JOURNAL OF CHEMICAL EDUCATION
Laboratory Experiment
pubs.acs.org/jchemeduc

Solvent-Free Synthesis and Fluorescence of a Thiol-Reactive Sensor for Undergraduate Organic Laboratories

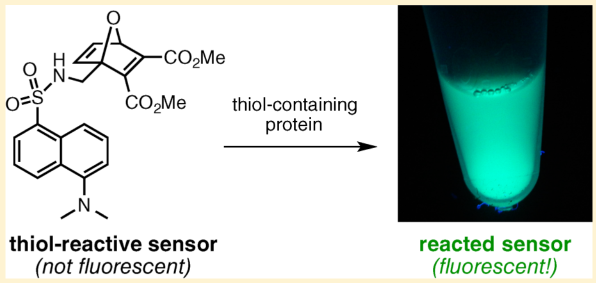
Anastasia L. Patterson,[†] Mary D. May,[†] Bryan J. Visser,[†] Alexander A. Kislukhin,[‡,§] and David A. Vosburg*,[†]

[†]Department of Chemistry, Harvey Mudd College, Claremont, California 91711, United States

[‡]Department of Chemistry, The Scripps Research Institute, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: A green organic laboratory experiment was developed in which students synthesize a sensor for thiols using a microscale, solventless Diels−Alder reaction at room temperature or 37 °C. The molecular probe is easily purified by column chromatography in a Pasteur pipet and characterized by thin-layer chromatography and NMR spectroscopy. The thiol-reactive sensor becomes intensely fluorescent upon exposure to thiols from *N*-acetylcysteine, bovine serum albumin, or human hair (pretreated with a reducing agent to reveal cysteine thiols in *α*-keratin). This fluorescence is observable even with micrograms of probe.



KEYWORDS: *Second-Year Undergraduate, Organic Chemistry, Laboratory Instruction, Hands-on Learning, Fluorescence Spectroscopy, Green Chemistry, Microscale Lab, NMR Spectroscopy, Organosulfur Compounds, Synthesis*

The phenomenon of fluorescence finds numerous applications in the life sciences and analytical chemistry. Fluorescence microscopy has contributed greatly to an understanding of the composition and structure of cells, and fluorescent sensors that can respond to changes in physiological states provide further insight into the dynamics of cellular processes.[1] The analytical applications of fluorescent sensors and probes include detection of natural and synthetic compounds in a variety of settings. Fluorometric sensors are typically much more sensitive than colorimetric sensors because the changes in emitted light are detected more readily than changes in absorbed light. Indeed, the detection of single molecules is possible using specialized fluorescence techniques.[2]

Due to their scientific importance and visual appeal, fluorescent sensors are ideally suited for laboratory experiments. Examples from this *Journal* include determining the critical micelle concentration of detergents,[3] the binding parameters of cyclodextrin complexes with solvatochromic dyes,[4,5] and the pH inside living cells.[6] There is currently great interest in developing undergraduate organic laboratory experiments featuring the synthesis of fluorogenic molecules.[7−9]

An undergraduate organic laboratory experiment is described involving a green, single-step synthesis and chromatographic purification of a fluorogenic oxanorbornadiene (OND) sensor, followed by tests of its reactivity with thiol-containing biomolecules (Scheme 1). The procedure was adapted from an article by Hong et al.[10] The probe itself is not strongly fluorescent because the electron-poor double bond of the OND quenches the fluorescence of the dansyl fluorophore.[11] Thiol addition removes this double bond, generating a fluorescent thiol adduct. The fluorescence quantum yield of the thiol adduct is up to 200-fold greater than that of the unreacted sensor. Exposure of the probe to thiols results in a rapid and dramatic burst in fluorescence that can be readily observed visually.[10] Reactions with other biologically relevant functional groups are substantially slower, with over 1000-fold selectivity for thiols over amines, but protein amines can also react with the OND sensor when thiol concentrations are low.[12]

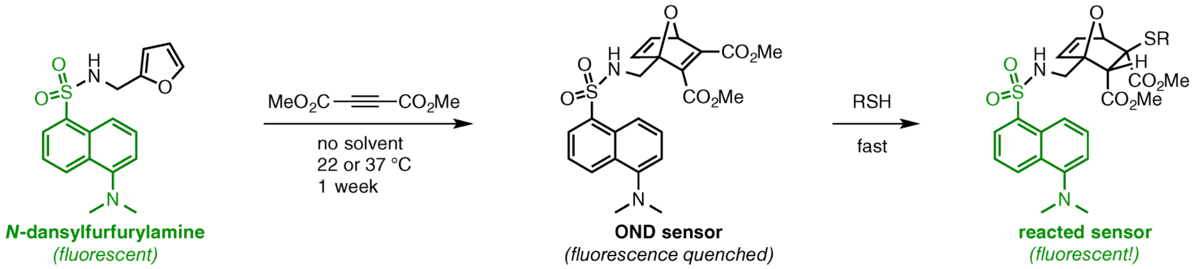
The pedagogic goals for this experiment were for students to predict the structure of their Diels−Alder product, to analyze the Diels−Alder reaction with green chemistry metrics, to detect a biomolecule with fluorescence, and to present their findings in a coherent written report.

■ EXPERIMENTAL OVERVIEW

N-Dansylfurfurylamine is prepared in advance by the instructor from dansyl chloride and 2-furfurylamine (see the Supporting Information and Hong et al.[10]). Students require 15 min at the end of one laboratory period to set up the Diels−Alder reaction and 3−4 h the following laboratory period for product purification and fluorescence tests. Each student reacts *N*-dansylfurfurylamine (16.5 mg, 0.05 mmol) with dimethyl acetylenedicarboxylate (3 equiv) at room temperature or 37 °C with no solvent and no stirring over a week. The crude OND sensor is analyzed on a thin-layer chromatography (TLC) plate with visualization under UV light and purified by silica gel column chromatography in a Pasteur pipet. The purified OND

Published: November 8, 2013

Scheme 1. Synthesis of OND Sensor and Reaction with Thiols



probe is analyzed by ^{1}H NMR spectroscopy, and then it is dissolved in acetonitrile for fluorescence tests with *N*-acetylcysteine, bovine serum albumin (BSA), and human hair pretreated with ammonium thioglycolate (perm salt).

■ HAZARDS

Dimethyl acetylenedicarboxylate is a lachrymator and a vesicant. Dansyl chloride is a skin and eye irritant and will cause inflammation or corneal damage. 2-Furfurylamine and ammonium thioglycolate are skin and eye irritants. Ethyl acetate, hexanes, triethylamine, and *N,N*-diisopropylethylamine are flammable and skin and eye irritants. *n*-Hexane in hexanes is a neurotoxin. Triethylamine and *N,N*-diisopropylethylamine may cause burns on the skin. Acetonitrile is flammable and corrosive. Deuterated chloroform is a cancer suspect agent and mutagen. Silica gel is an inhalation hazard. *N*-Dansylfurfurylamine, the OND sensor, and the thiol adduct should be treated with care, as their hazards are unknown. Gloves and protective eyewear should be worn for this experiment.

■ RESULTS AND DISCUSSION

The experiment has been run in the second semester by 11 second-year undergraduate organic chemistry students one time. The solventless Diels–Alder reaction was very reliable and consistently proceeded in 75–80% conversion after one week at room temperature.[13] Student yields of the purified OND sensor ranged from 30–70%, varying with each student's proficiency with column chromatography, rinsing of column fractions, and accidental spills. A yield of 30% (7 mg) was more than sufficient for ^{1}H NMR analysis and fluorescence testing. Sample student TLC, NMR, and fluorescence data are available in the Supporting Information. The observed fluorescence of the OND probe upon exposure to *N*-acetylcysteine (green) or BSA (blue-green) was remarkable and delighted students. Fluorescent labeling of reduced human hair worked well in some cases but not others; although a comprehensive study was not undertaken, lighter hair generally resulted in greater fluorescence (blue–green) than darker hair after reduction and treatment with the thiol-reactive sensor. A complementary method for covalently labeling hair with a thiol-reactive polymer followed by conjugation to a fluorescent probe has recently been reported,[14] and interested students could pursue additional resources on the chemistry of hair dyeing.[15]

With respect to green chemistry,[16] the small scale minimizes potential waste. Moreover, the Diels–Alder reaction proceeds with no solvent, no heating, and no stirring.[17,18]

The students' laboratory reports demonstrated that all four of the pedagogic goals of the experiment were met. The students correctly drew the structure of their Diels–Alder product, accurately analyzed the Diels–Alder reaction with green chemistry metrics, effectively detected a thiol-containing amino acid and protein with fluorescence, and coherently presented their findings in a written report.

■ CONCLUSIONS

This experiment was suitable for a typical undergraduate organic laboratory course. The thiol-reactive OND sensor was prepared by a solventless Diels–Alder reaction using inexpensive starting materials. The synthesis was simple and allowed instruction time to be devoted to purification and testing of the OND probe. The performance of the sensor was evaluated visually by a rapid rise of fluorescence upon addition of the thiols *N*-acetylcysteine and bovine serum albumin. Labeling of human hair provided an engaging real-world example of protein labeling. Combining the synthesis and fluorescence testing of the sensor exposed students to interdisciplinary applications.[19] Potential extensions of this experiment could include having students synthesize *N*-dansylfurfurylamine, further varying the thiol nucleophiles to observe different colors of fluorescence, and quantifying the fluorescence with a spectrophotometer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Instructions for students; notes for instructors; representative student photos, thin-layer chromatography plates, and NMR and fluorescence spectra. This material is available via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*D. A. Vosburg. E-mail: vosburg@hmc.edu.

Present Address

§Alexander A. Kislukhin is presently at the Department of Pharmacology, University of California—San Diego, La Jolla, California 92093, United States.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the 11 Harvey Mudd College (HMC) undergraduates who performed this experiment in the Chemistry 111 Organic Chemistry Laboratory course, Hayden Hatch for his enthusiastic participation, Stanislav Presolski and Vu Hong for fruitful discussions, and Marisa Hovlid for the first test sample of human hair. Support from the John Stauffer Charitable Trust, the HMC Chemistry Department, the National Institutes of Health grant 1F31CA165653 (A.A.K.), and M. G. Finn is gratefully acknowledged. This paper was

presented at the Spring 2012 National ACS Meeting in San Diego.

REFERENCES

(1) *The Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies*, 11th ed.; Johnson, I. D., Spence, M. T. Z., Eds.; Life Technologies: Carlsbad, CA, 2010.
(2) Zimmerman, J.; van Dorp, A.; Renn, A. Fluorescence Microscopy of Single Molecules. *J. Chem. Educ.* **2004**, *81*, 553−557.
(3) Goodling, K.; Johnson, K.; Lefkowitz, L.; Williams, B. W. Luminescent Characterization of Sodium Dodecyl Sulfate Micellar Solution Properties. *J. Chem. Educ.* **1994**, *71*, A8−A12.
(4) Crane, N. J.; Mayrhofer, R. C.; Betts, T. A.; Baker, G. A. Cyclodextrin Inclusion Complexes with a Solvatochromic Fluorescent Probe. *J. Chem. Educ.* **2002**, *79*, 1261−1263.
(5) Haldar, B.; Mallick, A.; Chattopadhyay, N. Supramolecular Inclusion in Cyclodextrins: A Pictorial Spectroscopic Demonstration. *J. Chem. Educ.* **2008**, *85*, 429−432.
(6) Ribou, A.-C.; Vigo, J.; Salmon, J.-M. C-SNARF-1 as a Fluorescent Probe for pH Measurements in Living Cells: Two-Wavelength-Ratio Method versus Whole-Spectral-Resolution Method. *J. Chem. Educ.* **2002**, *79*, 1471−1474.
(7) Miller, T. A.; Spangler, M.; Burdette, S. C. Lighting Up Protons with MorphFl, a Fluorescein−Morpholine Dyad: An Experiment for the Organic Laboratory. *J. Chem. Educ.* **2011**, *88*, 1569−1573.
(8) Key, J. A.; Li, M. D.; Cairo, C. W. A Fluorogenic Aromatic Nucleophilic Substitution Reaction for Demonstrating Normal-Phase Chromatography and Isolation of Nitrobenzoxadiazole Chromophores. *J. Chem. Educ.* **2011**, *88*, 98−100.
(9) Young, D. M.; Welker, J. J. C.; Doxsee, K. M. Green Synthesis of a Fluorescent Natural Product. *J. Chem. Educ.* **2011**, *88*, 319−321.
(10) Hong, V.; Kislukhin, A. A.; Finn, M. G. Thiol-Selective Fluorogenic Probes for Labeling and Release. *J. Am. Chem. Soc.* **2009**, *131*, 9986−9994.
(11) Guy, J.; Caron, K.; Dufresne, S.; Michnick, S. W.; Skene, W. G.; Keillor, J. W. Convergent Preparation and Photophysical Characterization of Dimaleimide Dansyl Fluorogens: Elucidation of the Maleimide Fluorescence Quenching Mechanism. *J. Am. Chem. Soc.* **2007**, *129*, 11969−11977.
(12) Kislukhin, A. A.; Higginson, C. J.; Hong, V.; Finn, M. G. Degradable Conjugates from Oxanorbornadiene Reagents. *J. Am. Chem. Soc.* **2012**, *134*, 6491−6497.
(13) Greater than 90% conversion of *N*-dansylfurfurylamine was achieved by placing the reaction in a 37 °C incubator for a week instead of room temperature; see the Supporting Information. The resulting student yields would be expected to be higher than those reported here, and product purification would be even simpler.
(14) Slavin, S.; Khoshdel, E.; Haddleton, D. M. Biological Surface Modification by "Thiol-Ene" Addition of Polymers Synthesised by Catalytic Chain Transfer Polymerisation (CCTP). *Polym. Chem.* **2012**, *3*, 1461−1466.
(15) Morel, O. J. X.; Christie, R. M. Current Trends in the Chemistry of Permanent Hair Dyeing. *Chem. Rev.* **2011**, *111*, 2537−2561.
(16) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998.
(17) For another excellent solventless Diels−Alder experiment, see: McKenzie, L. C.; Huffman, L. M.; Hutchison, J. E.; Rogers, C. E.; Goodwin, T. E.; Spessard, G. O. Greener Solutions for the Organic Chemistry Teaching Lab: Exploring the Advantages of Alternative Reaction Media. *J. Chem. Educ.* **2009**, *86*, 488−493.
(18) The Diels−Alder reaction conditions used by Hong et al. in ref 10 for the synthesis of this OND probe require ca. 3 h at 60−65 °C after boiling off CH_2Cl_2 solvent. Either the room temperature or 37 °C protocol described in this article offers obvious practical advantages for teaching laboratories.
(19) Enthusiastic instructors of advanced laboratory courses could extend this experiment to explicitly incorporate biological, analytical, or physical chemistry topics. Students interested in health professions may also appreciate that several functional groups in the sensor (heterocycle, sulfonamide, aromatic ring, amine, esters) are commonly found in pharmaceutical compounds.