Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Copper(II) Complexes for Cysteine Detection using ^{19}F Magnetic Resonance

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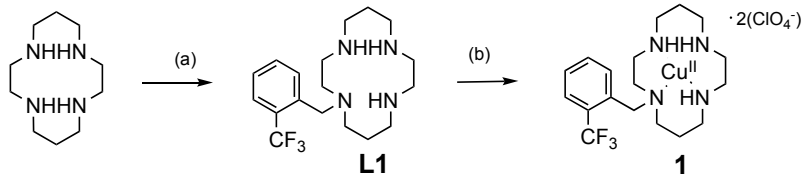
Materials and Methods

General Methods

All solvents and chemicals were purchased from Sigma-Aldrich, Arkpharm and Fisher Sci. and used as received. The ^{1}H, ^{13}C and ^{19}F NMR spectroscopic measurements were conducted in deuterated solvents from Cambridge Isotope Laboratories (Cambridge, MA), using an AGILENT MR 400 NMR spectrometer at 400, 100, 376 MHz, respectively. The chemical shifts for ^{1}H and ^{13}C NMR were calibrated to the solvent peak, while for ^{19}F NMR were calibrated to 5% TFA in D_2O (δ = -76.55 ppm). Walk-up LC-MS and high-resolution Electrospray Ionization (ESI) mass spectral analyses were performed by the Mass Spectrometry Facility of the Department of Chemistry at UT Austin. Electrochemistry experiments were carried out on a CHI 660D electrochemical workstation from the UT Austin Center for Electrochemistry. X-Ray crystallography was performed on an Agilent Technologies SuperNova Dual Source diffractometer using a μ-focus Cu Kα radiation source (λ = 1.5418.) with collimating mirror monochromators. EPR spectra were obtained with a Bruker Biospin EMXplus 114 X-band spectrometer equipped with a liquid nitrogen cryostat. MR images were collected on a Bruker BioSpin (Karlsruhe, Germany) Pharmascan 70/16 magnet with a BioSpec two-channel console and BGA-9s gradient coil in the Imaging Research Center at UT Austin.

Synthesis of 1



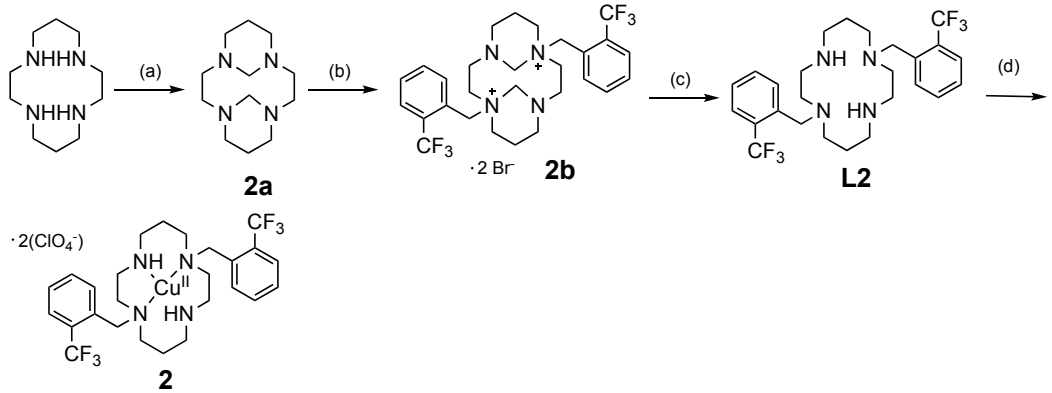
Scheme 1: (a) 1-(bromomethyl)-2-(trifluoromethyl)benzene, K_2CO_3, CH_3CN, 60 ˚C, overnight (60%); (b) $Cu(ClO_4)_2$, MeOH, r.t., overnight (40%)

1-(2-(trifluoromethyl)benzyl)-1,4,8,11-tetraazacyclotetradecane (L1). 1,4,8,11-tetraazacyclotetradecane (Cyclam) (0.228 g, 1.14 mmol) and potassium carbonate (K_2CO_3) (0.525 g, 3.8 mmol) were dissolved under a nitrogen environment with dry CH_3CN (100 mL). 2-

(Trifluoromethyl) benzyl bromide (0.1817 g, 0.76 mmol) was dissolved in CH_3CN (25 mL) and was added slowly into the solution. The mixture was refluxed at 60°C for 16 hrs. The mixture was filtered and washed with CH_3CN and concentrated. The product was purified by reverse phase column chromatography (1% CH_3CN in H_2O). (60%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.69 (s, 0H), 7.48 (d, *J* = 4.7 Hz, 1H), 6.73 – 6.62 (m, 5H), 6.62 (d, *J* = 14.2 Hz, 1H), 6.58 (s, 1H), 6.45 (t, *J* = 6.5 Hz, 1H), 2.82 (d, *J* = 4.6 Hz, 1H), 2.39 (d, *J* = 4.8 Hz, 2H), 2.23 – 2.16 (m, 1H), 2.13 (d, *J* = 6.2 Hz, 1H), 2.02 – 1.92 (m, 4H), 1.84 (d, *J* = 15.6 Hz, 3H), 1.02 – 0.89 (m, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -59.06. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.72, 137.49, 133.27, 132.37, 128.75, 127.32, 58.75, 55.77, 54.37, 53.64, 50.24, 50.04, 46.66, 46.37, 45.74, 25.65, 23.97, 19.18. HRMS (ESI$^+$): *m/z* 359.2423 $[M]^+$ calcd $[C_{18}H_{29}F_3N_4]^+$ 359.4612.

Copper complex 1. Compound **L1** (0.100 g, 0.279 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (0.103 g, 0.279 mmol) were dissolved in 3 mL of MeOH and was left stirring overnight at room temperature. A purple precipitate formed after 16hrs, then the solid was centrifuged down and the liquid was decanted. The solid was washed with ethyl ether three times and dried, leaving a light purple solid. (40%) HRMS (ESI$^+$): *m/z* 420.1565 $[M]^+$ calcd $[CuC_{18}H_{28}F_3N_4]^+$ 420.1562; *m/z* 210.5821 $[M]^{2+}$ calcd $[CuC_{18}H_{28}F_3N_4]^{2+}$ 210.5781.

Synthesis of 2



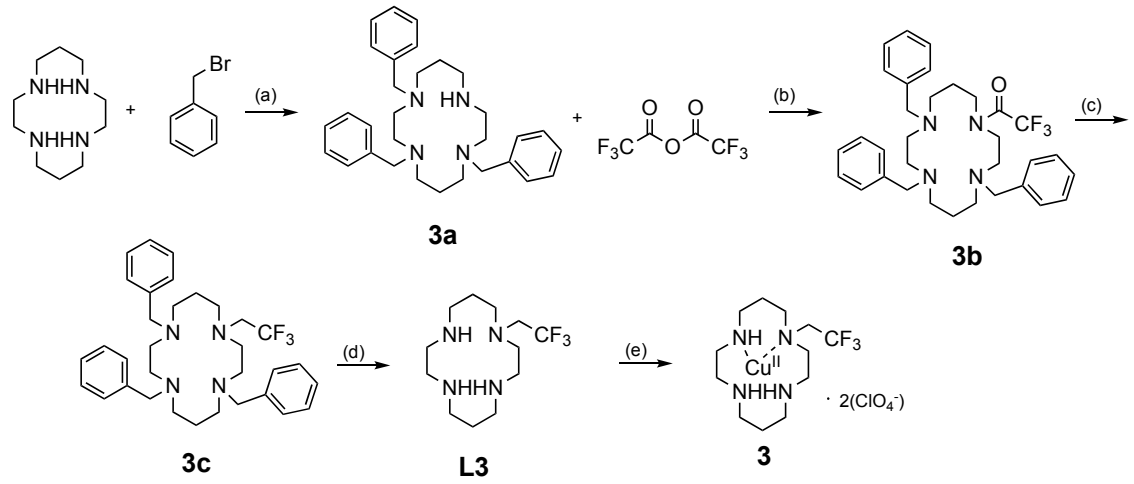
Scheme 2: (a) Formaldehyde (37%wt.), H_2O, 0 ˚C, 2 h (90%); (b) 1-(bromomethyl)-2-(trifluoromethyl)benzene, CH_3CN, r.t., 2 d (30%); (c) NaOH, r.t., 2 d (70%); (d) $Cu(ClO_4)_2$, MeOH, r.t., overnight (80%)

Cyclam bisformyl (2a) was synthesized using literature procedure.[1]

1,8-bis(2-(trifluoromethyl)benzyl)-1,4,8,11-tetraazacyclotetradecane (L2). Cyclam bisformyl **2a** (0.150 g, 0.668 mmol) was dissolved in minimal amount of dry CH_3CN (3mL). Then 2-(Trifluoromethyl) benzyl bromide (0.4 g, 1.67 mmol) was added slowly to the solution. The mixture was stirred for 2 days at room temperature. After two days a precipitate formed and was centrifuged down and the liquid was decanted. The solid was washed with minimal amount of CH_3CN and dried, without further purification the compound (**2b**) was used. Compound **2b** (0.120 g, 0.221 mmol) was dissolved in 3M NaOH (15 mL) and 1,4 Dioxane (5mL). The solution was stirred at room temperature for 24 hrs. After 24 hrs the product was extracted with $CHCl_3$ (5X). The organic layer was dried with sodium sulfate and concentrated to obtain a white powder. (70%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.79 (d, *J* = 7.8 Hz, 1H), 7.55 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 3.70 (d, *J* = 1.7 Hz, 2H), 2.82 – 2.68 (m, 4H), 2.68 – 2.56 (m, 4H), 1.81 (p, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 138.51, 131.56, 129.57, 126.43, 125.59, 125.53, 123.10, 67.07, 54.29, 51.65, 48.06, 47.73, 26.11. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -59.50. HRMS (ESI$^+$): *m/z* 517.2759 $[M]^+$ calcd $[C_{26}H_{34}F_6N_4]^+$ 517.2768.

Copper complex 2. Compound **L2** (0.0716 g, 0.139 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (0.051 g, 0.139 mmol) were dissolved in 3 mL of MeOH and was left stirring overnight at room temperature. A purple precipitate formed after 16hrs and the solid was centrifuged down and the liquid was decanted. The solid was washed with ethyl ether three times and dried giving a dark purple solid. (50%) HRMS (ESI$^+$): *m/z* 578.1898 [M]+ calcd $[CuC_{26}H_{33}F_6N_4]^+$ 578.1904; *m/z* 289.6000 $[M]^{2+}$ calcd $[CuC_{26}H_{33}F_6N_4]^{2+}$ 289.0952.

Synthesis of 3



Scheme 3: (a) CH_3CN, r.t., 24h (40%); (b) Et_3N, $CHCl_3$, r.t., 3h (50%); (c) $NaBH_4$, $BF_3{*}Et_2O$, Diglyme, 120°C, overnight (90%); (d) 10% Pd/C, H_2, AcOH, r.t., 2d (95%); (e) $Cu(ClO_4)_2$, MeOH, r.t, overnight (40%)

1,4,8-tribenzyl-1,4,8,11-tetraazacyclotetradecane (3a). 1,4,8,11-tetraazacyclotetradecane (Cyclam) (0.100 g, 0.499 mmol) was dissolved in 40 mL of methylene chloride and 2.7 equiv. of benzyl bromide (0.256 g, 1.5 mmol) was added. The solution was stirred for 2 days at room temperature. The solvent was evaporated and purified by reverse phase column chromatography (50% CH_3CN in H_2O) (40%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.63 (s, 4H), 8.48 (s, 2H), 7.38 – 7.20 (m, 15H), 3.76 (s, 2H), 3.64 (d, *J* = 15.4 Hz, 2H), 3.51 (s, 2H), 3.11 (t, *J* = 5.1 Hz, 2H), 3.00 (s, 2H), 2.84 (s, 2H), 2.78 (d, *J* = 16.4 Hz, 0H), 2.58 (s, 2H), 2.55 – 2.46 (m, 6H), 2.32 (t, *J* = 5.2 Hz, 2H), 1.92 (p, *J* = 6.0 Hz, 4H).

2,2,2-trifluoro-1-(4,8,11-tribenzyl-1,4,8,11-tetraazacyclotetradecan-1-yl)ethan-1-one (3b). Tri-substituted cyclam (**3a**) (0.055 g, 0.116 mmol) was dissolved in chloroform, then triethylamine (0.0295 g, 0.292 mmol) and trifluoroacetic anhydride (0.049 g, 0.234 mmol) were added. The reaction mixture was stirred at room temperature for 3 hours. The solvent was evaporated and purified by reverse phase column chromatography (50% CH_3CN in H_2O) (50%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.28 (dq, *J* = 15.2, 7.4 Hz, 15H), 3.67 (s, 2H), 3.58 – 3.38 (m, 6H), 2.69 (s, 6H), 2.60 (q, *J* = 12.4, 9.3 Hz, 4H), 2.40 (t, *J* = 5.8 Hz, 2H), 2.17 (s, 2H), 1.85

(s, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -69.22 (d, *J* = 20.9 Hz), -75.62.

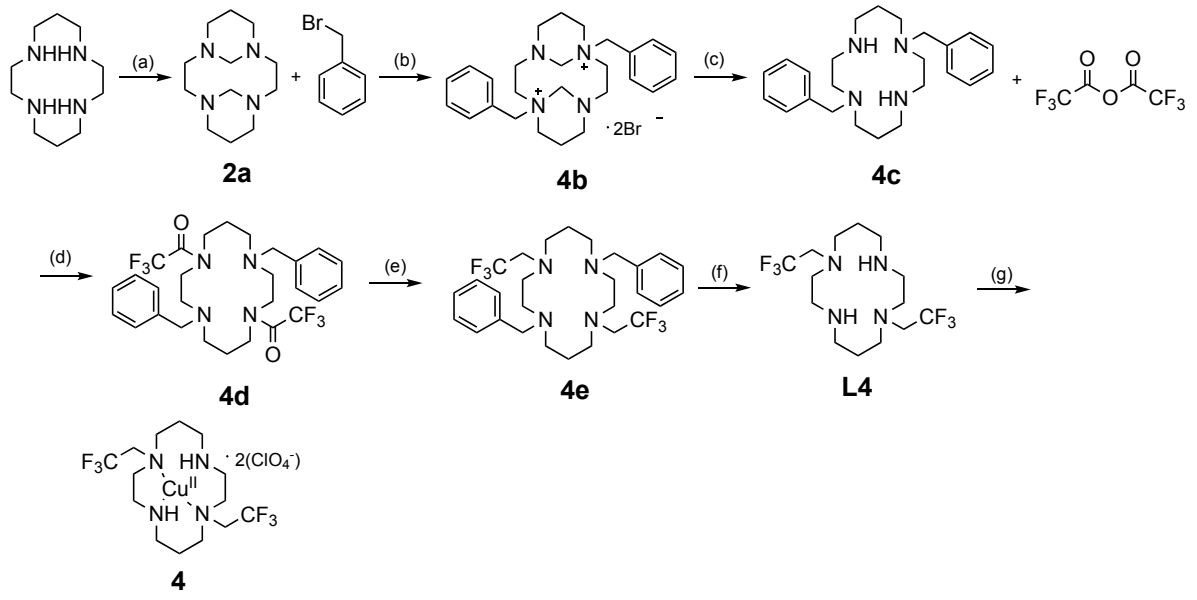
1,4,8-tribenzyl-11-(2,2,2-trifluoroethyl)-1,4,8,11-tetraazacyclotetradecane (3c). Compound **3b** (0.106 g, 0.188 mmol) and sodium borohyrdide (0.035 g, 0.940 mmol) were dissolved in diglyme and stirred. BF_3*Et_2O (0.133 g, 0.94 mmol) was diluted in diglyme and added dropwise into the stirring solution, while the escaping gas (B_2H_6) was passed through a solution of H_2O_2/H_2O/NaOH. Then the solution was heated up to 120°C and stirred overnight. Afterwards, the solution was cooled to room temperature and 5% sulfuric acid was added dropwise and the volatiles were evaporated in *vacou*. The residue was dissolved with 5% NaOH and the product was extracted with $CHCl_3$ (5x). The organic layers were combined and dried with sodium sulfate, filtered and evaporated. (80%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 – 7.17 (m, 15H), 3.51 (s, 2H), 3.45 (d, *J* = 7.4 Hz, 4H), 2.95 (q, *J* = 9.6 Hz, 2H), 2.80 (t, *J* = 6.3 Hz, 2H), 2.74 (t, *J* = 7.1 Hz, 2H), 2.63 – 2.52 (m, 6H), 2.50 (td, *J* = 7.0, 2.7 Hz, 4H), 1.78 – 1.65 (m, 4H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -69.48 (t, 3F).

1-(2,2,2-trifluoroethyl)-1,4,8,11-tetraazacyclotetradecane (L3). Compound **3c** (0.100 g, 0.181 mmol) was dissolved in glacial acetic acid and 10% Pd/C (0.015 g) catalyst was added under N_2. The system was evacuated and filled with H_2. The solution was stirred at room temperature for 2 days. After 2 days the mixture was filtered with celite to remove all of the catalyst and the filtrate was concentrated to give the final product. (90%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.09 (qd, *J* = 18.4, 16.4, 6.7 Hz, 1H), 2.82 – 2.63 (m, 7H), 2.63 (d, *J* = 4.9 Hz, 2H), 2.55 – 2.40 (m, 0H), 1.82 – 1.69 (m, 1H), 1.65 (ddd, *J* = 26.1, 11.6, 5.9 Hz, 1H), 1.28 (s, 1H), 1.23 (s, 12H), 1.19 (s, 3H), 1.06 (d, *J* = 18.2 Hz, 1H), 0.90 – 0.76 (m, 4H), 0.71 (dt, *J* = 14.9, 7.6 Hz, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -67.14 (t, 3F). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 54.85, 53.20, 50.84, 49.33, 48.62, 48.05, 47.36, 36.31, 27.98, 26.18, 22.67, 14.11.

Copper complex 3. Compound **L3** (0.0716 g, 0.139 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (0.051 g, 0.139 mmol) were dissolved in 3 mL of MeOH and was left stirring overnight at room

temperature. The solvent was concentrated and purified by reverse phase column chromatography (1% CH_3CN in H_2O) (40%) HRMS (ESI^+): *m/z* 344.123 [M]+ calcd [CuC26H33F6N4]+ 344.124;

Synthesis of 4



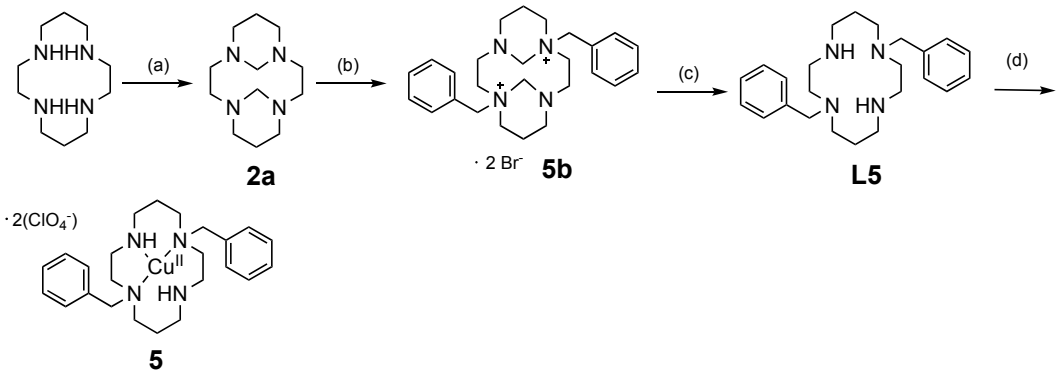
Scheme 4: (a) Formaldehyde (37%wt.) H_2O, 0°C-r.t., 3h (70%); (b) CH_3CN, r.t., overnight (90%); (c) 3M NaOH, r.t., overnight (80%); (d) Et_3N, CH_3Cl, r.t., 3h (60%); (e) $NaBH_4$, $BF_3{*}Et_2O$, Diglyme, 120°C, overnight (60%); (f) 10% Pd/C, H_2, AcOH, r.t., 2d (80%); (g) $Cu(ClO_4)_2$, MeOH, r.t., overnight (80%)

1,8-dibenzyl-1,4,8,11-tetraazacyclotetradecane (4c) was synthesized using literature procedure.[1]

1,8-bis(2,2,2-trifluoroethyl)-1,4,8,11-tetraazacyclotetradecane (L4) was synthesized using literature procedure.[2]

Copper complex 4. Compound **L4** (0.0606 g, 0.166 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (0.0615 g, 0.166mmol) were dissolved in 5 mL of MeOH and was left stirring overnight at room temperature. A purple precipitate formed and was centrifuged down and the liquid was decanted. The solid was washed with ethyl ether three times and dried giving a dark purple solid. (80%) HRMS (ESI^+): *m/z* 427.1358 $[M]^+$ calcd $[CuC_{18}H_{28}F_3N_4]^+$ 427.1280

Synthesis of 5



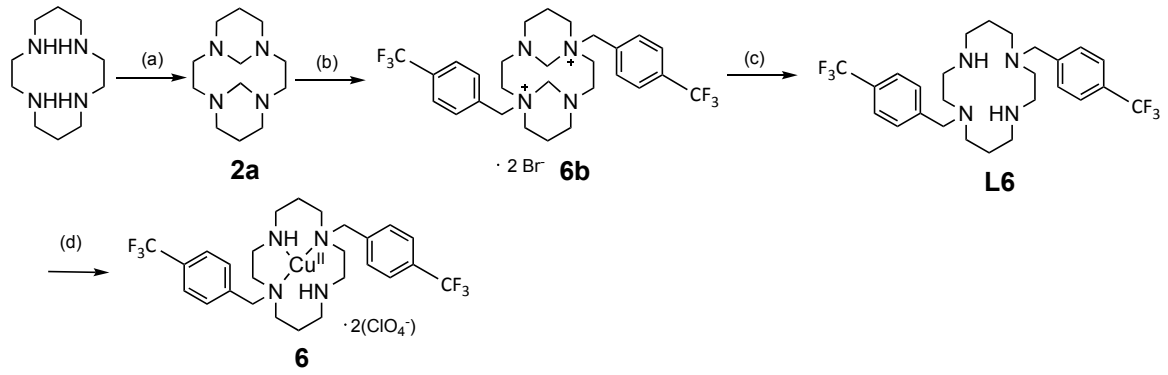
2a **5b** **L5** **5**

Scheme 5: (a) Formaldehyde (37%wt.), H_2O, 0 ˚C, 2 h (90%); (b) 1-(bromomethyl) benzene, CH_3CN, r.t., 2 d (70%); (c) NaOH, r.t., 2 d (80%); (d) $Cu(ClO_4)_2$, MeOH, r.t., overnight (80%)

1,8-dibenzyl-1,4,8,11-tetraazacyclotetradecane (L5). 2a (0.100 g, 0.446 mmol) was dissolved in minimal amount of dry CH_3CN (3mL). Then benzyl bromide 0.167 g, 0.98 mmol) was added slowly to the solution. The mixture was stirred for 1 day at room temperature. After a day, a precipitate formed and was centrifuged down and the liquid was decanted. The solid was washed with minimal amount of CH_3CN and dried, without further purification the compound (5b) was used. Compound 5b (0.210 g, 0.535 mmol) was dissolved in 3M NaOH (15 mL) and 1,4 Dioxane (5mL). The solution was stirred at room temperature for 24 hrs. After 24 hrs the product was extracted with $CHCl_3$ (5X). The organic layer was dried with sodium sulfate and concentrated to obtain a white powder. (80%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.27 – 7.07 (m, 10H), 3.63 (s, 4H), 2.73 (s, 3H), 2.62 (dt, *J* = 13.4, 5.3 Hz, 8H), 2.52 – 2.39 (m, 8H), 1.75 (q, *J* = 6.0, 5.4 Hz, 4H)

Copper complex 5. Compound **6c** (0.050 g, 0.131 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (0.048 g, 0.131 mmol) were dissolved in 3 mL of MeOH and was left stirring overnight at room temperature. A purple precipitate formed after 16hrs and the solid was centrifuged down and the liquid was decanted. The solid was washed with ethyl ether three times and dried giving a dark purple solid. (80%) LRMS (ESI$^+$): *m/z* 444.1358 $[M]^+$ calcd $[CuC_{24}H_{35}N_4]^+$ 444.1280

Synthesis of 6



2a **6b** **L6** **6**

Scheme 6: (a) Formaldehyde (37%wt.), H_2O, 0 ˚C, 2 h (90%); (b) 1-(bromomethyl)-4-(trifluoromethyl)benzene, CH_3CN, r.t., 2 d (50%); (c) NaOH, r.t., 2 d (70%); (d) $Cu(ClO_4)_2$, MeOH, r.t., overnight (80%)

1,8-bis(4-(trifluoromethyl)benzyl)-1,4,8,11-tetraazacyclotetradecane (L6). Cyclam bisformyl **2a** (0.080 g, 0.357 mmol) was dissolved in minimal amount of dry CH_3CN (3mL). Then 4-(trifluoromethyl) benzyl bromide (0.222 g, 0.927 mmol) was added slowly to the solution. The mixture was stirred for 2 days at room temperature. After two days a precipitate formed and was centrifuged down and the liquid was decanted. The solid was washed with minimal amount of CH_3CN and dried, without further purification the compound (6b) was used. Compound 6b (0.130 g, 0.240 mmol) was dissolved in 3M NaOH (15 mL) and 1,4 Dioxane (5mL). The solution was stirred at room temperature for 24 hrs. After 24 hrs the product was extracted with $CHCl_3$ (5X). The organic layer was dried with sodium sulfate and concentrated to obtain a white powder. (70%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53 (d, *J* = 8.0 Hz, 4H), 7.42 (d, *J* = 7.9 Hz, 4H), 3.73 (s, 4H), 2.74 (t, *J* = 5.3 Hz, 8H), 2.62 (s, 4H), 2.58 – 2.45 (m, 4H), 1.86 (s, 4H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.23 – -62.52 (t).

Copper complex 6. Compound **6c** (0.030 g, 0.058 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (0.022 g, 0.058 mmol) were dissolved in 3 mL of MeOH and was left stirring overnight at room temperature. A purple precipitate formed after 16 hrs and the solid was centrifuged down and the liquid was decanted. The solid was washed with ethyl ether three times and dried giving a dark purple solid. (80%) LRMS (ESI$^+$): *m/z* 578.1898 $[M]^+$ calcd $[CuC_{26}H_{33}F_6N_4]^+$ 578.1904.

X-ray crystallography. Crystals OF **1**, **2**, and **4** grew as clear, violet prisms by slow evaporation from a mixture of CH_3CN and water. The crystal for **1** was cut from a larger crystal and had approximate dimensions; 0.22 x 0.21 x 0.14 mm^3. The crystal for **2** had approximate dimensions; 0.40 x 0.29 x 0.23 mm^3. The crystal for **4** had approximate dimensions; 0.28 x 0.27 x 0.23 mm^3. The data were collected at -167 □C on a Nonius Kappa CCD diffractometer using a Bruker AXS Apex II detector and a graphite monochromator with MoK□ radiation (□ = 0.71073Å). Reduced temperatures were maintained by use of an Oxford Cryosystems 600 low-temperature device. A total of 645 frames of data were collected using □-scans with a scan range of 1.1□ and a counting time of 95 seconds per frame. Details of crystal data, data collection and structure refinement are listed in Table 1. Data reduction were performed using SAINT V8.27B. 1 The structures were solved by direct methods using Superflip 2 and refined by full-matrix least-squares on F2 with anisotropic displacement parameters for the non-H atoms using SHELXL-2013. 3 Structure analysis was aided by use of the programs PLATON98 4 and WinGX. 5 The hydrogen atoms bound to carbon atoms were calculated in idealized positions. The hydrogen atoms on N2 was observed in a ΔF map refined with an isotropic displacement parameter.

UV-Vis Spectroscopy. All UV-Vis spectroscopy was performed in a 6:4 HEPES Buffer to acetonitrile ratio (complexes **1** and **2**) or 100% HEPES buffer (complexes **3** and **4**). Data was collected from an Agilent Technologies Cary 60 UV-Vis at room temperature from 200 – 1000 nm, with a fast acquisition rate. The samples were prepared and transferred to a 3mL screw top quartz cuvette inside an anaerobic chamber, with a septum cap placed on top.

Selectivity Studies. All selectivity studies were performed on an Agilent Technologies Cary 60 UV-Vis at room temperature from 200-1000 nm. These studies were performed in a 6:4 HEPES buffer to acetonitrile ratio (complex **2**) or 100% HEPES buffer (complex **4**). 0.5 mM **2** and **4** were prepared inside a glovebag followed by different additions. For the amino acid competition studies, a 0.5 mM solution of complex **4** was prepared followed by an addition of 5 equivalents of different amino acids, and then a spectrum was acquired. Afterwards, 3 equivalents of cysteine were added and another spectrum was acquired. All of the preparation and additions were carried out inside a glovebag.

Cyclic Voltammetry (CV). Electrochemistry experiments were carried out on a CHI 660D electrochemical workstation. Cyclic voltammetry (CV) measurements for a 1 mM solution of the compound were recorded at 100 mV/s in a glovebox. A three-electrode cell was used, including a platinum electrode as working electrode, an Ag/Ag+ non-aqueous electrode as reference electrode (a 10 mM DMF solution of AgNO3 was used as the source of Ag+) and a platinum wire as auxiliary electrode. Bn_4NBF_4 (0.1 M) was used as the electrolyte and the spectra were calibrated by ferrocene.

EPR Spectroscopy. EPR spectra collected from Bruker Biospin EMXplus 114 X-band spectrometer equipped with a liquid nitrogen cryostat. The spectra of the samples were collected at room temperature. The samples were prepared in vials inside an anaerobic chamber and then transferred into 2 mm EPR tubes before acquisition.

^{19}F MR imaging. The magnetic resonance imaging experiments were performed on a Bruker BioSpin (Karlsruhe, Germany) Pharmascan 70/16 magnet with a BioSpec two-channel console and BGA-9s gradient coil. The RF coil was a quadrature single

resonance tunable T/R coil (Doty Scientific, Inc., Columbia, South Carolina, USA) with a resonant frequency of 282.2 MHz to correspond to ^{19}F at 7.0 T. Each element of the RF coil was tuned and matched with the samples loaded using a Morrwasfrequency sweeper (MorrwasInstruments, Inc. Ottawa, Ontario, Canada) while the complementary element was terminated with the receive chain of the instrument. All prescan adjustments and imaging was performed using product sequences and methods in ParaVision 6.0.1 (Bruker, vide supra). Samples were prepared in vials then transferred into eppendorf tubes. Specific scanning parameters are indicated in the caption of Figure S10.

Cell culture. Cell culture was performed in complete medium (Dulbecco's modified Eagle's Medium, supplemented with 2 mM L-glutamine, 1 mM Sodium Pyruvate, 10% heat inactivated fetal bovine serum, and antibiotics (200 U/cm3 penicillin and 200 µg/cm3 streptomycin) at 37 °C in atmosphere containing 5% CO2. HeLa cancer cells were grown in T-75 flasks. The following cell experiments were carried out at 80% cell confluence.

Cell uptake studies. Cell uptake studies were carried out in triplicate for complex **4** using the same conditions used for the in vitro NMR study (see below). After incubation of ~1 million HeLa cells with 0.5 mM **4**, cells were washed with 5 mL PBS buffer 2 times to remove the media and 2 mL of LCIS was added. Cells were scrapped with a cell scrapper transferred to Eppendorf tubes. The tubes were centrifuged for 5 min at 0.2 rcf and the media was removed and discarded. The cell pellet so obtained was digested with 1M NaOH (1 mL) and diluted with 2% (v/v) HNO_3 (4mL). The resulting mixture was filtered by 22 µm syringe filter and the clear filtrate was subjected

to inductively coupled plasma-optical emission spectrometry (ICP-OES) for determination of the whole copper content. The instrument was calibrated for copper by using standard solutions with copper level of 0, 10, 25, 50, 100, 250, 500, 1000 ppb, respectively.

Cell Viability. Cell toxicity studies were conducted using an MTT assay. HeLa cells were plated into 96 well plates (~7,000 cells per well). Cells were incubated for 18 hours under normal conditions. The next day, media was taken out and the cells were washed with 100 µL of PBS two times. After incubating cells with variable concentrations of **4** following the same procedure as for the in vitro NMR study, 15 µL of MTT (5mg/mL) solution was added to each well and incubated for 2 hours. Then 100 µL of DMSO was added to dissolve the MTT crystals, the plate was placed in a shaker for 15 minutes and the absorbance was recorded using a plate reader (570 nm filter).

In vitro ^{19}F NMR study. An in vitro 19F NMR test was carried out for **4** at a concentration of 0.5 mM. HeLa cells (~40 million) were used for this experiment under two conditions, one with no N-Acetylcysteine (NAC) (control) and one with 1 mM NAC. The stock solution of **4** was prepared at a 1 mM in sterile LCIS. The incubation temperature was set at 37 °C. For the control (no NAC), cells were grown in two T-150 flask until 80% confluence, then the cells were washed with PBS buffer twice and 8 mL of a 0.5 mM **4** solution along with 0.1% Pluronic F-127 were added to the cells. The cells were placed in a hypoxic chamber with a 0.1% O_2/0.3% CO_2/99.6% nitrogen atmosphere for 2 hours to allow for reaction to occur without reoxidation. After 2 hours, cells were washed with PBS buffer twice and 8 mL of phenol red-free media was added to the cells and placed in a 0.1% O_2/5% CO_2/ 94.9% nitrogen atmosphere incubator for

another two hours. After incubation, the media was removed and cells were washed with PBS twice and 4 mL of LCIS was added. Cells were transferred into Eppendorf tubes and centrifuged at 0.2 rcf for 5 min and the media was discarded. The cells were suspended in 100 µL of LCIS media and lysed with 300 µL of Celllytic M solution. The lysed cell solution was added to an NMR tube. A capillary of D_2O with 1 mM of 5-Fluorocytosine was put into the NMR tube for locking. For the 1 mM NAC group, HeLa cells were grown in two T-150 flasks under a 5% CO_2/95% air atmosphere (20% O_2) and incubated with 1 mM of NAC for 2 hours in 8 mL DMEM medium. After this the same process as the control was followed. ^{19}F NMR was performed on an Agilent 400 NMR spectrometer at 376 MHz.

Table S1. Bond Lengths for **1**

Atom	Atom	Length/Å	Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1	N1	1.501(3)	C10	N1	1.491(3)	N1	Cu1	2.101(2)
C1	C2	1.525(4)	C11	N1	1.511(3)	N2	Cu1	2.015(2)
C2	C3	1.516(4)	C11	C12	1.532(4)	N3	Cu1	2.026(2)
C3	N4	1.488(3)	C12	C17	1.399(4)	N4	Cu1	2.017(2)
C4	N4	1.493(3)	C12	C13	1.414(4)	O1	Cl1	1.4589(19)
C4	C5	1.511(4)	C13	C14	1.397(4)	O2	Cl1	1.450(2)
C5	N3	1.490(3)	C13	C18	1.500(4)	O3	Cl1	1.435(2)
C6	N3	1.488(3)	C14	C15	1.379(4)	O4	Cl1	1.434(2)
C6	C7	1.515(4)	C15	C16	1.383(4)	O6	Cl2	1.447(2)
C7	C8	1.520(4)	C16	C17	1.388(4)	O5	Cl2	1.456(2)
C8	N2	1.492(3)	C18	F3	1.342(4)	O7	Cl2	1.438(2)
C9	N2	1.480(3)	C18	F2	1.344(3)	O8	Cl2	1.438(2)
C9	C10	1.512(4)	C18	F1	1.356(4)			

Table S2. Bond Angles for **1**

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
N1	C1	C2	114.3(2)	C16	C17	C12	122.3(3)	C3	N4	Cu1	118.31(17)
C3	C2	C1	114.7(2)	F3	C18	F2	106.4(2)	C4	N4	Cu1	107.11(16)
N4	C3	C2	113.9(2)	F3	C18	F1	105.8(2)	N2	Cu1	N4	175.42(9)
N4	C4	C5	108.9(2)	F2	C18	F1	105.1(3)	N2	Cu1	N3	92.54(9)
N3	C5	C4	107.5(2)	F3	C18	C13	113.5(3)	N4	Cu1	N3	86.16(9)
N3	C6	C7	112.1(2)	F2	C18	C13	112.9(2)	N2	Cu1	N1	86.71(8)
C6	C7	C8	114.8(2)	F1	C18	C13	112.5(3)	N4	Cu1	N1	94.42(9)
N2	C8	C7	111.5(2)	C10	N1	C1	109.4(2)	N3	Cu1	N1	177.67(9)
N2	C9	C10	108.0(2)	C10	N1	C11	110.4(2)	O4	Cl1	O3	110.26(13)
N1	C10	C9	110.1(2)	C1	N1	C11	113.7(2)	O4	Cl1	O2	109.79(13)
N1	C11	C12	118.2(2)	C10	N1	Cu1	103.02(15)	O3	Cl1	O2	109.98(12)
C17	C12	C13	116.6(2)	C1	N1	Cu1	108.02(16)	O4	Cl1	O1	109.01(12)
C17	C12	C11	120.3(3)	C11	N1	Cu1	111.81(15)	O3	Cl1	O1	108.91(12)
C13	C12	C11	122.9(2)	C9	N2	C8	110.1(2)	O2	Cl1	O1	108.85(11)
C14	C13	C12	120.8(3)	C9	N2	Cu1	106.66(16)	O8	Cl2	O7	109.84(12)
C14	C13	C18	116.1(3)	C8	N2	Cu1	117.03(17)	O8	Cl2	O6	109.90(13)
C12	C13	C18	122.9(2)	C6	N3	C5	111.4(2)	O7	Cl2	O6	109.90(12)
C15	C14	C13	120.8(3)	C6	N3	Cu1	117.81(17)	O8	Cl2	O5	108.74(12)
C14	C15	C16	119.5(3)	C5	N3	Cu1	106.71(16)	O7	Cl2	O5	109.75(12)
C15	C16	C17	120.1(3)	C3	N4	C4	109.1(2)	O6	Cl2	O5	108.68(12)

Table S3. Bond Lengths for **2**

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Cu1	N2[1]	1.9855(11)	C4	C5	1.5259(19)
Cu1	N2	1.9855(11)	C5	N1[1]	1.4920(17)
Cu1	N1	2.1181(12)	C6	C7	1.5316(18)
Cu1	N1[1]	2.1182(12)	C7	C12	1.402(2)
F1	C13	1.3442(16)	C7	C8	1.412(2)
F2	C13	1.3468(17)	C8	C9	1.406(2)
F3	C13	1.3574(16)	C8	C13	1.507(2)
N1	C1	1.4919(16)	C9	C10	1.384(2)
N1	C5[1]	1.4920(17)	C10	C11	1.382(2)
N1	C6	1.5105(16)	C11	C12	1.392(2)
N2	C2	1.4857(17)	Cl1	O4	1.4292(13)
N2	C3	1.4878(17)	Cl1	O3	1.4378(12)
C1	C2	1.513(2)	Cl1	O2	1.4420(11)
C3	C4	1.515(2)	Cl1	O1	1.4514(10)

Table S4. Bond Angles for **2**

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
N2[1]	Cu1	N2	180.0	C12	C7	C6	117.41(13)
N2[1]	Cu1	N1	93.58(4)	C8	C7	C6	125.77(13)
N2	Cu1	N1	86.42(4)	C9	C8	C7	120.33(14)
N2[1]	Cu1	N1[1]	86.42(4)	C9	C8	C13	115.44(13)
N2	Cu1	N1[1]	93.58(4)	C7	C8	C13	124.23(12)
N1	Cu1	N1[1]	180.0	C10	C9	C8	121.22(14)
C1	N1	C5[1]	108.98(10)	C11	C10	C9	119.26(14)
C1	N1	C6	111.10(10)	C10	C11	C12	119.83(15)
C5[1]	N1	C6	112.61(10)	C11	C12	C7	122.67(14)
C1	N1	Cu1	102.32(8)	F1	C13	F2	105.92(12)
C5[1]	N1	Cu1	110.51(8)	F1	C13	F3	105.28(11)
C6	N1	Cu1	110.84(8)	F2	C13	F3	105.84(11)
C2	N2	C3	109.61(11)	F1	C13	C8	112.84(12)
C2	N2	Cu1	108.86(8)	F2	C13	C8	114.31(12)
C3	N2	Cu1	117.05(8)	F3	C13	C8	111.95(12)
N1	C1	C2	109.56(10)	O4	Cl1	O3	110.87(9)
N2	C2	C1	108.69(11)	O4	Cl1	O2	109.97(8)
N2	C3	C4	112.86(11)	O3	Cl1	O2	109.18(7)
C3	C4	C5	114.52(11)	O4	Cl1	O1	108.84(7)
N1[1]	C5	C4	114.64(11)	O3	Cl1	O1	109.07(7)
N1	C6	C7	117.07(10)	O2	Cl1	O1	108.88(7)
C12	C7	C8	116.65(12)				

Table S5. Bond Lengths for **4**

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Cu1	N1	2.121(2)	N2	C3	1.489(4)
Cu1	N1[1]	2.121(2)	N2	C7	1.475(4)
Cu1	N2[1]	1.985(3)	C1	C2	1.510(5)
Cu1	N2	1.985(3)	C3	C4	1.496(5)
F1	C1	1.311(5)	C5	C6	1.510(6)
F2	C1	1.318(5)	C6	C7[1]	1.510(5)
F3	C1	1.316(5)	Cl1	O1	1.403(4)
N1	C2	1.477(4)	Cl1	O2	1.420(3)
N1	C4	1.499(4)	Cl1	O3	1.431(3)
N1	C5	1.493(5)	Cl1	O4	1.417(3)

Table S6. Bond Angles for **4**

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
N1	Cu1	N1[1]	180.0	F1	C1	C2	114.2(3)
N2[1]	Cu1	N1	92.84(11)	F2	C1	C2	114.3(3)
N2[1]	Cu1	N1[1]	87.16(11)	F3	C1	F2	106.3(4)
N2	Cu1	N1[1]	92.84(11)	F3	C1	C2	109.1(4)
N2	Cu1	N1	87.16(11)	N1	C2	C1	120.3(3)
N2[1]	Cu1	N2	180.0	N2	C3	C4	109.5(3)
C2	N1	Cu1	108.07(18)	C3	C4	N1	110.4(3)
C2	N1	C4	113.1(3)	N1	C5	C6	114.1(3)
C2	N1	C5	112.8(3)	C7[1]	C6	C5	115.0(3)
C4	N1	Cu1	101.46(19)	N2	C7	C6[1]	113.5(3)
C5	N1	Cu1	110.9(2)	O1	Cl1	O2	111.2(2)
C5	N1	C4	110.0(3)	O1	Cl1	O3	110.0(2)
C3	N2	Cu1	108.0(2)	O1	Cl1	O4	109.7(3)
C7	N2	Cu1	118.4(2)	O2	Cl1	O3	109.57(19)
C7	N2	C3	110.6(3)	O4	Cl1	O2	109.2(2)
F1	C1	F2	105.8(4)	O4	Cl1	O3	107.2(2)
F1	C1	F3	106.7(3)				

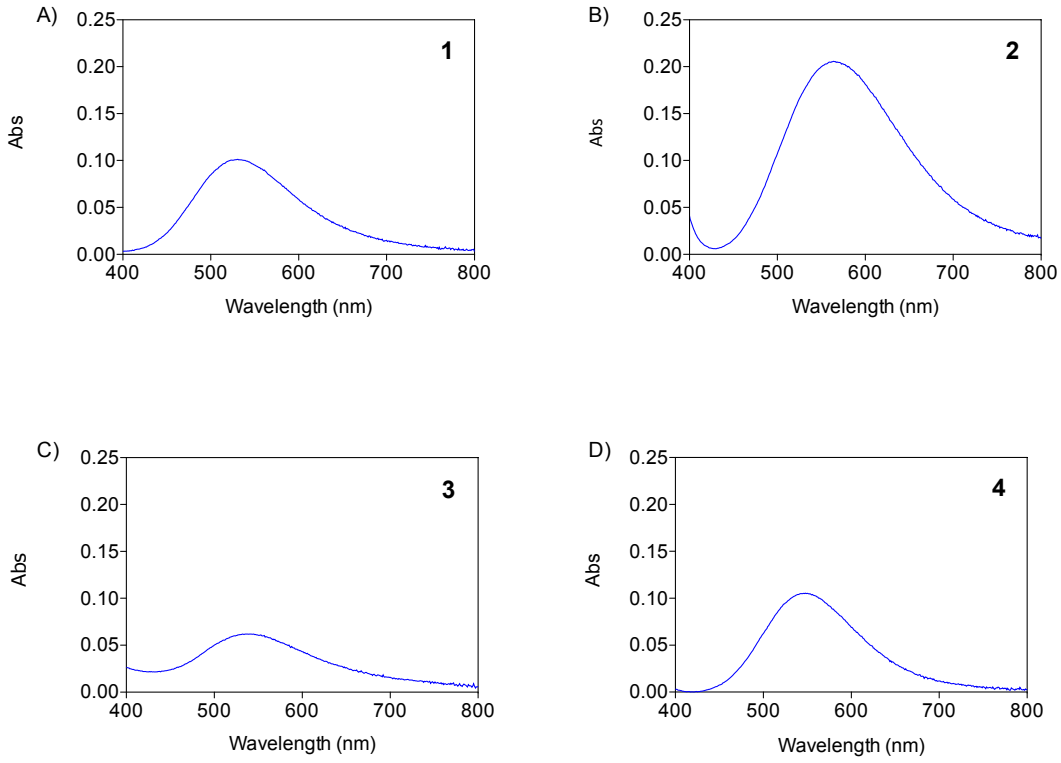


Figure S1. UV/Vis spectra of A) 0.5 mM **1** B) 0.5 mM **2** C) 0.5 mM **3** D) 0.5 mM **4**. **1** and **2** were dissolved in 6:4 HEPES: acetonitrile, **3** and **4** were dissolved in HEPES buffer.

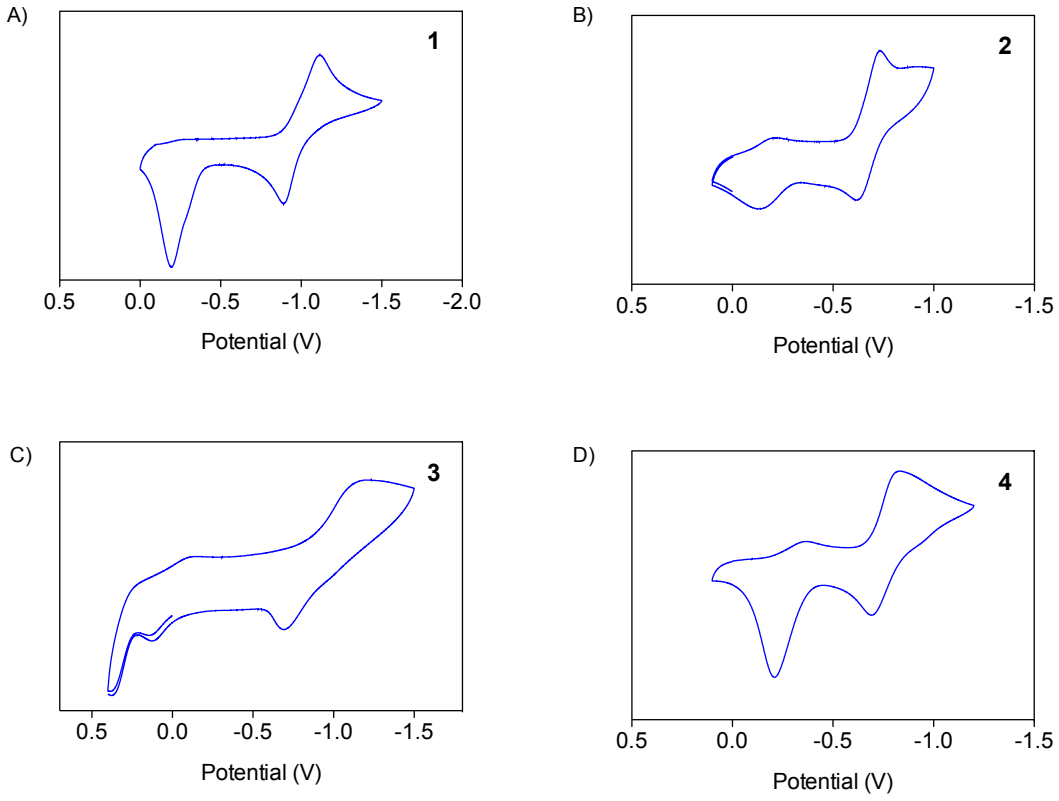


Figure S2. A) Complex **1**, B) Complex **2**, C) Complex **3**, D) Complex **4**; 1 mM in DMF, three-electrode cell; a platinum electrode as working electrode, an Ag/Ag+ non-aqueous electrode as reference and a platinum wire as auxiliary electrode. Bu_4NBF_4 (0.1 M) was used as the electrolyte and the spectra were calibrated by ferrocene.

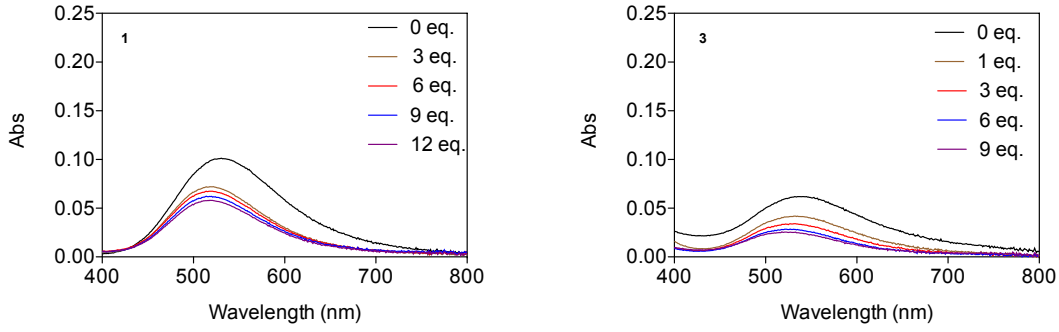


Figure S3. Titrations of **1** and **3** with cysteine. **1** was dissolved in 6:4 HEPES: acetonitrile, **3** was dissolved in HEPES buffer. Cysteine was dissolved in HEPES buffer (stock solution of 25 mM).

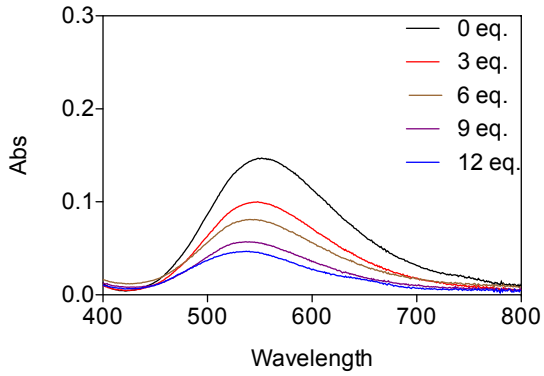


Figure S4. Titration of **6** with cysteine. This was performed in a 6:4 ratio of HEPES buffer: acetonitrile. Cysteine was dissolved in HEPES buffer (stock solution of 25 mM).

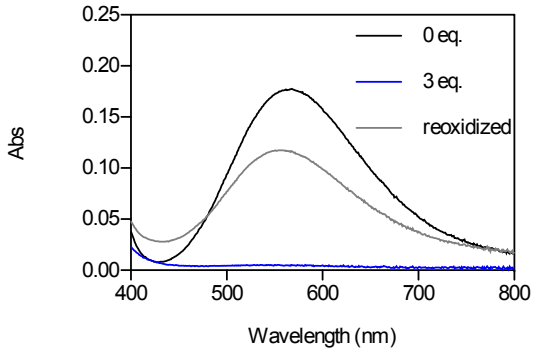


Figure S5. Reoxidation of **2** after cysteine reduction. This was performed in a 6:4 ratio of HEPES buffer: acetonitrile.

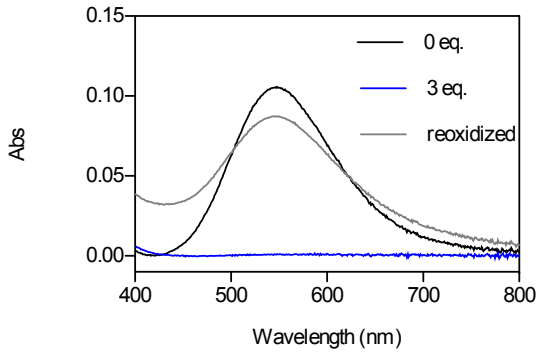


Figure S6. Reoxidation of **4** after cysteine reduction. This was performed in HEPES buffer.

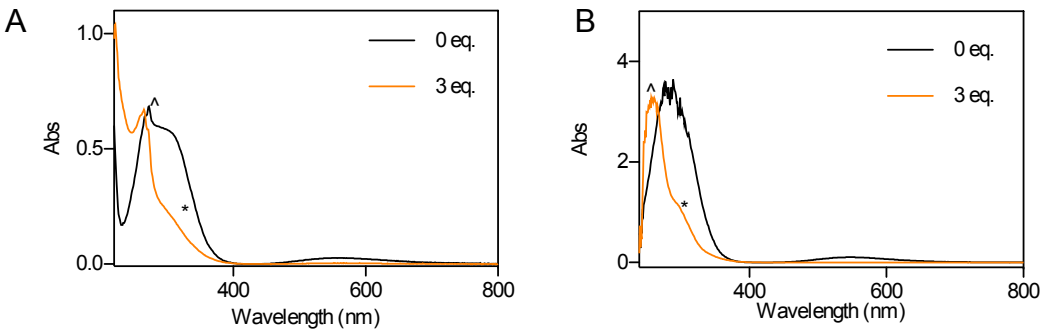


Figure S7. UV-Vis of complexes **2** (A) and **4** (B) before and after cysteine addition. The 300nm shoulder (*) and 260nm (^) peak are consistent with the Cu-Cys complex. **2** was dissolved in 6:4 HEPES: acetonitrile and **4** was dissolved in HEPES buffer.

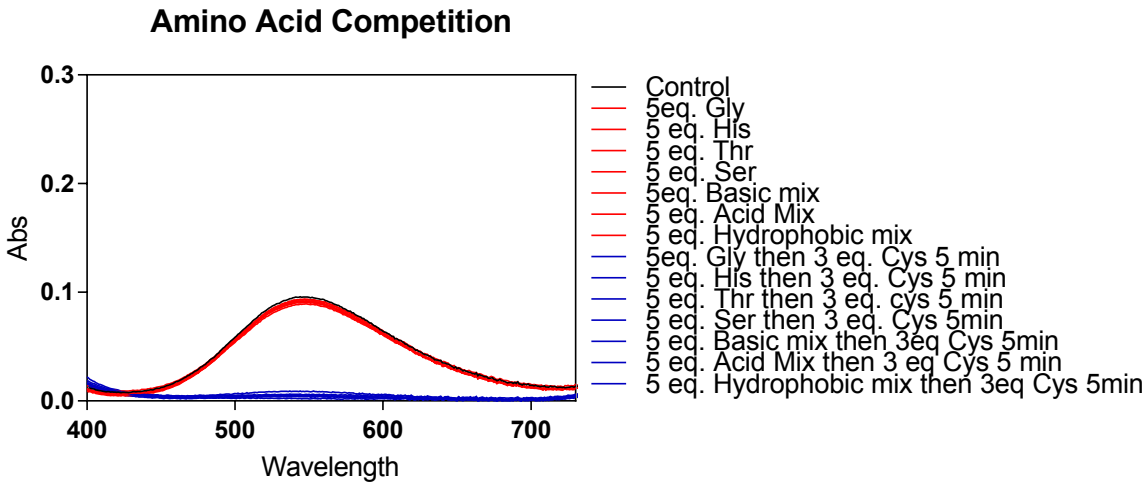


Figure S8. UV-Vis of complex **4** before and after amino acid addition. In red, 5 eq. of various amino acids (Glycine, histidine, threonine, serine, glutamate, aspartate, glutamine, asparagine, leucine, isoleucine, alanine, valine, and tryptophan). In blue, 5 eq. of various amino acids were added then 3 eq. of cysteine was added and incubated for 5 minutes. Basic mix contained asparagine and glutamine. Acid mix contained glutamate and aspartate. Hydrophobic mix contained leucine, isoleucine, alanine, valine, and tryptophan. **4** was dissolved in HEPES buffer to a 0.5mM concentration.

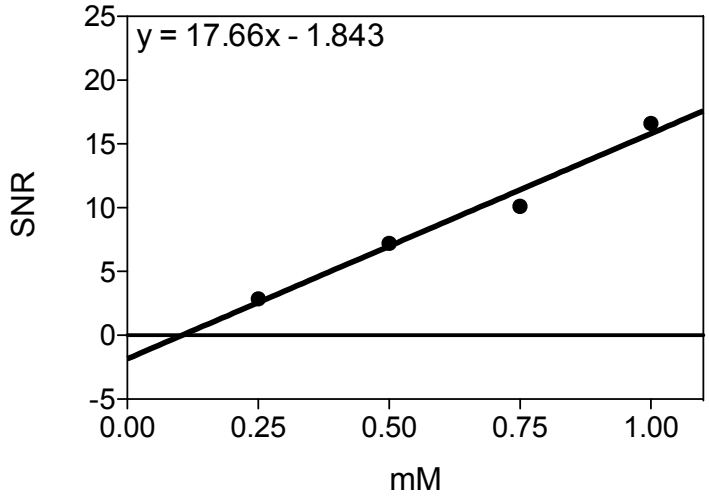


Figure S9. ^{19}F MRI Limit of detection graph of complex **4** treated with 3 equivalents of cysteine using four different concentrations (0.25 mM, 0.50 mM, 0.75 mM and 1.00 mM). Scanning parameters (RARE sequence): echo time: 14.99 ms, repetition time: 1200 ms, number of acquisition: 256, rare factor: 16, matrix size: 64 x 64, field of view: 40 x 40, slice thickness: 50 mm.

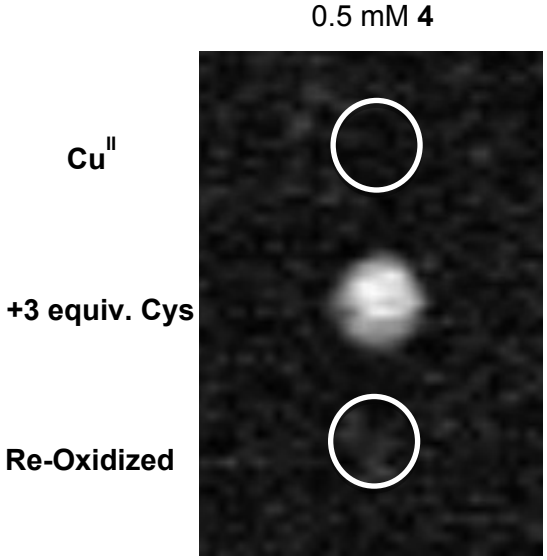


Figure S10. ^{19}F MR phantom images at 24 ºC on a 7.0 T MRI scanner. Complex **4** only in PBS buffer (pH 7.4) Top: 0.5 mM **4** ; Middle: 0.5 mM **4** with 3 equiv. of cysteine (1.5 mM of Cysteine) Bottom: 0.5 mM **4** in presence of 1.5 mM cysteine, re-oxidized for 1 hour. Scanning parameters (RARE sequence): echo time: 44.98 ms, repetition time: 1200 ms, number of acquisition: 1024, rare factor: 32, matrix size: 64 x 64, field of view: 40 x 40, slice thickness: 50 mm.

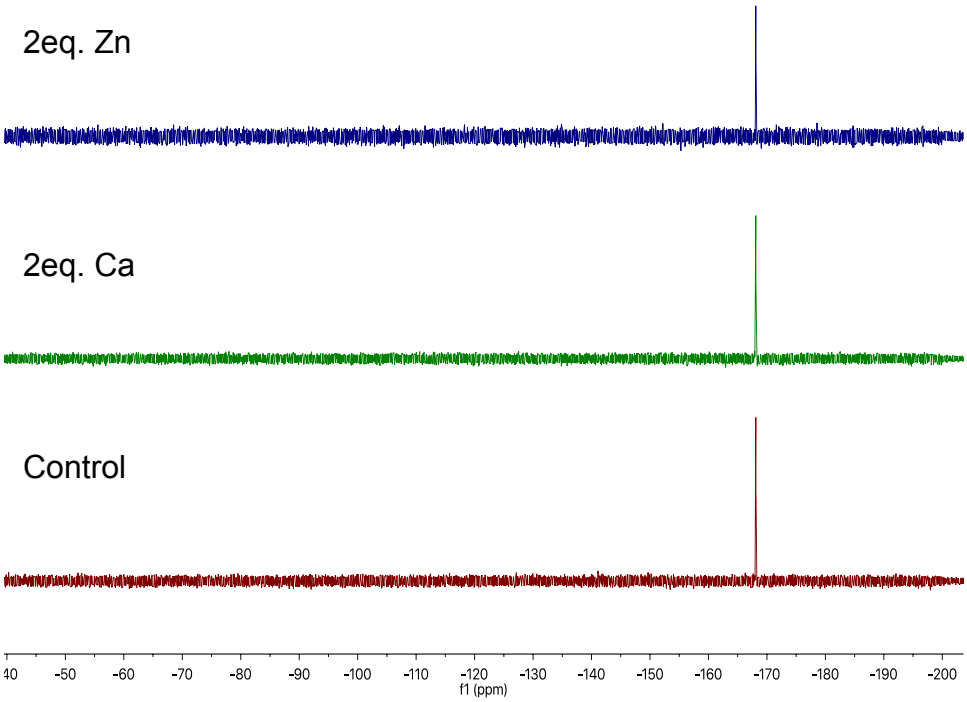


Figure S11: ^{19}F NMR of 1 mM of **4** in HEPES Buffer with 2 equivalents of $ZnCl_2$ (top), 2 equivalents of $CaCl_2$ (middle) and with no treatment (bottom)**.** 1 mM of 5-Fluorocytosine was added as reference (at -168 ppm).

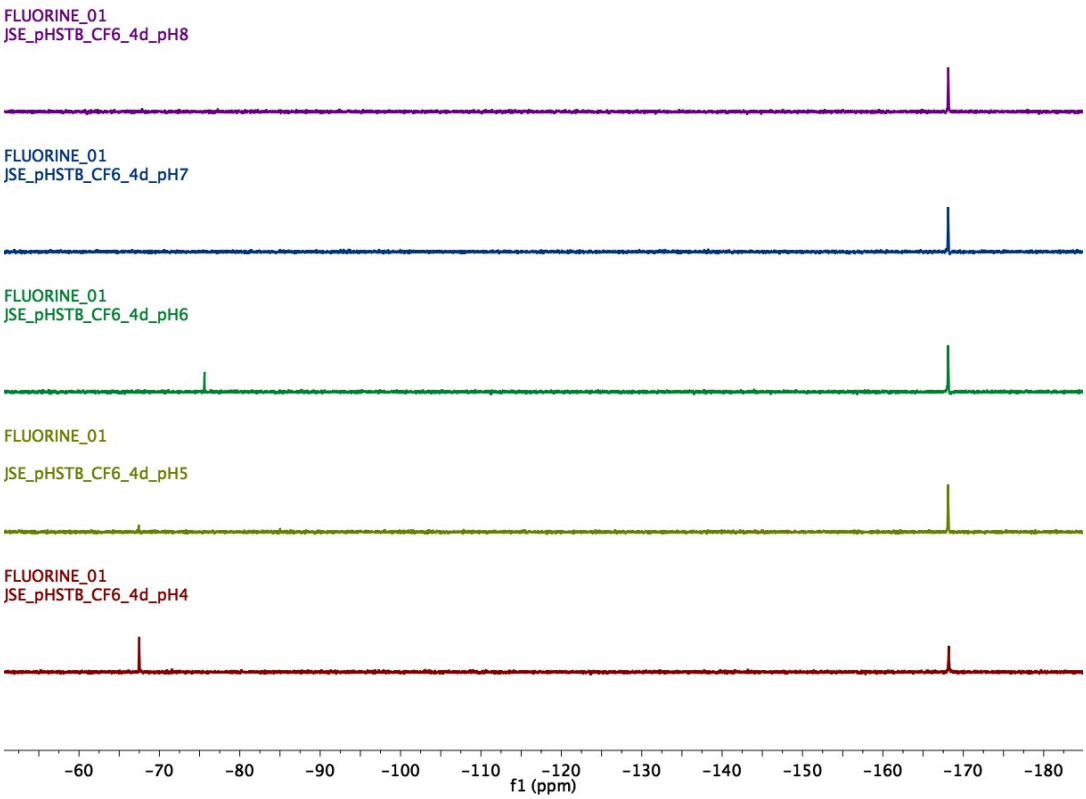


Figure S12: ^{19}F NMR of 1 mM of **4** in different pH buffer solutions**,** pH ranges from 4-8 (bottom to top). HEPES buffer used for pH 7 and 8, MES buffer used for pH of 6 and sodium acetate buffer used for pH 4 and 5. 1 mM of 5-Fluorocytosine was added as reference (at -168 ppm). In pH of 6 there is slight TFA contamination (at -75 ppm).

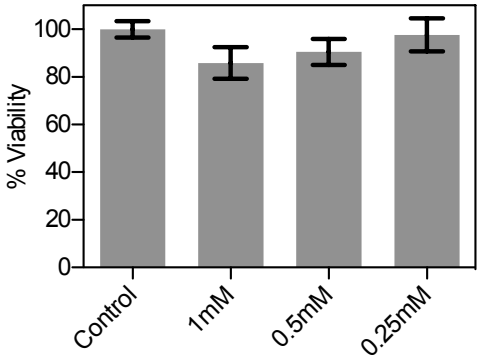
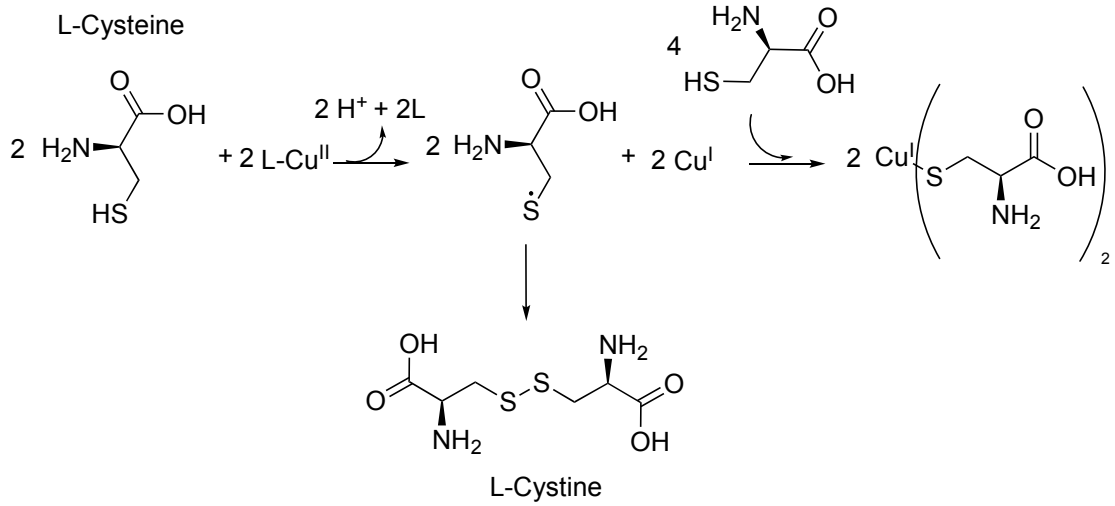
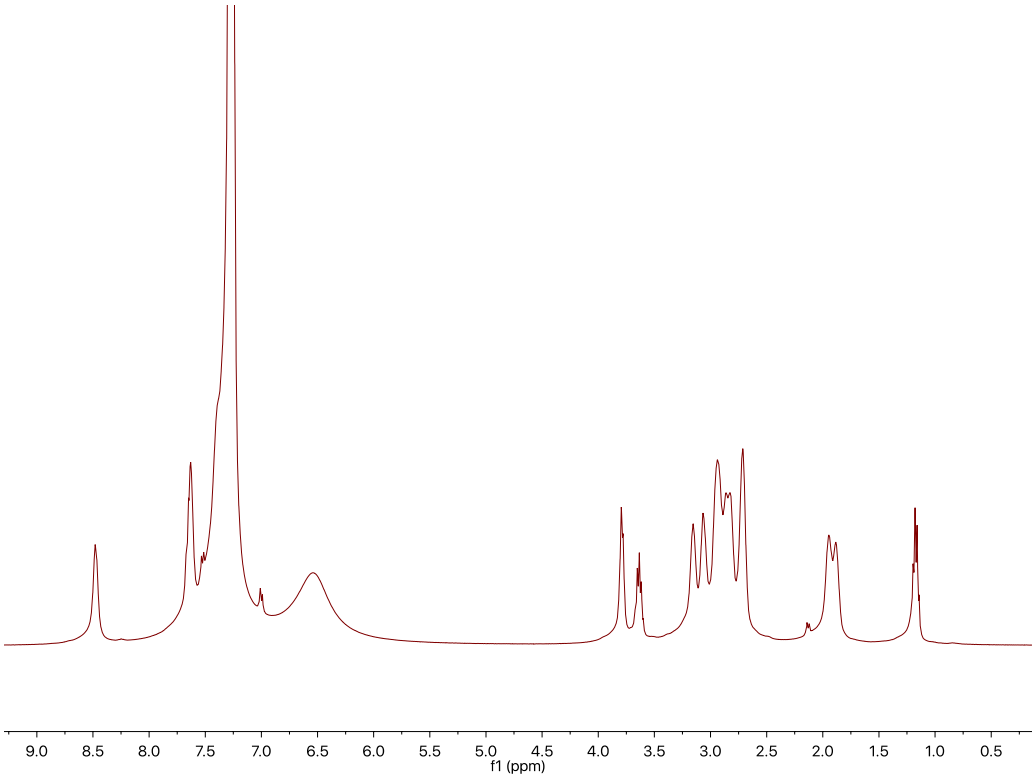


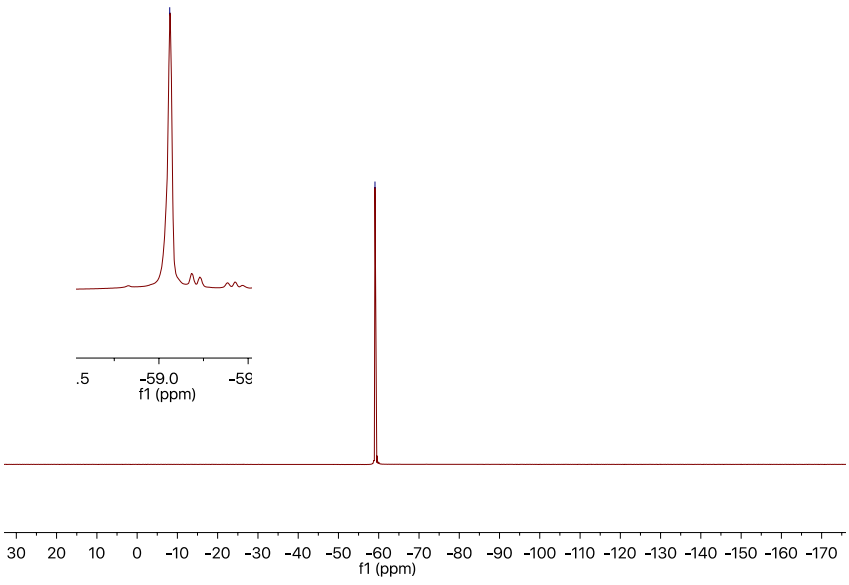
Figure S13: Cell viability as determined with MTT following incubation of cells with different concentrations of **4.**



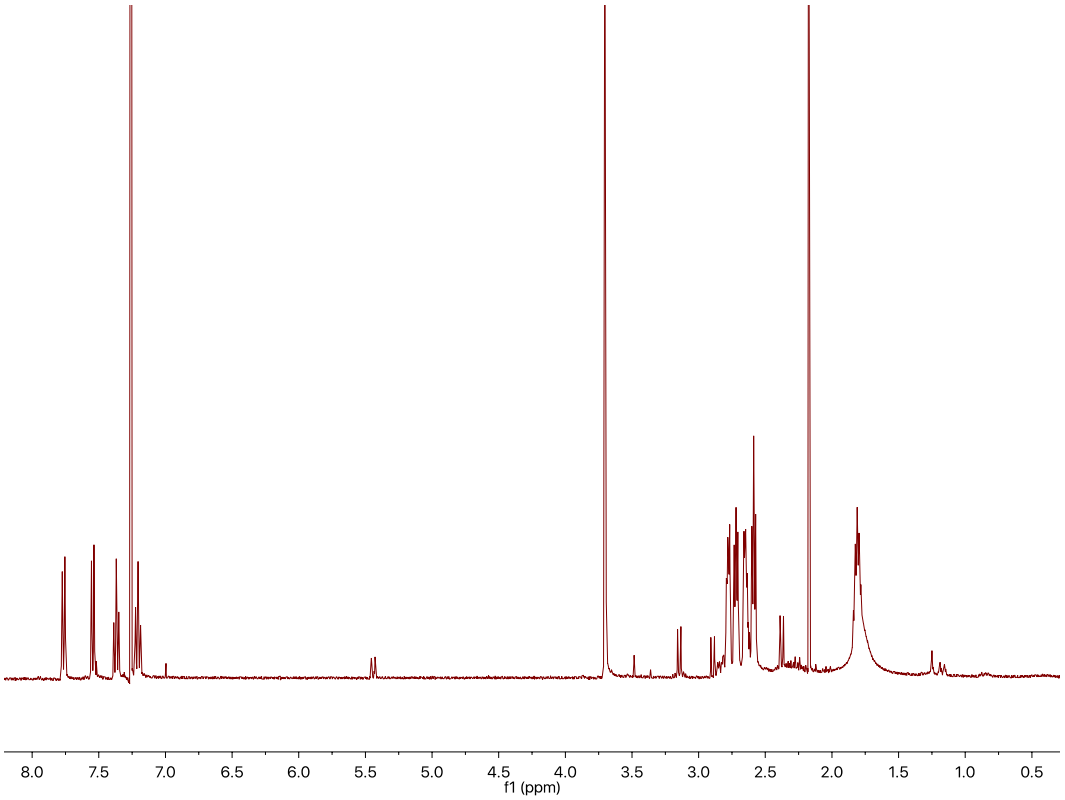
Scheme S7: Proposed mechanism of Cu(II) reduction by cysteine.



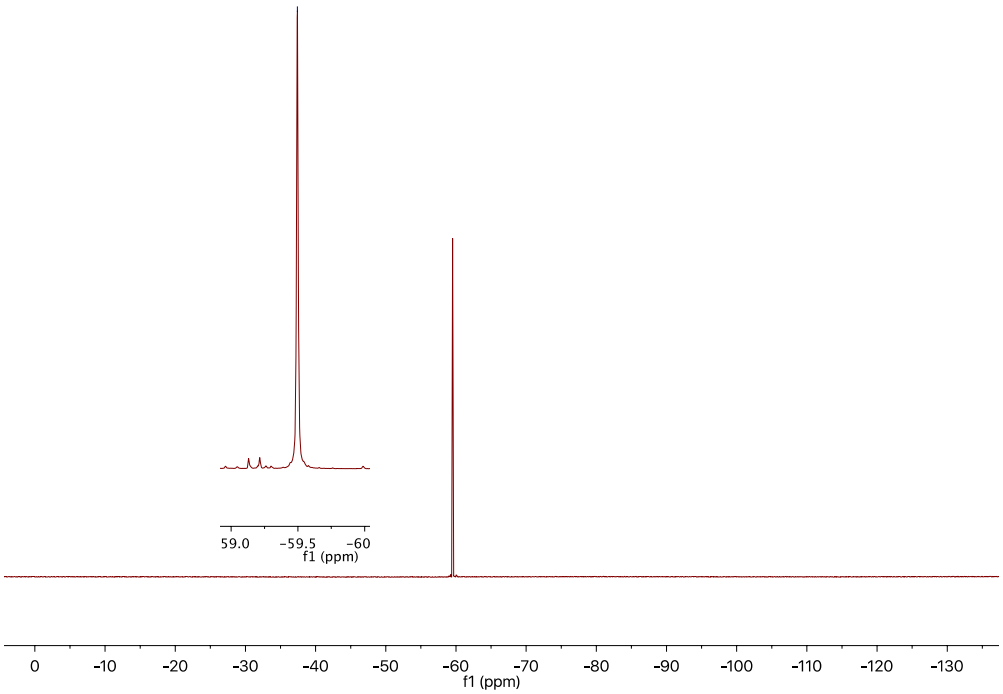
^{1}H NMR spectrum of **L1**



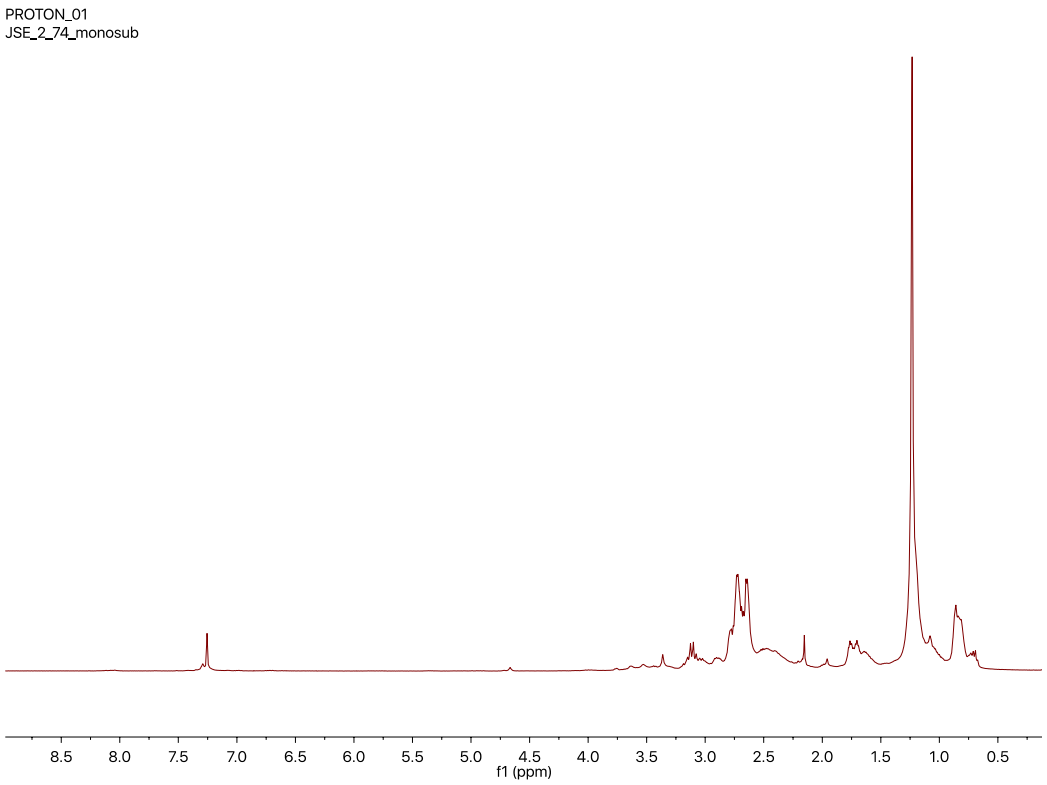
^{19}F NMR spectrum of **L1**



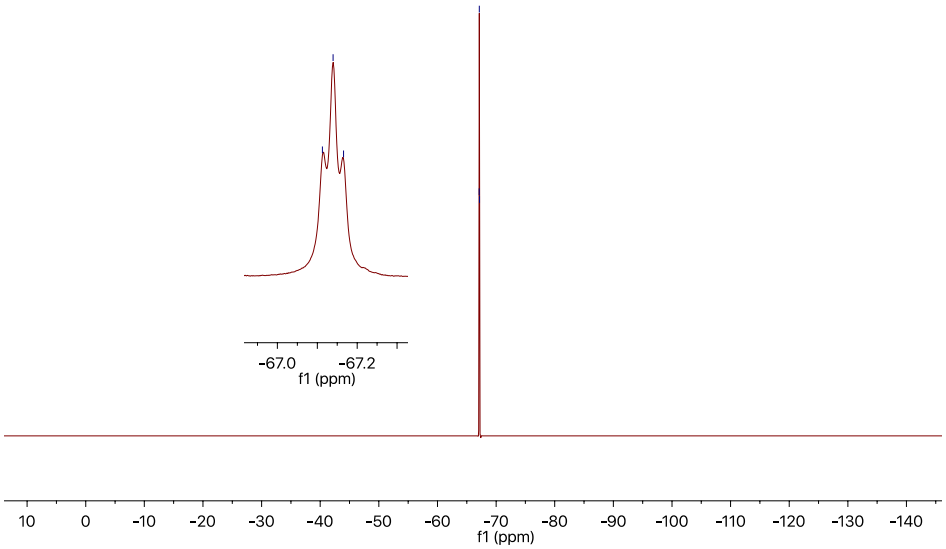
^{1}H NMR spectrum of **L2**



^{19}F NMR spectrum of **L2**



^{1}H NMR spectrum of **L3**



^{19}F NMR spectrum of **L3**

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