Measurement of Enantiomeric Excess by Kinetic Resolution and Mass Spectrometry**

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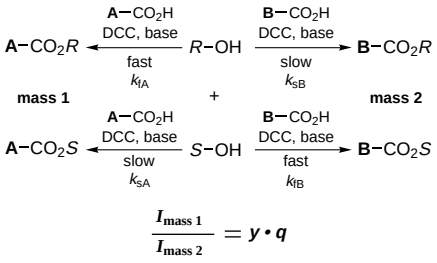
The powerful nature of combinatorial synthetic methods for the preparation of diverse libraries of small molecules has often made the analysis of such compounds the rate-limiting step in their application.[1] An intriguing target is the discovery of new enantioselective catalytic processes by the testing of large numbers of candidate catalysts.[2] Optimal use of combinatorial synthesis in this endeavor will require the high-throughput measurement of enantiomeric excesses on a small scale, a task for which current techniques are evolving.[1, 3] We describe here a method for the determination of enantiomeric excesses of alcohols and amines on the nanomole scale by diastereoselective derivatization and automated quantitative electrospray ionization mass spectrometry (ESI-MS).[4, 5] The capabilities of mass spectrometry for the analysis of component mixtures[4, 6] allows the chemical procedures and reagents used here to be simple and generally applicable.

The technique employs an equimolar mixture of pseudo-enantiomeric "mass-tagged" chiral acylating agents that differ in a substituent remote to the chiral center, such that the mass of the molecule is correlated to its absolute configuration. In general, the reactions of the enantiomers of any pair of chiral reagents will proceed with nonequal rate constants ($k_f > k_s$; f = fast, s = slow),[7] as shown in Scheme 1 for the reaction of



$$\frac{I_{\text{mass 1}}}{I_{\text{mass 2}}} = y \cdot q$$

Scheme 1. Generalized reaction of chiral alcohols with mass-tagged chiral acids in the presence of DCC and base. I = mass spectrum peak intensity, q = ionization correction factor, y = corrected intensity ratio.

alcohols with chiral mass-tagged acids **A**-CO_2H and **B**-CO_2H in the presence of 1,3-dicyclohexylcarbodiimide (DCC). The relative amounts of the product esters, measured in this case by mass spectrometry, can be used to determine the enantiomeric composition of the starting substrate using Equation (1)

$$\%\ ee = \left[\frac{(y-1)(s+1)}{(y+1)(s-1)}\right] \cdot 100 \quad \text{where} \begin{cases} s = \dfrac{k_f}{k_s} \\ y = \text{corrected intensity ratio} \end{cases} \qquad (1)$$

and two calibration measurements.[8] The method represents an elaboration of Horeau's procedure for the determination of the absolute configuration of secondary alcohols.[9] Although developed independently, it may also be regarded as an application of "parallel kinetic resolution" described recently by Vedejs and Chen.[10]

While several elegant nonenzymatic systems have been developed for kinetic resolution in acylation reactions,[10, 11] we employed the mass-tagged *N*-acylprolines **1a** and **1b**, along with DCC, as simple chiral acylating agents.[12] As discussed below, these structures were chosen not primarily for their ability to discriminate between nucleophile enantiomers, but rather because they are readily prepared and afford products which should be detected with high sensitivity in the electrospray technique. Although enantiomeric discrimination in the esterification of phenethyl alcohol (**2**) by these and related *N*-acylproline structures was only modest ($s = k_f/k_s = 2.0-2.6$ at room temperature), it proved to be sufficient to determine enantiomeric excesses using ESI-MS to measure small differences in the amounts of the mass-tagged products. Alcohols were analyzed by reaction with a 20-fold excess of an equimolar mixture of acids **1a** and **1b** in the presence of DCC and a catalytic amount of 4-dimethylaminopyridine (DMAP),[13] whereas amines were similarly derivatized with **1a**, **1b**, DCC, and 1-hydroxybenzotriazole. For these preliminary studies, 1 μmol of each alcohol and 10 nmol of each amine were used, but we have subsequently found that equal success may be achieved with 1–10 nmol (or less) of both types of substrates. After acylation, each mixture was simply evaporated, redissolved, diluted, and analyzed by ESI-MS in 5-μL injection aliquots, each of which contained no more than 50 pmol of the target esters or amides. Repeat injections were found to give reproducible intensity ratio to standard deviations of approximately 1–3 %.[14] We used two different instruments, one showing dominant $[M+H^+]$ ions and the other dominant $[M+Na^+]$ ions, both with good results.

Calibration must be performed on a racemic sample and a sample of known enantiomeric excess for each substrate structure. The observed mass ratio for the racemate defines a correction factor q which accounts for differences in electrospray ionization efficiencies between compounds differing by a methyl group. For the nonracemic calibration samples we chose enantiomerically pure (> 98 % *ee*) compounds available from commercial sources. The observed value of y (Scheme 1) and the known enantiomeric excess for the calibration sample are applied to a rearranged version of Equation (1)[15] to give s. The enantiomeric excess of other samples can then be measured from their observed MS intensity ratios, and the values of q and s obtained from calibration.

Figure 1 shows the results for nine secondary alcohols (**2**–**10**) and five primary and secondary amines (**11**–**15**) of varying structure, including aliphatic and aromatic substituents, each as samples of 20, 50, 70, and 90 % *ee*. Compound **15** is epibatidine, a potent nonopiod analgesic that has received

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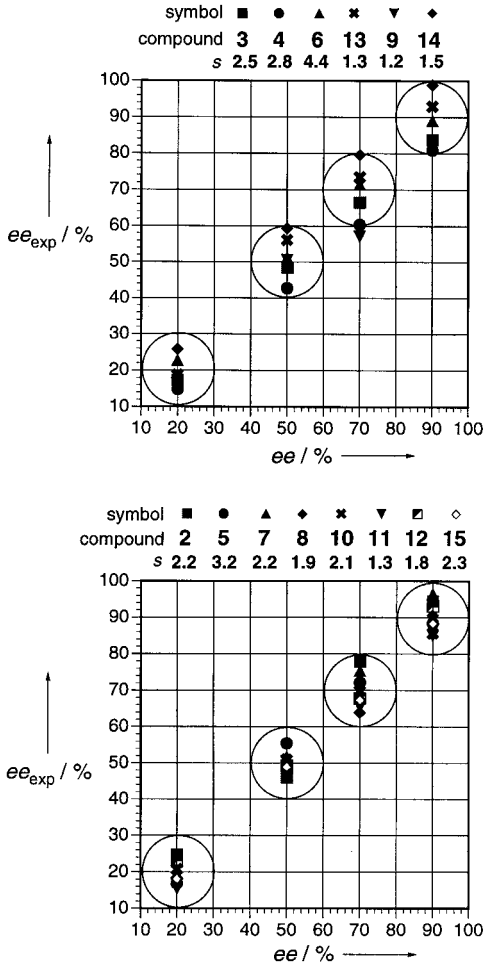
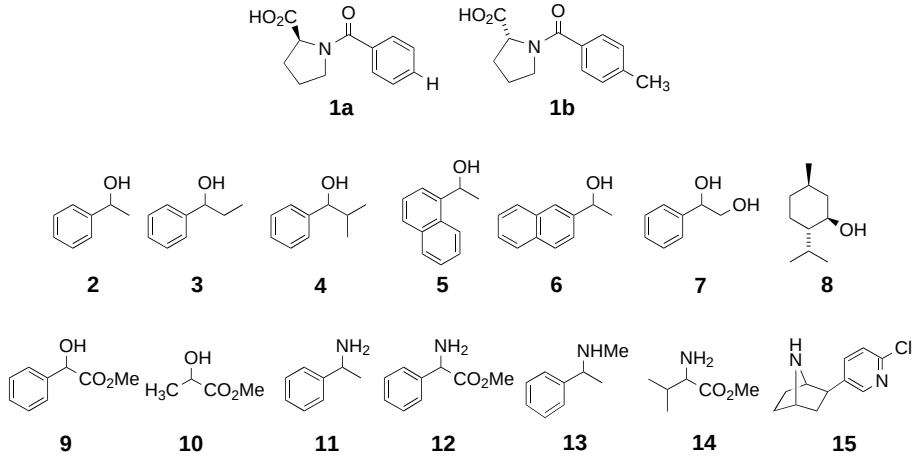


Figure 1. Plots of the actual versus measured enantiomeric excesses and *s* values for samples of **2**–**15** enriched in the *S* enantiomer (top) or the *R* enantiomer (bottom).



much recent attention.[16] Each point shown in Figure 1 is the averaged result of two independent acylation reactions per sample, each reaction being analyzed by three averaged ESI-MS injections. In every case but one (**9** at 70% *ee*), the measured value falls within 10% *ee* of the true value. In general, the greater the enantiomeric discrimination in the acylation process (*s* or 1/*s*), the better is the *ee* measurement. Remarkably, the mass spectrometer is sufficiently reliable to provide effective measurement of enantiomeric excesses for substrate–mass tag combinations showing k_f/k_s ratios as low as 1.2. Thus, the kinetic resolution process used to read the enantiomeric content of the desired species must proceed with an energy difference between competing diastereomeric transition states of only about 0.1 kcal mol^{-1}. Such a small energetic requirement means that a single pair of mass tags may be used to analyze many different types of structures, as observed here.[17]

The method for determining enantiomeric excesses described here has several distinctive features:

1) Because the necessary level of kinetic resolution is so low, it is likely that readily available chiral acids can be found for the analysis of chiral compounds of widely varying structures.

2) The technique can be reversed (mass-tagged chiral nucleophiles used to measure the enantiomeric composition of acylating agents) or used with many other bond-forming reactions as "reporter" processes, as long as they afford a small degree of kinetic resolution.

3) Instead of making use of known reactivity (the relative rate *s*) to measure unknown composition (*ee*), this approach may find utility for rapidly establishing structure–activity relationships by surveying reactivity (*s*) for compounds of known composition (structures of interest in racemic and enantiomerically pure form). Thus, the *s* values shown in Figure 1 reveal that better enantiomeric recognition occurs for the alcohols containing large aromatic groups (**5** and **6**), and highlights a potentially interesting difference between **4** and **9**, which are approximately the same size but undergo kinetic resolution with different efficiencies.

4) The method does not rely on chromatographic separation, and thus may be especially useful for cases in which chromatography is either ineffective or inconvenient.[18]

5) Since the mass-tagged chiral reagents are used in large excess and the desired masses can be selected from a complicated mass spectrum, this method is tolerant of reactive achiral impurities such as water and requires little or no purification.

6) The method is rapid,[19] amenable to automation, and usable for small amounts of substrate (10 nmol or less).[20] It is presently intended for the screening of asymmetric catalysts, for which purpose the determination of enantiomeric excess to ±10% is sufficient.

The development of new mass-tagged analytical reagents to improve the scope, sensitivity, and accuracy of the method is underway, as is its application to the screening of candidate catalytic asymmetric processes in a microtiter plate format.

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Experimental Section

Representative procedure for alcohols: A solution of alcohol (1 μmol in 50 μL of toluene) was placed in a small vial or microtiter plate. A mixture of **1a** (10 μmol) and **1b** (10 μmol) in 100 μL of CH_2Cl_2 was added followed by a mixture of DMAP (0.1 μmol) and DCC (10 μmol) in 50 μL of CH_2Cl_2. The vessel was sealed for 24 h to ensure complete reaction, although the method does not depend on reaching any particular percent conversion beyond what is necessary for detection of the desired esters. The solvent was then allowed to evaporate.

Representative procedure for amines: A solution of amine (10 nmol in 50 μL of toluene (THF in the case of **15**)) was placed in a 96-well microtiter plate. A mixture of **1a** (100 nmol) and **1b** (100 nmol) in 20 μL of CH_2Cl_2, 1-hydroxybenzotriazole (10 nmol) in 20 μL of THF, and DCC (100 nmol) in 20 μL of CH_2Cl_2 were sequentially added. The plate was sealed for 24 h, and the solvent was then allowed to evaporate. Two sets of identical reactions were set up for all samples, using the same stock solutions.

The reaction product obtained from each of the above procedures was taken up in 500 μL of methanol, and the precipitate was allowed to settle. For the alcohols, an aliquot of 5 μL of the supernatant was diluted to 1.0 mL before transfer to an HPLC autosampler vial. For the amines, an aliquot of 50 μL of the supernatant was diluted to 100 μL for analysis. The autosampler was used to deliver 5-μL samples to the electrospray mass spectrometer (Hewlett-Packard 1100 MSD). The flow rate of the mobile phase (methanol) was 0.5 mL min^{-1}, and samples were injected directly into the spectrometer at intervals of 2 min. A curtain gas of ultrapure nitrogen was pumped into the interface to aid desolvation of the charged droplets and to prevent particulate matter from entering the analyzer. The drying gas flow was set at a rate of 12 L min^{-1}. The drying gas temperature was set to 350 °C. The nebulizer pressure was set to 1810 Torr. A capillary voltage of 3500 V was applied to the interface sprayer to charge the sample droplets. The fragmentor was set at a relatively low potential (80 V) to minimize ion fragmentation. The data was acquired at a step size of 0.15 m/z, and was set to scan the relevant mass range. With this instrument and under these conditions, $[M+Na^+]$ ions were dominant. Samples **5**–**7** were analyzed in earlier runs on a Perkin-Elmer SCIEZ API100 single quadrupole LC-MS instrument (nebulizer gas 1.6 L min^{-1}, electrospray potential 4200 V, ultrapure nitrogen curtain gas at 1.6 L min^{-1}, 80 V orifice potential); under these conditions spectra were dominated by $[M+H^+]$ peaks. Each sample was analyzed with three averaged injections. A wash of 20 minutes at a flow rate of 2 mL min^{-1} was performed every 36 injections. Complete experimental details, including the preparation and characterization of mass-tagged reagents, are available in the supporting information.

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[12] The complementary set of acids, in which the *p*-tolyl and phenyl groups are switched, gave equivalent results.

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[13] Under DCC-mediated esterification conditions, we observe a small degree of epimerization of the *N*-acylprolines, but this does not interfere with the present application.
[14] The use of more concentrated samples, containing approximately 25 nmol of desired esters or amides along with the other components of the acylation reaction mixtures, showed poorer sensitivity and gave less accurate results (standard deviations for repeat injections of approximately 5%).
[15] $s = [\%\ ee(y+1) + 100(y-1)]/[\%\ ee(y+1) - 100(y-1)]$ for 100% *ee*, $s = y$.
[16] a) T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell, J. W. Daly, *J. Am. Chem. Soc.* **1992**, *114*, 3475–3478; b) J. W. Daly, *J. Nat. Prod.* **1998**, *61*, 162–172; c) Z. M. Chen, M. L. Trudell, *Chem. Rev.* **1996**, *96*, 1179–1193.
[17] For example, diol **7** was analyzed using the masses corresponding to its derived diesters containing two units of each mass tag (and ignoring diesters having one of each of the mass tags). It appears that the first derivatization of the primary alcohol occurs with little or no diastereoselectivity, whereas the secondary alcohol is esterified with $s \approx 2$. Thus, the analysis of chiral compounds such as diols containing multiple functionalities should also be feasible.
[18] Note, for example, that not all of the compounds analyzed here could have been accomodated by a single chiral HPLC or GC column. Of course, the techniques are related in that the chemical interactions responsible for molecular discrimination in chromatography are likely to be similar to those giving rise to diastereoselectivity in the derivatization process that preceeds mass spectrometry readout.
[19] Each mass spectrometry injection presently requires approximately two minutes. The analysis can be made more efficient by combining compounds of differing molecular weights in each injection.
[20] Our present sensitivity limit for quantitative ESI-MS of small molecules is approximately 500 fmol per μL.[6]

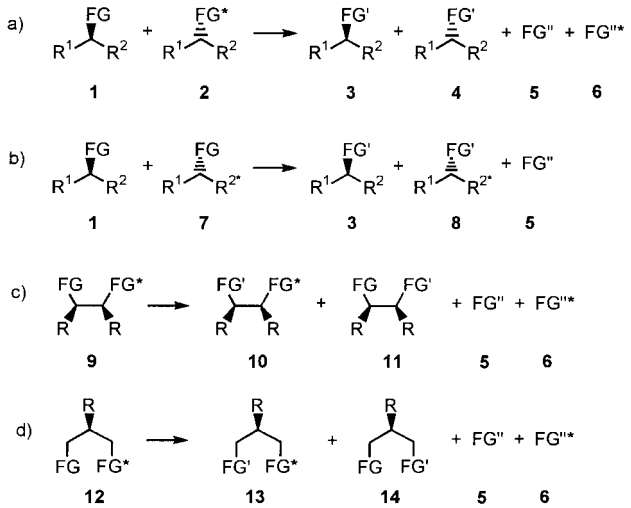
A Method for High-Throughput Screening of Enantioselective Catalysts**

Manfred T. Reetz,* Michael H. Becker, Heinz-Werner Klein, and Detlef Stöckigt

Whereas the principles of combinatorial chemistry are well established in pharmaceutical research,[1] extension to the area of catalysis is not as advanced.[2] One reason is that few general methods for high-throughput screening of heterogeneous and homogeneous catalysts have been devised. This applies all the more to enantioselective catalysts.[3] We have previously developed a screening system for the catalytic enantioselective hydrolysis of chiral *p*-nitrophenol esters in which the course of the reactions of the *R*- and *S*-configured substrates is monitored in a parallel manner by UV/Vis spectroscopy.[4] With the use of microtiter plates crude screening of about 800 different enantioselective catalysts is possible per day, in this case mutant lipases created by directed evolution.[4, 5] Accordingly, following expression of a library of mutant genes in *E. coli*/*P. aeruginosa*, the bacterial colonies on agar plates were collected and cultivated individually in the wells of microtiter plates, each supernatant containing a mutant lipase suitable for screening. By nature this particular screening system is restricted to chiral acids and cannot be used in the evaluation of asymmetric catalytic reactions involving chiral alcohols, diols, amines, amino alcohols, alkyl halides, or epoxides. Recently, IR thermography was introduced as a means to detect metal- or enzyme-catalyzed enantioselective reactions, but quantification still needs to be accomplished.[6]

We now describe a method based on electrospray ionization mass spectrometry (ESI-MS)[7] which enables the determination of enantioselectivity in about 1000 catalytic or stoichiometric asymmetric reactions per day. Two basically different stereochemical processes can be monitored by this approach, namely, kinetic resolution of racemates and asymmetric transformation of substrates which are prochiral due to the presence of enantiotopic groups.

The underlying principle is based on the use of isotopically labeled substrates in the form of *pseudo*-enantiomers or *pseudo*-prochiral compounds (Scheme 1).[8] The course of the asymmetric transformation—that is, the relative amounts of reactants and/or products—is detected by ESI-MS.[9, 10]



Scheme 1. a) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving cleavage of the functional groups FG and labeled functional groups FG*. b) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving either cleavage or bond formation at the functional group FG; isotopic labeling at R^2 is indicated by the asterisk. c) Asymmetric transformation of a *pseudo-meso* substrate involving cleavage of the functional groups FG and labeled functional groups FG*. d) Asymmetric transformation of a *pseudo*-prochiral substrate involving cleavage of the functional group FG and labeled functional group FG*.

In the case of kinetic resolution, compounds **1** and **2**, differing in absolute configuration and in labeling at the functional group FG*, are prepared in enantiomerically pure form and then mixed in a 1:1 manner, simulating a racemate (Scheme 1a). Following asymmetric functional group transformation (in an ideal kinetic resolution up to 50% conversion), true enantiomers **3** and **4** are formed, in addition to

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