Supporting Information for

Evaluation of Dicopper Azacryptand Complexes in Aqueous CuAAC Reactions and Their Tolerance Toward Biological Thiols

Thi V. Tran, Garret Couture, Loi H. Do*

Department of Chemistry, University of Houston, Houston, Texas, 77204

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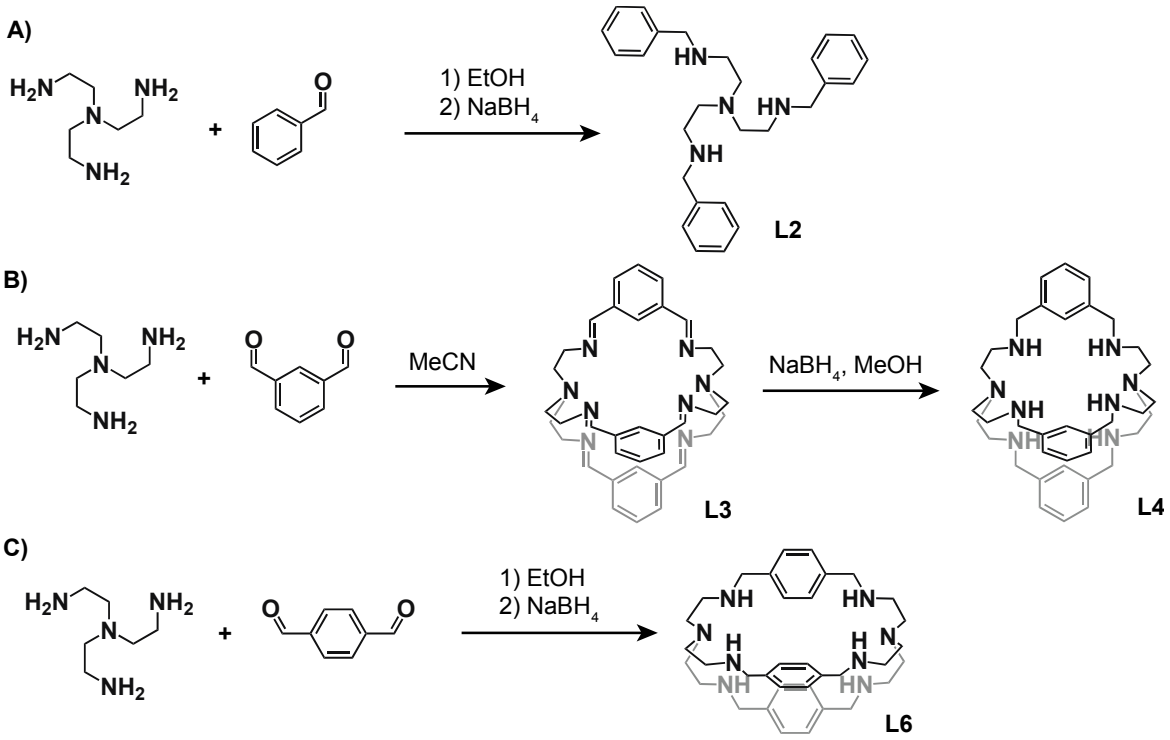
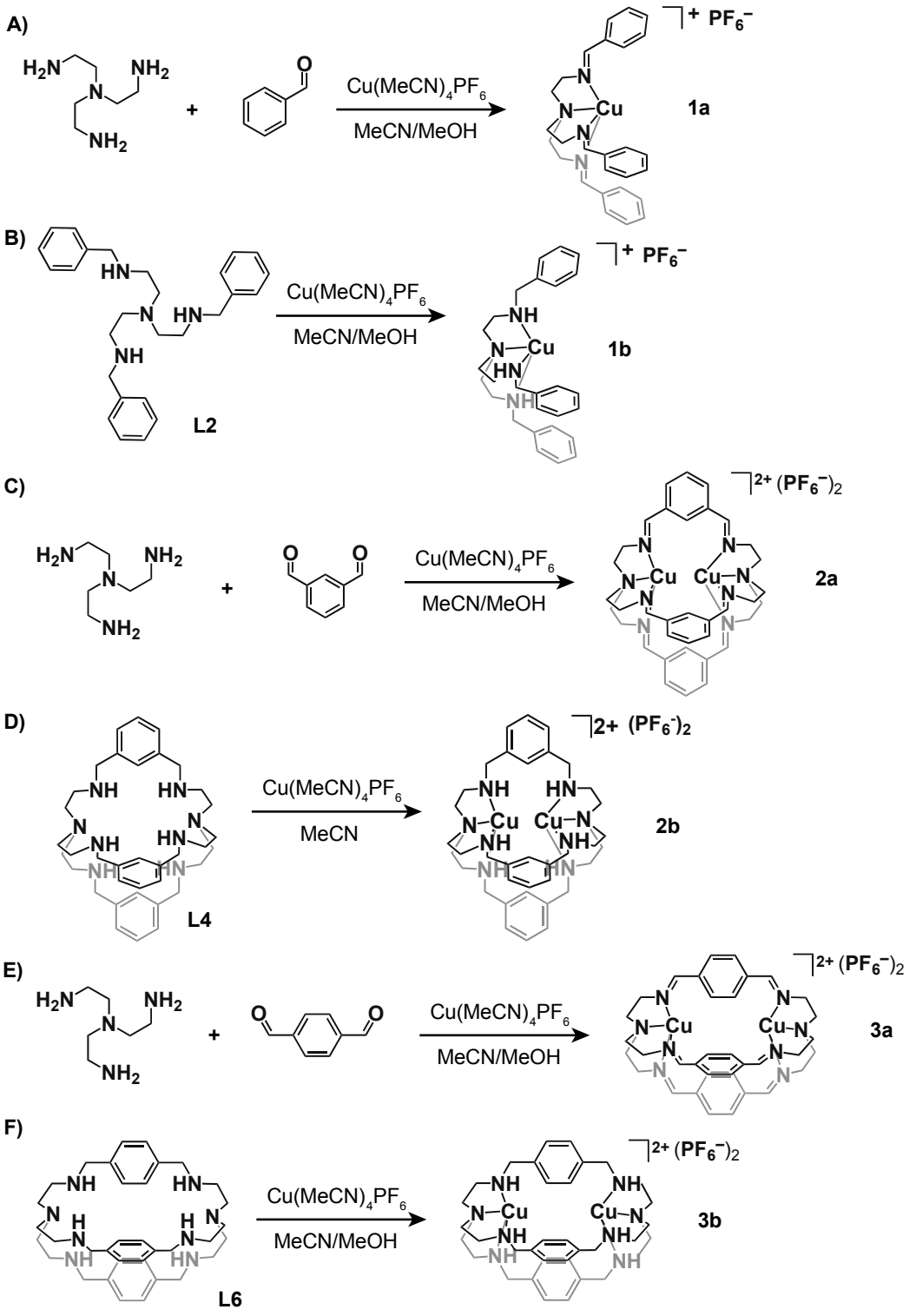
Experimental

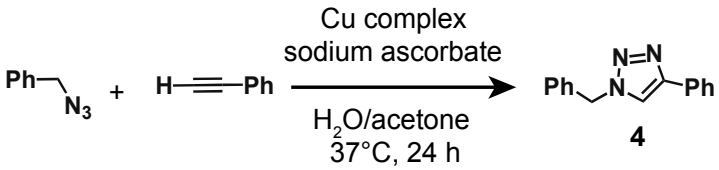


Scheme S1. Procedures for the synthesis of ligands **L2**, **L3**, **L4**, and **L6**.



Scheme S2. Procedures for the synthesis of copper complexes **1-3**.

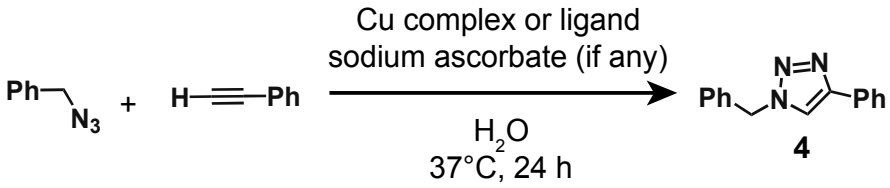
Table S1. Comparison of Cu Catalysts in Water/Acetone[a]



Entry	Cu Complex	GC Yield (%)[b]
1	**1a**	43
2	**1b**	6
3	**2a**	20
4	**2b**	95
5	**3a**	trace
6	**3b**	trace
7	$CuSO_4$	73

[a]Reaction conditions: benzyl azide (1.00 mmol), phenylacetylene (1.37 mmol), Cu (0.10 mmol, 10 mM), sodium ascorbate (1.50 mmol) in H_2O/acetone (3:2, 10 mL) at 37°C for 24 h. [b]Average yields from duplicate runs.

Table S2. Additional CuAAC Reactions[a]



Entry	Compound	Ascrobate	GC Yield (%)
1	**1a**	No	22
2	**1b**	No	99
3	**2a**	No	82
4	**2b**	No	99
5	**3a**	No	34
6	**3b**	No	44
7	**CuTBTA**	No	99
8	**L3** ligand	Yes	0
9	**TBTA** ligand	Yes	0

[a]Reaction conditions: benzyl azide (1.00 mmol), phenylacetylene (1.37 mmol), Cu (0.5 μmol) or ligand alone (0.5 μmol), sodium ascorbate (0.15 mmol, if any) in H_2O (10 mL) at 37°C for 24 h.

Synthesis and Characterization

Preparation of L2. This synthesis was modified from a literature procedure.[1] Benzaldehyde (3.0 g, 28.3 mmol) wad added to a mixture of tris(2-aminoethyl)amine (0.73 g, 5.0 mmol) in 100 mL of ethanol. The mixture was stirred under reflux overnight. It was then cooled to RT, treated with solid $NaBH_4$ (1.0 g, 26.4 mmol), and then continued to reflux for another 24 h. The volatiles were removed by rotary evaporation and the residues were dissolved in CH_2Cl_2 (100 mL). An aqueous solution of $NaHCO_3$ (10%, 50 mL) was added and the mixture was shaken. The organic layer was separated, washed with H_2O (100 mL), dried over Na_2SO_4, filtered, and then evaporated to dryness. The crude product was purified by column chromatography using basic alumina (Act. I, 50-200 mess) and eluted with CH_2Cl_2/MeOH/NEt_3 (95.5/4.0/0.5) to afford a light yellow oil (0.41 g, 1.1 mmol, 22%). The NMR spectra of the product matches those reported previously.[1] ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 7.35-7.20 (m, 15H), 3.73 (s, 6H), 2.66 (t, J_{HH} = 6Hz, 6H), 2.56 (t, J_{HH} = 6Hz, 6H).

Preparation of L3. This synthesis was modified from a literature procedure.[2,3] To a stirred solution of tris(2-aminoethyl)amine (20 mmol, 2.92 g) in MeCN (250 mL) was added dropwise a mixture of isophthalaldehyde (30 mmol, 4.03 g) in MeCN (150 ml) over a period of 1 h at RT. After stirring for additional 24 h, a large amount of a white precipitate had formed. The solid was isolated by filtration and then washed with Et_2O to afford the desired product (5.34 g, 9.1 mmol, 91%). The NMR spectra of the product matches those reported previously.[2,3] ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 8.18 (dd, J_{HH} = 7.7, 1.6 Hz, 6H), 7.57 (s, 6H), 7.52 (t, JHH = 8 Hz, 3H), 5.31 (m, 3H), 3.77-2.69 (m, 24H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 160.81, 136.94, 132.46, 129.10, 127.46, 60.09, 56.07.

Preparation of L4. This synthesis was modified from a literature procedure.[3] Solid $NaBH_4$ (1 g, 26.4 mmol) was added slowly portion-wise over 15 min to a stirred solution of **L3** (1.1 g, 1.88 mmol) in methanol (100 mL) at RT. The mixture was refluxed under nitrogen overnight. The solution was then cooled to RT and the solvent was removed by rotary evaporation. The residue was dissolved in CH_2Cl_2 (100 mL) and combined with aqueous $NaHCO_3$ (10%, 50 mL). The product was extracted into CH_2Cl_2, washed with H_2O (50 mL), and then dried over Na_2SO_4. Removal of solvent afforded a white sticky solid (0.51 g, 0.85 mmol, 45%). The NMR spectra of the product matches those reported previously.[3] ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) = 7.21-7.13 (m, 9H), 7.07 (s, 3H), 3.60 (m, 12H), 2.62-2.60 (m, 12H), 2.57-2.55 (m, 12H).

Preparation of L6. This synthesis was modified from a literature procedure.[4] Terephthaldehyde (2.01 g, 15.0 mmol) was dissolved in 150 mL of ethanol in a 500 mL three-neck round bottom flask. The flask was equipped with a reflux condenser on one neck and an addition funnel on another. The solution was heated to 78°C while tris(2-aminoethyl)amine (1.46 g, 10.0 mmol) in 50 mL of ethanol was added slowly dropwise using the addition funnel. The reaction mixture was then refluxed overnight. The next day, the solution was filtered to remove insoluble materials and the filtrate was transferred to another round bottom flask. The mixture was then treated with solid $NaBH_4$ (2 g, 10.5 equiv.). The solution was refluxed overnight and cooled to RT. The volatiles were removed by rotary evaporation and the residue was dissolved in CH_2Cl_2 (200 mL). The organic layer was combined with aqueous $NaHCO_3$ (10%, 100 mL) and shaken. The organic layer was separated, washed with H_2O (100 mL), dried with Na_2SO_4, removed solvent and recrystallized with toluene/hexane to afford solid white product (0.42 g, 0.7 mmol, 14%). NMR spectroscopic characterization of the product matches that measured previously.[4] ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 6.87 (s, 12H), 3.68 (s, 12H), 2.83-2.81 (m, 12H), 2.67-2.65 (m, 12H).

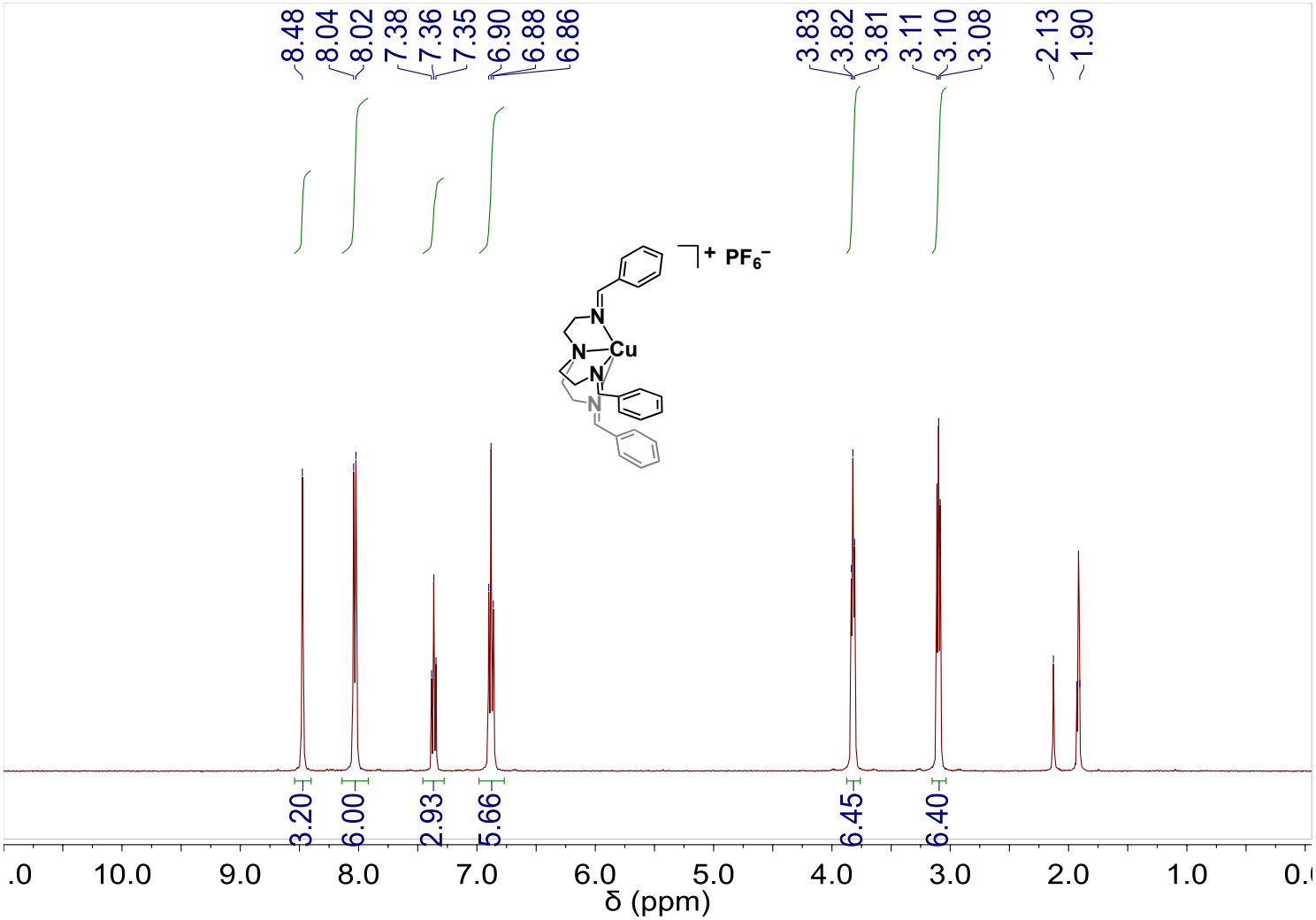


Figure S1. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of complex **1a**.

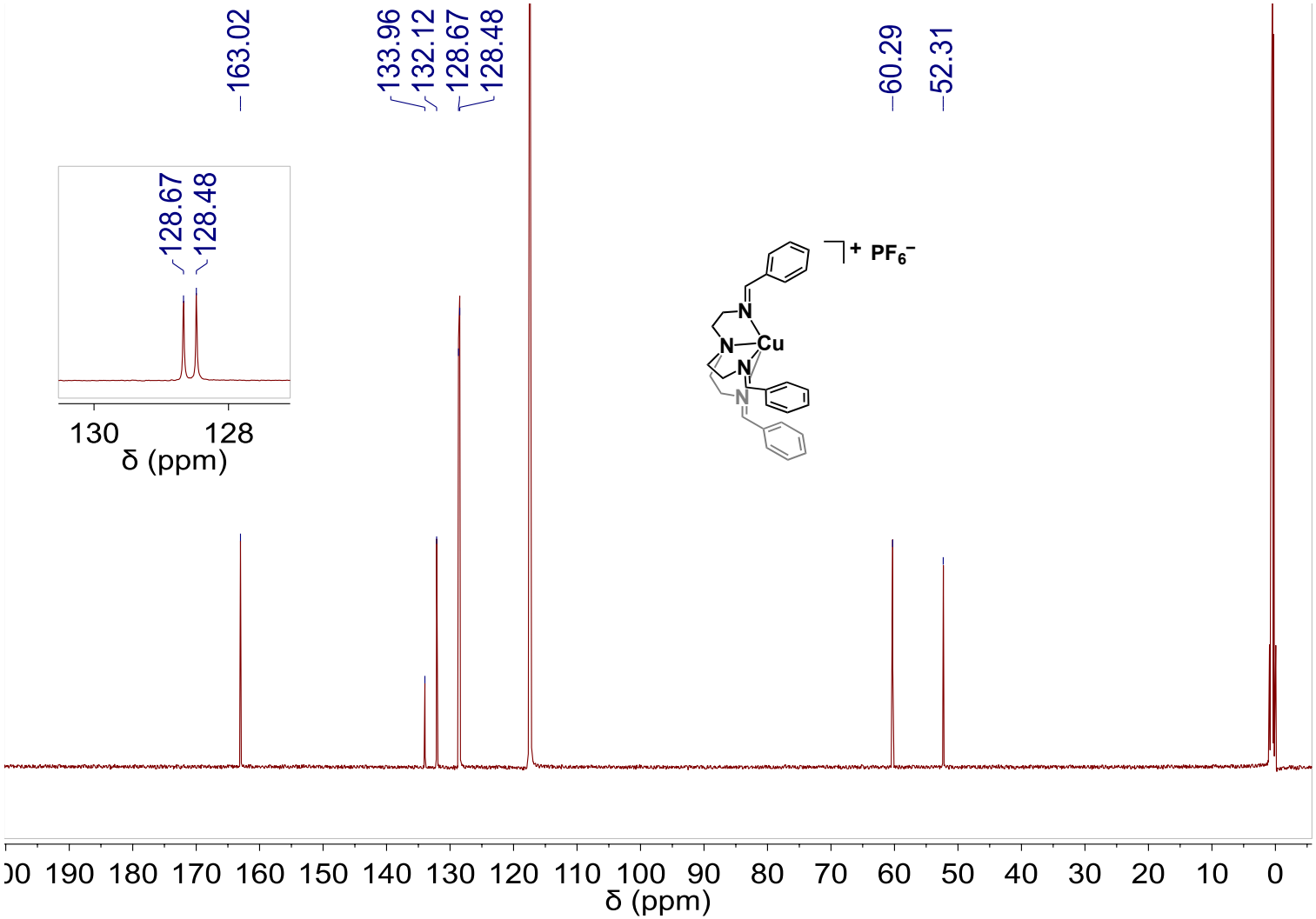


Figure S2. ^{13}C NMR spectrum (CD_3CN, 126 MHz) of complex **1a**.

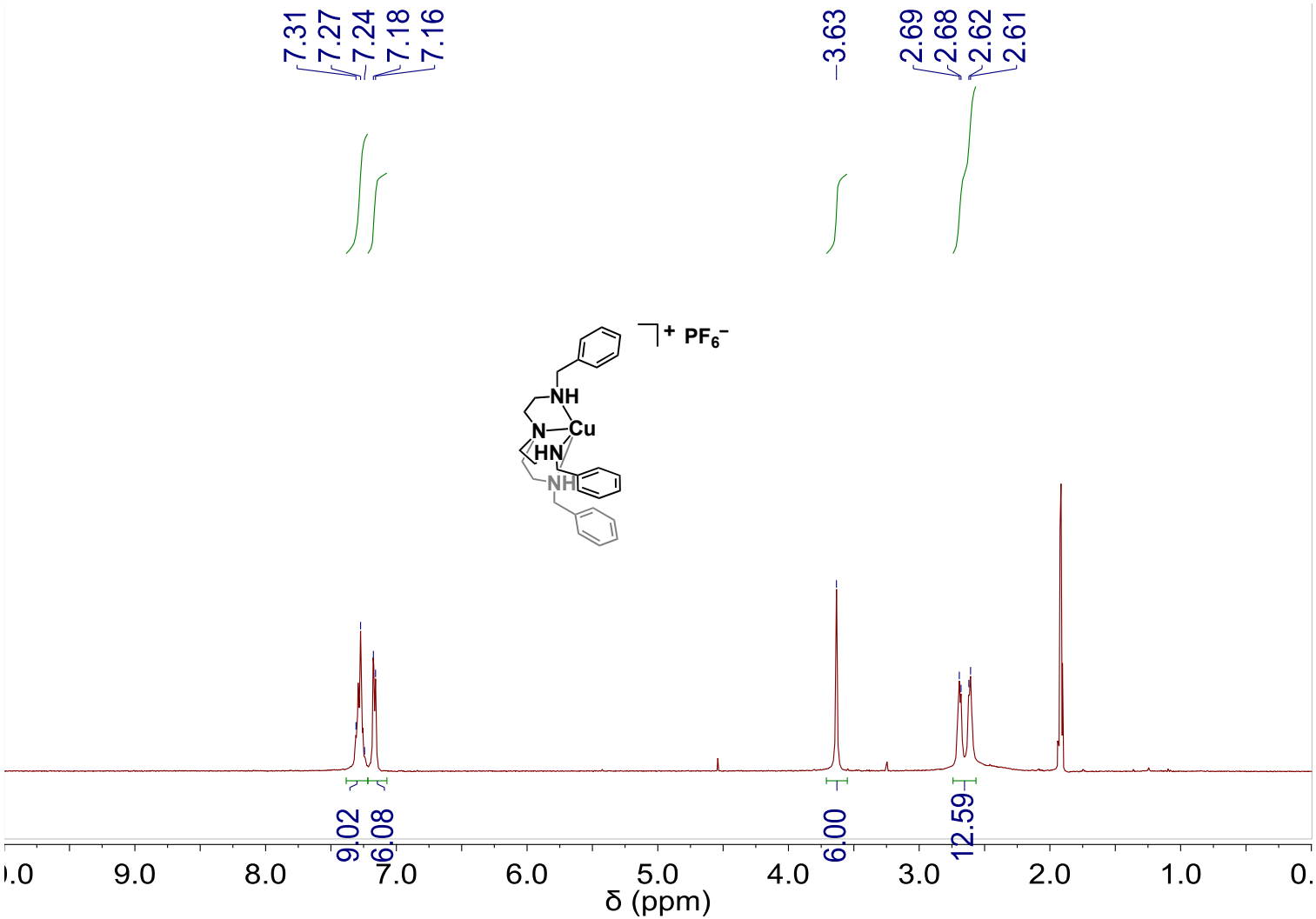


Figure S3. ^{1}H NMR spectrum (CD_3CN, 400 MHz) of complex **1b**.

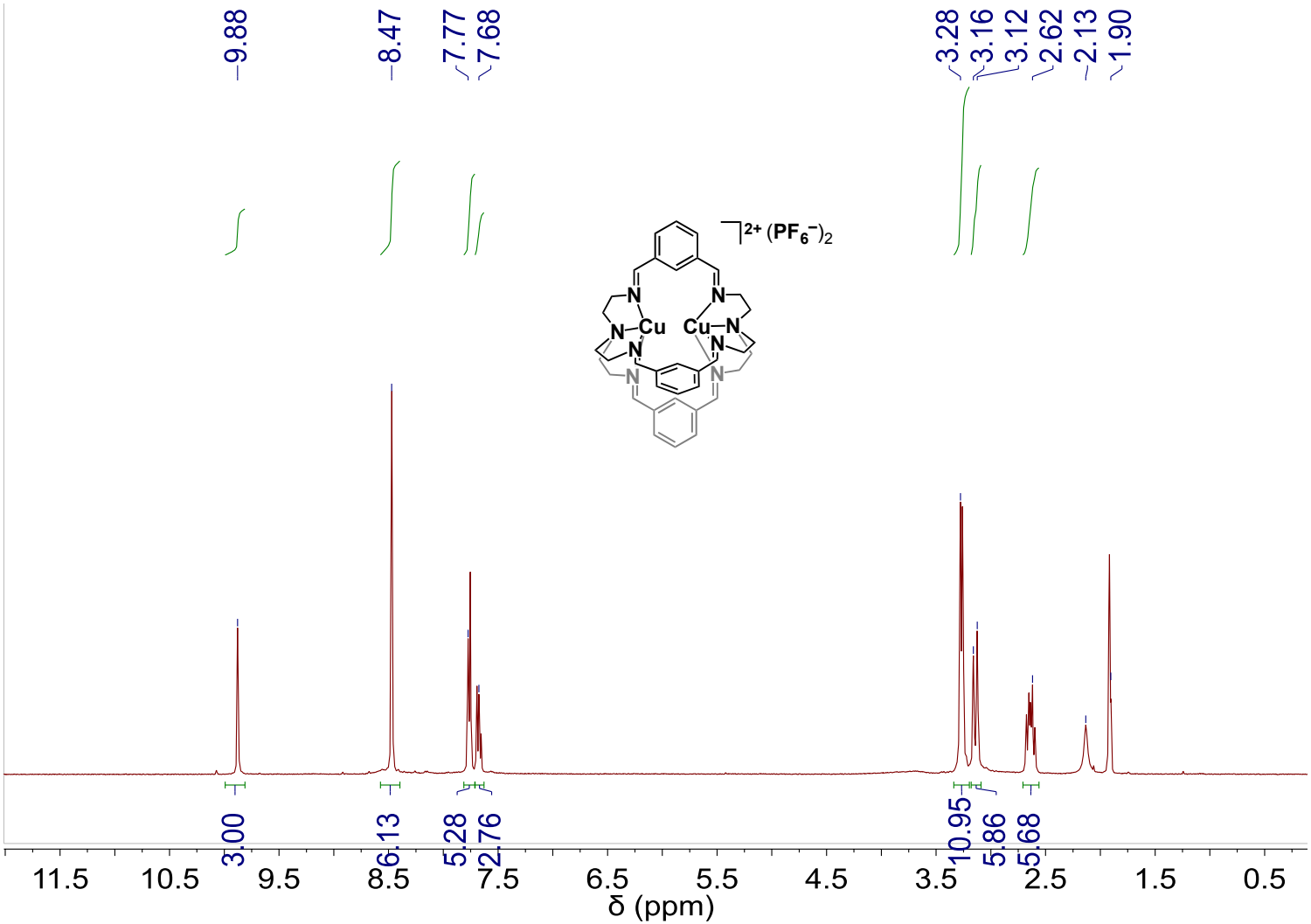


Figure S4. ^{1}H NMR spectrum (CD_3CN, 400 MHz) of complex **2a**.

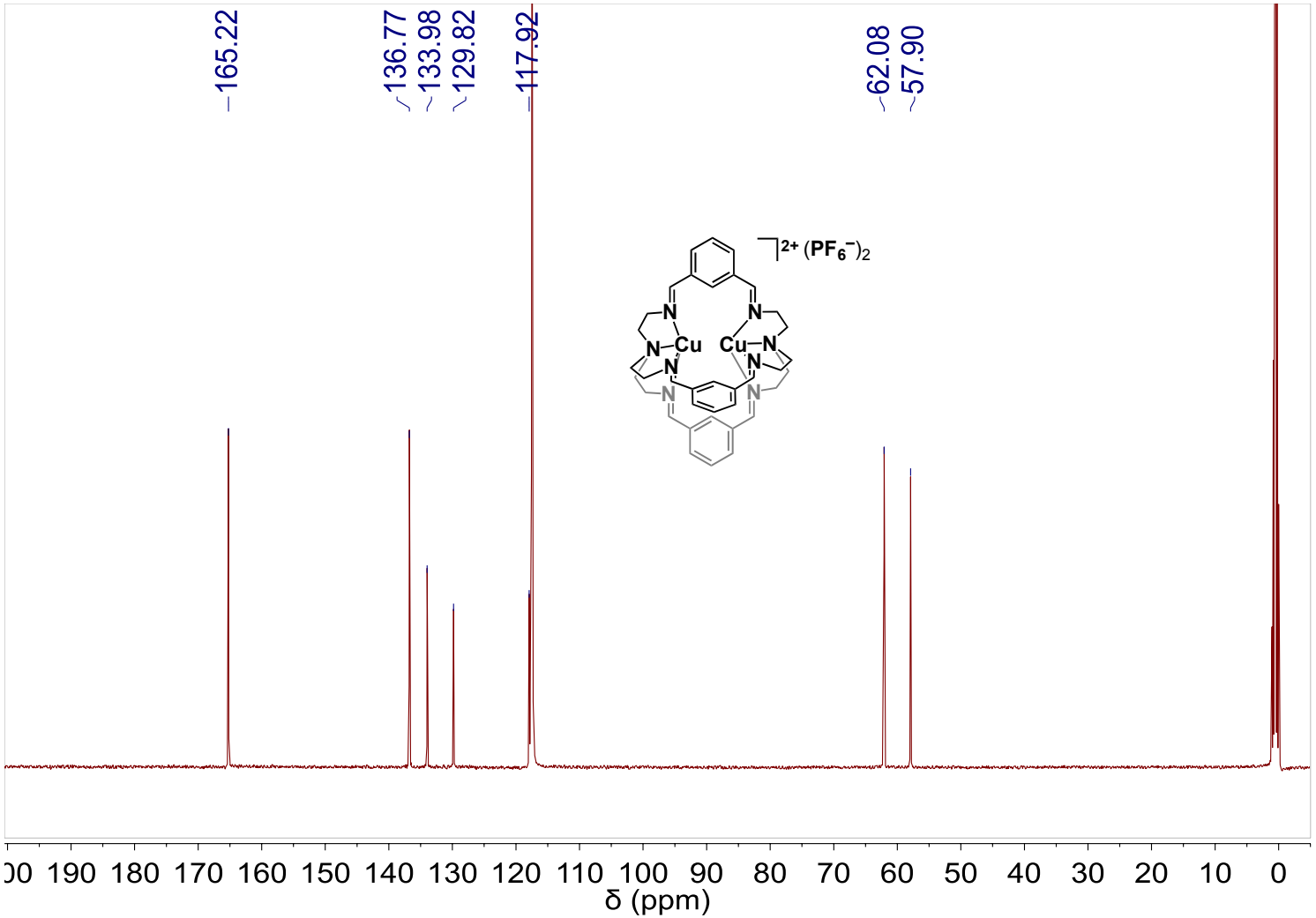


Figure S5. ^{13}C NMR spectrum (CD_3CN, 100 MHz) of complex **2a**.

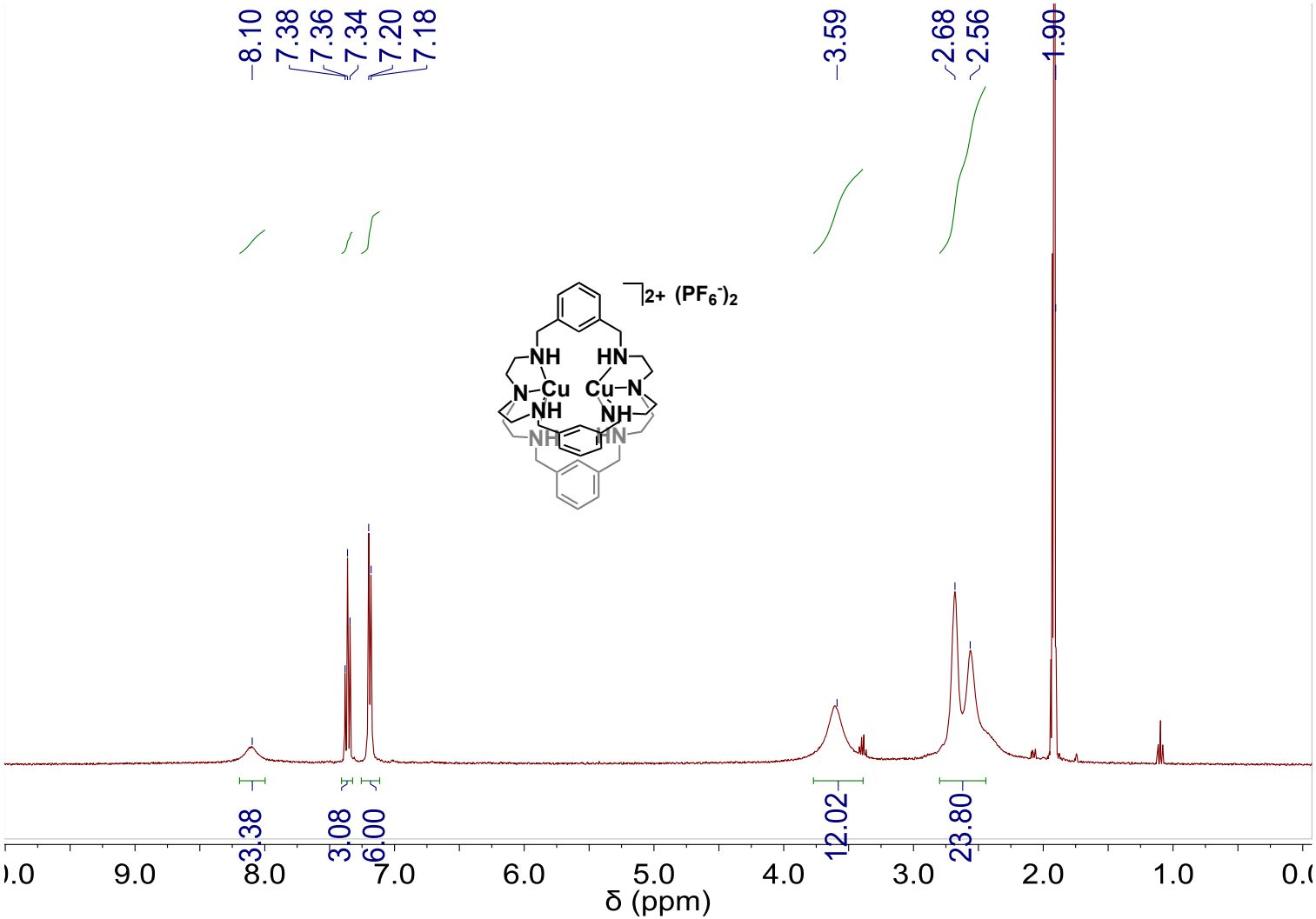


Figure S6. ^{1}H NMR spectrum (CD_3CN, 400 MHz) of complex **2b**. The broad peaks suggest that the complex undergoes dynamic structural changes in solution.

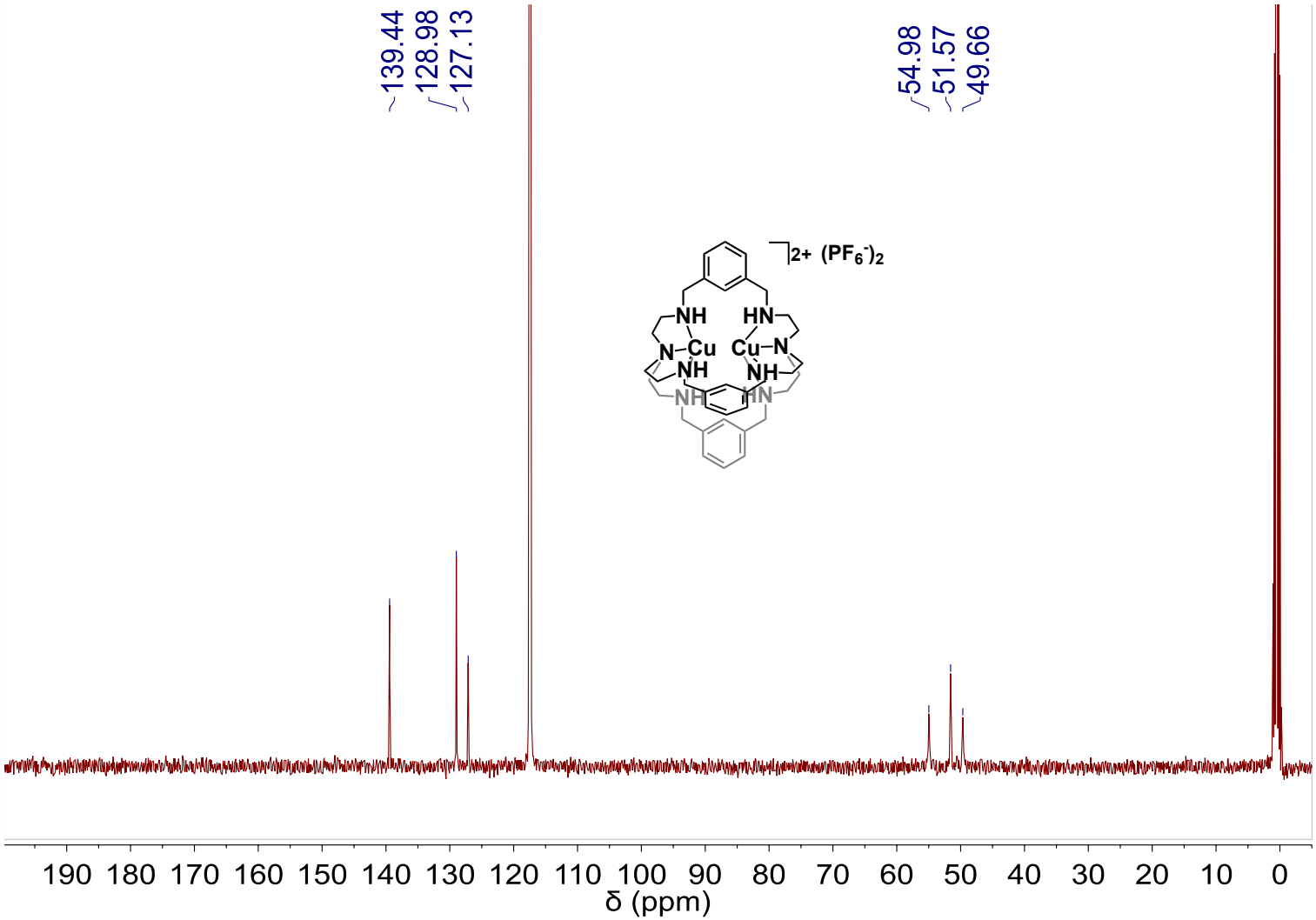


Figure S7. ^{13}C NMR spectrum (CD_3CN, 100 MHz) of complex **2b**.

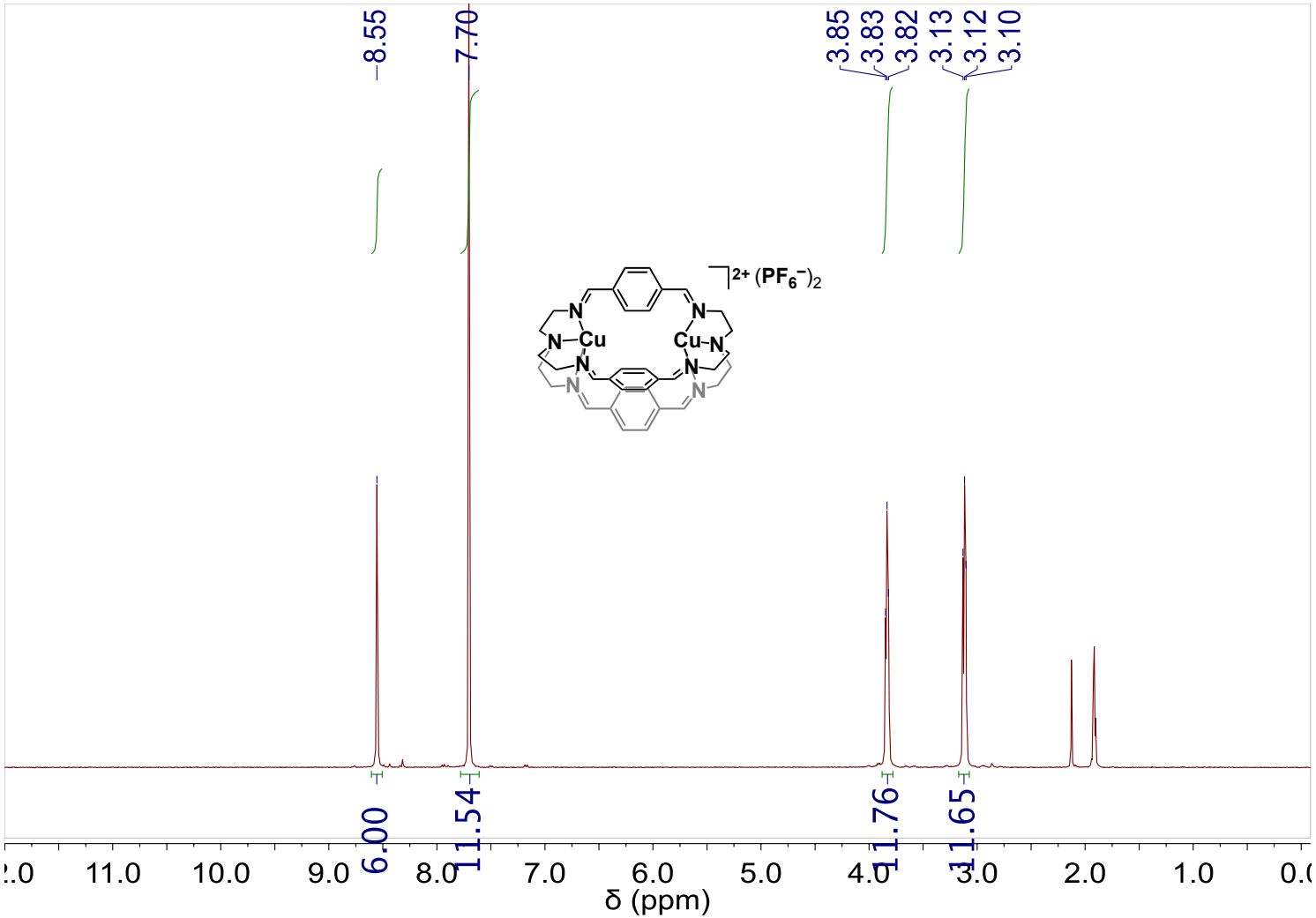


Figure S8. ^{1}H NMR spectrum (CD_3CN, 400 MHz) of complex **3a**.

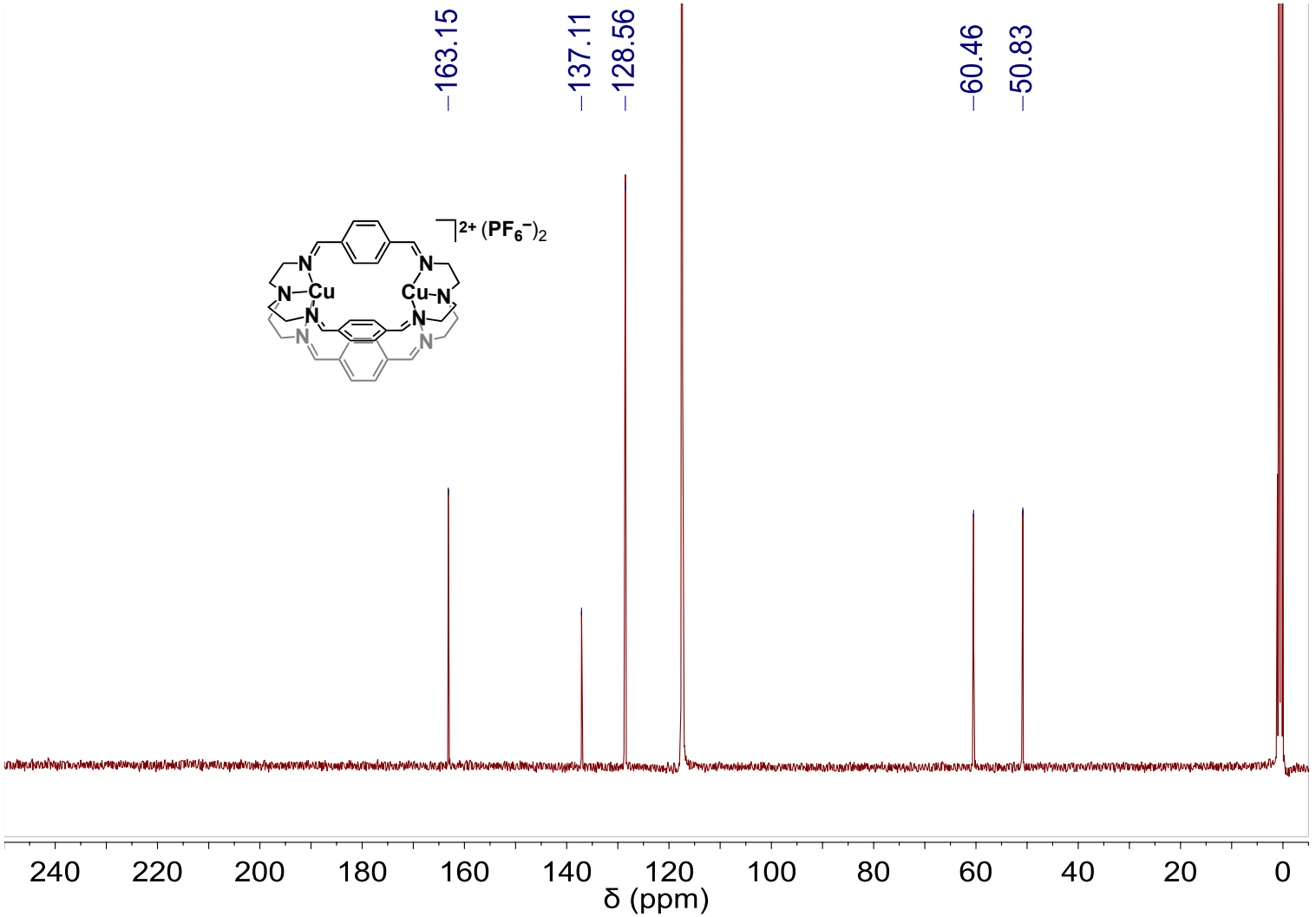


Figure S9. ^{13}C NMR spectrum (CD_3CN, 126 MHz) of complex **3a**.

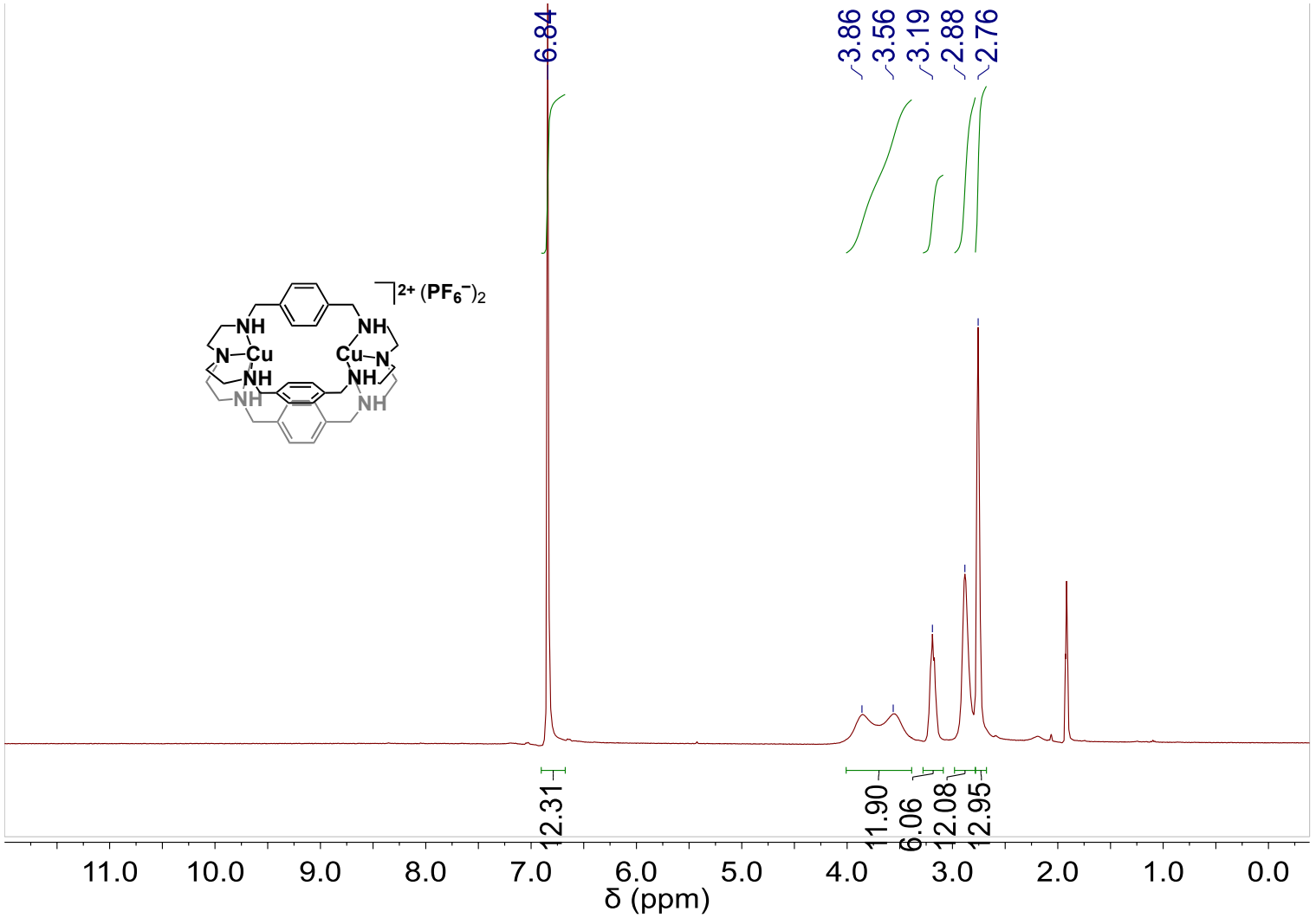


Figure S10. ^{1}H NMR spectrum (CD_3CN, 400 MHz) of complex **3b**. The broad peaks suggest that the complex undergoes dynamic structural changes in solution.

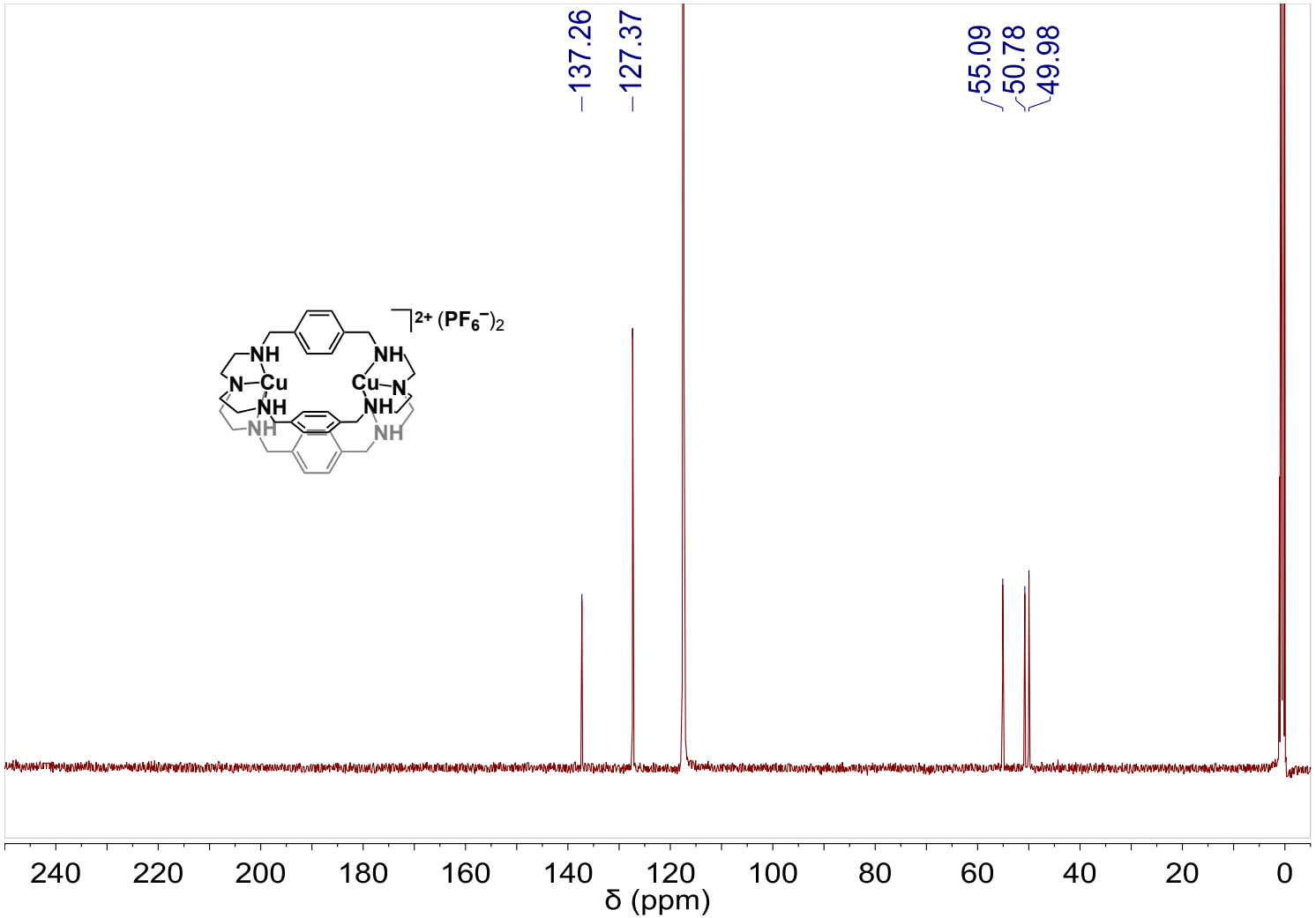


Figure S11. ^{13}C NMR spectrum (CD_3CN, 100 MHz) of complex **3b**.

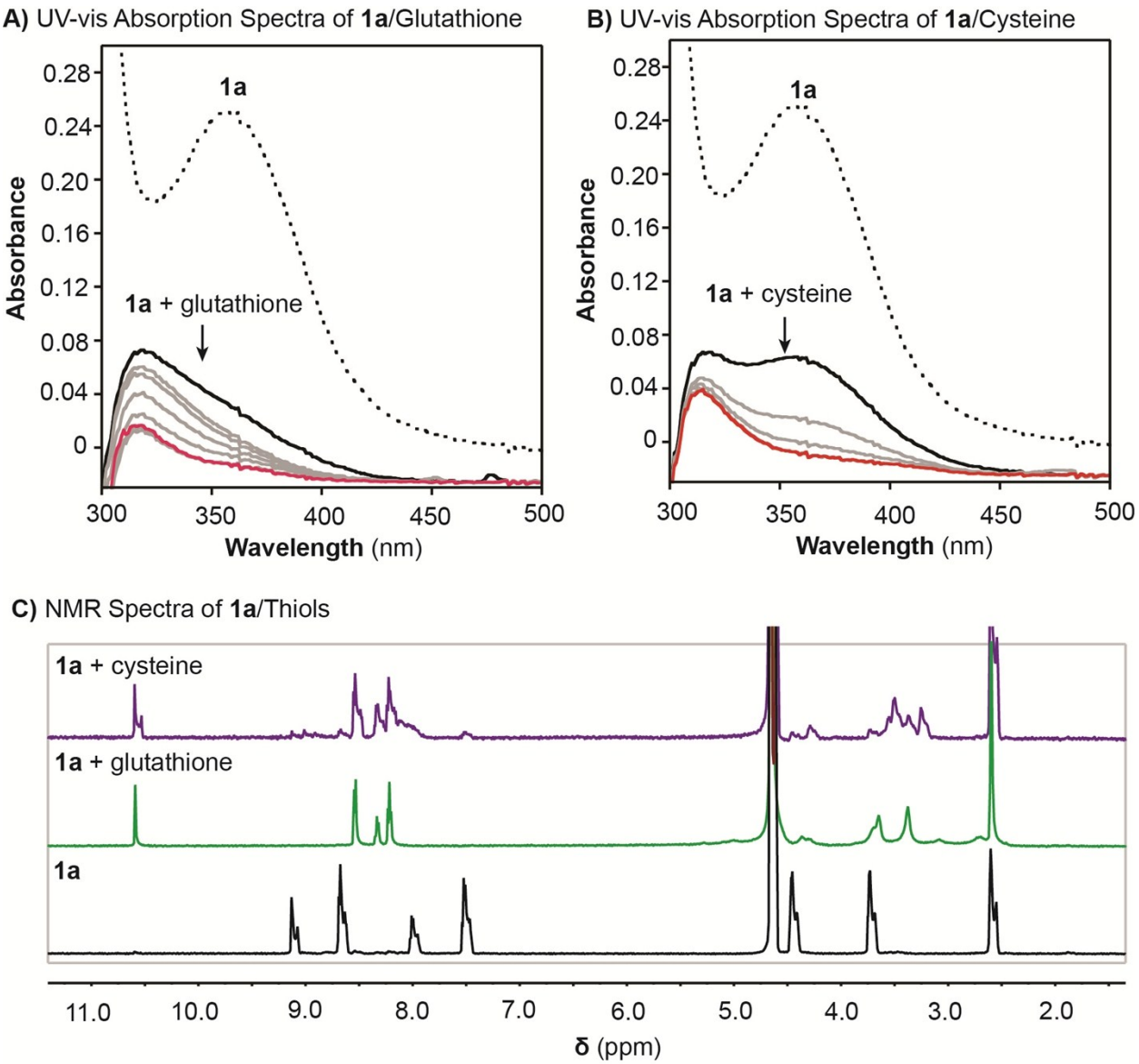


Figure S12. Reaction of complex **1a** (50 μM) with biological thiols (100 μM). Plots A and B show the UV-vis absorption spectra (H_2O) of **1a**/glutathione and **1a**/cysteine, respectively. The dotted trace shows the spectrum of **1a** without any additives. The solid black traces were obtained right after mixing **1a** with either glutathione or cysteine and the solid red traces were obtained after ~60-80 min. Plot C shows the NMR spectra (D_2O/CD_3CN (1:2), 600 MHz) of **1a**, **1a**/glutathione (1:2) and **1a**/cysteine (1:2) as indicated. The concentration of **1a** in the NMR sample was 6.7 mM.

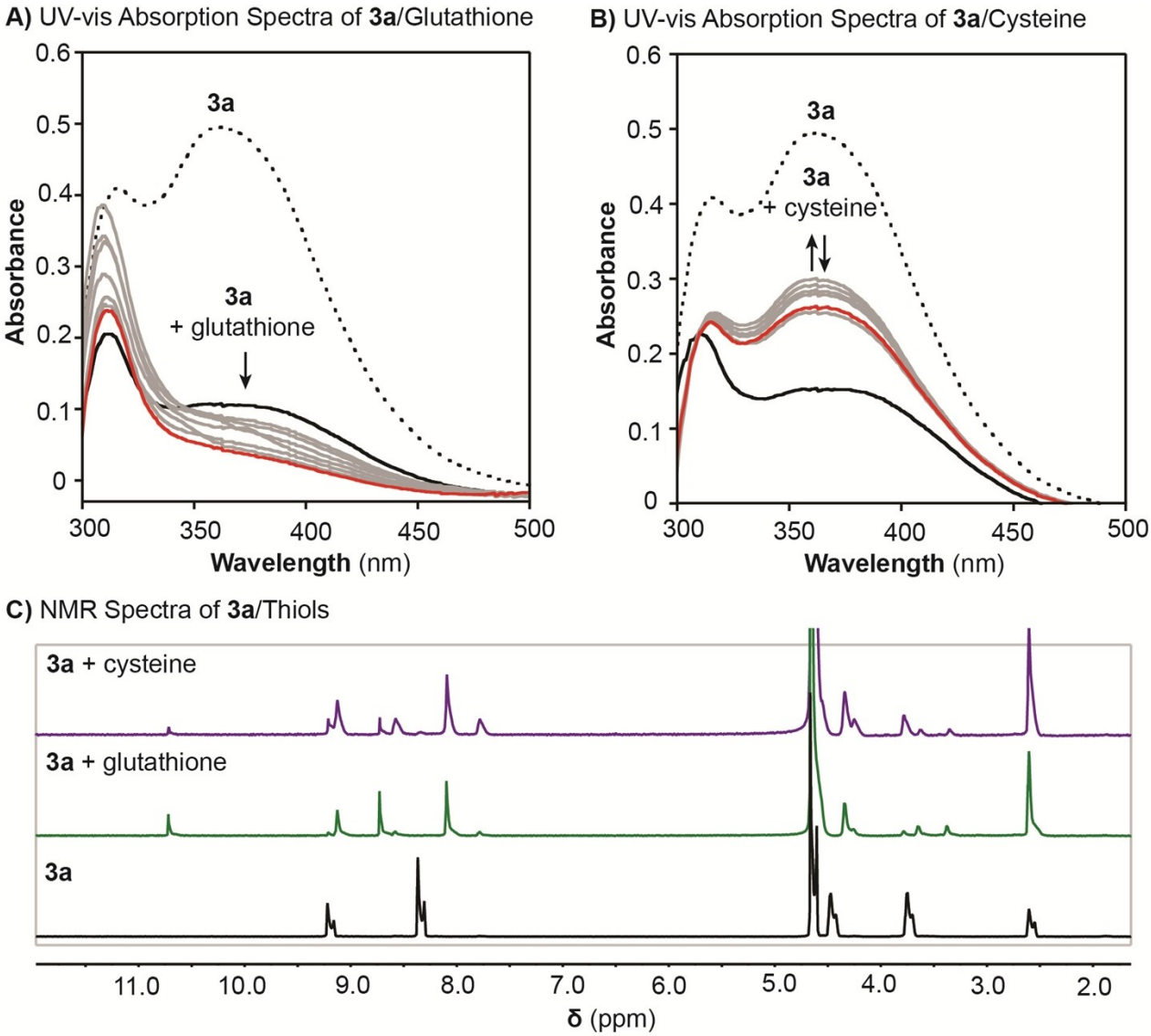


Figure S13. Reaction of complex **3a** (50 μM) with biological thiols (100 μM). Plots A and B show the UV-vis absorption spectra (H_2O) of **3a**/glutathione and **3a**/cysteine, respectively. The dotted trace shows the spectrum of **3a** without any additives. The solid black traces were obtained right after mixing **3a** with either glutathione or cysteine and the solid red traces were obtained after ~60-80 min. Plot C shows the NMR spectra (D_2O/CD_3CN (1:2), 600 MHz) of **3a**, **3a**/glutathione (1:2) and **3a**/cysteine (1:2) as indicated. The concentration of **3a** in the NMR sample was 6.7 mM.

References

(1) Charalambidis, G.; Ladomenou, K.; Boitrel, B.; Coutsolelos, A. G. *Eur. J. Org. Chem.* **2009**, *2009*, 1263-1268.
(2) MacDowell, D.; Nelson, J. *Tetrahedron Lett.* **1988**, *29*, 385-386.
(3) Möller, F.; Merz, K.; Herrmann, C.; Apfel, U. P. *Dalton Trans.* **2016**, *45*, 904-907.
(4) Whiteford, J. A. Bridged Polycyclic Compound Based Compositions for the Inibition and Amelioration of Disease. US 2008/0275141 A1, Nov. 6, 2008.