Two-Dimensional Deuterium NMR Spectroscopy of Chiral Molecules Oriented in a Polypeptide Liquid Crystal: Applications for the Enantiomeric Analysis through Natural Abundance Deuterium NMR

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Abstract: We describe several two-dimensional NMR experiments to facilitate the analysis of overcrowded proton-decoupled deuterium spectra of chiral molecules oriented in poly-γ-benzyl-L-glutamate liquid crystalline solutions. These 2D NMR experiments allow the correlation between the components of each quadrupolar doublet in the deuterium spectra and then their assignment on the basis of chemical shift. The 2D pulse sequences proposed in this work were developed using the Cartesian spin-operator formalism for spin $I = 1$ nuclei with a small quadrupolar moment, such as deuterons in high magnetic field. The features and analytical potentialities of each pulse sequence are discussed and compared. Illustrative applications of these sequences in natural abundance deuterium NMR spectroscopy using a 9.4 T magnetic field and standard NMR equipment are reported and examined. With this technique, it is demonstrated that quantitative measurements are possible within a precision of 10%.

Introduction

Over the past decade, there has been a considerable effort in the development of new NMR tools allowing the enantiomeric analysis of chiral organic molecules.[1,2] This challenge is mainly stimulated by the increasing interest in enantioselective reactions in the (bio)chemistry and quality control testing for chiral active ingredients in the pharmaceutical industry. Among the more recent and promising NMR methods, the use of a lyotropic chiral liquid crystal (organic solutions of poly-γ-benzyl-L-glutamate, PBLG) to discriminate enantiomers through deuterium, proton, carbon-13, or fluorine-19 has been developed in our laboratory.[3−7] This NMR method was shown convincingly to be suitable for a large variety of chiral materials, including compounds which are chiral by virtue of isotopic substitution and known to be very difficult to analyze with other analytical techniques.[3]

In a recent work, we reported the first cases of visualization of chiral molecules oriented in PBLG using proton-decoupled natural abundance deuterium NMR (NAD-NMR) spectroscopy.[8] Despite the low deuterium sensitivity (1.45×10^{-6} with respect to the proton), we showed the feasibility of this method using routine magnetic field strengths (5.87 T), a conventional high-resolution spectrometer, and a single-pulse sequence. In NAD-NMR, the scalar (J_{ij}) and dipolar (D_{ij}) couplings between two rare atoms are basically avoided (only about 2 molecules in 10^8 can yield it), and hence the spectra consist of a superposition of independent quadrupolar doublets corresponding to the different isotopomers in the mixture (isotopic filter).[8] The major interest of working with NAD-NMR is that all possible deuterated sites in the molecule can be simultaneously probed with no isotopic enrichment, thus increasing the probability of observing a chiral discrimination. However, the use of NAD-NMR can be a drastic disadvantage for large chiral molecules when the excessive overlapping of peaks yields undecipherable, overcrowded spectra. To resolve this limitation and enhance the analysis of NAD spectra, the use of two-dimensional autocorrelation deuterium NMR experiments is needed and provides a spectroscopic separation of all deuterated components of the mixture.[9] From the separated simple subspectra, we can then unambiguously identify all sets of quadrupolar doublets and directly measure their splittings. Despite the low sensitivity of deuterium nuclei, a short calculation actually indicates that 2D NAD-NMR experiments have sensitivities comparable with those of carbon-13 2D INADEQUATE experiments, thus showing that they may be feasible in practice.

The application field of these 2D autocorrelation NMR experiments can actually be successfully extended to perdeuterated molecules oriented in PBLG, because the $^2H-^2H$ splittings ($J_{ij} + 2D_{ij}$) are generally too small (1−2 Hz) to be measured in labeled materials. Except for some particular cases (methyl groups, for example) where they may be eventually observed, the scalar and dipolar couplings only contribute to

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the line width of the peaks. This situation results in the weakness of scalar couplings (≈42 times less than J_{H-H} couplings) and the weak degree of molecular orientation in PBLG (order parameter in the $10^{-4}-10^{-3}$ range), yielding very small residual dipolar couplings with a negative or positive sign.[10−12] Therefore, the ^{2}H spectra of perdeuterated molecules as well as NAD spectra consist of a superposition of individual quadrupolar doublets, thus suggesting that the overcrowded spectra analysis can be resolved in the same way.

The assignment of quadrupolar doublets in large oriented molecules through deuterium NMR was pioneered by Emsley and Turner in the case of a perdeuterated liquid crystal molecule. For this purpose, the use of a two-pulse sequence, $(\pi/2)_{\phi 1}-t_1/2-(\pi/2)_{\phi 2}-t_1/2-\mathrm{acq}(t_2)_{\phi r}$, was proposed.[13,14] However, the analysis and the assignment of quadupolar doublets in the 2D spectrum is possible only when the deuterons are significantly dipolar coupled to each other in the perdeuterated molecule. This sequence is, therefore, poorly adapted for perdeuterated chiral molecules dissolved in PBLG because the intensity of correlation peaks reflects the amplitude of the ^{2}H−^{2}H splittings, which are very weak in this medium. Furthermore, in NAD-NMR, the ^{2}H−^{2}H splittings do not exist, leading to the nonapplicability of this sequence. In another approach, Vega et al. have investigated the 2D deuterium NMR exchange spectroscopy in liquid crystalline solutions by using the general pulse sequence $(\pi/2)_{\phi 1}-t_1-(\theta)_{\phi 2}-\tau_m-(\theta)_{\phi 3}-\mathrm{acq}(t_2)_{\phi r}$.[15] Although the resulting 2D spectra have a COSY-like appearance, the cross-peaks appearing between the two components of each quadrupole doublet reflect the connectivity between exchanging spectral lines only. Finally, in a very recent work, Emsley and co-workers have introduced a new 2D experiment, referred to as DECOR, for *de*uterium *cor*relation, which correlates the carbon-13 spectrum with the deuterium spectrum and allows measurement and assignment of the quadrupolar couplings in oriented labeled materials.[16] This last 2D experiment cannot, however, be successfully applied in NAD-NMR spectroscopy, due to the extremely low probability in finding a deuterium coupled to a carbon-13 nucleus (only about 1 molecule in 10^6 can yield it).

To resolve specifically the analysis of NAD spectra as well as the deuterium spectra of poly- or perdeuterated molecules dissolved in PBLG, we propose applying a new class of 2D experiments, referred to as QUOSY, for *qu*adrupole *o*rdered *s*pectroscop*y*.[10] The basic idea of the QUOSY experiments is to obtain a 2D contour plot which enables us to correlate the components of each quadrupolar doublet on the proton-decoupled deuterium spectra (^{2}H−{^{1}H}). These 2D experiments make it possible to separate all quadrupolar doublets and then to assign each of them on the basis of chemical shift analysis by comparing ^{2}H and ^{1}H chemical shifts recorded in the isotropic phase.[9,10] In this work, three different types of 2D NMR spectroscopy, using the basic pulse sequences, were explored and proposed: 2D double-quantum experiments, 2D echo experiments, and 2D COSY-like experiments.

In the first part of this paper, we present and discuss several 2D experiments, leading to an improvement in the analysis of the overcrowded ^{2}H spectrum of molecules embedded in the PBLG phase. These sequences were developed using the Cartesian spin-operator formalism for an isolated spin $I = 1$ nucleus with a small quadrupolar moment, such as deuterons in high magnetic field.[17,18] To illustrate the potentialities of these 2D experiments, we investigate the case of perdeuterated 1-pentanol. This section is concluded with a brief discussion of the features and efficiency of the sequences presented. In the second part, we focus on 2D natural abundance ^{2}H−{^{1}H} NMR spectroscopy through the investigation of racemic and enriched mixtures of chiral compounds dissolved in the PBLG−$CHCl_3$ chiral nematic (N*) phase. We demonstrate that quantitative measurements of enantiomeric excess (ee) are possible within a reasonable precision.

Background

Some Aspects of Deuterium NMR Spectroscopy in the PBLG N* Phase. The proton-decoupled deuterium NMR spectrum of a molecule containing a single deuteron (spin $I = 1$) and dissolved in liquid crystalline solutions consists of a quadrupolar doublet separated by $\Delta\nu_Q = {}^3/_2(e^2Qq_{C-D}/h)S_{C-D}$, where (e^2Qq_{C-D}/h) is the quadrupole coupling constant (QCC) and S_{C-D} is the order parameter along the C−D axis.[10] In an oriented chiral medium such as PBLG, two enantiomers are generally differently ordered, and we may then expect to observe two different quadrupolar doublets, $\Delta\nu_Q^R$ and $\Delta\nu_Q^S$, centered at the same chemical shift. Note here that the chemical shift anisotropy difference between two enantiomers is generally too small to be measured in the ^{2}H−{^{1}H} spectra, due to both the small chemical shift anisotropy (CSA) of deuterium and the weak degree of ordering in PBLG.[3,4] In fact, the efficiency of the chiral discrimination by ^{2}H−{^{1}H} NMR in PBLG depends on both the ability of two enantiomers to interact differently with the polypeptide, which produces a differential ordering effect (DOE), and the relatively large magnitude of the deuterium QCC (≈170 kHz).[4,10] In other words, a small difference in the orientations of the *R* and *S* isomers can give a sufficiently large difference in their quadrupolar splitting ($\Delta\nu_Q^R - \Delta\nu_Q^S$), which allows us to visualize each of them in the spectrum and leads to the enantiomeric analysis of the mixture.[3,4,8] This method, therefore, offers both efficiency and simplicity.

For deuterium NMR in natural abundance and in perdeuterated molecules, the spectral analysis in PBLG is more complex, since in both cases the deuterium spectra consist of a superposition of quadrupolar doublets. Thus, for a racemic mixture of enantiomers possessing n nonequivalent deuterons, we can expect $2n$ doublets ($4n$ peaks) to be observed in the ^{2}H−{^{1}H} spectrum, assuming that all deuterated sites are discriminated and disregarding possible line overlaps or null quadrupolar splittings. This evaluation can be, however, reduced to $2n - 1$ doublets for molecules possessing an −OD or −ND group, because no chiral discrimination was (until now) observed for such as groups, due to the fast exchange of their deuteron. Finally, in NAD-NMR, the doublet number originating from organic cosolvent must be also accounted for.[8]

Another reason for interest in the PBLG liquid crystalline phase is its ability to discriminate the enantiotopic nuclei in

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prochiral groups.[3,19,20] Thus, two enantiotopic deuterons in a prochiral methylene group become magnetically nonequivalent, enabling us to distinguish between them through their $^2H-\{^1H\}$ NMR spectrum.[3,8] The *Re* and *Si* faces are actually discriminated by a difference in the bond order parameter of each C−D axis, S_{CD}, and hence it is possible to discriminate between *pro-R* and *pro-S* deuterons.[3,10,12] Consequently, the number of expected quadrupolar doublets is therefore equal to the number of nonequivalent deuterons, to which we must add a doublet for each enantiotopic deuteron in the molecules. Finally, due to the nonequivalence between two diastereotopic nuclei, two diastereotopic deuterons in a methylene group of a chiral molecule will lead to four doublets in the PBLG phase (when the enantiomers are discriminated and without peak overlapping), corresponding to the (*R*,*pro-R*), (*R*,*pro-S*), (*S*,*pro-R*), and (*S*,*pro-S*) deuterons of the *R* and *S* isomers, respectively. Such molecules, bearing a classical asymmetric carbon and an asymmetric carbon by virtue of isotopic substitution, are referred to as "*semi-isotopic*" diastereoisomers.

Experimental Section

Sample Preparation. All NMR samples investigated in this work were prepared using 100 mg of PBLG with DP = 534, MW ≈ 120 000 (purchased from Sigma), 100 mg of solute, and 350 mg of chloroform. The components of the mixture were weighed into a 5-mm-o.d. NMR tube which was sealed to prevent solvent evaporation. For efficient dissolution and mixing, the NMR samples were centrifuged back and forth until an optically homogeneous birefringent phase was obtained. It can be noted that the use of $CHCl_3$ for NAD-NMR applications is advantageous for various reasons. First, it dissolves a wide range of organic compounds and generally provides slightly viscous liquid crystalline phases, producing long apparent transversal relaxation times, and hence a good spectral resolution. The line widths of solutes measured on the $^2H-\{^1H\}$ spectra are usually 3−8 Hz. Second, it contains a single deuterated isotopomer, giving rise to a single additional quadrupolar doublet in the NAD spectrum.[8] Third, the number of deuterons per unit volume is not excessively large relative to those of the chiral solutes, thus minimizing the digitization problems associated with the dynamic range of the analog-to-digital converter (ADC).[21]

NMR Spectroscopy. The proton-decoupled deuterium experiments were performed on a Bruker DRX 400 high-resolution spectrometer equipped with a 5-mm-diameter inverse multinuclear probe operating at 61.4 MHz for deuterium. The NMR tubes were not spun along the magnetic field, and the temperature was controlled by the Bruker BVT 3000 temperature unit. Before the NMR spectra were measured, the sample was kept for about 0.5 h in the magnetic field in order to achieve a good thermal equilibration. The temperature of all samples was regulated carefully at 298.0 ± 0.1 K in order to keep a good long-term thermal stability. The field-frequency lock was not used, but the field stability of our spectrometer is good enough to allow for a long time accumulation without significant line broadening. The deuterium spectra were recorded with digital filtering and oversampling, thus enhancing the dynamic range of the ADC.[20−24] A 90° deuterium pulse of 20.5 μs was used. In all spectra, the protons were broad-band decoupled using the WALTZ-16 composite pulse sequence in order to remove the proton−deuterium scalar and dipolar couplings.[25]

All 2D NMR experiments presented in the following section were recorded using 1-s repetition time and 600-Hz spectral width in both dimensions. The spectral digitization was 512 (t_1) × 1024 (t_2) data points. Unless otherwise specified, the number of free induction decays added for each t_1 increment is 16. Except for the 2D phase-sensitive experiment, the 2D contour plots are presented in magnitude mode. Unshifted sine bell filtering in both dimensions prior to the double Fourier transformation was used. Other experimental parameters are given in the figure captions.

Results and Discussions

To illustrate the various 2D experiments described in this part, we have studied the case of the perdeuterated 1-pentanol dissolved in the PBLG−$CHCl_3$ phase. This prochiral compound of C_s symmetry possesses one methyl group and four prochiral methylene groups, yielding nine nonequivalent deuterons. Apart from the methyl group doublet, which can be easily identified (the most intense signals), the analysis of the spectrum based solely on the chemical shifts does not permit unambiguous assignment of each quadrupolar doublet in the spectrum and requires 2D experiments to be used. Note that, in this example, the CD_3 signal is not a single quadrupolar doublet but yields an unresolved structure, due to an exceptionally large $^2H-^2H$ dipolar coupling between deuterons of the methyl group.[10] However, this situation will never be encountered in NAD-NMR spectroscopy.

The 2D Double-Quantum Experiments. As previously pointed out, the deuterium NMR spectra in PBLG consist mainly of a superposition of deuterium subspectra. A solution to separate these subspectra can be achieved by two-dimensional double-quantum (DQ) NMR spectroscopy.[26,27] Originally developed for carbon-13 NMR and then for proton NMR, this approach can be extended to deuterium NMR if we generate and select the magnetization components which proceed via the coherence of order two ($p = 2$) in a spin $I = 1$ nucleus.[28,29] This approach is, therefore, identical to that involved in a dilute coupled spin system of two carbon-13 nuclei, with the important advantage in deuterium NMR that each deuteron in the sample will participate in the double-quantum spectrum.[30]

For the excitation and the detection of double-quantum coherence of spin $I = 1$, the classical pulse sequence $(\pi/2)_{\phi1}-\tau-(\pi)_{\phi2}-\tau-(\pi/2)_{\phi1}-t_1-(\alpha)_{\phi4}-\mathrm{acq}(t_2)_{\phi r}$ can be used. The sequence is identical to that used in any standard two-dimensional DQ experiment, and the phase cycling was not modified for spin $I = 1$ nuclei. Devoted to spins $I = 1$, we renamed this 2D experiment as Q-DQ, for *q*uadrupole *d*ouble-*q*uantum. Applying the Cartesian spin-operator formalism, we have determined the expression of the signal of one spin $I = 1$ nucleus during the acquisition period (t_2).[17,18] Disregarding all relaxation terms and phase factors, the expression $S(t_1,t_2)$ obtained after one scan, assuming that all phases were set along the *x* axis in the rotating frame, is

$$S(t_1,t_2) = A\{(^1/_2 \sin(2\alpha)\sin(4\pi\nu t_1) + i\sin(\alpha)\cos(4\pi\nu t_1)) \times (\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2] - \exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2])\} \quad (1)$$

where α is the reading pulse angle. This expression shows that,

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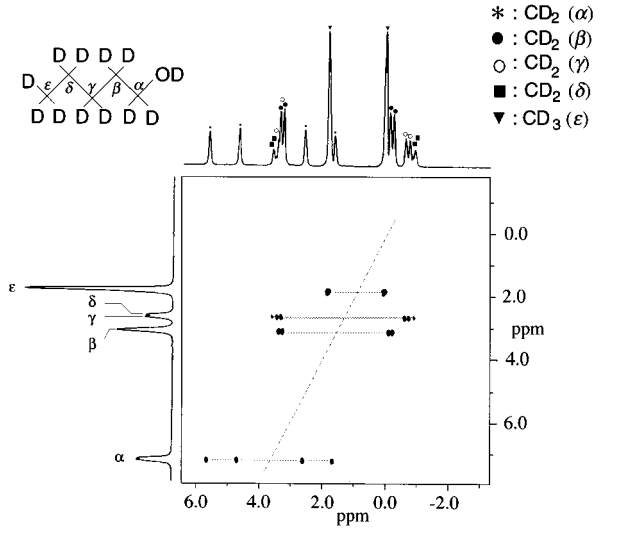

Figure 1. 2D Q-DQ spectrum of 1-pentanol-d_{12} obtained with τ = 1.45 ms; 64 free induction decays were added for each t_1 increment. The five slices parallel to the F_2 axis illustrate the five double-quantum transitions observed in the F_1 axis.

after a double Fourier transformation, the chemical shifts (ν) and the deuterium quadrupolar doublets ($\Delta\nu_Q$) corresponding to the single-quantum coherences appear in the F_2 dimension, while the double-quantum coherence associated with each deuterium nucleus appears at 2ν in the F_1 domain. From the calculation, we found that the amount of double-quantum coherence created for one single spin $I = 1$ nucleus is proportional to $\sin(2\pi\Delta\nu_Q\tau)$. Consequently, the condition for optimum transfer into double-quantum coherence is obtained with the delay $\tau = (2n + 1)/(4\Delta\nu_Q)$, where n is an integer (n = 0, 1, 2, ...). Finally, the α read pulse (α = 90°, 120°, or 135°) depends on the phase cycling employed and makes it possible to either optimize the double-quantum coherence or suppress the quadrature images in the F_1 axis.[31−33]

For our purpose, we have used the previous four-pulse sequence with an α read pulse = 90° and a phase cycle using 45° phase shifts.[34,35] The preparation time τ was set to 1.45 ms, corresponding approximately to the average value of residual quadrupolar splittings in the 1D spectrum. The 2D Q-DQ spectrum of 1-pentanol-d_{12} displayed in magnitude mode is reported Figure 1. Note that 2D Q-DQ spectra with peaks phased in pure absorption in both dimensions can be obtained using methods described in the literature,[36,37] but we have not applied them in the present work. As expected, each subspectrum straddles the skew diagonal of slope 2, each of them being located at twice their Larmor frequency (2ν) in the F_1 (double-quantum) dimension. The five slices parallel to the F_2 axis drawn in the 2D contour plot permit unambiguous identification of the respective components of each quadrupolar doublet, then assignment of each of them on the basis of chemical shifts. Thus, the subspectra associated with the α- and β-methylene groups clearly show two different doublets corresponding to the *pro-R* and *pro-S* deuterons in each of these groups. Note the strong prochiral discrimination (57.5 Hz) for the two deuterons of the α-methylene group. Because the chemical shift differences are doubled in the double-quantum spectrum, we are able to separate the signal between the δ- and γ-methylene groups.

Table 1. Four-Step Phase Cycles of the 2D Echo and COSY-like Experiments[a]

2D echo experiments						2D COSY-like experiments					
α = 90° (δ-resolved)			α = 180° (Q-resolved)			α = 90° (Q-COSY P)			α = 180° (Q-COSY)		
ϕ_1	ϕ_2	ϕ_r	ϕ_1	ϕ_2	ϕ_r	ϕ_1	ϕ_2	ϕ_r	ϕ_1	ϕ_2	ϕ_r
x	x	x	x	x	x	x	x	x	x	x	x
x	y	x	x	y	$-x$	x	y	x	x	y	$-x$
x	$-x$	x	x	$-x$	x	x	$-x$	x	x	$-x$	x
x	$-y$	x	x	$-y$	$-x$	x	$-y$	x	x	$-y$	$-x$

[a] The full phase cycling (16-steps cycle) is obtained by applying the CYCLOPS incrementation of all the pulse and receiver phases.

Disregarding the apodization effect, we can observe some differences in line intensity between the 1D single-quantum spectrum and the F_2 projection of the 2D spectrum. This effect originates in the dependence of the double-quantum coherence on the τ timing. Thus, for smaller (larger) values of τ, the strongest (smaller) quadrupolar doublets appear with greater intensity. Consequently, the choice of τ timing may reveal a serious challenge to the success of the experiment. One solution to avoid this effect involves the repetition of the experiment for different values of τ, to vary and average the relative intensities of the different subspectra. However, this procedure is very time-consuming and could not be reasonably applied in NAD-NMR, for which the sensitivity and the experiment time are two crucial problems. Therefore, although the Q-DQ experiment can be successfully applied to simplify the analysis of deuterium spectra, the condition for optimum transfer into double-quantum coherence actually restricts its use for quantitative measurements of enantiomeric excess.

The 2D Echo Experiments. To avoid the intrinsic inconveniences of the Q-DQ experiment, we have, in a second approach, used 2D echo experiments. One type of echo experiment, referred to as the solid echo or quadrupolar echo (QE), consists of refocusing the quadrupolar interaction, by using the following pulse sequence: $(\pi/2)_{\phi1}-\tau-(\pi/2)_{\phi2}-\tau-\mathrm{acq}(t_2)_{\phi r}$.[38−41] In partially aligned systems, the quadrupolar modulation provides the basis of the two-dimensional QE experiments, such as that proposed by Emsley and Turner and using the following pulse sequence: $(\pi/2)_{\phi1}-t_1/2-(\pi/2)_{\phi2}-t_1/2-\mathrm{acq}(t_2)_{\phi r}$.[13,14] In this particular experiment, the pulse sequence refocuses the quadrupolar couplings while the phase cycling is designed to suppress the chemical shifts after two scans, which enable the deuterium dipolar couplings to be specifically observed in the F_1 dimension. Although this experiment is poorly adapted to the analysis of molecules oriented in the PBLG, as pointed out in the introduction, it may be simply adapted for our purpose. Thus, by adding instead of subtracting the two first scans, it is possible to restore the chemical shift modulation during the t_1 period. We named this 2D deuterium NMR experiment the "δ-resolved" experiment. The phase cycling of the sequence is reported in Table 1. Disregarding all

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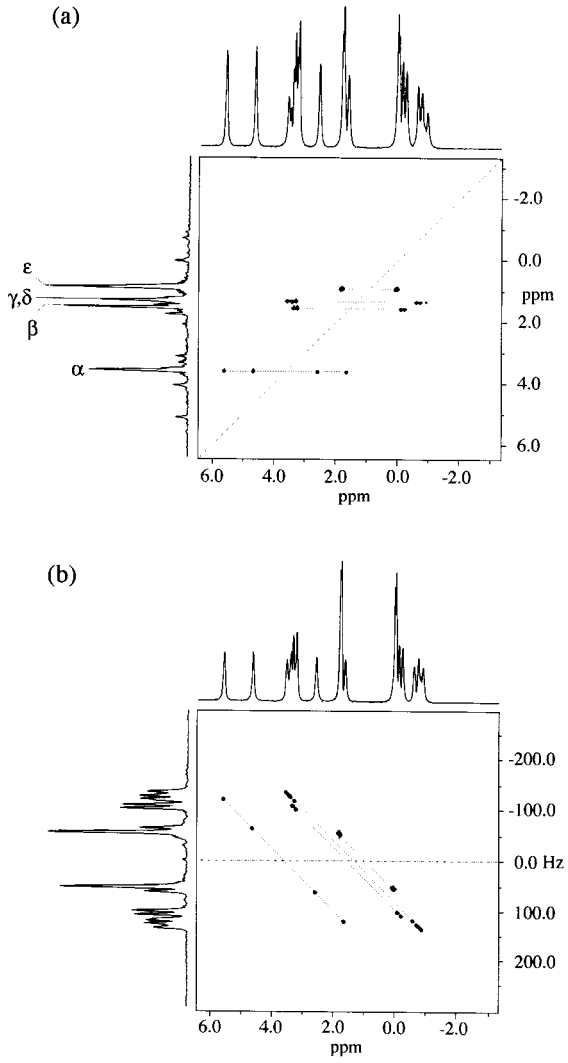

Figure 2. (a) 2D δ-resolved and (b) 2D Q-resolved spectra of 1-pentanol-d_{12} in the PBLG−$CDCl_3$ phase.

relaxation terms and phase factors, the expression for the signal during the acquisition period (t_2), after two scans, is

$$S(t_1,t_2) = A\{\exp[\mathrm{i}(2\pi\nu)\mathrm{t}_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[i(2\pi\nu)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2])\} \quad (2)$$

The expression for the signal during acquisition shows that only the chemical shift of each deuteron evolves during t_1. After a double Fourier transformation, the chemical shifts and the deuterium quadrupolar splittings, corresponding to the single-quantum deuterium spectrum, appear in the F_2 dimension, while the chemical shifts of each deuteron appear at ν in the F_1 domain. Note that the peaks in the F_1 dimension are *P*-peaks, and consequently the spectrum should be reversed in F_1 to obtain the correct chemical shift of each quadrupolar doublet. Because the experiment is based on a phase modulation during t_1 (after a single scan), the 2D contour plot should be displayed in magnitude mode to avoid the so-called "phase-twist" line shape.[26] The 2D δ-resolved spectrum of 1-pentanol-d_{12} is reported in Figure 2a. As expected, all quadrupolar doublets straddle the skew diagonal of slope 1, each of them being now located at their Larmor frequency (ν) in the F_1 dimension. The analysis of the 2D spectrum is identical to that in the 2D Q-DQ experiment; however, in this case, the chemical shift of all ^{2}H resonances is determined directly. In the 2D contour plot presented in Figure 2a, four slices parallel to the F_2 axis could be drawn. Contrary to the Q-DQ experiment, in which the chemical shift differences are doubled on the F_1 axis, we could not distinguish between the δ- and γ-methylene groups. Finally, we can observe some additional peaks in the F_1 projection. These resonances originate from further correlation peaks of weak intensity in the 2D spectrum (not presented on the 2D contour plot), which actually reveals deuterons that are dipolar and scalar coupled, as in the experiment of Emsley and Turner. Note that the intensity of correlation peaks reflects the amplitude of the ^{2}H−^{2}H splittings, and these will not be observed in NAD-NMR spectroscopy.

Another type of echo experiment, usually referred to as spin−echo, consists of refocusing the chemical shift interaction.[42−44] The idea is to obtain, after two Fourier transformations, the resonance frequencies and quadrupolar splitting constants of each deuteron separately on two distinct frequency axis. Although this experiment is similar to the 2D *J*-resolved spectroscopy used for spin $I = {}^1/_2$ nuclei, in our case the echoes are modulated by the quadrupolar couplings. This 2D experiment, referred to as a "Q-resolved" experiment, is achieved using the classical pulse sequence $(\pi/2)_{\phi 1}-t_1/2-(\pi)_{\phi 2}-t_1/2-\mathrm{acq}(t_2)_{\phi r}$. The phase cycling of the Q-resolved sequence is identical to that of the standard *J*-resolved experiment, as given in the Table 1.[35] Disregarding all relaxation terms and phase factors, the expression of the signal during the acquisition period, after one single scan, is

$$S(t_1,t_2) = A\{\exp[i(\pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[-i(\pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2]\} \quad (3)$$

From the expression for $S(t_1,t_2)$, we can see that the chemical shifts are refocused in the t_1 domain, while the chemical shifts and the quadrupolar splittings evolve during t_2. The development of the sequence using the Cartesian spin-operator formalism shows that, during the t_2 period, only single-quantum coherences are different from zero.[17,18] In other words, it means that all the initial longitudinal magnetization is converted into observable magnetization during acquisition, thus driving to maximum sensitivity. Such a feature is obviously highly valuable for applications in NAD-NMR. As with the δ-resolved, this experiment is based on the phase modulation in the F_1 dimension, and the 2D spectrum should be displayed in the magnitude mode so as to cancel out the phase-twist line shapes. The 2D Q-resolved spectrum of the 1-pentanol-d_{12} is reported in Figure 2b. After a double Fourier transformation, the quadrupolar doublets are centered on the frequency $F_1 = 0$ Hz and actually lie on the anticlockwise 45° diagonals. The four slices drawn in the 2D contour plot enable us to identify the different components of quadrupolar splittings in the spectrum. As with the 2D *J*-resolved, an interesting aspect of the 2D Q-resolved experiment is the possibility of producing a deuterium chemical shift spectrum by projecting cross sections along these 45° diagonals onto the F_2 axis.[26,30] This allows separation of the ν and $\Delta\nu_Q$ information and, consequently, determination of the chemical shifts of all ^{2}H resonances directly as for the δ-resolved spectrum. After the tilting process, the 2D spectrum can be symmetrized with respect to the $F_1 = 0$ axis in order to eliminate the undesirable artifacts.

The 2D COSY-like Experiments. Two-dimensional COSY-like experiments can be applied to establish the respective

(42) Hahn, E. L. *Phys. Rev.* **1950**, *50*, 580.
(43) Carr, H. Y.; Purcell, E. M. *Phys. Rev.* **1954**, *94*, 630.
(44) Meiboom, S.; Gill, D. *Rev. Sci. Instrum.* **1958**, *29*, 688.

components of each deuterium quadrupolar. The basic idea of these experiments is to obtain a 2D contour plot in which the correlation peaks appear along the main 45° diagonal, as in a classical 2D COSY spectrum. For this purpose, we propose applying two new sequences deriving from the 2D COSY experiment: the Q-COSY P and Q-COSY sequences. Considering the formal analogy between the *J*-resolved and Q-resolved experiments, we may expect a priori to correlate the components of each quadrupolar doublet using the well-known COSY pulse sequence $(\pi/2)_{\phi 1}-t_1-(\pi/2)_{\phi 2}-\mathrm{acq}(t_2)_{\phi r}$. This statement is, however, wrong in the case of the conventional phase-cycled COSY experiment for a spin $I = 1$ nucleus, because the cross-peaks are suppressed after two scans and only the diagonal peaks (*N*-peaks) are visible in the 2D spectrum.[26] In fact, this undesirable situation can be avoided by shifting the phase of the receiver by 180°, which eliminates the result of the first two phase-cycling steps. Disregarding all relaxation terms and phase factors, the expression of the signal during the t_2 period after *two scans* is

$$S(t_1,t_2) = A\{\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[i(2\pi\nu + \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2]\} \quad (4)$$

In this experiment, the diagonal peaks are actually eliminated, and only the cross-peaks correlating the components of each quadrupolar doublet (*P*-peaks) appear in the spectrum after a double Fourier transform. As the *P*-peaks have been selected by the phase cycling, the spectrum in the F_1 dimension should be reversed to obtain correlations along the main diagonal, as in the classical COSY spectra. This first COSY-like experiment is denoted as "Q-COSY *P*", where *P* refers to the type of correlation peaks, and its phase cycling is given in Table 1.

Another possible solution to obtain both the cancellation of diagonal peaks and the observation of cross-peaks consists of replacing the $\pi/2$ read pulse of the Q-COSY *P* sequence by a π pulse. The new pulse sequence that we now denoted as "Q-COSY" is, therefore, $(\pi/2)_{\phi 1}-t_1-(\pi)_{\phi 2}-\mathrm{acq}(t_2)_{\phi r}$. The phase cycling is given in Table 1. Disregarding all relaxation terms and phase factors, the expression of the signal during t_2 after one single scan is

$$S(t_1,t_2) = A\{\exp[-i(2\pi\nu + \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2] + \exp[-i(2\pi\nu - \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2]\} \quad (5)$$

Contrary to the Q-COSY *P* experiment, the diagonal peaks cancel out after a single scan, and only the "*N*-type" cross-peaks are observed in the 2D spectrum. Consequently, it is not necessary to reverse the spectrum in the F_1 dimension as in the Q-COSY *P* spectra. Furthermore, as in the Q-resolved experiment, only single-quantum coherences are different from zero during the acquisition after a single scan, thus driving to maximum sensitivity. This 2D experiment actually is more sensitive, by a factor of 2, than the Q-COSY *P* experiment. This feature of the Q-COSY sequence is a major advantage for observing molecules through NAD-NMR spectroscopy as already mentionned. However like the δ- or Q-resolved experiments, the Q-COSY *P* and Q-COSY experiments are based on phase-modulation in the F_1 dimension, and the 2D spectrum should be displayed in the magnitude mode. In Figure 3a, the 2D Q-COSY spectrum of the 1-pentanol-d_{12} in PBLG is reported. As expected, all quadrupolar doublets are distributed along the main 45° diagonal of the 2D contour plot, which enables separation of all quadrupolar doublets.

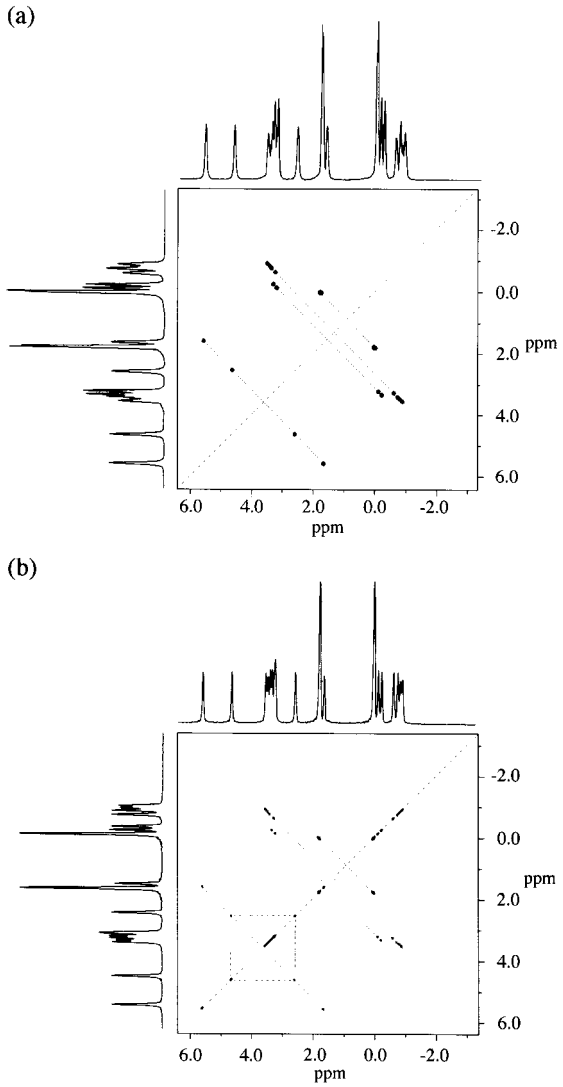

Figure 3. (a) Symmetrized 2D Q-COSY and (b) 2D Q-COSY Ph spectra of 1-pentanol-d_{12}. The Q-COSY Ph spectrum was recorded with eight scans for both echo and antiecho data sets (i.e., 16 scans for each t_1 increment) prior to the complex Fourier transformed according to the echo−antiecho mode. Note the enhancement of the resolution in the phase-sensitive spectrum.

It can be noted that the COSY-like spectra can be symmetrized with respect to the diagonal to remove some spurious signals and then tilted along the F_2 as in *J*-spectra, also giving a deuterium chemical shift spectrum in the F_2 dimension.[45] This data manipulation is actually advantageous in our case because on-diagonals peaks are absent in the 2D spectrum. After the tilt, all quadrupole doublets actually line up parallel to the F_1 axis and lead to slices located at the frequency 2ν in the F_2 axis, with respect to their true values (measured in F_1). This scaling factor is a consequence of the projection cross section theorem applied to a spectrum in which the chemical shifts were not refocused in the F_1 dimension.[26,46] Skew projection of the original spectrum on the F_2 axis allows us to scale both the quadrupolar splittings and the chemical shifts by an arbitrary factor. In our case, the scaling factors of the quadrupolar splittings (f_Q) and the chemical shifts (f_δ) obtained with the skew projection on the F_2 axis are respectively given by

(45) The tilting process can be achieved by using the computational algorithm developed for the *J*-RESOLVED experiments and usually implemented in the NMR software.

(46) Nagayama, K.; Bachmann, P.; Wütrich, K.; Ernst, R. R *J. Magn. Reson.* **1978**, *31*, 133.

$$f_Q = 1 - \cot(\varphi) \qquad \text{and} \qquad f_\delta = 1 + \cot(\varphi) \quad (6)$$

where the angle φ defines the direction of the skew projection on to the F_2 axis, which is usually referred to in the context of 2D *J*-spectra.[24] Thus, $\varphi = \pi/4$ (i.e., the classical tilting procedure) leads to $f_Q = 0$ and $f_\delta = 2$, while $\varphi = -\pi/4$, leads to $f_Q = 2$ and $f_\delta = 0$, which corresponds to the doubling of quadrupolar splittings and the elimination of the chemical shifts in the F_2 axis. The 2D spectrum after the conventional tilting procedure and their analysis is therefore formally comparable to that obtained on 2D Q-DQ spectra, except for the inversion of the F_2 and F_1 axes. Compared with the nontilted 2D spectrum, we gain a potential enhancement of the visibility of cross sections in the 2D spectrum and the ability to display each of them separately. Moreover, no severe distortions around the base of the peaks were observed in F_2 dimension. Two examples of symmetrized and tilted Q-COSY spectra will be presented and discussed in the last section of this paper.

One limitation of the Q-COSY sequences is that the 2D contour plot should be displayed in the magnitude mode to remove the phase-twist line shapes, which reduce both the spectral resolution and the S/N ratio.[26] It was, therefore, of interest to improve the basic 2D Q-COSY experiment, which gives spectra with peaks phased in pure absorption in both dimensions. No simple solutions directly derived from the Q-COSY or the Q-COSY *P* pulse sequence actually were obtained. The main reason is that these two sequences produce a phase modulation signal after a single scan, while an amplitude modulation is necessary to perform a phase-sensitive experiment. In our case, this amplitude modulation can be created if we enable discrimination between the signal of *N*- and *P*-peaks. For this purpose, we record the signal resulting from the pulse sequences $(\pi/2)_{x,x}-t_1-(\alpha)_{x,y}-\mathrm{acq}(t_2)_{x,-x}$ (odd experiment) and $(\pi/2)_{x,x}-t_1-(\alpha)_{x,y}-\mathrm{acq}(t_2)_{-x,-x}$ (even experiment), where α is set to $\pi/4$ and $3\pi/4$ successively in both sequences for each t_1 increment. The resulting signal from these experiments is then Fourier transformed using the echo−antiecho mode.[47,48] Note that, if the read pulse angle was only $\pi/4$ or $3\pi/4$, the *N*- and *P*-peak types would be obtained with different intensities, which could produce some unwanted artifacts in the 2D spectrum.[49] Disregarding all relaxation terms and phase factors, the expression of the *N*-peaks signal obtained for the odd experiments after four scans (2 + 2) is

$$\begin{aligned} S(t_1,t_2) = 2A\{&\exp[-i(2\pi\nu + \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[-i(2\pi\nu - \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2] + \exp[-i(2\pi\nu - \pi\Delta\nu_Q)t_1\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[-i(2\pi\nu + \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2]\} \end{aligned} \quad (7)$$

In the same way, the expression of the *P*-peaks signal obtained for the even experiments after four scans (2 + 2) is

$$\begin{aligned} S(t_1,t_2) = 2A\{&\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[i(2\pi\nu - \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2] + \exp[i(2\pi\nu - \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu + \pi\Delta\nu_Q)t_2] + \exp[i(2\pi\nu + \pi\Delta\nu_Q)t_1]\exp[i(2\pi\nu - \pi\Delta\nu_Q)t_2]\} \end{aligned} \quad (8)$$

Contrary to the Q-COSY *P* and Q-COSY experiments, the diagonal peaks and the cross-peaks are both observed in the 2D spectrum, and consequently the presentation of data is now very similar to the classical 2D COSY experiment. After two complex Fourier transformations using the echo−antiecho mode, we obtain a 2D contour plot where all peaks can be phased in pure absorption in both dimensions. We denoted this sequence as "Q-COSY Ph". The phase cycling is given in Table 2. The major interest of this sequence is the enhancement of the resolution compared with the magnitude mode, as shown on the 2D phased spectrum presented in Figure 3b. As the resolution is comparable with that of the 1D spectrum, we can observe the quadrupolar doublets of the β-, γ-, and δ-methylene groups more distinctly. Although the resolution attainable is higher than the equivalent magnitude mode spectrum, the global S/N ratio compared with the Q-COSY experiment is, however, reduced by a factor of $2^{1/2}$ (see Table 3). Actually, the resolution enhancement obtained with pure absorption phase peaks (S/N increased by a factor of $2^{1/2}$) does not permit compensating the lower sensitivity (factor of 2) of the Q-COSY Ph pulse sequence compared with the Q-COSY sequence (in terms of signal amplitude prior to the FT). In conclusion, although the Q-COSY Ph experiment is more interesting than the Q-COSY in terms of resolution, the phase-sensitive experiment is less adapted for NAD-NMR applications.

Table 2. Four-Step Phase Cycle of the Q-COSY Ph Experiments[a]

odd experiment			even experiment		
ϕ_1	ϕ_2[b]	ϕ_r	ϕ_1	ϕ_2[b]	ϕ_r
x	x	x	x	x	$-x$
x	y	$-x$	x	y	$-x$
x	$-x$	x	x	$-x$	$-x$
x	$-y$	$-x$	x	$-y$	$-x$

[a] The full phase cycling (16-steps cycle) is obtained by applying the CYCLOPS incrementation of all the pulse and receiver phases.[b] For each odd and even experiment, the detection pulse angle α is 45° and 135°, respectively.

To compare and discuss the analytical potentialities of these experiments, we have reported their respective NMR features in Table 3. Each of these experiments possesses both advantages and inconveniences in terms of resolution, sensitivity, and data presentation, and the choice of the sequence to be used depends mainly on the type and the quantity of organic compounds investigated. In this context, there is no doubt that the Q-COSY and Q-resolved experiments are the most adapted 2D techniques to applications in NAD-NMR or when the problem of sensitivity is crucial. On the other hand, the Q-COSY Ph experiments is the most efficient 2D approach when deuterated chiral materials are studied, since in this case the problem of spectral resolution is more important than that of the sensitivity.

Illustrative Examples in NAD-NMR

To explore and illustrate their potentialities in the field of the enantiomeric analysis, we present in this last section some applications of QUOSY experiments in NAD-NMR. We have previously pointed out that the Q-resolved and the Q-COSY experiments are the most convenient 2D experiments to analyze NAD spectra, and we limit the number of illustrative applications to these experiments.

Analysis of the (±)-1-Pentyn-3-ol through NAD-NMR. As the first example, we investigate the case of 1-pentyn-3-ol in racemic mixture dissolved in the PBLG−$CHCl_3$ phase. This chiral compound is interesting because it contains two dia-

(47) Davis, A. L.; Keeler, J.; Laue, E. D.; Moskau, D. *J. Magn. Reson.* **1992**, *98*, 207.
(48) Ancian, B.; Bourgeois, I.; Dauphin, J. F.; Shaw, A. *J. Magn. Reson.* **1997**, *125*, 348.
(49) Keeler, J.; Neuhaus, D. *J. Magn. Reson.* **1985**, *63*, 454.

Table 3. Comparative Table of Different 2D Autocorrelation Deuterium NMR Experiments

experiment features	Q-DQ	δ-resolved	Q-resolved	Q-COSY P	Q-COSY	Q-COSY Ph
α pulse angle	90°, 120°, or 135°	90°	180°	90°	180°	45° and 135°
minimum step number of the phase cycling	8	2	1	2	1	(2 + 2)
total step number of the phase cycling	32 or 64	16	16	16	16	(16 + 16)
number of quantums involved	2Q	1Q	1Q	1Q	1Q	1Q
phase-sensitive mode	possible	no	no	no	no	yes
refocalization period	yes	no	no	no	no	no
reverse in F_1	no	yes	no	yes	no	no
diagonal peaks	no	no	no	no	no	yes
symmetrization procedure	no	no	yes (after tilt)	yes	yes	yes
tilt procedure	no	no	yes	yes	yes	no
axis parallel to the cross sections in the 2D spectra	F_2	F_2	F_1 (after tilt)	F_1 (after tilt)	F_1 (after tilt)	
normalized sensitivity[a]	*c*	1	2	1	2	$2^{1/2}$
main characteristic of the sequence	not quantitive	low sensitivity	maximum sensitivity adapted to NA[b]	low sensitivity	maximum sensitivity adapted to NA[b]	maximum resolution

[a] The sensitivity of the δ-resolved experiment is used as reference. [b] NA: $^2H-\{^1H\}$ NMR in natural abundance. [c] The sensitivity of the experiment dependents on both α and τ parameters.

stereotopic nuclei associated with deuterons of the methylene group (case of *semi-isotopic* diasteoreisomers). For this group, four quadrupolar doublets centered on the same chemical shift are expected to be seen in the NAD-NMR spectrum, as already discussed. Consequently, we may a priori observe a maximum of 11 quadrupolar doublets, corresponding to 10 different chiral isotopomers and the solvent, but disregarding the −OD signal.

A first analysis of this 1D NAD spectrum, recorded using 60° pulses, 0.5 s recycling delay, and 150 000 scans with 1K data points,[8] enabled us to identify easily the two components of the $CHCl_3$ cosolvent. The quadrupolar splitting measured in the spectrum is 495 Hz. This value is typical for the sample concentration and the DP of PBLG used and facilitates both its identification and its assignment in the spectrum.[8] Note here that signals from PBLG itself do not appear in the NAD spectra, because of their large line widths compared to those of the solute and organic cosolvent. Apart from the solvent signals, the analysis of other peaks is, however, nontrivial, requiring the help of the 2D experiments.

The 2D NAD Q-COSY spectrum of (±)-1-pentyn-3-ol is presented in Figure 4. The 2D spectrum was acquired using 0.5 s repetition time and 2000 Hz of spectral width in both dimensions. A total of 448 transients were accumulated for each t_1 increment, leading to a total of 114 688 scans and a total acquisition time of 18 h. The data matrix of 256 (t_1) × 1024 (t_2) data points was weighted with 1 and 2 Hz exponential line broadening in t_1 and t_2, respectively, and zero-filled to 1024 (t_1) × 1024 (t_2) prior to 2D Fourier transformation. The 2D contour plot is shown after the tilting procedure, and the different deuterium subspectra are displayed above the 2D contour plot. As described previously, the chemical shifts of each deuteron appeared in the F_2 dimension with a scaling factor of 2 compared with those in the F_1 dimension. From each of them, we can observe the signals of the corresponding isotopomers in the mixture. Thus, the analysis of traces shows that the enantiomers are clearly discriminated on the methylene signals (four doublets), the chiral center, and the acetylenic group (two doublets), while the deuteron signals of the methyl group (CDH_2) show no discrimination. Finally, the least shielded trace, located at 7.3 ppm, corresponds to the chloroform signal. We can observe strong differences of the S/N ratios for each trace. This situation reflects both the number of equivalent isotopomers (for a given signal) and the fact that signals are or are not discriminated. Thus, the two doublets of the deuteron on the chiral center are 6 times less intense than the signal of the methyl

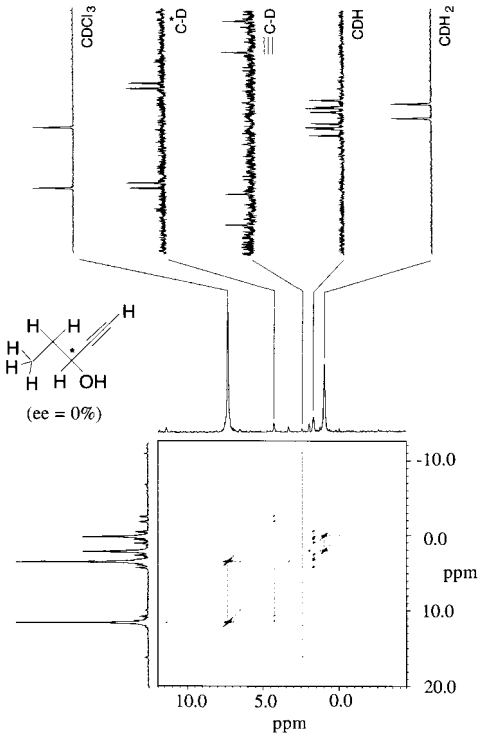

Figure 4. Tilted 2D spectrum of NAD Q-COSY experiment obtained for (±)-1-pentyn-3-ol in 18 h. The spectrum was symmetrized prior to performing the tilting procedure. Note that all isotopomers of the mixture are clearly visualized.

group which is not discriminated, hence the strong degradation of S/N observed on the corresponding trace. For the same reason, we can see on the subspectra that the intensity of the $CDCl_3$ doublet is very close to that of the methyl group. In this case, the percentage of deuterated isotopomers of chloroform is comparable with that found for isotopomers associated with the methyl group when no discrimination occurs. It may also be noted that the S/N ratio for the deuterium signal of the chiral center is 10 and that of the acetylenic group is around 6, while both groups are discriminated and the number of deuterons contributing to the peaks are equivalent. This difference in the S/N ratios is due to the fact that, in ordered media, the line widths increase with the magnitude of the quadrupolar splittings, leading to the reduction of line intensity compared with signals of same integral.

To compare the potential of the Q-COSY and Q-resolved experiments, we have recorded the 2D natural abundance $^2H-\{^1H\}$ Q-resolved experiment of (±)-1-pentyn-3-ol. The 2D spectrum was acquired with the same NMR conditions as described for the 2D Q-COSY experiment. The analysis of each trace mainly shows the same result as the Q-COSY experiment. However, the S/N ratio is lower than for the Q-COSY, as can be clearly seen on the subspectrum corresponding with the acetylenic group. This degradation of the S/N ratio for the weaker peaks most probably originates in a nonperfect 180° deuterium pulse in the Q-resolved sequence, while this undesirable situation was not observed for the Q-COSY. When the refocusing pulse flip angle actually differs slightly from 180°, we have checked that the spin−echo amplitude is not maximum after the second $t_1/2$ period and leads to a noticeable loss of observable magnetization.[50,51] For the Q-COSY sequence, this misadjustment is less important than in the Q-resolved because the 180° pulse is not a refocusing pulse but a read pulse. In this case, a nonideal 180° pulse flip mainly generates on-diagonal peaks in the 2D spectrum. We have also remarked small intensity distortions with offset. This can be understood in terms of offset effects and may possibly be circumvented using a composite pulse.[26]

Measurements of Enantiomeric Excess Using Q-Cosy Experiments. The final goal of this work is to demonstrate that measurements of enantiomeric excesses are possible using 2D QUOSY experiments. For this purpose, we have recorded the 2D NAD Q-COSY spectrum of 3-butyn-2-ol enriched in *S*-enantiomer (ee = 50%). The repetition time was 0.5 s, and the spectral width was of 2200 Hz in both dimensions. The 2D data matrix was acquired using 384 (t_1) × 1024 (t_2) data points and zero-filled 1024 (t_1) × 1024 (t_2) prior to 2D Fourier transformation. Six hundred forty transients were added per FID, leading to a total of 245 760 scans and an experiment time of 34 h. No filtering windows were applied. The tilted 2D contour plot and the traces for each subspectrum are shown in Figure 5.

The analysis of each trace shows an enantiomeric discrimination for the deuterons on the methyl group and on the chiral center of the molecule. Actually, the signal of the acetylenic group is also discriminated; however, the bad S/N ratio for the *S*-enantiomer doublet does not permit us to see its signal clearly from noise, as shown in the trace extracted from the 2D spectrum. Note here that the amount of *S*-enantiomer is 3.5×10^{-4} mol, i.e., 5.3×10^{-8} mol for the corresponding deuterated isotopomer. This situation perfectly illustrates the benefit of NAD-NMR spectroscopy, with which we can simultaneously probe all possible deuterated sites of the molecule and, therefore, determine the most suitable site in the molecule for the measurement of enantiomeric excess. The choice of site depends on both the S/N ratio and the difference in quadrupolar splittings. Obviously, the best sites for chiral discrimination would always be those groups which possesses the largest number of magnetically equivalent spins that contribute to a given doublet and the strongest difference of quadrupolar splittings. In this example, the signal from the methyl offers the best site for quantitative measurements. Thus, S/N ratios for the signal of the *R*- and *S*-enantiomers calculated are 37 and 80, respectively with a difference in quadrupolar splittings ($\Delta\nu_Q^R - \Delta\nu_Q^S$) equal to 70.1 Hz. The measured enantiomeric excess obtained by a simple peak integration of methyl group signals in the corresponding trace (averaged value on a series of integration) is 55 ± 10%, while the expected value was 50%. This experimental result shows that measurements of enantiomeric excess are possible through 2D NAD-NMR in PBLG within an accuracy of ±10% for reasonable signal-to-noise ratios. Such measurements provide, therefore, a very valuable estimation of the enantiomeric excess in the mixture without isotopic enrichment and using a reasonable quantity of chiral mixture. Besides, this technique allows determination of the best site for chiral discrimination in the molecule and, subsequently, choice of the most adequate synthetic strategy for an eventual introduction of a deuteron in the molecule.

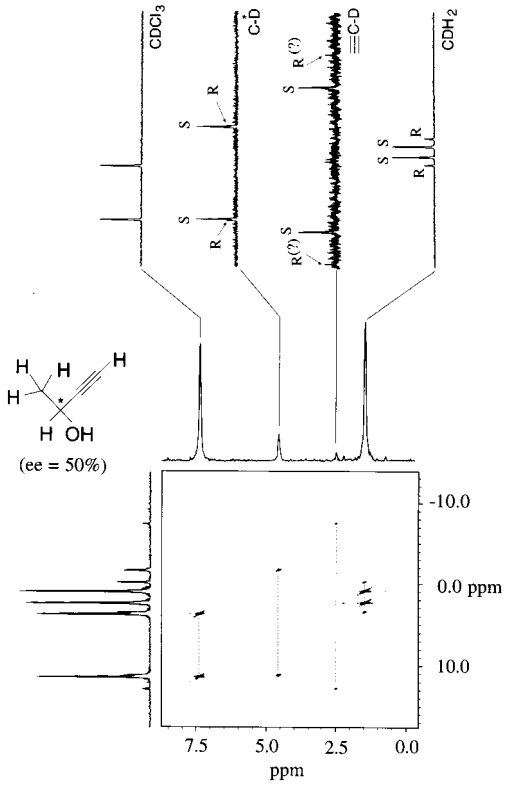

Figure 5. Tilted 2D contour plot of NAD Q-COSY spectrum of 3-butyn-2-ol mixture enriched in *S*-enantiomer (ee = 50%). The total number of scans is 245 760, and the experimental time is 34 h. The F_2 traces after tilting of the 2D data matrix are presented.

Conclusions

In natural abundance as well as in perdeuterated materials, the proton-decoupled deuterium spectra of a molecule dissolved in PBLG liquid crystalline solutions consist of a superposition of independent quadrupolar doublets. To simplify their analysis, we have developed several two-dimensional autocorrelation deuterium NMR experiments, referred to as QUOSY, which make it possible to correlate the two components of quadrupolar doublets. In this study, we have shown the interest of these sequences in the analysis of poly- and perdeuterated molecules and demonstrated their feasibility and their potential in natural abundance deuterium NMR spectroscopy, thus proposing a practical solution to DOE measurements in PBLG without site-specific isotopic labeling. These results also show that the measurement of enantiomeric excess in 2D NAD-NMR using a 9.4 T field and standard NMR equipment is possible within an accuracy of 10%, thus already providing a good evaluation of the ee. It is clear that, by using a very high magnetic field NMR spectrometer (17.6 T), it will be possible to record the spectra with shorter experimental times and to determine the enantiomeric excess with a higher and satisfactory precision.[8,52]

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In conclusion, the combination of QUOSY experiments and the NAD-NMR spectroscopy provides, therefore, a novel and interesting analytical method. We believe that the approach may be a methodology of choice soon in the field of enantiomeric analysis.

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