EMDee: An Enzymatic Method for Determining Enantiomeric Excess

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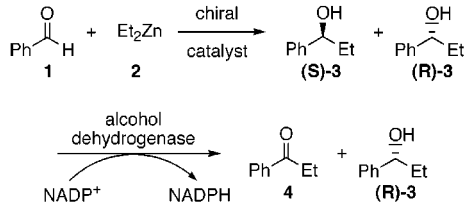
The techniques of combinatorial chemistry have recently been applied to the discovery of new asymmetric catalysts for a variety of organic transformations.[1] These techniques are useful for increasing the efficiency by which chiral ligands for metal catalysts are discovered since the design of these ligands is largely an empirical process, and there are currently no simple rules for reliably producing catalysts that give high stereoselectivity.[2] Using combinatorial methods, it is straightforward to generate thousands of potential asymmetric catalysts. However, screening these catalysts for stereoselectivity is currently the bottleneck in the process because it is time-consuming to measure the enantiomeric excess of so many samples. The most common analytical techniques for measuring ee, such as chiral GC and HPLC, generally require tens of minutes per analysis. Thus, they can be used to analyze hundreds of samples at a time, especially when employing an autosampler, but they become impractical for analyzing the thousands of catalysts that can be generated through combinatorial synthesis.

To bring the full power of combinatorial chemistry to bear on the problem of asymmetric catalysis, it will be necessary to develop new methods for the high throughput screening of enantiomeric excess. Several such methods have been reported in the recent literature. They rely on techniques that include kinetic resolution in conjunction with reaction microarrays[3a] or electrospray mass spectrometry (ESMS),[3b] the use of isotopically labeled *pseudo*-enantiomers with ESMS detection,[3c] IR detection of reactions using enantiomerically pure starting materials,[3d] capillary array electrophoresis using chiral host molecules,[3e] and HPLC using CD detection.[3f] In this communication we report on an enzymatic method for determining enantiomeric excess (EMDee) that can be used for the high throughput screening of asymmetric catalysts. In this method an enzyme is used to selectively process one enantiomer of a product from a catalytic reaction.

We have chosen the addition of diethylzinc to benzaldehyde as a test-bed for demonstrating EMDee. This is a well-studied reaction for which a variety of enantioselective catalysts have been developed.[4] The reaction yields 1-phenylpropanol **3**, and the (*S*) enantiomer of this product can be oxidized to the corresponding ketone using the (*S*)-aromatic alcohol dehydrogenase from *Thermoanaerobium sp.* (Scheme 1). The rate of the enzymatic oxidation can be conveniently monitored by observing formation of NADPH by UV spectroscopy at 340 nm. (*S*)-**3** is a good substrate for the enzyme with a K_M value of 6.4 ± 1.1 mM, while (*R*)-**3** is an inhibitor with a K_i value of 6.0 ± 1.5 mM. The Michaelis−Menten equation provides a direct relationship between the rate of this enzymatic oxidation and the concentrations of (*S*)-**3** and (*R*)-**3**. *Therefore, the rate of this oxidation can be used as a direct measure of the enantiomeric excess of samples of 1-phenylpropanol.*

Scheme 1



We have examined the rate of the enzyme-catalyzed oxidation of a number of samples of 1-phenylpropanol that range in composition from 100% (*S*)-**3** to 100% (*R*)-**3**. Figure 1 shows that there is an excellent correlation between sample % ee and reaction rate. Thus, EMDee is a reliable and fast method for determining the % ee of samples across the full range of stereochemical outcomes for the reaction of diethylzinc with benzaldehyde in the presence of chiral catalysts. To demonstrate the high throughput nature of this assay, 100 samples were analyzed in a 384-well format using a UV/fluorescence plate reader.[5] The sample volume in these runs was 100 μL, and each sample contained 1 μmol of 1-phenylpropanol. Data from a 30 min window were used to calculate the relative rates of these 100 reactions. On the basis of the data from these runs along with the data shown in Figure 1, we estimate that EMDee can be used to determine the % ee of samples with an accuracy of approximately ±10%. Thus, the method will be most useful as a preliminary high throughput screening technique for identifying the relatively small number of catalysts out of a library that show high stereoselectivity. Once these catalysts have been identified, their stereoselectivity can be measured more precisely, but more slowly, using chiral GC or HPLC.

To observe how EMDee functions with the products from actual catalytic reactions, we have examined the solvent dependence for the addition of diethylzinc to benzaldehyde using the previously reported catalysts **5** and **6**.[4e] It is known that catalyst

5 shows high stereoselectivity for formation of (*S*)-**3** with toluene as the solvent, while catalyst **6** has modest selectivity for formation of (*R*)-**3**. After the reactions were complete, they were subjected to an aqueous workup and without further purification were

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(5) See the Supporting Information.

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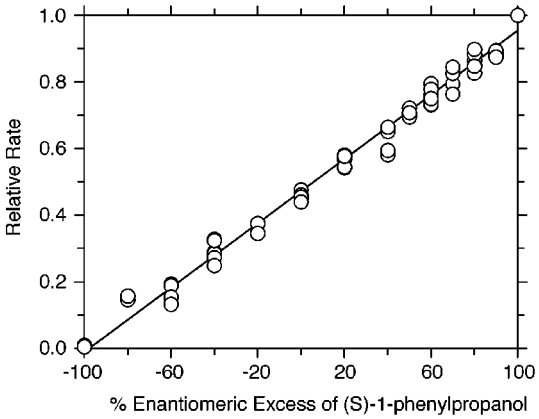

Figure 1. Plot of the initial rate of the enzyme-catalyzed oxidation of 1-phenylpropanol as a function of % ee. The solid line represents a fit of the data to the Michaelis−Menten formalism for competitive inhibition where [S] = [(*S*)-**3**] and [I] = [(*R*)-**3**]. The total alcohol concentration was maintained constant at 10 mM.

Table 1. Comparison of % ee of (*S*)-1-Phenylpropanol, as Determined by EMDee and Chiral GC, for Reactions Performed with **5** and **6** as Catalysts[a]

catalyst	solvent	% ee (EMDee)	% ee (GC)
5	toluene	90	95
5	CH_2Cl_2	88	84
5	THF	78	77
5	Et_2O	60	60
6	toluene	−37	−28
6	CH_2Cl_2	−58	−53
6	THF	−37	−35
6	Et_2O	−17	−18

[a] The values for catalyst **6** are listed as negative numbers because this catalyst yields the (*R*) enantiomer as the major product.

analyzed by both EMDee and chiral GC. For the analyses using EMDee, the % ee of 1-phenylpropanol was determined based upon the curve fit shown in Figure 1. The data in Table 1 show that there is a good agreement between the values obtained with the two different analyses. In all cases, the results from EMDee were within ±10% of those obtained with chiral GC. As expected, catalyst **5** shows good stereoselectivity in noncoordinating solvents such as toluene and methylene chloride, and poorer selectivity in coordinating solvents such as THF and diethyl ether. Catalyst **6** shows modest stereoselectivity in all solvents, with methylene chloride giving the highest % ee of (*R*)-**3**.

The alcohol dehydrogenase used in these studies has a high selectivity for (*S*)-aromatic alcohols, and does not process the (*R*) enantiomer to any appreciable extent under the assay conditions. Other substrates, or alcohol dehydrogenases from other sources, may show lower stereoselectivity. However, this does not limit the utility of EMDee. Previous studies, which also rely on kinetic resolution to separate enantiomers, have shown that even modest stereoselectivity in the kinetic resolution step is sufficient for accurate determinations of ee.[3a,b] Furthermore, a large number of alcohol dehydrogenases are commercially available, and many of these show broad substrate specificity along with good stereoselectivity.[6]

In the experiments described above, EMDee does not distinguish between catalyzed reactions that proceed with low stereoselectivity but high conversion, and high stereoselectivity but low conversion. In a practical sense this is not a major limitation because, if we are screening a library of thousands of potential catalysts, we are searching for catalysts that give both high stereoselectivity and high yields. These catalysts will give the highest rates in the EMDee assay. However, when designing asymmetric catalysts it is often useful to know which structures provide good stereoselectivity but poor yields. For these compounds the yield can sometimes be optimized by adjusting the reaction conditions. We have modified EMDee to provide information about both enantiomeric excess and extent of conversion. In a second set of assays, we have shown that the (*R*)-aromatic alcohol dehydrogenase from *Lactobacillus kefir*, which has the opposite stereoselectivity from the enzyme from *Thermoanaerobium sp.*, can be used to quantitate the amount of (*R*)-**3** that is present.[5] Thus, the extent of conversion can be calculated from the known amounts of (*R*)-**3** and (*S*)-**3**. A second approach to solving this problem might involve quantitating the residual benzaldehyde that remains after the reaction is terminated by reducing it to benzyl alcohol with an alcohol dehydrogenase in the presence of NADH or NADPH.

In summary, we have developed EMDee as a high throughput method for measuring enantiomeric excess that uses an enzyme to quantitate one stereoisomer in a mixture of enantiomers. The method can measure the % ee of samples that range from 100% (*S*) to 100% (*R*) with an accuracy of approximately ±10%. Furthermore, 100 samples can be processed in 30 min. We believe that this method, using other enzymes, will be applicable to analysis of a broad variety of products from catalytic asymmetric reactions. For example, alcohol dehydrogenases could be used to analyze the alcohol products from reactions such as other alkylations of aldehydes, hydrogenation of ketones, aldol reactions, nucleophilic ring opening of epoxides, and the kinetic resolution of alcohols. Lipases and esterases could be applied to the ester products from reactions such as allylic oxidations with *tert*-butyl peroxybenzoate, cyclopropanation of alkenes with alkyl diazoacetate, and the glyoxylate ene reaction, while acylases and proteases may be useful for analyzing amide products from the catalytic hydrogenation of *N*-acetyleneamines. Finally, EMDee will be useful for investigating aspects of asymmetric catalysis beyond the combinatorial synthesis of ligands. For example, it can be used to systematically examine the effects of various metals, solvents, and chiral and nonchiral additives[7] on the stereoselectivity of catalyzed reactions.

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Supporting Information Available: Procedures for the enzyme assays and data for assays performed with the UV plate reader and with the (*R*)-aromatic alcohol dehydrogenase (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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