Electronic Supplementary Material (ESI) for Chemical Communications.

A Dual-Responsive Probe for Detecting Cellular Hypoxia using ^{19}F Magnetic Resonance and Fluorescence Supporting Information

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Experimental Methods

General

Solvents and chemicals used were purchased and used as received from Sigma Aldrich, VWR, and Fisher Scientific. UV/Vis and fluorescence spectra were obtained using an Agilent Technologies Cary 6 UV-Vis Spectrophotometer and an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer, respectively. ^{1}H and ^{19}F NMR were performed using an AGILENT MR 400 NMR spectrometer at 400 MHz and 376 MHz, respectively. ^{13}C NMR was performed using a Bruker AVIII HD 500 at 125 MHz. Deuterated solvents were purchased from Cambridge Isotope Laboratories located in Cambridge, MA. ^{19}F MR images were obtained in the Biomedical Imaging Center at UT Austin using a Bruker BioSpin Pharmascan 70/16 magnet (Karlsruhe, Germany) with a BioSpec two-channel console and BGA-9s gradient coil. Reverse phase C18 chromatography was performed on a Biotage Isolera One. Low resolution (LR) and high resolution (HR) Electrospray Ionization (ESI) mass spectral analysis were conducted in the University of Texas (UT) Mass Spectroscopy Facility. Electrochemistry experiments were performed at the Center for Electrochemistry at UT Austin on a CHI 660D electrochemical workstation. Electron paramagnetic resonance (EPR) spectra were obtained with a Bruker Biospin EMXplus 114 X-band spectrometer. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed in the EWRE Lab in the Department of Civil, Architectural, and Environmental Engineering at UT Austin. Flow cytometry studies were conducted on a BD Accuri Flow Cytometer (BD Biosciences, San Jose, CA) using 20 mW laser and analyzed using Flowjo. Fluorescence microscopy images of HeLa cells were obtained on a Life Technologies EVOS FL Auto using GFP.

Electrochemistry

In an anaerobic chamber, A 1.0 mM **CuATSMF$_3$-Fl** sample was prepared in anhydrous DMF with 100 mM NBu_4PF_6. The solution was placed in a 20 mL scintillation vial. The CHI 660D electrochemical workstation was connected with a Pt working electrode, a 10mM Ag/Ag^+ reference electrode in DMF, and a Pt wire for the counter electrode. The cyclic voltammetry spectrum was obtained using a scan rate of 200 mV/s. Ferrocene ($E_{1/2}$ = 0.53 V vs. SCE) was then added for calibration (Figure S2). The CV was plotted in Prism and the $E_{1/2}$ of **CuATSMF$_3$-Fl** was determined.

^{19}F NMR Studies of CuATSMF$_3$-Fl Reduction

CuATSMF$_3$-Fl was transferred into an anaerobic chamber and dissolved in enough anhydrous d_6-DMSO to make an 8.33 mM stock solution. For the Cu^{2+} sample, 300 µL of the d_6-DMSO stock was mixed with 200 µL de-oxygenized HEPES (pH 7.2, 25 mM). For the reduced Cu^+ sample, a 10.5 mM $Na_2S_2O_4$ stock solution in de-oxygenated HEPES (pH 7.2, 25 mM) was used. 200 µL of the $Na_2S_2O_4$ stock was added to 300 µL of the 8.33 mM **CuATSMF$_3$-Fl** solution to reduce the complex. The brown Cu^{2+} complex in d_6-DMSO immediately turned bright yellow and fluorescent upon addition of $Na_2S_2O_4$. The samples were sealed and 32 ^{19}F scans were obtained with a two second relaxation delay. For acidic stability, a 0.3 mM **CuATSMF$_3$-Fl** solution in 3:2 anhydrous d_6-DMSO:50 mM HOAc/NaOAc (pH 5) containing excess $Na_2S_2O_4$ was prepared in an

anaerobic chamber, sealed, and scanned 256 times with a two second relaxation delay (Figure S1B).

Fluorescence Studies of CuATSMF_3-Fl Reduction

A 1.5 mM **CuATSMF_3-Fl** stock in DMSO was diluted 1:1000 into de-oxygenated HEPES (pH 7.2, 50 mM) in an anaerobic chamber. The sample was placed in an air tight cuvette and an initial fluorescence scan was recorded. Another 1.5 µM **CuATSMF_3-Fl** sample was prepared with 2 mM DTT in de-oxygenated HEPES (pH 7.2, 50 mM) and scanned. The reduction study was repeated with 2 mM GSH. The samples were excited with 497 nm and the emission was collected over 507 – 707 nm with 5 nm excitation and emission slit width.

Quantum Yield and pH Dependence Studies of CuATSMF_3-Fl

Serial dilutions of fluorescein and **CuATSMF_3-Fl** were prepared in 0.1M NaOH and HEPES (pH 7.2, 50 mM), respectively. The concentrated stock solution of **CuATSMF_3-Fl** contained 0.1% DMSO from a 1:1000 dilution. For every dilution of both samples, the absorbance (collected 300-800 nm) and fluorescence spectrum (excited 497 nm and collected 507-707 nm with 5 nm excitation and emission slit width) were collected. The fluorescence spectra were integrated and plotted against the absorbance, which was used to determine the quantum yield of **CuATSMF_3-Fl** against fluorescein (Figure S3). For pH dependence: A 1.5 mM **CuATSMF_3-Fl** was dissolved into various buffers (50 mM HOAc/NaOAc: pH 4,03, 4.52, 5.04; 50 mM MES: pH 5.53, 5.95, 6.44; 50 mM HEPES: pH 6.94, 7.46, 7.93) via 1:1000 dilution from DMSO stock and the fluorescence intensity for each sample was obtained, normalized, and plotted (Figure S1). The fluorescence was collected using the same parameters as the quantum yield studies. LCMS samples of pH 4.03 and pH 7.46 were submitted to check for stability and the MS showed the complex was intact.

EPR Studies of of CuATSMF_3-Fl Reduction

Three EPR samples were made, all of which were in 10% anhydrous DMSO in de-oxygenated HEPES (pH 7.2, 50mM): the first had 1 mM **CuATSMF_3-Fl**, the second had 1 mM **CuATSMF_3-Fl** with 1 eq. $Na_2S_2O_4$, and the third had 1 mM **CuATSMF_3-Fl** with 1000 eq. DTT. EPR spectroscopy at room temperature was ran on all three samples as well as $Na_2S_2O_4$ in 10% DMSO in HEPES and DTT in 10% DMSO in HEPES to confirm all relevant peaks were from the Cu^{2+} complex. EPR of 1mM **CuATSMF_3-Fl** in pure DMF can be seen in Figure S4.

^{19}F MR Imaging

In 500 µL Eppendorf tubes, 500 µL of 0.9, 1.8, 2.7, 3.6, and 4.5 mM **CuATSMF_3-Fl** samples in 3:2 DMSO:HEPES (pH 7.2, 50 mM) were prepared and excess $Na_2S_2O_4$ was directly added into each sample. With thorough mixing, the brown solution turned bright yellow and fluorescent. Phantom ^{19}F MR images were obtained using 1500 ms repetition time, 3.75 ms echo time, 800 averages, 3.748 ms echo spacing, rare factor of 32, 50 mm slice thickness, 64x64 matrix, one slice, and a 40x40 mm^2 field of view. Total scan time was 20 minutes. For Cu^{2+}, a 4.5 mM sample of **CuATSMF_3-Fl** in 3:2 DMSO:HEPES without any $Na_2S_2O_4$ was prepared and scanned.

Cell Culture Media

Cell culture media (hereafter "HeLa media" or "media") was prepared in a sterile fume hood by mixing 450 mL Dulbecco's Modified Eagle's Medium (DMEM, purchased from SigmaAldrich), 50 mL fetal bovine serum (FBS, purchased from SigmaAldrich), and 5 mL antibiotics (formulated with 5,000 U/mL penicillin and 2 mg/mL streptomycin, purchased from SigmaAldrich). The mixture was then sterile filtered and stored in the refrigerator (2-8 ^{o}C). All experiments were conducted at 37 ^{o}C with atmospheric air and 5% CO_2 for normoxic conditions and 0.1% oxygen, 94.9% nitrogen, and 5% CO_2 for hypoxic conditions. HeLa cells were grown to 80% confluency before proceeding with studies. HeLa cells were allowed to sit overnight in 0.1% oxygen 94.9% nitrogen, and 5% CO_2 to become hypoxic and media, PBS, and trypsin were kept under a nitrogen environment to de-oxygenate them. MTT assay was scanned on a ChroMate 4300 Plate Reader from Awareness Technology, Inc. (Palm City, Fl) at 570 nm.

Media Stability

A 30 mM stock solution of **CuATSMF$_3$-Fl** was prepared and diluted 1:1000 in phenol red free HeLa media and scanned on an Agilent Technologies Cary 6 UV-Vis Spectrophotometer immediately after the 30 μM solution was prepared. Then, the 30 μM **CuATSMF$_3$-Fl** solution was placed in a 37 ^{o}C incubator and the UV-Vis spectrum was obtained again after twenty-four hours. The spectra from time t = 0 hours and t = 24 hours are similar, thus showing that the complex is stable in HeLa media (Figure S5). The UV-Vis was baseline corrected with phenol red free HeLa media.

Cytotoxicity

Four-hour studies (Figure S7): HeLa cells were grown in two 96 well plates until 80% confluent. One of the plates was moved into a hypoxic chamber overnight once the HeLa cells were adhered. Then, varying concentrations of **CuATSMF$_3$-Fl** and **CuATSMF$_3$-FlAc** (0.1% DMSO in media; de-oxygenated media for hypoxic well plate) were added to the wells in batches of three. After four hours, the complexed media was removed, triply rinsed with DMEM, and replaced with 90% fresh media and 10% MTT in PBS (5 mg/mL). The cells were allowed to sit in normoxia three hours and then DMSO was added to each well. The plate was placed on an orbital shaker and absorbance values were obtained from a ChroMate 4300 plate reader and calibrated to wells that contained HeLa cells in media and HeLa cells in 0.1% DMSO in media as a control and vehicle, respectively.

Twenty-four-hour study (Figure S8): HeLa cells were grown in two 96 well plates until 80% confluent. One of the plates was moved into a hypoxic chamber overnight once the HeLa cells were adhered. Then, varying concentrations of **CuATSMF$_3$-Fl** and **CuATSMF$_3$-FlAc** (0.1% DMSO in media; de-oxygenated media for hypoxic well plate) were added to the wells in batches of three. After four hours, the complexed media was removed, triply rinsed with DMEM, and replaced with fresh media. The cells were allowed to sit for another twenty hours in normoxia. MTT in PBS (5 mg/mL) was added to each well and incubated for another three hours. Then, DMSO was added to each well. The plate was placed on an orbital shaker and absorbance values were obtained

from a ChroMate 4300 plate reader and calibrated to wells that contained HeLa cells in media and HeLa cells in 0.1% DMSO in media as a control and vehicle, respectively.

Cell Uptake

Cell uptake studies were performed on **CuATSMF$_3$-FlAc** under normoxic and hypoxic conditions. A 30 mM stock solution of **CuATSMF$_3$-FlAc** in sterile DMSO was prepared and diluted 1:1000 in media for 30 μM incubation. HeLa cells were incubated in a T75 flask with complex for one hour, washed with PBS, trypsinized, and centrifuged to obtain a cell pellet. A small aliquot of the trypsinized cells was diluted in trypan blue and counted on a hemocytometer. The pellet was dissolved in 2% (v/v) HNO_3 and subjected to ICP-OES to determine the copper concentration. The instrument used was calibrated with 0, 10, 25, 50, 100, 250, 500, and 1000 ppb copper in 2% (v/v) HNO_3. Control cells were incubated with sterile DMSO and all experiments were performed three times.

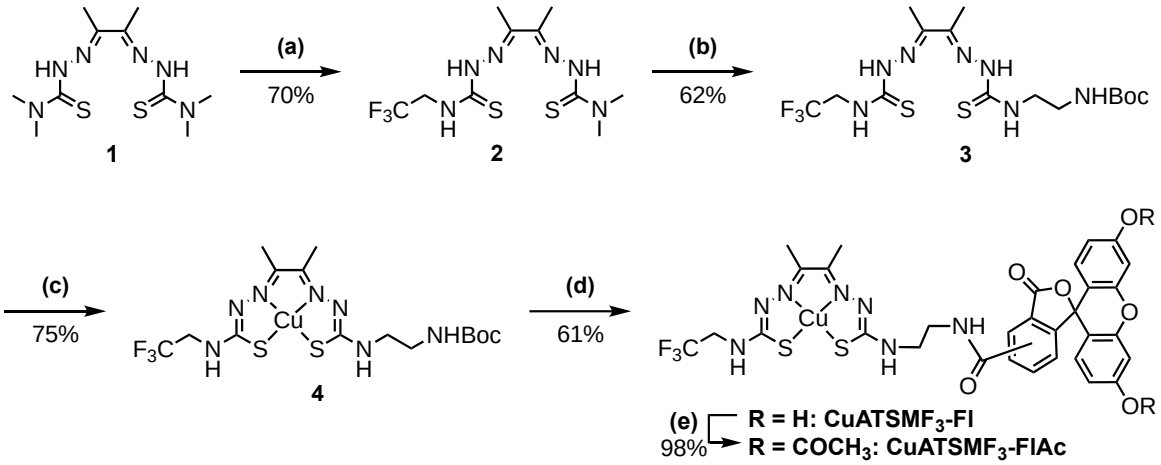
Flow Cytometry and EVOS Microscope Imaging

HeLa cells were grown on T25 flasks until 80% confluent, incubated with 30 μM **CuATSMF$_3$-FlAc** for one hour, washed with PBS, trypsinized, and centrifuged to obtain a cell pellet. The pellet was dissolved in Live Cell Imaging Solution (LCIS; purchased and used as received from Life Technologies) and cells were scanned using a BD Accuri Flow Cytometer. Triplicate samples of DMSO control and **CuATSMF$_3$-FlAc** in both normoxic and hypoxic conditions were obtained with an average of three scans per sample. Each scan measured 40,000 cells and GFP fluorescence was analyzed using a 488 nm laser to provide excitation, while collecting emitted photons through a 575/25 nm band pass filter. For EVOS microscope fluorescence images, cells were plated on to a 24 well plate and incubated with nothing (control), 0.1% DMSO in media (vehicle), and 30 μM **CuATSMF$_3$-FlAc** for one hour once cells were 80% confluent. All media was removed, cells were washed, and LCIS was added for imaging. Hypoxic and normoxic plates were imaged using de-oxygenated media for hypoxic conditions.

***In vitro* ^{19}F NMR**

HeLa cells were grown in four T150 flasks until 80% confluent. 60 μM **CuATSMF$_3$-FlAc** was added and cells were incubated at 37 ^{o}C for one hour. Complexed media was removed, washed with fresh media, then PBS, then trypsinized and centrifuged to obtain a cell pellet. The pellet was redissolved in PBS then centrifuged again to wash before adding CelLytic M (Sigma-Aldrich; purchased and used as received). The suspension was vortexed and lyophilized. The resulting solids were transferred into an anaerobic chamber and dissolved in anhydrous d_6-DMSO, transferred to NMR tubes, and sent to the NMR facility for 8192 ^{19}F scans at 500 MHz. The procedure above was repeated for two hypoxic conditions; however, de-oxygenated media, trypsin, and PBS were used for the 0.1% sample and media, trypsin, and PBS were left in a 2% oxygen environment for the 2% sample. All follow-up work for the 0.1% sample was performed in a sterile nitrogen environment glove bag and all follow-up work for the 2% sample was performed in a sterile hypoxic chamber with 2% oxygen.

Synthesis



Scheme S1. Synthesis of **CuATSMF$_3$-Fl** and **CuATSMF$_3$-FlAc**. Conditions: (a) 2,2,2-trifluoroethanamine hydrochloride, anhydrous DMSO, 70 ^{o}C, 1 h (70%); (b) *tert*-butyl (2-aminoethyl)carbamate, anhydrous DMSO, 70 ^{o}C, 2 d (62%); (c) Cu(OAc)$_2$·H$_2$O, DMF, room temperature, overnight (75%); (d) (1) TFA, H$_2$O, room temperature, overnight; (2) succinimidyl ester of 5-(and 6-)carboxyfluorescein, TEA, DMF, 31 ^{o}C, overnight, dark (61%); (e) acetic anhydride, pyridine, room temperature, 1 h, dark (98%).

Compound **2**
Compound **1** (364 mg, 1.2 mmol, 2 eq)[1] and 2,2,2-trifluoroethanamine hydrochloride (85.5 mg, 0.6 mmol, 1 eq) were added to a Schlenk flask. The air in the sealed flask was replaced with N_2, 2 mL of anhydrous DMSO was added with a syringe, and the flask was heated to 70°C for 1 hour. The solution was allowed to cool before filtering off excess **1** (230 mg) and the remaining filtrate was lyophilized to remove all solvent. The product was purified with C18 reverse phase chromatography using a 5% MeCN/95% H_2O/0.1% formic acid to 100% MeCN /0.1% formic acid in 12 minutes: R_t 6.1 min. All solvents were lyophilized off attaining a yellow product **2** (56 mg, 70% after recovery of **1**). We note that if compound **2** is left in aqueous solvent for an extended period of time, the hydrolyzed byproduct forms (Figure S13; LR ESI-MS (ESI$^-$, MeOH): calculated for $[C_7H_9F_3N_3OS]^-$ 241.1, found 241.1).
^{1}H NMR (400MHz, $CDCl_3$, 25 °C): δ 8.99 (br, 1H), δ 8.48 (s, 1H), δ 7.65 (t, 1H), δ 4.47 (m, 2H), δ 3.38 (s, 6H), δ 2.13 (s, 3H), δ 2.08 (s, 3H)
^{13}C NMR (125 MHz, d_6-DMSO, 25 ^{o}C): δ 181.73 (s), δ 179.80 (s), δ 149.97 (s), δ 148.95 (s), δ 124.72 (q, *J* = 280.2 Hz), δ 44.42 (q, *J* = 33.1 Hz), δ 42.37 (s), δ 11.75 (s), δ 11.24 (s)
^{19}F NMR (376 MHz, $CDCl_3$, 25 °C): δ -71.70 (t)
HR ESI-MS (ESI$^+$, MeOH): calculated for $[C_{10}H_{18}F_3N_6S_2]^+$ 343.0981, found 343.0986.

Compound **3**
Compound **2** (167.2 mg, 0.5 mmol, 1 eq) was added to a Schlenk flask with *tert*-butyl (2-aminoethyl)carbamate (93.9 mg, 0.6 mmol, 1.2 eq), sealed, and evacuated to replace the air with N_2. Then, 2 mL of anhydrous DMSO was added with a syringe and the flask

was heated to 70°C overnight. The following day, another 1.2 eq of *tert*-butyl (2-aminoethyl) was added and reacted for a second night. The solution mixture was lyophilized to remove all solvent. The product was purified with C18 reverse phase chromatography using a 5% MeCN/95% H_2O/0.1% formic acid to 100% MeCN /0.1% formic acid in 12 minutes: R_t 7.4 min. All solvents were lyophilized off yielding a yellow product **3** (139 mg, 62%). We note that if compound **3** is left in aqueous solvent for an extended period of time, the hydrolyzed byproduct forms (Figure S14).
^{1}H NMR (400MHz, $CDCl_3$, 25 °C): δ 8.87 (s, 1H), δ 8.74 (br, 1H), δ 8.63 (br, 1H), δ 7.64 (t, 1H), δ 4.95 (br, 1H), δ 4.50 (m, 2H), δ 3.81 (q, 2H), δ 3.45 (q, 2H), δ 2.30 (s, 3H), δ 2.12 (s, 3H), δ 1.41 (s, 9H)
^{13}C NMR (125 MHz, d_6-DMSO, 25 ºC): δ 179.81 (s), δ 178.08 (s), δ 156.17 (s), δ 149.71 (s), δ 147.85 (s), δ 124.71 (q, *J* = 280.2 Hz), δ 77.87 (s), δ 44.52 (s), δ 44.40 (q, *J* = 33.6 Hz), δ 28.19 (s), δ 11.98 (s), δ 11.79 (s)
^{19}F NMR (376 MHz, $CDCl_3$, 25 °C): δ -71.72 (t)
HR ESI-MS (ESI$^+$, MeOH): calculated for $[C_{15}H_{26}F_3N_7NaO_2S_2]^+$ 480.143458.2, found 480.1441.

Compound **4**
Under a N_2 atmosphere, **3** (70 mg, 0.15 mmol, 1 eq) was mixed with $Cu(OAc)_2 \cdot H_2O$ (42 mg, 0.21 mmol, 1.4 eq) in 4 mL of anhydrous DMF at room temperature overnight. The solvent was removed using a high-pressure vacuum and the product was purified with C18 reverse phase chromatography using a 5% MeCN/95% H_2O/0.1% formic acid to 100% MeCN/0.1% formic acid in 12 minutes: R_t 8.0 min. All solvents were freeze dried off affording a brown product **4** (60 mg, 75%).
HR ESI-MS (ESI$^+$, MeOH): calculated for $[C_{15}H_{25}CuF_3N_7O_2S_2]^+$ 519.0754, found 519.0767.

Compound **CuATSMF$_3$-Fl**
In a small round bottom flask under a N_2 environment, **4** (50 mg, 13 mmol, 1 eq) was added with 2 mL trifluoroacetic acid and four drops of deionized water. The reaction proceeded overnight at room temperature and the TFA and water were evaporated off. The remaining product was extracted in a hexane/ethanol mixture, with the intermediate product being in the ethanol solution. The ethanol was evaporated off and the resulting solid was dissolved in 4 mL DMF with the succinimidyl ester of 5-(and 6-)carboxyfluorescein (62 mg, 0.13 mmol, 1.2 eq; purchased and used as received from ThermoFisher Scientific) and triethylamine (18.3 µL, 0.13 mmol, 1.2 eq) and allowed to react overnight in dark at 31°C. The solvent was removed using a high-pressure vacuum and the product was purified with C18 reverse phase chromatography using a 5% MeCN/95% H_2O/0.1% formic acid to 100% MeCN /0.1% formic acid in 12 minutes: R_t 7.2 min. All solvents were freeze dried off affording a burnt orange **CuATSMF$_3$-Fl** (52mg, 61%).
HR ESI-MS (ESI$^+$, MeOH): calculated $[C_{31}H_{27}CuF_3N_7O_6S_2]^+$ 777.0707, found 777.0704 and 777.0710 (mixture of isomers, see Figures S22 and S23).

Compound **CuATSMF$_3$-FlAc**

CuATSMF$_3$-Fl (2.3 mg, 3 µmol, 1.0 eq) was dissolved in 1.25 mL pyridine and acetic anhydride (1 µL, 10 µmol, 3.3 eq) and reacted at room temperature for one hour in the dark. The solution was diluted in 0.02% acetic acid in deionized water, extracted with ethyl acetate, then dried with sodium sulfate. The product was purified with C18 reverse phase chromatography using a 5% MeCN/95% H_2O/0.1% formic acid to 100% MeCN /0.1% formic acid in 12 minutes: R_t 8.5 min. All solvents were freeze dried off affording a burnt orange **CuATSMF$_3$-FlAc** (2.5 mg, 98%).

HR ESI-MS (ESI$^+$, MeOH): calculated $[C_{35}H_{31}CuF_3N_7O_8S_2]^+$ 861.0918, found 861.0925.

Note on synthetic strategy:

The authors note that syntheses of free ligands ATSMF$_3$-Fl and ATSMF$_3$-FlAc were not possible using a similar synthetic route as outlined in Scheme S1 (with metalation occurring after deprotection of the boc group) as acidic conditions hydrolyzes the ATSM ligand scaffold when not complexed to Cu^{2+}.

Table S1. ^{19}F NMR chemical shifts and T_1 and T_2 values of **CuATSMF$_3$-Fl** compared to similar previously reported compounds.[1] ^{19}F T_1 and T_2 measurements were performed on a AGILENT MR 400 NMR spectrometer at 376 MHz.

Complex	Chemical Shift (ppm)	T_1 (ms)	T_2 (ms)
CuATSMF$_3$-Fl	-70.23	730	150
ATSM-F$_3$	-69.96	760	250
ATSM-F$_6$	-70.01	720	230

Table S2. Averaged flow cytometry data for 30 µM **CuATSMF$_3$-FlAc** in HeLa media and 0.1% DMSO in HeLa media (as a control) with one-hour incubation in hypoxic and normoxic conditions. Cells were grown in T25 flasks, incubated with complexed media for one hour, washed three times with fresh media, trypsinized, centrifuged, and resuspended in LCIS. De-oxygenated sterile HeLa media was used for hypoxic conditions.

HeLa Incubation Conditions	Fluorescence Intensity
Normoxic HeLa cells with 0.1% DMSO control	28,954.2
Hypoxic HeLa cells with 0.1% DMSO control	31,225.0
Normoxic HeLa cells with 30 µM **CuATSMF$_3$-FlAc**	1,759,435.5
Hypoxic HeLa cells with 30 µM **CuATSMF$_3$-FlAc**	2,911,054.2

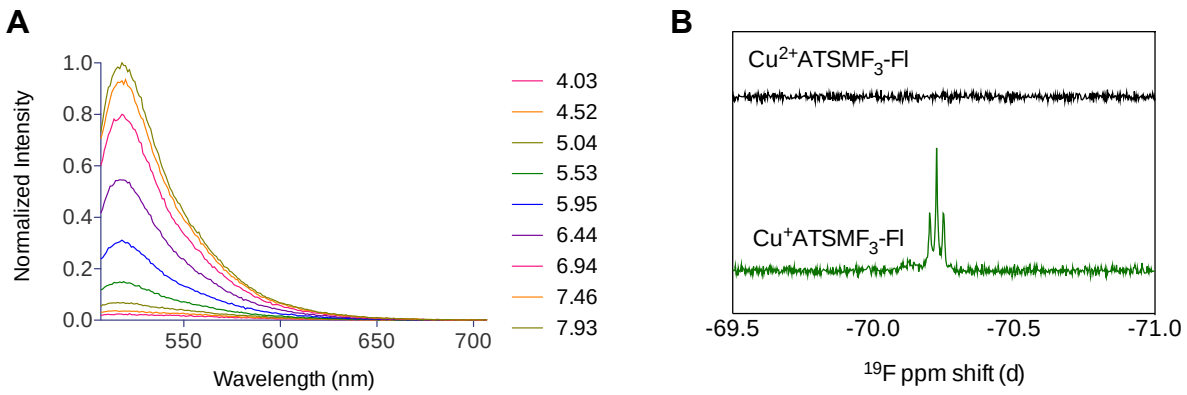


Figure S1. (A) pH dependence of 1.5 µM **CuATSMF$_3$-Fl** in various buffers (50 mM HOAc/NaOAc: pH 4,03, 4.52, 5.04; 50 mM MES: pH 5.53, 5.95, 6.44; 50 mM HEPES: pH 6.94, 7.46, 7.93). The samples were prepared with a 1:1000 dilution from a 1.5 mM stock in DMSO. The solution was excited with 497 nm and the emission was collected from 507 – 707 nm with 5 nm excitation and emission slit width. LCMS analysis of pH 4.03 and pH 7.46 samples indicated the complex was intact at both acidic and neutral pH. LCMS of **CuATSMF$_3$-Fl** in pH 2 media shows intact complex (Figure S23). (B) ^{19}F NMR spectra of 0.3 mM **CuATSMF$_3$-Fl** in the absence (top) and presence (bottom) of excess $Na_2S_2O_4$ performed in 3:2 d_6-DMSO:HOAc/NaOAc (pH 5.0, 50 mM).

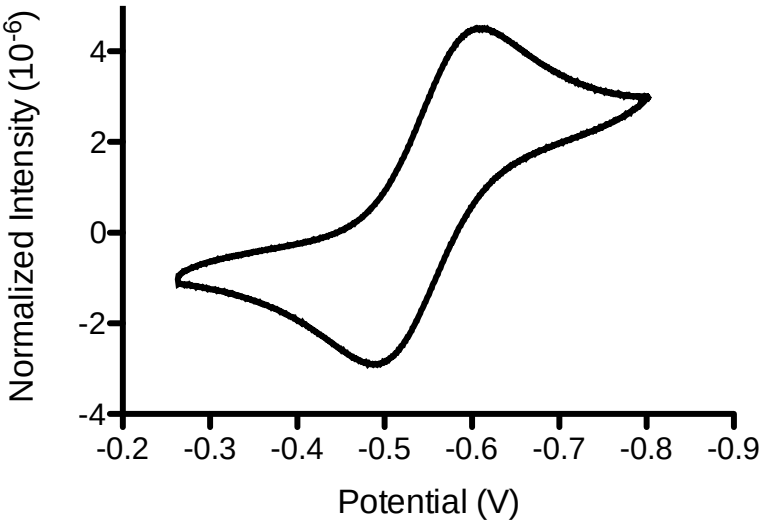


Figure S2. Cyclic voltammetry of 1 mM **CuATSMF$_3$-Fl** (vs. SCE) in dry DMF at 298 K. **CuATSMF$_3$-Fl** was dissolved with 10 mM $AgNO_3$ and 100 mM NBu_4PF_6 and scanned at 200 mV/s with -1.2 V against a Ag/Ag^+ reference (Ag^+ obtained from dissolved $AgNO_3$) and a Pt counter electrode. The spectrum was calibrated using ferrocene ($E_{1/2}$ = 0.53 V vs. SCE).

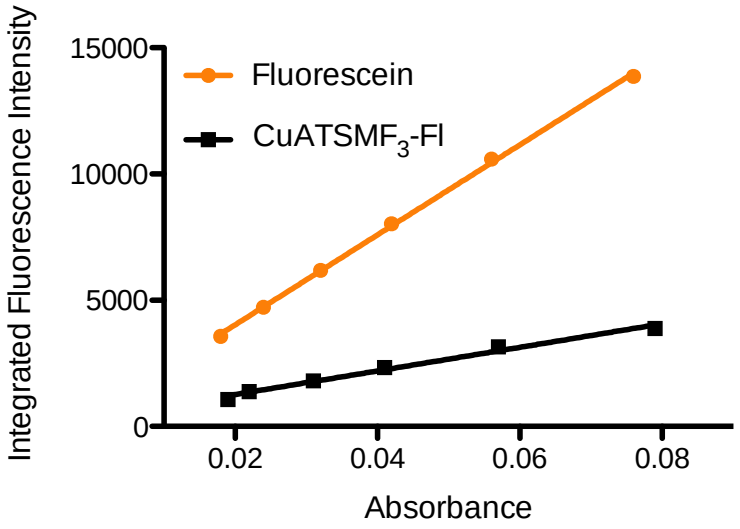


Figure S3. Quantum yield of **CuATSMF$_3$-Fl** (Φ = 0.196) with respect to fluorescein in 50 mM pH 7.4 HEPES buffer. For fluorescence, the emission spectra were collected over 507-707 nm with a 497 nm excitation (5 nm excitation and emission slit width).

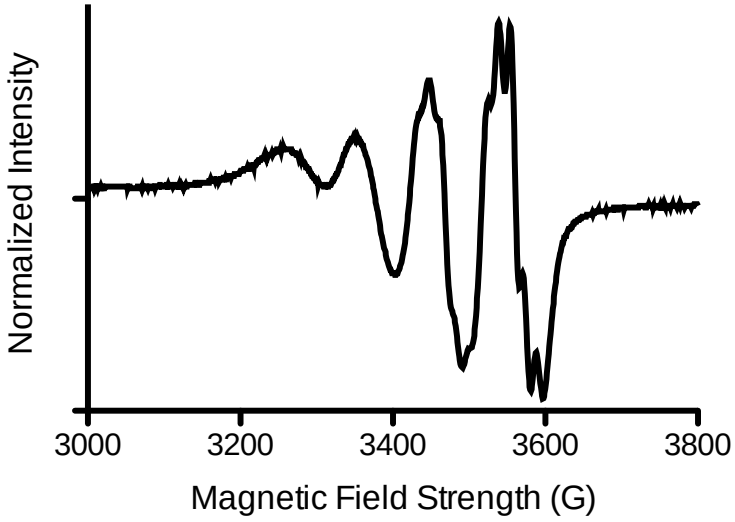


Figure S4. EPR of 1 mM **CuATSMF$_3$-Fl** in DMF at 298 K. The spectrum shows four peaks for Cu^{2+} with hyperfine splitting.[2]

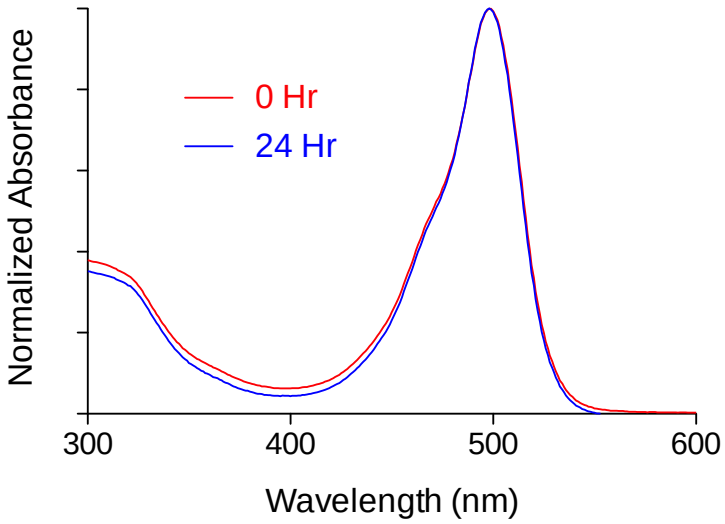


Figure S5. UV Vis stability of 30 µM **CuATSMF$_3$-FI** taken in phenol red free HeLa media at time t = 0 hr and t = 24 hr.

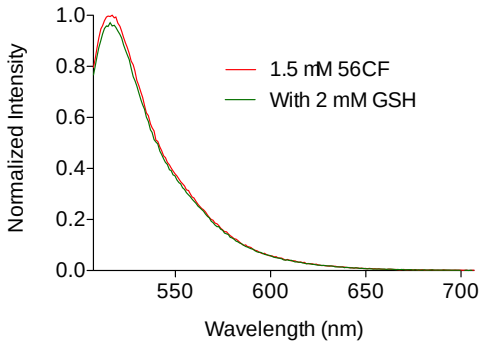


Figure S6. Fluorescence intensity measurements of 1.5 µM 5,6-carboxyfluorescein ("56CF") with 2 mM GSH in 50 mM pH 7.2 HEPES. Samples were excited at 497 nm and the fluorescence emission spectra were collected between 507 – 707 nm. No fluorescence increase due to glutathione is observed.

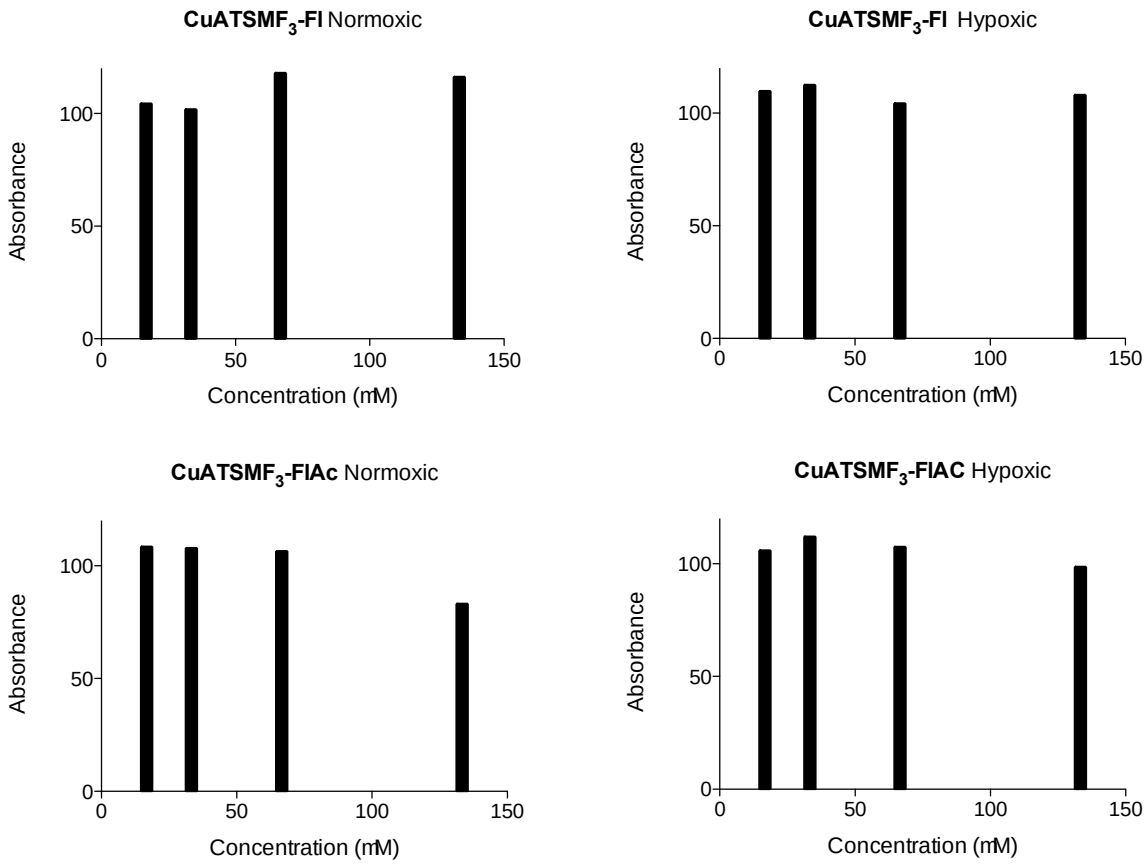


Figure S7. MTT Assay of **CuATSMF$_3$-Fl** and **CuATSMF$_3$-FlAc** (up to 133 µM) in HeLa cells incubated for four hours in normoxic and hypoxic conditions.

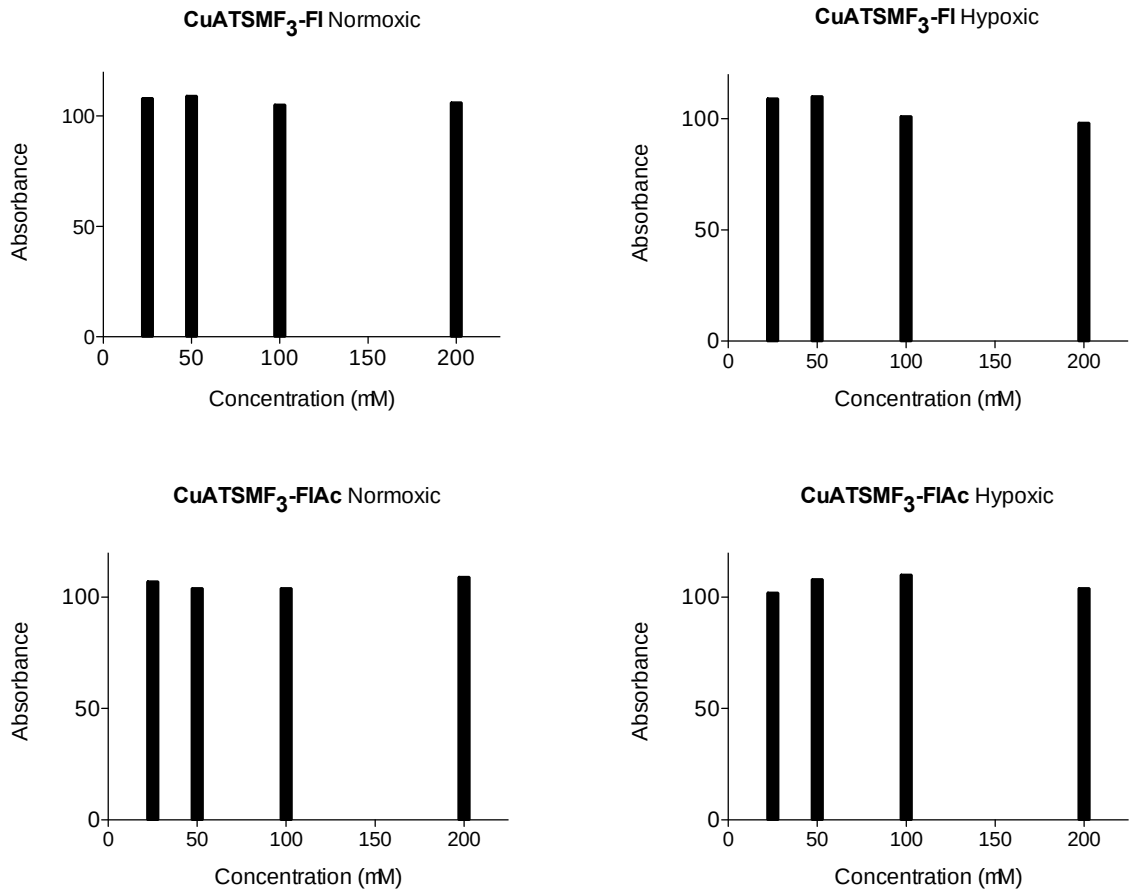


Figure S8. MTT Assay of **CuATSMF$_3$-Fl** and **CuATSMF$_3$-FlAc** (up to 200 µM) in HeLa cells incubated for four hours in normoxic and hypoxic conditions, washed, and kept in normoxia for twenty hours.

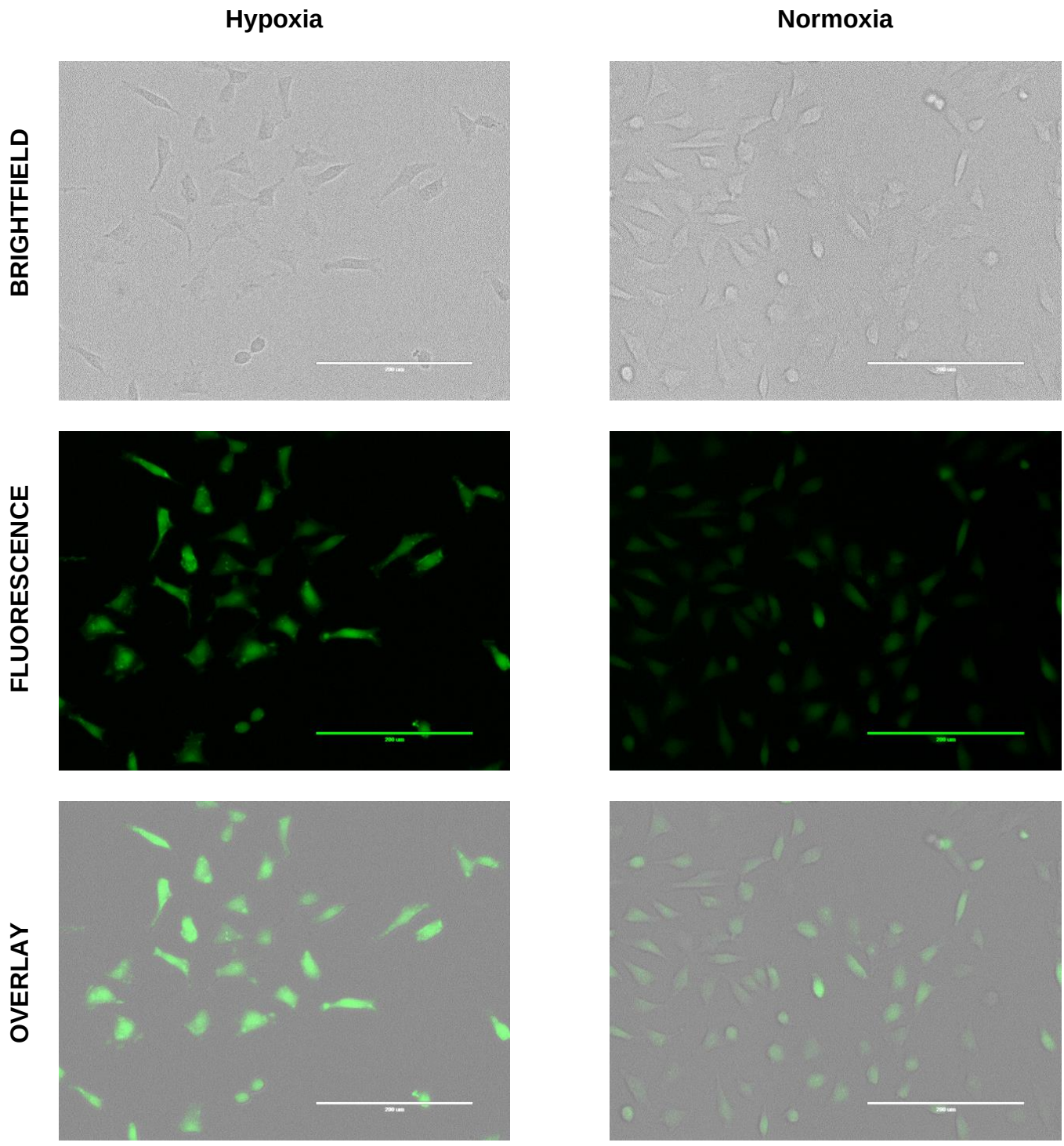


FIGURE S9. Magnified fluorescence images of hypoxic (left; brightfield top, fluorescence middle, overlay bottom) and normoxic (right) HeLa cervical cancer cells after one hour 30 µM incubation of **CuATSMF$_3$-FlAc**. Scale bar is 200 µm.

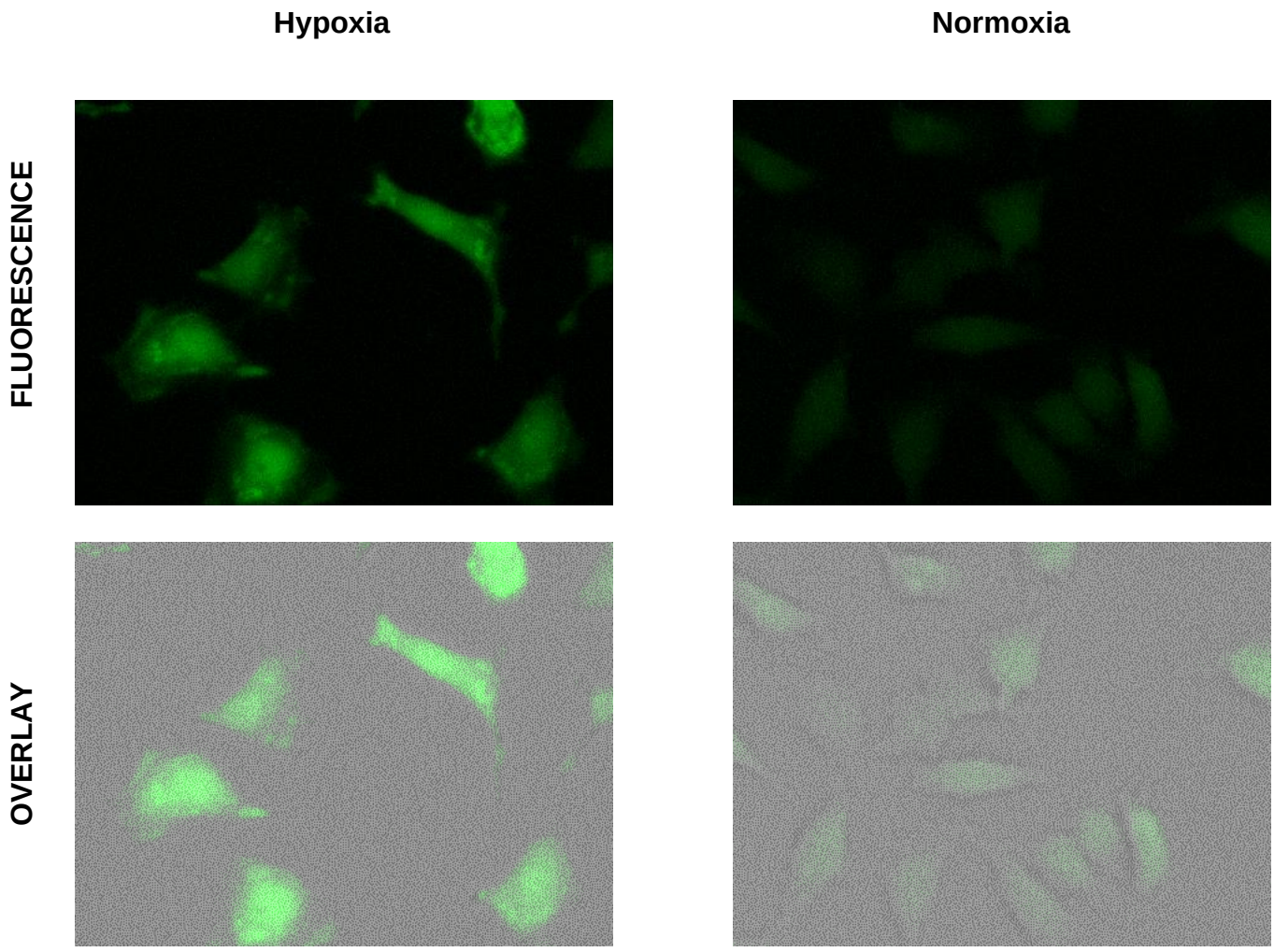


FIGURE S10. Zoomed in images of the magnified fluorescence images found in Figure S9 of hypoxic (left; fluorescence top, overlay bottom) and normoxic (right) HeLa cervical cancer cells after one hour 30 μM incubation of **CuATSMF$_3$-FlAc**.

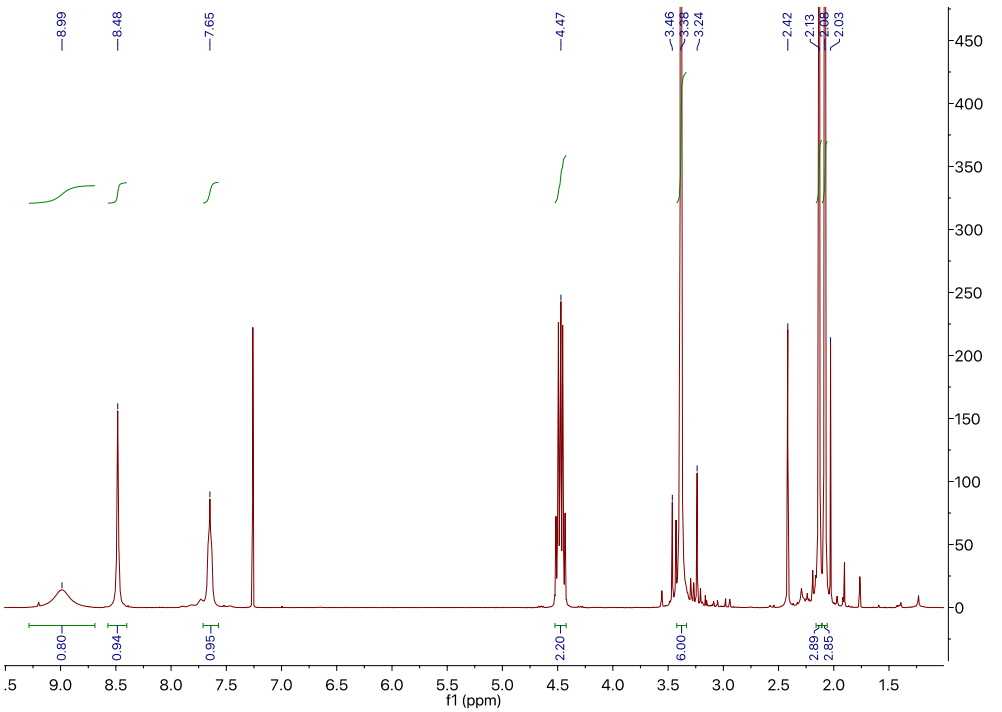


Figure S11. ^{1}H NMR of **2** in $CDCl_3$ at 25 °C. Some peaks (not integrated in figure; δ 2.03, δ 2.42, and δ 3.24; < 10%) are due to the hydrolyzed product of **2** that occurs during purification (see Figure S13 inset).

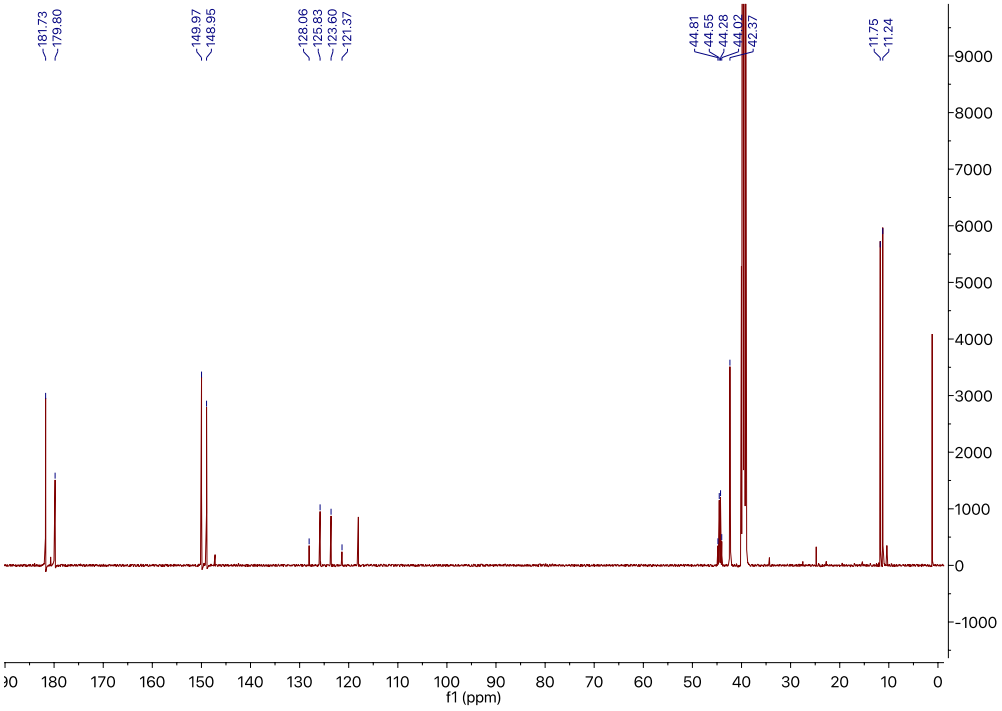


Figure S12. ^{13}C NMR of **2** in d_6-DMSO at 25 °C. Sample contains acetonitrile.

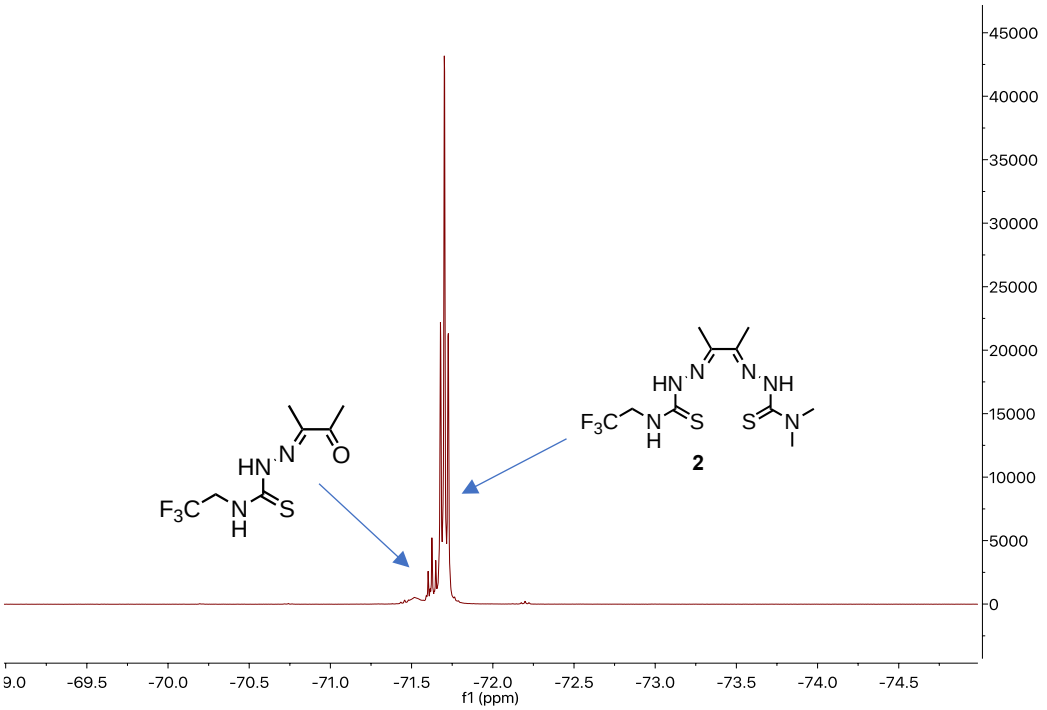


Figure S13. ^{19}F NMR of **2** (δ -71.70) in $CDCl_3$ at 25 °C. The small ^{19}F peak the left of the product peak is due to the hydrolyzed byproduct (inset; LR ESI-MS (ESI^-, MeOH): calculated for $[C_7H_9F_3N_3OS]^-$ 241.1, found 241.1).

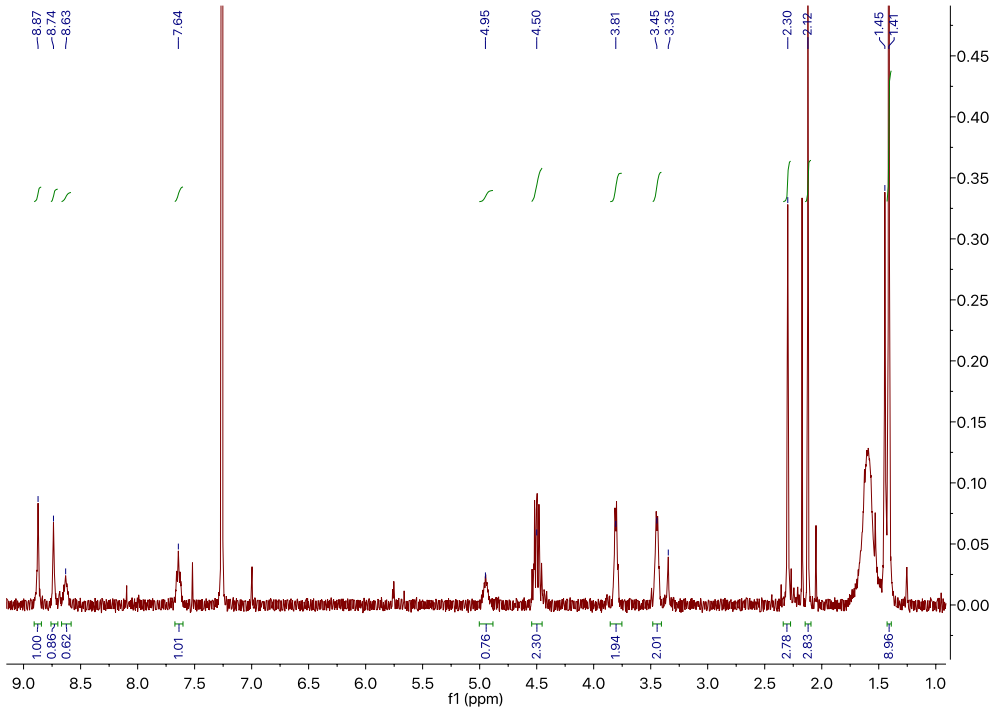


Figure S14. ^1H NMR of **3** in $CDCl_3$ at 25 °C. Contains H_2O, acetone, and ethyl acetate solvents. Peaks that were not integrated (δ 3.35 and δ 1.45) correspond to the hydrolyzed product.

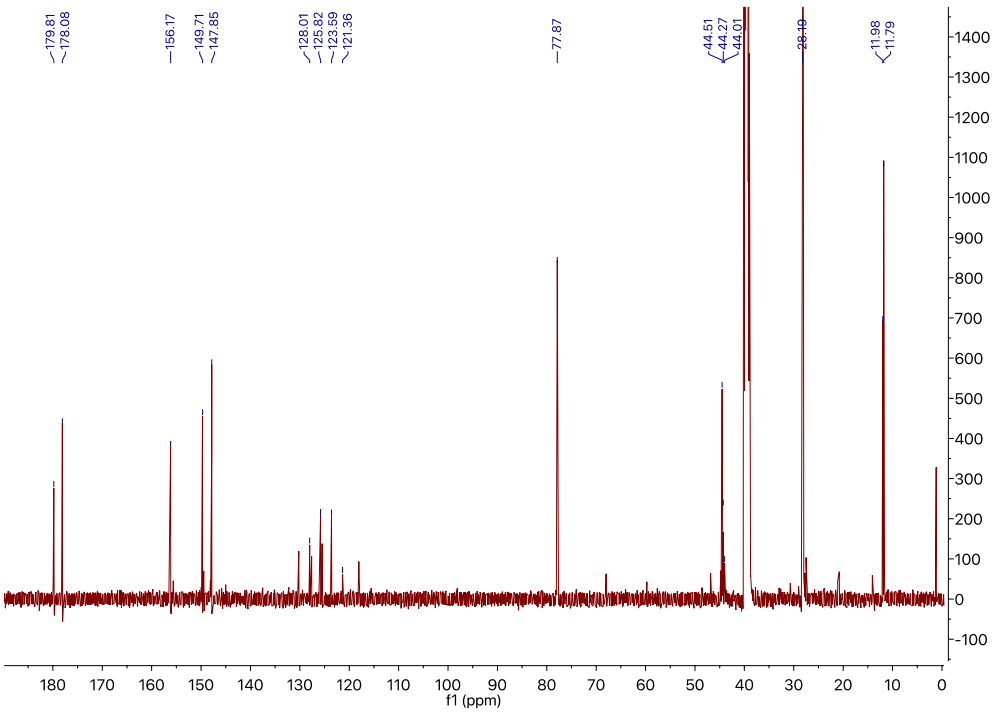


Figure S15. ^{13}C NMR of **3** in d_6-DMSO at 25 °C. Sample contains acetonitrile.

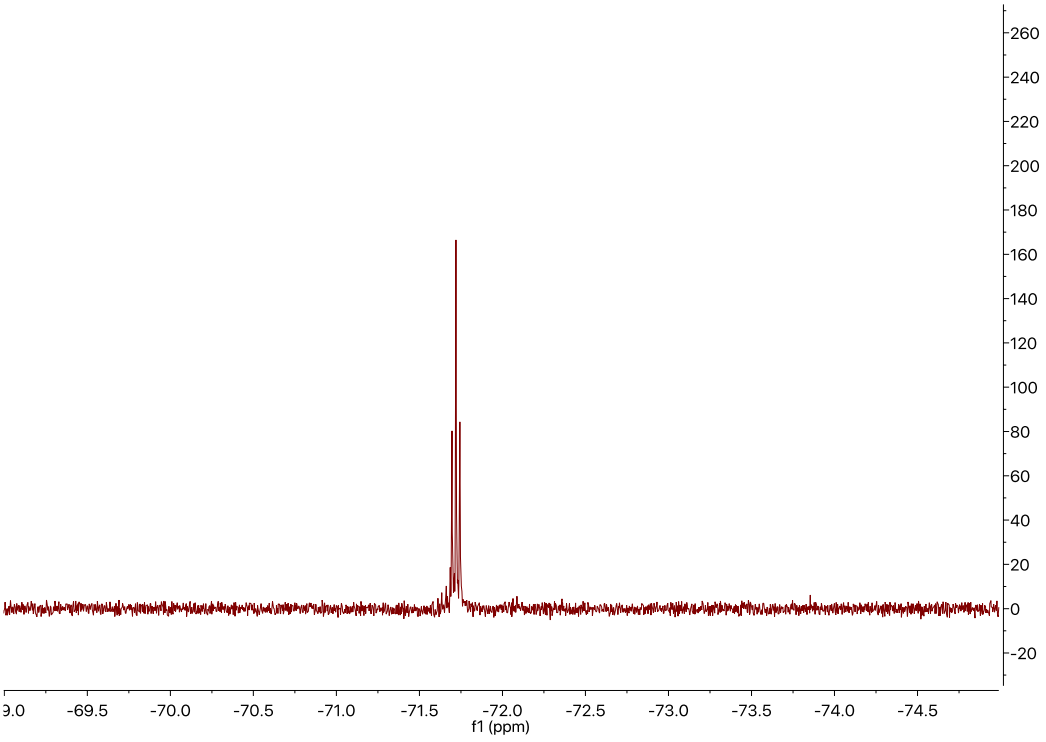


Figure S16. ^{19}F NMR of **3** (δ -71.72) in $CDCl_3$ at 25 °C.

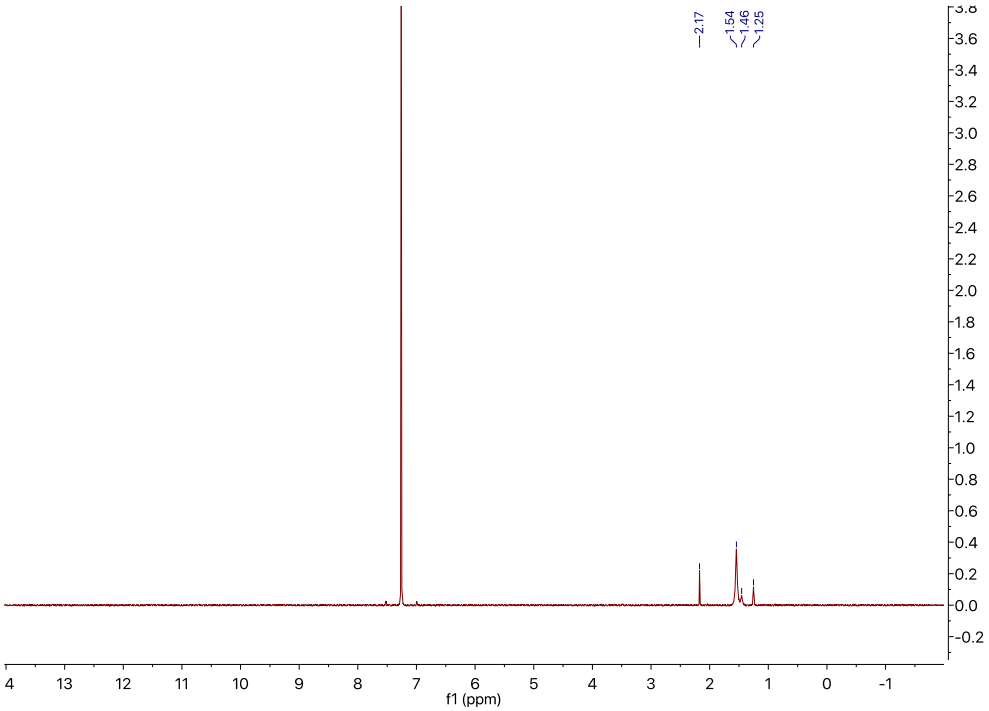


Figure S17. ^{1}H NMR of **4** in $CDCl_3$ at 25 °C. Sample contains H_2O, acetone, and some partially quenched peaks.

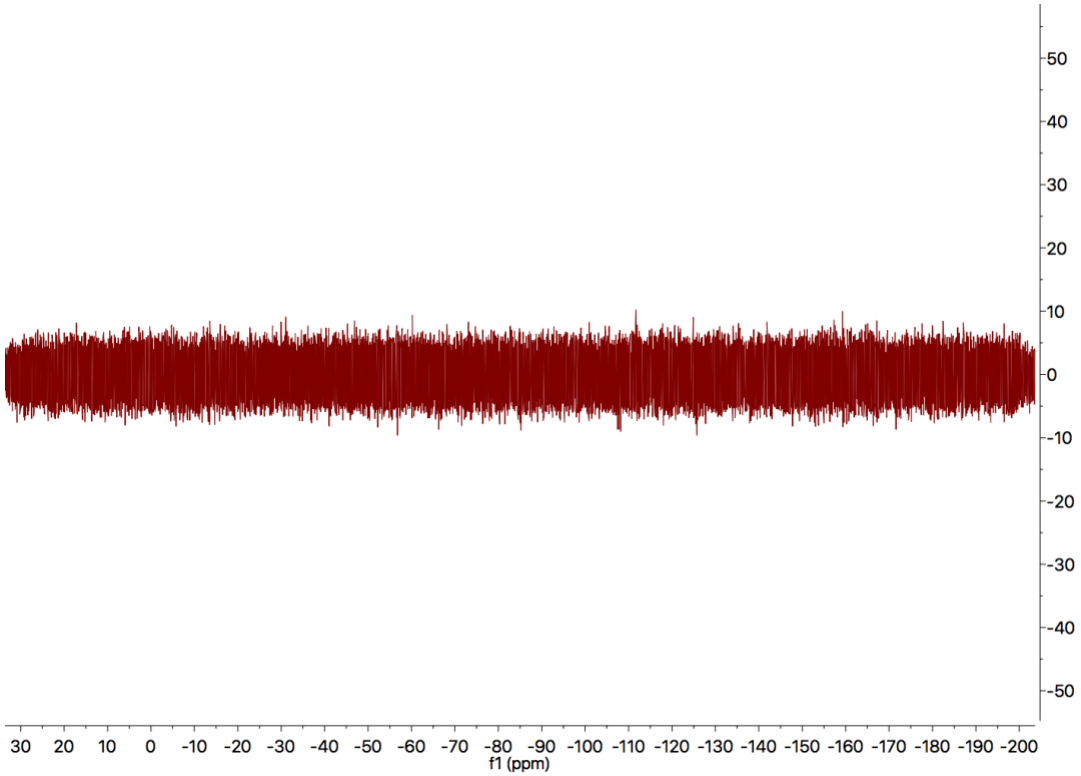
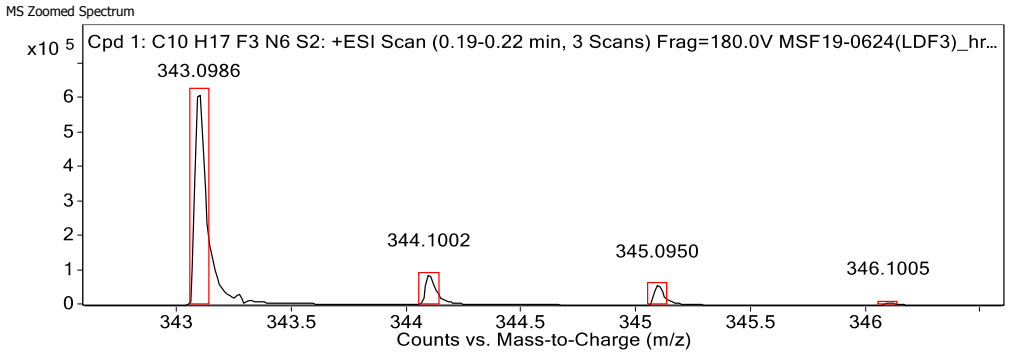


Figure S18. ^{19}F NMR of **4** in $CDCl_3$ at 25 °C.

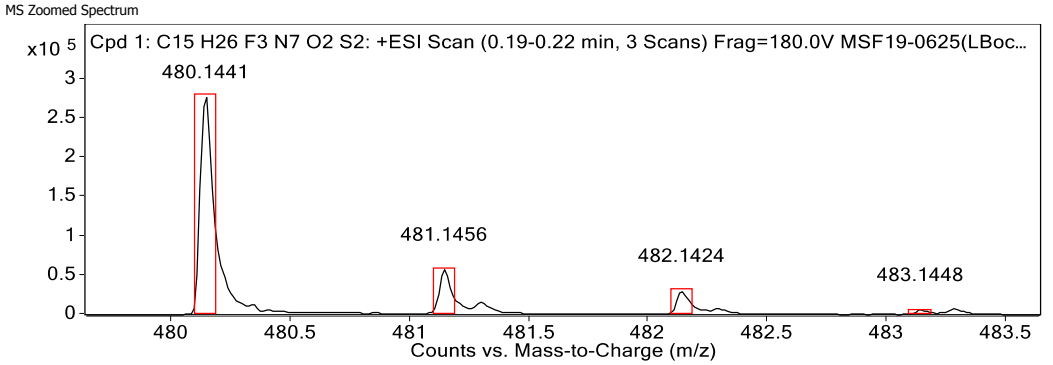


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
343.0986	343.0981	1	624319	C10H17F3N6S2	(M+H)+	-1.48
344.1002	344.1001	1	90094	C10H17F3N6S2	(M+H)+	-0.08
345.0950	345.0947	1	58072	C10H17F3N6S2	(M+H)+	-0.62
346.1005	346.0963	1	8325	C10H17F3N6S2	(M+H)+	-11.97
518.3696			1652757			

--- End Of Report ---

Figure S19. Flow injection analysis (FIA) ESI$^+$ HRMS of **2**.

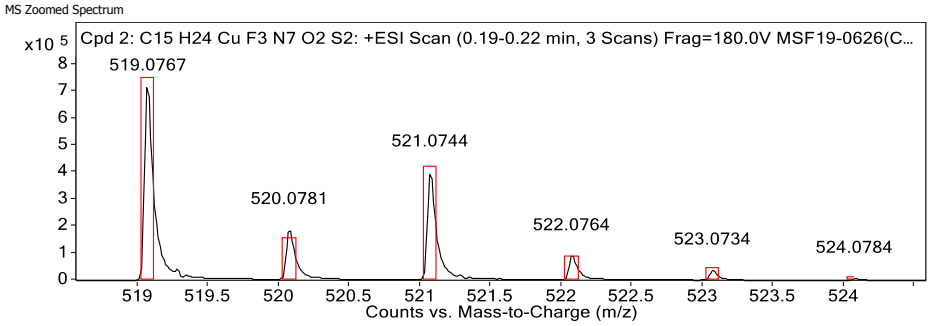


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
480.1441	480.1434	1	280284	C15H26F3N7O2S2	(M+Na)+	-1.57
481.1456	481.1457	1	57818	C15H26F3N7O2S2	(M+Na)+	0.13
482.1424	482.1411	1	30734	C15H26F3N7O2S2	(M+Na)+	-2.76
483.1448	483.1425	1	6404	C15H26F3N7O2S2	(M+Na)+	-4.68

--- End Of Report ---

Figure S20. FIA ESI$^+$ HRMS of [**3**+Na]$^+$.



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
519.0767	519.0754	1	727146	C15H24CuF3N7O2S2	(M+H)+	-2.5
520.0781	520.0777	1	188407	C15H24CuF3N7O2S2	(M+H)+	-0.85
521.0744	521.0735	1	401821	C15H24CuF3N7O2S2	(M+H)+	-1.88
522.0764	522.0756	1	91206	C15H24CuF3N7O2S2	(M+H)+	-1.43
523.0734	523.0713	1	38333	C15H24CuF3N7O2S2	(M+H)+	-3.97
524.0784	524.0727	1	7702	C15H24CuF3N7O2S2	(M+H)+	-10.82
525.0833	525.0701	1	2660	C15H24CuF3N7O2S2	(M+H)+	-25.02

--- End Of Report ---

Figure S21. FIA ESI$^+$ HRMS of **4**.

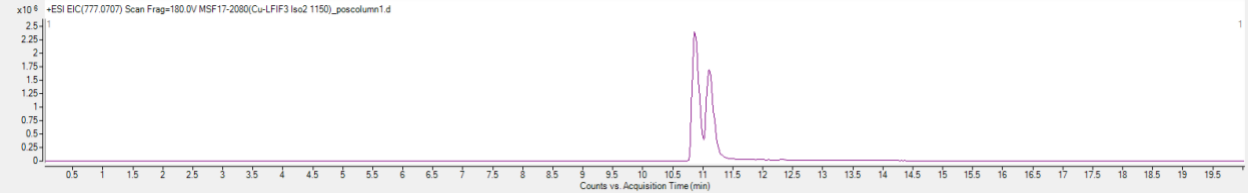
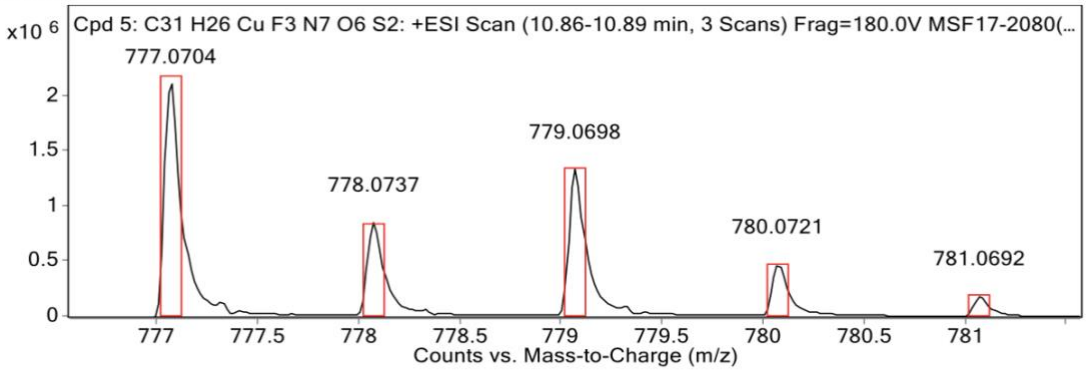


Figure S22. LCMS extracted ion chromatograph (EIC) trace for **CuATSMF$_3$-FI**, subtracting out the blank. Note: there are two isomers of the product present, accounting for both peaks.

MS Zoomed Spectrum

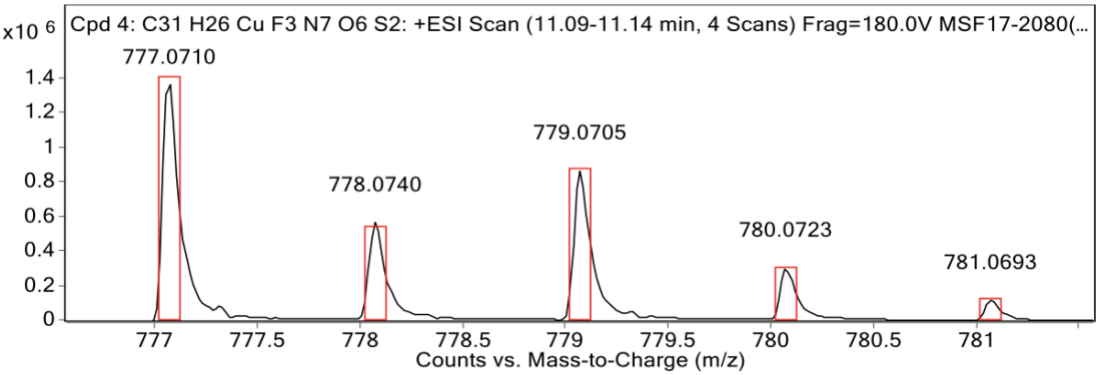


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
777.0704	777.0707	1	2138266	C31H26CuF3N7O6S2	(M+H)+	0.35
778.0737	778.0735	1	860619	C31H26CuF3N7O6S2	(M+H)+	-0.22
779.0698	779.0695	1	1341144	C31H26CuF3N7O6S2	(M+H)+	-0.33
780.0721	780.0717	1	480353	C31H26CuF3N7O6S2	(M+H)+	-0.46
781.0692	781.0696	1	191292	C31H26CuF3N7O6S2	(M+H)+	0.52
782.0692	782.0703	1	43976	C31H26CuF3N7O6S2	(M+H)+	1.46
783.0678	783.0699	1	10998	C31H26CuF3N7O6S2	(M+H)+	2.6
784.0683	784.0702	1	2473	C31H26CuF3N7O6S2	(M+H)+	2.49

--- End Of Report ---

MS Zoomed Spectrum

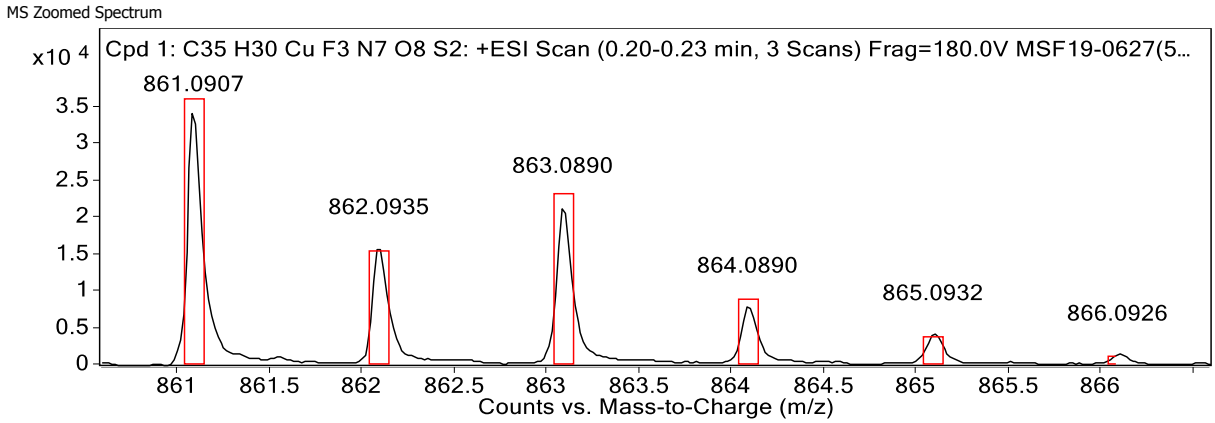


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
777.0710	777.0707	1	1378485	C31H26CuF3N7O6S2	(M+H)+	-0.38
778.0740	778.0735	1	569593	C31H26CuF3N7O6S2	(M+H)+	-0.67
779.0705	779.0695	1	869914	C31H26CuF3N7O6S2	(M+H)+	-1.22
780.0723	780.0717	1	307673	C31H26CuF3N7O6S2	(M+H)+	-0.73
781.0693	781.0696	1	120387	C31H26CuF3N7O6S2	(M+H)+	0.29
782.0704	782.0703	1	28822	C31H26CuF3N7O6S2	(M+H)+	-0.16
783.0684	783.0699	1	6665	C31H26CuF3N7O6S2	(M+H)+	1.87
784.0695	784.0702	1	1476	C31H26CuF3N7O6S2	(M+H)+	0.92

--- End Of Report ---

Figure S23. HRMS for both isomers of **CuATSMF$_3$-FI** found in Figure S22 with 0.1% formic acid in MeOH (pH ~2) as the mobile phase.



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
162.9739			289173			
861.0907	861.0918	1	34647	C35H30CuF3N7O8S2	(M+H)+	1.26
862.0935	862.0947	1	16023	C35H30CuF3N7O8S2	(M+H)+	1.31
863.0890	863.0909	1	21624	C35H30CuF3N7O8S2	(M+H)+	2.21
864.0890	864.0930	1	8072	C35H30CuF3N7O8S2	(M+H)+	4.64
865.0932	865.0914	1	4338	C35H30CuF3N7O8S2	(M+H)+	-2.05
866.0926	866.0921	1	1561	C35H30CuF3N7O8S2	(M+H)+	-0.58
867.1041	867.0919	1	1255	C35H30CuF3N7O8S2	(M+H)+	-14.04
868.0736	868.0924	1	585	C35H30CuF3N7O8S2	(M+H)+	21.56

--- End Of Report ---

Figure S24. FIA ESI$^+$ HRMS of **CuATSMF$_3$-FlAc**.

References

1. D. Xie, T. L. King, A. Banerjee, V. Kohli and E. L. Que, *J. Am. Chem. Soc.*, 2016, **138**, 2937-2940.
2. D. Xie, S. Kim, V. Kohli, A. Banerjee, M. Yu, J. S. Enriquez, J. J. Luci and E. L. Que, *Inorg. Chem.*, 2017, **56**, 6429-6437.