Using Experimental Spectroscopic Data to Guide and Validate Mechanisms in Catalyzed Aldol Reactions

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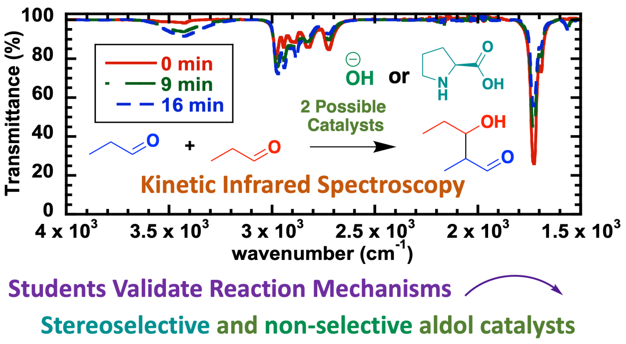
Abstract

Organic chemistry students typically struggle with reaction mechanisms; particularly in how they are proposed and justified. In this activity targeting second year organic undergraduates, students used infrared spectroscopy (IR) to track the reaction progress of two distinct aldol reactions and used polarimetry to analyze stereoselectivity of aldol catalysts. Students worked in two pairs, one focusing on the traditional hydroxide-catalyzed aldol reaction (two units of propionaldehyde combining via an enolate intermediate) and the other focusing on the enantioselective L-proline-catalyzed aldol reaction (propionaldehyde catalyzed by L-proline, showing an iminium intermediate). During the course of the lab period, students used IR spectra showing kinetic data and guided questions to propose and validate the reaction mechanisms. After the pairs of students analyzed their individual reactions, they formed groups of four to further analyze and compare the two mechanisms. This comparison of IR and polarimetry data allowed students to discuss both pathways and consider why chemists use different reaction conditions to reach the same product. The focus of this experiment is to improve the understanding of reaction mechanisms and the process by which scientists propose and justify mechanisms, while giving students practical experience with IR spectroscopy, polarimetry, and intermediate analysis.

Key Words: Organic Chemistry; Inquiry Based / Discovery Learning; Mechanism of Reactions; IR Spectroscopy; Second Year Undergraduate; Aldehydes / Ketones; Chirality/Optical Activity; Catalysis

Graphical Abstract



Introduction

Mechanisms are commonly used in organic chemistry to provide detailed understanding of how a reaction proceeds. Understanding a reaction mechanism is needed to optimize chemical processes.[1–3] Mechanisms have been a critical part of organic chemistry courses for many years, the American Chemical Society even specifies various reaction mechanisms as learning objectives for organic chemistry students.[4] Mechanisms have been a critical part of organic chemistry courses for many years.[5–8] Typically, the curved arrow formalism is used.[9] Previous research has shown that students do not properly understand the concepts driving arrow pushing, instead they employ memorization.[10–12] When asked synthetic questions, students develop mechanisms by connecting the reactant to the product through the most direct steps. Students often do not consider the feasibility of the chemistry or the chemical principles that guide a reaction.[12,13] New educational materials are needed to address this gap in students understanding of mechanisms. It is especially important that these educational materials discuss specifics of mechanism validation, in contrast to more traditional classroom settings where mechanisms would only be presented.

Several mechanism-probing experiments have been reported in the literature for undergraduate organic chemistry classes.[14–17] Traditionally, labs place significant focus on “cookbook” chemistry over learning the fundamental chemical principles that are needed for a deep understanding.[18] Some recently-developed labs go beyond this “cookbook” approach, and push students to develop mechanisms. However, many of these experiments provide the product to the students, in which case they could focus on getting to the product without considering the feasibility of the chemistry occurring or seeking evidence for their mechanism from experimental data.[12] Grant and Latimer developed a laboratory experiment using ^{1}H NMR and guiding questions to lead students to probe the mechanism that converts cholesterol into dibromocholesterol.[16] This is an equally fundamental reaction to the aldol reaction presented here, but NMR access can be difficult to obtain for large classes. A number of labs have also been developed focusing on the aldol reaction.[19–23] However, these labs focus on the use of complex catalysts to drive the reaction in an atypical way, either creating an enantioenriched product[20,23] or pushing students to elucidate the reactants based on the product’s spectroscopic data.[21] Yezierski et al. developed an upper division undergraduate laboratory that focuses on catalyst, enantioselectivity and characterization of aldol products.[24] The laboratory experiment developed here combines mechanistic analysis with classic carbonyl chemistry and catalysis. Additionally, this laboratory employs kinetic data from IR spectroscopy for students to develop their analytical skills. The combination of these foci push students to validate a mechanism guided by raw IR and polarimetry data, along with a scaffolded series of worksheets and questions.

The learning objectives for students in this laboratory experiment are:

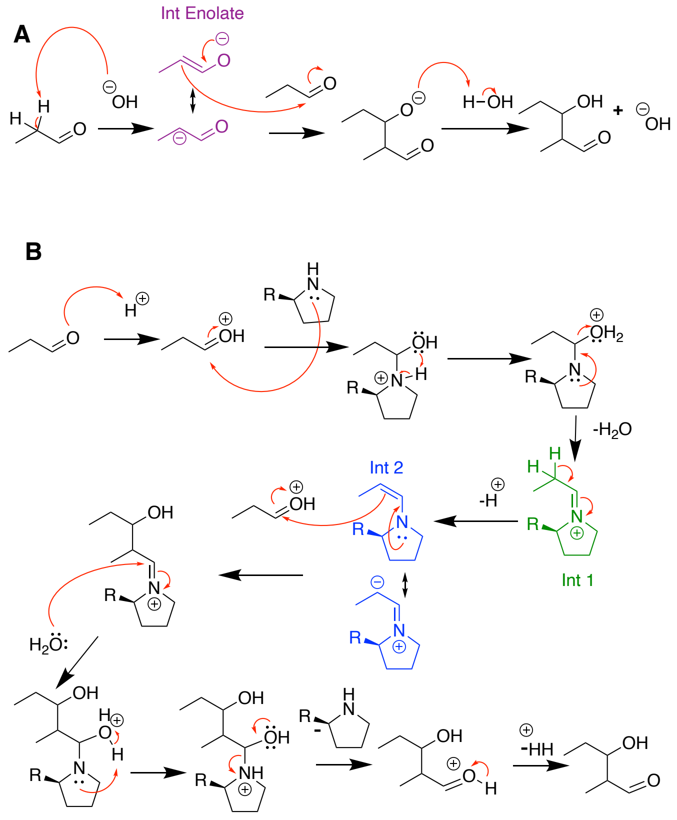
1. Analyze the IR spectra of a reaction taken at different times.
2. Validate and arrive at a mechanism consistent with experimental IR evidence.
3. Use polarimetry to analyze their products and compare two reactions.
4. Apply learned skills to analyze the benefits of various synthetic approaches to a given product.

This experiment was designed as a guided-inquiry experience, providing student pairs with minimal starting information and asking questions to lead them to synthesize their own knowledge. Two catalytic systems are considered, with half of student pairs working on hydroxide-catalyzed aldol reactions and half the student pairs working on L-proline-catalyzed aldol reactions. Student pairs propose a reasonable mechanism for their system based on existing chemistry knowledge and experimental data. To facilitate peer-peer interactions and learnings, students studying one catalytic system compare mechanisms and share knowledge with students working on the alternative catalytic system. Following the general principles of process oriented guided inquiry learning (POGIL), guided inquiry questions are used to prompt students as they develop their evidence-based reaction mechanisms.[25,26]

Background

In this experiment, students develop the mechanism for aldol reactions of propionaldehyde with distinct catalysts of either L-proline or NaOH, using IR spectra to identify intermediates. Intermediate analysis has been used by researchers to guide mechanistic chemistry[27–30] and validate postulated mechanisms. Here, the intermediates are expected to be in a steady state. The steady state intermediate concentrations, consistent with the typical IR vibration frequencies of the proposed intermediates, are measurable in this experiment, especially for the L-proline-catalyzed system. In this way the students are using experimental data to guide the development of a reasonable mechanism. The NaOH-catalyzed aldol reaction is typically covered in the second semester of undergraduate chemistry courses.[31] As indicated in Scheme 1A, the core mechanisms for this reaction occurs via α-hydrogen removal to form an enolate intermediate which then reacts with a second propionaldehyde molecule to form the aldol product.

The L-proline-catalyzed aldol reaction is not typically covered in organic chemistry textbooks.[5] The proposed mechanism, developed by the List and MacMillan groups,[32,33] is given in Scheme 1B. The initial step in the mechanism is the formation of a chiral iminium from the reaction of propionaldehyde with the L-proline catalyst. The iminium intermediate is in equilibrium with an enamine intermediate, which can react with a second molecule of propionaldehyde. The final step involves the expulsion of the L-proline, yielding a carbonyl and regenerating the catalyst.[32,33] It is important to note that students do not perform complete and definitive IR assignments in this activity, but rather identify peaks consistent with key functional groups in proposed intermediates, and use these intermediates to validate a mechanism. The carboxylic acid can reversibly form ester-based intermediates.[34] However, these are unlikely to lead to peaks that are clearly distinct from the carbonyl stretch of the product or propionaldehyde starting material.



Scheme 1 **A:** Proposed mechanism of NaOH-catalyzed aldol addition.[35] **B:** Proposed mechanism of L-proline-catalyzed aldol addition.[23] Int refers to intermediate.

Experimental Overview

This experiment consists of three major components: the pre-lab (see Student Handout in Supporting Information), the wet lab, and the guided inquiry questions and problem-solving activities. The laboratory is designed to be implemented prior to the discussion of aldol reactions in lecture and ideally after acidity of carbonyl compounds is presented. Implementing this lab before the aldol mechanism is taught is important as it allows students to explore the L-proline and NaOH mechanisms in the context of IR data, rather than based on content already covered in lecture on one specific reaction mechanism.

The pre-lab prepares students for the analysis they will be conducting and provides background information. The focus is on understanding what IR spectra represent and what a relative change in peak intensity indicates. Additionally, students are asked questions about the function of catalysts and to draw out related mechanisms, such as the mechanism for imine transformation to the aldehyde. Note that the facilitator may need to prompt the students to connect the pre-lab to the questions in the worksheets following the wet lab.

For the wet lab, students conduct either the NaOH-catalyzed reaction or the L-proline-catalyzed aldol reaction. The wet lab gives students access to the chemistry they are investigating including enantioselective reactions, but the key component of the activity is the guided inquiry questions. The reactions are stirred on ice for 5-10 minutes and then stored in a drawer until the next lab session. In the following lab session a short work up is performed and the products of the aldol reaction are analyzed by IR spectroscopy (Details in Supporting Information: Student Handout under “The Experiment”). After collecting the IR spectra, students performing the same experiment (NaOH or L-proline) combine their products and determine the specific rotation of the product. Combining products ensures a solution with sufficient concentration to have a measurable optical rotation. The students should find that the product from the NaOH catalyst is racemic, with negligible optical rotation, while the product from the L-proline catalyst is enantioenriched, with non-zero optical rotation. This is to highlight the importance of enantioselectivity as it relates to catalyst selection, as addressed in the post-lab questions (see Student Handout in Supporting Information). These polarimetry values are used to indicate a difference in the products, the precise enantiomer or specific rotation are not calculated in this experiment. All the students were able to extract and isolate their product, collect IR spectra, and contribute to the polarimetry sample.

In smaller class settings, where extended access (15-20 min) to IR spectrometers is possible for all students, the student groups can collect their own kinetic IR spectra in the wet lab (following the procedure at the end of the Student Handout). However, in larger class settings or ones where there is limited access to IR spectrometers the kinetic IR spectroscopic data available in Supporting Information can be used for kinetic analysis. To minimize evaporation during IR reaction monitoring, it is important that the aldol reaction mixture be maintained in a closed vessel. A fresh aliquot is deposited on the IR crystal for each measurement.

After the student lab pairs finished the wet lab portion (~30 minutes to set up the week before with ~1 hour to work up, with an additional 30-60 min if students are performing their own IR kinetic experiments), the NaOH and L-proline groups worked through Worksheet 1 (~3 hours). The students were formally paired with their lab partners, but collaboration was encouraged between all of the groups considering the same catalyst. Note: the NaOH and L-proline groups were separated in order to prevent students from comparing reactions before working on Worksheet 2. Worksheet 2 was completed the following week in lab (~2 hours) and involved a jigsaw method[36] of collaboration, pairing one L-proline group with one NaOH group to compare and analyze the two reaction mechanisms.

Facilitation

Before the class, students should receive the document Student Handout given as Supporting Information, which contains background, prelab questions, experimental procedures and post lab questions. The guided inquiry activities should occur immediately following the wet lab to facilitate connection between the experiment and the analysis, although it could also be performed in a later class session. Students who did the NaOH-catalyzed aldol wet lab should be given Worksheet 1_{OH} and IR tables and those who worked on L-proline-catalyzed reactions should be given Worksheet 1_p and IR tables (see Supporting Information). Kinetic IR spectra throughout the reaction are evaluated to observe products, changes in reactants and intermediates of the reaction. The guided inquiry activities have students focus on one catalyst and then share and compare their data-driven mechanisms with other students.

In the NaOH-catalyzed reaction the starting materials and products can be clearly identified from the IR spectra. The starting materials have a clear carbonyl peak (~1700 cm^{-1}), with the product exhibiting OH stretch bands (~3400 cm^{-1}) and changes in the carbonyl region (~1700 cm^{-1}). Some students may identify the peak at (~1550 cm^{-1}) as the C=C stretch of the enolate intermediate, using this to support their mechanism. In the L-proline system, the formation of OH stretch bands (~3400 cm^{-1}) is similar to the NaOH case. However, two key IR peaks appear, with one consistent with a C=N+ iminium (~1690 cm^{-1}) supporting Int 1 of Scheme 1B and the other consistent with the C=C of the enamine (~1640 cm^{-1}) supporting Int 2 of Scheme 1B. Only small shifts have been measured between imines and their iminium salts in IR spectra, therefore the peak at 1690 cm^{-1} is consistent with the iminium of Int 1, as an imine cannot form in this system.[37]

Following the principles of POGIL[38], students collaborate in teams of 2-4 on worksheets designed to guide them to construct their own description of the mechanism. There is at least one (ideally two per section of

25-30 students) facilitator present. The facilitator's primary role is to guide students through key concepts by asking questions rather than providing answers to the questions in the worksheet. In completing the worksheets, students draw on experimental data and their chemistry knowledge to develop and support their proposed mechanisms. Facilitators adapt their prompting and guidance to the needs of the student pairs to ensure progress without giving so much information that the learning is bypassed.

The experiment can be implemented through an in-person format with facilitation outlined above. Additionally, the provided IR spectra and polarimetry data can be used in remote settings, as outlined in the Supporting Information documents.

Hazards

Propionaldehyde (CAS #: 123-38-6) is a flammable and high odor chemical; it should not be removed from the fume hood during the lab. NaOH (CAS #: 1310-73-2) is a known caustic material that should be handled with care. If there is prolonged contact with NaOH, there is a high risk for burns, particularly the eyes. L-proline (CAS #: 147-85-3) is low risk but should be handled with care. Dichloromethane (DCM, CAS #: 75-09-2) is highly volatile and a potential carcinogen, inhalation should be avoided. DCM should be exclusively handled in the fume hood and solutions should be capped to minimize inhalation hazard. Sodium bicarbonate (CAS #: 144-55-8) will cause eye irritation on contact. Brine is not considered hazardous by the OSHA (Occupational Safety and Health Administration) Hazard Communication Standard.[39] Sample preparation should be done in a fume hood, with students wearing proper protection (safety goggles and nitrile gloves) during the wet lab.

Results and Discussion

The experiment was performed in summer of 2018 by 9 students in 1 section of a second semester non majors organic chemistry laboratory and in spring 2019 by 74 undergraduate students enrolled in 3 distinct sections of a second-semester, majors organic chemistry laboratory. This experiment was conducted before the aldol reaction was taught in the lecture course. The timing of the activity was chosen such that the students would arrive at the aldol mechanisms as part of the laboratory activity, rather than draw on aldol knowledge from their lecture course. Further, the use of the L-proline enantioselective catalyst,[32] not typically covered in the sophomore organic curriculum, allowed the students to investigate the impact of chiral catalysis and associated mechanisms beyond the standard reactions in the organic sequence. The Summer 2018 cohort identified the need to use approximately 3 hours for the Worksheet analysis, which was built into the Spring 2019 implementation. Additionally, the Summer 2018 cohort demonstrated that online discussion board approaches to Worksheet 2 are reasonable.

After completing wet lab experiments and mechanistic analysis through the worksheets, the students submit reports. Although students work in pairs, each student can submit their own report detailing their interpretation, although the laboratory could be implemented in a way that pairs only submit one report. It is possible that student responses can vary, even between pairs, therefore analysis at the individual student level is preferred. Asking students to submit reports as pairs would decrease the number of mechanisms to be analyzed, but would not capture any potential discrepancies in student responses. The student report consists of eight pre-lab questions, 12-13 questions from Worksheet 1, eight questions from Worksheet 2, and four post-lab questions (see Supporting Information, Student Handout).

This experiment focused on guiding students to connect experimental data with theoretical concepts to create a well-justified mechanism. The IR spectra allowed students to determine functional groups consistent with their intermediates and their products. Students had to combine this IR data and basic organic chemistry concepts (eg. nucleophilic/electrophilic interactions) to develop likely mechanistic steps.

With few exceptions students achieved these goals by proposing Int Enolate in Scheme 1A for the NaOH system, as well as Int 1 and Int 2 of Scheme 1B for the L-proline system.

For the NaOH-catalyzed reaction, many students attributed the enolate intermediate's formation to an IR peak observed near 1563 cm^{-1} in the kinetic NaOH IR spectra. The provided IR wavenumber table (See Supporting Information) indicates no likely functional groups that would present at that frequency, but it is a peak that appears during the reaction and disappears by the end of the reaction. For the L-proline catalyzed reaction, the majority of students correlated the peak wavenumbers at 1690 and 1640 cm^{-1} to Int 1 and 2, respectively, of the L-proline mechanism.

Overall, the vast majority of students proposed a NaOH-catalyzed aldol reaction that reasonably agreed with the one in Scheme 1A. Similarly, the vast majority of students identified the key aspects of the L-proline catalyzed aldol reaction. Typical mechanisms that could be expected are given in Figure S1 for the NaOH reaction, and Figure S2 for the L-proline reaction. Considering this laboratory was performed before the NaOH-catalyzed aldol is taught in lecture and the L-proline-catalyzed is not typically discussed, this indicates that students deduced reasonable mechanisms using experimental IR data and leveraging existing chemistry knowledge as opposed to just applying or transcribing mechanisms from a text.

In comparing the catalysts, the major difference was that the NaOH was a simpler mechanism, was not stereoselective and prone to elimination. The L-proline reaction was stereoselective and occurred in milder conditions, reducing likelihood of the elimination reaction. Students analyzed polarimetry data to note the stereoselectivity of the two catalysts. A small majority of the students understood the purpose of the polarimetry and correctly identified L-proline as stereoselective and NaOH as nonselective. Most students had not worked on specific rotation problems since the beginning of the first semester of organic chemistry lecture, with no polarimetry lab experience. This laboratory enabled students to gain hands on experience with asymmetric reactions, often for the first time.

Overall, the students were able to arrive at and validate mechanisms by connecting existing knowledge and experimental data. Some students struggled to appreciate the value of the guided inquiry basis of the lab, expecting a more product confirmation-type activity, nevertheless these students still engaged in mechanistic discussions. It is important to consider timing when implementing this lab. The L-proline-catalyzed aldol mechanism is more complex than the NaOH-catalyzed mechanism. Therefore, the NaOH groups are likely to finish their Worksheet 1 before the L-proline groups.

Summary

A new experiment was developed where students used IR spectroscopy and polarimetry data to propose and validate catalyzed aldol reaction mechanisms. Two distinct catalysts were used, the non-stereoselective NaOH and the stereoselective L-proline catalyst. The key focus of this laboratory was to use kinetic IR data to validate intermediates and guide mechanistic analysis and to use polarimetry to consider why different catalysts may be selected to drive a reaction. Overall, students were able to validate mechanisms using the experimental data.

Associated Content

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.XXXXXXX.

Supporting Information: Remote Instructions and Mechanisms (PDF)

Student Handouts- pre-lab information and laboratory instructions (DOCX)

Worksheets IR tables and Frequencies- guided inquiry questions for students to complete in-lab and IR tables (DOCX)

Facilitator's Notes- information for the lab instructors intended to assist students (DOCX)

NaOH IR Spectra Labeled- IR spectra for specific timepoints for the NaOH reaction (PDF)

L-proline IR Spectra Labeled- IR spectra for specific timepoints for the L-proline reaction (PDF)

Polarimetry Data Overlay-Polarimetry Data for Blank, L-Proline and NaOH catalyzed reactions (PDF)

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Notes

The authors declare no competing financial interest.

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References

(1) Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. Mechanistic Investigation of the Staudinger Ligation. *J. Am. Chem. Soc.* **2005**, *127* (8), 2686–2695.

(2) Bandar, J. S.; Pirnot, M. T.; Buchwald, S. L. Mechanistic Studies Lead to Dramatically Improved Reaction Conditions for the Cu-Catalyzed Asymmetric Hydroamination of Olefins. *J. Am. Chem. Soc.* **2015**, *137* (46), 14812–14818.

(3) Travis, C. R.; Mazur, L. E.; Peairs, E. M.; Gaunt, G. H.; Young, D. D. Mechanistic Investigation and Further Optimization of the Aqueous Glaser-Hay Bioconjugation. *Org. Biomol. Chem.* **2019**, *17* (13), 3396–3402.

(4) Committee on Professional Training. Organic Chemistry Supplement. **2015**, https://www.acs.org/content/dam/acsorg/about/governance/committees/training/2015-acs-guidelines-for-bachelors-degree-programs.pdf (Accessed Jun 15 2022)

(5) Morrison, R. T.; Boyd, R. N. *Organic Chemistry*; Allyn and Bacon: Boston, MA, 1959.

(6) Goldish, D. M. Let's Talk about the Organic Chemistry Course. *J. Chem. Educ.* **1988**, *65* (7), 603.

(7) Wheeler, D. M. S.; Wheeler, M. M. Trends in the Teaching of Organic Chemistry: A Survey of Some Textbooks. *J. Chem. Educ.* **1982**, *59* (10), 863–865.

(8) Ryles, A. P. Teaching A-Level Organic Chemistry Mechanisms: Some Suggestions. *Sch. Sci. Rev.* **1990**, *72* (258), 71–74.

(9) Bhattacharyya, G. From Source to Sink: Mechanistic Reasoning Using the Electron-Pushing Formalism. *J. Chem. Educ.* **2013**, *90* (10), 1282–1289.

(10) Galloway, K. R.; Stoyanovich, C.; Flynn, A. B. Students' Interpretations of Mechanistic Language in Organic Chemistry before Learning Reactions. *Chem. Educ. Res. Pract.* **2017**, *18* (2), 353–374.

(11) Flynn, A. B.; Ogilvie, W. W. Mechanisms before Reactions: A Mechanistic Approach to the Organic Chemistry Curriculum Based on Patterns of Electron Flow. *J. Chem. Educ.* **2015**, *92* (5), 803–810.

(12) Bhattacharyya, G.; Bodner, G. M. "It Gets Me to the Product": How Students Propose Organic Mechanisms. *J. Chem. Educ.* **2009**, *82* (9), 1402-1407.

(13) Flynn, A. B.; Featherstone, R. B. Language of Mechanisms: Exam Analysis Reveals Students' Strengths, Strategies, and Errors When Using the Electron-Pushing Formalism (Curved Arrows) in New Reactions. *Chem. Educ. Res. Pract.* **2016**, *18* (1), 64–77.

(14) Hessley, R. K. Computational Investigations for Undergraduate Organic Chemistry : Predicting the Mechanism of the Ritter Reaction. *J. Chem. Educ.* **2000**, *77* (2), 202–203.

(15) Wong, T. C.; Sultana, C. M.; Vosburg, D. A. A Green, Enantioselective Synthesis of Warfarin for the Undergraduate Organic Laboratory. *J. Chem. Educ.* **2010**, *87* (2), 194–195.

(16) Grant, A.; Latimer, D. Bromination and Debromination of Cholesterol : An Inquiry-Based Lab Involving Structure Elucidation , Reaction Mechanism , and 1 H NMR. *J. Chem. Educ.* **2003**, *80* (6), 670–671.

(17) Jarret, R. M.; New, J.; Patraitis, C. Electrophilic Aromatic Substitution Discovery Lab. *J. Chem. Educ.* **1995**, *72* (5), 457–459.

(18) Pickering, M. A Physical Chemist Looks at Organic Chemistry Lab. *J. Chem. Educ.* **1988**, *65* (2), 143–144.

(19) Wade, E. O.; Walsh, K. E. A Multistep Organocatalysis Experiment for the Undergraduate Organic Laboratory: An Enantioselective Aldol Reaction Catalyzed by Methyl Prolinamide. *J. Chem. Educ.* **2011**, *88* (8), 1152–1154.

(20) Afonso, C. A. M. ; Pereira, J. Asymmetric Aldol Reaction Induced by Chiral Auxiliary. *J. Chem. Educ.* **2006**, *83* (9), 1333–1335.

(21) Balija, A. M.; Reynolds, A. M. A Mixed-Aldol Condensation Reaction with Unknown Aldehydes and Ketones: Employing Modern Methods To Improve the Learning Process for Second-Year Undergraduate Organic Chemistry Students. *J. Chem. Educ.* **2013**, *90* (8), 2012–2014.

(22) Dicks, A. P. A Review of Aqueous Organic Reactions for the Undergraduate Teaching Laboratory *Green Chem. Lett. Rev.* **2009**, *2* (1), 9-21.

(23) Bennett, G. D. A Green Enantioselective Aldol Condensation for the Undergraduate Organic Laboratory. *J. Chem. Educ.* **2006**, *83* (12), 1871-1872.

(24) Torres King, J. H.; Wang, H.; Yezierski, E. J. Asymmetric Aldol Additions: A Guided-Inquiry Laboratory Activity on Catalysis. *J. Chem. Educ.* **2018**, *95* (1), 158–163.

(25) Hein, S. M. Positive Impacts Using POGIL in Organic Chemistry. *J. Chem. Educ.* **2012**, *89* (7), 860–864.

(26) Moog, R. S.; Spencer, J. N. *POGIL: An Overview*, 1st ed.; American Chemical Society, 2008.

(27) Root, K. S.; Hill, C. L.; Lawrence, L. M.; Whitesides, G. M. The Mechanism of Formation of Grignard Reagents: Trapping of Free Alkyl Radical Intermediates by Reaction with Tetramethylpiperidine-N-Oxyll. *J. Am. Chem. Soc.* **1989**, *111* (14), 5405–5412.

(28) Pai, E. F.; Schulz, G. E. The Catalytic Mechanism of Glutathione Reductase as Derived from X-Ray Diffraction Analyses of Reaction Intermediates *. *J. Biol. Chem.* **1983**, *258* (3), 1752–1757.

(29) Moctezuma, E.; Leyva, E.; Aguilar, C. A.; Luna, R. A.; Montalvo, C. Photocatalytic Degradation of Paracetamol: Intermediates and Total Reaction Mechanism. *J. Hazard. Mater.* **2012**, *243*, 130–138.

(30) Young, P. R.; Jencks, W. P. Trapping of the Oxocarbonium Ion Intermediate in the Hydrolysis of Acetophenone Dimethyl Ketals. *J. Am. Chem. Soc.* **1977**, *99* (25), 8238–8248.

(31) Brown, W. H.; Iverson, B. L.; Anslyn, E.; Foote, C. S. *Organic Chemistry*, 7th ed.; Cengage Learning, 2013.

(32) Northrup, A. B.; MacMillan, D. W. C. The First Direct and Enantioselective Cross-Aldol Reaction of Aldehydes. *J. Am. Chem. Soc.* **2002**, *124* (24), 6798–6799.

(33) List, B.; Lerner, R. A.; Barbas, C. F. Proline-Catalyzed Direct Asymmetric Aldol Reactions. *J. Am. Chem. Soc.* **2000**, *122* (10), 2395-2396.

(34) Schmid, M. B.; Zeitler, K.; Gschwind, R. M. NMR Investigations on the Proline-Catalyzed Aldehyde Self-Condensation: Mannich Mechanism, Dienamine Detection, and Erosion of the Aldol Addition Selectivity. *J. Org. Chem.* **2011**, *76* (9), 3005–3015.

(35) Perrin, C. L.; Chang, K.-L. The Complete Mechanism of an Aldol Condensation. *J. Org. Chem.* **2016**, *81* (13), 5631–5635.

(36) Warfa, A.-R. M. Using Cooperative Learning to Teach Chemistry: A Meta-Analytic Review. *J. Chem. Educ.* **2016**, *93* (2), 248–255.

(37) Childs, R. F.; Shaw, G. S.; Lock, C. J. L. Comparison of the Structure and Charge Delocalization in an Unsaturated Imine and Its Corresponding Iminium Salt. *J. Am. Chem. Soc.* **1989**, *111* (14), 5424–5429.

(38) Simonson, S. R. *POGIL: An Introduction to Process Oriented Guided Inquiry Learning for Those Who Wish to Empower Learners*; Stylus Publishing: Sterling, VA, 2019.

(39) Hess Corporation. Brine Solution- Safety Data Sheet.https://www.hess.com/docs/default-source/us-safety-data-sheets/brine_solution_sds_na2015_102618_final.pdf (Accessed Jun 15 2022)