Supporting Information for

Anti-Glioblastoma Gold(I)-NHC complex distorts mitochondrial morphology and bioenergetics to induce tumor growth inhibition

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Table of Contents	Page #s

Synthesis and General Experimental Details

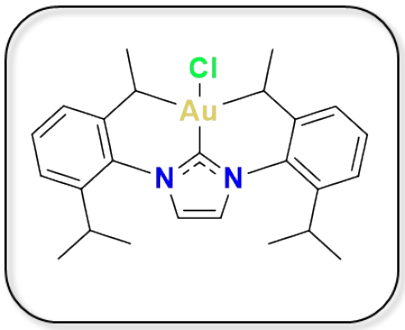
General Experimental Details: All reactions were carried under ambient conditions in air unless otherwise noted. Solvents were of ACS grade (Pharmco-Aaper) and used without further purification. The starting Au(I) precursor AuCl(NHC) was prepared from procedures reported in the literature.[1] Phenanthroline derivatives were purchased from Sigma Aldrich, TCI chemicals, Acros Organics, and Oakridge chemicals. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). NMR spectra were recorded on a Bruker Avance NEO 400 MHz spectrometer and samples calibrated for: ^{1}H NMR ($CDCl_3$ δ = 7.26 ppm, CD_3CN δ = 1.94 ppm, and DMSO-d_6 δ = 2.50 ppm), ^{13}C NMR ($CDCl_3$ δ = 77.36 and DMSO-d_6 δ = 49.00 ppm), ^{19}F NMR externally referenced to ($CFCl_3$ δ = 0.00 ppm. Aluminum backed silica-gel plates (20 ´ 20 cm2) were purchased from Silicycle (TLA-R10011B-323) and utilized for analytical thin-layer chromatography (TLC). Elemental analysis results were obtained from Atlantic Microlabs, Inc (Norcross, GA). 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.

X-ray Crystallography. Crystals of all complexes were grown from slow diffusion of Et_2O into a concentrated solution of DCM at room temperature. All crystals were mounted using polyisobutene oil on the end of a glass fiber, 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.[2] 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.[3] Space group determination and structure solution and refinement were carried out with SHELXT and SHELXL respectively.[4] 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. The structures, deposited in the Cambridge Structural Database, were checked for missed symmetry, twinning, and overall quality with PLATON, an R-tensor, and finally validated using CheckCIF.[5]

Electrochemical Studies. 5.0 mM solutions of **AuTri-C 3**, **AuTri-C 5** or **AuTri-C 6** were prepared by dissolving the compounds in DMSO. 0.1 M of NBu_4PF_6 was added as an electrolyte. Electrochemical measurements were performed at room temperature by using CH instruments 650E potentiostat, which contains 3-custom electrodes cell comprised of a 3 mm diameter glassy-carbon working electrode, platinum-wire counter electrode, and freshly anodized Ag/AgCl wire reference electrode. Ferrocene was used as an internal reference.

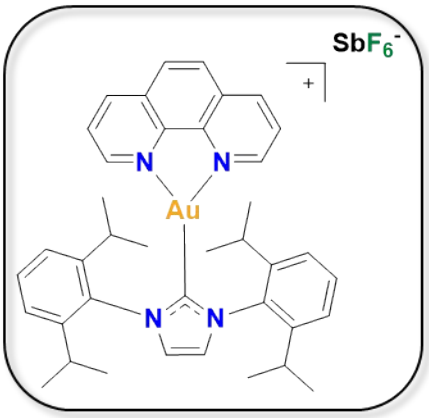
Synthesis of Au Complexes

Synthesis of AuCl(NHC):

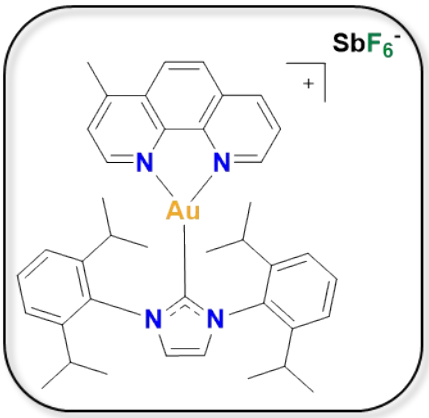


AuCl(NHC) was prepared according to reported procedure.[1] Briefly, a round bottom flask was charged with AuCl(tht) (740 mg, 2.3 mmol) and *N,N'*-Bis(2,6-diisopropylphenyl)imidazolium chloride (784.5 mg, 2.3 mmol) and dissolved in 10 mL of DCM. Sodium tert-butoxide (221 mg, 2.3 mmol) was added to the reaction solution, which turned black. This solution was left to stir for 2 hours at room temperature, after which the solution was filtered over a silica plug, yielding an orange liquid. The solution was concentrated *in vacuo* to a volume around 1–1.5 mL. The desired complex was precipitated out of solution by dropwise addition of the concentrated solution into hexane. The solid was dried in vacuo to afford a white solid. Yield 1.41 g, 98.7%. ^{1}H NMR (400 MHz, CD_3CN) δ = 7.58 (t, J = 16 Hz, 1H), 7.50 (s, 1H), 7.405 (d, J = 4 Hz, 2H), 2.565 (sept, J = 44 Hz, 2H), 1.31 (d, J = 8 Hz, 6H), 1.23 (d, J = 8 Hz, 2H).

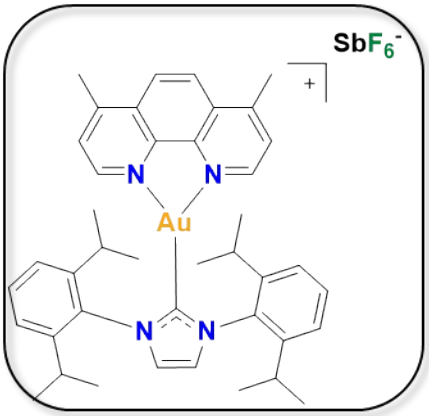
General Procedure for the Preparation of AuTri-C 1-6: All reactions were carried out under ambient conditions. Phenanthroline derivatives (0.048 mmol), and $AgSbF_6$ (0.053 mmol) were added to a round bottom flask and dissolved in 5 mL of DCM. AuCl(NHC) (0.048 mmol) was dissolved in 2.5 mL of DCM and added dropwise to the original solution. This reaction was left to stir for 30 minutes. After the reaction was completed, the mixture was placed in a 15 mL centrifuge tube and centrifuged at 2000 rpm for 1-2 minutes to pellet the inorganics. The supernatant was decanted into a round bottom flask and concentrated *in vacuo* to a volume around 1-1.5 mL. The desired compound was precipitated via the addition of Et_2O.



AuTri-C 1: Prepared as described in the general procedure. Phenanthroline (9 mg, 0.048 mmol), AuCl(NHC) (30 mg, 0.048 mmol) and silver hexafluoroantimonate (18 mg, 0.053) as a light-yellow solid. Yield: 32 mg, 88%. ^{1}H NMR (400 MHz, CD_3CN) δ = 8.79 (s, 1H), 8.15 (s, 2H), 7.67 (t, *J* = 8 Hz, 1H), 7.56 (s, 2H), 7.48 (d, *J* = 8 Hz, 2H), 7.725 (quint, *J* = 28 Hz, 2H), 1.265 (t, *J* = 12 Hz, 12H); ^{13}C NMR (101 MHz, CD_3CN) δ = 151.42, 146.50, 142.79, 139.42, 131.24, 130.43, 127.74, 125.17, 124.81, 29.11, 23.97, 23.50; ^{19}F NMR (376 MHz, CD_3CN) δ = -113.65, -117.76, -121.86, -125.98, -130.09, -134.20. EA calc: $C_{39}H_{44}AuF_6N_4Sb$: C, 46.77% H, 4.43% N, 5.59% Obs: $C_{39}H_{44}AuF_6N_4Sb$: C, 46.10% H, 3.96% N, 6.23%

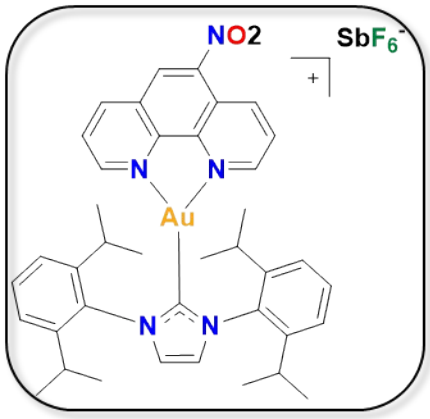


AuTri-C 2: Prepared as described in the general procedure. 4-dimethyl phenanthroline (9.5 mg, 0.048 mmol), AuCl(NHC) (30 mg, 0.048 mmol) and silver hexafluoroantimonate (18 mg, 0.053) as a light-yellow solid. Yield: 38 mg, 53%. ^{1}H NMR (400 MHz, CD_3CN) δ = 8.52 (d, *J* = 8 Hz, 1H), 8.17 (d, *J* = 8 Hz, 2H), 8.025 (d, *J* = 12 Hz, 1H), 7.77 (s, 2H), 7.70 (t, *J* = 16 Hz, 3H), 7.635 (d, *J* = 4Hz, 1H), 7.50 (d, *J* = 8 Hz, 4H), 2.80 (s, 3H), 2.755 (qu, *J* = 28 Hz, 4H), 1.29 (q, *J* = 8 Hz, 24H); ^{13}C NMR (101 MHz, CD_3CN) δ = 151.34, 146.77, 139.05, 135.46, 131.39, 127.63, 126.10, 125.21, 125.16, 125.03, 29.34, 24.17, 23.73, 19.14; ^{19}F NMR (376 MHz, CD_3CN) δ = -113.64, -117.75, -121.85, -125.97, -130.08, -134.19. EA calc: $C_{40}H_{46}AuF_6N_4Sb$: C, 47.31% H, 4.57% N, 5.52% Obs: $C_{40}H_{46}AuF_6N_4Sb \cdot 0.05\ CH_2Cl_2$: C, 46.90% H, 4.32% N, 5.77:

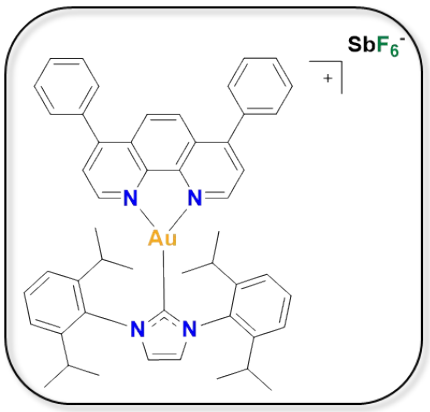


AuTri-C 3: Prepared as described in the general procedure. 4,7-dimethylphenanthroline (10 mg, 0.048 mmol), AuCl(NHC) (30 mg, 0.048 mmol) and silver hexafluoroantimonate (18 mg, 0.053) as a light-yellow solid.

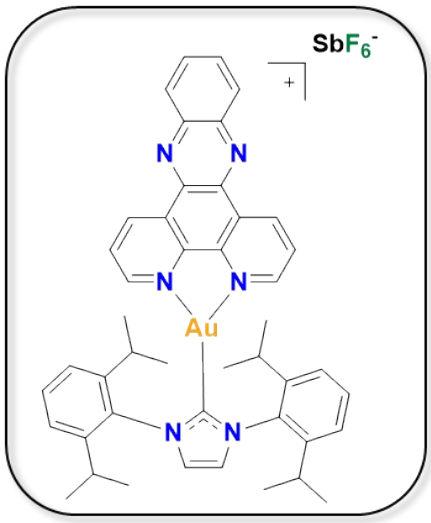
Yield: 40 mg, 57%. ^{1}H NMR (400 MHz, CD_3CN) δ = 7.40 (s, 1H), 7.33 (td, *J* = 8, 4 Hz, 1H), 7.15-7.19 (m, 2H), 6.14 (s, 2H), 3.79 (s, 3H), 3.66 (s, 6H); ^{13}C NMR (101 MHz, CD_3CN) δ = 151.02, 146.73, 142.71, 135.46, 131.32, 125.85, 125.12, 124.98, 123.70, 29.30, 24.15, 23.70, 21.91, 18.99; ^{19}F NMR (376 MHz, CD_3CN) δ = -113.65, -117.77, -121.88, -125.99, -130.09, -134.20. EA calc: $C_{41}H_{48}AuF_6N_4Sb$: C,47.83% H, 4.70% N, 5.44% Obs: $C_{41}H_{48}AuF_6N_4Sb$: C, 47.47% H, 4.76% N, 5.48%



AuTri-C 4: Prepared as described in the general procedure. 5-nitrophenanthroline (11 mg, 0.048 mmol), AuCl(NHC) (30 mg, 0.048 mmol) and silver hexafluoroantimonate (18 mg, 0.053) as a light-yellow solid. Yield: 20 mg, 38%. ^{1}H NMR (400 MHz, CD_3CN) δ = 9.025 (d, *J* = 12 Hz, 1H), 8.87 (s, 1H), 8.75 (d, *J* = 8 Hz, 1H), 7.915 (q, *J* = 12 Hz, 2H), 7.72 (q, *J* = 16 Hz, 4H), 7.51 (d, *J* = 8Hz, 4H), 2.685 (qu, *J* = 28 Hz, 4H), 1.285 (q, *J* = 12 Hz, 24H); ^{13}C NMR (101 MHz, CD_3CN) δ = 151.86, 146.52, 131.37, 126.29, 125.28, 124.89, 29.14, 24.03, 23.50; ^{19}F NMR (376 MHz, CD_3CN) δ = -113.67, -117.77, -121.90, -126.00, -130.11, -134.22. EA calc: $C_{39}H_{43}AuF_6N_5O_2Sb$: C, 44.76% H, 4.14% N, 6.69% Obs: $C_{39}H_{43}AuF_6N_5O_2Sb$: C, 44.89% H, 4.31% N, 6.70%.



AuTri-C 5: Prepared as described in the general procedure. bathophenanthroline (16 mg, 0.048 mmol), AuCl(NHC) (30 mg, 0.048 mmol) and silver hexafluoroantimonate (18 mg, 0.053) as a light-yellow solid. Yield: 28 mg, 74%. ^{1}H NMR (400 MHz, CD_3CN) δ = 7.82 (s, 1H), 7.635 (d, *J* = 4 Hz, 1H), 7.60 (d, *J* = 8Hz, 2H), 7.52-7.48 (m, 3H), 7.43 (t, *J* = 8Hz, 2H), 7.41 (s, 1H) 2.665 (qu, *J* = 28Hz, 4H), 1.205 (q, J = 12 Hz, 12H); ^{13}C NMR (101 MHz, CD_3CN) δ = 151.07, 146.76, 131.45, 130.13, 129.94, 129.50, 125.68, 125.43, 125.26, 125.04, 65.85, 29.34, 24.25, 23.70, 15.21; ^{19}F NMR (376 MHz, CD_3CN) δ = -113.63, -117.74, -121.86, -125.97, -130.07, -134.19. $C_{51}H_{52}AuF_6N_4Sb$: C, 59.09% H, 4.54% N, 4.86% Obs: $C_{51}H_{52}AuF_6N_4Sb \cdot 0.6\ C_4H_{10}O$: C, 53.68% H, 4.67% N, 4.94%.



AuTri-C 6: Prepared as described in the general procedure. Dipyridophenazine (13.5 mg, 0.048 mmol), AuCl(NHC) (30 mg, 0.048 mmol) and silver hexafluoroantimonate (18 mg, 0.053) as a light orange solid. Yield: 27 mg, 38%. ^{1}H NMR (400 MHz, CD_3CN) δ = 9.69 (d, *J* = 8 Hz, 2H), 8.405 (q, *J* = 12 Hz, 2H), 8.06 (q, *J* = 8 Hz, 2H), 7.93 (q, *J* = 16 Hz, 2H), 7.605 (q, *J* = 20 Hz, 2H), 7.525 (t, J = 12 Hz, 4H), 7.415 (t, *J* = 12 Hz, 2H), 3.065 (qu, J = 28 Hz, 4H) 1.64 (q, *J* = 16 Hz, 24H); ^{13}C NMR (101 MHz CD_3CN) δ = 152.93, 146.77, 142.90, 136.03, 132.47, 131.54, 129.86, 126.52, 125.38, 125.10, 64.38, 29.37, 28.05, 24.29, 23.73; ^{19}F NMR (376 MHz, CD_3CN) δ = -113.64, -117.75, -121.84, -125.98, -130.08, -134.19. EA calc: $C_{45}H_{46}AuF_6N_6Sb$: C, 48.97% H, 4.20% N, 7.62% Obs: $C_{45}H_{46}AuF_6N_6Sb$: C, 49.01% H, 4.22% N, 7.66%.

In Vitro Biological Assays

Cell Culture. Cells used were patient derived and grown in a humidified incubator at 37C with 5-10% CO_2. BT333 cells were grown in DMEM supplemented with 10% FBS, 1% amphotericin, and 1% penicillin/streptomycin. All supplements along with PBS and trypsin-EDTA were purchased from Corning Inc. and used as is.

Cell Viability of AuTri-C 1-6. The cell viability of all 6 complexes were performed in BT333 cell line. 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 DMEM. The cells were centrifuged at 2000 rpm for 10 minutes and the pellet washed carefully with 1 mL of PBS then suspended in 5 mL of the DMEM. 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. 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).

Solution Stability of AuTri-C Complexes. DMEM was warmed to 37 °C prior to use. 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. **AuTri-C 1-6** 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.

Reactivity of AuTri-C 5 with GSH. A 10.0 mM stock solution of **AuTri-C 5** in DMSO-d_6 was prepared. In a separate vial a 100 mM stock solution of L-glutathione in DMSO-d_6 was also prepared. Subsequently, equal volumes of both L-glutathione (0.5 mL) and **AuTri-C 5** (0.5 mL) from the stock solutions were combined in an NMR tube to form final concentrations of 5.0 mM **AuTri-C 5** and 50.0 mM L-glutathione, and ^{1}H NMR spectra were recorded at 0 h, 1 h, 6 h, 12 h, and 24 h.

Immunoblotting. Equal numbers of BT333 cells were seeded and treated with **AuTri-C 5** 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 Bovine Serum Albumin (BSA) in PBST for 1hr. The membranes were incubated overnight with the primary antibodies directed against OPA1, MFN1, DRP1, and beta-actin. Appropriate secondary antibodies were used accordingly. The membranes were placed in pierce enhanced chemiluminescence substrate and visualized with Bio-Rad imager. All antibodies used for this study were purchased from cell signaling technology.

Mitochondrial ROS (mtROS)

BT333 cells were plated in a six-well plate at 500,000 cells per well and allowed to adhere overnight. Cells were treated with **AuTri-C 5** for short period of 2 h at concentrations of 5 μM and 10 μM. After incubation time with test compound, cells were trypsinized and centrifuged to form pellets. Pellets were washed with PBS and resuspended in Mitosox dye solution (200 μL). This was transferred into FACS tube and incubated for 20mins followed by flow cytometry analysis.

Mitochondrial Membrane Potential (TMRE). BT333 cells were plated at a density of 500,000 cells/well using a 6 well plate and allowed to adhere overnight at 37°C. Compound **AuTri-C 5** was prepared as a stock in DMSO and added at a final concentration of 5 and 10 μM (< 1%

DMSO). The cells were treated 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 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 TMRE dye was prepared by adding 2 μL of dye into 7.998 mL of DMEM. Then, 100 μL/mL of working solution were added to the cells and incubated at 37 °C for 15 minutes. This was followed by analysis on a flow cytometer with 488nm excitation and appropriate emission filters.

Apoptosis

BT333 cells were seeded at a density of 500,000 cells/well in 6-well clear bottom plates. The cells were allowed to adhere overnight at 37C. **AuTri-C 5** was prepared as a stock (1 mM) in DMSO/DMEM mixture and added to the wells at concentrations of 5 and 10 μM and incubated for 24 h at 37°C. H_2O_2, used as a positive control, was prepared and treated in a separate well at a final concentration of 200 μM. After incubation, the media was collected into separate 15 mL tubes and the wells containing the cells were washed with 2 mL of PBS and transferred into the 15 mL tubes. The cells were detached by trypsinization and added to the tubes also. The tubes were centrifuged for 5 min to form pellets after which the supernatant was removed, and the pellets were resuspended in 300 μL of Annexin binding buffer. To each tube, 5 μL of Annexin V-FITC and 5 μL PI were added and incubated in the dark for 5 min before flow cytometry analysis.

Confocal Microscopy

Cells were seeded at 500,000 cells/mL on 35 mm glass-bottom Petri dishes and incubated overnight in DMEM at 37°C. After adhering overnight, media was removed and cells were washed with PBS twice before treatment with **AuTri-C 5** at 10 μM for 1 h, leaving a control dish untreated. After treatment with test compound, cells were washed three times with PBS followed by treatment with mitotracker red (200 nM) for 30 min after which the cells were washed three times and fixed with 4% PFA for 20 min. The cells were washed three times with PBS, after which Hoechst dye was added for 15 min to stain the nuclei. Cells were washed with PBS and mounted on the Nikon A1R confocal microscope with 60X oil objective. Images were analyzed with NIS viewer.

Bioenergetics Analysis with Seahorse XF96. Bioenergetics was analyzed via two methods,

pneumatic injection and pretreatment. BT333 cells were seeded at 30,000 cells/well for the Seahorse XF96 experiments. The cells were seeded a day prior to the experiment in a 100 µL volume per well and incubated overnight at 37°C. **AuTri-C 5** was prepared as a 1 mM stock in DMSO and phenol red free DMEM and diluted to working concentration with Seahorse XF96 assay buffer. The pneumatic injection assay was performed with the final injection concentrations of 5, 10, and 20 µM (<1% DMSO). The pretreatment occurred overnight at 0.1, 1, and 10 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 based on readings from a minimum of 3 wells.

In-Vivo Tumor Xenograft Monitoring

BALB/c nude mice were injected subcutaneously into the flank region (dorsolateral region) with 300,000 G9pCDH cells in 50 µL Matrigel. First measurements occurred at day 14 and treatment started 20 days after cell implantation, with 16.7 mg/kg body weight of **AuTri-C 5** (molar equivalent to 5 mg/kg of cisplatin) in a volume of 150 µL per mice, injected intraperitoneally (prepared from a stock solution of the **AuTri-C 5** at 10 mg/mL in DMSO, then diluted in 2% Tween 80 in PBS), and mice were then treated twice a week for 28 days. The first two weeks of the experiment are omitted (i.e., tumor growth phase before imaging and treatment). The control group was treated similarly with the vehicle. Fluorescence was monitored by the LifeScience Caliper software (v4.3), tumor volume was measured and calculated according to the following formula:

$$Volume(mm^3) = {([Width(mm)^2 \times Length(mm)])}/{2}$$

Supplementary Figures and Tables:

X-Ray Crystallographic Details:

Table S1: X-ray Crystallographic Parameters of **AuTri-C 1a**

X-ray Structural Data and Crystal Refinement

	AuTri-C 1a
Empirical Formula	$C_{39}H_{44}AuF_6N_4Sb$
Molecular Weight (g/mol)	1001.5
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Monoclinic, P2(1)/c
Unit Cell Dimensions (A), (o)	a =8.8161(2) Å alpha = 90 b = 27.2314(6) Å beta = 91.479(1) c = 15.8412(4) Å gamma = 90(1)
Volume	3801.81(15) $Å^3$
Z	4
Absorption Coefficient	4.628 mm^{-1}
F(000)	1960
Crystal Size (mm)	0.120 x 0.110 x 0.080
Theta Range	2.586 to 27.505
Completeness to Theta = 25.242	99.90%
F^2	1.044
Final R indices [I>2sigma(I)]	R1 = 0.0198, wR2 = 0.0383

Table S2: X-ray Crystallographic Parameters of **AuTri-C 1b**

X-ray Structural Data and Crystal Refinement	
Empirical Formula	**AuTri-C 1b** $C_{39}H_{44}AuF_6N_4Sb$
Molecular Weight (g/mol)	1001.5
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Monoclinic, P2(1)/c
Unit Cell Dimensions (A), (o)	a = 8.8234(2) A alpha = 90 b = 27.2096(5) A beta = 91.585(1) c = 15.8345(3) A gamma = 90
Volume	3800.11(13) $Å^3$
Z	4
Absorption Coefficient	4.630 mm^{-1}
F(000)	1960
Crystal Size (mm)	0.120 x 0.100 x 0.070
Theta Range	1.974 to 27.516
Completeness to Theta = 25.242	99.90%
F^2	1.037
Final R indices [I>2sigma(I)]	R1 = 0.0184, wR2 = 0.0388

Table S3: X-ray Crystallographic Parameters of **AuTri-C 2**

X-ray Structural Data and Crystal Refinement	
Empirical Formula	**AuTri-C 2** $C_{40}H_{46}AuF_6N_4Sb$
Molecular Weight (g/mol)	1015.52
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, P-1
Unit Cell Dimensions (A), (o)	a = 8.6432(2) A alpha = 86.9968(1) b = 14.4671(4) A beta = 89.402(1) c = 15.6498(3) A gamma = 87.477(1)
Volume	1952.19(88) $Å^3$
Z	2
Absorption Coefficient	4.507 mm^{-1}
F(000)	996
Crystal Size (mm)	0.130 x 0.110 x 0.080
Theta Range	1.970 to 27.523
Completeness to Theta = 25.242	99.90%
F^2	1.117
Final R indices [I>2sigma(I)]	R1 = 0.0171, wR2 = 0.0368

Table S4: X-ray Crystallographic Parameters of **AuTri-C 3**

X-ray Structural Data and Crystal Refinement	
Empirical Formula	**AuTri-C 3** $C_{41}H_{48}AuF_6N_4Sb$
Molecular Weight (g/mol)	1029.55
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, P-1
Unit Cell Dimensions (A), (o)	a = 8.8257(3) A alpha = 96.561(1) b = 14.2836(4) A beta = 90.466(1) c = 15.9439(4) A gamma = 94.096(1)
Volume	1991.4(1) $Å^3$
Z	2
Absorption Coefficient	4.420 mm^{-1}
F(000)	1012
Crystal Size (mm)	0.120 x 0.110 x 0.090
Theta Range	2.037 to 27.628
Completeness to Theta = 25.242	99.90%
F^2	1.056
Final R indices [I>2sigma(I)]	R1 = 0.0186, wR2 = 0.0367

Table S5: X-ray Crystallographic Parameters of **AuTri-C 4**

X-ray Structural Data and Crystal Refinement

Empirical Formula	**AuTri-C 4** $C_{39}H_{43}AuF_6N_5O_2Sb$
Molecular Weight (g/mol)	1046.5
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Monoclinic, P2(1)/c
Unit Cell Dimensions (A), (o)	a = 8.7865(2) A alpha = 90 b = 21.4256(5) A beta = 97.974(1) c = 21.0050(5) A gamma = 90
Volume	3916.08(16) $Å^3$
Z	4
Absorption Coefficient	4.501 mm^{-1}
F(000)	2048
Crystal Size (mm)	0.130 x 0.090 x 0.070
Theta Range	1.958 to 27.557
Completeness to Theta = 25.242	99.90%
F^2	1.048
Final R indices [I>2sigma(I)]	R1 = 0.0191, wR2 = 0.0375

Table S6: X-ray Crystallographic Parameters of **AuTri-C 5**

X-ray Structural Data and Crystal Refinement	
Empirical Formula	**Au Tri-C 5** $C_{106}H_{114}Au_2F_{12}N_8OSb$
Molecular Weight (g/mol)	2381.48
Temperature (K)	90.0(2)
X-ray Radiation (Å)	Mo Kα (0.71073 Å)
Crystal System, Space Group	Triclinic, P-1
Unit Cell Dimensions (A), (o)	a = 10.4178(4) A alpha = 106.482(1) b = 16.1533(4) A beta = 101.039(1) c = 16.1847(5) A gamma = 102.444(1)
Volume	2455.76(14) $Å^3$
Z	1
Absorption Coefficient	3.597 mm^{-1}
F(000)	1182
Crystal Size (mm)	0.200 x 0.200 x 0.100
Theta Range	2.079 to 27.500
Completeness to Theta = 25.242	100.0%
F^2	1.036
Final R indices [I>2sigma(I)]	R1 = 0.0165, wR2 = 0.0356

NMR Spectra of AuCl(NHC):

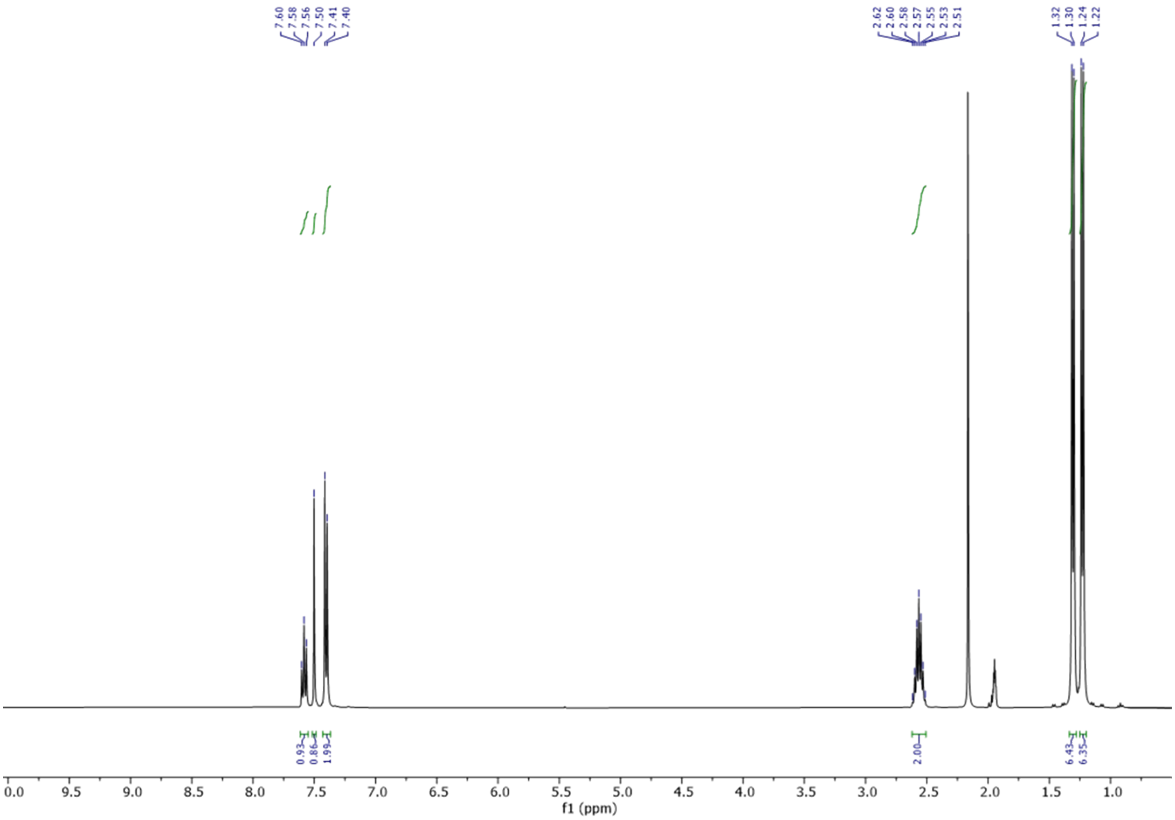


Fig. S1. ^{1}H NMR spectrum of **AuCl(NHC)** in CD_3CN at 298K.

NMR Spectra of AuTri-C 1-6:

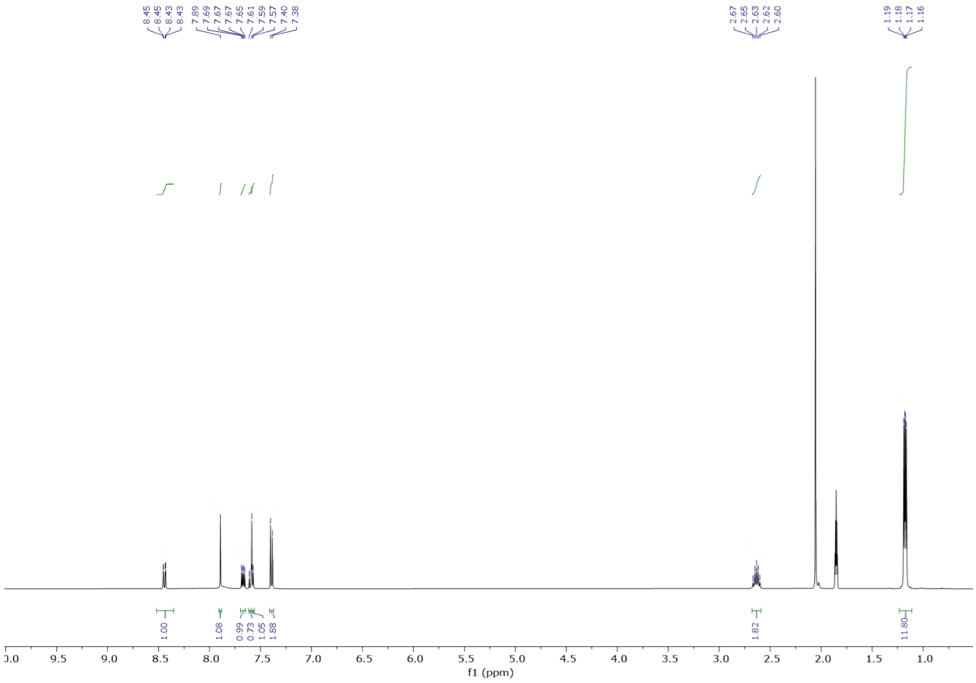


Fig. S2. ^{1}H NMR spectrum of **AuTri-C 1** in CD_3CN at 298K.

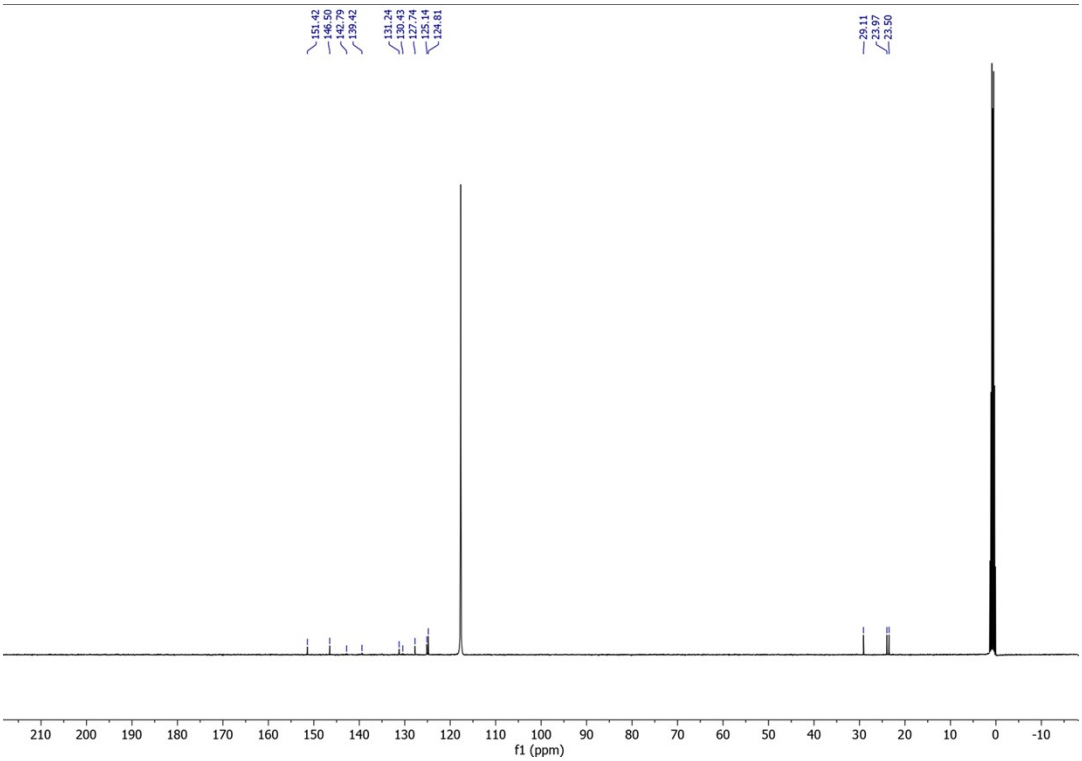


Fig. S3. ^{13}C NMR spectrum of **AuTri-C 1** in CD_3CN at 298K.

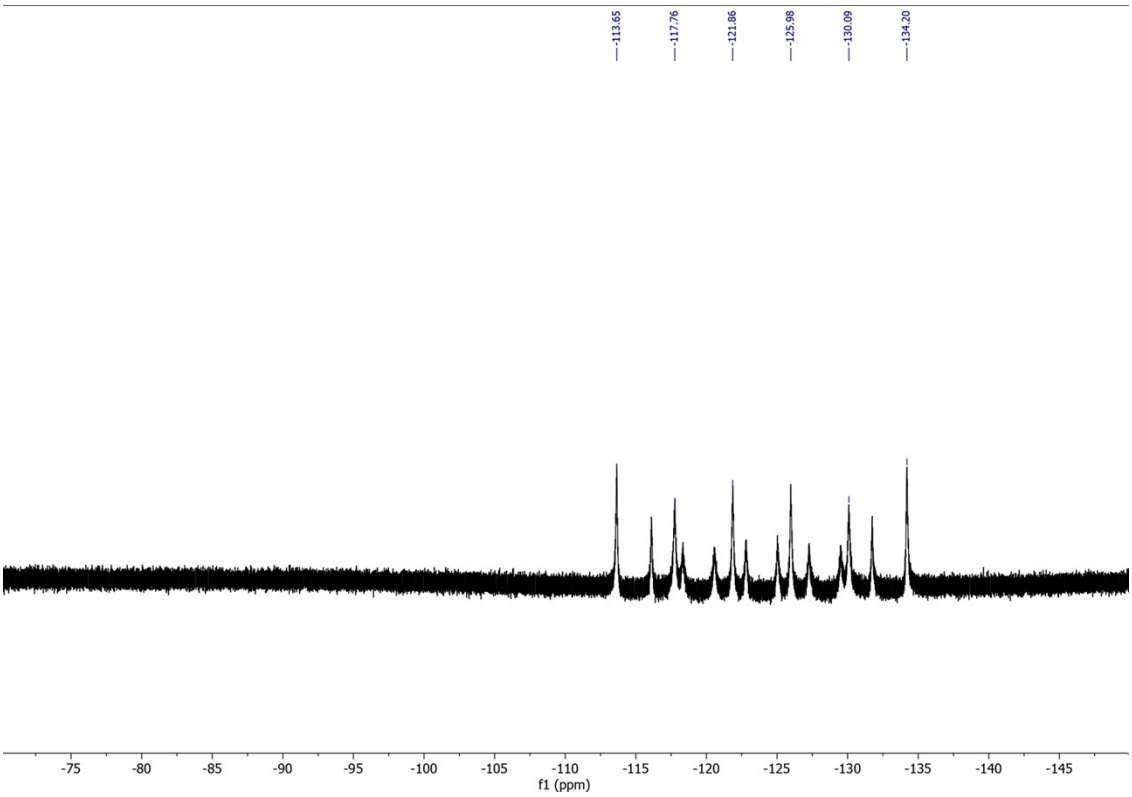


Fig. S4. ^{19}F NMR spectrum of **AuTri-C 1** in CD_3CN at 298K.

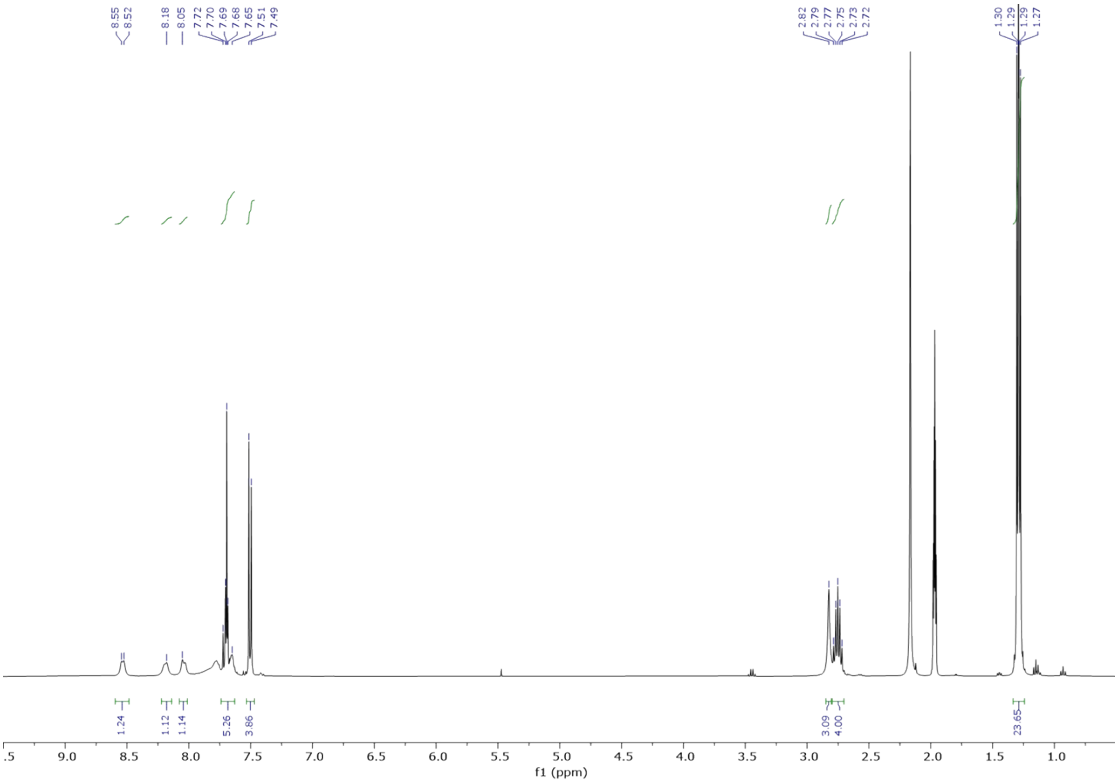


Fig. S5. ^{1}H NMR spectrum of **AuTri-C 2** in CD_3CN at 298K.

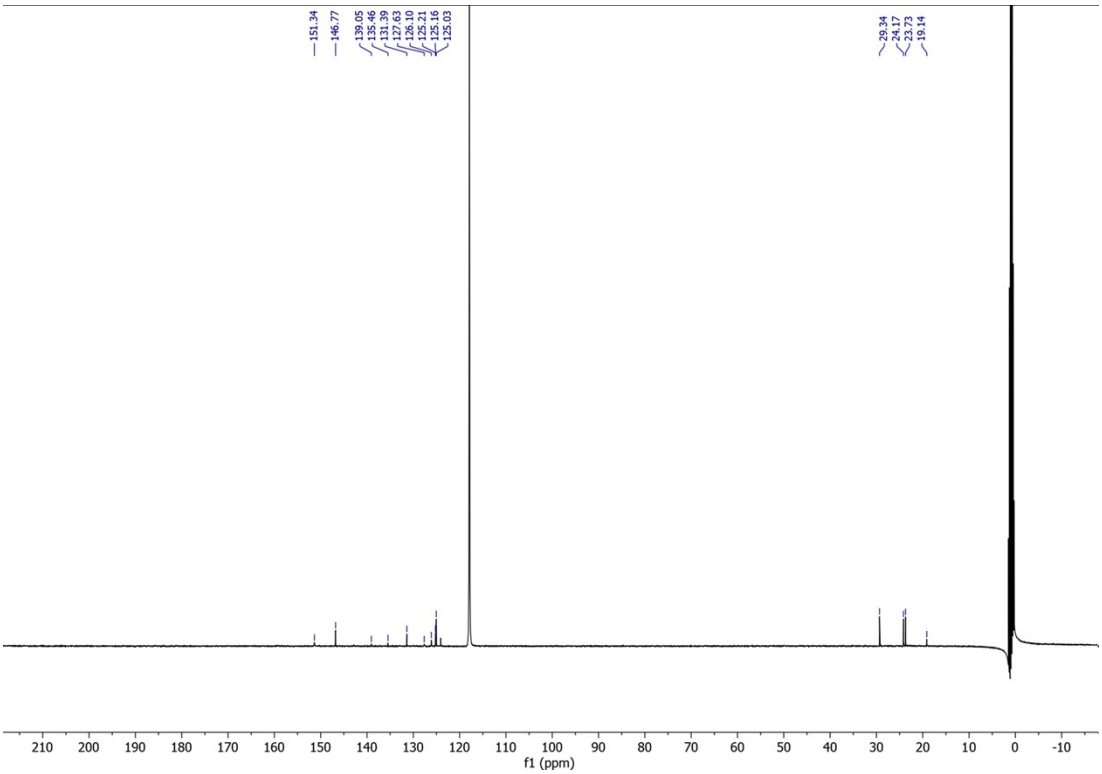


Fig. S5. ^{13}C NMR spectrum of **AuTri-C 2** in CD_3CN at 298K.

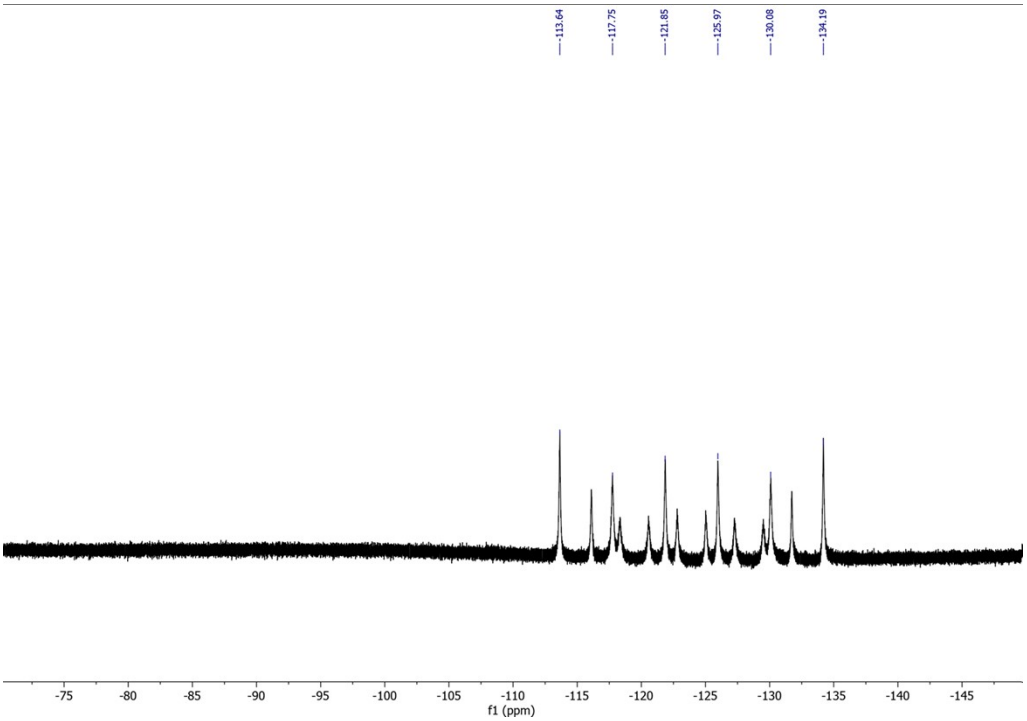


Fig. S6. ^{19}F NMR spectrum of **AuTri-C 2** in in CD_3CN at 298K.

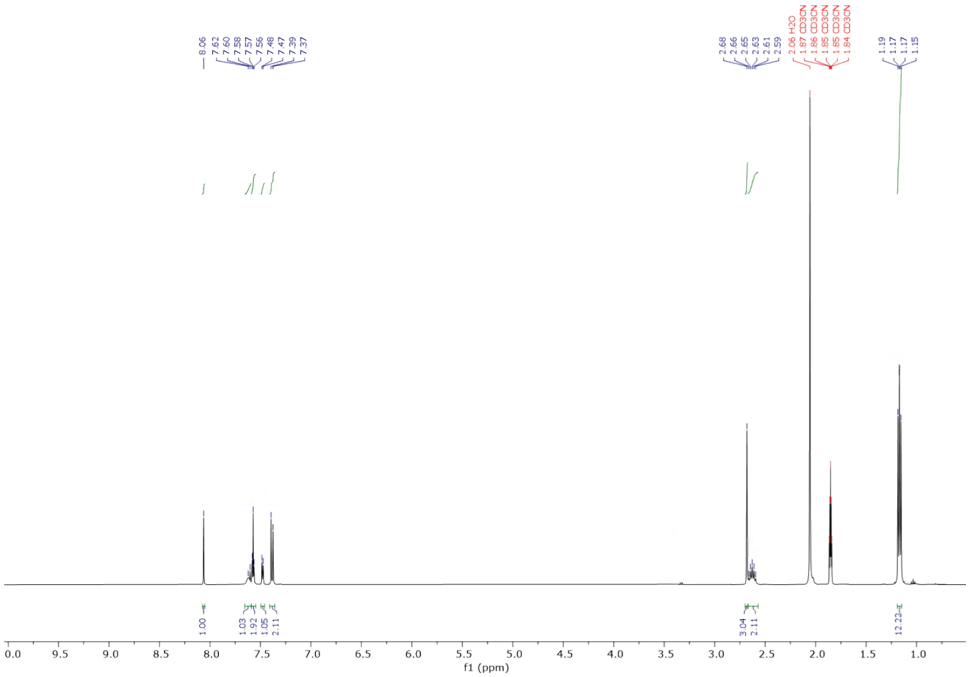


Fig. S7. ^{1}H NMR spectrum of **AuTri-C 3** in CD_3CN at 298K.

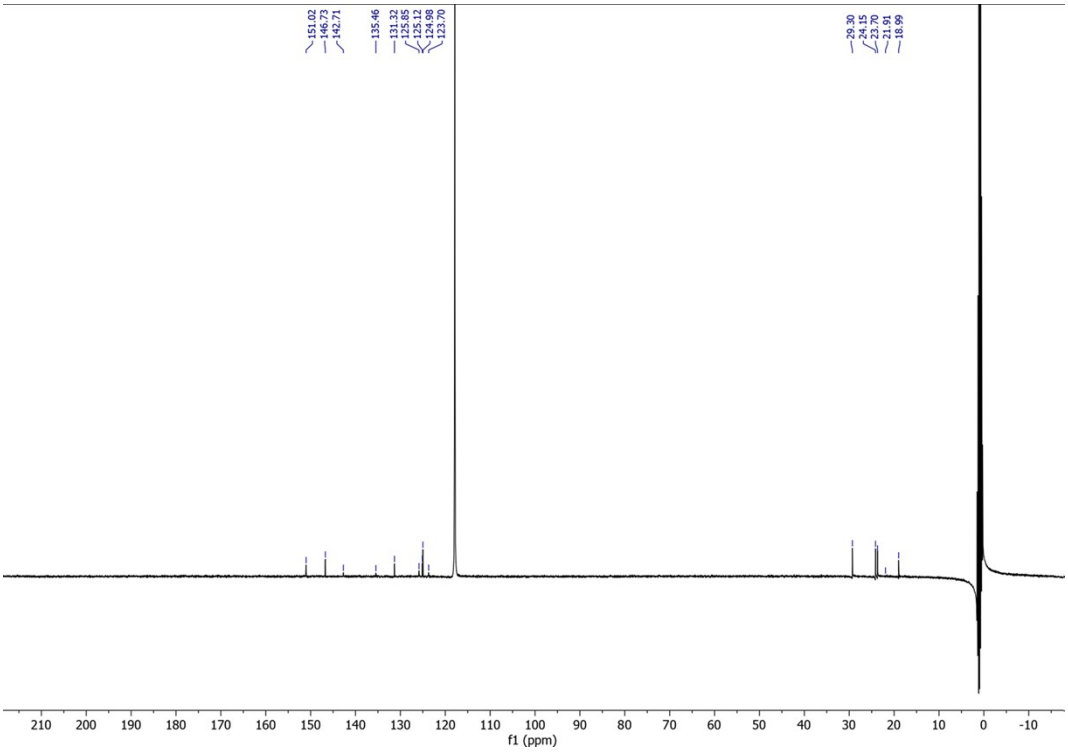


Fig. S8. ^{13}C NMR spectrum of **AuTri-C 3** in CD_3CN at 298K.

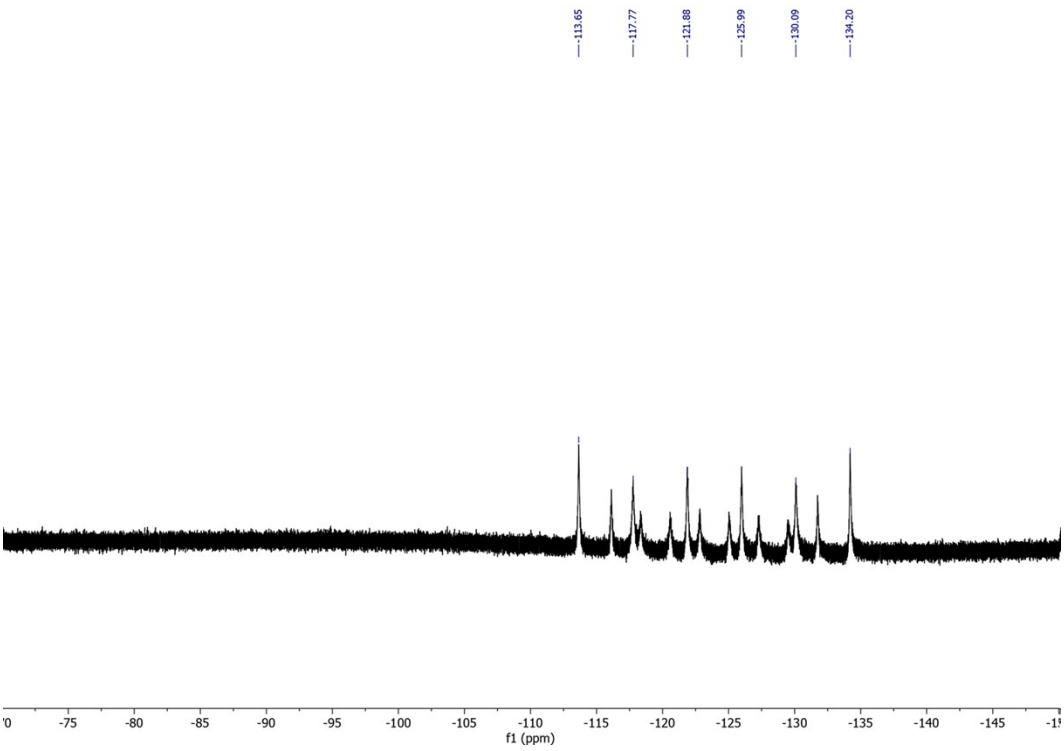


Fig. S9. ^{19}F NMR spectrum of **AuTri-C 3** in in CD_3CN at 298K.

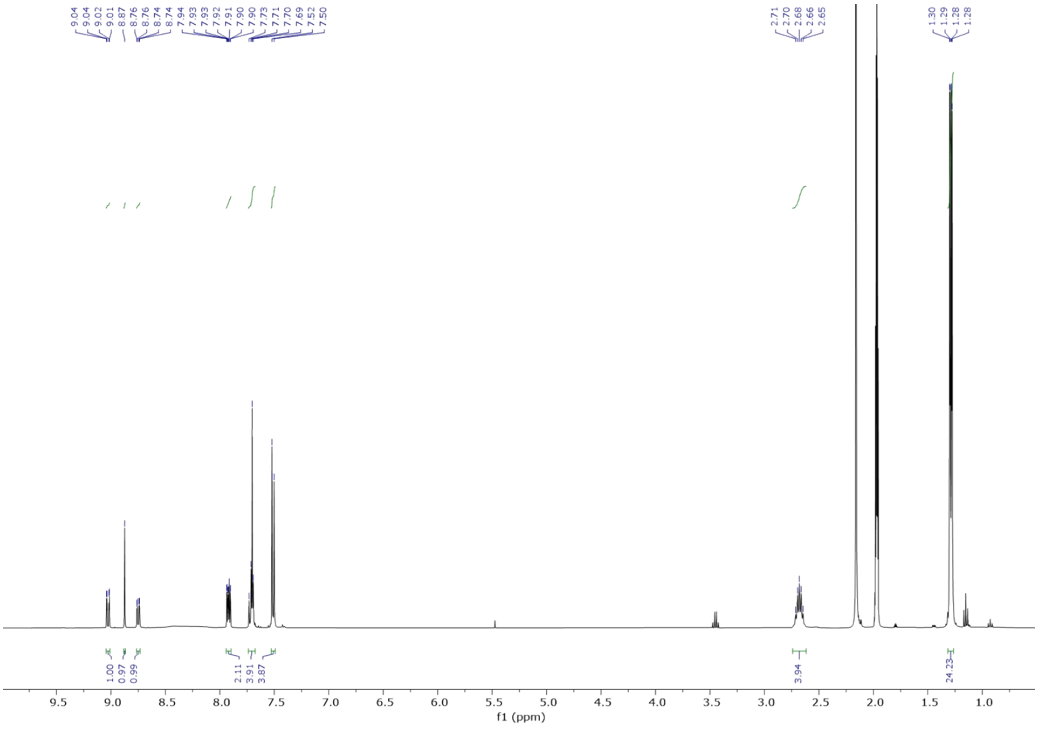


Fig. S10. ^{1}H NMR spectrum of **AuTri-C 4** in CD_3CN at 298K.

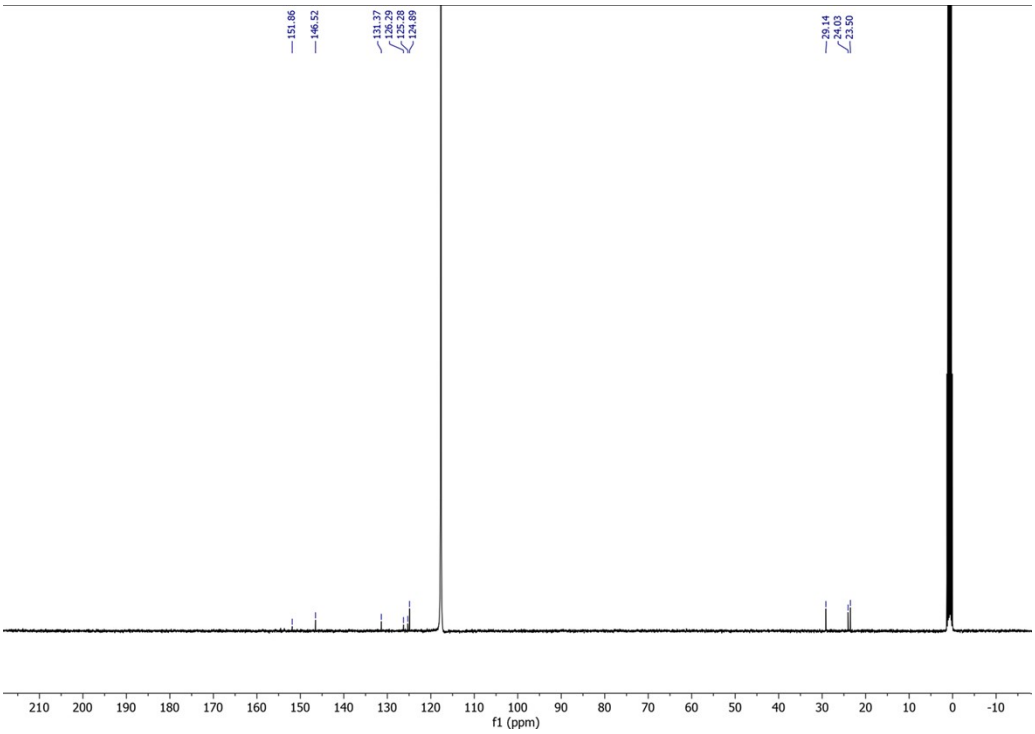


Fig. S11. ^{13}C NMR spectrum of **AuTri-C 4** in CD_3CN at 298K.

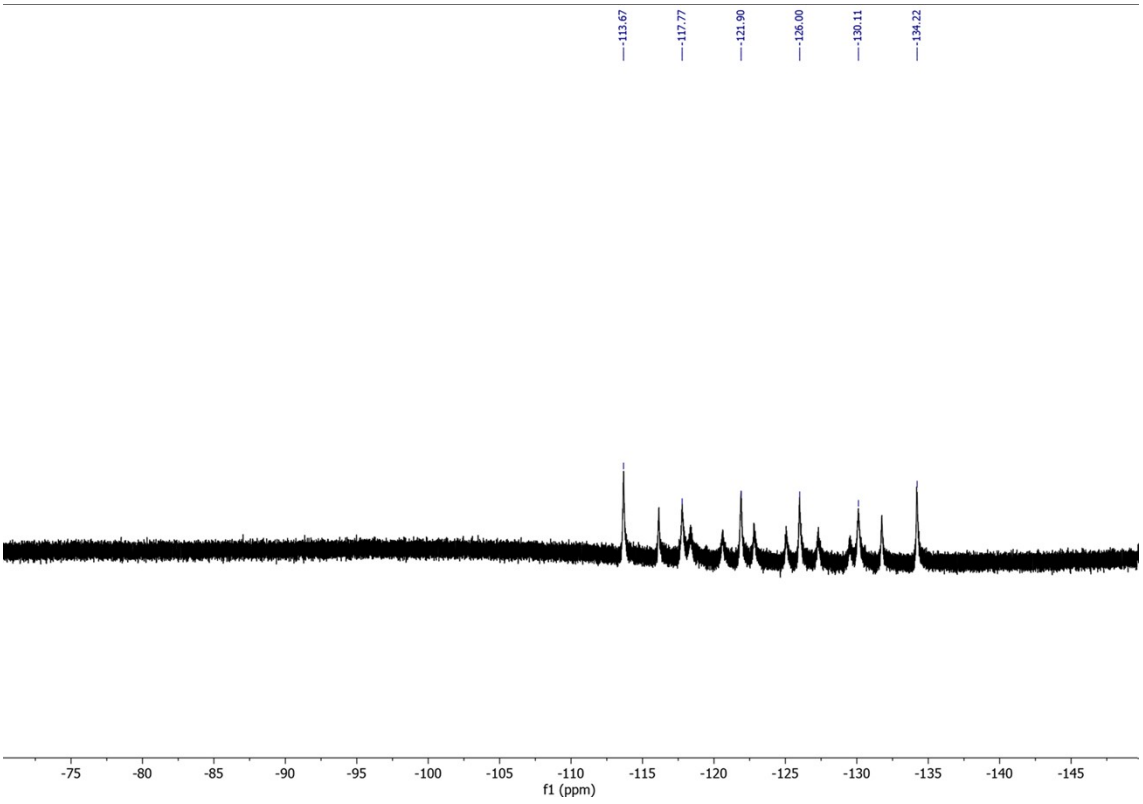


Fig. S12. ^{19}F NMR spectrum of **AuTri-C 4** in CD_3CN at 298K.

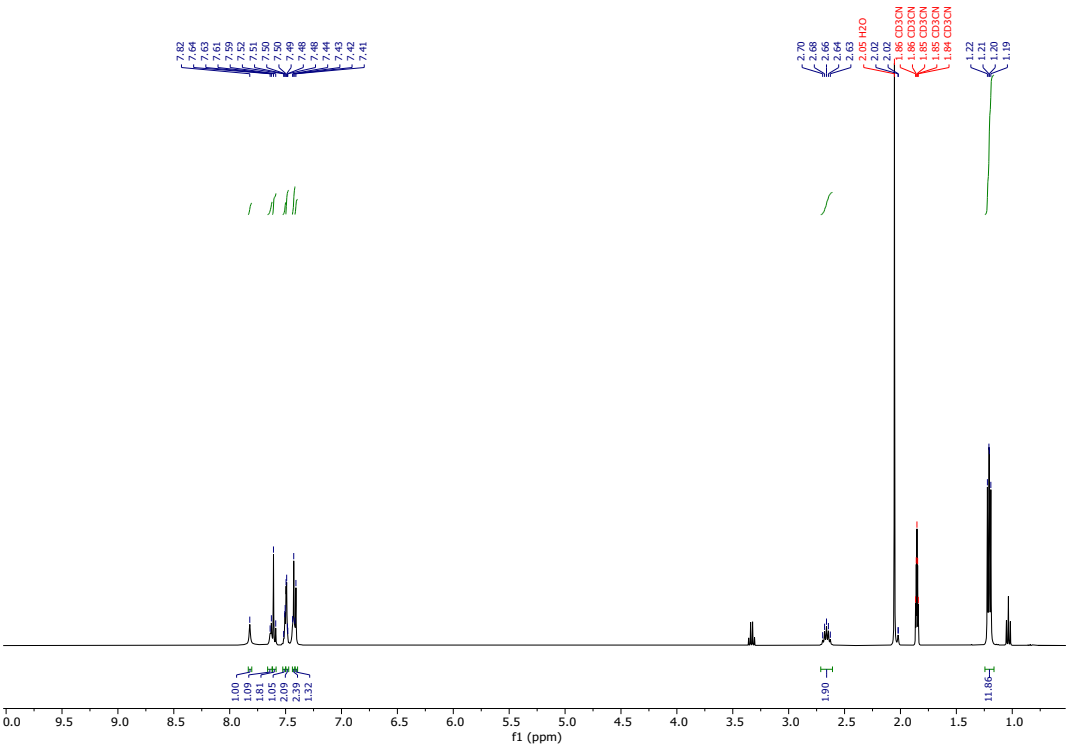


Fig. S13. ^{1}H NMR spectrum of **AuTri-C 5** in CD_3CN at 298K. Note the presence of ether is also observed in the crystal structure.

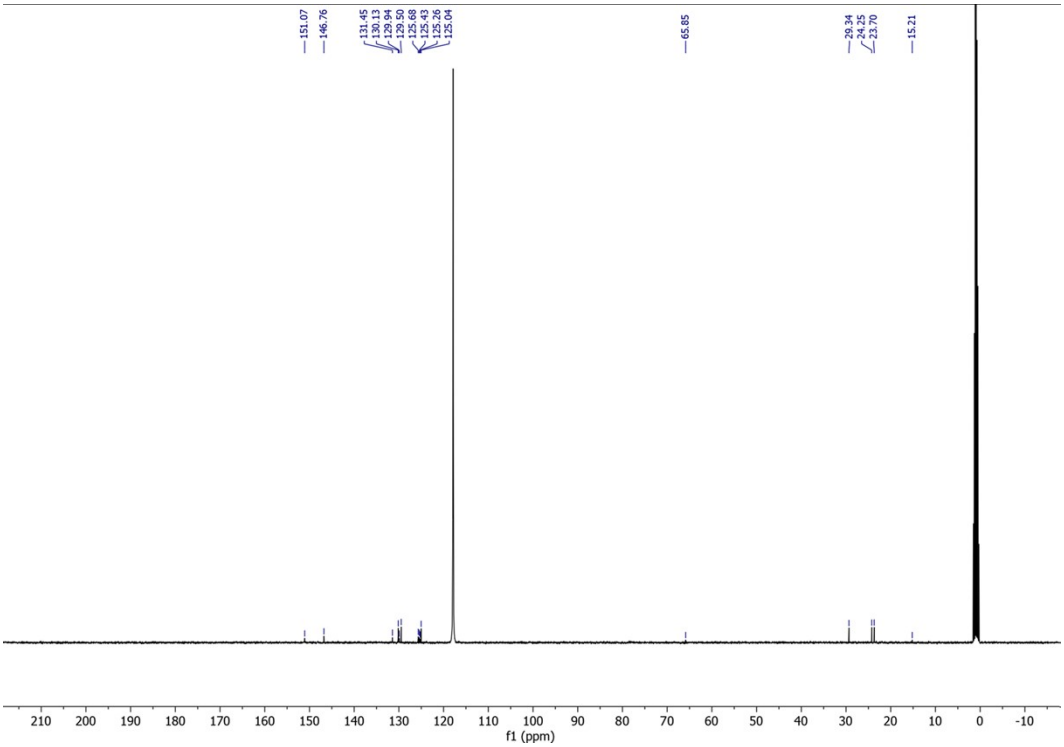


Fig. S14. ^{13}C NMR spectrum of **AuTri-C 5** in CD_3CN at 298K.

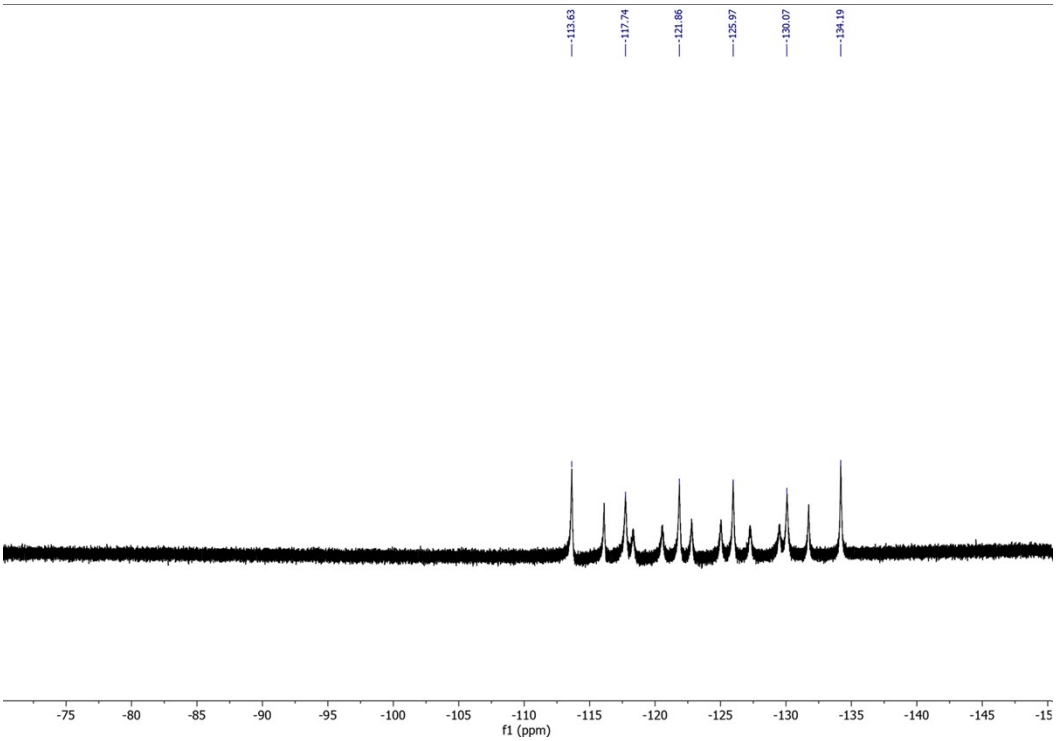


Fig. S15. ^{19}F NMR spectrum of **AuTri-C 5** in CD_3CN at 298K

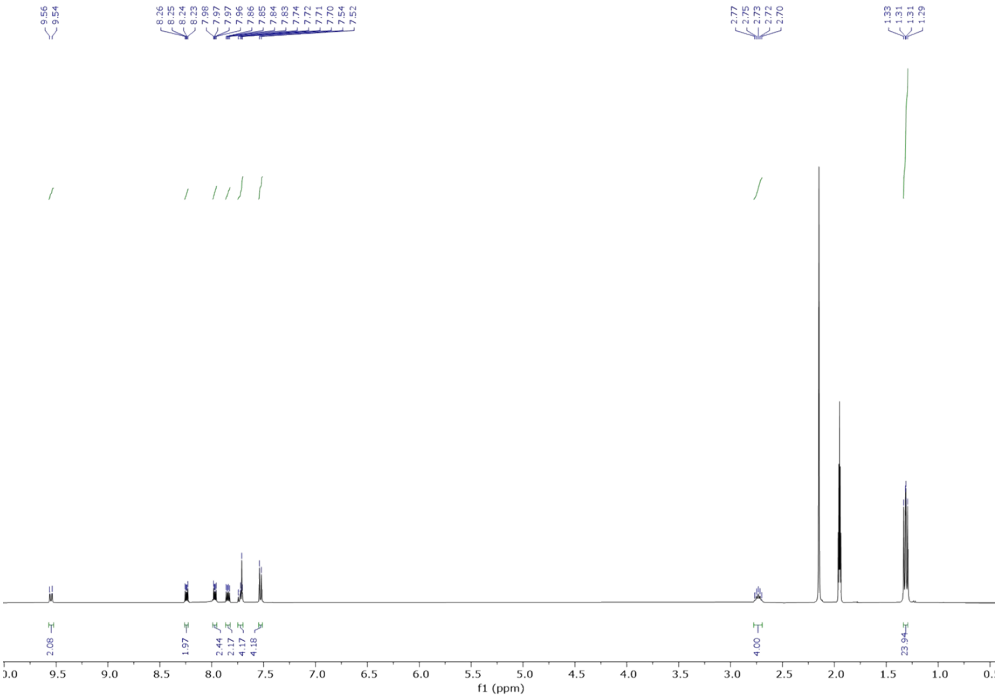


Fig. S16. ^{1}H NMR spectrum of **AuTri-C 6** in CD_3CN at 298K.

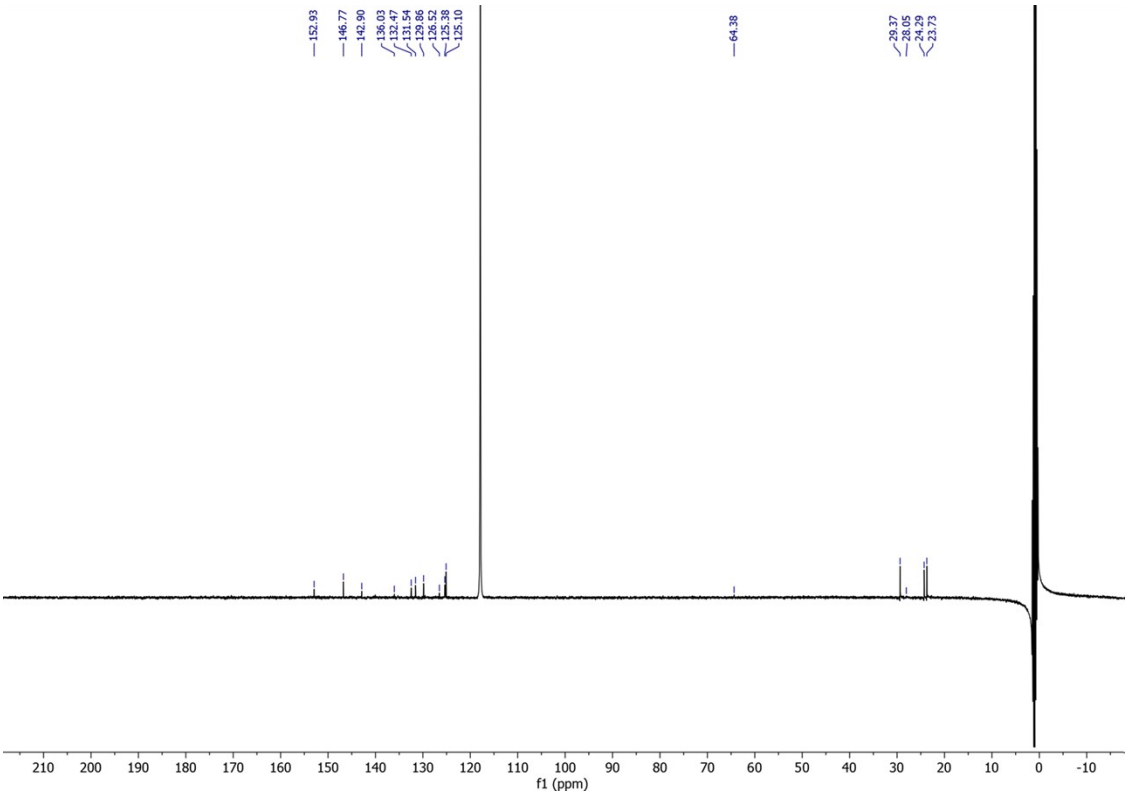


Fig. S17. ^{13}C NMR spectrum of **AuTri-C 6** in CD_3CN at 298K.

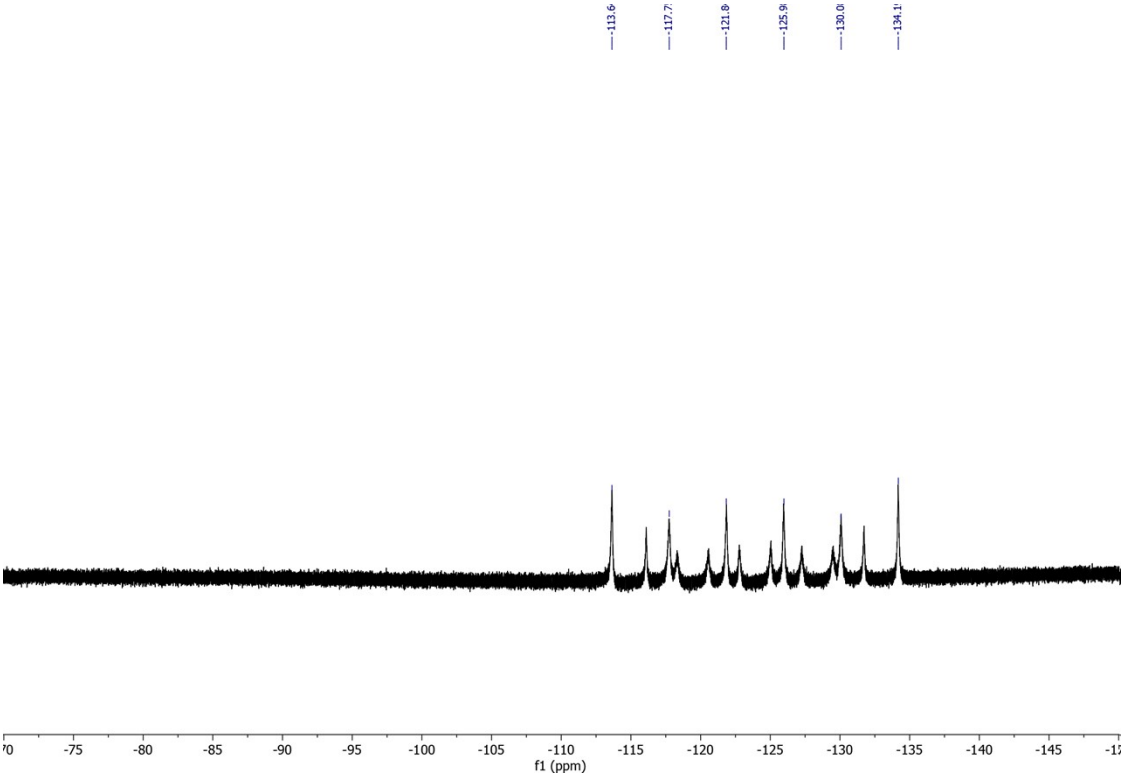


Fig. S18. ^{19}F NMR spectrum of **AuTri-C 6** in CD_3CN at 298K.

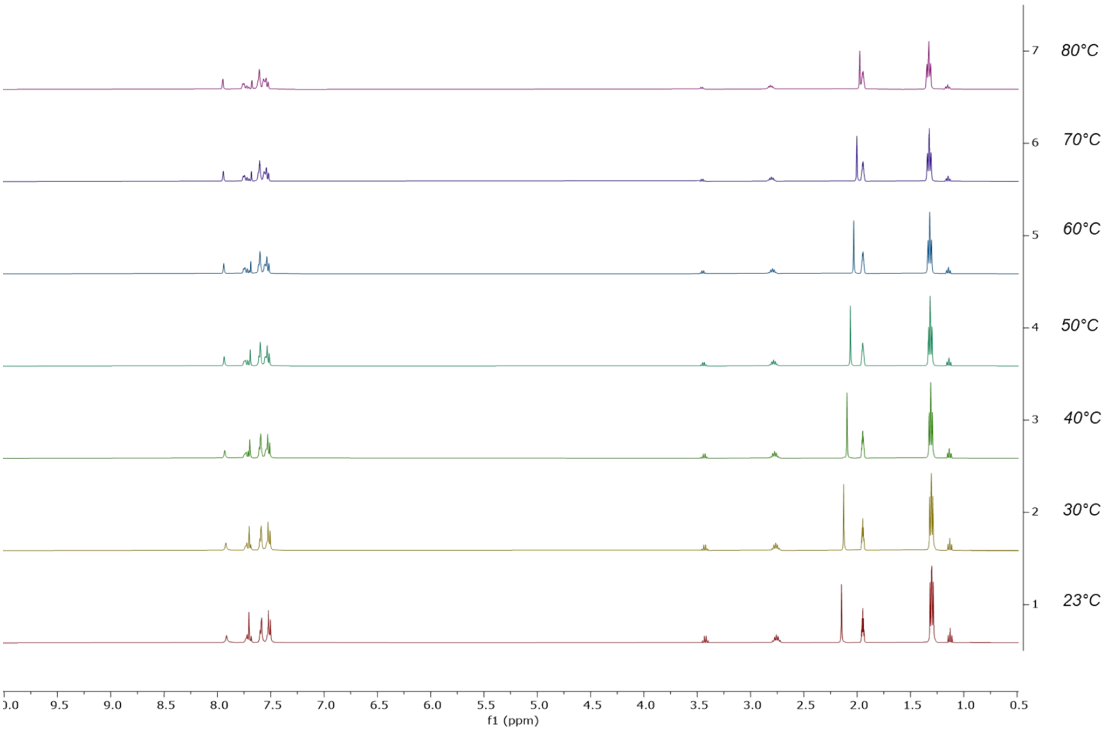


Fig. S19. Temperature dependent ^{1}H NMR spectrum of **AuTri-C 5** in CD_3CN at temperatures ranging from 298K-353K.

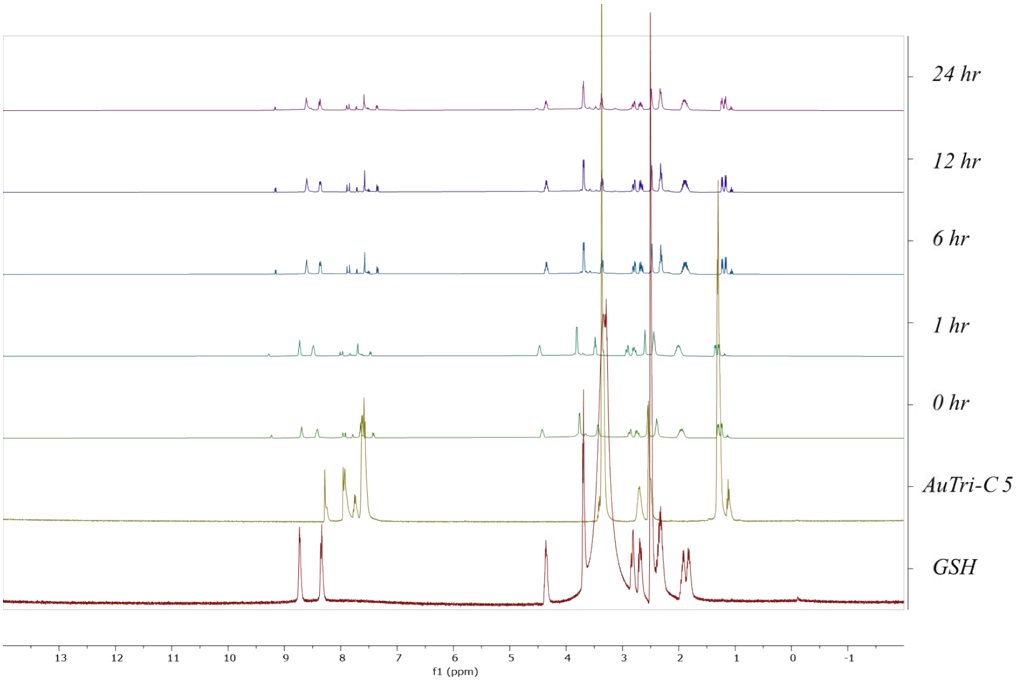


Fig. S20. ^{1}H NMR spectrum of 1:10 ratio **AuTri-C 5**:GSH in DMSO-d_6 at 298K.

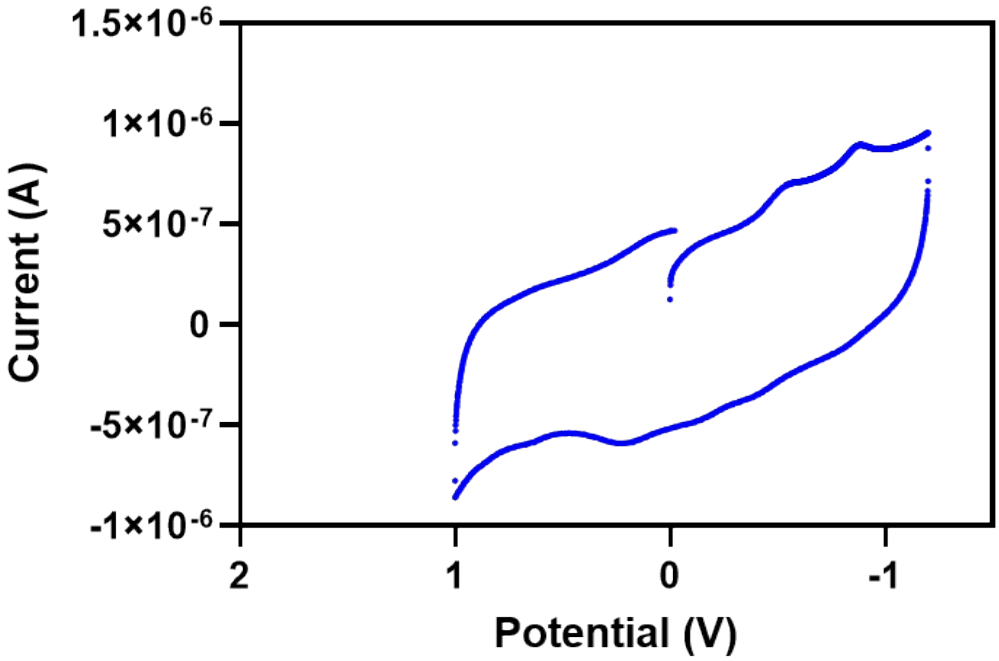


Fig. S21. Cyclic Voltammogram of 4,7-dimethylphenanthroline on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}).

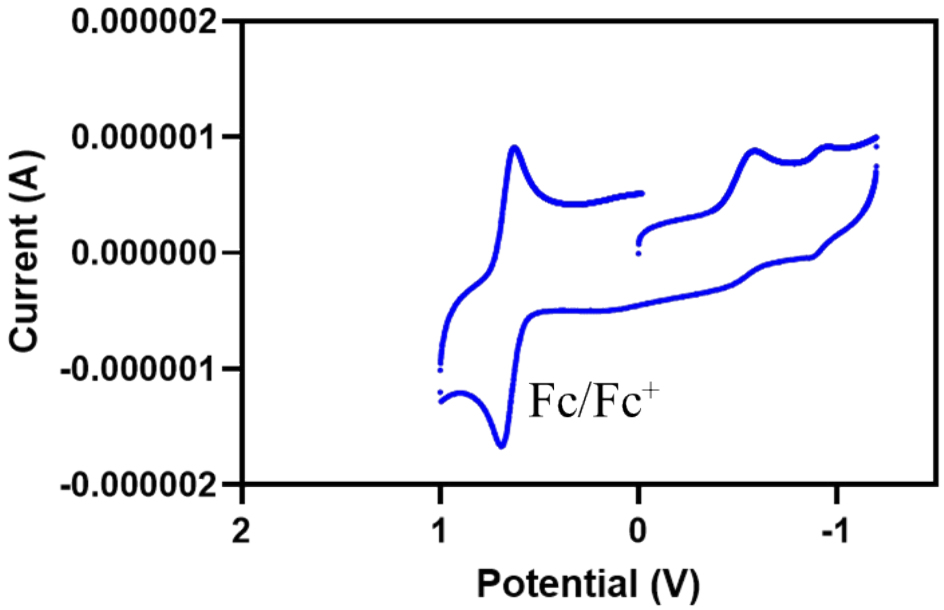


Fig. S22. Cyclic Voltammogram of 4,7-dimethylphenanthroline on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}) using Fc/Fc^+ redox couple as standard.

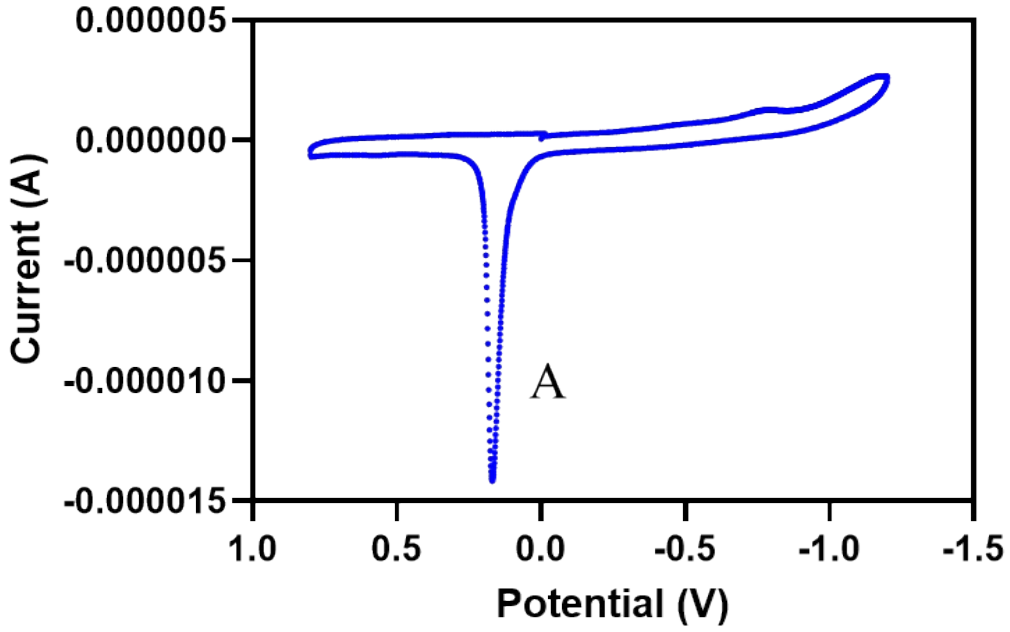


Fig. S23. Cyclic Voltammogram of **AuTri-C 3**. on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}).

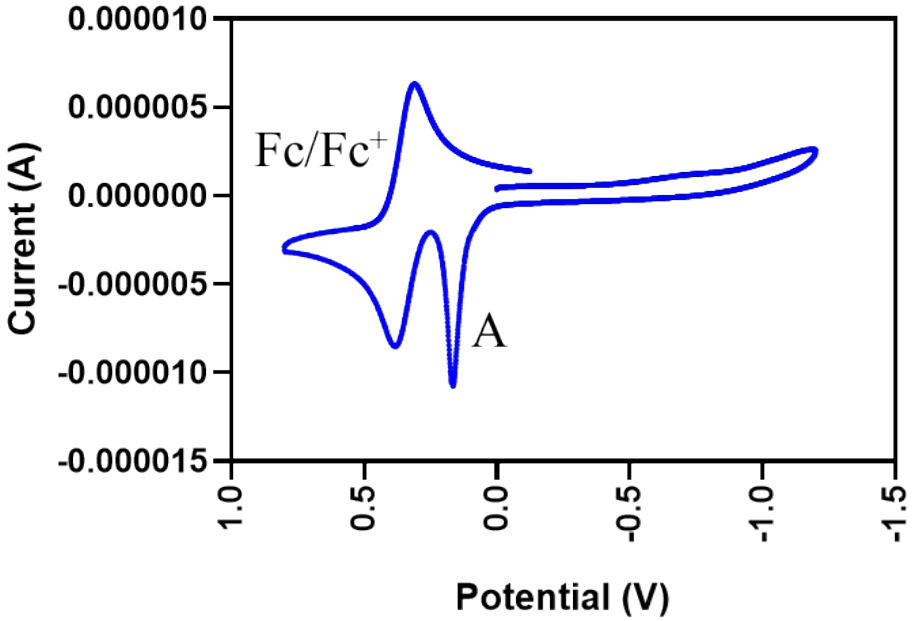


Fig. S24. Cyclic Voltammogram of **AuTri-C 3** on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}) using Fc/Fc^+ redox couple as standard.

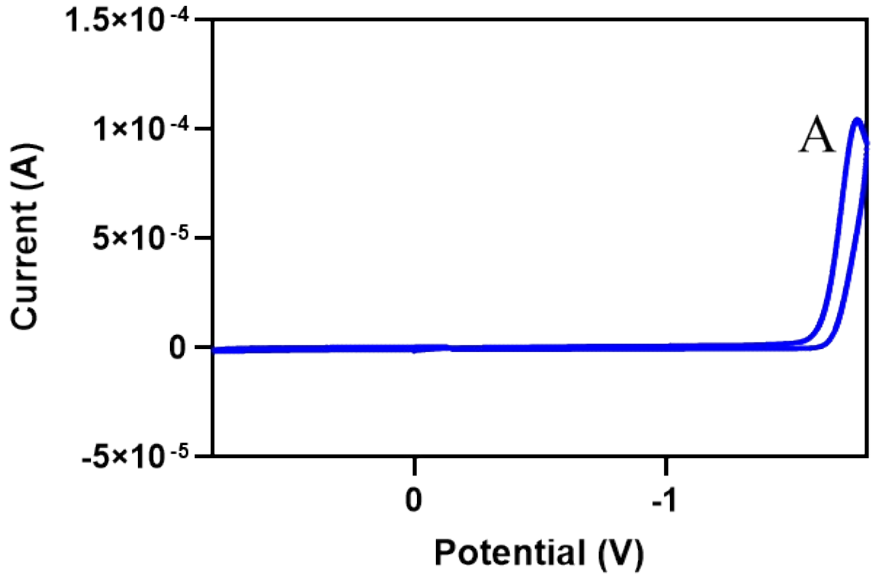


Fig. S25. Cyclic Voltammogram of bathophenanthroline on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}).

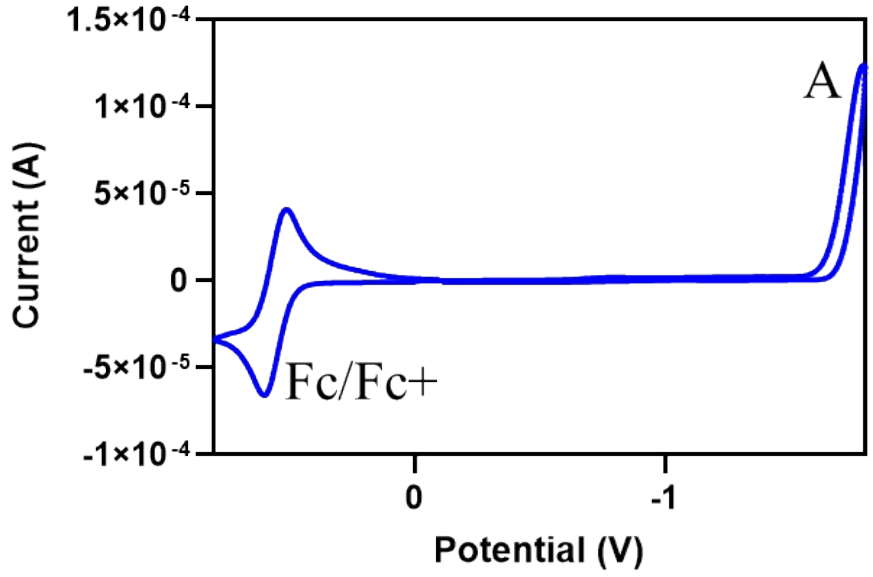


Fig. S26. Cyclic Voltammogram of bathophenanthroline on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}) using Fc/Fc^+ redox couple as standard.

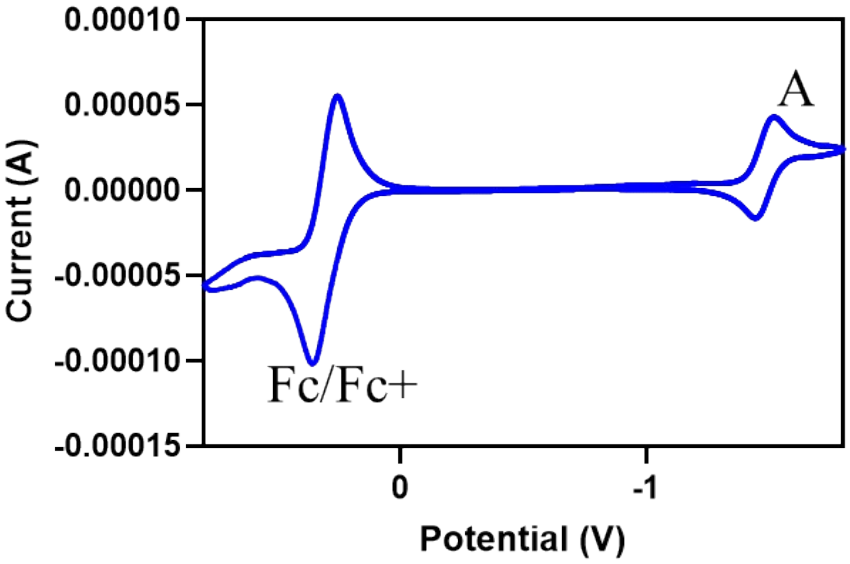


Fig. S27. Cyclic Voltammogram of **AuTri-C 5** on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}) using Fc/Fc^+ redox couple as standard.

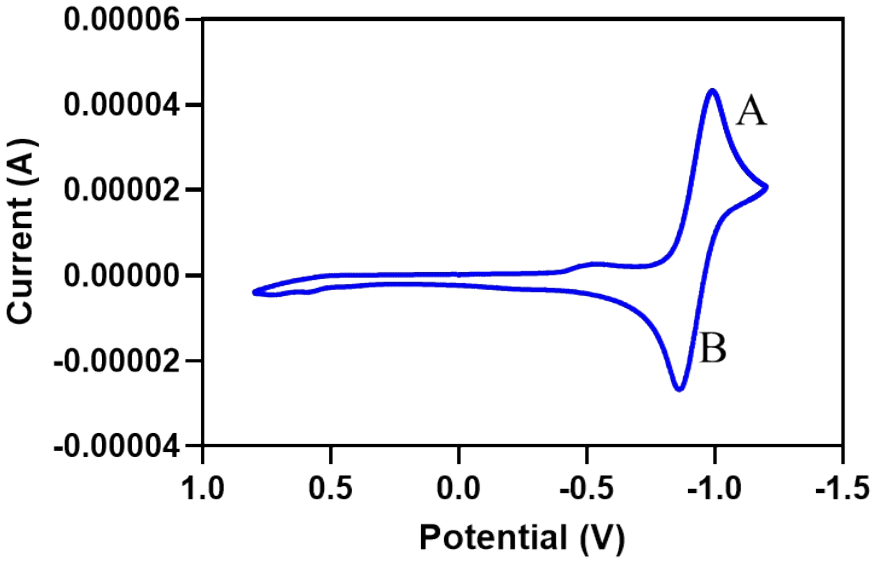


Fig. S28. Cyclic Voltammogram of dppz on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}).

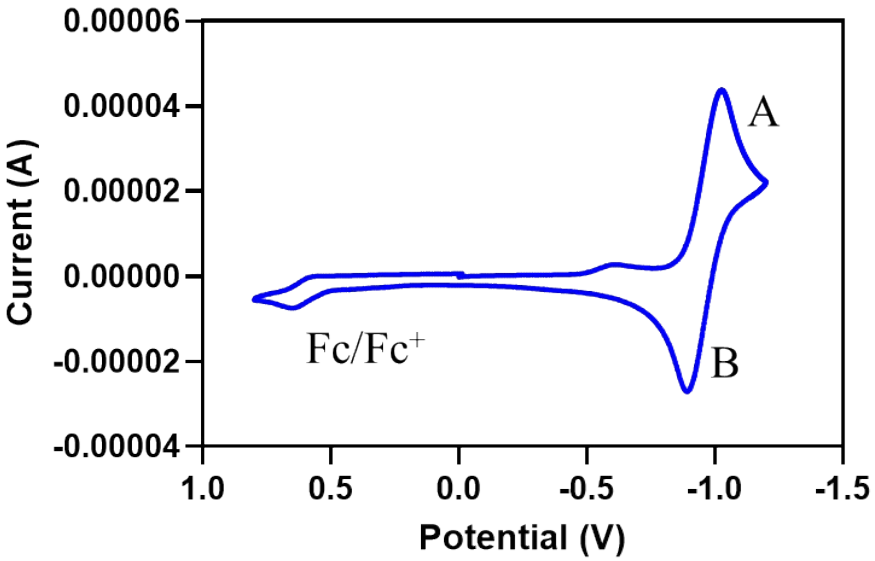


Fig. S29. Cyclic Voltammogram of dppz on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}) using Fc/Fc^+ redox couple as standard.

.

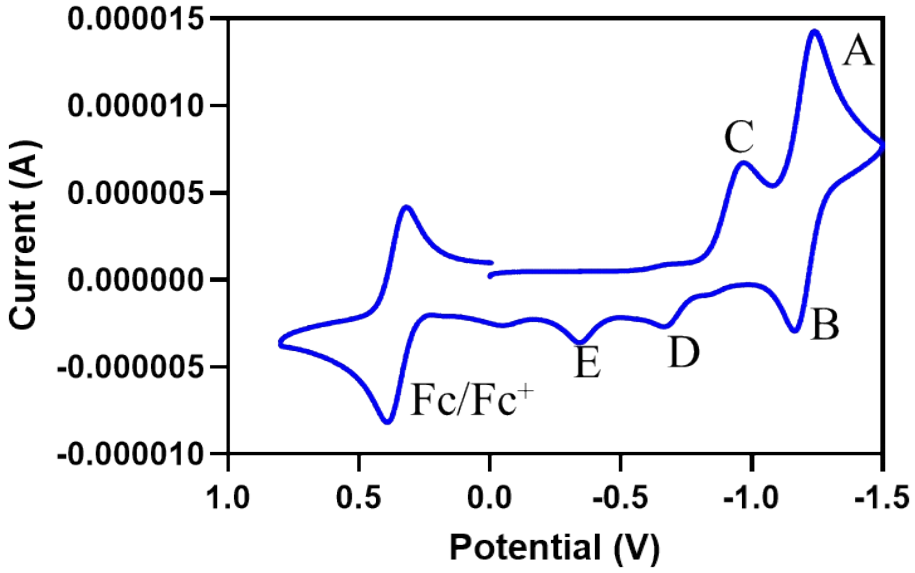


Fig. S30. Cyclic Voltammogram of **AuTri-C 6** on glassy carbon electrode (3 mm dia.) in a three-electrode configuration over 1 cycle in an acetonitrile/ambient atmosphere (supporting electrolyte: 0.1 M $N(Bu)_4PF_6$, counter electrode: Pt, reference electrode: Ag/AgCl, scan rate: 100 mV s^{-1}) using Fc/Fc^+ redox couple as standard.

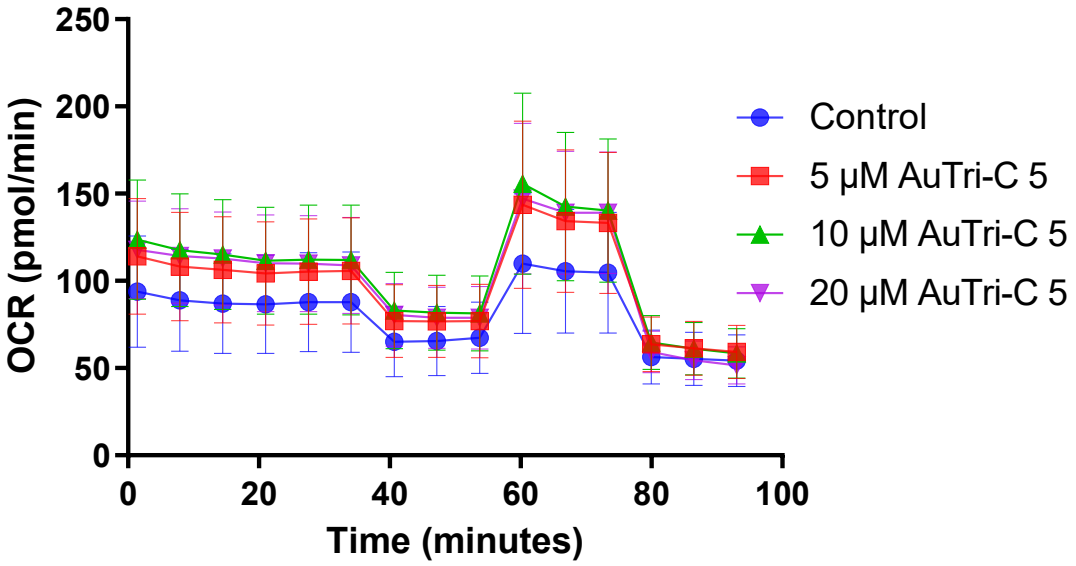


Fig. S28. Mitochondria stress test was performed with pneumatic injections of compound **AuTri-C 5** at concentrations ranging from (5–20 μM) and response to injections of oligomycin, FCCP, and antimycin A/rotenone. Data is plotted as the mean ± s.e.m (8 technical replicates).

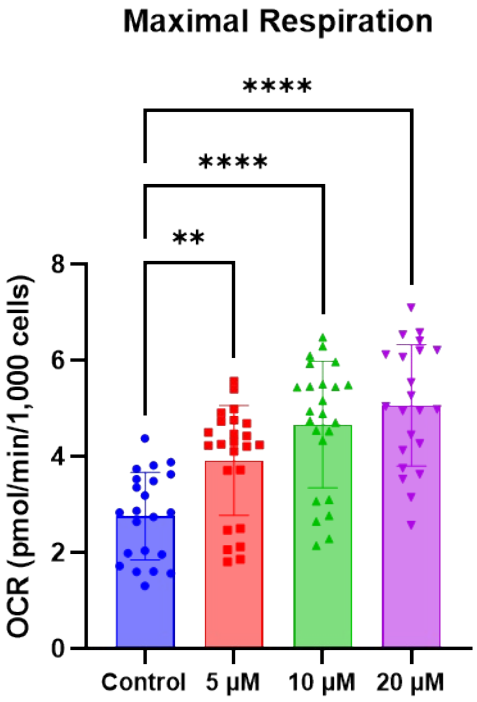


Fig. S29. Basal respiration data extrapolated from pneumatic injection mitochondrial bioenergetics assay. Data were analyzed via two-way ANOVA followed by Dunnett's multiple comparison test (**p < 0.01, ****p <0.0001).

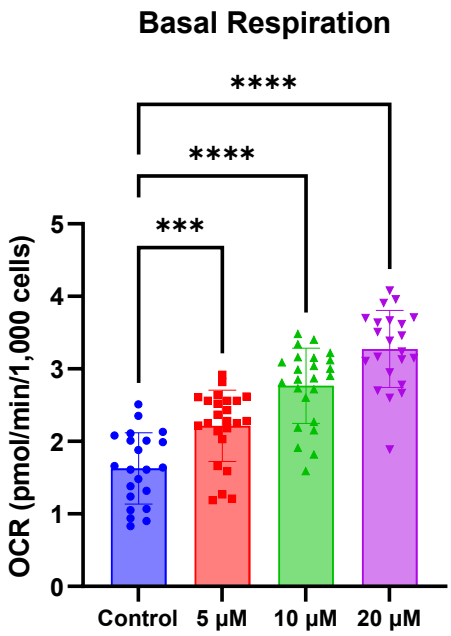


Fig. S30. Maximal respiration data extrapolated from pneumatic injection mitochondrial bioenergetics assay. Data were analyzed via two-way ANOVA followed by Dunnett's multiple comparison test (***p < 0.001, ****p <0.0001).

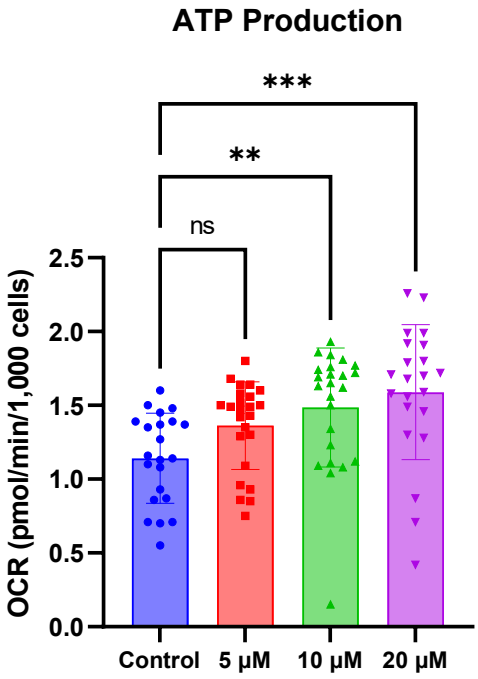


Fig. S31. ATP production data extrapolated from pneumatic injection mitochondrial bioenergetics assay. Data were analyzed via two-way ANOVA followed by Dunnett's multiple comparison test (**p < 0.01, ***p <0.001, ns – not significant).

References:

[1] A. Collado, A. Gómez-Suárez, A. R. Martin, A. M. Z. Slawin, S. P. Nolan, *Chem. Commun.* **2013**, *49*, 5541–5543.

[2] S. Parkin, H. Hope, *Journal of Applied Crystallography* **1998**, *31*, 945-953.

[3] M. Bruker (2012). Program name(s). Bruker AXS Inc., Wisconsin, USA. .

[4] aG. Sheldrick, *Acta Crystallographica Section C* **2015**, *71*, 3-8; bG. Sheldrick, *Acta Crystallographica Section A* **2008**, *64*, 112-122.

[5] aA. L. Spek, *Acta Crystallogr D Biol Crystallogr* **2009**, *65*, 148-155; bS. Parkin, *Acta Crystallographica Section A* **2000**, *56*, 317.