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Synthesis and Fluorescent Properties of a New Flavonoid Compound

Ashley L. Ingle
The University of Akron, ali17@zips.uakron.edu

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Ashley Ingle

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Abstract

Flavonoids are highly luminescent compounds that are used in diagnosing and treating certain diseases. They are composed of two aromatic rings (ring A and ring B) and a six-membered heterocyclic pyran ring (ring C) that contains a carbonyl and hydroxyl group, which are responsible for unique photophysical characteristics associated with flavonoids. The target flavonoids (compound 1, or **1**, and compound 2 or **2**) were synthesized, purified and characterized by NMR spectroscopy. The photophysical properties of the flavonoid were investigated in varying solvents by using UV-vis and fluorescence spectroscopy. Such properties include Excited State Intramolecular Proton Transfer (ESIPT), Intramolecular Charge Transfer (ICT), and solvatochromism. As expected, **1** was more red-shifted than **2** both for absorbance and emission. Compound **1** had the highest fluorescent intensity in toluene whereas **2** had the highest fluorescent intensity in acetonitrile.

Introduction

As a broad class of natural products, flavonoids are highly abundant pigments found in plants, which can give a wide range of luminescent colors when excited.[1] These pigments help to contribute to the color of the foods that are found in fruits and vegetables. Flavonoid-rich foods are thought to be very useful in the human body including treatment of certain diseases.[1] Chemical structure of a flavonoid includes two aromatic rings (ring A and ring B), and a six-membered heterocyclic pyran ring (ring C) as shown below.[2,3]

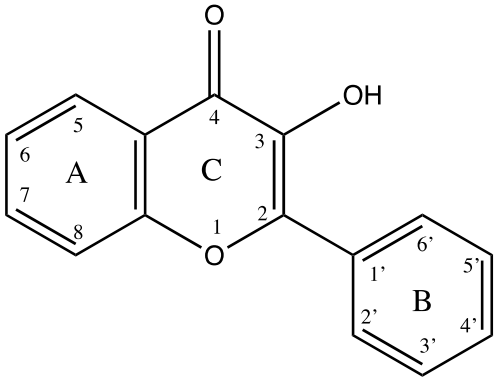
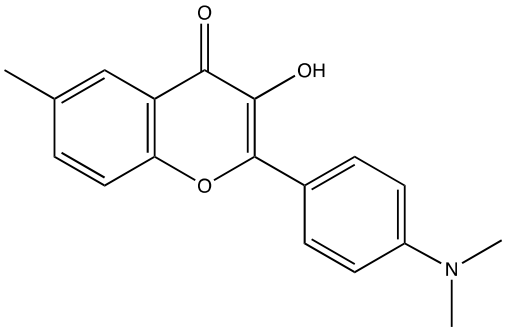
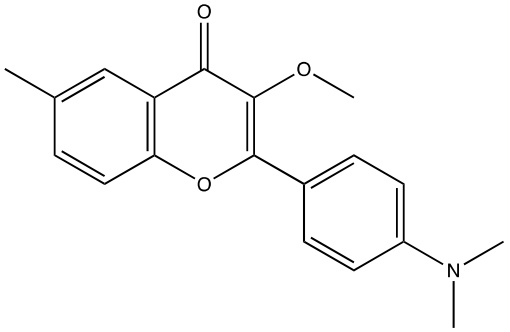


Figure 1: The basic structure of a flavonoid.[2]



2-(4-(dimethylamino)phenyl)-3-hydroxy-6-methyl-4*H*-chromen-4-one

Product 1



2-(4-(dimethylamino)phenyl)-3-methoxy-6-methyl-4*H*-chromen-4-one

Product 2

Figure 2: The structure of Product **1** and Product **2**.

(A) A Brief Overview of Fluorescence Properties.

When a molecule is irradiated with light, it absorbs a photon and causes an electron movement from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). This generates a so called "excited state". When a molecule in the excited state is returns to the ground state, the excess energy can be released by emitting light. The process that causes light emission can be classified as either fluorescence of phosphorescence.[4,5] The fluorescence is a photochemical process, during which a photon is produced when an excited molecule moves from an excited singlet (S_1 state in Figure 3) to

ground state (S_0 state). Although phosphorescence occurs in a similar process, it depends on the different electronic configuration of the excited state. In the phosphorescence process, the emission of a photon is due to relaxation of electron from an excited triplet state to a ground state (i.e. $T_1 \rightarrow S_0$).[4]

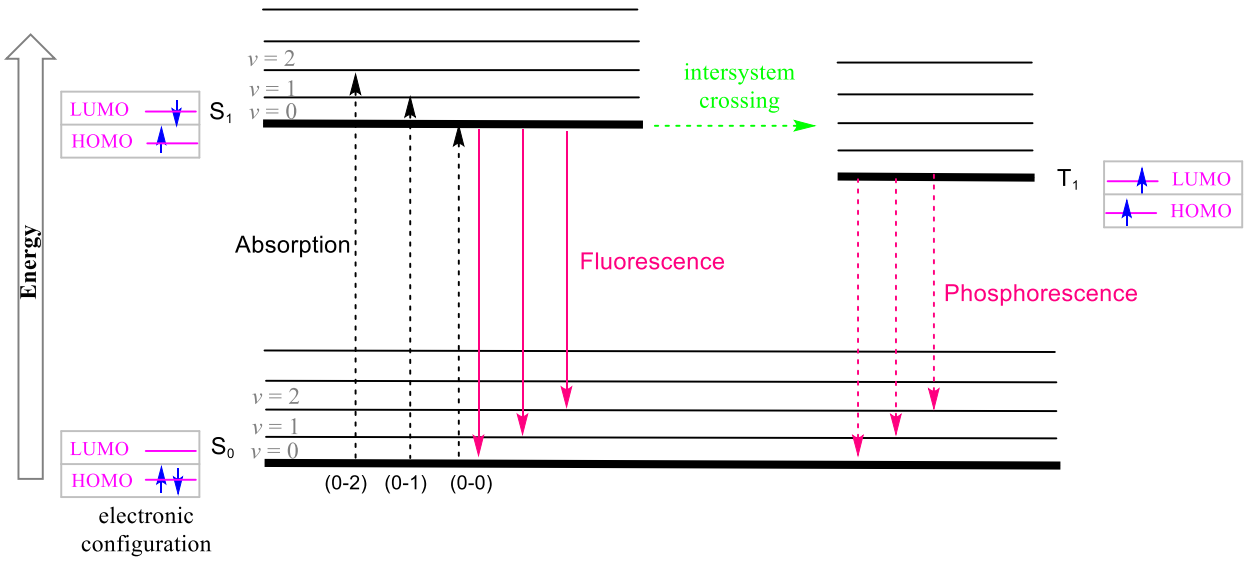


Figure 3: The simplified Jablonski diagram.[6]

The fluorescence can be better described by using the Jablonski diagram. Jablonski diagrams shows absorption and emission that occur during a reaction.[4] The ground, first, and second energy states are represented by S_0, S_1, and S_2, respectively.[4] Each energy state also includes multiple vibrational levels labeled 0, 1, 2, 3, and so on (not shown).[4] When an electron absorbs energy, it becomes excited and moves to a higher energy state. When the electron returns to the ground state, it releases energy in the form of a photon. This photon can be observed as fluorescence when placed under UV light.

When an electron is excited, it emits less energy than it absorbs and fluoresces at lower energies and longer wavelengths.[4] Quantum yield is described as the fraction of molecules that emit a photon after being excited.[7] It is used to evaluate the photophysical characteristics of a

fluorescent compound, as it provides a comparable measurement of fluorescent intensity and can be used to determine luminescent lifetimes.[7] Stokes shift, or the energy difference between absorption and emission, is another characteristic in all fluorescent compounds. There are several explanations for this phenomenon. One is the rate at which an electron drops to the lowest vibrational level of the excited energy state.[4] Different solvents, certain reactions, and energy transfers can also influence the magnitude of the Stokes shift.[4]

(B) Photophysical Processes in Flavonoids

Flavonoids illustrate several different fluorescent mechanisms that could influence their Stokes shift. One mechanism is excited state intramolecular proton transfer (ESIPT). This mechanism is dependent on the intramolecular hydrogen bonding, as shown in the excited normal form N* (Figure 4). The excited normal form N* can be quickly changed to its tautomer form T*.[8] When a molecule is exposed to $\hbar v$ light, it gains enough energy to be promoted to its excited state. For a conjugated molecule, such as a flavonoid, once it is in the excited state, the molecule has enough energy to transfer a proton from one atom to another, thus resulting in the structural change from a normal form to a tautomer form.[9] Because of this proton transfer in the excited state, the normal form of the flavonoid is responsible for the photon absorption, whereas the tautomer form for the emission. Due to this structural feature, the fluorescence of a flavonoid often exhibits a large Stokes shift.

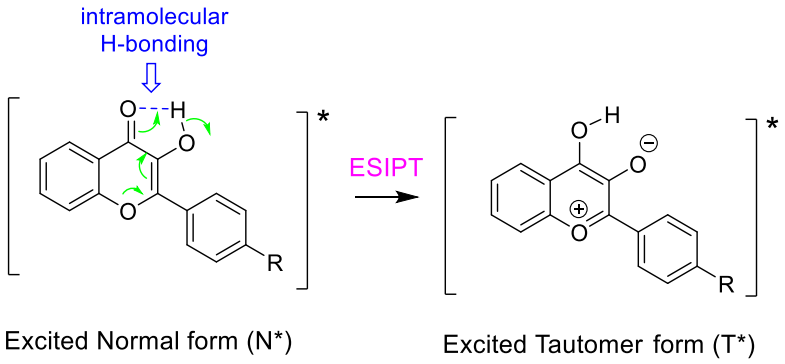


Figure 4. A flavonoid in the excited normal form (N*), which is capable of undergoing ESIPT to give its tautomeric form (T*).[10]

Another mechanism is internal charge transfer (ICT). This occurs when a flavonoid has both an electron donor and acceptor group in the compound with an aromatic structure.[11,12] A third mechanism that influences Stokes shift is solvatochromism. It is described as a change in solvents polarity that can affect the absorption and emission spectra of a compound.[13] The change is described as either negative or positive solvantochromism.[13] Negative solvatochromism occurs when the absorption bands shift towards a shorter wavelength whereas positive solvatochromism occurs when the bands shift towards a longer wavelength.[13] The direction of the spectral shift can be predicted based on which state the molecule is most stable.[13] Better stabilization in the ground state (relative to the first excited state) predicts negative solvatochromism, whereas better stability in the excited state predicts positive solvantochromism.[13]

The purpose of this experiment was to synthesize a flavonoid and observe how the change of the hydroxyl group to a methoxy group would alter photophysical properties. The solvtochromatic properties as well as their UV and fluorescence properties were be investigated.

Methods and Materials

Reagents

4-(Dimethylamino) benzaldehyde was purchased from Alfa Aesar. 2-Hydroxy-5-methylacetophenone was purchased Tokyo Chemical Industry. KOH and acetone were from Fischer Chemical. H_2O_2 and MeI were from EMD Chemicals. HCl was from Sigma Aldrich.

Flavonoid Synthesis and Purification

4-(Dimethylamino) benzaldehyde (6.76 mmol) was added to 2-Hydroxy-5-methylacetophenone (6.6813 mmol) in a minimal amount of ethanol, then aqueous KOH (2.8256 grams, 50.36 mmol) was added dropwise to the solution at 0°C. The mixture was stirred overnight while raising the temperature to 50°C. The solution was placed on ice and aqueous KOH (2.8256 grams, 50.36 mmol) was added dropwise to the solution at 0°C. H_2O_2 (2 mL of 30%) was added slowly. After stirring for 72 hours, the pH was tested and neutralized using 2M hydrochloric acid. The solution was filtered and the solid product was dried on vacuum. The solid product collected was dried and tested using NMR spectroscopy. The product was clean and 2-(4-(dimethylamino)phenyl)-3-hydroxy-6-methyl-4H-chromen-4-one (0.85 mmol) was transferred to a new round bottom flask with dry acetone and methyl iodide (9.16 mmol) was added. The reaction was run overnight. Reaction was monitored with TLC, once product was isolate and dried and purified by column chromatography on silica gel to give 2-(4-(dimethylamino)phenyl)-3-methoxy-6-methyl-4H-chromen-4-one, a yellow-orange solid with 11.8% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.11 (d, 2H, 2', 6'), 8.03 (s, 1H, 5), 7.43 (q, 2H, 7, 8), 6.79 (d, 2H, 3', 5'), 3.87 (s, 3H, OCH_3-3), 3.07 (s, 6H, $N(CH_3)_2$), 2.45 (s, 3H, Me-6).

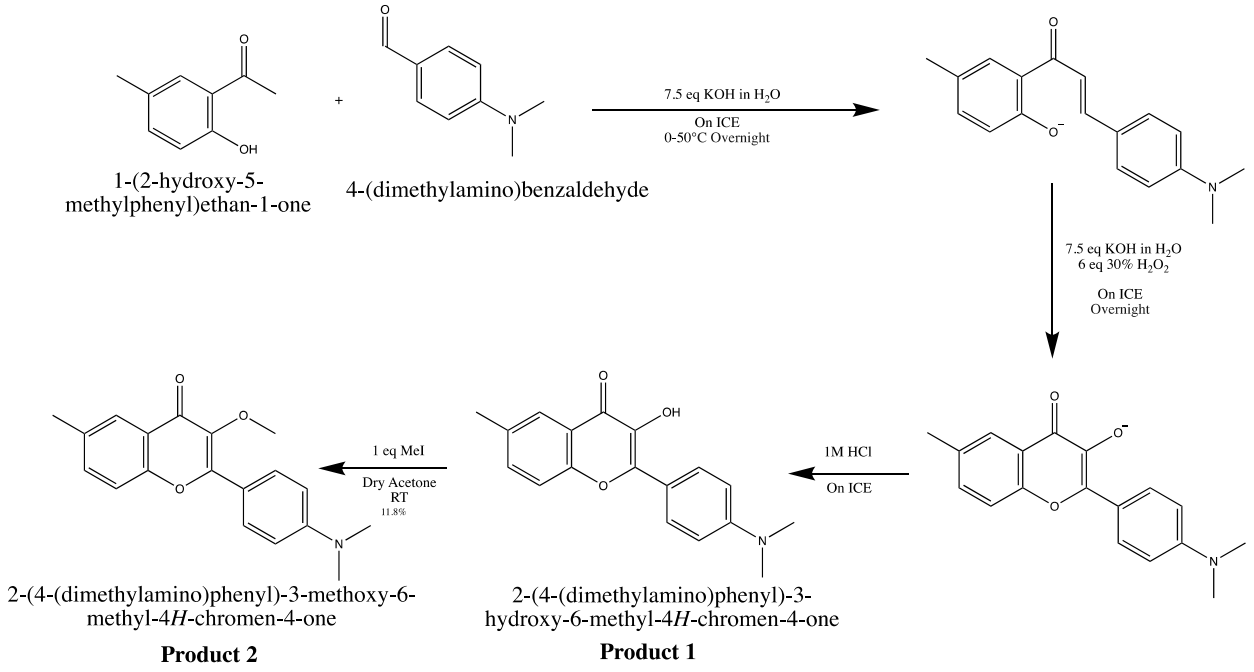


Figure 5: The synthesis of **1** and **2.**

Absorbance Studies

A 1mM solution of both the capped and uncapped flavonoid product was prepared in DMSO. Solutions of **1** and **2** were prepared in various solvents at 10 μM before measuring their emissions and absorbance. Each compound was tested in toluene, acetonitrile, DCM, methanol, nanopure water, and DMSO.

Results and Discussion

Flavonoid Synthesis and Purification

The flavonoids **1** and **2** were synthesized using 1-(2-hydroxy-5-methylphenyl)ethan-1-one and 4-(dimethylamino)benzaldehyde in a four-step reaction by Aldol Condensation. The product was purified using column chromatography on silica gel with 11.8% yield.

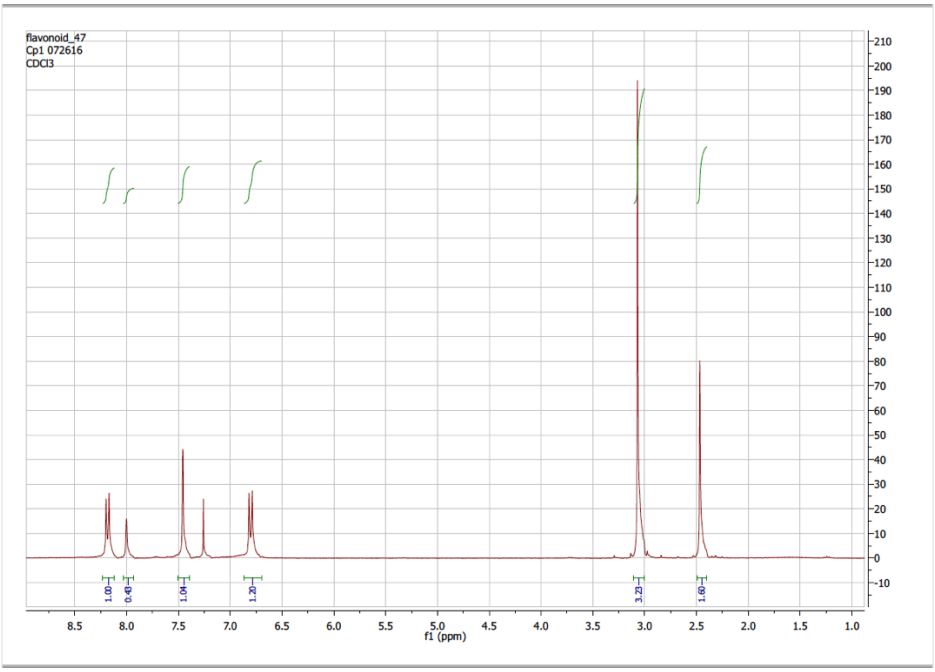


Figure 6: The NMR spectrum of **1** in $CDCl_3$. The singlet at 7.25 ppm was attributed to the residual protons from $CDCl_3$.

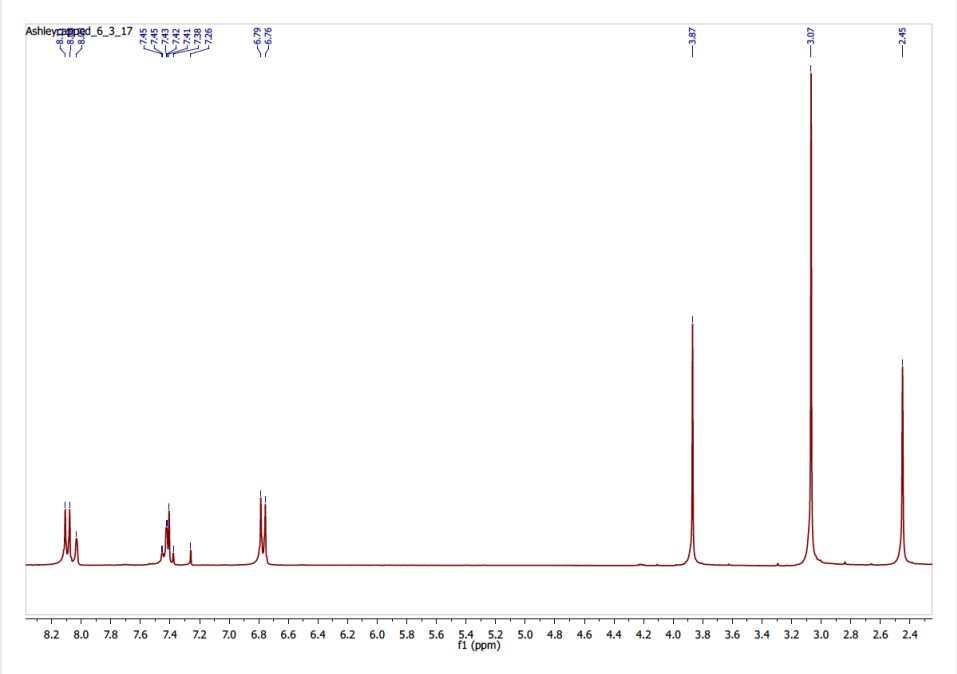


Figure 7: The NMR spectrum of **2** in $CDCl_3$. The singlet at 7.25 ppm was attributed to the residual protons from $CDCl_3$.

The integration of the signal on the NMR spectra were used to determine the number and position of each proton. The resonance at 2.45 ppm indicated the methyl group attached to carbon 6. The resonance at 3.07 ppm indicated the two methyl groups of the dimethylamine. The doublet at 6.79 ppm indicated each of the protons on carbons 3' and 5' in ring C. The doublet at 8.11 ppm indicated each of the protons on carbons 2' and 6'. The protons on carbons 7 and 8 appeared to be a quartet, however, the splitting pattern should be two separated doublets. The reason it showed as a quartet as due to poor resolution from the NMR instrument. The resonance at 8.03 ppm indicated the proton at carbon 5. The peak at 3.87 ppm indicated the protons on the methoxy group at carbon 3. The NMR spectra thus verified the structures of the synthesized **1** and **2**.

The spectra for **1** and **2** were very similar with only a few differences between them. First, the spectrum of **2** detected the methyl resonance at 3.87 ppm. Secondly, the chemical shift of the protons was slightly different in **2** than that in **1** because protection of the hydroxyl group caused a slight change in the chemical environment that is close to this group. Lastly, **1** had a hydroxyl group on ring C, but did not appear in the NMR spectra. It was likely that the hydroxyl proton of **1** was involved with the rapid proton exchange with trace amounts of water molecules, which made the hydroxyl proton signal too broad to detect.

Optical Properties of Flavonoids

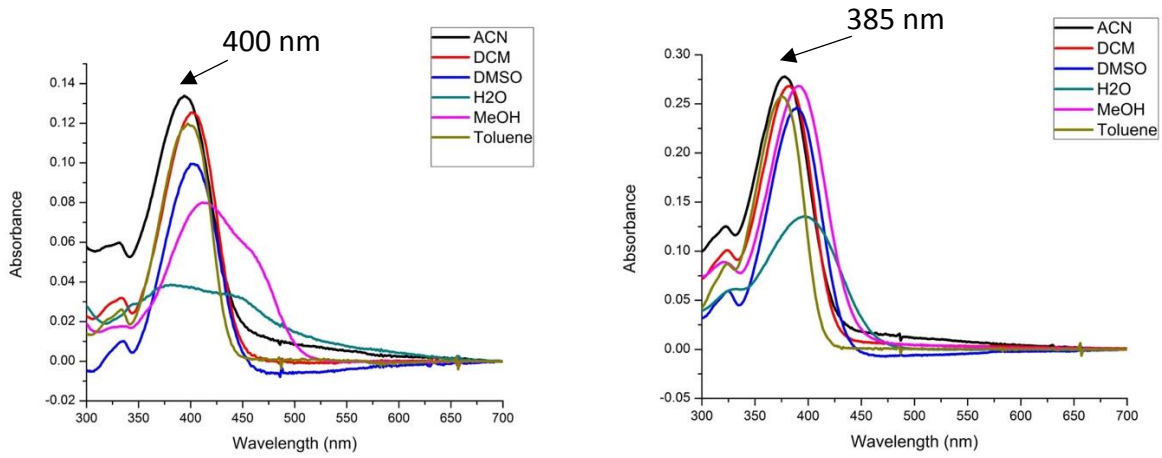


Figure 8: The absorbance spectra of **1** (left panel) and **2** (right panel) in various solvents.

UV-vis absorption of products **1** and **2** were examined in different solvents. When the two compounds were tested in the same solvent, both **1** and **2** exhibited very similar absorption profiles in aprotic solvents such as CH_3CN, CH_2Cl_2, toluene and DMSO. However, some spectral shift was observed in the protic solvents such as MeOH and water, revealing a new band at a longer wavelength. The absorption λ_{max} for **1** were near 400 nm, whereas the peaks for **2** were near 385 nm. Both **1** and **2** had the highest λ_{max} in acetonitrile.

Despite the small difference in their absorption ($\Delta\ \lambda_{max} \approx 15$ nm), compounds **1** and **2** exhibited quite different fluorescence. Compound **1** gave two fluorescence peaks, with one emission peak (λ_{em}) between 450-522 nm, whereas the other at 566-583 nm. In sharp contrast, compound **2** exhibited only one emission band with λ_{em} values between 445-495 nm. The spectral properties clearly indicated that compound **1** was more responsive to its environmental change, as shown in the different solvents. The drastic difference in fluorescence between **1** and

2 could be explained by considering that compound **1** could undergo ESIPT whereas **2** could not. Because of the ESIPT event (Figure 3), compound **1** could give two emission bands, with one peak (λ_{em} at 450-522 nm) being attributed to the normal form and the other peak ($\lambda_{em} \approx$ 445-495 nm) being attributed to its tautomer.

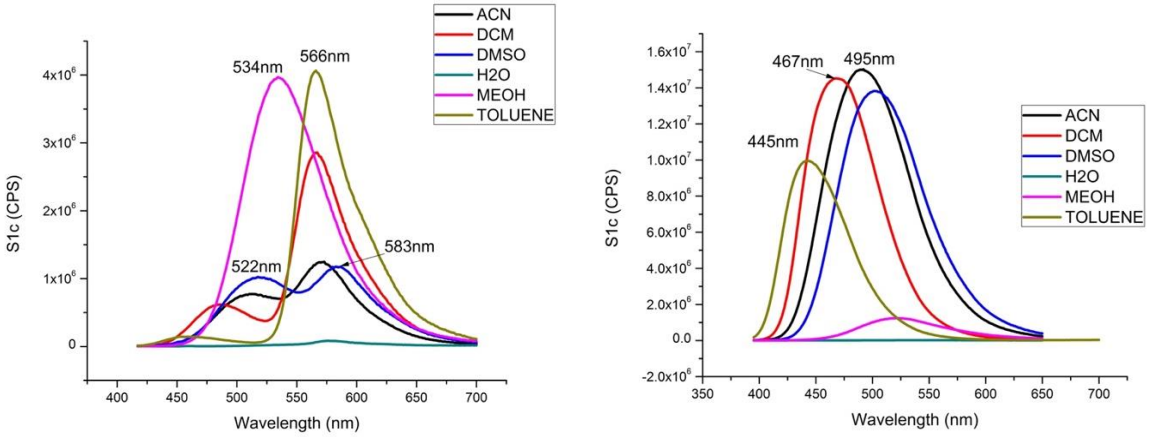


Figure 9: The emission spectra of **1** (left panel) and **2** (right panel) in various solvents.

It was noted that water completely quenched the fluorescence as expected for both **1** and **2**, due to the hydrogen bonding with water. As calculated by the difference between the absorbance λ_{max} and emission λ_{em}, compound **1** had a Stokes shift of roughly 166 nm, and **2** had a Stokes shift of roughly 110 nm. Both products underwent ICT process, by donation of the lone pair of electrons from the nitrogen to the carbonyl group, causing the molecule to give red-shifted emission when the excited flavonoid relaxed back to the ground state. Since compound **1** could also undergoes ESIPT, it had a greater Stokes shift than **2**.

Conclusion

The target flavonoids were successfully synthesized and analyzed by NMR spectrum to confirm the structure and purity. The hydroxyl form (i.e. flavonoid **1**) was synthesized first, and its hydroxyl group was masked by a methyl group to give flavonoid **2**. Both compounds were purified by using silica gel column chromatography, and different fractions were collected. NMR spectrum was used to determine which fractions contain the final product. The fractions containing the final product were examined by UV-vis and fluorescence spectroscopy in different solvents. Notable spectral difference in fluorescence was observed between **1** and **2**, showing that the hydroxyl group is essential for ESIPT that is responsible for the emission peak at longer wavelengths.

Acknowledgments

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Safety Appendix

Lab safety and instructions were provided by Keti Bertman, the graduate student overseeing this research. Personal Protective Equipment (PPE) were used at all times including gloves, safety glasses, and hair restrained when handling chemicals. All chemicals were disposed of in their appropriate waste containers. A mask was worn when handling silica. All work done was performed under the supervision of a graduate student in the lab. General lab and health protocols were maintained during this research.