Supporting Information

Red Si-rhodamine drug conjugates enable imaging in GFP cells

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General experimental procedures. Unless otherwise noted, reactions were carried out under an atmosphere of nitrogen or argon in air-dried glassware with magnetic stirring. Air- and/or moisture-sensitive liquids were transferred *via* syringe. Organic solutions were concentrated by rotary evaporation at 25 - 60 °C at 15-30 torr. Analytical thin layer chromatography (TLC) was performed using plates cut from glass sheets (silica gel 60 F-254 from Silicycle). Visualization was achieved under a 254 or 365 nm UV light and by immersion in an ethanolic solution of cerium sulfate, followed by treatment with a heat gun. Column chromatography was carried out as "Flash Chromatography" using silica gel G-25 (40-63 μM).

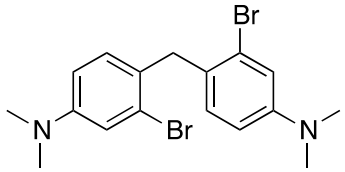
Materials. All reagents were obtained from commercial sources and used without further purification. Dry THF, MeOH, DCM and DMF were obtained from Aldrich.

Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded at 23°C on a Bruker 400 MHz spectrometer. Recorded shifts are reported in parts per million (δ) and calibrated using residual undeuterated solvent. Data are represented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad), coupling constant (*J*, Hz) and integration. LC-ESI-MS analysis and HPLC-purifications were performed on a Waters (Milford, MA) LC-MS system. For LC-ESI-MS analyses, a Waters XTerra® C18 5 μm column was used. For preparative runs, an Atlantis® Prep T3 OBDTM 5 μM column was used (eluents 0.1% TFA (v/ v) in water and MeCN; gradient: 0-1.5 min, 5-100% B; 1.5-2.0 min 100% B). Fluorescent images were collected using a DeltaVision microscope (Applied Precision, Issaquah, WA).

Chemical synthesis

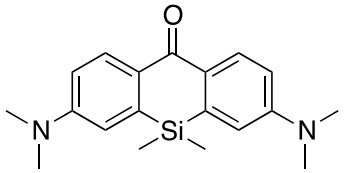
(Cyclopropanecarbonyl)piperazine-1-carbonyl]-4-fluorophenyl]methyl]-2H-phthalazin-1-one (compound **5**) was synthesized as described earlier[1].

4,4'-methylenebis(3-bromo-*N*,*N*-dimethylaniline) (1)



A solution of 3-bromo-N,N-dimethylaniline (400 mg, 2.00 mmol) and formaldehyde solution (880 μL) in AcOH (20 mL) was stirred at 85°C. 1hr later, the reaction mixture was cooled down to ambient temperature. AcOH was removed by evaporation and the crude product was treated with $NaHCO_3$ (sat) and 1N NaOH. Organic material was extracted with DCM three times. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The reaction mixture was purified with silica gel column chromatography (EA : Hex = 1:100 to 1:10) to give compound **1** (204 mg, 49.5%) as a white solid. ^{1}H NMR (400 MHz, $CDCL_3$) δ 7.25 (s, 1H), 6.93 (d, J = 2.7 Hz, 2H), 6.84 (d, J = 8.5 Hz, 2H), 6.58 (dd, J = 8.5, 2.7 Hz, 2H), 3.99 (s, 2H), 2.91 (s, 12H).; ^{13}C NMR (101 MHz, $CDCL_3$) δ 150.1, 130.9, 127.1, 125.7, 116.3, 111.9, 40.6, 40.0.; LRMS (ESI) m/z calcd for $C_{17}H_{20}Br_2N_2$ $[M+H]^+$ 411, found 411.

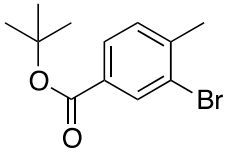
3,7-bis(dimethylamino)-5,5-dimethyldibenzo[*b*,*e*]silin-10(5*H*)-one (2)



To a solution of compound **1** (200 mg, 0.485 mmol) in THF (20 mL), a 1.3 M sec-BuLi solution (1.0 mL) in hexane was added dropwise. The resulting reaction mixture was stirred at -78°C for 30 min. A Si_2MeCl_2 (106 μL, 0.873 mmol) solution in THF (3.5 mL) was then added dropwise. The resulting reaction mixture was gradually warmed up to ambient temperature and stirred overnight. To a turbid solution, 12 mL of 1N HCl solution was added. The resulting blue reaction mixture was basified with $NaHCO_3$ (sat) and concentrated in vacuo. The resulting greenish color oil was diluted with acetone (3 mL) and stirred at -20°C. To the reaction mixture, $KMnO_4$ was added portionwise (6 x 30 mg) for 30 min, and stirred for 2h at the same temperature. The purple suspension was filtered through a Celite pad, washed with acetone and the yellow filtrate was evaporated. The reaction mixture was purified with silica gel column chromatography (Hex : DCM = 1 : 4) to give compound **2** (35 mg, 22.3%) as a white solid.

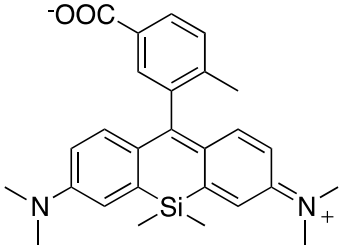
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 – 8.33 (m, 2H), 6.84 (dd, J = 9.2, 2.6 Hz, 2H), 6.79 (d, J = 2.6 Hz, 2H), 3.10 (d, J = 1.3 Hz, 12H), 0.47 (d, J = 1.3 Hz, 6H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.4, 151.6, 140.6, 131.8, 129.9, 114.4, 113.3, 40.2, -0.9.; LRMS (ESI) m/z calcd for $C_{19}H_{24}N_2OSi$ $[M+H]^+$ 325, found 325.

***tert*-butyl 3-bromo-4-methylbenzoate (3)**



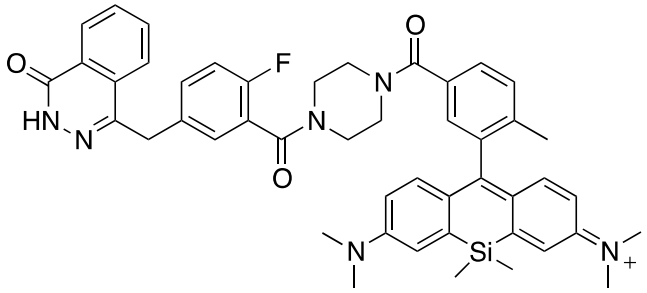
A solution of 3-Bromo-4-methylbenzoic acid (1.40 g, 6.51 mmol) and Di-tert-butyl dicarbonate (3.62 g, 16.6 mmol) and DMAP (180 mg, 1.47 mmol) in THF (10 mL) was refluxed overnight. 1 day later, the reaction mixture was cooled to ambient temperature and concentrated in vacuo. The resulting white solid was dissolved with $NaHCO_3$ (sat) and organic material was extracted with EA for three times. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The reaction mixture was purified with silica gel column chromatography (EA : Hex = 1 : 100 to 1 : 10) to give compound **3** (923 mg, 52.1%) as a clear oil. The purified compound was used for the next reaction without further characterization.

N-(10-(5-carboxy-2-methylphenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[b,e]silin-3(5H)-ylidene)-N-methylmethanaminium (4)



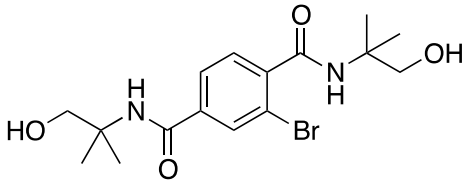
To a solution of compound **3** (200 mg, 0.741 mmol) in THF (5 mL), stirred at -78°C, a solution of *tert*-BuLi (450 μL, 0.765 mmol) in pentane was added and the reaction mixture was stirred at the same temperature for 1hr. After stirring, a solution of compound **2** (50 mg, 0.154 mmol) in THF (3 mL) was added dropwise to a reaction mixture. The reaction mixture was gradually warmed up to ambient temperature and stirred at the same temperature for 2 hr. 0.1N HCl aq. was added to the reaction mixture, and the resulting deep blue solution was gasified with $NaHCO_3$ (sat) and extracted with DCM 3 times. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting blue solid was used for the next reaction without further purification. The solution of blue solid in MeCN (2 mL) and 6N HCl aq. (8 mL) was stirred at 40°C for 1 hr. After cooling down to ambient temperature, the reaction mixture was basified with 0.1N NaOH to adjust the pH to 2-3 and then extracted with DCM three times. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The reaction mixture was purified with silica gel column chromatography (MeOH : DCM = 0 : 100 to 1 : 5) to give compound **4** (45 mg, 73.4%) as a deep blue solid. ^{1}H NMR (400 MHz, MeOD) δ 8.13 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.76 (d, *J* = 1.7 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 2.8 Hz, 2H), 7.07 (d, *J* = 9.6 Hz, 2H), 6.81 (dd, *J* = 9.7, 2.8 Hz, 2H), 3.38 (s, 12H), 2.13 (s, 3H), 0.66 (s, 3H), 0.64 (s, 3H).; ^{13}C NMR (101 MHz, MeOD) δ 169.25, 155.81, 149.54, 142.02, 140.18, 131.55, 131.21, 131.10, 128.35, 122.31, 115.38, 40.95, 19.61, -1.10, -1.27.; LRMS (ESI) *m*/*z* calcd for $C_{27}H_{30}N_2O_2Si^+$ $[M+H]^+$ 443, found 443.

N-(7-(dimethylamino)-10-(5-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazine-1-carbonyl)-2-methylphenyl)-5,5-dimethyldibenzo[b,e]silin-3(5H)-ylidene)-N-methylmethanaminium (6)



Compound **5** (10 mg, 0.027 mmol) was added to a solution of compound **4** (10 mg, 0.0225 mmol) and HBTU (10 mg, 0.0270 mmol) in DMSO (0.25 mL). The resulting reaction mixture was stirred at ambient temperature for 1 hr. Crude product was purified with standard HPLC to give compound **6** (6.6 mg, 37.0%) as a deep blue solid. ^{1}H NMR (400 MHz, MeOD) δ 8.34 (d, *J* = 7.7 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.87 – 7.75 (m, 2H), 7.57 – 7.43 (m, 3H), 7.38 – 7.31 (m, 3H), 7.21 (s, 1H), 7.16 – 7.01 (m, 3H), 6.82 – 6.69 (m, 2H), 4.35 (s, 2H), 3.85 – 3.49 (m, 8H), 3.33 (s, 12H), 2.07 (s, 3H), 0.60 (s, 3H), 0.58 (s, 3H).; LRMS (ESI) *m/z* calcd for $C_{47}H_{48}FN_6O_3Si^+$ $[M]^+$ 791, found 791.; HRMS (ESI) *m/z* calcd for $C_{47}H_{48}FN_6O_3Si^+$ $[M]^+$ 791.3536, found 791.3522.

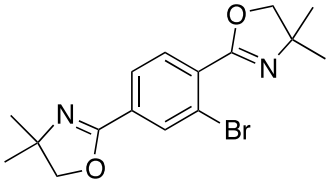
2-bromo-N1,N4-bis(1-hydroxy-2-methylpropan-2-yl)terephthalamide (7)



A solution of 2-Bromoterephthalic acid (750 mg, 3.06 mmol) in thionyl chloride (5 mL) with 5 drop of DMF was refluxed for 3 hr. After cooling to room temperature, thionyl chloride was removed by evaporation and dried under vacuum. The resulting residue was diluted with DCM (5 mL) and was added dropwise to a solution of 2-amino-2-methylpropan-1-ol and DIPEA in DCM (10 mL). The resulting reaction mixture was stirred at ambient temperature overnight. 1 day later, the resulting turbid solution was added to a $NaHCO_3$ (sat) aqueous solution. Organic material was extracted with EA three times and the combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Without further purification compound **7** (790 mg, 72.3%) was obtained as a white solid.

^{1}H NMR (400 MHz, DMSO) δ 8.04 (s, 1H), 7.87 – 7.78 (m, 2H), 7.71 (s, 1H), 7.42 (d, *J* = 7.9 Hz, 1H), 4.85 (t, *J* = 6.1 Hz, 1H), 4.80 (t, *J* = 6.0 Hz, 1H), 3.51 (t, *J* = 6.3 Hz, 4H), 1.31 (s, 12H).; 13C NMR (101 MHz, DMSO) 166.6, 164.4, 141.8, 137.2, 131.1, 128.3, 126.4, 118.6, 67.3, 67.0, 55.3, 23.5, 23.4.; LRMS (ESI) *m/z* calcd for $C_{16}H_{23}BrN_2O_4$ $[M-H]^-$ 385, found 385.

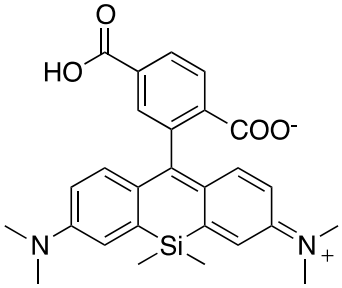
2,2'-(2-bromo-1,4-phenylene)bis(4,4-dimethyl-4,5-dihydrooxazole) (8)



A solution of compound **7** (500 mg, 1.29 mmol) in thionyl chloride (3 mL) was stirred at ambient temperature. After 1.5 hr of stirring, thionyl chloride was removed by evaporation and $NaHCO_3$ (sat) aqueous solution was carefully added to the reaction mixture. Organic material was extracted with EA three times and the combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The reaction mixture was purified with silica gel column chromatography (EA : Hex = 1:10 to EA only) to give compound **8** (392 mg, 54.9%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ 8.07 (d, *J* = 1.7 Hz, 1H), 7.89 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 4.14 (s, 2H), 4.11 (s, 2H), 1.31 (s, 6H), 1.29 (s, 6H).; ^{13}C NMR (101 MHz, DMSO) δ 159.5, 158.8, 132.2, 132.1, 131.5, 130.8, 126.7, 120.9, 78.8, 78.6, 68.1, 67.7, 28.1, 27.9.; LRMS (ESI) *m*/*z* calcd for $C_{16}H_{19}BrN_2O_2$ $[M+H]^+$ 351, found 351.

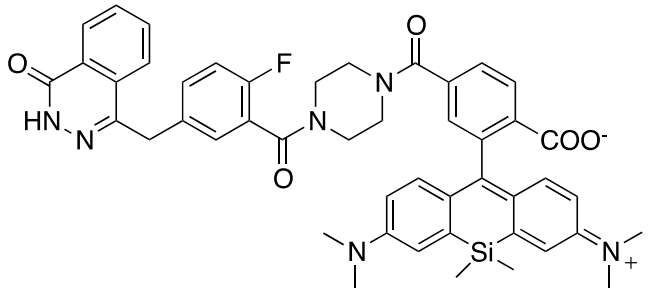
***N*-(10-(2,5-dicarboxyphenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[*b*,*e*]silin-3(5*H*)-ylidene)-*N*-methylmethanaminium (9)**



1.0 M tert-BuLi solution (0.3 mL) in cyclohexane was added dropwise at -78°C to a solution of compound **8** (129 mg, 0.379 mmol) in THF (5 mL). After 1hr stirring at the same temperature, a solution of compound **3** (30 mg, 0.093 mmol) in THF (5 mL) was added dropwise. The reaction mixture was gradually warmed up to ambient temperature and stirred at ambient temperature for 2 hr. The reaction mixture was cooled down with ice and AcOH (1 mL) was added to the reaction mixture dropwise. Solvent was evaporated and dried under vacuum. The resulting deep blue solid was dissolved with 6N HCl solution (12 mL) and stirred at 80°C for overnight. 1 day later, the resulting yellow turbid solution was cooled down to ambient temperature and the reaction mixture was added toward a $NaHCO_3$ (sat) (50 mL) aqueous solution. Organic material was extracted with DCM 4 times and the combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The reaction mixture was purified with silica gel column chromatography (MeOH : DCM = 0:100 to 1:10) to give compound **9** (25 mg, 57.1%) as a deep blue solid.

^{1}H NMR (400 MHz, MeOD) δ 8.21 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.84 (s, 1H), 7.04 (d, *J* = 2.9 Hz, 2H), 6.73 (d, *J* = 8.9 Hz, 2H), 6.64 (dd, *J* = 8.9, 2.9 Hz, 2H), 2.96 (s, 12H), 0.66 (s, 3H), 0.56 (s, 3H).; ^{13}C NMR (101 MHz, MeOD) δ 172.1, 156.3, 151.2, 138.0, 132.4, 131.2, 130.4, 129.1, 126.6, 126.4, 118.0, 114.9, 40.5, 0.3, -1.2.; LRMS (ESI) *m*/*z* calcd for $C_{27}H_{28}N_2O_4Si$ $[M+H]^+$ 473, found 473.

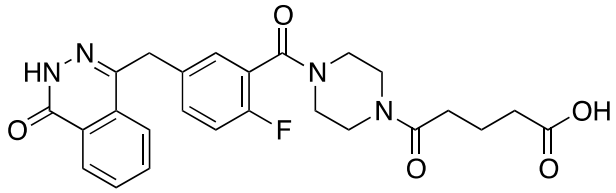
***N*-(10-(2-carboxy-5-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazine-1-carbonyl)phenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[*b*,*e*]silin-3(5*H*)-ylidene)-*N*-methylmethanaminiu (10)**



A solution of compound **9** (5 mg, 0.015 mmol), HBTU (6 mg, 0.015 mmol) and TEA (11 μL, 0.077 mmol) in DMF (200 μL) was incubated at ambient temperature. After 5 minutes of stirring, a solution of compound **5** (6 mg, 0.015 mmol) in DMF (200 uL) was added to the reaction mixture. The reaction mixture was stirred at ambient temperature for 1 hr. and was then purified using standard HPLC techniques to give compound **10** (2.0 mg, 15.8%) as a white solid.

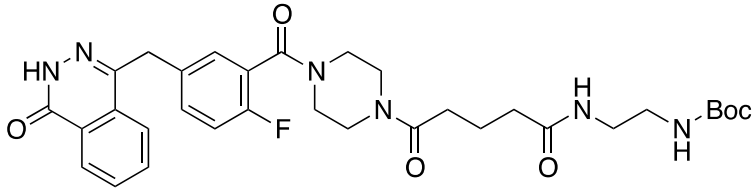
^{1}H NMR (400 MHz, MeOD) δ 8.44 – 8.30 (m, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.98 – 7.66 (m, 4H), 7.53 – 7.44 (m, 1H), 7.40 – 7.31 (m, 2H), 7.21 – 7.10 (m, 1H), 7.05 (d, *J* = 3.0 Hz, 2H), 6.81 – 6.52 (m, 4H), 4.37 (s, 2H), 3.93 – 3.43 (m, 8H), 2.96 (s, 12H), 0.65 (s, 3H), 0.56 (s, 3H).; LRMS (ESI) *m/z* calcd for $C_{47}H_{45}FN_6O_5Si$ $[M+H]^+$ 821, found 821.; HRMS (ESI) *m/z* calcd for $C_{47}H_{45}FN_6O_5Si$ $[M+H]^+$ 821, found 821.

5-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-5-oxopentanoic acid (11)



A solution of glutaric anhydride (56 mg, 0.49 mmol), 4-[[4-Fluoro-3-(piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one (150 mg, 0.41 mmol) and Triethylamine (171 μL, 1.23 mmol) in dichloromethane (2 mL) was stirred at ambient temperature. The reaction mixture was purified with silica gel column chromatography (MeOH : DCM = 0:100 to 1:5) to give compound **11** (160 mg, 81.3%) as a yellow oil. The purified compound was used for the next reaction without further characterization.

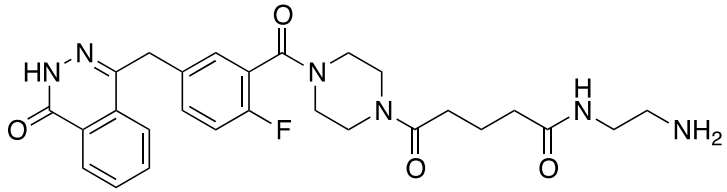
4-[[4-Fluoro-3-(4-(5-oxopentanamide)piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one (**12**)



A solution of compound **11** (140 mg, 0.29 mmol), N-Boc-Ethylenediamine (70 mg, 0.44 mmol) and Triethylamine (122 μL, 0.87 mmol) in DMF (3 mL) was stirred at ambient temperature for 1hr. The reaction mixture was purified with silica gel column chromatography (MeOH : DCM = 0:100 to 1:5) to give compound **12** (120 mg, 66.1%) as a clear sticky oil.

^{1}H NMR (400 MHz, Methanol-d) δ ^{1}H NMR (400 MHz, MeOD) δ 8.34 (d, *J* = 7.6 Hz, 1H), 8.04 – 7.90 (m, 2H), 7.89 – 7.76 (m, 2H), 7.35 – 7.52 (m, 2H), 7.15 (t, *J* = 8.8 Hz, 1H), 4.36 (s, 2H), 3.84 – 3.71 (dt, *J* = 26.1, 4.9 Hz, 2H), 3.70 – 3.61 (m, 2H), 356 – 3.45 (q, *J* = 7.6, 6.4 Hz, 2H), 3.35 – 3.08 (m, 6H), 2.44 (dt, *J* = 26.8, 7.3 Hz, 2H), 2.27 (q, *J* = 6.5 Hz, 2H), 1.97 – 1.83 (m, 2H), 1.42 (s, 9H).; LRMS (ESI) *m*/*z* calcd for $C_{32}H_{39}FN_6O_6$ $[M+H]^+$ 623, found 623.

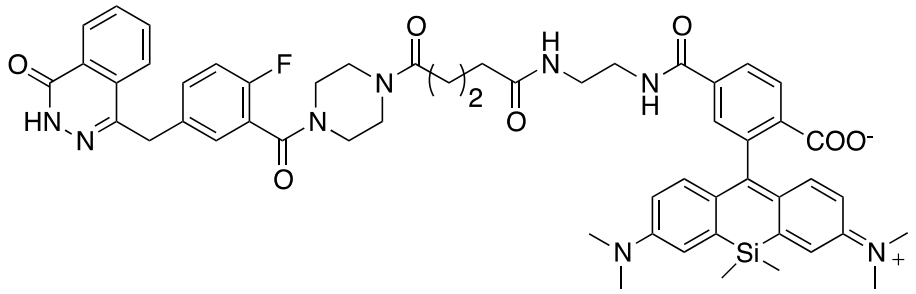
***N*-(2-aminoethyl)-5-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-5-oxopentanamide** (**13**)



A solution of compound **12** (90 mg, 0.187 mmol) in DCM and TFA (v:v = 3:1) was stirred at ambient temperature for 1hr and was then concentrated in vacuo. The reaction mixture was purified with silica gel column chromatography (MeOH : DCM = 0:100 to 1:5) to give compound **13** (74 mg, 75.6%) as clear sticky oil.

^{1}H NMR (400 MHz, MeOD) δ 8.43 (s, 1H), 8.38 (d, *J* = 7.5 Hz, 1H), 7.96 (t, *J* = 7.0 Hz, 1H), 7.85 (q, *J* = 8.7, 7.5 Hz, 2H), 7.51 (t, *J* = 6.2 Hz, 1H), 7.36 (t, *J* = 6.2 Hz, 1H), 7.17 (t, *J* = 9.0 Hz, 1H), 4.39 (s, 2H), 3.82 – 3.71 (m, 2H), 3.70 – 3.64 (m, 2H), 3.57 – 3.47 (m, 2H), 3.44 (t, *J* = 5.9 Hz, 2H), 3.05 (t, *J* = 5.9 Hz, 2H), 2.47 (dt, *J* = 25.3, 7.1 Hz, 2H), 2.30 (q, *J* = 6.7 Hz, 2H), 1.98 – 1.83 (m, 2H).; LRMS (ESI) *m*/*z* calcd for $C_{27}H_{31}FN_6O_4$ $[M+H]^+$ 523, found 523.

***N*-(10-(2-carboxy-5-((2-(5-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-5-oxopentanamido)ethyl)carbamoyl)phenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[*b*,*e*]silin-3(5*H*)-ylidene)-*N*-methylmethanaminium (14)**



A solution of compound **9** (5 mg, 0.015 mmol), HBTU (6 mg, 0.015 mmol) and TEA (11 μL, 0.077 mmol) in DMF (200 μL) was incubated at ambient temperature. After 5 minutes of stirring, a solution of compound **13** (8 mg, 0.015 mmol) in DMF (200 μL) was added to the reaction mixture, which was stirred at ambient temperature for 1 hr. The reaction mixture was purified using standard HPLC techniques to give compound **14** (3.3 mg, 32%) as a sky blue solid.

^{1}H NMR (400 MHz, MeOD) δ 8.45 (s, 1H), 8.36 (t, *J* = 6.8 Hz, 1H), 8.10 – 7.91 (m, 4H), 7.90 – 7.72 (m, 4H), 7.53 – 7.44 (m, 1H), 7.40 – 7.32 (m, 1H), 7.21 – 7.11 (m, 1H), 7.04 (d, *J* = 2.8 Hz, 2H), 6.70 (t, *J* = 9.0 Hz, 2H), 6.65 – 6.56 (m, 2H), 4.37 (d, *J* = 11.5 Hz, 2H), 3.78 – 3.57 (m, 2H), 3.56 – 3.36 (m, 8H), 3.27 – 3.16 (m, 2H), 2.96 (s, 12H), 2.39 – 2.12 (m, 4H), 1.82 (q, *J* = 8.1 Hz, 2H), 0.65 (s, 3H), 0.56 (s, 3H). LRMS (ESI) *m/z* calcd for $C_{54}H_{57}FN_8O_7Si$ $[M+H]^+$ 977, found 977.; HRMS (ESI) *m/z* calcd for $C_{54}H_{57}FN_8O_7Si$ $[M+H]^+$ 977.4176, found 977.4183.

***Enzyme assay*.**

A commercially available colorimetric assay (Trevigen, Gaithersburg, MD) was used to measure PARP activity in vitro in the presence of inhibitors. Olaparib and the Olaparib-derivatives (compound **6**, **10**, and **14**) (concentration from 10 μM to 1 nM) were incubated with 0.5 units PARP HSA for 10 minutes in histone-coated 96 well plates. All experiments were carried out in triplicate. Control samples did not contain inhibitor. All reaction mixtures were adjusted to a final volume of 50 uL and a maximum final concentration 0.4% DMSO in assay buffer. The remainder of the assay was performed according to the manufacturer's instructions. PARP activity was measured by absorbance at 450 nm in each well using a Safire[2] microplate reader (Tecan Group, Mannedorf, Switzerland). IC_{50} values were calculated using Prism software (GraphPad, La Jolla, CA).

Cell Lines

The MDA-MB-231 cell line was purchased from ATCC (Manassas, VA, USA) and the OVCA429 cell line was generously provided by Dr Michael Birrer (Massachusetts General Hospital, Boston, MA, USA). These cell lines were grown in RPMI media supplemented with 10% fetal bovine serum, 100 I.U. penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. HT1080 cells from ATCC were grown in DMEM supplemented with 10% fetal bovine serum, 100 I.U. penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine.

GFP expressing cell lines

GFP-H2B cells (HT1080 cell line) and GFP-PARP cells (MDA-MB-231 cell line) were constructed according to the previously reported procedure[2,3].

GFP-Mito cells (OVCA429 cell line)

Green Fluorescent Protein tagged to subunit VIII of cytochrome c oxidase (Mito-GFP) was utilized to identify mitochondria. Mito-GFP was generated by subcloning the mitochondrial targeting sequence and AcGFP1 from the pAcGFP1-Mito Vector (Clontech, Cat. # 632432) into the pLVX-mCherry-N1 Vector (Clontech, Cat. #632562) using BamHI and NotI restriction enzymes. Correct insertion of pAcGFP1-Mito was confirmed by sequencing the insert in its entirety.

Infectious lentiviral particles were produced in HEK-293T cells. Briefly, 293T cells at low passage number were plated in 10 cm dishes and cultured overnight at 37°C in a humidified chamber with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Tet-system approved fetal bovine serum (FBS, Clontech), 100 I.U. penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Cells were then transfected with a packaging plasmid mix of pMDL gag/pol, pRSV Rev, and pCMV-VSVg, (Addgene plasmids 12251, 12253, and 8454, respectively) and the pLVX-Mito-GFP target vector in a 1:1 ratio using the TransIT-LT1 Transfection Reagent (Mirus). The 293T cells were then incubated for approximately 72 h, at which point the supernatant was collected and filtered through a 0.45 μm cellulose acetate syringe filter. Supernatants were aliquoted into cryovials and snap frozen in liquid nitrogen before being stored at -80 °C.

To generate OVCA429 cells stably expressing the Mito-GFP reporter, cells were plated in 12 well plates and infected with lentiviral supernatants such that approximately 85% of cells were fluorescent. Cells were then sorted for positive fluorescence using a BD FACSAria (MGH

Pathology-Simches and CNY Flow Cytometry Core and Flow Image Analysis) followed by selection in 5 μg/ml puromycin. Cells were maintained in RPMI medium supplemented with 10% FBS, 100 I.U. penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 3 μg/ml puromycin.

Live cell fluorescence microscopic imaging of SiR-based CIDs.
OVCA429, HT1080 and MDA-MB-231 cells were plated at 5000 cells per well in 96-well black μ-clear bottom plates (Grenier Bio-One) and were grown for 48-72 hrs. On the day of imaging, cells were incubated with a final concentration of 10 μM (0.1% DMSO in growth media) of compound **6**, **10** and **14** for 30 min at 37°C. Cells were washed three times with media (3 min each) and live cells were imaged in a humidified environmental chamber of a DeltaVision microscope using a 40X objective.

Competition imaging experiment.
OVCA429 (GFP-Mito) cells were plated at 5000 cells per well in 96-well black μ-clear bottom plates (Grenier Bio-One) and were grown for 48 hrs. On the day of imaging, cells were incubated with final concentration of 800 and 400 nM (0.2% DMSO in growth media) of Olaparib (AZD-2281) for 30 min at 37°C. Without washing, cells were co-incubated with compound 14 (5 μM final concentration with 0.4% DMSO in growth media) and Olaparib (800 and 40 nM) for 30 min at 37°C. Cells were washed three times with media (5 min each) and live cells were imaged in a humidified environmental chamber of a DeltaVision microscope using a 20X objective.

Supplementary Figures

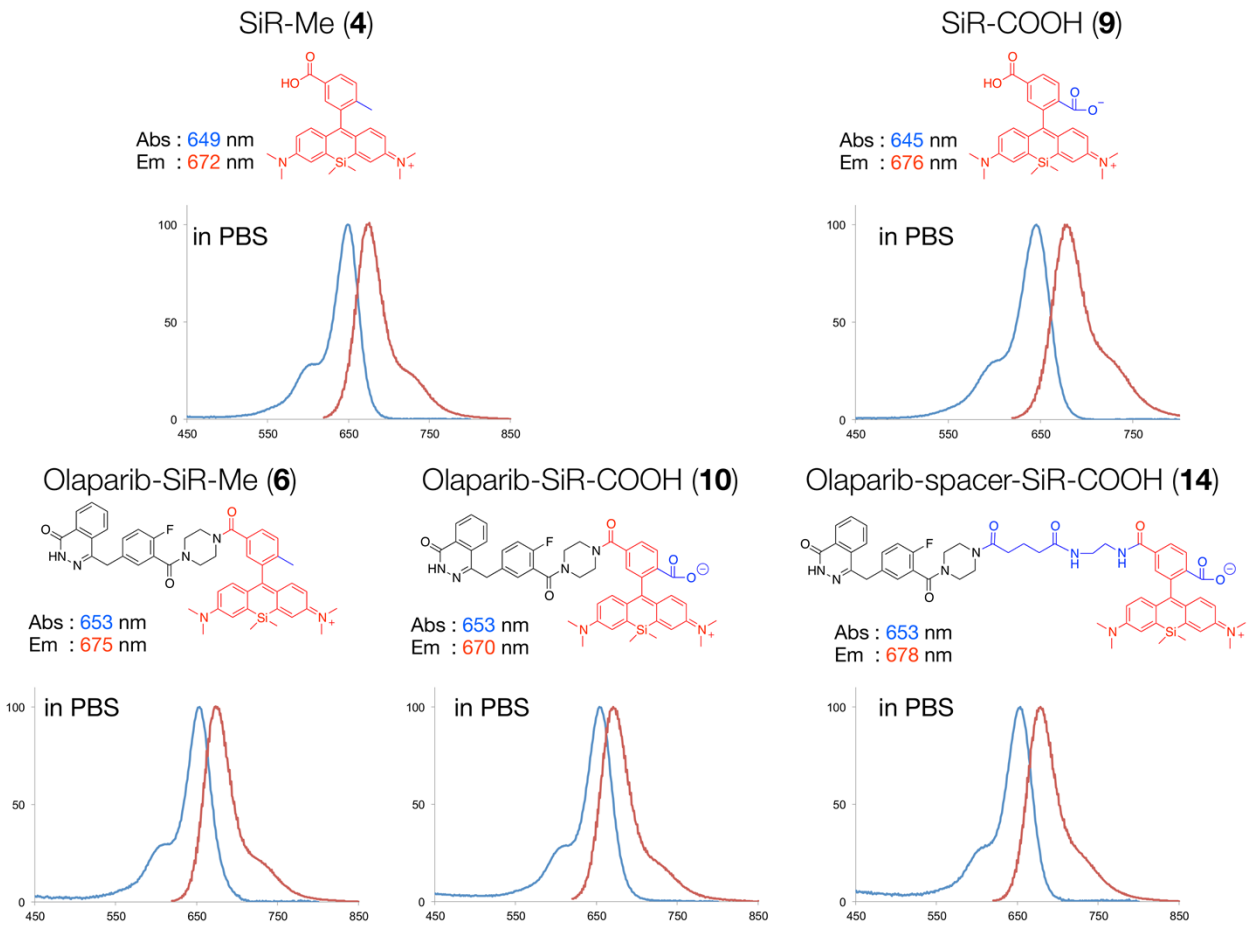
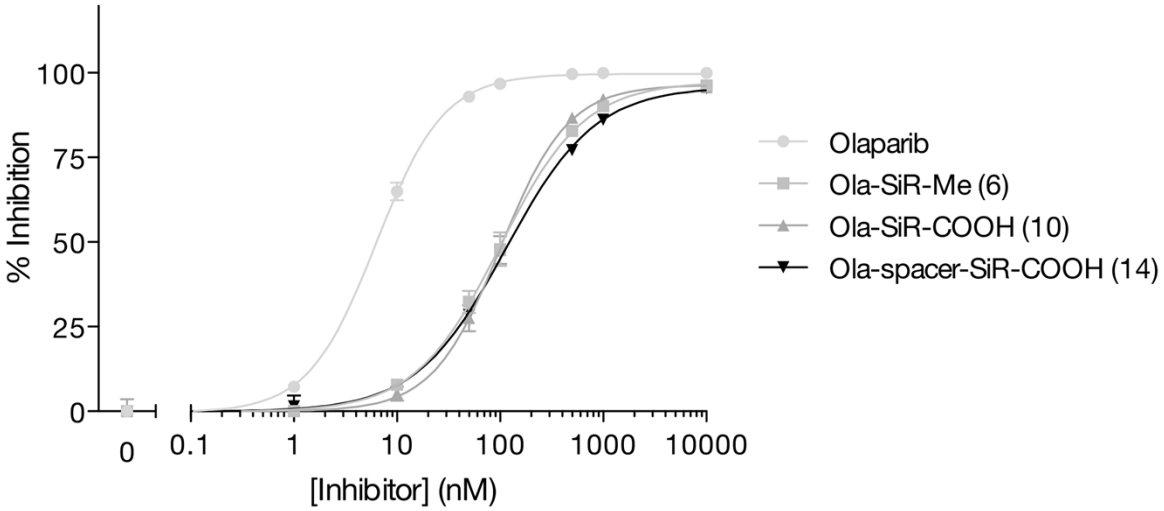


Fig. S1 Photophysical property of SiR derivatives in PBS.



Compound	IC_{50} (nM)	95% confidence Interval (nM)	R^2
Olaparib	6.3	5.9 to 6.7	0.99
Olaparib-SiR-Me (**6**)	99.2	89.9 to 109.5	0.99
Olaparib-SiR-COOH (**10**)	100.3	91.0 to 110.5	0.99
Olaparib-spacer-SiR-COOH (**14**)	112.9	103.7 to 122.8	0.99

Fig. S2 IC_{50} measurement of the three different companion imaging drugs against human recombinant PARP1 enzyme.

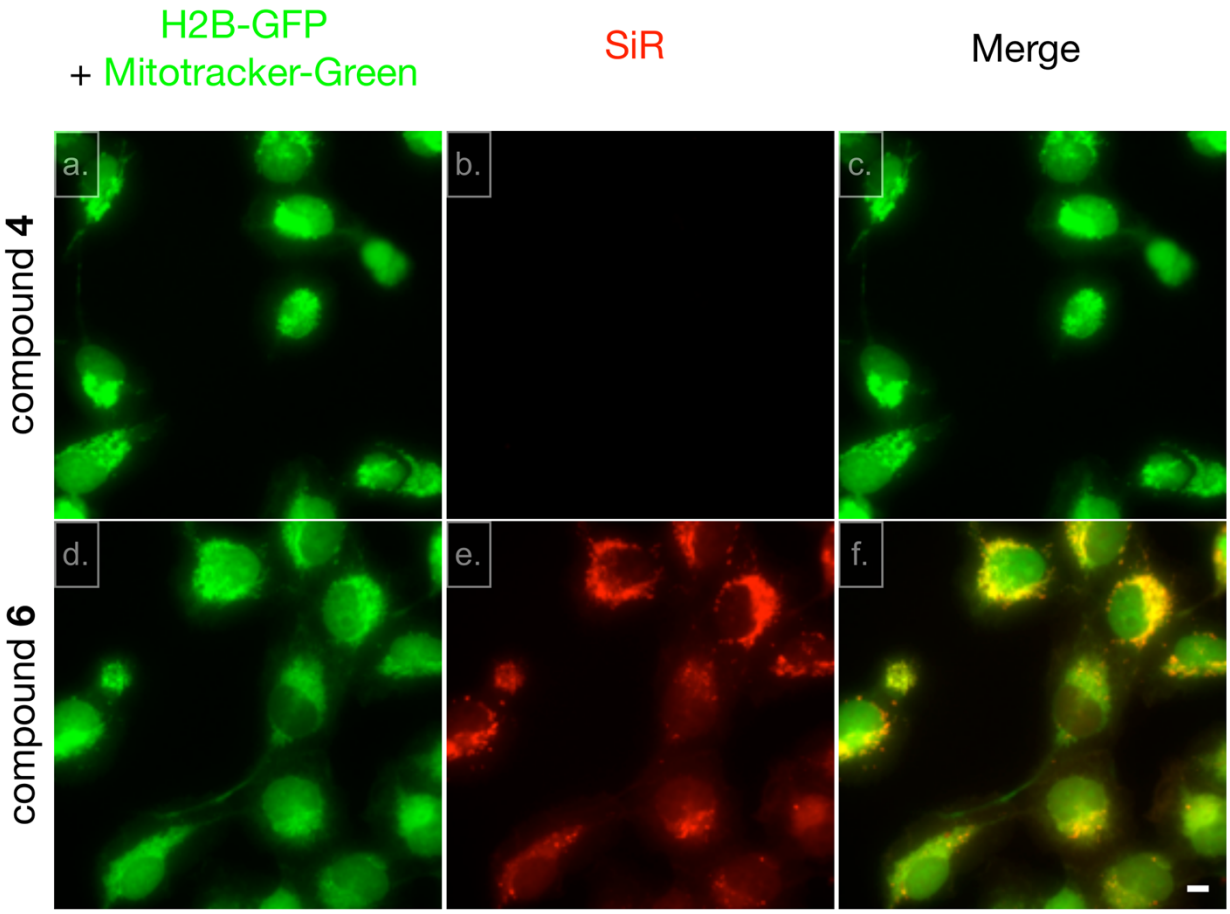


Fig. S3 Colocalization of Mitotracker green and **6** in live HT1080 cells expressing H2B-GFP. 10 μM **4** (a, b and c) or **6** (d, e and f) were incubated with HT1080 cells for 30 minutes in the presence of 100 nM of Mitotracker-green.

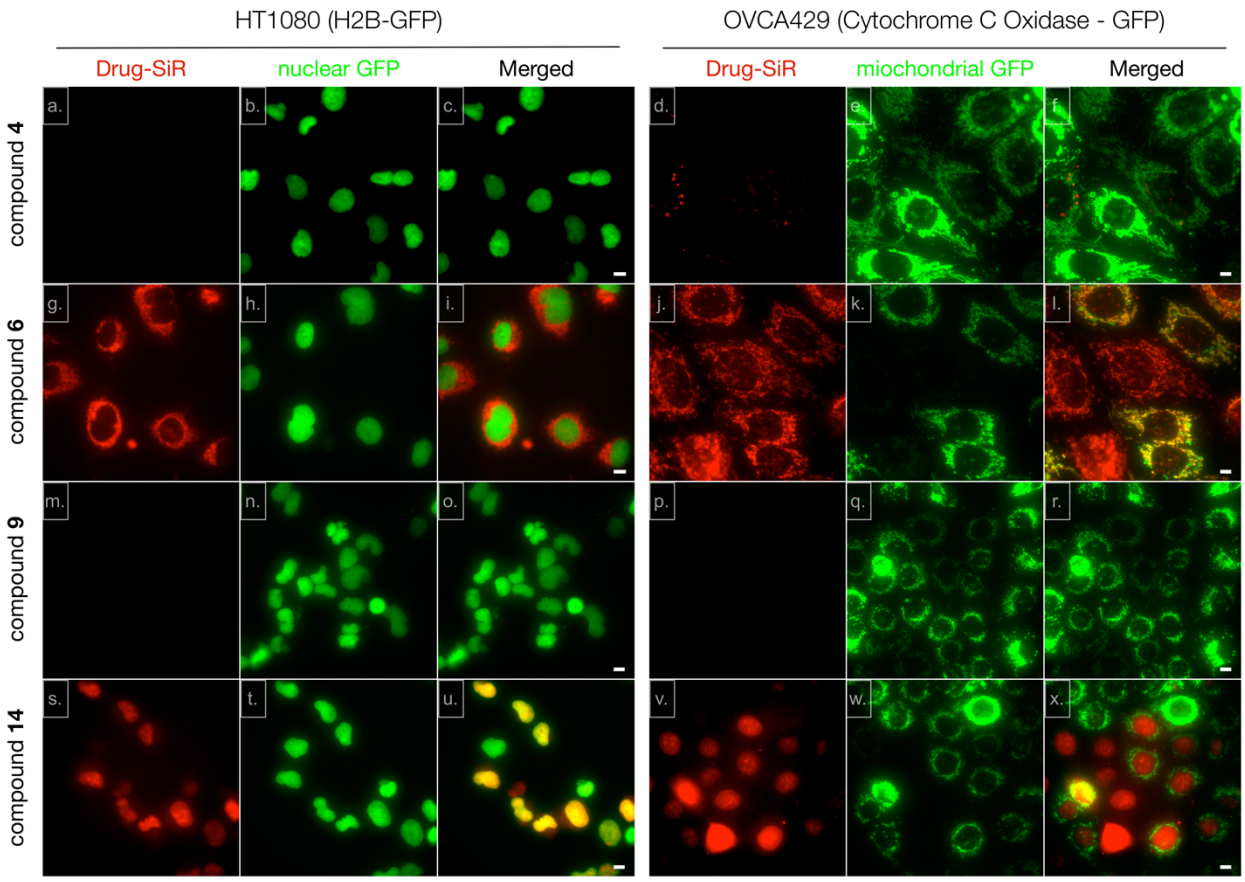


Fig. S4 Live cell imaging of HT1080 cells (expressing H2B-GFP; a-c, g-i, m-o and s-u) and OVCA429 cells (expressing cytochrome C Oxidase-GFP; d-f, j-l, p-r and v-x) with 10 µM **4** (a,b,c,d,e and f), **6** (g,h,i,j,k and l), **9** (m,n,o,p,q and r), and **14** (s,t,u,v,w and x). Cells were treated with appropriate compounds for 30 minutes. After washing with growth media three times for 3 minutes each, live cell images was collected using DeltaVision fluorescence microscope. Scale bar: 10µm.

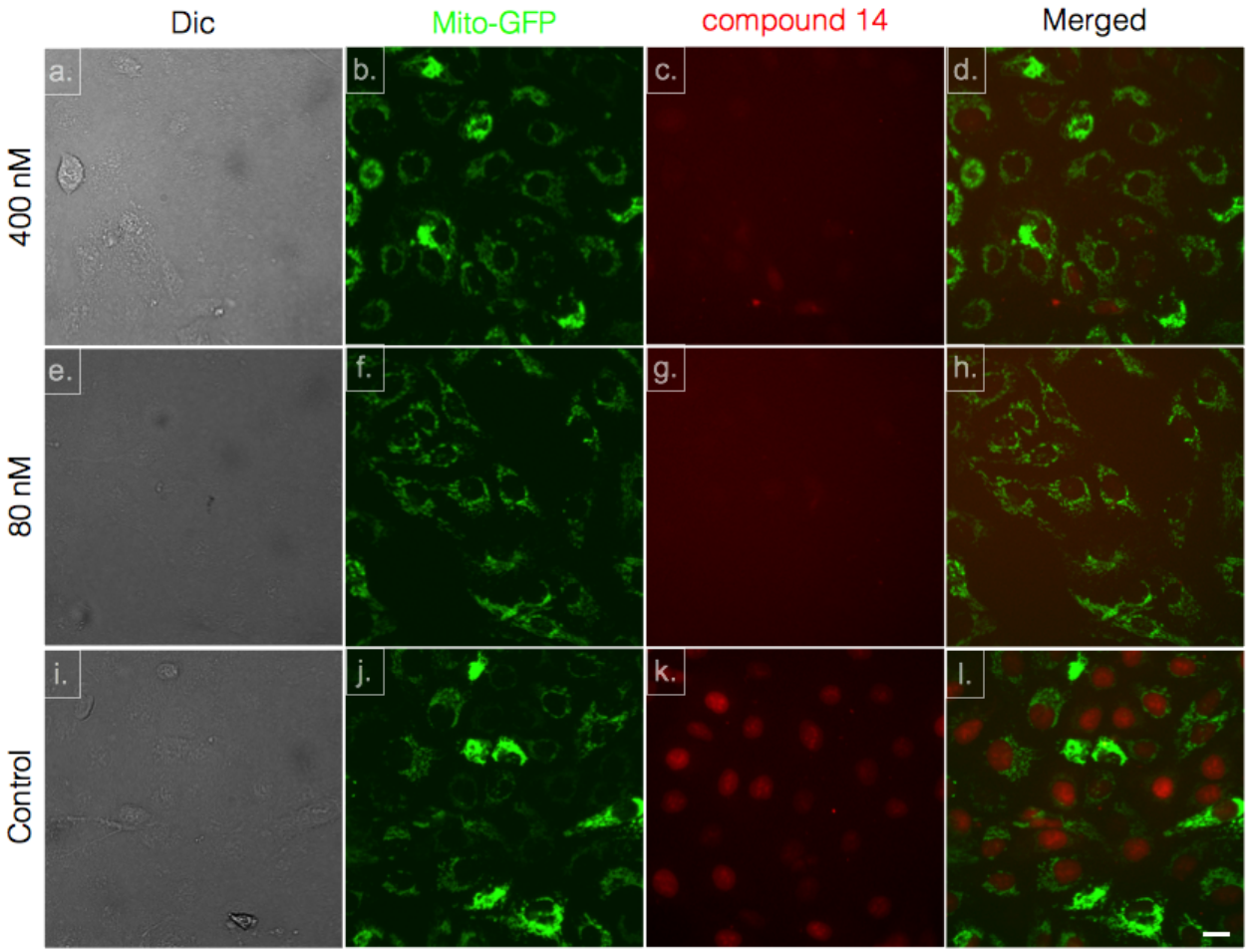
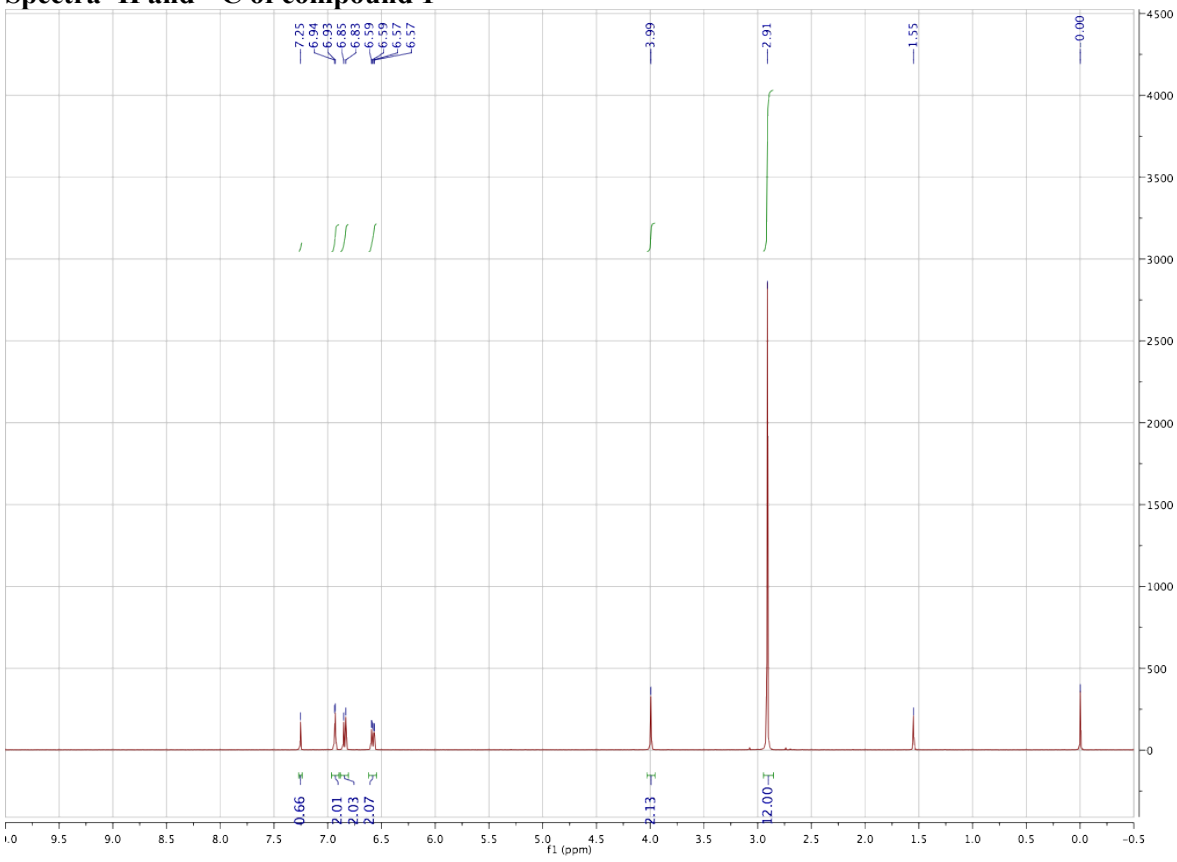
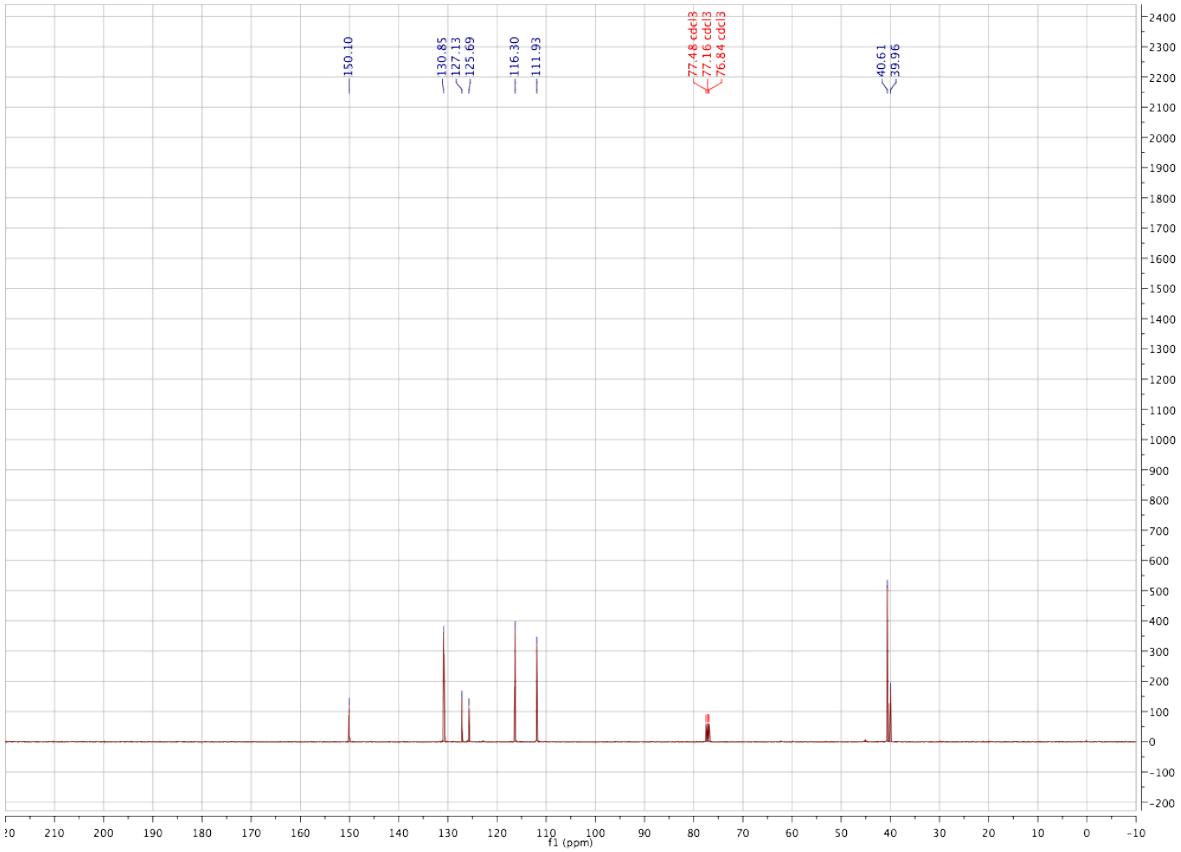


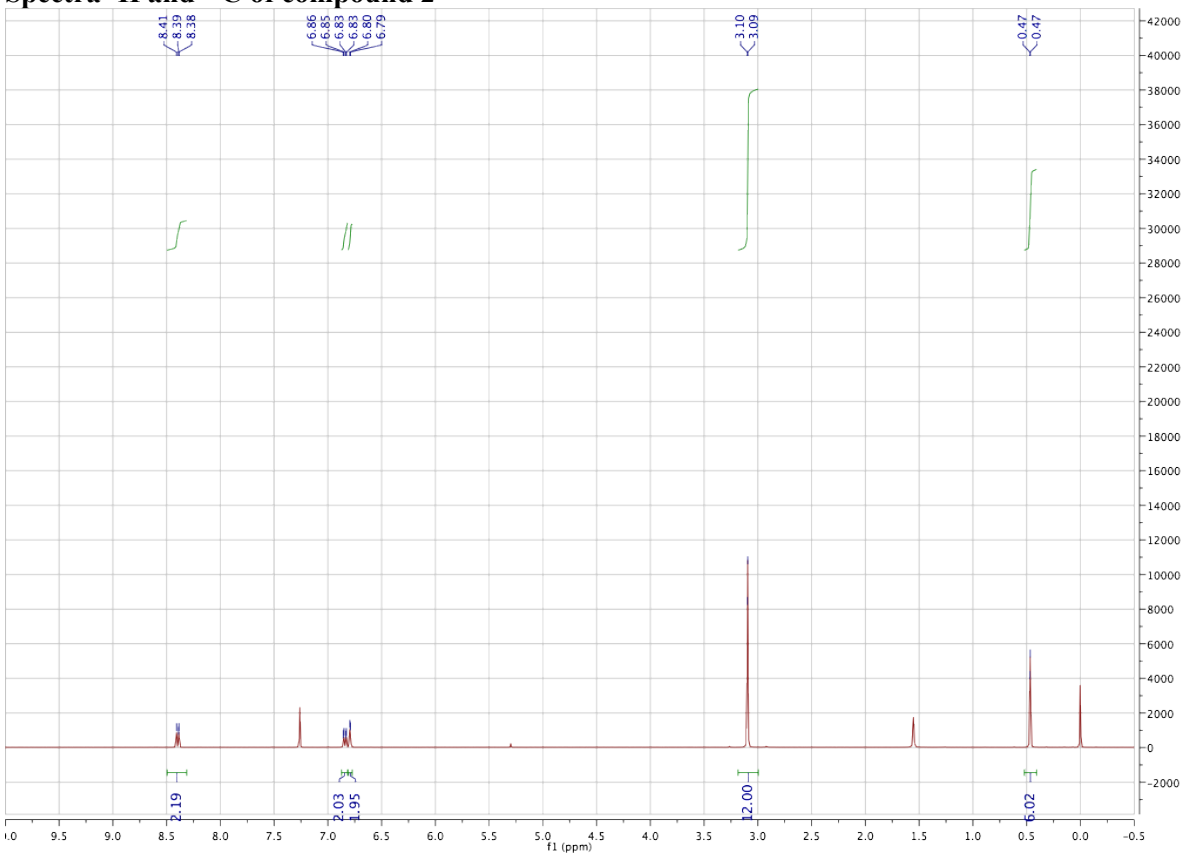
Fig. S5 Competition imaging experiment of OVCA429-mitoGFP. Cells were treated with 400 nM (a,b,c and d), 80 nM (e,f,g and h) of Olaparib or DMSO control (i,j,k and l) for 30 minutes. Without washing, cells were co-incubated with compound 14 (5 μM) and Olaparib for 30 minutes. After washing with growth media three times for 5 minutes each, live cell images was collected using DeltaVision fluorescence microscope. Scale bar: 20 μm.

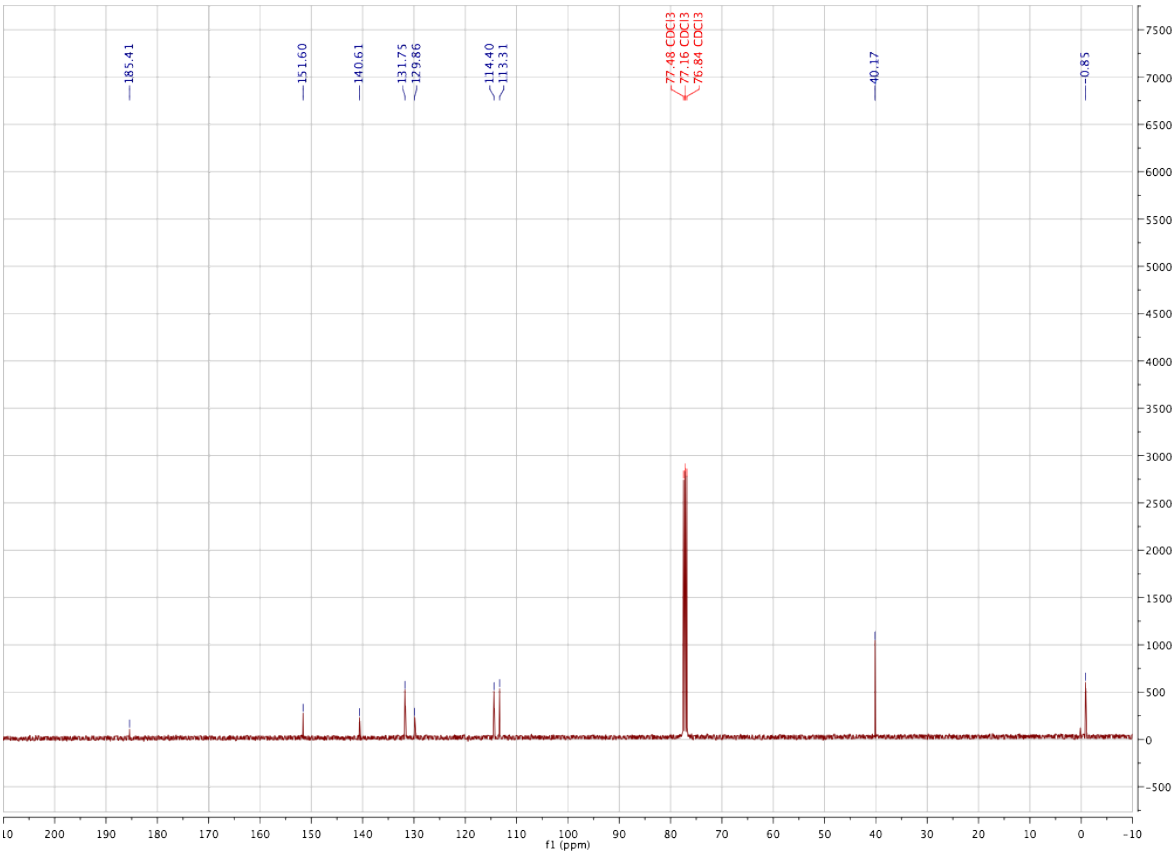
Spectra ^{1}H and ^{13}C of compound 1



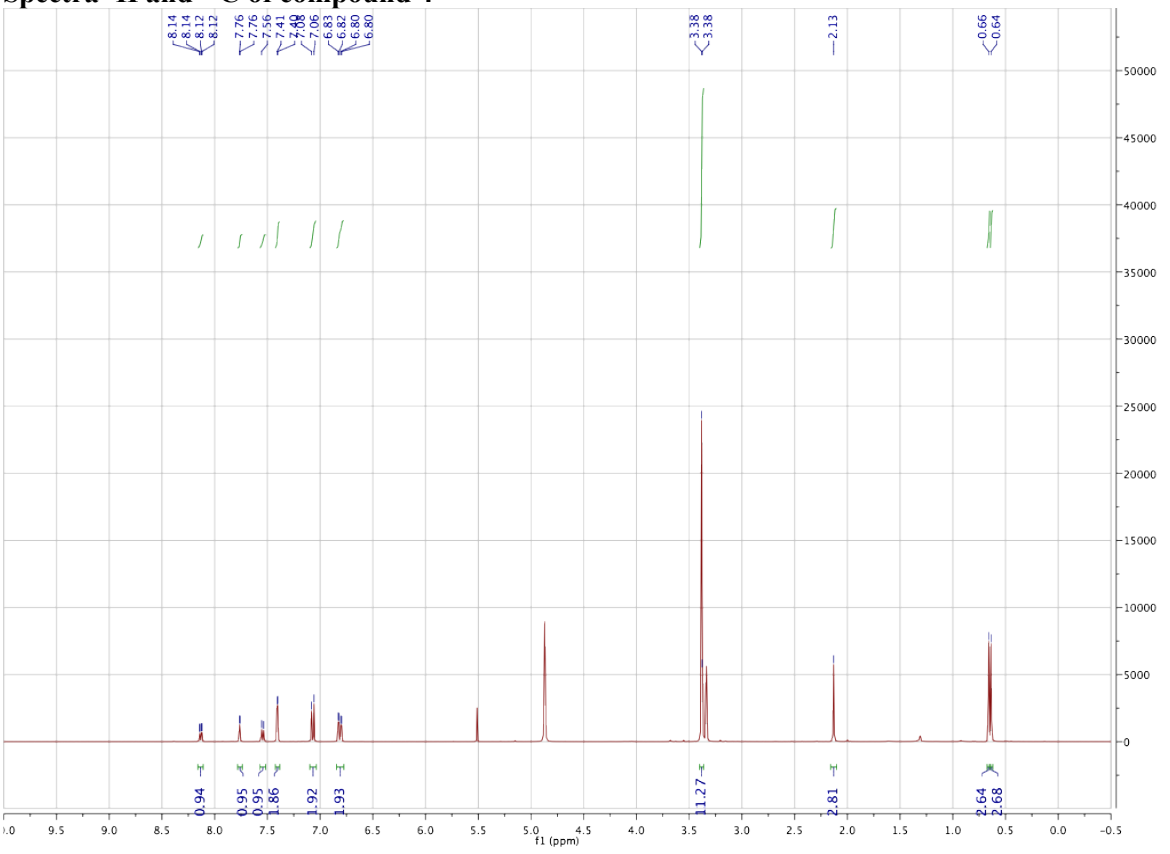


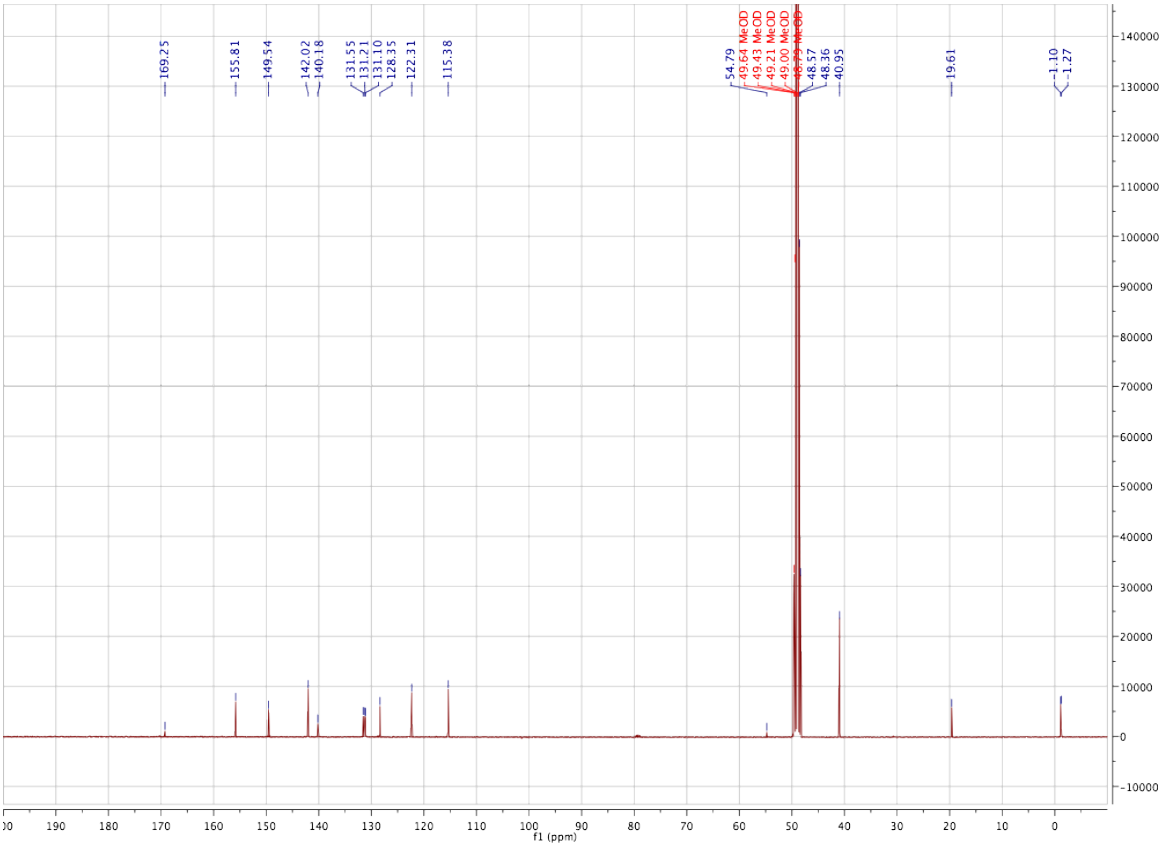
Spectra ^{1}H and ^{13}C of compound 2



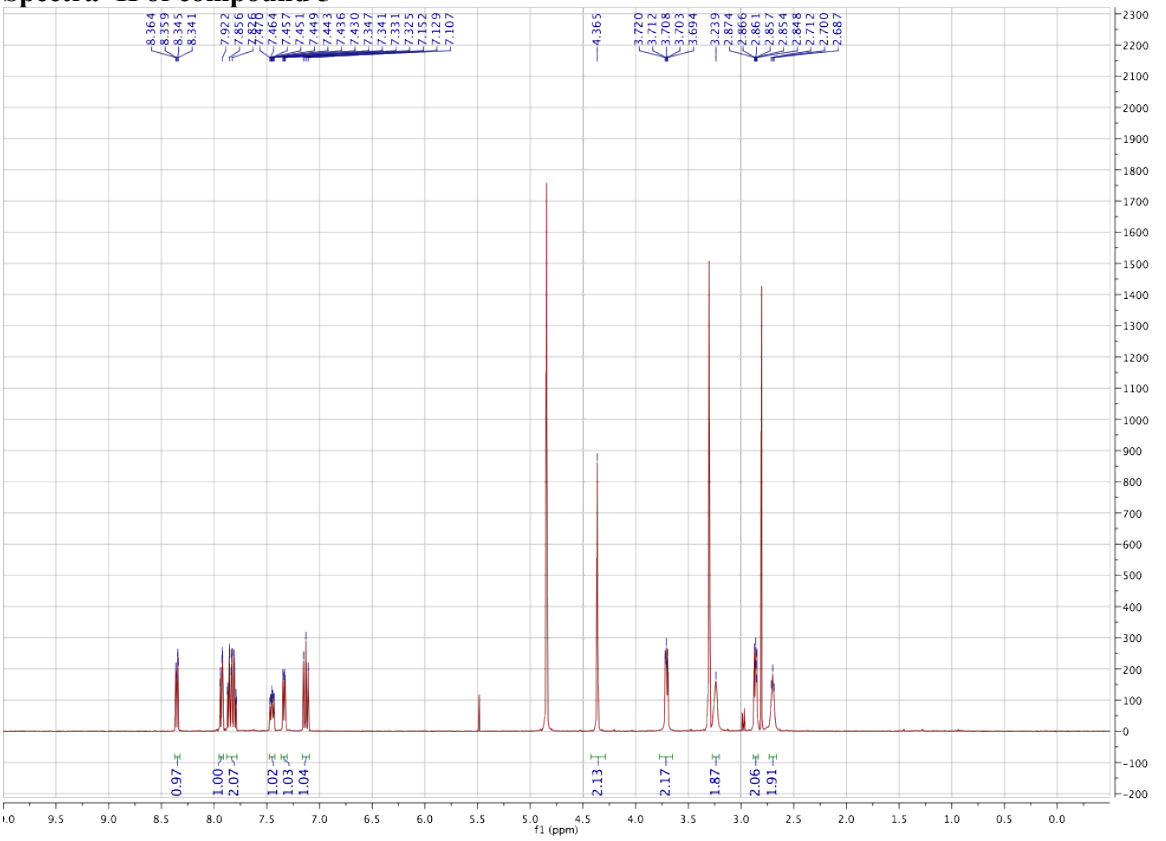


Spectra ^{1}H and ^{13}C of compound 4

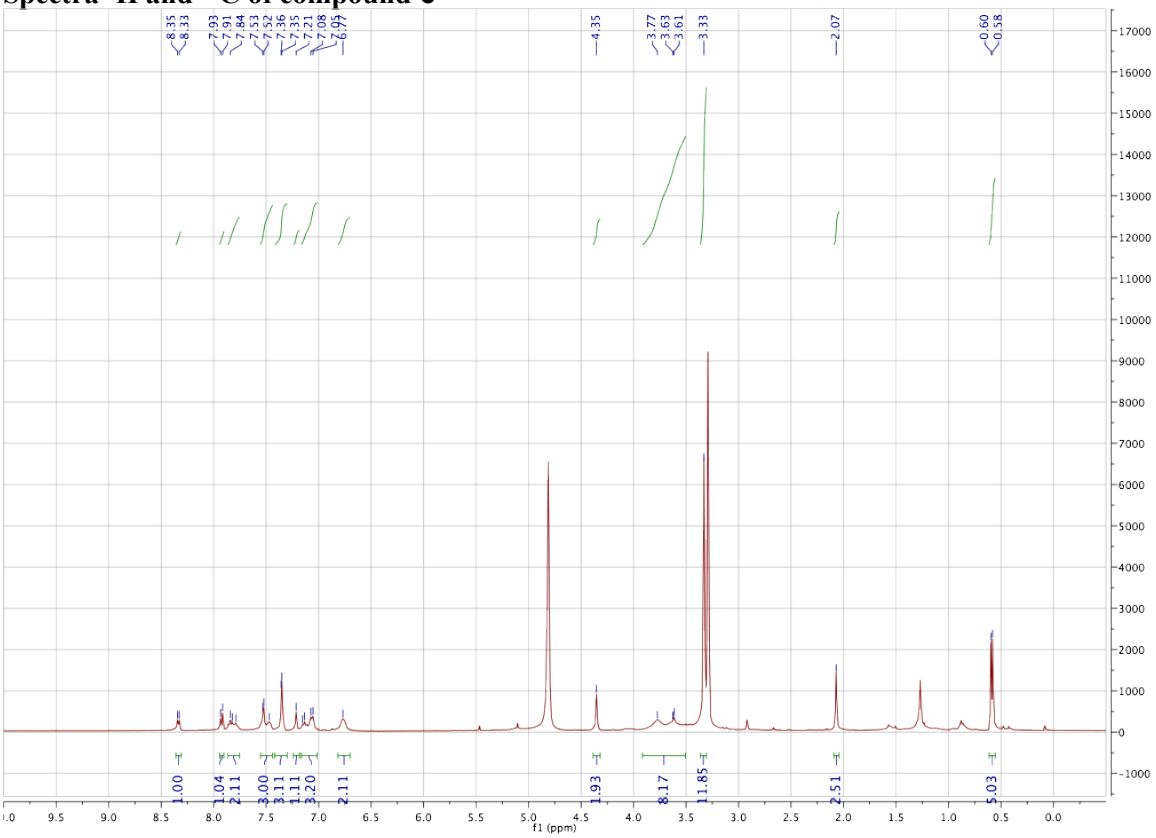




Spectra ^{1}H of compound 5



Spectra ^{1}H and ^{13}C of compound 6



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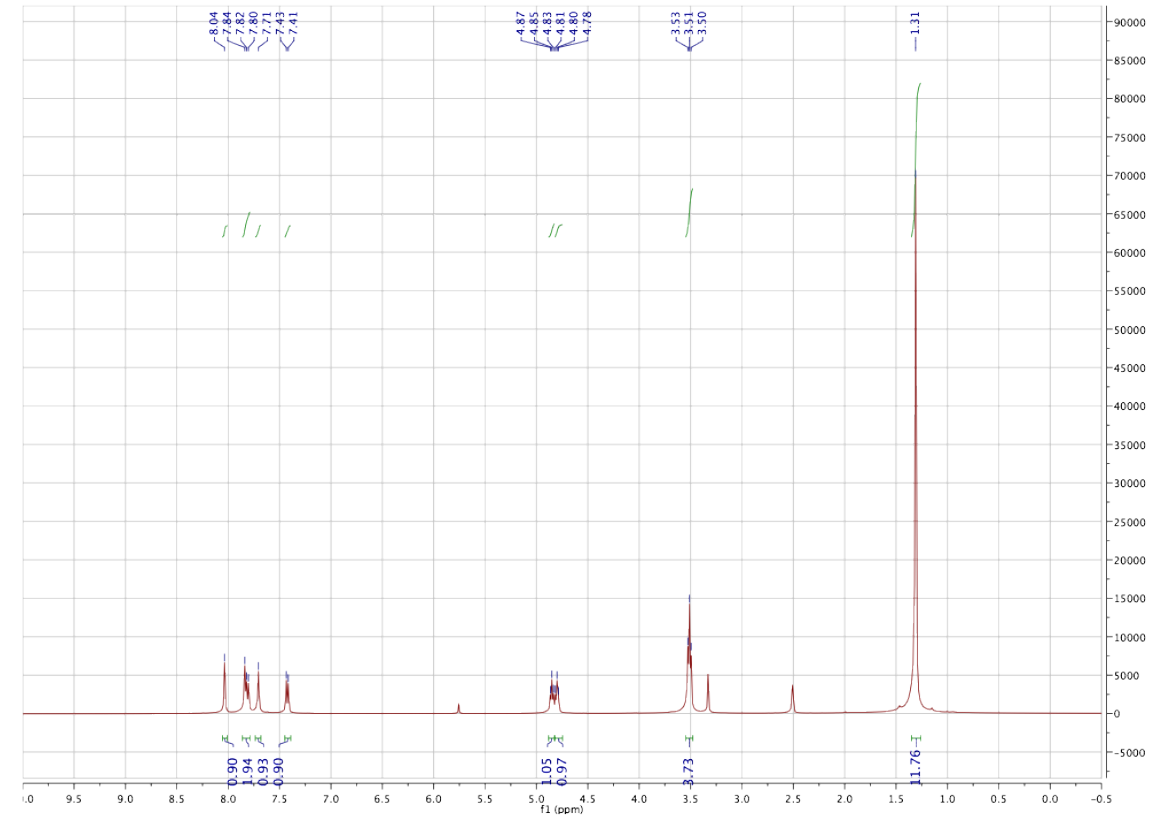
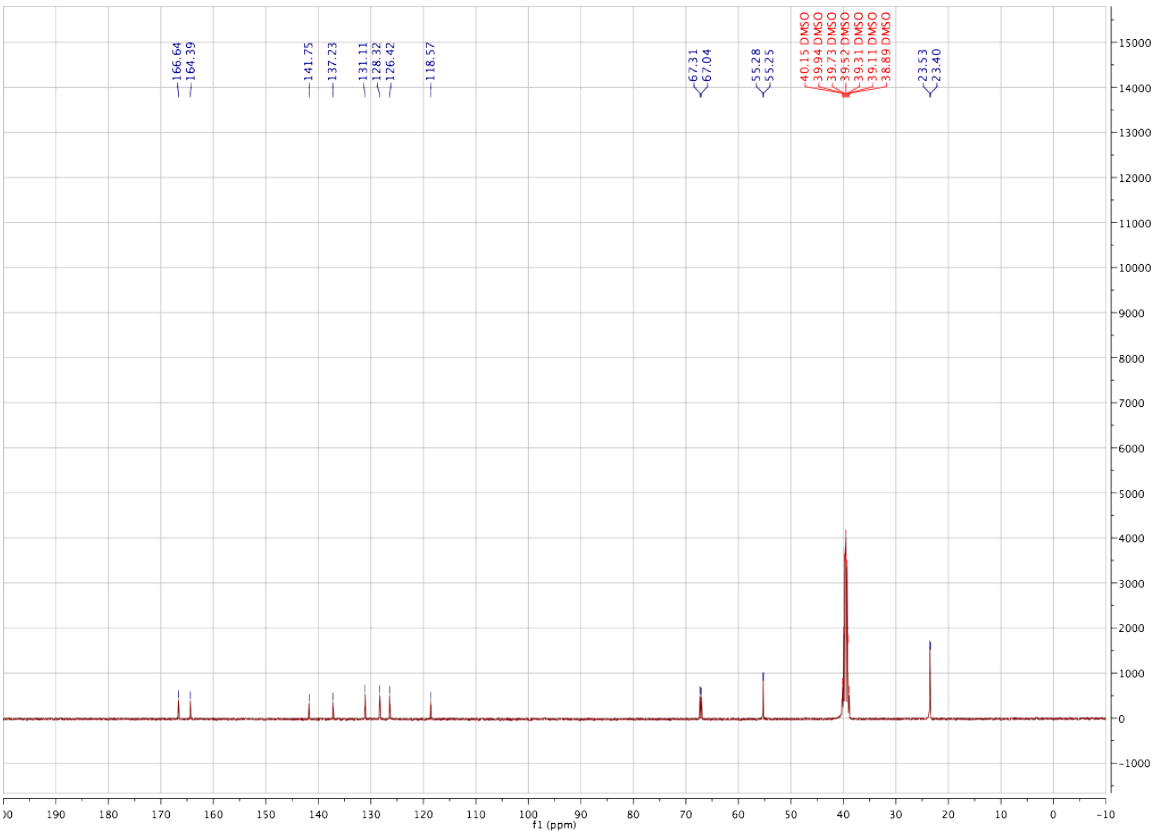
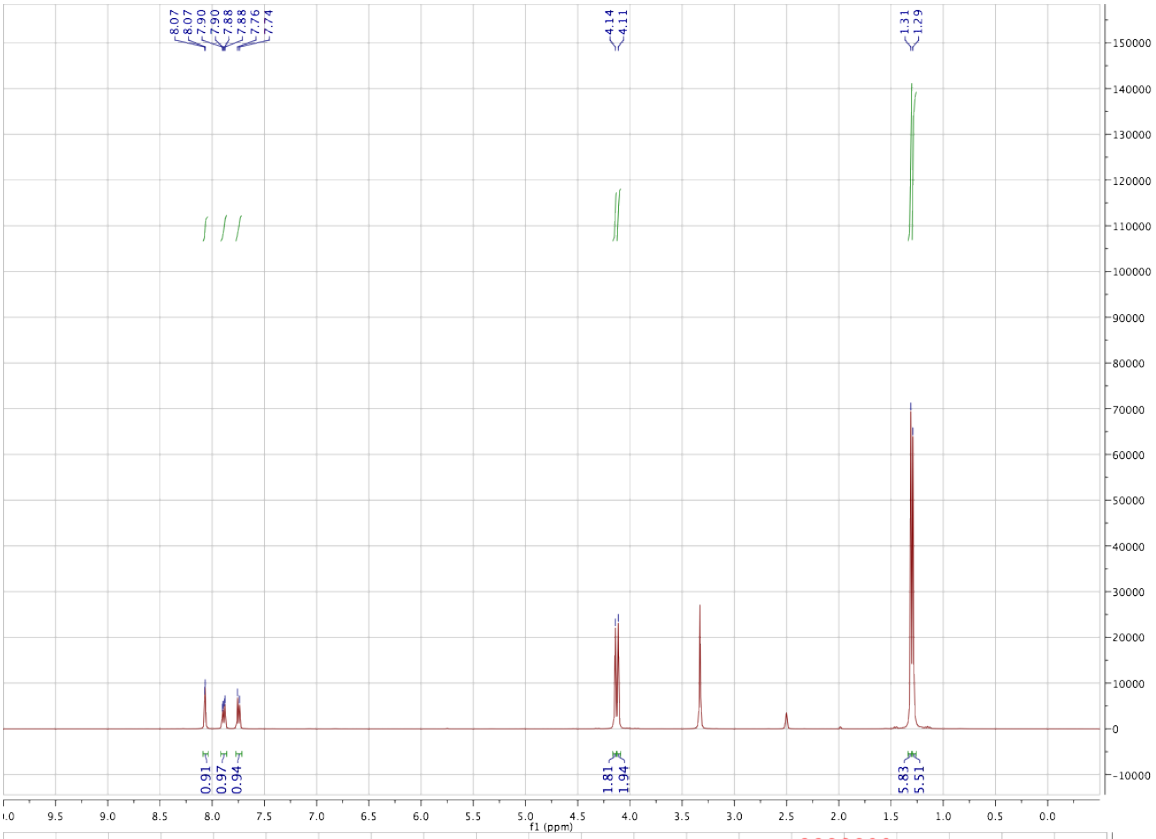
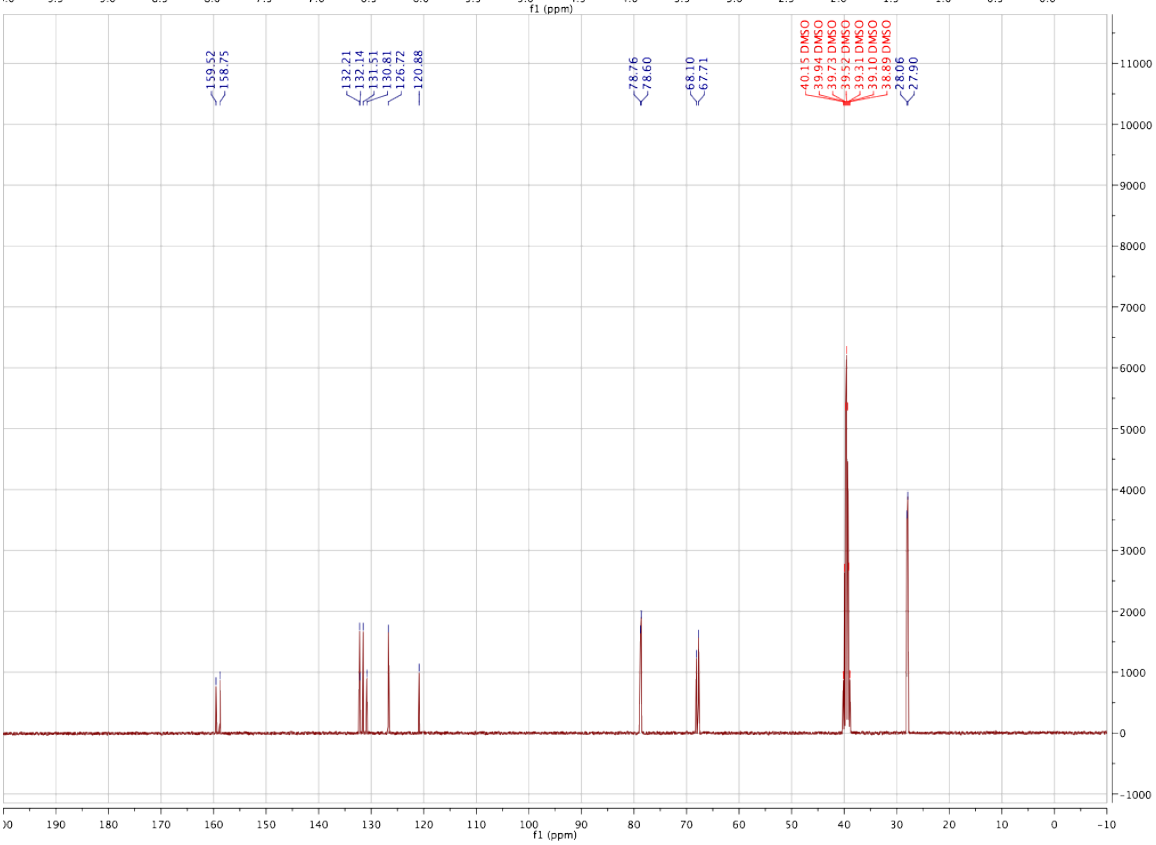


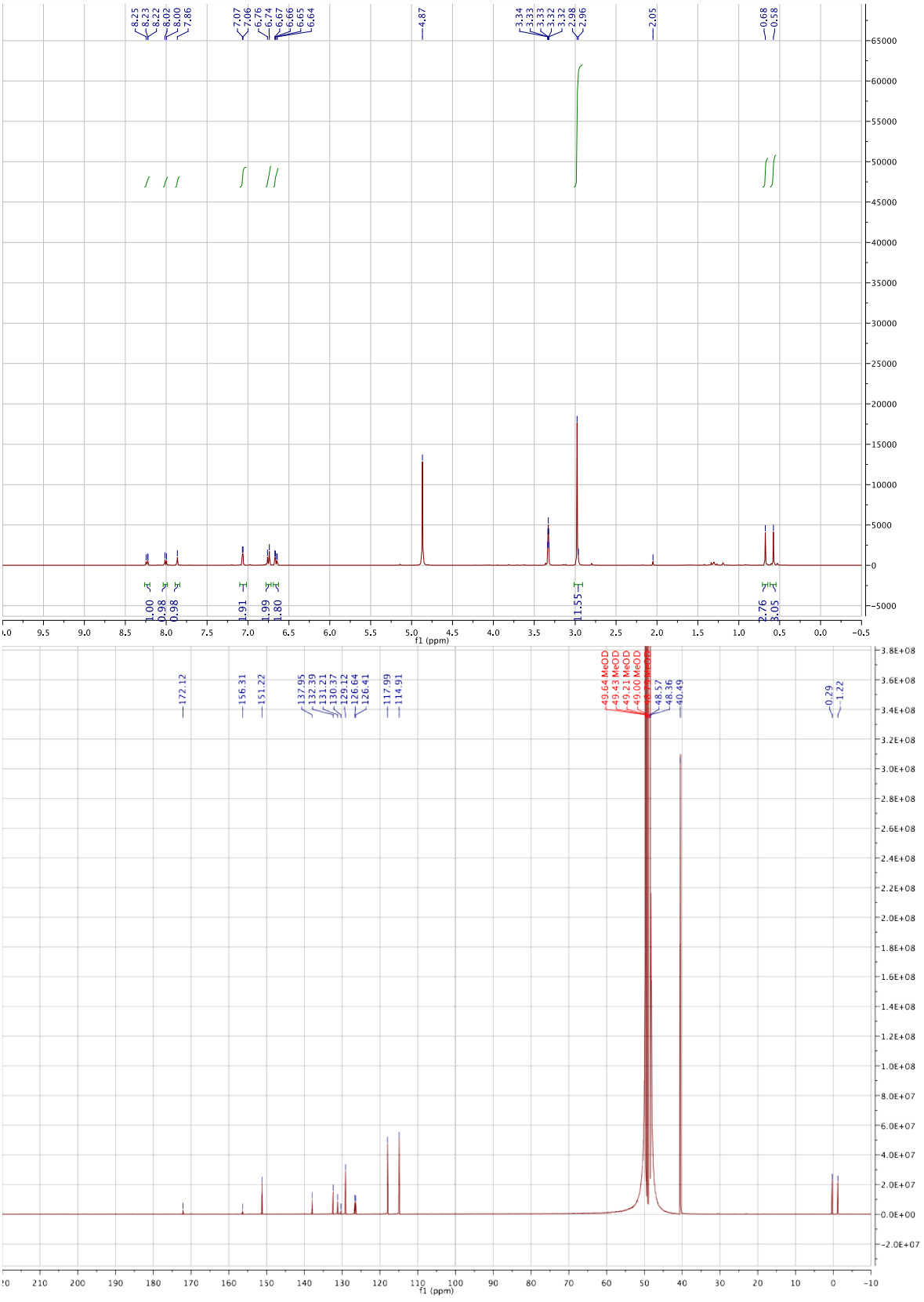


Spectra ^{1}H and ^{13}C of compound 8

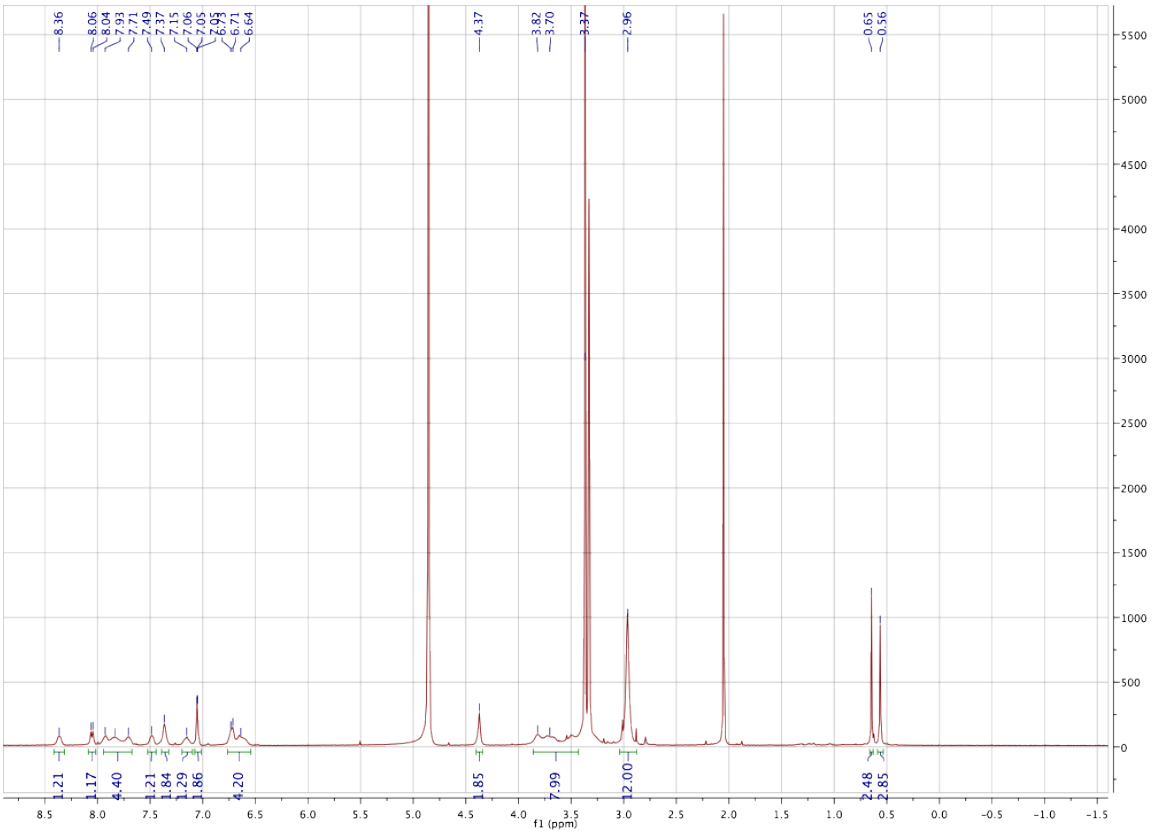




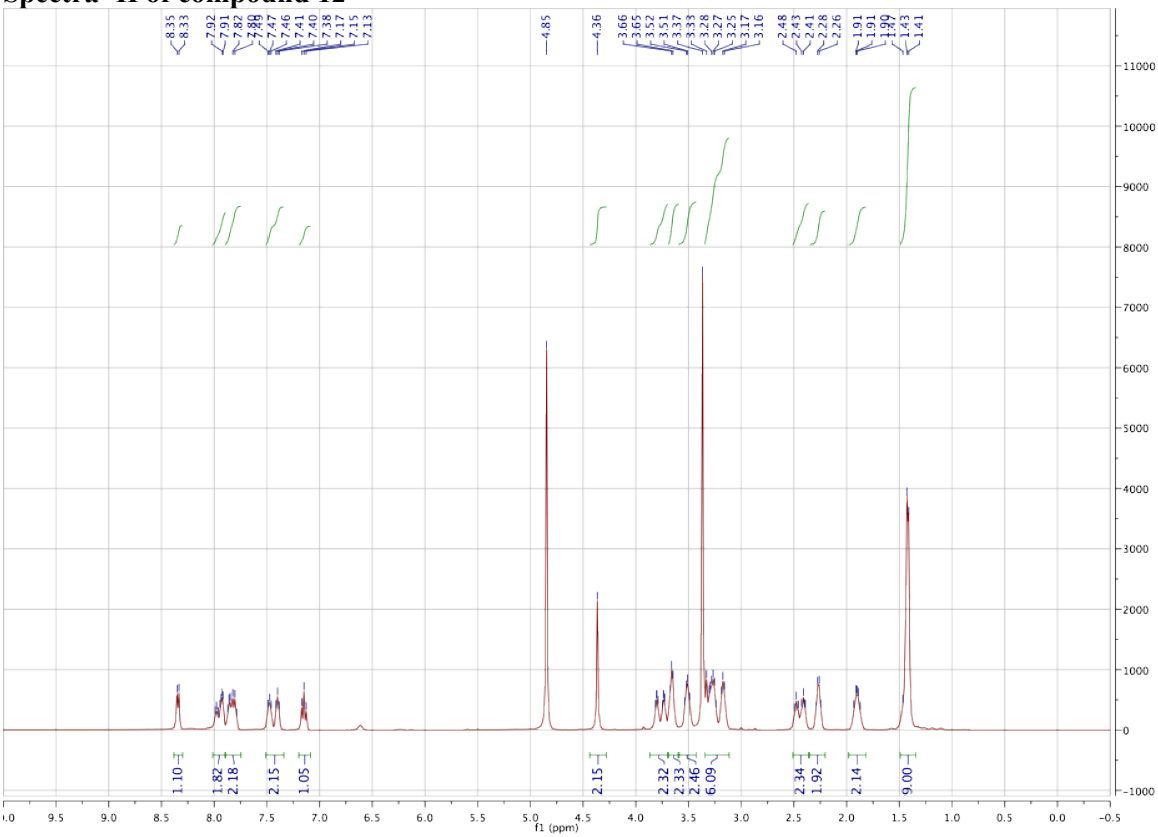
Spectra ^{1}H and ^{13}C of compound 9



Spectra ^{1}H of compound 10



Spectra ^{1}H of compound 12



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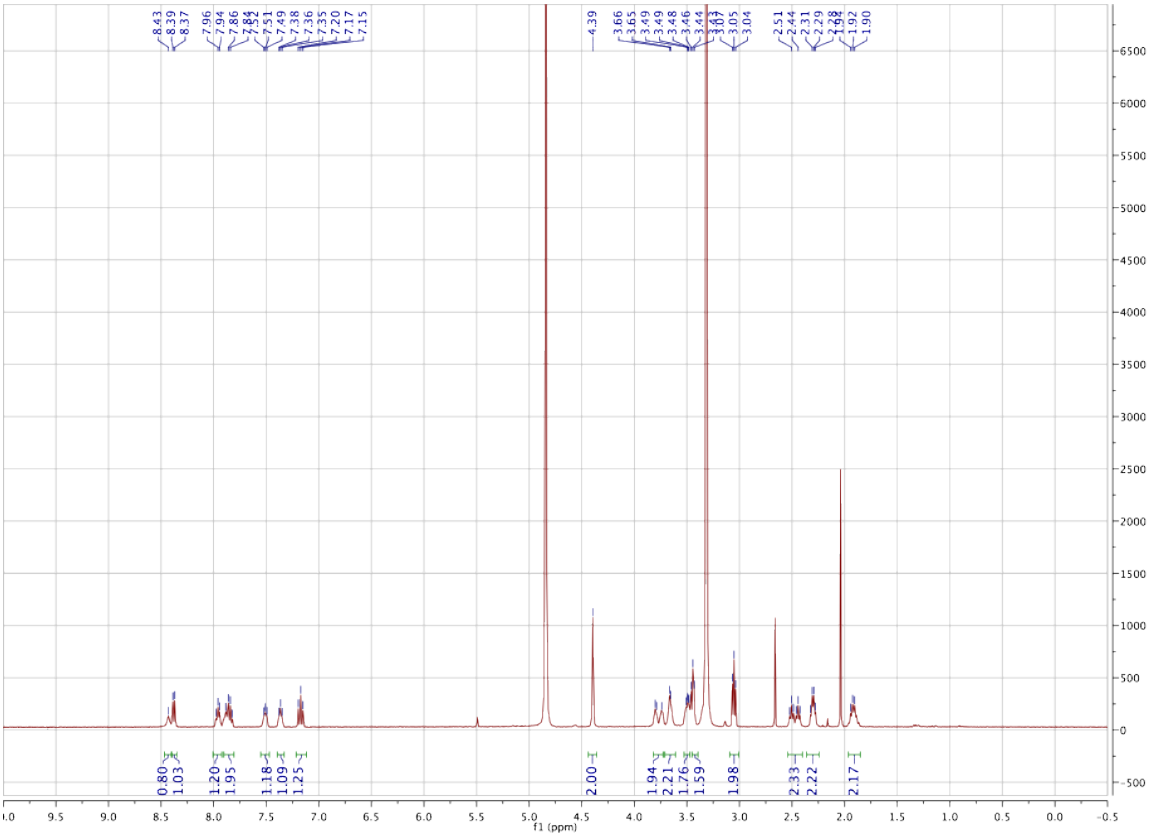
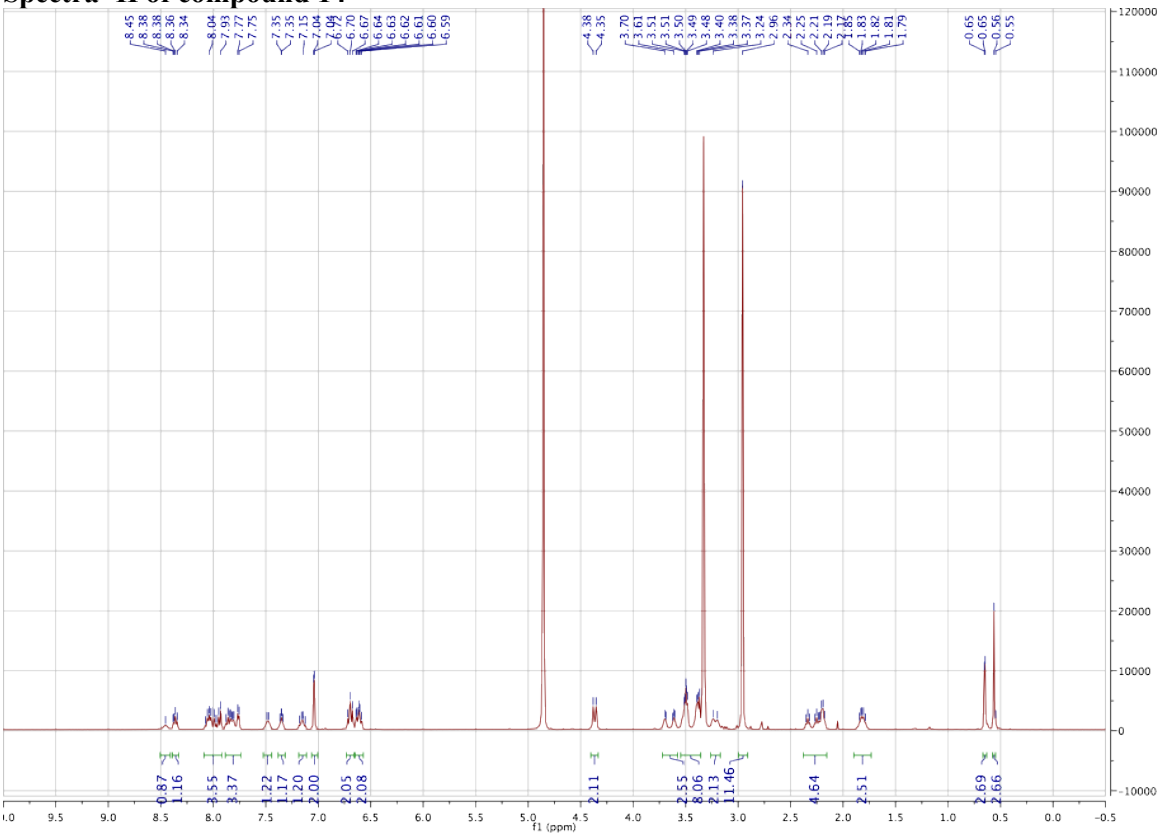


Spectra ^{1}H of compound 14



Reference

[1] K. A. Menear *et al.*, *J. Med. Chem.,* 2008, **51**, 6581.
[2] J. D. Orth, R. H. Kohler, F. Foijer, P. K. Sorger, R. Weissleder, T. J. Mitchison, *Cancer Res.,* 2011, **71**, 4608.
[3] T. Reiner, S. Earley, A. Turetsky, R. Weissleder, *Chembiochem,* 2010, **11**, 2374.