Disinction of enantiomers by NMR spectroscopy using chiral orienting media

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Abstract | NMR spectroscopy is a very important analytical tool in modern organic and inorganic chemistry. Next to the identification of molecules and their structure determination, it is also used for the distinction of enantiomers and the measurement of enantiomeric purity. This article gives a brief review of the techniques being developed for enantiomeric differentiation by virtue of chiral alignment media and their induction of enantiomerically dependent anisotropic NMR parameters like residual dipolar couplings. An overview of existing chiral alignment media, a brief introduction into the basic theory and measurement of the various anisotropic parameters, and several example applications are given.

1. Introduction

NMR spectroscopy is one of the most important analytical tools in modern organic and inorganic chemistry as it is the only tool that allows the determination of molecular structures at atomic resolution in solution. It is used to identify the constitution, conformation and configuration of countless molecules every day. However, the magnetic field used for the induction of the Zeeman splitting is *per se* achiral so that enantiomers have identical properties and therefore identical NMR spectra. For the distinction of enantiomers with NMR therefore special sample preparation is needed.

Most commonly used are chemical modifications with chiral substances of known chirality which leaves a diastereomeric pair in the end that can be distinguished with conventional NMR spectroscopy. The most popular example is the Mosher-ester and the many related compounds[1]. But the method relies on the existence of an appropriate functional group in the molecule of interest and especially when working with highly valuable compounds one doesn't necessarily want to irreversibly modify the substance.

Another possibility for the distinction of enantiomers is the orientation of the molecule of interest in a so-called chiral alignment medium. In this case, the molecule is partially aligned and anisotropic NMR parameters like residual quadrupolar couplings, residual dipolar couplings and residual chemical shift anisotropy can be measured[2–4]. As the orientation in a chiral alignment medium is different for the two enantiomers, resulting anisotropic parameters differ as well and the determination of enantiomeric purity is possible.

This article provides a brief overview of the existing chiral alignment media and how they can be used to distinguish enantiomers. After an introduction of the various chiral alignment media, individual anisotropic NMR parameters are introduced with the corresponding pulse sequences optimized for the differentiation of enantiomers. In addition, examples for enantiomeric distinction are summarized which demonstrate the width of

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potential applications. We also mention on the side the use of chiral lanthanide shift reagents.

Although we try to give an overview of the state of the art in chiral discrimination using chiral orienting media, the article is by no means complete. The field is progressing rapidly these days and new developments can be expected every day.

2. Chiral orienting media

The first partially aligned spectrum was published by Spence et al. in 1953 [5] and stayed widely unnoticed. Only the famous benzene spectrum in a liquid crystalline phase published by Saupe and Englert in 1963 [6] and the full theoretical explanation of the observed signals one year leater[7] started a whole new and very active field in NMR spectroscopy. Only a few years later, Sackmann first proposed[8] and then experimentally verified[9] the distinction of enantiomers within an optically active liquid crystalline phase.

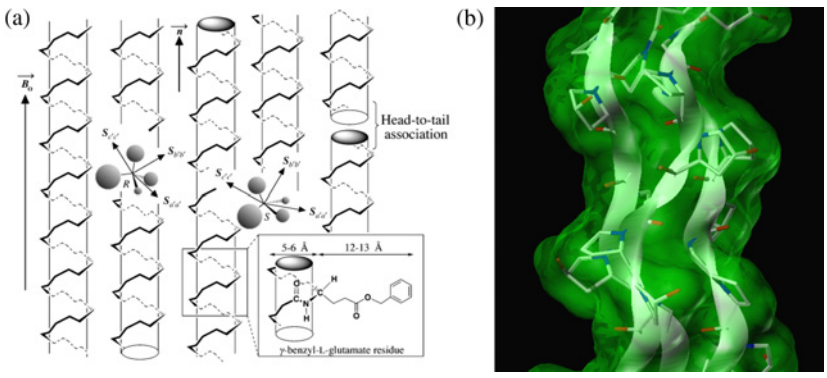
Today three kinds of alignment media are known, liquid crystalline phases, stretched polymer gels and alignment via paramagnetic centers, which all have their distinct behavior as is summarized in the following with the focus on chiral media with the ability to distinguish enantiomers.

2.1. *Chiral liquid crystalline phases*

Liquid crystalline phases were the first alignment media used for chiral distinction and are still the most widely used for organic solvents. First chiral liquid crystalline alignment media for the distinction of enantiomers were a mixture of cholesteryl chloride and cholesteryl myristate[9], a membranuous phase with optically active decyl-2-sulfate[10,11] and poly-γ-benzyl-L-glutamate (PBLG)[12,13]. Lateron further chiral phases were found with several cholesteric solvents[14,15], cesium *N*-dodecanoyl-L-alaninate[16], poly-γ-ethyl-L-glutamate (PELG) and poly-ε-carbobenzyloxy-L-lysin (PCBLL)[17], a lyotropic liquid crystal based on glucopon/buffered water/n-hexanol[18], amino acid-based anionic surfactants[19], and currently developed chiral polyguanidines[20]. While the cholesteric phases generally work with relatively narrow ranges with respect to temperature and solvent applicability, the poly(amino acids) PBLG, PELG, PCBLL and the polyguanidines can be used with most apolar organic NMR solvents like $CDCl_3$, CD_2Cl_2, or THF. In addition, PBLG can also be used with the polar organic solvent DMF[21] and in mixtures with up to 50% DMSO[22].

The most exceptional role for the chiral distinction of enantiomers is certainly held by the lyotropic liquid crystalline phases of PBLG. The first distinction of enantiotopic deuterons with PBLG was reported by Samulski[13] before the Courtieu-group used the same polymer to develop the large variety of tools for the distinction of enantiomers[21,23] (see section 3) which they applied to an even wider range of molecules (for some examples see section 4). Usually PBLG is applied in its L-form, but also the D-form, called PBDG, is available. With the two enantiomers in hand it was proven that a reversal of symmetry of both polymer and solute results in identical anisotropic NMR parameters[24,25]. It could also be shown that a 1:1 mixture of PBLG/PBDG does not result in a split spectrum, but in the corresponding spectrum of an achiral substance that cannot distinguish enantiomers anymore[24,26]. This is clear evidence that the effect of the alignment medium is highly averaged over time. The structure of PBLG is also quite well characterized to have an α-helical secondary fold (see Figure 1A), which matches the current opinion that a chiral secondary fold is

Figure 1: Helical secondary structures of PBLG and gelatin. (A) Ordered α-helices of the liquid crystalline phase of PBLG[21]; (B) the triple helix of gelatin.



needed to induce sufficiently different alignment properties to a solute molecule to be able to distinguish them.

Liquid crystalline alignment media, however, also have profound disadvantages, the most important of them being the existence of a lower limit of alignment. As could be shown recently[27], this limit correlates with the persistence length of the polymer chain used and the general rule applies that the longer the individual chains of the polymer are, the lower is the achievable lower limit of alignment. Only with special techniques like variable angle sample spinning (VASS)[28–30], which imply the use of specialized hardware, the alignment of solutes can be further reduced. Because of this limitation, most studies in liquid crystalline phases are limited to relatively small molecules and by using insensitive detection methods like natural abundance deuterium (NAD) detection (section 3.2).

Besides PBLG only PELG and PCBLL are frequently used as chiral liquid crystalline alignment media today. With the current development of chiral polyguanidines, however, there seems to be another, very cheap liquid crystal with similar properties when it comes to enantiomeric distinction.

It should be noted that the distinction of enantiomers can also be achieved with achiral liquid crystalline phases if a chiral additive is given to the sample. Pechine et al. showed, for example, that cyclodextrines can serve as chiral cages to differentiate the enantiomers of a racemic mixture of 1-deutero-1-phenylethanol[31].

2.2. *Stretched chiral polymer gels*

The measurement of anisotropic NMR parameters in stretched polymer gels has been introduced 1981 by Deloche and Samulski[32], but its primary use has been in the field of polymer science. This changed with the work of Tycko et al.[33] and Sass et al.[34] who introduced stretched polyacrylamide as alignment medium for biomacromolecules. Gels for the alignment with organic solvents have first been introduced in 2004 for apolar solvents like $CDCl_3$ and CD_2Cl_2 [35,36] and in 2005 for DMSO and other polar organic solvents[37–39]. The same year conventional gelatin was found to be the first chiral medium capable to distinguish enantiomers in pure water[40,41]. Several studies on enantiomers followed with the underlying polymer collagen[42] and gelatin in combination with a specially designed stretching apparatus [43–45] that allows to rapidly and freely scale the alignment to a desired strength[4,44,46].

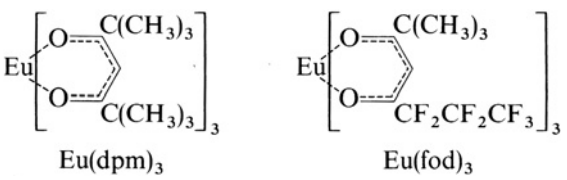
Gelatin and collagen form triple helices as a chiral secondary structure (Figure 1B) which apparently results in a stereospecific alignment of chiral molecules comparable to the α-helix of PBLG and other poly(amino acids). As with all stretched polymer gels no lower limit for the alignment strength applies which results in a higher flexibility of applicable pulse sequences and the more general use of dipolar couplings for the distinction of enantiomers. An extension to conventional gelatin was recently published with gelatin cross-linked by accelerated electrons, so-called e^--gelatin, which allows enantiomeric distinction also at temperatures above the melting temperature of conventional gelatin and with pure DMSO as the solvent [47]. A second water-compatible gel-based alignment medium was also introduced in 2009 with various types of carrageenan[48] as polysaccharide-based polymers with similar properties to gelatin.

The development of further polymer gel alignment media is in full swing right now and additional media can be expected soon for DMSO and aqueous solutions (M. Schmidt and C. Griesinger, unpublished results) and also apolar organic solvents (A. Krupp and M. Reggelin, unpublished results).

2.3. *Paramagnetic alignment*

The same year when anisotropic NMR parameters were first measured in stretched polymer gels it was also discovered by A. Bothner-By that paramagnetic compounds self-align in the magnetic field due to the strong magnetic moment of the paramagnetic centers[49]. In biomolecular NMR spectroscopy this effect is now used to induce residual dipolar couplings in proteins with so-called paramagnetic tags[50]. For the distinction of enantiomers, however, this approach is of no use since the alignment is too weak and would imply a chemical modification as with the Mosher-ester method. But there is a second effect that can be exploited for small organic compounds, the so-called contact and pseudocontact shifts with lanthanide shift reagents. Based on the seminal paper of Hinckley in 1969 [51], many different compounds have been designed to analyse stereochemical centers with the two most prominent being $Eu(dpm)_3$ (dpm = tris-dipivaloylmethanato)

Figure 2: The two most widely used lanthanide shift reagents.



$Eu(dpm)_3$ $Eu(fod)_3$

Figure 3: Coordinate systems. (A) In the laboratory frame spanned by x,y,z the static B_0-field always points along z and the solute is tumbling in space; (B) in an arbitrary reference frame of a rigid molecule a,b,c the magnetic field varies its direction due to molecular tumbling while the solute is fixed.

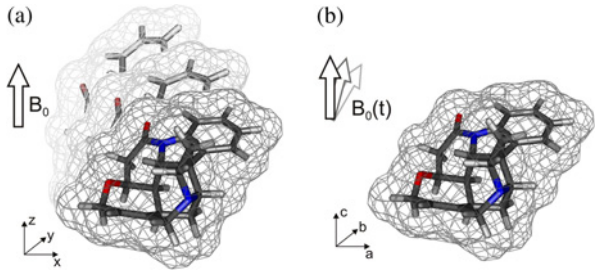
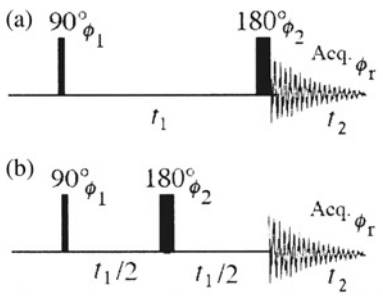


Figure 4: The Q-COSY (A) and Q-resolved (B) 2D pulse sequences for most efficient enantiomeric discrimination using deuterium quadrupolar couplings. For phase cycling and further experimental details the reader is referred to[55].



and Eu(fod)$_3$ (fod = tris-6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctanetonato) (Figure 2). The shift reagents need a functional group within the solute of interest to from a diastereomeric complex in fast exchange. The induced chemical shift changes are then different for enantiomers of the solute. Lanthanide shift reagents are not the topic of this article and we refer the more interested reader to[52] for comprehensive reading.

3. Distinction of enantiomers

The distinction of enantiomers in chiral orienting media is achieved by using the small differences in anisotropic NMR-parameters for enantiomeric resolution. After a very brief introduction of the concept of the alignment tensor, all three anisotropic parameters of diamagnetic molecules are discussed with the corresponding pulse sequences for an effective resolution of signals in the following.

3.1. *Averaging effects on partially aligned samples*

While solid state spectra contain the full anisotropic interactions, they are completely averaged out in isotropic samples. Partially aligned samples, on the other hand, have a more complex averaging behavior, which can most easily be described by the concept of a probability tensor or the more widely used alignment tensor, respectively.

For this concept it is necessary to leave the laboratory frame of reference in which the static magnetic field of the NMR spectrometer is fixed along the z-axis and the molecule is tumbling in space (Figure 3A). Instead, it is useful to go into an arbitrary frame of reference of the (rigid) molecule itself. In this reference frame with the cartesian axes named a,b,c the molecule is fixed in space while the magnetic field vector is changing its position (Figure 3B). The distribution of the magnetic field vector can then be described by the so-called probability tensor $\mathbf{P}$[53], which mathematically is represented by a (3×3) matrix with the time and ensemble averaged probabilities $P_{\alpha\beta}=\langle\cos\vartheta_\alpha\cos\vartheta_\beta\rangle$ with $\alpha,\beta=(a,b,c)$ to find the magnetic field at angles ϑ_a, ϑ_b, ϑ_c relative to the reference frame of the molecule. Because of the symmetry of the probability tensor along the diagonal and the condition for the probability distribution the condition $P_{aa}+P_{bb}+P_{cc}=1$ is always fulfilled. If the molecule tumbles freely in an isotropic solution, the probability tensor is furthermore represented by a diagonal matrix with the diagonal elements $P_{\alpha\alpha}=1/3$.

As one is usually only interested in the anisotropic contribution to a corresponding NMR parameter, it is more convenient to define the alignment tensor according to

$$\mathbf{A}=\mathbf{P}-1/3\ \mathbf{1}.$$

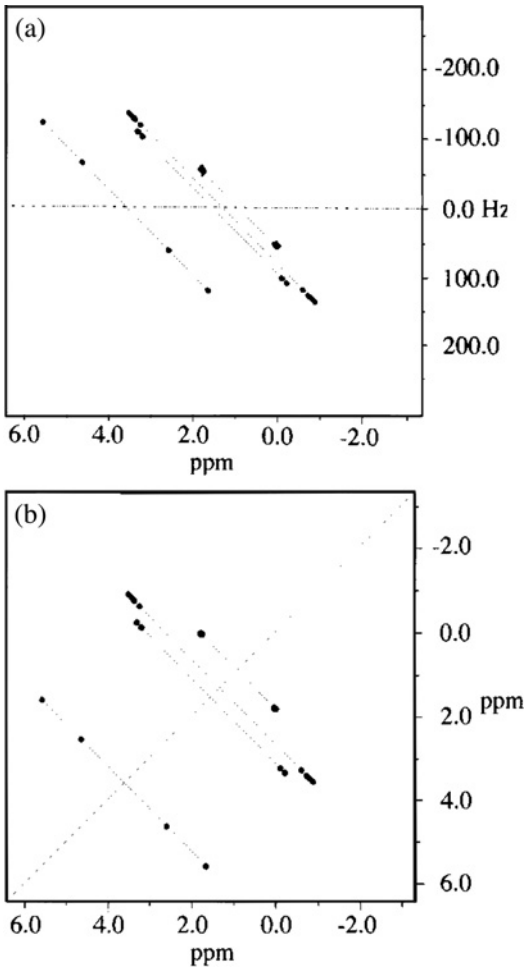
The alignment tensor has a vanishing trace $A_{aa}+A_{bb}+A_{cc}=0$. As will be seen in the following sections, the alignment tensor can directly be used to calculate the size of anisotropic NMR parameters in partially aligned samples. It should be noted that also the Saupe order matrix $\mathbf{S}$ is frequently used for the description of averaging effects which is related to the alignment tensor by $\mathbf{S}=3/2\ \mathbf{A}$.

If the alignment medium is chiral, two enantiomers, for simplicity referred to as the R- and S-form of a molecule in the following, will orient differently with the resulting individual alignment tensors $\mathbf{A}^R$ and $\mathbf{A}^S$.

3.2. *Residual quadrupolar couplings (RQCs)*

For the distinction of enantiomers with residual quadrupolar couplings a nuclear spin ≥ 1 with sufficiently small quadrupolar moment is needed, which narrows the applicable nuclei down to deuterium. Deuterium, on the other hand, is

Figure 5: Application of the Q-COSY (A) and Q-resolved (B) experiments from Figure 4 to 1-pentanol-d_{12} in PBLG/$CDCl_3$ [55].



available in practically all organic compounds, although at the very low natural abundance of ≈ 0.016 %. The measurement of RQC is therefore limited to at least partially deuterated molecules or to molecules for which a relatively large amount of sample is available.

A clear advantage of deuterium detection is the simple multiplet structure of resulting spectra, which makes them easy to analyze. If coupled protons are decoupled, natural abundance deuterium (NAD) spectra are doublets only split by the desired quadrupolar coupling.

Quadrupolar couplings occur because of the electric field gradient (EFG) induced by neighboring atoms which causes the quadrupolar moment to orient. In deuterium with only a single bound neighboring atom this EFG can be considered to be axially symmetric and equations simplify significantly. A detailed description of the theory of the quadrupolar coupling can be found e.g. in[54]. In the end, a resulting splitting $\Delta\nu_Q$ in the deuterium spectrum due to the quadrupolar coupling can be described by

$$\Delta\nu_Q = C_D \sum_{\alpha,\beta=a,b,c} A_{\alpha\beta} r_\alpha r_\beta \text{ with } C_D = \frac{eQ_D V_D}{h}$$

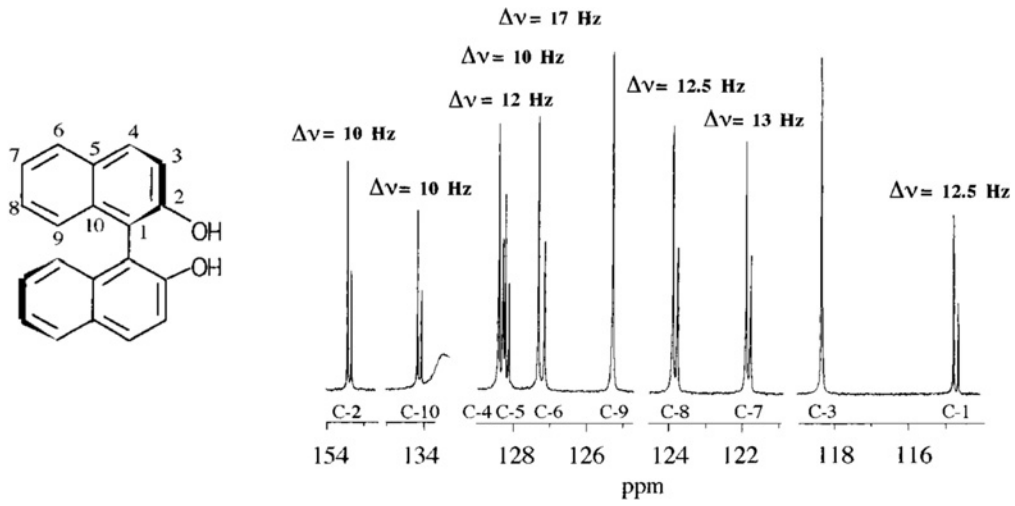
with the deuterium quadrupolar coupling constant C_D, the alignment tensor $\mathbf{A} = A_{\alpha\beta}$, the unity vector $\mathbf{r} = (r_a, r_b, r_c)$ in the direction from the deuterium to the directly bound atom in the frame of reference of the molecule, the deuterium electric quadrupolar moment eQ_D, the principal component of the EFG tensor V_D, and Planck's constant h. Typical values for the quadrupolar coupling C_D for carbon-bound deuteriums are 170 ± 5 kHz, 185 ± 5 kHz, and 210 ± 5 kHz for sp^3, sp^2, and sp hybridized carbons, respectively. The quadrupolar coupling therefore is quite strong, making it a very sensitive sensor to enantiomeric differences in alignment. It should be noted that the averaging according to the alignment tensor always implies for any anisotropic interaction that the measurable quantity, i.e. the splitting $\Delta\nu_Q$, can have both positive and negative sign and might even vanish completely. Since the alignment tensor $\mathbf{A}^R$ and $\mathbf{A}^S$ are generally different for two enantiomers in a chiral alignment medium, the resulting relative sizes of $\Delta\nu_Q^{R/S}$ can be used to differentiate them.

Regarding the actual acquisition of spectra, the most simple experiment for the detection of RQCs is a proton-decoupled deuterium 1D-spectrum. But the multitude of signals observed in some compounds makes it necessary to also consider 2D experiment. In Figure 4 the most useful experiments developed by the group of scientists around J. Courtieu are shown, the Q-COSY and Q-resolved experiments[55]. In the Q-COSY experiment resulting spectra have the quadrupolar doublet spanned along the antidiagonal and the chemical shift resolution along the diagonal. The Q-resolved experiment is equivalent to classical J-spectra in isotropic samples with the quadrupolar splitting in the indirect dimension and the chemical shift in the direct dimension. Corresponding examples are given in Figure 5.

3.3. *Residual chemical shift anisotropy (RCSA)*

Signals of isotropic liquid samples can be characterized by the isotropic chemical shift. In general, however, the chemical shift is a tensor $\sigma = \sigma_{\alpha\beta}$, which can be divided into an isotropic scalar σ^{iso} and the chemical shift anisotropy $\sigma_{\alpha\beta}^{\text{aniso}}$. With the effective averaging the chemical shift value

Figure 6: Distinction of the enantiomeric forms of 1,1′-bi-2-naphthol (see scheme to the left) in PBLG/DMF-d_7 using a ^{1}H-decoupled ^{13}C-1D-spectrum and differences in chemical shifts ($\Delta\nu$ indicated at each pair of signals). The weight in ee was 31% and integration determined 29.5% ee[57].



δ observed in a partially aligned sample can then be deduced to be

$$\delta = \sum_{\alpha,\beta=a,b,c} \sigma_{\alpha\beta} A_{\alpha\beta} = \sigma^{\text{iso}} + \sum_{\alpha,\beta=a,b,c} \sigma^{\text{aniso}}_{\alpha\beta} A_{\alpha\beta}.$$

Again the different alignment tensors $\mathbf{A}^R$ and $\mathbf{A}^S$ for the two enantiomers in a chiral alignment medium will lead to a different averaging and different observed averaged chemical shifts δ^R and δ^S. Nevertheless, the chemical shift anisotropy varies strongly among different nuclei and chemical surroundings and the discrimination of enantiomers is only possible in favorable cases.

The measurement of chemical shifts is trivial and can be done in any kind of spectrum with one or more chemical shift evolution periods. One of the most effective ways to measure enantiomeric excess is a proton-decoupled ^{13}C-1D spectrum (Figure 6)[56–58]. Due to the observed singlets with typically very narrow linewidth the discrimination power in this case is quite good for carbons with larger chemical shift anisotropy as e.g. aromatic, carbonyl, or olefinic carbons. Other nuclei with a generally high chemical shift anisotropy are ^{15}N, ^{31}P, and ^{19}F[59]. Whenever these nuclei are present in enantiomers, there is a good chance to be able to measure enantiomeric excess by simple integration of chemical shift separated signals. Protons with relative small chemical shift anisotropies and typically broad multiplets are usually not suited for enantiomeric discrimination via chemical shift differences.

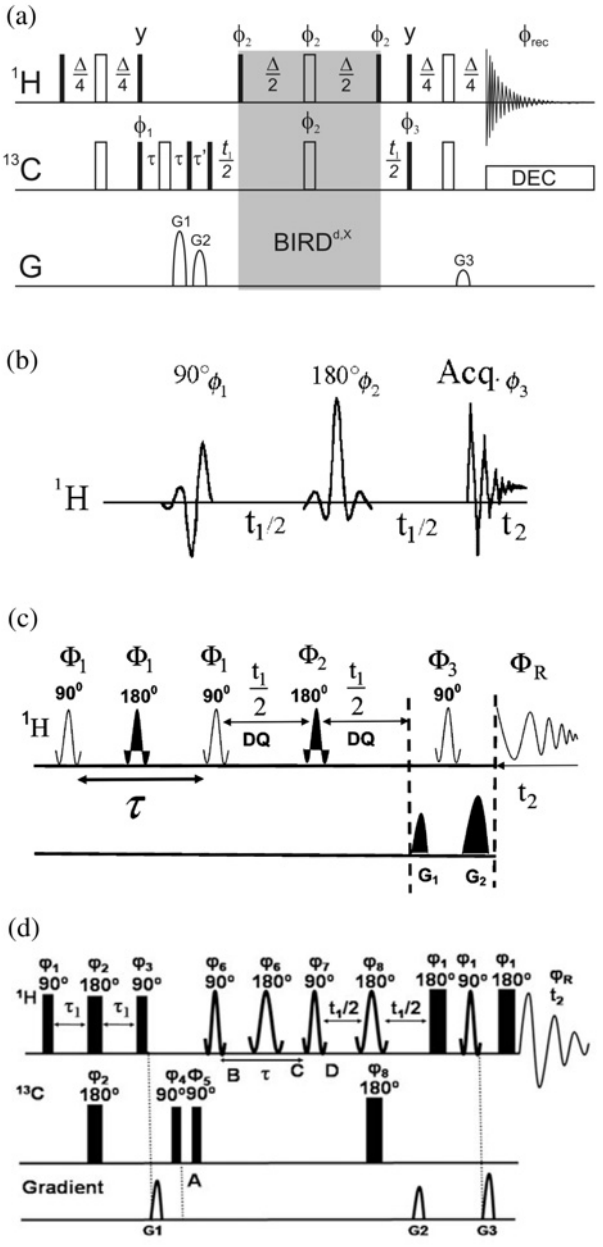
3.4. *Residual dipolar couplings (RDCs)*

Dipolar couplings are always active between two NMR-active nuclei close in space. As a spin can be seen as a classical magnet itself, its magnetic field adds up to the static B_0-field and leads to a change of the resonance frequencies of surrounding spins. Depending on whether the spin is oriented up or down, the corresponding contribution to the magnetic field is either additive or subtractive. Since up and down nuclear spins are almost equally populated, this leads to a split signal like in the case of scalar coupled spins. The size of the residual dipolar coupling is determined by

$$D_{IS} = d_{IS} \sum_{\alpha,\beta=a,b,c} A_{\alpha\beta} r_\alpha r_\beta \text{ with } d_{IS} = -\frac{\mu_0 \gamma_I \gamma_S \hbar}{8\pi^2 r^3}$$

with the dipole-dipole coupling constant d_{IS}, the alignment tensor $A_{\alpha\beta}$, the unity vector $\mathbf{r} = (r_a, r_b, r_c)$ along the vector between the dipolar coupled spins in the frame of reference of the molecule, the vacuum permeability μ_0, the gyromagntic ratios of the coupled nuclei γ_I and γ_S, the distance between the two coupled spins r, and the Planck's constant divided by 2π. The definition for the dipole-dipole coupling also varies in the literature by a factor 2 (e.g.[60–62]) or $1/2\pi$ (e.g. [63]). With the definition chosen here, the effective splitting observed in spectra corresponds to $2D_{IS}$ in the heteronuclear case and between $2D_{IS}$ to $3D_{IS}$ for homonuclear dipolar coupled spins, depending on their chemical shift differences. The residual dipolar coupling of a proton to its directly bound

Figure 7: Experiments for enantiomeric distinction based on residual dipolar couplings. (A) 2D-version of a JE-BIRD^{d,X}-HSQC experiment[40,41,69]; (B) the selective 2D-SERF experiment using shaped excitation and refocussing pulses[71]; (C) the selective DQ-SERF experiment[77] which can also be applied with higher order multiple quantum filtering[78]; (D) the corresponding CH-DQ-SERF experiment for filtering of heteronuclear double quantum[81]. For phase cycling and further experimental details we refer the reader to the references specified.



carbon, for example, is roughly $^1D_{CH} = 23$ kHz and therefore only about one order of magnitude smaller than the deuterium quadrupolar coupling. As before for RQCs and RCSA, a difference in the alignment tensor for the R- and S-form of an enantiomer will result in different RDCs.

When we look at the practical applicability of RDCs for enantiomeric distinction then RDCs seem to be the best compromise between the available sensitivity and the size of the anisotropic parameter. The experimental difficulty in this case is mainly determined by the multiplet widths of corresponding signals as decoupling is not as easily possible as in the case for measuring RQCs.

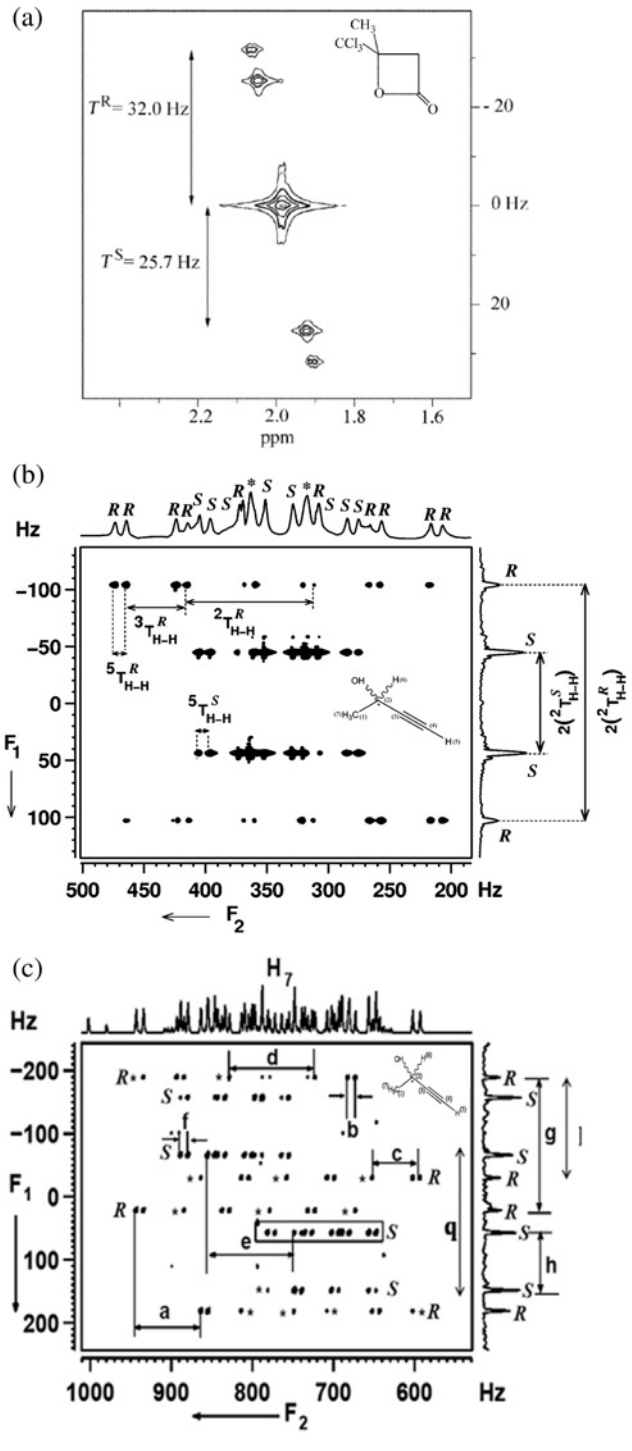
The most easily accessible RDCs are typically $^1D_{CH}$ couplings, which usually are acquired in HSQC or HMQC-type experiments like the CLIP-HSQC[64]. However, because of the necessity for the least possible signal widths for signal separation, more specialized methods have been developed. One class of pulse sequences relies on the introduction of a BIRD^{d,X}-filter[65–67] during an indirect evolution period. 2D methods have been introduced in[40,41] (see also Figure 7A) and generalized in[68]. A 3D-version for maximum coupling and chemical shift resolution has been developed for the measurement of $^1D_{NH}$-RDCs in proteins[69]. The corresponding experiments are the standard experiments in our laboratory, but they have the disadvantage that BIRD-filters only work for a narrow distribution of heteronuclear coupling constants and are therefore more applicable to gel-based chiral alignment media with no lower limit of alignment. An example for an application can be found in Figure 9.

A second class of pulse sequences uses selective pulses for effective decoupling. The methods have been recently summarized and we refer the interested reader to this article[70]. In Figure 7B, a simple ^{1}H selective refocusing experiment (SERF)[71,72] is shown with the corresponding example in Figure 8A.

Also homonuclear Lee-Goldberg-type decoupling schemes have been successfully exploited for its use in aligned samples[73–75].

A fourth class of high potential has recently been exploited by Suryaprakash in a long series of publications which uses multidimensional multiple quantum (MQ) filters for multiplet reduction. Homonuclear[76–78] as well as combined homo- and heteronuclear[79–81] MQ-filters can be used to dramatically reduce the multiplet components and allow the sign-sensitive measurement of RDCs with high accuracy. The experiments can generally be applied in a broadband[76,82,83] or frequency-selective fashion[77–80,84–88]. Because of the tilted spread of most multiplet components along the antidiagonal, signals corresponding to a single enantiomer can relatively easily be assigned in an enantiomeric mixture. In Figure 7C, D two example experiments are shown with the corresponding example spectra in Figure 8B, C.

Figure 8: Experimental examples for the 2D-SERF[71] (Figure 7B), the DQ-SERF[77] (Figure 7C), and the CH-DQ-SERF[81] (Figure 7D) experiments. Corresponding example molecules are given as small inlets. For further information regarding the indicated coupling constants the reader is referred to the cited references.



4. Applications

The applications of chiral alignment media for the distinction of enantiomers are plentiful. The majority of publications in this field were published by the group of scientists around Jacques Courtieu, who deserves special credit for the rich development of this field. Next to conventional enantiomeric distinction with RQCs[21,23,89–93], RDCs[25,40,41,43,45,47,56], and RCSA[21,56–59], there are a number of special symmetries and molecular classes that have been looked at. For example molecules with axial chirality[94], planar chirality[95], three-fold symmetries[96], rotational isomerism[97], invertomers[98], unlike/like stereoisomers[99], enantiotopic differentiation in molecules with C_s and C_{2v} symmetry[100], secondary alcohols[101], and even chiral alkanes[102] have been successfully studied.

An example for the distinction of enantiomers using chiral polymer-based alignment media is shown in Figure 9: The distinction of a mixture of L-alanine/D-alanine in gelatin and e^--gelatin. The alanine mixture by now has become the standard to characterize the ability to distinguish enantiomers in aqueous solutions by the use of RDCs. With strong alignment a simple 1D-experiment is sufficient to differentiate the two enantiomers and to measure enantiomeric excess by simple integration of the corresponding subsets of signals in this case with only few protons[42,43,45,47,48]. For molecules with more coupling partners, the multiplet width of the signals of interest would be broadened so much that a clean integration would not be possible. A more widely applicable way of distinguishing the two compounds with much lower alignment strength is the J-BIRD^{d,X}-HSQC introduced in Figure 7A, which reduces the multiplet width to only proton-proton two-bond couplings within CH_2 or CH_3 moieties. The resulting resolution in the indirect J-dimension can then be used to separate the two species independent of the multiplet width in the direct dimension. With e^--gelatin this way enantiomeric discrimination is possible up to 55°C. The decrease in resolution with increasing temperature is expected since both the polymer network and the solute molecule become more flexible and the weak binding events of the solute to the polymer get less specific.

An example showing the extreme power of enantiomeric differentiation by chiral alignment is presented in Figure 10 with the distinction of two enantiomeric chiral alkanes[102]. Because of the absence of a functional group the task would not be possible with any other NMR-based method. The chiral alkane possesses 10 proton/deuterium sites which, in a chiral alignment medium, will result in

Figure 9: The distinction of a mixture of L-alanine/D-alanine using stretched gelatin (A) and e⁻-gelatin (B–E). (A) With the JE-BIRD^{d,X}-HSQC experiment enantiomers can clearly be distinguished by the corresponding $^1D_{CH}$ couplings within the methyl groups. While weight in ee was 9%, the measured result was 7% ±3%[40,41]. (B–E) In e⁻-gelatin the distinction of enantiomers is possible up to approximately 60°C[47].

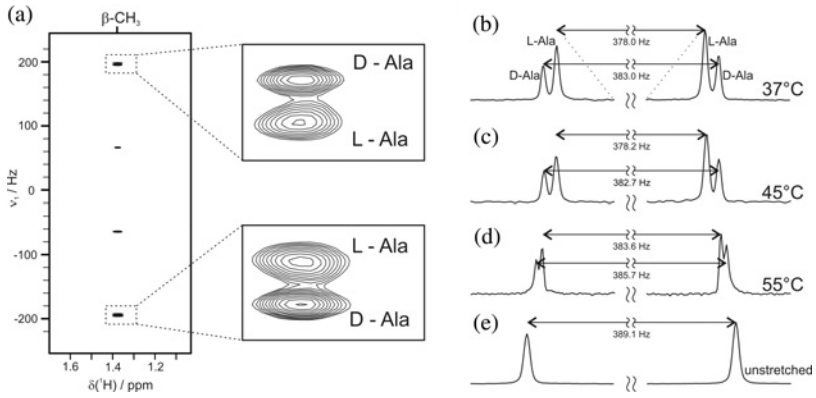
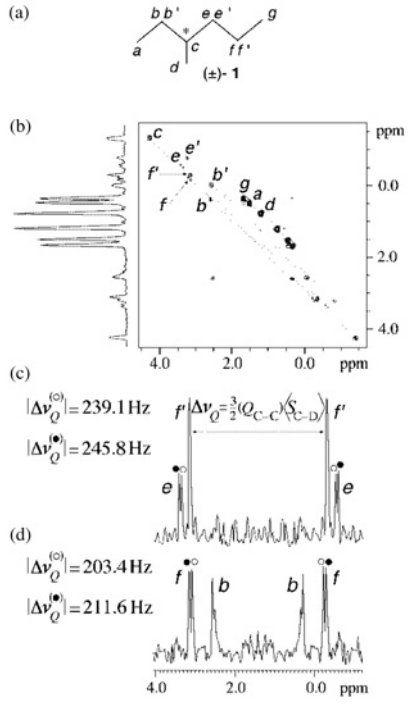


Figure 10: The distinction of a chiral alkane in PBLG/$CDCl_3$ [102]. The racemic mixture of compound (A) altogether shows 12 doublets in the Q-COSY spectrum for the ten hydrogens (B). Traces through deuterons e (C) and f (D) clearly indicate the distinction of the two enantiomeric species.

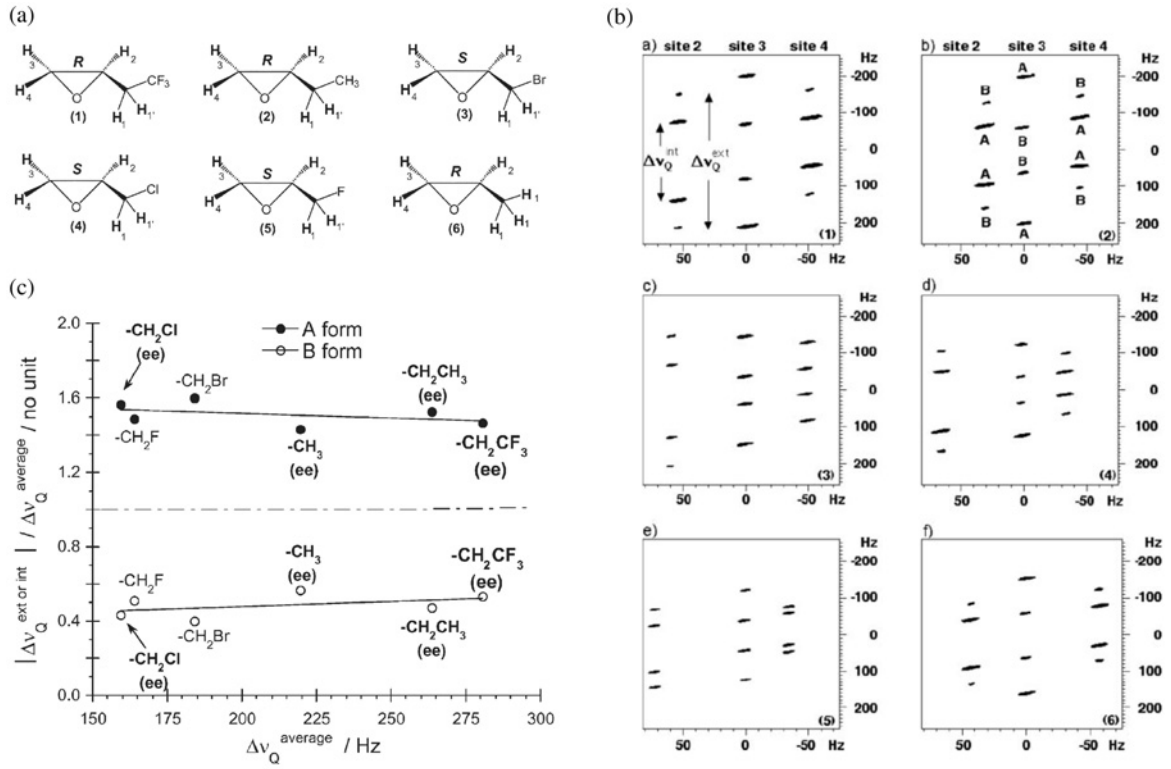


up to 20 multiplets. While no distinction would be possible in proton spectra with the large number of proton-proton couplings present in this case, the natural abundance deuterium (NAD) spectrum will lead to 20 doublets only. In the corresponding NAD-1D experiment, altogether 12 distinguishable doublets are observed. The signals, however, can only be assigned by the acquisition of a NAD Q-COSY. Traces along the antidiagonal of the Q-COSY clearly reveal differences for the e and f positions of approximately 6 and 8 Hz, respectively. These differences are so small that they most likely can only be seen with the NAD-approach.

If enantiomers can be distinguished by RDCs, the determination of absolute chirality should also be possible in principle. A general approach for this task has not yet been derived, but two examples using the conformational difference of enantiomers bound to the chiral alignment medium[103] and the comparison of experimental multiplet patterns with similar compounds of known chirality[104] were successful in determining the correct absolute chirality.

The summary of the empirical method for the determination of absolute chirality is as follows: the trifluoromethylated compound (1) in Figure 11A was synthesized in both racemic and enantiopure series, but the absolute configuration of the enantiopure series could not clearly be established from the synthetic pathway. On the other hand, compounds (2)–(6) of Figure 11A are commercially available in racemic or enantio-enriched mixtures. After the acquisition of NAD Q-COSY experiments for the whole series (Figure 11B), it turns out that all compounds have a very similar pattern for the enantiomers as indicated in one spectrum by the letters A and B. Plotting the

Figure 11: Determination of absolute chirality of an epoxide by comparison of deuterium quadrupolar couplings with similar compounds of known chirality[104]. (A) A-forms of the 6 enantiomeric pairs used in the study, from which the absolute chirality shall be determined for compound (1). Form B corresponds to the case where the substituent is behind the epoxide cycle. (B) The six corresponding natural abundance deuterium Q-COSY experiments with racemic mixtures of compounds (3) and (5) and A-form-enriched mixtures for (2),(4),(6). (C) The comparison of the normalized ratios of quadrupolar splittings clearly distinguishes A- from B-form and allows the secure determination of absolute chirality.



deuterium splittings divided by the average splittings of all compounds in a single graph clearly reveals the absolute configuration of the compound of question.

5. Conclusions

Chiral alignment media are now available for almost any kind of solvent, either as liquid crystalline phases or as stretched polymer gels. As the average orientation of two enantiomers differs if they are dissolved in such a chiral medium, the measurement of anisotropic NMR parameters like residual quadrupolar couplings, residual dipolar couplings or residual chemical shift anisotropies leads to spectra in which enantiomers can be distinguished and enantiomeric excess can be derived. The approach can be driven to such an extreme that fluoroalkanes and even chiral alkanes can be distinguished which is barely possible with any other technique. It was also shown that the absolute configuration can be determined by this approach if sufficiently similar molecules of known chirality are available.

The field currently is developing very fast and a number of new, improved or at least differently orienting, alignment media will be available during the next years and the measurement of enantiomeric excess will be widely applicable to most small to medium-sized organic molecules. Available pulse sequences with e.g. BIRD- or multiple quantum filters allow the distinction of enantiomers even in cases with very complex signals. At the same time computer methods for predicting alignment are progressing steadily and it must be assumed that in several years from now the determination of absolute chirality based on the differential alignment in an R- or S-configured alignment medium will be feasible in many cases.

In essence the chiral distinction of enantiomers using NMR spectroscopy in chiral orienting media is a non-trivial but powerful method for organic and inorganic chemists with which in particular ee

can be determined without chemical modification. With the improvements expected during the coming years it will soon become a standardized method applicable in most NMR analytic centers.

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