Supporting Information

Tumor Microenvironment Responsive "Head-to-Foot" Self-Assembly Nanoplatform for Positron Emission Tomography Imaging in Living Subjects

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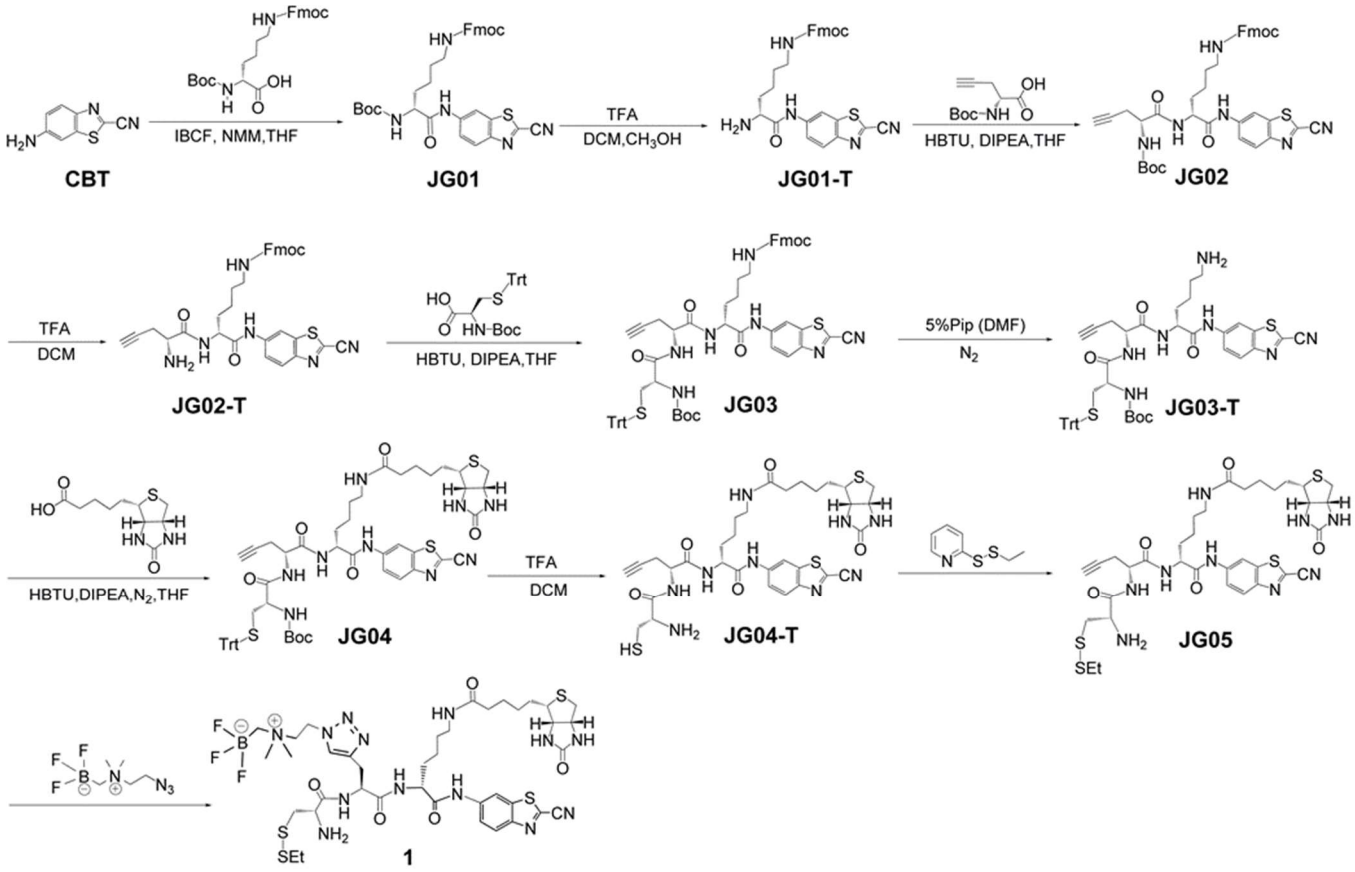
General methods

All chemicals of reagent grade were purchased from commercial sources and used without further purification. Phosphate buffered saline (PBS, 0.01 M, pH 7.4) was purchased from Sangon Biotech Co. Ltd. (Shanghai, China). The reagent 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT) used for cell lysis was purchased from Sigma (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM) and RPMI 1640 were purchased from Gibco Company (USA). Human cervical cancer cell line (Hela), human non-small-cell lung cancer (NSCLC) cell line (A549), human colon cancer cell line (HCT116) and normal human lung embryo cell line (WI-38) were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). BALB/c nude mice (18-20 g; 4-6 weeks old; SLAC Laboratory Animal Co. Ltd; Shanghai; China) were used for animal experiments. Mice were housed with free access to food and water and allowed ample time to acclimatize before the experiments. The tumor-bearing mice were established by subcutaneous injection of Hela or A549 cells (1×10^7) suspended in saline solution (100 μL) to the right flank of each nude mouse. The tumor was allowed to grow for around 3-4 weeks to reach the size of 0.5-1.0 cm in diameter for *in vivo* studies. All experiments involving animals were in accordance with the guideline for ethical review of animal welfare (GB/T 358922018, National Technical Committee for standardization of laboratory animals, China). The animal protocol of this study (No. JSINM2020-050) was approved by the Animal Care and Use Committee of Jiangsu Institute of Nuclear Medicine.

Elemental analysis was carried out using an analyzer (Vario EL III; Elementar; Germany). Electron spray ion mass spectra (ESI-MS) were determined using a Waters Platform ZMD4000 LC/MS (Waters, USA). High resolution mass spectrometry (HRMS) was determined on a MALDI SYNAPT MS (Waters, USA). Nuclear magnetic resonance spectrometer (Bruker DRX-400; Bruker; Germany) was used to obtain ^{1}H-NMR and ^{13}C-NMR spectra of samples dissolved in d_6-DMSO, and the chemical shift was referenced to tetramethylsilane (TMS). The high-performance liquid chromatography (HPLC) system was equipped with a pump (Waters 1525 HPLC; Waters; USA) and connected to reverse phase column (RP-C18; 4.6 × 250 mm; 10 um; Elite Analytical Instrument Company; Dalian; China), a UV detector (2487 dual wavelength absorbance; Waters; USA) as well as a radioactivity detector (Radiomatic 610TR; Perkin Elmer; MA; USA) which were operated by software programs Breeze (NY; USA) and proFSA (Perkin Elmer; USA). HPLC conditions for analysis and purification of the compounds were listed in Tables S2 and S3. The activity was counted using a γ counter (Packard-multi-prias; Perkin Elmer; USA). The size and size distribution of nanoparticles were determined by dynamic light scattering (DLS) method on a zeta sizer nano series (Malvern Instruments; United Kingdom). Transmission electron microscopy (TEM) was used to observe the formation of nanoparticles and measure the mean diameter of nanoparticles using the JEOL 2100 electron microscope (JEOL; Jappan). MicroPET imaging was performed on an Inveon scanner (Siemens, Germany).

Chemical synthesis and characterization of probe 1



Scheme S1. Synthesis route of probe **1**.

Synthesis of compound JG01

Isobutyl chloroformate (195 μL, 1.5 mmol, IBCF) and *N*-methyl morpholine (330 μL, 3.0 mmol, NMM) were added to Boc-Lys(Fmoc)-OH (702mg, 1.5 mmol) in dry tetrahydrofuran (5mL, THF). The reaction mixture was stirred for 1 h at 0 °C under nitrogen (N_2) and for another 6 h at room temperature. Then, the solution of 6-amino-2-cyanobenzothiazole (175 mg, 1.0 mmol, CBT) in THF (5 mL) was added and the reaction mixture was stirred for 1 h at 0 °C under N_2, followed by 12 h at room temperature. Hydrochloric acid (2 mL, 1 M) was added to the solution to quench the reaction. The crude product was extracted with ethyl acetate, washed with saturated sodium bicarbonate solution, and purified by silica gel chromatography with hexane/ethyl acetate (1:1) as eluents. **JG01** was obtained as a yellow solid (783 mg) with a yield of 83.5%.

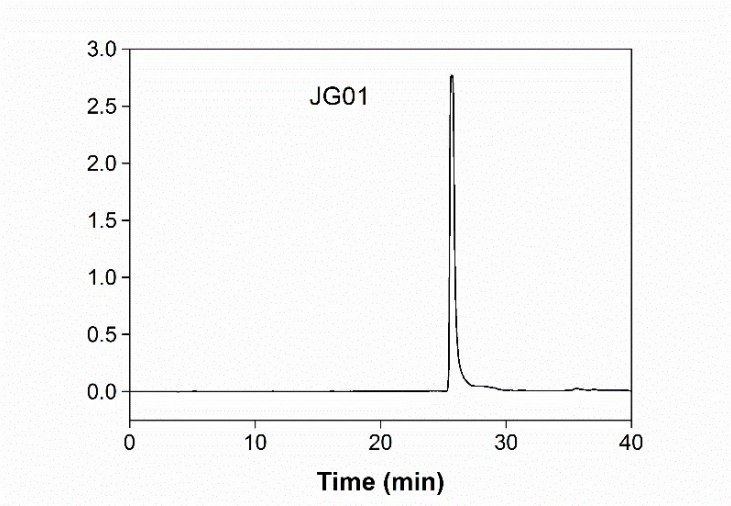


Figure S1. HPLC trace of compound **JG01**.

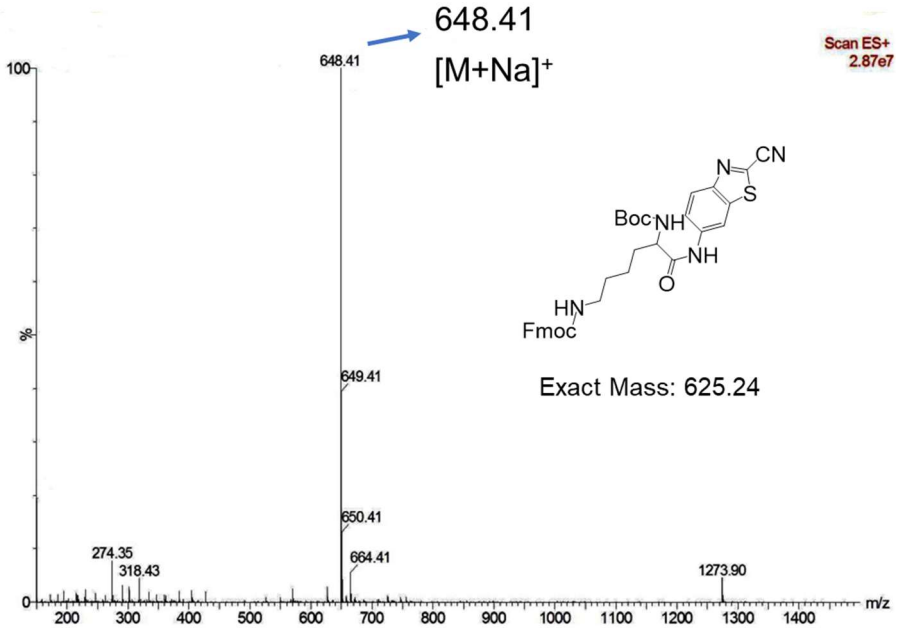


Figure S2. ESI-MS of compound **JG01**.

Synthesis of compound JG01T

JG01 was dissolved in 6mL of trifluoroacetic acid (TFA) and CH_2Cl_2 (DCM) (1:1, *v/v*) to remove the BOC group. Solvents were evaporated under reduced pressure and the crude product was precipitated with cold diethyl ether and collected by 5 min centrifugation (4000 r/min) to obtain a yellow solid (**JG01T**, 625 mg, 95%).

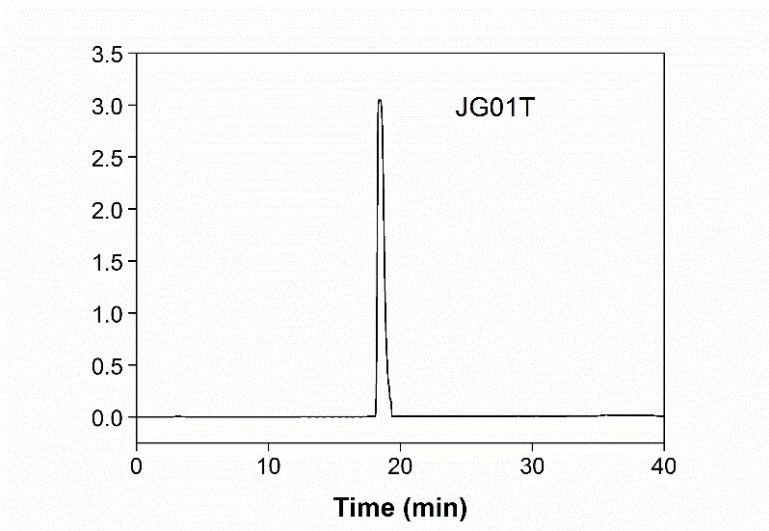
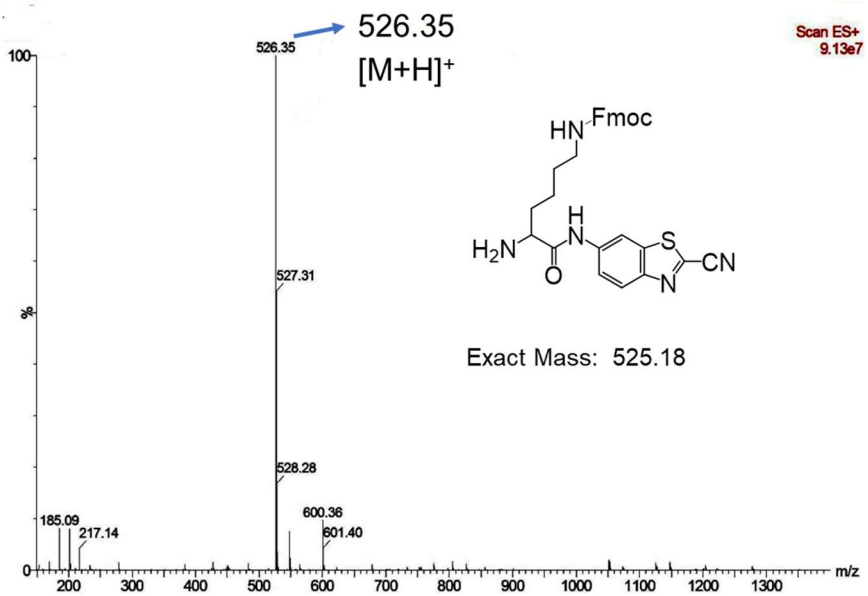


Figure S3. HPLC trace of compound **JG01T**.



FigureS4. ESI-MS of compound **JG01T**.

Synthesis of compound JG02

JG01T (625 mg, 1.2 mmol), *N*-Boc-D-propargylglycine (281 mg, 1.32 mmol), 2-(1H-benzotriazole-1-ly)-1,1,3,3-tetramethyluronium hexaflourophosphate (523 mg, 1.38 mmol, HBTU) and diisopropylethylamine (594 μL,3.6 mmol, DIPEA) in THF (5 mL) were stirred at room temperature for 1 h under N_2. The crude product was purified by silica gel chromatography (hexane/ethyl acetate, 1:1) to yield **JG02** as a white solid (657 mg, 76%).

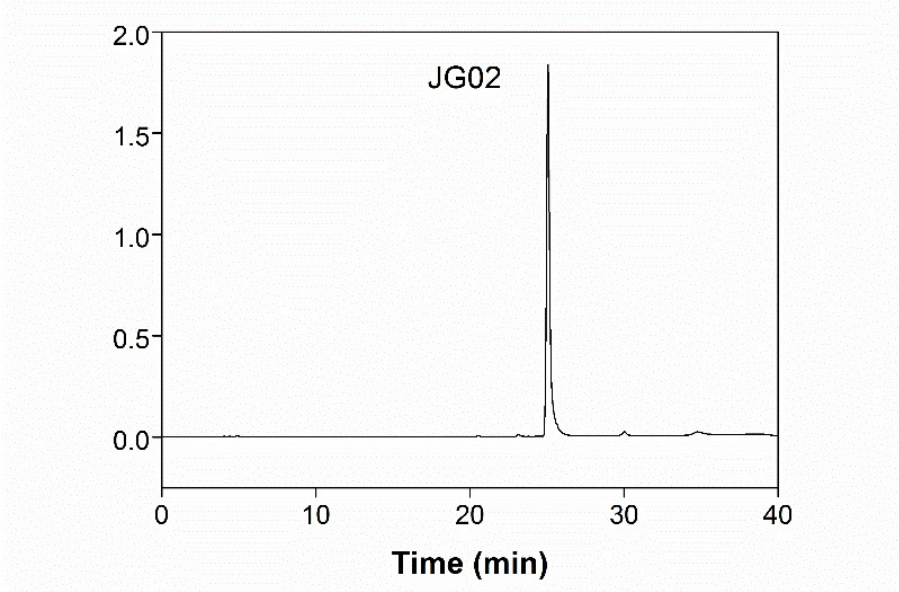


Figure S5. HPLC trace of compound **JG02**.

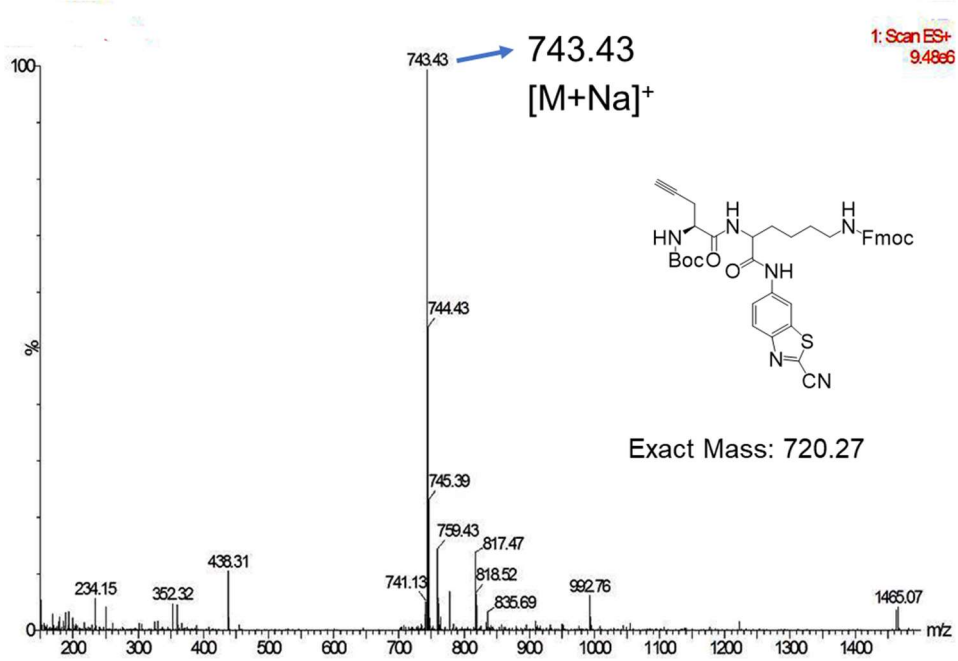


Figure S6. ESI-MS of compound **JG02**.

Synthesis of compound JG02T

The protecting group Boc was removed by treatment of **JG02** (657 mg, 0.91 mmol) with TFA/CH_2Cl_2 (8 mL, 1:1) at room temperature for 0.5 h. Then the solvent was removed under reduced pressure, and the crude product was precipitated with cold diethyl ether. Finally, **JG02T** (530 mg, 93.7%) was obtained after centrifugation (4000 r/min) for 5 min.

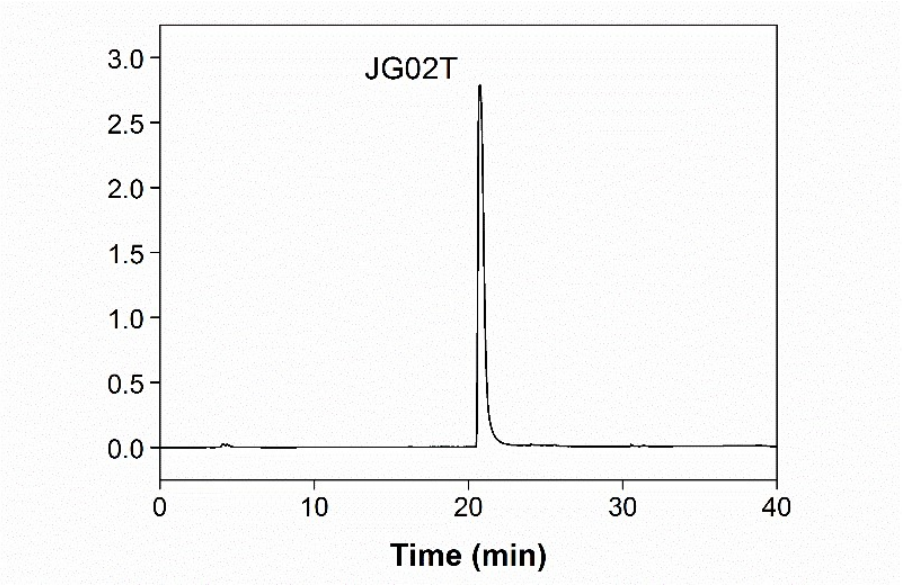


Figure S7. HPLC trace of compound **JG02T**.

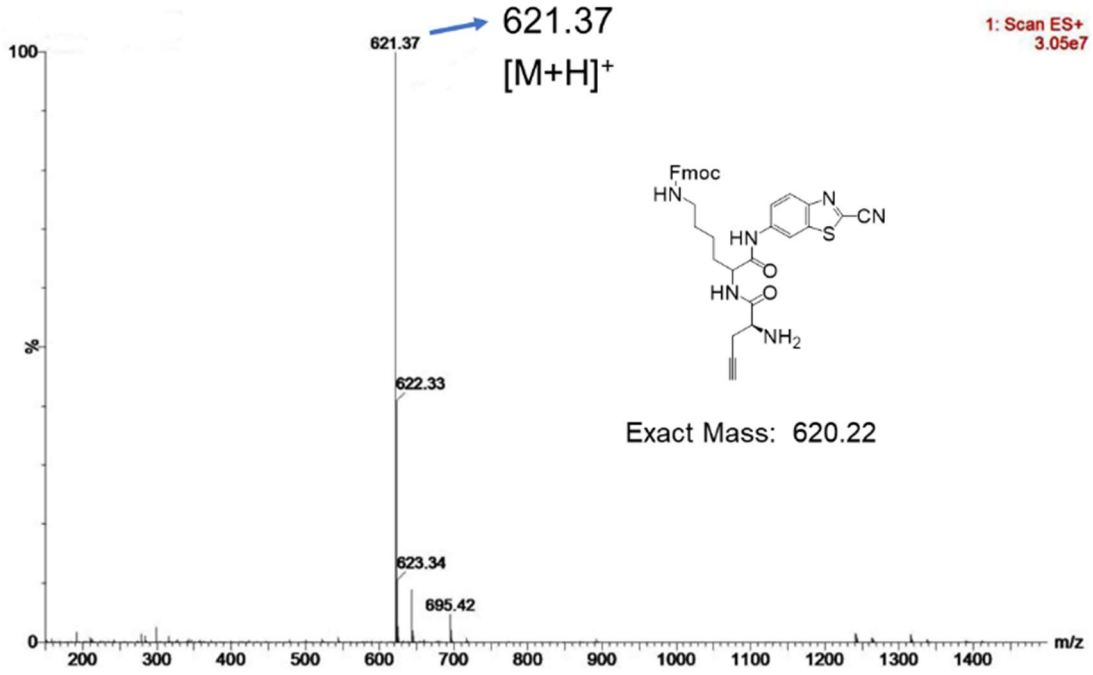


Figure S8. ESI-MS of compound **JG02T**.

Synthesis of compound JG03

JG02T (530 mg, 0.85 mmol), *N*-Boc-S-trityl-D-cysteine (435 mg, 0.94 mmol), DIPEA (420 μL, 2.55 mmol) and HBTU (370 mg, 0.98 mmol) were dissolved in THF (10 mL) and stirred at room temperature for 1 h under N_2. Solvent was evaporated and the crude product was purified by silica gel chromatography using hexane/ethyl acetate (2:1) as eluents to provide **JG03** (583 mg, 64%).

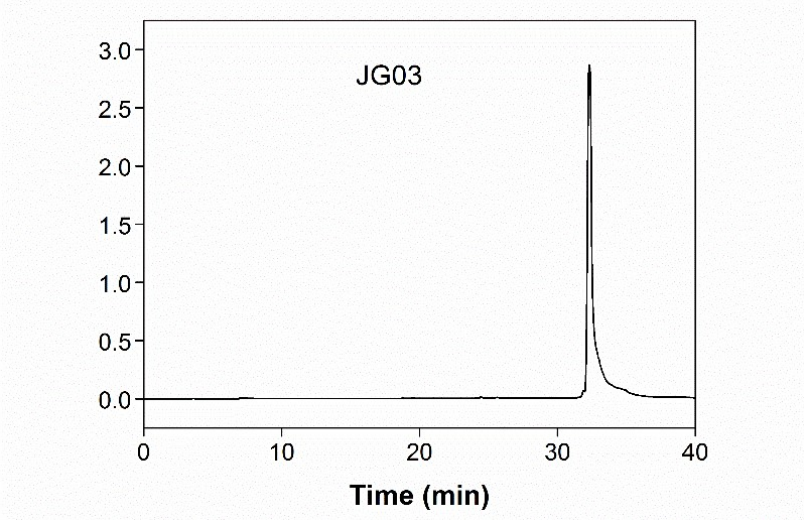


Figure S9. HPLC trace of compound **JG03**.

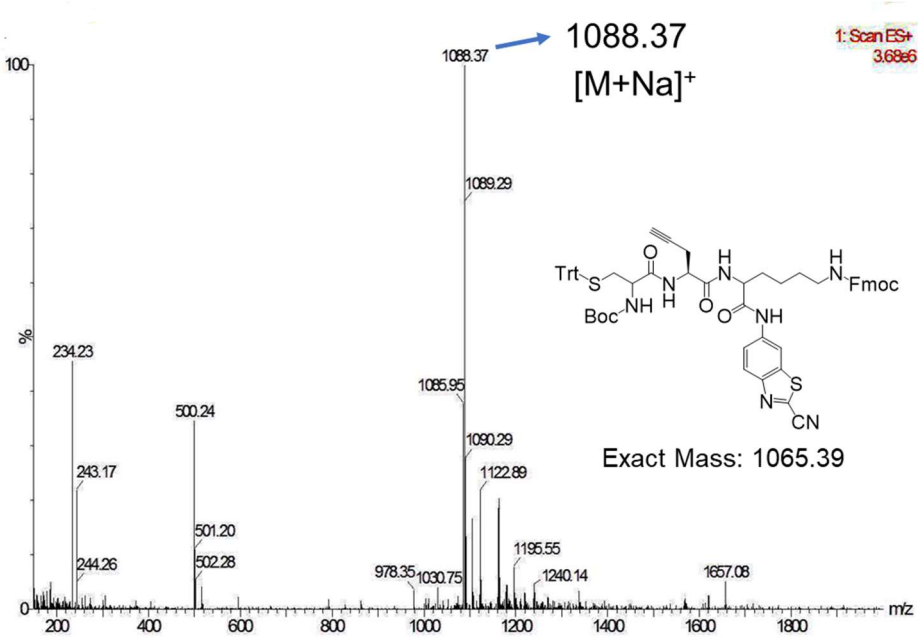


Figure S10. ESI-MS of compound **JG03**.

Synthesis of compound JG03T

JG03 (583 mg, 0.55 mmol) was dissolved in a solution of 5% piperidine in DMF (1.8 mL) under nitrogen atmosphere and the mixture was stirred for 15 minutes at room temperature. Then, HCl (1.1 mL, 1 M) was added dropwise to adjust pH to 5. The crude product was purified by preparative HPLC to yield **JG03T** as a white solid (185 mg, 40%).

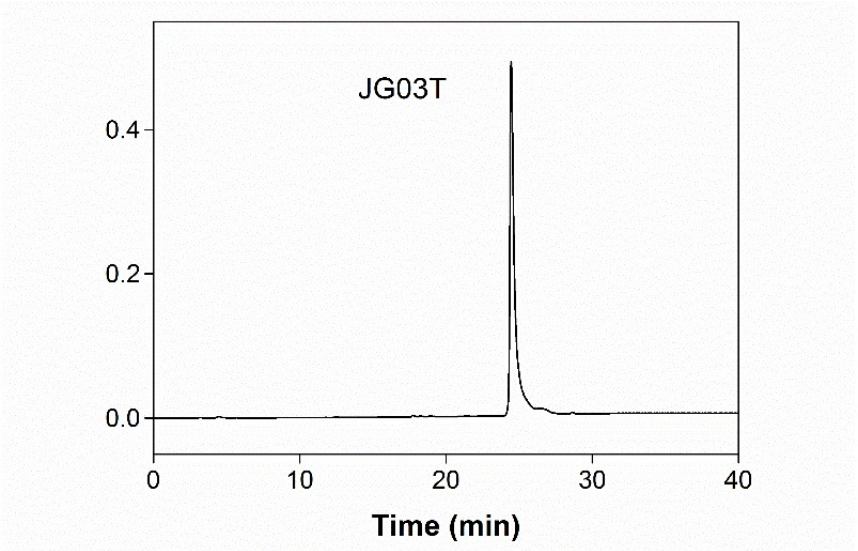


Figure S11. HPLC trace of compound **JG03T**.

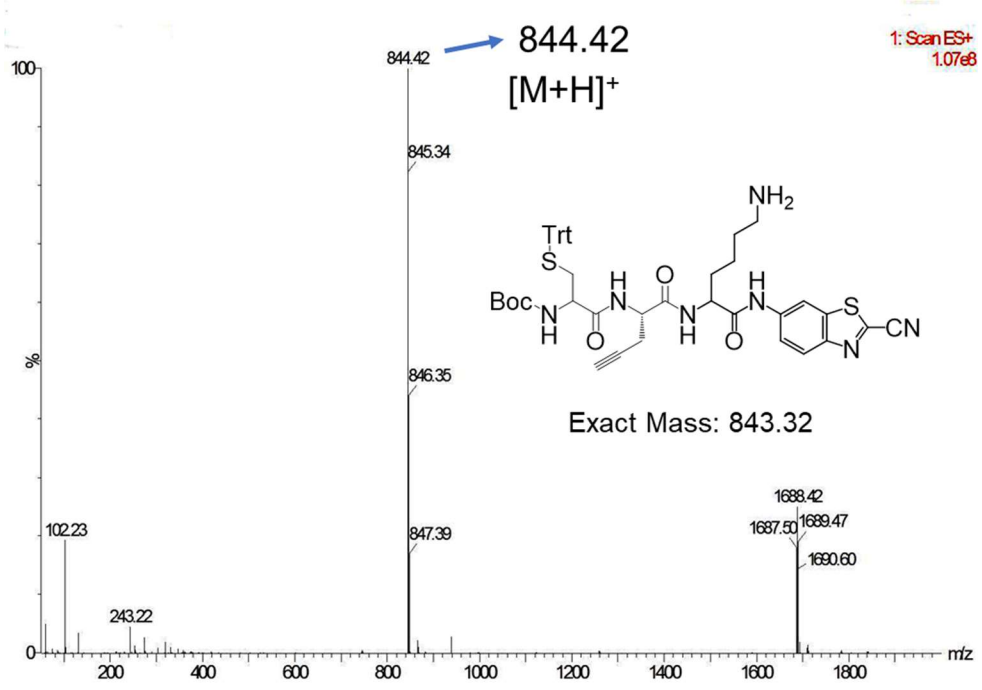


Figure S12. ESI-MS of compound **JG03T**.

Synthesis of compound JG04

To a solution of **JG03T** (185 mg, 0.22 mmol) in dry THF and DMF (1:1), D-biotin (64 mg, 0.26 mmol), HBTU (96 mg, 0.25 mmol) and DIPEA (110 μL, 0.66 mmol) were added. The mixture was stirred at room temperature for 1 h under N_2. Then the solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (DCM/MeOH, 10:1) to yield **JG04** as a white solid (136 mg, 58%).

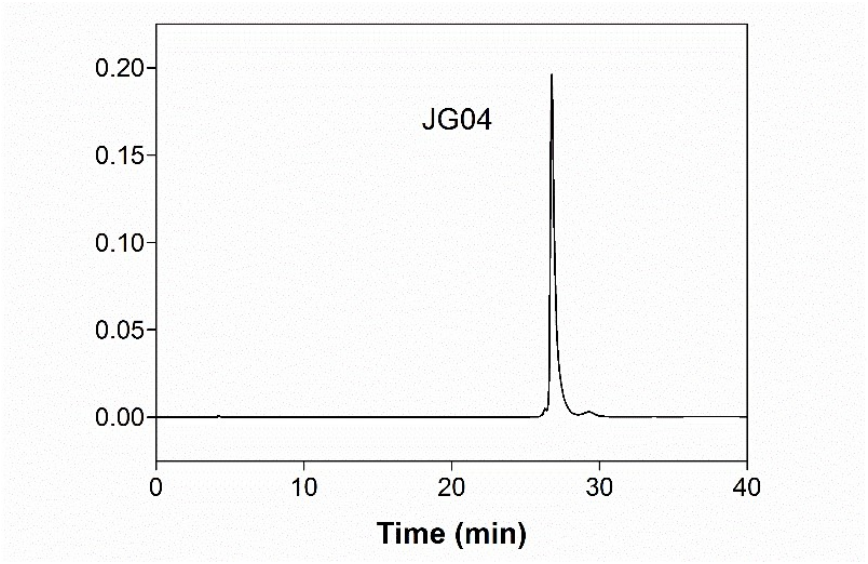


Figure S13. HPLC trace of compound **JG04**.

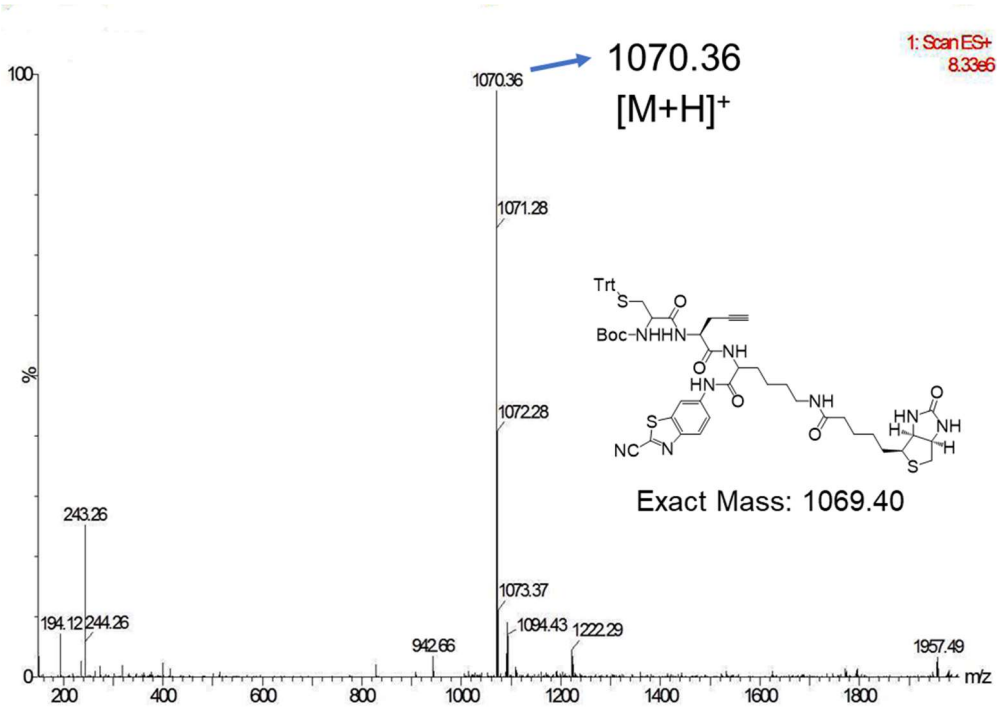


Figure S14. ESI-MS of compound **JG04**.

Synthesis of compound JG04T

Boc and Trt protecting groups were removed by treating **JG04** (136 mg, 0.13 mmol) with a solution (3 mL) of CH_2Cl_2, C_2H_3N and TFA (0.5:1:1.5) containing 2% of TIPS. After stirring at room temperature for 0.5 h, the solution was evaporated under reduced pressure, and the crude product was precipitated with diethyl ether. Finally, **JG04T** (86 mg, 93%) was obtained after centrifugation (4000 r/min) for 5 min.

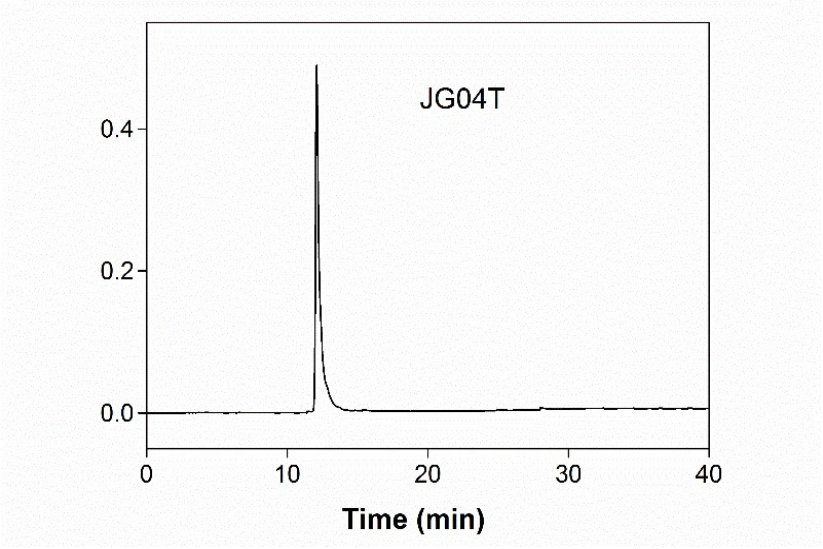


Figure S15. HPLC trace of compound **JG04T**.

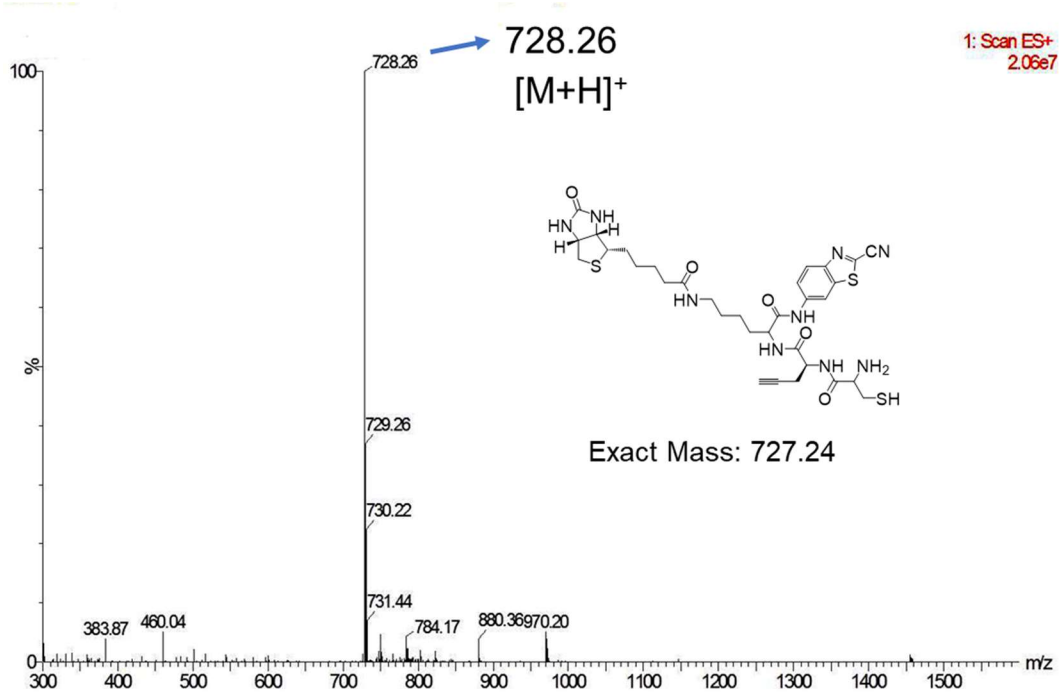


Figure S16. ESI-MS of compound **JG04T**.

Synthesis of compound JG05

JG04T (40 mg, 0.055 mmol) was dissolved in CH_3OH, and SEt (14 mg, 0.082 mmol) was added to the solution. The mixture was stirred at room temperature for 0.5 h under N_2. Then TIPS (1%) was added to the solution, and the mixture was stirred for another 0.5 h. Upon evaporation of the solvent, the crude product was precipitated with diethyl ether. **JG05** (41 mg, 95%) was obtained by centrifugation(4000 r/min) for 5 min.^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.93 (d, J = 8.9 Hz, 1H), 8.70 (d, J = 2.0 Hz, 1H), 8.58 (d, J = 7.8 Hz, 1H), 8.40 – 8.31 (m, 2H), 8.20 (d, J = 9.1 Hz, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.76 – 7.69 (m, 1H), 6.36 (d, J = 12.0 Hz, 2H), 4.61 (q, J = 7.1 Hz, 1H), 4.57 – 4.47 (m, 1H), 4.47 – 4.38 (m, 1H), 4.30 (dd, J = 7.7, 5.0 Hz, 1H), 4.15 – 4.12 (m, 1H), 3.15 (dd, J = 14.1, 5.1 Hz, 1H), 3.08 (d, J = 13.3 Hz, 1H), 3.02 (d, J = 5.9 Hz, 2H), 3.00 – 2.97 (m, 1H), 2.92 (t, J = 2.6 Hz, 1H), 2.83 (d, J = 5.1 Hz, 1H), 2.76 (d, J = 24.2 Hz, 1H), 2.66 (q, J = 7.2 Hz, 2H), 2.61 – 2.59 (m, 1H), 2.56 (s, 1H), 2.02 (t, J = 7.4 Hz, 2H), 1.81 – 1.68 (m, 2H), 1.51 – 1.45 (m, 2H), 1.43 – 1.39 (m, 2H), 1.34 – 1.28 (m, 2H), 1.25 (d, J = 8.9 Hz, 2H), 1.15 (t, J = 7.3 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 172.35, 171.61, 169.46, 167.28, 163.19, 148.18, 139.82, 137.12, 135.52, 125.29, 121.30, 113.99, 111.83, 80.34, 74.00, 61.53, 59.70, 55.87, 54.03, 52.26, 51.66, 38.66, 35.68, 32.06, 31.72, 29.34, 28.68, 28.49, 25.76, 23.22, 22.76, 14.46.

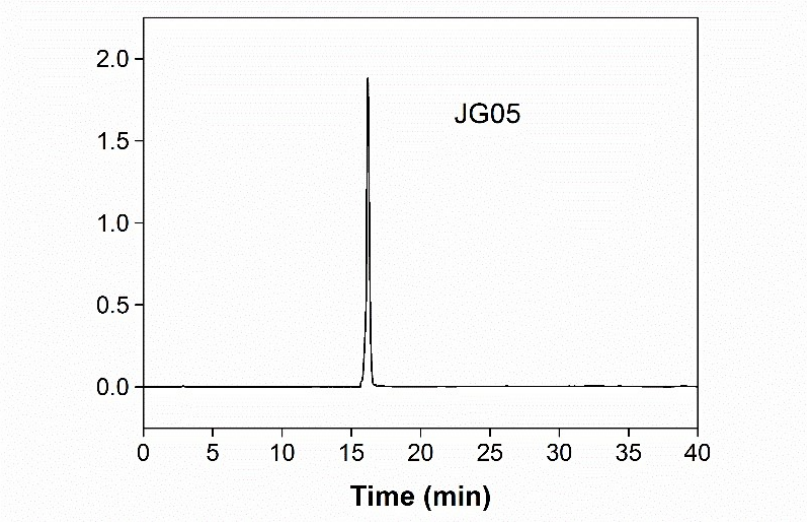


Figure S17. HPLC trace of compound **JG05**.

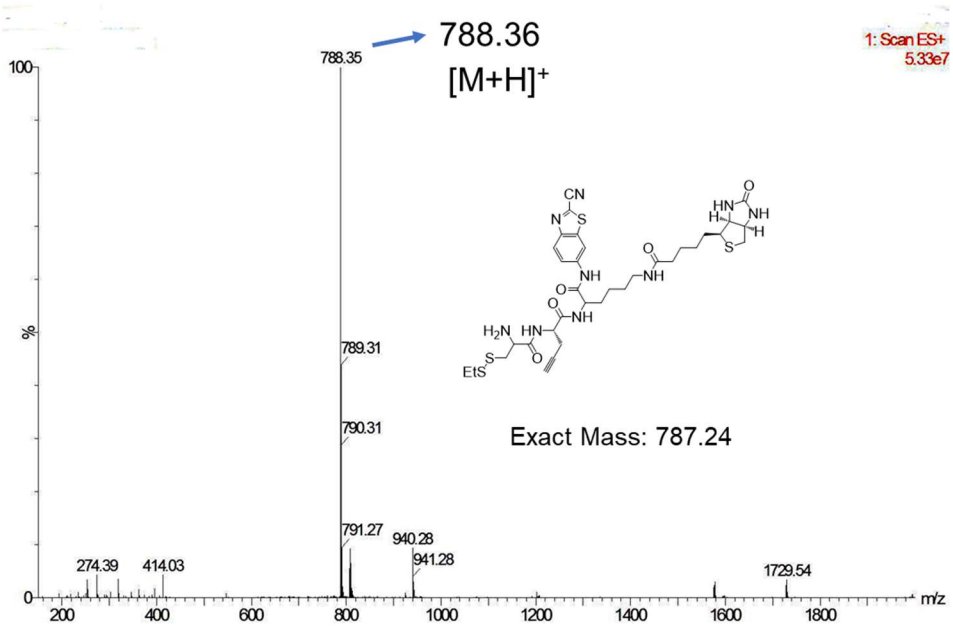


Figure S18. ESI-MS of compound **JG05**.

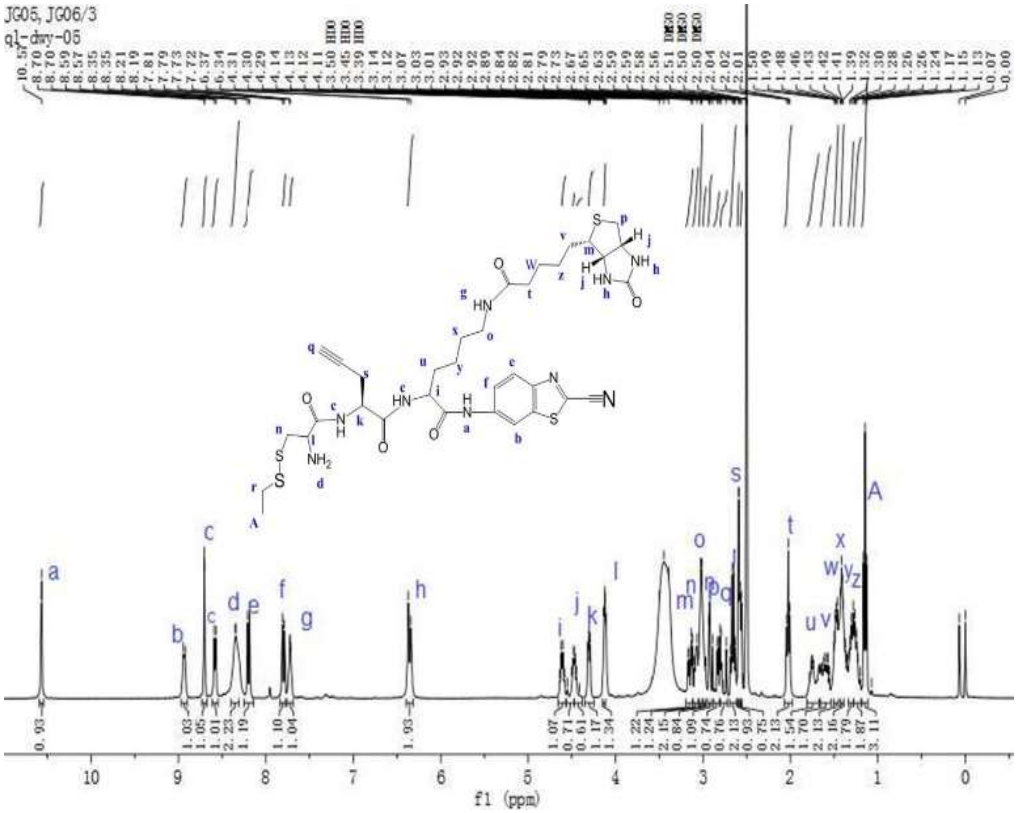


Figure S19. ^{1}H-NMR of compound **JG05**.

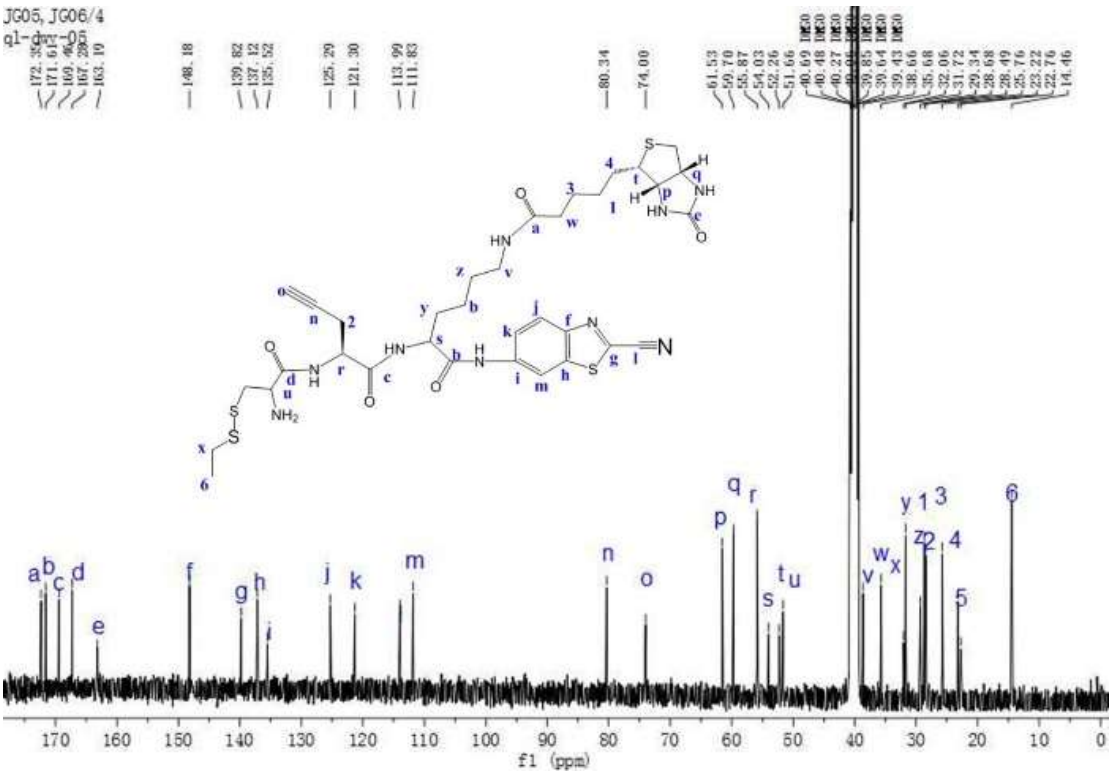


Figure S20. ^{13}C-NMR of compound **JG05**.

Synthesis of probe 1

JG05 (35 mg, 0.044 mmol), $AMBF_3$ (43 mg, 0.22 mmol) and tris(2-benzimidazolylmethyl)amines (1.9 mg, 0.0044 mmol, $(BimC_4A)_3$) were dissolved in a solution of DMF and H_2O (1:1, *v*/*v*) under N_2. Then, terakis(acetonitrile)copper(I) hexafluorophosphate (16.1 mg, 0.044 mmol) was added to the solution. The reaction mixture was stirred for 1 h at 45ºC and the solvent was removed under reduced pressure. The crude product was purified with preparative HPLC to give **1** (24 mg, 55%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, J = 8.0 Hz, 1H), 8.70 (d, J = 2.1 Hz, 1H), 8.44 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 5.0 Hz, 2H), 8.20 (d, J = 9.0 Hz, 1H), 7.96 (s, 1H), 7.79 (dd, J = 9.1, 2.1 Hz, 1H), 7.71 (t, J = 5.6 Hz, 1H), 6.36 (s, 2H), 4.85 (t, J = 7.1 Hz, 2H), 4.74 (q, J = 7.3 Hz, 3H), 4.30 (dd, J = 7.7, 4.9 Hz, 1H), 4.12 (dd, J = 7.8, 4.4 Hz, 2H), 3.74 (t, J = 7.1 Hz, 2H), 3.17 (dd, J = 14.0, 5.0 Hz, 1H), 3.10 – 3.05 (m, 2H), 2.99 (d, J = 8.2 Hz, 2H), 2.66 (q, J = 7.2 Hz, 2H), 2.57 (d, J = 12.4 Hz, 1H), 2.44 – 2.37 (m, 2H), 2.02 (t, J = 7.4 Hz, 2H), 1.79 – 1.61 (m, 2H), 1.57 (dd, J = 13.7, 7.4 Hz, 2H), 1.49 – 1.43 (m, 2H), 1.41 – 1.33 (m, 2H), 1.28 (q, J = 5.0, 2.9 Hz, 2H), 1.25 – 1.18 (m, 2H), 1.15 (t, J = 7.3 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 172.39, 171.58, 170.24, 167.18, 163.17, 158.67, 148.19, 143.23, 139.79, 137.12, 125.29, 124.20, 121.32, 111.85, 63.73, 61.52, 59.70, 55.86, 53.99, 53.75, 53.30, 51.74, 44.04, 38.66, 35.66, 31.99, 31.71, 29.32, 28.66, 28.47, 25.75, 23.13, 14.47.

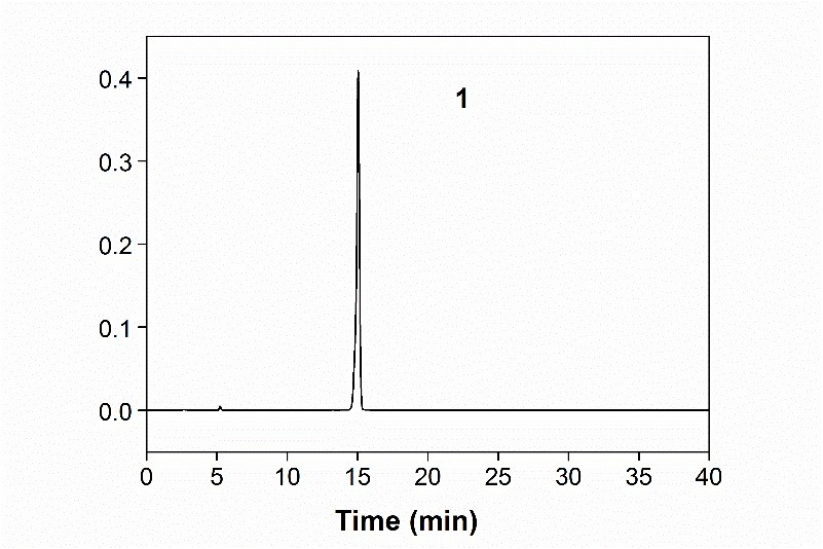


Figure S21. HPLC trace of probe **1**.

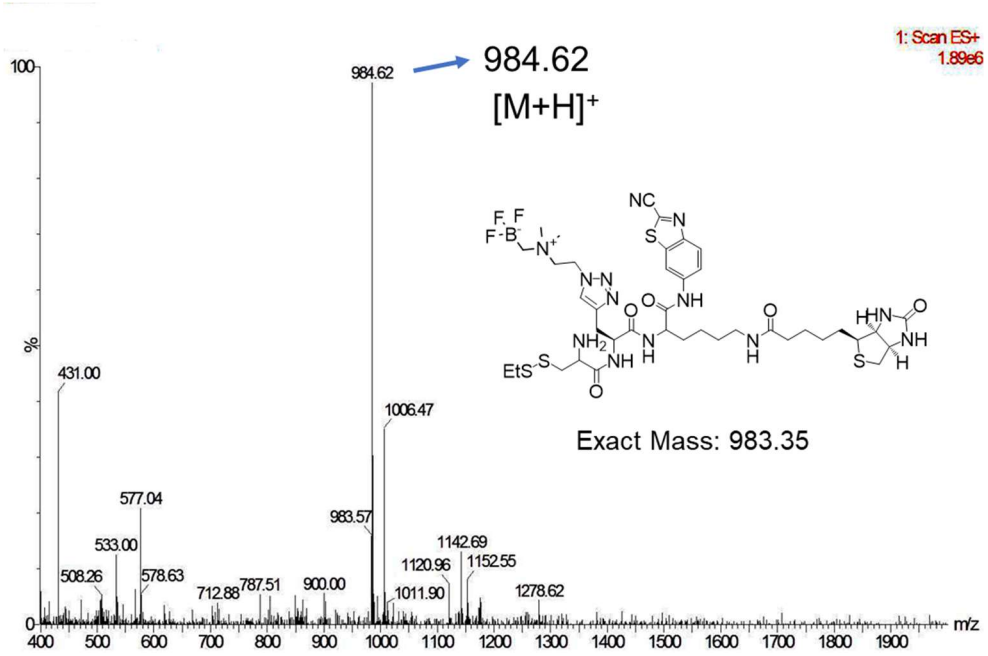


Figure S22. ESI-MS of probe **1**.

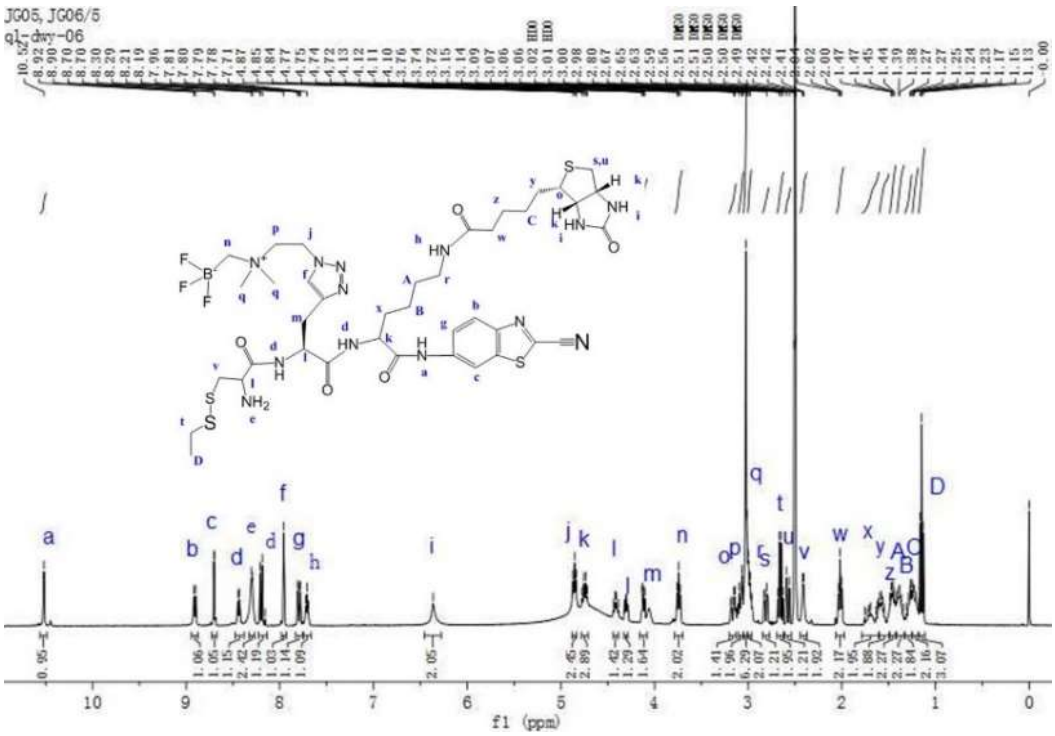


Figure S23. ^{1}H-NMR of probe **1**.

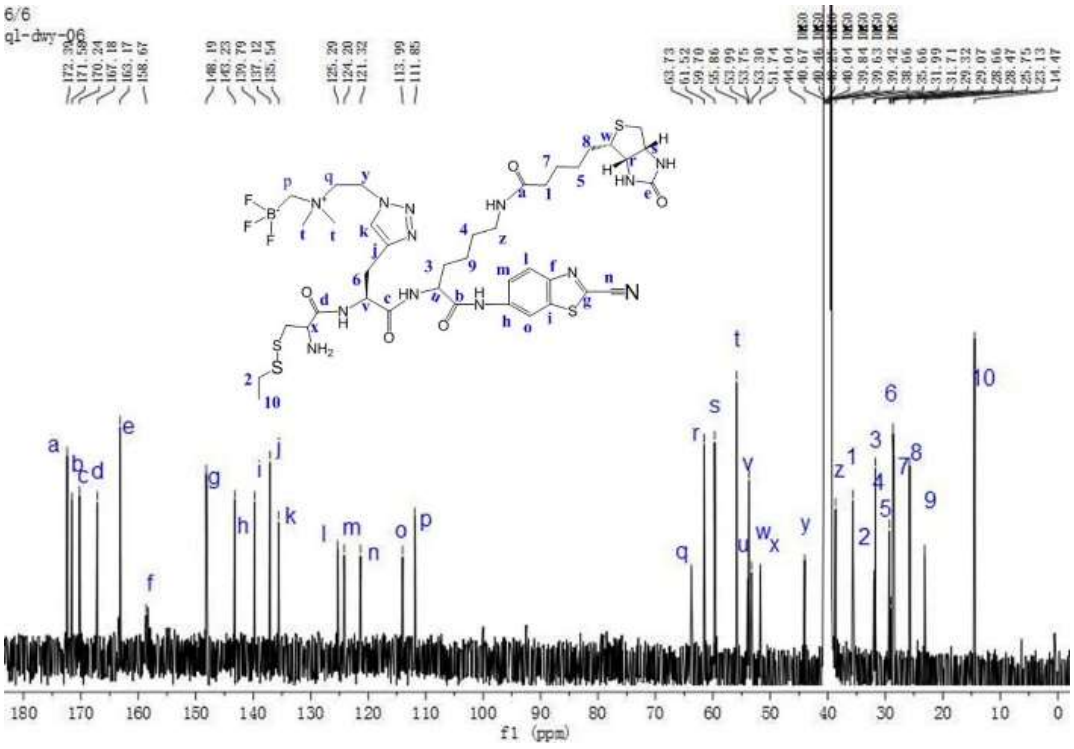
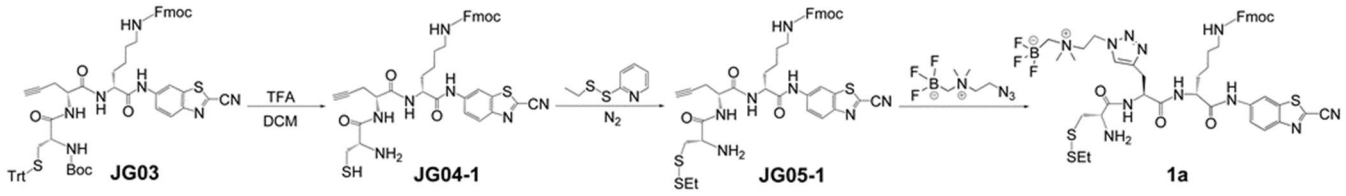


Figure S24. ^{13}C-NMR of probe **1**.

Chemical synthesis and characterization of control probe 1a



Scheme S2. Synthesis route of control probe **1a**.

Synthesis of compound JG04-1

JG03 (45 mg, 0.042 mmol) was dissolved in a mixture of CH_2Cl_2 and TFA (1:1) and stirred at room temperature for 0.5 h to remove the Boc protecting group. Upon evaporation of the solvent under reduced pressure, the crude product was precipitated with cold diethyl ether. Finally, **JG04-1** was obtained by centrifugation (4000 r/min) for 5 min as a white solid (30 mg, 98%).

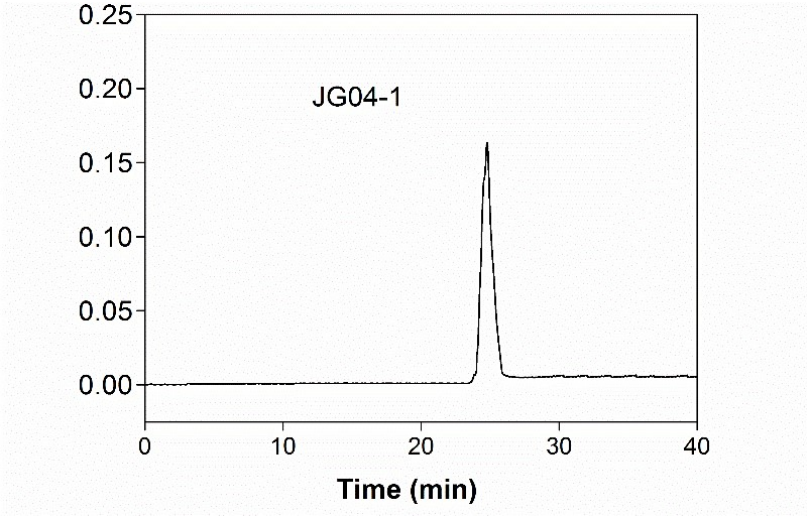


Figure S25. HPLC trace of compound **JG04-1**.

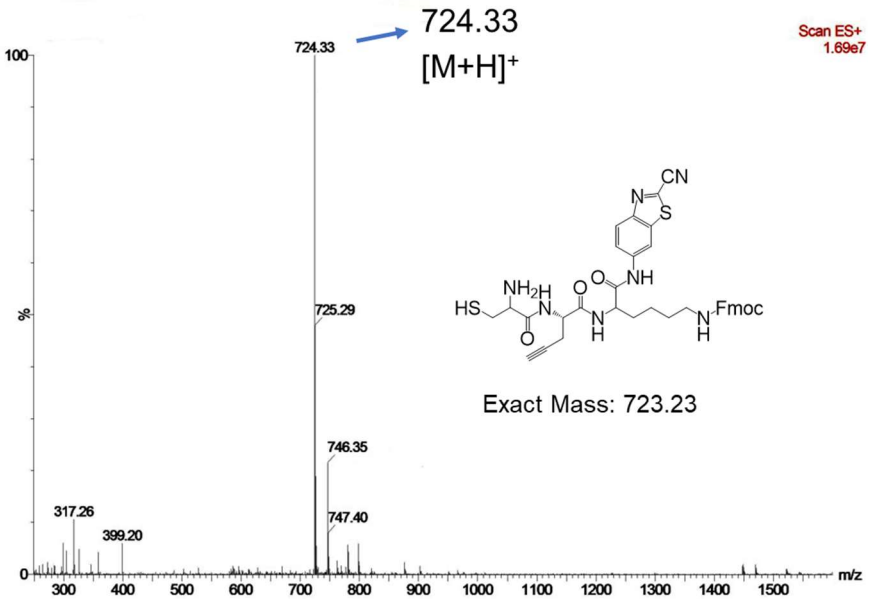


Figure S26. ESI-MS of compound **JG04-1**.

Synthesis of compound JG05-1

JG04-1 (30 mg, 0.041 mmol) was dissolved in CH_3OH, and SEt (11 mg, 0.062 mmol) was added to the solution. After stirring at room temperature for 0.5 h under nitrogen, TIPS (1%) was added to the solution, and the mixture was stirred for another 0.5 h. The solvent was evaporated under vacuum, and **JG05-1** (25 mg, 75%) was obtained by precipitation with diethyl ether and centrifugation (4000 r/min) for 5 min.

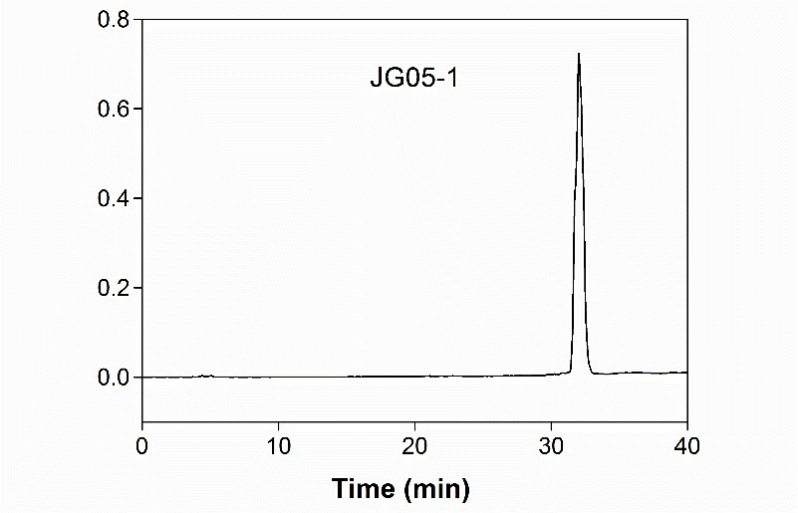


Figure S27. HPLC trace of compound **JG05-1**.

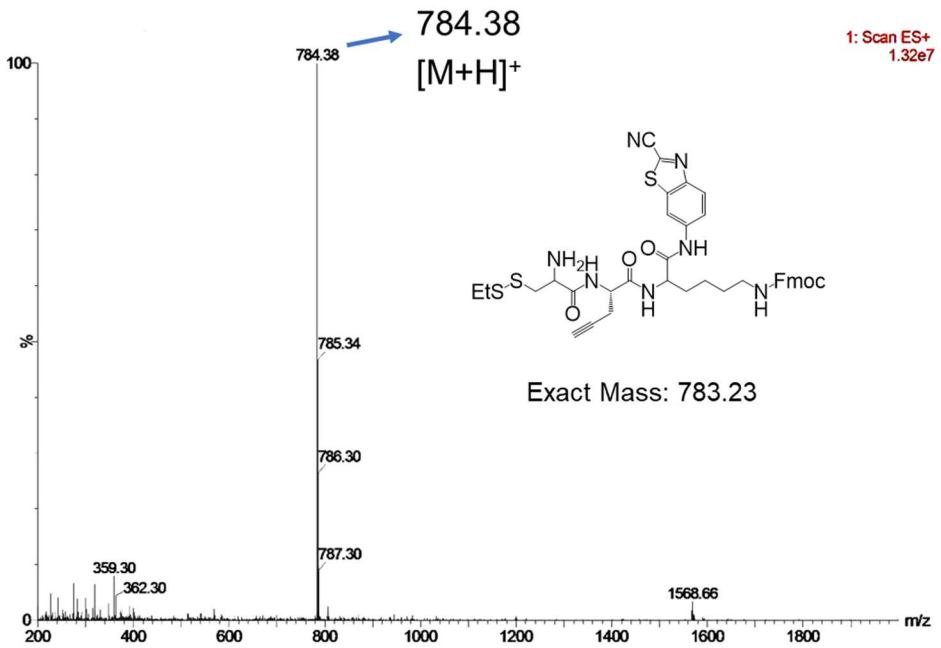


Figure S28. ESI-MS of compound **JG05-1**.

Synthesis of probe 1a

JG05-1 (25 mg, 0.032 mmol), $AMBF_3$ (31.3 mg, 0.16 mmol) and $(BimC_4A)_3$ (13.8 mg, 0.0032 mmol) were dissolved in the mixture solution of DMF and H_2O (6 mL, 1:1, *v/v*) under N_2. Terakis(acetonitrile)copper(I) hexafluorophosphate (11.6 mg, 0.032 mmol) was added to the solution, and the reaction mixture was stirred at 45°C for 1 h. Then the solvent was removed and the crude product was purified by preparative HPLC to afford **1a** (18 mg, 58%).^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.96 (d, J = 8.0 Hz, 1H), 8.71 (s, 1H), 8.49 (d, J = 7.6 Hz, 1H), 8.36 (s, 2H), 8.17 (d, J = 9.0 Hz, 1H), 7.98 (s, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.80 (d, J = 9.0 Hz, 1H), 7.64 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 7.27 (d, J = 5.5 Hz, 1H), 4.85 (t, J = 6.8 Hz, 2H), 4.75 (q, J = 7.3 Hz, 1H), 4.42 (q, J = 8.1 Hz, 1H), 4.25 (d, J = 6.9 Hz, 2H), 4.19 – 4.13 (m, 1H), 3.76 (s, 1H), 3.74 (s, 2H), 3.02 (s, 6H), 2.98 (s, 2H), 2.65 (q, J = 7.3 Hz, 2H), 2.51 (s, 4H), 2.42 (d, J = 4.5 Hz, 2H), 1.67 (d, J = 33.6 Hz, 2H), 1.50 – 1.35 (m, 2H), 1.23 (s, 2H), 1.14 (t, J = 7.3 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 167.24, 162.78, 148.14, 144.35, 143.26, 141.16, 139.81, 137.09, 128.03, 127.47, 125.55, 125.25, 124.23, 121.29, 120.56, 114.00, 111.78, 65.66, 63.77, 54.05, 53.65, 53.30, 51.69, 47.21, 44.01, 40.51, 39.44, 36.25, 32.00, 31.66, 31.24, 29.54, 29.06, 23.01, 14.45.

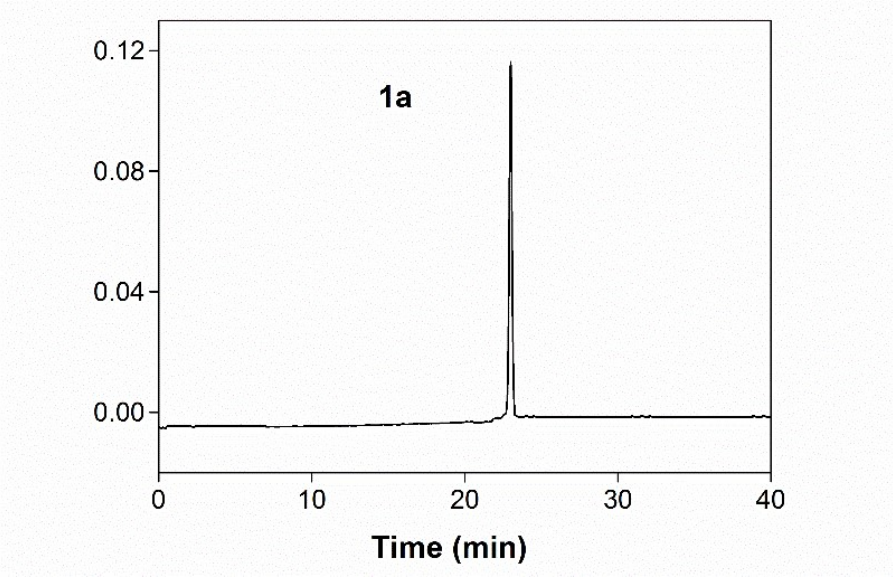


Figure S29. HPLC trace of probe **1a**.

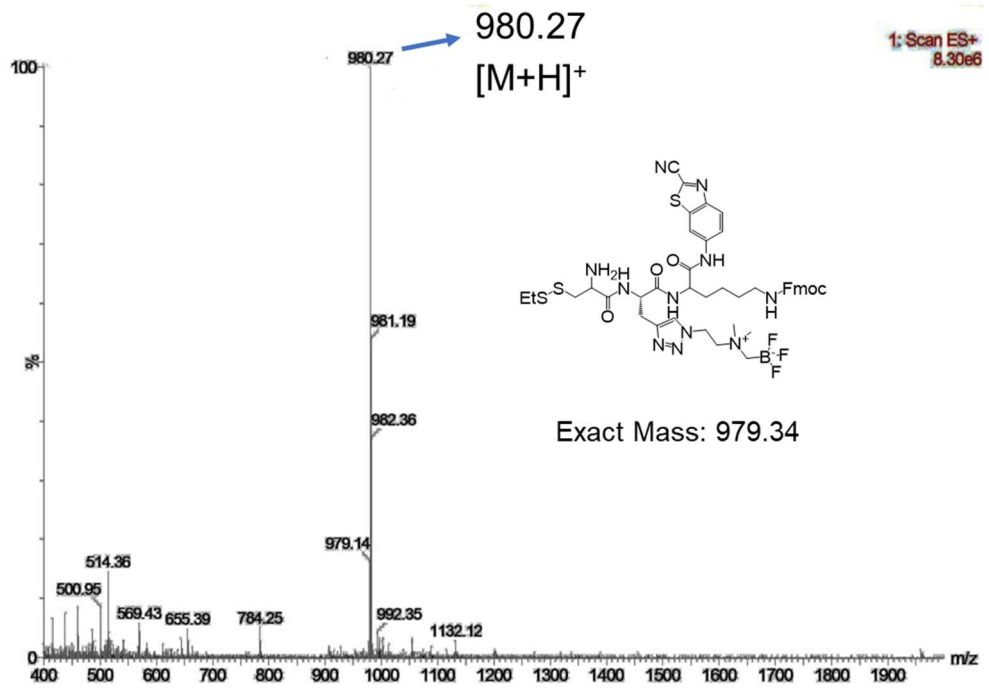


Figure S30. ESI-MS of probe **1a**.

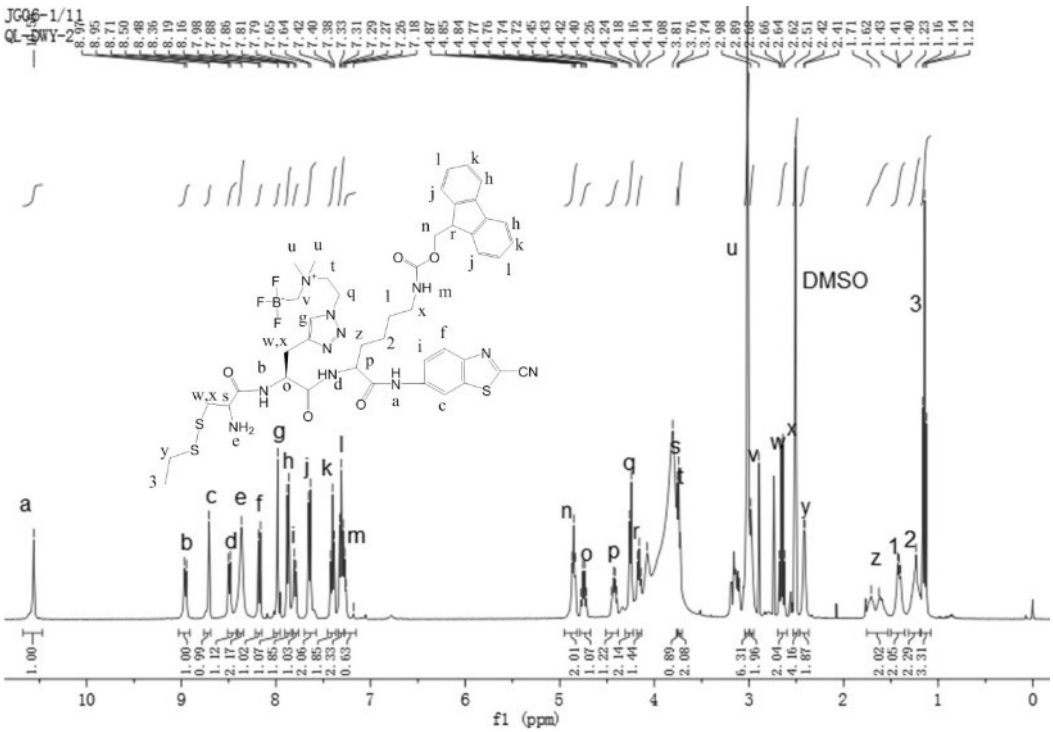


Figure S31. ^{1}H-NMR of probe **1a**.

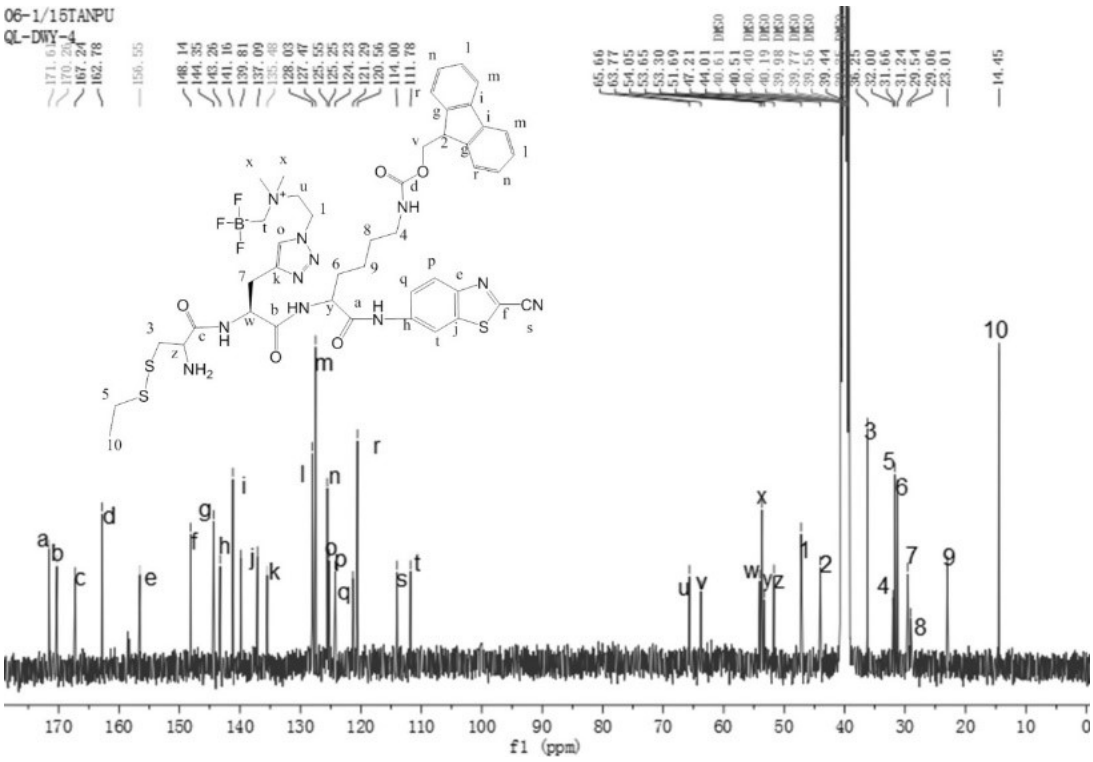
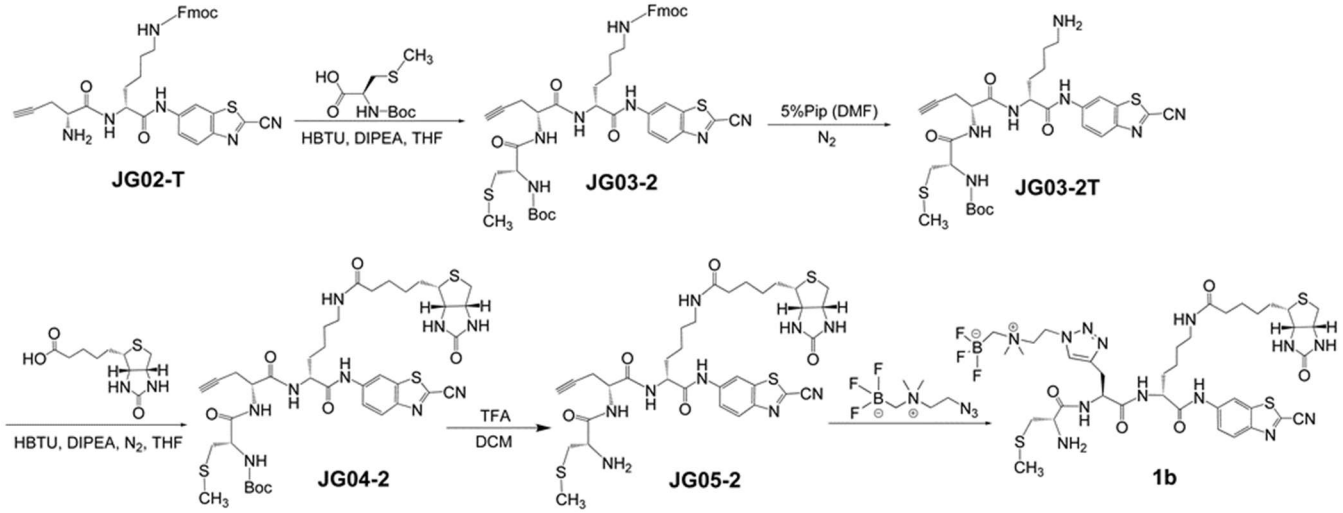


Figure S32.[13]C-NMR of probe **1a**.

Chemical synthesis and characterization of control probe 1b



Scheme S3. Synthesis route of control probe **1b**.

Synthesis of compound JG03-2

JG02T (260 mg, 0.42 mmol), *N*-Boc-S-Me-L-cysteine (118 mg, 0.50 mmol) and HBTU (183 mg, 0.48 mmol) were dissolved in THF (12 mL), and the solution was stirred at room temperature for 1 h under N_2. Afterwards the pH of the solution was adjusted to 7.4 with DIPEA and the solvent was removed under vacuum to yield **JG03-2** (166 mg, 47%).

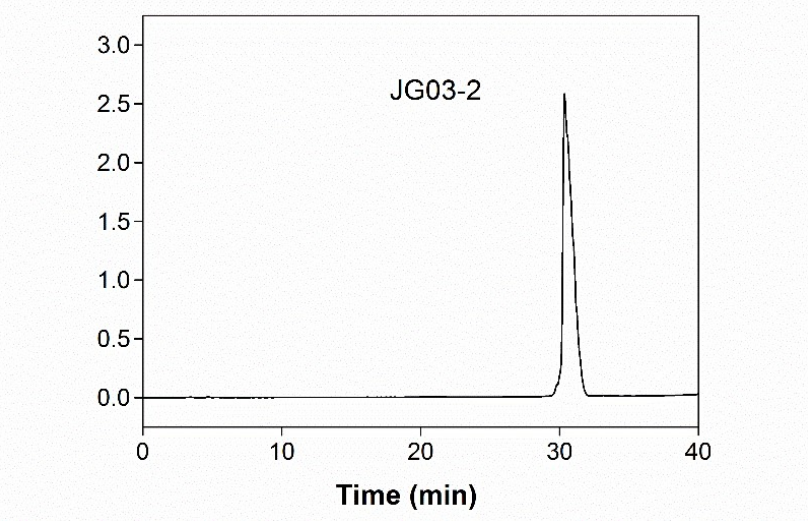


Figure S33. HPLC trace of compound **JG03-2**.

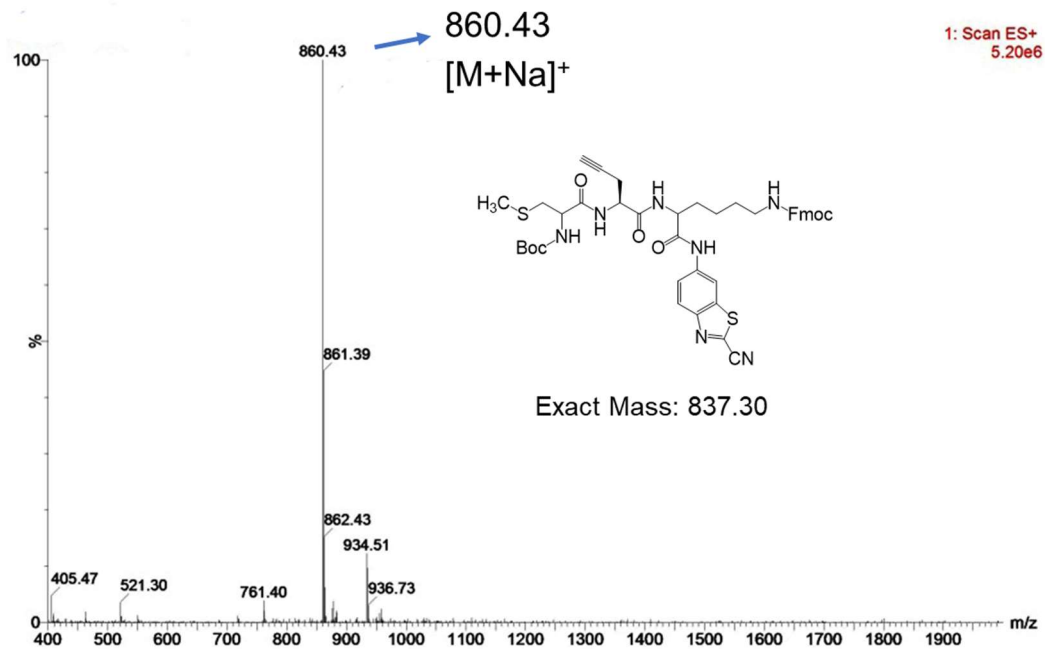


Figure S34. ESI-MS of compound **JG03-2**.

Synthesis of compound JG03-2T

Under N_2, **JG03-2** (166 mg, 0.198 mmol) was dissolved in DMF (1.8mL) containing 5% piperidine. The reaction mixture was stirred for 15 minutes at room temperature. Then HCl (1.2 mL, 1 M) was added dropwise to adjust pH to 5. Finally, the desired product **JG03-2T** (45 mg, 37%) was obtained after preparative HPLC purification.

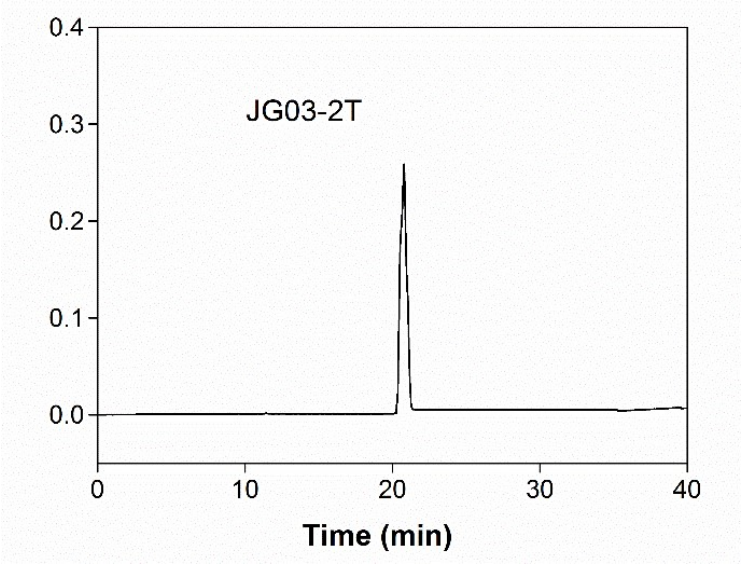


Figure S35. HPLC trace of compound **JG03-2T**.

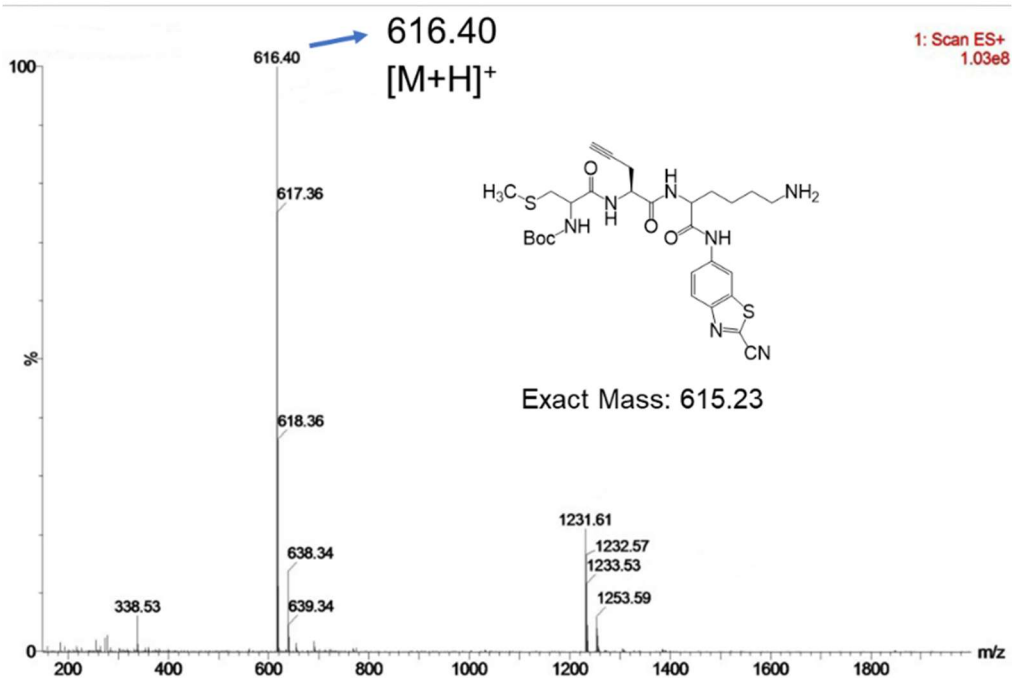


Figure S36. ESI-MS of compound **JG03-2T**.

Synthesis of compound JG04-2

To a solution of compound **JG03-2T** (45 mg, 0.073 mmol) in dry THF/DMF (1:1), D-biotin (22 mg, 0.088 mmol), HBTU (32 mg, 0.084 mmol) and DIPEA (36 μL, 0.219 mmol) were added, and the resulting solution was stirred at room temperature for 1 h under N_2. Solvent was removed under vacuum and the crude product was purified by silica gel column chromatography (DCM/MeOH, 10:1) to yield **JG04-2** (46 mg, 57%).

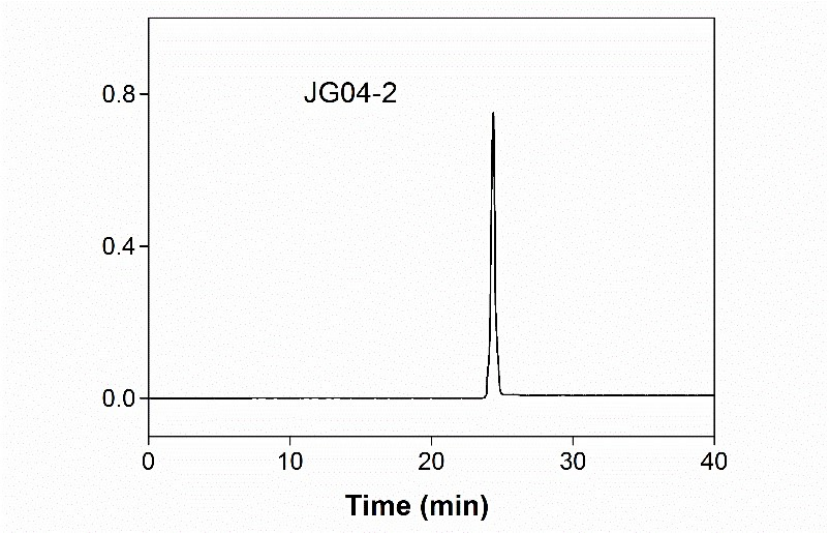


Figure S37. HPLC trace of compound **JG04-2**.

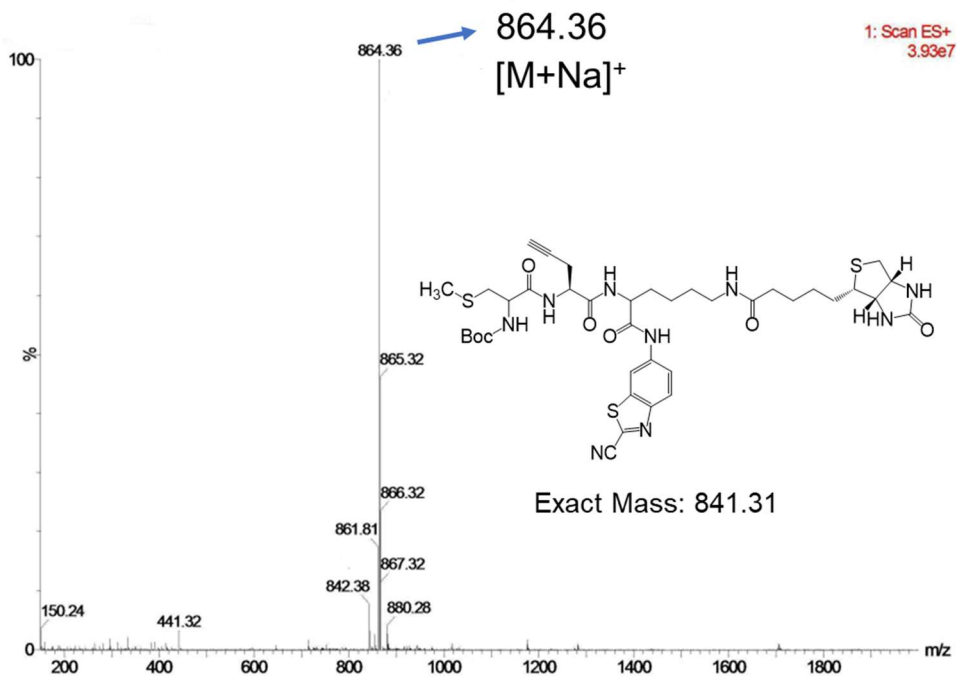


Figure S38. ESI-MS of compound **JG04-2**.

Synthesis of compound JG05-2

The Boc protecting group of **JG04-2** (46 mg, 0.055 mmol) was removed by treatment with a solution of 50% TFA in CH_2Cl_2 (6 mL) at room temperature for 0.5 h. Solvent was removed under vacuum, and the crude product was precipitated with cold diethyl ether, followed by centrifugation (4000 r/min) for 5 min to give **JG05-2** (40 mg, 98%)

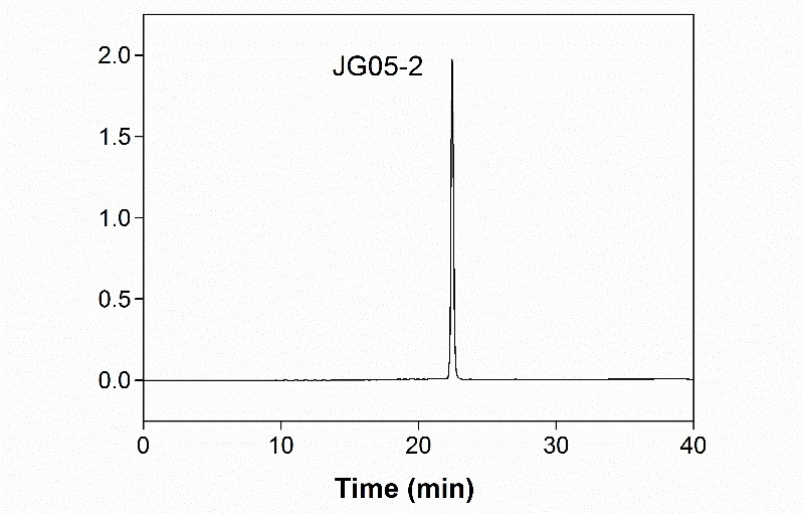


Figure S39. HPLC trace of compound **JG05-2**.

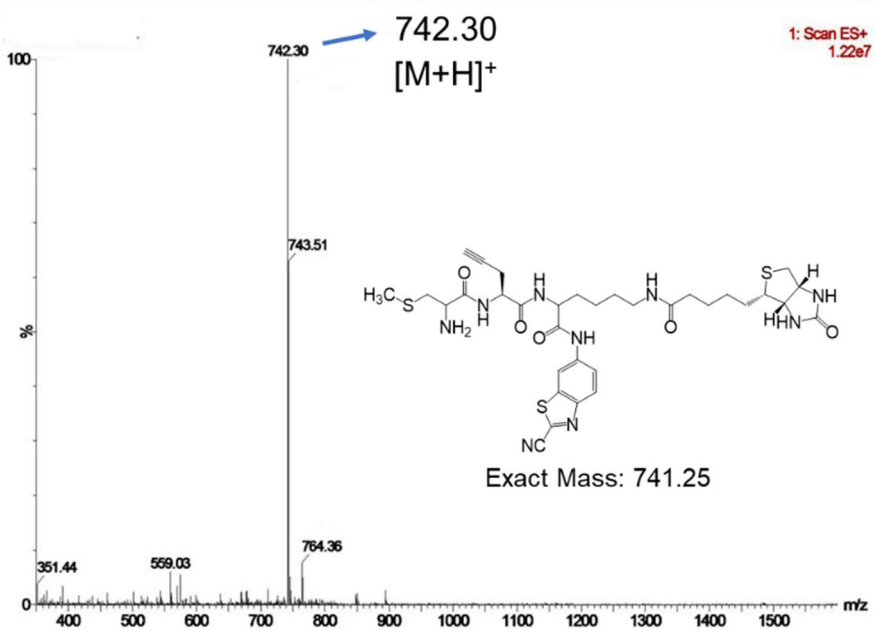


Figure S40. ESI-MS of compound **JG05-2**.

Synthesis of probe 1b

To a solution of **JG05-2** (40 mg, 0.054 mmol), $AMBF_3$ (52.7 mg, 0.27 mmol) and $(BimC_4A)_3$ (2.4 mg, 0.0054 mmol) in DMF/water (2:1), terakis(acetonitrile)copper(I) hexafluorophosphate (20 mg, 0.054 mol) was added. The reaction mixture was stirred at 45°C for 1 h under N_2. The crude product was purified by preparative HPLC to give compound **1b** as a white solid (24 mg, 47.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.89 (d, J = 8.1 Hz, 1H), 8.73 (s, 1H), 8.64 (d, J = 7.5 Hz, 1H), 8.21 (d, J = 8.9 Hz, 3H), 7.95 (s, 1H), 7.81 (d, J = 9.1 Hz, 1H), 7.76 (s, 1H), 6.41 (s, 2H), 4.86 (d, J = 6.9 Hz, 2H), 4.47 – 4.41 (m, 2H), 4.32 (d, J = 5.1 Hz, 1H), 4.17 – 4.07 (m, 1H), 3.99 (s, 1H), 3.73 (t, J = 7.1 Hz, 2H), 3.18 – 2.90 (m, 11H), 2.82 (d, J = 12.6 Hz, 2H), 2.58 (d, J = 12.6 Hz, 2H), 2.42 (d, J = 4.5 Hz, 2H), 2.04 (d, J = 15.6 Hz, 5H), 1.78 – 1.60 (m, 2H), 1.43 (dd, J = 20.1, 6.0 Hz, 6H), 1.27 (dd, J = 13.7, 7.4 Hz, 4H).^{13}C NMR (101 MHz, DMSO-d_6) δ 172.43, 171.75, 170.49, 167.67, 163.21, 162.78, 148.17, 143.23, 139.80, 137.14, 135.58, 125.31, 124.23, 121.30, 114.02, 111.84, 63.72, 61.51, 59.69, 55.86, 54.08, 53.72, 52.98, 51.40, 43.96, 38.64, 36.25, 35.66, 35.05, 31.91, 31.25, 29.52, 29.28, 28.66, 28.45, 25.76, 23.18, 15.11.

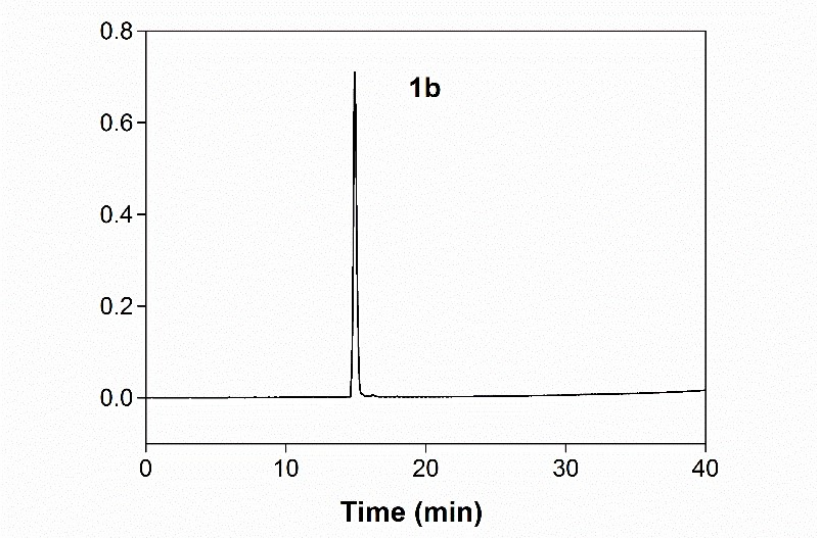


Figure S41. HPLC trace of probe **1b**.

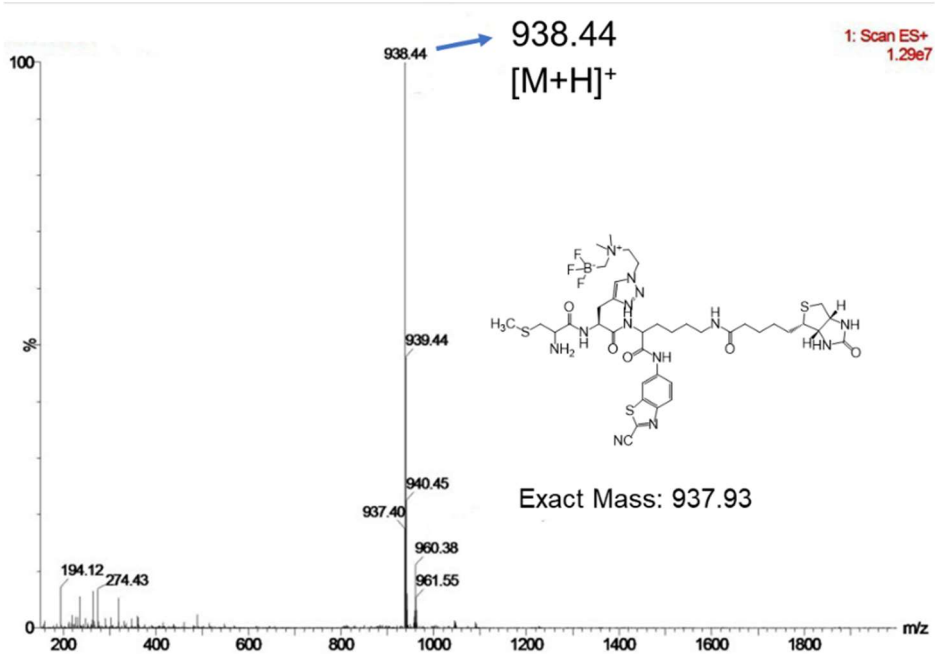


Figure S42. ESI-MS of probe **1b.**

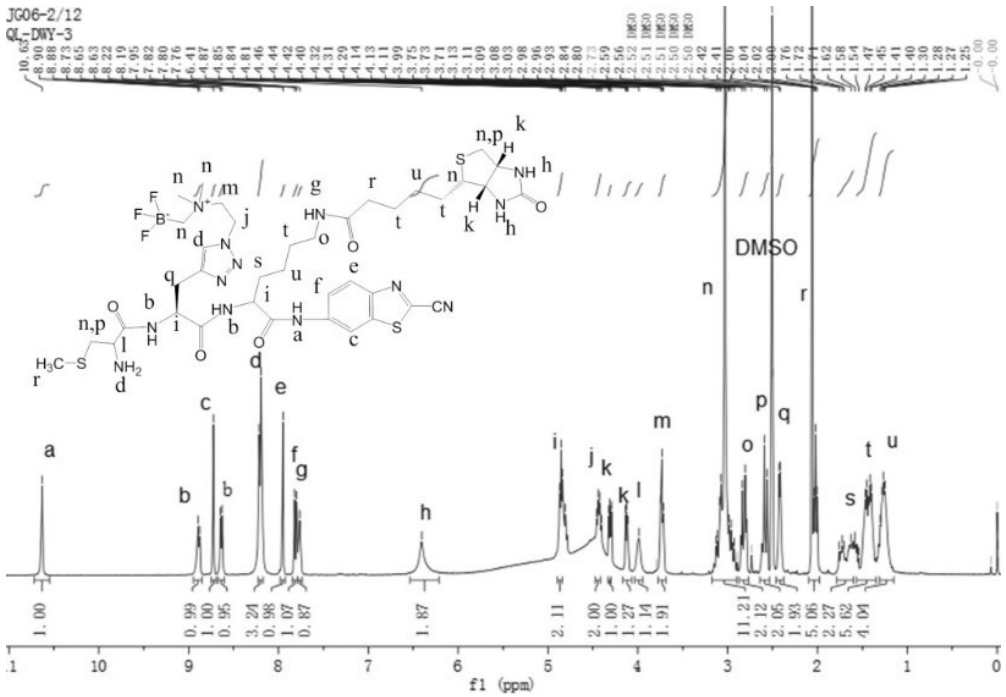


Figure S43. ^{1}H-NMR of probe **1b**.

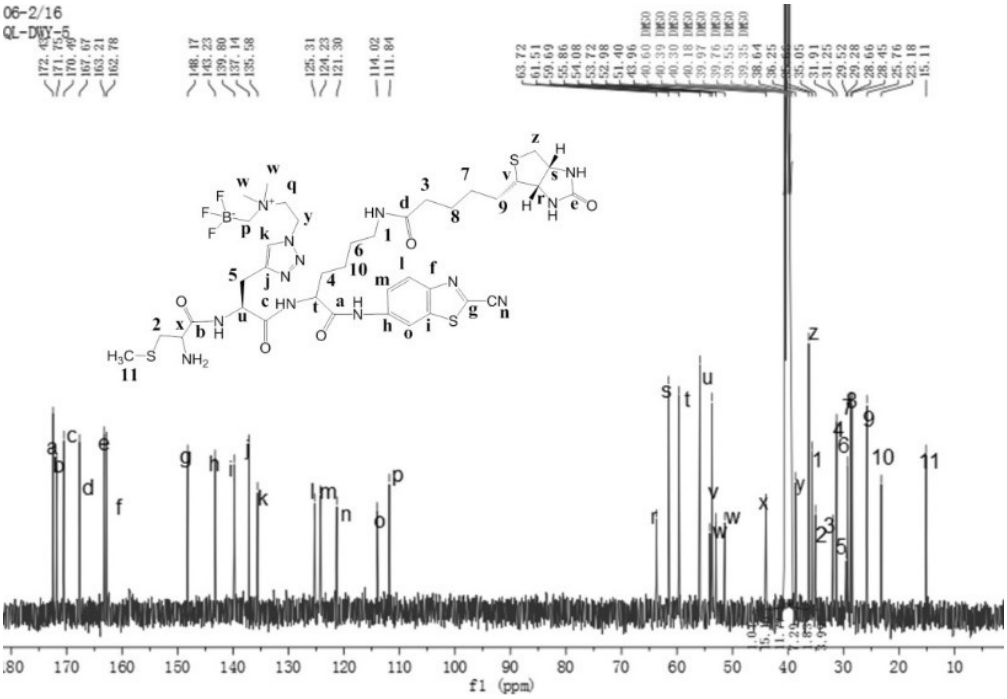


Figure S44. ^{13}C-NMR of probe **1b**.

Chemical synthesis and characterization of 1-Dimer

Nonradioactive probe **1** (2.5 μL, 25 mM) in DMF was added with an aqueous solution of TECP (25 μL, 25 mM). The resulting mixture was kept at room temperature for 1 h. A small portion of the solution was purified using HPLC to give pure **1-R**, and the rest of the mixture was diluted with PBS (250 μL). And the solution was incubated at 37 ℃ for 6 h to generate **1-Dimer**. The pure **1-Dimer** was obtained by purification of the reaction mixture with HPLC. The total reaction yield was about 77%.

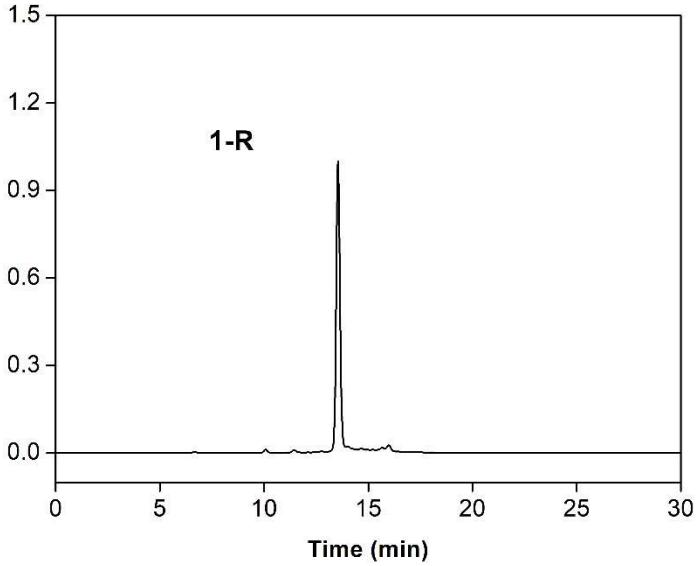


Figure S45. HPLC trace of pure **1-R**.

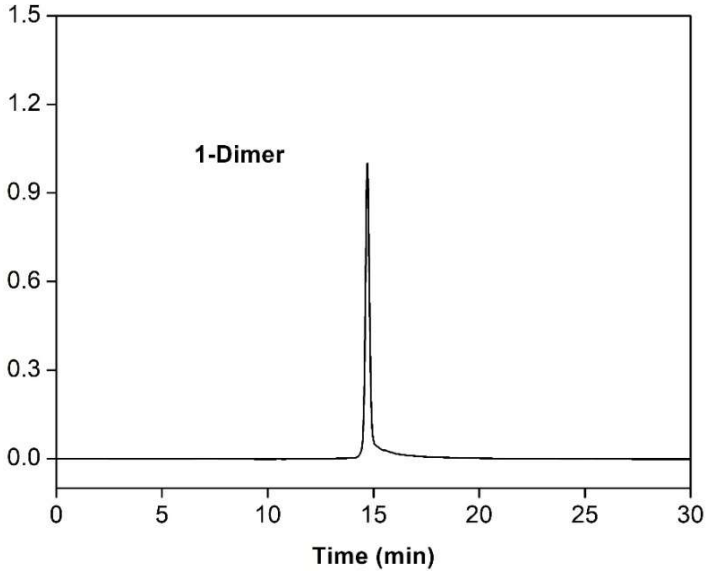


Figure S46. HPLC trace of pure **1-Dimer**.

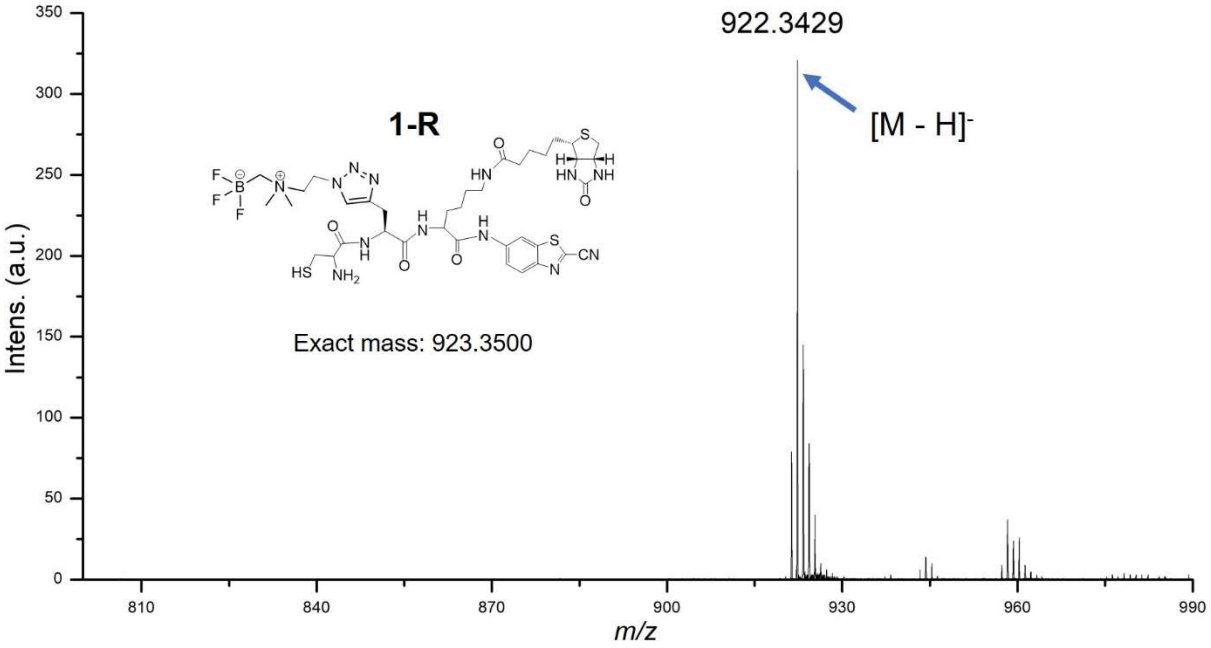


Figure S47. HRMS spectrum of pure **1-R**.

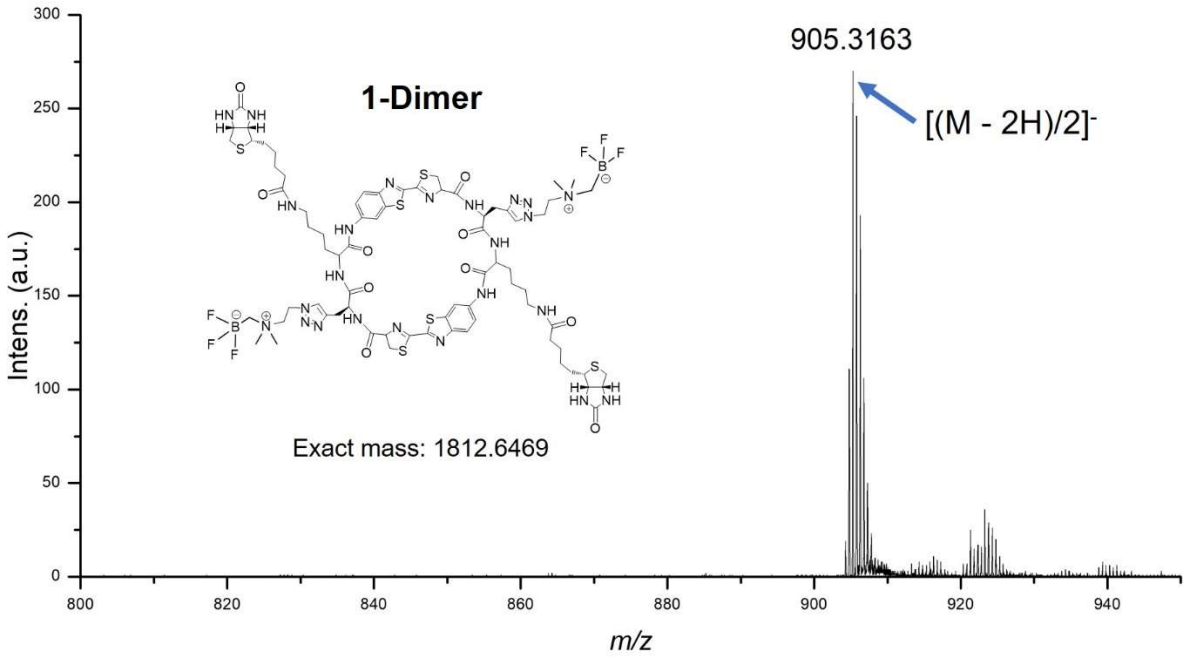


Figure S48. HRMS spectrum of pure **1-Dimer**.

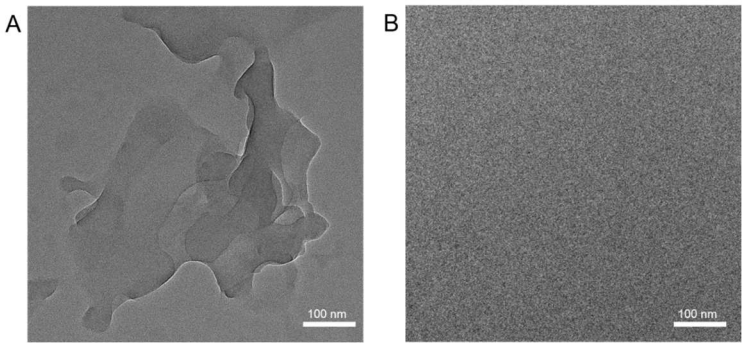
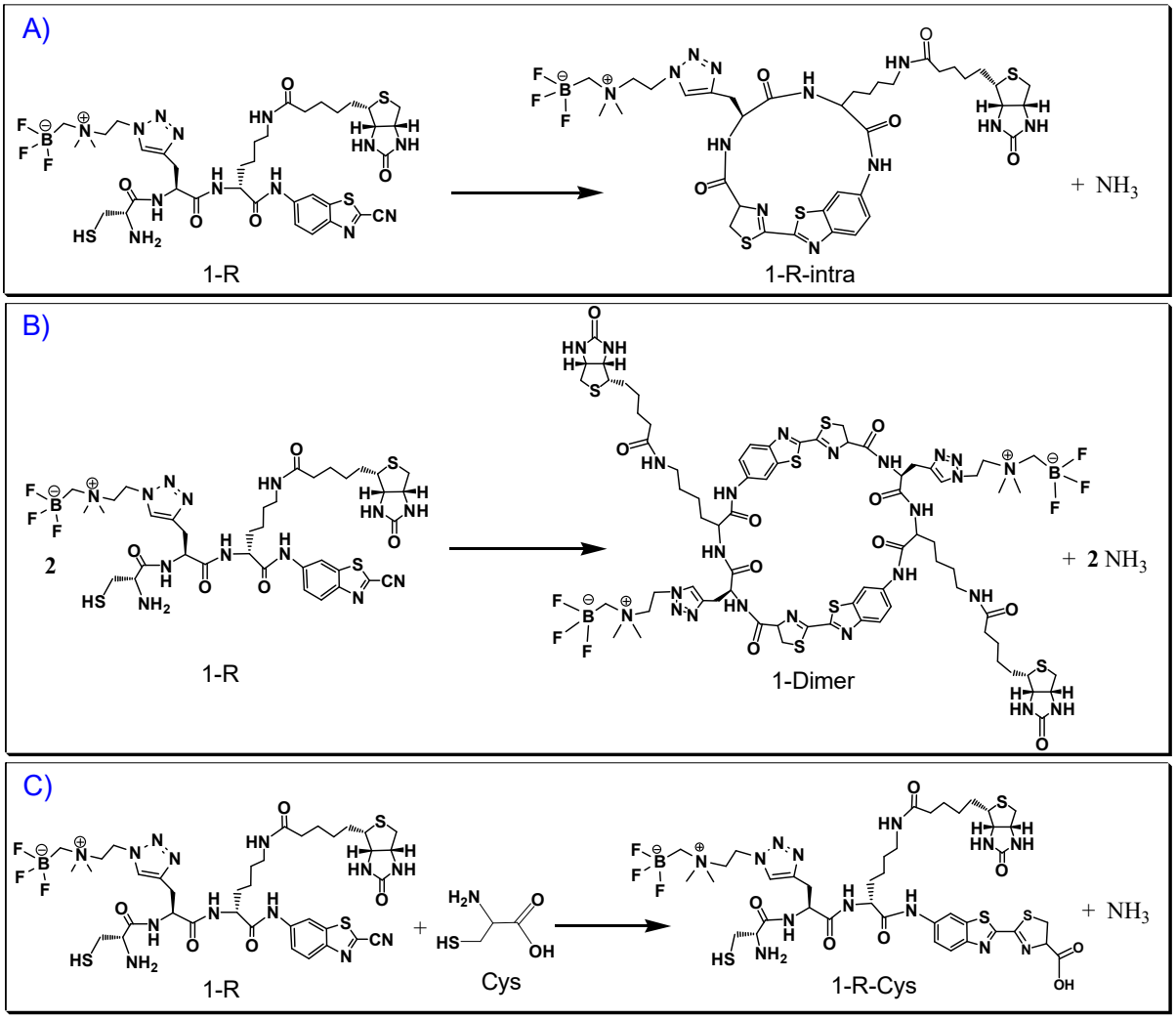


Figure S49. TEM images of (A) **1a-NP** and (B) **1b** in reducing environment.

Theoretical modeling



Scheme S4. Possible reaction mechanism of the target probe in living cells: (A) intramolecular and (B) intermolecular cyclization of monomer **1-R** *via* click condensation to yield macrocyclic oligomers **1-R-intra** and **1-Dimer**, or (C) intermolecular click condensation between **1-R** and free Cys to yield **1-R-Cys**.

According to the proposed possible reaction mechanisms of the target probe **1** in living cells (Scheme S1), the entropy changes (ΔS_T) and enthalpy changes (ΔH_T) for the possible intramolecular or intermolecular cyclization of reductive monomer **1-R** *via* click condensation to yield macrocyclic oligomers **1-R-intra** or **1-Dimer**, as well as possible click condensation between **1-R** and free cysteine to form the oligomer **1-R-Cys**, were calculated according to the following equations (1-6), respectively. The scale factor of 0.9614 for frequencies and ZPVE was imposed.

(1) $\Delta S_{T,\text{1-R-intra}} = (S^o_m)_{\text{1-R-intra}} + (S^o_m)_{\text{NH3}} - (S^o_m)_{\text{1-R}}$

(2) $\Delta S_{T,\text{ 1-Dimer}} = (S^o_m)_{\text{1-Dimer}} + 2\,(S^o_m)_{\text{NH3}} - 2\,(S^o_m)_{\text{1-R}}$

(3) $\Delta S_{T,\text{ 1-R-Cys}} = (S^o_m)_{\text{JG06T-Cys}} + (S^o_m)_{\text{NH3}} - (S^o_m)_{\text{ 1-R}} - (S^o_m)_{\text{Cys}}$

(4) $\Delta H_{T,\text{ 1-R-intra}} = (H^o_m + E + \text{ZPE})_{\text{1-R-intra}} + (H^o_m + E + \text{ZPE})_{\text{NH3}} - (H^o_m + E + \text{ZPE})_{\text{1-R}}$

(5) $\Delta H_{T,\text{ 1-Dimer}} = (H^o_m + E + \text{ZPE})_{\text{1-Dimer}} + 2(H^o_m + E + \text{ZPE})_{\text{NH3}} - 2(H^o_m + E + \text{ZPE})_{\text{1-R}}$

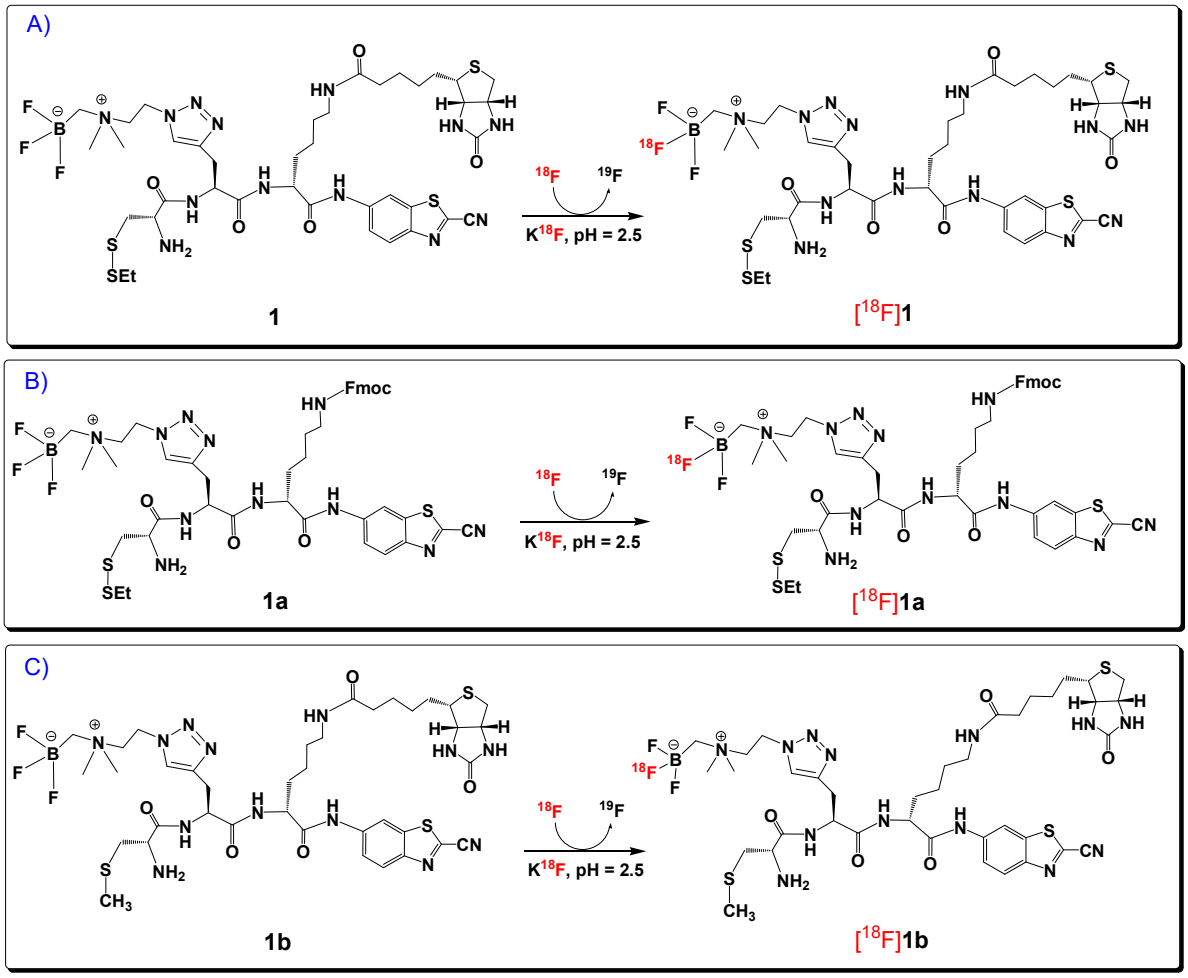
(6) $\Delta H_{T,\text{ 1-R-Cys}} = (H^o_m + E + \text{ZPE})_{\text{1-R-Cys}} + (H^o_m + E + \text{ZPE})_{\text{NH3}} - (H^o_m + E + \text{ZPE})_{\text{1-R}} - (H^o_m + E + \text{ZPE})_{\text{Cys}}$

Table S1. Thermodynamic properties and changes upon click condensation of probe **1** at different temperatures [a]

Compd.	T	$C^o_{p,m}$	S^o_m	H^o_m	ΔS_T	ΔH_T	ΔG_T	ln$k$
1-R	200	750.34	1333.02	89.78				
	298.15	1034.11	1685.40	177.33				
	400	1312.66	2029.05	297.13				
	500	1544.40	2347.70	440.36				
	600	1732.64	2646.52	604.55				
	700	1885.41	2925.48	785.71				
	800	2011.00	3185.70	980.73				
	900	2115.63	3428.79	1187.21				
	1000	2203.71	3656.38	1403.30				
Cys	200	101.60	331.02	13.24				
	298.15	132.87	377.45	24.75				
	400	162.34	420.72	39.83				
	500	186.39	459.62	57.30				
	600	205.90	495.39	76.95				
	700	221.87	528.36	98.37				
	800	235.19	558.88	121.24				
	900	246.47	587.25	145.34				
	1000	256.13	613.73	170.48				
NH_3	200	33.48	178.79	6.66				
	298.15	34.83	192.36	10.00				
	400	37.44	202.93	13.67				
	500	40.54	211.61	17.57				
	600	43.71	219.28	21.78				
	700	46.80	226.25	26.31				
	800	49.77	232.70	31.14				
	900	52.58	238.72	36.26				
	1000	55.22	244.40	41.65				
1-R-intra	200	699.52	1197.61	81.59	43.38	102.79	94.11	-56.60
	298.15	981.09	1529.26	164.03	36.22	101.02	90.22	-36.40
	400	1257.43	1857.04	278.33	30.92	99.19	86.82	-26.11
	500	1486.57	2163.10	415.91	27.01	97.44	83.93	-20.19
	600	1671.90	2451.12	574.17	23.88	95.72	81.39	-16.32
	700	1821.60	2720.49	749.11	21.26	94.03	79.14	-13.60
	800	1944.13	2971.99	937.59	18.99	92.32	77.12	-11.60
	900	2045.81	3207.02	1137.24	16.95	90.61	75.35	-10.07
	1000	2131.13	3427.11	1346.21	15.13	88.88	73.75	-8.87
1-Dimer	200	1411.71	2248.74	165.61	-59.72	-155.31	-143.37	86.22
	298.15	1972.04	2916.51	331.59	-69.57	-157.75	-137.01	55.27
	400	2523.72	3574.77	561.11	-77.47	-160.49	-129.50	38.94
	500	2982.28	4188.88	837.17	-83.30	-163.09	-121.44	29.21
	600	3353.80	4766.67	1154.64	-87.81	-165.58	-112.90	22.63
	700	3654.23	5307.02	1505.56	-91.44	-167.92	-103.91	17.86
	800	3900.25	5811.56	1883.68	-94.44	-170.18	-94.63	14.23
	900	4104.47	6283.09	2284.23	-97.05	-172.35	-85.01	11.36
	1000	4275.79	6724.66	2703.48	-99.30	-174.50	-75.20	9.05
1-R-Cys	200	817.54	1449.99	98.28	-35.26	-51.88	-44.83	26.96
	298.15	1128.80	1834.28	193.77	-36.21	-52.11	-41.32	16.67
	400	1434.07	2209.60	324.61	-37.24	-52.48	-37.59	11.30
	500	1687.27	2557.74	481.10	-37.97	-52.79	-33.81	8.13
	600	1892.35	2884.16	660.45	-38.47	-53.07	-29.99	6.01
	700	2058.33	3188.77	858.27	-38.82	-53.30	-26.13	4.49
	800	2194.47	3472.80	1071.12	-39.08	-53.51	-22.25	3.35
	900	2307.69	3738.00	1296.40	-39.32	-53.69	-18.31	2.45
	1000	2402.88	3986.21	1532.06	-39.50	-53.87	-14.37	1.73

[a]Unit: T/K, $C^o_{p,m}$/J·mol^{-1}·K^{-1}, S^o_m/J·mol^{-1}·K^{-1}, H^o_m/kJ·mol^{-1}, ΔS_T/J·mol^{-1}·K^{-1}, ΔH_T/kJ·mol^{-1}, ΔG_T/kJ·mol^{-1}. $\Delta G_T = \Delta H_T - T\Delta S_T = -RT\ln k$

^{18}F-Radiolabeling



Scheme S5. [^{18}F]Fluorination of probe **1**, **1a** and **1b**.

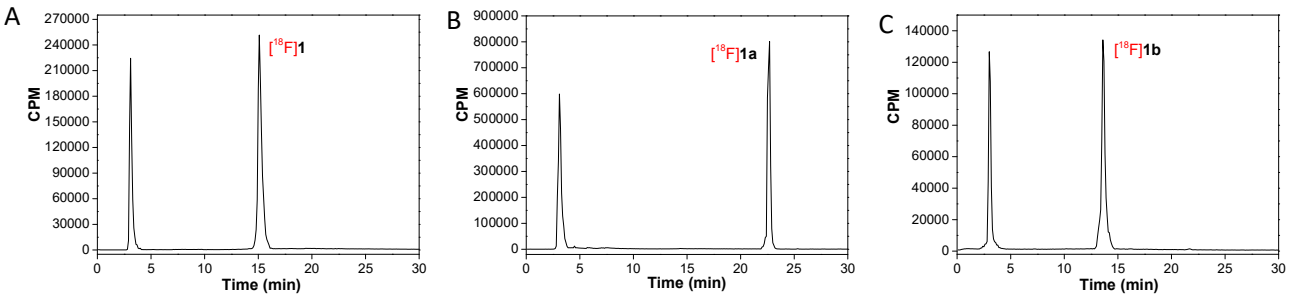


Figure S50. HPLC analysis of unpurified (A) [^{18}F]**1**, (B) [^{18}F]**1a**, and (C) [^{18}F]**1b**.

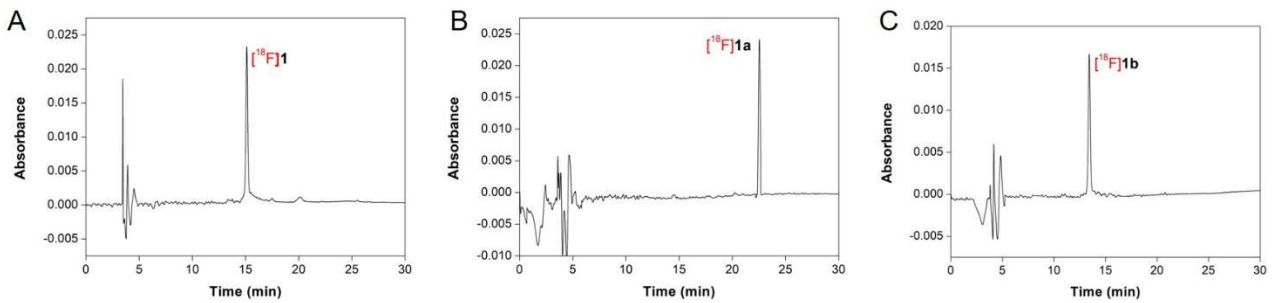


Figure S51. HPLC analysis (UV detector) of purified (A) [^{18}F]**1**, (B) [^{18}F]**1a**, and (C) [^{18}F]**1b.**

In vitro stability assay

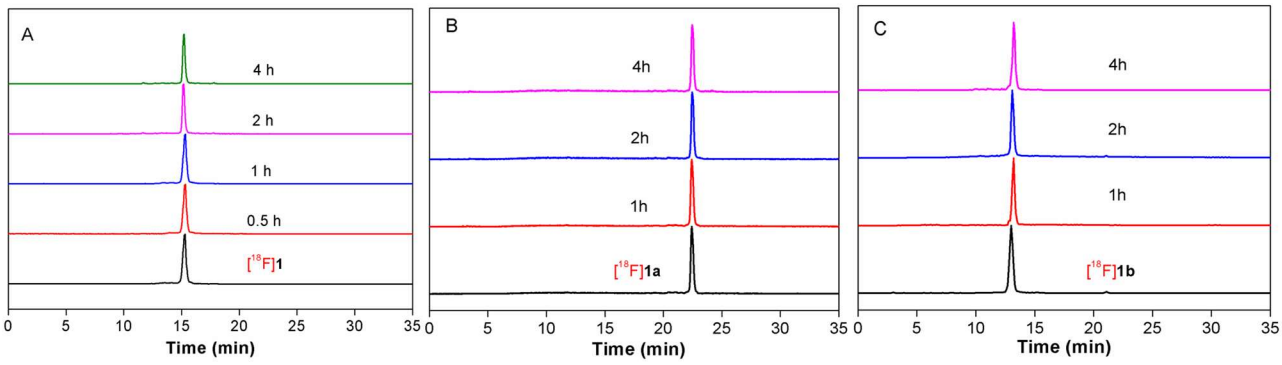


Figure S52. Stability assay of (A) [^{18}F]**1**, (B) [^{18}F]**1a** and (C) [^{18}F]**1b** in PBS for different time.

In vitro cytotoxicity assay

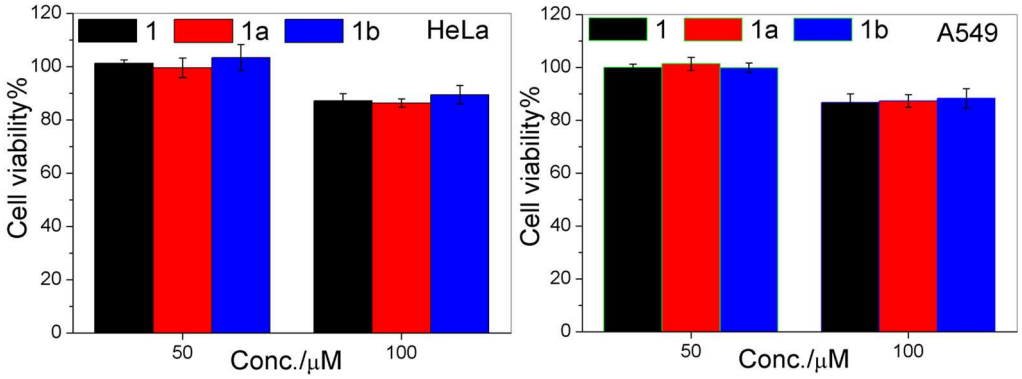


Figure S53. Cytotoxicity assay of nonradioactive probes **1**, **1a** and **1b** against HeLa (left) and A549 (right) cells.

Cellular uptake assay

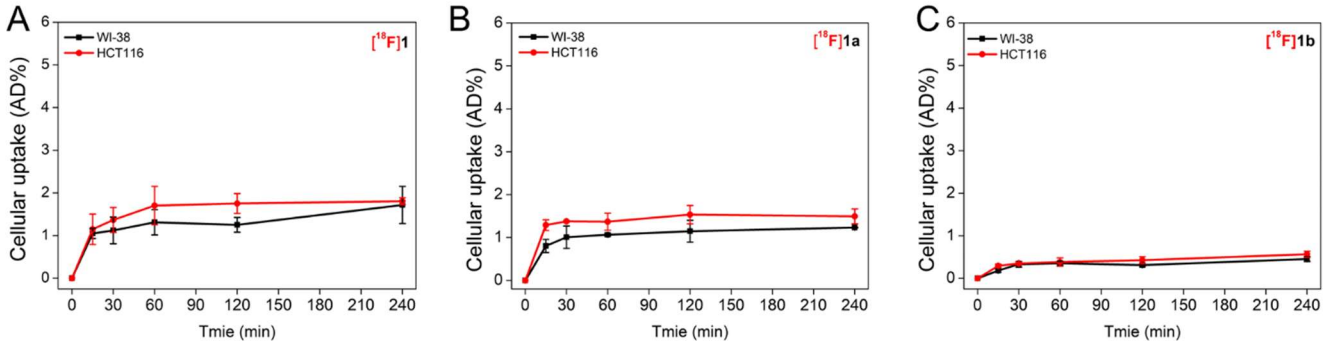


Figure S54. Cellular uptake of (A) [^{18}F]**1** (0.037 MBq, 4.8 × 10^{-3} nmol), (B) [^{18}F]**1a** (0.037 MBq, 3.5 × 10^{-3} nmol) and (C) [^{18}F]**1b** (0.037 MBq, 6.9 × 10^{-3} nmol) in BR-negative cells (WI-38 and HCT116).

In vivo stability assay

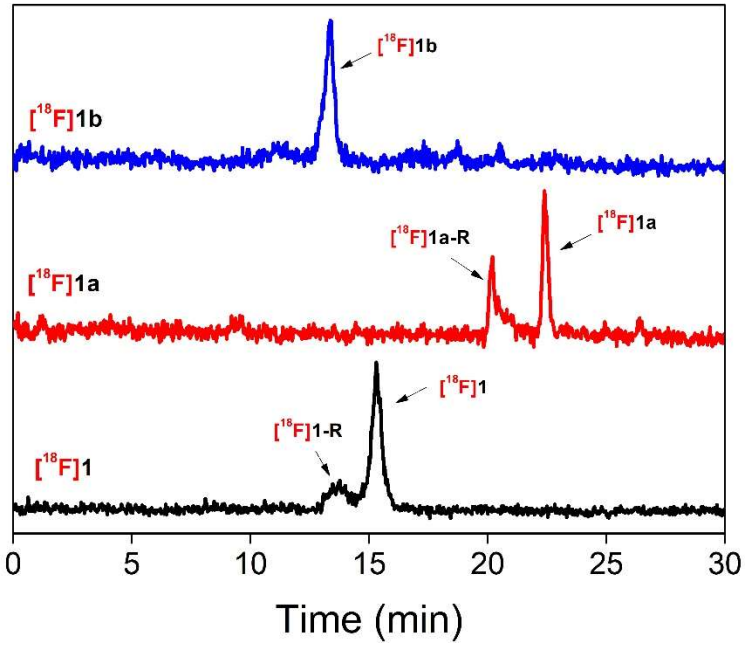


Figure S55. *In vivo* stability assay of [^{18}F]**1** (11.1 MBq, 1.43 nmol), [^{18}F]**1a** (11.1 MBq, 1.04 nmol) and [^{18}F]**1b** (11.1 MBq, 2.06 nmol) by injecting the radiotracer into mice *via* the tail vein.

Biodistribution study

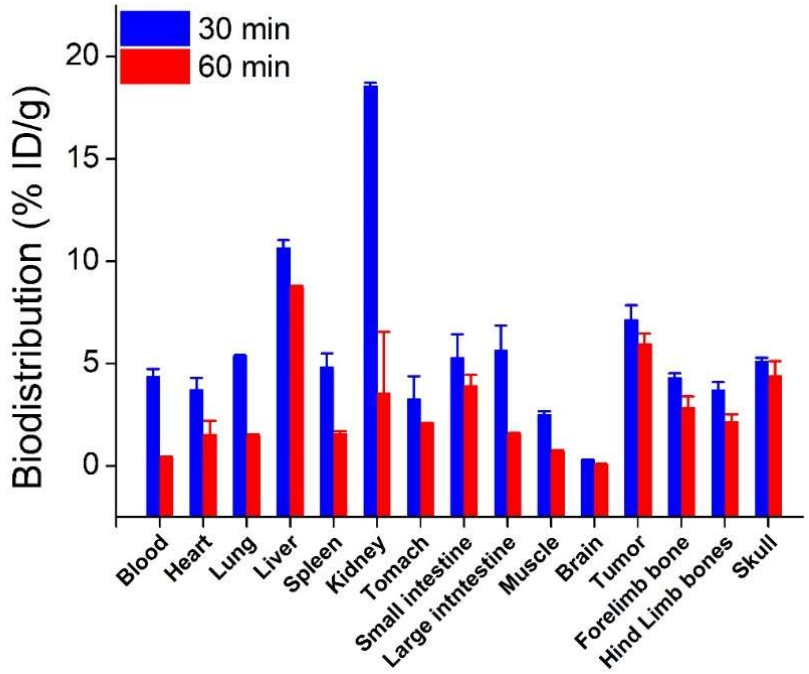


Figure S56. Biodistribution of [^{18}F]**1** (7.4 MBq, 0.95 nmol) in Hela-bearing mice at 0.5 h and 1 h post injection (% ID/g).

In vivo microPET imaging

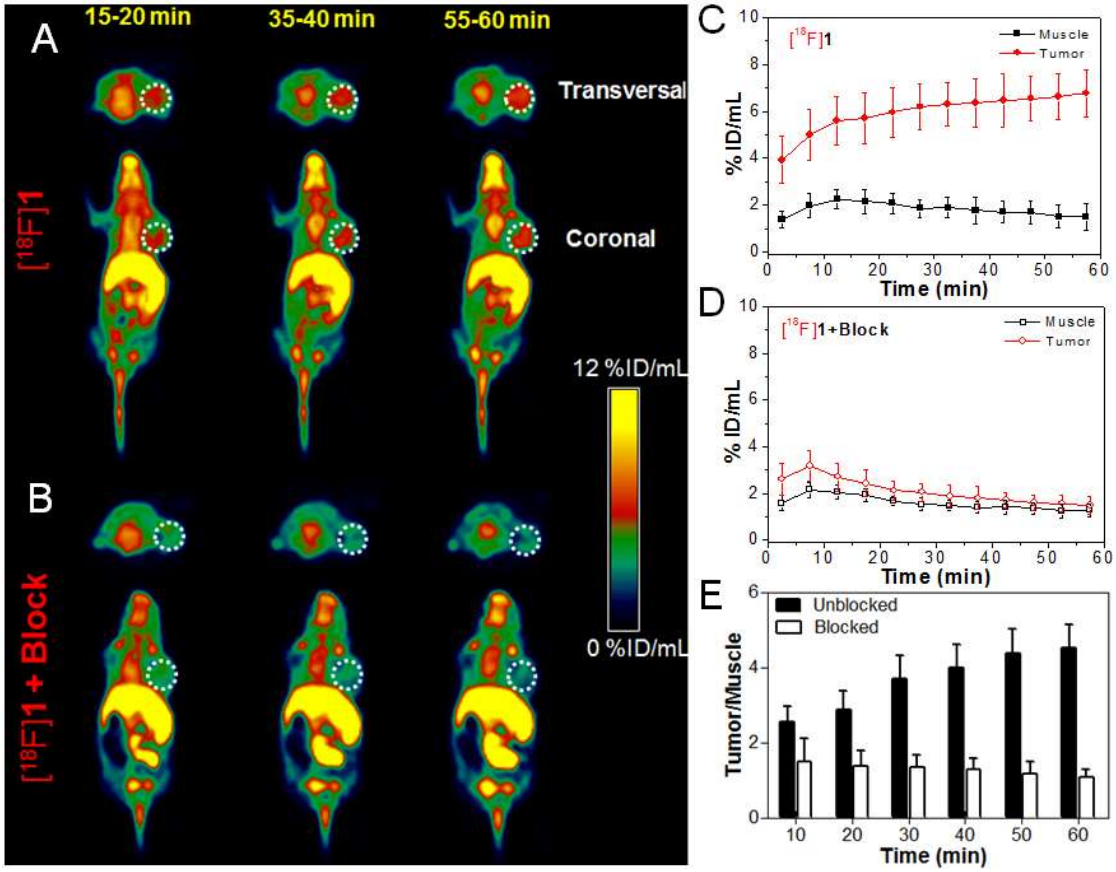


Figure S57. (A) MicroPET imaging of [^{18}F]**1** (7.4 MBq, 0.95 nmol in 150 μL saline) in A549 tumor-bearing mice at different time points post intravenous injection. (B) Mice were pretreated with biotin (100 μM, 150 μL) half an hour prior the administration of [^{18}F]**1** (7.4 MBq, 0.95 nmol in 150 μL saline). (C) Tumor and muscle uptake of [^{18}F]**1** at different time points. (D) Tumor and muscle uptake of [^{18}F]**1** in the blocking group at different time points. (E) Time course of tumor-to-muscle uptake ratio for [^{18}F]**1** in both groups.

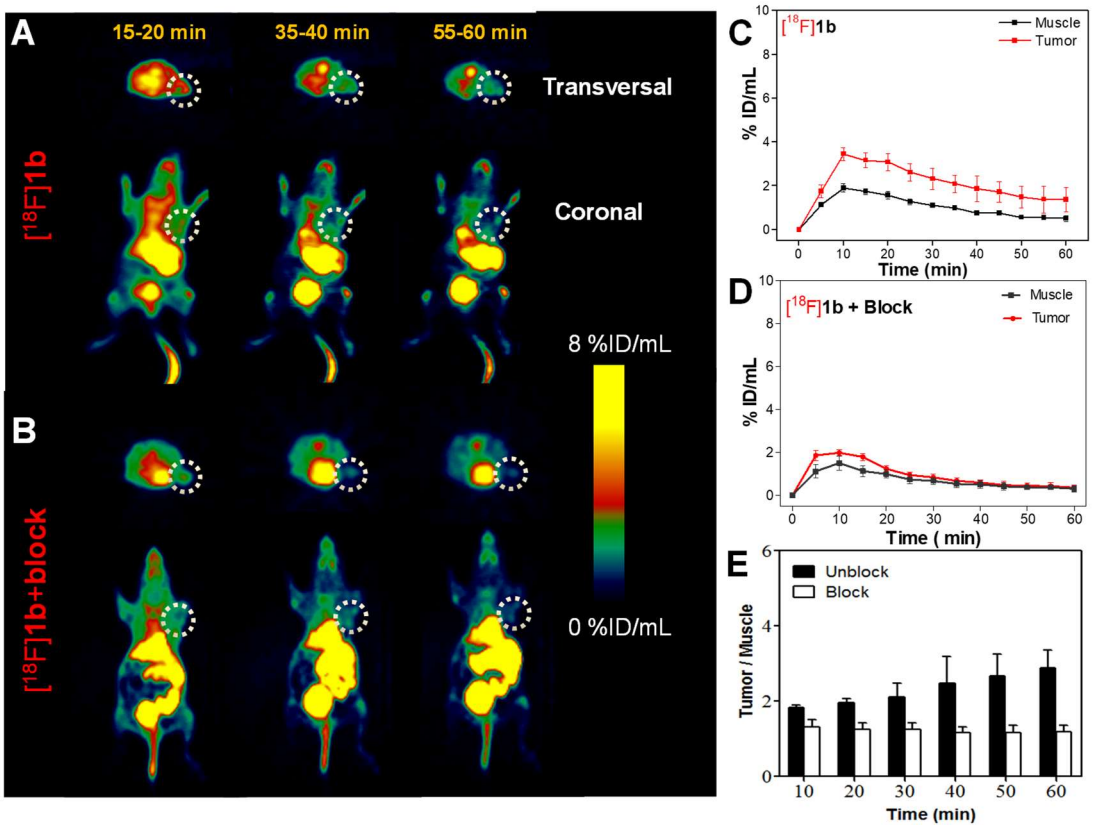


Figure S58. (A) MicroPET imaging of [^{18}F]**1b** (7.4 MBq, 1.37 nmol in saline 150 μL) in Hela tumor-bearing mice at different time points post intravenous injection. (B) Mice were pre-injected with biotin (100 μM, 150 μL) half an hour prior the administration of [^{18}F]**1b** (7.4 MBq, 1.37 nmol). (C) Time course of tumor and muscle uptake of [^{18}F]**1b**. (D) Time course of tumor and muscle uptake of [^{18}F]**1b** in the blocking group. (E) Time course of tumor-to-muscle uptake ratio for [^{18}F]**1b** in both groups.

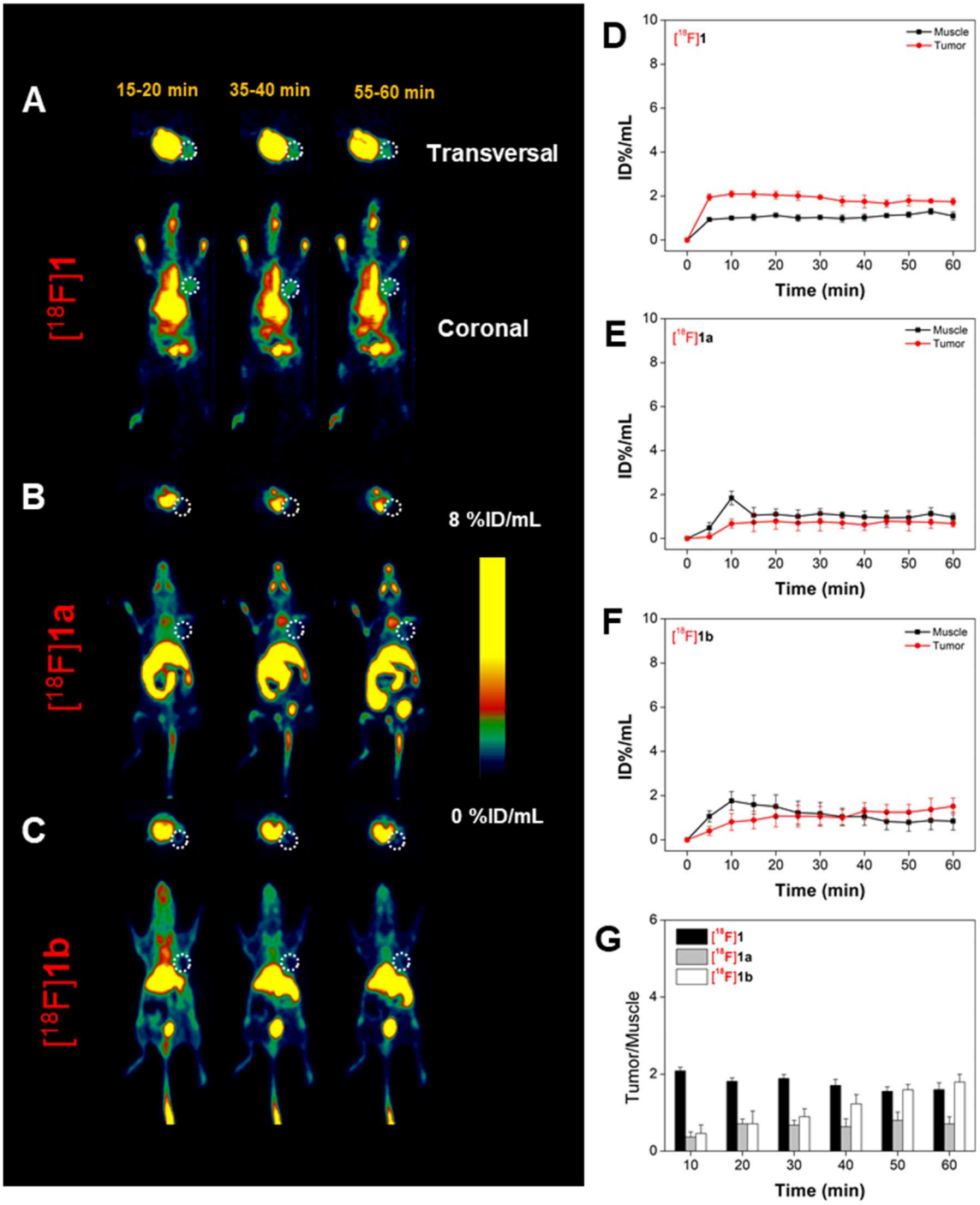


Figure S59. (A-C) MicroPET imaging of [^{18}F]**1** (7.4 MBq, 0.95 nmol in 150 µL saline), [^{18}F]**1a** (7.4 MBq, 0.70 nmo in 150 µL saline) and [^{18}F]**1b** (7.4 MBq, 1.37 nmol in 150 µL saline) in HCT116 tumor-bearing mice at different time points post injection. (D-F) Time course of the radiotracers ([^{18}F]**1,** [^{18}F]**1a** and [^{18}F]**1b)** uptake in tumor and muscle of HCT116-bearing mice. (G) Time course of tumor-to-muscle uptake ratio for [^{18}F]**1**, [^{18}F]**1a** and [^{18}F]**1b** in HCT116-bearing mice.

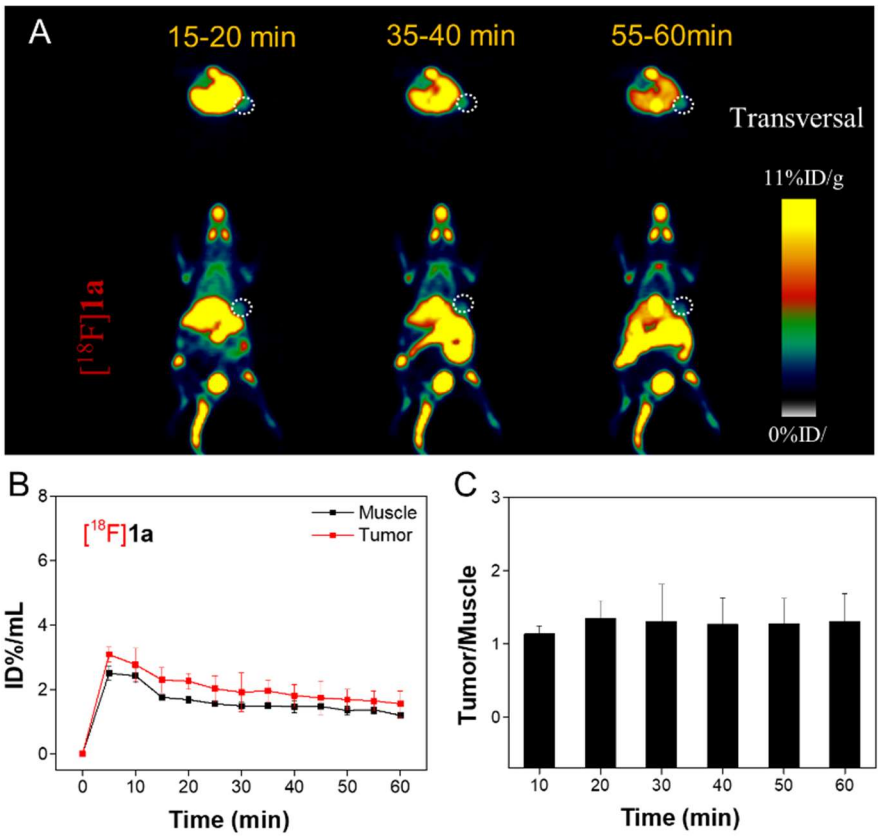


Figure S60. (A) MicroPET imaging of [^{18}F]**1a** (7.4 MBq, 0.70 nmol in 150 μL saline) in Hela tumor-bearing mice at different time points post injection. (B) Time course of radiotracer uptake in tumor and muscle of Hela-bearing mice. (C) Time course of tumor-to-muscle uptake ratio for [^{18}F]**1a** in Hela-bearing mice.

Detection of [^{18}F]1-Dimer in tumor.

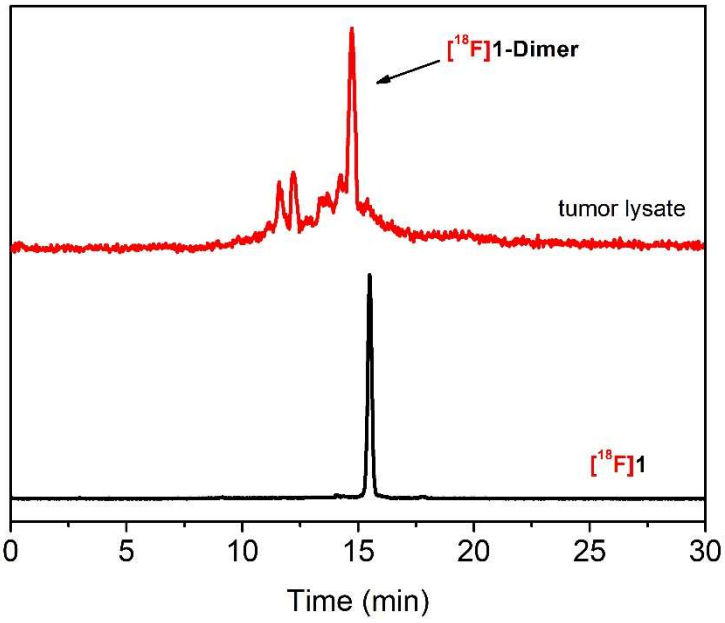


Figure S61. Radio-HPLC analysis of [^{18}F]**1-Dimer** in tumor lysates.

HPLC conditions

Table S2. HPLC conditions for analysis of compounds

Time/min	Flow (mL/min)	H_2O (0.1% TFA)%	MeCN (0.1% TFA)%
Initial	1	80	20
3	1	80	20
35	1	10	90
40	1	80	20

Table S3. HPLC conditions for purification of compounds

Time/min	Flow (mL/min)	H_2O (0.1% TFA)%	MeCN (0.1% TFA)%
Initial	3	80	20
3	3	80	20
35	3	10	90
40	3	80	20