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Using Enzyme Inhibition as a High Throughput Method to Measure the Enantiomeric Excess of a Chiral Sulfoxide

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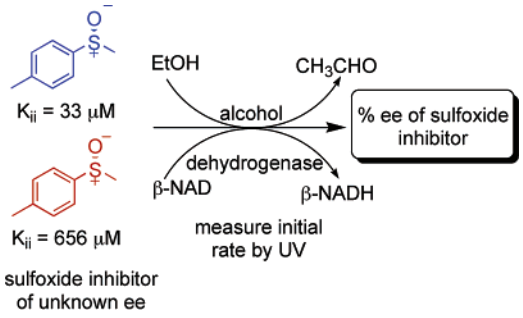
ABSTRACT



The enantiomeric excess of methyl *p*-tolyl sulfoxide can be determined in a high throughput format by measuring its ability to inhibit the alcohol dehydrogenase catalyzed oxidation of ethanol. The two enantiomers of the sulfoxide have very different inhibition constants for the enzyme. Thus, the initial rate of ethanol oxidation in the presence of the sulfoxide is correlated with the sulfoxide enantiomeric excess.

High throughput methods for measuring the enantiomeric excess (ee) of small molecules will have a significant impact on a variety of chemical problems, including the discovery of new enantioselective catalysts and the characterization and screening of chiral molecules for biological activity. Over the last several years, a number of such methods have been described in the literature that rely on chromatographic, spectroscopic, chemical, or biological tools to quantitate the concentrations of two enantiomers in a sample.[1,2] We recently reported a biological technique, called EMDee for an *E*nzymatic *M*ethod for *D*etermining ee, that uses an enzyme as an analytical tool to measure ee.[3,4] An enzyme is introduced into a sample of unknown stereochemical composition, where it catalyzes a reaction using only one of the enantiomers as a substrate. The initial rate of the enzyme-catalyzed reaction is correlated with the ee of the sample. We developed two examples of the assay. The first uses an

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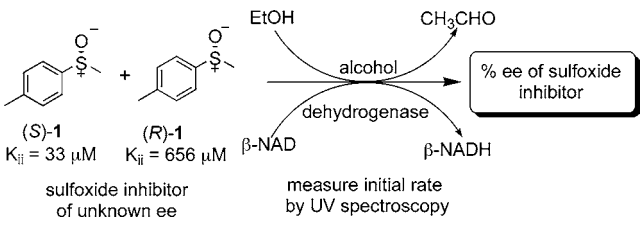
alcohol dehydrogenase to analyze chiral alcohols, while the second uses a lipase to analyze chiral esters. These and other parallel methods have a significant speed advantage over the traditional serial methods that have been used to measure ee, such as chiral HPLC and GC, optical rotation measurements, and NMR methods. As a result, these high throughput ee screens are powerful tools that can be used, in conjunction with combinatorial methods, to facilitate the discovery of new enantioselective catalysts for a variety of chemical transformations. Here, we report a new method that overcomes one of the major limitations of the original EMDee assay. In previous examples, the enzyme was required to both bind and catalyze a reaction with the analyte. In our current experiments, we remove the turnover restriction and demonstrate that EMDee works well with analytes in which the two enantiomers simply bind with different affinities to the enzyme and, thus, inhibit its activity to a different extent. These studies broaden the scope of the EMDee assay since there are a variety of chiral compounds that are enzyme inhibitors, and in most cases, the two enantiomers of these potential analytes have different affinities for their enzyme target.

We have chosen sulfoxide **1** as a proof of concept for this new EMDee procedure. Chiral sulfoxides have important applications in both chemistry and biology.[5] They have been used as chiral auxiliaries and as ligands for asymmetric catalysis and also in medicinal chemistry as anti-ulcer drugs and to prevent alcohol metabolism.[5b,6] For example, the anti-ulcer drug Nexium is a chiral sulfoxide. One of the most efficient routes to prepare chiral sulfoxides is the asymmetric catalytic oxidation of sulfides. While a number of enantioselective catalysts have been developed for this reaction, there is room for improved oxidation catalysts that avoid over-oxidation of the sulfide to the sulfone, that use lower ligand loadings and nontoxic metals and that provide higher yields and enantioselectivities.[7] High throughput methods to measure the ee of sulfoxides will facilitate the discovery effort.[8]

The assay procedure is outlined in Scheme 1. Plapp has shown that chiral sulfoxides are inhibitors of horse liver alcohol dehydrogenase (HLADH).[9] We examined sulfoxide **1** as an inhibitor of this enzyme using ethanol as the substrate.

Scheme 1. EMDee Procedure to Determine the Enantiomeric Excess of Methyl *p*-Tolyl Sulfoxide by Measuring its Inhibition of the Oxidation of Ethanol to Acetaldehyde Catalyzed by HLADH[a]



[a] Assay conditions: total concentration of sulfoxide **1** = 200 μM, 46 mM phosphate buffer (pH 7), [EtOH] = 50 mM, [β-NAD] = 15 mM, monitored by UV spectroscopy at 340 nm over 30 min.

The rate of the reaction was monitored by following the conversion of the cofactor β-NAD to β-NADH by UV spectroscopy. Both enantiomers of **1** are uncompetitive inhibitors, but (*S*)-**1** is a potent inhibitor with a K_{ii} value of 33 ± 3 μM, while (*R*)-**1** is a weak inhibitor with a K_{ii} value of 656 ± 66 μM. Because these are uncompetitive inhibitors, K_{ii} represents the dissociation constant of the sulfoxide from the HLADH/β-NADH/sulfoxide ternary complex.[9] Uncompetitive inhibition was confirmed using a combination of both Dixon and Cornish-Bowden analyses.[10]

Since there is a 20-fold difference between the K_{ii} values of the two enantiomers, we can measure the ee of the chiral sulfoxide by monitoring the initial rate of the HLADH-catalyzed oxidation of ethanol in the presence of a known concentration of this inhibitor. Reactions that are run in the presence of a higher concentration of (*S*)-**1** are slow, while reactions with (*R*)-**1** are fast. We used a modified form of the Michaelis−Menten equation for uncompetitive inhibition to correlate the initial rate of the enzymatic reaction in the presence of (*S*)-**1** and (*R*)-**1** with the ee of the inhibitor (eq 1). When the total sulfoxide and substrate concentrations, and the kinetic and inhibition constants are known, eq 1 can be used to convert initial rate data into ee values for the sulfoxide. In this particular example, the sulfoxide is an uncompetitive inhibitor of the enzyme. However, EMDee should be equally as effective with analytes that are competitive inhibitors.

$$v_i = \frac{V_{max}[\mathrm{EtOH}]}{K_m + [\mathrm{EtOH}]\left[1 + \left(\frac{[(S)\text{-}\mathbf{1}]}{K_{ii}\,(S)\text{-}\mathbf{1}} + \frac{[(R)\text{-}\mathbf{1}]}{K_{ii}\,(R)\text{-}\mathbf{1}}\right)\right]} \quad (1)$$

Figure 1 shows plots of the initial rate of the HLADH-catalyzed reaction in the presence of 200 μM sulfoxide **1**. We analyzed 84 samples that ranged from 100% *R* to 100% *S* using a 96-well UV plate reader. Each sample contained 36 nmol of sulfoxide, and initial rates were measured over a 30 min window. The solid line in each plot represents the theoretical curve generated using the modified form of the Michaelis−Menten equation for uncompetitive inhibition (eq 1). There is a good fit of the experimental data to the theoretical curve, demonstrating that inhibition rate profiles can be used to estimate ee of the sulfoxide inhibitor.

To demonstrate the utility of this assay for the analysis of products from catalytic enantioselective reactions, we screened

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(7) For a catalyst that uses a nontoxic oxidant and metal, see: Legros, J.; Bolm, C. *Chem.—Eur. J.* **2005**, *11*, 1086.
(8) Reetz, M. T.; Daligault, F.; Brunner, B.; Hinrichs, H.; Deege, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 4078.
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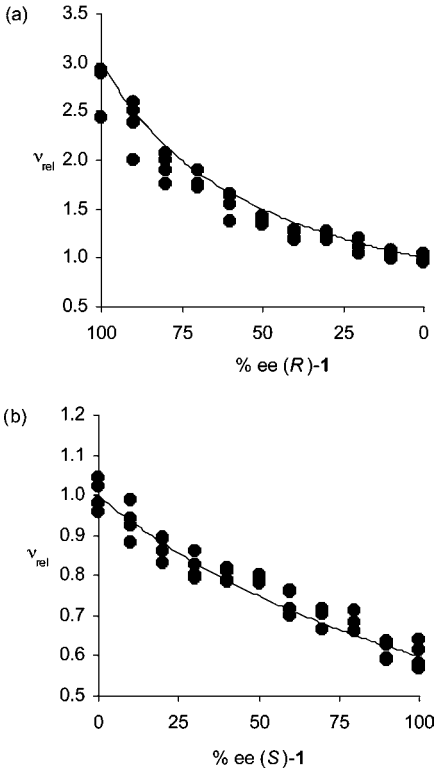
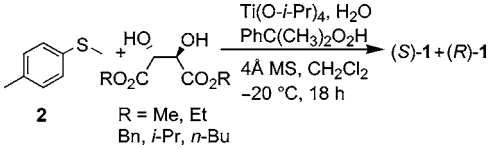


Figure 1. (a) Correlation between the relative initial rate of the HLADH-catalyzed oxidation of ethanol as a function of sulfoxide ee from 100 to 0% ee (*R*)-**1**. (b) Correlation between the relative initial rate of the HLADH-catalyzed oxidation of ethanol as a function of sulfoxide ee from 0 to 100% ee (*S*)-**1**. The solid line in each plot represents the theoretical curve generated using the Michaelis−Menten equation for uncompetitive inhibition (eq 1). Note that the *y*-axes in the two plots are drawn to different scales.

a series of tartrate esters as ligands in the enantioselective oxidation reaction reported by Kagan (Scheme 2).[11] These

Scheme 2. Enantioselective Oxidation of Sulfide **2** to Sulfoxide **1**



reactions use a stoichiometric amount of the tartrate ester as a chiral controller. The unquenched reaction mixtures were passed through a silica plug, and without further purification, the crude products were analyzed by EMDee and HPLC using a chiral column (Figure 2).[12] For the EMDee analyses,

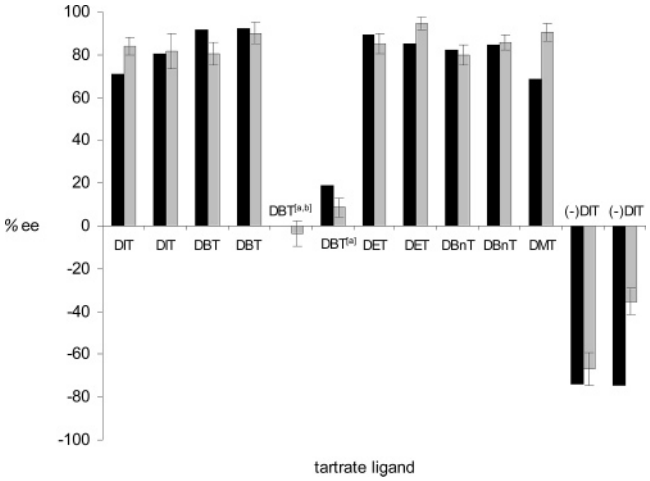


Figure 2. Comparison of ee values of sulfoxide (*R*)-**1** measured by chiral HPLC (black bars) and EMDee (gray bars). Samples of sulfoxide **1** were obtained from the enantioselective oxidation of sulfide **2** in the presence of the tartrate ester ligands listed on the *x*-axis. Abbreviations for tartrate esters: DIT = diisopropyl L-tartrate, DBT = di-*n*-butyl L-tartrate, DET = diethyl L-tartrate, DBnT = dibenzyl L-tartrate, DMT = dimethyl L-tartrate, (−)-DIT = diisopropyl D-tartrate. The gray bars represent the average of four separate EMDee measurements; the error bars correspond to ± one standard deviation from the mean. Negative values on the *y*-axis correspond to the ee of (*S*)-**1**: [a] 10 mol % of the DBT ligand was used in these reactions. [b] This sample was racemic by chiral HPLC.

eq 1 was used to calculate ee values from the initial rate data. All but one of the reactions was performed in duplicate. The EMDee assays are generally in good agreement with the ee values that were determined by HPLC, with a majority of the experiments showing less than ±10% difference between the two measurements. Like the other EMDee systems, this level of accuracy is sufficient for high throughput screening of large libraries of potential catalysts to identify those catalysts that give high levels of enantioselectivity. This much smaller subset of catalysts can then be further analyzed by more accurate, but slower methods, such as chiral HPLC. Interestingly, in our hands di-*n*-butyl tartrate provided the highest level of enantioselectivity in this reaction, slightly higher than that of the diethyl ester that was reported by Kagan. One of the (−)-DIT samples (far right of Figure 1) showed a large difference between the ee values determined by EMDee and HPLC. This difference is likely due to the fact that the EMDee assay shows its lowest level of sensitivity with samples near 100% ee (*S*)-**1**.

We would like to emphasize several important characteristics of this and other EMDee assays. (1) All of the enzymes that we use are commercially available and relatively inexpensive. (2) The assays are straightforward to set up. There is little development time since assay procedures for many enzymes are available in the literature. (3) The only piece of specialized equipment needed to implement the assay in its high throughput format is a 96- or 384-well UV

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(12) This isolation procedure does not lead to enantiomeric fractionation of sulfoxide **1**. See: Diter, P.; Taudien, S.; Samuel, O.; Kagan, H. B. *J. Org. Chem.* **1994**, *59*, 370.

or fluorescence plate reader. (4) The assays are high throughput; 84 samples can be analyzed in 30 min. (5) All of the EMDee assays that we have reported to date function well with crude samples generated from catalytic asymmetric reactions.

In these studies, we measured two kinetic constants (V_{max} and K_m) and two inhibition constants (K_{ii} for both (*R*)-**1** and (*S*)-**1**), so that we can use eq 1 to correlate relative rates with sample ee. However, for screening applications, where the goal is simply to identify the most stereoselective catalysts out of a large library, these measurements are not necessary. Samples that give the fastest and slowest initial rates in the enzyme assays correspond to the compounds with the highest ee values of both absolute configurations. Given the variety of chiral molecules that are either inhibitors or substrates for known enzymes, we believe that this method should be a useful tool for speeding the discovery of new asymmetric catalysts.

Supporting Information Available: Experimental details for the assays, Cornish-Bowden and Dixon plots for both enantiomers of sulfoxide **1**, general procedure for the enantioselective oxidation of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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