JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

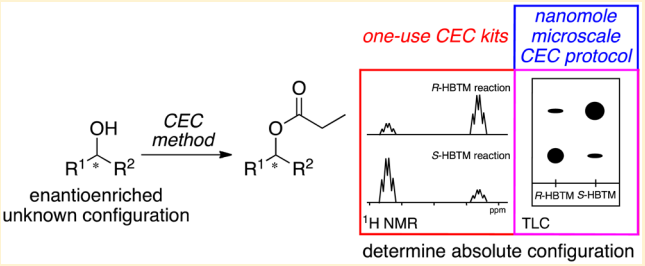
Nanomole-Scale Assignment and One-Use Kits for Determining the Absolute Configuration of Secondary Alcohols

Alexander J. Wagner, Shawn M. Miller,[†] Ryan P. King, and Scott D. Rychnovsky*

Department of Chemistry, University of California-Irvine, 1102 Natural Sciences II, Irvine, California 92697, United States

Supporting Information

ABSTRACT: Two different protocols were developed and optimized to address the need for (1) high sensitivity or (2) convenient utilization in the determination of the absolute configuration of secondary alcohols. The first protocol uses the competing enantioselective conversion (CEC) method to determine configuration on nanomole scale. Reactions were conducted with 145 nmol of the substrate using a 50 μL microsyringe as the reaction vessel, and the absolute configuration was assigned via qualitative determination of the fast reaction by thin-layer chromatography. This protocol resulted in a 50-fold reduction in material required from previous CEC method studies. The approach was evaluated with benzylic and β-aryl systems. The second protocol was optimized to address the needs of practicing medicinal chemists. A one-use CEC kit was developed, where the fast reaction was identified by ^{1}H NMR spectroscopy and thin-layer chromatography. The CEC reaction conditions developed for the microsyringe protocol and the one-use kit both displayed data consistent with pseudo-first-order kinetics in substrate. Therefore, the lower limit of sensitivity for the substrate is limited only by the ability to effectively detect the reaction conversions between alcohol substrate and ester product.



INTRODUCTION

The assignment of absolute configuration of stereogenic centers is a crucial task for the synthetic organic chemist with the continued rapid development of asymmetric strategies for the synthesis of organic molecules.[1] Several approaches have been reported to facilitate this process including the use of chiral derivatization reagents followed by NMR spectroscopy,[2] chiral-shift reagents,[3] the exciton chirality method with electronic circular dichroism,[4] vibrational circular dichroism coupled with density functional theory simulations,[5] specific rotation coupled with the use of Hartree−Fock and density functional theory simulations,[6] X-ray diffraction of single crystals,[7] enantiomeric pairs of molecularly imprinted polymers,[8] and detection of enantiomers of chiral molecules with microwave spectroscopy.[9]

Our laboratory has established the competing enantioselective conversion (CEC) method for the determination of absolute configuration of stereogenic centers. This method is a modern implementation of the Horeau method.[10] The enantioenriched compound of interest is reacted in parallel reactions with each enantiomer of a chiral kinetic resolution reagent over a given period of time. The fast (versus slow) reaction of the pair is discovered by analyzing conversion using one of several characterization methods. The identity of the fast reaction is then compared with an empirical mnemonic in order to assign the absolute configuration of the stereogenic center. The CEC method has been applied to secondary alcohols, oxazolidinones, lactams, thiolactams, and primary amines.[11−13] Characterization techniques used to determine the reaction conversion include ^{1}H NMR spectroscopy, thin-layer chromatography, and electrospray ionization-mass spectrometry (ESI-MS).

The CEC method for secondary alcohols uses the chiral acyl-transfer reagent homobenzotetramisole (HBTM), originally developed by Birman.[14] Propargyl and α-aryl secondary alcohols have previously been analyzed with ^{1}H NMR spectroscopy[11a] and thin-layer chromatography[11b] on micromole scale to assess the relatively fast and slow reactions with a quantitative or qualitative measure of reaction progress. After the fast reaction is determined, the predictive mnemonic for the HBTM system identifies the absolute configuration (Figure 1).[11] The CEC strategy was independently evaluated on secondary alcohols with Fu's planar-chiral DMAP catalysts by Derksen and co-workers.[15]

After the initial CEC protocol was developed, three key goals were investigated to expand the viability of the method for

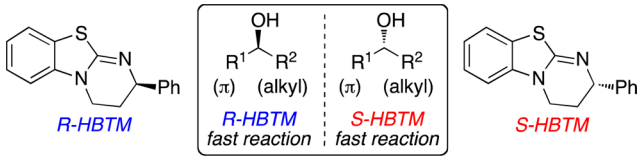


Figure 1. *R*-HBTM and *S*-HBTM acyl-transfer reagents and the mnemonic for determining the absolute configuration of secondary alcohols with the CEC method.

Received: April 12, 2016
Published: July 14, 2016

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

secondary alcohols: (1) reduce the amount of substrate required for the determination, (2) develop alternative CEC protocols based on reactions with pseudoenantiomeric reagents, and (3) expand the substrate scope.

One issue for the CEC method with secondary alcohols was the micromole scale at which the determinations were conducted. In the original report that used ^{1}H NMR spectroscopy, the micromole reactions were chosen as small-scale reactions that still achieved an effective signal-to-noise ratio to accurately assess a quantitative reaction conversion via peak integration.[11a] When thin-layer chromatography was introduced as a characterization technique, the micromole scale utilized was an artifact based on convenience to the user. Micromole scale afforded the possibility of both a qualitative assessment of relative reaction progress between the two reactions and a quantitative analysis of conversion to the ester product via ^{1}H NMR spectroscopy.[11b] However, for precious compounds including isolated natural products or complex intermediates in total synthesis, the use of even less material in the determination could prove beneficial. Our first goal was to develop a CEC method that used very small quantities of the secondary alcohol.

The second goal was to develop a CEC strategy for secondary alcohols based on competing reactions with pseudoenantiomeric reagents. Previous work in our group demonstrated the determination of absolute configuration of primary amines at nanomole scale using electrospray ionization-mass spectrometry as the characterization technique for the enantioselective transformations.[12] We initially investigated a CEC method for secondary alcohols that could be used with the highly sensitive ESI-MS characterization technique.

The third goal was to investigate the application of the CEC protocol utilizing HBTM to a broader substrate scope. Previously, we focused on enantioenriched α-aryl secondary alcohols due to a proposed interaction of the aryl system with the HBTM catalyst in the transition state during the acyl transfer, ultimately producing high enantioselectivity. The β-aryl alcohols might also provide selectivity and were considered good candidates to expand the substrate scope. Additionally, we rationalized that, based on the reported effectiveness of propargyl secondary alcohols, the hybridization at the α-position to the secondary alcohol would result in selectivity with the HBTM catalyst. Therefore, π-systems such as alkene or carbonyl groups might also serve to impart selectivity during the acyl transfer. Expanding the scope of the CEC method and improving its sensitivity would make the method more useful.

RESULTS AND DISCUSSION

Initial Studies with HBTM Salts. With a reduction in the scale of the analysis in mind, we first sought to determine the viability of using an ESI-MS protocol with the HBTM system. Analogous to the previously reported CEC method for primary amines utilizing stoichiometric acylation reagents, each enantiomer of HBTM could be converted to the corresponding acyl salt. The key difference between enantiomers would be the mass of the acyl group transferred from each HBTM reagent. By using different masses, two separate pathways for acylation could be monitored by ESI-MS analysis of the acylate products (Figure 2). The two different acyl groups selected were propionyl-d_5 with *R*-HBTM (**1**) and propionyl-H_5 with *S*-HBTM (**2**). These salts, with the same generic counterion X, would result in two esters formed with a mass difference of five units. The concept is illustrated in Figure 2, where a generic

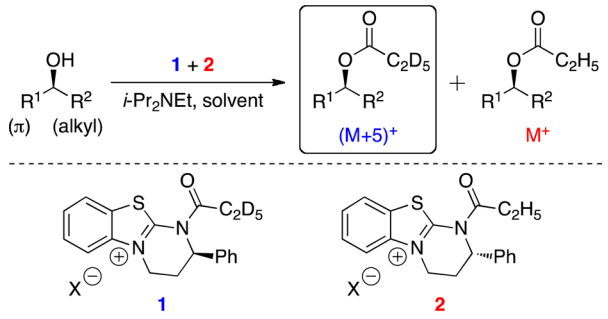
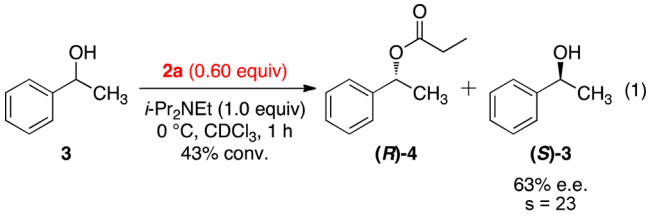


Figure 2. Proposed CEC method using D_5 and H_5 enantioselective acyl-transfer reagents with electrospray ionization-mass spectroscopy as the characterization technique.

enantioenriched alcohol is reacted with an equal amount of **1** and **2** in excess that leads to the formation of both esters. In accordance with the predictive mnemonic (Figure 1), one would expect that *R*-HBTM salt **1** would be the faster reacting of the two salts, and the resulting product should favor the $(M + 5)^+$ ester. The potential advantages of this method are the very high sensitivity of the ESI-MS analysis and that it requires only a single reaction.

The synthesis of a salt of an analog of HBTM (CF_3–PIP) was previously reported by Birman using acetyl chloride and a sodium salt of the counterion (SbF_6^-).[16] We used the same protocol to prepare propionyl salts (from propionyl chloride) of HBTM.[17] The HBTM salt with the NO_3^- counterion (**2a**) provided efficient and effective kinetic resolution of 1-phenylethanol **3** (eq 1).[18]



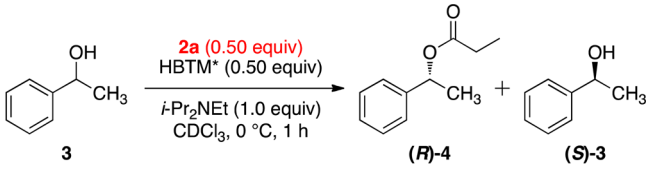
A number of other salts with different counterions were tested. With nonbasic counterions like SbF_6^- (**2b**), the reaction became much slower and the selectivity also declined. It is clear that the counterion in the original catalyzed reaction, $EtCO_2^-$, leads to a much faster reaction than any of the isolatable, less basic salts.[19] The stability of the salts was improved with nonbasic counterions. The NO_3^- salt **2a** was a compromise that gave reasonable reactivity and stability.

The next step was to analyze whether exchange of the propionyl group was possible between **1** and **2**. In the proposed ESI-MS protocol, salts **1** and **2** are in the same solution during the reaction. If propionyl exchange does occur, the propionyl-H_5 and propionyl-d_5 groups would scramble between each enantiomer of HBTM. Such an exchange would render the proposed protocol useless because the analysis is based on the $[M]^+/[M + 5]^+$ ratio with an assumption that each propionyl group comes exclusively from the corresponding HBTM salt. Therefore, **2a** was tested again via two kinetic resolutions of **3** (Table 1).

The test for propionyl exchange was conducted by adding "spectator" HBTM to an acylation using salt **2a**. An equimolar portion of the HBTM enantiomer was included in each reaction. In entry 1, the added HBTM matched the enantiomer of HBTM in salt **2a**. This resolution resulted in a conversion of

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253–6265

Table 1. Kinetic Resolution of Alcohol 3 via Enantioselective Acyl-Transfer of Salt 2a



entry	HBTM added	conversion[a] (%)	ee 3[b] (%)	s
1	S	34	42	16
2	R	34	6	1.4

[a]Conversion was determined via ^{1}H NMR spectroscopy. [b]Enantiomeric excess was calculated via analysis of chiral HPLC traces of recovered **3** on a Chiralpak AD column.

34% over 1 h with a selectivity factor of 16. When the other enantiomer, *R*-HBTM, was added with salt **2a** (entry 2), the reaction proceeded to the same reaction conversion of 34% over 1 h. However, the enantiomeric excess of the recovered starting material fell to 6% with a corresponding drop of the selectivity factor from 16 to 1.4. This experiment demonstrates that the exchange does indeed occur between the neutral HBTM reagent and the acylated-HBTM salt. The observed acyl exchange doomed the proposed ESI-MS strategy and led us to abandon it. We turned our focus to the catalytic CEC method to achieve our goals.

Microsyringe Nanomole-Scale Protocol. Among the analysis techniques previously utilized in our group, adapting the thin-layer chromatography protocol to nanomole-scale reactions appeared most promising. The original CEC protocol with TLC involves two parts: (1) the reactions in small vials on micromole scale followed by quenching and (2) the TLC analysis based on a small aliquot removed from each reaction solution. We previously reported that the TLC analysis displayed nanomole sensitivity. The quantity of substrate used in the CEC method, however, is dictated by the reaction protocol. Therefore, while nanomole sensitivity was displayed with the characterization technique, micromoles of substrate were used in the reaction component of the CEC method. The sensitivity of the CEC method would be dramatically increased if the reaction protocol were revised to only deliver enough material for TLC analysis.

To achieve similar reaction progress, the volume of the reaction would be reduced while maintaining similar reagent concentrations. We envisioned running reactions using a 50 μL microsyringe as the reaction vessel (Figure 3). Within the microsyringe, three separate stock solutions of equal volume would be drawn with an air bubble between each solution to prevent mixing while the solutions were measured. Then, the solutions would be mixed in a 600 μL glass vial and drawn back up into the microsyringe for the duration of the reaction. Dispensing the reaction solution into a vial containing methanol would quench the reaction and provide the final solution for TLC analysis. The outcome would be a significant reduction in the excess substrate used in the reactions.

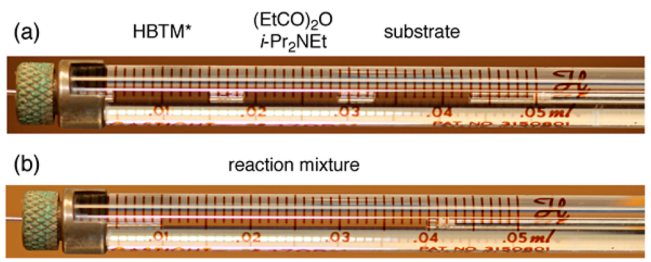


Figure 3. Nanomole-scale CEC reactions in microsyringes: (a) respective stock solutions drawn for use in one of two reactions in the CEC protocol; (b) the reaction mixture during the course of the reaction. The solutions are colorless but refract the background color.

Using microsyringes as the reaction vessels, several substrate loadings of alcohol (*R*)-**5** were analyzed with varying concentrations of HBTM, propionic anhydride, and *N*,*N*-diisopropylethylamine in order to gain an understanding of the reaction progress via qualitative analysis (Figure 4). Entry 1

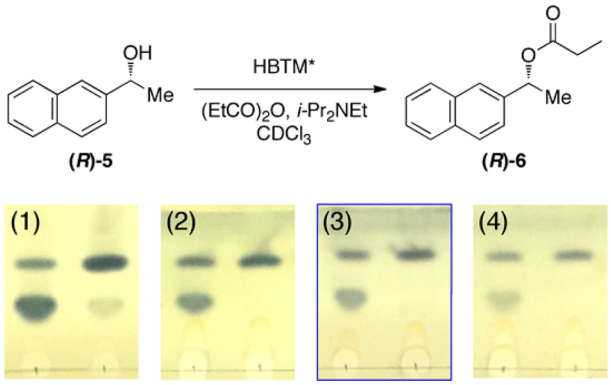


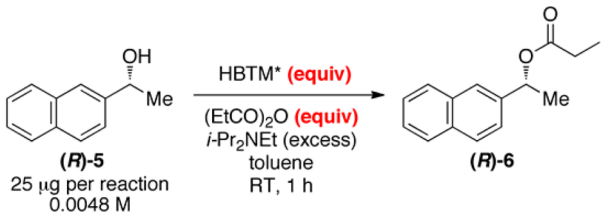
Figure 4. Initial investigations of reduced substrate loading of sample alcohol (*R*)-**5** with the CEC method and TLC. Substrate loadings per reaction were (1) 3000, (2) 290, (3) 145, and (4) 29 nmol of (*R*)-**5**. Left TLC lane, *R*-HBTM; right TLC lane, *S*-HBTM. Plates were eluted in 30% ethyl acetate in hexanes. Visualization was achieved by staining with PMA stain.

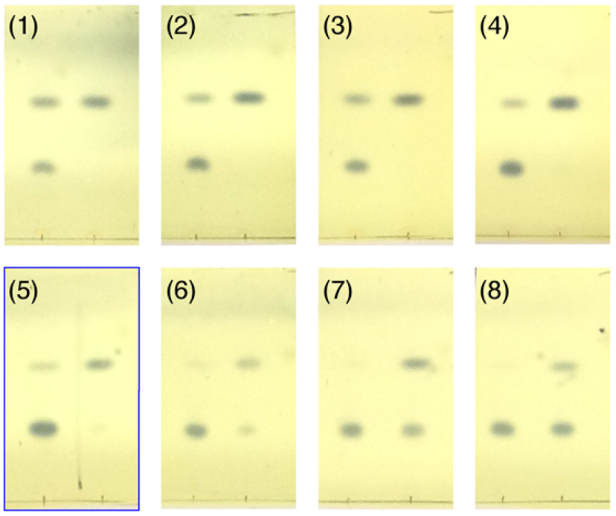
used the concentrations in a previously reported undergraduate laboratory experiment using the CEC method, because these concentrations were designed to minimize reagent costs without degrading the resultant TLC data, but reduced the volumes to only 10 μL of each of the stock solutions.[11c] As in the undergraduate experiment, toluene was used as the reaction solvent in order to reduce concentration changes due to evaporation during the reaction. Entry 1 conditions used 3000 nmol of substrate per reaction and showed clear differentiation of reaction progress for the lane containing *S*-HBTM on the right of the plate. A new set of stock solutions was prepared to enable approximately a 10-fold reduction of the concentration of (*R*)-**5**. Because of our previous findings of first order with respect to the catalyst, anhydride, and substrate in the kinetic investigations of the HBTM-mediated esterification of an enantiopure alcohol,[20] it seemed logical to offset the 10-fold reduction in substrate with an increase in the concentration of catalyst and anhydride. The result in entry 2, with 290 nmol of substrate in each reaction and a total additive volume of 30 μL, is a similar qualitative outcome with *S*-HBTM clearly designated as the fast reaction lane. Reduction in substrate loading to 145 nmol (entry 3) and 29 nmol (entry 4) resulted in a similar qualitative output with the *S*-HBTM lane proceeding as the fast lane. However, in entry 4, the spot densities appear significantly less distinctive. When considering the application of a general strategy, entry 3 was selected as a reliable substrate loading around which to build the CEC microscale system.

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

After selecting 145 nmol per reaction as the target substrate loading, a screen of equivalents of HBTM (0.10−1.0) and propionic anhydride (5.0−30) was conducted with the goal of identifying the best qualitative differentiation of reaction progress between HBTM reactions after a reaction time of 1 h (Table 2). All reaction volumes were 30 μL, and reaction progress was halted with 10 μL of methanol. TLC analysis was conducted with a 4 μL portion from each reaction. The 30 μL additive volume, composed of 10 μL portions from each stock solution was envisioned to minimize volume addition error by keeping a consistent volume value.[21]

Table 2. Screen of Equivalents of HBTM and Propionic Anhydride for Substrate (*R*)-5 at a Substrate Loading of 145 nmol per Reaction[a]





entry	$(EtCO)_2O$ (equiv)	HBTM* (equiv)	entry	$(EtCO)_2O$ (equiv)	HBTM* (equiv)
1	30	1.0	**5**	**15**	**0.20**
2	30	0.50	6	15	0.10
3	15	1.0	7	5.0	0.20
4	15	0.50	8	5.0	0.10

[a]Left TLC lane, *R*-HBTM; right TLC lane, *S*-HBTM. Plates were eluted in 30% ethyl acetate in hexanes. Visualization was achieved by staining with PMA stain. Optimal conditions are highlighted in bold.

Using 30 equiv of propionic anhydride, there was no substrate remaining in the lane with *S*-HBTM with both 1.0 equiv of HBTM (entry 1) and 0.50 equiv of HBTM (entry 2). With 15 equiv of propionic anhydride, there was again no substrate remaining in the lane with *S*-HBTM with both 1.0 equiv of HBTM (entry 3) and 0.50 equiv of HBTM (entry 4). The plates in entries 1−4 look nearly identical from a qualitative assessment of spot density, which is interesting with respect to the slow reaction lane given the significant change in the amount of anhydride and HBTM present. With 15 equiv of propionic anhydride and 0.20 equiv of HBTM (entry 5), a change in reaction conversion is apparent, as the spot density of (*R*)-**6** in the *R*-HBTM lane has decreased and there is now (*R*)-**5** present in the *S*-HBTM lane. As the equivalents of HBTM drop to 0.10 equiv (entry 6), the difference in spot density becomes less apparent in the *S*-HBTM lane. With 5 equiv of propionic anhydride, there was minimal product in the lane with *R*-HBTM with both 0.20 equiv of HBTM (entry 7) and 0.10 equiv of HBTM (entry 8). The eight entries offer a spectrum of reaction conversions of (*R*)-**5** to (*R*)-**6** for both HBTM reactions for the same time period. Of these options, entry 5 was selected as the ideal conditions because of the greatest contrast in spot density between the two reaction lanes.

After optimization of reaction conditions for substrates at 145 nmol and an additive concentration of 0.0048 M, a series of related structures were analyzed with the new microscale protocol after a reaction time of 1 h (Table 3).

Enantiomeric pairs in entries 1−2 and 3−4 showed opposite selectivity for the HBTM catalysts. In all four cases, there was a clear difference in reaction rate resulting in effective qualitative assessment of reaction conversion and identification of the fast reaction to assign configuration in accordance with the previously established mnemonic. The addition of a methoxy substituent also resulted in a clear difference in reaction conversion with the *S*-HBTM reaction, allowing for the assignment of the alcohol as back in the plane of the page (*R*).

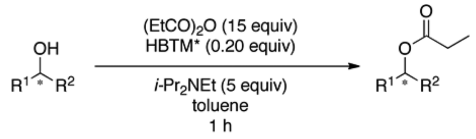
The most unusual of the aryl compounds considered was an intermediate in the synthesis of the anticancer drug Xalcori (crizotinib) by Pfizer. Crizotinib has been shown to be effective in the inhibition of mesenchymal-epithelial transition factor kinase. However, an 80-fold difference in potency was observed between each enantiomer (Figure 5).[22] Crizotinib gained FDA approval for treatment of non-small-cell lung cancers that express the anaplastic lymphoma kinase (ALK) after also showing effective inhibition of ALK. The intermediate in the synthesis of the enantiomer of crizotinib was analyzed with the microscale protocol (entry 6) and showed a clear preference in qualitative assessment of spot density for the *R*-HBTM reaction, which according the mnemonic indeed assigns the configuration as forward (*S*).

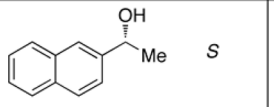
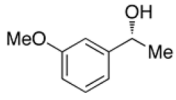
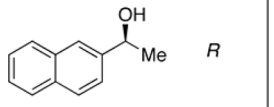
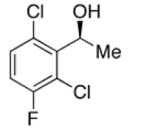
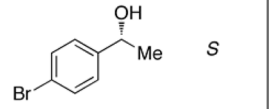
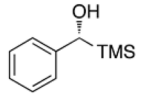
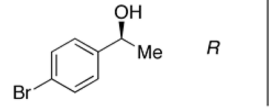
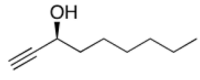
A new class of compounds was analyzed in entry 7, as α-(trimethylsilyl)benzyl alcohol also reacted in accordance with the mnemonic and the absolute configuration assignment correlated with the reported stereochemistry. Finally, entry 8 displayed the success of propargyl alcohols in the microscale system as well.

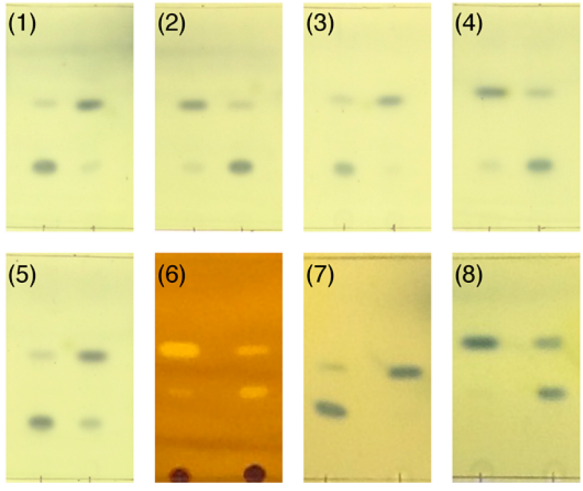
In an effort to continue to expand the substrate scope, a series of β-aryl secondary alcohols were also examined with the new microscale conditions (Table 4).[23] An initial screen revealed that the same reaction conditions could be utilized, but the reactions required 2 h before quenching and analysis. Of the five β-aryl alcohols containing only a phenyl ring (entries 1−3, 5, 8), four cases (entries 2, 3, 5, 8) revealed a positive assessment of the fast reaction via qualitative analysis. In all four cases, the fast reaction with *R*-HBTM matched the predictive mnemonic when π is considered the β-aryl system. Selectivity was not an issue even in diastereomeric entries 2 and 3, providing supporting evidence that the β-aryl system is indeed involved in recognition with the HBTM catalyst during acyl-transfer. The aromatic system in entry 5 was effectively recognized despite a bulky silyl protecting group on the primary alcohol adjacent to the secondary alcohol. Entry 8, the opposite stereochemistry from entries 1−7 for the alcohol being studied, revealed the fast reaction with *S*-HBTM in accordance with the

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

Table 3. Microsyringe CEC Method with Benzylic and Propargyl Secondary Alcohols



entry[a]		fast HBTM reaction	entry[a]		fast HBTM reaction
1		S	5		S
2		R	6		R
3		S	7		S
4		R	8		R



[a]Reactions were conducted with 145 nmol of substrate at an additive volume of 30 μL (0.0048 M) and halted with 10 μL of methanol. The fast reaction was determined by qualitative assessment of reaction conversion via TLC. Left TLC lane, *R*-HBTM; right TLC lane, *S*-HBTM. TLC solvent systems and stains for each entry are described in the Supporting Information.

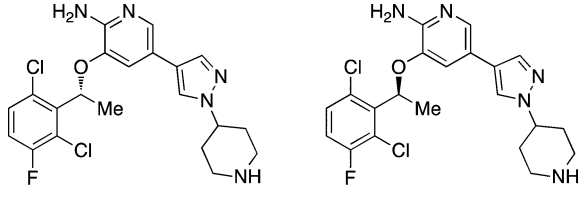
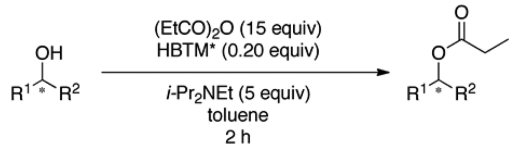
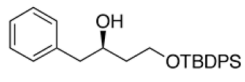
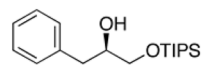
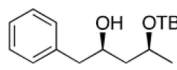
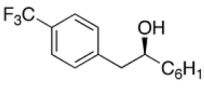
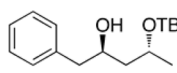
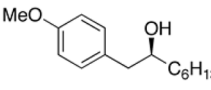
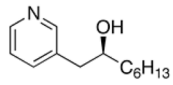
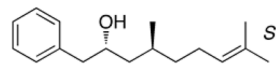


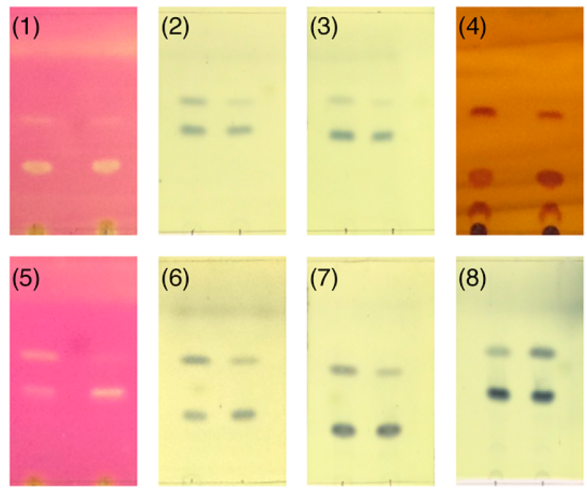
Figure 5. Crizotinib and the enantiomer of crizotinib with K_i values for c-MET kinase.

predictive mnemonic for assignment of absolute configuration. Additionally, entry 8 contained a nonheteroatomic stereogenic center and a long alkyl chain.

Table 4. Microsyringe CEC Method with β-Aryl Secondary Alcohols



entry[a]		fast HBTM reaction	entry[a]		fast HBTM reaction
1		–	5		R
2		R	6		R
3		R	7		R
4		R	8		S



[a]Reactions were conducted with 145 nmol of substrate at an additive volume of 30 μL (0.0048 M) and halted with 10 μL of methanol. The fast reaction was determined by qualitative assessment of reaction conversion via TLC. Left TLC lane, *R*-HBTM; right TLC lane, *S*-HBTM. TLC solvent systems and stains for each entry are described in the Supporting Information.

As previously seen in benzylic systems, electron-deficient (entry 6) and electron-rich (entry 7) β-aryl aromatic systems worked effectively with the HBTM CEC protocol, with the *R*-HBTM reaction qualitatively assigned as the fast reaction. Finally, a β-aryl system with pyridine was tested (entry 4). The assigned fast reaction behaved in the same fashion as the aforementioned systems, with *R*-HBTM as the fast reaction. All entries where a qualitative analysis of a fast reaction was made aligned with the previously established mnemonic for assignment of absolute configuration.

The developed microscale protocol represents a 50-fold reduction in substrate required for analysis of the CEC method via TLC. For a compound with a molecular mass between 100 and 500, this protocol uses between 29 and 145 μg accordingly

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

for the complete analysis. The microscale technique is appropriate for isolated natural products or other situations where the unknown alcohol is very precious.

Development of a One-Use CEC Kit. The previous section described a refined microscale protocol and an expanded substrate scope. This success led to a new goal: development of a commercially viable CEC kit. Initially, a set of three solutions (*R*-HBTM, *S*-HBTM, and a mixture of propionic anhydride and *N*-*N*-diisopropylethylamine) in toluene was proposed. The proposed target market was industrial research and development programs and academic research groups. A series of discussions and testing was initiated with Dr. Ryan Patman of Pfizer in an effort to better understand the potential of the CEC kit from chemists with industrial experience.

One of the first concerns about the kit was the solvent choice. While toluene was ideal for small volume measurements without significant volume loss due to evaporation, its dielectric constant and resultant poor compatibility with polar organic compounds greatly limited its utility in a medicinal chemistry setting. Another issue raised was the desirability of collecting both qualitative and quantitative data for the analysis. When industrial chemists were asked, the requirement for micromoles of material was not considered a problem. More important was the desire to record *quantitative* data for reaction conversion. A one-use kit was particularly convenient for the user and was preferred by the medicinal chemists polled.

The proposal for a commercially viable CEC kit was revised based on this feedback. The substrate loading in each reaction was adjusted to 8 μmol to accommodate ^{1}H NMR spectroscopic analysis. The total volume of the system was established at 550 μL with a 50 μL methanol quench, providing a slight excess of the volume required for standard NMR spectroscopy tubes. The solvent was switched from toluene to $CDCl_3$ to accommodate direct NMR analysis and solubility for a broader spectrum of organic compounds.

For convenience, the substrate would be added in a 100 μL portion. The protocol was optimized to use 20 μmol of substrate (5.0 mg for a substrate with a molecular mass of 250, for example) and 250 μL of $CDCl_3$ to ensure that two equal 100 μL portions could be withdrawn and added to each CEC kit vial.

The second problem to be resolved was the equivalents and concentration of CEC kit reagents. The optimized microscale protocol for secondary alcohols (0.2 equiv of HBTM, 15 equiv of propionic anhydride, 5 equiv of *N*,*N*-diisopropylethylamine) were applied to the new CEC kit parameters (8 μmol substrate and total additive volume of 550 μL) and the reaction conversion of alcohol **(*R*)-5** to propionate ester **(*R*)-6** via the CEC method was studied. The CEC kit represented a 3-fold increase in substrate concentration relative to the microscale protocol, so reactions were halted after 30 min instead of 1 h. Quantitative analysis by ^{1}H NMR spectroscopy revealed conversions for *R*-HBTM and *S*-HBTM of 15% and 98%, in alignment with the predictive mnemonic for assignment of absolute configuration. Quantitative analysis by GC-MS lead to nearly identical reaction conversions compared with the ^{1}H NMR spectroscopic data.[24] These concentrations were considered appropriate for the desired substrate loading of 8 μmol at an initial substrate concentration of 14 mM in the CEC kit.

Based on prior kinetic analysis,[20] the reaction system was expected to be pseudo-first-order in substrate. This expectation was tested by varying the substrate loading to give initial concentrations between 1.4 and 29 mM while maintaining the same CEC kit concentrations of HBTM, propionic anhydride, and *N*,*N*-diisopropylethylamine (Figure 6). If the hypothesis

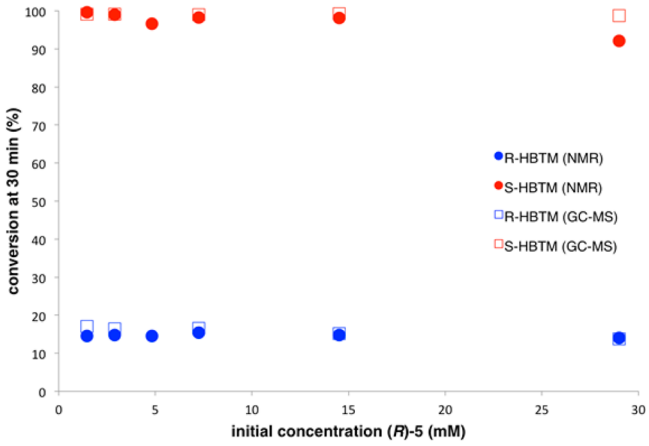


Figure 6. Alcohol **(*R*)-5** was solvated in $CDCl_3$ and then 100 μL was dispensed into each $CDCl_3$ CEC kit vial containing 450 μL of a solution of propionic anhydride, *N*,*N*-diisopropylethylamine, and HBTM. Conversion was determined via ^{1}H NMR spectroscopy and GC-MS analysis.

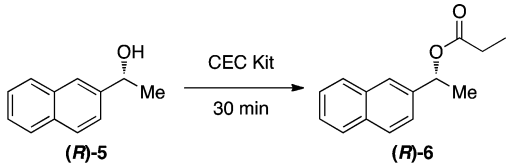
were correct, similar reaction conversions would be observed for both HBTM reactions over the span of the substrate concentrations. If incorrect, a change in the substrate concentration would result in a change in reaction conversion. After quantitative analysis of reaction conversion for the *R*-HBTM and *S*-HBTM reactions by both ^{1}H NMR spectroscopy and GC-MS, a plot of reaction conversion compared with initial substrate concentration over this 20-fold range revealed no significant change in reaction conversion for both the *R*-HBTM and *S*-HBTM reactions.[25] Thus, the reaction was confirmed to be pseudo-first-order in the secondary alcohol. These reaction conditions offer a significant advantage to the user compared with previous CEC protocols. The CEC kit will theoretically work with any quantity of substrate less than the recommended 20 μmol as long as the analytical method is capable of measuring the substrate conversion. The microscale protocol is more convenient for most situations with limited sample quantities, but the sensitivity of either procedure is limited only by the analytical method.

During testing, another issue raised was the possibility of developing CEC kits in solvents with higher dielectric constants in order to solvate increasingly polar pharmaceutical intermediates that displayed limited or no solubility in $CDCl_3$. CEC kits in THF-d_8, acetone-d_6, MeCN-d_3, DMF-d_7, and DMSO-d_6 were considered. THF-d_8, MeCN-d_3, and DMF-d_7 were ruled out because of the substantial cost associated with large volumes of these deuterated solvents. Acetone-d_6 and DMSO-d_6 were also ruled out because of the potential for water adsorption and possible side reactions occurring with the solvents in the CEC kit solutions when stored over long periods of time. In recognition of these issues, solvation of polar substrates in one of the aforementioned solvents followed by injection to a one-use CEC kit in $CDCl_3$ was considered (Table 5).

A general trend was observed using solvent mixtures created from injection of the substrate in different solvents to the CEC

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

Table 5. Testing Substrate-Loading Solvent Compatibility with $CDCl_3$ CEC Kit and Alcohol (*R*)-5



(*R*)-5 (*R*)-6

entry[a]	substrate loading solvent	conversion *R*-HBTM[b] (%)	conversion *S*-HBTM[b] (%)	fast reaction by TLC
1	toluene-d_8	18	**99**	*S*
2	$CDCl_3$	14	**98**	*S*
3	THF-d_8	11	**93**	*S*
4	acetone-d_6	14	**94**	*S*
5	MeCN-d_3	15	**89**	*S*
6	DMF-d_7	8	**83**	*S*
7	DMSO-d_6	8	**67**	*S*

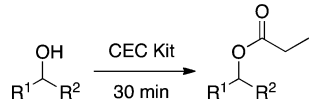
[a]Alcohol (*R*)-5 (20 μmol) was solvated in 250 μL of the loading solvent and then 100 μL was dispensed into each $CDCl_3$ CEC kit vial containing 450 μL of a solution of propionic anhydride, *N*,*N*-diisopropylethylamine, and HBTM. [b]Conversion was determined via ^{1}H NMR spectroscopy.

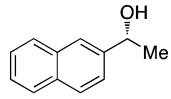
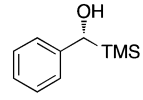
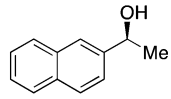
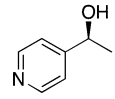
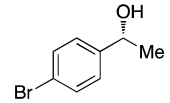
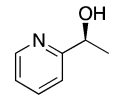
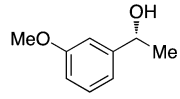
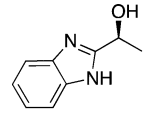
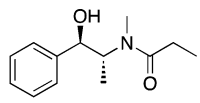
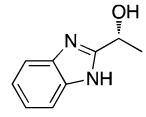
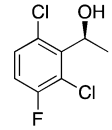
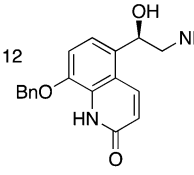
kit in $CDCl_3$. When loading substrates in toluene-d_8 (entry 1), $CDCl_3$ (entry 2), THF-d_8 (entry 3), and acetone-d_6 (entry 4), a range of conversion with *R*-HBTM was observed in 11−18% and a range of conversion with *S*-HBTM was observed in 93−99%. With DMF-d_7 (entry 6) as the loading solvent, a noticeable decrease in conversion with *R*-HBTM and *S*-HBTM of 8% and 83% was observed. For the highest dielectric constant tested, DMSO-d_6 (entry 7), the reduced conversion was more pronounced. Overall, with an increase in the dielectric constant of the substrate solvent, there was a general decrease in reaction conversion over the same 30 min time period.[26] However, a clear difference in reaction conversion was observed both qualitatively and quantitatively with the *S*-HBTM reaction proceeding as the fast reaction.[27] Therefore, for most substrates, the use of a standard CEC kit with $CDCl_3$, combined with an appropriate substrate solvent, should be satisfactory.

To confirm the validity of the kit with additional substrates, a series of benzyl and α-aryl secondary alcohols were examined with both a qualitative analysis of the fast reaction by TLC and a quantitative analysis of reaction conversion by ^{1}H NMR spectroscopy (Table 6).[28] Entries 1−7, previously tested in the microscale protocol, all proceeded effectively with the new CEC kit system. Qualitative and quantitative determinations of the fast reaction as *R*-HBTM (entries 2, 5, 6) and *S*-HBTM (entries 1, 3, 4, 7) to give configuration assignments in alignment with the predictive mnemonic were made. With the capability to assess reaction conversion quantitatively, entries 1−4 and 7 all progress with comparable reaction conversion (slow HBTM reaction, 10−26%; fast HBTM reaction, 92−98%) and therefore selectivity in the protocol. Entry 5 achieves a slightly lower reaction conversion for the fast reaction (76%) and for the slow reaction (7%). The fast reaction in sterically hindered entry 6, the intermediate for the enantiomer of crizotinib, proceeds with the lowest conversion of this group of compounds (48%), but is still operating at high selectivity, with the slow reaction proceeding to only 8% conversion.

α-Arylpyridines (entries 8−9) and benzimidizoles (entries 10−11) also proved amenable to quantitative determination of the fast reaction and subsequent alignment with the predictive

Table 6. CEC Kit Tested with Benzyl and α-Aryl Secondary Alcohols



entry[a]	HBTM* reaction conversion (%)[b]	entry[a]	HBTM* reaction conversion (%)[b]
1	 *R: 14* **S: 98**	7	 *R: 10* **S: 98**
2	 **R: 95** *S: 16*	8[c]	 **R: 78** *S: 35*
3	 *R: 26* **S: 92**	9[d]	 **R: 53** *S: 25*
4	 *R: 12* **S: 92**	10[e]	 *R: 97* *S: 53*
5	 **R: 76** *S: 7*	11[e]	 *R: 56* *S: 97*
6	 **R: 48** *S: 8*	12	 **R: 95** *S: 37*

[a]TLC images, solvent systems, and stains for each entry are included in the Supporting Information. The TLC fast reaction was determined by qualitative assessment of reaction conversion. [b]Conversion was determined via ^{1}H NMR spectroscopy. Bolded reaction conversions represent entries where qualitative assessments of the fast reaction by TLC were successful. [c]The substrate was loaded in DMSO-d_6, and the kit ran for 1 h. [d]The kit ran for 1 h. [e]The substrate was loaded in DMSO-d_6, and the kit ran for 10 min.

mnemonic. Entries 8 and 9 were also capable of qualitative assessment of the fast reaction by TLC. The difference in reaction conversion determined quantitatively by ^{1}H NMR for entries 10 and 11 was unable to be qualitatively visualized by TLC despite applying several different staining procedures. Lastly, entry 12 was considered with the CEC kit. Entry 12 is a key intermediate in the synthesis of batefenterol (TD-5959, GSK961081; Figure 7): a multivalent muscarinic antagonist and β_2-agonist (MABA) bronchodilator for treatment of moderate to severe chronic obstructive pulmonary disease (COPD).[29]

Entry 12 presented an interesting challenge because of the secondary amine also present in the molecule. We envisioned rapid acylation of the secondary amine, followed by the key esterification of the chiral secondary alcohol (Figure 8). After treatment in the CEC kit, loss of starting material was indicated by ^{1}H NMR spectroscopic analysis and TLC. Independent preparation of the amide through amidation of entry 12 confirmed the formation of the amide first.[30] Then, conversion of the newly formed amide to the propionate ester was measured to assess quantitative reaction conversion. Both qualitative and quantitative methods confirmed a faster reaction

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

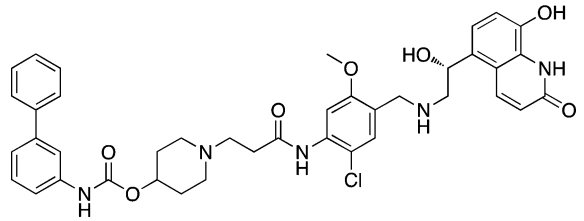


Figure 7. Batefenterol MABA bronchodilator containing structure of entry 12 (Table 6).

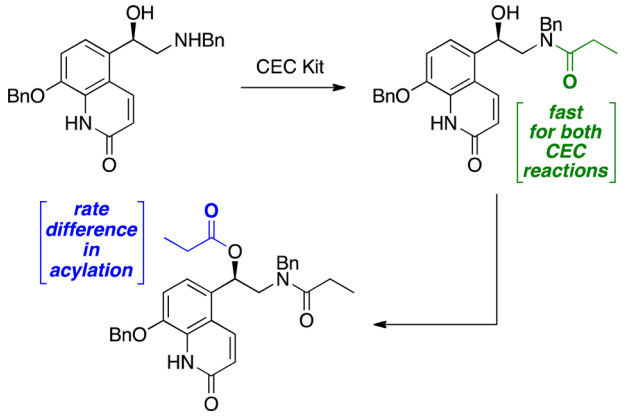


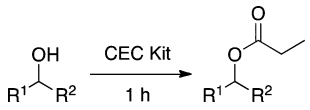
Figure 8. Proposed reactivity of entry 12 under CEC kit conditions.

with *R*-HBTM, indicating the stereocenter as forward (*R*) in accordance with the mnemonic and in alignment with the reported stereochemistry of the compound.

After testing a series of interesting benzyl and α-aryl secondary alcohols, we sought to analyze the utility of the CEC kit with additional substrate classes (Table 7).[28] The propargylic alcohol in entry 1 displayed the fastest reactivity observed for the CEC kit of this series, with the fast reaction reaching 99% conversion and the slow reaction at 63% after 30 min. To verify that the rate of reaction was the cause for the unusually high mismatched reaction conversion, the same compound was exposed to CEC kit conditions again (entry 2). In this second attempt, reaction progress was halted at 10 min. The fast reaction conversion was 93% and the slow reaction dropped to 39%. Next, an allylic alcohol was tested (entry 3). The reaction progress over 30 min proceeded to a lower reaction conversion compared with previous substrates and therefore was run for 1 h. A noticeable difference in reaction conversion was observed qualitatively and quantitatively, in alignment with the mnemonic for assignment of reaction conversion if the alkene is the π-group. Previously tested β-hydroxyesters proceed without selectivity between the HBTM reactions.[11a] Therefore, the inclusion of the allylic system appears to be the primary contributor to the observed reaction conversion difference. After discovering a substrate displaying selectivity with a simple π system directly connected to the stereocenter of study, α-hydroxyester substrates were considered. Methyl- and ethyl-lactate (entries 4 and 5) displayed a significant difference in reaction rate between the HBTM reactions culminating in a difference in reaction conversion after 10 min in alignment with the mnemonic for assignment of reaction conversion if the carbonyl group is applied in the mnemonic as π. Appending a benzyl group to the ester (entry 6) resulted in a similar outcome but with a decrease in reaction rate. Additionally, enantiomeric α-hydroxyamides were studied (entries 7 and 8). These compounds reacted significantly slower, achieving a reaction conversion of 35% for the fast reaction over 1 h. However, the slow reaction in both cases proceeded to far lower conversion, with reaction conversions of 7% and 3%. Again, if the carbonyl is assigned as π, the observed fast reaction aligns with the previously established mnemonic. Finally, four β-aryl secondary alcohols from the microscale studies were also tested (entries 9–12) with the CEC kit over 1 h and concluded with the *R*-HBTM reaction as the fast reaction according to both qualitative and quantitative analysis. This data also aligns with the conclusions drawn from the microscale studies for all four compounds. The conversion difference between fast and slow reactions was smallest in entry 9, with a β-pyridine group, but still produced a difference of 17% conversion. The studied substrates expand the secondary alcohols applicable to the CEC method with HBTM and the established mnemonic for assignment of absolute configuration.

One of the final questions about the CEC kit was the stability of the ingredients in $CDCl_3$ over time. In order to test this stability, a batch of kits was freshly prepared. The CEC kit was tested with (*R*)-**5** the same day. The kits were then stored in a −20 °C freezer. After six months, a kit from the same batch was removed, warmed to room temperature, and tested with (*R*)-**5**

Table 7. CEC Kit Tested with Propargylic, Allylic, α-Hydroxyester, α-Hydroxyamide, and β-Aryl Secondary Alcohols



entry[a]	HBTM* reaction conversion (%)[b]	entry[a]	HBTM* reaction conversion (%)[b]
1[c]	***R*: 99** *S*: 63	7	*R*: 35 *S*: 7
2[d]	***R*: 93** *S*: 39	8	*R*: 3 *S*: 35
3	*R*: 12 ***S*: 29**	9	***R*: 48** *S*: 31
4[d]	***R*: 94** *S*: 39	10	***R*: 34** *S*: 7
5[d]	***R*: 79** *S*: 35	11	***R*: 55** *S*: 24
6[c]	***R*: 94** *S*: 41	12	***R*: 25** *S*: 10

[a]The TLC fast reaction was determined by qualitative assessment of reaction conversion. TLC images, solvent systems, and stains for each entry are described in the Supporting Information. [b]Conversion was determined via ^{1}H NMR spectroscopy. Bolded reaction conversions represent entries where qualitative assessments of the fast reaction by TLC were successful. [c]The kit ran for 30 min. [d]The kit ran for 10 min.

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253–6265

(Figure 9). Comparison of the TLC plates qualitatively showed identical performance in each reaction lane and the assignment

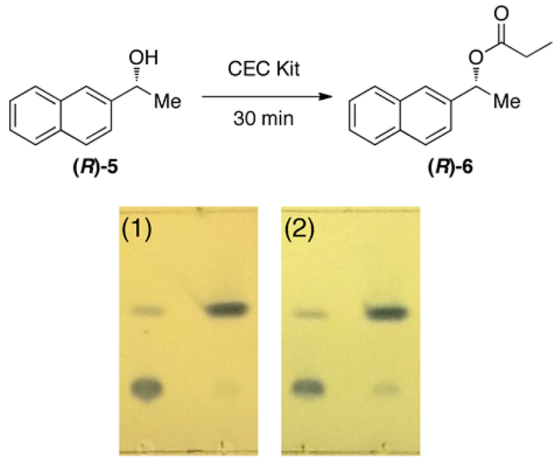


Figure 9. TLC plate images of CEC kits with (*R*)-**5** (1) the same day of CEC kit preparation and (2) after six months of storage in a freezer. Left TLC lane, *R*-HBTM; right TLC lane, *S*-HBTM. Plates were eluted in 30% ethyl acetate in hexanes. Visualization was achieved by staining with PMA stain. Reaction conversion via ^{1}H NMR spectroscopic analysis (%): (1) *R*-HBTM = 15, *S*-HBTM = 98; (2) *R*-HBTM = 16, *S*-HBTM = 96.

of the fast reaction was clearly made as the *S*-HBTM reaction. ^{1}H NMR spectroscopic data also confirmed this conclusion, with nearly identical reaction conversions between the *R*-HBTM and *S*-HBTM reactions for plate 1 and plate 2. Therefore, the CEC kit solutions appear stable when stored in a freezer over a six month period and display no noticeable degradation in performance.

After vetting the CEC kit system, we were still intrigued by the conclusions of Figure 6 and chose to investigate the influence of substrate alcohol concentration in the microscale procedure. Between the two processes, there was a 3-fold reduction in the concentration of HBTM, anhydride, and base in the microscale protocol. If the reaction shows saturation kinetics with respect to the anhydride, then the pseudo-first-order rate *constant* would be proportional to the HBTM concentration, and thus the observed rate *constant* would be about a factor of 3 smaller for the microscale procedure. A serial dilution of alcohol (*R*)-**5** was conducted to produce stock solutions varying between 7.26 and 0.907 mM, which resulted in a range of 72 to 9 nmol of substrate per reaction (Figure 10). Because of the scale and the poor detection of (*R*)-**5** at these concentrations via GC-MS, only qualitative data was accessed by TLC. However, the images of plates corresponding to entries 1−4 display comparable reaction conversion for all reaction lanes. This analysis supports the expectation that the microscale protocol displays pseudo-first-order kinetic behavior with the alcohol substrate. The limit of detection for effective recognition of the fast reaction of HBTM was 9 nmol per reaction for entry (*R*)-**5** in the PMA stain. However, the theoretical limit for the use of this protocol is based only on the ability to detect the reaction progress. An example of reduced-scale detection was recently illustrated by Poulter and co-workers using the microscale protocol with autoradiography on silica-TLC plates to determine the absolute configuration of hydroxysqualene synthesized in bacterial biosynthesis.[31]

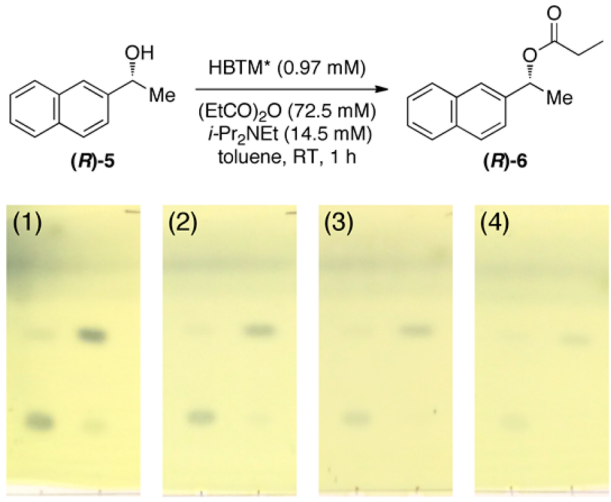


Figure 10. Variation in substrate loading of (*R*)-**5** between (1) 72, (2) 36, (3) 18, and (4) 9 nmol per reaction with microscale protocol concentrations of HBTM and anhydride at an additive reaction volume of 30 μL. Left TLC lane, *R*-HBTM; right TLC lane, *S*-HBTM. Plates were eluted in 30% ethyl acetate in hexanes. Visualization was achieved by staining with PMA stain.

CONCLUSION

A method for determining the absolute configuration of enantioenriched secondary alcohols with the competing enantioselective conversion (CEC) method on nanomole scale was described. Reactions were run with 145 nmol of material and an additive volume of 30 μL in a microsyringe. The absolute configuration was determined via qualitative analysis of the relatively fast reaction by thin-layer chromatography. This method represents over a 50-fold reduction in material required from previous CEC studies.

A one-use CEC kit was developed that would be appropriate for commercialization. The kit was designed for ^{1}H NMR spectroscopy and thin-layer chromatography assays. The CEC kit was shown to be stable over a period of six months and was effective when the substrate of interest was loaded in a variety of solvents with varying dielectric constants. The new CEC reaction conditions under both microscale and kit protocols are first order with respect to the alcohol. The substrate scope was extended to many new classes of secondary alcohols. Alcohol (*R*)-**5** was thoroughly investigated at varied substrate concentrations and produced effective data for recognition of the fast reaction with HBTM over a 889-fold difference in substrate loading per reaction (9−8000 nmol) between the two protocols. The limit of detection of the CEC method with both developed protocols is theoretically limited only by the ability to effectively analytically detect the alcohol substrate and the ester product.

EXPERIMENTAL SECTION

General Methods. All reactions were carried out under an atmosphere of air with anhydrous solvents unless otherwise noted. All glassware was oven-dried prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 298.0 K at 500 MHz. $CDCl_3$ was used as an internal reference for ^{1}H NMR (δ = 7.27) and ^{13}C NMR (δ = 77.16) spectra. Thin-layer chromatography (TLC) was performed on silica gel plates and visualized using various stains that are detailed for specific compounds. Stains were prepared according to literature procedure.[32]

Chemicals. All commercially available reagents and solvents were used without further purification unless otherwise noted. Hexanes,

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

ethyl acetate, toluene, methanol, propionic anhydride, *N*,*N*-diisopropylethylamine, toluene-d_8, $CDCl_3$, THF-d_8, acetone-d_6, MeCN-d_3, DMF-d_7, and DMSO-d_6, and **3** were purchased from various chemical suppliers. (***R***)-**5** was prepared according to literature procedure.[11c] In Table 3, entries 1−5 were synthesized according to literature procedures.[11c] Entry 6 was generously supplied by Pfizer, Inc., through Dr. Ryan Patman of Pfizer La Jolla.[22] Entry 7 was synthesized by Dr. Jennifer Cossrow in the Rychnovsky group.[33] Entry 8 was synthesized by Dr. Matthew Perry in the Rychnovsky group.[34] In Table 4, entries 1−8 were generously supplied by the Morken group.[23] In Table 6, entries 1−4, 6, and 7 were the same as those previously used in Table 3. Entry 5 was prepared by Dr. Angie Kim in the Rychnovsky group.[35] Entries 10 and 11 were prepared by Dr. Shawn Miller.[36] Entry 12 was generously supplied by Dr. Adam Hughes of Theravance Biopharma, Inc.[29] In Table 7, entries 1, 2, and 9−12 were the same as those previously used in Tables 3 and 4. Entry 3 was prepared by Dr. Michael Gesinski in the Rychnovsky group in accordance with literature precedent.[37] Entries 4 and 5 were purchased from Sigma-Aldrich. Entries 7 and 8 were prepared in accordance with literature procedure.[38]

General Preparation for Salts 2a and 2b. To a 25 mL oven-dried round-bottom flask with a magnetic stirbar was added *S*-HBTM (1 equiv). Methylene chloride was added (0.2 M). The reaction solution was cooled to 0 °C (ice bath), and propionyl chloride (1.10 equiv) was added dropwise. The bath was removed, and the reaction solution was allowed to warm to room temperature while stirring over 1 h. Acetonitrile (equal volume to methylene chloride) was added, followed by the sodium salt (1.05 equiv). The reaction mixture was refluxed for 3 h. The solution was filtered and concentrated under reduced pressure to give a solid. The crude salt was used without further purification.

*(S)-2-Phenyl-1-propionyl-1,2,3,4-tetrahydrobenzo[4,5]thiazolo-[3,2-a]pyrimidin-5-ium Nitrate (**2a**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.40−7.31 (m, 3H), 7.07 (d, J = 7.5 Hz, 2H), 6.64 (s, 1H), 5.11 (dd, J = 13.0, 4.0 Hz, 1H), 3.77 (td, J = 13.0, 4.0 Hz, 1H), 3.53 (ddd, J = 18.5, 14.0, 7.0 Hz, 1H), 3.27−3.17 (m, 1H), 2.75 (d, J = 14.5 Hz, 1H), 2.39 (ddd, J = 18.5, 14.0, 7.0 Hz, 1H), 1.12 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 160.2, 137.3, 136.4, 129.9, 129.7, 129.1, 127.9, 125.9, 125.2, 123.1, 115.1, 58.0, 42.2, 28.4, 27.2, 8.2.

*(S)-2-Phenyl-1-propionyl-1,2,3,4-tetrahydrobenzo[4,5]thiazolo-[3,2-a]pyrimidin-5-ium Hexafluoroantimonate (**2b**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 7.5 Hz, 1H), 7.80−7.76 (m, 2H), 7.71−7.66 (m, 1H), 7.46−7.38 (m, 3H), 7.05 (d, J = 7.0 Hz, 2H), 6.11 (s, 1H), 4.75 (dd, J = 13.5, 3.0 Hz, 1H), 3.81 (td, J = 14.0, 3.5 Hz, 1H), 3.14 (m, 1H), 3.01 (m, 1H), 2.81 (d, J = 15.0 Hz, 1H), 2.48−2.37 (m, 1H), 1.20 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.2, 160.1, 136.4, 136.3, 130.2, 129.8, 129.5, 128.2, 125.9, 125.0, 123.1, 114.5, 57.9, 41.5, 27.6, 26.0, 8.2.

Acylation with Salt 2a (Eq 1). 1-Phenylethanol (25.6 mg, 0.210 mmol) was added to a 20 mL scintillation vial with a stirbar. $CDCl_3$ (600 μL) and *N*,*N*-diisopropylethylamine (36.5 μL, 0.210 mmol) were added, and the reaction mixture was stirred at 0 °C (ice bath). Salt **2a** (48.6 mg, 0.126 mmol) was added, and the reaction was stirred for 1 h at 0 °C. The reaction was halted with the addition of methanol (85.0 μL). ^{1}H NMR spectral data of the crude reaction mixture revealed a reaction conversion to ester of 42.9%. The unreacted alcohol was recovered by column chromatography (9:1 hexanes/ethyl acetate) and analyzed by chiral HPLC (Chiralcel OD with OD guard, 5% *i*-PrOH in *n*-hexane, 23 bar, 1.0 mL/min, UV detection at 254 nm, t_R = 11.47 min, t_S = 14.06 min) 63.4% ee.

Acylation with Salt 2a and Added *S*-HBTM (Table 1, Entry 1). 1-Phenylethanol (24.9 mg, 0.204 mmol) was added to a 20 mL scintillation vial with a stirbar. $CDCl_3$ (600 μL), *N*,*N*-diisopropylethylamine (35.5 μL, 0.204 mmol), and *S*-HBTM (27.2 mg, 0.102 mmol) were added, and the reaction mixture was stirred at 0 °C (ice bath). Salt **2a** (39.3 mg, 0.102 mmol) was added, and the reaction was stirred for 1 h at 0 °C. The reaction was halted with the addition of methanol (82.6 μL). ^{1}H NMR spectral data of the crude reaction mixture revealed a reaction conversion to ester of 33.8%. The unreacted alcohol was recovered by column chromatography (9:1 hexanes/diethyl ether) and analyzed by chiral HPLC (Chiralcel OD with OD guard, 5% *i*-PrOH in *n*-hexane, 23 bar, 1.0 mL/min, UV detection at 254 nm, t_R = 10.42 min, t_S = 12.54 min) 42.4% ee.

Acylation with Salt 2a and Added *R*-HBTM (Table 1, Entry 2). 1-Phenylethanol (25.1 mg, 0.205 mmol) was added to a 20 mL scintillation vial with a stirbar. $CDCl_3$ (600 μL), *N*,*N*-diisopropylethylamine (35.8 μL, 0.206 mmol), and *R*-HBTM (27.4 mg, 0.103 mmol) were added, and the reaction mixture was stirred at 0 °C (ice bath). Salt **2a** (39.7 mg, 0.103 mmol) was added, and the reaction was stirred for 1 h at 0 °C. The reaction was halted with the addition of methanol (83.3 μL). ^{1}H NMR spectral data of the crude reaction mixture revealed a reaction conversion to ester of 33.7%. The unreacted alcohol was recovered by column chromatography (9:1 hexanes/diethyl ether) and analyzed by chiral HPLC (Chiralcel OD with OD guard, 5% *i*-PrOH in *n*-hexane, 23 bar, 1.0 mL/min, UV detection at 254 nm, t_R = 10.32 min, t_S = 12.46 min) 6.2% ee.

Preferred CEC Microscale Procedure (Tables 3 and 4). Preparation of Solutions. *Substrate.* To a 1 mL volumetric flask was added substrate (0.0145 mmol). The flask was filled with toluene to the line and mixed, generating a solution of the alcohol substrate (0.0145 M).

R-HBTM. To a 10 mL volumetric flask was added *R*-HBTM (7.7 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating the solution of *R*-HBTM (0.0029 M).

S-HBTM. To a 10 mL volumetric flask was added *S*-HBTM (7.7 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating the solution of *S*-HBTM (0.0029 M).

Propionic Anhydride/N,N-Diisopropylethylamine. To a 10 mL volumetric flask was added propionic anhydride (279 μL, 2.18 mmol) and *N*,*N*-diisopropylethylamine (126 μL, 0.723 mmol) via micropipette. The flask was filled with toluene to the line and mixed, generating a solution of propionic anhydride (0.218 M) and *N*,*N*-diisopropylethylamine (0.0723 M).

Preferred Microscale CEC Method. A 50 μL syringe was used to draw up, in sequence, air (5 μL), alcohol stock solution (10 μL), air (5 μL), HBTM stock solution (10 μL), air (5 μL), propionic anhydride/*N*,*N*-diisopropylethylamine stock solution (10 μL), and air (5 μL). The contents of the syringe were then mixed in a 600 μL amber glass vial placed inside a 1-dram vial, initiating the reaction. The mixed reaction solution was drawn back into the microsyringe and allowed to sit for 1 h (Table 3, entries 1−8) or 2 h (Table 4, entries 1−8). Then, the reaction solution in the microsyringe was ejected into methanol (10 μL) and analyzed by TLC as follows: To a TLC plate with two lanes was spotted the *R*-HBTM reaction (4.0 μL) and the *S*-HBTM reaction (4.0 μL) via micropipette. The plate was run, dried, stained, heated in an oven (160 °C and ∼1 min unless otherwise noted), and photographed. Solvent systems and TLC stains used for each entry are listed in the Supporting Information.

Preferred CEC One-Use Kit Preparation. *R-HBTM Vials.* To a 10 mL volumetric flask was added *R*-HBTM (9.5 mg, 0.036 mmol) followed by $CDCl_3$ (2.0 mL). To the solvated *R*-HBTM solution was added propionic anhydride (342 μL, 2.67 mmol) and *N*,*N*-diisopropylethylamine (155 μL, 0.890 mmol) via micropipette. The flask was filled to the line with $CDCl_3$, generating a solution of *R*-HBTM (0.0036 M), propionic anhydride (0.267 M), and *N*,*N*-diisopropylethylamine (0.0890 M). To oven-dried 1 mL amber vials was added *R*-HBTM stock solution (450 μL) via syringe. The vials were sealed under argon. A 10 mL volumetric batch was used to prepare 21 *R*-HBTM vials.

S-HBTM Vials. To a 10 mL volumetric flask was added *S*-HBTM (9.5 mg, 0.036 mmol) followed by $CDCl_3$ (2.0 mL). To the solvated *S*-HBTM solution was added propionic anhydride (342 μL, 2.67 mmol) and *N*,*N*-diisopropylethylamine (155 μL, 0.890 mmol) via micropipette. The flask was filled to the line with $CDCl_3$, generating a solution of *S*-HBTM (0.0036 M), propionic anhydride (0.267 M), and *N*,*N*-diisopropylethylamine (0.0890 M). To oven-dried 1 mL amber vials was added *S*-HBTM stock solution (450 μL) via syringe. The

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

vials were sealed under argon. A 10 mL volumetric batch was used to prepare 21 *S*-HBTM vials.

Kit Assembly. One sealed *R*-HBTM vial and one sealed *S*-HBTM vial, each containing 450 μL of their respective stock solutions, were placed in a 20 mL scintillation vial and capped under argon. The vial was then sealed with electric tape and stored in a freezer.

CEC One-Use Kit Procedure (Tables 6 and 7). *Preparation of Substrate Solution.* Substrate (0.020 mmol) was solvated in a deuterated solvent (250 μL) in a 1 dram vial. Masses used for each entry are included in the Supporting Information. The deuterated solvent was $CDCl_3$ unless otherwise noted.

Preferred One-Use Kit CEC Method. The resulting alcohol solution (100. μL) was dispensed to both the *R*-HBTM and *S*-HBTM CEC kit vials via microsyringe with a 1 min gap between additions. A needle was inserted to the CEC kit vial to equalize the pressure before addition of the alcohol solution. The solutions were agitated to ensure homogeneity and allowed to sit for 30 min (Table 6, entries 1−12) or 1 h (Table 7, entries 1−12). Methanol-d_4 (50. μL) was added via microsyringe, and the solution was again agitated to ensure homogeneity, halting the reaction progress. For TLC analysis, to a TLC plate with two lanes were spotted the *R*-HBTM reaction (4.0 μL) and the *S*-HBTM reaction (4.0 μL) via micropipette. The plate was run, dried, stained, heated in an oven (160 °C and ∼1 min unless otherwise noted), and photographed. Solvent systems and TLC stains used for each entry are listed in the Supporting Information. The quenched solution was then analyzed by ^{1}H NMR spectroscopy to assess reaction conversion via measurement of peak integration, traditionally of the proton geminal to the alcohol and ester functional groups on the substrate and product, respectively.

Optimization of Procedures: Initial Investigations of Reduced Substrate Loading of Sample Alcohol (*R*)-5 with the CEC Method and TLC from Figure 4. *Preparation of Solutions for Entry 1. (R)-1-(Naphthalen-2-yl)ethanol ((R)-5).* To a 5 mL volumetric flask was added (*R*)-1-(naphthalen-2-yl)ethanol (258.3 mg, 1.500 mmol). The flask was filled with toluene to the line and mixed, generating a solution of (*R*)-1-(naphthalen-2-yl)ethanol (0.3000 M).

R-HBTM. To a 2 mL volumetric flask was added *R*-HBTM (4.0 mg, 0.015 mmol). The flask was filled with toluene to the line and mixed, generating a solution of *R*-HBTM (0.0075 M).

S-HBTM. To a 2 mL volumetric flask was added *S*-HBTM (4.0 mg, 0.015 mmol). The flask was filled with toluene to the line and mixed, generating a solution of *S*-HBTM (0.0075 M).

Propionic Anhydride/N,N-Diisopropylethylamine. To a 10 mL volumetric flask was added propionic anhydride (769 μL, 6.00 mmol) and *N,N*-diisopropylethylamine (1045 μL, 6.000 mmol) via micropipette. The flask was filled with toluene to the line and mixed, generating a solution of propionic anhydride (0.600 M) and *N,N*-diisopropylethylamine (0.600 M).

Preparation of Solutions for Entries 2−4. (R)-1-(Naphthalen-2-yl)ethanol ((R)-5). To a 1 mL volumetric flask was added (*R*)-1-(naphthalen-2-yl)ethanol (5.0 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating a solution of (*R*)-1-(naphthalen-2-yl)ethanol (0.029 M).

R-HBTM. To a 2 mL volumetric flask was added *R*-HBTM (32.0 mg, 0.120 mmol). The flask was filled with toluene to the line and mixed, generating a solution of *R*-HBTM (0.060 M).

S-HBTM. To a 2 mL volumetric flask was added *S*-HBTM (32.0 mg, 0.120 mmol). The flask was filled with toluene to the line and mixed, generating a solution of *S*-HBTM (0.060 M).

Propionic Anhydride/N,N-Diisopropylethylamine. To a 10 mL volumetric flask was added propionic anhydride (1154 μL, 9.000 mmol) and *N,N*-diisopropylethylamine (1568 μL, 9.002 mmol) via micropipette. The flask was filled with toluene to the line and mixed, generating a solution of propionic anhydride (0.900 M) and *N,N*-diisopropylethylamine (0.900 M).

Optimization of the Microscale CEC Method. A 50 μL syringe was used to draw up, in sequence, air (5 μL), **(*R*)-5** stock solution (between 1 and 10 μL), air (5 μL), HBTM stock solution (10 μL), air (5 μL), propionic anhydride/*N,N*-diisopropylethylamine stock solution (10. μL), and air (5 μL). The volume of stock solution of **(*R*)-5** used was as follows: entry 1 (10 μL), entry 2 (10 μL), entry 3 (5 μL), entry 4 (1 μL). The contents of the syringe were then mixed in a 600 μL amber glass vial placed inside a 1 dram vial, initiating the reaction. The mixed reaction solution was drawn back into the microsyringe and allowed to sit for the remainder of a given time period: entry 1 (1 h), entry 2 (15 min), entry 3 (10 min), entry 4 (10 min). After the given time period, the reaction solution in the microsyringe was directly evaluated in the TLC analysis as follows: To a TLC plate with two lanes was spotted the *R*-HBTM reaction (3.0 μL) and the *S*-HBTM reaction (3.0 μL) via micropipette. The plate was run (30% ethyl acetate in hexanes), dried, stained (PMA), heated in an oven (160 °C, ∼ 1 min), and photographed.

Table 2 Experimental Information (Optimization of CEC Microscale Method). *Screening of Equivalents of HBTM and Propionic Anhydride for Substrate **(R)-5** at a Substrate Loading of 145 nmol per Reaction from Table 2. Preparation of Solutions for Entries 1−4. (R)-1-(naphthalen-2-yl)ethanol ((R)-5).* To a 2 mL volumetric flask was added (*R*)-1-(naphthalen-2-yl)ethanol (10.0 mg, 0.0581 mmol). The flask was filled with toluene to the line and mixed, generating a solution of (*R*)-1-(naphthalen-2-yl)ethanol (0.0290 M). To a 1 mL volumetric flask was added the solution of **(*R*)-5** (500 μL). The flask was filled with toluene to the line and mixed, generating the desired solution of **(*R*)-5** (0.0145 M).

R-HBTM. To a 2 mL volumetric flask was added *R*-HBTM (7.7 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating solution 1 of *R*-HBTM (0.014 M). To a new 2 mL volumetric flask was added solution 1 of *R*-HBTM (1.00 mL). The flask was filled with toluene to the line and mixed, generating solution 2 of *R*-HBTM (0.0072 M).

S-HBTM. To a 2 mL volumetric flask was added *S*-HBTM (7.7 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating solution 1 of *S*-HBTM (0.014 M). To a new 2 mL volumetric flask was added solution 1 of *S*-HBTM (1.00 mL). The flask was filled with toluene to the line and mixed, generating solution 2 of *S*-HBTM (0.0072 M).

Propionic Anhydride/N,N-Diisopropylethylamine. To a 10 mL volumetric flask was added propionic anhydride (558 μL, 4.35 mmol) and *N,N*-diisopropylethylamine (50.6 μL, 0.290 mmol) via micropipette. The flask was filled with toluene to the line and mixed, generating solution 1 of propionic anhydride (0.435 M) and *N,N*-diisopropylethylamine (0.0290 M). To a new 10 mL volumetric flask was added solution 1 of propionic anhydride and *N,N*-diisopropylethylamine (5.00 mL). The flask was filled with toluene and mixed, generating solution 2 of propionic anhydride (0.217 M) and *N,N*-diisopropylethylamine (0.0145 M).

Preparation of Solutions for Entries 5−8. R-HBTM. To a 10 mL volumetric flask was added *R*-HBTM (7.7 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating solution 3 of *R*-HBTM (0.0029 M). To a new 10 mL volumetric flask was added solution 3 of *R*-HBTM (5.00 mL). The flask was filled with toluene to the line and mixed, generating solution 4 of *R*-HBTM (0.0014 M).

S-HBTM. To a 10 mL volumetric flask was added *S*-HBTM (7.7 mg, 0.029 mmol). The flask was filled with toluene to the line and mixed, generating solution 3 of *S*-HBTM (0.0029 M). To a new 10 mL volumetric flask was added solution 3 of *S*-HBTM (5.00 mL). The flask was filled with toluene to the line and mixed, generating solution 4 of *S*-HBTM (0.0014 M).

Propionic Anhydride/N,N-Diisopropylethylamine. To a 10 mL volumetric flask was added propionic anhydride (279 μL, 2.18 mmol) and *N,N*-diisopropylethylamine (75.9 μL, 0.436 mmol) via micropipette. The flask was filled with toluene to the line and mixed, generating solution 3 of propionic anhydride (0.218 M) and *N,N*-diisopropylethylamine (0.0435 M). To a new 10 mL volumetric flask was added solution 3 of propionic anhydride and *N,N*-diisopropylethylamine (3.33 mL). The flask was filled with toluene to the line and mixed, generating solution 4 of propionic anhydride (0.0727 M) and *N,N*-diisopropylethylamine (0.0145 M).

CEC Method Combinations. The preferred microscale CEC method and TLC analysis (described above) was followed for the

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

entries in Table 2 with the following combinations: entry 1, (*R*)-**5** stock solution, solution 1 of each enantiomer of HBTM, solution 1 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 2, (*R*)-**5** stock solution, solution 2 of each enantiomer of HBTM, solution 1 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 3, (*R*)-**5** stock solution, solution 1 of each enantiomer of HBTM, solution 2 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 4, (*R*)-**5** stock solution, solution 2 of each enantiomer of HBTM, solution 2 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 5, (*R*)-**5** stock solution, solution 3 of each enantiomer of HBTM, solution 3 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 6, (*R*)-**5** stock solution, solution 4 of each enantiomer of HBTM, solution 3 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 7, (*R*)-**5** stock solution, solution 3 of each enantiomer of HBTM, solution 4 of propionic anhydride/*N*,*N*-diisopropylethylamine; entry 8, (*R*)-**5** stock solution, solution 4 of each enantiomer of HBTM, solution 4 of propionic anhydride/*N*,*N*-diisopropylethylamine.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00816.

^{1}H and ^{13}C NMR spectra of salt **2b** (X = SbF_6^-), ^{1}H NMR spectra and chiral HPLC traces for eq 1 and Table 1, TLC solvent systems and staining conditions for Tables 3 and 4, calibration curves for (*R*)-**5** and (*R*)-**6** with GC-MS, TLC plate images and ^{1}H NMR spectra for CEC kit reactions in Figure 6 and Table 5 and for entries in Tables 6 and 7, ^{1}H NMR spectra for CEC kit reactions in Figure 9, experimental information for Figure 10, and experimental information and TLC plate images for additional manipulations of microscale systems (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: srychnov@uci.edu.

Present Address

†Department of Chemistry, 505 South Mathews Avenue, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Dr. Ryan Patman for extensive discussion and feedback in the development of the CEC kit and through Pfizer, Inc., in La Jolla for the donation of several compounds for this study. We are also grateful to the chemists at Pfizer, Inc., in La Jolla for additional input on the CEC kits. Dr. Adam Hughes of Theravance Biopharma Inc. generously donated complex intermediate and late-stage compounds. The Morken group donated a significant number of β-aryl compounds that were used to expand the substrate scope of the CEC method. The National Science Foundation (Grants CHE 1152449 and CHE 1361998) provided support for the research discussed. Individual support was provided to support the progress of research in this work by the Department of Defense (DoD) through the National Defense Science & Engineering Graduate Fellowship (NDSEG) Program (A.J.W.), the National Science Foundation Graduate Fellowship Program (A.J.W.), the Allergan Undergraduate Research Fellowship (R.P.K.), and the UC Irvine Undergraduate Research Opportunities Program (R.P.K.).

■ REFERENCES

(1) (a) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; John Wiley & Sons, Inc.: Hoboken, NJ, 1994; pp 101−147 and 991−1105. (b) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17−118. (c) Wenzel, T. J.; Chisholm, C. D. *Chirality* **2011**, *23*, 190−214.

(2) (a) Wenzel, T. J. *Discrimination of Chiral Compounds Using NMR Spectroscopy*; John Wiley & Sons, Inc.: Hoboken, NJ, 2007; pp 1−181. For additional selected examples, see: (b) Louzao, I.; Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Commun.* **2010**, *46*, 7903−7905. (c) Pérez-Estrada, S.; Joseph-Nathan, P.; Jiménez-Vázquez, H. A.; Medina-López, M. E.; Ayala-Mata, F.; Zepeda, L. G. *J. Org. Chem.* **2012**, *77*, 1640−1652. (d) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549. (e) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519. (f) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096. (g) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Tetrahedron Lett.* **1989**, *30*, 3147−3150. (h) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296−1298. (i) For a review of the advanced Mosher method procedure, see: Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451−2458.

(3) (a) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2002**, *4*, 411−414. (b) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Tetrahedron Lett.* **2003**, *44*, 7489−7491. (c) Ghosh, I.; Zeng, H.; Kishi, Y. *Org. Lett.* **2004**, *6*, 4715−4718. (d) Ghosh, I.; Kishi, Y.; Tomoda, H.; Omura, S. *Org. Lett.* **2004**, *6*, 4719−4722. (e) Bian, G.; Fan, H.; Huang, H.; Yang, S.; Zong, H.; Song, L.; Yang, G. *Org. Lett.* **2015**, *17*, 1369−1372. (f) Seo, M.-S.; Kim, H. *J. Am. Chem. Soc.* **2015**, *137*, 14190−14195.

(4) For a review on the electronic circular dichroism exciton chirality method, see: (a) Harada, N.; Nakanishi, K.; Berova, N. In *Applications in Stereochemical Analysis of Synthetic Compounds, Natural Products, and Biomolecules*, 1st ed.; Berova, N., Polavarapu, P. L., Nakanishi, K., Woody, R. W., Eds.; Comprehensive Chiroptical Spectroscopy, Vol. *2*; John Wiley & Sons, Inc.: Hoboken, NJ, 2012; pp 115−166. For additional selected publications on electronic circular dichroism spectroscopy, see: (b) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257−263. (c) Gargiulo, D.; Cai, G.; Ikemoto, N.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 888−891. (d) Zhou, P.; Zhao, N.; Rele, D. N.; Berova, N.; Nakanishi, N. *J. Am. Chem. Soc.* **1993**, *115*, 9313−9314. (e) Mori, Y.; Sawada, T.; Sasaki, N.; Furukawa, H. *J. Am. Chem. Soc.* **1996**, *118*, 1651−1656. (f) Li, X.; Tanasova, M.; Vasileiou, C.; Borhan, B. *J. Am. Chem. Soc.* **2008**, *130*, 1885−1893. (g) Li, X.; Borhan, B. *J. Am. Chem. Soc.* **2008**, *130*, 16126−16127. (h) Tanasova, M.; Vasileiou, C.; Olumolade, O. O.; Borhan, B. *Chirality* **2009**, *21*, 374−382.

(5) For a review on using vibrational circular dichroism, see: (a) Freedman, T. B.; Cao, X.; Dukor, R. K.; Nafie, L. A. *Chirality* **2003**, *15*, 743−758. (b) Yang, G.; Xu, Y. *Top. Curr. Chem.* **2010**, *298*, 189−236. For an additional selected publication on the exciton chirality method in vibrational circular dichroism, see: (c) Taniguchi, T.; Monde, K. *J. Am. Chem. Soc.* **2012**, *134*, 3695−3698. For additional selected publications on vibrational circular dichroism, see: (d) Domingos, S. R.; Huerta-Viga, A.; Baij, L.; Amirjalayer, S.; Dunnebier, D. A. E.; Walters, A. J. C.; Finger, M.; Nafie, L. A.; de Bruin, B.; Buma, W. J.; Woutersen, S. *J. Am. Chem. Soc.* **2014**, *136*, 3530−3535. (e) Taniguchi, T.; Manai, D.; Shibata, M.; Itabashi, Y.; Monde, K. *J. Am. Chem. Soc.* **2015**, *137*, 12191−12194.

(6) For a review on determining absolute configuration with optical rotation, see: (a) Polavarapu, P. L. *Chirality* **2002**, *14*, 768−781. For selected publications on advances in calculations with optical rotation, see: (b) Mukhopadhyay, P.; Wipf, P.; Beratan, D. N. *Acc. Chem. Res.* **2009**, *42*, 809−819. (c) Kondru, R. K.; Wipf, P.; Beratan, D. N. *J. Phys. Chem. A* **1999**, *103*, 6603−6611.

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265

(7) For a review on determining absolute configuration with X-ray diffraction of single crystals, see: Flack, H. D.; Bernardinelli, G. *Chirality* **2008**, *20*, 681−690.
(8) Meador, D. S.; Spivak, D. A. *Org. Lett.* **2014**, *16*, 1402−1405.
(9) Patterson, D.; Schnell, M.; Doyle, J. M. *Nature* **2013**, *497*, 475−478.
(10) (a) 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.; Thieme: Stuttgart, Germany, 1977; Vol. 3, pp 51−94. (b) Horeau, A. *Tetrahedron Lett.* **1961**, *2*, 506−512. (c) Horeau, A. *Tetrahedron Lett.* **1962**, *3*, 965−969. (d) Horeau, A.; Kagan, H. B. *Tetrahedron* **1964**, *20*, 2431−2441. (e) Weidmann, R.; Horeau, A. *Tetrahedron Lett.* **1973**, *14*, 2979−2982. (f) Schoofs, A.; Horeau, A. *Tetrahedron Lett.* **1977**, *18*, 3259−3262. (g) Horeau, A.; Nouaille, A. *Tetrahedron Lett.* **1990**, *31*, 2707−2710. (h) Koenig, W. A.; Gehrcke, B.; Weseloh, G. *Chirality* **1994**, *6*, 141−147.
(11) For the CEC method with secondary alcohols, see: (a) Wagner, A. J.; David, J. G.; Rychnovsky, S. D. *Org. Lett.* **2011**, *13*, 4470−4473. (b) Wagner, A. J.; Rychnovsky, S. D. *J. Org. Chem.* **2013**, *78*, 4594−4598. (c) Wagner, A. J.; Miller, S. M.; Nguyen, S.; Lee, G. Y.; Rychnovsky, S. D.; Link, R. D. *J. Chem. Educ.* **2014**, *91*, 716−721.
(12) For the CEC method with primary amines, see: Miller, S. M.; Samame, R. A.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **2012**, *134*, 20318−20321.
(13) For the CEC method with oxazolidinones, lactams, and thiolactams, see: Perry, M. A.; Trinidad, J. V.; Rychnovsky, S. D. *Org. Lett.* **2013**, *15*, 472−475.
(14) Birman, V. B.; Li, X. *Org. Lett.* **2008**, *10*, 1115−1118.
(15) LeGay, C. M.; Boudreau, C. G.; Derksen, D. J. *Org. Biomol. Chem.* **2013**, *11*, 3432−3435.
(16) Li, X.; Liu, P.; Houk, K. N.; Birman, V. B. *J. Am. Chem. Soc.* **2008**, *130*, 13836−13837.
(17) Several salts containing different counterions were investigated. Salt **2a** was the most reactive and was used in these studies. ^{1}H and ^{13}C NMR characterization information for salts **2a** and **2b** are reported in the Experimental Section. Spectral data for the more stable SbF_6^- salt **2b** is reported in the Supporting Information.
(18) In ref 14, the catalytic asymmetric kinetic resolution of 1-phenylpropanol (0.25 M) in $CDCl_3$ with propionic anhydride (0.75 equiv), *N*,*N*-diisopropylethylamine (0.75 equiv), and *S*-HBTM (1 mol %) over 1 h reached 48% conversion with a selectivity factor of 26.
(19) Li, X.; Jiang, H.; Uffman, E. W.; Guo, L.; Zhang, Y.; Yang, X.; Birman, V. B. *J. Org. Chem.* **2012**, *77*, 1722−1737.
(20) (a) Wagner, A. J.; Rychnovsky, S. D. *Org. Lett.* **2013**, *15*, 5504−5507. (b) Wagner, A. J.; Rychnovsky, S. D. Correction to Kinetic Analysis of the HBTM-Catalyzed Esterification of an Enantiopure Secondary Alcohol. *Org. Lett.* **2013**, *16*, 4348.
(21) Studies on purposeful error in volume of stock solutions used and the qualitative effect on reaction conversion were conducted. All entries with purposeful error in volume measurements from stock solutions resulted in the same, correct, fast reaction lane. Equivalents of base were also invested and revealed no qualitative effect on the reaction conversion for CEC reactions. Summaries on both studies are included in the Supporting Information.
(22) Cui, J. J.; Tran-Dubé, M.; Shen, H.; Nambu, M.; Kung, P.-P.; Pairish, M.; Jia, L.; Meng, J.; Funk, L.; Botrous, I.; McTigue, M.; Grodsky, N.; Ryan, K.; Padrique, E.; Alton, G.; Timofeevski, S.; Yamazaki, S.; Li, Q.; Zou, H.; Christensen, J.; Mroczkowski, B.; Bender, S.; Kania, R. S.; Edwards, M. P. *J. Med. Chem.* **2011**, *54*, 6342−6363.
(23) For information on the synthesis and characterization of entries 1−8, see: Mlynarski, S. N.; Schuster, C. H.; Morken, J. P. *Nature* **2014**, *505*, 386−390.
(24) Calibration curve studies for (***R***)-**5** and (***R***)-**6** on GC-MS are included in the Supporting Information.
(25) TLC plate images of the *R*-HBTM and *S*-HBTM reactions for the samples in Figure 6 that were qualitatively analyzed to assess the fast reaction via spot density of substrate and product, as well as ^{1}H NMR spectra for each reaction, are included in the Supporting Information.
(26) A graph of the dielectric constant of the substrate-loading solvent compared with reaction conversion (%) for both the *R*-HBTM and *S*-HBTM reactions is included in the Supporting Information.
(27) TLC plate images of the *R*-HBTM and *S*-HBTM reactions for entries 1−7 of Table 5 that were qualitatively analyzed to assess the fast reaction via spot density of substrate and product, as well as ^{1}H NMR spectra for each reaction, are included in the Supporting Information.
(28) ^{1}H NMR spectra of *R*-HBTM and *S*-HBTM reactions for each entry are included in the Supporting Information. In certain cases, the methanol quench resulted in peak overlap with one of the proton peaks of interest in the ^{1}H NMR spectrum for reaction conversion analysis. In these cases, the reaction mixture was then concentrated under reduced pressure and resolvated in $CDCl_3$ for an additional ^{1}H NMR spectrum of the crude reaction mixture.
(29) Hughes, A. D.; Chen, Y.; Hegde, S. S.; Jasper, J. R.; Jaw-Tsai, S.; Lee, T.-W.; McNamara, A.; Pulido-Rios, M. T.; Steinfeld, T.; Mammen, M. *J. Med. Chem.* **2015**, *58*, 2609−2622.
(30) The amide was isolated after the substrate was treated with propionic anhydride and triethylamine at room temperature. Absolute mass data matched the mass of the amide. Spectral information comparing the amide to the CEC reactions, as well as the absolute mass data, is included in the Supporting Information.
(31) Pan, J.-J.; Ramamoorthy, G.; Poulter, C. D. *Org. Lett.* **2016**, *18*, 512−515.
(32) Pirrung, M. C. *The Synthetic Organic Chemist's Companion*; John Wiley & Sons, Inc.: Hoboken, NJ, 2007; pp 171−172.
(33) Rychnovsky, S. D.; Cossrow, J. *Org. Lett.* **2003**, *5*, 2367−2370.
(34) Perry, M. A.; Morin, M. D.; Slafer, B. W.; Rychnovsky, S. D. *J. Org. Chem.* **2012**, *77*, 3390−3340.
(35) Kim, A. I.; Rychnovsky, S. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 1267−1270.
(36) Miller, S. M. Ph.D. Dissertation, University of California−Irvine, Irvine, CA, 2014.
(37) Tan, C.-H.; Holmes, A. B. *Chem. - Eur. J.* **2001**, *7*, 1845−1854.
(38) Lewis, F. W.; Eichler, M. C.; Grayson, D. H. *Synlett* **2009**, *2009*, 1923−1928.

DOI: 10.1021/acs.joc.6b00816
J. Org. Chem. 2016, 81, 6253−6265