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Supporting Information for

Carbon-Phosphorous stapled Au(I) anticancer Agents via Bisphosphine Induced Reductive Elimination

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Experimental Section

General Information

All the solvents used in the work were purchased from Greenfield Global (ACS grade). Chemicals were purchased from Sigma-Aldrich and TCI chemicals and used without further purification. Tetrachloroauric(III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$) and was purchased from ACROS Organic, and the Au salt was stored under nitrogen atmosphere. A Clorox bleach was purchased from VWR. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Cayman Chemicals. All biological supplements for media, PBS and trypsin-EDTA were purchased from Corning Inc. and used as purchased. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). ^{1}H, ^{13}C (^{1}H-decoupled), and ^{31}P (^{1}H-decoupled) NMR spectra were recorded on a 500 MHz JEOL ECZr and Bruker Avance NEO 400 MHz spectrometer and samples calibrated for: ^{1}H NMR ($CDCl_3$ δ = 7.26 ppm), ^{13}C NMR ($CDCl_3$ δ = 77.16), ^{19}F NMR and ^{31}P NMR externally referenced to H_3PO_4 $\delta = 0.00$). Liquid chromatography mass spectra were obtained by direct flow injection (injection volume = 10 µL) using ElectroSpray Ionization (ESI) on an Advion Expression CMS MassExpress 6.7.15.1 mass spectrometer instrument in the positive mode coupled with 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). Elemental analysis results were obtained from Atlantic Microlabs, Inc (Norcross, GA). Removal of solvents in vacuo was performed using a Büchi rotay evaporator.

Synthesis and Characterization

Synthesis of dichloro(2-arylpyridine)gold(III)ligands: Synthesis of dichloro(2-phenylpyridine)gold(III), dichloro(2-benzylpyridine)gold(III), and dichloro(2-benzoylpyridine)gold(III) were prepared according to previous reported procedures.[11]

Synthesis of STG-1: To a 25 mL round bottom flask equipped with magnetic stir bar, dichloro(2-phenylpyridine)gold(III) (0.2069 g, 0.4902 mmol) and bis[(2-diphenylphosphino)phenyl]ether (0.2717 g, 0.5045 mmol) was suspended in 20 mL MeOH. The reaction was refluxed for 3 hours while stirring. Solvent was evaporated in vacuo to yield a tan solid. The crude product was redissolved in dichloromethane, and pure product was obtained by precipitation with diethyl ether. A white colored solid was obtained. Yield: 52.0 mg, 11.0%. ^{1}H NMR (400 MHz, DMSO) δ 8.53

(t, *J* = 6.9 Hz, 1H), 8.25 (d, *J* = 8.2 Hz, 1H), 8.17 – 8.07 (m, 1H), 7.90 (q, *J* = 9.4 Hz, 2H), 7.79 (t, *J* = 8.0 Hz, 1H), 7.75 – 6.91 (m, 29H), 6.57 (dd, *J* = 11.4, 7.6 Hz, 1H), 5.75 (dd, *J* = 8.3, 5.4 Hz, 1H), 3.17 (s, 2H). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO) δ 159.33, 157.91, 157.84, 150.09, 144.92, 141.31, 139.67, 139.53, 139.26, 136.93, 136.50, 134.53, 134.38, 133.97, 133.82, 133.60, 133.33, 133.05, 132.49, 130.50, 130.37, 130.14, 130.02, 129.88, 129.76, 129.29, 129.16, 126.00, 125.92, 125.72, 125.28, 125.05, 121.42, 119.82, 49.06, 40.62, 40.41, 40.20, 39.99, 39.78, 39.57, 39.36, 25.96. ^{31}P NMR (162 MHz, DMSO) δ 20.26. EA calc: $C_{47}H_{36}AuClNOP_2 \cdot 1.00\ CH_2Cl_2 \cdot 0.5\ Et_2O$: C, 57.35% H, 4.14% N, 1.34% Obs: $C_{47}H_{36}AuClNOP_2 \cdot 1.00\ CH_2Cl_2 \cdot 0.5\ Et_2O$: C, 57.02% H, 4.09% N, 1.43%

Synthesis of STG-2: To a 25 mL round bottom flask equipped with magnetic stir bar, dichloro(2-benzylpyridine)gold(III) (0.2208 g, 0.5063 mmol) and bis[(2-diphenylphosphino)phenyl]ether (0.2859 g, 0.5309 mmol) was suspended in 20 mL MeOH. The reaction was refluxed for 3 hours while stirring. Solvent was evaporated in vacuo to yield a yellowish/tan solid. The crude product was redissolved in dichloromethane, and pure product was obtained by precipitation with diethyl ether. A white colored solid with blue tint was obtained. Yield: 244.4 mg, 45.1%. ^{1}H NMR (400 MHz, DMSO) δ 8.34 (dd, *J* = 5.0, 1.7 Hz, 1H), 7.99 – 7.91 (m, 1H), 7.86 – 6.98 (m, 35H), 6.68 – 6.56 (m, 2H), 6.22 (dd, *J* = 8.3, 5.3 Hz, 1H), 3.86 (d, *J* = 15.8 Hz, 1H), 3.65 (s, 1H). $^{13}C\{^{1}H\}$ δ 160.46, 157.52, 149.49, 138.75, 137.13, 135.77, 134.60, 134.49, 134.17, 133.90, 130.85, 129.86, 123.58, 122.43, 43.07. ^{31}P NMR (162 MHz, DMSO) δ 20.30.

Synthesis of STG-3: To a 25 mL round bottom flask equipped with magnetic stir bar, dichloro(2-benzoylpyridine)gold(III) (0.1526 g, 0.3391 mmol) and bis[(2-diphenylphosphino)phenyl]ether (0.1738 g, 0.3227 mmol) was suspended in 20 mL MeOH. The reaction was refluxed for 3 hours while stirring. Solvent was evaporated in vacuo to yield a tan solid. The crude product was redissolved in dichloromethane, and pure product was obtained by precipitation with diethyl ether. A white/tan colored solid was obtained. Yield: 148.3 mg, 48.2%. ^{1}H NMR (400 MHz, DMSO) δ 8.74 (d, *J* = 4.8 Hz, 1H), 8.34 (t, *J* = 6.5 Hz, 1H), 8.07 (t, *J* = 7.7 Hz, 1H), 7.99 (t, *J* = 7.8 Hz, 1H), 7.92 (qd, *J* = 8.6, 4.5 Hz, 2H), 7.75 (dd, *J* = 16.5, 7.7 Hz, 1H), 7.70 – 7.26 (m, 26H), 7.18 (t, *J* = 7.7 Hz, 1H), 7.10 (dd, *J* = 13.9, 7.5 Hz, 2H), 6.64 – 6.54 (m, 1H), 6.22 (t, *J* = 6.9 Hz, 1H). $^{13}C\{^{1}H\}$ (101 MHz, DMSO) δ 192.57, 159.68, 157.84, 157.78, 152.74, 149.20, 138.62, 138.19, 138.12, 136.82, 135.30, 134.53, 134.38, 134.17, 134.03, 132.95, 132.72, 130.44, 130.30, 129.91, 128.10,

126.23, 126.13, 125.08, 122.20, 120.47, 119.90, 49.06, 25.96. ^{31}P NMR (162 MHz, DMSO) δ 25.60, 21.02.

Cyclic Voltammetry.

A solution of 100 mM $N(Bu)_4PF_6$ (as supporting electrolyte) in 10 mL DMSO was prepared and added to an electrochemical cell. The solution was purged for 15 minutes with N_2 gas to remove any dissolved O_2 or CO_2 present in the solvent. A background voltammogram of the electrolyte was then recorded. 1 mL of solution was removed from the electrochemical cell, to which DPEphos, STG-1, or STG-2 was dissolved in. The solution was reintroduced into the cell to reach a 5 mM final concentration of compound. The solution was purged for 5 minutes with N_2 gas to remove any O_2 or CO_2 introduced into the system. Cyclic voltammograms were then recorded at 0.1 V/s with a glassy carbon working electrode, platinum wire counter electrode, and Ag/AgCl reference electrode. Ferrocene was added in each sample as an internal reference. All measurements were performed with a CH instruments 650E potentiostat at room temperature.

X-ray Crystallography.

Crystals for compound **1** were grown at room temperature from a vapor diffusion of diethyl ether into dichloromethane. After carefully selecting an appropriate crystal through microscopic inspection using crossed polarizers, crystals were attached using polyisobutene oil on the end of a glass fiber, cooled through a cold gas stream of liquid nitrogen and diffraction collected using Bruker D8 Venture diffractometer with graded multilayer focused MoKα X-rays (λ = 0.71073 Å). APEX3 package[1-5] was used to correct Lorentz-polarization effects by integrating, scaling, merging, and correcting the raw data gotten from the diffractometer. Thereafter, the space group, structure solution and refinement were determined with SHELXT and SHELXL while the ellipsoid plots were drawn using SHELXTL-XP. [6-8] H atoms were found in difference Fourier maps, but subsequently included in the refinement using riding models, with constrained distances set to 0.95 Å ($C_{sp2}H$), 0.98 Å (RCH_3) and 0.99 Å (R_2CH_2). Water hydrogen atoms were refined using 1,2 and 1,3 distance restraints. To ensure satisfactory refinement for disordered groups in the structure, a combination of constraints and restraints were employed. The constraints (SHELXL command

EADP) were used to fix overlapping fragments. Restraints were used to ensure the integrity of ill-defined or disordered groups (SHELXL commands SAME, DFIX, SIMU, and ISOR). Refinement progress was checked using PLATON,[9] and by an R-tensor.[10] The final model was further checked with the IUCr utility CheckCIF. [9]

Crystals for compound **2** were grown at room temperature from a vapor diffusion of diethyl ether into dichloromethane. After selecting the appropriate crystal through microscopic inspection using crossed polarizers, crystals were attached using polyisobutene oil on the end of a glass fiber, cooled through a cold gas stream of liquid nitrogen and diffraction collected using Bruker D8 Venture diffractometer with graded multilayer focused CuKα X-rays (λ = 1.54178 Å). APEX5 package[2-5,10] was used to correct Lorentz-polarization effects by integrating, scaling, merging, and correcting the raw data gotten from the diffractometer. Thereafter, the space group, structure solution and refinement were determined with SHELXT and SHELXL while the ellipsoid plots were drawn using SHELXTL-XP.[6-8] Refinement progress was checked using PLATON,[9] and by an R-tensor.[10] The final model was further checked with the IUCr utility CheckCIF. [9]

Biological Characterization

Reactivity with L- Glutathione

L-glutathione (11.0 mg) and compound 1 (6.5 mg), were dissolved in 0.7 mL of DMSO-d^6 to reach final concentrations of 5 mM (GSH) and 1 mM (STG-1). The solution was transferred to a clean NMR tube and ^{1}H NMR spectroscopy was performed at time points of 0 h, 1 h, 2 h, 6 h, 12 h, and 24 h after incubation at 37 °C. ^{1}H NMR spectra were recorded with a Varian Unity 400 MHz NMR spectrometer. Data was referenced to the DMSO-d^6 peak at 2.50 ppm.

In Vitro Biological Assay

Cell Culture.

Breast, MDA-MB-468, MDA-MB-231, SUM-159, were generous gifts from Dr. Kathleen O'Connor at the University of Kentucky and MCF-7 was graciously provided by Dr Yadi Wu at the University of Kentucky; ovarian (OVCAR-3, A2780), and lung cancers (A549, H460, H1299) were purchased from American Type Culture Collection, ATCC. All cell lines in this study were

grown in a humidified incubator at 37 ℃ with 5% CO_2. MDA-MB-231, MDA-MB-468, and MCF-7 were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% amphotericin B. H460, A549, H1299, A2780 were maintained in RPMI 1640 supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% amphotericin B. OVCAR-3 was maintained in RPMI 1640 supplemented with 20% FBS and 10 μg/mL insulin. SUM-159 was maintained in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 1% amphotericin B, and 10 μg/mL insulin. All media and supplements were purchased from Corning Inc. PBS and trypsin-EDTA used for cell culture maintenance were also purchased from Corning Inc.

***In vitro* Cytotoxicity.**

Cytotoxicity of STG 1-3 was performed in MDA-MB-231, MDA-MB-468, MCF-7, OVCAR-3, SUM-159, H460, A549, H1299, and A2780 cancer cell lines. Cancer cells were harvested at 80% confluency via trypsinization. The cells were then suspended in 10 mL of media, centrifuged at 2000 rpm for 3 minutes, pellet removed, and the pellets were resuspended in 5 mL of media. Cell plating was carried out at a density of 3,000 cells/well in a 96-well clear bottom plate. The cells were allowed to adhere overnight in an incubator at 37 °C with 5% CO_2 before treating with compounds. All compounds were prepared fresh as 10 mM stock solutions in DMSO and diluted to a working concentration of 300 μM with media. Cells were treated at seven different concentrations by a 3x serial dilution starting at 100 μM for the highest concentration and DMSO control. The treated 96-well plates were incubated at 37 °C for 72 h with 5% CO_2. After incubation, media was removed and replaced with a 0.5 mg/mL solution of 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT dye) and incubated for 4 h at 37 °C with 5% CO_2. Dye was removed from each well and DMSO (100 μL) was added solubilize the formazan product. Plates were read using a Biotek Synergy H1 Plate Reader at 570 nm (peak absorbance) and the data plotted as mean ± SD using GraphPad Prism 10.2.0.

Seahorse XF Cell Mito Stress Test.

SUM-159 and OVCAR-3 cells (30,000 cells/wells, 100 μL) were plated in separate Seahorse XF96 plates and allowed to adhere overnight at 37 °C with 5% CO_2. A stock solution of STG-1 (1 mM) was prepared in DMSO and diluted to a working concentration of 100 μM with Seahorse XF96

assay buffer. The assay was performed using pneumatic injection, with injection concentrations of 10 μM, 5 μM, and 1 μM, and 0.1 μM. This was followed by injection of oligomycin (1.5 μM), FCCP (0.6 μM) and rotenone/ antimycin A (0.5 μM). Metabolic parameters were calculated based on the reading from a minimum of 16 wells and the data plotted as mean ± SD using GraphPad Prism 10.2.0.

Mitochondrial membrane potential (TMRE).

SUM-159 cells were plated at a seeding density of 500,000 cells/well in 6-well clear bottom plates and allowed to adhere overnight at 37 °C with 5% CO_2. A stock solution of compound 1 (10.81 mM) was prepared in DMSO and diluted to working concentrations of 5 μM and 10 μM with DMEM. Cells were treated for 3 hours at these concentrations. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was prepared as stock in DMSO and diluted to a working concentration of 100 μM with DMEM. Cells were incubated with CCCP for 1 hour as positive control. Cells were harvested with trypsin and rinsed with PBS to remove excess media, then pelleted by centrifugation. Tetramethylrhodamine ethyl ester (TMRE) was prepared by adding 2 μL of stock dye to 13.998 mL PBS. Cells were resuspended in 200 μL of diluted TMRE dye and pipetted through a cell strainer into 5 mL Falcon FACS tubes. Cells were incubated for 30 minutes with TMRE dye on ice followed by analysis with a BD Symphony A3 Cell Analyzer with 488 nm excitation and appropriate emission filters.

Mitochondrial ROS.

SUM-159 cells were plated at a seeding density of 500,000 cells/well and allowed to adhere overnight. A stock of STG-1 was made (10.81 mM) in DMSO and diluted to a working concentration of 5 μM and 10 μM in DMEM. After incubation with compound for 2 hours, media was removed, and cells were harvested with trypsin. Cells were rinsed with PBS and pelleted before being resuspended in 200 μL of MitoSOX Red solution (200 μL). The suspension of cells was then pipetted through a cell strainer into 5 mL Falcon FACS tubes. Cells were incubated for 30 minutes on ice before analysis with a BD Symphony A3 Cell Analyzer.

Apoptosis Analysis.

SUM-159 cells were plated at a seeding density of 500,000 cells/well and allowed to adhere overnight. A stock solution of compound 1 (10.81 mM) in DMSO was prepared, and diluted to working concentrations of 0.5 μM, 1 μM, and 2 μM with DMEM. Cells were treated with respective concentrations of compound and incubated for 24 hours at 37 °C with 5% CO_2. Cells were also treated with 200 μM H_2O_2 and incubated for 1 hour as a positive control. After incubation, media in each well was collected in separate 15 mL centrifuge tubes – followed by a rinse with 2 mL PBS that was collected in the same tubes. Cells were then harvested by trypsinization and collected into their respective tubes. Cells were pelleted by centrifugation, and the supernatant was discarded. Cells were resuspended in 300 μL Annexin binding buffer, followed by addition of 5 μL Annexin V-FITC and 5 μL propidium iodide. Samples were incubated for 10 minutes in the dark before analysis with a BD Symphony A3 Cell Analyzer.

Table S1. X-ray Parameters of STG-1 and STG-2

X-ray Structural Data and Crystal Refinement		
	STG-1	STG-2
Empirical Formula	$C_{52}H_{50}AuCl_4\ NO_3P_2$ [a]	$C_{48}H_{38}AuCl_2NOP_2$
Molecular Weight (g/mol)	1137.63	974.60
Temperature (K)	100.0(2) K	100.0(2) K
Wavelength	0.71073 Å	1.54178 Å
Crystal system, space group	Triclinic, P1	C2/c
Unit cell dimensions	a = 9.5743(4)Å, α = 69.840(1)° b = 10.8642(3) Å, β = 76.071(1)° c = 13.1587(5) Å, γ = 80.548(1)°	a = 27.5637(5) Å, α = 90° b = 10.7433(2) Å, β = 104.352(1)° c = 30.4501(5) Å, γ = 90°
Volume	1242.12(8) $Å^3$	8735.6(3) $Å^3$
Z, Calculated density	1, 1.521 Mg/m^3	8, 1.482 Mg/m^3
Absorption coefficient	3.283 mm^{-1}	8.421 mm^{-1}
F (000)	570	3872
Crystal size	0.170 x 0.100 x 0.070 mm	0.200 x 0.150 x 0.070 mm
Theta range for data collection	2.005 to 27.606°	3.310 to 74.799°
Limiting Indices	-12<=h<=12, -14<=k<=14, -17<=l<=17	-31<=h<=34, -13<=k<=13, -37<=l<=37
Reflections collected/unique	58572 / 11334 [R(int) = 0.0456]	53578/8803 [R(int) = 0.0323]
Completeness to theta (θ)	Θ = 25.242, 100.0%	θ = 67.679, 98.9%
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min transmission	0.862 and 0.759	0.587 and 0.355
Refinement method	Full-matrix least=squares on F^2	Full-matrix least=squares on F^2
Data/restraints/parameters	11334 / 21 / 592	8803 / 3 / 498
Goodness-of-fit on F^2	0.974	1.179
Final R indices [I>2σ(I)]	R1 = 0.0245, wR2 = 0.0425	R1 = 0.0386, wR2 = 0.0863
R indices (all data)	R1 = 0.0258, wR2 = 0.0429	R1 = 0.0394, wR2 = 0.0874
Extinction coefficient	n/a	0.000058 (5)
Largest diff. peak and hole	0.529 and -0.550 e. $Å^3$	0.887 and -1.179 e. $Å^3$
[a]The empirical formula contains one molecule CH_2Cl_2 , one molecule H_2O, and one molecule Et_2O.		

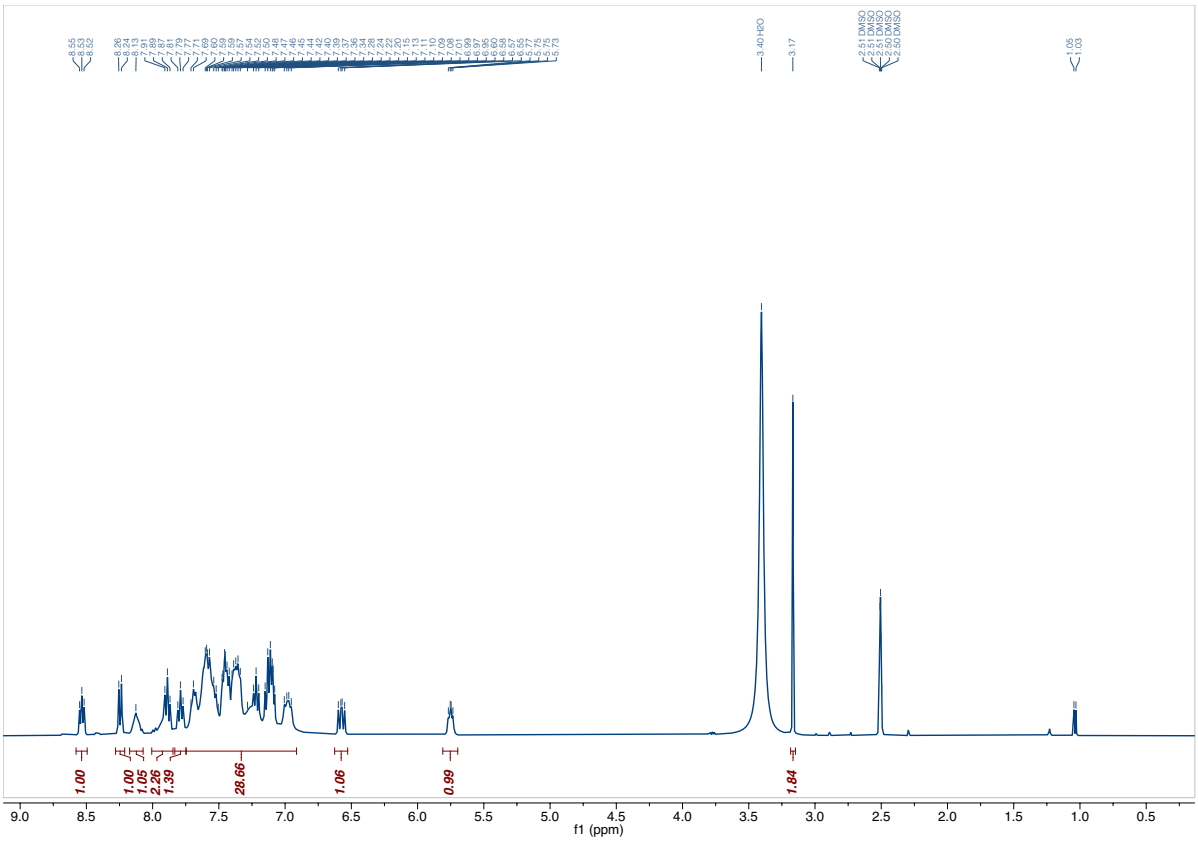

Fig. S1 ^{1}H NMR spectrum of STG-1 in DMSO-d^{6} at 298 K

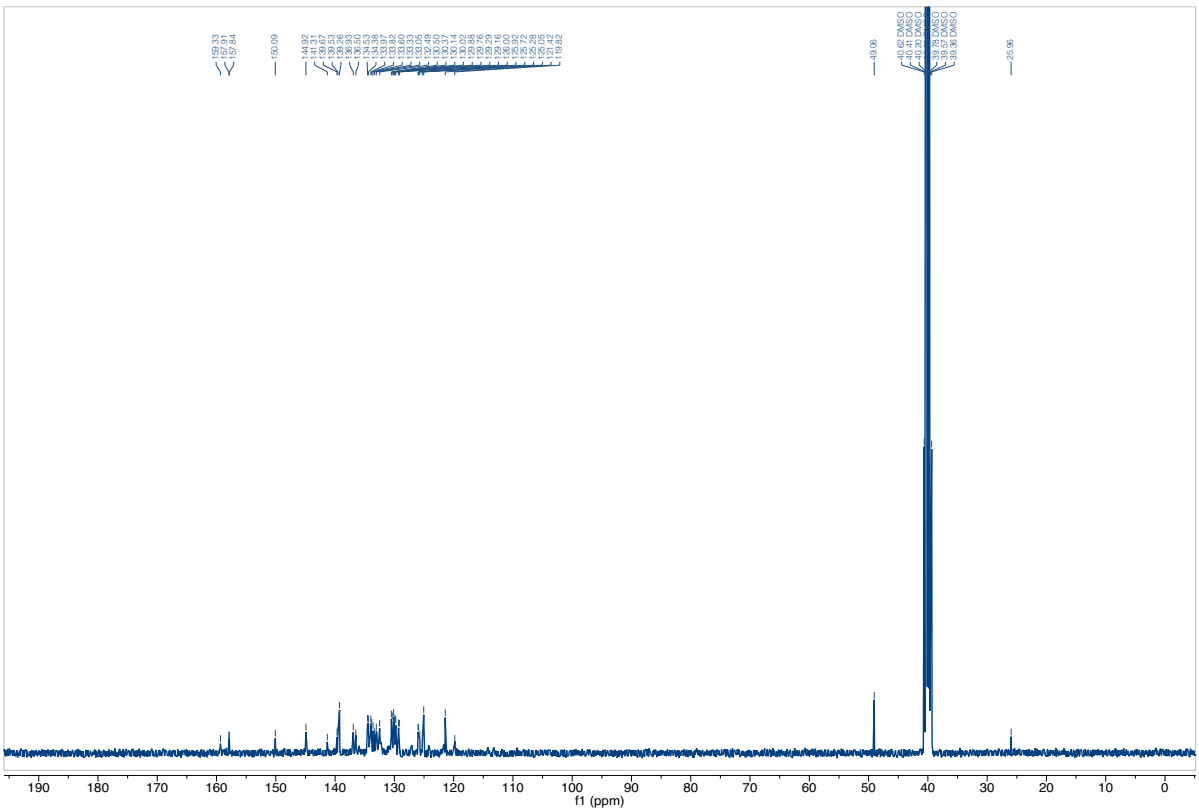

Fig. S2 ^{13}C NMR Spectrum of STG-1 in DMSO-d^{6} at 298 K

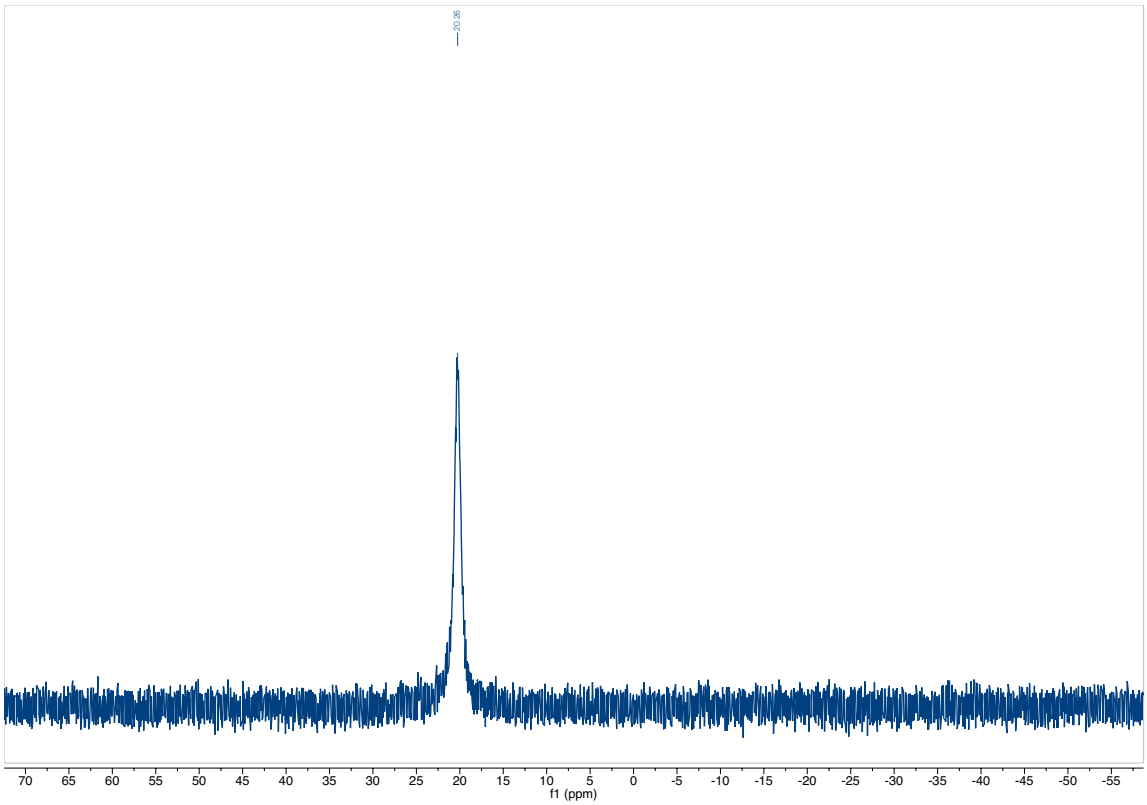

Fig. S3 ^{31}P NMR spectrum of STG-1 in DMSO-d^{6} at 298 K.

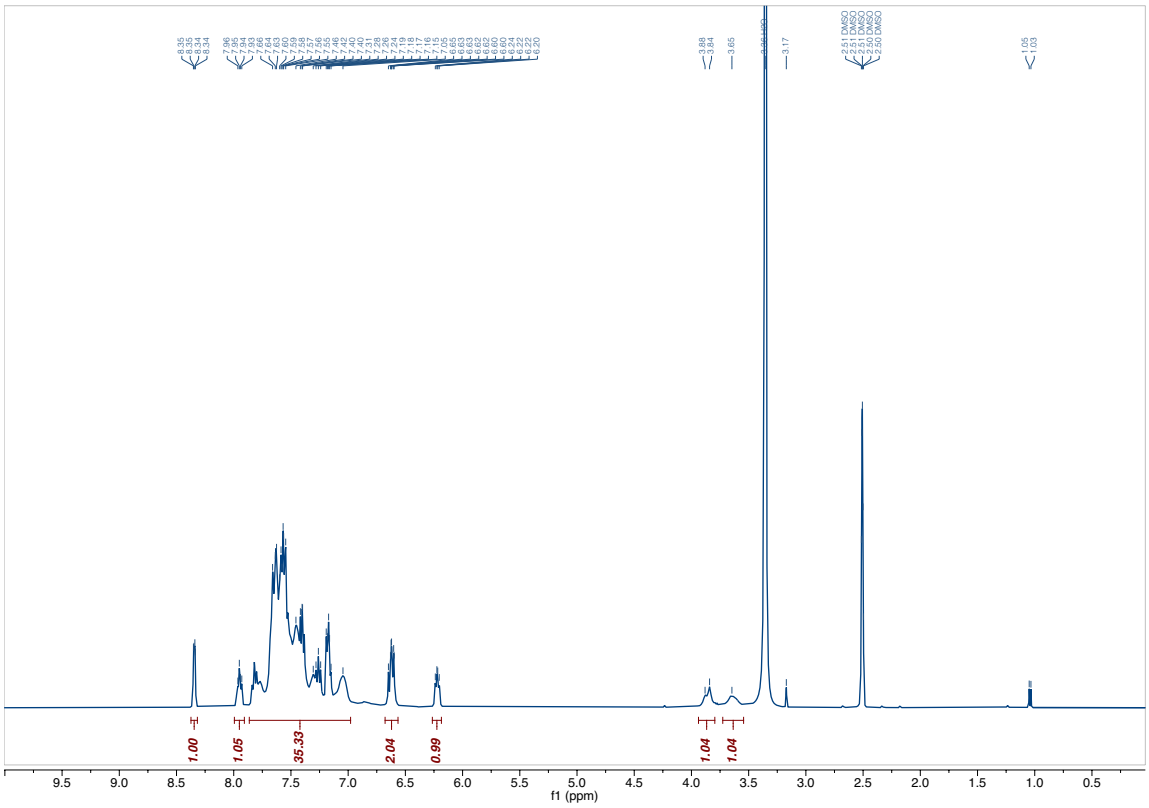

Fig. S4 ^{1}H NMR spectrum of STG-2 in DMSO-d^{6} at 298 K.

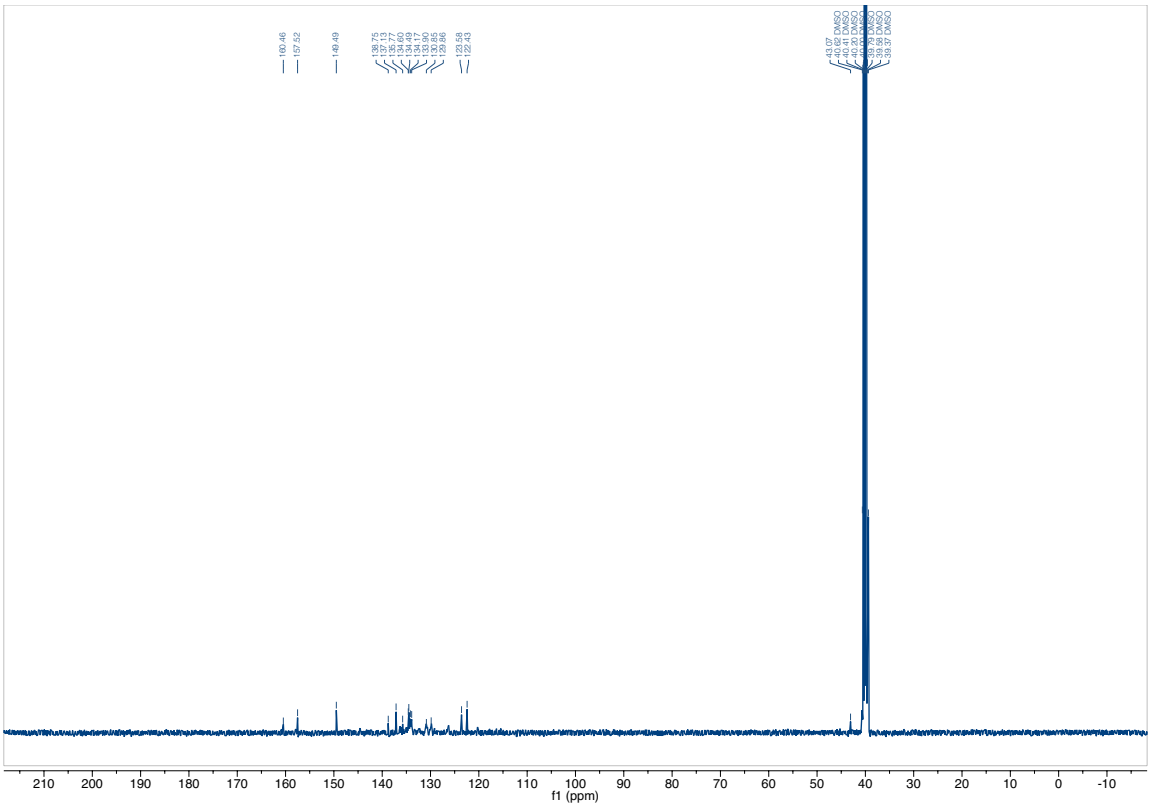

Fig. S5 ^{13}C NMR spectrum of STG-2 in DMSO-d^6 at 298 K.

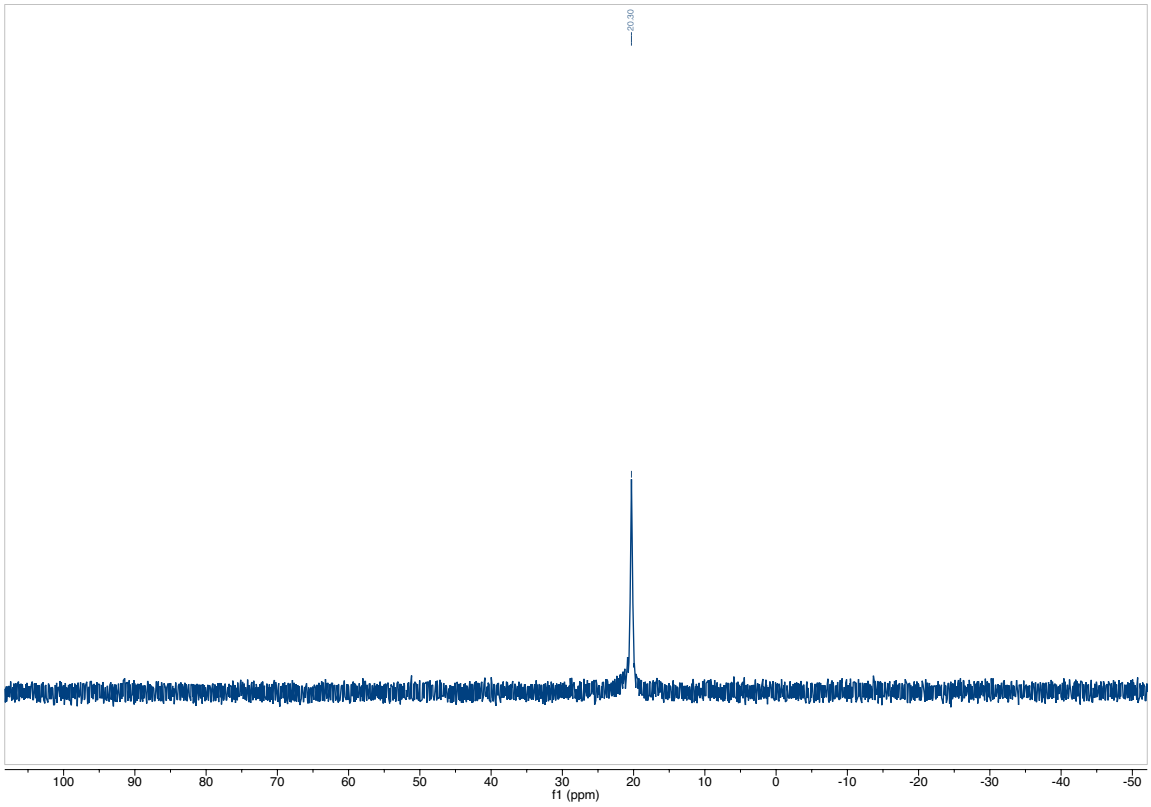

Fig. S6 ^{31}P NMR spectrum of STG-2 in DMSO-d^6 at 298 K.

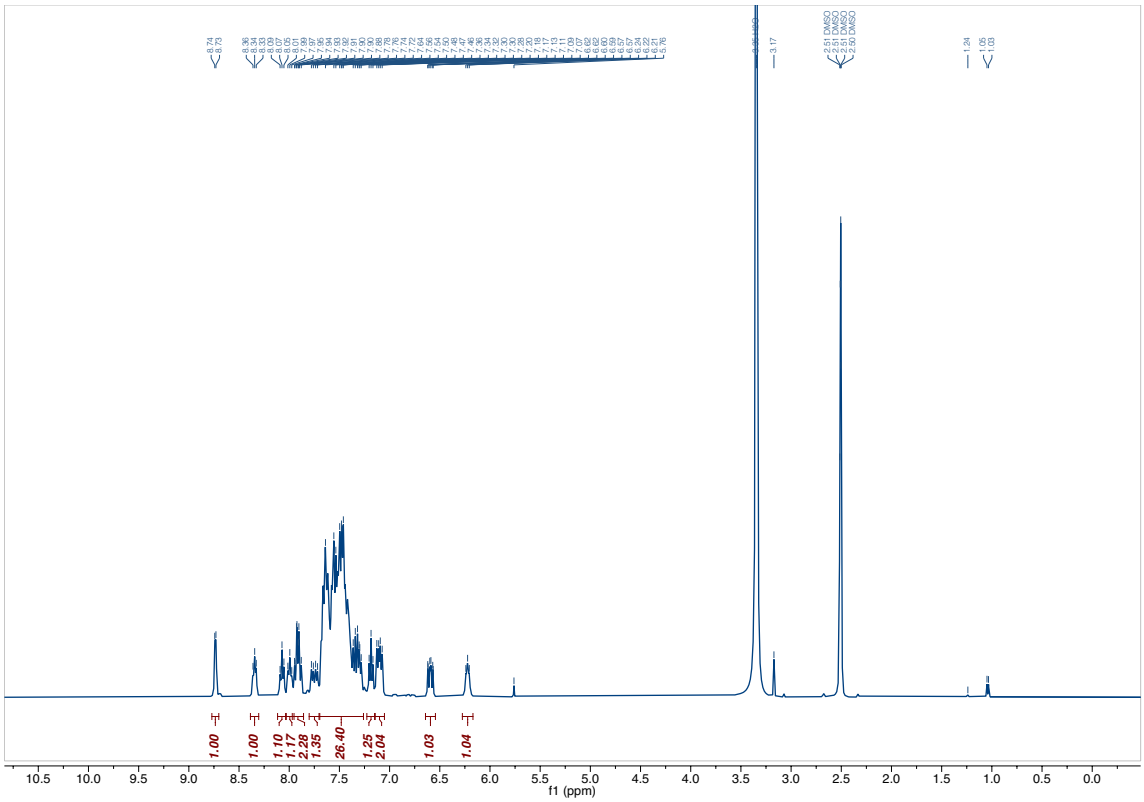

Fig. S7 ^{1}H NMR spectra of STG-3 in DMSO-d^{6} at 298 K.

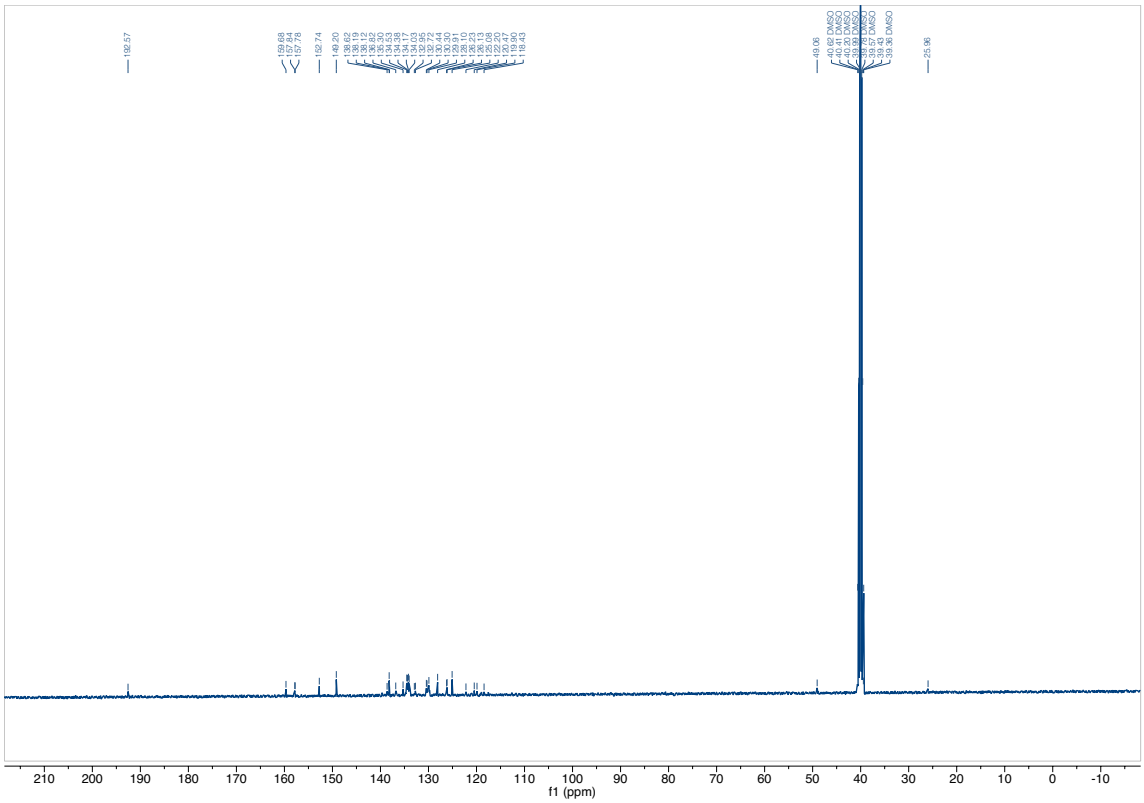

Fig. S8 ^{13}C NMR spectra of STG-3 in DMSO-d^{6} at 298 K.

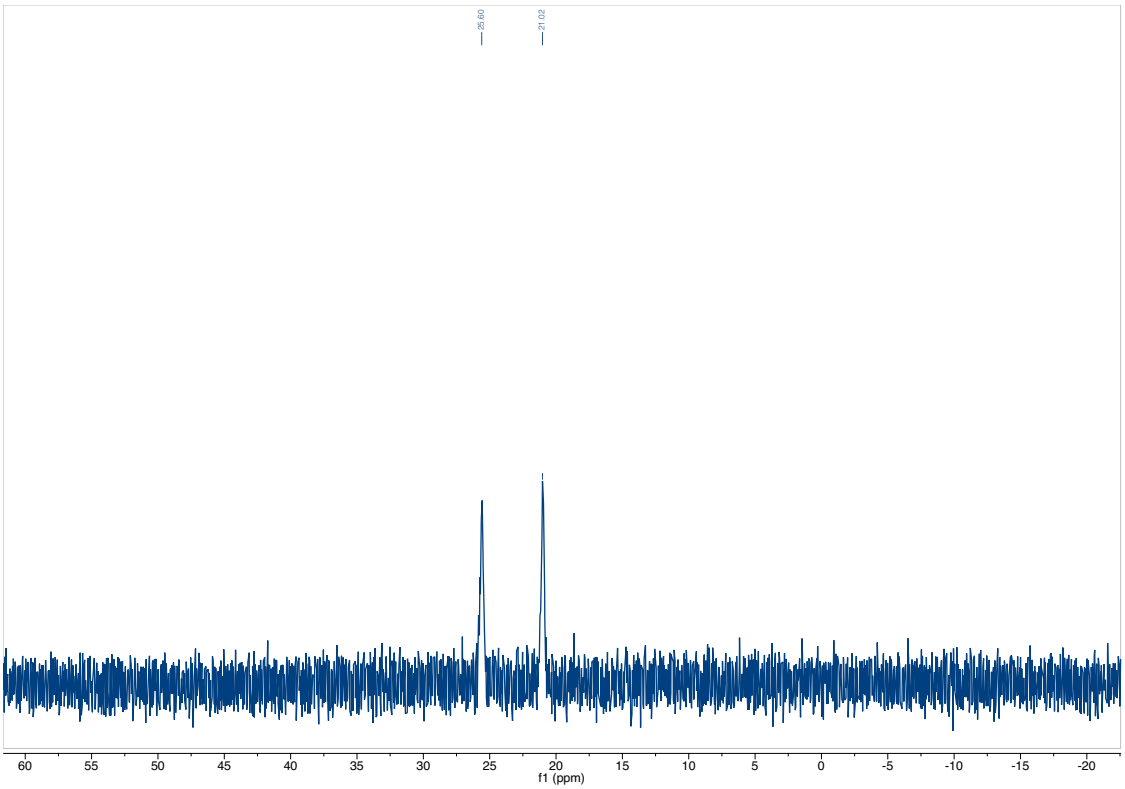

Fig. S9 ^{31}P NMR spectrum of STG-3 in DMSO-d^6 at 298 K.

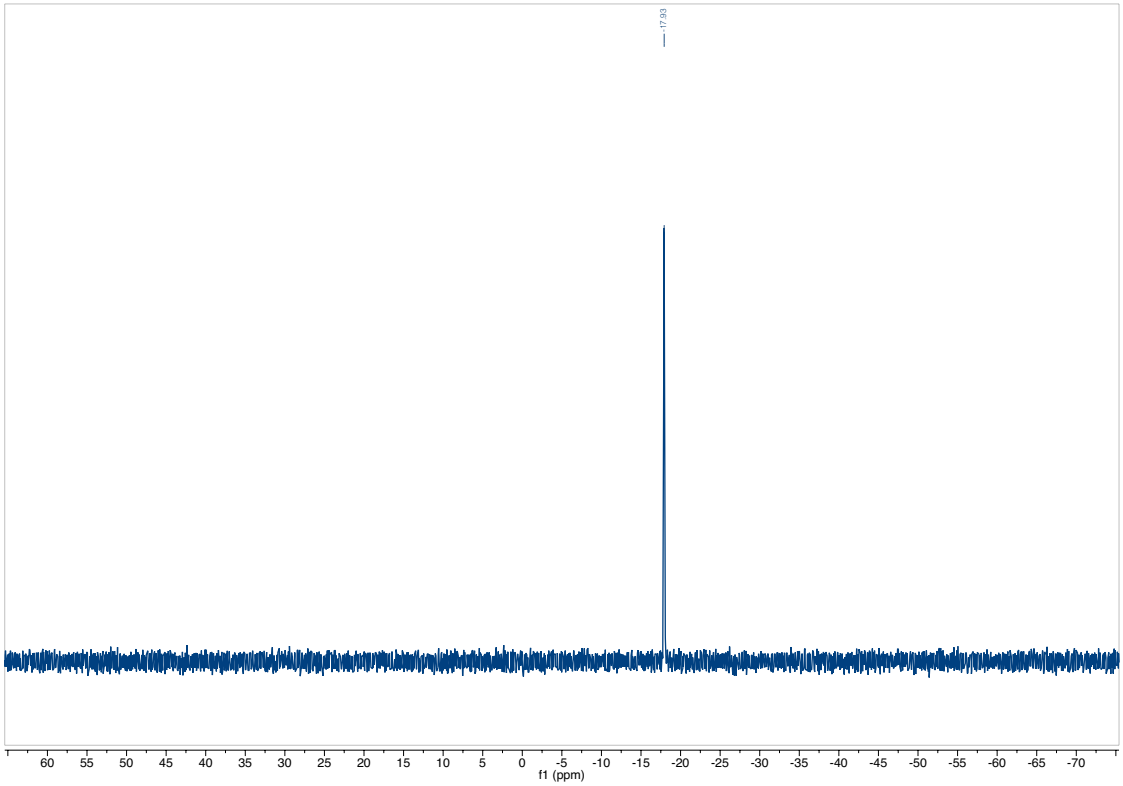

Fig. S10 ^{31}P NMR spectrum of DPEphos in DMSO-d^6 at 298 K.

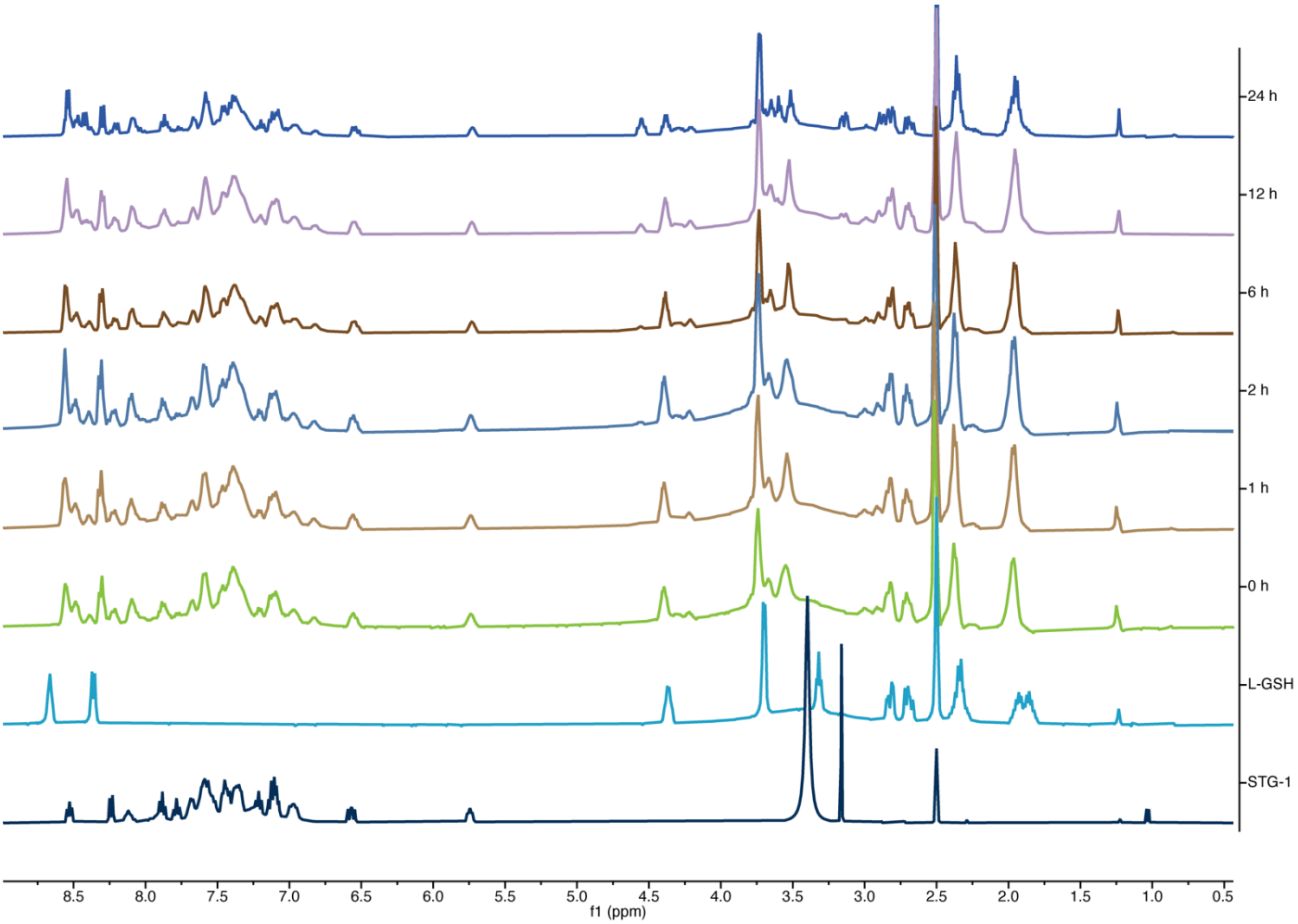

Fig. S11 ^{1}H NMR spectra of STG-1 (1mM) incubated with L-GSH (5 mM) at various time points. Spectra were recorded in DMSO-d^{6} and referenced to the DMSO-d^{6} ^{1}H chemical shift of 2.50 ppm.[13]

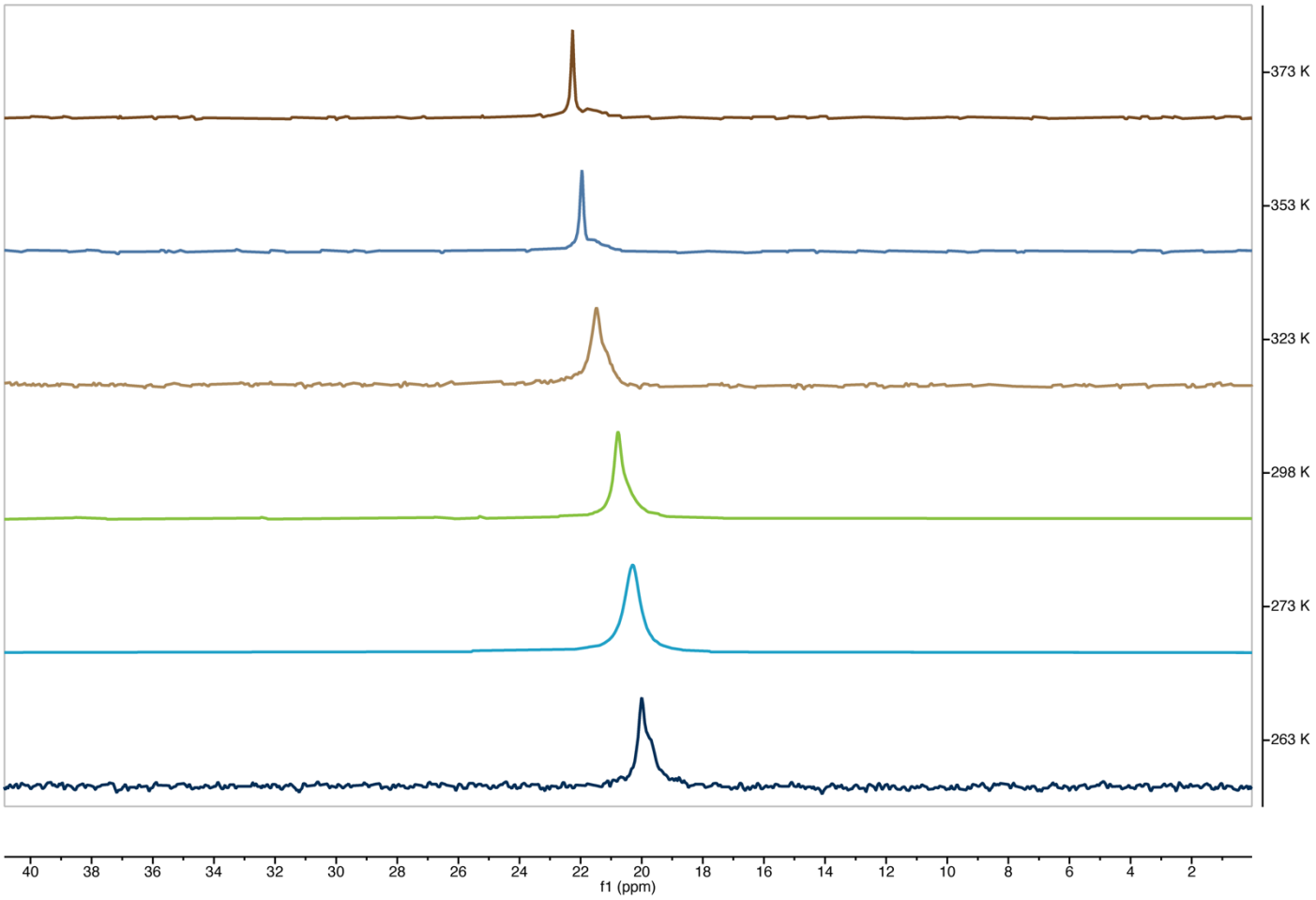

Fig. S12 ^{31}P NMR spectra of STG-1 in DMSO-d^6 (323 K- 373 K) and CD_3CN (263 K – 298 K) at variable temperatures.

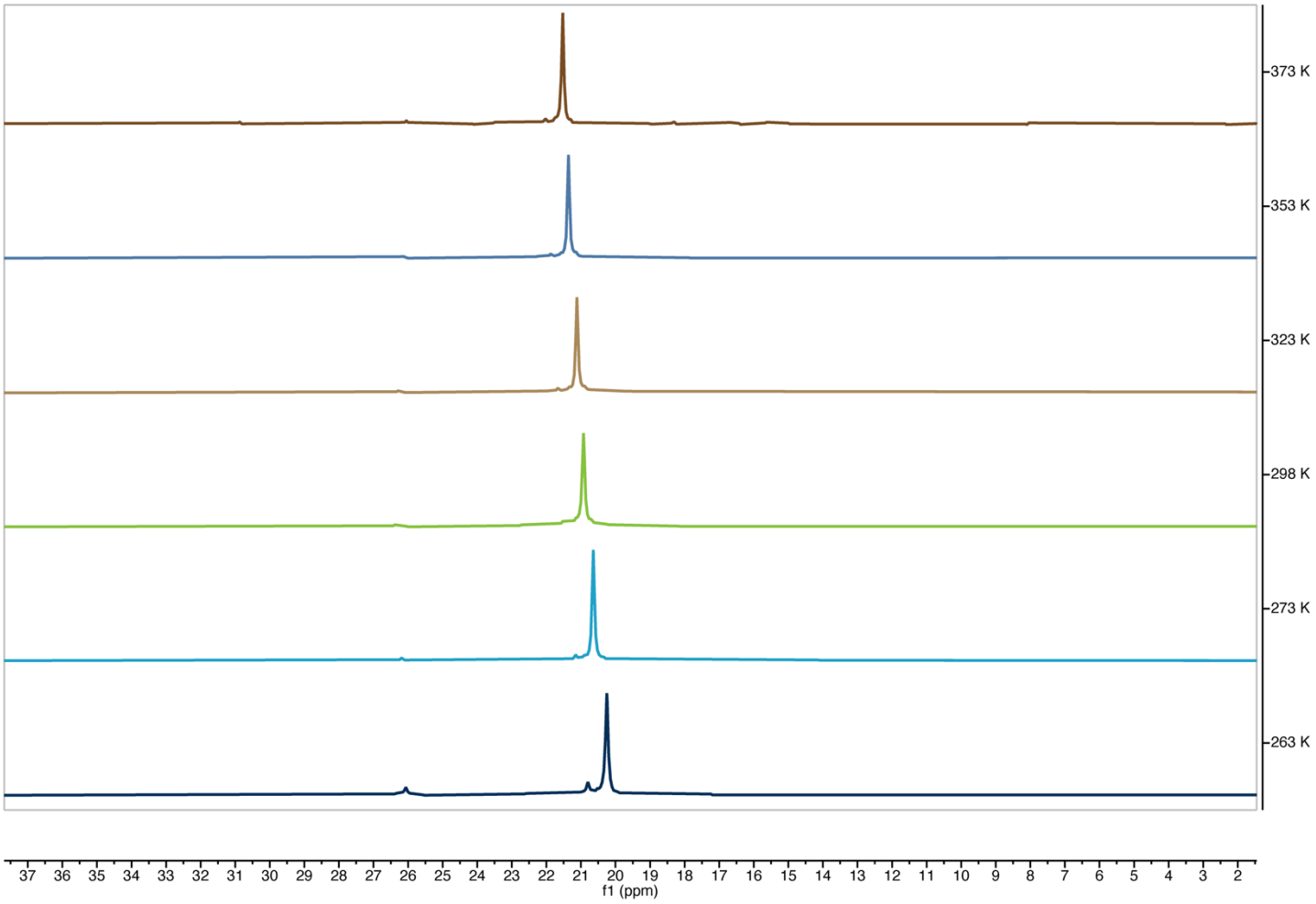

Fig. S13 ^{31}P NMR spectra of STG-2 in DMSO-d^6 (323 K- 373 K) and CD_3CN (263 K – 298 K) at variable temperatures.

HPLC Trace of Complexes

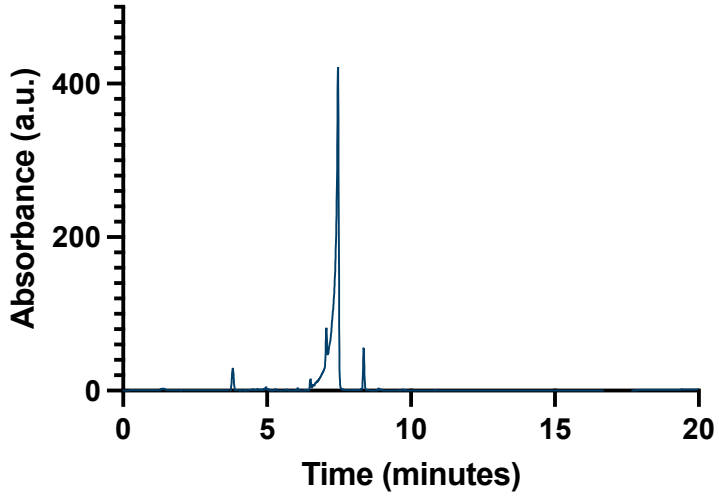

Fig. S14 HPLC of STG-1 (λ = 280 nm)

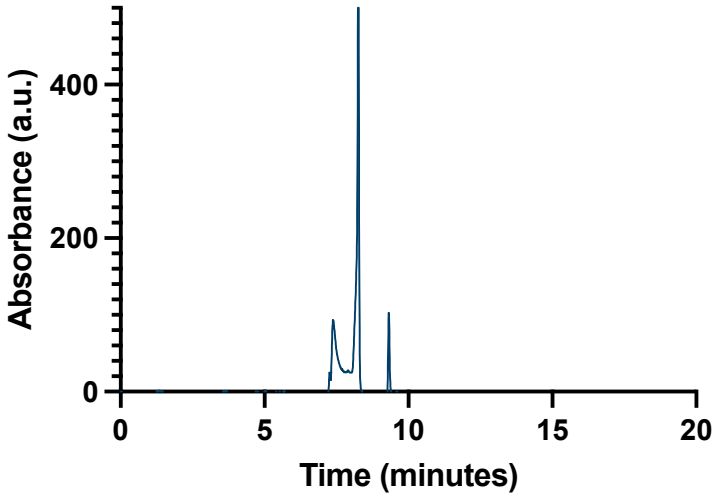

Fig. S15 HPLC of STG-2 (λ = 280 nm).

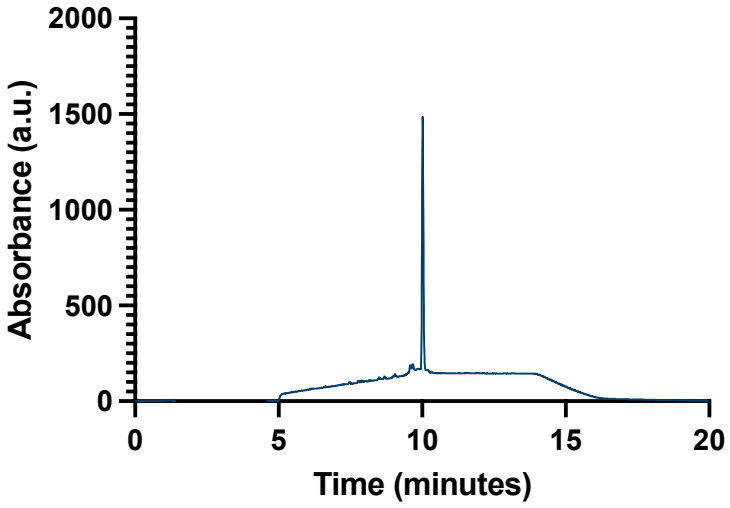

Fig. S16 HPLC of STG-3 (λ = 280 nm).

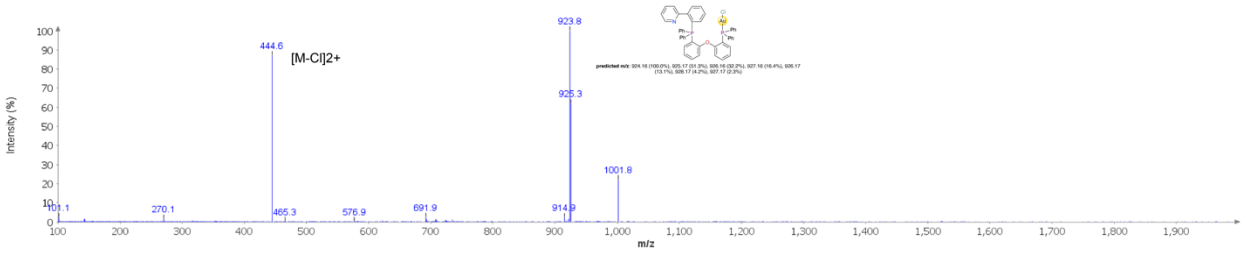

Fig. S17 ESI+ spectra of STG-1.

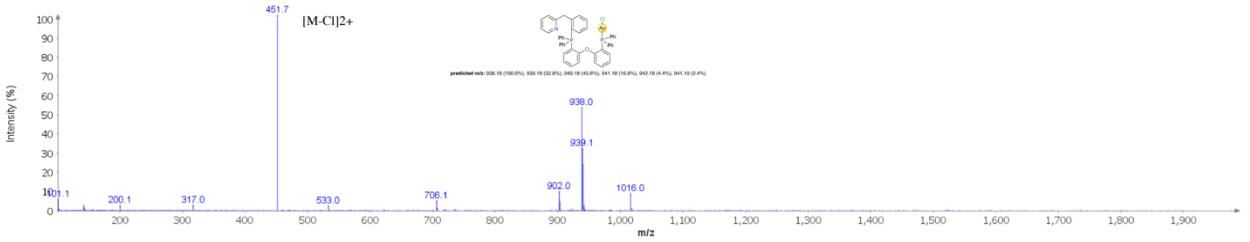

Fig. S18 ESI+ spectra of STG-2.

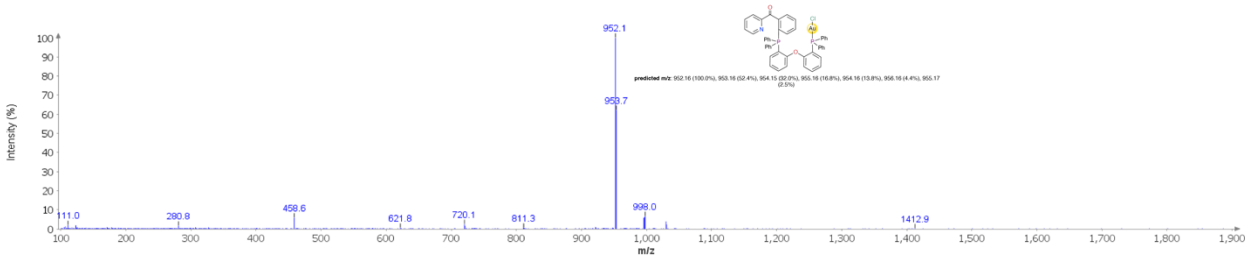

Fig. S19 ESI+ spectra of STG-3.

Electrochemical Characterization

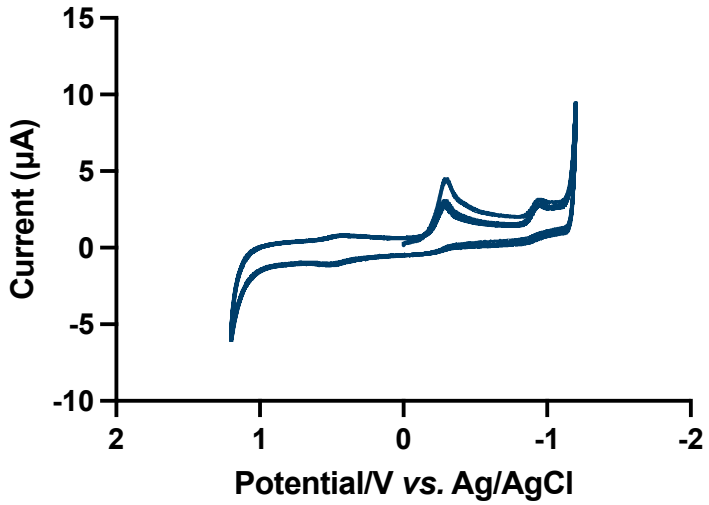

Fig. S20 Cyclic voltammogram of STG-1 with 9 sweep segments at 0.1 V/s.

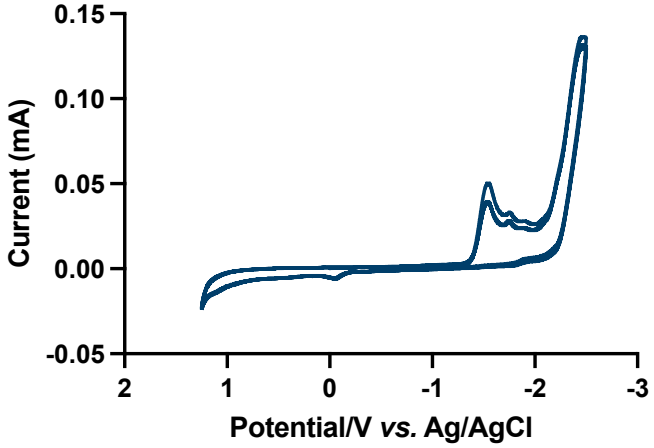

Fig. S21 Cyclic voltammogram of STG-2 with 9 sweep segments at 0.1 V/s.

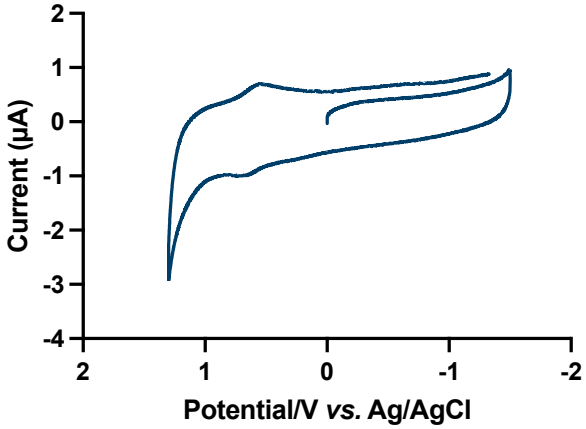

Fig. S22 Cyclic voltammogram of DPEphos with 3 sweep segments at 0.1 V/s.

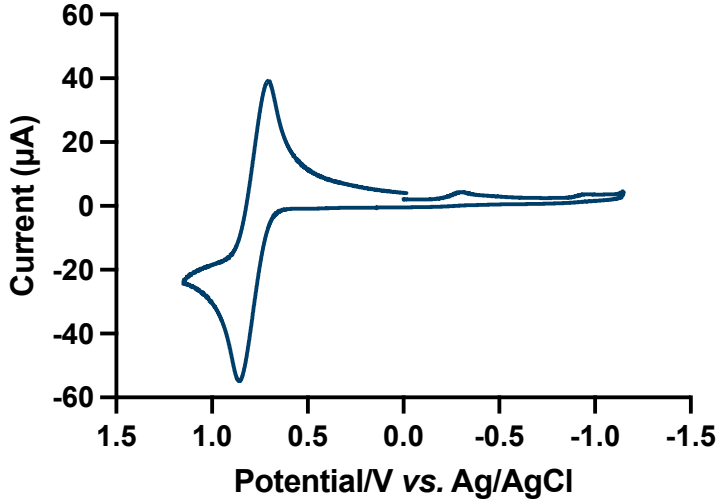

Fig. S23 Cyclic voltammogram of STG-1 with ferrocene reference.

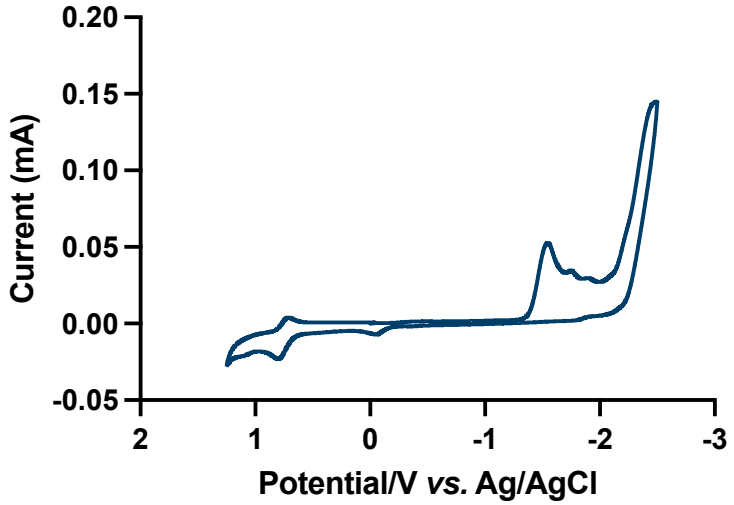

Fig. S24 Cyclic voltammogram of STG-2 with ferrocene reference.

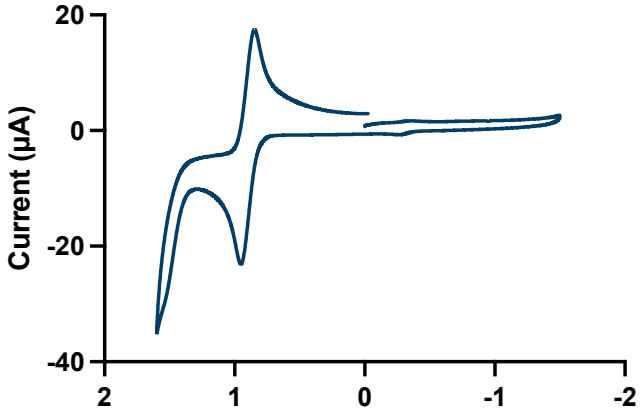

Fig. S25 Cyclic voltammogram of DPEphos with ferrocene reference.

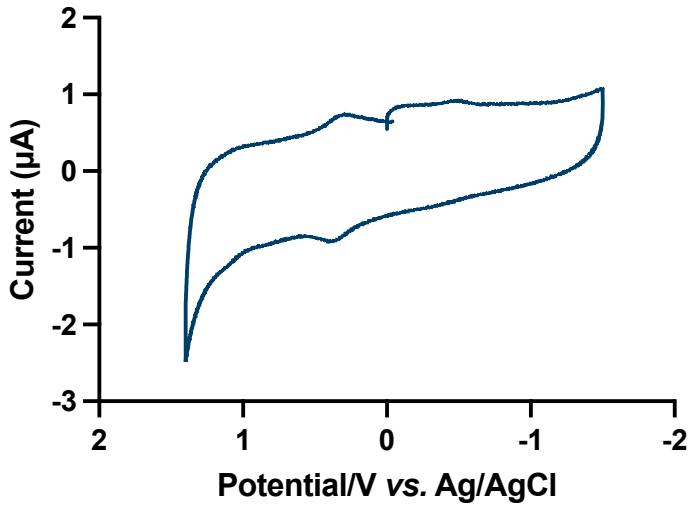

Fig. S26 Electrolyte background of 100 mM $N(Bu)_4PF_6$ used in the cyclic voltammetry measurements of STG-2.

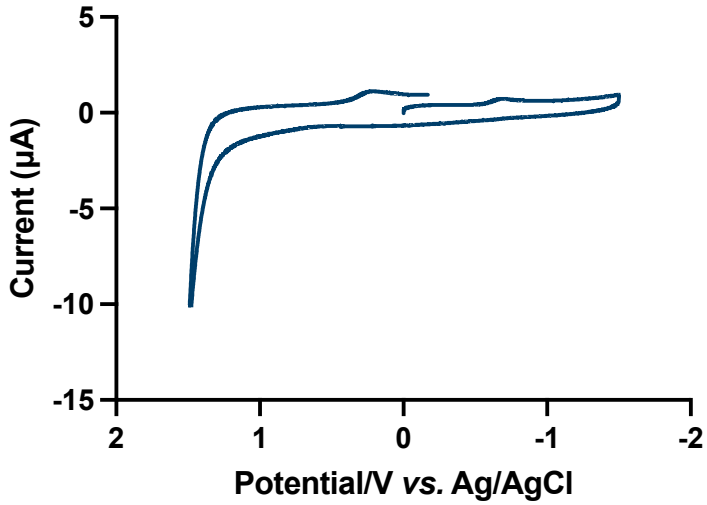

Fig. S27 Electrolyte background of 100 mM $N(Bu)_4PF_6$ used in the cyclic voltammetry measurements of DPEphos.

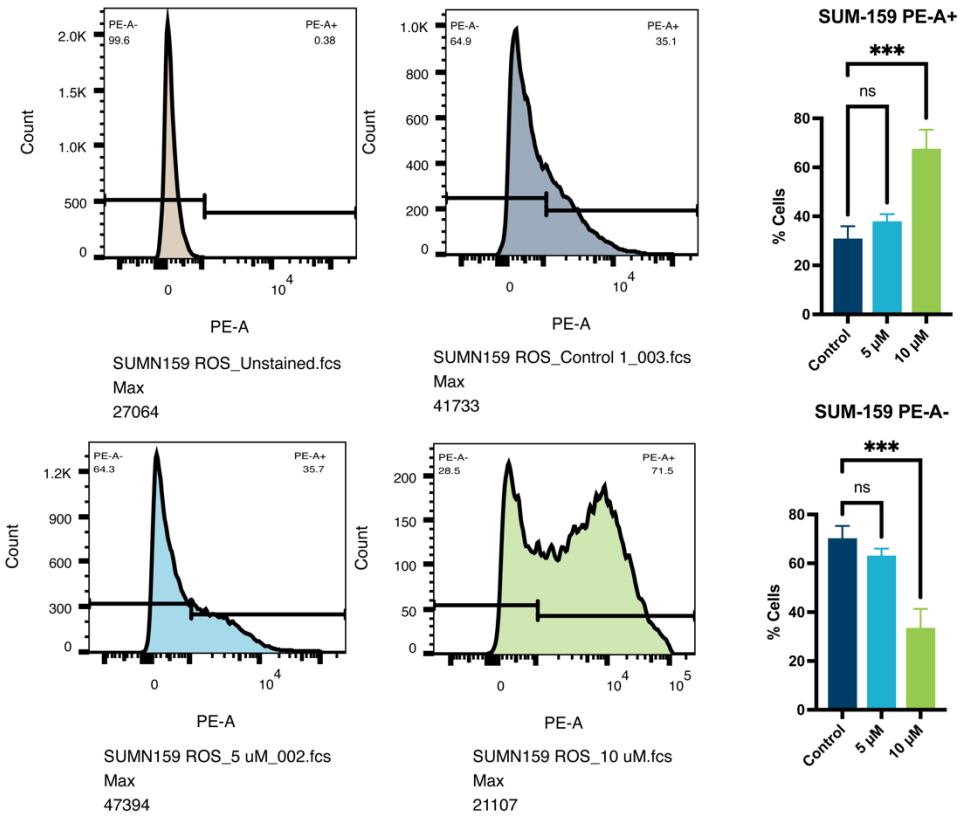

Fig. S28 Histograms of Unstained, control, 5 µM, 10 µM treatments from ROS. The bisector tool was used to quantify the percentage of cells in each population with PE-A intensity higher (PE-A+) or lower (PE-A-) than the maximum intensity of the unstained control. The mean percentage of cells amongst replicates was plotted and significance determined via one-way Anova with Dunnett's multiple comparisons test (***$p < 0.001$, ns – not significant).

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