

Supplementary Information

Toward the Development of Prochelators as Fluorescent Probes of Copper-Mediated Oxidative Stress

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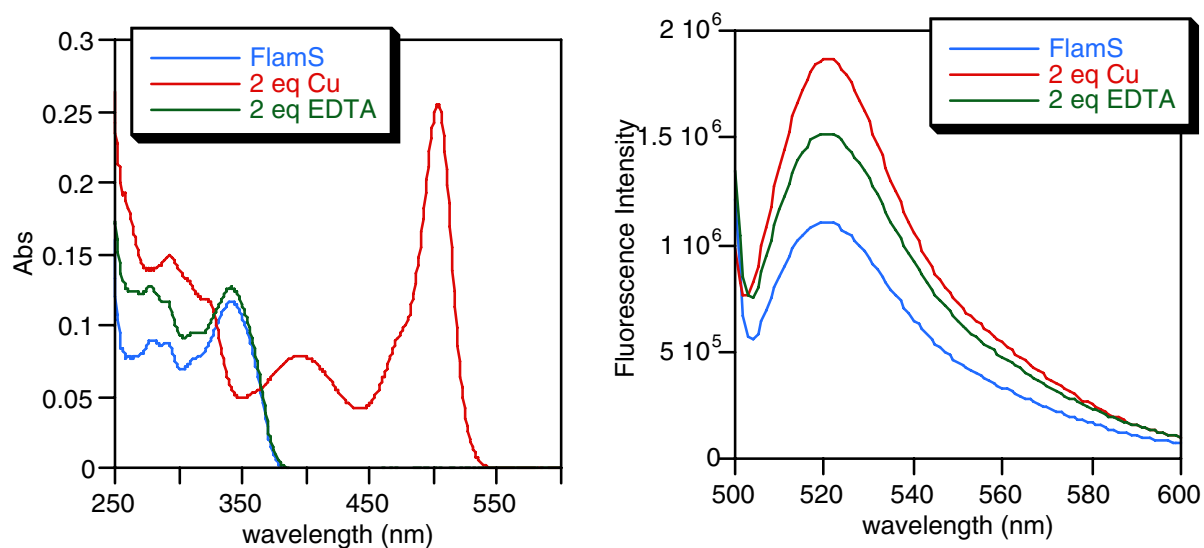


Figure S1. (Left) Addition of 2 equivalents of EDTA to a 10 μM solution of FlamS containing 20 μM Cu^{2+} in 60/40 HEPES/DMSO solution (10 mM, pH = 7.4) results in complete restoration of the absorbance spectrum of FlamS, indicating reversibility of the metal–ligand interaction. (Right) In contrast, the fluorescence intensity of the EDTA-treated sample does not return completely to the baseline spectrum of FlamS, retaining some residual signal. $\lambda_{\text{max}} = 495$ nm.

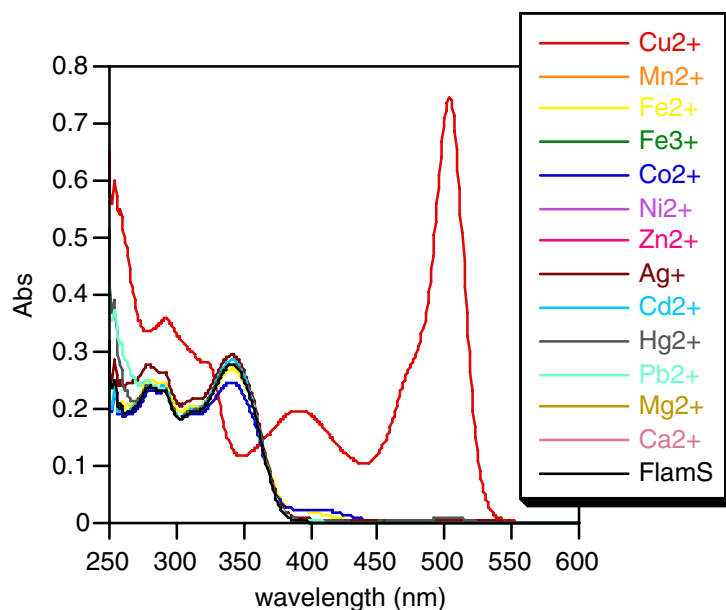


Figure S2. Aqueous solutions of Fe^{3+} , Fe^{2+} , Ni^{2+} , Zn^{2+} , Mn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Co^{2+} , Ag^{+} , Ca^{2+} and Mg^{2+} were titrated into solutions of 20 μM FlamS in 60/40 HEPES/DMSO (10 mM, pH = 7.4). Up to 2 equivalents of the transition metals were added, while Ca^{2+} and Mg^{2+} were titrated up to concentrations of 5 mM. Minimal changes are seen to the FlamS absorbance spectra with metal cations, with the exception being Cu^{2+} , which shows an intense band at 500 nm indicating an open spirolactam ring.

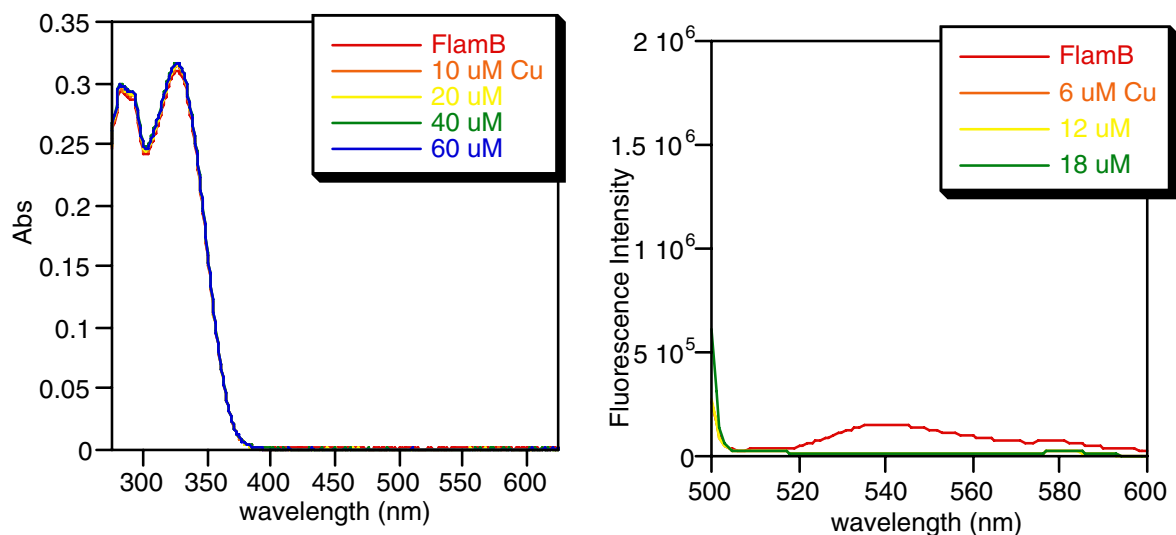


Figure S3. The boronic ester mask on FlamB was installed to prevent metal binding prior to H₂O₂ exposure. No metal-dependent spectral changes are observed with the absorbance (left) or emission spectra (right) when FlamB (20 μM or 6 μM, respectively) is titrated with Cu²⁺ up to 3 equivalents (shown above) in *organic* solvent (shown here: DMSO). $\lambda_{\text{max}} = 495 \text{ nm}$.

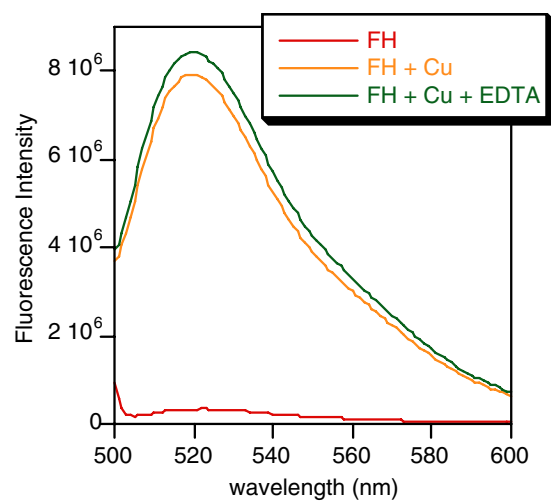


Figure S4. Emission spectra of FH (10 μM) in 60/40 HEPES/DMSO (10 mM, pH = 7.4) titrated with 1 equivalent of CuSO_4 and EDTA. $\lambda_{\text{max}} = 495 \text{ nm}$.

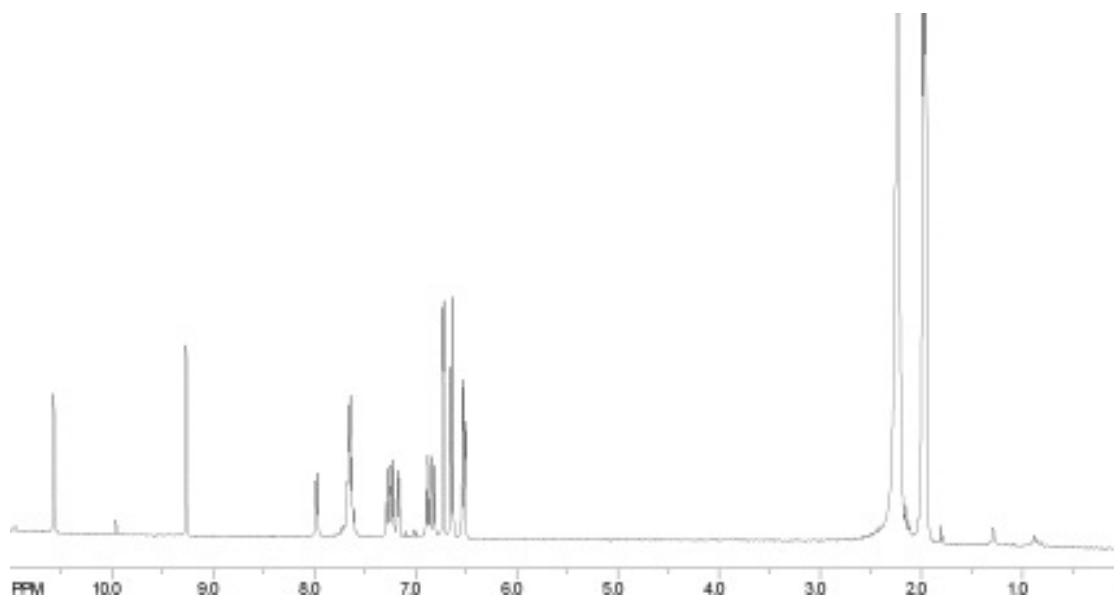


Figure S5. ¹H NMR spectra of **FlamS** (CD₃CN, 300 MHz).

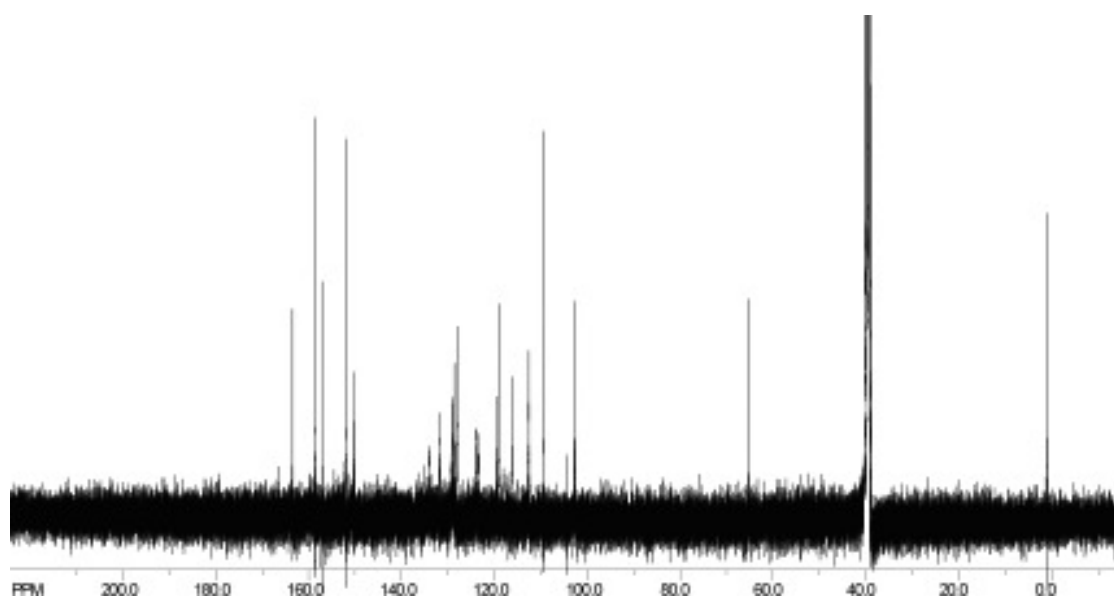


Figure S6. ¹³C NMR spectra of **FlamS** (CD₃CN, 100 MHz).

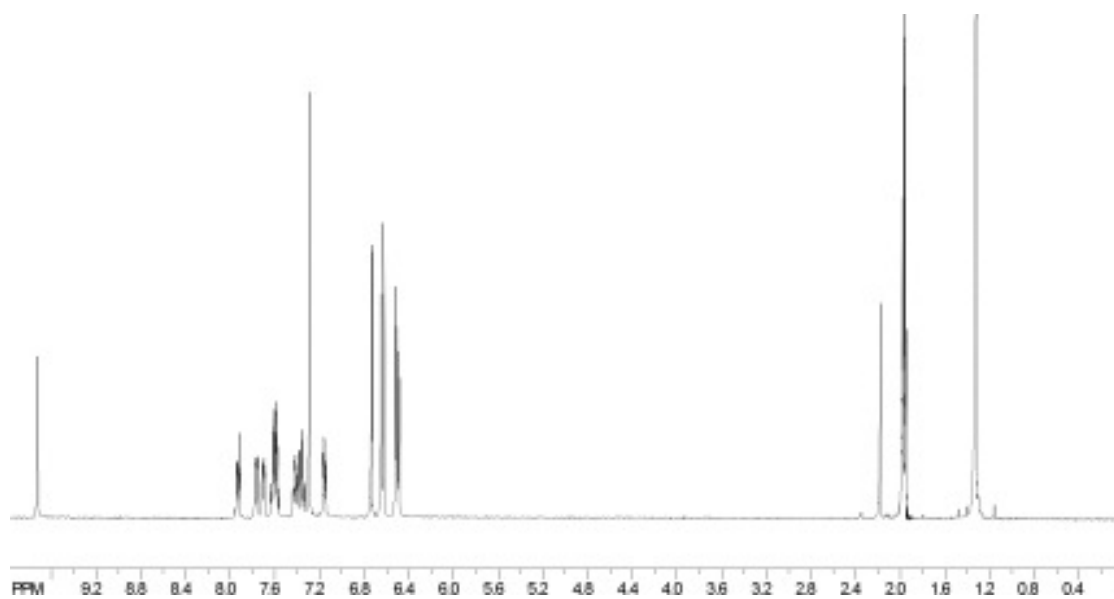


Figure S7. ¹H NMR spectra of **FlamB** (CD₃CN, 400 MHz).

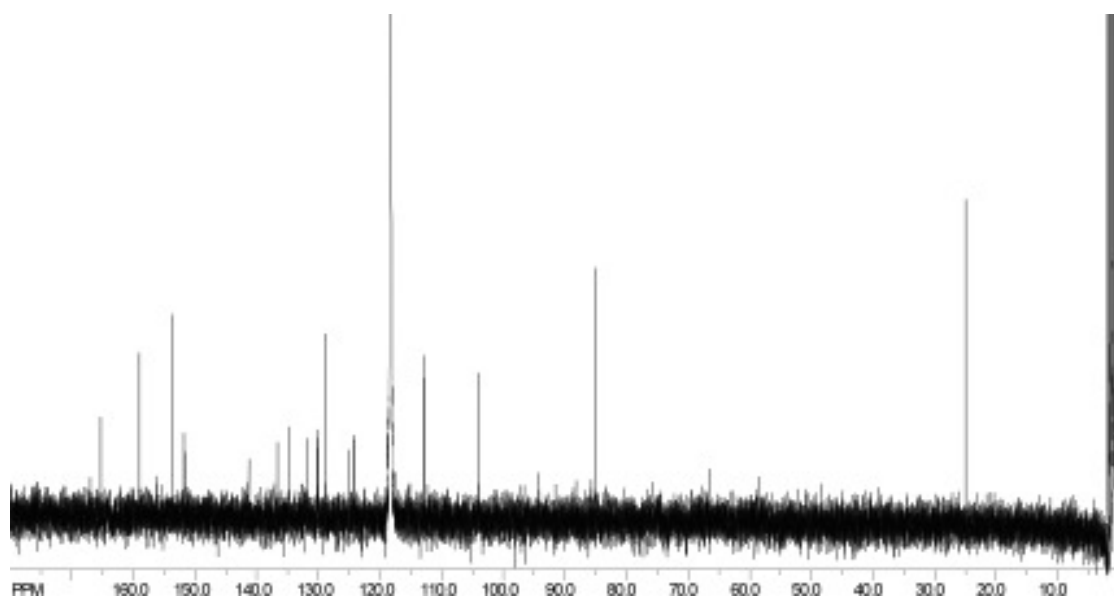


Figure S8. ¹³C NMR spectra of **FlamB** (CD₃CN, 100 MHz).

X-ray Data Collection and Structure Solution Refinement

Flam S crystals were obtained by boiling in acetonitrile and allowing the solution to cool slowly. Flam B were grown by slow evaporation of hexanes and methanol and Cu-FlamS from the evaporation of methanol and ethanol. The crystals were mounted on a Cryoloop and held in place by hardened Karo syrup.

X-ray intensity data from a colorless prism of FlamS were measured at 150(2) K on a Bruker SMART APEX diffractometer (Mo K α radiation, $\lambda = 0.71073 \text{ \AA}$).¹ Raw area detector data frame integration was performed with SAINT+ v6.45.¹ Final unit cell parameters were determined by least-squares refinement of 6388 reflections from the data set. Direct methods structure solution, difference Fourier calculations and full-matrix least-squares refinement against F² were performed with SHELXTL v6.14.1. The compound crystallizes in the space group P2₁/n as determined by the pattern of systematic absences in the intensity data. The asymmetric unit consists of one C₂₇H₁₈N₂O₅ molecule and a total of two acetonitrile-d₃ molecules of crystallization. One of the two CD₃CN molecules (N4, C30, C31) is disordered over two independent positions with refined populations A/B = 0.661(3) / 0.339(3) (constrained to sum to unity). Atoms of these groups were refined isotropically, and C31A/C31B was refined with common atomic coordinates and displacement parameters. All other non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms bonded to carbon were placed in geometrically idealized positions and included as riding atoms. The oxygen-bound hydrogen atoms were located in difference maps and refined freely. The deuterium atoms were accounted for during refinement to give the correct F.W. and d(calc).

Data for FlamB and Cu-FlamS were collected at 296 K and 100 K, respectively, on a Bruker Kappa Apex II CCD diffractometer equipped with a graphite monochromator and a Mo K α fine-focus sealed tube ($\lambda = 0.71073 \text{ \AA}$) operated at 1.75 kW power (50 kV, 35 mA). The detector was placed at a distance of 5.0 cm from the crystal. A total of 2655 frames were collected with a scan width of 0.5° and an exposure time of 10.0 and 30.0 sec/frame. The frames were integrated with the Bruker SAINT v7.12A software package¹ using a narrow-frame integration algorithm. Empirical absorption corrections were applied using SADABS v2.10 and the structure was checked for higher symmetry with PLATON v1.07. The structure was solved by direct methods with refinement by full-matrix least-squares based on F² using the Bruker SHELXTL Software Package. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms of sp² hybridized carbons and nitrogens were located directly from the difference Fourier maps; all others were calculated. The asymmetric unit of FlamB contains one C₃₃H₂₉BN₂O₆ molecule along with one H₂O and one CH₃OH. The asymmetric unit of Cu-FlamS consists of one [Cu₂Cl(FlamS)₂]Cl unit, where the outer-sphere chloride appears at two positions at half occupancy, both of which are disordered over two positions with refined populations of 0.3/0.2 and 0.35/0.15. There is also a NaOH with the Na at half occupancy and a disordered water molecule. Figures S8-S10 show fully labeled structures. Full lists of bond lengths and angles, atom coordinates, and anisotropic displacement parameters can be found in cif format in a separate file of Electronic Supporting Information.

¹ Bruker Analytical X-ray Systems, Inc., Madison, Wisconsin, USA.

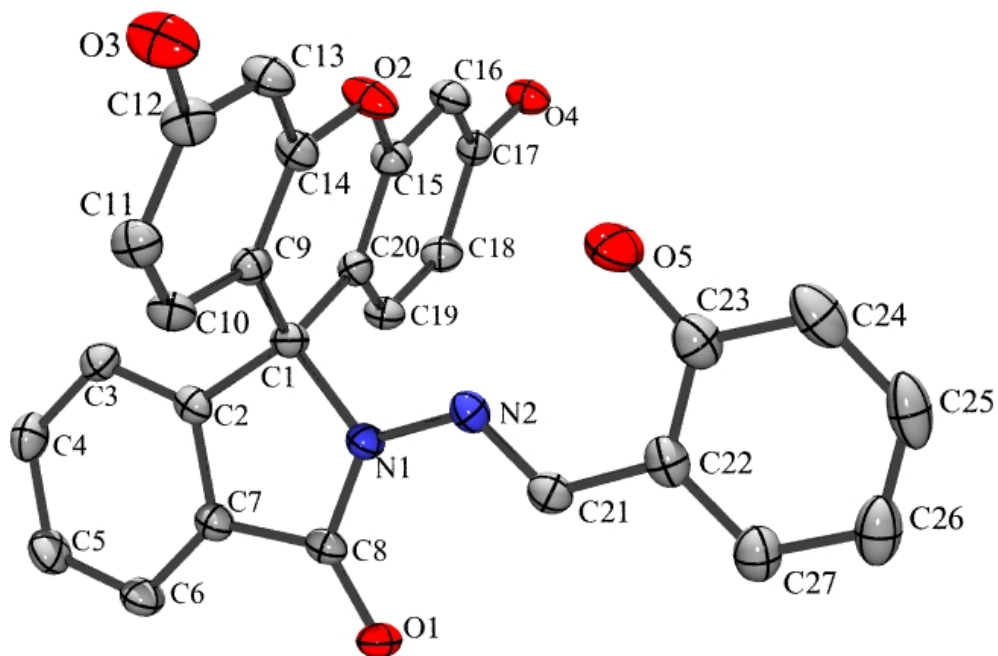


Figure S9. ORTEP diagram showing 50% thermal ellipsoid probability with full atom numbering scheme for FlamS. Hydrogen atoms and two acetonitrile molecules omitted for clarity.

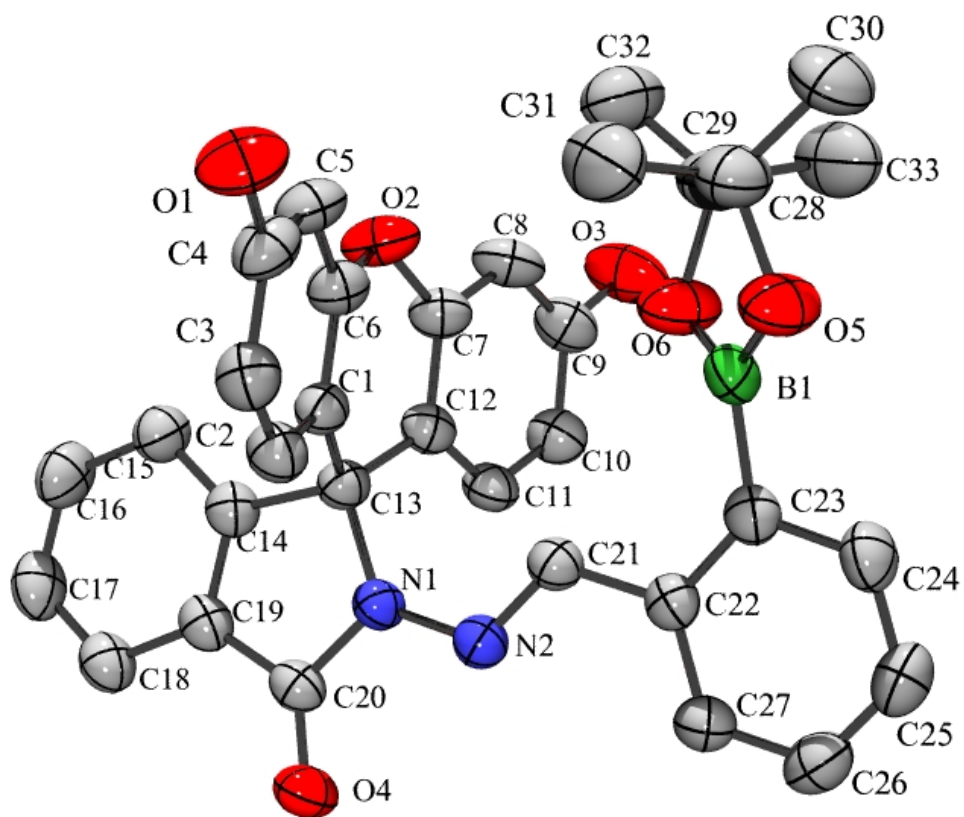


Figure S10. ORTEP diagram showing 50% thermal ellipsoid probability with full atom numbering scheme for FlambB. Hydrogen atoms and H₂O and CH₃OH omitted for clarity.

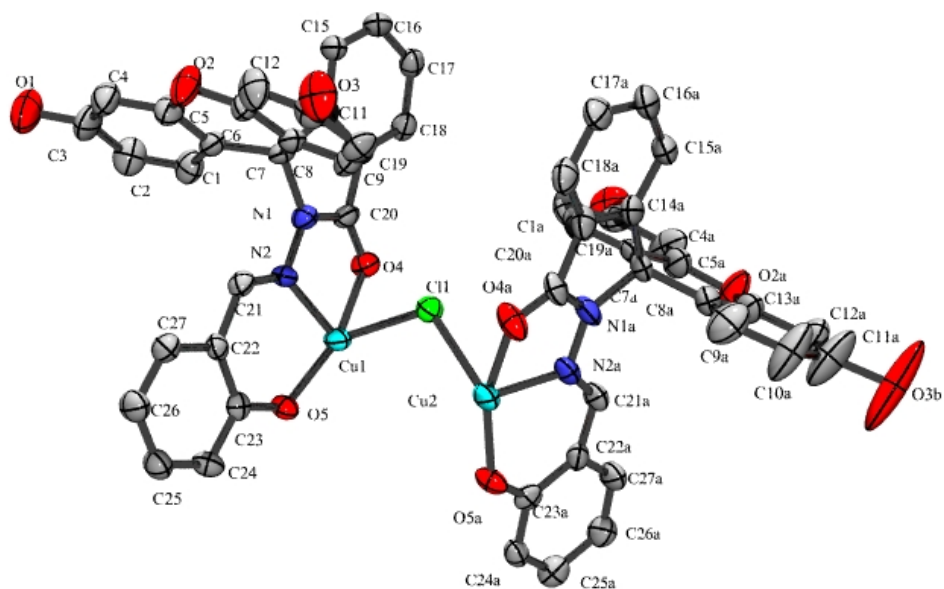


Figure S11. ORTEP diagram with 50% thermal ellipsoid probability showing asymmetric unit of $[\text{Cu}_2(\text{FlamS})_2\text{Cl}]^+$ with full atom numbering scheme. Hydrogen atoms and outer-sphere Cl^- , NaOH , and H_2O not shown.