

EXPERIMENT 2

ENANTIOSELECTIVE REDUCTION OF ACETOPHENONE AND DETERMINATION OF ABSOLUTE CONFIGURATION OF THE RESULTING ALCOHOL USING THE CEC METHOD

Reading Assignment: Smith, Chapter 20.6A, 20.6B, JOC reference: [Determination of Absolute Configuration Using Thin-Layer Chromatography](#)

Pre-lab questions:

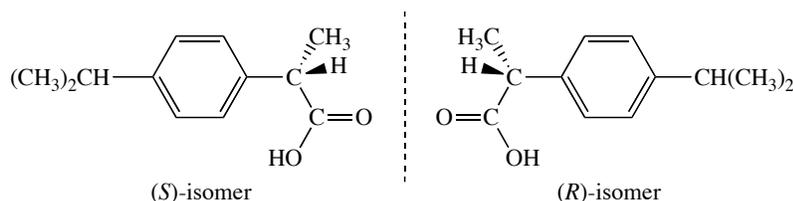
- 1) Comparing enantioselective carbonyl reduction using CBS reagent, and enantioselective biological reduction using an enzyme, what is an advantage and disadvantage for each?
- 2) Provide a mechanism for the CBS reaction with acetophenone.
- 3) Provide a mechanism for the HBTM-mediated esterification.
- 4) In the sample TLC provided for the CEC experiment, calculate the R_f for the alcohol and the R_f for the ester. Is the ester or the alcohol more polar?

In **Part 1** of this experiment, you will do an enantioselective reduction (AKA: asymmetric reduction) of acetophenone with **Corey-Bakshi-Shibata (CBS)** reagent to generate either *R*-(+)-1-phenylethanol or *S*-(-)-1-phenylethanol. In **Part 2** of this experiment, you will determine the absolute configuration of your alcohol using the **Competing Enantioselective Conversion (CEC)** method.

CBS Background

The ability to synthesize enantiomerically pure compounds is extremely important, especially when synthesizing biologically active compounds. Both the active sites of most enzymes and the receptor sites for the sense of smell are chiral. When drugs are metabolized in the body, often only a single enantiomer is metabolized. Ibuprofen, for example, is administered as a racemic mixture, but only the *S*-enantiomer is metabolized.

Ibuprofen:



Administering racemic drugs can be problematic, because the “inactive” form can sometimes be toxic.

Some examples:

Thalidomide: The *R*-(+)-enantiomer is effective for nausea, but the *S*-(-)enantiomer causes severe birth defects.

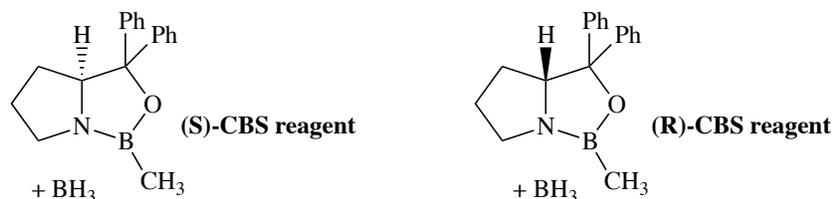
Ethambutol: One enantiomer is used to treat tuberculosis, and the other causes blindness.

Naproxen: One enantiomer treats arthritis pain, and the other causes liver poisoning.

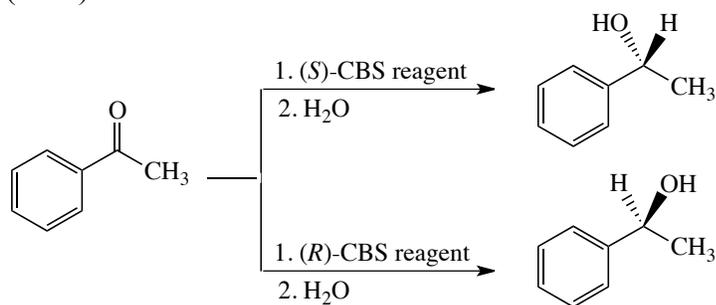
Methorphan: One enantiomer (levomethorphan) is a potent opioid pain-killer, while the other (dextromethorphan) is used as a cough suppressant.

To minimize these potential problems, if a pharmaceutical enters clinical trials as a racemate, both enantiomers must also be isolated and provided for testing. Thus, asymmetric reactions are very important.

The CBS reagent is an asymmetric catalyst derived from proline which is able to reduce a ketone enantioselectively to form one of two possible enantiomers. The selectivity is due to steric strain in the transition state which develops for one enantiomer but not the other.



Reduction of acetophenone affords either *R* or *S* 1-phenylethanol with high enantiomeric excess (% ee):



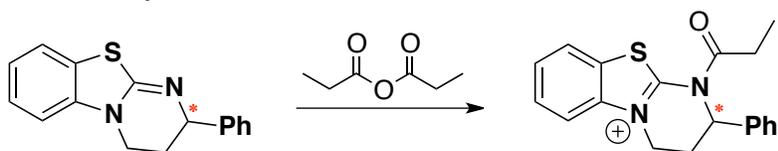
Working with a partner, you will reduce acetophenone with an unknown enantiomer of CBS reagent (you won't know if it is the *R*-CBS or *S*-CBS.) You will then determine the absolute configuration of your alcohol product using the CEC method, and, using this information, you will be able to determine which enantiomer of CBS reagent you were given.

CEC Background:

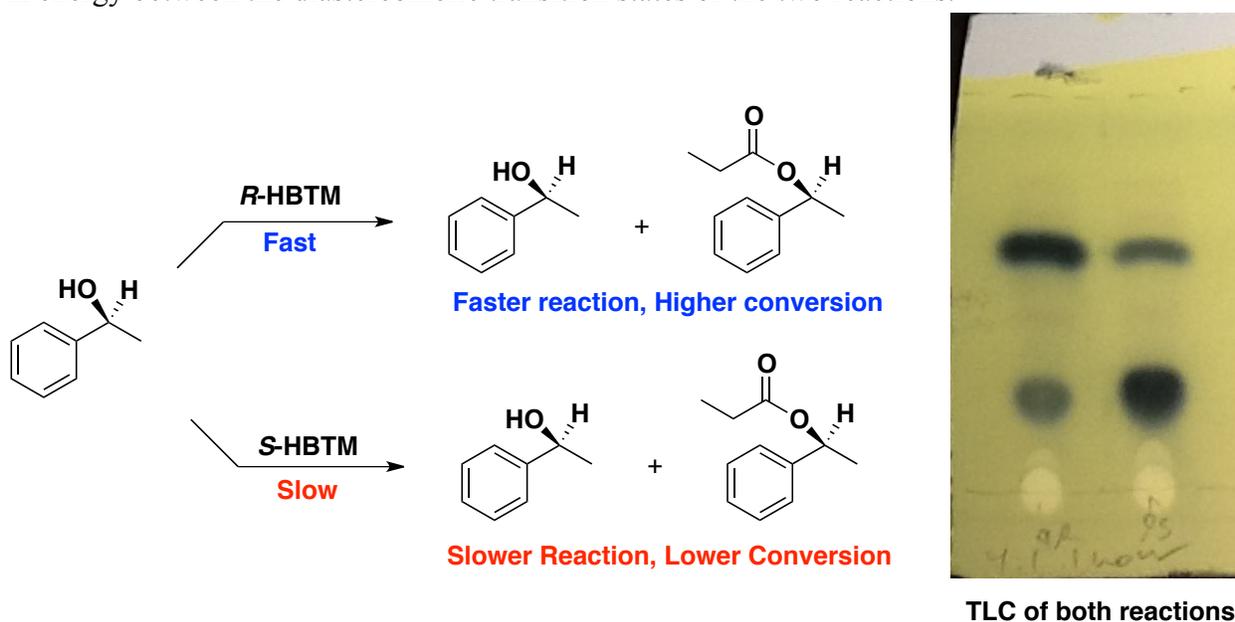
There are many ways to determine absolute configuration of chiral compounds, including X-ray crystallography, advanced Mosher method, vibrational circular dichroism, exciton chirality, lipase-catalyzed resolutions, and NMR spectroscopy using chiral shift reagents. We will be using the Competitive Enantioselective Conversion (CEC) to determine absolute configuration of your alcohol. This experiment is adapted from recent publication reported by the Rychnovsky group at UCI, and it uses parallel reactions of a chiral alcohol mixed with the R and S-enantiomers of homobenzoteramisole (HBTM), a chiral kinetic resolution catalyst.²



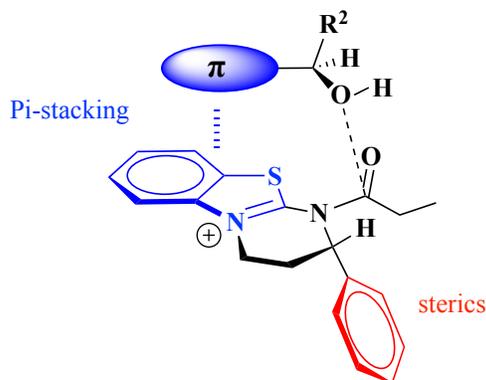
The overall reaction is an esterification of the alcohol that proceeds via an activated intermediate of the catalyst, which is shown below:



The alcohol will react faster with one enantiomer of the HBTM catalyst (the “matched” or “fast” case) than with the other HBTM catalyst (the “mismatched” or “slow” case) due to a difference in energy between the diastereomeric transition states of the two reactions.



The proposed transition state for the matched interaction between the activated (*S*)-HBTM catalyst and the (*R*)-secondary alcohol substrate is shown below:



Three factors operate here:

1. The phenyl ring highlighted in red blocks the alcohol from interacting with the catalyst from the bottom face due to steric hindrance.
2. The aromatic ring in the alcohol substrate and in the HBTM participate in pi stacking, an attractive non-covalent interaction between aromatic rings.
3. The alkyl group on the substrate (R^2) is pointed away from the catalyst to minimize steric interactions.

If the other enantiomer of the alcohol (*mismatched case*) were used with (*S*)-HBTM, these key interactions would be disrupted, resulting in a slower reaction due to the higher energy transition state.

The fast and slow reactions can be qualitatively determined by monitoring the reaction using TLC, and the percent conversion can be quantified by analyzing the ^1H NMR for each reaction. Once the “fast” reaction is determined, the absolute configuration of the alcohol can be determined using the following mnemonic:

Mnemonic for assigning absolute configuration:

If...	<i>R</i> -HBTM is Faster	<i>S</i> -HBTM is Faster
Then...	<p>(<i>S</i>)-configuration</p>	<p>(<i>R</i>)-configuration</p>

CAUTION

Diethyl ether and **tetrahydrofuran** are volatile organic solvents. They are also flammable. Use them in the hood and wear gloves when working with them.

Phosphomolybdic acid is a stain used with thin-layer chromatography. It is a skin irritant. Gloves should be worn when working with it. The major component of the stain is **ethanol**, which is flammable. Keep away from an open flame.

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

Propionic anhydride, **homobenzotetramisole (HBTM)**, and **methanol** are irritants. Wear gloves and avoid all contact with skin, eyes, and clothing.

Deuterated chloroform is a volatile carcinogenic organic solvent. It also passes through gloves rapidly. Use it in the hood and change gloves immediately if they come in contact with it.

CBS and **BH₃•THF** are flammable and water reactive. If in contact with water, they can produce a flammable gas that could ignite spontaneously.

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NOTE: Students will be working in pairs for both parts of this reaction.

Part 1. Enantioselective reduction of acetophenone with CBS

The CBS reaction has to be done in an inert atmosphere with dry glassware. We will be using a double-manifold Schlenk line for the CBS reaction.



In a double manifold Schlenk line, one manifold contains inert gas, and the other manifold is under vacuum. The manifold that is connected to the oil bubbler is the inert gas manifold, and

this usually, but not always in front of the vacuum manifold. *We will not be using the vacuum portion of the double manifold.* When the stopcock is horizontal, both manifolds are closed. When the stopcock is vertical with the black mark up, the inert atmosphere portion of the manifold will be open. We will be using nitrogen as the inert gas.

Procedure:

1. Working quickly, remove a 10 mL round bottom flask from the oven, add an oven dried small magnetic stir bar to it, and cap with a new rubber septa.
2. Once the flask is at around room temperature, attach it to the Schlenk line. This is done by sticking the needle at the end of the tubing through your septa. Purge your flask by opening the stopcock to nitrogen. If using a double manifold, the nitrogen line is the one that is attached to the oil bubbler.
3. Add .084 mL CBS (either R or S, 1M) to round bottom and then .830 mL BH_3 (1M in THF). Begin stirring solution.
4. Add .750 mL of the acetophenone solution to the round bottom dropwise. Allow reaction to stir for 30 minutes.
5. After 30 minutes, remove the septa and quench by adding 1 mL methanol slowly. Allow mixture to continue to stir. Once the bubbling stops, add 3 mL of 1N HCl and allow to stir for 5 minutes.
6. Add this solution to a 60 mL separatory funnel, add 5 mL DI water, and extract with 3 x 5 mL diethyl ether. Combined the organic layers and washed them with 5 mL DI water and then 5 mL brine. Dry over MgSO_4 and filter into a 50 mL round bottom flask. Concentrate on a rotovap.
7. Once mostly concentrated, transfer mixture in 50 mL round bottom into a 20 mL scintillation vial with ether, and rotovap thoroughly until no ether is left.

Part 2. Determination of absolute configuration of your alcohol using the CEC method

1. Measure 12 mg alcohol product and transfer to a new 1 dram vial (*dram vials are smaller than scintillation vials*), labeled R. Repeat for a second dram vial, labeled S.
2. Add .500 mL *S*-HBTM solution to the S vial and .500 mL *R*-HBTM solution to the R vial.
3. When ready to initiate the reaction, add .500 mL base + anhydride solution to S vial. Mark time of addition. Exactly 1 minute later, add .500 mL base + anhydride solution to R vial. Mark time of addition.

4. After exactly half an hour for each, take a TLC spotter and spot each solution on a TLC plate. Run TLC plate in 4:1 hexanes: ethyl acetate and stain with PMA. This stain requires heat, so have a hot plate or heat gun present.
5. After exactly 1 hour for each solution, quench with 50 μL deuterated methanol (CD_3OD). Spot each again, following the same procedure for running and staining. Comment on any differences between the TLC plate from 30 minutes vs. 1 hour.
6. Give your two dram vials to your TA to take an ^1H NMR of them.

Postlab write-up:

Summarize your conclusions for Part 1 and Part 2 in your final lab report. Be sure to include your two ^1H NMRs and a picture of your TLC plates with alcohol and ester labeled. Calculate % conversion for the two parallel CEC reactions (*show your work!*) and use your result to determine the absolute configuration of your chiral alcohol. Is your alcohol R- or S-? Based on the result of the CEC experiment, what enantiomer of CBS did you use?

Post-lab Questions:

- 1) If acetophenone was reduced using NaBH_4 rather than CBS reagent, a racemic mixture of (\pm)-1-phenylethanol would be obtained. How would you isolate pure R-(+)-1-phenylethanol from this mixture?
- 2) Can the results of the CEC experiment be used to determine %ee?
- 3) The CBS reduction had to be run in an inert atmosphere, but the isolation and subsequent reaction were performed in open air. *Why?*

Reference:

1. The CBS procedure is adapted from: E. J. Corey, C. J. Helal, *Angew. Chem. Int. Ed.* **1998**, *37*, 1986-2012.
2. The CEC procedure is adapted from: A. J. Wagner, S. M. Miller, S. Nguyen, G. Y. Lee, S. D. Rychnovsky, R. D. Link. *J. Chem. Educ.* **2014**, *91*, 716-712.