Supporting Information

Synthetic Control of Mitochondrial Dynamics: Developing three-coordinate Au(I) probes for Perturbation of Mitochondria Structure and Function

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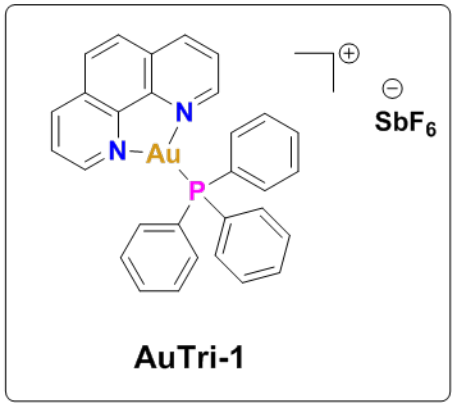
Synthesis and General Experimental Details

General Experimental Details: All reactions were carried under ambient conditions in air unless otherwise noted. Solvents were of ACS grade (Pharmco-Aaper) and used as is. The starting Au(I) complexes (ClAu(tht), $ClAuPPh_3$, $ClAuAsPh_3$, and ClAuSphos) were prepared from procedures in the literature.[1-4] Triphenylphosphine, triphenylarsine, and tetrahydrothiophene were purchased from Alfa Aesar. Sphos and $AgSbF_6$ were purchased from Strem Chemicals and stored under N_2. Phenanthroline (phen), 4,7-dimethylphenanthroline (dmphen), bethophenanthroline (bphen), and bipyridine (bpy) were all purchased from Matrix Scientific. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and JC-1 were purchased from Cayman Chemicals. NMR spectra were recorded on a Bruker Avance NEO 400 MHz spectrometer and samples calibrated for: ^{1}H NMR (CD_3CN δ = 1.94 ppm and DMSO-d_6 δ = 2.50 ppm), ^{13}C NMR (CD_3CN δ = 118.26 and 1.32 ppm and DMSO-d_6 δ = 49.00 ppm), and ^{31}P NMR externally referenced to H_3PO_4 δ = 0.00). High-resolution mass spectra (HRMS) were obtained by direct flow injection (injection volume = 2 μL) using ElectroSpray Ionization (ESI) on a Waters Synapt G2 HDMS instrument in the positive mode with a quadripole/TOF analyzer (UC Boulder). In addition to spectroscopic characterization, the purity of all compounds was assessed by RP-HPLC using an Agilent Technologies 1100 series HPLC instrument and an Agilent Phase Eclipse Plus C18 column (4.6 mm × 100 mm; 3.5 μm particle size). All compounds were found to be ≥97% pure.

Synthesis:

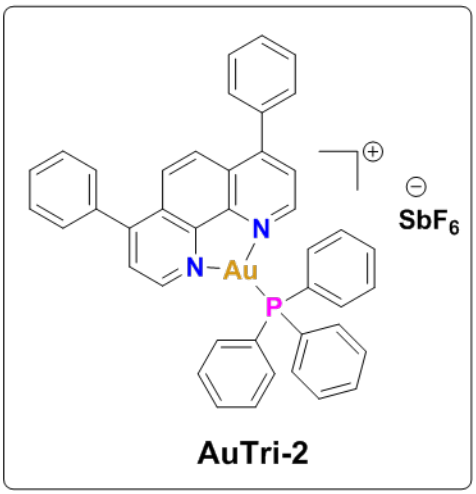
General Procedure for the Preparation of Au(I) Tricoordinate Complexes. To 5 mL of DCM was added 1 equivalent of the corresponding N^N bidentate ligand and 1.1 equivalents of $AgSbF_6$ in a 20 mL screw cap vial wrapped in aluminum foil. This mixture was stirred for 5 minutes at room temperature where it was then added to a solution of 1 equivalent of corresponding XAu(I)Cl (X = PPh_3, $AsPh_3$, and Sphos). The mixture was then stirred for 30 minutes at room temperature under the exclusion of light. The solution was then placed in 1.5 mL Eppendorf tubes and centrifuged at high RPM to pellet the $AgCl_{(S)}$. The supernatant was decanted into a round-bottom flask (cleaned with aqua regia) and concentrated *in vacuo* at 40 °C. Excess Et_2O was added to precipitate the corresponding tri-coordinate complex, which was further washed with excess Et_2O and vacuum dried under the exclusion of light. All complexes except **AuTri-4-6** are benchtop stable indefinitely. **AuTri-4-6** will slowly degrade into a purple looking solid at room temperature, indicating reduction of the gold metal center. In solution phase, (DMSO and MeCN), **AuTri-4-6** will rapidly decompose and deposit elemental gold on the glass vial in both air and under nitrogen atmosphere. Storage at +4 °C significantly improves the longevity of the complexes **AuTri-1-3** and **AuTri-7-10**.



AuTri-1

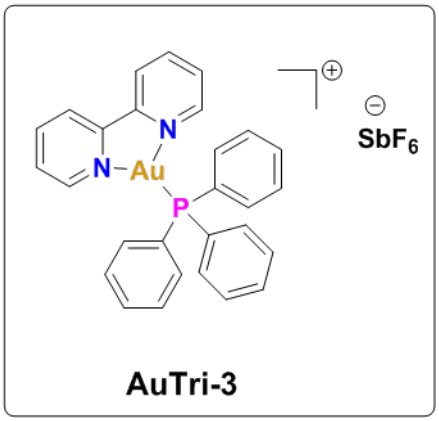
AuTri-1: ^{1}H NMR (400 MHz, MeCN-d_3) 9.25 (d, J = 4 Hz, 2H), 8.92 (d, J = 8 Hz, 2H), 8.30 (s, 2H), 8.20 (q, J = 8 Hz, 2H) 7.86 – 7.80 (m, 6H), 7.72 – 7.65 (m, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 152.35, 142.29, 140.43, 134.42, 134.28, 132.74, 132.71, 130.20, 130.08, 129.40, 128.06, 126.18; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 31.27. Purity was demonstrated to be >97% by RP-HPLC: R_f = 5.09 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min

(50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).



AuTri-2

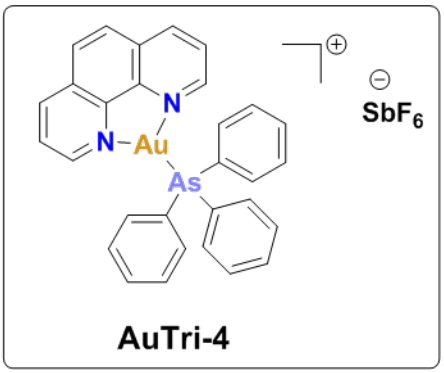
AuTri-2: ^{1}H NMR (400 MHz, DMSO-d_6) 8.13 (d, J = 8 Hz, 2H), 8.05 (s, 2H), 7.79 – 7.66 (m, 25H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 152.00, 151.53, 143.04, 136.30, 134.40, 132.73, 132.71, 130.21, 130.09, 129.58, 127.50, 126.35, 125.53; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 39.12. Purity was demonstrated to be >97% by RP-HPLC: R_f = 10.15 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min (50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).



AuTri-3

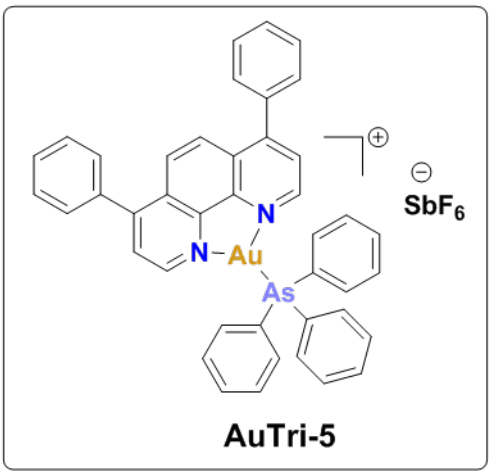
AuTri-3: ^{1}H NMR (400 MHz, MeCN-d_3) 8.81 (bs, 2H), 8.51 (d, J = 8 Hz, 2H), 8.27 (bs, 2H), 7.81 (bs, 2H) 7.60 – 7.51 (m, 15H); ^{13}C NMR (101 MHz, MeCN-d_3) δ = 142.29, 134.83, 133.84,

132.64, 132.46, 132.39, 131.61, 131.15, 130.77, 130.54, 127.50, 124.40, 124.10; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 30.75. Purity was demonstrated to be >97% by RP-HPLC: Rf = 4.77 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min (50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).



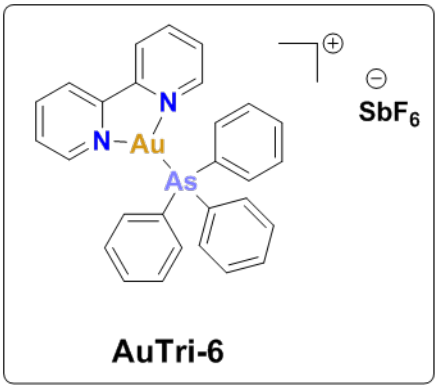
AuTri-4

AuTri-4: ^{1}H NMR (400 MHz, DMSO-d_6) 8.82 (d, J = 8 Hz, 2H), 8.59 (d, J = 8 Hz, 2H), 8.23 (t, J = 8 Hz, 2H), 7.77 (t, J = 8 Hz, 2H) 7.68 – 7.62 (m, 15H).



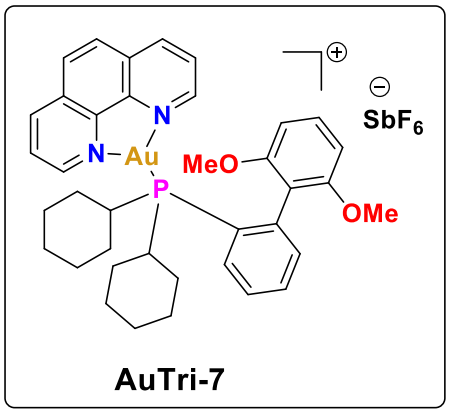
AuTri-5

AuTri-5: ^{1}H NMR (400 MHz, DMSO-d_6) 9.29 (d, J = 4 Hz, 2H), 8.14 (d, J = 4 Hz, 2H), 8.07 (s, 2H), 7.76 (t, J = 4 Hz, 5H) 7.75 – 7.63 (m, 20H).



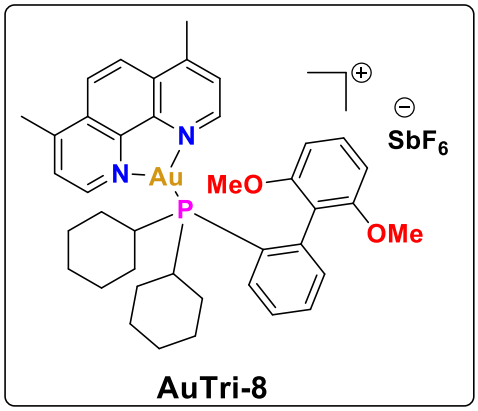
AuTri-6

AuTri-6: ^{1}H NMR (400 MHz, DMSO-d_6) 8.81 (d, J = 8 Hz, 2H), 8.59 (d, J = 8 Hz, 2H), 8.23 (t, J = 8 Hz, 2H), 7.77 (t, J = 8 Hz, 2H) 7.68 – 7.62 (m, 15H).



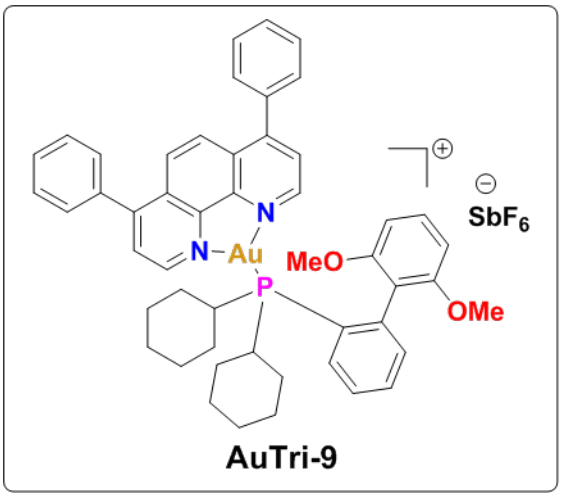
AuTri-7

AuTri-7: ^{1}H NMR (400 MHz, DMSO-d_6) 8.91 (d, J = 8 Hz, 4H), 8.30 (s, 2H), 8.20 (t, J = 8 Hz, 2H), 7.95 (t, J = 8 Hz, 1H), 7.55 (quint., J = 16 Hz, 2H), 6.96 (t, J = 4 Hz, 2H), 5.72 – 5.61 (m, 3H), 3.38 (s, 6H), 2.14 (bs, 2H), 1.85 (bs, 4H), 1.73 (bs, 4H), 1.46 – 1.34 (m, 10H); ^{13}C NMR (101 MHz, DMSO-d$_6$) δ = 156.13, 151.89, 151.54, 143.36, 143.23, 140.01, 139.04, 132.85, 132.34, 131.03, 127.82, 127.75, 125.76, 122.55, 105.64, 70.23, 32.02, 29.99, 26.24, 22.86, 21.87; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 39.09. Purity was demonstrated to be >97% by RP-HPLC: R_f = 5.22 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min (50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).



AuTri-8

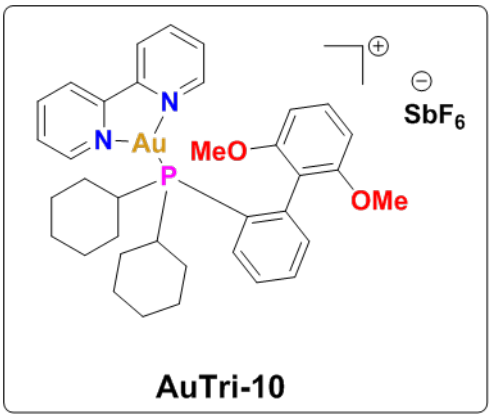
AuTri-8: ^{1}H NMR (400 MHz, DMSO-d_6) 8.71 (d, J = 8 Hz, 4H), 8.39 (s, 2H), 8.02 (t, J = 8 Hz, 2H), 7.94 (t, J = 8 Hz, 1H), 7.55 (quint., J = 16 Hz, 2H), 6.96 (t, J = 4 Hz, 1H), 5.76 – 5.61 (m, 3H), 3.39 (s, 6H), 2.95 (s, 6H), 2.16 (bs, 2H), 1.84 (bs, 4H), 1.72 (bs, 4H), 1.48 – 1.27 (m, 10H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 157.88, 151.11, 149.87, 142.87, 133.61, 133.53, 133.01, 132.97, 131.46, 129.75, 128.78, 128.16, 128.08, 126.23, 123.90, 103.32, 55.46, 32.03, 30.35, 26.80, 19.14; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 39.13. Purity was demonstrated to be >97% by RP-HPLC: R_f = 6.01 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min (50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).



AuTri-9

AuTri-9: ^{1}H NMR (400 MHz, DMSO-d_6) 8.93 (bs, 2H), 8.18 – 8.12 (m, 6H), 7.98 (t, J = 8 Hz, 2H), 7.94 (t, J = 8 Hz, 1H), 7.70 (t, J = 4 Hz, 11 H), 7.58 (quint., J = 16 Hz, 1H), 7.00 (t, J = 4 Hz, 1H), 5.86 – 5.67 (m, 3H), 3.44 (s, 6H), 2.20 (bs, 2H), 1.86 – 1.76 (m, 8H), 1.49 – 1.37 (m,

10H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 156.12, 152.47, 150.81, 143.28, 143.15, 140.39, 132.86, 132.78, 132.35, 132.31, 131.19, 128.80, 128.65, 127.83, 127.75, 126.46, 124.23, 122.61, 122.54, 106.15, 70.19, 31.88, 31.79, 29.80, 26.12, 22.72, 21.90; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 40.74. HRMS *(m/z)* calcd. 939.3354, found 939.3346 $[M - SbF_6]^+$, Purity was demonstrated to be >97% by RP-HPLC: R_f = 10.87 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min (50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).



AuTri-10

AuTri-10: ^{1}H NMR (400 MHz, DMSO-d_6) 8.58 (bs, 2H), 8.49 (d, J = 8 Hz, 2H), 8.24 (bs, 2H), 7.91 (quint., J = 16 Hz, 1H), 7.80 (bs, 2H), 7.54 (d, J = 8 Hz, 2H), 6.99 (t, J = 8 Hz, 1H), 6.46 (bs, 1H), 6.02 (d, J = 8 Hz, 2H), 3.43 (s, 6H), 2.03 (bs, 2H), 1.80 – 1.63 (m, 8H), 1.47 – 1.18 (m, 10H); ^{13}C NMR (101 MHz, MeCN-d_3) δ = 157.97, 133.63, 133.56, 133.04, 133.01, 131.67, 130.31, 130.14, 129.62, 128.20, 128.13, 126.77, 126.04, 135.60, 103.80, 55.53, 31.69, 30.06, 26.87, 26.77, 26.63, 26.32; ^{31}P NMR (162 MHz, DMSO-d_6) δ = 38.46. Purity was demonstrated to be >97% by RP-HPLC: R_f = 4.82 minutes using the following method: Flow rate: 1 mL/min; λ = 280 nm; Eluent A = H_2O with 0.1% TFA; Eluent B = MeOH with 0.1% TFA; Solvent Gradient: 0 – 3 min (50:50 H_2O:MeOH), 5 min (40:60 H_2O:MeOH), 7 min (30:70 H_2O:MeOH), 9 min (0:100 H_2O:MeOH), 10 min (20:80 H_2O:MeOH), 12 min until end of run (100:0 H_2O:MeOH).

Physical and Chemical Characterization

X-ray Crystallography. Crystals of all complexes were grown from slow diffusion of Et_2O into a concentrated solution of MeCN at room temperature. All crystals were mounted using polyisobutene oil on the end of a glass fibre, which had been mounted to a copper pin using an electrical solder. It was placed directly in the cold gas stream of a liquid nitrogen cryostat[5-6] A Bruker D8 Venture diffractometer with graded multilayer focused MoKα X-rays (λ = 0.71073 Å) was used to collect diffraction. Raw data were integrated, scaled, merged, and corrected for Lorentz-polarization effects using the APEX3 package.[7-9] Space group determination and structure solution and refinement were carried out with SHELXT and SHELXL respectively.[10-11] All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed at calculated positions and refined using a riding model with their isotropic displacement parameters (Uiso) set to either 1.2Uiso or 1.5Uiso of the atom to which they were attached. Ellipsoid plots were drawn using SHELXTL-XP.[12] The structures, deposited in the Cambridge Structural Database, were checked for missed symmetry, twinning, and overall quality with PLATON,[13] an R-tensor,[14] and finally validated using CheckCIF.[13] Complete X-ray structures can be found in Figures S1-S6 and corresponding complete crystallographic parameters in **Tables S1-S6**.

In Vitro Biological Assays

Cell Culture. All cell lines were purchased from ATCC and routinely grown in a humidified incubator at 37 °C with 5-10% CO_2. MDA-MB-468, MDA-MB-231, MDA-MB-175, MRC5, RPE-NEO, HCT116, and K562N were grown in DMEM supplemented with 10% FBS, 1% amphotericin and 1% penicillin/streptomycin. A2780 cells were grown in RPMI supplemented with 10% FBS, 1% amphotericin, and 1% penicillin/streptomycin, and 4 mM glutamine. All supplements along with PBS and trypsin-EDTA were purchased from Corning Inc. and used as is.

Cell Viability of AuTri-1-10 (Adherent Cell Lines). The cell viability of all 10 complexes were performed in MDA-MB-231. Additionally, **AuTri-9** was evaluated in the following: MDA-

MB-175, MA-MB-468, A2780, HCT116, OVCAR8 and MRC5. Cells were grown to confluency and trypsin was added to detach and harvest cells. The cells were washed with 2 mL of PBS and suspended in 10 mL of the appropriate media. The cells were centrifuged at 2000 rpm for 5 minutes and the pellet washed with 2 mL of PBS then suspended in 5 mL of the appropriate media. The cells were plated at a density of 2,000 cells/well in a 96-well clear bottom plate and allowed to adhere overnight at 37 °C with 5-10% CO_2. The compounds were prepared as a stock in DMSO and used fresh. The compounds were added at seven concentrations (< 1% DMSO) with a 3x serial dilution starting at 50 μM for the highest concentration and incubated at 37 °C for 72 h with 5-10% CO_2. 1% DMSO were added to control wells as the vehicle control. The medium was removed and a solution of MTT (100 μL, prepared by dissolving MTT at 5 mg/mL and diluting by 10x with DMEM) was added to each well and incubated for 4 h at 37 °C with 5-10% CO_2. The dye was removed from each well and 100 μL of DMSO was added to induce cell lysis. The plates were read using a Genios plate reader (λ = 570 nm). The experiment was performed in triplicate and data are plotted as the mean ± s.e.m. (n = 3). Full sigmoidal plots of **Autri-1-10,** phenanthroline, bathophenanthroline, bipyridine, dimethylphenanthroline, auranofin, cisplatin, and $NaSbF_6$ in MDA-MB-231 can be found in Figures S40-50.

Cell Viability of AuTri-9 (Suspended Cell Lines). The cell viability of **AuTri-9** was determined in K562. Cells were grown to confluency and centrifuged at 2000 rpm for 5 minutes to collect the cell pellet. The cells were washed with 5 mL of PBS, suspended in 5 mL of DMEM, and centrifuged again at 2000 rpm for 5 minutes to collect the pellet. The pellet was then washed with 2 mL of PBS and suspended in 5 mL of DMEM. The cells were plated at density of 2,000 cells/well in a 96-well white bottom plate. **AuTri-9** was prepared as a stock solution in DMSO and used fresh. The compound was added at seven concentrations (< 1% DMSO) with a 3x serial dilution starting at 50 μM for the highest concentration and incubated at 37 °C for 72 h with 5-10% CO_2. The cells were removed from the incubator and allowed to rest at room temperature for 30 minutes. To each designated well was then added 20 μL of CellTiter-Glo solution and orbitally shaken for 5 minutes and the luminescence (1000 ms integration and 150 ms gain) acquired on a Genios plate reader. The experiment was performed in triplicate. Data are plotted as the mean ± s.e.m. (n = 3).

Solution Stability of AuTri-9. All media were warmed to 37 °C prior to use. DMEM was supplemented with 10% FBS, 1% amphotericin and 1% penicillin/streptomycin, PBS (1X) was purchased from Fischer Scientific and used as it, and DMSO was purchased as ACS grade was purchased from Sigma Aldrich and used as is. All absorption spectra were recorded on a Shimadzu UV-1280 model instrument. Prior to each run, the instrument was blanked with the corresponding buffer/solvent. The solutions were incubated at 37 °C until used for absorption measurement. For DMEM, **AuTri-1-10** were prepared as a 1 mM stock in DMSO and diluted down to 50 μM with the corresponding medium. No precipitation was observed. The absorption spectra were recorded at each listed time interval. For DMSO and PBS, **AuTri-9** was prepared as a 1 mM stock in DMSO and diluted to 50 μM. No precipitation was observed. The absorption spectra were recorded at each listed time interval.

Reactivity of AuTri-9 with NAC and GSH. All media were warmed to 37 °C prior to use. DMEM was supplemented with 10% FBS, 1% amphotericin and 1% penicillin/streptomycin, PBS (1X) was purchased from Fischer Scientific and used as it, and DMSO was purchased as ACS grade was purchased from Sigma Aldrich and used as is. All absorption spectra were recorded on a Shimadzu UV-1280 model instrument. **AuTri-9** was prepared as a 1 mM stock in DMSO and diluted to 100 μM. Both NAC and GSH were dissolved in PBS (1 mM) and diluted to 100 μM with PBS. The solutions were added in the stated stoichiometric amounts and the absorption spectra recorded after 1 hour of incubation.

Immunoblotting. Equal numbers of RPE and MDA-MD-231 cells were seeded and treated with AuTri-9 for the indicated time points. Whole cell lysates were prepared using RIPA buffer, 1x protease inhibitor cocktail (Sigma), and 1x phosphatase inhibitor cocktails I and II (Sigma) and loaded by equal protein for SDS-PAGE. Protein concentrations in the cell lysates were determined with the Bradford protein assay reagent. Cell lysates containing equal amounts of protein were separated on a 4 – 20% SDS-polyacrylamide gel. Post separation, proteins were transferred to a nitrocellulose membrane and non-specific binding sites were blocked by treating with 5% nonfat dry milk. The membranes were incubated overnight with the primary antibodies directed against OPA1, MFF, MFN1, TOM20, SOD1, SOD2, NRF2, cytochrome c, and beta-actin. Appropriate secondary antibodies were used accordingly. Blots were visualized with Thermo Scientific Pierce

Supersignal West Dura Extended Duration Chemiluminescent Substrate on the LI-COR C-DiGit Chemiluminescent Western Blot Scanner (Lincoln, NE). Measurement of integrated density of protein bands was performed using ImageStudioLite software (LI-COR). Full immublots can be found in Figures S51-S55. The full ANOVA table can be found in Table S7.

Whole Cellular Uptake of AuTri-9.

For whole cellular uptake, MDA-MB-231 cells were seeded at a density of 1 x 10^6 cells/mL in a 6 well clear bottom plate with a volume of 2.5 mL and allowed to adhere overnight at 37 °C. Compounds were prepared as a stock in DMSO and added to each well at a final concentration of 1 μM (< 1% DMSO) and treated for 6 h. The cells were then collected by trypsinization and centrifuged at 2000 rpm for 5 minutes to form a pellet. The pellet was suspended in 1 mL of DMEM, transferred to a 1.5 mL Eppendorf tube, and centrifuged again at 2000 rpm for 5 minutes. The media were removed, washed with PBS twice, and the pellet stored at -20 °C until analysis. Prior to analysis, the pellets were suspended in 0.5 mL of concentrated HNO_3 and agitated for 1 minute. The solution was transferred to a 15 mL Falcon tube and then 4.5 mL of DI H_2O was added. The samples were then subjected to analysis with ICP-OES. Data is represented as the mean ± s.e.m. (n = 3). The standard curve can be found in supporting Figure S55.

Whole cellular uptake with pre-incubation of uptake inhibitors was performed as follows. MDA-MB-231 cells were seeded at a density of 1 x 10^6 in clear-bottomed six-well plates and incubated at 37 °C overnight. The cells were then pre-treated with the following inhibitors: NaN_3 (1 mM), methyl-β-cyclodextrin (5 mM), chlorpromazine hydrochloride (28 nM), wortmannin (50 nM), genistein (200 μM), for 1 hour. For the +4 °C sample, the cells were cooled to +4 °C prior to addition of **AuTri-9**. Following pre-treatment, the media were removed and washed with PBS (3 x 2 mL). The cells were then incubated with **AuTri-9** (5 μM for 24 hours, < 1% DMSO) at 37 °C and the +4 °C sample incubated at the same concentration at the indicated lower temperature. After treatment, the cells were collected both, the medium and tryspsinzed cells combined, centrifuged at 2000 rpm for 5 minutes to form a pellet. The pellet was suspended in 1 mL of DMEM, transferred to a 1.5 mL Eppendorf tube, and centrifuged again at 2000 rpm for 5 minutes. The media were removed, washed with PBS twice, and the pellet stored at -20 °C until

analysis. Prior to analysis, the pellets were suspended in 300 µL of 70% HNO_3. 100 µL aliquot of this stock was then diluted to 1 mL and subjected to GF-AAS. A calibration curve using Au in varying concentrations was performed. The same standard curve was used for data analysis as the previous experiment (Figure S56).

Subcellular Uptake of AuTri-9. The subcellular uptake of **AuTri-9** was conducted in a similar manner per the instructions from the RayBio® Nuclear Extraction Kit Protocol (RayBiotech, Inc.). MDA-MB-231 cells were seeded at a density of 10 x 10^6 in 100 mm petri dishes and incubated overnight at 37 °C. **AuTri-9** was added at 1 µM (< 1% DMSO) for 6 hours. Once the fractions had been isolated, the concentrate was subjected to GF-AAS analysis. Prior to analysis, a calibration curve using Au in varying concentrations was performed. Data is represented as the mean ± s.e.m. (n = 3). The same standard curve was used for data analysis as the previous experiment (Figure S56).

Mitochondrial Uptake of AuTri-9. The localization of Au content in mitochondria were performed by using Mitochondria Isolation Kit for Cultured Cells from ThermoFisher Scientific™.MDA-MB-231 cells were plated at a density of 20 x 10^6 in 100 mm petri dishes and incubated overnight at 37 °C. **AuTri-9** was added at a concentration of 1 µM (< 1% DMSO) for 6 hours. Once the fractions had been isolated, the concentrate was subjected to GF-AAS analysis. Prior to analysis, a calibration curve using Au in varying concentrations was performed. Data is represented as the mean ± s.e.m. (n = 3). The standard curve was used for data analysis as the previous experiment (Figure S57).

ROS Accumulation using DCF-DA. DCF-DA was purchased from Cayman Chemicals, stored at -20 °C, and used without light. DCF-DA was prepared as a 1 mM stock in DMSO and diluted to a working concentration of 20 µM with PBS. **AuTri-9** was prepared as a 1 mM stock in DMSO and diluted to a working concentration of 25 µM with PBS. MDA-MB-231 cells were grown to 85% confluency at 37 °C with 5-10% CO_2. The cells were trypsinized, washed with PBS, and aliquoted to a density of 3 x 10^5 cells, centrifuged, and suspended in 200 µL of a 20 µM DCF-DA solution prepared above. The cell suspensions were incubated at 37 °C for 30 minutes. **AuTri-9** was added (50 µL) to achieve a final volume 250 µL and a final concentration

of 5 μM of **AuTri-9** and analyzed using FACS (FITC channel, excitation 488 nm) at the given time intervals. After 4 hours, no response was observed as the population were dead (> 90%). Tert-butyl hydroperoxide was used as the positive control. Data were analyzed using BD CellQuest Pro and plotted using GraphPad Prism 6.

Quantitative Proteomics-DEP. MDA-MB-231 cells were seeded on petri dish (100 mm x 15 mm) and allowed to grow to 85% confluency. The cells were then treated with **AuTri-9** at a concentration of 1 μM for 12 h at 37 °C. Cells were harvested and sent to Creative Proteomics (Shirley, NY). Chemicals and Instrumentation used are as follows: TMT10plex Isobaric Label Reagent Set, Pierce Quantitative Colorimetric Peptide Assay, was purchased from Thermo Fisher Science. Triethylammonium bicarbonate buffer (1.0 M, pH 8.5 ± 0.1), Tris(2-carboxyethyl)phosphine hydrochloride solution (0.5 M, pH 7.0), iodoacetamide (IAA), formic acid (FA), acetonitrile (ACN), methanol, were purchased from Sigma (St. Louis, MO, USA). Trypsin from bovine pancreas was purchased from Promega (Madison, WI, USA). Ultrapure water was prepared from a Millipore purification system (Billerica, MA, USA). An Ultimate 3000 nano UHPLC system (Thermo Scientific, Waltham, MA) coupled online to a Q Exactive HF mass spectrometer (Thermo Scientific) equipped with a Nanospray Flex Ion Source (Thermo Scientific). Cell pellets were lysed by using 200 ul of RIPA lysis buffer including protease inhibitors, centrifuged at 12000 rpm for 15 min at 4℃and transfer the supernatant to a new EP tube and protein concentration determined by using BCA kit. Transfer 200 μL sample into a new microcentrifuge tube. To each sample tube, reduced by 10 mM TCEP at 56°C for 1 h, then alkylated by 20 mM IAA at room temperature in dark for 1h. Next, samples were added free trypsin into the protein solution at a ratio of 1:50, and the solution was incubated at 37°C overnight and lyophilized the extracted peptides to near dryness. Re-dissolve the sample with 100 mM TEAB. Samples were then labelled with peptide and fractionated. Samples were then analyzed by Nano LC-MS/MS. Nanoflow UPLC: Ultimate 3000 nano UHPLC system (ThermoFisher Scientific, USA); Nanocolumn：trapping column (PepMap C18, 100Å , 100 μm×2 cm, 5μm) and an analytical column (PepMap C18, 100Å, 75 μm×50 cm, 2μm); Loaded sample volume: 5 μL Mobile phase: A: 0.1% formic acid in water; B: 0.1% formic acid in 80% acetonitrile. Total flow rate : 250 nL/min LC linear gradient: a linear gradient from 5 to 7% buffer B in 2 min, from 7% to 20% buffer B in 80 min, from 20% to 40% buffer B in 35 min,

then from 40% to 90% buffer B in 4 min. Q Exactive HF mass spectrometer (Thermo Fisher Scientific, USA) Spray voltage: 2.2 kV Capillary temperature: 270°C MS parameters: MS resolution: 120000 at 200 m/z MS precursor m/z range: 300.0-1650.0 www.creative-proteomics.com 45-1 Ramsey Road, Shirley, NY 11967, USA Tel:1-631-275-3058 Fax:1-631-614-7828 www.creative-proteomics.com info@creative-proteomics.com MS/MS parameters: Product ion scan range: start from m/z 100 Activation Type: CID Min. Signal Required: 1500.0 Isolation Width: 3.00 Normalized Coll. Energy: 40.0 Default Charge State: 6 Activation Q: 0.250 Activation Time: 30.000 Data dependent MS/MS: up to top 15 most intense peptide ions from the preview scan in the Orbitrap.

Mitochondrial Membrane Potential (JC-1). MDA-MB-231 cells were plated at a density of 5 x 10^5 cells/plate using a glass bottom petri dish fitted with a #1.5 cover slip with a final volume of 1.5 mL and allowed to adhere overnight at 37 °C. Compound **AuTri-9** was prepared as a stock in DMSO and added at a final concentration of 10 μM (< 1% DMSO). The cells were treated for 1 h at this concentration. CCCP was prepared as a stock in DMSO and added at a final concentration of 5 μM and the cells treated for 15 minutes. This was used as a positive control. After the indicated treatment time, a working solution of the JC-1 dye (Cayman Chemicals) was prepared by adding 100 μL of dye into 900 μL of DMEM. Note: the working solution of JC-1 should always be prepared fresh and not stored for long-term use. Then, 100 μL/mL of DMEM were added to the cells and incubated at 37 °C for 20 minutes. Prior to imaging, the media was removed and replaced with room temperature PBS (2 mL). The cells were then visualized using confocal microscopy on a Nikon A1R Inverted Confocal Microscope. J-aggregates were imaged with (excitation/emission: 510/ 590 nm) and J-monomers with (excitation/emission: 488/525 nm).

Mitochondrial Metabolism Analysis with Seahorse XF96 Analysis. The optimum conditions for cell density and FCCP injection concentration were determined to be 30,000 cells/well and an FCCP injection concentration of 0.6 μM.[15] All Seahorse XF96 experiments with MDA-MB-231 were performed under these conditions. The cells were seeded the night prior to the experiment with a final volume of 100 μL and incubated overnight at 37 °C. Compound **AuTri-9** was prepared as a stock in DMSO and diluted to a working concentration of 200 μM with Seahorse

XF96 assay buffer and then subsequently serial diluted by 3x to achieve multiple concentrations. The assay was performed using a pneumatic injection method of **AuTri-9**, with the final injection concentrations of 0.1, 1, and 3 μM (< 1% DMSO). This was followed by injection of oligomycin (1.5 μM), FCCP (0.6 μM) and rotenone/ antimycin A (0.5 μM). The metabolic parameters are calculated as seen in the supporting information of the following papers.[16-17]

Transmission Electron Microscopy. MDA-MB-231 cells were seeded in 6 well plates and allowed to grow to 85% confluency. The cells were then treated with **AuTri-9** at a concentration of 10 μM (< 1% DMSO) for 1 h at 37 °C. Growth media were removed and immediately and added 3% glutaraldehyde at 4°C in 0.1 M Sorenson's or Cacodylate Buffer. After 5 minutes remove fix and replace with fresh fixative, leave for 45 min-1hr at 4°C, washed in 0.1 M buffer w/5% sucrose 4x, 5 minutes each. Post fix, the cells were stained with 1% OsO4 in 0.1 M buffer for 1 hr at 4°C and washed in 0.1 M buffer 1x. The cells were dehydrated in graded ethanols, 5 minutes each, from 50% through 100% and absolute ethanol (2x) at 4°C. After the last alcohol wash remove the residual alcohol with a small pipette and immediately add straight resin (14.5 g Eponate 12, 8.0 g DDSA, 7.15 g NMA, and 0.85 gm BDMA) to the dish. Swirl the dish and then remove as much of the resin, (which may contain residual alcohol), with a plastic pipette being careful not to disturb the cell layer. Then add straight resin 2x for 1-2 hours and place under the lamp, on a shaker. Pour off the 2nd straight resin wash, let the plate drain well (leaving a layer of resin on the bottom) and invert labeled beem capsules into each well that have had the conical ends removed. Place the smooth factory end down in the wells being careful not to move the capsules around too much and disturb the cell layer and polymerize overnight at 60°C. The following morning fill the beem capsules with fresh resin and polymerize for another 24 hours at 60°C. With the aid of a metal chemical spatula snap the capsules off the dishes when cool. The capsules were then sectioned using a fresh microtome. Transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) were performed on a Thermo Scientific Talos F200X microscope, operated at an accelerating voltage of 200 kV or/and 80 kV. Velox digital micrograph software was used to record TEM/STEM images, measure cell size, and measure changes in mitochondrial morphology.

In Vivo Biological Assays

Animal Facility and Standard of Care. Mice were placed in a facility accredited by the Institutional Animal Care and Use Committee (IACUC ID#: 2019-3183). Treatments were administered by intraperitoneal injection. The amount of anticancer agent was calculated on the basis of the average animal body weight. The Division of Laboratory Animal Resources of the University of Kentucky approved all animal protocols.

***In vivo* maximum tolerated dose using AuTri-9.** Six-week-old female Nu/Nu Nude mice were purchased from the Charles River Laboratories (Shrewsbury, MA) and quarantined for seven days. Animals were randomized into two treatment groups (n = 3) and given either PBS or **AuTri-9** at an equivalent dose of 10 mg/kg body weight through intraperitoneal injection (every other day, three-time per week, 7x). Mice were weighed every other day to monitor potential side effects and stress.

Supporting Figures and Tables:

X-Ray Crystal Structures: All atoms are drawn at the 50% probability level. H-atoms are omitted for clarity.

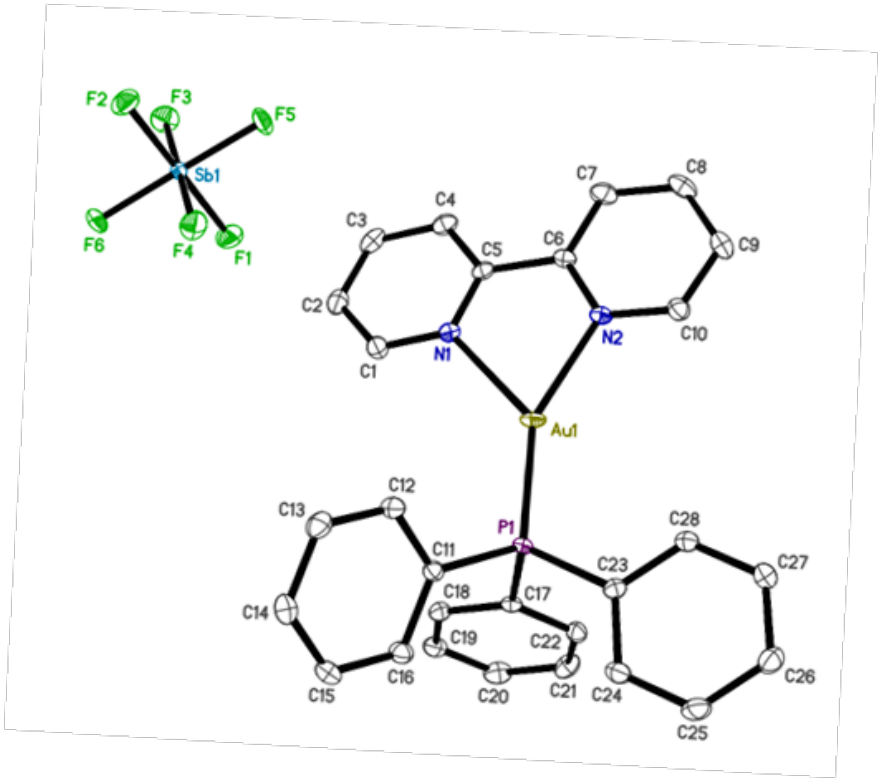


Figure S1: X-ray Structure of **AuTri-3**

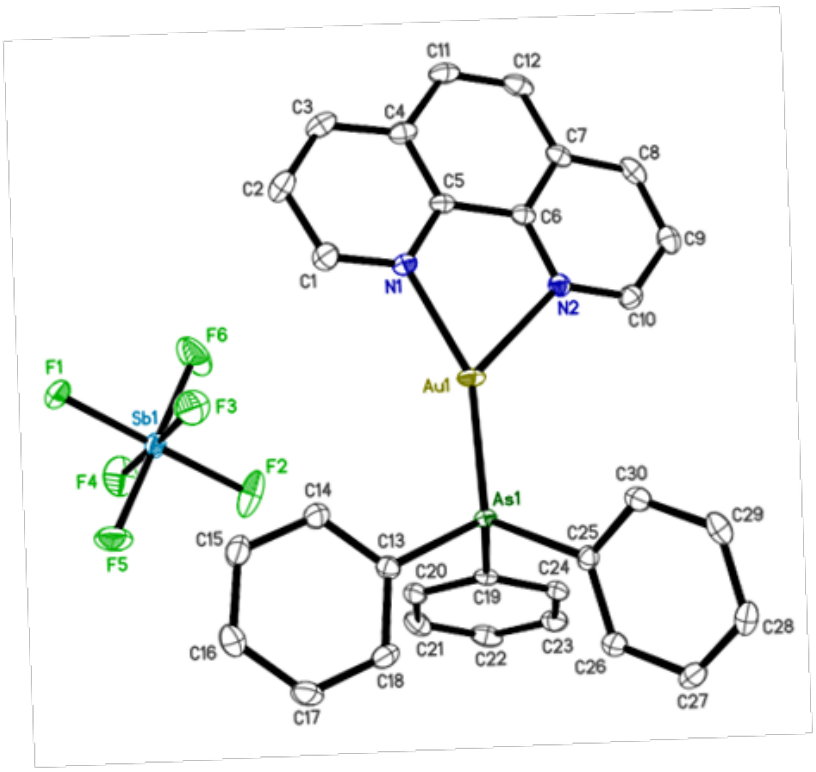


Figure S2: X-ray Structure of **AuTri-4**

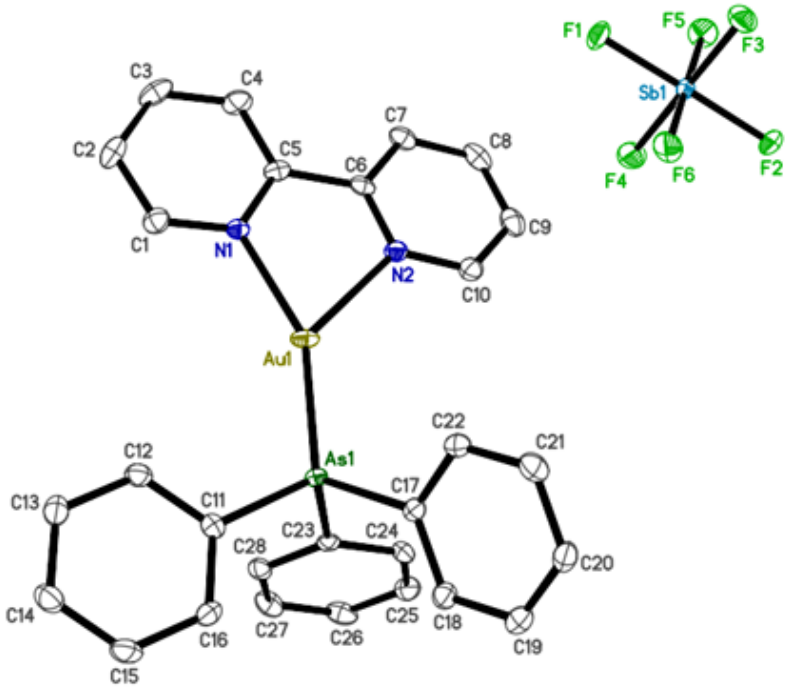


Figure S3: X-ray Structure of **AuTri-6**

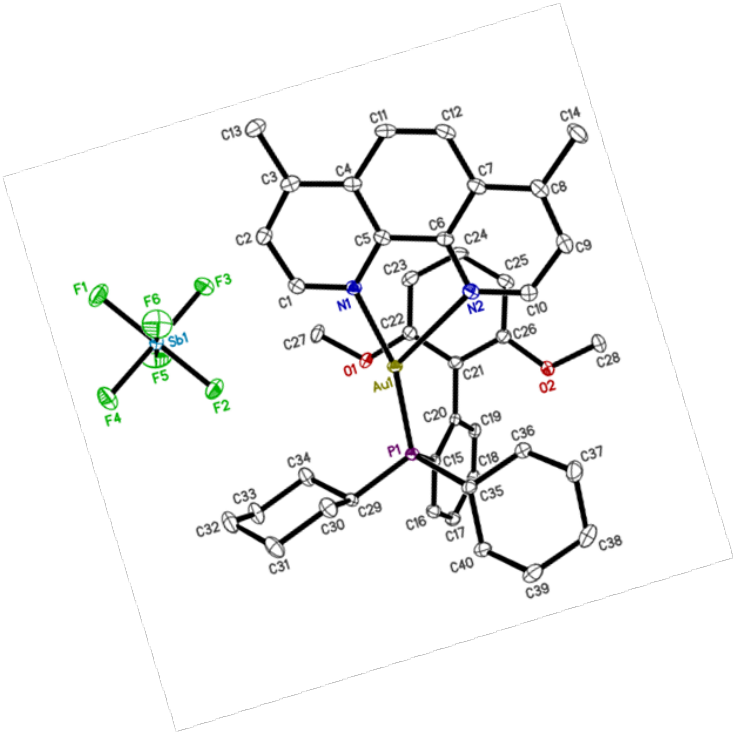


Figure S4: X-ray Structure of **AuTri-8**

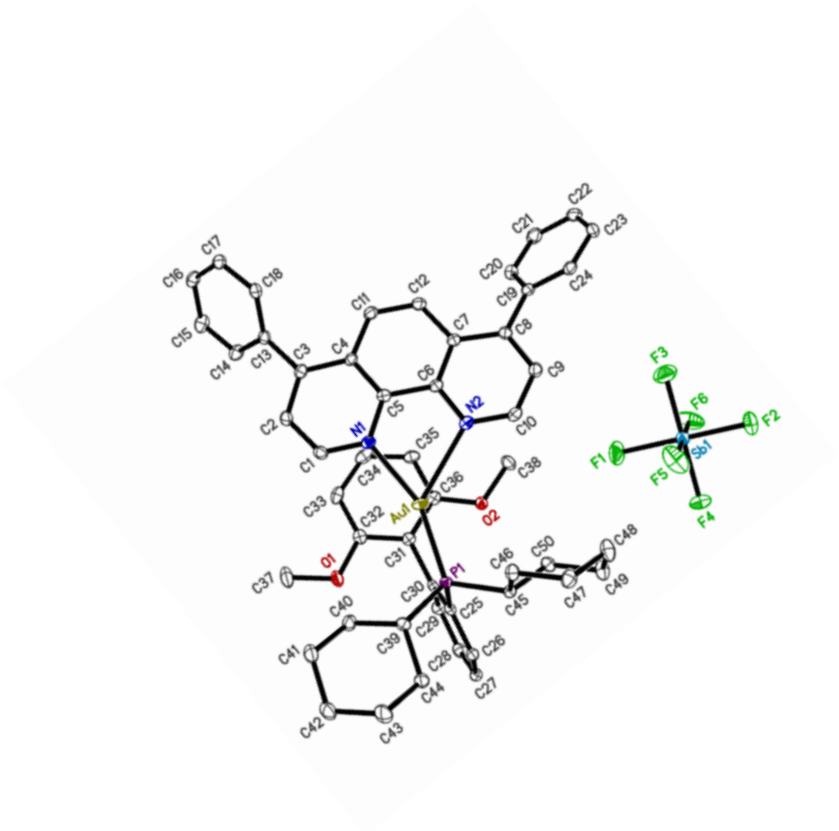


Figure S5: X-ray Structure of **AuTri-9**

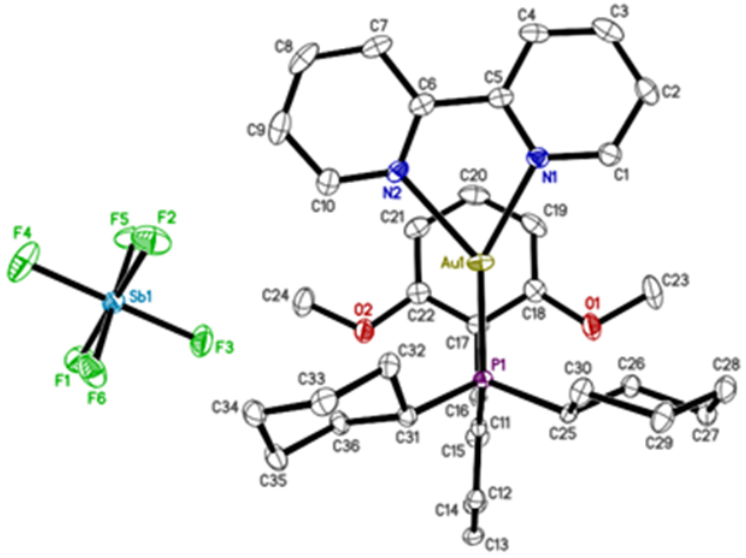


Figure S6: X-ray Structure of **AuTri-10**

X-Ray Crystallographic Details:

Table S1: X-ray Parameters of **AuTri-3**

X-ray Structural Data and Crystal Refinement

Empirical Formula	**AuTri-3** $C_{28}H_{23}AuF_6N_2PSb$
Molecular Weight (g/mol)	851.17
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, $P\bar{1}$
Unit Cell Dimensions (A), (o)	a =11.3162(2) Å alpha = 80.967(1) b = 11.3701(2) Å beta = 71.945(1) c = 11.5687(4) Å gamma = 72.244(1)
Volume	1344.58(4) $Å^3$
Z	2
Absorption Coefficient	2.102 mm^{-1}
F(000)	808
Crystal Size (mm)	0.080 x 0.070 x 0.050
Theta Range	2.298 to 27.493
Completeness to Theta = 25.242	99.8%
F^2	1.090
Final R indices [I>2sigma(I)]	R1 = 0.0122, wR2 = 0.0297

Table S2: X-ray Parameters of **AuTri-4**

X-ray Structural Data and Crystal Refinement

Empirical Formula	**AuTri-4** $C_{30}H_{23}AuF_6N_2AsSb$
Molecular Weight (g/mol)	919.14
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, $P\bar{1}$
Unit Cell Dimensions (A), (o)	a = 11.0384(2) Å alpha = 77.723(1) b = 11.6949(2) Å beta = 73.203(1) c = 12.1580(2) Å gamma = 72.939(1)
Volume	1413.83(4) $Å^3$
Z	2
Absorption Coefficient	2.159 mm^{-1}
F(000)	868
Crystal Size (mm)	0.120 x 0.110 x 0.090
Theta Range	2.462 to 27.521
Completeness to Theta = 25.242	99.9%
F^2	1.043
Final R indices [I>2sigma(I)]	R1 = 0.0155, wR2 = 0.0303

Table S3: X-ray Parameters of **AuTri-6**

X-ray Structural Data and Crystal Refinement

	AuTri-6
Empirical Formula	$C_{28}H_{23}AuF_6N_2AsSb$
Molecular Weight (g/mol)	895.12
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, $P\bar{1}$
Unit Cell Dimensions (A), (o)	a = 11.2990(2) Å alpha = 80.746(1) b = 11.4091(2) Å beta = 72.122(1) c = 11.6754(2) Å gamma = 72.527(1)
Volume	1362.41(4) $Å^3$
Z	2
Absorption Coefficient	2.182 mm^{-1}
F(000)	844
Crystal Size (mm)	0.090 x 0.070 x 0.040
Theta Range	2.293 to 27.481
Completeness to Theta = 25.242	99.9%
F^2	1.078
Final R indices [I>2sigma(I)]	R1 = 0.0220, wR2 = 0.0354

Table S4: X-ray Parameters of **AuTri-8**

X-ray Structural Data and Crystal Refinement

	AuTri-8
Empirical Formula	$C_{40}H_{47}AuF_6N_2O_2PSb$
Molecular Weight (g/mol)	1051.48
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Monoclinic, $P2_1/n$
Unit Cell Dimensions (A), (o)	a = 14.6761(4) Å alpha = 90 b = 17.2897(4) Å beta = 108.264(1) c = 16.0080(3) Å gamma = 90
Volume	3857.32(16) $Å^3$
Z	4
Absorption Coefficient	4.608 mm^{-1}
F(000)	2064
Crystal Size (mm)	0.110 x 0.100 x 0.080
Theta Range	2.559 to 27.487
Completeness to Theta = 25.242	99.9%
F^2	1.057
Final R indices [I>2sigma(I)]	R1 = 0.0162, wR2 = 0.0331

Table S5: X-ray Parameters of **AuTri-9**

X-ray Structural Data and Crystal Refinement

	AuTri-9
Empirical Formula	$C_{50}H_{51}AuF_6N_2O_2PSb$
Molecular Weight (g/mol)	1175.61
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, $P\bar{1}$
Unit Cell Dimensions (A), (o)	a = 10.6778(2) Å alpha = 91.4611(7) b = 14.6519(3) Å beta = 104.7596(7) c = 15.1311(3) Å gamma = 102.3939(7)
Volume	2227.73(8) $Å^3$
Z	2
Absorption Coefficient	4.000 mm^{-1}
F(000)	1160
Crystal Size (mm)	0.150 x 0.110 x 0.080
Theta Range	2.733 to 27.526
Completeness to Theta = 25.242	99.9%
F^2	1.076
Final R indices [I>2sigma(I)]	R1 = 0.0183, wR2 = 0.0364

Table S6: X-ray Parameters of **AuTri-10**

X-ray Structural Data and Crystal Refinement

	AuTri-10
Empirical Formula	$C_{36}H_{43}AuF_6N_2O_2PSb$
Molecular Weight (g/mol)	999.41
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Monoclinic, $P2_1/n$
Unit Cell Dimensions (A), (o)	a = 14.4178(3) Å alpha = 90 b = 15.4506(3) Å beta = 92.175(1) c = 16.0747(3) Å gamma = 90
Volume	3578.28(12) $Å^3$
Z	4
Absorption Coefficient	4.961 mm^{-1}
F(000)	1952
Crystal Size (mm)	0.110 x 0.080 x 0.040
Theta Range	2.828 to 27.482
Completeness to Theta = 25.242	99.9%
F^2	1.058
Final R indices [I>2sigma(I)]	R1 = 0.0202, wR2 = 0.0342.

NMR Spectra of AuTri-1-10:

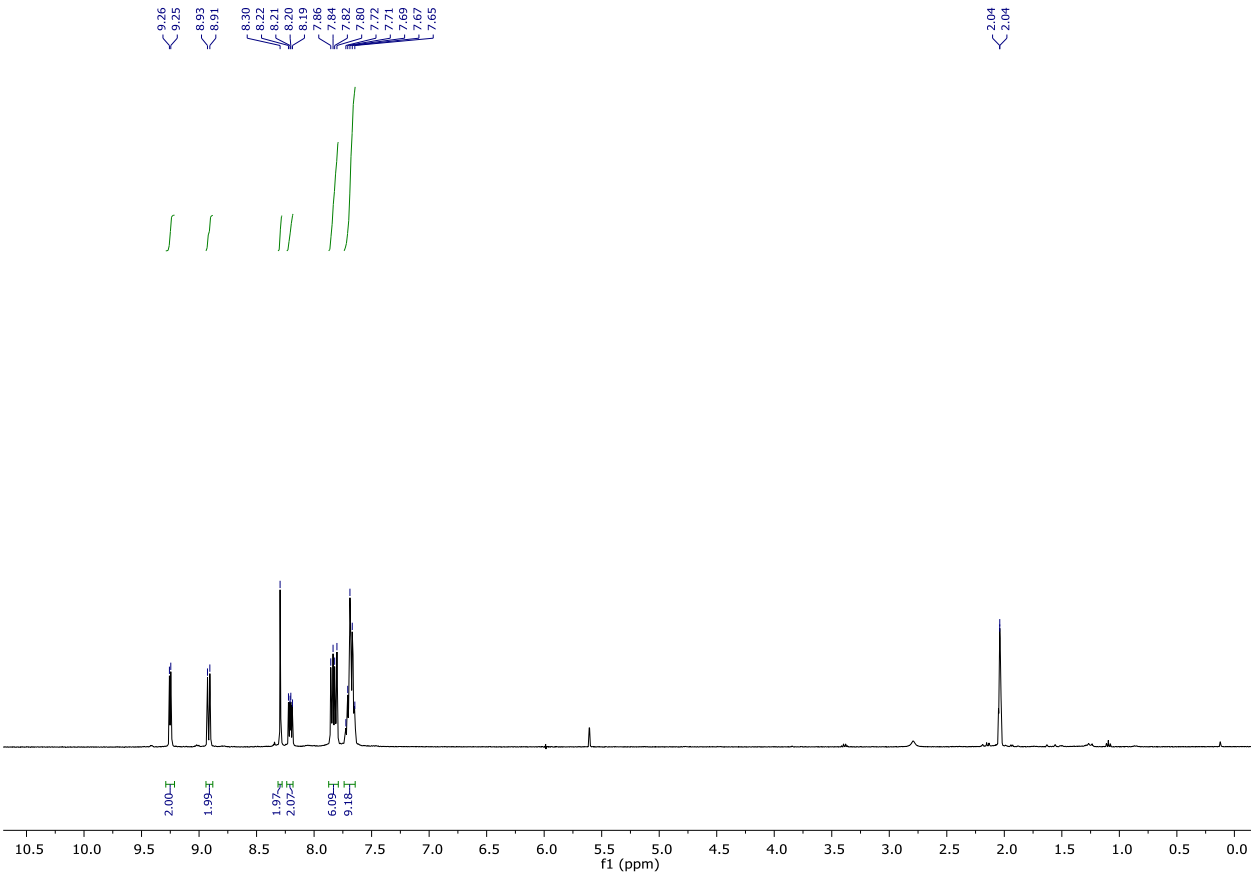


Figure S7. ^{1}H NMR spectrum of **AuTri-1** in CD_3CN at 298K.

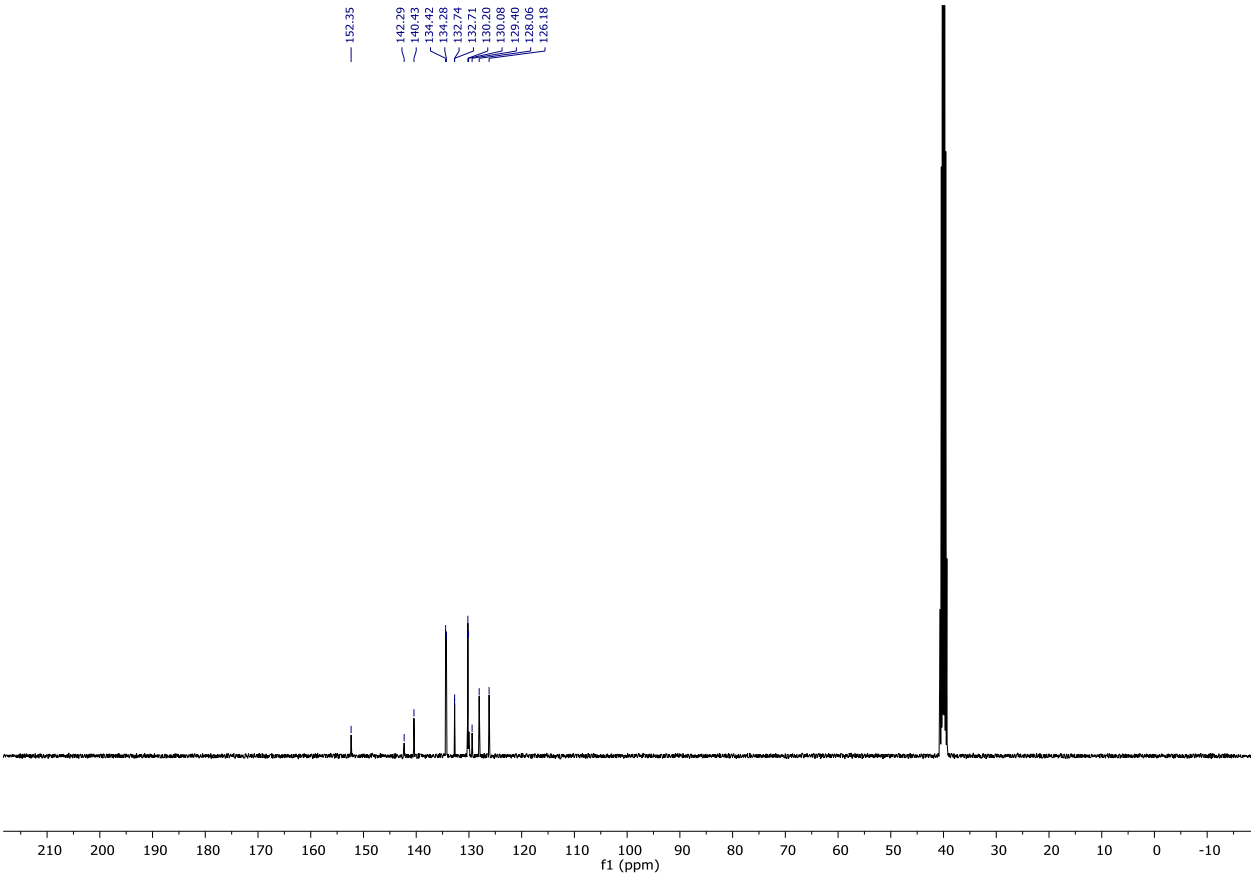


Figure S8. ^{13}C NMR spectrum of **AuTri-1** in DMSO-d_6 at 298K.

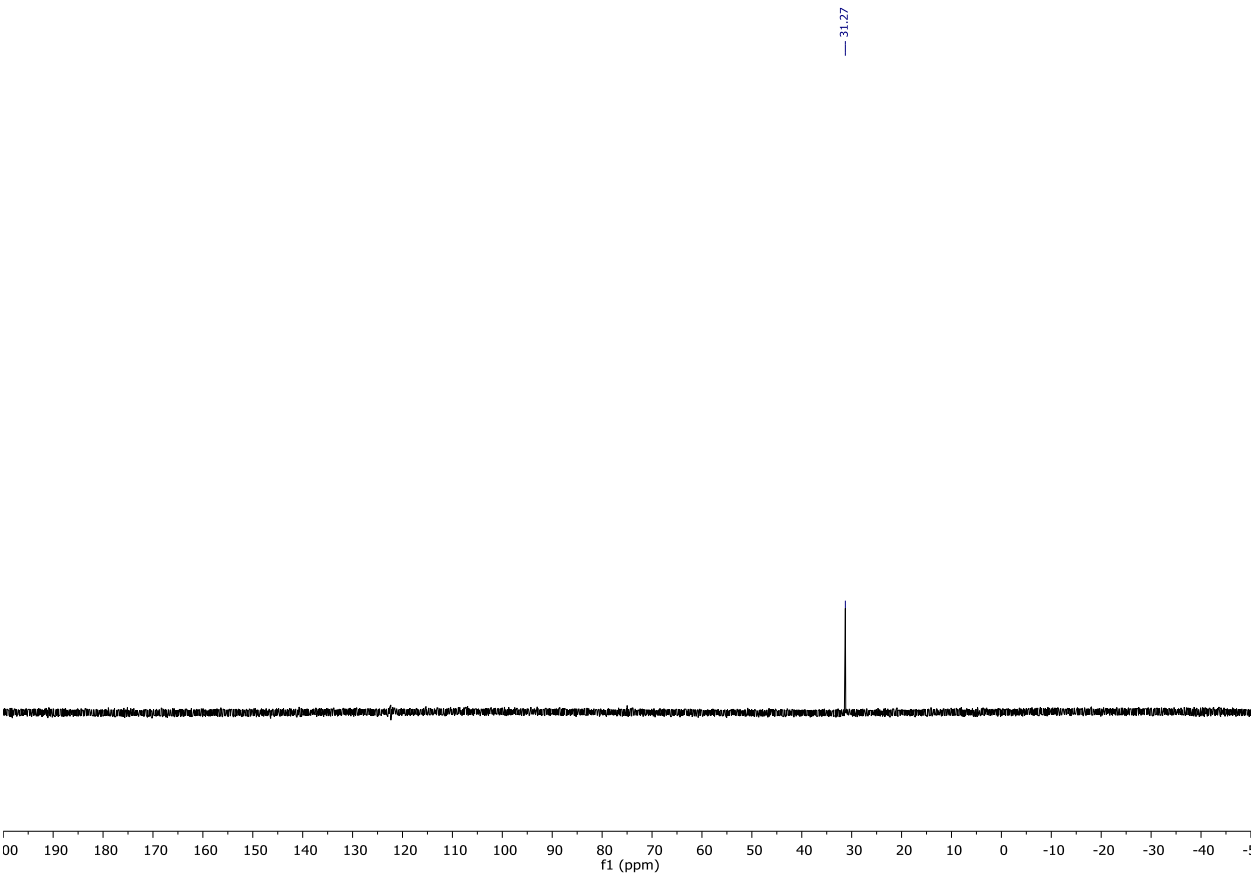


Figure S9. ^{31}P NMR spectrum of **AuTri-1** in DMSO-d_6 at 298K.

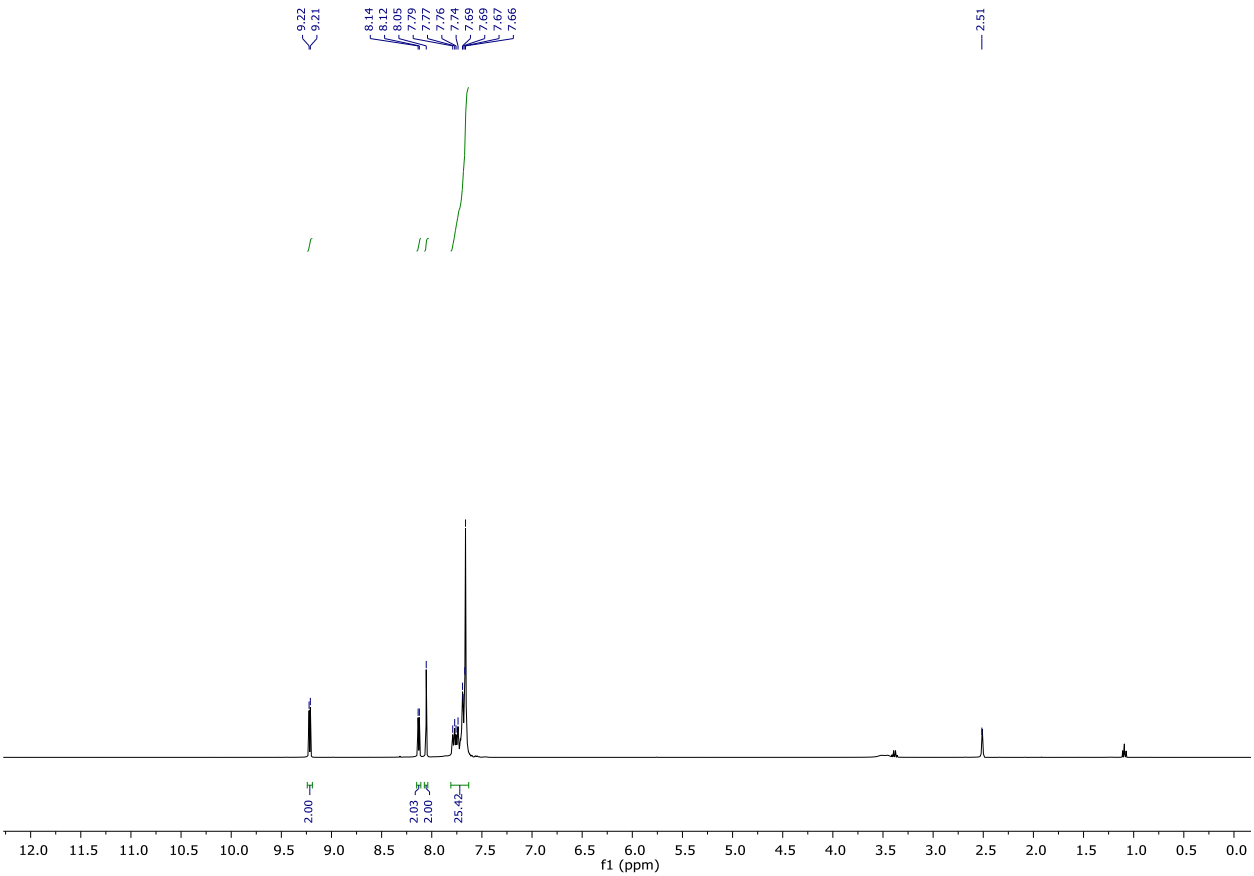


Figure S10. ^{1}H NMR spectrum of **AuTri-2** in DMSO-d_6 at 298K.

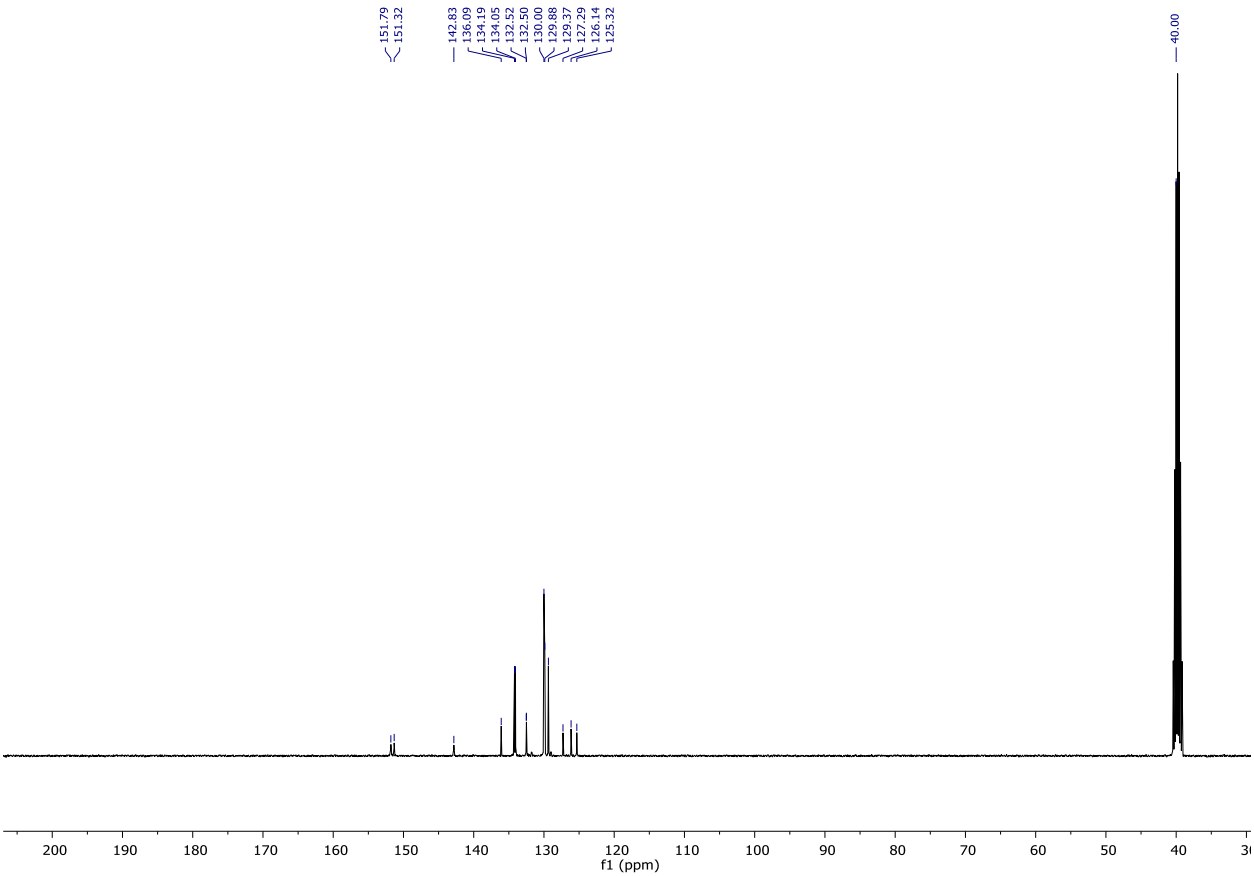


Figure S11. ^{13}C NMR spectrum of **AuTri-2** in DMSO-d_6 at 298K.

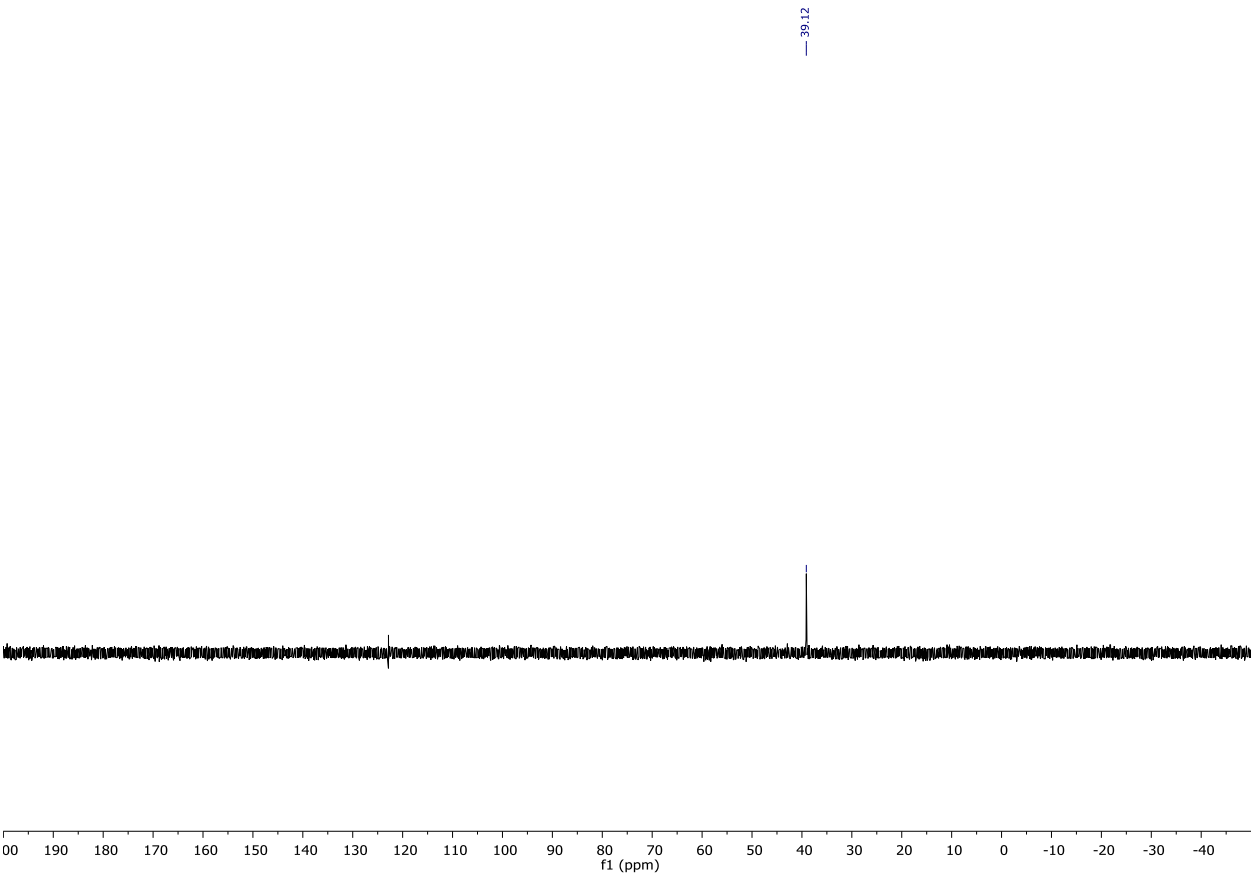


Figure S12. ^{31}P NMR spectrum of **AuTri-2** in DMSO-d_6 at 298K.

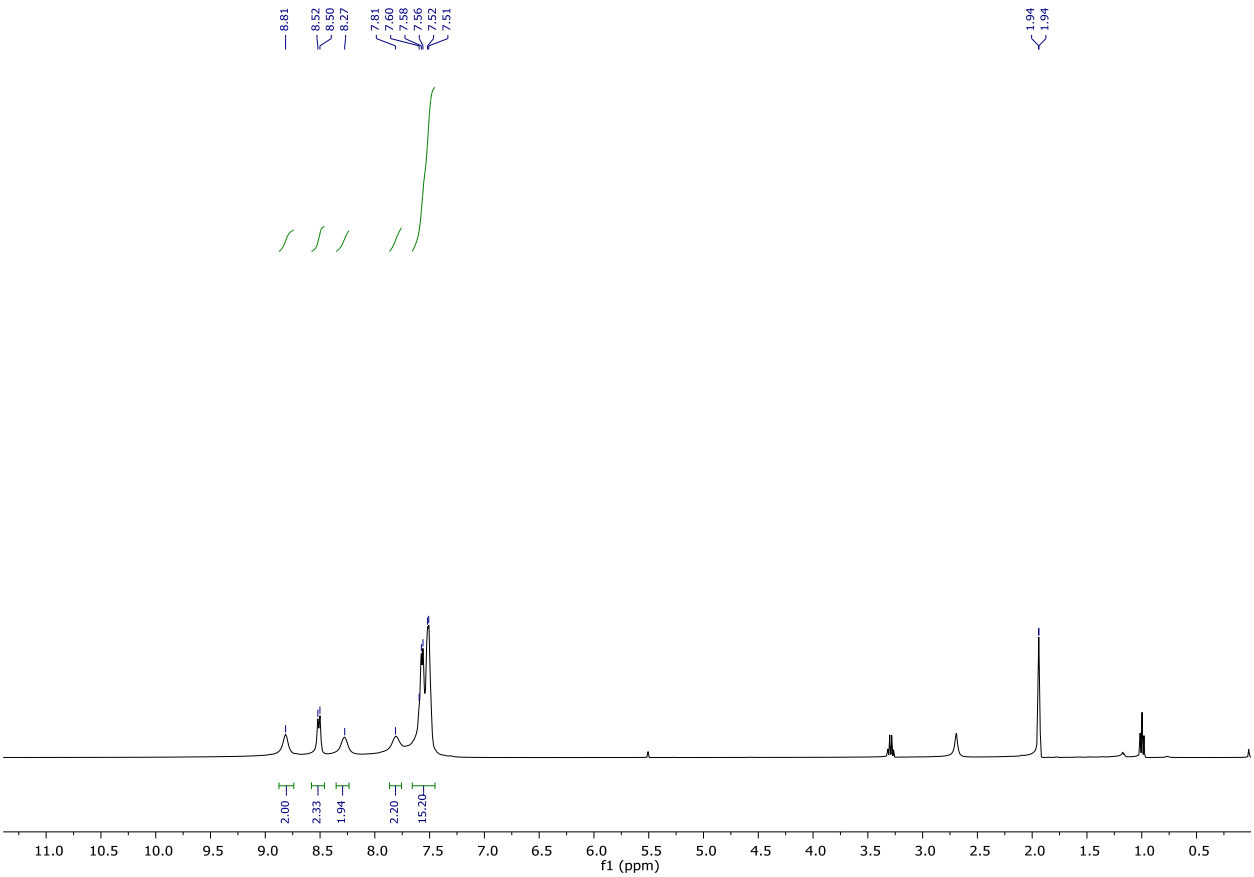


Figure S13. ^{1}H NMR spectrum of **AuTri-3** in CD_3CN at 298K.

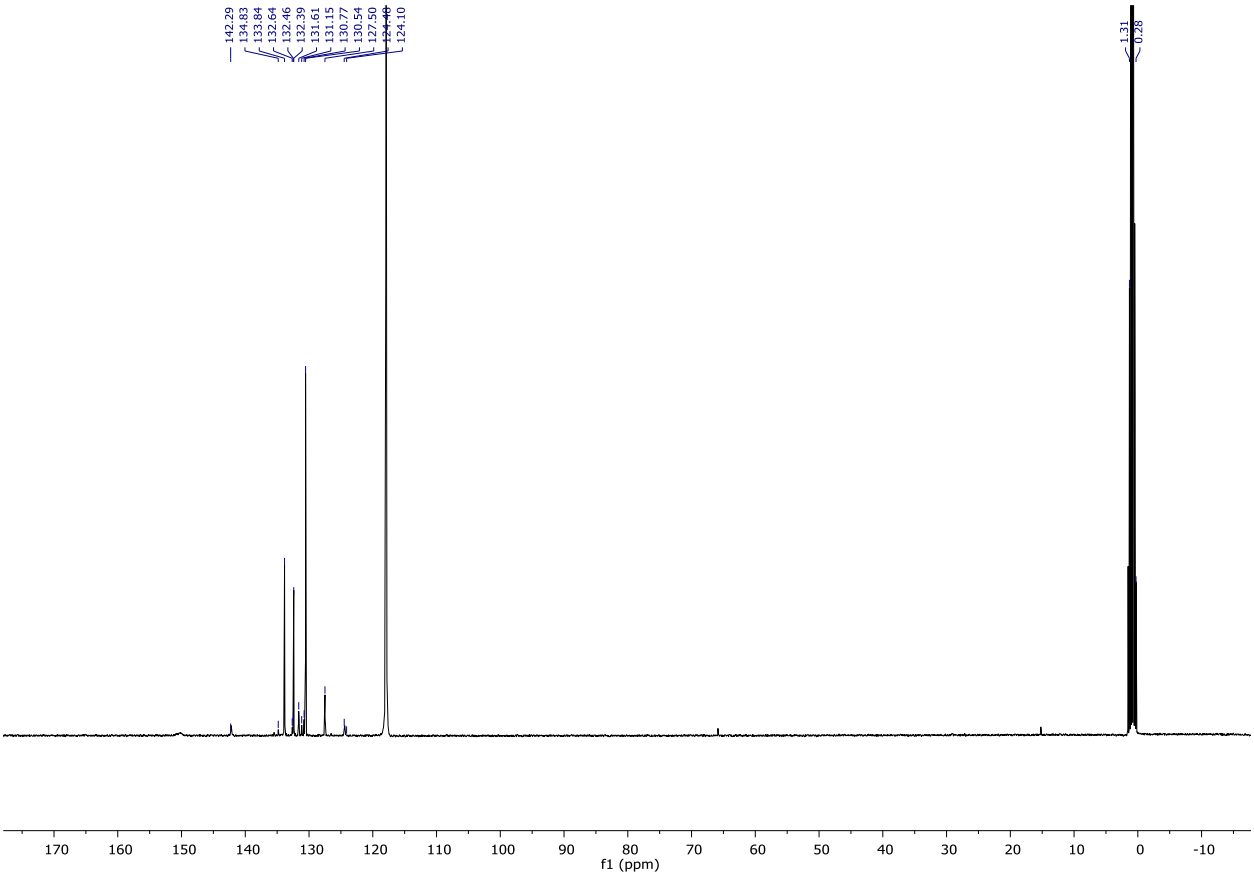


Figure S14. ^{13}C NMR spectrum of **AuTri-3** in DMSO-d_6 at 298K.

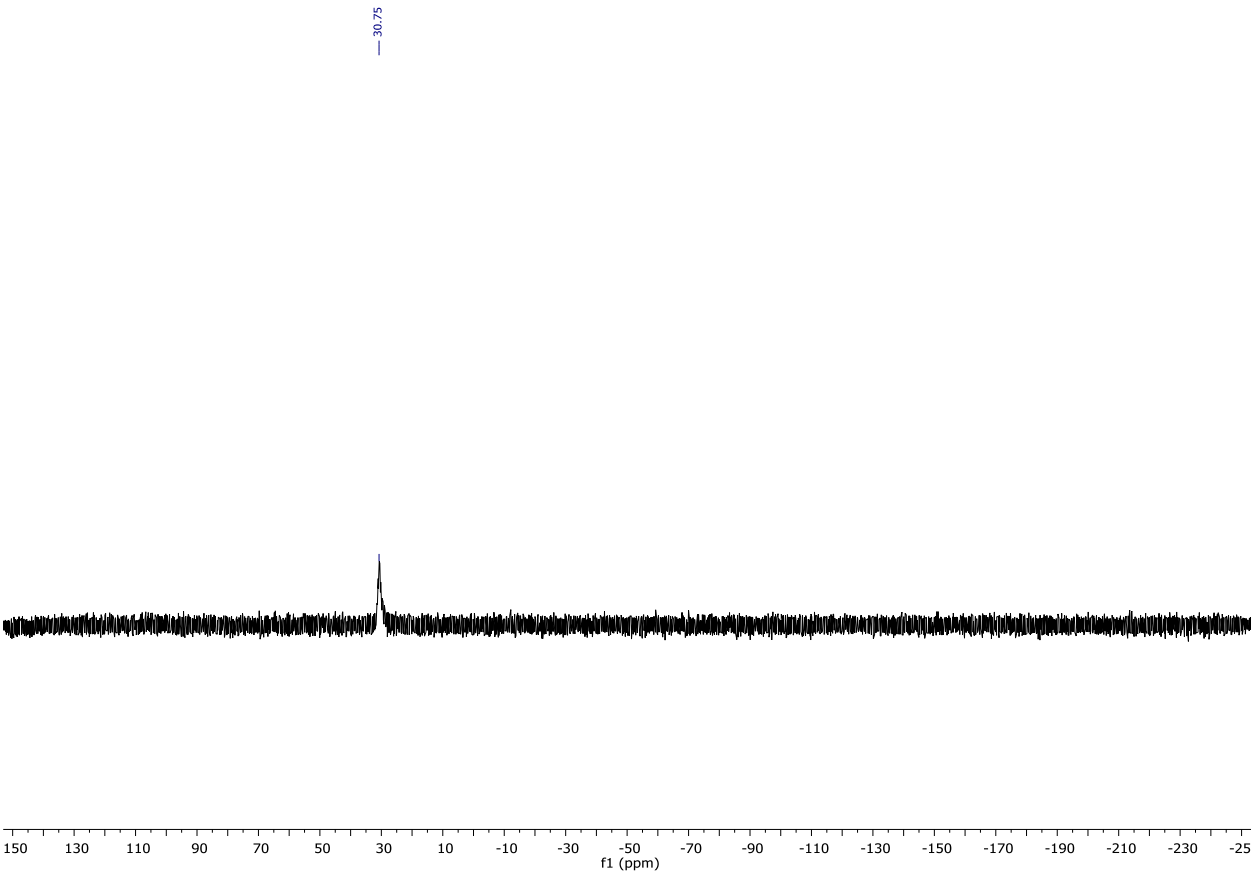


Figure S15. ^{31}P NMR spectrum of **AuTri-3** in DMSO-d_6 at 298K.

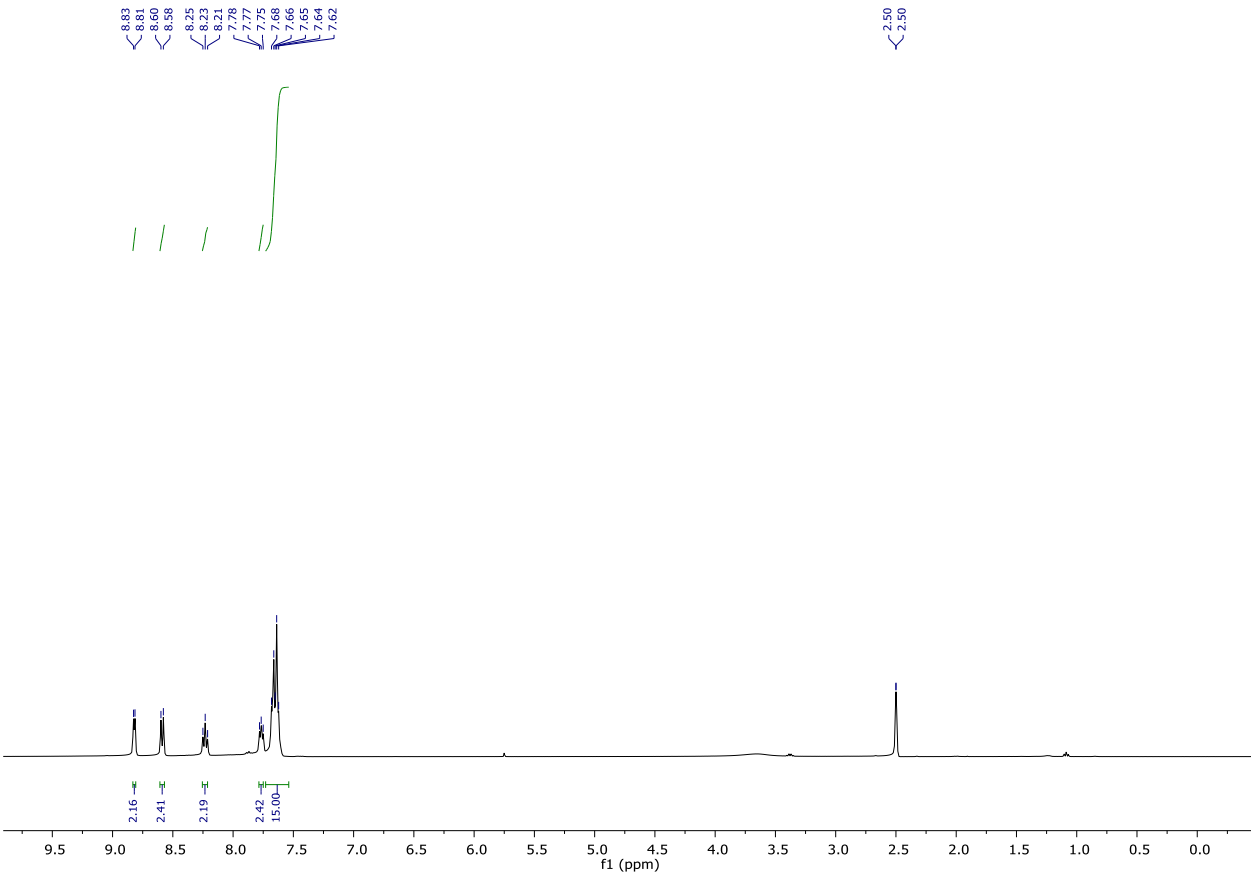


Figure S16. ^{1}H NMR spectrum of **AuTri-4** in DMSO-d_6 at 298K.

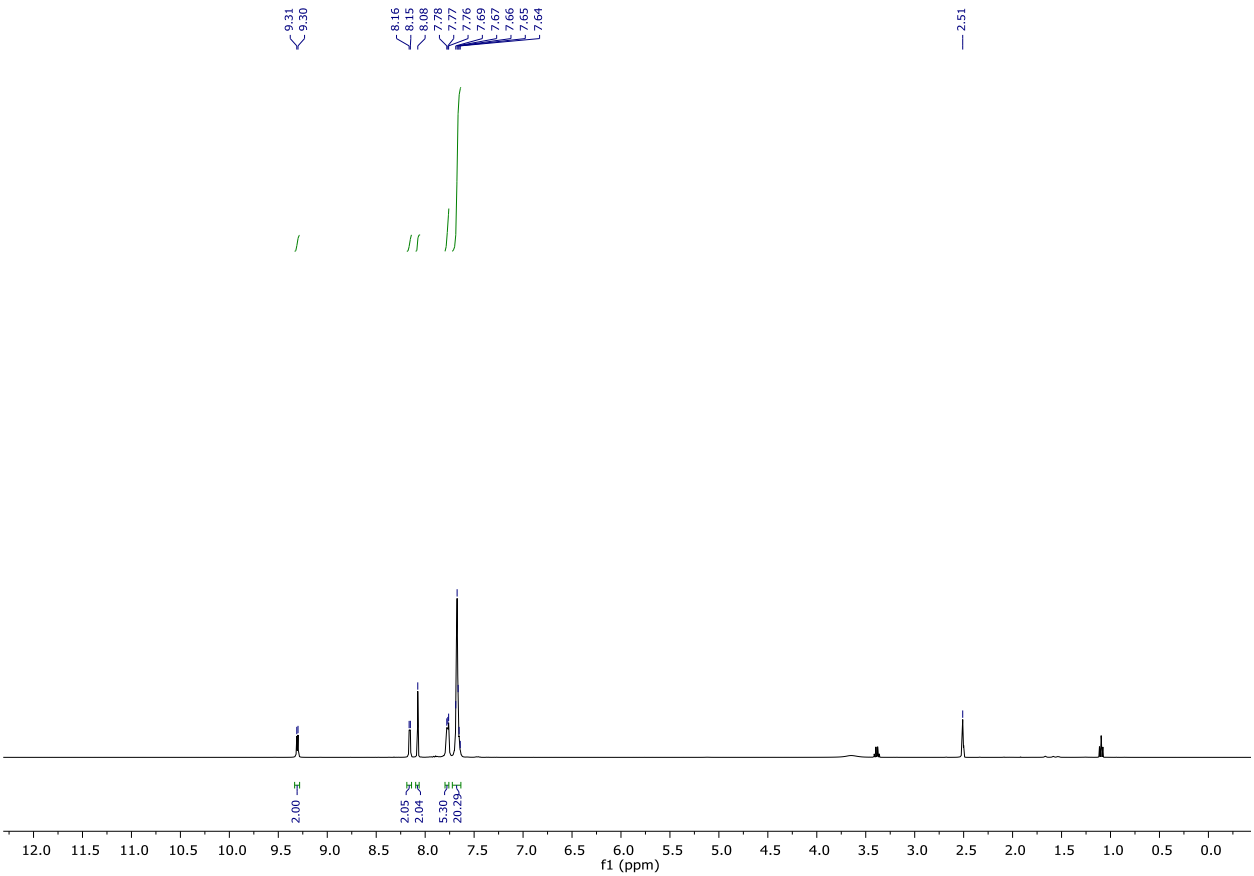


Figure S17. ^{1}H NMR spectrum of **AuTri-5** in DMSO-d_6 at 298K.

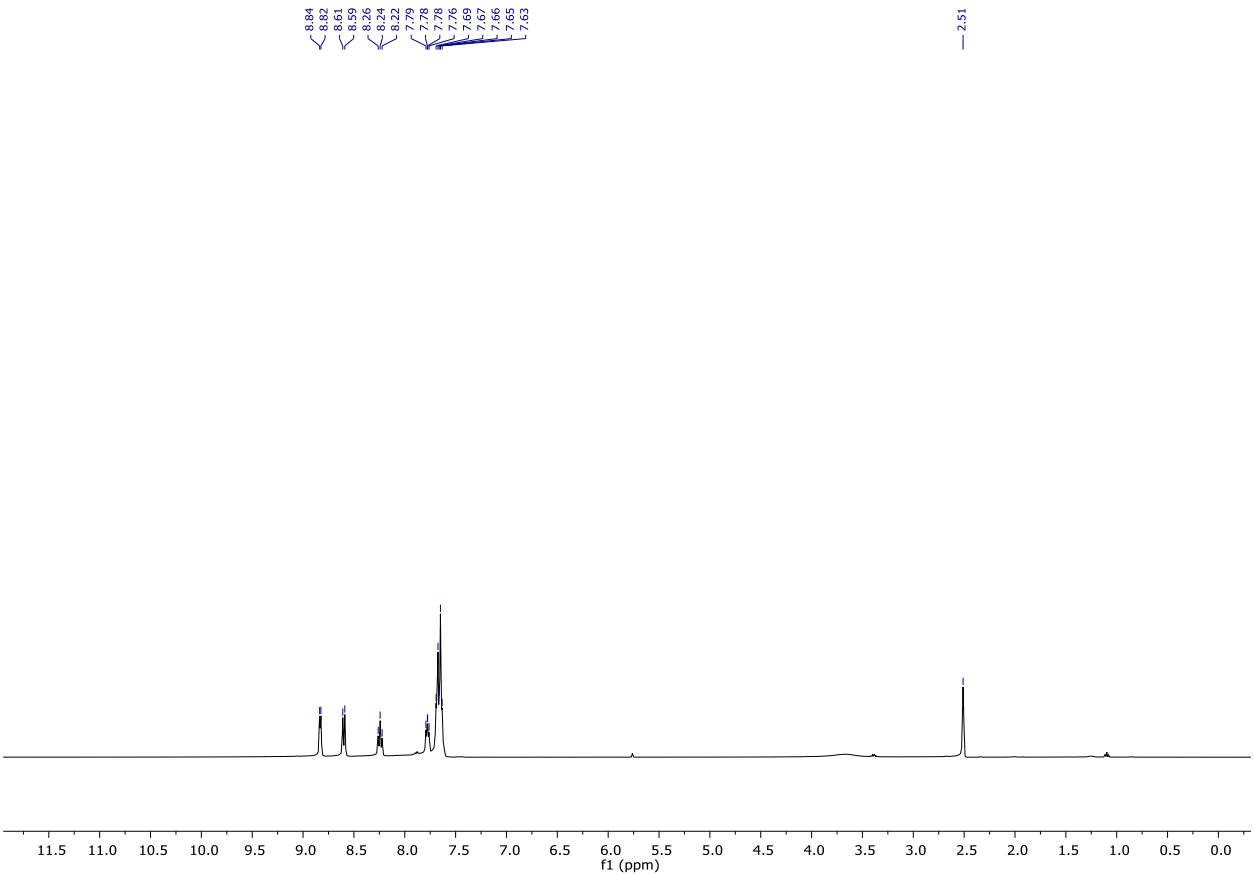


Figure S18. ^{1}H NMR spectrum of **AuTri-6** in DMSO-d_6 at 298K.

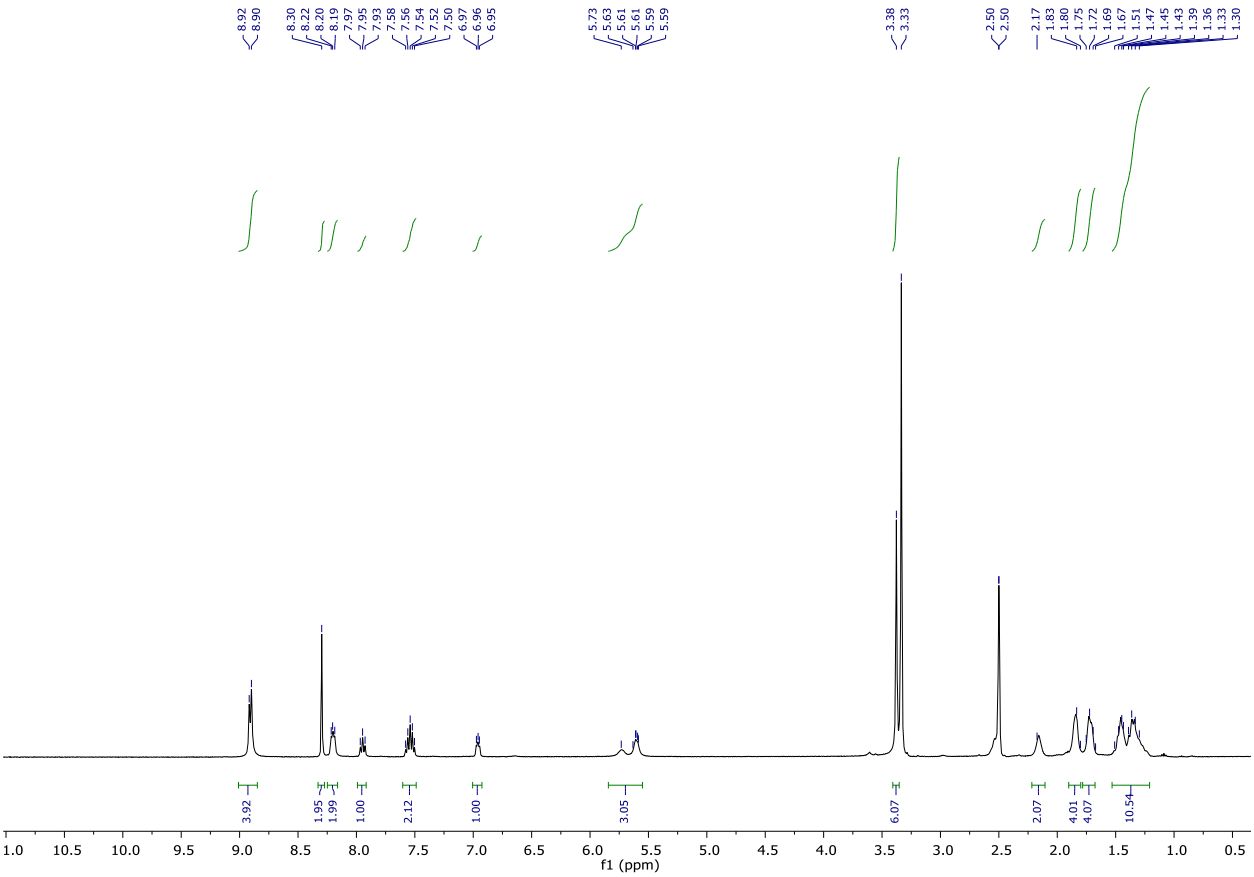


Figure S19. ^{1}H NMR spectrum of **AuTri-7** in DMSO-d_6 at 298K.

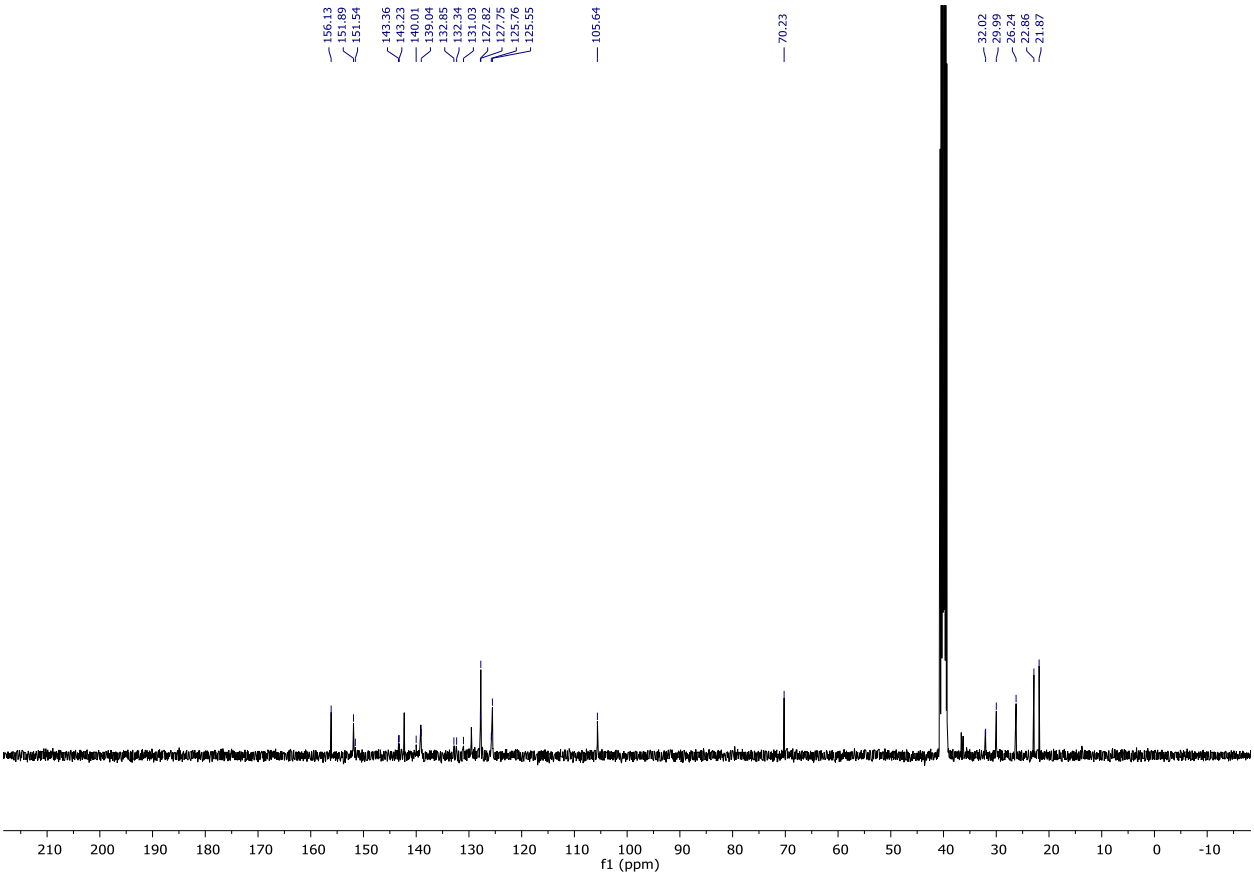


Figure S20. ^{13}C NMR spectrum of **AuTri-7** in DMSO-d_6 at 298K.

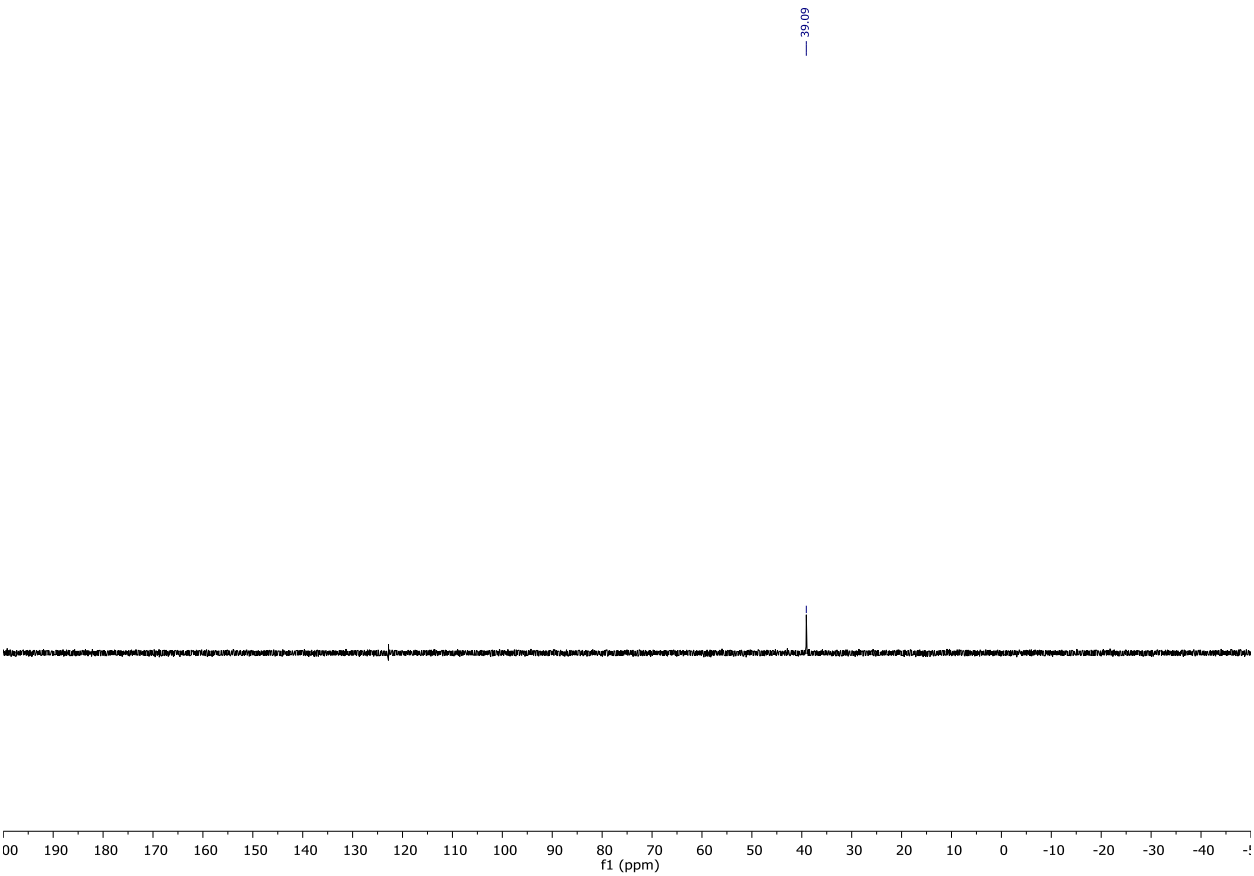


Figure S21. ^{31}P NMR spectrum of **AuTri-7** in DMSO-d_6 at 298K.

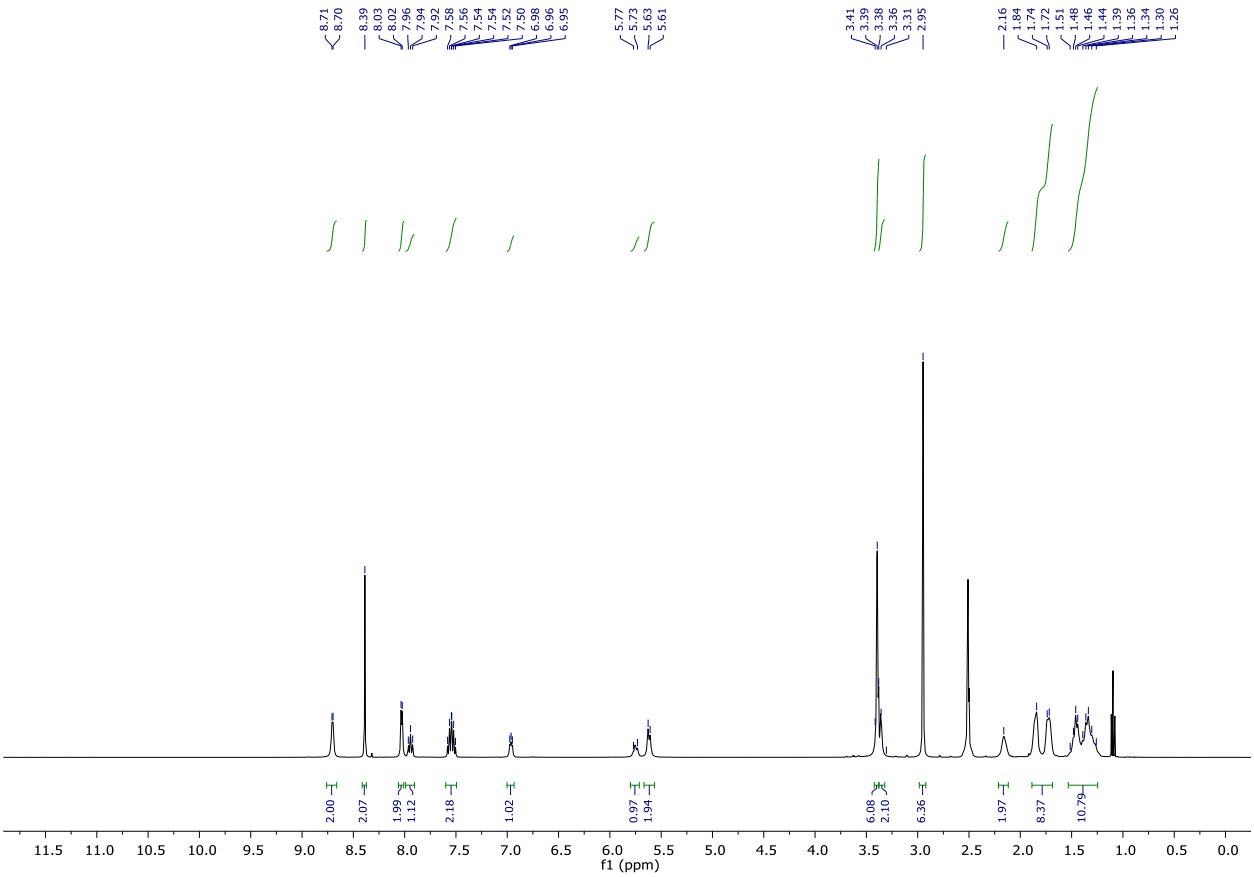


Figure S22. ^{1}H NMR spectrum of **AuTri-8** in DMSO-d_6 at 298K.

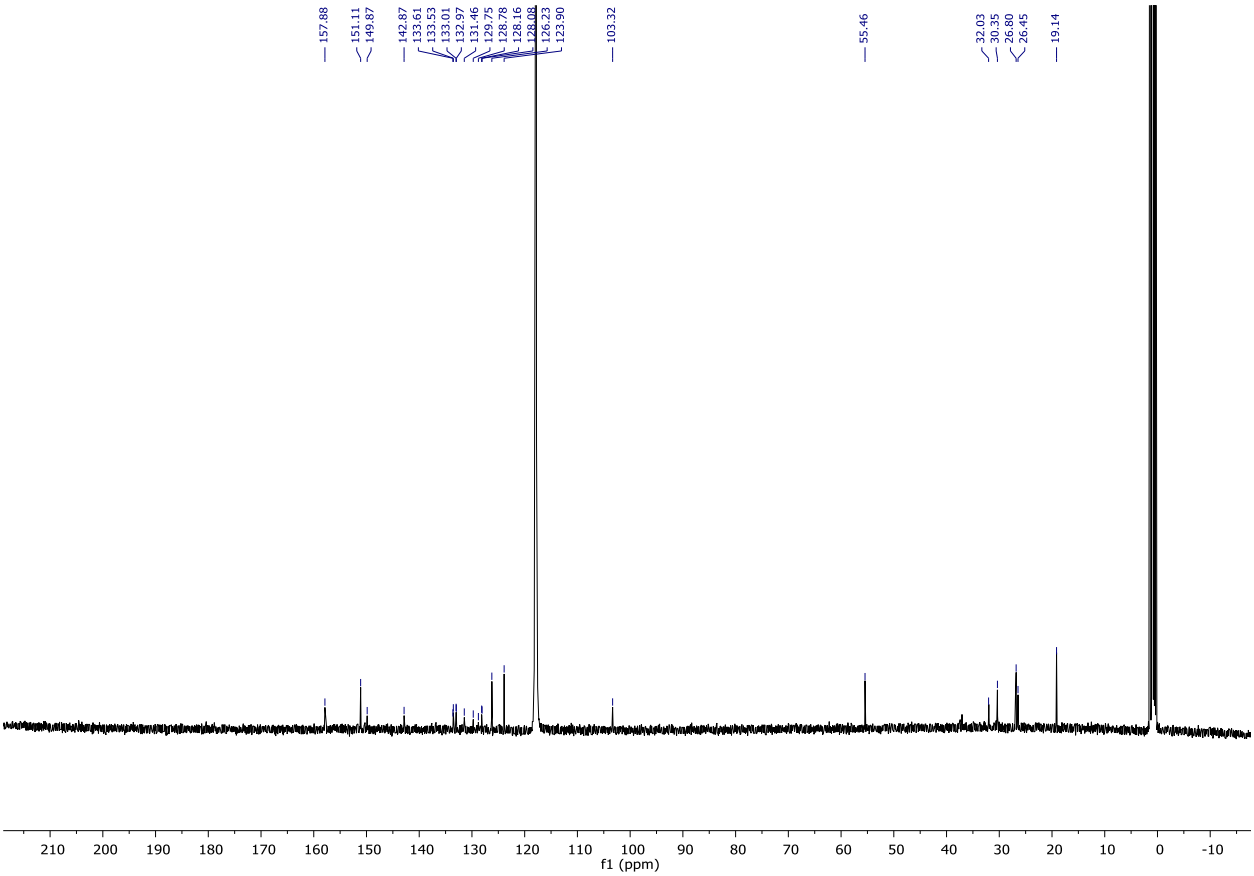


Figure S23. ^{13}C NMR spectrum of **AuTri-8** in DMSO-d_6 at 298K.

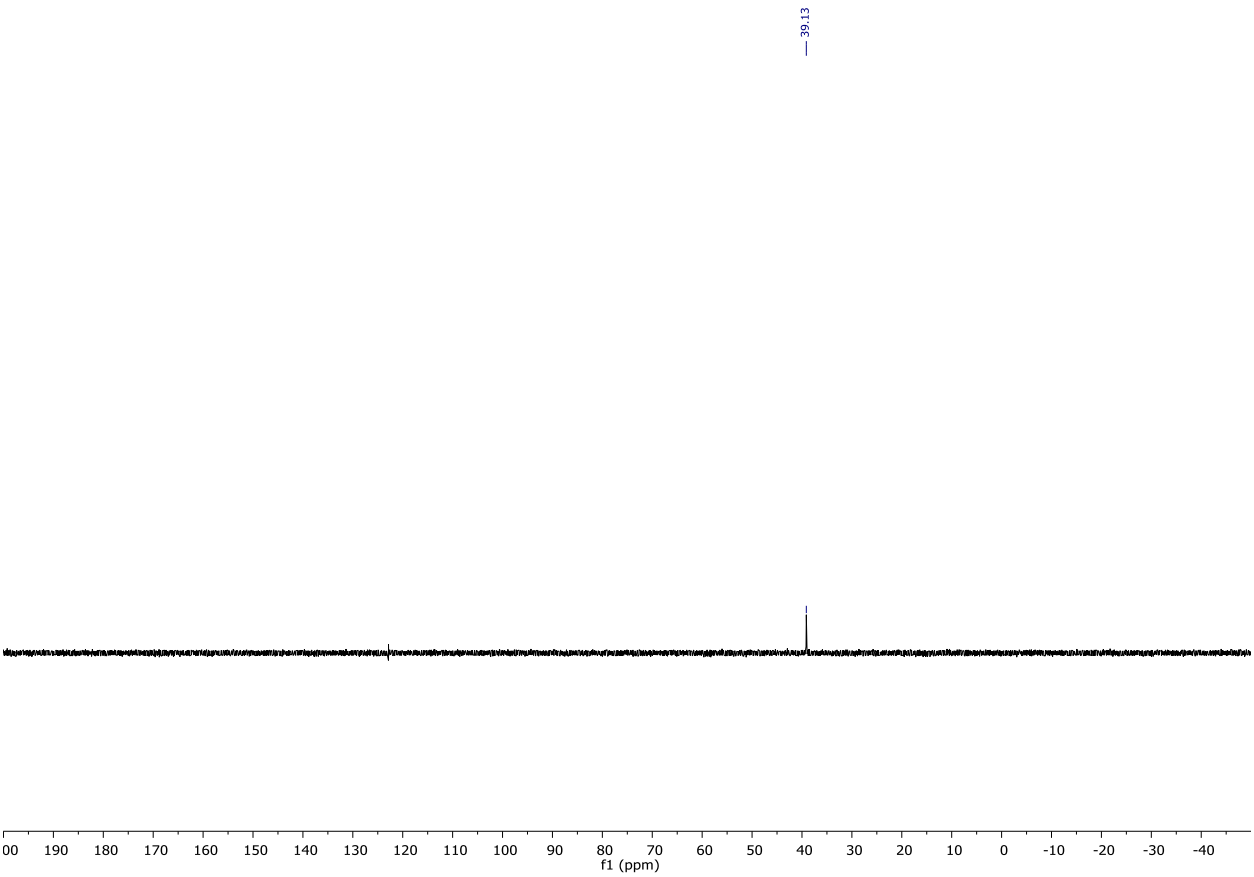


Figure S24. ^{31}P NMR spectrum of **AuTri-8** in DMSO-d_6 at 298K.

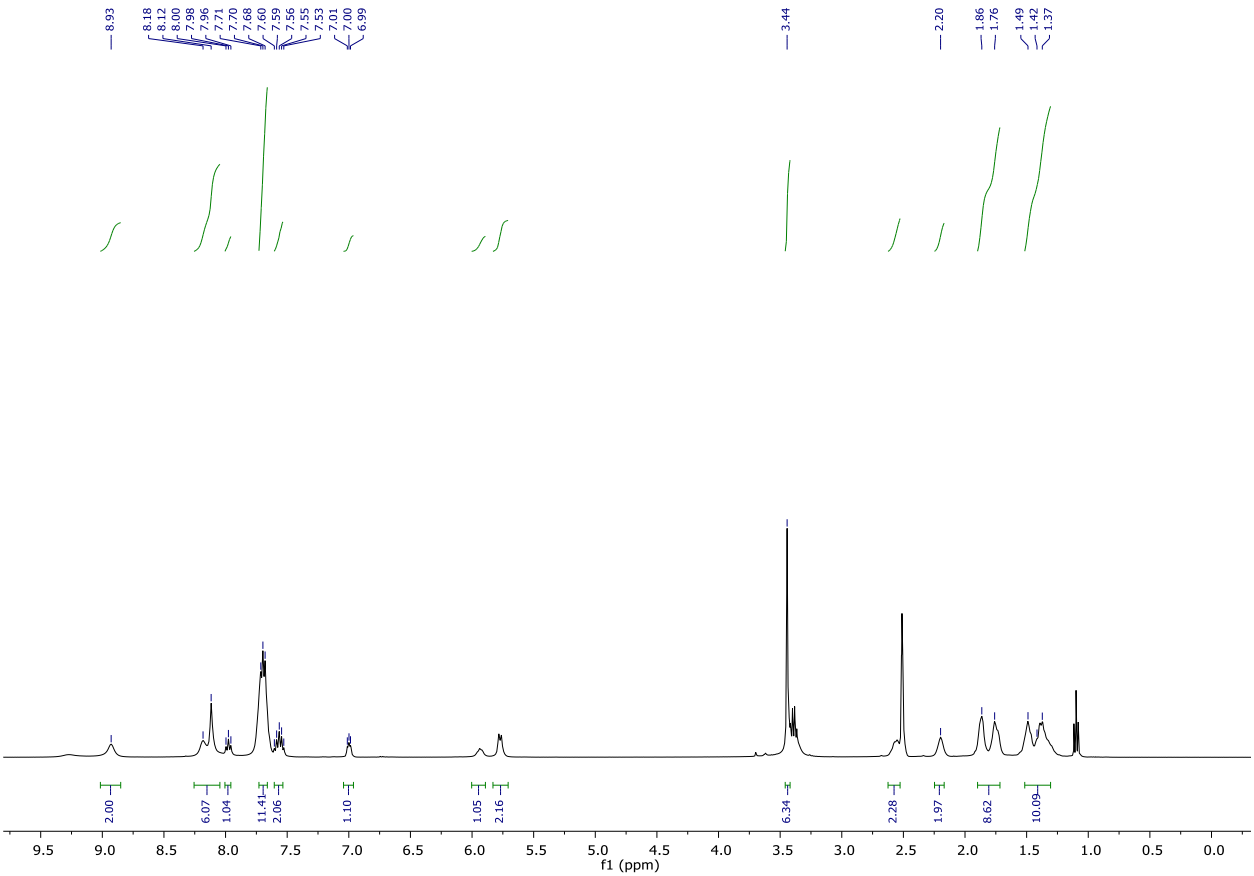


Figure S25. ^{1}H NMR spectrum of **AuTri-9** in DMSO-d_6 at 298K.

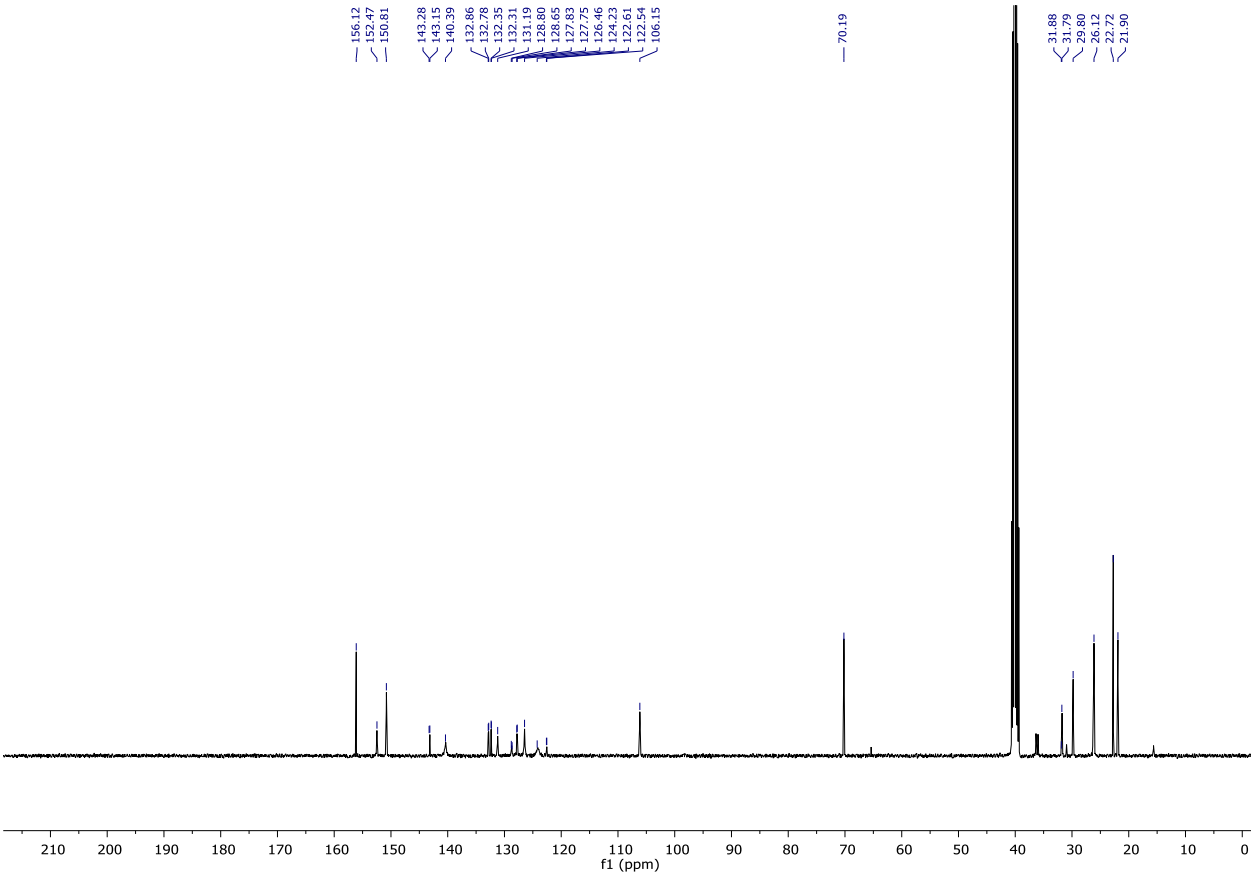


Figure S26. ^{13}C NMR spectrum of **AuTri-9** in DMSO-d_6 at 298K.

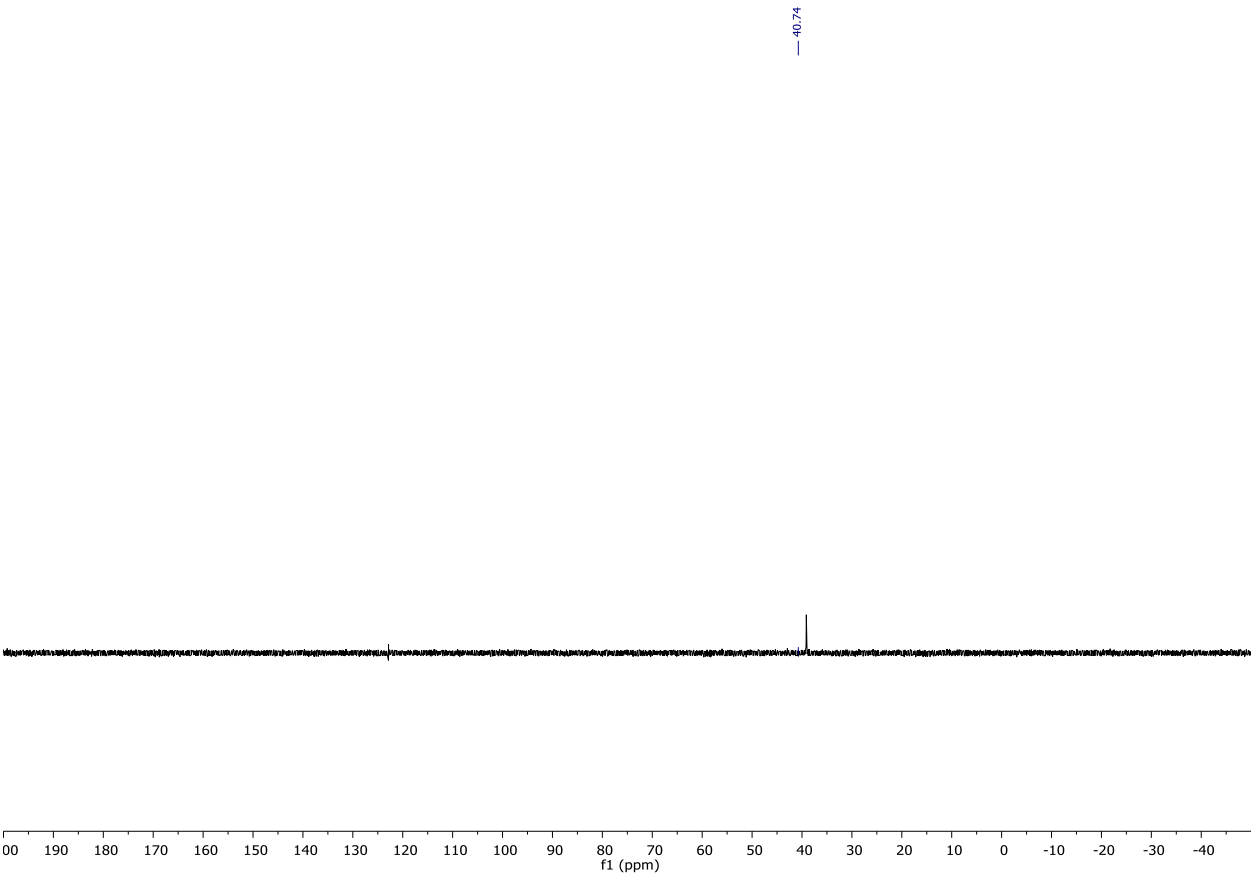


Figure S27. ^{31}P NMR spectrum of **AuTri-9** in DMSO-d_6 at 298K.

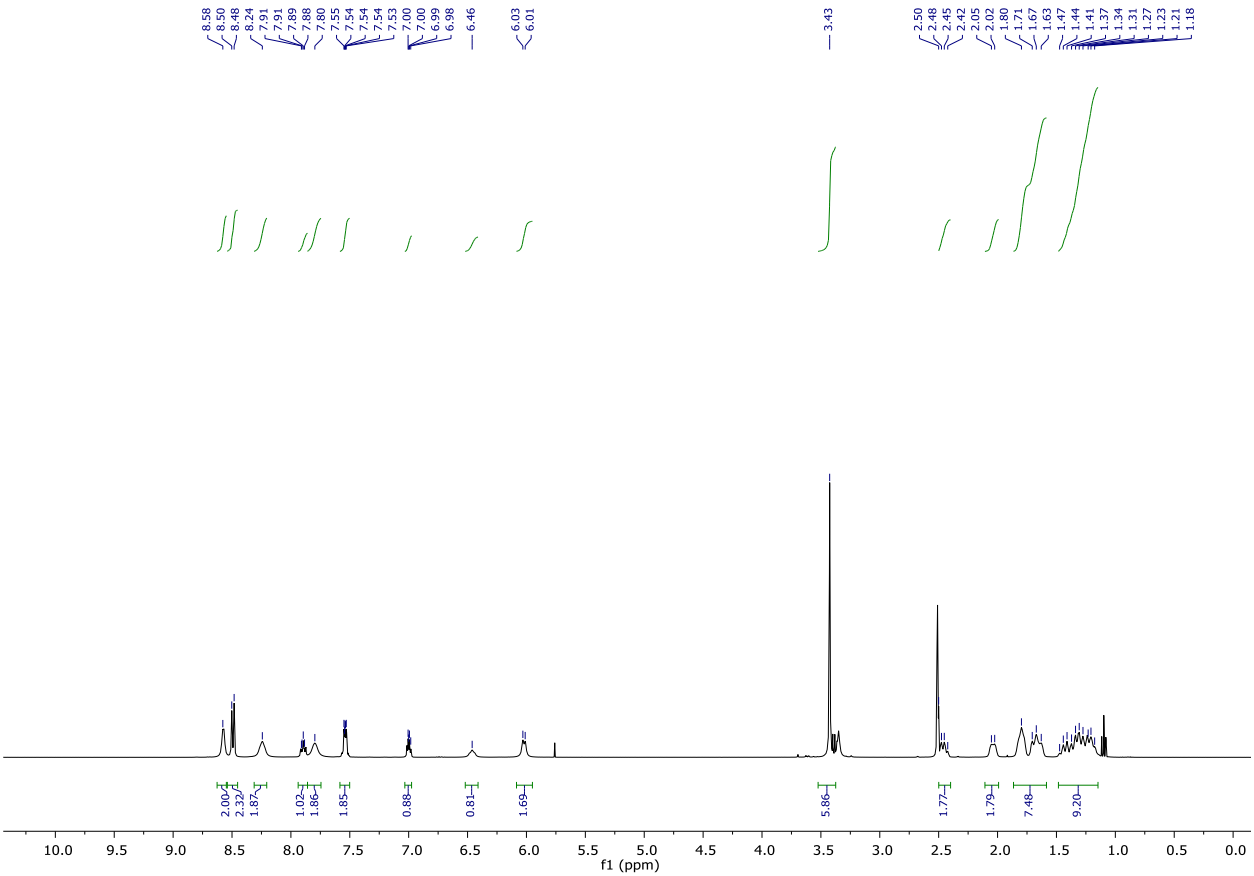


Figure S28. ^{1}H NMR spectrum of **AuTri-10** in DMSO-d_6 at 298K.

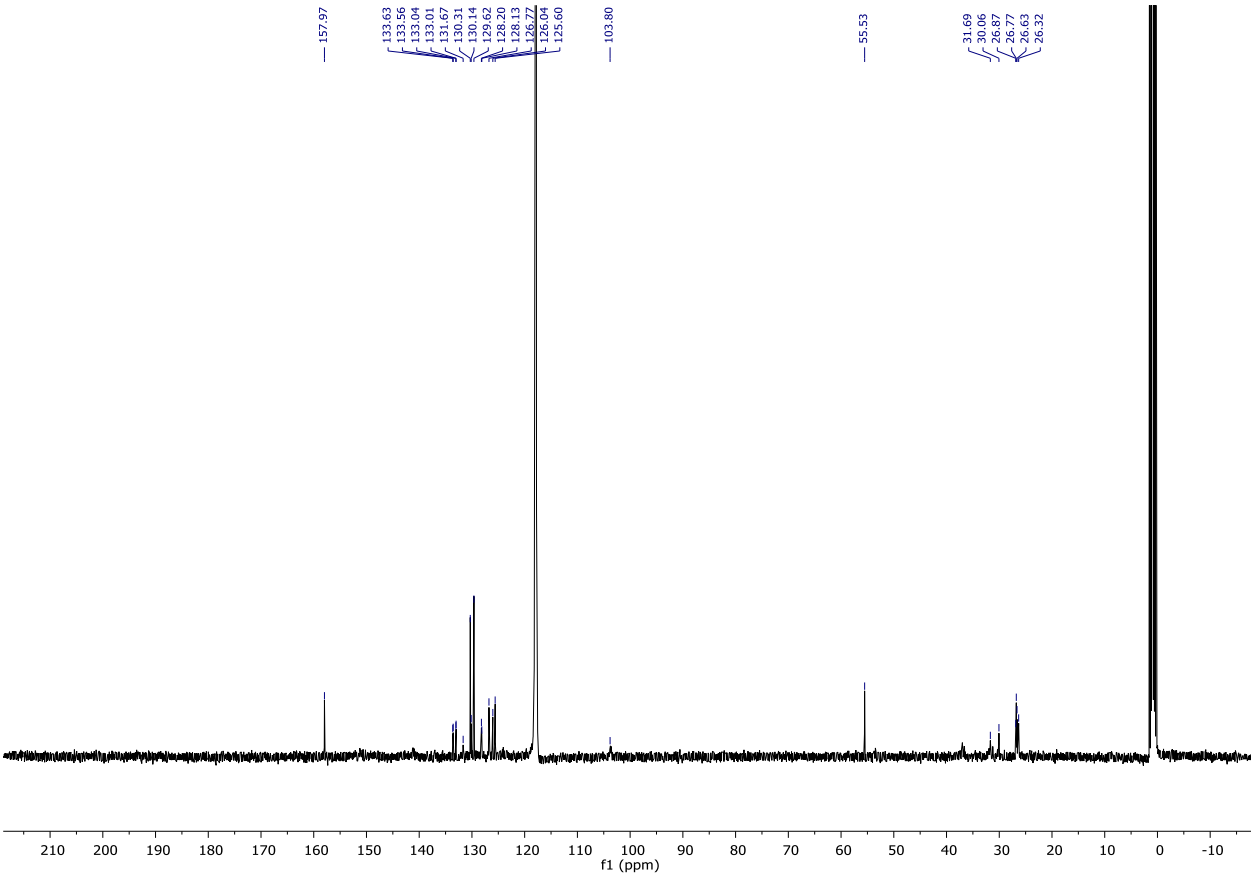


Figure S29. ^{13}C NMR spectrum of **AuTri-10** in DMSO-d_6 at 298K.

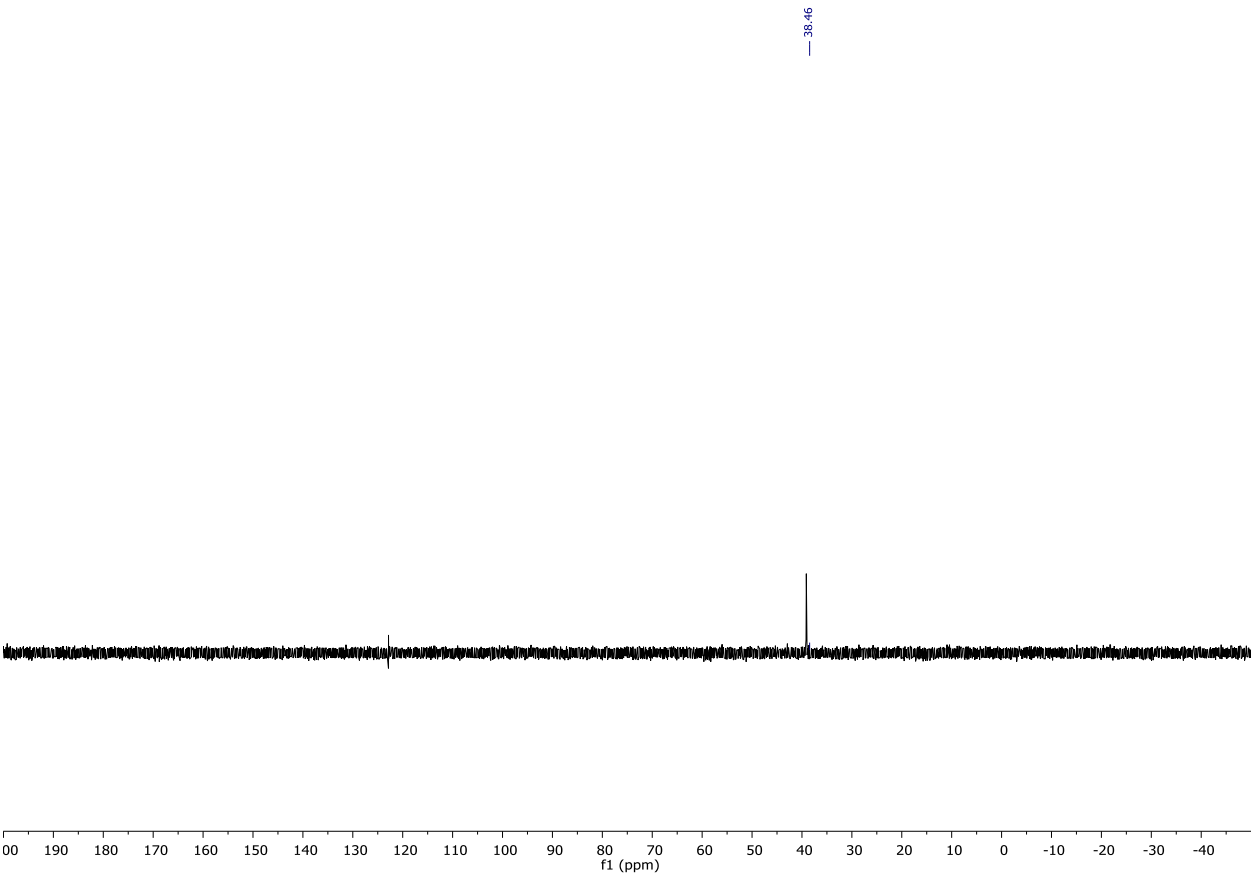


Figure S30. ^{31}P NMR spectrum of **AuTri-10** in DMSO-d_6 at 298K.

HRMS:

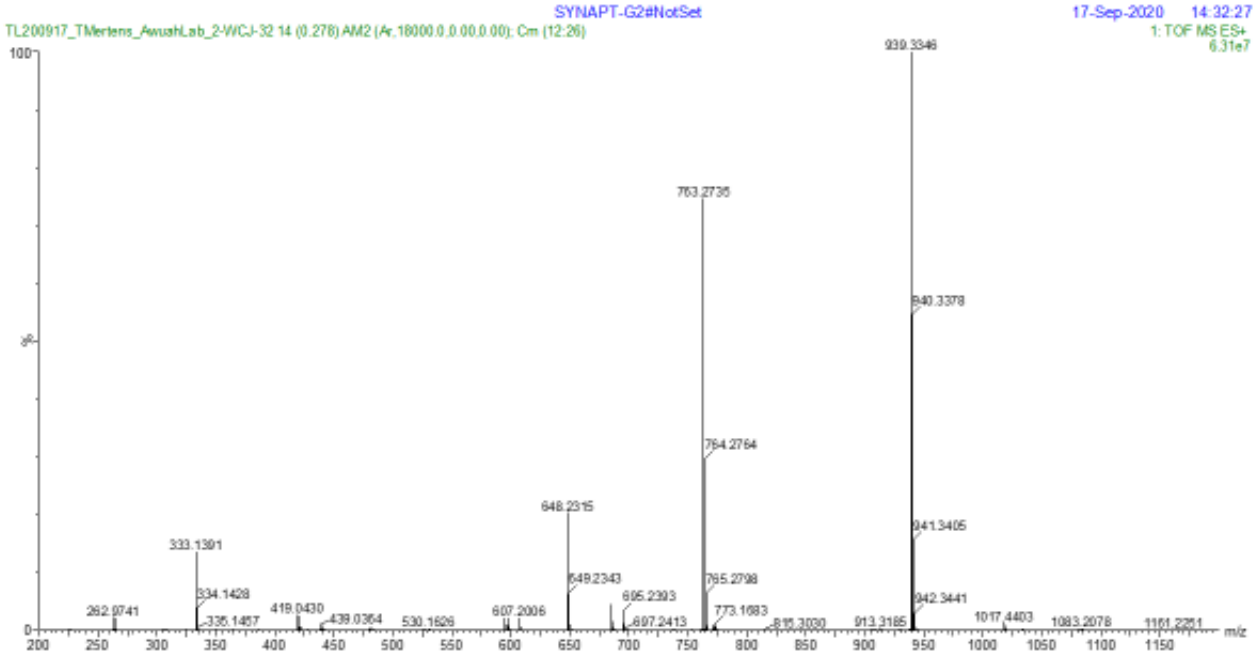


Figure S31. Full spectrum of HRMS of **AuTri-9 –** TOF MS ES$^+$

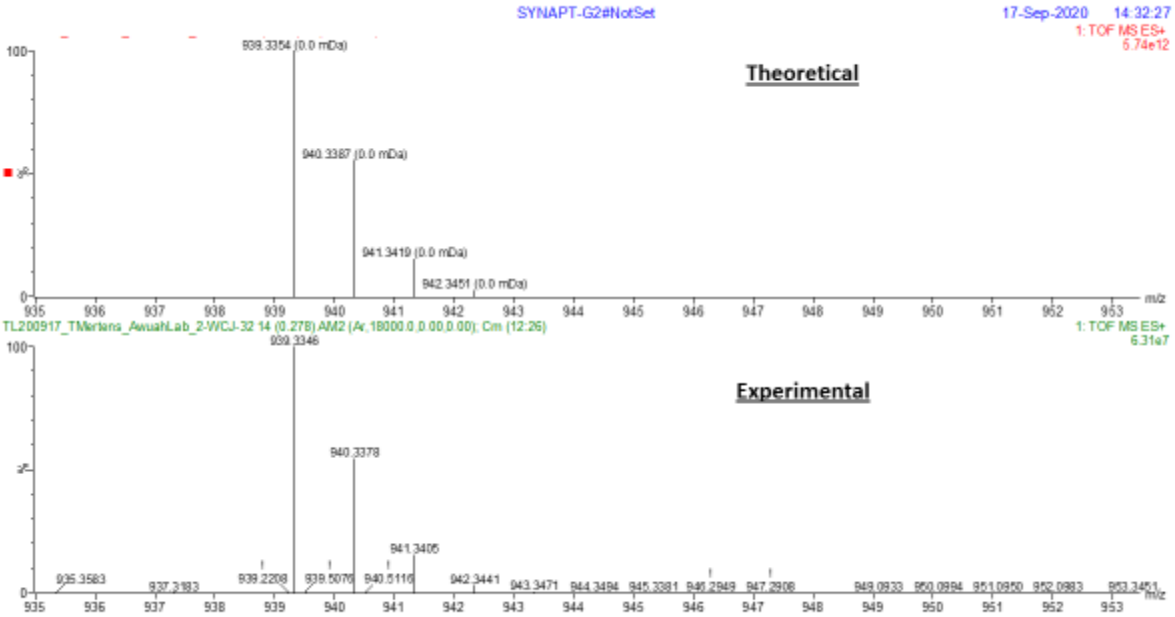


Figure S32. Experimental/Theoretical comparison of HRMS of **AuTri-9 –** TOF MS ES$^+$

HPLC Trace of AuTri-1-3 and AuTri-7-10:

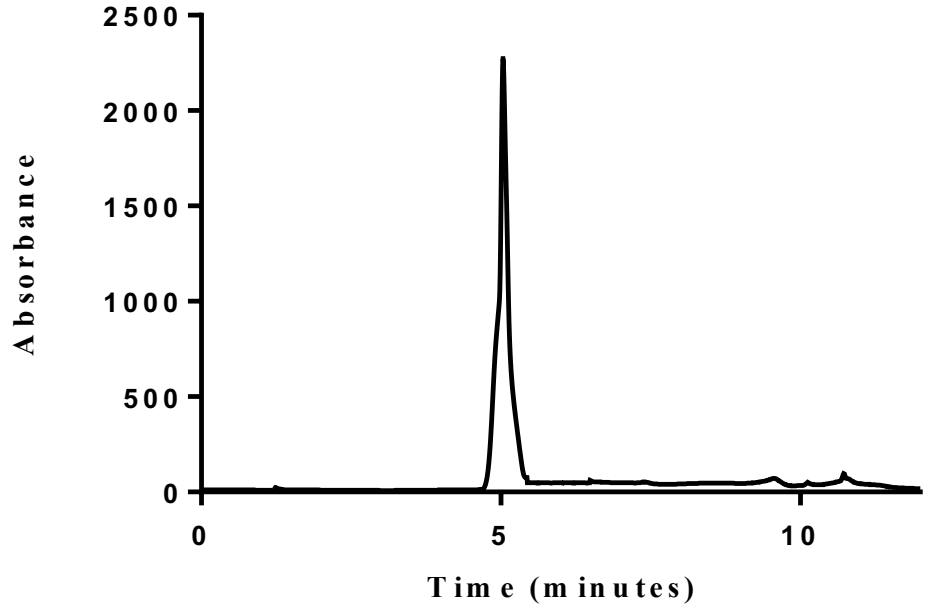


Figure S33. HPLC chromatogram of **AuTri-1,** (λ = 280 nm).

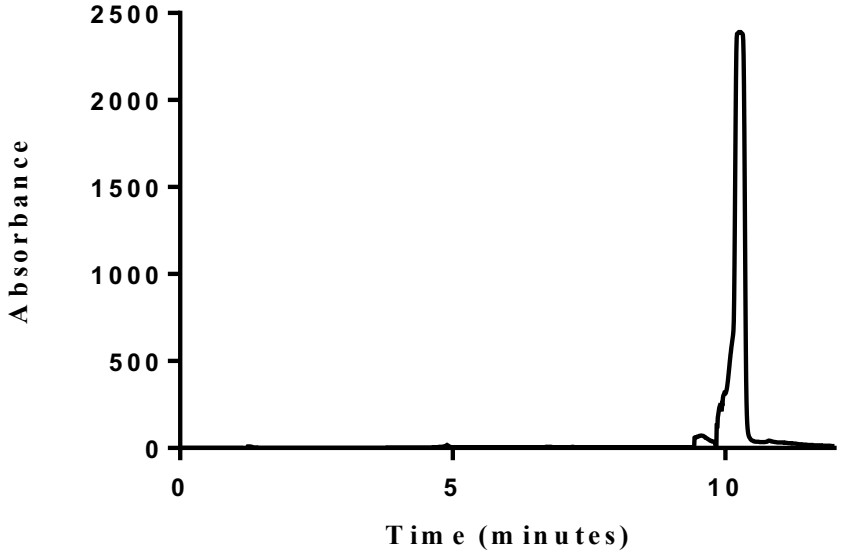


Figure S34. HPLC chromatogram of **AuTri-2,** (λ = 280 nm).

*Note: **AuTri-4-6** are unstable in solution due to the weak Au-As bond. Upon injection into HPLC the complexes undergo rapid reduction.

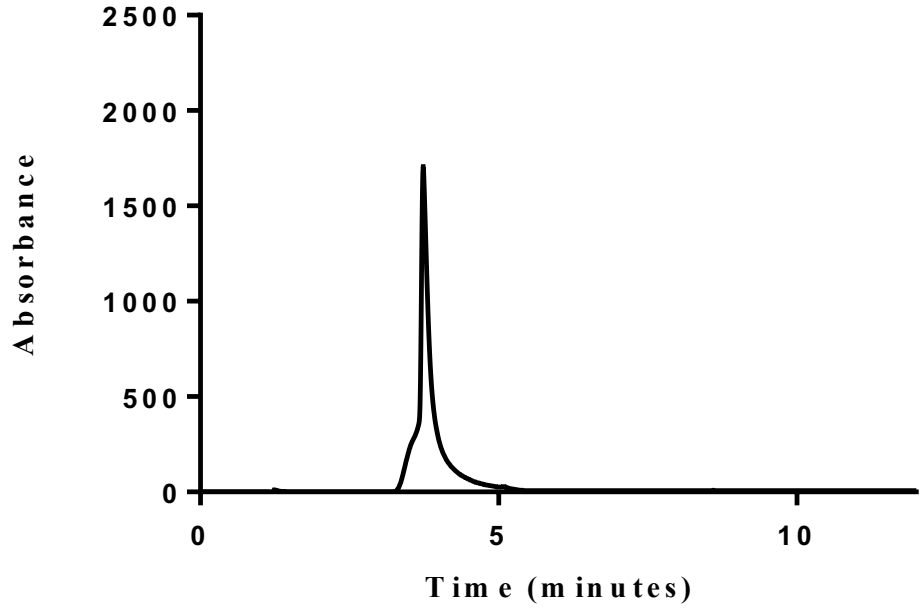


Figure S35. HPLC chromatogram of **AuTri-3,** (λ = 280 nm).

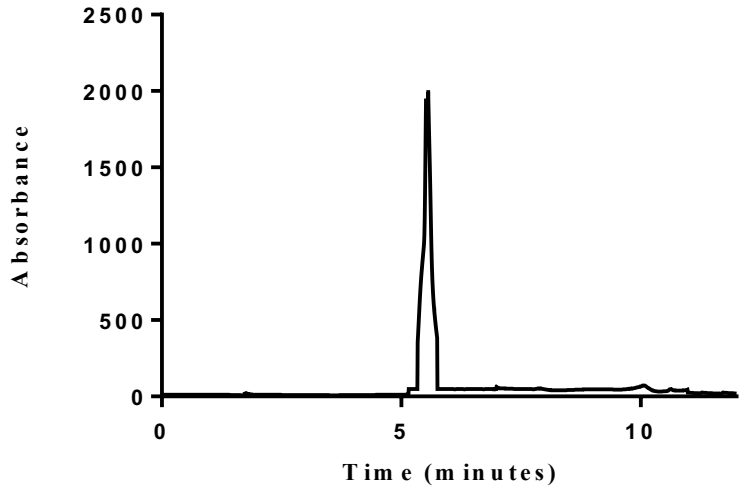


Figure S36. HPLC chromatogram of **AuTri-7,** (λ = 280 nm).

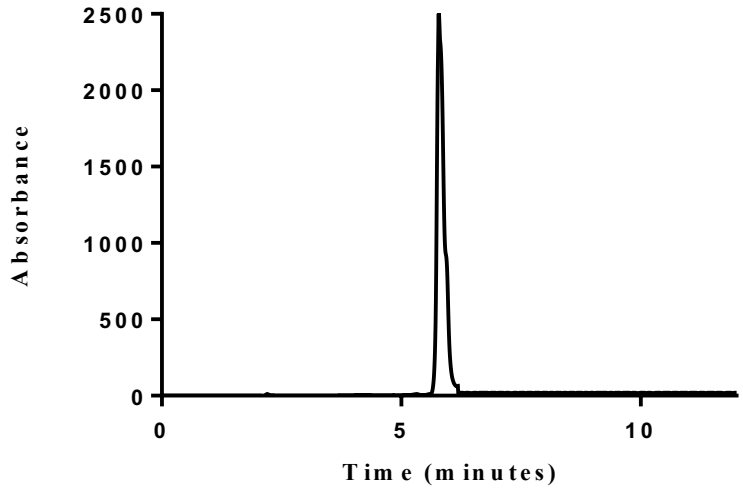


Figure S37. HPLC chromatogram of **AuTri-8,** (λ = 280 nm).

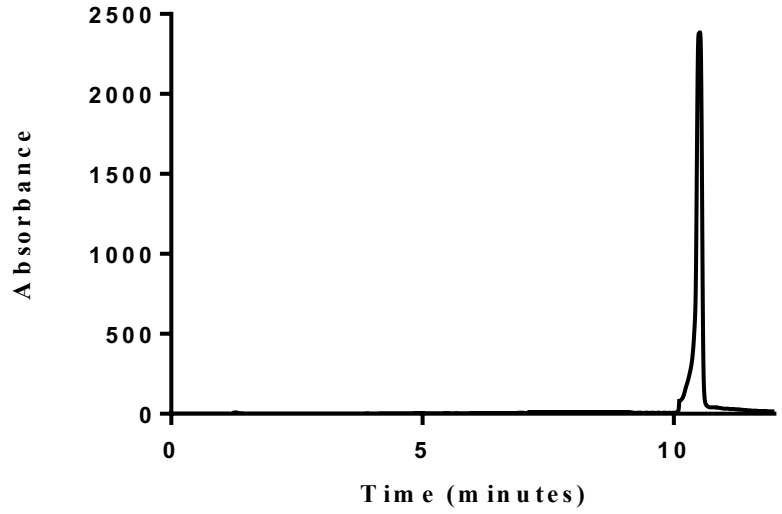


Figure S38. HPLC chromatogram of **AuTri-9,** (λ = 280 nm).

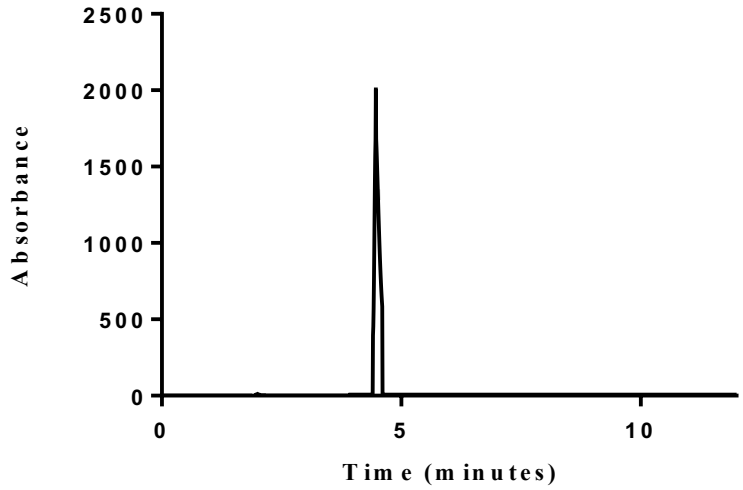


Figure S39. HPLC chromatogram of **AuTri-10,** (λ = 280 nm).

Cell Viability Sigmoidal Plot of AuTri-1-10 in MDA-MB-231:

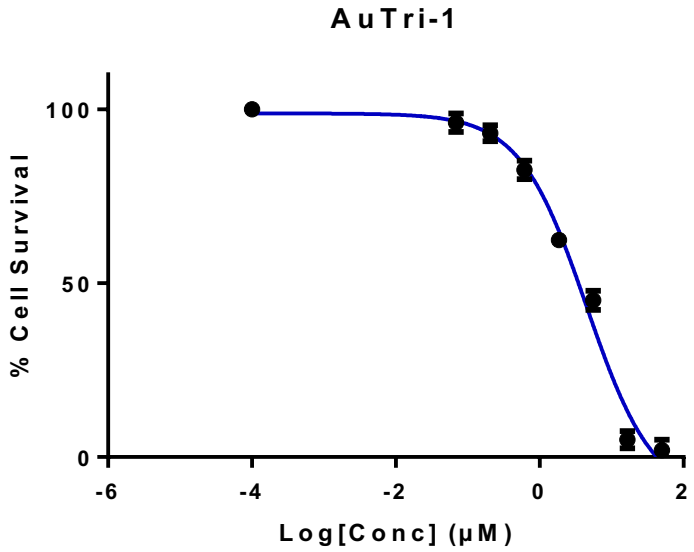


Figure S40. %Cell survival of **AuTri-1** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

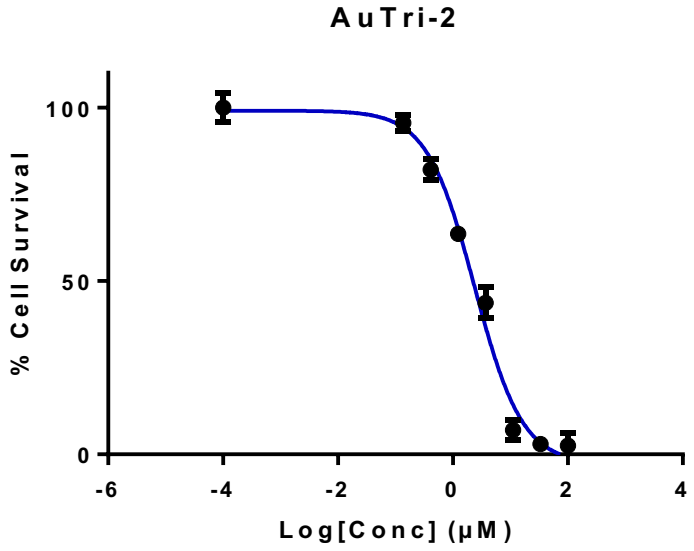


Figure S41. %Cell survival of **AuTri-2** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

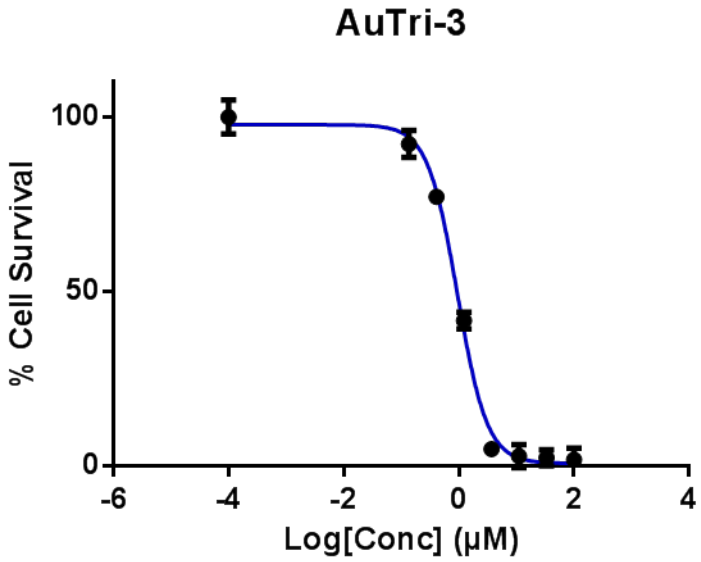


Figure S42. [%]Cell survival of **AuTri-2** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

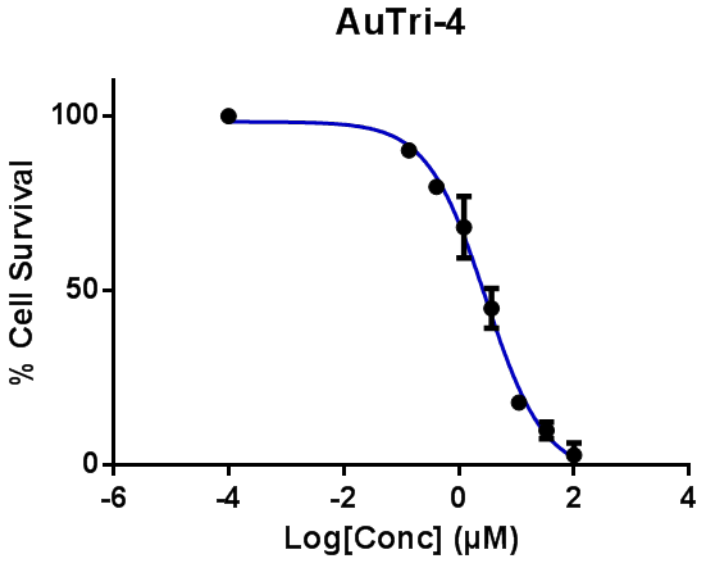


Figure S43. [%]Cell survival of **AuTri-4** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

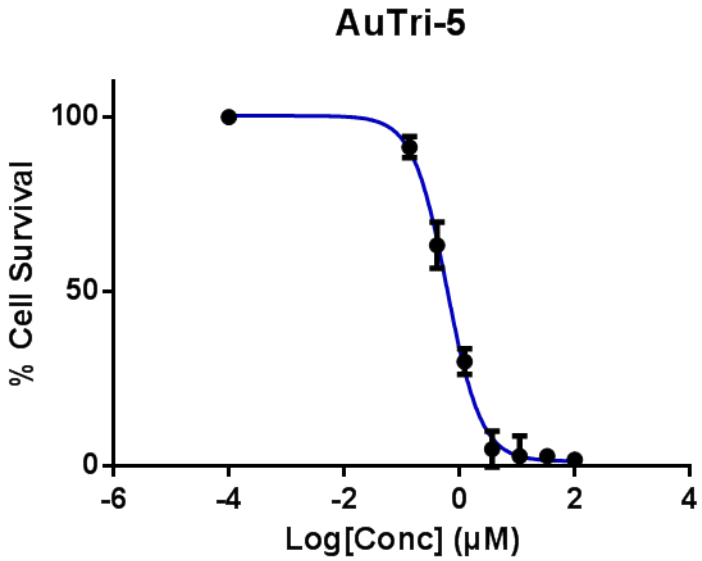


Figure S44. %Cell survival of **AuTri-5** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

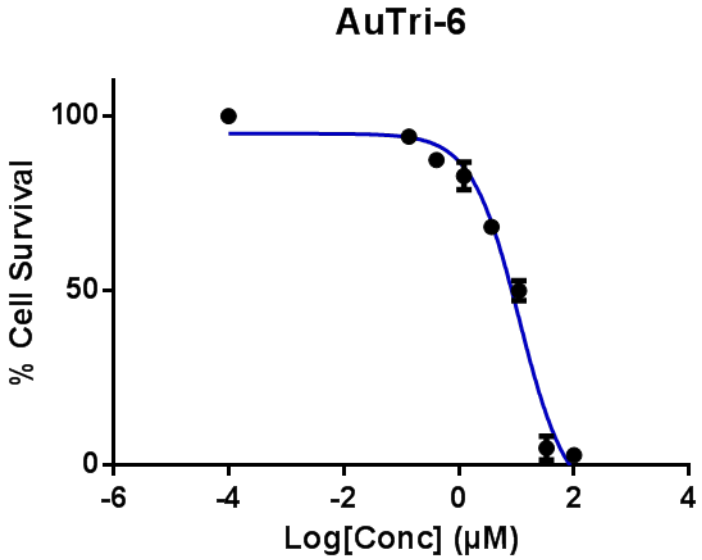


Figure S45. %Cell survival of **AuTri-6** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

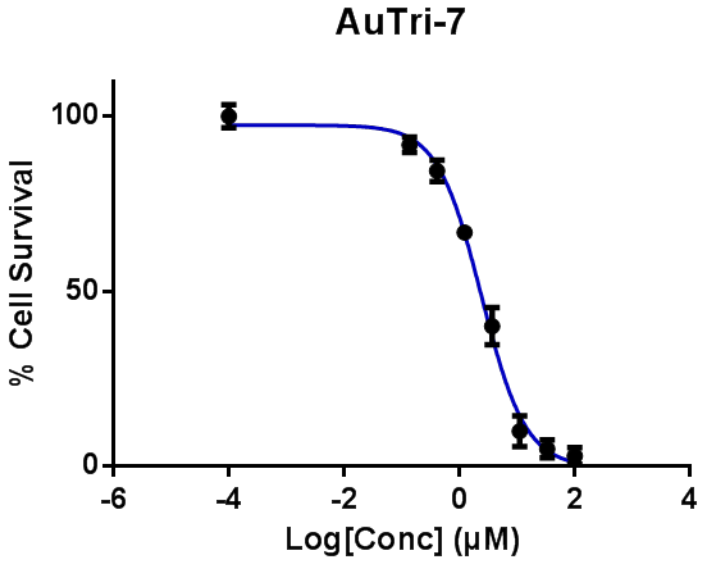


Figure S46. %Cell survival of **AuTri-7** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

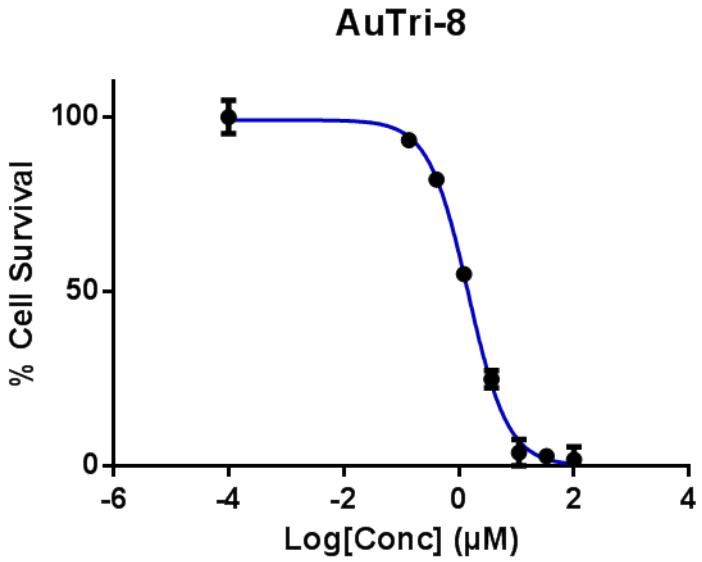


Figure S47. %Cell survival of **AuTri-8** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

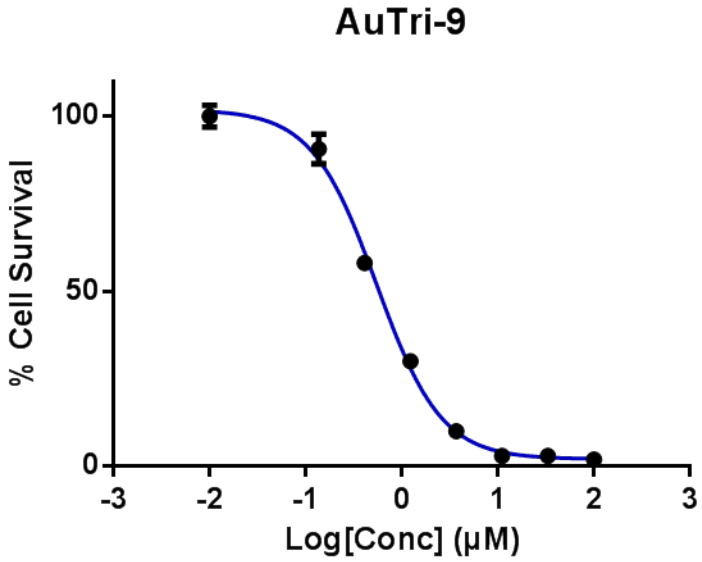


Figure S48. %Cell survival of **AuTri-9** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

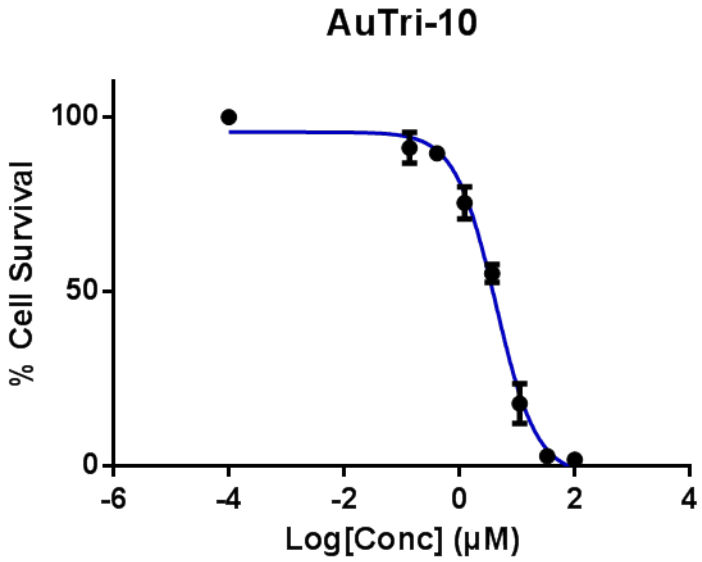


Figure S49. %Cell survival of **AuTri-10** in MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

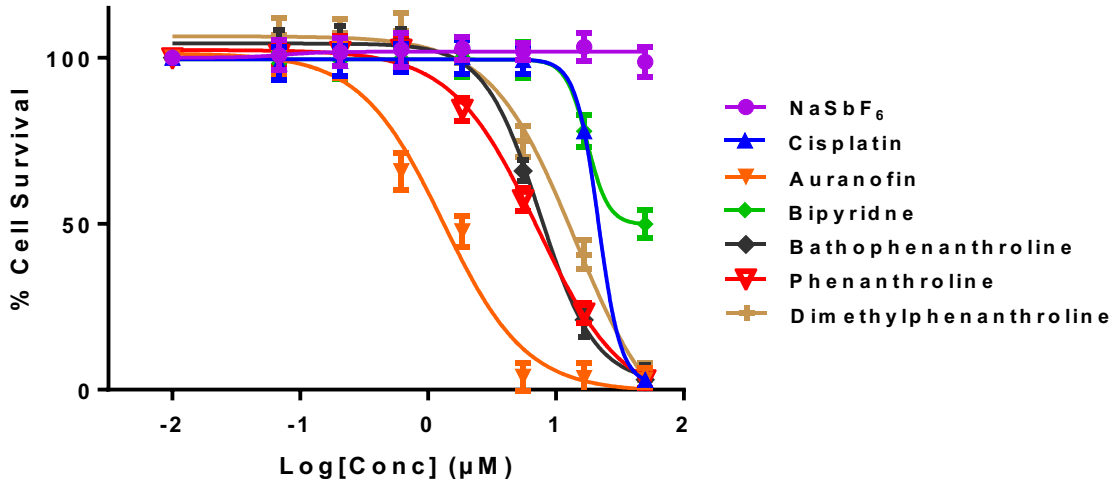


Figure S50. %Cell survival of bidentate ligands, Auranofin, Cisplatin, and $NaSbF_6$ as controls **in** MDA-MB-231, data are plotted as the mean ± s.e.m. (n = 3).

UV-Vis Absorbance of AuTri-1-8, and 10 in DMEM

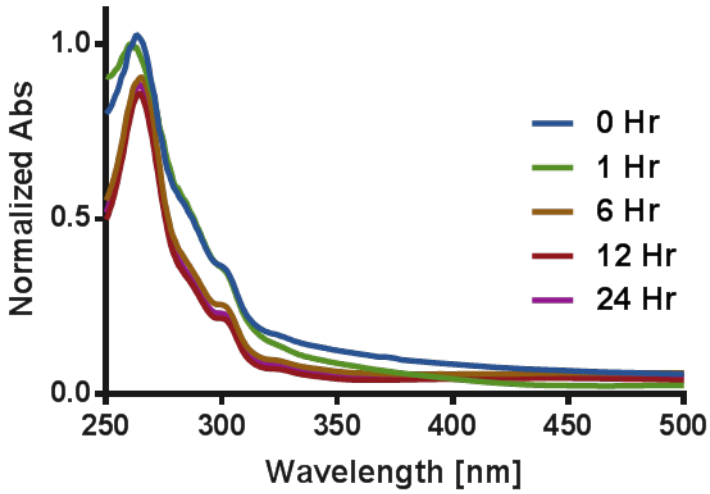


Figure S51. UV-Vis absorbance spectra of **AuTri-1** in DMEM at 37 °C over a 24 hour time period.

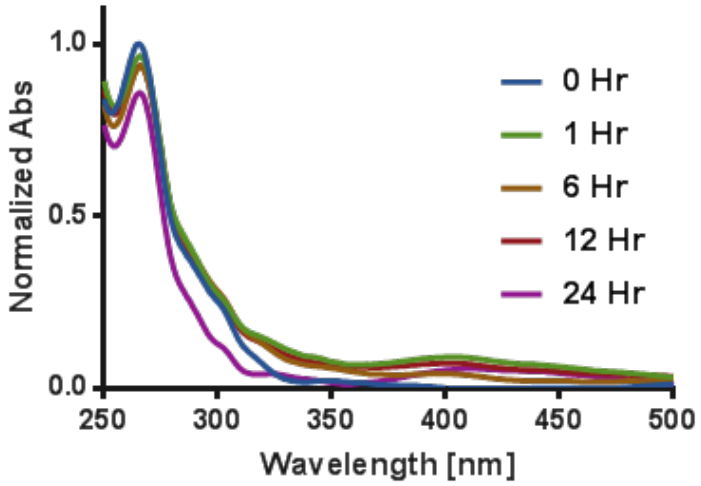


Figure S52. UV-Vis absorbance spectra of **AuTri-2** in DMEM at 37 °C over a 24 hour time period.

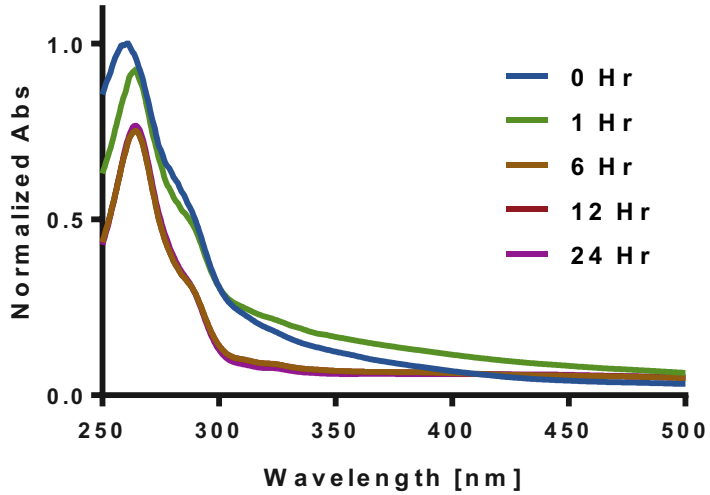


Figure S53. UV-Vis absorbance spectra of **AuTri-3** in DMEM at 37 °C over a 24 hour time period.

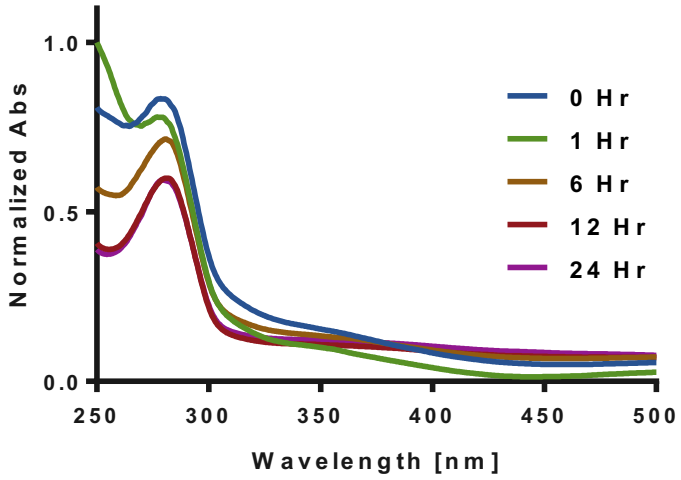


Figure S54. UV-Vis absorbance spectra of **AuTri-4** in DMEM at 37 °C over a 24 hour time period.

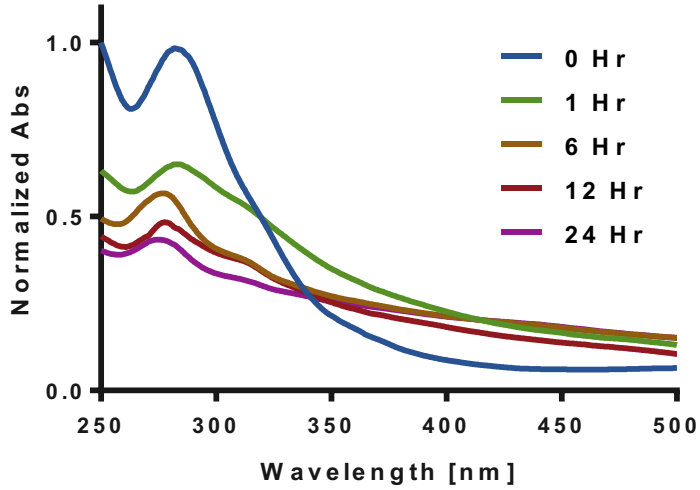


Figure S55. UV-Vis absorbance spectra of **AuTri-5** in DMEM at 37 °C over a 24-hour time period.

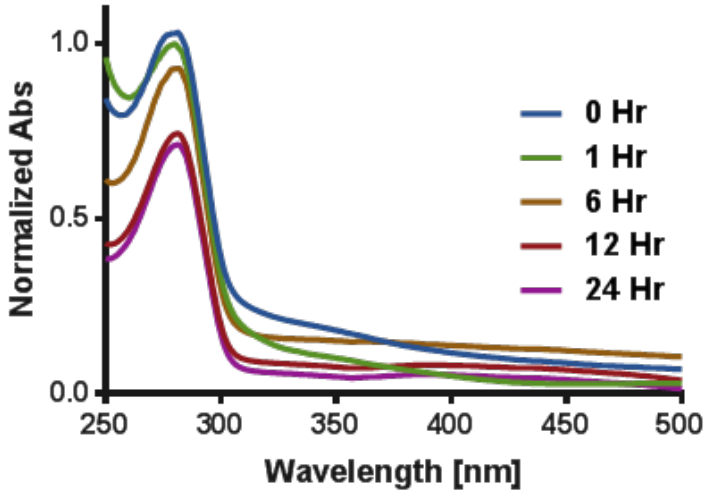


Figure S56. UV-Vis absorbance spectra of **AuTri-6** in DMEM at 37 °C over a 24-hour time period.

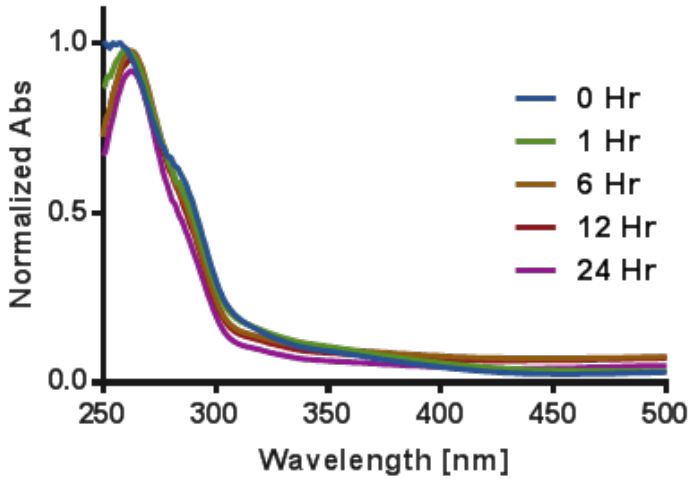


Figure S57. UV-Vis absorbance spectra of **AuTri-7** in DMEM at 37 °C over a 24-hour time period.

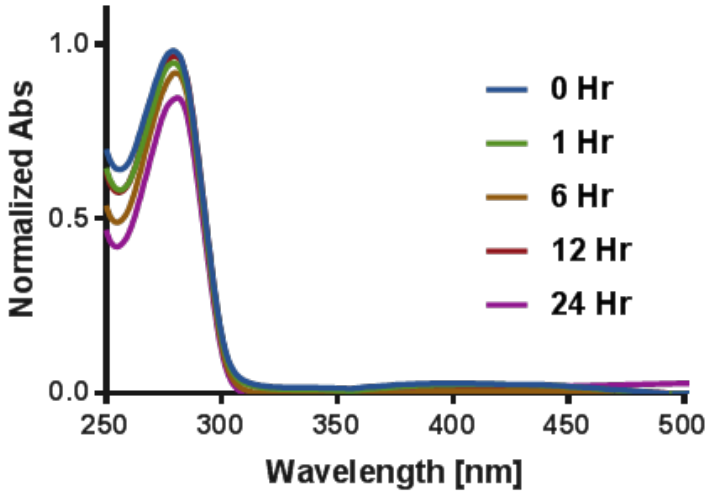


Figure S58. UV-Vis absorbance spectra of **AuTri-8** in DMEM at 37 °C over a 24-hour time period.

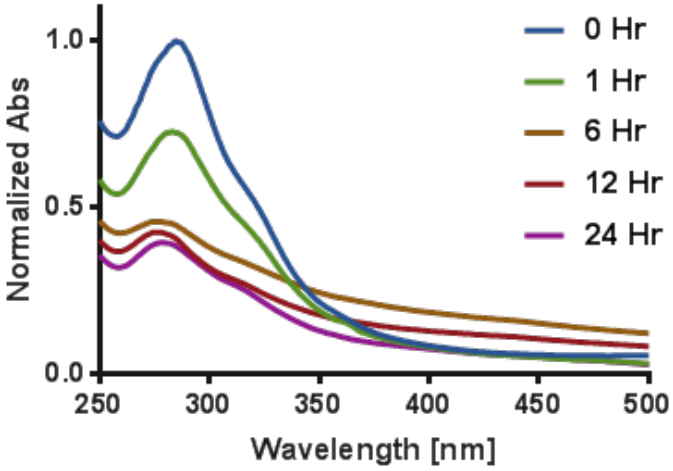


Figure S59. UV-Vis absorbance spectra of **AuTri-9** in DMEM at 37 °C over a 24-hour time period.

Immunoblots of AuTri-9 in MDAMB-231 and RPE-NEO:

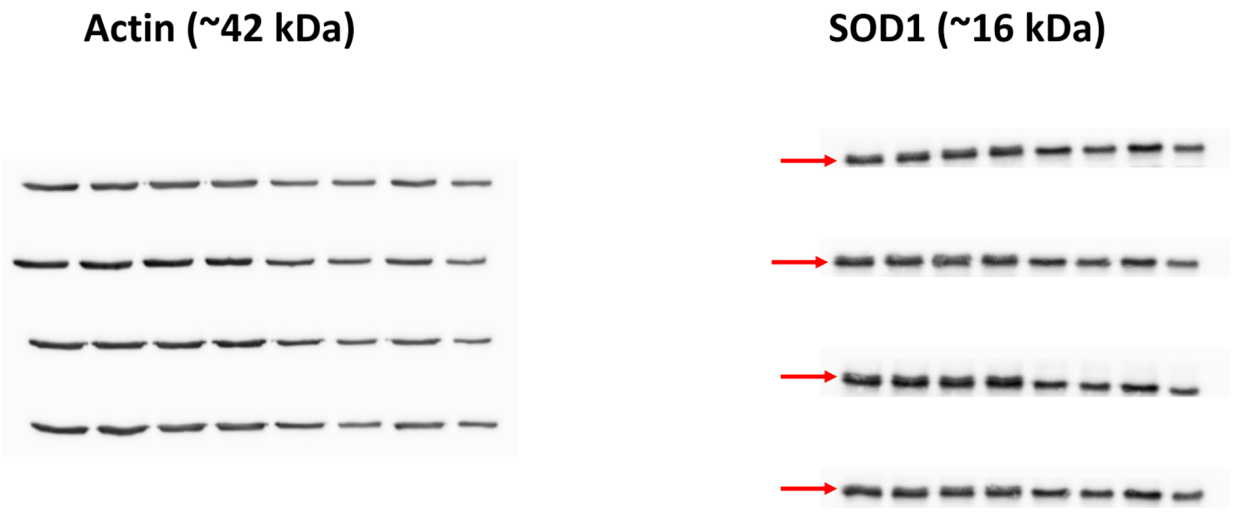


Figure S60. Full immunoblots of **AuTri-9**, actin and SOD1, n = 4.

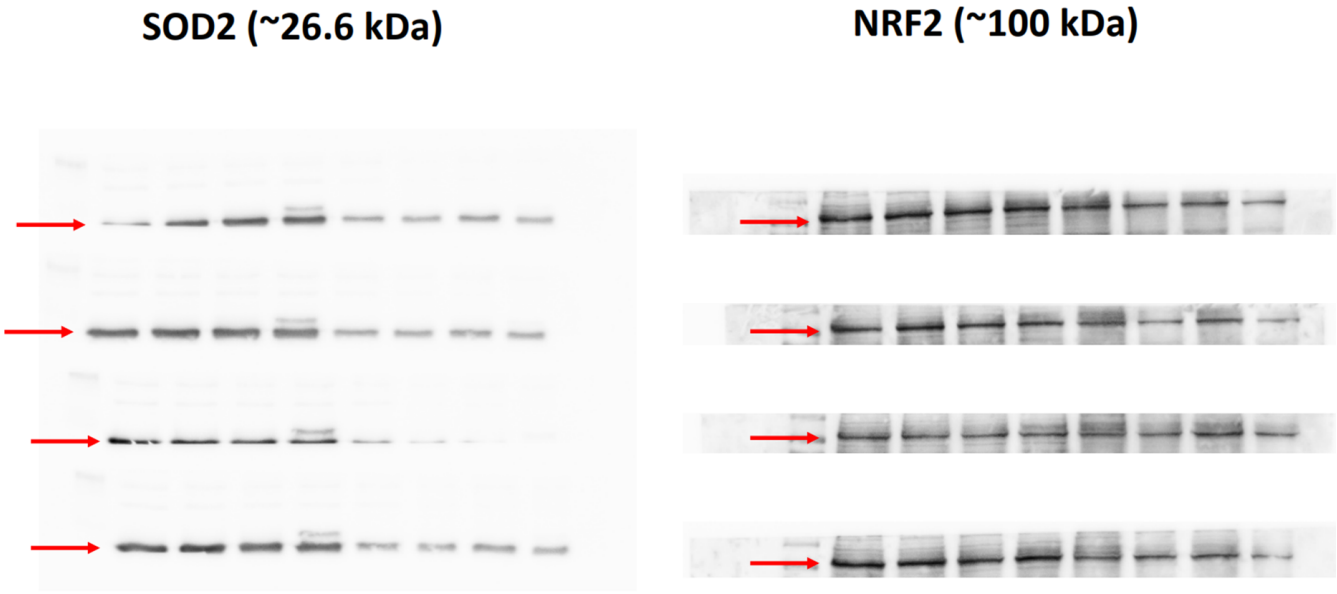


Figure S61. Full immunoblots of **AuTri-9**, SOD2 and NRF2, n = 4.

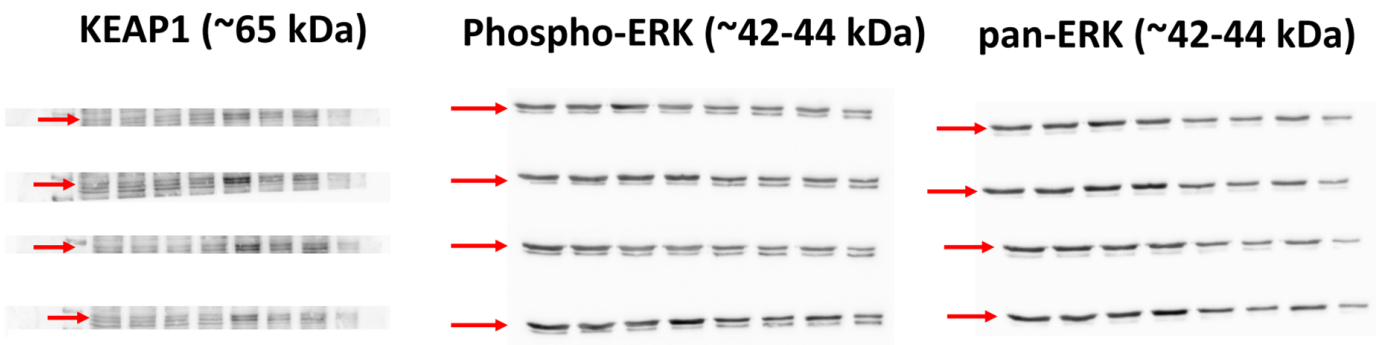


Figure S62. Full immunoblots of **AuTri-9**, KEAP1, p-ERK, and pan-ERK, n = 4.

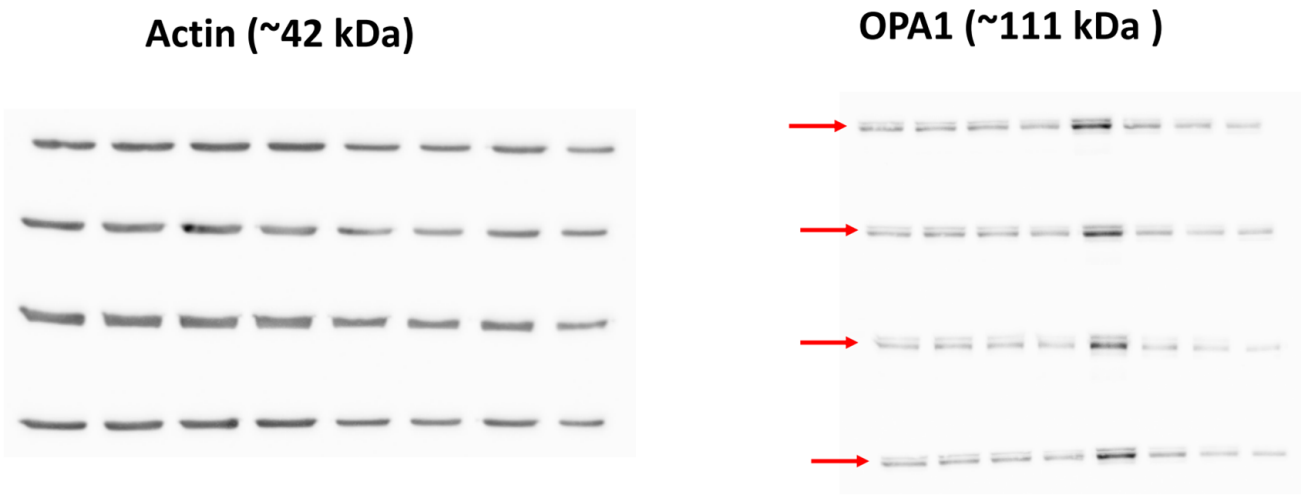


Figure S63. Full immunoblots of **AuTri-9**, actin and OPA1, n = 4.

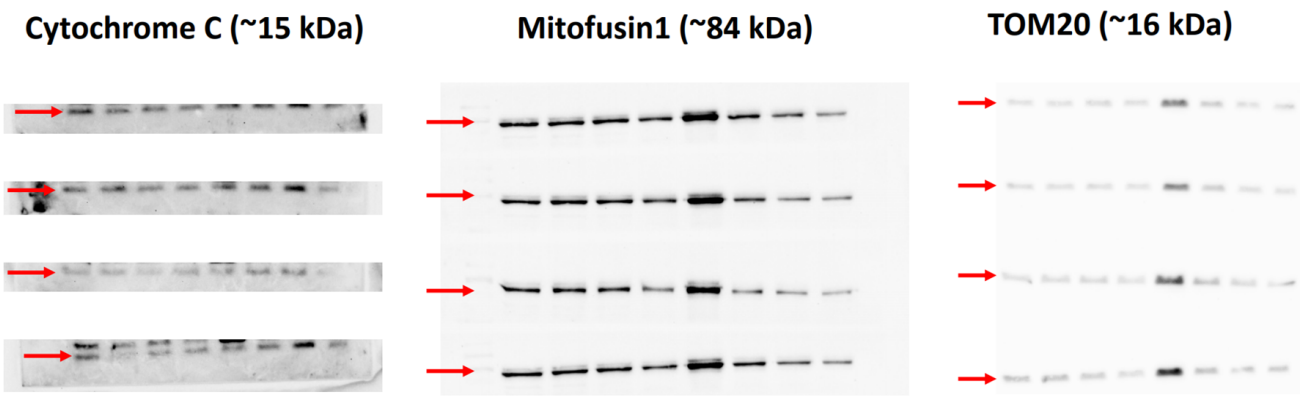


Figure S64. Full immunoblots of **AuTri-9**, Cytochrome C, MFN1 and TOM20, n = 4.

Table S7. Complete ANOVA table for Immunoblots for **AuTri-9**

ANOVA

(OPA1)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 24.46	**<0.001**
AuTri-9 exposure time (h)	F (3, 24) = 16.27	**<0.001**
Interaction	F (3, 24) = 15.19	**<0.001**
(Mitofusin 1)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 22.61	**<0.01**
AuTri-9 exposure time (h)	F (3, 24) = 32.57	**<0.01**
Interaction	F (3, 24) = 26.14	**<0.001**
(TOM20)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 64.61	**<0.001**
AuTri-9 exposure time (h)	F (3, 24) = 27.55	**<0.001**
Interaction	F (3, 24) = 26.94	**<0.001**
(SOD1)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 21.2	**<0.001**
AuTri-9 exposure time (h)	F (3, 24) = 0.9257	0.4434
Interaction	F (3, 24) = 0.8766	0.467
(SOD2)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 19.4	**<0.001**
AuTri-9 exposure time (h)	F (3, 24) = 0.5064	0.6815
Interaction	F (3, 24) = 0.4173	0.7422
(NRF2)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 21.2	0.7845
AuTri-9 exposure time (h)	F (3, 24) = 0.9257	0.7552
Interaction	F (3, 24) = 0.8766	0.8186
(KEAP1)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 73.01	**<0.001**
AuTri-9 exposure time (h)	F (3, 24) = 7.66	**<0.001**
Interaction	F (3, 24) = 8.767	**<0.001**
(Cytochrome C)	**F (DFn, DFd)**	**P value (Sig.)**
Cell type	F (1, 24) = 60.01	**<0.01**
AuTri-9 exposure time (h)	F (3, 24) = 5.451	**<0.01**
Interaction	F (3, 24) = 8.548	**<0.001**

Calibration Curves for GF-AAS of AuTri-9:

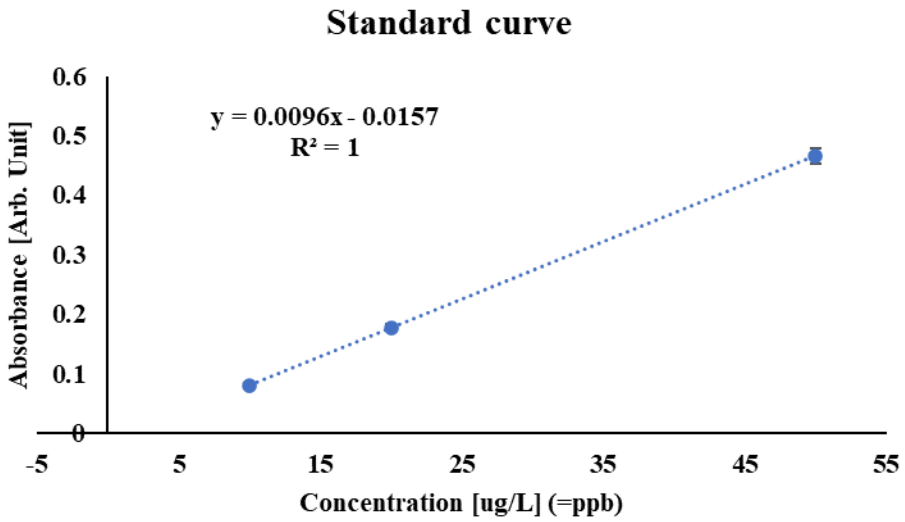


Figure S65. GF-AAS standard curve with Au concentrations of 10, 20 and 50 µg/mL. Data points are representative of triplicate and plotted as the mean ± s.d, n = 3.

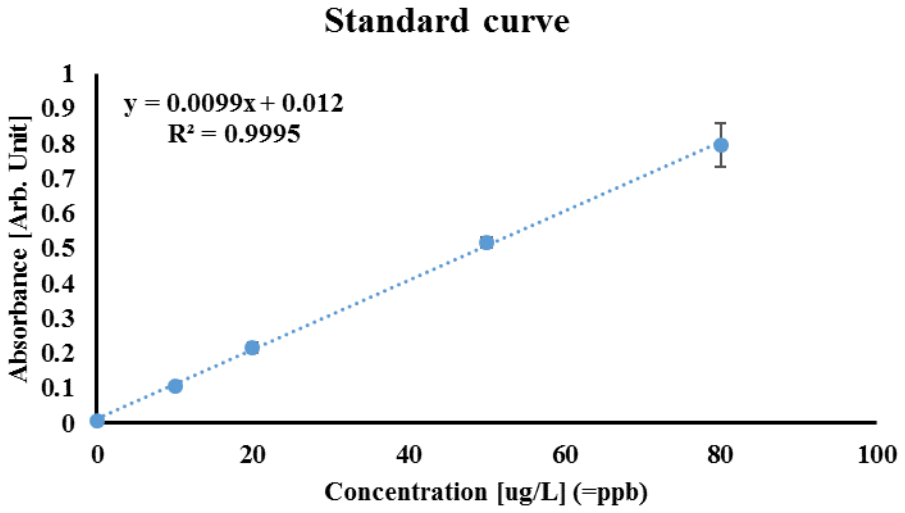


Figure S66. GF-AAS standard curve with Au concentrations of 0, 10, 20, 50, and 80 µg/mL. Data points are representative of triplicate and plotted as the mean ± s.d, n = 3.

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