

## Analytical Methods

### 2.1 The importance of analysis, and which method to choose

Central to the art and practice of stereoselective synthesis is the analysis of the outcome of the reaction. Given the results, we may compare them to our intent (or hope) and act accordingly. Additionally, we may try to understand the factors that governed the formation of the observed products.

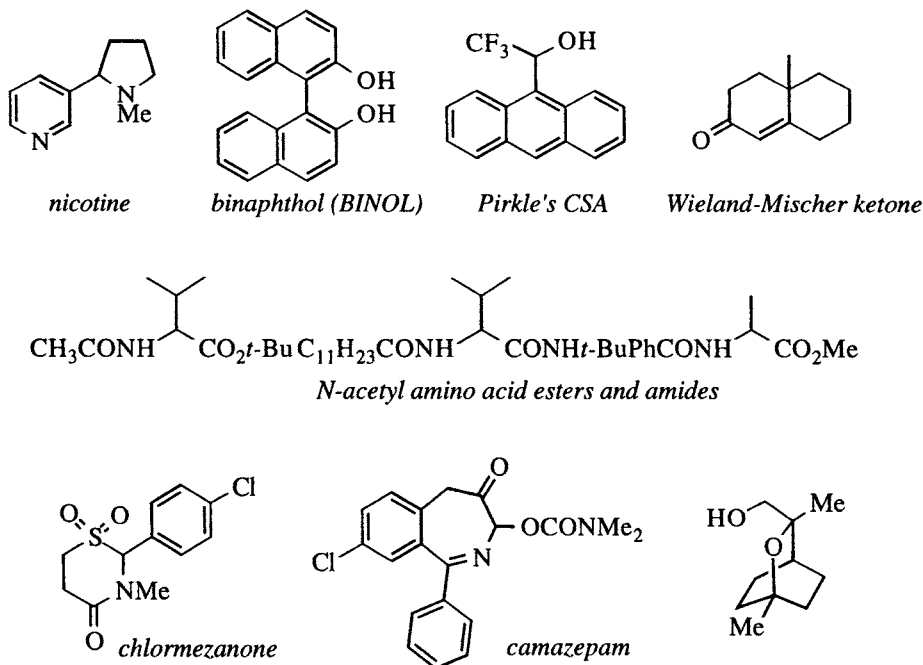
The separation of the enantiomorphous crystals of racemic sodium ammonium tartrate by Pasteur in 1848, and his observation that the two forms were optically active in solution, linked the concept of molecular chirality to optical activity [1]. When Emil Fischer began the first serious attempts at asymmetric synthesis in the latter 19th century, the polarimeter was the most reliable tool available to evaluate the results of an enantioselective reaction (by determination of optical purity), and it remained the primary tool for nearly 100 years. Only recently has analytical chemistry brought us to the point where we can say that polarimetry has been superseded as the primary method of analysis in asymmetric synthesis.

It is apparent from the preceding chapter that the analysis of enantiomers (by whatever means) addresses only part of the problem: often, a stereoselective reaction produces a mixture of diastereomers, and polarimetry is an inappropriate technique. Thus, asymmetric synthesis requires the means for the analysis of both enantiomeric and diastereomeric mixtures. Ultimately, the *ratio* of isomers and the *configuration* of each new stereocenter should be determined.

In choosing a technique for the analysis of a stereoselective reaction, a number of questions must be addressed:

1. How much material is available for the analysis, and what limits of detection are desired?
2. Are the stereoisomers enantiomeric or diastereomeric, and how many possible stereoisomers are there?
3. Do the products have a chromophore that might aid analysis by CD/ORD or UV spectroscopy, or detectability by HPLC?
4. Do the products have a functional group available for derivatization by a chiral or achiral reagent, or for interaction with a stationary phase in chromatography or with a chiral agent in solution?
5. If polarimetry is to be used for the analysis of enantiomers, is the specific rotation of the pure enantiomer known with certainty, or will it have to be determined?
6. Once the ratio of stereoisomers is determined, how will the configuration of each new stereocenter be assigned? Can the same method be used for the determination of product ratio *and* the assignment of configuration?

It should be recognized from the outset that an important aspect of any analysis of stereoisomer distribution is that the analysis reflect the ratio in the crude product without unintended enrichment by chromatographic means during sample purification and preparation. Most readers are probably aware that chromatography can separate diastereomers, so that care must be taken to insure that the diastereomer mixture being analyzed is the same as that produced in a reaction. In 1983, Crooks reported that *enantiomer enrichment* occurred upon chromatographic purification of a partly racemic nicotine sample [2]. Such enrichment has since been recorded for sulfoxides [3], amino acid derivatives [4-6], biaryls [5], alcohols [5,7], ketones such as the Wieland-Mischer ketone [8], and drugs such as chlormezanone and camazepam [5], and the list is growing (see Figure 2.1). The phenomenon is not observed with totally racemic samples. A likely explanation for this type of enrichment is that the chromatography is separating a heterochiral dimer of the analyte from the monomer (or a homochiral dimer). In support of this hypothesis, Matusch and Coors [5] showed that the phenomenon was more pronounced with higher column loadings (higher concentration). On the other hand, Dreiding and colleagues were unable to find any evidence for dimerization of the Wieland-Mischer ketone, either polarimetrically or spectroscopically [8]. In the latter case, it may be that the aggregation is taking place in the higher concentrations that occur at or near the surface of the stationary phase. *Thus, when preparing a sample for analysis, pooled fractions should include those eluting before and after the "main band" so as to minimize any adventitious stereoisomer enrichment.*

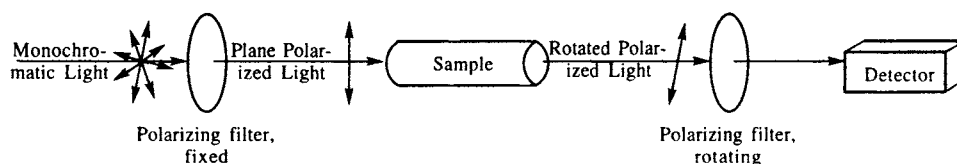


**Figure 2.1.** Compounds known to undergo enantiomeric enrichment upon chromatography on an achiral stationary phase such as silica gel [2-8].

Two types of analysis exist: those that separate the stereoisomers and those that do not. Polarimetry of course, fits the latter category. Separation is also not necessary for NMR analysis of a derivatized sample or with the aid of a chiral solvating agent or shift reagent. Chiral stationary phase (CSP) chromatographic techniques such as capillary GC or HPLC obviously *do* separate the analyte isomers, and may also facilitate the assignment of absolute configurations. The sections which follow describe the advantages and disadvantages of some of the more popular methods. If possible, more than one technique should be employed when a new synthetic method is being developed; use of procedures whose stereochemical consequences are well established may often rely on a single type of analysis.

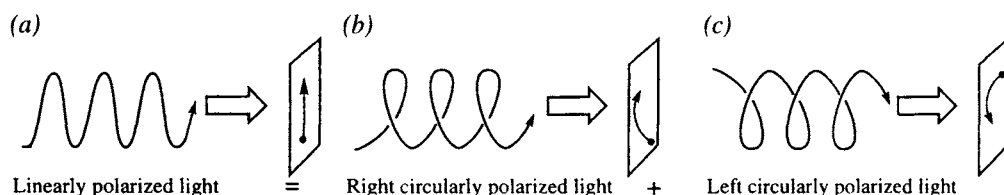
## 2.2 Polarimetry, the old-fashioned way<sup>1</sup>

Polarimetry measures the rotation of a plane of monochromatic polarized light after having passed through a sample, as shown schematically in Figure 2.2.



**Figure 2.2.** Schematic representation of a polarimeter.

It is not intuitively obvious why a chiral medium should have this effect, until the linearly polarized light beam, represented by the sine wave in Figure 2.3a, is broken down into the two circularly polarized components shown in Figures 2.3b and c.<sup>2</sup> When the linearly polarized beam passes through a perpendicular plane, the point of intersection moves along a line. When the circularly polarized beams pass



**Figure 2.3.** Representations of the waveforms of polarized light beams passing through a perpendicular plane.

through the same plane (the helices are moved without being rotated), the point of intersection describes a circle, moving either to the right or the left depending on the chirality sense of the helix. Note that the vector sum of the right- and left-circularly polarized beams equals the linearly polarized beam. The right- and left-circularly polarized beams are refracted equally by an achiral medium; that is, their refractive indices  $n_R$  and  $n_L$  (which measure change in velocity), are equal. As shown in Figure 2.4a, the vector sum of the two refracted circularly polarized beams remains in the plane of the incident polarized light, *i.e.*, the plane is not

<sup>1</sup> Monographs: [9-15].

<sup>2</sup> This analysis is an oversimplification. For a thorough treatment, see ref. [16].

rotated. In a medium where  $n_L$  and  $n_R$  are not equal, the two beams are shifted out of phase, and their vector sum is rotated out of plane by an angle,  $\alpha$ , as shown in Figure 2.4b. A medium which produces this effect is *circularly birefringent*. A solution of a pure enantiomer is circularly birefringent. In contrast, an equimolar mixture of two enantiomers will have an equal number of refractions to the right and left, and the net result will be  $\alpha = 0$ . Thus, a polarimeter cannot distinguish an achiral compound from a racemate. It was Pasteur's discovery of circular birefringence in solutions of enantiomorphous crystals of racemic sodium ammonium tartrate [1] that set the stage for the development of stereochemical theory by establishing the presence of chiral molecules in an optically inactive compound.



**Figure 2.4.** The effect on the transmitted plane by refraction of circularly polarized light beams relative to the incident plane of polarized light (dashed line). (a) When  $n_L = n_R$ , the vector sum (solid arrows) of the two circularly polarized beams (dashed arrows) remains in the same plane as the incident beam. (b) When  $n_L \neq n_R$ , the vector sum of the two waves is rotated  $\alpha^\circ$  away from the plane of the incident light.

To summarize, the differential refraction of right- and left-circularly polarized light by a chiral nonracemic substance results in the rotation of the plane of the sine wave that is the vector sum of the two circularly polarized beams. That the two circularly polarized beams should be refracted differently by a chiral substance is apparent if one considers their helicity and imagines the interaction of a helix with a chiral substance in the context of double asymmetric induction explained in the previous chapter: any chiral entity will interact differently with the two enantiomeric forms of another chiral entity. On a macroscopic scale, we can easily perceive with our right hand the difference between a right- and left-handed screw, just as a chiral molecule may detect the difference between right- and left-circularly polarized light. On the molecular scale, whether  $n_R$  and  $n_L$  differ enough to be measured depends on the system. If the 'refractivity'<sup>3</sup> of the various ligands around a stereocenter in a chiral molecule are nearly the same, the difference between  $n_R$  and  $n_L$  may be too small to detect, and no rotation will be observed. From a practical standpoint, it may be possible to change the wavelength to increase the difference in  $n_R$  and  $n_L$ .

<sup>3</sup> This term is used loosely, and is related to the polarizability of each ligand. Interestingly, before the days of IR and NMR spectroscopy attempts were made to quantify the refractive index of individual functional groups as a means of deducing structure. For a summary of 'Molar Refraction,' see S. Glasstone *Physical Chemistry*, Van Nostrand: Princeton (1946) pp. 528-534, and other texts of the same period.

In addition to polarimetry, other chiroptical properties may be useful for the assignment of absolute configuration, although they are rarely used to determine enantiomeric purity [9-12,17,18]. Optical rotatory dispersion, ORD, measures the optical rotation of a compound as a function of wavelength, and its theory is the same as for simple polarimetry described above. Circular dichroism, CD, is similar, but differs in that the substrate must have a chromophore that absorbs at the wavelengths employed. In this special case, the molar absorptivities (extinction coefficients) of the right- and left-circularly polarized beams are different. Thus, in addition to being out of phase, the vectors of the transmitted beams are also of unequal magnitude. As a result, the emergent beam no longer traverses a line, but describes an ellipse, and the emergent light is *elliptically polarized*. In the region of such a CD band, the ORD exhibits 'anomalous' behavior (a Cotton effect) due to the absorption. The mean wavelength between an ORD peak and trough [9] is close to the  $\lambda_{\text{max}}$  of the chromophore absorbing the light. It is not unusual for the ORD curve to change sign in such a region. Because ORD measures a rotation, it is theoretically finite at all wavelengths, but since CD measures a difference in absorption, it only occurs in the vicinity of an absorption band.

The degree of rotation observed in a polarimeter,  $\alpha$ , is dependent on the number of chiral species the light encounters on its passage through the sample chamber, as well as the wavelength of the light. Thus, analytical accuracy dictates strict control of a number of experimental parameters, such as temperature, concentration, light source, and path length. To minimize the effects of these variables and to increase the reproducibility, specific rotation,  $[\alpha]$ , is defined as:

$$[\alpha]_k^T = \frac{100\alpha}{l \cdot c}, \quad (2.1)$$

where  $T$  is the temperature,  $\lambda$  is the wavelength of the light (often the D lines of sodium at 589.0 and 589.6 nm and abbreviated simply 'D'),  $\alpha$  is the observed rotation,  $l$  is the sample path length in decimeters, and  $c$  is the concentration in grams per 100 milliliters of solution. To insure reproducibility, it is common practice to report the concentration and solvent along with the specific rotation, and the units are understood.<sup>4</sup> For example, if a solution of 0.014 g in 1.0 mL of ethanol solution afforded a measured rotation of  $+1.375^\circ$ , the specific rotation would be reported as:

$$[\alpha]_D^{25} +98 \text{ (} c = 1.4, \text{ EtOH) .}$$

This denotes a specific rotation of  $+98 \text{ deg}\cdot\text{mL/g}\cdot\text{dm}$  measured at the D line of sodium, temperature  $25^\circ\text{C}$ , at a concentration of 1.4 grams per 100 milliliters of ethanol. For pure liquids (or solids), the equation

$$[\alpha]_k^T = \frac{\alpha}{l \cdot \rho} \quad (2.2)$$

is used, where  $\rho$  is the density in grams per milliliter.

<sup>4</sup> It is incorrect to report specific rotation in "degrees."

Specific rotation was defined over 150 years ago, which accounts for the unusual units of path length and concentration: decimeters were used because a long path length was needed to get an accurate measurement, and mass was used instead of molecular weight because molecular weights were uncertain in the early 19th century. The D line of sodium was chosen because it is easily produced in a flame and is nearly monochromatic. Now that molecular weights are no longer an unknown, molecular rotation,  $[\Phi]$ , may be used instead of  $[\alpha]$ :

$$[\Phi]_{\lambda}^T = \frac{M[\alpha]}{100}, \quad (2.3)$$

where  $M$  is the molecular weight. Molecular rotation is commonly used in ORD.

Sign of rotation reflects absolute configuration (and is often used to assign it), and the magnitude of the rotation is used to determine the optical purity, usually expressed as a percent:

$$\% \text{ optical purity} = \frac{100[\alpha]_{\lambda}^T}{[\alpha_o]_{\lambda}^T}, \quad (2.4)$$

where  $[\alpha]_{\lambda}^T$  is the observed specific rotation, and  $[\alpha_o]_{\lambda}^T$  is the specific rotation of the pure enantiomer under identical conditions. The optical purity of an enantiomerically pure compound is 100%, and 0% for a racemate. Ideally, the specific rotation of a partly racemic mixture varies linearly with enantiomeric composition. Thus, a 3:1 mixture of a enantiomers whose  $[\alpha_o] = +98$  should exhibit  $[\alpha] = +49$ , and the optical purity would be 50%.

For a chiral compound, percent enantiomer excess (ee) is defined as:

$$\% \text{ enantiomeric excess} = 100 \frac{R-S}{R+S}, \quad (2.5)$$

where  $R$  and  $S$  represent the amounts of the two enantiomers. Thus, a 3:1 mixture of two enantiomers is 50% ee, which expresses the excess of one enantiomer over the racemate.

The optical purity is usually, *but not always*, equal to enantiomer excess. In order for the two to be equal, it is necessary that there be no aggregation. It is possible, for example, that a homochiral or heterochiral dimer (see Glossary, Section 1.6, for definitions) would refract the circularly polarized light differently than the monomer (or each other). In 1968 [19] Krow and Hill showed that the specific rotation of (*S*)-2-ethyl-2-methylsuccinic acid (85% ee) varies markedly with concentration, and even changes from levorotatory to dextrorotatory upon dilution. In 1969 [20], Horeau followed up on Krow and Hill's observation, and showed that the "optical purity" (at constant concentration) and enantiomer excess of (*S*)-2-ethyl-2-methylsuccinic acid were unequal except when enantiomerically pure or completely racemic. This deviation from linearity is known as *the Horeau effect*, and its possible occurrence should be remembered when determining enantiomeric purity by polarimetry.

For optical purity to accurately reflect enantiomeric purity, it is obvious that the sample must be free of any chiral impurities. It may not be as obvious that achiral

impurities can also cause significant error. For example, Yamaguchi and Mosher [21] showed that the specific rotation of enantiomerically pure 1-phenylethanol could be enhanced from  $[\alpha]_D^{20} +43.1$  ( $c = 7.19$ , cyclopentane) to  $[\alpha]_D^{20} +58.3$  ( $c = 2.64$ , cyclopentane) by the addition of 10.6 g/100 mL of acetophenone. Presumably, this enhancement is due to an interaction between the alcohol and the ketone, either through hydrogen bonding or hemiacetal formation.

In order to determine optical purity, it is necessary to know  $[\alpha_o]$ . In natural product synthesis, the rotation of the target is usually known, but the original authors may not have established that the isolated material was enantiomerically and chemically pure. One course of action is to resolve a sample and measure  $[\alpha_o]$  yourself. This may prove tedious, but it has the advantage of eliminating any ambiguities between sample and standard. Another possibility is to calculate  $[\alpha_o]$  by isotopic dilution or kinetic resolution.

In the isotopic dilution technique [22], an enantiomerically enriched sample of unknown %ee is diluted with a second, isotopically labelled sample of the same compound of known %ee (usually a racemate) and known isotope content. Measurement of the isotopic content after dilution, and comparison of rotations before and after dilution, allows extrapolation to rotation at 100% ee.

Kinetic resolution may be used to determine  $[\alpha_o]$  in two ways [23,24]. The results of two kinetic resolutions of a racemic compound, allowed to go to different (known) extents of conversion, can be used to calculate the specific rotation of the enantiomerically pure compound. Alternatively, one may use two 'reciprocal' kinetic resolutions: racemic A is resolved by B *and* racemic B is resolved by A. If the racemate is used in large excess in both cases, and the stereoselectivities of the resolutions are not too high,  $[\alpha_o]$  for A may be calculated if  $[\alpha_o]$  for B is known, or vice versa.

The discovery of the anomalies mentioned above are partly responsible for the declining popularity of polarimetry for the determination of enantiomer ratios. Even if the experimentalist is alert to these sources of error, the possibility still exists that an early determination of specific rotation, against which a new value must be compared, is itself in error. Thus, caution is advised. Nevertheless, if used carefully, polarimetry can provide a simple, efficient, and inexpensive method for the analysis of enantiomeric purity.

## 2.3 Nuclear magnetic resonance

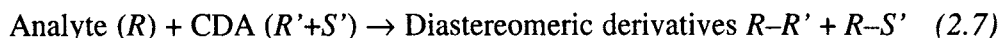
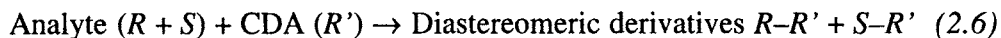
For the analysis of diastereomeric mixtures, NMR is an obvious choice, and derivatization of enantiomers with a chiral reagent can also be an excellent method of analysis (reviews: [25,26]). In the 1960s, a number of discoveries were made that facilitated the direct observation of diastereomeric and enantiomeric mixtures by NMR. The development of chiral derivatizing agents, lanthanide shift reagents, and chiral solvating agents made it possible to observe (and integrate) separate signals for enantiomers. The following discussions elaborate on each.

### 2.3.1 Chiral derivatizing agents (CDAs)

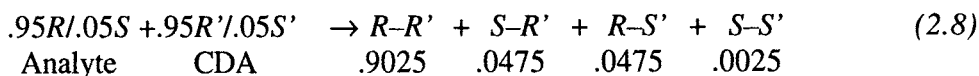
The derivatization of a mixture of enantiomers with a chiral reagent produces diastereomers that may be analyzed by NMR spectroscopy or by chromatography [27]. In order to be useful, a number of requirements must be met:

1. The CDA must be enantiomerically pure, or (less satisfactorily) its enantiomeric purity must be known accurately.
2. The reaction of the CDA with both enantiomers must go to completion under the reaction conditions, or (again less satisfactorily) the relative rate of reaction for each enantiomer must be known.
3. The CDA must not racemize under the derivatization or analysis conditions, and its attachment should be mild enough so that the substrate does not racemize either.
4. If analysis is by HPLC, the CDA should have a chromophore to enhance detectability. If analysis is by NMR, the CDA should have a functional group that gives a singlet and that is remote from other signals for easy integration.

The importance of the first point is evident if we consider the following reactions of an analyte with a CDA:



Equation 2.6 illustrates the derivatization of a mixture of *R* and *S* enantiomers of analyte with an enantiomerically pure derivatizing agent *R'*. If the reaction is complete for both enantiomers of analyte, the ratio of diastereomeric derivatives *R-R'* and *S-R'* will equal the ratio of enantiomers *R* and *S* of the analyte. Equation 2.7 shows how a CDA that is not enantiomerically pure can cause problems. If there is no kinetic resolution of the CDA by the analyte, the ratio of diastereomeric derivatives *R-R'* and *R-S'* will reflect the diastereomer ratio of the CDA. Note that *S-R'* (Eq. 2.6) and *R-S'* (Eq. 2.7) are enantiomeric and standard methods of analysis will not distinguish them. If both the analyte and the CDA are 90% ee (95:5 ratio of enantiomers), the four possible diastereomers will have the statistical ratio of .9025/.4075/.4075/.0025 (Eq. 2.8). Since the products are two diastereomeric racemates, combination of the enantiomers yields a .9050/.0950 ratio, or 81% ee.



Although a number of CDAs have been developed over the years [28], by far the most popular is Mosher's acid [29-31],  $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetic acid, abbreviated MTPA. It is commercially available in either enantiomeric form, and is used for the derivatization of alcohols and amines. Two recent reports [32,33] indicate that the enantiomeric purity of commercially available material may vary from 94 to 99.8% ee, and one might expect similar levels of enantiomeric purity from the original preparation [29]. The enantiomeric purity of MTPA may be determined by esterification of diacetone glucose and examination of the NMR [33], or more accurately by chiral stationary phase gas chromatography of the MTPA methyl ester [32].



Derivatization of chiral alcohols and amides of general structure RCHZR' (Z = OH or NH<sub>2</sub>) yields esters and amides that are frequently referred to as 'Mosher esters' or 'Mosher amides.' <sup>1</sup>H, <sup>13</sup>C, or <sup>19</sup>F NMR may be used to observe the diastereomeric derivatives [29,34]. Most commonly, the -OCH<sub>3</sub> is observed by <sup>1</sup>H NMR or the -CF<sub>3</sub> is observed by <sup>19</sup>F NMR. In most cases, one or the other of these nuclides will be well enough separated that accurate integration will be possible. In problematic cases, additional spectral dispersion may be obtained by adding a lanthanide shift reagent such as Eu(fod)<sub>3</sub> (fod = 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato ligand) to the NMR sample [21,35-37].<sup>5</sup>

Models have been proposed to correlate chemical shift data with absolute configuration [30]. In 1973, Mosher observed that derivatization of enantiomerically pure esters and amides with both enantiomers of MTPA and comparison of chemical shifts produced some interesting trends. With reference to

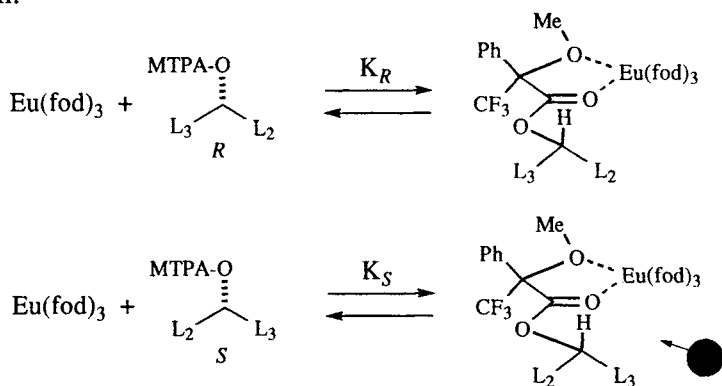
center is  $X > L_3 > L_2 > H$ , derivatization with both enantiomers of MTPA will give *R-R* (relative configuration *l*) and *S-R* diastereomers (relative configuration *u*), illustrated by the top two structures in Figure 2.5. By derivatizing a pure enantiomer with racemic MTPA, the absolute configuration may be determined. From the data gathered by Mosher and Yamaguchi, the following empirical rules can be stated:

1. The  $^1\text{H}$   $L_2$  signals of the *l* diastereomer will be upfield of the  $L_2$  signals of the *u* isomer.
2. The  $^1\text{H}$   $L_3$  signals and the  $^{19}\text{F}$   $\text{CF}_3$  signals of the *l* diastereomer will be downfield of the corresponding signals of the *u* isomer.

If an unequal mixture of enantiomers is present, symmetry considerations dictate that derivatization with only one MTPA enantiomer is necessary, since (Figure 2.5) derivatization of a racemate with one enantiomer of MTPA also produces an *l/u* mixture. Thus either the two left structures or the two right ones could be used to establish configurations. Again assuming the Cahn-Ingold-Prelog priority of the ligands around the unknown center is  $X > L_3 > L_2 > H$ , the following empirical rules apply:

1. If *R*-MTPA is used, the *R* configuration at the unknown stereocenter will give  $L_2$  signals upfield of the *S* diastereomer and  $L_3$  and  $\text{CF}_3$  signals downfield of the *S*-diastereomer.
2. If *S*-MTPA is used, the *S*-configuration at the unknown stereocenter will give  $L_2$  signals upfield of the *R* diastereomer and  $L_3$  and  $\text{CF}_3$  signals downfield of the *R*-diastereomer.

As a model for assignment of absolute configuration of Mosher esters and amides in the presence of  $\text{Eu}(\text{fod})_3$ , Yamaguchi proposed the equilibria shown in Figure 2.6. For both diastereomers, the europium is chelated by the MTPA carbonyl and methoxy oxygens. In the top case, the smaller carbinol ligand,  $L_2$ , is closest to the europium and is more deshielded than  $L_3$ . In the bottom case, (larger)  $L_3$  is closer to the europium, causing steric repulsion and disfavoring the equilibrium as drawn. Because of this repulsive interaction,  $K_R$  is larger than  $K_S$ , and the top ester-europium complex is more abundant. This model therefore predicts that the sterically less demanding ligand should be more deshielded in the *R* configuration.

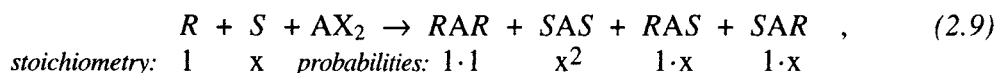


**Figure 2.6.** A predictive model of the equilibria between diastereomeric Mosher esters and a europium shift reagent.

In conclusion, two points must be emphasized. First, the rationales presented in Figures 2.5 and 2.6 are only models, and do not necessarily represent preferred conformations.<sup>6</sup> Second, it should be restated that in order for the CDA method to be accurate, any adventitious kinetic resolution in the derivatization must be quantitated or eliminated. For example, Heathcock has noted that MTPA derivatization of a racemic alcohol (0% ee) afforded a 1.7:1 mixture of Mosher esters (26% de) and the % ee determinations had to be corrected accordingly [42]. More recently, Svatos used a five-fold excess to force a derivatization to completion [43]. If the appropriate control experiments are done, derivatization with Mosher's reagent can be a very reliable method for determination of enantiomer ratios and absolute configuration of amines and alcohols. For the derivatization of ketones, chiral diols may be used [44], but similar control experiments should be undertaken.

### 2.3.2 Achiral derivatizing agents

Imaginative tricks can also be used to analyze enantiomeric mixtures. For example an achiral, bifunctional derivatizing agent may be used to randomly dimerize a mixture of enantiomers. If a statistical ratio can be proven in control experiments, the ratio of the chiral to meso diastereomers can be used to calculate the enantiomer composition [45]. Figure 2.7 shows several derivatizing agents that are available. In principle, the ratio could be determined by chromatographic or spectroscopic methods. <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P NMR provide a particularly facile method for the analysis of alcohols [45-47]. The following generic reaction indicates the process:



where *R* and *S* indicate the absolute configuration of the alcohols, *AX*<sub>2</sub> is the 'dimerization' reagent. *RAR* and *SAS* are a *d,l* pair, while *RAS* and *SAR* are meso. The latter may or may not be identical,<sup>7</sup> but it is necessary to recognize (statistically) that either an '*SAX + R*' or an '*RAX + S*' sequence would produce a meso isomer. If the *S/R* ratio is *x*, then the probability for the formation of the *d,l* pair is (1 + *x*<sup>2</sup>) and 2*x* for the meso isomer(s). Thus the *d,l*/meso ratio is given by

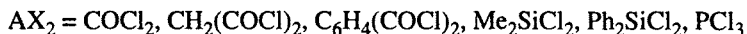
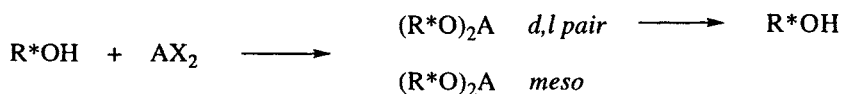
$$\frac{d,l}{meso} = y = \frac{1 + x^2}{2x} \quad . \quad (2.10)$$

Solving for *x* gives

$$x = \text{enantiomer ratio} = \frac{2y \pm \sqrt{4y^2 - 4}}{2} \quad . \quad (2.11)$$

There is an ambiguity in this determination in that the mathematics is oblivious to absolute configuration, hence the "±" in the quadratic formula. Thus, although *x* was defined as *S/R*, the solutions will be *S/R* and *R/S*.

<sup>7</sup> If 'A' is stereogenic in the products, the two will be diastereomers. For example, derivatization of alcohols with *PCl*<sub>3</sub> affords phosphonates in which the phosphorus is stereogenic and two meso isomers are produced [46].



**Figure 2.7.** Achiral derivatizing agents for the determination of enantiomer ratio and partial resolutions [45-47].

It is also of interest to note that, on a preparative scale, this method may be used to enrich the enantiomer ratio of a partially resolved racemate [45]. For example, dimerization of a 9:1 mixture (80% ee), followed by separation and then cleavage of the *d,l* isomer affords an 81:1 mixture of enantiomers (98.8% ee) with an 82% theoretical yield.

### 2.3.3 Chiral shift reagents (CSR)

The notion that enantiotopic groups would be anisochronous in a chiral environment was first suggested by Mislow in 1965 [27], and is the basis for the analysis of enantiomer ratios by chiral shift reagents and chiral solvating agents. Figure 2.6 illustrated that two *diastereomeric* complexes between an achiral lanthanide shift reagent and a ligand may afford differential deshielding of nuclides in the ligand. In these examples, enantiotopic nuclides were rendered diastereotopic (*i.e.*, placed in a chiral environment) by virtue of the MTPA derivatization. A simpler alternative is to use a chiral additive that renders the nuclei diastereotopic in a supramolecular complex. Two types of additive will be discussed: chiral shift reagents (CSR) and chiral solvating agents (CSA). Advantages of the CSR method are:

1. The chiral shift reagent need not be enantiomerically pure.
2. There can be no accidental resolution, deresolution, or racemization during a derivatization (but beware of enantiomer enrichment during sample purification, *vide supra*).
3. A wide range of functional groups can be analyzed with this technique, since all that is required is a Lewis basic atom to coordinate to the lanthanide.

Disadvantages are that absolute configurations cannot generally be determined without reference to a known sample, and that both enantiomers must be available to insure peak separation. An additional disadvantage has developed with the advent of high-field NMR spectrometers: the technique is not as effective at high fields, as explained below.

The first CSR was introduced by Whitesides in 1970 [48]. A 1973 monograph covered the details of lanthanide shift reagents [49], and two reviews have since covered chiral lanthanide shift reagents [50,51]. For our purposes, there are a few things we ought to know about shift reagents themselves, and about how they interact with ligands. Lanthanide shift reagents are *tris* complexes of  $\beta$ -diketonate ligands. For symmetrical, achiral diketones, the complexes exist as an equilibrating mixture of two enantiomeric forms, the  $\Delta$  and  $\Lambda$ . If the ligand is an unsymmetrical diketone, the  $\Delta$  and  $\Lambda$  isomers each exist as *cis* and *trans* (*fac*, *mer*) isomers. If the diketone ligand is chiral, the  $\Delta$  and  $\Lambda$  forms are diastereomers. Thus, a lanthanide (chiral) *tris*-(diketonato) complex is an equilibrating mixture of four diastereomers.

Assuming that coordination of an additional ligand is an outer sphere phenomenon, each face of each octahedron is a potential binding site. For reasons of symmetry, a cis complex (of an unsymmetrical diketone) has four unique binding sites and a

that there is also a temperature dependence hidden in Equation 2.12. Since  $k$  is related to energy of activation, the following proportionality holds:

$$\text{linewidth} = \delta\nu \propto H_0^2 e^{\Delta G^\ddagger/RT}, \quad (2.13)$$

which shows that linewidth is proportional (not only) to the square of the field strength, but also to the temperature. Although increasing the field of the spectrometer broadens the lines, raising the temperature tends to counteract this effect. Operationally, it is wise to conduct a CSR analysis on the lowest field spectrometer available; if line broadening is a problem, warming the sample may help [55-57]. Failing that, spin-echo techniques may be used to eliminate broadened lines [54].

The interaction of a ligand with a lanthanide complex may result in a change in chemical shift,  $\Delta\delta$ , for some of the nuclides of the ligand, especially those that are in the spatial vicinity of the coordinating atom. If the shift reagent and the ligand are chiral, there may be different lanthanide-induced shifts for corresponding nuclei in the two enantiomers of the ligand (nuclides that are enantiotopic by external comparison). This induced anisochrony (chemical shift difference) is  $\Delta\Delta\delta$ . Equation 2.14 illustrates a simplified view of the equilibration of a racemate with a chiral shift reagent, in which the equilibria of the CSR are ignored so that the CSR may be considered as a single species.



Two possible mechanisms have been suggested as the source of  $\Delta\Delta\delta$ :  $K_R \neq K_S$ , or  $(+)\text{-CSR}\cdot R$  and  $(+)\text{-CSR}\cdot S$  have different geometries [58]. It is likely that both of these mechanisms operate to differing extents in various systems. Regarding  $K_R$  and  $K_S$ , note that nuclei that are enantiotopic by internal comparison, such as the methylene protons of benzyl alcohol or the methyls of dimethyl sulfoxide, can be differentiated by CSRs [59]. Clearly no stability difference is required for inducing anisochrony. An important consequence of this fact is that the enantiomeric purity of compounds that are chiral by virtue of isotopic substitution (*e.g.*,  $\text{C}_6\text{H}_5\text{CHDOH}$ ) may be evaluated by this method (as well as by the CSA method described in the next section).

Since the spectrum observed is a time average of the free and CSR-bound ligand, the combination of the enantiomeric forms of a CSR with the enantiomeric forms of a ligand is a dynamic phenomenon. Because of this dynamic relationship, and in contrast to the 'static' derivatization discussed in the preceding section, the CSR need not be enantiomerically pure. Consider the two extremes: the CSR is enantiomerically pure, and the CSR is racemic.<sup>9</sup> Equation 2.14 illustrates the binding of an enantiomerically pure  $(+)\text{-CSR}$  with the two enantiomers of a ligand.<sup>10</sup> The observed spectrum is a time average of the spectrum of each free

<sup>9</sup> For simplicity, we will consider only homochiral *tris* complexes (*i.e.*, only complexes in which all three diketones have the same chirality sense). Dynamic exchange would in fact produce a number of heterochiral complexes, but on average their effects would cancel.

<sup>10</sup> A similar set of equilibria would result from analysis of an enantiomerically pure ligand by a racemic CSR (*cf.* Figure 2.5).

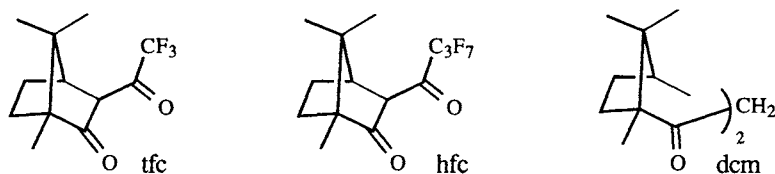
enantiomer and its (+)-CSR complex. On the other hand, if the CSR is racemic and both enantiomers of the ligand are present, Equation 2.15 applies. The spectrum of the *R* enantiomer would now be a time average of the free *R* enantiomer, the (+)-CSR·*R* complex, and the (–)-CSR·*R* complex. Likewise, the spectrum of the *S* enantiomer would be a time average of the free *S* enantiomer, the (+)-CSR·*S* complex, and the (–)-CSR·*S* complex. For reasons of symmetry (*e.g.*, (–)-CSR·*R* = (+)-CSR·*S*), the two time-averaged spectra will be identical, and the lanthanide-induced shifts will be the same (*i.e.*,  $\Delta\Delta\delta = 0$ ) if the CSR is 0% ee. An intermediate case, such as where the CSR is 80% ee, would produce a different time average, such that  $\Delta\Delta\delta$  would decrease, but the integral of the peaks corresponding to the two enantiomers of the ligand would be the same.



Lanthanides are ‘hard’ Lewis acids, and the best binding occurs with ligands that contain ‘hard’ Lewis basic atoms. Approximate binding affinities are primary amine > hydroxyl > ketone > aldehyde > ether > ester > nitrile [50]. Chiral shift reagents have also been used with sulfoxides, arsine sulfides, amino acids, and certain transition metal complexes [51]. Carboxylic acids decompose lanthanide diketonato complexes [58], and so they should be esterified before analysis. Other functional group interconversions may aid the analysis (increase  $\Delta\Delta\delta$ ) by changing the binding characteristics [50]. Such a change might be desirable in several circumstances. For example, weak binding of a sterically hindered hydroxyl might be increased by acetylation (the binding site becomes the more accessible ester carbonyl). In multifunctional molecules, it might be worthwhile to block binding at one site in order to improve binding at another. This might be accomplished by trifluoroacetylation of an alcohol or amine or by ketalization of a carbonyl.

Figure 2.9 illustrates the three ligands found in the most common and commercially available chiral shift reagents, and the abbreviations used for each. The *tfc* [60] and *hfc* [61] ligands are sold as the europium, ytterbium or praseodymium complexes, while the *dcm* ligand [58] is sold as the europium complex. Since  $\Delta\Delta\delta$  is a function of concentration, temperature, and ligand, a comparison of “resolving power” among the different reagents is difficult. Nevertheless, for 1-phenylethanol and 1-phenylethyl amine, the largest  $\Delta\Delta\delta$  for europium complexes was found for the *dcm* complex  $\text{Eu}(\text{dcm})_3$ ,<sup>11</sup> while  $\text{Eu}(\text{tfc})_3$  and  $\text{Eu}(\text{hfc})_3$  were about the same [51]. In choosing lanthanides, europium and ytterbium induce downfield shifts while praseodymium induces upfield shifts. Additionally, the three metals may also cause line broadening to differing extents [50]. For 1-phenylethanol and 1-phenylethyl amine,  $\text{Pr}(\text{hfc})_3$  induced larger shifts than  $\text{Eu}(\text{hfc})_3$ , and did so at lower concentration [51]. Still, the concensus appears to be that no single CSR is superior with all possible ligands.

<sup>11</sup> Since the *dcm* ligand is a  $C_2$ -symmetric  $\beta$ -diketone, there is no *cis/trans* (*fac/mer*) isomerization in the complex. As a result, the number of outer sphere coordination sites is reduced from 12 to 4 (two for the  $\Delta$  and two for the  $\Lambda$  isomers). Spectral averaging of fewer isomeric complexes may account for the larger differentiation by this ligand.



**Figure 2.9** The most common ligands for chiral shift reagents: trifluoroacetylcamphor (tfc) [60], heptafluorobutanoylcamphor (hfc) [61], and dicampholymethane (dcm) [58].

Fraser recommends the following experimental protocol [51]:

1. Try as many as four CSRs, the approximate order of capacity being  $\text{Eu}(\text{dcm})_3 > \text{Pr}(\text{hfc})_3 \approx \text{Yb}(\text{hfc})_3 > \text{Eu}(\text{hfc})_3$ .
2. Try changing the temperature. Lower temperature can have a substantial influence on lanthanide-induced shifts [58,61,62], while warming may sharpen lines [55].
3. If still unsuccessful, try derivatizing the ligand to make it a stronger, harder, Lewis base.

Before conducting a CSR study, the experimentalist should consult Sullivan's review for detailed experimental guidelines [50]. Briefly, the guidelines suggest: dry the substrate, the solvent, and the CSR (by sublimation if prepared fresh or over phosphorous pentoxide *in vacuo* if purchased); keep the substrate concentration low ( $\sim 0.1 - 0.25$  M); add the CSR (either as a solid or as a concentrated solution) in small increments, and filter the solution after each addition (the molar ratio needed for a good induced shift is rarely  $> 1:1$ , and too much lanthanide can broaden lines and even cause the induced shifts to decrease); re-shim the spectrometer after the CSR is added to compensate for the presence of the paramagnetic ions, and check for paramagnetic precipitates after the sample has been spinning for several minutes. Additionally, recall (*vide supra*) that the method is usually more effective at low field.

#### 2.3.4 Chiral solvating agents (CSA)

Mislow's 1965 suggestion [27] that enantiotopic nuclides would be anisochronous



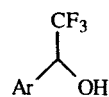
2. Anisochrony in CSAs is usually induced in enantiotopic groups of a ligand by the presence of an anisotropic moiety in the CSA, such as an aromatic ring (as opposed to a paramagnetic metal atom).
3. The induced chemical shift changes of diamagnetic CSAs are not usually as large as with CSRs, and the range of structural types that can be addressed is not as broad. An advantage of smaller  $\Delta\delta$  is that the effect of field strength on linewidth (*cf.* Equations 2.9 and 2.10) is not as problematic.
4. Because CSAs are diamagnetic, line broadening is not as much of a problem as with CSRs. Therefore, it is often possible to deduce enantiomer ratios by comparison of peak *height*, obviating the need for a complete separation of all lines in a shifted pair of multiplets.

As with CSRs, both enantiomers should be available to insure the presence of induced anisochrony.<sup>13</sup>

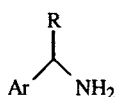
By far the most studied CSAs are the 1-(aryl)trifluoroethanols and the 1-(aryl)ethyl amines (aryl = phenyl, 1-naphthyl, 9-anthryl) that associate primarily through hydrogen-bonding mechanisms. Chiral acids are finding increased use for the analysis of amines as their diastereomeric salts,<sup>14</sup> although assignment of configuration is risky due to aggregation and other dynamic phenomena [64]. Figure 2.10 lists several of the readily available CSAs along with some of the structural types with which they have been used for determination of enantiomer excess and absolute configuration.<sup>15</sup>

The equilibria that describe the 1:1 interactions of a CSA and a pair of enantiomeric solutes (Equation 2.16) is similar to the one used to explain shift reagents (Equation 2.11). An important distinction is that we *assumed*, for the sake of simplicity, that the CSR was a single species. For chiral solvating agents, that

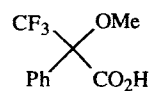
The first rationale for “recognition”<sup>16</sup> of enantiomers was the three-point model proposed by Easson and Stedman in 1933 to explain the interaction of racemic drugs with biological receptors [66]. A similar model was proposed by Ogston in 1948 to explain the enantioselectivity of enzyme reactions [67]. These simplistic models proposed three simultaneous *binding* interactions to explain enzyme enantio-specificity. Similarly, the best rationale for understanding induced anisochrony in enantiomers is based on three interactions, although all three do not have to be binding [64].



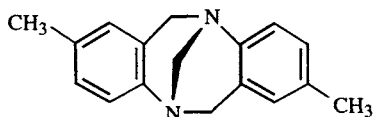
Hydroxy esters [68]  
Arylalkylamines [72]  
Amino esters [76]  
Oxiranes [79]  
Lactones [81,82]  
Phosphine oxides,  
Amineoxides,  
RS(=O)XR, X = N, O, S [86]  
Sulfoxides [87,88]



Sulfoxides [69]  
Phosphine oxides [73]  
*sec*-Benzylic alcohols [77,78]  
*N*-Phthalimido amino acids [80]  
2-(Aryl)carboxylic acids [83,84]  
Hydroxy esters [85]

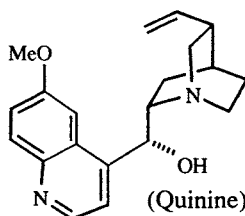


*tert*-Amines [70,71]  
Diamines [74,75]



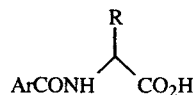
(Tröger's base)

*sec*- and *tert*-  
Benzylic alcohols [89]



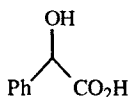
(Quinine)

Binaphthyls,  
*sec*-Benzylic amines [90]

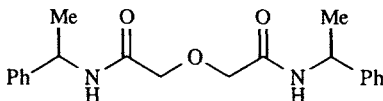


R = Ph, *i*-Bu

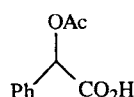
Diamines,  
Amino esters,  
Amino alcohols [74]  
Benzodiazepinones,  
Naphthamides,  
Lactones [91]



Diamines,  
Amino esters,  
Amino alcohols [74]



Amides [92]



Amines,  
Amino alcohols [93]

**Figure 2.10** Common chiral solvating agents and some classes of compounds they have been used with. For a more complete listing, see ref. [65].

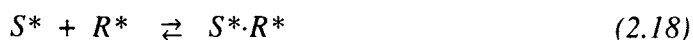
<sup>16</sup> The anthropomorphic notion that a chiral molecule can somehow ‘recognize’ or ‘discriminate’ the chirality sense of another chiral molecule is a convenience that is used commonly, realizing that it is the observer that does the recognizing, not the molecules [64].

Figure 2.11 illustrates the principle with two specific binding interactions and a third, which provides the anisotropy for enantiomer discrimination.<sup>17</sup> In this example, there are two hydrogen bond donors in the CSA: the hydroxyl and the benzylic hydrogen (which has been rendered acidic by the neighboring trifluoromethyl group). Suppose for example, that the solute is dibasic and binds preferentially such that OH and B<sub>1</sub> interact and CH and B<sub>2</sub> interact. Note that the solute substituent that is syn to the aryl group on the CSA will be shielded relative to the other (R<sub>2</sub> on the left and R<sub>1</sub> on the right). *This is the third point required for discrimination of enantiomers.* Because of the shielding cone above the aromatic ring, the time-averaged spectrum will have R<sub>2</sub> at higher field in the absolute configuration on the left. If the preference of the two bonding interactions between the CSA and the solute are known, then the absolute configuration of the CSA can be used to determine the absolute configuration of the solute. Once again, there need be no difference in stability between the two solvates, since protons that are enantiotopic by internal comparison can also be differentiated by CSAs [91]. Detailed models for the assignment of absolute configuration have only been made for the 1-(aryl)trifluoroethanols and the 1-(aryl)ethyl amines, and the reader is referred to Pirkle's review for further details [64].



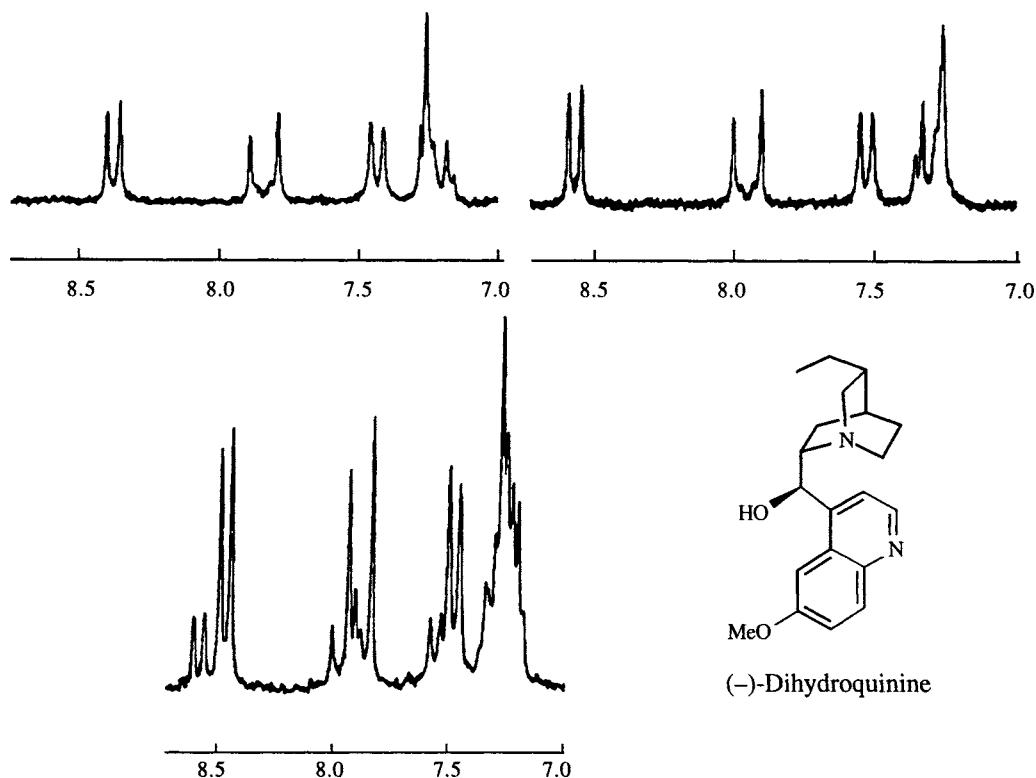
**Figure 2.11** The interaction of a 1-aryltrifluoroethanol chiral solvating agent and the two enantiomers of a dibasic solute.

Although much of the usefulness of CSAs is for analysis of enantiomeric excess, the principles described above may manifest themselves in other ways. For example in 1969, Williams *et al.* demonstrated that dihydroquinine can serve as a chiral solvating agent for itself [94]. Thus, the NMR spectrum of the racemate and the (–)-enantiomer were *not* superimposable, and a 3:1 mixture of enantiomers exhibited anisochronous signals for several enantiotopic protons. The spectra of racemic, 100% ee, and 50% ee dihydroquinine are shown in Figure 2.12. Clearly, the three spectra are different. Although the spectra of both the pure enantiomer and the racemate are similar and easily interpreted, they do not match. The spectrum of the partially enriched enantiomer is unexpectedly complex. Assuming binary association, the phenomenon can be understood in terms of the following equilibria,



where  $R^*$  and  $S^*$  represent the two enantiomers. Since the molecule also contains an anisotropic perturbing function, anisochrony of the homochiral ( $S^* \cdot S^*$ ) and the

<sup>17</sup> This example illustrates two *binding* interactions, although one attractive and one repulsive interaction would also suffice, so long as a third is present as well.



**Figure 2.12** Portion of the 100 MHz NMR spectrum of dihydroquinine. Top left, natural (–)-enantiomer; top right, racemate; bottom left, 3:1 mixture of the two enantiomers (50% ee). Reprinted with permission from ref. [94], copyright 1969, American Chemical Society.

heterochiral ( $S^*.R^*$ ) dimers ensues. The observed spectrum is a time-average of the species from Equations 2.17-2.19 that are present in the samples. The pure enantiomer represents the time average of the species in Equation 2.17 (or 2.19) alone, whereas the racemate represents the time average of all three equilibria. In the present case, these time-averaged spectra are not identical. When the enantiomers are not present in equal amounts, the weighting factors for each equilibrium's contribution to the time averaged spectrum are unequal. For example, if the  $S^*$  enantiomer predominates, the primary interactions will be equilibria 2.17 and 2.19, and the time-averaged spectra for the enantiomers are different ( $S^*$ ,  $S^*.S^*$ ,  $S^*.R^*$  vs.  $R^*$ ,  $S^*.R^*$ ). In other words, the spectrum of enantiomerically enriched dihydroquinine is the result of 'self-induced nonequivalence' whereby the enantiomer in excess acts as the CSA for both enantiomers of the racemate.

Williams also noted [94] that the spectral anomalies of the dihydroquinine enantiomer, racemate, and various mixtures were solvent dependent ( $\text{CH}_3\text{OD}$  reduced the anisochrony, as did  $O$ -acylation) and became identical at high dilution. In synthesis, such anomalies should be remembered when interpreting spectra and when comparing spectra of synthetic materials with literature data if one is enantiomerically pure and the other is fully or partly racemic, or if the spectra were recorded at different solute concentrations.

## 2.4 Chromatography

Asymmetric reactions and processes give rise to two kinds of stereoisomeric products: diastereomers and enantiomers. The physical separation of these isomers with simultaneous analysis of isomer distribution (peak integration) is an excellent way to determine the selectivity of a reaction. For the analysis of *diastereomers*, standard chromatographic techniques suffice, although the chromatographic method should be accompanied by another technique that determines the configuration of the new centers. Diastereomer analysis also ensues in cases of double asymmetric induction, and the configuration of known centers in the reactants may be used as a point of reference for determination of the new stereocenter(s) by NMR or X-ray.

Early methods for chromatographic analysis of *enantiomers* called for derivatization with a chiral reagent. This is a method that is still used, although the problems of kinetic resolution discussed in Section 2.3.1 should be recalled when planning such an analysis. A much more appealing method is the direct separation of enantiomers by gas or liquid chromatography on a chiral stationary phase (CSP). The growing popularity of this method is evidenced by the number of monographs published on the subject in the last few years [95-102]. This method has a number of appealing features:

1. No kinetic resolution arises as a result of double asymmetric induction in a chiral derivatization scheme, although care must still be taken to avoid enantiomer enrichment (or depletion) during workup (*cf.* Figure 2.1 and accompanying discussion).
2. The order of enantiomer elution for a given class of compounds is often known, so that enantiomeric purity and absolute configuration can be determined simultaneously.
3. The sensitivity of GC or HPLC detectors are such that very small amounts of analyte, as little as a few micrograms under favorable circumstances, may be analyzed. This is far below the limits of detection in polarimetry and NMR.
4. Integration of chromatographic peaks is usually much more accurate than measurement of rotations or integration of NMR peaks. Therefore chromatography is the method of choice when accuracy is important, and is especially applicable to the analysis of samples of high enantiomeric purity.

stationary phase is a liquid and the mobile phase is a gas, while in HPLC the stationary phase is a solid and the mobile phase is a liquid. In the extreme, a sample that does not interact with the stationary phase is eluted in the amount of time it takes the mobile phase to travel the column,  $t_0$ . Samples that *do* interact with the stationary phase will obviously take longer. Their retention may be expressed as  $t_R$  (retention time),  $V_R$  (retention volume), or  $\kappa'$  (capacity ratio).

The latter is defined as

$$\kappa' = \frac{A_s}{A_m} \quad , \quad (2.20)$$

where  $A_s$  and  $A_m$  represent the amount of solute in the stationary and mobile phases, respectively. Thus the capacity ratio is the equilibrium constant for the partitioning of the analyte between the mobile and stationary phases. The capacity ratio can also be expressed in terms of retention times,

$$\kappa' = \frac{t_R - t_0}{t_0} \quad , \quad (2.21)$$

where  $t_0$  is the retention time of an unretained compound, usually visible as the solvent front (see Figure 2.13).

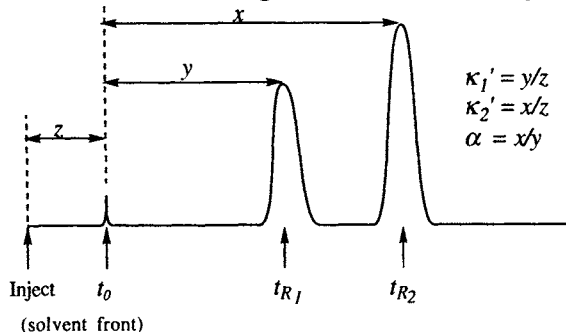
For two peaks to be 'resolved' chromatographically, the capacity ratios,  $\kappa_1'$  and  $\kappa_2'$ , must be different. For analytical purposes, two interdependent chromatographic properties must be considered: the chromatographic separability factor,  $\alpha$ , and the resolution,  $R_S$ . The chromatographic separability factor is defined as

$$\alpha = \frac{\kappa_2'}{\kappa_1'} \quad , \quad (2.22)$$

where  $\kappa_1'$  and  $\kappa_2'$  are the capacity ratios of the first and second eluting peaks, respectively. Combination of Equations 2.18 and 2.19 gives

$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0} \quad , \quad (2.23)$$

where  $t_{R1}$  and  $t_{R2}$  are the retention times of the first and second eluting peaks, respectively. Using Equations 2.21 and 2.22, capacity ratios and separability factors can be easily obtained from a chromatogram, as shown in Figure 2.13.



**Figure 2.13** A hypothetical chromatogram, showing the retention time of an unretained compound ( $t_0$ ), the retention times of two analytes,  $t_{R1}$  and  $t_{R2}$ , and the relationship of these quantities to the capacity ratios,  $\kappa_1'$  and  $\kappa_2'$ , and the chromatographic separability factor,  $\alpha$ .

Because the capacity ratios reflect the equilibrium between two analytes and the stationary phase, the separability factor,  $\alpha$ , is directly related to the free energy difference between the analyte-stationary phase complexes, according to

$$\Delta\Delta G = -RT\ln\alpha. \quad (2.24)$$

Rearrangement gives

$$\alpha = e^{-\Delta\Delta G/RT}, \quad (2.25)$$

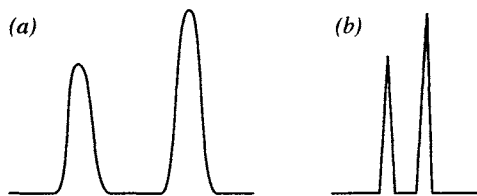
which is the same exponential relationship described in the previous chapter (*cf.* Equations 1.1 and 1.2). For the interaction of chiral stationary phases with enantiomers, the complexes are diastereomeric; in order for separation to occur, they must *not* be isoenergetic.<sup>19</sup> Separability factors of 1.1 are common in CSP chromatography, which translates to a free energy difference,  $\Delta\Delta G$  (at 25° C), of only 56 cal/mole (*cf.* Figure 1.3)! It is the *amplification* of this difference during the chromatographic process that accounts for the separation.

Resolution,  $R_s$ , of chromatographic peaks is the ratio of the peak separation to the average peak width:

$$R_s = \frac{2(t_{R2} - t_{R1})}{w_1 + w_2}, \quad (2.26)$$

where  $w_1$  and  $w_2$  are the widths of the first and second peaks, respectively. Thus, the resolution is dependent on both the separation factor,  $\alpha$ , and the column efficiency (number of theoretical plates). As shown in Figure 2.14, the same resolution may give rise to two closely spaced narrow peaks or to two broader peaks that are more widely separated.

Racemization of either the analyte or the chiral stationary phase may give rise to peak coalescence, but the two are easily distinguished by their appearance, as shown in Figure 2.15. Over time, racemization of the CSP may occur, and this will reduce



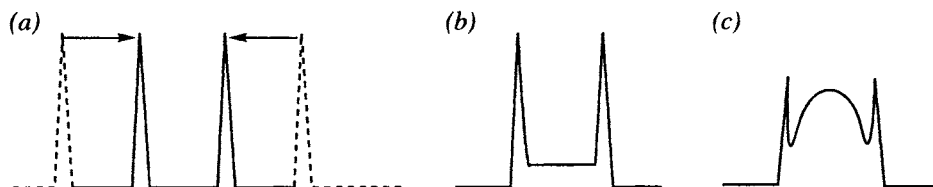
**Figure 2.14** Hypothetical chromatograms of identical resolution. (a) Large  $\alpha$  on a low efficiency column. (b) Smaller  $\alpha$  on a high efficiency column.

the separation factor,  $\alpha$ . If racemization of a CSP is possible (such as with an amino acid derived CSP), it is wise to periodically run a standard to check for peak coalescence. Another type of peak coalescence is due to racemization of the analyte on the column [104,105]. The appearance of such a phenomenon depends on the relative rates of racemization and separation [105]. The two extremes are fast and

<sup>19</sup> Recall that the diastereomeric complexes of enantiomers with CSRs or CSAs *may* be isoenergetic and still exhibit anisochrony (Sections 2.3 and 2.4).

slow racemization, relative to the separation. Fast racemization would yield a single sharp peak, and extremely slow racemization would go undetected. Intermediate cases might appear as a trough between the peaks, or a hump, as shown in Figure 2.15b and c [105].

The historical origins of CSP chromatography are early in the 20th century when it was observed that certain dyes were enantioselectively adsorbed onto biopolymers such as wool [106-108]. Although there were isolated instances of chromatographic resolutions earlier,<sup>20</sup> development of CSP chromatography as a useful tool did not take place until capillary gas chromatography (GC) and high



**Figure 2.15** Hypothetical chromatograms showing peak coalescence due to racemization. (a) Racemization of the chiral stationary phase causes the peaks to move closer together ( $\alpha$  decreases, ultimately to zero if the CSP is racemic). (b) and (c) Racemization of the analyte on the column may produce a trough between the enantiomer peaks, as in (b), which may grow to a hump, as in (c), or even a single peak, depending on the relative rates of racemization and separation [105].

performance liquid chromatography (HPLC) were popularized in the 1970s. The separation of enantiomers on a CSP requires the formation of diastereomeric adsorbate 'complexes' between the analyte and the CSP. A number of CSPs have come into use, but only in a few cases has detailed work been done to rationalize the relative stabilities of the diastereomeric adsorbates. Indeed, the energy differences that are required for enantiomer separation on an efficient column are so small ( $\sim 50$  cal/mole, *vide supra*) that caution is advised in overinterpreting enantiomer 'recognition' models.

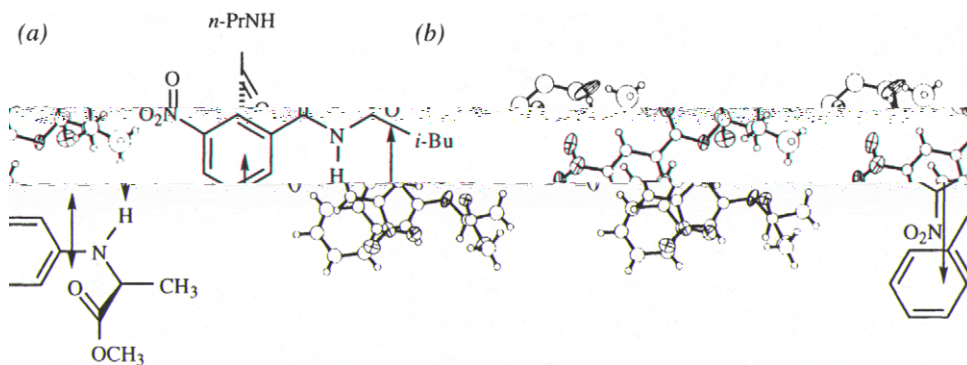
In 1952, Dalglish [111] extended the 3-point model [66,67] to CSP chromatography, to explain the separation of amino acid enantiomers by paper chromatography. Dalglish postulated a 3-point *attraction*, which now seems to be somewhat oversimplified. More recently, Pirkle has argued that, although three points are required, all need not be attractive [112]. At least one, however, must be stereochemically dependent. A detailed study of chiral solvating agents (*vide supra*) has led to fairly exact models of 3-point solvation to explain the chemical shift effects of the CSA. Immobilization of one or the other of the CSA components on silica gel produced separations having the same order of elution as expected based on the selectively solvated species in the NMR experiment. For example, *N*-(3,5-dinitrobenzoyl)leucine amide bonded to silica gel shows a high degree of selectivity ( $\alpha = 9.7$ ) for the enantiomers of methyl *N*-(2-naphthyl)alaninate [113]. The converse is also true: *N*-(2-naphthyl)alanine ester as a CSP shows a high affinity for *N*-(3,5-dinitrobenzoyl)leucine derivatives [114]. The model for the complexation of

<sup>20</sup> For more detailed accounts of the early history of CSP chromatography see ref. [95,109,110].



these two species involves an aromatic  $\pi$ -stacking interaction and two hydrogen bonds, as indicated in Figure 2.16a. This model is supported by intermolecular NOE enhancements in  $\text{CDCl}_3$  [113], and an X-ray crystal structure of the bimolecular complex [115]. The latter is illustrated in Figure 2.16b, which confirms the three interactions proposed earlier [113,114], and which provides strong experimental support for the model. Although detailed models of other CSP separations have not been as extensively studied as the Pirkle systems, it is likely that they also conform to some variant of the 3-point rule [112,116].

In CSP-GC and CSP-HPLC, there are only a few categories of chiral selectors used as stationary phases. There is a broader variety of CSP columns on the market for HPLC than for GC, but most types have been investigated in both media. For the most up to date information, literature from vendors of CSP columns should be



**Figure 2.16** Supramolecular complex of *N*-(3,5-dinitrobenzoyl)leucine *n*-propyl amide and methyl *N*-(2-naphthyl)alaninate. (a) Schematic representation of the three recognition points deduced from NOE data [113]. (b) Stereoview of a bimolecular crystal. The orientation of the two species concurs with solution NOE data. Reprinted with permission from ref. [115].

consulted. In the US, Regis, J. T. Baker, and Daicel have a variety of HPLC columns to choose from, and they have published some useful tables on selecting the correct column for a given application [117,118]. A recent review also lists a number of compound types that are resolvable on various CSPs [119]. Similar information on GC columns and applications is available from Applied Science or Supelco, and Souter's monograph [95] also contains an extensive listing of CSPs for GC, and the types of compounds separated by each. An expedient method of finding a solution to a separation problem may be the use of computerized databases. In addition to the standard databases such as those offered by *Chemical Abstracts*, Roussel and Piras have constructed a database dedicated to the enantiomeric resolution of racemic mixtures by HPLC [120].

Here, only general categories of chiral stationary phases will be mentioned.<sup>21</sup> One of the more popular types of GC and HPLC columns use donor-acceptor interactions such as those illustrated in Figure 2.16 for enantiomer separation.

<sup>21</sup> Lough's monograph gives a particularly thorough coverage of CSP types for HPLC [97], while Souter's slightly older text is particularly good for GC [95].

Types of donor-acceptor interactions are hydrogen bonding,  $\pi$ -stacking, dipole stacking, etc. Derivatization is usually required for both GC and HPLC applications.

In HPLC, a large variety of analyte types have been resolved on columns packed with derivatized cellulose. Cellulose acetate, benzoate, and carbamate derivatives provide CSPs that will separate a very broad range of analyte types, although a single CSP may not have broad applicability to a wide variety of analytes, and derivatization may be required. Separation is achieved by donor-acceptor interactions, with inclusion phenomena sometimes playing a secondary role.

Microcrystalline cellulose triacetate, cyclodextrin- and crown ether-derived CSPs, as well as some chiral synthetic polymers, achieve enantiomer separation primarily by forming host-guest complexes with the analyte; in these cases, donor-acceptor interactions are secondary. Solutes resolved on cyclodextrins and other hydrophobic cavity CSPs often have aromatic or polar substituents at a stereocenter, but these CSPs may also separate compounds that have chiral axes. Chiral crown ether CSPs resolve protonated primary amines.

Chiral ligand exchange chromatography utilizes immobilized transition metal complexes that selectively bind one enantiomer of the analyte, which is usually an amino acid.

Proteins such as bovine serum albumin, immobilized to silica, achieve enantiomer separation primarily *via* hydrophobic and electrostatic interactions. Although the protein-based CSP columns have low capacity and preparative use is impossible, these phases offer the analyst the convenience of being able to resolve a broad spectrum of analytes with a single column.

## 2.5 Summary

Over seventy five years after the van't Hoff - Le Bel theory of the asymmetric carbon atom was introduced, Bijvoet and colleagues established for the first time the absolute configuration of a chiral molecule, sodium rubidium tartrate, using anomalous dispersion of X-rays [121].<sup>22</sup> Recall that Emil Fischer's assignment of the absolute configuration of D- and L-tartrate was arbitrary and for the first half of the twentieth century was hypothetical. Thus, the assignment of the absolute configuration of all known chiral molecules is predicated on this single method. More commonly, it is used to establish molecular constitution and relative configurations. This is a very powerful tool [122] that should not be overlooked by the synthetic practitioner, although its implementation is usually left to a specialist.

The simplest methods for analysis are polarimetry and NMR, when applicable. Advances in chromatographic science continue apace, and it is likely that further advancements will be made to ease the burden of analysis. Chromatography is the

<sup>22</sup> It is interesting that tartrate was the first resolved compound [1] as well as the first compound whose absolute configuration was established; it is fitting that this seminal work was done at the *van't Hoff* Laboratories of the University of Utrecht. Given the role tartaric acid played in the establishment of the field of stereochemistry, it was perhaps inevitable that it would also play a major role in asymmetric synthesis, as will be seen in a number of examples throughout this book.



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