Synthesis of Self-Immolative Rhodamine Based Theranostic Agent

Jonathan Alexander, Alessandra Rivera, Nathaniel Garrison, and Herman Nikolayevskiy*
University of San Francisco, Department of Chemistry, San Francisco, CA 94117

Abstract

Theranostic agents, a class of molecules that simultaneously serve both a diagnostic and therapeutic function, enable *in vivo* imaging for early diagnosis and targeted drug delivery within a single entity. Due to recent advances in the synthesis and photophysical properties of the rhodamine scaffold, rhodamine dyes present a promising new direction for theranostic research. Toward the goal of exploring rhodamine dyes as self-immolative prodrugs for theranostic applications, we have synthesized a rhodamine precursor via a telescoped radical bromination/hydrolysis**.** Upon completion of the full rhodamine synthesis, we will explore and optimize the kinetics of its "turn-on" fluorescence and drug release *in vitro*.

Theranostic Agents

- Many cancers go undetected until late-stage, when prognosis is poor[1]; thus early detection is vital to improving patient outcomes[2]
- Theranostic enables simultaneous *in vivo* imaging for early diagnosis and targeted drug delivery within a single entity[3]
- Single-molecular theranostic agents (SMTA) offer the benefit of synthetic modularity, higher membrane permeability, easier renal clearance, and a cheaper cost of production[4]

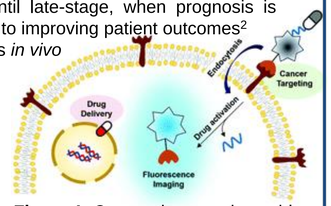


Figure 1. Cancer theranostic enables both diagnostic imaging and drug delivery[7]

Self Immolative Molecules

- They consist of three parts: self-immolative spacers, trigger moieties, and a substrate such as drug[5]
- Upon stimulus, cleavage of the trigger moiety occurs along with a cascade of disassembly reactions which result in the release of the substrate[6]

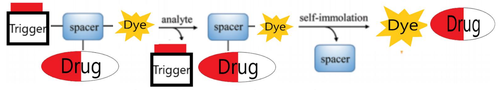
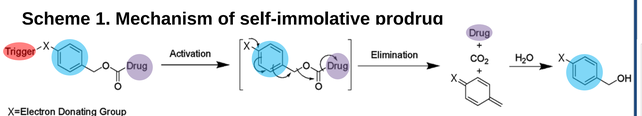


Figure 2. Overview of a self-immolative prodrug process

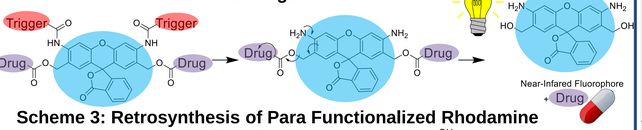
Scheme 1. Mechanism of self-immolative prodrug



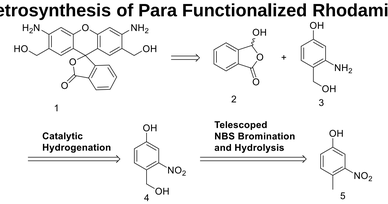
Rhodamine

- The goal of this research is to explore rhodamine dyes as self-immolative prodrugs for theranostic applications
- We aim to develop an efficient synthesis of polysubstituted rhodamines and optimize the kinetics of "turn-on" fluorescence and drug release in vitro

Scheme 2. Example application and mechanism of self-immolative rhodamine based theranostic agent



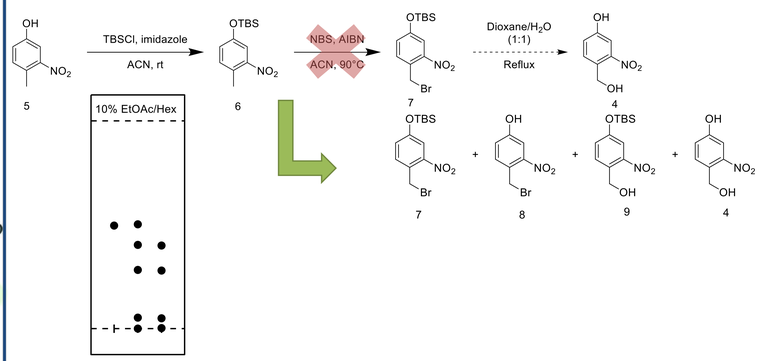
Scheme 3: Retrosynthesis of Para Functionalized Rhodamine



Results and Discussion

Failed NBS Radical Bromination

Scheme 4: Failed radical bromination due to hydrolysis



- Originally, we attempted to obtain phenol **4** via a radical bromination to yield bromide **7**. This would be followed by hydrolysis with dioxane/water.
- TLC and ^{1}H NMR from the attempted NBS bromination suggested the product quickly underwent hydrolysis to form a mixture of products, including the originally desired alcohol **4** as the major product (figure 3)
- This inspired us to experiment with a telescoped bromination and hydrolysis (scheme 5)

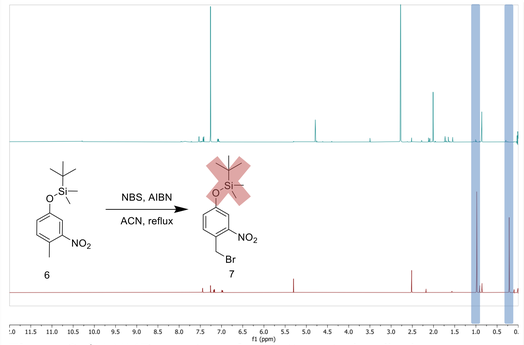
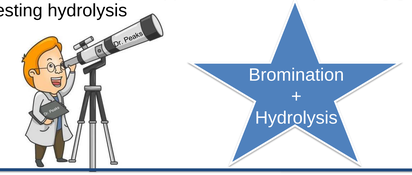
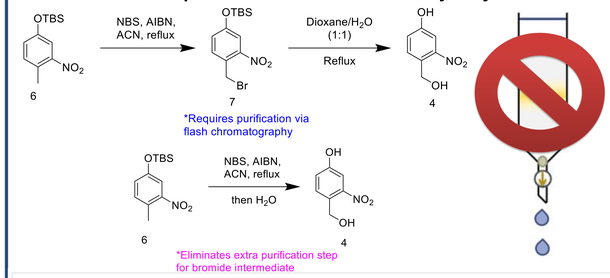


Figure 3. ^{1}H NMR spectrum from attempted radical bromination, showing disappearance of TBS protecting group, suggesting hydrolysis



Scheme 5. Telescoped radical bromination and hydrolysis



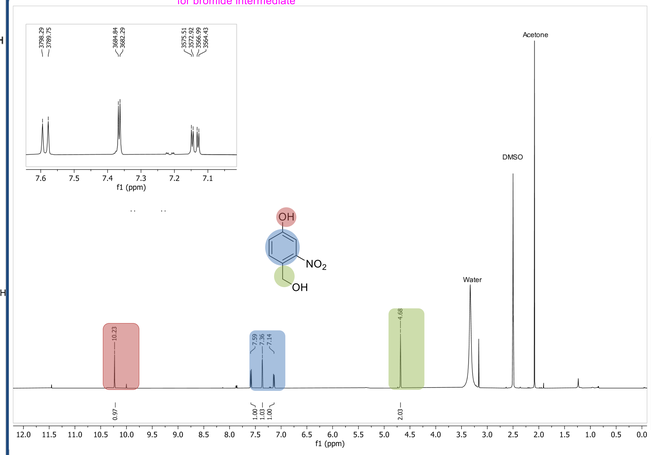
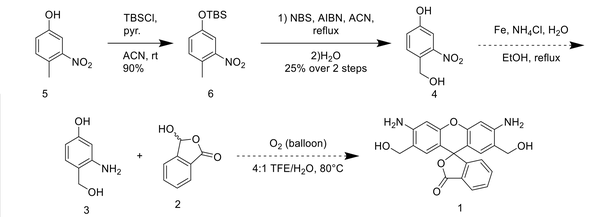


Figure 4. ^{1}H NMR spectra from telescoped NBS radical bromination and hydrolysis

Telescoped NBS Bromination and Hydrolysis

- Telescoping is a technique in organic synthesis in which a reactant is subjected to successive chemical reactions in one flask.
- By telescoping the NBS bromination and hydrolysis, an intermediate workup and purification step is avoided (scheme 5)
- The product of the telescoped reaction was confirmed via ^{1}H NMR (figure 4) and mass spectrometry (figure 5)

Scheme 6. Synthesis of para-functionalized Rhodamine



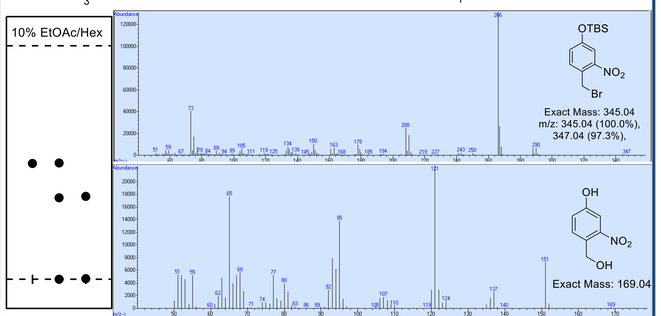
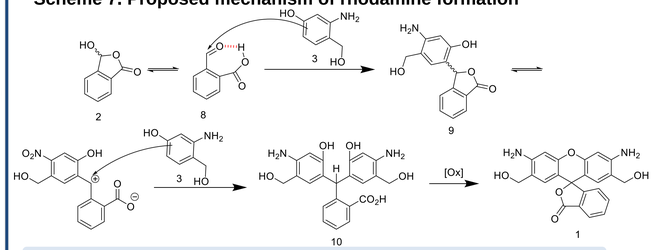


Figure 5. TLC and mass spectra from telescoped NBS radical bromination and Hydrolysis

Future Work

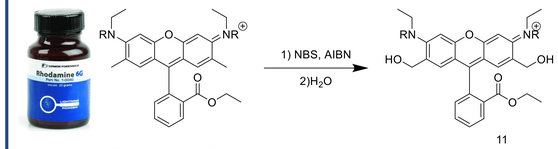
Completion of the Rhodamine Synthesis

Scheme 7. Proposed mechanism of rhodamine formation



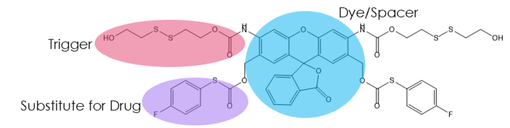
Derivatization of Commercially Available Rhodamines

Scheme 8. Functionalization of Rhodamine 6G



Rhodamine 6G

Testing Kinetics of Self-Immolative Process



- Test the kinetics of the self-immolative drug release mechanism by attaching a fluorinated reporter to the labile thiocarbonate and a disulfide-based trigger to the aniline.
- Monitor the progress of the self-immolation reaction via the release of the fluorinated motif using ^{19}F-NMR
- Based on the data collected from these tests, the optimal isomer will be further modified to improve the time it takes for the drug to be released

Conclusions

- We have synthesized a para-rhodamine precursor in 2 steps and 22.5% overall yield
- Developed a telescoped radical bromination and hydrolysis that creates a more efficient synthesis of the rhodamine scaffold that eliminates an extra workup and purification step

Acknowledgments

- USF Chemistry Department
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