Electronic Supplementary Information

Superoxide-responsive quinone methide precursors (QMP-SOs) for proximal protein labeling to study superoxide biology

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Supplementary Methods

Materials and reagents for chemical synthesis

Diphenylphosphinic chloride (D109429) and sodium ascorbate (S105026) was from Aladdin. 2-(2-Methoxyethoxy)ethan-1-amine (BD69049), 5-iodopent-1-yne (BD257417) and trifluoromethanesulfonic anhydride (BD40907) were from BLDPharm. Triethylamine AR (MC20130) and *N,N′*-disuccinimidyl carbonate (MC00747) were from Dieckmann. 3,4-Dihydroxybenzaldehyde (D807224), 3-bromoprop-1-yne and 4-nitrophenyl chloroformate (N822729) were from Macklin. 1,2-Dithiolane-3-Valeroylamide 98% (S29908) was purchased from Yuen Yip. 2-(2-Methoxyethoxy)ethanol (QE-8523) and 4-hydroxybenzaldehyde (QA-5829) were from Combi-Block. Menadione 98% (M104154) and *β*-nicotinamide adenine dinucleotide lithium salt ≥95% (N196978) were from Aladdin. Tris-Hydroxypropyltriazolylmethylamine (THPTA) (BD00811998) was from BLDPharm. Ammonium iron(II) sulfate hexahydrate (A837811) and Peroxynitrite (Calbiochem®, 516620) were from Calbiochem. Sodium hypochlorite solution (S828471), *tert*-butyl hydroperoxide solution (B802372) and xanthine were from Macklin. Potassium dioxide (278904) was from Sigma-Aldrich.

Materials and reagents for biological experiments

Tris-Hydroxypropyltriazolylmethylamine (THPTA) (BD00811998) was from BLDPharm. ProteaseMAX™ Surfactant, Trypsin Enhancer (V2072) was from Promega. Paraformaldehyde solution 4% in PBS (sc-281692) and MnTBAP chloride (c-221954A) were from Santa Cruz Biotechnology. Superoxide dismutase bovine, lyophilized powder (S9697), xanthine oxidase from bovine milk (X4376) and superoxide dismutase–polyethylene glycol (S9549) were from Sigma-Aldrich. Dihydroethidium (D1168), RIPA Lysis and Extraction Buffer (89900), Pierce Protease and Phosphatase Inhibitor Mini Tablets (EDTA-free; A32961) and Pierce™ TMTsixplex™ Protein Quantitation Kit and Label Reagent Sets (90066) were from Thermo Fisher Scientific.

Antibodies and reagents for immunoblotting

Oxidized-DJ1 antibodies (ab169520) and NOX2/gp91phox antibodies (ab129068) were obtained from Abcam. DJ-1 antibodies (#5933), Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (#4370), p44/42 MAPK (Erk1/2) (137F5) antibody (#4695), phospho-MEK1/2 (Ser217/221) (41G9) antibody (#9154), MEK1/2 (D1A5) antibody (#8727), LC3B (D11) antibody (#3868), p62 antibody (#5114), phospho-Akt (Ser473) (D9E) antibodies (#4060), Akt (pan) (C67E7) antibody (#4691), phosphor-p38 MAPK (Thr180/Tyr182) antibody (#9211), p38 MAPK antibody (#9212), PARP antibody (#9542),

GAPDH (D16H11) antibody (#5174), SOD2 (D3X8F) antibody (#13141), Lamin A/C (4C11) antibody (#4777), COX IV (3E11) antibody (#4850), anti-rabbit IgG, HRP linked antibody (#7074), anti-mouse IgG, HRP linked Antibody (#7076) and 20× LumiGLO® Reagent and 20× Peroxide (#7003) were obtained from Cell Signal Technology. PVDF membranes (1620177) and trans-blot turbo 5x transfer buffer (10026938) were from Bio-Rad.

Physical measurements and instrumentation

^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra were collected on Bruker AVANCE NEO 600 MHz spectrometer at the Department of Chemistry at the University of Hong Kong. All chemical shifts are reported in the standard δ notation of parts per million relative to residual solvent peak as an internal reference. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets; tt, triplet of triplets. UV-Vis absorption and microplate fluorescence were recorded on Perkin Elmer Victor 3 (Molecular Devices). UV-Vis absorption in solution was measured on NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific). Gel-electrophoresis was performed using the electrophoresis chamber from Bio-Rad or the XCell4 SureLock™ Midi-Cell electrophoresis system from Invitrogen™ (Thermo Fisher Scientific). In-gel fluorescence images were recorded on the ChemiDoc MP Gel Imaging system (Bio-Rad). Protein transfer was performed using the Trans-Blot Turbo Transfer System (BioRad).

The selectivity and reactivity of QMP-SOs with ROS in aqueous buffer solutions were measured by liquid chromatography-mass spectrometry on a Waters Autopurification System using a SunFire C18 HPLC column (50 × 4.6 mm with 5 μm diameter particles; Waters). Separation was achieved by a gradient elution from 5% to 100% MeCN in water (constant 0.1 vol % formic acid) over 4 min, isocratic elution with 100% MeCN (with 0.1 vol % formic acid) from 4 to 8 min, and returning to 5% MeCN in water (with 0.1 vol % formic acid) and equilibrated for 2 min. The data was analysed using MassLynx™ software by calculating the area under the curve.

Confocal fluorescence microscopy imaging was performed with a Zeiss laser scanning microscope 880 with a 20× water-immersion objective lens using ZEN 2.3 (Black Version) software (Carl Zeiss). Hoechst 33342 was excited with a 405 nm diode laser, and emission was collected on a META detector between 371 and 507 nm. Fluor 545 was excited by a 561 nm diode laser, and emission was collected on a META detector between 576 and 683 nm. Image analysis was performed using ImageJ. A region of interest (ROI) was created around individual cells, and cellular fluorescence intensity was measured. The reported average cellular fluorescence intensity was determined by averaging the measured

intensity from 30 different cells from 3 different biological replicates/group. Statistical analyses were performed with a two-tailed Student's t-test (MS Excel).

Proteomics data were collected by the Orbitrap Fusion Tribrid Lumos mass spectrometer (ThermoFisher) at the Centre for PanorOmic Sciences, LKS Faculty of Medicine.

Chemical synthesis

Compound 1.

Triethylamine (TEA; 228.5µL, 1.64 mmol) was added to 4-hydroxybenzaldehyde (100 mg, 0.82 mmol) in dry dichloromethane (5 mL). At 0 ºC, diphenylphosphinic chloride (156.3µL, 0.82mmol) was diluted in dichloromethane and added portion-wise to the solution mixture. The reaction mixture was stirred at 0 ºC for 1 h. The reaction was quenched with water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine solution, dried with anhydrous $MgSO_4$, and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (2:1, v/v) as eluent, yielding (7c) (109.2 mg, 20%). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.89 (1H, s), 7.91-7.86 (4H, m), 7.79 (2H, d, J = 8.64 Hz), 7.57 (2H, td, J = 7.6, 1.28 Hz), 7.50-7.46 (4H, m), 7.38 (2H, d, *J*=8.4Hz). MS (ESI$^+$): *m/z* 323 ([M+H]$^+$).

Compound 2.

Compound **1** (68.8 mg, 0.196 mmol) was dissolved in THF (250 µL) and diluted in methanol (2.75 mL). At 0 ºC, $NaBH_4$ (4.2 mg, 0.11 mmol) was added to the solution mixture with vigorous stirring. The solution mixture was stirred at 0 ºC for 30 min and then quenched with water. Any organic volatile was removed by evaporation under reduced pressure, and the aqueous layer was extracted with dichloromethane. The dichloromethane layer was washed with brine solution, dried with anhydrous $MgSO_4$, and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (1:1, v/v) as eluent, yielding compound **2** (75.5mg, 69%). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.89 (4H, m), 7.54 (2H, t, *J*=7.36), 7.49-7.44 (4H, m), 7.23 -7.18 (4H, m), 4.61 (2H, s). MS (ESI$^+$): *m/z* 325 ([M+H]$^+$).

QMP-SO-1.

N,N'-Disuccinimidyl carbonate (178.5 mg, 0.69 mmol) was added to **2** (75.5 mg, 0.23 mmol) in dry dichloromethane (3 mL). Diluted TEA (97.0 µL, 0.69 mmol) was added dropwise to the solution mixture and the reaction mixture was stirred at room temperature overnight. The reaction was then quenched with water and extracted with dichloromethane. The dichloromethane layer was washed with

$NaHCO_3$ and brine, dried with anhydrous $MgSO_4$ and filtered. The volatile organic solvent was removed by evaporation under reduced pressure. The dried organic layer was redissolved in dry dichloromethane. 2-(2-Methoxyethoxy)ethan-1-amine (31.6 µL, 0.26 mmol) was added to the solution mixture, followed by the dropwise addition of TEA (64.4 µL, 0.47 mmol). The mixture was allowed to react at room temperature overnight. After the reaction, any volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/ methanol (49:1, v/v) as eluent, yielding QMP-SO-1 (44.3mg, 40%). ^{1}H NMR (600 MHz, Chloroform-d) δ= 7.90-7.87 (4H, m), 7.54 (2H, t, J = 8.28, 7.56 Hz), 7.48-7.45 (4H, m), 7.26-7.19 (4H, m), 5.28 (1H, d), 5.28 (1H, d, J = 21.5Hz), 5.00 (s, 2H), 3.59 (t, J-4.08, 4.68 Hz, 2H), 3.54 (t, J-4.92, 4.86, 2H), 3.52 (t, J = 4.80, 4.26Hz, 2H), 3.39-3.37 (2H, m), 3.36 (1H, s). ^{13}C NMR (151 MHz, Chloroform-d) δ=156.33, 150.64, 132.93, 132.52, 131.79, 130.84, 129.59, 128.63, 120.73, 71.82, 70.18, 65.91, 59.04, 40.84. MS (ESI$^+$): *m/z* 470 ([M+H]$^+$)

Compound 4b.

K_2CO_3 (500 mg, 3.62 mmol) was added to 3,4-dihydroxybenzaldehyde (500 mg, 22.5 mmol) in acetone (20 mL). 5-iodopent-1-yne (439 µL, 3.62 mmol) was added dropwise to the solution mixture with vigorous stirring. The solution was allowed to react at room temperature overnight. After the reaction, the undissolved solid was filtered off. The filtrate was then evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (5:1, v/v) as eluent, yielding compound **4b** (237 mg, 32.0%). ^{1}H NMR (600 MHz, Chloroform-d) δ=9.85 (s,1 H), 7.45 (d, *J*=1.98 Hz, 1 H), 7.42 (dd, *J*=1.98, 8.22 Hz, 1 H), 6.98 (1H, d, *J*=8.22 Hz), 5.81 (1H, s), 4.28 (2H, t, *J*=6.12 Hz), 2.44 (2H, dt, *J*=2.64, 6.69 Hz), 2.10 (1H, d, *J*=12.96 Hz), 2.05 (1H, t, *J*=2.64 Hz). MS (ESI$^+$): *m/z* 205 ([M+H]$^+$).

Compound 5b.

Triethylamine (TEA; 40.6 µL, 0.291 mmol) was added to (6) (59.4 mg, 0.29 mmol) in dry THF (3 mL). At 0 °C, diphenylphosphinic chloride (83.3 µL, 0.437 mmol) was diluted in dry THF and added portion-wise to the solution mixture. The reaction mixture was stirred at 0 °C for 3 h. The reaction was quenched with water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine solution, dried with anhydrous $MgSO_4$, and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (2:1, v/v) as eluent, yielding (7) (98.7 mg, 84%). ^{1}H NMR (600 MHz, Chloroform-d) δ=9.78 (1H, s), 7.95-7.91 (4H, m), 7.81 (1H, t, *J*=1.71 Hz), 7.63 (1H, q, *J*=3.31 Hz), 7.56-7.53 (2H, m), 7.49-7.46 (4H, m), 7.00 (1H, d, *J*=8.45 Hz), 4.15 (2H, t, *J*=6.09 Hz), 2.39-2.36 (2H, m), 2.01-1.96 (3H, m). MS (ESI$^+$): *m/z* 405 ([M+H]$^+$).

Compound 6b.

Compound **5b** (98.7 mg, 0.24 mmol) was dissolved in THF (250 μL) and diluted in methanol (2.75 mL). At 0 °C, $NaBH_4$ (5 mg, 0.13 mmol) was added to the solution mixture with vigorous stirring. The solution mixture was stirred at 0 °C for 30 min and then quenched with water. Any organic volatile was removed by evaporation under reduced pressure, and the aqueous layer was extracted with dichloromethane. The dichloromethane layer was washed with brine solution, dried with anhydrous $MgSO_4$, and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/Methanol (30:1, v/v) as eluent, yielding compound **6b** (46.6 mg, 47%). ^{1}H NMR (600 MHz, Chloroform-d) δ=7.93-7.89 (4H, m, J=4.14 Hz), 7.50 (2H, q, J=4.96 Hz), 7.43-7.41 (4H, m), 7.35 (1H, s), 7.03 (1H, d, J=8.28 Hz), 6.82 (1H, d, J=8.34 Hz), 4.47 (2H, s), 4.02 (2H, t, J=6.09 Hz), 2.98 (1H, s), 2.37-2.34 (2H, m), 1.99 (1H, t, J=2.61 Hz), 1.94 (2H, t, J=6.52 Hz). MS (ESI$^+$): *m/z* 407 ([M+H]$^+$).

QMP-SO-C5-alkyne.

N,N'-Disuccinimidyl carbonate (88.1 mg, 0.34 mmol) was added to **6b** (46.6mg, 0.115 mmol) in dry dichloromethane (3 mL). Diluted TEA (47.9 μL, 0.34 mmol) was added dropwise to the solution mixture and the reaction mixture was stirred at room temperature overnight. The reaction was then quenched with water and extracted with dichloromethane. The dichloromethane layer was washed with $NaHCO_3$ and brine, dried with anhydrous $MgSO_4$ and filtered. The volatile organic solvent was removed by evaporation under reduced pressure. The dried organic layer was redissolved in dry dichloromethane. 2-(2-Methoxyethoxy)ethan-1-amine (15.6 μL, 0.13 mmol) was added to the solution mixture, followed by the dropwise addition of TEA (31.8 μL, 0.23 mmol). The mixture was allowed to react at room temperature overnight. After the reaction, any volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/ methanol (25:1, v/v) as eluent, yielding QMP-SO-C5-alkyne (24.9 mg, 37%). ^{1}H NMR (600 MHz, Chloroform-d) δ=7.95-7.91 (4H, m), 7.53-7.51 (2H, m), 7.46-7.43 (4H, m), 7.37 (1H, s), 7.03 (1H, d, J=8.33 Hz), 6.84 (1H, d, J=8.36 Hz), 5.22 (1H, s), 4.92 (2H, s), 4.04 (2H, t, J=6.03 Hz), 3.60 (2H, t, J=4.26 Hz), 3.54 (4H, t, J=5.23 Hz), 3.37 (5H, s), 2.39-2.36 (2H, m), 1.99-1.95 (3H, m). ^{13}C NMR (151 MHz, Chloroform-d) δ=156.34, 149.72, 139.95, 132.36. 131.80, 129.36, 128.46, 125.22, 122.02, 113.18, 83.24, 71.84, 69.08, 66.87, 59.05, 40.82, 28.10, 15.13. ^{31}P NMR (202 MHz, Chloroform-d) δ=31.04. MS (ESI$^+$): *m/z* 552 ([M+H]$^+$).

Compound 4a.

The synthesis of **4a** followed a procedure similar to that of **4b** except that 3-bromoprop-1-yne (1 g, 7.2mmol) was used instead of 5-iodopent-1-yne. Other reagents were scaled accordingly. The crude

product was purified by column chromatography on silica gel using hexane/ethyl acetate (5:1, v/v) as eluent, yielding **4a** (303.9 mg, 23.8%). ^{1}H NMR (600 MHz, Chloroform-d) δ=9.86 (1H, s), 7.47 (1H, d, J=1.95 Hz), 7.44 (1H, dd, J=1.96, 8.26 Hz), 7.11 (1H, d, J=8.26 Hz), 7.08 (1H, d, J=8.02 Hz), 5.71 (1H, s), 4.86 (2H, d, J=2.39 Hz), 2.61 (1H, t, J=2.40 Hz). MS (ESI^{+}): *m/z* 177 ([M+H]$^{+}$).

Compound 5a.

The synthesis of **5a** followed a procedure similar to that of **5b** except that **4a** (303.9 mg, 1.73 mmol) was used instead of **4b**. Other reagents were scaled accordingly. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (49:1, v/v) as eluent, yielding **5a** (426 mg, 66%). ^{1}H NMR (600 MHz, Chloroform-d) δ= 9.81 (1H, s), 7.98-7.94 (4H, m), 7.90 (1H, t, J=1.68 Hz), 7.65 (1H, q, J=3.34 Hz), 7.56-7.53 (2H, m), 7.48-7.45 (4H, m), 7.10 (1H, d, J=8.46 Hz), 4.74 (2H, d, J=2.40 Hz), 2.58 (1H, t, J=2.37 Hz). ^{13}C NMR (151 MHz, Chloroform-d) δ=190.13, 153.68, 140.58, 132.61, 131.91, 131.15, 130.63, 130.23, 128.54, 126.98, 123.86, 113.65, 56.59, 29.71. ^{31}P NMR (202 MHz, Chloroform-d) δ 32.57. MS (ESI^{+}): *m/z* 377 ([M+H]$^{+}$).

Compound 6a.

The synthesis of **6a** followed a procedure similar to that of **6b**, except that **5a** (451mg, 1.25 mmol) was used instead of **5b**. Other reagents were scaled accordingly. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (49:1, v/v) as eluent, yielding compound **6a** (223.5mg, 47%). ^{1}H NMR (600 MHz, Chloroform-d) δ=7.97-7.93 (4H, m), 7.51 (2H, t, J=2.69 Hz), 7.45-7.42 (5H, m), 7.06 (1H, q, J=3.18 Hz), 6.95 (1H, d, J=8.40 Hz), 5.29 (1H, s), 4.62 (2H, d, J=2.34 Hz), 4.52 (2H, s), 2.53 (1H, t, J=2.37 Hz). ^{31}P NMR (202 MHz, Chloroform-d) δ=31.83. MS (ESI^{+}): *m/z* 379 ([M+H]$^{+}$).

QMP-SO-C3-alkyne.

The synthesis of QMP-SO-C3-alkyne followed a procedure similar to that of QMP-SO-C5-alkyne, except that compound **6a** (98.5 mg, 0.419 mmol) was used instead of **6b**. Other reagents were scaled accordingly. After reaction with *N*,*N*'-disuccinimidyl carbonate. 50.1 mg of the intermediate compound was taken to react with 2-(2-aminoethoxy)ethan-1-ol (14.5 μL, 0.144 mmol). The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (2:1, v/v) as eluent, yielding QMP-SO1 (21.3mg, 42%). ^{1}H NMR (600 MHz, Chloroform-d) δ= 7.96-7.93 (4H, m), 7.50 (3H, t, J=3.61 Hz), 7.44-7.42 (4H, m), 6.98 (1H, d, J=8.06 Hz), 6.91 (1H, d, J=8.32 Hz), 5.72 (1H, s), 4.95 (2H, s), 4.63 (2H, d, J=2.01 Hz), 3.75-3.71 (2H, m), 3.60-3.55 (4H, m), 3.38 (2H, q, J=4.86 Hz), 3.23 (1H, s). ^{13}C NMR (151 MHz, Chloroform-d) δ=156.45, 148.05, 140.42, 132.47, 131.94, 128.47, 124.38, 121.63, 114.18, 78.11, 75.94, 72.51, 70.08, 65.36, 61.71, 56.68, 41.01. ^{31}P NMR (202 MHz, Chloroform-d) δ=31.82. MS (ESI^{+}): *m/z* 494 ([M+H]$^{+}$).

Compound 8.

TEA (317.8 µL, 2.28 mmol) was added to **4a** (200 mg, 1.14 mmol) in dry dichloromethane (6 mL). Trifluoromethanesulfonic anhydride (286.5µL, 1.7mmol) was diluted in dichloromethane and added portion-wise to the solution mixture at -78 ºC. The reaction mixture was stirred at -78 ºC for 1 h. The reaction was quenched with water and extracted with dichloromethane. The dichloromethane layer was washed with brine solution, dried with anhydrous $MgSO_4$, and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (3:1, v/v) as eluent, yielding **8** (171 mg, 49%). ^{1}H NMR (600 MHz, Chloroform-d) δ=9.91 (1H, s), 7.90 (1H, dd, J = 8.52, 1.93 Hz), 7.79 (1H, d, J = 1.91 Hz), 7.34 (1H, d, J = 8.52 Hz), 4.91 (2H, d, J = 2.42 Hz), 2.63 (1H, t, J-2.41). MS (ESI$^+$): *m/z* 309 ([M+H]$^+$).

Compound 9.

The synthesis of **9** followed a procedure similar to that of **7b** except that compound **8** (129 mg, 0.419 mmol) was used. Other reagents were scaled accordingly. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (2:1, v/v) as eluent, yielding (8b) (90.5mg, 70%). ^{1}H NMR (600 MHz, Chloroform-d) δ 7.33 (1H, d, J = 6.74 Hz), 7.29 (1H, d, J = 1.73 Hz), 7.18 (1H, d, J-8.46), 4.80 (2H, d, *J*=2.38 Hz), 4.67 (2H, d, *J*=5.74 Hz) , 2.56 (1H, t, *J*=2.38 Hz) , 1.72 (1H, t, *J*=5.86 Hz). MS (ESI$^+$): *m/z* 311 ([M+H]$^+$).

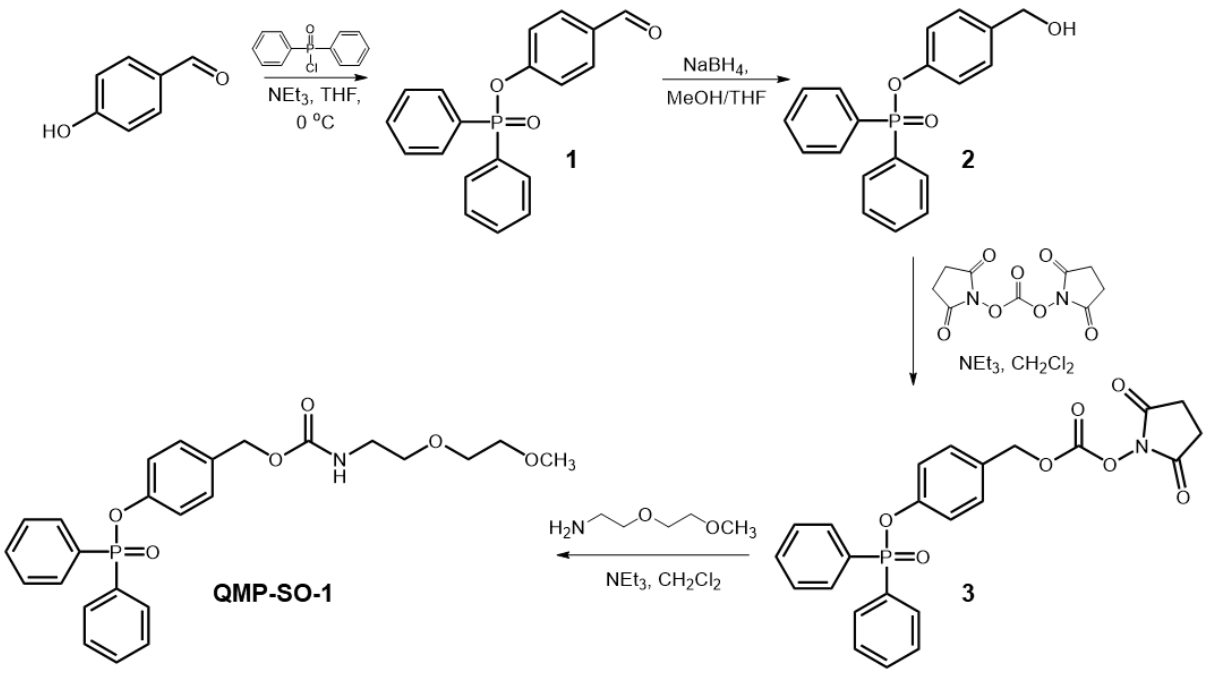
QMP-SO-OTf-alkyne.

TEA (52.1 µL, 0.374 mmol) was added to compound **9** (58.1 mg, 0.187 mmol) in dry dichloromethane (6mL). At 0 ºC, 4-nitrophenyl chloroformate (45.3 mg, 0.224 mmol) was added to the solution mixture and the reaction mixture was stirred overnight. The reaction was then quenched with water and extracted with dichloromethane. The dichloromethane layer was washed with brine, dried with anhydrous $MgSO_4$, and filtered. The volatile organic solvent was removed by evaporation under reduced pressure. The dried organic layer was redissolved in dry dichloromethane. 2-(2-methoxyethoxy)ethan-1-amine (46.6 µL, 0.374 mmol) was added to the solution mixture, followed by the dropwise addition of TEA (52.13 µL, 0.374 mmol). The mixture was allowed to react at room temperature overnight. The reaction solution was then quenched with water and extracted against dichloromethane. The dichloromethane layer was washed with $NaHCO_3$ and brine, dried with anhydrous $MgSO_4$ and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (49:1, v/v) as eluent, yielding QMP-SO-OTf-alkyne (35.7 mg, 42%). ^{1}H NMR (600 MHz, Chloroform-d) δ=7.34 (1H, q, *J*=3.36 Hz), 7.28 (1H, d, *J*=1.86 Hz), 7.17 (1H, d, *J*=8.46 Hz), 5.30 (1H, d, *J*=6.12 Hz), 5.04 (2H, s), 4.80 (2H, d, *J*=2.34 Hz), 3.61 (2H, q, *J*=2.94 Hz), 3.56 (2H, t, *J*=5.01 Hz), 3.53 (2H, q, *J*=3.00 Hz), 3.40 (5H, q, *J*=5.26 Hz), 2.56 (1H, t, *J*=2.34Hz). ^{13}C NMR (151 MHz, Chloroform-d) δ=156.11, 149.02, 138.78, 130.95, 122.69, 119.77, 117.65, 114.59, 71.85, 70.18, 65.20, 59.06, 56.86, 40.92, 29.70. MS (ESI$^+$): *m/z* 456 ([M+H]$^+$).

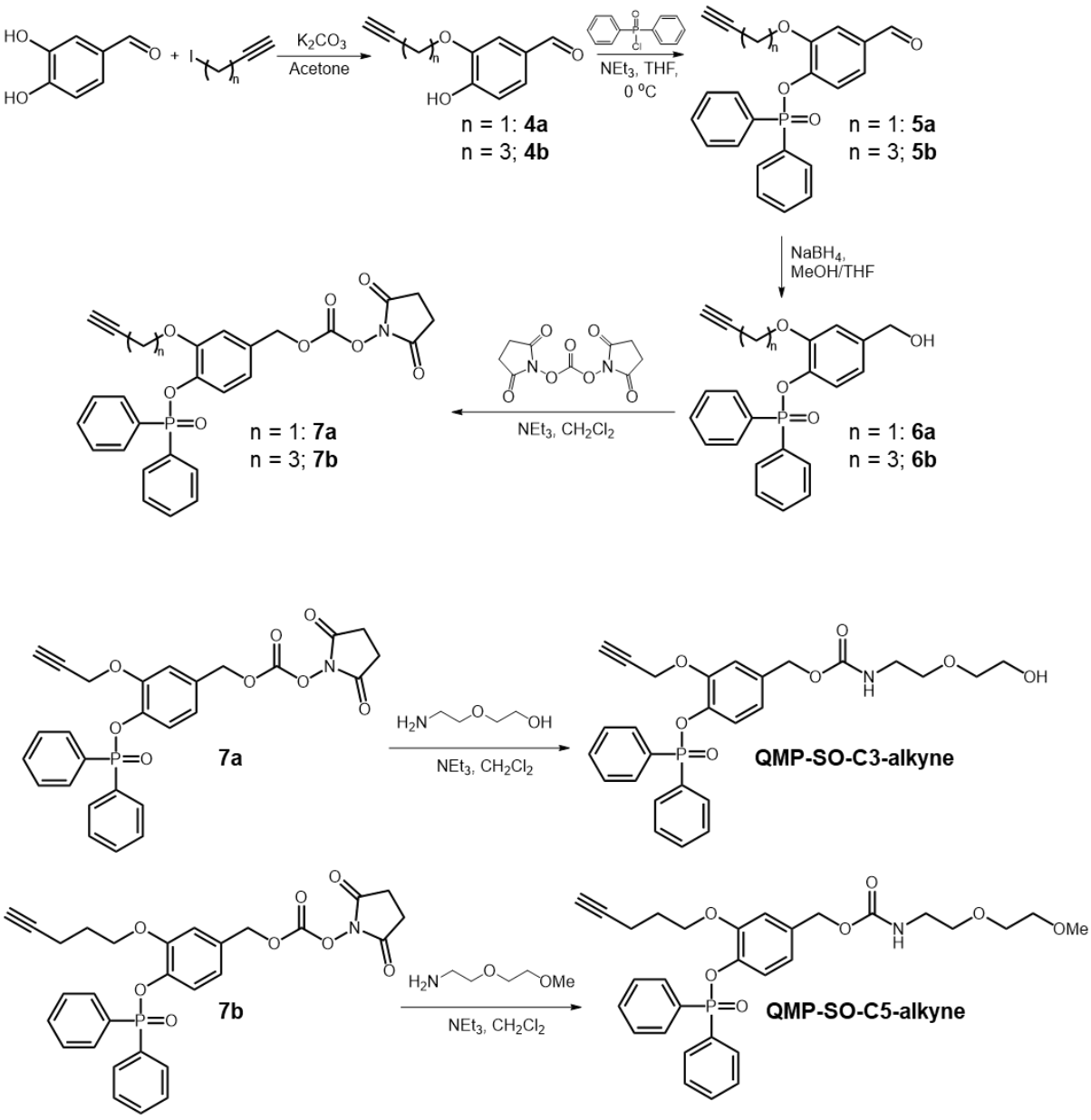
DL-Lipoamide

DL-lipoamide was synthesised by reducing DL-5-(1,2-dithiolan-3-yl)valeramide with $NaBH_4$, as reported in the literature.[1] DL-5-(1,2-Dithiolan-3-yl)valeramide (200 mg, 0.97mmol) was suspended in methanol (4 mL) and water (1 mL). $NaBH_4$ (200 mg, 5.28 mmol) was dissolved in water (1 mL) and added to the solution mixture at 0 °C. The reaction mixture was stirred for 45 min until the solution turned clear. The solution mixture was then acidified with dilute hydrochloric acid and extracted with dichloromethane. The dichloromethane layer was dried by anhydrous $MgSO_4$ and filtered. The volatile organic solvent was evaporated under reduced pressure. The crude product was purified by crystallisation using toluene/ hexane (2.5:1) and yielded DL-lipoamide (165 mg, 82%). ^{1}H NMR (600 MHz, Chloroform-d) δ=5.33 (2H, d, J=54.55 Hz), 2.96-2.90 (1H, m), 2.77-2.64 (2H, m), 2.25 (2H, t, J=7.40 Hz), 1.94-1.88 (1H, m), 1.78-1.76 (1H, m), 1.69-1.64 (6H, m).

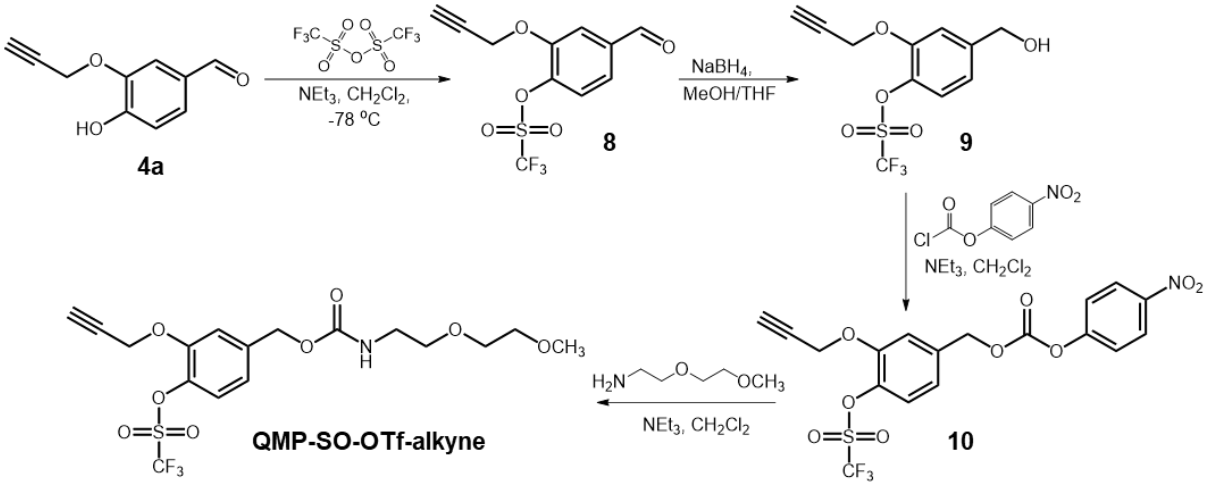
(1) Håkansson, A. P.; Smith, A. W. Enzymatic Characterization of Dihydrolipoamide Dehydrogenase from Streptococcus Pneumoniae Harboring Its Own Substrate*. *Journal of Biological Chemistry* **2007**, *282* (40), 29521–29530. https://doi.org/10.1074/jbc.M703144200.



Scheme S1. Synthetic scheme for QMP-SO-1.



Scheme S2. Synthetic scheme for QMP-SO-C3-alkyne and QMP-SO-C5-alkyne.



Scheme S3. Synthetic scheme for QMP-SO-OTf-alkyne.

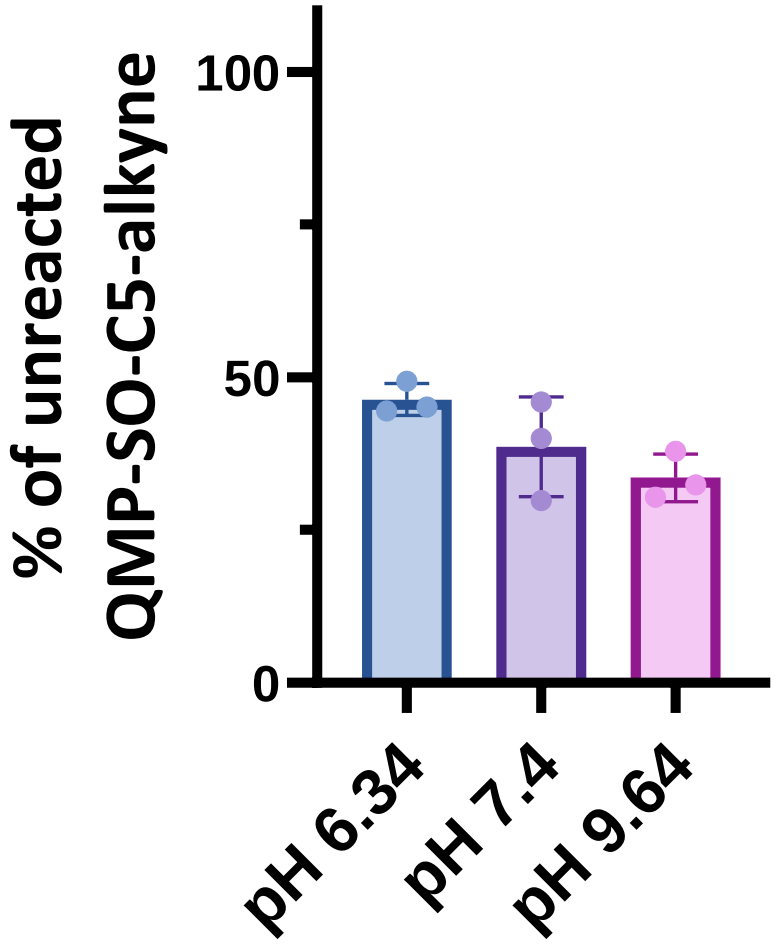


Figure S1. Percentage of unreacted QMP-SO-C5-alkyne in the solution mixture with KO_2 at different pHs, as measured by LC-MS experiments after incubation for 15 min. The slightly higher amount of unreacted probe at pH 6.34 can be attributable to the lower stability of KO_2 in acidic buffer solutions, resulting in less superoxide to react QMP-SO-C5-alkyne.

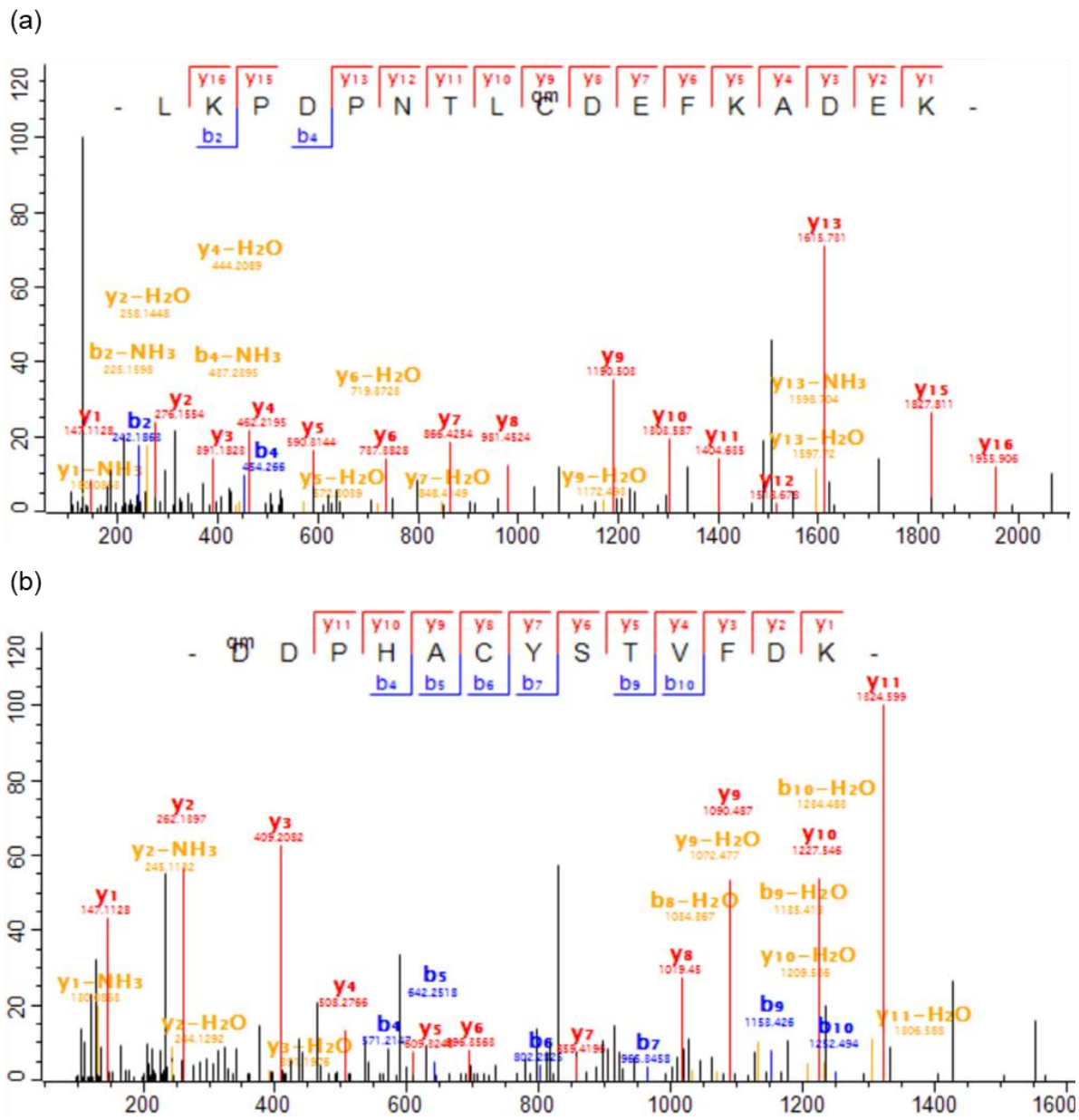


Figure S2. Representative MS/MS showing the QMP-SO-1-induced modifications on (a) Cys and (b) Asp of BSA respectively.

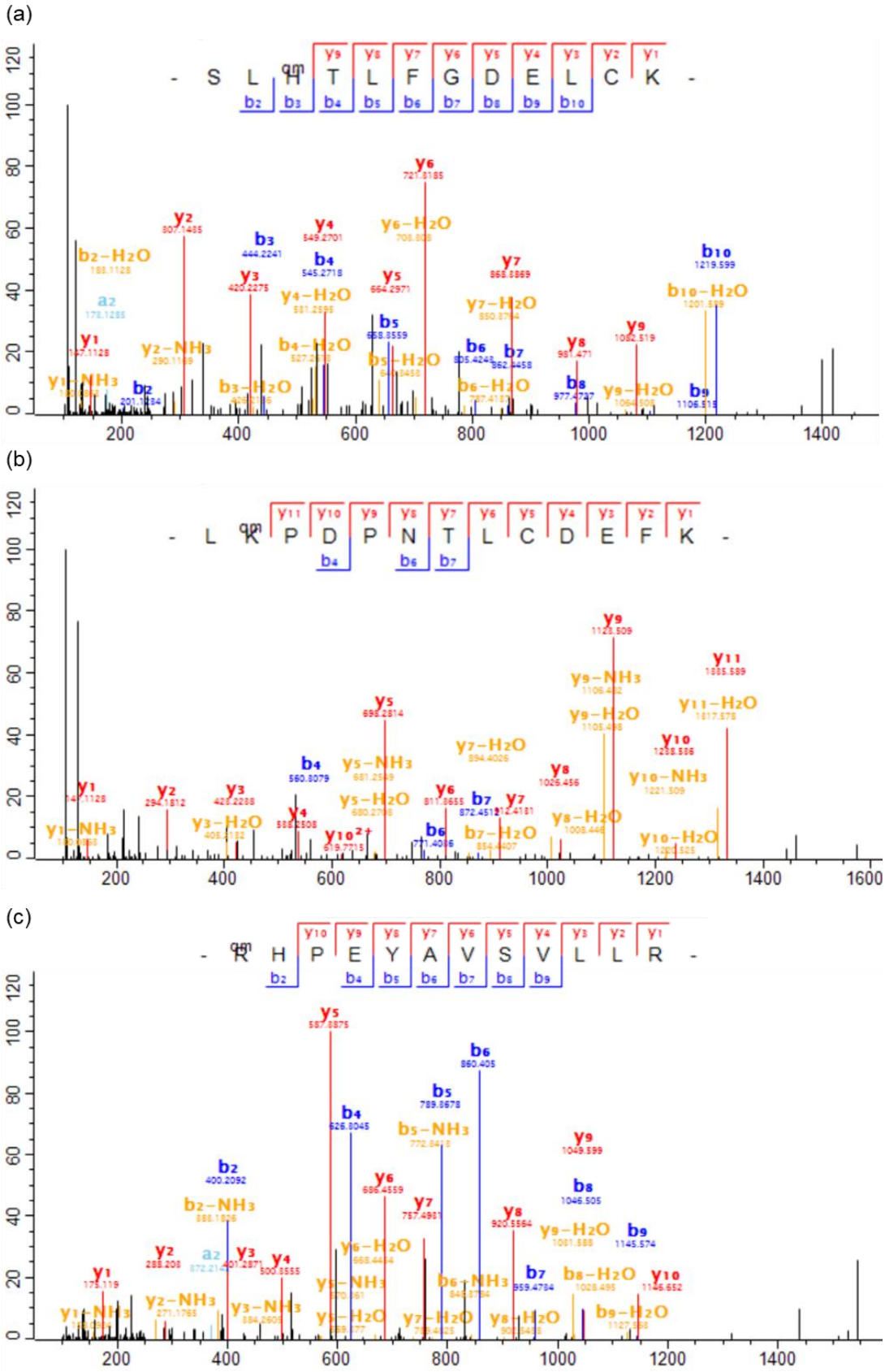


Figure S3. Representative MS/MS showing the QMP-SO-1-induced modifications on (a) His, (b) Lys and (c) Arg of BSA respectively.

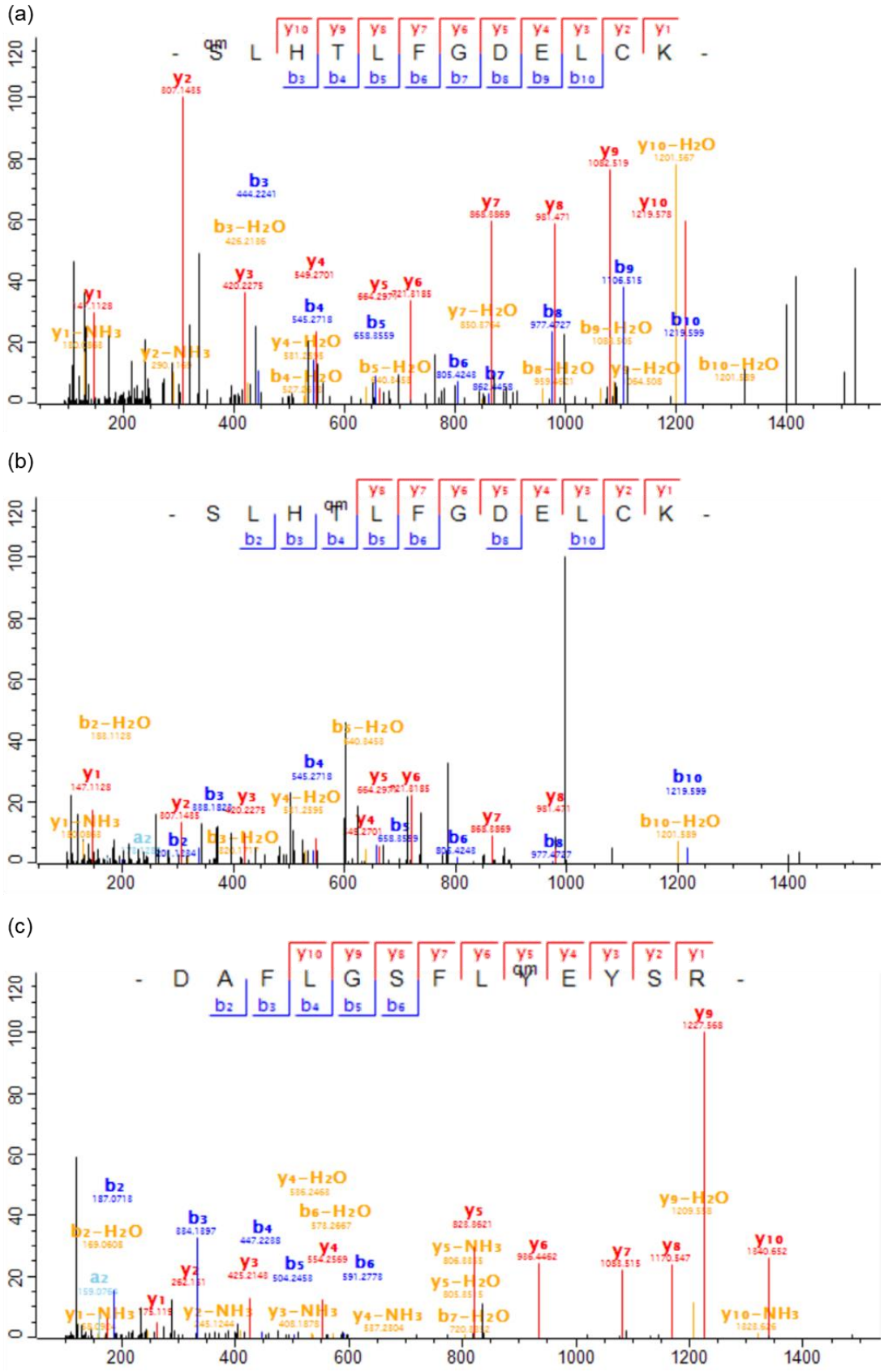


Figure S4. Representative MS/MS showing the QMP-SO-1-induced modifications on (a) Ser, (b) Thr and (c) Tyr of BSA respectively.

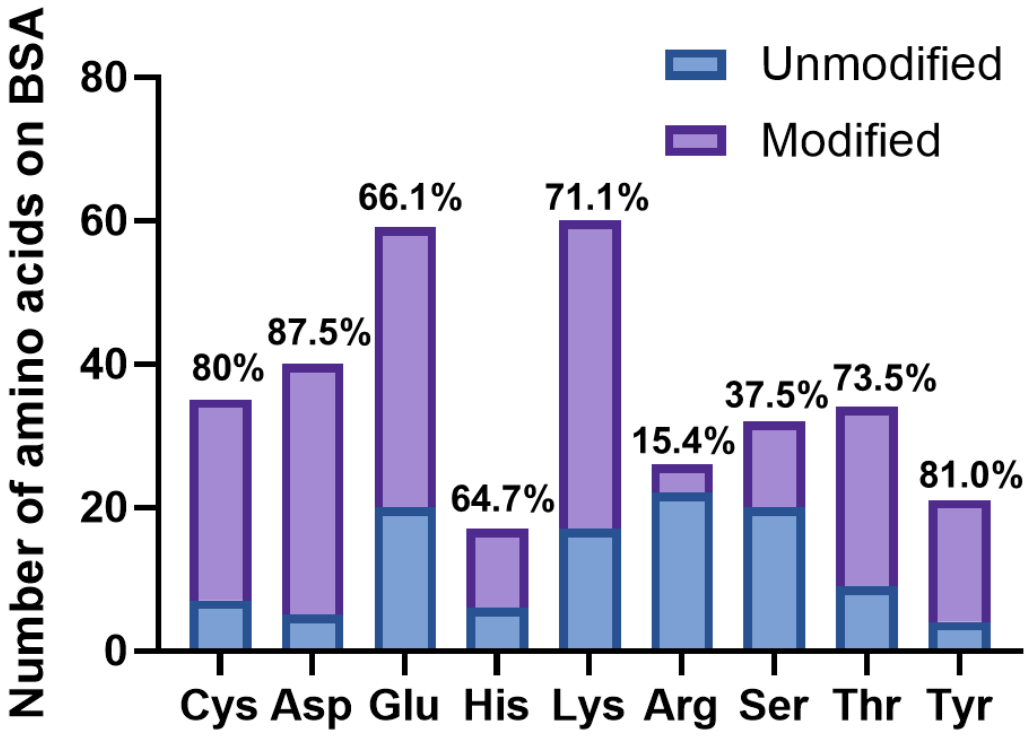


Figure S5. The number of modified and unmodified nucleophilic amino acids on BSA by QMP-SO-1 in the presence of KO_2, as found in the shotgun MS experiment.

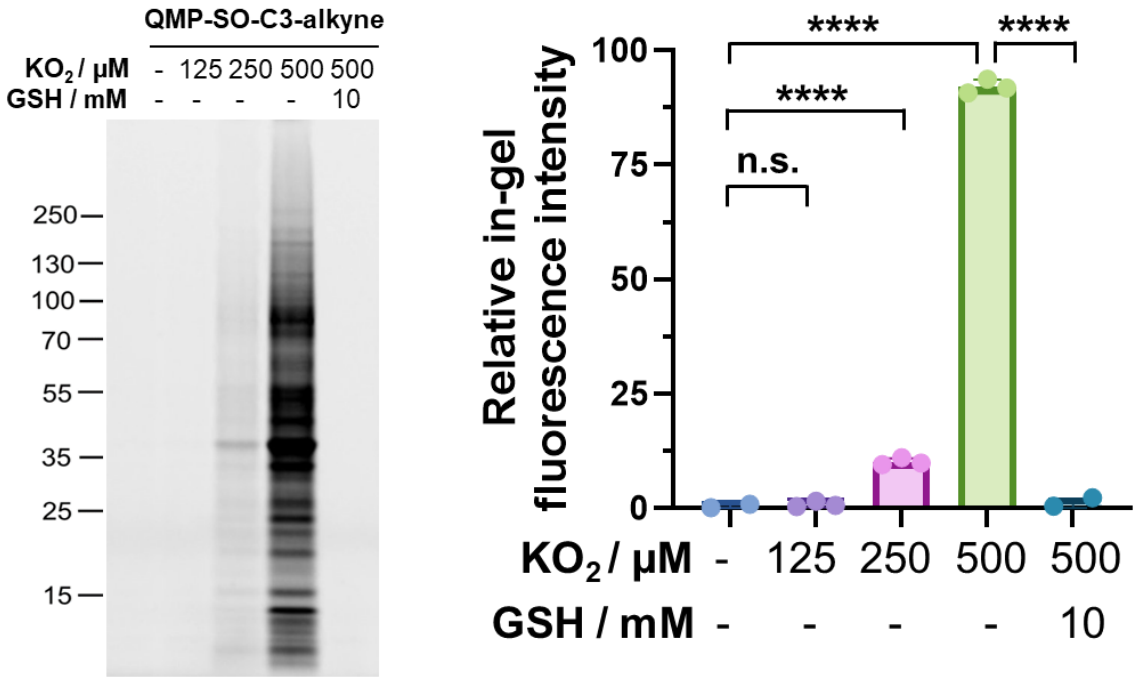


Figure S6. HepG2 cell lysates (50 µg) in PBS were incubated with QMP-SO-C3-alkyne (10 µM), KO_2 and/or GSH at the indicated concentrations for 2 h. Excess reagents were removed by acetone precipitation at -20 °C overnight, and the precipitated proteins were re-dissolved in PBS. The labeled proteins were then reacted with azide-fluor 545 (25 µM) by copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, boiled with sampling buffer and read out by in-gel fluorescence after SDS-PAGE. Quantified data were shown in average±SD (n=3 replicates/group). Statistical analysis using a two-tailed Student's t-test. ****p<0.0001; n.s. = not significant.

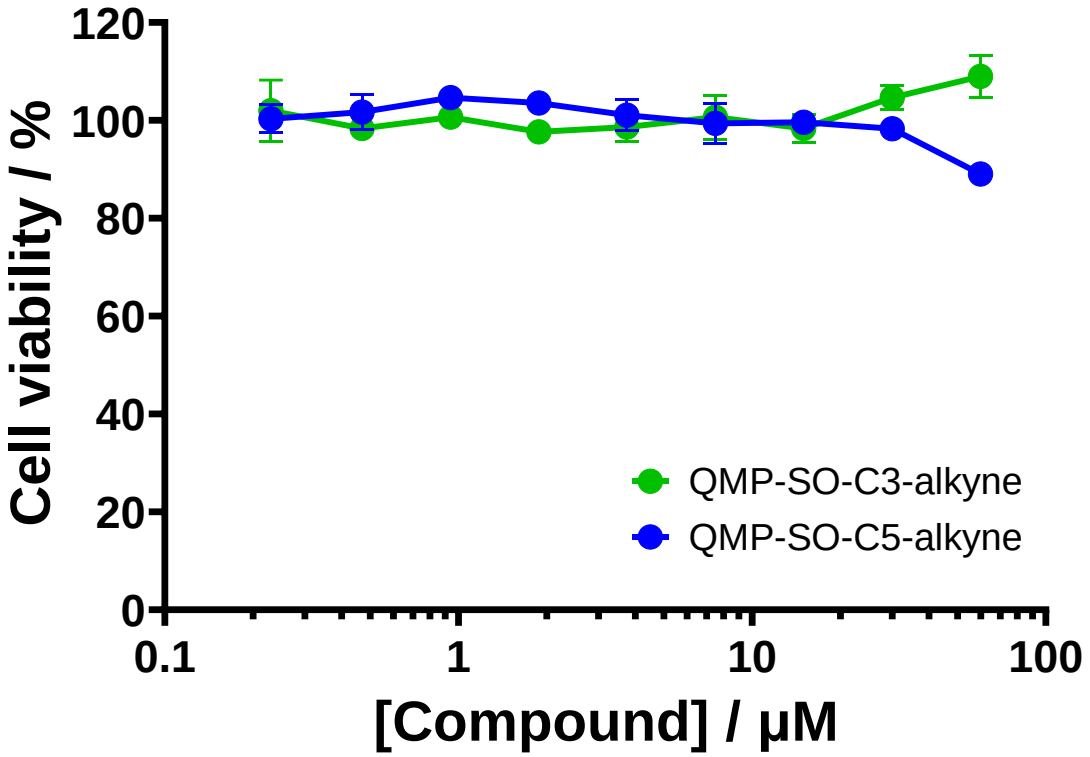


Figure S7. MTT assay to study the viability of HepG2 cells incubated with QMP-SO-C3-alkyne and QMP-SO-C5-alkyne in complete medium for 4 h.

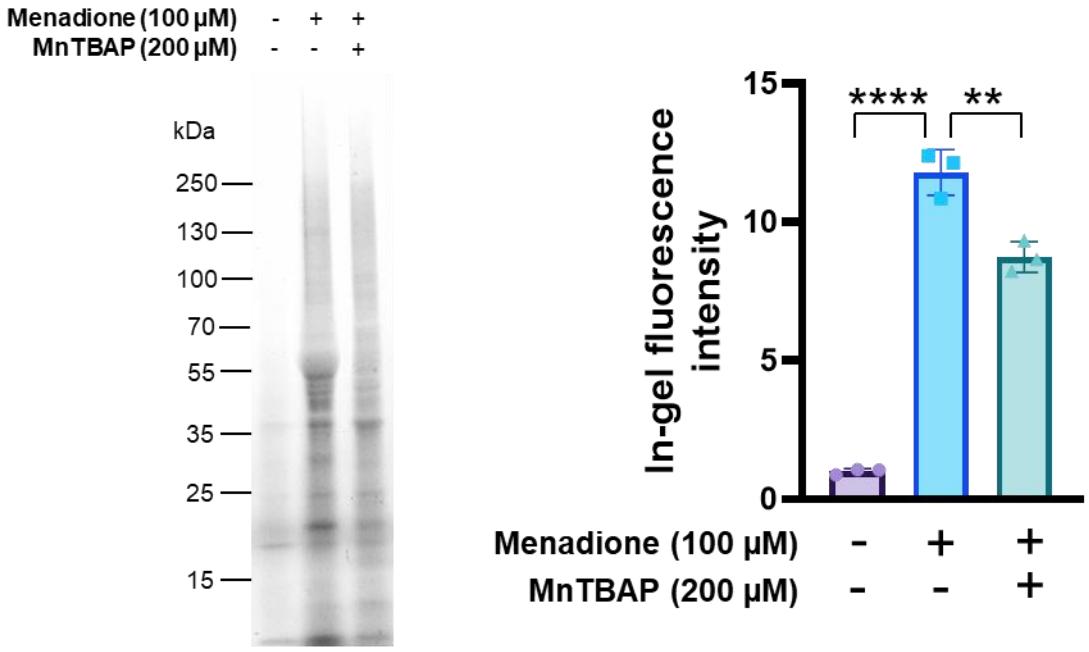


Figure S8. Live HepG2 cells were pretreated with solvent vehicle or MnTBAP (200 µM) in complete medium for 12 h, followed by the incubation with QMP-SO-C5-alkyne (10 µM), menadione and/or MnTBAP for 4 h in complete medium. The cells were washed with PBS and lysed by sonication in PBS. After protein assay and normalization, the cell lysates were reacted with azide-fluor 545 (25 µM) by CuAAC. The labeled proteins were then boiled with sampling buffer, separated by SDS-PAGE and visualized by their in-gel fluorescence. Quantified data were shown in average±SD (n=3 replicates/group). Statistical analysis using a two-tailed Student's t-test. **p<0.01 and ****p<0.0001.

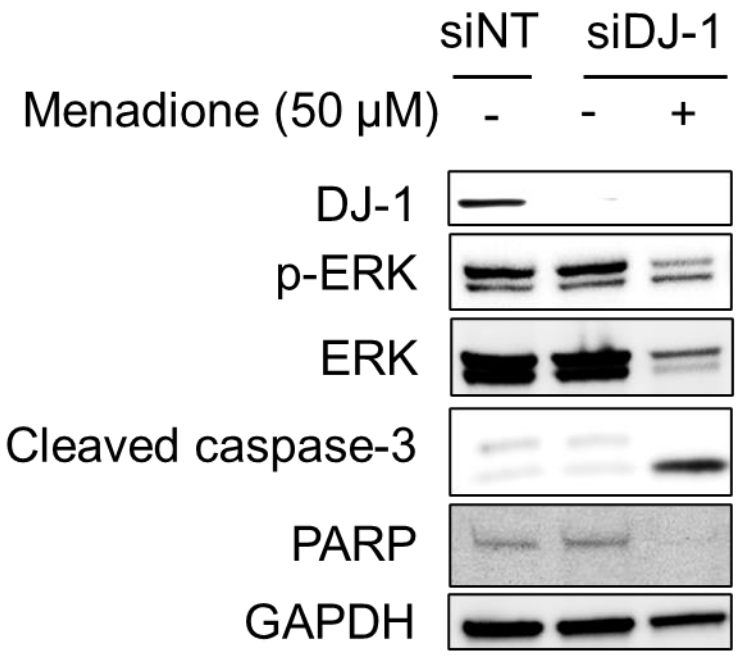


Figure S9. Genetic manipulation of DJ-1 was achieved by transfection of HepG2 cells using Dharmafect 4 and non-targeting (NT) siRNA (50 nM; Horizon, #D-001810-10-20) or DJ-1 siRNA (50 nM; Horizon, #005984-00-0020). After 48 h, the cells were incubated with solvent control or menadione (50 μM) in complete medium for 2 h, followed by cell lysis and protein normalization. The protein mixtures were then subjected to SDS-PAGE and immunoblotting.

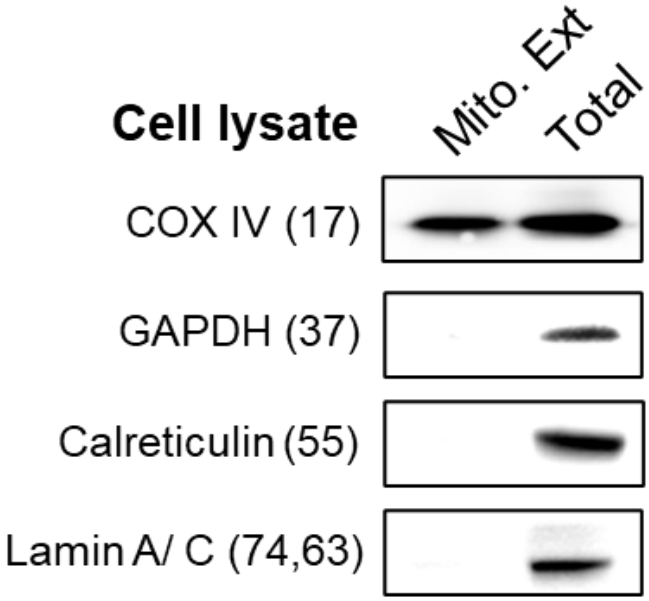
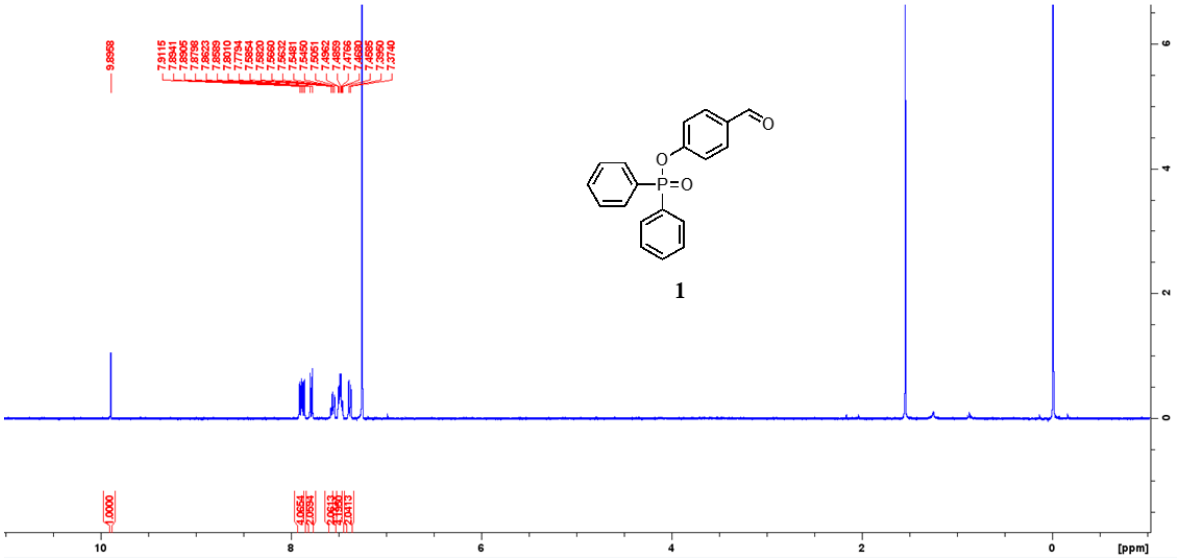


Figure S10. Immunoblotting to investigate levels of mitochondrial, cytosolic, endoplasmic reticulum and nucleus markers, COX IV, GAPDH, calreticulin and lamin A/C respectively, in the mitochondrial extract of HepG2 cells.

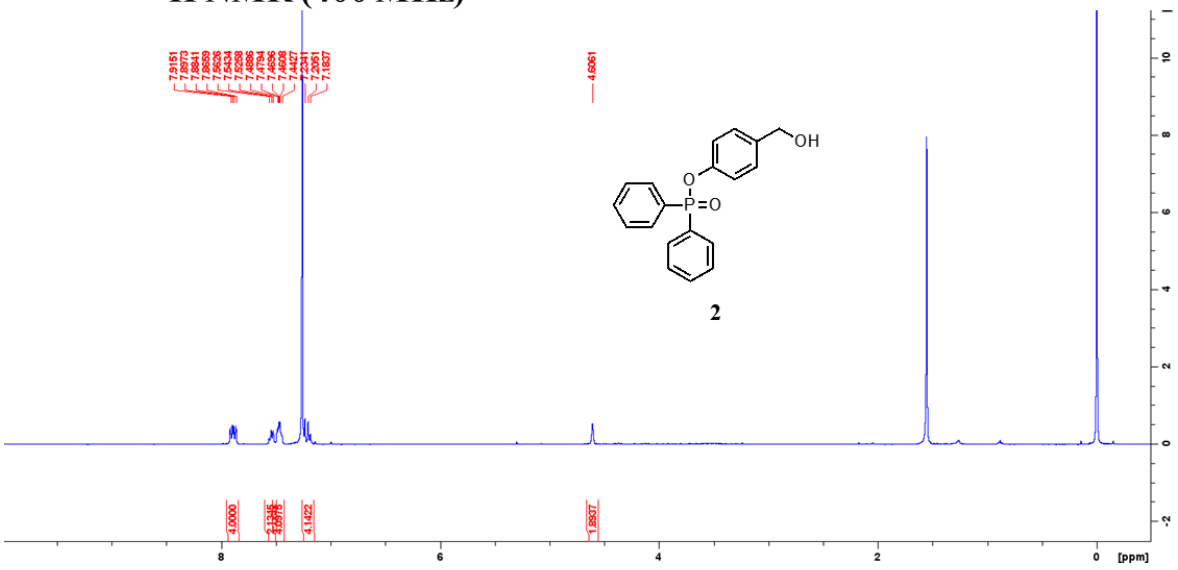
NMR Spectra of the synthesized compounds

^{1}H NMR (400 MHz)



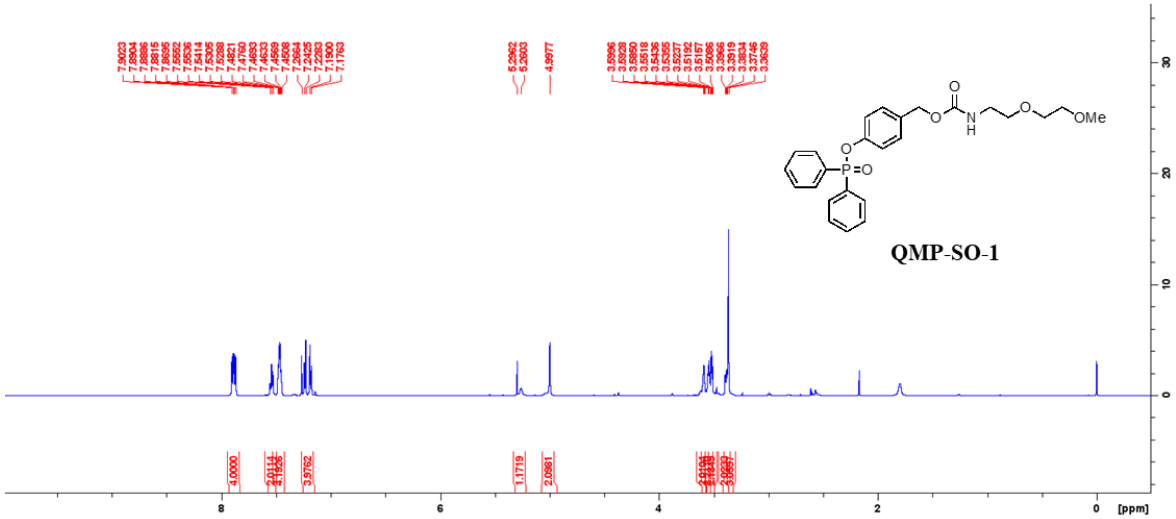
1

^{1}H NMR (400 MHz)

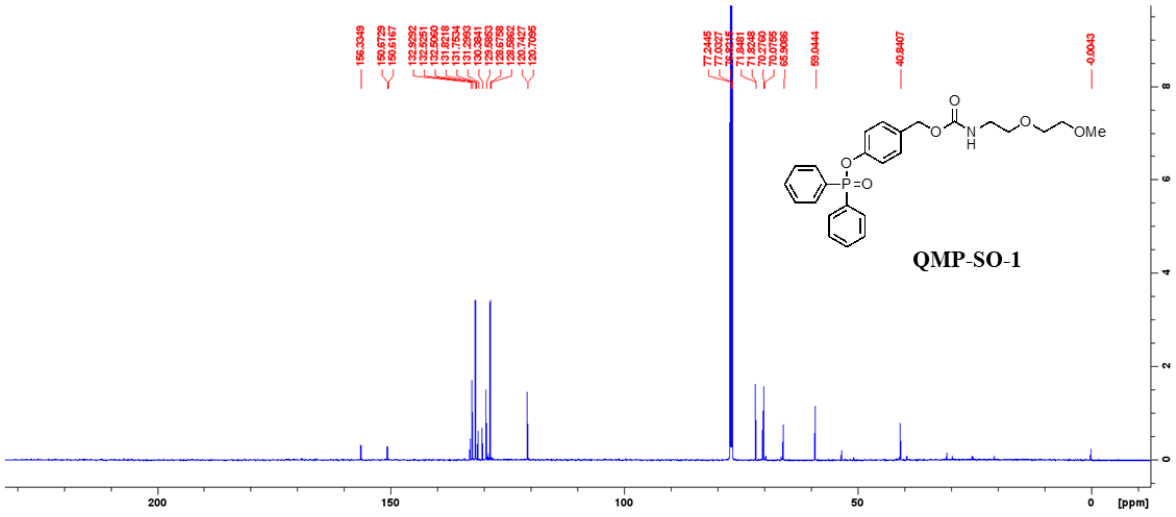


2

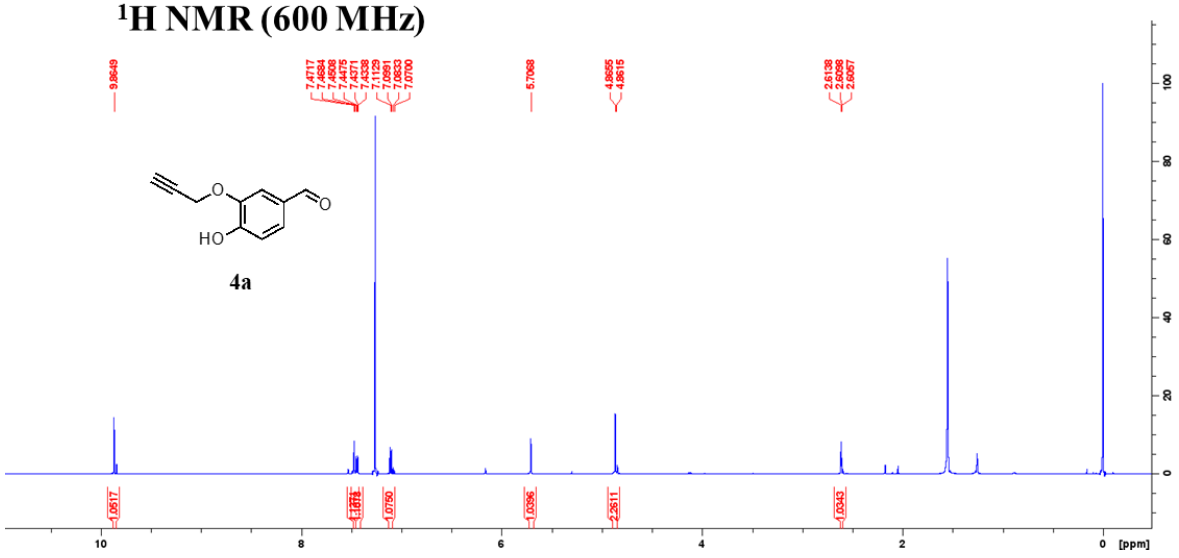
^{1}H NMR (600 MHz)



^{13}C NMR (151 MHz)

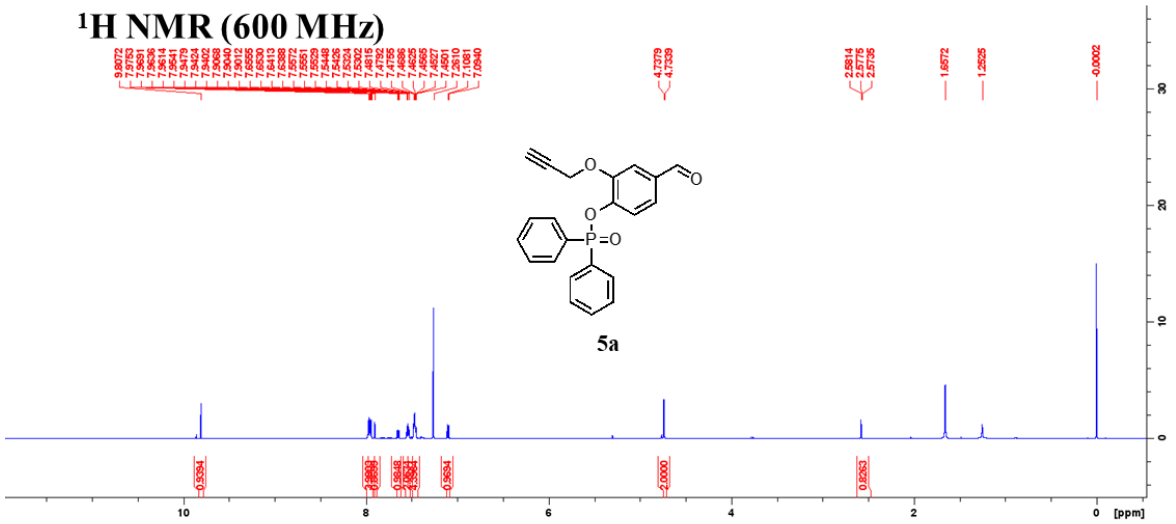


^{1}H NMR (600 MHz)

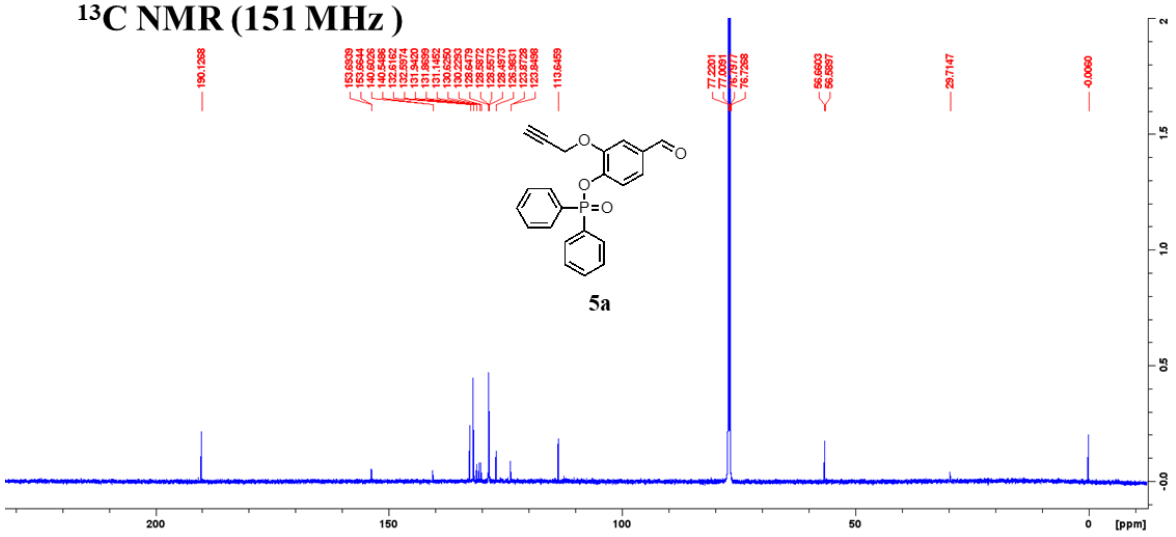


4a

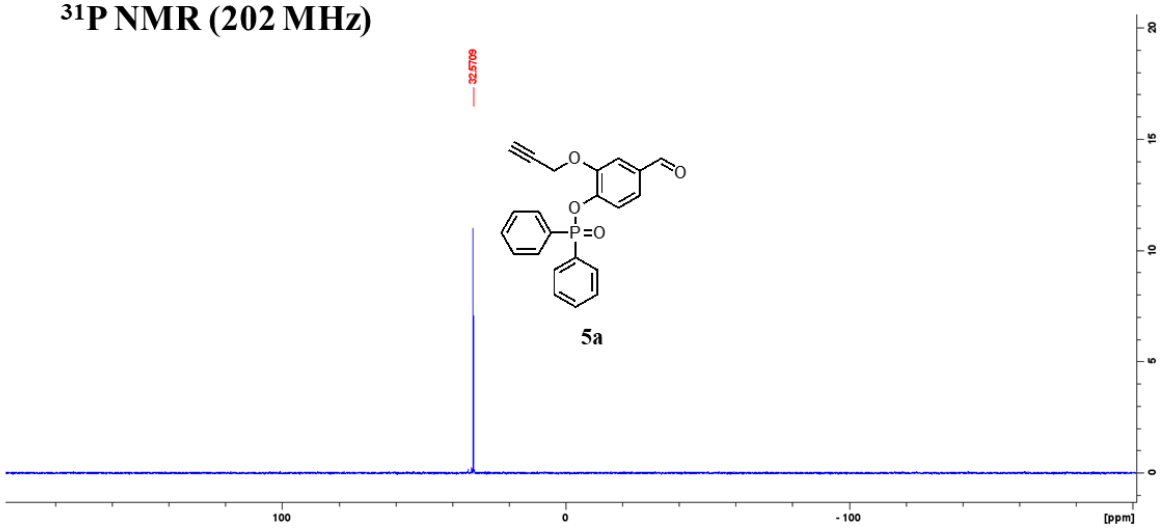
^{1}H NMR (600 MHz)



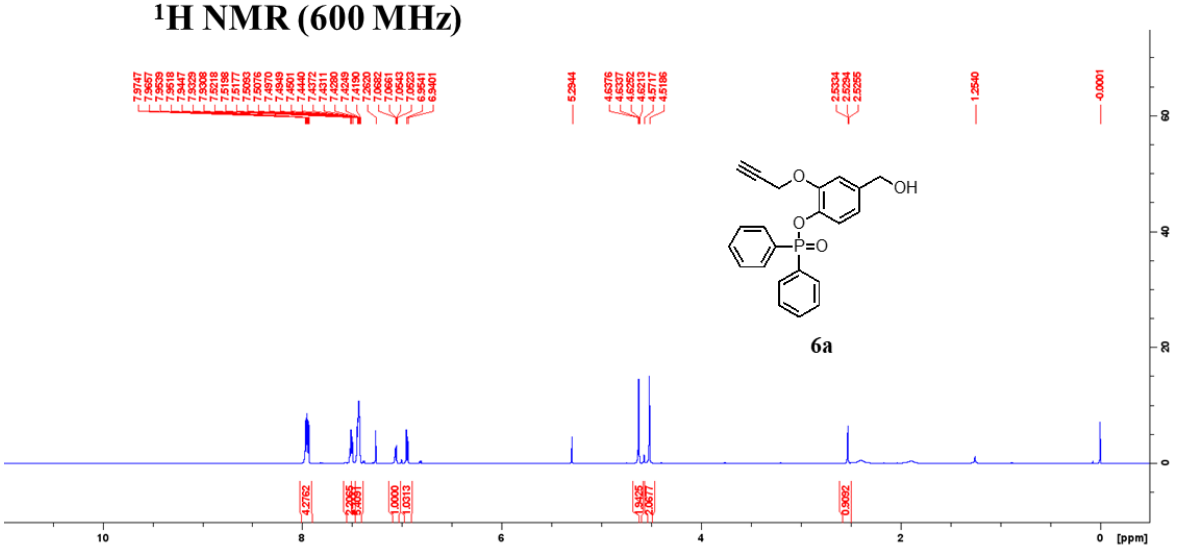
^{13}C NMR (151 MHz)



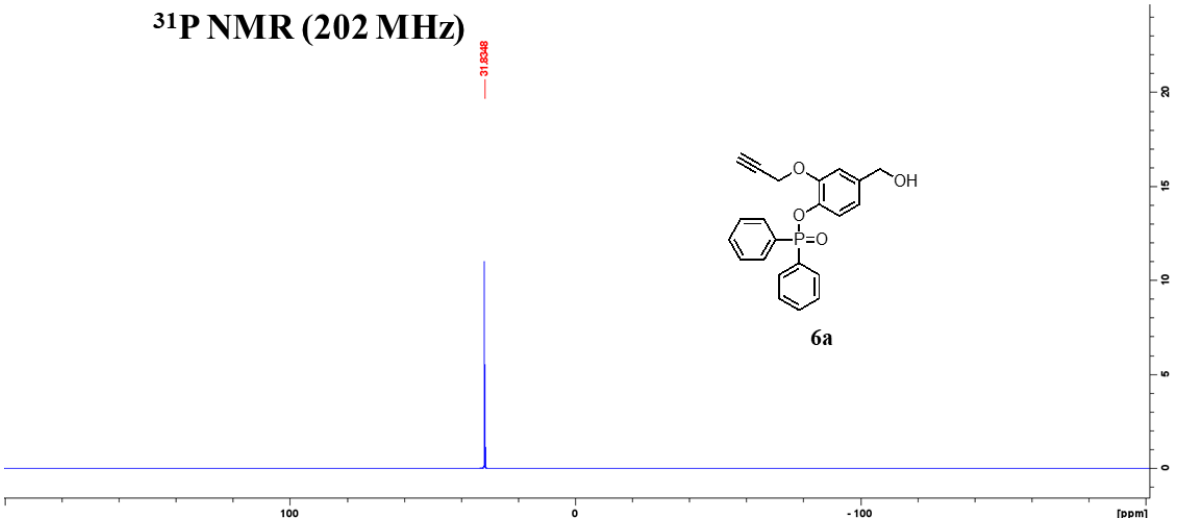
^{31}P NMR (202 MHz)



^{1}H NMR (600 MHz)



^{31}P NMR (202 MHz)



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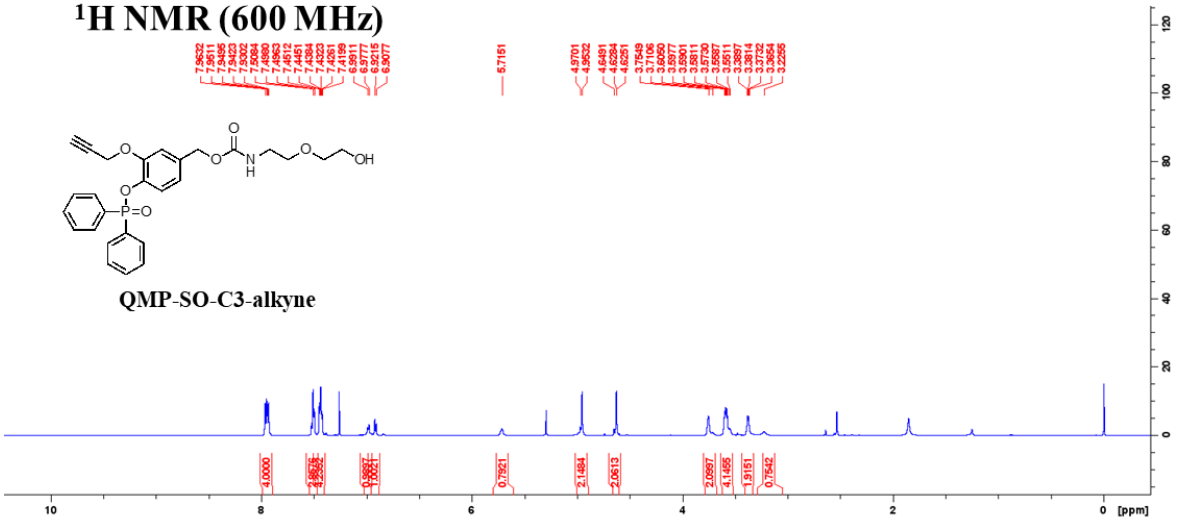

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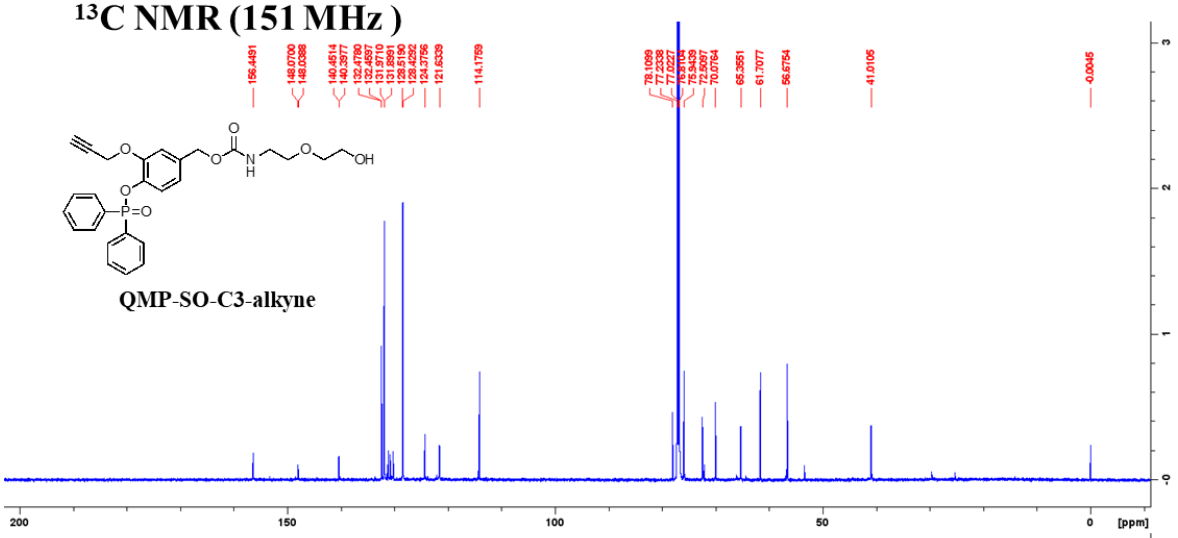
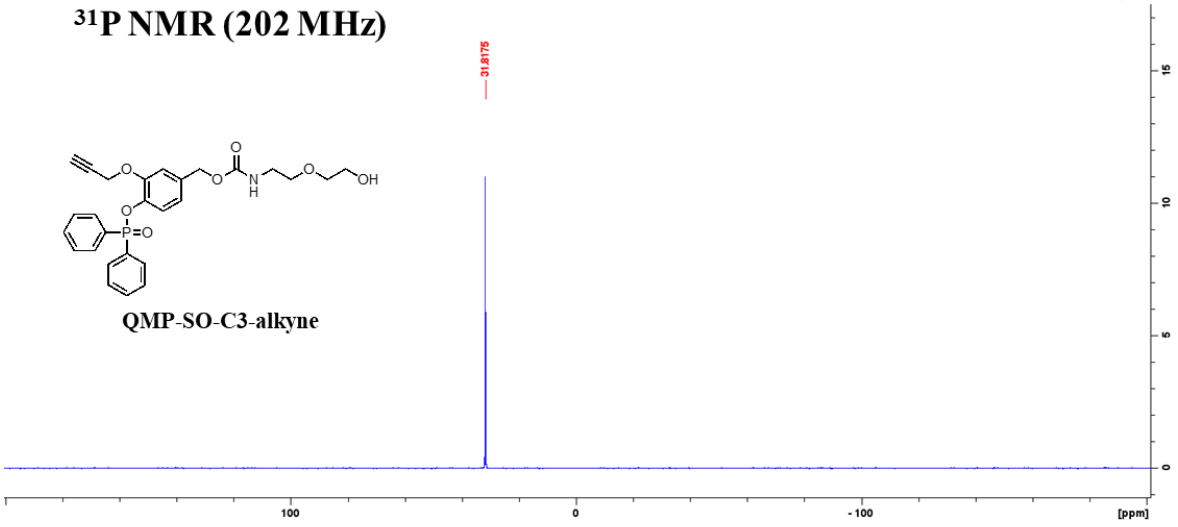
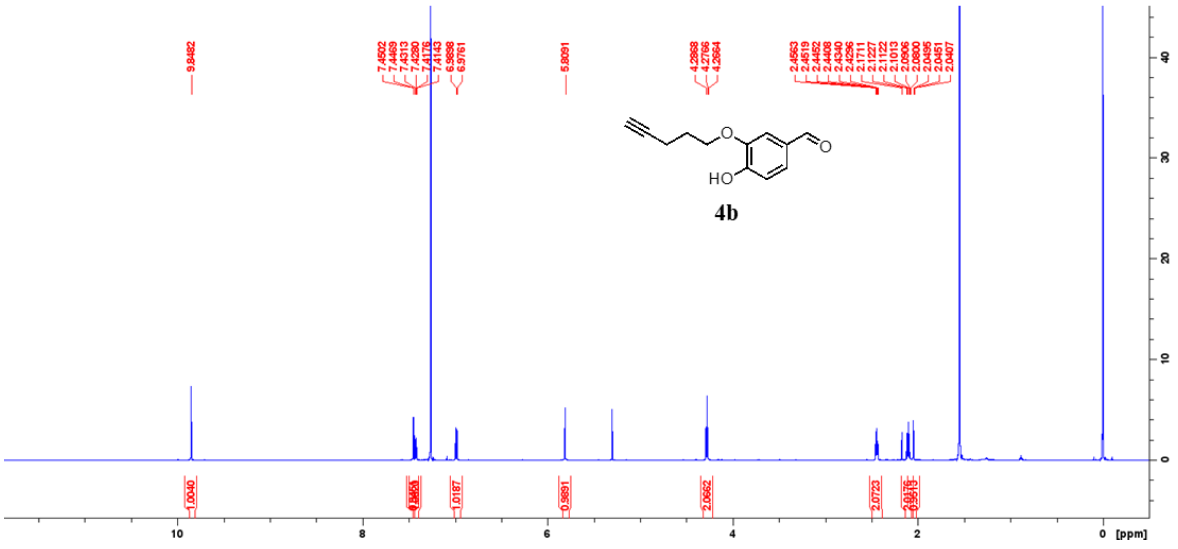


^{31}P NMR (202 MHz)

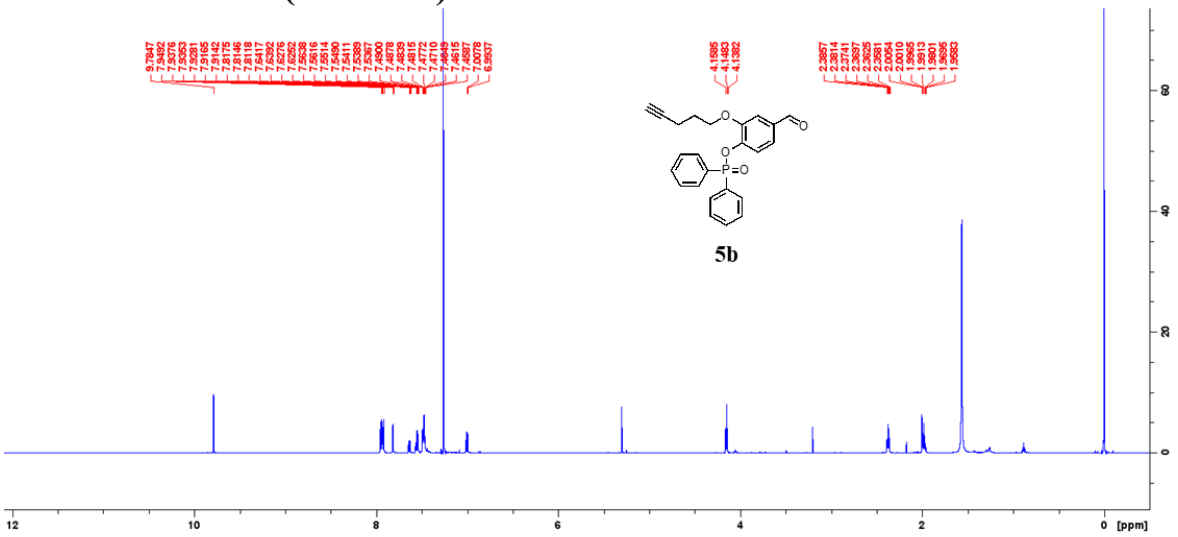


^{1}H NMR (600 MHz)

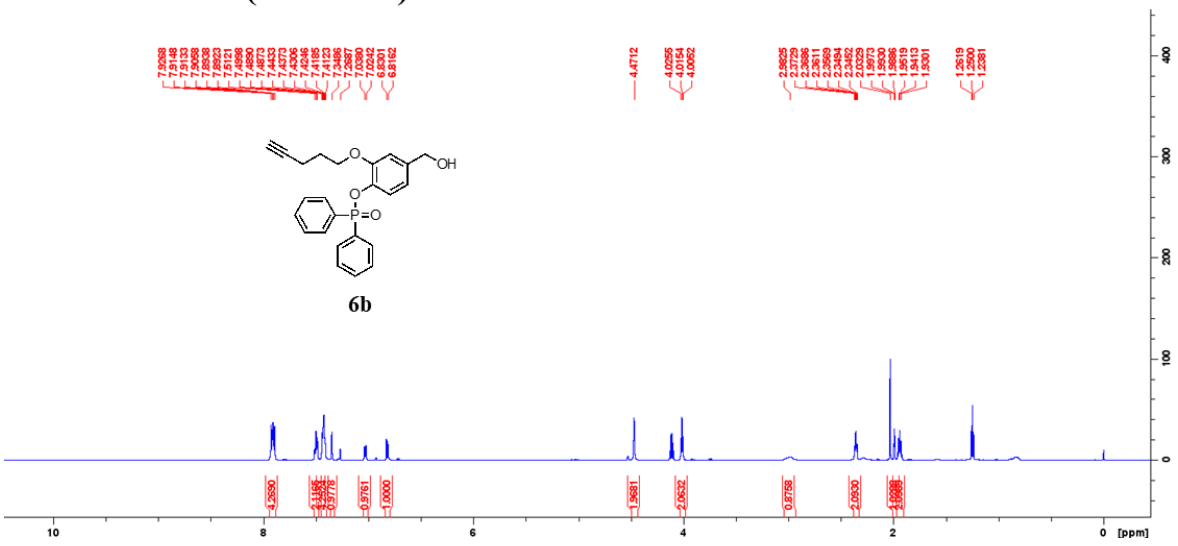


4b

^{1}H NMR (600 MHz)

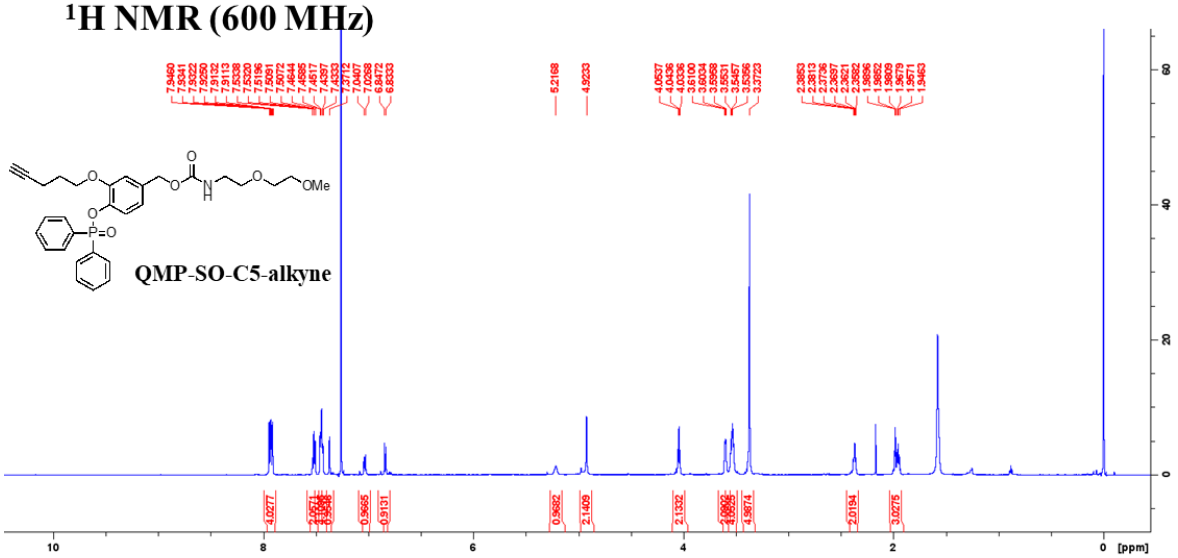


^{1}H NMR (600 MHz)

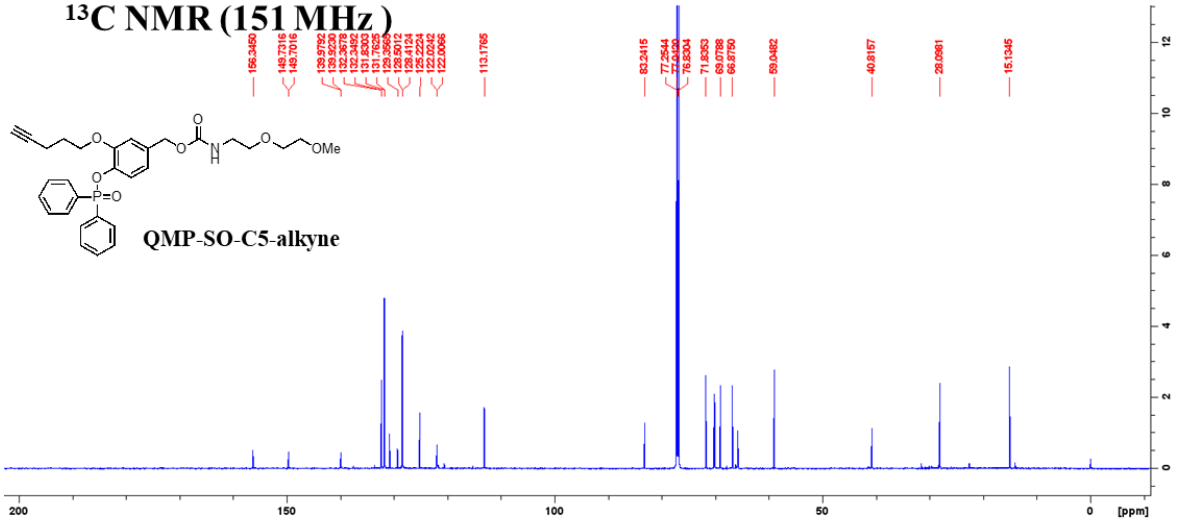


6b

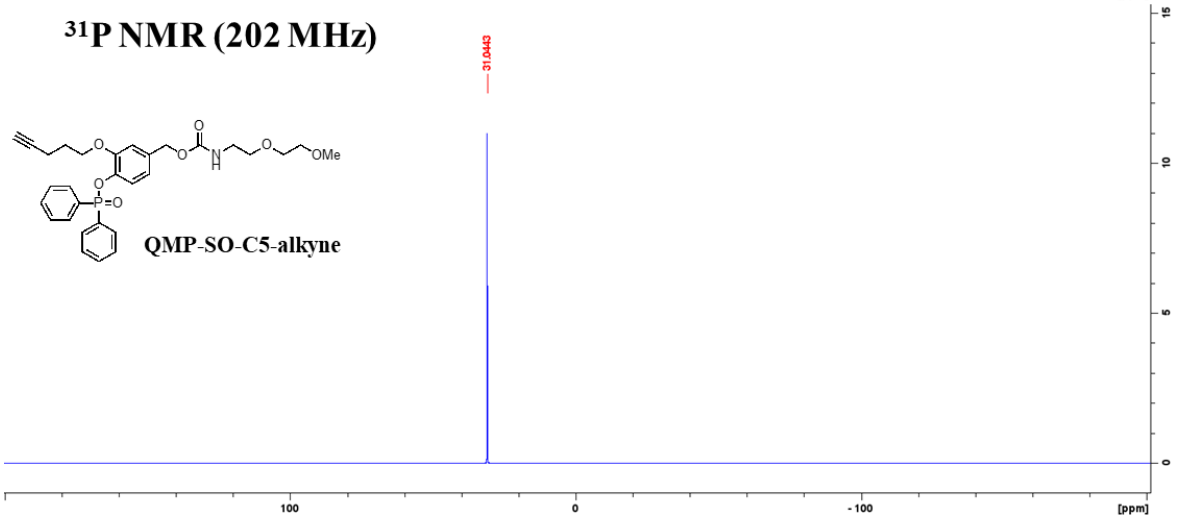
^{1}H NMR (600 MHz)



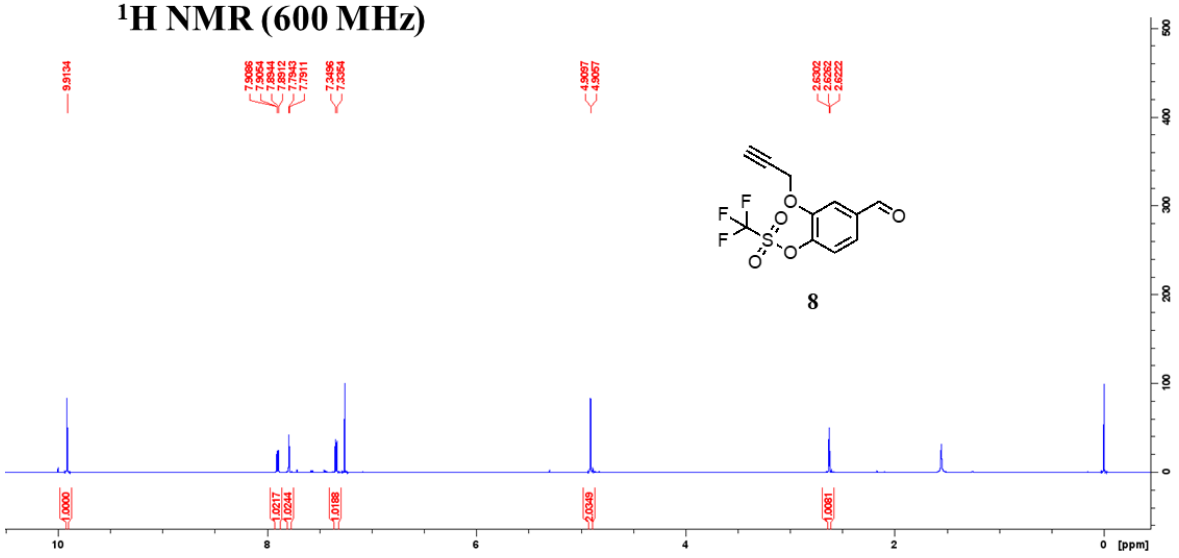
^{13}C NMR (151 MHz)



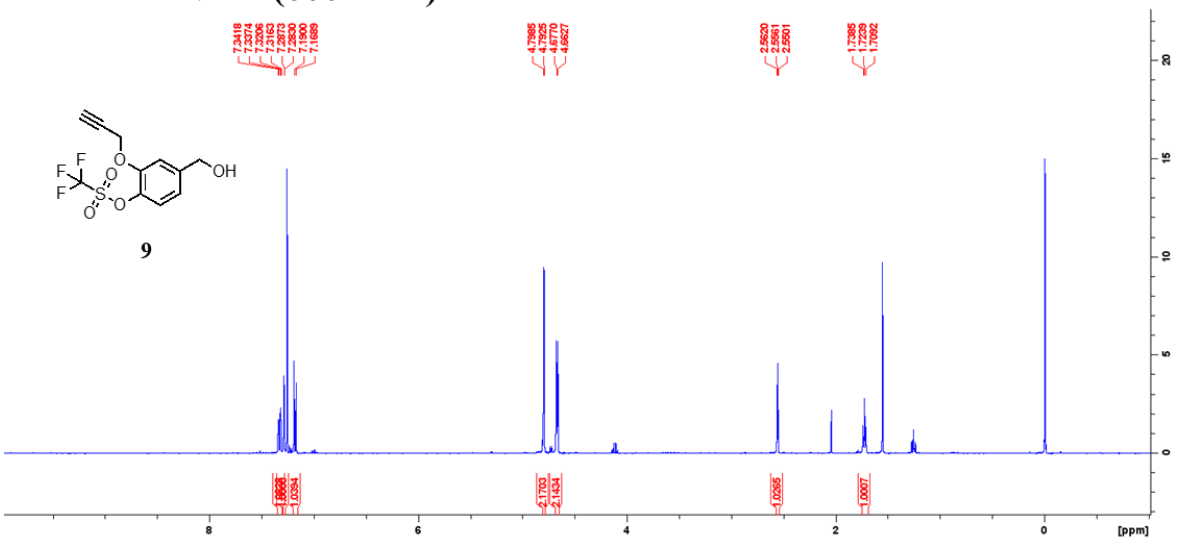
^{31}P NMR (202 MHz)



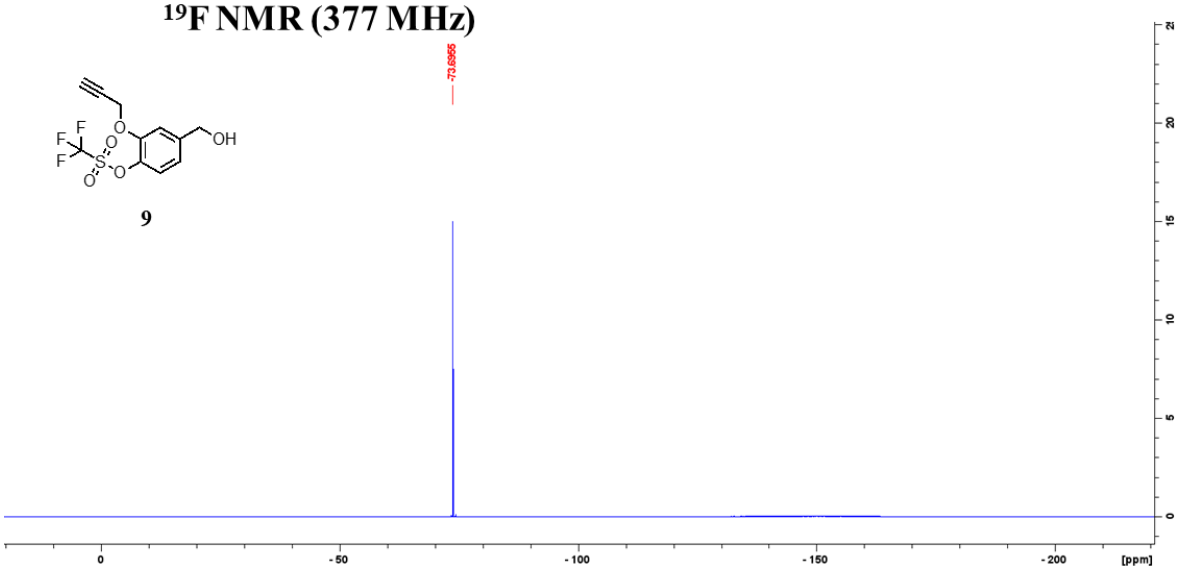
^{1}H NMR (600 MHz)



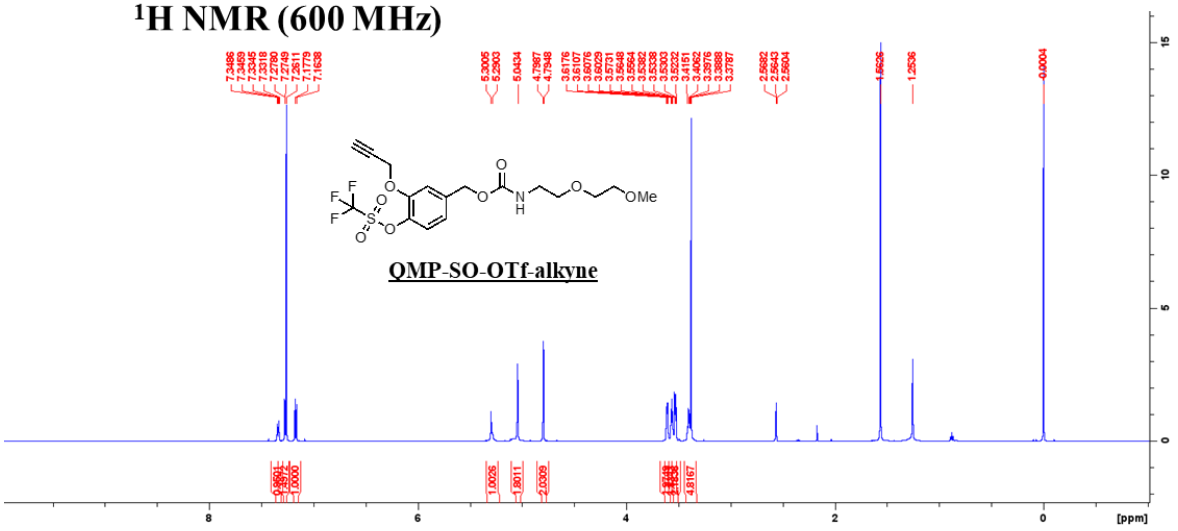
^{1}H NMR (600 MHz)



^{19}F NMR (377 MHz)



^{1}H NMR (600 MHz)



^{13}C NMR (151 MHz)