Discovery-based Experiments in the Organic Chemistry Lab: The S_N2 Conversion of Phenylalanine to Phenyllactic Acid

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Chemistry experiments with an "element of discovery" are often more engaging for students than expository or "cookbook" versions where every student executes the same procedure and hopes for the same outcome. At Miami University Middletown, we've designed a discovery-based approach to an organic synthesis of phenyllactic acid from phenylalanine. Previously-published experiments focus on the elucidation of the reaction mechanism utilizing either the L- or D-phenylalanine reactant. In our version, students are given one of three unlabeled starting materials (L-phenylalanine, D-phenylalanine, or D,L-phenylalanine) and must deduce which form they receive based upon the characterization of their product. Students learn the importance of complementary analyses, coupled with an understanding of the reaction mechanism, as they solve their experimental puzzle.

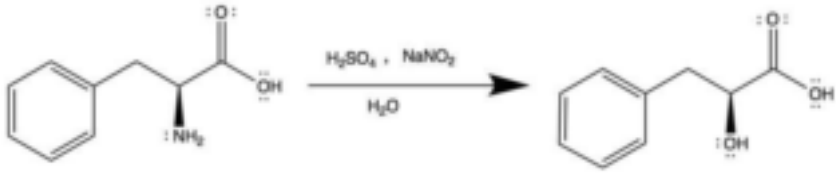
Introduction to the Experiment

Many sophomore-level organic chemistry labs include chemical syntheses followed by the analysis and characterization of isolated products (Lehman, 2009). These experiments generally complement reaction mechanisms and theories taught in the accompanying lecture course. Not surprisingly, experiments with an "element of discovery" are more engaging for students than simply following a recipe. In discovery-based labs, students often solve experimental puzzles which may differ from student-to-student. These types of experiments readily interest students since they typically include an element of investigation (Domin, 1999).

An excellent example of an experiment that lends itself to the discovery approach is the double S_N2 conversion of phenylalanine to phenyllactic acid (2-hydroxy-3-phenylpropanoic acid), as shown in Figure 1. The experiment can be used to teach acid/base, nucleophilic substitution, and diazotization reactions, all of which occur in the conversion of the amino group ($-NH_2$) to the hydroxyl group (-OH). The phenyllactic acid product can be analyzed using a variety of methods including melting point, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR)

spectrometry, and polarimetry. In addition, the experiment is safe, economical to execute, and well-suited to an undergraduate organic chemistry lab course.

Figure 1. Reaction for the conversion of L-phenylalanine to L-phenyllactic acid.



The reaction is run under aqueous conditions and does not require specialized safety considerations or equipment; although, students should use goggles, disposable gloves, and laboratory fume hoods. The phenylalanine starting material and other reagents are easily obtained and reasonably priced for an organic synthesis experiment, and the required laboratory equipment includes standard glassware and fixtures (magnetic stir plates, vacuum aspirators, fume hoods). For the analysis of the phenyllactic acid product, most organic teaching labs are outfitted with an infrared (IR) spectrophotometer and equipment for measuring melting points. For labs that do not have access to a polarimeter, the Vernier Chemical Polarimeter is an affordable option that is reliable and easy to use with undergraduate students.

An important feature of the experiment is the phenylalanine reactant. Phenylalanine is an essential amino acid that exists in several forms. It is a chiral compound, meaning it displays the property of "handedness." The L-enantiomer is one of the twenty amino acids naturally found in human proteins. The other enantiomer is designated D and has no nutritional value. A mixture of the two forms in equal concentrations (the racemic form or racemate) is designated D,L. All are non-toxic amino acids, provide a good illustration of the concept of the "chiral pool," and are commercially available.

Previously-published experiments focus on the elucidation of the reaction mechanism using either the L- or D-phenylalanine reactant. The original work by Van Draanen and Hengst uses the L-enantiomer and emphasizes the characterization of the product using NMR (Van Draanen & Hengst, 2010). An internet search turned up a different approach taken at Indiana University where students use several analytical tools to determine the mechanism of the reaction, again starting with L-phenylalanine (Indiana

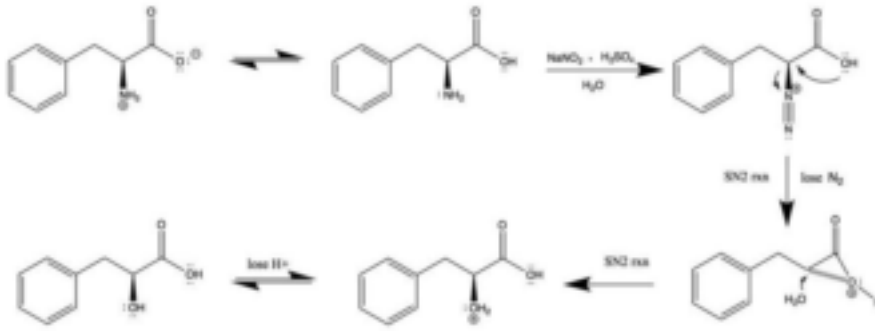
University, 2015). Students on the main campus of Miami University (Oxford) run the reaction with either the L or D form. They use mixed melting point determinations and polarimetry to characterize their product and deduce the mechanism of the reaction (Novak, Hershberger, & Galloway, 2014).

Our Discovery-based Approach

The availability and affordability of the three stereoisomeric forms of phenylalanine sparked our interest in developing a discovery-based version of the experiment. Specifically, students are given one of three unlabeled starting materials (L-phenylalanine, D-phenylalanine, or D,L-phenylalanine) and must deduce which form they receive based upon the characterization of their phenyllactic acid product. Students learn the importance of complementary analyses and draw upon their understanding of the reaction mechanism and its stereospecificity, in order to solve their experimental puzzle.

The mechanism of the conversion of phenylalanine into phenyllactic acid involves a double S_N2 substitution reaction, as illustrated in Figure 2. Initially, diazotization of phenylalanine converts the amino group into a good leaving group. The first intramolecular substitution reaction is rapid, evolving N_2 gas to form a strained lactone. The second intermolecular substitution reaction occurs over a 24-hour period to yield the phenyllactic acid product. The net result is the chirality or “handedness” of the starting material is preserved meaning L-phenylalanine yields L-phenyllactic acid and D-phenylalanine yields D-phenyllactic acid. Not surprisingly, the D,L starting material yields the D,L product.

Figure 2. Mechanistic steps for the conversion of L-phenylalanine to L-phenyllactic acid.



Experimentally, the reaction is easy to execute, and several visual clues indicate the conversion proceeds as expected. The enantiomeric L-phenylalanine (or D-phenylalanine) reactant readily dissolves in an acidic aqueous solution. After dissolution, the reaction mixture is chilled since the diazotization step needs to be run slowly under cool conditions. As the reaction proceeds, bubbles of nitrogen gas are observed, as shown in Figure 3. Once the sodium nitrite ($NaNO_2$) reagent is completely added, the reaction mixture is warmed to room temperature. The crystalline L-phenyllactic acid (or D-phenyllactic acid) product often begins to precipitate from the solution after 15-30 minutes (Figure 4). After 24 hours, the enantiomeric product is ready to isolate and characterize.

Figure 3. Reaction flask during the diazotization step, indicated by the evolution of N_2 gas.

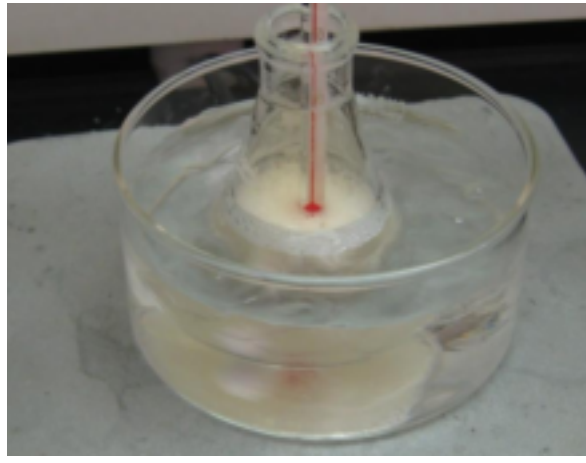
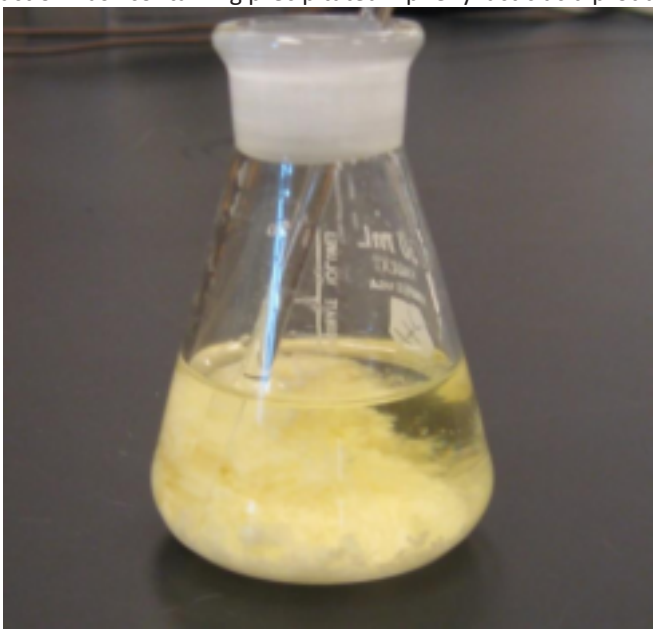


Figure 4. Reaction flask containing precipitated L-phenyllactic acid product.



The design of our “discovery-based” experiment involved several developmental challenges. The synthesis, isolation, and characterization of either L- or D-phenyllactic acid works very well, starting with the L- or D-form of phenylalanine. However, the reaction with the D,L-racemate was not as straightforward. The solubility properties of the D,L-phenylalanine reactant and the D,L-phenyllactic acid product differ from either of the pure enantiomers, as is often observed for chiral compounds. Isolation of the racemic product using the same approach used for the pure enantiomers proved to be challenging because D,L-phenyllactic acid does not readily precipitate from the reaction solution. And in order to keep the identity of the reactants a mystery, all students need to execute the same experimental procedure. Fortunately, we discovered that prolonged cooling of the D, L-reaction mixture yielded crystalline product in 3-4 days. Therefore, unbeknownst to the students, all of their reaction flasks are refrigerated for a week between the running of the reaction and the isolation of the product. Because the total number of students in our organic lab is relatively small, this “behind-the-scenes” experiment modification is easy to accommodate.

Solving the Experimental Puzzle

In our design of this experiment, students are given the mechanism of the reaction and recognize its high stereospecificity. What they don't know is which starting material they will be assigned. They must identify the form of the phenylalanine reactant – either L, D, or D,L – based upon the characterization of their phenyllactic acid product.

In our lab, analyses used to characterize the product include melting point determination, infrared (IR) spectroscopy, and polarimetry. Infrared (IR) analysis helps students confirm that the desired functional group transformation has occurred, but it does not help them identify the specific form of their reactant. Melting point measurements are useful for distinguishing either the L- or D-enantiomers of phenyllactic acid from the D,L-racemate. Polarimetry, an optical technique for the analysis of chiral compounds, is used to differentiate the L- and D-enantiomers. For the pure L- or D-forms of phenyllactic acid, the observed rotation of plane-polarized light differs in the direction, or sign, of rotation but not in the absolute magnitude. A 1:1 mixture of the two, which is the racemate, is optically inactive and does not rotate plane-polarized light. Distinguishing features of these analyses for the three possible products are outlined in Table 1 (Novak, Hershberger, & Galloway, 2014; Van Draanen & Hengst, 2010).

The combination of analyses is complementary and required for students to confirm the identity of their unknown reactant. In addition, pure samples of all three products are available in the lab, and students can design their own mixed melting point determinations to confirm the "handedness" of their starting material.

Table 1. Analytical characterization of phenyllactic acid.

Product Formed	**Infrared (IR) Spectroscopy Key Spectral Features**	**Melting Point**	**Specific Rotation, [α] (acetone, D line, 20 °C)**
L-phenyllactic acid	υ(OH) of alcohol at 3440 cm^{-1}; υ(OH) and υ(C=O) of carboxylic acid at ~2900 cm^{-1} and 1723 cm^{-1}, respectively	124-125 °C	-26.9°
D-phenyllactic acid	same	124-125 °C	+26.9°
D,L-phenyllactic acid (racemate)	same	94-95 °C	0° not optically active

Theoretical and Experimental Concepts Learned

This experiment serves to illustrate and reinforce several important organic chemistry concepts. For example, the effect of acid/base reactivity on the water solubility of organic compounds is observed. Specifically, phenylalanine is a positively-charged species under acidic conditions and is readily soluble in the aqueous reaction solution. In contrast, phenyllactic acid is neutral, or uncharged, under the same conditions and is not appreciably soluble. Because it precipitates from the aqueous reaction mixture, isolation of the product and workup of the reaction is simplified.

The diazotization reaction illustrates the concept of forming an excellent leaving group, nitrogen gas, from a poor leaving group, the amino group, in order to facilitate the first substitution reaction. The product of the first substitution reaction is a lactone, or cyclic ester, which is a functional group prevalent in natural product chemistry.

Lastly, the mechanism of both the intramolecular and intermolecular S_N2 reactions involves an inversion of configuration at the carbon undergoing substitution. Therefore, the double S_N2 reaction leads to a net retention of configuration. This means the L-form of the phenylalanine reactant yields the L-form of the phenyllactic acid product. The high degree of stereospecificity is one of the most important features of the mechanism, and this experiment offers students an excellent opportunity to observe it, especially when they compare their results to others in the class.

Experimental Logistics and Student Results

In our course, this experiment is completed over a series of three lab periods. During the first week, students synthesize the phenyllactic acid product, following the procedure outlined by van Draanen and Hengst (2010). However, in order to isolate sufficient product for polarimetry, the scale of the reaction is doubled. Between the first and second weeks, the product precipitates from the reaction solution, with refrigeration required for the D,L-racemate. Product is isolated by vacuum filtration in the second lab period and left to dry for analysis the following week. Since the synthesis and isolation procedures are relatively quick, students are running an additional experiment during those weeks. During week three, students complete the analysis of their phenyllactic acid product. In our lab, we have two IR spectrophotometers (Thermo Scientific Nicolet iS5), two polarimeters (Vernier Chemical Polarimeter with Labquest II), and twelve Mel-Temps for measuring melting points. Since the analyses are independent and students must complete all three to determine the

stereoisomeric form of their product, and by analogy their unknown reactant, students circulate among the instruments without too much time spent waiting on equipment.

After incorporating this discovery-based approach for the conversion of phenylalanine to phenyllactic acid into our course, we have had three lab sections, with a total of fifty students working in pairs, successfully complete this experiment. Student yields of product typically fall between 40-50% which is sufficient to complete the analyses and obtain meaningful results. In all cases, the melting points measurements clearly indicate relatively pure enantiomeric or racemic products, consistent with the literature and our development work. Additionally, the measurements of optical activity have allowed students to either confirm the D,L-racemate or clearly distinguish between the L- and D-forms of phenyllactic acid.

Students who start the reaction with racemic phenylalanine typically notice some differences as they run the experiment and in the behavior of the racemic phenyllactic acid product, as compared to either pure L- or D-phenyllactic acid. In particular, students who use pure enantiomeric reactants often observe product starting to precipitate as they complete the synthesis reaction, during the first lab period of the experiment. In contrast, as we had observed, the racemic product does not readily precipitate which is clearly noticed by all students and especially those running that particular version of the reaction. Also, racemic phenyllactic acid is less soluble in acetone, the solvent used for polarimetry, than either enantiomer, as is also noticed. Students are particularly observant of how well the experiment is going for the class as a whole, given some of these differences and the challenge of determining their experimental unknown.

Conclusions

We've been very pleased with how well this experiment has worked in our organic chemistry lab course. At our institution, the number of students per section is limited to twenty-four, and we typically have one section of lab each semester. By design, we have students execute this experiment in pairs which facilitates discussion and collaboration as students work together to determine the identity of their unknown reactant. Having students pair up also helps with the sharing of analytical instrumentation and minimizes time spent waiting in line. In our experimental approach, the addition of D,L-phenylalanine as a reactant

requires the refrigeration of those reaction flasks in order to precipitate the racemic product. The "behind-the-scenes" instructor involvement is more feasible at a smaller institution, such as ours, as compared to a program with hundreds of students enrolled in organic chemistry lab. Most importantly, the addition of an "element of discovery" to this published experiment has helped our students learn from their fellow students' observations and conclusions, as much as their own. We encourage educators in similar academic environments to take advantage of opportunities to include discovery-based lab experiences in their courses.

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