Proton-Decoupled Carbon-13 NMR Spectroscopy in a Lyotropic Chiral Nematic Solvent as an Analytical Tool for the Measurement of the Enantiomeric Excess†

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Abstract: Organic solutions of poly-γ-(benzyl-L-glutamate) (PBLG) generate a sufficient differential ordering effect (DOE) to discriminate enantiomers using proton decoupled carbon-13 NMR in natural abundance. Discrimination between enantiomers is observed through the carbon-13 chemical shift anisotropy (CSA) differences. This method is successfully applied to a large number of chiral molecules including a case of axial chirality and offers the advantage that no labeling or chemical modification of molecules is needed. In most cases, the chemical shift differences are large enough to measure the enantiomeric excess with accuracy. We show that this new tool is an attractive and powerful alternative to the existing enantiomeric analytical techniques.

1. Introduction

In view of the growing importance of asymmetric synthesis in the pharmaceutical industry, the development of new and convenient nuclear magnetic resonance (NMR) spectroscopic techniques for the enantiomeric analysis is an exciting challenge.[1,2] Recently, we developed a new NMR tool for the measurement of enantiomeric excesses. It consists of using a chiral lyotropic liquid crystal based on a mixture of an organic solvent and Poly-γ-(benzyl-L-glutamate) (PBLG) as NMR solvent.[3−8] We have shown that the spectral discrimination between enantiomers in such an oriented medium originates from a difference in their ordering, thus affecting all order dependent NMR interactions such as quadrupolar splittings, dipolar couplings and chemical shift anisotropies.[6,9] First, we have focused on the deuterium quadrupolar interaction on labeled chiral molecules as it is the strongest NMR interaction and consequently the most sensitive to the differential ordering effect (DOE) of enantiomers.[5,6] Although synthetic methods for introducing deuterium in chiral molecules are numerous and well documented, it is clear that it is not always possible or easy to do and thus may become a limitation of this technique.[4,6,8] Whenever deuteration is difficult, other anisotropic NMR interactions may be used.[6,7] The second strongest anisotropic interaction is the residual dipolar coupling. Unfortunately, the magnitude and the number of dipole−dipole couplings are such that the proton or carbon-13 spectra are often not resolved when the number of interacting nuclei is large. Apart from some rather simple molecules this interaction will not be very efficient to use for chiral discrimination.

In this paper, we present an extended experimental study of the visualization of enantiomers through proton-decoupled carbon-13 NMR (^{13}C-{^{1}H}) in natural abundance. In this case, enantiomer discrimination is observed only through a difference of carbon-13 chemical shift anisotropies (CSA). Enantiomeric excesses are then determined by integration of the NMR resonance intensities for each enantiomer. To explore the capabilities and the limits of this analytical method, a large collection of chiral compounds including a case of axial chirality were investigated and analysed in details. All experimental parameters that affect the quality of spectra are presented and discussed herein.

2. Theoretical Considerations

When embedded in a liquid crystal solvent, solute molecules are partially ordered. Consequently the resonance NMR frequency ν_i of any nucleus i contains an anisotropic contribution σ_i^{aniso} such as[9]

$$\nu_i = -\frac{\gamma}{2\pi}(1 - \sigma_i^{iso} - \sigma_i^{aniso})B_o \qquad (1)$$

In this eq, $\sigma_i^{iso} = (\sigma_{iaa} + \sigma_{ibb} + \sigma_{icc})$ is the isotropic average of the shielding tensor expressed in a molecule fixed frame (a,b,c). The anisotropic contribution σ_i^{aniso} is expressed as[9]

$$\sigma_i^{aniso} = \frac{2}{3}\sum_{\alpha,\beta=a,b,c} \sigma_{i\alpha\beta} \cdot S_{\alpha\beta} \qquad (2)$$

where $S_{\alpha\beta}$ are the elements of the second rank molecular

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ordering tensor,[10] the sum runs over $\alpha,\beta \equiv$ (a,b,c) the axis of the molecular frame, and $\sigma_{i\alpha\beta}$ are the elements of the electronic shielding tensor at nucleus i in the same frame.

This NMR measurable quantity, σ_i^{aniso}, is not trivial to analyze as from eq 2 it depends both on the elements of the shielding tensor and on the molecular order parameters, $S_{\alpha\beta}$. In order to consider the various factors governing σ_i^{aniso}, it is more convenient to recast eq 2 into[9]

$$\sigma_i^{aniso} = \frac{2}{3}S_{aa}\left[\sigma_{iaa} - \frac{1}{2}(\sigma_{ibb} + \sigma_{icc})\right] + \frac{1}{3}(S_{bb} - S_{cc})(\sigma_{ibb} - \sigma_{icc}) + \frac{2}{3}S_{ab}\sigma_{iab} + \frac{2}{3}S_{ac}\sigma_{iac} + \frac{2}{3}S_{bc}\sigma_{ibc} \quad (3)$$

For each carbon-13 nucleus i, it exists a frame (a'_i,b'_i,c'_i), where the chemical shift tensor is diagonal. Consequently the previous relationship can be rewritten as[9]

$$\sigma_i^{aniso} = \frac{2}{3}S_{a'_ia'_i}\left[\sigma_{a'_ia'_i} - \frac{1}{2}(\sigma_{b'_ib'_i} + \sigma_{c'_ic'_i})\right] + \frac{1}{3}(S_{b'_ib'_i} - S_{c'_ic'_i})(\sigma_{b'_ib'_i} - \sigma_{c'_ic'_i}) \quad (4)$$

with

$$S_{a'_ia'_i} = \sum_{\alpha,\beta=a,b,c} < \cos\phi_\alpha^{a'i} \cos\phi_\beta^{a'i} > S_{\alpha\beta} \quad (5)$$

where $\phi_\alpha^{a'i}$ are the angle between the molecular frame and the principal axis system of carbon i and the brackets denote an average on intramolecular motions.

In the relationship 4, $\sigma_{a'_ia'_i} - (\sigma_{b'_ib'_i} + \sigma_{c'_ic'_i})/2$ corresponds to the anisotropy of the electronic shielding, $\Delta\sigma_i$, while $(\sigma_{b'_ib'_i} - \sigma_{c'_ic'_i})$ may be seen as the asymmetry of the electronic shielding of carbon i. An examination of this eq shows clearly that $\Delta\sigma_i$, the asymmetry term $(\sigma_{b'_ib'_i} - \sigma_{c'_ic'_i})$, as well as the differential ordering effect of enantiomers, $(S^R_{a'_ia'_i} - S^S_{a'_ia'_i})$ and $[(S^R_{b'_ib'_i} - S^S_{c'_ic'_i}) - (S^R_{b'_ib'_i} - S^S_{c'_ic'_i})]$, should both be large in order to observe any frequency difference, $\Delta\nu = \nu^R_i - \nu^S_i$, between the NMR signals of nucleus i for each enantiomer.

Consequently, all factors which increase either the DOE, such as electrostatic interaction, or the electronic shielding anisotropy of carbons, such as the nature of the substituents bonded to nucleus i or its hybridization state, will increase the magnitude of σ_i^{aniso}. Such an anisotropic NMR interaction cannot be used efficiently with ^{1}H NMR, because the proton CSA is known to be very small in general and it is difficult to observe it experimentally. As a matter of fact we have seen it clearly in only a single case when studying the (±)-2-bromopropionic acid.[5] Conversely, the chemical shift anisotropy of carbon-13 may be quite large, and lines are usually narrow when proton decoupling is applied. Thus, we can expect to visualize readily distinct resonances for each enantiomer using ^{13}C-{^{1}H} NMR. In this case the CSA may be used reliably to discriminate enantiomers and to measure an enantiomeric excess through conventional integration. These features are depicted in Figure 1. Furthermore, the parameters governing the strength of the carbon-13 CSA are well known: $|\Delta\sigma_i|$ mainly increases with the electronegativity of the substituents and the hybridization of the carbon atom: $\Delta\sigma$ (sp) > $\Delta\sigma$ (sp^2) > $\Delta\sigma$ (sp^3).[9,10] Consequently, carbons belonging to ethynyl, aryl, carbonyl, or carboxyl groups are likely to give better results than aliphatic carbons. Carbon-13 is not, of course, the only possible nuclei as ^{19}F or ^{77}Se are also known, among others, to have a large

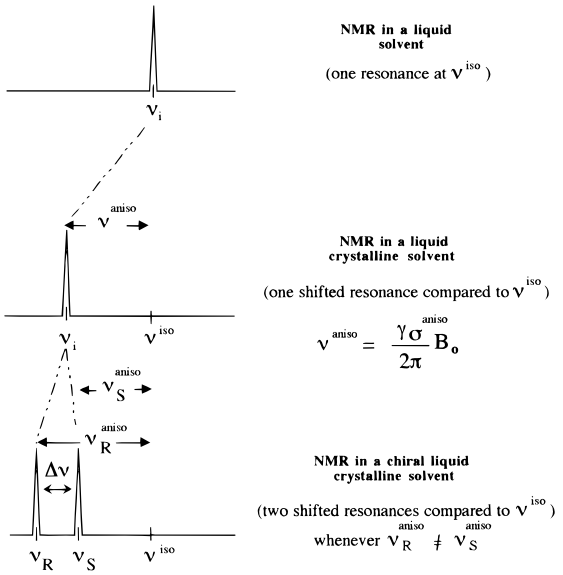

Figure 1. Principle of the enantiomeric discrimination that may be observed on the proton-decoupled carbon-13 NMR spectra in a chiral liquid−crystalline solvent. On the scheme, σ^{aniso} is assumed negative. The *R* and *S* assignment is arbitrary. Note that the important quantity for the chiral discrimination is $\Delta\nu$.

CSA. However this work focuses only on ^{13}C NMR because it is the most commonly used nucleus, after the proton, in organic chemistry.

Finally, eq 1 shows that the resonance frequencies depend directly upon the magnetic field strength B_o. Consequently, the use of NMR spectrometers operating at high magnetic field increases the measured separation, $\times$f4$\nu^R_i - \nu^S_i\times$f4, and improves the chiral discrimination.

3. Experimental Section

PBLG fibers can be dissolved in various helicogenic deuterated organic solvents such as dichloromethane, chloroform, dimethylformamide, dioxane, or 1,1,1-trichloropropane, which are convenient solvents for a wide variety of enantiomer mixtures. In all cases, the organic solvent must both dissolve homogeneously the polymer and preserve its helical structure.[11] Although the sample preparation was already reported elsewhere, here we will outline a typical procedure.[5,7] Chiral compounds (20−80 mg) are dissolved in about 500 mg of organic solvent and added to 80−100 mg of the polymer, which has been directly weighted into a 5 mm NMR tube. Several studies for the optimization of this ternary mixture have shown that the optimal NMR results are obtained for samples prepared with a concentration in PBLG which can vary between 12−25% by weight. For the sake of clarity, the following notation (*x* mg of solute/*x*′ mg of PBLG /*x*″ mg of solvent/% by weight of PBLG) will be used in this text. Under these conditions, the total volume of the sample is optimal compared to the length of the coil of a 5 mm diameter dual probe. Finally, it is recommended to wait for a slow dissolution of the compounds in the NMR tube and then to centrifuge the sample in both directions until an optically homogeneous birefringent phase is obtained. We must note that in the case of DMF or dioxane, it is necessary to heat the sample in order to dissolve the polymer. Even though the carbon-13 chemical shift anisotropies are much greater than for the proton, the chemical shift differences measured in the spectra are typically just a few Hertz. Therefore, a good homogeneity of both sample composition and magnetic field is required to obtain an optimal enantiomeric discrimination. Under the above conditions described, line widths of 2−4 Hz are commonly observed in the proton decoupled carbon-13 spectra.

The average molecular weight of PBLG is an important factor for the enantiomeric discrimination. The sample viscosity depends strongly

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on the molecular weight, the concentration of the polymer, and the nature of the organic solvent used. At a given PBLG concentration and with the same organic solvent, the fluidity of the phase is greater for a low molecular weight, which leads to a better spectral resolution. Therefore we recommand using a PBLG whose degree of polymerization (DP) is in the range of 350−500.

NMR experiments were performed on a Bruker AM-400 high resolution spectrometer operating at a frequency of 100.62 MHz for carbon-13 and using a 5 mm diameter $^{1}H/^{13}C$ dual probe. The deuterated organic solvent provides a convenient lock signal. However, the sample temperature has to be very carefully regulated in order to furnish a long term stability of the lock signal frequency. The temperature was generally maintained at or near 300 K through the Bruker BVT 1000 temperature regulation system, and the samples were spun at about 20 Hz. In the case of DMF solvent, it was necessary to work at higher temperature (above 305 K) to reach a liquid−crystalline phase. The NMR probe was always tuned and matched for each sample before recording the spectra. In order to avoid sample overheating, broad-band proton decoupling was applied using the WALTZ-16 composite pulse sequence (1 W of RF power).[12] The carbon-13 interferograms were acquired using a pulse angle of ∼30°, a recycle delay of ∼2 s and 16 or 32 k of data points. Zero filling was applied to increase the digital resolution. Under these conditions, only 1000−2500 scans, i.e., about 1−3 h of acquisition, were usually necessary to obtain spectra with a good signal-to-noise (S/N) ratio. Finally, in all the spectra which are shown in this work, no apodization, elimination of points from the free induction decays, or filtering prior to the Fourier transformation was applied to enhance the spectral appearance.

4. Results

The first case of enantiomeric discrimination using carbon-13 NMR in natural abundance was observed on the (±)-2-bromopropionic acid in a racemic mixture, where frequency differences of 4.5 and 3.5 Hz were measured on the carboxyl and methyl carbons, respectively.[6] Following this result, we explored the reliability of this technique, investigating a large collection of chiral molecules.

The experimental results obtained for series of alcohols, carboxylic acids, and heterocyclic compounds are presented in Tables 1−3. In these tables, we have reported all the carbon sites where two different carbon-13 resonances were clearly visible and the chemical shift differences expressed in ppm and Hz. The carbon-13 signals were assigned from their isotropic chemical shifts which are only slightly modified by the anisotropic part. The listed chemical shifts, δ_1, are assigned to the most unshielded carbon signal for each discriminated site. Unless otherwise specified, the compounds were investigated in racemic mixtures, using deuterochloroform as organic solvent.

From an inspection of these tables, several comments about the experimental results may be made. First, the chemical shift measured in the anisotropic phase are very close to those observed in the isotropic solvent. Consequently, the assignment of the carbon-13 resonances is easy, and the comparison with the isotropic spectrum readily reveals the carbons of the chiral molecule which are discriminated.

Second, a chemical shift difference of around 4−8 Hz is generally observed between carbon-13 resonances, allowing the measurement of enantiomeric excess by NMR resonance integration or using a deconvolution procedure. This difference reaches 39 Hz in the case of the C-2 of (±)-oct-1-yn-3-ol, the spectrum of which is shown in Figure 2. Using this molecule we checked the quantitative aspect of this method and analyzed the precision that can be reached in measuring of the enantiomeric excess using a 400 MHz spectrometer. We recorded the spectrum on 59.9 mg of a mixture of (±)-oct-1-yn-3-ol enriched in the *R* enantiomer (ee = 87.3%). Note that the quantity of *S* enantiomer is then only 3.8 mg, i.e., 3×10^{-5} mol. The signals of carbons C-1 and C-2 are also presented in Figure 2. A reasonable signal-to-noise ratio is obtained considering the ee's determination. The experimental enantiomeric excess determined on the spectrum by line integration is 86%, i.e., an experimental error within 2% of the true value. Generally, we

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Table 1. Data for Alcohols

Structure	Entry	Site [a]	δ_1 [b] (ppm)	$\delta_1 - \delta_2$ [c] (ppm)	$\nu_1 - \nu_2$ [d] (Hz)
[e] 31% ee (-)	**1**	C4	133.98 (-)	0.07	7
		C3	118.68 (-)	0.08	7.5
	2	C1	145.35	0.07	7
		C6	143.43	0.06	6
		C2	128.34	0.07	6.5
		C4	126.81	0.05	5
		C5	124.85	0.08	8.5
		C3	124.38	0.09	8.5
		C7	75.85	0.07	6.5
		C8	35.50	0.04	4
	3	C3	146.13	0.05	5
		C4	128.43	0.04	4
		C6	127.49	0.05	5.5
		C5	125.44	0.03	3
		C2	69.96	0.04	4
		C1	24.99	0.03	2.5
[e] 72% ee (*S*)	**4**	C2	85.27 (*S*)	0.25	25.5
		C1	71.59 (*S*)	0.18	18
		C4	24.19 (*R*)	0.03	3
	5	C3	58.24	0.02	2
		C4	23.96	0.03	3
	6	C2	84.16	0.39	39
		C1	71.86	0.27	27.5
		C4	37.55	0.03	3
		C5	31.34	0.02	1.5
		C6	24.66	0.02	1.5

[a] Carbon sites where two signals were observed. [b] Chemical shift of the most unshielded carbon-13 signal expressed in ppm. [c,d] Chemical shift differences expressed in ppm and in Hz, respectively. [e] Enantiomeric excess.

Table 2. Data for Carboxylic Acids

Structure	Entry	Site [a]	δ_1 [b] (ppm)	$\delta_1 - \delta_2$ [c] (ppm)	$\nu_1 - \nu_2$ [d] (Hz)
	1	C4	136.16	0.02	2.5
		C7	129.49	0.06	6
		C2	81.98	0.06	6
		C3	57.05	0.02	2
	2	C1	180.22	0.06	6
		C4	139.95	0.05	5
		C6	128.66	0.08	8
		C5	127.59	0.07	7
		C7	127.43	0.03	3
*	**3**	C1	174.18	0.04	4.5
		C3	21.83	0.03	3.5

[a−d] See the notes of Table 1. * Spectrum recorded in [PBLG/CD_2Cl_2] phase.

Table 3. Data for Heterocycles

Structure	Entry	Site [a]	δ_1 [b] (ppm)	$\delta_1 - \delta_2$ [c] (ppm)	$\nu_1 - \nu_2$ [d] (Hz)
*	**1**	C1	61.72	0.01	0.5
		C2	47.78	0.01	1
*	**2**	C1	101.83	0.01	1
		C4	68.29	0.02	2
		C3	41.82	0.01	1
[e] * 40% ee (*R*)	**3**	C1	164.44 (S)	0.02	2
		C3	76.53 (S)	0.02	2

[a−e] See the notes of Table 1. * Spectrum recorded in [PBLG/CD_2Cl_2] phase.

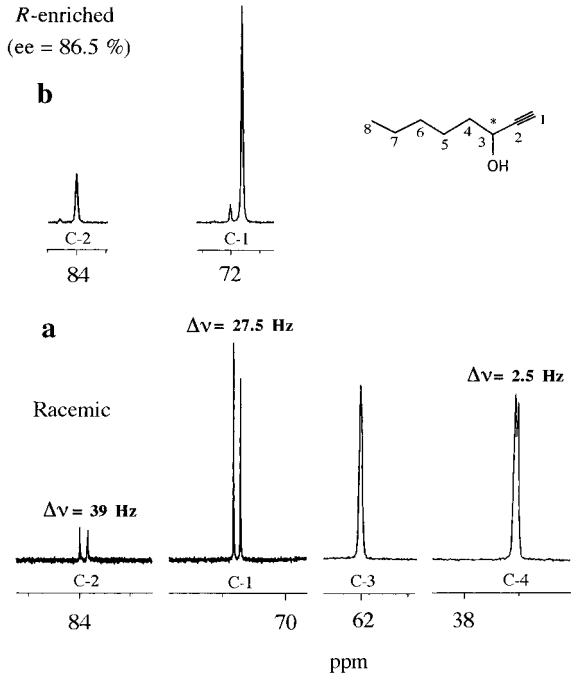


Figure 2. (a) Expansion of the ^{13}C-{^1H} spectrum of (±)-oct-1-yn-3-ol (54/100/555/14.1%) dissolved in PBLG/$CDCl_3$ at 300 K. Two thousand scans were added. Note the large $\Delta\nu$ differences on the sp carbons. (b) ^{13}C-{^1H} spectrum of C-1 and C-2 of an *R*-enriched sample (87.3% ee) (59.9/80/521/12.1%). The spectrum was recorded with 5000 scans and 16 k of data points. The recycle delay was 2 s. The measured ee through integration was 86%.

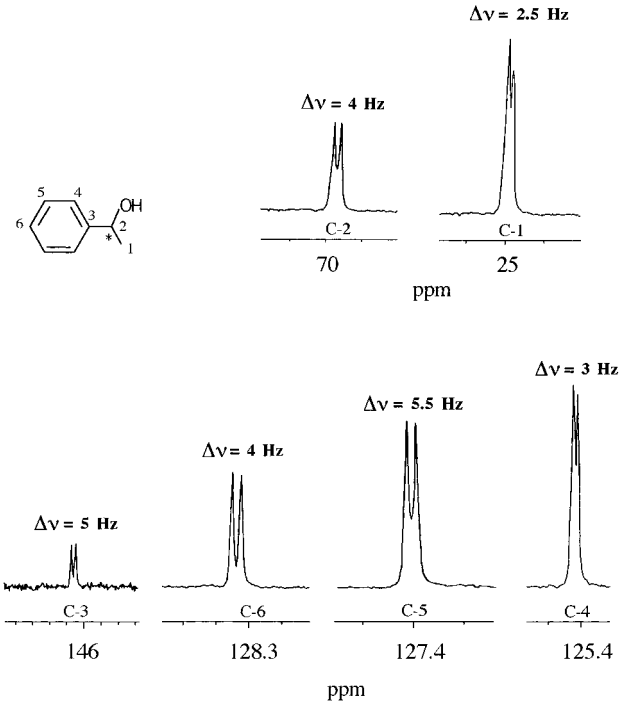


Figure 3. ^{13}C-{^1H} spectrum of (±)-1-phenylethanol (54/100/581/13.6%) dissolved in PBLG/$CDCl_3$ at 300 K. All carbon signals are split.

estimate that the accuracy on the enantiomeric excess measurements is about 5% of the true value when the spectrum is recorded and processed using standard conditions.[13,14] We estimate that the technique is fully suitable for high enantiomeric excess up to about 95% (at 400 MHz), but the use of NMR spectrometers operating at higher magnetic field obviously will enhance the sensitivity of the method.

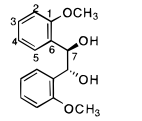
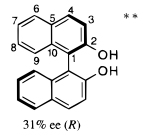
Third, compared to the ^2H-{^1H} spectroscopy of monodeuterated molecules, the ^{13}C-{^1H} spectroscopy provides numerous observation sites simultaneously. In an ideal case every carbon atom in the molecule can be potentially discriminated, while only one observation site is needed for detecting and measuring the enantiomeric excess. This situation was typically encountered in (±)-1-phenylethanol (Table 1, entry 3) and presented in Figure 3. In this example, we can observe that all carbons of the molecule are discriminated, while in most cases, the resonance separations were only observed for a few carbons of the solute.

Finally, we may note that proton decoupled carbon-13 NMR was successfully applied on different kinds of chiral molecules (aromatic, aliphatic, alicyclic, heterocyclic molecules), thus allowing for scanning a very large variety of chiral materials.

In order to further explore the capabilities of discrimination of the method, we have attempted to visualize the enantiomers of chiral diols. This application seemed interesting to us since this class of chiral compounds has been frequently used as asymmetric inducing agents.[15,16] No general analytical methods to visualize these enantiomers have been reported.[17] We have therefore studied (±)-*o*-methoxy-1,2-diphenyl-1,2-ethanediol in a racemic mixture. For this compound, chiral gas chromatographic methods using cyclodextrin as stationary phase were applied unsuccessfully. To our knowledge, only HPLC method involving particular conditions of elution with hexane/propan-2-ol (49:1) have been described in the literature to discriminate these enantiomers.[17] The results obtained in the PBLG / $CDCl_3$ system at 300 K were successful since *R* and *S* enantiomers were clearly separated up to 31 Hz for the C4 carbon (Table 4, entry 1). This result indicates that the method can provide a competitive alternative to other techniques for this class of chiral materials. A more detailed study about the visualization of chiral diols is presently under way.

Among the various possible diols, we decided to investigate the case of the atropoisomerism provided by the binaphthyl derivatives.[18] For this purpose, the proton-coupled carbon-13 spectrum of (±)-1,1′-bi-2-naphthol mixture enriched in *R*

Table 4. Data for Diols

Structure	Entry	Site [a]	δ_1 [b] (ppm)	$\delta_1 - \delta_2$ [c] (ppm)	$\nu_1 - \nu_2$ [d] (Hz)
	1	C1	156.90 (*S,S*)	0.24	24
		C6	128.56 (*S,S*)	0.18	18
		C3	128.38 (*S,S*)	0.21	21.5
		C5	128.07 (*S,S*)	0.17	17.5
		C4	120.45 (*S.,S*)	0.31	31
		C2	110.03 (*S,S*)	0.15	15
 31% ee (*R*)	2	C2	154.37 (*R*)	0.10	10
		C10	135.18 (*R*)	0.10	10
		C4	129.44 (*R*)	0.12	12
		C5	129.25 (*R*)	0.10	10
		C6	128.35 (*R*)	0.17	17
		C8	124.94 (*R*)	0.12	12.5
		C7	122.95 (*R*)	0.13	13
		C1	115.71 (*R*)	0.12	12.5

[a–e] See the notes of Table 1. **Spectrum recorded in [PBLG/DMF-d_7] phase.

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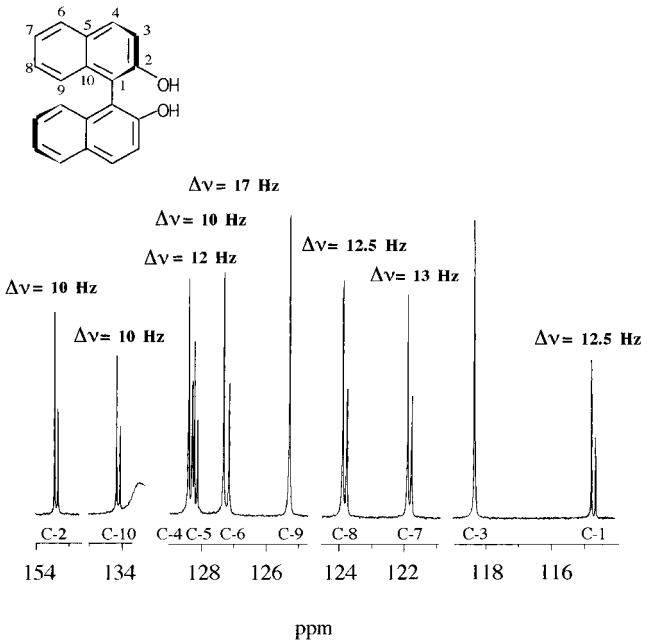

Figure 4. ^{13}C-{^1H} spectrum of 1,1′-bi-2-naphthol in PBLG/DMF-d_7 at 313 K. The sample is enriched with the *R* component (31% ee) and the mixture made of 57.5 mg of chiral solute (57.5/134/258/29.8%). The average measured ee through integration was 29.5%.

enantiomer (ee = 31%) dissolved in the PBLG/DMF-d_7 (57.5/134/258/29.8%) phase was recorded at 300 K (Table 4, entry 2). Since this compound is only slightly soluble in mixtures of PBLG and chlorinated organic solvents, we have used the PBLG/DMF-d_7 system as the chiral liquid-crystalline phase. The ^{13}C-{^1H} spectrum is presented in Figure 4. Eight carbons of the molecule exhibit two different signals with chemical shift differences varying from 10 to 17 Hz. Such separations allowed an easy measure of the enantiomeric excess of the sample by peak integration. The value of the ee determined as the average of the measures made on each discriminated carbon site was 29.5% with a standard deviation of 3%. This result illustrates that the method is well suited to discriminate the chiral molecules of that type.

Among other potential applications of this method, the possibility to discriminate chiral molecules possessing an asymmetric heteroatom, such as sulfur or phosphorus nuclei is a very interesting challenge. Therefore, we have attempted to distinguish between the enantiomers of chiral sulfur derivatives.[20,21] It is known that many of them are difficult to discriminate with classical analytical NMR methods involving chiral shift reagents for example. For this purpose, a sulfoxide, a sulfoximine, and a sulfilimine derivative were investigated using carbon-13 NMR, and the results are reported in Table 5. As an illustration, the spectrum of (±)-*S*-methyl-*S*-*P*-tolyl-*N*-tosylsulfoximine in a racemic mixture dissolved in the PBLG/$CDCl_3$ system at 13.6% is presented in Figure 5. Remarkably, the ^{13}C-{^1H} spectrum of this compound clearly exhibits four doublets belonging to carbons of the aromatic rings and one doublet for a methyl group, with $\Delta\nu^{R,S}$ values up to 11 Hz (Table 5, entry 3). Similar results were observed in the case of the (±) methyl-*P*-tolyl-*N*-sulfoxide and the *S*-methyl-*S*-*P*-tolyl-*N*-

Table 5. Data for Chiral Sulfur Compounds

Structure	Entry	Site [a]	δ_1 [b] (ppm)	$\delta_1 - \delta_2$ [c] (ppm)	$\nu_1 - \nu_2$ [d] (Hz)
[e] 26% ee (*R*)	1	C2	142.55 (*R*)	0.05	5
		C5	141.55 (*R*)	0.07	7
		C4	129.81 (*R*)	0.05	5
		C3	123.34 (*R*)	0.05	5
		C1	43.26 (*R*)	0.02	2
	2	C7	143.40	0.05	5
		C9	130.43	0.06	6
		C3	128.66	0.09	9
		C2	125.79	0.06	6.5
		C8	125.67	0.1	10
	3	C7	145.93	0.11	11
		C10	135.43	0.07	7.5
		C9	130.32	0.06	6.5
		C8	127.52	0.07	7.5
		C6	46.56	0.03	3

[a–e] See the notes of Table 1.

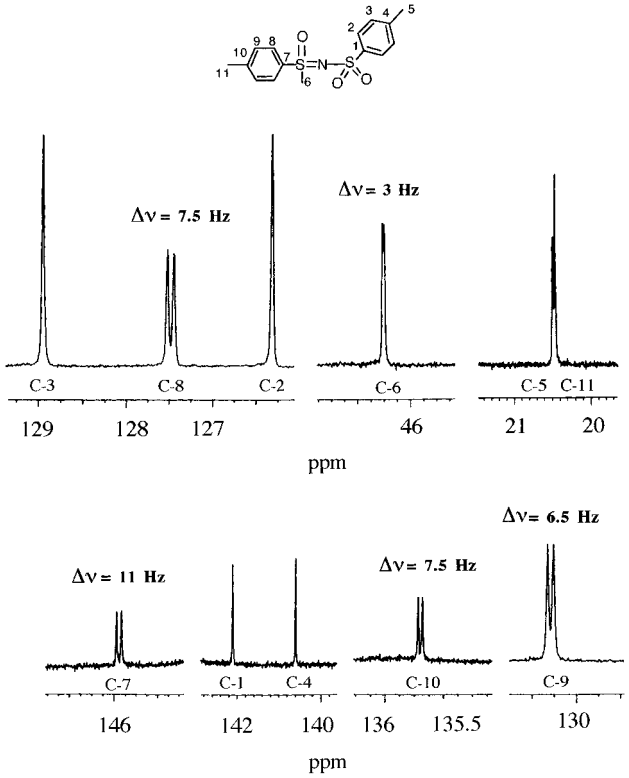

Figure 5. ^{13}C-{^1H} spectrum of racemic (±)-*S*-methyl-*S*-*P*-tolyl-*N*-tosylsulfoximine (63/99/545/14.0%) dissolved in the PBLG/$CDCl_3$ system at 300 K. 1000 scans were added. The best separations is observed on the sp^2 carbon of the molecule.

tosylsulfilimine in a racemic mixture dissolved in the PBLG/$CDCl_3$ system at 14% (see Table 5, entries 1 and 2). Here again the large chemical shift differences between the enantiomers allow unambiguously measurement of an enantiomeric excess for the first compound.

In the Introduction, we mentioned that significant line broadening of the resonances in the proton-carbon-13 spectra are observed when the number of protons in the chiral molecule becomes large. In such cases, we reach the limits of the applicability of the proton-coupled carbon-13 NMR similar to the situation which is encountered in proton NMR spectroscopy.[9,11] Nevertheless, in the case of chiral compounds exhibiting an isolated methyl group far removed from other protons in the molecule, it may be convenient to record the proton-coupled carbon-13 spectrum in order to detect the separation of enantiomers through a difference in the residual dipolar couplings.[7] In order to illustrate this fact, we present

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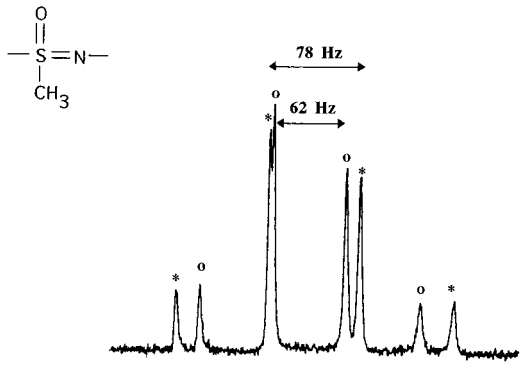

Figure 6. Doubled quartet associated to the methyl (C6) group of (±)-*S*-methyl-*S*-*P*-tolyl-*N*-tosylsulfoximine observed on the proton-coupled carbon-13 spectrum at 300 K. Fifteen hundred scans were added. The peaks due to each enantiomer are labeled by (*o*) and (*). Proton decoupling was applied during the relaxation delay period in order to gain the Nuclear Overhauser Effect.

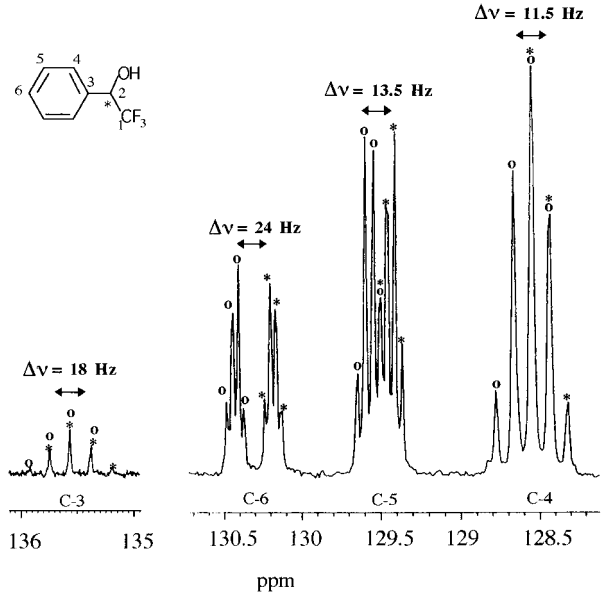

Figure 7. ^{13}C-{^{1}H} spectrum of (±)-1-deutero-1-phenyl-2,2,2-trifluoroethanol (65/100/406/17.5%) dissolved in PBLG/CD_2Cl_2 at 300 K. Twenty-two scans were added. The peaks due to each enantiomer are labeled by (*o*) and (*), but the attribution for each carbon is arbitrary.

in Figure 6 an expansion of the proton coupled carbon-13 spectrum of (±)-*S*-methyl-*S*-*P*-tolyl-*N*-tosylsulfoximine. Here we observe good discrimination of the signals belonging to the isolated methyl group bonded to the sulfur atom (two quartets).

Another interesting class of compounds is provided by chiral fluorinated derivatives that have been involved in numerous synthetic developments since the last few years. In particular, they are useful as molecular probes in the elucidation of biochemical processes and in drug design.[22] It was of interest to check if the method was applicable for this class of molecules. For this purpose, we recorded the spectra of several fluorinated chiral compounds as reported in Table 6. In all cases, the discrimination of the enantiomers was observed through a difference of chemical shift anisotropy. In Figure 7 the proton-decoupled carbon-13 spectrum of the (±)-1-deutero-1-phenyl-2,2,2-trifluoroethanol (Table 6, entry 7) is presented. We may note the very large separations of the enantiomer signals for each of the aromatic carbons through a difference of $\Delta\sigma$ up to 24.5 Hz. The chemical shifts differences measured in this

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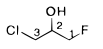
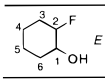
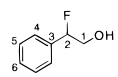
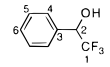
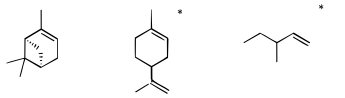
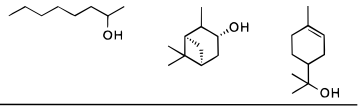
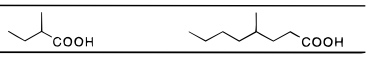
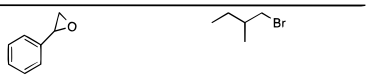
Table 6. Data for Chiral Fluorinated Compounds

Structure	Entry	Site [a]	δ_1 [b] (ppm)	$\delta_1 - \delta_2$ [c] (ppm)	$\nu_1 - \nu_2$ [d] (Hz)	$T_{(C\text{-}F)_1}$ [e] (Hz)	$T_{(C\text{-}F)_2}$ [f] (Hz)
	**1**	C2	90.63	0.01	10	211.5	214.4
	**2**	C1[g]	83.30	ns	ns	181.7	173.9
			83.32	0.04	4	171.9	177.6
		C2	69.62	0.02	23	14.9	19.4
		C3	44.31	0.03		0.0	5.2
	**3**	C1	93.28	0.02	2	159.2	162.8
	**4**	C2	96.41	0.03	3	172.1	178.4
		C1	72.85	0.01	1	14.8	16.8
	**5**	C3	136.51	0.06	6	25.2	24.2
		C6	128.58	0.07	7	ns	ns
		C4	125.67	0.04	4	8.8	7.5
		C2	94.80	0.07	7	148.1	187.6
		C1	66.24	0.03	3	18.5	25.0
	**6**	C3	135.67	0.17	17	17.9	18.3
		C6	130.46	0.24	24	3.8	3.5
		C5	129.61	0.13	13.5	5.2	4.8
		C4	128.64	0.11	11.5	11.4	11.5
		C1	125.13	ns	ns	250.1	236.6

[a–d] See the notes of Table 1. [e] ns means that no separation was observed on the fluorine-coupled carbon spectrum. [f] Dipolar splitting measured on the fluorine-coupled carbon spectrum: $T_{CF} = J_{CF} + 2D_{CF}$. [g] Two differents interpretations of the spectrum are possible.

Table 7. Compounds Where No Chiral Discrimination Was Observed

Hydrocarbons	
Alcohols	
Carboxylic acids	
Other compounds	

* Spectrum recorded in [PBLG/CD_2Cl_2] phase.

example are much larger than those obtained for the non-fluorinated analogue (Table 1, entry 3). This seems to indicate that the DOE is considerably enhanced by the presence of the trifluoromethyl group in the molecule. This intriguing result is currently being studied to assess its origin by investigating several similar examples. The quartets observed in the spectrum with proton decoupling arise from the carbon-fluorine scalar and dipolar couplings, thus providing another order-sensitive interaction to measure.

5. Discussion

The proton decoupled carbon-13 NMR spectroscopy in a chiral liquid crystalline solvent was applied successfully to visualize enantiomers and measure the chiral purity on various compounds. However, the experimental results showed that in some cases no enantiomeric discrimination was observed. All the molecules in which we failed to detect any chiral discrimination are presented in Table 7. These results indicate that the method is not entirely general. Therefore it is useful to attempt to rationalize the important factors favoring the chiral discrimination of enantiomers via this technique.

As presented in the theoretical part, the correlation between the magnitude of σ_i^{aniso} and the hybridization state or the nature

of substituents were in general observed experimentally. The effect of the electronegativity of substituents on σ_i^{aniso} was obvious in the case of the monofluorinated compounds such as the 2-fluorocyclohexanol where only the carbons bonded to heteroatoms displayed a line separation (Table 6, entry 4). Similarly, the effect of carbon hybridization state was clearly evident with the chiral acetylenic compounds for which $\Delta\nu^{R,S}$ values from 18 to 39 Hz were observed for the sp carbons (Table 1, entries 4 and 6). In general, chiral discrimination is larger for sp and sp^2 than for sp^3 carbons.

However, some examples seem to be exceptions from this general rule because the magnitude of the electronic shielding anisotropy, $\Delta\sigma$, is not the only parameter involved. The ordering parameters, $S_{u_iu_i\ (u\equiv a',b',c')} = \langle(3\cos^2\theta^z{}_{a'i} - 1)/2\rangle$, where $\theta^z{}_{a'i}$ is the angle between the magnetic field and one of the principal axis of the CSA tensor for a given carbon i, also plays an important role on the σ_i^{aniso} values. It must be emphasized that the function $(3\cos^2\theta - 1)/2$ is rather flat when $\theta \approx 0°$ and $\theta \approx 90°$. For these particular angles a small difference $\theta^R - \theta^S$ will correspond to a negligible DOE, $S^R - S^S$, thus leading to a failure of the chiral discrimination. A typical example is given by (±)-trimethylsilane-1-butyn-3-ol (Table 1, entry 5) where no separations on the sp carbon atoms were visible, whereas a small difference is observed on the sp^3 carbons. This molecule most probably adopts a position where the triple bond orients parallel to the helix axis. Consequently, a small variation from θ^R to θ^S does not affect the order parameters enough. Another example is given by (±)-*S*-methyl-*S*-*P*-tolyl-*N*-tosyl-sulfoximine where we can observe that all the carbons in one of the aromatic rings (C-7, C-8, C-9, C-10) were distinguished, while no visible separations were obtained for the other one.

At this point it is worthwhile to review the factors influencing the DOE. Analysis of carbon-13 data as well as those obtained in deuterium seem to indicate that the electrostatic interactions between the chiral solutes and the polypeptide helix play an important role in the chiral recognition mechanism.[4−6] Among these interactions, hydrogen bonds may be considered as the strongest involved. van der Waals forces might also have an effect depending on the polarity of the solute. Besides, geometrical factors have to be considered when discussing the differential ordering effect and the mechanisms of chiral recognition.[23] Actually, it seems that the PBLG liquid crystal systems might be seen as acting like any natural biopolymer which gives rise to an helical structure such as proteins, DNA, or polysaccharides.[4] The classical models involved for enzyme enantioselectivity might furnish a good starting point to understand the chiral discrimination phenomenon involved with PBLG.[24] In these models, the molecular shape recognition mechanisms plays an important role, and it should be the case with the PBLG system. Consequently molecules with a large shape asymmetry will probably exhibit a large DOE in this medium and this should be a favorable situation.[25]

Even if we cannot presently give a definitive answer to the general mechanism of enantiomeric selectivity in this chiral liquid-crystalline solvent, we can offer the following remarks from this discussion: chiral molecules having sp or sp^2 carbons, polar groups that may give strong electrostatic interactions, and a large shape asymmetry can most likely be discriminated with this technique. Conversely, the probability of observation of an enantiomeric separation on hydrocarbons or weakly polar compounds will be *a priori* small.

6. Conclusion

In this present study, we investigated the potential of proton decoupled natural abundance carbon-13 NMR spectroscopy for the observation of enantiomers of chiral molecules dissolved in the [PBLG]/[organic solvent] chiral liquid crystal. The visualization of each optical isomer was achieved through a difference in the chemical shift anisotropies of the carbon-13 resonances. The best results were obtained for sp^2 or sp carbons. The quantitative determination of enantiomeric purity was made using peak integration. The method is characterized by its simplicity and adequate sensitivity, providing a useful new NMR analytical tool. Besides, it has the valuable advantage that no labeling or any chemical modification of the chiral material is needed.

The method is applicable to a wide range of chiral materials bearing various functional groups or chiral molecules with an asymmetric heteroatom. In addition, the result obtained with the (±)-1,1′-bi-2-naphthol suggests that this technique may be extended to investigate *planar or axial chirality*. These applications are currently under study.

This analytical method can be considered as a competitive alternative to classical chiroptical, chromatographic, or NMR techniques. However, as we have emphasized, this method does not work for some cases, in particular for hydrocarbons.

The examination of the experimental results allowed review of the most important factors in the chiral discrimination, thus helping to predict better whether the two enantiomers of a given chiral molecule could be visualized using ^{13}C-{^1H} NMR in PBLG. However, we still need to improve our general understanding of chiral recognition mechanisms in PBLG in order to be able to assign absolute configurations.

Finally, it is important to emphasize that the method is nondestructive since the chiral solutes can be extracted from the liquid-crystalline phase using classical procedures.

Acknowledgment. The authors gratefully thank Prof. H. B. Kagan, Dr. O. Rian, Dr. W. Smadja, and Dr. A. Haudrechy, who kindly provided the samples of chiral sulfur, acetylenic, and diol derivatives, respectively, and Prof. J. W. Emsley and Prof. A. Loewenstein for stimulating discussions.

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