Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information
For

Fluorescence *off-on* reporter for real time monitoring of gemcitabine delivery to the cancer cells

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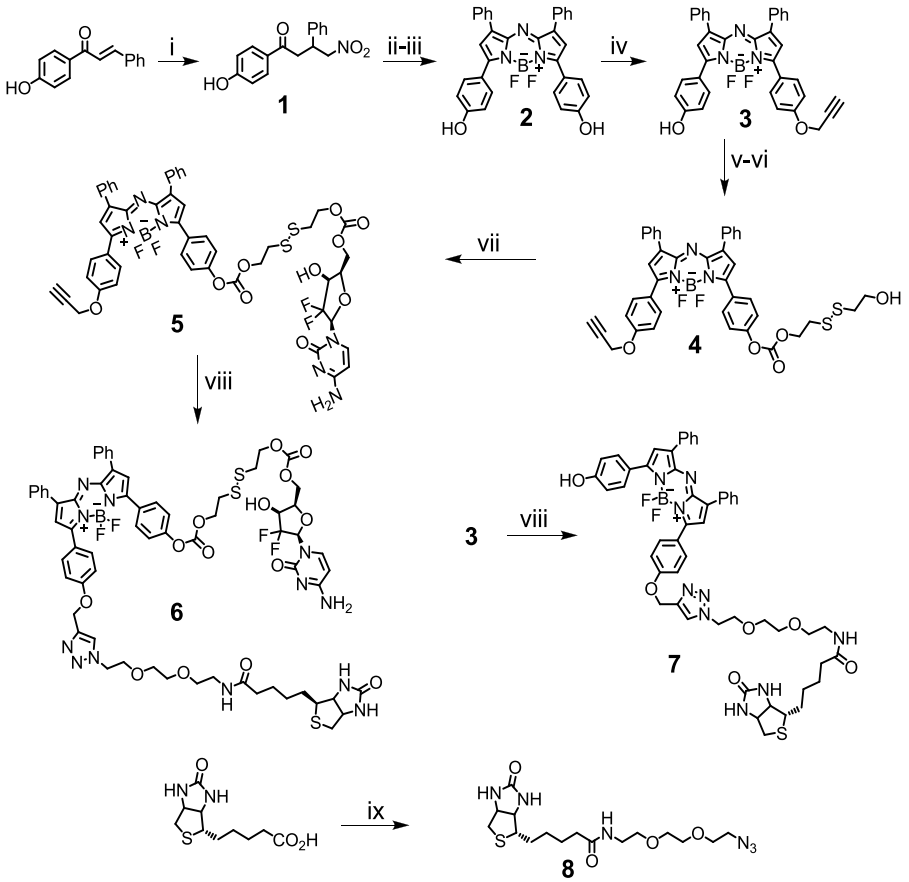
Materials, Methods and Instrumntations: *4*-Hydroxychalcone (Alfa-aesar), *N*,*N*-diethyl amine (Aldrich), nitromethane (Alfa-aesar), BF_3:OEt_2 (Aldrich), ammonium acetate (Aldrich), 2-hydroxyethyl disulfide (Aldrich), DMTr-chloride (Alfa-aesar), EDCI (Carbosynth), DMAP (Alfa-aesar), TEA (Aldrich), DIPEA (Aldrich), 4-nitrophenyl chloroformate (TCI), gemcitabine (Carbosynth), DMF (Aldrich), ethanol (Ducsan), DCM (Ducsan), THF (J. T. Baker) were used without further purification. The compound **1**-**3** were synthesized followed by previously reported procedure.[1] Column chromatography was performed using silica gel 60 (70–230 mesh) as stationary phase. Analytical thin layer chromatography was performed using 60 silica gel (precoated sheets with 0.25 mm thickness). The mass spectra were obtained on an IonSpec HiResESI mass spectrometer. NMR spectra were collected on a 400MHz spectrometer (AS400, Varian, USA).

Synthesis of Compounds:



Scheme 1. Synthesis of BODIPY based theranostic prodrug **6**.

1-(4-Hydroxyphenyl)-4-nitro-3-phenylbutan-1-one (1): This compound was synthesized followed by reported procedure;[1] yield: 72%

BF_2 Chelate of [5-(4-hydroxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl)-3-phenylpyrrol-2-ylidene]amine (2): This compound was synthesized followed by reported procedure;[1] yield: 37% in two steps.

BF_2 Chelate of 4-{4-phenyl-5-[3-phenyl-5-(4-prop-2-ynyloxyphenyl)-pyrrol-2-ylideneamino]-1H-pyrrol-2-yl}phenol (3): This compound was synthesized followed by reported procedure;[1] yield: 38.5%

Synthesis of Compound 4: To aDCM solution (10 mL) of mono-O-DMTr-2-hydroxyethyl disulfide (456 mg, 1.0 mmol), phosgene (20% in toluene; 2.0 mL, 4.04 mmol) and DIPEA (0.5 mL, 5 mmol) were added at -10 ^oC and stirred for 2h, then unreacted phosgene was removed by purging of argon gas for 15-20 min. Then **3** (284 mg, 0.5 mmol) in DCM was added at 0^oC and continued to stirr at rt for 12h. The reaction miaxture was diluted with water and extracted in EtOAc. The organic layer was dried over anhydrous Na_2SO_4. The crude compound was treated with AcOH (80%) in DCM (20%) for 24h. The AcOH was quenched with $NaHCO_3$ solution and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4. The crude compound was passed through silica column chromatography using EtOAc/Hex (1:1) as eluent to afford 150 mg (40%) of **4**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.05 (m, 8H); 7.42 (m, 6H); 7.30 (d, *J* = 8.32 Hz, 2H); 7.08 (br, 2H); 6.93 (s, 1H); 4.7(s, 2H); 4.54 (t, *J* = 6.72 Hz, 2H); 3.03 (t, *J* = 6.78 Hz, 2H); 2.90 (t, *J* = 7.78 Hz, 2H); 2.58 (s, 1H); 2.21(s,

1H). ^{13}C-NMR (100 MHz, $CDCl_3$): 160.53, 160.31, 156.06, 153.26, 152.58, 146.43, 144.79, 144.73, 142.81, 132.68, 132.21, 132.18, 132.07, 131.22, 131.28, 131.14, 130.11, 129.62, 129.48, 128.82, 124.49, 121.33, 119.72, 118.48, 115.37, 78.87, 76.52, 66.78, 60.46, 56.10, 41.73, 36.82. ESI-MS *m/z* (M+1) calcd 747.18, found 747.30.

Synthesis of Compound 5: To a DCM (5.0 mL) solution of **4** (0.5 mmol) 4-nitrophenyl chloroformate (201.56 mg, 1.0 mmol), DIPEA (258 mg, 2.0 mmol) and catalytic amount of pyridine were added at 0 ^{o}C and stirred for 2h at rt. Then the reaction mixture was concentrated under vacuo. The crude residue was dissolve in 5.0 mL DMF. To this solution gemcitabine (262 mg, 1.0 mmol) in DMF (2.0 mL) and TEA (0.5 mL) were added and continued to stirr for 12h. After completion of reaction, the reaction mixture was diluted in water. The compound was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4. The crude compound was passed through silica column chromatography using DCM/MeOH (9:1) as eluent to afford 50 mg (10%) of **5**. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.12 (br, 8H); 7.46 (br, 11H); 7.18 (d, *J* = 6.54 Hz, 1H); 6.18 (s, 1H); 5.74(d, *J* = 7.54 Hz, 1H); 5.23 (m, 1H); 4.95 (m, 1H); 4.32 (br, 8H); 3.47 (dd, J_1 = 10.54 Hz, J_1 = 4.54 Hz, 2H); 3.32 (t, *J* = 8.57 Hz, 2H); 2.89 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): 160.39, 160.08, 156.00, 153.68, 153.08, 152.49, 146.20, 131.92, 130.98, 129.69, 128.61, 124.34, 121.86, 119.55, 118.42, 115.91, 78.05, 76.26, 66.55, 66.46, 55.93, 36.54, 29.43. ESI-MS *m/z* (M-1) calcd 1035.24 found 1035.40.

Synthesis of Compound 6: To a MeOH (2.0 mL) solution of **5** (103.5 mg, 0.01 mmol) were added sodium ascorbate (10 mol%) and **8** (40 mg, 0.01 mmol). The reaction mixture as degassed for 15min by purging argon gass. Then 2.0 mg (0.002 mmol) of $CuSO_4$ in 0.5 mL water was added to the reaction mixture. The reaction was continued for 4h. Then the crude reaction mixture was directly passed through silica column chromatography using DCM/MeOH (8.5:1.5) as eluent to afford 30 mg (50%) of **6**. ^{1}H-NMR (400 MHz, DMSO-d_6)**:** δ 8.26 (s, 2H); 8.14 (br, 4H); 7.87 (br, 2H); 7.76 (m, 3H); 7.48 (m, 8H); 7.34 (m, 2H); 6.42 (m, 1H), 6.37 (m, 1H); 6.10 (d, *J* = 8.73 Hz, 1H); 5.83 (m, 1H); 5.78 (m, 1H); 5.28 (t, *J* = 7.89 Hz, 1H); 4. 74 (t, *J* = 9.89 Hz, 4H); 4.47 (t, *J* = 6.89 Hz, 4H); 4. 09 (t, *J* = 9.89 Hz, 4H); 3.74 (br, 8H); 3.63 (m, 5H); 3.16 (m, 7H); 1.93 (d, *J* = 4.14 Hz, 2H); 1.53 (m, 2H); 1.21 (m, 4H). ^{13}C-NMR (400 MHz, DMSO-d_6)**:**170.40, 168.24, 159.52, 156.23, 153.43, 151.35, 151.32, 150.25, 145.54, 135.55, 131.21, 131.065, 130.90, 116.64, 116.17, 111.16, 110.65, 109.44, 101.26, 78.23, 70.72, 70.45, 70.22, 68.31, 66.63, 60.42, 55.60, 55.63, 50.67, 41.59, 40.00, 36.67, 36.82, 28.34, 27.51. ESI-MS *m/z* (M+1) calcd 1437.42 found 1437.50.

Synthesis of 7: To a MeOH (4.0 mL) solution of **3** (235.5 mg, 01.0 mmol) were added sodium ascorbate (10 mol%) and **8** (200 mg, 0.5 mmol). The reaction mixture as degassed for 15min by purging argon gass. Then 2.0 mg (0.008 mmol) of $CuSO_4$ in 0.5 mL water was added to the reaction mixture. The reaction was continued for 4h. Then the crude reaction mixture was directly passed through silica column chromatography using DCM/MeOH (8.5:1.5) as eluent to afford 30 mg (50%) of **6**. ^{1}H-NMR (400 MHz, DMSO-d_6)**:** δ 8.26 (s, 2H); 8.14 (br, 8H); 7.56 (br, 8H); 7.26 (m, 2H); 6.92 (d, *J* = 9.89 Hz , 2H); 5.23 (s, 1H); 4. 54 (t, *J* = 9.89 Hz, 1H); 4.37 (m, 1H); 4. 09 (t, *J* = 9.89 Hz, 1H); 3.48 (br, 6H); 3.33 (m, 3H); 3.16 (m, 2H);3.06 (m, 2H); 1.99 (t, *J* = 4.14 Hz, 2H); 1.53 (m, 2H); 1.21 (m, 4H). ^{13}C-NMR (400 MHz, $CDCl_3$): 170.40, 159.52, 153.43, 151.35, 151.32, 150.25, 145.54, 135.55, 131.21, 131.065, 130.90, 116.64, 116.17, 111.16, 110.65, 109.44, 101.26, 78.23, 70.72, 70.45, 70.22, 66.63, 60.42, 55.60, 55.63, 50.67, 41.59, 40.00, 36.67, 36.82, 28.34, 26.87. ESI-MS *m/z* (M+1) calcd 967.38 found 968.39.

Synthesis of 8: D-Biotin (244 mg, 1.0 mmol) in 5.0 mL DMF were added EDCI (191.73 mg, 1.0 mmol), DMAP (61 mg, 0.5 mmol) at rt. Then 2-(2-(2-azidoethoxy)ethoxy)ethanamine (174 mg, 1.0 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 200 mg (75%) of **8**. ^{1}H-NMR (400 MHz, DMSO-d_6)**:** δ 7.78 (s, 1H); 6.43 (s, 2H); 4.23 (d, *J* = 14.14 Hz, 2H); 3.51 (m, 6H); 2.98 (s, 2H); 2.73 (m, 1H); 2.51(m, 1H), 2.01 (t, *J* = 4.11 Hz, 2H); 1.55

(m, 4H); 1.23 (br, 2H). ^{13}C-NMR (400 MHz, DMSO-d_6)**:** 175.21, 162.21, 147.76, 108.21, 62.01, 55.21, 50.01, 39.23, 36.6, 28.34, 26.72. ESI-MS *m/z* (+1) calcd 401.19 found 401.19.

UV-vis. and fluorescence spectroscopy

Stock solutions of **6** and biologically relevant analytes, including thiols and metal ions, were prepared in triple-distilled water. Absorption spectra were recorded on an 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 (3.0 mL volume). Excitation was provided at 730 nm with excitation and emission slit widths at 3 nm. All spectroscopic measurements were performed under physiological conditions (at 37°C in PBS buffer, pH 7.4).

Cell culture and cellular imaging

Human lung adenocarcinoma 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 Ligandat 37 °C under 5 % CO_2 for 15 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.The cells were seeded on 24-well plates and stabilized for overnight. Compounds **6** were applied to the cells to monitor their uptake and drug release as discussed in the main text above. In some experiments, the cells were incubated with media containingLyso- or ER tracker prior to treatment with **6**. Then the cells were briefly washed with 1 ml of PBS and were then treated with **6** in PBS. After incubation, residual quantities of **6**that were not taken up in the cells were removed by washing the cells three times with PBS before the cells were placed in 1 ml of a PBS solution. Fluorescence images were taken using a confocal laser scanning microscope (Zeiss LSM 510, Zeiss, Oberko, Germany).

MTT assay

The cell viability assay was measured using 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT).To test the effects of **5**, **6** and gemcitabine, the cells were seeded at 1 x 10^4cells/well on a 96 well plate. After proper treatment for the experiments, the cells were incubated for 72 hrs at 37℃ and then the media were removed. MTT solution (0.05 mg/ml) was added to each well and incubated for 2hs at 37℃ to let the cells form formazan crystals. The solution was removed and 100 ㎕ DMSO was added per well to dissolve the crystals. The amount of the crystals was measured based on the absorbance at 570 nm using a microplate reader (Molecular Devices, VERSAMAX microplate reader).

^{1}H-NMR, ^{13}C-NMR, ESI-MS and LCMS Spectra

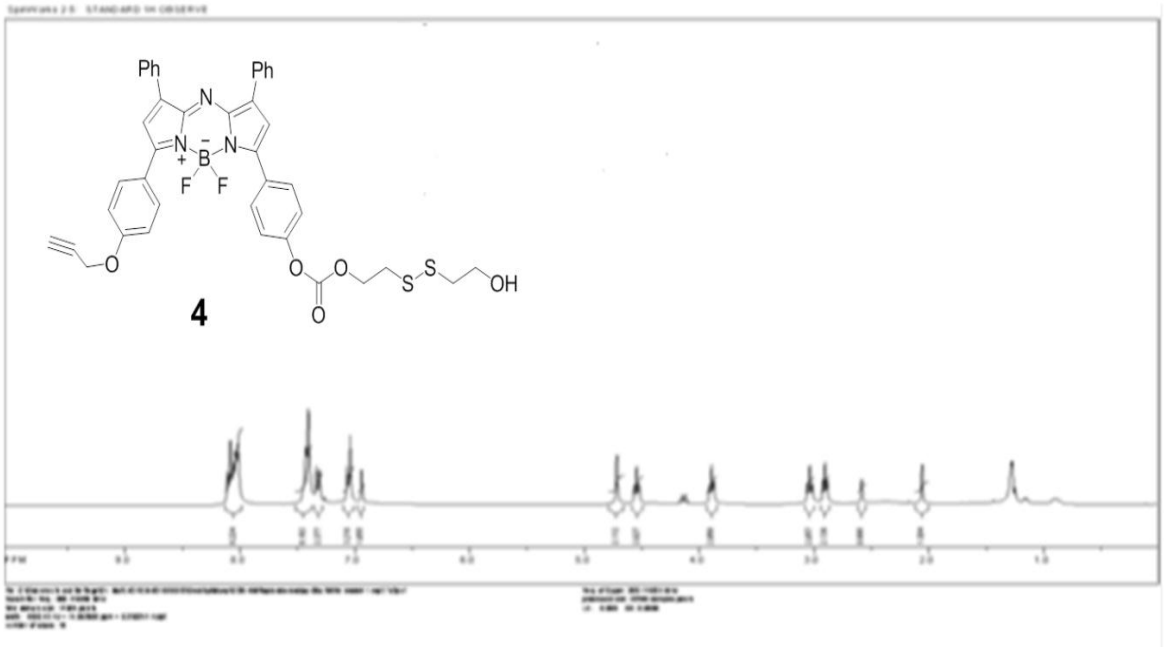


Figure S1. ^{1}H-NMR spectrum of **4**.

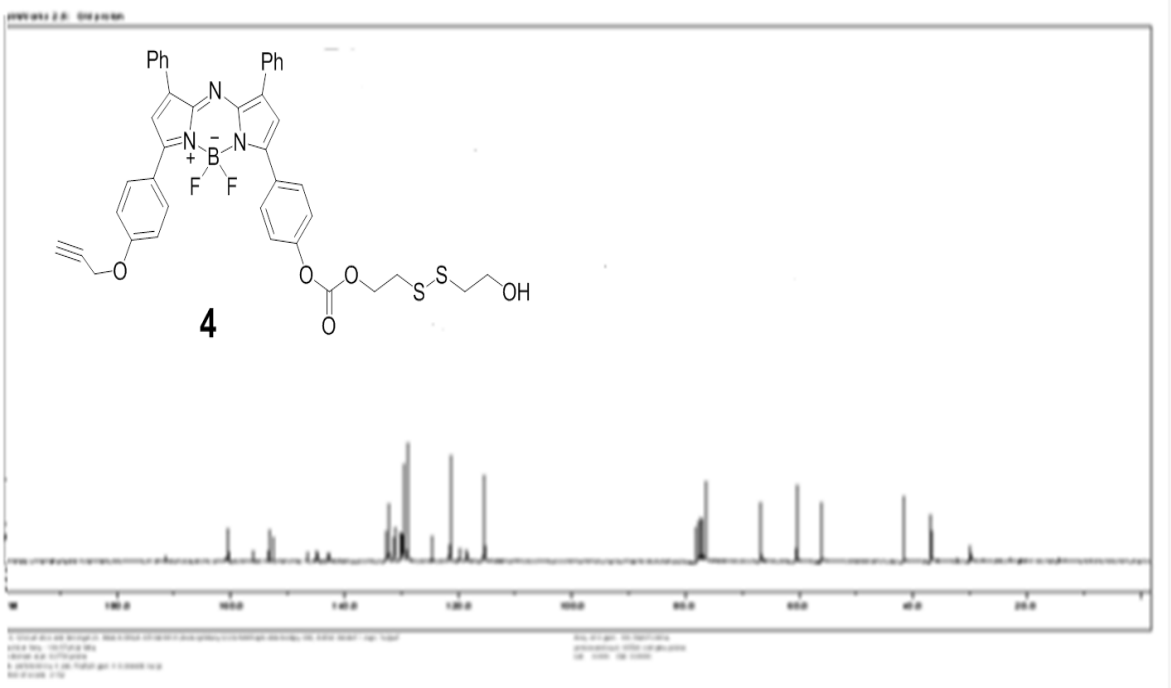


Figure S2. ^{13}C-NMR spectrum of **4**.

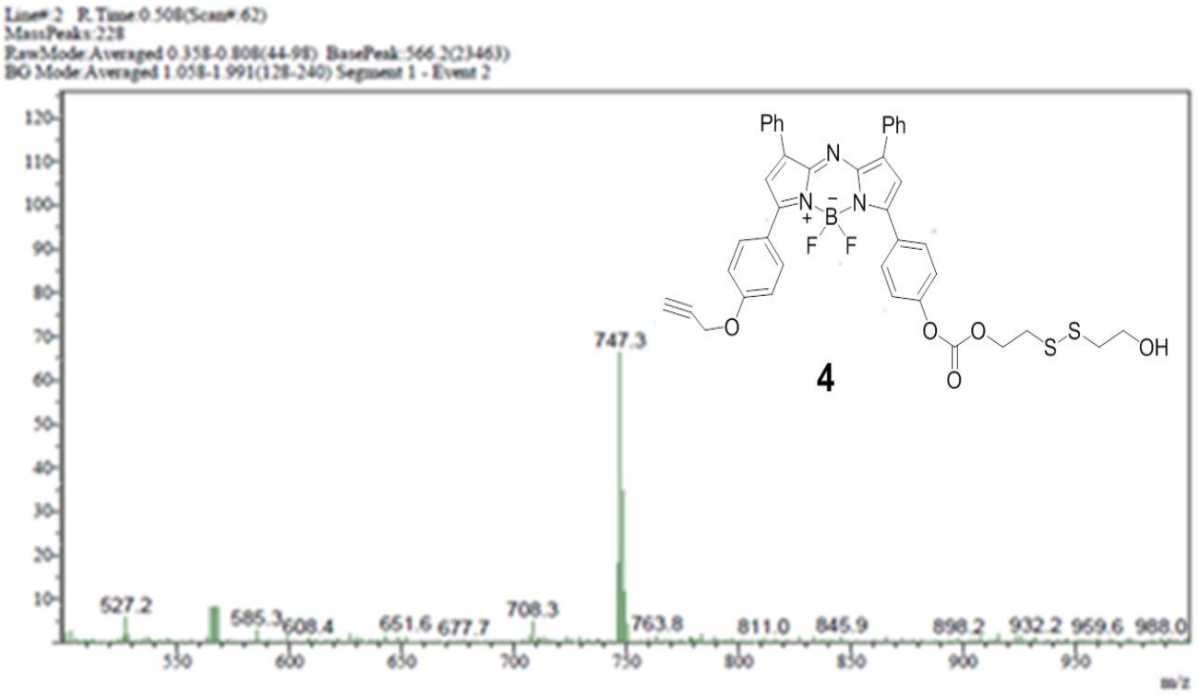


Figure S3. Mass spectrum of **4**.

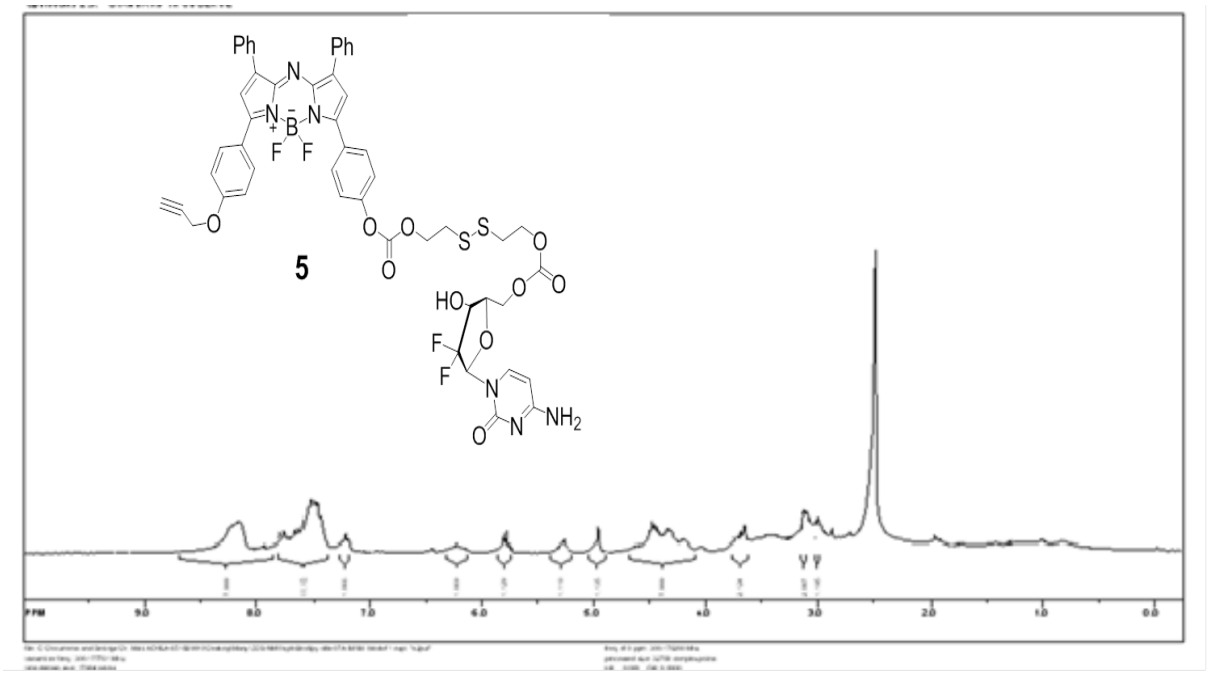


Figure S4. ^{1}H-NMR spectrum of **5**.

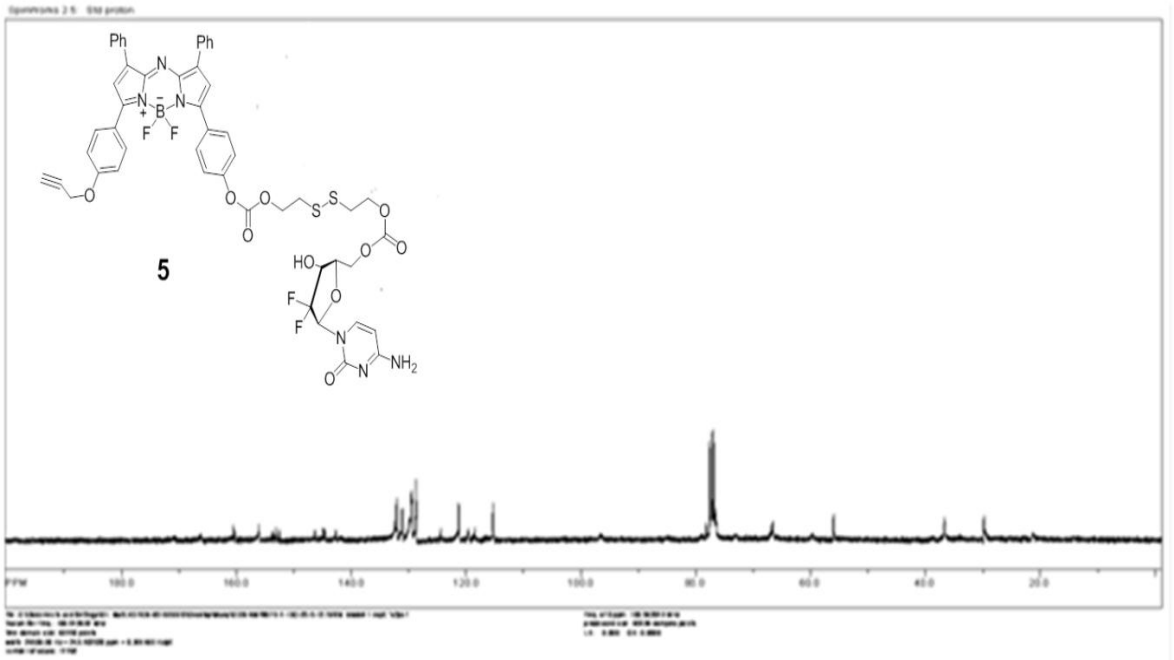


Figure S5. ^{13}C-NMR spectrum of **5**.

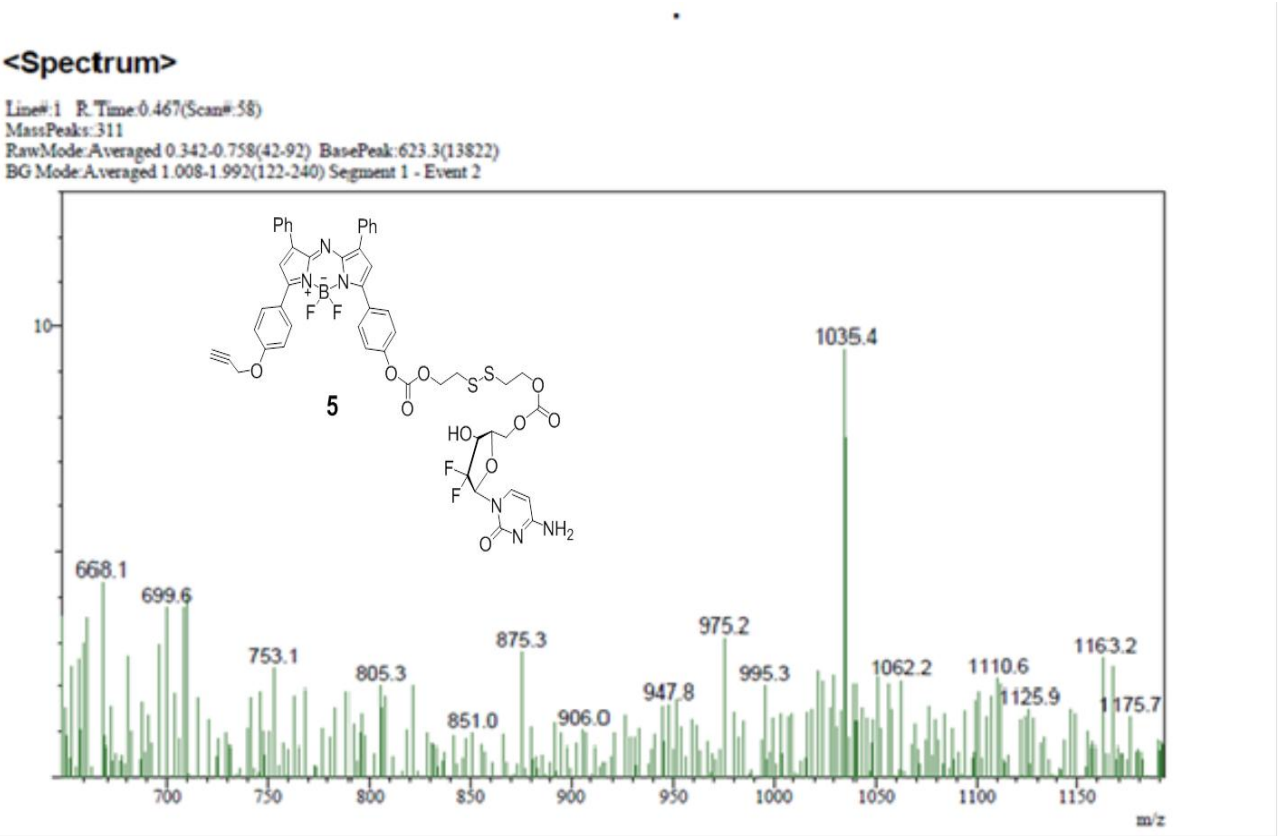


Figure S6. MS spectrum of **5**.

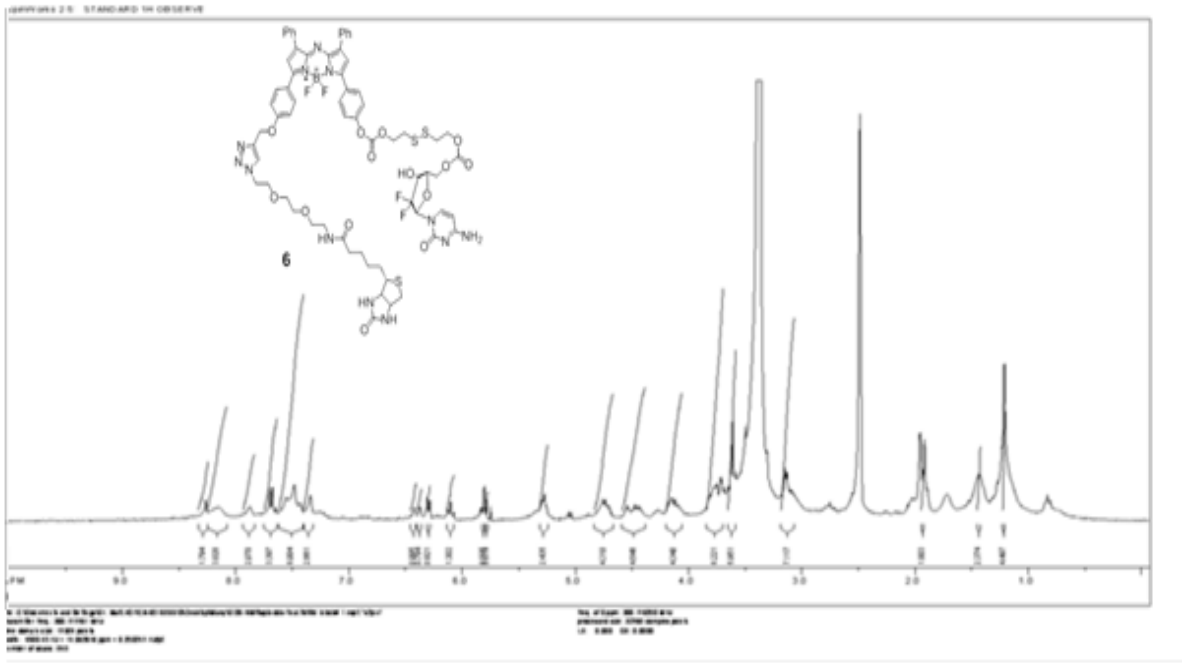


Figure S7. ^{1}H-NMR spectrum of **6**.

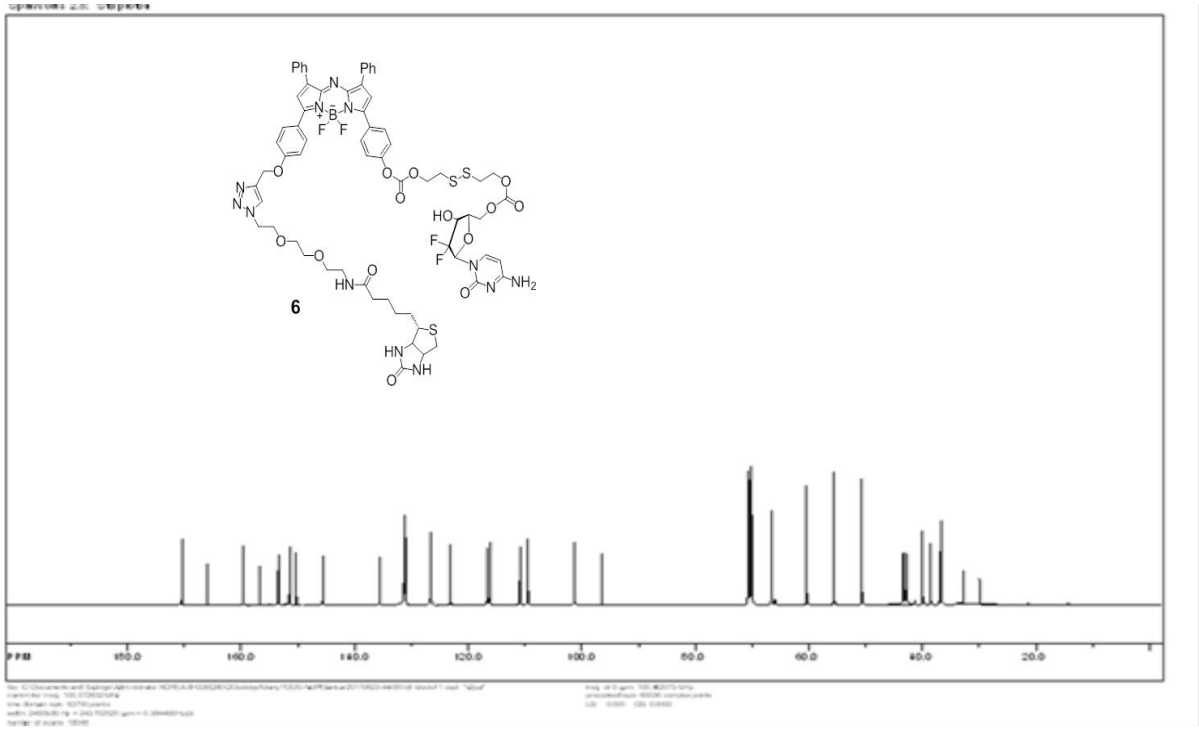


Figure S8. ^{13}C-NMR spectrum of **6**.

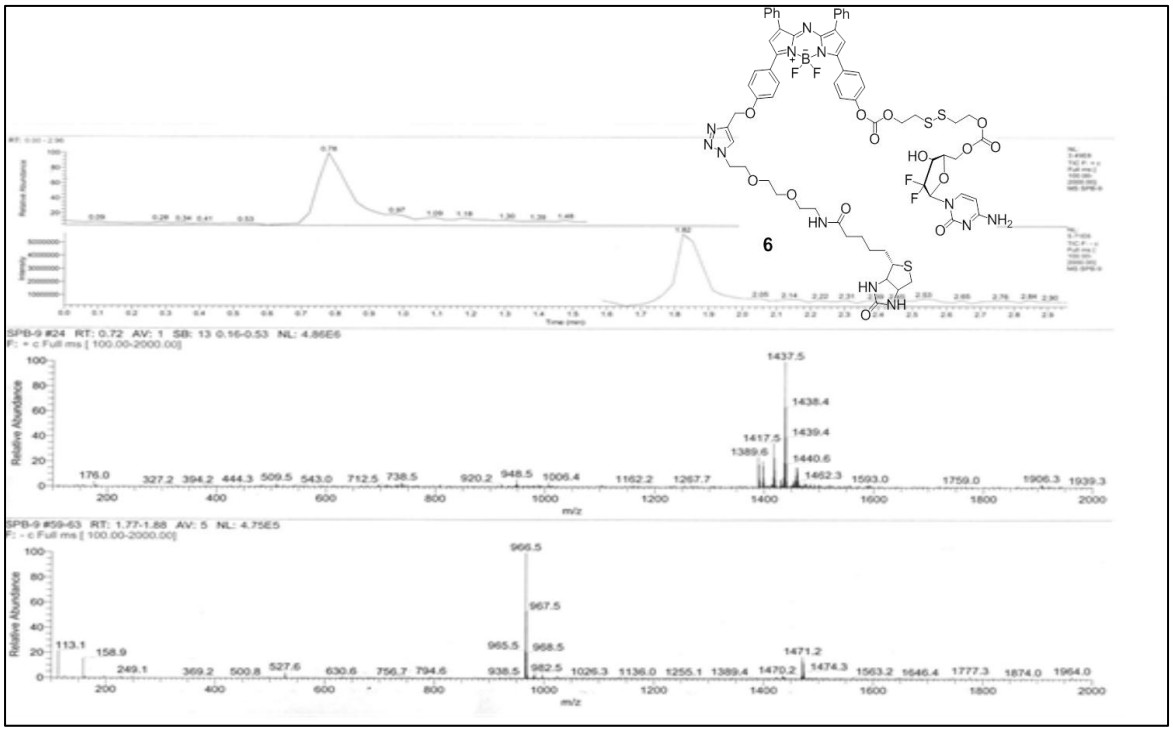


Figure S9. MS spectrum of **6**.

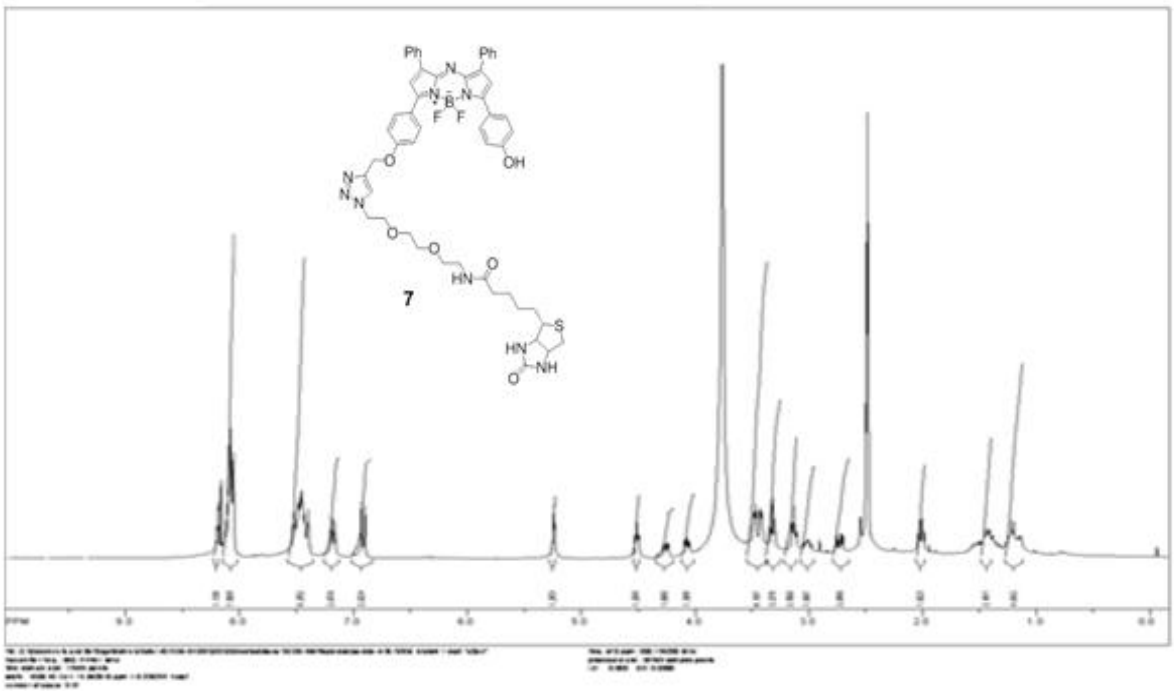


Figure S10. ^{1}H-NMR spectrum of **7**.

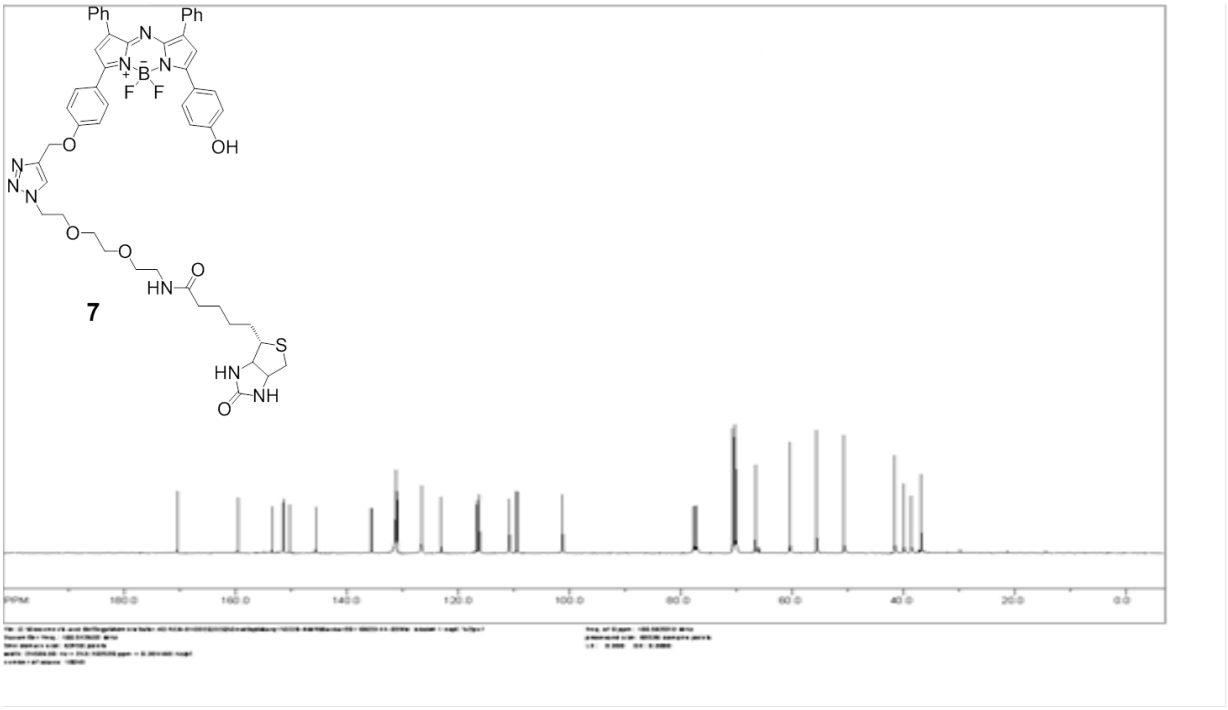


Figure S11. ^{13}C-NMR spectrum of **7**.

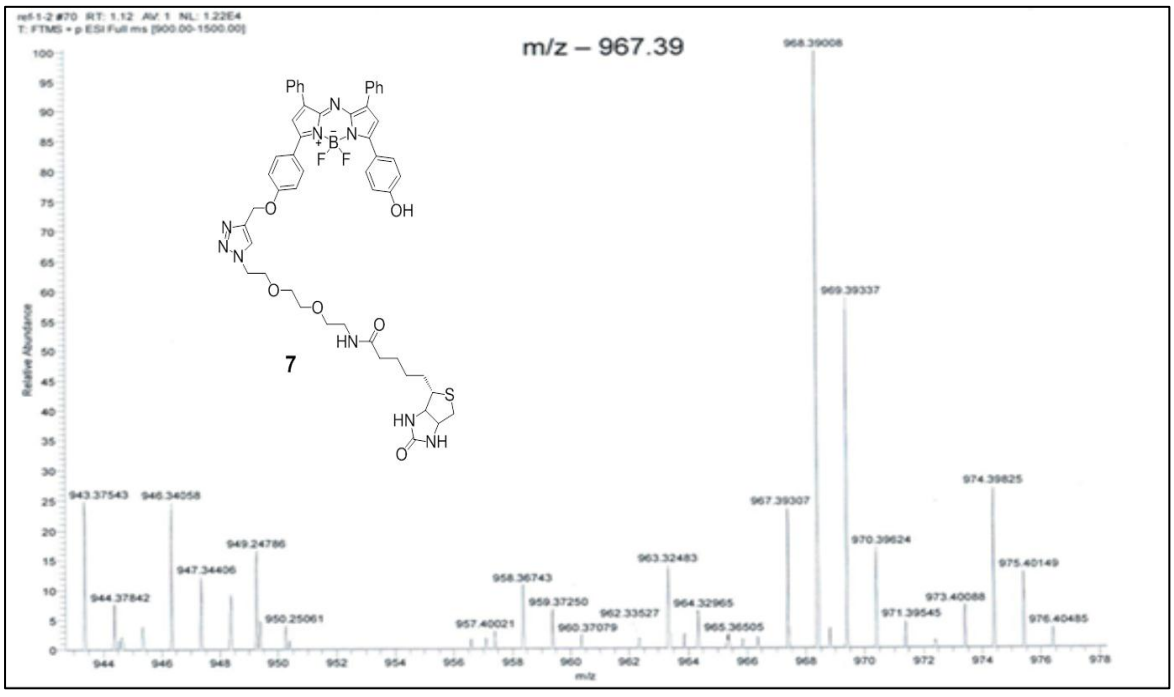


Figure S12. MS- spectrum of **7**.

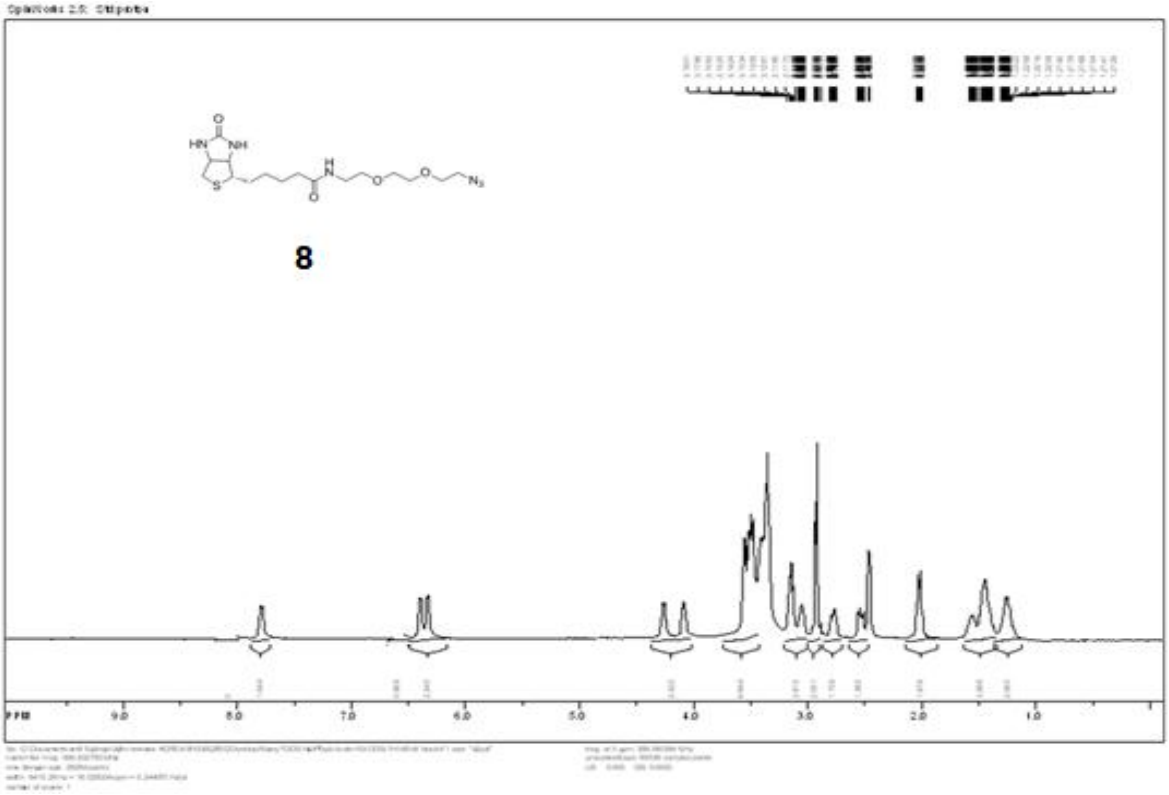


Figure S13. ^{1}H-NMR spectrum of **8**.

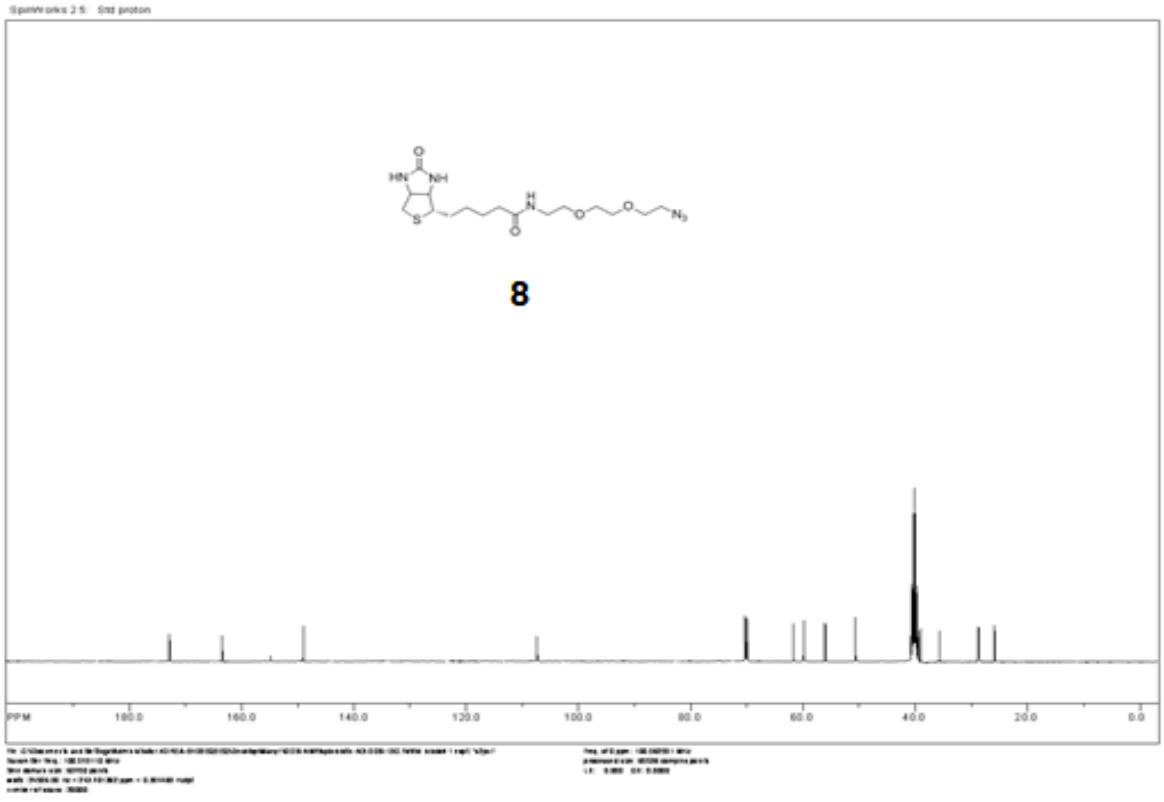


Figure S14. ^{13}C-NMR spectrum of **8**.

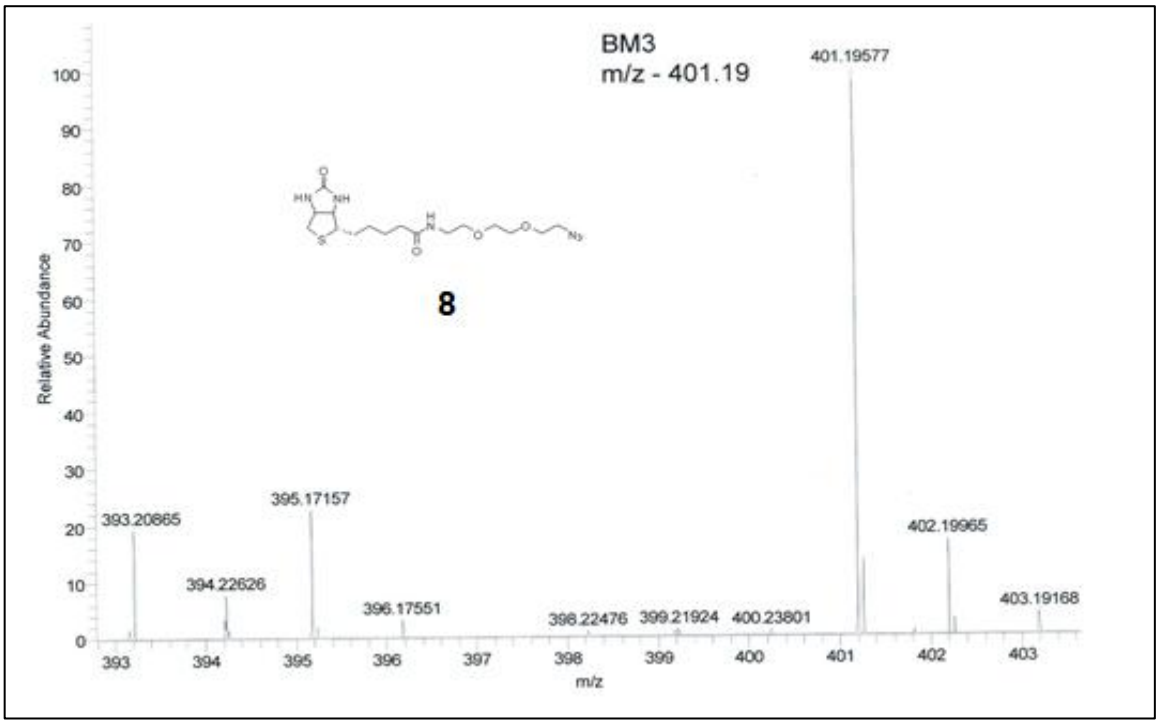


Figure S15. MS- spectrum of **8**.

Additional absorption and fluorescence studies.

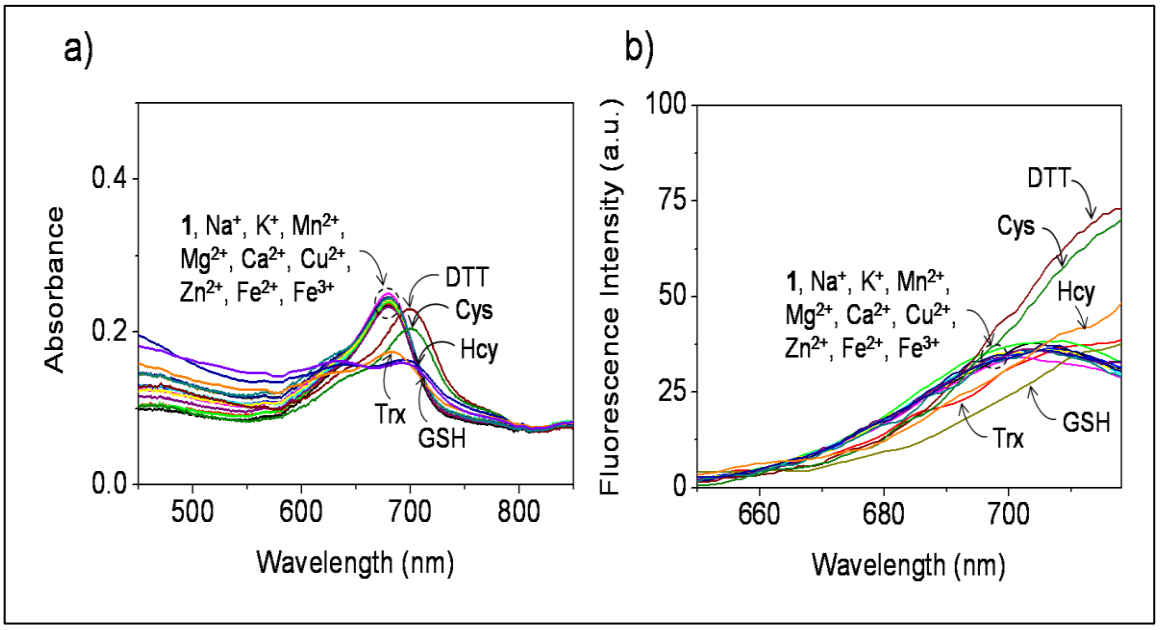


Figure S16. Absorption (a) and Fluorescence response (b) of **6** for various analytes. All the spectra were acquired 30min after addition of DTT at 37 ^{o}C in PBS buffer (pH 7.4) with excitation at 700 nm.

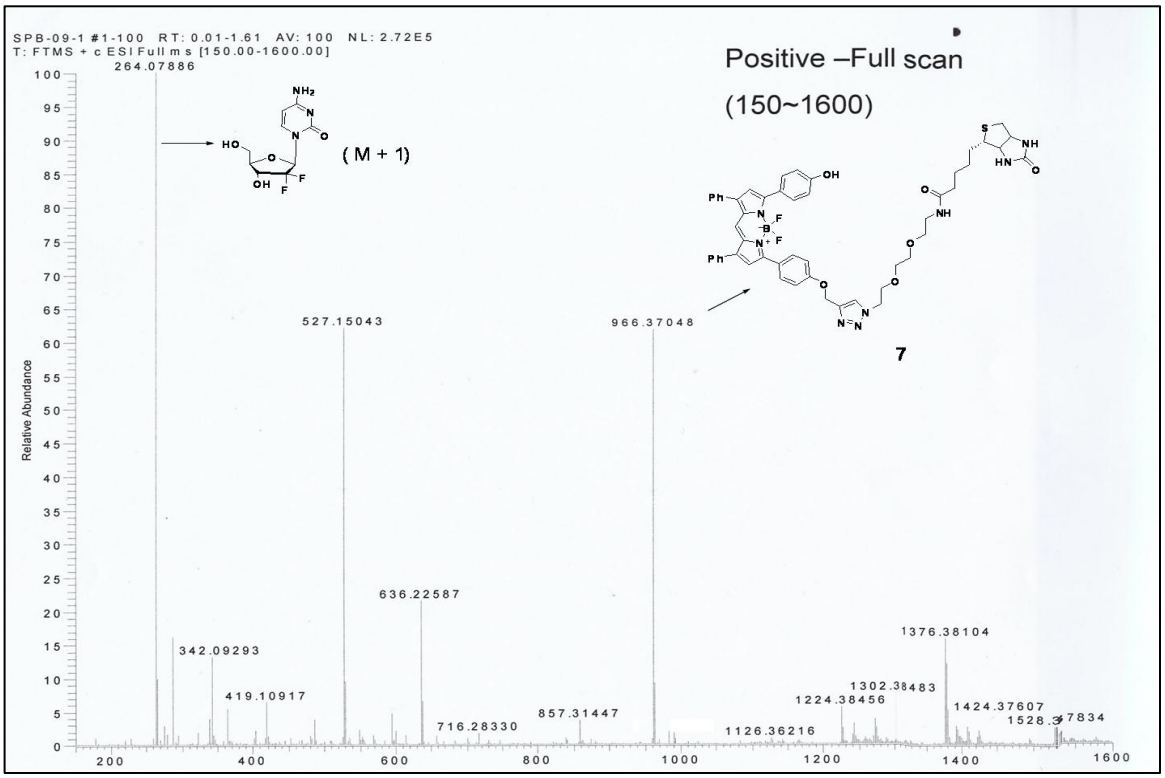


Figure S17. Mass spectrum of **6** (5μM) after treatment with GSH (5 mmol) for 2h at 37 °C.

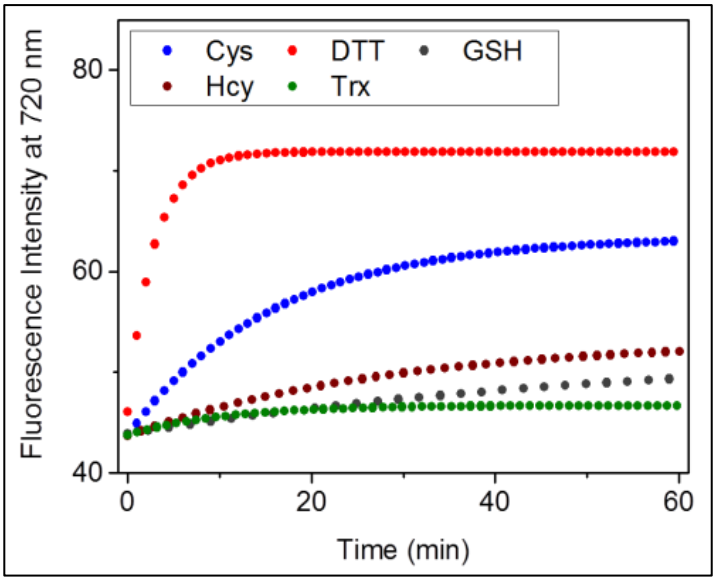


Figure S18. Changes in fluorescence intensity at 720 nm as function of time with various thiols such as Cys, HCys, GSH (5.0 mM), and Trx (5.0 μM). All the spectra were acquired 30 min after addition of DTT at 37 °C in PBS buffer (pH 7.4) containing 4% (v/v) of DMSO and 0.4% (v/v) of Chremophore EL with excitation at 700 nm.

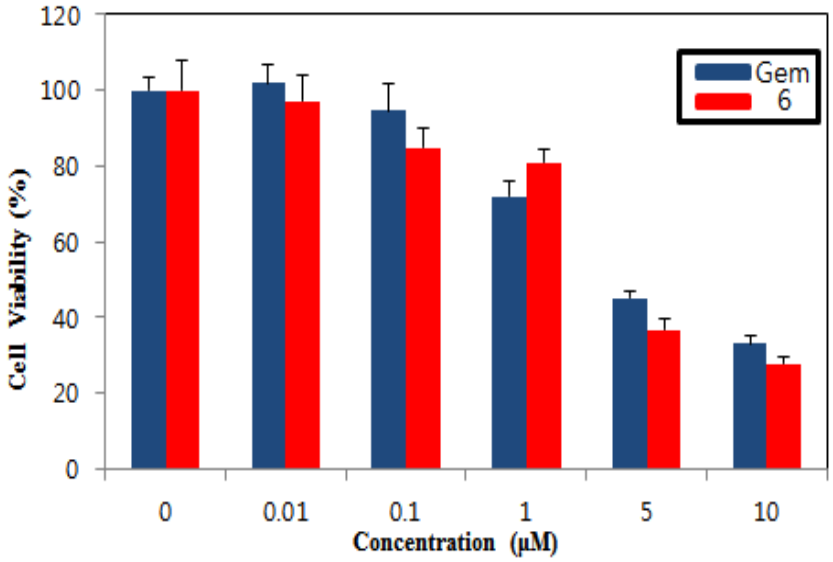


Figure. S19. Cell viability assay of **6** and Gem(gemcitabine) on A549 lines. All compounds were incubated with the cells for 72 h, and the cell viability observed *via* MTT assay.

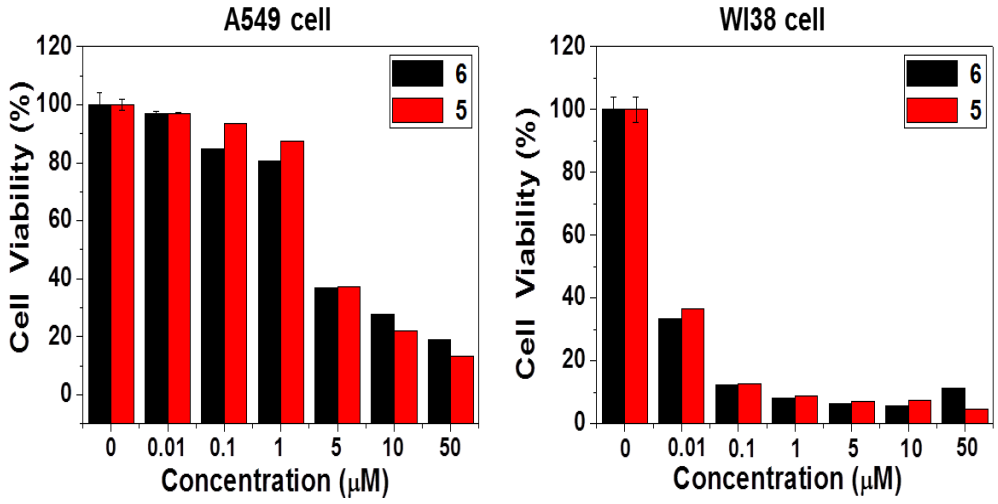


Figure S20. Cell viability assay of **6** and **5** on A549 and WI38 cell lines. All compounds were incubated with the cells for 72 h, and the cell viability observed *via* MTT assay.