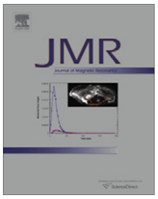
Journal of Magnetic Resonance 210 (2011) 24–30

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr



SERF-filtered experiments: New enantio-selective tools for deciphering complex spectra of racemic mixtures dissolved in chiral oriented media

Jonathan Farjon, Denis Merlet *

Equipe de RMN en Milieu Orienté, ICMMO, UMR 8182, Bat. 410, Univ. Paris-Sud, 91405 Orsay Cedex, France

ARTICLE INFO

Article history:
Received 2 November 2010
Revised 1 February 2011
Available online 4 March 2011

Keywords:
Semi-selective pulses
Enantio-selection
PBLG chiral oriented media
Selective refocusing experiments
Racemic mixtures

ABSTRACT

The use of chiral liquid crystals as NMR solvent is a powerful tool to visualize and quantify enantiomers. This technique is much more robust than traditional liquid state techniques. Recent developments have been performed to simplify proton spectra obtained in these solvents which are usually complex to analyze. In the different published sequences the enantiomeric differentiation can be visualized but none of them rely the different ^{1}H–^{1}H couplings to have a coherent set for each enantiomer especially in cases of racemic mixtures. In this work to overcome such limitations, we present a new NMR pulse sequence using semi-selective pulses to assign for each enantiomer the different ^{1}H–^{1}H couplings. The aim is to obtain the signal of only one enantiomer, through a first Selective ReFocussing (SERF) filtering block implemented for the enantio-selection followed by a second SERF block for measuring a chosen coupling. The whole sequence is called ^{1}H SERF-filtered-SERF. These techniques permit for the first time to assign all dipolar couplings to each enantiomer of racemic mixtures dissolved in chiral oriented media. We demonstrate that using this experiment it is possible to visualize enantio-differentiation even in the case where the multiplets are not resolved in the SERF experiments, pushing back the limits of the enantiomeric visualization.

1. Introduction

In many fields of application like pharmacology, materials sciences, biology and catalysis, the determination of the relative and absolute stereochemistry is essential. The elucidation of the stereochemistry requires the knowledge of the molecular conformation as well as the configuration of asymmetric stereocenters. Moreover, the determination of enantiomeric excesses is necessary before commercializing an active drug, a polymeric material or a catalyst.

In these domains, NMR using chiral liquid crystal as a solvent is a method of choice to differentiate and quantify enantiomers [1,2]. In such medium, enantiomers are not oriented in the same manner due to solute–solvent interactions. The NMR interactions depending to the molecular orientation namely chemical shift anisotropies, quadrupolar couplings for nuclei with $I > 1/2$ and dipolar couplings D_{ij} are then different for enantiomers. In other words, enantiomers have not the same NMR spectrum using a chiral anisotropic medium. The dipolar couplings are the most popular anisotropic measurable to determine the geometry of the solute [3]. They are directly linked to the molecular geometry and to orientational order parameters $S_{\alpha\beta}$. For a rigid moiety of the studied molecule, the mathematical relationship is:

$$D_{ij} = -\frac{\mu_o \hbar \gamma_i \gamma_j}{8\pi^2 . r_{ij}^3} \sum_{\alpha,\beta=x,y,z} \cos\theta_{ij}^{\alpha} \cos\theta_{ij}^{\beta} S_{\alpha\beta} \qquad (1)$$

where i and j are the two nuclei which interact. γ_i and γ_j are the magnetogyric ratio of i and j nuclei. θ_{ij}^{α} is the angle between the ij internuclear direction and the axes of the reference frame $\alpha \in (x,y,z)$. $S_{\alpha\beta}$ are second rank elements of the Saupe tensor. Note that in this medium, the coupling measured on the spectrum is the total coupling, T_{ij}, which is $T_{ij} = J_{ij} + 2 \cdot D_{ij}$ for an isochronous nuclei ($\delta_i \neq \delta_j$) in the case of a first order spectrum and $T_{ij} = 3 \cdot D_{ij}$ for isochronous nuclei ($\delta_i = \delta_j$) [5]. Usually ^{1}H and ^{13}C spectra of enantiomeric mixtures are hard to analyze or undecipherable in chiral liquid crystalline media such as a binary mixture composed on poly-γ-benzyl-L-glutamate (PBLG) and an organic solvent [4,5]. These spectra are the sum of very large unresolved signals (Fig. 1) because numerous short and long range total couplings enlarge signals and make analysis really difficult. Furthermore, due to the chirality of the medium, the signals of each enantiomer are potentially different. Thus, at each chemical shift the enantio-differentiation is obtained but unfortunately many transitions are overlapped that render analysis undecipherable.

Numerous NMR techniques have been developed to make easier spectral analysis especially the use of selective pulses [6,7]. They were intensively implemented either for simplifying spectra of complex mixtures, or for deciphering overcrowded spectra of small to averaged sized compounds like polysaccharides [8–10]. More

---

* Corresponding author. Fax: +33 1 69 15 81 05.
E-mail address: denis.merlet@u-psud.fr (D. Merlet).

1090-7807/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2011.02.002

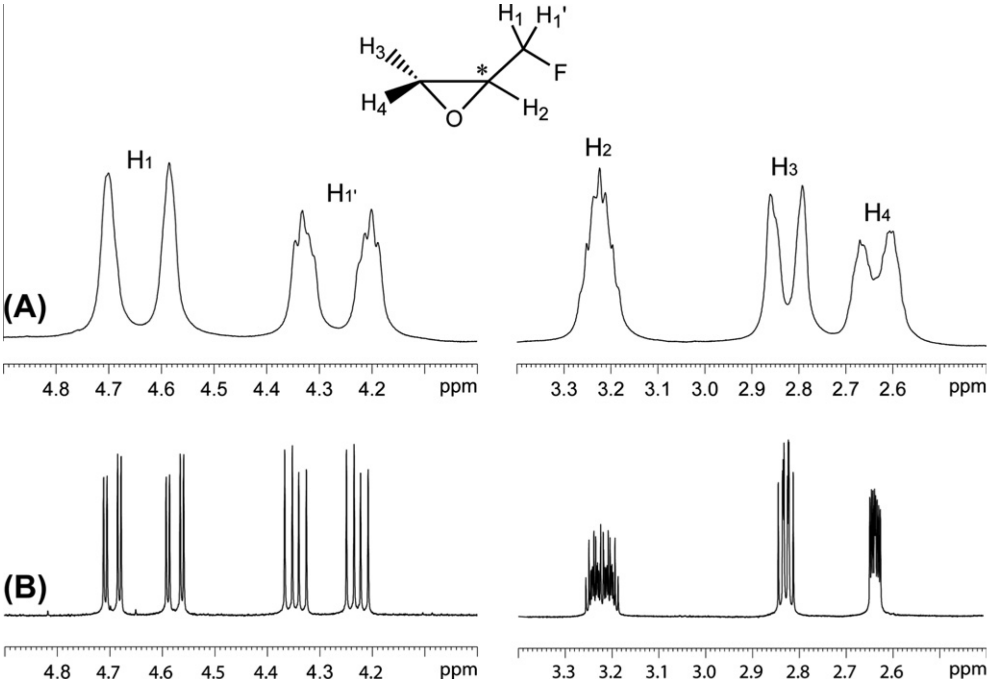


Fig. 1. (A) 400 MHz proton spectrum of a racemic mixture of epifluorhydrin drawn on the top in PBLG/CHCl$_3$ and (B) in deuterated chloroform.

recently their utility has been shown for disentangling also complex spectra obtained for enantiomeric mixtures dissolved in chiral oriented media like the ones composed of a liquid crystalline mesophase such as poly-γ-Benzyl-L-glutamate (PBLG) dissolved in an organic solvent. NMR experiments such as Selective ReFocussing experiment (SERF) [11,12] and Heteronuclear Selective ReFocusing experiment (HETSERF) [13], use selective refocusing techniques. They permit to let evolve only one coupling during the t_1 time of 2D experiments simplifying the signal in the F_1 dimension. They allow discriminating and quantifying enantiomeric mixtures. In this case, the enantiomeric excesses can be determined. The assignment of the different signals can be trivially done on enriched mixtures but the enantiomers have signals with the same intensity in racemic mixtures. Then it is not possible to assign a set of couplings to one configuration. This is a serious limitation as the dipolar couplings are necessary to determine the orientational order of a solute and to access to the magnitude of the enantio-differentiation. More recently, soft-COSY [14], *z*-COSY [15], β-COSY [16] and CESS-COSY [17] experiments have also shown their potential for simplifying spectral analysis of enantiomeric mixtures. Among them, the 1D ^{1}H selective versions of COSY experiments have been an efficient tool for detecting a single enantiomer ^{1}H spectrum [18]. As far as the spectral analysis and the accuracy on the measurement of ^{1}H–^{1}H couplings is concerned such techniques can be employed. However, in case of unresolved multiplet, these techniques failed for discriminating enantiomers. To overcome limitations of previous methods, we propose a new set of experiments baptized SERF-filtered experiments.

2. Results and discussion

The pulse sequence of the ^{1}H SERF-filtered SERF experiment is presented in Fig. 2. It allows connecting ^{1}H–^{1}H couplings two by two, and then permits to get a coherent ensemble of NMR anisotropic data for each enantiomer.

For better understanding the effect of this pulse sequence, let's consider a system of three spins H$_i$, H$_j$ and H$_k$. The aim is to correlate T_{HiHj} the total coupling between H$_i$ and H$_j$ with T_{HjHk} the total coupling between H$_j$ and H$_k$ for a given enantiomer.

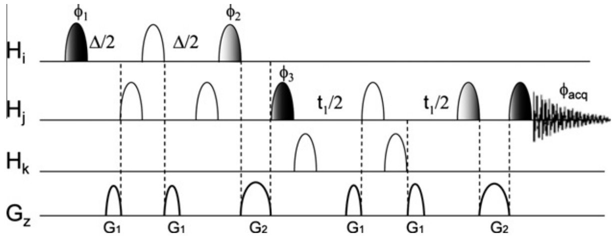


Fig. 2. Pulse sequence of the ^{1}H SERF-filtered SERF experiment. Open shapes are 180° refocusing pulses, black shapes are 90° excitation pulses, and grey shapes are 90° reversal selective pulses. Field gradient pulses (G_z) are black circled semi-ellipsoid. BURP [19] and Gaussian cascades [20] shaped pulses have been tested and shown similar results. All phases are *x* except $\phi_1 = x, -x, x, -x$, $\phi_2 = y$, $\phi_3 = x, x, -x, -x$ and $\phi_{acq} = x, -x, -x, x$. Field gradient pulses are applied along the *z* axis with 500 μs duration for G_1 (5 G/cm), and 1 ms for G_2 (25 G/cm).

The pulse sequence starts with a first 90° selective pulse, exciting the H$_i$ proton, then a selective echo on H$_i$ and H$_j$ allows converting the $-H_{iy}$ magnetization term into $2H_{ix}H_{jz}$ single quantum antiphase coherence. Then two selective 90° pulses on H$_i$ and H$_j$ convert $2H_{ix}H_{jz}$ into $2H_{iz}H_{jy}$. Between them, a purging *z* gradient pulse is used to filter out the $-H_{iy}$ coherence, in order to transfer the magnetization from H$_i$ to H$_j$ nucleus. After the H,H INEPT block, a selective echo or SERF lets evolve the T_{HjHk} couplings during the incremental t_1 time of a 2D experiment. Finally a *z* gradient filter is implemented to only retain the cosine term during t_1 for getting phase sensitive signals in F_1 and F_2 dimensions of the resulting 2D spectrum after double Fourier transform.

The equation of the FID signal without considering relaxation contributions is:

$$
\begin{aligned}
S(t_1,t_2) = {} & \frac{A}{2}\sin(\pi\cdot T_{\mathrm{HiHj}}\Delta)\cdot\cos(\pi\cdot t_1\cdot T_{\mathrm{HjHk}})\cdot[\exp(-i\cdot t_2(2\pi\nu_{\mathrm{Hj}} \\
& + \pi T_{\mathrm{HiHj}})) - \exp(-i\cdot t_2(2\pi\Delta\nu_{\mathrm{Hj}} + \pi T_{\mathrm{HiHj}})] \\
& \times \prod_{l\neq i}\cos(\pi\cdot t_2 T_{\mathrm{HjHi}}) \qquad (2)
\end{aligned}
$$

where ν_{Hj} is the H_j frequency of precession and T_{HjHl} total couplings between H_j and other protons, H_l, of the studied molecule except H_i.

After a double Fourier transform and phase corrections, it gives a 2D spectrum in pure absorption by using the sequential quadrature procedure (QSEQ).

Note that the amplitude of the signal is depending on the $\sin(\pi \cdot \Delta T_{HiHj})$ term. Assuming a different total coupling T_{HiHj} for each enantiomer, the modulation is different. Thus, choosing carefully the value of Δ, will permit to select the signals of a chosen enantiomer.

To test the ability of the SERF-filtered SERF experiment to select a desired enantiomer, an enriched mixture of propylene oxide (*S* enantio-enriched ee = 21%) dissolved in a liquid crystalline medium composed on PBLG/$CHCl_3$ solvent has been used. The 2D SERFph spectrum between H_3 and H_4 is presented in Fig. 3. It shows two doublets in F_1 dimension centered on zero due to the chemical shifts refocusing, with $T^{R}_{H3H4} = 21.5$ Hz and $T^{s}_{H3H4} = 15.0$ Hz. In F_1 dimension it is easy to visualize the enantio-differentiation that is not the case in F_2 dimension. Note the assignment of the different signals is trivial due to the enantiomeric excess.

In the following, the difference between T_{H3H4} total couplings of each enantiomer will allow selecting a given enantiomer. In this case, it is necessary to maximize the magnitude of the signal of one enantiomer (for instance the enantiomer A) and minimize the signal of the other one (enantiomer B). To determine the optimal delay Δ for keeping the signal of the A enantiomer: $\Delta^{\text{opt-A}}_{\text{H3H4}}$, we have searched to maximize the sine modulation factor (see Eq. (2)) difference F^{diff} giving by:

$$F^{\text{diff}} = \left\| \sin\left(\pi \cdot T^{A}_{\text{H3H4}}\Delta\right) \right| - \left| \sin\left(\pi \cdot T^{B}_{\text{H}_3\text{H}_4}\Delta\right) \right\| \text{with A, B the enantiomers}$$

The goal is to obtain Δ values that make reach F^{diff} to 1 as the sine factor of the chosen enantiomer shall be close to 1 and the other one close to zero.

In Fig. 4, are presented the magnitude of the sine modulation and their difference for the enriched mixture of propylene oxide. In order to cancel the *S* enantiomer signals from the spectrum, the optimal Δ delay: $\Delta^{\text{opt-}R}_{\text{H3H4}}$ need to be fixed to 66.7 ms. A modulation factor of 98 % could be theoretically reached for the *R* enantiomer signals and the S enantiomer signals should be null. Contrariwise, for vanishing the *R* enantiomer: $\Delta^{\text{opt-}S}_{\text{H3H4}}$ delay need to be set to 92.6 ms. A modulation factor of 94 % of the *S* enantiomer signals will be obtained and it remains 2% of the *R* enantiomer signals.

In Fig. 5A is presented the SERF-filtered SERF spectrum obtained for the H_4–H_3–H_2 spin system with $\Delta^{\text{opt}-R}_{\text{H3H4}} = 66.7$ ms. Only the doublet with T_{H3H2} = 4.8 Hz associated to the H_3–H_2 spin system of the *R* enantiomer is clearly visible on F_1 dimension. No signals of the *S* enantiomer appear on the spectrum. In F_2 dimension, the $T^{R}_{H3H4} = 21.5$ Hz can be measured through an antiphase doublet (see F_2 row projection). On the contrary, optimizing the INEPT block for $\Delta^{\text{opt}-S}_{\text{H3H4}} = 92.6$ ms leads to the vanishing of *R* signals (Fig. 5B) and only a doublet with T_{H3H2} = 7.4 Hz can be detected on F_1 dimension. The very good selection of each enantiomer can be seen in Fig. 5C where are overlaid the positive F_1 projections of the standard SERFph and the two SERF-filtered SERF experiments respectively. In the same manner, SERF-filtered SERF experiments have been successfully applied on the H_3–H_4–H_2 spin system for assigning T_{H4H2} to each enantiomer (see Fig. 5 D, E and F).

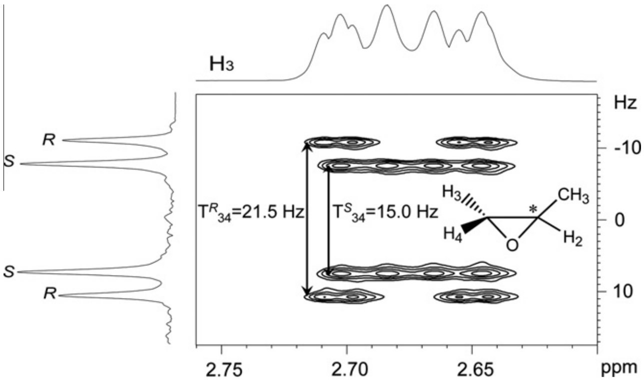


Fig. 3. 2D SERFph spectrum to measure the couplings between H_3 and H_4 of an enriched mixture of propylene oxide dissolved in a chiral liquid crystal phase composed on PBLG/$CHCl_3$.

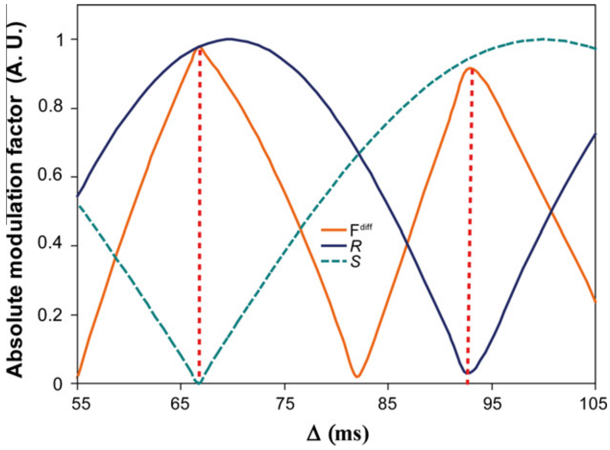


Fig. 4. Evolution of the absolute modulation sine factor depending on T_{34} (see Eq. (2)), for each enantiomer (blue and green) and their difference: F^{diff} (in orange) against the Δ delay for the enantio-enriched propylene oxide. Red lines are mentioning Δ optimal values for selecting a given enantiomer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As shown with the propylene oxide sample, ^{1}H-SERF-filtered SERF experiment allows to assign total couplings to each enantiomer of enriched mixtures. In order to demonstrate the efficiency of the SERF-filtered SERF experiment, a racemic mixture of epifluorhydrin in PBLG/$CHCl_3$ has been studied.

Again to determine the optimal Δ delays in the SERF-filtered SERF, it is necessary to first access the amplitude of couplings using a SERFph experiment. In Fig. 6A the SERFph spectrum of (±)-epifluorhydrin in PBLG/$CHCl_3$ to measure T_{H3H4} couplings is presented. Two doublets are visualized corresponding to the A and B enantiomers with the values T^{A}_{34} = 29.0 Hz and $T^{B}_{34} = 19.0$ Hz. The same experiment has been done for the T_{H3H2} determination as shown in Fig. 6B. One singlet and one doublet are observed on the F_1 projection; again we can visualize the enantio-differentiation on this internuclear direction. Unfortunately the SERFph experiment is not efficient to know whether T_{H3H2} coupling of the A enantiomer is 0 or 3.3 Hz. In order to answer the question, the SERF-filtered-SERF experiment has been used. Thus, we have determined an optimal Δ delay to remove signals of the A enantiomer ($\Delta^{\text{opt-}B}_{\text{H3H4}}$) through the HH INEPT block and the optimal Δ delay to remove signals of the B enantiomer ($\Delta^{\text{opt-}A}_{\text{H3H4}}$).

From the values of T_{H3H4} the delays are determined to be: $\Delta^{opt-A}_{\text{H3H4}} = 52.6$ ms and $\Delta^{\text{opt-}B}_{\text{H3H4}} = 34.5$ ms (Fig. 7). They have been used in the SERF-filtered SERF experiments and the spectra are presented in Fig. 8A and B.

On the F_1 projection of Fig. 8A, we can clearly visualize a singlet. As the experiment has been optimized using the $\Delta^{\text{opt-}A}_{\text{H3H4}}$, this signal corresponds to the A enantiomer. On the contrary, a 3.3 Hz doublet is visualized on the F_1 projection of the SERF-Filtered SERF spectra

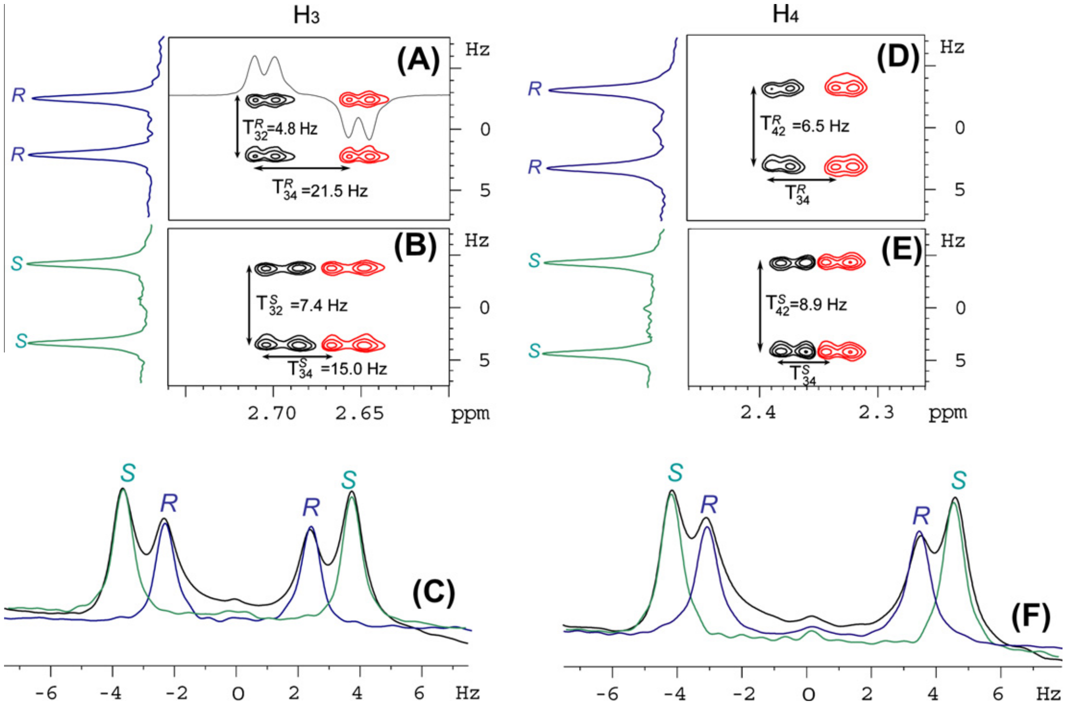


Fig. 5. SERF spectra on an *S* enriched mixture of propylene oxide dissolved in a chiral liquid crystal phase composed on PBLG/CHCl$_3$. 2D SERF-filtered-SERF spectra selective on H$_4$–H$_3$–H$_2$ protons, optimized for (A) $\Delta^{opt\text{-}R}_{H3H4}$ = 66.7 ms (B) $\Delta^{opt\text{-}S}_{H3H4}$ = 92.6 ms. (C) Superimposition of positive F$_1$ projections of (A) (blue), (B) (green) and the one of the 2D H$_3$–H$_2$ SERFph (black). SERF-filtered-SERF spectra selective on the H$_3$–H$_4$–H$_2$ optimized for (D) $\Delta^{opt\text{-}R}_{H3H4}$ = 66.7 ms, (E) $\Delta^{opt\text{-}S}_{H3H4}$ = 92.6 ms. (F) Superimposition of positive F$_1$ projections of (D) (blue), (E) (green) and the one of the 2D H$_4$–H$_2$ SERFph (F) (black). Red signals are negative and black ones are positive on the 2D spectra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

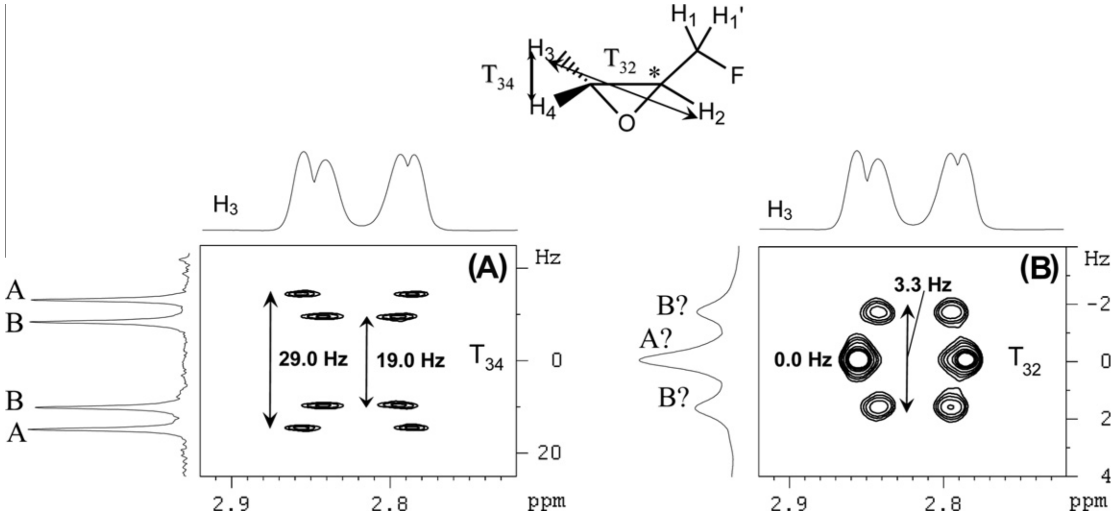


Fig. 6. 2D SERFph spectra selective (A) on H$_3$ and H$_4$, (B) on H$_3$ and H$_2$ of a racemic mixture of epifluorhydrin dissolved in a mesophase composed on PBLG/CHCl$_3$.

(Fig. 8B) obtained using the $\Delta^{opt\text{-}B}_{H3H4}$ delay, and then this signal corresponds to the B enantiomer.

The selection of a given enantiomer allows strongly enhancing the spectral resolution for signals on F_1 dimension. For example, on the 2D SERFph optimized for $H_3H_{1'}$ couplings (Fig. 8F), the visualization of the enantio-differentiation in F_1 is not easy since the natural linewidth of signals is large. Moreover, the precise measurement of $H_3H_{1'}$ couplings is not possible anymore. Using the SERF-filtered experiments, the measurement of $H_3H_{1'}$ could be done with a higher resolution and therefore accuracy (Fig. 8D and E). In the same manner, SERF-filtered experiments have shown their efficiency for measuring close H_4H_1 (Fig. 8J and K) and H_4H_2 (Fig. 8G and H) couplings.

Due to the periodicity of the sine function, numerous Δ^{opt} delays can be used to select each enantiomer. In order to prevent losses in sensitivity by relaxation during the enantio-selective filter, only the smallest optimum delays have been chosen.

However, the robustness of this experiment has been tested for selecting a given enantiomer for small coupling. In this case longer Δ delay need to be used and even at delay longer than 170 ms, good results have been obtained (data not shown). In Fig. 9A and B are presented the SERFph spectrum obtained for the $H_4H_{1'}$ and

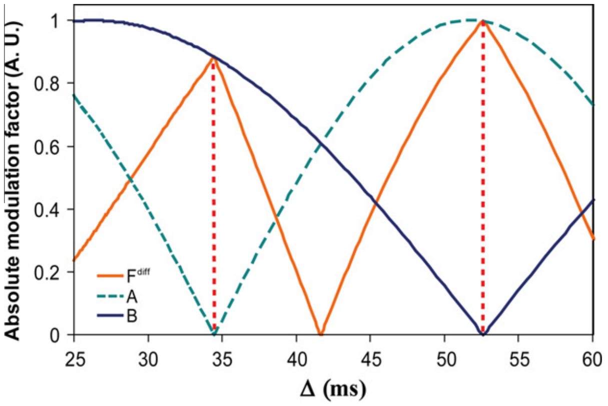


Fig. 7. Evolution of the absolute modulation sine factor for the T_{34} coupling (see Eq. (2)), for each enantiomer (blue and green) and their difference: F^{diff} (in orange) depending on the Δ value for the epifluorhydrin solutes. Red lines are mentioning Δ optimal values for selecting a given enantiomer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$H_1H_{1'}$ coupling. In these two experiments, no enantiomeric differentiations are visualized.

By comparing the results obtained with the SERF-filtered SERF experiments, very close total couplings can be assigned and measured between H_4 and $H_{1'}$ (Fig. 9B and C), but also between H_1 and $H_{1'}$ (Fig. 9F and G). This oversimplification comes from the combination of the field inhomogeneities refocussing during t_1 time of SERF sequences with the enantiomeric selection of the SERF-filtered SERF version. It allows gaining an average factor of 2 in resolution comparing with the SERFph experiment. It is now possible to assign total couplings only separated by 0.60 Hz with an average accuracy close to 0.3 Hz that has not been possible to measure precisely with the standard SERFph experiment.

SERF-filtered SERF experiments permit to separate and to assign total couplings for each A, B enantiomer, if there is at least one coupling on which an enantio-differentiation is measurable with the SERF experiment. Therefore it makes easier the analysis of racemic mixture, here the (±)-epifluorhydrin, in a chiral oriented medium (Table 1).

3. Conclusion

In this paper, we have developed a new NMR sequence called SERF-filtered SERF. This corresponds to modulate the amplitude of the SERF experiment by the evolution of another coupling during a first preparing step of this experiment. This technique is very efficient to oversimplify spectra and allows assigning total couplings to each enantiomer of racemic but also enantio-enriched mixtures. The ^{1}H version allows enhancing the spectral resolution of SERFph spectra by an average factor of 2, especially in case of very close couplings that could not be separated with SERFph experiments. SERF-filtered SERF experiments permit for the first time to get for each enantiomer, especially of racemic mixtures, a coherent ensemble of total couplings and therefore the dipolar couplings. The latters are mandatory for accessing the order matrix of *R* and *S* enantiomers in order to determine orientational order.

4. Experimental

4.1. Sample preparation

The two liquid–crystalline NMR samples investigated in this work were prepared using standard procedure [4]. The sample of propylene oxide was made of 11 mg of *S* enantiomer and 42 mg of the racemic mixture (ee = 21%) dissolved in 100 mg of PBLG (MW = 168,000, purchase from SIGMA), and 684 mg of dry $CHCl_3$. The sample of epifluorhydrin is a racemic mixture and

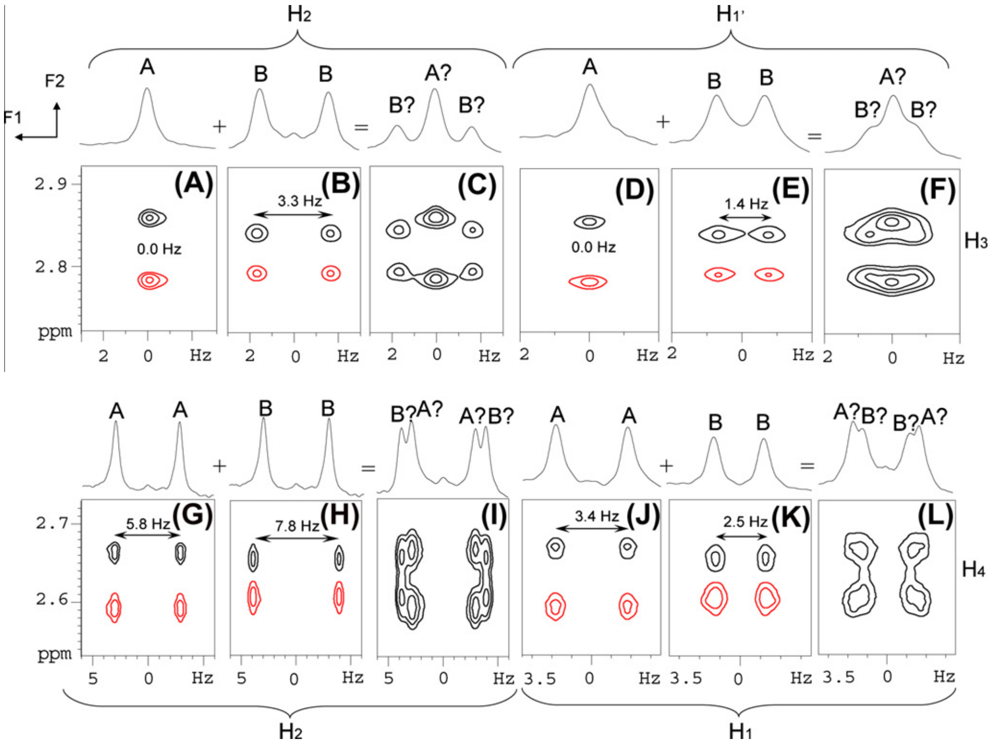


Fig. 8. SERFph and SERF-filtered SERF spectra recorded at 400 MHz for a racemic mixture of epifluorhydrin dissolved in PBLG/$CHCl_3$ for different H_i–H_j total couplings for the enantiomers. (A), (D), (G), (J) SERF-filtered SERF optimized for A enantiomer with a $\Delta^{\text{opt-}A}_{\text{H3H4}}$ = 52.6 ms, (B), (E), (H), (K) SERF- filtered SERF optimized for B enantiomer with a $\Delta^{\text{opt-}B}_{\text{H3H4}}$ = 34.5 ms. (C), (F), (I), (L) SERFph spectra.

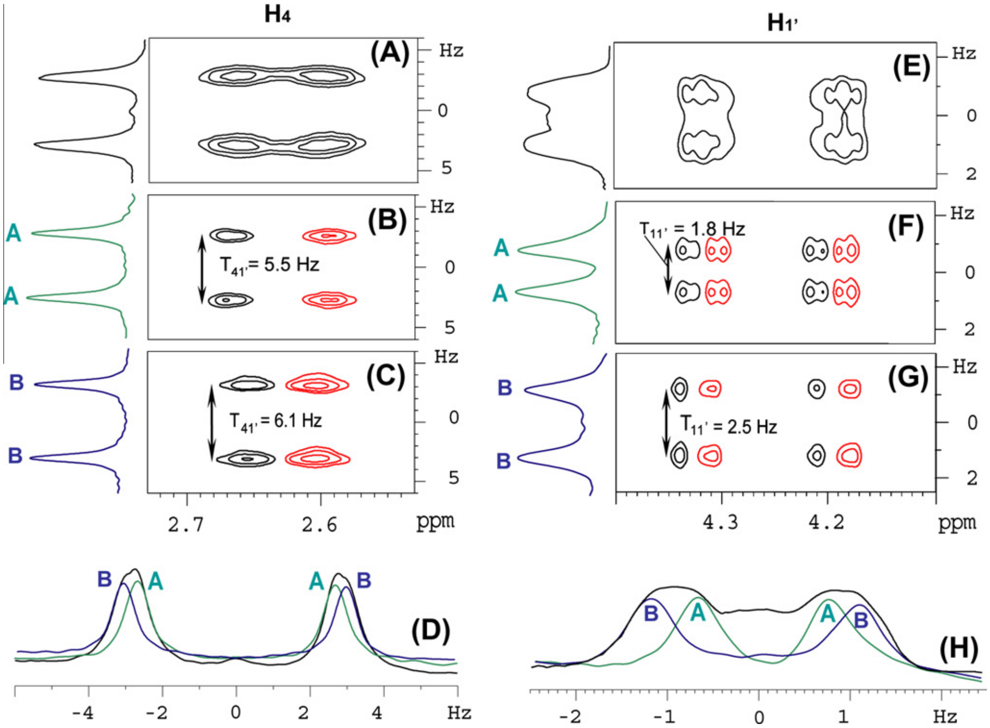


Fig. 9. SERFph experiments applied on (±)-epifluorhydrin in PBLG/CHCl$_3$: (A) the SERFph between H$_4$ and H$_{1'}$, (B) SERF-filtered SERF on H$_3$–H$_4$–H$_{1'}$ using Δ^{opt-A}_{H3H4} = 52.6 ms, (C) SERF-filtered SERF on H$_3$–H$_4$–H$_{1'}$ using Δ^{opt-B}_{H3H4} = 34.5 ms, D) Overlay between (A) and (B), (C) F$_1$ positive projections. (E) the SERFph between H$_{1'}$ and H$_1$, (F) SERF-filtered SERF experiments on H$_2$–H$_{1'}$–H$_1$ using $\Delta^{opt-A}_{H2H1'}$ = 96 ms (G) using $\Delta^{opt-B}_{H2H1'}$ = 147 ms. (H) Overlay between (E) and (F), and (G) F_1 positive projections.

Table 1
Table of total couplings extracted and assigned from the SERF-filtered SERF experiments, for each enantiomer of a racemic mixture of epifluorhydrin dissolved in a PBLG/CHCl$_3$ liquid crystalline medium.

i–*j*	Number of bonds	T_{ij} (A) in Hz	T_{ij} (B) in Hz
H$_4$–H$_3$	2	29.0	19.0
H$_1$–H$_{1'}$	2	1.8	2.5
H$_2$–H$_3$	3	0.0	3.3
H$_2$–H$_4$	3	5.8	7.8
H$_1$–H$_2$	3	5.5	4.0
H$_{1'}$–H$_2$	3	10.4	6.8
H$_3$–H$_1$	4	1.5	1.5
H$_3$–H$_{1'}$	4	0.0	1.4
H$_4$–H$_1$	4	3.4	2.5
H$_4$–H$_{1'}$	4	5.5	6.1

commercially available. The sample is composed on 54 mg of epifluorhydrin, dissolved in 100 mg of PBLG and 678 mg of dry CHCl$_3$. All 5 mm o.d. NMR tubes are screw thread tubes to avoid solvent evaporation and are centrifuged back and forth until an optically homogeneous birefringent phase was obtained.

4.2. NMR spectroscopy

Proton 1D and 2D NMR spectra in oriented solvents were performed at 9.4 T at 304 K on a high-resolution Bruker DRX 400 spectrometer using a Broad Band Inverse probe equipped with a *z* field gradient coil and a standard variable-temperature unit (BVT 3000). All 2D spectra were obtained with 64–128 t_1 increments and zero filled to 1024 points in the F_1 dimension. 2048 points have been used zero filled to 4096 points in the F_2 dimension. No digital filtering was applied prior the double Fourier transform. Other experimental details can be found in the legend of figures. For SERF and SERF-filtered experiments, the excitation, refocalization, and time reversal excitation pulses have the same time. 67 ms for the BURP semi-selective pulses corresponding to 60 Hz selective window centered on chosen protons have been used. Moreover, gaussian cascades G4, Q5, 83 ms (60 Hz) and Q3, 41.5 ms (60 Hz) can be used as well.

Acknowledgments

J.F. gratefully thanks J.P. Baltaze and A. Dos Santos for their daily technical assistance.

References

[1] K. Kobzar, H. Kessler, B. Luy, Stretched gelatin gels as chiral alignment media for the discrimination of enantiomers by NMR spectroscopy, Angew. Chem., Int. Ed. 44 (20) (2005) 3145–3147.
[2] J.W. Emsley, J.C. Lindon, NMR Spectroscopy using Liquid Crystal Solvents, Pergamon Press, 1975.
[3] N. Tjandra, A. Bax, Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium, Science 278 (5340) (1997) 1111–1114.
[4] C. Aroulanda, M. Sarfati, J. Courtieu, P. Lesot, Investigation of the enantioselectivity of three polypeptide liquid-crystalline solvents using NMR spectroscopy, Enantiomer 6 (2001) 281.
[5] M. Sarfati, P. Lesot, D. Merlet, J. Courtieu, First successful enantiomeric discrimination of chiral alkanes using NMR spectroscopy, Chem. Commun. (2000) 2069.
[6] S. Berger, NMR techniques employing selective radiofrequency pulses in combination with pulsed field gradients, Prog. Nucl. Magn. Res. 30 (1997) 137–156.
[7] R. Freeman, Shaped radiofrequency pulses in high resolution NMR, Prog. Nucl. Magn. Res. 32 (1998) 59–106.
[8] D. Uhrın, P.N. Barlow, Gradient-enhanced one-dimensional proton chemical-shift correlation with full sensitivity, J. Magn. Reson. 126 (1997) 248–255.
[9] J.M. Nuzillard, Application of biselective refocusing soft pulses to the simplification of heteronuclear correlation spectra, J. Magn. Reson. 190 (2008) 292–297.
[10] B. Plainchont, A. Martinez, S. Tisse, J.-P. Bouillon, J.-M. Wieruszeski, G. Lippens, D. Jeannerat, J.-M. Nuzillard, An alternative scheme for the multiplexed acquisition of 1D and 2D NMR spectra, J. Magn. Reson. 206 (2010) 68–73.
[11] J. Farjon, D. Merlet, P. Lesot, J. Courtieu, Enantiomeric excess measurements in weakly oriented chiral liquid crystal solvents through 2D H-1 selective refocusing experiments, J. Magn. Reson. 158 (2002) 169–172.

[12] L. Beguin, N. Giraud, J.M. Ouvrard, J. Courtieu, D. Merlet, Improvements to selective refocusing phased (SERFph) experiments, J. Magn. Reson. 199 (2009) 41–47.
[13] J. Farjon, J.P. Baltaze, D. Merlet, P. Lesot, J. Courtieu, Heteronuclear selective refocusing 2D NMR experiments for the spectral analysis of enantiomers in chiral oriented solvents, Magn. Reson. Chem. 42 (2003) 594–599.
[14] R.P. Uday, B. Baishya, N. Suryaprakash, Separation and complete analyses of the overlapped and unresolved ^{1}H NMR spectra of enantiomers by spin selected correlation experiments, J. Phys. Chem. A 112 (2008) 5658–5669.
[15] R.P. Uday, N. Suryaprakash, Application of z-COSY experiment and its variant for accurate chiral discrimination by1 H NMR, J. Magn. Reson. 202 (2010) 217–222.
[16] R.P. Uday, N. Suryaprakash, Band selective small flip angle COSY: a simple experiment for the analyses of 1 H NMR spectra of small chiral molecules, J. Magn. Reson. 195 (2008) 145–152.
[17] N. Nath, N. Suryaprakash, Demixing of severely overlapped ^{1}H NMR resonances and interpretation of anomalous intensity pattern of dipolar coupled A3 spins in a weakly aligning medium, J. Magn. Reson. 207 (2010) 190–196.
[18] N. Nath, N. Suryaprakash, Selective detection of single-enantiomer spectrum of chiral molecules aligned in the polypeptide liquid crystalline solvent: transition selective one-dimensional H-1-H-1 COSY, J. Magn. Reson. 202 (2010) 34–37.
[19] H. Geen, R. Freeman, Band-selective radiofrequency pulses, J. Magn. Reson. 93 (1991) 93–141.
[20] L. Emsley, G. Bodenhausen, Gaussian pulse cascades – new analytical functions for rectangular selective inversion and in-phase excitation in NMR, Chem. Phys. Lett. 165 (6) (1990) 469–476.