Kristen Fox Chem 213
Synthetic #2 FFR

Green Synthesis of Fluorescein Dye

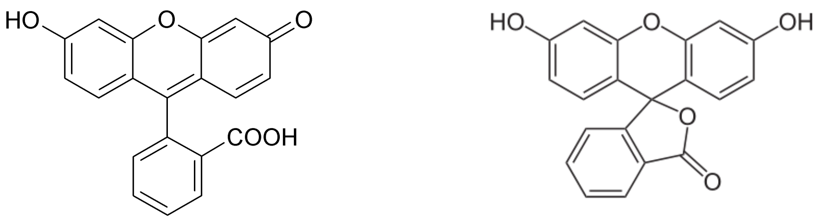
Introduction

Fluorescein is a fluorescent dye that has a significant impact in the fields of medicine and science. An everyday utilization of fluorescein is an additive to food dye known as Yellow No. 7. Being a fluorescent dye, it absorbs energy at a wavelength of λ_{max}496nm and its electrons then move to an excited state at the next energy level. The electrons then move down to the lower energy level and energy is then emitted as a fluorescence photon at a wavelength of 570-490 nm.[2] This range of emitted light on a wavelength emission spectrum corresponds with green light, which is the color fluorescein appears when emitted. Due to its fluorescent properties, fluorescein is commonly used in fluorescent microscopy to observe cell structure and their components. Additional uses are fluorescent tagged labeling on antibodies, which target specified areas in a cell and aid in locating desired components[1].

The purpose of the experiment is to synthesize fluorescein dye while adhering to the concept of Atom Economy. Atom Economy is a method of expression how efficiently a particular reaction makes use of the reactant atoms. In an ideal chemical reaction, the amount of starting material would equal the amount of product with no waste. [3]

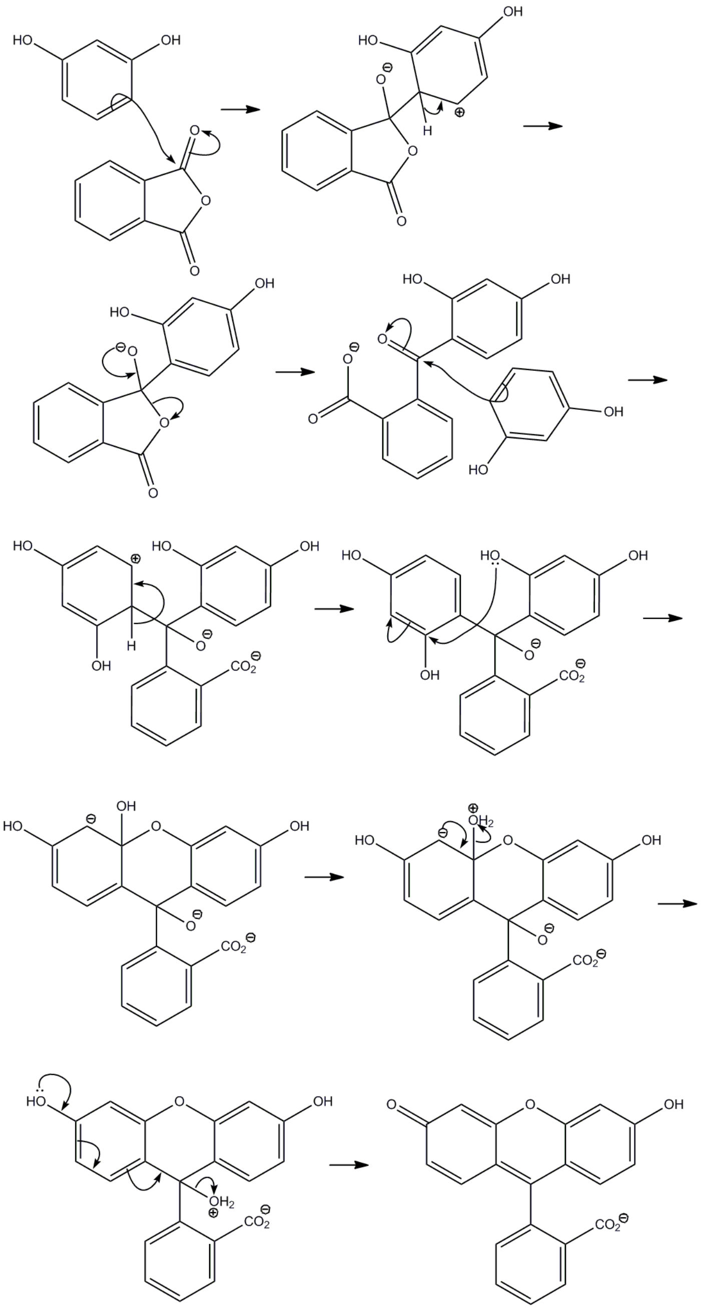
Fluorescein is synthesized by reacting resorcinol and phthalic anhydride with an acid catalyst. The reaction proceeds via two Fridel Crafts reactions. Near a neutral pH, derivatives of fluorescein exist in the carboxylic acid(1) and the closed lactone(2) forms. The product is then analyzed through 60MHz 1H and 400MHz 1H NMR instrumentation.

Scheme 1



(1) (2)

Scheme 2. Mechanism of Synthesis of Fluorescein



http://www.chem.ucla.edu/harding/IGOC/R/reaction_mechanism.html

The mechanism for the synthesis of fluorescein dye begins with an electron pair on the oxygen of resorcinol moving down to create a double bond on the benzene ring. This causes the double bonds on the ring to rearrange and a pair of electrons to attack the carbonyl group on the acid activated phthalic anhydride. An electron pair on the negatively charged oxygen moves down and kicks the single bonded oxygen off. A second resorcinol then attacks the carbonyl group of the compound creating a larger compound. The negatively charged oxygen then deprotonates a hydrogen adjacent to the positively charged carbon. An electron pair on a hydroxide ion attacks a carbon attacked to a hydroxyl group on the compound and kicks off an electron pair to create a negative charge on the ring. These electrons then move down and kick off a water group off. Finally, electrons on a hydroxyl group kick down and cause a change in double bonds on the ring, causing a water group to leave, creating the final product, fluorescein.

Experimental

Fluorescein Dye. Resorcinol (145mg, 1.32 mmol), phthalic anhydride (100mg, .3 mmol), 8M sulfuric acid (3 drops) were added and heated in sand bath to 180^{O}C. After running for 30 minutes, the solution cooled to room temperature. Acetone (5ml) was added and stirred for 10 minutes until it becomes a dark yellow color. Additional acetone (10 ml) was added until solid dissolved. The progress was monitored by TLC (80% ethyl acetate/ 20%). The solvent was evaporated to yield dark orange thick oil like substance. The residue was purified by column chromatography (80% ethyl acetate/20% hexanes) with thirty 10 ml fractions. Monitor the fraction with TLC and combine fractions that contain product. Solvent was evaporated to yield a solid orange powder. (.08g, 36%) [mp 314-316^{O}C]; ^{1}H NMR (60 MHz, DMSO) d ppm 7.88 (t, 1H), 7.75 (d, 1H), 7.48 (d, 1H), 7.2 (d, 1H), 6.8 (s, 1H), 6.2 (d, 1H); ^{1}H NMR (400 MHz, DMSO), d (ppm) 8.1 (d, 1H) ,7.89

(s, 1H), 7.77 (t ,1H), 7.68 (d 1H), 7.67 (t, 1H), 7.5 (t 1H), 7.4 (d 1H), 7.25 (d, 1H), 6.85; UV/Vis λ_{max} 500 nm

Results and Discussion

The synthesis of fluorescein dye from resorcinol and phthalic anhydride with an acid catalyst proceeded through two subsequence Freidel Crafts reactions. The goal of the experiment was to synthesis the product through the idea of Green Chemistry. Green Chemistry, also known as Atom Economy, is a chemical reaction that does not waste any atoms, meaning to amount of reactants equals the amount of product. The final product was then purified through column chromatography and monitored by TLC to determine purity to yield a solid orange powder. The Rf value of the TLC product was .67. Compared to the .83 Rf value of the starting material resorcinol standard, the data concludes the product was successfully purified.

Melting point was a useful tool to determine the purity and identification of the synthesized compound. The literature melting point of fluorescein is 314-316^{O}C. The experimental melting point determined in lab was 220-225^{O}C. This difference in melting points is due to impurities located in the product, which depress the melting point value.

UV/Vis spectroscopy was a useful technique taken of the product to determine the absorbance of a particular sample. The product gave off an absorbance of 500 nm, which corresponds with the range of green light, the fluorescent color fluorescein gives off.

NMR ^{1}H 60 MHz and ^{1}H 400 MHz was also used to analyze and determine the synthesized product(Figure 3,4,5 Supplemental information). The 400 ^{1}H MHz NMR shows a peak at 8.1 ppm which splits into a doublet. A singlet is found at 7.8 ppm that corresponds with the hydroxyl group coming off the ring. Many aromatic hydrogens are found which correspond with the many hydrogens coming off the numerous benzene rings located in the product fluorescein. Those aromatic hydrogens are found at 7.8 with a triplet, 7.7 split into a doublet, and 7.7 ppm split into a triplet. Other can be located at 7.6, which is split into a triplet, 7.5 which is split into a doublet, and 7.4 ppm which is also split into a doublet. A total of 8 hydrogens are present in the 400 MHz ^{1}H NMR. Water appeared on the NMR as an impurity in the 60 M between 7-8 ppm as a bump on the graph.

NMR, UV/Vis, and melting point all aided to determine the purity and identification of the synthesized compound. These analyzes showed the compound was not completely pure and contained impurities that depressed the melting point. The impurity water appeared on the 60 ^{1}H MHz NRM as a large bump between 7-8ppm. Fluorescein theoretical yield was calculated to be .22g. The actual yield synthesized of fluorescein was calculated to be 36% with .08g collected. Although the yield is not ideal, it is acceptable. Possible error in the experiment could be allowing the temperature to exceed 200^{O}C and burning the compound when heating in the sand bath. Future goals would be to synthesize more product to get a more successful percent yield.

Fluorescein dye is an extremely useful product that can be synthesized in a green manor through multiple freidel crafts reactions. It is used as a fluorescent dye for labeling and visualizing cells and their components such as fibrous networks and proteins and aids in the fields of medical and science. The synthesized product was finally analyzed by melting point, UV/Vis, 60 ^{1}H MH NMR, and 400 ^{1}H MHz NMR.

References

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