Supplementary Information

Dual-Responsive Probe and DNA Interstrand Crosslink Precursor Target the Unique Redox Status of Cancer Cells

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Experiment protocols

Absorbance/fluorescence measurements. The stock solution of probe **10** (10 μM) in anhydrous DMSO was freshly prepared, and used to make the test solution (1 μM) for UV-Vis absorbance/fluorescence assays by mixing stock solution with PBS buffer (pH 7.4, 10 mM) to reach a total volume (3 mL). The UV-Vis spectra were recorded on a HITACHI U-3900 spectrophotometer (PMT voltage: Auto; Slit width: 1 nm), while the fluorescence signal was captured using a HITACHI F-7000 fluorescence spectrophotometer (EX WL: 395 or 455 nm; EX slit: 5.0 nm; EM slit: 5.0 nm; PMT voltage: 500 V).

Cell culture and fluorescence microscopic imaging. HULEC-5A, H1299 were cultured in RPMI 1640 medium (VivaCell Biosciences), 293T, 786-O cells were cultured in DMEM medium (VivaCell Biosciences) supplied with 10% FBS, 1% NEAA (100 ×, SKU:01-340-1B) and 100 μg/mL penicillin-streptomycin (Yeasen 60162ES76). Cells were passaged every 2-3 days, and maintained in a humidified incubator at 37 ℃ with 5% CO_2. For fluorescence imaging assay, cells were seeded in confocal dishes (35 mm, glass bottom size 20 mm) at a density of 80,000 cells/mL (2 mL), and incubated for 10 h. After treating with DMSO (0.5%, v/v) or probe **10** (10 μM) at 37 ℃ for 5 h, the medium was discarded and the cells were washed with PBS buffer (1 mL × 2). The fluorescence images were captured on a Zeiss LSM 800 Confocal Laser Scanning Microcopy equipped with a Plan-Apochromat 63×/1.40 Oil DIC M27. Excitation/Detection settings: GSH detection: λ_{ex} = 353 nm, λ_{det} = 450~470 nm; H_2O_2 detection: λ_{ex} = 493 nm, λ_{det} = 520~540 nm; FRET detection: λ_{ex} = 353 nm, λ_{det} = 520~540 nm.

ICL formation analysis. The 5'-FAM labeled ODN **15a** (3.2 μM) was annealed with its complementary strand **15b** (1.5 equiv) by heating at 95 ℃ for 5 min in 200 μL reaction solution (PBS buffer, pH 7.4, 10 mM potassium phosphate, 100 mM NaCl), and slowly cooling down to room temperature. The stock solution of **14** was freshly prepared at desired concentration. The reaction solution was prepared by mixing the stock solution of **14** (6 μL, final concentration: 2 mM) with duplex **15** (final concentration: 0.5 μM), GSH (2 mM), H_2O_2 (5 mM) and PBS buffer to reach a final volume of 20 μL (pH 7.4, 10 mM potassium phosphate, 100 mM NaCl). After incubation for desired time, the reaction was quenched with 90% formamide loading buffer, and analyzed via 20% denaturing polyacrylamide gel electrophoresis (PAGE, acrylamide/bisacrylamide = 19:1, 45% urea). The ICL yields were quantified by Sapphire Biomolecular Imager equipped with Sapphire Capture Software.

EVE trapping for ICLs formation. Competitive inhibition experiments for DNA ICL formation were conducted via ethyl vinyl ether (EVE) trapping assay. The EVE stock solutions, as appropriate for the desired concentration (1~1000 mM), were added to the ODN (0.5 μM) reaction mixture as described above. The resulting reaction mixture was incubated at room temperature for 24 h (note: the trapping reaction was carried out at room temperature instead of 37 ℃ as the boiling point of EVE is 35.6 ℃), quenched with

90% formamide loading buffer, and analyzed by 20% denaturing PAGE.

Maxam-Gilbert sequencing. 5' FAM-labeled ODN **15a** (2.5 μM, 20 μL) was added to formic acid (50 μL), and the resulting reaction mixture was incubated at room temperature for 8 min. Stop buffer (150 μL) (25 μg /mL calf thymus DNA, 0.3 M NaOAc, pH 5.2, 0.1 mM EDTA) was added to quench the reaction, followed by the addition of anhydrous ethanol (-20 ℃, 660 μL). The mixture was placed at -80 ℃ for 30 min, and then the ODN was pelleted out by centrifugation (13000 rpm, 5 min). The supernatant was discarded, and the residue was washed with ethanol (-20 ℃, 60 μL), and dried via SpeedVac. The obtained residue was treated with piperidine (1.0 M, 60 μL) at 90 ℃ for 30 min, dried by SpeedVac, solubilized in loading buffer (90% formamide), and analyzed by 20% denaturing PAGE.

Heat stability study of ICLs. To the reaction mixture obtained from DNA ICL reaction at the optimized conditions, 10 μL sodium acetate (1.0 M, pH = 5.2) and 900 μL of cold absolute ethanol were added. The resulting mixture was placed at -80 ℃ for 30 min, and centrifuged at 13000 rpm for 5 min. The supernatant was removed, and the pellet was washed with ethanol (-20 ℃, 60 μL), and dried in SpeedVac. The pellet was re-dissolved in water (60 μL) and equally divided into three portions. One portion was used as control, while the second and third portions were heated at 90 ℃ in the presence of PBS buffer (pH 7.4, 10 mM) and piperidine (1.0 M) for 30 min, respectively. The solvent was removed by SpeedVac, and the samples were solubilized in loading buffer (90% formamide), and analyzed by 20% denaturing PAGE.

The hydroxyl radical sequencing. The reaction solution containing 5'-FAM-labeled ODN **15a** (1 μM), sodium phosphate (10 mM), NaCl (100 mM), EDTA (0.1 mM), sodium ascorbate (1 mM), $(NH_4)_2Fe(SO_4)_2$ (50 μM), and H_2O_2 (2 mM) with a total volume of 20 μL was incubated at room temperature for 3 min. The reaction mixture was quenched with thiourea (100 mM, 10 μL), and dried by SpeedVac. The residue was treated with piperidine (1.0 M) at 90 ℃ for 30 min. After removing the solvent using SpeedVac, the sample was solubilized in loading buffer (90% formamide), and subsequently analyzed by 20% denaturing PAGE.

Cytotoxicity assay. Cytotoxicity of precursor **14** was determined by standard MTT assay. Cells were seeded into 96-well plates at a density of 3,000 cells/well (200 μL) and incubated for 12 h. Precursor **14** was dissolved in DMSO with a concentration of 20 mM, which was further serially diluted with DMSO for 8 times (each dilution reduces the drug concentration by half). The prepared drug solutions (1 μL) were then added to appropriate wells accordingly, and incubated at 37 °C for 24 h. Then, the filter-sterilized 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS solution (5 mg/mL, 10 μL) was added to each well, and incubated for an additional 4 h. The media was discarded, and DMSO (100 μL) was added to each well to dissolve formazan crystals for 30 min. The absorbance at 570 nm was recorded by a full wavelength microplate spectrophotometer, with the background at 630 nm was subtracted. The percentage of

cells survival was determined as the ratio of the absorbance of the treated cells to that of untreated control cells.

Cell cycle assay. H1299 lung cancer cells were seeded in a dish (6 cm) at a density of 12,0000 cells/mL (4 mL), and incubated at 37 ℃ with 5% CO_2 overnight (~16 h). The cells were then treated with DMSO (0.5%, v/v) or various concentrations of **14** (5, 10, 20 μM) at 37 ℃ for 24 h. Afterwards, the cells were harvested using 0.25% trypsin-EDTA, and centrifuged at 2000 rpm for 3 min. The cell pellets were washed with cold PBS twice, and fixed with 70% ethanol at 4 ℃ overnight. After centrifugation and washing with ice-cold PBS, 100 μL ribonuclease A was added to suspend the cell pellets. The cell suspension was incubated at 37 ℃ for 30 min, after which propidium iodide (PI) (400 μL) was added. The resulting mixture was incubated at 4 ℃ for an additional 30 min, which was then analyzed by detecting propidium iodide fluorescence using NovoCyte Flow Cytometer (Agilent).

Cell apoptosis assay. H1299 lung cancer cells were seeded in a culture dish (6 cm) at a density of 12,0000 cells/mL (4 mL), and incubated at 37 ℃ incubator with 5% CO_2 overnight (~16 h). DMSO (0.1%, v/v) or **14** in DMSO with desired concentrations (5, 10, 20 μM) was added to corresponding dishes. The cells were then incubated for 24 h, and harvested using 0.25% trypsin-EDTA. The cells were collected and centrifugated at 1000 rpm for 5 min. The cell pellet was washed by ice-cold PBS twice, and stained with Annexin V-FITC Apoptosis Detection Kit (Bioss, BA00101) according to the manufacturer's instructions. Briefly, the cell pellets were resuspended in 1× binding buffer (100 μL) (diluted by DDH_2O), followed by the addition of Annexin V-FITC (5 μL) and propidium iodide staining solution (5 μL). The resulting mixture was incubated at room temperature for 15 min, diluted with 1× binding buffer (400 μL), and placed on ice. The sample was determined via detecting the FITC and PI fluorescence intensity using NovoCyte Flow Cytometer Systems (Agilent).

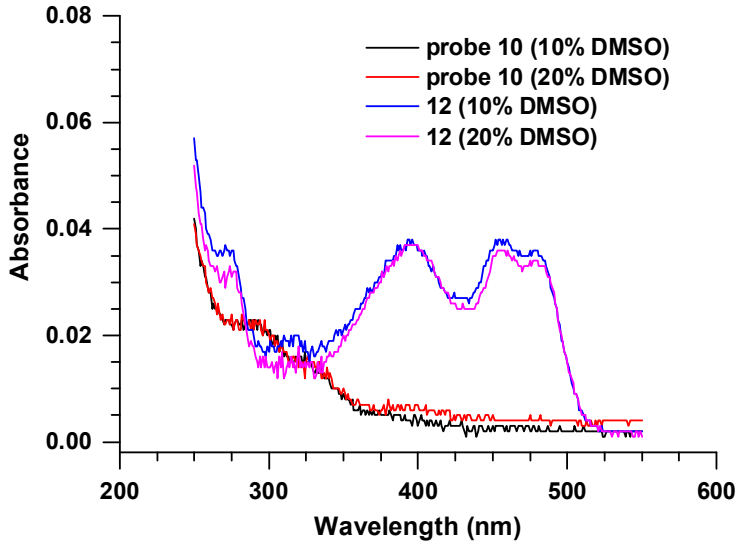


Figure **S1**. UV-Vis absorption spectra of probe **10** (1 μM) and product **12** (1 μM) in PBS buffer (pH7.4, 10 mM) containing 10% or 20% DMSO. Probe **10** (1 μM) and product **12** (1 μM) can completely dissolve in PBS buffer containing 10% DMSO.

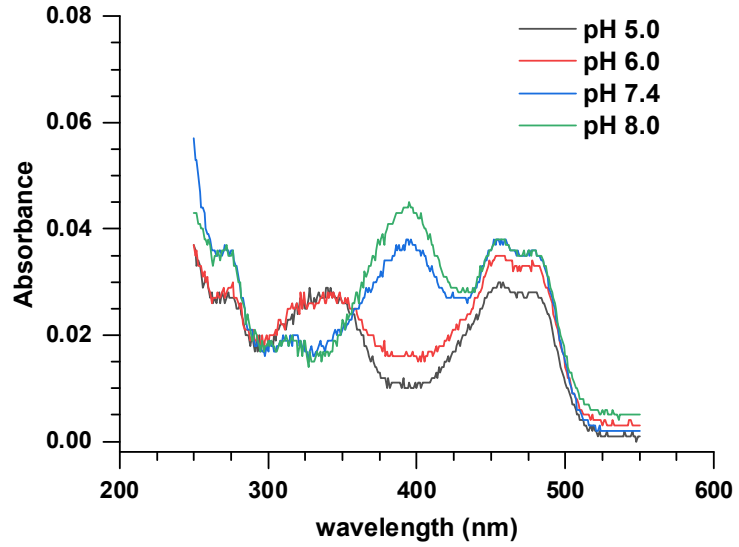


Figure **S2**. UV-Vis absorption spectra of **12** (1 μM) in PBS buffers with different pH values. Obvious absorbance at 390 nm and 455 nm was observed in PBS buffer (10 mM, 10 % DMSO) with pH 7.4 or 8.0, which favors the fluorescence detection.

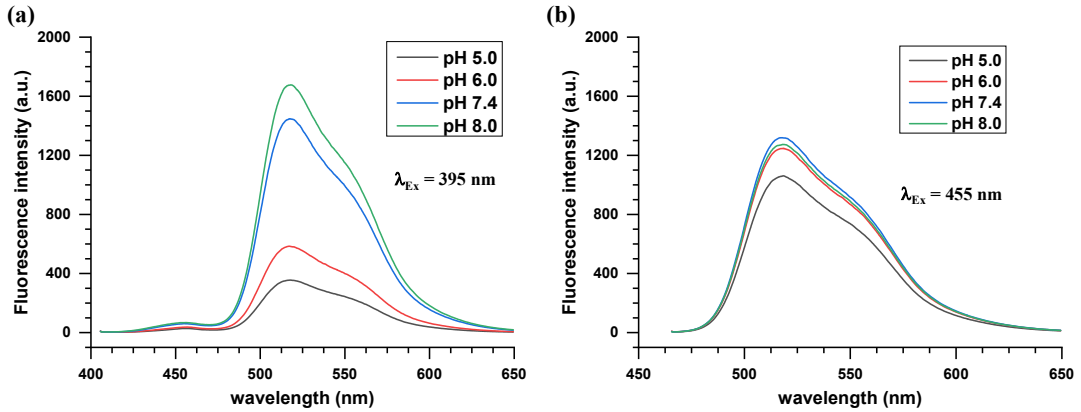


Figure **S3**. Fluorescence emission spectra of product **12** (1 μM) in PBS buffers (10 mM, pH 7.4, 10% DMSO) with various pH. (a) λ_{ex} = 395 nm; (b) λ_{ex} = 455 nm. Significant fluorescence signal of **12** was observed upon excitation with light at 395 nm or 455 nm in PBS buffer (pH 7.4 or 8.0).

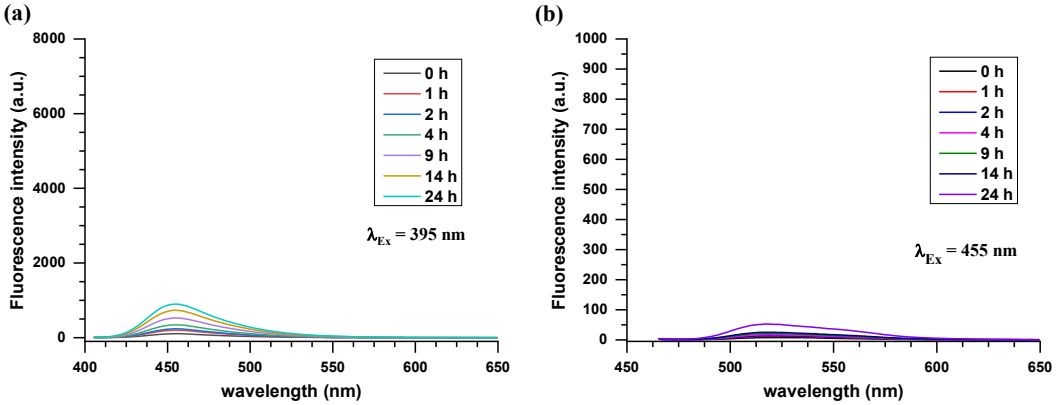


Figure **S4**. Stability of probe **10** in PBS buffer characterized by UV-Vis spectra (10 mM, pH 7.4, 10% DMSO). Probe **10** (1 μM) was incubated at 37 ℃ for different durations, and the fluorescence emission spectra was recorded. (a) λ_{ex} = 395 nm; (b) λ_{ex} = 455 nm.

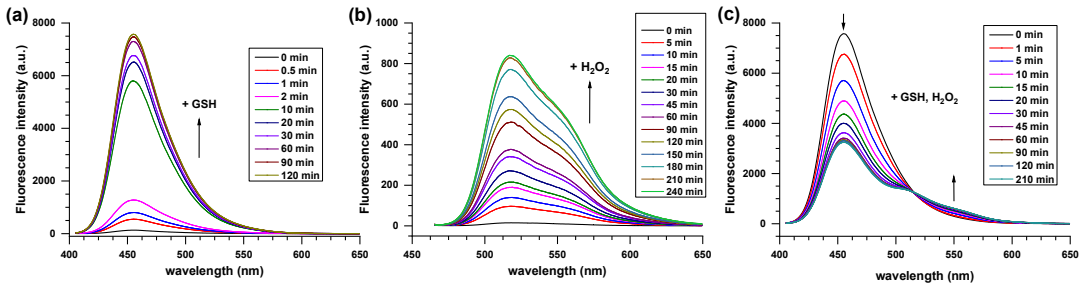


Figure **S5**. Fluorescence response of probe **10** (1 μM) towards GSH (1 mM) and H_2O_2 (1 mM). (a) Probe **10** was treated with GSH for desired time and the fluorescence signal was detected (λ_{ex} = 395 nm); (b) Probe **10** was treated with H_2O_2 for desired time (λ_{ex} = 455 nm); (c) Probe **10** incubated with GSH for 90 min was treated with H_2O_2 for desired time (λ_{ex} = 395 nm).

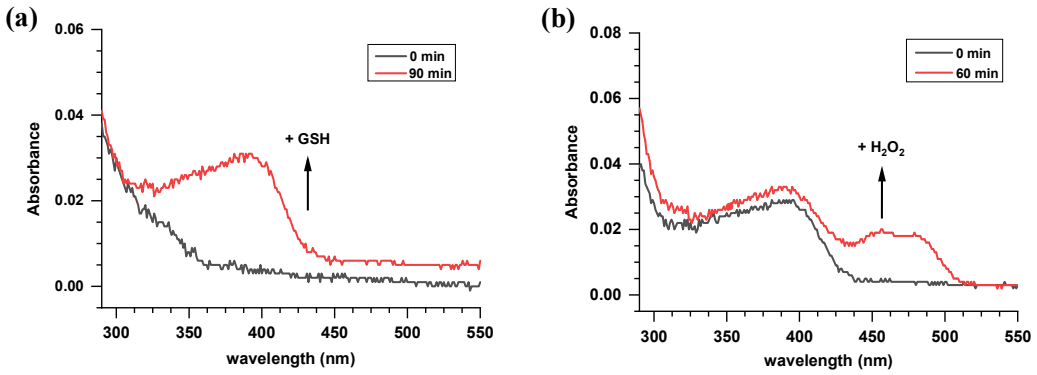


Figure **S6**. UV absorbance spectra of probe **10** (1 μM) upon treatment with GSH (1 mM) (a), or H_2O_2 (1 mM) (b), in PBS buffer (10 mM, pH 7.4, 10% DMSO) for different durations.

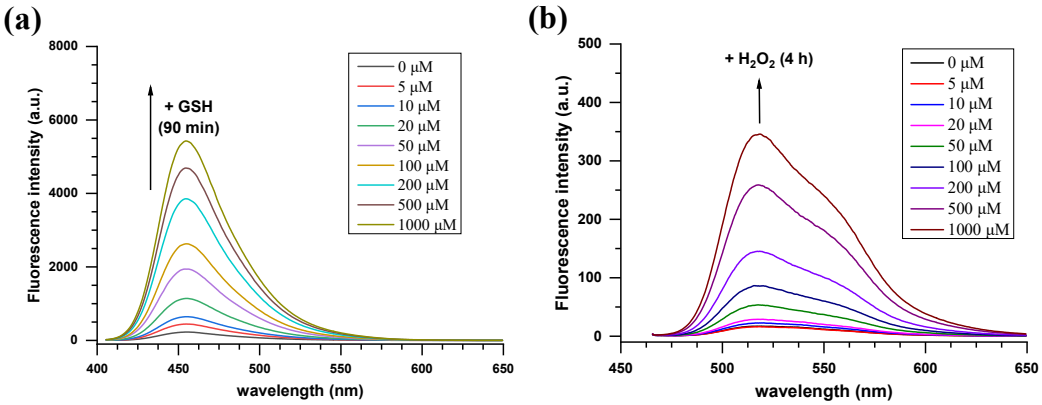


Figure **S5**. Concentration-dependent fluorescence response of probe **10** (1 μM) toward GSH or H_2O_2 in PBS buffer (pH 7.4, 10 mM, 10 % DMSO). (a) Probe **10** was treated with GSH (0~1 mM) for 90 min (λ_{ex} = 395 nm); (b). Probe **10** was treated with H_2O_2 (0~1 mM) for 4 h (λ_{ex} = 455 nm).

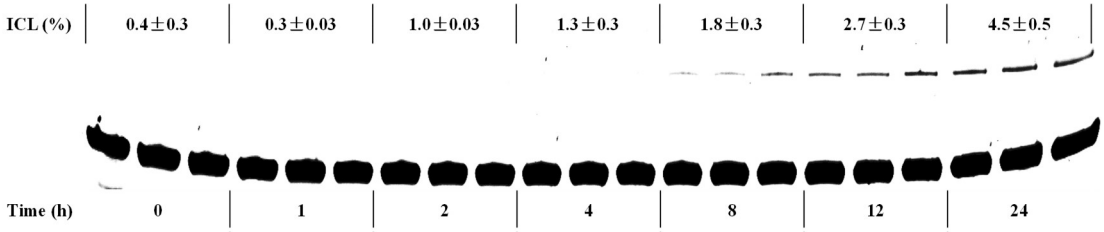


Figure **S8**. Time-dependent ICL formation induced by **14**. Concentration of duplex **15** is 0.5 μM. That is 2 mM for **14** or GSH, and 4 mM for H_2O_2.

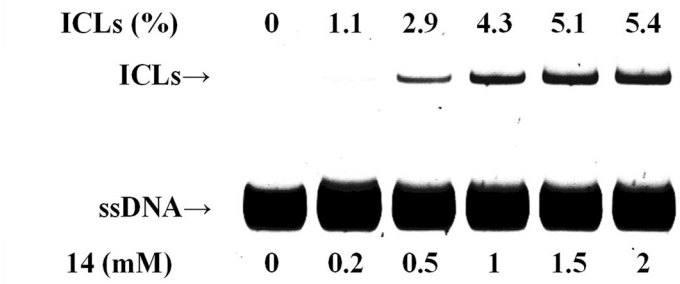


Figure S**9**. Concentration-dependent ICL formation induced by **14** (Higher concentration of **14** was not achieved due to its solubility in buffer solution. The ratio of GSH/**14** is 1:1, and the ratio of H_2O_2/**14** is 2:1).

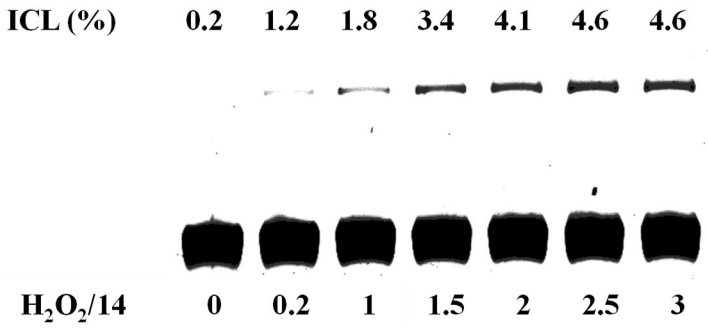


Figure S**10**. H_2O_2/**14** ratio-dependent ICL formation induced by **14** (the concentration of **14** and GSH is 2 mM).

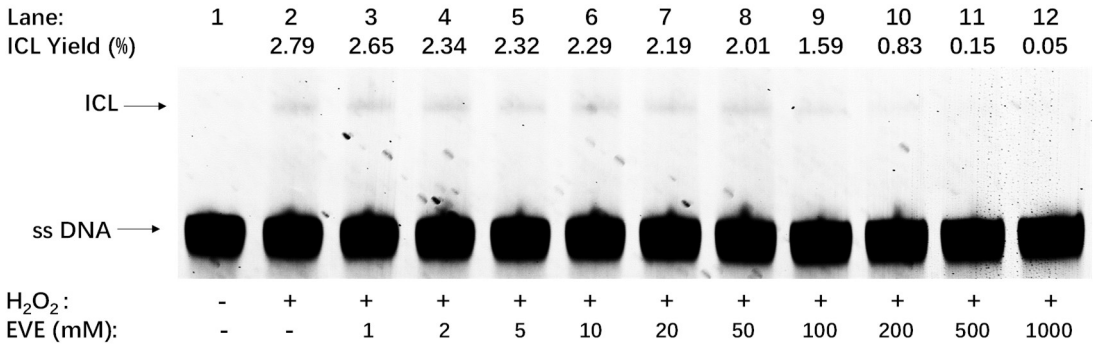


Figure **S11**. The influence of EVE on DNA ICL formation. (Note：the reaction was carried out at room temperature as the boiling point of EVE is 35.6 ℃)

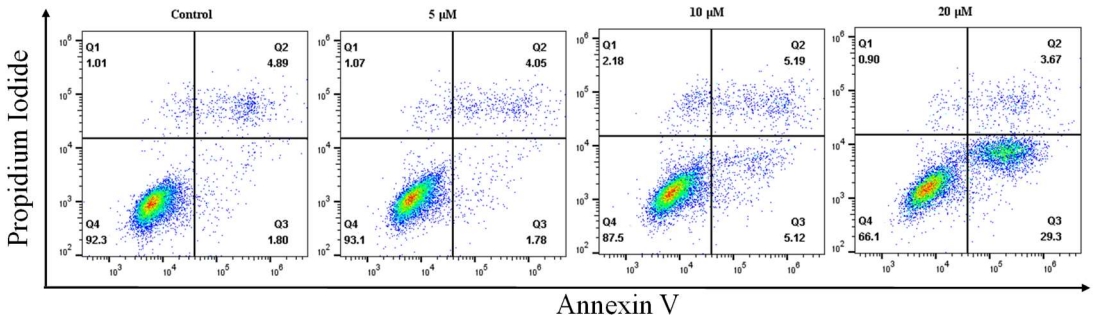
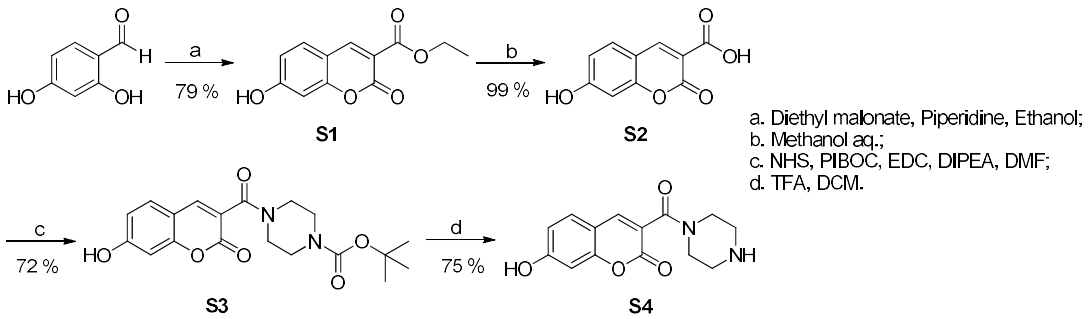


Figure **S12**. Cell apoptosis analysis of H1299 cells treated with **14** at desired concentrations for 24 h.



Ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate (**S1**). To a 100 mL flask equipped with a reflux condenser, 2,4-dihydroxybenzaldehyde (2.76 g, 20 mmol) and diethyl malonate (6.41 g, 40 mmol) in ethanol (10 mL) were added, followed by the addition of piperidine (1 mL). The reaction mixture was refluxed for 6 h, and cooled to 0 ℃. The resulting mixture was acidified with 0.5 M HCl (aq) to produce a light yellowish precipitate, which was collected by filtration to obtain **S1** as a light yellowish solid (6 g, 25.6 mmol, 79 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 7.76 (d, J = 8.6 Hz, 1H), 6.85 (dd, J = 8.6, 2.2 Hz, 1H), 6.74 (d, J = 2.2 Hz, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H).

7-Hydroxy-2-oxo-2H-chromene-3-carboxylic acid (**S2**). To a 50 mL flask, **S1** (0.78 g, 3.2 mmol) in 2 M NaOH (aq, 10 mL) was added. The reaction mixture was stirred at room temperature for 12 h, which was then acidified by 2 M HCl (aq) to pH=2~3 to produce precipitate. The precipitate was collected by filtration to yield **S2** as a light yellowish solid (0.66 g, 3.2 mmol, 99 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H), 7.73 (d, J = 8.6 Hz, 1H), 6.83 (dd, J = 8.6, 2.3 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H).

Tert-butyl 4-(7-hydroxy-2-oxo-2H-chromene-3-carbonyl)piperazine-1-carboxylate (**S3**). **S2** (206 mg, 1 mmol), 1-hydroxypyrrolidine-2,5-dione (290 mg, 2.5 mmol), *tert*-butyl piperazine-1-carboxylate (424 mg, 2.5 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro (288 mg, 1.5 mmol) were dissolved in DMF (3 mL), followed by the addition of DIPEA (0.1 mL). The reaction mixture was stirred at room temperature for 12 h, and quenched with H_2O (10 mL). The resulting mixture was extracted with dichloromethane (3 × 10 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4. After removing solvent, the residue was purified by silica chromatography with a gradient of petroleum ether/ethyl acetate (1:1 to 1:3) to obtain **S3** as a slight yellowish oil (270 mg, 0.72 mmol, 72 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.60 (d, J = 8.6 Hz, 1H), 6.84 (dd, J = 8.5, 2.3 Hz, 1H), 6.76 (d, J = 2.2 Hz, 1H), 3.37 (s, 8H), 1.41 (s, 9H).

7-Hydroxy-3-(piperazine-1-carbonyl)-2H-chromen-2-one (**S4**). To a solution of **S3** (300 mg, 0.8 mmol) in dichloromethane (6 mL), trifluoroacetic acid (1.2 mL) was added slowly. The reaction mixture was stirred at room temperature for 1 h, after which the solvent was removed. The residue was purified by silica chromatography with dichloromethane/methanol (5:1) to yield **S4** as a white solid (190 mg, 0.69 mmol, 61 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.15 (s, 1H), 7.62 (d, J = 8.6 Hz, 1H), 6.86

(dd, J = 8.5, 2.3 Hz, 1H), 6.78 (d, J = 2.2 Hz, 1H), 3.69 (d, J = 80.9 Hz, 4H), 3.37 (s, 1H), 3.14 (d, J = 22.9 Hz, 4H).

4-(2,4-Dihydroxybenzoyl)isophthalic acid (**2**). To a 500 mL flask equipped with a reflux condenser, 1,3-dioxo-1,3-dihydroisoben-zofuran-5-carboxylic acid (19.2 g, 0.1 mol), resorcinol (22.0 g, 0.2 mmol) and methanesulfonic acid (100 mL) were added. The reaction mixture was stirred at 80 ℃ overnight, and was then poured into ice-water mixture (500 mL) under vigorous stirring to precipitate an orange solid. The solid was collected by filtration, and 2 M NaOH was added till the solid dissolved. Neutralized with HCl (aq, 12 M) to obtain 5(6)-carboxyfluorescein as an orange precipitate. The precipitate was collected by filtration to obtain a crude solid, which was then dissolved in NaOH (50 wt.%, 200 mL) and stirred at 80 ℃ overnight. When the color of the reaction mixture changed from dark red to colorless, crushed ice (300 mL) was added to quenched the reaction. Followed by the addition of HCl (12 M) to adjust the pH of the mixture to 1~2. The resulting mixture was placed at 4 ℃ overnight, and was filtered to obtain a mixture of 4-(2,4-dihydroxybenzoyl)isophthalic acid and 2-(2,4-dihydroxybenzoyl)-terephthalic acid as a light yellowish solid. The two was further separated by recrystallization, where the mixture was dissolved in methanol (100 mL), followed by addition of H_2O (3 L) slowly, the resulting mixture was placed at room temperature for 5 days to afford **2** as a brownish crystal (5.31 g, 17.6 mmol, 18 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 10.77 (s, 1H), 8.51 (d, J = 1.7 Hz, 1H), 8.22 (dd, J = 7.9, 1.7 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H), 6.95 (d, J = 8.8 Hz, 1H), 6.33 (d, J = 2.3 Hz, 1H), 6.29 (dd, J = 8.7, 2.4 Hz, 1H).

4-(6-Hydroxy-3-oxo-3H-xanthen-9-yl)isophthalic acid (**3**). In a 500 mL flask, **2** (6 g, 19.8 mmol) and resorcinol (2.4 g, 21.8 mmol) in methanesulfonic acid (75 mL) were added. The reaction mixture was stirred at room temperature overnight. The resulting reaction mixture was poured to H_2O (4 ℃, 300 mL) under vigorous stirring, filtered to obtain a yellowish crude solid. The crude solid was purified by precipitation as follows. After dissolving the crude solid in NaOH (aq, 2 M), the reaction mixture was subsequently adjusted with HCl (12 M) until lots of solid precipitated. Filtered and reprecipitated in the same way as described above to afford **3** as a yellow solid (7.3 g, 19.3 mmol, 98 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 2H), 8.40 (t, J = 1.0 Hz, 1H), 8.30 (dd, J = 8.0, 1.6 Hz, 1H), 7.45 – 7.33 (m, 1H), 6.72 (d, J = 2.3 Hz, 2H), 6.62 (d, J = 8.7 Hz, 2H), 6.56 (dd, J = 8.7, 2.3 Hz, 2H).

Dimethyl 4-(6-methoxy-3-oxo-3H-xanthen-9-yl)isophthalate (**4**). To a solution of **3** (3.6 g, 9.57 mmol) and anhydrous K_2CO_3 (6.61 g, 47.8 mmol) in DMF (30 mL), CH_3I (6.79 g, 2.98 mL, 47.8 mmol) was added. The reaction was stirred at room temperature for 24 h. Quenched with water (150 mL), and extracted with DCM (2 × 150 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4. After removing solvent, the residue was purified by silica chromatography with dichloromethane/methanol (40:1) to afford **4** as a light yellow solid (3 g, 7 mmol, 75 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.81 (d, J = 1.7 Hz, 1H), 8.31 (dd, J = 7.9, 1.8 Hz,

1H), 7.36 (d, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.76 (d, *J* = 8.9 Hz, 1H), 6.73 (d, *J* = 9.7 Hz, 1H), 6.68 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.49 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 3.96 (s, 3H), 3.85 (s, 3H), 3.62 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 184.26, 164.39, 163.76, 163.36, 157.74, 153.37, 148.31, 137.95, 132.35, 131.20, 130.78, 130.01, 129.82, 128.95, 128.82, 127.54, 116.40, 113.36, 112.74, 104.89, 99.45, 55.05, 51.78, 51.68. HRMS-ESI (+) (m/z): $[M + H]^+$ calcd. for $C_{24}H_{19}O_7^+$, 419.1125; found: 419.1124.

3'-Hydroxy-6'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5-carboxylic acid (**5**). To a 100 mL flask, **4** (3 g, 7.17 mmol) in a mixture of methanol (15 mL) and NaOH (aq, 10 wt.%, 15 mL) was added. The reaction mixture was stirred at room temperature for 3 h. After removing methanol, the resulting solution was diluted with water (20 mL), neutralized with HCl (12 M) to pH 5, and extracted with ethyl acetate (3 × 100 mL). The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated to afford **5** as an orange solid (2.6 g, 6.7 mmol, 93 %), which was used for next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.57 (s, 1H), 10.22 (s, 1H), 8.43 (d, *J* = 1.0 Hz, 1H), 8.31 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.48 – 7.35 (m, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.76 – 6.72 (m, 2H), 6.70 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.66 (d, *J* = 8.7 Hz, 1H), 6.58 (dd, *J* = 8.7, 2.4 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.78, 165.98, 161.13, 159.69, 156.05, 151.79, 151.67, 136.13, 132.89, 129.26, 129.13, 126.63, 125.53, 124.52, 112.83, 111.92, 110.27, 108.74, 102.21, 100.79, 82.96, 55.62. HRMS-ESI (+) (m/z): $[M + H]^+$ calcd. for $C_{22}H_{15}O_7^+$, 391.0812; found: 391.0817.

2,5-Dioxopyrrolidin-1-yl 3'-methoxy-3-oxo-6'-(((trifluoromethyl)sulfonyl)oxy)-3H-spiro[isobenzofuran-1,9'-xanthene]-5-carboxylate (**7**). To a 100 mL flask, **5** (1 g, 2.56 mmol)，N-Hydroxysuccinimide (445 mg, 3.84 mmol) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride (737 mg, 3.84 mmol) in DMF (20 mL) were added. The reaction mixture was stirred at room temperature overnight. Quenched with water (100 mL), and extracted with ethyl acetate (3 × 100 mL). The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated to obtain a residue. The residue was then dissolved in DMF (20 mL), followed by the addition of N-Phenyl-bis(trifluoromethanesulfonimide) (3.66 g, 10.2 mmol) and DIPEA (1.66 g, 2.23 mL, 12.8 mmol) sequentially. The resulting reaction mixture was stirred at room temperature for 2 h. After removing solvent, the residue was purified by silica chromatography with petroleum ether/ethyl acetate (3:1) to afford **7** as a light yellowish solid (412.6 mg, 0.67 mmol, 26 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, *J* = 1.5 Hz, 1H), 8.47 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.73 (t, *J* = 1.5 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.26 (d, *J* = 1.5 Hz, 2H), 7.01 (d, *J* = 2.6 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 6.77 (dd, *J* = 8.9, 2.6 Hz, 1H), 3.85 (s, 3H), 2.94 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.10, 166.80, 161.57, 160.75, 157.29, 151.27, 151.25, 149.90, 136.81, 131.12, 129.59, 127.12, 127.03, 126.78, 125.78, 119.77, 118.73, 117.36, 116.58, 112.71, 110.72, 109.25, 100.92, 81.56, 55.77, 25.56. HRMS-ESI (+) (m/z): $[M + H]^+$ calcd. for $C_{27}H_{17}F_3NO_{11}S^+$, 620.0469;

found: 620.0471.

2,5-Dioxopyrrolidin-1-yl 3'-methoxy-3-oxo-6'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-5-carboxylate (**8**). To a solution of **7** (600 mg, 0.97 mmol), bis(pinacolato)diboron (1.23 g, 4.84 mmol), palladium (II) acetate (43.5 mg, 0.19 mmol), and 2-(dicyclohexylphosphino)-biphenyl (272 mg, 0.77 mmol) in anhydrous 1,4-dioxane (15 mL) under Ar atmosphere, DIPEA (1 g, 1.35 mL, 7.75 mmol) was added using syringe. The reaction mixture was stirred at 110 ℃ for 2 h, solvent was removed, and the residue was purified by silica chromatography with petroleum ether/ethyl acetate (3:1) to yield **8** as a white foam (300 mg, 0.5 mmol, 52 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 1.6 Hz, 1H), 8.49 – 8.41 (m, 1H), 7.59 (s, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.42 – 7.35 (m, 1H), 6.98 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 2.5 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 6.72 (dd, J = 8.8, 2.6 Hz, 1H), 3.84 (s, 3H), 2.94 (s, 4H), 1.30 (s, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.89, 170.61, 167.51, 161.98, 161.23, 158.37, 152.11, 150.50, 137.24, 129.99, 128.49, 127.49, 127.43, 127.12, 126.10, 123.00, 120.94, 112.78, 109.82, 101.34, 84.67, 82.76, 73.97, 56.20, 26.06, 25.05. HRMS-ESI (+) (m/z): $[M + H]^+$ calcd. for $C_{32}H_{29}BNO_{10}^+$, 598.1879; found: 598.1881.

3-(4-(3'-Methoxy-3-oxo-6'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-5-carbonyl)piperazine-1-carbonyl)-2H-chromen-7-yl 2,4-dinitrobenzenesulfonate (**10**). To a 10 mL flask under Ar atmosphere, **S4** (46.8 mg, 0.14 mmol) in DMF (0.5 mL) was added, followed by the addition of DIPEA (31.5 mg, 43.5 μL, 0.24 mmol). The reaction mixture was stirred at room temperature for 10 min. Then, a solution of **8** (50 mg, 0.08 mmol) in DMF (1 mL) was added and stirred at room temperature for 3 h. Quenched with H_2O (30 mL) and extracted with ethyl acetate (3 × 20 mL). The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in dichloromethane (1 mL), followed by addition of DIPEA (10.2 mg, 13.8 μL, 0.08 mmol). The reaction mixture was stirred at room temperature under Ar atmosphere for 10 min, then a solution of 2,4-dinitrophenylsulfonyl chloride (21.1 mg, 0.08 mmol) in dichloromethane (1 mL) was added. The resulting reaction mixture was stirred at room temperature for 2 h, solvent was removed, the residue was purified by silica chromatography with petroleum ether/ethyl acetate (1:5) to obtain **10** as a white solid (42 mg, 0.042 mmol, 51 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.63 (dd, J = 8.9, 2.3 Hz, 1H), 8.32 (d, J = 8.7 Hz, 1H), 8.27 (s, 1H), 8.08 (s, 1H), 7.84 (dd, J = 12.9, 8.2 Hz, 2H), 7.58 (s, 1H), 7.45 (s, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.25 (dd, J = 8.6, 2.3 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.92 (s, 1H), 6.82 (s, 1H), 6.73 (s, 1H), 3.83 (s, 3H), 3.79 – 3.39 (m, 8H), 1.30 (s, 12H).^{13}C NMR (101 MHz, DMSO-d_6) δ 168.45, 168.16, 163.13, 161.81, 157.60, 154.53, 153.61, 152.11, 150.88, 150.55, 148.59, 142.07, 138.33, 134.90, 134.08, 131.41, 131.10, 129.96, 129.63, 128.14, 126.22, 125.50, 124.80, 123.90, 122.96, 121.75, 121.70, 119.17, 118.79, 112.68, 110.96, 110.58, 101.34, 84.65, 82.36, 56.18, 25.06. HRMS-ESI (+) (m/z): $[M + H]^+$ calcd. for $C_{48}H_{40}BN4O_{17}S^+$, 987.2197; found: 597.2216.

4-Methoxy-2,6-bis((phenylselanyl)methyl)phenyl 2,4-dinitrobenzenesulfonate (**14**).

A solution of **13** (1.1 g, 2.38 mmol) in DCM (20 mL) was cooled to 0 ℃, followed by the addition of triethylamine (530.2 mg, 0.73 mL, 5.24 mmol). After stirring at 0 ℃ for 10 min, 2,4-dinitrobenzenesulfonyl chloride (823.9 g, 3.09 mmol) was added slowly. The reaction mixture was warmed to room temperature, and stirred for another 3 h. Quenched with water (100 mL), extracted with dichloromethane (2 × 100 mL). The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica chromatography with a gradient of petroleum ether/dichloromethane (5:1 to 1:1) to produce **14** as an orange solid (1.5 g, 2.17 mmol, 91 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.44 (s, 1H), 8.23 (d, *J* = 1.4 Hz, 2H), 7.25 – 7.19 (m, 4H), 7.16 – 7.09 (m, 6H), 6.48 (s, 2H), 3.93 (s, 4H), 3.51 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 156.89, 149.25, 147.25, 137.63, 134.03, 132.96, 131.96, 131.52, 129.03, 128.11, 126.47, 125.71, 119.36, 114.35, 54.44, 25.62. HRMS-ESI (+) (m/z): $[M + H]^+$ calcd. for $C_{27}H_{22}N_2NaO_8SSe_2^+$, 716.9320; found: 716.9320.

NMR Spectra of Compounds

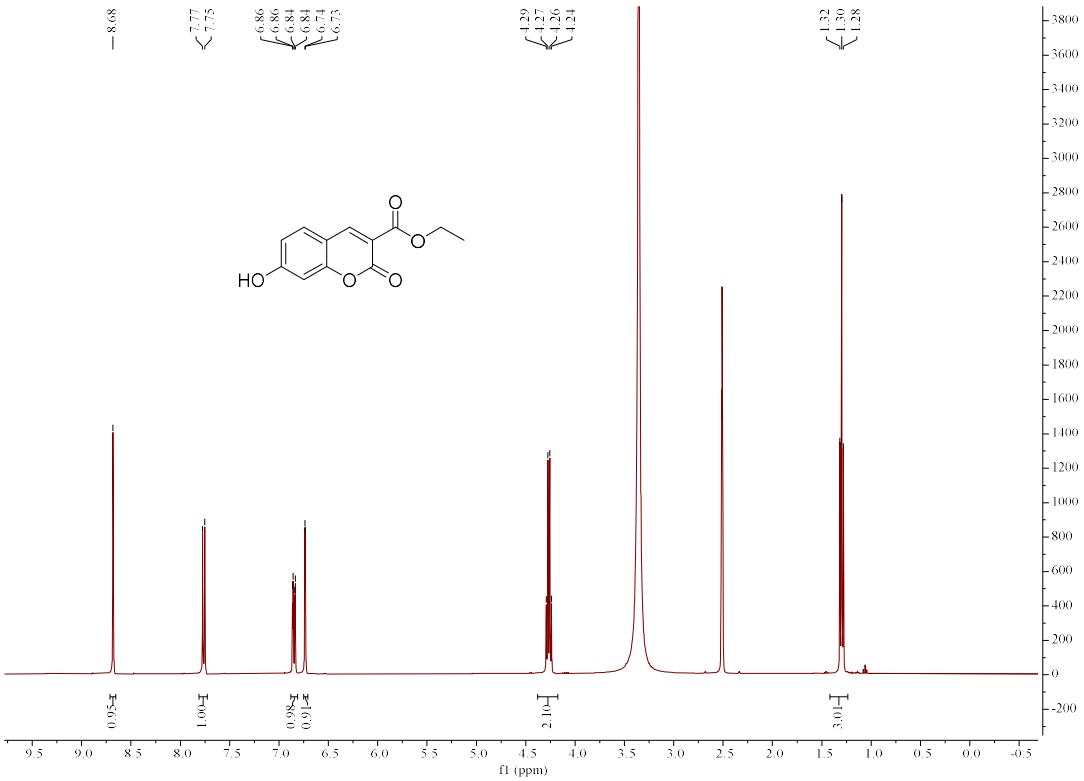


Figure **S13**. [1]H-NMR spectrum of **S1**.

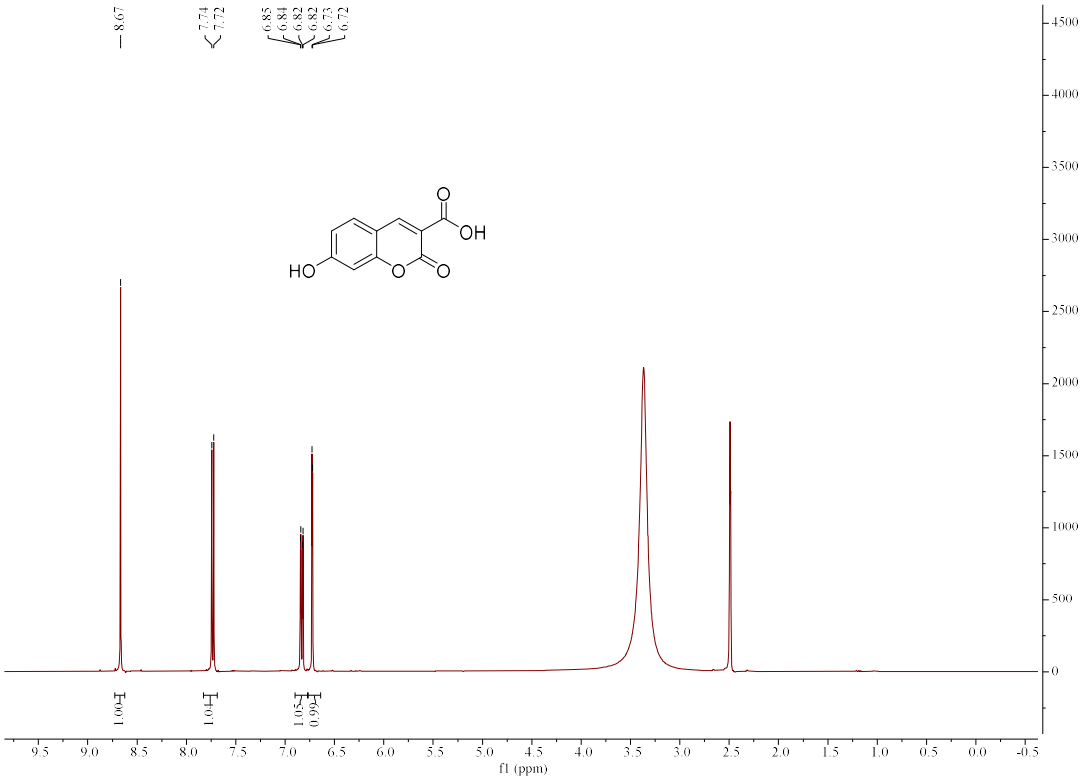


Figure **S14**. [1]H-NMR spectrum of **S2**.

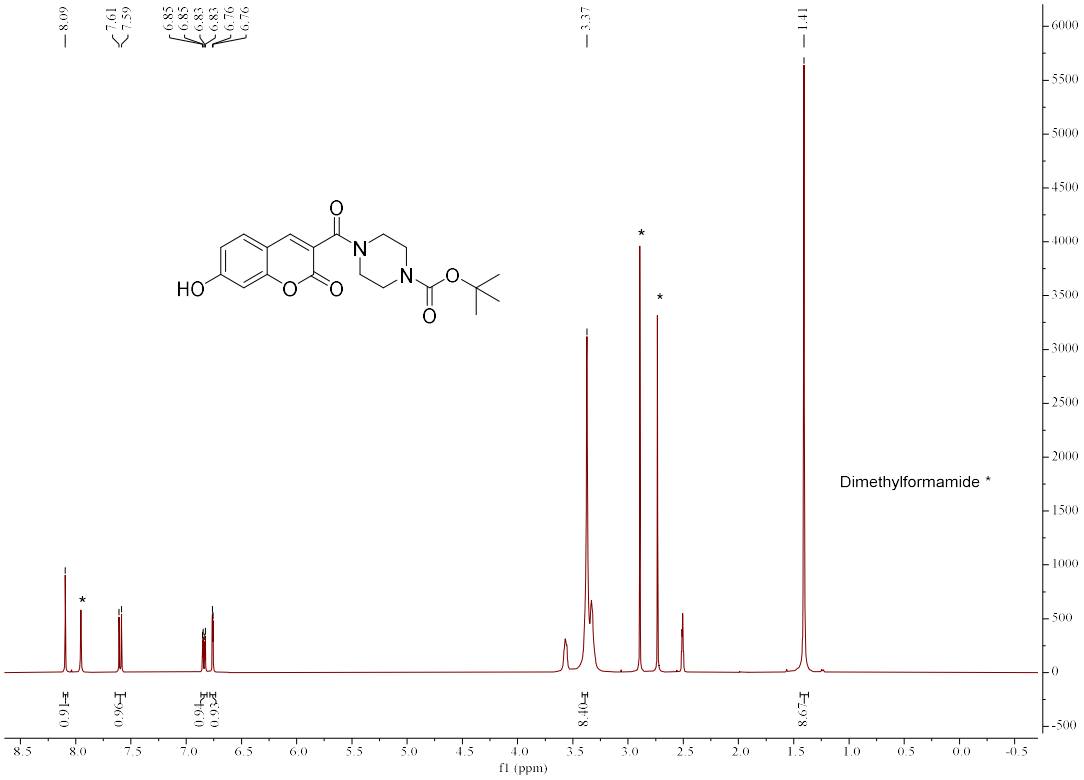


Figure **S15**. [1]H-NMR spectrum of **S3**.

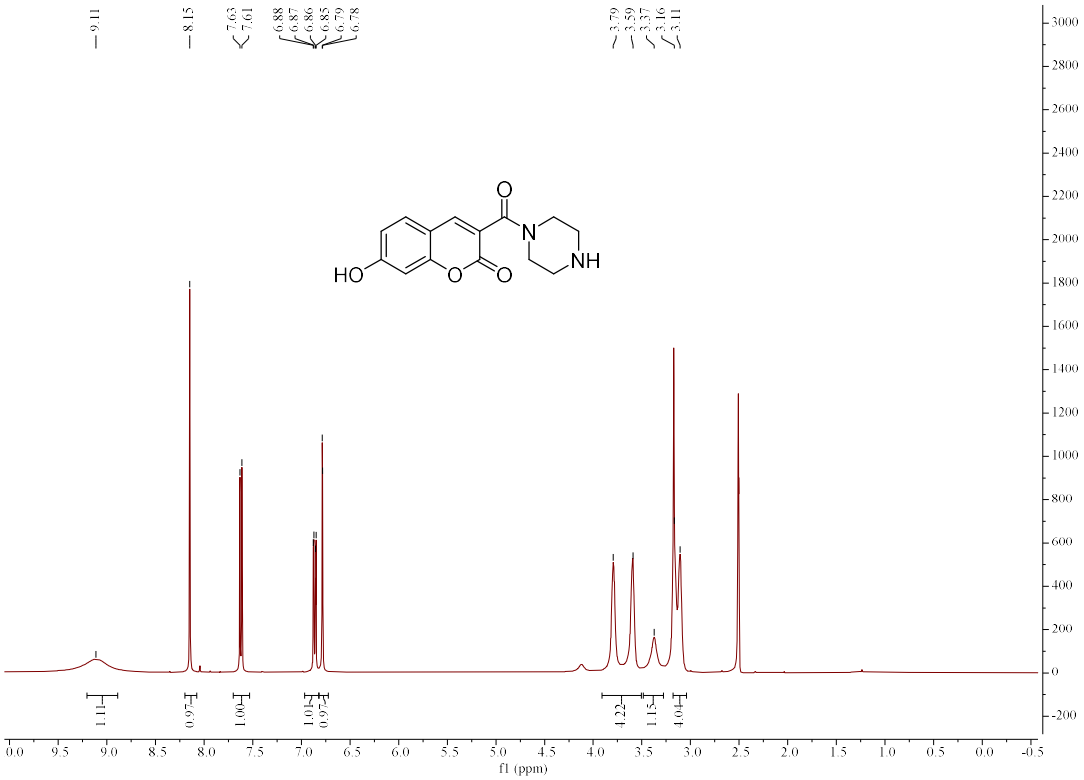


Figure **S16**. [1]H-NMR spectrum of **S4**.

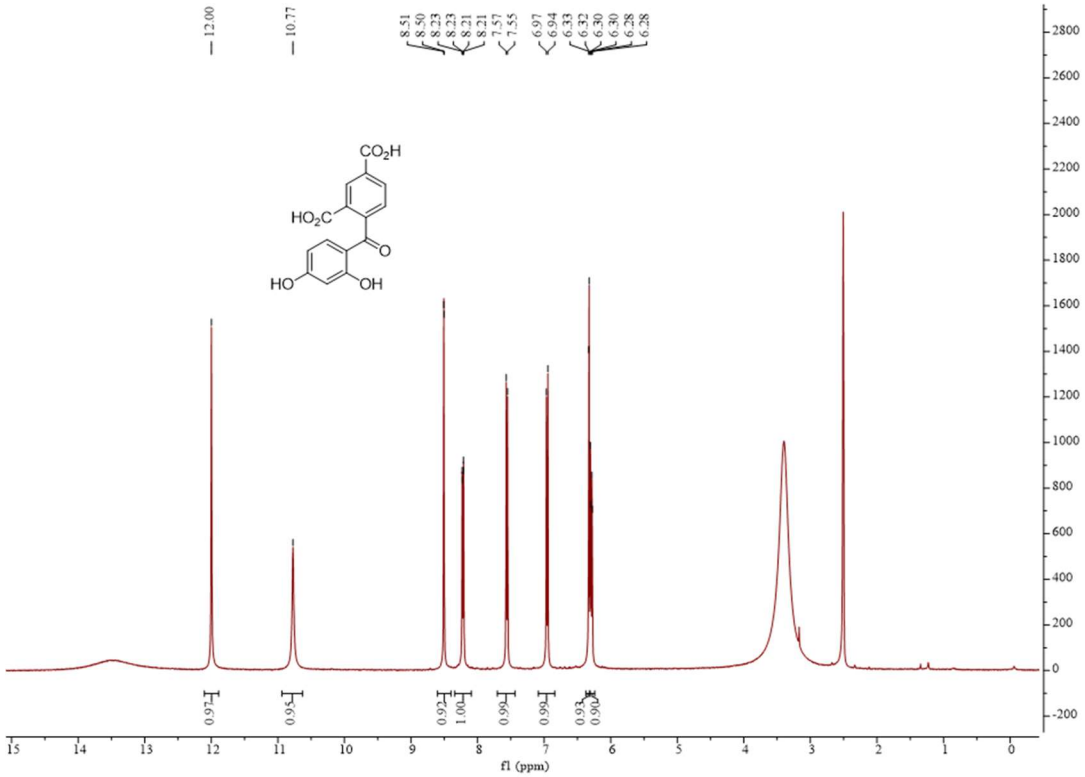


Figure **S17**. ^{1}H-NMR spectrum of **2**.

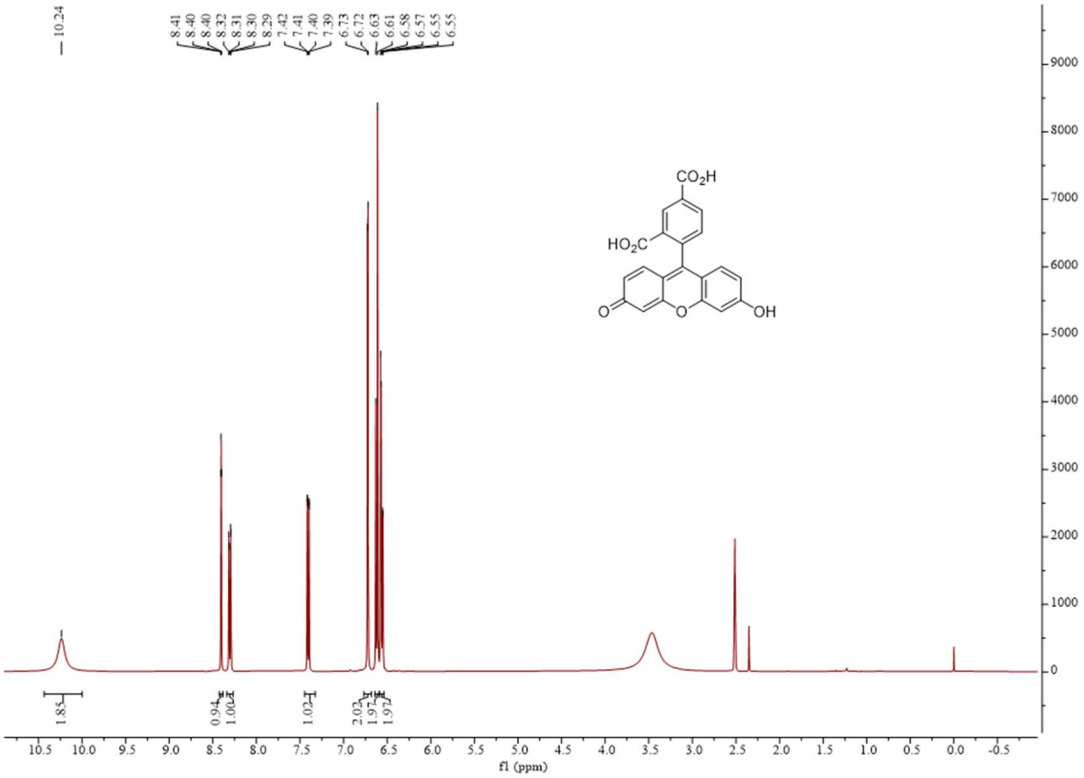


Figure **S18**. ^{1}H-NMR spectrum of **3**.

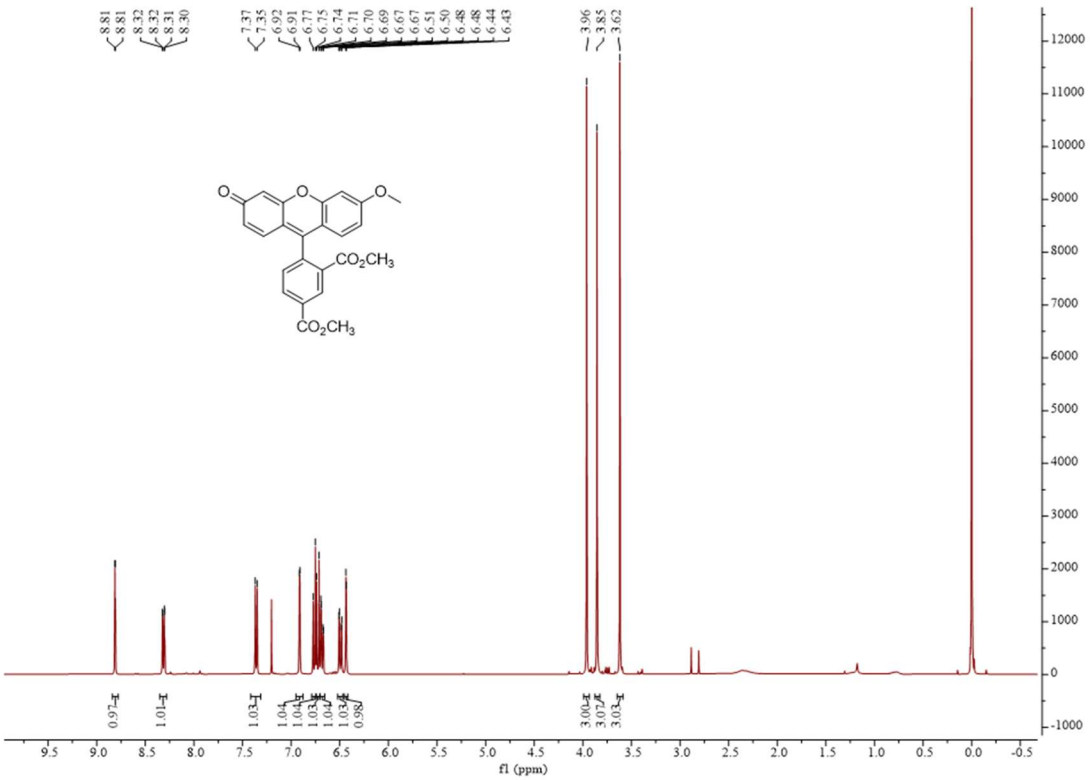


Figure **S19**. [1]H-NMR spectrum of **4**.

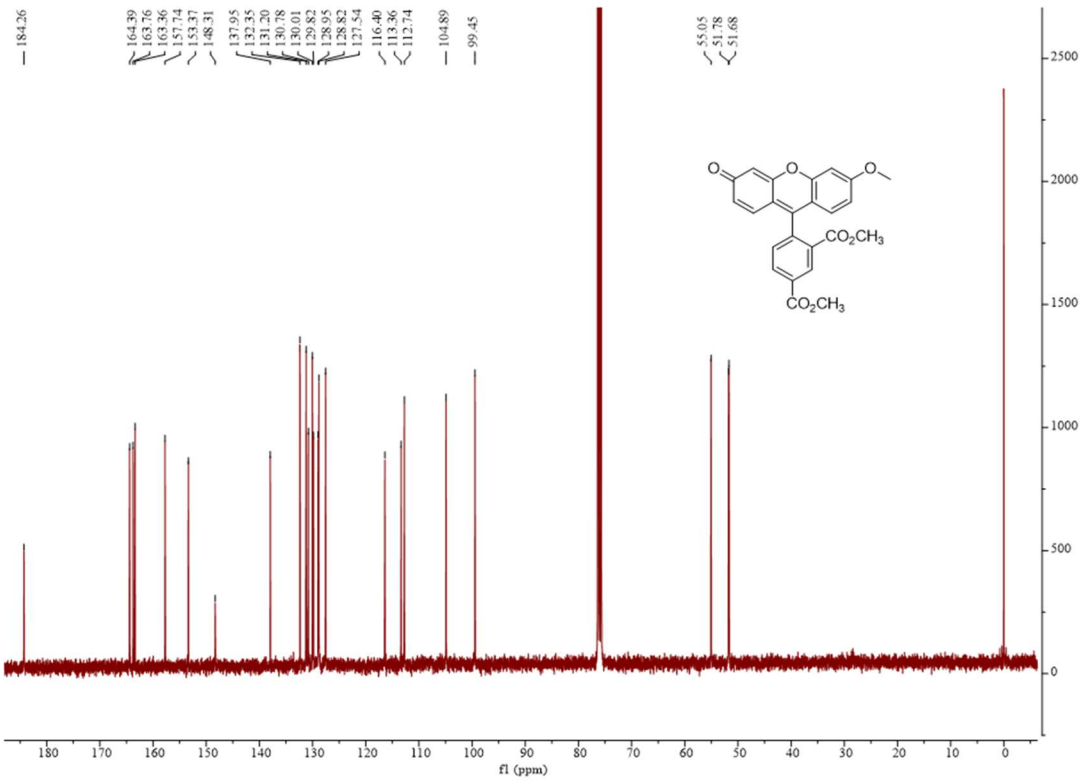


Figure **S20**. [13]C-NMR spectrum of **4**.

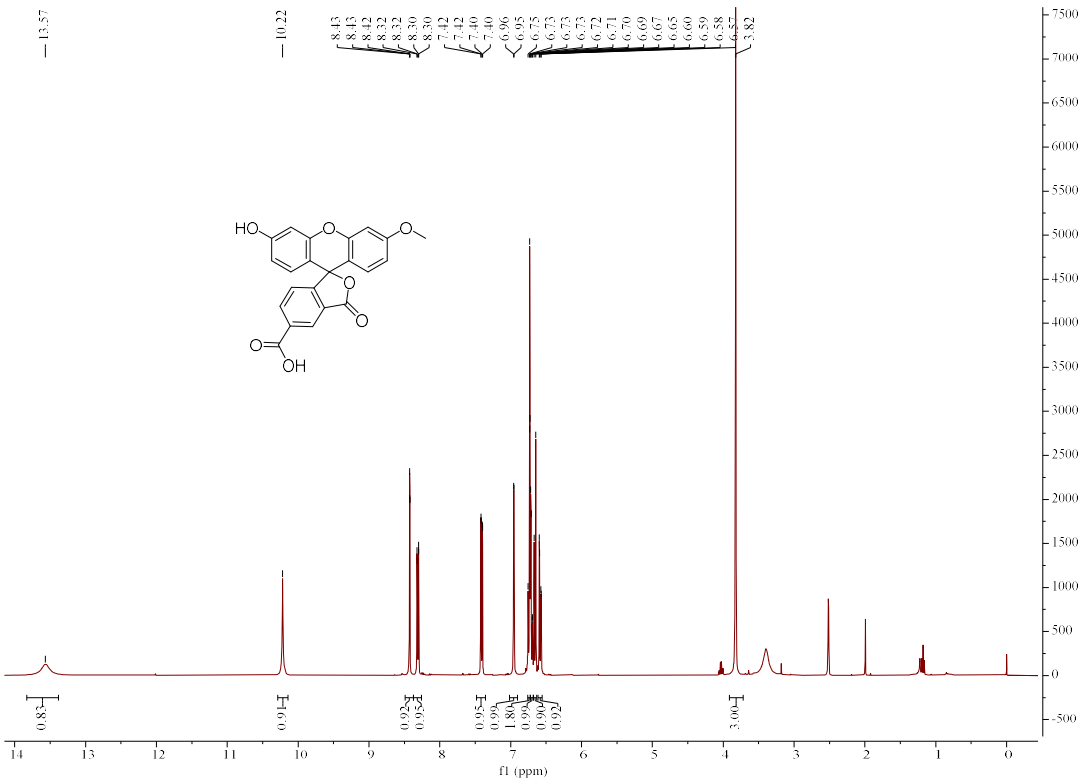


Figure **S21**. [1]H-NMR spectrum of **5**.

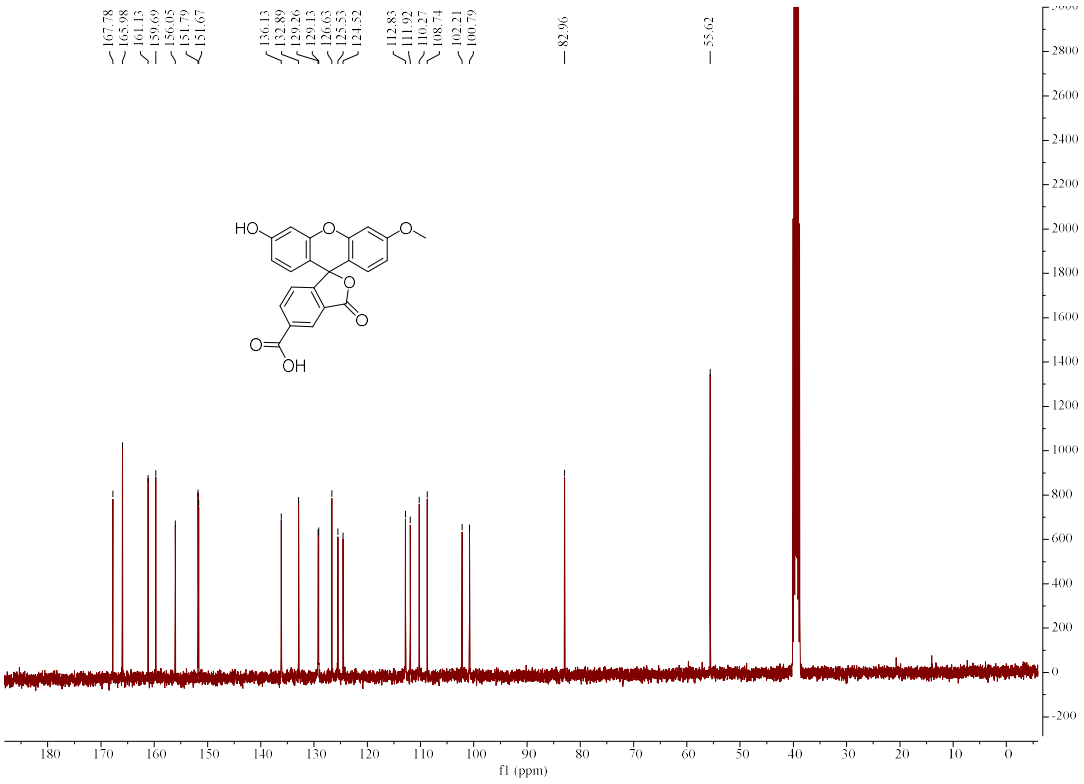


Figure **S12**. [13]C-NMR spectrum of **5**.

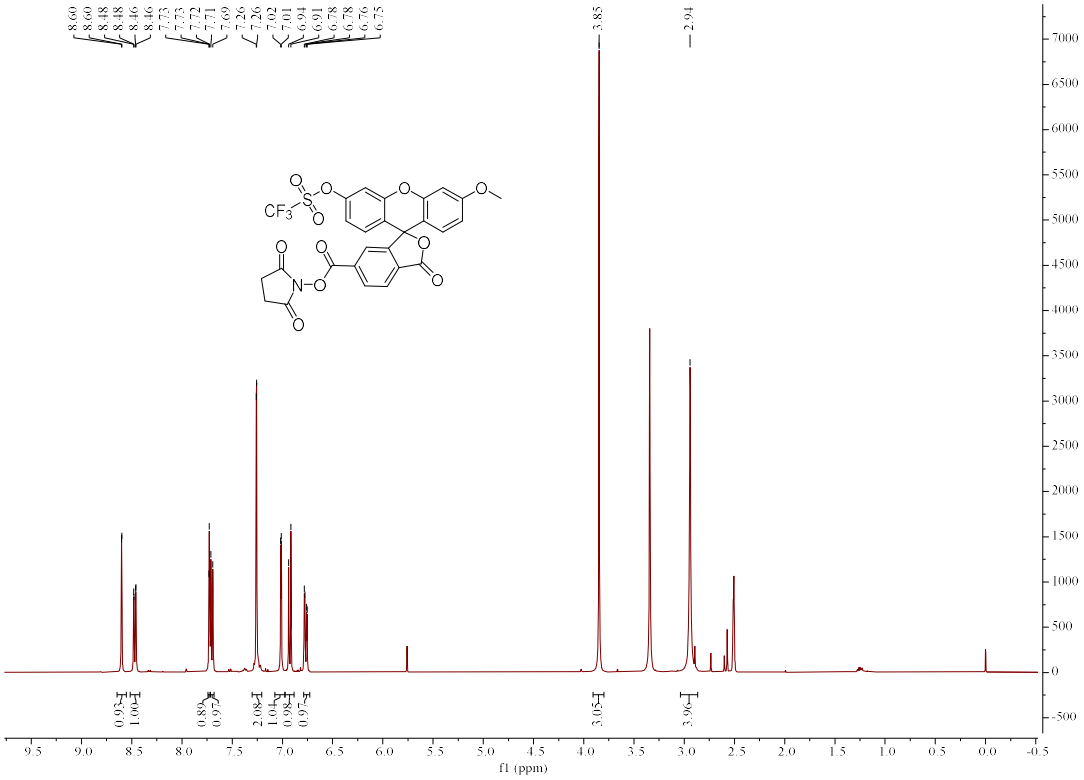


Figure **S23**. ^{1}H-NMR spectrum of **7**.

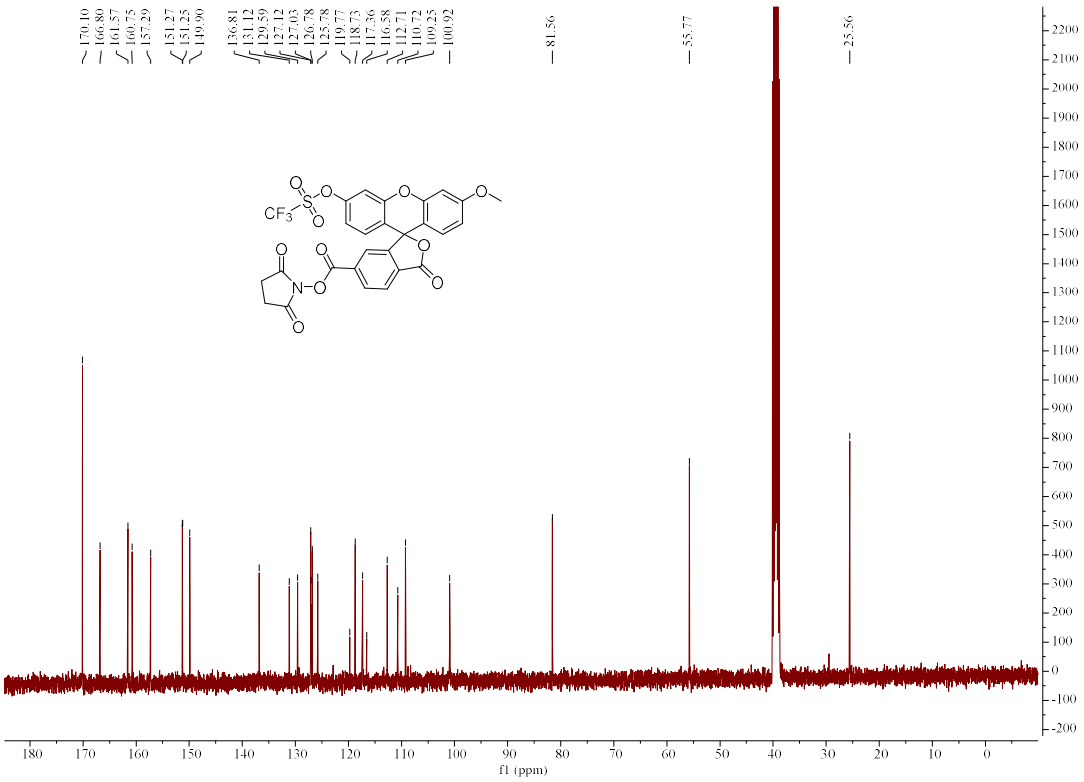


Figure **S24**. ^{13}C-NMR spectrum of **7**.

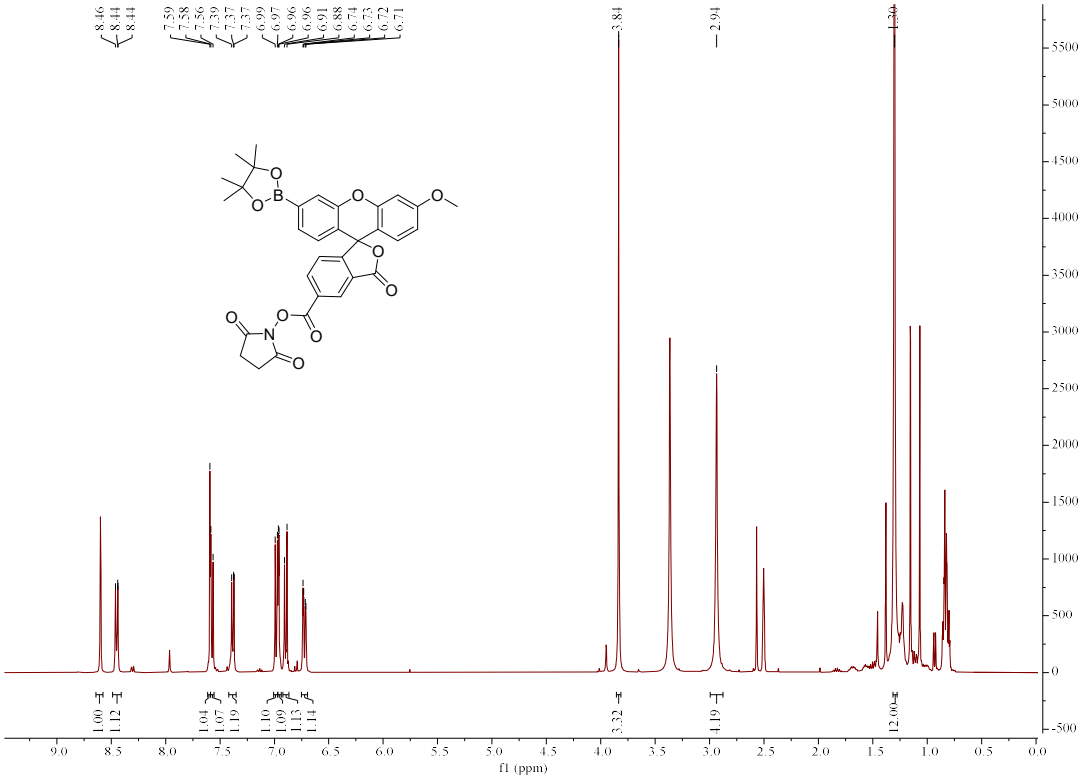


Figure **S25**. ^{1}H-NMR spectrum of **8**.

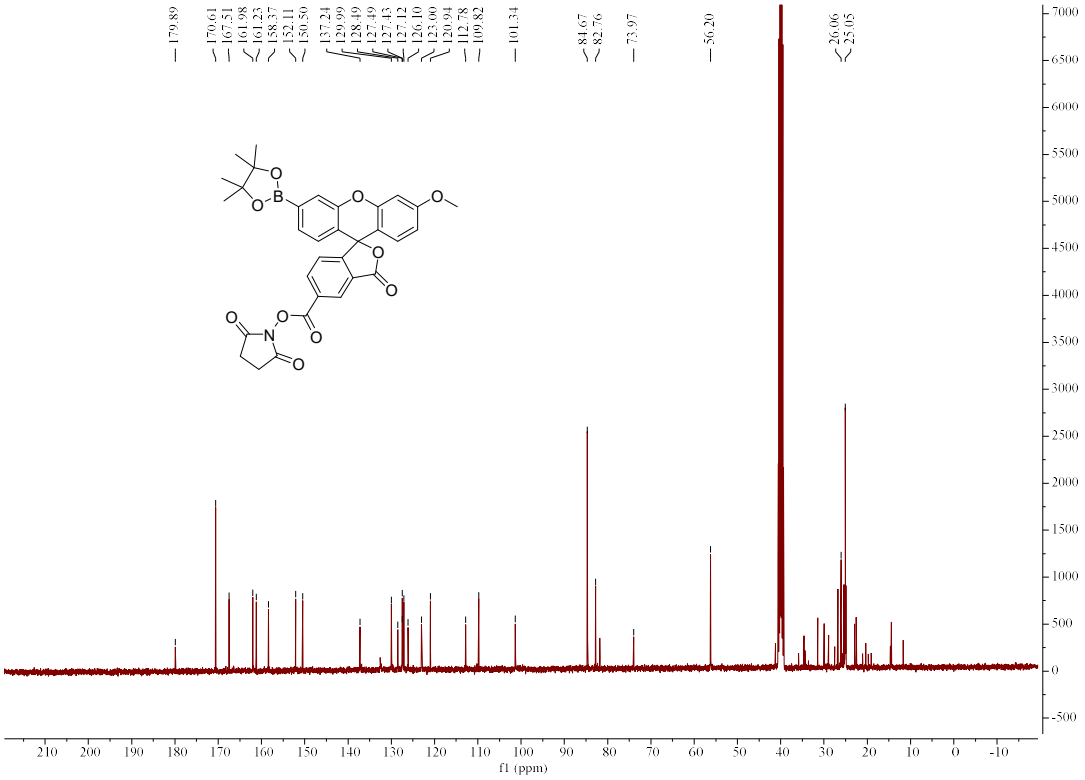


Figure **S26**. ^{13}C-NMR spectrum of **8**.

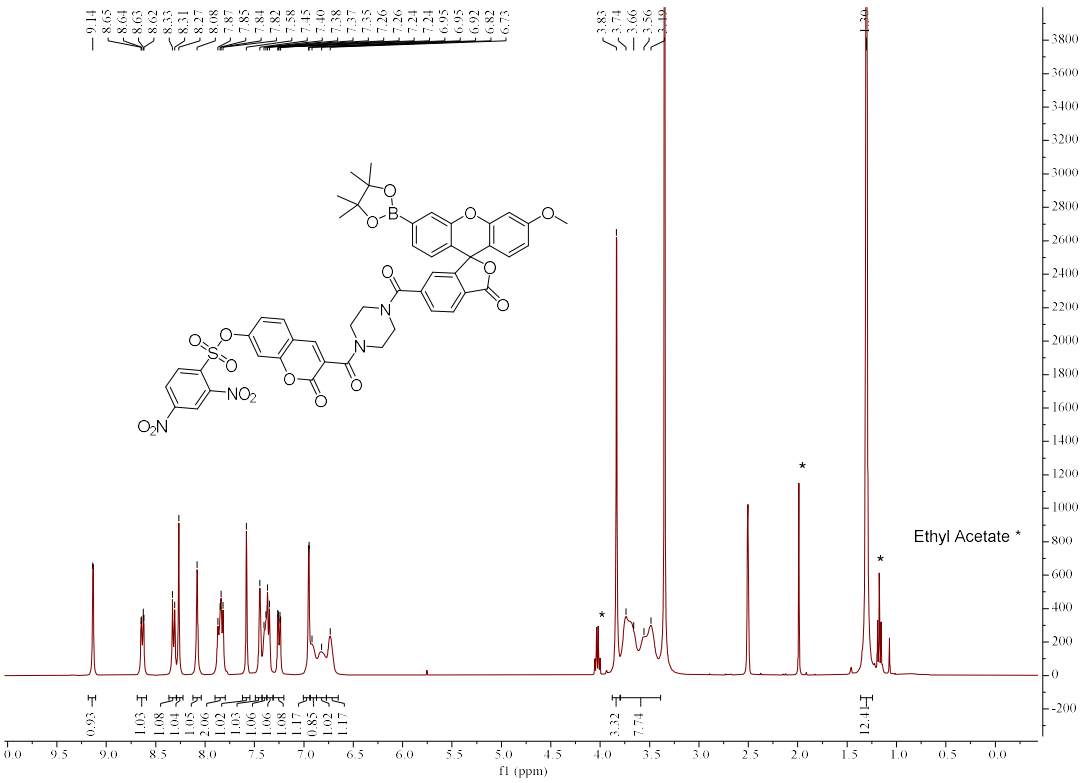


Figure **S27**. ^{1}H-NMR spectrum of **10**.

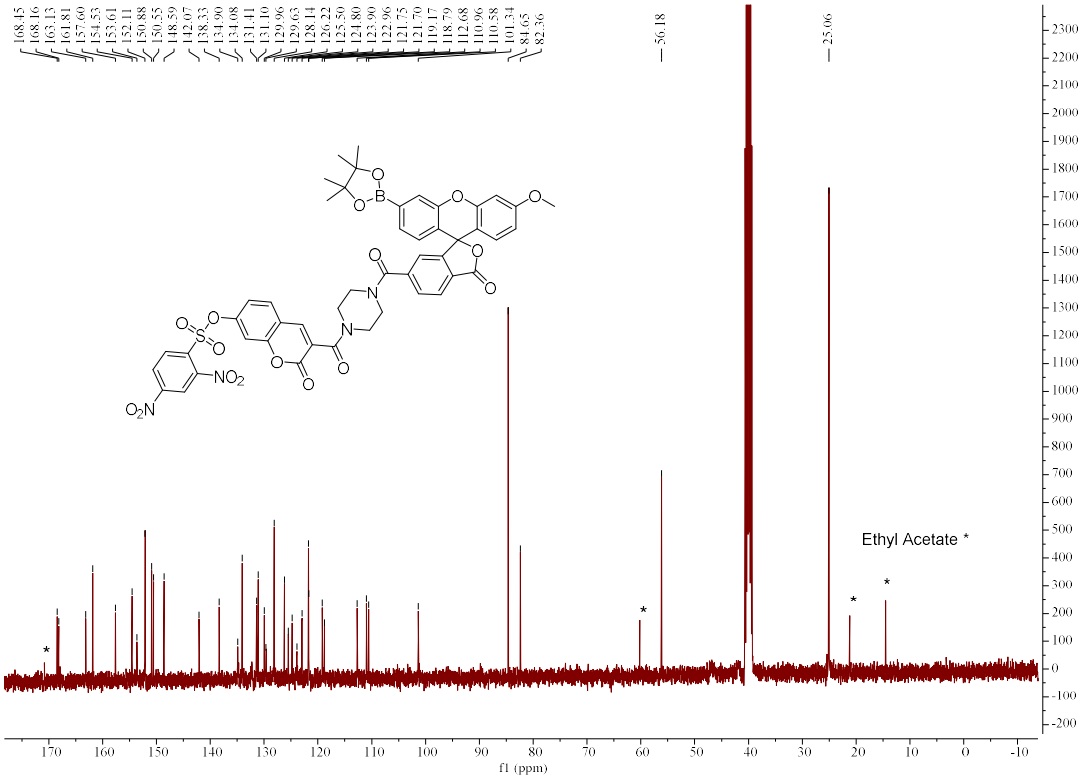


Figure **S28**. ^{13}C-NMR spectrum of **10**.

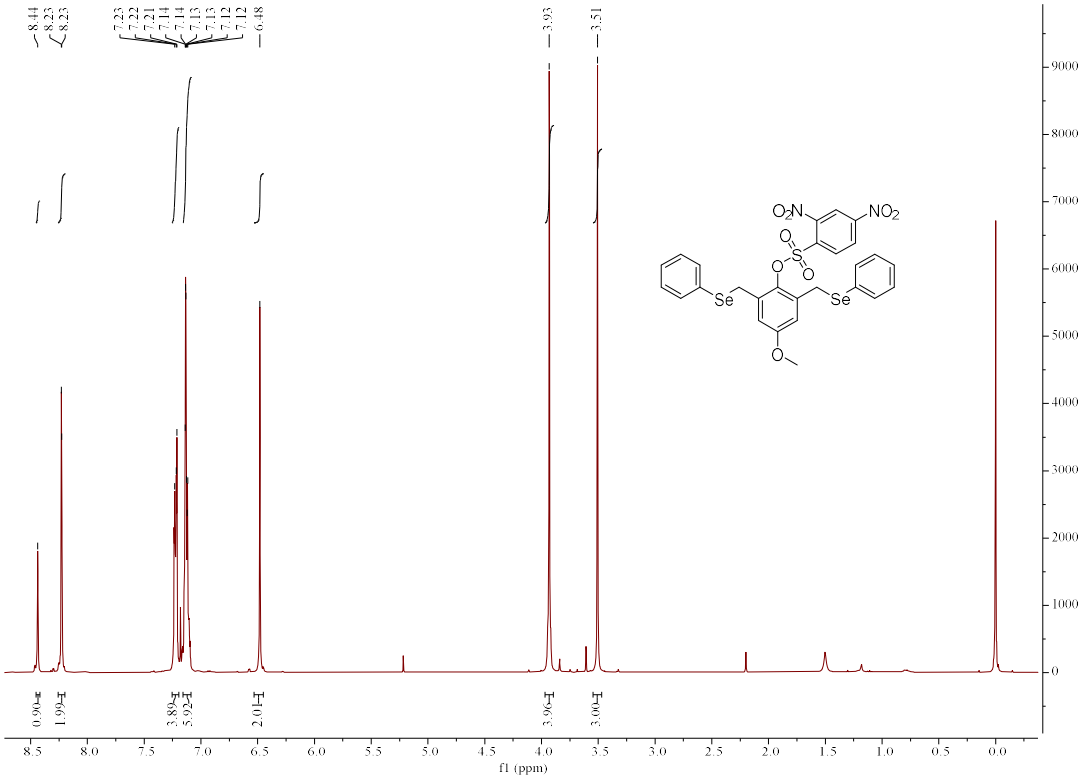


Figure **S29**. ^{1}H-NMR spectrum of **14**.

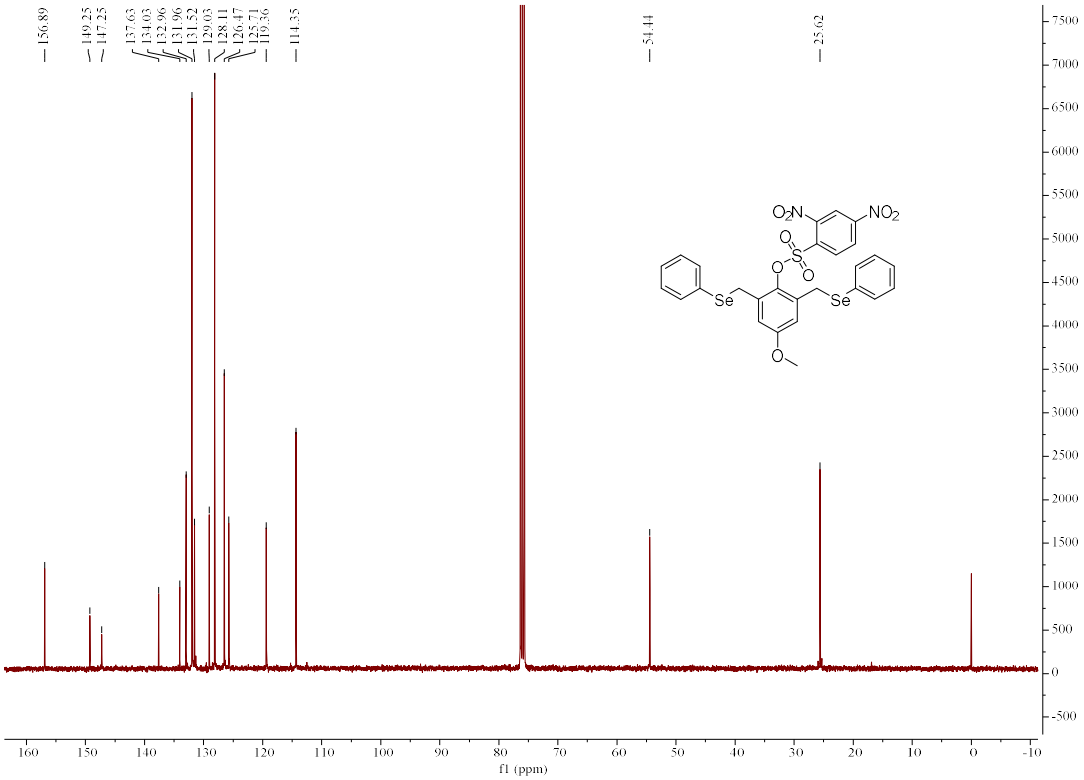


Figure **S30**. ^{13}C-NMR spectrum of **14**.

HRMS spectra of compounds

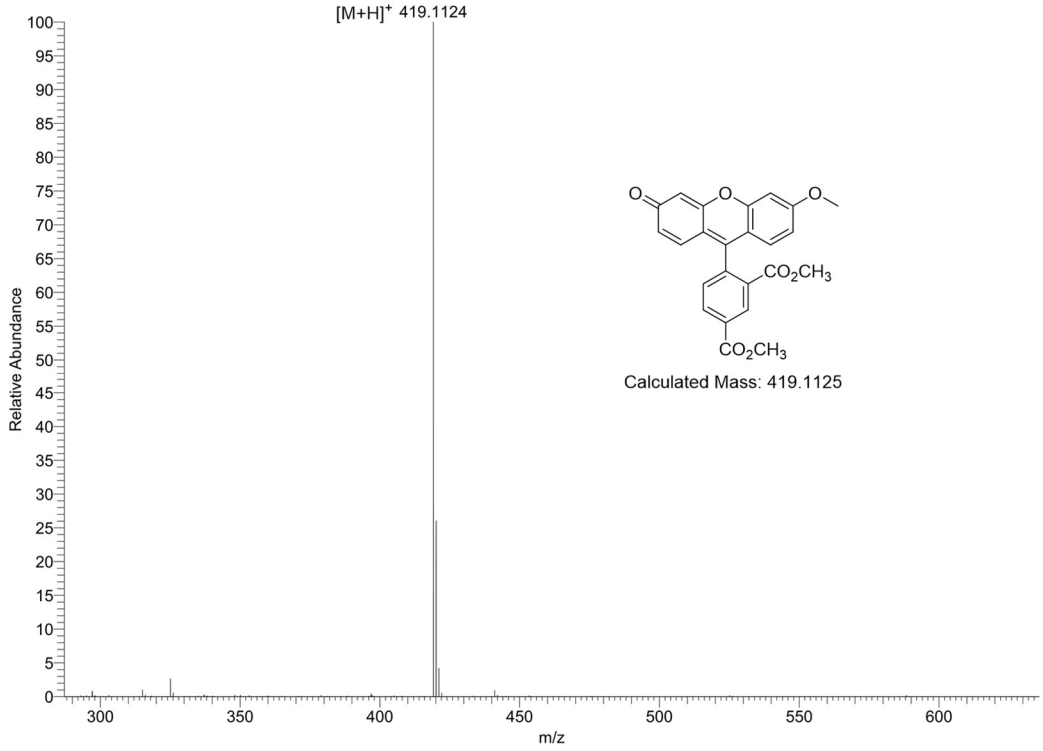


Figure **S31**. HRMS(ESI) spectrum of **4**.

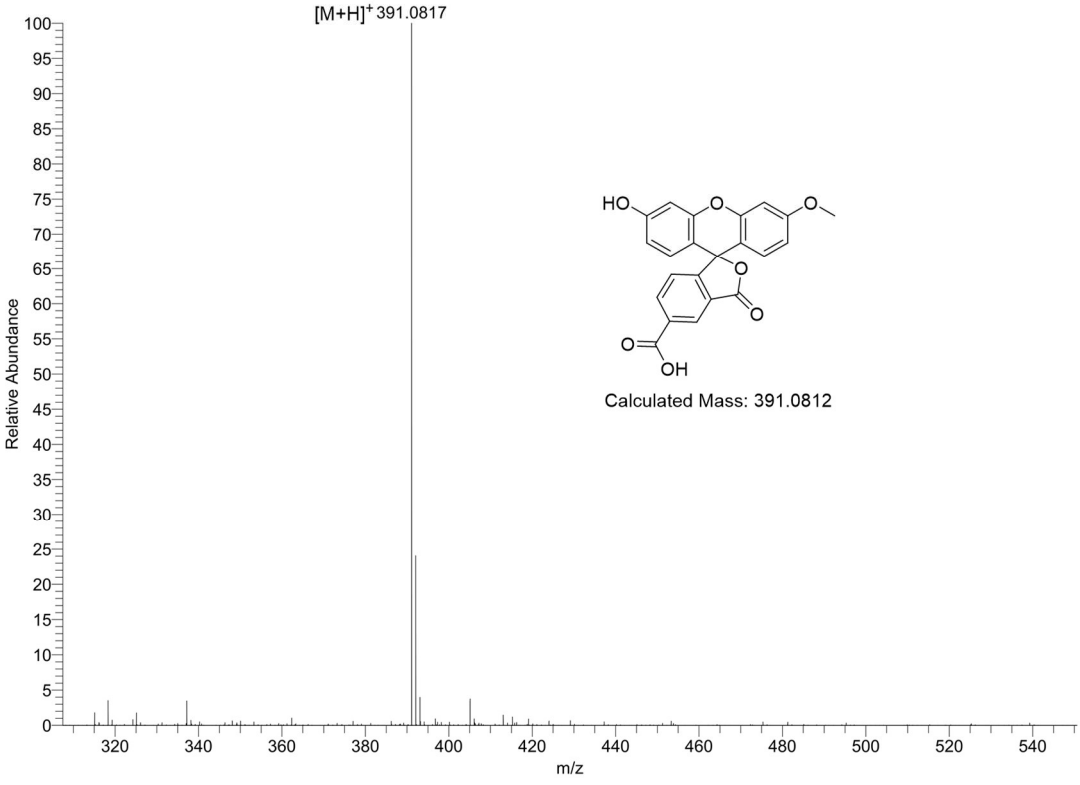


Figure **S32**. HRMS(ESI) spectrum of **5**.

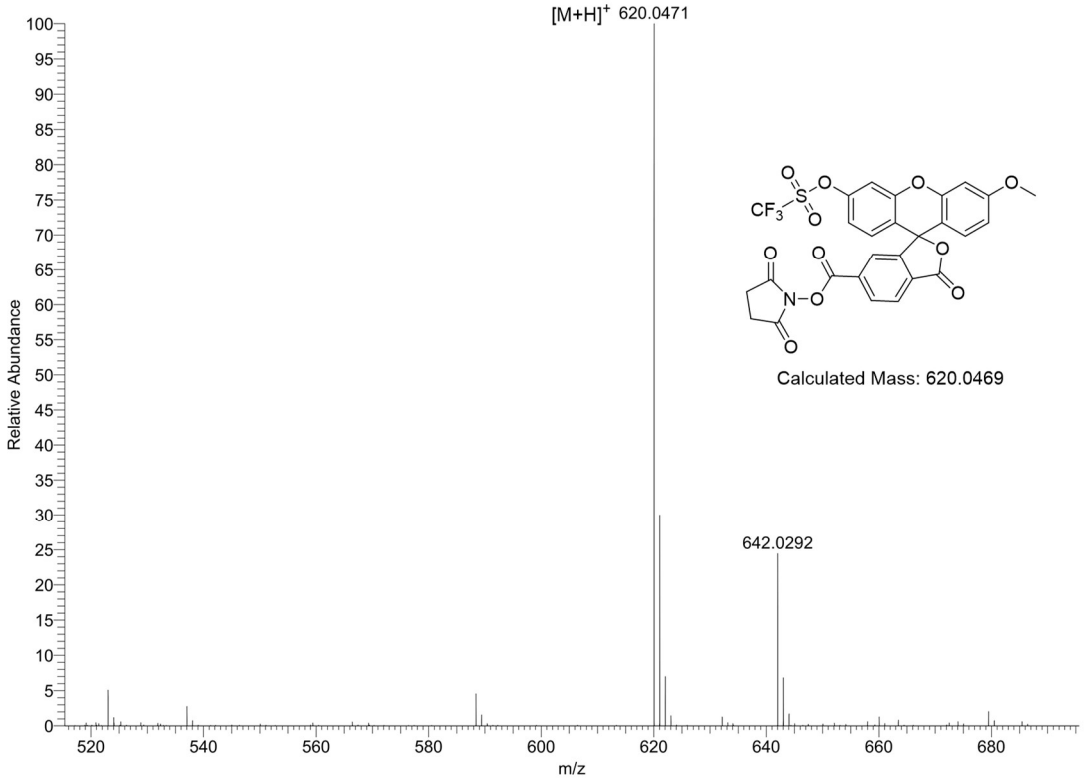


Figure **S33**. HRMS(ESI) spectrum of **7**.

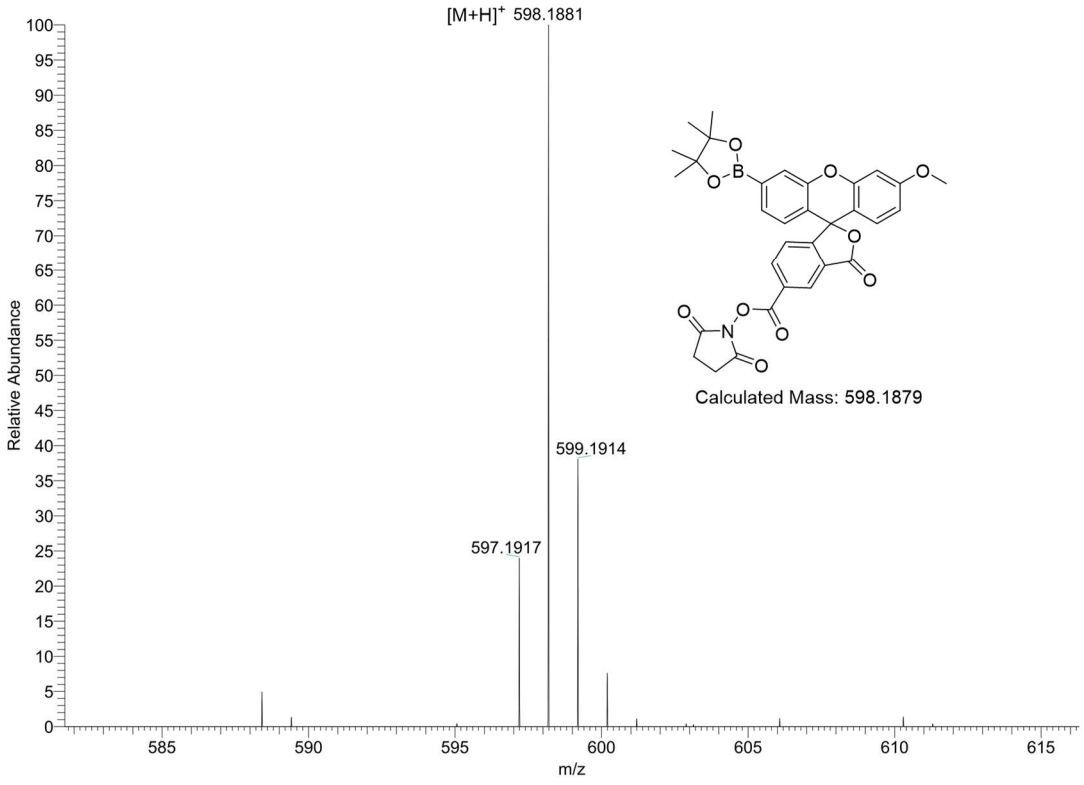


Figure **S34**. HRMS(ESI) spectrum of **8**.

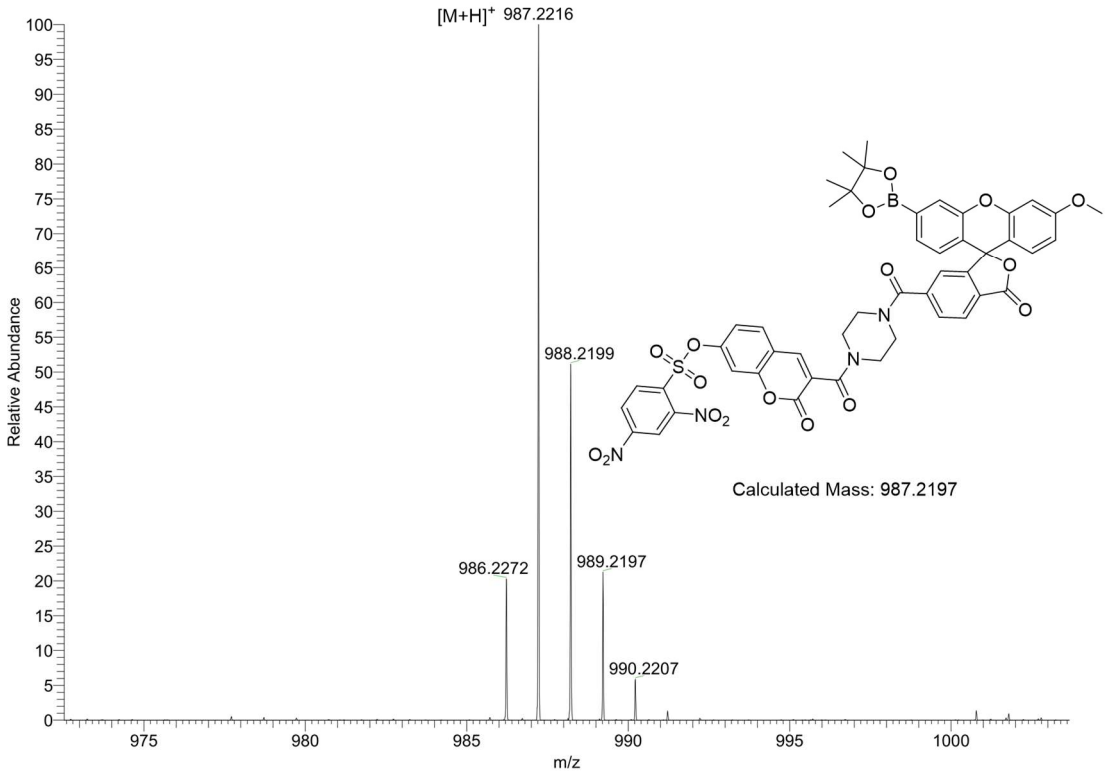


Figure **S35**. HRMS(ESI) spectrum of **10**.

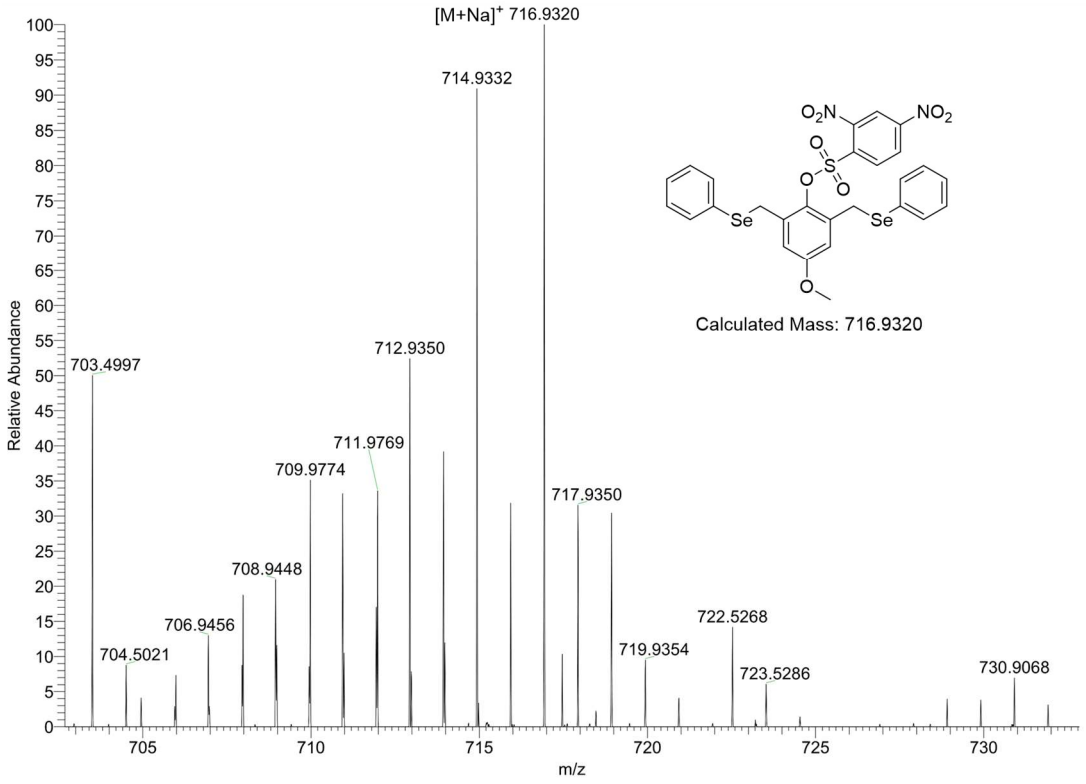


Figure **S36**. HRMS(ESI) spectrum of **14**.