Anticancer Gold(III)-bisphosphine Complex Alters Mitochondrial Electron Transport Chain to Induce *In Vivo* Tumor Inhibition

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Experimental Details

Materials and instrumentation. All chemicals were purchased from Sigma-Aldrich and used without further purification. Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) was purchased from NANOPARTZ and stored in a glovebox or dessicator before use as received. ACS grade solvents were purchased from Pharmco-Aaper and used without further purification or drying. Deuterated solvents were purchased from Cambridge Isotope Laboratories and used as received. Silica gel for column chromatography (Silicycle, P/N: R10030B SiliaFlash®F60, Size: 40-63 μm, Canada) was purchased from Silicycle. Aluminum backed silica-gel plates (20 × 20 cm2) were purchased from Silicycle (TLA-R10011B-323) and utilized for analytical thin-layer chromatography (TLC).

All reactions were insensitive to air or moisture, as a result, they were carried out under standard atmospheric conditions without air-sensitive techniques or drying agents. Reactions were carried out in round-bottom flasks or scintillation vials equipped with Teflon-coated magnetic stir bars for stirring non-homogenous reaction mixtures. Reactions were monitored by NMR and TLC, and the TLC plates visualized under low-wavelength light (254 nm) or stained with iodine on Silica. All compound purification was performed using silica-gel chromatography, employing CombiFlash® Rf+ Lumen, Teledyne ISCO. Filtrations were carried out using medium-porosity ceramic funnels. Removal of solvents in vacuo was performed using a Büchi rotary evaporator and further drying was achieved via a Schlenk line at ~120 mTorr using a dynamic vacuum pump.

^{1}H, ^{13}C (^{1}H-decoupled), and ^{31}P (^{1}H-decoupled) NMR spectra were recorded on a Varian Unity 400 MHz NMR spectrometer with a Spectro Spin superconducting magnet at the University of Kentucky NMR facility in the Department of Chemistry. Chemical shifts in ^{1}H and ^{13}C NMR spectra were internally referenced to solvent signals (^{1}H NMR: $CDCl_3$ at δ = 7.26 ppm; 13C NMR: $CDCl_3$ at δ = 77.16 ppm), and those in ^{31}P NMR spectra, which were run in $CDCl_3$, were externally referenced to 85% H_3PO_4 in D_2O at δ = 0 ppm.

High-resolution mass spectra (HRMS) were obtained using a Waters Synapt G2 HD mass spectrometer. Samples were directly injected into the instrument at 50 μL/min and ionized with ElectroSpray Ionization (ESI) in the positive mode. The source parameters were: capillary = 2.8 kV, sampling cone = 40, extraction cone: 5.0, source temperature = 80 °C, desolvation temperature = 150 °C, and desolvation gas flow = 500L/h. Mass spectrometry experiments and analysis were conducted at the Central Analytical Laboratory at the University of Colorado, Boulder.

In addition to spectroscopic characterization, bulk purity of all new compounds was assessed by combustion elemental analysis for C, H, N. Elemental analysis was carried out at the Atlantic Microlab (Norcross, GA) using Perkin Elmer 2400 Series II Autoanalyzers and Carlo Erba Model 1108 Analyzers. Instrument specifications list a precision of ± 0.3 percent.

X-ray Crystallography. Crystal for **AuPhos-82. 83, 84** and **85** were grown at 4 °C or room temperature from a vapor diffusion of either DMF into diethylether or acetone into THF solution of AuPhos compounds. Suitable crystals were selected by microscopic examination through crossed polarizers, mounted on a fine glass fiber in polyisobutane oil, and cooled to 90 K under a stream of nitrogen. A Bruker-AXS D8 Venture dual microsource diffractometer was used to collect the diffraction data using Mo$K\alpha$ radiation (λ = 0.71073 Å) from the crystal. The raw data were integrated, scaled, merged, and corrected for Lorentz-polarization effects using the APEX3 package[1]. Absorption correction was performed by SADABS[2-3] within APEX3. Space group determination and structure solution were carried out with SHELXT, and refinement used SHELXL-2017[4-5]. 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 (U_{iso}) derived from the atom to which they were attached. The structures, deposited in the Cambridge Structural Database, were checked with an R-tensor[6], by PLATON[7], and were further validated using CheckCIF[7]. See Figure 1 and Table S1-S4 for structure details.

Stability experiment of AuPhos-89 with L-GSH (HPLC chromatogram). CH_3CN (ACS reagent grade) solvent was degassed overnight using a sonicator and then filtered with a 0.22 μm pore size filter paper before use. All spectra were recorded using an Agilent Technologies 1100 series HPLC instrument and an Agilent Phase Eclipse Plus C18 column (4.6 mm x 100 mm; 3.5 μm particle size). AuPhos-89 and glutathione were prepared as 1 mM stock in DMSO and water, respectively. Equimolar amounts were mixed and the solutions were measured at 0, 1, 3, and 24 h by HPLC. The parameters used in the HPLC were as follows: flow rate, 1 mL/min; λ = 280 nm; eluent A = H_2O with 0.1 % TFA; Eluent B = CH_3CN with 0.05 % HCOOH; Solvent gradient: 0 min (100:0 H_2O:CH_3CN), 10 min (0:100 H_2O:CH_3CN), 15 min (100:0 H_2O:CH_3CN).

Reactivity with L-GSH (^{1}H NMR spectroscopy). Stock solutions comprised of a 1 mL, 20 mM solution of AuPhos-89 in DMSO-d_6. The solution of L-GSH (20 mM) was sonicated for 5 minutes to dissolve the reagent in DMSO-d_6. 1 mL of each solution were mixed in a 1:1 ratio to produce a final concentration of 10 mM in DMSO-d_6. The solution was then analyzed by 1H NMR spectroscopy. The solution was further analyzed at different time intervals, t = 1 h, 6 h, 12 h, and 24 h.

Cell Lines and Cell Culture Conditions. All cell lines (OVCAR8, A2780, H460, and MDA-MB-231) were cultivated in RPMI or DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% amphotericin. All cells were grown at 310K in a humidified atmosphere containing 5% CO_2.

Cell viability assay. Various established human ovarian, lung, and breast cancer cell lines were seeded in 96-well plates (4000 cells/well) and were incubated with RPMI 1640 or DMEM (100 µL) supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% amphotericin for 24 h and at 37 °C. They were then treated with AuPhos compounds at increasing concentrations for 72 h from stock solutions following serial dilutions, in which AuPhos stock solutions were prepared in DMSO/media mixture (% v/v = 20:80). Thereafter, cellular viability was assessed via the established crystal violet colorimetric assay. In brief, cells were fixed with 50 µL of a 1% glutaraldehyde solution and placed in an incubator (37 °C, 5% CO_2) for 1 hour. The plates were washed with water by running them under a gentle flow of tap water. The plates were air-dried, and crystal violet reagent was added to each well and allowed to incubate at room temperature for 20 min. The plates were washed with water and dried again. Methanol (200 µL/well) was added to each well, and the measurements of absorbance were subsequently performed using a Biotek Synergy H1 Plate Reader at 570 nm (peak absorbance).

Whole-cell uptake studies. OVCAR8 cells and MDA-MB-231 (5×10^5) were seeded in a 6-well plate and incubated overnight for attachment. Cells were then incubated with the test compounds (1 µM or 5 µM) in fresh media (RPMI or DMEM) and subsequently incubated for a given time (15 h for OVCAR8 and 18 h for MDA-MB-231, respectively.) at 37 °C. The media were then removed, and cells were collected via trypsinization. Cells were then washed with PBS (3 x 1 mL). The cells were digested by adding 0.1-0.2 mL of 70% HNO_3, and briefly sonicated. The cell solution was then diluted to an appropriate concentration using DI water. The gold content was analyzed by GF-AAS to obtain the whole cell uptake after quantification.

Mitochondrial uptake assay. To measure the mitochondrial uptake, MDA-MB-231 and NCM 460 cells (20×10^6) were treated with 1 or 5µM of AuPhos-89 at 37 ºC for 12 h or 18 h. The medium was removed, and the cells were washed with PBS solution (1 mL × 3), harvested, and centrifuged. The mitochondria extraction kit (ThermoFisher Scientific) was used to extract the mitochondria of the cells according to the manufacturer's protocol. The separated mitochondria pellets were mineralized with 70% HNO_3 and then diluted with DI water as needed. The gold content was analyzed by GF-AAS. Cellular gold levels were expressed as pmol of Au per million cells.

Differential Gene Expression using RNA-Sequencing. MDA-MB-231 cells were seeded in petri-dishes (100 mm x 15 mm) and allowed to grow to 85% confluency. The cells were then treated with AuPhos-89 at a concentration of 1 μM for 12 h at 37 °C. Cells were harvested and 1 x 10^7 cells were collected. High quality RNA was isolated using RNA Qiagen kit following manufacturer's protocol and subsequently sent to Novogene® for RNA-sequencing and analysis. Prior to analysis samples were required to pass three tests before library construction: 1) nanodrop for RNA purity (OD_{260}/OD_{280}), 2) agarose gel electrophoresis for RNA integrity and potential contamination, and 3) Agilent 2100 check RNA integrity. Next, the NEB library was constructed from mRNA enrichment and fragmentation, followed by reverse transcription, second strand cDNA synthesis, end repair, addition of adaptor, and finally amplification with PCR. After library construction, qPCR was used to accurately quantify the library effective concentration (> 2 nM), in order to ensure the library quality. Raw reads were removed *via* the following parameters: 1) remove reads containing adaptors, 2) remove reads containing N > 10% (N represents bases that could not be determined), 3) the Qscore (Quality value) of over 50% bases of the read was ≤ 5. Novogene® then uses STAR to accomplish the mapping reads to the reference genome. Gene expression level is then estimated by the abundance of transcripts (count of sequencing) that mapped to genome or exon where read counts are proportional to gene expression level, gene length and sequencing depth. Samples are then subjected to analysis using Pearson's correlation coefficient and principal component analysis for statistical significance.

Mitochondrial Membrane Potential (JC-1). MDA-MB-231 cells were plated at a density of 5 x 10^5 cells/plate in a 6 well plate and allowed to adhere overnight at 37 °C. AuPhos-89 was prepared as a stock in DMSO/DMEM (% v/v=20:80) and added at a final concentration of 10 μM. The cells were incubated (37 °C, 5% CO_2) for 1 h at this concentration. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was prepared as a stock in DMSO and added at a final concentration of 100 μM, and the cells were treated for 1 h. It was used as a positive control. After the indicated treatment time, a working solution of the JC-1 dye (Cayman Chemicals, Cat. #15003) 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 working solution of the JC-1 dye was added to the cells and incubated at 37 °C for 20 minutes. The cells were harvested and resuspended in 2mL PBS. The cells were analyzed on a flow cytometer with 488 nm excitation and appropriate emission filters. Gated cells, excluded debris and the CCCP-treated sample was used to performed standard compensation.

Mitochondrial Metabolism Analysis with Seahorse XF96 Analysis. The initial step of Seahorse XF96 analysis included optimization of the cell density. In this stage, isolated mitochondria (30 μg) or MDA-MB-231 cells were seeded at a range of densities from 2000 cells/well to 100,000 cells/well, followed by optimization of the FCCP injection concentration used (0.6 μM of 1.2 μM). The optimum conditions were determined to be 30,000 cells/well and an FCCP injection concentration of 0.6 μM. 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. AuPhos-89 was prepared as a stock in DMSO/DMEM (%v/v=20:80) and diluted to a working concentration of 100 μM with Seahorse XF96 assay buffer and then subsequently serial diluted by 10x to achieve multiple concentrations. The assay was performed using a pneumatic injection method of AuPhos-89, with the final injection concentrations of 0.01, 0.1, 1, and 10 μM. This was followed by injection of oligomycin (1.5 μM), FCCP (0.6 μM) and rotenone/ antimycin A (0.5 μM).

Cell Cycle Analysis. MDA-MB-231 cells were seeded at a density of 5 x 10^5 cells/well in a 6-well clear bottom plate with a final media volume of 2 mL and allowed to adhere overnight 37 °C. AuPhos-89 was prepared fresh as a stock in DMSO/DMEM (% v/v=20:80) and added at a final concentration of 1 μM with a final volume of 2.5 mL. Cells were treated with AuPhos-89 for time periods of 24 h, 48 h, and 72 h. After the desired treatment period, the medium was removed and added to a 15 mL Falcon tube. The wells were washed with 5 mL of PBS and added to the Falcon tube. The cells were trypsinized (1 mL) and added 5 mL of fresh DMEM. All media were combined, and the tube centrifuged at 2000 rpm for 5 minutes to collect the pellet. The medium was decanted, and the pellet suspended in 1 mL of PBS, which was then transferred to a 1 mL Eppendorf tube, centrifuged at 2000 rpm for 5 minutes and suspended in 70% EtOH/PBS solution. These solutions were stored at 4 °C until ready for analysis. Once all treatments had been collected, the cells were collected by centrifuging at 2000 rpm for 5 minutes. The cells were washed twice with PBS (1 mL) and suspended in a 50 μL of RNase solution (100 μg/mL) and 200 μL of a 50 μg/mL PI solution. The solutions were then filtered through a 5 mL polystyrene round-bottom tube fit with a cell-strainer cap. The samples were then analyzed with FACS.

Apoptosis Analysis. MDA-MB-231 cells were seeded at a density of 5 x 10^5 cells/well in a 6-well clear bottom plate with a final media volume of 2 mL. The cells were allowed to adhere overnight at 37 °C. A stock of AuPhos-89 was prepared fresh in DMSO/DMEM (% v/v=20:80) and added to the desired well at a concentration of 1 μM with a final volume of 2.5 mL and incubated for 15 h at 37 °C. A stock of H_2O_2 was prepared in PBS and the cells treated at a final concentration of 2 mM for 3 hours and used as positive control. When ready for analysis, medium was removed and the wells washed with 5 mL of PBS. The cells

were trypsinized (1 mL), 5 mL of DMEM were added to each well, and total volume collected and centrifuged to pellet the cells. The cells were resuspended in 2 mL of fresh media, counted, and reconstituted to a concentration of 1 x 10^5 cells/mL. The cells were centrifuged again, and the pellet suspended in 500 µL of Annexin binding buffer. To each sample was added 5 µl of Annexin V-FITC and 5 µl PI and incubated in the dark at room temperature for 5 minutes. The samples were then subjected to FACS analysis.

TMT-based Quantification Analytical data. MDA-MB-231 cells were seeded on a petri dish (100 mm x 15 mm) and allowed to grow to 85% confluency. The cells were then treated with AuPhos-89 at a concentration of 1 µM for 12 h at 37 °C. Cells were harvested, and 1 x 10^7 cells were collected. Cell pellets were lysed using 200 ul of RIPA lysis buffer, including protease inhibitors, and centrifuged at 12000 rpm for 15 min at 4℃. And the supernatant was transferred to a new EP tube. Protein concentration was determined using a 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. Alkylated by 20 mM IAA at room temperature in the dark for 1h, add free trypsin into the protein solution at a ratio of 1:50, and the solution was incubated at 37°C overnight. The extracted peptides were lyophilized to near dryness. Re-dissolve the sample with 100 mM TEAB. Immediately before use, equilibrate the TMT Label Reagents to room temperature. Add 20 µL of anhydrous acetonitrile to each tube, and allow the reagent to dissolve for 5 minutes with occasional vortexing. Briefly centrifuge the tube to gather the solution. Transfer the samples to the TMT Reagent vial and incubate the reaction for 1 hour at room temperature. Add 8µL of 5% hydroxylamine to the sample and incubate for 15 minutes to quench the reaction. And combine samples at equal amounts in a new microcentrifuge tube. Fractionation of the labeled peptides with 6 components using HPLC. The LC and MS used are as follows: Nanoflow UPLC: Ultimate 3000 nano UHPLC system (ThermoFisher Scientific, USA), 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. The 6 raw MS files were analyzed and searched against the HUMAN protein database based on the samples' species using Maxquant (1.5.6.5). The parameters were set as follows: the protein modifications were carbamidomethylation (C) (fixed), oxidation (M) (variable); the enzyme specificity was set to trypsin; the maximum missed cleavages were set to 2; the precursor ion mass tolerance was set to 10 ppm, and MS/MS tolerance was 0.6 Da. Only high confident identified peptides were chosen for downstream protein identification analysis. In total, 2052 proteins were identified for this project (Table 3.1). Proteins of relative quantitation were divided into two categories. A quantitative ratio over 1.5 was considered up-regulation, while a quantitative ratio of less than 1/1.5 was considered as down-regulation.

Animals. Female, 5 week-old BALB/c mice were purchased from Charles River Laboratoires (Wilmington, MA). All mice were quarantined for 1 week prior to use and kept in micro-isolator cages (four mice per cage) in a temperature- and humidity-controlled environment as per the Division of Laboratory Animal Research (DLAR) of University of Kentucky. All mice were maintained in a pathogen-free environment under the care of DLAR of University of Kentucky. Our study was performed in compliance with the NIH guidelines (NIH Publication No. 85-23 Rev. 1985) for the care and use of laboratory animals and all experimental procedures were monitored and approved by the Institutional Animal Care and Use Committee (IACUC) of University of Kentucky (USA).

In vivo experiment of AuPhos-89. 10 female BALB/c mice (5 weeks) were received from Charles River Laboratories (Wilmington, MA), and they had an acclimation period of one week before implanted with 1,000,000 4T1 cells subcutaneously on their flanks. Three days post-implantation, the mice were administered 10 mg/kg AuPhos-89 retro-orbital intravenously (IV), 0.1 mL/mouse formulated as 1% DMSO, 10% Kolliphor, and 89% PBS. The control group was injected with a PBS solution containing 1% DMSO and 1% Kolliphor. AuPhos-89 injection, tumor-size/body-weighing measurement were performed three days a week, and mice were euthanized 19 days later. (n=5 for AuPhos-89, and n=5 for vehicle control)

In vivo comparative experiment of AuPhos-89 and cisplatin. Mice were implanted with 2,000,000 4T1 cells subcutaneously on their flanks. Five days post-implantation, the mice were administered 10 mg/kg AuPhos-89 and 3 mg/kg cisplatin intraperitoneally (IP). For AuPhos-89, 0.2 mL/mouse was formulated as 1.5% DMSO, 12% Kolliphor, and 86.5% PBS, and cisplatin was dissolved in 100% PBS. The control group was injected with a PBS solution containing 1.5% DMSO and 12% Kolliphor. Injection and tumor-size/body-weight measurement was performed three days a week. (n=5 for AuPhos-89, n=3 for cisplatin, and n=4 for vehicle control).

Hematoxylin and eosin (H&E) staining. The mice used in the *in vivo comparative experiment of AuPhos-89 and cisplatin* were sacrificed at day 14 post tumor cells(4T1) injection. Mice organs (heart, lung, liver, kidney, spleen, and tumor) were fixed in freshly prepared paraformaldehyde (4% in PBS), the fixation time was 24 hours. And those processed for paraffin sectioning. The organs sections of 5 μm were stained with H&E staining and used for histological examination of the organs and tumor. A total of 5 sections per tissues (spanning the full depth of the organ) were examined and photographed using a Nikon Eclipse 55i microscope.

Tissue biodistribution of AuPhos-89. With four mice inoculated with 4T1 (1,000,000 cells), after 20 days, AuPhos-89 was inserted into mice by IV administration (0.1 mL, 10 mg/kg). Each tissue was obtained by sacrificing the mice (n=2) 1 and 24 hours later. The thus obtained tissues were boiled for 5 hours at 60 °C with 70% nitric acid (0.5 ml) and then boiled again at 60 °C for 10 minutes by adding 35% hydrogen peroxide (0.5 ml). The solution turned yellow and diluted as needed to measure the gold content using a Graphite Furnace Atomic Absorption Spectrometer. Before measuring all samples, the standard solution curves were measured (Figure S44).

Chemical synthesis of intermediate and final compounds

Dichloro(2-benzoylpyridine)gold(III)

In a 30 ml pressure vessel, 2-benzoylpyridine (0.126 g, 0.686 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.228 g, 0.579 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 18 h at 130 °C. The precipitate was then vacuum filtered and washed with water to afford an off-white solid (0.175 g, 67 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (d, J = 5.8 Hz, 1H), 8.55 (t, J = 7.7 Hz, 1H), 8.36 (d, J = 7.8 Hz, 1H), 8.09 (t, J = 6.7 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.69 (dd, J = 6.5, 2.8 Hz, 1H), 7.47 (ddd, J = 6.0, 3.9, 1.8 Hz, 2H).

Dichloro(2-benzylpyridine)gold(III)

In a 30 ml pressure vessel, 2-benzylpyridine (0.085 g, 0.500 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.197 g, 0.500 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 7 hours at 130 °C. The precipitate was then vacuum filtered and washed with water to afford an off-white solid (0.179 g, 82 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (d, J = 5.9 Hz, 1H), 8.26 (t, J = 7.6 Hz, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.71 (t, J = 6.7 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 7.0 Hz, 1H), 7.18 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 7.4 Hz, 1H), 4.62 (d, J = 15.2 Hz, 1H), 4.35 (d, J = 15.2 Hz, 1H).

Dichloro(2-phenylpyridine)gold(III)

In a 30 ml pressure vessel, 2-phenylpyridine (0.076 g, 0.492 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.194 g, 0.492 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 22 h at 130 °C. The precipitate was then vacuum filtered and washed with water to afford an off-white solid (0.156 g, 75 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (d, J = 6.0 Hz, 1H), 8.40 (dd, J = 6.5, 1.3 Hz, 2H), 8.00 – 7.94 (m, 1H), 7.85 – 7.73 (m, 2H), 7.48 (t, J = 7.5 Hz, 1H), 7.42 – 7.33 (m, 1H).

Dichloro(benzo[h]quinoline)gold(III)

In a 250 ml round bottom flask, benzo[h]quinoline (0.370 g, 2.06 mmol) was dissolved in CH_3CN (15 mL), and $HAuCl_4 \cdot 3H_2O$ (0.750 g, 1.90 mmol) in distilled water (15 mL) was added. A yellow precipitate formed. After stirring for 2 h, the precipitate was filtered. And then it was placed in a ceramic mortar and placed in the oven set at 185 °C for 3 days to afford a brown solid (0.610 g, 72 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (d, J = 5.7 Hz, 1H), 9.00 (d, J = 8.2 Hz, 1H), 8.15 – 8.03 (m, 4H), 7.92 (d, J = 7.8 Hz, 1H), 7.74 (t, J = 7.9 Hz, 1H).

Dichloro(2-(p-tolyl)pyridine)gold(III)

In a 30 ml pressure vessel, 2-(p-tolyl)pyridine (0.082 g, 0.485 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.191 g, 0.485 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 26 h at 130 °C. The precipitate was then vacuum filtered and washed with water to afford an off-white solid (0.186 g, 88 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (d, J = 5.9 Hz, 1H), 8.35 (d, J = 5.3 Hz, 2H), 7.85 (d, J = 7.8 Hz, 1H), 7.72 (dt, J = 8.6, 4.1 Hz, 1H), 7.61 (s, 1H), 7.30 (d, J = 7.8 Hz, 1H), 2.40 (s, 3H).

Dichloro(4-(2-pyridyl)benzaldehyde)gold(III)

In a 30 ml pressure vessel, 4-(2-pyridyl)benzaldehyde (0.103 g, 0.560 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.206 g, 0.523 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 23 h at 130 °C. The precipitate was then vacuum filtered and washed with water to afford an off-white solid (0.165 g, 70 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 9.59 – 9.53 (m, 1H), 8.54 (d, J = 7.9 Hz, 1H), 8.46 (t, J = 7.7 Hz, 1H), 8.29 (s, 1H), 8.22 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.87 (t, J = 6.6 Hz, 1H).

Dichloro(2-phenoxypyridine)gold(III)

In a 30 ml pressure vessel, 2-phenoxypyridin (0.090 g, 0.526 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.204 g, 0.517 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 22 h at 130 °C. The precipitate was then vacuum filtered and washed with water to afford a white-pink solid (0.124 g, 55 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (d, J = 5.6 Hz, 1H), 8.42 (t, J = 7.6 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.69 (t, J = 6.5 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 9.2 Hz, 2H), 7.23 (t, J = 7.0 Hz, 1H).

Dichloro(2-anilinopyridine)gold(III)

2-bromopyridine (200 mg, 1.266 mmol), aniline (117.86 mg, 1.266 mmol), t-BuONa (364.988 mg, 3.798 mmol), $Pd_2(dba)_3$ (57.69 mg, 0.063 mmol), and RuPhos (39.415 mg, 0.066 mol) were loaded into a Schlenk tube equipped with a Teflon-coated magnetic stir bar. The mixture was evacuated and back-filled with nitrogen in three cycles. The flask was then placed into a preheated oil bath and stirred for 48 h at 80 °C. After completion of reaction, the flask was allowed to cool to room temperature. The mixture was purified by column chromatography gradient 10-20 % ethyl-acetate:hexane to afford the desired product (off-white solid, 190.2 mg, 89.1 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1H), 8.14 (s, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.55 (t, J = 8.7 Hz, 1H), 7.23 (s, 2H), 6.91 – 6.81 (m, 2H), 6.74 (d, J = 6.7 Hz, 1H). 13C NMR (101 MHz, DMSO) δ 156.39, 147.69, 142.20, 137.64, 129.02, 120.81, 118.49, 114.67, 111.09.

In a 30 ml pressure vessel, 2-anilinopyridin (0.0946 g, 0.556 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.2125 g, 0.540 mmol) were dissolved in distilled water (10 mL). The reaction mixture was stirred for 45 minutes at 130 °C. The precipitate was then vacuum filtered and washed with water to afford a dark brown solid (0.1634 g, 69 % yield), which could then be used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.82 (d, *J* = 6.5 Hz, 1H), 7.97 (t, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.11 (dd, *J* = 17.5, 7.4 Hz, 2H), 7.02 (t, *J* = 7.6 Hz, 1H).

AuPhos-82: (2-anilinopyridine)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(2-anilinopyridine)gold(III) (0.082 g, 0.186 mmol) was placed in a 100 mL of round bottom flask and 10 mL of chloroform was added, the solution turned dark brown. 1,2-Bis(diphenylphosphino)benzene (0.085 g, 0.189 mmol) was added, the solution turned dark yellow instantly. The solution was stirred for about 16 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf+ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.63 (s, 1H), 8.39 (d, *J* = 7.7 Hz, 1H), 8.04 (s, 2H), 7.99 – 7.82 (m, 3H), 7.81 – 7.73 (m, 4H), 7.70 – 7.46 (m, 7H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.29 (s, 1H), 7.20 – 6.99 (m, 5H), 6.86 (s, 2H), 6.70 (s, 2H), 6.48 (dd, *J* = 18.2, 5.8 Hz, 2H), 6.15 (t, *J* = 6.6 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 153.70, 147.10, 140.44, 136.76, 136.74, 136.20, 136.15, 136.06, 136.00, 135.59, 135.51, 135.09, 135.06, 135.02, 134.99, 134.89, 134.76, 134.26, 134.24, 134.16, 133.61, 132.70, 129.93, 129.80, 128.60, 124.76, 124.67, 120.19, 117.21, 116.26, 116.22. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 49.16 (d, *J* = 11.3 Hz), 41.93. HRMS (ESI) (m/z): calcd. for $C_{41}H_{33}AuClN_2P_2$ $[M\text{-}Cl]^+$ 847.1473, found: 847.1469. Anal. Calcd. for $C_{41}H_{33}AuCl_2N_2P_2$ 1.1H_2O: C, 54.51; H, 3.93; N, 3.1. Found: C, 54.41; H, 3.82; N, 3.08.

AuPhos-83: (2-phenoxypyridine)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(2-phenoxypyridine)gold(III) (0.099 g, 0.226 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added. 1,2-Bis(diphenylphosphino)benzene (0.108 g, 0.242 mmol) was added, the solution turned yellow instantly. The solution was stirred for about 12 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf^+ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (s, 1H), 8.05 (dd, *J* = 12.8, 7.6 Hz, 2H), 7.82 (t, *J* = 9.9 Hz, 3H), 7.75 – 7.61 (m, 4H), 7.59 – 7.27 (m, 15H), 7.13 (s, 2H), 6.96 (dt, *J* = 27.9, 6.6 Hz, 2H), 6.81 (t, *J* = 6.8 Hz, 2H), 6.64 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.41, 153.41, 136.09, 136.00, 135.89, 135.58, 135.47, 135.34, 134.56, 134.51, 134.42, 134.33, 134.29, 134.20, 134.13, 133.64, 133.53, 133.07, 132.41, 129.68, 129.57, 129.29, 129.17, 129.05, 128.92, 127.42, 126.80, 126.70, 126.52, 125.98, 124.31, 123.64, 123.14, 122.56, 121.14, 120.91, 120.85, 118.59, 112.25. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 52.90 (d, *J* = 5.3 Hz), 52.63 (d, *J* = 5.0 Hz). HRMS (ESI) (m/z): calcd. for $C_{41}H_{32}AuClNOP_2$ $[M\text{-}Cl]^+$ 848.1313, found: 848.1309. Anal. Calcd. for $C_{41}H_{32}AuCl_2NOP_2$ $0.7H_2O$: C, 54.89; H, 3.75; N, 1.56. Found: C, 54.87; H, 3.76; N, 1.58.

AuPhos-84: (2-benzylpyridine)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(2-benzylpyridine)gold(III) (0.098 g, 0.224 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added. 1,2-Bis(diphenylphosphino)benzene (0.109 g, 0.244 mmol) was added, the solution turned yellow instantly. The solution was stirred for about 15 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf^+ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.43 (t, *J* = 4.6 Hz, 1H), 8.27 (d, *J* = 7.4 Hz, 1H), 7.93 (dd, *J* = 28.6, 7.7 Hz, 5H), 7.79 (dt, *J* = 14.6, 7.4 Hz, 4H), 7.67 (t, *J* = 7.2 Hz, 1H), 7.64 – 7.51 (m, 6H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.36 (t, *J* = 7.2 Hz, 1H), 7.25 (s, 4H), 7.02 (t, *J* = 7.2 Hz, 1H), 6.91 (s, 2H), 6.81 (dd, *J* = 13.5, 7.7 Hz, 3H), 6.70 (s, 1H), 6.58 (s, 1H), 4.92 (d, *J* = 14.1 Hz, 1H), 4.08 (d, *J* = 14.7 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.55, 149.21, 148.10, 142.44, 136.44, 136.37, 135.87, 135.76, 135.64, 135.57, 135.47, 135.36, 135.30, 135.19, 135.17, 134.79, 134.68, 134.64, 133.82, 133.78, 133.74, 133.71, 133.33, 133.29, 133.24, 133.14, 133.11, 132.75, 132.64, 130.53, 130.47, 130.30, 130.18, 130.15, 130.03, 129.93, 129.80, 129.77, 129.65, 128.18, 128.14, 127.83, 127.75, 125.02, 124.50, 123.79, 123.75, 123.01, 122.35, 118.81, 49.45. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 50.02, 41.06. HRMS (ESI) (m/z): calcd. for $C_{42}H_{34}AuClNP_2$ $[M\text{-}Cl]^+$ 846.1520, found: 846.1528. Anal. Calcd. for $C_{42}H_{34}AuCl_2NP_2$ $1.95H_2O$: C, 54.97; H, 4.16; N, 1.53. Found: C, 54.91; H, 4.22; N, 1.62.

AuPhos-85: (2-benzoylpyridine)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(2-benzoylpyridine)gold(III) (0.091 g, 0.202 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added. 1,2-Bis(diphenylphosphino)benzene (0.095 g, 0.212 mmol) was added, the solution turned yellow instantly. The solution was stirred for about 5 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf$^+$ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.62 (d, *J* = 3.8 Hz, 1H), 8.16 (t, *J* = 7.3 Hz, 1H), 8.08 – 7.93 (m, 4H), 7.92 – 7.77 (m, 3H), 7.64 (dt, *J* = 14.2, 6.5 Hz, 7H), 7.58 – 7.47 (m, 7H), 7.39 (ddd, *J* = 32.2, 15.0, 7.4 Hz, 4H), 7.26 (s, 1H), 7.16 (dq, *J* = 15.1, 7.5 Hz, 3H), 6.93 (dd, *J* = 14.1, 7.9 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 196.44, 196.41, 159.84, 158.81, 155.29, 148.68, 138.71, 138.64, 138.16, 138.13, 137.20, 137.02, 136.94, 136.72, 136.66, 136.40, 136.36, 136.31, 136.29, 135.97, 135.95, 135.85, 135.57, 135.52, 135.46, 135.41, 135.34, 135.02, 135.00, 134.80, 134.70, 134.58, 134.54, 134.51, 134.35, 134.30, 134.28, 134.08, 134.01, 133.98, 133.89, 133.82, 133.73, 133.69, 133.64, 133.61, 133.49, 133.30, 133.21, 132.21, 132.14, 131.38, 130.97, 130.86, 130.26, 130.16, 130.00, 129.35, 129.24, 128.97, 128.88, 128.82, 128.72, 126.37, 126.28, 124.99, 124.29, 124.16, 123.82, 123.70, 122.76, 122.20, 119.80, 119.22. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 55.09 (d, *J* = 7.5 Hz), 53.47 (d, *J* = 7.1 Hz). HRMS (ESI) (m/z): calcd. for $C_{42}H_{32}AuClNOP_2$ $[M\text{-}Cl]^+$ 860.1313, found: 860.1321. Anal. Calcd. for $C_{42}H_{32}AuCl_2NOP_2$ 1.2H_2O: C, 54.84; H, 3.79; N, 1.52. Found: C, 54.94; H, 3.78; N, 1.53.

AuPhos-86: (benzo[h]quinoline)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(benzo[h]quinoline)gold(III) (0.080 g, 0.179 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added, the solution turned dark brown. 1,2-Bis(diphenylphosphino)benzene (0.084 g, 0.188 mmol) was added, the solution turned dark yellow instantly. The solution was stirred for about 22 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf$^+$ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 – 8.02 (m, 3H), 7.97 – 7.85 (m, 5H), 7.81 (t, *J* = 8.5 Hz, 1H), 7.68 (dq, *J* = 17.1, 8.7, 7.7 Hz, 10H), 7.41 (dd, *J* = 13.7, 7.9 Hz, 2H), 7.29 (s, 2H), 7.21 (dd, *J* = 7.6, 4.7 Hz, 2H), 7.02 (s, 7H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 152.98, 151.72, 146.87, 137.72, 137.63, 136.87, 136.61, 136.54, 136.24, 136.21, 136.17, 136.15, 136.08, 135.94, 135.63, 135.58, 134.15, 134.04, 133.96, 133.76, 133.73, 133.67, 133.65, 133.13, 133.01, 130.33, 130.21, 129.29, 129.17, 127.93, 126.98, 126.97, 125.53, 124.93, 121.76. ^{31}P NMR (162 MHz, Chloroform-d) δ 52.38 (d, *J* = 11.4 Hz), 51.33 (d, *J* = 11.4 Hz). HRMS (ESI) (m/z): calcd. for $C_{43}H_{32}AuClNP_2$ $[M\text{-}Cl]^+$ 856.1364, found: 856.1367. Anal. Calcd. for $C_{43}H_{32}AuCl_2NP_2$ 1.75H_2O: C, 55.89; H, 3.87; N, 1.52. Found: C, 55.77; H, 3.95; N, 1.64.

AuPhos-87: (2-(p-tolyl)pyridine)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(2-(p-tolyl)pyridine)gold(III) (0.084 g, 0.192 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added. 1,2-Bis(diphenylphosphino)benzene (0.089 g, 0.199 mmol) was added, the solution turned yellow instantly. The solution was stirred for about 14 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf$^+$ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 7.97 (m, 3H), 7.80 (dt, *J* = 17.3, 8.6 Hz, 5H), 7.67 (dd, *J* = 15.5, 6.3 Hz, 6H), 7.58 – 7.35 (m, 6H), 7.30 (s, 4H), 7.22 – 7.09 (m, 4H), 7.00 (d, *J* = 7.9 Hz, 1H), 6.79 – 6.67 (m, 2H), 2.11 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 147.01, 137.69, 136.43, 136.34, 136.08, 136.06, 135.63, 134.17, 134.14, 134.06, 133.95, 133.68, 133.65, 133.45, 133.34, 130.29, 130.29, 130.18, 129.86, 129.73, 129.28, 129.19, 128.32, 125.38, 124.84, 121.99, 121.28, 120.48, 21.33. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 52.08 (d, *J* = 12.0 Hz), 51.51 (d, *J* = 12.1 Hz). HRMS (ESI) (m/z): calcd. for $C_{42}H_{34}AuClNP_2$ [M-Cl]$^+$ 846.1520, found: 846.1513. Anal. Calcd. for $C_{42}H_{34}AuCl_2NP_2$ 1.7H_2O: C, 55.24; H, 4.13; N, 1.53. Found: C, 55.20; H, 4.10; N, 1.59.

AuPhos-88: (4-(2-pyridyl)benzaldehyde)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(4-(2-pyridyl)benzaldehyde)gold(III) (0.077 g, 0.172 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added. 1,2-Bis(diphenylphosphino)benzene (0.082 g, 0.182 mmol) was added, the solution turned pale pink instantly. The solution was stirred for about 13 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf$^+$ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.78 (s, 1H), 8.05 (d, *J* = 30.8 Hz, 3H), 7.94 (d, *J* = 9.9 Hz, 1H), 7.83 (dd, *J* = 12.3, 7.5 Hz, 6H), 7.77 – 7.64 (m, 7H), 7.51 (dd, *J* = 15.9, 8.4 Hz, 4H), 7.31 (s, 4H), 7.16 (s, 4H), 6.91 (s, 1H), 6.80 (s, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 191.32, 155.80, 147.47, 138.14, 136.95, 136.69, 136.62, 136.35, 136.13, 135.99, 135.97, 135.75, 135.70, 134.49, 134.46, 134.09, 133.98, 133.91, 133.88, 133.39, 133.27, 130.38, 130.27, 130.13, 130.00, 129.66, 129.57, 128.80, 125.00, 124.44, 123.52, 122.09, 121.73, 121.04. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 53.02 (d, *J* = 9.6 Hz), 52.69 (d, *J* = 9.4 Hz). HRMS (ESI) (m/z): calcd. for $C_{42}H_{32}AuClNOP_2$ [M-Cl]$^+$ 860.1313, found: 860.1282. Anal. Calcd. for $C_{42}H_{32}AuCl_2NOP_2$ 1.4H_2O: C, 54.73; H, 3.81; N, 1.52. Found: C, 54.73; H, 3.85; N, 1.55.

AuPhos-89: (2-phenylpyridine)[1,2-Bis(diphenylphosphino)benzene]gold(III) dichloride

Under normal atmospheric conditions, dichloro(2-phenylpyridine)gold(III) (0.152 g, 0.360 mmol) was placed in a 100 mL round bottom flask and 10 mL of chloroform was added, the solution turned brown. 1,2-Bis(diphenylphosphino)benzene (0.170 g, 0.380 mmol) was added, the solution turned yellow instantly. The solution was stirred for about 11 minutes. The solution was monitored by TLC in 5% MeOH in CH_2Cl_2 as an eluent. Separation of compound was achieved via flash chromatography using CombiFlashR Rf^+ Lumen with 5:95/MeOH:CH_2Cl_2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 – 7.95 (m, 3H), 7.78 (d, *J* = 8.7 Hz, 5H), 7.65 (dd, *J* = 17.7, 6.2 Hz, 7H), 7.52 – 7.34 (m, 5H), 7.21 – 7.01 (m, 6H), 6.83 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.23, 147.10, 140.32, 137.78, 136.33, 136.26, 136.06, 136.01, 135.91, 135.17, 135.14, 134.20, 133.99, 133.90, 133.68, 133.33, 133.23, 130.61, 130.52, 130.24, 130.22, 130.15, 130.12, 129.87, 129.75, 129.65, 129.57, 127.41, 125.25, 124.68, 122.42, 121.88, 121.16, 120.86. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 51.94 (d, *J* = 13.0 Hz), 51.84 (d, *J* = 11.3 Hz). HRMS (ESI) (m/z): calcd. for $C_{41}H_{32}AuClNP_2$ $[M\text{-}Cl]^+$ 832.1364, found: 832.1365. Anal. Calcd. for $C_{41}H_{32}AuCl_2NP_2$ $1.7H_2O$: C, 54.77; H, 3.97; N, 1.56. Found: C, 54.76; H, 3.99; N, 1.53.

NMR spectra data

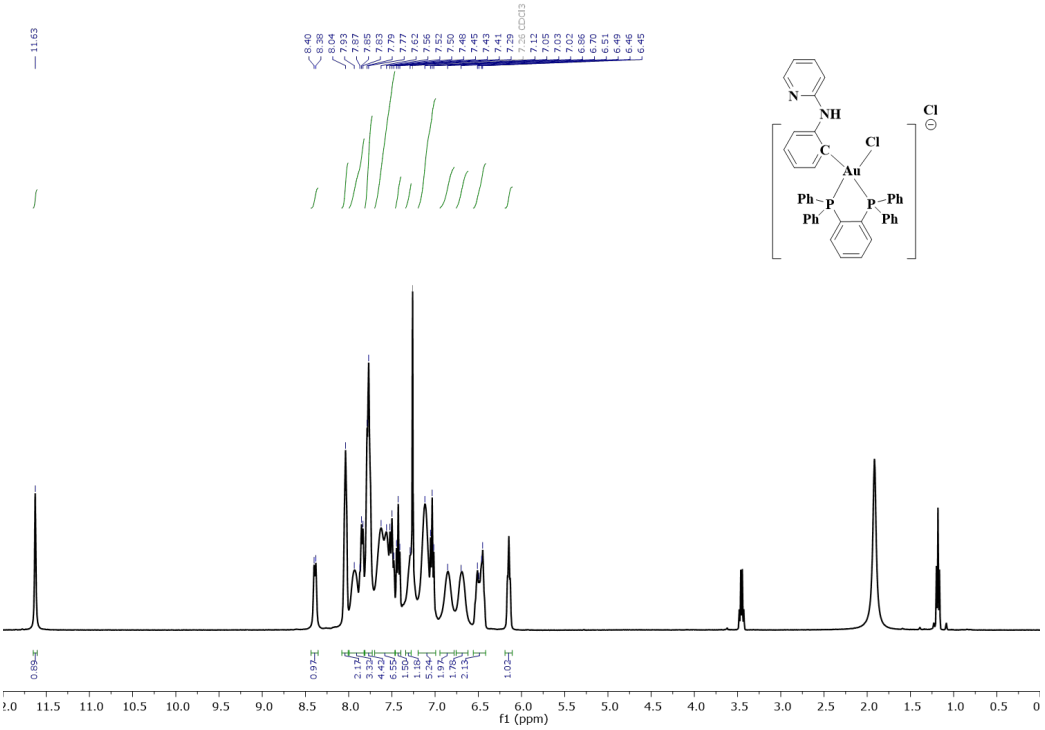

Figure S1. ^{1}H NMR spectrum of complex AuPhos-81 in $CDCl_3$ at 298K

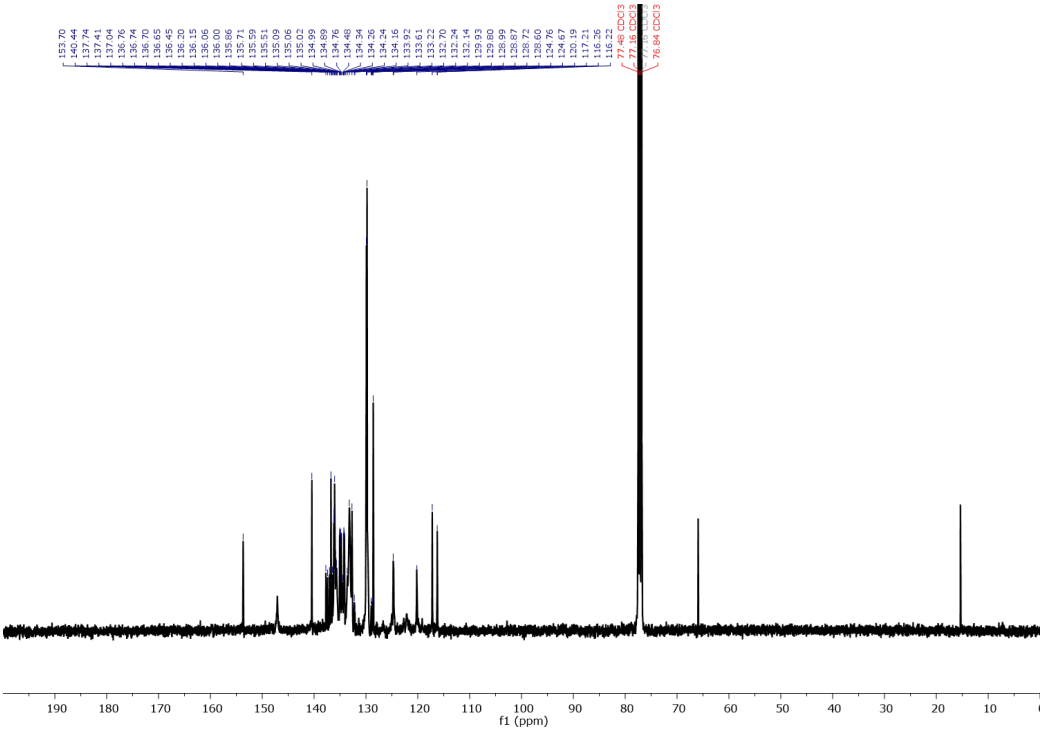

Figure S2. ^{13}C{^{1}H} NMR spectrum of complex AuPhos-81 in $CDCl_3$ at 298K, impurity(ether) at 66 & 15 ppm

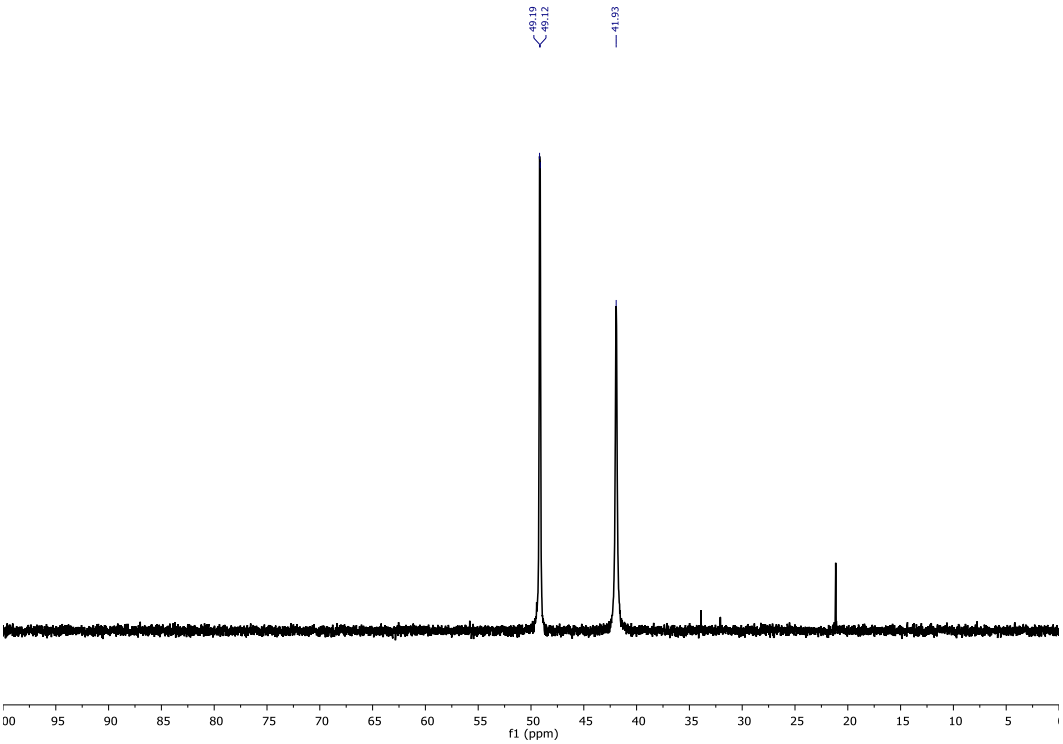

Figure S3. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-81 in $CDCl_3$ at 298K, impurity at 34, 32 & 21 ppm

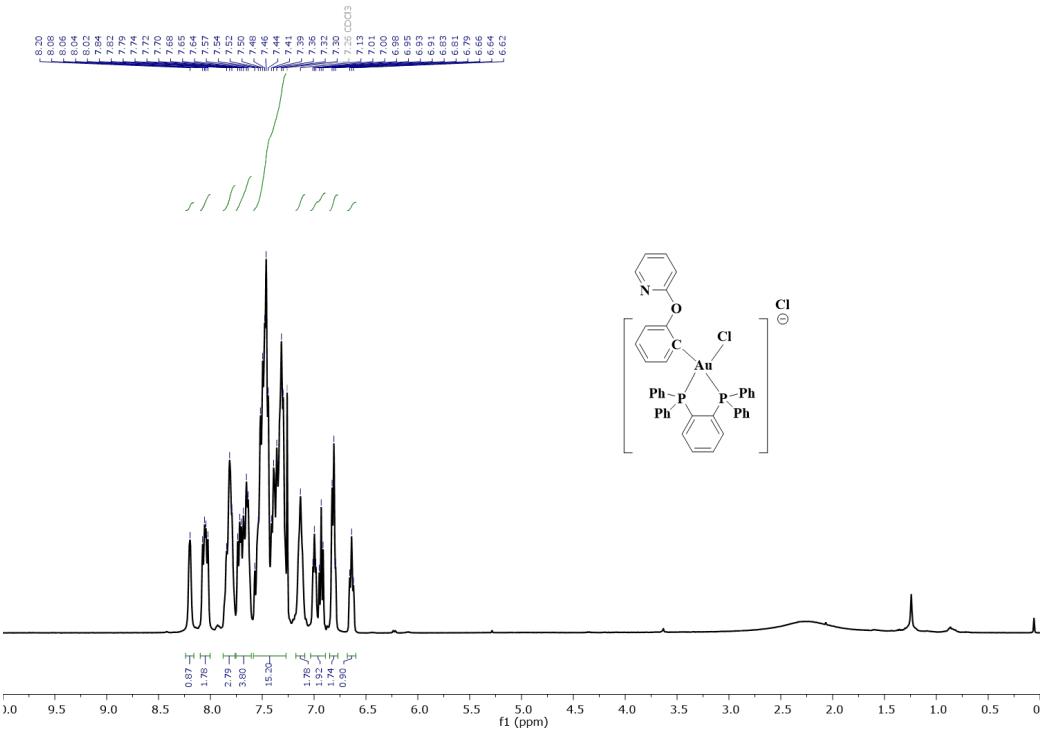

Figure S4. ^{1}H NMR spectrum of complex AuPhos-82 in $CDCl_3$ at 298K

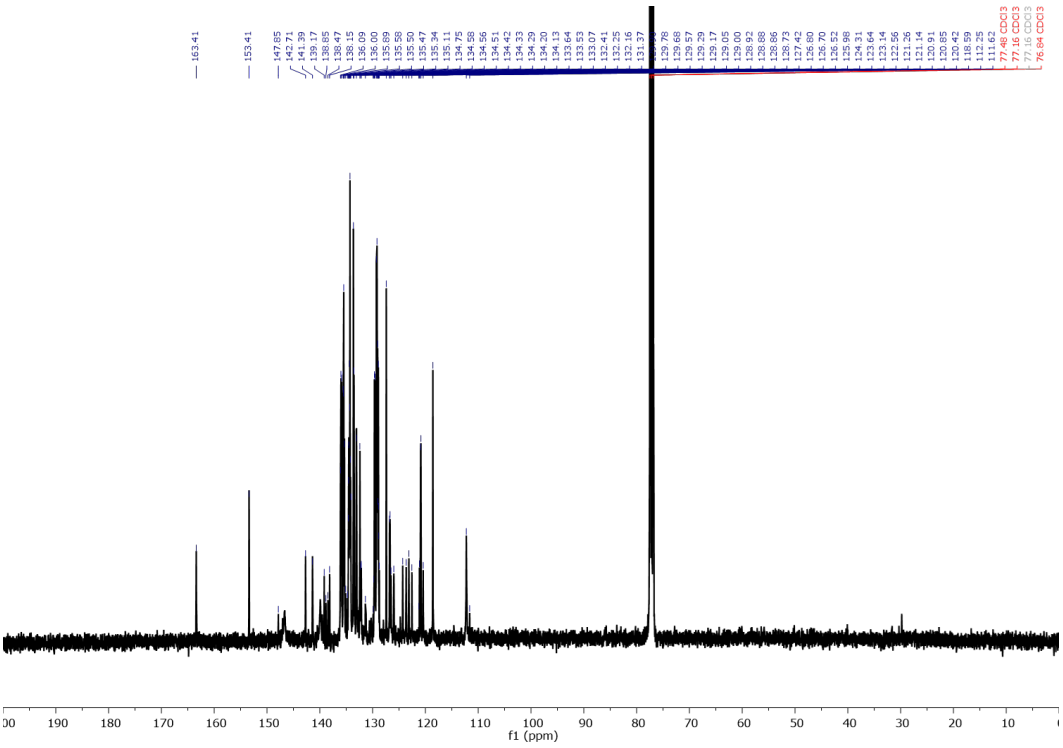

Figure S5. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-82 in $CDCl_3$ at 298K

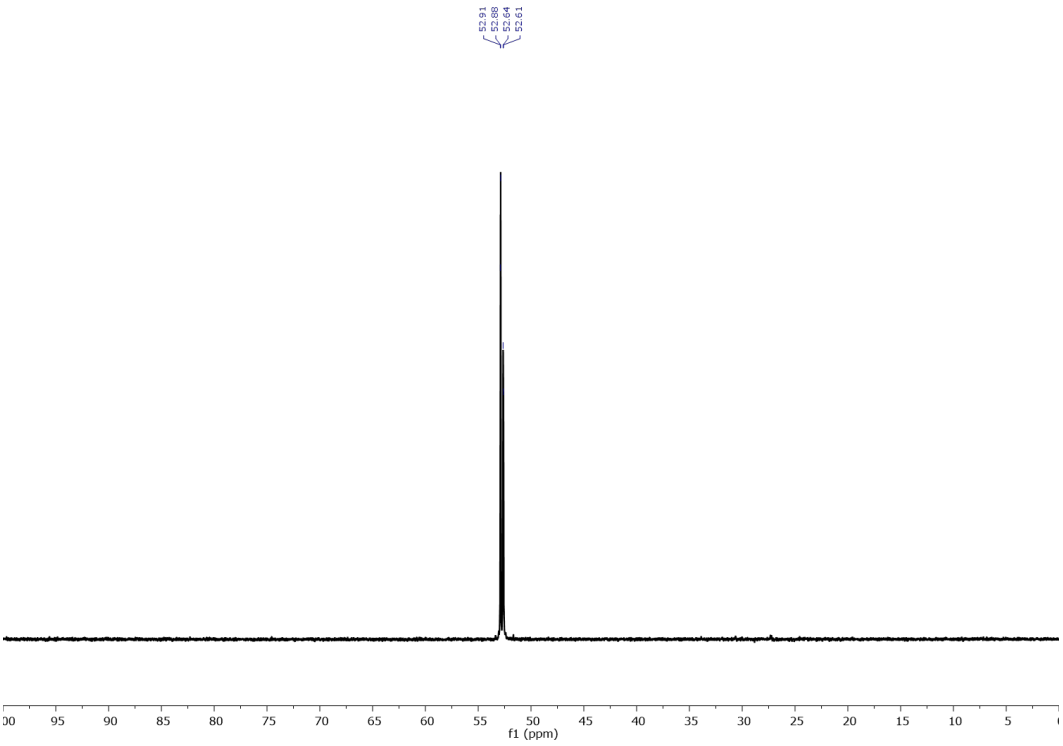

Figure S6. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-82 in $CDCl_3$ at 298K

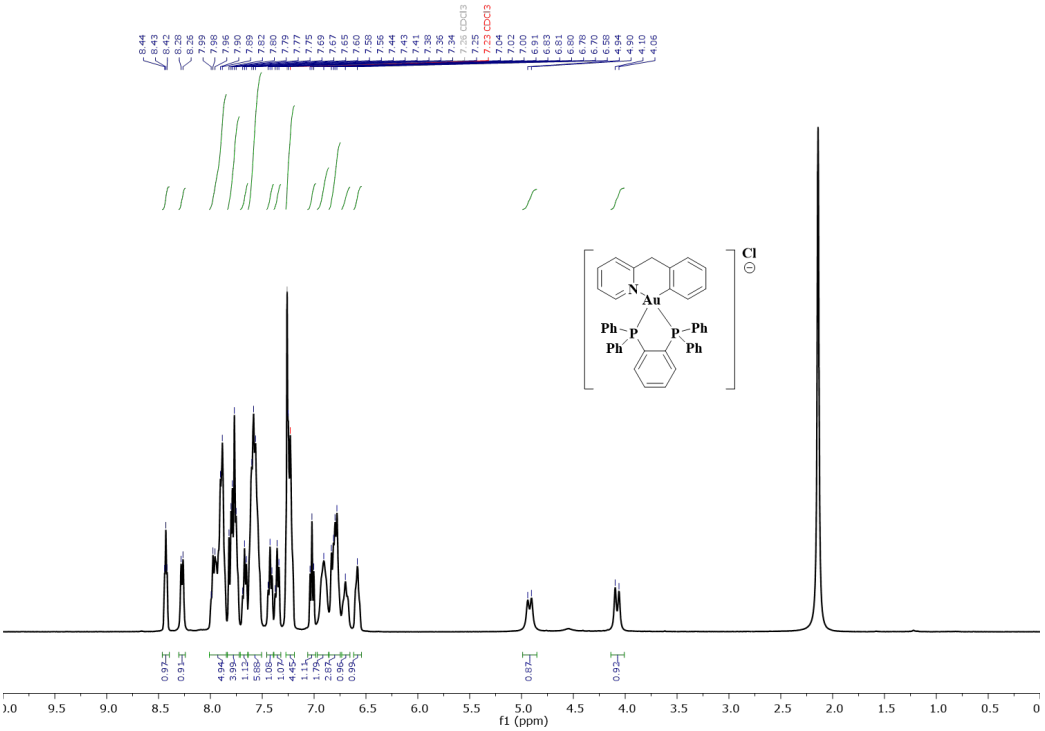

Figure S7. ^{1}H NMR spectrum of complex AuPhos-83 in $CDCl_3$ at 298K

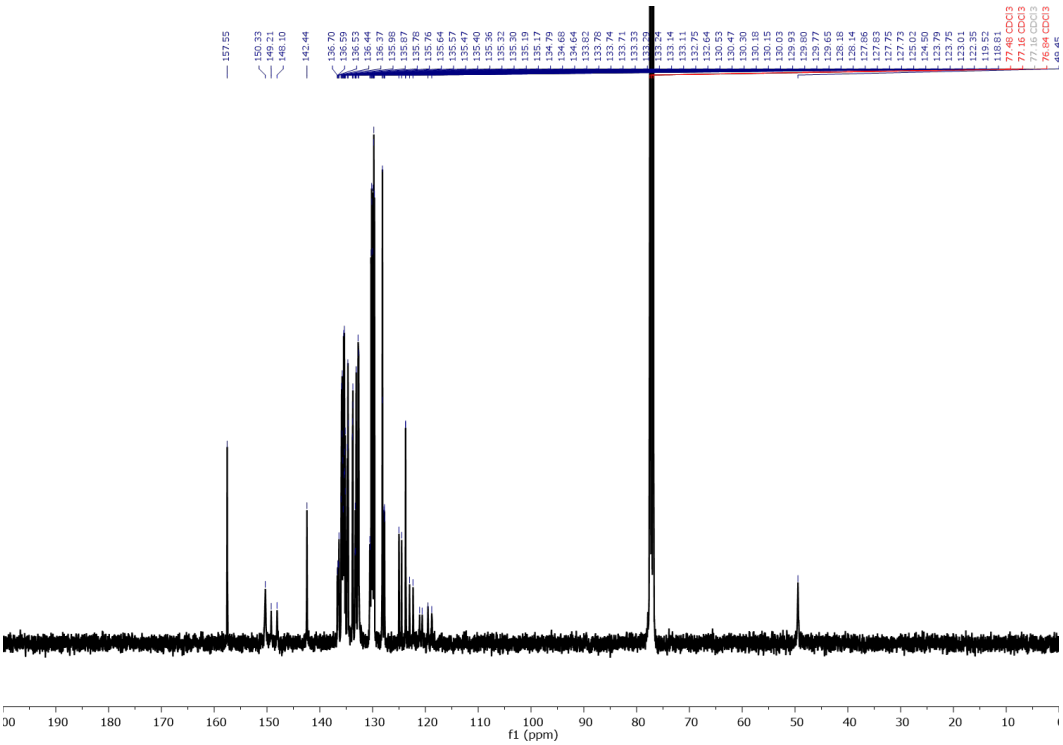

Figure S8. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-83 in $CDCl_3$ at 298K

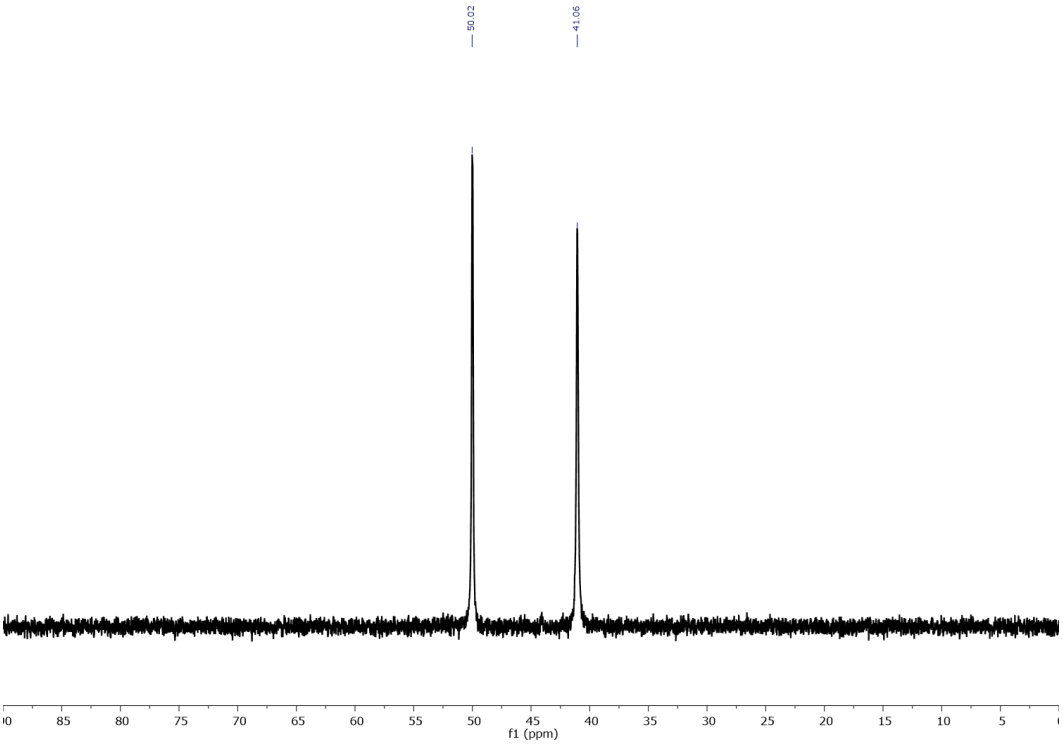

Figure S9. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-83 in $CDCl_3$ at 298K

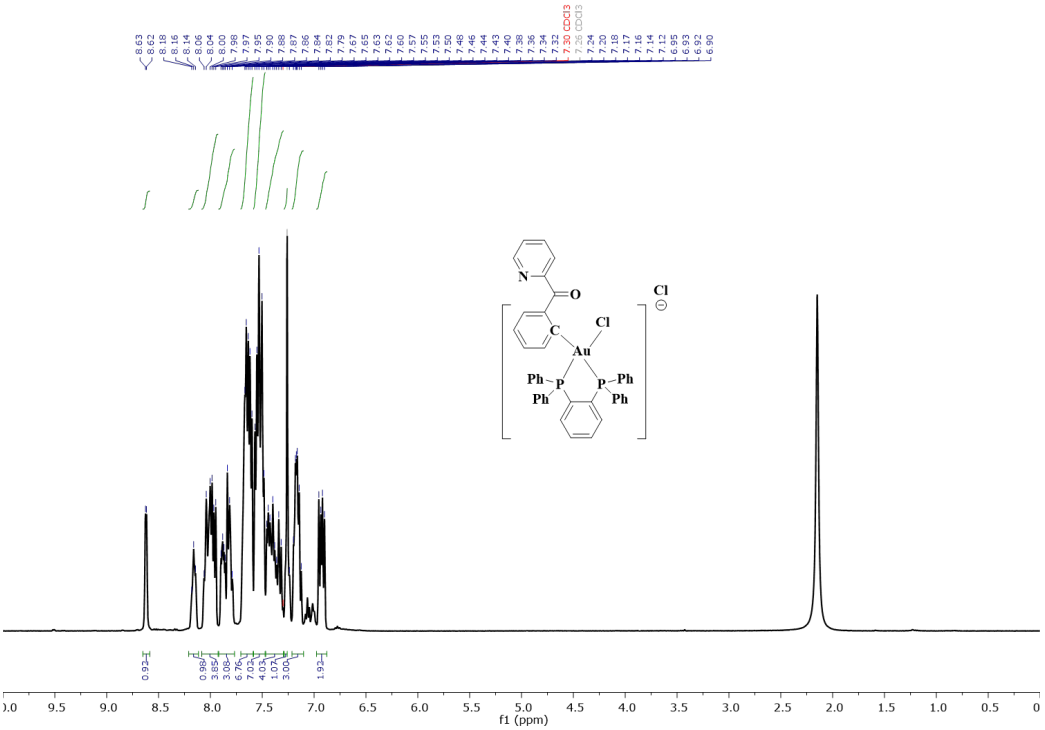

Figure S10. ^{1}H NMR spectrum of complex AuPhos-84 in $CDCl_3$ at 298K

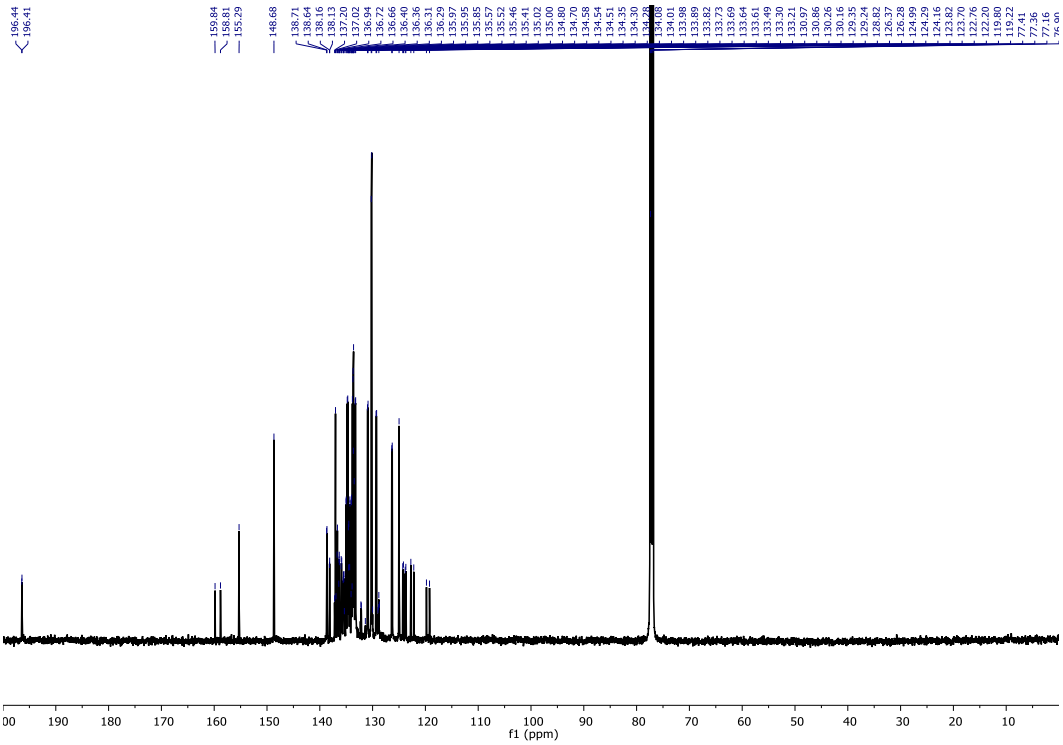

Figure S11. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-84 in $CDCl_3$ at 298K

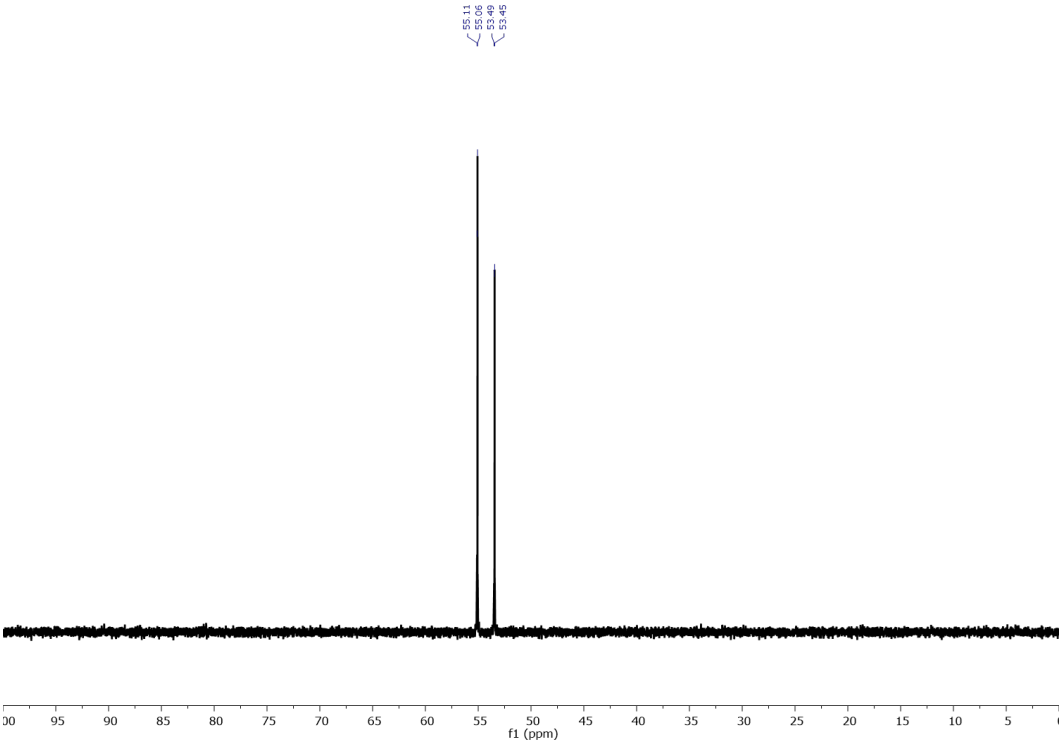

Figure S12. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-84 in $CDCl_3$ at 298K

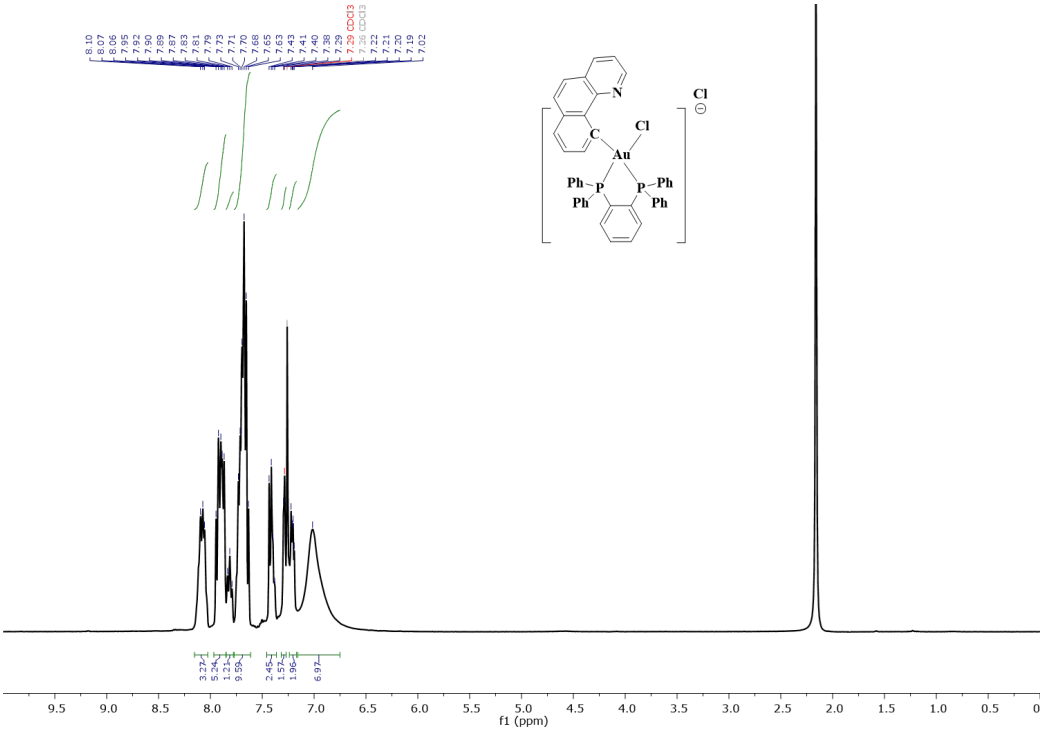

Figure S13. ^{1}H NMR spectrum of complex AuPhos-85 in $CDCl_3$ at 298K

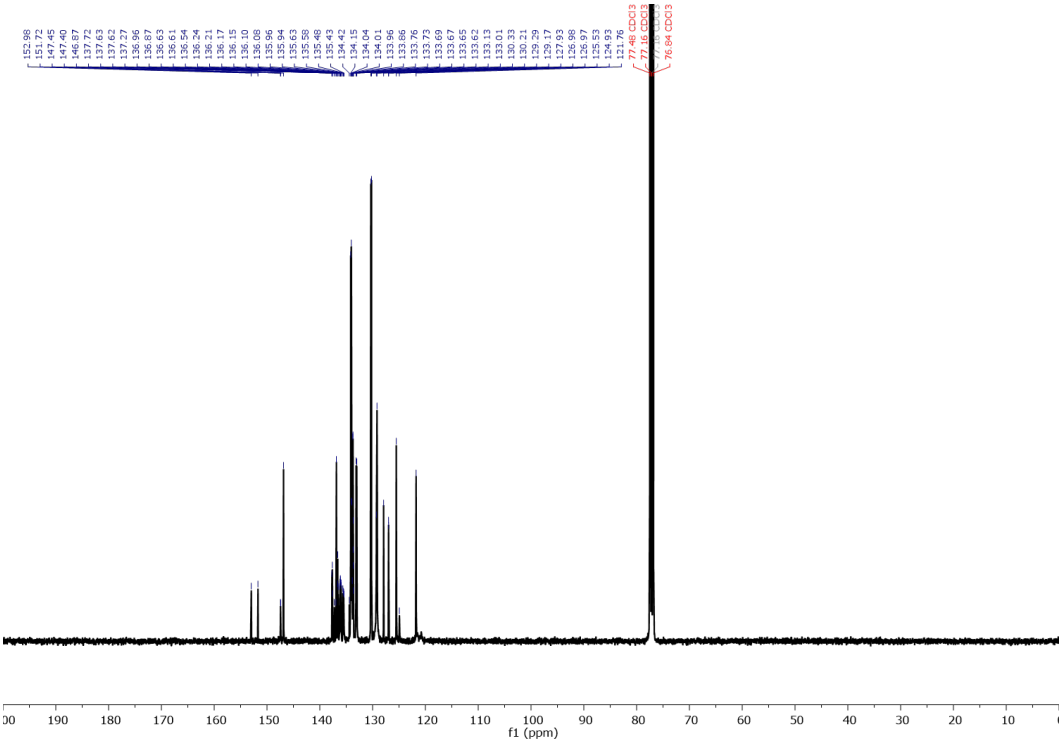

Figure S14. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-85 in $CDCl_3$ at 298K

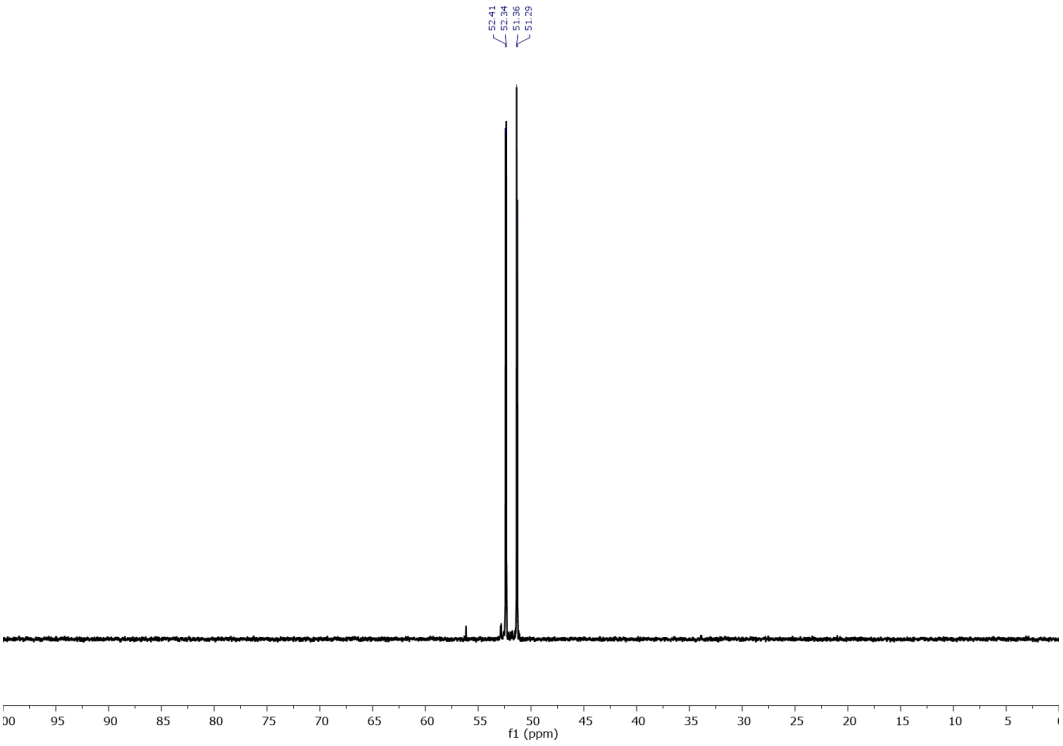

Figure S15. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-85 in $CDCl_3$ at 298K

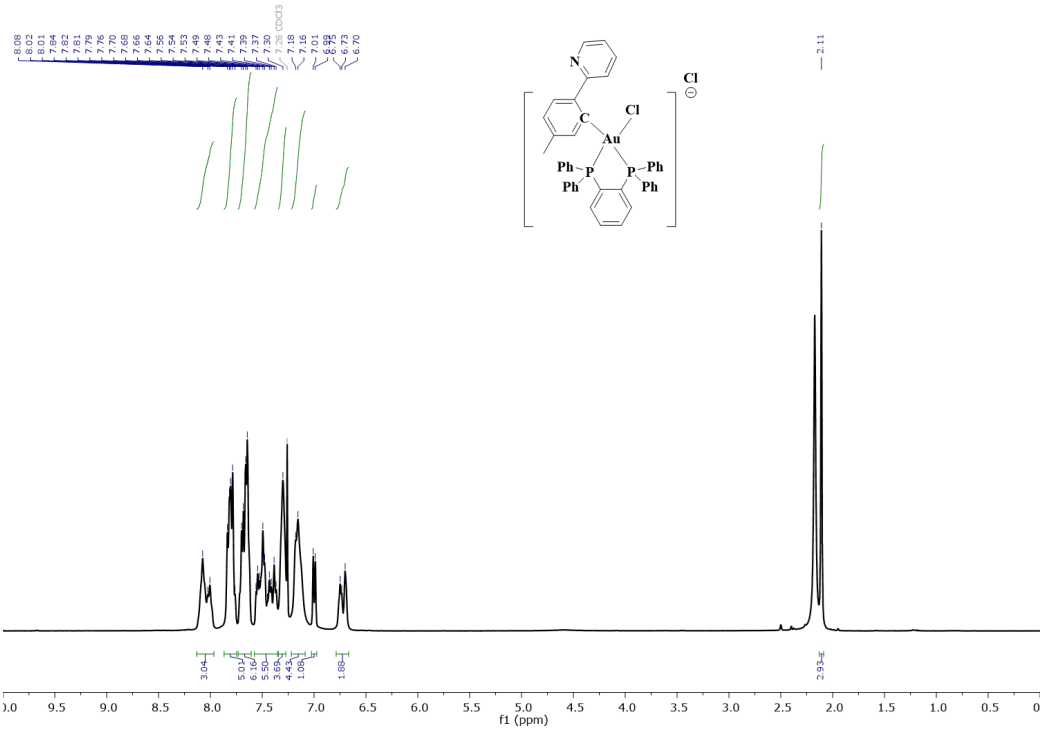

Figure S16. ^{1}H NMR spectrum of complex AuPhos-86 in $CDCl_3$ at 298K

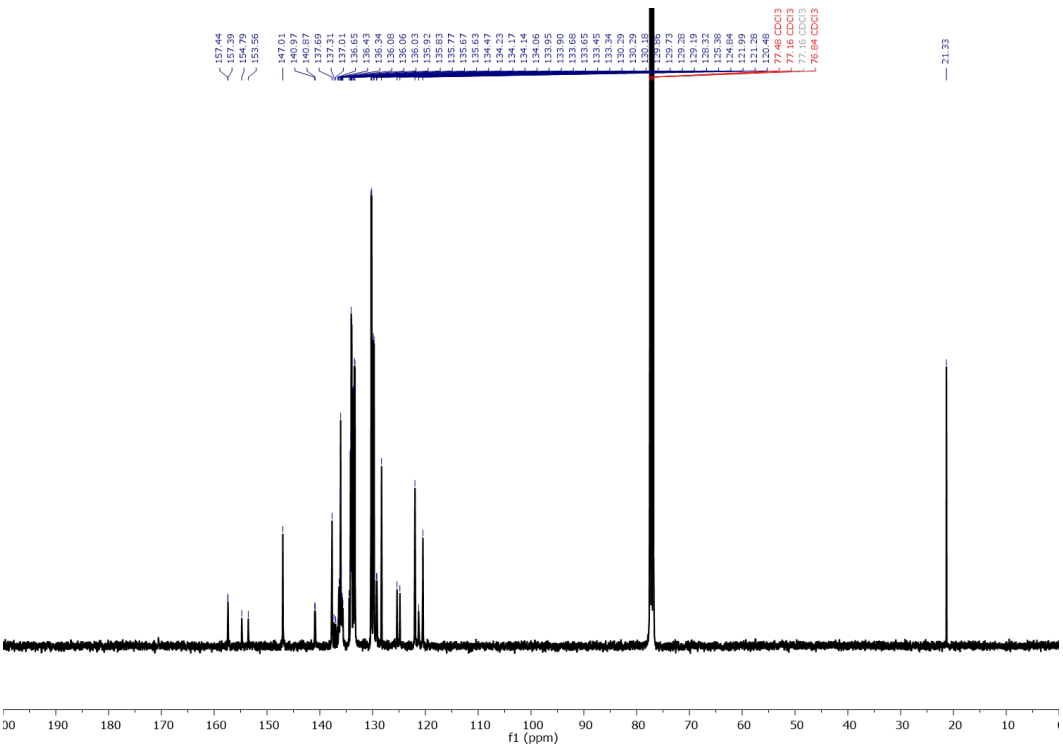

Figure S17. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-86 in $CDCl_3$ at 298K

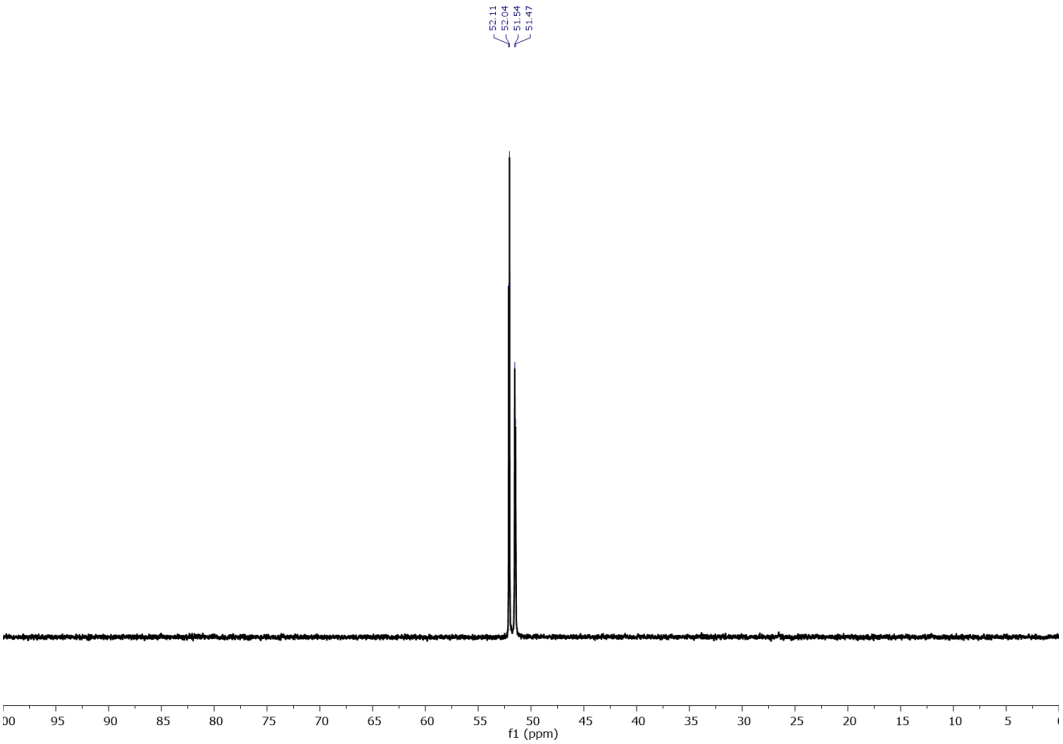

Figure S18. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-86 in $CDCl_3$ at 298K

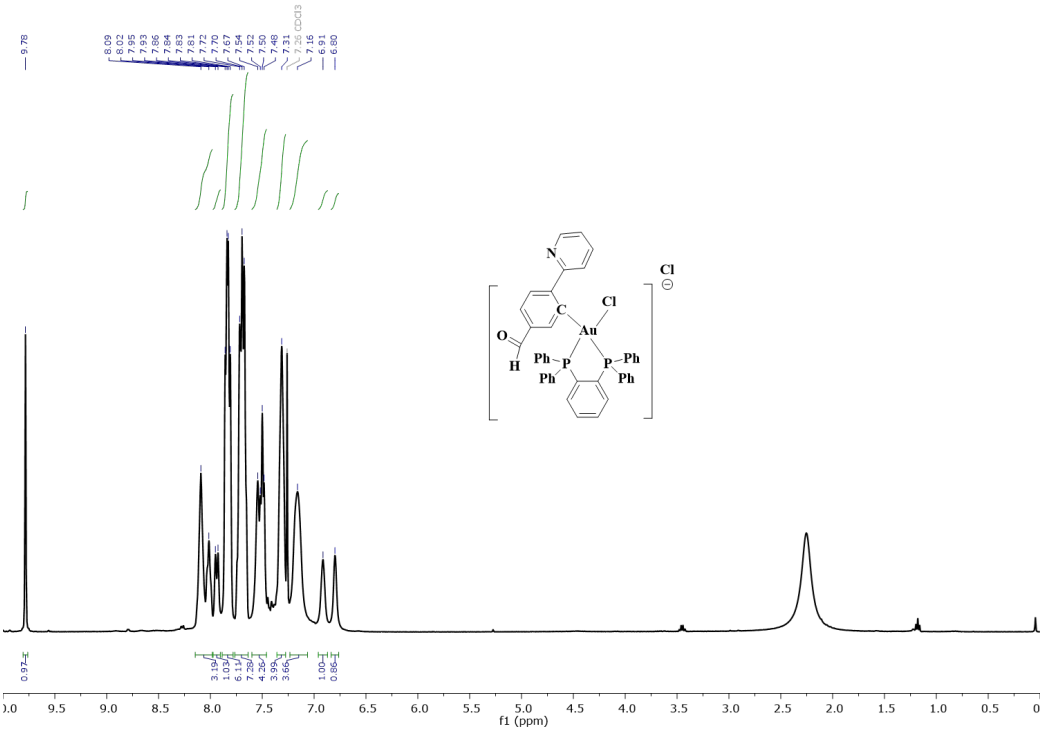

Figure S19. ^{1}H NMR spectrum of complex AuPhos-88 in $CDCl_3$ at 298K

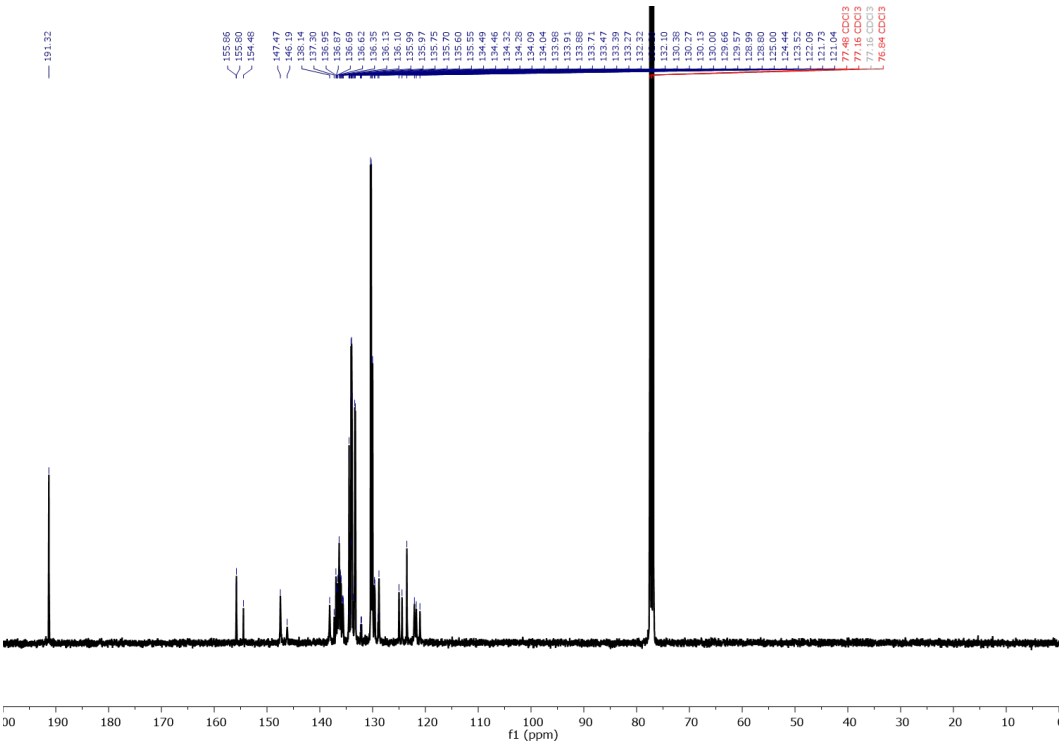

Figure S20. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-88 in $CDCl_3$ at 298K

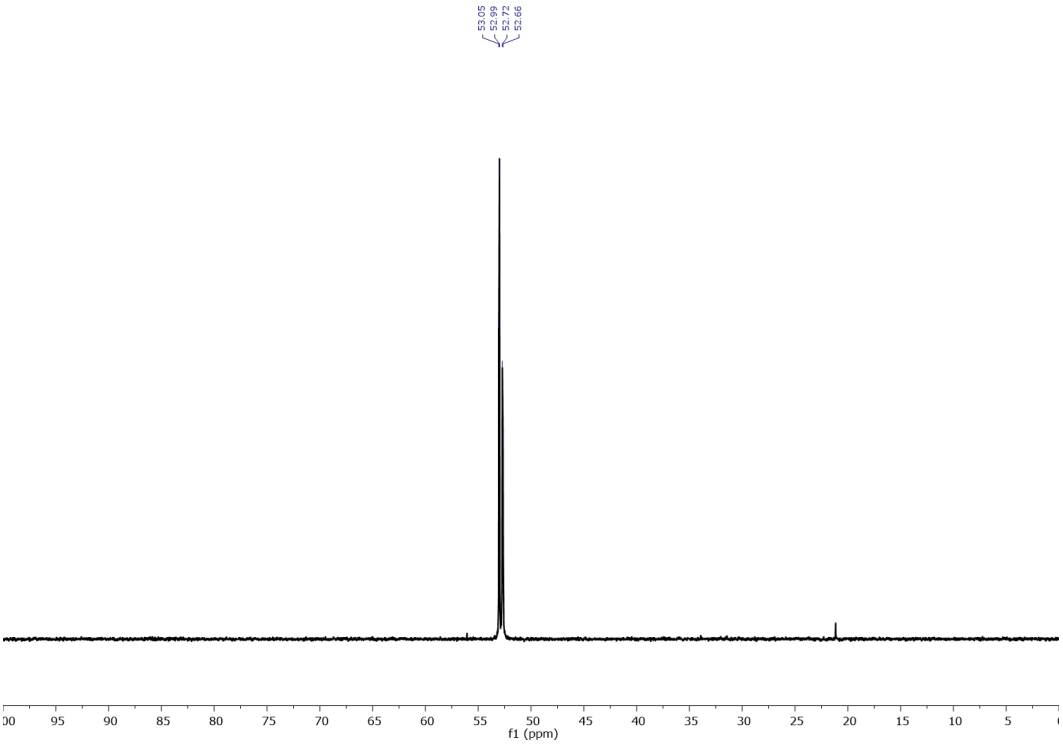

Figure S21. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-88 in $CDCl_3$ at 298K, impurity at 21 ppm

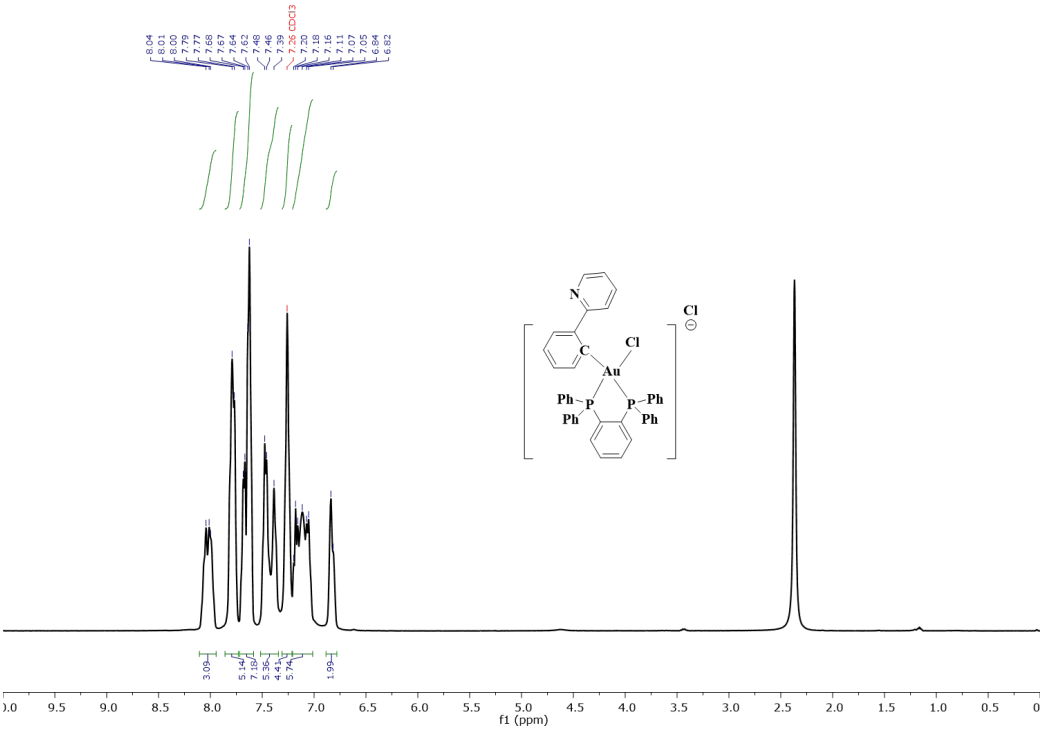

Figure S22. ^{1}H NMR spectrum of complex AuPhos-89 in $CDCl_3$ at 298K

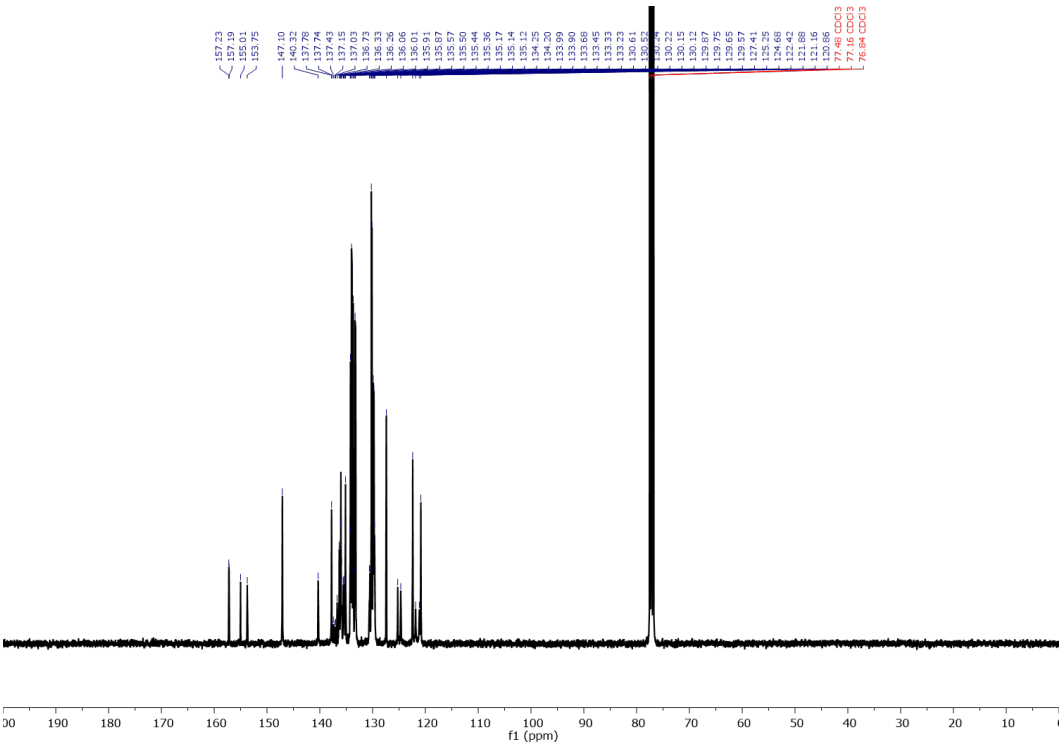

Figure S23. $^{13}C\{^{1}H\}$ NMR spectrum of complex AuPhos-89 in $CDCl_3$ at 298K

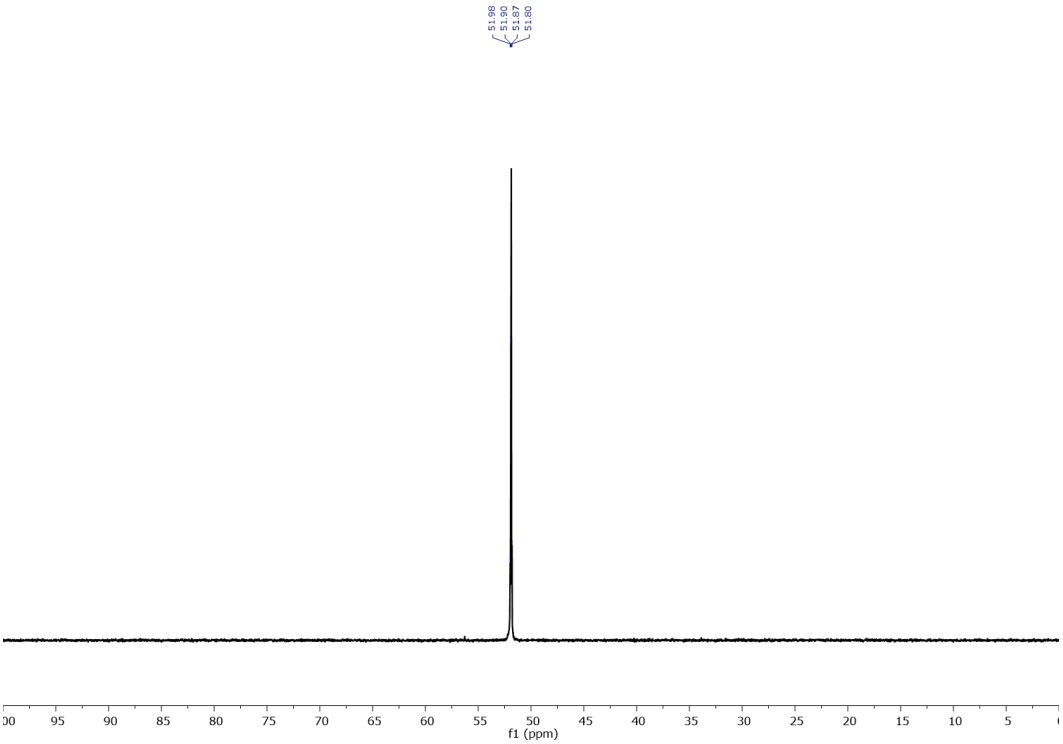

Figure S24. $^{31}P\{^{1}H\}$ NMR spectrum of complex AuPhos-89 in $CDCl_3$ at 298K

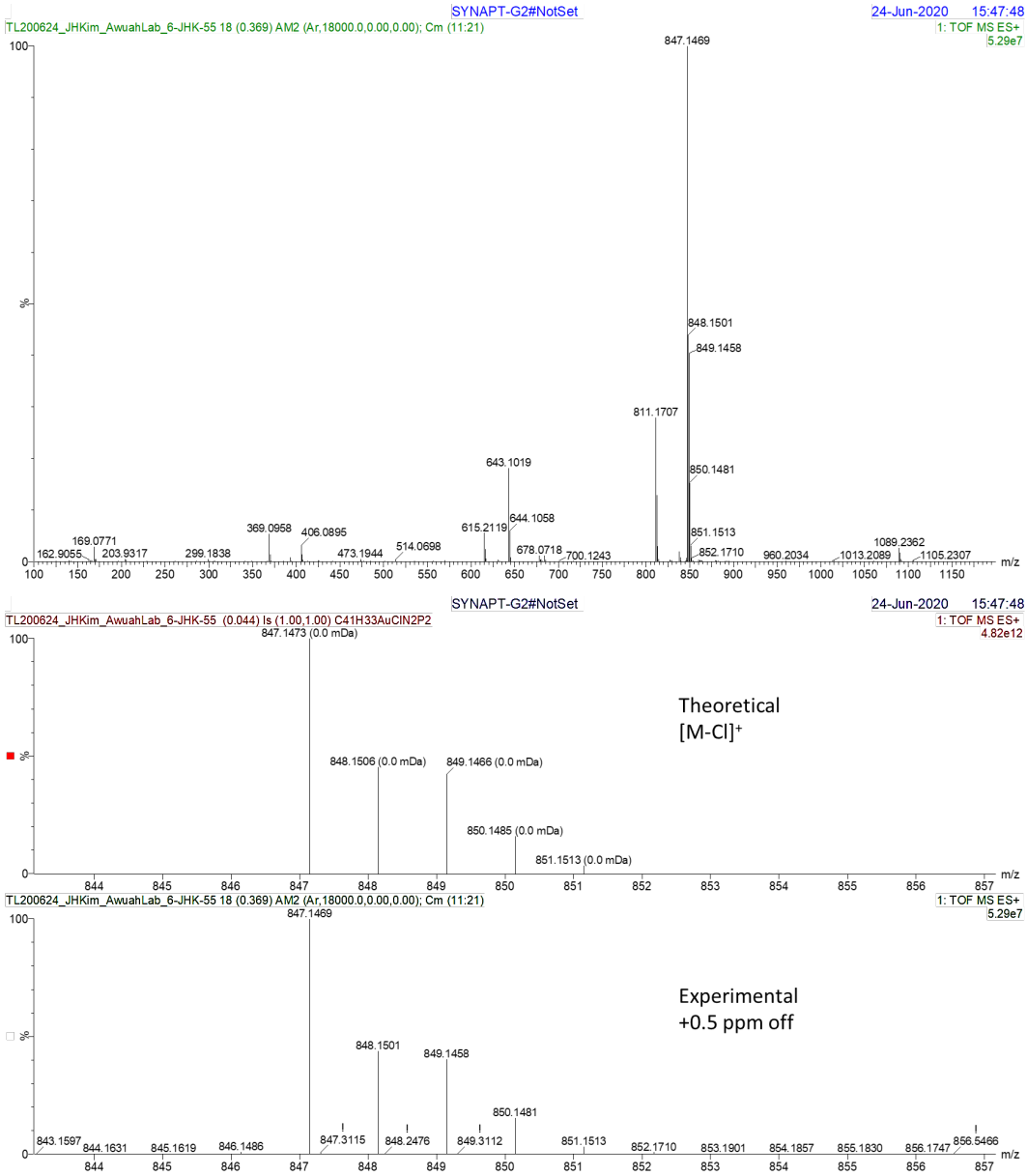

Figure S25. HRMS (ESI) of compound AuPhos-81

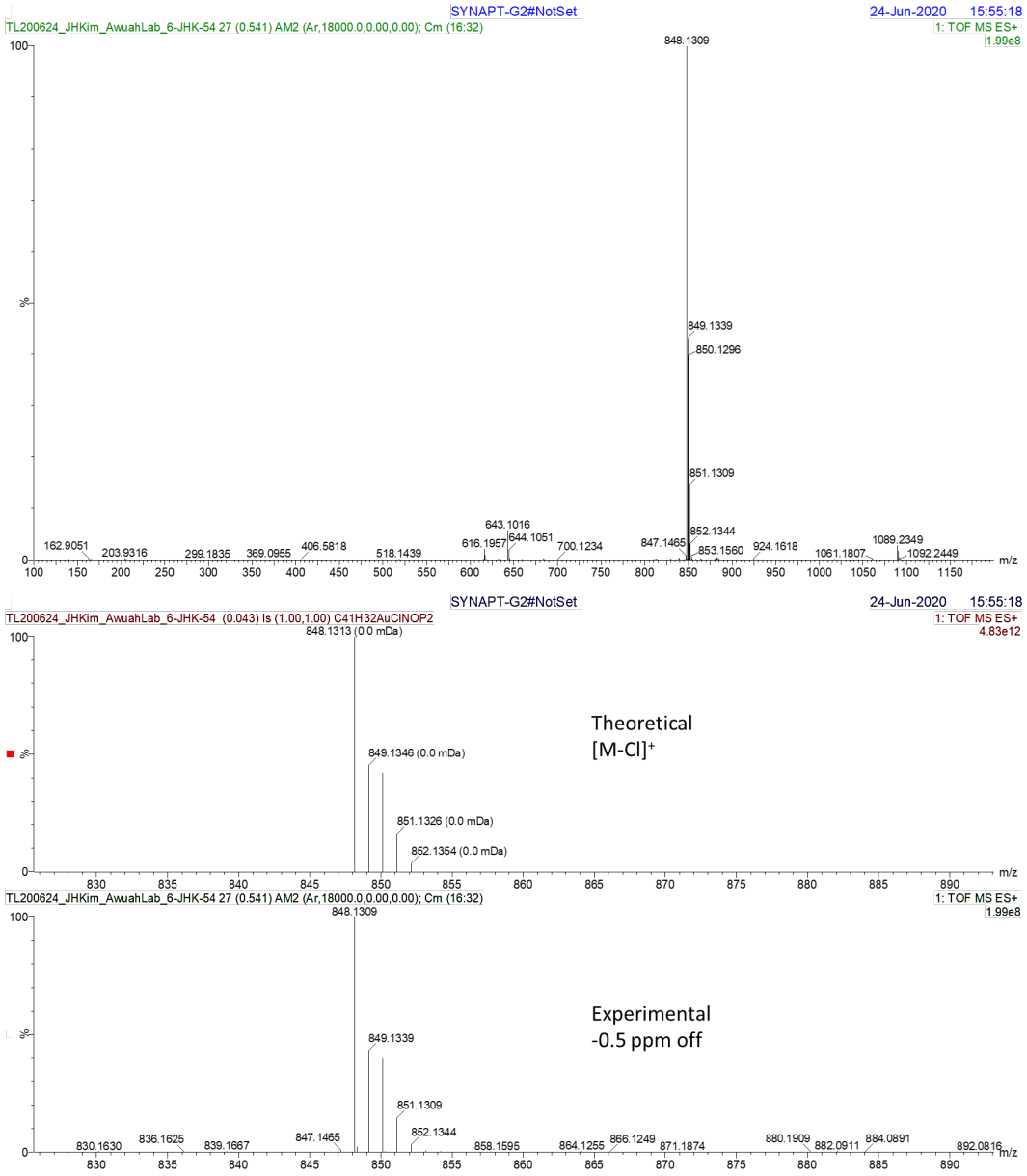

Figure S26. HRMS (ESI) of compound AuPhos-82

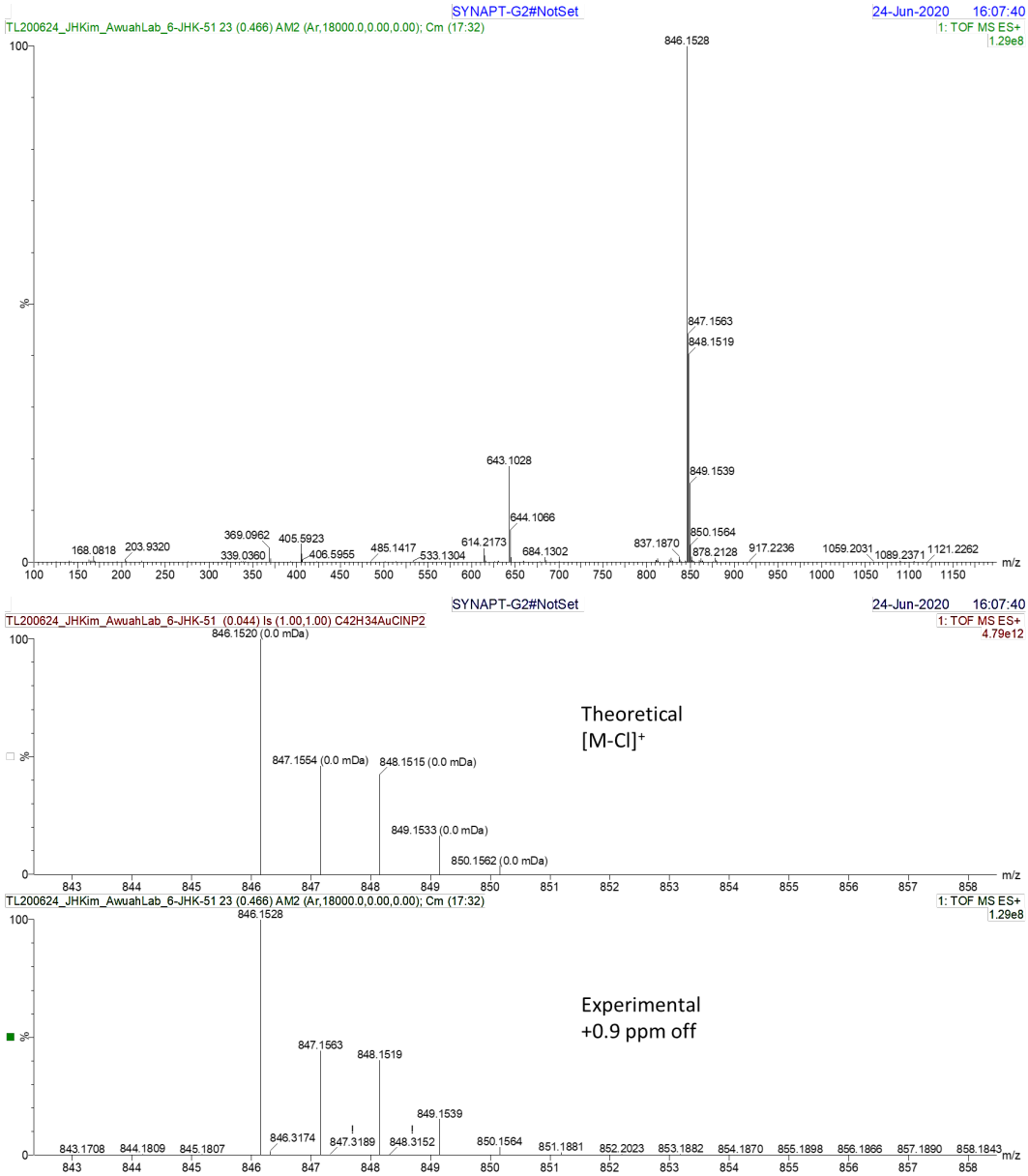

Figure S27. HRMS (ESI) of compound AuPhos-83

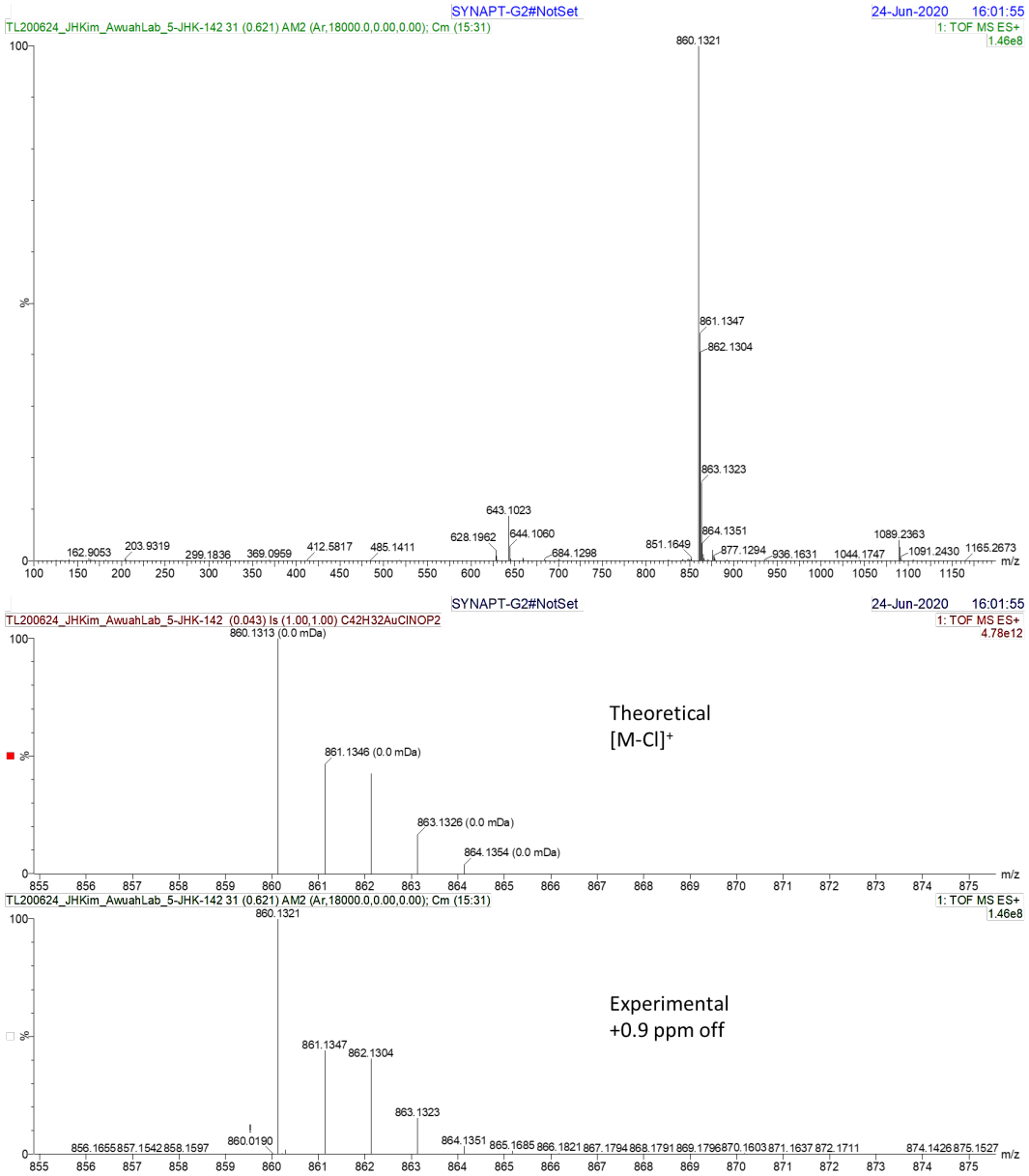

Figure S28. HRMS (ESI) of compound AuPhos-84

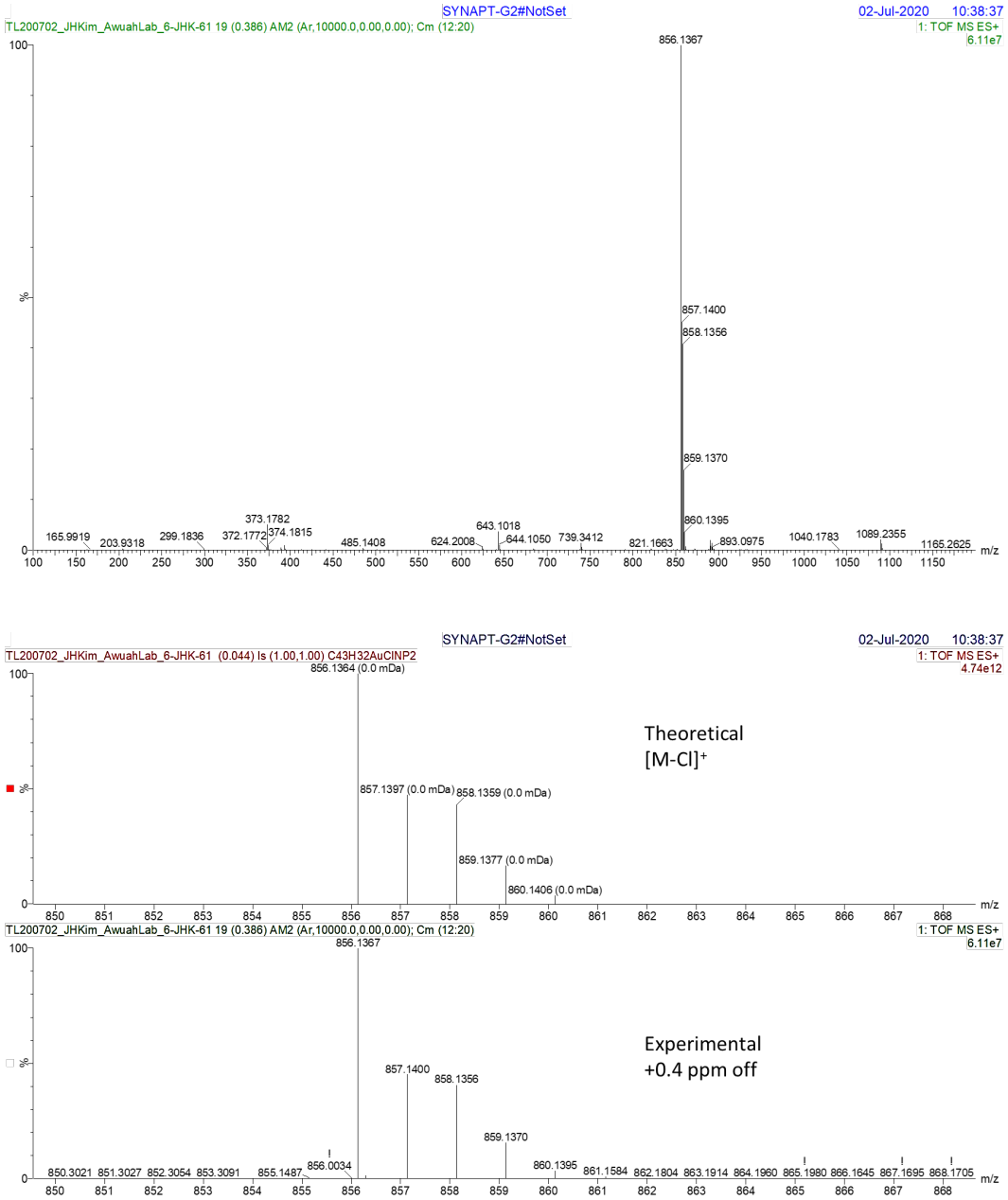

Figure S29. HRMS (ESI) of compound AuPhos-85

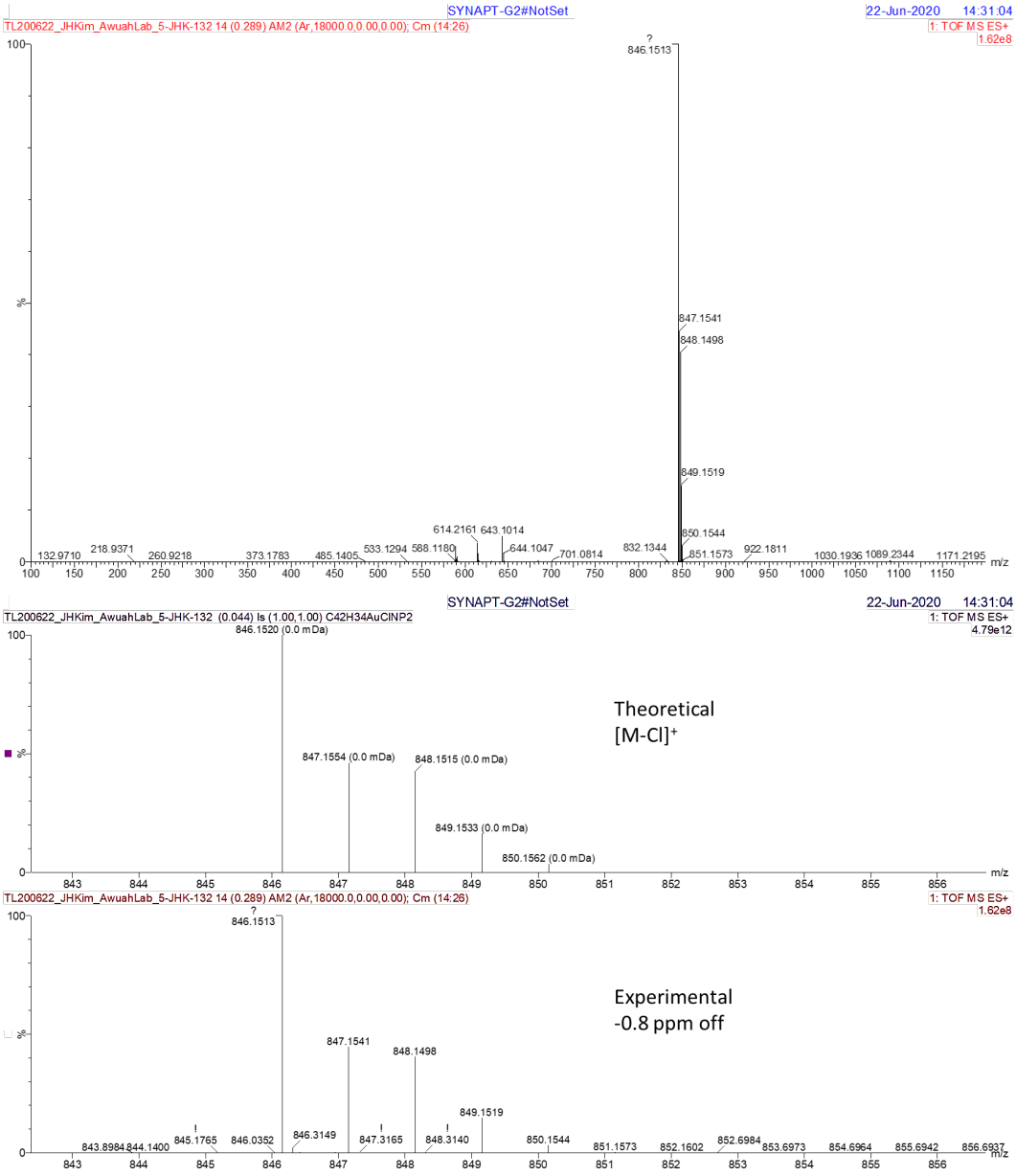

Figure S30. HRMS (ESI) of compound AuPhos-86

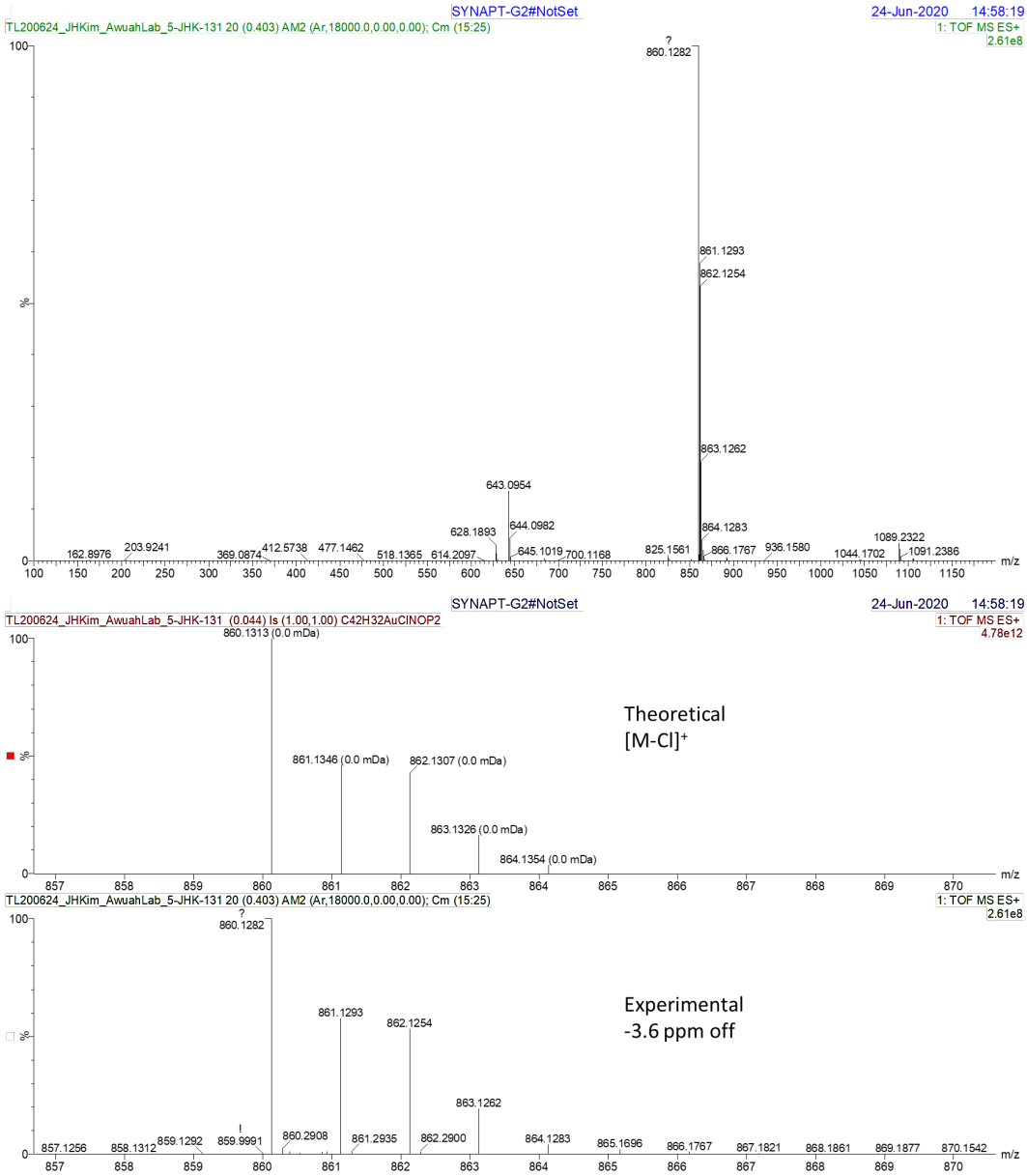

Figure S31. HRMS (ESI) of compound AuPhos-88

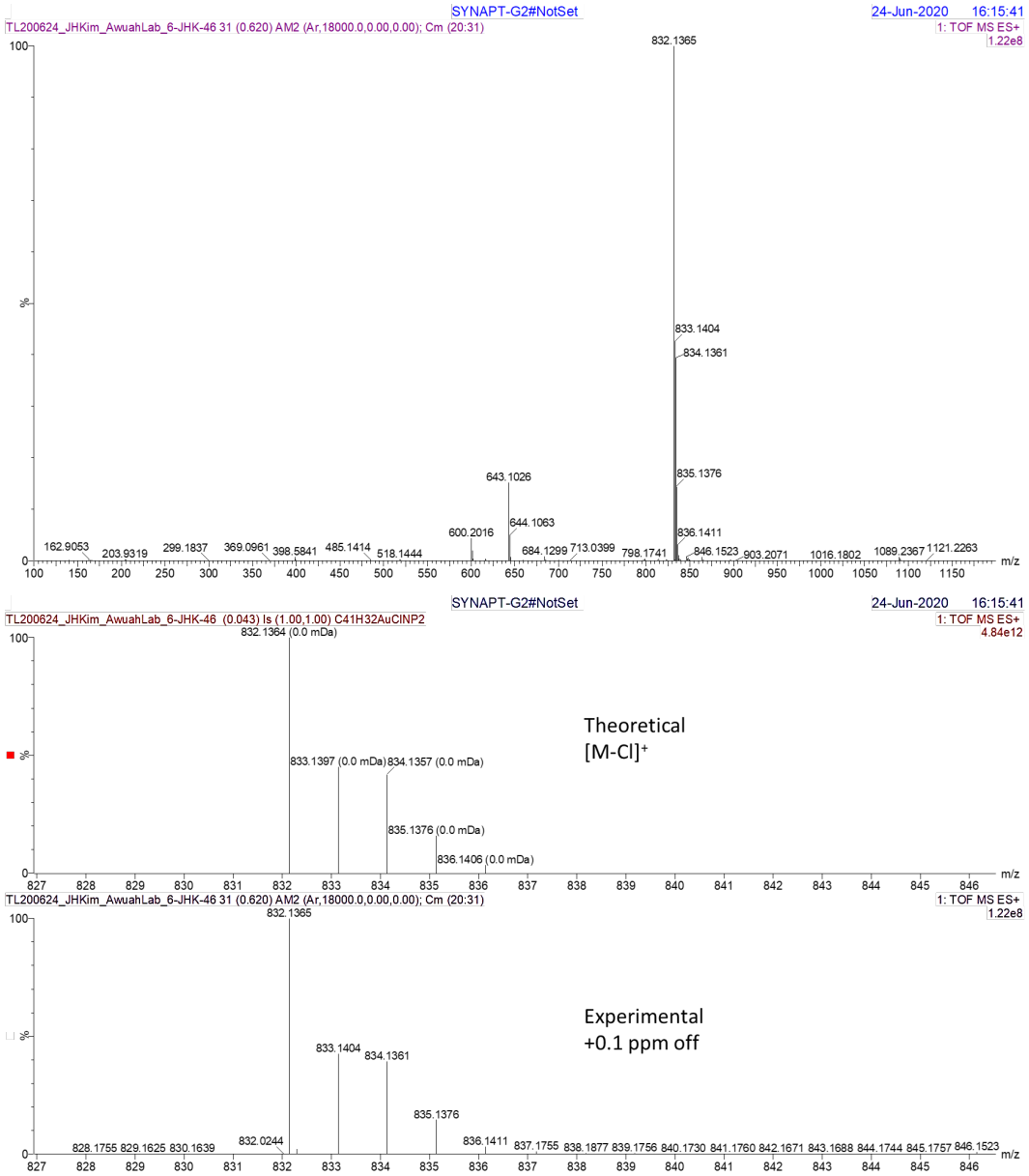

Figure S32. HRMS (ESI) of compound AuPhos-89

Supplementary Figures

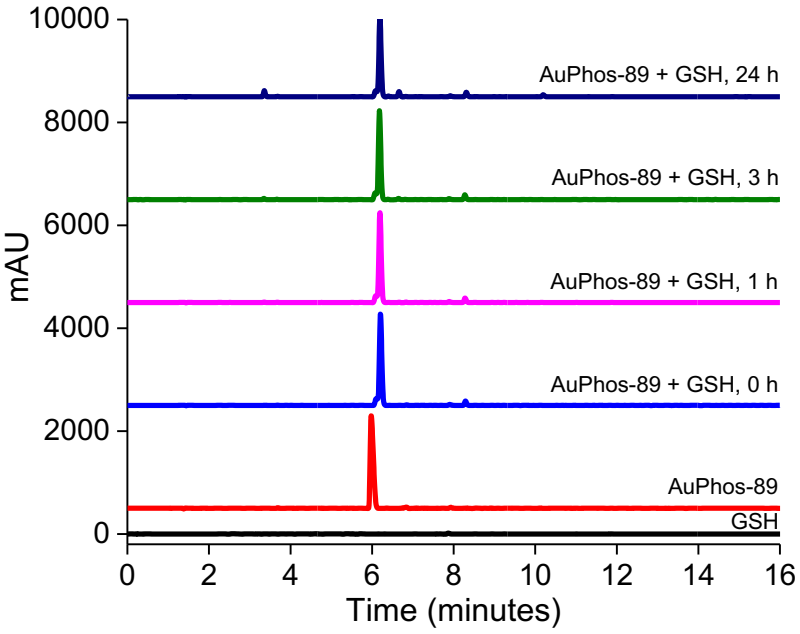

Figure S33. HPLC chromatogram of AuPhos-89 + GSH. (λ = 280 nm).

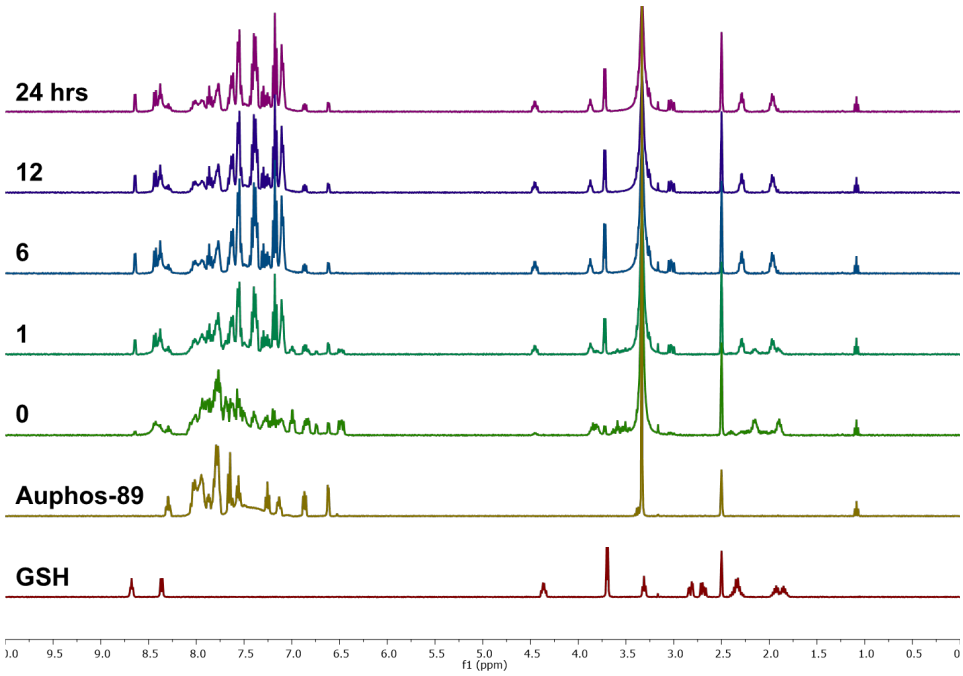

Figure S34. ^{1}H NMR spectra (DMSO-d_6) of AuPhos-89 (20 mM), GSH (20 mM), and AuPhos-89 / GSH (10 mM).

Table S1. IC_{50} values for AuPhos compounds across a panel of cell lines. Cells were seeded at a density of 4,000 cells/well and treated for 72 h. IC_{50} values are plotted as the mean ± SD (n = 3). Full dose response curves can be found in the supporting information (Figure S35-S38).

	IC_{50}(μM)			
	MDA-MB-231 (breast)	H460 (lung)	A2780 (ovarian)	OVCAR8 (ovarian)
AuPhos-82	0.22±0.20	1.04±0.17	0.34±0.04	0.85±0.06
AuPhos-83	0.22±0.07	0.27±0.06	0.22±0.01	0.89±0.14
AuPhos-84	0.24±0.16	0.76±0.15	0.25±0.06	1.24±0.14
AuPhos-85	0.26±0.06	0.34±0.07	0.15±0.03	0.64±0.11
AuPhos-86	0.29±0.04	0.34±0.07	0.33±0.04	0.82±0.44
AuPhos-87	0.45±0.19	0.71±0.09	0.18±0.04	0.82±0.76
AuPhos-88	0.37±0.21	0.40±0.03	0.27±0.02	0.85±0.07
AuPhos-89	0.45±0.14	0.72±0.16	0.34±0.07	1.16±0.04

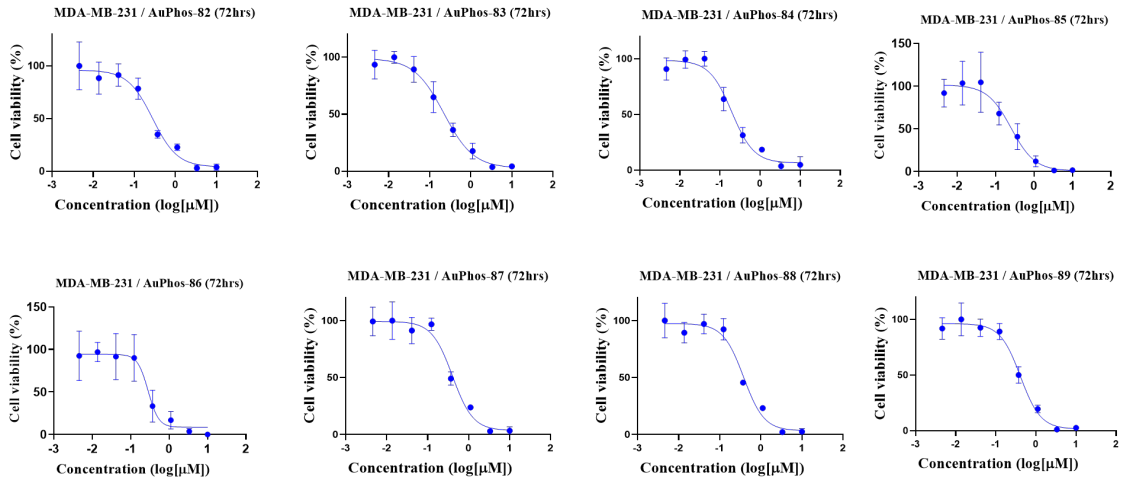

Figure S35. Evaluation of cytotoxicity for AuPhos compounds in MDA-MB-231

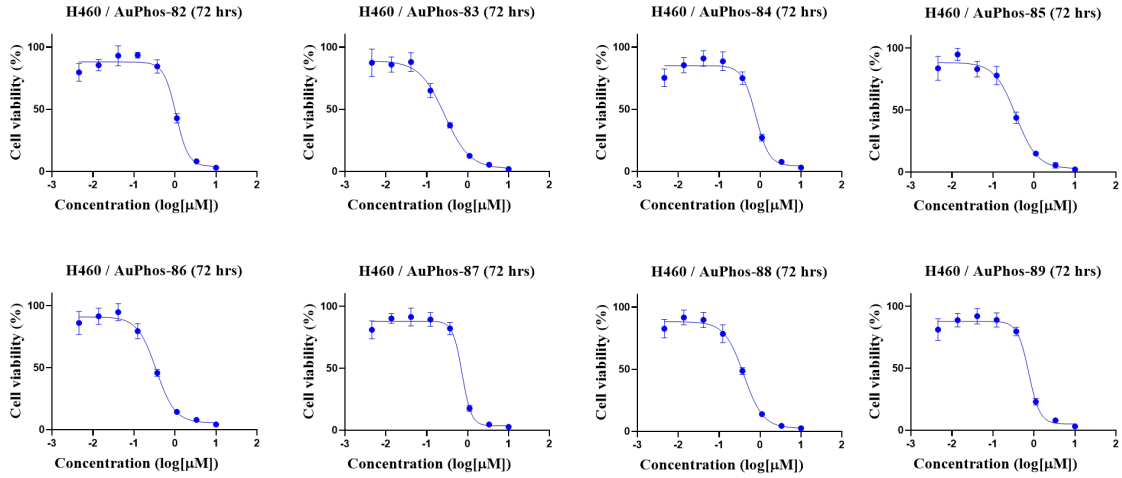

Figure S36. Evaluation of cytotoxicity for AuPhos compounds in H460

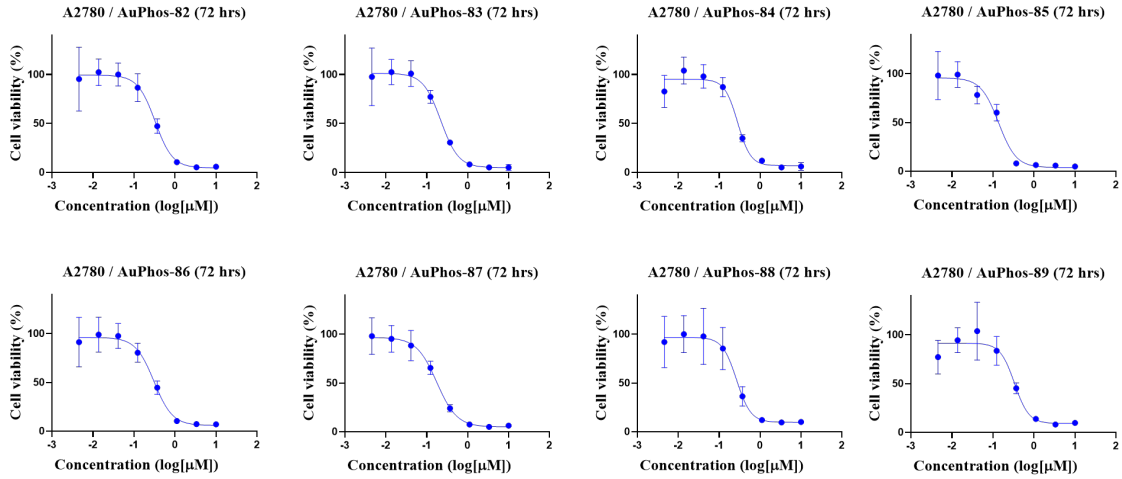

Figure S37. Evaluation of cytotoxicity for AuPhos compounds in A2780

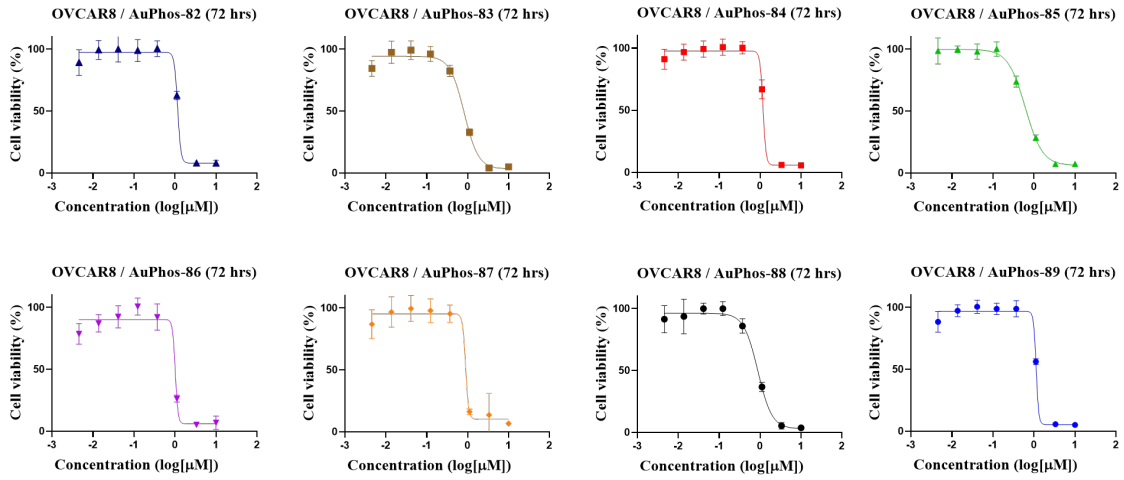

Figure S38. Evaluation of cytotoxicity for AuPhos compounds in OVCAR8

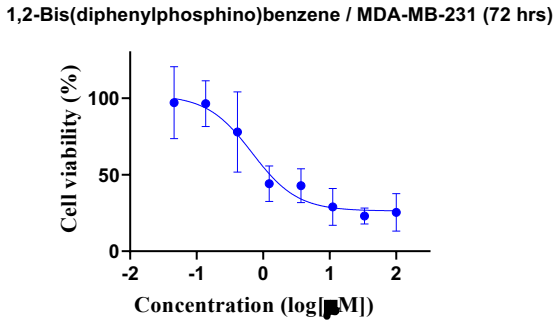

Figure S39. Evaluation of cytotoxicity for 1,2-Bis(diphenylphosphino)benzene compounds in MDA-MB-231, IC_{50} is 1.37 ± 0.45 μM. Compared with the AuPhos compounds (Table S1), it shows less cytotoxicity of ~6 times and confirms the AuPhos compound's superior activity.

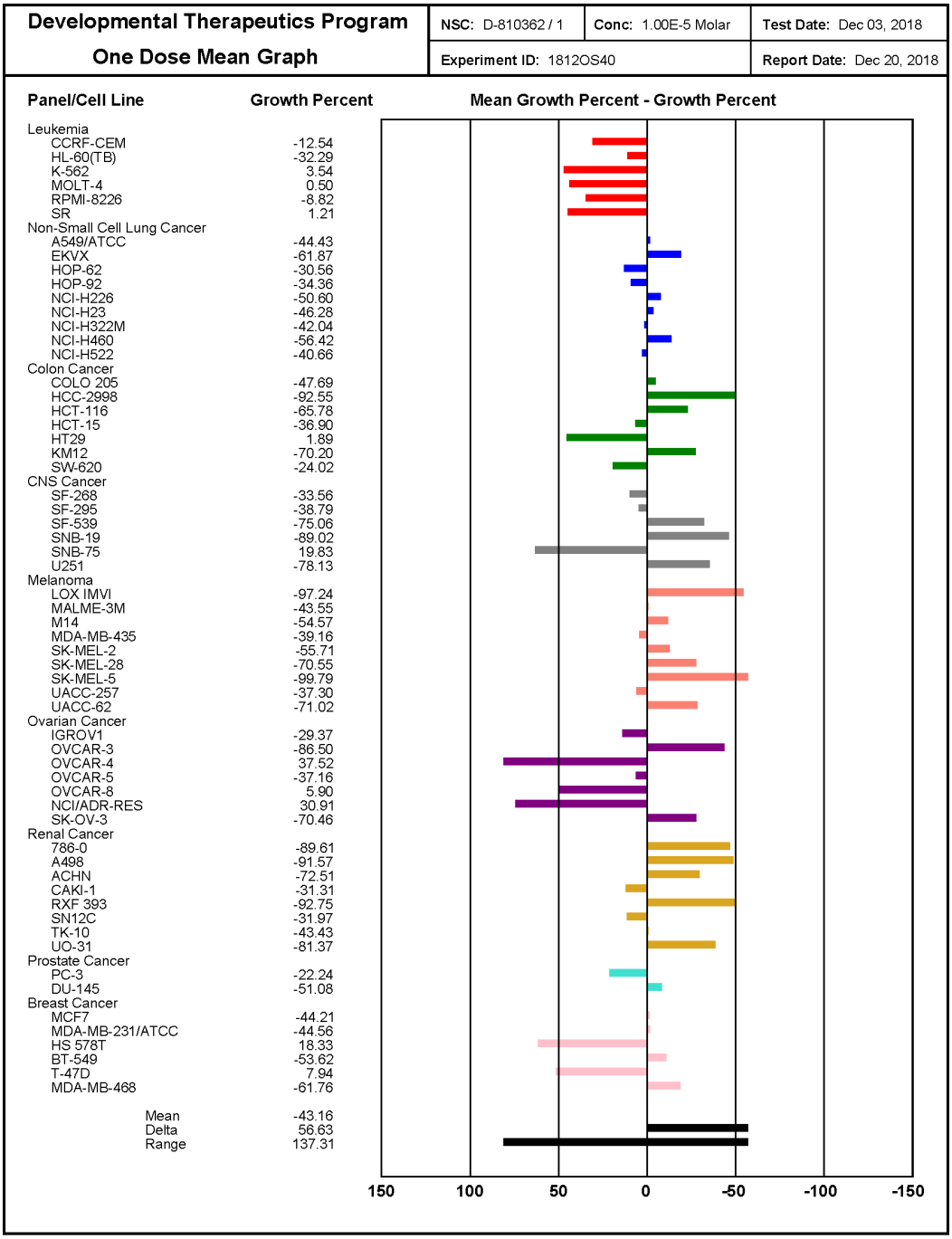

Figure S40. NCI-60 screening result of AuPhos-83

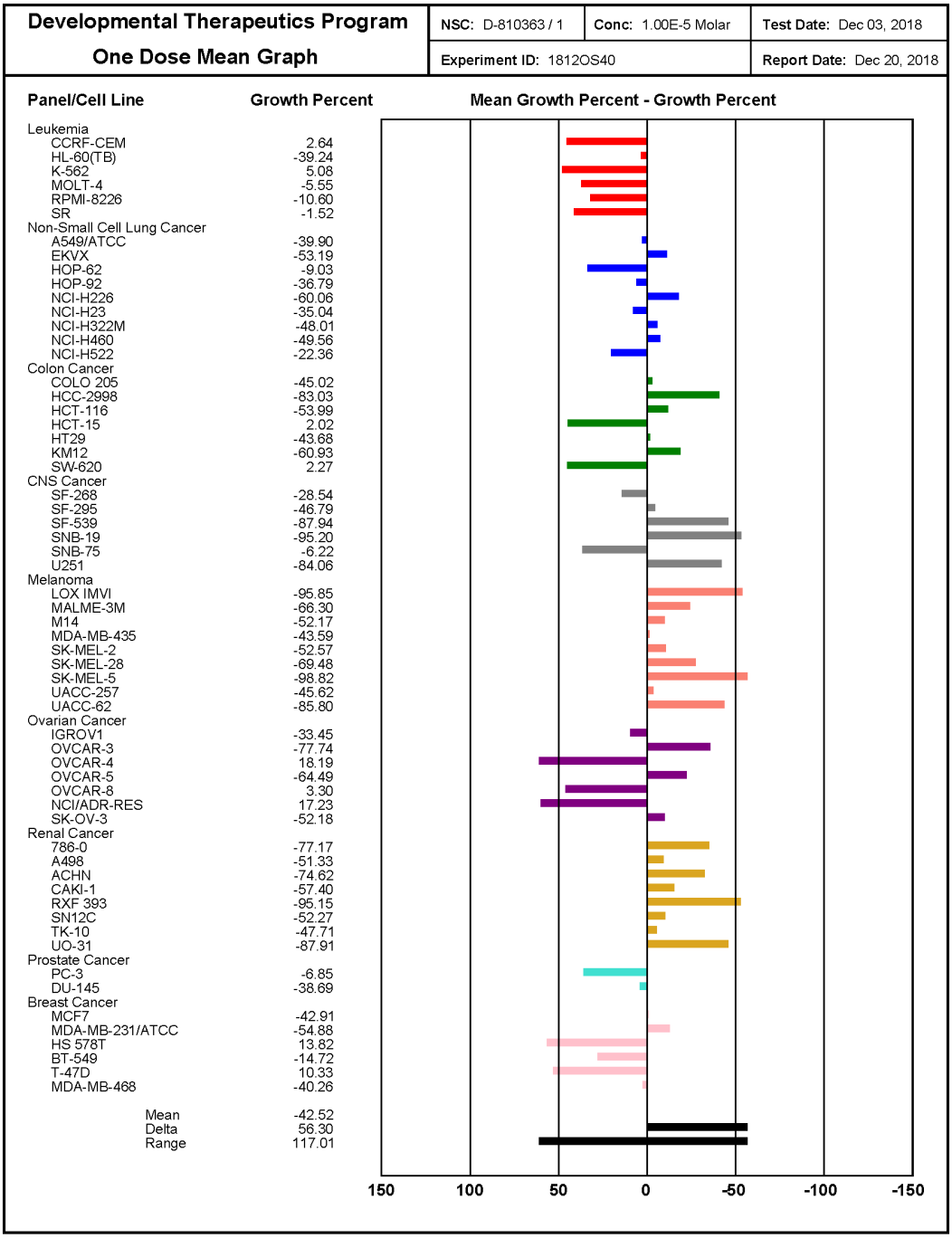

Figure S41. NCI-60 screening result of AuPhos-84

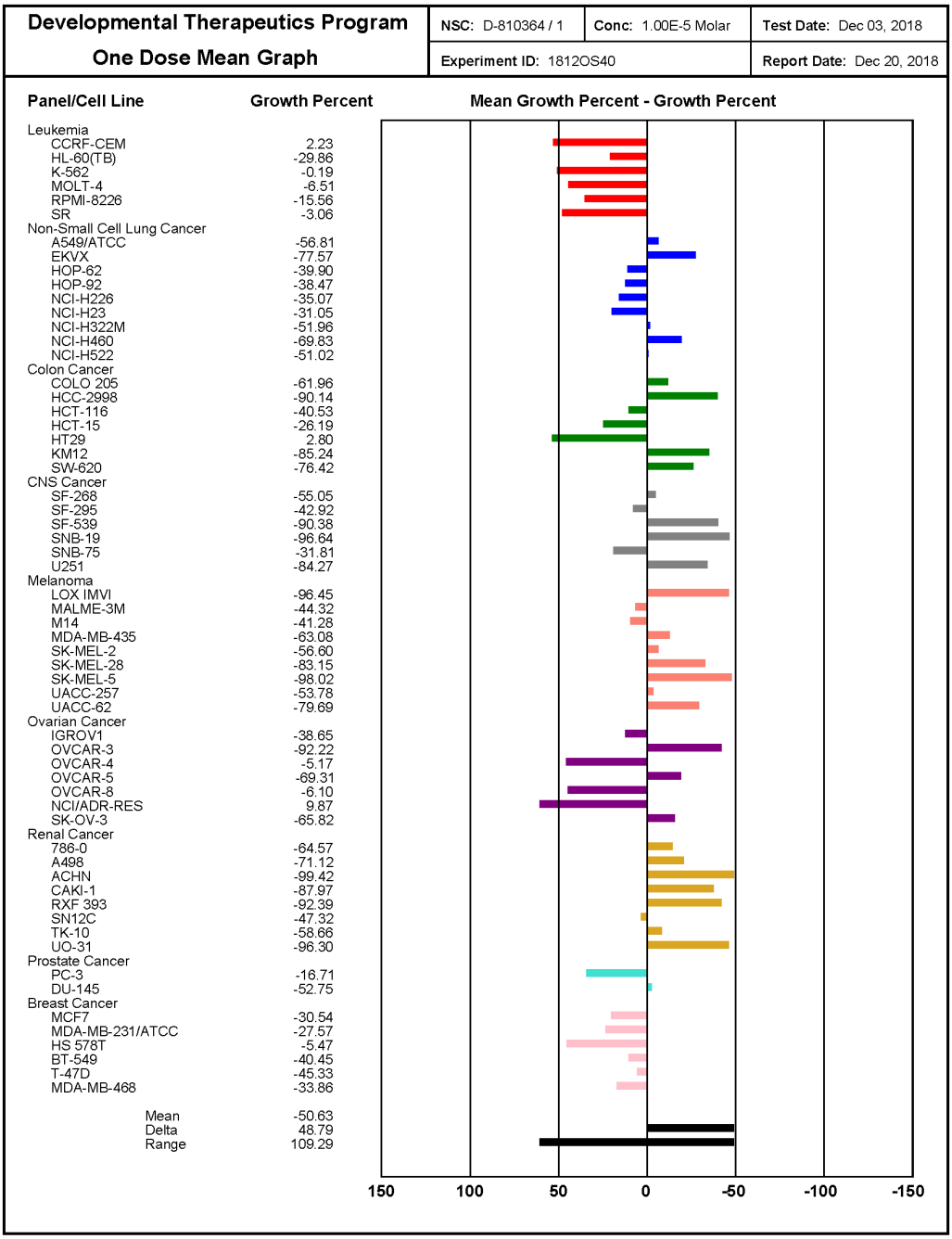

Figure S42. NCI-60 screening result of AuPhos-89

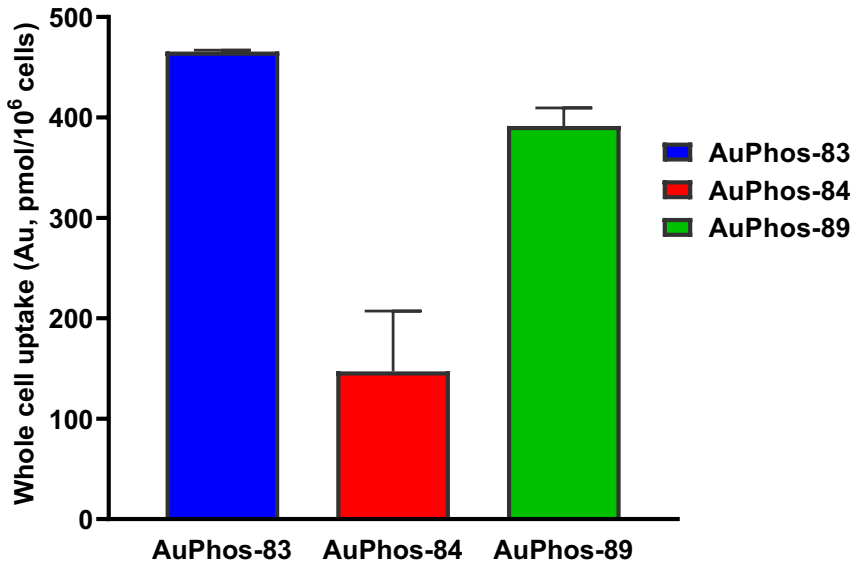

Figure S43. Whole cell (OVCAR8) uptake results from auranofin (5 μM) and complexes AuPhos-83, 84, and 89 (10 μM). Cells were incubated with compounds for 15 h.

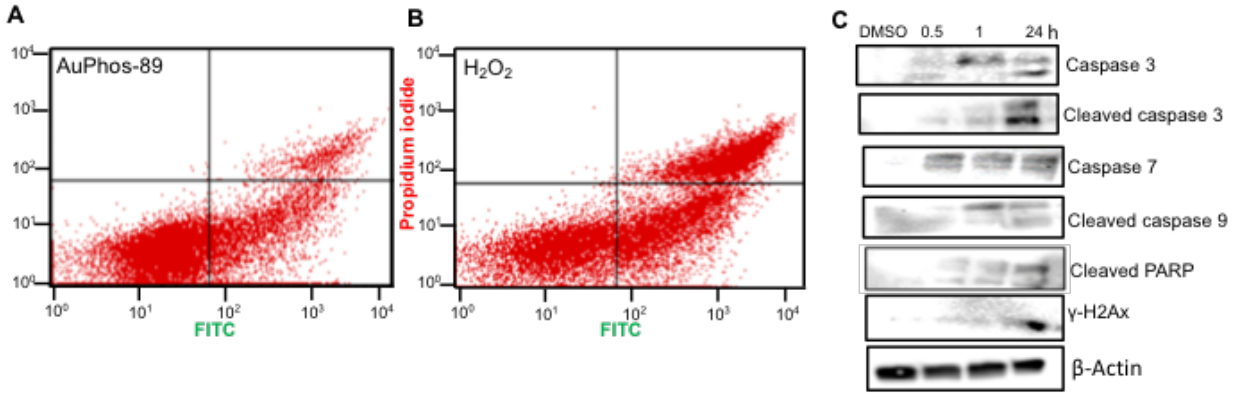

Figure S44. AuPhos induces apoptosis in breast cancer cells. A. Quadrants displaying apoptotic population of MDA-MB-231 within 15 h of treatment with AuPhos-89 (1 μM). Cells were seeded at a density of $5x10^5$ per well. B. Quadrants displaying apoptotic population of MDA-MB-231 within 3 h of treatment with H_2O_2 (2 mM). C. Western blotting of apoptotic markers following the exposure of AuPhos-89 (1 μM) at indicated times. Cells were seeded at a density of $5x10^5$ per well. Data is representative of three individual experiments. H_2O_2 was used a positive control.

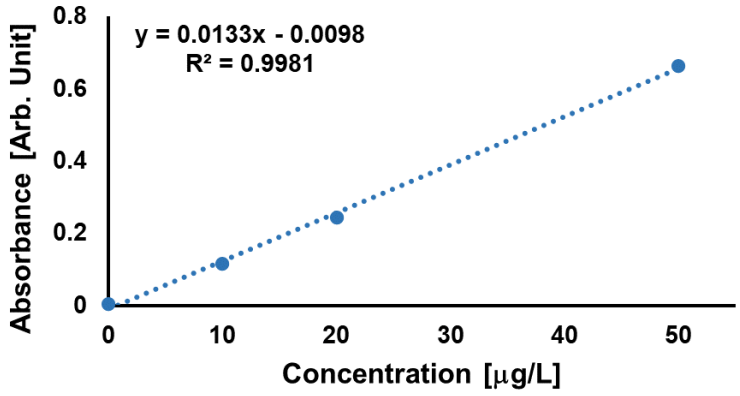

Figure S45. Standard curve of GFAAS.

Table S2. Crystal data and structure refinement for compound AuPhos-81.

	AuPhos-81
Empirical formula	$C_{41}H_{33}AuCl_2N_2P_2$
Formula weight	883.5
Temperature	90.0(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 10.4667(2) Å alpha = 90°
	b = 16.4519(4) Å beta = 96.182(1)°
	c = 23.6049(6) Å gamma = 90°
Volume	4041.06(16) $Å^3$
Z, Calculated density	4, 1.452 Mg/m^3
Absorption coefficient	3.881 mm^{-1}
F(000)	1744
Crystal size	0.160 x 0.120 x 0.110 mm
Theta range for data collection	2.054 to 27.520°
Limiting indices	$-13\leq h\leq 12$, $-21\leq k\leq 21$, $-30\leq l\leq 30$
Reflections collected / unique	68155 / 9298 [R(int) = 0.0394]
Completeness to theta = 25.242	100.00%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.000 and 0.000
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9298 / 0 / 435
Goodness-of-fit on F^2	1.087
Final R indices [$I>2\sigma(I)$]	$R_1 = 0.0180$, $wR_2 = 0.0402$
R indices (all data)	$R_1 = 0.0207$, $wR_2 = 0.0409$
Extinction coefficient	0.00053(4)
Largest diff. peak and hole	0.572 and -0.724 $e.Å^{-3}$

Table S3. Crystal data and structure refinement for compound AuPhos-82.

	AuPhos-82
Empirical formula	$C_{44}H_{39}AuCl_2N_2O_2P_2$
Formula weight	957.58
Temperature	90.0(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/n$
Unit cell dimensions	a = 10.4749(2) Å alpha = 90°
	b = 16.5319(4) Å beta = 93.007(1)°
	c = 23.0359(7) Å gamma = 90°
Volume	3983.63(17) $Å^3$
Z, Calculated density	4, 1.597 Mg/m^3
Absorption coefficient	3.947 mm^{-1}
F(000)	1904
Crystal size	0.150 x 0.120 x 0.020 mm
Theta range for data collection	2.096 to 27.502°
Limiting indices	$-13\leq h\leq 12$, $-21\leq k\leq 21$, $-29\leq l\leq 29$
Reflections collected / unique	57806 / 9157 [R(int) = 0.0560]
Completeness to theta = 25.242	100.00%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.746 and 0.632
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9157 / 56 / 523
Goodness-of-fit on F^2	1.035
Final R indices [$I>2\sigma(I)$]	$R_1 = 0.0249$, $wR_2 = 0.0419$
R indices (all data)	$R_1 = 0.0371$, $wR_2 = 0.0451$
Extinction coefficient	0.00030(3)
Largest diff. peak and hole	0.635 and -0.649 $e.Å^{-3}$

Table S4. Crystal data and structure refinement for compound AuPhos-83.

	AuPhos-83
Empirical formula	$C_{48}H_{46}AuCl_2NO_2P_2$
Formula weight	998.66
Temperature	90.0(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 8.9901(2) Å alpha = 90 deg.
	b = 17.6443(4) Å beta = 95.938(1) deg.
	c = 27.1668(7) Å gamma = 90°
Volume	4286.19(18) $Å^3$
Z, Calculated density	4, 1.548 Mg/m^3
Absorption coefficient	3.671 mm^{-1}
F(000)	2000
Crystal size	0.130 x 0.110 x 0.100 mm
Theta range for data collection	2.309 to 27.522°
Limiting indices	$-11 \leq h \leq 11$, $-22 \leq k \leq 22$, $-35 \leq l \leq 35$
Reflections collected / unique	67263 / 9841 [R(int) = 0.0605]
Completeness to theta = 25.242	99.90%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.694 and 0.621
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9841 / 85 / 526
Goodness-of-fit on F^2	1.067
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0296$, $wR_2 = 0.0534$
R indices (all data)	$R_1 = 0.0463$, $wR_2 = 0.0566$
Extinction coefficient	n/a
Largest diff. peak and hole	0.675 and -0.814 $e.Å^3$

Table S5. Crystal data and structure refinement for compound AuPhos-84.

	AuPhos-84
Empirical formula	$C_{42}H_{32}AuCl_2NOP_2$
Formula weight	896.49
Temperature	90.0(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P_1
Unit cell dimensions	a = 12.0282(6) Å alpha = 97.253(2)°
	b = 16.7453(8) Å beta = 95.135(2)°
	c = 20.3891(11) Å gamma = 90.405°
Volume	4056.8(4) $Å^3$
Z, Calculated density	4, 1.468 Mg/m^3
Absorption coefficient	3.868 mm^{-1}
F(000)	1768
Crystal size	0.140 x 0.090 x 0.070 mm
Theta range for data collection	2.206 to 27.530°
Limiting indices	$-15\leq h\leq 15$, $-21\leq k\leq 21$, $-26\leq l\leq 26$
Reflections collected / unique	44289 / 44289 [R(int) = ?]
Completeness to theta = 25.242	96.00%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.746 and 0.650
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9841 / 85 / 526
Goodness-of-fit on F^2	1.058
Final R indices [$I>2\sigma(I)$]	$R_1 = 0.0529$, $wR_2 = 0.1550$
R indices (all data)	$R_1 = 0.0727$, $wR_2 = 0.1696$
Extinction coefficient	n/a
Largest diff. peak and hole	1.939 and -2.846 $e.Å^{-3}$

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