Determining the antioxidant activity of small metal-binding ligands that target agents known to lead to neurodegenerative diseases

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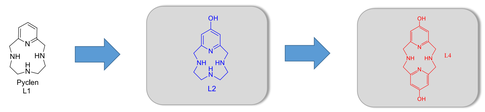
Abstract

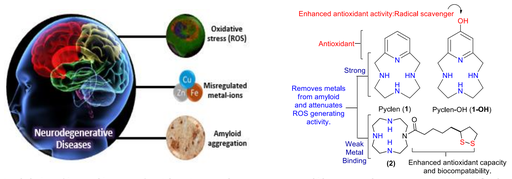
Oxidative stress in the brain is a known contributor to the development of neurodegenerative diseases, including Alzheimer's. The focus of this project is to target the amyloid-β plaque formations and reactive oxygen species (ROS) derived from misregulated metal-ions that lead to disease-causing oxidative stress. The present investigation is measuring the antioxidant reactivity of the new molecule L4. L4 contains two radical scavenging pyridol groups along with a metal-binding nitrogen rich ligand system. It was hypothesized that increasing the number of pyridol groups in our small molecule library would increase the radical scavenging activity, which in turn may provide cells protection from oxidative stress. The radical scavenging ability of L4 was quantified using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay and this was compared to other radical scavenging small molecules to evaluate the effect of the additional radical scavenging group on the antioxidant activity. The interaction of L4 with redox active metal-ions such as copper(II) was also evaluated to show the molecule's ability to target misregulated metal-ions in diseased tissues.



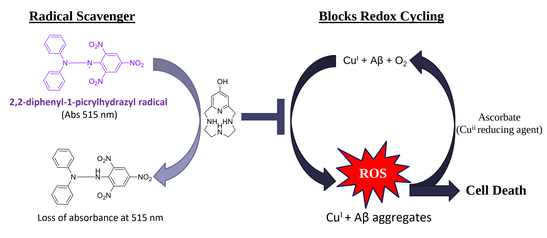
Introduction

ROS and Antioxidant balance in the body is an important element in the development of a variety of diseases, including neurodegenerative disorders. There are many factors that contribute to this careful balance and finding therapeutics to help regulate this balance is a major part of this investigation.

When developing a therapeutic agent, several aspects must be considered and investigated to confirm that the agent is stable, water soluble, available in the gram scale, biologically compatible in the sense that it is non-toxic and can cross the BBB. Additionally, in the case of neurodegenerative diseases like Alzheimer's, the agent should have the functional abilities to break apart amyloid, prevent aggregate formation, and control the production and balance of ROS.



The small metal binding ligands that make up our library have many of these characteristics and capabilities. The goal is to find ways to amplify these characteristics, specifically the antioxidant radical scavenging and metal-binding capabilities, in order to decrease the dosage necessary to obtain significant results.

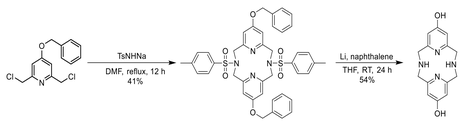


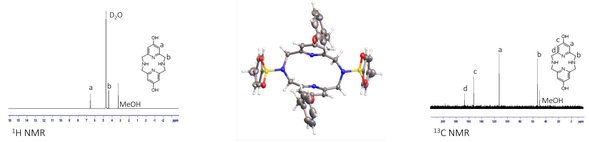
Characterization and Evaluation of L4

L4 Hypothesis and Synthesis

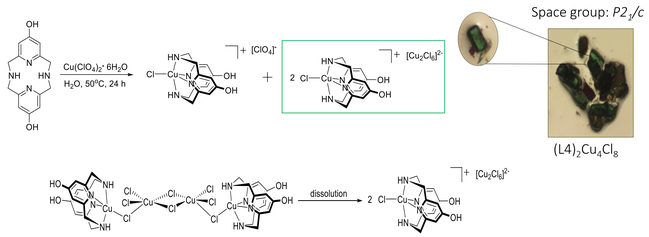
Hypothesis: By doubling the number of pyridol groups on the metal-binding macrocyclic ligand, we should increase the radical scavenging activity and, therefore, the antioxidant capabilities of the molecule.

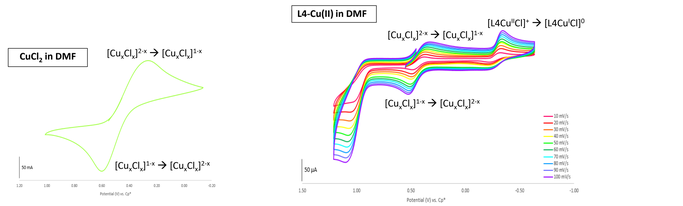
Synthesis





Electrochemistry of L4-Cu (II): Confirmation of Metal-Binding



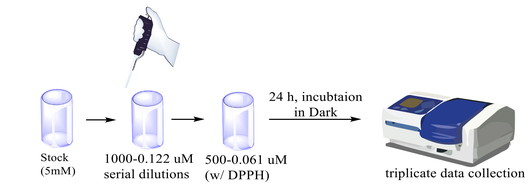


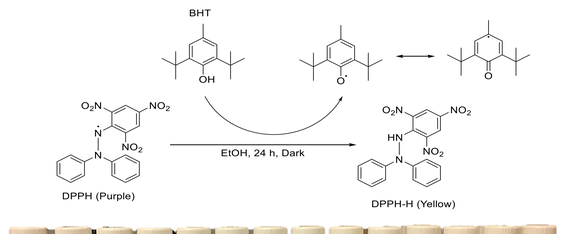
- Conditions
  - 0.1 M TBAP
  - 0.265 mM L4-Cu(II) in DMF
  - referenced to Cp*
- Analogous redox events in DMF
  - Dissolved crystals of L4-Cu(II)
  - Dissolved $CuCl_2$
- Further evidence that dimer breaks apart in solution

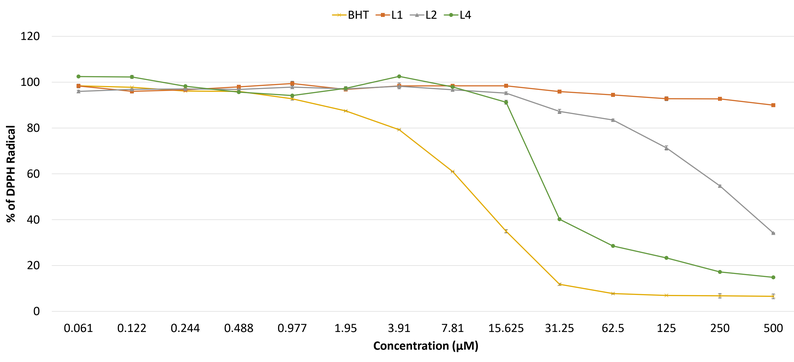
	Redox Event	E_{pc} (V)	E_{pa}(V)	$E_{1/2}$ (V)	ΔE_p (V)
From dissolved $CuCl_2$	$[Cu_xCl_a]^{2-x} \rightarrow [Cu_xCl_a]^{1-x}$	0.263	0.601	0.432	0.864
From dissolved L4-Cu(II)	$[Cu_xCl_a]^{2-x} \rightarrow [Cu_xCl_a]^{1-x}$	0.328	0.528	0.428	0.856
From dissolved L4-Cu(II)	$[L4Cu^{II}Cl]^{+} \rightarrow [L4Cu^{I}Cl]^{0}$	0.344	-	-	-

L4 Radical Scavenging and Antioxidant Activity

A DPPH assay was utilized to quantify and analyze the radical scavenging capabilities of the L4 molecule in comparison to L1 and L2, as well as the positive control, butylated hydroxyl toluene (BHT). BHT is a natural antioxidant that has radical scavenging capabilities via the hydroxyl functional group. DPPH is a compound made up of stable radical molecules and is used because of the observable and measurable change in absorbance that occurs when reacted with antioxidant compounds. The rate of reaction between DPPH in solution and the ligand of interest in varied concentrations can be observed and measured using UV-VIS spectroscopy.







Conclusion and Future Directions

The preliminary results of the DPPH assay showed that the L4 molecule had a higher percentage of radical scavenging at the 0.5 mM concentration point as compared to the L1 and L2 molecules, suggesting that our hypothesis was correct. Additional runs of the assay must be run for better comparison and with the addition of higher concentration points to be able to compare more potent doses of the ligands. We will also run the assay as a time-study to see the relative rates of radical scavenging for the library of ligands. In the future, we would also like to run two other assays, the ABTS and CCA assay, which help quantify the radical scavenging ability in comparison to other known antioxidants as well as observe the copper and iron metal-binding and redox blocking capabilities of the ligands, respectively.

References

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