Lab Documentation

Instructors' Notes for Chiral Compounds and Green Chemistry: Reduction Of A Ketone By Sodium Borohydride And Baker's Yeast

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CAS Registry Numbers and Amounts of Chemicals Needed for Chemical Reduction Experiments

Chemical	*CAS Number*	*Experiment 1*	*Experiment 3*
alumina	[1344-28-1]	------	~1.4 g
chloroform-*d*	[865-49-6]	1 mL	1 mL
dichloromethane	[75-09-2]	70 mL	8 mL
ethanol	[64-17-5]	40 mL	------
ethyl acetate	[141-78-6]	------	60 mL
ethyl acetoacetate	[141-97-9]	5.0 g	0.44 g
hydrochloric acid (1M)	[7647-01-0]	30 mL	15 mL
magnesium sulfate	[7487-88-9]	~1.7 g	~1.7 g
sodium borohydride	[16940-66-2]	1.5 g	0.5 g
sodium chloride (saturated aqueous solution)	[7647-14-5]	------	40 mL
sodium hydrogencarbonate (saturated aqueous solution)	[144-55-8]	------	40 mL
(L)-tartaric acid	[87-69-4]	------	2.0 g
tetrahydrofuran	[109-99-9]	------	15 mL

Hazards

As with any hydride reaction, hydrogen gas is evolved during the course of the reaction. Hydrogen must be kept away from ignition sources to avoid explosions. The organic solvents are all flammable. Tetrahydrofuran is an irritant. Deuterated chloroform used to make samples for NMR is highly toxic and a cancer suspect agent. Hydrochloric acid and sodium borohydride are corrosive and tartaric acid is an irritant. Standard chemical safety precautions should be practiced at all times.

Equipment Needed for Chemical Reduction Experiments

Each student will require equipment to run the reactions, do aqueous extractions, and remove solvents under reduced pressure. Specifically, Experiment One will require (per experiment): a 100-mL round-bottomed flask, a 50- or 100-mL graduated cylinder, an ice bath, stir bar, stir plate, a rotary evaporator, a 60- or 125-mL separatory funnel, 2 beakers that hold at least 50 mL, an NMR tube, weighing paper, 3 glass Pasteur pipettes with bulbs, and, optionally, filter paper and a small funnel to remove magnesium sulfate and a second 100- or 250-mL round-bottomed flask. Experiment Three will require (per experiment): 25-mL, 50-mL and 250-mL round-bottomed flasks,

a rubber septum for the 250-mL flask, a 25- or 50-mL graduated cylinder, an ice bath, stir bar, stir plate, a rotary evaporator, a 60- or 125-mL separatory funnel, 2 beakers that hold at least 50 mL, an NMR tube, weighing paper, 5 glass 5.75" Pasteur pipettes with bulbs, a small piece of cotton to fit into one pipette, and, optionally, filter paper and a small funnel to remove magnesium sulfate. Access to a ^{1}H NMR spectrometer is necessary if students will obtain spectra of their compounds. A polarimeter and/or gas chromatography set-up is necessary for determining enantiomeric excesses for the reactions, unless a chiral NMR shift reagent approach is used instead. Information for the gas chromatography and optical rotation analysis is given below.

OPTICAL ROTATION ANALYSIS OF ETHYL 3-HYDROXYBUTYRATE ENANTIOMERS

If a chiral GC column is not available, each student can calculate the percent conversion of their sodium borohydride/tartaric acid reduction from their ^{1}H NMR spectrum. From this information, the amount of the reduced product that is in the sample can be calculated using the assumption that the molecular weights of both starting material and products are the same. Since the starting material is achiral, the percent enantiomeric excess can be calculated from the observed optical rotation. (A polarimeter cell that accepts one milliliter of material or less is optimal.) The major enantiomer from the reduction has an optical rotation value of opposite sign of that of (L)-tartaric acid. This calculation yielded approximately the same enantiomeric excess as that from gas chromatography analysis. We used a Jasco DIP-370 digital polarimeter.

GAS CHROMATOGRAPHY ANALYSIS OF ETHYL 3-HYDROXYBUTYRATE ENANTIOMERS

Both the yeast reduction and the sodium borohydride/tartaric acid reduction products were analyzed by GC. The yeast reduction gas chromatograms showed the enantiomerically-pure ethyl 3-hydroxybutyrate, in addition to a variety of side products. The chemical reduction chromatograms displayed a ~ 2:1 ratio of the ethyl 3-hydroxybutyrate enantiomers as well as some starting material.

Gas Chromatograph: HP 5890 Series II
Head Pressure: 80 kPa
Aux. Gas (nitrogen) 30 cc/min
He: 80 cc/min
Split: 100:1

Injector Oven Temperature: 200 °C
Detector Oven Temperature: 250 °C

Column Oven Temperatures:
120 °C (2.14 min, no separation of the ethyl 3-hydroxybutyrate enantiomers)
100 °C (3.20 min, 3.27 min)
80 °C (6.09 min, 6.32 min)

Ethyl (R)-(-)-3-hydroxybutyrate elutes last.

Authentic samples were obtained from Sigma-Aldrich.
Ethyl 3-hydroxybutyrate: Catalog No. E3,060-3 [5405-41-4]
Ethyl (R)-(-)-3-hydroxybutyrate: Catalog No. 34,732-9 [24915-95-5]

Chiral Column Information:
Chiraldex Capillary GC Column
Beta Cyclodextrin Dimethyl
20 m x 0.25mm

Advanced Separation Technologies
37 Leslie Court
P.O. Box 297
Whippany, NJ 07981

The URL for their homepage is http://www.astecusa.com.

Chiral Compounds and Green Chemistry: Reduction Of A Ketone By Sodium Borohydride And Baker's Yeast

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PURPOSE

To synthesize both chiral and achiral versions of ethyl beta-hydroxybutyrate from ethyl acetoactate using three different protocols. The procedures are designed to introduce the concepts of chiral catalysis and green chemistry.

BACKGROUND READING FROM PAVIA ET AL.'S *INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES: A MICROSCALE APPROACH* (HARCOURT COLLEGE PUBLISHING: 1999, 3RD EDITION)

- Reduction using sodium borohydride: pages 267-270.
- Yeast reduction: pages 279-281.
- Microscale column chromatography: pages 669-692.
- Polarimetry: pages 731-737.

Also, a review of the section about stereochemistry in your organic chemistry textbook would be helpful.

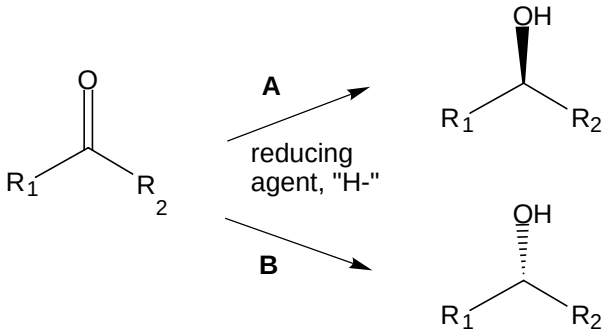
INTRODUCTION

The importance of chiral compounds. The entire biological world is pervaded with chiral compounds—compounds that have a handedness to their structure. Therefore, the design of molecules that can specifically interact with biological targets often benefits from the inclusion of stereogenic centers. In fact, many important therapeutics such as the vancomycin antibiotics and the large class of steroid drugs contain one or more stereogenic carbons. The handedness of a drug is so critical to its biological activity that now the Food and Drug Administration requires drugs to be sold as single enantiomers unless each enantiomer is tested separately and shown to be safe. This regulation has been a large driving force in developing the field of chiral synthesis and separations. Chiral separation technologies (for instance, see the work of Prof. Armstrong in this department), chiral catalysts that only form a single enantiomer of the desired product and thereby avoid chiral separations (for instance, see the work of Prof. Woo here), and enzymatic technologies or engineered biosynthetic pathways to produce chiral starting materials and products (for instance see the work of Prof. Pohl here) are extremely active areas of research around the world in both academic and corporate labs.

Green chemistry. Another extremely active area of research that overlaps with research in chiral compounds is that in "green" chemistry. What is green chemistry? Green chemistry is chemistry that looks at an entire process with an eye toward minimizing its negative environmental impact. To these ends, green chemists try to 1) minimize waste, 2) design safe (nontoxic, nonexplosive, etc.) chemical syntheses, 3) explore alternative solvents, 4) minimize energy usage (for instance, design reactions conducted at ambient pressure and temperature), 5) utilize renewable resources whenever possible, 6) design products that break down after disposal into safe nonpersistant substances, and 7) discover reactions that require catalytic rather than stoichiometric reagents. [1] Chemists are constantly trying to invent and discover new reactions and methods that improve known reaction hazards or circumvent harmful processes entirely. However, no process is perfect. Therein lies the challenge.

How can chiral compounds be made from achiral compounds using the principles of green chemistry? Nature is the ultimate source of chirality. Chemists can borrow enzymes and screen their ability to catalyze new reactions or even use genetic engineering to design new optimal protein catalysts for a particular reaction. [2] These enzymes can then be used either in a less stable purified form or, if substrate

access is not a problem, enzymes can be used while still contained in the cell that made the protein. An alternative method is to use chiral compounds, such as glucose or malic acid, available from such processes in the design of a chemical reaction. The chiral compound can either be covalently or noncovalently attached to the starting material, thereby biasing the resulting reaction in favor of a desired enantiomer, or be attached to a reagent, thereby biasing its reaction with the achiral substrate.



The chemical reduction of ketones to alcohols. The reduction of a ketone to an alcohol is a classic example of a reaction that has the potential to produce a chiral compound from an achiral starting material. Currently the most common way of accomplishing this transformation chemically is to use a metal hydride reducing agent such as sodium borohydride ($NaBH_4$) or lithium aluminum hydride ($LiAlH_4$). Ketones (pictured on the left) in which R_1 and R_2 are identical will produce an alcohol that is achiral. However, if R_1 and R_2 are different and achiral, the resulting alcohols will be chiral. Because sodium borohydride and lithium aluminium hydride are not chiral reagents, the alcohol that is produced in reactions with those reagents will always be racemic. The transition states leading to each isomer of the alcohol are enantiomeric and of equal energy. This means that the activation energy for reaction pathways A and B will be identical. The two enantiomers will form at the same rate yielding a racemic product (a 1:1 mixture of each enantiomer).

The reduction of ketones to form nonracemic alcohols. If a source of chirality is introduced into the system then the transition states leading to the two isomers of the alcohol become diastereomeric and of different energy. Reaction pathways A and B will no longer be identical. Therefore, different amounts of the two stereoisomers of the alcohol can be formed. A source of chirality can be introduced into the reaction mixture in many ways. For example, a chiral reducing agent or a chiral solvent can be employed in the reaction. Chemists in recent years have devised a number of chiral reducing agents and much research is in progress to optimize chiral reductions using new combinations of ligands, solvents, and reducing agents. For ultimate practicality, the principles of green chemistry have to be kept in mind, too. Two experiments below describe the chemical reduction of a ketone both in the presence and the absence of a chiral compound.

The enzymatic reduction of ketones to alcohols. Biocatalysis is often thought of as an environmentally friendly alternative to the use of harsher chemicals. Chemists in recent years have devised a number of chiral reducing agents, but few of them are as efficient as the enzymatic reducing agents found in nature. One experiment will introduce you to the methods of using a benign organism—Baker's yeast—to carry out a synthetic organic transformation. Enzymes, which are protein catalysts, can also be isolated from organisms such as yeast and used directly to carry out a desired reaction. The use of whole organisms or individual enzymes is desirable as the chemical reactions happen at ambient temperature and pressure, but they also usually require large amounts of water that must be properly disposed as waste.

For this series of experiments, the reduction of a ketoester with and without the use of a chiral additive will be investigated. Two chiral reagents will be examined to compare the procedures: an enzyme contained within Baker's yeast[3] and a chiral tartaric acid ligand complexed to sodium borohydride.[4] Afterwards the reaction yield will be determined along with the enantiomeric excess and absolute configuration of the alcohols that are formed. Which method most closely achieves the "green" ideal?

TIMETABLE FOR THE EXPERIMENTS

In order to complete this series of experiments, you will need to plan your time carefully. You should try to keep to the following timetable. You will carry out the three experiments in groups of two people each and then compare the resulting products from all three experiments. Each group may choose how to divide the tasks.

Monday, February 19	Initial briefing and set up the reductions (working in pairs): Experiment 1: Sodium borohydride reduction of ethyl acetoacetate. Experiment 2: Yeast-catalyzed reduction of ethyl acetoacetate.
Wednesday, February 21	Experiment 1: Analysis of the $NaBH_4$ reduction. Experiment 2: Continue the yeast-catalyzed reduction. Experiment 3: Set up sodium borohydride reduction using chiral ligand.
Monday, February 26	Experiment 2: Work-up and isolation of the yeast reduction product(s). Experiment 3: Work-up and isolation of the chiral reduction.
Wednesday, February 28	Experiments 2 and 3: Characterization of the yeast and chiral sodium borohydride reduction product(s). Compare enantiomeric excesses.

CAUTIONS!!

As with any hydride reaction, hydrogen gas is evolved during the course of the reaction. Hydrogen must be kept away from ignition sources to avoid explosions. The organic solvents are all flammable. Tetrahydrofuran is an irritant. Deuterated chloroform used to make samples for NMR is highly toxic and a cancer suspect agent. Hydrochloric acid and sodium borohydride are corrosive and tartaric acid is an irritant. Standard chemical safety precautions should be practiced at all times.

PRELAB QUESTIONS

1) What is the boiling point of ethanol at ambient pressure and temperature? Tetrahydrofuran? Ethyl acetoacetate? The desired alcohol product?

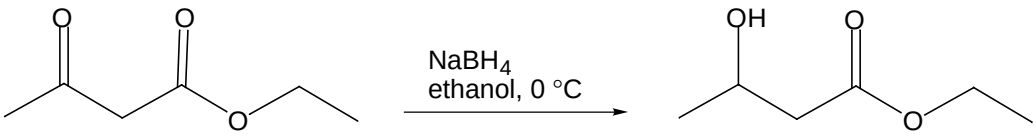
2) The addition of acetic acid to sodium borohydride will cause the displacement of one or more hydrides from each boron atom with an acetate ion. The resulting borohydride reducing agent is less reactive than the parent sodium borohydride. What does tartaric acid do in the sodium borohydride reduction to cause a chiral reaction environment for the ketone reduction reaction?

3) What are the specific rotations of pure (R)-ethyl 3-hydroxybutanoate, (S)-ethyl 3-hydroxybutanoate and (L)-tartaric acid?

4) Assume your reduction reaction converted 80% of the starting material, ethyl acetoacetate, to give a product mixture of (R)-ethyl-3-hydroxybutyrate and (S)-ethyl-3-hydroxybutyrate. Using a 1 dm long polarimeter cell, you find the optical rotation of the mixture to be +8.5° with a concentration of 0.5 g/mL (total material, not just product). Find the percent enantiomeric excess of the dominant enantiomer.

5) Could you use $LiAlH_4$ for the reductions of ethyl acetoacetate rather than $NaBH_4$?

EXPERIMENT 1: Reduction of Ethyl Acetoacetate with Sodium Borohydride

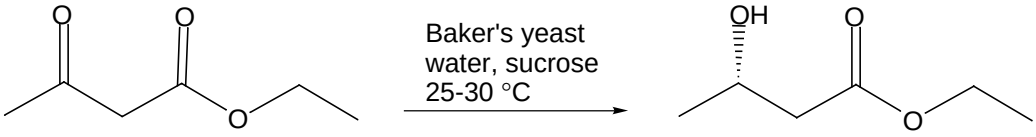


Add sodium borohydride (1.5 g, 40 mmol, MW 37.83) to ethanol (25 mL) in a 100-mL round-bottomed flask, and cool the resulting mixture to 0 °C. To this mixture add a solution of the ethyl acetoacetate (5.0 g, 38 mmol, MW 130.14) in ethanol (15 mL), and stir the resulting solution at 0 °C for 15 minutes, then at room temperature for 15 minutes.

Evaporate the solvents on a rotary evaporator, and suspend the resulting white solid in dichloromethane (30 mL). Add 1 M hydrochloric acid (30 mL) drop-wise to quench the reaction. (**CAUTION:** The addition of HCl will cause frothing and will release hydrogen gas.) Add the hydrochloric acid slowly and while the flask is on ice. Separate the organic layer. Extract the aqueous layer two times with dichloromethane (20 mL). Combine the organic layers and dry using magnesium sulfate. Filter off the magnesium sulfate and evaporate the solvent using a rotary evaporator with a water bath temperature no higher than 30 °C. Record the actual yield, ^{1}H NMR spectrum, IR spectrum, and observed rotation of the product. Calculate the specific rotation and percent yield.

CAUTION: As with any hydride reaction, hydrogen gas is evolved during the course of the reaction. Hydrogen must be kept away from ignition sources to avoid explosions.

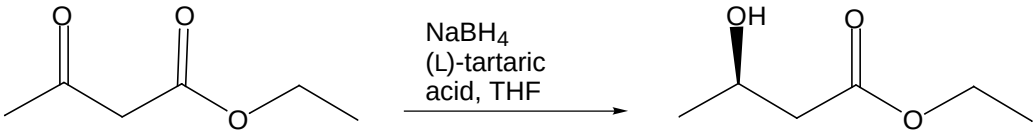
EXPERIMENT 2: Chiral Reduction of Ethyl Acetoacetate with Baker's Yeast



In this experiment we will use the enzymes found in Baker's yeast to reduce ethyl acetoacetate to S-(+)-ethyl 3-hydroxybutanoate. This compound is a very useful synthetic building block.

This experiment is described on pages 278-281 of your laboratory textbook.

EXPERIMENT 3: Chiral Reduction of Ethyl Acetoacetate by Sodium Borohydride and (L)-Tartaric Acid



Day one: Place a magnetic stir bar in a 50-mL round-bottomed flask and add approximately 15 mL of tetrahydrofuran (THF). Add sodium borohydride (0.50 g, 13 mmol, MW 37.83) to the flask and begin

stirring. To the suspension, add (L)-tartaric acid (2.0 g, 13 mmol, MW 150.09) and stir for 15 minutes. Cool the flask on an ice bath and add ethyl acetoacetate (0.44 g, 3.4 mmol, MW 130.14). Remove the flask from the ice bath and stir for 1 hour.

Quench the reaction with 15 mL of 1 M hydrochloric acid. Recall that addition of the acid will cause violent frothing and the formation of hydrogen gas. The acid should be added drop-wise while the flask is on an ice bath and the reaction is stirring. After adding the HCl, remove the flask from the ice bath and stir the solution for 10 minutes.

Extract the solution two times with ethyl acetate (30 mL). If your separatory funnel is not large enough to allow sufficient mixing of the aqueous wash with your organic layer, you may need to separate the extracts into two portions. Wash the extracts with saturated aqueous sodium bicarbonate solution (40 mL) and separate the layers. Wash the organic layer with saturated aqueous sodium chloride solution (40 mL) and again separate the layers. Aqueous layers should be kept until you are certain the product is in the organic layer. Dry the organic layer with magnesium sulfate. Filter off the magnesium sulfate and transfer the solution to a 250-mL round-bottomed flask. Cap with a rubber septum and store until the next lab period.

Day Two: Use a rotary evaporator to remove the solvent. Using a 5.75" Pasteur pipette, prepare a microscale column with neutral alumina as the absorbent and dichloromethane as the eluent. (See pages 678-685 of your laboratory textbook for background.) Place a small piece of cotton in the pipette and carefully push it to the bottom. The cotton should allow liquid to freely move through the column. Fill the pipette with approximately 1.5" of alumina. Add dichloromethane (2 mL) to the column allowing it to drain through the alumina until the solvent surface is just above the alumina surface. This fraction of dichloromethane can be properly discarded. Prepare a 25-mL round-bottomed flask to collect the product. Transfer the product from the 250-mL round-bottomed flask to the wet column. Rinse the round-bottomed flask with dichloromethane (1-2 mL) to dissolve any remaining product and add this to the column. Allow the mixture to flow through the column until the solvent level just reaches the alumina. Add dichloromethane (2 mL) to the column and again allow the mixture to pass through the column as before. Repeat with another portion of the solvent (2 mL) to elute the product from the column. Use a rotary evaporator to remove the solvent.

Day three: Characterize the starting material/product mixture by ^{1}H NMR. Estimate the amount of starting material that is present. (You may want to get a NMR spectrum of the starting material for ease in comparison.) Take an optical rotation and calculate the percent enantiomeric excess based on the concentration of the alcohol present in the sample.

ISSUES TO ADDRESS IN YOUR FINAL LAB REPORT

1) Compare and contrast the sodium borohydride/tartaric acid reduction with the yeast reduction. Which process is more “green”? Include an evaluation of all waste (including aqueous), chemical yields, yield of a single enantiomer, safety, and energy efficiency.

2) What methods of chemical characterization can you use to characterize the presence of an enantiomerically pure compound? Do the ^{1}H NMR spectra of the racemic mixtures look the same as those of an enantiomerically pure compound? Optical rotations? IR?

REFERENCES

1) For a leading reference describing green chemistry, see: Hjeresen, D. L.; Schutt, D. L.; Boese, J. M. “Green Chemistry and Education.” *J. Chem. Educ.* **2000,** *77,* 1543.

2) For reviews on biocatalysis and biotransformations, see the articles contained in the special issue of *Nature*, **2001**, *409*, 225-257 and Sime, J. T. “Applications of Biocatalysis to Industrial Processes.” *J. Chem. Educ*. **1999**, *76*, 1658. For an example of the genetic engineering of Baker’s yeast to produce an organism with greater stereoselectivity in reducing beta-ketoesters see: Rodriguez, S.; Kayser, M. M.; Stewart, J. D. “Highly Stereospecific Reagents for Beta-Keto Ester Reductions by Genetic Engineering of Baker’s Yeast.” *J. Am. Chem. Soc.* **2001**, *123*, 1547-1555.

3) Seebach, D.; Sutter, M. A.; Weber, R. H.; Züger, M. F. “Yeast Reduction of Ethyl Acetoacetate: (*S*)-(+)-Ethyl 3-Hydroxybutanoate.” *Org. Synth.* **1984**, *63*, 1.

4) Yatagai, M.; Ohnuki, T. “Asymmetric Reduction of Functionalized Ketones with a Sodium Borohydride—(L)-Tartaric Acid System.” *J. Chem. Soc. Perkin Trans. I.* **1990**, 1826.

Determining Enantiomeric Excesses by Gas Chromatography using a Chiral Column

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PURPOSE

To analyze the percent of each enantiomer of ethyl 3-hydroxybutanoate that was formed in the reductions of ethyl acetoacetate by yeast and by chiral hydride chemistry. The procedure introduces the concept of separating enantiomers of a compound by chiral chromatography that relies on noncovalent interactions with a chiral support.

BACKGROUND READING FROM PAVIA ET AL.'S *INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES: A MICROSCALE APPROACH* (HARCOURT COLLEGE PUBLISHING: 1999, 3RD EDITION)

• Gas chromatography: pages 711-726.

The development of methods to synthesize a single enantiomer of a compound relies on the accurate determination of the enantiomeric excess of the products of a reaction. Several options are commonly used. If the optical rotation of the desired product is known, then polarimetry can be used to determine the enantiomeric excess of your sample. Optical rotation measurements are sensitive to impurities, however. Alternatively, a chiral shift reagent can be added to a NMR sample (see pages 282-285 of your laboratory textbook) to alter the chemical shifts of one enantiomer compared to another. This latter method relies on the interaction of one enantiomer with a chiral compound. Determination of enantiomeric excesses by this method requires reasonable differences in the chemical shifts of the two enantiomers complexed with the chiral shift reagent. This method is often accurate and does not require a pure sample, but is time consuming when a variety of synthetic conditions want to be assayed.

A method that lends itself to high-throughput screening of synthetic methods is the separation of enantiomers by their differential interactions with a chiral medium. Many columns that are packed with a chiral material are commercially available for attachment to separations instruments such as gas chromatographs. A desired sample can be passed through a chiral column and tested for the desired separation. Since each component of the racemic mixture that is loaded onto the column will interact differently with the chiral column media, the enantiomers will be retained on the column for different lengths of time. Several columns often have to be tested to get the desired separation of enantiomers that allows integration of the peaks of the chromatograph. Well-resolved peaks for each enantiomer can be integrated and the values compared to obtain the percent of each enantiomer in the applied sample. The inability to separate enantiomers with commercially available chiral columns fuels current efforts to develop new chiral packing materials.

A cyclodextrin (a cyclic molecule containing six to ten glucose units) packed column media has been shown to separate a racemic mixture of ethyl 3-hydroxybutanoate using a gas chromatograph. Obtain gas chromatograms using this column of the purified products of both the yeast reduction and the tartaric acid/sodium borohydride reduction. Integrate the peaks and calculate the percent yield of each enantiomer and the enantiomeric excess. Compare these values to those obtained by polarimetry measurements.