GR: Supporting Information

Targeted Delivery of a γ-Glutamyl Transpeptidase Activatable Near-Infrared-Fluorescent Probe for Selective Cancer Imaging

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Figure S1. MALDI-TOF Spectrum of the reaction solution of probe **1** with GGT, found: m/z = 1289.2478 ($[M]^+$), which is identical to that of probe **2** (calcd. For $C_{64}H_{78}ClN_{12}O_{13}S^+$ $[M^+]$: 1289.5215).

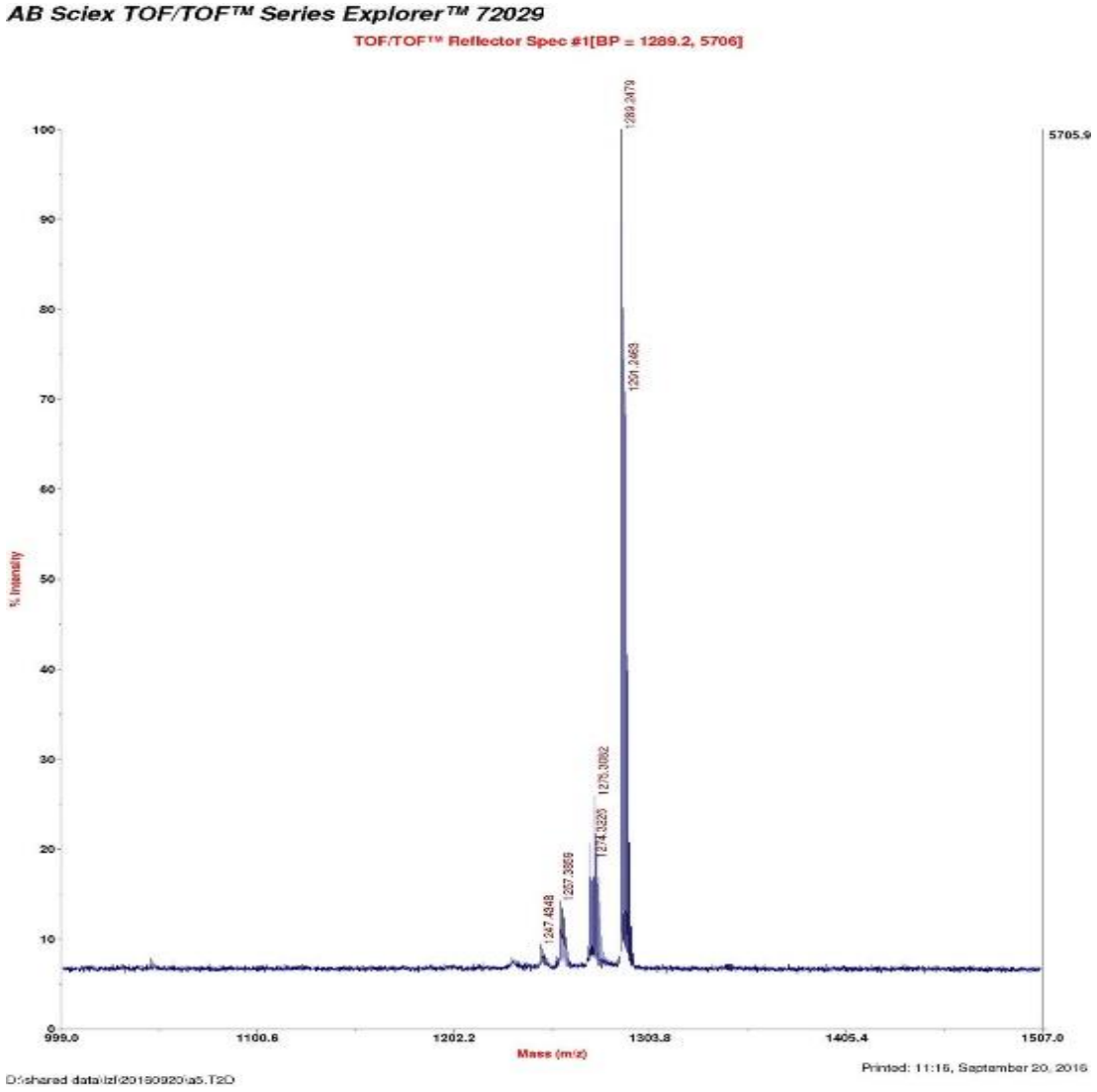


Figure S2. Analysis of probe **1-ctrl** towards GGT activation *in vitro*. (a) UV-Vis absorption and (b) fluorescence spectra of probe **1-ctrl** (5 μM) following incubation with GGT (100 U/L) at 37 ºC for 60 min. Fluorescence spectra were measured by synchronous fluorescence scanning (λ_{ex} = 675-835 nm, offset = 15 nm). (c) HPLC analysis of probe **1-ctrl** before (black) and after (red) incubation with GGT (100 U/L) at 37 °C for 60 min.

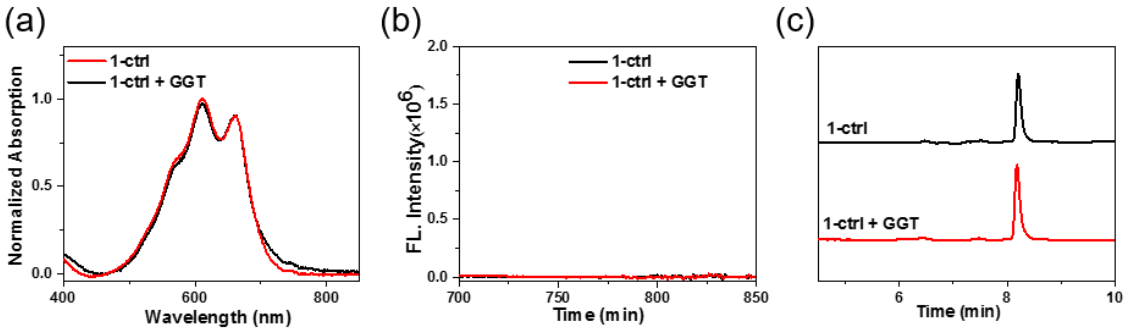


Figure S3. Kinetic evaluation for the GGT enzyme-catalyzed reaction. (a) Fluorescence intensity changes of different concentration of probe **1** vs. time upon incubation with 10 U/L GGT. (b) Linear fitting curve of fluorescence intensity (λ_{em} = 720 nm) with the concentration of probe **2**. Error bars represent standard deviation (*n* = 3). (c) Plots of concentration of probe **2** released from probe **1** vs. time under different probe concentrations.

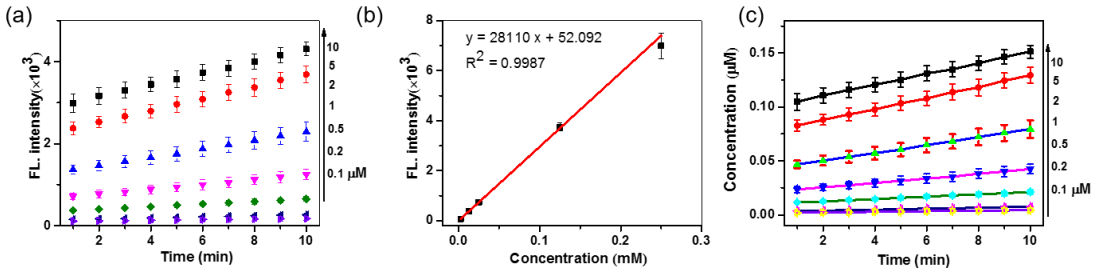


Figure S4. (a) Fluorescence analysis of U87MG cells after incubation with 5 μM probe (red) or probe **1-ctrl** (black) at 37 ºC for 60 min. (b) HPLC analysis of incubation medium of U87MG cells after incubation with 5 μM probe (red) or probe **1-ctrl** at 37 °C for 60 min.

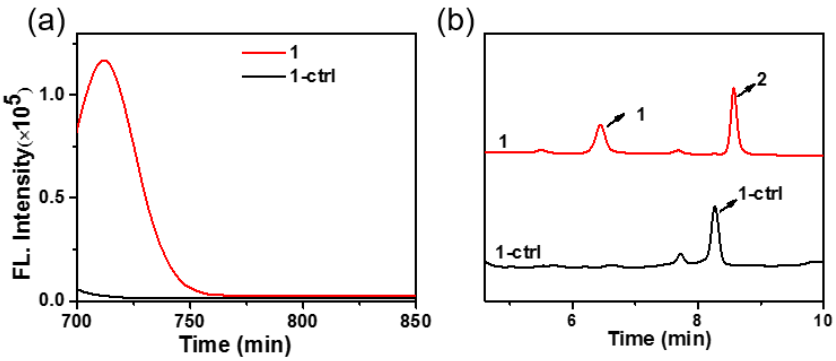


Figure S5. Qualification of GGT content in different with AMC-Glu. (a) Fluorescence spectra of AMC-Glu (5 μM) following incubation with varying concentration of GGT at 37 ºC for 60 min. (b) Linear fitting curve of fluorescence intensity (λ_{em} = 440 nm) with the concentration (*c*) of GGT from 0.2-8.0 U/L. (c) Fluorescence spectra of AMC-Glu (5 μM) following incubation with U87MG, HEK293, and HUVEC cell lysates at 37 ºC for 60 min. (d) The corresponding fluorescence intensity of different cell lysates samples indicated in (c).

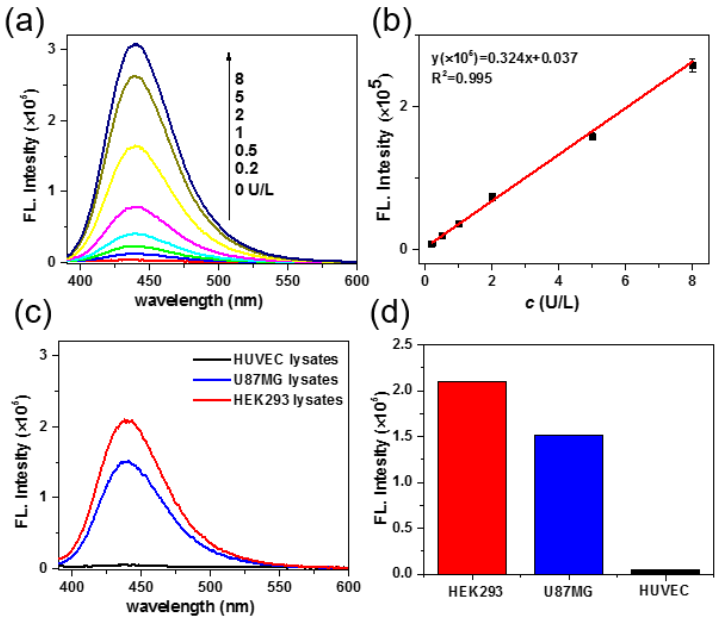


Figure S6. Fluorescence imaging of U87MG cancer cells, HEK293 normal cells, HUVEC normal cells with GANP. Cells were incubated with GANP (2 μM) for 30 min.

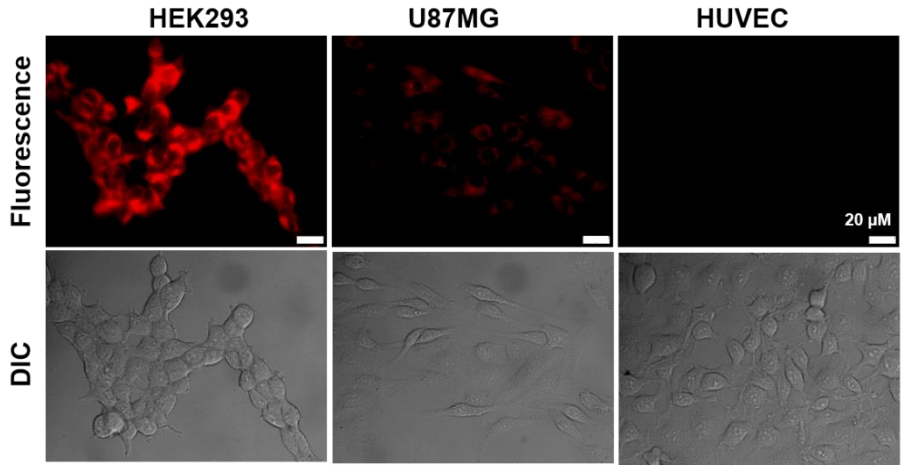


Figure S7. Fluorescence imaging of GGT *in vivo*. (a) The longitudinal change of tumor-to-background ratio of U87MG tumor-bearing mice receiving i.v. in-jection of probe **1** (25 μM, 150 μL), **1-ctrl** (25 μM, 150 μL), probe **1** with i.t. injection of GGsTop (5 mM, 100 μL), or probe **1** with i.t. injection of free c-RGD (2 mM, 100 μL). (b) Comparison of the tumor-to-background ratio at 4 h as indicated in (a). Values are mean ± SD (n = 3). ** $P < 0.01$. (c) The indicated ROI locations of tumor and background.

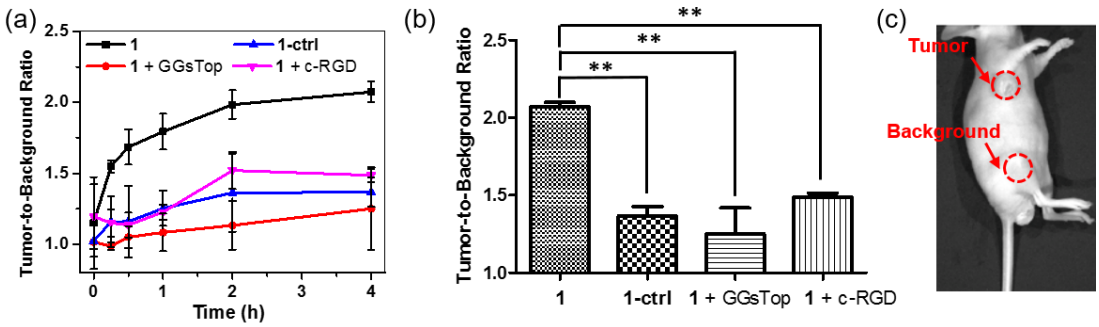


Figure S8. Fluorescence imaging of GGT in healthy mice. (a) Longidudinal fluorescence imaging of healthy nude mice receiving i.v. in-jection of probe **1** (25 μM, 150 μL). (b) *Ex vivo* fluorescence images of indicated organs resected from healthy mice 2 h after i.v. in-jection of probe **1** (25 μM, 150 μL). The results showed that probe **1** could be delivered into liver and produce bright NIR fluorescence in the livers.

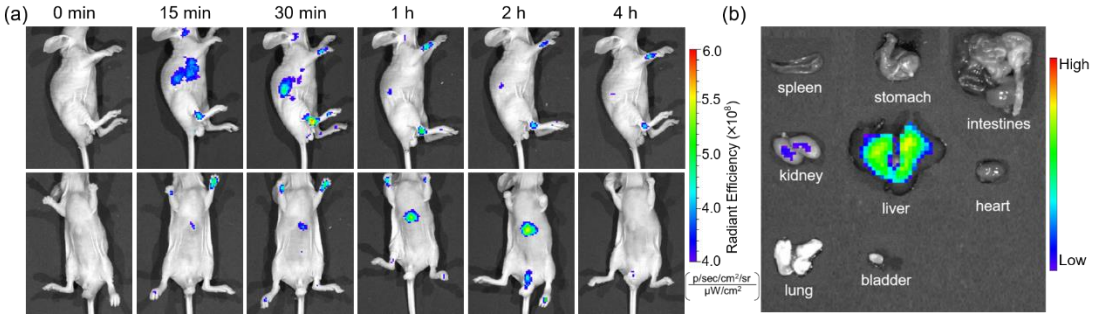


Table S1. Kinetic parameters of probe **1**, GANP and AMC-Glu.

	probe **1**	**GANP**	**AMC-Glu**
K_m **(μM)**	1.91 ± 0.45	1.26 ± 0.09	3.23 ± 0.37
v_{max} **(nM s^{-1})**	0.109 ± 0.004	0.0970 ± 0.0003	0.967 ± 0.008
k_{cat} **(s^{-1})**	0.00502± 0.00018	0.00447 ± 0.00001	0.0445 ± 0.0003
k_{cat}/K_m **(M^{-1} s^{-1})**	2624 ± 404	3520 ± 18	13800 ± 17

Table S2. Comparison of the GGT detection among different probes.

Probe	λ_{ex} **(nm)**	λ_{em} **(nm)**	**LOD**	K_m	**Type**	**Ref.**
1	496	525	N.D.[a]	145 μM	FL[b]	1
2	490	635	57 mU/L	10.27 μM	FL	2
3	585	615	5.6 mU/L	7.64 μM	FL	3
4	362	473	210 mU/L	17.64 μM	FL	4
5	680	708	500 mU/L	7.01 μM	FL	5
6	450	601	N.D. [a]	18.76 μM	FL	6
7	360	472	N.D. [a]	15.17 μM	FL	7
8	460	557	150 mU/L	N.D.	FL	8
9	408	537	760 mU/L	N.D.	FL	9
10	440	565	160 mU/L	N.D.	FL	10
11			0.66 mU/L	1.17 μM	BL [c]	11
12	390	490	33 mU/L	6.95μM	FL	12
13			192 mU/L 442 mU/L	12.57 18.42	BL	13
14	680	720	3.6 mU/L	1.26 μM	FL	14
probe **1**	680	720	2.9 mU/L	1.85 μM	FL	This work

[a] Not Determined.

[b] Fluorescence.

[c] Bioluminescence.

Experimental Section

Chemicals and Instrumentation. GGT from equine kidney, GGT inhibitor 3-[[(3-amino-3-carboxypropyl) methoxyphosphinyl] oxy] benzeneacetic acid (GGsTop) and human recombinant Cathepsin B were obtained from R&D systems. Human recombinant MMP-2 was obtained from Sino Biological Inc. *N*-Fmoc-$_L$-glutamic acid 1-allyl ester (Fmoc-Glu-OAll) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU) were obtained from GL Biochem（Shanghai）Ltd. 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. The stock solution of probe **1** was prepared by dissolving in dimethyl sulphoxide (DMSO) and stored at -20 °C. 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 Avance III 400 spectrometer. High-performance liquid chromatography (HPLC) was carried out on Thermo Scientific Dionex Ultimate 3000 with CH_3CN/H_2O (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 images of cells and tissue slices were acquired with an Olympus IX73 fluorescent inverted microscope or a Leica TCS SP5 confocal laser scanning microscope. The *in vivo* fluorescence imaging was performed with an IVIS Lumina XR III system (E_x/E_m = 660/710 nm), and the fluorescence intensity was quantified by region-of-interest measurement using Living image software (PerkinElmer).

Enzyme Kinetic Studies of Probe 1 and AMC-Glu 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 (0.200, 0.400, 1.00, 2.00, 4.00, 10.0 and 20.0 μM) was prepared in 100 μL GGT enzymatic reaction buffer (PBS buffer, 1x, pH 7.4) and placed in a 96-well black plate. The reactions were initiated upon addition of 100 μL GGT (20 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). The amount of probe **2** in each well was determined by a standard curve under the same conditions. 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 the Sensitivity towards GGT. To evaluate the sensitivity of probe **1** towards GGT, probe **1** (5 μM) was incubated with varying concentration of GGT (0-100 U/L) in PBS buffer at 37 °C for 60 min, and the resulting fluorescence ranging from 690 to 850 nm were recorded with excitation from 675 to 835 nm by fluorescence synchronous scanning. The detection limit was calculated from $3\sigma/k$, where σ represents the standard deviation of 11 blank measurements and k represents the slope of fluorescence intensity towards GGT concentration.

Determination of the Specificity towards GGT. Probe **1** (5 μM) in a 96-well black plate was incubated with 100 U/L GGT, 1.0 μg/mL Cathepsin B, 5 nM MMP-2, or 100 U/L GGT pretreated with its inhibitors, GGsTop (100 μM). For comparison, probe **1-ctrl** was incubated with or without 100 U/L GGT. The fluorescence intensity at 720 nm in each well was recorded on a microplate reader every five minutes, and last for 60 min ($\lambda_{ex/em}$ = 680/720 nm).

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, probe **1** (5 μM) in the corresponding solution was incubated at

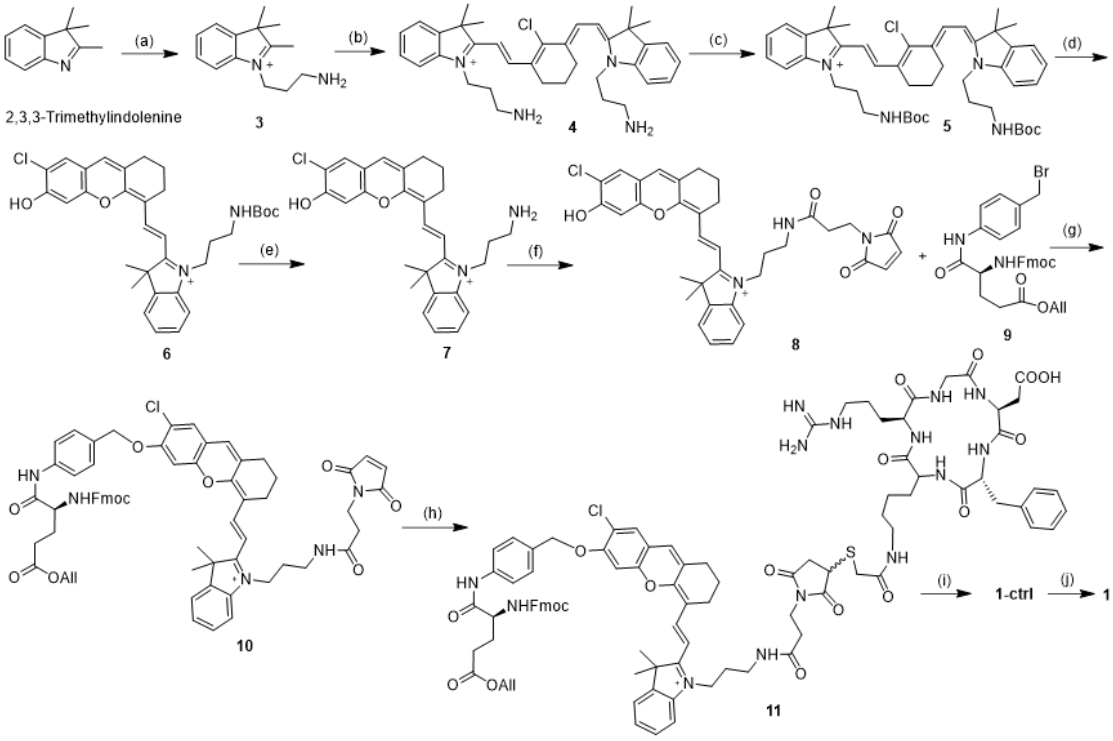
37 °C for 60 min, and the fluorescence spectra of incubation solutions were recorded with excitation from 675 to 835 nm by fluorescence synchronous scanning.

Cell Culture. U87MG, HEK-293, HUVEC cells were grown in high-glucose DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin and incubated in a 5% CO_2 humidified incubator at 37 °C.

Detection of GGT activity in U87MG, HEK293, HUVEC Cell Lysates with AMC-Glu. Cells with a confluence of ~90% were digested and adjusted to a density of 8.0 $\times 10^6$ cells/mL. Then, 1 mL of cell suspension was centrifuged at 1,000 rpm for 4 min. After the removal of supernatant, 150 μL cold RIPA buffer was added and the mixture was placed on ice for 45 min to lyse the cells. The cell lysates were obtained after centrifuging at 1,4000 g for 15 min. Then, the activity of GGT in each cell lysate was measured by incubation with the established GGT substrate AMC-Glu (10 μM) at 37 °C for 1 h. To obtain the linear correlation curve between AMC-Glu and GGT, varying concentration of GGT was placed in cold RIPA buffer for 45 min and then incubated with AMC-Glu (10 μM) at 37 °C for 1 h. The resulting fluorescence was measured with $\lambda_{ex/em}$ =380/440 nm.

U87MG Cell Lysates Studies with Probe 1 and 1-ctrl. The detached U87MG cells (~4.0 $\times 10^6$ cells) were incubated with probe **1** or **1-ctrl** (5 μM, 200 μL) at 37 °C for 60 min. Then, the incubation cells were centrifuged a speed of 1,4000 g for 15 min, and the supernatant was collected for fluorescence and HPLC analysis.

Chemical synthesis of Probes 1 and 1-ctrl



Scheme S1. Synthesis of probes **1** and **1-ctrl**. Reaction conditions: (a) 3-Bromopropylamine hydrobromide, 100 ºC, 4 h, 67.1%; (b) 2-Chloro-1-formyl-3-(hydroxymethylene), 1-Butanol/benzene (7/3), 135 ºC, 4 h, 74.5%; (c) Di-tert-butyl dicarbonate, TEA, EtOH; (d) 4-Chloro-1,2-Dihydroxybenzene, TEA, DMF, 75 ºC. (e) 50% TFA in DCM, 0 ºC, 43.8% for three steps. (f) N-Succinimidyl 3-maleimidopropionate, TEA, THF, 2 h, 90.8%. (g) $KHCO_3$, 18-crown-6, KI, acetone, 40 ºC, 46.7%. (h) c-RGD-SH, DMF. (i) 5% Piperidine in DMF, 30.0% for two steps. (j) $Pd(PPh_3)_4$, $PhSiH_3$, DMF, 15.1%.

Synthesis of compound 4. Firstly, compound **3** was synthesized according to the procedure reported previously (*J. Am. Chem. Soc.* **2012**, *134*, 13510-13523). Brieftly, 2,3,3-Trimethylindolenine (3.18 g, 20.0 mmol), and 3-Bromopropylamine hydrobromide (5.25 g, 24.0 mmol) were mixed and stirred at 100 ºC for 4 h. The crude product **3** was crystallized from acetone/methanol (5:1) to afford a pink solid (2.91g, yield 67.1%). Then, a mixture of 2-chloro-1-formyl-3-(hydroxymethylene) (200 mg, 1.16 mmol) and compound **3** (687 mg, 3.17 mmol) were dissolved in 30 mL

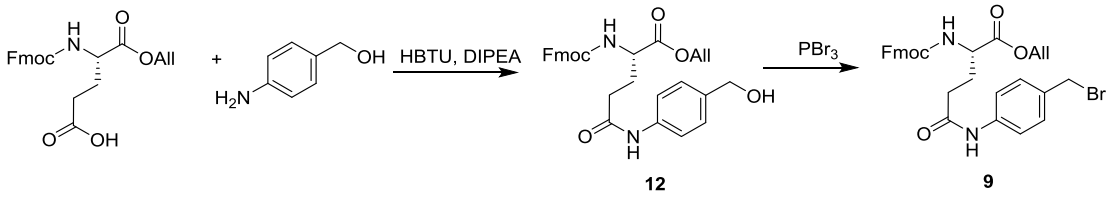
1-Butanol/benzene (7:3), and the solution was stirred under reflux to remove water by Dean-Stark trap. The reaction solution was kept stirring at 75 ºC for another 6 h. After removing the solvent under vacuum, and the residue was purified by silica gel flash chromatography using CH_2Cl_2/CH_3OH (10:1 to 1:1) as an eluent to obtain compound **4** as a green solid (492 mg, yield 74.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27-8.32 (m, 2H, H-14), 7.95 (t, J = 5.7 Hz, 4H, H-13), 7.66-7.69 (m, 2H, H-12), 7.57 (d, J = 7.9 Hz, 2H, H-11), 7.44-7.48 (m, 2H, H-10), 7.31 (t, J = 7.4 Hz, 2H, H-9), 6.38-6.42 (m, 2H, H-8), 4.38 (t, J = 7.4 Hz, 4H, H-7), 3.01 (h, J = 5.7 Hz, 4H, H-6), 2.77-2.79 (m, 4H, H-5), 2.05 (p, J = 7.4 Hz, 4H, H-4), 1.86 (p, J = 6.3 Hz, 2H, H-3), 1.66-1.71 (m, 12H, H-1, H-2:). ^{13}C NMR (100 MHz, DMSO-d_6) δ C-18: 172.39, C-17: 148.25, C-16: 143.34, C-15: 141.88, C-14: 141.05, C-13: 128.58, C-12: 126.55, C-11: 125.26, C-10: 122.62, C-9: 111.47, C-8: 101.73, C-7: 49.06, C-6: 41.22, C-5: 36.39, C-4: 27.51, C-3: 26.06, C-2: 25.15, C-1: 20.40. MS: calcd. For $C_{36}H_{46}ClN_4^+$ [M^+]: 569.3406; HRMS found: m/z 569.3247.

Synthesis of NIR fluorophore 7. To a solution of compound **4** (492 mg, 0.86 mmol) in EtOH, Di-tert-butyl dicarbonate (322 mg, 1.73 mmol) and TEA (242 μL, 1.73 mmol) were added. The mixture was stirred at r.t. for 3 h. The solvent was then removed under vacuum, and the residue was dissolved in ethyl acetate and washed with 1M HCl, water, brines and dried by anhydrous Na_2SO_4. The solvent was then removed to afford the compound **5**, which was used directly for next step. 4-Chloro-1,2-Dihydroxybenzene (477 mg, 5.29 mmol) and TEA (1.06 mL) were dissolved in 3.0 mL anhydrous DMF, and the solution was stirred at r.t. under nitrogen atmosphere for 10 min, to which compound **5** in 1.0 mL anhydrous DMF was added dropwise. After addition, the solution was heated to 75 ºC and kept stirring at 75 ºC for another 5 h. The solvent was evaporated, and the residue was purified by silica gel flash chromatography using CH_2Cl_2/CH_3OH (50:1) as an eluent to afford compound **6** as a blue-green solid, which was followed by reaction with a mixture DCM/TFA (1:1, 10 mL) at r.t. for 1 h to remove the Boc groups. After removing the solvent under vacuum, cold Et_2O was added, and the precipitates were collected after filtration to

afford the NIR fluorophore (**7**) as a blue solid (174 mg, yield 43.8% for three steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ H-18: 8.58 (d, J = 14.8 Hz, 1H), H-17: 7.98 (t, J = 5.6 Hz, 2H), H-16: 7.75-7.79 (m, 1H), H-14~15: 7.74-7.68 (m, 2H), H-13: 7.53-7.57 (m, 1H), H-11~12: 7.45-7.50 (m, 2H), H-10: 7.14 (s, 1H), H-9: 6.56 (d, J = 14.9 Hz, 1H), H-8: 4.48 (t, J = 7.4 Hz, 2H), H-7: 3.07-2.95 (m, 2H), H-5~6: 2.67-2.74 (m, 4H), H-4: 2.03-2.10 (m, J = 7.6 Hz, 2H), H-1~3: 1.76-1.85 (m, 8H). ^{13}C NMR (400 MHz, DMSO-d_6) δ C-27: 177.85, C-26: 160.98, C-25: 157.45, C-24: 152.82, C-23: 145.61, C-21~22: 142.39, 141.79, C-20: 133.33, C-16~19: 129.30, 128.50, 127.45, 127.29, C-15: 123.19, C-14: 118.70, C-12~13: 115.29, 114.75, C-11: 113.40, C-10: 104.54, C-9: 103.60, C-8: 50.80, C-7: 42.53, C-6: 36.77, C-5: 28.83, C-4: 27.96, C-3: 25.98, C-2: 23.97, C-1: 20.35. MS: calcd. For $C_{28}H_{30}ClN_2O_2^+$ [M^+]: 461.1990; HRMS found: m/z 461.1868.

Synthesis of compound 8. Compound **7** (230 mg, 0.496 mmol), TEA (68 μL, 0.496 mmol) and N-Succinimidyl 3-maleimidopropionate (264 mg, 0.991 mmol) were dissolved in 7.0 mL anhydrous THF, and the mixture was stirred at r.t. under nitrogen atmosphere for 2 h. After the reaction was completed, the solvent was removed, and the reside was purified by silica gel flash chromatography using CH_2Cl_2/CH_3OH (50:1 to 25:1) as an eluent to give compound **8** as a blue solid (276 mg, yield 90.8%).^{1}H NMR (400 MHz, DMSO-d_6) δ H-22: 11.84 (s, 1H), H-21: 8.56 (d, J = 14.9 Hz, 1H), H-20: 8.19 (t, J = 5.5 Hz, 1H), H-19: 7.77-7.79 (m, 1H), H-17~18: 7.67-7.69 (m, 2H), H-16: 7.56 (td, J = 7.7, 1.2 Hz, 1H), H-15: 7.45-7.49 (m, 1H), H-14: 7.42 (s, 1H), H-13: 7.11 (s, 1H), H-12: 7.00 (s, 2H), H-11: 6.55 (d, J = 15.0 Hz, 1H), H-10: 4.40 (t, J = 7.4 Hz, 2H), H-9: 3.59-3.62 (m, 2H), H-8: 3.18 (q, J = 6.5 Hz, 2H), H-7 & H-6: 2.69 (dt, J = 20.3, 6.1 Hz, 4H), H-5~7: 2.34-2.38 (m, 2H), H-4: 1.88-1.95 (m, 2H), H-4 & H-3: 1.79-1.85 (m, 2H), H-2 & H-1: 1.75 (s, 6H). ^{13}C NMR (400 MHz, DMSO-d_6) δ C-32: 177.34, C-30~31: 170.74, 169.59, C-29: 160.09, C-28: 156.46, C-27: 152.21, C-26: 144.95, C-24~25: 141.97, 141.29, C-23: 134.53, C-22: 132.22, C-18~21: 128.87, 128.00, 127.05, 126.98, C-17: 122.76, C-16: 117.87, C-13~15: 114.85, 114.03, 113.08, C-12: 104.45, C-11: 103.08, C-10: 50.35, C-9: 42.78, C-8:

40.01, C-7: 35.88, C-5~6: 33.94, C-4: 28.36, C-3: 27.35, C-2: 23.41, C-1: 19.83. MS: calcd. For $C_{35}H_{35}ClN_3O_5^+$ [M^+]: 612.2260; HRMS found: m/z 612.2086.



Scheme S2. Synthesis of intermediate 9.

Synthesis of compound 9. Fmoc-Glu-OAll (1.00 g, 2.44 mmol), HBTU (1.12 g, 2.44mmol) and DIPEA (849 μL, 4.88 mmol) were dissolved in 10.0 mL anhydrous THF. The solution was stirring under ice bath, and 4-aminobenzyl alcohol (360 mg, 2.93 mmol) was then added. The reaction mixture was stirred at 0 ºC for 10 min, and then kept stirring at r.t. for another 2 h. The solvent was then removed under vacuum, and the residue was dissolved in ethyl acetate and washed with 1M HCl, water, brines and dried by anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (100:1 to 75:1) as an eluent to afford compound **12** as a yellow solid (929 mg, yield 74.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ H-18: 9.91 (s, 1H), H-16~17: 7.84-7.95 (m, 3H), H-15: 7.73 (d, J = 7.4 Hz, 2H), H-14: 7.49-7.55 (m, 2H), H-13: 7.42 (t, J = 7.4 Hz, 2H), H-12: 7.34 (td, J = 7.5, 1.2 Hz, 2H), H-11: 7.19-7.26 (m, 2H), H-10: 5.86-5.95 (m, 1H), H-9: 5.31 (dq, J = 17.3, 1.7 Hz, 1H), H-8: 5.21 (dq, J = 10.5, 1.5 Hz, 1H), H-7: 5.10 (t, J = 5.7 Hz, 1H), H-6: 4.60 (dt, J = 5.4, 1.6 Hz, 2H), H-3~5: 4.11-4.61 (m, 4H), H-2: 2.44 (t, J = 7.5 Hz, 2H), H-1: 1.84-1.98 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ C-22: 171.86, C-21: 169.95, C-20: 156.14, C-19: 143.73, C-18: 140.69, C-16~17: 137.78, 137.08, C-15: 132.34, C-12~14: 127.62, 127.05, 126.87, C-11: 125.21, C-10: 120.10, C-9: 118.70, C-8: 117.69, C-7: 65.67, C-6: 64.83, C-5: 62.57, C-4: 53.46, C-3: 46.57, C-2: 32.40, C-1: 26.19. MS: calcd. For $C_{30}H_{30}N_2O_6Na^+$ [$(M+Na)^+$]: 537.2002; HRMS found: m/z 537.1844.

Compound **12** (528 mg, 1.03 mmol) from last step was dissolved in 5.0 mL anhydrous THF, and PBr_3 (146μL, 1.54 mmol) was added under ice bath. The mixture was stirring at 0 ºC for another 2 h. The mixture was added to a saturated $NaHCO_3$

solution (10 mL) and then diluted with 50 mL H_2O, followed by extracting with ethyl acetate (50 mL) for three times. The organic phases were combined and dried by anhydrous Na_2SO_4. The solvent was then removed and the residue was rapidly purified by flash chromatography on silica gel using petroleum/ethyl acetate (10:1 to 5:1) as eluent to afford compound **9,** which was used directly for the synthesis of compound **10**.

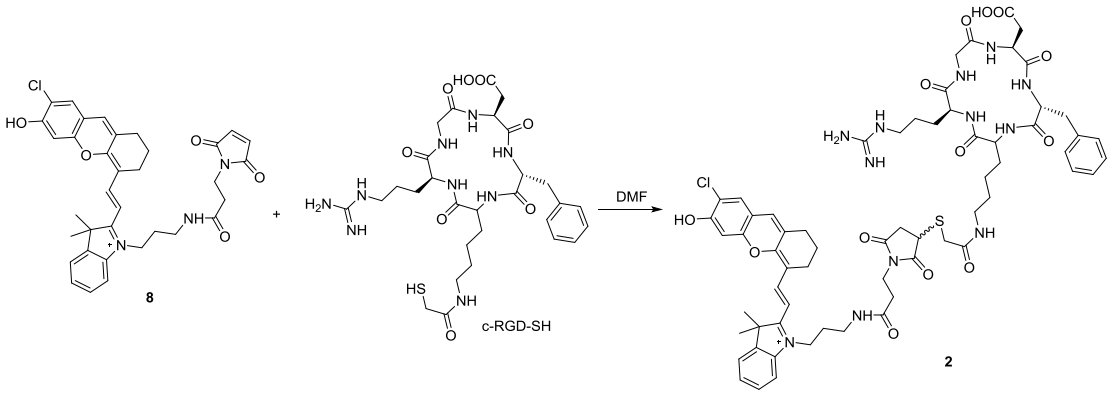
Synthesis of compound 10. Compound **8** (37.5 mg, 0.061 mmol), **9** (150 mg, 0.26 mmol), $KHCO_3$ (16.5 mg, 0.12 mmol), 18-crown-6 (32.3 mg, 0.12 mmol) and KI (135 mg, 0.61 mmol) were mixed in 5.0 mL acetone, and the resulting mixture was stirred at 40 ºC under nitrogen for 15 h. After removal of the solvent, the residue was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (100:1 to 40:1) as eluent to afford the compound **10** as a blue solid (29.9 mg, yield 46.7%), ^{1}H NMR (400 MHz, DMSO-d_6) δ H-38: 10.08 (s, 1H), H-37: 8.54 (d, J = 14.9 Hz, 1H), H-36: 8.17 (t, J = 5.5 Hz, 1H), H-33~35: 7.82-7.90 (m, 4H), H-29~32: 7.67-7.75 (m, 6H), H-28: 7.54-7.58 (m, 1H), H-26~27: 7.47-7.51 (m, 3H), H-22~25: 7.28- 7.43 (m, 6H), H-21: 7.00 (s, 2H), H-20: 6.57 (d, J = 15.1 Hz, 1H), H-19: 5.83-5.93 (m, 1H), H-18: 5.36 (s, 2H), H-17: 5.26-5.32 (m, 1H), H-16: 5.16-5.20 (m, 1H), H-15: 4.56-4.58 (m, 2H), H-12~14: 4.09-4.34 (m, 4H), H-11: 3.59-3.62 (m, 2H), H-10: 3.16 -3.20 (m, 2H), H-8~9: 2.64-2.72 (m, 4H), H-6~7: 2.34-2.48 (m, 4H), H-1~5: 1.76-2.15 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ C-53: 177.81, C-49~52: 171.80, 170.73, 170.33, 169.61, C-48: 159.67, C-46~47: 156.11, 155.89, C-45: 152.16, C-39~44: 144.96, 143.69, 142.21, 141.22, 140.66, 139.33, C-38: 134.52, C-37: 132.30, C-36: 131.21, C-29~35: 130.07, 128.88, 128.68, 128.16, 127.59, 127.33, 127.02, C-28: 125.16, C-27: 122.82, C-23~26: 120.08, 119.01, 118.74, 117.64, C-22: 115.73, C-21: 114.22, C-20: 113.26, C-19: 105.11, C-18: 101.99, C-17: 70.97, C-16: 65.66, C-15: 64.81, C-14: 53.39, C-13: 50.55, C-12: 46.54, C-11: 42.93, C-10: 40.01, C-9: 35.88, C-7~8: 33.94, 33.74, C-6: 32.42, C-5: 28.45, C-4: 27.42, C-3: 26.09, C-2: 23.49, C-1: 19.71. MS: calcd. For $C_{65}H_{63}ClN_5O_{10}^+$ [M^+]: 1108.4258; HRMS found: m/z 1108.3939.

Synthesis of probe 1-ctrl. Compound **10** (20.0 mg, 0.018 mmol) and c-RGD-SH

(16.0 mg, 0.024 mmol) were dissolved in 2 mL DMF and stirred at r.t. for 1 h. Then, 100 μL piperidine was added in the reaction solution, and stirred at r.t. for 10 min to remove the Fmoc group. Probe **1-ctrl** was obtained as a blue solid after purification by preparative HPLC (8.44 mg, yield 30 %). MS: calcd. For $C_{79}H_{96}ClN_{14}O_{16}S^+$ [M^+]: 1563.6532; HRMS found: m/z 1563.6129.

Synthesis of probe 1. Probe **1-ctrl** (15.0 mg, 0.009 mmol), $Pd(PPh_3)_4$ (11.5 mg, 0.010 mmol) and $PhSiH_3$(4.32 mg, 0.040 mmol) were dissolved in 1 mL DMF, and the solution was stirred at r.t. for 1 h to remove the Alloc group. After reaction, the mixture was purified by preparative HPLC to afford probe **1** as a blue solid, (2.07 mg, yield 15.1 %,). MS: calcd. For $C_{76}H_{92}ClN_{14}O_{16}S^+$ [M^+]: 1523.6219; HRMS found: m/z 1523.5828;

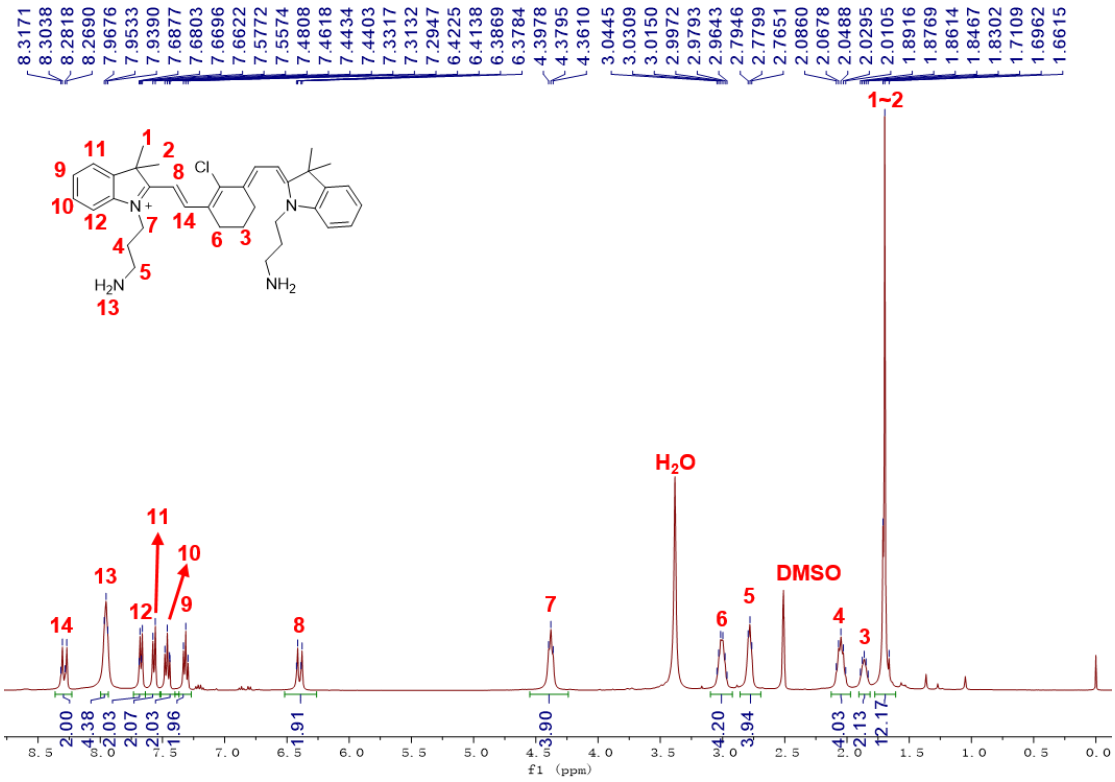
Chemical synthesis of Probe 2

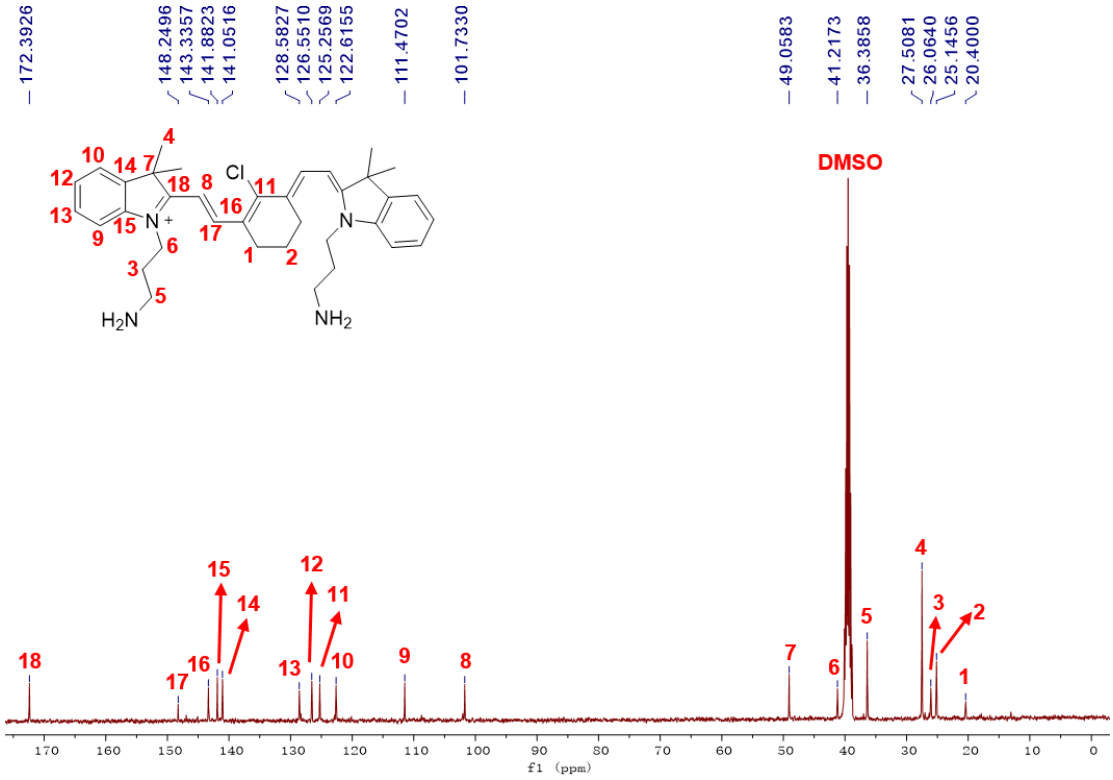


Scheme S3. Synthesis of probe **2**.

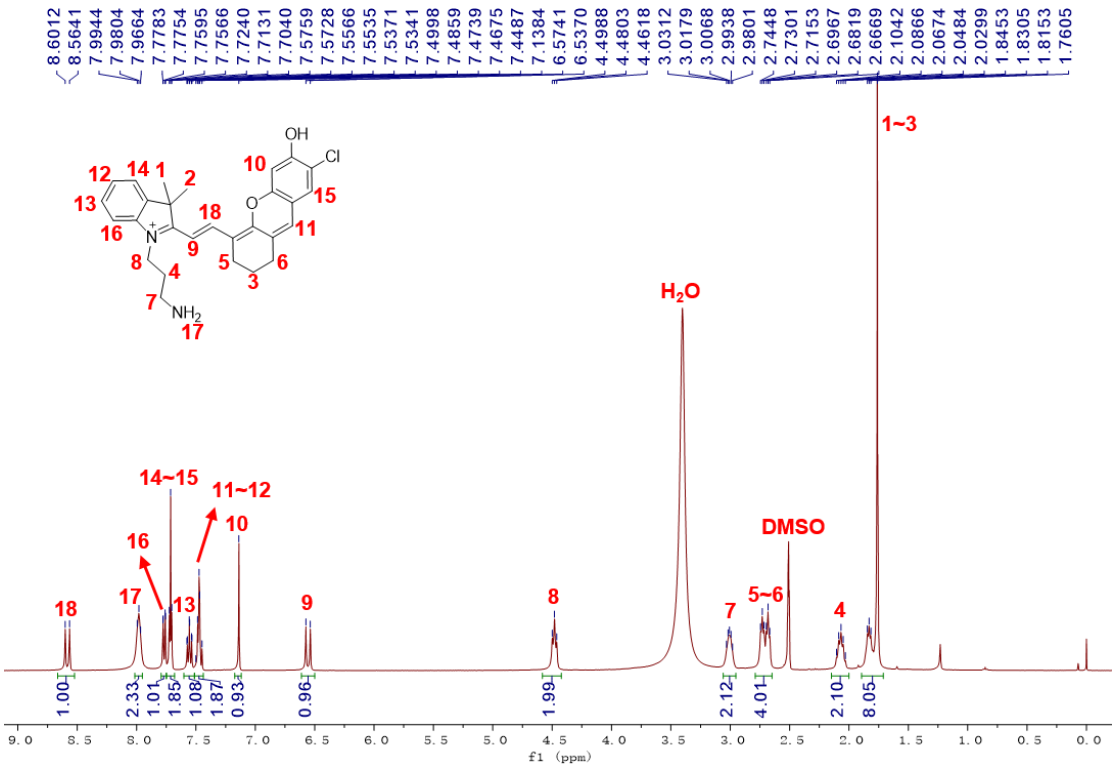
Compound **8** (5.0 mg, 0.008 mmol) and c-RGD-SH (8.0 mg, 0.012 mmol) were dissolved in 2 mL DMF and stirred at r.t. for 1 h. After the reaction was completed, the mixture was purified by preparative HPLC to afford probe **2** as a blue solid, (7.75 mg, yield 75.2 %,), MS: calcd. For $C_{64}H_{78}ClN_{12}O_{13}S^+$ [M^+]: 1289.5215; HRMS found: m/z 1289.5990.

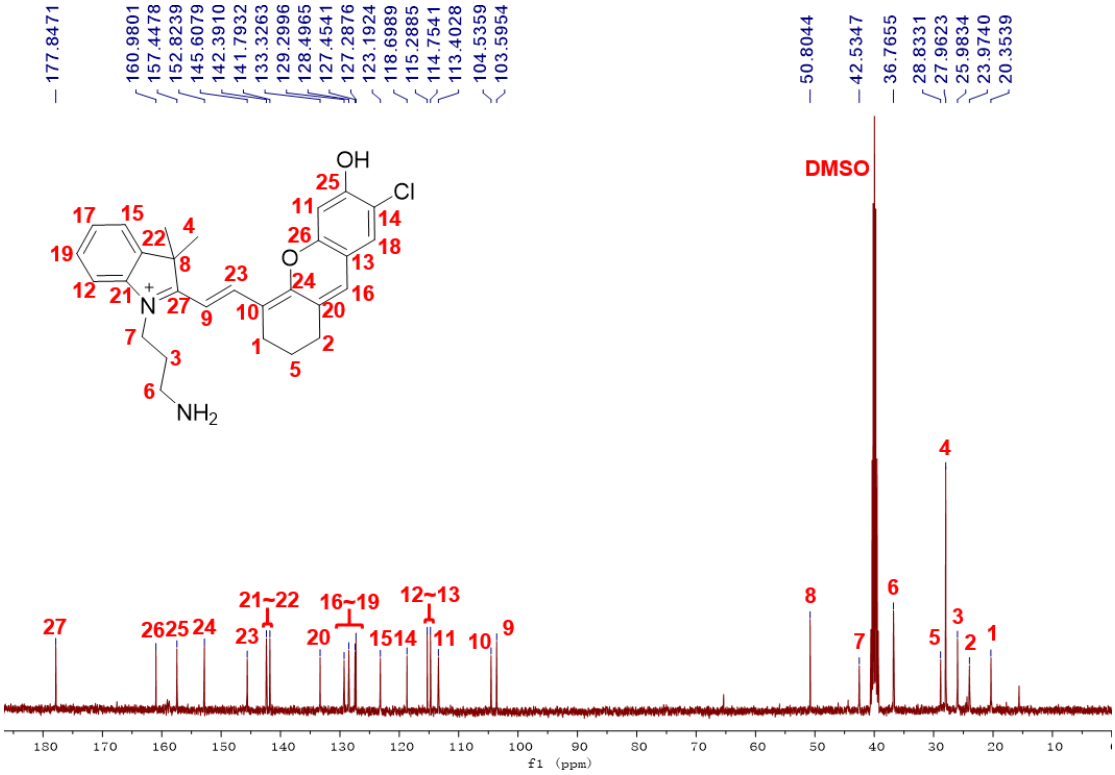
^{1}H-NMR and ^{13}C-NMR spectra of compound **4** (DMSO-d_6).



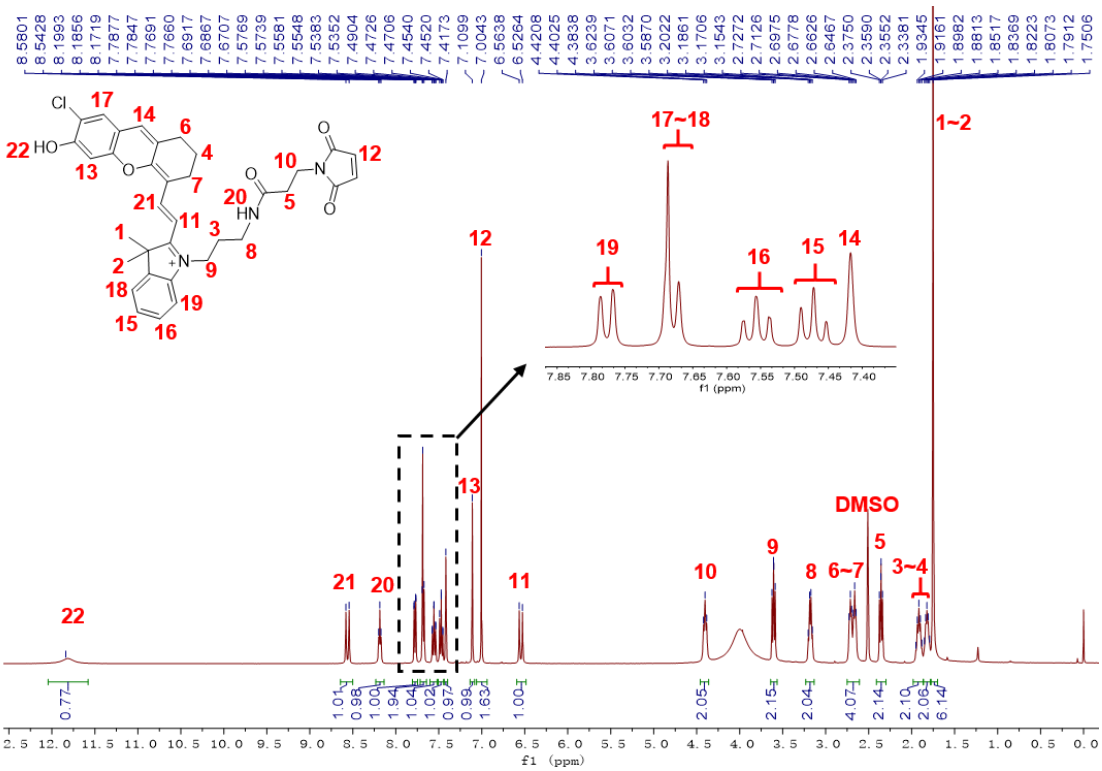


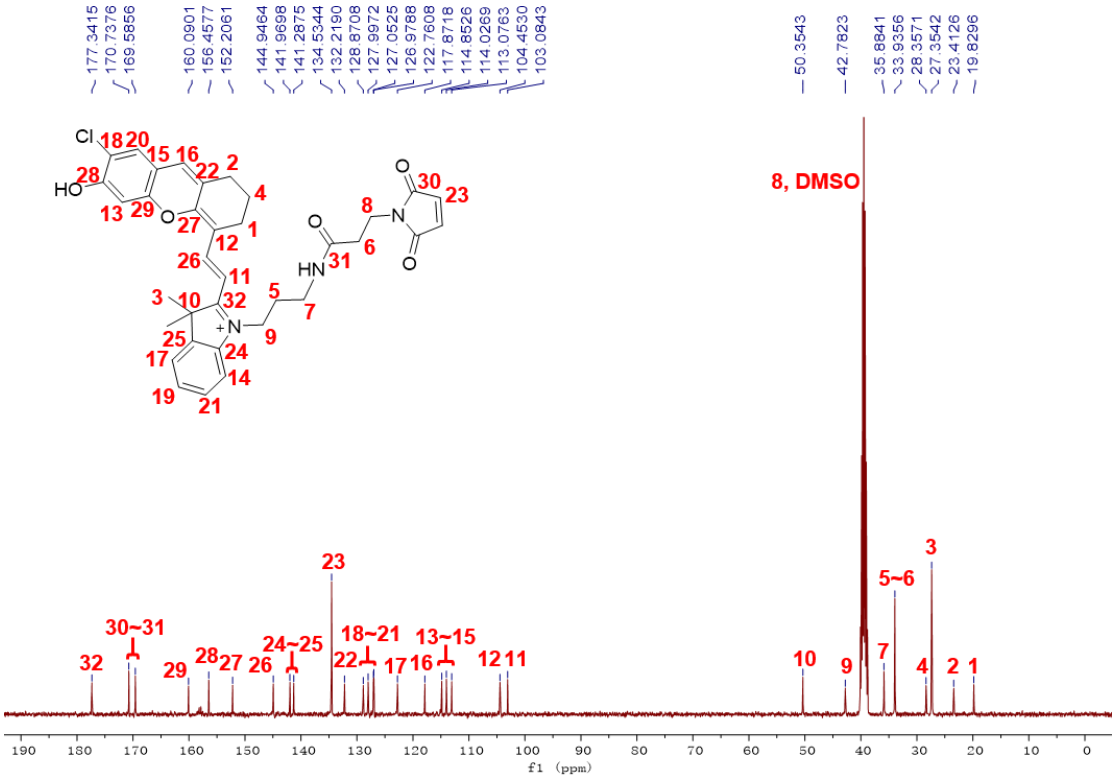
^{1}H-NMR and ^{13}C-NMR spectra of compound **7** (DMSO-d_6).



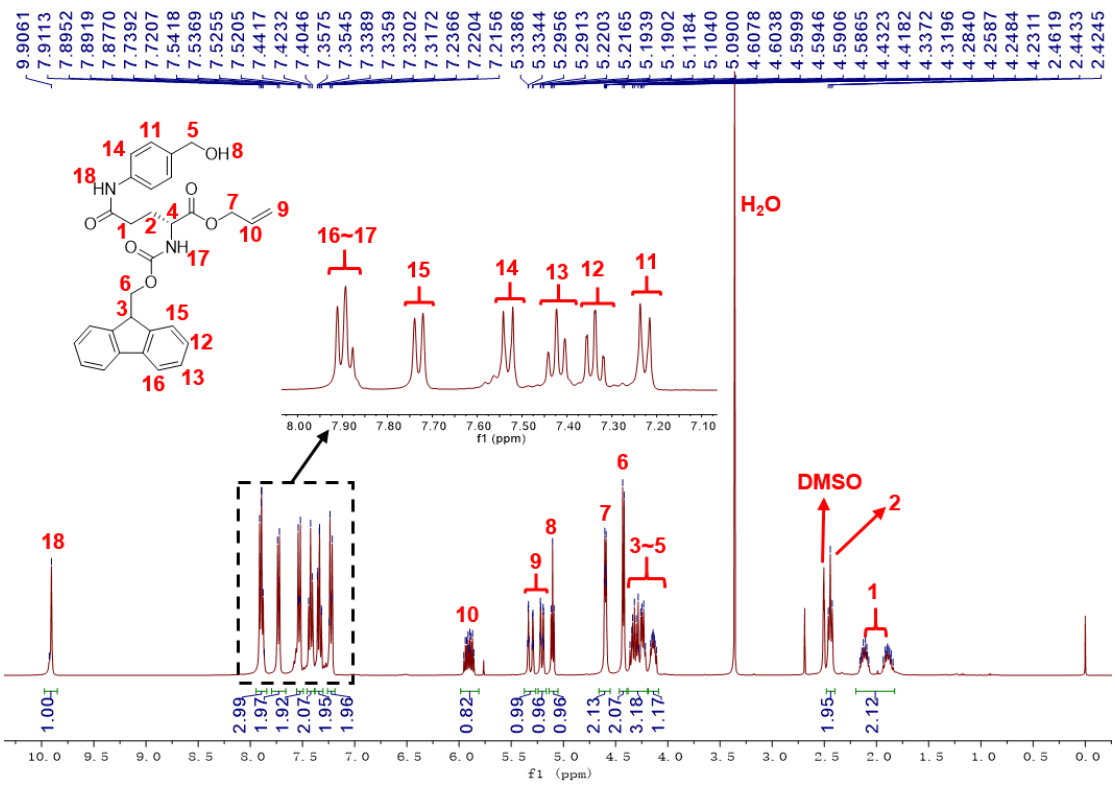


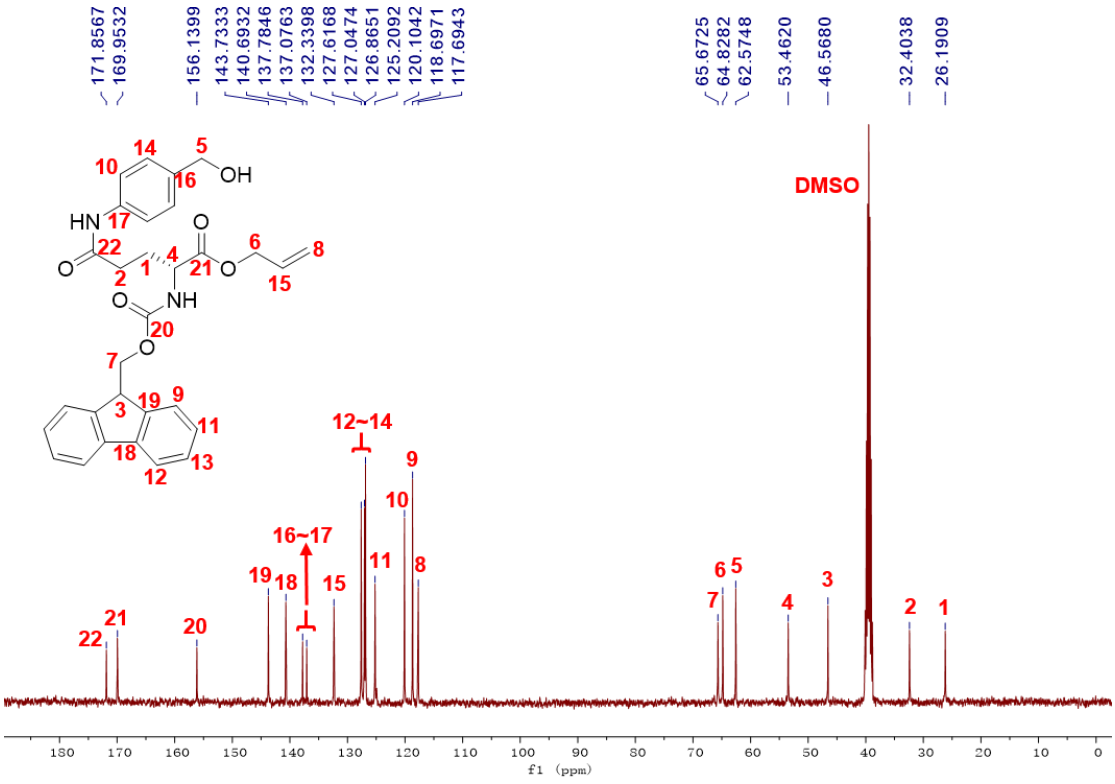
^{1}H-NMR and ^{13}C-NMR spectra of compound **8** (DMSO-d_6).



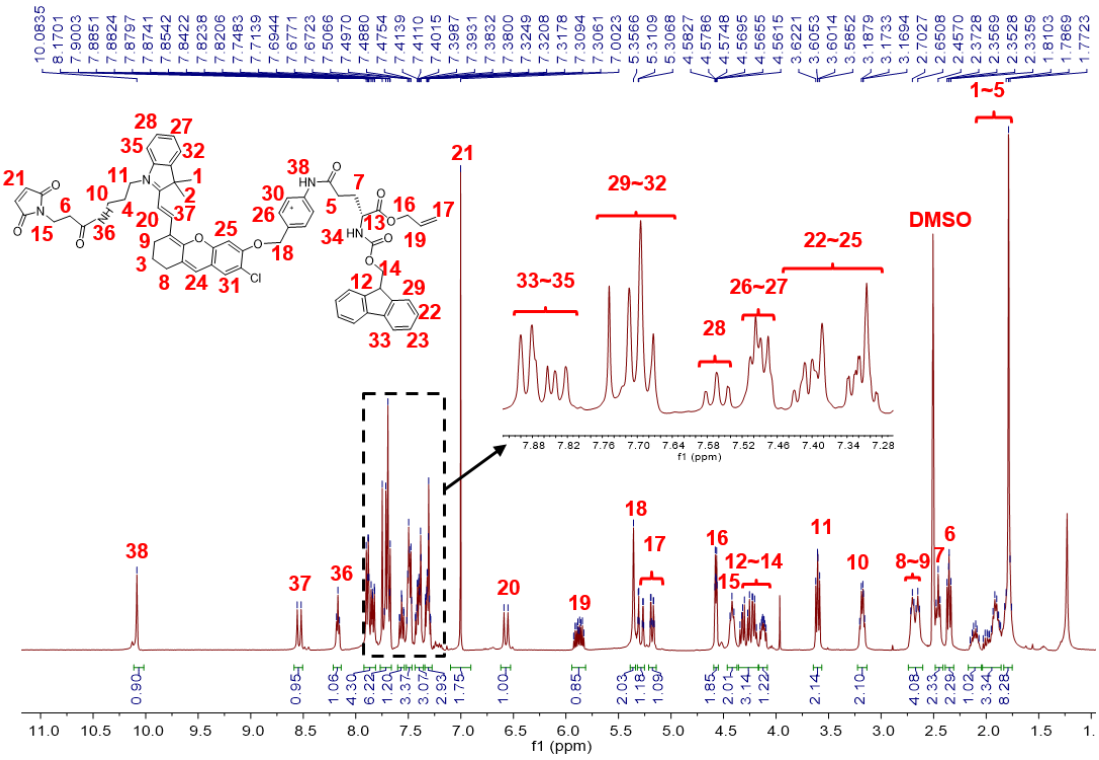


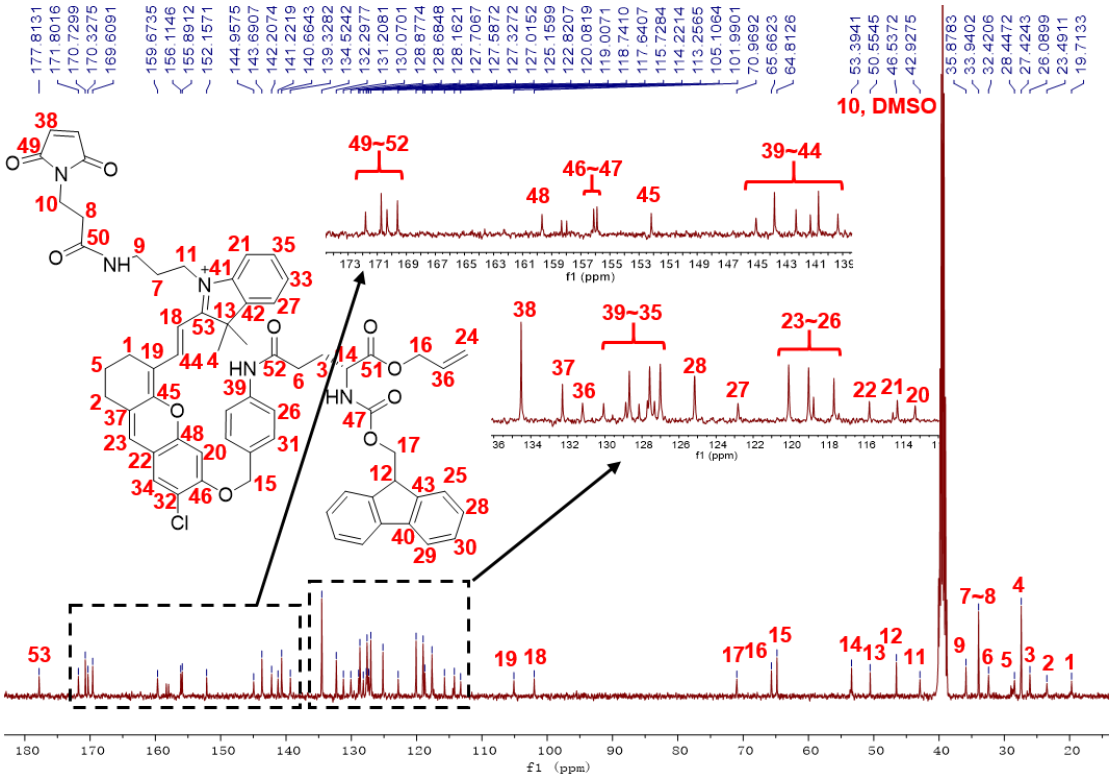
^{1}H-NMR and ^{13}C-NMR spectra of compound **12** (DMSO-d_6).



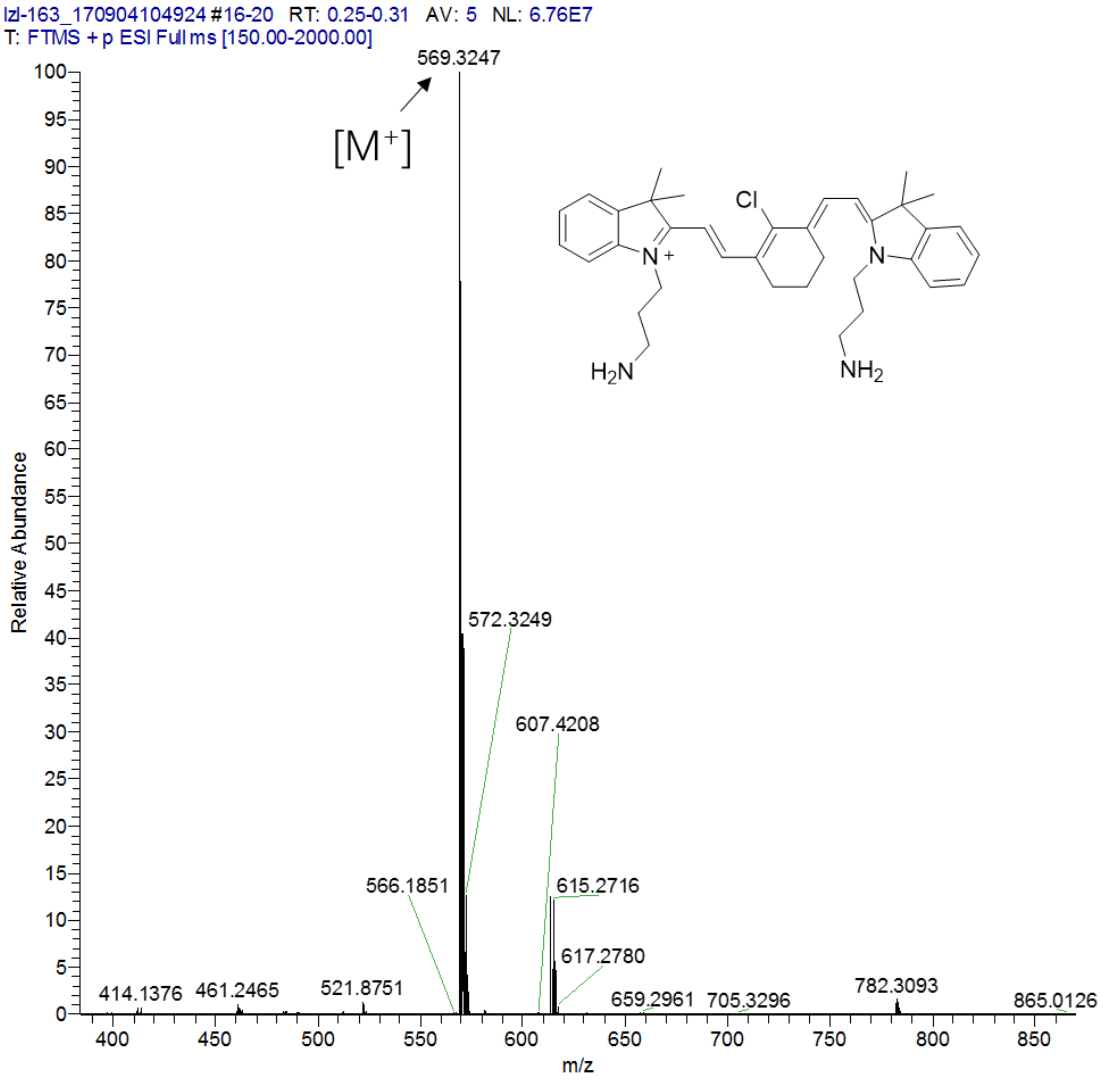


^{1}H-NMR and ^{13}C-NMR spectra of compound **10** (DMSO-d_6).

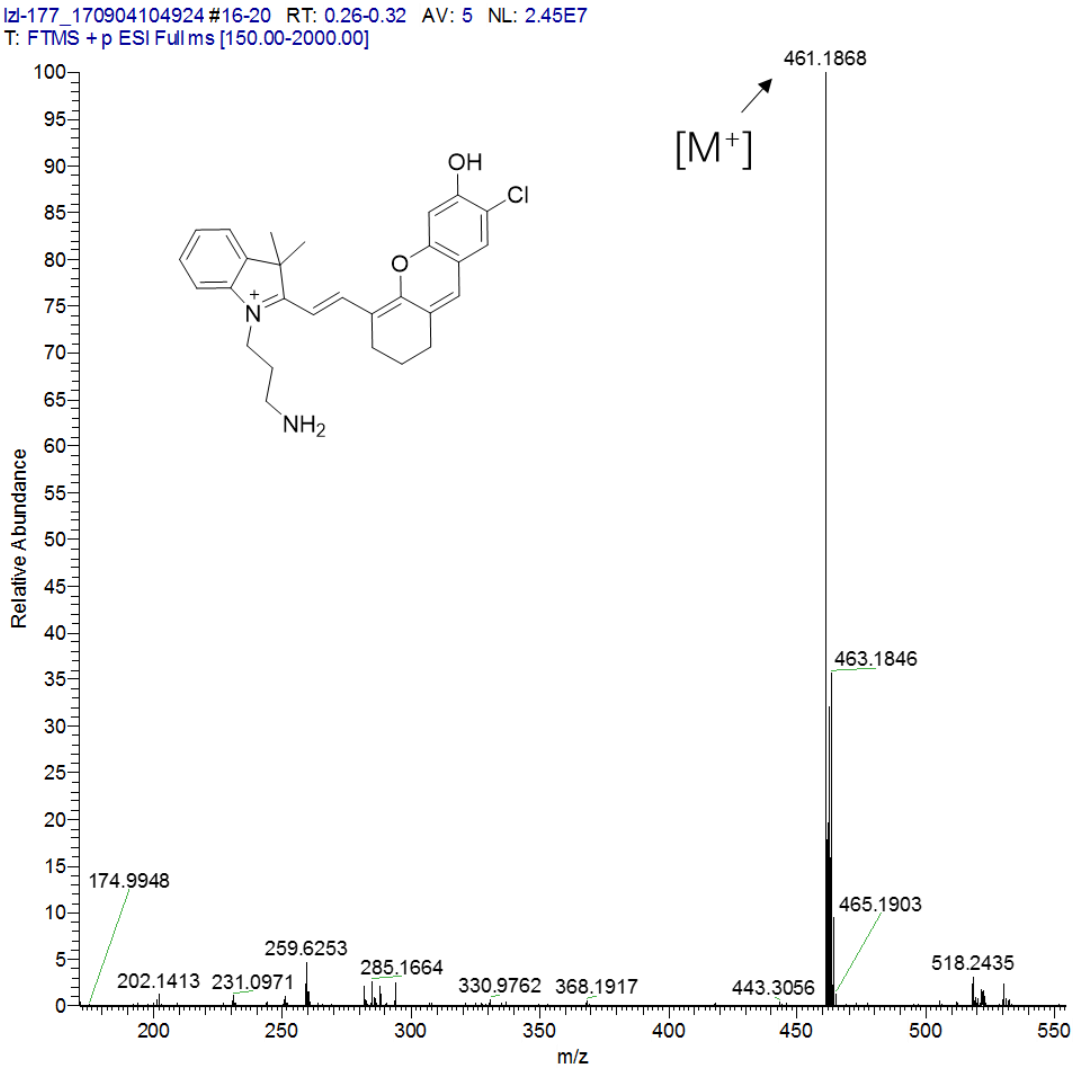




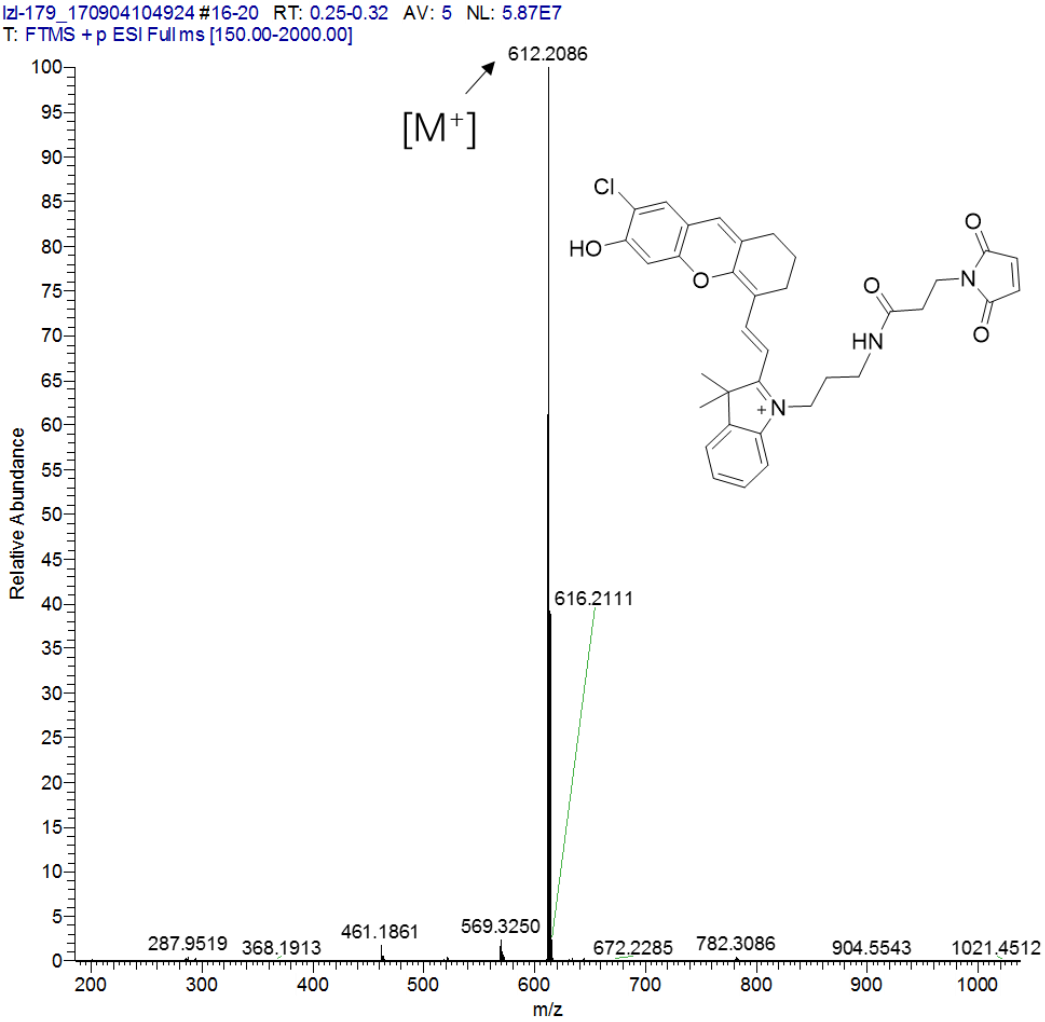
HRMS spectrum of compound **4**.


lzl-163_170904104924 #16-20 RT: 0.25-0.31 AV: 5 NL: 6.76E7
T: FTMS + p ESI Full ms [150.00-2000.00]
569.3247
[M+]
572.3249
607.4208
566.1851
615.2716
617.2780
414.1376
461.2465
521.8751
659.2961
705.3296
782.3093
865.0126
Relative Abundance
m/z
Cl
N+
N
H2N
NH2

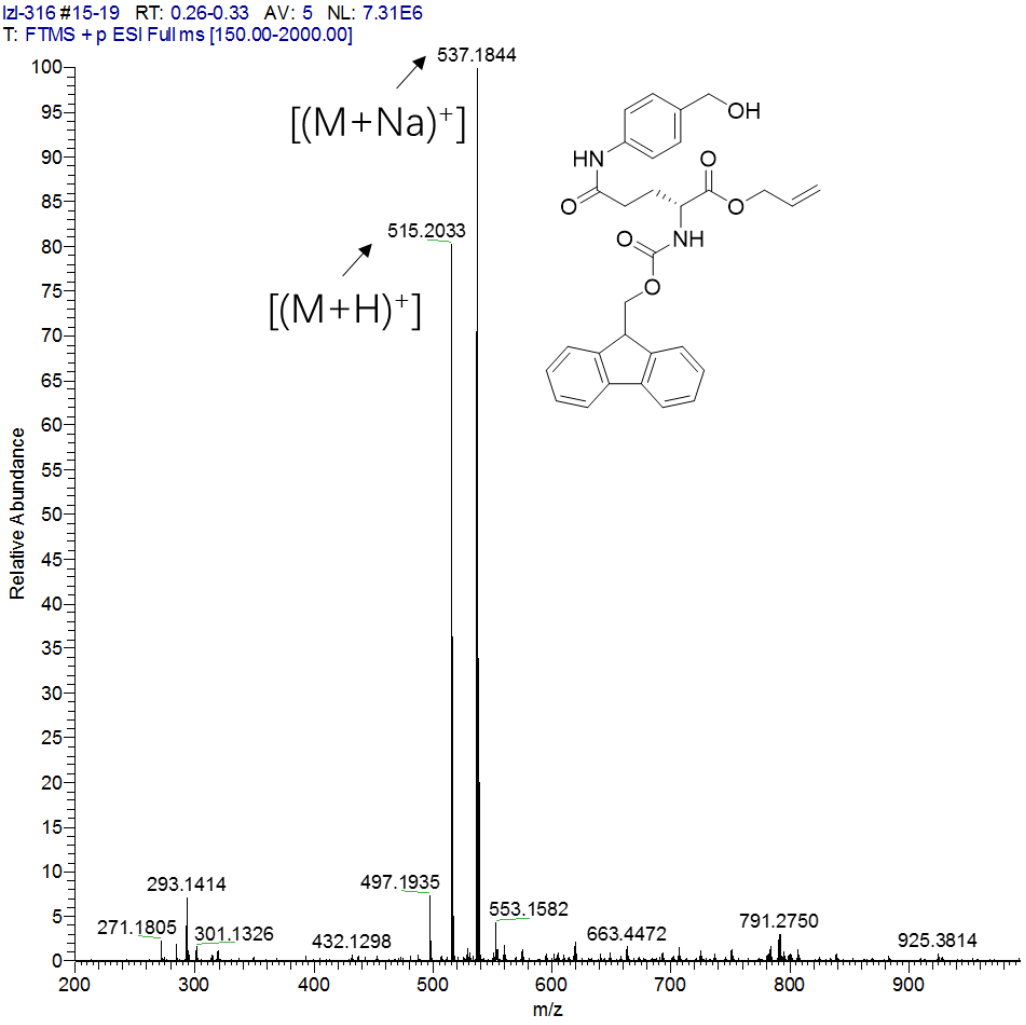
HRMS spectrum of **compound 7**.



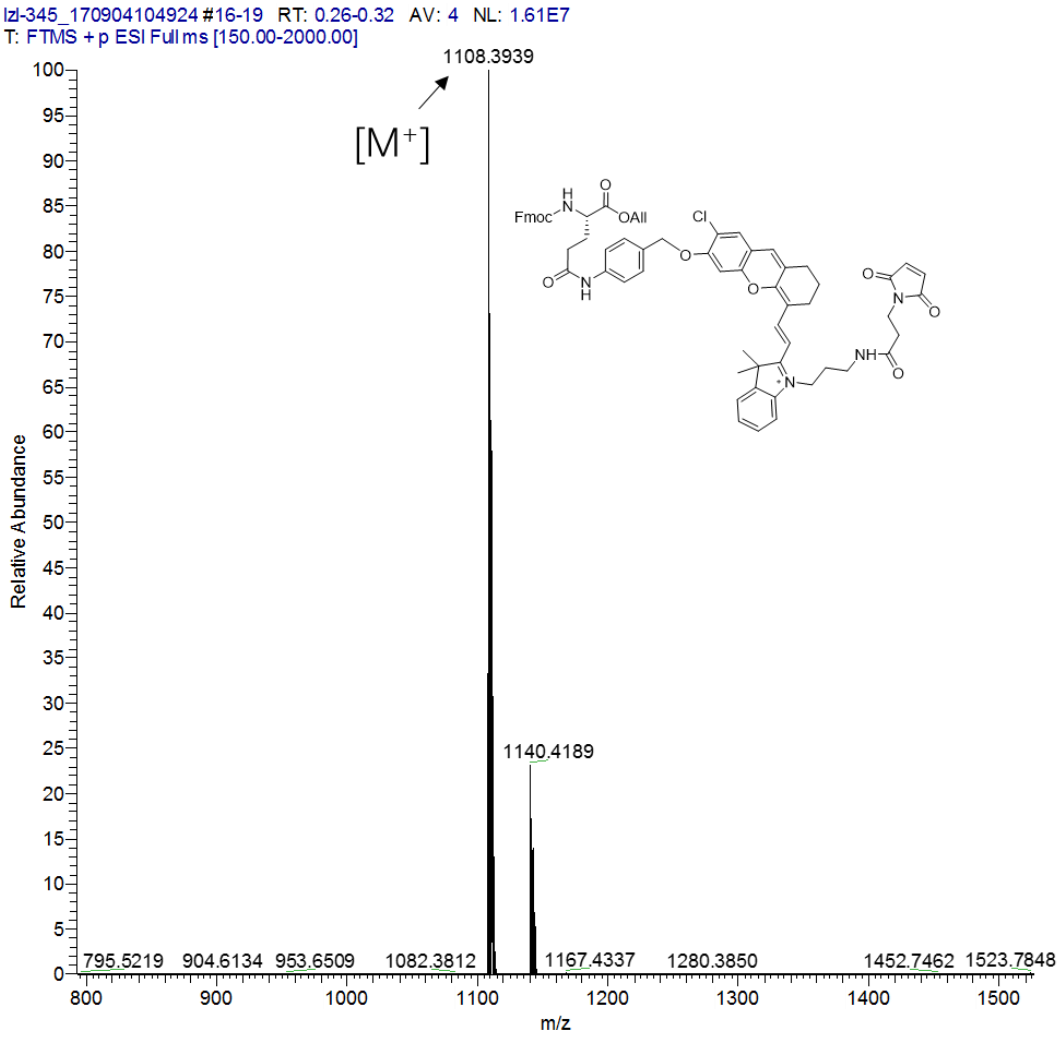
HRMS spectrum of **compound 8**.



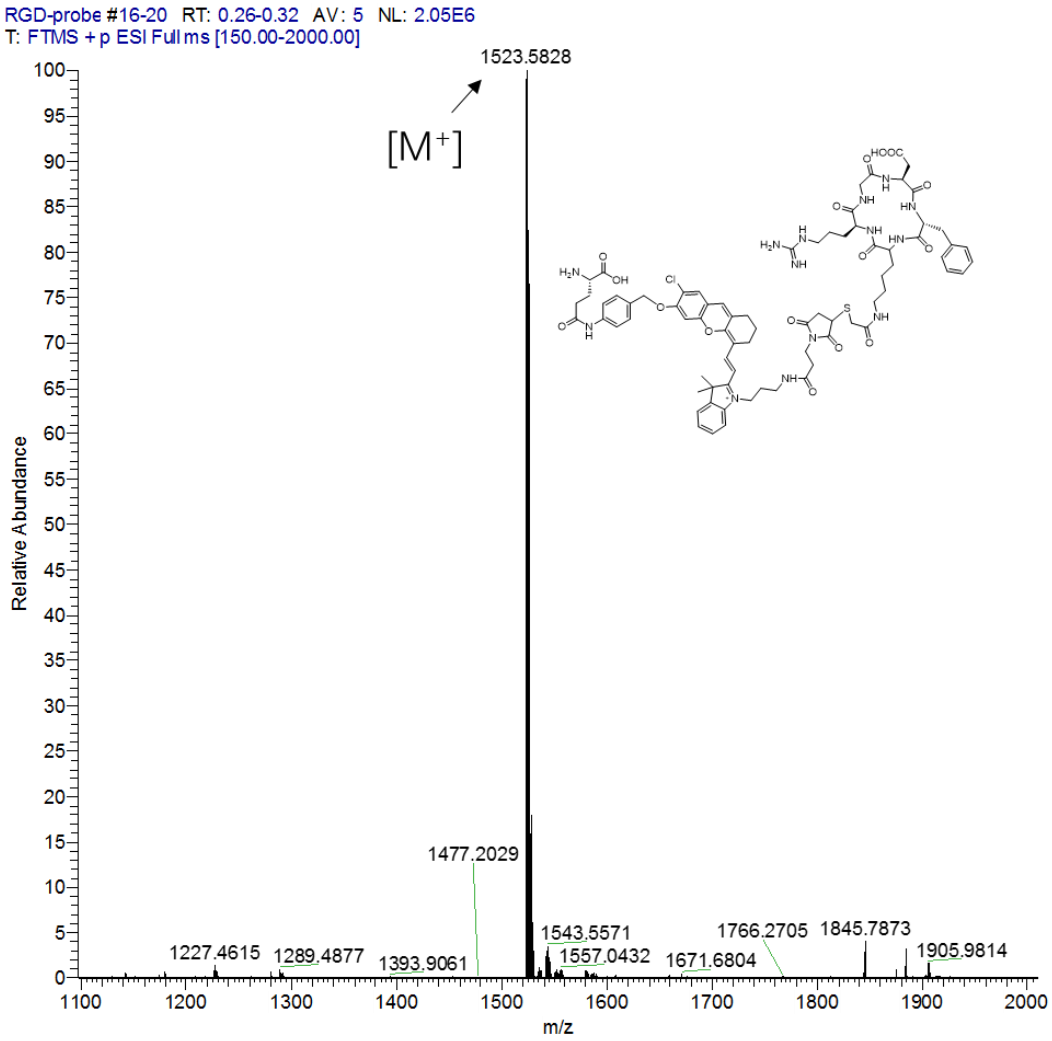
HRMS spectrum of **compound 12**.



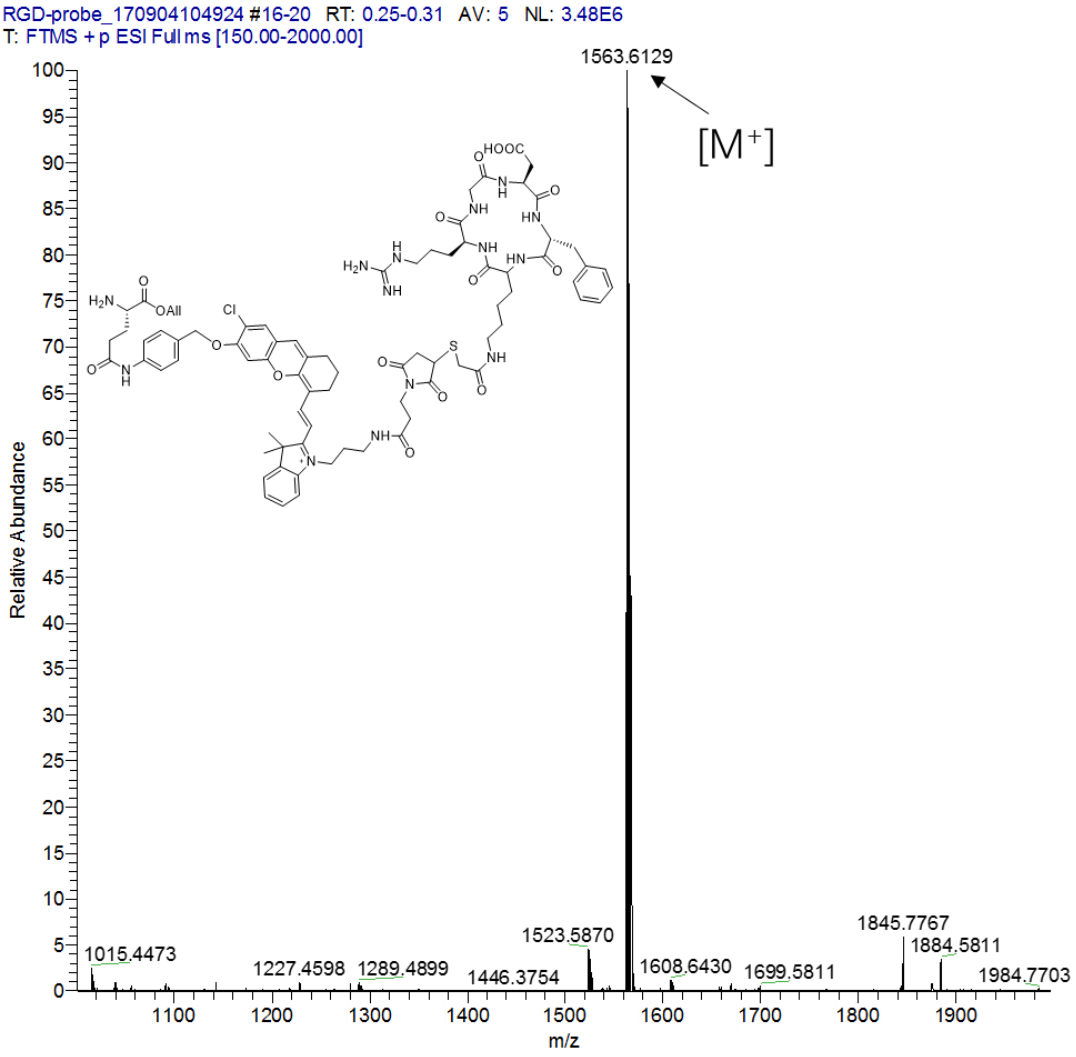
HRMS spectrum of **compound 10**.



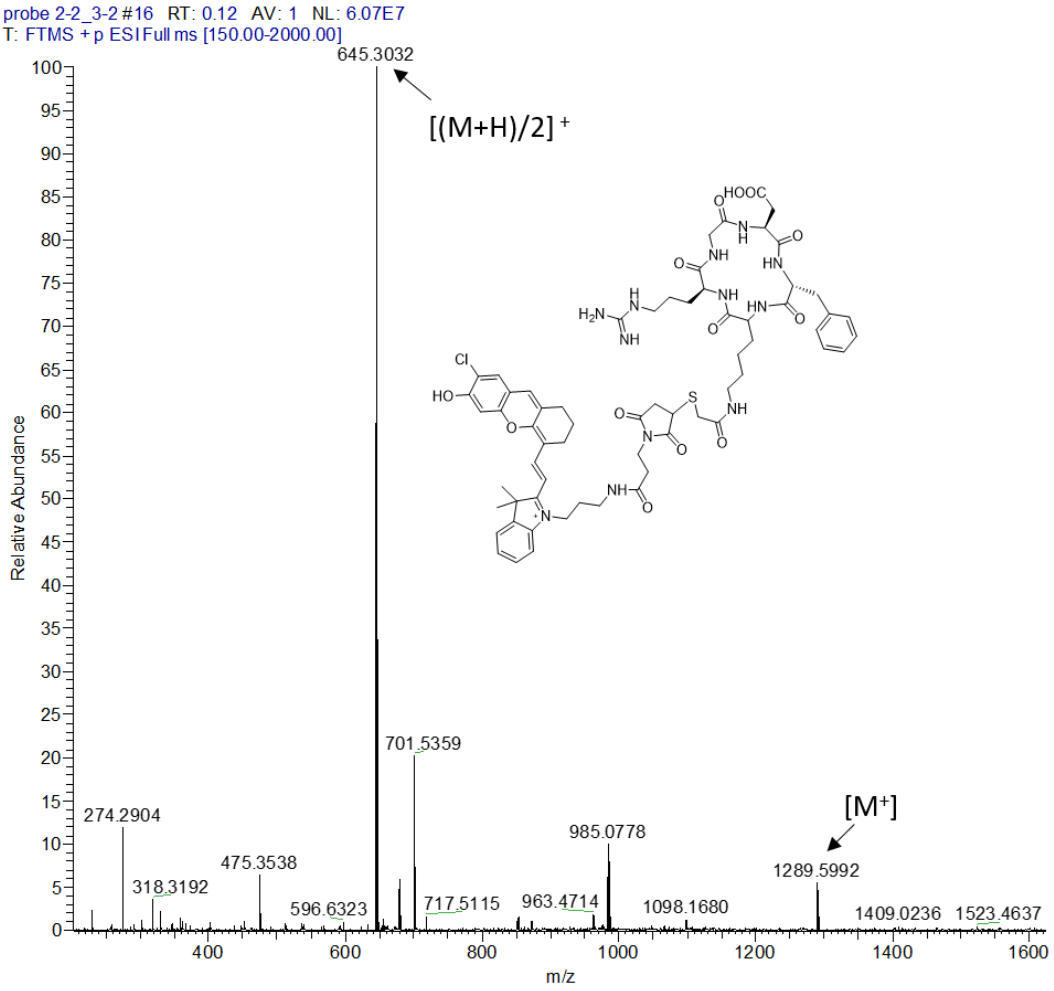
HRMS spectrum of probe **1**.



HRMS spectrum of probe **1-ctrl**.



HRMS spectrum of probe **2**.



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