

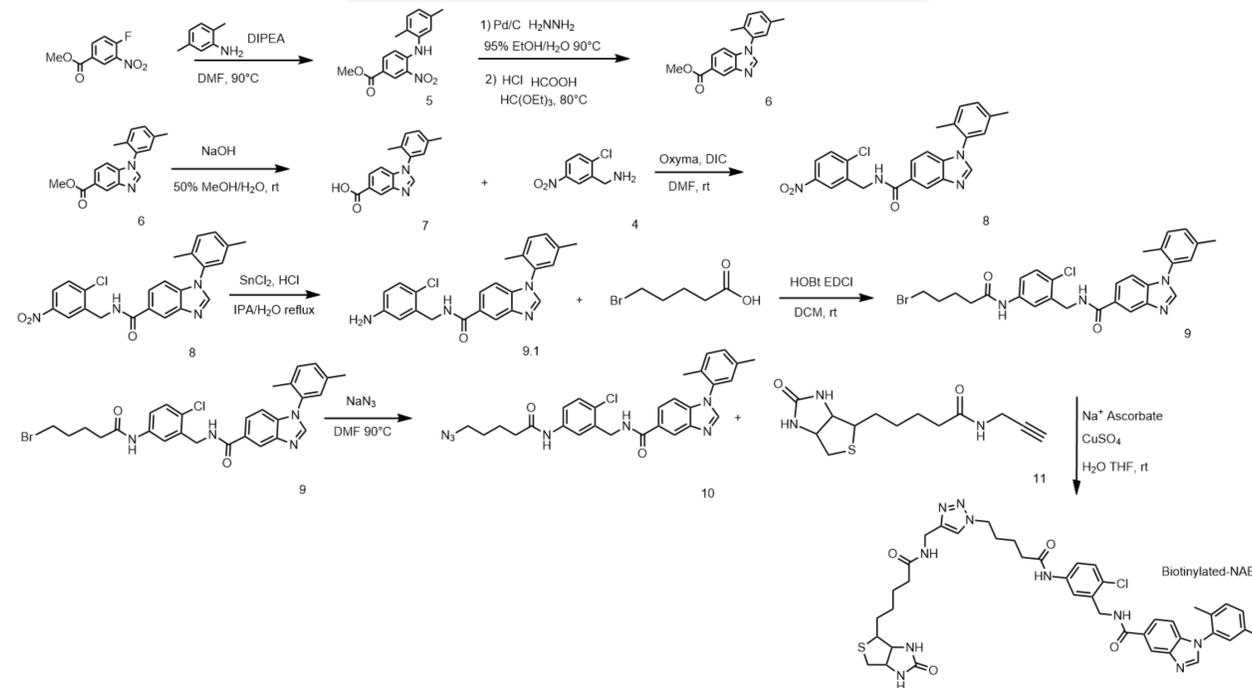
The Synthesis of Biotinylated-NAB2 for Nedd4-Tau AUTAC Molecule for Proximity Ubiquitination

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Abstract

Ubiquitin is a 76-amino acid peptide that is necessary for regulating many cellular processes. Ubiquitination occurs through a three-enzyme cascade of Ub-activating enzymes (E1), Ub-conjugating enzymes (E2), and Ub ligases (E3). The attachment of Ubiquitin to a protein is responsible for signaling DNA repair, protein degradation, and cell cycle regulation, to name a few. In disease-affected neurons, there are Lewy bodies that contain and express an E3 ligase, Nedd4. This ligase is responsible for the addition of ubiquitin to target proteins after ubiquitination. To isolate Nedd4, small-molecule binding is needed. N-aryl benzimidazole (NAB) compounds show successful binding to Nedd4 ligase by surface plasmon resonance. An analog compound of NAB, NAB2, has shown the best binding affinity and efficiency. To better study this binding between Nedd4 and NAB2, biolayer interferometry studies using a biotinylated-NAB2 can be used.

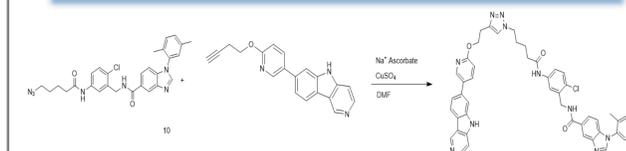
Proposed Synthetic Route



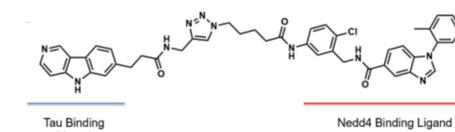
Conclusions

With Methyl-4-fluoro-3-nitrobenzoate, an S_NAr reaction occurs to attach 2-5-dimethylaniline (80%), followed by a nitro group reduction and benzimidazole cyclization (17%). A hydrolysis reaction converts the methoxy group to a hydroxyl group (78%) to allow for an amide bond formation (58%). The nitro group reduction to an amine (65%) is to allow for a second amide bond formation with 5-bromovaleric acid (18%). Using the bromovaleryl linker, an azido group is formed through S_N2 chemistry (32%). Copper-click on azido using alkyne functional group (91%).

Directions for Future Research

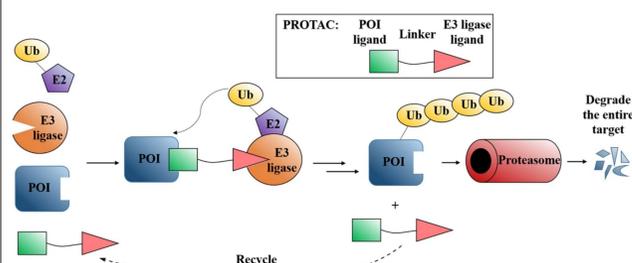


- Theoretical reaction using copper (I) click chemistry is shown above with the azido intermediate and alkyne functional group. Experiments to synthesize alkyne species are ongoing.
- Test purified Nedd4 and biotinylated-NAB2 on a streptavidin tip binding. Using biolayer interferometry affinity binding, gather signal output data.
- Design of a Nedd4-Tau AUTAC molecule for degradation



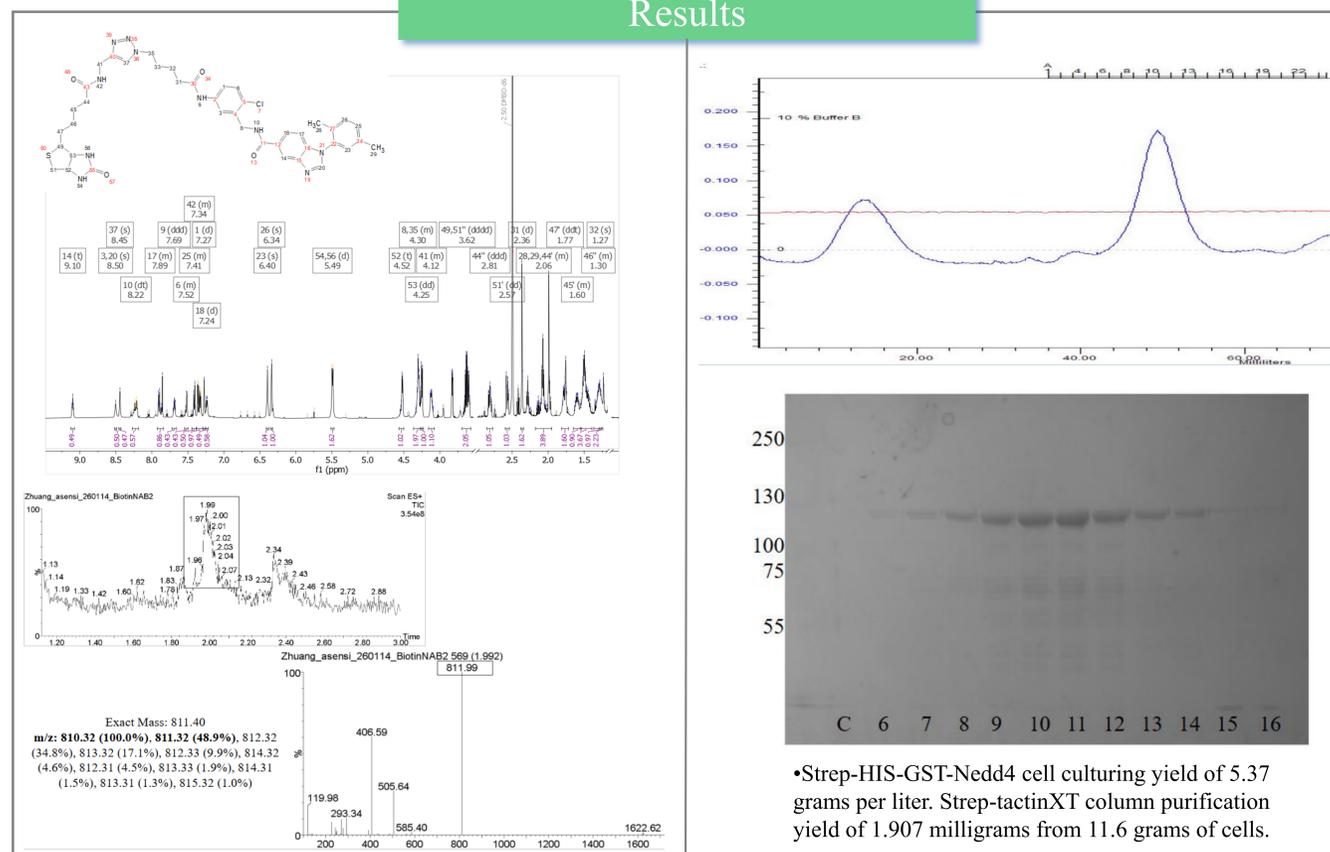
Purpose & Background

- Tauopathies are neurodegenerative diseases caused by Tau protein aggregation. Tau exists in six isoforms. Alzheimer's disease is a combination of the 3 and 4 isoform microtubule-binding repeat domain aggregates.
- Tau contains 44 lysine (K) residues with 5 sites commonly observed to be ubiquitinated in Alzheimer patients. However, the proteasomal degradation fails and aggregated Tau builds up and becomes neurotoxic.



- AUTAC (Autophagy-Targeting Chimera) binds E3 ligase Nedd4 that builds K63-linked polyubiquitin chains to re-tag these residues for autophagy.

Results



References

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