ENVIRONMENT SENSITIVE PROBES BASED ON SQUARIC ACID SCAFFOLDS

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ENVIRONMENT SENSITIVE PROBES BASED ON SQUARIC ACID SCAFFOLDS

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ABSTRACT

Environment-sensitive probes are desirable molecules due to their ability to respond to changes in media. These responses can be measured via photophysical properties such as absorbance and emission, as factors such as polarity and viscosity change. In this project, we investigated how functionalized squaric acid scaffolds, specifically with 8-aminoquinoline moieties, would serve as probes measuring changes in the local media. Four squaric acid derivatives were synthesized, however it was found that only the 1,3-disubstituted squaric acid derivative was a probe sensitive to both polarity and viscosity.

<u>ACKNOWLEDGMENTS</u>

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1. INTRODUCTION

1.1. Environment Sensitive Probes: General Considerations

Organic dyes are molecule whose photophysical properties are sensitive towards external stimuli such as temperature, viscosity, or polarity. Such responses to changes in their environment make these class of compounds of particular interest and use due to their abilities to potentially serve as environment sensitive probes.[1] There are various mechanisms through which these probes can operate, such as charge transfer, isomerization, aggregation, or conformational change.[1] In all cases, a distinct form of the probe is produced that has a set of photophysical properties different than the set prior to the sensing mechanism.[1] While all these mechanisms remain valid and significant, this project focuses on conformational changes as the process through which the environment sensitive probes operate. The internal rotation of covalently linked moieties allows the probe to realize various conformations, such as planar and twisted forms (Figure 1). These planar and twisted conformations could have unique photophysical profiles as a result of an internal rotation due to environmental changes.
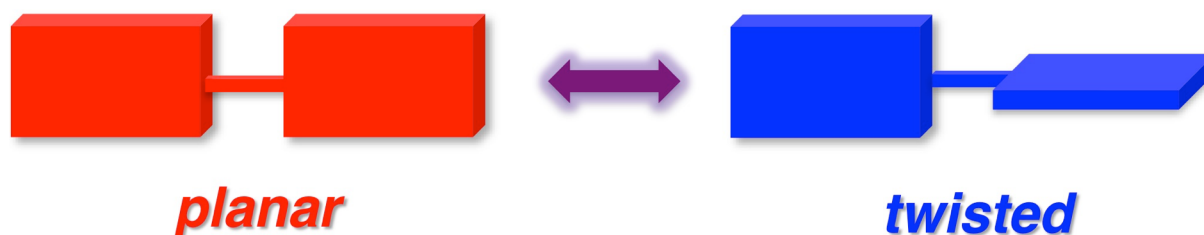

planar   *twisted*

Figure 1. Planar and twisted forms of an environment-sensitive probe, which is operating under a conformational change regime.

The photophysical properties of interest are absorption and emission. These properties are a result of the excitation of a molecule to an excited state and consequent relaxation to the ground state (Figure 2). Absorption can be distinguished from emission by the fact that absorption only includes the process of excitation, while emission is excitation as well as relaxation to the ground state. Additionally, the lifetime of the molecule, or the amount of time spent in the excited state, should also be considered.
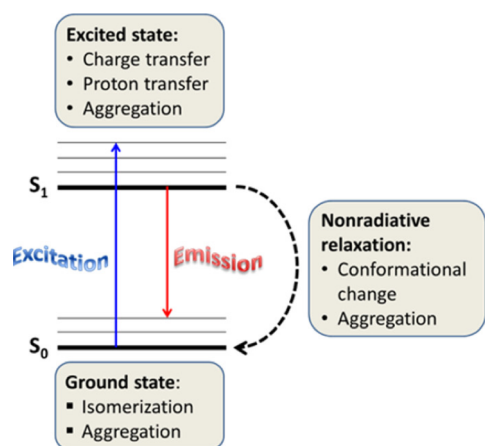

Figure 2. Processes of absorption and emission as a result of excitation of a molecular probe. Adopted from Ref. 1.

1.2. Environment Sensitive Probes: Developments from Dzyuba Research Group

In the past, the Dzyuba research group utilized BODIPY-type scaffolds for the development of several small molecule-based environment-sensitive probes (Figure 3).[2] These fluorophores, which operate under a conformational change regime, have been shown to be viable molecular viscometers for a variety of media, such as molecular solvents, ionic liquids, and more importantly, a number of biologically relevant systems.[2]
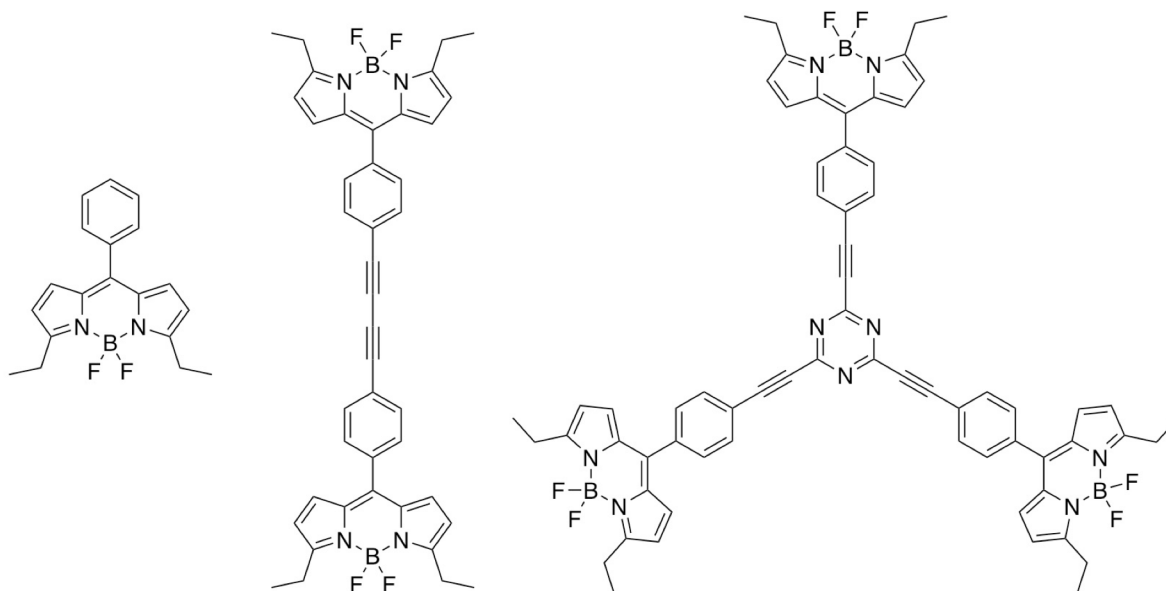
Figure 3. Examples of BODIPY-based viscometers that operate under a conformational change regime.

The main goal towards the design and synthesis of these probes was to utilize modular, facile, and short syntheses, preferably in less than five to six steps. Despite interesting properties and functions of these BODIPY probes, it appeared to be challenging to introduce multiplexing capabilities onto BODIPY scaffolds, while preserving short, modular and facile synthetic sequences towards these probes.

1.3. Squaric acid, squaramides, semi-squaraines, and squaraines

In order to expand on the structural and functional diversity of small molecule-based environment-sensitive probes with multiplexing capabilities, we decided to
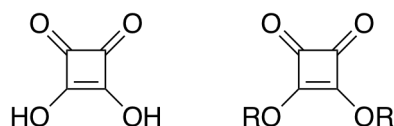

Figure 4. General structures of squaric acid (left) and alkyl squarates (right). R = alkyl group, *i.e.,* Me, Et, or n-Bu.

explore dyes based on squaric acid (Figure 4). Squaric acid, or 3,4-dihydroxy-3-cyclobutene-1,2-dione, is an aromatic organic compound aptly named due to its symmetrical, four-membered ring and high acidity, having pKas of 0.5 and 3.5.[2]

Although squaric acid was first synthesized in 1959,[2] the full potential of this moiety started to unfold only a few decades later. Squaric acid and its derivatives have a diverse range of applications, including those in the field of supramolecular, medicinal and bioorganic chemistries.[2] More specifically, squaramides, which could be prepared in one step from alkyl squarates with various nucleophiles, were found to be viable inhibitors of enzymes such as glyoxalase I and pyruvate dehydrogenase,[3] as well as having the potential to be an antimalarial agents.[4]

Of particular interest are squaraine dyes, which are organic compounds based on squaric acid cores that are show strong and narrow absorption and emission bands in the near infrared (NIR) range.[5] These unique, and highly desirable photophysical properties arise from a planar structure with extended electron conjugation throughout.[5] Coupled in part with synthetic accessibility of these dyes, the coveted photophysical properties contribute to ever expanding applications of squaraines.[7] Some specific and recent applications include serving as biotin receptor probes in cancer cells,[6] uses in *in vivo* fluorescence and photoacoustic imaging,[7] as well as serving as components of solar cells.[8]

In this research, the synthesis and application of several dyes based on squaric acid scaffolds were envisioned (Figure 5). The choice of 8-aminoquinoline as a functional moiety for the derivatization of squaric acid's scaffold was driven by several factors. Specifically, quinoline's nitrogen provides a possibility for further

functionalization. In other words, quaternarization of the nitrogen provides a possibility for so-called "ionic liquid" type compounds. This could have applications such as soft materials, whereas protonation of the nitrogen might yield a pH-sensitive probe.
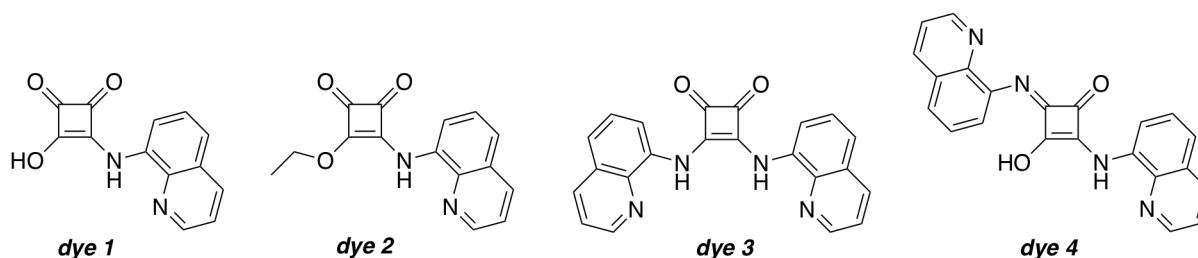

Figure 5. 8-aminoquinoline-containing squaric acid congeners

2. RESULTS AND DISCUSSION

2.1. Synthesis of dyes based on squaric acid scaffolds

The functionalization of squaric acid-scaffolds is typically done upon condensation reactions of nucleophiles, such as amines, for example, with either squaric acid or its derivatives (Scheme 1).

R_1 = H, alkyl (*i.e.*, Me, Et, Bu)
R_2 = alkyl, aryl
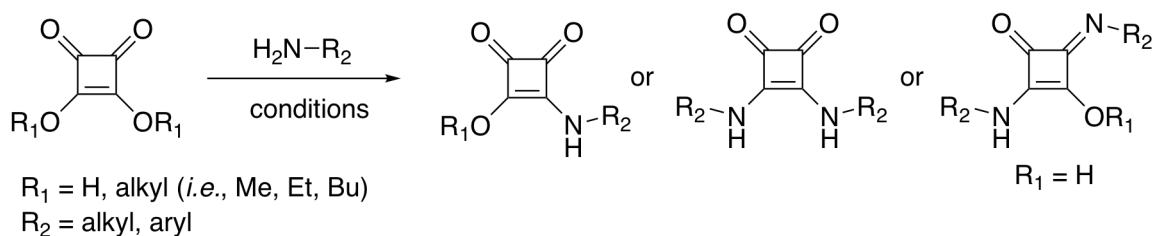
Scheme 1. General syntheses of squaric acid derivatives using amine-based nucleophiles.

In general, depending on the conditions, such as solvents, additives, amount and nature of amine nucleophiles and R-groups, monosubstituted products, squaramides, or squaraines could be obtained (Scheme 1). It should be noted that efficiencies of these

reactions vary greatly, as yields from poor (< 10 %) to good (> 70 %) were reported. Furthermore, there is also a great deal of variation in regard to isolation and purification of the products. Although many accounts utilize filtration and multiple washing of the precipitate with several solvents as the default techniques, recrystallization and column chromatography have also been reported. In this research, in order to prepare **dyes 1 –**
4, modified literature procedures for the synthesis of specific classes of squaric acid derivatives have been utilized.

Synthesis of **dye 1** was accomplished using a condensation of 8-aminoquinoline and squaric acid in water under reflux (Scheme 2).
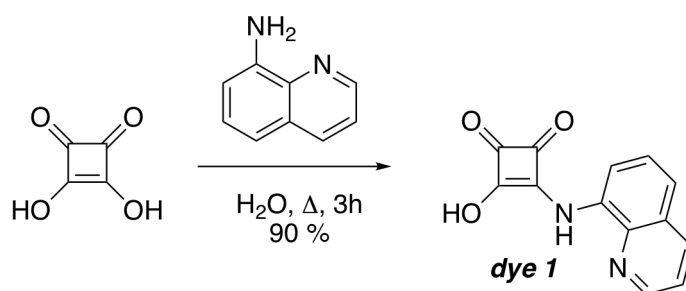
Scheme 2. Synthesis of **dye 1**.

Although this compound was reported in literature, no spectral characterization was provided. **Dye 1** was easily isolated using simple filtration of the reaction mixture. Optimization studies, conducted in order to increase the yield of the reaction, revealed that increasing the number of amine equivalents to from 1 to 2 to 3 had only a marginal effect on the reaction's yield. Interestingly, when 10 equivalents of 8-aminoquinoline were used, no product was obtained, and the majority of the amine was recovered. Arguably, the high acidity of the OH-groups of squaric acid was responsible for the acid-base reaction, which removed the nucleophilicity of the amine, and prevented the

formation of **dye 1**. Some additional experimentation revealed that **dye 1** could also be obtained upon reaction of squaric acid with 8-aminoquinoline in glacial acetic acid at room temperature (Scheme 3).
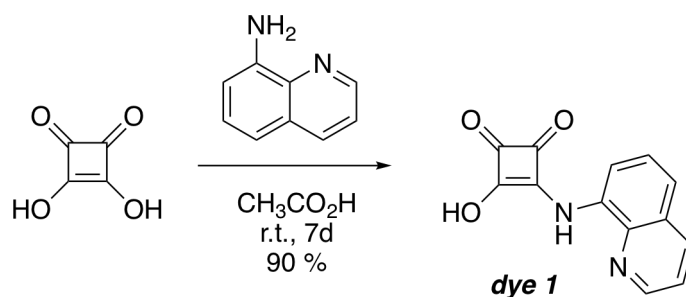
Scheme 3. Room-temperature synthesis of **dye 1**.

Although the efficiency of this novel modification (in regard to reaction time) is not particularly impressive, the fact that this transformation could be accomplished at room temperature suggests that the scope could be expanded to include temperature sensitive compounds as well.
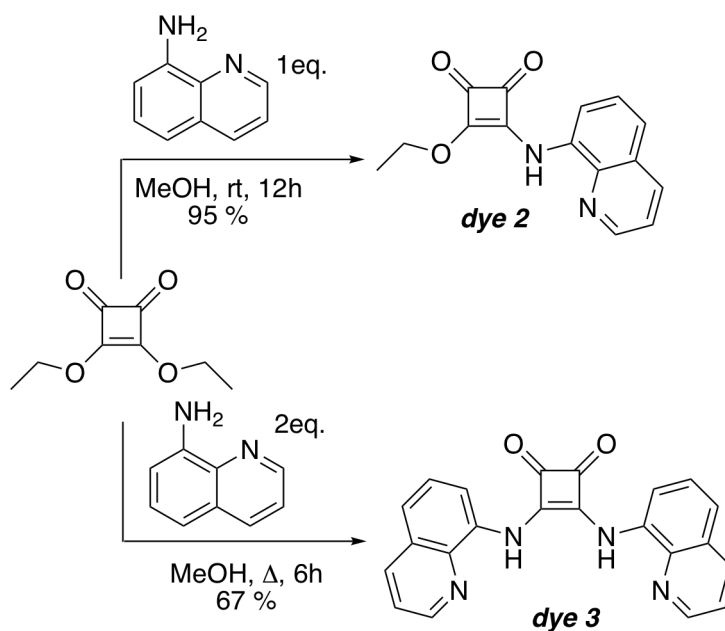

Scheme 4. Syntheses of **dye 2** (top) and **dye 3** (bottom).

Next, synthesis of **dye 2** was conduced using equimolar amounts of 3,4-diethoxy-3-cyclobutene-1,2-dione (Figure 4) in methanol (Scheme 4A). Isolation of pure product was accomplished by simple filtration of the precipitated **dye 2** out of the reaction mixture.

Interestingly, it was found that the synthesis of **dye 3** could be accomplished in a similar manner by simply adjusting the number of equivalents of 8-aminoquinoline to 2, and conducting the reaction under reflux (Scheme 4B). This small alteration is particularly interesting since synthesis of squaramides is typically accomplished in the presence of various additives, such as Zn(OTf)$_2$. Yet it appeared that **dye 3,** as well as a few other squaramides, which have been prepared in our laboratory (not shown), can be synthesized without these additives without significant impact on the yield. Thus, it appears that the use of additives might not be necessary, which should lead to more efficient and viable methodology in terms of costs.

Finally, **dye 4** was prepared using Dean-Stark distillation (Scheme 5). However, unlike the cases of **dyes 1 – 3**, pure **dye 4** could not be isolated upon simple filtration and washing protocols, which included repetitive use of solvents with various polarities. In addition, any attempts to improve both the yield and the purity of the product by adjusting the number of equivalents of the reactants, nature of solvents, or reaction times failed to give pure product. Pure **dye 4** was obtained upon recrystallization from
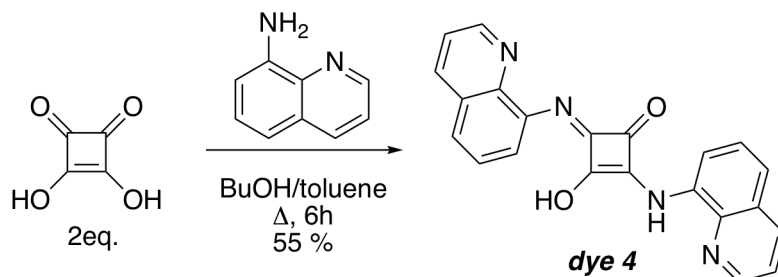
Scheme 5. Synthesis of **dye 4**.

either CF_3CH_2OH (TFE) or CH_3CH_2OH (EtOH). Considering the relative cost of these solvents, the more viable CH_3CH_2OH was chosen as the preferred choice of recrystallization solvent for purification of **dye 4**.

2.2. Photophysical properties of squaric acid-based dyes

2.2.1. Dyes 1 – 4 as polarity probes

Solvatochromic properties, or the dependence of photophysical properties on the property of the media, have been established for various squaraines and squaramides,[14] Yet, specific correlations, which would provide some predictability, could rarely be found in literature. Also, no solvatochromic behavior for **dyes 1 – 4** have been reported.

The absorbance spectra of the four isolated compounds were measured in solvents of various polarities. In general, any correlation between the absorption maxima, for example, and the polarity of the media, would produce a so-called solvatochromic probe. This means that the photophysical characteristics of the probe could be used to assess the polarity of the media, without knowing the identity and/or composition of the media. Ideally, a linear correlation between the peak absorbance value of the squaric acid derivatives in solution as a function of solvent polarity should be the most desirable due to ease of use. Furthermore, a least squares regression is an attractive tool due to its simplicity as well as it allowing for the prediction for the behavior of the potential probes.

The monosubsituted squaric acid, **dye 1** (Figure 6) showed no linear correlation between the absorbance maximum and the polarity of the solvent, as seen by the low R^2 value (Figure 6, inset). However, the plot does indicate that the absorbance

spectrum is changing as the polarity of the solvent changes, but without a significant pattern.
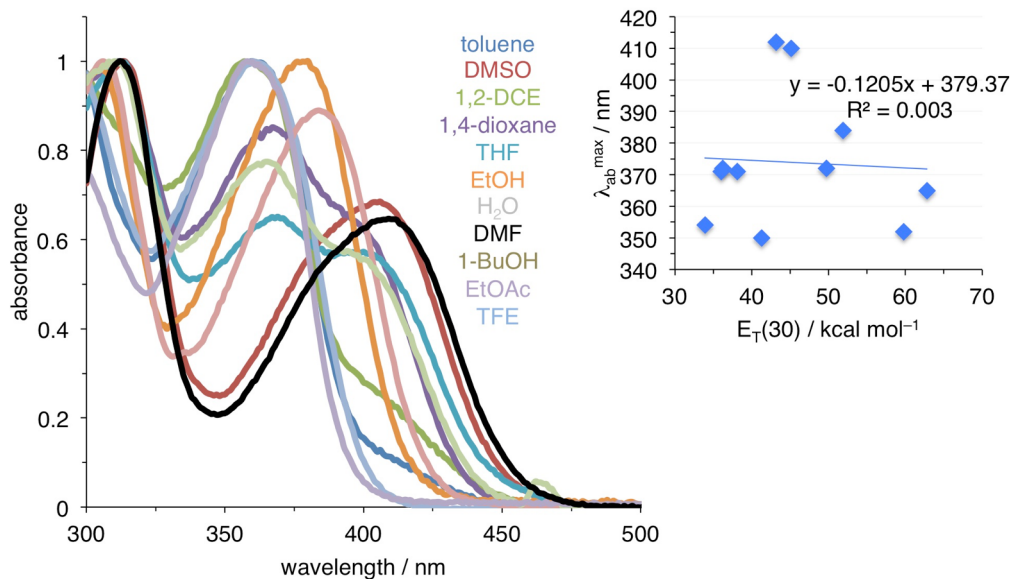

Figure 6. Normalized absorbance spectra of **dye 1** in solvents of various polarities. Inset: absorbance maximum values of **dye 1** as a function of solvent polarity.

The absorbance of the monosubsituted ethoxy squaric acid, **dye 2** (Figure 7) was measured under the same conditions, and it yielded a better linear correlation than the
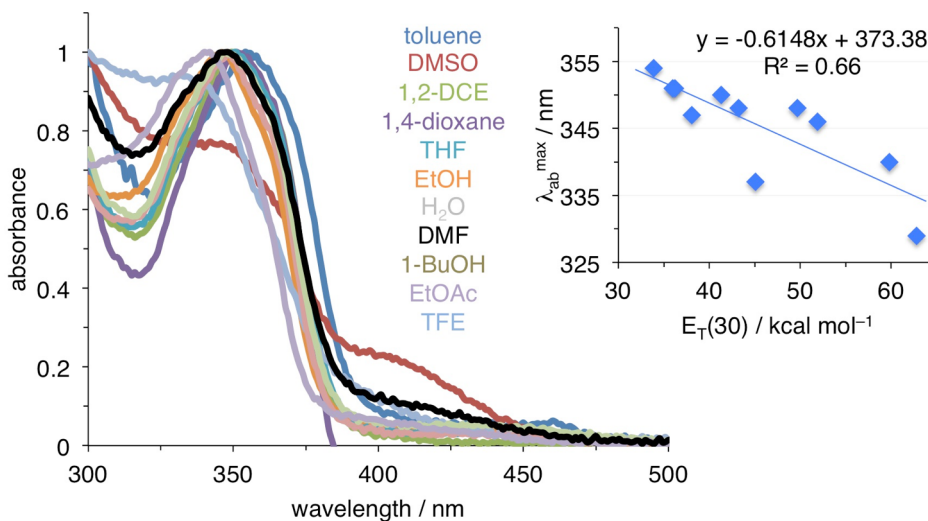

Figure 7. Normalized absorbance spectra of **dye 2** in solvents of various polarities. Inset: absorbance maximum values of **dye 2** as a function of solvent polarity.

monosubstitued squaric acid, however the R^2 value was still considerably low, i.e., not suitable for practical use. As the complexity of the squaric acid scaffold increased, we obtained a better linear correlation with the 1,2-disubstituted squaric acid, **dye 3** (Figure 8).
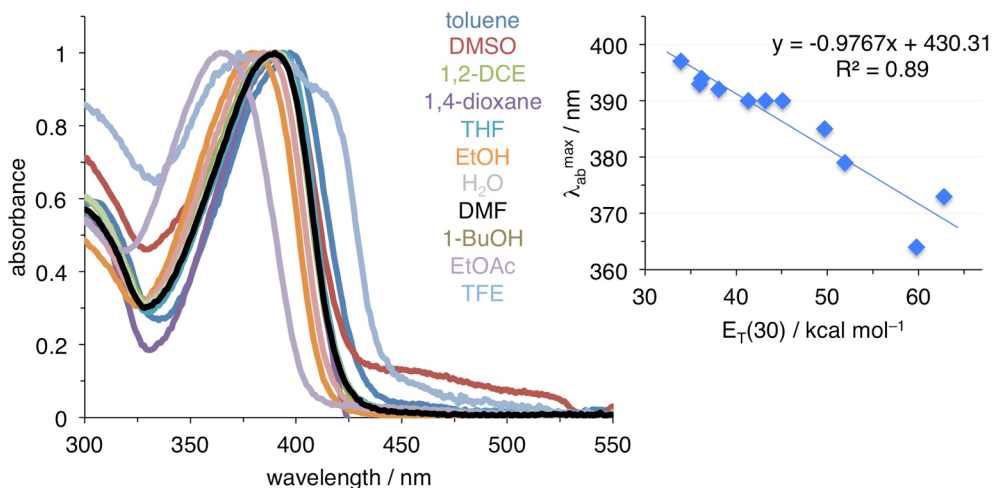

Figure 8. Normalized absorbance spectra of **dye 3** in solvents of various polarities. Inset: absorbance maximum values of **dye 3** as a function of solvent polarity.

However, the 1,3-disubstituted squaric acid, **dye 4**, exhibited the best linear correlation (Figure 9). This demonstrated that the gradual adjustment of the substituents and their placement on the same squaric acid scaffold leads to the design of a polarity sensitive probe. It should be noted that from the synthetic chemistry point of view the simplicity of synthesis was maintained throughout these adjustments.
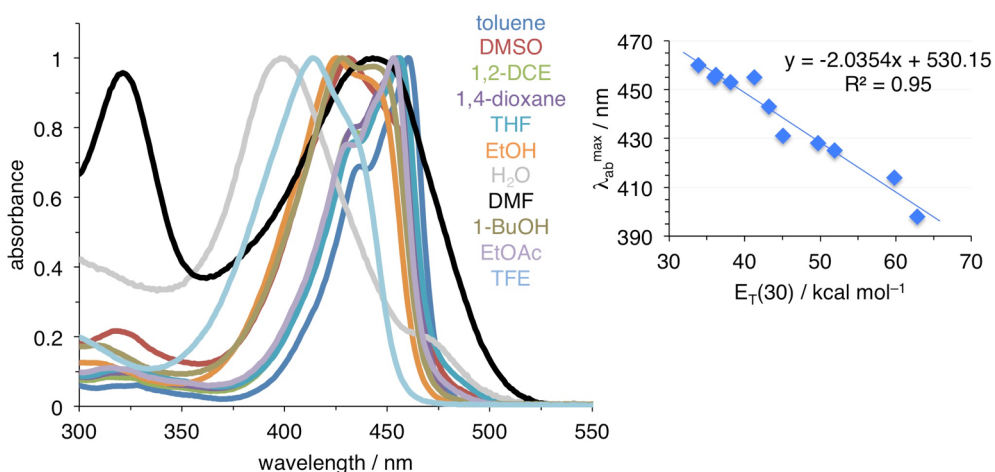
Figure 9. Normalized absorbance spectra of **dye 4** in solvents of various polarities. Inset: absorbance maximum values of **dye 4** as a function of solvent polarity

Many applications of dyes based on squaric acid scaffold are based on their attractive emission properties. Thus, a possibility of utilizing emission of **dyes 1 – 4** for sensing and monitoring environmental properties was explored. It appeared that **dyes 1** and **2** are non-emissive in a number of solvents tested. **Dyes 3** and **4** revealed some dependency of the emission intensity on the polarity of solvents (Figure 10), albeit without any useful trends and correlations.
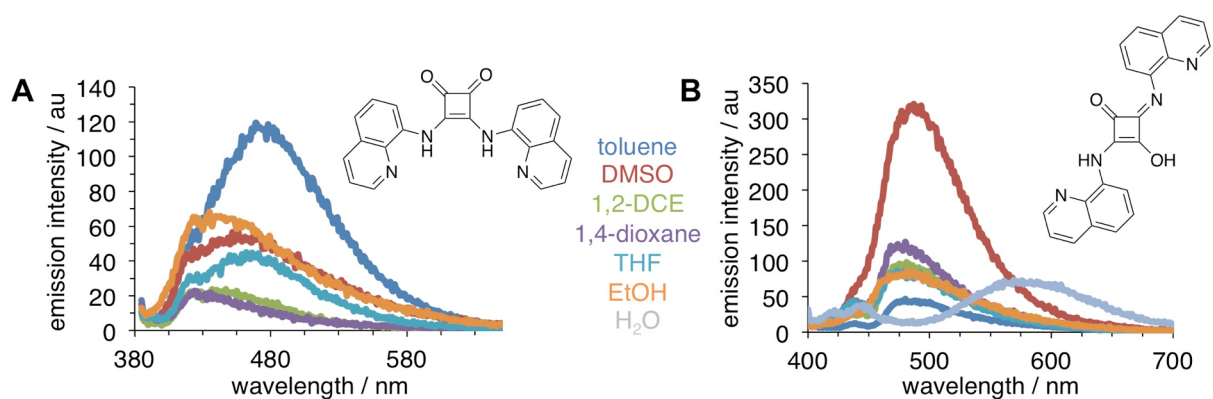

Figure 10. Emission intensities of **dye 3** (A) and **dye 4** (B) in solvents of various polarities.

However, in a set of alcohols, a linear correlation between emission intensity and viscosity of the solvents was noted (Figure 11). The result indicated that **dye 3** and **dye 4** might be used as molecular viscometers. Thus, more detailed studies have been initiated.
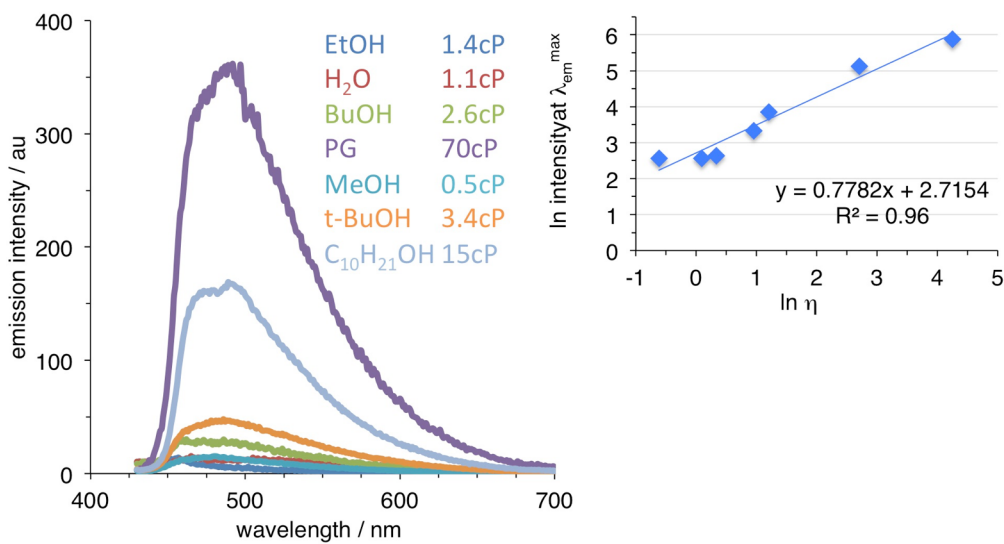

Figure 11. Emission spectra of **dye 4** in alcohols of various viscosities. Inset: emission intensity as a function of alcohols viscosity.

2.2.2. Dyes 1 – 4 as molecular viscometers

It should be noted that the use of squaraines as molecular viscometers has been relatively limited.[15] Arguably, given the ease of synthesis and modular approaches to various structurally and functionally diverse squraines, there might be a lot of potential in exploring the ability of squaraines to act as molecular viscometers.

Using a typical set of methanol/glycerol mixtures, where viscosity is modulated by varying the ratio between more viscous glycerol and less viscous methanol, good correlations between emission intensities of **dye 3** and **dye 4** were obtained (Figure 12).
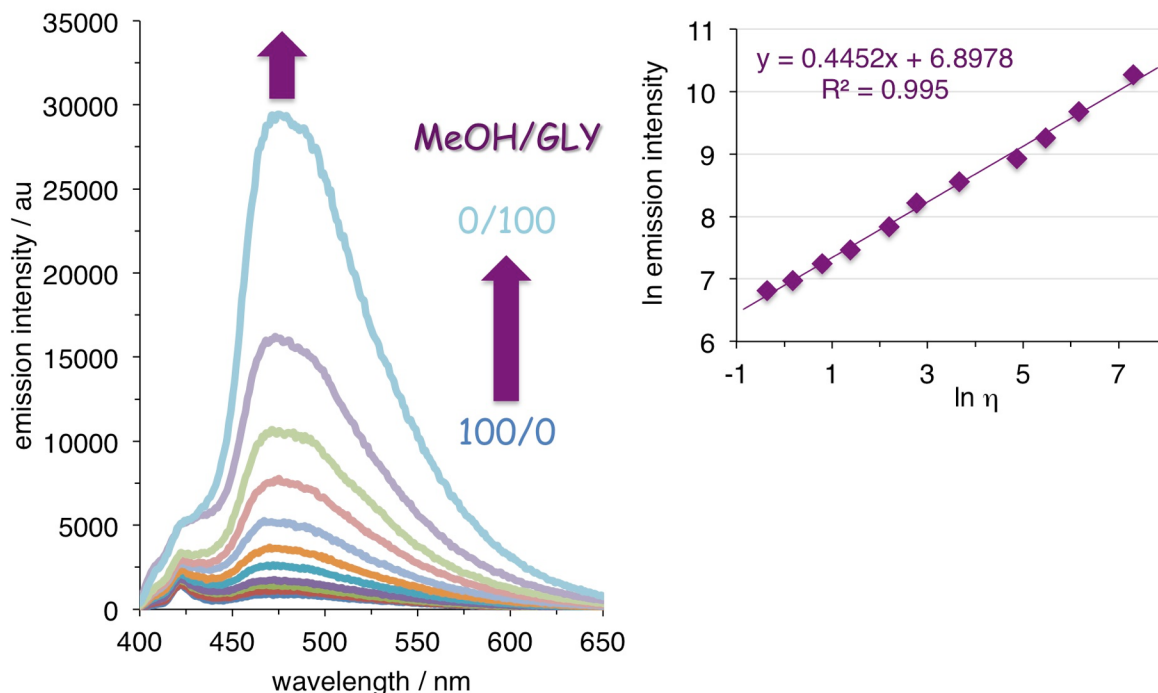
Figure 12. Emission spectra of **dye 4** in methanol (MeOH) – glycerol (GLY) mixtures. Inset: emission intensity of dye 4 (at λ_{em}^{max} = 470 nm) as a function of media's viscosity.

The observed linear correlations with slopes above 0.1 clearly indicated that these dyes are rotors. Arguably, the internal rotation produces a number of conformers with planar and twisted forms of this squaraine as extremes (Figure 13).
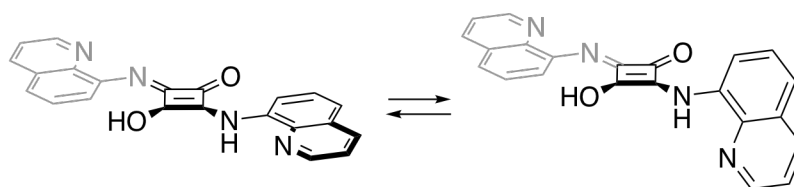

Figure 13. Planar (left) and twisted (right) forms of **dye 4**. See **Figure 1** for a more general representation.

A correlation between fluorescence lifetimes of **dye 4** and media's viscosity was explored (Figure 14). It should be noted that unlike emission intensity, fluorescence lifetimes are much less prone to the effects associated with dye's concentration. Thus,

fluorescence lifetimes could be regarded as precise and unambiguous photophysical parameter of the dye that is used for assessing physical properties of the media.
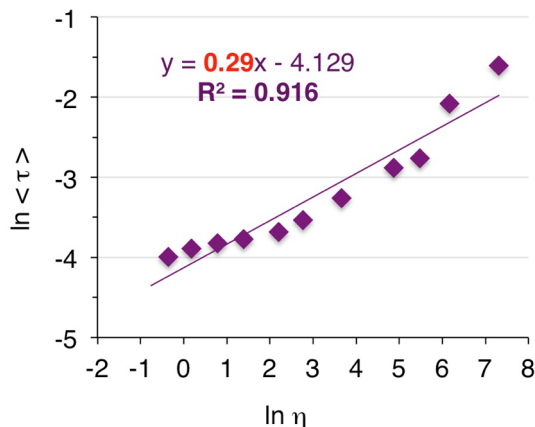

Figure 14. Fluorescence lifetimes, τ, (average amplitude weighted) of **dye 4** as a function of media's viscosity. Media: MeOH/GLY mixtures with viscosities in the 0.7 – 1500 cP range.

The observed linear correlation between fluorescence lifetimes (i.e., average amplitude weighted lifetimes) of **dye 4** and the media's viscosity suggested that this dye could be used as a viable molecular viscometer. Further studies are ongoing in the laboratory of Professor Gryczynski to determine the effects of temperature and pH on the fluorescence lifetimes as well.

Collectively, the aforementioned photophysical studies should establish the multiplexing potential of **dyes 1 – 4** as environment sensitive probes.

3. EXPERIMENTAL PART

### 3.1. Materials and methods

All reagents and solvents were purchased from the commercial courses (Sigma-Aldrich, Acros, and TCI) and were used as received. NMR spectra were acquired on

Bruker (400 MHz) spectrophotometer, and the chemical shifts are reported in ppm (δ) from the residual DMSO peak (2.51ppm). For dye 4, 13C NMR was acquired in CF_3CO_2H due to low solubility DMSO, which prevented the observation of resonances from the quaternary carbons. Multiplicities are reported as: s – singlet, d – doublet, t – triplet, m – multiplet, dd – doublet of doublets. High-resolution mass spectra (HRMS-ESI) were acquired at the Mass Spectrometry Facility, Louisiana State University.

All photophysical studies, *i.e.,* absorbance, emission, and fluorescent lifetimes were performed in the laboratory of Professor Zygmunt Gryzynski (TCU – Department of Astronomy and Physics).

3.2. Synthesis of squaric acid derivatives

Dye 1: A round bottom flask was charged with stirring bar, water (20 mL), squaric acid (0.158 mg, 1.39 mmol), 8-aminoquinoline (0.040 mg, 0.28 mmol), and it was subjected to reflux for 3 hours. Upon cooling, the precipitate was filtered, washed with hot water, and dried under vacuum to give **dye 1** as a red solid (65 mg, 97 % yield).
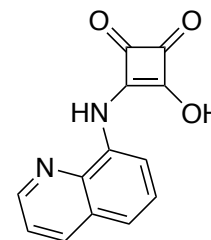
^{1}H NMR (DMSO-d$_6$): δ 10.13 (s, 1H), 8.97 (m, 1H), 8.46 (m, 1H), 8.25 (m, 1H), 7.65 (m, 3H).

^{13}C NMR (DMSO-d$_6$): δ 189.49, 185.53, 171.67, 149.58, 138.29, 137.25, 134.79, 128.64, 127.51, 122.87, 122.37, 116.32.

HRMS (ESI; $C_{13}H_8N_2O_3$) m/z: calc. for (M+H) 241.0608; found: 241.0601.

Dye 2: A round bottom flask was charged with a stirring bar, methanol (1 mL), 3,4-diethoxy-3-cyclobutene-1,2-dione (146 microliters, 0.995 mmol), and 8-aminoquinoline (142.0 mg, 0.9850 mmol). The reaction mixture was vigorously stirred at room temperature for 12 hours. Next, the reaction mixture was filtered, and the solid was washed with diethyl ether to give **dye 2** as beige solid (175.3 mg, 66 % yield).
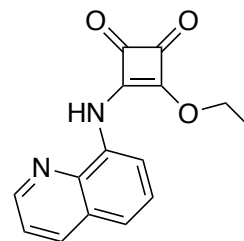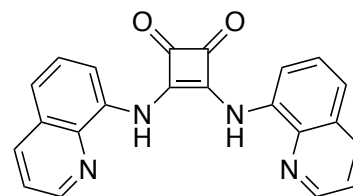
^{1}H NMR (DMSO-d_6): δ 8.95 (dd, J = 4.4, 1.6 Hz, 1H), 8.44 (dd, J = 8.4, 1.6 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.64 (m, 2H), 4.94 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H).

^{13}C NMR (DMSO-d_6): δ 188.05, 184.85, 179.32, 170.30, 150.13, 139.77, 136.99, 133.93, 128.68, 127.06, 124.42, 122.87, 119.04, 70.14, 16.05.

HRMS (ESI; $C_{15}H_{12}N_2O_3$) m/z: calc. for (M+H) 269.0921; found: 269.0914.

Dye 3: A round bottom flask was charged with a stirring bar, methanol (2 mL), 3,4-diethoxy-3-cyclobutene-1,2-dione (0.3 mg, 2 mmol), and 8-aminoquinoline (0.590 g, 4.09 mmol). The reaction mixture was stirred under refl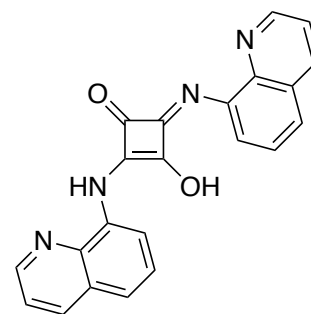ux for 12 hours. Next, the reaction mixture cooled, filtered, and the solid was washed with diethyl ether to give **dye 3** as a pale yellow solid (128 mg, 17 % yield).

^{1}H NMR (DMSO-d_6): δ 11.48 (s, 2H), 9.05 (m, 2H), 8.46 (m, 2H), 8.10 (m, 2H), 7.69 (m, 6H).

^{13}C NMR (DMSO-d_6): δ 183.39, 167.02, 149.80, 139.64, 137.07, 135.05, 128.80, 127.27, 123.23, 122.69, 119.13.

HRMS (ESI; $C_{22}H_{14}N_4O_2$) m/z: calc. for (M+H) 367.1190; found: 367.1180

Dye 4: A round bottom flask was charged with a stirring bar, squaric acid (0.5025 g, 4.406 mmol), 8-aminoquinoline (1.3337 g, 9.2502 mmol), n-butanol (10 mL), and toluene (10 mL). The reaction mixture was stirred under reflux using Dean-Stark apparatus for 4 hours. Next, the reaction mixture cooled, filtered, and the solid was washed with diethyl ether. The resulting solid was recrystallized from ethanol to give **dye 4** as a bright yellow solid (0.2587 g, 16 % yield).

^{1}H NMR (DMSO-d$_6$): δ 10.86 (s, 2H), 8.99 (d, J = 2.8 Hz, 2H), 8.48 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H), 7.70 (m, 3H).

^{13}C NMR (CF$_3$CO$_2$H): δ 180.34, 171.79, 151.33, 147.11, 134.90, 134.75, 133.00, 132. 62, 132.03, 127.83, 127.61, 124.83.

HRMS (ESI; $C_{22}H_{14}N_4O_2$) m/z: calc. for (M+H) 367.1190; found: 367.1182.

4. CONCLUSIONS

From this project, we concluded that molecular probes can be used to assess properties of their media, such as polarity and viscosity. Futhermore, based on the absorbance and emission spectra, the 1,3-disubstituted squaric acid derivate with 8-aminoquinnoline moieties is a polarity sensitive probe and molecular viscometer. This was achieved by increasing the complexity of the squaric acid scaffold, while maintaining a facile and concise synthesis throughout the process. Based on the results

with the 8-aminoquionline moieties, this project has been extended to the 2,3,4,5, and 6-aminoquinoline isomers using the same approach.

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