DNA Binding, Photoswitching Nanoprobes

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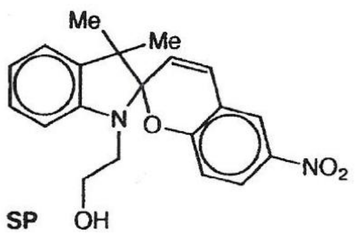
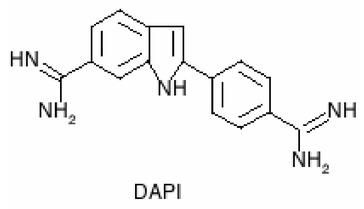
Introduction

The need to analyze what happens within a cell has become exceedingly important, but accurate imaging has been an obstacle in that endeavor. The goal of this research was to make a molecular probe that can bind to DNA (using a fluorescent DNA dye, DAPI, which binds to the minor grooves of the double-helix structure of DNA) and by using a spiropyran photoswitching molecule that also fluoresces, changes from colorless to purple, and can be used to image the targeted DNA within the cell.



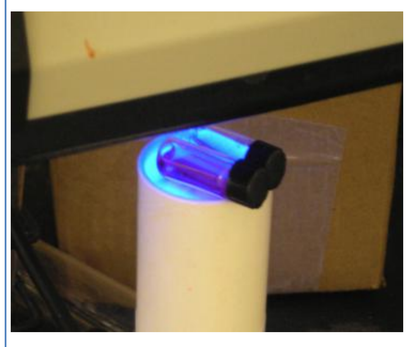


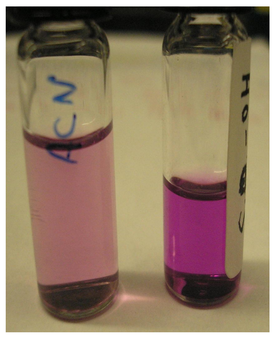
Applications

- High-resolution cell imaging
- Cytometry
- Chromosome staining

Accurate targeting and bright images are needed for measurements within the cell, both of which can be accomplished with this synthesized probe. All of these applications can be quantitatively measured and reported using various forms of microscopy or spectroscopy.

SP molecule irradiated with UV light switches color.



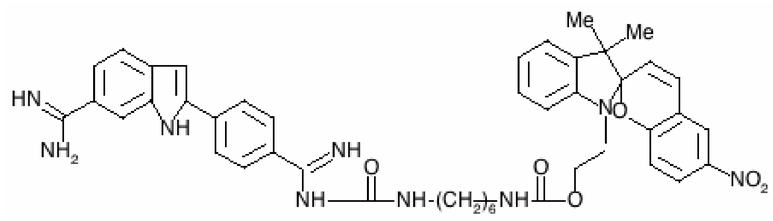


Method

The project was comprised largely of organic synthesis, purification, and characterization techniques. Three steps of synthesis were needed to yield the photoswitching nanoprobes. Variable conditions and apparatus were used to complete the project including molecular sieves, nitrogen atmosphere to protect from moisture, variable temperatures and concentrations, and preventing active groups of the linking molecule (1,6 Diisocyanatohexane) from polymerizing.

1. Synthesis of spiropyran derivative SP.
2. Attaching linking molecule to SP.
3. Attaching DAPI to opposite end of linking molecule.

The final product is depicted below.



In order to track progress during the many reactions Thin Layer Chromatography (TLC) was used to monitor reactions and purification. Filtration, washing, and flash column chromatography were used to purify the products.

Nuclear Magnetic Resonance (NMR) was an essential characterization technique during the research. NMR provided a good indication to whether or not the desired product, during the many steps, had been synthesized.

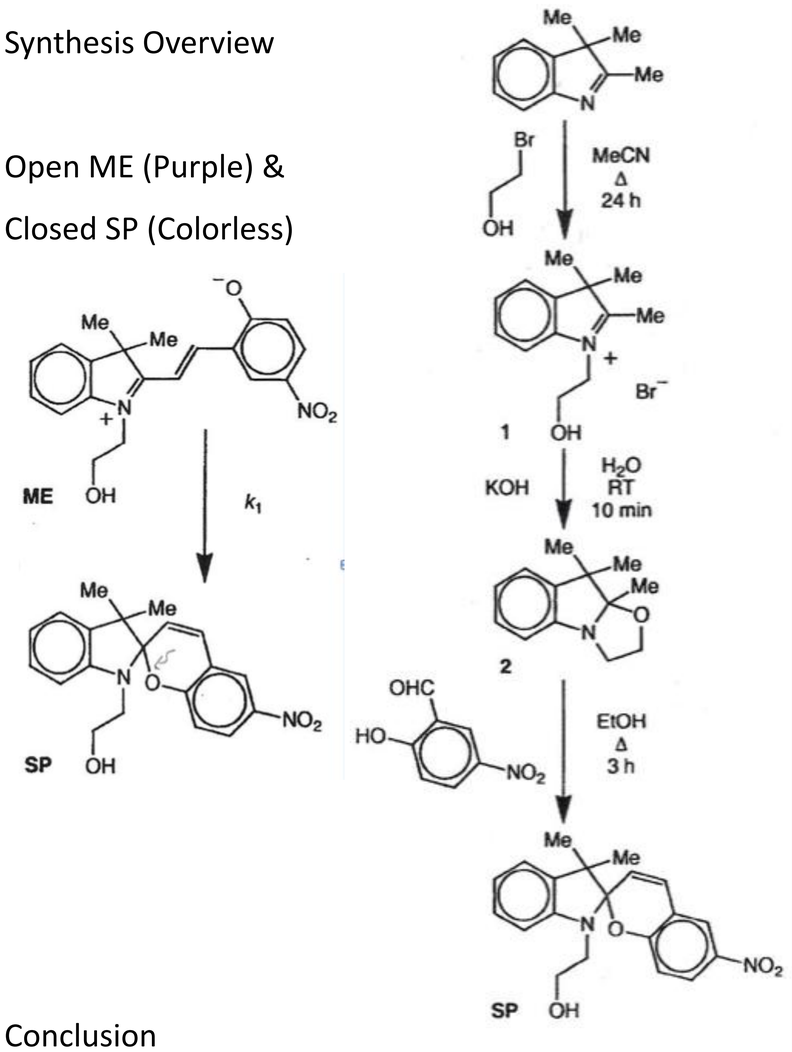
Future Directions

The next phase would be to utilize the photoswitching molecular probes for quantitative in vitro or in vivo DNA imaging and other applications. Either the DAPI or SP molecule can be further functionalized and attached to other biomolecules and be used in a myriad of other applications, such as photoswitching nanoparticles, targeted delivery in disease treatment, and high-resolution cell imaging among others.

Synthesis Overview

Open ME (Purple) &

Closed SP (Colorless)



Conclusion

The work that was done this summer was successful and fulfilling. The research project that was planned and discussed with the faculty advisor was accomplished in just 10 weeks, and largely completed alone with some guidance. Dr. Li's lab and the Department of Chemistry benefitted from all of the research that the REU students completed, and more work is yet to be done.

Thank you to Dr. Li, and to all who who have helped me.

This work was supported by the National Science Foundation's REU program under grant number 0851502.