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# In Vivo Targeted Delivery of Antibodies into Cancer Cells with pH-Responsive Cell-Penetrating Poly(disulfide)s

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## Supporting Information

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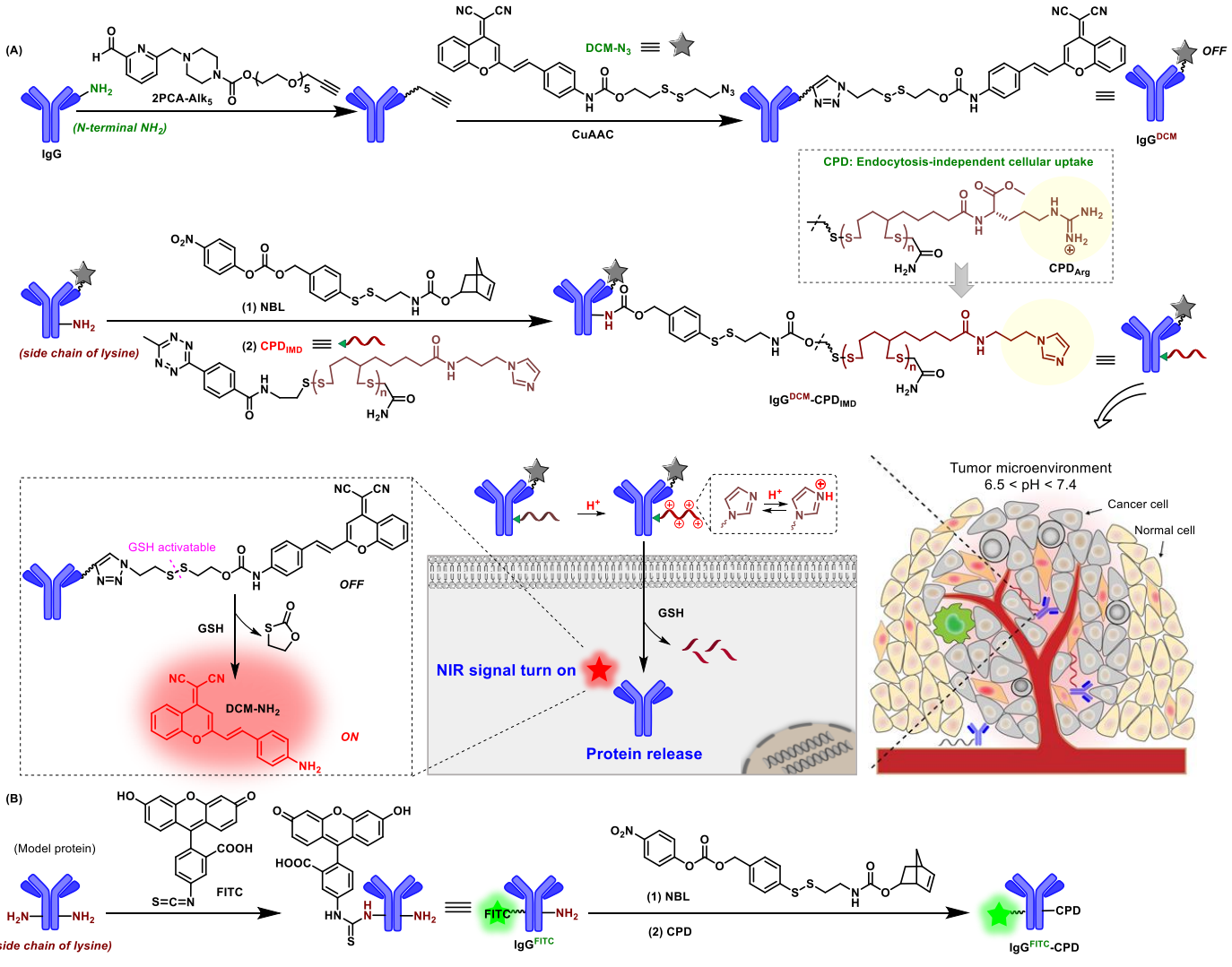
## 1. General information

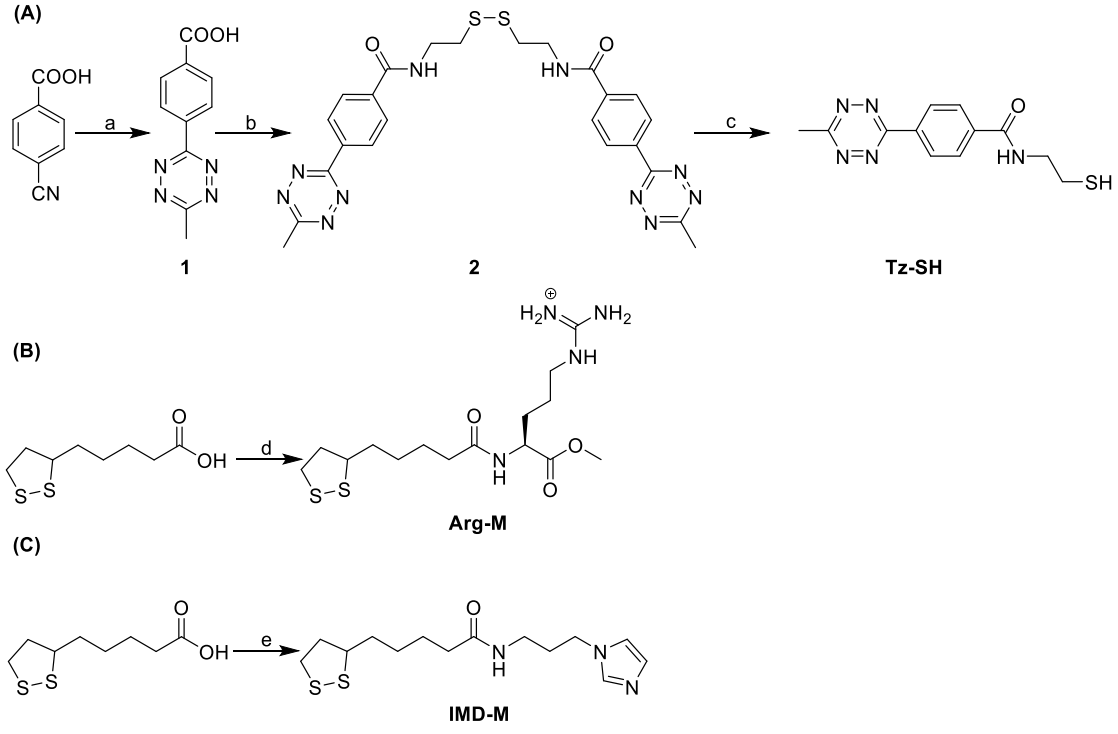
All chemicals were purchased from commercial vendors and used without further purification. Special reaction conditions including nitrogen atmosphere would be noted and reaction progress was monitored with thin layer chromatography (TLC) on silica plate (Qingdao Bangkai Hi-tech Materials Co, HSGF 254) by UV light or appropriate staining. Flash column chromatography was carried out using silica gel 60F (Qingdao Haiyang Chemical co., Ltd). All **$^{1}$H NMR** and **$^{13}$C NMR** spectra were carried out from on a BRUKER AVIII 500M (500 MHz). Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference ($^{1}$H: chloroform, δ 7.26 ppm; methanol, δ 3.31 ppm; DMSO, δ 2.50; $^{13}$C: chloroform, δ 77.16; methanol, δ 49.0; DMSO, δ 39.52). **$^{1}$H NMR** data was reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets or overlap of nonequivalent resonances, dq = doublet of quartets), coupling constant and integration. Mass spectra were recorded on Agilent 6200 Series TOF and 6500 Series LC-MS. Analytical GPC was carried out on Waters1525 & Agilent PL-GPC220 with Waters™ ultra-hydrogel linear column (PL aquqgel-OH MI*ED 8 um) and a Waters™ RI 2414 detector. Buffer with deionized water (0.2 M $NaNO_3$, 0.01 M $NaH_2PO_4$, pH = 7) was used as the mobile phase at the flow rate of 1 mL/min. In gel fluorescence scanning was imaged with Amersham Typhoon Multifunctional Laser Imager (GE Amersham) equipped with a solid state multi wavelength illuminator ($\lambda_{ex}$ = 473 nm; $\lambda_{ex}$ = 532 nm; $\lambda_{ex}$ = 635 nm; $\lambda_{ex}$ = 685 nm; $\lambda_{ex}$ = 785 nm). Confocal images were acquired either on OLYMPUS IX83-FV3000 Confocal Microscope System equipped with Leica HCX PL APO 40×/0.85 Dry CORR CS, 405 nm diode laser, argon ion laser, white laser (470 nm to 670 nm, with 1 nm increments, with 8 channels AOTF for simultaneous control of 8 laser lines, each excitation wavelength provides 1.5 mV), a PMT detector ranging from 420 nm to 700 nm for steady state S2 fluorescence or on Zeiss LSM 800 with Airyscan Confocal Microscope System. The concentrations of protein were determined using Bicinchoninic Acid Kit (purchased from Beyotime Biotechnology (P0011)). Fluorescence spectra were determined by a microplate reader (Infinite® M1000 Pro from Tecan). Fluorescence-activated cell sorting (FACS) analysis was performed on ACEA NovoCyte™ equipped with ultra-high detection sensitivity (FSC: 0.5 μm, SSC: 0.2 μm, FITC < 75MESF, PE < 50MESF). Human immunoglobulin G (IgG, human plasma, 16-16-090707) was purchased from Shanghai Acmec Biochemical Co., Ltd. All other reagents were in analytical grade and was obtained from commercial vendors.




**Scheme S1.** (A) Design of targeted delivery of antibodies into cancer cells with pH-responsive **CPD$_{IMD}$** together with the NIR indicator furnishing (**DCM-N$_3$**) to track the cellular uptake of the antibody. (B) **IgG$^{FITC}$** was used as the "always-on" model to track the distribution of the protein.

## 2. Chemical synthesis

### 2.1 CPD preparation




**Scheme S2.** (a) Zinc trifluoromethanesulfonate, 4-cyanobenzoic acid, hydrazine hydrate, $NaNO_2$, $CH_3CN$, 60 °C, 24 h, 60%; (b) EDC·HCl, cystamine dihydrochloride, triethylamine, $CHCl_3$, overnight, room temperature (r.t.), 90%; (c) TCEP, dimethyl formamide (DMF), 30 min, r.t.; (d) Methyl L-argininate dihydrochloride, DIEA, CDI, DMF, 4.5 h, r.t., (e) N-(3-aminopropyl)-imidazole, DIEA, CDI, DMF, 18 h, r.t.

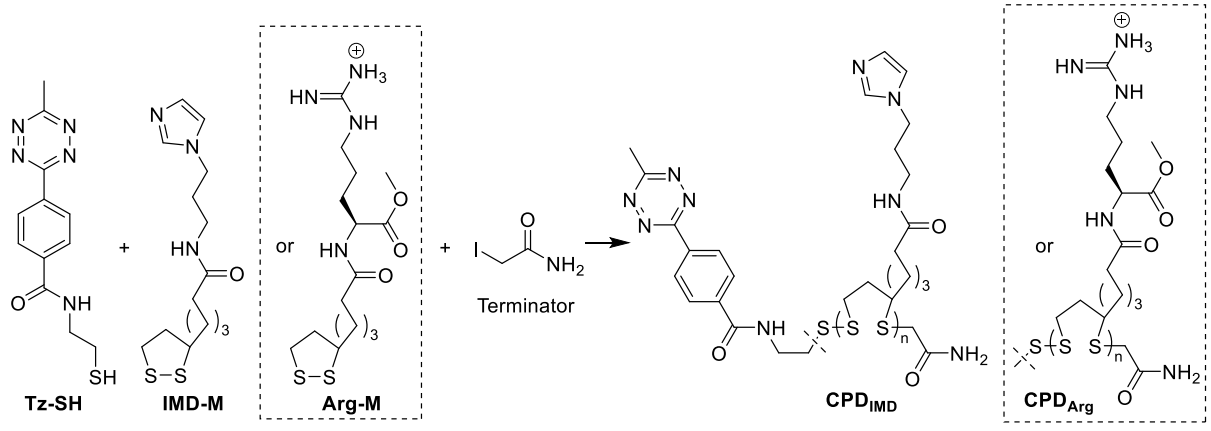
***Tz-SH*.** The initiator for CPDs (**Tz-SH**) was prepared following published procedures.[1]

***Arg-Monomer (Arg-M).*** **Arg-M** was synthesized according to polished protocol.[2] CDI (3.24 g, 20 mmol) was added into a solution of α-lipoic acid (4.12 g, 20 mmol) in anhydrous DMF (10 mL) and the mixture was stirred at r.t. under $N_2$ atmosphere for 1 h. After running through a cotton filter, a mixture of methyl L-argininate dihydrochloride (2.61 g, 10 mmol) and DIEA (1740 μL, 10 mmol) in anhydrous DMF (10 mL) was added into the above solution and stirred at r.t. under $N_2$ atmosphere for 3.5 h. To the reaction mixture was added $Et_2O$ (200 mL) dropwise. The emulsion was centrifuged (2 min, 4400 rpm) and the yellow oil at the bottom was then washed with $CH_2Cl_2$/$Et_2O$ mixture (1:2, 10 mL × 5). **Arg-M** was obtained as pale yellow sticky solid after drying the residue in vacuo. **$^1$H NMR** (500 MHz, Methanol-$d_4$) δ 4.42 (dd, $J$ = 9.1, 5.1 Hz, 1H), 3.72 (s, 3H), 3.58 (dq, $J$ = 9.0, 6.4 Hz, 1H), 3.25 – 3.08 (m, 5H), 2.51 – 2.42 (m, 1H), 2.29 (td, $J$ = 7.4,

1.3 Hz, 2H), 1.90 (dtt, *J* = 13.6, 6.9, 3.6 Hz, 2H), 1.81 – 1.57 (m, 7H), 1.53 – 1.42 (m, 2H). **MS** (ESI): m/z $[M]^+$ calcd 376.53, found 376.63.

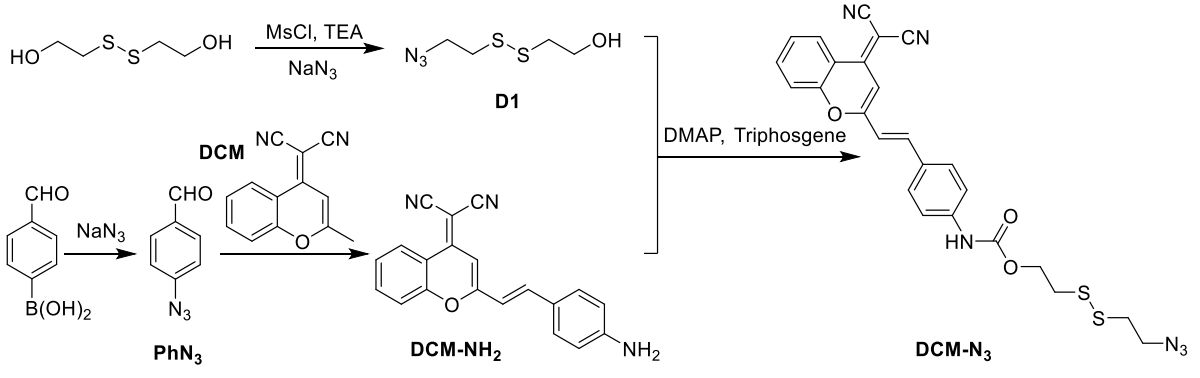
***IMD-Monomer (IMD-M).*** To a solution of α-lipoic acid (4.12 g, 20 mmol) in anhydrous DMF (10 mL), CDI (3.24 g, 20 mmol) was added and the mixture was stirred at r.t. under $N_2$ atmosphere for 1 h. After adding N-(3-aminopropyl)-imidazole (1.25 g 10 mmol) and DIEA (1740 µL, 10 mmol) dissolved in anhydrous DMF (10 mL), the mixture was stirred at r.t. under $N_2$ atmosphere overnight. The reaction mixture was extracted by ethyl acetate (EA; 200 mL) and saturated $NaHCO_3$ solution (250 mL) twice, then the organic layer was washed by $Et_2O$. After running through a cotton filter the organic layer was dried in vacuo to obtain the sticky pale yellow solid. **$^1$H NMR** (500 MHz, Chloroform-*d*) δ 7.49 (s, 1H), 6.97 (d, *J* = 34.2 Hz, 2H), 6.77 (s, 1H), 3.96 (t, *J* = 6.9 Hz, 2H), 3.52 (dq, *J* = 8.9, 6.3 Hz, 1H), 3.20 (q, *J* = 6.4 Hz, 2H), 3.17 – 3.01 (m, 2H), 2.41 (dtd, *J* = 13.0, 6.6, 5.3 Hz, 1H), 2.15 (t, *J* = 7.2 Hz, 2H), 1.96 (p, *J* = 6.8 Hz, 2H), 1.86 (dt, *J* = 12.9, 6.8 Hz, 1H), 1.70 – 1.54 (m, 5H), 1.47 – 1.35 (m, 2H). **$^{13}$C NMR** (126 MHz, Chloroform-*d*) δ 173.00, 136.01, 127.16, 118.62, 55.64, 43.90, 39.42, 37.68, 35.22, 33.74, 30.26, 28.07, 24.71, 21.35. **HRMS** (ESI): m/z $[M]^+$ calcd 314.1355 found 314.1355.

***Synthesis of CPDs.*** All CPD polymers were synthesized according to previously published protocols[3] (Scheme S2). Briefly, stock solutions of the monomer (**IMD-M** or **Arg-M**, 2 M in DMF), initiator (**Tz-SH**, 50 mM in DMF), terminator (iodoacetamide, 0.5 M in $H_2O$, freshly prepared), TCEP (125 mM in $H_2O$) and TEOA buffer (1 M, pH = 7.0) were prepared. The initiator was first generated in situ, by mixing 50 µL of the initiator precursor (**2**) stock solution, 50 µL of the TCEP solution with 50 µL DMF and shaking vigorously for 30 min at room temperature. Subsequently, 350 µL of DMF, 400 µL of TEOA buffer mixture and 100 µL of monomer stock solution were added to the reaction mixture. The polymerization reaction was quenched by addition of 19 mL of the terminator stock solution. The resulting polymer was purified with a NAP$^{TM}$-5 desalting column (GE Healthcare) against $H_2O$ in the same day, following protocols provided by the vendor. The elution was lyophilized and kept in -20 °C.




**Scheme S3.** Preparation of CPDs.

## 2.2 NIR probe (DCM-$N_3$) preparation




**Scheme S4.** Synthesis of the GSH-activatable fluorogenic probe **DCM-$N_3$**.

***Synthesis of compound D1.***[4] 2-hydroxyethyl disulfide (814 mg, 4.74 mmol) and 0.79 mL of triethylamine (TEA) were dissolved in 3 mL of tetrahydrofuran (THF). Methanesulfonyl chloride (543 mg, 4.74 mmol) in 1.5 mL of THF was added dropwise to the mixture. The reaction was stirred vigorously at r.t. for 0.5 h, followed by the addition of 20 mL of water. The product was extracted three times (3 × 20 mL) with EA. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. DMF (3.5 mL) was used to dissolve the condensate and sodium azide (308 mg, 4.74 mmol) was added to the mixture. The slurry was stirred at 60 °C overnight, followed by the addition of 40 mL of water. The product was extracted three times (3 × 15 mL) with EA. The combined organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (Hexane/EA = 4/1, v/v) to give **D1** as colorless oil (326 mg, 38.3% yield).

**$^1$H NMR** (500 MHz, Chloroform-*d*) δ 3.89 (t, J = 5.8 Hz, 2H), 3.60 (t, J = 6.7 Hz, 2H), 2.90 – 2.82 (m, 4H), 2.18 (s, 1H).

***Synthesis of 4-azidobenzaldehyde (PhN$_3$).***[5] 4-acetylphenylboronic acid (1500 mg, 10 mmol) was added into the mixture of sodium azide (900 mg, 15 mmol) and copper acetate monohydrate (200 mg, 1 mmol) in methanol (60 mL). The reaction was stirred under an argon atmosphere for 3 h at 55 °C. The solvent was removed under reduced pressure, and then the crude product was purified by silica gel chromatography with petroleum ether as the eluent to afford the desired product as faint yellow oil (798 mg): Yield 58.04%. **$^1$H NMR** (500 MHz, Chloroform-*d*) δ 9.84 (s, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 8.5 Hz, 2H).

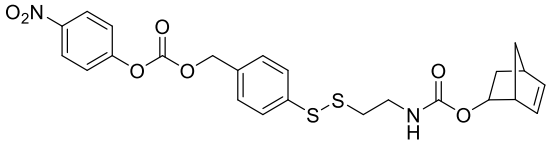
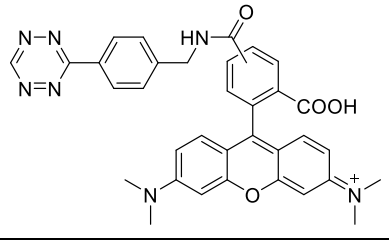
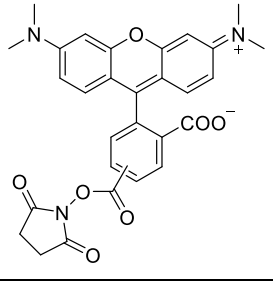
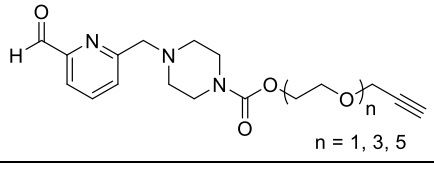
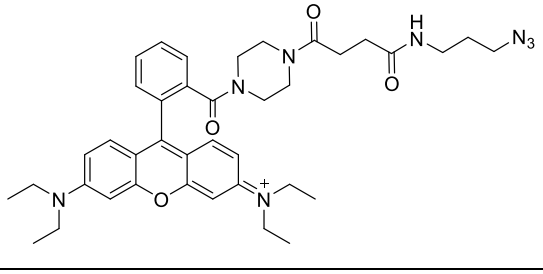
***Synthesis of DCM-NH$_2$.***[5] **DCM** (443 mg, 2.11 mmol; synthesized according to the literature[6]) and **PhN$_3$** (306 mg, 2.11 mmol) were dissolved in toluene (20 mL) with acetic acid (0.34 mL) and piperidine (0.68 mL). Then the mixture was refluxed for 15 h under an argon atmosphere. The solvent was removed under reduced pressure, and then the crude product was purified by silica gel chromatography using $CH_2Cl_2$ as the eluent to afford **DCM-NH$_2$** as deep red solid (120 mg): Yield 16.6%. **$^1$H NMR** (500 MHz, DMSO-*d6*) δ 8.71 (d, J = 9.7 Hz, 1H), 7.90 – 7.85 (m, 1H), 7.76 (d, J = 9.4 Hz, 1H), 7.63 (d, J = 15.7 Hz, 1H), 7.57 (t, J = 8.4 Hz, 1H), 7.47 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 15.8 Hz, 1H), 6.85 (s, 1H), 6.60 (d, J = 8.6 Hz, 2H), 6.01 (s, 2H).

***Synthesis of DCM-N$_3$.***[4] **D1** (300 mg, 1.67 mmol), triethylamine (174 mg, 1.69 mmol) and triphosgene (166mg, 0.56 mmol) were dissolved in anhydrous $CH_2Cl_2$ (6.6 mL). The reaction was stirred at r.t. for 2 h, then the solvent and gas were removed carefully. A solution of **DCM-NH$_2$** (150 mg, 0.5 mmol) and DMAP (200 mg, 1.64 mmol) in anhydrous $CH_2Cl_2$ was added into the above mixture and then triethylamine (230 μL) was added dropwise. After stirring overnight at r.t., the solvent was evaporated and the resulting residue was purified by silica gel chromatography (PE/EA = 5/3) to afford **DCM-N$_3$** (28 mg): Yield 10.8%. **$^1$H NMR** (500 MHz, DMSO-$d_6$) δ 10.07 (s, 1H), 8.72 (dt, *J* = 8.3, 2.3 Hz, 1H), 7.91 (ddq, *J* = 8.6, 7.2, 1.5 Hz, 1H), 7.81 – 7.74 (m, 1H), 7.73 – 7.63 (m, 3H), 7.63 – 7.53 (m, 3H), 7.35 (dd, *J* = 16.0, 7.2 Hz, 1H), 6.96 (d, *J* = 7.5 Hz, 1H), 4.37 (t, *J* = 6.3 Hz, 2H), 3.64 (t, *J* = 6.5 Hz, 2H), 3.08 (t, *J* = 6.3 Hz, 2H), 2.98 (t, *J* = 6.5 Hz, 2H). **$^{13}$C NMR** (126 MHz, DMSO-$d_6$) δ 158.46, 153.05, 141.19, 138.50, 135.36, 129.22, 129.13, 126.12,

124.62, 119.03, 118.26, 117.59, 117.10, 115.94, 106.24, 62.20, 59.69, 49.15, 36.85.

### 2.3 Synthesis of other compounds

**Table S1.** Summary of probes used in the current study. They were either purchased or synthesized based on literature: **NBL**[3], **Tz-TMR**[7], NHS-Rhodamine (#46406, Thermo Scientific), **2PCA-Alk$_n$**[8], **TER-N$_3$**[8].

| Probe | structure |
|---|---|
| ***NBL*** |  |
| ***Tz-TMR*** |  |
| ***NHS-Rhodamine*** |  |
| ***2PCA-Alk$_n$*** |  |
| ***TER-N$_3$*** |  |

## 3. <u>Biological experiments</u>

### 3.1 Characterization of CPDs and the NIR probe (DCM-N$_3$)

***CPDs*:** Analytical GPC was used to determine the molecular weight of **$CPD_{Arg}$** or **$CPD_{IMD}$** polymer after calibration with standard molecular weight makers (Figure S1A/B). Briefly, analytical GPC was carried out on Waters1525 & Agilent PL-GPC220 with Waters™ Ultra hydrogel linear column (PL aquqgel-OH MI*ED 8 um) and Waters™ RI 2414 detector. Buffer with deionized water (0.2 M $NaNO_3$, 0.01 M $NaH_2PO_4$, pH=7) was used as the mobile phase at the flow rate of 1 mL/min. The concentration of CPDs was calculated by UV-Vis measurements of the specific tetrazine absorbance at 520 nm, during which tetrazine disulfide (**2**) of different concentrations was used to generate the calibration curve (Figure S1C).

***NIR probe*:** Absorption and fluorescence spectra were measured on the microplate reader. GSH was purchased from Aladdin (G105426-10g) and used to study the spectral properties of the NIR probe. With $\lambda_{max}$ = 490 nm according to the absorption spectra, following fluorescence experiments were done upon excitation at this wavelength. It was reported that 10 μM of the NIR probe (DCM-S) can be fully turned on by GSH (2.5 mM; representative for GSH level inside cancer cells) within 20 min,[5] so **DCM-$N_3$** or **$IgG^{DCM}$** was incubated with GSH for 0.5 h in the photophysical studies.

### 3.2 Labeling of native proteins

***$IgG^{FITC}$-NBL***: Cargo proteins were labeled by FITC and NBL sequentially following published protocols[13b] with some modifications: to 50 μL of $NaHCO_3$ (1 M, pH = 9.2) and 400 μL of IgG (10 mg/mL in PBS) was added 50 μL of 2.5 mM FITC at 4 °C with shaking overnight. Unreacted FITC was removed by dialysis and the labeled protein was kept at -20 °C. The concentration of the obtained FITC-labeled IgG (**$IgG^{FITC}$**) was determined on a NanoDrop™. Then **$IgG^{FITC}$** (225 μL of 10 μM) was reacted with 2.5 μL of 20 mM **NBL** in $NaHCO_3$ (100 mM, pH = 9.2) for 2 h at room temperature before the excess **NBL** was removed by G-25 column (GE Healthcare Life Science) to give **$IgG^{FITC}$-NBL**. In order to check the labelling efficiency, **Tz-TMR** was used to click with protein labeled by **NBL** followed by SDS-PAGE/in-gel fluorescence scanning (no DTT was added when dealing with the traceless tag **NBL**; Figure S2A). The labeling ratio of IgG/**NBL** was calculated to be 2:1 according to the fluorescence of TMR (Figure S2B) following reported protocol.[13c]

***$IgG^{DCM}$-NBL***: 6 μL of 10 mM **2PCA-$Alk_5$** [23b] was added into a solution of 25 μL of $NaHCO_3$ (1 M, pH = 9.2) and 250 μL of IgG (10 mg/mL in PBS) with shaking for 36 h at 37 °C. The protein labeled by **2PCA-$Alk_5$** was purified by dialysis and then reacted with **DCM-$N_3$** (50 μL of 100 μM, protein/**DCM-$N_3$** = 2/1) in

PBS Buffer (1×, pH = 7.5) containing 10% DMSO with shaking for 8 h at 37 °C. Excess **DCM-$N_3$** and other small molecules were removed by dialysis and G-25 Column. The labeling ratio of IgG/**DCM-$N_3$** was calculated to be around 1 according to the fluorescence of **DCM-$N_3$**. The resulting **$IgG^{DCM}$** was further labeled by **NBL** as mentioned above to give **$IgG^{DCM}$-NBL**. All protein samples were stored at -20 °C before use.

***$IgG^{FITC/DCM}$-CPD***: Protein either labeled by FITC or **DCM-$N_3$** (**$IgG^{FITC}$-NBL** or **$IgG^{DCM}$-NBL**) was conjugated to tetrazine-containing CPDs (**$CPD_{IMD}$**/**$CPD_{Arg}$**) upon click with **NBL** under similar conditions as reported.[13b] 2 μM (final concentration) of **$IgG^{FITC}$-NBL**/**$IgG^{DCM}$-NBL** was reacted with 5 μM (final concentration) of CPDs for 2 h at room temperature. And then 2 μL of above solution was added into 200 μL DMEM (fetal bovine serum-free; pH = 6.5 or 7.5 respectively) for 0.5 h at room temperature before applying to cells for live-cell imaging. PBS used here was the same as that for cell culture.

### 3.3 Cell culture and cell viability assay

According to the standard procedure, HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and SW620 cells in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100.0 mg/L streptomycin and 100 IU/mL penicillin in a humidified atmosphere of 5% $CO_2$ at 37 °C. All reagents were purchased from commercial sources. For cytotoxicity assay, HeLa cells were seeded at $1 \times 10^4$ cells/well in a 96-well plate and cultured in 100 μL of media overnight. After removing the media, cells were washed thrice with PBS and treated with 100 μL of IgG-CPD at different concentrations in DMEM. Cells were incubated for 1 h at 37 °C before the media was removed. Cells were then washed thrice with PBS containing 0.1 mg/mL heparin and kept in DMEM for additional 24 h. The cell viability was then evaluated by Cell Counting Kit-8 (CCK-8; Beyotime-C0037, Shanghai, China) assay.

### 3.4 Confocal laser scanning microscopy (CLSM) for live-cell imaging

HeLa cells were cultured in 4-chamber glass bottom dish (D35C4-20-1-N, Cellvis) and grown until 50~60%. After medium removal and washed by PBS, HeLa cells were treated with 2 μL of 2 μM IgG-CPD (freshly prepared in 1 × PBS, pH = 7.4; final concentration at 20 nM) in 200 μL of DMEM at pH 6.5 or 7.5. At the same time, CPD-free **IgG-NBL** was utilized as negative controls. The cells were incubated for 1.5 h at 37 °C

before washing with PBS (containing 0.1 mg/mL heparin) for five times. HeLa cells were further co-stained with Hoechst for additional 15 min. Subsequently, 400 μL of DMEM (FBS-free) was re-introduced into the cells, which were then imaged by CLSM (FITC, $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 520 nm; **DCM**, $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 665-740 nm. Nuclear-staining Hoechst: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 440-470 nm).

### 3.5 Fluorescence-activated cell sorting (FACS) analysis

HeLa cells were seeded in 6-well plates and were cultured overnight in DMEM supplemented with 10% FBS and antibiotics at 37 °C in 5% $CO_2$ atmosphere. After removing the medium, cells were washed by PBS, then serum-free DMEM containing **IgG$^{FITC}$-CPD** with pH = 6.5 or 7.5 was added. After incubation at 37°C for 2 h and removal of the medium, cells were washed with PBS thrice, trypsinized, collected, and re-suspended in fresh PBS buffer. Samples were analyzed by flow cytometry (BECKMAN COULTER: ACEA NovoCyteTM and Cytomic FC 500MCL) using 488 nm laser (10,000 cells were counted for each event; in duplicate).

### 3.6 *In vivo* protein delivery

Animals were maintained under the guidelines of the National Institute Guide for the Care and Use of Laboratory Animals and all studies with animals were approved by the Institutional Animal Care and Use Committee of Zhejiang University. The 6-8-week-old BALB/nude mice were purchased from Zhejiang Academy of Medical Sciences. Three mice were used for each imaging group in following experiments.

The nude mice were injected with SW620 cells ($1 \times 10^6$ cells in 100 μL of saline solution) subcutaneously on the right thigh. When the tumor grew up to 10×10×10 $mm^3$, mice were in abrosia for 6 h before IgG-CPDs were injected intratumorally (5 mg/kg) or via rail vein (1 mg/kg). Mice were anesthetized by isoflurane before imaging and in vivo imaging was taken at different time points after injection. Mice were sacrificed after in vivo imaging and the major organs including heart, kidney, spleen, lung, stomach, liver and tumor were excised and washed by PBS. Imaging of the organ was obtained as mentioned above (FITC: $\lambda_{ex}/\lambda_{em}$ = 490/520 nm), and imaging of the whole body was obtained as mentioned above (FITC: $\lambda_{ex}/\lambda_{em}$ = 490/520 nm; **DCM**: $\lambda_{ex}/\lambda_{em}$ = 500/680~720 nm). Mice treated with or without **IgG$^{FITC}$-CPD$_{Arg}$/CPD$_{IMD}$** were sacrificed after in vivo imaging and the tumor was excised and washed by PBS. Then the tumor was embedded in paraffin

before the central part of each tumor was sectioned into 10 μm-thick slices. Representative confocal images of the tumor section were obtained with the CLSM (FITC: $\lambda_{ex}/\lambda_{em}$ = 490/520 nm).

## 4. Supplementary figures

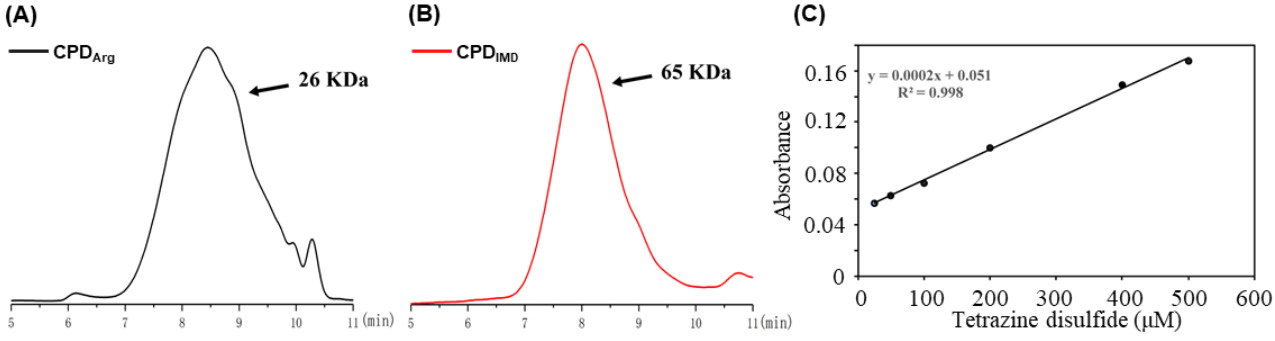



**Figure S1.** Characterization of CPDs. Representative GPC profiling of (A) **CPD$_{Arg}$** and (B) **CPD$_{IMD}$**. (C) UV-Vis absorption ($A_{520nm}$) of tetrazine disulfide (**2**) at different concentrations (25, 50, 100, 200, 400, 500 μM) to generate the standard curve (y = 0.0002 x +0.051, $R^2$ = 0.998) for calculating CPD concentration.

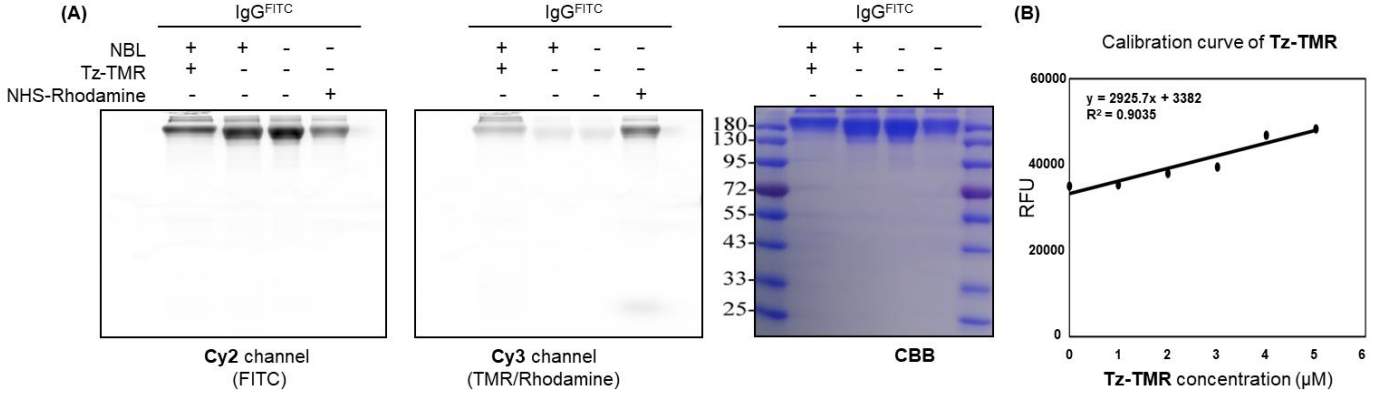



**Figure S2.** Conjugation efficiency of **NBL** with IgG. (A) Proteins labelled with FITC were reacted with **NBL** as follows: 225 μL of 10 μM protein was reacted with 2.5 μL of 20 mM **NBL** in $NaHCO_3$ (100 mM, pH = 9.2; total volume of 250 μL) for overnight at 4 °C. The reaction mixture was clicked with 12.5 μM of **Tz-TMR** (in PBS, pH = 7.4; structure shown in Table S1) for 1 h at room temperature. 5-fold pre-chilled acetone was added to precipitate the protein out from the excess **Tz-TMR**, and the resulting protein was resuspended in PBS, separated by SDS-PAGE and visualized by in-gel fluorescence scanning (FL) as well as Coomassie brilliant blue staining (CBB). The NHS-Rhodamine was use as the positive control. (B) The linear relationship between the fluorescence intensity of **Tz-TMR** and its concentration. The labeling degree of

**NBL** (clicked with **Tz-TMR**) on IgG was estimated by determining the *Dye* (i.e., **Tz-TMR**): *Protein* ratio (as **2 : 1**) according to the fluorescence of dye following reported protocol[9]. To be noted, excess dyes were removed before calculation.

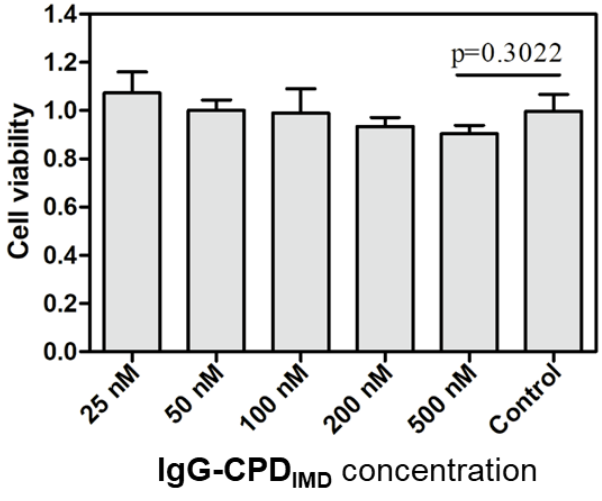



**Figure S3.** CCK-8 viability assay of HeLa cells upon treatment with different concentrations of **IgG-CPD$_{IMD}$** after incubation for 60 min at 37 °C. Cells were washed and incubated for another 24 h before test. Error bars were obtained from triplicate.

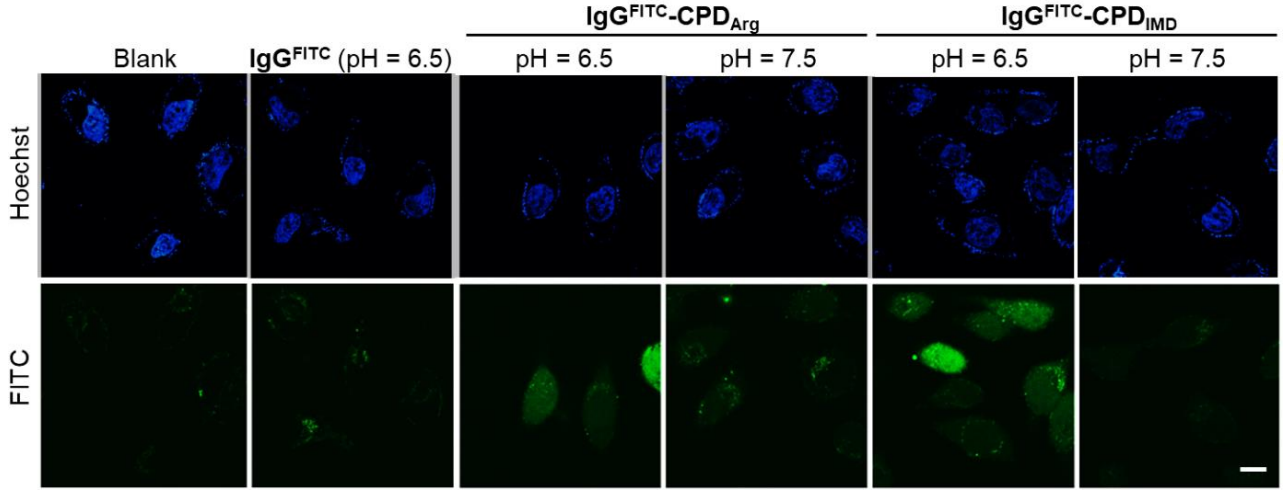



**Figure S4.** Delivery of FITC labelled IgG by **CPD$_{IMD}$** in comparison with **CPD$_{Arg}$**. CLSM images of HeLa cells incubated with buffer (blank)/**IgG$^{FITC}$**/**IgG$^{FITC}$-CPD$_{Arg}$**/**IgG$^{FITC}$-CPD$_{IMD}$** (20 nM of IgG) in DMEM medium at pH = 6.5 or 7.5 for 2 h before imaging. Cells were co-stained with Hoechst 33342 for nuclei. $\lambda_{ex}$ = 350 nm, $\lambda_{em}$ = 461 nm. Scale bar = 20 μm.

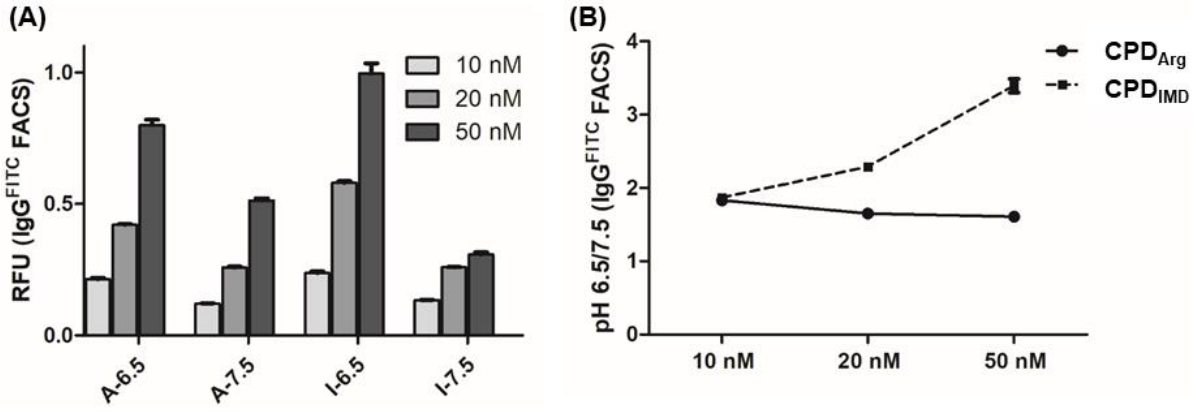



**Figure S5.** FACS quantification of **IgG$^{FITC}$** uptake in living HeLa cells with **CPD$_{IMD}$**/**CPD$_{Arg}$** at pH = 6.5 or 7.5. HeLa cells from a 6-well plate were treated with different concentrations (10, 20, or 50 nM of the protein) of **IgG$^{FITC}$-CPD$_{Arg}$** (**A** for short in the graph)/**IgG$^{FITC}$-CPD$_{IMD}$** (**I** for short) in DMEM at pH = 6.5 or 7.5 for 2 h, followed by FACS analysis. (A) The amount of IgG$^{FITC}$ delivered into the cell was indicated by the average fluorescence intensity of FITC. Here the fluorescence intensity was normalized to the strongest signal (set as 1.0). (B) The ratio of the average FITC signal at pH 6.5 to that at pH 7.5 was calculated for both **CPD$_{IMD}$** and **CPD$_{Arg}$**.

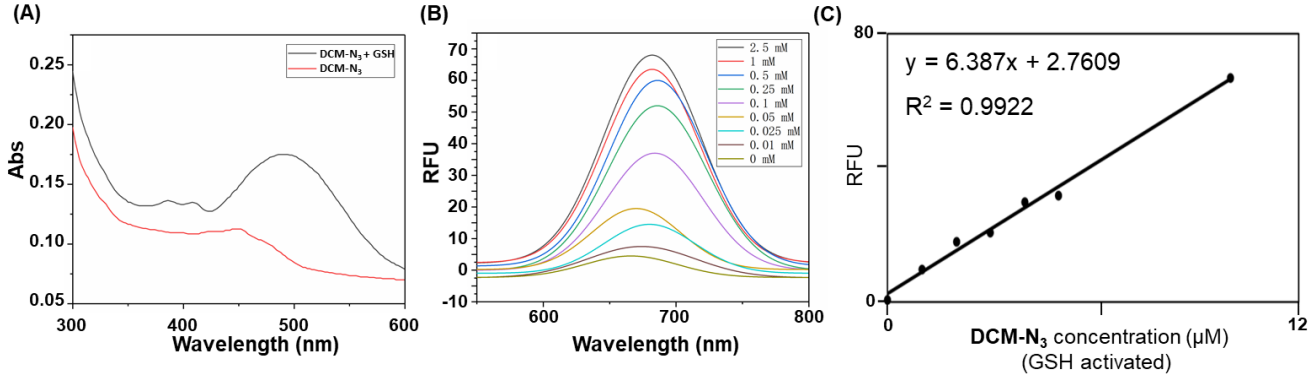



**Figure S6.** Photophysical properties of **DCM-N$_3$** (10 μM) in a solution of DMSO/PBS (pH=7.4, 10 mM; 50/50, v/v). (A) Absorption spectra of **DCM-N$_3$** with or without GSH (2.5 mM). (B) Fluorescence spectra of **DCM-N$_3$** upon the addition of different concentrations of GSH (2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0 mM). All spectra were recorded after incubation for 30 min at room temperature with $\lambda_{ex}$ = 490 nm (consistent with confocal and in vivo experiments). (C) The linear relationship between the fluorescence intensity of **DCM-N$_3$** (activated by 2.5 mM GSH for 0.5 h) and the concentration. The labeling ratio of **DCM-N$_3$** on IgG was estimated by determining the *Dye* (i.e., activated **DCM-N$_3$**): *Protein* ratio (as 1 : 1) according to the

fluorescence of dye following reported protocol[9]. To be noted, excess dyes were removed before test, and **IgG$^{DCM}$** was pre-incubated with 2.5 mM GSH for 0.5 h to turn-on **DCM-N$_3$**.

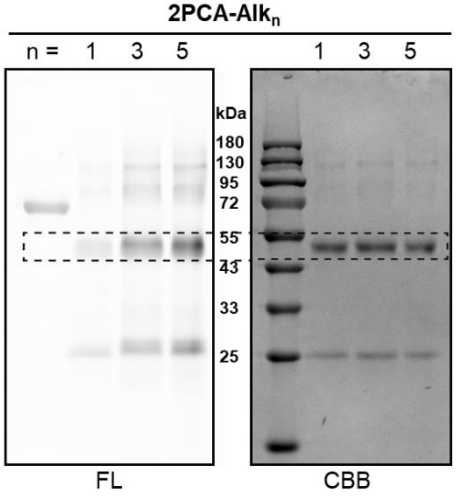



**Figure S7.** Labeling efficiency of **2PCA-Alk$_n$** (n = 1, 3, 5 for the PEG length; structures shown in Table S1) towards IgG as estimated by reaction with **TER-N$_3$** (structures shown in Table S1) followed by SDS-PAGE/in-gel fluorescence scanning (left) and CBB staining (right). **2PCA-Alk$_5$** was chosen for N-terminal labeling of IgG.

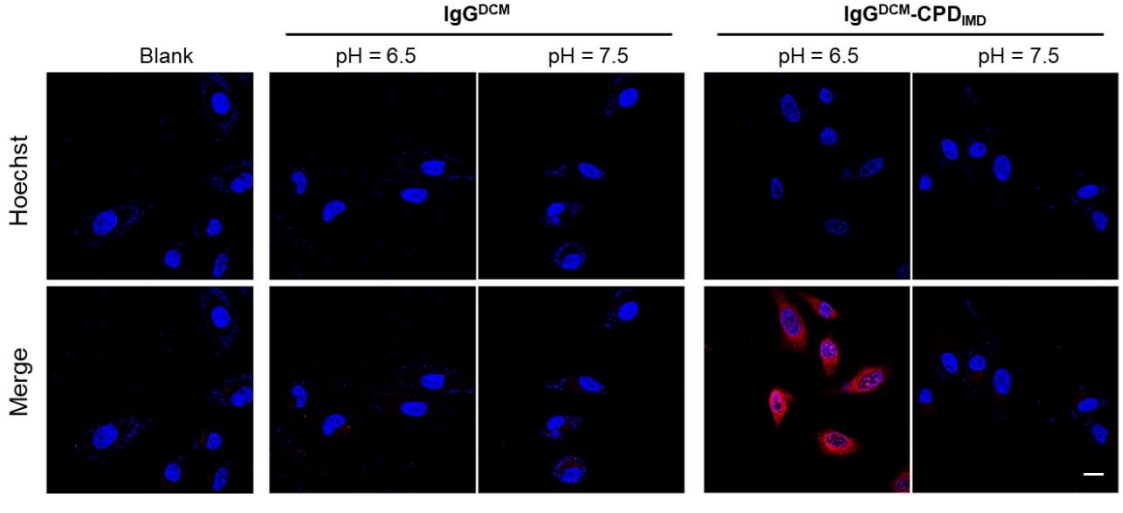



**Figure S8.** CLSM images of HeLa cells showing intracellular delivery of **IgG$^{DCM}$**. HeLa cells were treated with buffer (blank)/**IgG$^{DCM}$**/**IgG$^{DCM}$-CPD$_{IMD}$** (20 nM of IgG) in DMEM medium at pH = 6.5 or 7.5 for 3 h

before imaging. Cells were co-stained with Hoechst 33342 (for nuclei). $\lambda_{ex}$ = 490 nm, $\lambda_{em}$ = 680 nm. Scale bar = 20 μm.

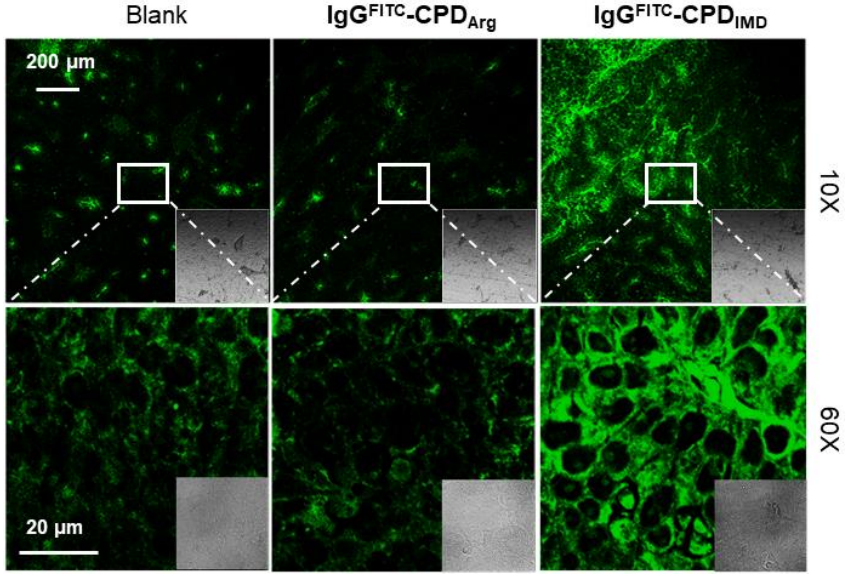



**Figure S9.** CLSM of tumor slices extracted from the mice after 2-h treatment of **IgG$^{FITC}$-CPD$_{Arg}$** or **IgG$^{FITC}$-CPD$_{IMD}$** (5 mg/kg) via intravenous injection together with the blank group (mice injected with buffer only). $\lambda_{ex}$ = 490 nm, $\lambda_{em}$ = 520 nm. Part of the figure was also shown in the maintext.

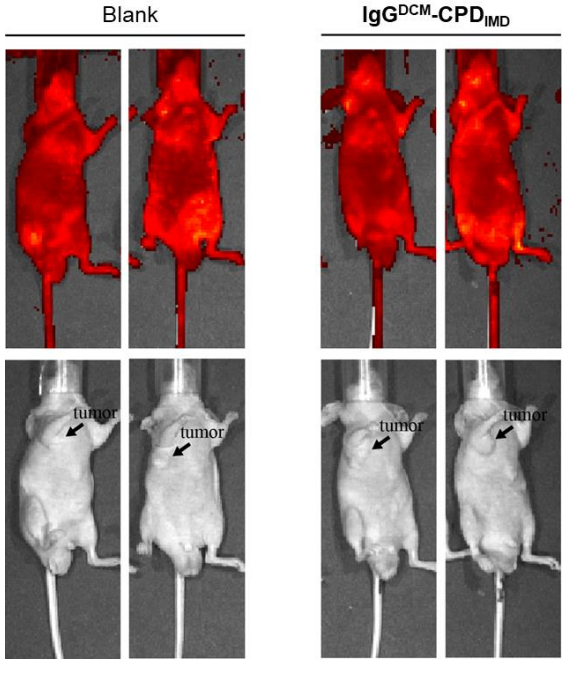



**Figure S10**. In vivo imaging of SW620 tumor-bearing mice after tail intravenous injection of buffer (blank)

or **IgG$^{DCM}$-CPD$_{IMD}$** (1 mg/kg) for 5 h. $\lambda_{ex}$ = 500 nm, $\lambda_{em}$ = 680 nm.

## 5. References


(1) Fu, J.; Yu, C.; Li, L.; Yao, S., Intracellular Delivery of Functional Proteins and Native Drugs by Cell-Penetrating Poly(disulfide)s. *J. Am. Chem. Soc.* **2015**, *137* (37), 12153-12160.

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(5) Wu, X.; Sun, X.; Guo, Z.; Tang, J.; Shen, Y.; James, T.; Tian, H.; Zhu, W., In Vivo and in Situ Tracking Cancer Chemotherapy by Highly Photostable NIR Fluorescent Theranostic Prodrug. *J. Am. Chem. Soc.* **2014**, *136* (9), 3579-3588.

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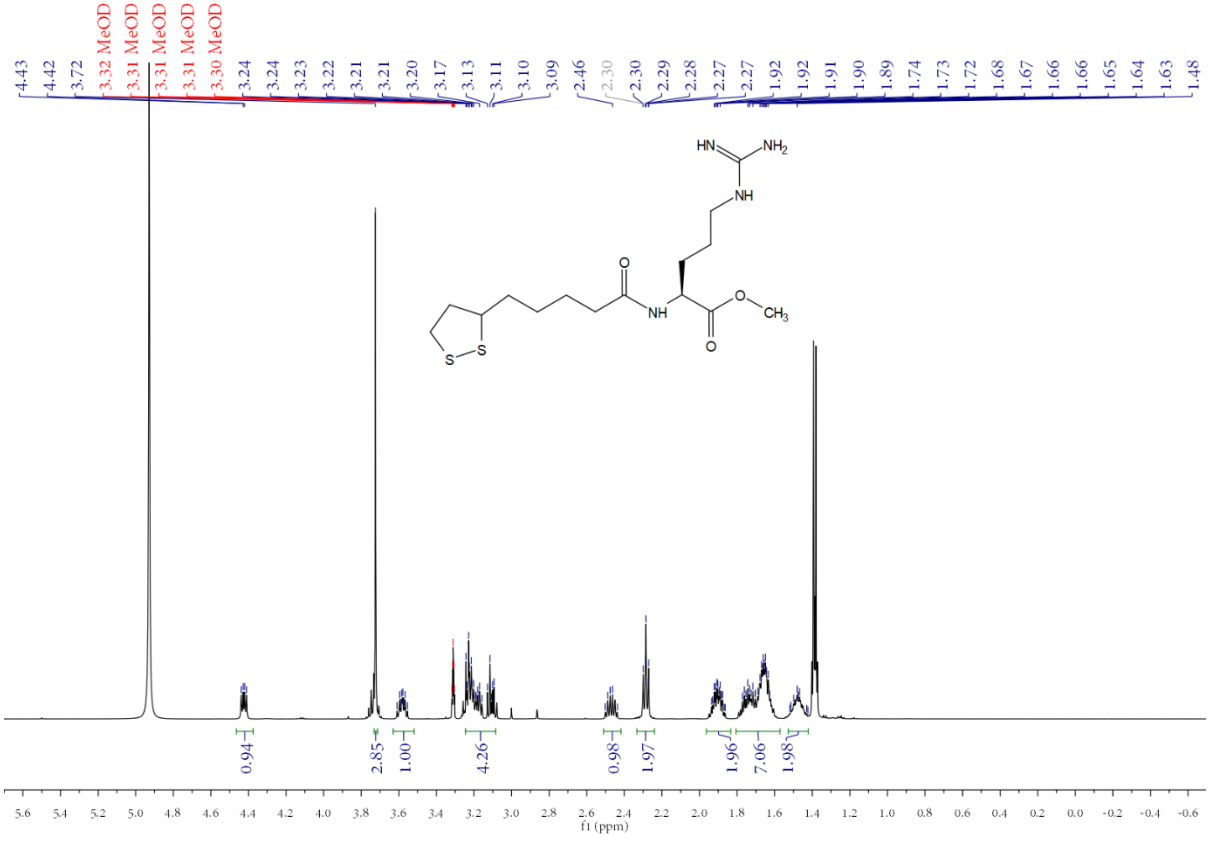
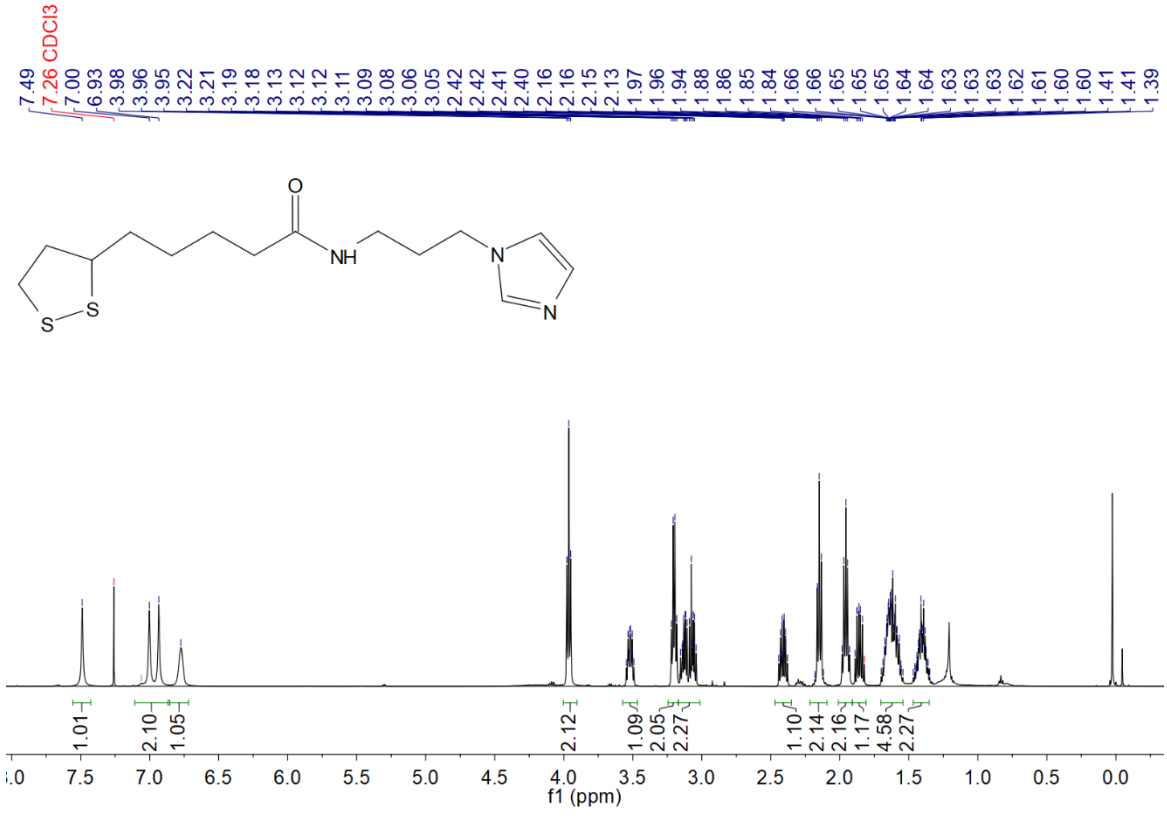
(8) Wang, W.; Zhang, Y.; Zhao, H.; Zhuang, X.; Wang, H.; He, K.; Xu, W.; Kang, Y.; Chen, S.; Zeng, S.; Qian, L. *Chem. Sci.*, **2021**, *12*, 13477-13482.

(9) Liew, S.; Zhang, C.; Zhang, J.; Sun, H.; Li, L.; Yao, S., Intracellular Delivery of Therapeutic Proteins through N-terminal Site-Specific Modification. *Chem. Commun.* **2020**, *56* (77), 11473-11476.

## 6. NMR and HRMS spectra

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3.12
3.12
3.11
3.09
3.08
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3.05
2.42
2.42
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1.39
O
NH
N
N
S
S
1.01
2.10
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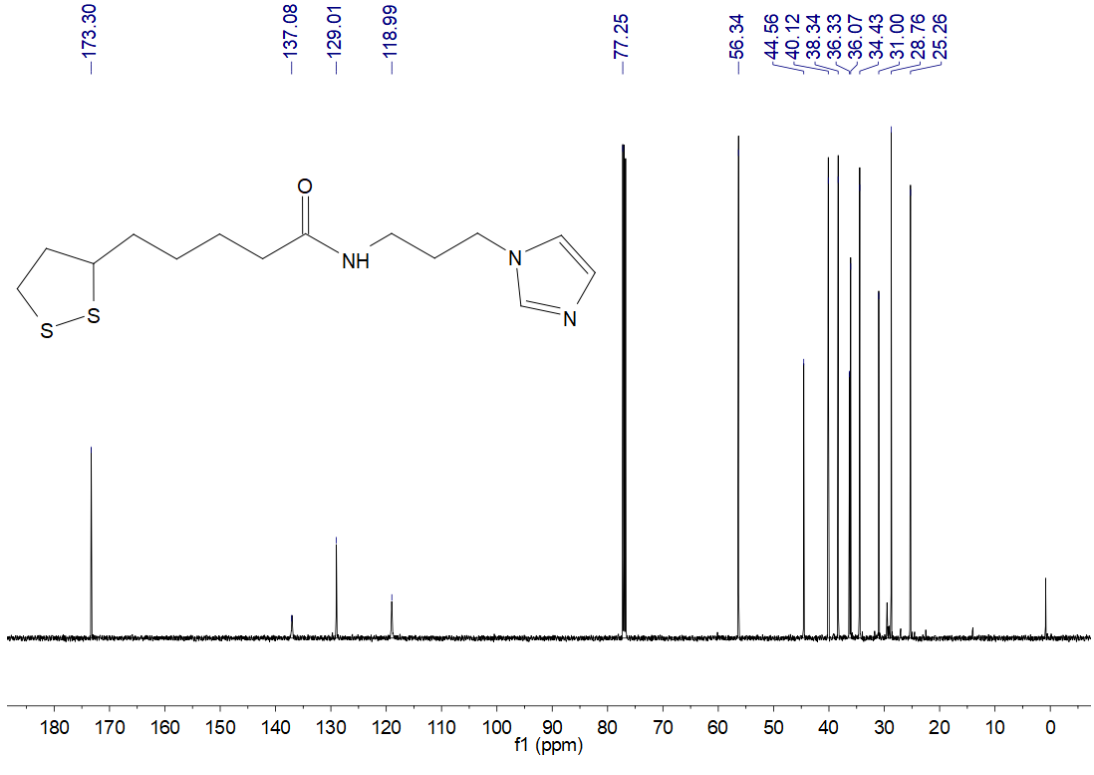
1.17
4.58
2.27
f1 (ppm)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O
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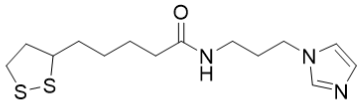
N
N
S
S
f1 (ppm)



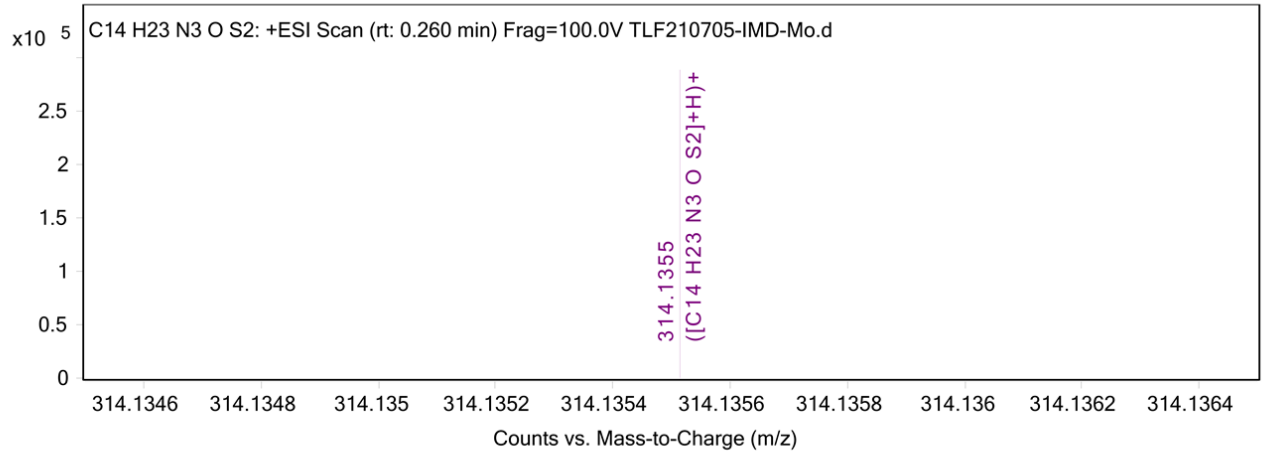

IMD-M

**ESI-HRMS**: m/z $[M+H]^+$ calcd, 314.1355; found, 314.1355.

**Spectra**

---

| Fragmentor Voltage | Collision Energy | Ionization Mode |
|---|---|---|
| 100 | 0 | ESI |

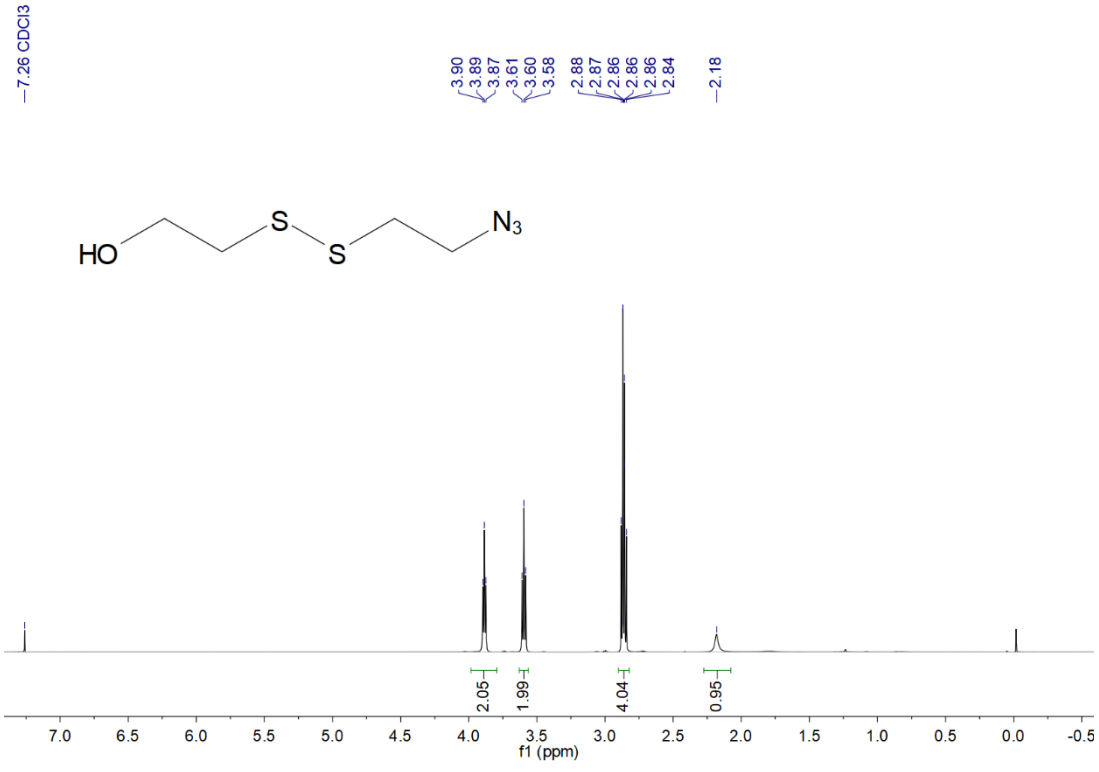



**Formula Calculator Element Limits**

| Element | Min | Max |
|---|---|---|
| C | 0 | 100 |
| H | 0 | 200 |
| O | 0 | 50 |
| N | 0 | 10 |
| S | 0 | 2 |

**Formula Calculator Results**

| Formula | Best | Measured Mass | Tgt Mass | Diff (ppm) | Score |
|---|---|---|---|---|---|
| C14 H24 N3 O S2 | True | 314.1355 | 314.1355 | 0.33 | 99.92 |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HO
S
S
N3
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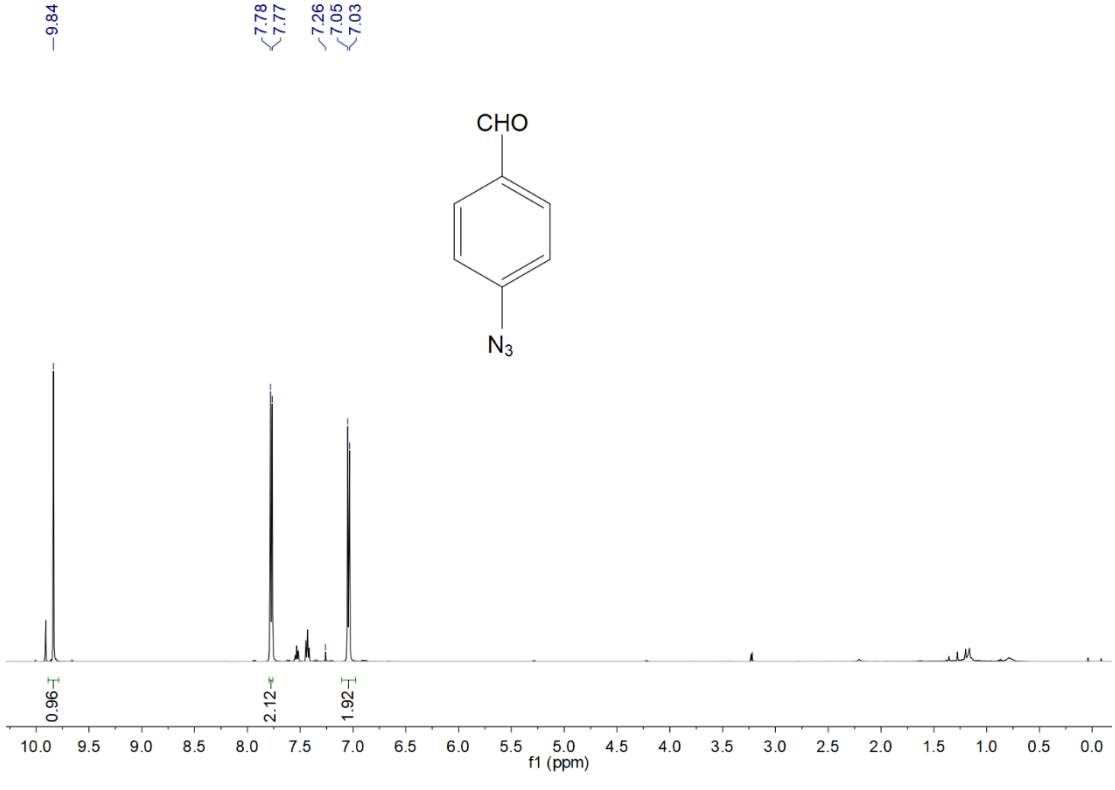
0.95
f1 (ppm)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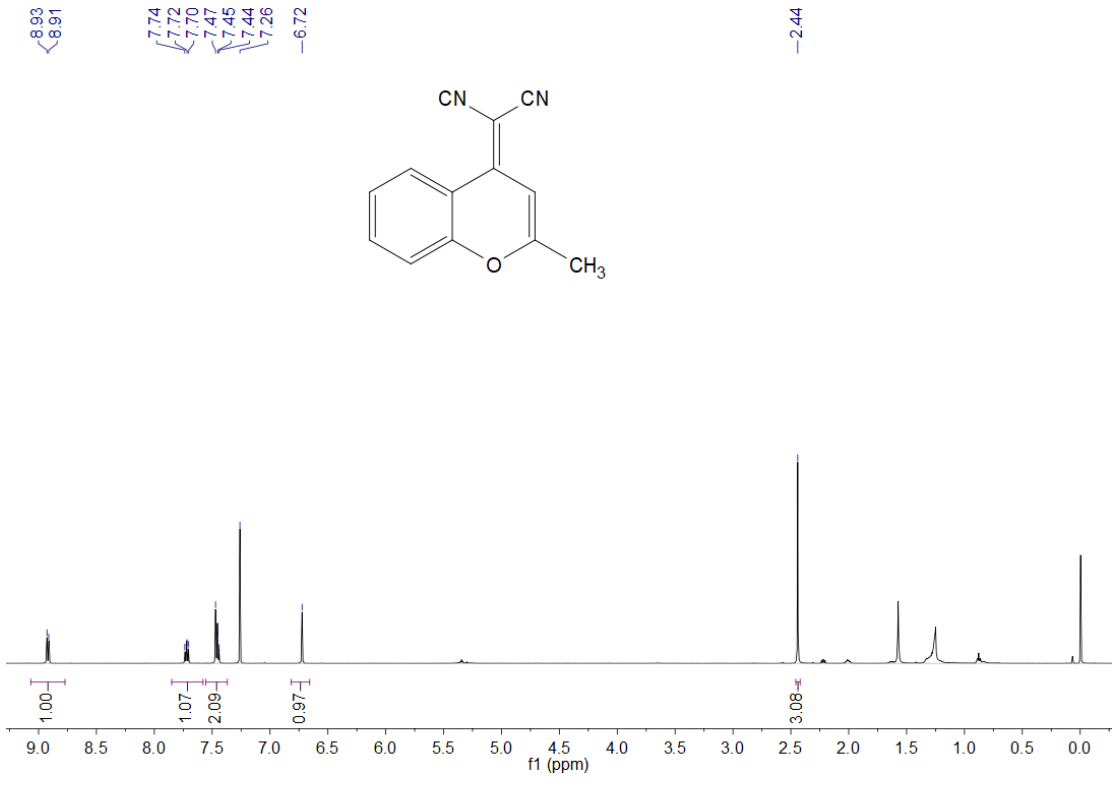
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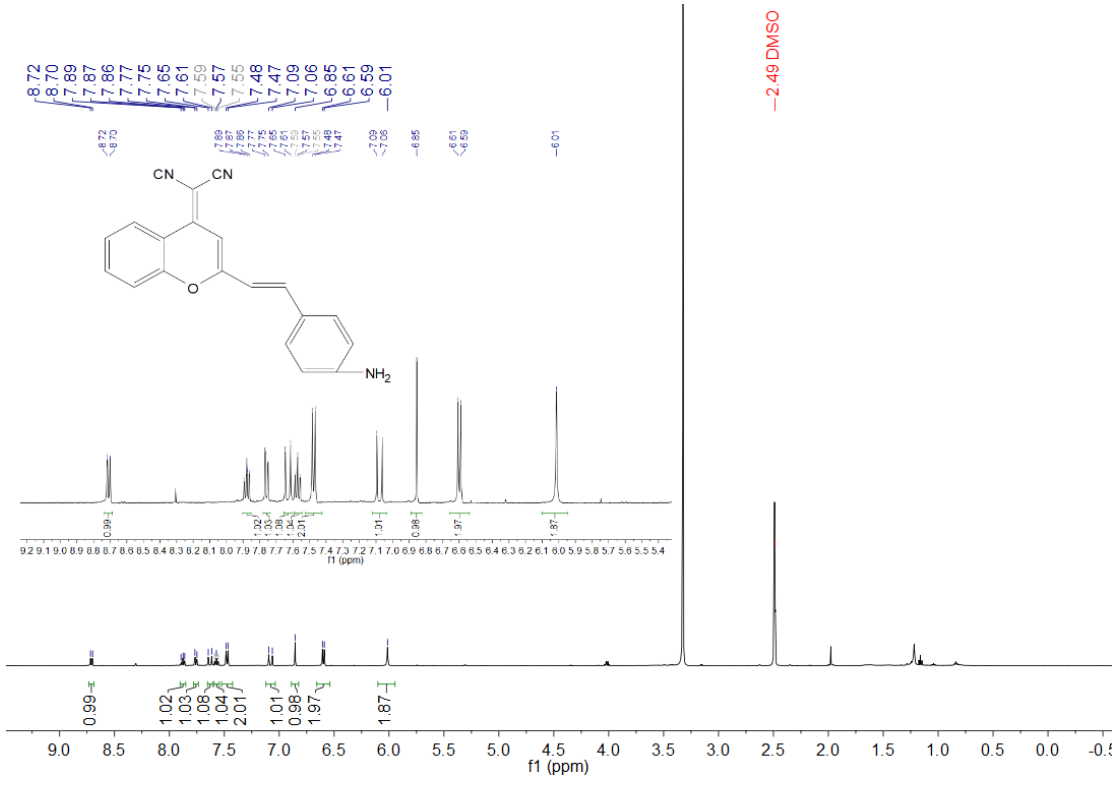
f1 (ppm)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7.09
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6.61
6.59
6.01
2.49 DMSO
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CN
O
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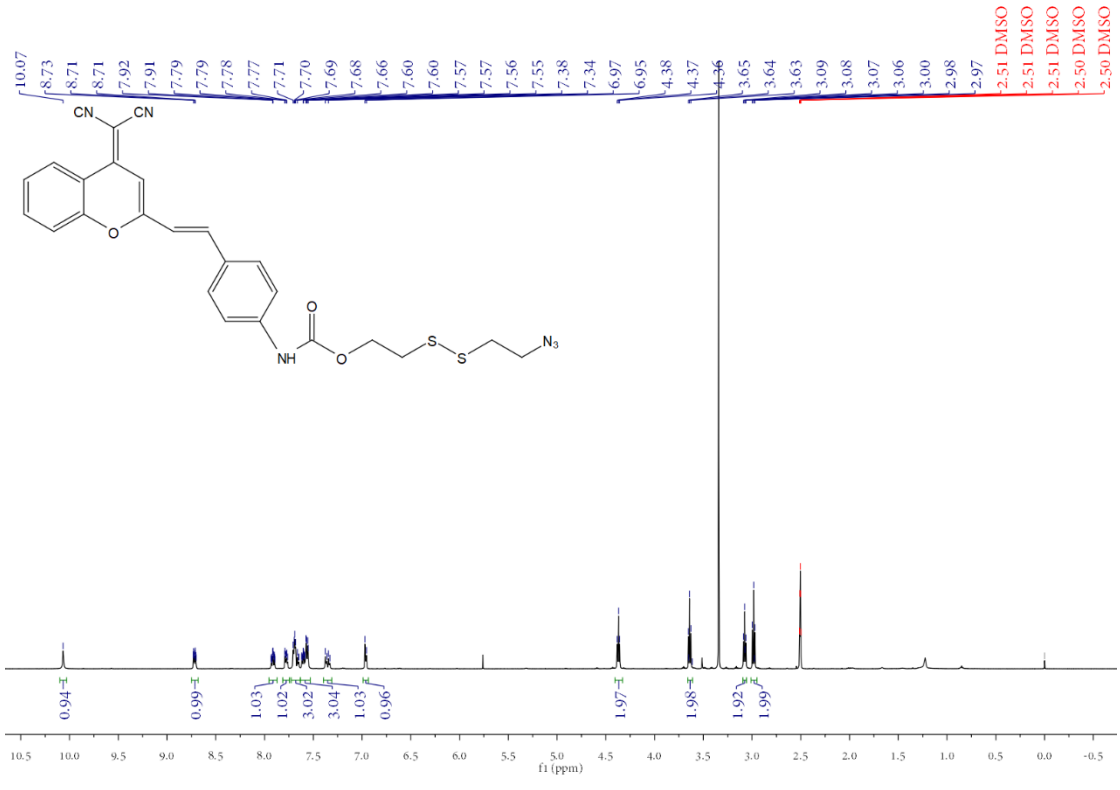
1.87
f1 (ppm)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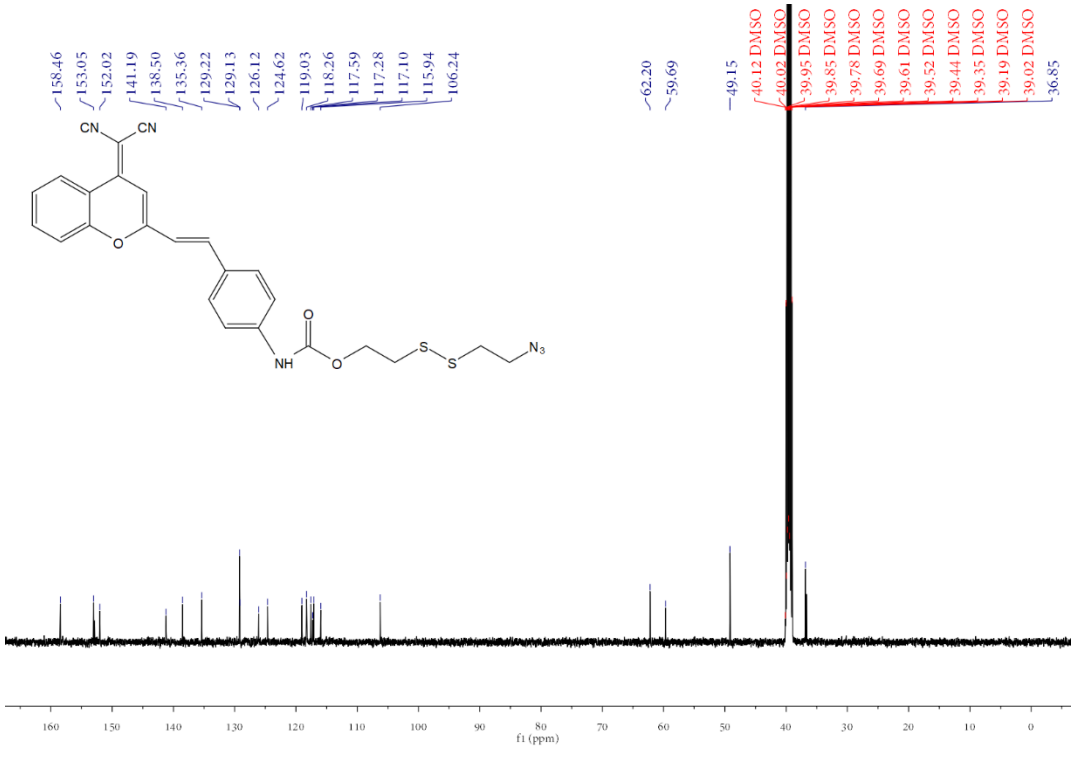
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39.02 DMSO
36.85
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CN
O
NH
O
O
S
S
N3
f1 (ppm)