Supporting Information

Noninvasive ratiometric fluorescence imaging of γ-glutamyltransferase activity using an activatable probe

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Figure S1. ^{1}H-NMR spectra of compound **4** ($CDCl_3$)

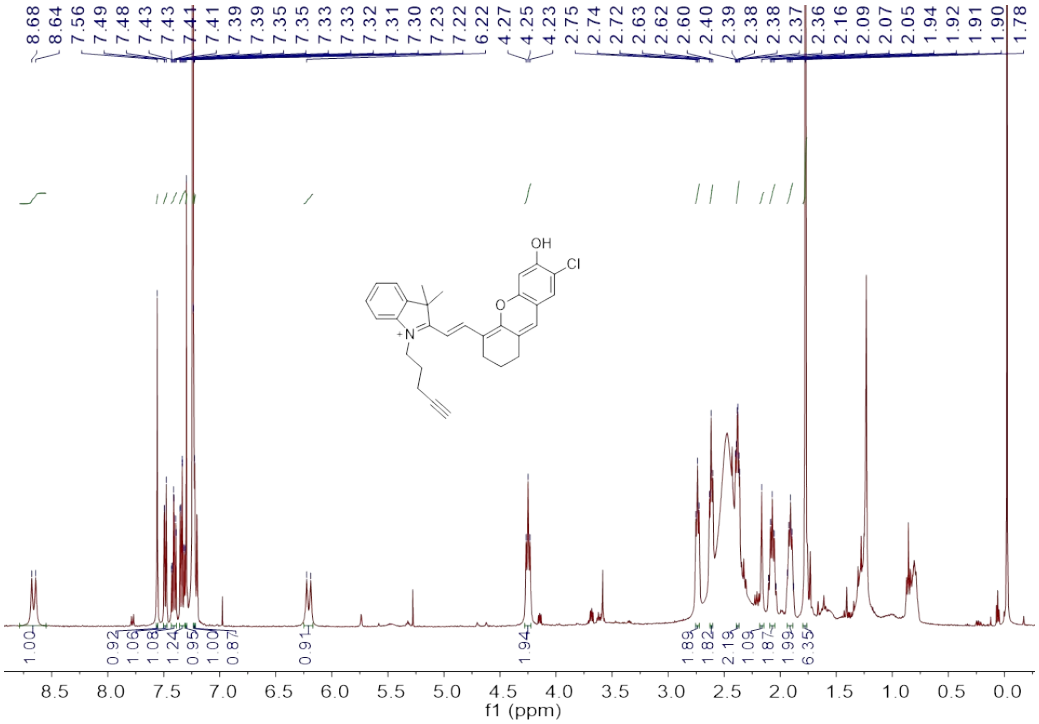


Figure S2.^{13}C-NMR spectra of compound **4** ($CDCl_3$)

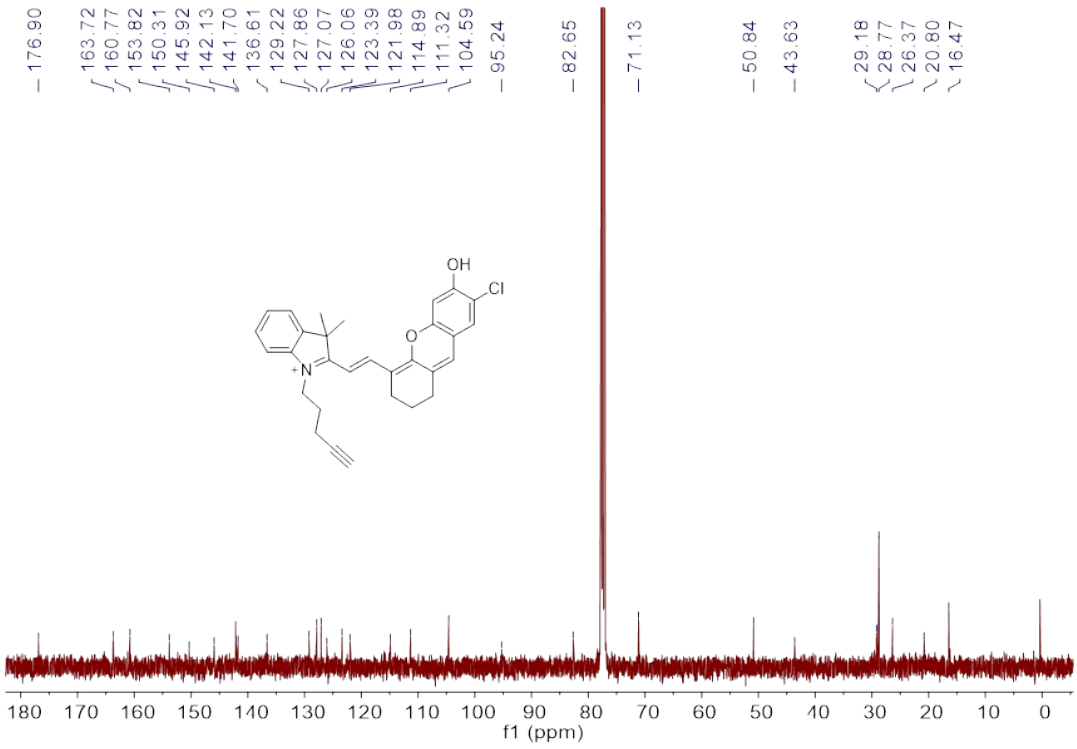


Figure S3. ^{1}H-NMR spectra of compound **6** ($CDCl_3$)

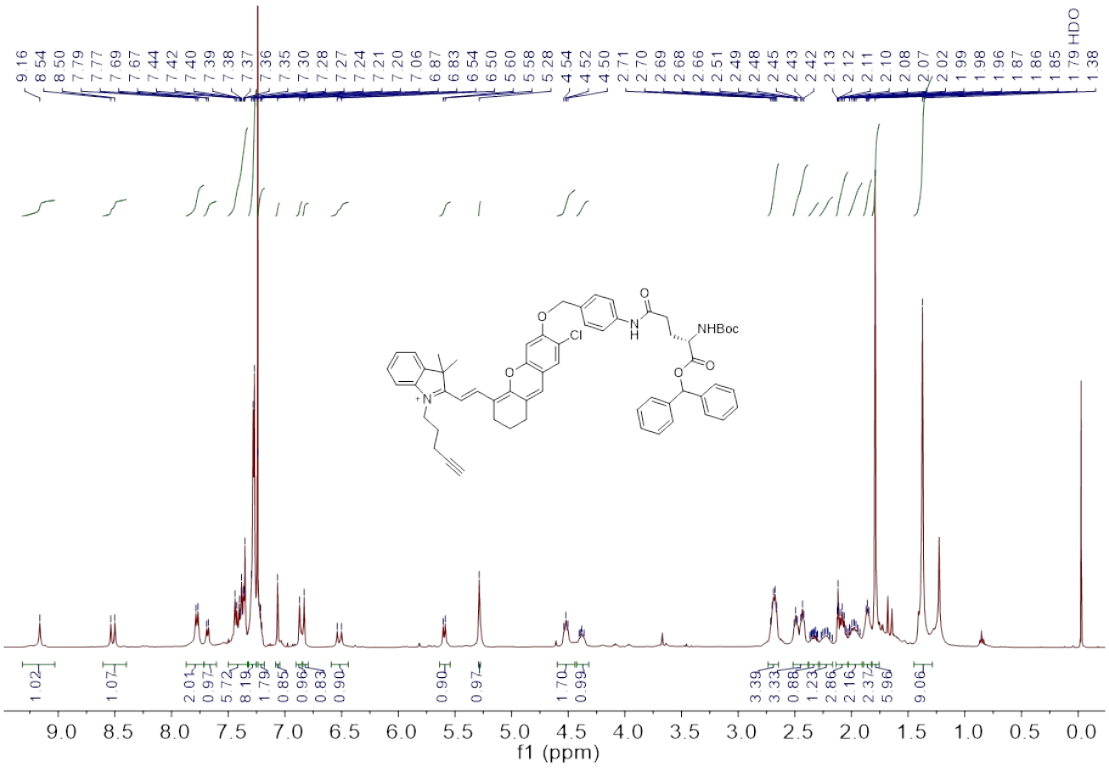


Figure S4.^{13}C-NMR spectra of compound **6** ($CDCl_3$)

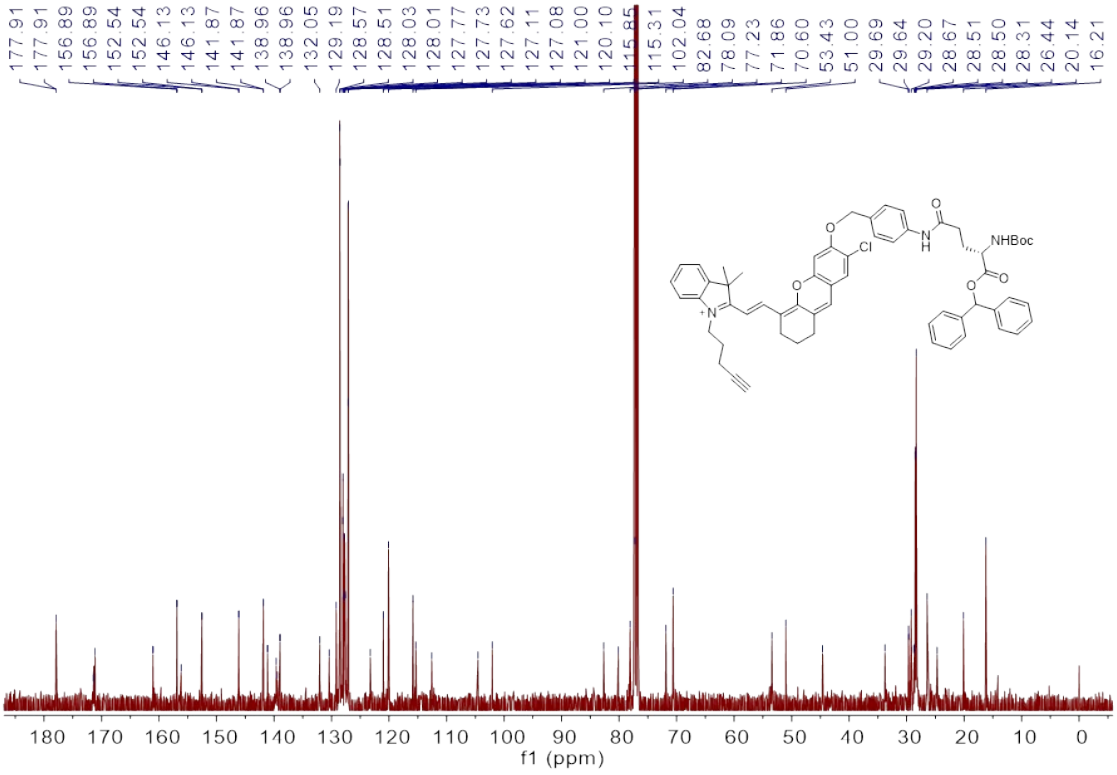


Figure S5. ^{1}H-NMR spectra of compound **7** (DMSO-d_6)

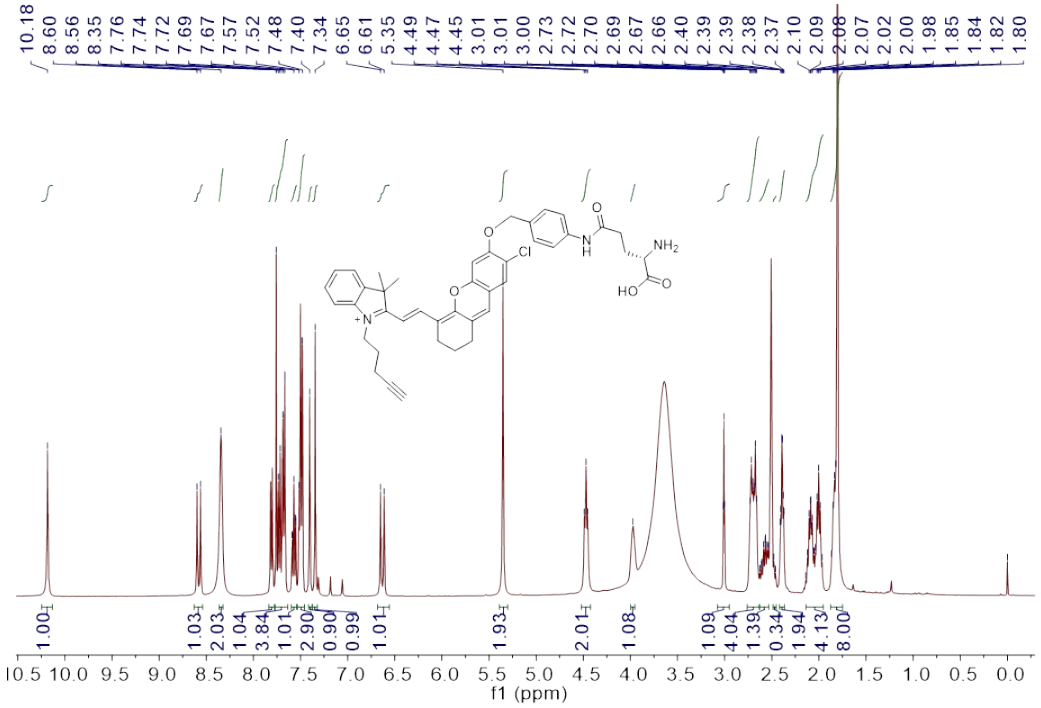


Figure S6. ^{13}C-NMR spectra of compound **7** (DMSO-d_6)

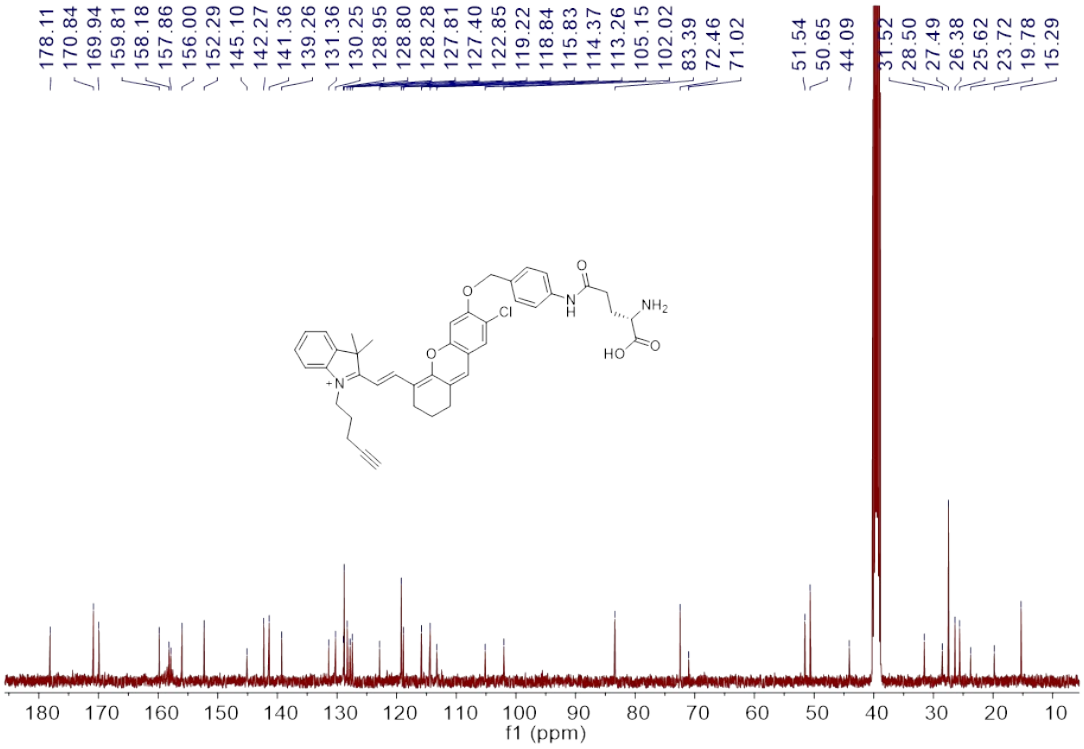


Figure S7. ^{1}H-NMR spectra of probe **1** (DMSO-d_6)

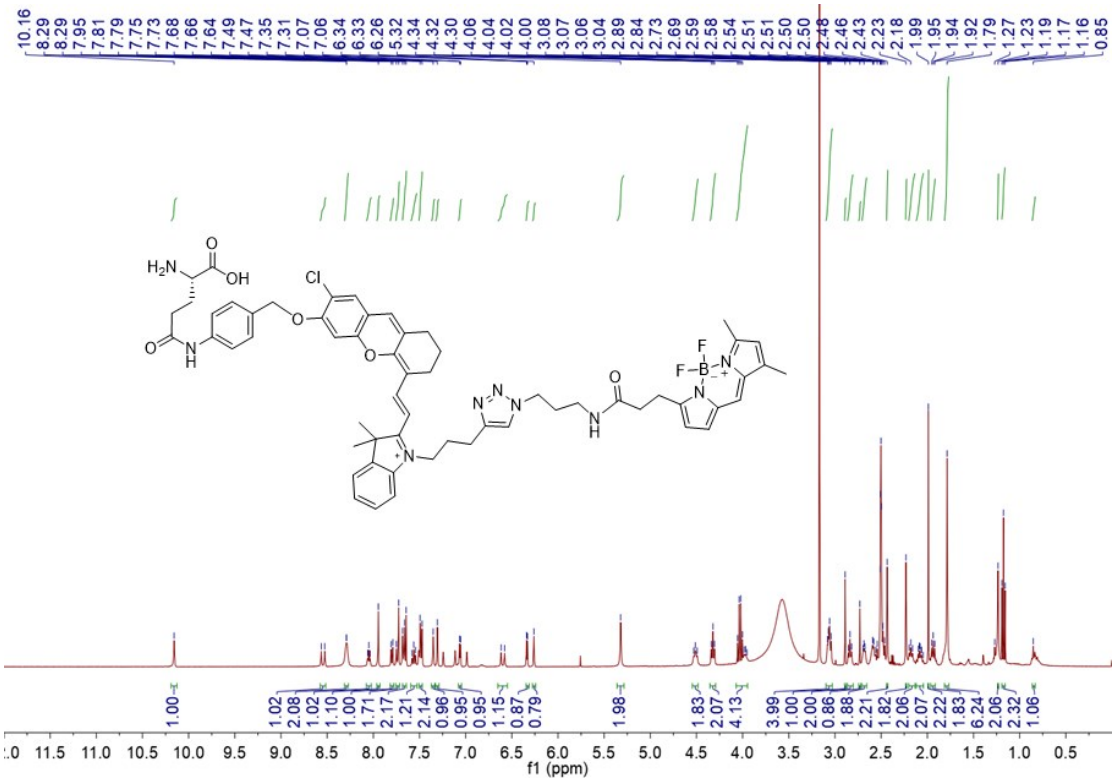


Figure S8. HRMS spectrum of compound **4**

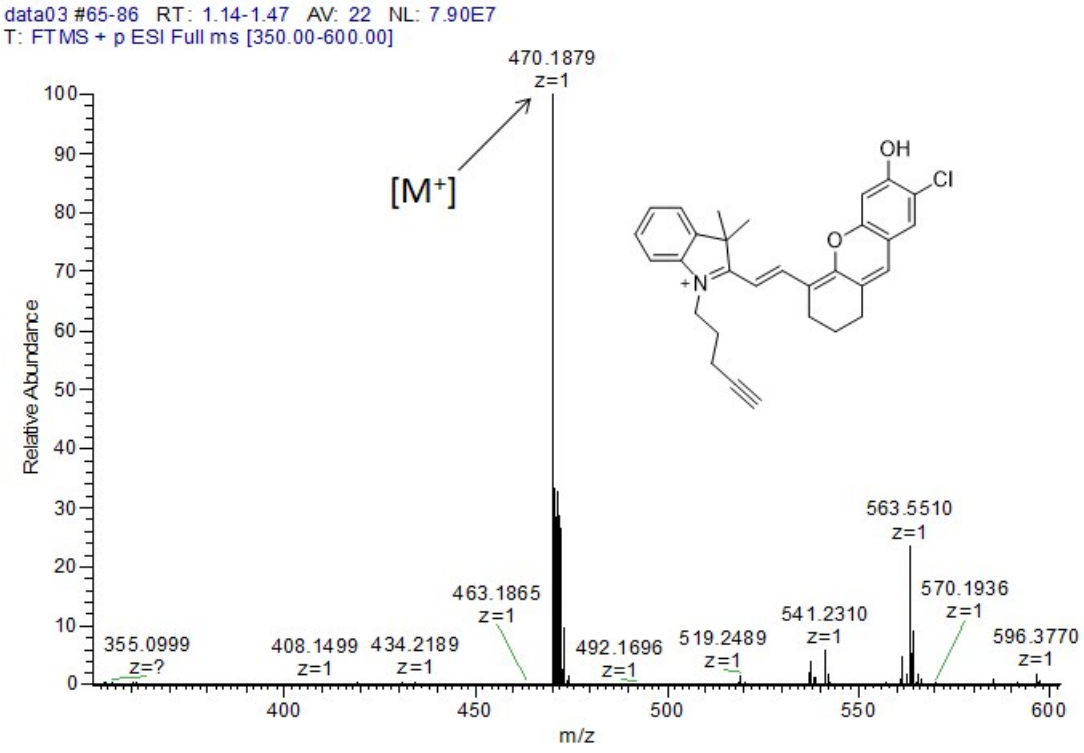


Figure S9. HRMS spectrum of compound **6**:

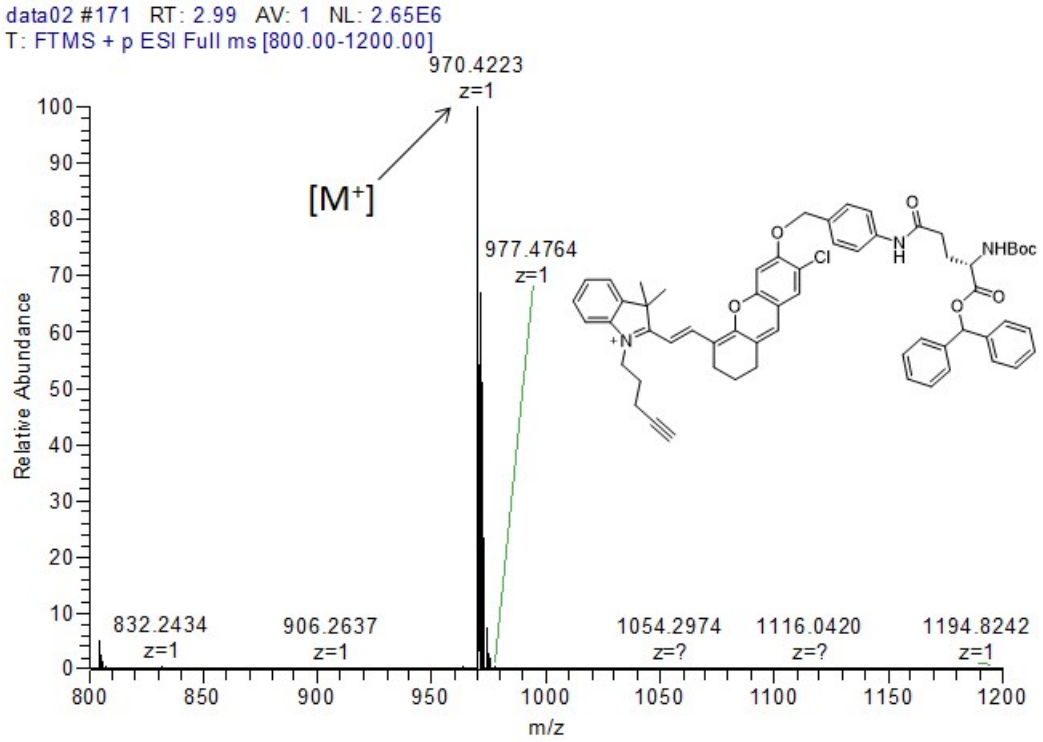


Figure S10. HRMS spectrum of compound **7**:

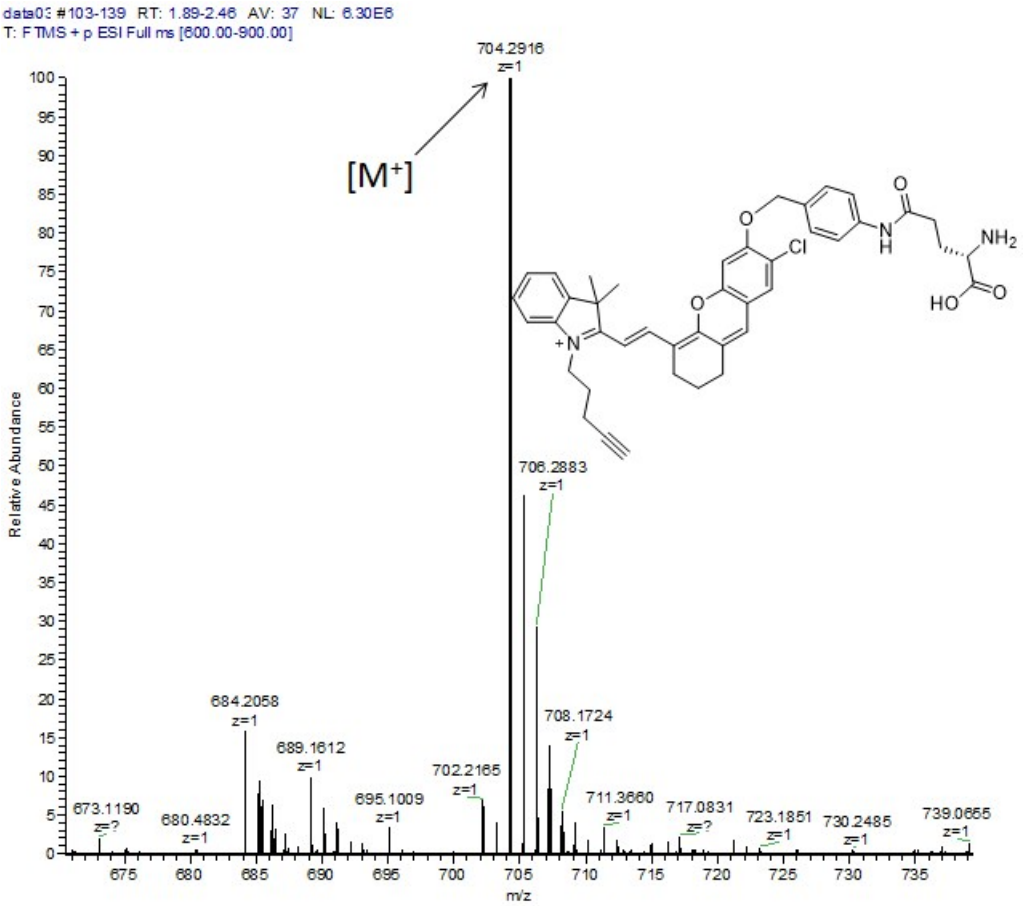


Figure S11. HRMS spectrum of probe **1**:

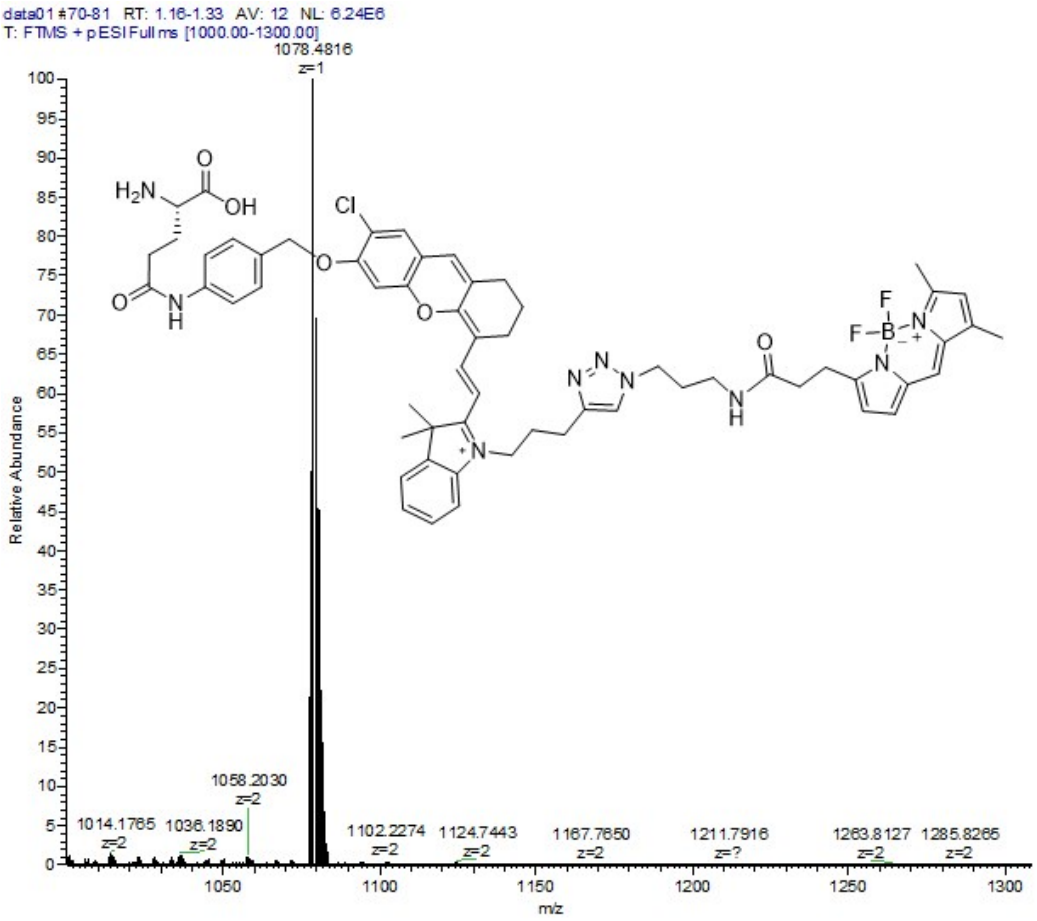


Figure S12. HRMS spectrum of the product **2**. MS: calcd. For $C_{47}H_{50}BClF_2N_7O_3^+$ [M^+]: 844.3719; HRMS found: m/z 844.3783.

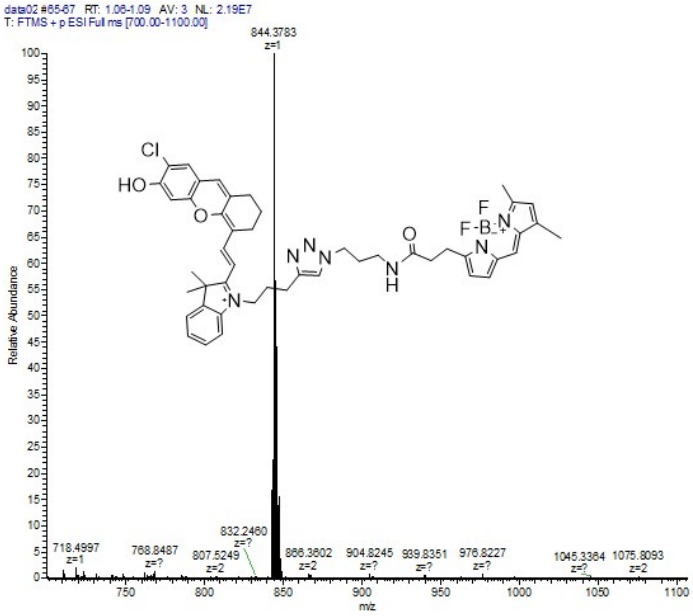


Figure S13. Kinetic evaluation for the GGT enzyme-catalyzed reaction. (a) Plots of **2** concentrations released from probe **1** vs. time under different probe concentrations. (b) Michaelis-Menten plots of **1** with GGT.

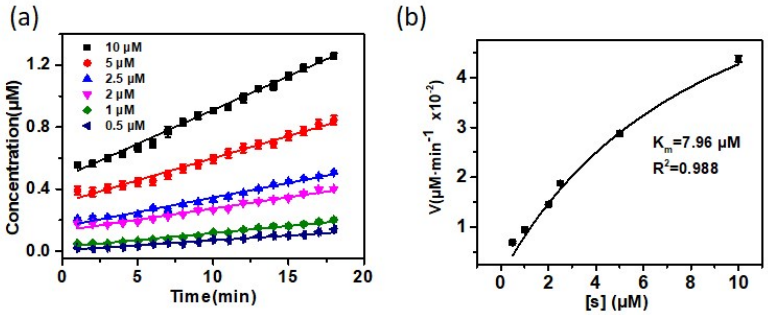


Figure S14. Stability test. (a) Time-dependent ratiometric fluorescence ratio ($I_{735/517}$) changes of probe **1** (5 μM) following incubation in PBS buffer (pH=7.4) (A), DMEM (B), PBS buffer (pH=7.4) containing 10% mice serum (C) or DMEM containing 10% FBS (D), PBS buffer containing 500 U/L GGT (E) for different time. Error bars represent standard deviation (n = 3). (b) HPLC analysis of probe **1** (5 μM) after 5 h incubation with conditions indicated in (a).

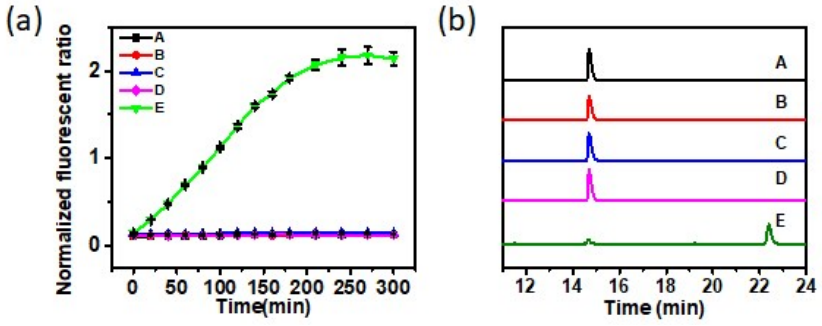


Figure S15. Viability of HeLa cells. The cells were incubated with probe **1** at 0, 0.5, 1, 2, 5, 10 μM for 24 h, and the cell viability was determined by MTT assay. Error bars are standard deviation (n = 3).

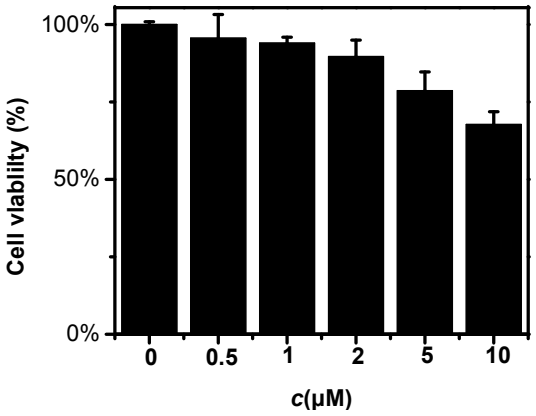


Figure S16. Optimization the incubation conditions for GGT detection with probe **1** based on fluorescence imaging. (a) Fluorescence imaging of HeLa cells following incubation with probe **1** (2 μM) for 0, 15, 30, 45, 60 and 120 min. Scale bar: 20 μm. (b) The intracellular ratiometric fluorescence ratio of the corresponding fluorescence images in (a).

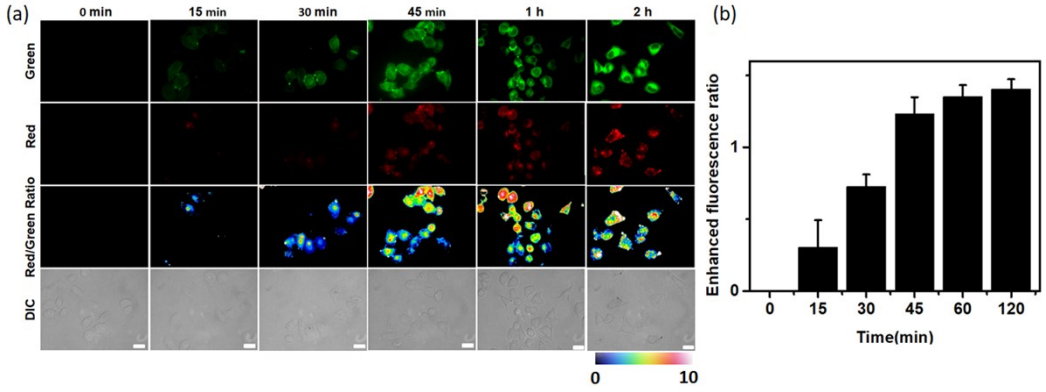


Figure S17. Concentration-dependent fluorescence imaging of HeLa cells incubated with probe **1** (a) Fluorescence imaging of HeLa cells incubated with 0.2, 0.5, 1, 2, 3 and 5 μM probe **1** for 2 h. Scale bar: 20 μm. (b) The intracellular fluorescence ratio of the corresponding fluorescence images in (a).

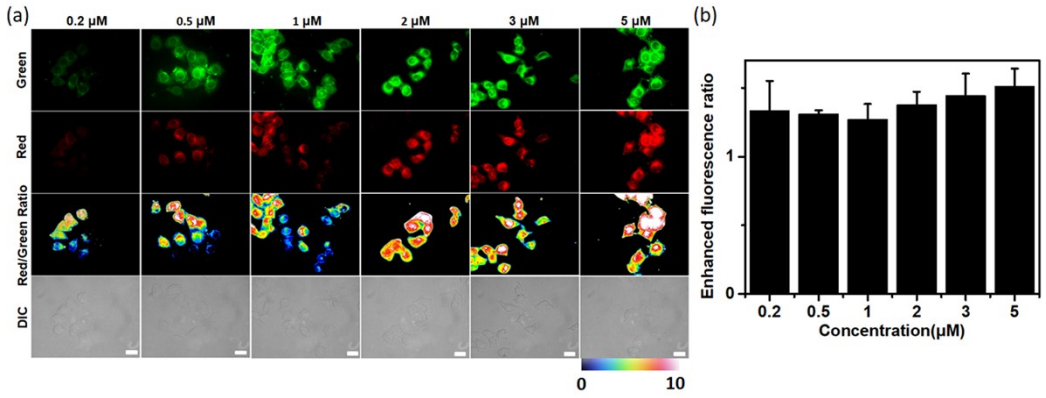


Figure S18. Ratiometric fluorescence imaging of exogenous GGT using probe **1**. (a) Longitudinal fluorescence images (BODIPY: $\lambda_{ex}/\lambda_{em}$= 480/520 ± 20 nm; mCy-Cl: $\lambda_{ex}/\lambda_{em}$= 660/710 ± 20 nm) of a nude mouse receiving subcutaneous injection of probe **1** (10 μM, 50 μL, left) (left) or probe **1** (10 μM, 50 μL) together with GGT (1 U/mL, right). The whole body fluorescence images at BODIPY and mCy-Cl channels were both acquired at 0 min, 10 min, 15 min, 20 min, 30 min, 40 min, 1 h, 1.5 h and 2 h. The ratiometric fluorescence images were produced by dividing the fluorescence intensity of mCy-Cl to that of BODIPY in both injection sites. (b) Plot of normalized fluorescence intensity of BODIPY, (c) mCy-Cl, and (d) fluorescence ratio (mCy-Cl/BODIPY) in both injection regions.

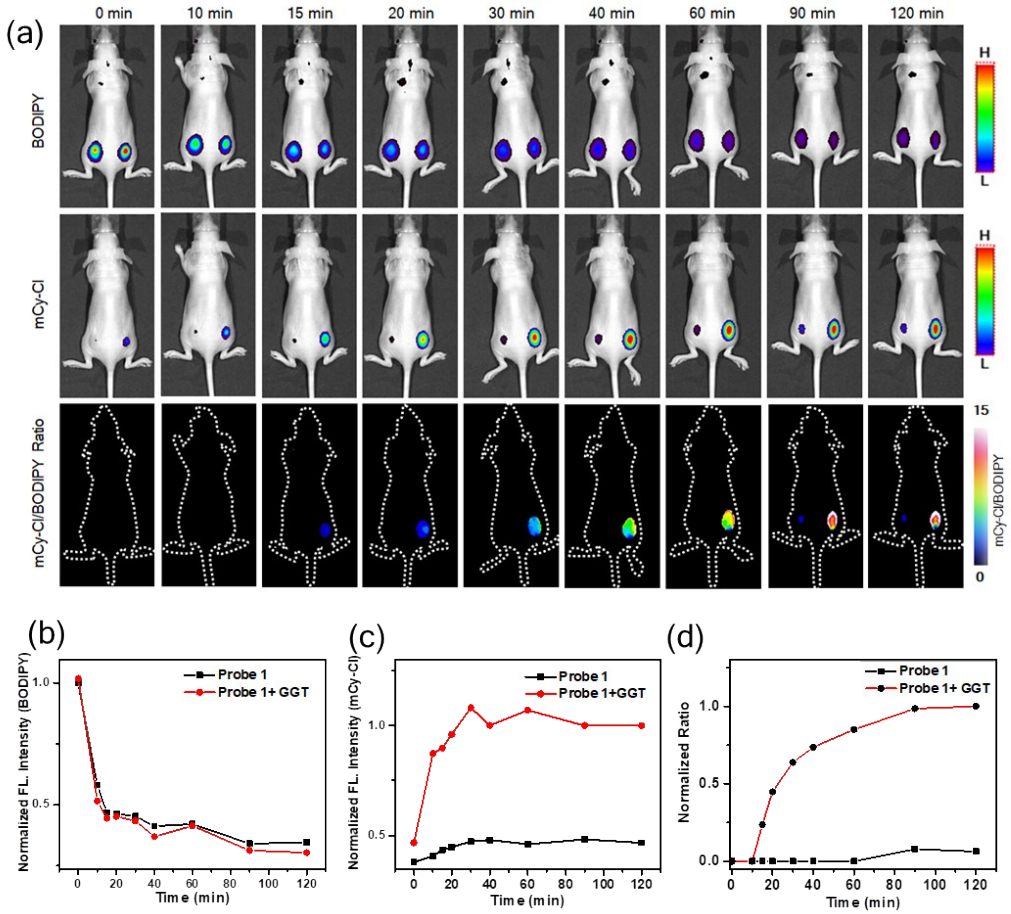
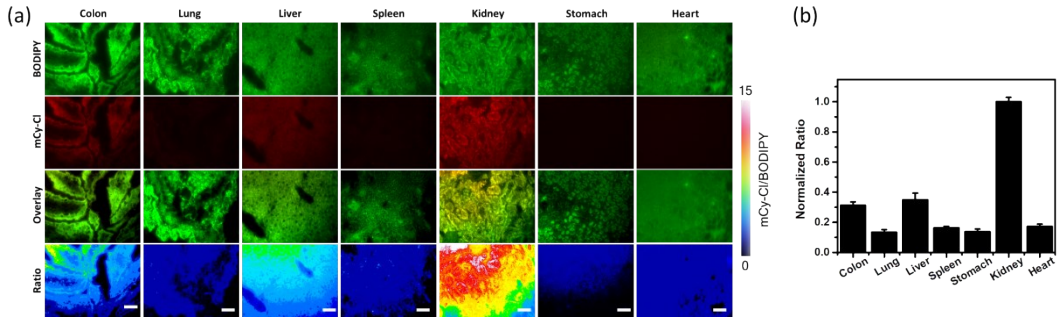


Figure S19. Fluorescence imaging of GGT activity of mouse tissue slices. The tissue slices (4 μm) incubated with probe 1 (2 μM) in DMEM for 2 h, BODIPY (pseudo green, λ_{ex} = 482 ± 12.5 nm, λ_{em} = 510-550 nm); mCy-Cl (pseudo red, λ_{ex} = 650 ± 22.5 nm, λ_{ex} = 690-750 nm). The scale bars are 100 μm. (b) The intracellular ratiometric fluorescence ratio of the corresponding fluorescence images in (a).

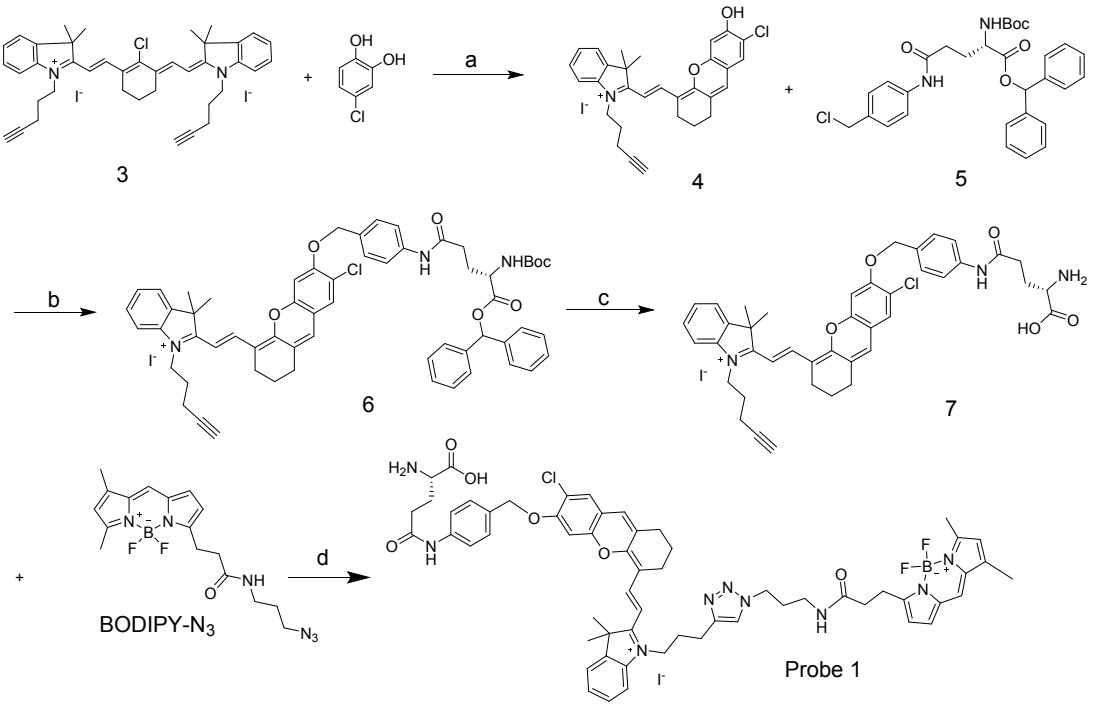


Experimental Procedures

General materials and methods: GGT from equine kidney, GGT inhibitor 3-[[(3-amino-3-carboxypropyl) methoxyphosphinyl] oxy] benzeneacetic acid (GGsTop) and caspase 3 were obtained from R&D systems. Human recombinant MMP-2 was obtained from Sino Biological Inc. Boc-l-glutamic acid 1-tert-butyl ester (Boc-Glu-OtBu) and O-(benzotriazol-1-yl)-*N*, *N*, *N*', *N*'-tetramethyluronium hexafluorophosphate (HBTU) were obtained from GL Biochem（Shanghai）Ltd. BODIPY-N_3 fluorescent dye was obtained from Lumiprobe. 4-Aminobenzyl alcohol was obtained from J&K. All other chemicals were purchased from qualified reagent supplies with analytical reagent grade and used without further purification. GGT was dissolved in water and stored at −80 °C.

The ^{1}H and ^{13}C NMR spectra were acquired on a 400 MHz Bruker Advance III 400 spectrometer. High-performance liquid chromatography (HPLC) was carried out on Thermo Scientific Dionex Ultimate 3000 with CH_3CN/H_2O (0.1 % CF_3COOH) as the eluents. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) and high-resolution mass spectrometry (HRMS) analysis were conducted on AB SCIEX 4800 Plus MALDI TOF/TOF™ mass spectrometer and LTQ Orbitrap XL™ liquid chromatography/mass spectrometry system, respectively. The UV/Vis spectra were measured with an Ocean Optics Maya 2000 Pro spectrometer. The fluorescence spectra were measured with a HORIBA Scientific Fluoromax-4 spectrofluorometer with a 1 cm quartz cuvette. Fluorescent microscopy images of cells and tissue slices were acquired with an Olympus IX73 fluorescent inverted microscope. The images of solution before and after reaction was performed with a IVIS Lumina XR III system.

Chemical synthesis of Probes 1



Scheme S1 Synthesis of the probe **1**. Reaction conditions: (a) TEA, DMF, 75 °C, 4 h, 95.6%; (b) $KHCO_3$, KI, 18-crown-6, acetone, 40 °C, 12 h, 56.2%; (c) DCM/TFA/TIPSH=87.5/10/2.5, 0 °C 5 h, 95%. (d) $CuSO_4$, THPTA, Vc, H_2O/DMF=3/7, r.t., 4 h, 75.6%

Synthesis of compound 3.

Compound **3** containing the alkyne groups was synthesized according to our previously reported procedure (*J. Am. Chem. Soc.,* **2020**, *142*, 6, 2787-2794). Briefly, to a solution of 2, 3, 3-trimethylindolenine (10 mmol) and 5-chloro-1-pentyne (10 mmol) in 8 mL acetonitrile was added KI (22 mmol), the reaction mixture was kept reflux for 24 h. After filtration, the solvent was removed under reduced pressure and the residue was recrystallization with acetone to afford white powder. Without further purification, the white powder were dissolved in 40 mL n-butyl alcohol/benzene (7/3, v/v), to which 2-chloro-3-(hydroxyMethylene)cyclohex-1-enecarbaldehyde (2 mmol) was added. The reaction mixture were kept stirring and reflux at 135 °C for 6 h. After the reaction, the solvent was removed under reduced pressure, and the residue was purified by silica gel flash chromatography with an eluent of DCM/MeOH (10/1) to afford compound **3** as green solid with metallic luster.

Synthesis of compound 4.

4-Chloro-1, 3-benzenediol (146 mg, 1.02 mmol), TEA (329 μL) were dissolved in 15.0 mL anhydrous DMF and stirred at room temperature (r.t.) under nitrogen atmosphere for 10 min. Compound **7** (200 mg 0.34 mmol) dissolved in 2.0 mL anhydrous DMF was added dropwise. After addition, the reaction solution was heated to 75 ºC and kept stirring at 75 ºC for 6 h. The solvent was evaporated, and the crude product was purified by silica gel flash chromatography using CH_2Cl_2/CH_3OH (100:1→10:1) as an eluent to give compound **4** as a blue green solid (95.6% 134 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 14.3 Hz, 1H), 7.56 (s, 1H), 7.49 (d, *J* = 7.3 Hz, 1H), 7.41 (td, *J* = 7.7, 1.1 Hz, 1H), 7.36 – 7.31 (m, 1H), 7.30 (s, 1H), 7.24 – 7.23 (m, 1H), 7.22 (s, 1H), 6.21 (d, *J* = 14.4 Hz, 1H), 4.25 (t, *J* = 7.3 Hz, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 2.62 (t, *J* = 5.9 Hz, 2H), 2.40 – 2.36 (m, 2H), 2.16 (s, 1H), 2.10 – 2.04 (m, 2H), 1.94 – 1.88 (m, 2H), 1.78 (s,

6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.90, 163.72, 160.77, 153.82, 150.31, 145.92, 142.13, 141.70, 136.61, 129.22, 127.86, 127.07, 126.06, 123.39, 121.98, 114.89, 111.32, 104.59, 95.24, 82.65, 71.13, 50.84, 43.63, 29.18, 28.77, 26.37, 20.80, 16.47. MS: calcd. For $C_{30}H_{29}ClNO_2^+$ [M^+]: 470.1881. HRMS found: m/z 470.1879.

Synthesis of compound 6.

Compound **5** (98 mg 0.22mmol), compound **4** (50 mg, 0.11 mmol), $KHCO_3$ (22 mg, 0.22 mmol), 18-Crown-6 (58 mg, 0.22 mmol), KI (183 mg, 1.1 mmol) dissolving in acetone (5 ml) and the resulting mixture was stirring at 40 ºC under nitrogen for 24 h. Then, ethyl acetate (30 mL) was added, and the resulting solution was washed with 1M HCl, water, brines and dried by anhydrous Na_2SO4. After removal of the solvent, the residue was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (50:1) as eluent to afford the compound **6** as a blue solid (58.9 mg, 42.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.52 (d, *J* = 14.8 Hz, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.68 (d, *J* = 7.1 Hz, 1H), 7.44 – 7.35 (m, 6H), 7.30 – 7.27 (m, 8H), 7.23 – 7.19 (m, 2H), 7.06 (s, 1H), 6.87 (s, 1H), 6.83 (s, 1H), 6.52 (d, *J* = 14.8 Hz, 1H), 5.59 (d, *J* = 8.0 Hz, 1H), 5.28 (s, 1H), 4.52 (t, *J* = 6.9 Hz, 2H), 4.40 – 4.35 (m, 1H), 2.71 – 2.66 (m, 3H), 2.51 – 2.42 (m, 3H), 2.37 – 2.30 (m, 1H), 2.27 – 2.17 (m, 1H), 2.13 – 2.05 (m, 3H), 2.04 – 1.93 (m, 2H), 1.86 (t, *J* = 4.0 Hz, 2H), 1.79 (s, 6H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.91, 177.91, 171.40, 171.13, 171.13, 161.05, 161.05, 156.89, 156.89, 156.14, 152.54, 152.54, 146.13, 146.13, 141.87, 141.87, 141.12, 141.12, 139.63, 139.46, 138.96, 138.96, 132.05, 130.41, 129.19, 128.57, 128.51, 128.03, 128.01, 127.77, 127.73, 127.62, 127.11, 127.08, 123.25, 121.00, 120.10, 115.85, 115.31, 112.59, 104.53, 102.04, 82.68, 80.14, 78.09, 77.23, 71.86, 70.60, 53.43, 51.00, 44.61, 33.76, 29.69, 29.64, 29.20, 28.71, 28.67, 28.51, 28.50, 28.31, 26.44, 24.69, 20.14, 16.21. MS: calcd. For $C_{60}H_{61}ClN_3O_7^+$ [M^+]: 970.4193. HRMS found: m/z 970.4223

Synthesis of compound 7.

Compound **6** (10.0 mg) was followed by deprotection of the Boc and O^{t}Bu group with a mixture DCM/TFA/TIPSH=87.5/10/2.5 for 3 h. Then, compound **7** was obtained after purification by preparative HPLC as a blue solid. (6.1 mg 80.7%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.58 (d, *J* = 15.0 Hz, 1H), 8.35 (s, 2H), 7.81 (d, *J* = 7.5, 1H), 7.77 – 7.64 (m, 4H), 7.59 – 7.55 (m, 1H), 7.52 – 7.48 (m, 3H), 7.40 (s, 1H), 7.34 (s, 1H), 6.63 (d, *J* = 15.0 Hz, 1H), 5.35 (s, 2H), 4.47 (t, *J* = 7.3 Hz, 2H), 3.97 (s, 1H), 3.01 (t, *J* = 2.6 Hz, 1H), 2.73 – 2.66 (m, 4H), 2.62 – 2.46 (m, 2H), 2.41 – 2.37 (m, 2H), 2.14 – 1.96 (m, 4H), 1.87 – 1.80 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.11, 170.84, 169.94, 159.81, 158.18, 157.86, 156.00, 152.29, 145.10, 142.27, 141.36, 139.26, 131.36, 130.25, 128.95, 128.80, 128.28, 127.81, 127.40, 122.85, 119.22, 118.84, 115.83, 114.37, 113.26, 105.15, 102.02, 83.39, 72.46, 71.02, 51.54, 50.65, 44.09, 31.52, 28.50, 27.49, 26.38, 25.62, 23.72, 19.78, 15.29. MS: calcd. For $C_{42}H_{43}ClN_3O_5^+$ [M^+]: 704.2886. HRMS found: m/z 704.2916.

Synthesis of probe 1

Compound **7** (3.8 mg), BODIPY-N_3 (2.0 mg), Vitamin C (5.3 mg), THPTA (2.32 mg) were mixed in DMF/H_2O = 7/3 (5 mL), r.t, 3h. Finally, Probe **1** was obtained after purification by preparative HPLC as a blue solid.(4.2 mg 72.4 %).^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 8.54 (d, *J* = 15.0 Hz, 1H), 8.29 (d, *J* = 4.0 Hz, 2H), 8. 06-8.03 (m, 1H), 7.94 (s, 1H), 7.80 (d, *J* =

7.4 Hz, 1H), 7.75 (d, *J* = 7.4 Hz, 1H), 7.72 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.64 (s, 1H), 7. 58-7. 54 (m, 1H), 7. 51-7. 47 (m, *J* = 8.6 Hz, 3H), 7.35 (s, 1H), 7.30 (s, 1H), 7.06 (d, *J* = 4.0 Hz, 1H), 6.59 (d, *J* = 15.1 Hz, 1H), 6.33 (d, *J* = 4.0 Hz, 1H), 6.26 (s, 1H), 5.32 (s, 2H), 4.61-4.44 (m, 2H), 4.34-4.30 (m, 2H), 3.13 – 3.02 (m, 4H), 2.89 (s, 1H), 2.89-2.84 (m,, 2H), 2.73 (s, 1H), 2.71 – 2.65 (m, 2H), 2.61 – 2.55 (m, 2H), 2.49-2.47(m,1H), 2.43 (s, 3H), 2.23 (s,3H), 2.20 – 2.14 (m, 2H), 2.11 – 2.04 (m, 2H), 1.99 (s, 3H), 1.96 – 1.88 (m, 2H), 1.78 (s, 6H), 1.23 (s, 3H). MS: calcd. For $C_{59}H_{64}BClF_2N_9O_6^+$ [M^+]: 1078.4724 HRMS found: m/z 1078.4816.

General Procedure for the Detection of GGT with Probe 1.

The stock solution (5 mM) of probe **1** was prepared by dissolving an appropriate amount of probe **1** in dimethyl sulfoxide (DMSO) and stored at −20 °C. The work solution of probe **1** (20 μM) was prepared by diluting an appropriate volume of stock solution of probe **1** into enzyme reaction bu□er (1× PBS bu□er, pH 7.4, 10% DMSO, 0.1% Tween@20) for 2 h. Probe **1** (20 μM, 100 μL) was mixed with 100 μL of enzyme assay bu□er containing GGT, and the mixed reaction solution was then incubated at 37 °C. The GGT-triggered reaction of probe **1** was analyzed by HPLC, the UV−vis absorption spectra and fluorescence spectra. The UV−vis absorption spectra were collected from 400 to 900 nm. The fluorescence spectra from 465 to 815 nm with excitations from 450 to 800 nm were recorded by fluorescence synchronous scanning.

Enzyme Kinetic Studies of Probe 1 towards GGT.

For enzyme kinetic evaluation, we incubated different concentrations of probe with a constant concentration of GGT enzyme. Briefly, probe **1** at different concentration (1.00, 2.00, 4.00, 5.00, 10.0 and 20.0 μM) was prepared in 100 μL GGT enzymatic reaction buffer (1×PBS buffer, pH 7.4, 10 % DMSO, 0.1% Tween@20) and placed in a 96-well black plate. The reactions were initiated upon addition of 100 μL GGT (100 U/L). The fluorescence intensity in each well during the first ten minutes was recorded with a Spark™ 10M Multimode Microplate Reader ($\lambda_{ex}/_{em}$ = 680/720 nm). Kinetic values, including K_m, v_{max}, were calculated according to Michaelis-Menten equation which was described as: $v = v_{max}[S]/(K_m+[S])$ and k_{cat}, k_{cat}/K_m were further calculated from these two parameters.

Determination of Sensitivity towards GGT:

To evaluate the sensitivity of probe **1** towards GGT, fluorescence response of probe **1** to GGT at varying concentrations (0–50 U/L) was investigated. The reaction solutions were kept at 37 °C for 90 min, and the fluorescence spectra from 465 to 815 nm with excitations from 450 to 800 nm were recorded by fluorescence synchronous scanning. The linear-fitting curve of the fluorescence ratio (I735/517) versus the concentration of GGT from 0.5 to 50 U/L was obtained. The detection limit was calculated from 3s/k, where σ represents the standard deviation of 11 blank measurements and k represents the slope of fluorescence intensity towards GGT concentration.

Determination of Specificity towards GGT:

The specificity towards GGT was investigated by incubating probe **1** (5 μM) in GGT assay buffer with 100 U/L GGT, 1.0 μg/mL caspase 3, 5 nM MMP-2, 1.25 mM Cys, 10 mM GSH or 100 U/L GGT pretreated with its inhibitors, GGsTop (100 μM). The reaction solutions were kept

at 37 °C for 90 min, and the fluorescence spectra from 465 to 815 nm with excitations from 450 to 800 nm were recorded by fluorescence synchronous scanning.

Stability Evaluation:

The stability of probe **1** in PBS buffer (pH 7.4), high-glucose Dulbecco's modified Eagle's medium (DMEM), PBS buffer (pH 7.4) containing 10% mice serum, or DMEM containing 10% fetal bovine serum (FBS) at 37°C was investigated. Briefly, 5 μM probe **1** was prepared with corresponding buffer to get a 200 μL solution and the fluorescence intensity in each solution was monitored every 20 minutes with a microplate reader ($\lambda_{ex}/_{em}$=680/735 nm and $\lambda_{ex}/_{em}$= 480/517 nm), which last for 5 h. After that, each solution was analyzed with HPLC.

Cell Culture:

OVCAR5, HeLa, U87MG and HUVEC cells were obtained from purchased from Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). All these cells were routinely tested to exclude infection with mycoplasma. OVCAR5 cells were grown in high-glucose RPMI-1640 medium; HeLa, U87MG, HUVEC cells were grown in high-glucose DMEM. All the mediums were supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin and all cells are incubated in a 5 % CO_2 humidified incubator at 37°C.

Cytotoxicity studies:

HeLa cells were seeded on flat-bottomed 96-well plates (5000 cells/well) and incubated at 37 °C for 24 h. Varying concentrations of probe **1** (0, 0.5, 1, 2, 5,10 μM) in the medium were then added. After being incubation for 24 h, MTT solution (50 μM, 1× in PBS) was added into each well. The cells were kept at 37 °C for another 4 h, and the medium containing unreacted MTT in each well was then removed carefully. The resulting purple crystals in the wells were dissolved by addition of 150 μL DMSO. The absorbance (OD) of formazan at 490 nm in each well was recorded on a microplate reader (Tcan). The absorbance of cells without any treatment ($OD_{control}$) were used as the control, and the percentage of cell viability in each treatment was calculated by dividing OD to $OD_{control}$. Every experiment was repeated three times.

Fluorescence imaging on cells:

HeLa, U87MG and HUVEC cells (5×10^4) were seeded on a glass-bottom cell culture dish, and kept growing overnight. Probe **1** (2 μM) in FBS free DMEM medium were added into cells and incubated at 37 °C for 2 h. OVCAR5 cells keep other conditions unchanged, change the medium to 1640 medium. To inhibit GGT activity, cells were pretreated with GGsTop (100 μM) for 30 min, and then incubated with probe **1** (2 μM) at 37 °C for 2 h. After being washed with cold PBS (1×) for three times, fluorescence images were captured on an Olympus IX73 fluorescent inverted microscope for BODIPY (pseudo green, λ_{ex} = 482 ± 12.5 nm, λ_{em} = 510-550 nm) and mCy-Cl (pseudo red, λ_{ex} = 650 ± 22.5 nm, λ_{ex} = 690-750 nm). Fluorescence ratio images (Red/Green) from the two channels were constructed using Ratio Plus plugin in ImageJ software (NIH). Average fluorescence intensity ratios are measured by ImageJ.

Animals and Tumor Models

BALB/c female mice at 5-6 weeks old were purchased from the Model Animal Research Center

(MARC) of Nanjing University (Nanjing, China). All animal experiments were performed in compliance with the Guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Nanjing University, and approved by the Animal Ethical and Welfare Committee (AEWC) at Nanjing University. To establish subcutaneous HeLa tumors, 2×10^6 HeLa cells suspended in 50 μL of 50 v/v% mixture of matrigel in suspended DMEM were injected subcutaneously into the selected positions of nude mice. The tumors were allowed to grow for around 3−4 weeks to reach the size of around 100 mm^3 before euthanized.

Ratiometric fluorescence imaging of GGT in resected mouse organ tissue slices

The organs was excised from euthanized mice and freeze at −80°C. Then the frozen tissue was cut flat using a vibrating-blade microtome to obtain 4 μM thickness slices. The thickness slices were cultured with 2 μM probe **1** at 37°C for 2 h. After being washed with cold PBS (1×) for three times, fluorescence images were captured on an Olympus IX73 fluorescent inverted microscope for BODIPY (pseudo green, λ_{ex} = 482 ± 12.5 nm, λ_{em} = 510-550 nm) and mCy-Cl (pseudo red, λ_{ex} = 650 ± 22.5 nm, λ_{ex} = 690-750 nm). Fluorescence ratio images (Red/Green) from the two channels were constructed using Ratio Plus plugin in ImageJ software (NIH).

Fluorescence Imaging of GGT in HeLa Tumor Tissue Slices

HeLa tumor was excised from euthanized mice and freeze at −80°C. Then the frozen tumor tissue was cut flat using a vibrating-blade microtome to obtain 4 μM thickness slices. The thickness slices were pretreated with or without 100 mM GGsTop for 1 h and then cultured with 2 μM probe **1** at 37°C for 2 h. After washing with PBS (1×) for three times, 10 $\mu g \cdot mL^{-1}$ DAPI was added and incubated for another 30 min. After being washed with cold PBS (1×) for three times, fluorescence images were captured on an Olympus IX73 fluorescent inverted microscope for DAPI (pseudo blue, λ_{ex} = 365 ± 22.5 nm, λ_{em} = 420-460 nm); BODIPY (pseudo green, λ_{ex} = 482 ± 12.5 nm, λ_{em} = 510-550 nm) and mCy-Cl (pseudo red, λ_{ex} = 650 ± 22.5 nm, λ_{ex} = 690-750 nm). Fluorescence ratio images (Red/Green) from the two channels were constructed using Ratio Plus plugin in ImageJ software (NIH).

Fluorescence imaging of GGT activity in mice

For non-invasive fluorescence imaging of GGT activity in subcutaneous injection models, 50 μL saline solution containing 10 μM probe **1** with or without GGT (100 U/mL, 0.5 μL) were subcutaneously injected in the right and left hind leg of mouse, respectively. Whole body fluorescence images were acquired immediately, 10 min, 15min, 20 min, 30 min, 40min, 1 h, 1.5h and 2 h post injection. For fluorescence imaging of endogenous GGT activity in living mice bearing subcutaneous HeLa tumors, mice were i.t. injected with probe **1** (20 μM) in 50 μL buffer (1× PBS bu□er, pH 7.4, 10% DMSO, 0.1% Tween@20). Whole body fluorescence images were acquired immediately, 10 min, 30min, 1 h and 2 h post injection. The whole-body fluorescence images at indicated time point were acquired on an IVIS Lumina XR III imaging system, using a 480 nm excitation filter and a 520 nm emission filter for BODIPY; a 660 nm excitation filter and a 710 nm emission filter for mCy-CL. The fluorescence intensities were quantified by the region of interest (ROI) measurement using Living Image Software (4.5.2, PerkinElmer, MA, U.S.A).