Enzymatic Resolution of 1-Phenylethanol and Formation of a Diastereomer: An Undergraduate 
\(^1\)H NMR Experiment To Introduce Chiral Chemistry

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Stereochemistry is usually introduced in the first semester of undergraduate organic chemistry and is a crucial concept for organic chemistry (racemization, S\(_\text{N}2\) reactions), biochemistry (peptides, proteins, helicity of B- and Z-DNA), and inorganic chemistry (chiral catalysis). Unfortunately, students often struggle with the abstract concepts of stereochemistry. There are many experiments described in this Journal and others that attempt to enhance student understanding of stereochemistry (1–22). An organic laboratory experiment is presented that focuses on stereochemistry by introducing students to stereoselective enzyme reactions, resolution of enantiomers, and NMR analysis of diastereomers.

This experiment uses an enzyme for the preparation of an enantiopure product from a racemic precursor. Acylase I from Aspergillus melleus (27), which catalyzes the transformation of a range of aromatic alcohols to esters (24) by transferring an acyl group, is used. A racemic mixture of 1-phenylethanol is reacted with vinyl acetate. The reaction is shown in Scheme 1 and reflects the stereoselectivity of the enzyme. The reaction follows a greener reaction route (23) and is ∼80% less expensive than the formation of Mosher diastereomers (5). It is also carried out in hexane, which is more suitable for many organic molecules than the aqueous environment most enzymes require (24).

Previously described enzymatic methods include the stereospecific, catalyzed reactions of penicillin acylase derivatives (25) and their use in the chiral resolution of isomers (26). Students benefit from the hands-on experience and observe firsthand how enzymes can resolve racemic compounds. To determine which enantiomer the enzyme preferred, the students derivatize the unconverted alcohol(s) to a set of diastereomeric esters using a chiral carboxylic acid and a coupling reagent.

The experiment is divided into two parts and conducted over three, laboratory periods 1 week apart. Part 1 is the stereoselective formation of an ester from a racemic alcohol by acylase I, followed by the separation of the product ester and the unreacted alcohol and part 2 is the formation of a diastereomeric compound from the unreacted alcohol and use of \(^1\)H NMR spectroscopy to determine the chirality of the unreacted alcohol.

**Experimental Details**

**Synthesis**

In part 1, a racemic mixture of 1-phenylethanol was reacted with vinyl acetate, catalyzed by acylase I, in hexane for 1 week at room temperature. No stirring was required and the reaction occurred by simply storing the mixture for 1 week at the student’s lab stations. If the reaction continued longer than 7 days, increasing quantities of the (S)-enantiomer were consumed, complicating the analysis. After the reaction was complete, the alcohol and ester products were separated by silica gel column chromatography (monitored by TLC).

In part 2, students carried out three reactions to investigate the stereochemistry of the reaction in part 1. The unreacted 1-phenylethanol was derivatized with (R)-(−)-acetoxyphenylacetic acid ((R)-(−)-O-acetoxy-2-phenyl-2-ethanoic acid) in the presence of a coupling reagent, ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and a catalyst, 4-dimethylaminopyridine (4-DMAP), to prepare the ester (Scheme 2). Students then reacted the racemic starting alcohol with (R)-(−)-acetoxyphenylacetic acid to make the pair of diastereomeric esters. Finally, they reacted the enantiopure (R)- or (S)-alcohol with (R)-(−)-acetoxyphenylacetic acid to make the diastereomeric ester. Proton NMR analysis was used to assess the stereochemistry of the products.

The details of the experimental setup are provided in the supporting information.

**NMR Analysis**

The \(^1\)H NMR (300 MHz) spectrum from the reaction of the racemic starting alcohol with (R)-(−)-acetoxyphenylacetic acid is shown in Figure 1. The two diastereomers can be distinguished by NMR as the methyl protons in the 1-phenylethyl group of the diastereomers appear as two doublets at 1.5 ppm. An expansion of the 1-3 ppm region of the \(^1\)H NMR spectrum of a 50:50 mixture of diastereomers (Figure 2A) shows the benzylic methyl group from the two diastereomers to have slightly different chemical shifts. The same region from a spectrum of the diastereomer derived from commercially available (R)-phenylethanol (Figure 2B) shows the doublet at ∼1.5−1.6 ppm is attributable to the (R,R) diastereomer, whereas the doublet at ∼1.4−1.5 ppm comes from the (S,R) diastereomer.

The \(^1\)H NMR (90 MHz) spectrum of the ester (Figure 3) from part 1 (Scheme 1) indicates that the majority (approximately 3:1) of the unreacted alcohol was the (S)-enantiomer leading to the (S,R)-diastereomer, indicating the enzyme preferred (R)-(−)-1-phenylethanol. The student data showed 76%
In the Laboratory

Optical Purity

Students determined the optical purity of their unreacted alcohol by measuring its specific rotation and comparing to a purchased standard, (R)-1-phenylethanol (99.9% from Aldrich). The specific rotation of the standard was +43.03 and the resolved alcohol -25.02 (both in methanol), giving an optical purity of 58% (S)-enantiomer by polarimetry.

Hazards

1-Phenylethanol, vinyl acetate, dichloromethane, and deuteriochloroform are cancer suspect agents. 4-Dimethylaminopyridine (DMAP) is highly toxic. EDC, 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride is an irritant. These materials must be used in small quantities in fume hoods. Students should use chemically resistant gloves and protective eye wear.

Organization of the Group Work

The students each perform the enzyme-catalyzed transesterification with the racemic alcohol (part 1). Each student in a group of 3–4 then either reacts the (R)-enantiomer of the alcohol, the experimentally unreacted (S)-enantiomer (collected from all group members), or the racemic mixture with the acetoxphenylacetic acid so that each group had a complete set of complexes to analyze (part 2). This allows students to gain enough information to determine which enantiomer reacted preferentially with the enzyme in part 1. The weekly experimental schedule for the laboratory exercise is

Week 1: Each student reacts racemic 1-phenylethanol with vinyl acetate using acylase I from A. melleus as a catalyst. This reaction takes 10–15 min to prepare and can be performed at the end of another lab experiment session. If possible, students should come in during the course of the week to test the reaction mixture via TLC and observe the disappearance of the starting alcohol peak and the increase in the product ester peak.
In the Laboratory

Week 2: Each student performs TLC analysis of their reaction mixture and then separates the alcohol and ester by microscale column chromatography. Each fraction is analyzed by TLC, and pure fractions are combined. The students in each group are then assigned an alcohol:

Student 1: Reacts the racemic alcohol with \((R)-(\_\_)-\)ace-toxyphenylacetic acid to make the pair of diastereomeric esters.

Student 2: Reacts the unreacted alcohol with \((R)-(\_\_)-\)acetoxyphenylacetic acid to make the diastereomeric ester.

Student 3: Reacts the enantiopure \((R)\)- or \((S)\)-alcohol with \((R)-(\_\_)-\)acetoxyphenylacetic acid to make the diastereomeric ester.

For larger groups: Additional students can replicate another student.

Week 3: Students collect and share \(^1\)H NMR spectra and analyze their results to determine which enantiomer of 1-phenyl-ethanol was the preferred substrate in the enzyme reaction. Student 1 obtains a \(^1\)H NMR spectrum showing the doublets from both diastereomeric esters (Figure 1). The \(^1\)H NMR spectrum from student 2 shows the doublet from the ester of the unreacted alcohol (Figure 3). Student 3 has an \(^1\)H NMR of the ester from the pure, known \((R)\)- or \((S)\)-alcohol (Figure 2B).

From this data, the group can deduce which enantiomer of the starting alcohol was consumed by the enzyme and calculate by how much it was preferred.

Costs

An attractive aspect of this enzyme-facilitated enantiomeric resolution is its low cost. On the basis of catalog prices from commercial suppliers, the average cost per student is about $3.50, exclusive of solvents, TLC plates, and other common reagents. The majority of the cost is \((R)-(\_\_)-\)acetoxyphenylacetic acid, EDC, and DMAP at about $2.75 per student. In comparison, on a similar scale using an acid chloride, the experiment costs approximately $43 per student (Sigma-Aldrich Handbook of Fine Chemicals 2007–2008).

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Literature Cited


Supporting Information Available

Student handout; notes for the instructor. This material is available via the Internet at http://pubs.acs.org.