Supplementary Information

Rapid GSH Detection and Versatile Peptide/Protein Labelling to Track Cell Penetrating using Coumarin-Based Probes

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Experimental Methods.

General Methods. Dimethylformamide (DMF) and dichloromethane (DCM) were distilled over CaH_2 prior to anhydrous reactions. All other commercially available reagents were directly used without further purification. The reactions were carried out under a positive pressure of argon atmosphere, and the reaction progress was monitored by TLC on Silica Gel G-25 UV254 (0.25 mm). Column flash chromatography was employed to purify desired compounds, and the ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). The NMR spectra of compounds were recorded on a Bruker Avance III 400 MHz spectrometer. The UV-Vis absorption spectra and fluorescence emission spectra were recorded on a JASCO V-770 spectrophotometer (scan speed: 1200 nm/min) and HITACHI F-7000 fluorescence spectrophotometer (scan speed: 1200 nm/min; EX slit: 2.5 nm; EM slit: 2.5 nm) respectively. Fluorescence quantum yields were determined using 4-methylumbelliferone (0.63, 0.01 M, phosphate buffer (pH 10)) and 7-Methoxycoumarin-4-acetic acid (0.18, 0.01 M, CH_3OH) as the standards. The PBS buffer used in the study contains 10 mM potassium phosphate and 100 mM NaCl. The stability of probes was characterized using UV-Vis absorption spectra upon incubation of probes (50 μM) at room temperature in PBS buffer (pH 7.2, 10% DMSO) for varied time. The effects of solution pH on fluorescence intensity enhancement were determined after the reaction between probes (50 μM) and GSH (50 μM) in PBS buffer (10% DMSO) with pH values at 6, 7.2, or 8 at room temperature for 2 min. The selectivity of probes towards analysts were investigated by determining fluorescence intensity enhancement after the reaction of probes (50 μM) with distinct analysts (50 μM), which was performed in PBS buffer (pH 7.2, 10% DMSO) at room temperature for 2 min. The fluoresce images of cells or zebrafish embryos were obtained on a Zeiss LSM 800 Confocal Laser Scanning Microcopy using an oil immersion objective (Plan Apochromat 63x/1.40 Oil DIC M27), and acquired using ZEN lite from Zeiss Microscopy. The intensity of fluorescence signal in cells or zebrafish embryos was analyzed using ImageJ 1.53e (Fiji).

Determining limit of detection (LOD). The limit of detection was obtained using fluorescence assay (triplicate). The fluorescence spectra of probes were recorded after incubation of probes (50 μM) with GSH (varied concentration) in PBS buffer (pH 7.2, 10% DMSO) at room temperature for 2 min. The fluorescence intensity standard deviation of blank sample was obtained by at least three measurements. The fluorescence intensity at the maximum emission wavelength was plotted over a concentration of GSH to afford the slop. The LODs of probes towards GSH were calculated using the equation: Limit of Detection = 3σ/K. The σ is the fluorescence intensity standard deviation of blank measurement, and K is the slop between the fluorescence intensity of reaction mixture versus the concentration of GSH.

Cell culture. A549 lung cancer cells were purchased from Procell Life Science & Technology Co., Ltd.. A549 lung cancer cells were grown in Roswell Park Memorial Institute medium (RPMI-1640). RPMI-1640 medium was supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate. All cells were grown as adherent monolayers in a humidified atmosphere of 5% CO_2-95% air at 37 ℃.

Detection of GSH in live A549 cells. A549 lung cancer cells were seeded at confocal dishes (size 35 mm) and cultured at 37 ℃ until 80% confluent. The cells were pre-incubated with or without N-Ethylmaleimide (NEM, desired concentration) at 37 ℃ for 1 h. After washing with PBS buffer (pH 7.2, 10 mM potassium phosphate and 100 mM NaCl) for three times, the cells were incubated with probe **4** (10 μM, 0.1% DMSO) at 37 ℃ for 2 min. (Incubation with prolonged time resulted in negligible fluorescence signal increase.) The medium was replaced with new PBS buffer (pH 7.2) for three times, and fluorescence images were captured using a Zeiss LSM 800 Confocal Laser Scanning Microcopy (Excitation: 405 nm; Emission: 400-600 nm.).

Detection of cellular protein labelling during GSH detection. A549 lung cancer cells were incubated with or without probe **4** (10 μM, 0.1% DMSO) at 37 ℃ for 2 min. The medium was replaced with new PBS buffer (pH 7.2) for three times. The proteins in the cells were analyzed using SDS-PAGE gel (5% and 12%). Visualize the

proteins by fluorescence (FL) imaging on a spectro line UV-viewing cabinet with the excitation at 365 nm. The Coomassie brilliant blue (CBB) staining was used to detect all proteins in polyacrylamide gels (PAGE). The fluorescence and CBB stained images were captured using a smartphone camera.

Detection of GSH in Zebrafish embryos. Zebrafish embryos aged within 48 h were incubated with probe **4** (10 μM, 0.1% DMSO) at 28.5 °C for varied time. Zebrafish embryos was washed with PBS buffer (pH 7.2) for three times prior to fluorescence imaging captured by a Zeiss LSM 800 Confocal Laser Scanning Microcopy (Excitation: 405 nm; Emission: 400-600 nm.).

Bioconjugation of Cys-TAT with 4. The cell penetrating peptide (CPP) Cys-TAT (47-57) was dissolved in a mixture of water-acetonitrile (4:1, v/v) in a quartz fluorescence cell. To the solution, the probe **4** was added, and the final concentration of Cys-TAT (47-57) and **4** is both 100 μM. The reaction mixture was placed at room temperature. The reaction progress was detected in a U.V. Cabinet with long-wave light (365 nm). The reaction solution with the reaction time for 2 min was collected and evaporated to dryness under vacuum. The residue dissolving in 10% MeOH was analyzed by ESI-MS on an Orbitrap LC-MS system (Q Exactive Focus high resolution mass spectrometer, ESI).

Monitoring locations of labelled TAT 6 in A549 cells. TAT conjugate product **6** (final concentration in cell medium: 10 μM, 0.1% DMSO) was added to the A549 lung cancer cells seeded at confocal dishes (size 35 mm) with about 80% confluent. Cells were incubated at 37 ℃ for 30 min, washed with PBS (1 mL × 3), fixed by 4% paraformaldehyde (500 μL) and permeabilized in 0.1% Triton X-100 for 15 min. After washing with PBS (1 mL × 3), cells were stained using ethidium bromide (EB) (10 μL, 100 μg/μL). The fluorescence images were captured on a Zeiss LSM 800 Confocal Laser Scanning Microcopy (EB channel: laser wavelength: 488 nm, detection wavelength: 450~700; Coumarin channel: laser wavelength: 405 nm, detection wavelength: 400~600 nm).

Fluorescent labeling of Cys-bearing proteins (H3 and BSA) using probe 4. A mixture of H3 or BSA (100 μM) and probe **4** (100 μM) in the PBS buffer (0.1 mM, pH 7.2, 10% acetonitrile) was incubated at room temperature for 2 min. The fluorescent labeling progress could be monitored in in a U.V. Cabinet with long-wave light (365 nm). The resulted solution was transferred to Amicon® Ultra-0.5 centrifugal filter (3 K), rinsed with a mixture of water-acetonitrile (9:1, v/v) and centrifuged (14,000 × g, 5 min) for 5 times. The residue was redissolved into a mixture of water-acetonitrile (9:1, v/v, the concentration of labeled protein is about 100 μM). The solution can be directly used for further ESI-TOF-MS and SDS-PAGE analysis.

Fluorescent labeling of Cys-free proteins (H2B) using Traut's agent and probe 4. A mixture of H2B (100 μM) and Traut's reagent (5 eq., 500 μM) in the PBS buffer (100 nM, pH 6.8) containing 2 mM EDTA was incubated at 37 ℃ for 1 h. The reaction solution was transferred to Amicon® Ultra-0.5 centrifugal filter (3 K), and centrifuged (14,000 × g, 5 min) to remove excessive Traut's reagent. After rinsing with a mixture of water-acetonitrile (9:1, v/v) and centrifuge (14,000 × g, 5 min) for 5 times, the residue was redissolved into PBS buffer (0.1 mM, pH 7.2, 10% acetonitrile). The solution was incubated with probe **4** (100 μM) at room temperature for 2 min. The resulted solution was directly used for SDS-PAGE analysis.

Cellular uptake of fluorescent labelled Cys-TAT/H3 in A549 cells. To a solution of Cys-TAT (47-57)/H3 protein in a mixture of water-DMSO (9:1, v/v), the probe **4** was added, and the final concentration of Cys-TAT (47-57) and **4**/H3 are both 1 mM. The mixture was incubated at room temperature for 2 min to afford fluorescent **4**-Cys TAT/H3 conjugate. The reaction solution was transferred to Amicon® Ultra-0.5 centrifugal filter (3 K for Cys TAT, and 10 K for H3), and centrifuged (14,000 × g, 5 min) to remove possibly unreacted probe. The residue was rinsed with a mixture of water-CH_3CN (9:1, v/v) and centrifuged (14,000 × g, 5 min) for 5 times. The residue was redissolved into water with a concentration of 1 mM. The solution of fluorescent **4**-Cys TAT conjugate product (final concentration in cell medium: 10 μM) was added

to the A549 lung cancer cells seeded at confocal dishes (size 35 mm) with about 80% confluent. They were incubated at 37 ℃ for varied time, and washed with PBS buffer for three times. The cells were fixed by 4% paraformaldehyde (500 μL) for 30 min prior to fluorescence imaging on a Zeiss LSM 800 Confocal Laser Scanning Microcopy (Excitation: 405 nm; Emission: 400-600 nm.).

The fluorescent H3 conjugate product was diluted with transfer buffer (pH 7.3, 20 mM Hepes, 110 mM potassium acetate, 5 mM sodium acetate, 0.5 mM EGTA) and protein inhibitor (2 mM DTT, 10 mM AEBSF, 8 μM aprotinin, 0.5 mM bestatin, 0.2 mM leupeptin, 0.15 mM pepstatin A, 0.1 mM PMSF) (final concentration in cell medium: 10 μM). After washing with transfer buffer (sterilized, 500 μL × 3), the solution of fluorescent H3 conjugate product was added to the A549 lung cancer cells seeded at confocal dishes (size 35 mm) with about 80% confluent. They were incubated at 37 ℃ for varied time, washed with transfer buffer for three times and fixed in 4% paraformaldehyde (in transfer buffer) prior to fluorescence imaging on a Zeiss LSM 800 Confocal Laser Scanning Microcopy (Excitation: 353 nm; Emission: 400-600 nm.). To study the effect of lysine addition on H3-binding, the lysine (varied concentrations) and labelled H3 histone were added and incubated for 30 min prior to further treatments following similar protocol as above.

SDS-PAGE analysis to check fluorescent labeling of proteins. The protein solution (about 100 μM) was mixed with 2X SDS-PAGE loading buffer (pH 6.8, 100mM Tris-HCl, 4％ SDS, 12％ glycerol, 2％ β-ME, 0.008％ bromophenol blue) at a ratio of 1:1 (v/v). The mixture was heated at 95 °C for 10 min in a heat block to denature the protein. After cooling to the room temperature, the mixture was centrifuged (14,000 × g, 5 min), and the supernatant was loaded on SDS-PAGE gel. Run the SDS-PAGE gel at 80 V until the bromophenol blue dye reaches the bottom of the gel. Visualize the labeled proteins by fluorescence (FL) imaging on a spectro line UV-viewing cabinet with the excitation at 365 nm. The Coomassie brilliant blue (CBB) staining was used to detect all proteins in polyacrylamide gels (PAGE). The fluorescence and CBB stained images were captured using a smartphone camera.

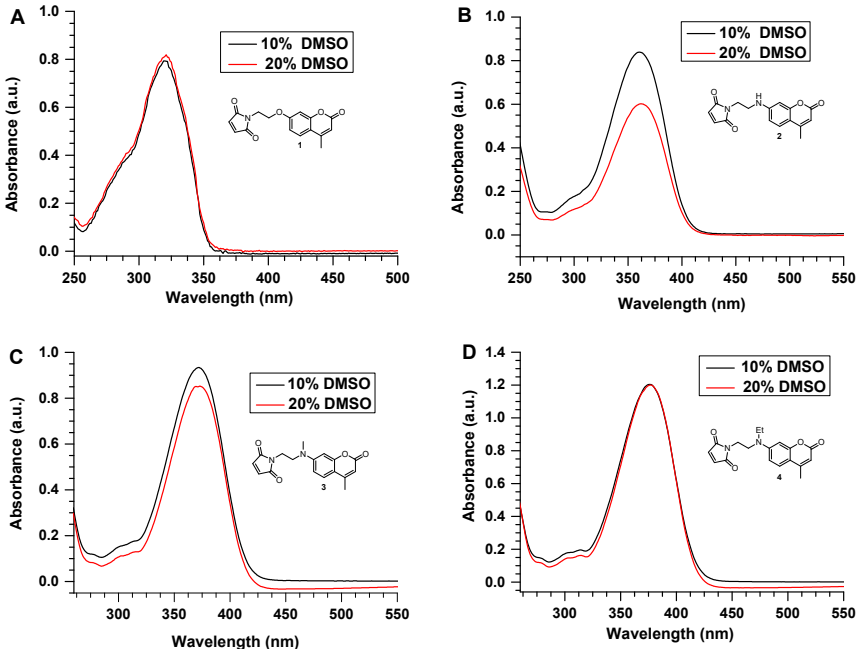


Figure S1. Determine the solubility of probes **1-4** (50 μM) in PBS buffer (pH 7.2, 10% DMSO vs 20% DMSO) by UV-Vis absorption spectra.

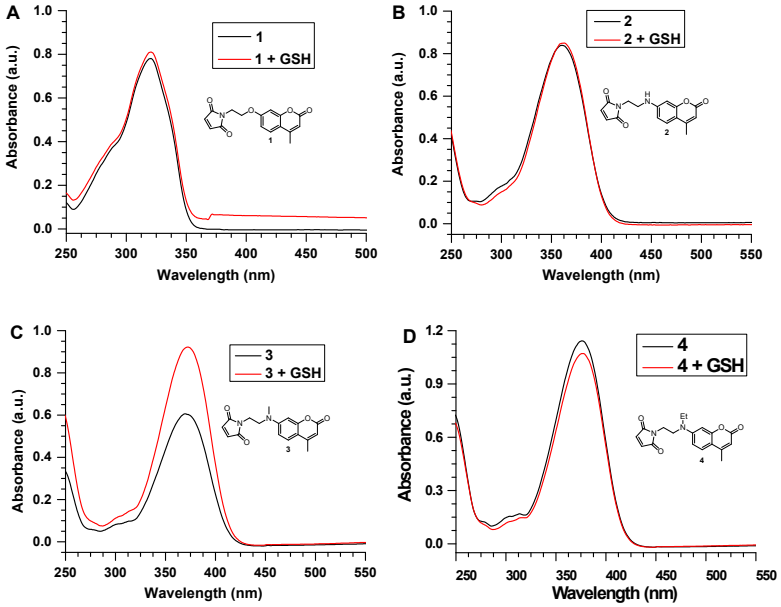


Figure S2. UV-Vis absorption spectra of probes **1-4** (50 μM) before and after reacting with GSH (50 μM, 2 min) in PBS buffer (pH 7.2, 10% DMSO).

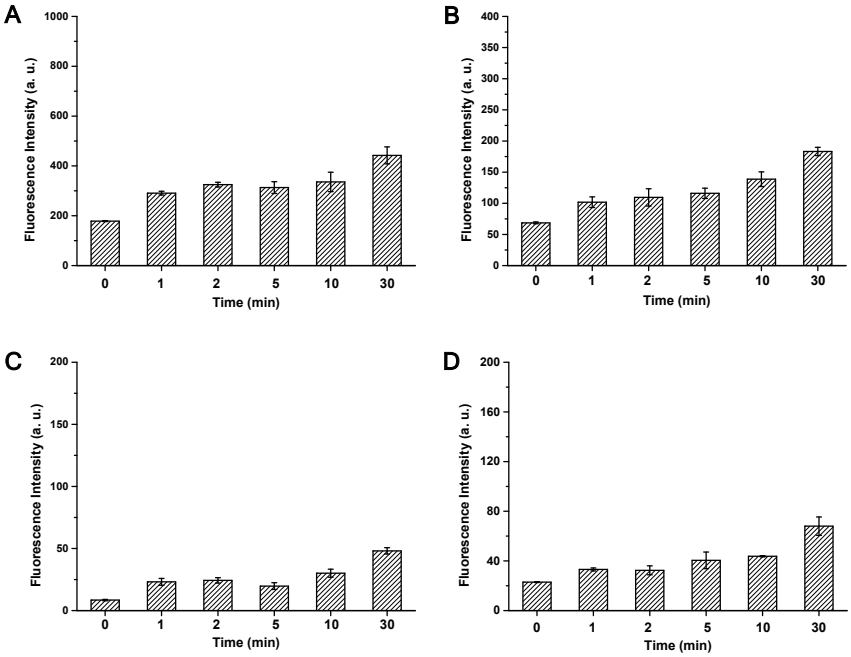


Figure S3. The stability of probes **1**-**4** in PBS buffer (pH 7.2, 10% DMSO). Fluorescence intensities of probes (50 μM) at the maximum emission wavelength were recorded at varied time. The experiments were performed in triplicate, and data were expressed as the mean ± standard deviation. (Excitation: 320 nm for **1**; 360 nm for **2**; 380 nm for **3** and **4**; Emission: 380 nm for **1**; 450 nm for **2**; 460 nm for **3** and **4**; slit width: 2.5 nm.)

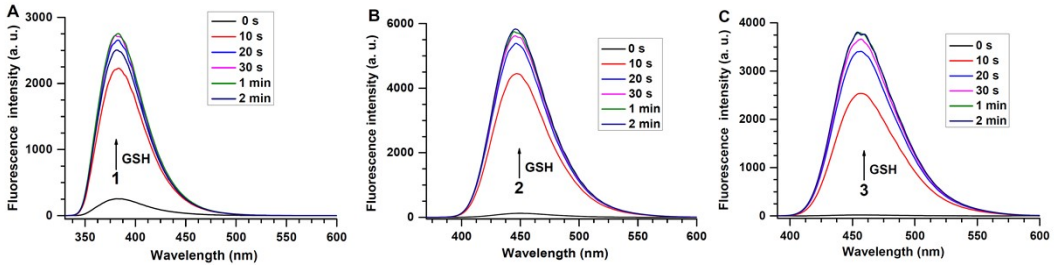


Figure S4. Fluorescence response of probes (**1**-**3**, 50 μM) towards GSH (50 μM) for varied reaction time. Fluorescence intensities of probes (50 μM) at the maximum emission wavelength were recorded upon reaction with GSH (50 μM) for desired time in PBS buffer (pH 7.2, 10% DMSO). (Excitation: 320 nm for **1**; 360 nm for **2**; 380 nm for **3** and **4**; Emission: 380 nm for **1**; 450 nm for **2**; 460 nm for **3**; slit width: 2.5 nm.)

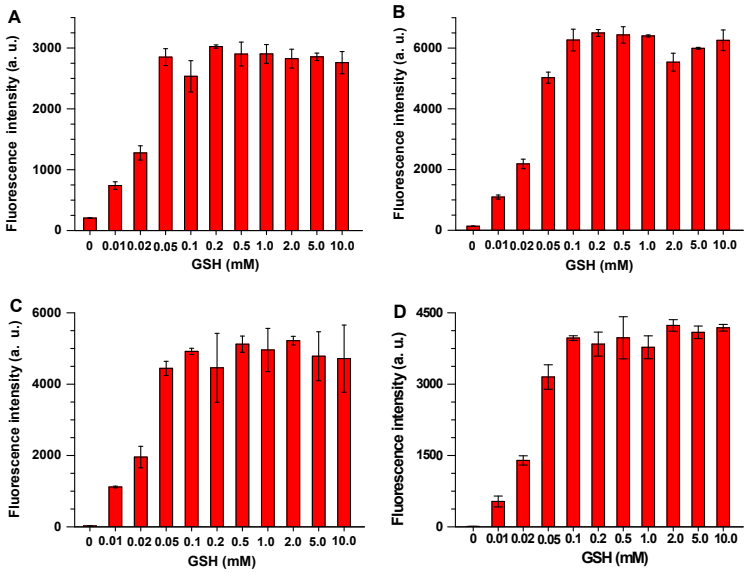


Figure S5. Fluorescence response of probes (**1**-**4**, 50 μM) towards GSH (varied concentration). Fluorescence intensities of probes (50 μM) at the maximum emission wavelength were recorded upon reaction with GSH (0-10 mM) for 2 min in PBS buffer (pH 7.2, 10% DMSO). The experiments were performed in triplicate, and data were expressed as the mean ± standard deviation. (Excitation: 320 nm for **1**; 360 nm for **2**; 380 nm for **3** and **4**; Emission: 380 nm for **1**; 450 nm for **2**; 460 nm for **3** and **4**; slit width: 2.5 nm.)

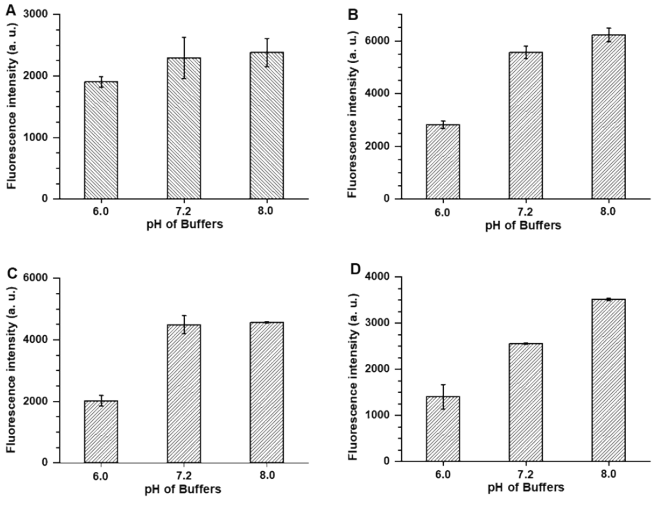


Figure S6. Fluorescence response of probes (**1**-**4**, 50 μM) towards GSH (50 μM) in PBS buffers with different pH values. The experiments were performed at room temperature for 2 min in triplicate, and data were expressed as the mean ± standard deviation. (Excitation: 320 nm for **1**; 360 nm for **2**; 380 nm for **3** and **4**; Emission: 380 nm for **1**; 450 nm for **2**; 460 nm for **3** and **4**; slit width: 2.5 nm.)

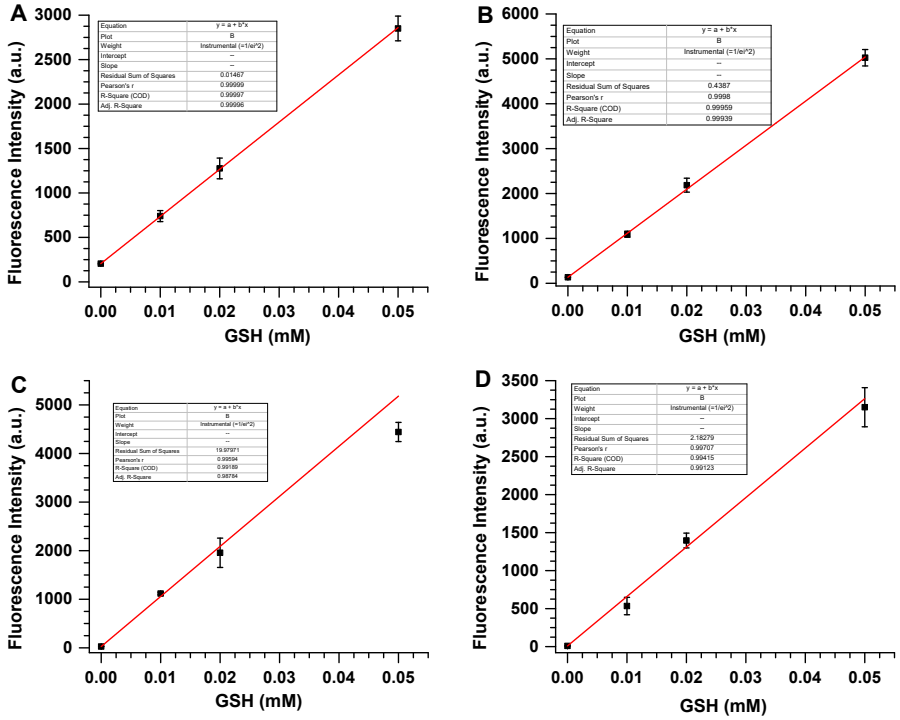


Figure S7. Determine the limit of detection of probes towards GSH using fluorescence assay. The fluorescence intensities of probes (**1**-**4**, 50 μM) at the maximum emission wavelength were recorded upon reaction with GSH (0-50 μM) for 2 min in PBS buffer (pH 7.2, 10% DMSO). The experiments were performed in triplicate, and data were expressed as the mean ± standard deviation. (Excitation: 320 nm for **1**; 360 nm for **2**; 380 nm for **3** and **4**; Emission: 380 nm for **1**; 450 nm for **2**; 460 nm for **3** and **4**; slit width: 2.5 nm.)

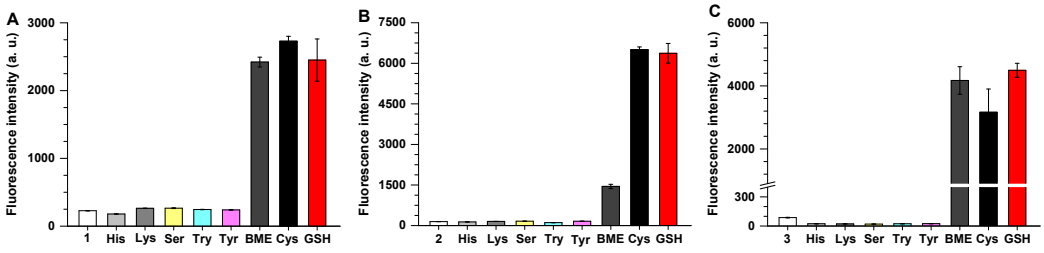


Figure S8. The selectivity of probes (**1**-**3**, 50 μM) towards different analytes (50 μM). Fluorescence intensities of probes (50 μM) were recorded upon their reaction with various analytes (50 μM) at room temperature for 2 min in PBS buffer (pH 7.2, 10% DMSO). The experiments were performed in triplicate, and data were expressed as the mean ± standard deviation. (Excitation: 320 nm for **1**; 360 nm for **2**; 380 nm for **3** and **4**; Emission: 380 nm for **1**; 450 nm for **2**; 460 nm for **3** and **4**; slit width: 2.5 nm.)

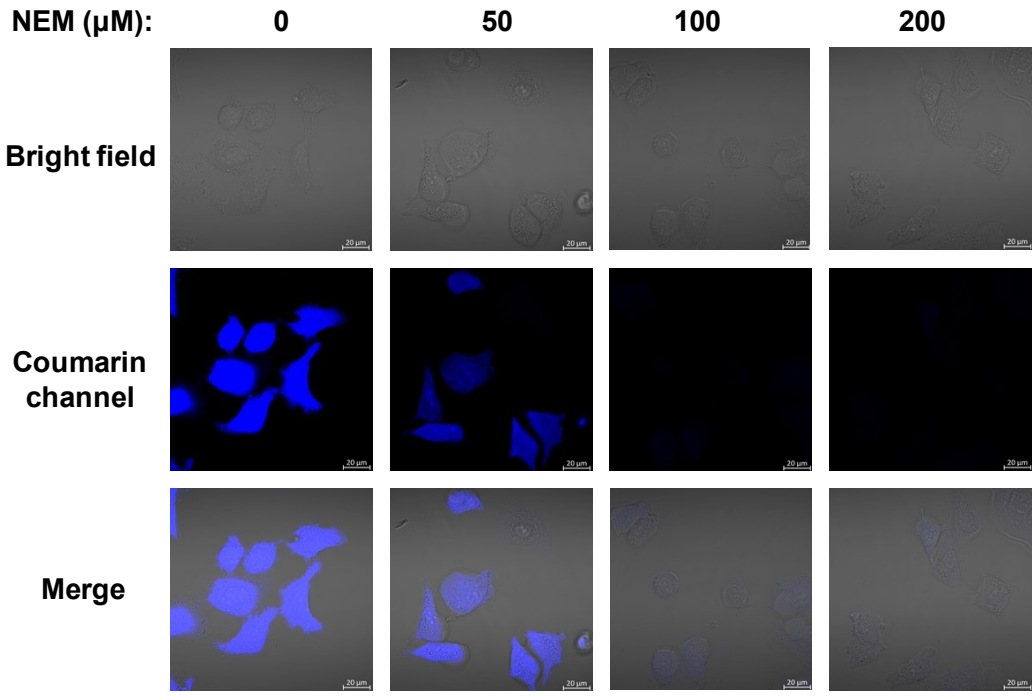


Figure S9. Imaging GSH in live A549 lung cancer cells using the probe **4**. The cells were pretreated with NEM (varied concentration, GSH inhibitor) at 37 ℃ for 1 h. Then, the cells were incubated with probe **4** (10 μM) at 37 ℃ for 2 min. The cells were washed with PBS buffer for three times before confocal microscope imaging analysis (Coumarin-channel: Ex 405 nm/Em 400-600 nm; scale bars, 20 μm).

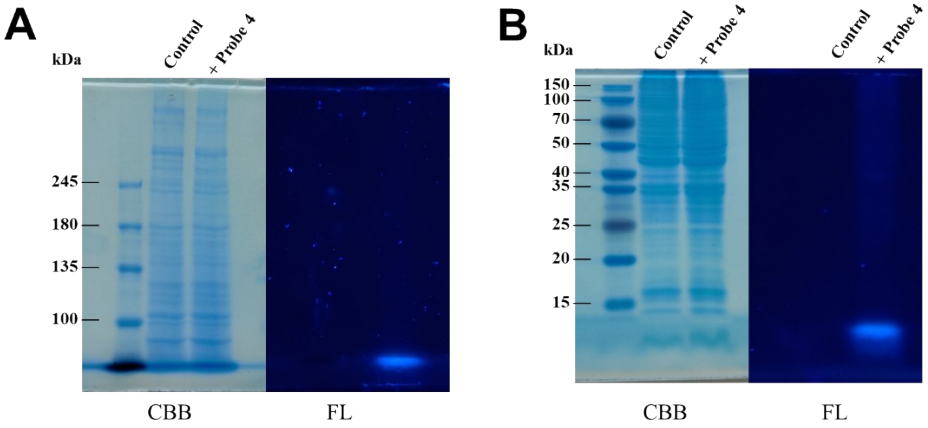


Figure S10. Analysis of cellular protein labelling during GSH detection using SDS-PAGE (A, 5%; B, 12%). The A549 cells were treated with or without probe **4** (10 μM, incubation for 2 min) at 37 ℃. The cells were washed with PBS buffer three times, and the lysates were analyzed by SDS-PAGE, followed by Coomassie brilliant blue (CBB) stain or fluorescence (FL) imaging (Excitation: 365 nm).

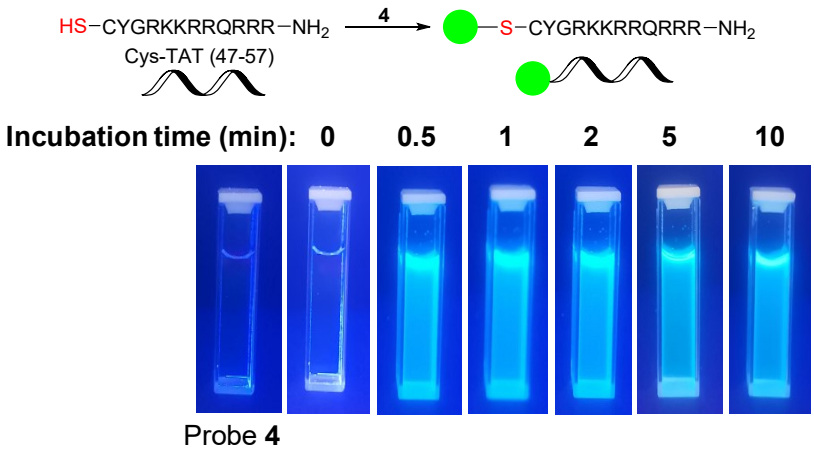


Figure S11. Monitor the fluorogenic reaction between Cys-TAT and **4** by fluorescence assay. Cys-TAT (47-57) (1 mM) was incubated with probe **4** (1 mM) at room temperature in PBS buffer (pH 7.2, 10% DMSO), affording fluorescent conjugate **6**. The progress of the reaction was detected under a spectroline UV-viewing cabinet with the excitation at 365 nm.

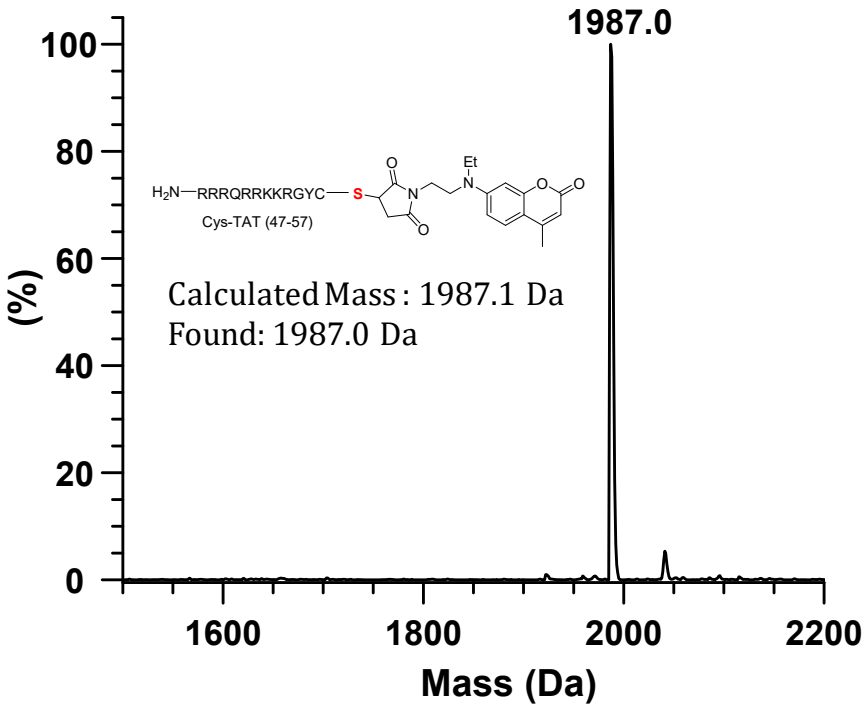


Figure S12. ESI-TOF-MS spectrum of fluorescent conjugate **6**. (Calculated: 1987.1 Da, Found: 1987.0 Da).

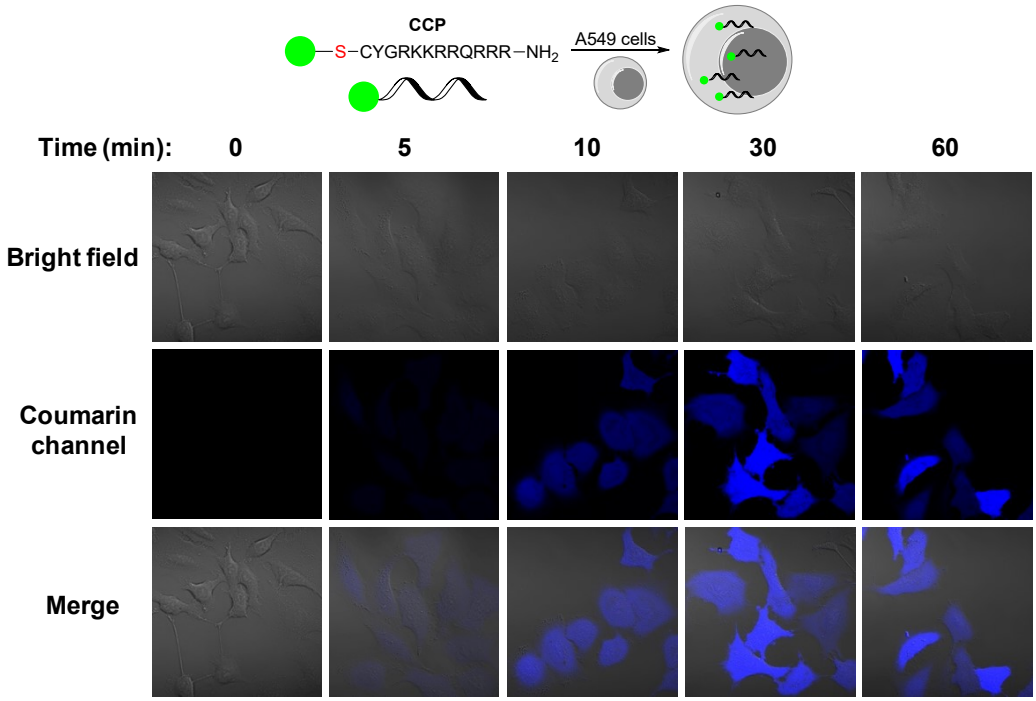


Figure S13. Imaging fluorescent conjugate **6**'s cellular uptake by A549 cells. The briefly purified bioconjugated product **6** (about 10 μM) was added to A549 cells at 37 ℃. Upon incubation for desired time, the cells were washed with PBS buffer three times before confocal microscope imaging analysis (Ex 405 nm/Em 400-600 nm).

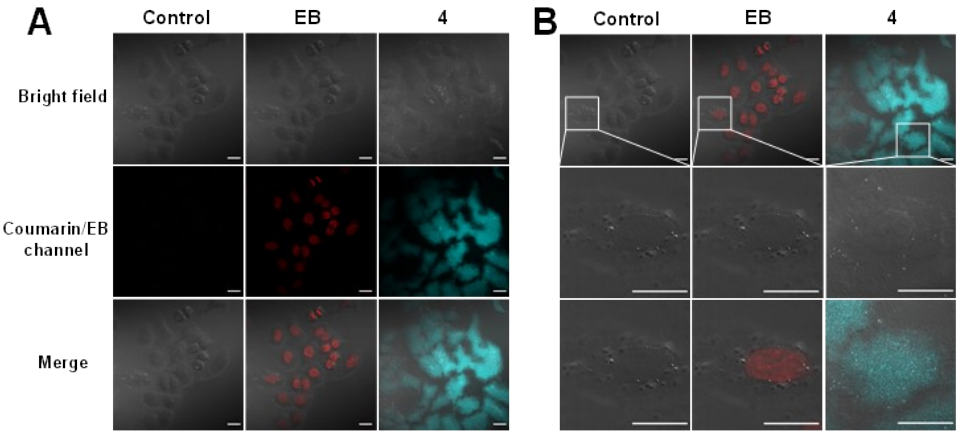


Figure S14. Analysis of location of labelled TAT **6** in A549 cells (A) and in single cells (B). The briefly purified bioconjugated product **6** (about 10 μM) was added to A549 cells at 37 ℃. Upon incubation for 30 mins, the cells were washed with PBS buffer three times, fixed by 4% paraformaldehyde (500 μL) and stained with ethidium bromide (EB) before confocal microscope imaging analysis (scale bars, 20 μm).

Cys-free Histone H2B:
MPEPAKSAPAPKKGSKKAVTKTQKKDGKKRRKSRKESYAIYVYKVLKQVHP
DTGISSKAMSIMNSFVNDVFERIAGEASRLAHYNKRSTITSREIQTAVRLLLPG
ELAKHAVSEGTKAVTKYTSAK

Cys-bearing Histone H3:
MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALR
EIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEASEAYLVG
LFEDTNLCGIHAKRVTIMPKDIQLARRIRGERA

Scheme S1. Sequences of Cys-free Histone H2B and Cys-bearing Histone H3 protein. The Cys (C) residue in the sequence was marked as red.

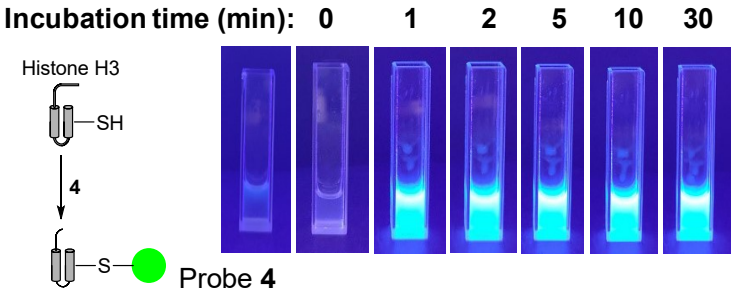


Figure S15. Monitor the fluorogenic reaction between Cys-bearing histone H3 protein and **4** by fluorescence assay. Histone H3 protein (1 mM) was incubated with probe **4** (1 mM) at room temperature in PBS buffer (pH 7.2, 10% DMSO). The progress of labeling reaction was detected under a spectroline UV-viewing cabinet with the excitation at 365 nm.

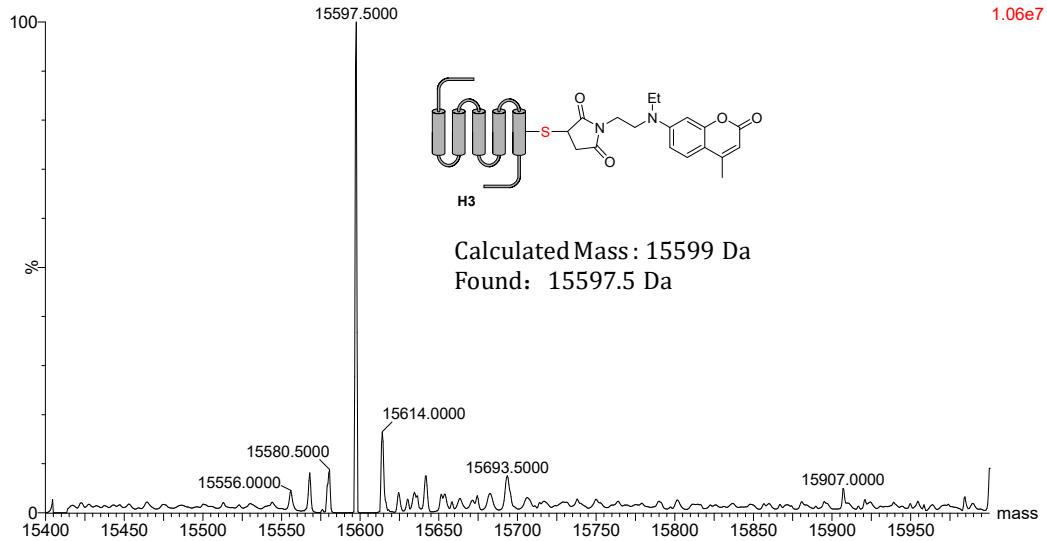


Figure S16. ESI-TOF-MS spectrum of labeled histone H3 protein. (Calculated: 15599 Da, Found: 15597.5 Da).

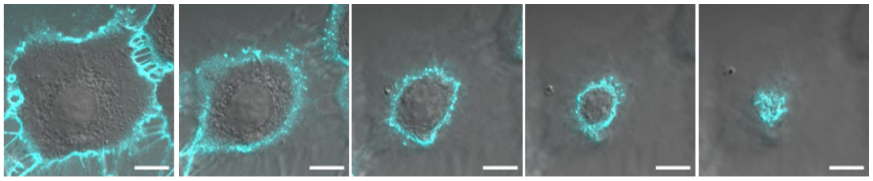


Figure S17. Continuous z-stack scan of A549 cells treated with labelled H3. The cells upon incubation with labelled H3 (same protocol as in Figure 6) was scanned at specific z-stack depths (scale bars, 10 μm).

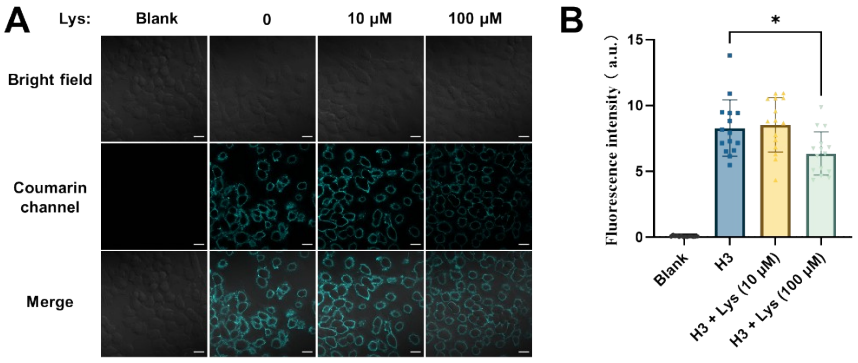


Figure S18. Effect of lysine addition on H3 binding with membranes. The intensity of fluorescence signal on cell membranes were recorded (A) and analyzed upon addition of the lysine in cell culture media (scale bars, 20 μm).

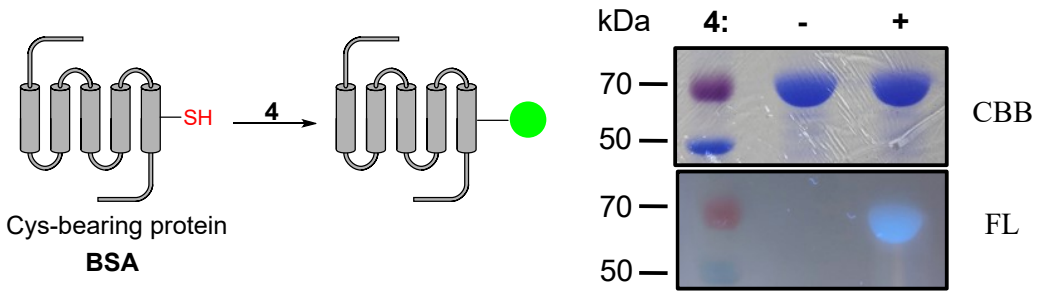
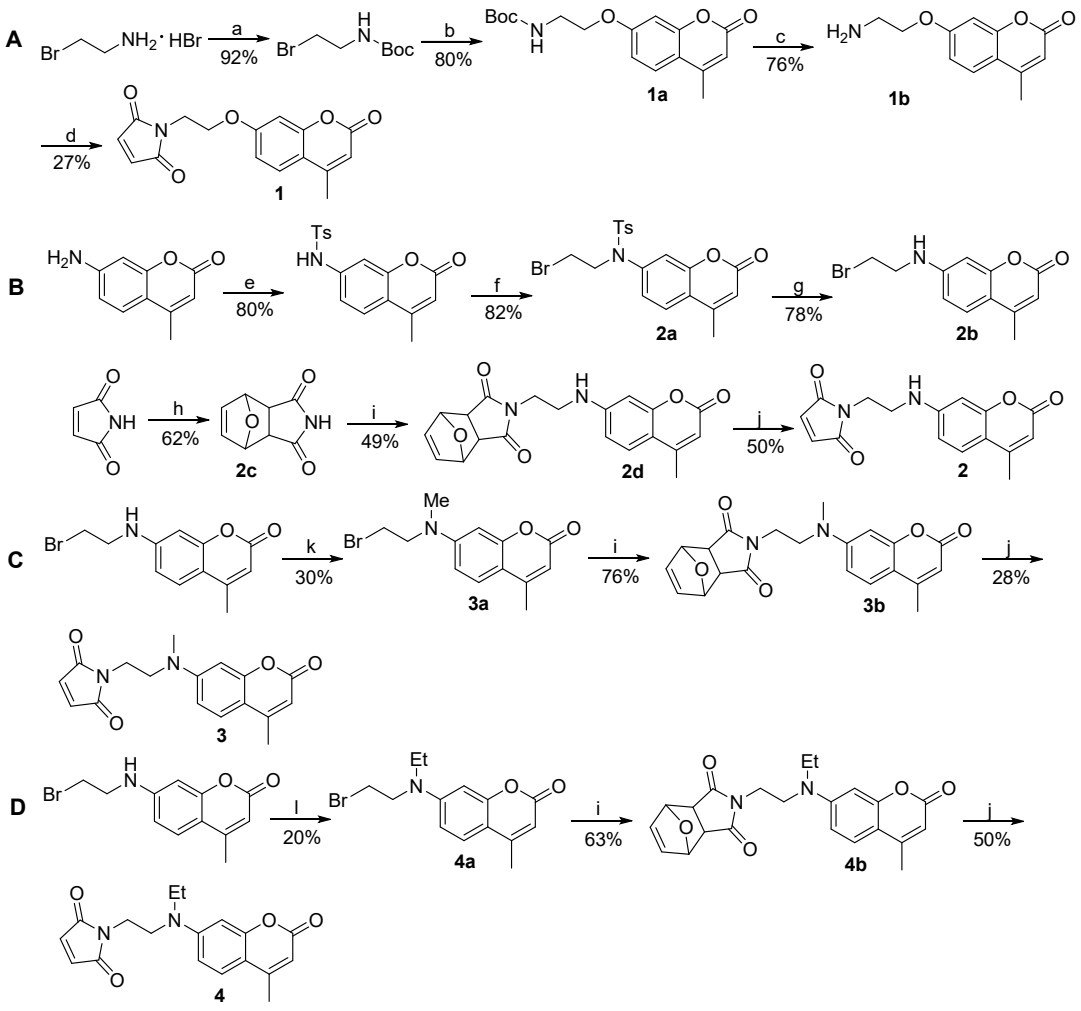
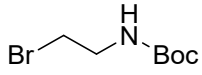


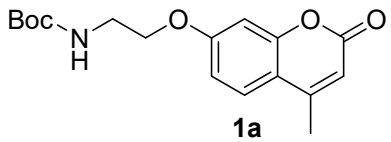
Figure S19. Fluorogenic labeling of Cys-bearing protein BSA using probe **4**. The Cys-bearing protein (100 μM) was incubated with/without probe **4** (100 μM) at room temperature for 2 min. The samples were analyzed by SDS-PAGE, followed by fluorescence (FL) imaging (Excitation: 365 nm) or Coomassie brilliant blue (CBB) stain.



Scheme 2. Synthetic route of probes **1**-**4**.

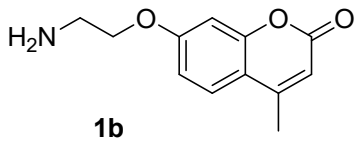


Tert-butyl (2-bromoethyl)carbamate. To a solution of 2-bromoethan-1-amine hydrogen bromide (1.0 g, 4.9 mmol) in methanol (60 mL), di-tert-butyl dicarbonate (2.13 g, 9.8 mmol) and triethylamine (7 mL) were added. The reaction mixture was stirred at 60 ℃ for 1 h, and then continued to stir at room temperature for another 14 h. The solvent was removed under reduced pressure. The residue was diluted by dichloromethane (50 mL), washed with 1 M HCl (30 mL) and brine (20 mL). The organic phase was dried over anhydrous sodium sulfate. The solvent was removed under vacuum to provide the product as a colorless oil (1.0 g, 92%). ^{1}HNMR (400 MHz, DMSO-*d6*) δ 7.11 (s, 1H), 3.41 (d, J = 5.6 Hz, 2H), 3.27 (d, J = 6.3 Hz, 2H), 1.38 (s, 9H).



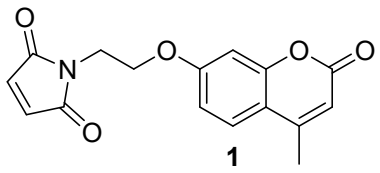
1a

Tert-butyl (2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethyl)carbamate (**1a**). To a solution of 7-hydroxy-4-methyl-2H-chromen-2-one (0.6 g, 1.7 mmol) in acetone (30 mL), potassium carbonate (0.51 g, 3.72 mmol) was added. The reaction mixture was refluxed for 1 h. The tert-butyl (2-bromoethyl)carbamate (0.83 g, 3.72 mmol) was added. The resulting mixture was continued to reflux for another 18 h. After cooling to room temperature, the solvent was removed under reduced pressure. Water (20 mL) was added to the residue, and the suspension was extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with saturated brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:4 to 1:1, v/v) to give the desired product **1a** as a white solid (870 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.48 (d, J = 8.8 Hz, 1H), 6.86 (dd, J = 8.8, 2.5 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.14 (s, 1H), 5.02 (s, 1H), 4.07 (t, J = 5.1 Hz, 2H), 3.56 (dd, J = 9.9, 5.1 Hz, 2H), 2.39 (s, 3H), 1.44 (s, 9H).



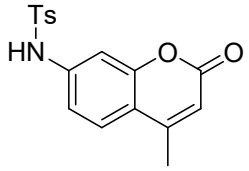
1b

7-(2-Aminoethoxy)-4-methyl-2H-chromen-2-one (**1b**). To a solution of **1a** (0.1 g, 0.3 mmol) in dichloromethane (10 mL), trifluoroacetic acid (1.7 mL) was added slowly. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. The residue was diluted with dichloromethane (20 mL) and washed with water (10 mL × 3). The organic layer was washed with saturated brine, dried over anhydrous sodium sulfate and concentrated under vacuum to yield **1b** as a white solid (50 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.49 (d, J = 8.8 Hz, 1H), 6.87 (dd, J = 8.8, 2.5 Hz, 1H), 6.82 (d, J = 2.4 Hz, 1H), 6.14 (d, J = 0.8 Hz, 1H), 4.05 (t, J = 5.1 Hz, 2H), 3.13 (t, J = 5.1 Hz, 2H), 2.40 (s, 3H).

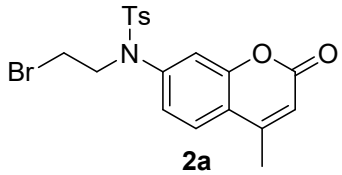


1

1-(2-((2-Oxo-2H-chromen-7-yl)oxy)ethyl)-1H-pyrrole-2,5-dione (**1**). To a solution of **1c** (0.1 g, 0.46 mmol) in acetic acid (5 mL), maleic anhydride (0.09 g, 0.92 mmol) was added. The reaction mixture was refluxed for 8 h. After cooling to room temperature, $NaHCO_3$ aqueous solution (60 mL) was added slowly. The mixture was extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with 1 M HCl (30 mL) and brine (20 mL), and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to give **1** as a white solid (40 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.49 (d, J = 8.8 Hz, 1H), 6.82 (dd, J = 8.8, 2.5 Hz, 1H), 6.77 – 6.75 (m, 1H), 6.75 (s, 2H), 6.13 (d, J = 0.9 Hz, 1H), 4.20 (dd, J = 12.1, 6.4 Hz, 2H), 3.97 (t, J = 5.7 Hz, 2H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*) δ 169.4, 160.1, 160.1, 154.1, 151.4, 133.3, 124.6, 113.0, 111.5, 111.2, 100.5, 64.1, 35.8, 17.7. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{16}H_{13}NO_5^+$, 300.0866; found: 300.0866.

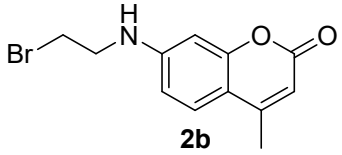


4-Methyl-N-(4-methyl-2-oxo-2H-chromen-7-yl)benzenesulfonamide. To a solution of 7-amino-4-methyl-2H-chromen-2-one (1.75 g, 1.0 mmol) in dichloromethane (10 mL), 4-toluenesulfonyl chloride (2.3 g, 12.3 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (dichloromethane:CH_3OH = 20:1) to afford the product as a white solid (3.0 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.76 (m, 2H), 7.47 (d, J = 8.4 Hz, 1H), 7.28 (s, 1H), 7.12 (s, 1H), 7.08 (d, J = 2.3 Hz, 1H), 7.05 (d, J = 2.0 Hz, 2H), 6.19 (s, 1H), 2.39 (s, 3H), 2.37 (s, 3H).



2a

N-(2-bromoethyl)-4-methyl-N-(4-methyl-2-oxo-2H-chromen-7-yl)benzenesulfonamide (**2b**). To a solution of 4-methyl-N-(4-methyl-2-oxo-2H-chromen-7-yl)benzenesulfonamide (1.65 g, 50 mmol) in CH_3CN (30 mL), 1,2-dibromoethane (9.3 g, 500 mmol) and Cs_2CO_3 (4.9 g, 150 mmol) were added. The mixture was refluxed for 6 h. After cooling to room temperature, the solvent was removed under reduced pressure. H_2O (20 mL) was added to the residue, and the suspension was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL), and then dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo offered **2a** as a white solid (1.9 g, 85%).^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.61 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 1.7 Hz, 1H), 7.48 (d, J = 1.9 Hz, 1H), 7.29 (t, J = 1.2 Hz, 1H), 7.27 (d, J = 2.1 Hz, 1H), 7.24 (d, J = 2.2 Hz, 1H), 6.89 (d, J = 2.1 Hz, 1H), 6.31 (q, J = 1.4 Hz, 1H), 3.92 (t, J = 7.2 Hz, 2H), 3.41 (t, J = 7.2 Hz, 2H), 2.44 (s, 6H).

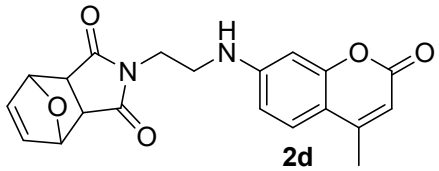


2b

7-((2-Bromoethyl)amino)-4-methyl-2H-chromen-2-one (**2b**). A solution of **2a** (1.0 g, 35.5 mmol) in concentrated sulfuric acid (10 mL) was stirred at room temperature for 1 h. The mixture was carefully and slowly poured into water and neutralized with saturated sodium bicarbonate aqueous solution. The mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to give **2b** as a yellow solid (0.6 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.40 (dd, J = 8.6, 1.8 Hz, 1H), 6.62 (ddd, J = 8.7, 4.0, 2.4 Hz, 1H), 6.53 (dd, J = 5.8, 2.4 Hz, 1H), 6.02 (t, J = 1.5 Hz, 1H), 3.64 – 3.57 (m, 3H), 3.36 (t, J = 6.7 Hz, 1H), 2.35 (s, 3H).

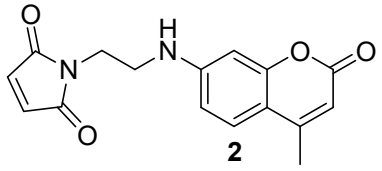
2c

3a,4,7,7a-Tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**2c**). The solution containing furan (9.7 g, 0.14 mol) and maleimide (13.6 g, 0.14 mol) in toluene (160 mL) was heated at 90 ℃ for 12 h. When the mixture was cooled to room temperature, white solid was precipitated. The suspension was filtered and washed with toluene (20 mL × 3). The filtrate was dried in air to afford **2c** as a white solid (14.6 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 8.23 (s, 1H), 6.52 (t, *J* = 1.0 Hz, 2H), 5.31 (t, *J* = 1.0 Hz, 2H), 2.89 (s, 2H).



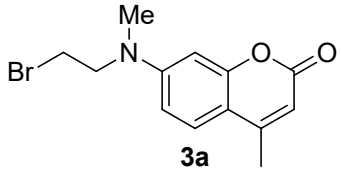
2d

2-(2-((4-Methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**2d**). To a solution of **2b** (0.56 g, 2.0 mmol) in DMF (10 mL), **2c** (0.66 g, 4.0 mmol) and K_2CO_3 (0.83 g, 6.0 mmol) were added. The reaction mixture was heated at 50 ℃ for 2 h. After cooling to room temperature, the residue was diluted with ethyl acetate (50 mL). The mixture was washed with water (20 mL) and saturated brine. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 2:1) to give **2d** as a yellow solid (0.36 g, 49%).^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.38 (d, *J* = 8.7 Hz, 1H), 6.67 (s, 1H), 6.51 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.48 (s, 2H), 6.39 (d, *J* = 2.2 Hz, 1H), 5.87 (s, 1H), 5.06 (s, 2H), 3.42 (t, *J* = 7.0 Hz, 2H), 3.16 (t, *J* = 6.7 Hz, 2H), 2.86 (s, 2H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ176.5, 160.6, 155.6, 153.7, 151.8, 136.5, 126.1, 110.2, 109.2, 107.8, 96.4, 80.3, 47.2, 36.6, 18.0. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{20}H_{18}N_2O_5^+$, 367.1288; found: 367.1287.



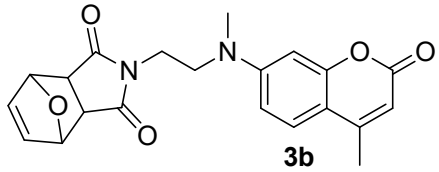
2

1-(2-((4-Methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-1H-pyrrole-2,5-dione (**2**). A solution of **2d** (0.3 g, 0.82 mmol) in anisole (10 mL) was stirred at 150 ºC for 20 min. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to afford **2** as a yellow solid (122 mg, 50%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.44 (d, J = 8.7 Hz, 1H), 7.02 (s, 2H), 6.56 (dd, J = 8.7, 2.3 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 5.94 (s, 1H), 3.54 (t, J = 5.9 Hz, 2H), 3.32 (t, J = 11.5, 5.7 Hz, 2H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ170.9, 160.5, 155.5, 153.6, 151.8, 134.4, 125.9, 110.1, 108.9, 107.6, 96.2, 35.9, 17.9. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{16}H_{14}N_2O_4^+$, 299.1026; found: 299.1024.



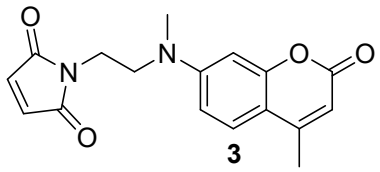
3a

7-((2-Bromoethyl)(methyl)amino)-4-methyl-2H-chromen-2-one (**3a**). To a solution of **2b** (0.32 g, 1.15 mmol) in CH_3CN (10 mL), potassium carbonate (1.25 g, 1.5 mmol), tetrabutyl ammonium bromide (0.06 g, 0.1 mmol) and methyl iodide (2.7mL, 2.0 mmol) were added. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the solvent was removed under reduced pressure. Water (20 mL) was added to the residue, and the suspension was extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with saturated brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2) to yield **3a** as a yellow solid (270 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.42 (d, J = 8.9 Hz, 1H), 6.62 (dd, J = 8.9, 2.6 Hz, 1H), 6.51 (d, J = 2.6 Hz, 1H), 5.99 (d, J = 1.4 Hz, 1H), 3.80 – 3.76 (m, 2H), 3.23 (dd, J = 8.6, 7.1 Hz, 2H), 3.07 (s, 3H), 2.34 (s, 3H).



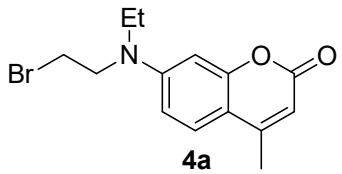
3b

2-(2-(Methyl(4-methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**3b**). To a solution of **3a** (0.15 g, 0.5 mmol) in anhydrous N, N-dimethylformamide (5 mL), furan protected maleimide **2c** (0.16 g, 1 mmol) and potassium carbonate (207 mg, 1.5 mmol) were added. The suspension was stirred at 50 °C for 2 h. Water (20 mL) was added to the residue, and the suspension was extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with saturated brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to give **3b** as a yellow solid (110 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.40 (d, J = 8.9 Hz, 1H), 6.68 (dd, J = 8.9, 2.6 Hz, 1H), 6.50 – 6.48 (m, 1H), 6.47 (s, 2H), 5.96 (d, J = 1.0 Hz, 1H), 5.22 (s, 2H), 3.68 (t, J = 6.7 Hz, 2H), 3.57 (t, J = 6.7 Hz, 2H), 3.01 (s, 3H), 2.74 (s, 2H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*) δ 175.1, 161.0, 154.6, 151.8, 150.5, 135.6, 135.5, 124.5, 109.1, 108.7, 107.8, 97.4, 79.8, 48.0, 46.4, 37.5, 34.7, 17.5. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{21}H_{20}N_2O_5^+$, 381.1445; found: 381.1442.



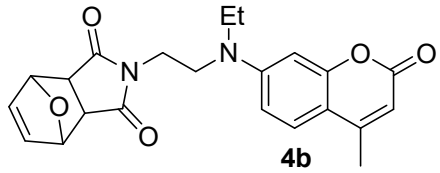
3

1-(2-(Methyl(4-methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-1H-pyrrole-2,5-dione (**3**). A solution of **3b** (0.1 g, 0.3 mmol) in anisole (5 mL) was stirred at 150 °C for 20 min. After cooling to the room temperature, the solvent was removed under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to obtain **3** as a yellow solid (25 mg, 28%).^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.32 (d, J = 8.9 Hz, 1H), 7.18 (s, 1H), 6.55 (d, J = 5.2 Hz, 2H), 6.42 (d, J = 2.5 Hz, 1H), 5.90 (d, J = 0.9 Hz, 1H), 3.65 (t, J = 6.5 Hz, 2H), 3.52 (t, J = 6.5 Hz, 2H), 2.94 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*) δ 169.5, 160.9, 154.7, 151.8, 150.5, 135.6, 133.2, 124.6, 109.0, 108.6, 107.6, 97.3, 80.0, 48.7, 37.2, 33.6, 17.5. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{17}H_{16}N_2O_4^+$, 313.1183; found: 313.1181.



4a

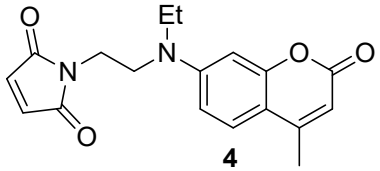
7-((2-Bromoethyl)(ethyl)amino)-4-methyl-2H-chromen-2-one (**4a**). To a solution of **2b** (0.32 g, 1.15 mmol) in CH_3CN (10 mL), potassium carbonate (1.25 g, 1.5 mmol), tetrabutyl ammonium bromide (0.06 g, 0.1 mmol) and iodoethylane (2.7mL, 2.0 mmol) were added. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the solvent was removed under reduced pressure. Water (20 mL) was added to the residue, and the suspension was extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with saturated brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2) to give **4a** as a yellow solid (210 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.42 (d, J = 8.9 Hz, 1H), 6.59 (dd, J = 9.0, 2.6 Hz, 1H), 6.50 (d, J = 2.6 Hz, 1H), 5.98 (d, J = 1.4 Hz, 1H), 3.72 (dd, J = 9.1, 7.2 Hz, 2H), 3.45 (q, J = 7.1 Hz, 2H), 3.21 (dd, J = 9.1, 7.2 Hz, 2H), 2.34 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H).



4b

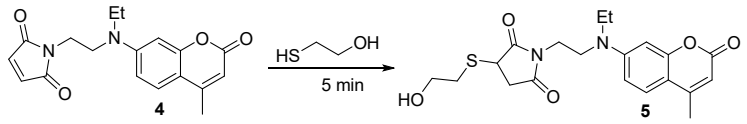
2-(2-(Ethyl(4-methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**4b**). To a solution of **4a** (0.1 g, 0.33 mmol) in anhydrous N, N-dimethylformamide (5 mL), furan protected maleimide **2c** (0.11 g, 0.66 mmol) and potassium carbonate (138 mg, 1 mmol) were added. The suspension was stirred at 50^{o}C for 2 h. After cooling to room temperature, the solvent was removed under reduced pressure. Water (20 mL) was added to the residue, and the suspension was extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with saturated brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to give **4b** as a yellow solid (60 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.42 (m, 1H), 6.74 (d, J = 6.7 Hz, 1H), 6.72 (d, J = 2.6 Hz, 1H), 6.56 (d, J = 9.9 Hz, 2H), 5.95 (s, 1H), 5.25 (s, 2H), 3.68 (t, J = 7.1 Hz, 2H), 3.49 (t, J = 6.7 Hz, 2H), 3.38 (m, 2H), 2.82 (s, 2H), 2.32 (s, 3H), 1.17 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*) δ 175.1, 161.0,

154.8, 151.8, 149.2, 135.5, 124.8, 109.0, 108.5, 107.8, 97.3, 79.9, 46.5, 46.0, 44.4, 34.8, 17.5, 11.0. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{22}H_{22}N_2O_5^+$, 395.1607; found: 395.1602.



4

1-(2-(Ethyl(4-methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-1H-pyrrole-2,5-dione (**4**). A solution of **4b** (0.1 g, 0.3 mmol) in anisole (5 mL) was stirred at 150°C for 20 min. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc:Hexane = 1:2 to 1:1, v/v) to offer **4** as a yellow solid (45 mg, 50%).^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.42 (d, *J* = 8.9 Hz, 1H), 6.71 (s, 2H), 6.69 (d, *J* = 2.6 Hz, 1H), 6.56 (d, *J* = 2.6 Hz, 1H), 5.97 (s, 1H), 3.75 (t, *J* = 7.1 Hz, 2H), 3.55 (t, *J* = 7.1 Hz, 2H), 3.40 (q, *J* = 7.1 Hz, 2H), 2.34 (s, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*) δ169.5, 161.0, 154.8, 151.8, 149.2, 135.6, 133.3, 109.0, 108.5, 107.7, 97.2, 80.0, 47.7, 46.8, 44.2, 33.8, 17.4, 11.0. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{18}H_{18}N_2O_4^+$, 327.1339; found: 327.1337.



4 **5**

1-(2-(Ethyl(4-methyl-2-oxo-2H-chromen-7-yl)amino)ethyl)-3-((2-hydroxyethyl)thio)pyrrolidine-2,5-dione (**5**). To a solution of **4** (32 mg, 1 mmol) in dichloromethane (5 mL), 2-mercaptoethanol (96 mg, 1.2 mmol) was added slowly. The reaction mixture was stirred at room temperature for 5 min. The solvent was removed under vacuum to yield **5** as a yellow oil (40 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.42 (d, *J* = 8.9 Hz, 1H), 6.72 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.56 (d, *J* = 2.6 Hz, 1H), 5.97 (d, *J* = 1.5 Hz, 1H), 5.29 (s, 1H), 3.88 (dt, *J* = 6.6, 4.6 Hz, 2H), 3.82 (dd, *J* = 9.2, 4.2 Hz, 1H), 3.76 – 3.70 (m, 2H), 3.54 (t, J = 7.0 Hz, 2H), 3.42 (q, *J* = 7.2 Hz, 2H), 3.15 – 3.05 (m, 2H), 2.85 – 2.77 (m, 1H), 2.48 (dd, *J* = 18.8, 4.1 Hz, 1H), 2.33 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*) δ 176.5, 173.4, 161.0,

154.8, 151.8, 149.4, 124.8, 109.1, 108.6, 107.7, 97.3, 60.8, 45.7, 44.1, 38.4, 35.3, 35.2, 34.7, 28.7, 17.4, 11.0. HRMS-ESI (+) (m/z): $[M + H]^+$ Calcd for $C_{20}H_{24}N_2O_5S^+$, 405.1479; found: 405.1481.

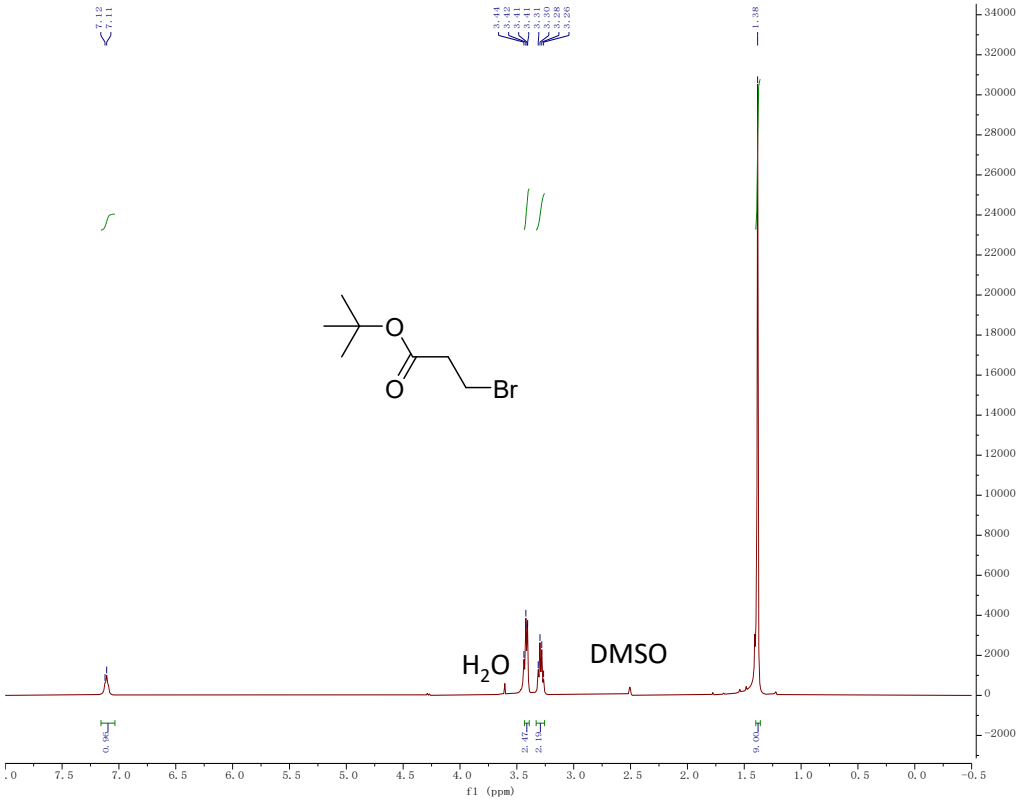


Figure S20. ^{1}H-NMR spectrum of tert-butyl (2-bromoethyl)carbamate ($CDCl_3$-*d*).

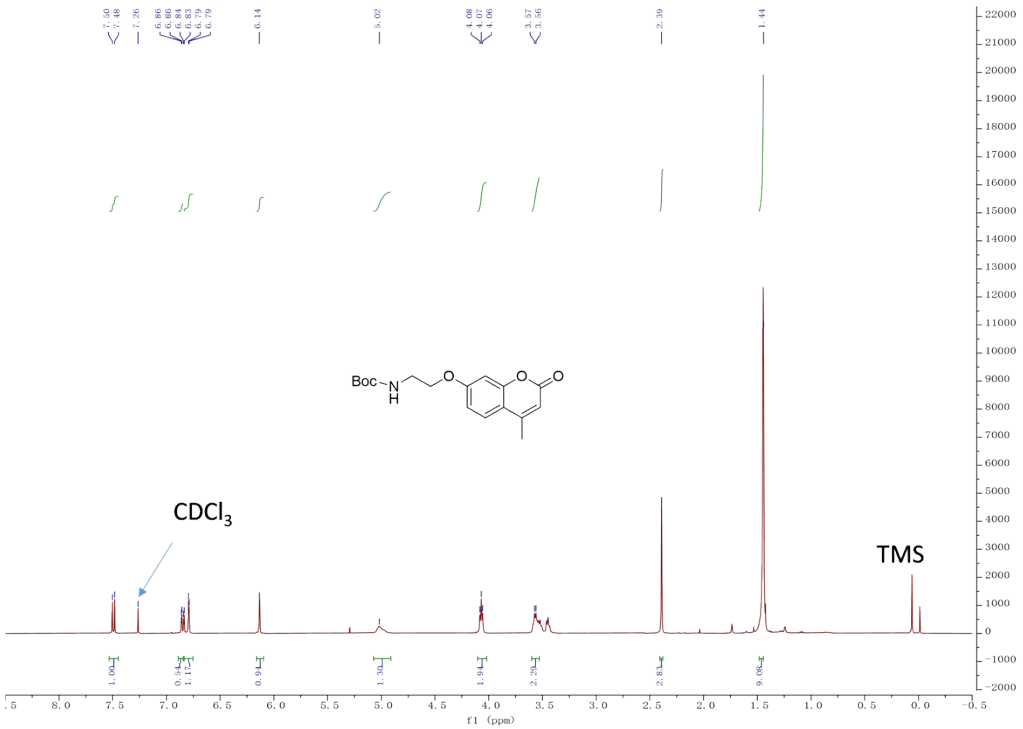


Figure S21. ^{1}H-NMR spectrum of **1a** ($CDCl_3$-*d*).

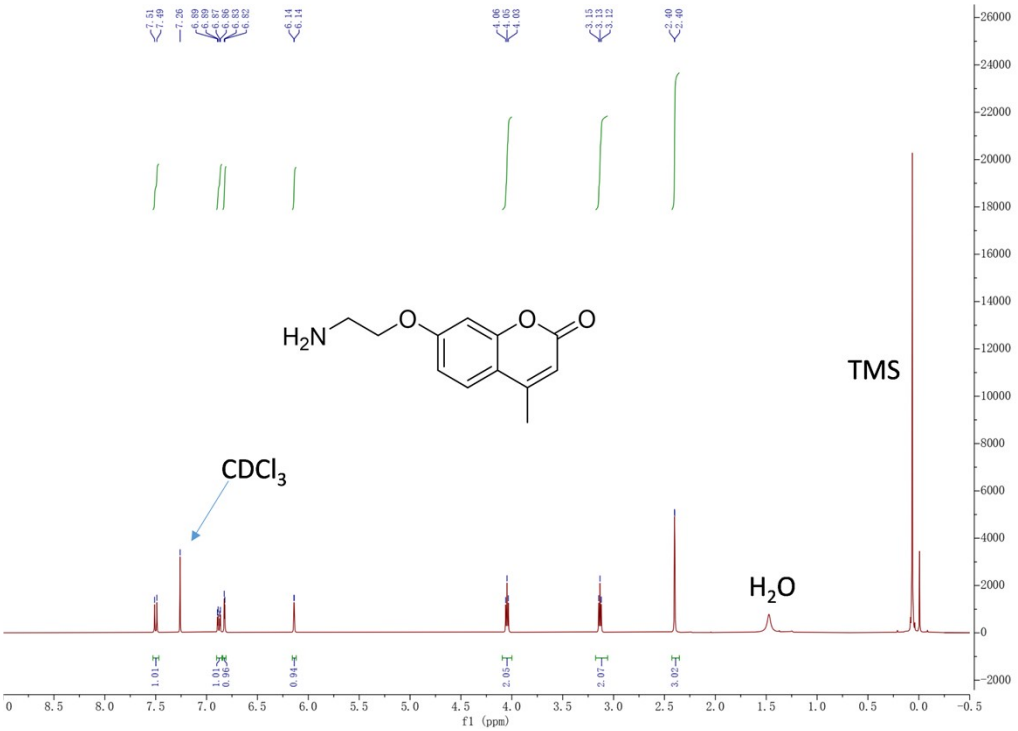
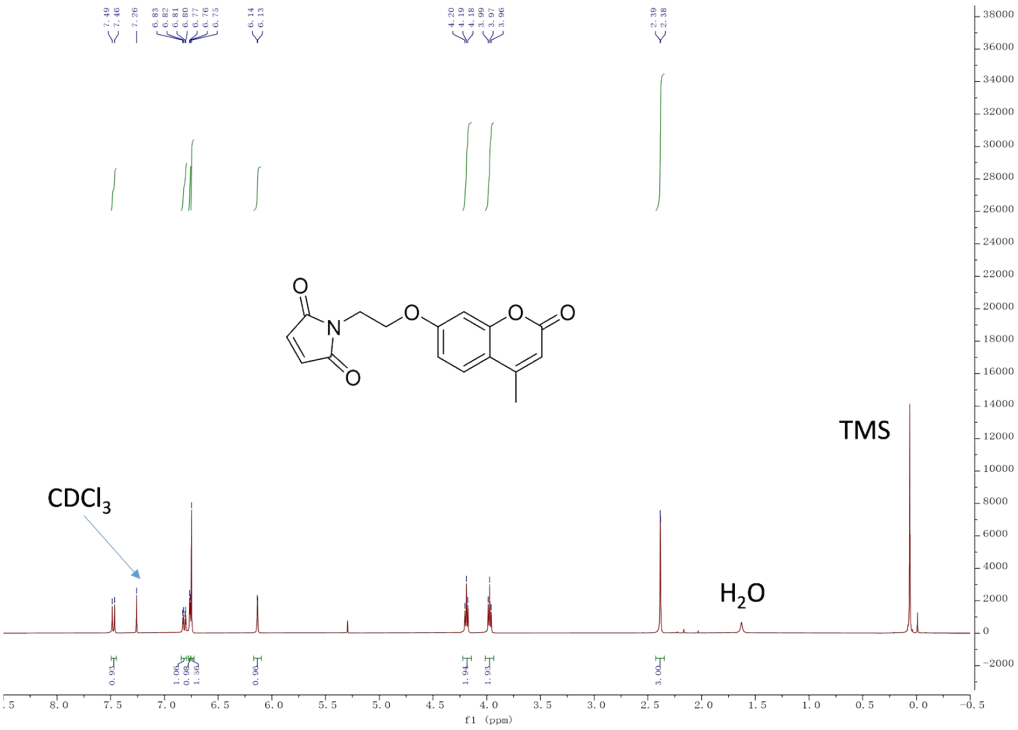


Figure S22. ^{1}H-NMR spectrum of **1b** ($CDCl_3$-*d*).



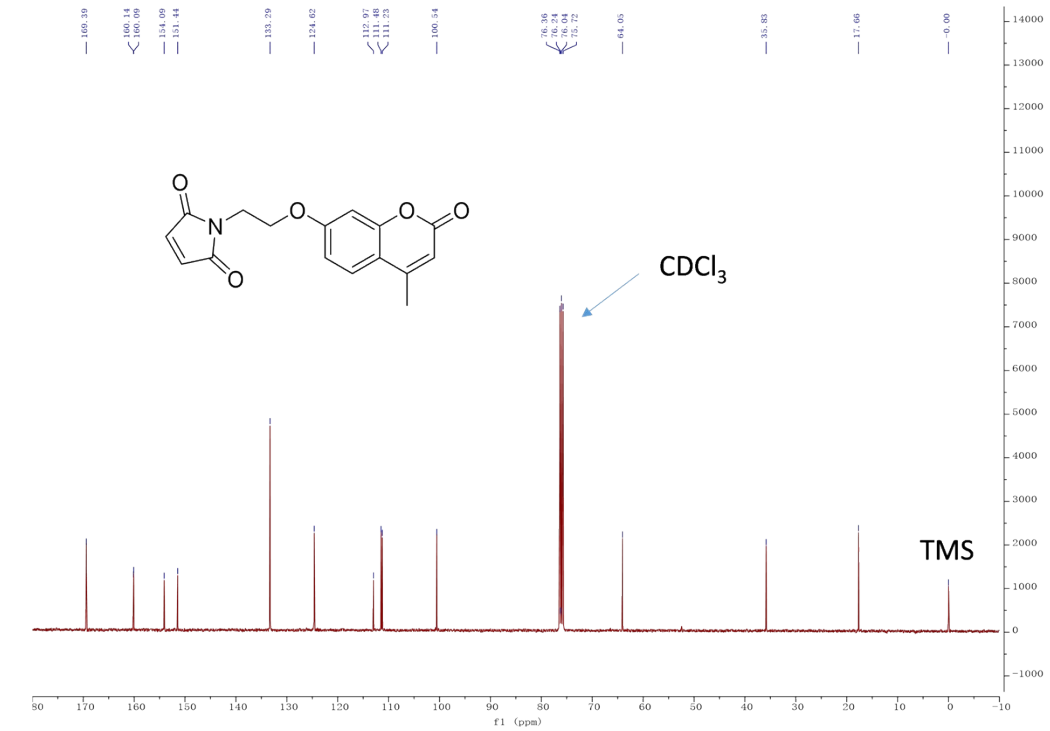


Figure S23. ^{1}H NMR and ^{13}C NMR spectra of probe **1** ($CDCl_3$-*d*).

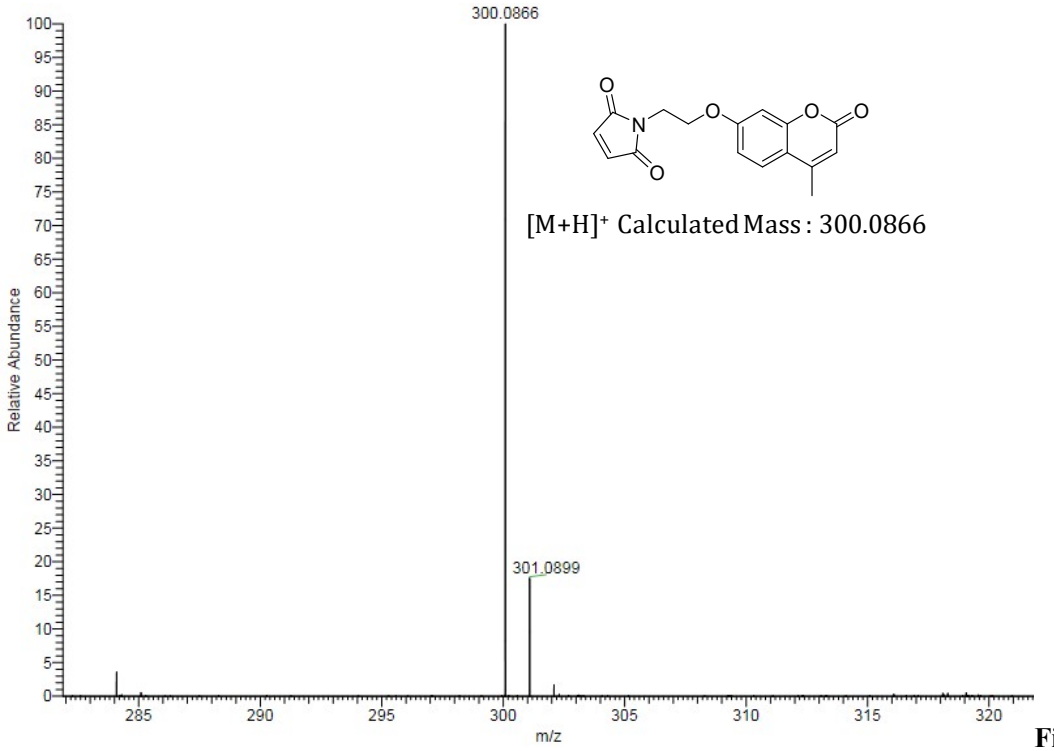


Figure S24. ESI-TOF-MS spectrum of probe **1**.

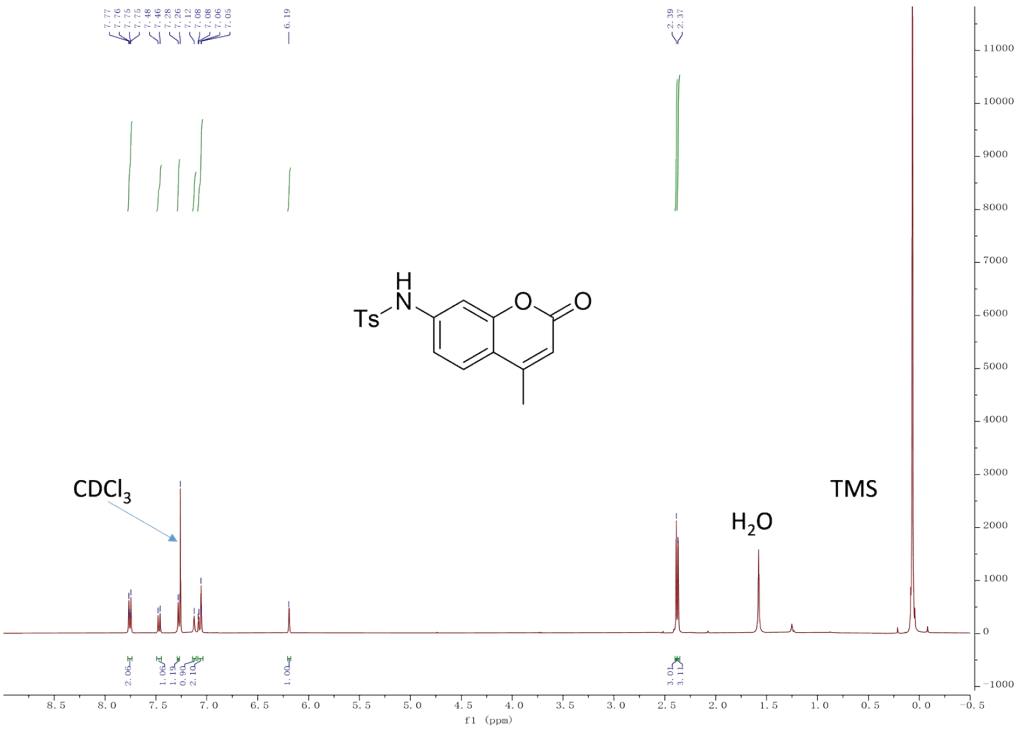


Figure S25. ^{1}H-NMR spectrum of 4-Methyl-N-(4-methyl-2-oxo-2H-chromen -7-yl)benzenesulfonamide ($CDCl_3$-*d*).

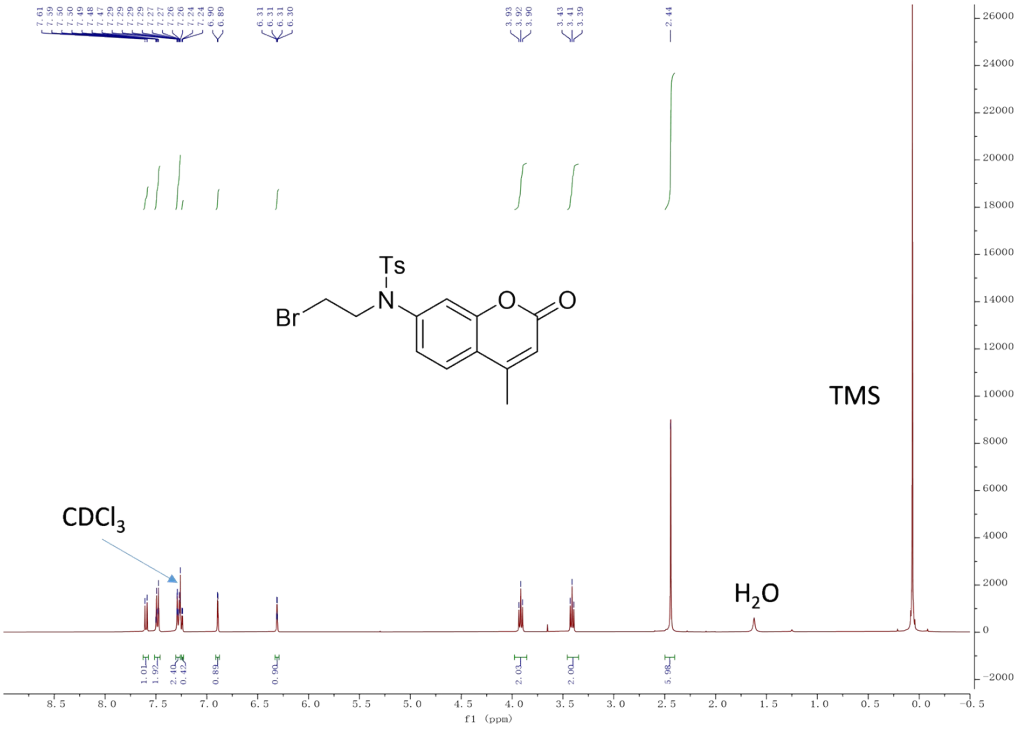


Figure S26. ^{1}H-NMR spectrum of **2a** ($CDCl_3$-*d*).

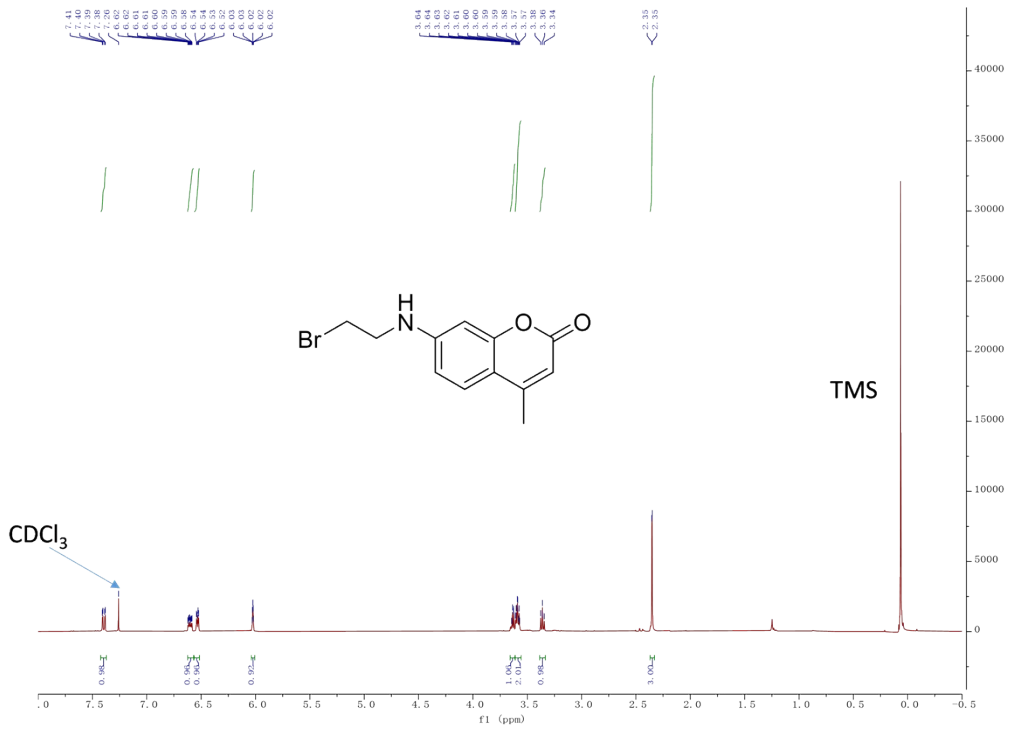


Figure S27. ^{1}H-NMR spectrum of **2b** ($CDCl_3$-*d*).

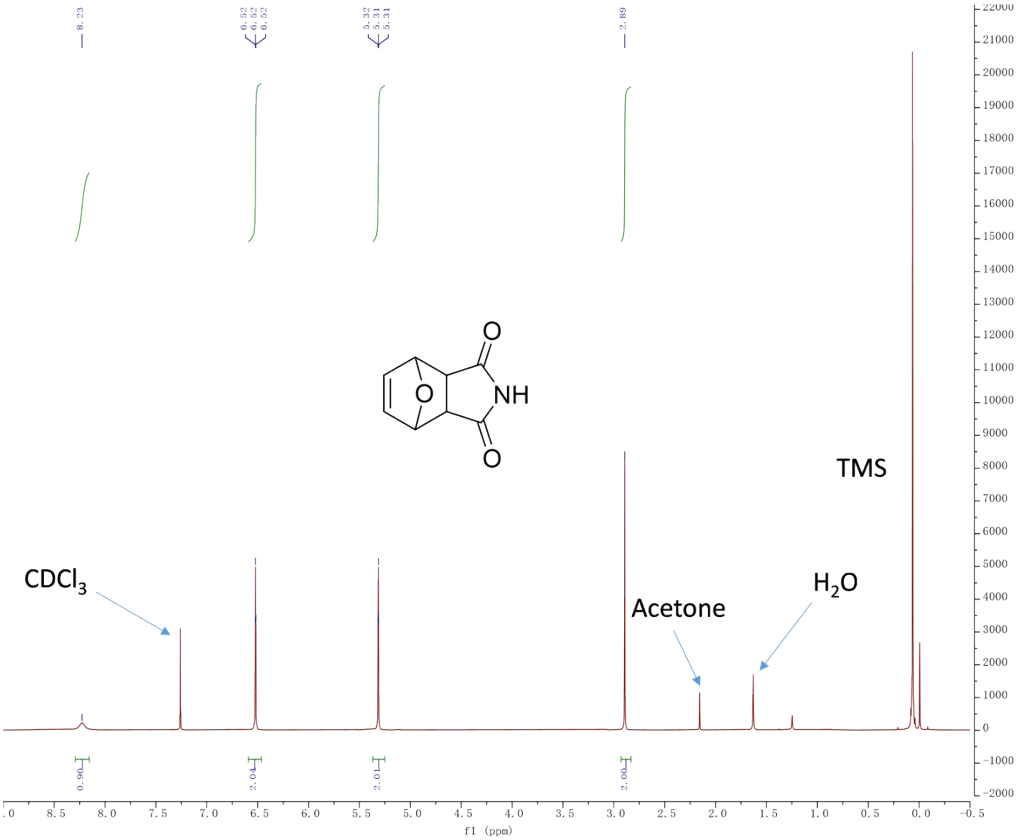


Figure S28. ^{1}H-NMR spectrum of **2c** ($CDCl_3$-*d*).

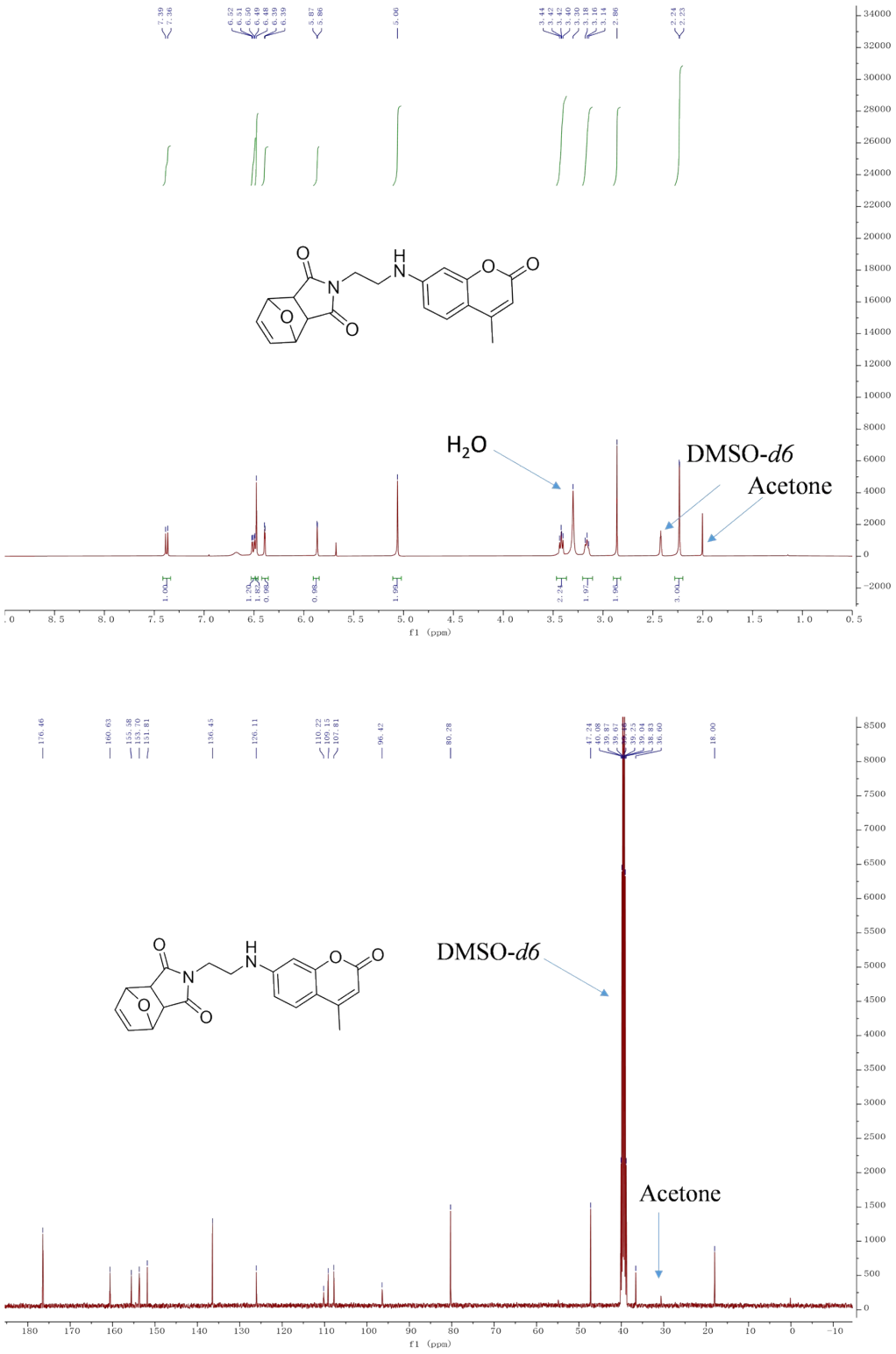


Figure S29. ^{1}H NMR and ^{13}C NMR spectra of **2d** (DMSO-*d6*).

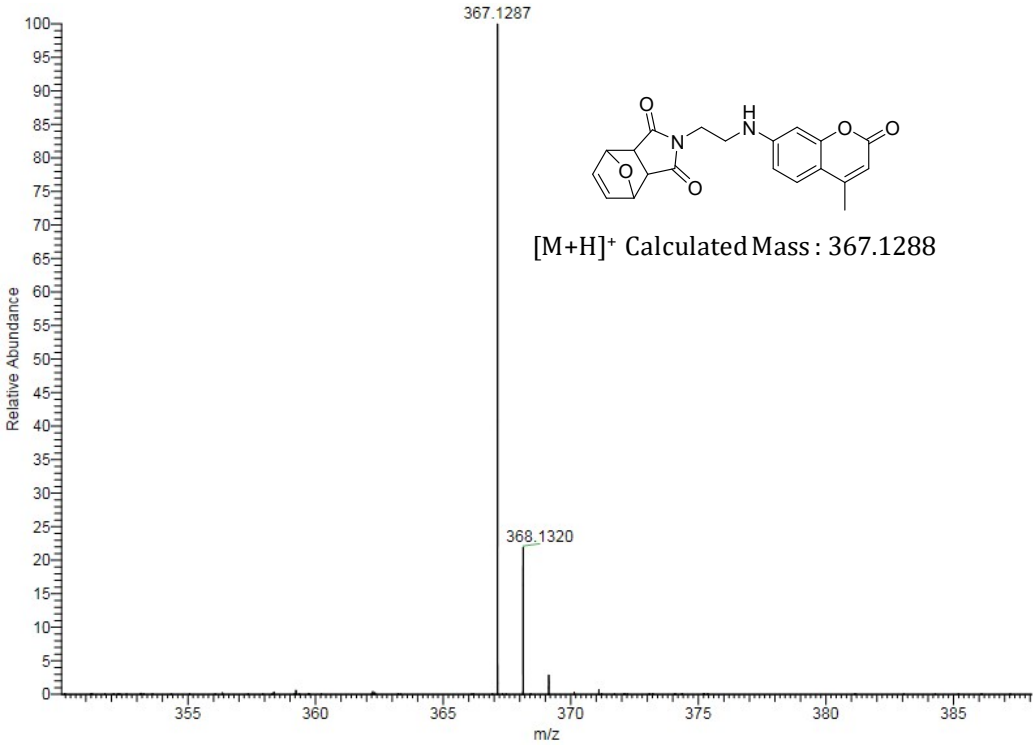
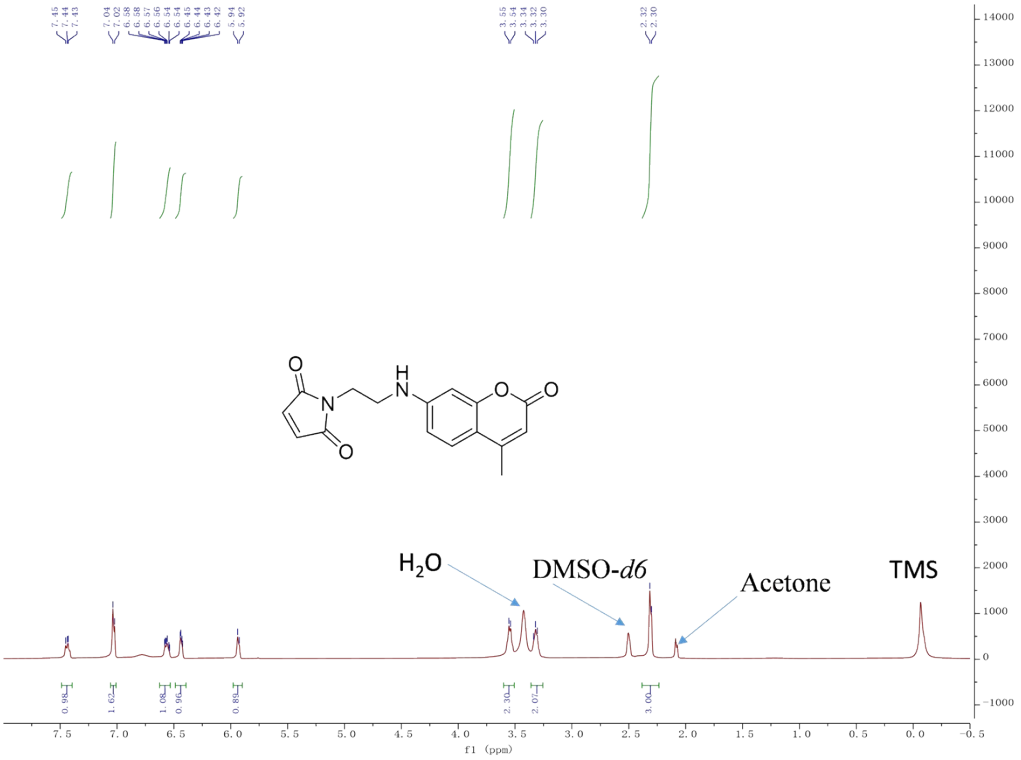


Figure S30. ESI-TOF-MS spectrum of **2d**.



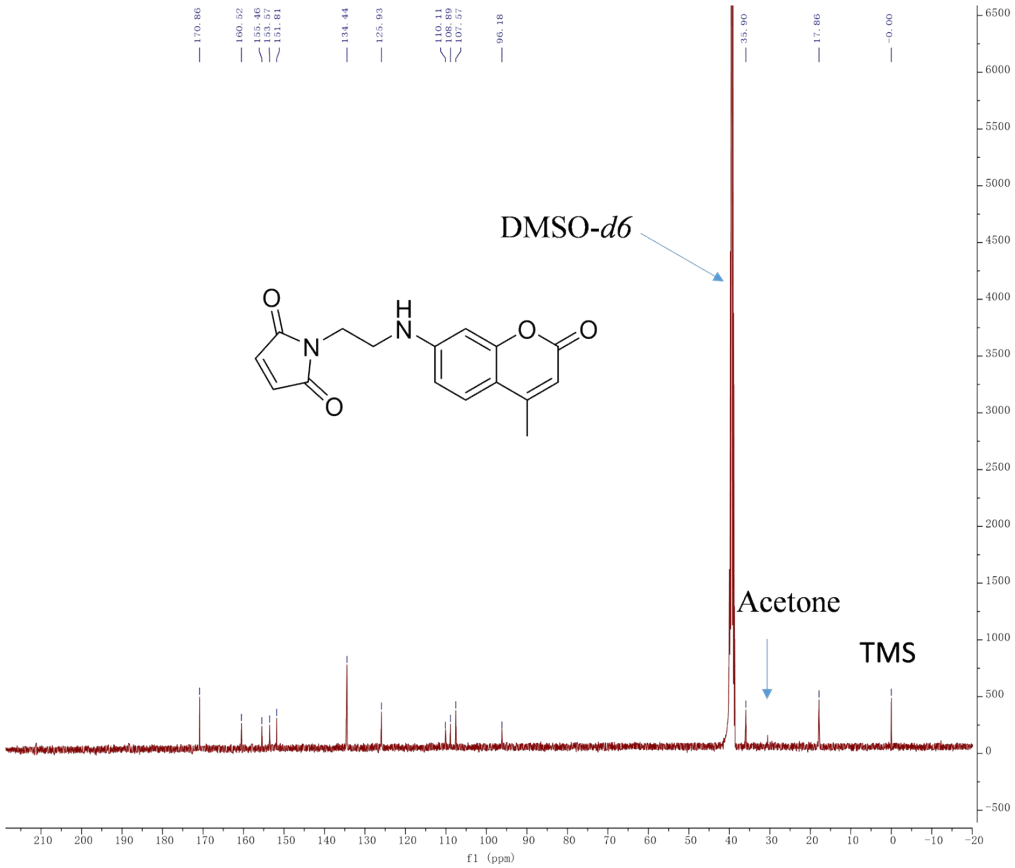


Figure S31. ^{1}H NMR and ^{13}C NMR spectra of probe **2** (DMSO-*d6*).

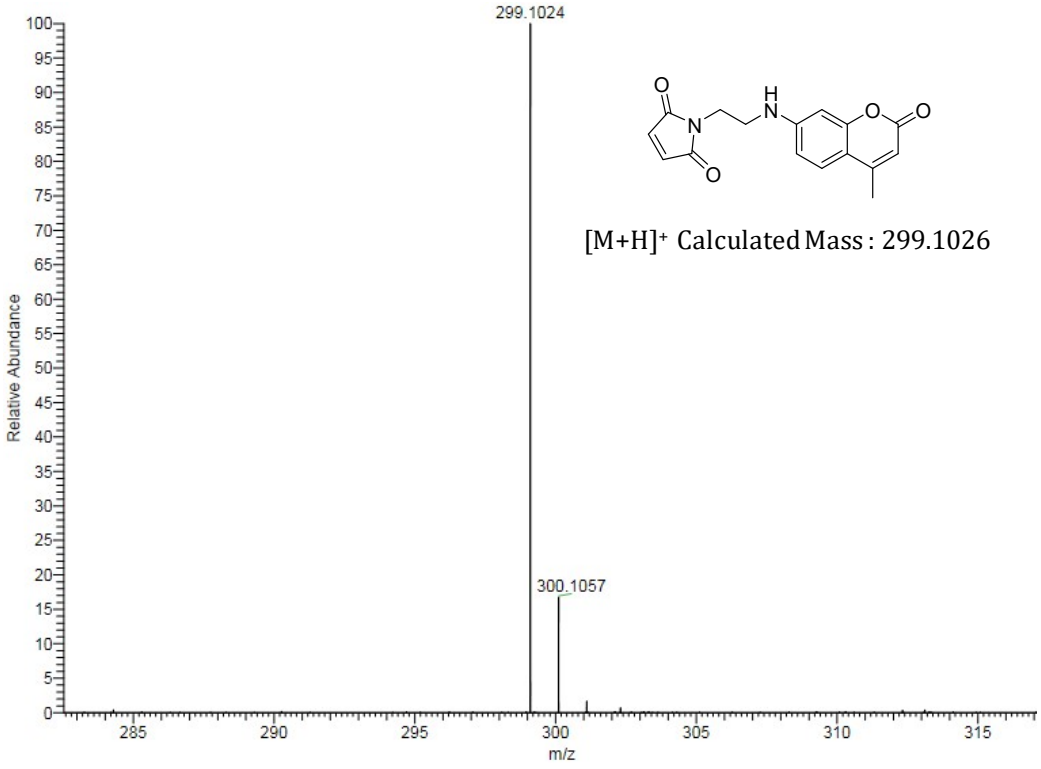


Figure S32. ESI-TOF-MS spectrum of probe **2**.

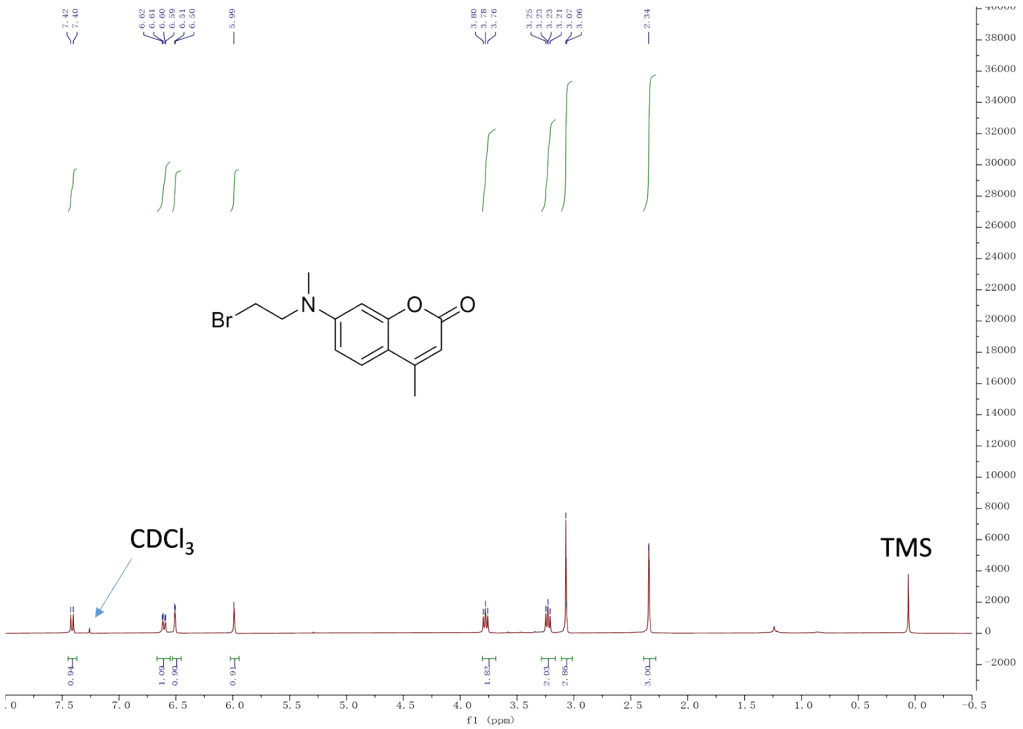
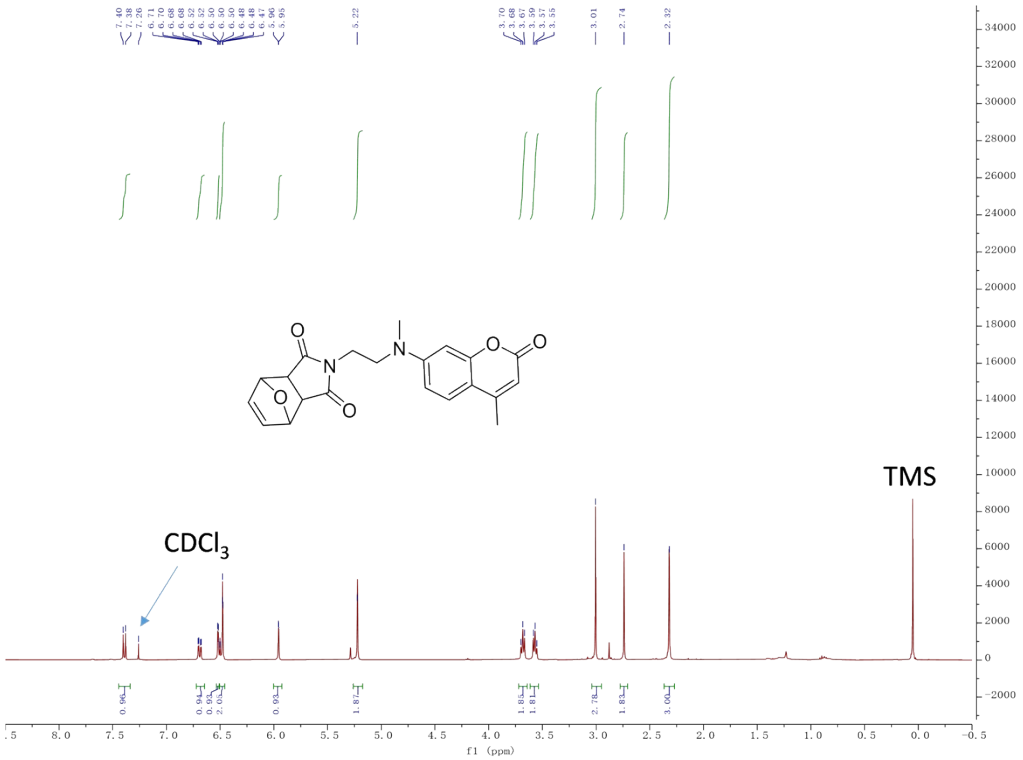


Figure S33. ^{1}H-NMR spectrum of **3a** ($CDCl_3$-*d*).



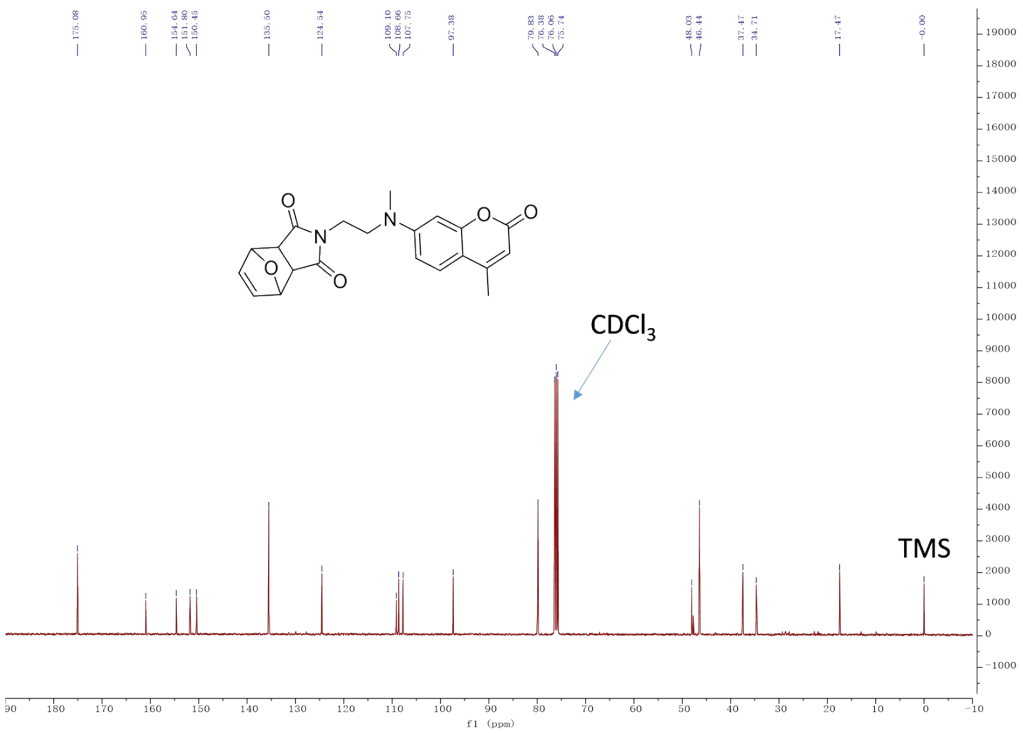


Figure S34. ^{1}H NMR and ^{13}C NMR spectra of **3b** ($CDCl_3$-*d*).

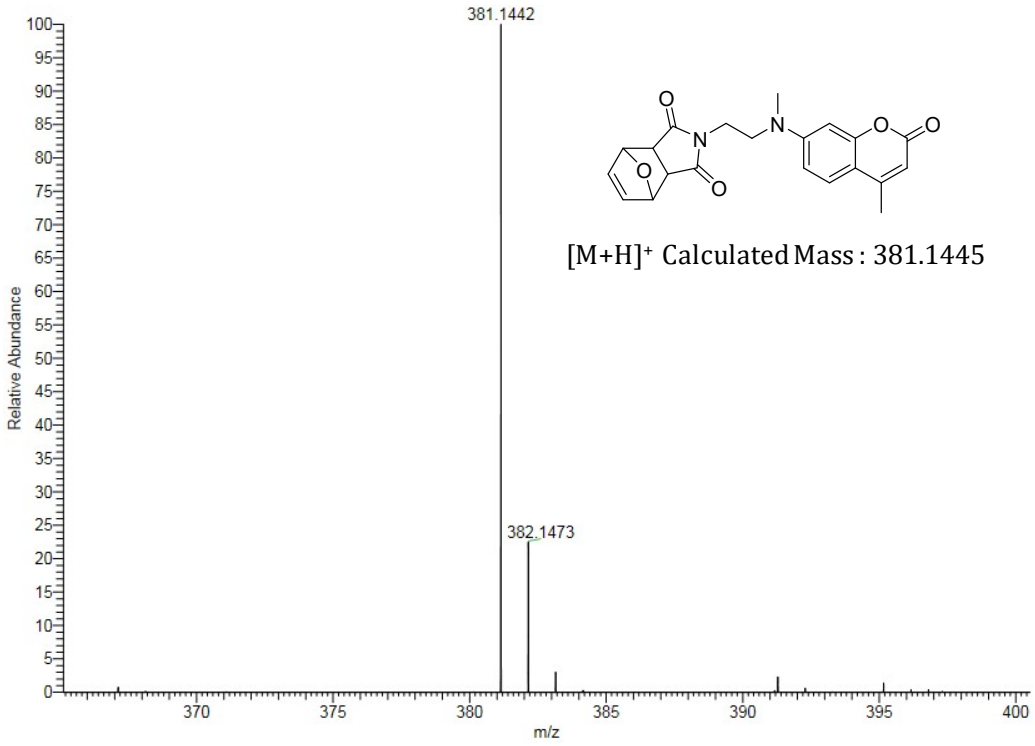


Figure S35. ESI-TOF-MS spectrum of **3b**.

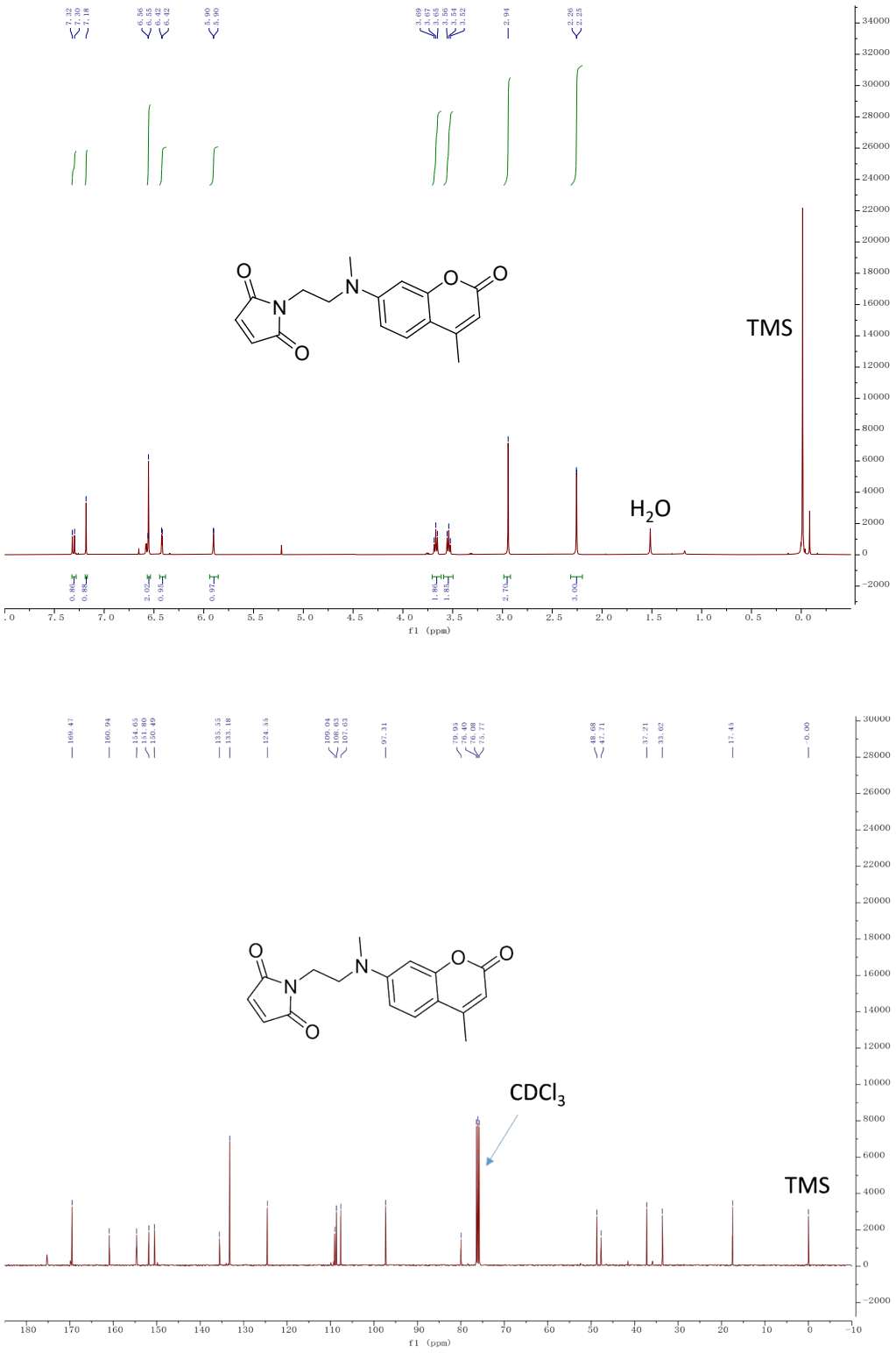


Figure S36. ^{1}H NMR and ^{13}C NMR spectra of probe **3** ($CDCl_3$-*d*).

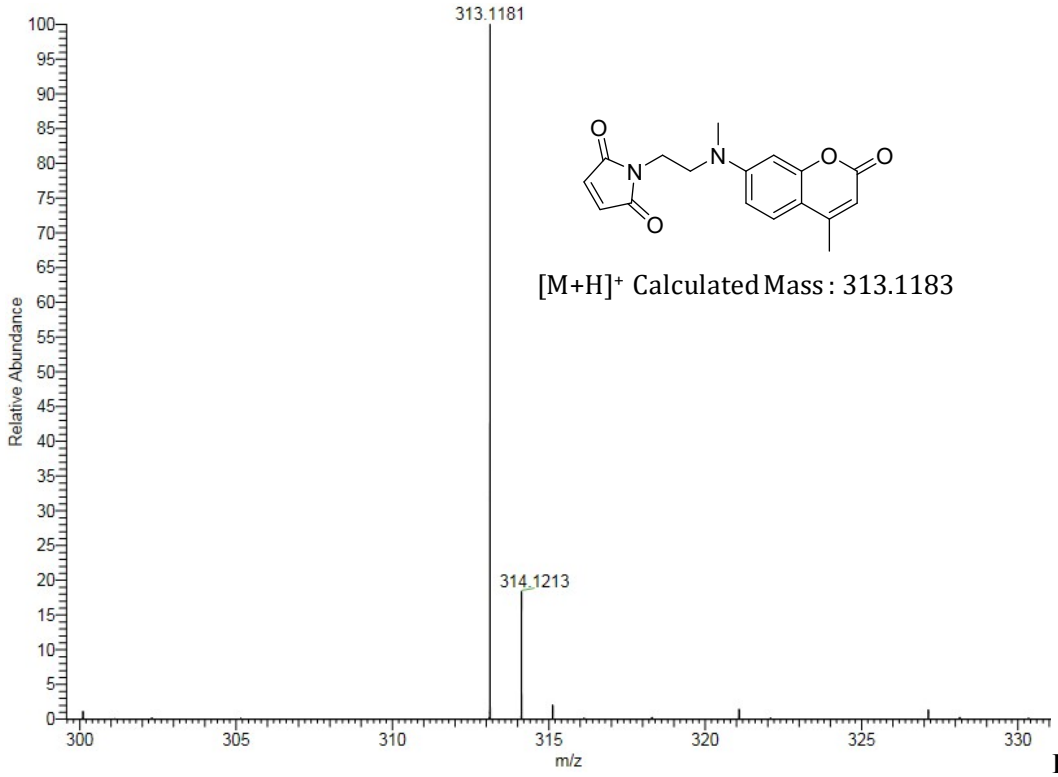


Figure S37. ESI-TOF-MS spectrum of probe **3**.

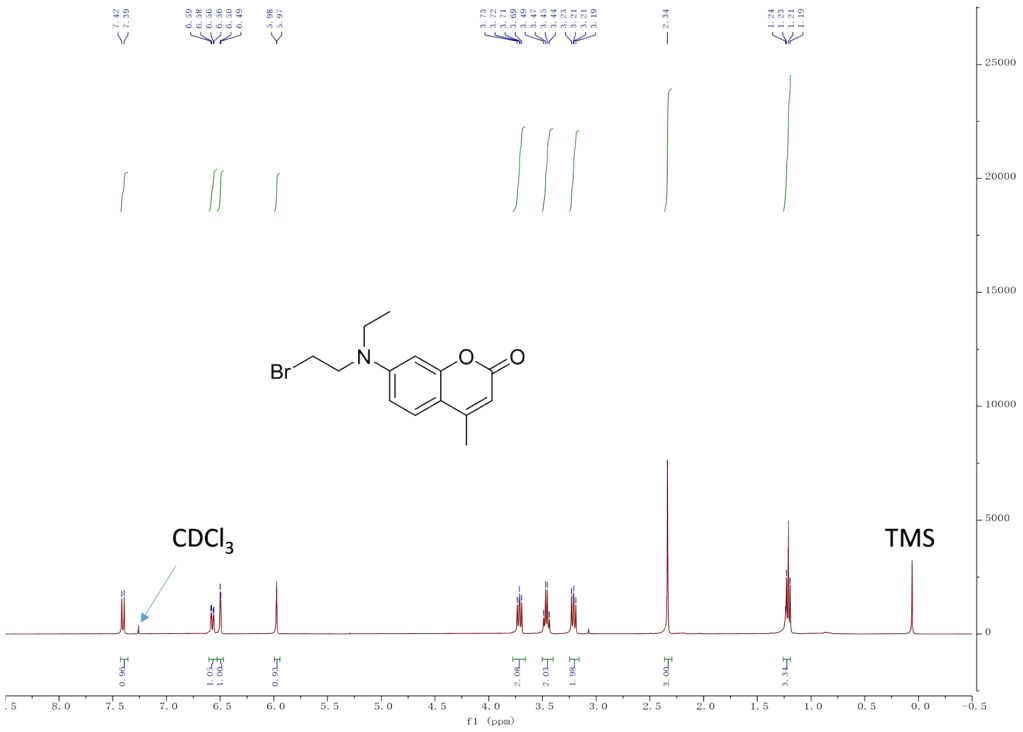


Figure S38. ^{1}H-NMR spectrum of **4a** ($CDCl_3$-*d*).

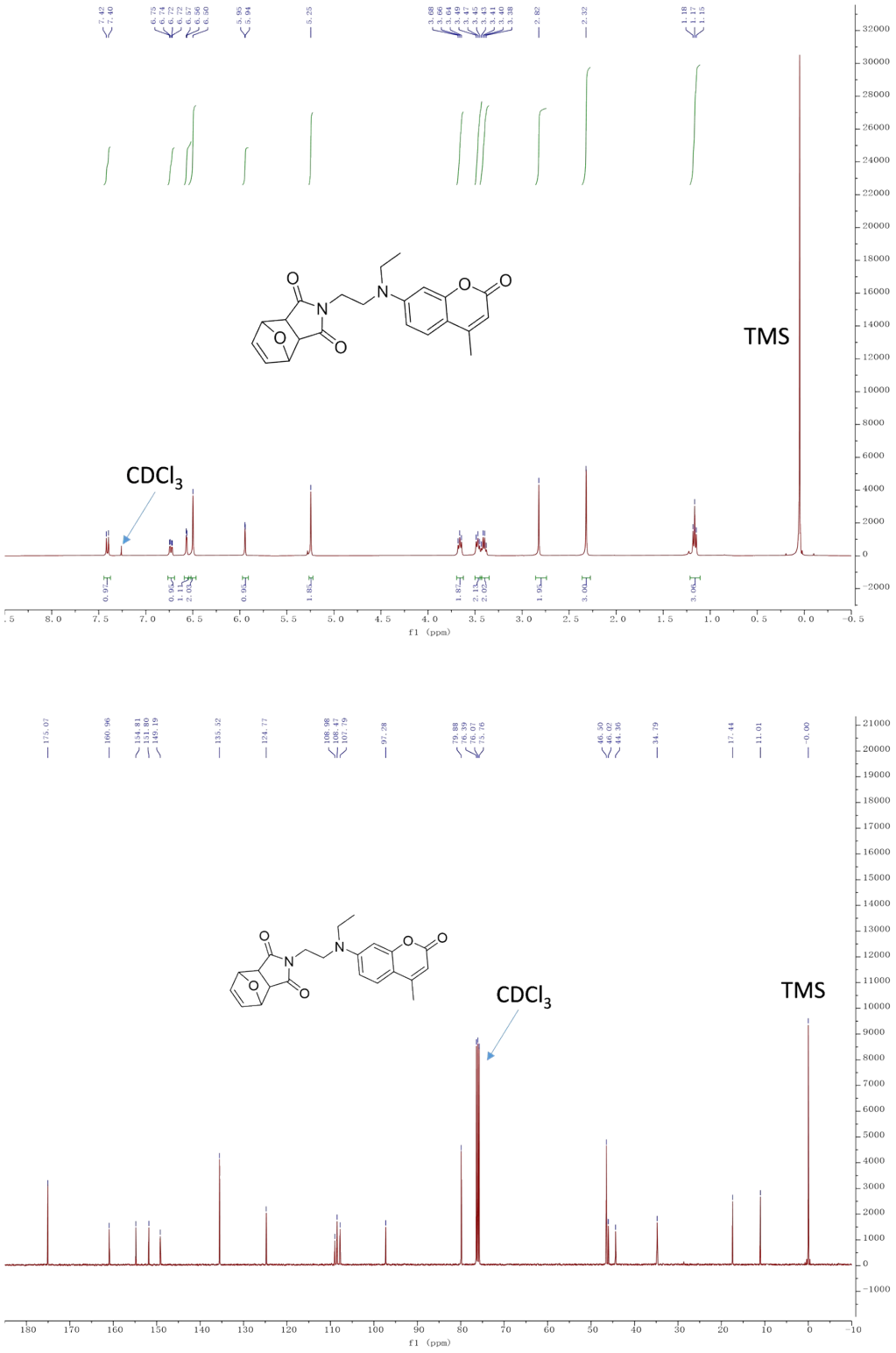


Figure S39. ^{1}H NMR and ^{13}C NMR spectra of **4b** ($CDCl_3$-*d*).

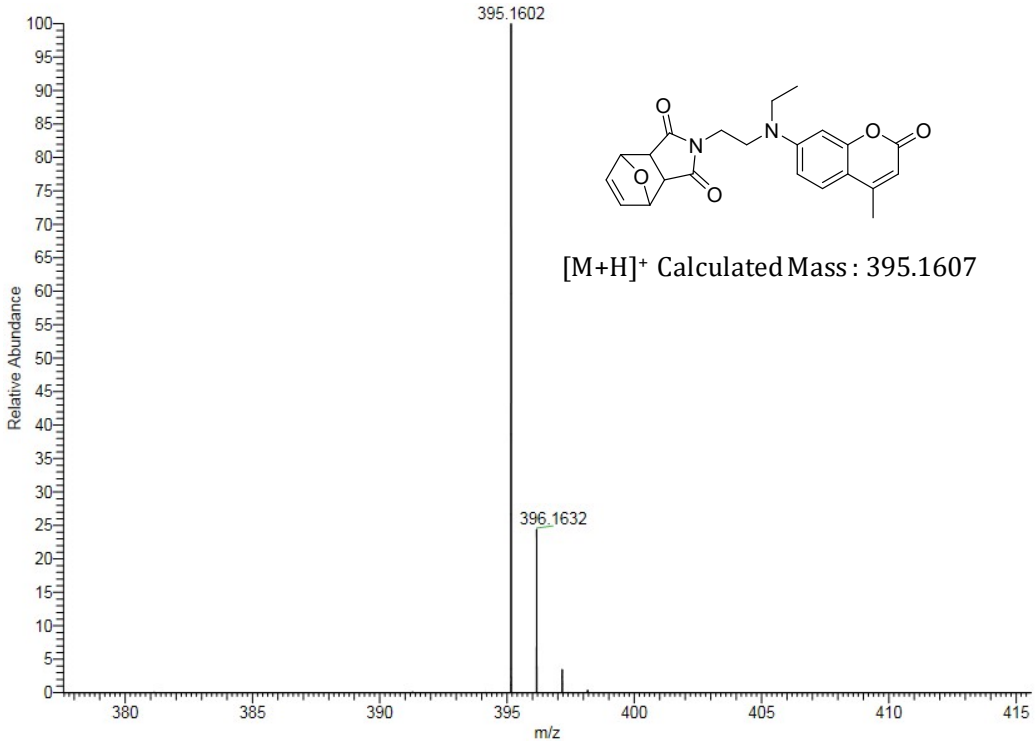
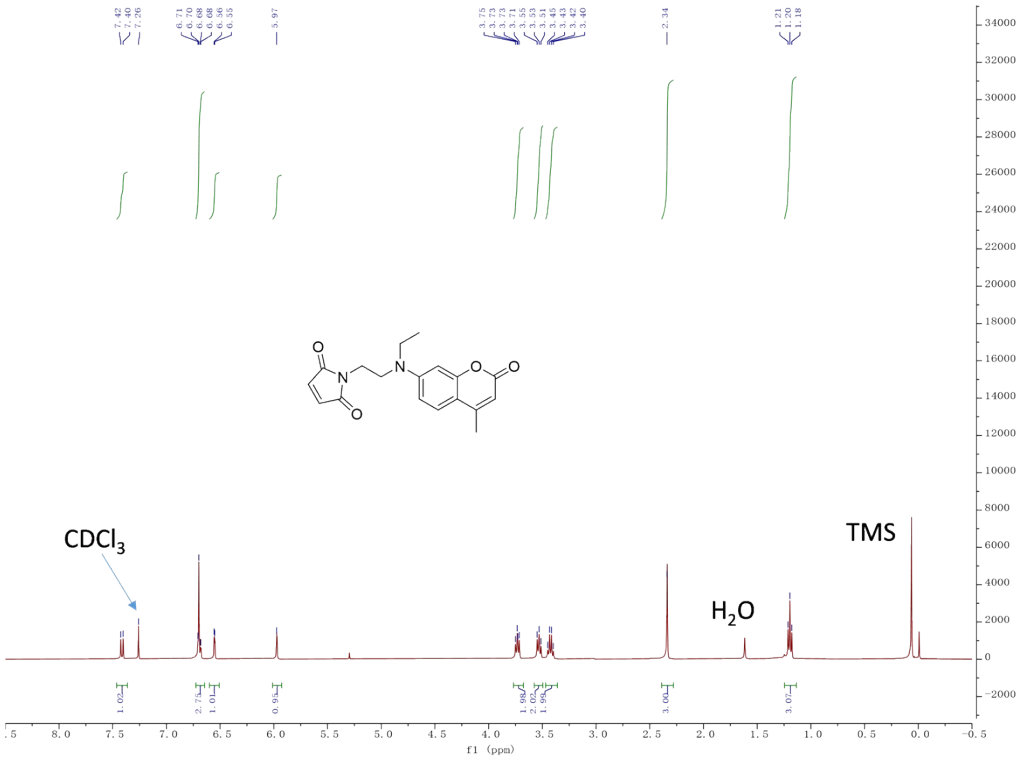


Figure S40. ESI-TOF-MS spectrum of **4b**.



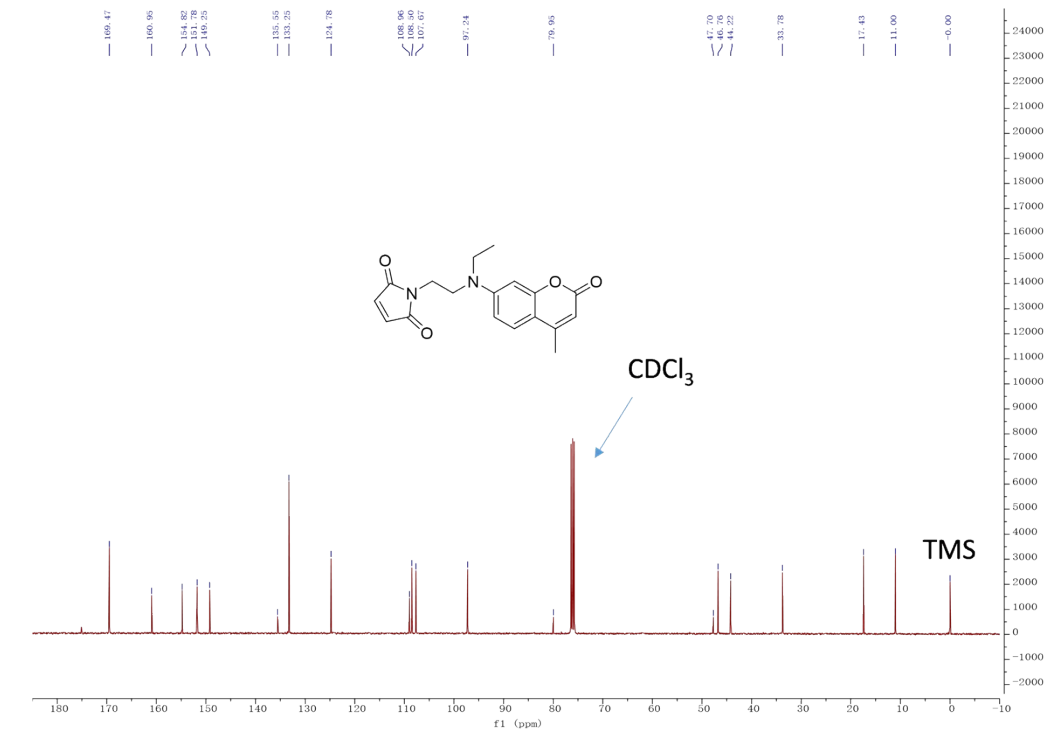


Figure S41. ^{1}H NMR and ^{13}C NMR spectra of probe **4** ($CDCl_3$-*d*).

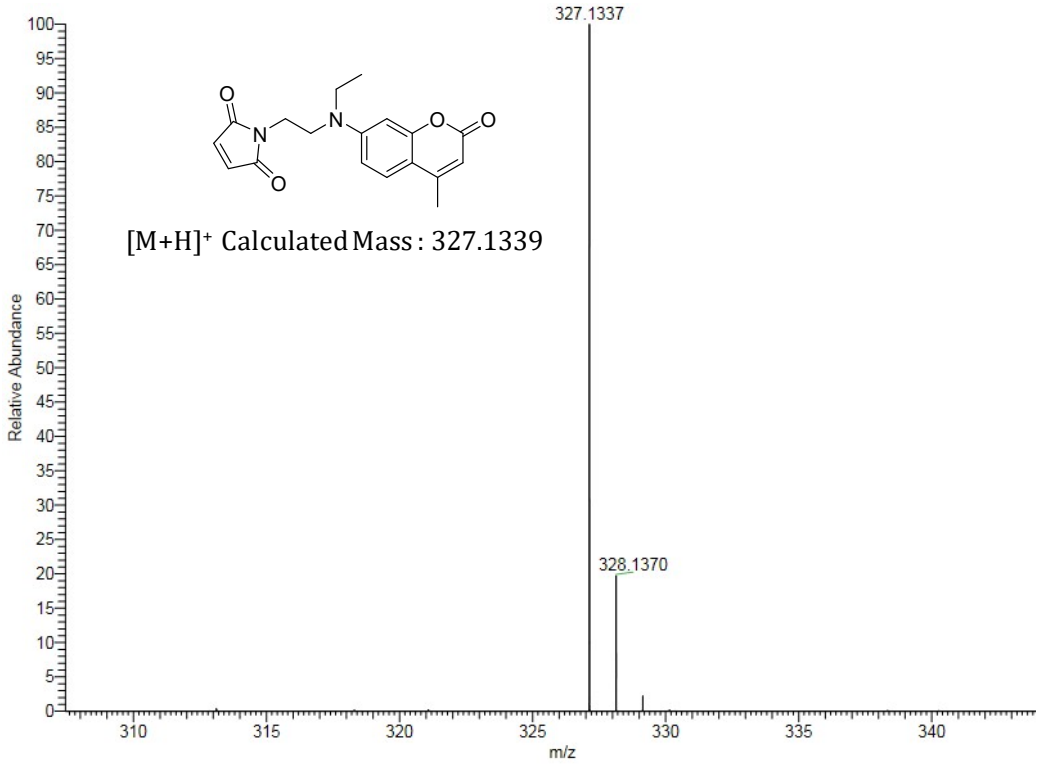


Figure S42. ESI-TOF-MS spectrum of probe **4**.

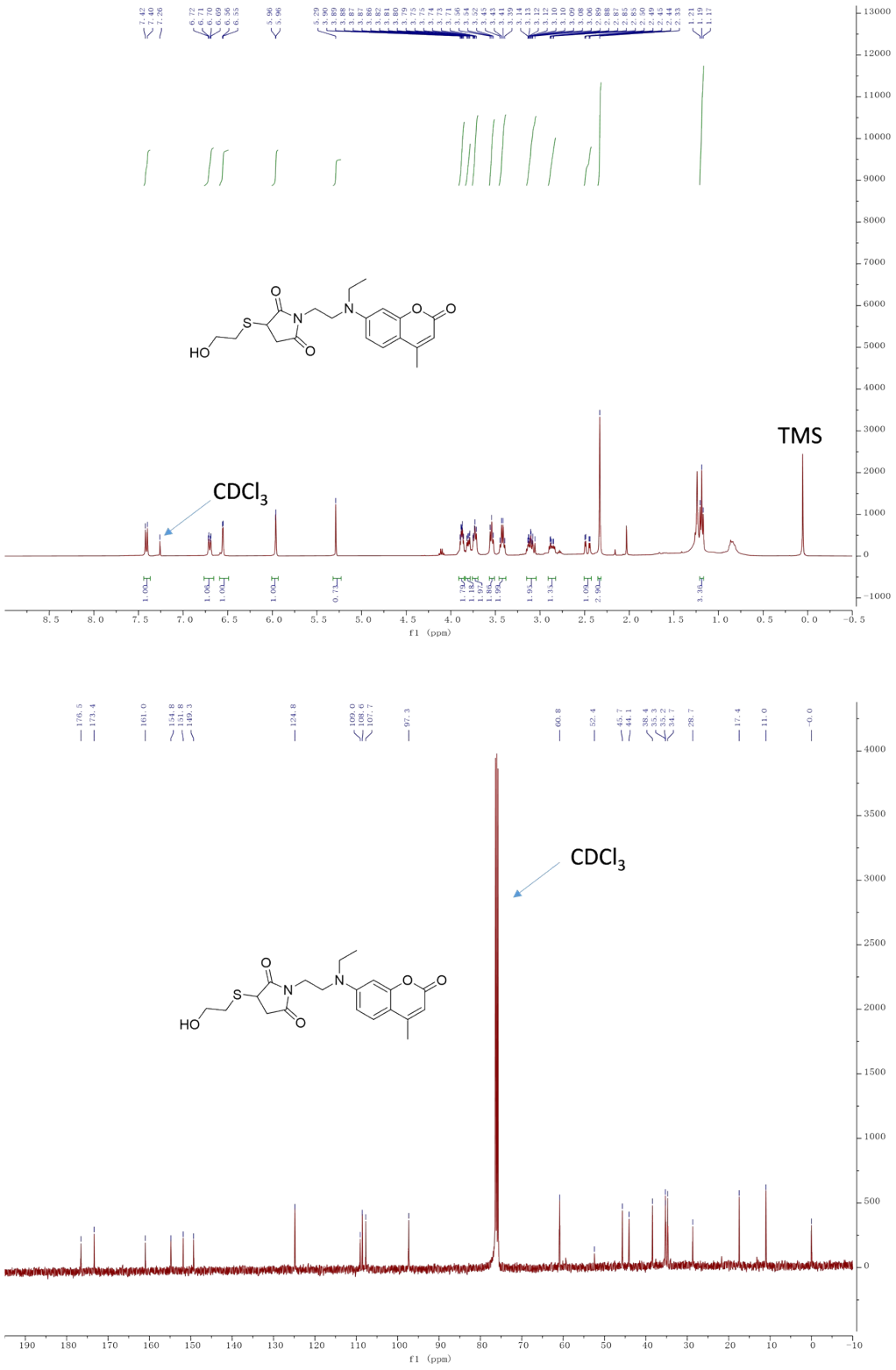


Figure S43. ^{1}H NMR and ^{13}C NMR spectra of **5** ($CDCl_3$-*d*).

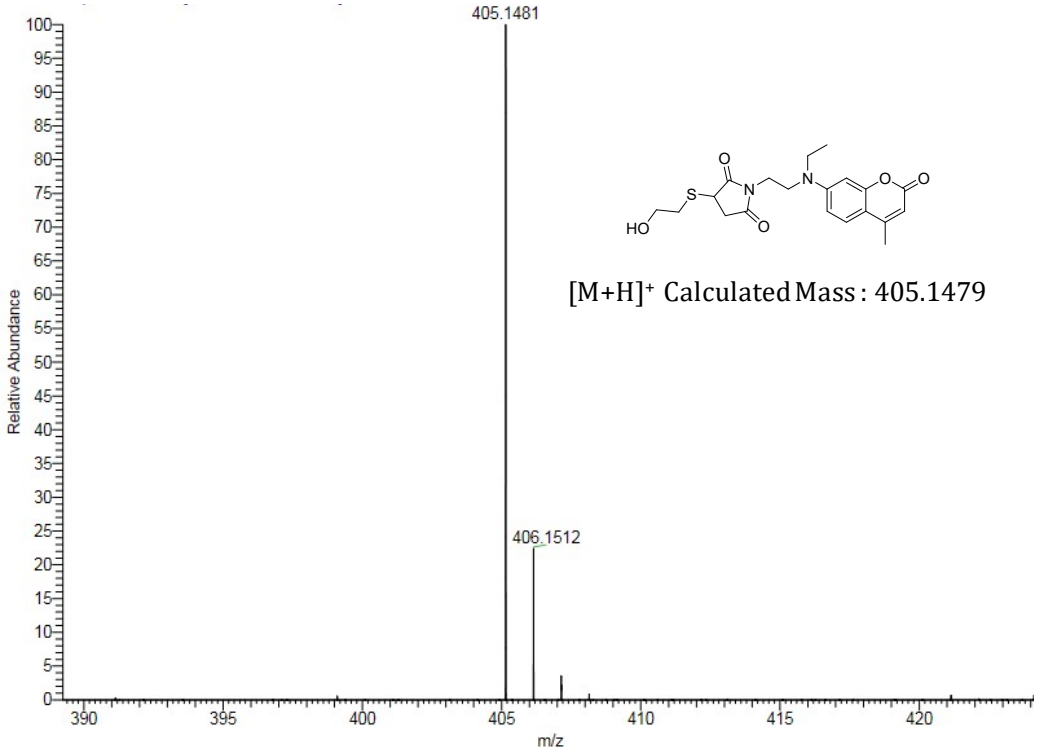


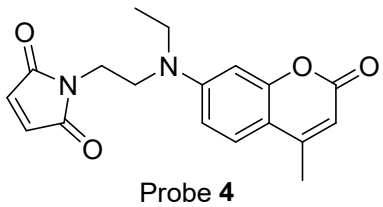
Figure S44. ESI-TOF-MS spectrum of **5**.

Computational Methods.

The Gaussian 16 package was used for all geometry optimizations, which were determined using the ωB97XD/def2TZVP level[1]. Gibbs free energy and enthalpy were calculated at 298.15K.

Coordinates of Calculated Structures.

Probe **4**:



Probe **4**

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Standard orientation:

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3	6	0	1.597395	-1.521851	0.236202
4	6	0	2.864069	-0.956034	0.064850
5	6	0	2.913834	0.430809	-0.081527
6	6	0	1.779165	1.220642	-0.057117
7	6	0	4.104404	-1.694661	0.029327
8	6	0	5.255063	-1.016361	-0.139633
9	6	0	5.299300	0.426268	-0.286913
10	8	0	4.088938	1.082939	-0.248305
11	8	0	6.286675	1.088797	-0.437733
12	6	0	4.091139	-3.183718	0.179867
13	7	0	-0.621559	1.410460	0.129617
14	6	0	-1.902084	0.841524	0.467050
15	6	0	-2.612552	0.251928	-0.754161
16	7	0	-3.851790	-0.393699	-0.390465
17	6	0	-3.940750	-1.685241	0.105100
18	6	0	-5.395968	-1.929502	0.371121
19	6	0	-6.071589	-0.832952	0.061480
20	6	0	-5.104823	0.203757	-0.429662
21	8	0	-3.015309	-2.431974	0.279109
22	8	0	-5.334962	1.326168	-0.786922
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24	6	0	-0.292078	3.660637	1.143357
25	1	0	-0.493421	-1.269559	0.375294
26	1	0	1.504867	-2.594723	0.346741
27	1	0	1.917969	2.285978	-0.157335
28	1	0	6.214723	-1.512521	-0.174176
29	1	0	5.099757	-3.589602	0.130501
30	1	0	3.648107	-3.471177	1.135830
31	1	0	3.492357	-3.646177	-0.607769
32	1	0	-2.529416	1.623655	0.899589
33	1	0	-1.791557	0.071779	1.232858
34	1	0	-1.972717	-0.484044	-1.240605
35	1	0	-2.845284	1.038117	-1.472041
36	1	0	-5.756635	-2.870622	0.755765
37	1	0	-7.131103	-0.640323	0.125651
38	1	0	-1.555221	3.130646	-0.518370
39	1	0	0.144427	3.058764	-0.889878
40	1	0	-0.280012	4.726174	0.908705
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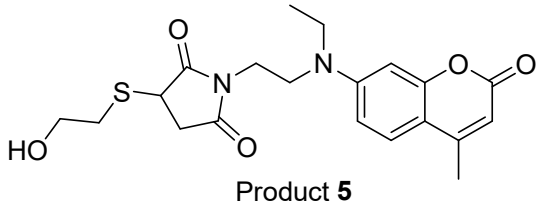
42	1	0	0.674283	3.391629	1.570876

Product **5**:



Product **5**

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Standard orientation:

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3	6	0	2.319964	1.431844	-0.102229
4	6	0	3.701410	1.219203	-0.136962
5	6	0	4.132532	-0.103566	-0.029486
6	6	0	3.253047	-1.161483	0.108363
7	6	0	4.696883	2.255769	-0.274147
8	6	0	5.996878	1.905186	-0.288615
9	6	0	6.437619	0.528087	-0.171509
10	8	0	5.448991	-0.422370	-0.046097
11	8	0	7.575355	0.150228	-0.174242
12	6	0	4.273251	3.686155	-0.395911
13	7	0	0.984521	-1.974541	0.267720
14	6	0	-0.422406	-1.733974	0.466944
15	6	0	-1.174734	-1.571107	-0.856424
16	7	0	-2.563418	-1.236531	-0.630691
17	6	0	-2.984013	0.054110	-0.364633
18	6	0	-4.478325	0.005041	-0.061601
19	6	0	-4.886901	-1.425904	-0.409851
20	6	0	-3.585194	-2.172351	-0.605065
21	8	0	-2.256049	1.010284	-0.405103
22	8	0	-3.427518	-3.355528	-0.739721
23	16	0	-5.427446	1.269365	-0.942790
24	6	0	-5.650889	2.578202	0.292252
25	6	0	-4.420682	3.008572	1.083469
26	8	0	-4.131946	2.133846	2.160808
27	6	0	1.419178	-3.359024	0.284238

28	6	0	1.828364	-3.852843	1.666881
29	1	0	0.368729	0.636621	0.027417
30	1	0	1.934823	2.439740	-0.189978
31	1	0	3.679067	-2.148108	0.205502
32	1	0	6.787714	2.635245	-0.388133
33	1	0	5.136703	4.341814	-0.491445
34	1	0	3.632992	3.825958	-1.269588
35	1	0	3.698748	3.994385	0.480282
36	1	0	-0.577092	-0.849084	1.086702
37	1	0	-0.847300	-2.578060	1.013833
38	1	0	-1.144400	-2.498785	-1.426396
39	1	0	-0.725917	-0.773779	-1.447772
40	1	0	-4.572181	0.204088	1.006060
41	1	0	-5.443526	-1.477602	-1.347770
42	1	0	-5.490201	-1.910830	0.354622
43	1	0	-6.451862	2.296696	0.980424
44	1	0	-6.014241	3.418320	-0.303053
45	1	0	-4.580728	4.027598	1.453979
46	1	0	-3.538367	3.007468	0.445800
47	1	0	-4.767259	2.288958	2.861148
48	1	0	2.235438	-3.488921	-0.427958
49	1	0	0.593996	-3.966199	-0.094206
50	1	0	2.141169	-4.897276	1.622591
51	1	0	2.654125	-3.262915	2.065777
52	1	0	0.992575	-3.778130	2.365012

Reference:

1. Gaussian 16, Revision C.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.