Recent Advances in the Measurement of Enantiomeric Excesses

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Abstract: The elaboration of an ever increasing number of chiral catalysts or reagents demands fast and sensitive methods to measure enantiomeric excesses of the products. This review discusses the various ways to solve these problems. Mass spectrometry, chromatographic or chiroptical methodologies, fluorescence, liquid crystals, enzymatic methods, and immunoassays, are some of the technologies which may be used. Kinetic resolution is often a convenient and complementary tool for analysing a mixture of enantiomers.

Keywords: asymmetric catalysis; catalyst screening; enantiomeric excess; fast screening; kinetic resolution; *pseudo*-racemic

1 Introduction

The use of a polarimeter to measure enantiomeric excesses (ee's) is very old, and can be traced back to Pasteur's time. The common expressions "optical purity" or "optical yield" reflect well the fundamental importance of the specific rotations and the chiroptical methods used in studies related to enantiomers. With the introduction of spectroscopic and chromatographic methods, more accurate analyses of a mixture of enantiomers could be achieved.[1,2,3] The development of combinatorial chemistry made it possible to create libraries of asymmetric catalysts.[4,5,6] Their screening involves parallel assays, where one needs to quickly determine the enantiomeric excesses on tiny amounts of products. This explains the renewed interest in the measurement of ee's.

In this article we will not focus on the high-throughput screening of asymmetric catalysts (which has been fully reviewed recently by Reetz[6,7]). We will rather concentrate on the exclusive problem of measuring the enantiomeric excess of a mixture of enantiomers (whatever is its origin) which is available in only a small amount.

2 Modern Version of Some Conventional Methods

2.1 General Remarks

Measurements of enantiomeric excess by use of chiral stationary phases such as in GC or HPLC, or in capillary array electrophoresis (CAE) have been considered to be able to handle only a limited number of samples per day. The development of new technology has shortened times for analysis.[8a,8b] Reetz et al. showed in studies on the directed evolution of enantioselective enzymes that GC and also CAE are powerful tools for high-throughput ee determinations.[6] In addition to GC and CAE, methods involving diastereomeric interaction have

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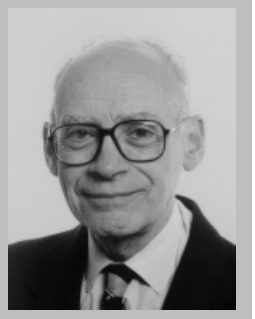


Henri B. Kagan was born in Boulogne-Billancourt (France) in 1930. He graduated from the Sorbonne and Ecole Nationale Supérieure de Chimie de Paris in 1954. He prepared his Ph. D. under the supervision of Dr. J. Jacques. He joined Prof. A. Horeau at the Collège of France in Paris in 1962 as a research associate. In 1965 he worked with Prof. T. Mabry at the University of Texas, Austin. He joined the Université Paris-Sud, Orsay in 1968. He is emeritus Professor of Université Paris-Sud since 1999. He is member of the French Academy of Sciences. H. B. Kagan developed investigations in various areas, such as asymmetric synthesis, asymmetric catalysis, lanthanide reagents (for example, diiodosamarium). His awards include the Prelog Medal, the August-Wilhelm-von-Hofmann Medal, the Chirality Medal, the Nagoya Medal of Organic Chemistry, the Medal of the RSC, the Tetrahedron Prize, the 2001 Wolf Prize for Chemistry (shared with K. B. Sharpless and R. Noyori) and the 2002 Grand Prix de la Fondation de la Maison de la Chimie (shared with H. Yamamoto).

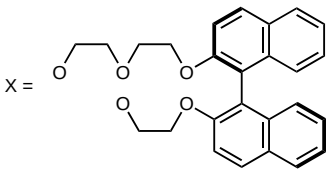
Masaki Tsukamoto was born in Yokohama (Japan) in 1970. He studied chemistry at Nagoya University where he obtained his M. Sc. (1994) and Ph. D. (1999) under the supervision of Profs. R. Noyori and M. Kitamura. He then moved to Research Center for Materials Science at the same university in Prof. M. Kitamura's group as a research fellow of the Japan Society for the Promotion of Science (JSPS). Since September 2001, he has been working with Prof. H. B. Kagan at Université Paris-Sud, Orsay as a post-doctoral fellow. He is currently involved in the study of non-linear effects and the development of new catalysts.

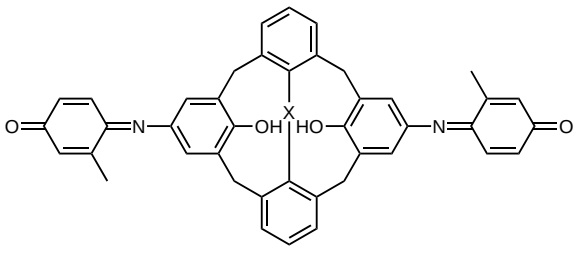
been reported. One promising way is visible ee recognition with chiral chromogenic host molecules. The use of on-bead substrates is also effective in terms of product separation from the reaction mixture. Chiroptical properties and liquid crystals are methods without the use of diastereomeric interactions.

2.2 Chromogenic Host Molecules

Visual recognition between enantiomers can be one of the simple and fast measurements of enantiomeric excess. If the interaction between a chiral host molecule and one or the other of the enantiomers of the guest molecule (sample) causes different UV-visible spectral changes, visual discrimination will be possible. Chromogenic host molecules which have phenol moieties were reported for that purpose. This type of host-guest interaction is based on the study of the enantiomeric recognition of amines by various chiral macrocyclic molecules.[9]

One example is a calixarene derivative which contains an enantiopure 1,1′-binaphthyl subunit and two indophenol chromophores.[10] Kubo et al. found that the red ethanol solution of **1** was immediately changed to blue-violet upon addition of (*R*)-phenylglycinol, (*R*)-**2**, giving a new absorbance band around 650 nm. In the experiment with the other enantiomer, (*S*)-**2**, the solution remained red with almost no detectable changes in the spectrum. Merely 0.1 – 1% of host molecule **1** referred to **2** enables us to determine the concentrations of (*R*)-**2** even in the presence of (*S*)-**2**. Thus, a satisfactory calibration curve was obtained in the range of 0 – 33% ee of (*S*)-**2**. It was suggested that enantiomer recognition takes place in the cavity involving the ether oxygens of the macro-ring and phenolate of the indophenol and that the OH group of amine **2** participates in the chiral recognition by hydrogen bonding with the phenolate of the indophenol. Enantiomeric discrimination is also possible between *R* and *S* enantiomers of phenylalaninol salt **3** or phenylglycine (**4**).





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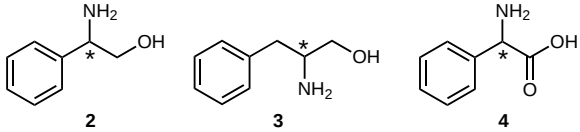
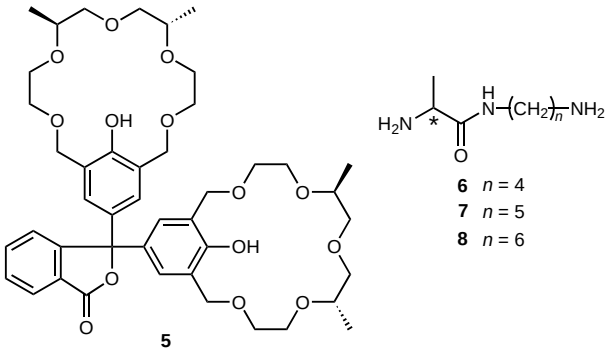


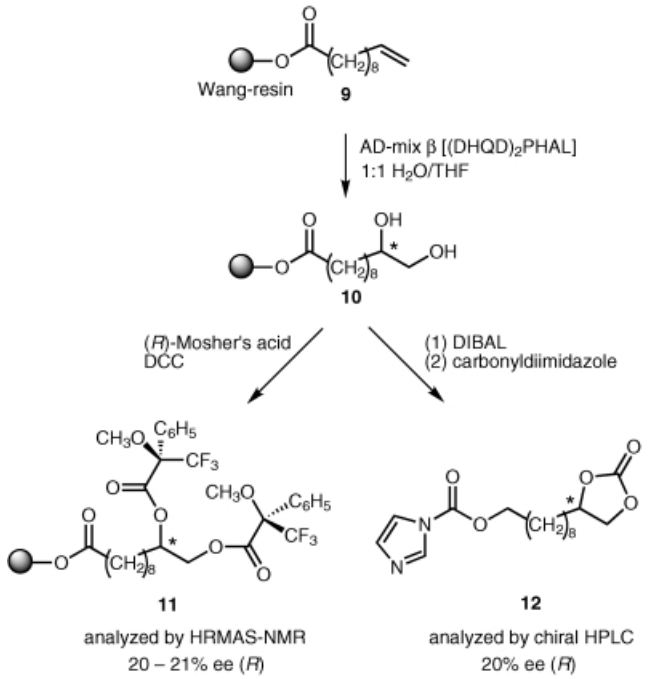
2 **3** **4**

Another example reported by Fuji et al. is the case of phenolphthalein derivatives.[11] They synthesized a series of chiral host molecules such as **5** and examined the interactions between host molecules and alanine derivatives, **6**, **7**, and **8**, by using UV-visible titration in the presence of a large excess of *N*-ethylpiperidine. In the case of host **5**, the molar absorption coefficient around 570 nm with alanine derivative (*R*)-**8** is 1.9 times larger than that for (*S*)-**8**. A significant difference was also observed between the *R* and *S* enantiomers of **7**. Consequently, the combination of host **5** with guest (*R*)-**7** or (*R*)-**8** gave a purple color, whereas no color development was observed between **5** and (*S*)-**7** or (*S*)-**8**. Thus, the absolute configuration of the alanine derivatives could be easily determined visually. A colored complex of host **5** with alanine derivative (*R*)-**8** in the presence of *N*-ethylpiperidine was proposed. The distance between the two amino groups is also crucial for the color development since neither the *S* nor the *R* enantiomer of diamine **6** gave a color in the presence of host **5**.[12]



2.3 On-Bead ee Measurements

There are many cases of polymer-bounded heterogeneous catalysts, however, only few examples were reported of the alternate situation of the asymmetric transformation of a polymer-bound substrate followed by on-bead measurement of enantiomeric excess. Berkessel et al. studied the Sharpless asymmetric dihydroxylation[13] for polymer-bound olefins and measured the enantiomeric excess of the products with and without destruction of the bead.[14] One example is shown in Scheme 1. A prochiral olefin **9** was the substrate connected to a Wang-resin support. After the quantitative (96% conversion) dihydroxylation of **9** with AD-mix β containing $(DHQD)_2PHAL$ in a 1:1 mixture of H_2O and THF, the diol **10** on bead was derivatized to the bis-Mosher's ester **11** and the enantiomeric excess was measured by ^{13}C NMR spectroscopy using high-resolution magic angle spinning (HRMAS). The observed result by this method was 20 – 21% ee with *R* configuration. For comparison with this NMR method, the enantiomeric excess was also analyzed by chiral

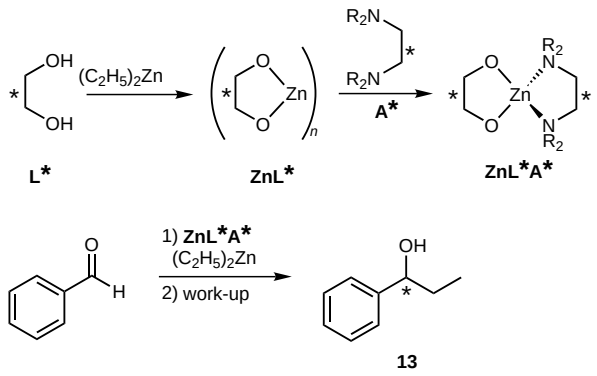


Scheme 1. Determination of enantiomeric excess in Sharpless dihydroxylation of a polymer-bound olefin.

HPLC. First, the resin of diol **10** was reductively cleaved by DIBAL and the resulting triol was treated with carbonyldiimidazole. HPLC analysis of the cyclic carbonate **12** showed 20% ee which was consistent with the value obtained with HRMAS NMR method. There are some interesting points. First, polymeric supports effected yield and ee of the reaction. When the polymer support of **9** was replaced with TentaGel S-OH®, asymmetric dihydroxylation under the same conditions gave the corresponding diol in 45% ee and 44% yield. Secondly, the enantiomeric excesses of diols obtained in solid phase synthesis tend to have lower values compared to that obtained in solution phase synthesis. Although this method needs derivatization and full assignment of the ^{13}C NMR signals of the bis-Mosher's ester, on-bead determination of ee is a promising methodology in this area.

2.4 Chiroptical Properties

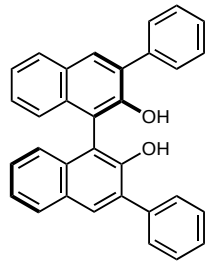
So far, measurements of enantiomeric excess of chiral compounds which have been conducted by use of chiral HPLC or GC require peak separations of both of enantiomers (sometimes difficult to achieve). Combinations of polarimetry, circular dichroism (CD), and liquid chromatography (LC) are one of the trends of ee determinations.[15] Mikami et al. showed that detecting CD made it possible to determine the ee by use of HPLC on non-chiral stationary phases.[16,17] The method requires simultaneous monitoring of the CD signal ($\Delta\varepsilon$) and the UV absorption (ε) in HPLC on non-chiral



Scheme 2. Asymmetric activation of diol-zinc catalysts by nitrogen ligands.

stationary phases at a fixed wavelength in a flow system to obtain the anisotropy factor ($g = \Delta\varepsilon/\varepsilon$).[18] As shown in Scheme 2, these authors investigated the enantioselective addition of diethylzinc to benzaldehyde catalyzed by zinc-diol complexes **ZnL***A* which produces chiral 1-phenylpropanol (**13**). First, it was confirmed that the *g* factor is proportional to the enantiomeric excess of (*S*)-**13** and that there is no concentration effect. Thus, on the basis of this system, the authors screened alkylation reactions with a series of diol-zinc catalysts prepared *in situ* by mixing diol (**L***), diamine (**A***), and diethylzinc (Scheme 2). Finally, the optimized conditions were found, which afforded quantitatively (*S*)-**13** of 97 – 99% ee in CH_2Cl_2 between –78 to –20 °C with a combination of **14** and **15** (2 – 10 mol %). Reetz et al. applied this analytical method for determination of the enantiomeric excess of 1-phenylethanol obtained by either reductase-catalyzed reduction of acetophenone or lipase-catalyzed kinetic resolution of racemic 1-phenylethyl acetate.[19]

Raman optical activity was also examined for the determination of ee's. Spencer et al. investigated the applicability of scattered circularly polarized Raman optical activity (SCP ROA) to the ee measurement of α-pinene.[20] Since the signal strength in SCP ROA is proportional to the ee's of α-pinene, quantification is possible. Similarly, incident circular polarization Raman optical activity (ICP ROA) was performed to identify 100% and 98% ee of menthol.[21]



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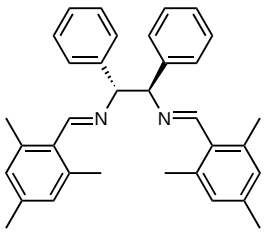

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2.5 Liquid Crystals

Liquid crystals (LC's) may act as a color indicator of molecular chirality. When a small amount of an optically active molecule is added to a nematic liquid crystal, a cholesteric phase is induced with the helical pitch (*p*) which is expressed by the Equation (1).[22]

$$p = (c \cdot \beta \cdot ee)^{-1} \quad (1)$$

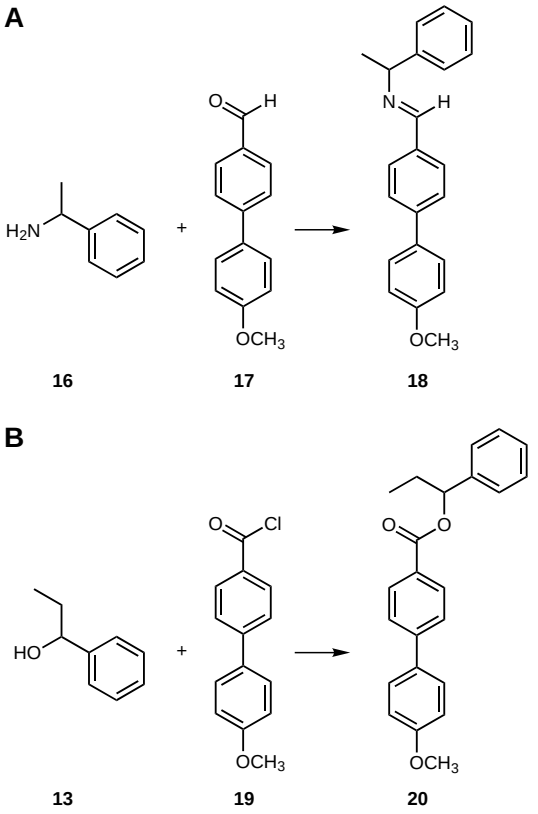
Here, *c* (wt %), β, and *ee* are the concentration, the helical twisting power, and the enantiomeric excess of the molecule (dopant), respectively. The induced cholesteric phase which is oriented by a linearly rubbed polyimide-covered glass plate can reflect light of wavelength $\lambda(\alpha)$ at the angle of the incident light (α) relative to the normal of the surface. $\lambda(\alpha)$ is proportional to the pitch and the mean refractive index (*n*).

$$\begin{aligned}\lambda(\alpha) &= n \cdot p \cdot \cos[\sin^{-1}(\sin \alpha/n)] \\ &= n(c \cdot \beta \cdot ee)^{-1} \cdot \cos[\sin^{-1}(\sin \alpha/n)]\end{aligned} \quad (2)$$

Color induction is possible when $\lambda(\alpha)$ lies in the region of the wavelength of the visible light. Feringa et al. succeeded in visualization of the enantiomeric excess of amine **16** and alcohol **13** by derivatizing to appropriate dopants and selecting the suitable liquid crystalline host material (Scheme 3).[23] As the host, the authors choose E7 which consists of a *para*-alkoxy-substituted cyanobiphenyl unit, and derivatized **16** or **13** was selected as a dopant with achiral mesogenic units which have a similar structure to that of the host.

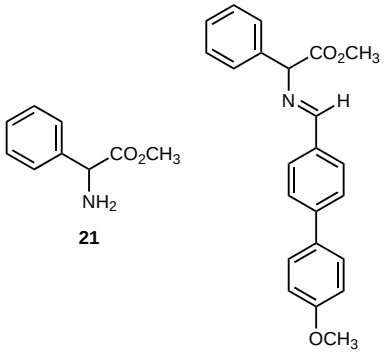
Thus, as shown in Scheme 3, (*S*)-1-phenylethylamine [(*S*)-**16**] was condensed with aldehyde **17**, and consequently the resulting imine **18** showed cholesteric textures with E7 with colors ranging from red to violet depending on the concentration of **18** (10 to 19 wt %). Interestingly, amine **16** itself does not show the cholesteric textures. In the same manner, imine **18** derived from amine **16** with various ee were prepared, and the reflected wavelength $\lambda(45°)$ of doped E7 was measured. The wavelength of the maximum reflection [$\lambda(45°)$] depends linearly on the ee of imine **18**, ranging from purple for 100% ee to red for 50% ee at the concentration of 18.9 wt % of **18**. Only microgram quantities of chiral samples are required. The same color indication is also possible for the chiral alcohol **13** by derivatization to the ester **20** with acid chloride **19**. Under these experimental conditions, the following characteristics were noted: (1) samples with ee values lower than 50% do not show any color, (2) the color depends on both ee and conversion of the sample.

Color indication for the full range of enantiomeric excess would be informative, if it were possible. Recently, Feringa et al. optimized the conditions and also succeeded in the study of methyl phenylglycine (**21**).[24] Thus, **21** was reacted with **17** to give the



Scheme 3. Derivatization of chiral amine **16** and alcohol **13** with achiral mesogenic units.

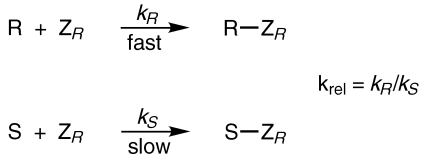
corresponding imine **22**. Instead of doping with 18.5% of analyte, a 1:3 mixture of enantioimpure analyte and enantiomerically pure (*S*)-**22** was used as dopant of E7 and the wavelength of reflection $\lambda(45^\circ)$ was also measured as a function of the ee of the analyte. Doped LC phases showed a red glow at –100% ee [(*R*)-**22**], yellow at 0% ee, and, violet at 100% ee [(*S*)-**22**], respectively. Since the ee is just determined by measurement of the wavelength of maximum reflection, this method is fast and suitable for ee determination in the screening of chiral catalysts.



3 Kinetic Resolution

3.1 General Remarks

Kinetic resolution of a racemic or partially resolved mixture may occur under the influence of a catalyst or an achiral reagent, the mathematical aspects are well known (reviews: Refs.[25,26,27]). The reaction involves the transformation of a racemic mixture (R, S) by a chiral reagent Z_R, as symbolized in Scheme 4, where $k_{rel} = k_R/k_S$ is the stereoselectivity factor *s* of the process (R substrate assumed here to be the fast one). The reaction may be, for example, the kinetic resolution of a racemic alcohol by an enantiopure acid chloride.



Scheme 4. Kinetic resolution of a racemic mixture (R + S) by an enantiopure reagent Z_R.

If the reaction is first-order in substrate and catalyst, Equation (3) can be used. It relates the conversion (*c*) to the ee of recovered starting material (ee_{sm}). Equation (4) relates *c* and ee of the product (ee_{prod}) when the product is chiral.

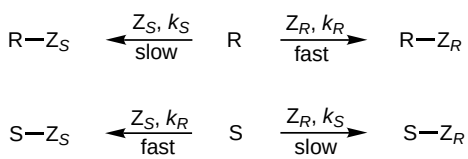
$$s = \frac{\ln\left[(1-ee_{sm})(1-c)\right]}{\ln\left[(1+ee_{sm})(1-c)\right]} \quad (3)$$

$$s = \frac{\ln\left[1-c(1+ee_{prod})\right]}{\ln\left[1-c(1-ee_{prod})\right]} \quad (4)$$

It has been pointed out by Horeau that kinetic resolution can be a tool to calculate enantiomeric excesses and maximum specific rotations of compounds.[28,29] This approach has been discussed by Schoofs and Guetté in a review article.[30]

Let us consider the use of kinetic resolution for the analysis of the ee of a small amount of compound (R, S). It is the kinetic resolution of a racemic reagent ($Z_R + Z_S$) that will indirectly provide the desired information. The general process is indicated in Scheme 5.

The stereoselectivity factor $s = k_R/k_S$ is involved both in the couple of reactions [R/Z_R, R/Z_S] as well as [S/Z_S, S/Z_R]. It can be measured easily by a preliminary reaction between racemic substrate (R, S) and racemic reagent Z at any conversion, as explained in Ref.[25]. Here, *s* is



Scheme 5. Kinetic resolution between an enantioimpure mixture (R + S) and a racemic reagent ($Z_R + Z_S$).

equal to the diastereomeric ratio [R-Z_R + R-Z_S]/[S-Z_S + S-Z_R]. The enantiomeric excess of substrate (R, S) is given by Equation (5), where *y* refers to the experimental ratio which characterizes the ratio of products deriving from the Z_R and Z_S reagents.

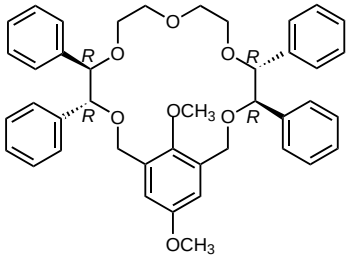
$$ee(\%) = \frac{(y-1)(s+1)}{(y+1)(s-1)} \times 100 \qquad (5)$$

Equation (5) applies when a large excess of racemic reagent $Z_R + Z_S$ is used, meaning that it will remain practically racemic in composition during the whole course of the reaction. Then the *y* ratio of [R-Z_R + S-Z_R] and [S-Z_S + R-Z_S] is easily calculated, using $er = [R]/[S]$ and *s* as parameters. One finds $y = (s \cdot er + 1)/(er + s)$. Since $ee = (1 - er)/(1 + er)$ it becomes Equation (5).

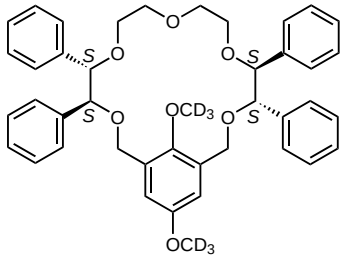
Consider a racemic reagent as a "*pseudo*-racemic" system where one of the enantiomers, Z_R for example, has been specifically labeled at a remote position with no modification of its reaction rate in the kinetic resolution. Then Equation (5) applies and *y* is easily measured by a spectroscopic method such as MS or fluorescence (*vide infra*). When the compound to be analysed is enantiomerically pure, Equation (5) gives $y = s$, the ratio of diastereomers is equal to the stereoselectivity factor. If the compound is racemic, one finds $y = 1$ (equimolar amounts of the two diastereomers). Equation (5) is valid only for $s \approx 1$. Even if *s* is small, e.g., $s = 2$, 50% ee will lead to $y = 1.40$ which is easily measured. In the subsequent sections we describe the various ways to prepare *pseudo*-racemic reagents able to lead to an easy detection of the diastereomeric ratio *y*.

3.2 Measurement of ee by Kinetic Resolution (Host-Guest Chemistry) Combined to Mass Spectrometry

Sawada et al. measured the enantiomeric excesses of amino acids ester salts (AA) in the presence of chiral host compounds.[31] The method is based on the enantiomeric recognition of primary amines by chiral crown derivatives.[9] They prepared a solution of the amino acids ester salts and a 2-fold excess of a 1:1 mixture of the host compounds, (*R*,*R*,*R*,*R*)-**23** and [$^2H_6$]-(*S*,*S*,*S*,*S*)-**23**. The relative peak intensity of the diastereomeric host-guest complex ions, [(*R*,*R*,*R*,*R*)-**23** + AA]$^+$ and {[$^2H_6$]-(*S*,*S*,*S*,*S*)-**23** + AA}$^+$, in FAB mass spectrometry, was plotted against the ee of the amino acids ester salts. Since there is a linear relationship between the two values, it is possible to deduce the ee's of unknown samples with a standard deviation of $\pm 5\%$. The relative peak intensity slightly depends on the concentration of the host and guest solution. The method is applicable to the ee determination of hydrochloride esters of phenylglycine, aspartic acid, asparagine, and valine. There is no difference in the relative intensity in the case of 1-phenylethylamine hydrochloride, indicating that the CO_2R group plays a crucial role in chiral recognition with the host compound.

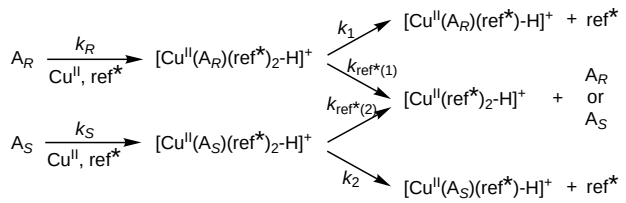


(*R*,*R*,*R*,*R*)-**23**



[$^2H_6$]-(*S*,*S*,*S*,*S*)-**23**

The studies by Cooks et al.[32–36] and by Lebrilla et al.[37–39] are based on the kinetic resolution of a diastereomeric mixture. Cooks et al. reported a method to determine the enantiomeric excess of amino acids by using the kinetics of competitive unimolecular fragmentations of trimeric Cu(II)-bound complexes.[32,33] The experiments were conducted by mixing the sample with copper(II) chloride and a chiral amino acid as a chiral reference compound, ref*, in aqueous methanol. The fragmentation path is depicted in Scheme 6. First, two enantiomers, A_R and A_S of analyte A, are interacted with Cu(II) ion and ref* to form diastereomeric ions, [$Cu^{II}(A_R)(ref^*)_2$-H]$^+$ and [$Cu^{II}(A_S)(ref^*)_2$-H]$^+$, through electrospray ionization. Then, these complexes undergo collision-induced dissociation in a quadrupole ion trap to give [$Cu^{II}(A_R)$(ref*)-H]$^+$, [$Cu^{II}(A_S)$(ref*)-H]$^+$, and [Cu^{II}(ref*)$_2$-H]$^+$ by the loss of the neutral reference



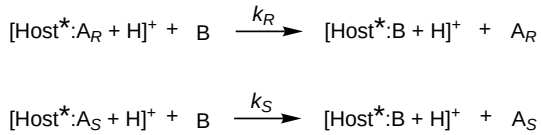
Scheme 6. Measurement of ee by a Cu(II) complex.

compound ref* and A_R or A_S, respectively. The relative abundance ratio R defined as Equation (6) reflects the ratio of A_R and A_S.

$$R=\frac{[Cu^{II}(A)(ref^*)-H]^+}{[Cu^{II}(ref^*)_2-H]^+} \quad (6)$$

R values were measured as a function of ee of A, giving the calibration curve $\ln R = a \cdot (\%\ \text{ee}) + b$ (where a and b are constants). This means that the natural logarithm of R is proportional to the enantiomeric excess of A, allowing us to determine the ee of an unknown sample with a high accuracy ($R^2 > 0.999$). As chiral references, aromatic amino acids such as tyrosine and tryptophane are effective. This methodology was applied for the measurement of the enantiomeric excess of peptides,[34] α-hydroxy acids [Co(II) was used as a metal ion],[35] as well as chiral drugs such as norepinephrine, ephedrine, and atenolol.[36]

MS analysis of pharmaceutical compounds was similarly conducted by Grigorean and Lebrilla.[37] As shown in Scheme 7, the cyclodextrin host (Host*) and a pharmaceutical compound (A) were mixed in a 1:1 ratio and the solutions were electrosprayed. The mixture of diastereomeric protonated complexes, [Host*:A + H]$^+$, was produced in the gas phase. The nature of the interaction in the gas-phase inclusion complexes was discussed in detail.[38] Reaction with a gaseous amine (either achiral or chiral) B afforded the analyte A and a new protonated complex with the amine. Peak intensities of the two protonated complexes, [Host*:A + H]$^+$ and [Host*:B + H]$^+$ were detected by Fourier transform mass spectroscopy and the exchange rate was measured. Since the first-order rate constant is dependent on the enantiomeric excess of the analyte A, quantification of enantiomeric excess is possible. As pharmaceutical



Scheme 7. Guest-exchange reaction in the gas phase.

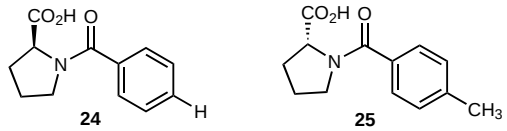
compounds, DOPA, amphetamine, ephedrine, and penicillamine were studied with combinations of amines including *n*-propylamine, (*R*)-1-amino-2-propanol, ethylenediamine, and 1,3-diaminopropane. The first-order plots of exchange reactions obtained with DOPA and penicillamine showed a break in the curve indicating that at least two species are involved in the whole reaction. A selectivity value, k_R/k_S, close to unity tends to give larger error. Determinations of ee's of amino acids were also conducted by this method.[39]

3.3 Kinetic Resolution with "Mass-Tagged" *Pseudo*-Racemic Reagents Combined to Mass Spectrometry

Horeau's method of the determination of the absolute configuration of secondary alcohols has been widely used for many years.[28] It is based on the kinetic resolution of racemic 2-phenylbutyric acid PhCH(Et)-CO_2H (as its anhydride in pyridine). The absolute configuration of the alcohol was obtained by considering the configuration of the recovered 2-phenylbutyric acid after hydrolytic work-up or by the relative stereochemistry of the major ester. The ee of the recovered 2-phenylbutyric acid and the diastereomeric excess (de) of the major ester are indicative of the amount of stereoselectivity of the kinetic resolution that has occurred. In 1990 Horeau adjusted his method to a microscale level (100 μg of alcohol), by using a 1:1 mixture of (*S*)-PhCH(Et)CO_2H and (*R*)-PhCH(CH_2CH_2D)CO_2H, a labeled *pseudo*-racemic acid.[29] MS analysis of the mixture of diastereomeric esters (ratio of peak intensities at 120 and 119) indicated which was the faster-reacting 2-phenylbutyric acid. Hence the absolute configuration of the alcohol was available by applying the general stereochemical rule established by Horeau.[28] The above ratio of peak intensities also may give indirect access to the enantiomeric excess of the residual 2-phenylbutyric acid, but this was not investigated by the authors.

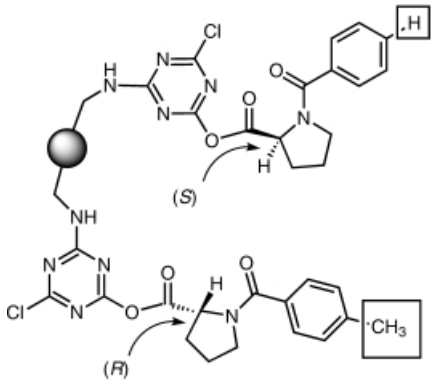
Finn et al. described in 1999 an elegant method to measure enantiomeric excesses of alcohols or amines.[40] The authors used as "mass-tagged" acylating agents the two acids **24** and **25** derived from (*S*)- and (*R*)-proline, respectively. The two reagents differs by a mass of 14 (CH_2). The 1:1 mixture of these two acids (the *pseudo*-racemic reagent) was taken in 20-fold excess in dichloromethane in the presence of DCC and a small amount of DMAP for acylation of alcohols, while amines were similarly treated but with 1-hydroxybenzotriazole replacing DMAP. This procedure allowed one to work down to 1 – 10 nmol of substrate. After reaction, each mixture was evaporated, diluted in a suitable solvent, and injected for analysis by the electrospray technique, the ESI-MS method. The relative amounts of acyl derivatives (esters or amides) related to tagged and

untagged acylating agents **24** and **25** were measured either on the $[M+H]^+$ or $[M+Na]^+$ peaks. The ratio of intensity peaks *y* (after correction for ionization of each compound) was introduced into Equation (5) to calculate the enantiomeric excess of the alcohol or amine. There was a good agreement between known and measured values of enantiomeric excesses, the accuracy has been estimated to $\pm 10\%$. The method is rapid, may be automatized, and works on a very small amount of compound. Alcohols such as PhCH(OH)R (R = Me, Et, *i*-Pr, etc.) or menthol as well as many amines [PhCH(NH)Me, phenylglycine methyl ester, etc.] were amongst the substrates whose ee's have been measured. Of course, the method should be also applicable to the measurement of enantiomeric excesses of acids by using a mass-tagged *pseudo*-racemate of chiral alcohols or amines. As discussed previously, a large *s* factor is not needed. But a calibration is necessary to measure the *s* factor. This can be done by using the racemic substrate. A calibration is also necessary to get the ionization correcting factor.



24 **25**

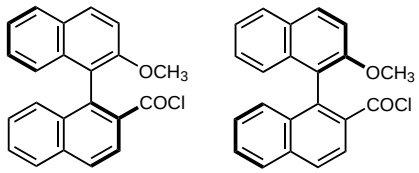
Mass-tagged chiral activated esters on derivatized polystyrene of type **26** have been used (containing 0.80 mmol/g).[41] On the same bead are introduced equimolar amounts of the two *pseudo*-enantiomeric activated esters. The supported acylating reagent is in a large excess with respect to the mixture of enantiomeric amines. After reaction (catalyzed by DMAP), the resulting solution of the mixture of amides was evaporated and some methanol added. A direct injection and ESI-MS of each aliquot gave reliable intensity ratios, providing enantiomeric excesses which fall within 10% of the actual ee value. Many amines have been screened by this procedure. The speed of the ESI-MS analysis can be increased by combining samples of reactions performed on various amines of different molecular weights.



26

3.4 Measurement of ee by Kinetic Resolution Combined to CD

Recently Finn et al. developed an untagged racemic acylating reagent [(*S*)-**27**, (*R*)-**27**].[42] The kinetic resolution of the reagent was achieved by various enantioimpure alcohols, as in the Horeau method.[28,29] The enantiomeric excesses of the alcohols were measured by the CD of the resulting mixture of diastereomeric esters. The method also applies even if $s \cong 1$.



(*S*)-**27** (*R*)-**27**

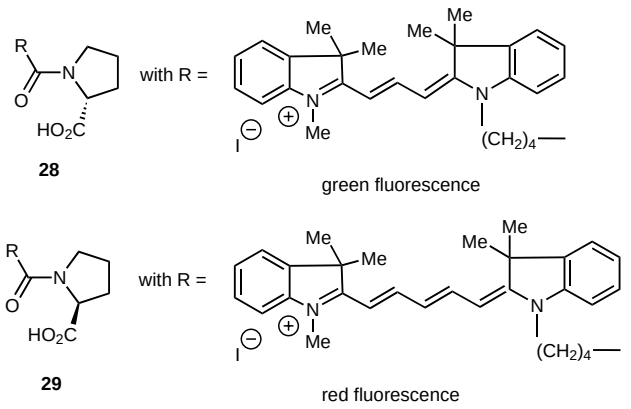
3.5 Enzymatic Method

It has long been known that enzymatic methods are useful for the ee measurement of some compounds. For example, the enantiomeric excess of α-amino acids can be measured on a small scale by using fully enantiospecific enzymes such as oxidases or decarboxylases.[43] The production of molecular oxygen or carbon dioxide gas precisely allows one to evaluate the ee of the initial sample. Recently Seto et al. developed a new enzymatic method for determining the ee (EMDee) of secondary alcohols and applied it to the addition of diethylzinc to benzaldehyde (*cf.* Scheme 2) catalyzed by oxazolidines.[44] The *S*-enantiomer of the resulting 1-phenylpropanol (**13**) was selectively oxidized (kinetic resolution) to the corresponding ketone with $NADP^+$ as a cofactor by using an (*S*)-aromatic alcohol dehydrogenase. The formation of NADPH, measured by UV spectroscopy, gives access to the initial rate of the enzymatic oxidation which, in turn, is linearly correlated with the ee of **13** over the full range of ee established by a calibration curve. The accuracy has been estimated to be approximately $\pm 10\%$. This method made it possible to analyse 100 samples in 30 min. Although the rate also depends on the conversion of the alkylation, this assay is fast and reliable for screening asymmetric reactions. It may be expected that the ee of classes of compounds other than alcohols could be measured by a similar enzymatic approach.

3.6 Measurement of ee by Kinetic Resolution Combined to Fluorescence

Shair et al. adapted DNA microarray technology to the measurement of the ee's of thousands of samples and named it reaction microarrays.[45] The method had been

evaluated for the specific case of α-amino acids. An amine-functionalized glass slide was allowed to react with the carboxylic group of *N*-Boc-amino acids at specific locations on the slide (in arrays). The remaining free amino groups on the surface were blocked by acetylation. Finally, the amino groups of the α-amino acids were deprotected by removing the Boc residue. In this way, microarrays of spots of $\cong 10^{-11}$ moles of amino acids were placed on the surface (by automatic manipulations). The ee's of the various amino acids were deduced from the reactions with a *pseudo*-racemic reagent involving two enantiomers labeled with different fluorescent reporters. One labeled enantiomer **28** was a derivative of (*R*)-proline while the *pseudo*-enantiomeric reagent **29** labeled with a different fluorophore was prepared from (*S*)-proline. The immobilized amino acids (with free NH_2) of unknown ee's on the glass slide are treated with an excess of the *pseudo*-racemic reagent. During the coupling reaction a parallel kinetic resolution occurs at each spot on the surface, consuming non-equimolar amounts of the two *pseudo*-enantiomeric reagents. The fluorophore in **28** gave a green emission by excitation at 532 nm, while the fluorophore in **29** gave a red fluorescence emission (excitation at 635 nm). Equivalent emission intensities of the two systems provided a yellow color. The enantiomeric excess of the immobilized proline can be calculated by using general Equation (5) for kinetic resolution where *y* is the measured fluorescent intensity ratio at each spot. A normalization factor *z* has been introduced to correct for non-equivalent fluorescent intensities for equimolar amounts of the two fluorophores. The stereoselectivity factor *s* can be calculated in a preliminary experiment using a sample of proline of known ee. As already noticed,[40] even modest values of *s* allow one to obtain accurate determinations of the ee. In conclusion, the color of each spot (after laser excitation at 532 nm and 635 nm) immediately informs one of the ee and the absolute configuration of the immobilized amino acid. This method has been applied to many kinds of α-amino acids. Of course, achiral glycine always led to yellow spots, because of the equivalent incorporation of the yellow and green fluorophores. The accuracy has been estimated at ±10% ee. An example of high-throughput analysis with the measurement of the ee's of 15,552 samples of proline deposited on a glass slide was described by the authors.



Scheme 8. Enantiomeric reagents tagged by different fluorophores.

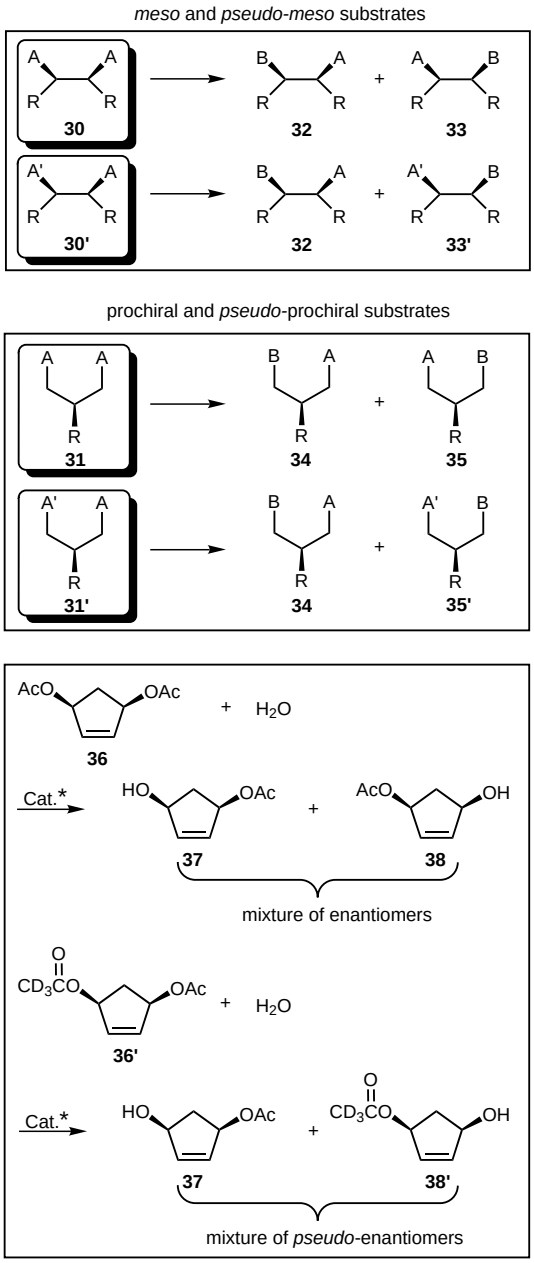
3.7 Measurement of ee by Kinetic Resolution Combined to Immunoassay

Immunoassay was recently established as a powerful tool for ee determination by Mioskowski et al. during their investigations on the enantioselective synthesis of mandelic acid by asymmetric transfer hydrogenation of benzoyl formic acid.[46] Monoclonal antibody mAb-8 binds (*S*)-mandelic acid stereoselectively, which decreases in absorbance, thus the ee measurement is possible. The calibration curve was obtained and the accuracy was estimated to ±9% ee. In the same manner, the yield can be determined by use of an *anti*-racemate antibody which binds the two enantiomers of mandelic acid. Based on this approach, the authors optimized the conditions of the asymmetric synthesis of mandelic acid by high-throughput screening.

3.8 *Pseudo*-Prochiral or *Pseudo*-Racemic Substrates

Research on new catalysts for a given enantioselective reaction always involves the ee measurement of the same mixture of enantiomers. A method has been proposed by Reetz et al. to quickly screen the ability of a catalyst to transform *meso* or prochiral substrates (**30** and **31**, respectively) with two functional groups (as diester **36**) into a chiral product (**32** or **34**, respectively) by selective reaction at one of the two enantiotopic functional groups (Scheme 9).[47] For this purpose, the substrates **30** and **31** were replaced by related chiral compounds **30′** and **31′** where one group A was isotopically labeled. Then the selective reaction at one of the two enantiotopic functional groups can be easily analysed by the product distribution, as illustrated in Scheme 9 in the case of the lipase-catalyzed hydrolysis of *meso*-1,4-diacetoxy-2-cyclopentene (**36**). The ee analysis was done by ESI-MS, with measurements of the $[M + Na]^+$ peaks of species **37** and **38′**. The relative peak intensities of **36′**, **37**, and **38′** give access to the conversion and to the *pseudo*-enantiomeric excesses of the product (monoacetate).

The method is valid only if the isotopic label is suitably located in order to not give rise to an isotopic effect during the reaction. This can be checked by some control experiments. The synthesis of enantiopure **30′** or **31′** is a prerequisite condition for applying this approach, which should be extendible to many types of asymmetric



Scheme 9. Labeled prochiral substrates for enantioselective synthesis.

reactions. The authors also developed the use of isotopically labeled *pseudo*-racemic esters to study the efficiency of chiral catalysts in the hydrolytic kinetic resolution of racemic esters.

Kinetic resolution of activated racemic amino acids by chiral amine has been also investigated by Still et al.[48] They labeled each enantiomer with colored dyes.

4 Conclusion

In the past decade there has been a renewed interest in approaches for measuring enantiomeric excesses. Modern technologies allow us to work on small scale in various ways. Chiroptical and enzymatic methods remain valid when adapted to micro-quantities. Visual indication of enantiomeric excesses by color changes is also a promising approach, for example, by using liquid crystals. Analysis by mass spectroscopy of mixtures of *pseudo*-enantiomers or *pseudo*-diastereomers generated by kinetic resolution with a *pseudo*-racemic reagent as well as diastereomers obtained by host-guest chemistry is a powerful tool. The alternative methods, use of labeled *pseudo*-prochiral or *pseudo-meso* substrates, are also very attractive for the study of a given enantioselective transformation. Kinetic resolution with a *pseudo*-racemic reagent (with mass or fluorescent tags) combined to MS or fluorescence detection of the products gave sensitive methods to measure ee's. All these approaches are compatible with ee screening in the high-throughput evaluation for new catalysts, as well as the ee measurement of tiny amounts of materials coming from various origins. One can expect to see many new developments in the near future.

Added in Proof

Pu et al. demonstrated a new enantioselective fluorescent sensor for mandelic acid.[49]

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[8] For example, see Ref.[8a] for rapid chiral GC analysis and Ref.[8b] for the convenient combined use of chiral HPLC and MS. a) M. Ostermeier, J. Prieß, G. Helmchen, *Angew. Chem. Int. Ed.* **2002**, *41*, 612–614; b) C. J. Welch, B. Grau, J. Moore, D. J. Mathre, *J. Org. Chem.* **2001**, *66*, 6836–6837.

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