Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Rational Design of Biotin-Disulfide-Coumarin Conjugates: A Cancer Targeted Thiol Probe and Bioimaging

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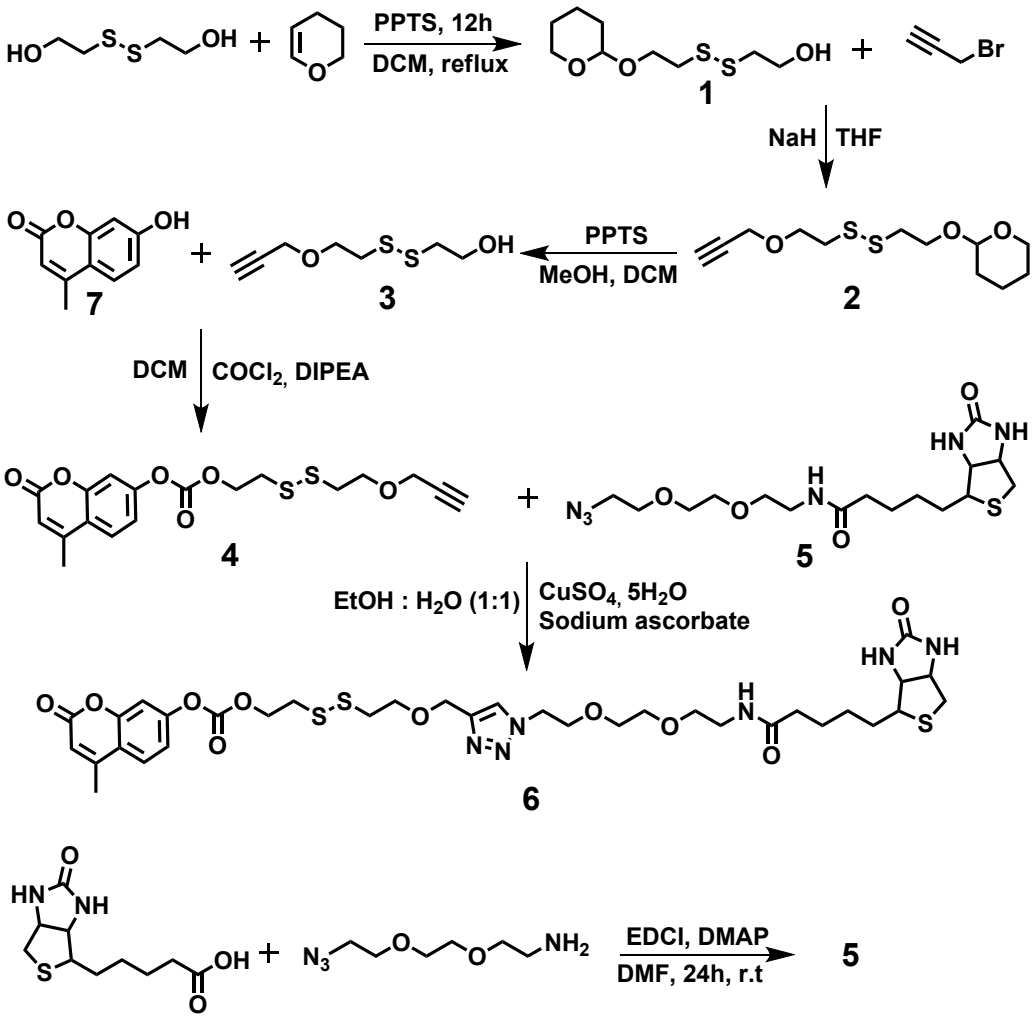
Contents

Synthetic Materials and Methods: Chemicals used in this project were purchased from Aldrich, Alfa-Aesar, Carbosynth, TCI, and Ducsan without further purification. Silica gel 60 (Merck, 0.063-0.2 mm) was used for column chromatography. Analytical TLC was performed using Merck 60 F254 silica gel (precoated sheets, 0.25 mm thick). ^{1}H and ^{13}C NMR spectra were collected in $CDCl_3$, DMSO (Cambridge Isotope Laboratories, Cambridge, MA) on Varian 300 and 400 MHz spectrometers. All chemical shifts are reported in ppm value using the peak of residual proton signals of TMS as an internal reference. The mass spectra were obtained on an Ion Spec Hi-Res mass spectrometer.

Synthesis of Compounds:



Scheme 1. Synthetic routes of **1** to **6**.

Synthesis of Compound 1: The 2, 2'-dithiodiethanol (5 g, 0.1 mol), 3, 4-dihydropyran (1.36 g, 0.05 mol) and pyridinium *p*-toluenesulfonate (PPTS, 33 mg) were dissolved in dry CH_2Cl_2 (60 mL). The reaction mixture was heated under reflux for 12h in an N_2 atmosphere. After cooling down to room temperature, the solvent was removed under vacuum and the resulting mixture was purified by column chromatography using EtOAc / Hexane (2:8) as eluent. Product **1** was isolated as a colourless oil (2.5g, yield, 32.28%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.67–4.64 (m, 1H), 4.08–3.93 (m, 2H), 3.89–3.84 (m, 2H), 3.72–3.64 (m, 1H), 3.54–3.46 (m, 1H), 2.94–2.83 (m, 4H), 2.42 (t, *J* = 6.3 Hz, 1H) 1.88–1.79 (m, 1H), 1.77–1.70 (m, 1H), 1.64–1.50 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz): 99.3, 66.2, 62.6, 60.4, 41.7, 39.0, 30.7, 25.6, 19.6.

Synthesis of Compound 2: The THP-protected alcohol **1** (2.5 g, 8.0 mol) and NaH (2.52 g, 0.08 mol) were suspended in dry THF (300 mL) and the suspension was stirred at 0℃ for 1h. Propargyl bromide (6.21 g, 0.04 mol) was added drop wise (as an 80% wt solution in toluene) and the reaction mixture was left to stir at room temperature overnight. After quenching with MeOH, CH_2Cl_2 (300 mL) was added to the reaction mixture and the organic layers were washed with H_2O. The organic layer was then dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. The resulting mixture was purified by column chromatography using EtOAc / Hexane (1:9) as eluent. Product **2** was isolated as a yellow oil (1.1 g, yield, 37.98%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.64–4.62 (m, 1H), 4.19–4.18 (d, *J* = 2.5 Hz, 2H), 4.01–3.96 (m, 1H), 3.92–3.86 (m, 3H), 3.81 (t, *J* = 6.6Hz, 2H), 2.97–2.89 (m, 4H), 2.46 (t, *J* = 2.5 Hz, 1H), 1.86–1.74 (m, 1H), 1.71–1.66 (m, 1H), 1.64–1.52 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz): 99.2, 79.6, 75.0, 68.3, 66.1, 62.5, 58.4, 39.2, 38.6, 30.8, 25.6, 19.6.

Synthesis of Compound 3: The compound **2** (1.1 g, 2.4 mol) and PPTS (126 mg) were dissolved in CH_2Cl_2 and MeOH (1:1, 400 mL). The reaction mixture was heated under reflux overnight in an N_2 atmosphere. After cooling down to the room temperature, the solvent was removed under vacuum and the resulting mixture was purified by column chromatography using EtOAc / Hexane (2:8) as eluent. The product **3** was isolated as a light yellow oil (0.5 g, yield, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.17 (d, *J* = 2.4 Hz, 2H), 3.85 (t, *J* = 5.9 Hz, 2H), 3.77 (t, *J* = 6.4 Hz, 2H), 2.89 (t, *J* = 6.4 Hz, 2H), 2.85 (t, *J* = 5.9 Hz, 2H), 2.46 (t, *J* = 2.4 Hz, 1H), 1.99 (t, *J* =5.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100MHz): 79.5, 75.1, 68.3, 60.5, 58.5, 41.6, 38.6.

Synthesis of Compound 4: Compound **3** (0.5 g, 1.30 mmol) was dissolved in distilled DCM (30 mL) in round bottom flask and placed in an ice path under nitrogen gas. Phosgene in toluene (2.00 mL) was added with a syringe. *N, N*-Diisopropylethylamine (1.13 mL, 6.50 mmol) was slowly added with a syringe. After 50 minutes of stirring, the mixture was purged with argon for 30 minutes to eradicate the remaining phosgene gas. 7-Hydroxy-4-methylcoumarin (137 mg, 0.78 mmol) dissolved in 5 mL of distilled DCM with few drops DMF was added to the mixture. The mixture was stirred overnight. The mixture was evaporated and vacuumed. 100 mL of distilled water and 100 mL of ethyl acetate was added, where the organic part was extracted. After removing the solvent, the product was purified by column chromatography using EtOAc / Hexane (2:8) as eluent. The product **4** was isolated as yellow oil (0.5 g, yield, 49%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.61 (d, *J* = 8.6Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.17 (dd, J_1 = 2.4 Hz J_2 = 2.2 Hz, 1H), 6.28 (s, 1H), 4.54 (t, *J* = 6.7 Hz, 2H), 4.21 (t, *J* = 2.7 Hz, 2H), 3.81 (t, *J* =

6.3 Hz, 2H), 3.05 (t, *J* = 6.5 Hz, 2H), 2.96 (t, *J* = 6.42 Hz, 2H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 100MHz): 160.6, 154.3, 153.3, 152.9, 152.0, 125.7, 118.3, 117.6, 114.9, 110.2, 79.5, 75.1, 68.2, 67.0, 58.5, 38.9, 37.0, 19.0. ESI – MS: calcd for $C_{18}H_{18}O_6S_2$, 394.46; found: 394.0.

Synthesis of Compound 5: D-Biotin (463 mg, 1.89 mmol) in 5.0 mL DMF were added EDCI (294.08 mg, 1.89 mmol), DMAP (105.20 mg, 0.86 mmol) at rt. Then 2-(2-(2-azidoethoxy)ethoxy)ethanamine (300 mg, 1.72 mmol) was added and continued to stirr for 6h. After completion of reaction, the reaction mixture was diluted with water then extarcted with EA. The organic layer was dried over sodium sulfate. The crude product was passed through silica column chromatography using DCM/MeOH (9:1) as eluent to afford 300 mg (45%) of **5**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.50–4.45 (m, 1H), 4.30–4.27 (m, 1H), 3.74–3.64 (m, 4H), 3.60–3.53 (m, 2H), 3.45–3.34 (m, 2H), 3.22–3.20 (m, 1H), 2.95–2.90 (m, 2H), 2.78–2.69 (m, 2H), 2.26–2.20 (m, 2H), 1.74–1.58 (m, 2H), 1.50–1.40 (m, 2H), 1.30–1.21 (m, 2H). ^{13}C NMR ($CDCl_3$, 100MHz): 172.9, 163.4, 70.3, 70.2, 69.9, 69.8, 61.7, 59.9, 56.1, 50.6, 40.7, 39.1, 35.8, 28.9, 28.7, 25.9. ESI – MS: calcd for $C_{16}H_{28}N_6O_4S$, 400.19; found: 423.25.

Synthesis of Compound 6: To a EtOH : MeOH (2 : 1, 3.0 mL) solution of **4** (40 mg, 0.101 mmol), were added **5** (36 mg, 0.115 mmol) and sodium ascorbate (20 mol%). The reaction mixture as degassed for 15 min by purging argon gas. Then 1.26 mg (5 mol %) of $CuSO_4$, $5H_2O$ in 0.5 mL water was added to the reaction mixture. The reaction was continued for 3h. Then the crude reaction mixture was directly passed through silica column chromatography using DCM/MeOH (9:1) as eluent to afford 30 mg (38%) of **6**. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.61 (d, *J* = 9.48 Hz, 1H), 7.22–7.21 (m, 1H), 7.17 (d, *J* = 2.46 Hz, 1H), 7.14 (d, *J* = 2.03 Hz, 1H), 6.51 (s, 1H), 4.66 (s, 2H), 4.54 (t, *J* = 5.10 Hz, 2H), 4.35 (t, *J* = 6.99 Hz, 2H), 3.87 (t, *J* = 4.58 Hz, 2H), 3.77 (s, 4H), 3.59–3.53 (m, 4H), 3.50 (t, J = 4.78 Hz, 2H), 3.40 (t, J = 4.58 Hz, 2H), 3.15-3.10 (m, 1H), 3.00 (t, J = 6.21 Hz, 2H), 2.94-2.89 (m, 2H), 2.43 (s, 3H), 2.19 (t, *J* = 6.58 Hz, 2H), 2.02 (t, *J* = 6.58 Hz, 2H), 1.79 (s, 2H), 1.65 (t, *J* = 7.79 Hz, 2H), 1.41 (t, *J* = 7.37 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100MHz): 173.5, 163.8, 155.7, 154.3, 153.3, 152.9, 152.1, 125.8, 124.2, 124.1, 118.3, 117.6, 114.9, 110.2, 70.6, 70.2, 69.5, 68.7, 67.0, 65.9, 63.6, 62.0, 66.3, 55.7, 55.2, 50.4, 40.8, 39.4, 38.8, 37.1, 28.2, 25.8, 19.0. ESI–MS: calcd for $C_{34}H_{46}N_6O_{10}S_3$, 794.24; found: 795.40.

Spectroscopic Materials and Methods. Stock solutions of biologically relevant analytes [thiols, Val, Tyr, Thr, Tau, Ser, Pro, Phe, Met, Lys, Leu, Ile, His, Gly, Gluc, Glu, Gln, Asp, Asn, Arg, Ala, Trp, Zn(II), Na(I), Mg(II), K(I), Fe(III), Fe(II), Cu(II) and Ca(II)] were prepared in triple-distilled water. Stock solutions of **6** were also prepared in triple-distilled water. All spectroscopic measurements were performed under physiological conditions (PBS buffer containing 5% of DMSO, pH 7.4, 37°C). Absorption spectra were recorded on a S-3100 (Scinco) spectrophotometer, and fluorescence spectra were recorded using an RF-5301 PC spectrofluorometer (Shimadzu) equipped with a xenon lamp. Samples for absorption and emission measurements were contained in quartz cuvettes (3 mL volume). Excitation was provided at 325 nm with excitation and emission slit widths of 1.5 and 3 nm, respectively.

Two-Photon Fluorescence Microscopy. Two-photon fluorescence microscopy images of labeled cells and tissues were obtained with spectral confocal and multiphoton microscopes (Leica TCS SP2) with ×10, ×40 dry and ×100 oil objectives, numerical aperture (NA) = 0.30, 0.75 and 1.30. The two-photon fluorescence microscopy images were obtained with a DM IRE2 Microscope (Leica) by exciting the probes with a mode-locked titanium-sapphire laser source (Coherent Chameleon, 90 MHz, 200 fs) set at wavelength 740 nm and output power 1260 mW, which

corresponded to approximately 5 mW average power in the focal plane. To obtain images at 450−600 nm range, internal PMTs were used to collect the signals in an 8 bit unsigned 512 × 512 and 1024 × 1024 pixels at 800 and 400 Hz scan speed, respectively.

Preparation of Cell Culture. Human lung carcinoma epithelial cell (KCLB, Seoul, Korea) were cultured in RPMI (WelGene Inc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL). Two days before imaging, the cells were passed and plated on glass-bottomed dishes (MatTek). All the cells were maintained in a humidified atmosphere of 5/95 (v/v) of CO_2/air at 37 °C. For labeling, the growth medium was removed and replaced with RPMI without FBS. The cells were treated and incubated with compound **6** at 37 °C under 5 % CO_2 for 20 min. The cells were washed three times with phosphate buffered saline (PBS; Gibco) and then imaged after further incubation in colorless serum–free media for 15 min. Fluorescence images were taken using a confocal laser scanning microscope (Zeiss LSM 510, Zeiss, Oberko, Germany).

Cell Viability. We used CCK-8 kit (Cell Counting Kit-8, Dojindo, Japan) for cell viability studies.

Additional absorption and fluorescence studies:

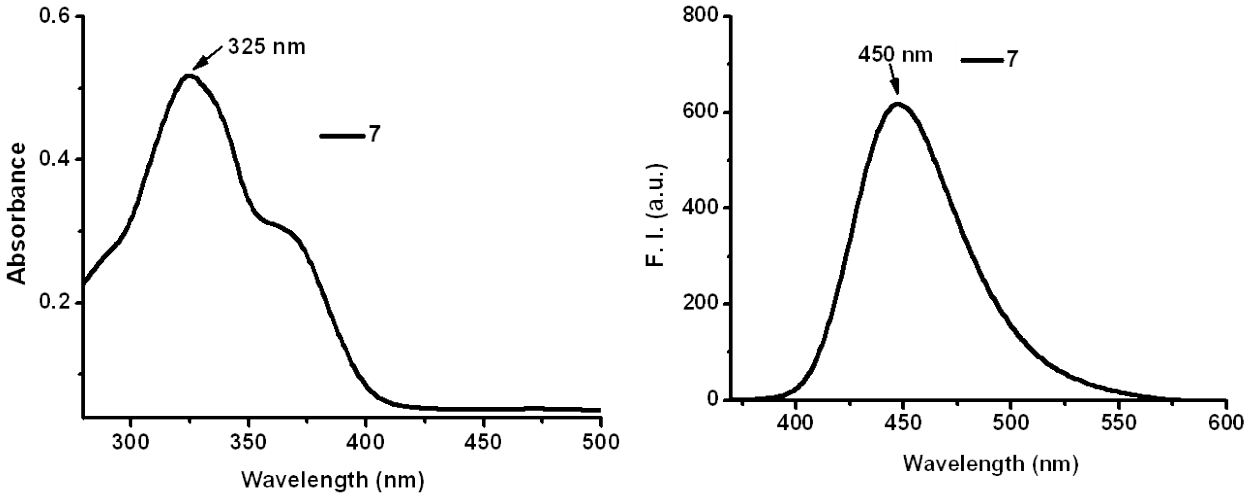


Figure S1. Absorption and Fluorescence spectra of **7** (5.0 μM). All spectra were recorded in 5% DMSO & 95% PBS buffer (pH 7.4) at 37 ℃.

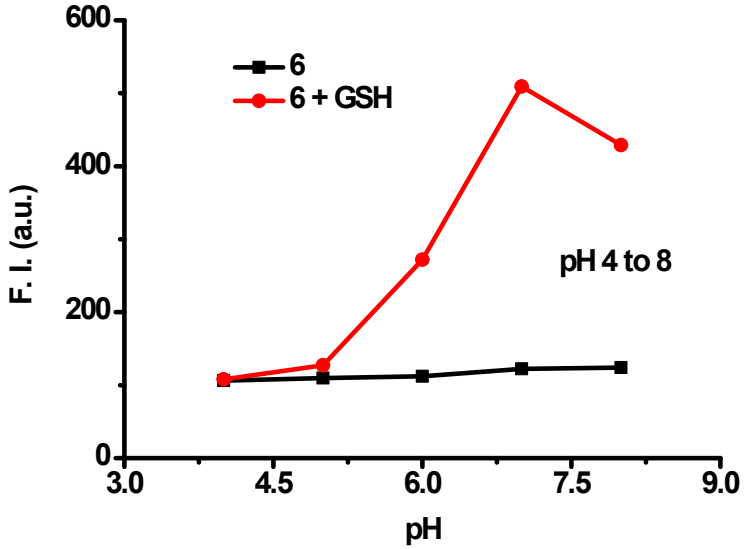


Figure S2. Fluorescence response of **6** (5.0 μM) with and without GSH (5.0 mM) as a function of pH. All spectra were recorded in 5% DMSO & 95% PBS buffer (pH 7.4) at 37 ˚C. Excitation was effected at 325 nm.

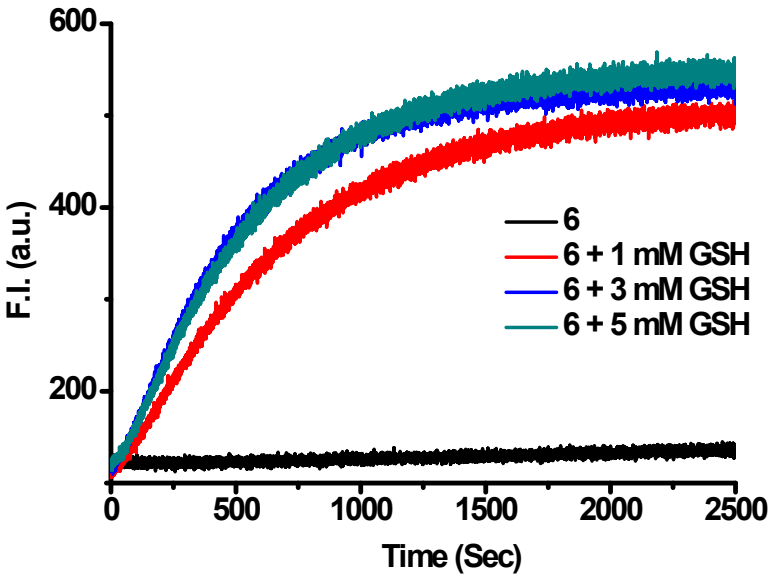


Figure S3. Temporal behavior of the fluorescence from the reaction of **6** (5.0 μM) with GSH (0.0 mM – 5.0 mM). Excitation was effected at 325 nm. All data were measured in PBS buffer (pH 7.4) containing 5% DMSO & 95% PBS buffer (pH 7.4) at 37 ℃.

Additional cell imaging data:

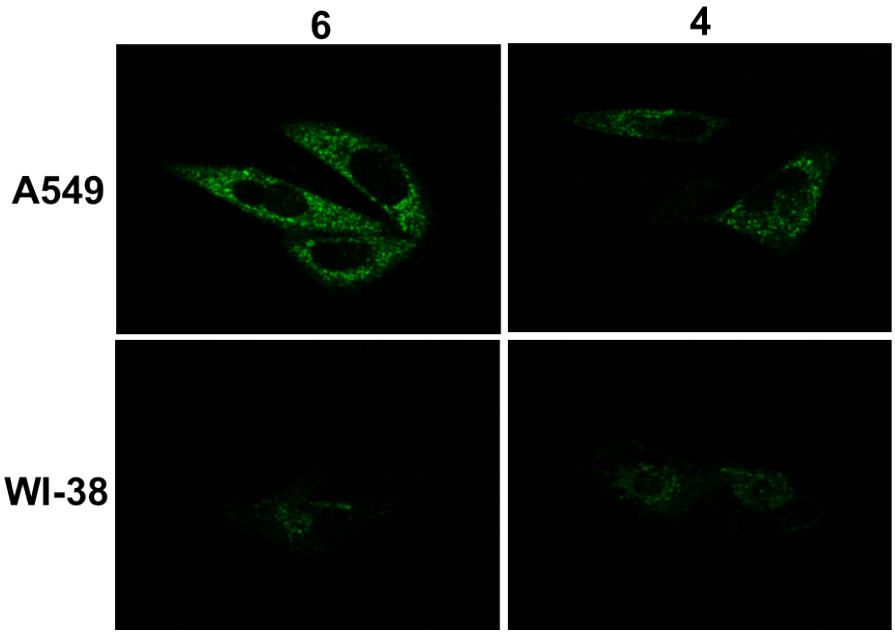


Figure S4. Confocal laser fluorescence microscopic images of A549 and WI-38 cells treated with **6** and **4** for 20 min at 37 ˚C. Cell images were obtained using two photon excitation wavelengths of 740 nm, and emission wavelengths of 400–550 nm, respectively.

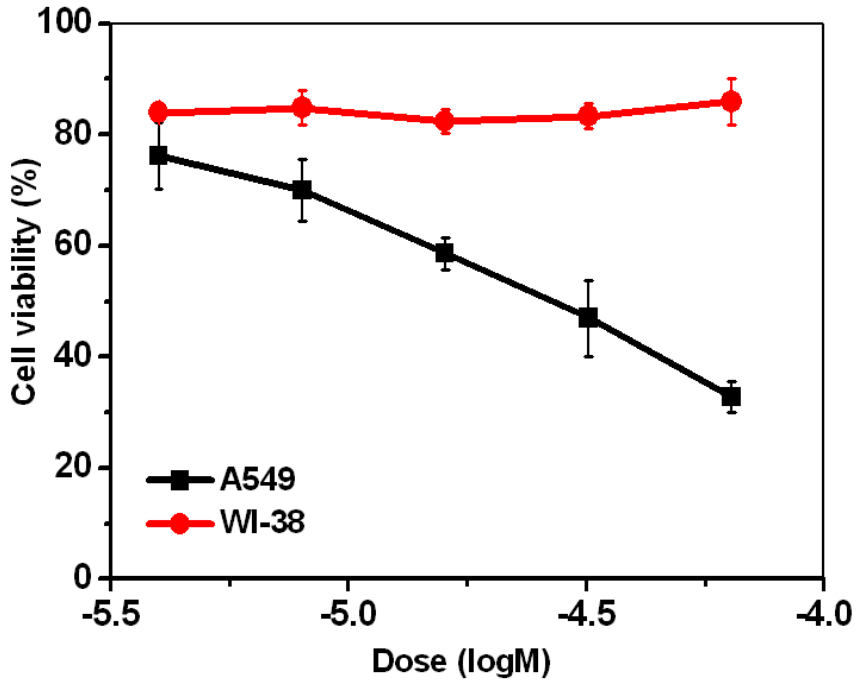


Figure S5. Cell viability data of **6** with A549 and WI-38 cell lines. The cell lines were treated with DMSO. The indicated doses of Ligand (4, 8, 16, 32, and 64 μM) for 1 days.

^{1}H–NMR, ^{13}C–NMR, and ESI–MS Spectra:

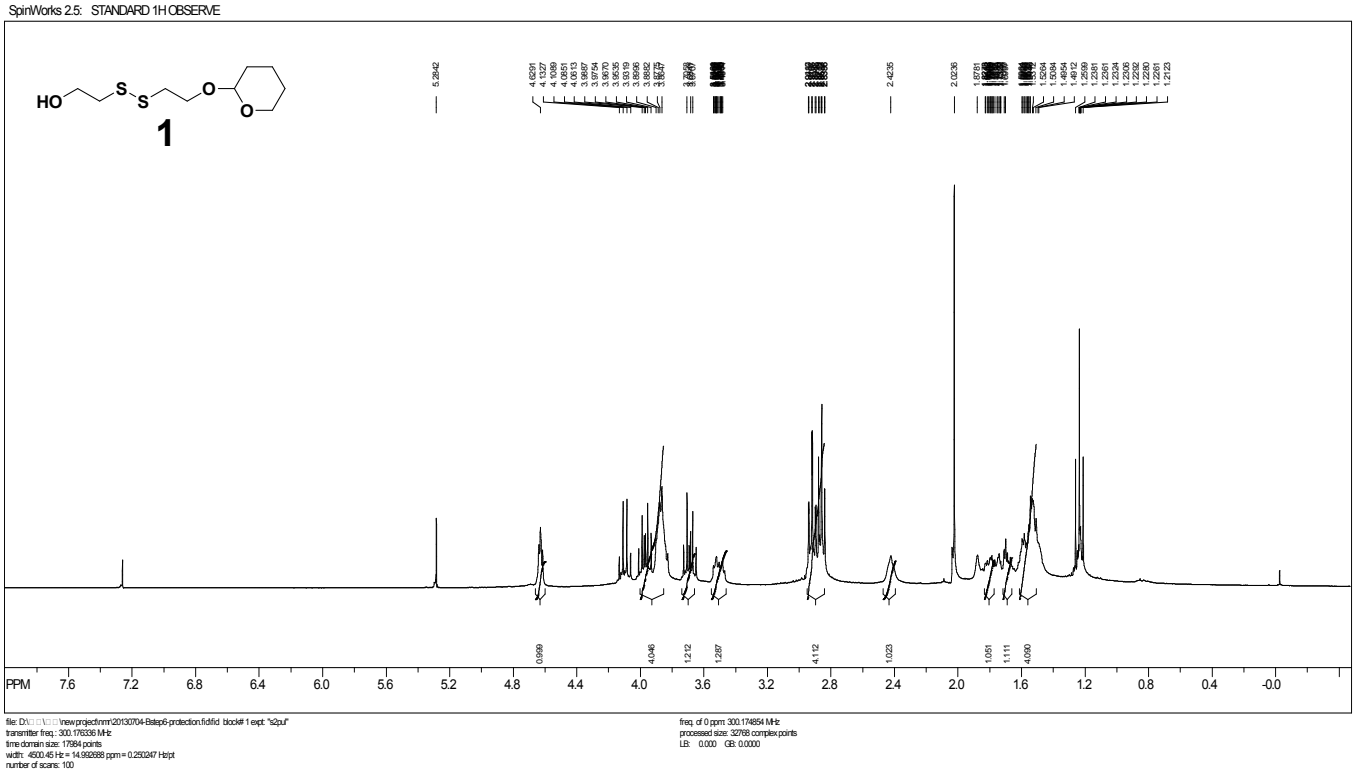


Figure S6. ^{1}H–NMR spectrum of **1** recorded in $CDCl_3$.

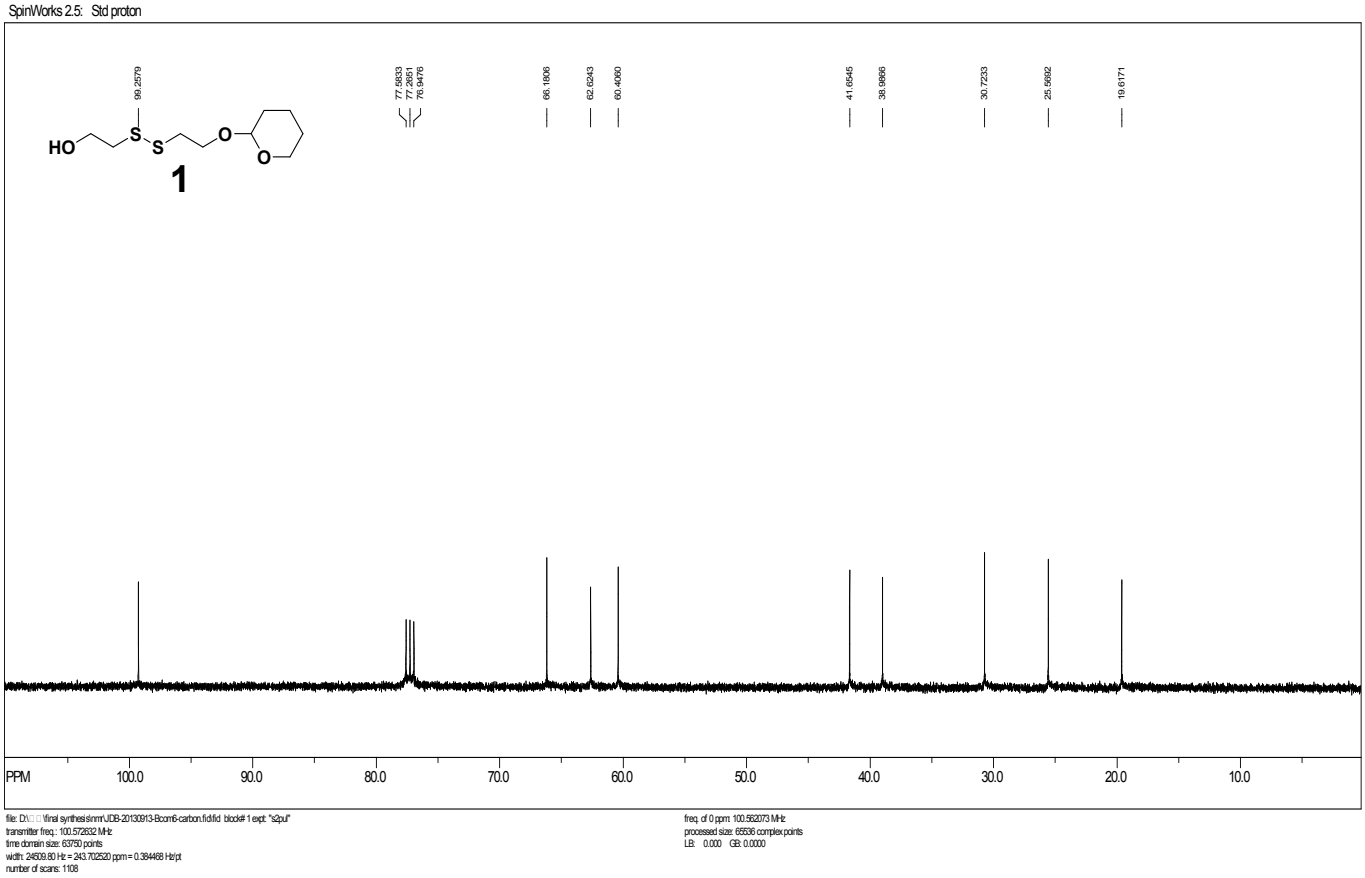


Figure S7. ^{13}C–NMR spectrum of **1** recorded in $CDCl_3$.

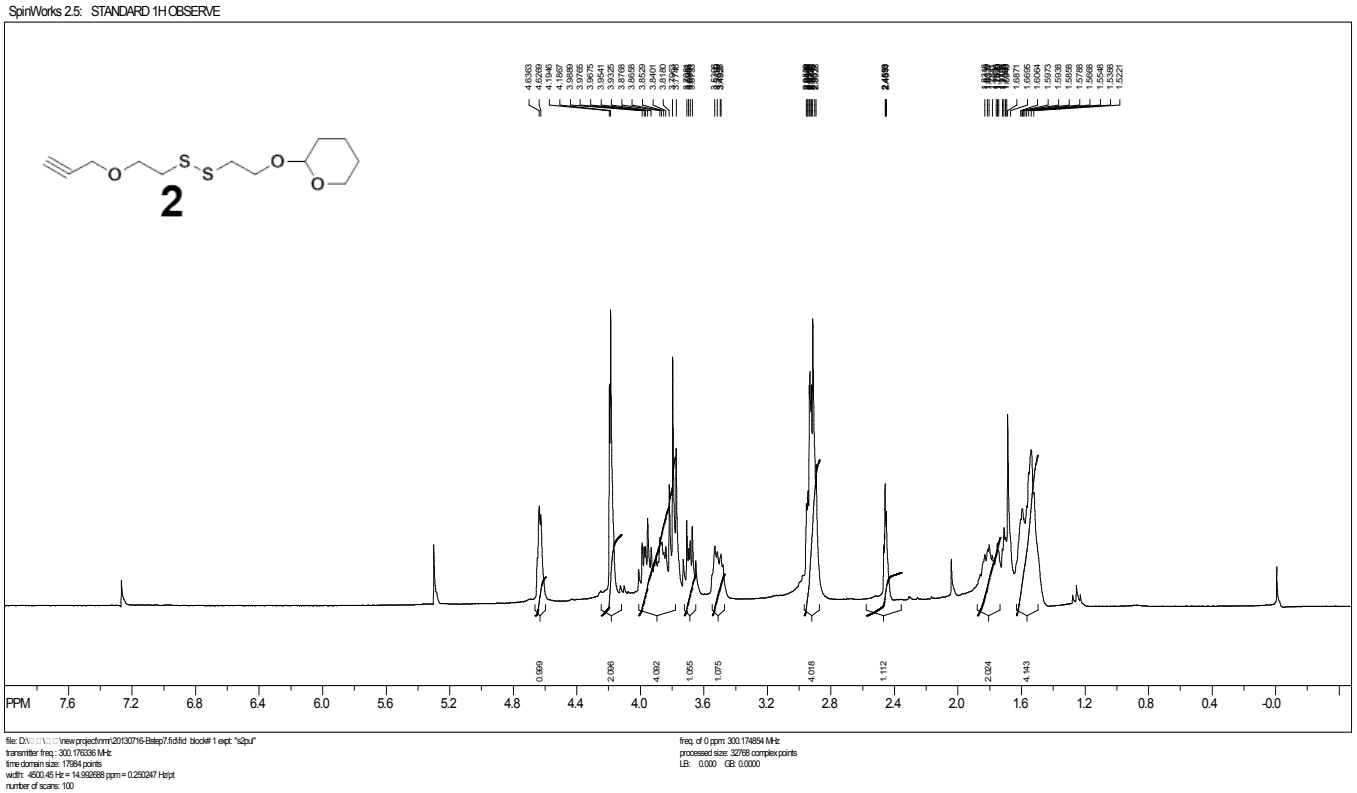


Figure S8. ^{1}H–NMR spectrum of **2** recorded in $CDCl_3$.

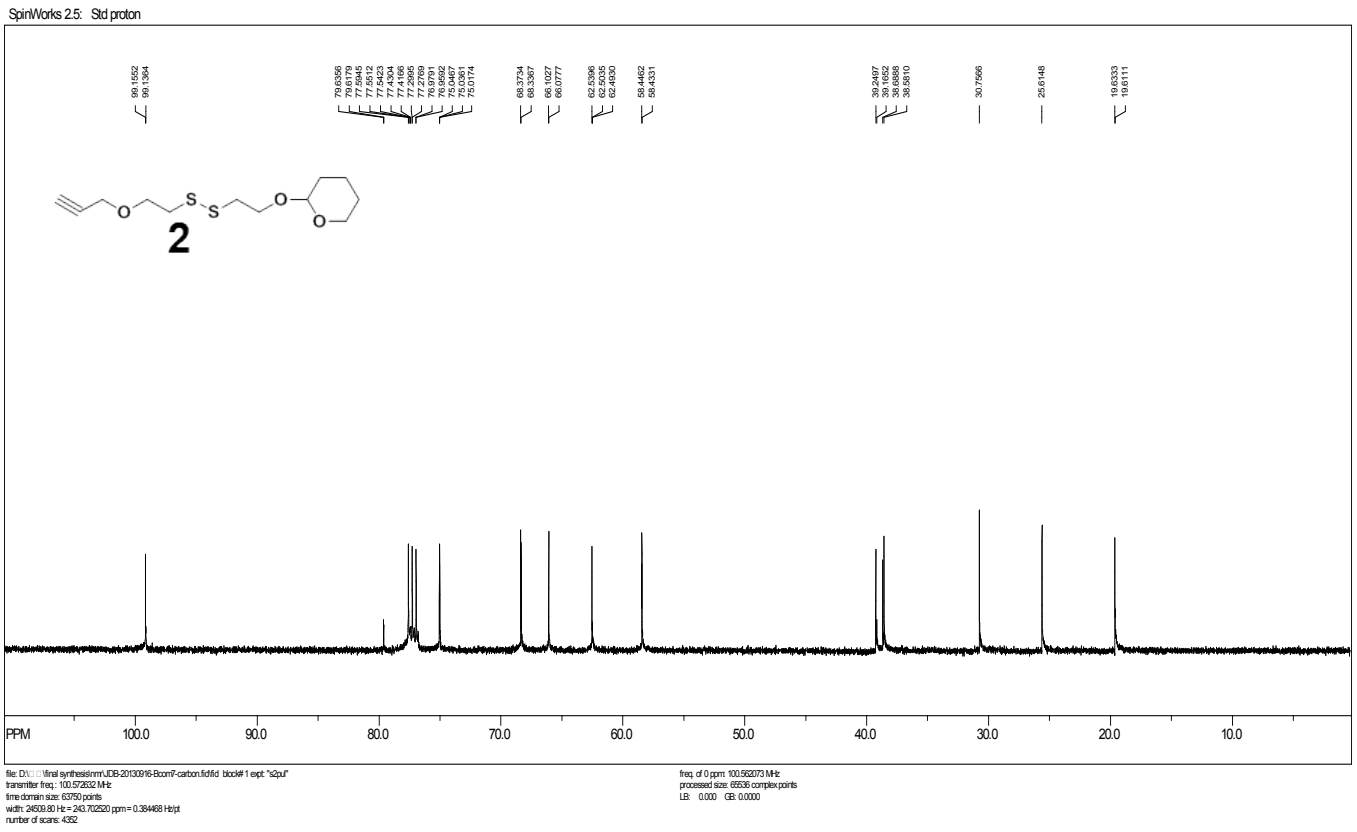


Figure S9. ^{13}C–NMR spectrum of **2** recorded in $CDCl_3$.

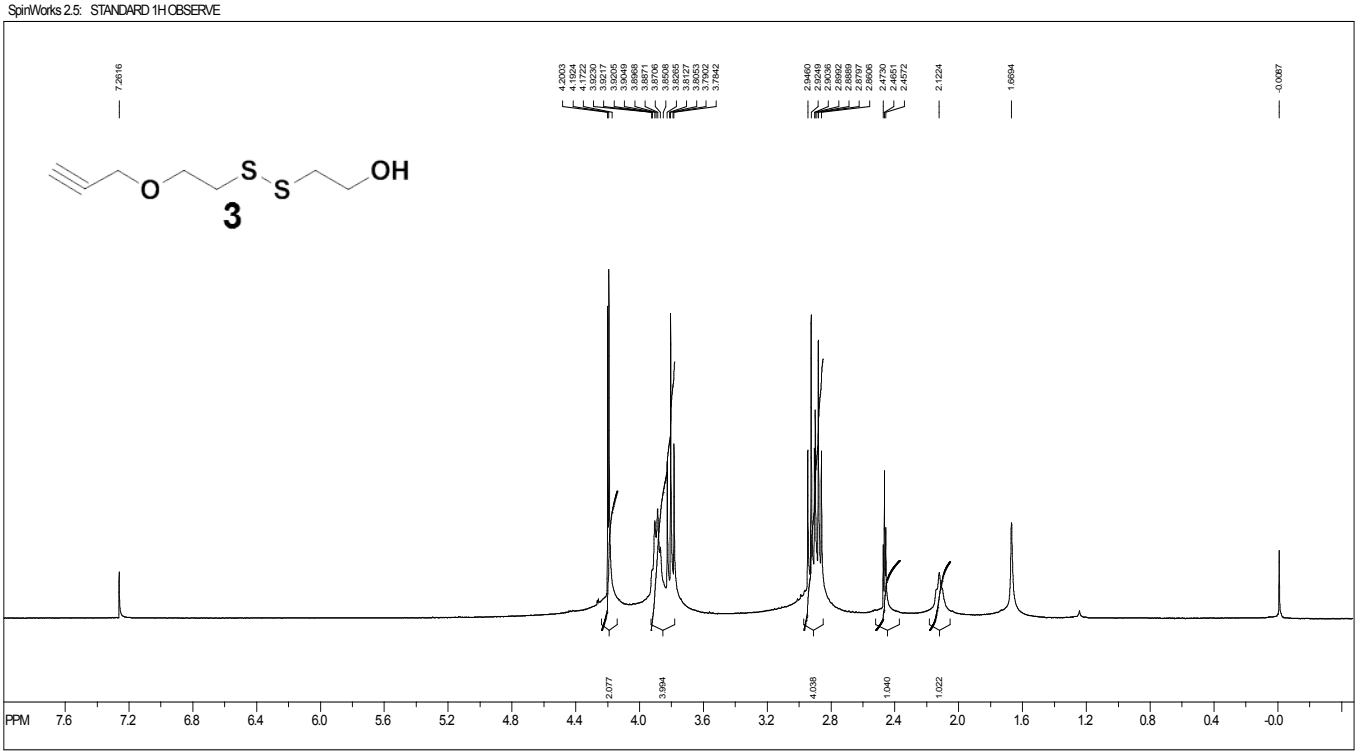


Figure S10. ^{1}H–NMR spectrum of **3** recorded in $CDCl_3$.

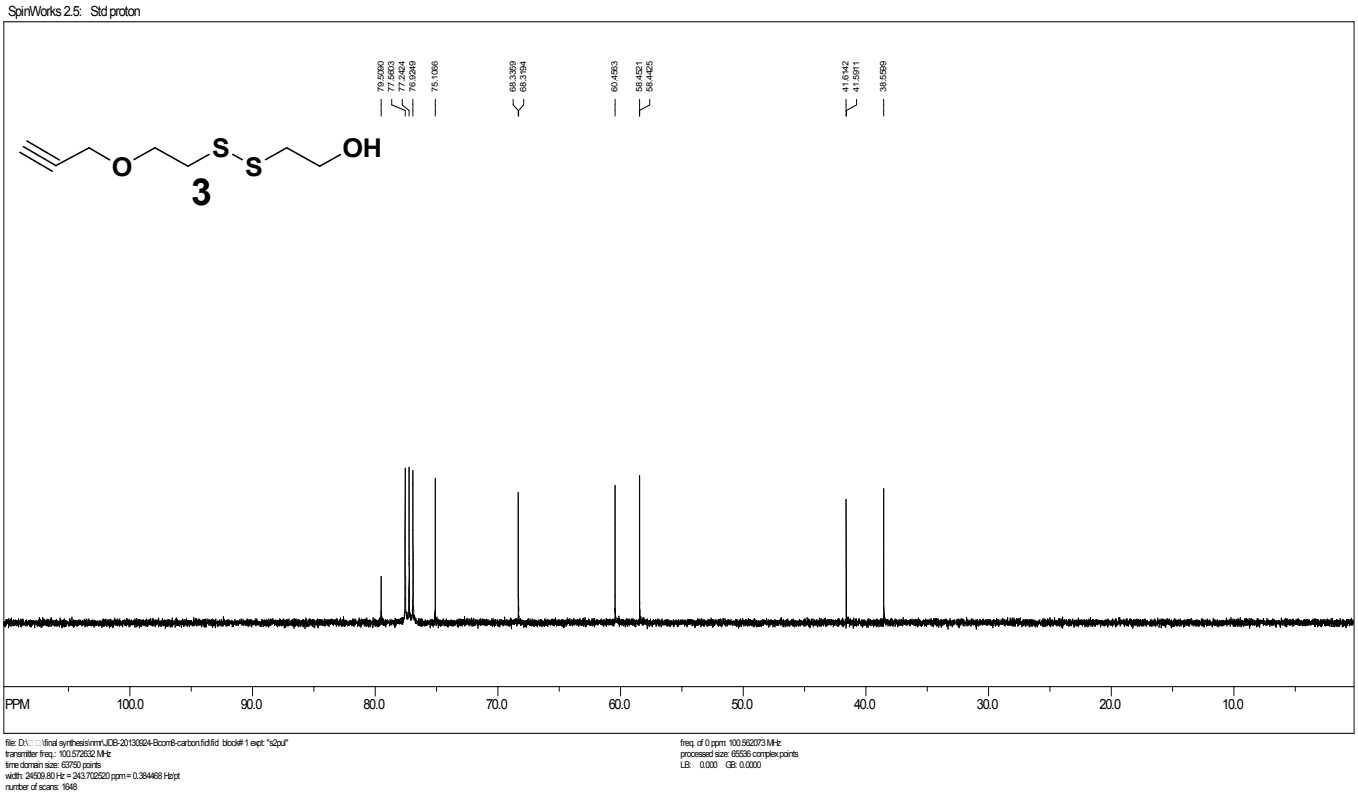


Figure S11. ^{13}C–NMR spectrum of **3** recorded in $CDCl_3$.

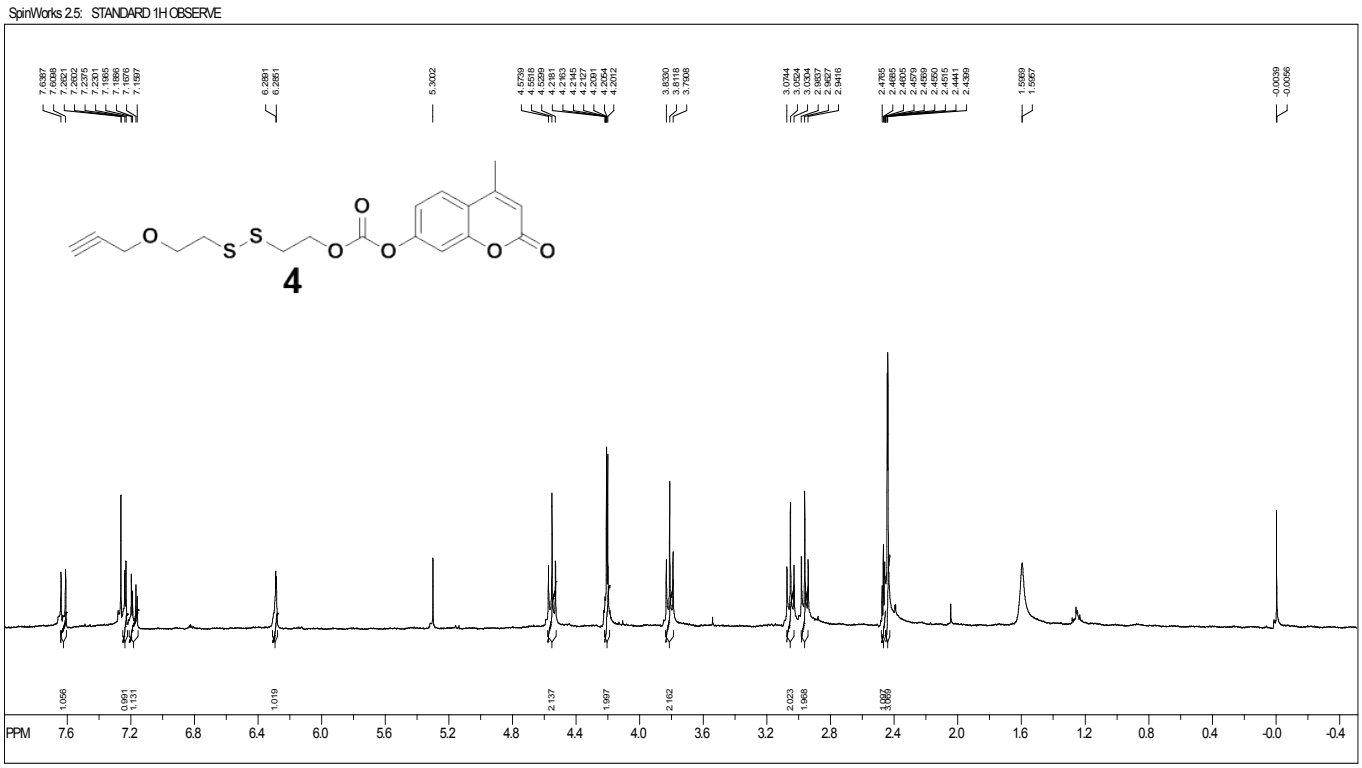


Figure S12. ^{1}H–NMR spectrum of **4** recorded in $CDCl_3$.

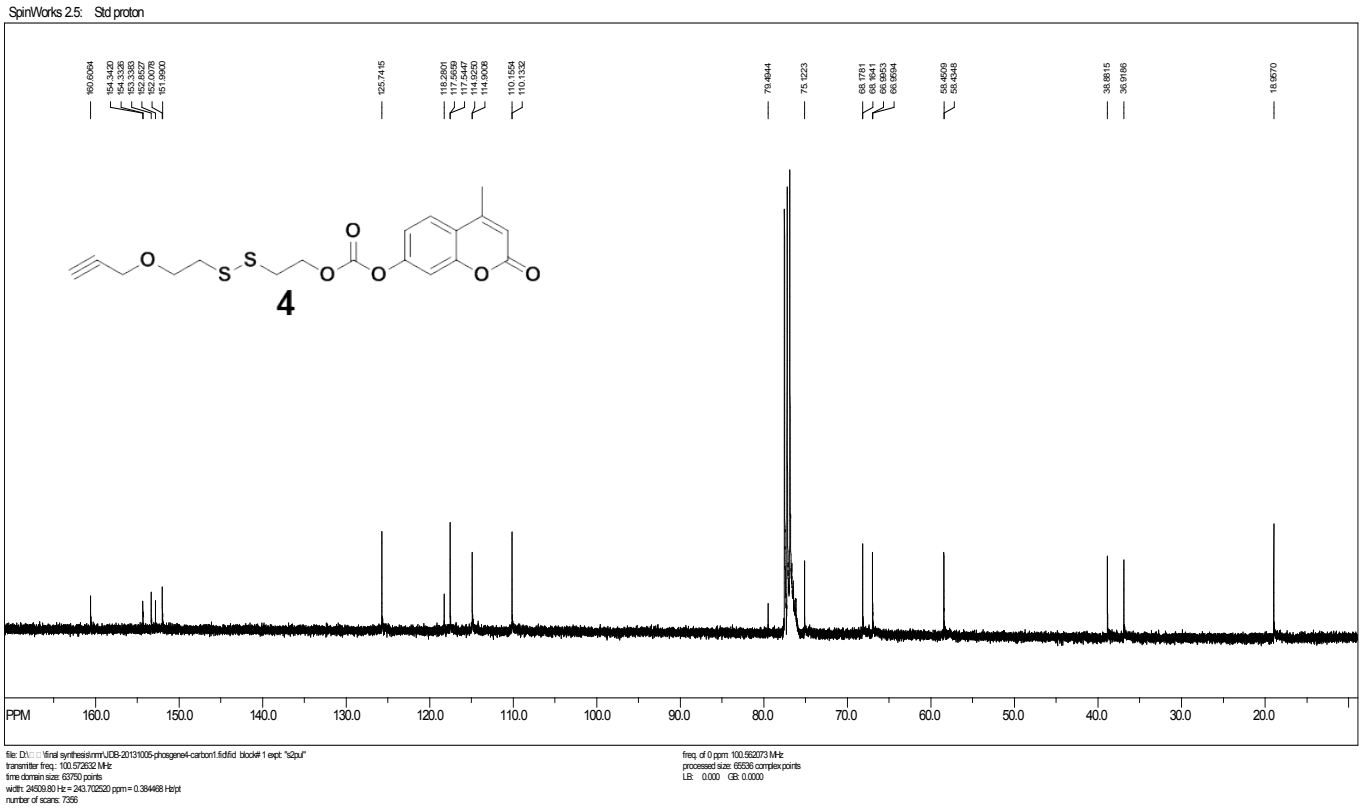


Figure S13. ^{13}C–NMR spectrum of **4** recorded in $CDCl_3$.

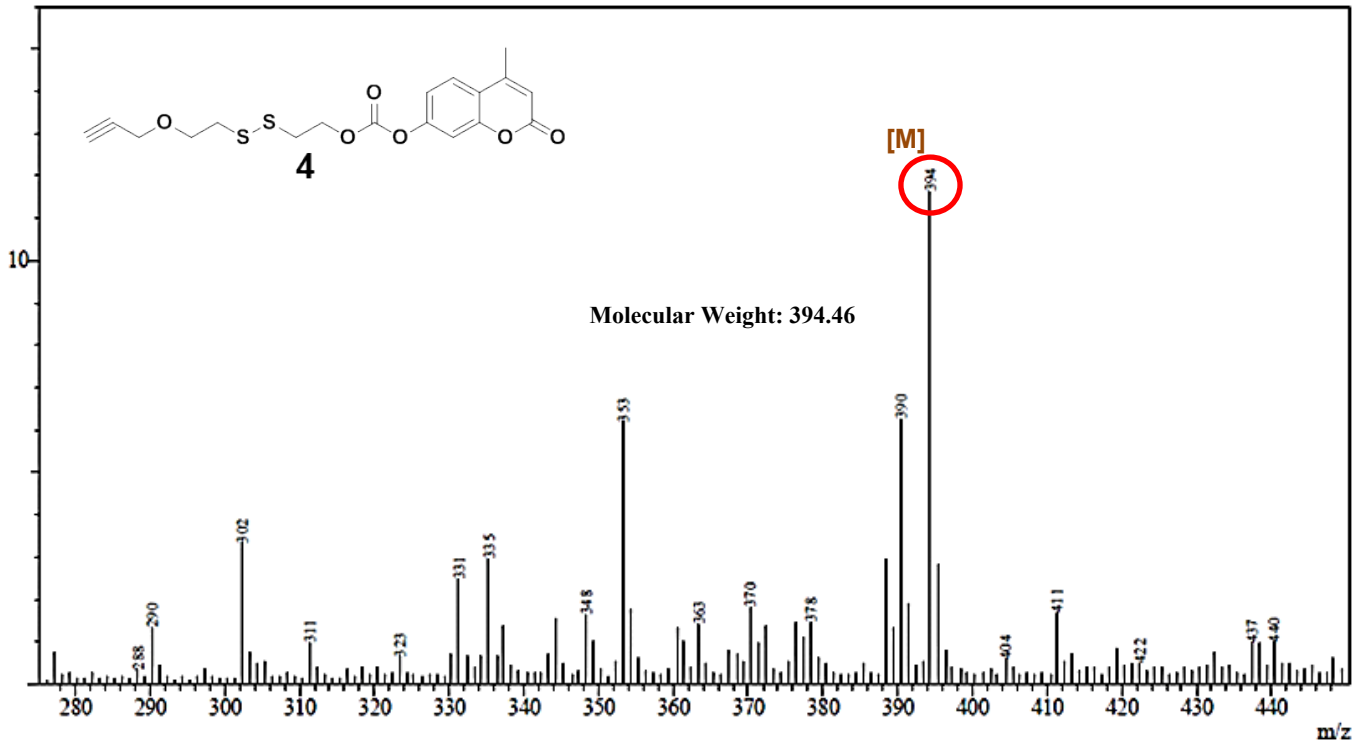


Figure S14. ESI–MS spectrum of **4**.

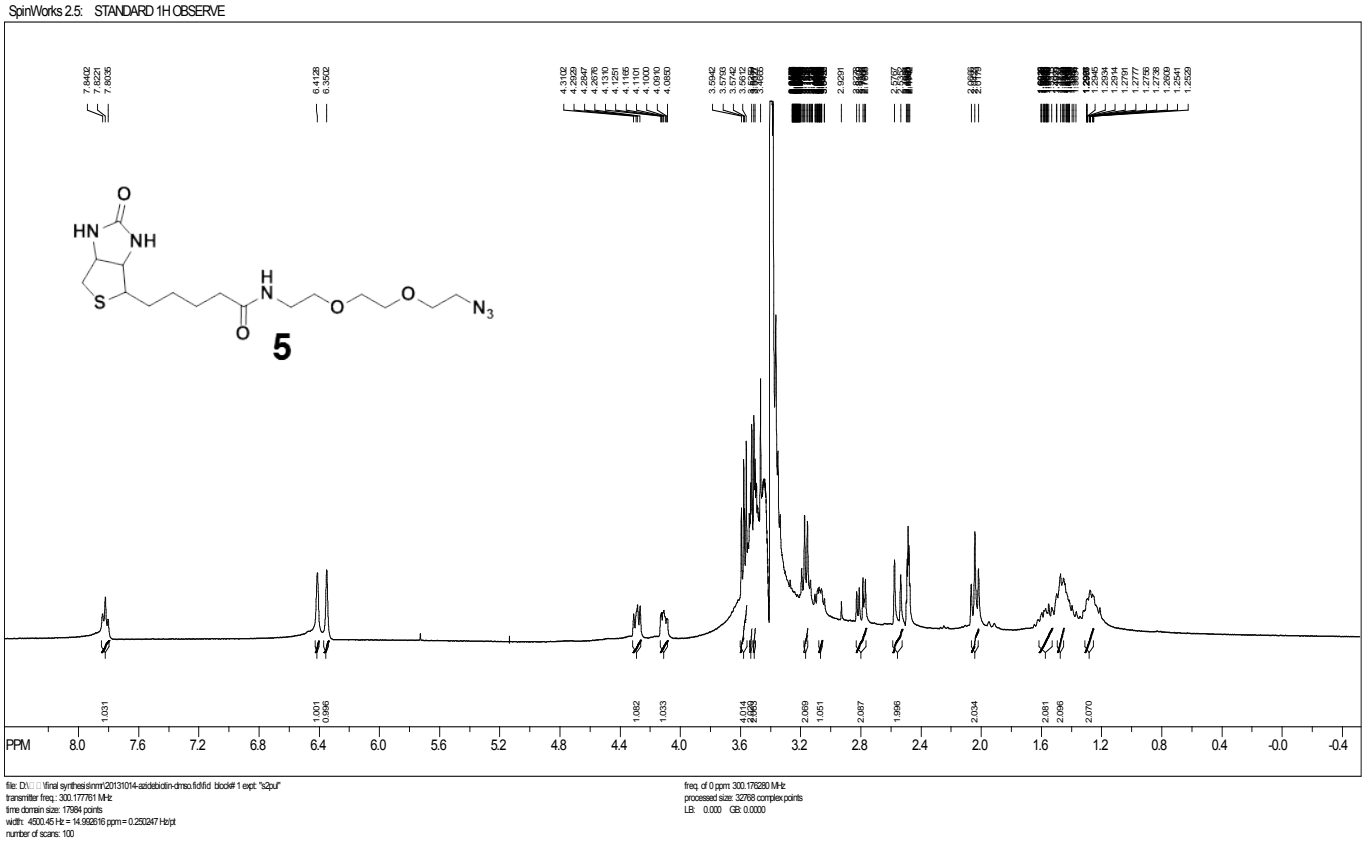


Figure S15. ^{1}H–NMR spectrum of **5** recorded in $CDCl_3$

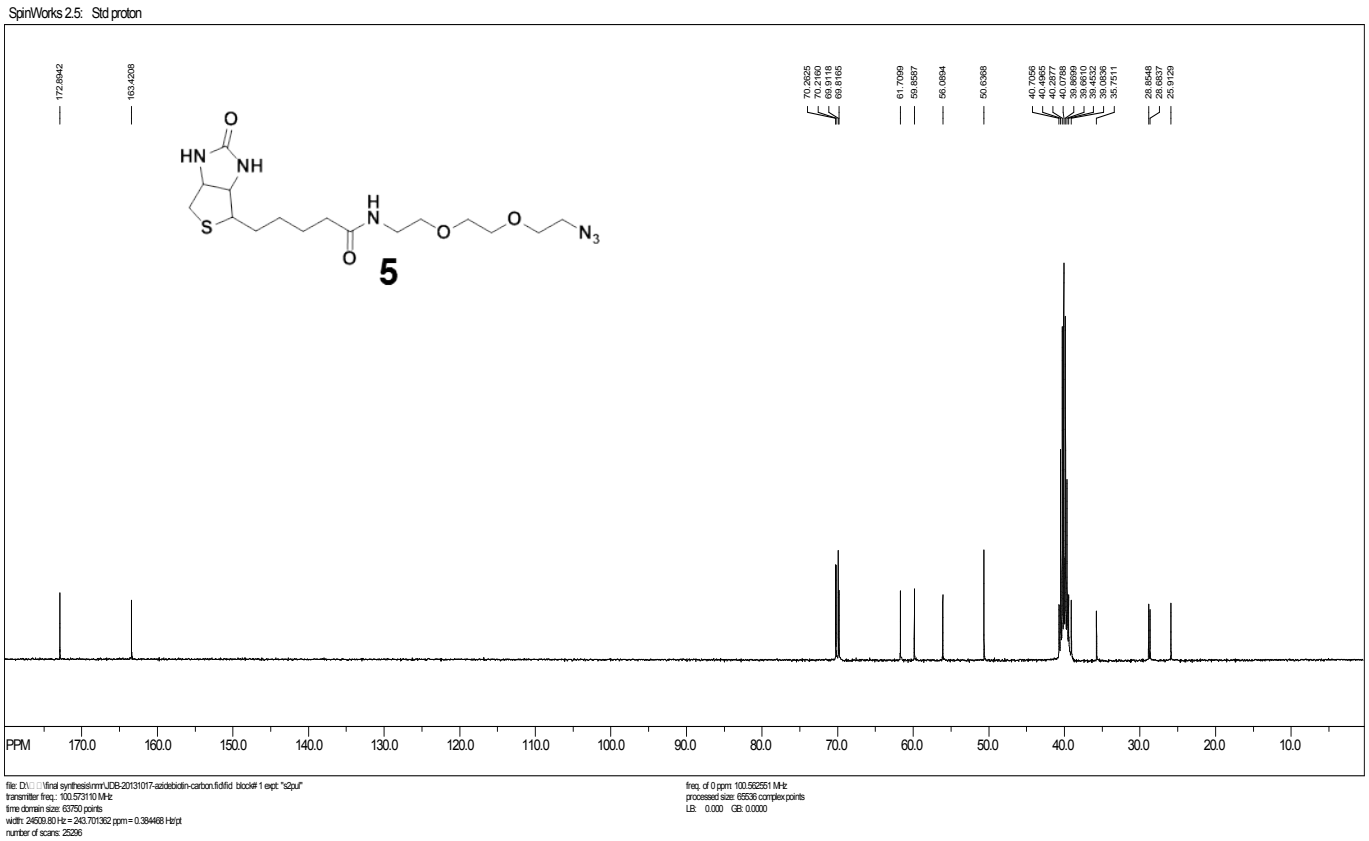


Figure S16. ^{13}C–NMR spectrum of **5** recorded in $CDCl_3$.

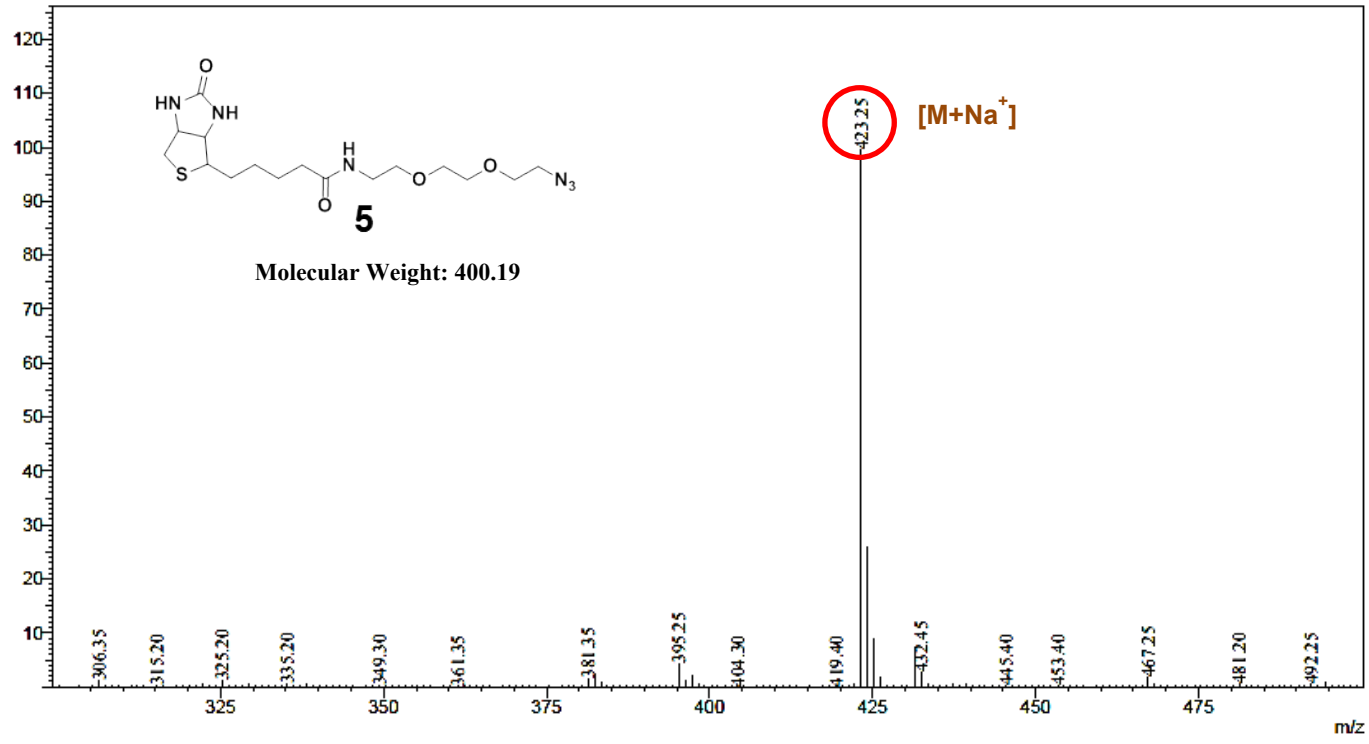


Figure S17. ESI-MS spectrum of **5**.

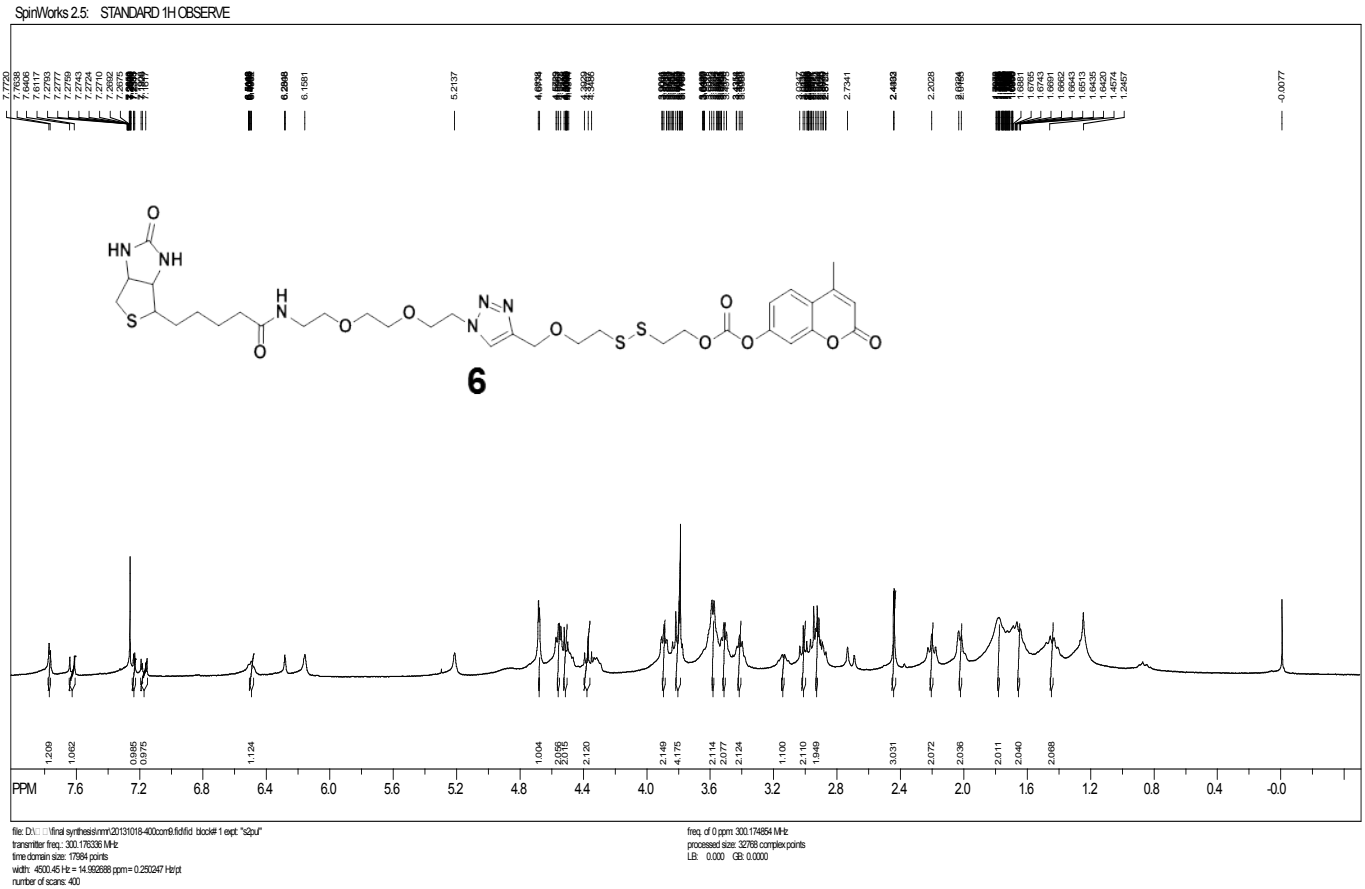


Figure S18. ^{1}H–NMR spectrum of **6** recorded in DMSO-d$_{6}$.

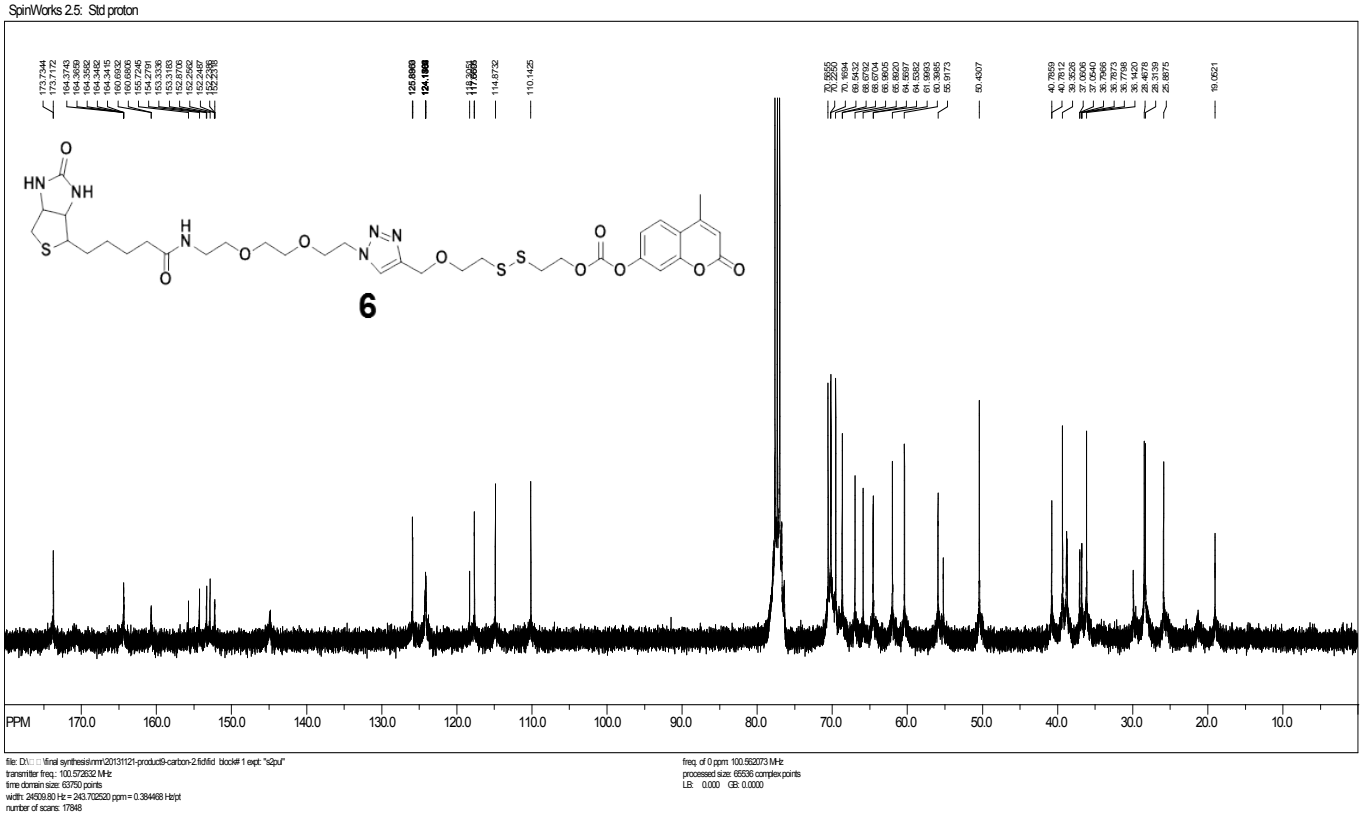


Figure S19. ^{13}C–NMR spectrum of **6** recorded in DMSO-d$_{6}$.

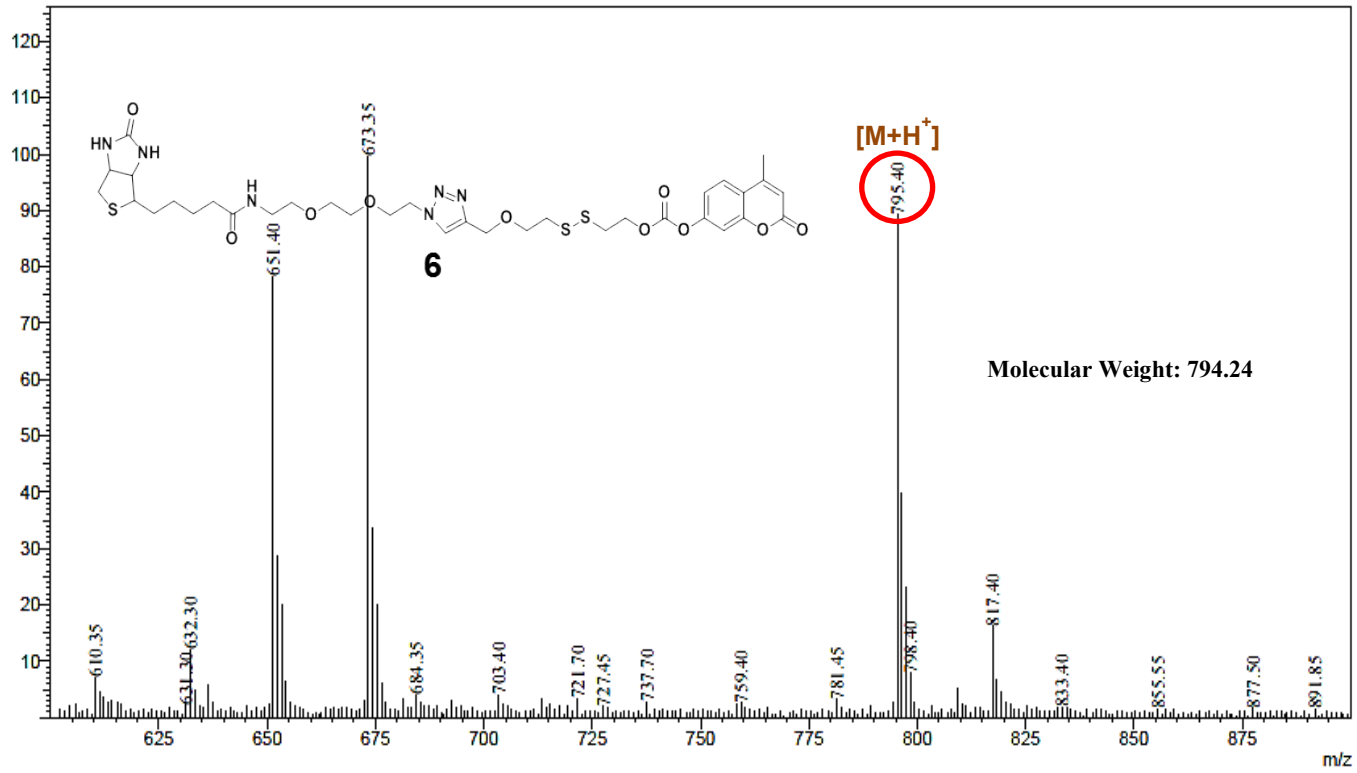


Figure S20. ESI-MS spectrum of **6**.

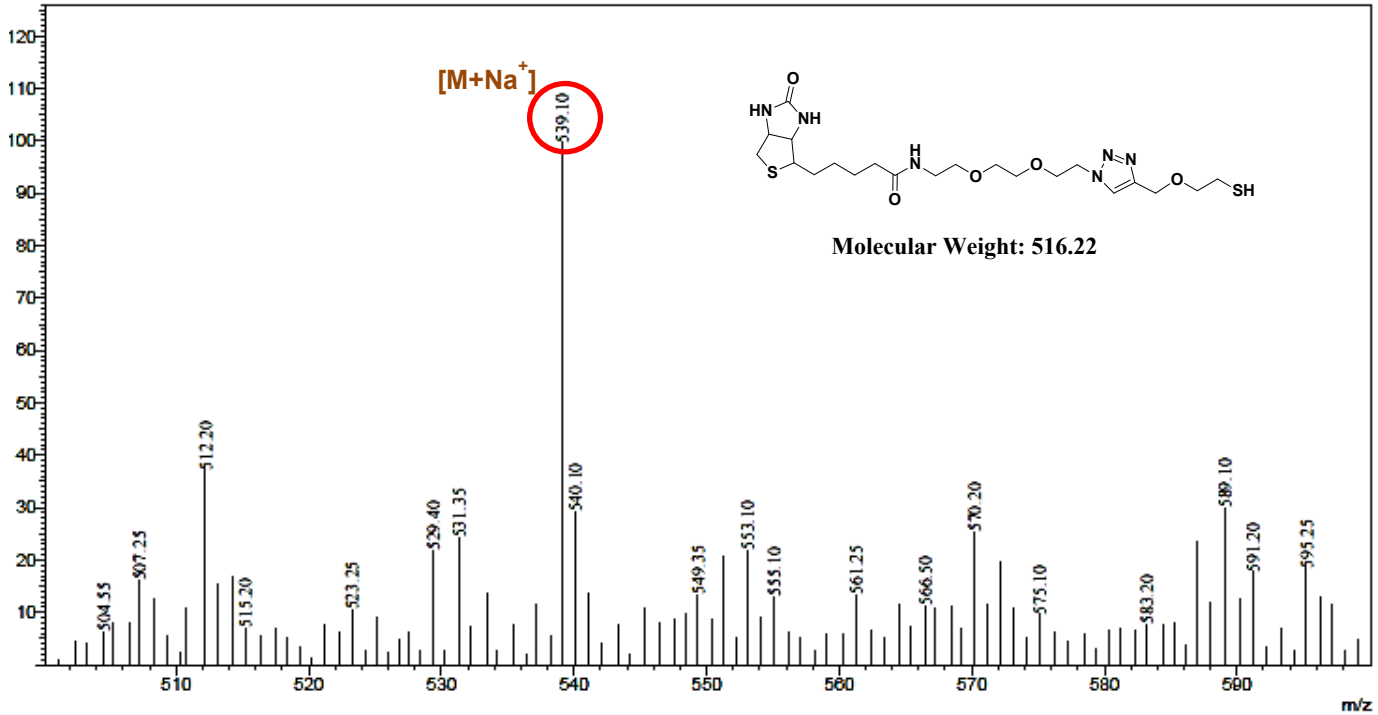


Figure S21. ESI-MS spectrum of the product from the reaction of **6** with GSH.

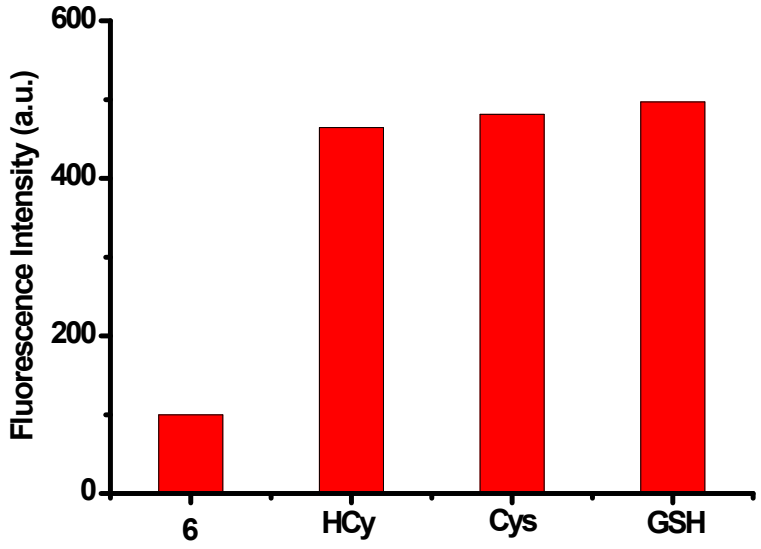


Figure S22. Change of fluorescence intensity of **6** (5.0 μM) with GSH (5.0 mM), Cys (5.0 mM), and Hcy (5.0 mM). All spectra were recorded in 5% DMSO & 95% PBS buffer (pH 7.4) at 37 ˚C. Excitation was effected at 325 nm.