INTRODUCTION

1

Nuclear magnetic resonance (NMR) spectroscopy is one of the most common methods used to determine enantiopurity and assign the absolute configuration of chiral compounds. The strategy that has been most exploited, as first recognized by Raban and Mislow in 1965 [1], is to use an enantiopure chiral reagent to distinguish a pair of enantiomers through the formation of nonequivalent diastereomeric complexes. With the diastereomeric complexes, the resonances of enantiotopic nuclei become anisochronous and may split into two resonances, one for the (*R*)-derivative and one for the (*S*)-derivative of the analyte. The area of the two resonances can be used to determine enantiopurity. The enantiopure probe molecule functions as either a chiral derivatizing agent (CDA) or a chiral solvating agent (CSA). Furthermore, the association of an enantiopure compound with a prochiral molecule with nuclei that are enantiotopic by internal comparison (e.g. the methyl groups of 2-propanol) renders these nuclei nonequivalent such that distinct resonances are often observed in the NMR spectrum. Classifying chiral metal compounds as either CDAs or CSAs is sometimes difficult. What is important is whether the analyte molecule undergoes fast or slow exchange with the metal center. Strategies based on different packing orders for a pair of enantiomers, such as it occurs in liquid crystals or solid-state systems, have also been used for chiral analysis in NMR spectroscopy.

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1.1. CHIRAL DERIVATIZING AGENTS

CDAs form a covalent bond with a reactive moiety of the analyte. Many CDAs are available for the analysis of carboxylic acids, alcohols, and amines, although strategies for preparing derivatives of many other functional groups will be described as well throughout the text. There are two potential concerns with the application of CDAs when determining enantiopurity. One is the possibility of kinetic resolution, which involves a situation where one enantiomer reacts faster with the CDA than the other. If the reagents are not allowed to react for a long enough time, the proportion of the two diastereomers will not be equivalent to the proportion of the two enantiomers in the original mixture. Kinetic resolution is significant when determining enantiopurity, but it is not significant if the CDA is being used to assign the absolute configuration of an enantiopure analyte such as a natural product.

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A second concern with CDAs is that no racemization occurs during the derivatization reaction. This can be significant whether it happens to the analyte or the CDA. With some CDAs for which unacceptable levels of racemization did occur, further study was undertaken to develop reaction conditions that minimize or eliminate racemization. When pertinent, these studies are described in the text.

A general understanding is that CDAs used for determining the enantiopurity of an analyte should be 100% enantiopure. A method for using CDAs that are less than 100% enantiopure has been described. The enantiopurity of the reagent must first be accurately measured using an appropriate method. A set of equations is provided in the report to determine the enantiopurity of an unknown from the known purity of the chiral reagent [2].

Many CDAs incorporate moieties, such as aryl rings, that produce specific and predictable perturbations in the chemical shifts of the resonances of the analyte. In such cases, the changes in chemical shifts in the spectrum of an enantiopure analyte in the derivatives with the (*R*)- and (*S*)-enantiomers of the CDA can be used to assign absolute configuration. In other situations, moieties on the analyte may cause specific and predictable perturbations of the chemical shifts of resonances of the CDA. If so, these can be used to assign absolute configuration as well.

Another procedure that is often used with CDAs or CSAs is to look for the presence of specific trends in the chemical shifts that correlate with the absolute configuration of the analyte. The assumption is that if the trends are consistent among a series of compounds with known configurations, then they will be consistent for an unknown analyte with a similar structure. Empirical trends such as these have been observed in many situations and are described where appropriate throughout the text.

An alternative, although much less-used, derivatizing strategy involves a self-coupling reaction of a chiral molecule. The self-coupling of two chiral molecules leads to the formation of a mixture of *meso* (*R*,*S*) and *threo* [(*S*,*S*)/(*R*,*R*)] derivatives. Assuming these species exhibit distinct resonances in the NMR spectrum, the areas of the different resonances depend on the enantiopurity of the analyte [3]. A recent example is a generalized procedure for determining the enantiopurity of 2-phenylpropionic acid and other profens. A stereospecific *N*,*N*′-dicyclohexylcarbodiimide coupling produces a statistical mixture of diastereoisomeric chiral ((*R*,*R*) and (*S*,*S*)) and *meso* ((*R*,*S*) and (*S*,*R*)) anhydrides. The ratio of the anhydrides in the ^{1}H NMR spectrum can be related to the initial enantiopurity. The reaction can be done in an NMR tube in about 2 min. Because the coupling is stereo random, the reaction does not need to go to completion. The method is more accurate for samples with moderate-to-high enantiomeric excess than those closer to racemic proportions [4].

1.2. CHIRAL SOLVATING AGENTS

CSAs associate with the analyte through non-covalent interactions as shown in Eqs 1.1 and 1.2 for the (*R*) and (*S*) forms of an analyte (A). This can involve dipole–dipole, ion-pairing, and π–π interactions. Steric effects are also important in the recognition properties of many CSAs. The choice of solvent is an important parameter when using a particular CSA.

Organic-soluble CSAs are often more effective in nonpolar solvents that cannot effectively solvate the polar groups of the CSA and analyte. Water-soluble CSAs, which are often organic macrocyclic compounds, usually rely on hydrophobic effects to promote the interaction or insertion of a hydrophobic portion of the analyte within the hydrophobic cavity of the CSA.

$$\mathrm{CSA} + (R) - \mathrm{A} = \mathrm{CSA} - (R) - \mathrm{A} \quad K_R \tag{1.1}$$

$$\mathrm{CSA} + (S) - \mathrm{A} = \mathrm{CSA} - (S) - \mathrm{A} \quad K_S \tag{1.2}$$

CSAs generally undergo fast exchange with analytes. With fast exchange, the NMR spectrum is a weighted average of the proportion of bound and unbound analyte. Resonances of the analyte double with the presence of chiral recognition. If slow exchange and enantiodifferentiation occur, and not all of the analyte is bound to the CSA, three resonances are observed for a particular nucleus in the NMR spectrum. One is for the unbound analyte. The other two are for the bound forms of the (*R*)- and (*S*)-isomers of the analyte. Sometimes the resonances of the analyte or CSA are broadened, which occurs if the system has an intermediate rate of exchange. In such cases, it may be possible to speed up the exchange to acceptable levels by warming the sample, while still retaining enantiodifferentiation.

CSAs are most often used to determine enantiopurity. There are instances, though, in which the interaction of the CSA with the analyte is understood with enough specificity to assign absolute configuration. Similar to the use of CDAs, the relative magnitudes of perturbations in chemical shifts with the (*R*) and (*S*) forms of the CSA are used in assigning absolute configuration. There are also other CSAs where empirical trends that correlate with absolute configuration are noted for compounds with similar structures. Unlike CDAs, when measuring enantiopurity with a CSA, it is not necessary to have 100% enantiopurity for the chiral reagent. What is needed is sufficient recognition to cause nonequivalence in the spectra of the enantiomers so that the resonances can be accurately integrated.

Chiral recognition with a CSA can occur from two mechanisms. One is that the CSA complexes with the (*R*)- and (*S*)-isomers of the analyte are diastereomers, and similar nuclei in the two analyte enantiomers may have different chemical shifts. The other is that the two enantiomers of the analyte often have different association constants (K_R and K_S for Eqs 1.1 and 1.2) with the CSA, such that the time-averaged solvation environments are different. In many cases, both mechanisms likely contribute to some extent to the nonequivalence that is observed in the NMR spectrum.

When using CSAs for enantiodifferentiation in NMR spectroscopy, it is usually best to record a series of spectra, often referred to as a titration, with increasing concentration of the CSA relative to the analyte. As the resonances shift position in the spectrum, they may often overlap with other resonances of the CSA or analyte. Recording a series of spectra better ensures that a spectrum with unobscured splitting of one of the resonances is observed. In some CSA–analyte pairs, resonances may exhibit broadening over one region of the titration because of an intermediate exchange rate, whereas broadening is not observed in other regions.

The spectra in Figure 1.1 illustrate many of the observations that may occur in the titration of an analyte with a CSA. The series of spectra in Figure 1.1 show the resonances for the diastereotopic methylene protons of 3-amino-3-cyclohexylpropionic acid (**1.1**), a β-amino acid (10 mM), with increasing concentrations (1–20 mM) of (18-crown-6)-2,3,11,12-tetracarboxylic acid (**1.2**) (Section 8.2.2). One observation is that the resonances of the hydrogen labeled H_A show very large enantiodifferentiation during the titration and these signals can be readily used to determine enantiopurity. Furthermore, the H_A resonance for one enantiomer undergoes a large perturbation in chemical shift, whereas the H_A resonance for the other enantiomer undergoes a negligible perturbation in chemical shift. Another observation is that the resonances of the other methylene hydrogen labeled H_A' show only a small degree of enantiodifferentiation during the titration. It is obvious that there are some concentration ratios where the resonances of H_A overlap with H_A' in the spectrum (Figure 1.1f and g). Another important observation is the broadening of the H_A resonance in the spectrum in Figure 1.1d and e where the concentration of crown ether is 3 and 4 mM. Finally, a careful inspection of the spectra show that the 3-bond coupling

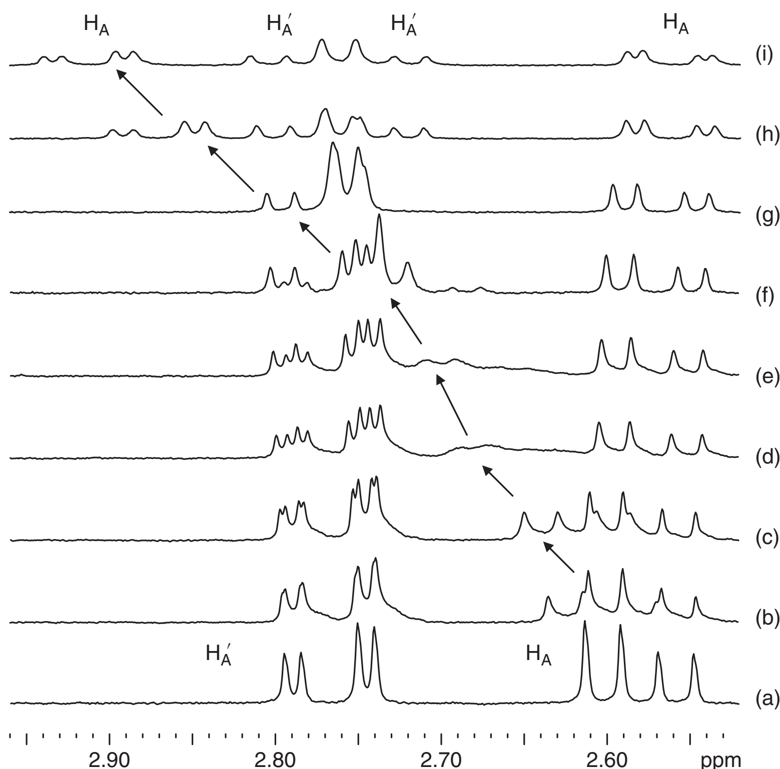
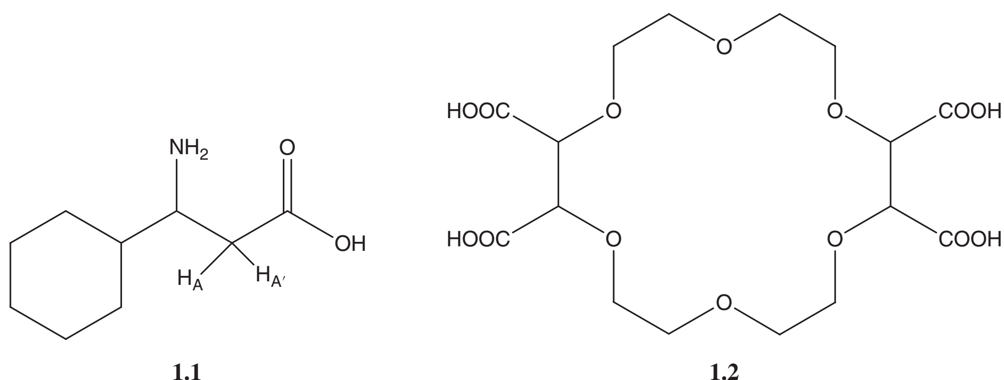


Figure 1.1 ^{1}H NMR spectra (400 MHz, methanol-d_4, 23°C) of the (a) α-methylene hydrogen atoms of 3-amino-3-cyclohexylpropionic acid (**1.1**) (10 mM) with (18-crown-6)-2,3,11,12-tetracarboxylic acid (**1.2**) at (b) 1 mM, (c) 2 mM, (d) 3 mM, (e) 4 mM, (f) 5 mM, (g) 10 mM, (h) 15 mM, and (i) 20 mM. (Ref. [5]. Reproduced with permission from Elsevier.)

constants between the H_A and H'_A protons and the neighboring methine proton change over the series of spectra. Presumably, binding of **1.1** to the crown ether alters the rotational motion about the single C—C bonds in the analyte, thereby accounting for the change in coupling constants [5].



When enantiodifferentiation with a CSA is dominated by structural differences in the CSA–analyte diastereomers, it is often desirable to force the reaction toward formation of complexes to maximize enantiodifferentiation in the NMR spectrum. This is commonly achieved by using a large excess of the CSA relative to the analyte and/or by cooling the sample to promote complex formation.

The situation can be much more complex when differences in the association constants of the two enantiomers with the CSA dominate enantiodifferentiation in the NMR spectrum. In this case, at low ratios of CSA-to-analyte, the stronger binding enantiomer has larger perturbations in chemical shifts. As the concentration of CSA is raised, more of the weaker binding enantiomer begins to associate with the CSA and its resonances undergo perturbations in chemical shifts. It is possible in a titration with a CSA that a resonance may split, eventually re-coalesce, and then even reverse the order as both enantiomers bind and the diastereomeric nature of the complexes becomes most important in influencing the chemical shift. A detailed analysis of this situation has been published [6]. The potential for this behavior means that spiking a CSA–analyte mixture with one of the pure enantiomers to assign configurations to the resonances is risky unless a full titration experiment has been performed in advance [6].

An example of the unusual concentration effects that can occur with CSAs is seen in Figure 1.2 for the methine proton of mandelic acid (**1.3**) with macrocycle **1.4** ($n = 3$) (Section 8.2.9). Association of **1.4** with **1.3** involves an acid–base neutralization with ion-pairing and hydrogen-bonding interactions. The large enantiodifferentiation at relatively low concentrations of **1.4** and diminishment of enantiodifferentiation as the concentration is raised likely reflects a transition from a 2 : 1 mandelic acid–macrocycle complex to a 1 : 1 complex. But it also could involve a chiral recognition process dominated by differences in association constants of (*R*)- and (*S*)-mandelic acid with **1.4** [7].

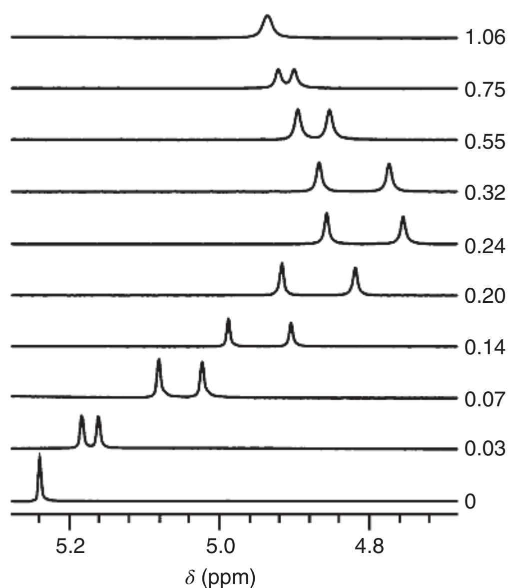
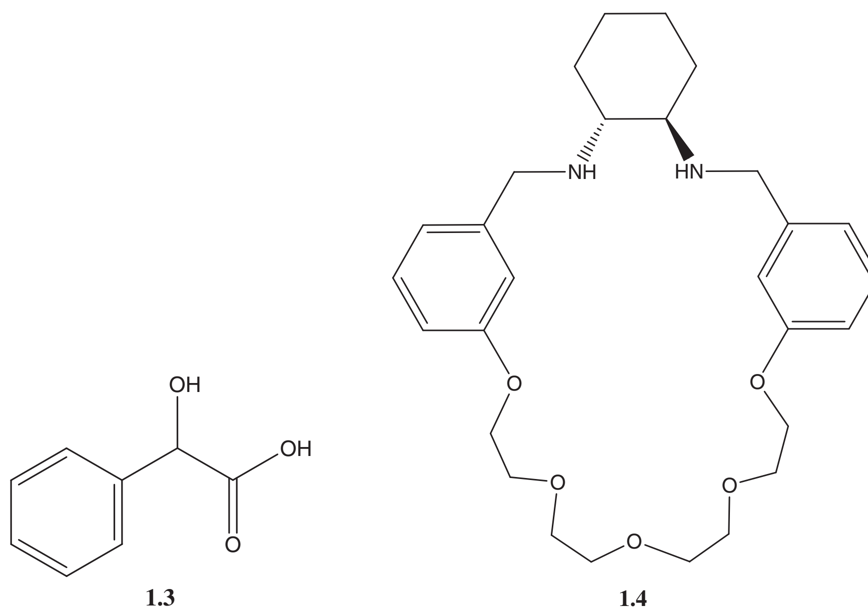


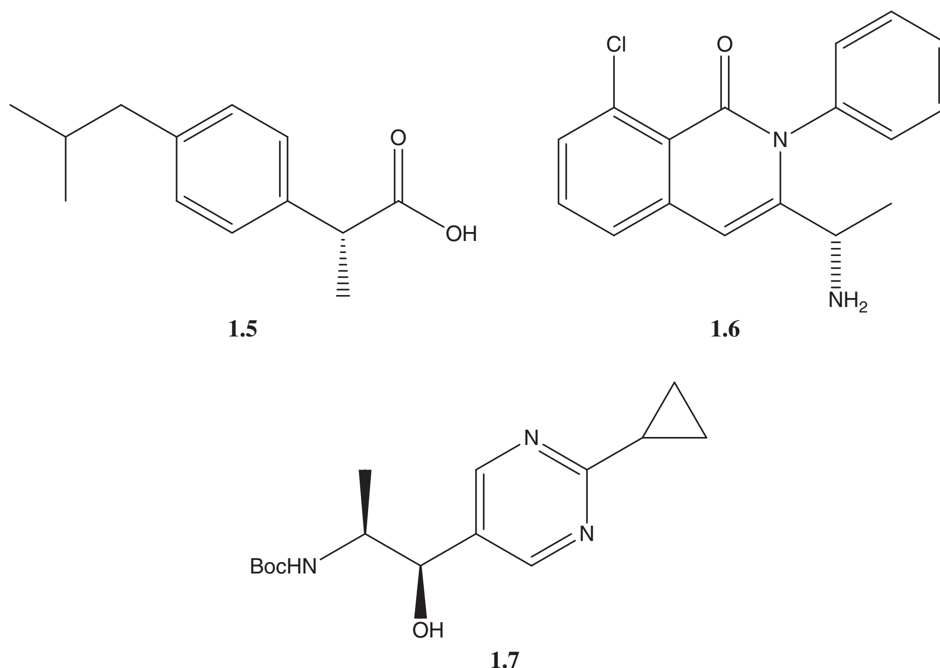
Figure 1.2 ^{1}H NMR spectra (300 MHz, $CDCl_3$) of the methine resonance of mandelic acid (**1.4**) with increasing amounts of macrocycle **1.3**. (Ref. [7]. Reproduced with permission from American Chemical Society.)



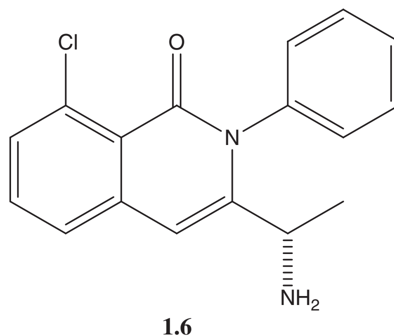
A common situation when performing chiral analysis using NMR spectroscopy is to have anisochronous splitting of one or more resonances, but the multiplet nature of the resonances leads to incomplete differentiation and overlap. Sometimes it is still possible to use partially differentiated resonances for determining enantiopurity, whereas

other times the overlap is too severe for a reliable analysis. A similar problem results with broadened resonances. This can occur if the association of a CSA with an analyte has an intermediate exchange rate. Broadening is also common when paramagnetic lanthanide-containing reagents are used. The result is that relatively sharp signals (e.g. a methyl singlet) are often monitored for chiral differentiation. Also, resonances closer to the stereocenter often exhibit larger enantiodifferentiation and are better to use for determining enantiopurity.

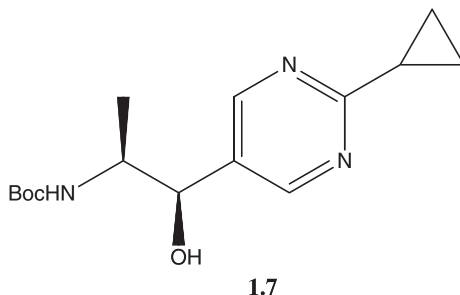
CSAs are typically used to determine the enantiopurity of an analyte in a sample where there is some amount of each enantiomer. A method to determine whether a CSA will be effective for determining whether an enantiopure compound isomerizes on standing has been described. To demonstrate the utility of the method, ^{1}H chemical shifts in the spectrum of (*R*)-ibuprofen (**1.5**) were measured in the presence of 20 equivalents of (*R*)- and (*S*)-*tert*-butylphenylphosphinothioic acid (Section 7.1.1.3). If certain resonances of the analyte have different chemical shifts in the two samples, it means that analysis of the sample of ibuprofen on standing can be measured in the presence of either the (*R*)- or (*S*)-isomer of the CSA to determine whether any racemization takes place. The utility of the method is also demonstrated on mixtures of *tert*-butylphenylphosphinothioic acid with amine **1.6** and 1,1′-binaphthyl-2,2′-diylphosphoric acid (Section 7.1.1.9) with amino alcohol **1.7** [8].



1.5



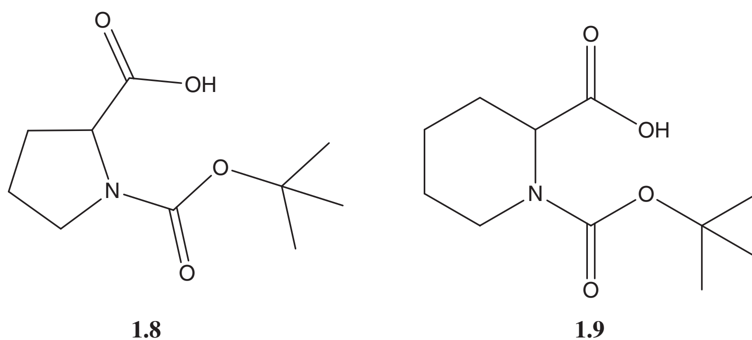
1.6



1.7

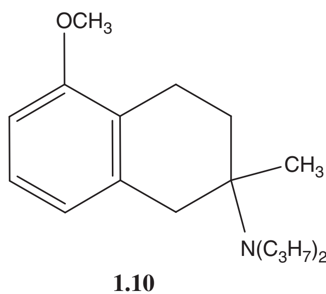
A generalized pattern recognition procedure using principal component analysis and a least square support vector machine has been described for determining enantiomeric purity using NMR methods. The method does not require enantiodifferentiation in the spectrum but examines differences in the chemical shifts of three solutions with different enantiomeric purities. The utility of the method is demonstrated

for a mixture of quinine (Section 6.12.1) with *N*-Boc-protected amino acids alanine, proline (**1.8**), and piperidine-2-carboxylic acid (**1.9**). Chemical shift data from three solutions are used to generate loading coefficients that are then used to determine the enantiomeric purity of samples with unknown compositions. Because enantiodifferentiation is not needed in the spectrum, catalytic amounts of the chiral reagent can be used [9].



1.8 **1.9**

NMR spectroscopy can typically be used to analyze mixtures where the minor enantiomer is as low as 1–5%. A problem with the analysis of highly enriched solutions is obtaining accurate integration of the resonance of the minor enantiomer. The methoxy resonance of 5-methoxy-2-methyl-2-dipropylaminotetralin (**1.10**) is enantiodifferentiated in the presence of 1,1′-binaphthyl-2,2′-diylphosphoric acid (Section 7.1.1.9) in acetone-d_6. In addition to integrating the two methoxy signals to determine enantiopurity, another procedure involves comparing the area of the ^{13}C satellite peak of the methoxy resonance of the major enantiomer to the area of the methoxy resonance of the minor enantiomer. Knowing that ^{13}C is 1.108% abundant, quantification of the minor enantiomer in highly enriched samples is more accurate since two peaks of more similar area are being compared [10].



1.10

The magnitude of enantiodifferentiation of a resonance is often denoted as a $\Delta\Delta\delta$ value. The resonance of the analyte has an initial chemical shift δ. Addition of a CSA will perturb the chemical shift of the resonance and the difference between the new and original chemical shift is $\Delta\delta$. If the resonance shows anisotropy with a separate peak for each of the enantiomers, the difference in chemical shifts between these two peaks is $\Delta\Delta\delta$. However, a $\Delta\Delta\delta$ value does not indicate whether the resonances are fully differentiated because of the multiplet nature of many resonances.

It has been proposed that the use of an enantioresolution quotient *E* (Eq 1.3) is a better measure of chiral differentiation than $\Delta\Delta\delta$.

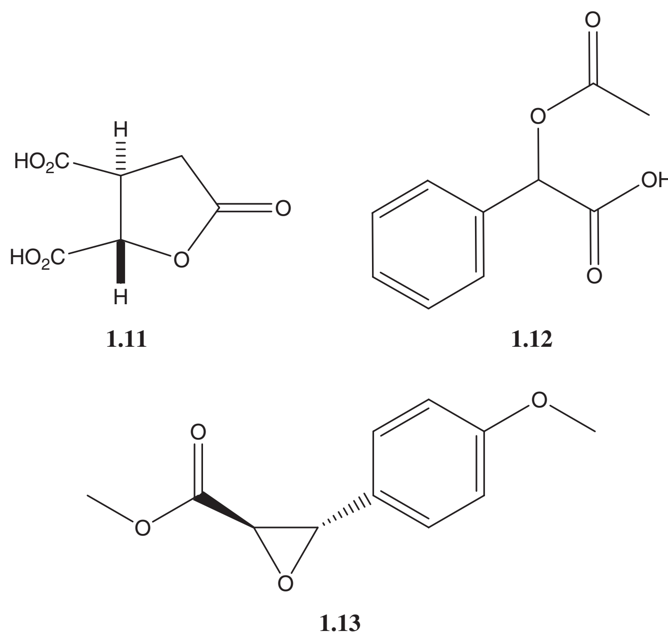
$$E = \frac{|\Delta\Delta\delta|}{3W} \tag{1.3}$$

W in Eq 1.3 is the width of a resonance at one-tenth of its height. The *E* value is a better indicator than $\Delta\Delta\delta$ of whether two multiplets are fully separated or still overlap in the NMR spectrum: $E=0$ (no enantiodifferentiation), $0 < E < 1$ (partial enantiodifferentiation), $E \cong 1$ (moderate enantiodifferentiation), $E >> 1$ (high enantiodifferentiation) [11]. Whether the use of *E* will be widely adopted by investigators in the field remains to be determined.

1.3. NMR METHODS TO IMPROVE THE QUALITY OF DATA WITH CSAs AND CDAs

A number of pulse sequences that can be used to simplify NMR spectra and eliminate peak overlap have been developed. Many of these are referred to as "pure-shift" methods, where the basic strategy is to use either a one- or a two-dimensional (2D) pulse sequence that eliminates coupling and collapses partially resolved multiplet resonances into two singlets in one of the dimensions.

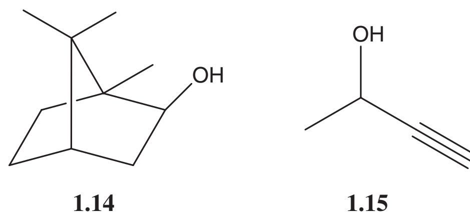
The utility of a heteronuclear multiple quantum correlation (HMQC) method to remove overlapping proton resonances has been demonstrated using quinine (Section 6.12.1) as a CSA with DL-isocitric lactone (**1.11**), ibuprofen (**1.5**), *O*-acetylmandelic acid (**1.12**), and *trans*-3-(4-methoxyphenyl)glycidic acid methyl ester (**1.13**). The overlap in proton resonances can be completely resolved by differences in the carbon chemical shifts, demonstrating the utility of HMQC spectra for chiral analysis [12].



1.11 **1.12**

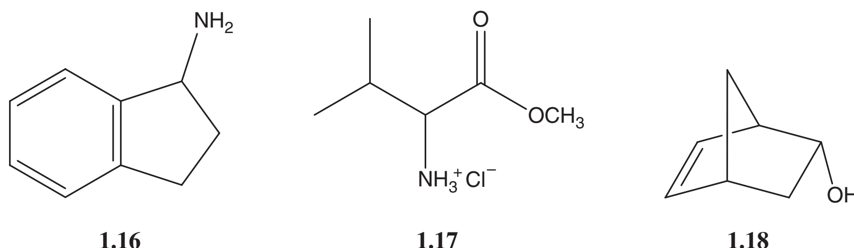
1.13

A ^{1}H δ-resolved 2D broadband homodecoupled proton NMR method to visualize the spectrum of each enantiomer has been reported. The method uses semi-selective RF pulses combined with a *z*-field gradient pulse. Different selective echoes occur in various parts of the sample. Detection of subtle chemical shift differences between enantiomers is possible along the diagonal of the 2D map. The utility of the method is demonstrated on a mixture of the Eu(III) tris-β-diketonate of 3-trifluoroacetyl-D-camphor ($Eu(tfc)_3$) (Section 9.2) and 2-methylisoborneol (**1.14**) and 3-butyn-2-ol (**1.15**) in a liquid crystal system consisting of poly(γ-benzyl-L-glutamate) (PBLG) (Section 10.2) in chloroform-*d* [13].



1.14 **1.15**

Suryaprakash and coworkers have reported several procedures for simplifying ^{1}H NMR spectra [14–16]. The most recent of these involves a 2D resolved total correlation spectroscopy (RES-TOCSY) pulse sequence. The method involves a selective excitation of one nucleus (H_i) in each enantiomer or diastereomer. During the evolution, additional pulses decouple H_i from other protons, resulting in evolution of only its chemical shift. Separation of the resonances occurs in the indirect dimension and the separation of all enantiodifferentiated peaks are scaled to the separation of H_i. The direct dimension exhibits both the chemical shift and the coupling. The utility of this pulse sequence is demonstrated with four chiral systems: (i) the iminoboronate ester formed by reacting formylphenylboronic acid with (*R*)-2,2′-hydroxy-1,1′-binaphthalene (BINOL) and 2-aminobutane (Section 7.3.1), (ii) BINOL (Section 4.14.1) as a CSA with 1-aminoindane (**1.16**), (iii) (18-crown-6)-2,3,11,12-tetracarboxylic acid (Section 8.2.2) with valine methyl ester hydrochloride (**1.17**), and (iv) $Eu(tfc)_3$ (Section 9.2) with *exo*-norborneol (**1.18**). The method is effective for simplifying complex, overlapped spectra and with severely broadened spectra [16].

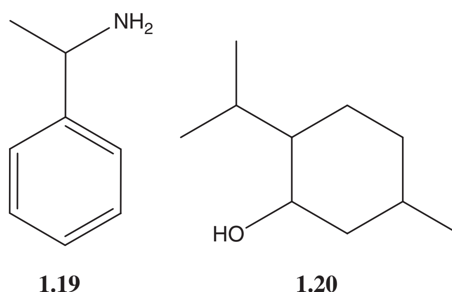


1.16 **1.17** **1.18**

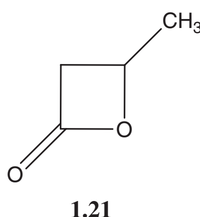
An ultrahigh resolution pure-shift NMR technique called PSYCHE (pure shift yielded by Chirp excitation) uses low flip angle swept-frequency pulses in the presence of a weak magnetic field gradient. This method can be used for better sensitivity or elimination of artifact signals. The method yields an order of magnitude improvement over existing pure-shift methods [17].

A frequency-selective one-dimensional pure-shift ^{1}H NMR experiment has been described. The method is a homodecoupled version of the regular one-dimensional single pulsed-field-gradient echo scheme. A particular frequency is selected for analysis, and a sensitive TOCSY transfer from another isolated proton resonance allows the visualization of singlets with other overlapping resonances. The utility of the method is demonstrated on mixtures of β-cyclodextrin (Section 8.1.2) with ibuprofen (**1.5**) and 2,2,2-trifluoro-1-(9-anthryl)ethanol (Section 4.1) with 1-aminoindane (**1.16**). The method can measure multiple resonances using band-selective or multiple frequency pulses provided the excited nuclei are not *J*-coupled [18].

A method for obtaining pure-shift ^{1}H NMR spectra using homonuclear band-selective decoupling (HOBS) in both the F_1 and F_2 dimensions has been described. The method eliminates overlapping peaks by decoupling sidebands that can compromise other pure-shift methods. HOBS used in this method results in more sensitivity than the PSYCHE method, which uses broadband decoupling. The utility of the method is demonstrated on mixtures of $Eu(tfc)_3$ (Section 9.2) and 1-phenylethylamine (**1.19**) (methine resonance) and menthol (**1.20**) (methine resonance of the isopropyl group). Because only partially resolved resonances are needed, pure-shift measurements allow lower concentrations of $Eu(tfc)_3$ to be used, which reduces broadening in the spectrum [19].

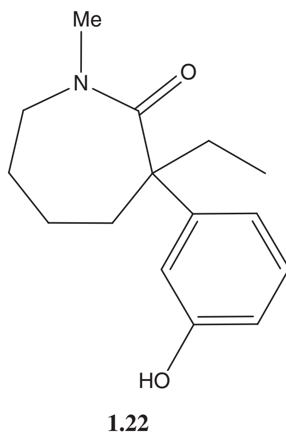


A band-selective homonuclear decoupling experiment in the F_1 and F_2 dimensions that is conceptually similar to the HOBS pulse sequence [19] for obtaining pure-shift spectra has been described. The method is more sensitive than prior HOBS method because it used 90° pulses instead of small angle mixing pulses. The utility of the method is demonstrated on mixtures of 2.2′-dihydroxy-1,1-binaphthalene (Section 4.14.1) with β-butyrolactone (**1.21**), β-cyclodextrin (Section 8.1.2) with ibuprofen (**1.5**), $Eu(tfc)_3$ (Section 9.2) with menthol (**1.20**), and PBLG/chloroform (Section 10.2) with 3-butyn-2-ol (**1.15**) [20].



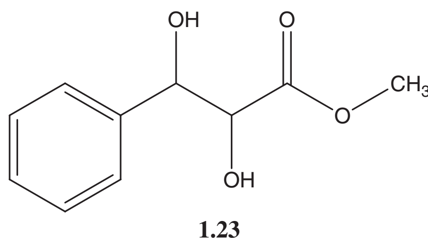
1.21

A method for obtaining pure-shift heteronuclear single quantum correlation spectra that allows for simultaneous ^{1}H and ^{13}C NMR enantiodifferentiation has been reported. The regular ^{1}H and ^{13}C spectra of lactam **1.22** with (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (Section 4.1) in chloroform-*d* are very complex and most signals cannot be differentiated. The method described in this report allows enantiodifferentiation of many more resonances (15 of the 16 proton resonances; 10 of the 11 protonated carbon resonances) in **1.22** [21].



1.22

A procedure for examining isolated scalar-coupled spin systems to improve enantiodifferentiation has been reported. The procedure nullifies the scalar couplings and adds up all the chemical shifts for the protons in the coupled spin systems for each of the enantiomers. Excitation and detection of appropriate highest quantum coherence yields the measureable difference in the frequencies between two transitions, one for each enantiomer. The F_2 cross section at each of the frequencies yields an enantiopure spectrum. The result is to enhance the separation of differentiated peaks. One drawback is that the projections into the multiquantum directions are not as precise, so determinations of enantiopurity are compromised. The utility of the method is demonstrated on mixtures of (18-crown-6)-2,3,11,12-tetracarboxylic acid (Section 8.2.2) with amines and Eu(tfc)$_3$ (Section 9.2) with 1-phenylethylamine (**1.19**) and methyl-2,3-dihydroxy-3-phenyl propionate (**1.23**) [22].



1.23

A one-dimensional TOCSY procedure for selectively observing only a few coupled protons in a complex ^{1}H NMR spectrum has been described. The utility of the method is demonstrated on the α-methoxyphenylacetic acid (MPA) (Section 2.4) derivatives of several secondary alcohols. One or more clear signals are needed for the procedure to work.

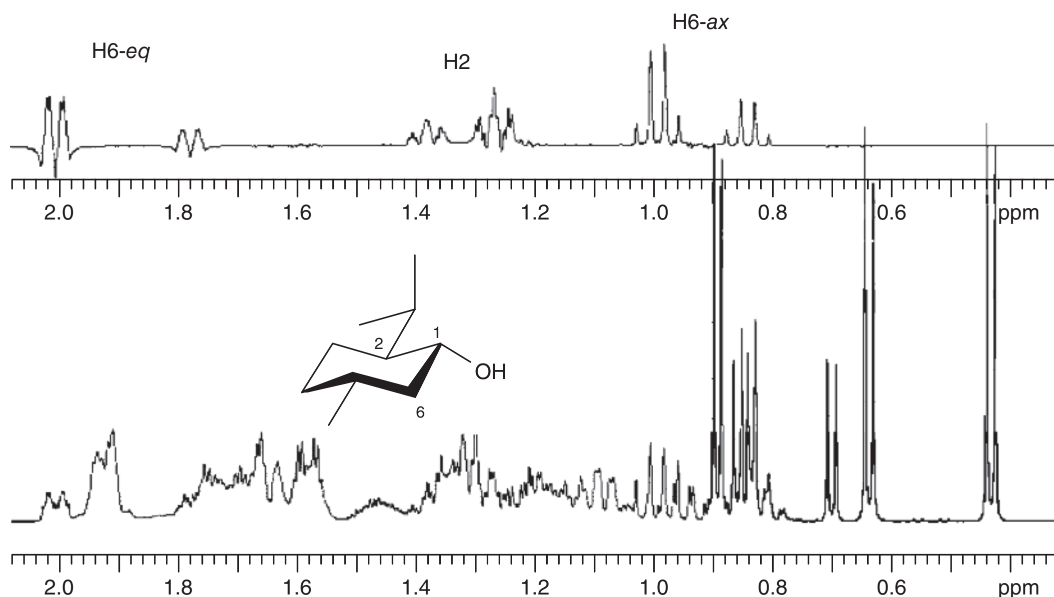
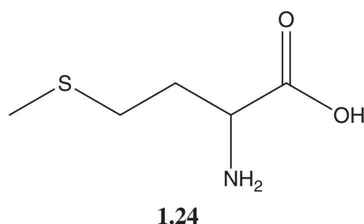


Figure 1.3 ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the menthol derivative with MPA. Bottom spectrum is the complete spectrum. Top spectrum is a TOCSY 1D spectrum with selective excitation of H1 in the MPA esters. (Ref. [23]. Reproduced with permission from American Chemical Society.)

In this report, the α-proton on the MPA moiety is excited with a short mixing time that reveals only the nearby protons in the coupled network. Figure 1.3 shows the simplification that can be achieved in a complex spectrum of the MPA ester of menthol (**1.20**). Lengthening the mixing time brings in more protons in the same coupling network into the spectrum. A one-dimensional nuclear Overhauser effect spectroscopy (NOESY) experiment can be used to observe protons out of the coupling network [23].

NMR diffusion measurements are often used by investigators to study aspects of CSA–analyte mixtures. Standard diffusion measurements are time-consuming, and the experiment must be repeated multiple times with varying gradient strengths or diffusion delays. An ultrafast method for measuring diffusion rates in a single scan has been described. The method relies on an ultrafast pulsed-field-gradient spin echo sequence and shortens the analysis time by one to three orders of magnitude compared to conventional NMR diffusion measurements [24].

The use of dynamic nuclear polarization (DNP) to enhance the sensitivity of ^{13}C signals in chiral recognition studies has been demonstrated. The method is demonstrated on a mixture of (18-crown-6)-2,3,11,12-tetracarboxylic acid (Section 8.2.2) with a ^{13}C-labeled methionine (carbonyl group) (**1.24**). Electron spin polarization is transferred to the ^{13}C label on a frozen sample at 1.4 K. Using a microwave oven, the frozen sample is quickly dissolved in deuterium oxide at room temperature and rapidly transferred to the spectrometer for analysis. Signal can be detected only for the duration of the spin–lattice relaxation time. The method is not yet suitable for practical applications, and the report represents a proof-of-concept for the use of DNP sensitivity gains [25].



1.24

The use of chiral liquid crystals and gels for chiral differentiation often provide highly complex NMR spectra that are challenging to interpret. Sections 10.2.6 and 10.2.9 provide a discussion of additional NMR methods that facilitate the interpretation and assignment of spectra in these partially aligned systems.

1.4. OVERVIEW OF CHIRAL REAGENTS AND METHODOLOGIES

The ensuing chapters describe a diverse variety of CDAs and CSAs that have been developed for use in NMR spectroscopy. Published review articles have described different aspects of CSAs [26, 27], CDAs [28], the use of NMR spectroscopy to assign absolute configurations [29, 30], the use of chiral fluorine-containing reagents for the determination of enantiopurity [31], and the use of NMR spectroscopy for chiral analysis [32–34]. More recently, NMR methods for the assignment of the absolute configuration of sulfoxides [35], hydroxyphosphonates, aminophosphonates [36], and bioactive compounds [37] have been reviewed. A comprehensive review on the use of NMR reagents for assigning the absolute configuration of a wide variety of compound classes with oxygen-, nitrogen-, sulfur-, and phosphorus-containing functionalities has been published [38]. NMR reagents for the chiral recognition of ethers have been reviewed [39]. Other recent review articles have covered general categories of CDAs [40–42], CSAs [40, 42, 43], metal complexes [40, 42], chiral liquid crystals, and other aligning media [40, 42]. The ^{1}H nucleus is most commonly used for spectral analysis in chiral differentiation studies. However, other NMR active nuclei can be used as well. A recent review article describes many studies where ^{19}F, ^{31}P, ^{13}C, and ^{77}Se spectra are used for chiral analysis [44].

A prior edition of this book was published in 2007 [45]. That volume provides a comprehensive overview of the first 40 years of work using NMR spectroscopy for chiral differentiation. This volume is a complement to the first edition, presenting important findings and chiral reagents from the first 40 years of work in this field and integrating in new findings and reagents over the past decade. Many reagents discussed in the first volume that had been applied on a limited basis and that have had no additional studies over the past decade are not included herein.

An important family of reagents described in Chapter 2 are aryl-containing carboxylic acids, the most well-known of which is α-methoxy-α-trifluoromethylphenylacetic acid (MTPA). These are mostly used as CDAs for the assignment of absolute configuration of analytes such as alcohols and amines. Shielding by the aromatic ring of the CDA in the resulting diastereomeric complexes is used to make the assignment. While MTPA is the most well-known of these reagents, as will be discussed in Chapter 2, there are other reagents that are recommended for the analysis of certain classes of compounds.

Chapter 3 describes other carboxylic acids that have been used either as CDAs or as CSAs. Certain of these reagents, such as camphanic acid, have proven to be useful for distinguishing the *pro-R* and *pro-S* positions of α-deuterated primary alcohols. Several reagents based on axially chiral systems are also discussed.

Hydroxy-containing compounds, as described in Chapter 4, have been widely exploited for chiral analysis in NMR spectroscopy. This includes the application of 2-(9-anthryl)-2,2,2-trifluoroethanol, one of the most widely used CSAs ever developed. Shielding by the anthryl group of this reagent can also be used to assign the absolute configuration of certain classes of analytes. Alcohol reagents are also used as CDAs, especially in the analysis of carboxylic acids. Certain diols and glycosides have been used as effective CDAs for ketones and secondary alcohols, respectively. Axially chiral compounds, such as BINOL, have been used as effective CDAs or CSAs with suitable analytes.

Primary, secondary, and tertiary amines have been used as CDAs and CSAs as described in Chapter 5. 1-Phenylethylamine, the first compound ever used as a CSA and 1-(1-naphthyl)ethylamine have been used extensively used to analyze carboxylic acids and other compounds as well. Phenylglycine methyl ester hydrochloride is another important reagent for assigning the absolute configuration of carboxylic acids. Some amine reagents have been exploited as CDAs for the analysis of aldehydes and ketones. Certain diamine reagents have proven to be useful reagents for chiral analysis by NMR spectroscopy.

As described in Chapter 5, chemical shift data measured with the CSAs *N*,α-dimethylbenzylamine and bis-1,3-methylbenzylamine-2-methylpropane have been used to construct ^{13}C and ^{1}H NMR databases for all of the configurations of particular structural motifs. The pattern of the chemical shifts for the known configurations that best matches that of an unknown can be used to determine stereochemistry. The method is especially well suited to the assignment of structural motifs within complex natural products.

Chapter 6 describes a collection of chiral reagents that encompass a variety of compound classes. These include reagents with amide, lactam, aldehyde, ketone, isocyanate, and heterocyclic ring functionalities. Quinine, which has a variety of functional groups that influence its association with a number of compound classes, is a broadly applicable CSA. Many of these other reagents have been studied on a limited basis and apply to specific types of analytes, although some of the reagents are soluble analogs of widely applicable chiral liquid chromatographic phases and are effective with a variety of compound classes. Certain of the reagents described in Chapter 6 are used as CSAs and associate through combinations of dipole–dipole and π–π interactions. Other ketone, aldehyde, and isocyanate reagents are utilized as CDAs for particular classes of analytes.

Reagents specifically designed to incorporate phosphorus, selenium, boron, and silicon atoms are described in Chapter 7. One broadly applicable CSA described in this chapter is *tert*-butylphenylphosphinothioic acid. ^{31}P and ^{77}Se NMR spectra are often measured with the phosphorus and selenium reagents. The singlet resonances and greater dispersion in ^{31}P and ^{77}Se spectra facilitate the analysis of enantiopurity. The majority of the reagents described in this chapter are used as CDAs. In the case of phosphorus, boron, and silicon-containing reagents, the reactions usually involve addition of the analyte at the heteroatom to form diastereomeric complexes. There are some examples where the selenium atom is

incorporated as a spectroscopic probe rather than a reactive center. An important set of chiral cationic and anionic phosphorus-based reagents that form ion pairs with ionic analytes is also described in this chapter. The anionic reagents are especially useful in the analysis of cationic metal complexes, although organic cations can be analyzed as well.

Another versatile strategy for effective chiral recognition, as described in Chapter 8, is through the use of chiral macrocycles and receptor compounds. Cyclodextrins have been the most widely studied family of macrocycles in chiral NMR applications. Cyclodextrins can be derivatized either selectively or randomly at the different hydroxyl groups providing a range of host compounds of varying solubility and chiral recognition properties. In the aggregate, these cyclodextrin derivatives have the potential to function as CDAs for a broad array of analytes.

Crown ethers are another common group of macrocycles that are used with primary amines, although it has been shown that the only commercially available chiral crown ether for NMR studies is also an effective CSA for secondary amines. Calixarenes and resorcinarenes are less studied in NMR applications but offer interesting potential for future development and applications. There are also many specialized macrocyclic and receptor compounds that have been described that exhibit chiral recognition toward a specific class of compounds.

The use of metal complexes for chiral recognition in NMR spectroscopy is an area that has received considerable attention. The importance of paramagnetic lanthanide shift reagents within the entire field of chiral NMR analysis cannot be underemphasized. Although the use of chiral lanthanide shift reagents is mostly described in Chapter 9, the utilization of lanthanide species as a means of enhancing enantiodifferentiation of other NMR reagents is described in other chapters of the book. The utilization of lanthanide shift reagents has diminished as more investigators have obtained access to higher-field NMR spectrometers. One reason is that the enhanced dispersion caused by addition of a paramagnetic lanthanide is often no longer necessary. The other is that the line broadening caused by the paramagnetic ions is more pronounced at higher field strengths.

Chiral reagents based on diamagnetic metal complexes of palladium, platinum, rhodium, and silver have significant applications as well. These metals are especially effective at bonding to soft Lewis bases, thereby broadening the scope of compound classes amenable to chiral analysis by NMR spectroscopy. Among these, a rhodium dimer of α-methoxy-α-trifluoromethylphenylacetic acid is noteworthy. The exceptionally large shielding of analyte nuclei caused by the porphyrin rings of metal complexes of cobalt, zinc, and ruthenium has been exploited in NMR spectroscopy. A number of specialized reagents involving other metal species are described in Chapter 9.

One of the more intriguing developments in recent years, which is described in Chapter 10, involves the use of chiral liquid crystals and gels for chiral NMR differentiation. Chiral liquid crystals and gels undergo partial ordering in an applied magnetic field. A pair of (*R*)- and (*S*)-enantiomers often adopt a different packing order relative to the magnetic field when dissolved in these phases. The different packing order can lead to different dipolar coupling or quadrupolar splitting for the enantiomers, which causes distinct resonances. Since no specific interactions need to occur between the chiral aligning media and analyte, this method is potentially amenable to any chiral analyte, including aliphatic

hydrocarbons. Solid-state NMR studies offer some of the same potential as liquid crystals but have been used to far less of an extent.

Chapter 11 provides a summary of the most noteworthy CSAs and CDAs for chiral NMR studies. Areas where more work in the field of chiral NMR analysis is desirable are described. Finally, recent theoretical work has demonstrated that it is possible to probe the chirality of compounds using only instrumental NMR methods without the need of an enantiopure reagent. Recent work in this interesting area is described.