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High-Throughput Enzymatic Method for Enantiomeric Excess Determination of O-Acetylated Cyanohydrins

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High-throughput methods are becoming increasingly recognized for the discovery of new catalysts for asymmetric synthesis.[1] By the use of combinatorial techniques for ligand preparation, large modular catalyst libraries are conveniently accessible.[2] Catalyst diversity may be further increased by the use of achiral or chiral additives affecting the catalyst structures[3] or by using mixtures of different ligands.[4]

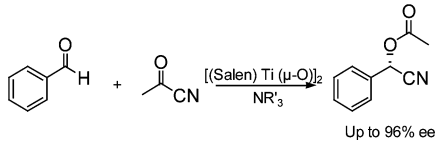
The bottleneck in the development of parallel screening methods for the assessment of catalyst performance is the lack of general techniques for efficient enantiomeric excess determinations. The most commonly used methods for analysis of the enantioselectivity are GC and HPLC on chiral stationary phases, but these methods are time-consuming, require serial analyses, and can only handle a limited amount of samples per day. To overcome these limitations, several techniques have emerged which have solved these problems for certain types of products.[5] Most of these are based on a selective transformation of one of the enantiomers and use UV−vis spectroscopy,[6] IR thermography,[7] circular dichroism,[8] mass spectroscopy,[9] or fluorescence[10] for detection.

Enzymatic methods for determining the enantiomeric excess (EMDee) have been used in a few cases.[6c,f,h,j,k,7] The principle of EMDee is to convert a mixture of enantiomers to a mixture of chemically different species by selective enzymatic transformations, which enables measurement of the enantiomeric excess by simple chemical analyses. A disadvantage of the methods reported so far is that they require control of product concentration and sample volume. Another drawback is that it is commonly not possible to simultaneously determine the yield and/or conversion and enantioselectivity of a catalytic reaction. This has been accomplished only in some rare cases using enzymes[6h,j] or catalytic antibodies.[6e]

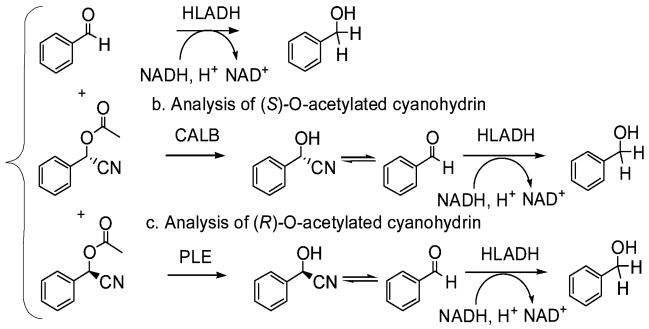
Enantiomerically pure cyanohydrins serve as highly versatile synthetic building blocks.[11] Much effort has therefore been devoted to the development of catalytic systems for enantioselective cyanation of aldehydes and prochiral ketones.[12] We have recently found a highly efficient catalytic system employing dual Lewis acid−Lewis base activation[13] for the preparation of highly enantioenriched O-acetylated cyanohydrins from aldehydes and acetyl cyanide (Scheme 1).[14] The reactions produce high yields of the desired compounds without concomitant formation of byproducts. By varying the reaction conditions, in particular, the Lewis base consisting of achiral or chiral amines, yields and selectivities in the catalytic reaction can be optimized. The methodology employed for the catalyst development is highly suitable for high-throughput screening since both the Lewis acid and Lewis base can easily be varied.

Herein, we describe an enzymatic method for the simultaneous determination of enantioselectivity, yield, and conversion of starting material in reactions with benzaldehyde using a combination of one selective enzyme and one nonselective enzyme. In contrast to earlier developed methods,[6c] the enantiomeric excess in this method is calculated from relative reaction endpoints rather than relative reaction rates, making it more robust and suitable for downscaling.

Scheme 1. Dual Lewis acid−Lewis base Catalyzed Addition of Acetyl Cyanide to Benzaldehyde



Scheme 2. One-Pot Step-by-Step Analysis of Unreacted Benzaldehyde (a), (*S*)-O-Acetylated Cyanohydrin (b), and (*R*)-O-Acetylated Cyanohydrin (c)



In the metal-catalyzed reaction, a scalemic mixture of the chiral O-acetylated cyanohydrin is obtained. For the analysis, a method based on the quantification of benzaldehyde using NADH and horse liver alcohol dehydrogenase (HLADH) was developed. Unreacted benzaldehyde is first reduced with NADH, which absorbs at 340 nm, producing unabsorbing NAD^+ (Scheme 2), allowing the amount of benzaldehyde to be determined by UV spectroscopy.

This is followed by enzymatic hydrolysis of acetylated cyanohydrin using *Candida antarctica* lipase B (CALB), which has a very high *S*-enantioselectivity for this substrate.[15] The free cyanohydrin of the *S*-product is in equilibrium with benzaldehyde and can, therefore, be analyzed in the same way as unreacted aldehyde, that is, by NADH reduction. Unselective hydrolysis using pig liver esterase (PLE) finally affords the free cyanohydrin of the second enantiomer, which is again reduced and analyzed using NADH. The change in absorbance due to consumption of NADH in the three consecutive steps can thus be used for determination of remaining starting material and the enantiomeric excess of the product. Since all analyses are performed using the same aliquot and only ratios between the different signals are used to calculate the enantiomeric excess and conversion, a precise knowledge of concentration and volume is not required. Knowing the sample volume, the total yield can also be determined. The protocol used results in a simple and robust method.

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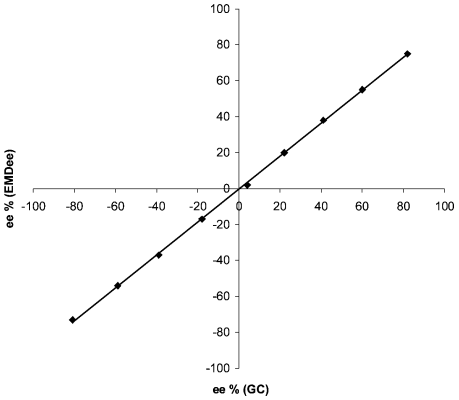

Figure 1. Plot of the enantiomeric excess values measured by GC versus those measured by EMDee. Negative enantiomeric excess values in this plot correspond to an excess of the *S*-enantiomer, while positive values denote an excess of the *R*-enantiomer. Linear regression analysis gave the following equation: EMDee = 0.92GCee + 0.3, $r2 = 0.9996$.

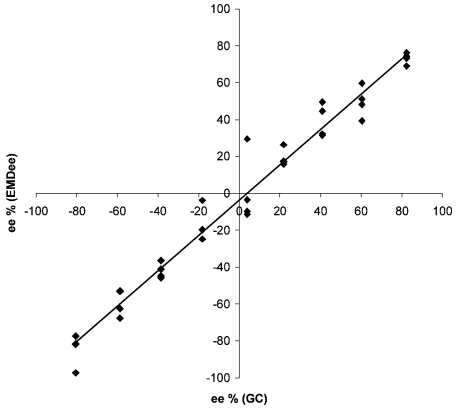

Figure 2. Plot of the enantiomeric excess values measured by GC versus those measured by EMDee in microtiter plates. Negative enantiomeric excess values in this plot correspond to an excess of the *S*-enantiomer, while positive values denote an excess of the *R*-enantiomer. Linear regression analysis gave the following equation: EMDee = 0.96GCee − 3.6, $r2 = 0.97$.

Samples with different enantiomeric excess values were prepared by mixing crude reaction mixtures of high enantiomeric excess, *S*- and *R*-major, in different ratios and subjected to the above enzymatic analysis. To validate the method, the samples were also analyzed by GC using a chiral column. For the initial study, using standard cuvettes (1 cm, 2 mL) and a spectrophotometer, excellent correlation between analyses using enzymes and GC was obtained (Figure 1).

To demonstrate the efficiency of this method, that is, its high-throughput character, samples were analyzed on a microtiter plate using a UV plate reader. Good correlation between the GC analyses and our EMDee was obtained (Figure 2). The precision of the enantiomeric excess determination was fairly good (±10%). This shows that the method can be used for high-throughput screening for discovery of catalysts exhibiting good activity and enantioselectivity.

We have thus access to an enzymatic method for rapid screening of reactions producing O-acetylated cyanohydrins. The crude reaction mixtures can be used directly, without any pretreatment, such as filtration, and analyzed accurately. Since our method, in contrast to previous enzymatic enantiomeric excess determinations, is based on the measurement of relative reaction endpoints, it is independent of exact concentrations, and accurate measurements of volumes are therefore not required, which makes this procedure suitable for downscaling. Preliminary experiments show that, although the method does not seem to be applicable to the analysis of aliphatic O-acetylated cyanohydrins,[16] it can be adapted to products derived from other aromatic aldehydes as well as to products obtained using other α-ketonitriles.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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