Synthesis of a Cargo-Linked Peroxynitrite Cleavable Monomer

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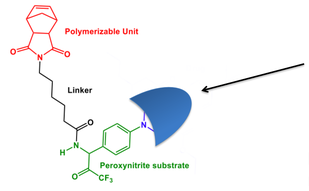
ABSTRACT

Reactive oxygen species (ROS) responsive probes are of growing interest in the field of biomedicine. Because of its extreme reactivity and selectivity over other endogenous ROS, peroxynitrite is a promising target for the purpose of detecting and targeting the site of diseased tissue, such as a myocardial infarct. In this study, we worked towards the synthesis of a peroxynitrite-responsive cleavable monomer, which upon activation is expected to release a bound cargo; in our study, this cargo was a therapeutic small molecule drug or a fluorescent tag. The probe consists of a polymerizable moiety, a linker molecule, a peroxynitrite substrate, and a cargo which possesses a 2˚ amine. In the drug-linked monomer, it was determined that the 1,4-dihydropyridine amine was likely not nucleophilic enough for a Buchwald-Hartwig (C-N) amination. In switching gears to a monomer linked to a more nucleophilic fluorophore, 5(6)-carboxyrhodamine, initial protection and coupling steps were completed. Synthesis of the new monomer is ongoing. Given successful responsiveness to peroxynitrite, this probe can be polymerized and assembled into nanoparticles used for the effective detection of peroxynitrite production in diseased tissue, and may be altered to carry different cargos, such as drugs, labels, and targeting moieties.

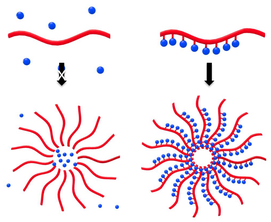


Cargo: drug molecule, fluorophore, contrast agent, targeting peptide

Figure: General structure of peroxynitrite responsive cleavable monomer.

Background of ROS Responsive Materials

The Gianneschi Lab specializes in synthesizing responsive polymers and drug-loaded particles. Covalently bound cargos offer several advantages over encapsulated cargos in nanoparticle formulations.



Advantages of Covalently Bonded Cargos
• High density packing
• No leakage during storage of molecules
• Tuned responsiveness and activated release

Figure: Model demonstrates the efficiency of polymer with covalently bonded cargo (Right) as opposed to trapped free-floating cargo (Left)

Drug-Loaded Peroxynitrite Responsive Monomer

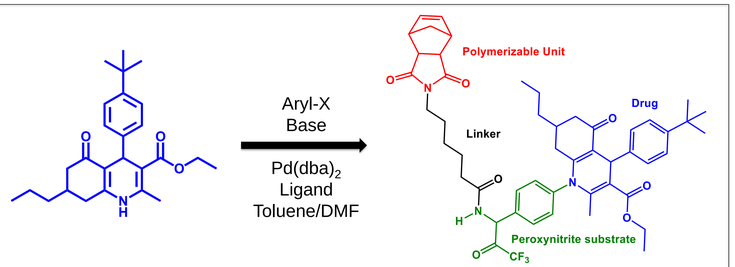


Figure: Amine drug molecule (left) and peroxynitrite-responsive cleavable monomer bound to drug molecule (right).

Synthesis of Drug

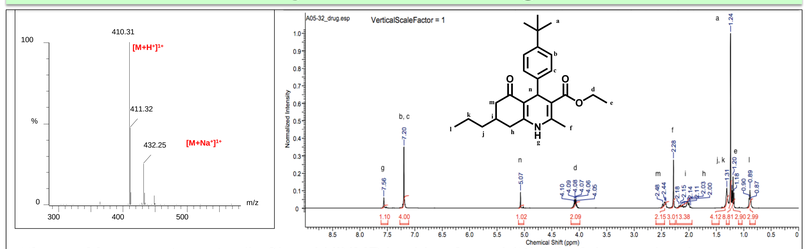


Figure: Mass spectrometry (Left) and H'NMR in chloroform-d (Right) of drug molecule

Screen of Buchwald-Hartwig Reaction

Reagent	Catalyst	Ligand				Aryl-X	Amine	Base	Product
Identity	$Pd(dba)_2$	(R)-BINAP	XantPhos	Xphos	RuPhos	I-Phe	Drug	Cs_2CO_3	Drug-Phe
eq	0.05	0.1	0.1	0.2	0.2	1	1.2	1.4	1
# aliquots	4	1	1	1	1	4	4	4	4

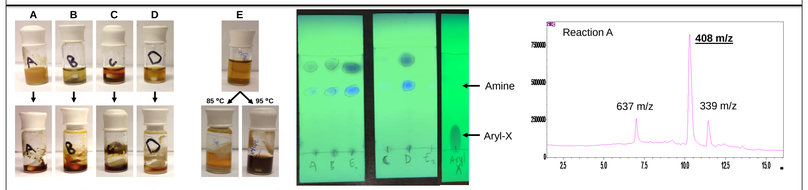


Figure: Screen of Buchwald-Hartwig Reactions (Top), color change in reaction viles indicates the activation of the catalyst by the ligand (Left), TLC and LCMS reveal consumption of Aryl-X but excess Amine drug remaining and no product material. (Right)

Modified Reaction Scheme

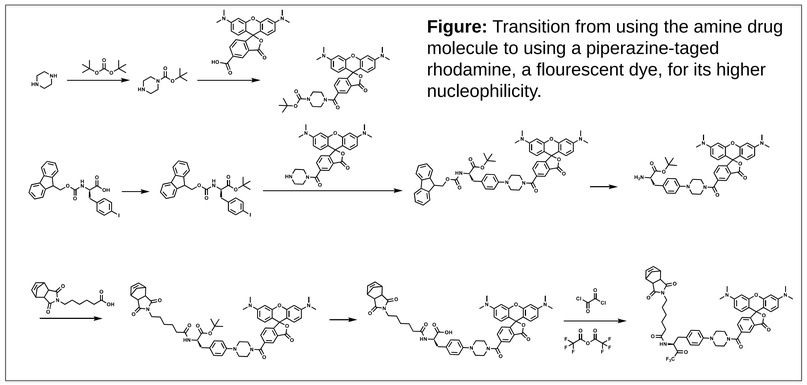


Figure: Transition from using the amine drug molecule to using a piperazine-taged rhodamine, a flourescent dye, for its higher nucleophilicity.

Protection of Piperazine

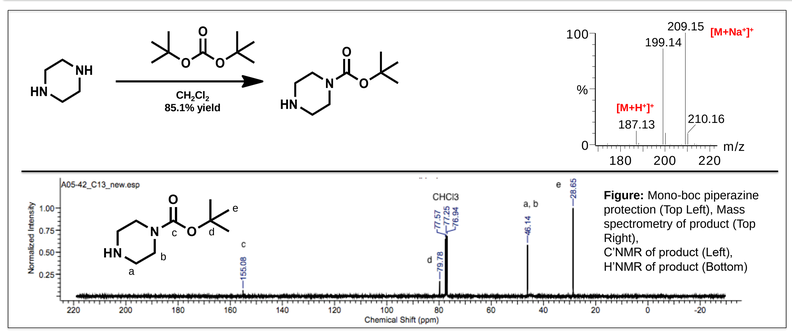
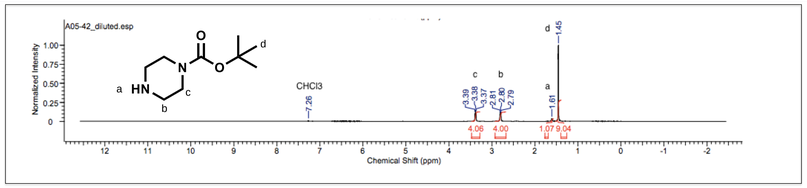


Figure: Mono-boc piperazine protection (Top Left), Mass spectrometry of product (Top Right), C'NMR of product (Left), H'NMR of product (Bottom)



Protection of Fmoc-(4-iodo)-Phe-OH

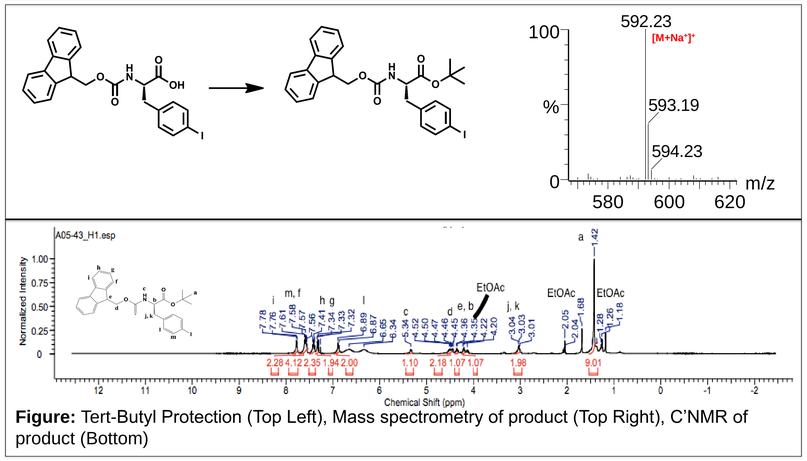


Figure: Tert-Butyl Protection (Top Left), Mass spectrometry of product (Top Right), C'NMR of product (Bottom)

Synthesis of Rhodamine

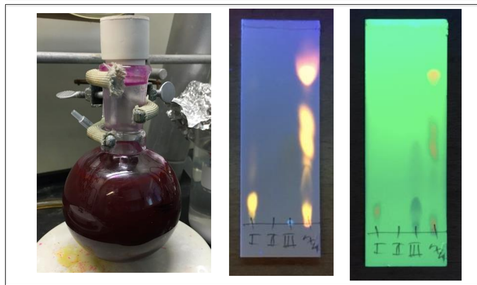


Figure: On-going reaction for the synthesis of rhodamine-piperazine, (Left), TLC Analysis under 365/254 nm UV light of the starting reagents and crude reaction solution (Middle and Right)

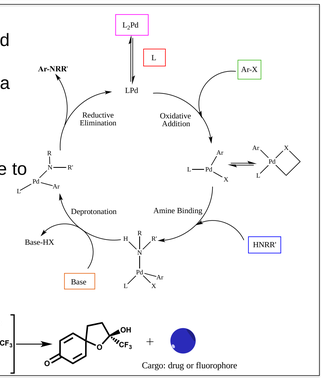
Conclusions and Future Directions

Conclusions:

- Synthesized amine drug molecule with optimal purity and recrystallization
- Completed one screen of Buchwald-Hartwig reaction in a vacuum-sealed glove box environment with the catalyst $Pd(dba)_2$ and four ligands: Xphos, XantPhos, RuPhos, (R)BINAP
- Transitioned from using weak nucleophilic drug molecule to synthesizing a highly nucleophilic secondary amine, rhodamine

Future:

- Complete full screen of Buchwald-Hartwig reaction, incorporating several different reagents
- Test responsivity of final probe in the presence of peroxynitrite



References

1. Yang, D.; Sun, Z.-N.; Peng, T.; Wang, H.-L.; Shen, J.-G.; Chen, Y.; Tam, P. K.-H., Synthetic Fluorescent Probes for Imaging of Peroxynitrite and Hypochlorous Acid in Living Cells. In *Live Cell Imaging: Methods and Protocols*, Papkovsky, B. D., Ed. Humana Press: Totowa, NJ, 2010; pp 93-103.
2. 1. Peng, T.; Yang, D., HKGreen-3: A Rhodol-Based Fluorescent Probe for Peroxynitrite. *Organic Letters* **2010,** *12* (21), 4932-4935.
3. 1. Surry, D. S.; Buchwald, S. L., Dialkylbiaryl phosphines in Pd-catalyzed amination: a user's guide. *Chemical Science* **2011,** *2* (1), 27-50.