JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

pubs.acs.org/jchemeduc

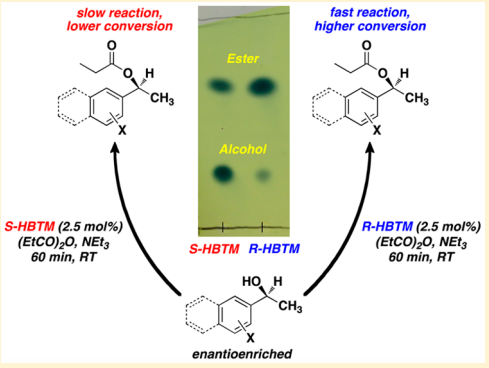
Undergraduate Laboratory Experiment To Determine Absolute Configuration Using Thin-Layer Chromatography

Alexander J. Wagner, Shawn M. Miller, Steven Nguyen, Ga Young Lee, Scott D. Rychnovsky,* and Renée D. Link*

Department of Chemistry, University of California—Irvine, Irvine, California 92697, United States

Supporting Information

ABSTRACT: A new undergraduate organic chemistry laboratory experiment has been developed to determine the absolute configuration of enantioenriched secondary alcohols with the competing enantioselective conversion (CEC) method. The CEC method uses both enantiomers of a chiral kinetic resolution reagent in parallel reactions to identify the fast-reacting reagent and thus the configuration of the alcohol. Students working in pairs are given one of three enantioenriched secondary alcohols with an unknown absolute configuration. They assign the molecular structure using the corresponding ^{1}H NMR spectrum and determine the absolute configuration via the CEC method. The parallel reactions are run at room temperature for 1 h using small quantities of solvent, substrate, and catalyst. Students use thin-layer chromatography (TLC) to analyze the parallel reactions and determine the fast reaction qualitatively. The free program ImageJ is used with a picture of the TLC plate to carry out a quantitative analysis of reaction conversion. Data collected in the spring 2013 laboratory course (n = 1036) show that 93.6% of students determined the absolute configuration of their unknown correctly with just a qualitative analysis of the TLC plate. This experiment provides a platform for discussions of stereochemistry, mechanism, kinetics, energy diagrams, and transition states.



KEYWORDS: *Second-Year Undergraduate, Organic Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Problem Solving/Decision Making, Catalysis, Enantiomers, Kinetics, Stereochemistry, Thin Layer Chromatography*

One of the most difficult concepts in introductory undergraduate organic chemistry laboratories is the idea of chirality and its application to understanding stereochemistry.[1−3] A laboratory experiment was developed that highlights the overarching topic of chirality using a benchtop chemistry protocol that involves problem-solving and critical thinking.[4,5] The experimental content covers a variety of learning styles (visual, audio, reading/writing, and kinesthetic) as well as both a qualitative and a quantitative analysis. An effective experiment topic for discussing chirality and stereochemistry is the determination of absolute configuration of a stereocenter in a chiral molecule. The use of chemical reactions for this determination also provides the opportunity for additional discussions about the kinetics, thermodynamics, mechanism, and transition state of the reaction.

BACKGROUND

Absolute Configuration

With the development of asymmetric transformations and improvements in the isolation of single enantiomers of natural products came a need to be able to identify and determine the absolute configuration of the enantioenriched stereocenter(s) of interest.[6−9] A variety of methods have been developed for the determination of absolute configuration including the advanced Mosher method,[10−12] vibrational circular dichroism,[13] exciton chirality,[14] NMR spectroscopic chiral shift reagents,[15−18] lipase-catalyzed resolutions,[19] and X-ray crystallographic analysis.[20] A number of chiral derivatization methods have been transformed into undergraduate experiments, where students convert a single enantiomer into two diastereomers and analyze the diastereomeric products by NMR spectroscopy.[21−23] While effective, the NMR spectroscopic analysis can be complicated for introductory undergraduate organic laboratories.

The Competing Enantioselective Conversion Method

The Rychnovsky group recently reported the competing enantioselective conversion (CEC) method for the determination of absolute configuration of stereogenic centers.[24−27] The CEC method is a modern implementation of the Horeau method.[28] The CEC method utilizes parallel reactions, where the substrate of interest is subjected to reaction conditions that facilitate a chemical transformation (Figure 1A). The transformation is mediated by the use of chiral kinetic resolution reagents. Each reaction contains one enantiomer of the kinetic resolution reagent. The difference in the rate of the transformation between the two parallel reactions results

Published: April 2, 2014

dx.doi.org/10.1021/ed4004996 | J. Chem. Educ. 2014, 91, 716−721

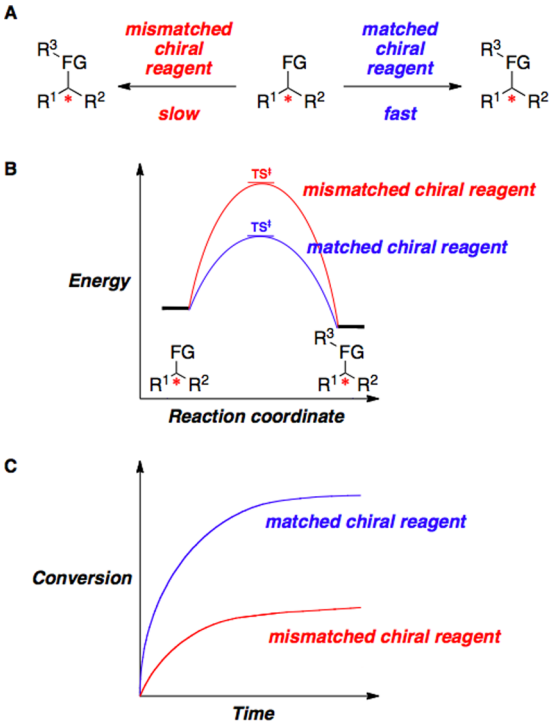


Figure 1. (A) General depiction of the competing enantioselective conversion method; FG = functional group; * = stereogenic center. (B) Energy diagram corresponding to the parallel reactions highlighted in panel A. (C) Kinetic analysis based on the energy diagram measuring conversion for each reaction.

from the difference in energy between the diastereomeric transition states of the enantioenriched substrate with each enantiomer of the kinetic resolution reagent (Figure 1B). The result is a difference in reaction conversion between the two parallel reactions (Figure 1C). The "fast" and "slow" reactions can be determined based on reaction conversion. Then, with the use of an empirical mnemonic developed through testing a number of substrates with known configurations, the absolute configuration can be assigned.

Given the importance of chirality and stereochemistry to the undergraduate organic chemistry curriculum, a modified procedure for the CEC method was developed that is applicable to an undergraduate laboratory experiment. Secondary alcohols were chosen as the functional group to use for this experiment because of their affordability and importance in chemistry. Additionally, the CEC method for secondary alcohols uses the homobenzotetramisole (HBTM) catalyst[29] (Figure 2) at very low molar equivalents, which is beneficial for

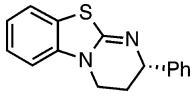


Figure 2. The (*S*)-HBTM catalyst developed by Birman.[29]

a laboratory experiment with a large number of students. The HBTM catalyst is commercially available.[30] HBTM can also be made through a two-pot procedure, which is described in the Supporting Information. The catalyst can be stored for months at room temperature in a desiccator without noticeable decomposition. Thin-layer chromatography is used as the characterization technique because it is a cost-effective and easily scalable technique that is commonly employed in the undergraduate organic chemistry laboratories.

EXPERIMENTAL SECTION

This experiment is suitable for the second semester of the introductory organic chemistry laboratory series. The time required for the experiment is 2 h for the benchtop chemistry and 1 h for the subsequent analysis of the data. Each pair of students is given a stock solution of an unknown enantioenriched secondary alcohol (**1**, **2**, or **3**) in toluene and the matching ^{1}H NMR spectrum of the unknown alcohol (Figure 3).[31] The unknown compounds are spectroscopically

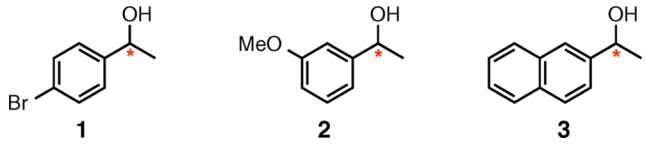


Figure 3. Enantioenriched alcohols used in the experiment. Both the *R* and *S* enantiomers of each alcohol were used as unknowns. The * symbol represents a stereogenic center.

differentiated by their aromatic protons and the methoxy group found only on compound **2**. Three additional stock solutions in toluene [(*S*)-HBTM with triethylamine, (*R*)-HBTM with triethylamine, and propionic anhydride] are prepared for student use. Student groups are tasked with (i) determining the molecular structure of the unknown using the ^{1}H NMR spectrum and (ii) determining the absolute configuration of the unknown using the CEC method with thin-layer chromatography (TLC).

Each group is given two vials (A and B). A solution of the unknown alcohol in toluene is added to both vials. The (*S*)-HBTM solution is added to vial A and the (*R*)-HBTM solution is added to vial B. Both reactions are initiated by the addition of the propionic anhydride solution. Sixty minutes after the anhydride is added, methanol is added to vial A and vial B to halt the reaction progress.

A TLC plate is prepared with two lanes, labeled *a* and *b*. Each lane is spotted with the reaction solution in the corresponding vial. The plate is run, stained with phosphomolybdic acid (PMA) stain, and visualized by heating the plate in an oven. A qualitative analysis of the fast reaction is made by each student individually. The plate is photographed and analyzed quantitatively by each student individually using the free spot-density analysis program ImageJ.[32] The fast reaction is found quantitatively via reaction conversion. Using the mnemonic in Figure 4, the absolute configuration is determined for both the qualitative and quantitative results based on the enantiomer of HBTM present in the fast reaction. A detailed

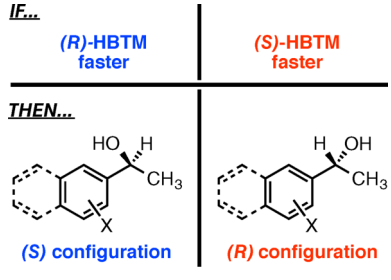


Figure 4. Mnemonic for assigning absolute configuration.

dx.doi.org/10.1021/ed4004996 | J. Chem. Educ. 2014, 91, 716−721

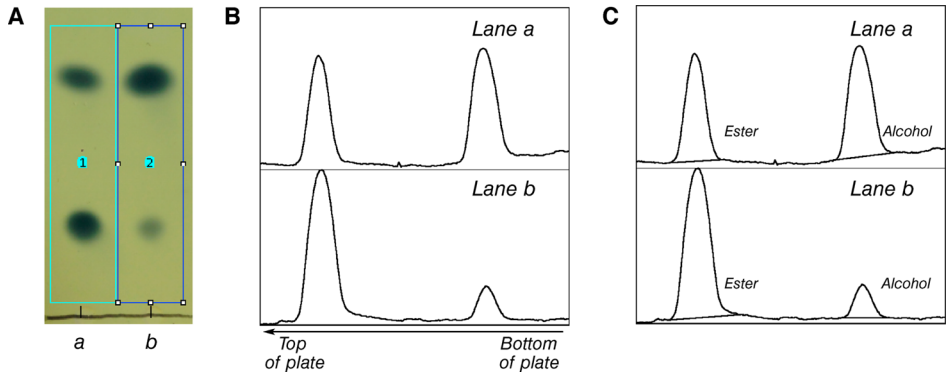
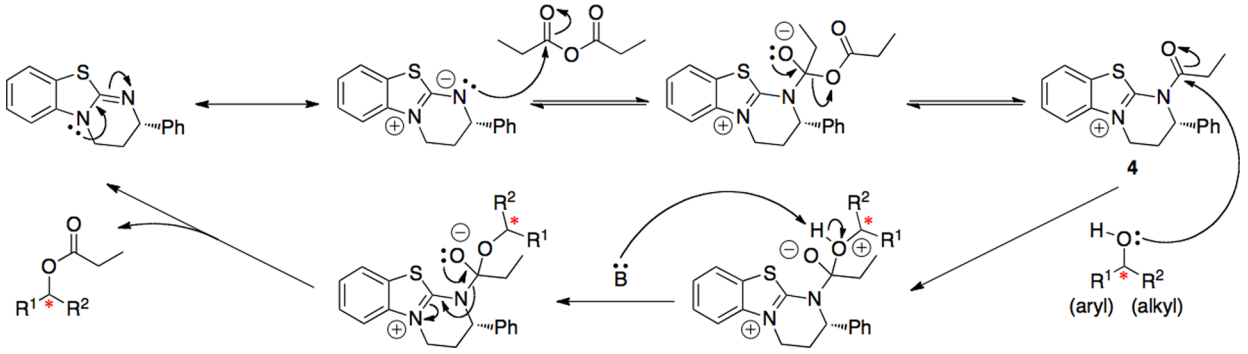


Figure 5. (A) Student TLC plate from experiment. (B) ImageJ readout of TLC plate lanes. (C) Area of peaks from manual integration of peaks corresponding to spots from the reaction in vials A and B. Lane *a*: ester −10777, alcohol −13270. Lane *b*: ester −18806, alcohol −2889.

Scheme 1. Proposed Mechanism for the HBTM-Mediated Esterification



description of the experiment is available in the Supporting Information.

HAZARDS

Toluene, ethyl acetate, hexanes, and methanol are volatile organic solvents. They are also flammable. *n*-Hexane, a major component of hexanes, is a neurotoxin. Use all solvents in the hood and wear gloves when working with them. Phosphomolybdic acid stain is a skin irritant and an oxidizer. The major component of the stain is ethanol, which is flammable. Keep away from an open flame. Triethylamine is an irritant and is pungent. Use only in the hood. Propionic anhydride, homobenzotetramisole (HBTM), the unknown alcohols, and the unknown ester products are irritants. Wear gloves and avoid contact with skin, eyes, and clothing.

DISCUSSION

A representative student result of the TLC plate from the experiment is shown in Figure 5A. The spot at the higher R_f is the ester. The lane with a higher spot density of the ester qualitatively appears to be in lane *b*, which contains the (*R*)-HBTM. Using the predictive mnemonic, the absolute configuration of the alcohol was then determined to be *S*. A readout of the lanes taken from ImageJ is shown in Figure 5B. When the peaks are manually integrated (Figure 5C), the reaction conversion can be found for each lane. In this example, the conversion of the reaction in vial A was calculated to be 45% while the conversion of the reaction in vial B was 87%. Therefore, the quantitative analysis determined that reaction in vial B was the fast reaction, and this result matched the qualitative analysis. A video link describing the use of ImageJ for this experiment is included in ref 32.

Mechanism

The mechanism of the transformation (Scheme 1) is hypothesized to occur through an activated catalyst intermediate (**4**).[33] The first step is acylation of the catalyst. This activated intermediate then interacts with the substrate alcohol. After a proton transfer with base, the tetrahedral intermediate collapses to give the ester product and to regenerate the HBTM catalyst. Thorough kinetic studies have been done with the HBTM system that corroborate the proposed mechanism and acyl-HBTM intermediate **4**.[34]

Transition State

Given that the formation of the activated intermediate can occur equally with either enantiomer of the HBTM catalyst, the reason for the difference in the rate of reaction between the parallel reactions is the diastereoselective interaction of the activated intermediate with the secondary alcohol. The proposed transition state for the matched interaction is shown in Figure 6.[29,33]

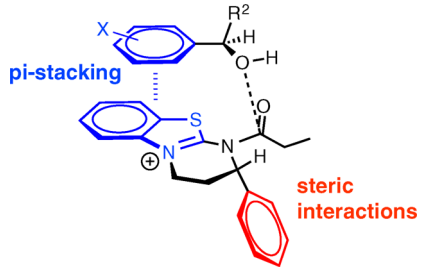


Figure 6. Proposed transition state of matched interaction between activated (*S*)-HBTM catalyst and the *R* secondary alcohol substrate.

dx.doi.org/10.1021/ed4004996 | J. Chem. Educ. 2014, 91, 716−721

The phenyl ring highlighted in red serves to block the alcohol from interacting with the catalyst from the bottom face, as drawn. Therefore, the alcohol should prefer to interact with the catalyst from the top face. Additionally, the aromatic systems highlighted in blue are participating in pi stacking. This interaction is one of the recognition points between the substrate and the catalyst and helps to dictate selectivity. The alkyl group (R^2) on the substrate is pointed away from the catalyst to minimize steric interactions. Then, the orientation of the alcohol determines the reactivity. Here, the *R* enantiomer is shown with the hydroxyl group positioned directly above the carbonyl of the activated amide on the catalyst, leading to the fast reaction. If the other enantiomer of the alcohol (mismatched) is considered, these key interactions would be disrupted, resulting in the slow reaction.

^{1}H NMR Spectroscopic Analysis

The ^{1}H NMR spectra given to the students are interpreted with an introductory organic chemistry understanding in mind. Students differentiate between the three possible unknowns based on the type of aromatic group present on the compound, the position of any substituents on the aromatic ring, and whether the substituent on the aromatic ring contains additional hydrogens. Students determine their unknown by ruling out the other two possible compounds. The logic used in ruling out structures and confirming the student's unknown structure was included in their lab report. Further discussion on the NMR spectral analysis can be found in the Supporting Information.

■ ANALYSIS OF STUDENT DATA

This experiment was performed in the spring 2013 quarter of an introductory organic chemistry laboratory course with 1036 students in 58 laboratory sections. A data sheet was designed for every student to fill out that included their unknown number, the qualitative analysis, integration values from ImageJ, the quantitative analysis, and a drawn structure of the unknown with the experimentally determined absolute configuration. Thirty-nine students did not turn in a lab report, so the total number used in the statistical analysis of the collected data set was 997 (Table 1).

Table 1. Analysis of Student Laboratory Conclusions

Student Conclusion	Yes (%)[a]	No (%)[a]	Omitted (%)[a]
Correct qualitative analysis of TLC	933 (93.6)	64 (6.4)	
Correct quantitative analysis via ImageJ	916 (91.9)	81 (8.1)	
Correct structure via ^{1}H NMR	842 (84.5)	63 (6.3)	92 (9.2)
Correct configuration on structure	737 (73.9)	68 (6.8)	192 (19.3)

[a]The percentages were based on the number of reports received (997); thirty-nine students did not turn in a report.

As a part of both the qualitative and quantitative analysis, students determined the fast reaction, the enantiomer of HBTM that was in the fast reaction, and the absolute configuration of their unknown using the mnemonic in Figure 4. Analysis of the student data revealed that 93.6% of students correctly identified all three parts of the qualitative analysis and 91.9% of students correctly identified all three parts of the quantitative analysis.

The statistical analysis also showed that 84.5% of students correctly identified the molecular structure of their unknown based on the given NMR spectroscopic data and 73.9% of students drew the correct configuration on the structure. One of the main reasons for the reduced percentages in the structure data was that a considerable number of students did not draw a structure (9.2%) or did not draw a configuration (19.3%) on the sheets that were analyzed.

The major error for those students (64) with incorrect qualitative analyses (Table 2) was misinterpretation of the TLC plate. This likely occurred because of a misunderstanding of which spot was the ester and which spot was the alcohol in each reaction lane. Labeling errors, such as switching the enantiomers of HBTM for reactions in vials A and B or mislabeling the vials A and B, also occurred. There were a small number of students that misused the mnemonic in Figure 4. An additional error was the incorrect use of ImageJ followed by matching the incorrect quantitative data to the qualitative information.

Table 2. Student Errors for Incorrect Qualitative Analyses

Error	Number of Students
Misinterpreted TLC plate	19
Conclusions match provided TLC plate, but not unknown number (switched HBTM enantiomers for reactions in vials A and B)	10
Claims both reactions went to completion	9
Performed ImageJ analysis incorrectly and matched quantitative result to qualitative result	8
Used mnemonic incorrectly	8
Mislabeled fast reaction	4
Claims reactions did not run	3
Unclear, may have guessed	3
Total	64

Conversion data from the correct quantitative ImageJ integration data were also collected for each enantiomer of the three different secondary alcohols, and the averages from the entire population of data are shown in Table 3. In every case, there was comparable conversion data between the enantiomeric sets for each alcohol.

Table 3. Student ImageJ Conversion Data

Unknown	Reaction A Conversion (%)	Reaction B Conversion (%)
R-**1**	85	50
S-**1**	49	91
R-**2**	84	43
S-**2**	46	79
R-**3**	89	47
S-**3**	46	87

■ CONCLUSION

An introductory undergraduate organic chemistry laboratory experiment was developed for determining the absolute configuration of secondary alcohols using the competing enantioselective conversion method. This is the first reported experiment in the *Journal* to determine the absolute configuration of an unknown substrate without preparing

dx.doi.org/10.1021/ed4004996 | J. Chem. Educ. 2014, 91, 716−721

diastereomers and without using NMR spectroscopy. The experiment used small quantities of substrate, solvent, and catalyst as well as a simple analytical technique (TLC) commonly used in a typical undergraduate organic curriculum. Both qualitative and quantitative analyses were included to determine the fast reaction, which was then used for the determination of absolute configuration of the unknown compound. A ^{1}H NMR spectrum was included to allow a student to determine the molecular structure of the unknown as well. The experiment provided a platform for discussions on chirality, stereochemistry, spectroscopy, energy diagrams, transition states, mechanism, and kinetics.

■ ASSOCIATED CONTENT

Supporting Information

Laboratory experiment handout, experiment grading rubric, stockroom preparation sheet, list of chemicals and equipment required, results table used for collecting data, student learning outcomes, and resources for additional discussion. Experimental procedures for the synthesis of the HBTM catalyst and the enantioenriched secondary alcohols. This material is available via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: (S.D.R.) srychnov@uci.edu, (R.D.L.) rlink@uci.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the students and teaching assistants in the 51LC Organic Chemistry Laboratory course during the spring 2013 quarter for their participation in the development of this new laboratory experiment. We also thank Organic Chemistry Stockroom Manager Lukas Holt and the rest of the stockroom staff for their assistance. Support was provided by the National Science Foundation (CHE 1152449), as well as by the Department of Defense (DoD) through the National Defense Science & Engineering Graduate Fellowship (NDSEG) Program (A.J.W.) and the National Science Foundation Graduate Fellowship Program (A.J.W.).

■ REFERENCES

(1) Brand, D. J.; Fisher, J. Molecular structure and chirality. *J. Chem. Educ.* **1987**, *64*, 1035−1038.
(2) Collins, M. J. Demonstrating chirality: Using a mirror with physical models to show non-superimposability of chiral molecules with their mirror images. *J. Chem. Educ.* **2001**, *78*, 1484−1485.
(3) Abraham, M.; Varghese, V.; Tang, H. Using Molecular Representations To Aid Student Understanding of Stereochemical Concepts. *J. Chem. Educ.* **2010**, *87*, 1425−1429.
(4) Jacob, C. Critical Thinking in the Chemistry Classroom and Beyond. *J. Chem. Educ.* **2004**, *81*, 1216−1223.
(5) Lyle, K. S.; Robinson, W. R. Teaching Science Problem Solving: An Overview of Experimental Work. *J. Chem. Educ.* **2001**, *78*, 1162−1163.
(6) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; Wiley-Interscience: Hoboken, NJ, 1994; pp 101−147 and 991−1105.
(7) Seco, J. M.; Quiñoá, E.; Riguera, R. The Assignment of Absolute Configuration by NMR. *Chem. Rev.* **2004**, *104*, 17−118.
(8) Wenzel, T. J.; Chisholm, C. D. Assignment of absolute configuration using chiral reagents and NMR spectroscopy. *Chirality* **2011**, *23*, 190−214.
(9) Cahn, R. S.; Ingold, C.; Prelog, V. Specification of Molecular Chirality. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385−415.
(10) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. *J. Org. Chem.* **1969**, *34*, 2543−2549.
(11) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(12) For a review of the advanced Mosher method procedure, see: Hoye, T. R.; Jeffrey, C. S.; Shao, F. Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons. *Nat. Protoc.* **2007**, *2*, 2451−2458.
(13) For a review on using vibrational circular dichroism, see: Freedman, T. B.; Cao, X.; Dukor, R. K.; Nafie, L. A. Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism. *Chirality* **2003**, *15*, 743−758.
(14) For a review on the electronic CD exciton chirality method, see: Harada, N.; Nakanishi, K.; Berova, N. Electronic CD Exciton Chirality Method: Principles and Applications. In *Comprehensive Chiroptical Spectroscopy, Vol. 2: Applications in Stereochemical Analysis of Synthetic Compounds, Natural Products, and Biomolecules*; Berova, N.., Polavarapu, P. L., Nakanishi, K.., Woody, R. W., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, 2012; pp 115−166.
(15) Kobayashi, Y.; Hayashi, N.; Kishi, Y. Toward the Creation of NMR Databases in Chiral Solvents: Bidentate Chiral NMR Solvents for Assignment of the Absolute Configuration of Acyclic Secondary Alcohols. *Org. Lett.* **2002**, *4*, 411−414.
(16) Kobayashi, Y.; Hayashi, N.; Kishi, Y. Application of chiral bidentate NMR solvents for assignment of the absolute configuration of alcohols: scope and limitation. *Tetrahedron Lett.* **2003**, *44*, 7489−7491.
(17) Ghosh, I.; Zeng, H.; Kishi, Y. Applications of Chiral Lanthanide Shift Reagents for Assignment of Absolute Configuration of Alcohols. *Org. Lett.* **2004**, *6*, 4715−4718.
(18) Ghosh, I.; Kishi, Y.; Tomoda, H.; Omura, S. Use of a Chiral Praseodymium Shift Reagent in Predicting the Complete Stereostructure of Glisoprenin A. *Org. Lett.* **2004**, *6*, 4719−4722.
(19) Jing, Q.; Kazlauskas, R. J. Determination of absolute configuration of secondary alcohols using lipase-catalyzed kinetic resolutions. *Chirality* **2008**, *20*, 724−735.
(20) For a review on X-ray crystallographic analysis, see: Flack, H. D.; Bernardinelli, G. The use of X-ray crystallography to determine absolute configuration. *Chirality* **2008**, *20*, 681−690.
(21) Allen, D. A.; Tomaso, A. E.; Priest, O. P.; Hindson, D. F.; Hurlburt, J. L. Mosher Amides: Determining the Absolute Stereochemistry of Optically-Active Amines. *J. Chem. Educ.* **2008**, *85*, 698−700.
(22) Saba, S.; Clarke, D. D.; Iwanoski, C.; Lobasso, T. Using NMR to Probe the Regio- and Sterochemistry of the Hydration of 1-Hexene. *J. Chem. Educ.* **2010**, *87*, 1238−1241.
(23) Fenton, O. S.; Sculimbrene, B. R. A Web-Lab Approach to Stereochemistry Using ^{31}P NMR Spectroscopy. *J. Chem. Educ.* **2011**, *88*, 662−664.
(24) For the CEC method with secondary alcohols using ^{1}H NMR spectroscopy, see: Wagner, A. J.; David, J. G.; Rychnovsky, S. D. Determination of Absolute Configuration Using Kinetic Resolution Catalysts. *Org. Lett.* **2011**, *13*, 4470−4473.
(25) For the CEC method with oxazolidinones, lactams, and thiolactams using ^{1}H NMR spectroscopy, see: Perry, M. A.; Trinidad, J. V.; Rychnovsky, S. D. Absolute Configuration of Lactams and Oxazolidinones Using Kinetic Resolution Catalysts. *Org. Lett.* **2013**, *15*, 472−475.
(26) For the CEC method with primary amines using mass spectrometry, see: Miller, S. M.; Samame, R. A.; Rychnovsky, S. D. Nanomole-Scale Assignment of Configuration for Primary Amines Using a Kinetic Resolution Strategy. *J. Am. Chem. Soc.* **2012**, *134*, 20318−20321.

(27) For the CEC method with secondary alcohols using thin-layer chromatography, see: Wagner, A. J.; Rychnovsky, S. D. Determination of Absolute Configuration of Secondary Alcohols Using Thin-Layer Chromatography. *J. Org. Chem.* **2013**, *78*, 4594−4598.
(28) Horeau, A. Determination of the Configuration of Secondary Alcohols by Partial Resolution. In *Stereochemistry, Fundamentals and Methods*; Fiaud, J., Horeau, A., Kagan, H. B., Eds.; Georg Thieme: Stuttgart, 1977; Vol. *3*, pp 51−94.
(29) Birman, V. B.; Li, X. Homobenzotetramisole: An Effective Catalyst for Kinetic Resolution of Aryl-Cycloalkanols. *Org. Lett.* **2008**, *10*, 1115−1118.
(30) The HBTM catalyst is commercially available from Sigma-Aldrich (R-HBTM, L511730; S-HBTM, L511862).
(31) Both enantiomers of compounds **1**, **2**, and **3** were synthesized based on the following procedure: Ohtani, T.; Nakatsukasa, H.; Kamezawa, M.; Tachibana, H.; Naoshima, Y. Enantioselectivity of *Candida antarctica* lipase for some synthetic substrates including aliphatic secondary alcohols. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 53−60.
(32) ImageJ spot-density program can be downloaded free of charge at http://rsbweb.nih.gov/ij/ (accessed Mar 2014). There is a tutorial video for the students hosted on the UCIReplay servers. http://replay.uci.edu/media/public/spring2013/How_to_use_ImageJ_-_Flash_%28Large%29_-_20130425_10.12.58PM.html (accessed Mar).
(33) For a computational analysis of the transition state of a similar system, see: Li, X.; Liu, P.; Houk, K. N.; Birman, V. B. Origin of Enantioselectivity in CF_3-PIP-Catalyzed Kinetic Resolution of Secondary Benzylic Alcohols. *J. Am. Chem. Soc.* **2008**, *130*, 13836−13837.
(34) For a detailed kinetic study with the HBTM catalyst, see: Wagner, A. J.; Rychnovsky, S. D. Kinetic Analysis of the HBTM-Catalyzed Esterification of an Enantiopure Secondary Alcohol. *Org. Lett.* **2013**, *15*, 5504−5507.