JOURNAL OF CHEMICAL EDUCATION

pubs.acs.org/jchemeduc

Demonstration

Watch It Happen: Teaching Esterification and Hydrolysis with Fluorescein Diacetate

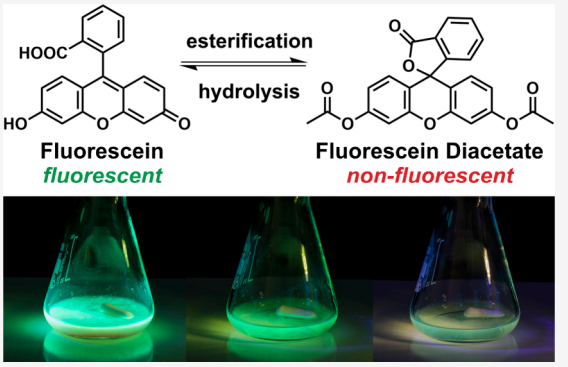
Alexander Paul Fritz, Dominik Diekemper, and Stefan Schwarzer*

Cite This: *J. Chem. Educ.* 2025, 102, 5010−5014

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Classic esterification experiments often engage students through their characteristic aromas but typically lack a direct visual indicator of reaction progress. This experiment addresses that gap with a simple, rapid, and visually observable method for teaching both esterification and ester hydrolysis. By converting fluorescein into its nonfluorescent ester, students can visually track the reaction progress under UV light. The esterification is complete within approximately 10 min, making it well-suited for classroom or laboratory settings with limited time. The product, fluorescein diacetate, is obtained in high yield and good purity through simple precipitation and filtration. Subsequent basic hydrolysis demonstrates the reverse reaction, with the reappearance of fluorescence providing a vivid real-time indicator of ester cleavage.



KEYWORDS: *high school/introductory chemistry, organic chemistry, demonstrations, esters, mechanisms of reactions*

ESTER SYNTHESIS: A VISUAL TWIST

Banana, blueberry, and mango—these familiar fruity aromas can be attributed to esters, a class of compounds frequently encountered as flavor additives in food products. In chemistry education, ester synthesis is often introduced through aroma-based experiments,[1,2] leveraging students' prior olfactory experience for engagement. While these reactions strongly appeal to the sense of smell,[3−6] they offer limited visual cues, making them ideal for highlighting chemical structure−property relationships beyond purely optical observation.

In contrast, reactions that produce visible effects, such as a color or fluorescence change, are more convenient to observe collectively and can enhance student engagement, especially in demonstration settings.[7] Visual reactions are also particularly valuable when time, equipment, or safety considerations limit the feasibility of hands-on experiments, as they still allow all students to follow and understand the chemical transformation.

Despite this potential, esterification reactions with a strong visual component remain scarce in the literature and teaching practice. Janssens et al.[8] described the visual observation of catalyst recovery after esterification, while Kam et al.[9] reported a color-based extraction of a synthesized ester. However, to our knowledge, no published experiment offers the direct visual monitoring of an esterification suitable for classroom demonstrations.

To address this gap, we present a simple and fast esterification experiment that combines the familiar concept of ester formation with a fascinating visual effect: the conversion of fluorescein into fluorescein diacetate (FDA) (Figure 1). This reaction proceeds within minutes and can be conducted using readily available materials and reagents,

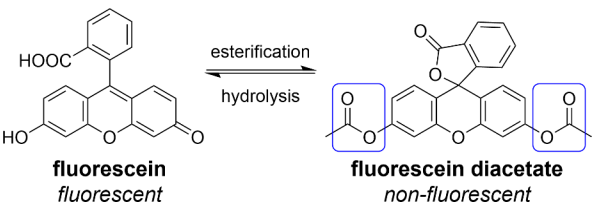
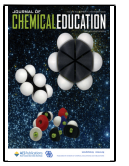


Figure 1. Structures of fluorescein (left) and fluorescein diacetate (right). The ester groups formed during the esterification are marked in blue.

making it highly suitable for use in school laboratories. While fluorescein exhibits intense green fluorescence under UV light, its diacetylated ester FDA is nonfluorescent, resulting in a visible decrease in fluorescence as the esterification progresses. The reversibility of the esterification reaction can also be demonstrated by subsequently hydrolyzing the synthesized FDA in sodium hydroxide solution, resulting in the return of fluorescence.

Received: August 13, 2025
Revised: September 30, 2025
Accepted: October 20, 2025
Published: October 27, 2025



https://doi.org/10.1021/acs.jchemed.5c01150
J. Chem. Educ. 2025, 102, 5010−5014

Fluorescein is a well-known xanthene dye with an intense green emission around 515 nm under UV or blue light. Its strong fluorescence, large Stokes shift, and high quantum yield (up to 0.95) make it widely used in analytical research, education, and applications such as tracer studies or ophthalmic diagnostics.[10−12] Its phenolic groups allow easy derivatization, for example to fluorescein diacetate, a non-fluorescent, cell-permeable ester commonly used in cell biology to assess membrane permeability and esterase activity.[13]

Fluorescein has been proposed multiple times as a cost-effective and visually engaging chemical for educational use and has already been employed in various classroom demonstrations involving fluorescence spectroscopy and pH-dependent fluorescence quenching.[14,15]

EXPERIMENTAL PROCEDURE

The following section presents an approach for visualizing the described process. In the fluorescein−FDA system, two consecutive experiments can be performed. First, the esterification of fluorescein to form FDA is demonstrated. Subsequently, the reverse reaction—hydrolysis of FDA back to fluorescein—is presented. A video of the experiment is available in the Supporting Information.

Materials

The required materials include fluorescein, acetic anhydride, concentrated sulfuric acid or *p*-toluenesulfonic acid monohydrate, sodium hydroxide solution (10 wt %), a magnetic stir bar, a hot plate, a 25 mL Erlenmeyer flask, a 100 mL Erlenmeyer flask, a 100 mL beaker, pipettes, a funnel and paper filter, a large test tube, and a UV flashlight with a wavelength of 365 or 395 nm.

It is essential to use the free acid form of fluorescein and not the sodium salt (uranine) for the esterification. Acetic anhydride is a regulated precursor chemical under drug control legislation in some countries. For this demonstration, only small amounts are required (5 mL per experiment), and acquisition must comply with local regulations.

Synthesis of Fluorescein Diacetate via Esterification of Fluorescein

In a fume hood or well-ventilated laboratory, 5 mL of acetic anhydride is added to a 25 mL Erlenmeyer flask containing a magnetic stir bar. Then 0.05 g of fluorescein is added, resulting in the formation of a red suspension. Using a pipet, no more than one small drop of concentrated sulfuric acid is added as a catalyst. As a safer alternative to sulfuric acid, 0.05 g of *p*-toluenesulfonic acid monohydrate can be used instead. Subsequently, the flask is covered with a watch glass, placed on a hot plate and heated to 120 °C while stirring. The reaction mixture turns into a yellow solution that exhibits intense bluish-green fluorescence under UV light. If the mixture turns dark orange instead, too much sulfuric acid was added, resulting in the decomposition of fluorescein. As the target temperature is reached and the reaction proceeds, the fluorescence gradually diminishes and almost completely disappears after approximately 6 min (Figure 2).

The reaction mixture is then poured into a 100 mL beaker containing 50 mL of water and stirred until no phase separation is visible and a colorless precipitate forms. The solid is collected by filtration using a paper filter and washed with water. The resulting product is FDA, obtained as a colorless to slightly yellow solid (Figure 3) in an isolated yield of 80%. The presence of FDA was confirmed by ^{1}H NMR spectroscopy (Figure S1).

Figure 2. From left to right: reaction mixture after 1 min at 120 °C, after 2 min, and after 5 min (under UV light).

Figure 3. Fluorescein and fluorescein diacetate.

Hydrolysis of Fluorescein Diacetate to Fluorescein

A large test tube is fixed to a stand using a clamp for better visibility (alternatively, a test tube rack may be used). After filling it with a 10 wt % sodium hydroxide solution, a small amount (spatula tip) of solid FDA is added. Under UV light, the formation of bright-green fluorescent streaks is observed as particles disperse (Figure 4). Particles remaining at the surface exhibit a distinct color change to red, indicating the hydrolysis of FDA back to fluorescein (Figure 5).

Fluorescein further reacts with sodium hydroxide to form the sodium salt of fluorescein (uranine), which increases its water solubility. As a control experiment, FDA can be added to a test tube containing only water, in which no fluorescence is observed. The hydrolysis can be done at room temperature and does not require a fume hood.

HAZARDS

For safety reasons, protective goggles, gloves, and a lab coat should be worn. Fluorescein may cause minor skin and eye irritation. Acetic anhydride is corrosive and must be handled in a well-ventilated laboratory or a fume hood. Concentrated sulfuric acid is highly corrosive and poses severe risks to skin and eyes. *p*-Toluenesulfonic acid may cause eye and skin irritation. Sodium hydroxide solution poses severe risks to skin and eyes. Heating on a hot plate (120 °C) carries a risk of thermal burns. UV flashlights emit ultraviolet radiation, which can be harmful to the eyes, so direct exposure should be avoided. Given these considerations, the experiment is best suited as a teacher-led demonstration.

When the esterification reaction mixture is poured into water, an aqueous solution containing acetic acid and the acid catalyst is formed. During hydrolysis, an aqueous sodium hydroxide solution containing the sodium salt of fluorescein is obtained. Both solutions must be neutralized and collected in the appropriate waste containers.

RESULTS AND DISCUSSION

Based on existing literature[16−18] and our own investigations, the reaction pathway shown in Figure 6 is proposed.

https://doi.org/10.1021/acs.jchemed.5c01150
J. Chem. Educ. 2025, 102, 5010−5014

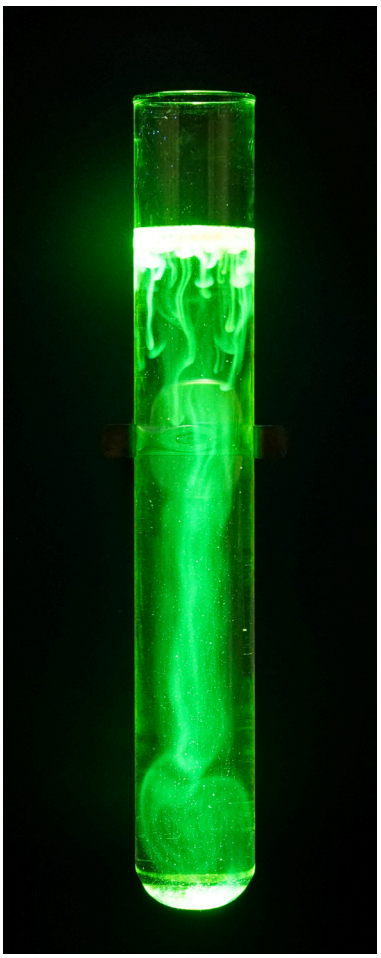


Figure 4. Hydrolysis of fluorescein diacetate to fluorescein in a sodium hydroxide solution under UV light.

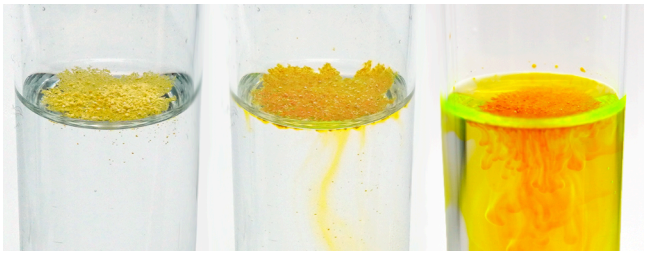


Figure 5. From left to right: fluorescein diacetate immediately after addition to sodium hydroxide solution, after 2 min, and after 5 min.

Fluorescein exhibits low solubility in acetic anhydride, which initially leads to the formation of a red suspension. Upon addition of the acid catalyst, the protonated form of fluorescein is generated, which dissolves well in acetic anhydride and results in a yellow solution which shows intense bluish-green fluorescence under UV light.[17] The yellow coloration is attributed to the delocalized positive charge within the pyrylium-type ring system.[16]

The protonated form of fluorescein has two hydroxyl groups, which undergo esterification with acetic anhydride via the acid-catalyzed esterification mechanism (Figure 7). This reaction

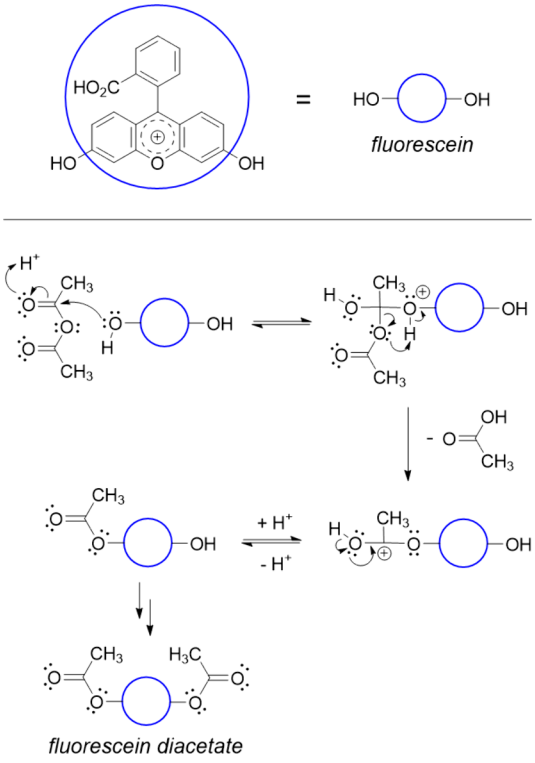


Figure 7. Reaction mechanism of the esterification of fluorescein with acetic anhydride under acidic conditions. Fluorescein is stylized as a circle with two hydroxyl groups for the sake of clarity.

yields the protonated form of fluorescein diacetate, which shows only weak fluorescence. However, it still appears yellow due to the presence of the same pyrylium ring system.[16]

When the reaction mixture is poured into water, fluorescein diacetate is deprotonated, resulting in the precipitation of the neutral, colorless and nonfluorescent form of the compound. The ester is colorless due to the break of the π conjugation by the lactone ring.[19]

PEDAGOGICAL CONSIDERATIONS

A key pedagogical advantage of this experiment is that esterification and hydrolysis can be directly and visually monitored, providing an immediate and engaging representation of a fundamental chemical transformation. Color changes, fluorescence, or other optical effects allow for collective

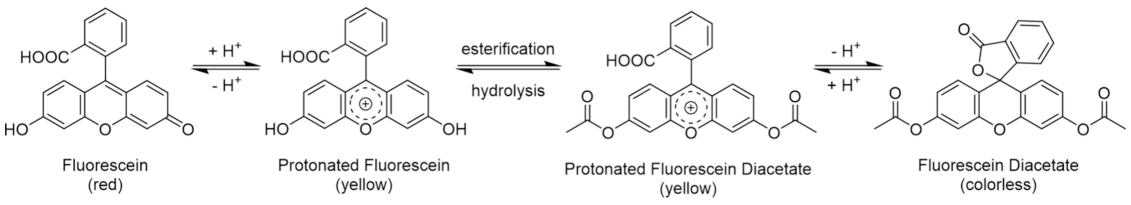


Figure 6. Proposed reaction pathway of the esterification of fluorescein to fluorescein diacetate.

https://doi.org/10.1021/acs.jchemed.5c01150
J. Chem. Educ. 2025, 102, 5010−5014

observation in classroom or lecture hall settings, making them well-suited for demonstration experiments where individual sensory engagement (such as smelling) is impractical. In addition, visual transformations can make real-time changes during a chemical reaction more apparent, thereby providing opportunities for learners to follow the reaction's progress and potentially strengthen their understanding of how macroscopic phenomena relate to molecular-level processes.[7]

For use in a high school setting, it is sufficient to discuss the simplified reaction mechanism shown in Figure 7. The detailed structure of fluorescein, the protonated forms, and the reason why its diacetate derivative is nonfluorescent do not need to be addressed at this level.

While the reaction with acetic anhydride differs slightly from the Fischer esterification typically taught in schools (where carboxylic acids are used instead of anhydrides) the fundamental steps remain the same: protonation of the carbonyl oxygen, nucleophilic attack of the alcohol on the carbonyl carbon, tautomerization, elimination of a small molecule (in this case, acetic acid instead of water), and deprotonation of the carbonyl oxygen. This offers a valuable opportunity to encourage students to transfer their knowledge of Fischer esterification and apply it to a new context, thereby promoting mechanistic thinking over rote memorization.

In introductory undergraduate organic chemistry courses, this experiment can be extended to cover more advanced concepts such as delocalized π systems and tautomerism. The loss of fluorescence upon esterification provides an example of how molecular structure influences electronic properties: esterifying the phenolic hydroxyl groups prevents the formation of the quinoid, thereby locking the molecule into its lactone structure, which in turn disrupts the extended π conjugation responsible for fluorescence (Figure 6).[17] Furthermore, both the esterification and hydrolysis can be quantitatively monitored through fluorescence intensity measurements, allowing for kinetic studies.[20] Since the esterification also proceeds at a reduced temperature of 60 °C within 50 min, the reaction can be performed in a temperature-controlled fluorimeter cuvette, providing a potential application in a physical chemistry laboratory.

CONCLUSIONS

This experiment provides a valuable addition to the chemistry curriculum by combining the well-established topic of esterification with a visually striking and easily interpretable fluorescence change, allowing students to monitor the reaction progress in real time. The short reaction time, high yield, and simple handling make it ideally suited for use in classrooms or laboratory settings with limited time and resources. The subsequent hydrolysis provides a vivid demonstration of ester cleavage and reinforces the reversibility of esterification reactions. In addition, the experiment may provide opportunities for mechanistic thinking by prompting students to connect their knowledge of Fischer esterification with a related but slightly different mechanism. Such engagement could contribute to developing a deeper conceptual understanding and enhance students' ability to apply reaction mechanisms beyond rote memorization.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.5c01150.

Video of the experiment (MP4)

^{1}H NMR spectrum of synthesized fluorescein diacetate and UV−vis spectra (PDF)

AUTHOR INFORMATION

Corresponding Author

Stefan Schwarzer − *Department of Chemistry Education, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany;* orcid.org/0000-0002-4134-5684; Email: stefan.schwarzer@uni-tuebingen.de

Authors

Alexander Paul Fritz − *Department of Chemistry Education, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany;* orcid.org/0009-0007-0049-8012

Dominik Diekemper − *Department of Chemistry Education, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany;* orcid.org/0000-0003-0916-475X

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jchemed.5c01150

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Rebecca Seitz for her assistance with the experimental procedure, Tim Fiedler for suggesting the use of *p*-toluenesulfonic acid as an alternative catalyst, and Hannah Röhrig for her assistance in obtaining the pictures and video of the experiment.

REFERENCES

(1) Bromfield-Lee, D. C.; Oliver-Hoyo, M. T. An Esterification Kinetics Experiment That Relies on the Sense of Smell. *J. Chem. Educ.* **2009**, *86* (1), 82.

(2) McCullagh, J. V.; Hirakis, S. P. Synthesis of the Commercial Fragrance Compound Ethyl 6-Acetoxyhexanoate: A Multistep Ester Experiment for the Second-Year Organic Laboratory. *J. Chem. Educ.* **2017**, *94* (9), 1347−1351.

(3) Marino, M. A.; Wisnewski, F.; Alexis, A.; Reilly, C. Multisensory Learning in the Undergraduate Chemistry Lab: The Case for Smell. *J. Chem. Educ.* **2025**, *102*, 2592.

(4) Steele, J. H.; Bozor, M. X.; Boyce, G. R. Transmutation of Scent: An Evaluation of the Synthesis of Methyl Cinnamate, a Commercial Fragrance, via a Fischer Esterification for the Second-Year Organic Laboratory. *J. Chem. Educ.* **2020**, *97* (11), 4127−4132.

(5) Reilly, M. K.; King, R. P.; Wagner, A. J.; King, S. M. Microwave-Assisted Esterification: A Discovery-Based Microscale Laboratory Experiment. *J. Chem. Educ.* **2014**, *91* (10), 1706−1709.

(6) Ji, Q.; El-Hamdi, N. S.; Miljanić, O. Š. Scent Transmutation: A New Way To Teach on Chemical Equilibrium, Distillation, and Dynamic Combinatorial Chemistry. *J. Chem. Educ.* **2014**, *91* (6), 830−833.

(7) Diekemper, D.; Pölloth, B.; Schwarzer, S. From Agricultural Waste to a Powerful Antioxidant: Hydroxytyrosol as a Sustainable Model Substance for Understanding Antioxidant Capacity. *J. Chem. Educ.* **2021**, *98* (8), 2610−2617.

(8) Janssens, N.; Wee, L. H.; Martens, J. A. Esterification Reaction Utilizing Sense of Smell and Eyesight for Conversion and Catalyst Recovery Monitoring. *J. Chem. Educ.* **2014**, *91* (6), 876−879.
(9) Kam, C. M. T.; Levonis, S. M.; Schweiker, S. S. A Visual Organic Chemistry Reaction: The Synthesis of 4-Amino-3-nitrobenzoic Acid Methyl Ester via Fischer Esterification. *J. Chem. Educ.* **2020**, *97* (7), 1997−2000.
(10) Martin, M. M.; Lindqvist, L. The pH dependence of fluorescein fluorescence. *J. Lumin.* **1975**, *10* (6), 381−390.
(11) Alharbi, A. H.; Khan, S. Antimicrobial, antioxidant, cell imaging and sensing applications of fluorescein derivatives: A review. *Anal. Biochem.* **2024**, *688*, 115479.
(12) Sehrawat, S.; Yadav, R. K.; Pratap Singh, A. A review on derivatives of fluorescein aldehydes and their applications. *Mater. Today Proc.* **2022**, *61*, 1093−1099.
(13) Kramer, D. N.; Guilbault, G. G. A Substrate for the Fluorometric Determination of Lipase Activity. *Anal. Chem.* **1963**, *35* (4), 588−589.
(14) Blitz, J. P.; Sheeran, D. J.; Becker, T. L.; Danielson, N. D. Classroom Demonstrations of Concepts in Molecular Fluorescence. *J. Chem. Educ.* **2006**, *83* (5), 758.
(15) Matsuoka, M.; Akitsu, T. Hydrolysis of 2-Chloro-2-methylpropane—Demonstration Using the Quenching of Fluorescence from Fluorescein. *J. Chem. Educ.* **2021**, *98* (3), 941−945.
(16) Markuszewski, R.; Diehl, H. The infrared spectra and structures of the three solid forms of fluorescein and related compounds. *Talanta* **1980**, *27* (11), 937−946.
(17) Sjöback, R.; Nygren, J.; Kubista, M. Absorption and fluorescence properties of fluorescein. *Spectrochim. Acta, Part A* **1995**, *51* (6), L7−L21.
(18) Zanker, V.; Peter, W. Die prototropen Formen des Fluoresceins. *Chem. Ber.* **1958**, *91* (3), 572−580.
(19) Yan, F.; Fan, K.; Bai, Z.; Zhang, R.; Zu, F.; Xu, J.; Li, X. Fluorescein applications as fluorescent probes for the detection of analytes. *TrAC, Trends Anal. Chem.* **2017**, *97*, 15−35.
(20) Johnson, R. J.; Hoops, G. C.; Savas, C. J.; Kartje, Z.; Lavis, L. D. A Sensitive and Robust Enzyme Kinetic Experiment Using Microplates and Fluorogenic Ester Substrates. *J. Chem. Educ.* **2015**, *92* (2), 385−388.

https://doi.org/10.1021/acs.jchemed.5c01150
J. Chem. Educ. 2025, 102, 5010−5014