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High-throughput methods for the development of new catalytic asymmetric reactions

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Chiral, single enantiomer pharmaceuticals have become increasingly more important. Therefore, research aimed at providing new methods for their selective preparation has taken on an even greater importance. One of the most efficient strategies for the synthesis of non-racemic, chiral molecules is asymmetric catalysis. There are many variables involved in the discovery of a new catalytic asymmetric transformation; hence, methods for the rapid screening of large numbers of catalysts have been developed. Herein, these techniques and strategies for the rapid discovery of novel asymmetric catalysts are reviewed.

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▼ Until several years ago, the use of diversity approaches in solving problems outside of drug discovery had not been fully recognized and embraced by the chemical community. [1] Since then, combinatorial strategies have been applied successfully in a broad range of areas, including polymer chemistry [2], materials science [3] and molecular recognition. [4] In particular, the development of new catalytic reactions exemplifies the considerable advances that have been made in applying diversity-based problem solving techniques in a new research area [5–10]. In this regard, this review focuses on the development of HTS methods for the discovery of new catalytic reactions with a special emphasis on asymmetric transformations.

The need for enantioenriched compounds

As the preparation of chiral, enantiomerically pure pharmaceutical agents has become more important, the need for new synthetic methods for their efficient preparation has also increased [11]. In 2001, single-enantiomer pharmaceuticals accounted for 36% of the US$410 billion drug industry [12]. Marketing estimates for drug manufacturing suggest that the need for chiral materials in pharmaceutical preparations will continue to rise with a 9.4% annual growth between 2000 and 2005. The introduction of novel asymmetric methods can offer effective new avenues for the preparation of the enantiomerically pure drugs of the future.

Notwithstanding the value of other techniques for generating enantiomerically enriched compounds, catalytic asymmetric reactions hold particular promise for providing desired products with the greatest efficiency and least waste. Whereas resolution strategies can often lead to significant material loss, and auxiliary strategies can add additional steps to a synthetic sequence, catalytic asymmetric transformations can, in theory, yield the desired enantiomerically enriched targets directly in the presence of just a small amount of a chiral additive (i.e. catalyst). Unfortunately, the list of reliable catalytic asymmetric transformations that chemists have at their disposal for addressing their synthetic challenges is still fairly limited. Not surprising, the development of useful new reactions continues to be a fruitful and active area of research.

Combinatorial versus traditional methods for asymmetric catalyst discovery

Traditionally, catalysts with new reactivities have been developed by an iterative process involving catalyst design, synthesis of the desired system, testing of the system and subsequent modification of the design until an acceptable level of reactivity and selectivity is obtained. High-throughput, or combinatorial approaches can, in principle, accelerate the discovery and development of new catalysts by synthesizing and testing many catalyst

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modifications simultaneously. Not only does this modification to the reaction development protocol enable a more rapid catalyst-discovery process, it can also provide substantial mechanistic insight into a transformation (by way of the volume of data generated), as well as facilitating serendipitous discovery.

Crucial to the evaluation of a catalyst library is the ability to analyze quickly and accurately the efficiency of the reaction [13,14]. Asymmetric catalysis poses an additional hurdle because enantioselectivity must be measured in addition to reaction conversion. Individual asymmetric catalysts can lead to either antipode of a desired product; therefore, the ability to screen mixtures of catalysts becomes unreliable because of the difficulty in differentiating between mixtures of highly selective catalysts providing equal and opposite antipodes of the desired products, and non-selective catalysts yielding products with little selectivity. Because of this limitation in examining mixtures, screening for enantioselectivity typically entails a parallel, spatially segregated approach. This reaction-screening format has its limitations and, in particular, depending on the number of variables, can lead to a large number of parallel reactions that all require evaluation for reactivity and/or selectivity.

The rapid or simultaneous assessment of many reactions can be a limiting factor in using high-throughput strategies for catalyst development. Fortunately, numerous ingenious methods have been brought to bear on the evaluation problem. Instead of discussing recent advances in new reaction development by reaction type or screening format, this review will focus on the analytical strategies employed to evaluate reaction efficiencies under the unique demands of HTS formats.

Chromatographic methods for HTS

Although inherently serial in design, one of the most common methods for screening asymmetric reactions involves chiral chromatography. Both chiral HPLC and gas-liquid chromatography (GLC) have been used in HTS because of their accuracy in determining both reaction conversion and enantioselectivity, in addition to being easily automated.

In 1998, Porte and co-workers developed a novel phosphine oxazoline ligand for an asymmetric Pd-mediated allylic substitution reaction featuring HTS with chiral HPLC as the means to evaluate each reaction (eqn 1, Fig. 1a) [15]. Parallel reactions followed by automated chiral HPLC analysis enabled the screening of various ligands (synthesized in a combinatorial fashion to allow for diversity), solvents, ligand to metal ratios and additives. Ultimately, a catalyst system was found that gave 78% yield and 90% enantiomeric excess (ee) of the desired alkylated product.

Recently, Long *et al.* reported a catalytic, enantioselective hetero-Diels Alder reaction (eqn 2, Fig. 1b) [16]. Several diol ligands with $Ti(OiPr)_4$ as a Lewis acid were screened for the formal (4 + 2) addition of Danishefsky's diene to aromatic aldehydes. Thirteen diols in binary combinations with all other ligands (104 reactions) were screened by chiral HPLC. Under solvent-free conditions, highly efficient ligand–metal loading as low as 0.05 mol% were identified, with the ligand illustrated providing the cycloaddition products in >90% yield with ee's to 99%.

Using a HPLC assay to screen peptide-based ligands, Sculimbrene and Miller recently succeeded in developing a catalyst to enantioselectively phophorylate a partially benzyl-protected inositol leading to an efficient synthesis of D-*myo*-inositol-1-phosphate (eqn 3, Fig. 1c) [17]. Although known routes to this molecule involved numerous steps and the use of chiral auxiliaries, the screening of several peptide-based catalysts provided an effective catalyst (2 mol% loading, 65% yield, >98% ee) that leads to the target molecule in a more efficient and direct manner. Because of the function of inositol phosphates as secondary messengers, and the previous difficulties in their synthesis [18], this application nicely highlights the potential of HTS methods for the development of new catalytic asymmetric transformations.

In 1998, Sigman and Jacobsen reported a catalyst for the asymmetric hydrocyanation of imines (the asymmetric Strecker reaction) discovered through HTS of chiral Schiff base ligands (eqn 4, Fig. 1d) [19]. The process was initiated by solid-phase screening of an amino acid-urea-diamine-salicylaldehyde ligand with 11 different metals. The results indicated that the reaction without a metal salt gave the best result (59% conversion, 19% ee). In the second phase, 48 ligands were synthesized on solid phase with varying amino acid R groups, chiral diamines and salicylaldehydes. A few lead ligands were extracted from this library and, after classical ligand optimization, a third round of screening was conducted on 132 ligands. The resulting ligand yielded chiral amino cyanides with yields from 65–92% and ee's from 70–91%.

Limitations and strategies for optimizing HPLC and GLC screening

Although the use of chiral HPLC and GLC has been well established, they are limited in the number of reactions that can be screened per day. A fairly rapid chiral HPLC run can require 10 minutes and often longer, which translates to only 144 reactions screened per day per instrument. A few solutions to eliminate this potential bottleneck have been proposed. Reetz developed a method for chiral GLC where long enantiomer resolution times are taken advantage

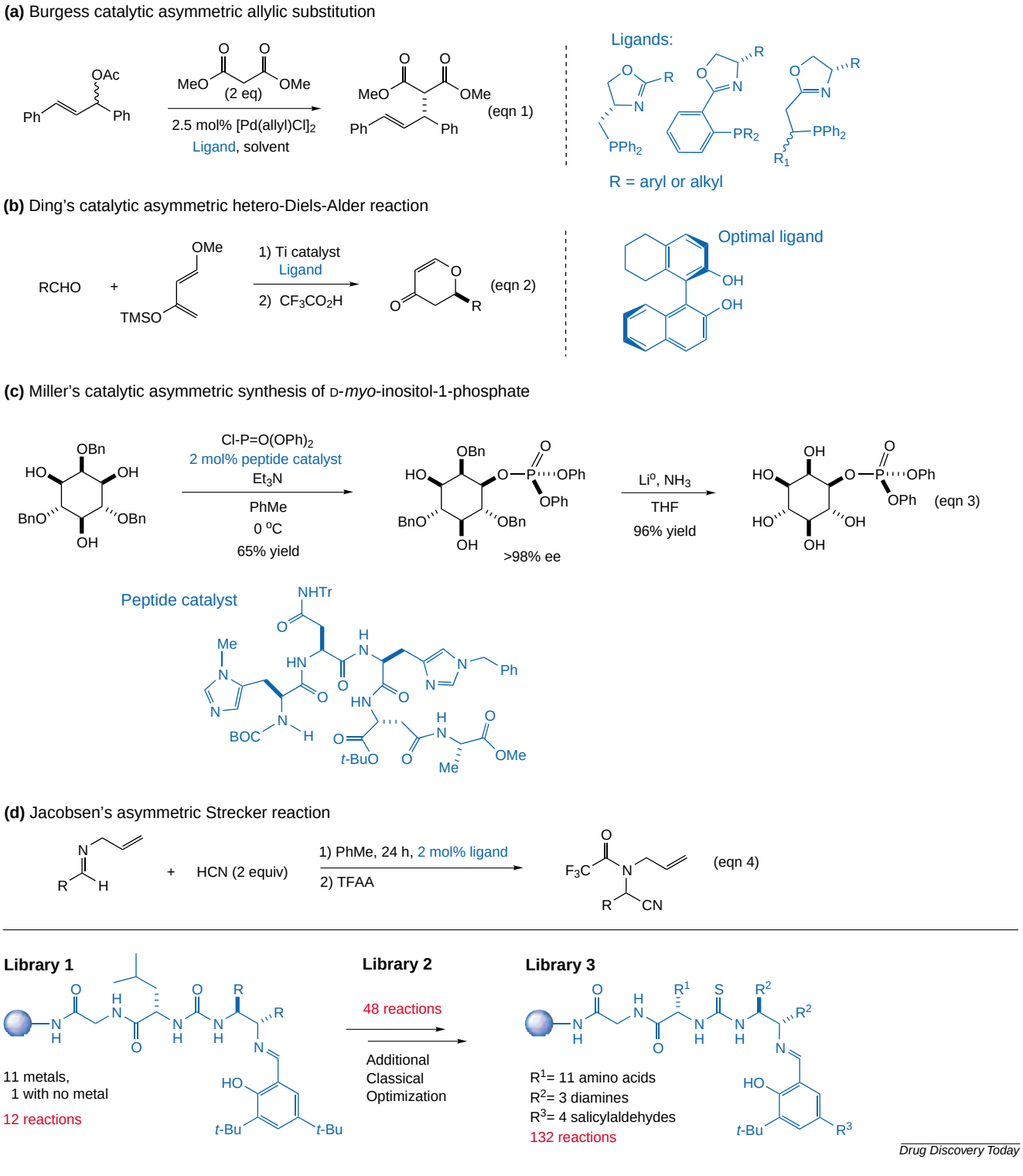

Figure 1. Catalytic anantioselective reactions developed using HPLC and/or GLC for evaluating reaction selectivity and reactivity. Abbreviation: TFAA, trifluoroacetic anhydride.

of by overlaying the GLC runs [20]. This overlap enables up to 700 reactions to be screened per day using an isothermal run and specialized software. Another path to maximize GLC time is to separate multiple compounds in parallel runs. Although this requires the ability to separate each individual compound in each GLC run, it has been used

successfully by Brouwer and co-workers to monitor the asymmetric addition of diethylzinc to different aldehydes [21].

To help handle assay limitations in catalyst synthesis and reaction screening, our laboratory (in collaboration with our colleague Amir Hoveyda and his group) employs sampling strategies to reduce the total number of reactions to be run and evaluated [22]. The peptide-Schiff base catalyst, initially described by Inuoe, was considered most suitable for this diversity-based approach to new reaction discovery [23]. The chiral ligand structure depicted in Figure 2 is modular and easily synthesized, either in solution or on solid phase using standard peptide-coupling procedures. The large number of commercially available amino acids and substituted salicylaldehydes (or other functionalized benzaldehydes) enables a diverse range of ligands to be synthesized. For example, if 20 salicylaldehydes and 20 different amino acids were varied at each position, 8000 (20^3) different ligands would result (not including metal, solvent, additive and temperature screening). Assuming a 20 minute HPLC or GLC run, only 72 reactions could be monitored per day, leading to the requirement of at least 111 machine-days of analysis for this hypothetical, yet modest library.

To circumvent this assay limitation, a positional screening method to sample ligand diversity was adopted and used in the discovery of a competent catalyst for the asymmetric opening of *meso* epoxides by cyanide (eqn 5, Fig. 2). [24] Through this methodology, only one module of the ligand is varied; the others are kept constant. In the optimization of the reaction illustrated in eqn 5, the first step was to screen AA [1] (10 amino acids) while keeping the rest of the ligand constant. This small ligand library lead to a catalyst that offered an increase in selectivity from 26–56% ee by switching AA [1] of the peptide ligand from L-Val to L-Tle (L-tert-leucine). In the second library, L-Tle is kept at AA [1] while the L-Phe at AA^2 (16 amino acids) was varied, resulting in L-Thr(O*t*-Bu) as the optimal amino acid (63% ee). Finally, the salicylaldehyde module was varied (13 aldehydes), with 3-fluorosalicylaldehyde yielding 86% ee. From 2080 possible ligands ($10 \times 16 \times 13$), less than 2% of the possibilities ($10 + 16 + 13 = 39$ ligands) were synthesized and screened for a selective catalyst to be discovered. Although this method assumes no co-operativity between modules (which might or might not be the case), the goal is not necessarily to find the best ligand out of the 2080 possibilities, but simply to find a competent ligand for the catalytic asymmetric reaction as efficiently as possible.

The sampling strategy of this modular peptide ligand has proven fruitful, as illustrated by the number of new asymmetric reactions that have been developed using this protocol (Fig. 2, eqns 6–10) either collaboratively, or independently by Amir Hoveyda and his group. These reactions include the asymmetric Strecker synthesis of aryl [25] and alkenyl [26] amino nitriles and acids (eqn 6), the catalytic, asymmetric, three-component synthesis of aryl and alkyl amines (eqn 7) [27], the formation of non-racemic tertiary alcohols via asymmetric addition of cyanide to ketones (eqn 8) [28], the conjugate addition of dialkylzincs to allylic phosphates to yield non-racemic tertiary and quaternary centers (eqn 9) [29], and the asymmetric conjugate addition of dialkylzincs to cyclic [30] and linear enones (eqn 10) [31].

UV–visible spectroscopy

To circumvent the limitations of serial chromatography, several groups have taken advantage of the wide availability of UV–visible plate readers. As plate readers measure the absorbance of light through a 96-well (or more) plate, clever techniques were needed to adopt this instrument towards screening for enantioselectivity.

In 1997, Reetz reported the *in vitro* evolution of an enzyme to create a selective lipase for resolution of racemic esters [32]. The enzyme chosen was the lipase from the bacterium *Pseudomonas aeruginosa*, which in the wild type catalyzes the hydrolysis of the nitrophenol ester with a selectivity of only 2% ee. Mutagenesis of the DNA representing the enzyme using error-prone PCR [33] resulted in a library of ~1000 new enzymes. By measuring the rate of enzyme hydrolysis of separated enantiomers of the substrate [k (S) and k (R)] as measured by the absorbance of *para*-nitrophenol over time, new enzymes with improved selectivity could be found rapidly (eqns 11,12; Fig. 3a). The improved DNA for the more selective enzymes was then remutated and rescreened. After only four rounds of mutation and screening, an enzyme that afforded the desired compound with an ee of 81% was found. Because ~4000 reactions had to be evaluated, neither HPLC nor GLC were realistic options for screening. Reetz has further developed this method for identifying enzymes that are selective for generating the opposite antipode [34], as well as improving the original enzyme through additional directed-evolution techniques [35]. A related method was developed by Reymond and co-workers, where the substrates of each reaction were enzymatically converted to a fluorescent product, which was used to evaluate a catalytic antibody library for the chiral resolution of acetate esters [36,37].

An enzymatic method for determining enantiomeric excess (EMDee) using the absorbance of nicotinamide adenine dinucleotide phosphate (NADPH) was developed by Abato and Seto (eqn 13, Fig. 3b) [38]. Using the well-established enantioselective reaction of diethylzinc to aldehydes as a test reaction, Seto analyzed the chiral alcohol products

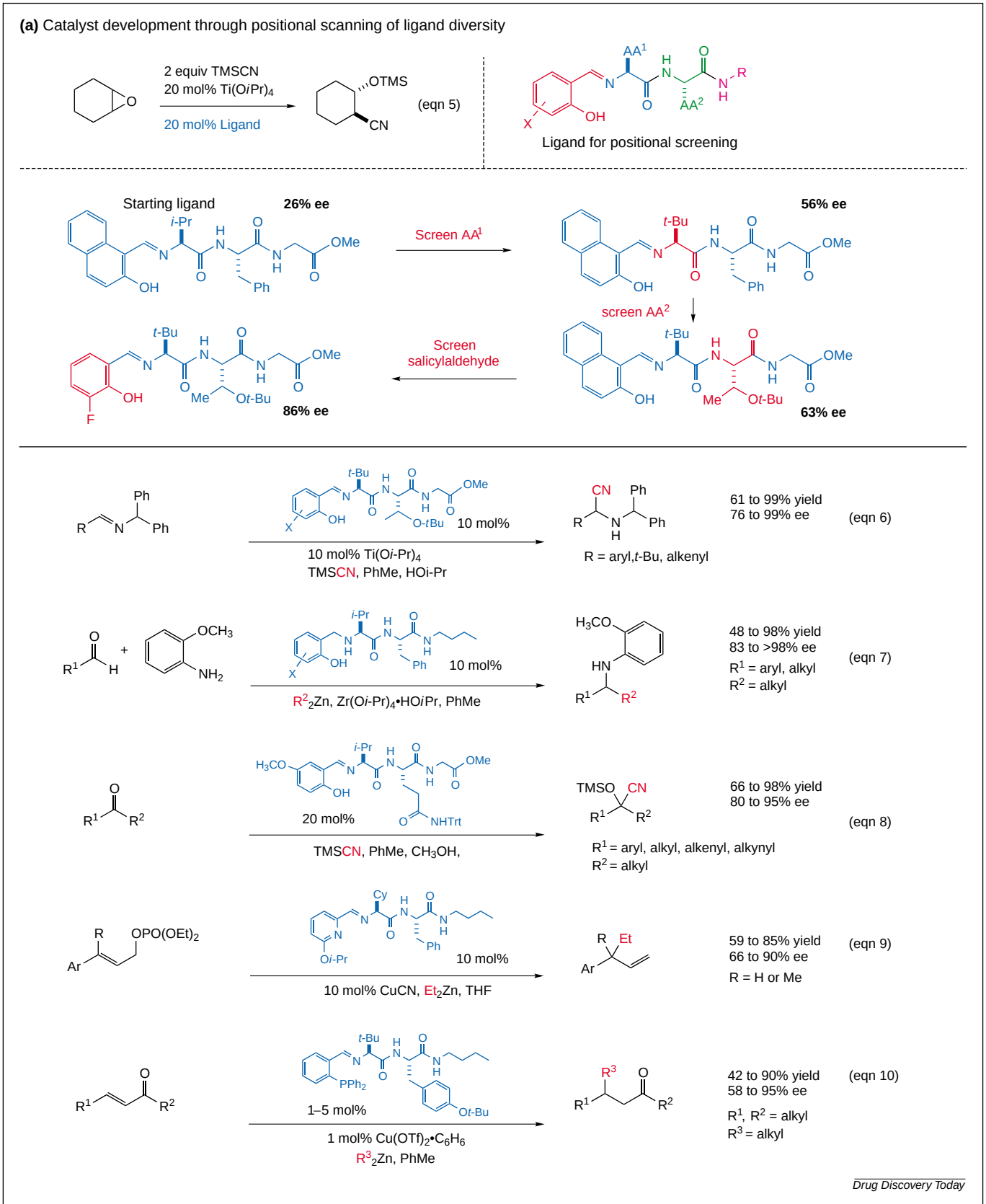

Figure 2. Examples of new catalytic asymmetric reactions developed through positional screening of peptide ligands. Abbreviations: THF, tetrahydrofuran; TMSCN, trimethylsilyl cyanide.

by treating them with alcohol dehydrogenase and NADP+. The (S) enantiomer is a known substrate for the enzyme, whereas the (*R*) enantiomer is a known inhibitor. As both the K_m and K_i are known, the ee can be determined using the Michaelis–Menton equation. Therefore, by monitoring the appearance of NADPH (which absorbs at 340 nm) over time, reactions can be monitored easily using standard 96-well plate readers. The reaction is limited because the desired products must be substrates for a known enzyme with a product or side product that is detectable by UV–vis methods, and only reactive and selective catalysts are identified. Although this places some limitations on the technique, the authors point out that a large number of alcohol dehydrogenases are available and because of the ability to screen large numbers of reactions, the most interesting reactions are those that proceed with both high conversion and enantioselectivity. The authors claim that 100 readings can be taken in 30 minutes, with an error of less than 10% when compared to chiral GLC.

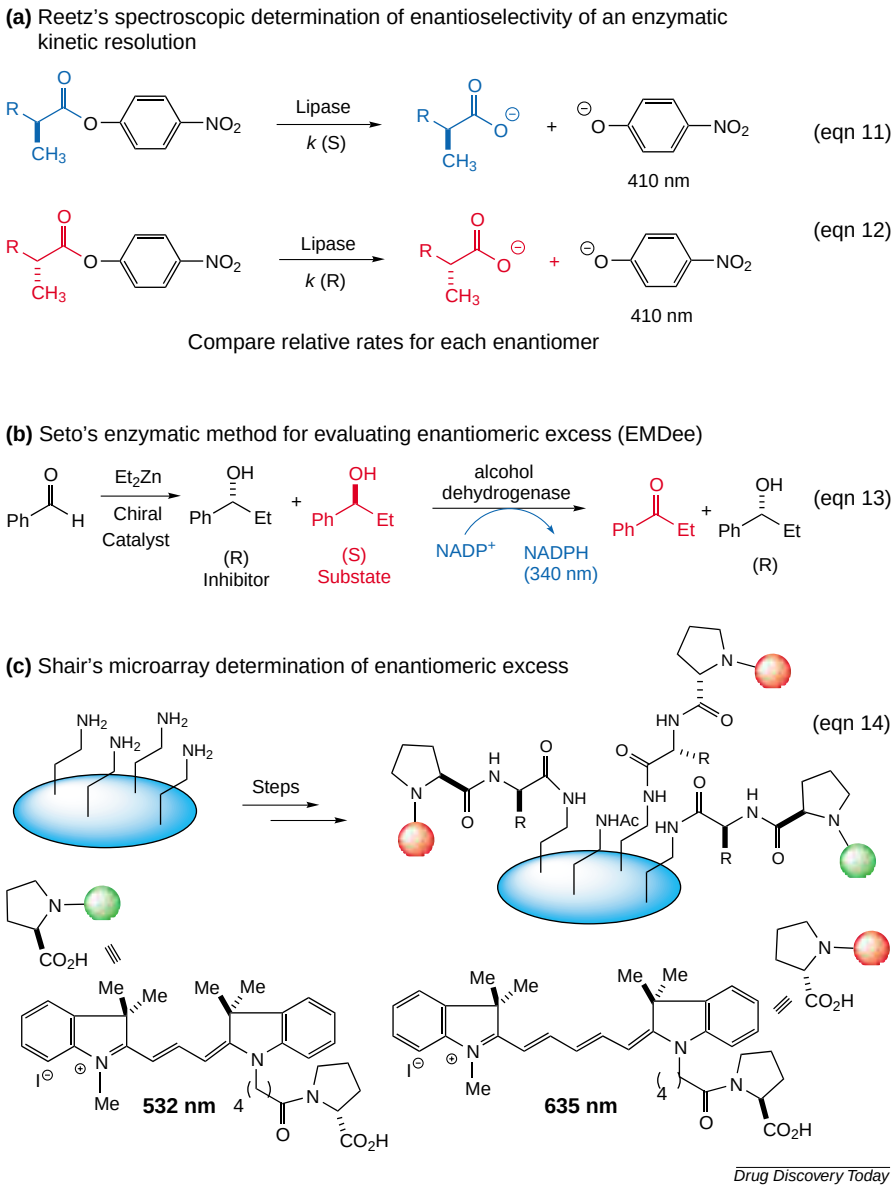

Figure 3. Kinetic resolutions evaluated through parallel, high-throughput spetroscopic methods.

Screening of microarrays

A similar strategy was used by Berkowitz *et al.* to discover new catalysts for intramolecular allylic aminations [39]. Berkowitz uses a biphasic system where the catalytic allylic amination reaction occurs in the upper, organic layer, with the detection system (oxidation of ethanol to acetaldehyde by alcohol dehydrogenase with production of NADH) occurring in the lower, aqueous layer. The use of a biphasic system was crucial in this case because the catalysts used are highly colored. By having the spectrophotometer beam pass only through the aqueous layer, interference from the colored catalysts is avoided.

Recently, Shair and co-workers have taken advantage of DNA microarray technology to rapidly and efficiently screen thousands of samples (eqn 14, Fig. 3c) [40]. Using automated contact printing methods, nanoliter volumes of various *N*-protected amino acids are chemoselectively printed on amine functionalized glass slides in a spatially defined manner (step 1). Unreacted amines were capped as acetamides (step 2), followed by deprotection to yield free amines (step 3). The resulting spots are 140 μm in diameter, enabling up to 75,000 samples per 25 × 75 mm glass slide. The detection technique relies on kinetic resolution of pseudoenantiomers of *R* and *S* prolines that are tethered separately to different fluorescent reporters, one of which is excited at 532 nm, the other at 635 nm (step 4). Because the two enantiomers of proline react at different rates with different enantiomers of the amino acids, the predominance of one fluorescent output over the other

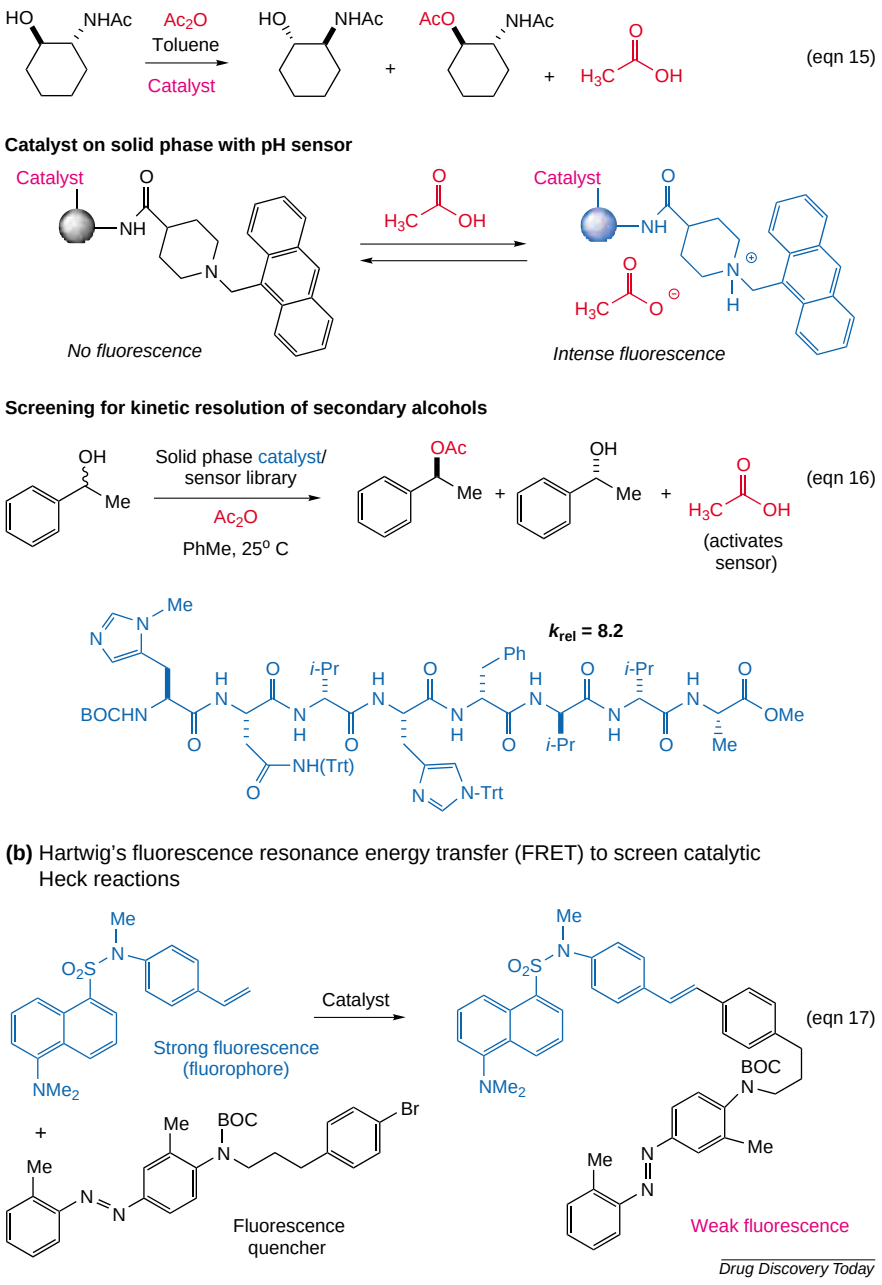

Figure 4. Using fluorescence to evaluate reactivity and selectivity in acylations and Heck reactions.

can be screened for, and the ee calculated. In practice, 15,552 samples were screened in 48 hours. The standard error of the measured to actual ee was <8% in most cases.

Other fluorescent approaches

Another resourceful approach to screening large numbers of catalysts was developed by Jarvo and Miller [41]. The Miller group is interested in the discovery of minimal peptide catalysts for various asymmetric transformations: one reaction is the resolution of racemic alcohols using acetic anhydride as an acylating agent. Because neither the products nor side products were chromophores, detection by UV–vis methods could appear difficult. Miller took advantage of the by-product of the reaction, acetic acid, as an indirect probe (Fig. 4a, eqn 15) [42].

Miller's peptide-based catalysts are easily synthesized on solid phase. By linking a fluorescent pH sensor on the same beads, a simple acid sensitive indicator for reaction rate is produced. Non-reactive beads appear dark, whereas reactive beads fluoresce when viewed under a fluorescent microscope. Control experiments proved that the acetic acid produced by one bead does not 'leak' to non-catalytic beads. Using a split and pool method of synthesis, ~100,000 octapeptide catalysts were synthesized [43]. A portion of the library was randomly screened (~1000 beads per hour) and 27 of the brightest beads were manually selected, washed and subjected to a kinetic resolution reaction. The beads with the highest selectivity were then deconvoluted by Edman degradation, resynthesized and subject to the reaction conditions. The resulting octapeptide catalyst was found to have a k_{rel} of 8.2 ($k_{rel} = k_{fast}/k_{slow}$; eqn 16, Fig. 4). Further screening of a directed library towards the optimal ligands resulted in a catalyst with a k_{rel} value of 20 for the resolution of *sec*-phenylethanol. Using similar methodology, catalysts for the resolution of tertiary alcohols have also been developed [44].

A non-asymmetric but important catalytic room-temperature Heck reaction was developed by Hartwig. using fluorescence resonance energy transfer (FRET) as a screening method (eqn 17, Fig. 4b) [45]. FRET occurs when the emission band from one molecule overlaps with the

absorbance of another, resulting in a net quenching of fluorescence. The molecules have to be close in space (2–8 nm) for the quenching to occur; therefore, the presence of quenching indicates that the molecules are linked covalently. Reactions are monitored by a fluorescence reader by measuring the amount (or lack) of fluorescence; 96 phosphine ligands were screened with CpPd(allyl) as a palladium source. Screening results from FRET were shown to be similar to HPLC analysis. The 15 best were then evaluated further with various solvents and from these screens, two phosphine ligands with $Pd(dba)_2$ yielded coupling products with aryl bromides in excellent yields (88–99%) at room temperature. Although this is not an asymmetric reaction, one could imagine cases where this screening strategy could be applied to the development of enantioselective transformations.

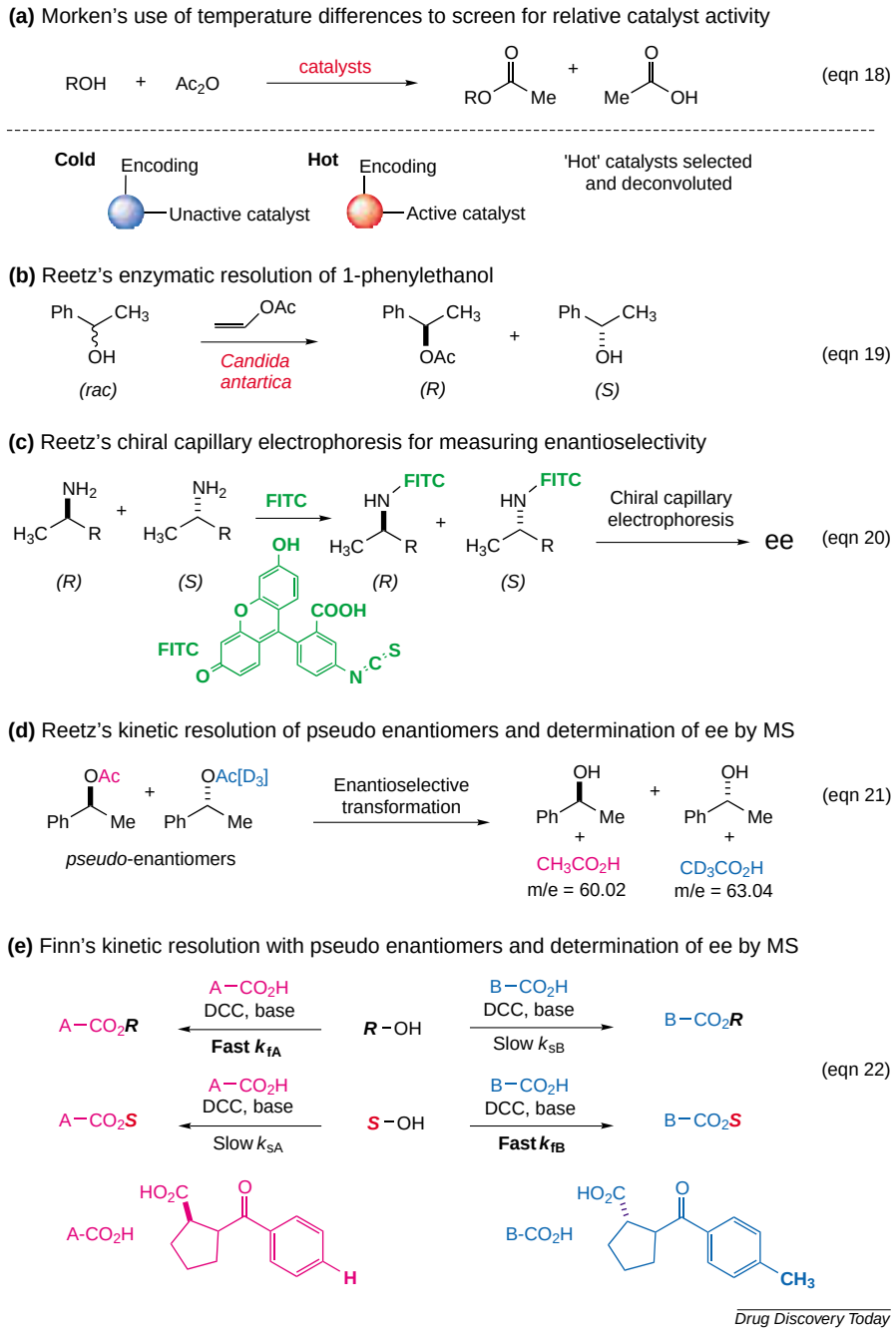

Figure 5 Evaluation kinetic resolutions using thermography, chiral capillary electrophoresis and MS.

Infra-red spectroscopy

Solid phase synthesis of catalysts lends itself to the preparation of a large number of compounds. In 1998, Taylor and Morken used an infra-red (IR) technique for parallel screening of a large immobilized library of catalysts (Fig. 5a, eqn 18) [46]. The technique relies on the differences in heat generated by the various catalysts in a particular reaction (both exothermic and endothermic reactions can be studied) [47]; differences as small as 0.02°C are reported to be detectable by the IR camera. Chloroform was chosen as the solvent because it was necessary for the beads to float for IR detection. An encoded library using split and pool synthesis with binary tags [48] was synthesized and screened for acylation activity. From a library of ~7000 beads, 23 of the most active were chosen and deconvoluted: of these, 21 were one of two catalysts, both of which were found to be catalytically competent after resynthesis.

The same concept was used in an asymmetric approach by Reetz, who monitored the kinetic resolution of racemic alcohols by a lipase isolated from *Candida antartica*. The lipase is known to select for the *(R)* enantiomer with 99% selectivity (eqn 19, Fig. 5b) [49]. By running separate reactions with known concentrations of racemic, all *(S)* and all *(R)* alcohol, the reactions were monitored over time for heat generation. The experiment showed the largest

amount of heat generated for the *(R)* alcohols, followed by the racemic alcohols, with the *(S)* alcohols remaining cool.

Capillary electrophoresis

The separation of enantiomers by chiral capillary electrophoresis was first developed in 1985 by Gassman and co-workers [50]. Reetz has developed a system where up to 7000 reaction mixtures can be analyzed per day. As indicated in eqn 20 (Fig. 5c), the fluorescein thiourea of *(R)* and *(S)* amines of various enantiopurities were separated on chiral capillary electrophoresis. The data correlated closely (all within 4%) with the corresponding chiral GLC data [51,52].

Chiroptical methods

Chiroptical methods of enantiomer determination are based on the ability of chiral compounds to rotate polarized light [53]. Both circular dichroism (CD) and optical rotation fall into this category. When used as a detector for an HPLC, both can be used to provide ee values for a sample. In general, HPLC conditions are faster for non-chiral separations (versus chiral) leading to higher possible throughput samples on the HPLC.

In 1994, Bertucci *et al.* developed a method for the determination of enantiomeric excess by non-chiral HPLC equipped with a dual CD–absorption detector [54]. The absorption detector simply measures the absorption (ε), whereas the CD detector measures the CD at the same wavelength (Δε). To eliminate the effect of concentration in the sample, the anisotropy factor g is used to measure ee, where $g = \Delta\varepsilon/\varepsilon$. Excellent correlation between data obtained from chiral HPLC and achiral HPLC coupled to the CD–absorption detector was obtained.

Mikami has taken advantage of this technique to analyze data from a screen of chiral catalysts for the catalytic asymmetric addition of diethylzinc to aldehydes [55]. Several chiral diols and chiral amines were screened as chiral ligands. The retention time of the desired alcohol was only 3 minutes; therefore, a rapid HPLC run could be used to determine the ee of the reaction. Analyses as short as 1.5 minutes per run were also reported in some cases [56]. Reetz has also published a similar method for determining ee in the catalytic asymmetric reduction of ketones where 700–900 samples could be analyzed per day [57].

Mikami developed an analogous method using optical rotation as a detector instead of CD [58]. This is advantageous because, whereas CD relies on compounds with a chromophore, optical rotation does not, enabling the monitoring of any chiral compound. Therefore, by measuring the optical rotation per refractive index (given the value e), enantiopurity can be measured. A linear dependency of e and enantiomeric excess was found, as well as proof of independence of e on overall concentration.

Mass spectrometry

At first thought, MS does not seem to be applicable to the determination of enantiomeric excess. Despite this, several clever methods have been developed to determine the ee of a reaction using this technique. The use of MS is desirable because runs are fast, facilitating a high-throughput analysis where only a small amount of material is necessary.

Reetz reported a method that uses MS to determine the difference between pseudoenantiomers (different isotopes used for each enantiomer) resolved by an enantioselective transformation (eqn 21, Fig. 5d) [59]. Because the products of the reaction of each pseudoenantiomer has a different molecular mass, simple integration of the resulting spectrum provides accurate ee data. Various mixtures of the pseudoenantiomers were synthesized, and the ee determined by both chiral GC and MS were in close agreement. One thousand measurements can be made per day. In addition, although electrospray ionization (ESI-MS) was used, matrix-assisted laser desorption–ionization (MALDI) also proved effective.

Siuzdak and Finn developed a method that combines kinetic resolution of the product with pseudoenantiomers followed by MS (eqn 22, Fig. 5e) [60]. First, the rate of reaction (k) with each enantiomer of the alcohol (*R*-OH, *S*-OH) with the two pseudoenantiomers (A-CO_2H, B-CO_2H) was determined. The mixture of alcohols was then reacted with an excess of the pseudoenantiomers and dicyclohexylcarbodiimine (DCC) as a dehydrating agent. The samples were then analyzed by ESI-MS: the majority of the ee data from the MS was found to be within 10% of actual values. A $k_f/k_s = 1.2$ was found to be sufficient for successful ee determination.

Conclusions

As the importance of enantiomerically pure, chiral pharmaceutical agents grows, new efficient methods for their synthesis will be in increasing demand. High-throughput methods enable a rapid catalyst discovery process for new asymmetric reactions. As described, several methods have been developed for the efficient synthesis and screening of new catalysts in the introduction of a variety of asymmetric transformations. Each reaction is unique, so different methods have been developed to exploit the properties of the various reaction products and by-products as indicators of enantioselectivity. High-throughput methods have already yielded several useful catalysts and are likely to continue

making significant contributions, especially considering the growing acceptance and adoption of this strategy in the pursuit of new and efficient transformations.

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