Determining Enantiomeric Purity by NMR:

Deuterium 2D NMR at Natural Abundance in Weakly Oriented Chiral Liquid Crystals

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Introduction

The NMR spectra of chiral solutes in chiral liquid crystalline solvents have an extremely rich information content. First, anisotropic observables not detectable in the spectra of isotropic samples can be detected, e.g., the chemical shift anisotropy, ($\Delta\sigma_i$), the spin-spin coupling anisotropy (ΔT_{ij}), and the quadrupolar splitting ($\Delta\nu_{Qi}$) for nuclei with spin $I > 1/2$, which is averaged to zero in the liquid state [*1*]. Second, in a chiral environment enantiomers may give different NMR spectra from which the enantiomeric composition can be obtained by peak integration. This situation arises when differences in the enantioselective interactions between the *S* and *R* isomers and the chiral liquid crystalline phase result in sufficient differential ordering effects (DOE) so that the isomers can be discriminated by their order-sensitive NMR observables. Consequently, NMR spectroscopy in chiral liquid crystal solvents has important potential in the field of enantiomeric analysis. To facilitate this alternative approach, when analytical NMR in an isotropic medium fails to provide the desired information, we have developed several NMR methods based on the use of weakly oriented, chiral liquid crystals made by dissolving a synthetic polypeptide, poly-γ-benzyl-L-glutamate (PBLG), in an organic solvent such as $CHCl_3$, DMF, or THF [*2-3*].

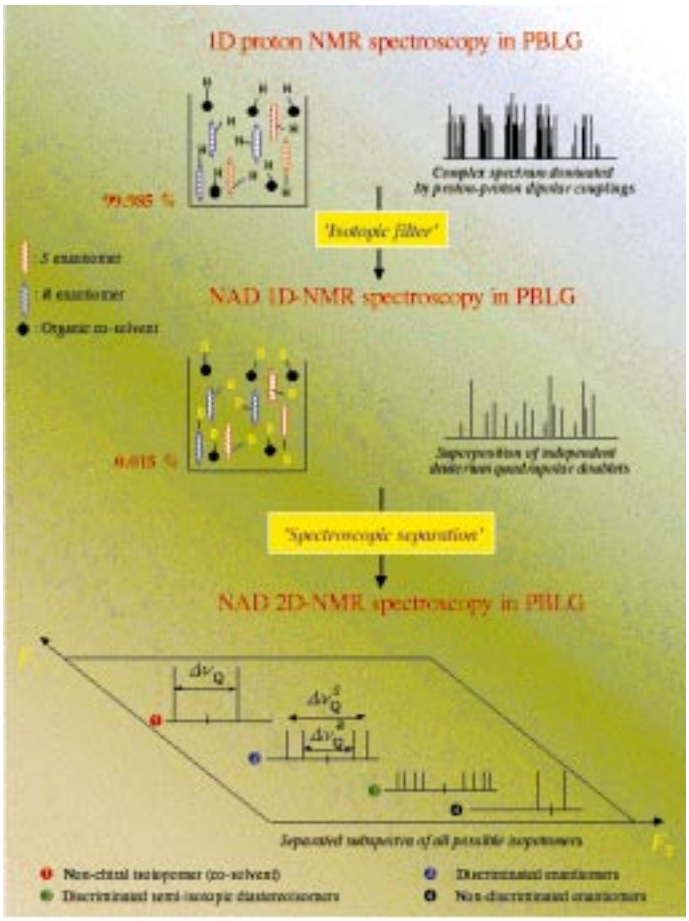

Fig. 1: *Principle of enantiomeric discrimination using natural-abundance deuterium (NAD) NMR spectroscopy in a chiral liquid crystal environment.*

A rough classification of the sensitivity of the various anisotropic interactions towards the DOE of enantiomers indicates that $|\Delta\nu_{Qi}| > |\Delta T_{ij}| > |\Delta\sigma_i|$. Consequently in the earliest studies, we focused on chiral discrimination using deuterated materials. Although numerous synthetic methods for selective deuteration are available, it is clear that this may not always be possible, desirable, or easy to do. However, we have shown recently that it is possible to routinely record natural-abundance deuterium (NAD) NMR spectra of enantiomers with satisfactory quality on DRX 400 or AM 250 spectrometers equipped with a 5-mm broadband inverse (BBI) probehead [*4,5*]. Consequently, it appeared to us that the enantiomeric differentiation of chiral compounds using NAD-NMR is quite feasible with standard NMR equipment, despite the very low sensitivity of deuterium in natural abundance.

We present here three specific examples illustrating the latest developments and applications of this promising approach. In particular, we will demonstrate that unsaturated and saturated chiral hydrocarbons can be differentiated using NAD NMR in PBLG. For optimal performance this work was carried out with a DRX 400 spectrometer equipped with a high-resolution selective deuterium probehead and fluorine lock channel, designed and built by Bruker SA to explore the analytical potential of the technique.

Enantiomer discrimination using NAD-NMR in PBLG

Background

Theoretically, the proton-decoupled deuterium NMR spectrum of two monodeuterated enantiomers which orient differently in a chiral ordered environment, such as a PBLG liquid crystalline phase, should show two quadrupolar doublets. For the *S* enantiomer the separation between the peaks of a doublet for a given deuterium D_i, the quadrupolar splitting in Hertz, is given by Eqs. 1 and 2. For the *R* enantiomer the same equations apply with the superscript *S* replaced by *R*.

$$\Delta\nu^{S}_{Qi} = (3/2)\, K_{C\text{-}D}\, S^{S}_{C\text{-}Di} \qquad (1)$$

$$K_{C\text{-}D} = [e^2 Q_{Di}\, q_{C\text{-}Di} / h] \qquad (2)$$

The term $K_{C\text{-}D}$ is the deuterium quadrupolar coupling constant, and it is assumed that the quadrupole tensor is axially symmetric about the C-D bond. $K_{C\text{-}D}$ varies between 170 to 210 kHz, depending on the hybridization state of the carbon bonded to a given deuteron [*1*].

$S_{C\text{-}Di}$ is the order parameter of the $C\text{-}D_i$ internuclear axis relative to the magnetic field axis and can be different for the *S* and *R* enantiomers. The relatively large magnitude of $K_{C\text{-}D}$ for the C-D bond means that, even though differences between the order parameters for *R* and *S* isomers may be small, their quadrupolar splittings $\Delta\nu_{Qi}$ may be sufficiently different so that the two doublets can be resolved.

At natural abundance the ^{2}H-^{2}H spin-spin couplings are not detected. Thus, when all scalar and dipolar couplings with protons are eliminated through decoupling, the NAD spectra in the PBLG phase consist of superpositions of independent quadrupolar doublets corresponding to all non-equivalent deuterons in each of the enantiomers [*4-6*]. Disregarding quadrupolar doublets originating from the co-solvent, we can expect up to 2*n* doublets (4*n* peaks) in the NAD spectrum for a racemic mixture of enantiomers possessing *n* non-equivalent deuterons, if there is no signal overlap, if all quadrupolar splittings are non-zero, and if all deuterated chiral isotopomers are discriminated. This number is, however, reduced to 2(*n-k)* doublets for molecules possessing *k* exchangeable -OD or -ND groups; chiral discrimination is not possible for such groups, due to fast exchange of their deuterons.

The largest quadrupolar splittings observed in PBLG solutions have approximately the same magnitude as the deuterium chemical shift range. Thus, for large chiral molecules the identification of two components for each quadrupolar doublet is generally not straightforward due to overlap of numerous peaks [*5,6*]. Consequently, one-dimensional NAD-NMR spectra do not exhibit the approximately symmetrical character observed for strongly ordered liquid crystals for which the correlation between two components is usually trivial. However, with weak ordering each doublet can be assigned on the basis of its chemical shift. To facilitate the analysis of overcrowded NAD spectra, we have developed several proton-decoupled deuterium 2D NMR experiments referred to as QUOSY (for QUadrupole Ordered SpectroscopY). Among them, the 2D autocorrelation experiment named *Q*-COSY was found to be the most suitable and useful 2D sequence for NAD-NMR in terms of signal sensitivity [*5*]. The pulse sequence (90°-t_1-180°-t_2.) is formally analogous with some sequences applied for spin-1/2 systems, but it cannot be simply compared to them [*6*]. To correct for the effects of non-ideal pulses, which lead to a degradation of the S/N ratio and undesirable on-diagonal peaks in the *Q*-COSY spectrum, we have replaced each single pulse in the basic two-pulse sequence by a composite pulse, providing greater tolerance to imperfections and off-resonance effects [*6*]. The *Q*-COSY pulse program for AVANCE spectrometers is given at the end of this article.

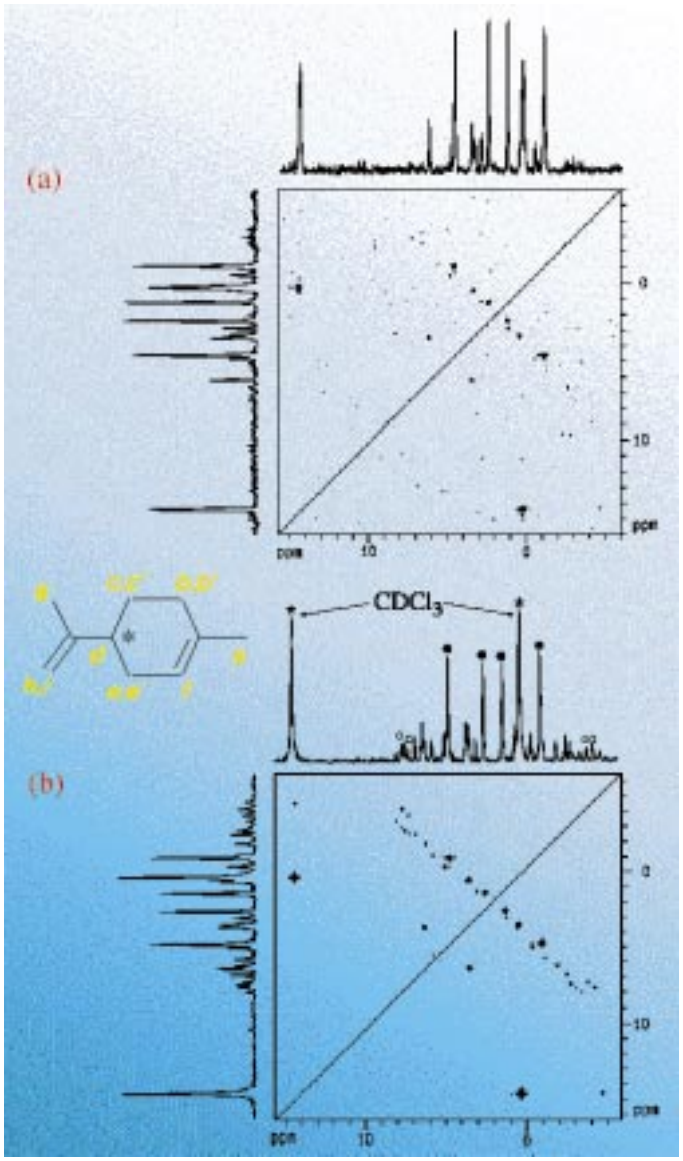

Fig. 2: *61.4 MHz (9.4 T) 2D NAD Q-COSY spectrum of (±)-limonene using (a) a 5-mm BBI probehead and (b) a selective deuterium probehead with the same experimental conditions. The data matrix is 256 (t_1) × 1400 (t_2) data points. An exponential window function was applied in both dimensions.*

NMR sample preparation

Suitable samples for NAD-NMR in PBLG require 50 to 100 mg of the investigated material, 80 to 100 mg of PBLG, and 350-500 mg of co-solvent in a 5-mm NMR tube to give an optimal volume for a 5-mm selective probehead. The NMR tube should be sealed to avoid solvent evaporation, and the sample is then mixed back and forth until a homogeneous birefringent phase is obtained. In this study we have used PBLG (Sigma Corp.) with a degree of polymerization DP = 562 (MW = ca. 120 kDa) and dry chloroform as co-solvent. Chloroform is the most efficient solvent for NAD NMR in PBLG. It dissolves a large range of organic compounds and the linewidths of the signals from the chiral solutes are usually small (3-10 Hz). Also, chloroform contains only one deuterated isotopomer, giving rise to a single additional and easily recognizable quadrupolar doublet in the NAD spectrum (**Fig. 1**). A further advantage of chloroform as co-solvent is that the number of deuterons *per* unit volume is not excessively large relative to those of the chiral solutes, thus minimizing potential dynamic range problems for data acquisition [*7*].

Spectrometer requirements

1D and 2D NAD-NMR experiments in PBLG can be performed on any multinuclear spectrometer and no special hardware is required. Nevertheless, the use of a high magnetic field for good chemical shift dispersion and a selective ^{2}H probe for optimal sensitivity at natural abundance is recommended. In the work presented here, we used a Bruker DRX 400 high-resolution NMR spectrometer and a 5-mm selective deuterium probe (61.4 MHz) equipped with a ^{1}H-decoupling channel and ^{19}F lock (376.5 MHz) developed by Bruker SA. Actually, we found that, with our equipment and environment, there were no significant differences in linewidths or S/N between spectra recorded with and without the fluorine lock. However, when long-term magnetic field drift is comparable to the linewidths or when external field perturbations are significant, a field-frequency lock on a fluorine signal is absolutely essential for good performance. A few drops of $CFCl_3$ (ca. 10-20 mg) are sufficient and can be added to the co-solvent without perturbing the PBLG phase.

The NAD-NMR experiments presented here were recorded with digital filtering and oversampling to maximize dynamic range and S/N [*8,9*], and the WALTZ-16 composite pulse

sequence was used to decouple protons. Note that proton decoupling in NAD-NMR applications does not require more power than for isotropic samples because the residual ^1H-^2H dipolar couplings remain small. The NMR tube was not spun in the magnet, and the temperature of the sample was regulated at 298.0 ± 0.1 K by the Bruker BVT 3000 temperature unit. Unless specified otherwise in the text or figure legends, the NAD 2D *Q*-COSY experiments were recorded using 320 transients for each t_1 increment with a relaxation delay of 0.5 s. All 2D spectra were zero-filled to 1024 (t_1) × 2048 (t_2) points prior to 2D FT and then symmetrized.

doublets if the spectra of all nonequivalent deuterated isotopomers are resolved. In addition, the relatively low chemical shift dispersion of deuterium signals in this kind of cycloalkene suggests that the 2D *Q*-COSY experiment will be required to clearly identify the two components for each quadrupolar splitting.

Fig. 2 presents 2D NAD-NMR spectra of (±)-limonene in PBLG recorded using the BBI **(a)** and the selective deuterium probe **(b)** with the same acquisition conditions. The liquid-crystalline NMR sample contained 100 mg of racemic mixture, 100 mg of PBLG and 350 mg of $CHCl_3$. The pulse sequence used generates phase-twisted lineshapes; therefore, a magnitude-mode transform was used for the 2D contour plot shown. One might argue that 2D experiments giving pure absorption peaks in both dimensions should be more valuable in terms of spectral resolution and S/N ratio. However, we have shown that the phase-sensitive variant of the *Q*-COSY sequence, referred to as *Q*-COSY Ph, is *less* sensitive by a factor $2^{1/2}$ compared *Q*-COSY itself [5]. The resolution enhancement obtained with pure absorption phase peaks would increase S/N by a factor $2^{1/2}$; however, the pulse sequence for phase-sensitive 2D *Q*-COSY Ph results in a factor of 2 loss in signal amplitude in the acquired data.

Comparison of the two spectra in Fig. 2 confirms the definite advantage of using a selective deuterium probehead (the inner rf coil for ^2H provides a better filling factor and higher coil quality factor compared to the outer coil in an inverse probehead). A significant gain in sensitivity (S/N per mg, inversely proportional to the 90° pulse length) is observed, thus allowing the detection of the smallest deuterium signals in the spectrum [7]. This is a very important point when the molecule of interest does not possess methyl groups which provide strong signals from 3 equivalent deuterons. Furthermore, the observation of all deuterium signals in the 2D spectra may facilitate spectral analysis and avoid ambiguities in assignments and chiral discrimination.

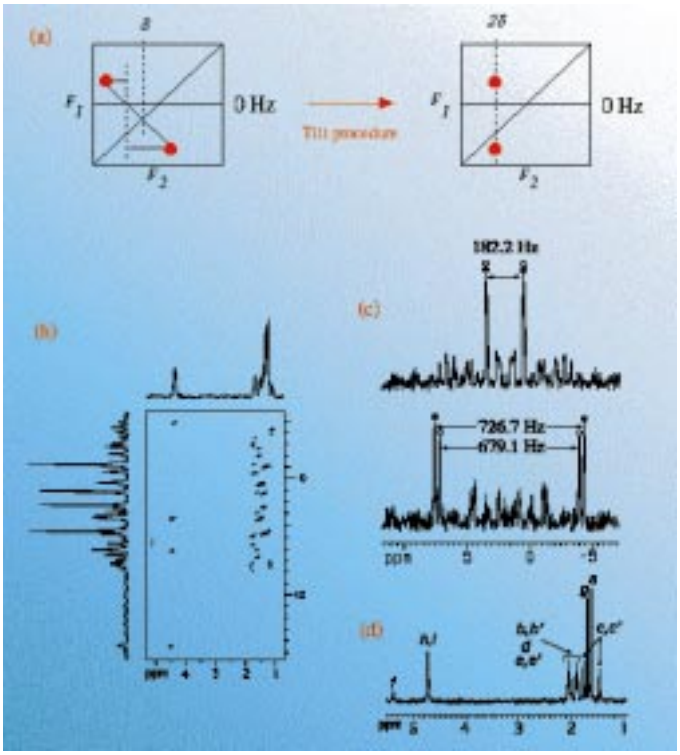

Fig. 3: *(a) Principle of the tilt procedure as applied to a Q-COSY spectrum. (b) tilted Q-COSY spectrum of (±)-limonene. (c) Columns from the tilted spectrum show the quadrupolar doublets corresponding to the two diastereotopic deuterons c and c'; only one shows a chiral discrimination. (d) 1D isotropic NAD-NMR spectrum of (±)-limonene at 298 K.*

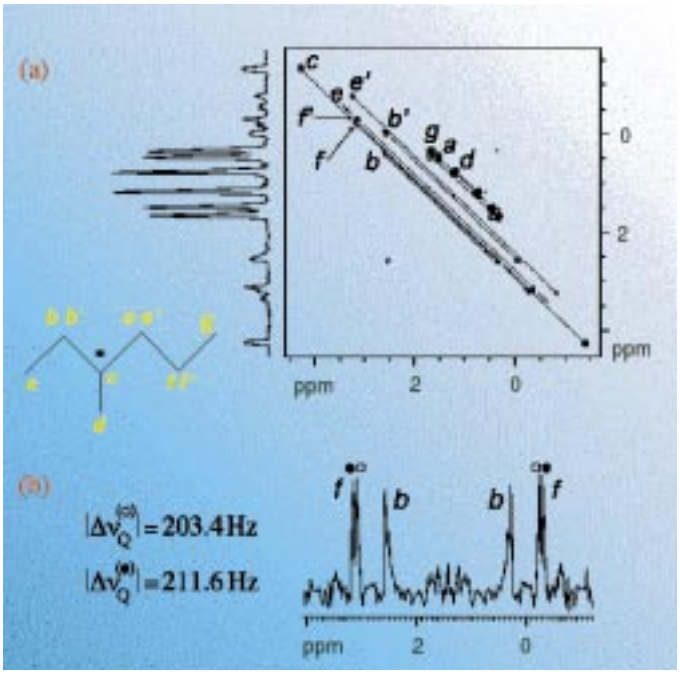

Fig. 4: *61.4 MHz 2D NAD Q-COSY spectrum of (±)-3-methylhexane recorded using 300 (t_1)×1700 (t_2) data points. A Gaussian window function was used in both dimensions, and the matrix was symmetrized. The chloroform doublet is not shown in the spectrum.*

Results and Discussion

Enantiomer differentiation of chiral hydrocarbons in PBLG

A racemic mixture of limonene, a well-known chiral intermediate for natural product synthesis, was investigated. This apolar, semi-rigid molecule was an interesting candidate for testing the potential of the technique for studying unsaturated hydrocarbons. Although this chiral semi-rigid cycloalkene is the simplest chiral *p*-menthane monoterpene ($C_{10}H_{16}$), we can expect to detect a maximum of 2×12 = 24 quadrupolar

Apart from the quadrupolar doublet from $CDCl_3$ (* in the projection of Fig. 2b), we observed 17 clearly resolved quadrupolar doublets. A first analysis revealed that several deuterated sites can be discriminated. For instance, the existence of two quadrupolar doublets, centered at 1.5 ppm (open circles in Fig. 2b) shows chiral discrimination ($|\Delta\nu^{\delta}_{Qi} - \Delta\nu^{R}_{Qi}|$ = 78 Hz) for the corresponding deuteron, while the intense doublets centered at 1.8 and 1.9 ppm (methyl groups, filled circles in Fig. 2b) are not differentiated (one doublet for each methyl group).

To confirm this analysis, we have recorded the NAD 2D *Q-COSY* spectrum of (±)-limonene in a racemic mixture of PBLG and PBDG (the enantiomer of PBLG) in $CHCl_3$ [*10*]. In this case the *S* and *R* isomers were in fast diffusional exchange between PBLG and PBDG environments, and this averaging process eliminated the chiral discrimination. Experimentally, we observed a single quadrupolar doublet (instead of two) centered at 1.5 ppm, and identical doublets for the methyl groups, thus confirming our hypothesis. The complete assignment of all doublets is not trivial since the deuterium chemical shift dispersion in this cycloalkene is rather small. To facilitate the analysis of 2D *Q*-COSY spectrum, it is possible to perform a 45°-tilt of the data about the horizontal $F_1 = 0$ axis, as in a *J*-resolved 2D experiment, as shown schematically in **Fig. 3a**. Following this manipulation, all quadrupolar doublets line up parallel to the F_1 axis with F_2 coordinates equal to $2\delta_D$ [5]. Thus, the F_2 projection spectrum represents a deuterium chemical shift spectrum, and F_1 slices can be used to observe the quadrupolar doublets for each isotopomer in the mixture.

Note here that the scale labeling the F_2 axis of the tilted *Q*-COSY in **Fig. 3b** has been adjusted to show the correct chemical shifts. The tilt algorithm in the AVANCE software was designed for *J*-resolved experiments and does (yet) provide the capability of automatically relabeling the F_2 axis for *Q*-COSY applications (e.g. reduce the effective SW by a factor of 2).

From the various columns extracted from the tilted 2D spectrum, it is trivial to observe the two components of each quadrupolar doublet and to measure their residual splittings (**Fig. 3c**). Furthermore, we can compare the deuterium chemical shift spectrum in the F_2 axis with the 1D NAD-NMR spectrum of limonene recorded in the isotropic phase (**Fig. 3d**). This facilitates the assignment of numerous quadrupolar doublets on the basis of their chemical shifts, assuming that the deuteron chemical shifts in PBLG are similar to the isotropic proton shifts measured in neat $CHCl_3$. In this example, we could show unambiguously that enantiomer differentiation was possible for only one of the diastereotopic deuterons, c and c' (**Fig. 3c**). This emphasizes the versatility of chiral discrimination between two chiral isotopomers and demonstrates the ability of the 2D NAD-NMR to probe all possible deuterons in a molecule.

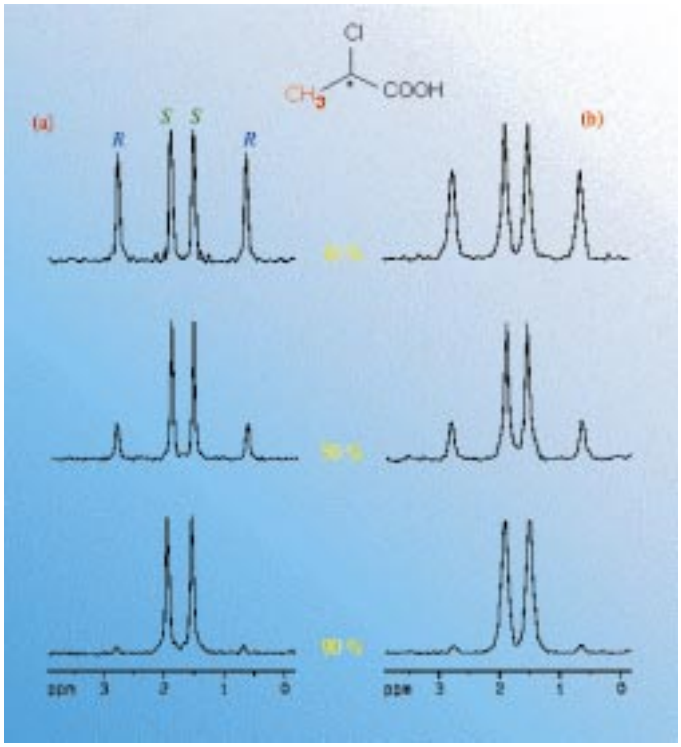

Fig. 5: *61.4 MHz 2D NAD Q-COSY experiment for 2-chloropropionic acid in PBLG was recorded for three samples with known enantiomeric excess (S-R = 10, 50 and 90%). The 2D data matrix contained 280 (t_1)×1600 (t_2) data points, no window function was applied, and the final matrix was tilted and symmetrized. The quadrupolar doublets for the methyl group are shown and were obtained: (a) from a single matrix column, or (b) from the sum of several adjacent columns.*

We turn now our attention to the case of (±)-3-methylhexane in the PBLG phase. In contrast to the previous molecule, this simple saturated alkane does not possess functional groups; consequently, none of the known chemical derivatization techniques can be applied [*11*]. Also, this molecule is not a good candidate for the application of chiral shift reagents since it does not possess a defined complexation site. To the best of our knowledge, all isotropic NMR techniques have failed to discriminate such flexible chiral alkanes (no results were found in the literature), but the resolution of enantiomers by capillary gas chromatography using cyclodextrin derivatives as the chiral stationary phase was described in 1990 [*12*]. In **Fig. 4**, we present the 2D NAD-NMR spectra of (±)-3-methylhexane in PBLG using the selective deuterium probehead. The liquid-crystalline NMR sample contained 80 mg of racemic material, 81 mg of PBLG (DP = 562(50%) and 1078(50%)) and 445 mg of $CHCl_3$. In the 2D spectrum we observed 12 different quadrupolar doublets for 10 nonequivalent sites. This indicates that chiral discrimination is achieved for two deuteron sites. This was confirmed by the NAD 2D *Q-COSY* spectrum of (±)-3-methylhexane in a racemic mixture of PBLG and PBDG in $CHCl_3$ where the expected 10 quadrupolar doublets were observed. We were able to prove that deuterons *e* and *f* are discriminated (see **Fig. 4**), while deuterons *b*, *b'* and *c* show broad peaks, indicating a weak chiral differentiation. As in the case of limonene, no chiral discrimination was detected on the methyl signals (*a*, *d*, *g*) [*13*]. This study with 3-methylhexane demonstrates conclusively that even flexible, chiral molecules without polar functional groups can be discriminated in the PBLG phase. Thus, the method is general and applicable to both polar and apolar chiral molecules. The results presented here also suggest that shape recognition may play a significant role for chiral discrimination in PBLG, in particular, for compounds lacking polar groups.

Measurements of enantiomeric excesses using NAD-NMR in PBLG

To explore the potential and limitations of this approach for quantitative analysis, we have recorded the 2D NAD *Q*-COSY spectrum of 2-chloropropionic acid in PBLG for three samples with known enantiomeric excess (*ee*) of 10 ± 1%, 50 ± 1% and 90 ± 1%. The liquid-crystalline NMR samples were prepared from 100 mg of chiral material (*R*/*S* ratios of 45/55, 25/75, and 5/95 mg), 100 mg of PBLG and 350 mg of $CHCl_3$. In **Fig. 5**, we present the quadrupolar doublets for the methyl group, as extracted from the tilted 2D NAD *Q*-COSY spectrum. The three spectra at the left represented single F_1 slices for the three different samples (**Fig. 5a**), while the spectra at the right represent sums of several F_1 slices (**Fig. 5b**). In both series, we can visualize unambiguously the enantiomeric discrimination ($|\Delta\nu^{S}_{Qi} - \Delta\nu^{R}_{Qi}| = 115$ Hz) and the enantiomeric purity can be determined with sufficient accuracy by simple peak integration.

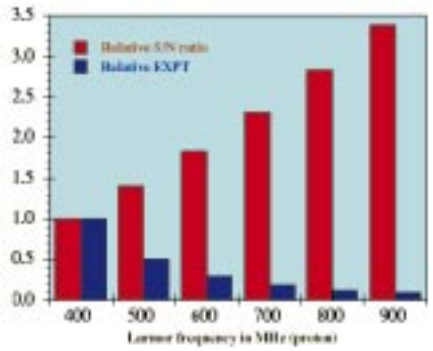

Fig. 6: *Theoretical frequency dependence of the S/N ratio for a constant experiment time EXPT (red) and of the EXPT required for a constant S/N (black), expressed relative to the values at 400 MHz (9.4 T).*

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;Q-cosy
;avance-version
;2D quadrupolar correlation with proton decoupling
;using composite pulse
;D. Merlet, B. Ancian, J. Courtieu & P. Lesot
;J. Am. Chem. Soc. 121, 5249 (1999)
;D. Merlet, M. Sarfati, B. Ancian, J. Courtieu & P. Lesot
;Phys. Chem. Chem. Phys. 2, 2283 (2000)

#include <Avance.incl>

"p2=p1*2"
"d11=30m"
"d0=3u"

1 ze
  d11 pl12:f2
  d11 cpd2:f2
2 d1
3 p1 ph1
  p1 ph2
  d0
  p1 ph3
  p2 ph4
  p1 ph3
  go=2 ph31
  d1 wr #0 if #0 id0 zd
  lo to 3 times td1
exit

ph1=0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3
ph2=1 1 1 1 2 2 2 2 3 3 3 3 0 0 0 0
ph3=3 1 0 2 0 2 1 3 1 3 2 0 2 0 3 1
ph4=0 2 1 3 1 3 2 0 2 0 3 1 3 1 0 2
ph31=0 0 2 2 1 1 3 3 2 2 0 0 3 3 1 1

;pl1 : f1 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD decoupling
;p1 : f1 channel -  90 degree high power pulse
;d11: delay for disk I/O                                  [30 ms]
;d1 : relaxation delay; 1-5 * T1
;d0 : incremented delay (2D)                              [3 usec]
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;in0: 1/(1 * SW) = 2 * DW
;nd0: 1
;NS: 4 * n
;DS: 16
;td1: number of experiments
;MC2: QF
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Fig. 7: *AVANCE Pulse program for Q-COSY with ^{1}H decoupling.*

Note that chiral differentiation was even larger for the deuteron of the asymmetric carbon ($|\Delta\nu^S_{Qi} - \Delta\nu^R_{Qi}|$ = 215 Hz), but the weaker S/N ratio (one deuteron instead of three for the methyl group) makes these signals less attractive for quantitative analysis. Thus, NAD-NMR spectroscopy allows us to simultaneously probe all possible deuterated sites of a molecule and determine which site is most suitable for determination of the enantiomeric excess. The optimal site should have a well-resolved chemical shift, a number of equivalent spins for a maximum in S/N ratio, and a sufficiently large difference in quadrupolar splittings for complete resolution of *R* and *S* signals.

The results presented here show that the technique allows us to detect relatively small *ee*'s as well as large *ee*'s (up to 90%). In the latter situation, the amount of *R* enantiomer was 45.9 µmol, corresponding to 6.89 nmol for natural abundance deuterium at the stereocenter and 20.7 nmol for deuterium on the methyl group (3 equivalent deuterons). It should be noted that the apparent linewidths increase for the spectra summed over several slices (Fig. 5b) as a result of the altered lineshape created by the tilt procedure, but this should not significantly affect the measurement of *ee*. The average *ee*'s calculated from several integrations of the methyl group signals in the three spectra of Fig. 5a are 12%, 49% and 91%. These values agree closely with the expected values and, therefore, provide an acceptable measurement of the *ee*'s. The same *ee* values were obtained using the summed spectra of Fig. 5b. Thus, under the conditions described here (9.4 T, 5-mm selective ^{2}H probehead, overnight measurement), we find that *ee* can be determined with an accuracy of ca. 5 - 15% of the true value with ca. 1 mmol of the enantiomeric mixture using 2D NAD-NMR in PBLG. The technique provides a simple, rapid determination of the best site for chiral discrimination in the molecule so that, if greater precision is necessary, the most promising synthesis strategy for selective deuteration can be chosen.

Prospects

The results described here illustrate the feasibility and potential of NAD-NMR spectroscopy at 9.4 T using a selective deuterium probehead and provide a practical solution to DOE measurements in PBLG without the need for site-specific isotopic labeling. At higher magnetic field strength, it should be possible to acquire adequate NAD spectra in a much shorter time or to determine the enantiomeric excess with higher precision. The S/N ratio increases approximately as $B_0^{3/2}$ for a given measurement time EXPT, while EXPT is proportional to B_0^{-3} for a given S/N ratio. as shown in **Fig. 6**. Thus, for NAD-NMR at 115.1 MHz (17.6 T or 800 MHz for ^{1}H), the S/N ratio should increase by a factor of 2.8 for the same EXPT (relative to 9.4 T). Conversely, the EXPT would decrease by a factor 6.6 for the same S/N ratio. Another cheaper and more effective solution for improving the S/N ratio of NAD-NMR would be the use of a selective deuterium cryogenic probehead. Indeed, cryoprobes have been shown to provide a factor of 3 - 4 gain in S/N compared with standard high-resolution probeheads [*14*]. Consequently, the use of a cryoprobe and higher field would provide an order-of-magnitude improvement in S/N, allowing a more accurate determination of enantiomeric excess, or a significant decrease in the amount of chiral material required.

Conclusion

The differentiation of enantiomers and their stereochemical characterization is the ultimate challenge in asymmetric synthesis, and requires methodologies which can be successfully applied to a wide range of chemical types. In this respect, NAD-NMR spectroscopy of chiral compounds in liquid-crystalline PBLG solutions provides an efficient and convenient analytical tool for resolving and characterizing a large number of the possible chiral isotopomers. Of major interest is the fact that the polypeptide helices in the liquid-crystalline phase are able to interact enantioselectively with both polar and apolar chiral compounds to give sufficiently large differences in the order parameters and the NMR-detectable quadrupolar splittings for *R* and *S* forms. This direct analytical method is an attractive alternative to classical chiroptical and chromatographic techniques or other NMR methods for investigating small to medium-size chiral molecules. Undoubtedly, ultra-high magnetic field strengths and cryoprobe technology will further enhance the potential of this technique in the near future.

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