High Throughput Screening Methods for Asymmetric Synthesis

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Abstract: Combinatorial and high throughput methods have recently been applied to chemical reaction development as it greatly increases the reactivity "space" available to the chemist. Recent focus has been the development of detection methods, particularly those directed towards enantioselectivity. Progress in this area has been reviewed with particular emphasis placed on recent enantioselective methods.

Keywords: High throughput screening, combinatorial chemistry, parallel synthesis, reaction screening, asymmetric reaction screening, enantioselective reaction screening.

INTRODUCTION

The advent of combinatorial chemistry has revolutionized drug development. Rather than synthesizing compounds one at a time, randomized libraries are now prepared and tested. Compounds showing desired properties are then identified and pursued. The process is essentially an "analog" one, as opposed to traditional "one-at-a-time" scanning, which is somewhat of a "digital" process [1]. High throughput processes can operate much faster than traditional ones, but add the extra burden of information extraction.

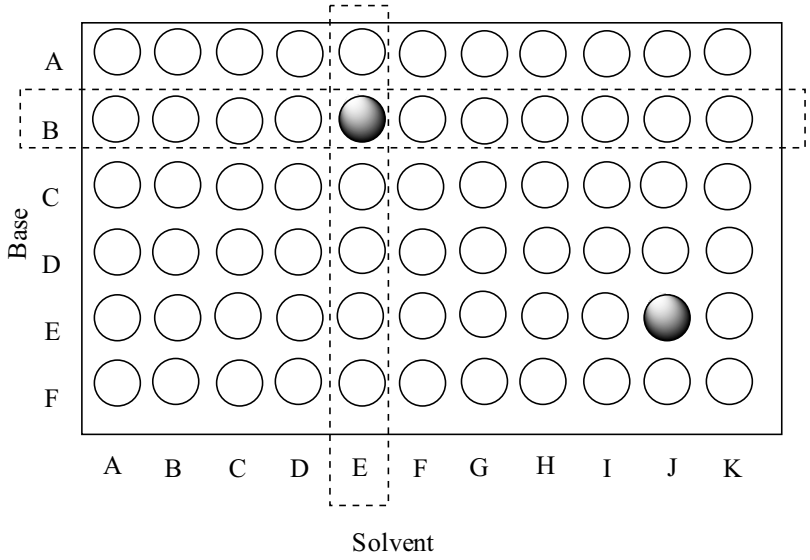

Fig. (1).

High throughput principles have been more recently applied to reaction discovery and development. Rather than optimize a process by performing a series of test reactions one-at-a-time, libraries of reactions or catalysts are prepared and scanned all at once. As shown in Fig. (**1**), a chemist performing reaction discovery on a plate rather than in a flask would examine simultaneously a selection of variables, for example solvent and base as shown. Other parameters can be changed by adding plates or "stacks" varying one factor over each plate or stack of plates. The process is analogous to traditional methods development. A chemist changing one variable at a time effectively examines one row and one column, changing first one parameter then the other. In the example shown, this would result in the identification of optimal solvent E and base B. High throughput screening carries out the same process, but examines all combinations simultaneously. This process can identify the same results as traditional methods, and also has the power to look at combinations that otherwise would not be examined (solvent J with base E). The main disadvantages are accuracy and the difficulties associated with handling the massive amounts of data generated. The time required to analyze the outcome of large numbers of reactions is a particular challenge. In high throughput methods, rather than analyze reaction products, one searches for a "readout" of desired reactivity. In recent years, chemists have become increasingly aware of the power of high throughput methods as evidenced by the increasing number of successful implementations of the concept. One major barrier to the method however, remains the extraction of information from the large number of samplings. This account summarizes recent advances in this deconvolution phase for high throughput investigations of chemical reactivity.

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Traditional analysis methods, like gas and high performance liquid chromatography, mass spectrometry and nuclear magnetic resonance spectrometry have been modified in various ways for this purpose. In addition, techniques commonly used in biology and biochemistry have also been adapted to the screening of organic reagents and catalysts. Other techniques more familiar to the organic chemist, such as thin-layer chromatography or infrared spectrometry have also found applications in combinatorial screening. Moreover, a number of more rapid technologies have recently been implemented for the simultaneous assessment of reactivity such as colorimetric methods, TLC and fluorescence. In the last few years, the challenge has been to amend these high-throughput methods to enantioselectivity screening.

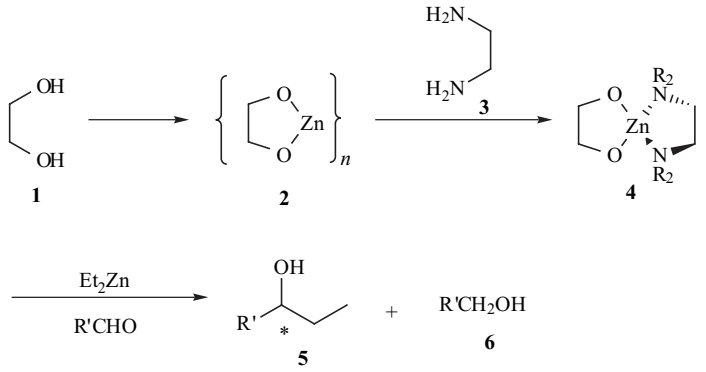
Excellent reviews have been written on the subject of high throughput screening for chemical reaction development. Publications by Reetz [2b] and Finn [2c] are particularly insightful. However, the last few years have seen a tremendous growth, particularly in the development of enantioselective screening methods, which deserve a closer look.

ADAPTATIONS OF GAS CHROMATOGRAPHY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

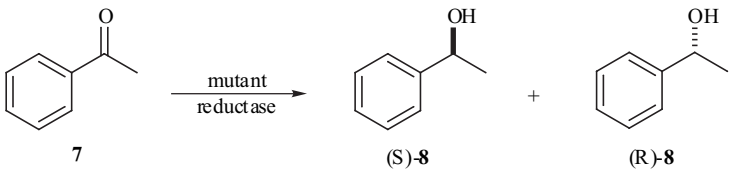
Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) are two of the most commonly used methods for substrate purification and identification in the laboratory. When the need for high-throughput screening arose, many scientists turned to variations of these methods because of their reliability and accuracy. Interesting results were obtained by Gennari [3], Liskamp [4], Burgess [5], Wolf [6] and Jacobsen [7] by analyzing reaction mixtures in parallel using automated GC. This screening method was not truly high-throughput however, as each sample required at least 15 minutes to analyze. A significant improvement made to GC methods in terms of rapidity was designed by Reetz [8]. Two GC instruments, equipped with chiral columns, were connected to a prep-and-load sample manager and a computer. Using this unit, the yield and enantiomeric excess of a mixture could be determined in about 2 minutes provided that the separation parameters (solvent, temperature, etc) were optimized for each new chiral compound.

HPLC has proven to be a more versatile method for the rapid screening of a variety of samples. Mikami [9a] used chiral columns on an HPLC to quantify enantiomers, calculate the enantiomeric excess of reactions and identify interesting catalysts. The disadvantage of this method was the time required (up to 45 minutes per run). The Mikami group therefore decided to employ a simple, achiral column for their separations using circular dichroism (CD) and UV-VIS detection systems, and applied this technology to optimize diol-zinc catalysts for asymmetric additions (Scheme **1**). The tandem use of UV and CD detectors in place of chiral columns was originally proposed by Mason and co-workers [10] then further elaborated by Salvadori [11] and Mannshreck [12]. From UV and CD measurements, the anisotropy factor g could be calculated: $g = \Delta\varepsilon/\varepsilon$, where $\Delta\varepsilon$ was the CD signal and ε the absorption. The method was based on the assumption that the g-factor was linear with respect to the enantiomeric excess and was independent of concentration. If the substrates formed dimers or aggregates, these approximations would no longer be valid and so these properties needed to be investigated for each new compound and reaction. Because of the use of UV/Vis detection, simple aliphatic products could not be used.

In 2000, the Mikami group refined their technique [9b] and adapted their method for aliphatic alcohols by substituting a refractive index unit (RIU) for the UV-Vis detector. The optical rotation per RIU approach could be used for both aliphatic and aromatic substrates requiring approximately three minutes per sample. When working with RIU, the g-factor could no longer be calculated since the absorption ε, was not measured. Instead, the "e-factor", defined as the ratio of the RIU to optical rotation [9c], was used to reduce run time to 1.5 minutes per sample.



Scheme 1.



Scheme 2.

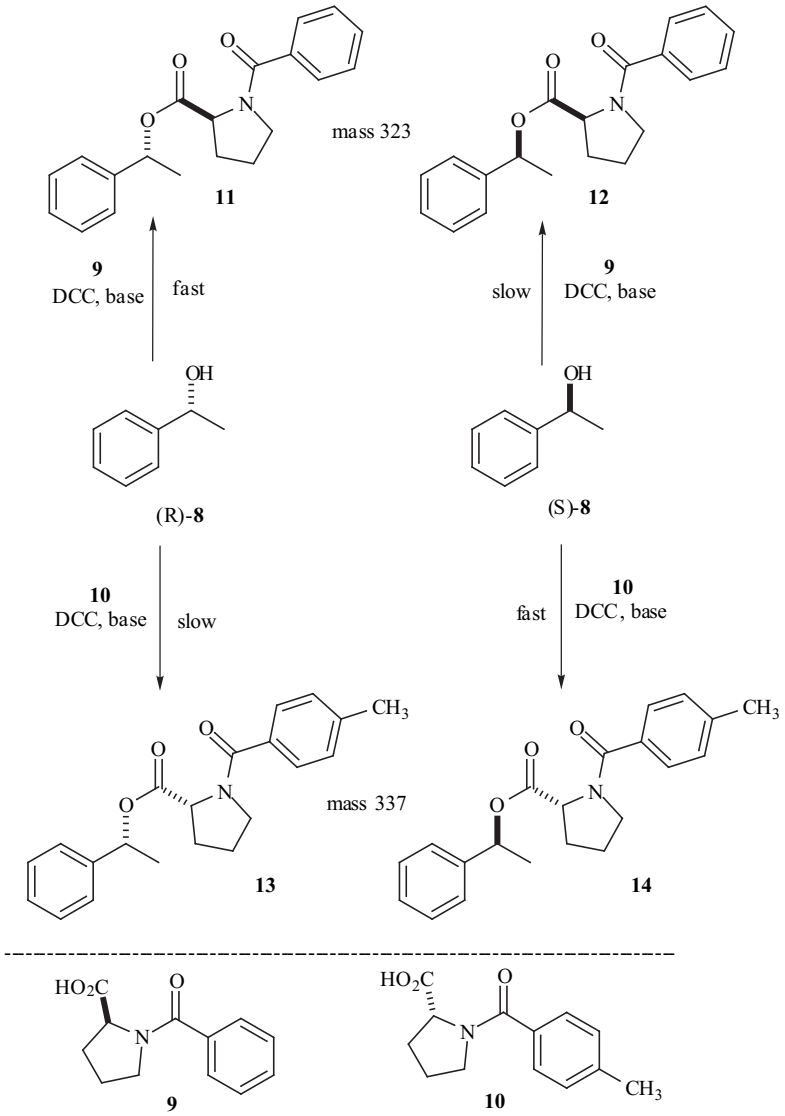
Mikami's method was used by Reetz [13] to successfully measure the ee and yield for the asymmetric reduction of prochiral ketones (**7**) with mutant reductases and the asymmetric hydrolysis of acetates with mutant lipases (Scheme **2**). In this paper it was suggested that the use of UV-Vis together with CD might be sufficient for enantioselectivity determinations on certain systems, however no experimental results were presented to demonstrate this possibility.

GC and HPLC methods are very general and accurate tools; familiar to most practicing organic chemists, however they are very slow and can be considered low throughput methods. Because of their widespread presence, these methods have been extremely valuable in many of the accounts of high throughput chemistry development in which small libraries were screened [3-7,8].

MASS SPECTROMETRY

Although mass spectrometry on its own provides no chiral information, asymmetry can be introduced though the formation of diastereomeric complexes or adducts, thus adapting mass spectrometry for the determination of enantiomeric excess. Suizdak and Finn [14] developed a mass spectrometric method inspired by Horeau [15] for the determination of the enantiomeric excess of alcohols and amines in a mixture. Mixtures of chiral alcohol (**8**) were reacted with chiral mass-tagged acids (**9** and **10**) in the presence of DCC and base. The mass-tagged acids differed in small substituents that were distal to the chiral centre. Because there was a small kinetic resolution during the acylation, the mass of the product could be correlated to its configuration and therefore used to determine the optical purity. The enantiomeric excess could be calculated from equation **1** using the measured rates of esterification for each substrate enantiomer with the labelled pseudoenantiomeric acids and the intensity of each peak observed. The precision and accuracy of this method were quite good, within 1 to 3% of the actual values.

Reetz [16] took a different approach in which one substrate enantiomer was isotopically labelled and used to prepare a pseudo-racemic mixture with the unlabelled

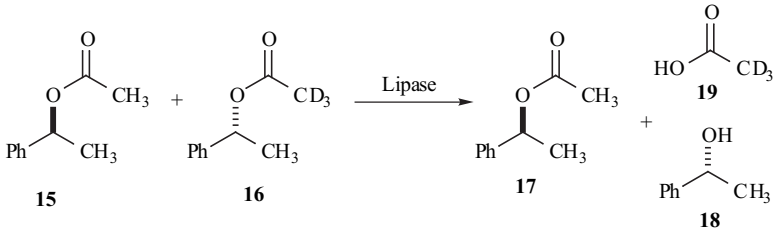


Scheme 3.

$$\%ee = \left[\frac{(y-1)(s+1)}{(y+1)(s-1)}\right] X\ 100$$

$$y = \text{corrected intensity ratio}$$

$$s = \frac{k_{fast}}{k_{slow}} \qquad (1)$$



Scheme 4.

enantiomer. This mixture then underwent an asymmetric functional group transformation and kinetic resolution. One of the products of the reaction was deuterium labelled; the other was not, leading to a detectable difference in molecular mass that could be measured by mass spectrometry (Scheme **4**). Using electrospray ionization (ESI-MS) or matrix-assisted laser desorption/ionization (MALDI) the enantiomeric excess was calculated but not the reaction yield. This method was also used to monitor the asymmetric transfer of prochiral substituents. However, this was a somewhat slow (1.5 minutes per sample), laborious and equipment intensive method that was not applicable to systems that did not have enantiotopic faces. Quick kinetic studies of the labelled and non-labelled substrates had to be performed beforehand to ensure that there was no secondary isotope effect.

The addition of a parallel eight-channel multiplexed ESI-MS system [17] considerably increased the speed of analysis. Using this method, a sample could be analyzed approximately every 9 seconds. The additional equipment was somewhat expensive and so the cost of implementing this technology had to be considered.

Mass spectroscopy, like GC and HPLC, is a general method that probes directly the composition of the reaction mixture and allows for precise structure identification. In general, the existing methods are relatively slow and demanding in terms of equipment and maintenance. Asymmetric detection relies on kinetic resolution and so experiments are necessary to validate each new method.

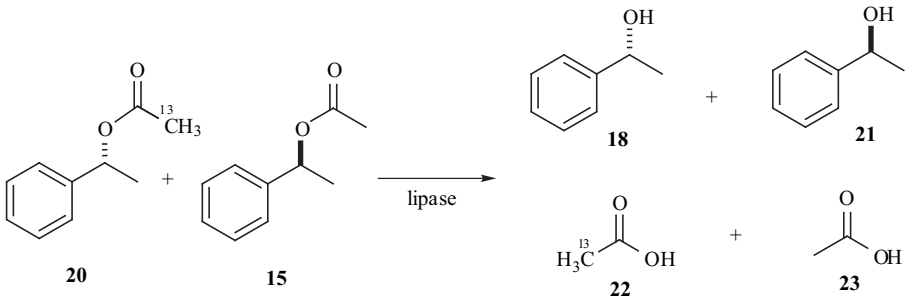
NUCLEAR MAGNETIC RESONANCE

Generality and structure identification are the main advantages of NMR-based methods. The analysis conditions are also very mild and permit the detection of compounds that would be degraded in other methods. The automation of an NMR instrument together with the visual assessment of spectra [18] has been used in high throughput screening, but the method suffered from long analysis times. The introduction of a flow cell permitted the automated analysis of each sample in about 11 minutes but large volumes (240 μL) of sample were required [18].

The low sensitivity of ^{13}C NMR and the lack of automated proton-based structure verification methods also restrict the use NMR methods for high-throughput screening. Schröder [19] addressed this issue by using HSQC spectroscopy together with automated pattern recognition [20]. Molecules were treated as agglomerations of substructures and analyzed as linear combinations of units to identify spectral patterns typical of the substructures. The predicted spectral patterns for a given molecule were then compared to the actual NMR spectrum and a ratio of integrals established. With this method, each sample could be analyzed in about 10 minutes.

The analysis time for NMR assays was dramatically reduced in two approaches taken by Reetz [21]. The first was based on ^{13}C labelling of only one of the enantiomers (Scheme **5**). The ^{13}C label was placed on an isolated methyl group that would give uncoupled proton resonances. The non-labelled enantiomer gave a singlet in the ^{1}H spectrum whereas the same group of the ^{13}C labelled enantiomer resonated as a doublet. This worked very well for chiral alcohols and amines since one enantiomer could be easily acylated with ^{13}C-labelled acetyl chloride and the other with non-labelled acetyl chloride. A pseudo-racemic mixture could be used to measure the enantiomeric excess (or selectivity factor E [21,22]) that was calculated by separately integrating the doublet and the singlet. The reaction yield could also be measured by integration of a characteristic, general product peak common to both pseudo-enantiomers and normalizing with an internal standard. Integration and quantitative analysis were done by software and used to establish enantiomeric excess values that were within 2% of the GC measurements. Using this method samples could be analyzed in about 1 minute.

The disadvantage of this method was that it could not be used to measure the enantiomeric excess of the products of reactions on prochiral compounds without enantiotopic faces, like previous mass spectrometric methods [16]. Consequently, a second, slightly different method was developed for these types of substrates. Diastereomers were formed by coupling the substrates with chiral reagents or complexing agents that produced chiral hydrogen bonds. Since diastereomers could be resolved by NMR, the enantiomeric excess was calculated by integrating the desired peaks. The enantiomeric excess values were within 5% of the GC assessments and samples were analyzed in about 1 minute. These two methods were very similar to the Mass



Scheme 5.

Spectrometry techniques introduced earlier by Reetz and indeed spring from the same principle. The MS-based system was faster but the NMR system was more precise.

With NMR screening the advantage of precise structure identification was offset by low throughput, and the need for sizeable, expensive equipment. The requirement for flow-cells necessitates a large amount of product due to the poor sensitivity of NMR, a serious disadvantage since combinatorial synthesis operates optimally on very small volumes for economical and ecological reasons. Finally, when organometallic reagents were analyzed, a prepurification was necessary to eliminate paramagnetic compounds.

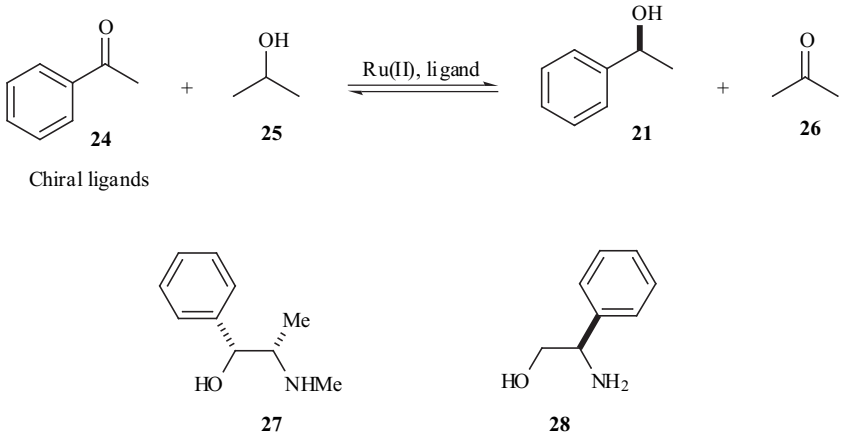
INFRARED SPECTROMETRY

The first example of the use of automated infrared spectrometry for screening was reported by Sofia [23] using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). This method was ideally suited for high-throughput applications as it required no sample preparation, was very rapid and could be easily automated. The solid-phase reduction of azido monosaccharides to amino monosaccharides on grafted polystyrene resins was monitored using this new qualitative technique. Each measurement took approximately 30 seconds; however, since spectra were evaluated manually, the actual analysis required longer and was not suited for large libraries.

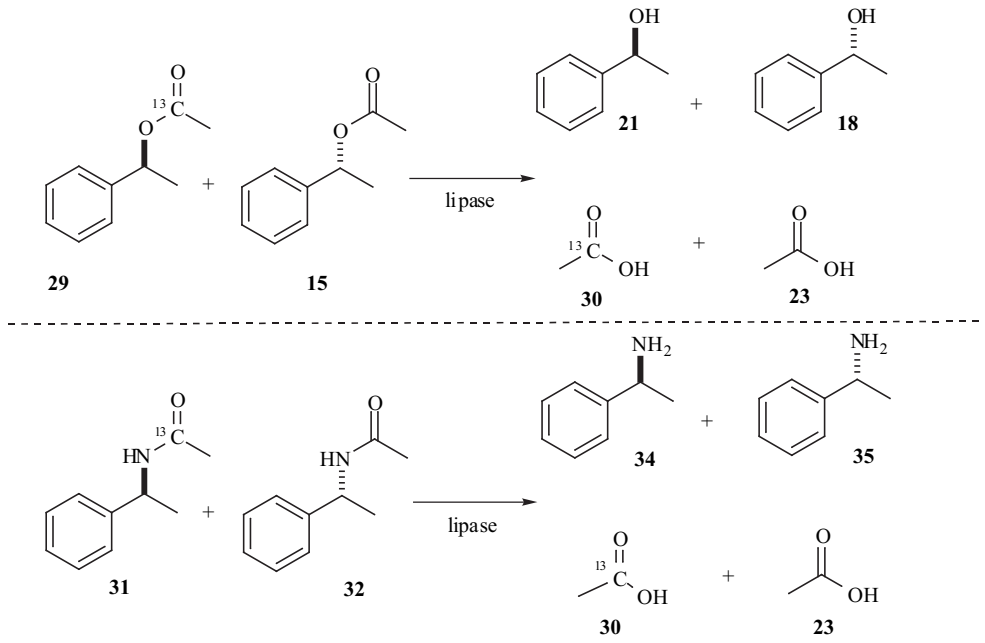
Three years later, van Leeuwen [24] developed a new screening technique to measure the enantiomeric excess of chiral hydrogen-transfer catalysts in the reduction of acetophenone using ephedrine **27** or phenylglycinol **28** as ligands (Scheme **6**). A single measurement was sufficient to calculate the enantiomeric excess of each mixture by subtracting a reference spectrum from each sample spectrum. The resulting difference spectra gave flat lines for non-selective catalysts and large peaks for active or highly selective catalysts. Manual comparison of the spectra taken gave an approximation of the enantiomeric excess achieved for the particular ketone used. Although very few analyses were performed, it was suggested that with automation this method could measure enantiomeric excess in less than 40 seconds per sample. It required sequential analysis however, and was only applicable if the reagent and the product had easily distinguishable absorbances.

Shortly after this publication, a method for truly parallel screening using FTIR imaging was introduced by Caruthers [25]. The coupling of a focal plane array detector and an FTIR spectrometer enabled the user to simultaneously obtain spectral information for several samples achieving a resolution of 8 cm^{-1}. The spectra were collected in 6 seconds and analyzed automatically by computer. Ultimately, the goal was to achieve automated development of a predictive model using chemistry "rules" and experimental data. So far, this has not been fully realized because the knowledge extraction required large amounts of high-quality data.

A very similar set-up was used recently by Reetz [26]. Unlike the previous method, samples in standard 96, 384 or 1536 well formats were brought one by one into the focus of the IR beam. The spectra were then analyzed automatically according to a selected evaluation method such as peak integration allowing for the direct calculation of



Scheme 6.



Scheme 7.

concentration using the Beer-Lambert law. To adapt this technology to the measurement of enantiomeric excess, substrate carbonyl groups were labelled with ^{13}C using commercially available reagents (Scheme **7**). The carbonyl functionality was chosen for this in part because it gave intense and characteristic vibrational bands in a region of the IR spectrum free from other absorbances. The introduction of a ^{13}C label shifted the carbonyl stretching vibration to lower frequencies by 40 to 50 wavenumbers, allowing for the detection of pseudoenantiomeric substrates, as shown in Scheme **7**.

Mixtures containing various concentrations of the ^{13}C-labelled compound together with the non-labelled enantiomer were prepared and used as unknowns. The ee's of these mixtures were measured on the FTIR set-up using a resolution of 8 cm^{-1} giving values within 7 % of those determined by GC. This method was not only accurate; it was also fairly rapid analyzing a sample in 8.9 seconds. One of the main advantages for the application to reaction mixture scanning was that workup and drying were not necessary, unless solvent interference was experienced. On the other hand, a calibration curve was required for every sample and the molar absorption coefficient ε had to be calculated for each enantiomer.

BIOLOGICAL METHODS

Numerous methods have been developed for the screening of reactions relevant to organic chemistry using biological substrates such as enzymes and antibodies. These techniques so far have limited applications but can be quite efficient in a high-throughput sense. The Quick-E Test from Kazlauskas [27] simulated competition to measure the enantioselectivity of lipases or esterases. Absorbance measurements were taken at regular time intervals during the first 15 seconds of separate reactions of each pure enantiomer. The initial rates of hydrolysis were calculated from the increases in absorbance but no information was provided about stereoselectivity. The enantiomeric ratio E could not be obtained directly from the ratio of these initial rates of reaction because it ignored the competition for binding between the two isomers. To reintroduce competition, a reference compound, resorufin tetradecanoate, was added. The ratio of the rates of hydrolysis measured separately for each pure enantiomer (equation **3**) against the rate for the reference compound gave E (equation **4**). Screening was done using duplicate lanes on a single plate, with each enantiomer being the substrate in its own lane. With this technique, the enantiomeric selectivity of an enzymatic resolution could be obtained in about 60 seconds.

$$E = \frac{(k_{cat}/K_M)_{\text{fast enantiomer}}}{(k_{cat}/K_M)_{\text{slow enantiomer}}} \quad (2)$$

Approximation

$$\text{selectivity} = \frac{(k_{cat}/K_M)_{(S)\text{-enantiomer}}}{(k_{cat}/K_M)_{\text{reference}}} = \frac{v_{(S)\text{-enantiomer}}\,[\text{reference}]}{v_{\text{reference}}\,[(S)\text{-enantiomer}]} \quad (3)$$

$$E = \frac{\dfrac{(S)\text{-enantiomer}}{\text{reference}}\ \text{selectivity}}{\dfrac{(R)\text{-enantiomer}}{\text{reference}}\ \text{selectivity}} \quad (4)$$

The Kazlauskas group later published a different method for hydrolase screening [28]. The acid produced by hydrolysis was detected with a pH indicator whose concentration was measured by UV/VIS spectrometry. If the buffer and the substrate had the same pKa values (within 0.1 units), the absorbance increase was a direct measurement of the reaction rate and calibration curves were not necessary. From the ratio of initial reaction rates, E could be approximated. These were not "true" E values, since the

calculation did not account for competition between the two isomers for the binding site, but they could provide a reasonable approximation appropriate for high-throughput screening. Only data from the first 3 to 4 minutes of each reaction was used so screening time could be considerably reduced. In total, 72 hydrolases could be analyzed in about 180 minutes, which corresponded to about 2 minutes per enzyme.

Reaction rate calculation

$$Q = \frac{C_B}{C_{In}} \times \frac{1}{\Delta\varepsilon_{404\,nm}} \quad (5)$$

$$\text{rate} = \frac{dA}{dt} \times Q \times \text{reaction volume} \times 10^6 \quad (6)$$

$\frac{dA}{dt}$ = rate of indicator absorbance change

Q = buffer factor

C = molar concentration of buffer (B) and indicator (In)

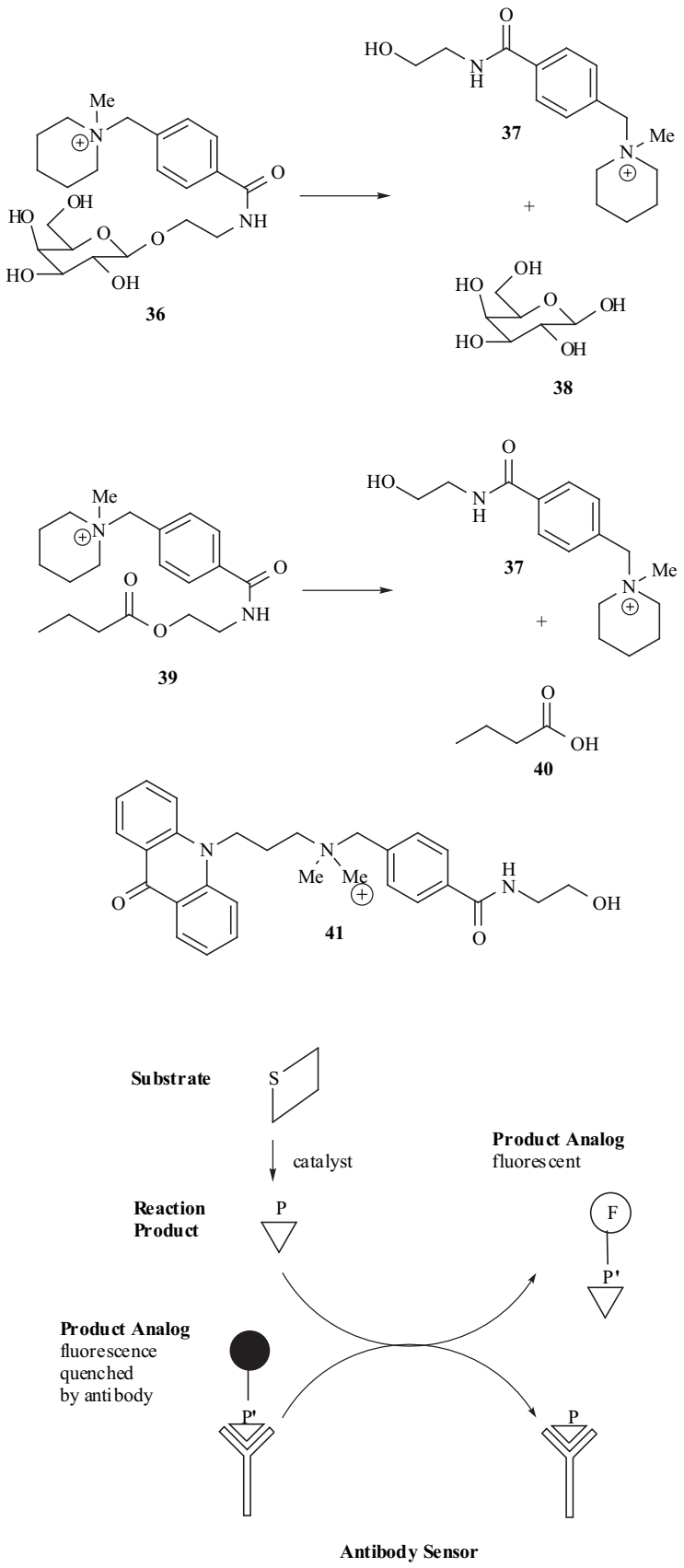
$\Delta\varepsilon_{404\,nm}$ = difference in extinction coefficient between the protonated and unprotonated indicator.

An alternative technique similar to *cat*-ELISA (catalyst enzyme-linked immuno-sorbent assay) [29] was introduced by Reymond [30] using an "antibody sensor" to follow the reaction progress. This antibody sensor was comprised of a product-specific antibody bound to a fluorescently-labelled product analog. In the bound state, fluorescence of the product analog was quenched by the antibody. Fluorescence was possible only when this product analog was released into solution. As the true reaction product was formed, it displaced this analog from the antibody, resulting in an increase in fluorescence. The method was tested by monitoring the rate of hydrolysis of **36** by β-galactosidase and **39** by esterases (Scheme **8**). Both compounds released **37** as the product of hydrolysis and this compound displaced the fluorescent product analog **41** from the antibody. When bound to the antibody, fluorescence of the acridone moiety in **41** was quenched, but fluorescence at 445 nm was possible when **41** was unbound in solution. Using a calibration curve, the concentration of **41** could therefore be obtained from the intensity of emission. Although many different types of reactions could be monitored using this technique, there were several variables that needed to be optimized. The antibody had to be capable of quenching the tag fluorescence, the reactions had to occur in a buffered aqueous environment, and a proper fluorescent product analog had to be found.

Wagner and Mioskowski [31] used a similar idea to develop a high-throughput screening method for the enantioselective catalytic reduction of ketones. Using competitive enzyme immunoassays (EIA) [32], the yield as well as the enantiomeric excess of the reaction could be measured for "thousands" of catalysts per day. After the reaction had occurred, two EIA runs were performed. In one of those runs, the crude reaction mixture was added to a specific *anti*-product antibody linked to a solid support, together with an enzyme-product conjugate. This antibody was not stereoselective and so both enantiomers of the product would displace the enzyme-product conjugate and the yield of the reaction could be obtained. A second assay was performed using an enantiospecific antibody, allowing for determination of the enantiomeric excess. The concentration of enzyme-product conjugate in solution could be determined by absorbance and the signal was related to the bound enzyme activity. The absorbance decreased as more enzyme-product conjugate was released into solution. The difference between the absorbance at any given time and the initial absorbance was proportional to the concentration of product in the liquid phase. With this screening method, the enantioselective reduction of an α-keto acid by hydrogen transfer using chiral metal complexes was optimized. The hydrogen source, as well and the metal catalyst and ligand were varied and a 100 % yield of (S)-mandelic acid in 81 % ee was achieved. For these analyses, the calculated enantiomeric excesses were within 9% of the values obtained by HPLC. Of critical importance in this technique was the choice of antibodies with appropriate binding specificity. The antibody must not bind to the reactant, only to the product. Monoclonal antibodies raised against hapten H3 were used in this analysis but this type of antibody would not suit every application and must be optimized for every new reaction.

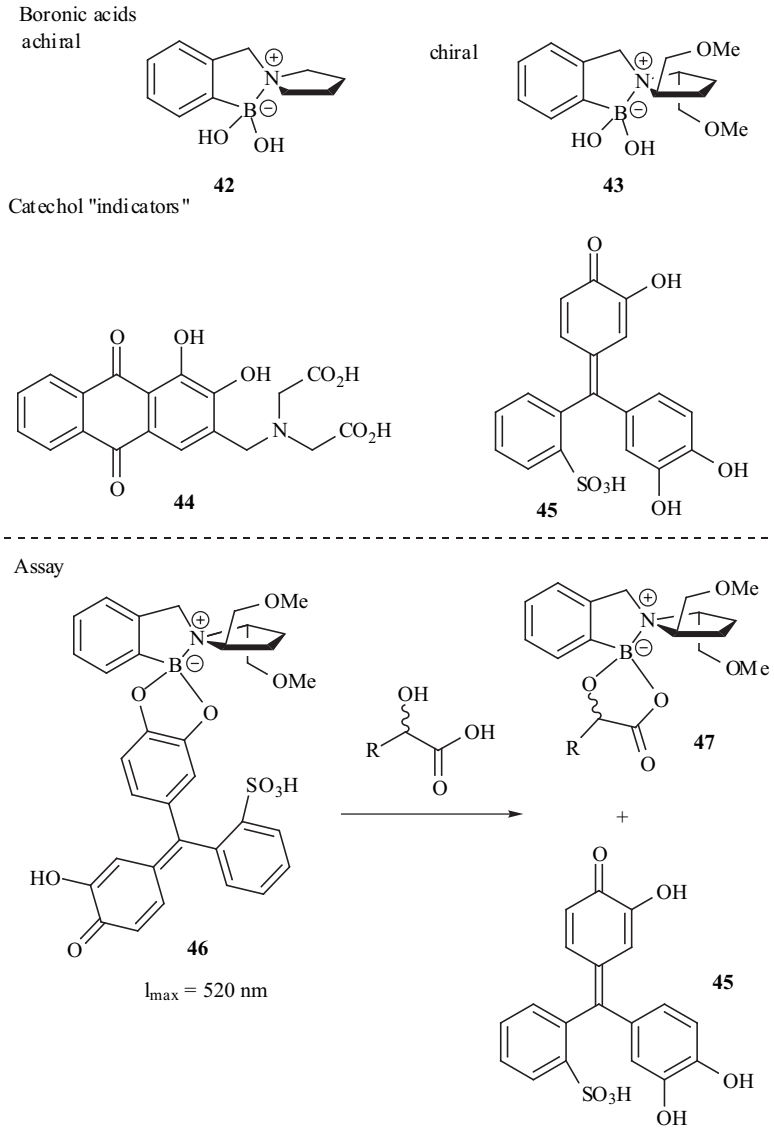
Recently, a new high-throughput indicator-displacement assay (IDA) was reported by Anslyn [33] for the analysis of α-hydroxyacids. Boronic acids bound to catechols served as "receptors" for α-hydroxyacids (Scheme **9**). When achiral boronic acids such as **42** were used, the receptors bound both enantiomers without preference. But when chirality was introduced into the receptor, the displacement of "indicator" catechols **44** and **45** from D- and L-**43** was enantioselective. To measure this selectivity, the absorbance of the receptor-indicator complex was monitored. It was observed that, because of the different absorbances of **46** and **45** that solutions of pure enantiomers of a particular compound at a given concentration had distinct UV absorbances. The difference in absorbance could be as large as 0.27. In solutions containing a mixture of the two enantiomers, the difference in absorbance could be correlated with the enantiomeric excess. In practice, two independent measurements were necessary to obtain the concentration and ee of the products. First, an IDA was performed with an achiral receptor, a combination of **45** and **42**, and the concentration of α-hydroxyacid was calculated from the solution absorbance using the Beer-Lambert law. Then, a second IDA was done using a chiral receptor, **43**. The enantiomeric excess could then be calculated, without the need for a calibration curve. Concentrations calculated were within 10 % and ee values within 20 % of the actual values.

The Bornscheuer group used a different tactic in the development of their enzymatic high-throughput screening method [34]. The activity and enantiomeric selectivity of hydrolases (lipases and esterases) were measured using the coupled enzymatic conversion shown in Scheme **10**. Acetic acid released by hydrolysis of **48** was fed through a cascade of enzymatic reactions generated by a cheap test-kit for food analysis [34]. Initial rates of acetic acid formation could be monitored by measuring the increase in absorption at 340

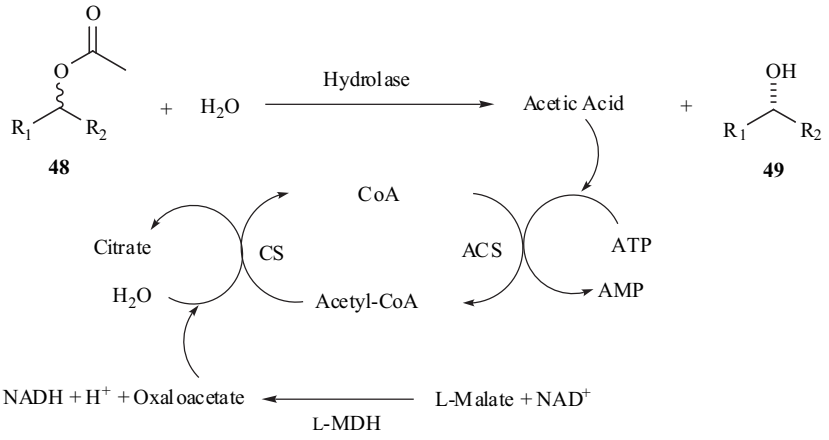


Scheme 8.

nm caused by the change in concentration of NADH. In order to calculate the enantiomeric ratio for the enzymatic reaction, solutions of pure R and S esters were necessary. The ratio of their initial hydrolysis rates, obtained from

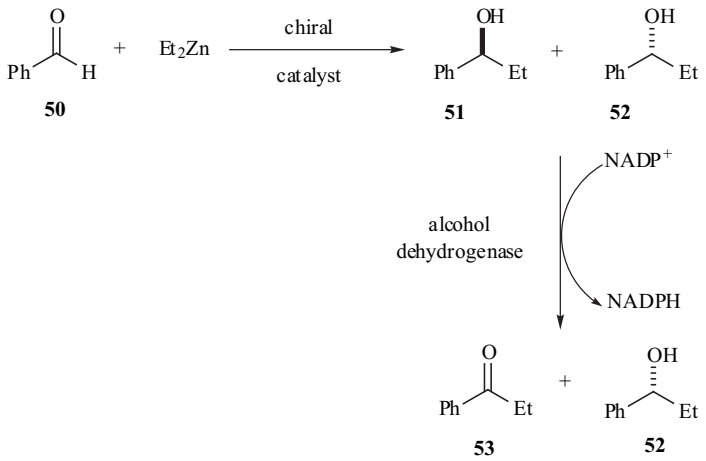


Scheme 9.



Scheme 10.

absorbance measurements, was used to approximate the enantiomeric ratio of the enzyme, as in the method of Kazlauskas [28]. Comparisons between the approximate enantiomeric ratios calculated and the "true" values measured by GC showed only small discrepancies. Each 96-well plate was screened in 3 to 4 minutes so an individual reaction could be analyzed in about 7 seconds. This technique was not restricted to lipases and esterases. It could also be used to screen other enzymes producing acetic acid, such as proteases and amidases.



Scheme 11.

EMDee, a very interesting enzymatic method for ee assessment, was developed by Seto [35]. This technique used the relationship, derived from the Michaelis-Menten equation for competitive inhibition, between reaction rate and the concentration of each enantiomer formed. To test this methodology, the researchers chose to look at the addition of diethylzinc to benzaldehyde. The products of this addition, a mixture of (R) and (S) 1-phenylpropanol (**51** and **52**), were reacted with an alcohol dehydrogenase that selectively oxidized one of the enantiomers, as shown in Scheme **11**. The rate of this oxidation was directly proportional to the enantiomeric excess of 1-phenylpropanol. This was demonstrated with samples 1-phenylpropanol of varying composition, from 100% **51** to 100% **52**. The correlations between observed reaction rates and known enantiomeric excesses were excellent. Oxidation rates were measured by monitoring the concentration of NADPH produced using a UV/fluorescence plate reader at 340 nm for 30 minutes. 100 samples were analyzed at a time, meaning that each reaction was assessed in 18 seconds achieving an accuracy of ±10 % ee compared to values obtained with chiral GC. The alcohol dehydrogenase used in these studies, *Thermoanaerobium sp*., had a very high selectivity for (S)-aromatic alcohols. Other enzymes exhibiting modest stereoselectivity during the kinetic resolution would potentially be appropriate for accurate ee determinations [14,35].

One of the drawbacks to EMDee was that it could not distinguish between reactions that proceeded with low stereoselectivity and high conversion, or that proceeded with high stereoselectivities and low conversion. To solve this problem, a second set of assays was performed using *Lactobacillus kefir*, an (R)-aromatic alcohol dehydrogenase, to quantitate the amount of **52** present. Since the amounts of both enantiomers were known, the extent of the conversion could be calculated. It was also suggested that this could be done *via* quantitation of the residual benzaldehyde by reduction to benzyl alcohol using an alcohol dehydrogenase together with NADH or NADPH. These extra steps considerably complicated the method. EMDee was therefore suitable for screening very large libraries of catalysts that were both highly active and highly selective, but the method could be problematic if applied to asymmetric catalyst design.

These enzyme and antibody-based methods could be quite efficient for screening the particular system they were designed for; however, they so far have few applications in organic chemistry because the types of reactions that could be screened were very limited. However biological methods have long experience in high throughput scanning and are robust, reliable and highly selective.

CAPILLARY ARRAY ELECTROPHORESIS

Capillary electrophoresis with chiral stationary phases has been used to determine enantiomeric purity for nearly two decades [36]. This slow sequential method was recently modified for the Human Genome Project to increase throughput. Capillary array electrophoresis (CAE), in which bundles of capillaries operate in parallel, gave hope for the potential use of this method in the high-throughput screening of organic reactions. The first example of high-throughput organic reaction monitoring with CAE was made by Yeung [37] who used a homebuilt prototype that combined a commercial 96-capillary system with a UV detector. The analysis was carried out in organic solvents, which meant that the sample could be injected directly, without purification, dilution or quenching. Crude mixtures from the reaction block were used directly to reduce both the risk of contamination and the volume necessary for analysis. Each plate analysis took 90 minutes, including 30 minutes of capillary cleaning, which translates to almost one minute per sample. However, it was discovered that the capillaries could be truncated by 75% while still affording good resolution. With 12.5 cm columns, each 96-well plate could be analyzed in 15 minutes, plus cleaning time.

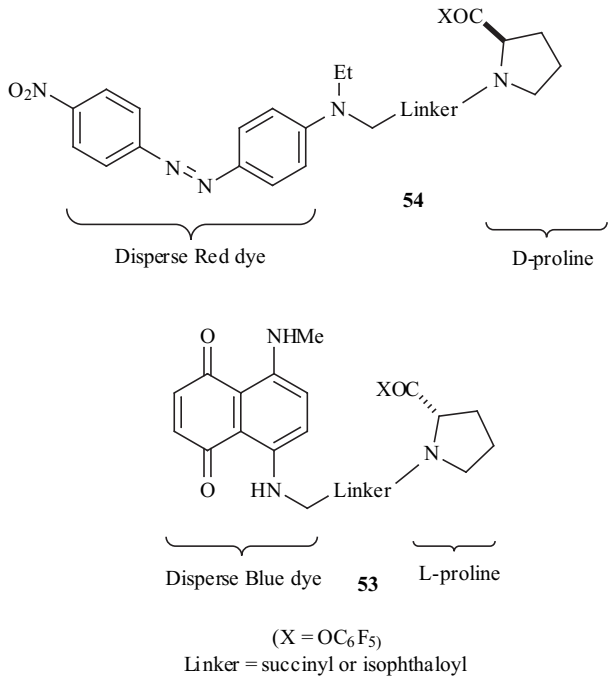
A different method for the screening of enantioselective catalysts with CAE was introduced very soon after the publication by Yeung. Reetz [38] used CAE together with cyclodextrins as chiral sensors to measure enantiomeric excess. These sensors interacted with the enantiomers, forming reversible diastereomeric complexes that had different electrophoretic motilities. Capillary array electrophoresis did not behave like single-capillary systems however, and several parameters had to be optimized in order to obtain reproducible results. In single-capillary systems, a polyacrylamide coating is normally used but, when used in CAE, these coatings gave unstable, non-reproducible runs.

Linear polyacrylamide was added to the electrolyte to improve the reproducibility. The best results were obtained with an electrolyte consisting of γ-cyclodextrin dissolved in 2-(N-cyclohexylamino)ethanesulfonic acid (CHES buffer) together with linear polyacrylamide. Using this mixture, the ee measurements corresponded well to those obtained by GC. The separation took 19 minutes per plate, or about 12 seconds per sample. Products were detected after separation by laser induced fluorescence and so a fluorescent tag had to be attached to the substrate to permit detection, necessitating an additional processing step.

Capillary array electrophoresis required very little product, barely any solvent and no high pressure pumps or valves. The columns were quite durable and the chiral phases were cheap to produce. Unfortunately, the instrumentation was expensive and very bulky. The main disadvantage though was the extensive optimization required for quality analyses. An excellent review has been written by Rizzi on chiral separation by electrophoresis [39]. Although it does not discuss high-throughput screening, this paper thoroughly covers the optimization process of chiral capillary electrophoresis.

COLORIMETRIC ASSAY

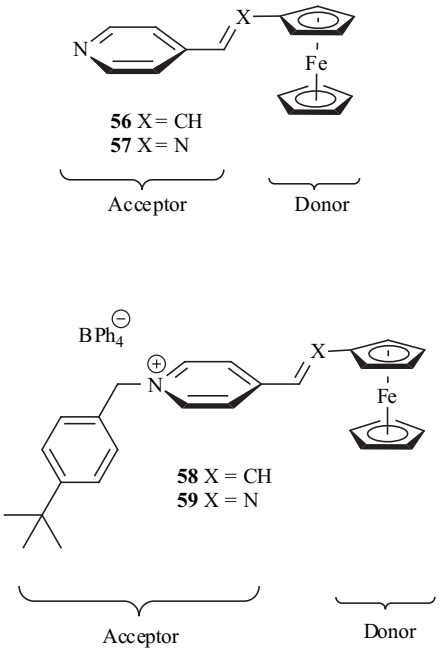
Several methods have been reported using dyes and other colored products in various manners to screen libraries of reactions. Most of these assays required visual identification and even manual collecting of the more active samples. They therefore are so far best suited for academic applications of high-throughput screening. The importance of solid phase in the combinatorial synthetic process encouraged the development of enantioselectivity screening methods for resin-bound products. Such a technique was developed by Still [40] for assessing chiral sensors attached to polystyrene beads using enantiomeric probes labelled with colored dyes.



Scheme 12.

As shown in Scheme **12**, L-proline was bound through a linker to the Disperse Blue dye and D-proline to the Disperse Red dye. A 50:50 mixture of these substrates was treated with various chiral sensors. In this study, the sensors were chiral amines that reacted by acylation or salt formation with **54** and **53** respectively. After a predetermined amount of time, the reaction was stopped and the beads were washed with DMF. The recovered beads were either red or blue if the binding between the probe and the sensor was highly enantioselective and brown if the binding was not selective. The more selective beads were then removed using a low-powered microscope and characterized. Analysis of the microscopic image of selected beads with a color CCD camera provided the enantiomeric excess, with an accuracy of ±5%.

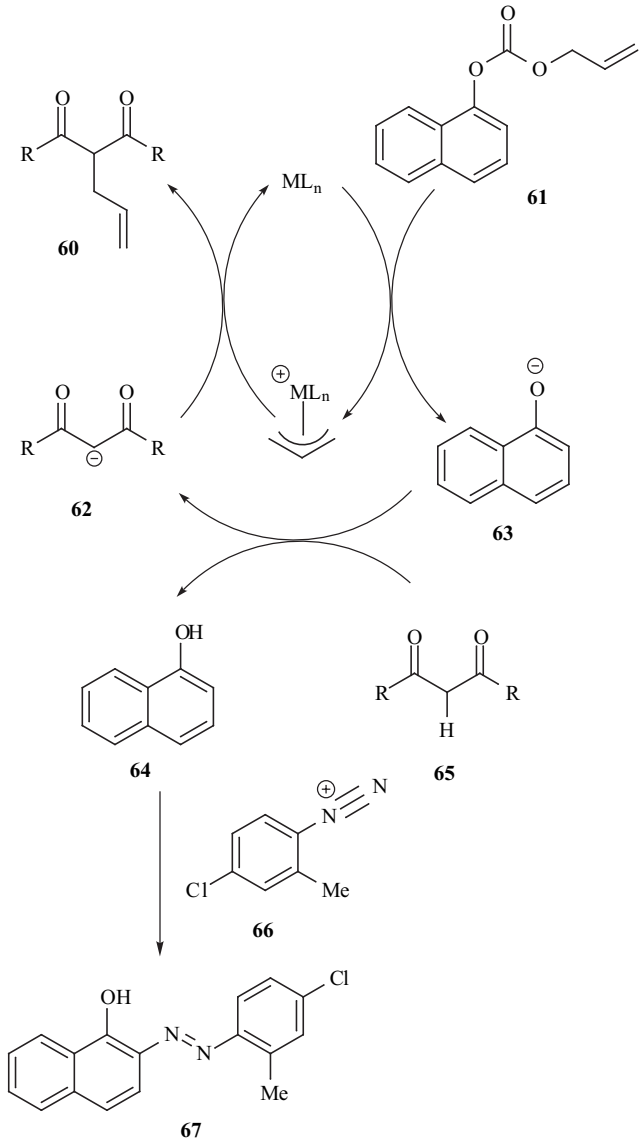
Dyes were also used in a very different technique for catalyst screening devised by Crabtree [41]. Reactive dyes composed of a donor (D) and an acceptor (A) group linked by a C=C or C=N functionality (Scheme **13**) could undergo a color change (bleaching) if the connection between the D and A groups was severed. This property could be used to monitor catalyst activity in alkene and imine hydrosilation since the reaction saturated the C=C and C=N bonds, preventing electron movement between the donor and the acceptor. The brightly colored dyes would then turn yellow. The rate of bleaching was recorded manually and images were taken by digital camera. Both the "initial" and "final" color changes were noted. These corresponded to 40% and 95% conversion respectively since it was a visual assay. Using their methodology, the Crabtree group discovered that $[Pd(Ar_2PC_6H_4CH_2)OAc]_2$, a Heck reaction catalyst, was quite efficient in promoting hydrosilation. The potential of this method was seriously restricted because of the required visual screening. Automation of the detection method however could make it a very promising technique.



Scheme 13.

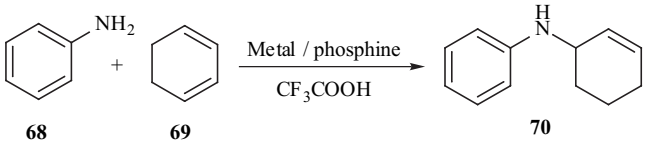
Yet another dye-based colorimetric screening method was reported by Morken and Lavastre in 1999 [42] for the

optimization of allylic alkylation catalysts as shown in Scheme **14**. Various metal salts and ligands were placed in the wells of microtiter plates then **65** was added to the mixtures. After two hours diazonium salt **66**, Fast Red dye, was added to the solutions. In the more effective catalytic conditions, the 1-naphthol by-product (**64**) reacted with Fast Red dye to give **67**, a bright orange azo product. Catalyst activity was assessed visually but it was suggested that parallel UV analysis could be employed and would be particularly useful to differentiate catalysts of similar activity. With this screening method, the first non-phosphane iridium catalysts for allylic alkylation were discovered. The main disadvantage was that this technique could not easily be applied to other reactions.



Scheme 14.

In 2001, Hartwig reported two qualitative visual assays developed for the screening of transition metal catalysts promoting hydroamination. The first method was aimed specifically at optimizing catalysts for the reaction of primary or secondary amines with acrylic acid derivatives [43]. Solutions of $[Na_2Fe(CN)_5NO{\bullet}2H_2O]$ and acetaldehyde became temporarily blue in the presence of secondary amines. The consumption or production of secondary amines in a reaction could therefore be followed visually with these tracers. The second method was very similar but was designed for the hydroamination of aniline and dienes [44] in the presence of a transition metal catalyst and TFA. In an acidic environment, furfural reacted with 2 equivalents of aniline **68** and, by condensation and ring opening, gave a red product. This reaction did not occur with allylic amines like **70** and so was perfectly suited to monitor the disappearance of aniline **68**. The more reactive catalysts caused a faster disappearance of the red color.



Scheme 15.

These qualitative visual assays could just distinguish between highly active catalysts and relatively inactive ones. The methods were only applicable to the screening of very small libraries but in that context they provided useful information about the optimal conditions for hydroaminations. Colorimetric methods provided a rapid, reliable assay for very specific reactions. Their range of application is so far narrow but they were suitable for screening catalyst activity in small libraries. Some strongly colored organometallic compounds may create problems in colorimetric assays by interference with the desired chromophores.

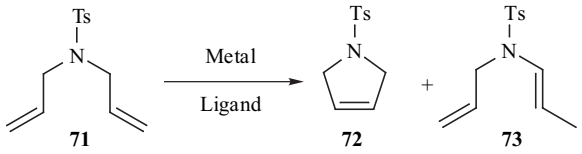
THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is ubiquitous in organic chemistry, because of its simplicity and wide range of application. High-throughput applications of TLC offer the same advantages. Detection can be performed visually, by UV illumination or even mass spectrometry, allowing for considerable flexibility in the choice of substrates.

The first reported application of thin-layer chromatography in high-throughput screening was in biocatalyst screening [45]. Acridone-tagged reagents were used in various enzyme-catalyzed reactions in 96-well microtiter plates. Small samples of the crude reaction mixture were applied to a TLC plate using an automated multichannel pipetter and dried under vacuum for 10 minutes. The samples were then preconcentrated by eluting for 2.5 cm using polar solvents and dried again for 10 minutes under vacuum. Elution with the separating solvent then took place. The preconcentration steps could be avoided if HP-TLC plates with preconcentration zones were used; however these had a tendency to break during loading. The acridone moiety caused blue fluorescence upon UV radiation so the products and reagents could easily be detected on the plate. Substrates were identified visually by their retention factor R_f. Already, this was a reliable, sensitive and versatile screening method.

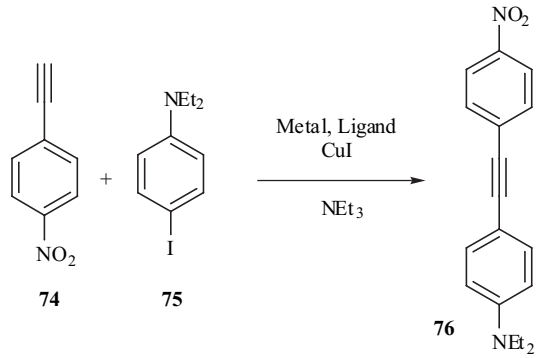
TLC screening was a particularly valuable tool when several elements were varied in the analysis, as demonstrated recently by Lavastre [46]. Combinations of metal-containing reagents and ligands, with and without metal salts, together

with $N_2CHSiMe_3$, were tested as ring-closing metathesis catalysts (Scheme **16**). After the reaction, the mixtures exhibited very different colors because of the presence of trace impurities and it would have been quite challenging to analyze them by spectrometric methods. A 1 μL aliquot of each well was transferred to the TLC plate using a 12-channel pipette. After elution, the plates were analyzed visually and the products identified by their R_f. An interesting spot was discovered with $[Ir(COD)Cl]_2/PPh_3$ which was later identified as **73**, the product of isomerization of one of the double bonds of **71**. This would not have been noticed with most screening methods.



Scheme 16.

Shortly after, important contributions to TLC-based high-throughput screening were introduced by Salo [47] and Lavastre [48]. Salo first reported the use of various surface sampling probes coupled to mass-spectrometers for the quantitative analysis of TLC spots. Mass spectrometry, however, was a rather slow method and was very equipment intensive. In comparison, the Lavastre approach was more economical and practical. The spots were analyzed with image analysis software normally used in the life sciences. Images of the TLC plates were captured with an office scanner or simple digital camera and the image analysis software converted the 2-D image into a 3-D volume by integrating the area and intensity of each spot. To test this method, catalysts for the Sonogashira coupling reaction were screened. Reactions were carried out in 96-well microtiter plates then aliquots were deposited on TLC plates. The desired product **76** was orange and could therefore be detected without UV visualization or chemical staining. Calibration curves were prepared for **76** and a good correlation was obtained with independent measurements. The starting materials, **74** and **75** were colorless and did not interfere with the image analysis. As expected, palladium catalysts were the most active but the first ruthenium-based catalyst for the Sonogashira coupling was discovered. It was not the most active catalyst, giving only 46% conversion but its identification exemplifies the power of high throughput in reaction discovery.



Scheme 17.

This technique could also be applied to the analysis of colorless compounds if UV irradiation was used during image capture. The accuracy and reproducibility of these measurements were also very good, with less than 10% deviation from the calibration curve.

Thin-layer chromatography was a very promising method, especially for the high-throughput screening of organometallic compounds. These types of reactions frequently must undergo purification steps before analysis by GC, HPLC or NMR (to remove paramagnetic compounds), but with TLC analysis no prepurification steps were required. The main advantages of this method were low cost, simplicity, a very broad application spectrum and limited or no development time.

INFRA-RED THERMOGRAPHY

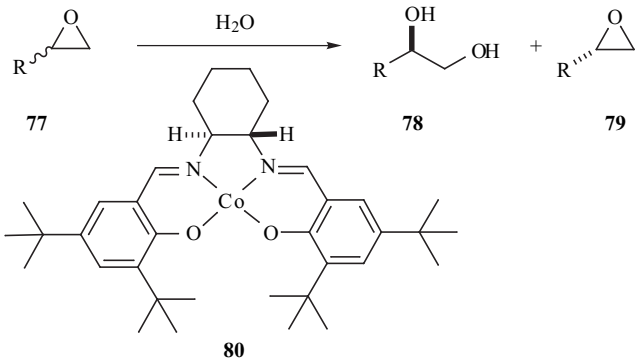
The rapidity and universality of infra-red thermography screening methods make them very appealing to researchers. Most chemical reactions proceed with a change in enthalpy that can be detected by an increase or decrease in temperature. It is therefore assumed in this assay that the change in heat detected is related to product formation.

In 1998, Morken [49] reported a two-dimensional infrared thermography method that could measure rates in an array of reactions. This method relied on the fact that the temperature change in a catalytic reaction is proportional to the product of the turnover frequency and the enthalpy of reaction. If catalysts in a library are analyzed under identical reaction conditions, the most active catalysts will cause the largest temperature perturbations. In this analysis, an IR camera was used to monitor the heat evolved. The catalysts were carried on beads placed together in a solution of substrate and additives. During the reaction, the more active catalyst beads exhibited a small (~1 °C) temperature increase relative to the solvent that was easily detected by IR. The most "active" beads could then be selected and decoded. This technique was very general and could be applied to the screening of most libraries. The only setback was that interference from the solvent prevented the observation of temperature changes for submerged beads. Chloroform was therefore added to increase the solution density and force the beads to the surface.

This method was soon modified by Reetz [50] to analyze reactions in microtiter plates. After the substrates were combined, the plate was shaken for 5 seconds then 250 images were recorded during 5 seconds with an IR camera. The readings were taken in the centre of each well to avoid reflections from the top part of the vessel walls. As a demonstration, measurements of activity were taken for catalysts such as **80** in the kinetic resolution of racemic epoxides by enantioselective ring-opening reactions (Scheme **18**) [51]. It was found that **80** was the most active catalyst and reacted selectively with **77**. These observations corresponded well to the results previously obtained by Jacobsen [51] in laboratory-scale reactions.

The same methodology was later applied to a mildly endothermic reaction, ring-closing olefin metathesis, and proved to be just as reliable [52].

An extremely interesting and potentially very powerful development from researchers at GlaxoSmithKline was the observation that when Reetz' method was applied to the



Scheme 18.

enantioselective enzymatic acetylation of 1-phenylpropanol, the enantiomeric excess of the products was not only proportional to the initial rates of reaction, but also to the areas under the temperature curves [53].

When using IR thermography, several factors could cause problems with the image recorded. If there were secondary emissions or reflected radiations, the image could be disturbed and provide false information. To avoid these issues, Maier [54] recommended the use of a PtSi-based camera, instead of the usual focal plane array camera. A linear correction was applied to the detector response and a reference spectrum was subtracted from all images in order to get the temperature differences caused by the catalytic activities. After removal of the subtraction artifacts, thermography proved to be a powerful technique for the high-throughput screening of heterogeneous catalysts.

A scale-transparent adaptation of infrared thermography was first reported by Leugers in 2001 [55]. To avoid internal reflection and other undesirable interferences, the bottoms of the microtiter plates were replaced with IR-transparent materials. Detection could be done by transmission providing improved spectral resolution. This technique was used in an assay conceived by Klein [56] in which scale-transparent IR thermography was used as an initial screen. Since it was a very rapid technique that gave indirect information about reactivity, IR thermography was coupled to a slower but more precise analytical instrument equipped with a positionable sampling capillary. The most active catalysts were identified with an IR camera then picked up by the "sniffing" capillary and injected into a mass spectrometer. Thus, only the most active and useful mixtures were thoroughly analyzed, providing both speed and quality.

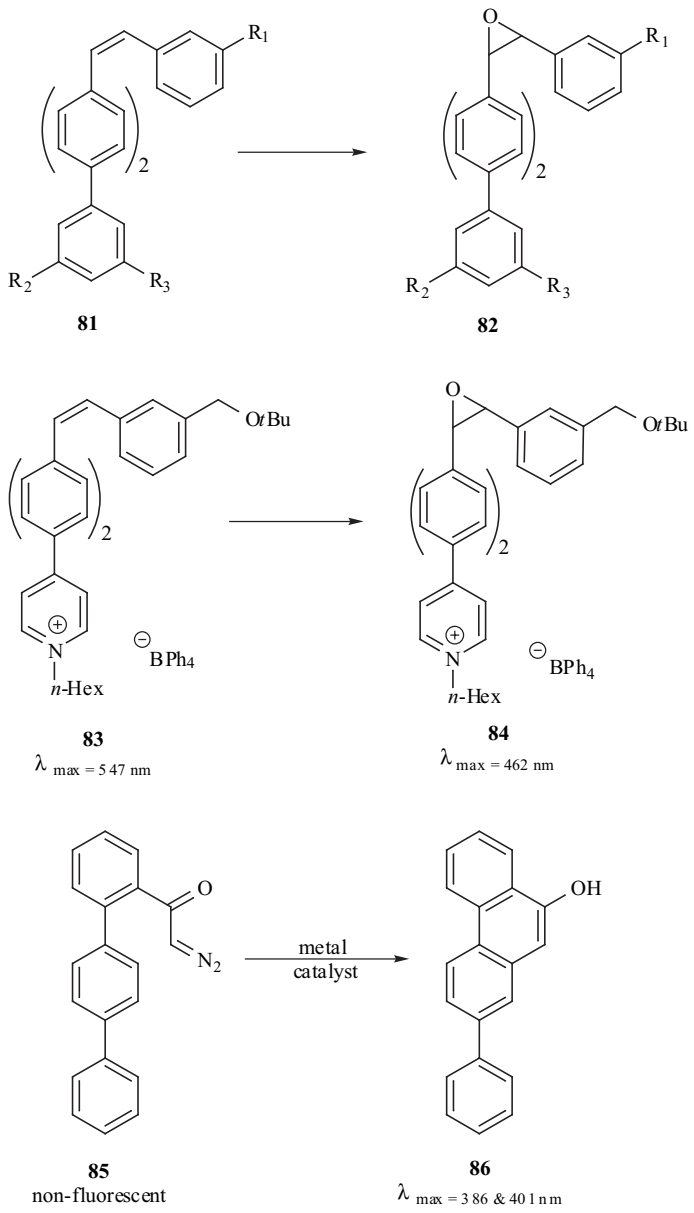
Sutherland proposed the use of multiplexed arrays of thermistors to monitor reaction temperatures in catalytic reactions [57]. The samples were placed in 96-well plates and temperatures were monitored for 10 seconds as a reference. The catalysts were then added and the plates were put in an incubator, shaken to mix the reagents and positioned so that the thermistors were over the centre of each well. Temperature changes were monitored as resistance changes. When the reference was subtracted from the run measurements, the more active catalysts could easily be detected. As an example, the slightly exothermic β-lactamase-catalyzed hydrolysis of penicillin G to penicilloate was studied. The microtiter plate was loaded with β-lactamase in phosphate buffer and equilibrated at 37 °C before the addition of penicillin. The highest temperature reached was related to the amount of enzyme contained in each well. The same reaction was also analyzed in the presence of a β-lactamase inhibitor, potassium clavulanate. It was found that inhibition could be observed with this technique and it was even possible to compile a crude ranking of inhibitory efficiency. Thermistor arrays represent an interesting alternative to IR thermography, mainly because of the increased sensitivity (100 μK *vs*. 10 mK for IR).

Since it did not probe directly the composition of the reaction mixture, IR thermography could not indicate if the desired product was being formed. It was however a very rapid and efficient method for the approximation of catalytic activity for a particular target. When catalyst discovery is the goal, IR thermography may not be the best option because heat changes could result from side-reactions or unexpected reactivity. Infrared thermography will perhaps find its niche in the high-throughput optimization of industrial processes rather than the screening of large, diversified catalyst libraries [55].

FLUORESCENCE

Many of the most versatile and fast screening methods utilize fluorescence detection because of the high sensitivity of the process. Fluorescence methods have been applied to biological and catalyst high-throughput screening and several aspects have already been covered in the biological methods section. In this section, the emphasis is placed on the screening of synthetic and organometallic catalysts by fluorescence.

In 2001, Sames reported a high-throughput screening assay for atom transfer catalysis [58]. It was not intended to be a general assay as it was designed for this sole purpose. Specific reagents were chosen for their fluorescent properties, which were significantly altered upon carbene or oxygen atom transfer, as illustrated in Scheme **19**. Changes in fluorescence were caused by a shortening or lengthening of the conjugated π-system that could be measured with a simple fluorescence plate reader. To study catalysts for epoxide formation, terphenyls **81** were chosen. The emission maximum of **81** was blue-shifted and the emission intensity increased by as much as a factor of 2.5 as a result of the reaction. In **83**, the pyridine moiety increased the π-delocalisation and shifted the emission into the visible range. These probes were intensely fluorescent so as little as 2-3% conversion could be detected. Carbene insertion was studied with slightly different terphenyl probes. Terphenyl diazoketone **85** was not fluorescent but upon metal-catalyzed carbene insertion, the highly fluorescent phenanthrol **86** was formed. This assay was very sensitive, detecting less than 1% conversion. The yields measured corresponded, within 5%, to the yields measured by NMR. The reactions were carried out in 1536-microwell plates. One bead was placed in each well and then the solvent, probes and other reagents were added. The plates were placed in a vapour chamber for a determined period of time then scanned with a fluorescence plate reader. A 1536-well plate could be scanned in 5 minutes, corresponding to 0.2 seconds per sample. The most

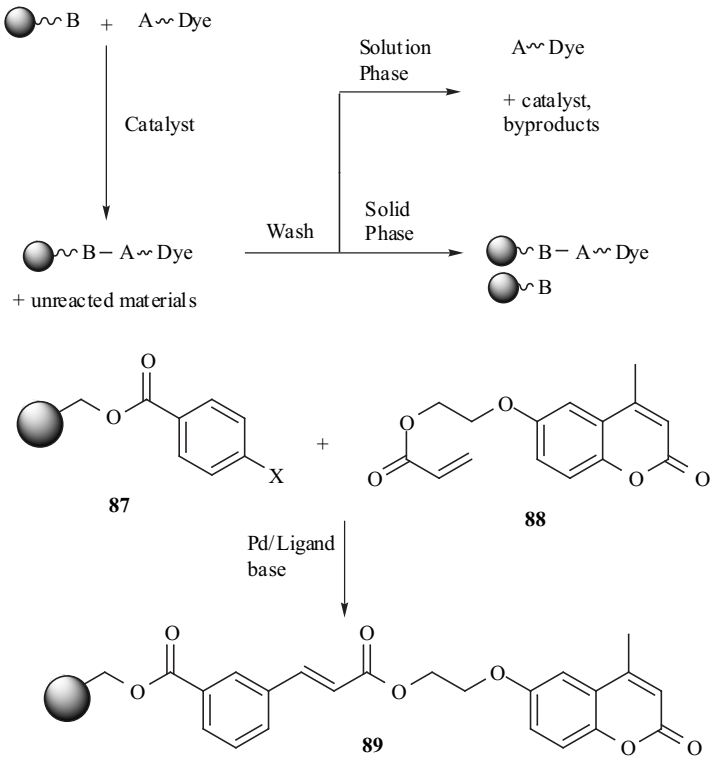


Scheme 19.

time-consuming step was the manual distribution of beads on the plate.

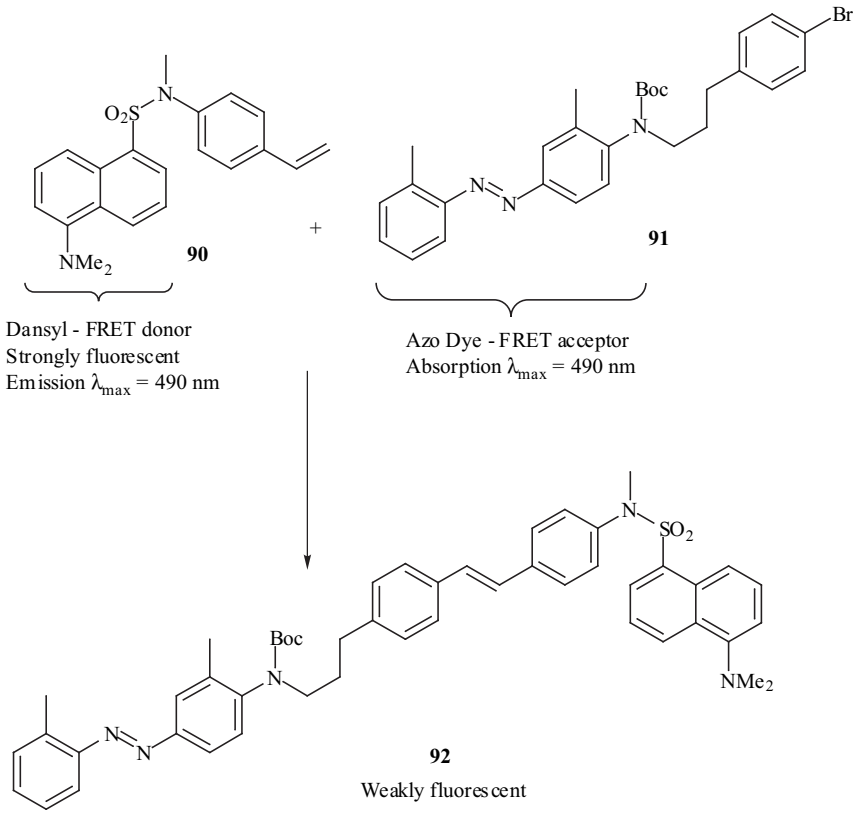
Hartwig reported one of the first fluorescence-based screening methods with applications in organic chemistry for the optimization of Heck coupling reactions [59]. This process was chosen because there was a need to develop ligands that could promote the coupling of aryl chlorides and electron-rich aryl bromides at low temperatures. One of the reagents was bound to a fluorophore and the other was attached to a cross-linked polystyrene resin bead. The success of a coupling reaction was indicated by the appearance of fluorescence on the solid support after washing and drying as shown in Scheme **20**. The aryl halide reagent was attached to Wang resin [60] and acrylate was linked by an alkyl group to 4-methyl-7-hydroxycoumarin, a fluorescent tag. Reactions were carried out in vials placed in an aluminium heating block preheated to 100 °C. After workup and several solvent washes, the resins were dried and irradiated under a hand-held UV lamp. Pictures were recorded but the degree of fluorescence was evaluated visually. Because this method provided no information about selectivity, the ligands that appeared to be the most reactive in the fluorescence screen were used in laboratory-scale experiments. This method was reliable and produced the discovery of two ligands for the Heck coupling of aryl halides and aryl chlorides: di(*tert*-butylphosphino)ferrocene and tri(*tert*-butyl)-phosphine. It was considerably faster than GC or HPLC but was a qualitative assay. The use of resins could also have been problematic with fluorescence detection since there was a risk of the resin altering the fluorescent properties of the dye.

Hartwig took a slightly different direction and devised a general, rapid and quantitative screening assay using Fluorescence Resonance Energy Transfer (FRET) [61]. FRET had been effectively used to measure biocatalyst activity but it had never been adapted for homogeneous



Scheme 20.

catalysis. The FRET phenomenon occurs when the emission band of one fluorophore overlaps with the absorption band of another. The molecule that absorbed at higher energy was called the FRET donor. When excited, the emission it produced was quenched by the FRET acceptor. In certain conditions, this emission was inversely proportional to the mole fraction of each molecule. Therefore in a reaction in which a covalent bond was formed between the acceptor and



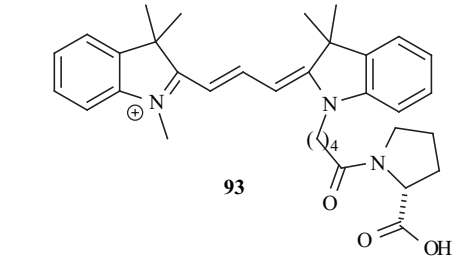
Scheme 21.

donor molecules, the yield could be deduced from fluorescence measurements using a calibration curve. To generate this curve, solutions containing different ratios of product and starting materials were prepared and the mole fraction of product was plotted against the measured fluorescence intensity. This assay was perfectly suited to screen ligands for the Heck reaction. When the molecules were separated, the emission was not quenched but as coupling occurred, there was a drop in fluorescence due to the proximity of the donor and acceptor groups. For this assay, a dansyl fluorophore (donor) was covalently bound to a styrenyl group and the quencher, an azodye, was tethered to an aryl bromide as shown in Scheme **21**. The reactions were performed in an aluminium reaction block with a 96-well glass plate. After 15 hours at 70 °C, aliquots were taken, diluted and analyzed on a fluorescent plate reader. Each reaction could be analyzed in 1 second, a tremendous improvement over the fluorescent bead method presented earlier. Yields determined by the FRET method differed at most by 10% from the HPLC derived values. Again, two very active catalysts were discovered with this assay. At room temperature, 1-adamantyl-di-*tert*-butyl phosphine and $Ph_5FcP(t\text{-}Bu)_2$ palladium complexes were found to catalyze the Heck coupling of aryl bromides.

This same methodology was later used to discover catalysts and optimize reaction conditions for the arylation of cyanoacetates [62] and amines [63]. In the first case, the optimized reaction conditions provided the first general conditions for the arylation of cyanoacetates. With some ligands, the reaction could even be carried out at room temperature. The arylation of amines, on the other hand, had already been thoroughly studied but it was shown that the yields measured with the FRET method agreed with those obtained by conventional analysis.

The FRET method was general, inexpensive and was not equipment intensive. Reaction yields could be measured rapidly (1 second) and accurately. It was superior to GC, HPLC, NMR, and colorimetric methods but did not give any information about regio- or stereoselectivity. The method was perfect for the discovery of highly active catalysts for coupling reactions but in some cases, the synthesis of the large fluorescent molecules was necessary.

An extension of Still's method [40] was reported by Shair in 2001 that adapted DNA microarray technology for the measurement of enantiomeric excess [64]. To evaluate the methodology, the composition of mixtures of α-amino acids was studied. N-Boc-protected amino acids were arrayed and covalently bound to an amine-functionalised glass slide by automated contact printing. Only a very small amount of amino acid was required for each spot, less than 10^{-11} mol. The remaining uncoupled amine functionalities on the glass plate were then acetylated and the amino acids were then deprotected. The only free amino functionalities were therefore those of the amino acids. Chiral fluorescent probes were then coupled with these free amines giving a parallel kinetic resolution. The probes used were Cy3 fluorophore bound to D-proline (**93**) and Cy5 bound to L-proline (**94**). Each of these preferentially bound to one enantiomer of the α-amino acid providing the necessary kinetic resolution. The enantiomeric excess of the original α-amino acid mixture could be expressed as the ratio of fluorescent intensities of pseudoenantiomeric fluorophores that emitted at different wavelengths. Irradiation with an automated laser scanner caused the excitation of Cy3 at 532 nm and Cy5 at 635 nm. The relation between enantiomeric excess and fluorescence intensity for each spot was calculated from the equations shown.



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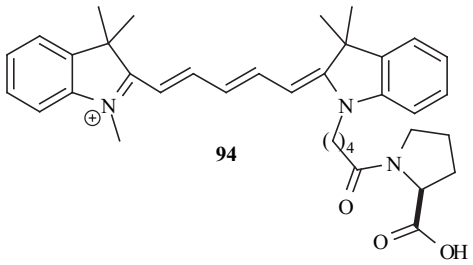


94

$$\%\,ee = \left[\frac{(x-1)(s+1)}{(x+1)(s-1)}\right] \times 100\%$$

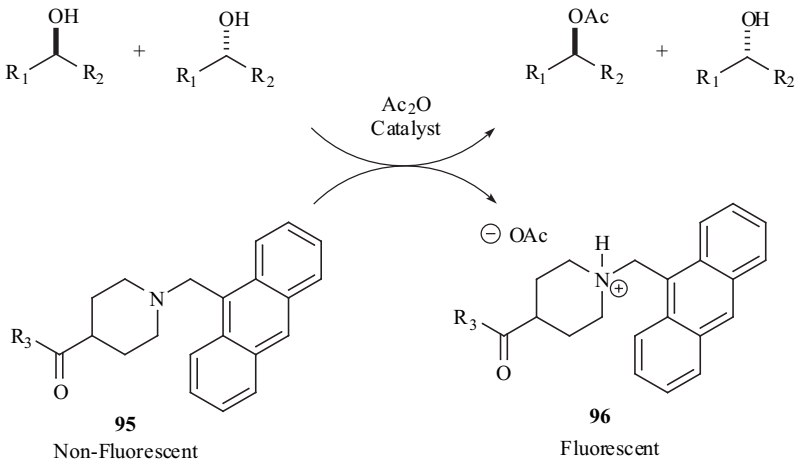
$$x = \frac{I_{fluor,\mathbf{93}}}{I_{fluor,\mathbf{94}}} \times \frac{1}{z}$$

$$s = \frac{k_{fast}}{k_{slow}}$$

Scheme 22.

Although only small kinetic differences were necessary, higher amounts of resolution translated to more accurate enantiomeric excess values. The average measured enantioselectivities were within 10 % of the real values. This was a very rapid screening method, as each spot was analyzed in about 11 seconds, and the method could also be applied to a variety of conditions (inert atmosphere, anhydrous, etc).

One of the most interesting and versatile screening methods of determining enantiomeric excess was first reported by Miller in 1999 [65]. In earlier studies with asymmetric acylation catalysis it was noticed that the more selective catalysts also caused the fastest reactions [66], an assumption that served as the basis for a screening assay. Selectivity was expressed as k_{rel}, which was the ratio of reaction rates for the two enantiomers of a compound in a resolution reaction. The catalyzed acylation reactions studied produced one equivalent of acetic acid per catalytic turnover. By monitoring the concentration of acetic acid in solution, the catalytic activity could be indirectly measured. To detect the production of acetic acid, a pH-sensitive fluorophore, **95**, was added to the solutions along with the reagents. This aminomethylanthracene (AMA) was not fluorescent as a free amine but became intensely fluorescent when protonated, as shown in Scheme **23**. The emission intensity was then



Scheme 23.

measured at regular intervals with a fluorescence plate reader and the resulting plot was interpreted as a direct readout of the evolution of acetic acid with time. Each catalyst was screened separately against both pure enantiomers of the starting material. The higher the ratio of initial reaction rates between enantiomers of a compound, the more selective the catalyst was assumed to be. It was a very rapid assay that could screen 1000 catalysts in an hour. Unfortunately, pure enantiomers of the substrate alcohols were required for the calculation of k_{rel}.

In a later version of the assay, the fluorescent sensor **95** and the peptide-based catalyst were bound to the same polystyrene bead [67] The various catalysts on these special solid supports were analyzed in separate vessels, as well as combined in the same reaction vessel (split and pool), giving essentially the same results. Diffusion of acetic acid between beads was considerably slower than the reaction monitoring. With this technique, Miller studied the use of small conformationally-constrained peptides as mimics of acylases. These oligopeptides, synthesized randomly by split-and-pool, were tested for the kinetic resolution of secondary [67] and tertiary [68] alcohols. After an initial screen, a new, biased catalyst library was built to optimize the more selective candidates. When the reactions were repeated on a larger scale, the selectivity was often somewhat different from the bead measurements but the screen was still a good approximation of selectivity. Several general and selective peptide catalysts were thus discovered for the kinetic resolution of alcohols.

Later, catalyst-functionalised beads were placed in a polymer matrix that allowed diffusion of the substrates between the beads [69] The sensor was no longer bound to the solid support, but was incorporated into the gel so that the more active catalysts induced fluorescence of the gel in their vicinity. The advantage of this method was the separation of the fluorescent sensor from the catalyst.

Fluorescence-based assays are very promising for enantioselectivity and activity screening because of their excellent sensitivity and versatility. These methods have been extensively used and have considerably improved in rapidity over the last few years. Miller's gross approximation for the measurement of enantiomeric excess, although it has to be tested for every new compound, provides possibly one of the most useful high-throughput enantioselectivity screening advance. Simply searching for the most active catalysts in a primary screen, then following up with a more precise method in a secondary screen of only the most active examples greatly reduces the searching required.

CONCLUSION

The initial attempts to modify traditional methods for high-throughput screening have had some success. Despite impressive and creative approaches, NMR, GC, HPLC, and MS remain low-throughput and equipment-intensive methods. They do provide precise information about each reaction mixture but, as a general rule, the increased precision is time-consuming. Although some may criticize the lack of accuracy in many of the more rapid techniques and defend the use of these more traditional instruments, it should be stressed that this is not the goal of high-throughput screening. Gross approximations must be made in order to obtain results in a timely manner. More accurate methods may be quite useful and indispensable in secondary screening, after an initial, more rapid evaluation of the library.

The most promising methods for high-throughput screening are possibly IR thermography, CAE, TLC and fluorescence. IR thermography is quite rapid but does not give any indication as to whether the desired reaction is indeed occurring and it has not yet been adapted to enantioselectivity assays. CAE is extremely accurate and has proven itself as a high throughput method in the success of the human genome project. It is equipment intensive and may require extensive tuning however. TLC is simple, cheap, easily understood and robust. Recent automations and the use of digital imaging suggest that this method has a lot of promise in combinatorial-type reaction screening. Fluorescence methods indicate clearly if the expected process is being achieved and can be used for enantioselectivity purposes. They are extremely sensitive but require tuning and optimization.

There will most likely never be a general high-throughput screening method for evaluating reactivity and stereoselectivity in all situations. But with so many

techniques offered to the chemist, it should be possible to screen most libraries very rapidly and considerably speed up the discovery and optimization of new reagents and catalysts. The best compromise of speed and accuracy today seems to be the use of a fast method like TLC or fluorescence in a primary screen, then following up with a more precise method such as HPLC in a secondary screen. In this way, one can rapidly identify the most promising new chemical methods, and use precise measurements to fully optimize them.

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