in CH_3CN (5 mL) at 0 °C. TEA (68.0 μL, 49.6 mg, 493 μmol) were added into the solution. The solution was stirred at rt for 12 hours. After removal of the solvent, the residue was purified by column chromatography (SiO_2, $CHCl_3$) to give a white crystal **8** (162 mg, 333 μmol, 65%). ESI-HRMS m/z found 509.0912, calcd 509.0908 ($[C_{24}H_{23}ClN_2O_5S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.41 (1H, d, *J* = 18.3 Hz), 3.60 (1H, d, *J* = 18.3 Hz), 3.64 (2H, d, *J* = 6.3 Hz), 3.80 (3H, s), 4.38 (1H, d, *J* = 12.0 Hz), 4.48 (1H, d, *J* = 12.0 Hz), 4.92 (1H, d, *J* = 4.8 Hz), 5.19 (2H, s), 5.81 (1H, dd, *J* = 9.0, 4.5 Hz), 5.97 (1H, d, *J* = 8.7 Hz), 6.87 (2H, dt, *J* = 8.7, 3.0 Hz), 7.25–7.38 (7H, m).

^{1}H-NMR spectrum of compound **8**

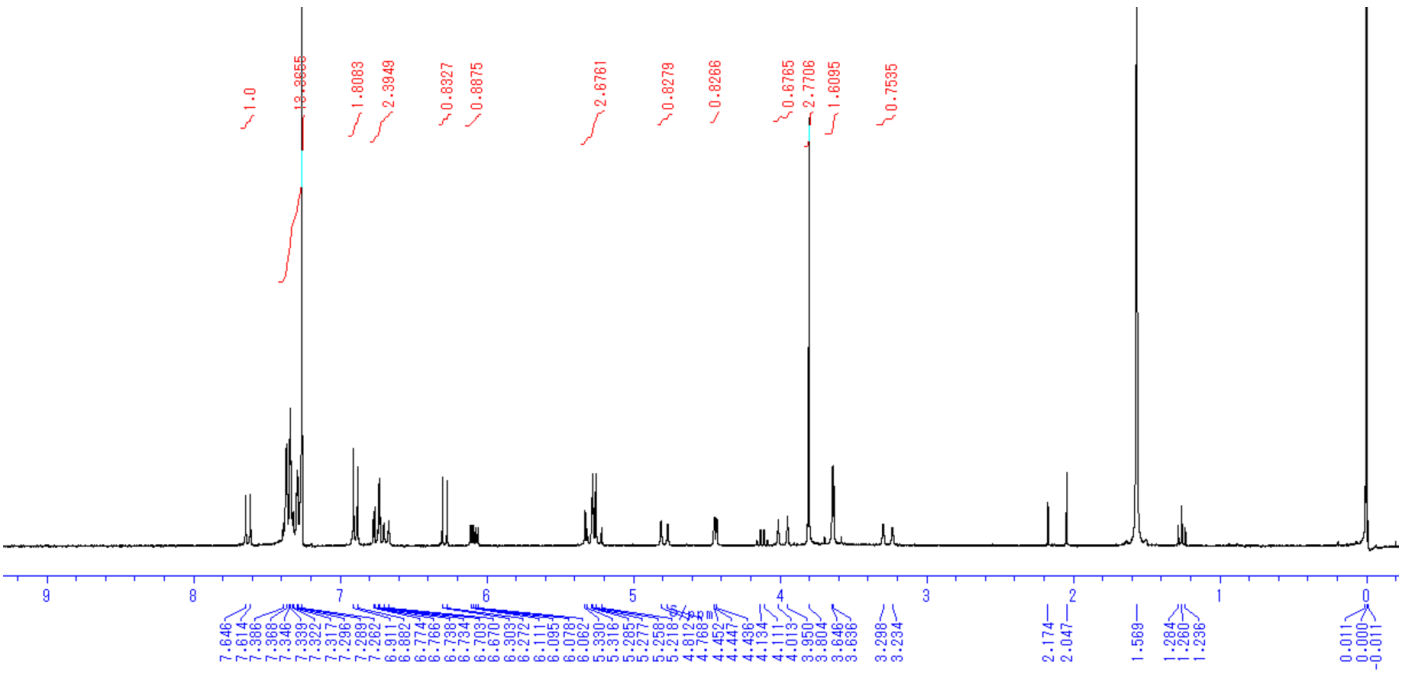


Synthesis of compound 9

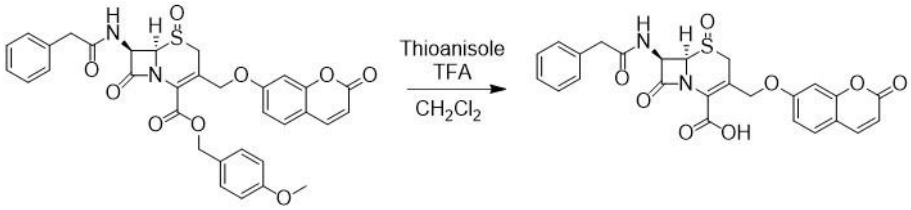


Sodium iodide (700 mg, 4.66 mmol) was added into a solution of compound **8** (237 mg, 487 μmol) in acetone (12 mL). The mixture was stirred at rt for an hour, followed by removal of the solvent *in vacuo*. The residue was diluted with ethyl acetate (80 mL) and the organic layer was washed with water (100 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated. After dried *in vacuo* at rt for an hour, the residue was applied for next reaction without further purification. Umbelliferone (158 mg, 970 μmol) and K_2CO_3 (202 mg, 1.46 mmol) were added into a solution of the fore-mentioned crude in CH_3CN (10 mL). The mixture was stirred at rt for 2 hours. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 100 / 1) to give a crude of umbelliferone conjugate (358 mg) as a yellow oil. The crude was dissolved in CH_2Cl_2 (20 mL). After cooling to 0 ºC, *m*CPBA (86.7 mg, 327 μmol) was added into the solution. After the reaction at 0 ºC for 20 minutes, the solution was diluted with $CHCl_3$ (80 mL) and washed with saturated $NaHCO_3$ (100 mL), water (100 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 50 / 1) to give a pale orange solid **9** (89.5 mg, 142 μmol, 29%). ESI-HRMS m/z found 651.1406, calcd 651.1408 ($[C_{33}H_{28}N_2O_9S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.27 (1H, d, *J* = 19.2 Hz), 3.64 (2H, d, *J* = 3.0 Hz), 3.80 (3H, s), 3.98 (1H, d, *J* = 18.9 Hz), 4.44 (1H, dd, *J* = 4.8, 2.0 Hz), 4.79 (1H, d, *J* = 13.2 Hz), 5.24 (1H, d, *J* = 12.0 Hz), 5.30 (1H, d, *J* = 11.7 Hz), 5.31 (1H, d, *J* = 13.8 Hz), 6.09 (1H, dd, *J* = 9.9, 4.2 Hz), 6.29 (1H, d, *J* = 9.3 Hz), 6.69 (1H, d, *J* = 9.9 Hz), 6.73–6.77 (2H, m), 6.90 (2H, d, *J* = 8.7 Hz), 7.29–7.39 (7H, m), 7.63 (1H, d, *J* = 9.6 Hz).

^{1}H-NMR spectrum of compound **9**

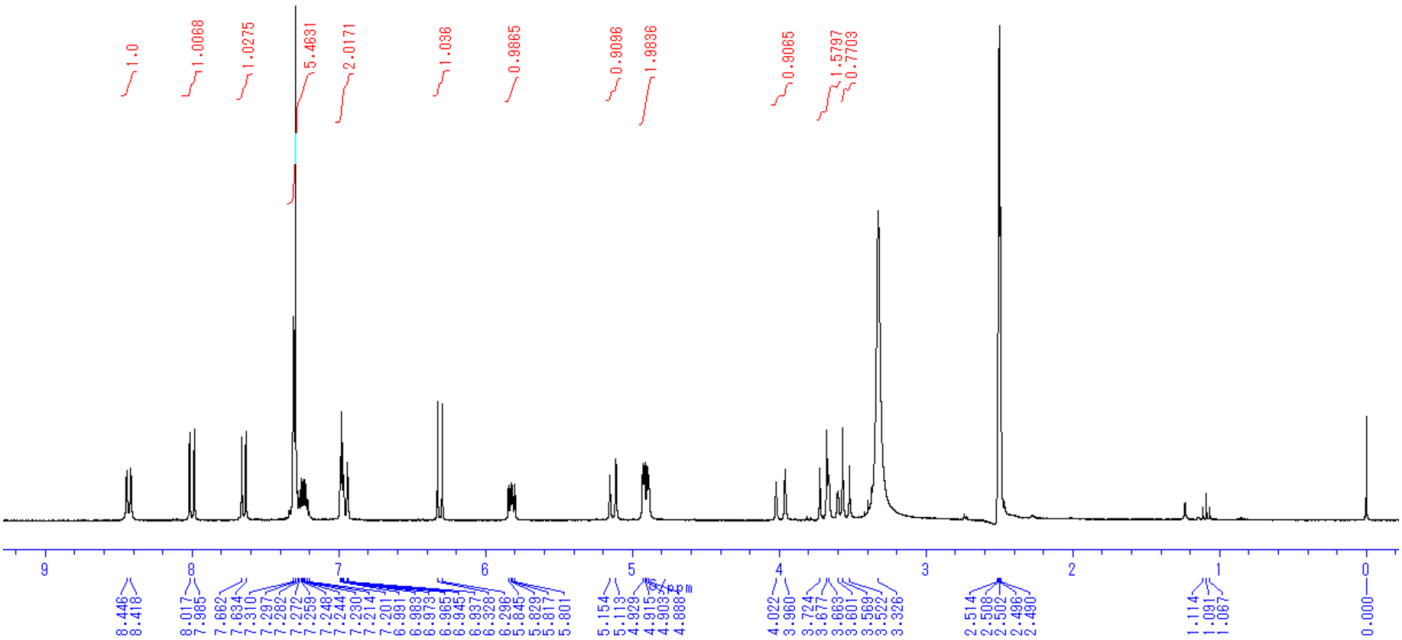


Synthesis of Bn-SoxLC

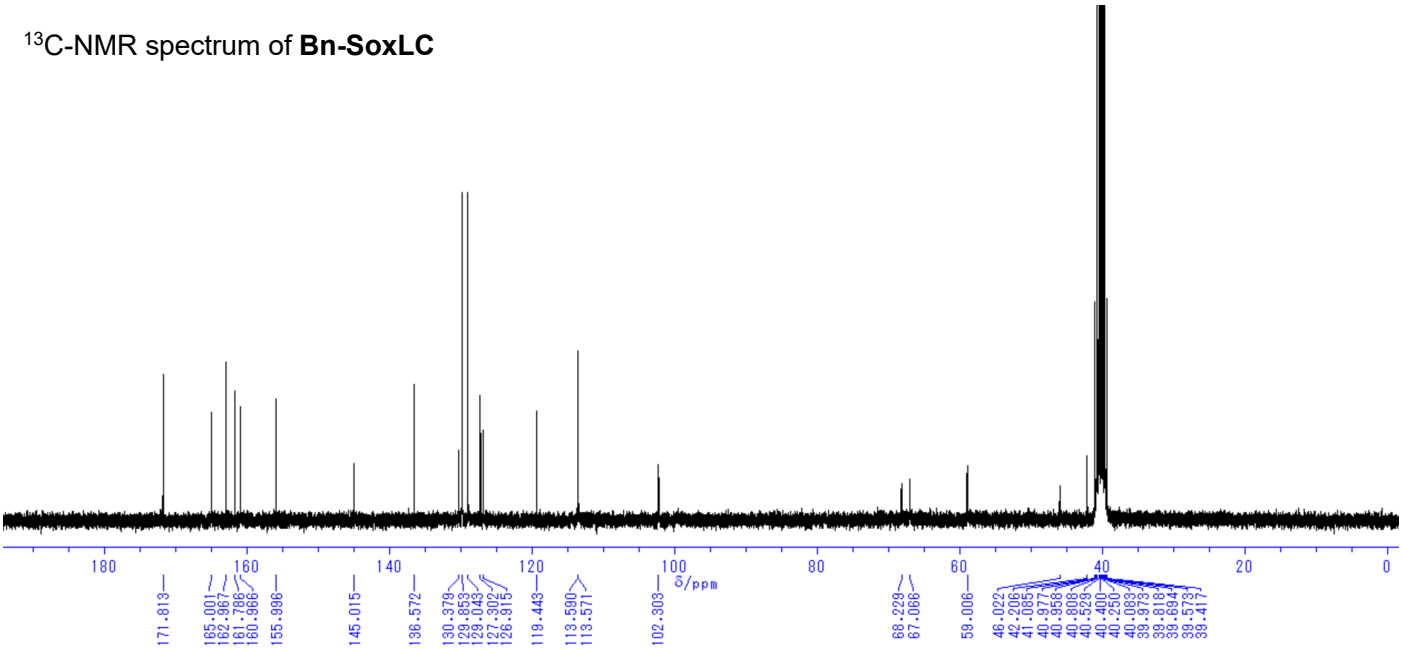


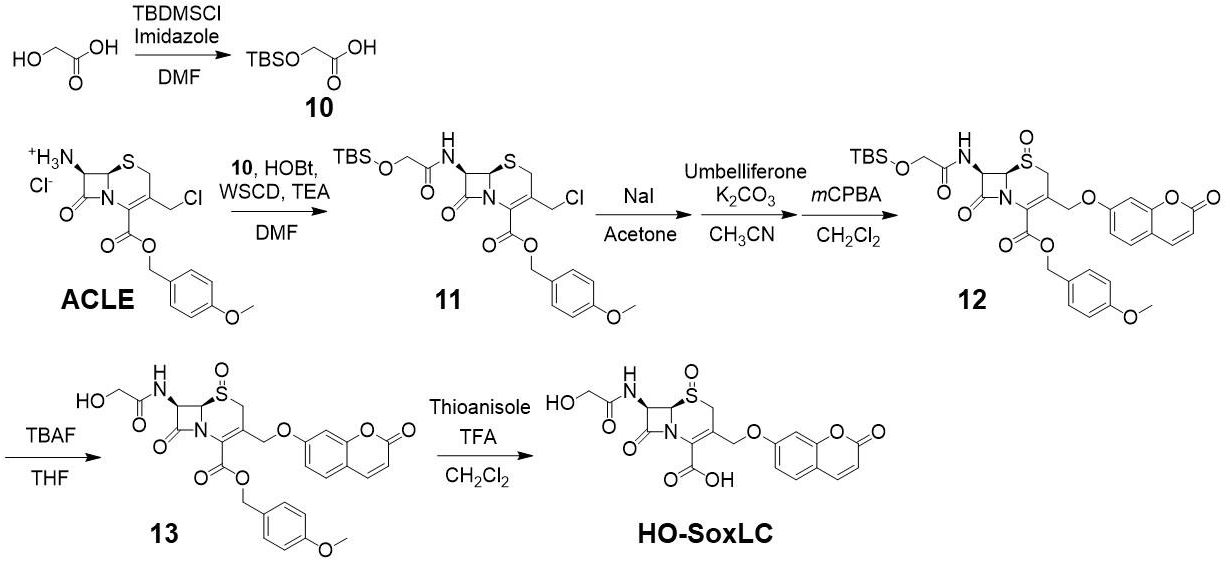
Compound **9** (40.0 mg, 63.6 μmol) was dissolved in CH_2Cl_2 (3 mL). Thioanisole (600 μL) and TFA (1.8 mL) were added into the solution at 0 °C. The solution was stirred at 0 °C for 4 hours. After addition of cold diethyl ether (12 mL), the precipitate was collected by the centrifugation (21500 g × 1 min) and the supernatant was removed. Addition of cold diethyl ether (6 mL) followed by the centrifugation step was repeated 3 times to wash the precipitate. The residue was dried *in vacuo* to give a pale orange solid **Bn-SoxLC** (28.2 mg, 55.5 μmol, 87%). ESI-HRMS m/z found 531.0835, calcd 531.0833 ($[C_{25}H_{20}N_2O_8S+Na]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.55 (1H, d, *J* = 14.1 Hz), 3.63 (1H, d, *J* = 18.6 Hz), 3.70 (1H, d, *J* = 14.1 Hz), 3.99 (1H, d, *J* = 18.6 Hz), 4.91 (2H, dd, *J* = 8.1, 4.5 Hz), 5.13 (1H, d, *J* = 12.3 Hz), 5.82 (1H, dd, *J* = 8.4, 4.8 Hz), 6.31 (1H, d, *J* = 9.6 Hz), 6.94–6.99 (2H, m), 7.23–7.31 (5H, m), 7.65 (1H, d, *J* = 8.4 Hz), 8.00 (1H, d, *J* = 9.6 Hz), 8.43 (1H, d, *J* = 8.4 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 41.09, 42.21, 46.02, 59.01, 67.07, 68.23, 102.30, 113.57, 119.44, 126.92, 127.30, 129.04, 129.85, 130.38, 136.57, 145.02, 156.00, 160.97, 161.79, 162.98, 165.00, 171.81. The physical properties are consistent with that of the reported data.[S2]

^{1}H-NMR spectrum of **Bn-SoxLC**

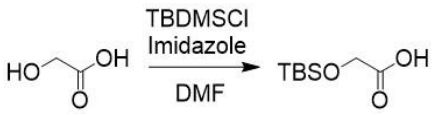


^{13}C-NMR spectrum of **Bn-SoxLC**



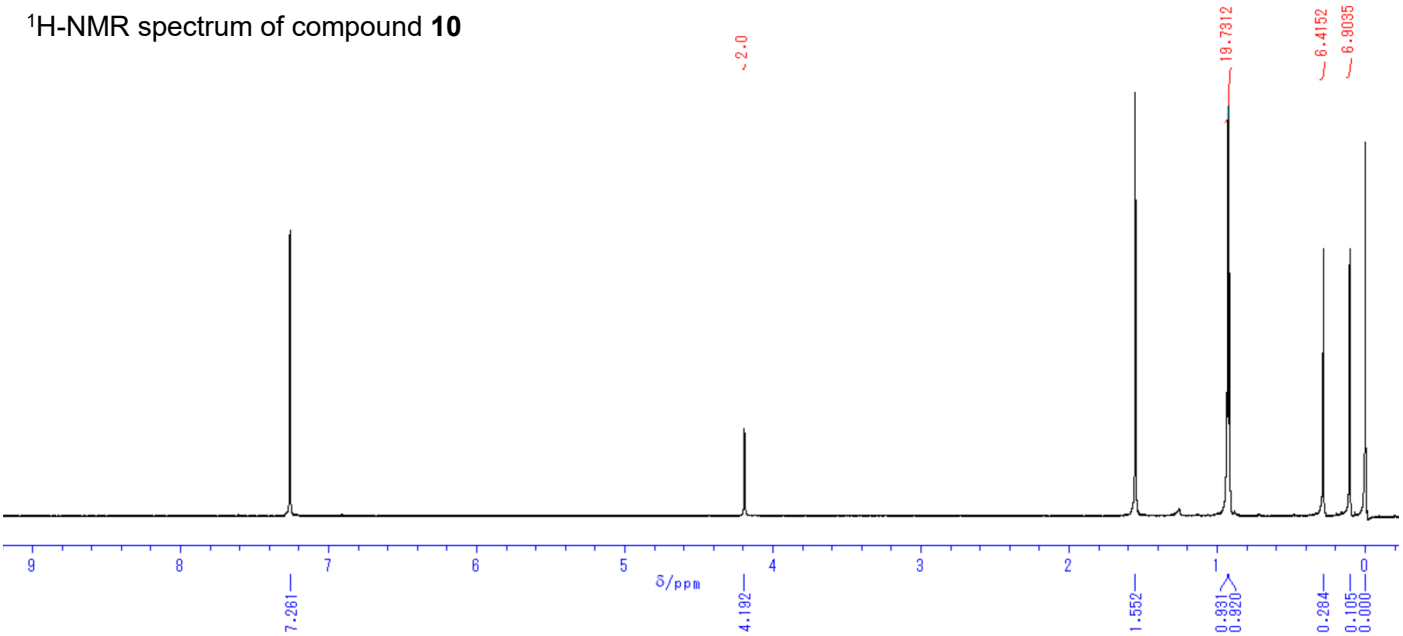


Synthesis of compound 10

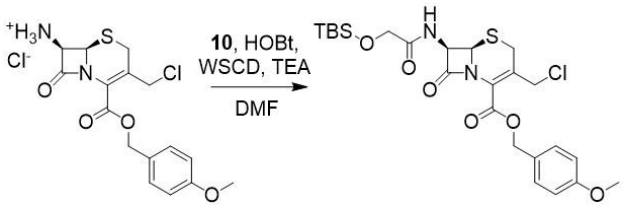


TBDMSCl (*tert*-butyldimethylsilyl chloride, 2.8 g, 18.5 mmol) and imidazole (1.7 g, 25.0 mmol) were added to a solution of glycolic acid (1.0 g, 13.1 mmol) in DMF (15 mL). The reaction was stirred at rt for 3 hours. After the dilution with ethyl acetate (80 mL), the organic layer was washed with water (100 mL × 2), and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, $CHCl_3$) to give a clear oil crude **10** (185 mg, including TBDMSOH 1 eq. to compound **10**, **10**: 574 μmol, 4.4%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.28 (6H, s), 0.93 (9H, s), 4.21 (2H, s); including TBDMSOH, 0.11 (6H, s), 0.92 (pH, s).

^{1}H-NMR spectrum of compound **10**

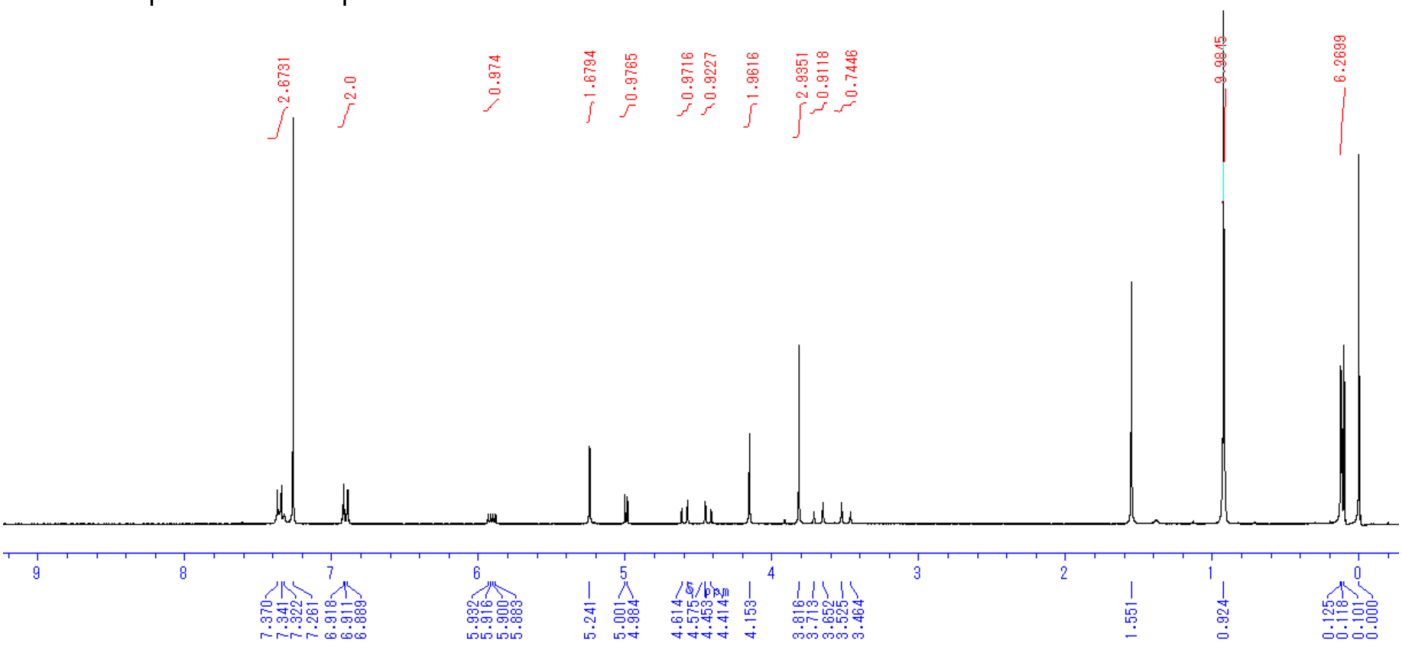


Synthesis of compound 11

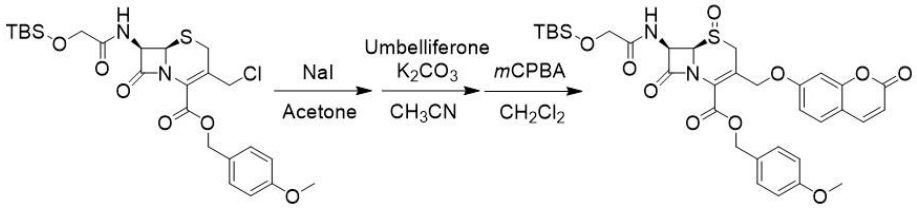


ACLE·HCl (280 mg, 691 μmol), HOBt·H_2O (190 mg, 1.45 mmol), and crude **10** (160 mg, **10**: 497 μmol) were dissolved in DMF (5.5 mL). WSCD·HCl (177 mg, 923 μmol) and TEA (95.9 μL, 70.0 mg, 692 μmol) were added to the solution. The solution was stirred at 0 °C for 4 hours. After dilution with ethyl acetate (100 mL), the organic layer was washed with saturated $NaHCO_3$ (100 mL), water (100 mL), and brine (40 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 100 / 1) to give a clear oil **11** (196 mg, 362 μmol, 73%). ESI-HRMS m/z found 563.1409, calcd 563.1409 ($[C_{24}H_{33}ClN_2O_6SSi+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.12 (6H, d, *J* = 2.4 Hz), 0.92 (9H, s), 3.50 (1H, d, *J* = 18.3 Hz), 3.68 (1H, d, *J* = 18.3 Hz), 3.82 (3H, s), 4.15 (2H, s), 4.43 (1H, d, *J* = 11.7 Hz), 4.59 (1H, d, *J* = 11.7 Hz), 4.99 (1H, d, *J* = 5.1 Hz), 5.24 (2H, s), 5.91 (1H, dd, *J* = 9.9, 5.1 Hz), 6.90 (2H, d, *J* = 8.7 Hz), 7.33 (1H, d, *J* = 8.1 Hz), 7.35 (2H, d, *J* = 8.7 Hz).

^{1}H-NMR spectrum of compound **11**

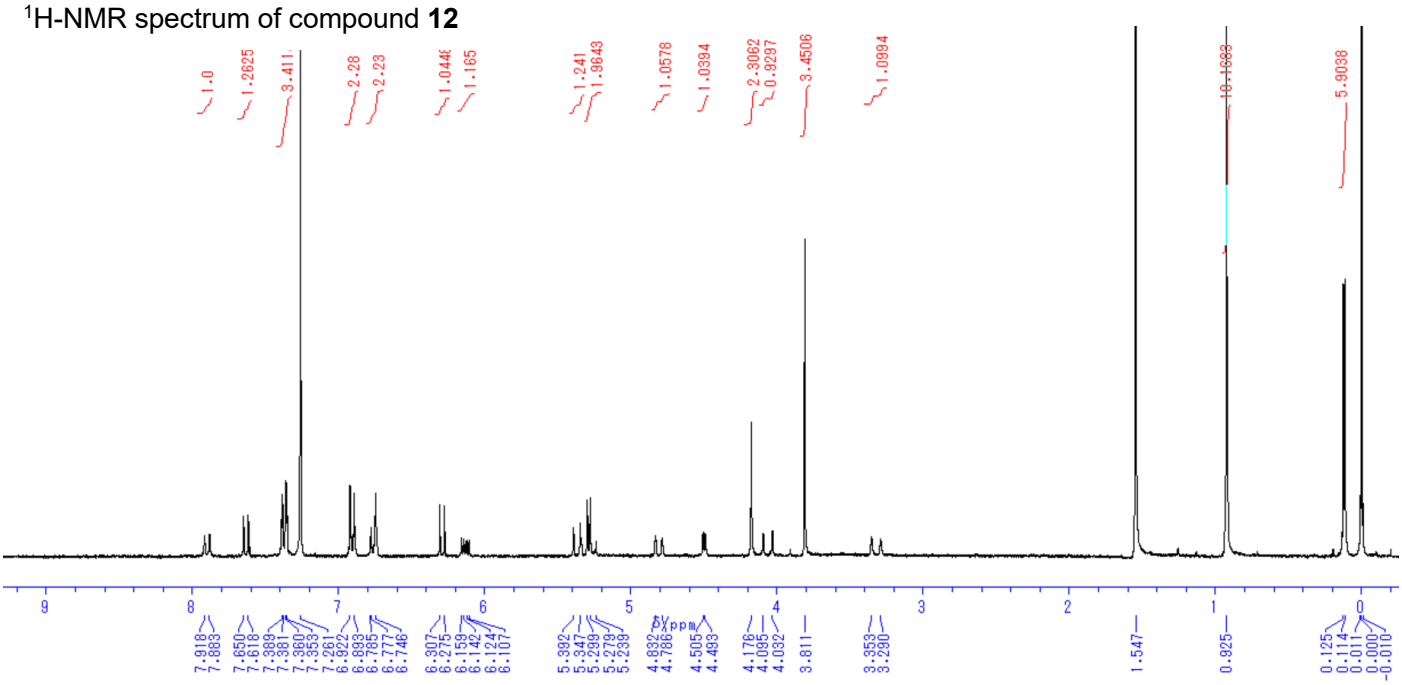


Synthesis of compound 12

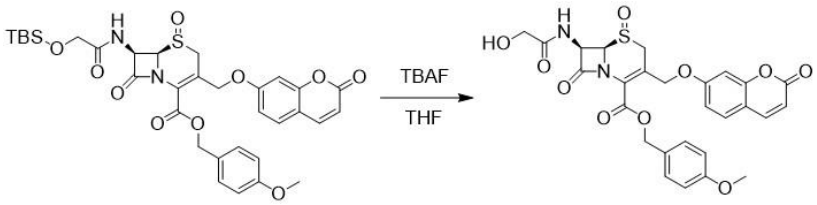


Sodium iodide (500 mg, 2.68 mmol) was added to a solution of compound **11** (196 mg, 362 μmol) in acetone (15 mL). The mixture was stirred at rt for 30 min, followed by removal of the solvent *in vacuo*. The residue was dissolved in ethyl acetate (100 mL) and the organic layer was washed with water (100 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. After drying *in vacuo* at rt for an hour, the residue was used for the next reaction without further purification. Umbelliferone (117 mg, 722 μmol) and K_2CO_3 (150 mg, 1.08 mmol) were added into a solution of the fore-mentioned crude in CH_3CN (9 mL). The mixture was stirred at rt for an hour. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 100 / 1) to give a crude of the umbelliferone-conjugate as a yellow oil. The oil was dissolved in CH_2Cl_2 (10 mL). After cooling to 0 °C, *m*CPBA (38.2 mg, 155 μmol) was added to the solution. After stirring at 0 °C for 20 minutes, the solution was diluted with $CHCl_3$ (90 mL) and washed with saturated $NaHCO_3$ (100 mL), water (100 mL), and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 100 / 1) to give a clear amorphous **12** (46.4 mg, 67.9 μmol, 19%). ESI-HRMS m/z found 705.1906, calcd 705.1909 ($[C_{33}H_{38}N_2O_{10}SSi+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.12 (6H, d, *J* = 3.3 Hz), 0.93 (9H, s), 3.32 (1H, d, *J* = 18.9 Hz), 3.81 (3H, s), 4.06 (1H, d, *J* = 18.9 Hz), 4.18 (2H, s), 4.50 (1H, d, *J* = 3.6 Hz), 4.81 (1H, d, *J* = 13.8 Hz), 5.26 (1H, d, *J* = 12.0 Hz), 5.33 (1H, d, *J* = 12.0 Hz), 5.37 (1H, d, *J* = 13.5 Hz), 6.13 (1H, dd, *J* = 10.5, 5.1 Hz), 6.29 (1H, d, *J* = 9.6 Hz), 6.75 (1H, s), 6.76 (1H, d, *J* = 9.3 Hz), 6.91 (2H, d, *J* = 8.7 Hz), 7.35–7.39 (3H, m), 7.63 (1H, d, *J* = 9.6 Hz), 7.90 (1H, d, *J* = 10.5 Hz).

^{1}H-NMR spectrum of compound **12**

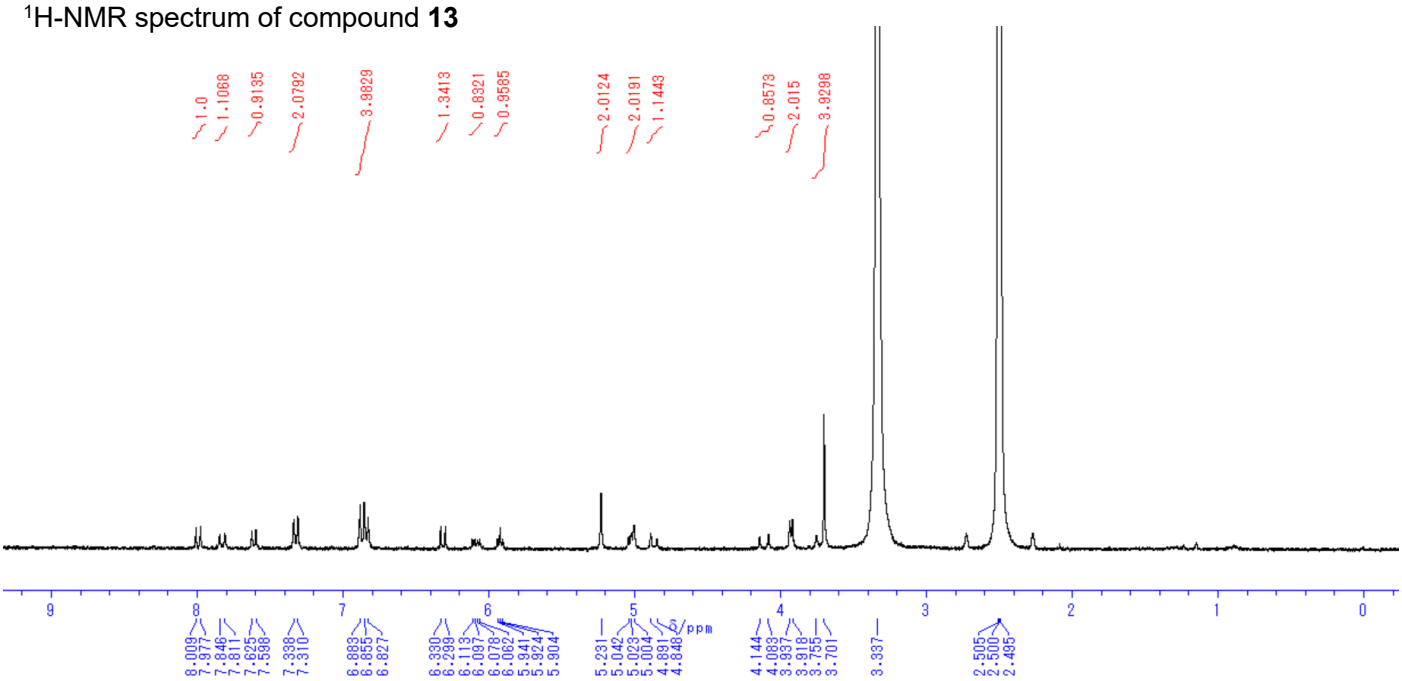


Synthesis of compound 13

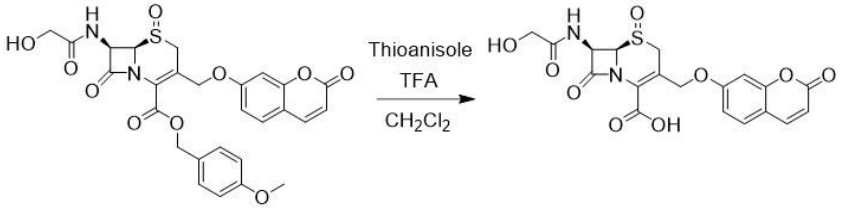


1M Tetrabutylammonium-fluoride solution in THF (100 µL) was added into a solution of compound **12** (44.2 mg, 64.2 µmol) in THF (1.5 mL). The solution was stirred at 0 °C for 15 min and stirred at rt for 15 min. The solution was directly loaded to a silica-gel column chromatography and purified ($CHCl_3$ / methanol = 50 / 1). The isolated fractions were dried *in vacuo* to give a white solid **13** (21.7 mg, 38.2 µmol, 59%). ESI-HRMS m/z found 591.1045, calcd 591.1044 ($[C_{27}H_{24}N_2O_{10}S+Na]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.70 (3H, s), 3.73 (1H, d, *J* = 16.2 Hz), 3.93 (2H, d, *J* = 5.7 Hz), 4.11 (1H, d, *J* = 18.3 Hz), 4.87 (1H, d, *J* = 12.6 Hz), 5.00–5.04 (2H, m), 5.23 (2H, s), 5.92 (1H, t, *J* = 5.6 Hz), 6.09 (1H, dd, *J* = 10.5, 4.8 Hz), 6.32 (1H, d, *J* = 9.3 Hz), 6.83–6.88 (4H, m), 7.32 (2H, d, *J* = 8.4 Hz), 7.61 (1H, d, *J* = 8.1 Hz), 7.83 (1H, d, *J* = 10.5 Hz), 7.99 (1H, d, *J* = 9.6 Hz).

^{1}H-NMR spectrum of compound **13**

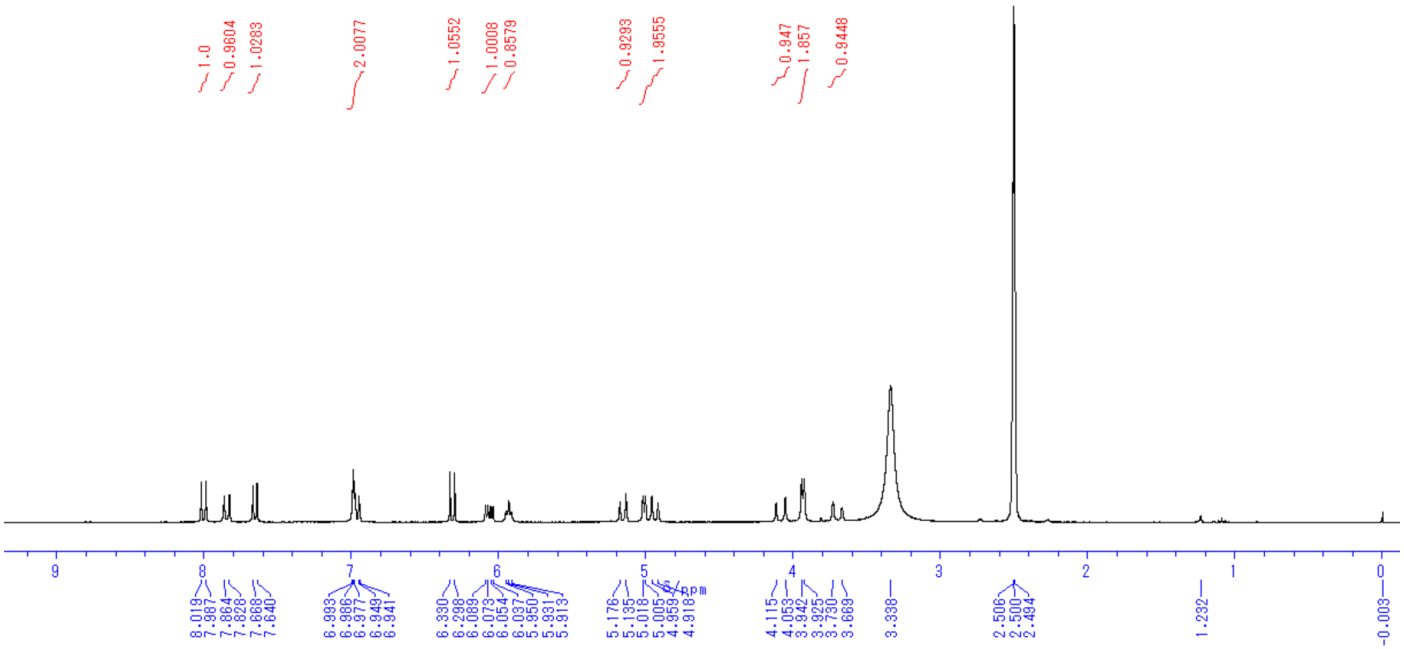


Synthesis of HO-SoxLC

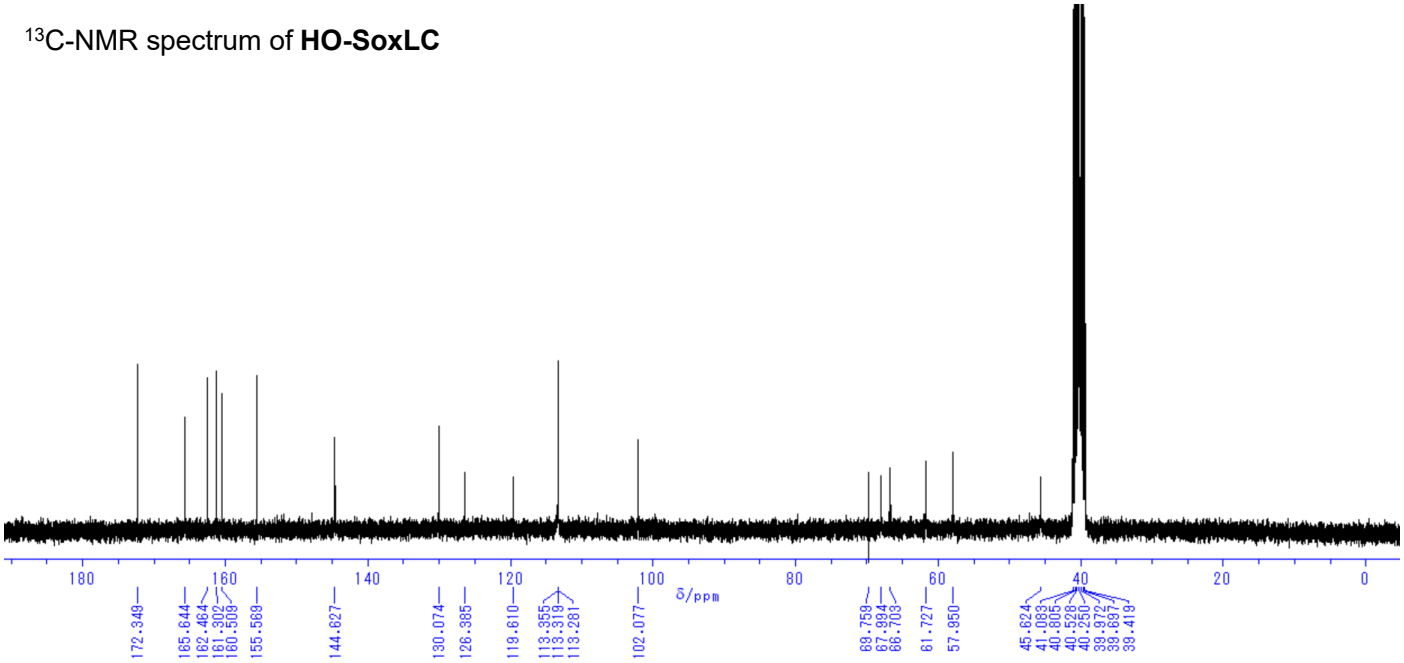


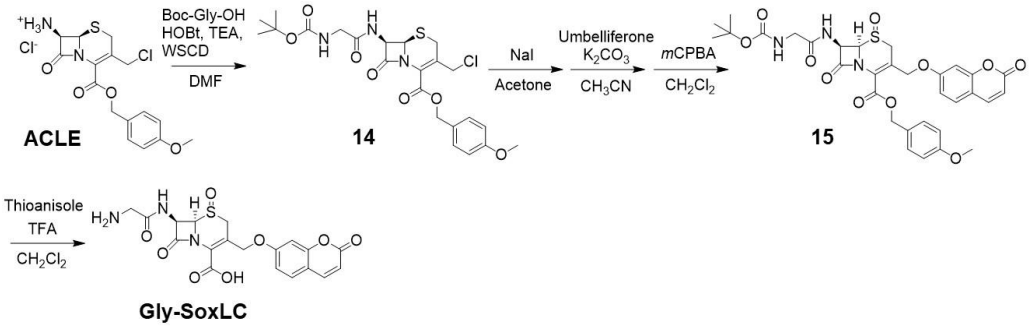
TFA (0.8 mL) and thioanisole (252 µL) were added to a solution of compound **13** (15.2 mg, 26.7 µmol). The solution was stirred at 0 ºC for 4 hours. After addition of cold diethyl ether (6 mL), the precipitate was collected by centrifugation (10000 g × 1 min) and the supernatant was removed. The residue was suspended in cold diethyl ether (2 mL) and centrifuged, followed by the removal of the supernatant. The procedure was repeated 3 times and the residue was dried *in vacuo* to give a white solid **HO-SoxLC** (15.2 mg, quant). ESI-HRMS m/z found 471.0471, calcd 471.0469 ([$C_{19}H_{16}N_2O_9S$+Na]$^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.70 (1H, d, *J* = 18.3 Hz), 3.93 (2H, d, *J* = 5.1 Hz), 4.09 (1H, d, *J* = 18.6 Hz), 4.94 (1H, d, *J* = 12.3 Hz), 5.01 (1H, d, *J* = 3.9 Hz), 5.16 (1H, d, *J* = 12.3 Hz), 5.93 (1H, t, *J* = 5.4 Hz), 6.06 (1H, dd, *J* = 10.8, 5.1 Hz), 6.32 (1H, d, *J* = 9.6 Hz), 6.94–6.99 (2H, m), 7.65 (1H, d, *J* = 8.4 Hz), 7.85 (1H, d, *J* = 10.8 Hz), 8.01 (1H, d, *J* = 9.6 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 45.62, 57.95, 61.73, 66.70, 67.99, 69.76, 102.08, 113.28, 113.32, 119.61, 126.39, 130.07, 144.63, 155.57, 160.51, 161.30, 162.46, 165.64, 172.35.

^{1}H-NMR spectrum of **HO-SoxLC**

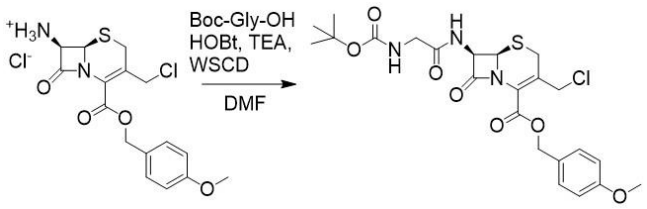


^{13}C-NMR spectrum of **HO-SoxLC**



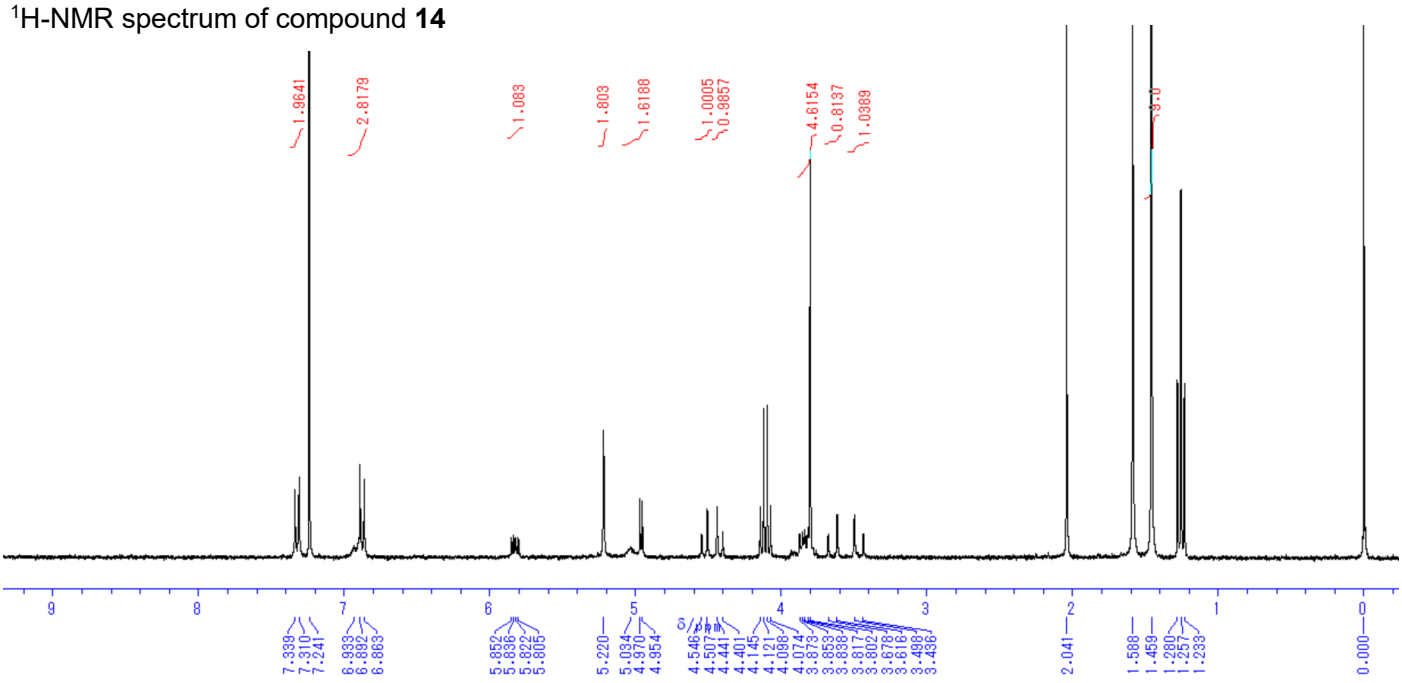


Synthesis of compound 14

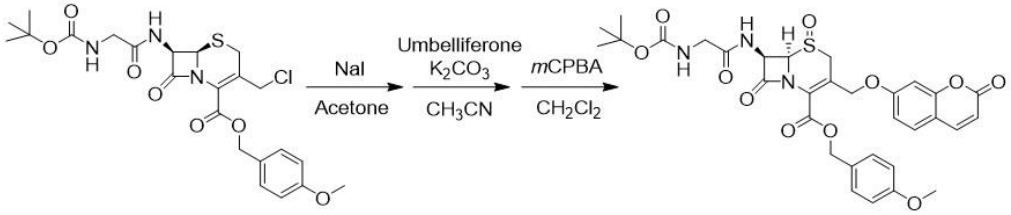


ACLE·HCl (98 mg, 242 μmol), HOBt·H_2O (64.8 mg, 479 μmol), and Boc-Gly-OH (48.2 mg, 275 μmol) were dissolved in DMF (1 mL). WSCD·HCl (58.2 mg, 303 μmol) and TEA (34.2 μL, 25.0 mg, 247 μmol) were added to the solution. The solution was stirred at 0 °C for 3 hours. After dilution with ethyl acetate (80 mL), the organic layer was washed with saturated $NaHCO_3$ (100 mL), water (100 mL), and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, hexane / ethyl acetate = 1 / 1) to give a clear oil **14** (105 mg, 200 μmol, 83%). ESI-HRMS m/z found 548.1228, calcd 548.1229 ($[C_{23}H_{28}ClN_3O_7S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.46 (9H, s), 3.47 (1H, d, *J* = 18.6 Hz), 3.65 (1H, d, *J* = 18.6 Hz), 3.80 (3H, s), 3.82–3.87 (2H, m), 4.42 (1H, d, *J* = 12.0 Hz), 4.53 (1H, d, *J* = 11.7 Hz), 4.96 (1H, d, *J* = 4.8 Hz), 5.03 (1H, br), 5.22 (2H, s), 5.83 (1H, dd, *J* = 9.3, 5.1 Hz), 6.88 (2H, d, *J* = 8.7 Hz), 6.93 (1H, br), 7.32 (2H, d, *J* = 8.7 Hz).

^{1}H-NMR spectrum of compound **14**

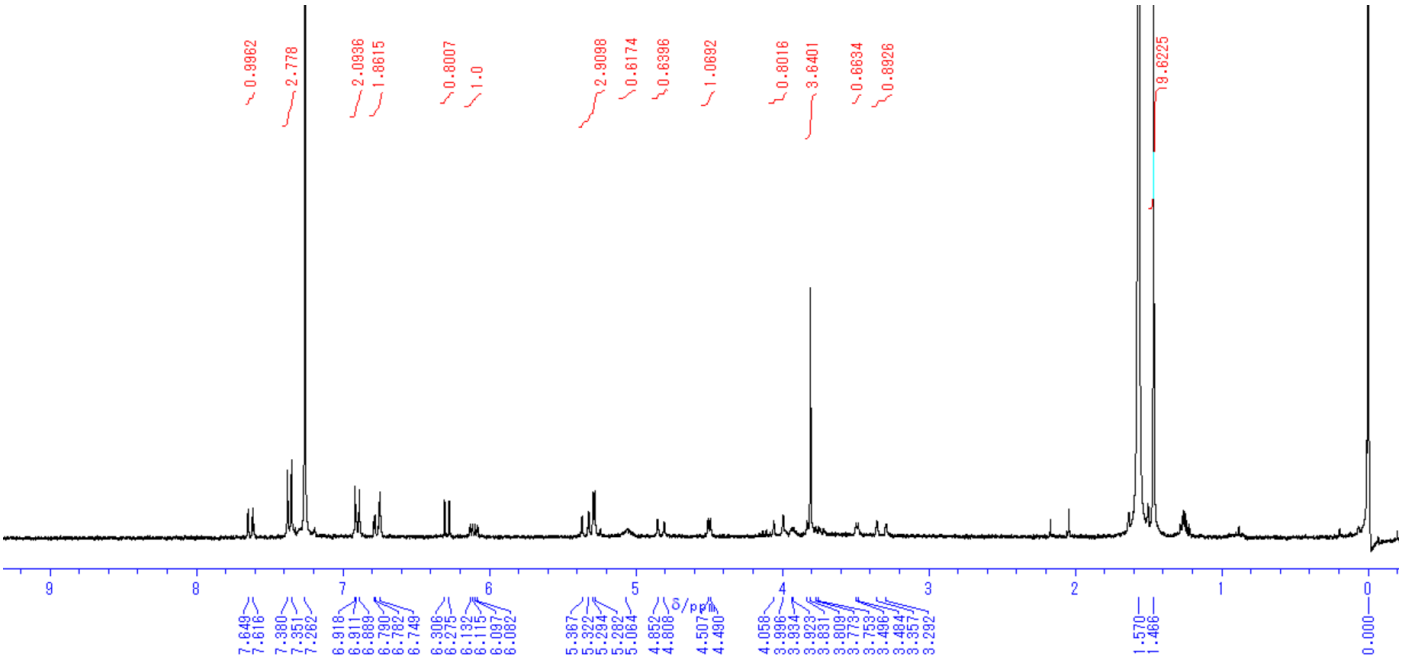


Synthesis of compound 15

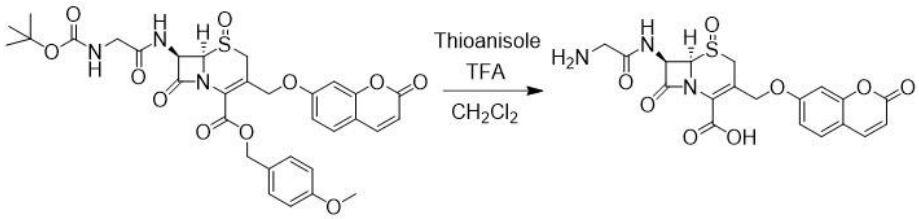


Sodium iodide (240 mg, 1.60 mmol) was added to a solution of compound **14** (84.3 mg, 160 μmol) in acetone (2 mL). The mixture was stirred at rt for an hour, followed by removal of the solvent *in vacuo*. The residue was dissolved in ethyl acetate (80 mL) and the organic layer was washed with water (100 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated. After drying *in vacuo* at rt for an hour, the residue was used for the next reaction without further purification. Umbelliferon (49.3 mg, 304 μmol) and K_2CO_3 (63.2 mg, 457 μmol) were added to a solution of the fore-mentioned crude in CH_3CN (2 mL). The mixture was stirred at rt for an hour. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 50 / 1) to give a crude of umbelliferone conjugate (72.4 mg) as a yellow oil. The crude was dissolved in CH_2Cl_2 (8 mL). After cooling to 0 °C, *m*CPBA (30.2 mg, 122 μmol) was added to the solution. After stirring at 0 °C for 20 min, the solution was diluted with $CHCl_3$ (80 mL) and washed with saturated $NaHCO_3$ (50 mL), water (50 mL), and brine (15 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol = 50 / 1) to give a white crystal **15** (27.4 mg, 41.0 μmol, 26%). ESI-HRMS m/z found 690.1724, calcd 690.1728 ($[C_{32}H_{33}N_3O_{11}S+Na]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 3.32 (2H, d, *J* = 19.5 Hz), 3.49 (1H, d, *J* = 3.6 Hz), 3.81 (3H, s), 3.93 (1H, br), 4.03 (2H, d, *J* = 18.6 Hz), 4.50 (1H, d, *J* = 5.1 Hz), 4.83 (1H, d, *J* = 13.2 Hz), 5.06 (1H, br), 5.29 (2H, d, *J* = 3.6 Hz), 5.35 (1H, *J* = 13.5 Hz), 6.10 (1H, dd, *J* = 9.9, 4.5 Hz), 6.29 (1H, d, *J* = 9.3 Hz), 6.75–6.79 (2H, m), 6.90 (2H, d, *J* = 8.7 Hz), 7.36 (2H, d, *J* = 11.7 Hz), 7.63 (1H, d, *J* = 9.9 Hz).

^{1}H-NMR spectrum of compound **15**

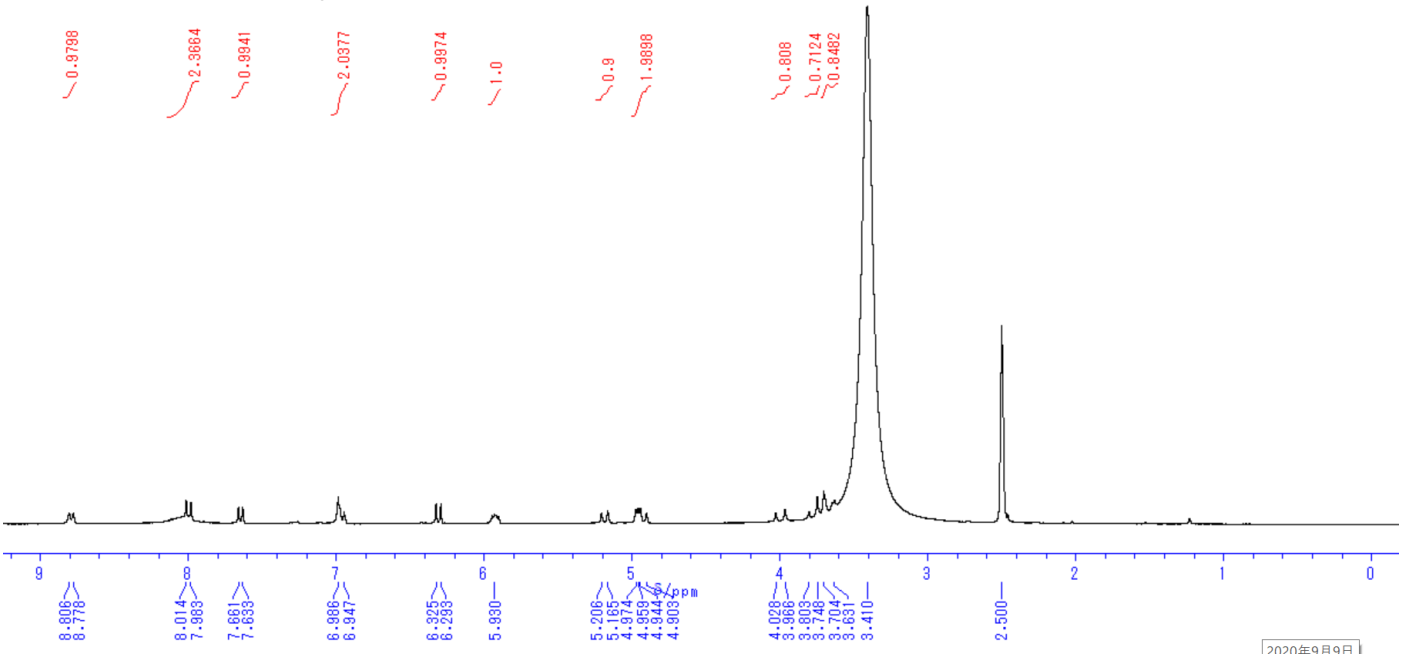


Synthesis of Gly-SoxLC

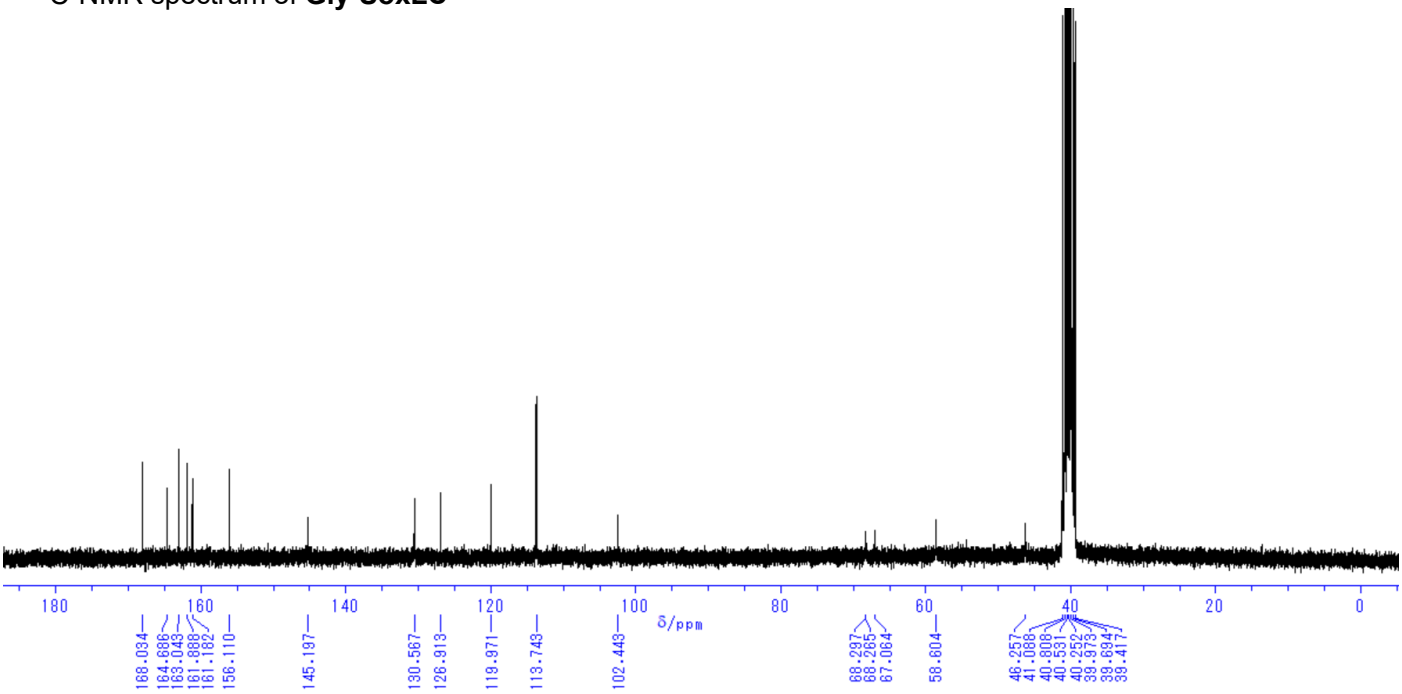


Compound **15** (27.4 mg, 41.0 μmol) was dissolved in CH_2Cl_2 (3 mL). Thioanisole (600 μL) and TFA (1.8 mL) were added to the solution at 0 °C. The solution was stirred at 0 °C for 4 hours. After addition of cold diethyl ether (12 mL), the precipitate was collected by the centrifugation (21500 g × 1 min) and the supernatant was removed. Addition of cold diethyl ether (6 mL) followed by the centrifugation step was repeated 3 times to wash the precipitate. The residue was dried *in vacuo* to give a white solid **Gly-SoxLC** (10.3 mg, 18.3 μmol, 45%). ESI-HRMS m/z found 448.0808, calcd 448.0809 ($[C_{19}H_{17}N_3O_8S+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 3.63–3.80 (3H, m), 4.00 (1H, d, *J* = 18.6 Hz), 4.92 (1H, d, *J* = 12.3 Hz), 4.96 (1H, d, *J* = 4.5 Hz), 5.19 (1H, d, *J* = 12.3 Hz), 5.93 (1H, dd, *J* = 8.1, 5.4 Hz), 6.31 (1H, d, *J* = 9.6 Hz), 6.97 (2H, s+d, *J* = 11.7 Hz), 7.65 (1H, d, *J* = 8.4 Hz), 8.00 (1H, d, *J* = 9.3 Hz), 8.02 (2H, br), 8.79 (1H, d, *J* = 8.4 Hz). ^{13}C-NMR (75 MHz, DMSO-*d6*) δ 46.26, 58.60, 67.06, 68.30, 102.44, 113.74, 119.97, 126.91, 130.57, 145.20, 156.11, 161.18, 161.89, 163.04, 164.69, 168.03.

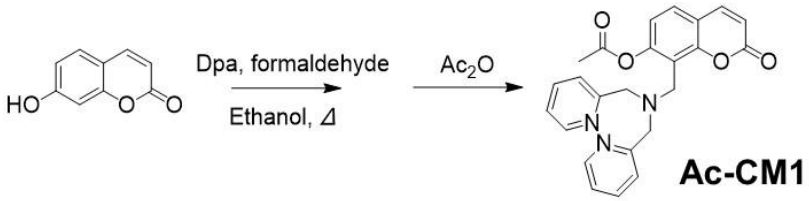
^{1}H-NMR spectrum of **Gly-SoxLC**



^{13}C-NMR spectrum of **Gly-SoxLC**

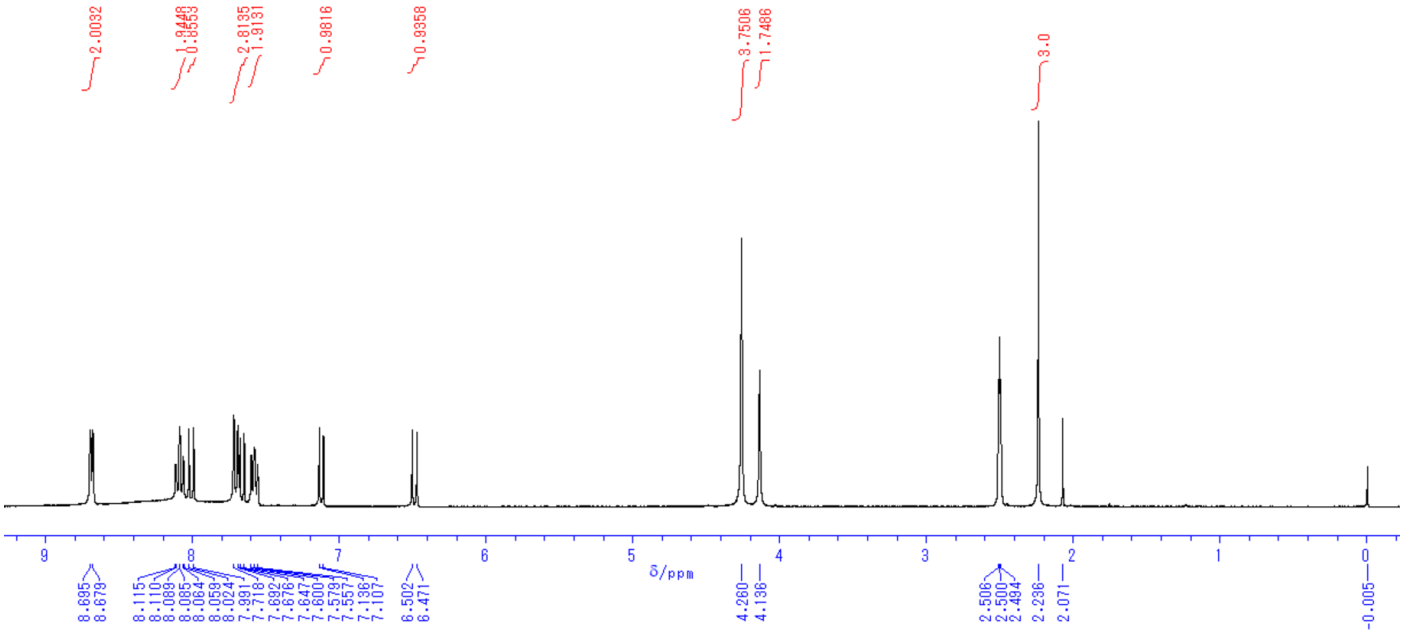


Synthesis of Ac-CM1



The synthesis was conducted according to the reported method.[S4] Formaldehyde solution (37% in water including 10% methanol as a stabilizer, 280 μL) was added into a solution of bis(2-pyridylmethyl)amine (135 mg, 675 μmol) in ethanol (20 mL). After the solution was refluxed at 80 °C for 30 min, a solution of umbelliferone (98.0 mg, 605 μmol) was added and the mixture was stirred at 70 °C for 15 hours. The solvent was removed *in vacuo* and the residue was purified by column chromatography (SiO_2, $CHCl_3$ / methanol / acetic acid = 100 / 4 / 1). After removal of the solvent, the residue was dissolved in acetic anhydride (Ac_2O, 1 mL). The solution was stirred at rt for 2 hours. The solution was diluted with a mixture (9 mL) of CH_3CN / water = 2 / 1 and filtered. The solution was purified by HPLC (COSMOSIL, 5C18-ARII, 20 × 250 mm, flow rate = 4.0 mL/min, solvent A: CH_3CN (0.1% TFA), solvent B: water (0.1% TFA), gradient (Time (min), B (%)) = (0, 95) (30, 70) (31, 0) (36, 0), retention time 25 min) to give a white amorphous Ac-CM1 (138 mg, 331 μmol, 55%). ESI-HRMS m/z found 416.1605, calcd 416.1605 ($[C_{24}H_{21}N_3O_4+H]^+$). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 2.24 (3H, s), 4.14 (2H, s), 4.26 (4H, s), 6.49 (1H, d, *J* = 9.3 Hz), 7.12 (1H, d, *J* = 8.7 Hz), 7.58 (2H, t, *J* = 6.3 Hz), 7.66 (1H, d, *J* = 8.7 Hz), 7.71 (2H, d, *J* = 7.8 Hz), 8.01 (1H, d, *J* = 9.9 Hz), 8.09 (2H, td, *J* = 7.5, 1.5 Hz), 8.69 (2H, d, *J* = 4.8 Hz). The physical properties are consistent withs the reported data.[S4]

^{1}H-NMR spectrum of **Ac-CM1**



Fluorescence measurements

Fluorescence experiments were conducted with a solution (100 μL) of the fluorescent probe (10 μM) in 50 mM HEPES buffer (pH 7.4, 100 μL), 1% (v/v) DMSO in a 96-well plate (EIA/RIA Plate, 96 well half area, No-lid, flat bottom, non-treated, black polystyrene, Corning REF-3694) at 37 °C. All stock solutions of probes were prepared at 10 mM in DMSO. Each stock of metal ion was prepared at 10 mM in distilled water and the stock was diluted with 50 mM HEPES buffer before use. GSH stock solution was prepared at 100 mM in 50 mM HEPES buffer (pH 7.4). The fluorescence was monitored at different time, 5–60 min after the addition of metal ion using plate reader SpectraMax iD5 (Molecular Devices, Dpa-SoxLC λ_{ex} = 390 nm, λ_{em} = 470 nm, ZnAF-2 λ_{ex} = 485 nm, λ_{em} = 520 nm, Zinpyr-1 λ_{ex} = 485 nm, λ_{em} = 630 nm, Ac-CM1 λ_{ex} = 390 nm, λ_{em} = 510 nm). ZnAF-2 (CDX-Z0006-M001-1) and Zinpyr-1 (CDX-Z0001-M020) were purchased from AdipoGen Life Sciences, Ltd.

Enzymatic reactivity of the substrates for β-lactamase (AmpC)

Nitrocefin (Cayman Chemical, #15425), Bn-SoxLC, and Dpa-SoxLC were dissolved in each DMSO at 10 mM. Crude β-lactamase (from *Enterobacter cloacae* Type IV P99, lyophilized powder, 0.2–0.6 units/mg protein, SIGMA-Aldrich, P4524-100UN) was dissolved in 10 mM phosphate buffer (pH 6.0, 50 mM NaCl) at 1 mg/mL. After the β-lactamase stock solution was added to a probe solution, the solution was incubated at 37 °C and the absorbance of nitrocefin product (A_{486}) or the fluorescence (λ_{ex} = 390 nm, λ_{em} = 520 nm) was monitored every 20 sec for 10 min. Measurement conditions: PBS buffer (pH 7.4), 1% (v/v) DMSO, β-lactamase final conc. 0.01 mg/mL, [Nitrocefin] = 10, 30, 50, 70, 100 μM, [Bn-SoxLC / Dpa-SoxLC] = 1, 3, 5, 7, 10 μM, total volume 100 μL, Nitrocefin: TPP, cell culture 96-well plate, flat bottom, clbt, #92696, Bn-SoxLC/Dpa-SoxLC: 96-well half-area black flat bottom plate, Corning REF-3694.

Kinetics of the zinc-catalyzed reaction

Fluorescence intensities at different substrate concentration ([Dpa-SoxLC] = 2, 4, 8, 12, 16 μM) were monitored every 3 min for 30 min. The concentrations of the products were calculated using standard samples of umbelliferon. The plot (reaction rate vs Dpa-SoxLC concentration) was analyzed by non-linear approximation and the K_m and V_{max} were calculated by the approximation formula ($V = -0.0000164097 \times [\text{Dpa-SoxLC}]^2 + 0.0007535114 \times [\text{Dpa-SoxLC}]$, $R^2 = 0.9978$). The catalytic reaction was evaluated at 5 μM $ZnCl_2$ with the different concentrations of Dpa-SoxLC (3–50 μM) in 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, 2 mM GSH. In the same way, the fluorescent response of ZnAF-2 (0.5–10 μM) was evaluated at the typical concentration of 1 μM $ZnCl_2$ in 50 mM HEPES buffer (pH 7.4), 1% (v/v) DMSO, 2 mM GSH. All experiments were carried out at 37 °C with 96-well half-area black flat bottom plate, Corning REF-3694.

HPLC analyses of the zinc-catalyzed reaction of Dpa-SoxLC

The reaction solution was prepared with the following conditions, [Dpa-SoxLC] = 1.4 mM, [$ZnCl_2$] = 1.4 mM, 2.5% CH_3CN (we avoided using DMSO which interferes with the absorption analysis), 50 mM HEPES buffer (pH 6.0 or 7.4 or 8.0). The solution was incubated at 36 °C for 1 hour or 3 hours. Umbelliferone stock solution was prepared in CH_3CN and a total of 1.4 μmol was injected for the HPLC analysis (Figure 3c). The compound elution was monitored by absorbance λ_{abs} = 220 nm or 325 nm. The HPLC analyses were conducted with ODS-column (Waters, C18-100Å, μ-Bondasphere, 7.8 × 300 mm). HPLC condition: flow rate = 4.0 mL/min, solvent A: CH_3CN (0.1% TFA), solvent B: water (0.1% TFA), gradient (Time (min), B (%)) = (0, 100) (10, 100) (15, 90) (45, 75) (48,0) (50,0).

Calculation of Detection limit (DL)

The solutions are almost same to other experiments except for the contents of zinc and GSH. Fluorescence intensities were monitored in different conditions ([GSH] = 0, 1, 2 mM) at reaction time, 0, 5, 15, 30, 45, 60 min after the addition of different concentrations of zinc ([$ZnCl_2$] = 0, 0.625, 1.25, 2.5, 5 μM). The DL parameters (Table S2) were calculated using the following formula,

$$DL = \frac{3\sigma}{m\,(t)}$$

σ is the standard deviation of fluorescence intensities at 5 min in a metal-free solution of the probe (n = 3)

m (t) is the linear approximation of the fluorescence intensity vs zinc-concentration plots at the different reaction times (t, min after addition of zinc ion)

Evaluation of pH Effect in the zinc-catalyzed reaction

50 mM HEPES buffer was prepared at different pH (pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5). Fluorescence experiments were carried out with solutions of Dpa-SoxLC (10 μM) in 50 mM HEPES buffer at the various pHs (100 μL, 1% DMSO) with or without $ZnCl_2$ (10 μM). The fluorescence was monitored at different times, 5–60 min after the

addition of metal ion. All experiments were carried out at 37 °C with 96-well half-area black flat bottom plate, Corning REF-3694. Fluorescence measurements: λ_{ex} = 390 nm, λ_{em} = 470 nm.

Cell Culture

HeLa cells were cultured in high‐glucose Dulbecco's modified Eagle's medium (DMEM; Nacalai Tesque, #08458-45) supplemented with 5 % fetal bovine serum (FBS; CCP-FBS-BR-500, #P180803 (Cosmo Bio Co. Ltd.)), 50 μg/mL Kanamycin sulfate (Meiji Seika Pharma, #46204854), 50 U/mL Penicillin G potassium (Meiji Seika Pharma, #45397387), and 50 μg/mL Streptomycin sulfate (Meiji Seika Pharma, #45844665) at 37 °C under a humidified atmosphere of 5 % CO_2 in air. A subculture was performed every two to three days from confluent cultures using a trypsin–ethylenediaminetetraacetic acid (EDTA) solution (Fujifilm Wako Pure Chemical Co., #208-17251).

Fluorescence imaging using confocal microscopy LSM700

For the fluorescence bioimaging, cells (5.0×10^4 cells mL^{-1}) were cultured in 500 μL DMEM for 24 hours in each compartment with a 35 mm glass-bottomed dish (CellVIEW, 4 compartments, sterile, Greiner bio-one, #627870). After washed twice with 500 μL PBS, the cells were incubated with each probe ([Dpa-SoxLBC] = 3 μM, [Dpa-LBC] = 3 or 5 μM, [ZnAF-2 DA] = 3 or 5 μM) in 500 μL HBS medium (20 mM HEPES, 107 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 11.5 mM D-(+)-Glucose (Nacalai Tesque, #16805-35), pH 7.4) for 30 min. After washed once with 500 μL HBS medium, the cells were treated with 1–50 μM ZnPT for 5–120 min followed by the observation of cells by confocal microscopy (×40, Carl Zeiss, LSM700). Fluorescence channel: For Dpa-SoxLBC or Dpa-LBC, λ_{ex} = 405 nm, λ_{em} = 420–530 nm, For ZnAF-2-DA, λ_{ex} = 488 nm, λ_{em} = 510–630 nm). We also confirmed no fluorescence using HeLa cells cultured in DMEM for these two channels (data not shown). For the TPEN inhibition, cells were incubated in HBS medium containing both 50 μM ZnPT and 200 μM TPEN for 30 min. All treatment was conducted in CO_2 incubator (37 °C, 5% CO_2). ZnAF-2 DA (CDX-Z0008-M001-1) was purchased from AdipoGen Life Sciences, Ltd.

Cell viability check after the incubation with Dpa-LBC

Cells (1.0×10^5 cells mL^{-1}) were cultured in 100 μL DMEM for 24 hours in each well (TPP, cell culture 96-well plate, flat bottom, clbt, #92696). After removal of the medium by pipetting and washed with PBS (100 μL), the cells were incubated in HBS medium containing different concentrations [Dpa-LBC] = 0–20 μM for 1 hour. After removal of the medium, the cells were incubated in DMEM containing 10% WST-8 Kit (Kishida Chemical Co. Ltd., #260-96160). After the treatment for 2 hours, the produced water-soluble formazan was measured using the absorbance λ_{abs} = 450 nm.

Fluorescence imaging using HeLa cells pre-treated with *N*-methylmaleimide (NMM)

Cells (5.0×10^4 cells mL^{-1}) were cultured in 500 μL DMEM for 48 hours in a 35 mm glass-bottomed dish (CellVIEW, 4 compartments, sterile, Greiner bio-one, #627870). After washing twice with 500 μL HBS medium, the cells were

incubated with 3 μM ZnAF-2 DA and 100 μM NMM in 500 μL HBS medium, 1.3% (v/v) DMSO for 30 min. After washing twice with 500 μL HBS medium, the cells were treated with 0, 5, or 50 μM ZnPT for 30 min followed by the observation of cells by confocal microscopy (×40, Carl Zeiss, LSM700). Fluorescence channel: λ_{ex} = 488 nm, λ_{em} = 510–630 nm).

References

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