Supporting Information

Logic-Gated Approach for Targeted Delivery and Site-Selective Activation of Photothermal Agents in Precision Cancer Treatment

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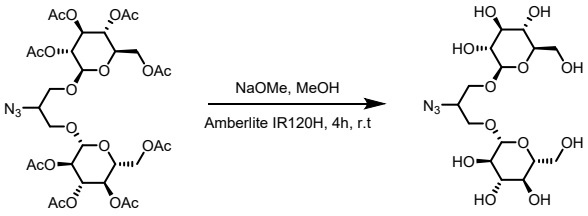
Materials. Materials were purchased from commercial vendors and used without further purification. Thin layer chromatography (TLC) was performed on glass-backed TLC plates precoated with silica gel containing an UV254 fluorescent indicator (Macherey-Nagel). TLCs were visualized with a 254/365 nm UV hand-held lamp (UVP). Flash silica gel chromatography was performed using 0.04 – 0.063 mm 60 M silica (Macherey-Nagel). Deuterated solvents were purchased from Cambridge Isotope Laboratories. Thermo Fisher Scientific: n-BuOH, DCM, THF, DMF, acetone, acetonitrile, acetic anhydride, galacial acetic acid, sulfuric acid, ethyl acetate, molecular sieves 4 Å-8+12 (ca 2 mm) beads, ferric chloride, phosphate saline buffer (Corning), and toluene. The following chemicals were purchased from Oakwood Chemicals: 4-dimethylaminopyridine (DMAP), hydroxyethyl disulfide, potassium carbonate, sodium sulfate (anhydrous), ammonium acetate, sodium ascorbate, sodium thiosulfate anhydrous, potassium carbonate, triflic acid, propargyl bromide, p-hydroxybenzaldehyde, 2,6-dichlorophenol, nitromethane, 2,6-pyridinemethanol, 2-aminomethylpyridine, sodium borohydride, trichloroacetonitrile, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), sodium azide, imidazole, 2-aminopropane-1,3-diol. The following chemicals were purchased from VWR: potassium hydroxide. The following chemicals were purchased from Sigma-Aldrich: phosgene solution (15 wt% in toluene), Amberlite IR120H, tetrakis(acetonitrile)copper(I) hexafluorophosphate, boron trifluoride etherate, palladium (II) tetrakis, acetyl chloride, thionyl chloride, sulfuryl chloride, and agarose. The following chemicals were purchased from AK Scientific:

camptothecin, 1,3-dimethylbarbituric acid (DBA). Methanol was purchased from Macron Fine Chemicals. The following chemicals were purchased from Alfa Aesar: triethylamine, allyl bromide, ethylenediamine. Iodine was purchased from TCI, and ethanol 200 proof was purchased from Decon Chemicals Inc. L-glucose was purchased from Aaron Fine Chemicals. Lysotracker Green®, Mitotracker Green®, and ERtracker Green® were acquired from Thermo Fischer. The following chemicals were purchased from Generic: sodium methoxide, tris(3-hydroxypropyltriazolylmethyl)amine.

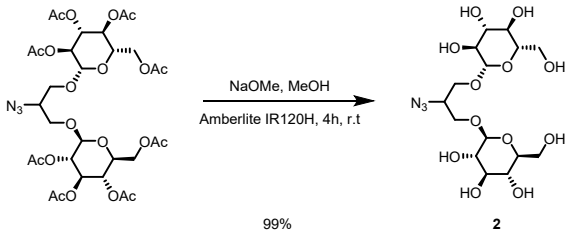
Instruments and Software. ^{1}H and ^{13}C NMR spectra were acquired on Varian 500, Carver B500, or B600 Bruker NEO spectrometers. The following abbreviations were used to describe coupling constants: singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), multiplet (m), and broad singlet (bs). Spectra were visualized and analysed using MestReNova (version 10.0). High resolution mass spectra were acquired with a Waters Q-TOF Ultima ESI mass spectrometer. Fluorescence spectra were acquired on a QuantaMaster-400 scanning spectrofluorometer with micro fluorescence quartz cuvettes (Science Outlet). Cells were counted using the Countess II FL Cell Counter (Invitrogen, Thermo Fisher Scientific). All the photothermal studies were conducted using an APT Lighting Science & Technology Co. 808nm Infrared IR Dot Laser Module. Thermal images and real-time temperature were collected with a TOOLTOP ET11S thermal camera multimeter. All other data analysis was performed using Microsoft Excel or Origin. Photoacoustic imaging was performed using the MSOT InVision 128 (iThera Medical). Reported values correspond to mean PA signals (Mean Pixel Intensity, MSOT a.u.) in regions of interest (ROIs) of equal area. Fluorescence imaging was performed using the CRi Maestro In-Vivo Fluorescence Imaging System. Images were analyzed using ImageJ and reported as the mean pixel intensity in ROIs of equal area.

Synthetic Procedures. Thin-layer chromatography (TLC) was performed on glass-backed TLC plates precoated with silica gel containing an UV254 fluorescent indicator (Macherey-Nagel). TLCs were visualized with a 254/365 nm UV hand-held lamp (UVP). Flash silica gel chromatography was performed using 0.04–0.063 mm 60 M silica (Macherey-Nagel). All glassware used under anhydrous reaction conditions were flame-dried under vacuum and cooled immediately before use. Acetal-protected azido mvGlu, acetal-protected l-azido mvGlu, and aza-BODIPY were prepared with published procedures. [1, 2]



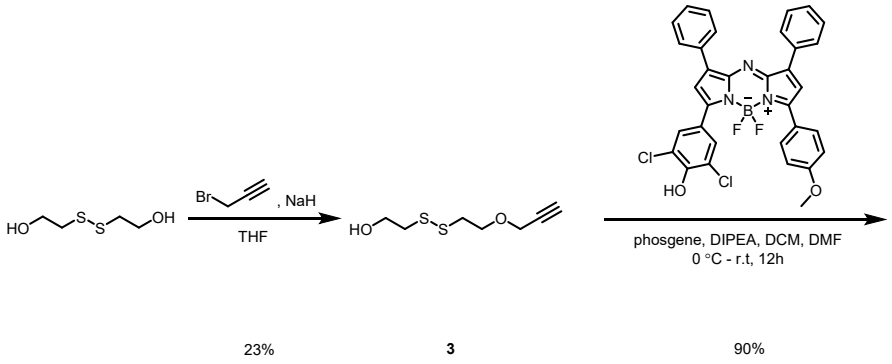
Scheme S1.

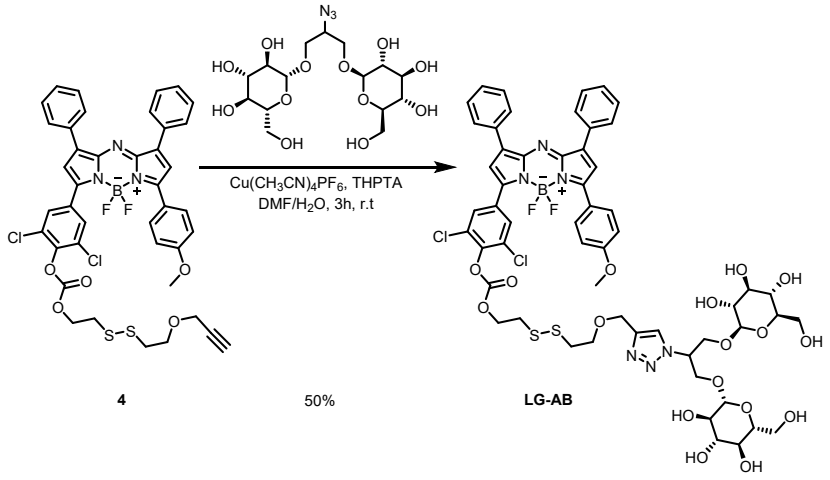
Azido mvGlu (1). Acetal-protected azido mvGlu (200 mg, 0.257 mmol, 1 equiv.) was dissolved in anhydrous methanol (6 ml) under a nitrogen atmosphere. A solution of NaOMe (35.4 mg, 0.643 mmol, 2.5 equiv.) in anhydrous methanol (6 ml) was then added. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 4 hours. The solid was filtered out followed by the addition of Amberlite IR120H. The solvent was removed via rotary evaporation to obtain **1** as a white solid (112 mg, 0.254 mmol, 99%). ^{1}H NMR (500 MHz, D_2O) δ 4.49 (dd, *J* = 8.1, 2.1 Hz, 2H), 4.09 (dd, *J* = 11.0, 3.4 Hz, 1H), 3.96 – 4.03 (m, 2H), 3.92 (dd, *J* = 12.4, 2.3 Hz, 2H), 3.87 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.79 (dd, *J* = 11.0, 6.6 Hz, 1H), 3.72 (dd, *J* = 12.3, 5.8 Hz, 2H), 3.44 – 3.51 (m, 4H), 3.36 – 3.41 (m, 2H), 3.29 (t, *J* = 8.0 Hz, 2H) ^{13}C NMR (125 MHz, D_2O) δ 102.8, 102.3, 75.9, 75.6, 73.0, 69.6, 68.6, 60.7.



Scheme S2.

L-azido mvGlu (2). Acetal-protected L-azido mvGlu (200 mg, 0.257 mmol, 1 equiv.) was dissolved in anhydrous methanol (6 ml) under a nitrogen atmosphere. A solution of NaOMe (35.4 mg, 0.643 mmol, 2.5 equiv.) in anhydrous methanol (6 ml) was then added. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 4 hours. The solid was filtered out followed by the addition of Amberlite IR120H. The solvent was removed via rotary evaporation to obtain **2** as a white solid (112 mg, 0.254 mmol, 99%). ^{1}H NMR (500 MHz, D_2O) δ 4.50 (d, *J* = 7.9 Hz, 2H), 3.68 – 4.10 (m, 9H), 3.46 – 3.53 (m, 4H), 3.40 (t, *J* = 9.4 Hz, 2H), 3.31 (t, *J* = 8.7 Hz, 2H) ^{13}C NMR (125 MHz, D_2O) δ 102.8, 102.3, 76.0, 75.6, 73.0, 69.6, 68.7, 60.7.





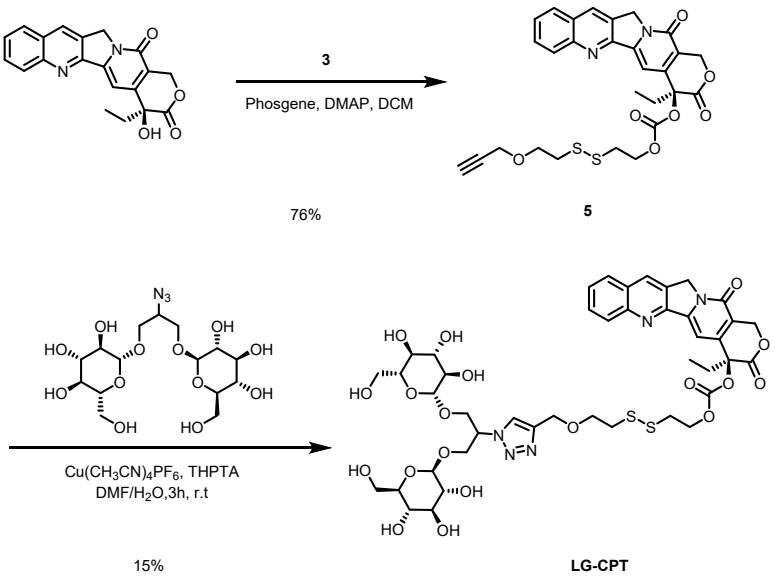
Scheme S3.

Compound 3. A flame-dried two-necked flask was charged with hydroxyethyl disulfide (7.6 g, 49.3 mmol, 2.0 equiv.), propargyl bromide (80 wt.% in toluene, 2.65 ml, 24.65

mmol, 1.0 equiv.) and anhydrous THF (75 ml) under a nitrogen atmosphere. NaH (60% dispersion in mineral oil, 1.63 g, 37 mmol, 1.5 equiv.) was added in three successive batches at 0 °C within 2 hours. The resulting reaction mixture was then warmed to room temperature and stirred under a nitrogen atmosphere for 8 hours. Followed by the addition of a few drops of H_2O, the mixture was filtered and dried via rotary evaporation. The crude oil residue was purified via column chromatography (SiO_2, 1:5 v/v EtOAc/Hexanes) to give the desired product **3** as a light-yellow oil (23%, 1.1 g, 5.7 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.20 (d, *J* = 1.4 Hz, 2H), 3.91 (q, *J* = 6.4 Hz, 2H), 3.81 (t, *J* = 6.4 Hz, 2H), 2.93 (t, *J* = 6.2 Hz, 2H), 2.89 (t, *J* = 5.6 Hz, 2H), 2.46 (t, *J* = 2.2 Hz, 1H), 1.99 (t, *J* = 6.2 Hz, 1H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 79.5, 75.0, 68.3, 60.4, 58.4, 41.6, 38.5.

Compound 4. A flame-dried flask was charged with **3** (23.56 mg, 122.5 µmol, 3.0 equiv.) and anhydrous CH_2Cl_2 (2.5 mL) under a nitrogen atmosphere. A solution of phosgene (15 wt.% in toluene, 0.233 mL, 367.5 µmol, 9 equiv.) and DIPEA (79.16 mg, 612.45 µmol, 15 equiv.) was added slowly at 0 °C. The resulting reaction mixture was stirred at 0 °C under a nitrogen atmosphere for 50 minutes. Anhydrous CH_2Cl_2 (3 ml) was added. The mixture was charged with dry nitrogen for 20 minutes. A solution of aza-BODIPY (25 mg, 40.83 µmol, 1.0 equiv.) in anhydrous CH_2Cl_2 (2.5 mL) was added to the reaction mixture followed by the addition of two drops of DMF. After overnight stirring (~15 hours), the reaction was concentrated under reduced pressure and the crude oil residue was purified via column chromatography (SiO_2, 1:1 v/v toluene/CH_2Cl_2) to give the desired product **4** as a dark-green solid (90%, 30.6 mg, 36.84 µmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 2H), 8.06 – 8.08 (m, 2H), 8.03 (d, *J* = 7.0 Hz, 2H), 8.00 (s, 2H), 7.44 – 7.49 (m, 5H), 7.16 – 7.20 (m, 2H), 7.06 (d, *J* = 9.0 Hz, 2H), 6.89 (s, 1H), 4.60 (t, *J* = 6.8 Hz, 2H), 4.21 (d, *J* = 2.4 Hz, 2H), 3.92 (s, 3H), 3.82 (t, *J* = 6.4 Hz, 2H), 3.09 (t, *J* = 6.7 Hz, 2H), 2.98 (t, *J* = 6.4 Hz, 2H), 2.47 (t, *J* = 2.4 Hz, 1H), 2.36 (s, 1H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 163.1, 151.4, 150.9, 147.6, 146.3, 144.5, 144.0, 141.5, 132.8, 132.5, 131.8, 130.3, 129.7, 129.6, 129.3, 129.2, 129.0, 128.9, 128.8, 128.4, 125.4, 123.0, 120.7, 117.6, 114.9, 79.5, 75.0, 68.1, 67.6, 58.4, 55.8, 38.9, 36.8, 22.2, 21.6.

LG-AB. A round-bottom flask was charged with **4** (30 mg, 36.12 μmol, 1.0 equiv.), $Cu(CH_3CN)_4PF_6$ (13.46 mg, 36.12 μmol, 1.0 equiv.), THPTA (15.69 mg, 36.12 μmol, 1.0 equiv.), DMF (3 ml), and H_2O (1 ml) under a nitrogen atmosphere. A solution of **1** (23.91 mg, 54.18 μmol, 1.5 equiv.) in H_2O (0.5 ml) was added. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 3 hours. Then, the solvent was removed via rotary evaporation and purified via reverse-phase chromatography (C18, 100% H_2O → 75% acetonitrile/H_2O) to afford the product as a green solid (23 mg, 18.06 μmol, 50%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.34 (d, J = 10.6 Hz, 2H), 8.21 – 8.25 (m, 5H), 8.13 (d, J = 8.7 Hz, 2H), 7.93 (s, 1H), 7.52 – 7.60 (m, 5H), 7.43 – 7.47 (m, 1H), 7.23 (d, J = 10.6 Hz, 2H), 5.13 – 5.20 (m, 2H), 5.07 (t, J = 6.5 Hz, 2H), 4.91 – 4.95 (m, 2H), 4.75 (q, J = 8.7 Hz, 2H), 4.46 – 4.61 (m, 5H), 4.20 – 4.26 (m, 5H), 4.08 – 4.17 (m, 5H), 3.93 (s, 3H), 3.72 (t, J = 7.6 Hz, 2H), 3.40 – 3.69 (m, 4H), 3.17 (d, J = 6.4 Hz, 3H), 2.92 – 3.00 (m, 3H), 2.29 (t, J = 8.8 Hz, 3H) ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.7, 163.2, 143.2, 139.8, 133.2, 132.1, 129.5, 129.3, 128.9, 128.8, 128.0, 124.3, 115.0, 113.7, 103.3, 103.2, 76.9, 76.5, 76.5, 73.3, 73.3, 72.3, 72.0, 70.0, 69.9, 67.9, 67.7, 67.5, 63.3, 62.8, 61.1, 61.0, 60.3, 60.2, 56.0, 40.1, 37.7, 36.0, 29.0. ESI HR-MS: Calculated for $C_{56}H_{60}BCl_2F_2N_6O_{17}S_2$ $[M+H]^+$ m/z 1271.2895, found 1271.2892.

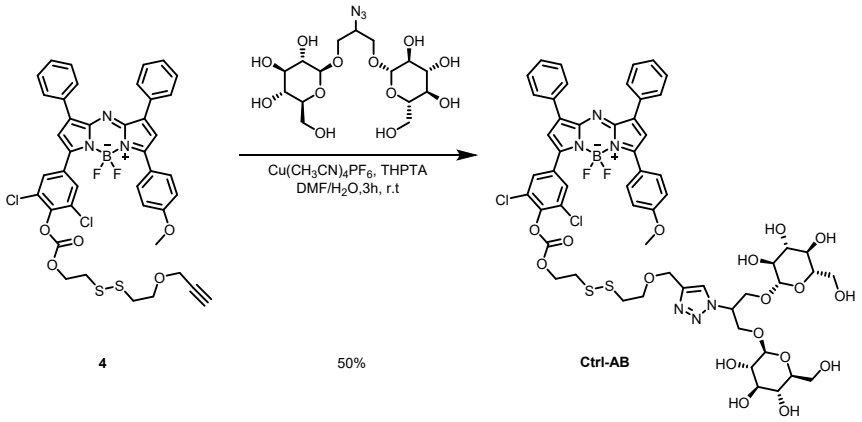


Scheme S4.

Compound 5. A flame-dried flask was charged with camptothecin (0.20 g, 0.57 mmol, 1.0 equiv.) and anhydrous CH_2Cl_2 (16 mL) under a nitrogen atmosphere. A solution of phosgene (15 wt.% in toluene, 0.54 mL, 0.86 mmol, 1.5 equiv.) was added slowly. A solution of 4-dimethylaminopyridine (0.35 g, 2.87 mmol, 5.0 equiv.) in anhydrous CH_2Cl_2 was then added dropwise. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 1.5 hours. A solution of **3** (0.09 g, 0.57 mmol, 1.0 equiv.) in anhydrous CH_2Cl_2 (4 mL) was added dropwise to the reaction mixture. After overnight stirring (~15 hours), the reaction was concentrated under reduced pressure and the crude oil residue was purified via column chromatography (SiO_2, 1:9 v/v MeOH:EtOAc) to give the desired product as an off-white solid (76%, 230 mg, 0.43 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 7.4 Hz, 1H), 7.83 – 7.86 (m, 1H), 7.66 – 7.70 (m, 1H), 7.34 (s, 1H), 5.71 (d, *J* = 17.2 Hz, 1H), 5.39 (d, *J* = 17.0 Hz, 1H), 5.26 – 5.34 (m, 2H), 4.33 – 4.42 (m, 2H), 4.13 (d, *J* = 2.4 Hz, 2H), 3.72 (t, *J* = 6.4 Hz, 2H), 2.93 – 2.97 (m, 2H), 2.87 (t, *J* = 6.2 Hz, 2H), 2.44 (t, *J* = 2.4 Hz, 1H), 2.12 – 2.33 (m, 2H), 1.01 (t, *J* = 7.5 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 157.5, 153.6, 152.5, 149.1, 146.6, 145.8, 131.3, 130.9, 129.9, 128.6, 128.3, 128.3, 120.5, 96.1, 79.5, 78.1, 75.0, 68.1, 67.2, 66.8, 58.3, 50.2, 38.8, 36.7, 32.1, 29.8, 7.8.

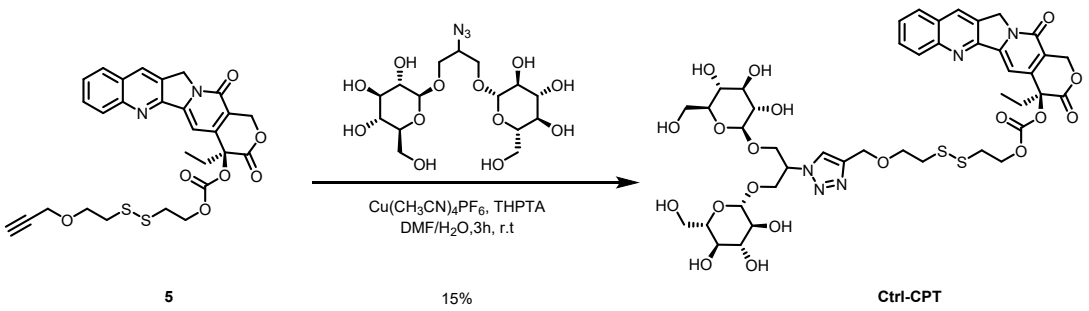
LG-CPT. A round-bottom flask was charged with **5** (25 mg, 44.12 μmol, 1.0 equiv.), $Cu(CH_3CN)_4PF_6$ (1.6 mg, 4.4 μmol, 0.1 equiv.), THPTA (1.9 mg, 4.4 μmol, 0.1 equiv.), DMF (3.75 ml), and H_2O (1 ml) under a nitrogen atmosphere. A solution of **1** (29.2 mg, 66.18 μmol, 1.5 equiv.) in H_2O (0.5 ml) was added. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 4 hours. Then, the solvent was removed via rotary evaporation and purified via HPLC to afford the product as a white solid (6.7 mg, 6.6 μmol, 15%). ^{1}H NMR (500 MHz, 1:1 v/v D_2O:CD_3CN) δ 8.95 (s, 1H), 8.17 (d, J = 8.6 Hz, 1H), 8.04 – 8.07 (m, 2H), 7.89 (t, J = 8.0 Hz, 1H), 7.73 (t, J = 7.6 Hz,1H), 7.39 (s, 1H), 5.66 (d, J = 16.8 Hz, 1H), 5.47 (d, J = 16.8 Hz, 1H), 5.24 (s, 2H), 5.09 – 5.11 (m, 1H), 4.35 – 4.46 (m, 6H), 4.25 – 4.30 (m, 2H), 3.79 – 3.83 (m, 3H), 3.58 – 3.63 (m, 4H), 3.31 – 3.41 (m, 4H), 3.24 – 3.26 (m, 2H), 3.16 (t, J = 8.0 Hz, 2H), 2.97 (t, J = 6.0 Hz, 2H), 2.82 (t, J = 6.2 Hz, 2H), 2.17 – 2.28 (m, 2H), 1.89 (d, J = 47.1 Hz, 1H), 1.01 (t, J = 7.4 Hz, 3H) ^{13}C NMR (150 MHz, 1:1 v/v D_2O:CD_3CN) δ 169.5, 158.7, 154.4, 149.0, 147.4, 147.2, 133.2, 131.9, 130.3, 129.6, 129.4, 129.1, 125.5, 103.9, 103.5, 97.6,

79.4, 77.0, 76.7, 74.0, 70.7, 70.7, 69.2, 68.9, 67.7, 67.4, 64.0, 62.0, 61.9, 61.7, 51.5, 38.7, 37.4, 31.8, 8.0. ESI HR-MS: Calculated for $C_{43}H_{54}N_5O_{19}S_2$ $[M+H]^+$ m/z 1008.2854, found 1008.2831.



Scheme S5.

Ctrl-AB. A round-bottom flask was charged with **4** (30 mg, 36.12 μmol, 1.0 equiv.), $Cu(CH_3CN)_4PF_6$ (13.46 mg, 36.12 μmol, 1.0 equiv.), THPTA (15.69 mg, 36.12 μmol, 1.0 equiv.), DMF (3 ml), and H_2O (1 ml) under a nitrogen atmosphere. A solution of **2** (23.91 mg, 54.18 μmol, 1.5 equiv.) in H_2O (0.5 ml) was added. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 3 hours. Then, the solvent was removed via rotary evaporation and purified via reverse-phase chromatography (C18, 100% H_2O → 75% acetonitrile/H_2O) to afford the product as a green solid (23 mg, 18.06 μmol, 50%).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, J = 8.5 Hz, 2H), 8.23 (s, 2H), 8.11 – 8.20 (m, 4H), 7.90 (s, 1H), 7.51 – 7.57 (m, 5H), 7.41 – 7.46 (m, 1H), 7.21 (d, J = 8.5 Hz, 2H), 5.05 – 5.12 (m, 2H), 4.93 – 4.98 (m, 3H), 4.78 – 4.81 (m, 1H), 4.47 – 4.60 (m, 7H), 3.94 – 4.22 (m, 4H), 3.92 (s, 3H), 3.81 – 3.87 (m, 3H), 3.64 – 3.73 (m, 5H), 3.49 (q, J = 5.3 Hz, 2H), 3.08 – 3.10 (m, 5H), 3.00 – 3.05 (m, 2H), 2.92 – 2.98 (m, 4H) ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.7, 163.3, 150.9, 149.0, 147.2, 145.4, 143.5, 139.8, 133.2, 132.2, 131.1, 130.5, 129.5, 129.0, 128.1, 123.8, 122.0, 115.0, 103.4, 103.1, 77.0, 76.6, 73.3, 72.3, 72.0, 70.0, 67.8, 67.5, 63.4, 62.8, 62.4, 61.1, 60.9, 60.3, 56.0, 37.8, 37.7, 36.0, 29.1. ESI HR-MS: Calculated for $C_{56}H_{60}BCl_2F_2N_6O_{17}S_2$ $[M+H]^+$ m/z 1271.2895, found 1271.2909.



Scheme S6.

Ctrl-CPT. A round-bottom flask was charged with **5** (25 mg, 44.12 μmol, 1.0 equiv.), $Cu(CH_3CN)_4PF_6$ (1.6 mg, 4.4 μmol, 0.1 equiv.), THPTA (1.9 mg, 4.4 μmol, 0.1 equiv.), DMF (3.75 ml), and H_2O (1 ml) under a nitrogen atmosphere. A solution of **2** (29.2 mg, 66.18 μmol, 1.5 equiv.) in H_2O (0.5 ml) was added. The resulting reaction mixture was stirred at room temperature under a nitrogen atmosphere for 4 hours. Then, the solvent was removed via rotary evaporation and purified via HPLC to afford the product as a white solid (6.7 mg, 6.6 μmol, 15%). ^{1}H NMR (500 MHz, 1:1 v/v D_2O:CD_3CN) δ 8.61 (s, 1H), 8.18 (d, J = 8.6 Hz, 1H), 8.05 – 8.09 (m, 2H), 7.90 (t, J = 7.6 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.41 (s, 1H), 5.66 (d, J = 16.8 Hz, 1H), 5.47 (d, J = 16.8 Hz, 1H), 5.27 (s, 2H), 5.09 – 5.11 (m, 1H), 4.43 – 4.47 (m, 3H), 4.35 – 4.41 (m, 3H), 3.79 – 3.85 (m, 3H), 3.31 – 3.42 (m, 6H), 3.16 (t, J = 8.4 Hz, 2H), 2.97 (t, J = 6.0 Hz, 2H), 2.82 (t, J = 6.2 Hz, 2H), 2.11 – 2.28 (m, 5H), 1.91 (d, J = 42.6 Hz, 1H), 1.01 (t, J = 7.4 Hz, 3H) ^{13}C NMR (150 MHz, 1:1 v/v D_2O:CD_3CN) δ 169.6, 158.7, 154.4, 152.9, 149.0, 147.4, 147.2, 133.2, 131.9, 130.3, 129.6, 129.1, 125.5, 120.2, 103.9, 103.5, 97.6, 79.4, 77.0, 76.7, 74.0, 72.7, 72.7, 70.6, 68.9, 67.4, 64.0, 62.0, 61.5, 59.0, 51.5, 38.6, 37.4, 33.1, 31.8, 8.0. ESI HR-MS: Calculated for $C_{43}H_{54}N_5O_{19}S_2$ $[M+H]^+$ m/z 1008.2854, found 1008.2843.

Photophysical Characterization. Extinction coefficients and fluorescence quantum yields were acquired in experimental triplicates. Extinction coefficients were measured by performing serial dilutions of the compounds in 1:1 v/v MeCN:PBS (pH = 7.4) within the linear range (absorbance values between 0.05-1.5). Reported fluorescence quantum yields are relative quantum yields compared to dimethoxy aza-BODIPY (Φ=0.36, chloroform). The compounds were titrated into acetonitrile or 1:1 MeCN/PBS (pH = 7.4) such that their absorbance values were kept below 0.1 to prevent secondary absorbance events.

PA Imaging in Tissue-Mimicking Phantoms. Tissue phantoms were prepared by mixing agarose (750 mg) in milliQ water (49 mL). The solution was heated in a microwave oven in 30 second intervals until a viscous, homogeneous gel was formed. 2% milk (1 mL) was then added to the warm solution and mixed. The gel was transferred to 50 mL syringes with their tips removed. Plastic straws were put into the gel using a custom syringe holder. The phantom was allowed to harden for at least 30 minutes and then submerged in milliQ water within a 50 mL falcon tube. Sample solutions of LG-AB and AB (5 μM) were prepared in PBS, pipetted into plastic tubing, and sealed shut with hot glue. PA measurements were taken at 5 nm intervals (660 - 1000 nm) and sample spectra were subtracted from background spectra of the buffer.

PTT in vitro. Different concentrations of AB or LG-AB were prepared by diluting 5 mM of stock solution in DMSO with PBS. 0.2 ml of each solution was pipetted to a 96-well plate. The 808 nm NIR laser was placed 5 cm above the wells. Temperatures were measured with a TOOLTOP thermometer every minute. Thermal images were taken every other minute.

GSH in vitro Turn-on. The initial fluorescence signal (550 to 850 nm) of LG-AB (5 μM in 1:1 v/v MeCN:PBS, pH = 7.4) was measured before the addition of glutaththione with 1:1 v/v MeCN:PBS (pH = 7.4). After addition, the cuvette was sealed and incubated for one hour at 37 °C. Final measurements were recorded, and the fluorescence fold turn-on was calculated by dividing the final fluorescence intensity by the initial fluorescence intensity.

LG-AB pH Stability Assay. The initial fluorescence signal (550 to 850 nm) of LG-AB (5 μM in 1:1 v/v MeCN:PBS, pH = 7.4) was measured before the addition of 1:1 v/v

MeCN:PBS at pH 4.0, 7.4 or 10.0. After addition, the cuvette was sealed and incubated for one hour at 37 °C. Final measurements were recorded, and the fluorescence fold turn-on was calculated by dividing the final fluorescence intensity by the initial fluorescence intensity.

In vitro Selectivity Assay. The initial fluorescence signal (550 to 850 nm) of LG-AB (5 μM in 1:1 v/v MeCN:PBS, pH = 7.4) was measured before the addition a panel of metal ions (100 μM), reactive oxygen species (50 μM) or amino acids (100 μM). After addition, the cuvette was sealed and incubated for one hour at 37 °C. Final measurements were recorded, and the fluorescence fold turn-on was calculated by dividing the final fluorescence intensity by the initial fluorescence intensity.

Cell Culture. A549 lung cancer cells and 4T1 murine breast cancer cells were acquired from ATCC. A549 cells were cultured in Ham's F-12K Medium containing 10% FBS and 1% penicillin streptomycin solution, and 4T1 cells were cultured in RPMI-1640 Medium containing 10% FBS and 1% penicillin streptomycin solution. Cells were incubated at 37 °C with 5% CO_2. Experiments were performed in 4-well plates (Nuc Lab-Tek Chambered Coverglass, Thermo Scientific) or 24, 48, 96 well plates (Nuclon Delta Surface Flat Bottom, Thermo Scientific).

MTT Cytotoxicity Assay. A 96-well plate was seeded with 8,000 A549 or 4T1 cells per well and incubated at 37 °C with 5% CO_2 until ~50% confluent (12 hours). The media was removed and replaced with fresh serum medium (200 μL) containing DMSO vehicle control (n = 6) or LG-AB (0.1, 0.5, 1, 10, or 50 μM) (n = 6). The cells were incubated under the abovementioned conditions for 12 hours. The media was then removed and replaced with a 20:1 mixture of PBS and (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 5 mg/mL stock in PBS). The cells were incubated for 3 hours under the same conditions, then the media was removed, and replaced with DMSO (100 μL). The absorbance of each well was recorded at 555 nm on a SpectraMax M2 plate reader. Percent viability was calculated relative to the vehicle control.

Trypan Blue Cytotoxicity Assay. A 24-well plated was seeded with 50,000 A549 or 4T1 cells per well and incubated at 37 °C with 5% CO_2 until ~90% confluent (36 hours). The media was removed and replaced with fresh serum medium (200 μL) containing DMSO

vehicle control (n = 6) or LG-AB (50 μM) (n = 6). The cells were incubated under the abovementioned conditions for 12 hours. Then the media was removed, and the cells underwent trypsinization. The cell suspension was diluted with fresh media and mixed with Trypan Blue solution 0.4% (w/v) in PBS in a 1:1 ratio. Viabilities were measured using the Countess II FL Cell Counter (Invitrogen, Thermo Fisher Scientific).

Cellular Uptake Studies. 4-well borosilicate plates were prepared with poly-L-Lysine coating and seeded with 100,000 cells per well and incubated at 37 °C with 5% CO_2 for 24 hours (~60% confluent). Serum-free media containing LG-AB (2 μM), LG-AB (2 μM) + 15 mM Glucose, Ctrl-AB (2 μM), or vehicle (DMSO) were prepared. Competition glucose wells were preincubated with 15 mM glucose in serum-free media for 30 minutes. Then the media was removed from each well, and each experimental condition was added and allowed to incubate for 30 minutes under the same conditions. The media was removed and replaced with PBS. Cells were imaged using an EVOS FL epifluorescence microscope with a Cy7 filter cube.

Quantification of Cell Imaging Data. In FIJI (ImageJ), ROIs were drawn around representative cells and the integrated density (area x mean gray value) was measured. The total cell fluorescence was calculated by subtracting the integrated density of the cell by the background integrated fluorescence. 20 cells were measured in each picture taken. Values are reported as the average (± SD) of all replicates.

PTT in Live Cells. T75 flasks (Thermo Scientific) of 4T1 cells (~90% confluent) were treated with serum media containing LG-AB (40 μM), LG-AB (40 μM) + 15 mM Glucose, Ctrl-AB (40 μM), LG-AB (40 μM) + 0.5 mM buthionine sulfoximine (BSO), LG-AB (40 μM) + 0.5 mM BSO + 15 mM Glucose, or vehicle (DMSO) for 2 hours. Competition BSO groups were preincubated with 0.5 mM BSO for 24 hours, glucose groups were preincubated with 15 mM glucose for 30 minutes. Then the media was removed, and the cells underwent trypsinization. Cell populations were counted using the Countess II FL Cell Counter to ensure transfer the same number of cells to each Eppendorf tube. The extra media were removed, and the cells were irradiated with the 808 nm NIR laser for 10 minutes. 1 ml of PBS was added to make a diluted cell suspension which then mixed

with Trypan Blue solution 0.4% (w/v) in PBS in a 1:1 ratio. Viabilities were measured using the Countess II FL Cell Counter.

Cell Lysis Studies. T75 flasks (Thermo Scientific) of 4T1 cells (~90% confluent) were treated with serum media containing LG-AB (40 μM), LG-AB (40 μM) + 0.5 mM BSO for 1 hour. Competition BSO groups were preincubated with 0.5 mM BSO for 24h. Cell populations were counted using the Countess II FL Cell Counter to ensure transfer the same number of cells to each Eppendorf tube. The extra media were removed, and 0.5 ml of 1:1 v/v MeCN:PBS (pH = 7.4) were added followed by 2 minutes of sonication. After being centrifuged at 14,000 rcf for 10 minutes, the absorption spectrum was measured with UV-Vis.

Localization Studies with LG-AB. 4-well borosilicate plates were prepared with poly-L Lysine coating and seeded with 10,000 cells per well and incubated at 37 °C with 5% CO_2 for 24 hours (~60% confluent). Serum-free media containing LG-AB (10 μM) was prepared. Cells were incubated for 30 minutes with LG-AB. The cells were washed 1x with PBS and incubated with the relevant organelle tracker according to the manufacturer recommended protocol. Cells were imaged using an EVOS FL epifluorescence microscope.

LogD$_{7.4}$ Measurements. LogD measurements were conducted in technical triplicate and 5 experimental replicates. Samples at a known concentration (n = 3) were added to Eppendorf tubes containing PBS (pH = 7.4, 500 μL) and octanol (500 μL). The Eppendorf tubes were each vortexed for 30 seconds and subsequently subjected to microcentrifuge for 1 minute. Then the PBS and octanol layers were pipetted into a clear 96-well plate. The absorbance values were taken using a SpectraMax M2 plate reader. The log of (dye absorbance in octanol/dye absorbance in PBS) was calculated and averaged across different concentrations to generate the log$D_{7.4}$.

Wound-healing assay. A 48-well plated was seeded with 30,000 A549 or 4T1 cells per well and incubated at 37 °C with 5% CO_2 until ~90% confluent (36 hours). A wound was created by scratching the well with a 200 μL plastic pipette tip. Cells were treated with LG-CPT (1 μM) or vehicle (DMSO) for 24h. During this time, the wounds were monitored via brightfield microscopy (20x magnification) at various time points between 0 and 24 h

after the wound was introduced. The experiment was performed in triplicate (n = 3) and the standard deviations of the obtained mean values were used for error evaluation.

Live-Subject Statement. All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of the University of Illinois at Urbana–Champaign, following the principles outlined by the American Physiological Society on research animal use. The University of Illinois is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). USDA registration number: 33-R-0029 NIH animal assurance number: D16-00075 (A3118-01) (expiration 6/30/25) AAALAC: #00766. Accredited campuswide since 2001.

In vivo Imaging. Before all imaging, hair on the bottom half of mice was removed via an electric razor and depilatory cream. A background scan was taken of each mouse before injection. 1 mg/kg of LG-AB was injected (Retro-orbital) into the mice. Fluorescence imaging was performed using the CRi Maestro In-Vivo Fluorescence Imaging System. Images were analyzed using ImageJ and reported as the mean pixel intensity in ROIs of equal area.

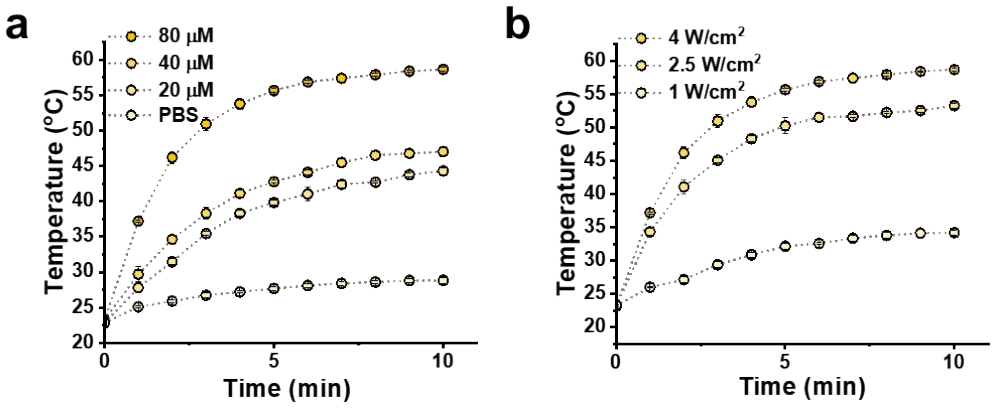


Figure S1. (a) Temperature variation of different concentrations of AB upon the irradiation of 808nm at different times. (b) Temperature variation of AB (80 μM) upon different power of irradiation at different times.

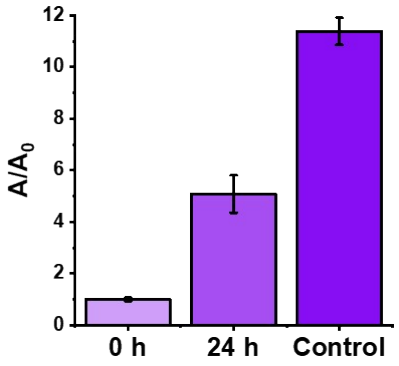


Figure S2. Time dependent turn-on with 0.1 mM GSH. Positive control is 10 mM GSH 1 hour incubation.

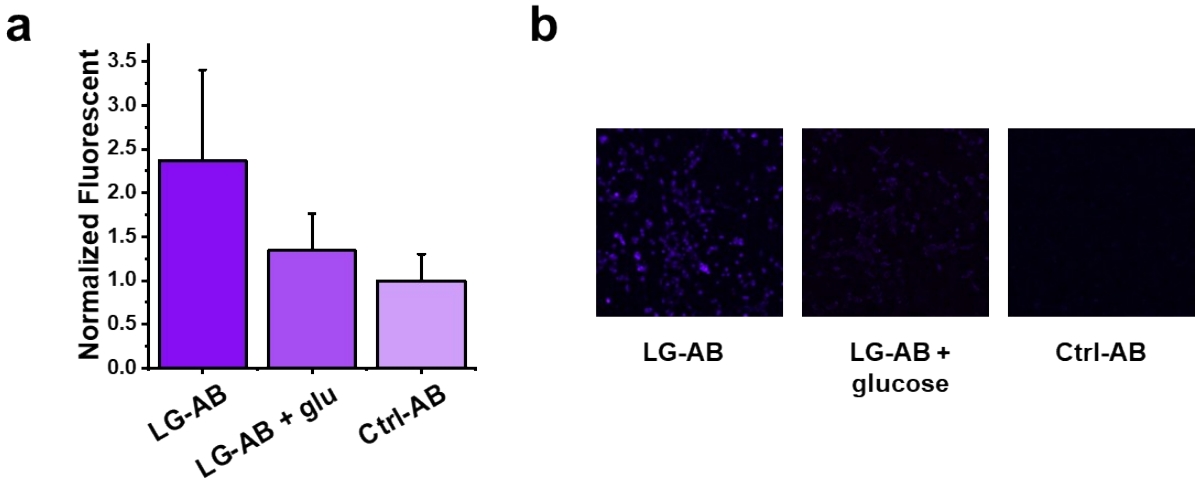


Figure S3. (a) Representative fluorescent images of 4T1 cells treated with LG-AB, LG-AB + glucose, or Ctrl-AB.(b) Quantified data from panel (a).

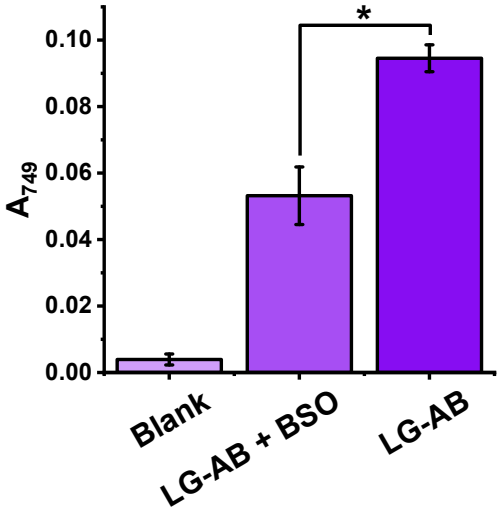


Figure S4. Absorption at 749 nm (turn-over product AB) of the cell lysis treated with different conditions.

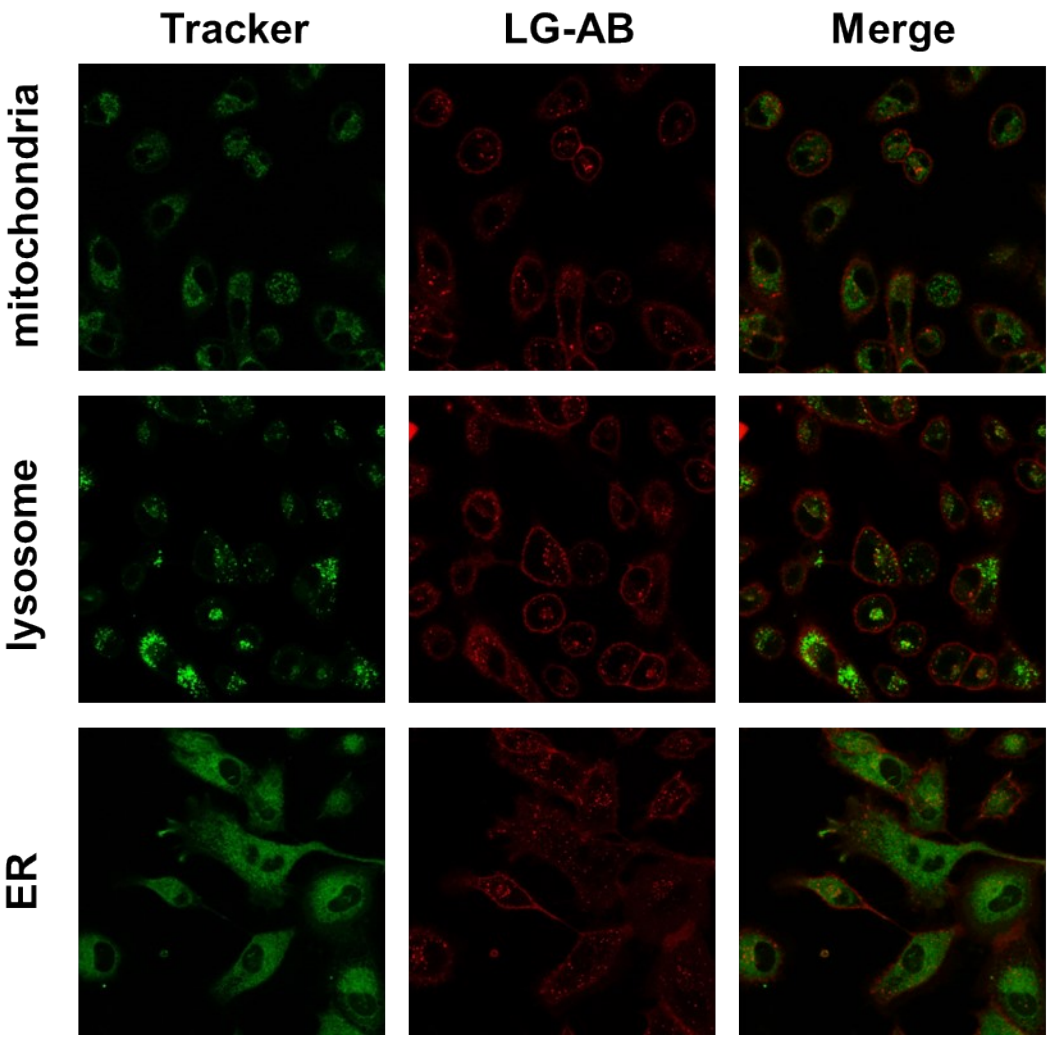


Figure S5. Localization studies of LG-AB (red) with MitoTracker Green® (green), Lysotracker Green® (green), or ER Tracker Green® (green) in A549 cells.

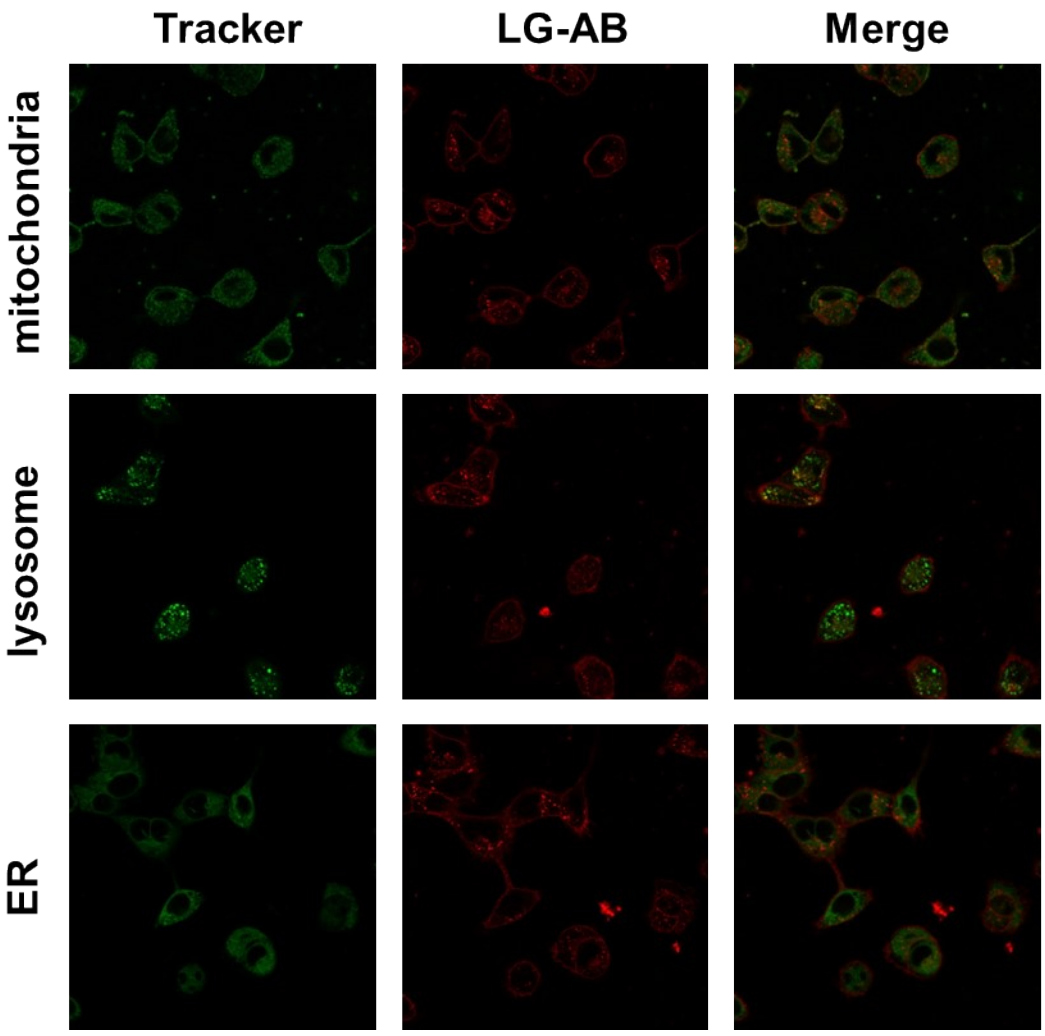


Figure S6. Localization studies of LG-AB (red) with MitoTracker Green® (green), Lysotracker Green® (green), or ER Tracker Green® (green) in 4T1 cells.

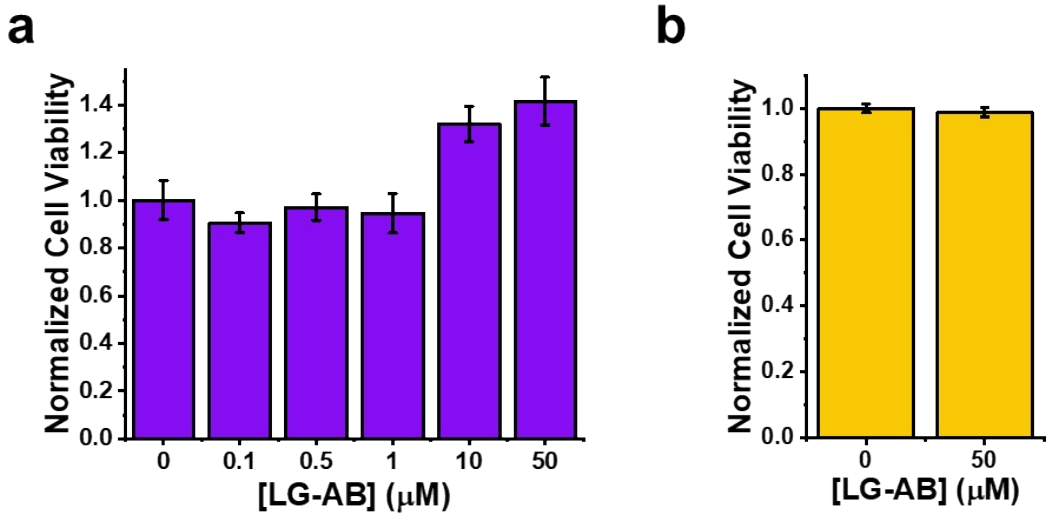


Figure S7. (a) LG-AB MTT viability assay in A549 cells (b) LG-AB Trypan Blue viability assay in A549 cells.

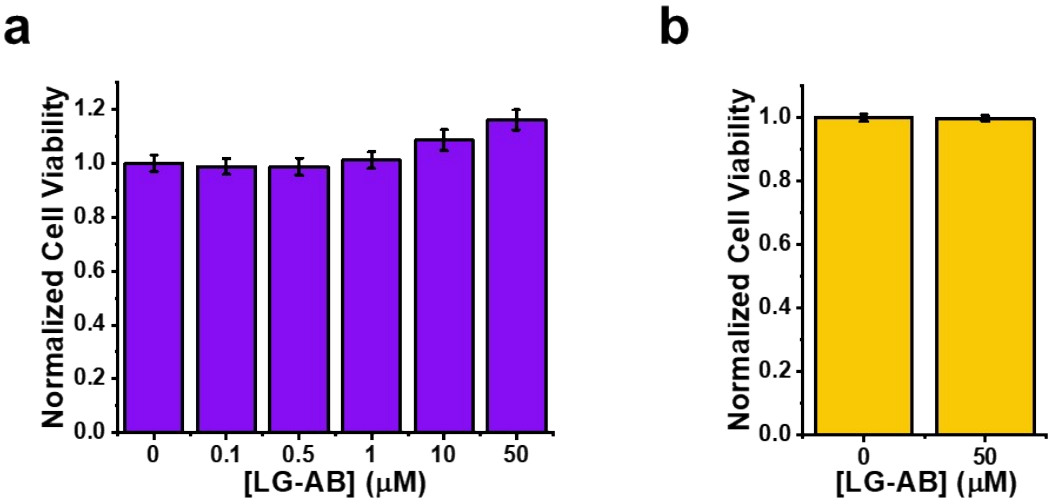


Figure S8. (a) LG-AB MTT viability assay in 4T1 cells (b) LG-AB Trypan Blue viability assay in 4T1 cells.

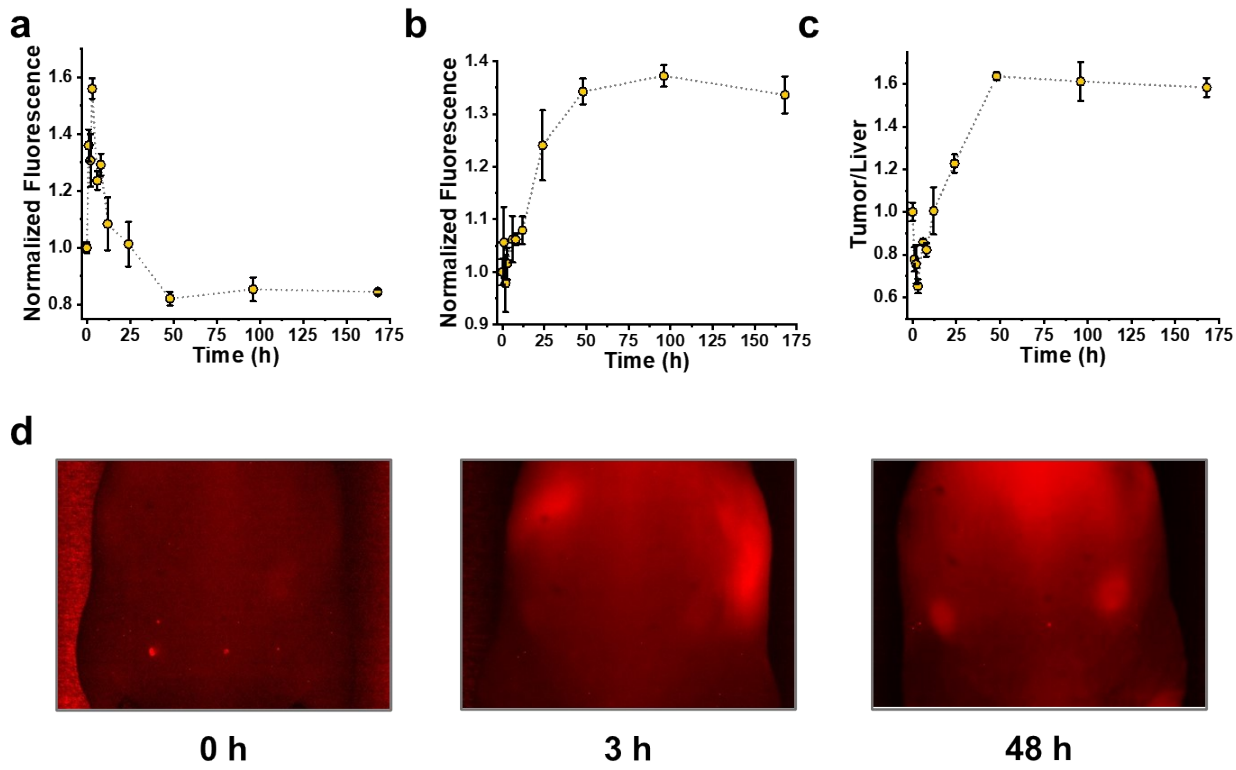


Figure S9. (a) Normalized fluorescent intensity of the liver in 7 days (b) normalized fluorescent intensity of the tumors in 7 days (c) tumor to liver fluorescent intensity ratio in 7 days (d) selected fluorescent images from the study.

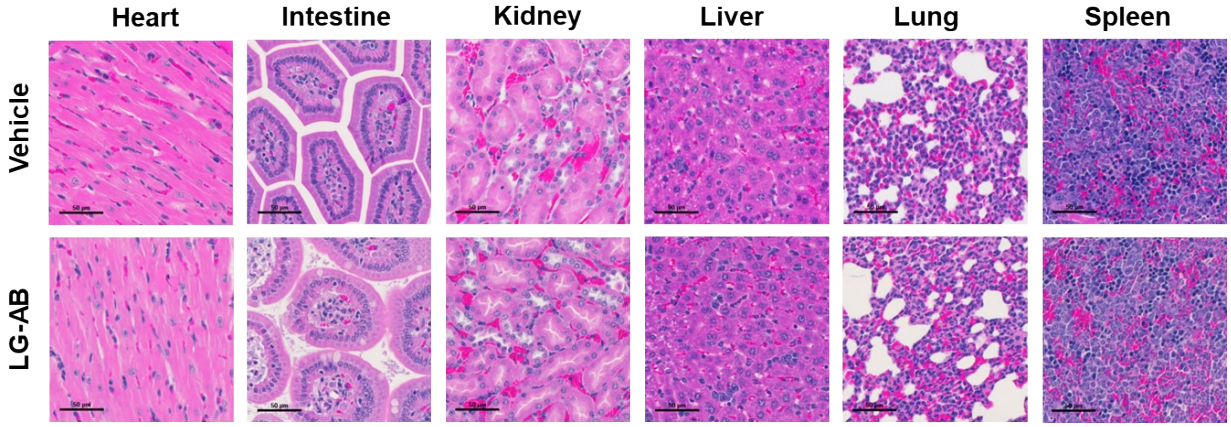


Figure S10. H&E staining figures of the major organs from the LG-AB in vivo treatment study.

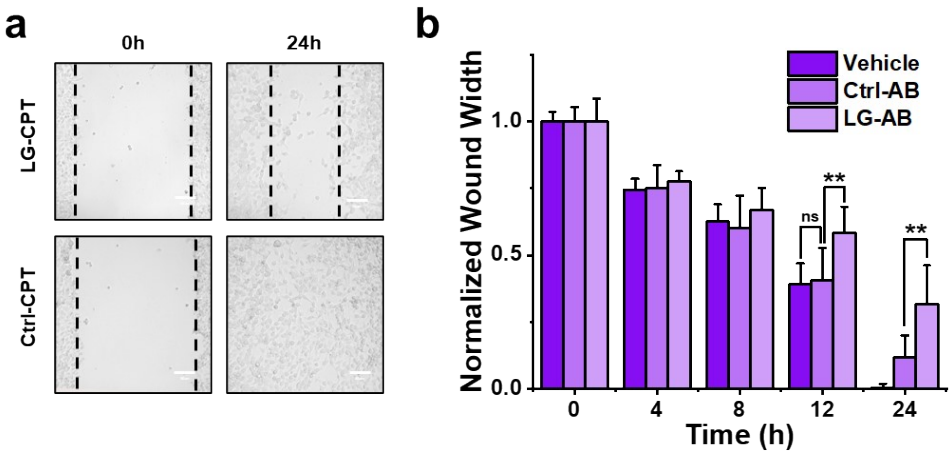


Figure S11. (a) Representative images of 4T1 wound-healing assay. (b) Quantified data from panel (a). Data are presented as mean values ± S.D. ($n = 3$ independent experiments).

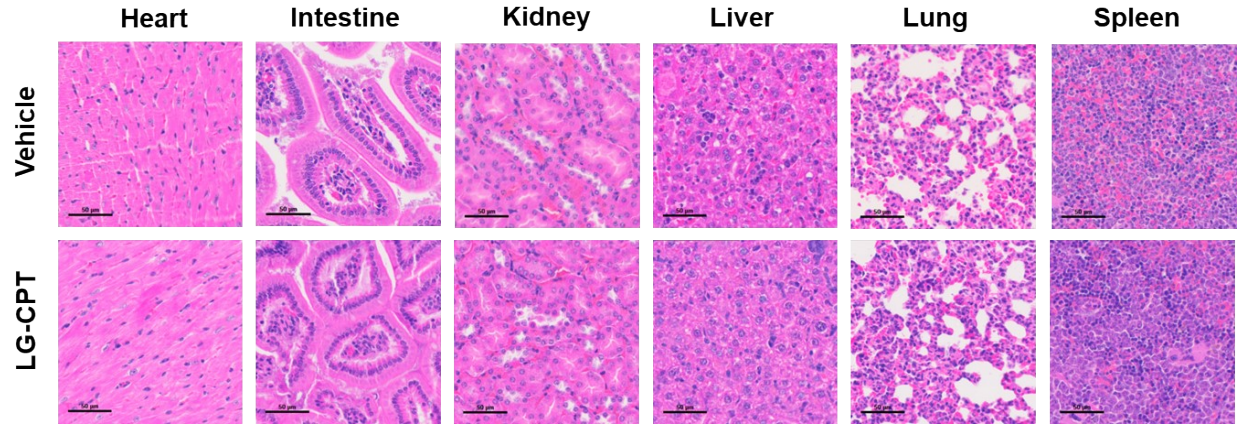


Figure S12. H&E staining figures of the major organs from the LG-CPT in vivo treatment study.

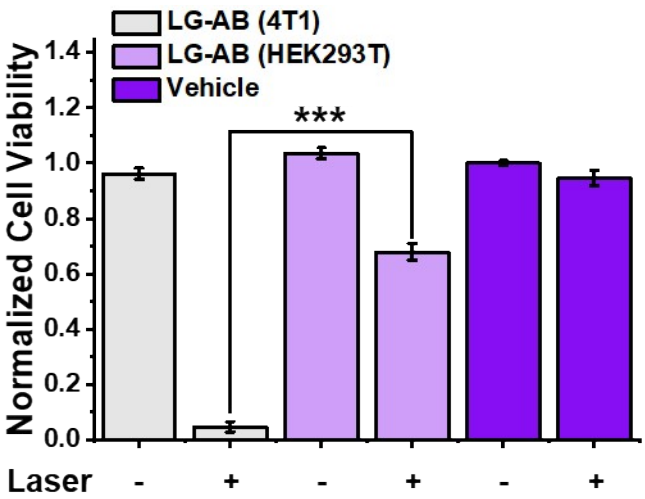
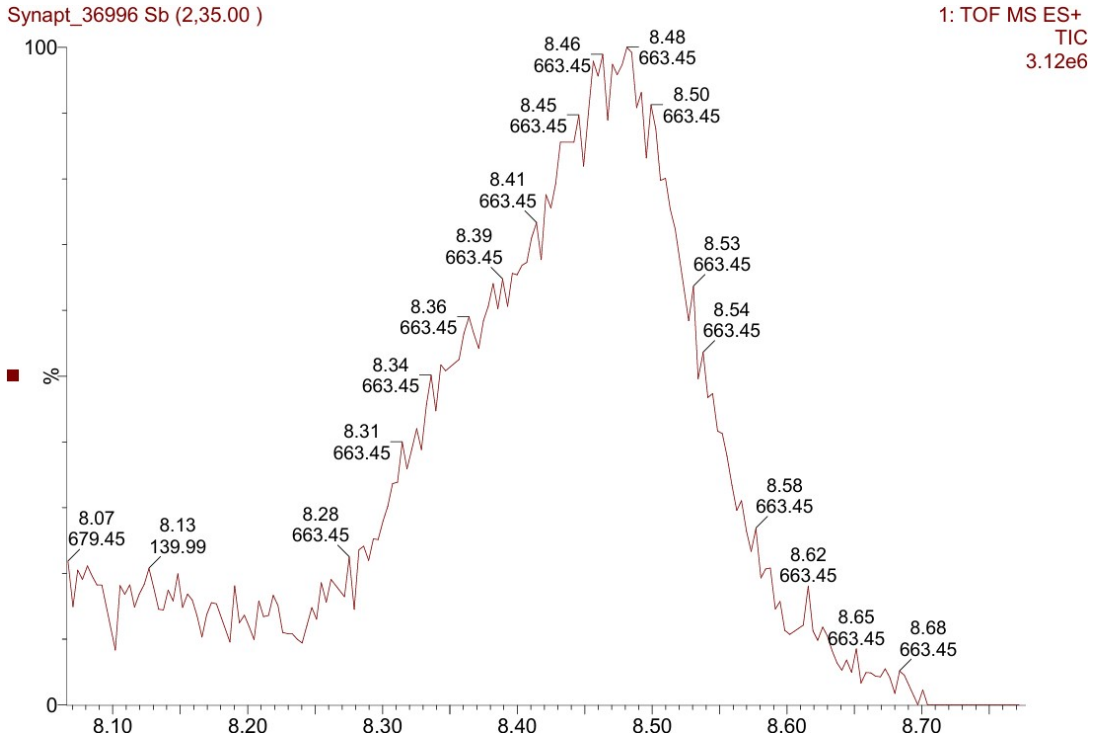


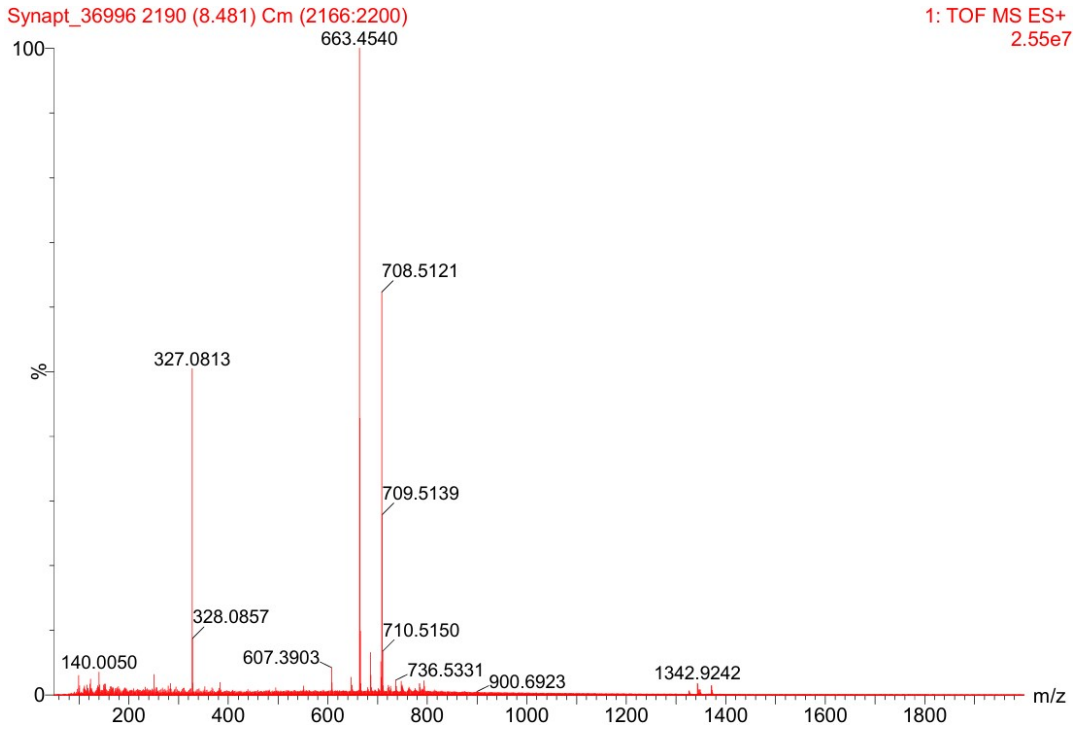
Figure S13. Cytotoxicity assays of HEK293T human embryonic kidney cells and 4T1 murine breast cancer cells under different conditions with and without 808 nm irradiation for 10 min. Data are presented as mean values ± SD (n = 3 independent experiments).

Liquid Chromatography Data.

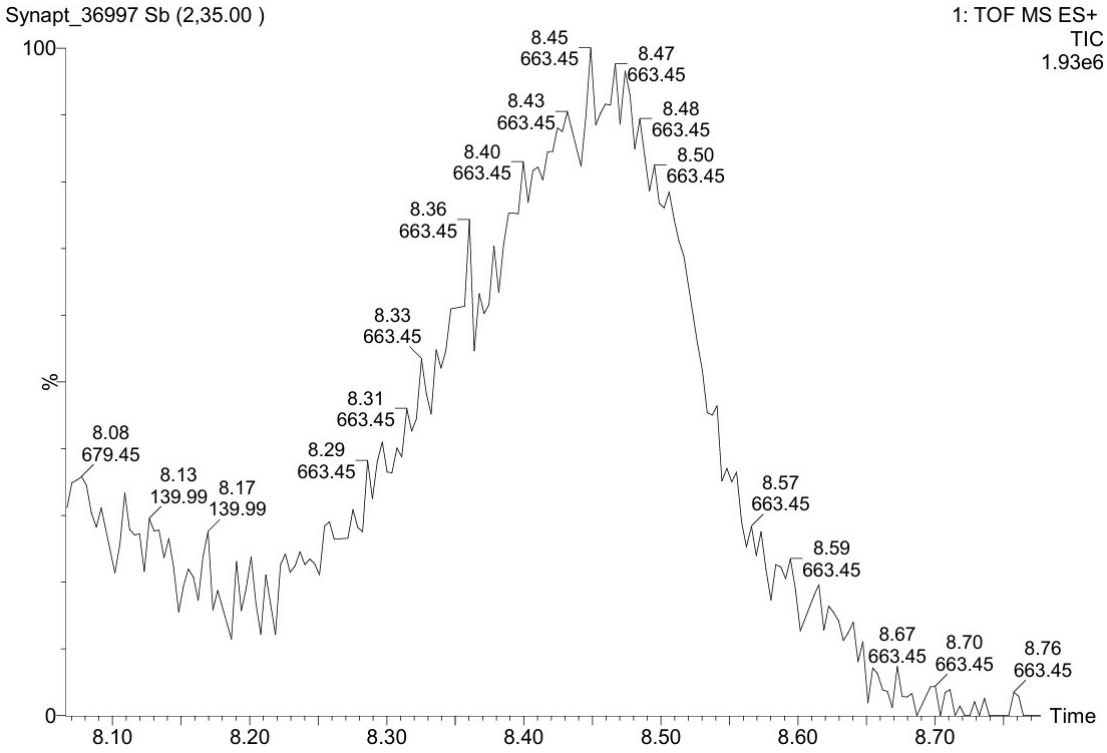
LC-HRMS analysis of reported compounds are reported below. Products were separated on a CORTECSTM UPLC C18 column (1.6 µm, 2.1 by 50 mm) with a linear gradient using a combination of solvent A (95% water, 5% acetonitrile, 0.1% formic acid) and solvent B (95% acetonitrile, 5% water, 0.1% Formic acid) at a flow rate of 0.4 mL/minute. Linear gradient protocol in minutes: 0 (90% A), 0.5 (90% A), 3 (90% A), 5 (40% A), 6.5 (10% A), 8 (0% A), 8.1 (90% A), 10 (90% A). Time of gradient was 10 minutes.



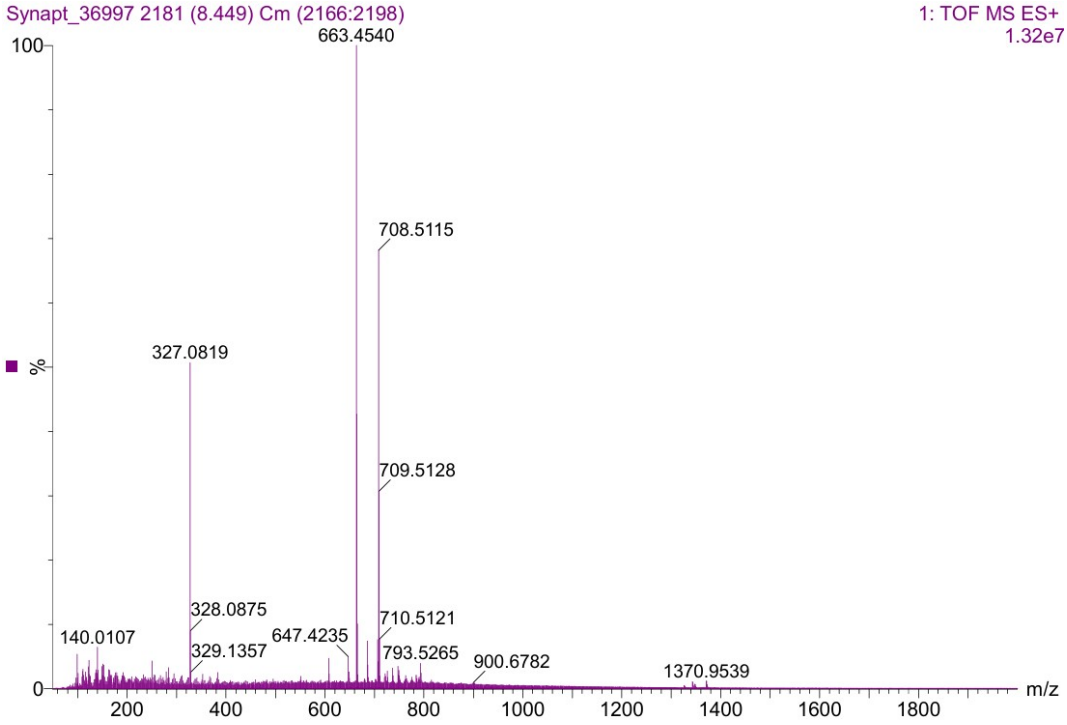
LC TIC trace of AB.



Combined MS of AB.

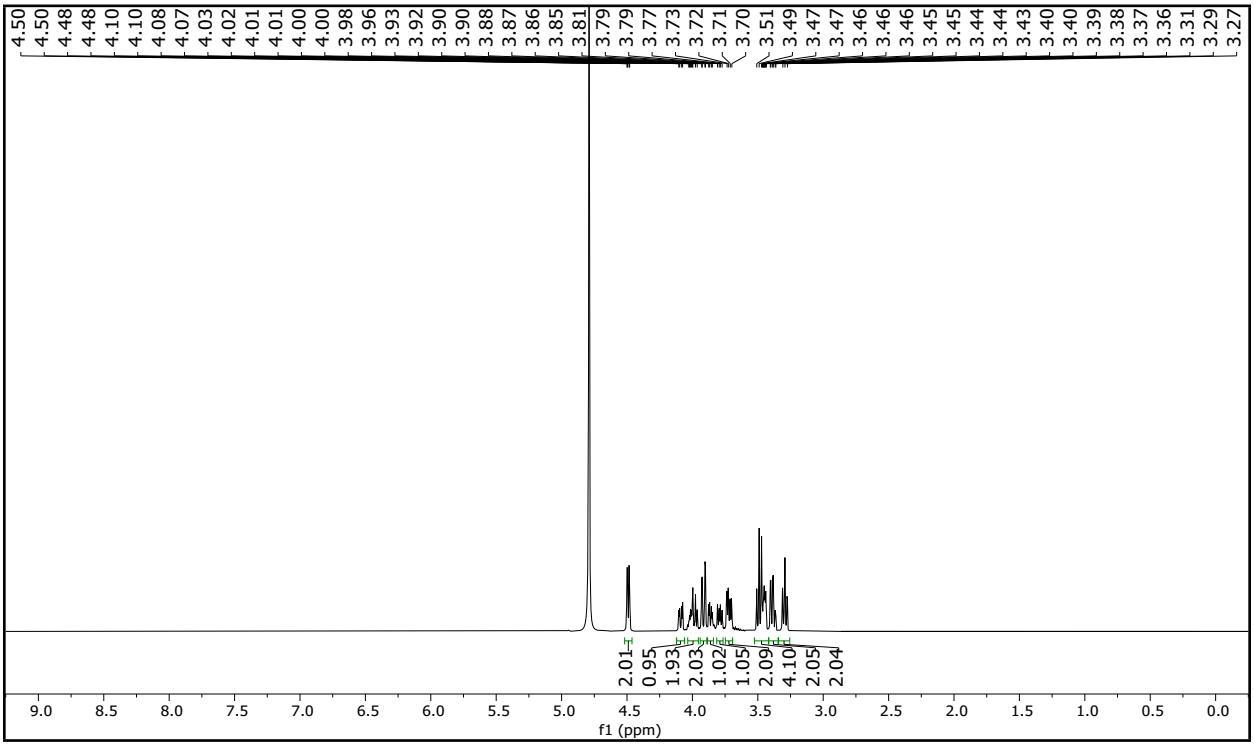


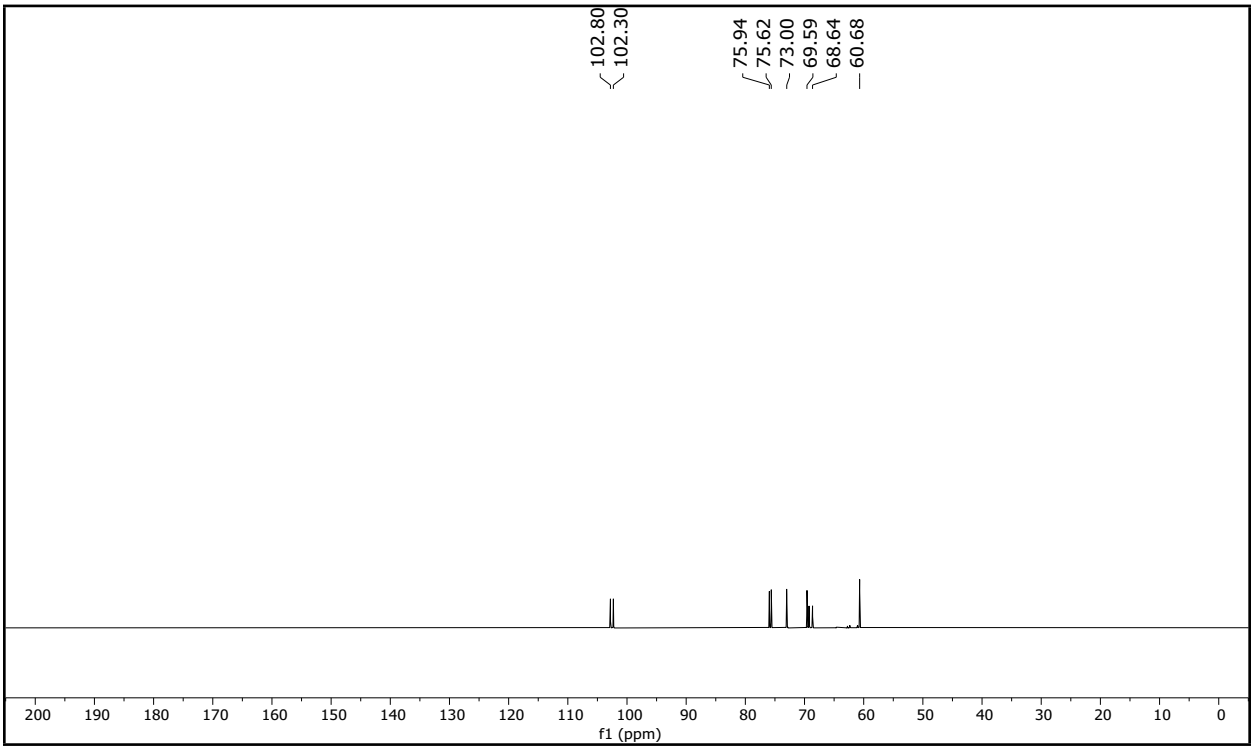
LC TIC trace of LG-AB + GSH (10 mM, incubated at 37 °C for 2 hours).



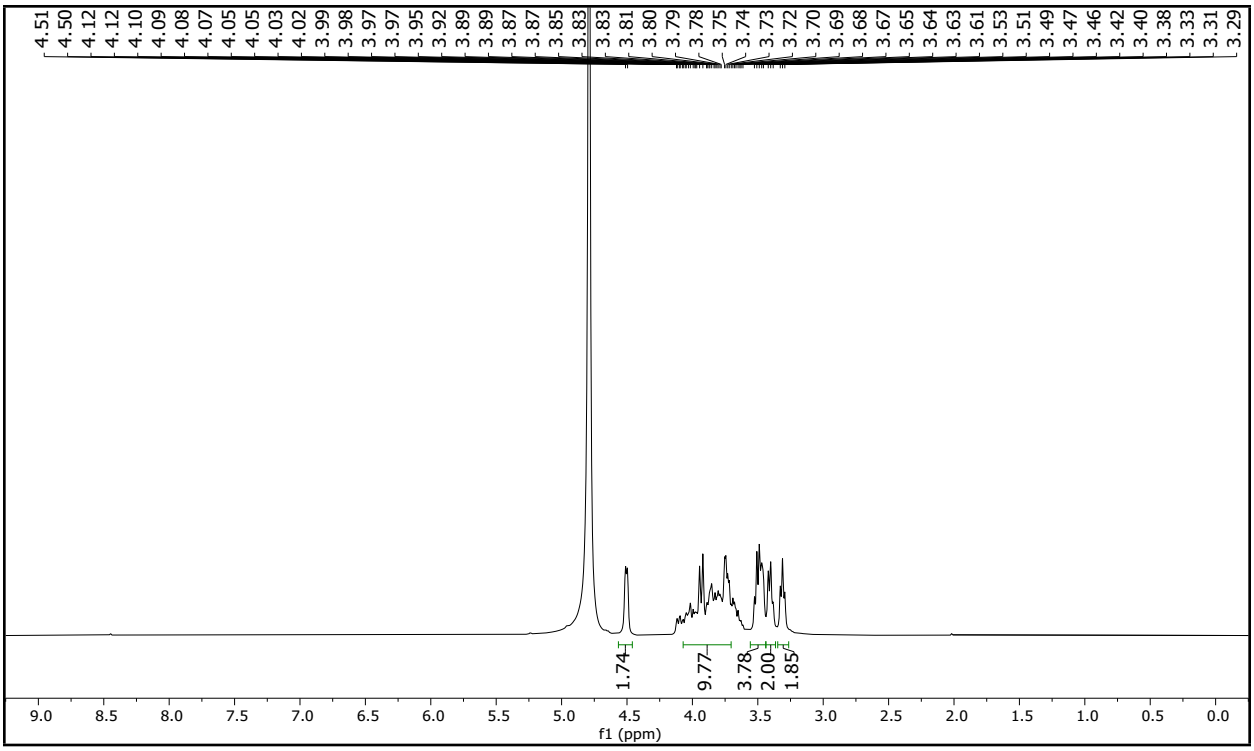
Combined MS of LG-AB + GSH (10 mM, incubated at 37 °C for 2 hours).

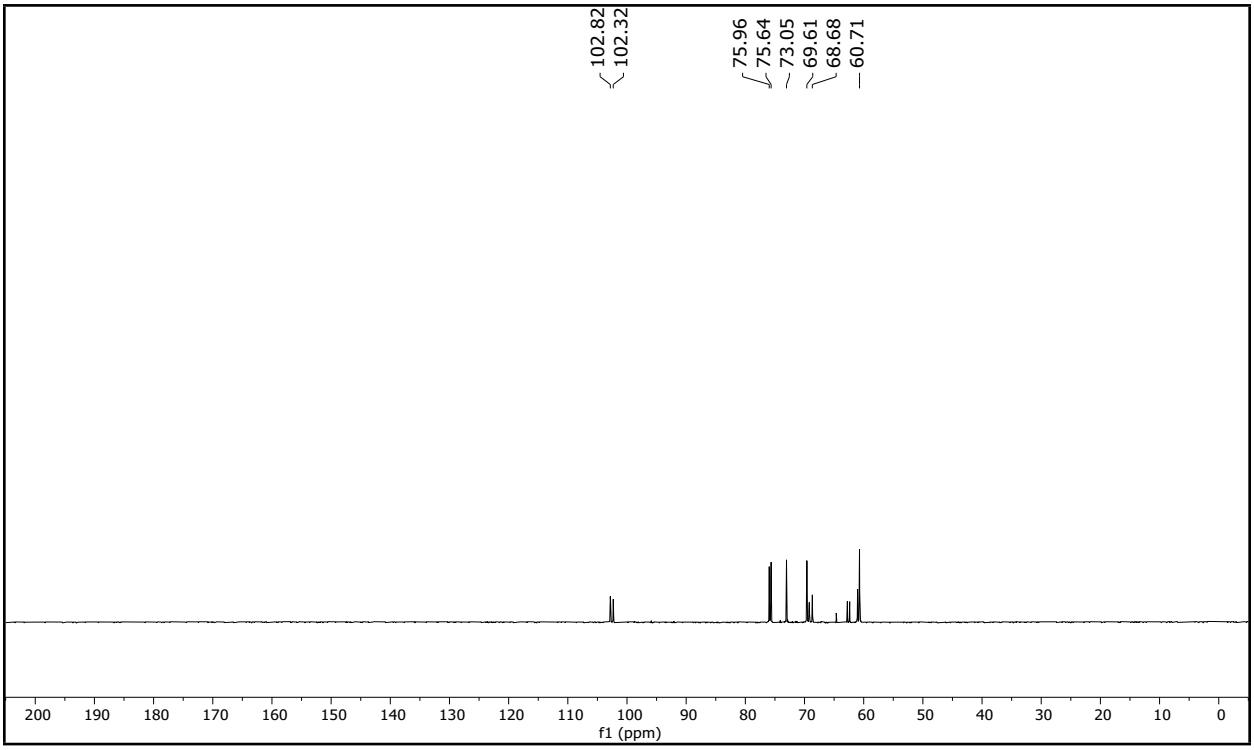
NMR Spectroscopic Data.



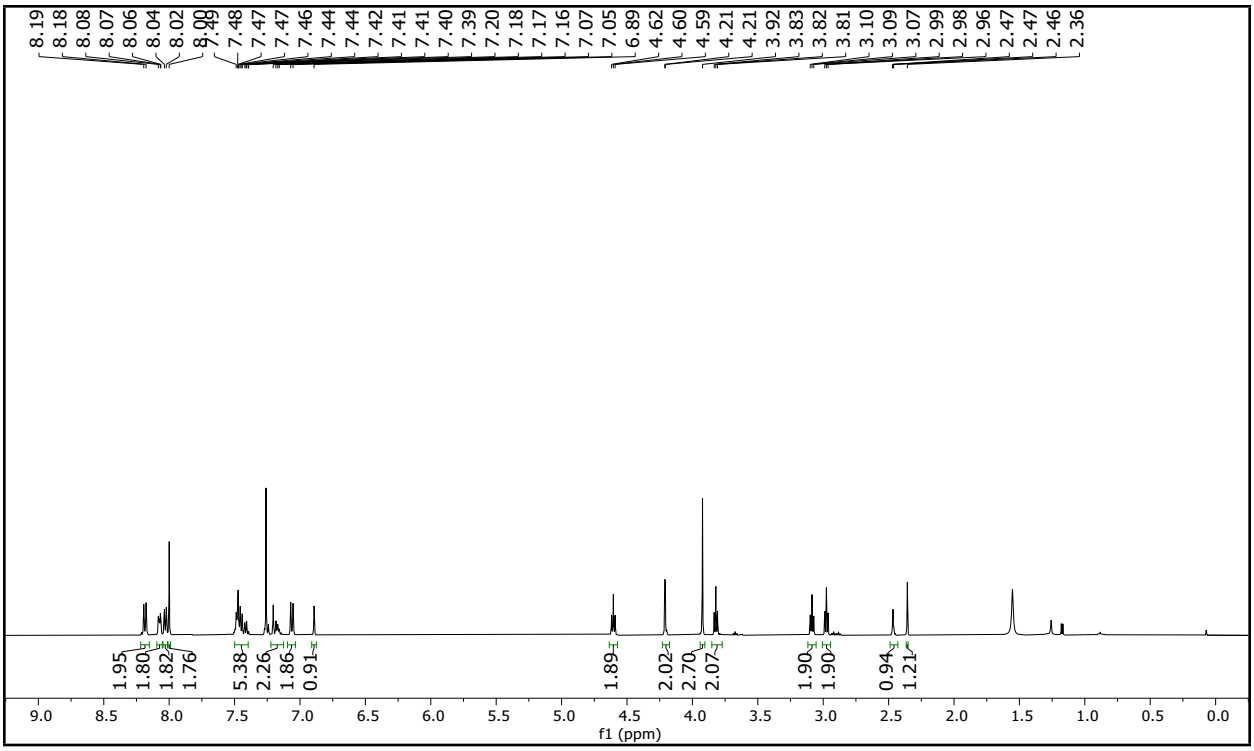


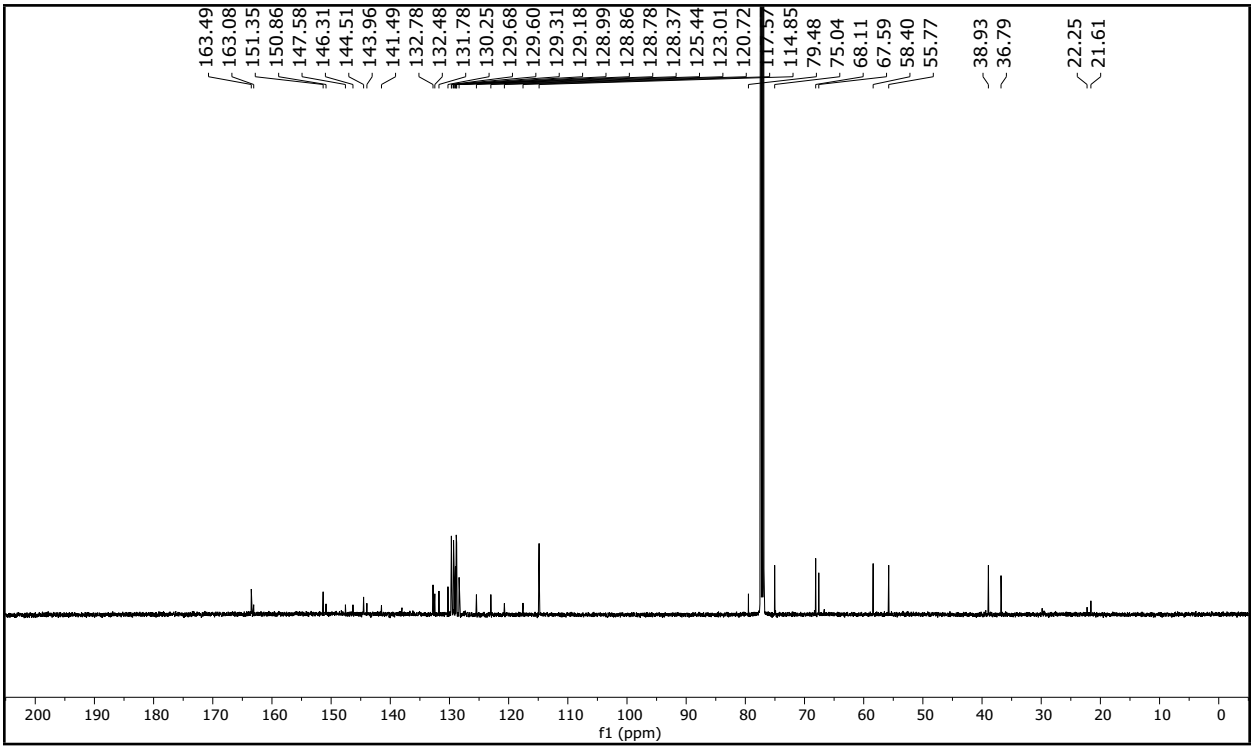
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) spectra of **azido-mvGlu (1)**



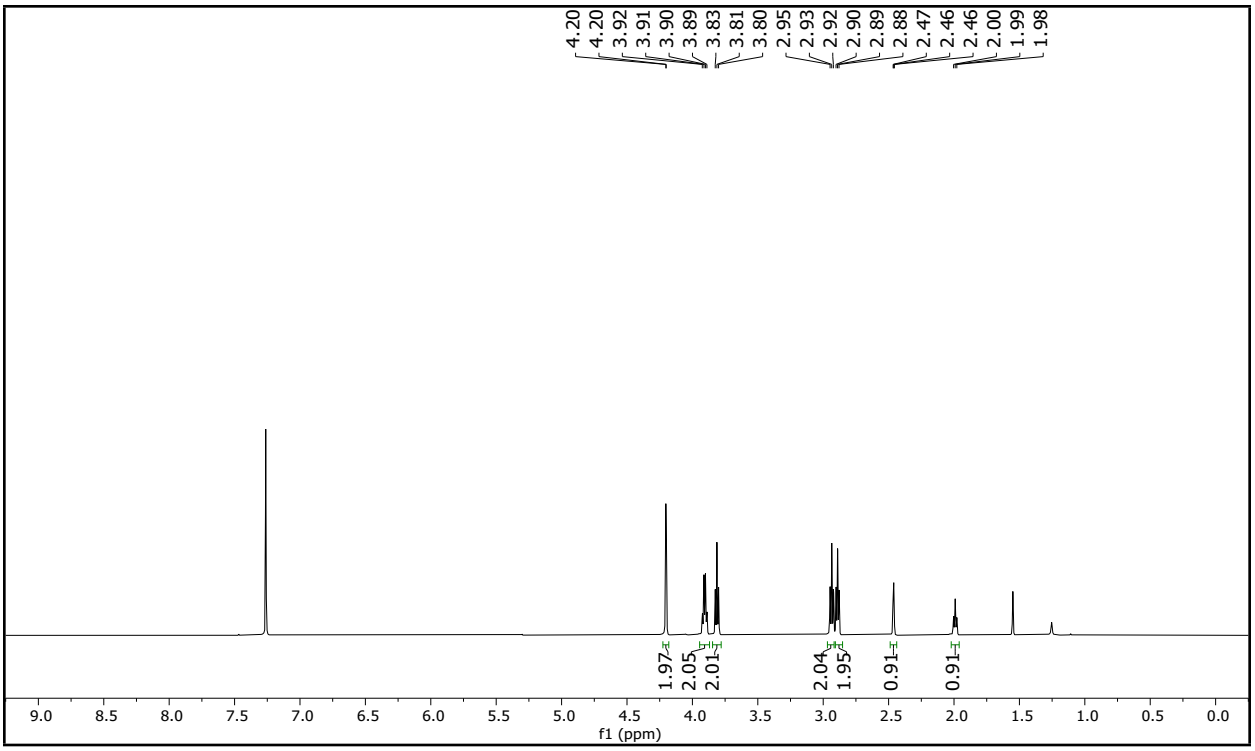


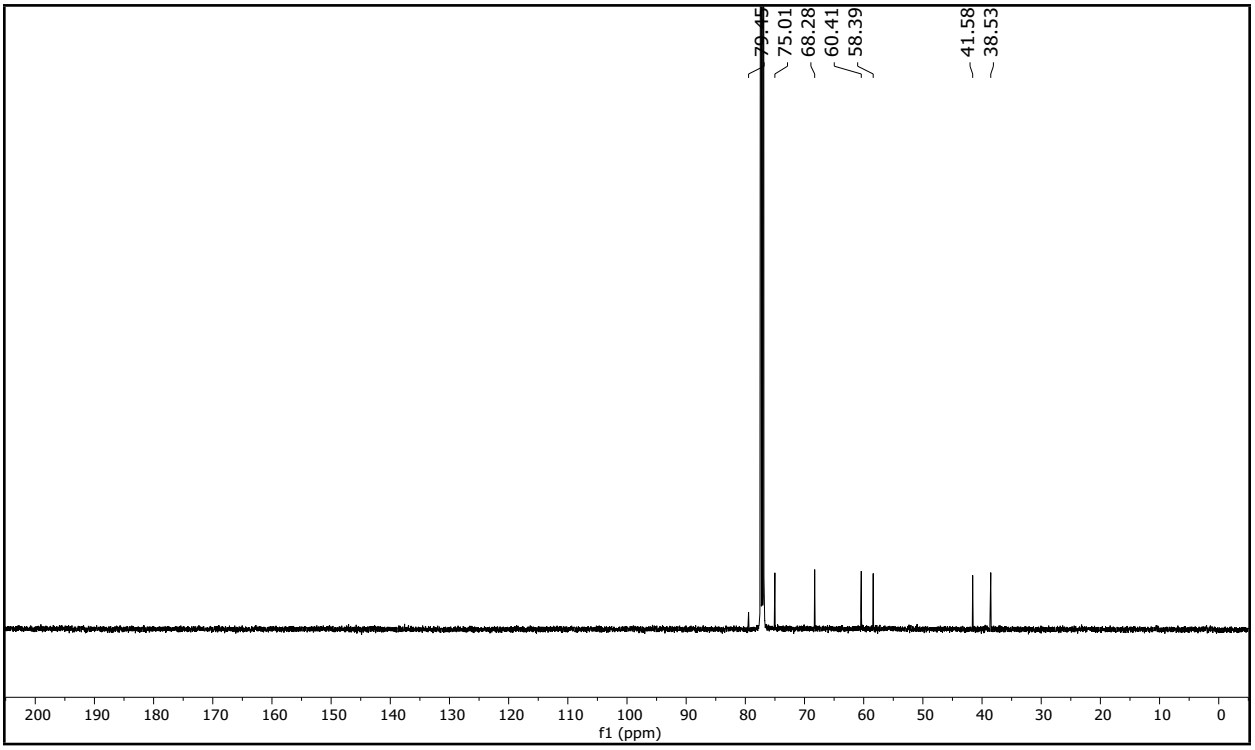
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) spectra of **l-azido-mvGlu (2)**



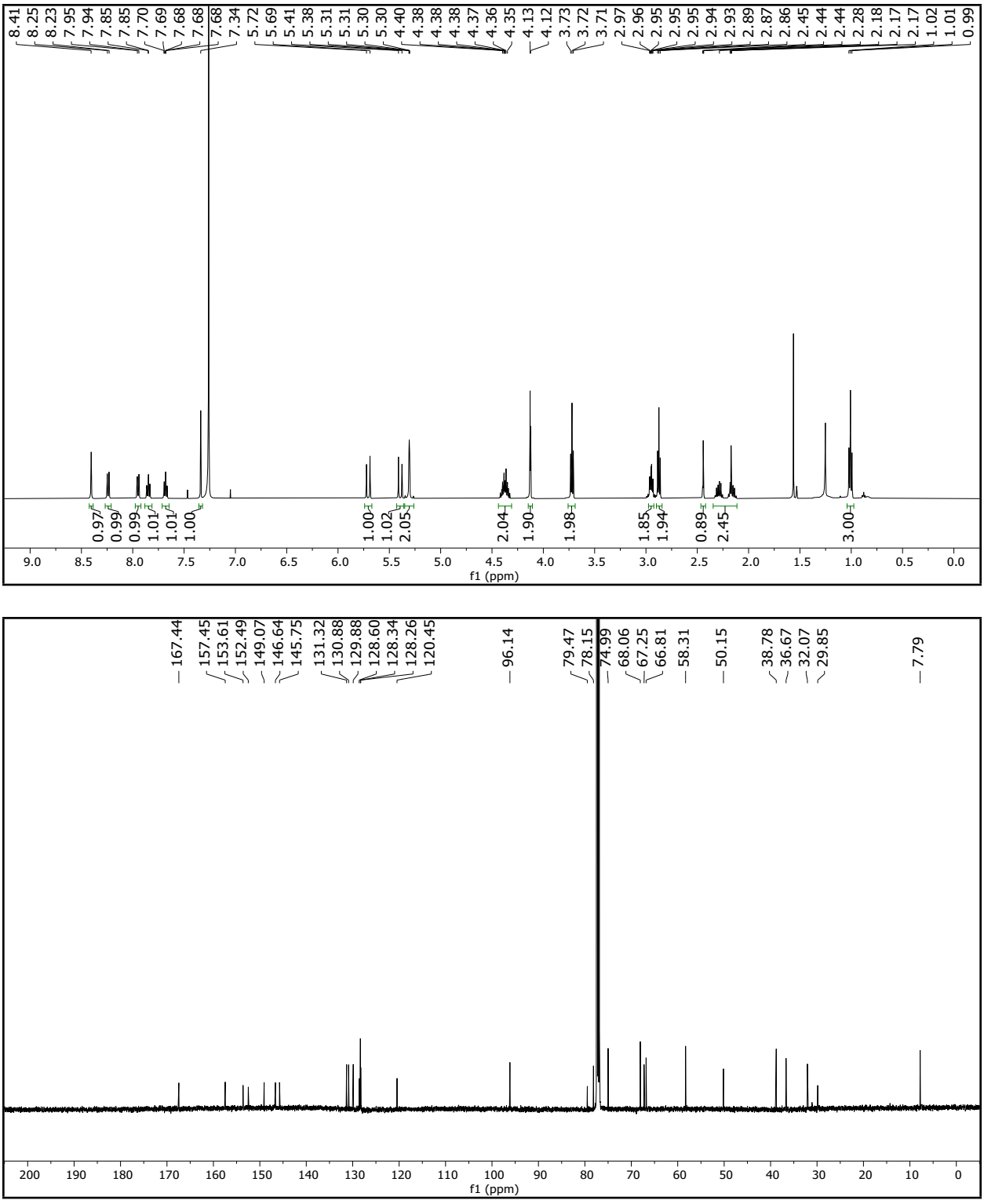


^{1}H NMR (500 MHz, $CHCl_3$) and ^{13}C NMR (125 MHz, $CHCl_3$) spectra of **Compound 3**

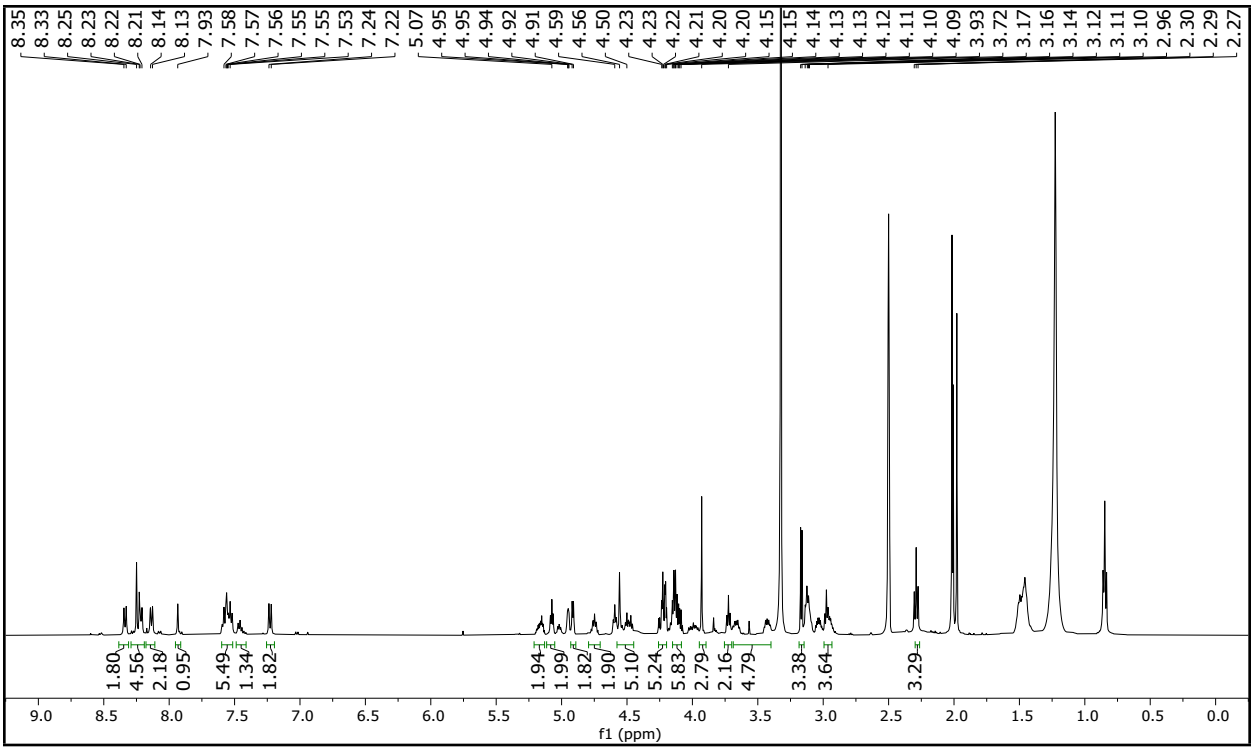




^{1}H NMR (500 MHz, $CHCl_3$) and ^{13}C NMR (125 MHz, $CHCl_3$) spectra of **Compound 4**



^{1}H NMR (500 MHz, $CHCl_3$) and ^{13}C NMR (125 MHz, $CHCl_3$) spectra of **Compound 5**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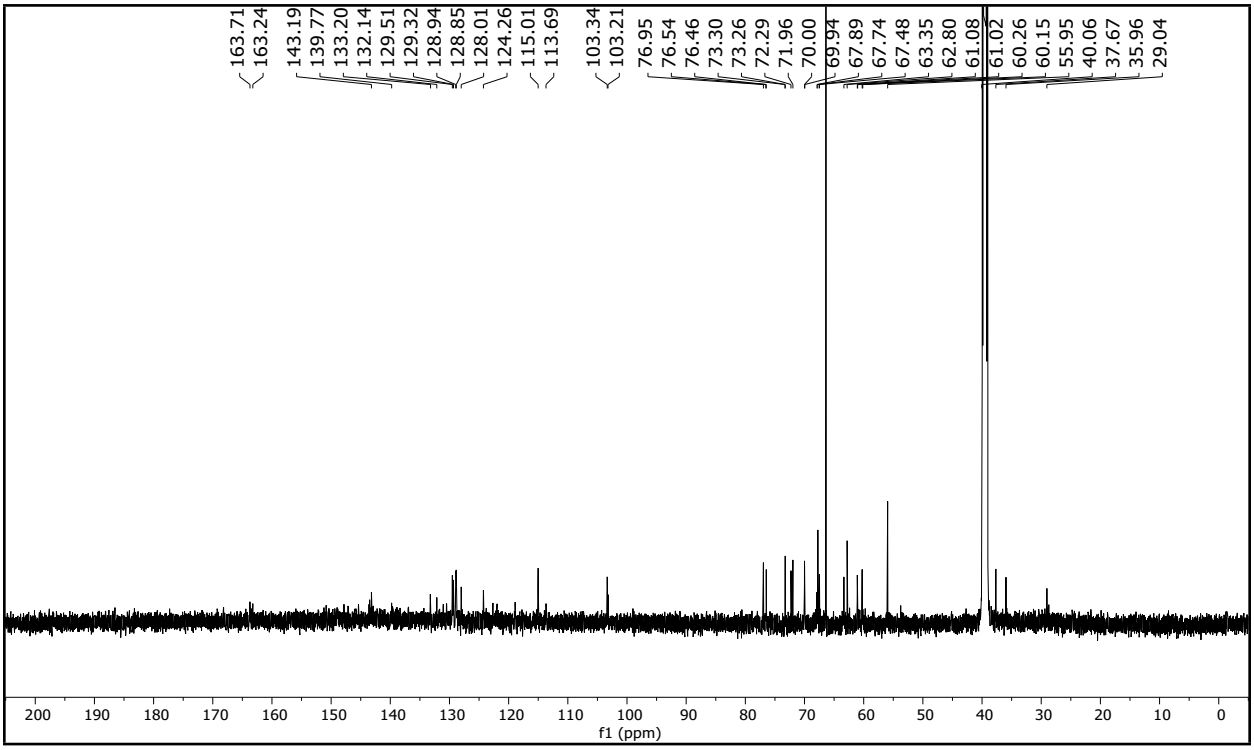
1.80 4.56 2.18 0.95 5.49 1.34 1.82 1.94 1.99 1.82 1.90 5.10 5.24 5.83 2.79 2.16 4.79 3.38 3.64 3.29
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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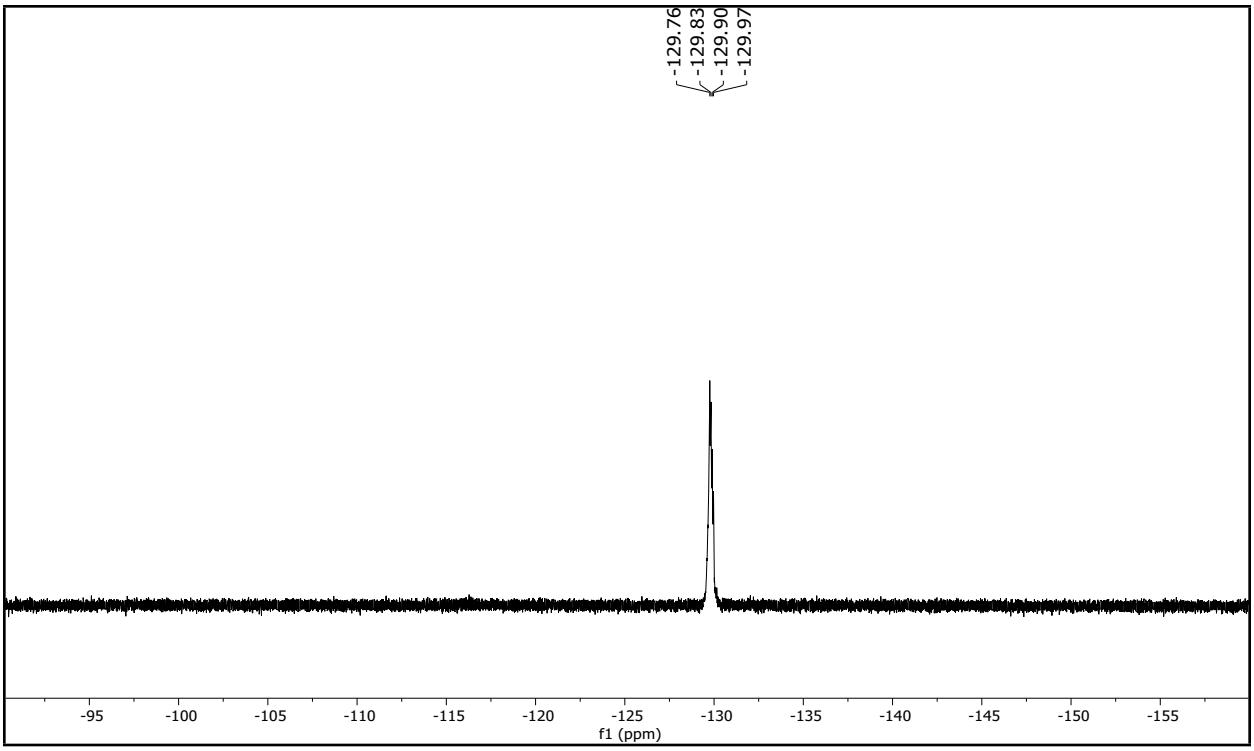
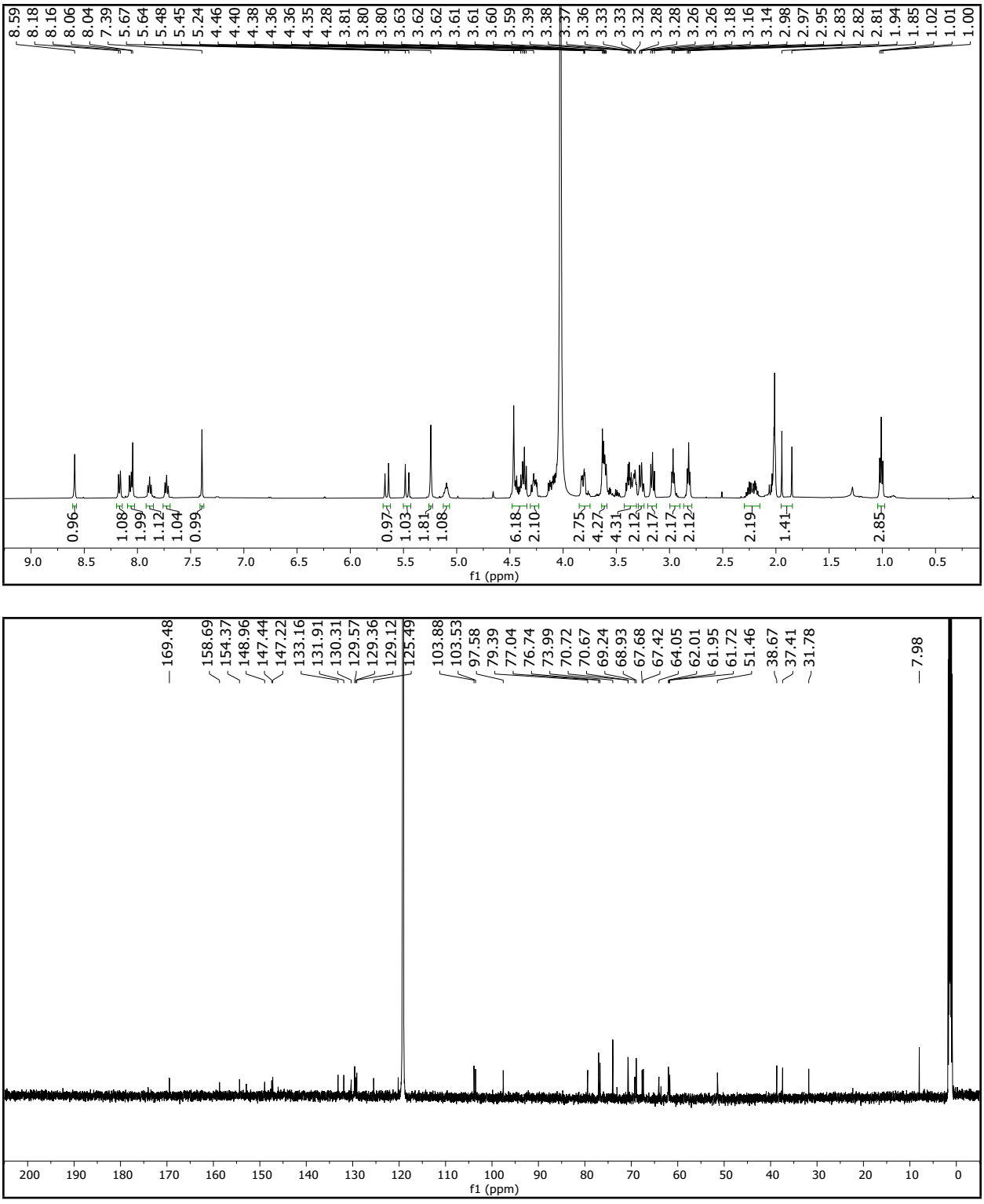
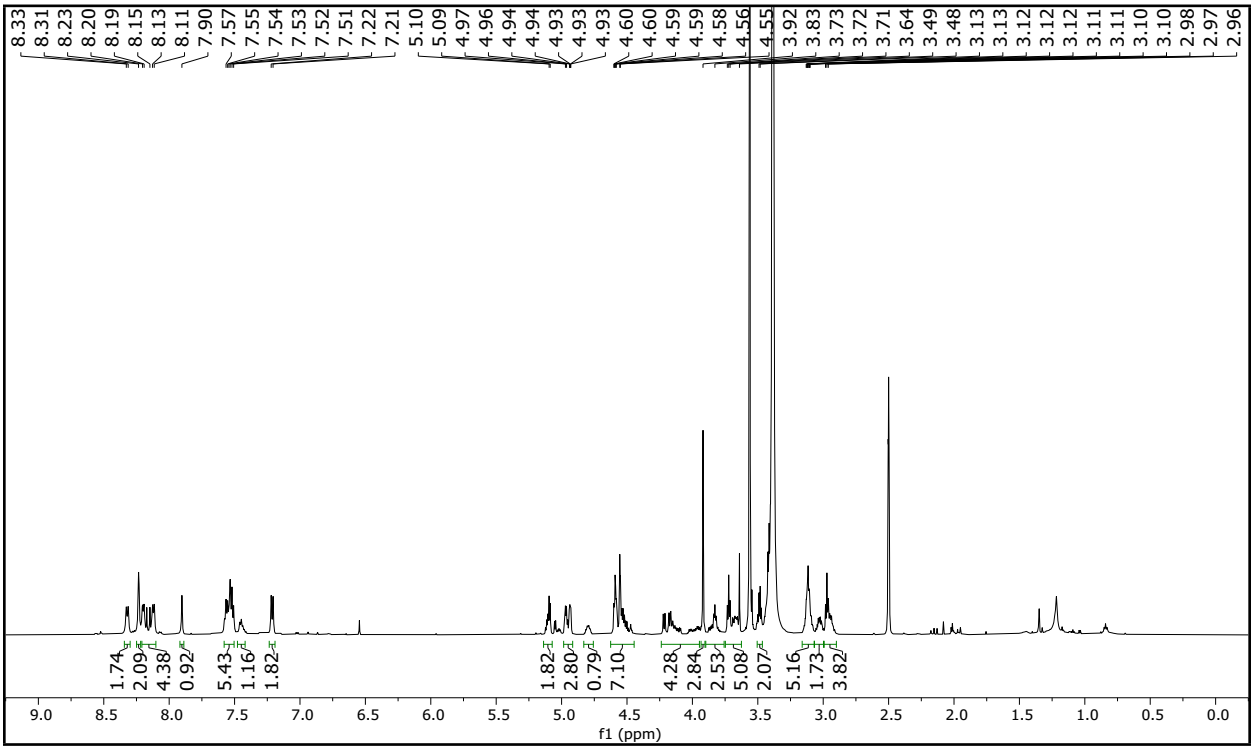


^{1}H NMR (600 MHz, DMSO-d_6), ^{13}C NMR (150 MHz, DMSO-d_6) and ^{19}F NMR (470 MHz, DMSO-d_6) spectra of **LG-AB**



^{1}H NMR (500 MHz, 1:1 v/v D_2O:CD_3CN) and ^{13}C NMR (150 MHz, 1:1 v/v D_2O:CD_3CN) spectra of **LG-CPT**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1.74 2.09 4.38 0.92 5.43 1.16 1.82 1.82 2.80 0.79 7.10 4.28 2.84 2.53 5.08 2.07 5.16 1.73 3.82
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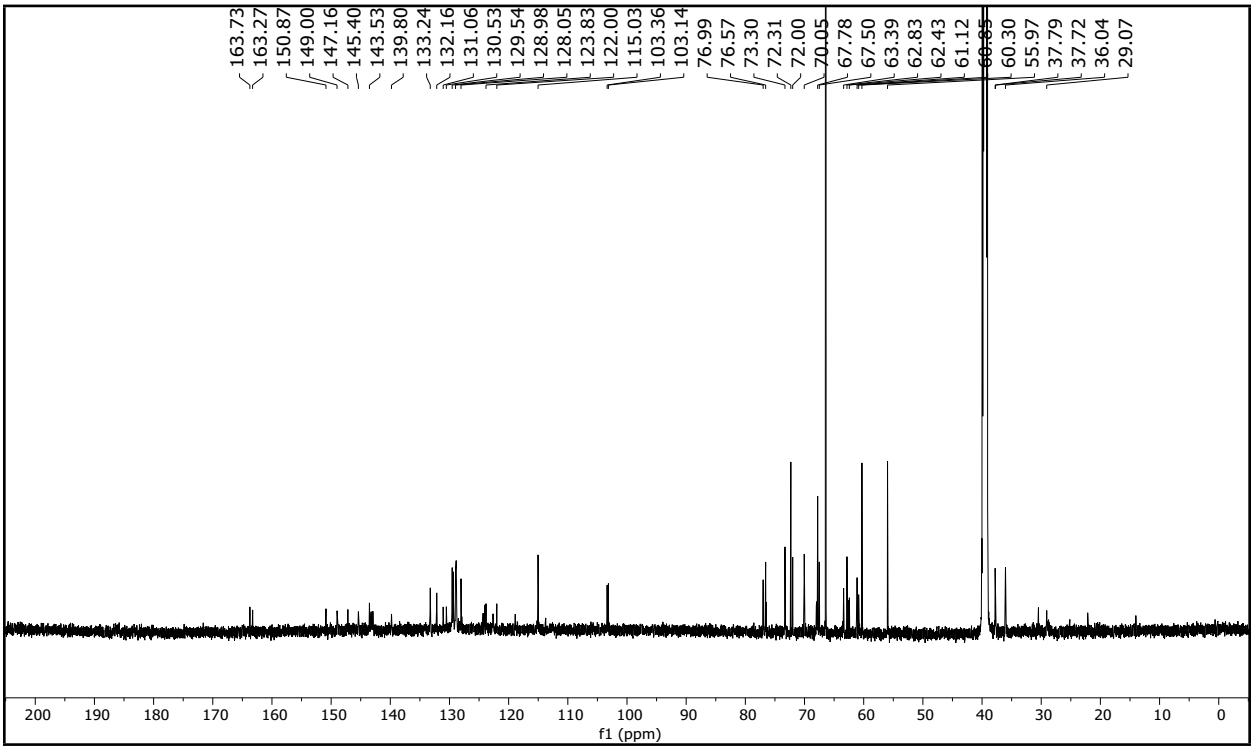
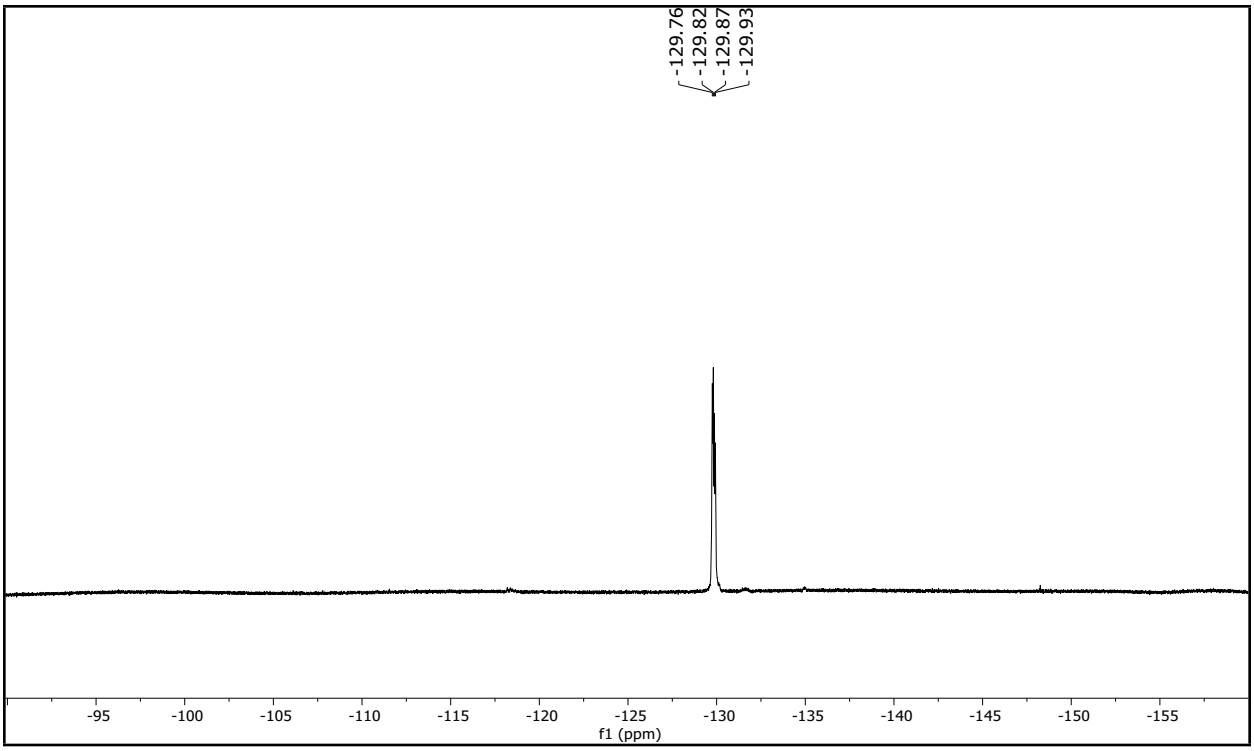
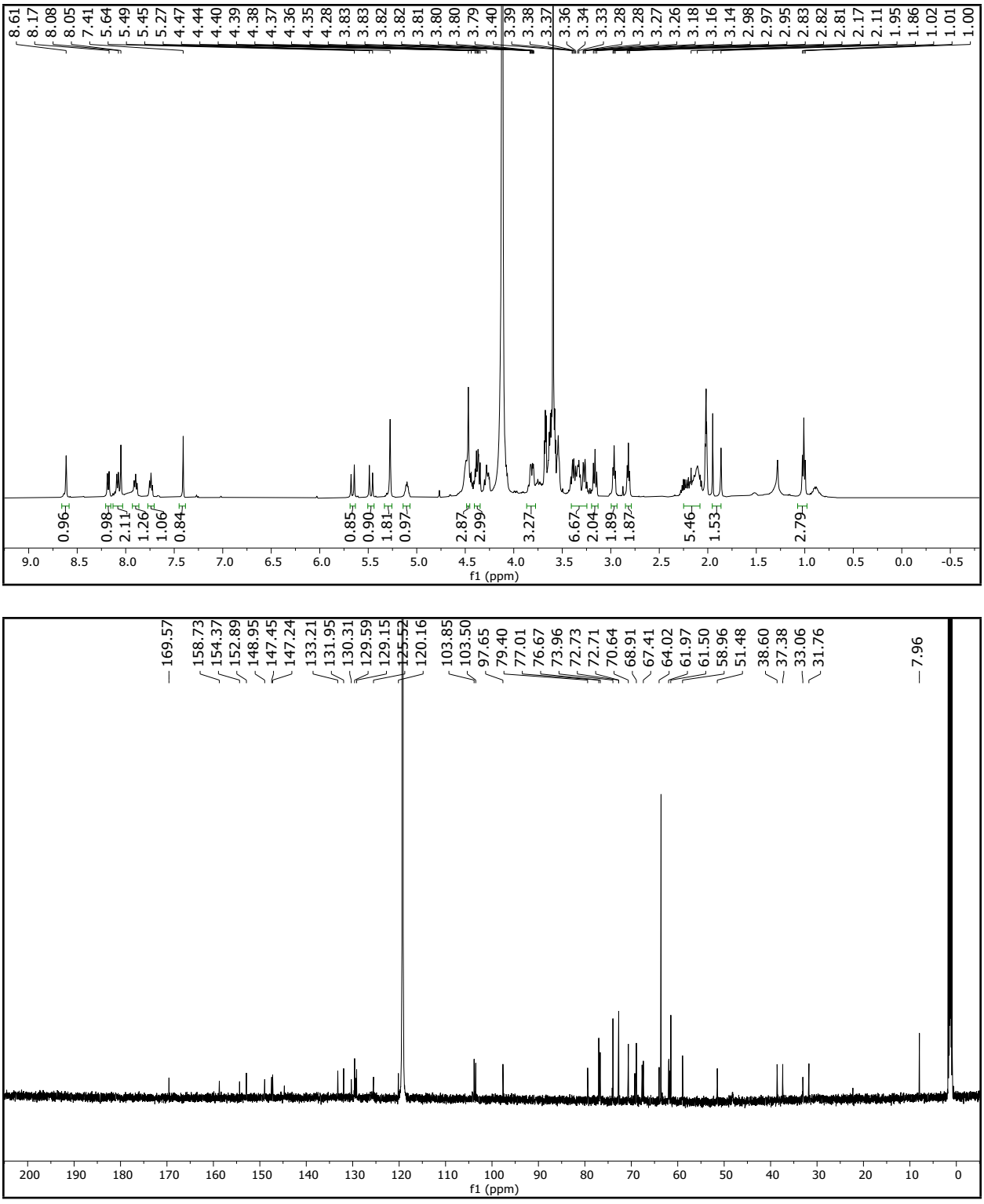
f1 (ppm)


163.73 163.27 150.87 149.00 147.16 145.40 143.53 139.80 133.24 132.16 131.06 130.53 129.54 128.98 128.05 123.83 122.00 115.03 103.36 103.14 76.99 76.57 73.30 72.31 72.00 70.05 67.78 67.50 63.39 62.83 62.43 61.12 60.85 60.30 55.97 37.79 37.72 36.04 29.07
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



^{1}H NMR (600 MHz, DMSO-d_6), ^{13}C NMR (150 MHz, DMSO-d_6) and ^{19}F NMR (470 MHz, DMSO-d_6) spectra of **Ctrl-AB**



^{1}H NMR (500 MHz, 1:1 v/v D_2O:CD_3CN) and ^{13}C NMR (150 MHz, 1:1 v/v D_2O:CD_3CN) spectra of **Ctrl-CPT**

References:

1) Amanda K. East, et al *Journal of the American Chemical Society* **2023** 145 (13), 7313-7322. DOI: 10.1021/jacs.2c13489

2) Hao Li, et al *Journal of the American Chemical Society* **2015** 137 (50), 15628-15631. DOI: 10.1021/jacs.5b10504