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## Synthesis and Characterization of a Model Multi-Copper Oxidase

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# Synthesis and Characterization of a Model Multi-Copper Oxidase

Joshua Gavin, Dr. Brian Johnson

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## Introduction

Multicopper oxidases are present in many biological systems, examples being laccase in plants and ceruloplasmin in vertebrates. Laccases catalyze the oxidation of organic substrates, such as the single-electron oxidation of phenols. Ceruloplasmin oxidizes ferrous ions in blood to the ferric state – supporting iron transport – and is linked to Wilson's disease. Both these processes are coupled to the reduction of dioxygen to water, taking place at a tri-nuclear copper site.

The mechanism for the four-electron reduction of dioxygen proves elusive, however. There are multiple possible configurations for the binding of dioxygen in this site, as well as the structure of the intermediates. The goal of this research is to synthesize a model of the tri-nuclear copper site that will provide insight into the mechanism of action for these multicopper oxidases.

The model **3** is based off of the chelating ligand tris(2-pyridylmethyl)amine, a compound shown to bind copper in a tridentate manner using nitrogen donors, similar to the histidine residues used in these oxidases. Additionally, the model uses a triethylbenzene derivative that drives the nitrogen donors to be in close proximity to each other, creating the tri-copper center. This model will be used as a tool for determining the structure of the dioxygen-bound species present in tri-nuclear copper oxidases.

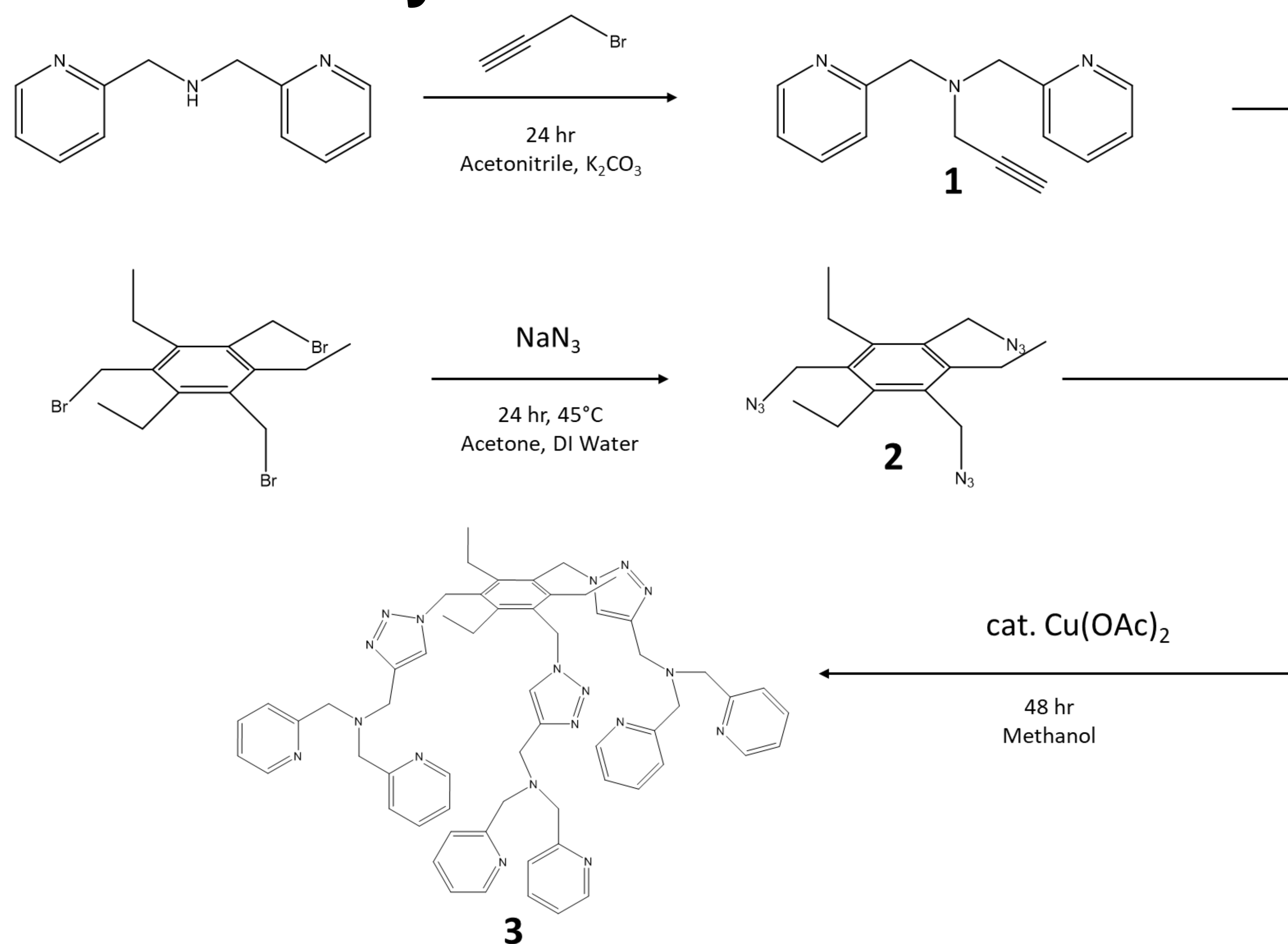
## Methods

**N, N-bis(2-picoyl)-N-propargylamine (1)**<sup>1</sup>: Potassium carbonate (2.75 g), acetonitrile (20 mL), Di-(2-picoyl)amine (0.55 mL) and propargyl bromide (80% in toluene, 0.45 mL) were stirred for 24 hours in nitrogen atmosphere (monitored by TLC: MeOH  $R_f=0.67$ ). The reaction mixture was filtered through celite and concentrated under reduced pressure, yielding a thick brown oil. Mixture was purified by column chromatography (neutral alumina, activated charcoal; mobile phase 0-10% EtOAc in DCM), and dried under reduced pressure to produce a golden oil.

**1,3,5-tris(azidomethyl)-2,4,6-triethylbenzene (2)**<sup>2</sup>: Behind a blast shield, 1,3,5-tribromo-2,4,6-triethylbenzene (0.270g, 0.886 mmol), acetone (20 mL), sodium azide (0.286g, 4.430 mmol) were stirred, and was diluted with deionized water until mixture became creamy white. After stirring for 24 hours at 45 °C under nitrogen atmosphere the reaction was placed in ice bath, precipitating the product out of solution. The product was extracted with TBME and dried under reduced pressure, yielding a white crystalline precipitate.

**1,3,5-tris(4-(N,N-bis(2-picoyl))-1-triazole)-2,4,6-triethylbenzene (3)**<sup>3</sup>: **2**, **1** (0.80g, 2.92 mmol), methanol (30 mL), and Copper (II) Acetate (0.15 M, 0.9 mL) were stirred for 48 hours (monitored by TLC: 75:25 hexanes:ethyl acetate  $R_f$ : 0.56) at room temperature. Mixture was concentrated under reduced pressure, purified via column chromatography (basic alumina, activated charcoal; 5% methanol in ethyl acetate) and purified under reduced pressure, yielding a golden yellow solid.

## Synthetic Scheme



## Discussion

The azide-alkyne Huisgen cycloaddition to form ligand **3** proceeded to only partial completion, determined due to skewed integration levels on <sup>1</sup>H-NMR of corresponding peaks. The product instead formed primarily mono- and di-substituted product. This type of reaction is commonly proceeded under elevated temperatures<sup>3</sup>, so the absence of heat did not bring the reaction to completion. In addition, procedures have not incorporated sodium ascorbate, which has been noted in cited literature. These two factors are expected to cause incomplete substitution on **2**.

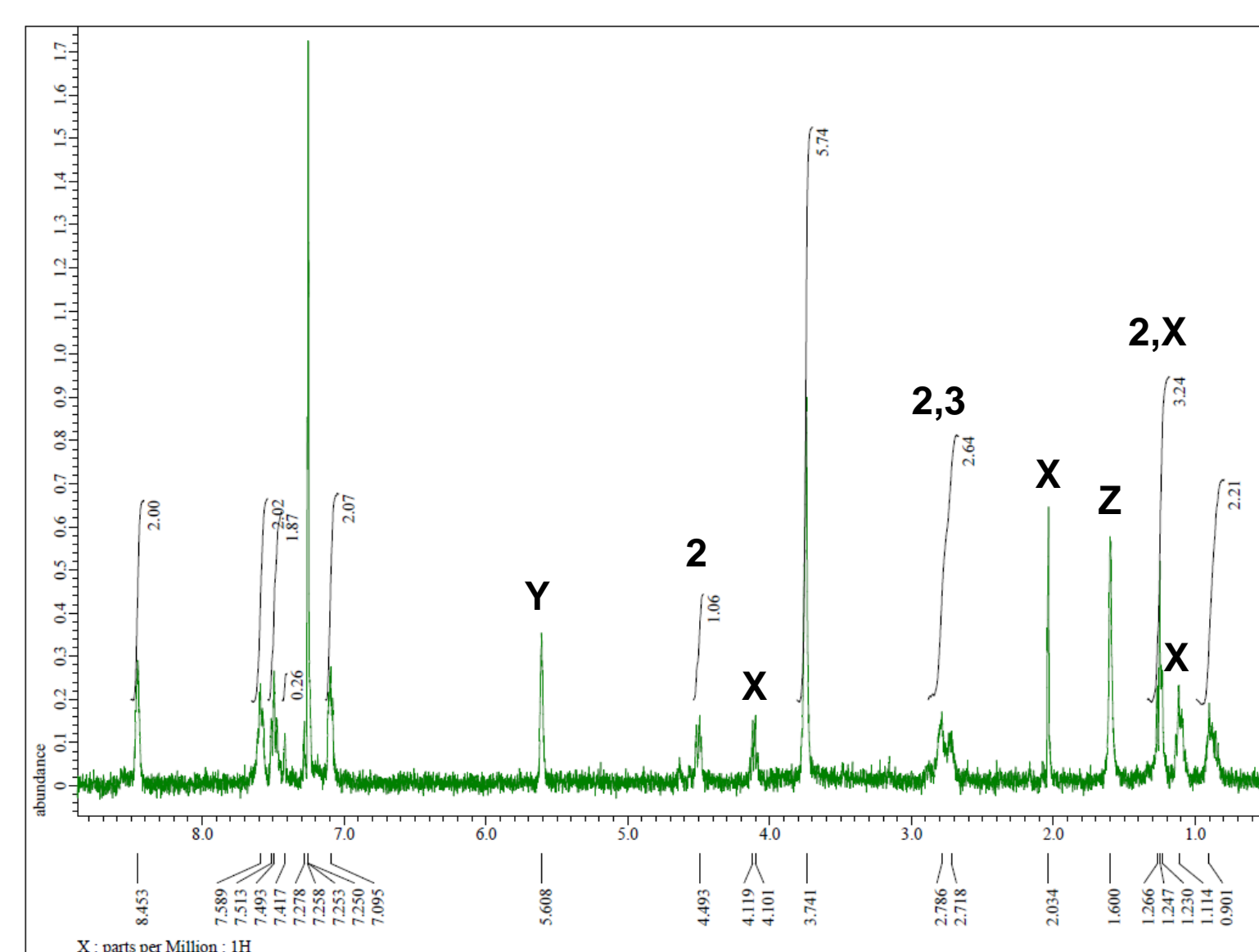
## Future Work

- Improve synthesis of **3** by testing ascorbate content and heat
- Complexation with copper (I)
- Crystallization of ligand:copper complex

## Acknowledgements

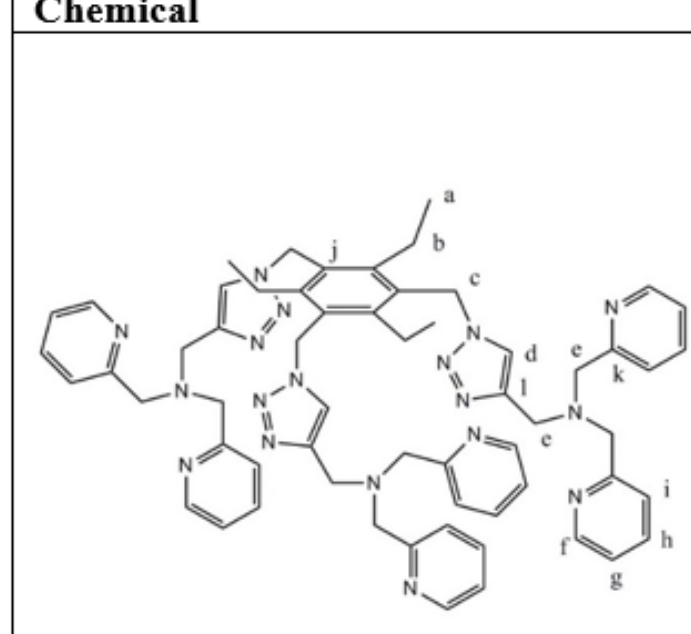
- CSBSJU Undergraduate Research and Experiential Learning
- My former partner Simone Creed, for her work improving the first step of the synthesis
- NSF S-STEM Grant Award Number DUE-1059730 for supporting my education in chemistry through the FoCuS chemistry scholarship

## Results

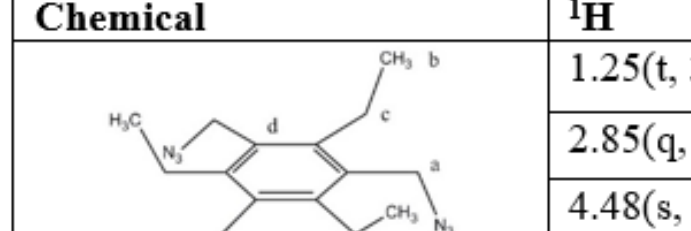


**Figure 1:** <sup>1</sup>H-NMR of **3** in CDCl<sub>3</sub> (X=EtOAc; Y=Dichloromethane; Z=H<sub>2</sub>O). Integration of 2.79 ppm is higher than 7-8.5 ppm peaks due to partial substitution of **2**.

**Table 1:** <sup>1</sup>H-NMR table for **3**

Chemical	<sup>1</sup> H
	0.79(t, 3H)
	2.77(q, 2H)
	3.63(comp, 6H)
	5.58(s, 2H)
	7.08(d, 2H)
	7.44(t, 2H)
	7.54(s, 1H)
	7.58 (t, 2H)
	8.44(d, 2H)
	–
	–
	–

**Table 2:** <sup>1</sup>H-NMR table for **2**

Chemical	<sup>1</sup> H
	1.25(t, 3H)
	2.85(q, 2H)
	4.48(s, 2H)
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