ARE GREEN ROUTES TO RED MOLECULES REAL? SUSTAINABILITY AND COST-EFFECTIVENESS STUDIES ON THE SYNTHESIS OF HIGH-VALUE INFRARED EMITTING MATERIAL

by

Hannah Sachs

Submitted in partial fulfillment
of the requirements for Departmental Honors in
the Department of Chemistry & Biochemistry
Texas Christian University
Fort Worth, Texas

May 5, 2025

ARE GREEN ROUTES TO RED MOLECULES REAL? SUSTAINABILITY AND COST-EFFECTIVENESS STUDIES ON THE SYNTHESIS OF HIGH-VALUE INFRARED EMITTING MATERIAL

Project Approved:

Supervising Professor: Sergei Dzyuba, Ph.D.
Department of Chemistry & Biochemistry

Kayla Green, Ph.D.
Department of Chemistry & Biochemistry

Sarah Newton, M.F.A.
School for Classical & Contemporary Dance

ABSTRACT

Chemicals compounds that are either in high demand and/or have many valuable applications could be considered high-value chemicals. Arguably, the profitable benefits of high-value molecules run the risk of outweighing both cost and environmental concerns that result from their synthesis, isolation, and purification. As these products have ever-increasing scopes of prized applications that could further inspire high probabilities of mass production, the necessity of defining increasingly sustainable and environmentally benign yet still facile and financially efficient syntheses become exponentially important.

Squaraine dyes are versatile, fluorescent, organic small molecules with spectral profile in the infrared region, a key characteristic that contributes to their identification as broadly and diversely applicable molecules in biological and energy-based processes. Such applications place them in the category of high-value chemicals. Their synthesis as outlined in literature, however, often relies on the use of toxic and volatile organic solvents. This research outlines our group's efforts to develop quantitative, easy to use metrics for assessing synthetic routes to squaraine dyes, driven by defining greener routes. Our sustainability analysis, as driven by a focus on waste production and cost-effectiveness, will furthermore outline the importance of thorough and detailed reporting of synthetic procedures, which is a universally applicable concept for estimating and understanding the impact of high value chemical's syntheses.

INTRODUCTION

Small organic molecules are used across nearly all modern sciences, their presence valued in chemical, biological, materials, and energy-related work. One can image that a small organic compound (i.e., a molecule with molecular weight less than 1000 g/mol) with promising pharmacological application towards a devastating disease would have its placement on the market a priority regardless of the cost of production, amount of waste generated from its synthesis, or the demand it presents on any other resources. On a similar note, creating a molecule capable of detecting early onset neurodegeneration, for example, would be relentlessly pursued for both its medicinal benefit as well as its profitability in both production and for society in general. Furthermore, a compound with the capacity for efficiently harvesting sunlight and aiding in its transformation into electricity would be exceptionally prized in the name of decreasing societal dependance on fossil fuels and sparing natural resources. However, the profitable benefits of such high-value molecules run the risk of outweighing both cost and environmental concerns that result from their synthesis, isolation, and purification. Importantly, as these molecules/materials have prized applications that inspire high probabilities of mass, large-scale production (i.e., kg or ton scale), the necessity of defining increasingly sustainable and environmentally benign yet still facile and financially efficient syntheses for these molecules becomes exponentially important.

There are current gaps of inefficiency within chemistry sustainability assessments. This is a broadly applicable problem but one that stands even more notable in the context of high-value molecules. While the potential profits from placing these compounds on the market are attractive, it is non-negotiable that these compounds be brought to increased production scale responsibly, meaning there must be an awareness on the synthetic costs, both environmental and financial.

This has already partially been asserted through the efforts of green chemistry, which guide the chemical communities' desire to minimize waste and move chemistry into a more sustainable future. Defining sustainable production of high-value molecules can be achieved through the employment of thorough and encompassing sustainability metrics. It could be argued that currently available metrics are not allowing effective analysis of syntheses due to the existence of some gaps and space for vague interpretation in metric parameters. Furthermore, the absence of some synthetic details provided in literature presents an additional obstacle which prevents meaningful comparison of synthetic procedures.

This research is focused on identifying and determining the cost and waste production associated with a high-value chemical synthesis, outlining these as the leading metrics for defining sustainability and environmental impacts. Selecting a relatively narrow lens of metrics for sustainability assessment can be justified as follows: if a chemical is to be brought to large scale production, cost is the parameter of utmost importance to the buyer (both industrial and societal), and the amount of waste generated during synthesis will present the greatest impact to the environment and therefore, society.

Environmental-factor (E-factor) (eq. 1) is a well-established and widely used metric[1], leaving it without the need for significant alterations. This parameter is easy to use and presents information of interest on waste production in a singular number, assuming all amounts from synthesis, isolation, and purification steps can be accounted for.

$$\mathrm{E-factor} = \frac{\text{total mass of waste (g)}}{\text{mass of product (g)}} \qquad \text{(eq. 1)}$$

Navigating cost assessment is unfortunately not as straightforward. Literature accounts often have qualitatively driven estimates for the costs of molecule production, based on assumptions that compounds are more or less expensive depending on the natural availability of precursors. For example, costs of metal catalysts are typically judged by the nature of the metal. Gold, palladium, and ruthenium are expensive, therefore catalysts containing these metals are expensive. Conversely, iron, copper, and zinc are inexpensive, hence their respective catalysts are inexpensive. However, the specific nature (i.e., oxidation state) of the metal, as well as the nature of the ligand that will complex with the metal could be even more important factors determining the cost. It is not unusual for iron-containing catalysts, for example, to be more expensive than those that contain palladium. To remedy such situations, Cost of Academic Methodology (CAM) was proposed (eq. 2).[2]

$$aA + bB \xrightarrow{cC} P$$

A = reactants; B = reagents; C = catalysts

a,b,c = number of equivalents

P = product

$$CAM = \frac{\Sigma\,(aA+bB+cC)(\$\,mol^{-1})}{1\ or\ yield} \qquad \text{(eq. 2)}$$

It could be argued that one metric accounting for waste production (E-factor)[1] and another accounting for financial demand (CAM)[2], should allow for the qualitative assessment of novel green approaches to the synthesis of high-value molecules.

Since the Dzyuba group synthetic efforts are focused on design and synthesis of fluorescence molecules with broad ranges of applications[3], squaraine dyes were chosen as an example of a high-value chemicals to be used for development of metrics that would provide facile, sufficient, and robust quantitative assessments of cost and waste production.

RESULTS AND DISCUSSION

The term “green routes” refers to increasingly sustainable, environmentally and financially conscious approaches to producing organic molecules. The distinction of organic molecules is due to organic syntheses often employing organic solvents which have long been a complication of sustainability as they may be toxic and volatile and tend to be insoluble in water.[4] Sustainability studies have become increasingly top-of-mind since the 1980’s and now have been transformed under the umbrella of green chemistry.[1] In general, green chemistry can be understood as an effort to minimize waste and the use of toxic and/or hazardous solvents and reagents while also efficiently using all materials in the process of chemical manufacturing and application.[1,5] Specifically, green chemistry has been defined by 12 principles (Table 1)[1], which are used as guiding statements that highlight the various ways the chemistry community is approaching the endeavor of sustainability.

Table 1: The 12 Principles of Green Chemistry[1]

1. Waste prevention not remediation	7. Renewable rather than depleting raw material
2: Atom efficiency	8. Shorter synthesis (avoid derivatization)
3: Less hazardous/toxic materials	9. Catalytic rather than stoichiometric reagents
4: Safer products by design	10. Design products for degradation
5: Innocuous solvents and auxiliaries	11. Analytical methods for pollution prevention
6. Energy efficient by design	12. Inherently safer processes

The most notable principles in the context of our research are principles one, three, and twelve which emphasize reducing waste efficiently and using increasingly benign chemicals.

The prevalence of this topic within the current chemistry climate is also evident in the number of journals, which are advanced by many major publishers and chemical societies, in which green chemistry is the focus. Examples include *Green Chemistry* (published by the Royal Society of Chemistry), *Green Chemistry Letters and Reviews* (published by Taylor & Francis), *ACS Sustainable Chemistry & Engineering* (published by the American Chemical Society), *Current Research in Green and Sustainable Chemistry* (published by Elsevier), and *ChemSusChem* (*Sus* stands for Sustainability, published by Wiley).

Our investigation into sustainability is particular towards squaraine dyes as high-value molecules. One characteristic that can evaluate a molecule to such standard is their ability to emit light in the infrared (IR) or near-IR range of the electromagnetic spectrum (Figure 1), where the near-IR range refers to the wavelengths around 700nm and upward.

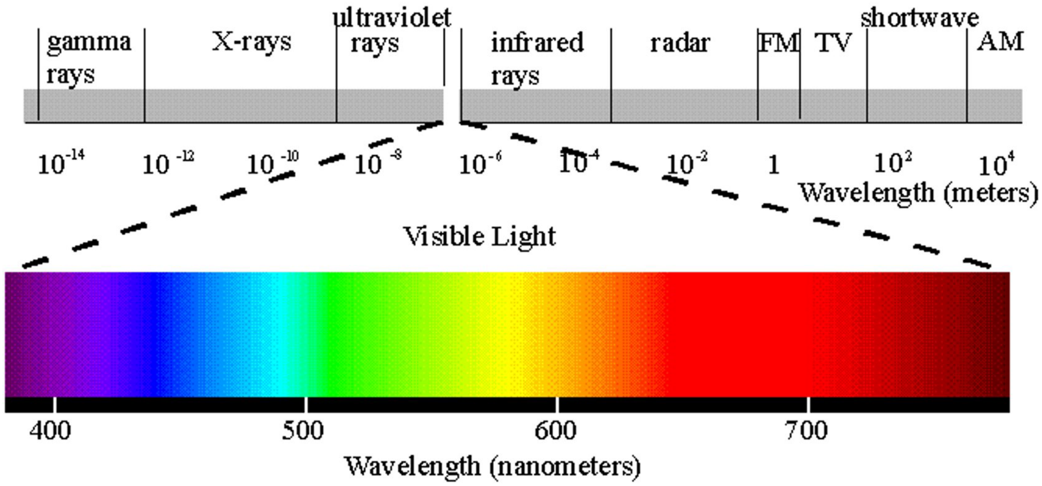


Figure 1. The Electromagnetic Spectrum. Adopted from https://ozonedepletiontheory.info/what-is-radiation/

Thus, this definition is responsible for our label of “red molecules”. Examples of materials that feature this attractive near-IR emission are BODIPY and squaraine dyes (Figure 2). Both dyes have been investigated within the Dzyuba lab since their favorable qualities having led to the establishment of diverse and valuable applications for both.[3,6,7,8,9,10] However, squaraine dyes have more recently been brought into our group’s research focus and a few of their applications are worth noting, especially in the context of our highlighting them as models for high-value chemical driven evolvement of sustainability studies.

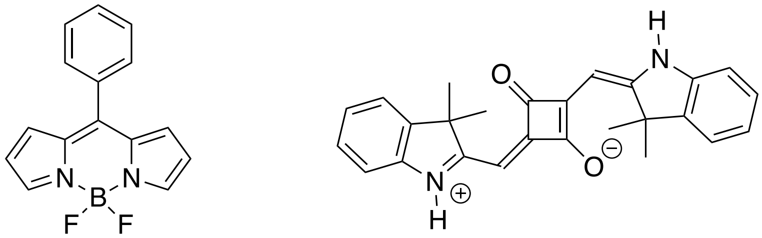


Figure 2. General examples of BODIPY (left) and squaraine (right) dyes.

One significant utilization of squaraine dyes is within photodynamic therapy (PDT). As a light-based therapy which uses a photosensitizer to induce the destruction of tumor cells, PDT is an attractive alternative to traditional cancer treatments made possible through a photosensitizer's production of a highly reactive oxygen species (ROS).[6,11] Once the sensitizer has been transferred into the body and accumulated upon the tumor cells, using the sensitizer’s suitable wavelength then irradiates the target tissue and stimulates production of ROS.[11] Interaction with ROS can lead biological structures to experience an interruption in function which then inspires cell death.[11] The study of squaraine dyes serving in the role of photosensitizer for PDT has been unraveling for years and developed to in-vivo studies.[11] A 2008 study on male swiss albino mice with skin cancer demonstrated squaraines dye's success in inducing tumor-destruction.[12,13] In complementary approaches, squaraine dyes have found success in photothermal therapy (PTT) and bioimaging.[6]

Successful application of squaraine dyes within photovoltaic devices, including organic photovoltaic (OPV) cells, perovskite solar cells, and dye-sensitized solar cells, is also of note.[14] Solar cells are an attractive product for the conversion of solar energy into electricity.[14] Within studies on OPV cells, squaraines have been recognized as a novel, increasingly facile electron donor entity compared to the previous standard oligomers and polymers. Both symmetric and asymmetric squaraine dyes have been found to demonstrate properties that are promising of the enhancement of OPV cell performance.[14]

Most of the applications of squaraine dyes relate to their emission properties which warrants discussion on the general concepts behind this characteristic. In general, the emission of light is known as luminescence (Figure 3).[15,16,17] Upon exposure to light (photon), excitation from the ground state (S_0) to one of the higher energy excited states (S_1 or S_n) occurs. Following this

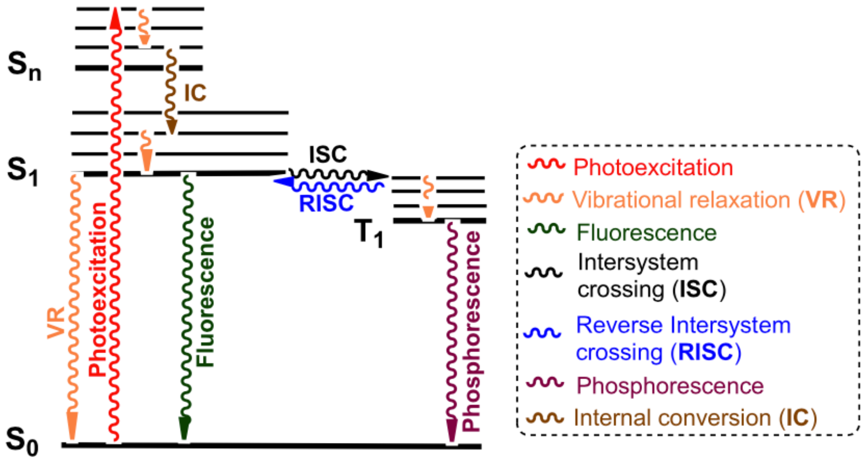
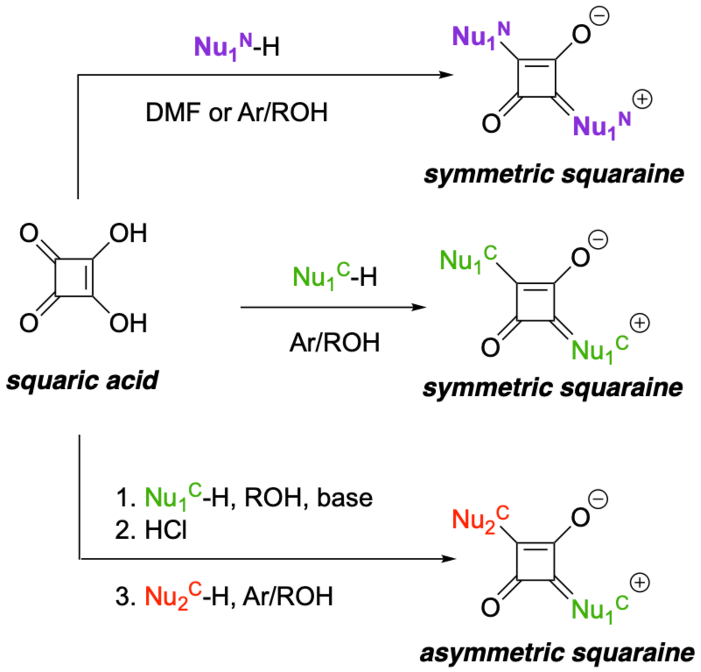


Figure 3. The Jablonski diagram as a graphical representation of luminescence. [16]

photoexcitation, the return of the molecules to its ground level (S_0) occurs through the emission of energy in the form of light (photon) as well as through non-radioactive pathways. Depending on the specific structural features of the molecule, the emission from the excited state could result in either in fluorescence emission or phosphorescence emission (Figure 3). Fluorescence emission occurs on a rapid timescale (an order of nanoseconds) with the shorter wavelengths of the process offering a notable degree of precision.[15,16] Phosphorescence is characterized by a longer lifespan, most often seen within glow in the dark products, where "glow" is maintained for a longer period (typically on the scale of seconds or minutes).[15] Of our interest is fluorescence, which is cemented as a valuable tool across fields, with several current efforts existing to harness and tune the light emission for areas of intrigue. One development, fluorescent proteins, makes way for nuanced investigation into biological processes.[17] Another approach, fluorescent gels, create real-time monitoring and visual feedback.[16]

The relative simplicity of synthetic routes for squaraine dyes relies on the efficient condensation reaction (i.e., smaller molecules reacting to form a larger structure) of readily available squaric acid with nucleophiles. This synthesis allows for structural and functional diversity of nucleophiles, which provides facile access to symmetrical (i.e., two nucleophiles on the squaraine core are the same) and asymmetrical (i.e., two nucleophiles on the squaraine core are different) scaffolds (Scheme 1).[3]



Scheme 1. General representative syntheses of squaraine dyes using squaric acid as the starting material. Nu^C and Nu^N refer to carbon-based and nitrogen-based nucleophiles respectively. Color coding highlights different structures of the nucleophiles. Ar = aromatic solvent (e.g., toluene), ROH = alcohol (e.g., n-butanol).

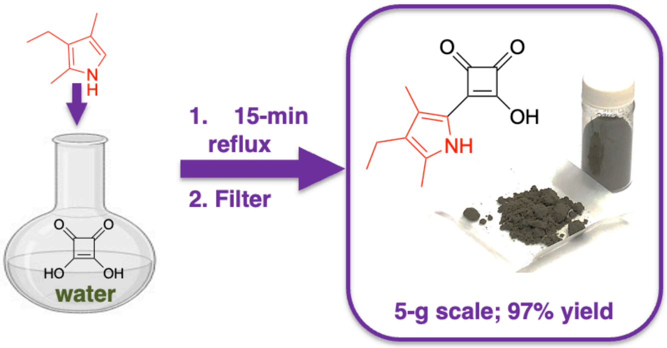
In addition to operationally facile syntheses (Scheme 1),[3] isolation and purification of squaraine dyes can be oftentimes achieved through filtration, recrystallization and/or washing. A variety of solvent systems have been employed, most often at raised temperatures, with the most popular solvents being mixtures of aromatic hydrocarbons (e.g., toluene or benzene) and alcohols (e.g., n-butanol), however both types of solvents are hazardous, toxic, and flammable.[3]

One of the first places to begin in defining novel green routes for the production of high-value molecules is to employ sustainability metrics to create quantitative points of analysis. The roughly three decades of efforts towards studies on greenness has resulted in a multitude of metrics (Table 2)[1], which serve to assess sustainability through various lenses.

Table 2: Some of the mass-based metrics for measuring greenness of chemical processes[1]

E-factor	**Atom Economy (AE)**
$\mathrm{E-factor} = \dfrac{\text{total mass of waste}}{\text{mass of product}}$	$\mathrm{AE} = \dfrac{\text{MW of product}}{\text{sum of MWs of reactants/reagents}}\ x\ 100\%$
Mass Intensity (MI)	**Process Mass Intensity (PMI)**
$\mathrm{MI} = \dfrac{\text{total mass used in the process}}{\text{mass of product}}$	$\mathrm{PMI} = \dfrac{\text{total mass used in the process (incl. } H_2O\text{)}}{\text{mass of product}}$
Reaction Mass Efficiency	**Mass Productivity**
$\mathrm{RME}(\%) = \dfrac{\text{mass of product x 100}}{\text{total mass of reactants}}$	$\mathrm{MP}(\%) = \dfrac{\text{mass of product x 100}}{\text{total mass (incl solvents)}}$
Carbon Economy (CE)	**Effective Mass Yield (EMY)**
$\mathrm{CE}(\%) = \dfrac{\text{carbon in product x 100}}{\text{total carbon in reactants}}$	$\mathrm{EMY}(\%) = \dfrac{\text{mass of product}}{\text{mass of hazardous reactants}}$

Within the Dzyuba group, some of these metrics have been employed to define the greenness of a novel synthesis developed for a semi-squaraine dye that was used to prepare a library of asymmetrical squaraine dyes.[18] It was discovered that an alkylated pyrrole (i.e., kryptopyrrole) could almost quantitatively react with squaric acid within15 minutes of reflux in water (Scheme 2).[18] This method was found to be more efficient when compared to literature accounts, thus prompting application of green chemistry metrics to provide quantitative comparison since the simple use of water is not sufficient for claiming the process as green (or greener). By using standard metrics, such as E-factor, atom economy, mass intensity, and process mass intensity (Table 2), quantitative comparison to the best literature protocol was conducted (Scheme 2).



Green Chemistry Metrics	our procedure	best literature procedure	Comment
E-factor	0.2	6.2	0 is ideal
Atom Economy (AE)	92.4	63.0	100 is ideal
Mass Intensity (MI)	3.1	75.6	lower number is desired
Process Mass Intensity (PMI)	46.9	82.3	lower number is desired

Scheme 2: Synthesis of semi-squaraine synthon and the metrics used for the quantitative comparison to literature procedure.[18]

As previously described, there are a number of sustainability metics to choose from (Table 2), however we define waste and financial demand to be of the utmost importance in outlining the cost of a synthesis. Thus, we choose to move forward with one of the earliest sustainability metrics developed, the Environmental-factor (E-factor) (eq. 1) introduced in 1992.[1,5] This metric's evaluative efficiency and ease of use have distinguished it as a widely adopted metric, accepted worldwide in both academia and industry. The E-factor is easily applied to multi-step processes which is facilitative of offering holistic assessments. The ideal E-factor is zero, aiming to honor the first of the 12 principles of green chemistry (Table 1). Notably, through the E-factor's history, water has been a point of conversation as at one point, it was excluded for fear of skewing results. However, current industrial applications trend towards including water in acknowledgment of the processes required for its disposal or reuse.[1] This outlines the very reason inefficiency exists within sustainability studies, variability in interpretation and prioritization can result in details of the synthetic process being left out. In this research, all materials that are used to produce squaraine dyes will be included in the E-factor calculations.

In conjugation with waste, cost could be of utmost importance in outlining sustainability. A more recently proposed metric that seeks to quantify a financial side of sustainability is the Cost of Academic Methodology (CAM).[2] CAM was developed to initiate conversation on quantitative assessment of the financial demands posed by reagents and catalysts within the academia (eq. 2).[2]

There are several details excluded from the original CAM metric due to the authors decision to make it more user-friendly.[2] Specifically, CAM excludes the cost of standard reagents (e.g., Et_3N, i-Pr_2NEt, NaOH, KOH, HCl, AcOH, etc.) as well as the cost of solvents since their typical cost of 5 $\$ \ mol^{-1}$ should make their contributions to CAM negligible. Additionally, costs of isolation and purification, reaction scale, and reaction time were left out as they are not routinely accounted for or expressed clearly within publications. Finally, material hazards and associated additional shipping costs were further disregarded due to inability to locate and/or estimate those costs.[2]

The exclusion of selected costs within literature by reasoning of negligible contribution or standardization, while potentially making the assessment easier, might leave gaps within the evaluation. For example, volumes of solvents involved in purification steps can quickly add up, which can override their seemingly cheap cost. Specifically in column chromatography, a solvent can quickly become a dominating factor if multiple purification columns are required to obtain pure product. Additionally, the case of recrystallization can similarly demand larger quantities of solvents when repetitive recrystallizations are necessary for desirable purity. In regard to squaraine dyes, it was noted that although in many instances these dyes could be isolated by precipitation in water followed by washing with water and/or organic solvents to obtain pure product, the amounts of solvents (typically in 100-800 ml range) required for those washes were far greater than the amounts of the obtained dyes (typically in 20-100 mg range). Extrapolating such syntheses to larger (i.e., kg or greater) scales could lead to the use of thousands of liters of water and/or other solvents.

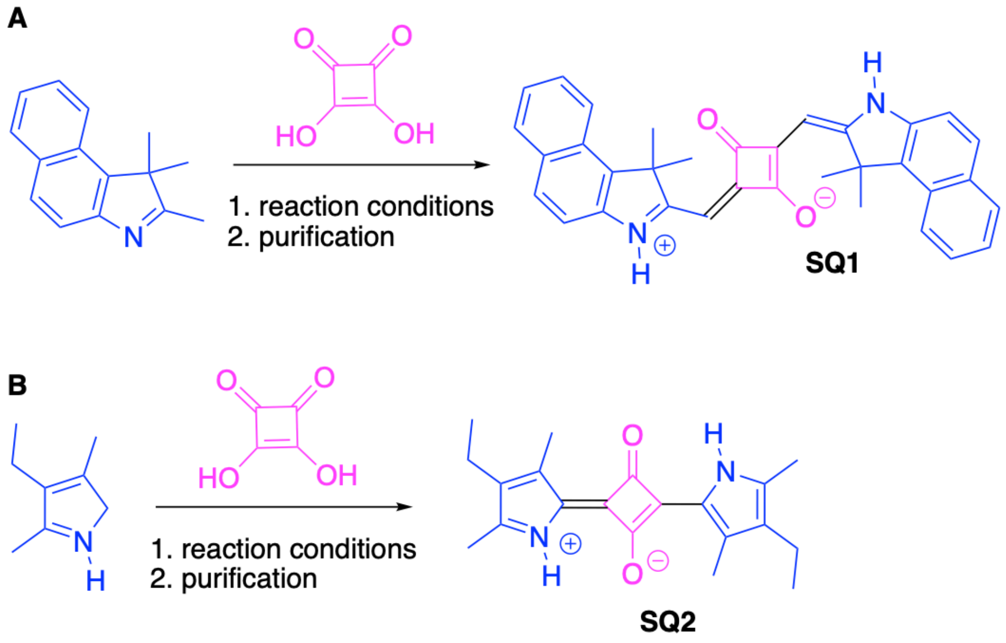
Due to the aforementioned concerns, it was decided to bring forth Cost of Academic Methodology – All Inclusive (CAM-AI) metrics. Simply put, justifiable exclusion from sustainability assessment does not mean those costs cease to exist in practice. So, while there is success in the development of sustainability driven efforts, progress is needed in the rigor of these analyses, in how accurately they define the demand of synthetic processes, so we do not run the risk of leaving holes in the financial, sustainable, and efficiency evaluations of the synthetic methodology.

In the case of CAM-AI (eq. 3), we seek to account for more details of the synthetic process by including all solvents and accounting for the cost of materials that are used in both purification and isolation steps. Just as with CAM, a lower number is desired for CAM-AI.

$$\mathrm{CAM-AI} = \frac{\sum \text{cost of all chemicals \& solvents (\$ mol}^{-1}\text{)}}{\text{yield of product}} \qquad \text{(eq. 3)}$$

Overall, E-factor and CAM-AI are the metrics of emphasis for our sustainability assessment, addressing waste and financial demand respectively and accounting for facets of the synthetic processes that, at some point in the future, might be of interest in an industrial setting. It is important to note that other metrics remain important, like yield, atom economy, and carbon efficiency, however E-factor and CAM-AI were given greater importance in this research. The two are deemed required and sufficient parameters in evaluating chemical production of squaraine dyes as examples of high-value chemicals.

This lens for sustainability assessment was used to assess greenness and cost of synthesis of two squaraine dyes, i.e., **SQ1** and **SQ2** (Scheme 3).



Scheme 3. A: synthesis of **SQ1**; **B**: synthesis of **SQ2**.

Aside from synthetic accessibility, i.e., both **SQ1** and **SQ2** could be prepared from commercially available starting materials, through using some of the routes that have been tested in the Dzyuba laboratory these dyes have also shown a number of valuable applications. **SQ1** has proven applications as a pH sensor (blue-shift in absorption was observed as a function of increasing basicity) as well as a metal ion sensor (ON-OFF fluorescence response was observed as a function of increasing concentrations of Cu^{2+} and Fe^{3+} ions) (Figure 3).[19]

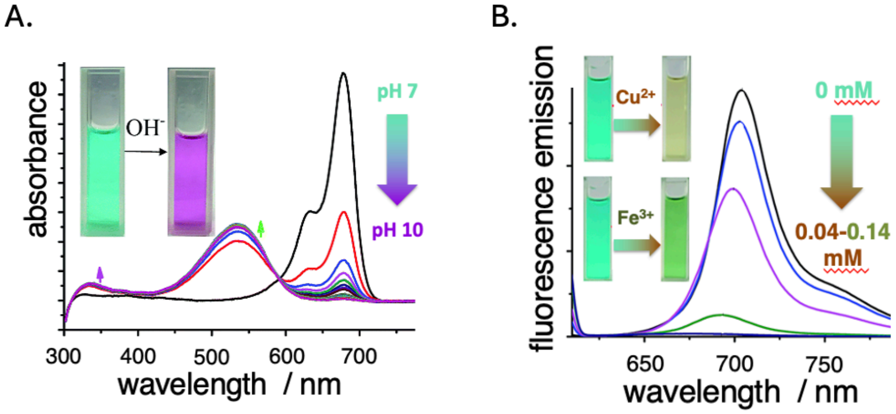


Figure 3: Applications of **SQ1**. **A:** pH-sensing capabilities of **SQ1. B:** ON-OFF Cu^{2+} and Fe^{3+}) sensing capabilities of **SQ1**. Adopted from ref. 20.

SQ2 was first reported in 1965[20], yet its unique properties have been brought to light only recently. It was shown to be a dual-state emitter, with emission covering ca. 600 nm range in solution and ca. 800 nm range in the solid state, as well as a molecular viscometer (Figure 4).[21]

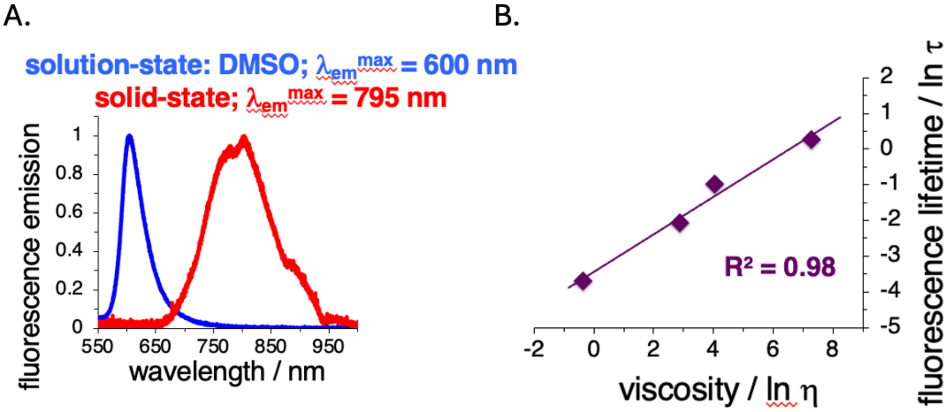


Figure 4: Applications of **SQ2. A:** dual-state emission capabilities of **SQ2.** B: molecular viscometer capabilities of **SQ2.**

In order to inspire effective progress, the synthesis, purification, and isolation were all viewed as opportunities for assessment of waste generation and cost-associated with the synthesis of **SQ1** and **SQ2**. While squaraine dye synthesis is facile, it typically uses toluene–n-butanol mixtures as the solvent system. Due to hazards associated with these solvents, novel, increasingly green alternatives were explored. Specifically, these include using glycols (ethylene glycol, polyethylene glycol), as greener alternatives[22] as well as solvent-free approaches[23].

The mechanics of isolation and purification were also explored as it is within these steps that the squaraine dye synthetic process can demand the most solvents through use of column chromatography, washing, extraction, or recrystallization. Alongside these efforts of evolvement, the literature methods were repeated to generate an encompassing assessment.

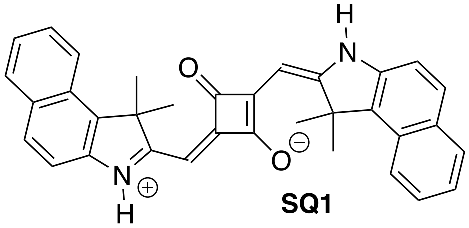
The cost of all chemicals was obtained using the information provided on the websites of the major chemical manufacturers, including Aldrich, TCI-America, VWR and ThermoFisher. The price of the highest quantity available for the specific compound was used and converted to \$/mol.

If more than one manufacturer provided the same material, only the lower cost option was considered.

The sustainability analysis of **SQ1** began with performing the synthesis under various conditions (Table 4). Traditionally used toluene and n-butanol solvent system (entry 1) was coupled with isolation and purification achieved through precipitation of **SQ1** using petroleum ether, followed by washing the solid dye with ample amounts of organic solvents, such as diethyl ether, small amounts of ethyl acetate, and finally petroleum ether to obtain pure material. The E-factor for this process was found to be 715, while CAM-AI was 22,102.

It should be noted that the literature preparation of **SQ1**[19] which relied on the use of column chromatography (although the quantities of solvent and solvents were not specified) proved inefficient in obtaining any meaningful (more than 10 mg) amounts of **SQ1**.

Table 4. E-factor and CAM-AI analysis of various syntheses of **SQ1.**



SQ1

Entry	Reaction Conditions	Purification methods	E-factor	CAM – AI ($/mol)
1	toluene/n-butanol dean-Stark, 7h, reflux	precipitation by organic solvents followed by wash with organic solvents	715	22,102
2	polyethylene glycol 150°C, 5h	precipitation by water followed by wash with water	3,287	28,668
3	polyethylene glycol 150°C, 5h	precipitation by water followed by chromatography	2,962	75,256
4	solvent-free, 150°C, 2.5h	wash with organic solvents	688	177,617

These numbers are informative as they compare to the results of our novel green routes. Compared to the polyethylene glycol solvent system paired with the purification method of precipitating then washing with copious amounts of water to obtain pure product (entry 2), entry 1 has a significantly lower E-factor in coordination with a lower CAM-AI.

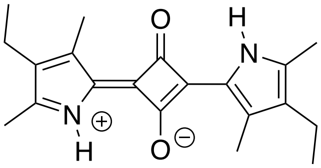
Similar results were obtained when the polyethylene glycol solvent system was paired with purification of precipitation, washing, and chromatography (entry 3). While using the minimum amount of solvent, column chromatography was used to obtain pure **SQ1**. Both E-factor and CAM-AI for entry 3 are over three times larger than the values defined for entry 1. It should be noted that in general, the use of column chromatography for purification of organic compounds is not considered environmentally friendly nor cost-effective due to its demand of large volumes of solvents alongside large amounts of sorbents to obtain pure materials. Based on a number of purifications done in our laboratory, for a given quantity of product placed on the column the amount of sorbent needed can be 200 times larger, while the amount of solvent could be 1000 times larger. Therefore, without compromising the purity of the desired dye, special attention was placed on utilizing minimum required amounts of both sorbents and solvents in this research. Additionally, solvent systems were tested to assure optimum separation while providing minimum retention to the sorbent. For example, dry packing of the column with the sorbent was utilized (as opposed to commonly used wet packing methods where the sorbents are packed as a slurry in the solvent) for purification of **SQ1** using column chromatography. These considerations are the most like reasons for entry 3's purification method being comparable with the precipitation/wash method of entry 2, which could often be viewed as more environmentally preferrable than column chromatography.

The only novel green route that had lower, albeit comparable, E-factor than entry 1 was the solvent-free approach (entry 4). However, the CAM-AI for entry 4 was a multitude of eight times larger due to a relatively lower product yield.

Collectively, the above assessment (Table 4) clearly outlined waste and financial inefficiencies as neither of entries 2, 3, or 4 compared favorably to entry 1 despite using what are typically perceived to be green solvent and green purification routes.

Next, the sustainability analysis of **SQ2** was completed (Table 5). The literature preparation that utilized toluene and n-butanol solvent system (entry 1) was assessed, finding an E-factor of 874 and CAM-AI of 23,982. Similar to **SQ1**, the literature preparation of **SQ2** lacked sufficient details to allow for the calculation of both E-factor and CAM-AI[24] and thus it was repeated with some alterations.

Table 5: E-factor and CAM – AI analysis of various syntheses of **SQ2**.



SQ2

Entry	Reaction Conditions	Purification methods	E-factor	CAM – AI ($/mol)
1	toluene/n-butanol Dean-Stark, 7h, reflux	precipitation with organic solvents followed by wash with organic solvents	874	23,982
2	ethylene glycol 150℃, 15 min	precipitation with water followed by wash with water	1,001	9,245
3	water, reflux, 7h	wash with water followed by recrystallization from organic solvents	322	17,898
4	grinding, 150℃, rt	recrystallization from organic solvent	57	10,651

Using ethylene glycol as a novel solvent system (entry 2) provided a lower CAM-AI value than that of entry 1, although it also defined a slightly higher E-factor. Noteworthy, however, is that this approach is the most facile as **SQ1** was obtained in only 15 min (entry 2). This also highlights the balance between reaction efficiency, waste production, and associated costs.

When water was used as a solvent system for the reaction, some 3-fold decrease in E-factor, coupled with a more than 2-fold decrease of CAM-AI was noted (entry 3). Yet, the lowest E-factor for **SQ2** synthetic routes came from the grinding reaction (i.e., mechanochemical conditions, the starting materials are simply grinded with a mortar and pestle) (entry 4). This system was also found to have a lower value of CAM-AI than that of entry 1, though it was slightly higher than the CAM-AI of entry 2. Despite efficiencies provided by this mechanochemical approach, its scope might be limited to only very reactive nucleophiles, such as kryptopyrrole. In support of this notion, all attempts to use mechanochemistry to obtain **SQ1** (or other related dyes) failed.

When one considers that the ideal E-factor is zero and the lowest possible number is desired for CAM-AI, the above results (Tables 4 and 5) for both squaraine dyes, **SQ1** and **SQ2**, obtained and purified using various approaches, outline high degrees of waste production as well as high costs. If one further considers the molecular weights of these dyes, the CAM-AI would be related to obtaining only 324 g and 497 g of **SQ1** and **SQ2**, respectively. Thus, further research would be required to realize more efficient synthetic and purification routes for large-scale (i.e., above 10 kg) production of these dyes.

CONCLUSIONS

Investigation of various synthetic approaches coupled with several isolation and purification procedures in the production of squaraine dyes, as examples of high-value chemicals, revealed the value of quantitative metric systems to assess waste and cost associated with these dyes. Our analyses have indicated that greenness, implemented by the use of water, green solvents, mechanochemistry, and what deem to be environmentally benign purifications, still resulted in appreciable waste accumulation and high costs. This suggests that despite perceived progress towards greener synthesis of these dyes, more work is required in the future.

The results of this initial research also aim to spark conversation on implementing better reporting practices within chemical literature, such that assessments of newly designed routes could be easily completed and compared to reported methods. Specifically, in the hopes of a truly greener future for chemistry, gaps of information must be minimized in literature, especially in the context of quantities of solvents used during the isolation and purification processes (i.e., column chromatography and recrystallization). By providing as much information as possible, we present each other the opportunity to accurately navigate the process of designing increasingly sustainable routes to chemicals that can benefit society.

ACKNOWLEDGEMENTS

I would like to thank Harley Jacobs for the results obtained during the synthesis of SQ2 Dye. I would also like to acknowledge the Professor D.E. Minter Endowment Fund for Undergraduate Research and the Dean's Research initiative Grant for financial support of this work.

REFERENCES

1. Sheldon, R.A. Metrics of green chemistry and sustainability: past, present, and future. *ACS Sustainable Chem. Eng*. **2018,** 6, 32.

2. Berger, O.; Winter, K.R.; Sabourin, A.; Dzyuba, S.V.; Montchamp, J.-L. On the cost of academic methodologies. *Org. Chem. Front.* **2019**, *6*, 2095.

3. Ta, D.D.; Dzyuba, S.V. Squaraine-based optical sensors: designer toolbox for exploring ionic and molecular recognitions. *Chemosensors* **2021**, *9*, 302.

4. Lipshutz, B.B.; Ghorai, S. Transitioning organic synthesis form organic solvents to water. what's your E-factor? *Green Chem*. **2014**, *16*, 3660.

5. Dun, P.J. The importance of green chemistry in process research and development *Chem. Soc. Rev*. **2012**, *41*, 1452.

6. Ilina, K.; MacCuaig, W.M.; Laramie, M.; Jeouty, J.N.; McNally, L.R.; Henary, M. Squaraine dyes: Molecular design for different applications and remaining challenges *Bioconjug. Chem.* **2020**, *31*, 194213.

7. Favret, J.M; Dzyuba, S.V. Synthetic approaches toward phosphorus-containing BODIPY and squaraine dyes: enhancing versatility of small-molecule fluorophores *Molecules* **2025**, *30*, 116.

8. Samantha, S.; Lai, K.; Wu, F.; Liu, Y.; Cai, S; Yang, X.; Qu, J.; Yang, Z. Xanthene, cyanine, oxazine and BODIPY: the four pillars of the fluorophore empire for super-resolution bioimaging *Chem. Soc. Rev.* **2023**, *52*, 7197.

9. Dzyuba, S.V. Bodipy Dyes as Probes and Sensors to Study Amyloid-β-Related Processes *Biosensors* **2020**, *10*, 192.

10. Requena, S.; Ponomarchuk, O.; Castillo, M.; Rebik, J.; Brochiero, E.; Borejdo, J.; Gryczynski, I.; Dzyuba, S.V.; Grycznski, Z.; Grygorczyk, R.; Fudala, R. Imaging viscosity of intragranular mucin matrix in cystic fibrosis cells *Sci. Rep.* **2017**, *7*, 16761.

11. Avirah, R.R; Jayaram, D.T.; Adarsh, N.; Ramaiah, D. Squaraine dyes in PDT: from basic design to *in vivo* demonstration. *Org. Biomol. Chem.* **2012**, *10*, 911.

12. Abraham,A,; Gayathri, D. D.; Cibin, T.R.; Ramaiah, D. Two different approaches in cancer therapy – using photosensitizer and natural product *Proc. SPIE–Int. Soc. Opt. Eng.* **2010**, *7576*, 757603.

13. Gayathri, D.D.; Cibin, T.R.; Ramaiah, D.; Abraham, A. Bis(3,5–diiodo–2,4,6–trihydroxyphenyl) squaraine:a novel candidate in photodynamic therapy for skin cancer models in vivo *J. Photochem. Photobiol. B* **2008**, *92*, 153.

14. Chen, G.; Sasabe, H.; Igarashi, T.; Hong, Z.; Kind, J. Squaraine dyes for organic photovoltaic cells *J. Mater. Chem. A* **2015**, *3*, 14517.

15. Lakowicz, J.R. *Principles of fluorescence spectroscopy (third edition)*, Springer, 2006

16. Belmonte-Vazquez, J.L.; Amdor-Sanchez Y.A.; Rodriguez-Cortes L.A.; Rodriguez-Molina B. Dual-state emission (DSE) in organic fluorophores: design and applications *Chem. Mater.* **2021**, *33*, 7160.

17. Greenwald, E.C.; Sohum, M.; Zhang, J. Genetically encoded fluorescent biosensors illuminate the spatiotemporal regulation of signaling networks. *Chem. Rev.* **2018**, *11, 8,* 11707.

18. Ta, D. D.; Favret, J.M.; Dzyuba, S.V. Facile synthesis of pyrrolyl-containing semisquaraines in water as precursors for non-symmetric squaraines *Compounds* **2023**, *3*, 17.

19. Wang,Y,; Wang, C.; Xue, S.; Zhang, Q.; Li, Z.; Xu, S. Highly selective and sensitive colorimetric and fluorescent chemosensor of Fe^{3+} and Cu^{2+} based on 2,3,3-trimethylnaphto[1,2-*d*] squaraine *RSC. Adv.* **2016**, *6*, 6540.

20. Treibs, A.; Jacob, K. Cyclotrimethine dyes derived from quadratic acid [1,2-dihydroxycyclobutenedione]. *Angew. Chem. Int. Ed.* **1965**, *4*, 694.

21. Ta, D.D.; Lee, B.; Jablonska, A.; Burnett, W.L.; Sagoo, R.; Pham, D.; Ceresa, L.; Chavez, J.L.; Kimball, J.D.; Powell, C.B.; Coffer, J.L.; Green, K.N.; Gryczynski, I.; Gryczynski, Z.; Janesko, B.G.; Dzyuba, S.V. Symmetric bis-kryptopyrrol-2-yl squaraine as a molecular viscometer and dual-state emitter. **2024-2025**, *unpublished results*.

22. Ta, D.D.; Rodriguez, E.; Dzyuba, S.V. Glycols as novel solvents for synthesis of squaraine dyes. *Tetrahedron Green Chem.* **2024**, *3*, 100042.

23. Zappimbulso, M.; Capozzi, M.A.M.; Porcheddu, A.; Farinola, G.M.; Punzi, A. Solvent-free reactions for the synthesis of indolenine-based squaraines and croconanes: comparison of thermal heating, mechanochemical milling, and IR irradiation, *ChemSusChem* **2021**, *14*, 1363.

24. Balcerak, A.; Kwiatkowska, D.; Kabatc, J. Novel photoinitiators based on difluoroborate complexes of squariane dyes for radical polymerization of acrylates upon visible light. *Polym. Chem.* **2022**, *13*, 220.