Review Article

Emerging Methods for the Rapid Determination of Enantiomeric Excess

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ABSTRACT   Methods for enantiomeric excess determination using a variety of spectroscopic techniques are summarized. Particular attention is paid to techniques that have promise for application to problems of combinatorial catalyst discovery but have not yet been so employed. *Chirality 14:534–540, 2002.*   © 2002 Wiley-Liss, Inc.

This review is meant to introduce the reader to some developing methods for the rapid determination of the enantiomeric content of organic molecules, particularly those with promise for application to the discovery of new or improved asymmetric organic reactions. The history of the very young and exciting field of combinatorial development of *asymmetric* catalysts has been beautifully described by Reetz[1] in an article with which readers are encouraged to acquaint themselves. For the most part, I focus here on methods which have not yet been applied directly to the asymmetric catalytic challenge, but which may be worth considering in this regard. An emphasis on speed, allowing "high-throughput" measurements to be made on large numbers of reactions, means that relatively little attention will be paid to many of the well-established methods for the measurement of enantiomeric excess (ee), although these may provide the basis for more rapid techniques. Such "classical" methods include the covalent synthesis and detection of diastereomers using enantiomerically pure derivatizing agents,[2–6] the detection of transient diastereomeric interactions by NMR using chiral shift reagent[6,7] or chiral solvating agents,[8–10] and the use of chiral stationary phases in gas and liquid chromatography.[11–13] The last technology—HPLC and GC over chiral stationary phases—constitutes the present state of the art in terms of accuracy and generality, if not speed. Certain intriguing variations have been developed for specialized applications.[14,15]

The combinatorial development of novel (but not necessarily enantioselective) catalysts—involving screening for *activity* as the primary analytical task—is an older and somewhat more established enterprise, recently described by a number of authors.[16–21] Although more difficult than detecting the binding of candidate compounds to a target (the fundamental measurement underlying many types of combinatorial screening for drug discovery), the determination of catalyst activity is being addressed in true high-throughput fashion using detectors that can work in parallel. The physical observable is typically absorbance, flu-

orescence, heat, or the presence of the product of interest as revealed by mass spectrometry, the latter requiring highly specialized engineering to render in array format.[19]

In contrast, the combinatorial discovery of *asymmetric* catalysts requires the detection of enantiomers or a signal coupled to the selective production (or destruction) of enantiomers—a much tougher problem. With the exception of biocatalyst screening by Reetz,[1] these efforts have involved relatively limited numbers of catalyst candidates and thus the more traditional ways of measuring asymmetric induction have proven to be sufficient. The striking success of these endeavors (usually involving hundreds of reactions, compared to the tens or even hundreds of *thousands* of compounds typically employed in a primary screen for lead compounds in drug discovery) attests both to the insightfulness of the investigators and the great promise inherent in the field. In this article I hope to look ahead a bit and discuss the emerging techniques that will allow the combinatorial probing of enantioselective catalytic reactions to reach its full potential. As with all things combinatorial, the *analytical* challenges are the most daunting.

DIRECT DETECTION OF ENANTIOMERS

It would seem that the simplest strategy to adopt for the rapid determination of ee would be to directly detect the signature property of chiral molecules—their ability to rotate plane polarized light. The measurement of optical rotation and/or circular dichroism (CD) has two problems that make it difficult to employ in a practical manner: relatively low sensitivity and a correspondingly low tolerance

of impurities, particularly chiral ones. However, recent technical developments are overcoming these difficulties.

Circular dichroism detectors are now commercially available for use with HPLC instruments.[22] The use of CD spectroscopy for the direct measurement of ee has reached a very efficient stage in the hands of the Reetz and Mikami groups. The former showed that rapid HPLC separation of chiral product from achiral starting material could be coupled with the use of an in-line CD detector to provide measurement of ee in 1–2 min per sample.[23] The Mikami laboratory earlier described similar screening of combinatorial catalyst arrays in slightly over 3 min per sample,[24] and continues to refine the technique (<1.5 min per sample).[25]

A quantum leap in sensitivity has been achieved by Bornhop, who has described a microinterferometry method to obtain polarimetric information from picomolar amounts of chiral compounds held in a capillary tube of the type used for GC and HPLC analysis.[26,27] While an applicable device based on this technology has not yet been invented, the fundamental method holds great promise for bringing the direct measurement of optical purity to the necessary levels of scale, speed, and generality so as to be useful in high-throughput screening.

CD DETECTION OF DERIVATIVES IN WHICH AN ACHIRAL UNIT IS ADDED

While not yet generally applied to the rapid determination of ee, the technique of exciton-coupled circular dichroism, developed chiefly by Nakanishi, Berova, and co-workers, provides intense chiroptical signals for suitable compounds. Having long been used for the determination of absolute configuration,[28–30] the method requires the presence or installation of chromophoric groups on the chiral structure of interest, allowing measurements to be made on very small amounts of material.[31–33] Alcohols and amines, which can be decorated with chromophores by acylation, are therefore the most common analytes, but improvements continue to be made in the application of the technique to different classes of substrates.[34–36]

The phenomenon of *induced circular dichroism* is being exploited, often with porphyrin derivatives,[37,38] by the Nakanishi and Inoue groups, among others. The potential practical benefits include extending the detection of chirality to compounds lacking strong chromophores and offering partial solutions to the inconvenient requirement of making tailored derivatives of most chiral analytes for analysis by exciton-coupled CD. For the Inoue molecule, a bis(zinc octaethylporphyrin) derivative (**1a**, Scheme 1), binding of a chiral analyte (usually an amine) induces a screw-like displacement of one porphyrin ring with respect to the other in the adduct **1b**.[39,40] This establishes a chiral diporphyrin conformation that is readily characterized by CD and assigned to the absolute configuration of the amine. The tethered tetraarylporphyrin derivative **2a** (Scheme 1) of Nakanishi and co-workers operates similarly, with the exception that an achiral "carrier" structure (such as the diamine shown) is used to ferry the chiral analyte (alcohol
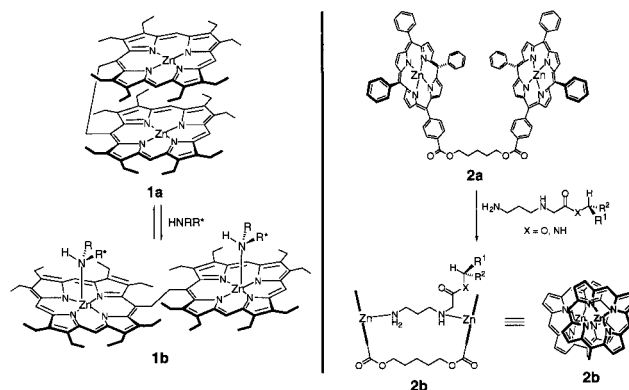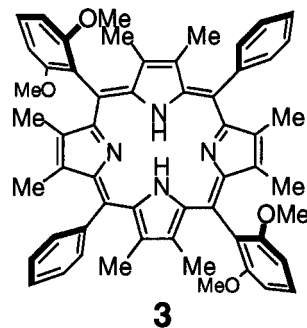

Scheme 1.

or amine) into the diporphyrin pocket, establishing a twisted conformation upon bridging of the two metal centers.[41,42] As with traditional exciton-coupled CD spectroscopy, both of these systems have been employed primarily for the determination of absolute configuration, but they hold some promise for the quantification of ee.

A conceptually distinct and clever use of porphyrins to report the interaction with a chiral analyte (in this case, carboxylic acids) is found in the "chiral memory molecule" of Aida and co-workers (Scheme 2) (**3**).[43,44] This nonplanar freebase porphyrin is a rapidly interconverting mixture of conformational enantiomers, but protonation locks the compound into its saddle-shaped structure. When the acid is chiral, a nonequal population of diastereomeric salts form. Remarkably, when the chiral acid is exchanged with an achiral one, the protonated porphyrin does not racemize, providing a persistent CD signal deriving from the previous interaction with the chiral acid.

ABSORPTION SPECTROSCOPY AS A READOUT OF CHIRAL INTERACTIONS

One may obtain information about catalytic asymmetric reactions, particularly kinetic resolutions, by separately examining the reactions of the resolved enantiomers of the substrate with the chiral catalyst. In this way the chiral analytical challenge is eliminated, replaced by the requirement to prepare pure enantiomers of the substrate in


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Scheme 2.

sufficient quantity to be useful for the discovery of a better catalyst for making them! When one wishes to pay this price, the best screening techniques are those that take maximum advantage of the situation by being amenable to true high-throughput implementation; electronic (UV-visible) spectroscopy is certainly the easiest analytical technique to employ in these circumstances. Applications to asymmetric catalyst discovery, using either chromophores built into the starting materials or generated from a product of the reaction in quantifiable amounts, are included in the review by Reetz.[1]

The physical separation of enantiomers using a chiral stationary phase in HPLC or GC is, as mentioned above, the most widely used method for determining ee. Some modifications have been made to speed this process, bringing it closer to true "high throughput" status. For example, selective-ion monitoring has been employed to eliminate the problem of overlap of starting material and product peaks eluting from a chiral HPLC column. This provided a relatively rapid (5 min per sample) HPLC-MS assay for ee of diarylcarbinols made by enantioselective yeast-catalyzed reduction of the ketone.[45] Capillary electrophoresis of arrayed reactions using multiple bundled columns has been performed in the presence of chiral electrolyte additives to achieve enantiomer separation and thereby the potential for true high-throughput screening.[46]

If one wishes to avoid the need to separate enantiomers, the challenge is then to connect a nonchiral signal such as absorption with a chirality-sensitive chemical probe of the analyte. In other words, the production of enantiomeric excess can be sensed with a "reporter" molecule—an approach that is conceptually related to the application of "Mosher esters" or related techniques. An intriguing example using uv-visible absorption spectroscopy has been provided by the group of Kubo with the development of a binaphthyl-based "chiral chromogenic receptor" (Scheme 3) (**4**).[47,48] Hydrogen-bonding interaction of β-amino alcohols or amino acids with the calixarene phenolic residues induces a chirality-dependent change in the absorbance spectrum of the structure. With proper quantitation and analyte purity, the method is adaptable to the determination of ee.
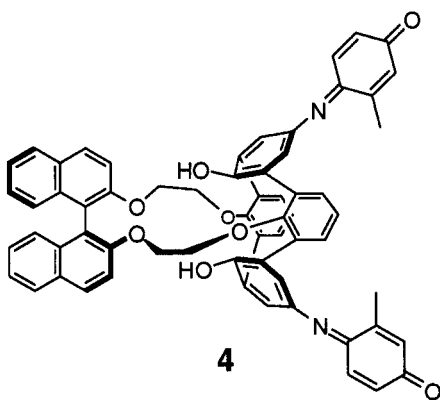
MASS SPECTROMETRY DETECTION OF CHIRAL INTERACTIONS FOR DETERMINATION OF ENANTIOMERIC PURITY

Mass spectrometry (MS) is also a technique that provides no chiral information but is attractive to high-throughput applications because of its wide scope (many different types of molecules can be detected without the need for a chromophore), high sensitivity, tolerance of impurities (peaks other than the masses of interest can be ignored), and potential for speed. Chiral information can be introduced in a variety of ways to measure ee several of which are becoming useful in practical applications. Each, of course, relies upon the creation of diastereomers or diastereomeric interactions. The first detection of a chirality-dependent effect on mass spectrometry signals was described in 1977,[49] and such phenomena have been actively pursued since that time.[50]

Elegant methods have been developed independently by the research groups of Cooks and Wan. The former relies on the difference in rates of dissociation of chiral ligands from gas-phase copper complexes generated in situ from the analyte of interest, copper ion, and an enantiopure "reference" compound.[51] An example is shown in Figure 1. The necessary cationic ternary copper complex is prepared in situ, electrosprayed and "isolated" in the gas phase by mass selection using a quadrupole mass spectrometer.[52] This complex exists in two diastereomeric forms, the relative abundance of which depends on the enantiomeric content of the analyte. Subsequent collisional excitation in an ion trap induces dissociation of either the neutral reference or analyte species. The relative rates of these processes are usually different for the two diastereomers, and therefore the intensity ratio of the resulting fragment complexes depends linearly on the enantiomeric content of the analyte. Calibration by use of the racemate and a sample of known ee is required. The method has been used with amino acids as the reference compounds and amino alcohols,[51] α-hydroxyacids,[53] and amino acids[54] as the analytes. The technique of Wan and coworkers is very similar, relying noncovalent interactions between
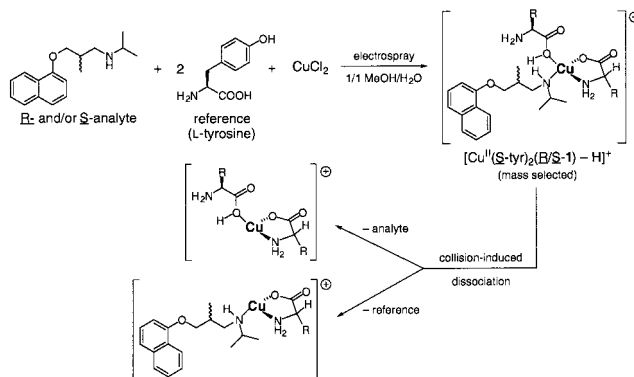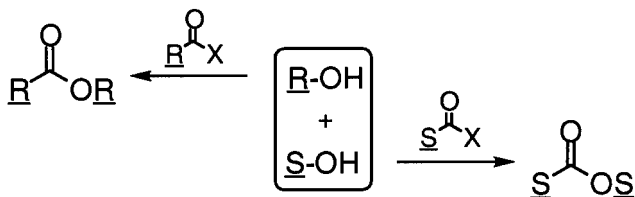

Scheme 3.

Fig. 1. The Cooks gas-phase "kinetic method" for ee determination; the detailed nature of the structures shown are speculative, and not specified by Cooks et al.

analytes and reference compounds in the absence of a central metal ion.[55,56] Further developments in this area will be greatly assisted by fundamental studies of the energetics of such interactions, such as those recently performed using molecular beams.[57]

KINETIC RESOLUTION IN THE SERVICE OF CHIRAL DETECTION

The ability of a chiral reagent or catalyst to selectively derivatize one enantiomer of a substrate in the presence of the other is, of course, of tremendous importance to asymmetric synthesis. If one knows (or can easily measure) the enantiomeric selectivity of the reagent or catalyst, then such a process can also be used to determine the enantiomeric content of the substrate. As a hypothetical example, consider Scheme 4, showing the treatment of an enantiomeric mixture of alcohols, R-OH and S-OH, with enantiomeric acylating agents. If kinetic resolution is perfect, reaction will occur only in "matched" sets, say R-alcohol reacting with R-acylating agent and S-alcohol with S-acylating agent at exactly the same rate. The product esters are also enantiomeric, but the acyl component can be chosen to facilitate the detection of the relative amounts of products, and thus the enantiomeric ratio of the starting alcohol. Two situations have been developed: 1) the derivatizing agent is employed in a "tagged" form, and must therefore be prepared as a mixture of "pseudo-enantiomers"; and 2) the derivatizing agent is a true racemate which incorporates a functional group that gives a dominating chiroptical signal. In the former case, the necessary chiral information is encoded into the chemical agent, whereas in the latter case the chiral information is extracted from the product mixture.

Kinetic resolution is never found to be the "perfect" process, shown in Scheme 4. Instead, each enantiomer of the substrate (analyte) undergoes reaction with both enantiomers of the derivatizing agent, characterized by "matched" (fast) and "mismatched" (slow) rates. Scheme 5 shows the more complete scenario. If one knows the kinetic resolution selectivity, defined as the relative rates of the competing fast and slow processes (k_f/k_s, in the kinetic resolution literature, often termed s or E), then a measurement of the relative amounts of the products from R and S derivatizing agents will give the relative amounts of the starting analyte. Such a measurement is often enabled by choosing derivatizing agents which give rise to distinct signals, most commonly mass, uv-visible absor-
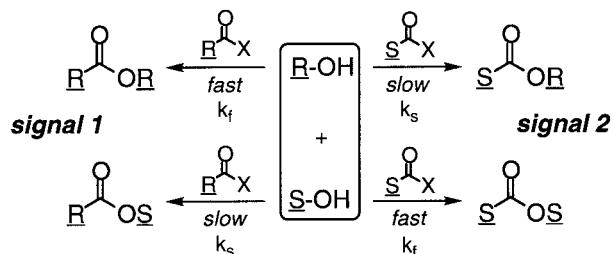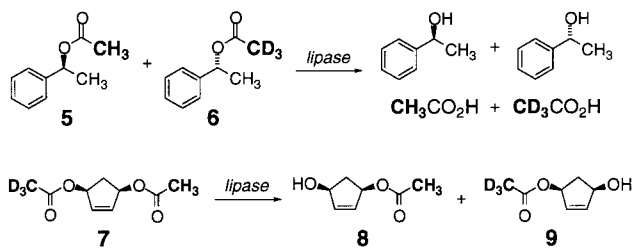

Scheme 5.

bance, or fluorescence. When such experiments are performed with equimolar amounts of derivatizing agents, the mathematics are particularly simple.[58] Such situations have been described and exploited for asymmetric synthesis by Vedejs and Chen as cases of "parallel kinetic resolution"[59] and were used by Horeau as the basis for the "Horeau method" of absolute configuration assignment.[60]

We have taken advantage of kinetic resolution in a mass spectrometry-based method (MSEED = mass spectrometry enantiomeric excess determination) using both soluble[61] and resin-bas[62] mass-tagged chiral acylating agents. The salient feature of the technique is that electrospray ionization MS provides sufficient precision in the determination of *relative* peak intensities to allow reliable measurements to be made even when the kinetic resolution selectivity of the derivatization process is poor. It can therefore be applied to many different types of analytes, using a variety of different types of reactions to perform the kinetic resolution step. Shair and co-workers have employed a similar type of kinetic resolution assay by attaching fluorescent tags, instead of mass tags, to the pseudo-enantiomeric acylating agents.[63] The ability to perform fluorescence measurements in arrays allows the screening of thousands of samples for high enantiomeric content.

Reetz et al.[64] have employed the complementary approach of encoding mass information into the substrates of enantioselective reactions in order to screen for kinetic resolution selectivity. This work is therefore conceptually similar to the tagging of enantiomers with dyes and examining their separate rates of reaction with a reagent or catalyst of interest, mentioned above. However, mass labels have the outstanding advantage of being nonperturbing (keeping the chemistry of the labeled compound the same as the chemistry of the "real" target) and more generally applicable. Thus, as shown in Scheme 6, a mixture of the pure enantiomers **5** and **6**, mass-tagged and of opposite absolute configuration, are subjected to hydrolysis by a chiral catalyst, in this case a selection of lipases. Kinetic resolution can be easily and rapidly quantified by the measurement of the relative amounts of **5** and **6** remaining, as well as acetic acid and acetic acid-d$_3$ produced. Furthermore, the pseudo-meso compound **7** reveals the enantioselectivity of the lipase catalyst by measurement of the ratio of **8** and **9** obtained. This method also requires the preparation of substrate (**7**) in enantiomerically pure form.

Hattori, Finn, and co-workers have also applied the scenario of Scheme 5 to examples in which no kinetic resolu-

Scheme 4.

Scheme 6.

tion selectivity is observed in the derivatization (acylation) step.[68] In this case, chiral information is provided by a combination of factors: a binaphthyl unit is employed in the acylating agent to provide a strong CD signal, but that signal must be modulated by the chirality of the analyte. Thus, when no kinetic resolution is obtained ($s = k_f/k_s = 1.0$), equal amounts of "signal 1" and "signal 2" compounds are produced, and there is no CD signal if the CD spectra of the products are *completely* determined by the binaphthyl component alone. However, in the majority of cases examined the CD spectrua with *R*CO*R*'s and *R*CO*S*'s ("signal 1" compounds) are slightly different. Likewise, the CD spectra of *S*CO*R* and *S*CO*S* ("signal 2" compounds) are slightly different from each other and of opposite sign to the

"signal 1" pair. Thus, a residual CD signal is obtained for the product mixture, which can be related to the ee of the starting analyte when proper calibrations are performed.

Lastly, we have taken the first steps to bring mass spectrometry methods for ee determination to true high-throughput status by marrying our kinetic resolution technique with a rapid chip-based MS sampling method. DIOS (desorption/ionization on silicon) mass spectrometry utilizes MALDI (matrix assisted laser desorption/ionization) instrumentation to analyze small molecules from porous silicon sample plates.[65–67] The porous silicon chips can be photopatterned to make arrays of MS-active spots, onto which submicroliter amounts of analyte solution can be deposited. More than 100 spots per 4 cm^2 chip can be easily created, separately addressed, and analyzed.

Figure 2 shows the protocol, sample data, and results for the analysis of two aromatic carbinols by a combination of the MSEED and DIOS techniques (Shen et al., unpubl.). The observed values are well within the ±10% error range characteristic of MSEED, but the MS data was acquired at a rate of 1 min per sample. Data acquisition rates of 5–10 sec per sample are now routine for DIOS (Shen and Siuzdak, unpubl.), which should be applicable to any MS-based method. We are currently optimizing this rapid technique for measuring enantiomeric excess and will fully describe the details in due course.
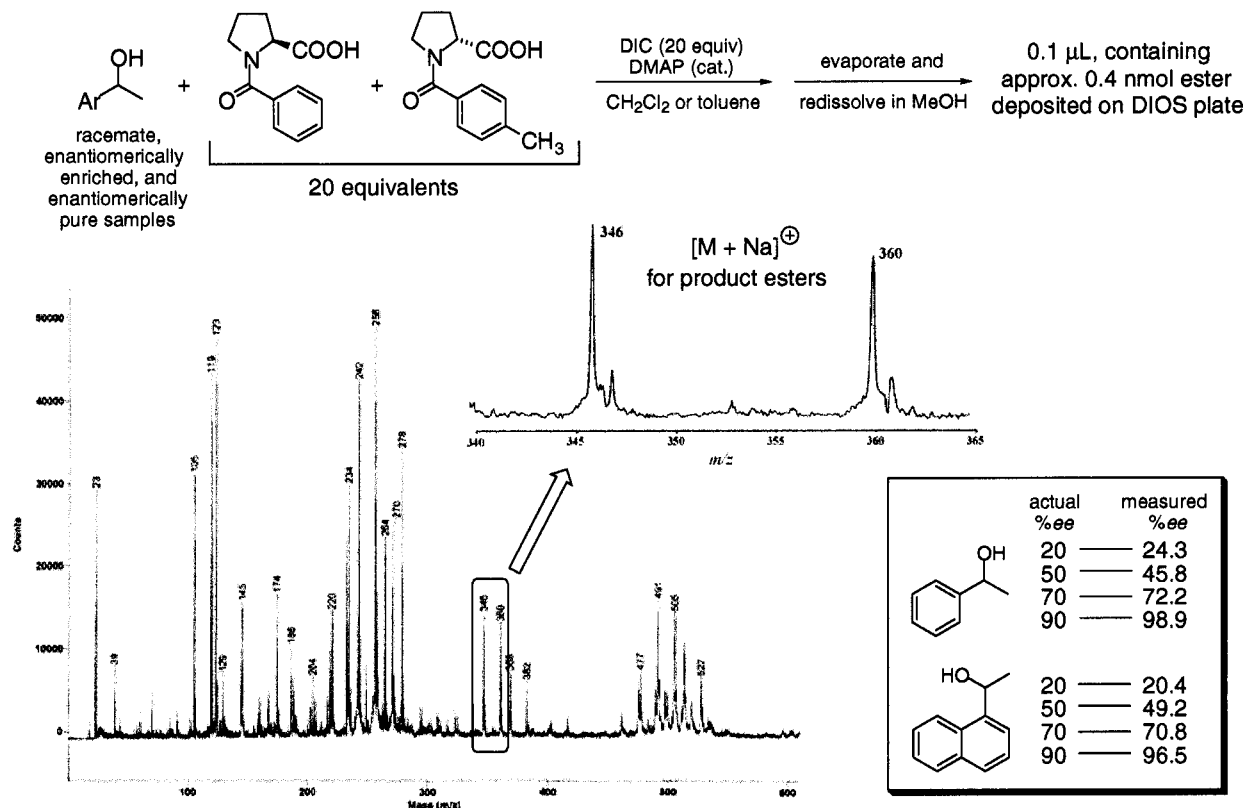

Fig. 2. MSEED-DIOS determination of the enantiomeric content of two secondary arylcarbinols. (*top*) Summary of experimental protocol. (*bottom*) Sample DIOS-MS spectrum for one of the *sec*-phenethyl alcohol samples, with an expansion of the section containing the two peaks of interest. (*lower right*) Summary of the results.

OUTLOOK

It is apparent from the work described here and elsewhere[1] that, while the determination of ee has been the "slow step" in many combinatorial approaches to catalyst discovery, there is unlikely to be a single technique that will answer all such problems. The field must develop to the point at which different types of measurements achieve validation in practical applications, so that the appropriate analytical technique can be smoothly implemented by newcomers with good ideas for novel engines of enantioselectivity. It is also true that enhanced speed and reliability in ee determination will enable ever more sophisticated and practical efforts in reaction optimization, as distinct from reaction discovery. The day is approaching when the industrial process chemist, searching for better yield and asymmetric induction in an enantioselective step of a large-scale synthesis, will mount a fast and extensive combinatorial survey of catalysts, solvents, temperatures, and additives to find just the right conditions for the *particular* transformation of interest. The mechanistic chemist, too, will be empowered to look more widely and deeply for structure–activity relationships involving enantioselectivity. At that point, catalyst discovery will involve searches for both generally applicable and highly tailored systems, and mechanistic insights will abound.

ACKNOWLEDGMENTS

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