Synthesis of a Near-Infrared Emitting Squaraine Dye in an Undergraduate Organic Laboratory

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Supporting Information

Abstract: Squaraines are a class of organic fluorophores that possess unique photophysical properties, including strong near-infrared absorption and emission. The synthesis of many squaraines involves the condensation of an electron-rich aromatic ring with squaric acid. These reactions are generally refluxed overnight in a benzene–butanol solvent mixture. Reported herein are modifications to the synthesis of a squaraine dye that allowed the experiment to be performed in an undergraduate laboratory setting. The desired compound was formed after a 2-h reflux, using toluene as a co-solvent rather than the more toxic benzene. Moreover, the photophysical properties of the synthesized squaraine compound were analyzed in a separate laboratory period and led to important pedagogical opportunities about the absorption and fluorescence of organic compounds.

Keywords: Upper-Division Undergraduate, Organic Chemistry, Laboratory Instruction, Hands-On Learning Manipulatives, Dyes/Pigments, Green Chemistry, NMR Spectroscopy, Synthesis, Thin Layer Chromatography

Squaraines are a diverse class of organic fluorophores that have characteristic narrow absorption and emission bands with high extinction coefficients. They have been utilized in a variety of applications, including as chemosensors for metal ions and thiols, as indicators of membrane polarity, and as guests encapsulated in aromatic macrocycles. Squaraines have also been used extensively for biological imaging.

Although squaraines have been known since at least 1965, they have rarely been studied at the undergraduate level. Searching both the JCE Index online database and the annotated List of laboratory experiments for the keyword “squaraine” provided no prior work on the synthesis of squaraines in educational settings. This is likely due to the long reaction times reported for the synthesis of squaraines. Squaraines such as compound 1 (Figure 1) are synthesized from the condensation of electron-rich aromatic compounds with squaric acid (3,4-dihydroxycyclobut-3-ene-1,2-dione) (compound S) (Scheme 1).

These condensation reactions are typically performed by refluxing the reactants for 16–24 h in a mixed solvent system (either benzene/n-butanol or 2-propanol/tri-n-butyl orthoformate). Nonsymmetrical squaraines (compound 2) and other squaraine derivatives (i.e., compound 3) are also well-known.

Squaraines have a number of potential resonance forms, including the zwitterionic form shown in Figure 1 and Scheme 1. Other possible resonance forms include a polymethine-like form (structure 1', Figure 1) and a biradical form (structure 1''), with some literature suggesting that structure 1’ is the most accurate depiction. Squaraine dyes have been used for a number of fluorescence and imaging applications. For example, Smith and co-workers reported that a squaraine probe could be used to image a bacterial infection in living mice. Squaraines have also been used as energy acceptors in combination with conjugated polymer donors. The reported energy transfer scheme resulted in nearly 100-fold amplification of the squaraine’s fluorescence from polymer excitation compared to exciting the squaraine directly. The adapted procedure reported herein (adapted from ref 11) allowed advanced undergraduate students to synthesize one example of a squaraine dye and to study its photophysical properties.

Pedagogic Value

The synthesis of a squaraine compound introduced the concept of organic dyes to advanced undergraduate organic students. Many colored compounds the students had encountered in their previous undergraduate laboratories were transition-metal complexes, although some organic dyes (i.e., pH indicators) have also been used. In this experiment, the students were introduced to the idea that organic compounds can function as bright, fluorescent dyes, and the applications of these dyes were also discussed.

Another pedagogical opportunity relates to the unique structure of the squaraine core. Although multiple resonance forms of squaraine can be drawn, one option is a zwitterionic structure, with a plus 2 charge on the central ring and a negative charge on each oxygen, as shown in structure 1 (Figure 1). This resonance form gains substantial stability from the aromaticity of the cyclobutene ring, which has two π electrons (4n + 2, with n = 0). The students had learned about the concept of aromaticity in their lecture course, but had not seen...
many examples of aromatic compounds. A discussion of the aromaticity of the squaraine core thus provided multiple instructional opportunities.

A final pedagogical opportunity in the reported experiment is that the procedure for the synthesis of dibenzylaniline represents an example of green chemistry. The reaction was conducted in an aqueous solvent system rather than in more toxic organic solvents. Students benefited from a discussion of the principles of green chemistry.

The specific goals of this experiment are (1) for the students to successfully synthesize an organic fluorophore; (2) for the students to learn about the photophysical properties of the fluorophore by measuring its absorbance and fluorescence spectrum; and (3) to introduce the students to applications of the squaraine fluorophores. This project was performed in three laboratory periods near the end of the semester. These consecutive laboratory periods allowed the students to synthesize the squaraine compound (in two 4-h sessions) and fully characterize the compound by $^1$H NMR, UV−vis, and fluorescence spectroscopy (one 4-h session).

The synthetic sequence reported herein was performed by nine students in an upper-level undergraduate advanced organic laboratory course. Seven students successfully met the first goal by synthesizing the squaraine dye. All the students learned about the photophysical properties of the squaraine (goal 2). Finally, students fabricated hybrid nanoparticles that contained a related squaraine dye and measured the energy transfer from an organic polymer to the squaraine dye. This application of squaraine dyes successfully accomplished goal 3.

**EXPERIMENTAL OVERVIEW**

Aniline (compound 6, 1.0 equiv) and benzyl bromide (compound 7, 2.2 equiv) were dissolved in sodium bicarbonate in water (0.55 M), with a catalytic amount of sodium dodecyl sulfate (SDS; 0.010 equiv) (Scheme 2). The reaction mixture was refluxed at 80 °C for 1.5 h, or until the TLC showed complete consumption of starting material. Aniline 4a was extracted with ethyl acetate and crystallized from hexanes as tan microcrystals in 50−75% yield.

Dibenzylaniline (compound 4a, 1.0 equiv) was mixed with squaric acid (compound 5, 0.50 equiv) and dissolved in a 1:1 toluene/n-butanol mixture (Scheme 3). The reaction was heated to reflux with azeotropic removal of water. After 2 h, the reaction mixture was cooled to room temperature and bright green microcrystals of pure product formed spontaneously. These crystals were filtered and washed with 2-propanol to yield the desired product in yields ranging from 8 to 35%.

**REQUIRED EQUIPMENT AND INSTRUMENTATION**

This experiment required access to $^1$H NMR, IR, UV−vis, and fluorescence spectrometers for a thorough analysis of the squaraine product. If any of these instruments are not available, then the instructor may rely on the remaining instruments to analyze the squaraine product, although this will affect the level of analysis and discussion possible.
HAZARDS

Benzy1 bromide [CAS: 100-39-0] is a strong lachrymator and should only be used in a well-ventilated fume hood. It causes eye, skin, and respiratory tract irritation. Aniline [CAS: 62-53-3] is a combustible liquid with a characteristic odor that may cause irritation. n-Butanol [CAS: 71-36-3] is a flammable liquid and vapor that causes severe eye irritation. Toluene [CAS: 108-88-3] is highly flammable with a characteristic odor. Ethyl acetate [CAS: 141-78-6] and n-hexane [CAS: 110-54-3] are flammable and volatile and should be used with caution. Chloroform [CAS: 67-66-3] and deuterated chloroform [CAS: 865-49-6] are highly toxic and suspected carcinogens. Appropriate personal protective equipment should be used at all times, and the reagents should only be handled in a well-ventilated fume hood. MSDS sheets are freely available from Sigma-Aldrich.33

RESULTS AND DISCUSSION

The first step of the reaction was the synthesis of disubstituted aniline (Scheme 2). This reaction proceeded in high yield (50–75%). The first compound formed was the monosubstituted product ($R_1 = 0.16$ in 95:5 hexanes/ethyl acetate), and students should be aware that this is not their final product ($R_1$ of final product $= 0.32$ in 95:5 hexanes/ethyl acetate). Compound 4a is also commercially available (CAS: 91-73-6) and relatively inexpensive.

The second step was the synthesis of the squaraine dye, which crystallized spontaneously as bright green crystals (Scheme 3). This reaction proceeded in moderate yields (comparable to the literature-reported values of 35%).11 Another potential product that may contribute to these moderate yields is the monosubstituted squaraine product (with only one phenyl substituent). This amorphous solid displays a characteristic pink-red color. Even low yields, however, were sufficient for the photophysical analysis, as students needed only a few milligrams of their final product to obtain chloroform solutions of the desired concentrations.

In the third part of this experiment, students measured the absorbance and fluorescence spectra of dilute (0.50 mg/mL) and concentrated (10 mg/mL) squaraine solutions in chloroform. The dilute solution (bright blue color) had a sharp absorption peak around 630 nm (in the red spectral region). The concentrated solution had a broader absorption peak with an additional shoulder around 570 nm. The shoulder is due to the formation of H-aggregates34 that absorb in that region. Squaraine aggregates are discussed extensively in the literature.35–38 Students learned how this absorption spectrum correlated with the reddish-blue color of the concentrated solution. Both solutions had a strong fluorescence signal (from 600 nm excitation), with a maximum around 670 nm (Figure 2).

Both steps of the synthesis are very robust. The first reaction was run in open air, using an aqueous solvent system. The second reaction was also performed in open air with no deleterious effect on the reaction yield. Small amounts of moisture were also tolerated in the second reaction, because the water was removed with a Dean–Stark trap.

SUMMARY

This experiment was tested by nine students in an advanced organic laboratory during the fall 2011 semester. All of the students obtained pure dibenzylaniline 4a (Scheme 2) in yields ranging from 50% to 75%. Seven of the nine students obtained the desired squaraine product in 8–35% yield. The other two students failed to obtain any of the desired squaraine product, which may be a result of inefficient heating (by using a setup that was too large for the amount of material).

Instructors should be aware of the following problems: in the first step, the first product formed is the monosubstituted aniline ($R_1 = 0.16$ in 95:5 hexanes/ethyl acetate). Students should be aware that this is not the desired end product ($R_1 = 0.32$ in 95:5 hexanes/ethyl acetate). Students may also observe unreacted benzy1 bromide by TLC and should not confuse that UV-activative solvent with their desired product. Dibenzylaniline also melts at relatively low temperatures; if students use mild heating during rotary evaporation of the organic solvents, they may have to let the flask sit at room temperature until the product resolidifies.

Obtaining a good 1H NMR spectrum of the squaraine product is fairly challenging and requires a relatively dilute solution (should look blue to the naked eye). Students who attempted to collect 1H NMR spectra with concentrated samples (those that look red to the naked eye) generally observed only solvent peaks, which is due to the tendency of the squaraine to form aggregates. Representative student data (1H NMR, IR, UV−vis, and fluorescence spectra) can be seen in the accompanying Supporting Information.

CONCLUSION

The two-step synthesis of a squaraine dye is an interesting and robust experiment that can easily be performed in an advanced undergraduate laboratory. It introduces students to new concepts, including the fluorescence of organic compounds and the synthesis of organic compounds using green chemistry principles. The resulting squaraine can be used for a number of interesting applications, including the fabrication of hybrid fluorescent nanoparticles (i.e., similar to the type formed in ref 39).

ASSOCIATED CONTENT

Supporting Information

Synthesis details, student handout, notes for instructor, idealized spectra of all compounds, representative student spectra of all synthesized compounds. This material is available via the Internet at http://pubs.acs.org.
Laboratory Experiment

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**