PROPOSAL TO LOYOLA UNIVERSITY NEW ORLEANS
UNIVERSITY COMMITTEE ON INTERNAL GRANTS
Marquette Faculty Fellowship Proposal

Name: CJ Stephenson

College/Department: CAS/ Chemistry and Biochemistry

Rank: Associate Professor

Title of Project: Rotational barrier study of novel compounds that can function as nano-scale switches and sensors

Year of Project: **Summer 2020**

Amount Requested: $3,700

Plan for Fellowship Funds
- ☐ Salary Reimbursement/Stipend
- X Project Reimbursement (provide budget justification in narrative)
- ☐ Both (provide budget justification in narrative)

Project Summary (100 words):

The project goal is to create compounds with potential function as memory storage molecules, open/close gates for drug delivery, and biological sensors for the detection and study of cancer and as parts of nano-machines. The proposed work is related to specific testing needed to confirm that the compounds we are using are heat stable. The data collected on how heat affects the compounds is itself very important and will be presented and prepared for publication in the fall. The data obtained will also allow us to investigate the sensing potential of these compounds as part of future studies.

Where will the results be published, exhibited or performed? Published: Journal or Organic Chemistry or Journal or Physical Organic
Presented: American Chemical Society regional Meeting Fall 2020

What other sources of funding (internal and external) have you identified for this project?
August Elmer, Jr. Distinguished Professorship in Chemistry

List years and amounts of prior Loyola University faculty grants (for the last three years):

2017, Bobet Fellowship, "Evaluation of sensor/analyte interactions for a Rhodamine Based Chiral Sensor developed in our lab," Supported 1 student, conference travel, and supplies. Funding source: Loyola University New Orleans College of Humanities and Natural Sciences Dean's office. Amount awarded: $2,700. PI: Clifton J. Stephenson.

2016, Bobet Fellowship, "Formation and study of the interactions of rhodamine based sensors," Supported 1 student, conference travel, and supplies. Funding source: Loyola University New Orleans College of Humanities and Natural Sciences Dean's office. Amount awarded: $2,700. PI: Clifton J. Stephenson.

2016, Faculty Research Grant, "Formation and testing of Novel Fluorescent compounds for sensing," Loyola University New Orleans. Amount awarded: $3,500. PI: Clifton J. Stephenson.

Does your research involve human subjects? ____ Yes __X___ No.
If yes, funding for this project is contingent on receiving IRB approval. If you have IRB approval prior to submitting your proposal, please attach the approval memo to your application. If you do not have IRB approval at the time of your submission, please complete the IRB protocol as soon as possible after your proposal submission.

I have submitted the Employee Conflict of Interest Disclosure Form. __Y___

I have read and understand the University's reimbursement policy. __Y___

Narrative Description of Project:

See attached

MARQUETTE FELLOWSHIP PROPOSAL TO
LOYOLA UNIVERSITY NEW ORLEANS
UNIVERSITY COMMITTEE ON INTERNAL GRANTS

SIGNATURES

Name: CJ Stephenson

College/Department: CAS/Chemistry and Biochemistry

Rank: Associate Professor

Chair/Professorship: August Elmer, Jr. Distinguished Professor in Chemistry

Date Submitted: 11/22/2019

Title of Project: Rotational barrier study of novel compounds that can function as nano-scale switches and sensors

[signature] 11/22/2019
Applicant Signature Date

DEPARTMENT CHAIR/DEAN'S SIGNATURE (if applicable)

I have reviewed the application and acknowledge that the department supports this project.

William Walkenhorst [signature] 11/22/19
Department Chair (Print Name) Signature Date

I have reviewed the application and acknowledge that the college supports this project.

URIEL QUESADA [signature] 11/22/19
Dean (Print Name) Signature Date

Rotational barrier study of novel compounds that can function as nano-scale switches and sensors

The goal of this project is to create and test novel compounds that undergo structural changes under stimuli such as heat and acidity. These compounds are referred to as a molecular switch. Molecular switches often change in such a manner that the new shape is maintained after the stimulus is removed. This affords compounds that can function as memory storage molecules, open/close gates for drug delivery, biological sensors for the detection and study of cancer,[1-5] and as parts of nano-machines (a structure made of only a few molecules that can do work). The proposed work will add essential innovation to this field and help the PI quickly publish this important work.

Herein, is proposed work related to a specific type of testing needed to confirm that the compounds we are using are stable and that they will function as switches and sensors. The data collected on how heat affects the compounds is itself very important and will be published as a part of a collaboration with a professor at Xavier. The data obtained through this project will also allow us to investigate the sensing potential of these compounds as part of future studies.

The compounds used to make these switches are derivatives of the dye rhodamine B. Rhodamine B can exist in both a colorless, non-fluorescent (off) form and a colored, fluorescent (on) form (**Figure 1a**). (Fluorescence is the ability of a compound to give off light when exposed to certain types of light.) The ability for rhodamine derivatives to transition between a non-fluorescent and a fluorescent form provides an ideal mechanism for detecting environmental changes. Molecules that can change from no signal (off) to a strong signal (on) can make very sensitive sensors. For these compounds to function as the desired sensors and switches they must be chiral. Chiral means that the pair of compounds is almost identical but is not imposable mirror images. Human hands are chiral because they are mirror images but are not the same. For molecules, the property of being chiral is important for interacting with may biological molecules such as proteins and DNA.

To use these compounds as the desired molecular switches and sensors the top rectangle must be asymmetric and the blue circle must be locked onto one side at room temperature (**Figure 1b**). The main focus of this project will be to see how long it takes for the blue circle to change sides when heated. The amount of energy needed to move the blue ball to the other side of the molecule is known as the rotational barrier. We will study how structural changes affect this barrier. Knowing how the structure will affect the barrier will allow us to make better sensors and switches. This is important information to the scientific community that will be readily publishable upon its completion.

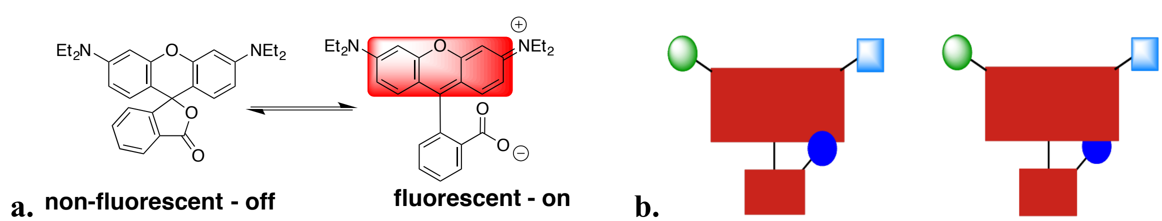
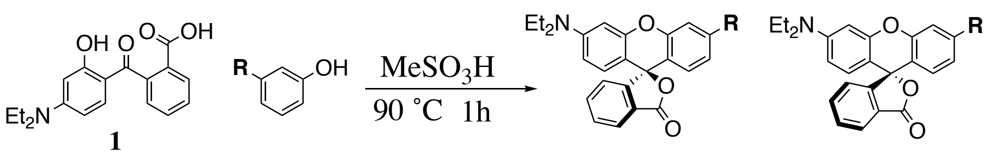


Figure 1a. The two forms of rhodamine. Conversion from one form to the other can be used as a signal for sensors. **1b.** Cartoon showing that asymmetric forms of rhodamine can switch between two different states.

This project will focus on three specific aims. **Aim 1**: Form and purify the remaining compounds in the set we are testing. **Aim 2**: Perform tests to carefully measure the rotational barriers. **Aim 3**: Use the acquired data and computer models to complete a manuscript related to this work and submit to a high-impact journal. My research group has already made progress towards these aims, and the Marquette fellowship would allow me to complete the work needed to publish our results related to forming and testing the rotating aspect of these important sensors. This work will be done with the assistance of student researchers. Research students learn important lab skills such as making and purifying compounds. Students who work on the project will be coauthors on publications and presentations. Student research experiences help students prepare for graduate school and careers.

My research group has already produced a range of prototype fluorescent switches that have potential as sensors. The approach used for forming these derivatives will involve forming novel rhodamine derivatives starting from two initial materials, phthalic anhydride (**1**) and substituted phenols (**Scheme 1**).[6-8] Compound **1** is commercially available and will be heated in methylsulfonic acid with a series of compounds to form the asymmetric rhodamine derivative. My research group has made, crystalized, and published some of these compounds and is working on publishing the other derivatives.[9] The rotational barriers not published. Once compounds all compounds in Scheme 1 are made and purified their rotational barrier will be measured. My group will then examine the role different structural elements have in raising and lowering the rotational barrier.



Scheme 1. Scheme for the formation of an asymmetric rhodamine derivative that will function as a highly fluorescent sensor. Compounds made: R= F, Cl, Br, I, MeO. Compounds to make: NO_2, Ph, OAc.

The rotational barrier is tested by heating the compounds in conditions where they can rotate and then recording the rate of change by measuring the rate of conversion from state **A** to state **B** at set time intervals (Figure 2). Previous attempts at measuring these barriers have been complicated by non-ideal conditions. Last summer we developed a much better method and now have preliminarily measured the rotational barrier for three derivatives and confirmed our results with computer models. We need more sensitive equipment to finish our studies. This equipment will be purchased by spring 2020 and will allow the completion of this project in summer 2020 if the funds requested are provided. The completion of this novel work is critical to expanding the range of rhodamine sensors available to those using these compounds for bio-imaging.

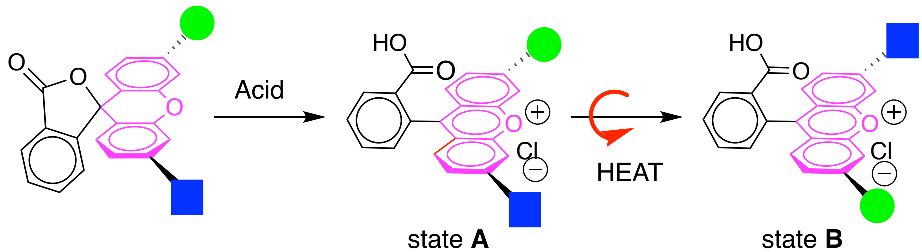


Figure 2: Depiction of the rotation of rhodamine derivative to convert from states **A** to **B** with heat. When heated the pink potion flips over to create state **B**.

Finally, the completion of the rotational barrier studies will provide the needed information for the presentation and publication of this data. This rotational barrier data will be compared to computer models being developed by a collaborator at Xavier. This comparison will afford important information on how the structure changes the rotational barrier and how the rotation happens. This important information will be published in a high-quality, peer-reviewed journal.

The data attained through this grant will be presented at a major regional chemistry conference that will be hosted in New Orleans in the fall of 2020. This conference will allow both myself and the students who work on the project to present the data. Presenting this data at conferences is an important part of meeting others in my field and finding potential grant and journal reviewers. Student presentations help them stand out as they prepare to enter a graduate program.

The funds requested herein will provide student researcher salary and supplies. These funds are an important part of the completion, publication, and presentation of the proposed work (see timeline). Recent grant reviews I have received have suggested that having more publications in my field will greatly increase my chances of state and federal funding. This grant would afford me the opportunity to publish the proposed work in advance of the 2020 fall grant deadlines. The data obtained through this project will then be used to help us form sensors and molecular switches that may have applications to studying cancer, drug delivery, and nanotechnology.

The requested funds will pay a Loyola student to work with me part-time throughout the summer and buy the supplies needed to complete the work described above. Student involvement in this work is not only critical to the completion of the project, but is very important training for the students preparing for graduate school or medical school, and also make students more competitive for these programs.

References:

1. Y. Zhou and J. Yoon, *Chem Soc Rev*, 2012, **41**, 52-67.
2. J. Yin, Y. Hu and J. Yoon, *Chem Soc Rev*, 2015, **44**, 4619-4644.
3. J. Y. Xin Zhang, Juyoung Yoon, *Chem Rev*, 2014, **114**, 4918-4959.
4. L. Pu, *Accounts Chem Res*, 2012, **45**, 150-163.
5. K. W. W. Bentley, C., *J Org Chem*, 2014, DOI: 10.1021/jo500959y.
6. M. Beija, C. A. M. Afonso and J. M. G. Martinho, *Chem Soc Rev*, 2009, **38**, 2410-2433.
7. H. B. Yu, Y. Xiao and H. Y. Guo, *Org Lett*, 2012, **14**, 2014-2017.
8. M. J. Uddin and L. J. Marnett, *Org Lett*, 2008, **10**, 4799-4801.
9. C. J. Stephenson, J. T. Mague, N. Kamm, N. Aleman, D. Rich, Q.-N. Dang and H. V. Nguyen, *Acta Crystallographica Section E Crystallographic Communications*, 2017, **73**, 327-333.

Marquette Faculty Fellowship – Time Table

CJ Stephenson

Title: **Rotational barrier study of novel compounds that can function as nano-scale switches and sensors**

This work will take place in three phases.

Phase I. Complete the measuring of the rotational barrier for compounds available in my lab. (Three – four weeks)

Phase II Evaluate data and make any other compounds needed to complete for comparing set of rational barrier. (One - two weeks).

Phase III. Work with student research and collaborator to write drafts of the manuscript and prepare presentations for fall American Chemical Society Conference in New Orleans. Three to four weeks)

Marquette Faculty Fellowship – Budget

CJ Stephenson

Title: **Rotational barrier study of novel compounds that can function as nano-scale switches and sensors**

Total request: $3,700

Student stipends: $2600
200 hours (25 hours a week for eight weeks) of research student work at $13.00 an hour.

The money will be used to pay one student to work on this project. Student research assistants are needed to make rapid research progress and are an important part of students learning.

Supplies: $1100

- $400 Solvents and compounds
- $400 Separations materials
- $300 glassware, filters, and vials

These supplies are needed to for the testing described herein.

CENTER FOR FACULTY INNOVATION
FINAL PROJECT REPORT - UNIVERSITY COMMITTEE ON INTERNAL GRANTS

If you have any questions, please contact Tarana, the internal grant coordinator at (504) 864-7055. Please forward the completed report to lawrence@loyno.edu.

Name: CJ Stephenson

College and Department: Arts and Sciences, Chemistry

Type of Award Received: ___ Marquette Faculty Fellowship

___ Faculty Development Grant

__X_ Faculty Research Grant

Project Title: Formation and testing of novel fluorescent compounds for sensing

Report

Describe the activities performed under the grant. Explain how these activities contributed to fulfilling the goals of the grant as specified in your application.

Over the summer Dayla Rich, Nathalie Aleman, and Victoria Odom worked to synthesize and purify compound **7** and **8** (Scheme 1). Dayla focused on the separation of compound **7**. **7** consist of two compounds that are mirror images. Separating mirror images is difficult, but as necessary for preliminary testing. After Dayla separated these compounds, Nathalie was able to do preliminary test that showed evidence of chiral sensing (Figure 1). This result is a pivotal piece of information that will be used as preliminary data for an upcoming grant submission.

Scheme 1. Synthesis of compounds

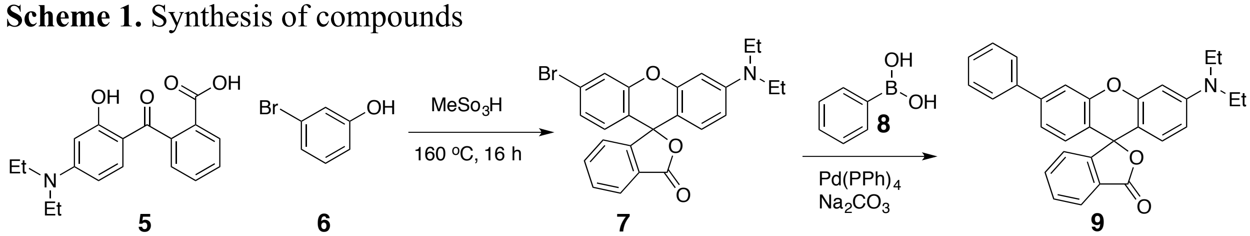
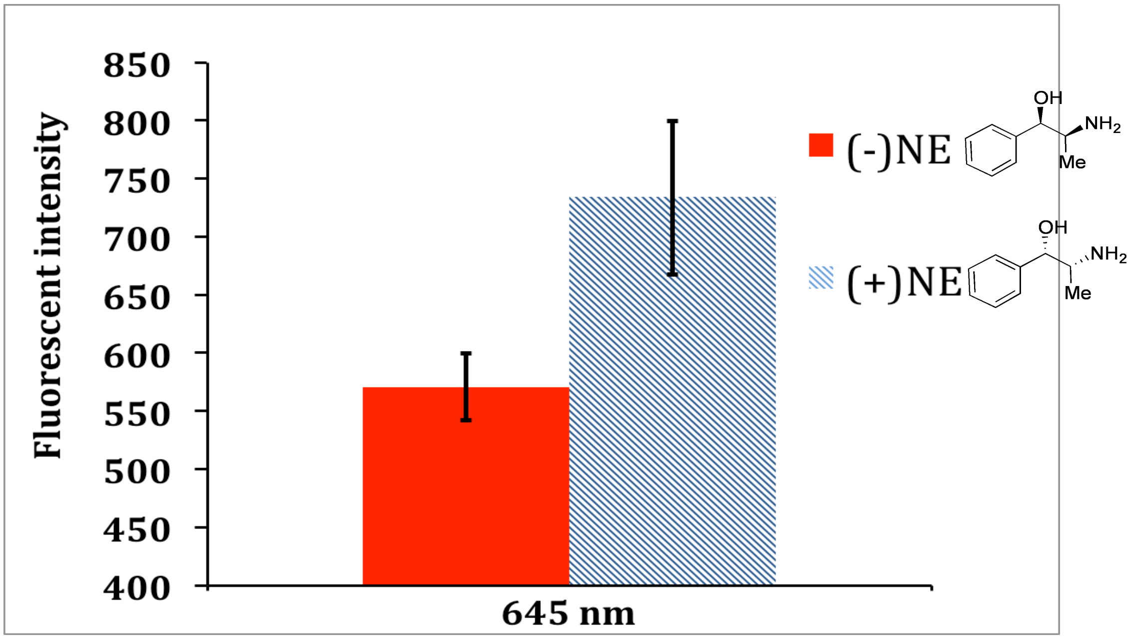


Figure 1. Key test showing sensing potential of compound **7.**

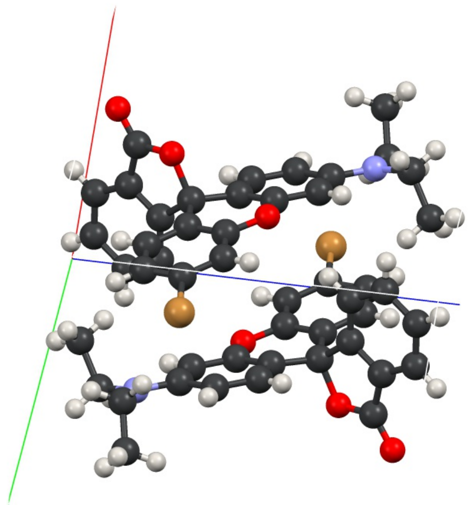


Additionally Dayla and Nathalie were able to grow several different crystal structures of the compound **7** (Figures 2 a,b). These structures are going to submitted for publication this fall. The manuscript was sent to our collaborator earlier this week.

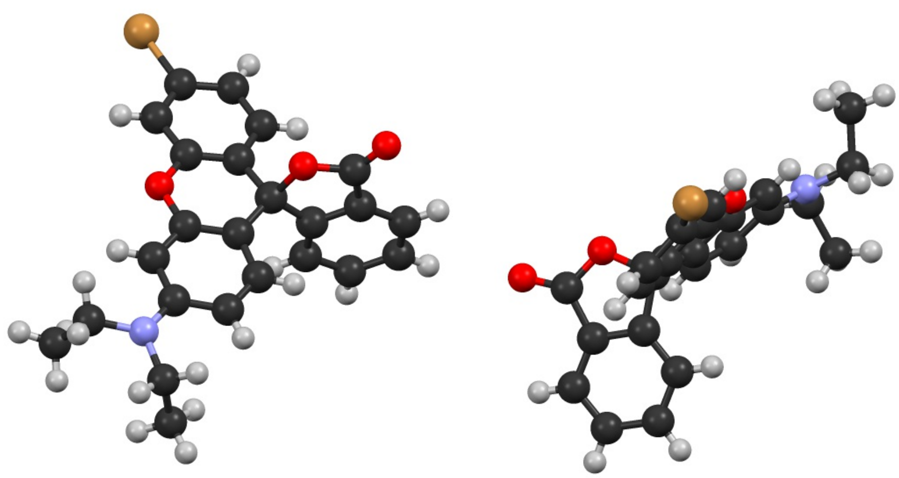
The work described above competed phase I and II and this data was presented at an international conference this summer and will be presented again this fall at a regional chemistry meeting. Dalyla and Nathalie will each present a poster and I will give a talk related to this work.

Phase III is still in progress and we expect the crystal structure paper to be submitted this fall.

Figure 2 a,b. Two different crystal structures of compound **7**. These structures will be submitted as part of a publication to be submitted this fall.



a.



b.

Were any goals and/or objectives not completed? If yes, please explain.

Phase III is still in progress and we expect the paper to be submitted this fall.

What were the outcomes/products from the grant? (Please provide citations if available.)

The data produced from this grant was described above. This work has resulted in one poster at an international conference. In late October, this data will be presented and the South East Regional Meeting of the American Chemical Society. Dayla and Nathalie will present posters and I will present a talk.

Development of Chiral Sensors Based on the Supramolecular Interactions of Rhodamine Derivatives with Organic Analytes; Justin Romaire,[a] Dayla Rich,[a] Nathaniel Kamm,[a] Nathalie Aleman,[a] Michael Maher,[b] and Clifton J. Stephenson. International Symposium on Macrocyclic and Supramolecular Chemistry, July 10-14, 2016, Seoul, South Korea.

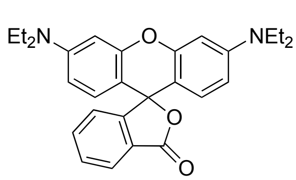
What are your plans to build upon the work that you did under this grant?

Dayla and Nathalie are continuing this work this fall. We hope to finish more testing and then submit a second paper based on this project. Additionally, the results of this project are being used as critical preliminary data for and upcoming Board of Regents grant that will be submitted by November 7th.

The funds provided by the Marquette fellowship allowed me to spend nine weeks this summer working with Loyola students on the formation of a fluorescent sensor for detecting stress. This funding allowed my research group and me to focus on the formation of the mechanophore pre-polymer, which is a key goal of this fluorescent stress sensor project. We were able to form a mechanophore pre-polymer and make significant progress on the formation of a second mechanophore pre-polymer. We were also able to start the initial testing with these compounds. Additionally, the data collected this summer will be presented by students at the spring meeting of the American Chemical Society.

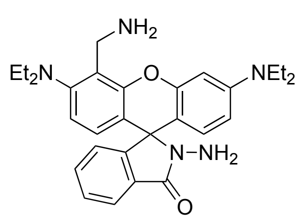
Rising junior Farihah Haque worked with me on this project for 8 weeks at about 35 hours a week. Additionally, rising senior Justin Romaire was helping with the mechanophore project part-time. This report will focus on three aspects of this project that were worked on this summer. 1) Formation of a rhodamine compound that will function as a mechanophore. 2) Formation of a fluorescein compound that will function as a mechanophore. 3) Preliminary testing of the mechanical properties for compounds similar to the mechanophores we are forming.

1) Initial attempts to form a mechanophore focused on the modification of rhodamine B (**1**) to form a mechanophore pre-polymer (**2**) that could be used to form multiple types of polymers. (Figure 1). The first step of this process was adding a modifiable group to the fluorescent portion of the dye to form compound **3** (scheme 1). Compound **3** was formed and fully characterized by Justin. Farihah then used compound **3** to try multiple paths for forming the rhodamine mechanophore (**2**). Method A involved forming the reactive amine on the top group and then adding the second amine group, while method B involved adding the second amine and then forming the reactive amine at the top group (Scheme 1). We were able to confirm that intermediate **4** was formed but were not able to fully purify it due to its reactive nature. When compound **2** was formed via intermediate **4**, we obtained a mixture of products that was very difficult to separate; therefore, method B was attempted. Method B did yield a pure compound for intermediate **5**, but when compound **2** was formed from **5**, mixed fractions were again obtained. Also of note, this is the first known synthesis of compound **5**. I feel that we have made significant progress towards forming this rhodamine-based mechanophore. We have learned a lot about the potential difficulties of this process and this knowledge will allow us to move forward with both this and other similar mechanophores. Methods for forming pure compound **2** are still of interest to our group and we hope to solve this problem this academic year.



1

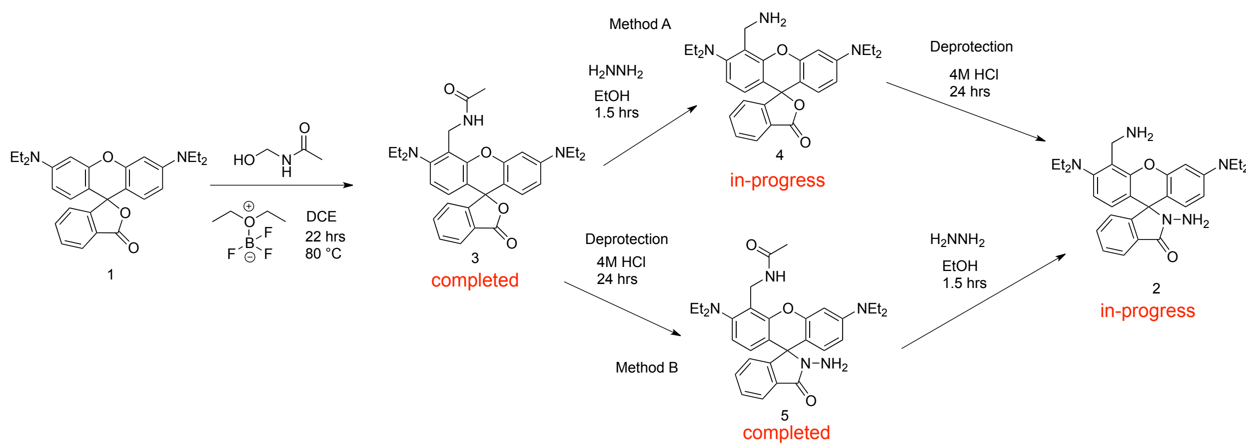
Rhodamine B



2

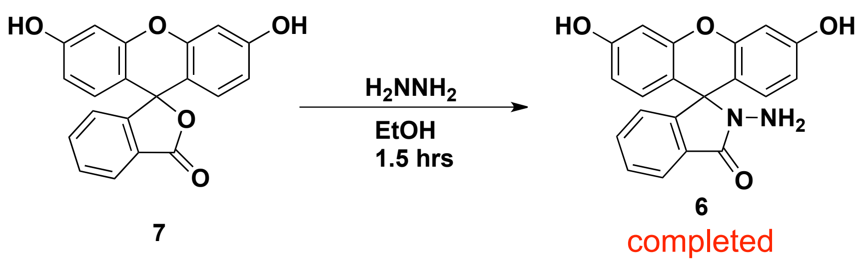
Rhodamine mechanophore pre-polymer

Figure 1: Structure of rhodamine B starting material (**1**) and mechanophore target (**2**)



Scheme 1: Formation of rhodamine mechanophore pre-polymer

2) The problems experienced with formation of the rhodamine mechanophore inspired us to attempt to form mechanophore (**6**) with a similar dye called fluorescein (**7**) (Scheme 3). Fluorescein is potentially advantageous because the two OH groups on the upper ring should be reactive enough to be used for forming a mechanophore. Farihah has been able to form, purify and characterize compound **6**, and we are currently attempting to make two different type of mechanophore polymers with **6**.



Scheme 2: Formation of fluorescein mechanophore pre-polymer

3) Mechanophores are known to open when intense sonic vibrations are applied.[1] In order to show that the dyes we are working with do exhibit this mechanical property, several different rhodamine derivatives were tested. A fixed amount of the rhodamine derivatives were dissolved in two different solvents. The solutions were then tested with a high frequency sonic probe and any change in color was observed (Table 1, Figure 2). These tests did show a color change for some of the derivativeswhich demonstrated that the rhodamine derivative should be able to function as a mechanophore. Additionally, this test provided information of how to conduct more rigorous testing later on.

Table 1: Changes in color of compound by sonication

Compound	**Solvent**	**Change**
Rhodamine Amide (**3**)	Acetonitrile	No change
Rhodamine B Base (**1**)	Acetronitrile	Pink to slightly darker pink
Rhodamine B Base (**1**)	Toluene	No change
Rhodamine B Hydrazide	Acetonitrile	Mostly colorless to light pink
Rhodamine B Hydrazide	Toluene	Colorless to slight yellow tint
Rhodamine B Amide Hydrazide (**2**)	Acetontrile	No change
Rhodamine B Amide Hydrazide (**2**)	Toluene	No change

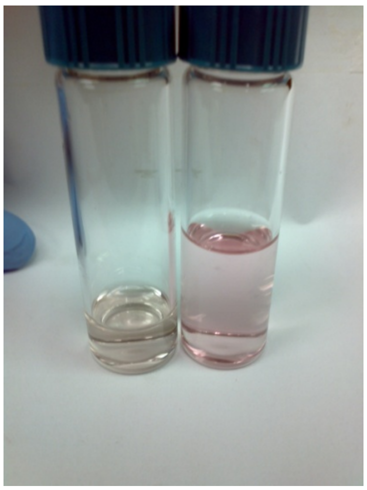


Figure 2: Rhodamine B hydrazide before (left) and after (right) sonication. Solvent is acetonitrile.

Conclusions

The Marquette Fellowship enabled me to work with current Loyola undergraduates to make strong progress towards the formation of a fluorescent sensor for stress detection. This semester Farihah Haque is continuing to work on forming mechanophore pre-polymers, and a new research student, Zoe Glick, has started working on forming a polymer with the fluorescein compound (**6**). Thank you for your time and support.

1. S. L. Potisek, D. A. Davis, N. R. Sottos, S. R. White and J. S. Moore, *J Am Chem Soc*, 2007, **129**, 13808-+.