2D-NMR Strategy Dedicated to the Analysis of Weakly Ordered, Fully Deuterated Enantiomers in Chiral Liquid Crystals

Philippe Lesot,*,[†] Muriel Sarfati,[†] Denis Merlet,[†] Bernard Ancian,[‡]
James W. Emsley,[§] and Bakir A. Timimi[§]

*Contribution from the Laboratoire de Chimie Structurale Organique, CNRS UMR 8074,
ICMMO, Bât. 410, Université de Paris-Sud, 91405 Orsay, France, the Université Paris
VII-Denis Diderot, Département de Chimie, 2 Place Jussieu, 75251 Paris Cedex 05, France, and
the University of Southampton, Department of Chemistry,
Highfield Southampton SO17 1BJ, United Kingdom*

Received October 16, 2002; Revised Manuscript Received March 4, 2003

Abstract: Methods for the assignment of the quadrupolar doublets in the deuterium NMR spectra of weakly ordered, perdeuterated or partially deuterated enantiomers dissolved in chiral liquid crystals are described which use robust 2D correlation NMR experiments. To overcome a lack of resolution in deuterium tilted *Q*-COSY 2D spectra in such materials, we propose and explore a correlation 2D sequence which is based on deuterium−carbon 2D correlation spectroscopy. The technique results in a $^{13}C-^2H$ contour plot and allows the full resonance assignment of overcrowded deuterium 1D spectra using carbon−deuterium correlations. The ^2H autocorrelation and $^{13}C-^2H$ correlation experiments are applied in the case of a racemic mixture of 2-ethylhexanoic acid-d_{15} dissolved in a polypeptidic chiral oriented solvent. The performance and the limits of both techniques are presented and discussed. For the last step of the assignment procedure, we propose a simple method for obtaining two coherent sets of quadrupolar splittings, one for each enantiomer.

Introduction

Deuterium NMR spectrocopy is a powerful tool for the investigation of orientational ordering, conformational dynamics, and molecular structure of molecules dissolved in liquid crystalline phases.[1] However, the quadrupolar doublet assignment of perdeuterated or partially (nonselectively) deuterated molecules becomes very difficult as the number of nonequivalent deuterium sites in a molecule increases. This situation is particularly complex when the molecules are chiral and are dissolved in a chiral liquid crystal (CLC). In such phases the two enantiomers have different orientational ordering.[2] Consequently each of them has its own spectrum,[3] and the deuteriums in CD_2 groups are nonequivalent since they are diastereotopic. A number of different NMR strategies has been developed for the general problem of the assignment of quadrupolar doublets. Thus, Emsley and Turner applied a spin−echo method to the assignment of the doublets in fully deuterated, thermotropic liquid crystals.[4] In these strongly oriented molecules, the magnitude of the quadrupolar splittings is large compared with

the ^2H chemical shift dispersion, and almost symmetrical spectra result from which the identification of the components for each quadrupolar doublet is rather trivial, but their assignment is not. The solution proposed by these authors relied on the observation of cross-peaks in the 2D spectrum which arise because there is a dipolar coupling between adjacent deuteriums in the molecule. A complete assignment was achieved by assuming the assignment of one site in the molecule. A similar, but in fact more efficient, method was recently proposed, which is a COSY-type experiment and was dubbed as *Q*-COSY.[5] This method was applied to the case of weakly ordered enantiomer solutes in the chiral nematic phase obtained by dissolving a polypeptide, such as poly-γ-benzyl-L-glutamate (PBLG) or poly-ϵ-carbo-benzyloxy-L-lysine (PCBLL) in a solvent such as $CHCl_3$.[5,6] In these phases the quadrupolar splittings have approximately the same magnitude as the deuterium chemical shift dispersion. Consequently, the 1D-NMR spectra are no longer symmetrical, and the assignment of the quadrupolar doublets can be made on the basis of the ^2H chemical shifts, but the identification of each component of the doublet becomes more difficult. The 2D *Q*-COSY experiment easily identifies the corresponding components in each quadrupolar doublet, provided that the deuterium

---

* To whom correspondence should be addressed: Dr. Philippe Lesot, e-mail: philesot@icmo.u-psud.fr; fax: 33 (0)1-69-15-47-70.
† Université Paris-Sud (Paris XI).
‡ Université Denis Diderot (Paris VII).
§ University of Southampton.

(1) Emsley, J. W.; Lindon, J. C. *NMR Spectroscopy Using Liquid Crystal Solvents*; Pergamon Press: Oxford, 1975.
(2) Lesot, P.; Merlet, D.; Rantala, T. P.; Jokisaari, J.; Emsley J. W.; Courtieu J. *J. Phys. Chem. A* **1997**, *101*, 5719.
(3) Sarfati, M.; Lesot, P.; Merlet, D.; Courtieu, J. *Chem. Commun.* **2000**, 2069.
(4) Emsley J. W.; Turner, D. L. *Chem. Phys. Lett.* **1981**, *82*, 447; Emsley, J. W. In *Nuclear Magnetic Resonance of Liquid Crystals*; Emsley, J. W., Ed.; Reidel: Drodrecht, 1985; p 398.
(5) (a) Merlet, D.; Ancian, B.; Courtieu J.; P. Lesot P. *J. Am. Chem. Soc.* **1999**, *121*, 5249. (b) Lesot, P.; Sarfati, M.; Merlet, D.; Courtieu, J.; Ancian, B.; Brevard, C. *Bruker Rep.* **2001**, *149*, 29.
(6) Aroulanda, C.; Sarfati, M.; Courtieu, J.; Lesot, P. *Enantiomer* **2001**, *6*, 2081.

chemical shifts are sufficiently well dispersed, and has been successfully applied to simplify the analysis of congested natural abundance deuterium 1D-spectra.[5,7] Another approach was suggested by Emsley and co-workers, who proposed a 2D deuterium−carbon correlation NMR technique, referred to as DECOR, and illustrated its usefulness by applying it to assign the quadrupolar doublets in the thermotropic 5CB (4-*n*-pentyl-4′-cyanobiphenyl).[8] The method correlates the deuterium doublets with the chemical shift of the attached ^{13}C, and now there is a much greater resolving power as a consequence of carbon shift dispersions being much larger than those of deuterium. In the DECOR sequence a cross-polarization (CP) step is applied to transfer uniformly the magnetization from deuteriums to carbons.[9] The deuterium resonances are split by the quadrupole interaction, and hence to satisfy the Hartmann−Hahn condition it is necessary to sweep the ^{2}H frequency ("ramp") in the CP step. More recently, Sandström and Zimmermann have described a heteronuclear multiple-quantum 2D experiment based on the standard HMQC scheme to also investigate deuterated oriented materials.[10] The advantage is that it is not necessary to use a CP ramp for magnetization transfer between ^{2}H and ^{13}C spins, considerably simplifying the setup of the experiment. However, it is necessary to decouple the deuteriums from the carbons, and this can be a problem when the ^{13}C−^{2}H total spin−spin coupling (dipolar and scalar) becomes large in strongly ordered liquid crystalline solvents.

We describe here a new 2D experiment for making the assignment of quadrupolar doublets in weakly ordered solutes. This is also a ^{13}C−^{2}H correlation experiment, but it is based on the HETCOR method, which uses an INEPT transfer of polarization from the deuterium to the carbon-13 nucleus. This approach is compared with the *Q*-COSY 2D experiment in the study of very crowded deuterium 1D spectra. As an illustrative example we investigate a chiral, perdeuterated acid, 2-ethylhexanoic acid-d_{15} (EHA) as a racemic mixture dissolved in an organic solution of PBLG. We also present a simple method to distinguish between the quadrupolar doublets for two enantiomers and hence to obtain two sets of coherent data, one for each enantiomer.

Experimental Section

Synthesis. The perdeuterated (±)-2-ethylhexanoic acid was synthesized by a catalytic deuterium exchange method using D_2O in a slightly alkaline condition at high temperature and pressure.[11] A racemic mixture of 2-ethylhexanoic acid (7.5 g), sodium hydroxide pellets (3 g), platinum on carbon (10%, 2.5 g), and D_2O (80 g) was heated and stirred in a stainless steel high-pressure vessel at 180 °C for 7 days. After a careful neutralization of the mixture, the perdeuterated acid was isolated by extraction with ether. The pure perdeuterated acid (∼6.5 g) was finally obtained by distillation. The level of deuteration was found by proton NMR to be ∼90%.

NMR Spectroscopy. The chiral liquid crystalline NMR samples were prepared using about 100 mg of PBLG (commercially available from Sigma) with a MW ∼112 000, 50 mg of *per*deuterated (±)-2-
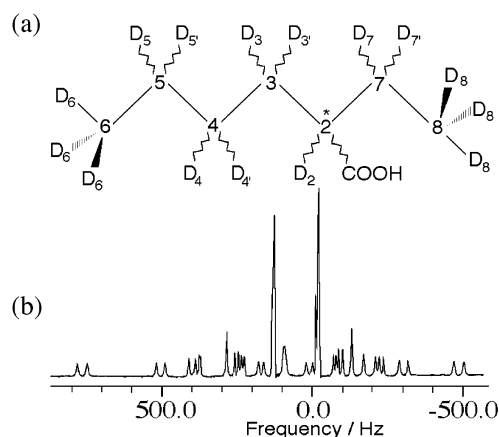
(7) (a) Lesot, P.; Merlet, D.; Loewenstein, A.; Courtieu, J. *Tetrahedron: Asymmetry* **1998**, *9*, 1871. (b) Parenty, A.; Campagne, J.-M.; Aroulanda, C.; Lesot, P. *Org. Lett.* **2002**, *4*, 1663
(8) (a) Auger, C.; Lesage, A.; Caldarelli, S.; Hodgkinson P.; Emsley, L. *J. Am. Chem. Soc.* **1997**, *119*, 12000. (b) Auger, C.; Lesage, A.; Caldarelli, S.; Hodgkinson P.; Emsley, L. *J. Phys. Chem. B* **1998**, *102*, 3718.
(9) Hodgkinson, P.; Auger C.; Emsley, L. *J. Chem. Phys.* **1998**, *109*, 1874.
(10) (a) Sandström, D.; Zimmermann, H. *J. Phys. Chem. B* **2000**, *104*, 1490. (b) Sandström, D.; Zimmermann, H. *Mol. Phys.* **2002**, *100*, 1935.
(11) Zimmermann, H. *Liq. Cryst.* **1989**, *4*, 591.

Figure 1. (a) Numbering system of the atoms of perdeuterated EHA. (b) 61.4 MHz ^{2}H-{^{1}H} 1D spectrum of the EHA in racemic mixture dissolved in the PBLG/CHCl$_3$ phase at 300 K using a simple one-pulse sequence. A Gaussian filtering (LB = −2 Hz, GB = 80%) was applied to enhance the spectral resolution.

ethylhexanoic acid, and 350 mg of organic cosolvent directly weighed into a 5 mm o.d. NMR tube. All NMR tubes were sealed to avoid solvent evaporation and centrifuged back and forth until an optically homogeneous birefringent phase was obtained. Full details of the method can be found in ref 3. The 1D and 2D experiments were performed at 9.4 T on a Bruker DRX 400 high-resolution spectrometer equipped with an inverse triple nuclei probe (TXI) operating at 61.4 MHz for deuterium and 100.6 MHz for carbon. The temperature of the sample was maintained by the standard variable temperature unit (BVT 3200), and the tube was not spun. All experiments have been recorded at 300 K. The 2D matrixes were zero-filled to 1k (t_1) × 2k (t_2) data points prior to the double Fourier transformation, and the spectra were displayed in magnitude mode. To remove residual proton−deuterium scalar and dipolar couplings in the molecule, the protons were broadband decoupled using the WALTZ-16 composite pulse sequence.[3] For the carbon−deuterium correlation 2D experiments, the deuterium channel (lock) of the probe was used. Other experimental NMR parameters or details are given in the figure captions.

Results and Discussion

Figure 1 shows the deuterium 1D spectrum of a racemic mixture of EHA dissolved in the PBLG/CHCl$_3$ phase at 300 K. The (±)-EHA is approximately 90% deuterated, and so the spectrum was recorded with proton decoupling to cancel out any ^{2}H−^{1}H scalar and dipolar couplings. The chiral nature of the molecule means that the two diastereotopic deuterons in each CD$_2$ group are potentially nonequivalent and hence will have different quadrupolar splittings.[5] Disregarding the carboxylic group, there are one CD site, four CD$_2$ sites, plus the two CD$_3$ groups so that there are 11 nonequivalent deuterium sites in each enantiomer, making a maximum of 22 quadrupolar doublets in total in the racemic mixture. In practice there are only 36 resolved peaks, and so there is either some overlapping of peaks or some sites do not have a resolvable difference between the two enantiomers.

Deuterium *Q*-COSY 2D Experiments. First we show the result of applying the deuterium *Q*-COSY 2D experiment.[5,12] The pulse scheme of this experiment is shown in Figure 2a. Although the *Q*-COSY 2D experiment presents a formal analogy with some sequences applied for spin $I = 1/2$, it cannot be simply compared with them because it has been specifically

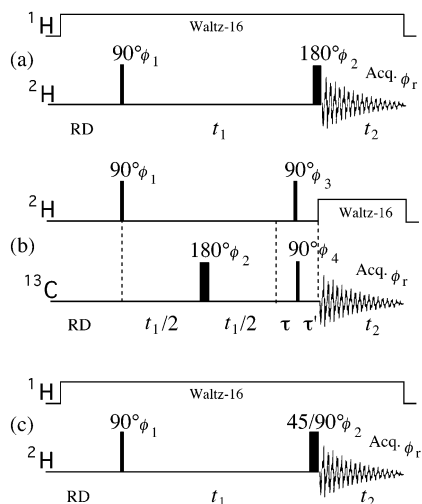(12) Merlet, D.; Sarfati, M.; Ancian, B.; Courtieu J.; Lesot, P. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2283.

Figure 2. Basic pulse schemes for deuterium 2D NMR spectroscopy of weakly ordered, deuterated solutes: (a) ^{2}H Q-COSY 2D experiment. (b) ^{2}H−^{13}C HETCOR 2D experiment with ^{13}C decoupling during evolution and ^{2}H decoupling during acquisition. (c) ^{2}H−^{2}H COSY 2D experiment. The full phase cycling of sequence (a) and its variant using composite pulses can be found in ref 5b. For sequences b and c, the phase cycling used is identical to that provided in the literature.[13]

designed for spin $I = 1$. In this sequence, the first pulse creates single quantum coherences that evolve during the t_1 period. The 180° pulse produces a total coherence transfer between "intra-nuclear" single quantum coherences that evolve again during the detection period t_2, and consequently it cannot create internuclear correlations.[5,12] Consequently for ^{2}H−^{2}H coupled systems, the Q-COSY spectrum is a sum of independent quadrupolar doublets, and hence no cross correlation peaks are observable on the 2D map. After a basic one-step phase cycle and disregarding all relaxation terms and phase factors, the expression for the signal is

$$S(t_1, t_2) = A\{\exp[-i(\omega_D + \omega_Q)t_1] \times \exp[i(\omega_D - \omega_Q)t_2] + \exp[-i(\omega_D - \omega_Q)t_1] \times \exp[i(\omega_D + \omega_Q)t_2]\} \quad (1)$$

for a single deuterium atom. In eq 1, $\omega_D = 2\pi\nu_2$H and $\omega_Q = \pi\nu_Q$ denote the deuterium frequency and quadrupolar splitting, respectively.

After the second Fourier transformation, a 2D map is obtained in which only the quadrupolar doublets are visible and distributed along the main 45° diagonal, enhancing the visibility of the 2D spectrum. As diagonal peaks are absent in the 2D Q-COSY, it is possible to tilt the data as in a *J*-resolved 2D experiment. In this case, all the quadrupolar doublets line up parallel to the F_1 axis with a scaling factor of 2 in the F_2 dimension.[5,12] This data manipulation produces a deuterium chemical shift spectrum in the F_2 dimension and allows the separation and the extraction of the signals of all the inequivalent deuterons in the molecule.

In Figure 3a the tilted 2D spectrum of the ^{2}H Q-COSY experiment obtained for the (±)-EHA is displayed. As expected, the quadrupolar doublets centered on their respective chemical shifts, δ ^{2}H, line up parallel to the F_1 dimension, while the spectrum in the F_2 dimension is formally identical to the ^{2}H-$\{^1$H$\}$ spectrum recorded in an isotropic solvent, as shown in Figure 3b. The magnitude of the deuterium chemical shifts means that some of the quadrupolar doublets can be tentatively
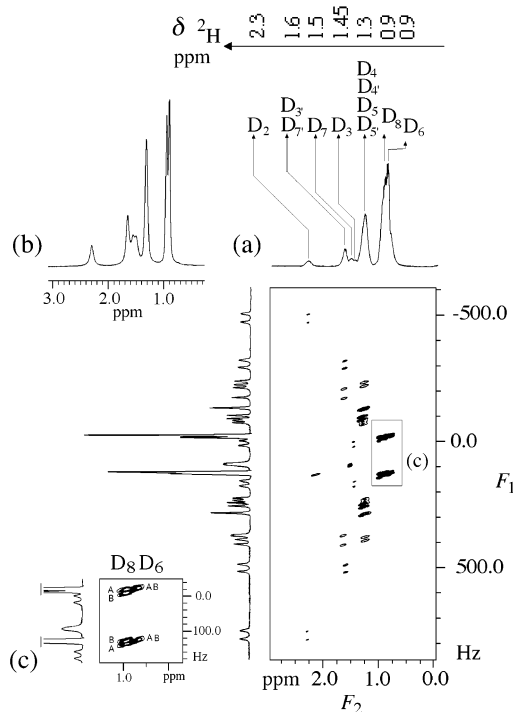

Figure 3. (a) Tilted 2H Q-COSY 2D map obtained for the (±)-EHA in 2 h. To overcome undesirable residual on-diagonal peaks in the Q-COSY, each pulse has been replaced by a composite pulse which has a greater tolerance to imperfections.[12] The spectrum was symmetrized prior to the tilt procedure. The spectrum was acquired as a 300 (t_1) × 1344 (t_2) data matrix with 16 scans per t_1 increments and identical spectral widths in the two dimensions. Sine-bell filtering was used in the F_1 and F_2 dimensions to enhance the resolution of the magnitude spectrum. In F_1 and F_2 dimensions are displayed the ^{2}H-$\{^1$H$\}$ 1D spectrum. (b) Isotropic ^{2}H-$\{^1$H$\}$ spectrum of (±)-EHA recorded in CDCl$_3$. (c) Expansion on the region of methyl groups.

assigned. For instance the lowest field signal corresponds to the deuteron located on the asymmetric carbon, while the most shielded can be safely assigned to one of the methyl groups. However in this example the exact assignment of methyl groups 6 and 8 based only on analysis of the Q-COSY spectrum is not possible. Also the correct assignment of the diastereotopic deuterium nuclei in CD$_2$ groups is not straightforward. This situation is very specific for flexible molecules for which such an assignment can be particularly puzzling. The main reason is that the chemical shift difference between two diastereotopic deuterons belonging to the same carbon atom is not easy to predict, and their signals can overlap with other resonances coming from other CD$_2$ groups. Thus we can see that at least six distinct quadrupolar doublets participate to the peak located around 1.3 ppm. Finally the analysis of columns of the 2D spectrum indicates that several deuterated sites show a chiral discrimination, since two or more doublets are centered on the same or very similar chemical shifts on the 2D map. This is clearly the case for the deuteron at site 2, while the most shielded methyl group 6 (vide infra) does not exhibit spectral enantio-discrimination. However here again the difficult task is to determine unambiguously if the diastereotopic deuterons show a spectroscopic enantiodiscrimination or not.

Deuterium−Proton and Proton−Deuterium 2D Correlation Experiments. The chemical shift range for the deuteriums in EHA is rather small and limits the usefulness of the Q-COSY experiments in this case. To overcome this limitation, we turn

our attention to direct and inverse heteronuclear 2D correlation experiments. It could be argued here that for partially or statistically deuterated molecules, deuterium−proton or proton−deuterium correlation experiments are more interesting because we benefit from a very sensitive nucleus, the proton. This remark is a priori judicious, but not true. Indeed attempts to perform these heteronuclear correlation 2D experiments produced a dramatic loss of deuterium signal compared with simple 1D measurements. The main reason is that the small magnitude of deuterium−proton scalar and dipolar couplings in very weakly oriented solutes requires unacceptably large delays for the transfer periods in the NMR sequences with respect to the deuterium T_1 and T_2 relaxation times.

Deuterium−Carbon 2D Correlation Experiments. The HMQC 2D experiment proposed by Sandström and Zimmermann has an overall sensitivity, under ideal NMR conditions, proportional to[14]

$$S/N \propto \{(\gamma_{13_C})^{5/2}\}\{1 - \exp(- T/T_1^{13_C})\} \qquad (2)$$

where γ_{13C} is the gyromagnetic ratio of the carbon nucleus. The sensitivity is optimized by having $T > T_1^{13C}$, the longitudinal relaxation time of the carbon atoms, which can be unfavorably long, thus resulting in very long experimental times.

To overcome this disadvantage, we have explored a direct heteronuclear correlation 2D experiment based on the classical HETCOR pulse sequence which uses a single INEPT transfer step.[14,15] The pulse scheme of this experiment is given in Figure 2b. Although this pulse sequence has been used extensively for recording carbon−proton 2D maps in isotropic solvents, its application for analyzing solutes in oriented media have not been explored so far, to the best of our knowledge.

The global sensitivity for this pulse sequence under ideal conditions is proportional to[14]

$$S/N \propto \{\gamma_{2_H}(\gamma_{13_C})^{3/2}\}\{1 - \exp(- T/T_1^{2_H})\} \qquad (3)$$

where γ_{2H} is the gyromagnetic ratio of deuterium nuclei and T_1^{2H} is its longitudinal relaxation time. Compared to eq 2, the sensitivity depends now on the longitudinal relaxation time of the deuterium spins, T_1^{2H}, whereas the first term is reduced by a factor $\gamma^2_H / \gamma^{13}_C$. This loss in the first term is balanced by T_1^{2H}, which is usually much shorter than T_1^{13C}.[15] This allows a shorter recycling time to be used between subsequent scans, and therefore the total time for $^2H−^{13}C$ HETCOR 2D experiments can be significantly reduced. This situation is very advantageous for weakly ordered compounds because the values of the T_1^{2H} are generally below 1 s. There is another advantage of the HETCOR-type experiment in that the line width in the F_1 dimension is less than that for the HMQC type. This is because in the latter experiment the line width is mainly determined by the relaxation rate of multiple quantum coherence, which often leads to broadening since multiple quanta usually relax faster than single quantum coherences.[16]

During the variable evolution period t_1 that follows the first ^2H $\pi/2$ pulse, quadrupolar-modulated single quantum coherences evolve, while the $^{13}C−^2H$ total spin−spin couplings, T_{CD}, are refocused by the central ^{13}C π pulse. The removal of couplings by refocusing has the additional advantage of requiring much less average power than broad-band decoupling. The coherences are converted into detectable ^{13}C magnetization during the polarization transfer step that is made by simultaneous application of the ^2H and ^{13}C $\pi/2$ pulses. Finally with the broad-band, deuterium, composite pulse decoupling the signal during t_2 evolves only under the ^{13}C chemical shift. After a two-step phase cycle and disregarding all relaxation terms and phase factors, one obtains the following expression for the time domains t_1 and t_2

$$S(t_1, t_2) \propto \{\exp(i\omega_C\tau') \times \sin(2\omega_{CD}\tau') \times \sin(\omega_{CD}\tau)\} \times$$
$$\{\sin[(\omega_D \pm \omega_Q)\tau] \times \cos[(\omega_D \pm \omega_Q)t_1] +$$
$$\cos[(\omega_D \pm \omega_Q)\tau] \times \sin[(\omega_D \pm \omega_Q)t_1]\} \times \{\exp(i\omega_C t_2)\} \quad (4)$$

for a coupled deuterium−carbon pair. In this equation $\omega_D = 2\pi\nu_{2H}$, $\omega_C = 2\pi\nu_{13C}$, $\omega_Q = \pi\nu_Q$, and $\omega_{CD} = \pi T_{CD}$ where T_{CD} is the $^{13}C−^2H$ total spin−spin coupling, $T_{CD} = J_{CD} + 2D_{CD}$. The double Fourier transform of this signal gives a 2D map in which the ^{13}C chemical shifts in the F_2 dimension are correlated to the ^2H quadrupolar splittings centered on their respective chemical shifts in the F_1 dimension. As previously the quadrupolar doublets line up parallel to the F_1 axis, but are now centered on ^{13}C chemical shifts. Actually the 2D contour plot presents a formal analogy with the tilted deuterium Q-COSY spectrum, because we obtain a sum of independent quadrupolar doublets in the F_1 dimension, but offers a better reading of quadrupolar doublets because of the larger ^{13}C chemical shift dispersion.

Figure 4 shows the $^2H−^{13}C$ correlation spectrum of ($\pm$)-EHA obtained in magnitude mode after 15 h of recording time using a 128 (t_1) × 1600 (t_2) data matrix with 512 scans per t_1 increment, and a repetition time of 0.712 s. The ^2H and ^{13}C transmitter frequencies have been set at the center of the ^2H and ^{13}C spectrum to minimize the spectral width required in both dimensions. The optimization for the length of the refocalization delays τ and τ' (before and after the deuterium to carbon-13 magnetization transfer) is not simple (see Figure 2b).[17] The optimum choice for τ for a given one-bond $^{13}C−^2H$ pair should be in principle be equal to $1/2[^1T_{C-D}]$ with the total spin−spin coupling $^1T_{C-D} = {}^1J_{C-D} + 2[^1D_{C-D}]$. However accounting for the dispersion of directly connected $^{13}C−^2H$ total spin−spin couplings, it is suitable to use an approximate average of the values of the different $^1T_{C-D}$. This was done by assuming that the $^1J_{C-D}$ is ~20 Hz and positive, as all $^1J_{C-H}$ have positive sign.[2,18] The evaluation of $|^1D_{C-D}|$ is also possible assuming that the ratio $|\Delta\nu_Q^{C-D}/^1D_{C-D}|$ is fixed by the quadrupolar and dipolar constants at about 80. Finally the choice of τ' depends on the length of τ, and a good signal amplitude for all C−D multiplicities is obtained when the ratio τ/τ' is set to 3. The 2D

(13) Braun, S.; Kalinowski, H.-O.; Berger, S. *150 and More Basic NMR Experiments*; Wiley-VCH: Weinheim, 1998.
(14) Ernst, R. R.; Bodenhausen G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, 1987.
(15) Freeman R.; Morris, G. *Chem. Commun.* **1978**, 684; Bax, A.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 501.

(16) Norwood, J.; Boyd J.; Heritage J. E.; Soffe, N.; Campbell, I. D. *J. Magn. Reson.* **1990**, *87*, 488; Bax, A.; Ikura, M.; Kay, L. E.; Torchia, D. A.; Tschudin, R. *J. Magn. Reson.* **1990**, *86*, 304.
(17) Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press: Dordretcht, 1984.
(18) Kalinowski, H. O.; Berger S.; Braun, S. *Carbon-13 NMR Spectroscopy*; Wiley and Sons: Chichester, 1984.
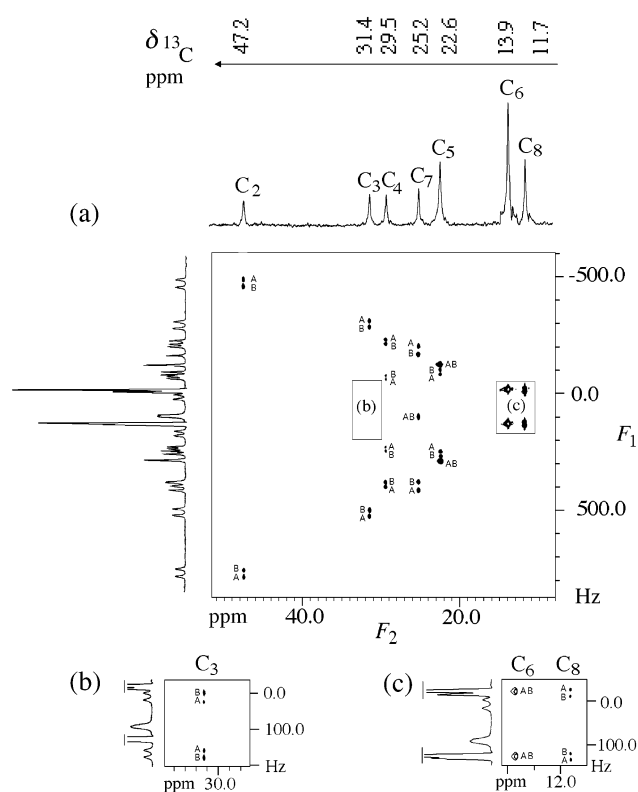
Figure 4. (a) ^{2}H$-^{13}$C heteronuclear 2D map of ($\pm$)-EHA recorded in 15 h. The 2D spectrum was acquired in magnitude mode as a 128 (t_1) × 1600 (t_2) data matrix with 512 scans per t_1 increments. The refocusing delays τ and τ' are fixed to 12.5 ms and 4.2 ms. A Gaussian (LB$_1$ = −5 Hz, GB$_1$ = 40%) and Lorentzian (LB$_2$ = 10 Hz) filtering was used in the F_1 and F_2 dimensions, respectively. In the F_2 dimension is displayed the 2D projection while the deuterium 1D spectrum is given in the F_1 dimension. (b) Expansion (intensity multiplied by a factor of 4) of inner quadrupolar doublets associated with C-3. (c) Expansion (intensity divided by a factor of 8) of the region of the methyl groups. Note the inversion of chemical shift of C-6 and C-8 compared with that of D-6 and D-8 (see the expansion c given in Figure 3).

experiment presented in Figure 4 was recorded using an average value of 40 Hz for |$^1T_{C-D}$|, yielding a delay τ equal to 12.5 ms.

The weak signals located around the carbon-13 resonances of the methyl groups 6 and 8 correspond to residual CDH$_2$ and CD$_2$H isotopomers, meaning that the molecule is not uniformly 100% deuterated at the different carbon sites with the level of deuteration being less at the methyl than the methylene sites. These extra signals do not complicate the spectral analysis in this example and could be eliminated by proton decoupling. For this reason the ^{2}H$-^{13}$C HETCOR experiment can be used for randomly deuterated molecules, which are usually easier to synthesize than either those deuterated at a specific site only, or which are fully deuterated. We also note that correlations are not observed to the quaternary carbon (not shown), as expected if one-bond magnetization-transfer processes dominate.

As previously the signal at lowest field corresponds to the asymmetric carbon, but a comparison with the Q-COSY 2D spectrum shows the undeniable advantage of ^{2}H$-^{13}$C correlation 2D experiments for resolving and assigning the other peaks. First the analysis of the methyl groups is clearly improved as their difference in ^{13}C chemical shift is now 2.2 ppm instead of less than 0.1 ppm in the ^{2}H Q-COSY spectrum. Second, the CD$_2$ group resonances are also much more dispersed. The peak located at 22.6 ppm can be confidently assigned to the CD$_2$

group at position 5, since this is the position least affected by the presence of the COOH group. The assignment of the methylene carbons at positions 3, 4, and 7 is less straightforward because the electron-withdrawing effect of the carboxylic group and the chemical shift are not simply related. However, the full and unambiguous assignment of the ^{13}C spectrum can be accomplished using the well-known ^{13}C$-^{13}$C INADEQUATE experiment.[14,17] Although possible,[3,19] this experiment on the sample of EHA dissolved in PBLG is not required because the ^{13}C chemical shifts are expected to be similar to those measured for a sample dissolved in an isotropic solvent.[3,20] The INADEQUATE 2D spectrum performed on the protonated molecule in CDCl$_3$, (not presented), enables us to easily assign all carbons of the molecule with the result given in Figure 4. Note that the ^{2}H$-^{13}$C correlation spectrum reveals that the two enantiomers of EHA dissolved in the PBLG solution have identical ^{13}C chemical shifts. This is because the chemical shift anisotropy (CSA) for sp^3 hybridized carbons is usually too small to be detected in the carbon-13 spectrum of a sample dissolved in PBLG, at least using a 9.4 T magnetic field. However, a large ^{13}C chemical shift difference is expected for enantiomers in chiral acetylenic or ethylenic compounds because of the large CSA for these carbons[20,21] and hence should be observed using the ^{2}H$-^{13}$C HETCOR experiment. Such an example would be the first spectroscopic enantiodiscrimination using simultaneously two different nuclei and a single 2D spectral map. This challenge is currently underway.

It is now possible to assign all the deuterium quadrupolar doublets in the ^{2}H spectrum and then to use this to assign the deuterium chemical shifts obtained in the F_2 dimension of the tilted Q-COSY spectrum. It should be noted that a simple correlation does not exist between the deuterium and carbon-13 chemical shifts. For instance, the ^{13}C chemical shifts of C-6 and C-8 are inverted compared with their ^{2}H chemical shifts. This can be clearly established because only deuterons in methyl group 8 exhibit two quadrupolar doublets (see expansion in Figure 3c and 4c). As expected it is trivial to correlate the signal of two diastereotopic deuterons with the corresponding carbon. This situation allows a simple and direct analysis of all deuterated sites showing a spectroscopic enantioseparation. For instance, the C-4 carbon is correlated to four quadrupolar doublets, proving that both diastereotopic deuterons are spectroscopically enantiodiscriminated. Only three quadrupolar doublets are detected for the deuterium nuclei attached to C-7; one of them has an intensity of two deuteriums and a quadrupolar splitting near to zero. This means that for both enantiomers one of two diastereotopic C$-$D bond directions is on average at an angle of 54.7° (magic angle) to the mesophase director.[3] Finally we can see that the deuterons of methyl group 6 do not exhibit spectral chiral distinction. The deuterium and carbon-13 assignments as well as the quadrupolar splittings for the nonequivalent deuterons are listed in Table 1.

Deuterium$-$Deuterium COSY 2D Experiments. In contrast to molecules embedded in nonchiral liquid crystals, for chiral solutes in a CLC it is necessary to separate the quadrupolar

(19) Sandström, D.; Summamen, K. T.; Levitt, M. H *J. Am. Chem. Soc.* **1994**, *116*, 9357; Sandström, D.; Levitt, M. H. *J. Am. Chem. Soc.* **1996**, *118*, 6966.
(20) Meddour, A.; Berdagué, P.; Hedli, A.; Courtieu J.; Lesot, P. *J. Am. Chem. Soc.* **1997**, *119*, 4502.
(21) Madiot, V.; Lesot, P.; Grée, D.; Courtieu, J.; Grée, R. *Chem. Commun.* **2000**, 169.

Table 1. Spectral Data for the Two Enantiomers of (±)-EHA Measured in the ^{2}H-{^{1}H} 1D Spectrum Recorded at 300 K in the PBLG/CHCl$_3$ Phase

groups	CD	CD$_2$	CD$_2$	CD$_2$	CD$_2$	CD$_3$	CD$_3$
δ ^{13}C, ppm	47.14	31.43	29.50	25.15	22.61	13.86	11.70
δ ^{2}H, ppm	2.26	1.61/1.46	1.28	1.61/1.51	1.27	0.85	0.90
spectral discrimination	y	y/y	y/y	y/n	n/y	n	y
$\|\Delta\nu_Q^A\|$,[a] Hz	1289	838/140	626/299	620/0[b]	416/333	147	161
$\|\Delta\nu_Q^B\|$,[a] Hz	1222	780/180	598/316	544/0[b]	416/359	147	139
$\|\Delta\Delta\nu_Q\|$, Hz	67	58/40	28/17	76/0	0/26	0	22
assignment	2	3/3′	4/4′	7/7′	5/5′	6	8

[a] The *S* and *R* descriptors are replaced by the arbitrary notation A and B. The measurement for the nonzero quadrupolar splittings is estimated at 2 Hz. [b] The precision on the values is around 5 Hz.

splittings into two sets of coherent data, one for each enantiomer. This is possible because two enantiomers do not have any common couplings, and consequently the 2D map obtained will have no connecting cross-peaks between them. In a liquid crystalline sample the deuterons of perdeuterated solutes are coupled through dipolar as well as scalar interactions, and hence they can, in principle, be correlated to each other. This was demonstrated first by Emsley and Turner in the case of an achiral molecule, and here we describe an alternative to their 2D experiment which is much simpler to analyze and interpret.[4]

Numerical simulations have been done using the NMRSIM program (supplied by Bruker Biospin) and demonstrated that the spin-1/2 COSY 2D experiment (see Figure 2c) can also be efficiently applied to establish the respective correlations between deuterium nuclei sharing a common spin–spin coupling. The presence of the quadrupolar interaction means that for two nonequivalent deuterium nuclei sharing a spin–spin coupling, each of the four components of the quadrupolar doublets have connecting cross-peaks in the COSY 2D map. For our purpose the conventional phase-cycling initially designed for spin $I = 1/2$ nuclei is suitable and either a 45° or a 90° read pulse can be used. The full analytical expression for the NMR signal during t_1 and t_2, after a basic two-step phase cycle for the COSY-45 and COSY-90 2D experiments for two coupled deuterons D$_i$ and D$_j$, is reported in Supporting Information.

Compared to the *Q*-COSY 2D map, the 1D deuterium spectrum is expected to be observed on the main diagonal of a spin-1 COSY 2D experiment, and autocorrelation peaks appear displaced from the other diagonal by the chemical shifts.[5] The primary difference between the spin-1 COSY-45 and COSY-90 experiments is that autocorrelation peaks are not observed in the 2D contour plot when the read pulse is 90°. The reason is that these peaks are suppressed after two scans and only the diagonal peaks and ^{2}H–^{2}H correlation peaks are visible in the 2D spectrum.[5] However, the intensity of the correlation peaks depends mainly on the magnitude of ^{2}H–^{2}H dipolar couplings and are not affected by the value of the read pulse angle. An important point to note here is that the observation of cross-correlation peaks in COSY type experiments does not require the ^{2}H–^{2}H total spin–spin couplings to be resolved in the 1D spectrum.[22] For compounds dissolved in the PBLG system, it is usually the case that even the largest total spin–spin couplings, which are usually those between geminal deuterons, are not resolved. Illustrations of the analytical potential of spin-1 COSY 2D experiments for our specific purpose are also proposed in the Supporting Information.
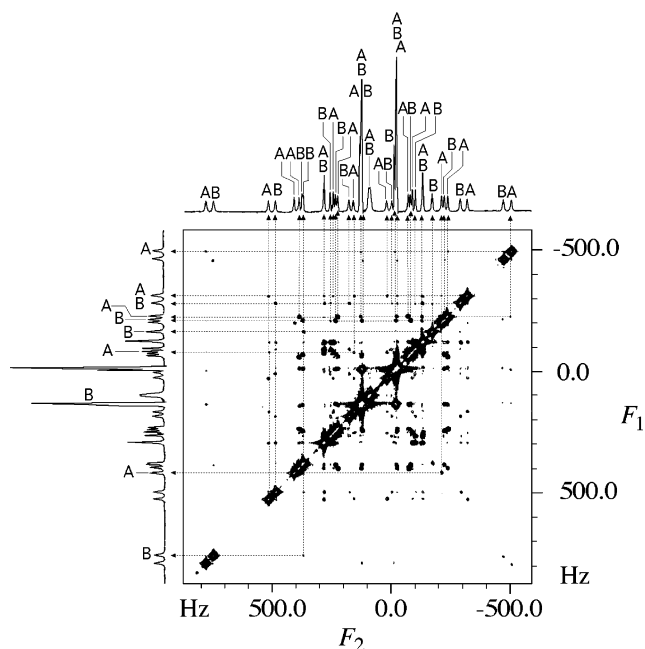
(22) Vuister, G. W.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 7772.

Figure 5. Symmetrized ^{2}H–^{2}H COSY-90 2D map of (±)-EHA recorded in 4.5 h. The 2D spectrum was acquired in magnitude mode as a 300 (t_1) × 1340 (t_2) data matrix with 96 scans per t_1 increment and identical spectral widths in the two dimensions. Sine-bell filtering was applied in both dimensions. The separation of two enantiomers is based on the analysis of correlation peaks.

Figure 5 shows the ^{2}H–^{2}H COSY-90 2D spectrum of (±)-EHA in the PBLG/CHCl$_3$ phase. As the intensity of the correlation peaks are very weak, we have displayed a very low level contour plot. For such a level, we can see some residual autocorrelation peaks which are not theoretically expected, but arise because the read pulse angle is not exactly 90°. These additional peaks are not at all cumbersome for the spectral analysis, and they might be removable by using a composite pulse.[14]

As described for the simulated 2D spectra, the analysis of various correlations enabled us to assign the various quadrupolar doublets corresponding to each enantiomer. This assignment for each enantiomer noted A and B is listed in Table 1. We have now two coherent sets of quadrupolar splittings, that can be used for further calculations. However, such an experiment does not enable us to determine the absolute configuration of each enantiomer, and hence the assignment of the *S* and *R* descriptor to each set of data is not possible in a racemic mixture to date.[2]

Conclusion

We report the possibility through robust 2D NMR experiments to discriminate and identify correctly the quadrupolar

doublets given by each enantiomer for selectively, randomly, or fully deuterated chiral solutes oriented in polypeptide chiral liquid crystals, thus facilitating their spectral analysis. As experimentally demonstrated here, the $^2H-^{13}C$ heteronuclear 2D method, furnishes an elegant and useful way to overcome the lack of ^2H chemical shift dispersion in tilted *Q*-COSY 2D spectra. In addition we present a simple 2D strategy to attribute the quadrupolar doublets for two enantiomers and hence obtain two sets of coherent data, one for each enantiomer, that can be confidently used for further calculations. In particular these data can now be confidently used to test various models of the orientational order and conformational distribution in this chiral, flexible molecule.[23] This theoretical work is currently underway.

The sample used in this work was highly enriched in deuterium. This was necessary in order to achieve an acceptable signal-to-noise ratio in a reasonable experimental time. It would be clearly be an advantage to be able to do the same experiments using samples containing deuterium at natural abundance. This is likely to be a practical possibility using a NMR cryoprobe in conjunction with a higher magnetic field. Keep in memory that the S/N ratio is proportional to $(B_o)^{3/2}$ for a given experimental time (EXPT) while the EXPT is proportional to $1/(B_o)^3$ for a given S/N ratio. In addition, the current results obtained with cryoprobes show a very significant gain of the signal sensitivity (about a factor 4) compared with standard high resolution probes. To give a simple comparison, calculations indicate that the sensitivity of a 600 MHz spectrometer equipped a selective cryogenically cooled probe would be equivalent to a 1500 MHz spectrometer equipped with a standard probe! No doubts that the NMR methods presented here will then become more widely applicable for the study of the structure and conformation of chiral molecules.

Supporting Information Available: The NMR analytical expressions of the spin-1 COSY-90 and spin-1 COSY-45 pulse sequences and their corresponding schematic 2D maps are reported in the Supporting Information. Illustrations of the analytical potential of spin-1 COSY 2D experiments in the field of the enantiomeric analysis is also proposed. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

(23) Lesot, P.; Merlet, D.; Sarfati, M.; Courtieu, J.; Zimmermann, H.; Luz, Z. *J. Am. Chem. Soc.* **2002**, *124*, 10071.

JA021277X