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Visualisation of enantiomers via insertion of a BIRD module in X–H correlation experiments in chiral liquid crystal solvent

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Abstract

Several ^{13}C–^{1}H NMR techniques are derived simplifying the visualisation of enantiomers in chiral ordering solvents. They proceed through various heteronuclear 2D experiments where a bilinear rotation decoupling sequence (BIRD) is inserted in the middle of the t_1 evolution period. In this way, the small couplings are refocused while the large couplings are preserved. The methods allow extracting precise values of one-bond carbon-proton residual dipolar couplings for each enantiomer out of unresolved proton-coupled ^{13}C or carbon-coupled ^{1}H spectra. Illustrative examples are analysed and discussed using various pulse sequences.
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1. Introduction

NMR using chiral liquid crystal solvents has been shown a powerful technique to visualise enantiomers [1–3]. The basis of the technique is that chiral solutes do not order identically when dissolved in such media due to the diastereomeric interactions with the molecules of the ordering solvent [4]. Consequently, all the order sensitive NMR interactions are different for enantiomers, namely, residual dipolar couplings, quadrupolar splittings and chemical shift anisotropies. This technique has proven to be efficient for a very large variety of molecules and nuclei [1,2].

In preceding papers, we have shown that proton-carbon 13 dipolar couplings could provide excellent results when proton NMR fails to differentiate enantiomers or gives poor results [5]. However, the sensitivity of these proton-coupled ^{13}C experiments is small and the visualisation of the enantiomers is often not trivial due to the numerous long-range dipolar couplings. As an example, the ^{13}C spectrum of 1,2-propylene carbonate in PBLG/$CDCl_3$ liquid crystal is presented in Fig. 1. Obviously, it is not possible to identify enantiomers in this 1D spectrum and to make any accurate measurement of couplings constants.

One possibility to simplify such spectra is to use heteronuclear selective refocusing experiments, HETSERF [6]. This method requires selective excitation of one isolated proton and this is not always possible. Another technique is to use ^{1}H homonuclear decoupling during acquisition. But this requires the use of multiple pulse sequences synchronised on the sampling frequency that modifies the heteronuclear dipolar couplings [7].

Another possibility was demonstrated in a recent work [8,11]. It consists in selecting one kind of heteronuclear coupling by introducing a bilinear rotation decoupling module (BIRD) in the middle of the t_1 evolution period of a *J*-resolved experiment. Due to the BIRD, only large one-bond heteronuclear couplings evolve during t_1, giving rise to very simple patterns to analyse. The present work explores and demonstrates the efficiency of various BIRD inserted heteronuclear ^{1}H–^{13}C 2D experiments. We will demonstrate here that these various experiments provide valuable NMR tools for analyzing mixture of enantiomers. Illustrative examples will be presented in order to show the efficiency of the method and we will discuss about the potentialities of the different sequences.

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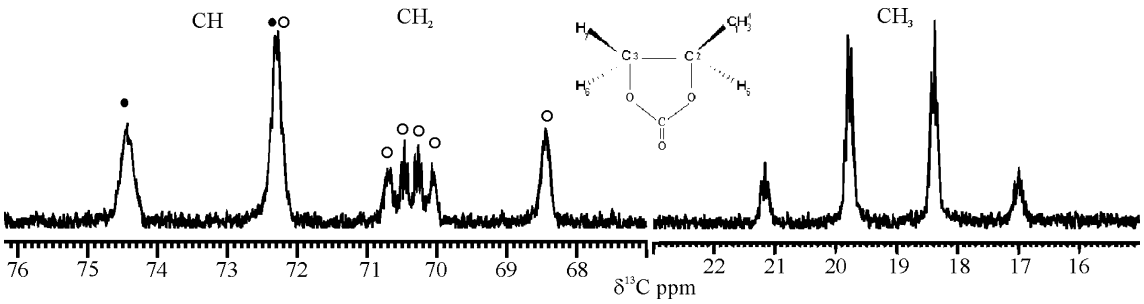

Fig. 1. 100.6 MHz proton-coupled carbon-13 1D spectrum of the 1,2-propylene carbonate dissolved in the PBLG-$CDCl_3$ phase, recorded at 304 K. The peaks with solid circles (●) correspond to the doublet of the CH group and the open circles (○) correspond to the CH_2 group. Note that long-range couplings prevent separate observation of enantiomers signals.

2. Theoretical background

To simplify the ^{13}C spectra we analyse the effect of the bilinear rotational decoupling module presented in the Fig. 2 [9].

In isotropic medium, the average Hamiltonian for an heteronuclear spin system ($I = {}^1H$, $S = {}^{13}C$) made of protons I_1, $I_{1'\ldots}$ directly bonded to the ^{13}C and having long-range coupling with protons $I_m, I_{m'\ldots}$ can be written as [10]

$$\sigma(t) \xrightarrow{\pi S_x} \sum \xrightarrow{\pi J_{I_1 S}\tau 2I_1^y S^z} \sum \xrightarrow{\pi J_{I_m S}\tau 2I_m^y S^z} \sum \xrightarrow{\pi J_{I_m I_1}\tau 2I_m^y I_1^y} \sum \xrightarrow{\pi J_{I_m I_{m'}}\tau 2I_m^y I_{m'}^y} \sum \xrightarrow{\pi J_{I_1 I_{1'}}\tau 2I_1^y I_{1'}^y} \sigma(t+\tau) \quad (1)$$

where $\sigma(t)$ and $\sigma(t+\tau)$ are the density matrices before and after the BIRD module, respectively. When the delay τ is taken equal to $1/J_{I_1 S}$ the Hamiltonian reduces to [10]

$$\sigma(t) \xrightarrow{\pi \sum I_1^y} \xrightarrow{\pi S^v} \sigma(t+\tau) \quad (2)$$

Where v stands for either x or y for, respectively, an odd or even number of protons ^1H directly bonded to the ^{13}C (CH, CH_2 or CH_3). If the BIRD module is incorporated in the middle of the incremented t_1 time, the average Hamiltonian involved during this evolution time reduced to

$$\overline{\mathbf{H}}^{(e)} = \sum \mathbf{H}_m + \sum \mathbf{H}_{I_1 S} + \sum \mathbf{H}_{I_1 I_{1'}} + \sum \mathbf{H}_{mm'} \quad (3)$$

Where $\mathbf{H}_m$ is the mth proton chemical shift interaction, and the various $\mathbf{H}_{ij}$'s are the coupling interactions between nuclei i and j. In weakly oriented medium, all the scalar couplings, J's, must be replaced by the total couplings, T's, which are the sum of the scalar and the double of the dipolar couplings

$$T_{ij} = J_{ij} + 2D_{ij}. \quad (4)$$

It is important to note that whenever the density matrix is involving either S or I_1 coherences at the beginning of the t_1 evolution time, the F_1 spectrum will exhibit only the one-bond coupling between I_1 and S and eventually the $I_1 - I_{1'}$ geminal couplings, thus simplifying considerably the spectral patterns. Several experiments can do this and can be classified as ^1J-resolved experiments. They must be studied separately depending on their preparation sequence as presented in Fig. 3. All the results are summarised in Table 1 that gives the density operator just before t_1 in term of product operators, and average Hamiltonian during the BIRD inserted t_1 evolution period for the various sequences used in this work.

All these experiments will not be quantitative in general due to the dependence of the signal intensities to the total coupling which will not be the same for enantiomers. The assignments have been checked using quantitative experiments such as SERF or HETSERF experiments [6,12].

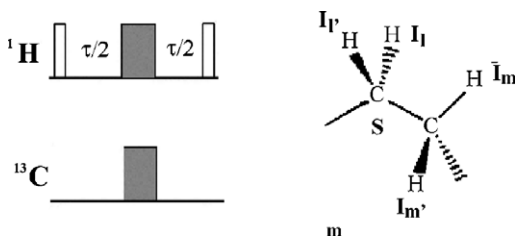

Fig. 2. Pulse sequence of the BIRD module used. Thick-grey and thin-white rectangles represent 180° and 90° pulses, respectively. All the pulses are applied along the x axis. On the right, the nomenclature of the various type of nuclei in the spin system under study.

3. Results and discussion

The simplest way to use the BIRD module is to introduce it in the middle of the t_1 time after an excitation pulse on the ^{13}C channel, giving the experiment presented in the Fig. 3A and named the ^{13}C-J-resolved-BIRD. In this case, only the directly bonded ^{13}C–^1H couplings modulate the signal in t_1. This has been applied in the case of 1,2-propylene carbonate in PBLG/$CDCl_3$ medium. The 2D spectrum is presented in Fig. 4.

We can see clearly the enantiomeric differentiation on this spectrum for all the carbon sites, differentiation which was not visible on the 1D carbon-13 spectrum in Fig. 1. The different single bond dipolar couplings can be measured accurately for each enantiomer and this is of prime importance to study the differences in the order parameters of chiral molecules.

This experiment has the following disadvantages. First, it has a poor sensitivity because only carbon-13 coherences

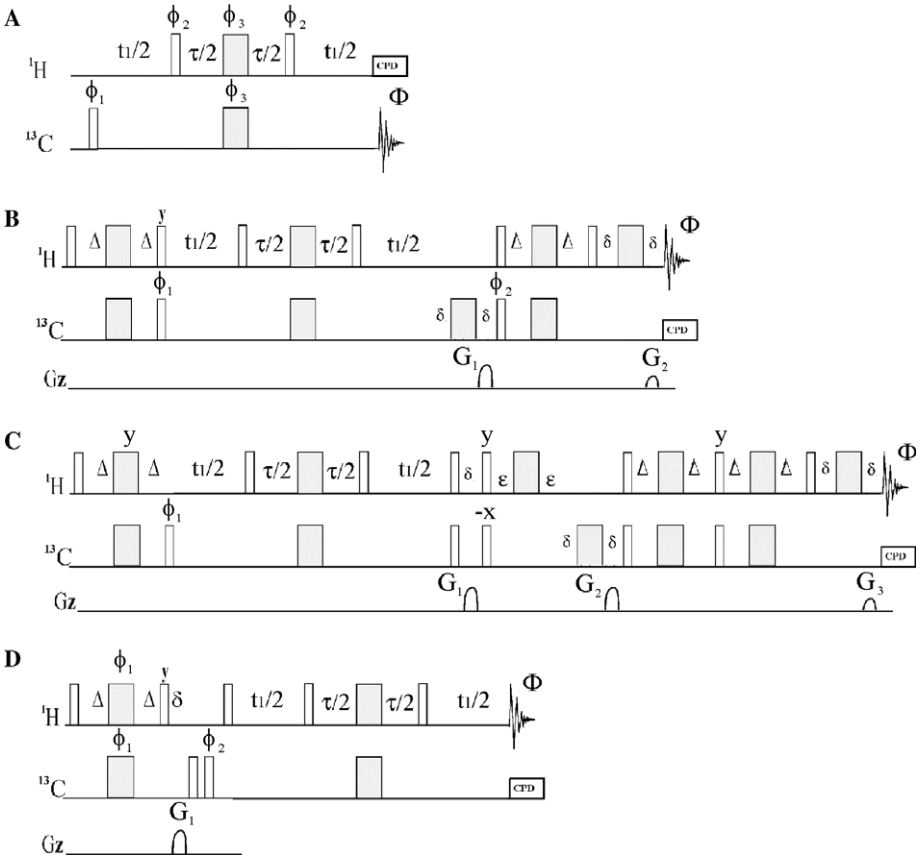

Fig. 3. Different experiments incorporating a BIRD module and phase cycle used (without precision the phase is x): 3-A-^{13}C-J-resolved-BIRD ($\phi_1 = x, x, x, x, y, y, y, y$; $\phi_2 = x, x, -x, -x$; $\phi_3 = x, x, x, x, -x, -x, -x, -x$; $\Phi = x, x, x, x, -y, -y, -y, -y$) 3-B-$J$-HSQC-BIRD, ($\phi_1 = x, -x$; $\phi_2 = x, x, -x, -x$; $\Phi = x, -x, -x, x$; the gradient ratio is $G_1/G_2 = 4$) 3-C-J-HMQC-BIRD, ($\phi_1 = x, -x$; $\Phi = x, -x$; the gradient ratio are $G_2/G_3 = 4$ and $G_3/G_1 = 1$) [13] 3-D-^{1}H-J-HSQC-BIRD, ($\phi_1 = x, x, -x, -x$; $\phi_2 = x, -x$; $\Phi = x, -x$).

Table 1
Average Hamiltonian for the different experiments evolving during t_1

Sequence	Product operator describing the system just before t_1	Average Hamiltonian $\overline{\mathbf{H}}$ evolving during t_1 (elimination of the terms commuting with the state of the system)
^{13}C-J-resolved-BIRD	S^y	$\overline{\mathbf{H}} = \sum \pi T_{I_1S} t_1 2I_1^z S^z$
J-HSQC BIRD	$2I_1^z S^y$	$\overline{\mathbf{H}} = \sum \pi T_{I_1S} t_1 2I_1^z S^z$
J-HMQC BIRD	$2I_1^y S^y$	$\overline{\mathbf{H}} = \pi T_{I_1S} t_1 2I_1^z S^z + \pi T_{I_1I_{1'}} t_1 2I_1^z I_{1'}^z$
^{1}H-J-HSQC BIRD	$2I_1^x S^z$	$\overline{\mathbf{H}} = \pi T_{I_1S} t_1 2I_1^z S^z + \pi T_{I_1I_{1'}} t_1 2I_1^z I_{1'}^z$

evolve during t_1 and t_2. Second, a long repetition cycle must be used due to the long relaxation time of carbon-13. Third, we cannot assign the different couplings to a specific proton in the methylene group. Consequently it is important to develop other experiments, based on an inverse detection of carbon-13 like HSQC experiment, for instance. This sequence is represented in Fig. 3B and named J-HSQC-BIRD. In this case, we have proton detection, and only the ^{13}C–^{1}H one-bond couplings modulate the carbon single quantum coherences during t_1 evolution. This experiment is slightly different from the HSQC-BIRD sequence published by Feher et al. In our case the ^{13}C chemical shifts and field inhomogeneities are refocused to improve the resolution in F_1 dimension [13]. This experiment has been realised on propylene oxide sample dissolved in PBLG/$CDCl_3$ mesophase and is shown in Fig. 5.

We can see again the enantiomeric differentiation on all the carbon sites of the compound. Note that methylene groups exhibit a single doublet, which gives access only to the sum of the couplings with the two directly bonded protons. Using this sequence, it is not possible to determine the heteronuclear single bond dipolar coupling for each of the diastereotopic protons. Consequently, it is impossible to know whether the two couplings are equal or not. To overcome this limitation, Kover et al. developed a BIRD version of HMQC, (Fig. 3C). In this sequence the density matrix contains only carbon-proton multiple quantum coherences at the beginning of the t_1 time. Consequently,

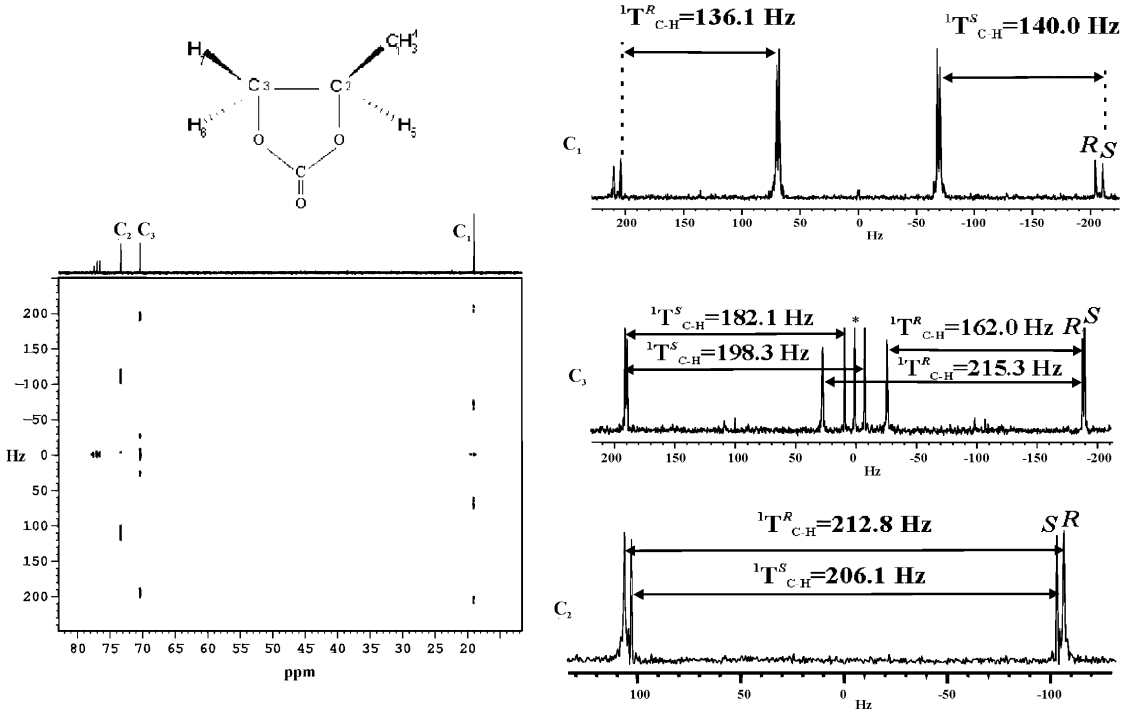

Fig. 4. 2D ^{13}C-*J*-resolved-BIRD map on 1,2-propylene carbonate in PBLG/$CDCl_3$ phase. On the right, the F_1 projections for the different ^{13}C chemical shift of the solute. The asterisks correspond to artefacts due to pulse imperfections. 16 scans per t_1 increment were used. The spectrum width was 500 Hz in F_1.

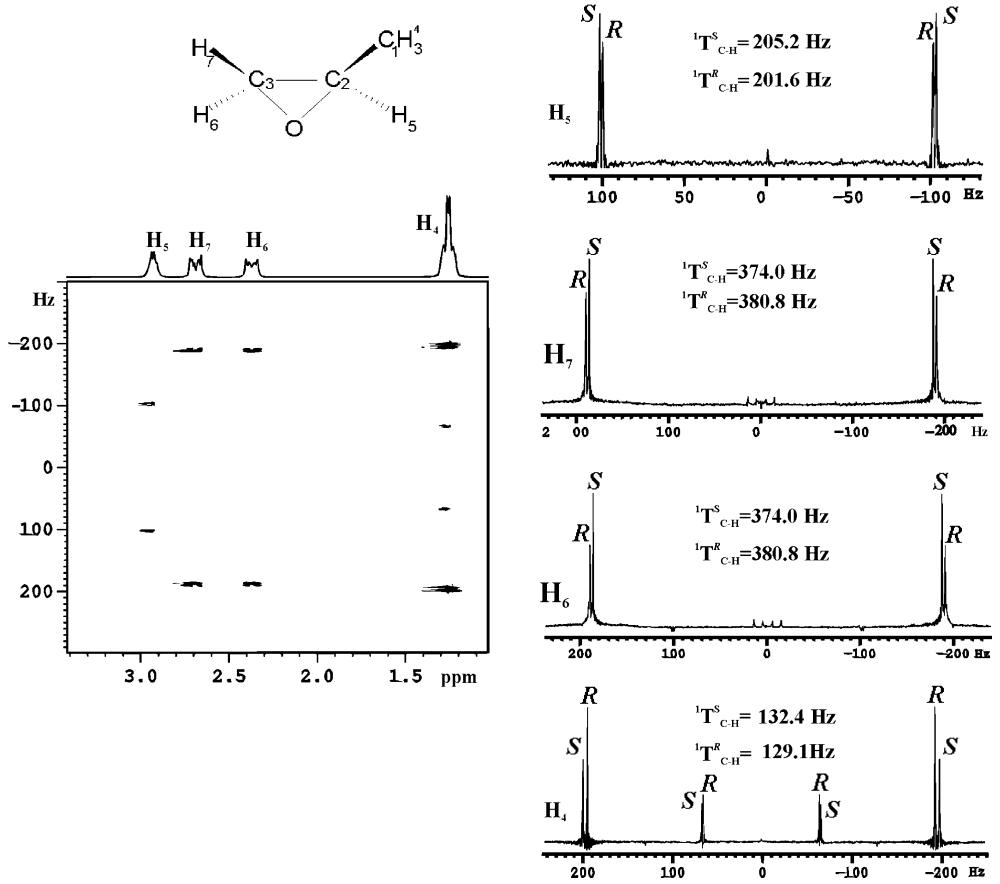

Fig. 5. *J*-HSQC-BIRD experiment on propylene oxide in PBLG/$CDCl_3$ phase. The F_1 rows for the different protons are shown. Note also the normal proton 1D spectrum along the projection in F_2. The spectrum width was 600 Hz in F_1.

only the passive couplings can evolve i.e., no modulated signal for a CH group, but a doublet of doublet for a CH_2 group made of two diastereotopic protons [14]. The two passive couplings are the geminal proton–proton coupling and the ^{13}C–^{1}H coupling with the proton of the methylene that is not involved in the multiple quantum coherence. This experiment realised on the propylene oxide sample is presented in Fig. 6.

The protons of the CH_2 groups are diastereotopic and they have different chemical shifts as seen in the 2D map. The enantiomeric differentiation is visible on this spectrum as there are two doublets of doublet in F_1 at each diastereotopic proton chemical shift column. With this experiment, we have access to the geminal proton couplings and these also are clearly different for the enantiomers. This last piece of data was not obtained using the *J*-HSQC-BIRD experiments.

Nevertheless, few disadvantages can be found for this experiment. First no modulation appears on CH signals. Consequently one needs to do two experiments, *J*-HSQC-BIRD for CH and CH_3's and *J*-HMQC-BIRD for CH_2's. Second, it is necessary to be extremely careful in the spectral analysis because the carbon-proton coupling measured at the proton chemical shift of proton H_i in a H_i–C–H_j group is actually the coupling with the other proton, $^{1}T_{CHj}$, in the same methylenic group and conversely. In Fig. 6 for instance, the column at H_7 gives access to the C_3–H_6 coupling, and the column at H_6 allows measuring C_3–H_7 coupling.

To overcome this problem, we have developed a new sequence called ^{1}H-*J*-HSQC-BIRD pictured in Fig. 3D. As in many 2D heteronuclear experiments, the pulse sequence starts with a period of polarisation transfer achieved here via an INEPT experiment. At the end of the second delay Δ, the antiphase proton magnetisation is converted into longitudinal two-spin order by the 90° ^{1}H pulse applied along the *y* axis. This spin polarisation is not affected by the field gradient that act as a z purge to eliminate undesired coherences not oriented along the *z* axis or arising from proton attached on ^{12}C. After the gradient, the two-spin order is back converted into the antiphase proton magnetisation by the final 90° ^{1}H pulse just before the t_1 evolution period. During the BIRD inserted t_1 evolution the antiphase proton magnetisation evolves under the effects of the heteronuclear ^{13}C–^{1}H couplings and eventual geminal proton–proton couplings. The sequence ends with the acquisition of the ^{1}H signal with broadband ^{13}C decoupling during t_2. This sequence is performed in phase-sensitive mode in order to obtain pure absorption peaks after 2D FT transform. Note that proton chemical shifts and field inhomogeneities are refocused thus improving the resolution in the indirect dimension. Consequently, small frequency differences can be detected improving the chance to visualise enantiomers in chiral liquid crystal.

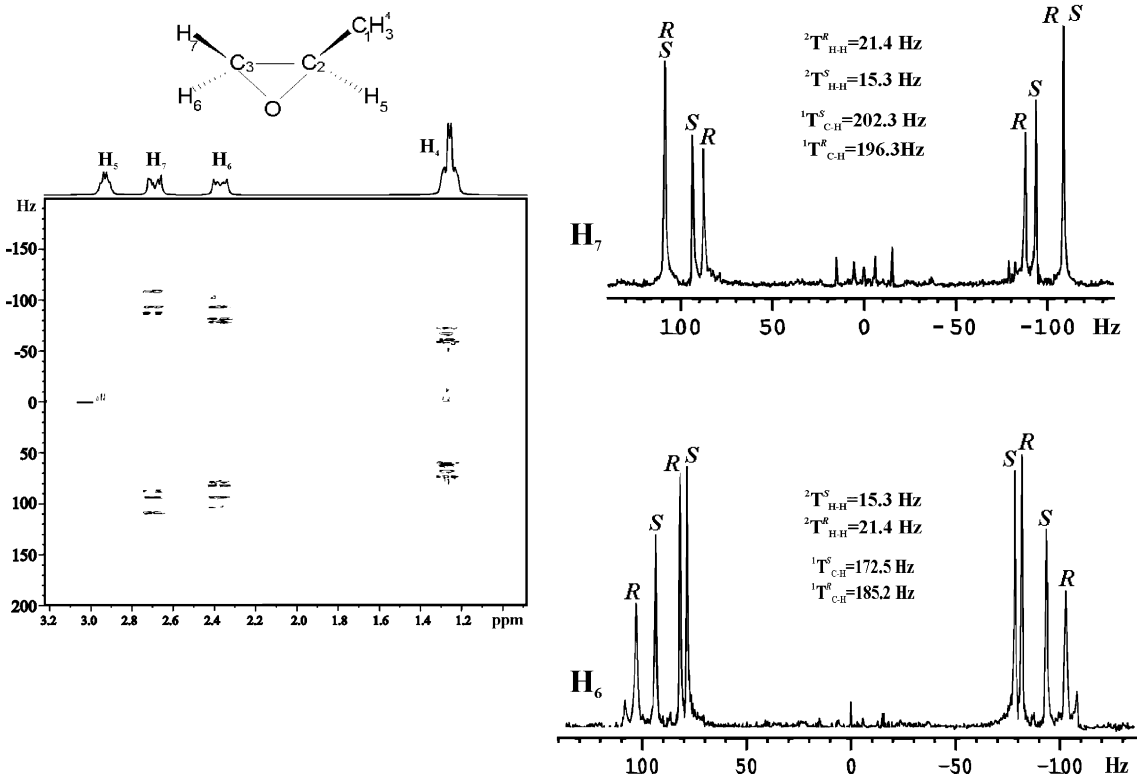

Fig. 6. *J*-HMQC-BIRD experiment on propylene oxide in PBLG/$CDCl_3$ phase. The F_1 projections for the different methylene protons are shown. The spectrum width was 400 Hz in F_1.

To illustrate the utility of this sequence, let us consider the example of the propylene oxide molecule dissolved in the PBLG/$CDCl_3$ phase. The spectrum is presented in Fig. 7.

The 2D spectrum shows the normal proton spectrum in the F_2 dimension and simple patterns in the F_1 dimension. For the CH group the splitting is only due to the one-bond heteronuclear dipolar couplings ($^1T_{C-H}$), one for each enantiomer. The CH_2 group displays a doublet of doublet due to both the one-bond heteronuclear coupling ($^1T_{C-H}$) and the geminal total coupling ($^2T_{H-H}$), observed for each enantiomer. As only proton single quantum coherences evolve during t_1, the active coupling measured in F1 domain at the chemical shift of a proton H_i in a H_i–C–H_j group is this time the $^1T_{CHi}$ heteronuclear one-bond coupling, contrary to the *J*-HMQC-BIRD experiment.

The methyl group signal in Fig. 7 is rather complex but it becomes easy to analyse when the τ delay of the BIRD is adjusted to the total coupling of the CH_3 group, $^1T_{CH} = 130$ Hz (instead of 200 Hz), as can be seen in Fig. 8. The spectrum then appears as a doublet of triplet due to the one-bond heteronuclear ^{13}C–^1H coupling and to the geminal dipolar proton couplings among the A_3 spin system. The F_1 projection in Fig. 8 is made of two doublets of triplet, one for each enantiomer. The enantiodifferentiation can be clearly visualised on this signal.

This pulse sequence is advantageous because one experiment is enough to obtain all the one-bond heteronuclear coupling simultaneously for the three types of spin system, CH, CH_2, and CH_3. This is not the case for the other sequences. Furthermore, homonuclear couplings between geminal protons can be measured with a very good precision among methylene and methyl protons in the anisotropic medium, thus driving to the dipolar couplings whenever the scalar couplings are known.

4. Conclusions

In this work, we have shown the effect of a BIRD module installed in the middle of the t_1 evolution time of various heteronuclear 2D experiments. The aim was to make possible the visualisation of enantiomers dissolved in a chiral liquid crystal solvent on the basis of the one-bond homonuclear ^{13}C–^1H dipolar couplings. The BIRD sequence was incorporated after creating either ^{13}C single quantum in phase coherences, or ^{13}C heteronuclear single

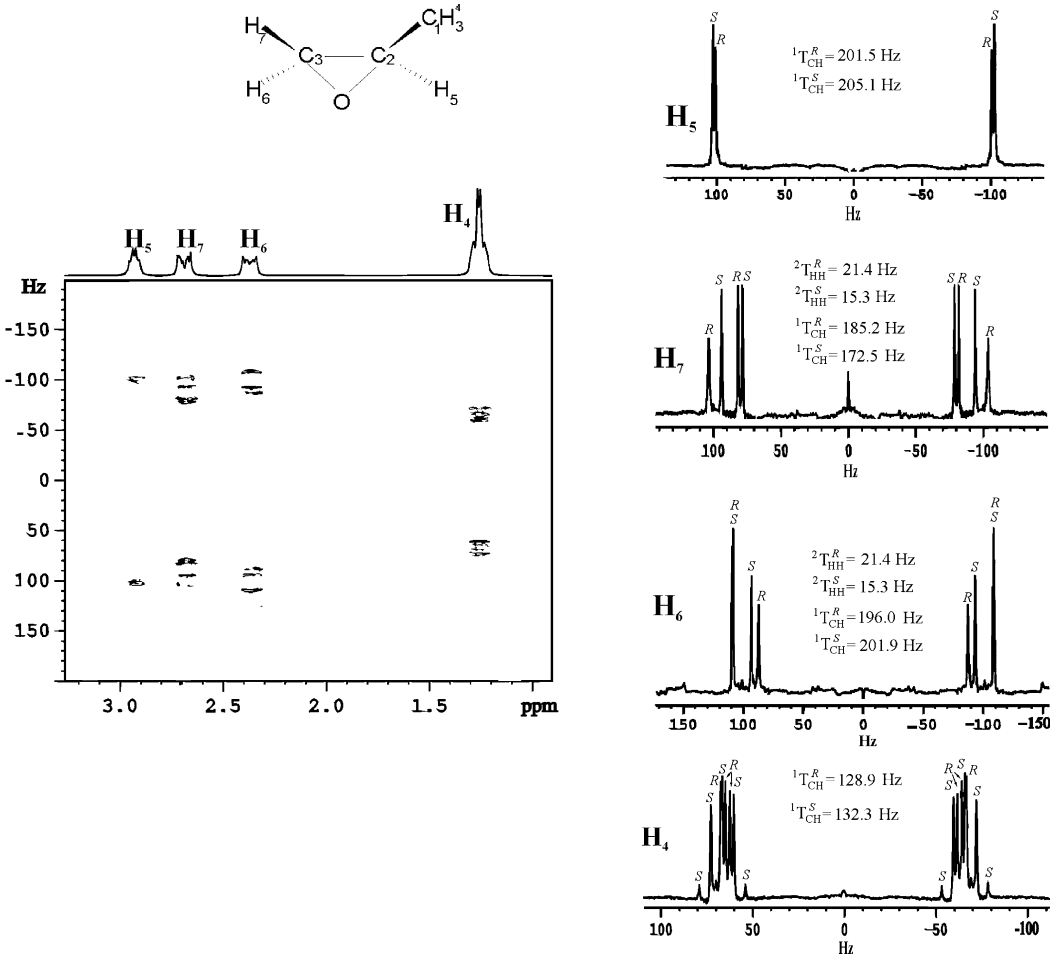

Fig. 7. The 2D ^1H-*J*-HSQC-BIRD spectrum of the propylene oxide dissolved in the PBLG-$CDCl_3$ phase, recorded at 304 K.On the top, the normal 1D proton spectrum. On the right, the F_1 projections at the different proton chemical shift. The spectrum width was 400 Hz in F_1.

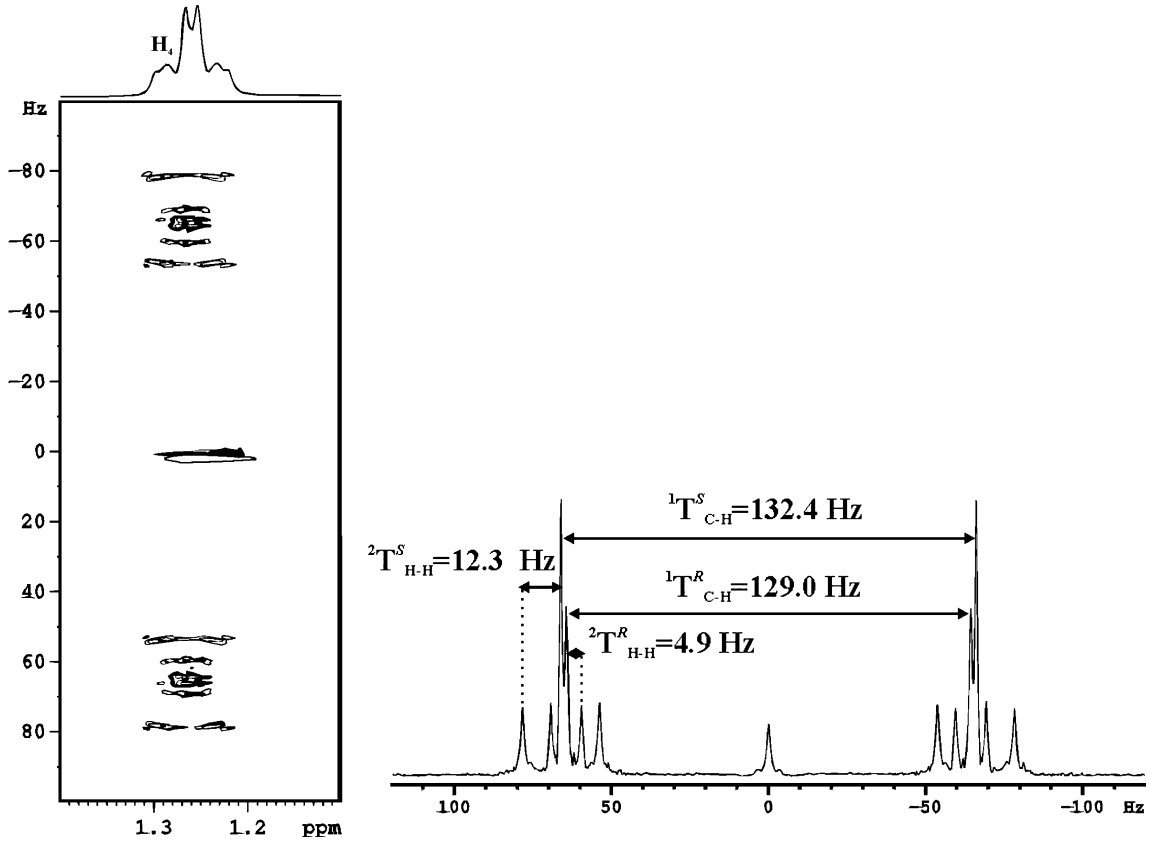

Fig. 8. Methyl part of the ^{1}H-*J*-HSQC-BIRD experiment on propylene oxide in PBLG/$CDCl_3$ phase with the $\tau = 1/{}^{1}T_{CH}$ delay corresponding to a heteronuclear coupling of ${}^{1}T_{CH} = 130$ Hz. The F_1 projection for the methyl protons is shown on the right. The spectrum width was 400 Hz in F_1.

quantum anti-phase coherences, or heteronuclear multi-quantum coherences or ^{1}H heteronuclear single quantum anti-phase coherences, thus giving rise to the ^{13}C-*J*-resolved-BIRD, *J*-HSQC-BIRD, *J*-HMQC-BIRD, or ^{1}H-*J*-HSQC-BIRD sequences. In all of these experiments, the only heteronuclear coupling which participate to the coherence evolutions are the one-bond ^{13}C–^{1}H couplings. The effect of the BIRD is to cancel out all the long-range heteronuclear couplings, thus simplifying the spectral patterns and giving the opportunity to measure precise values of the single bond total couplings. In addition, in the case of *J*-HMQC-BIRD and ^{1}H-*J*-HSQC-BIRD sequences we have access also to the geminal ^{1}H–^{1}H couplings.

We have demonstrated that the various experiments greatly simplify the visualisation of any enantiomeric differentiation. Furthermore, in all the experiments the magnetic field inhomogeneities are refocused thus improving the resolution in the indirect dimension.

The new ^{1}H-*J*-HSQC-BIRD sequence seems to be the most suitable experiment for the following reasons:

- The sensitivity is higher than in ^{13}C-*J*-resolved-BIRD
- It permits to measure separately the two ^{13}C–^{1}H couplings in a CH_2 group with diastereotopic protons in contrast with the *J*-HSQC-BIRD where we have only access to the sum of those couplings,
- It allows measuring in the same experiment the heteronuclear couplings among CH, CH_2 and CH_3 groups which is not possible with the *J*-HMQC-BIRD.
- It allows measuring the geminal couplings among CH_2 and CH_3.

These techniques should be of interest to all people involved in measuring precise values of residual dipolar coupling out of intricated or unresolved spectra in ordered media.

5. Experimental section

5.1. Sample preparation

The liquid-crystalline NMR samples investigated in this work were prepared using standard procedure [15]. The carbonate sample was made of 100 mg of PBLG (DP 782, Sigma), 30.6 mg and 21.2 mg of *R* and *S* carbonate enantiomers, respectively (Sigma), and 633 mg of $CDCl_3$. The epoxyde NMR sample was made of 100 mg of PBLG (DP 782, Sigma), 20.8 mg and 31.9 mg of *R* and *S* propylene oxide enantiomers, respectively (Sigma), and 695 mg of $CDCl_3$. The 5 mm o.d. NMR tubes were sealed to avoid solvent evaporation and then centrifuged head to tail until an optically homogeneous birefringent phase was obtained.

5.2. NMR spectroscopy

All the NMR spectra were performed on a high-resolution Bruker Avance 400 MHz spectrometer (9.4 T) using a 5 mm broad band inverse probe equipped with a z field-gradient coil and a 5 mm ^{1}H–^{13}C dual probe for the ^{13}C-J-resolved-BIRD experiment. The temperature was controlled at 304 K using a standard variable-temperature unit (BVT 3000). Unless otherwise specified, the 2D experiments were recorded using 2048 (TD_2)*1024(TD_1) data matrix and 8 scans *per* t_1 increment. The large number of t_1 increment, 1 K, was necessary to take advantage of the very effective narrowing of the lines in the F_1 dimension. The relaxation delays were 2 s. Otherwise specified, the BIRD τ delays were equal to 2.5 ms corresponding to a ^{13}C–^{1}H total coupling of 200 Hz and in all the experiments: $\Delta = \tau/4$.

Zero filling to 2048 data points was applied in the first dimension and exponential filtering of 1 Hz was applied in the second dimension prior double Fourier transform. All the experiments are phase-sensitive and the signals were phased in both dimensions. Further technical details will be found in the figure captions.

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