Electronic Supplementary Information

Development of a kit-like radiofluorinated biomolecule leading to a controlled self-assembly of ^{18}F nanoparticles for a smart PET imaging application

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General

All chemicals were purchased as reagent grade 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 glioblastoma cell lines (U87MG) and human colorectal cancer cell lines (HCT116) were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). BALB/c nude mice (18-20 g; 4-6 week 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 U87MG cells (5×10^6) suspended in PBS (100 μL) in the right shoulder of each nude mouse. The tumors were 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 procedures and animal protocols were approved by the Animal Care and Ethnics 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). Nuclear magnetic resonance spectrometers (^{1}H-NMR and ^{13}C-NMR, Bruker DRX-400; Bruker; Germany) were used to obtain spectra of samples dissolved in d_6-DMSO, and the chemical shifts were referenced to tetramethylsilane. The high performance liquid chromatography (HPLC) system was equipped with a pump (Waters 1525 HPLC; Waters; USA), 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) and a radioactivity detector (Radiomatic 610TR; Perkin Elmer; MA; USA) which were operated by software programs Breeze (NY; USA) and proFSA (Perkin Elmer; USA). The radioactivity was counted using a γ counter (Packard-multi-prias;

Perkin Elmer; USA). Dynamic light scattering (DLS) was measured on a Zeta Sizer Nano Series (Malvern Instruments; United Kingdom). Transmission electron micrography (TEM) image was obtained on a JEOL 2100 electron microscope (JEOL; Jappan) operating at 200 kV. Micro-PET imaging was performed on an Inveon scanner (Siemens, Germany). Cerenkov luminescence imaging was performed using IVIS Spectrum (Perkin Elmer; MA; USA).

Chemical synthesis and characterization of 1-Cold

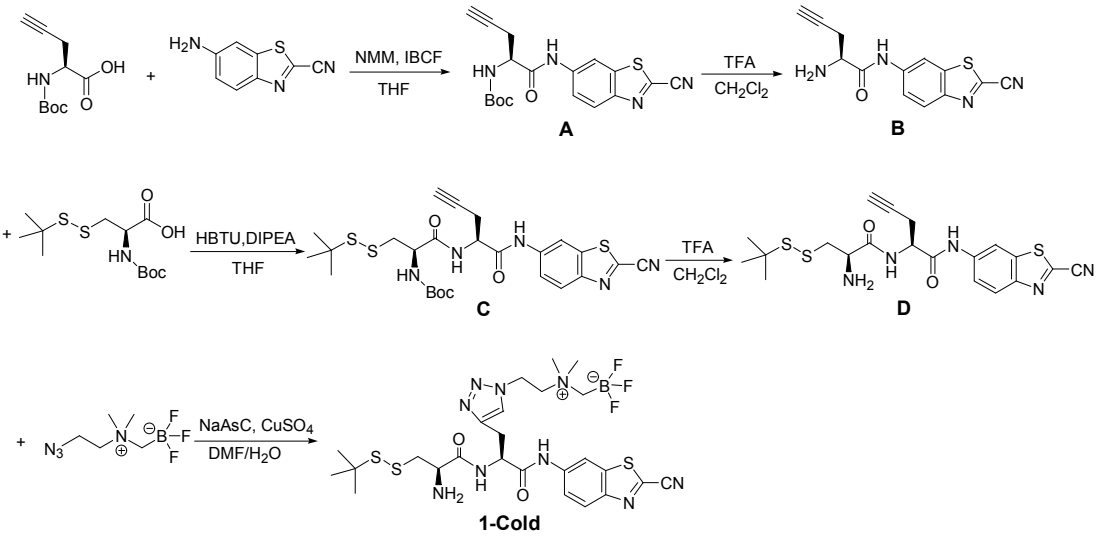

Fig. S1. Synthesis of the precursor **1-Cold.**

Synthesis of the compound **A**

Starting from 2-cyano-6-aminobenzothiazole (NH_2-CBT), compound **A** was obtained according to the method reported previously.[1] The isobutyl chloroformate (390 μL, 3.0 mmol, 1.5 eq) and 4-methylmorpholine (NMM) (660 μL, 6.0 mmol, 3 eq) was separately added to N-Boc-propargylglycine (768 mg, 3.6 mmol, 1.8 eq) in dry THF (8.0 mL) at 0 °C under N_2 and the reaction mixture was stirred for 2 hours. 2-Cyano-6-aminobenzothiazole (350 mg, 2.0 mmol, 1 eq) in THF (6 mL) was added to the reaction mixture at 0 ºC. After further reaction at room temperature overnight, the solvent was removed under vacuum. The residue was dissolved in ethyl acetate (EtOAc) (50 mL) and washed with water and aqueous $NaHCO_3$ (50 mL × 3). The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel chromatography with the eluent of Hexane :

EtOAc = 1:1 to get the compound **A** (676 mg, yield: 91%). ESI-MS calcd for $C_{18}H_{18}N_4O_3SNa^+$ ([M+Na]$^+$) 393.10, found 393.13.

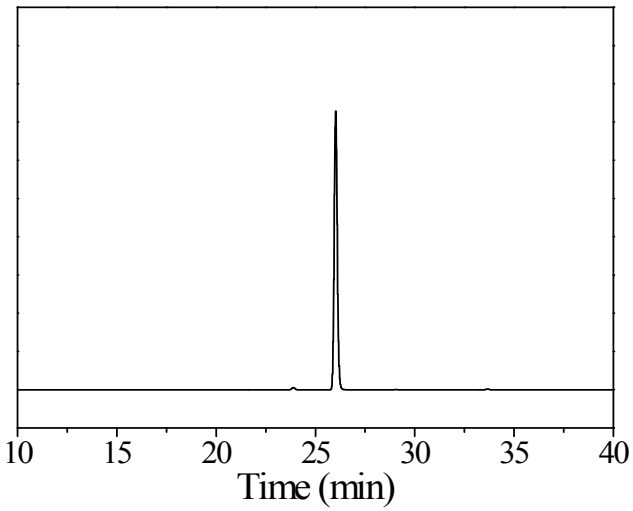

Fig. S2. HPLC trace of purified compound **A**.

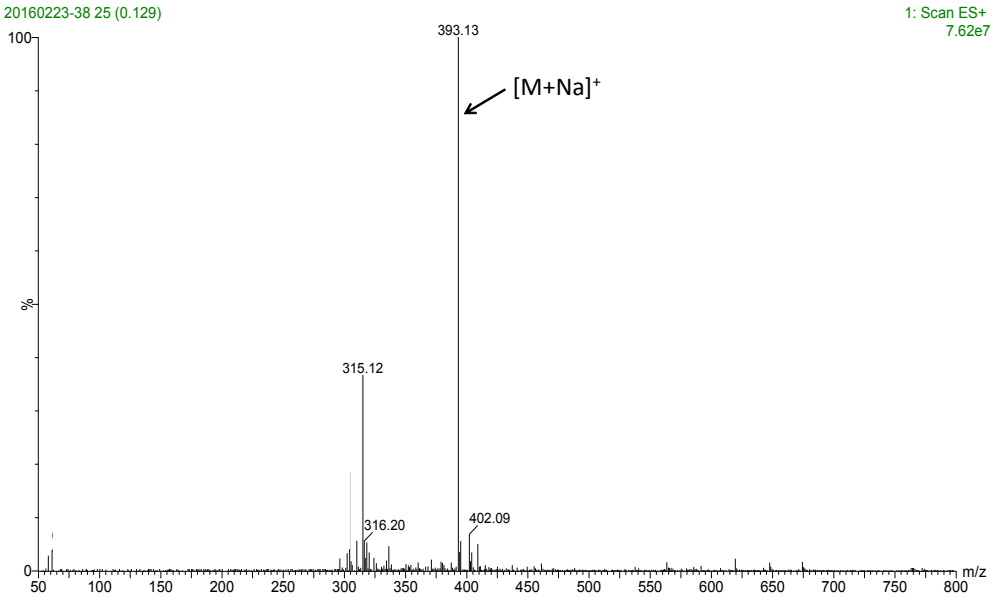

Fig. S3. ESI-MS of compound **A**.

Synthesis of the compound **B**

To remove the protecting group of Boc, TFA (6 mL) was added to a solution of compound **A** (670 mg, 1.6 mmol) in DCM (6 mL) and stirred for 1 hour at room temperature. Then the solvent was evaporated under reduced pressure and precipitated from the cleavage solution with cold diethyl ether. The crude product was purified with preparative HPLC to give the desired product **B** (461 mg, yield: 94 %). ^{1}H NMR (400 MHz, d_6-DMSO), δ (ppm): 11.06 (s, 1H), 8.73 (s, 1H), 8.52 (s, 1H), 8.25 (d, J =

8.00 Hz, 1H), 7.79 (d, J = 8.00 Hz, 1H), 4.20 (t, J = 8.00 Hz, 1H), 3.17 (s, 1H), 2.88 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO), δ(ppm): 166.9, 148.6, 138.8, 137.2, 136.3, 125.5, 121.4, 114.0, 112.6, 77.8, 76.2, 52.1, 21.5; ESI-MS calcd for $C_{13}H_{11}N_4OS^+$ ($[M+H]^+$) 271.07, found 271.14.

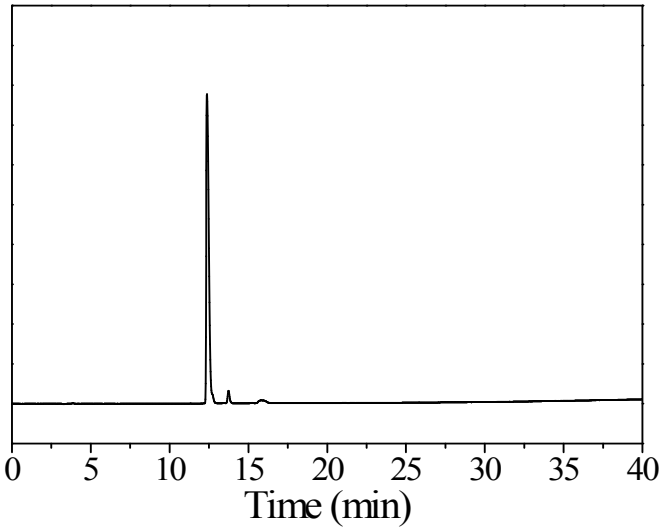

Fig. S4. HPLC trace of purified compound **B**.

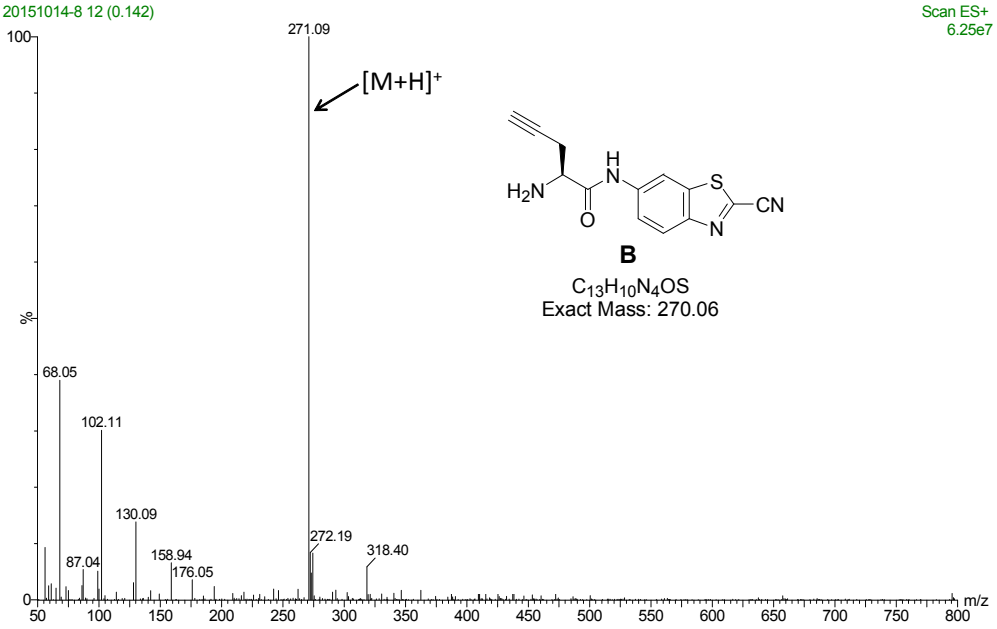

Fig. S5. ESI-MS of compound **B**.

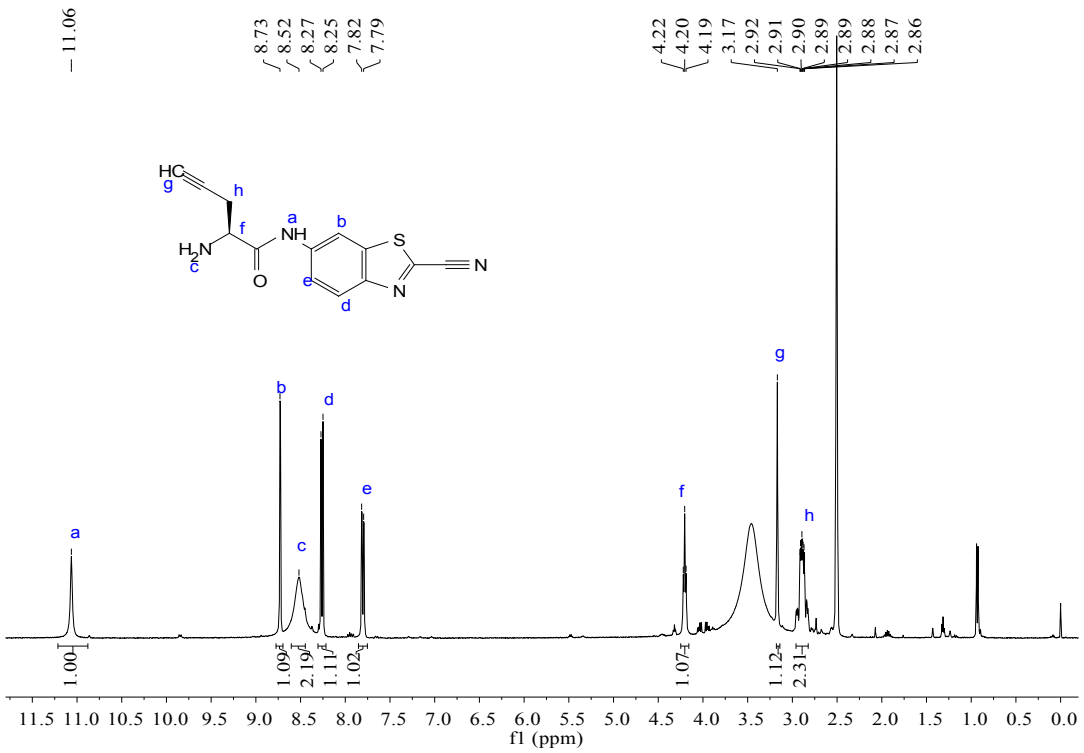

Fig.S6. [1]H NMR of compound **B**.

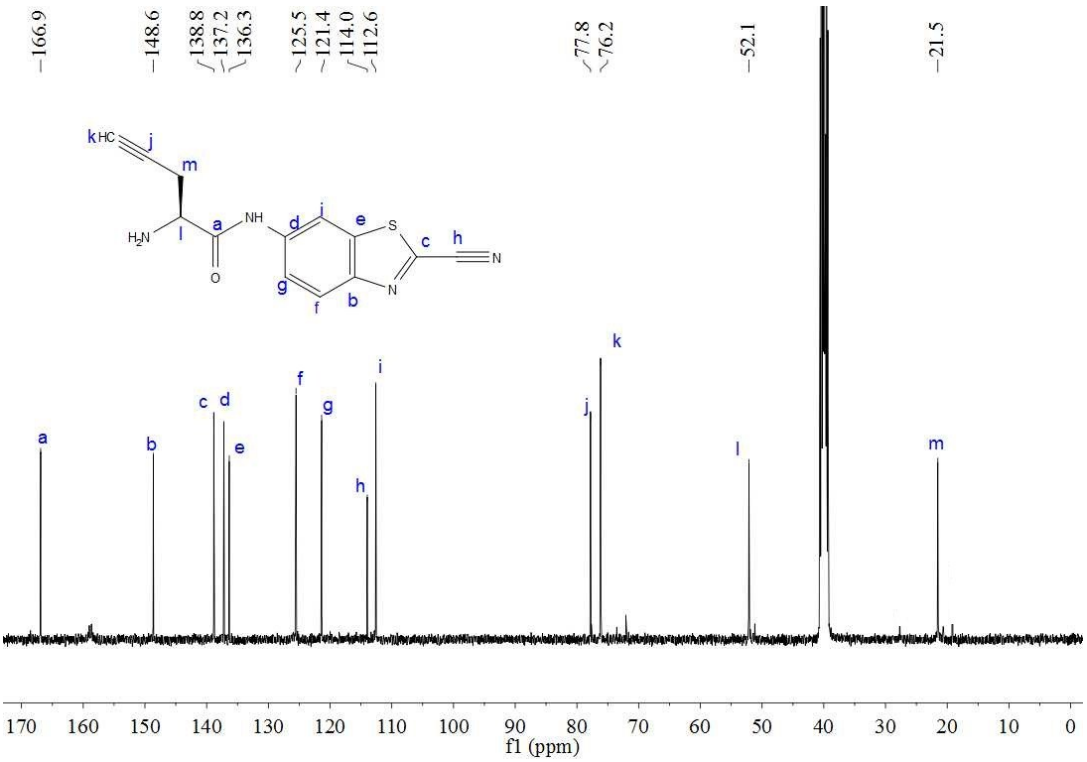

Fig. S7. [13]C NMR of compound **B**.

Synthesis of the compound **C**

To a solution of compound **B** (368 mg, 1.4 mmol, 1eq) in dry THF (5 mL), Boc-S-tert-butylmercapto-L-cysteine (463 mg, 1.5 mmol, 1.1 eq), HBTU (594 mg, 1.15eq) and DIPEA (562 μL, 2.5 eq) were added. The resulting solution was stirred for 2 hours at room temperature and then evaporated under reduced pressure. The crude product was purified by silica gel chromatography (Hexane : EtOAc = 1.5:1) to yield the compound **C** (457 mg, yield: 60%). ^{1}H NMR (400 MHz, d_6-DMSO), δ (ppm): 10.53 (s, 1H), 8.73 (s, 1H), 8.44 (s, 1H), 8.22 (d, J = 8.00 Hz, 1H), 7.81 (d, J = 8.00 Hz, 1H), 7.17 (s, 1H), 4.65 (s, 1H), 4.27 (s, 1H), 3.07 (s, 1H), 2.93 (m, 2H), 2.68 (m, 2H), 1.38 (s, 9H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, d_6-DMSO), δ (ppm) 170.9, 169.5, 155.8, 148.3, 139.4, 137.1, 135.8, 125.3, 121.4, 114.0, 112.2, 80.3, 79.0, 73.9, 54.4, 52.9, 48.2, 41.8, 30.0, 28.6, 22.2; ESI-MS calcd for $C_{25}H_{31}N_5O_4S_3Na^+$ ($[M+Na]^+$) 584.14, found 584.15.

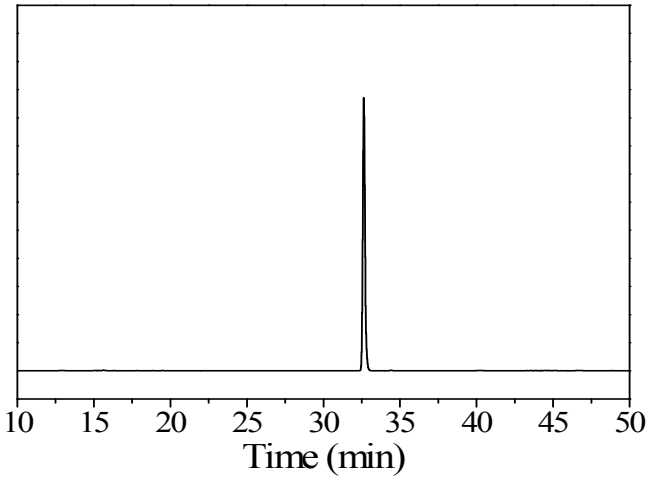

Fig. S8. HPLC trace of purified compound **C**.

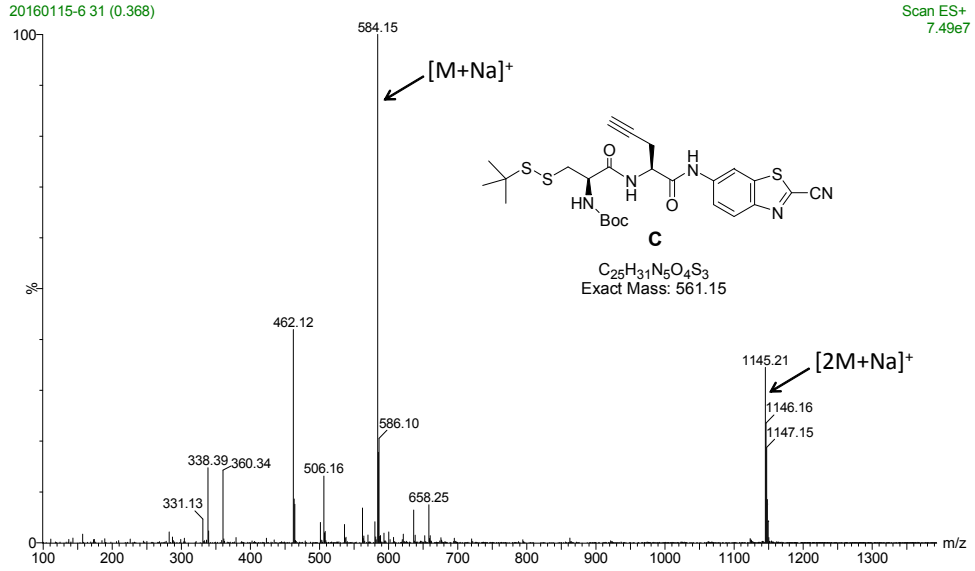

Fig. S9. ESI-MS of compound **C**.

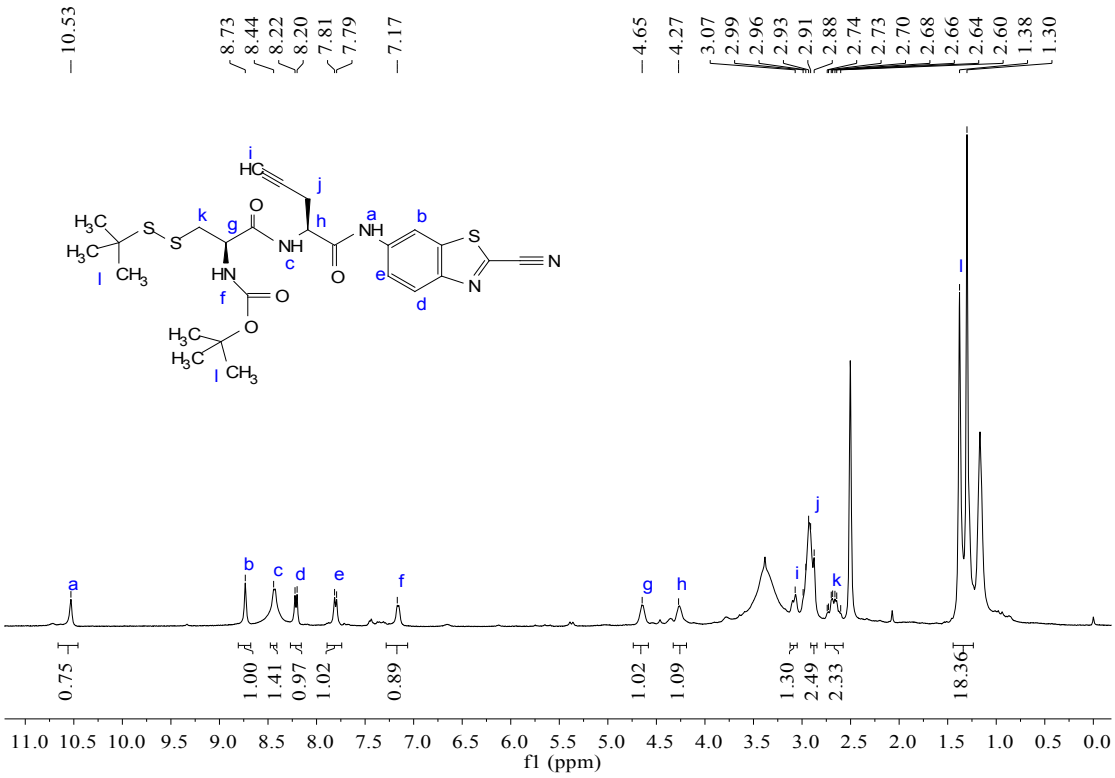

Fig. S10. ^{1}H NMR of compound **C**.

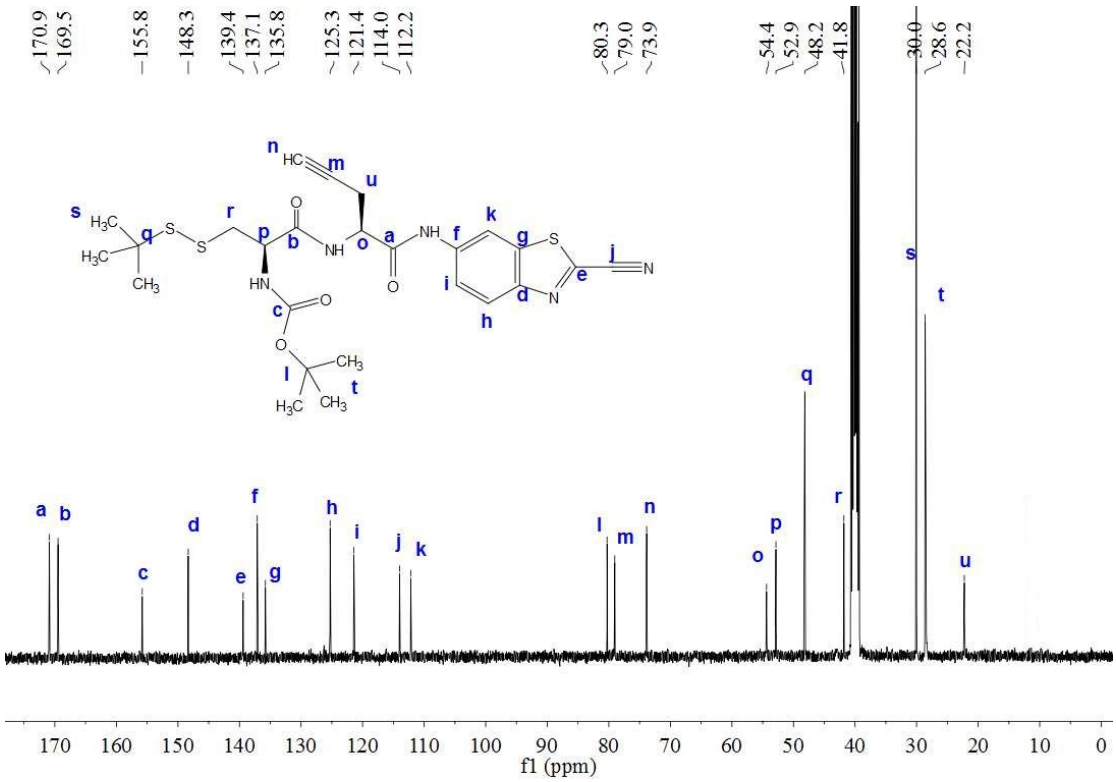

Fig.S11.^{13}C NMR of compound **C**.

Synthesis of the compound **D**

To remove the Boc group, TFA (3 mL) was added to the solution of compound **C** (280 mg, 0.5 mmol) in DCM (3 mL). The resulting solution was stirred at room temperature for 1 hour and then the solvent was evaporated under reduced pressure and precipitated from the cleavage solution with cold diethyl ether. The crude product was purified with preparative HPLC to give the desired product **D** (230 mg, yield:94%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 10.85 (s, 1H), 9.26 (s, 1H), 8.73 (s, 1H), 8.43 (d, J = 8.00 Hz, 2H), 8.24 (d, J = 8.00 Hz, 1H), 7.84 (d, J = 8.00 Hz, 1H), 4.77 (m, 1H), 4.16 (t, J = 8.00 Hz, 1H), 3.18 (m, 2H), 2.96 (s, 1H), 2.72 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, d_6-DMSO), δ (ppm): 169.2, 167.4, 148.4, 139.4, 137.1, 135.9, 125.4, 121.4, 114.0, 112.2, 79.9, 74.2, 53.1, 52.0, 48.6, 41.8, 29.9, 22.5; ESI-MS calcd for $C_{20}H_{24}N_5O_2S_3^+$ ([M+H]$^+$) 462.11, found 462.25.

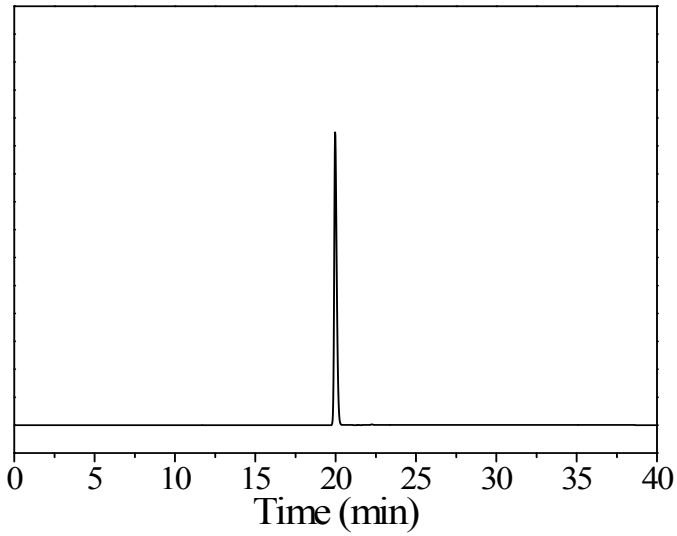

Fig. S12. HPLC trace of purified compound **D**.

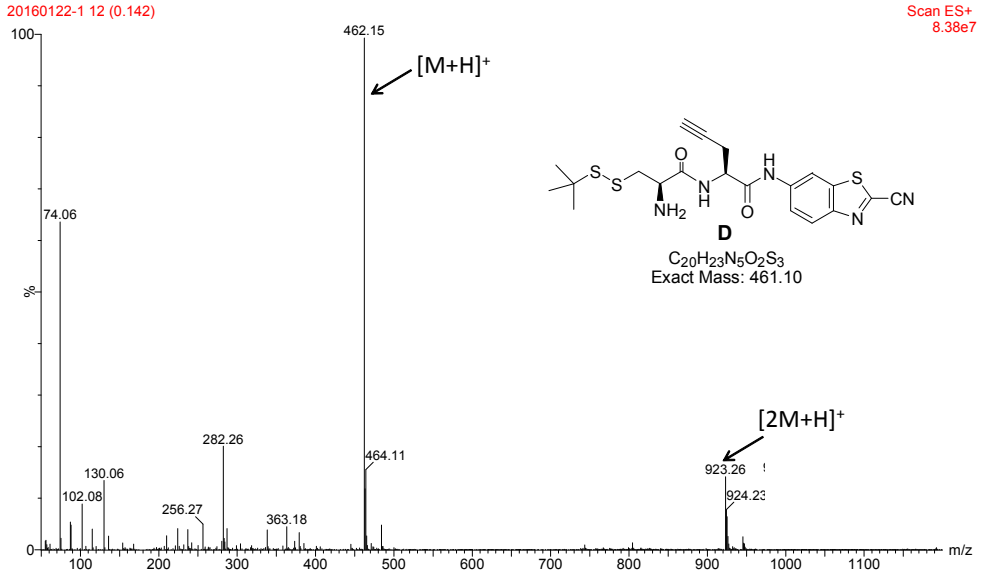

Fig. S13. ESI-MS of compound **D**.

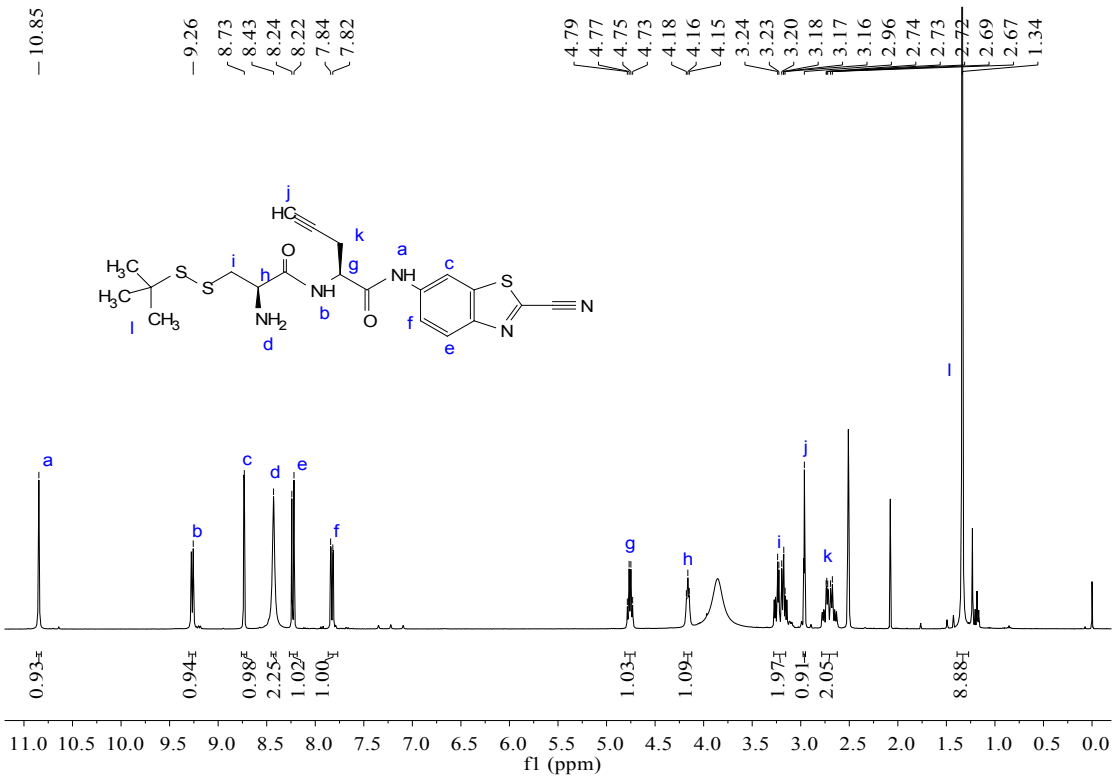

Fig. S14. [1]H NMR of compound **D**.

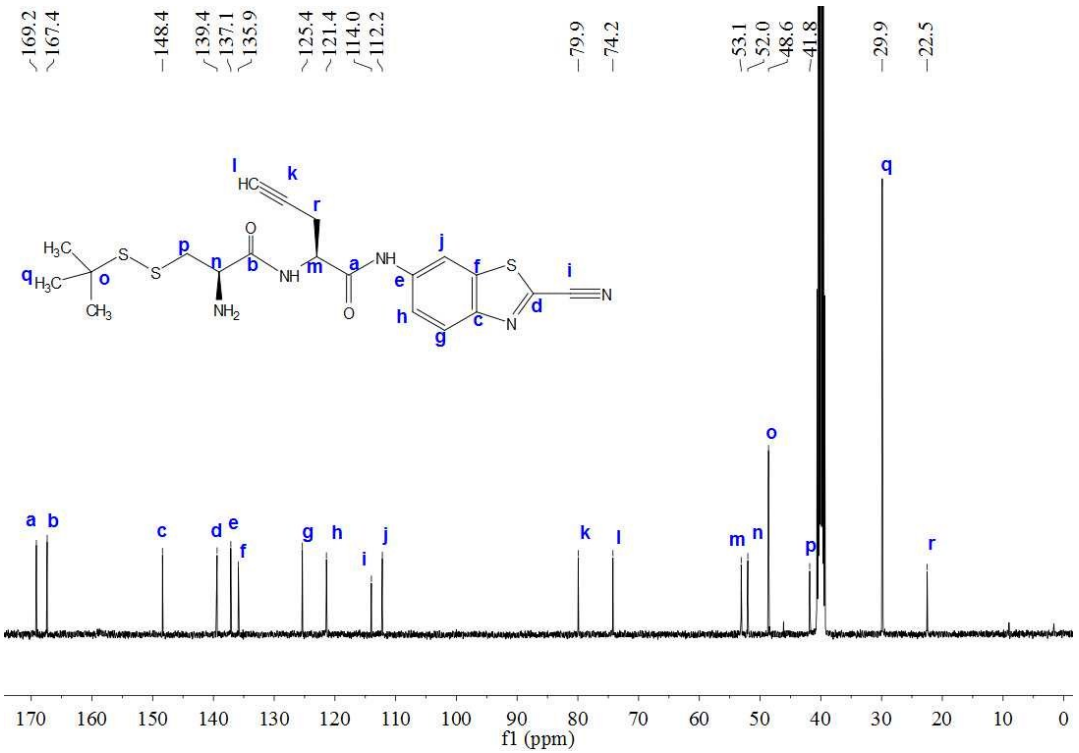

Fig. S15. [13]C NMR of compound **D**.

Synthesis of the compound **1-Cold**

The intermediate of 2-azidoethyl-N,N-dimethylammoniomethyltrifluoroborate

($AMBF_3$) was obtained according to the method reported previously.[2] To a solution of compound **D** (210 mg, mmol, 1 eq) in DMF (5 mL), $AMBF_3$ (135 mg, mmol, 1.5 eq) was added. Then sodium ascorbate (183 mg, 2 eq) and $CuSO_4$ (23 mg, 0.2 eq) in water and HEPES (0.2 M, 1.0 mL) was added. The resulting solution was heated to 45 ℃ for 2 hours. The crude product was purified by preparative HPLC to get the compound **1-Cold** (224 mg, 75 %). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 8.48 (s, 1H), 8.00 (d, *J* = 8.00 Hz, 1H), 7.89 (d, 1H), 7.59 (d, *J* = 8.00 Hz, 1H), 4.11 (t, *J* = 8.00 Hz, 1H), 3.71 (t, *J* = 8.00 Hz, 2H), 3.27 (m, 1H), 3.22 (m, 2H), 3.13 (m, 2H), 3.02 (t, *J* = 8.00 Hz, 2H), 2.98 (s, 6H), 2.40 (d, *J* = 4.00 Hz, 2H), 1.28 (s, 9H); ^{13}C NMR (101 MHz, CD_3OD), δ (ppm): 169.9, 167.4, 148.6, 138.8, 138.7, 136.6, 135.7, 124.6, 121.2, 121.1, 112.7, 111.9, 63.3, 54.4, 53.9, 52.6, 48.3, 47.0, 44.1, 40.7, 28.8, 27.6; ^{19}F NMR (386 MHz, CD_3OD), δ (ppm) 76.7; ESI-MS calcd for $C_{25}H_{36}BF_3N_9O_2S_3^+$ ($[M+H]^+$) 658.22, found 658.23.

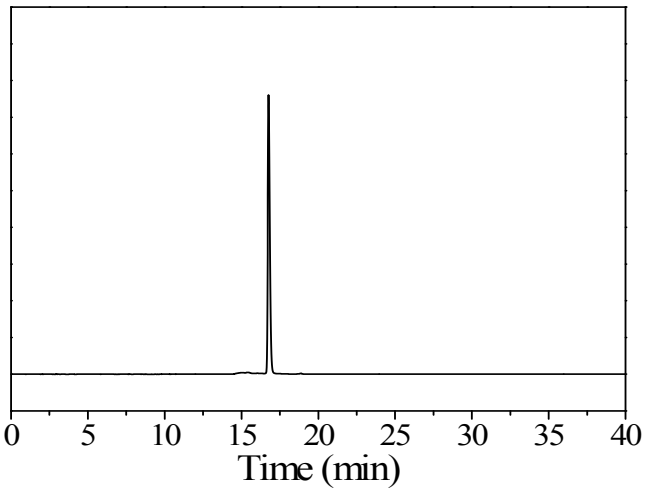

Fig. S16. HPLC trace of purified compound **1-Cold**.

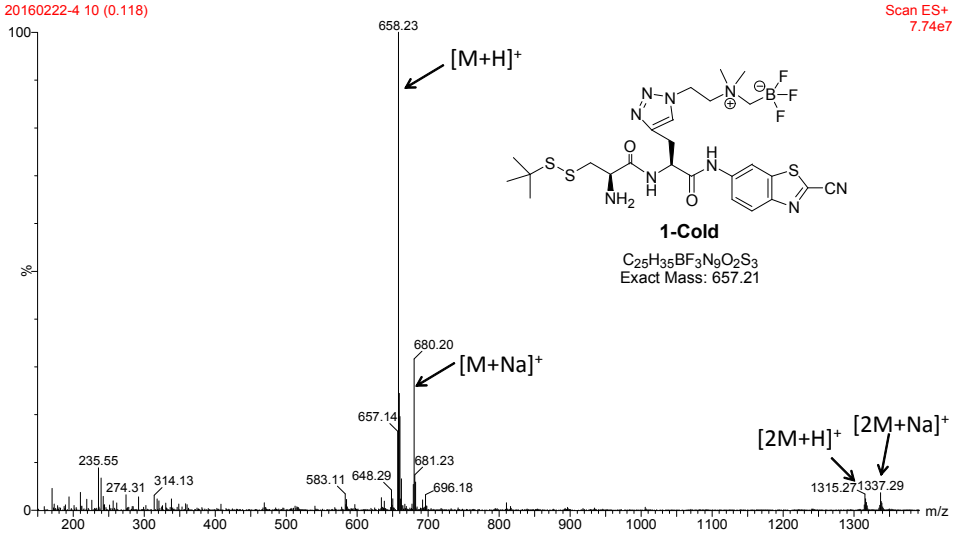

Fig. S17. ESI-MS of compound **1-Cold**.

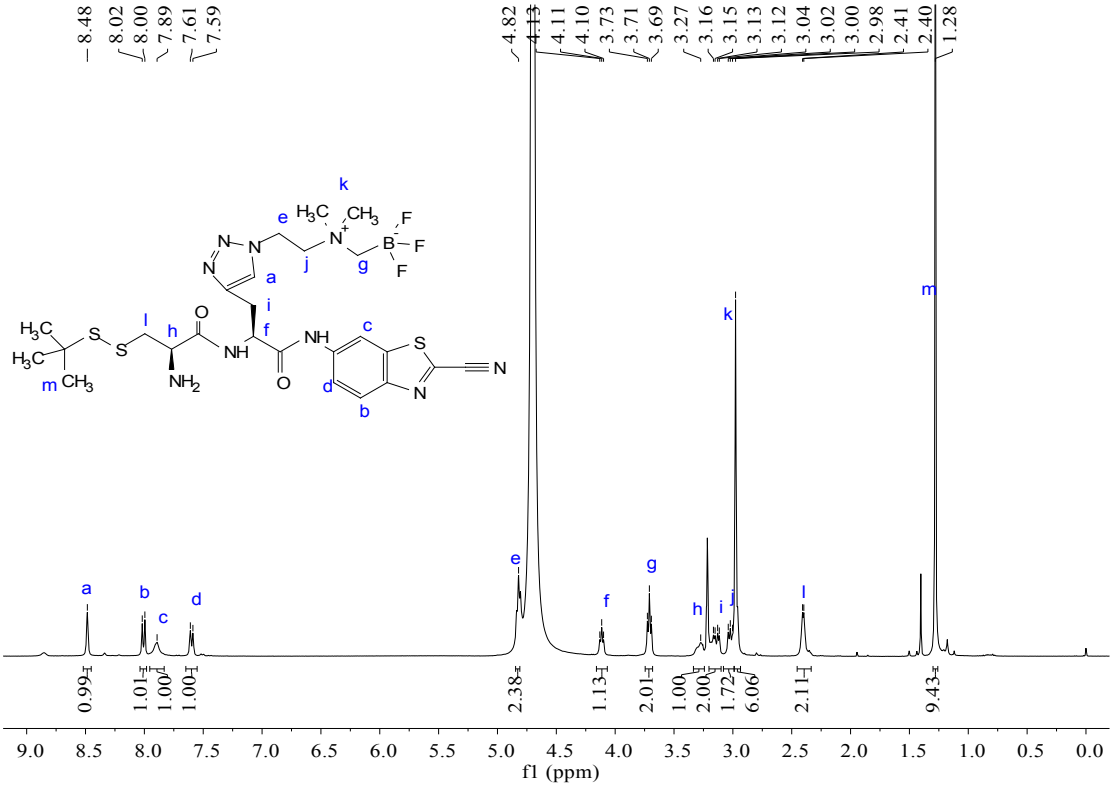

Fig.S18. ^{1}H NMR of compound **1-Cold**.

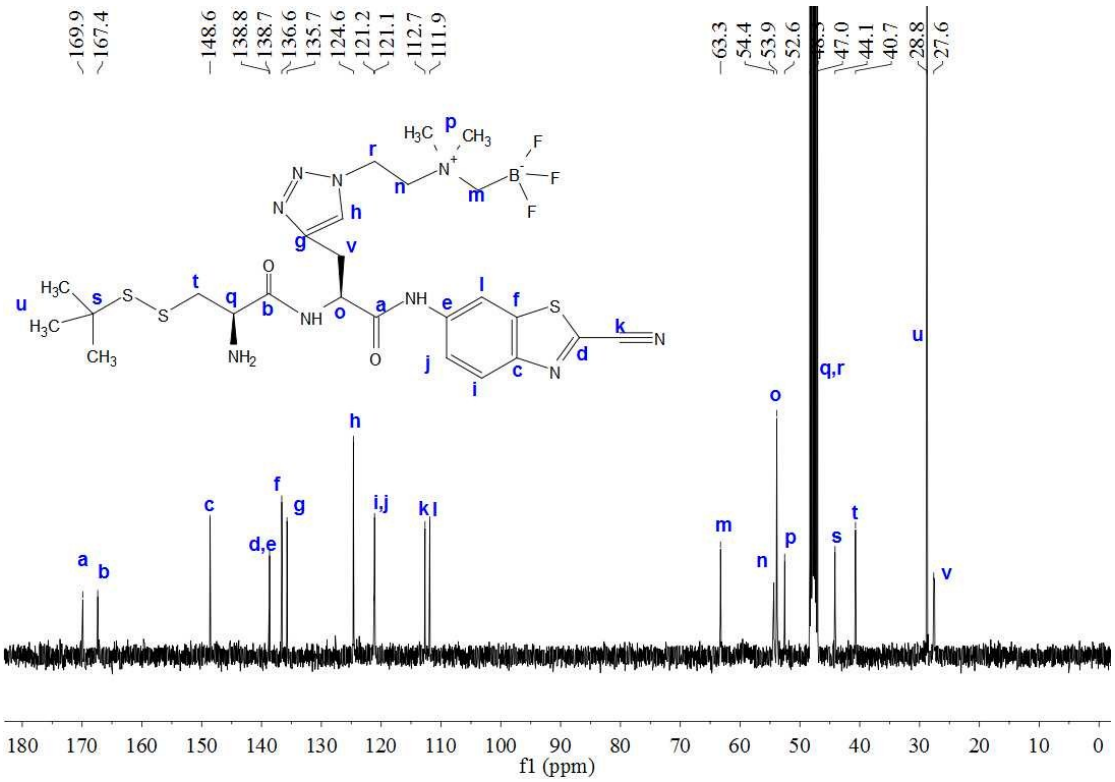

Fig. S19. ^{13}C NMR of compound **1-Cold**.

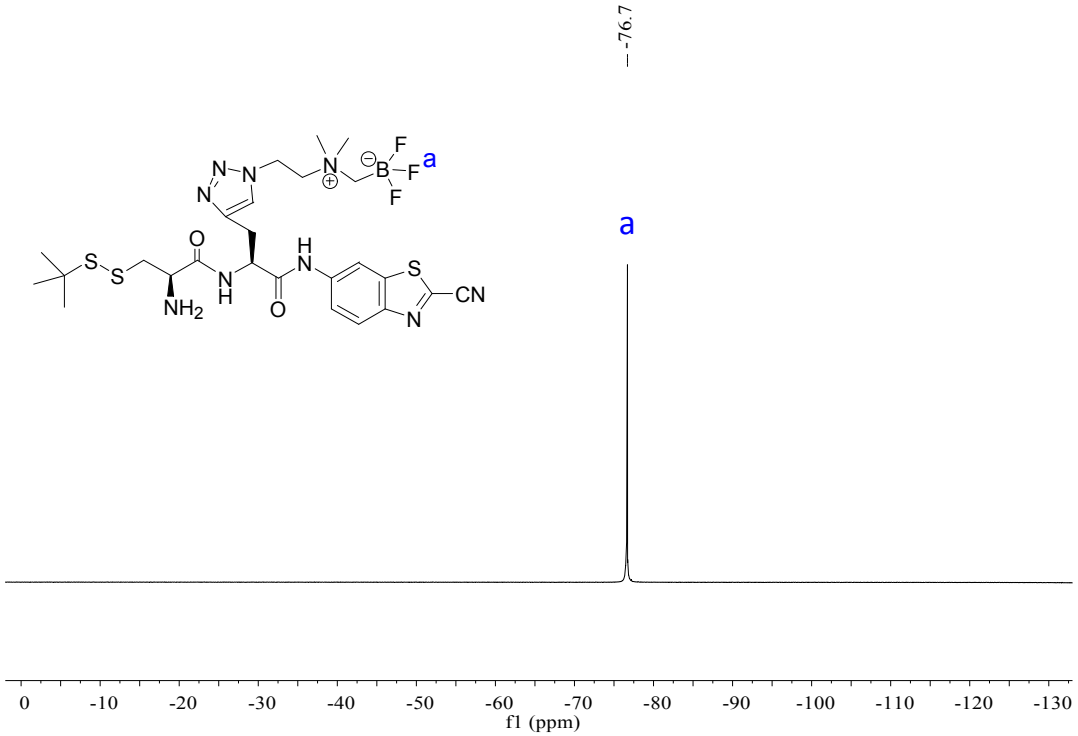

Fig. S20. ^{19}F NMR of compound **1-Cold**.

Characterization of disulfide reduction-triggered macrocyclization in vitro

To evaluate whether disulfide reduction of the probe **1-Cold** could trigger intramolecular cyclization, TECP·HCl (66 μL, 50 mM) was added to the solution of **1-Cold** (100 μL, 3.3 mM) in citrate buffer (pH = 3.0). The mixture was fixed to 330 μL with the buffer and incubated for 1 h at room temperature and then analyzed by the HPLC. After that, the pH value of the mixture was adjusted to 7.4 with sodium orcarbonate. Each experiment was performed independently at least three times. A similar experiment was performed by adding GSH or U87MG cell lysates (66 μL, 50 mM) to **1-Cold** (100 μL, 3.3 mM), respectively. The mixtures were fixed to 330 uL and incubated in phosphate buffer (10 mM PB, pH = 7.4) containing 2% DMF (v/v) for 4 h. The reaction mixture was analyzed by HPLC and LC-MS.

It was observed that the solution of **1-cold** was still clear with reducing agent TCEP at pH = 3. However, the reduced product was formed according to the analysis of HPLC and LC-MS. The intermediate became a turbid dispersion quickly after the pH value was adjusted to 7.4, since the major fractions of cyclized products were formed. The same phenomenon was observed when **1-Cold** was incubated with the reducing agent GSH or U87MG cell lysates under physiological environment (pH = 7.4).

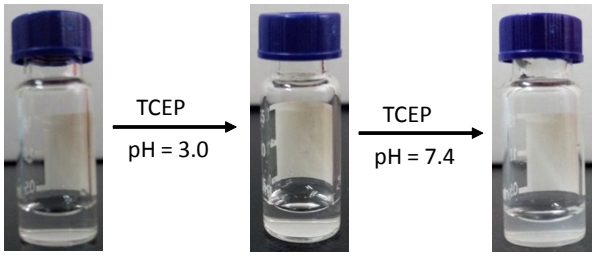

Fig. S21. Incubation of **1-Cold** with TCEP at different pH values.

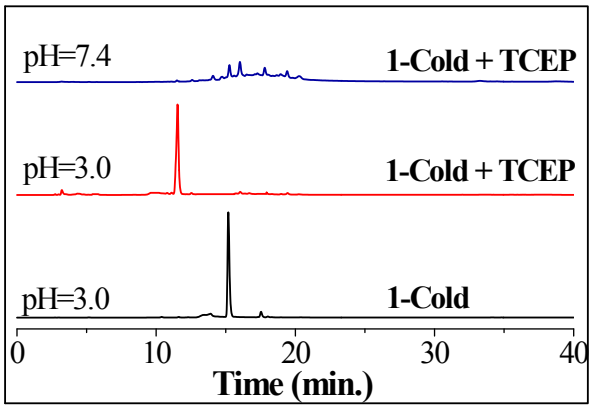

Fig. S22. HPLC traces of **1-Cold** incubated with reducing agent TCEP in aqueous solution. Bottom: **1-Cold** alone at pH = 3; middle: reduced intermediate formed following TCEP reduction at pH = 3; top: cyclized products formed following incubation of **1-Cold** with TCEP at pH = 7.4.

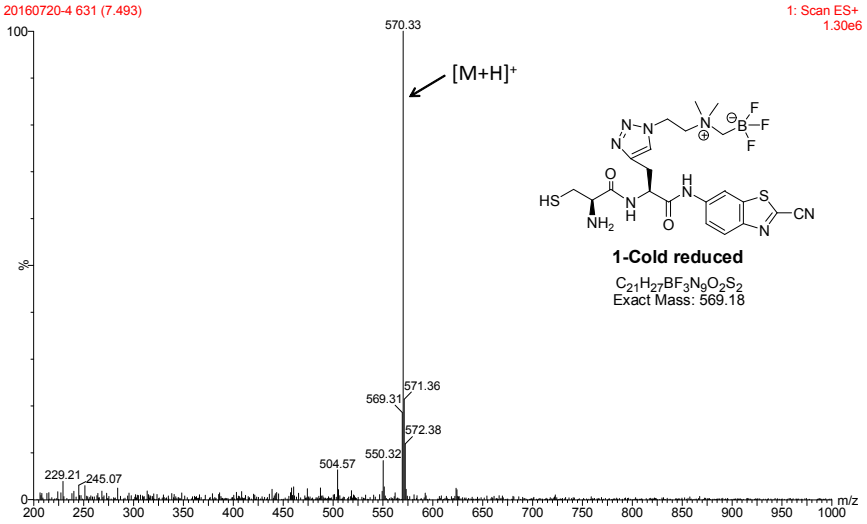

Fig. S23. ESI-MS of **1-Cold** incubated with TCEP at pH 3.0.

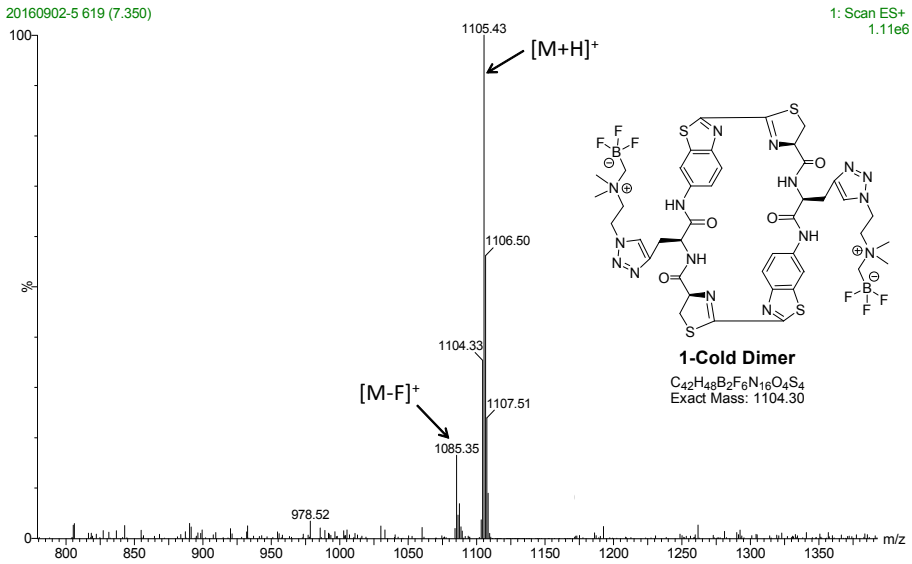

Fig. S24. ESI-MS of **1-Cold** incubated with TCEP at pH 7.4.

Stability of 1-Dimer

The stability of **1-Dimer** in the mixture of DMSO and PBS (pH = 7.4) (v/v=1:1) was determined under 37 °C at different time. **1-Dimer** (100 nmol) was dissolved in the mixed solvent (500 uL) of DMSO and PBS, and incubated under 37 °C. At different incubation time (30 to 240 min.), a small sample was removed for purity analysis by HPLC at 254 nm.

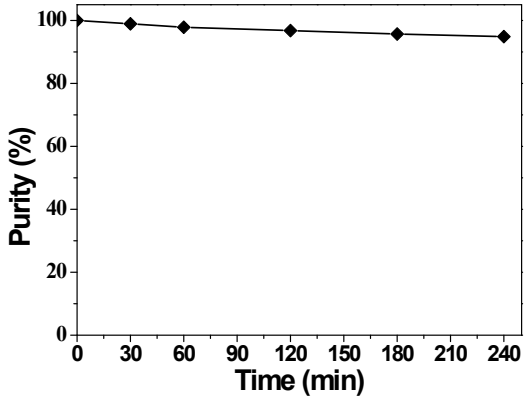

Fig. S25. Stability of **1-Dimer** in the mixture of DMSO and PBS (pH = 7.4) (v/v=1:1) incubated at 37 °C.

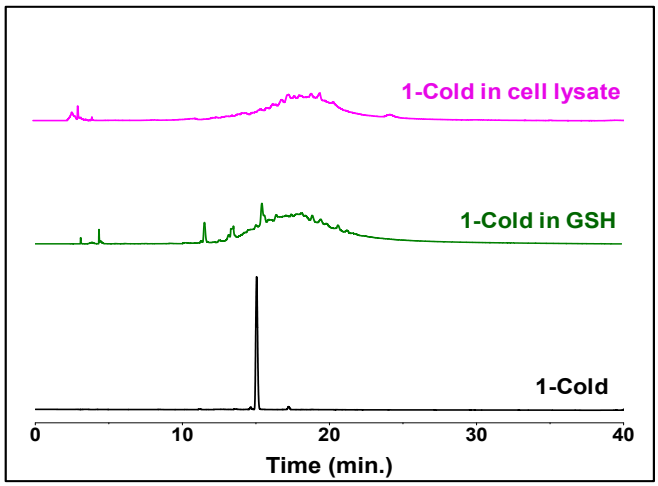

Fig. S26. HPLC traces of **1-Cold** under reducing conditions. Bottom: **1-Cold** alone; middle: cyclized products formed following incubation of **1-Cold** with GSH at pH = 7.4.; top: cyclized products formed following incubation of **1-Cold** with U87MG cell lysates at pH = 7.4.

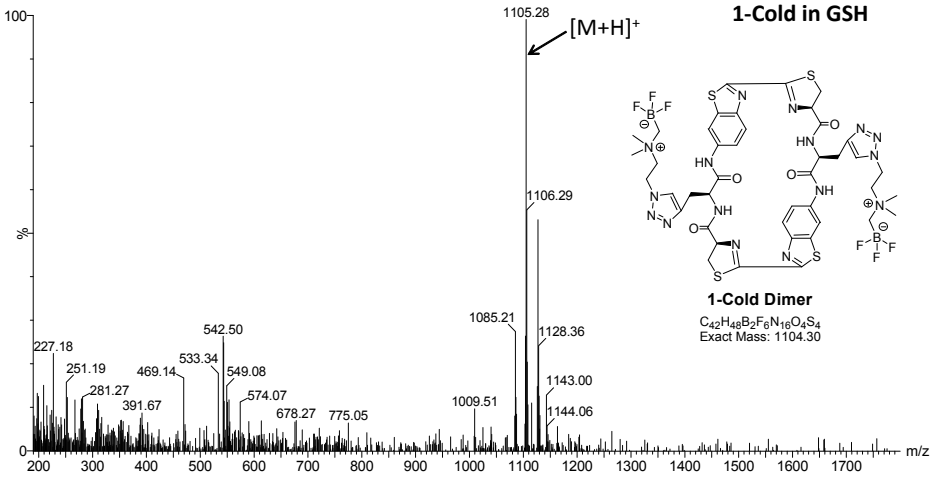

Fig. S27. ESI-MS of **1-Cold** incubated with GSH at pH 7.4.

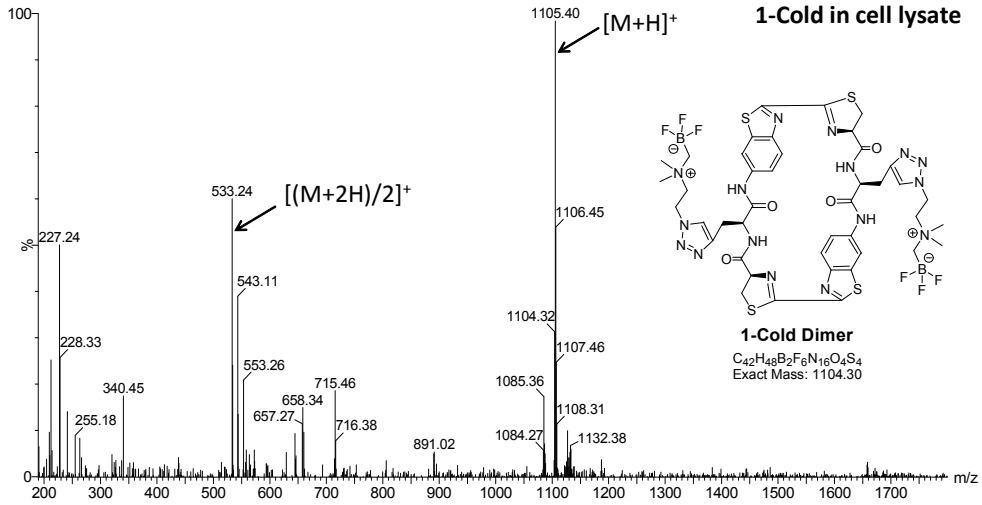

Fig. S28. ESI-MS of **1-Cold** incubated with U87MG cell lysate at pH 7.4.

Determination of the dimerization of 1-Cold and radioactive 1 in tumor-bearing mice

1-Cold (5 nmol) was dissolved in the mixed solvent (50 uL) of DMSO and saline (v/v=1/1), and then it was injected into the tumor directly. After 4 h, the tumor was collected and weighted. The tumor tissue (~250 mg) was homogenized by an electronic homogenate machine in RIPA lysis buffer (3 mL). Then, DMSO (3 mL) was added to the mixture and homogenized once again. The supernatant was obtained by centrifugation at 14,000 ×g for 5 min and analyzed by ESI-MS. The dimer formed in the tumor was detected according to the ESI-MS analysis, indicating that the dimerization of **1-Cold** took place in vivo.

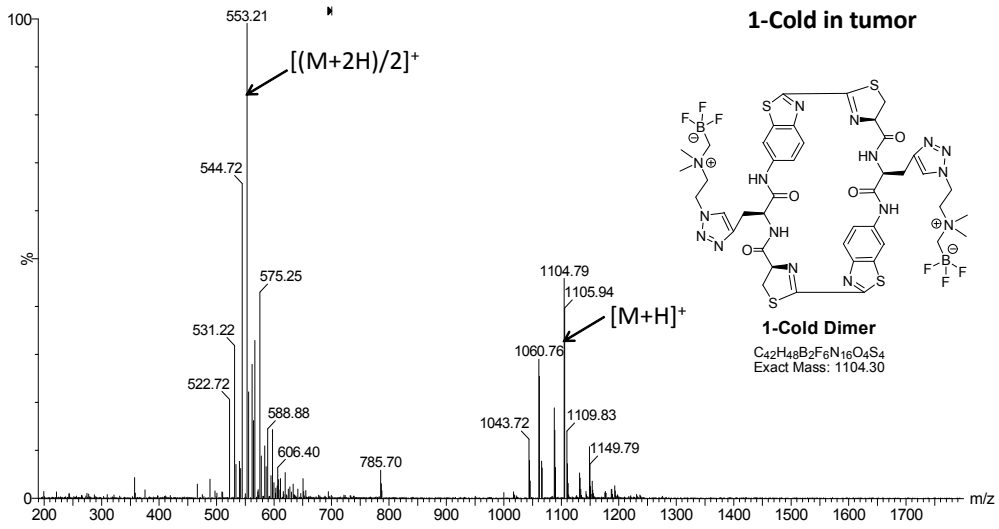

Fig. S29. ESI-MS of **1-Cold** in U87MG tumor-bearing mice.

Characterization of the nanoparticles of the self-assembled condensation products of 1-Cold

The condensation products were obtained with **1-Cold** (100 μM) in phosphate buffer (pH 7.4, 0.2 M) containing TCEP (400 μM) and incubated at room temperature for 1 h. The size distribution of the aggregated nanoparticles was determined by dynamic light scattering (DLS). Also, the samples were prepared by casting one drop of the solution on carbon-coated copper grids for the transmission electron microscope (TEM) measurement.

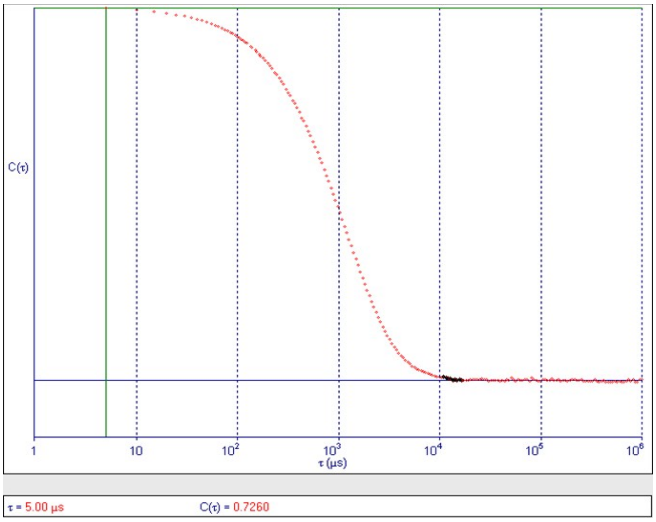

Fig. S30. Fitting results of the normalized autocorrelation function for **1-Dimer** in PBS (pH = 7.4) at T = 298 K.

Biocompatibility of 1-Cold and 1-Dimer

To evaluate the biocompatibility of the precursor **1-Cold**, its cytotoxicity against human glioblastoma cell lines U87MG was evaluated by the conventional MTT assay. Firstly, 5×10^3 suspended cells per well was seeded into 96-well plates. The plates were incubated at 37 °C under 5% CO_2 for 24 h to allow cells attachment. The compound **1-Cold** was dissolved in DMSO (0.1%) and diluted with DMEM to different concentrations (12.5, 25, 50 and 100 μM, respectively). Then cells were exposed to various concentrations of **1-Cold** and cultured at 37 °C under 5% CO_2 for 3 h, 6 h, 12 h, and 24 h, respectively. Then, MTT solution (5 mg/mL, 20 μL) was added to each well. After plates were cultured at 37 °C for another 4 h, the supernatants were removed and DMSO (150 μL) was added to each well for dissolving the formazan crystal. Then the absorbance of each well was measured at the wavelength of 470 nm using a microplate reader (BioTek Instruments, Inc. Vermont, USA). All tests were carried out in 6 repeats for at least three independent experiments. The cell viability was determined by the following equation, viability (%) = (mean absorbance of the experimental group / mean absorbance of the control group) × 100%. Similarly, the cytotoxicity of **1-Dimer** (the nanoparticles) against U87MG was also assessed by using the same protocol.

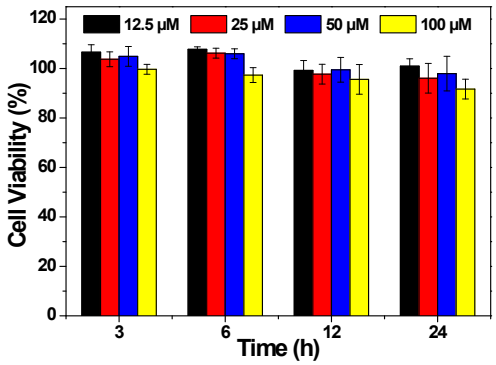

Fig. S31. Cytotoxicity study of **1-Cold** against U87MG cells.

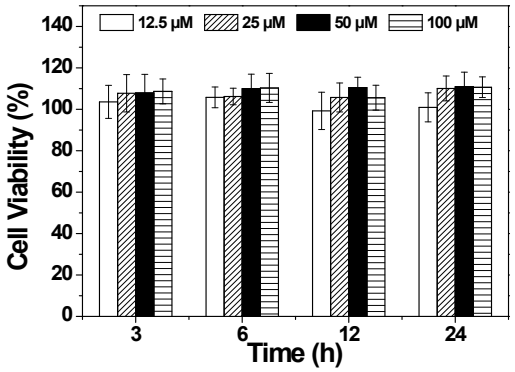

Fig. S32. Cytotoxicity study of **1-Dimer** against U87MG cells.

Stability of 1-Cold in pyridazine-HCl buffer

The stability of the precursor **1-Cold** in pyridazine-HCl buffer (pH = 2.0-2.5) was determined at different temperatures. At first, **1-Cold** (100 nmol) was dissolved in pyridazine-HCl buffer (500 uL). Then the mixtures were heated at 50, 60, 70, 80, 90 and 100 °C for 30 min, respectively. A small sample was removed for quality control analysis by HPLC at 254 nm.

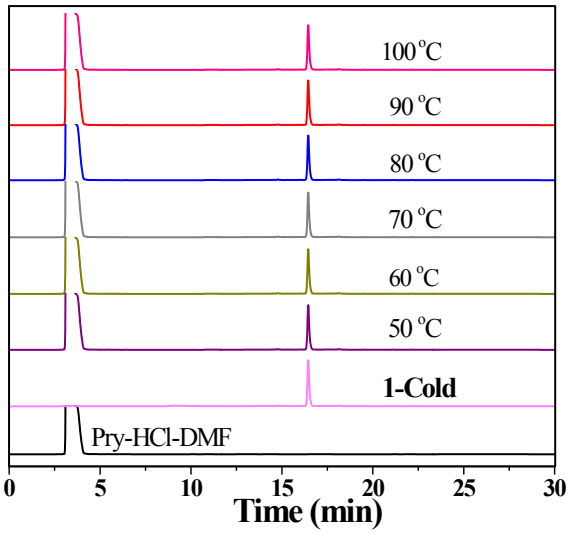

Fig. S33. HPLC traces of **1-Cold** incubated in pyridazine-HCl buffer (pH = 2.0-2.5) at different temperatures (UV at 254 nm).

[^{18}F]fluorination of 1-Cold

For radiolabelling, a wet no carrier added (NCA) solution of [^{18}F]fluoride ion was used directly following trapping. A QMA column was used to trap NCA [^{18}F]fluoride ion efficiently, which was directly eluted with 300-700 μL PBS into a reaction tube. Then the precursor (100 nmol) in DMF (20 μL) was added to the tube. The reaction was kept at 80 °C for 30 min. The reaction mixture was diluted with water (20 mL) and loaded onto a C18 light Sep-Pak cartridge, and free [^{18}F]fluoride ion was removed by washing thrice with deionized water (10 mL). The desired probe **1** was then eluted off the column with ethanol (0.5 mL), and diluted with saline for further use. A small sample was removed for quality control by radio-HPLC.

The radiolabelling was firstly optimized for the reaction conditions including reaction temperature and heating time. In terms of the influence of reaction

temperature, the radiochemical yield (RCY) under different temperature (30, 40, 50, 60, 70, 80, 90, and 100 °C) was determined. As for the influence of the reaction time, the radiochemical yield (RCY) at different incubation time (0-120 min) was measured under the fixed reaction temperature of 80 °C.

The in vitro stability of the probe **1** (100 μCi) diluted in saline (100 μL) was evaluated in the presence of PBS (0.1 M, pH = 7, 1 mL) and mouse serum (1 mL), respectively. Then the solutions were incubated at 37 °C. Subsequently, samples (50 μL) were removed at different incubation time for the radiochemical purity (RCP) analysis by HPLC to evaluate the stability of the probe **1**.

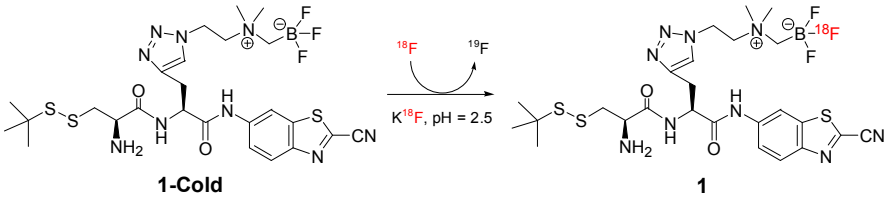

Fig. S34. One-step ^{18}F-fluorination of **1** from the precursor **1-Cold**.

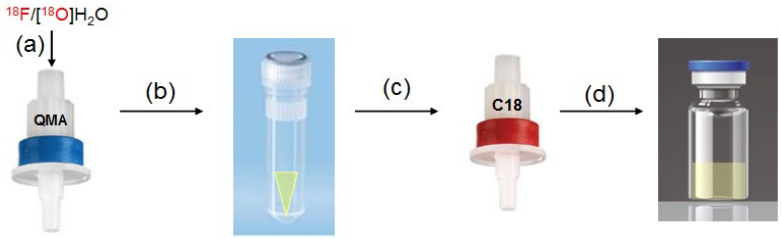

Fig. S35. Radiosynthesis and purification of the probe **1**. (a) Loading of [^{18}F]fluoride to the QMA cartridge, and then elution of cartridge with PBS to tube. (b) Adding [^{18}F]fluoride (200 mCi) in PBS to the tube containing precursor (100 nmol), and then incubate at 80 °C for 20 min. (c) Diluting the reaction mixture with water and loading onto a C18 light cartridge, washing the cartridge with water (10 mL) twice. (d) Formulation of the final product in saline containing <10% EtOH.

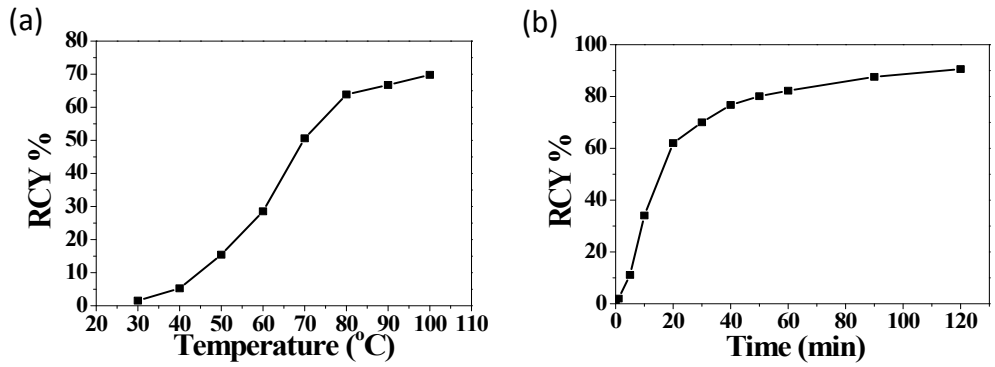

Fig. S36. Optimized reaction conditions for the [^{18}F]fluorination of **1-Cold**: (a) Effect of different reaction temperature; (b) Effect of different reaction time.

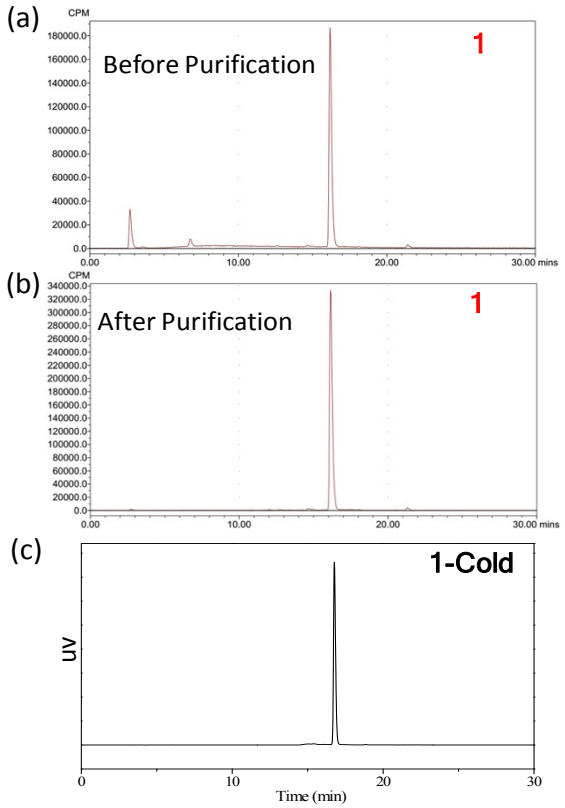

Fig. S37. HPLC traces of the reaction mixture of **1** (radiochromatograph) (a), purified **1** (radiochromatograph) (b) and **1-Cold** (c, absorbance at 254 nm), respectively.

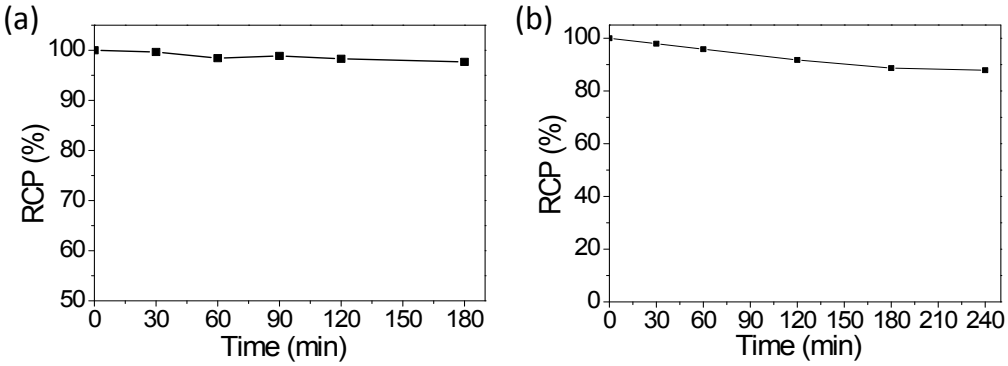

Fig. S38. Stability of the probe **1** in PBS (a) and mouse serum (b) at 37 °C.

Determination of partition coefficient (log *P*)

Octanol/water partition coefficient was determined for the probe **1** at pH = 7.0 by measuring the distribution of the radiotracer in n-octanol and PBS. n-Octanol (500 μL) was added into a polypropylene tube which was filled with the radiotracer (500 μL) in distilled water (pH 7.0). Then the tube was centrifuged (4,000 g, 5 min) to separate the two layers after oscillating on a vortex mixer at 25 °C. The radioactivity of samples (100 μL) in each layer was measured with a γ counter. The partition

coefficient (log *P*) was calculated using the equation of log *P* = log (Co/Cw), where Co and Cw were the radioactivity of the tracer in the n-octanol layer and that in the water layer, respectively. The result was average of the data from at least three independent experiments and expressed as mean ± SD.

Cell uptake studies

Human glioblastoma cell lines U87MG and human colorectal cancer cell lines HCT116 were cultured in DMEM, containing 10% (v/v) fetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel) in a 37 °C incubator with 5% CO_2. The cell uptake studies were performed in triplicates using the following protocol. U87MG or HCT116 cells were plated into 6-well plates (5×10^5 cells/well) and incubated overnight for cells attachment. Then the cells were washed 3 times with PBS, and fresh serum-free medium (500 μL) containing the probe **1** (1 μCi) was added to each well. Then the cells were incubated at 37 °C for 30, 60, 120 and 240 min, respectively. At each predetermined time, the medium was removed by aspiration and the cells were rinsed twice with ice-cold PBS. Subsequently, the cells were lysed with 0.1 M NaOH. The cell lysates and subsequent eluents of PBS were collected, and the radioactivity was measured using a γ counter and corrected for decay. The uptake rate was described as %AD (the percentage of the total added radioactivity dose) and normalized to per 5×10^5 cells.

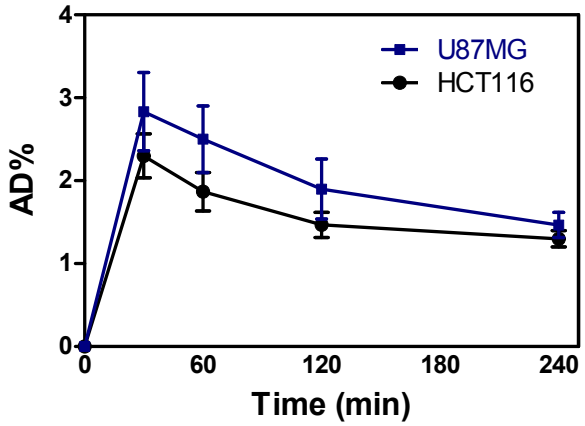

Fig. S39. Cell uptake of the probe **1** in cancer cells U87MG and HCT116.

PET imaging

PET images were acquired by an Inveon Dedicated micro-PET scanner (Siemens). In all experiments, the mice were anesthetized with isoflurane (2% isoflurane in oxygen

at a flow rate of 2 L/min). For imaging of the GSH in the living mice, the probe **1** alone (~100 μCi) and the probe **1** (~100 μCi) with GSH (10 mM) in physiological saline (100 μL) were intramuscularly injected into the left leg and the right leg of the normal mice, respectively. For imaging of the U87MG tumor-bearing mice, the probe **1** (~100 μCi, in 100 μL physiological saline) was administrated by intravenous injection. For blocking experiment, the thiol inhibitor N-ethylmaleimide (NEM) (1 mg, 100 μL) was initially injected 10 minutes in advance. PET images were acquired in a list mode date for 60 min. The PET data were histogrammed into 12 frames and each frame was reconstructed with the OSEM3D/MAP algorithm using micro-PET Manager (version 6869, Siemens). The reconstructed pixel size was 0.78×0.78×0.80 mm on a 128×128×159 image matrix. All PET images were corrected for decay but not for attenuation. Each image analysis was performed using ASIPRO software (Siemens). To characterize the accumulation of the radiotracer in the tumor, region-of-interest (ROI) analysis was performed manually by visualizing the tumor site as it appeared as bumps under the skin to identify the radioactivity originating in the tumor region.

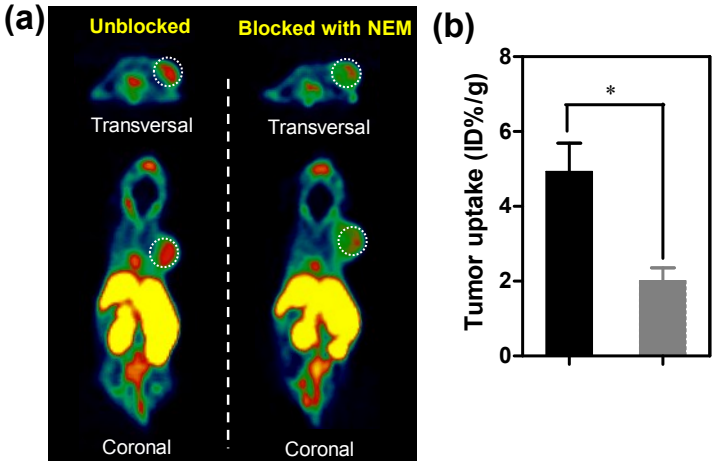

Fig. S40. PET imaging of nude mice bearing U87MG tumor at 10-15 min post injection of **1** alone (left) and **1** with thiol inhibitor NEM (right), respectively.

Cerenkov luminescence imaging (CLI)

Probe **1** (~100 μCi, in 100 μL physiological saline) was intramuscularly injected into the hind legs of the mice. After injection, the mice were anesthetized with isoflurane (2%). Then cerenkov luminescence imaging (CLI) was performed using IVIS Spectrum. CLI signal was expressed in units of photons per cm ^{2} per second per steradian ($P \cdot cm^{-2} \cdot s^{-1} \cdot sr^{-1}$).

Biodistribution studies

The probe **1** (~150 μCi, 150 uL) was injected via the tail vein of anesthetized U87MG tumor-bearing nude mice (n = 3 per group). At the time point of 10, 30, and 60 min post injection of the radiotracer, animals were sacrificed respectively. Interested organs were collected and weighed, and 200 mL of blood was taken from the carotid artery. The radioactivity of each sample was measured using a γ counter to determine the percentage of radioactivity incorporated into each tissue. Predefined standard (150 μL) was diluted and also counted for data normalization to injected dose. Data were expressed as the percent injected dose per gram of tissue (%ID/g) in Fig. S41.

To further detect the formation of radioactive **1-Dimer** in vivo, the tumor tissue at 30 min post injection of **1** was homogenized by an electronic homogenate machine in RIPA lysis buffer (3 mL). Then, DMSO (3 mL) was added to the mixture and homogenized once again. The supernatant was obtained by centrifugation at 14,000 ×g for 5 min and analyzed by radio-HPLC. The formation of cyclized products at 30 min post injection of **1** in U87MG tumor was demonstrated on the basis of radio-HPLC analysis (Fig. S42).

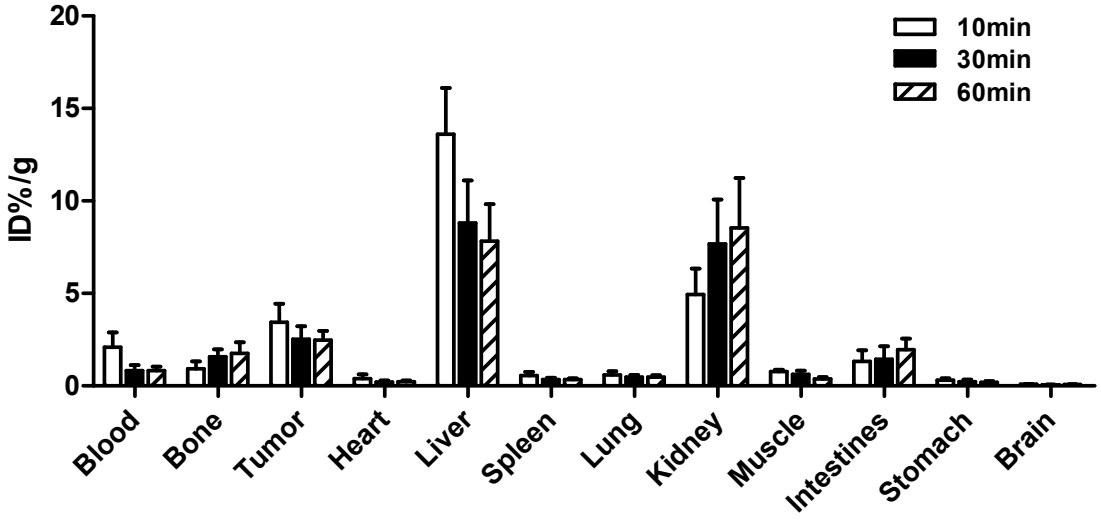

Fig. S41. Biodistribution of probe **1** in U87MG tumor-bearing mice at different time points (mean ±SD, n = 3, %ID/g).

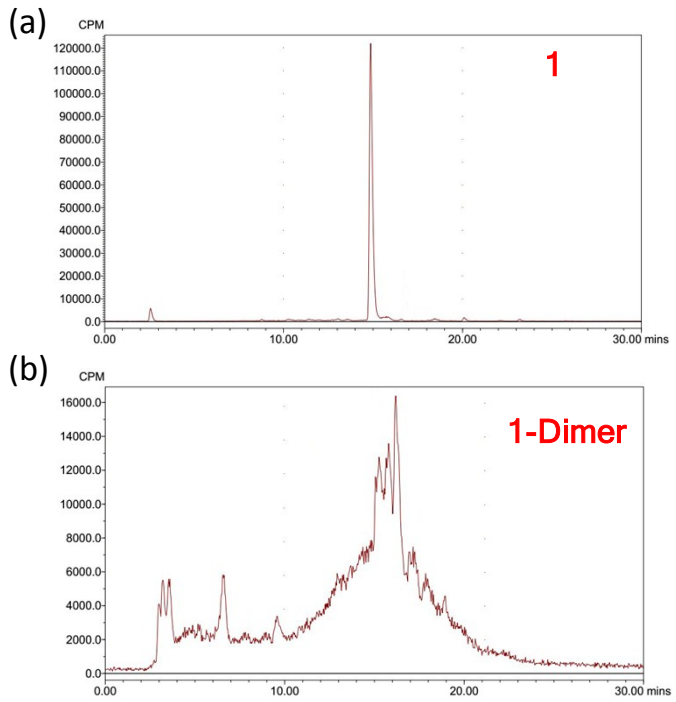

Fig. S42. Radio-HPLC traces of the radiotracer 1 before injection (a) and the cyclized products formed at 30 min post injection of 1 in U87MG tumor (b).

Determination of GSH in different organs of tumor-bearing mice

Total glutathione (GSH) content was determined with GSH and GSSG assay kit (Beyotime Biotech Inc., Nantong, China) according to the manufacturer's protocol. Briefly, the tissues of liver, tumor and muscle (~50 mg) were homogenized by an electronic homogenate machine in 0.5 mL of solution provided by the kit, respectively. The samples (~50 mg) were suspended in 0.15 mL of solution provided by the kit, frozen in the liquid nitrogen and then thawed at a water bath (37 ºC) for two times. The supernatants obtained from the cell or tissue homogenization by centrifugation at 10,000 ×g for 10 min at 4 ºC were used for the determination of total GSH content according to the manufacturer's instruction.

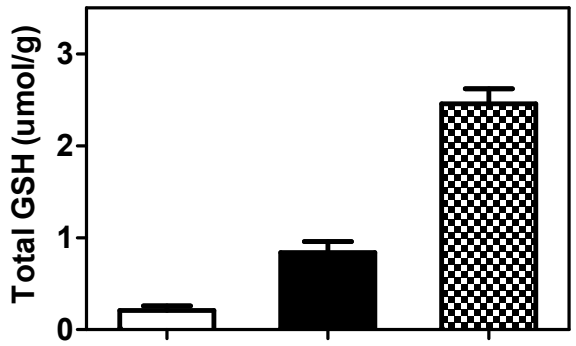

Fig. S43. Total glutathione (GSH) content in different organs of tumor-bearing mice.

HPLC conditions for the analysis and purification.

Table S1. Analytical HPLC condition for all the compounds.

Time (minute)	Flow (mL/min.)	H_2O(0.1% TFA)%	CH_3CN(0.1 %TFA)%
0	1.0	80	20
3	1.0	80	20
35	1.0	10	90
40	1.0	80	20

Table S2. HPLC condition for the purification of compound **B**.

Time (minute)	Flow (mL/min.)	H_2O(0.1% TFA)%	CH_3CN(0.1% TFA)%
0	3.0	80	20
3	3.0	80	20
10	3.0	75	25
20	3.0	55	45
25	3.0	30	70
35	3.0	80	20

Table S3. HPLC condition for the purification of compound **D**.

Time (minute)	Flow (ml/min.)	H_2O(0.1% TFA)%	CH_3CN(0.1% TFA)%
0	3.0	80	20
3	3.0	80	20
15	3.0	56	44
20	3.0	56	44
25	3.0	52	48
30	3.0	52	48
35	3.0	10	90
40	3.0	80	20

Table S4. HPLC condition for the purification of **1-Cold**.

Time (minute)	Flow (mL/min.)	H_2O(0.1% TFA)%	CH_3CN(0.1% TFA)%
0	3.0	80	20
3	3.0	80	20
10	3.0	64	36
15	3.0	64	36
20	3.0	63	37
25	3.0	63	37
30	3.0	30	70
35	3.0	80	20

References

[1]. Angew. Chem. Int. Ed. 2013, 52, 10511-10514.

[2]. Angew. Chem. Int. Ed. 2014, 53, 11876-11880.