SYNTHESIS OF ENANTIOPURE ALCOHOLS AND ESTERS USING A LIPASE-BASED KINETIC RESOLUTION

REACTIONS: Reduction of a ketone, acetylation of an alcohol, and a kinetic resolution using a lipase.

TECHNIQUES: Reflux, TLC, extraction, liquid chromatography, HPLC, $^1$H NMR, polarimetry.

We like to integrate what you learn in your chemistry courses so the compounds you will be synthesizing and separating will be used for Chem 51LC in the Absolute Configuration lab. The compounds made by the students in this experiment will be used for future Chem 51LC classes.

In Part A of this experiment, you will synthesize the ester required for the kinetic resolution by first reducing a ketone with sodium borohydride and then acetylation the resultant alcohol using acetic anhydride.

In Part B of this experiment, you will perform a kinetic resolution of the racemic ester using a lipase derived from Candida antarctica, a fungus. In nature, lipases are used to hydrolyze, or break down, fats. Due to the high specificity with which enzymes perform their function, they can be an excellent way to convert a racemic mixture of enantiomers into a set of two, separate, enantioenriched compounds. The lipase you will use selectively converts the R-ester into the R-alcohol, while the S-ester is left intact. After performing this reaction, you will then separate the alcohol and the ester using flash column chromatography.
In Part C of this experiment, you will characterize the enantioenriched compounds using \(^1\)H NMR and polarimetry.

Background: Enzymes are specially suited to do particular reactions in a catalytic manner. They have evolved to be very efficient at their processes and this can be attributed to the way in which they bind, capture, and transform their substrates. The lipase enzyme hydrolyzes ester bonds through three active site amino acid residues called “the catalytic triad”. The residues are Ser153, Asp177, and His264. Given the image below of the active site of lipase, what types of factors are important for governing the enantioselectivity of this hydrolysis?

![Active Site of Lipase](image)

Efficient Use of Lab Time:
You will have a total of four lab periods in which to complete the entire lipase project. Good stopping points are noted throughout the experimental section. All necessary materials will be provided in each of the four lab periods. It is up to you to decide how much of the project you can complete in each lab period.

READING ASSIGNMENT:
- This handout for procedure.
- Supplementary information on enzyme resolution in Janice Gorzynski Smith (3rd ed), Section 28.3B. (Note: This section refers to resolution of amino acids instead of esters. The general concepts are the same.)
- Supplementary information on reduction reactions in Janice Gorzynski Smith (3rd ed), Section 20.4A-B,
- Polarimetry Technique 16.6 Pgs 203-211
- HPLC Technique 18.9 Pgs 253-256
- Review esterification of an alcohol with an anhydride, in general. Details of the specific esterification reaction used in this project will be discussed in class.

PRE-LAB ASSIGNMENT:
- Complete all portions of pre-lab notebook work according to the guidelines.
- Rewrite the procedure in your lab notebook using your own words.
- Sapling assignment.
CAUTION

Sodium borohydride is harmful and all contact with skin or eyes should be avoided! Sodium borohydride is also water reactive and may cause fires if exposed to it. Keep away from water unless specifically instructed!

Dichloromethane is toxic, an irritant, absorbed through the skin, and harmful if swallowed. Wear gloves and wash your hands thoroughly after handling it. Avoid contact with skin and eyes. Dichloromethane is a suspected carcinogen when inhaled in large quantities.

Acetic anhydride and methanol are irritants. Wear gloves and avoid all contact with skin, eyes, and clothing.

Dimethylaminopyridine is hazardous and acts as a permeator and skin irritant. Gloves must be worn at all times and extreme caution exercised when using this material.

Triethylamine is a skin irritant and is quite pungent. Use only in the hood!

Acetophenone derivatives are harmful if ingested. Avoid contact with eyes or skin.

EXPERIMENTAL:

PART A: SYNTHESIS OF RACEMIC ESTER

Add 1.00 g of your ketone, 3.0 mL dichloromethane and 3.0 mL methanol to a 25 mL round bottom flask containing a stirbar and stir until the solid is dissolved. Add 5.0 mmol sodium borohydride to the reaction mixture. You should see immediate gas evolution. Allow the mixture to stir for thirty minutes. Determine whether the reaction is complete using TLC (30% ether/hexanes) and visualize the TLC with UV light. (What types of compounds can be visualized using UV light?) If the reaction is complete, slowly add 5.0 mL saturated aqueous ammonium chloride solution and allow the mixture to stir for a few minutes until most gas evolution ceases. (What should you do if the reaction is not complete?) Separate the organic layer from the mixture using a separatory funnel and extract the remaining aqueous layer twice with 5.0 mL portions of dichloromethane. Combine the organic extracts and dry them over sodium sulfate. Decant the solution into a clean 50 mL Erlenmeyer flask. Rinse the sodium sulfate left behind with 5.0 mL dichloromethane and add the rinse to the reaction vessel. Remove a small aliquot of the solution and place it into a small Erlenmeyer flask to use a TLC reference later. (What is an aliquot?)

Cool the Erlenmeyer flask containing your solution to 0 °C using an ice bath. Add 2.0 equivalents triethylamine (relative to original ketone) and 0.5 mmol DMAP. Slowly add 3.0 equivalents acetic anhydride (relative to original ketone), remove the Erlenmeyer flask from the ice bath and allow the reaction mixture to stir for 1 hour while warming to room temperature. Confirm reaction completion using TLC. If the reaction is complete, slowly add 10 mL saturated aqueous ammonium chloride solution. Pour the mixture into a separation funnel, shake thoroughly and then separate the organic layer. Wash the organic layer with 10 mL saturated aqueous sodium bicarbonate solution and then with 10 mL H₂O. Dry the solution over sodium sulfate, decant or filter into a tared 50 mL Erlenmeyer flask, rinse the sodium sulfate 3 times with 2 mL portions of dichloromethane and concentrate using an air stream. If you are not immediately moving
on to the next step, submit your product in a labeled vial to your TA for use in the next lab period. What data should you record at this point? (STOPPING POINT)

PART B: KINETIC RESOLUTION OF RACEMIC ESTERS AND RECOVERY OF ENANTIONENRICHED COMPOUNDS

(Note: You can perform the column chromatography step either in the same lab period as the kinetic resolution or in the next lab period. If you are planning to perform chromatography in the same lab period you should prepare the column and silica gel during the 120 minutes that the kinetic resolution mixture is stirring.)

Obtain the scintillation vial containing your ester product. For every 50 mg of ester you are using, add 1 mL of pH 7 aqueous buffer to a 50 mL Erlenmeyer flask. Stir with a stirbar until the ester dissolves. Next, for every 50 mg of ester you are using, add 14 mg of lipase enzyme to the vial. Increase the stirring rate to create a suspension of the enzyme in the solution and allow the solution to stir for 120 minutes at room temperature. The enzyme will not dissolve. (Efficient stirring is crucial for this step. WHY?)

Once 120 minutes have passed, vacuum filter the buffer solution into a 50 mL filter flask through a thin pad of Celite (a filtration aid) on filter paper in a Hirsch funnel. Rinse the Celite pad with 20 mL water and then twice with 10 mL portions of diethyl ether. Pour the filtrate into a 125 mL separatory funnel and shake thoroughly. Remove the aqueous layer and then collect the organic layer in a separate flask. Extract the aqueous layer twice more using 20 mL portions of diethyl ether. Combine the organic extracts, dry over sodium sulfate, decant into a tared 250 mL beaker, rinse the sodium sulfate with 10 mL diethyl ether twice and remove the diethyl ether using an air stream. What data should you record here?

High Performance Liquid Chromatography (HPLC):
To determine the percent conversion of the starting material an HPLC sample must be prepared. Your TA will provide specific sample preparation and sample submission instructions. Samples must be prepared before you move on to the separation of ester and alcohol using liquid chromatography (described below).

If you are not proceeding directly to separation of products by liquid chromatography, use a small quantity of diethyl ether to transfer your enzyme resolution product mixture to a scintillation vial for storage. Evaporate solvent using an air stream. Give the labeled vial to your TA. (STOPPING POINT)

Liquid (Column) Chromatography
Assemble the column and secure in a straight upright position using a clamp. Pour approximately 35 mL of silica gel into a 100 mL beaker in the hood and add enough 30% diethyl ether/ 70% hexanes solution to create a slurry. (You will need to create this solvent mixture yourself from diethyl ether and hexanes.) Carefully pour the slurry into the column. With a beaker underneath the column, open the stopcock and allow the
solvent to flow out of the column with the packed silica gel being left behind. Accelerate this process by carefully using an air line to push the solvent through the column. Repeat this process until all of the silica gel is in the column. Solvent pushed through the column at this point is not contaminated with any other compounds and can be reused. Drain the column until a centimeter of solvent remains above the top of the silica gel and then close the stopcock.

When you are ready to separate the components of your crude reaction mixture, open your column’s stopcock and allow the solvent to drain to the level of the silica gel. Using a long glass pipette, carefully transfer your crude product mixture to the top of the column and allow the column to again drain to the level of the silica gel. Once all of your crude mixture is transferred, rinse the beaker or scintillation vial with a small amount of ether and transfer the rinse to the top of the column. Once the solvent in the column has drained again to the level of the silica gel, fill the column with the eluent and begin collecting fractions in test tubes. Collect ~15 fractions using small test tubes (fill test tubes almost full, but be careful not to let them overflow) while refilling the column with fresh solvent as necessary. As before, this process can be accelerated using an air line. Take a TLC plate with five lanes using every third fraction to determine if both the ester and the alcohol have eluted. If not, continue taking fractions until the alcohol has finished eluting.

Collect the alcohol fractions in a tared scintillation vial. Rinse the test tubes with a small amount of ether and concentrate via air stream. If you are not immediately moving on to Part C, label the vial and submit to your TA for use in the next lab period.

Collect the ester fractions in a tared scintillation vial. Rinse the test tubes with a small amount of ether and concentrate via air stream.

(What data should you record at this point?)

If you have any mixed fraction containing both ester and alcohol, you can combine these mixed fractions, evaporate most of the solvent, and repeat the chromatography process. (STOPPING POINT)

PART C: CHARACTERIZATION OF COMPOUNDS USING ¹H NMR AND POLARIMETRY

You will be analyzing the enantiopurity of the separated products by polarimetry and the purity of the separated products by ¹H NMR spectroscopy. Polarimetry will be conducted on the combined products for your lab section, but you will conduct your own NMR experiment for your individual products. You will need 100 mg for the NMR sample. Prepare your NMR sample as described below. Then provide the rest of your product to your TA in vials CLEARLY LABELED with your name and the structure of the compound contained in the vial. BE CAREFUL! At no point should ester and alcohol samples be mixed!
More details on polarimetry are provided in a separate handout.

Preparation of $^1$H NMR sample:
NMR spectra should be obtained for both the alcohol and the ester.

Transfer about 100 mg of material into a small beaker or Erlenmeyer flask. Add about 0.5 mL of CDCl$_3$ (deuterated chloroform), mix thoroughly and pipette the solution into an NMR tube. Cap the tube and run an NMR experiment (ask TA for help). Be sure to clearly label your NMR tubes. You might need to run your NMR experiment outside of your normal lab time.

If you are able to complete your NMR experiment before your 4$^{th}$ lab period of the project, recover the products from your NMR samples by pouring the contents of the NMR tube into a tared scintillation vial and evaporating the CDCl$_3$ with an air stream. Clearly label the vial and give the recovered product to your TA to be included with the polarimetry sample for your lab section.

Collection of Final Products
Your final alcohol and ester products will be collected for use in future 51LC classes. Follow specific instructions provided by your TA and in the NMR room for recovery of compounds from NMR samples after the 4$^{th}$ lab period of the project and submission of final product vials. BE VERY CAREFUL TO KEEP ALL VIALS, NMR SAMPLES, AND POLARIMETRY SAMPLES CLEARLY LABELED AT ALL TIMES! DO NOT MIX ALCOHOL AND ESTER SAMPLES!