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Communication

Enantiomeric excess measurements in weakly oriented chiral liquid crystal solvents through 2D ^{1}H selective refocusing experiments

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Abstract

In this article, a simple and robust method is proposed for simplifying the analysis of proton spectra of molecules dissolved in weakly oriented chiral media. The NMR approach investigated is based on the use of proton selective refocusing 2D experiments (SERF) to measure proton–proton dipolar couplings from unresolved lines. This technique is applied to the case of enantiomers dissolved in chiral polypeptide liquid crystals. It is shown that an accurate determination of enantiomeric excess is possible within a short experimental time.
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Keywords: NMR; Chiral liquid crystal; SERF; Enantiomeric excess; Orientation

1. Introduction

The design and synthesis of pure optically active compounds are two great challenges in modern organic and inorganic (bio)-chemistry. Hence the determination of enantiomeric excesses (*ee*'*s*) of an unknown mixture is a prominent task. In this aim, numerous NMR strategies have been developed and reported in literature [1]. One of the most recent methods in this field consists of recording the NMR spectrum of two enantiomers dissolved in weakly oriented chiral liquid crystals [2,3]. To date the best spectroscopic results have been obtained using lyotropic liquid crystals based on organic solutions of homo-polypeptides such as poly-γ-benzyl-L-glutamate (PBLG) or poly-ε-carbobenzyloxy-L-lysine (PCBLL), dissolved in various co-solvents such as $CHCl_3$, CH_2Cl_2, DMF... [3,4]. Chiral recognition is possible in these anisotropic media because two enantiomers have different orientational orders. This difference in the molecular orientations is revealed through the order sensitive anisotropic part of NMR interactions, namely chemical shift anisotropies, dipolar couplings, and quadrupolar splittings for $I > 1/2$ nuclei. Thereby, the spectral enantiodiscrimination can be visualised using NMR spectra of various nuclei at natural abundance level or after labelling the compounds to be studied [3–7]. Due to its sensitivity and to the variety of the nuclei that can be used, this method has proven to be efficient, powerful and much more general than the conventional methods employed in laboratories (chiral solvating agents, chiral-lanthanide shift reagents, etc.) [1].

Nevertheless, most of the previous investigations were achieved using either ^{13}C or ^{2}H NMR, and very few results reported the use of proton NMR in this field of chiral analysis in polypeptidic liquid crystals [3,8]. Although advantages of ^{1}H NMR are numerous, proton spectra of enantiomer mixtures in such media are often impossible to analyse because only large unresolved lines centred on the chemical shift of the different protons are obtained. This fact originates mainly from the very large number of long and short-range dipolar couplings and from the doubling of the spectrum, one for each enantiomer. Consequently, even using considerable post-processing, the visualisation of optical isomers through proton NMR in polypeptide liquid crystals remains limited to very small molecules. In other words, standard ^{1}H NMR in chiral liquid crystals does not provide a convenient analytical tool for enantiomeric analysis.

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To overcome the above-mentioned problem, we were interested in a general and robust tool which could simplify ^{1}H spectra of compounds dissolved in these weakly oriented chiral solvents. Such an experiment would extent to proton NMR this powerful method of enantiomeric discrimination. In this work we demonstrate that ^{1}H selective refocusing 2D experiments, SERF, does provide such a tool. The efficiency of the method will be illustrated on two examples where enantiomeric excesses are measured.

2. Experimental

2.1. Sample preparation

The two liquid-crystalline NMR samples investigated in this work were prepared using standard procedure. The sample of 1,2-dibromopropane and 3-methyl-4,4,4-trichlorobutyric-β-lactone were made of 138 and 100 mg of PBLG with MW ~120,000, 80 and 100 mg of enantiomer mixture, 551 and 350 mg of dry $CHCl_3$, respectively. Details on the method and sample preparation can be found in literature [7]. Note PBLG is commercially available from Sigma and all 5 mm o.d. NMR tubes were sealed to avoid solvent evaporation and centrifuged back and forth until an optically homogeneous birefringent phase was obtained.

2.2. NMR spectroscopy

Proton 1D and 2D NMR spectra in oriented solvents were performed at 9.4 T on a high-resolution Bruker DRX 400 spectrometer equipped with a standard variable-temperature unit (BVT 3000). All 2D spectra were obtained with 128 t_1 increments and zerofilled to 512 points in the F_1 dimension. No digital filtering was applied prior the double Fourier transform. Other experimental details can be found in the legend of figures.

3. Results and discussion

A few years ago, Fäcke and Berger have developed an efficient 2D NMR method for the measurement of a single specific proton to proton spin–spin coupling in isotropic media [9]. This approach involving selective radiofrequency (RF) pulses is referred to as the selective refocusing experiment (SERF). It is based on the well known *J*-resolved 2D experiment but uses selective and doubly selective RF excitations. The pulse sequence that we have applied with chiral liquid crystal solvents is presented in Fig. 1. The first selective RF pulse selects the ^{1}H signal to be observed during the time domain, t_2, whereas the doubly selective 180° RF pulses let only evolve the desired coupling during the evolution time, t_1,

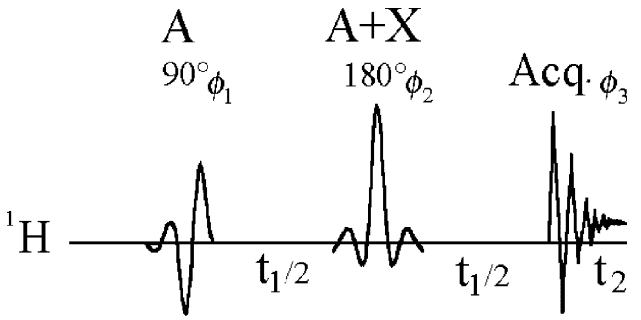

Fig. 1. Basic pulse scheme of the SERF 2D experiment. The first selective-excitation 90° pulse uses an EBURP-2 shaped pulse while the doubly selective 180° pulse uses the REBURP shaped pulse. The 4-steps phase cycling of the SERF sequence used was $\phi_1 = \phi_{acq.} = x, -x, -x, x$ and $\phi_2 = x, x, x, x$.

all the other couplings being refocused by this second selective excitation. In other words, if the 90° selective RF pulse is applied on a proton A, and if the 180° selective RF pulses are applied simultaneously on A and another proton X coupled to A, one gets on the 2D map the signal corresponding to A with the full coupling multiplicity in the F_2 dimension and only the coupling between A and X in the F_1 domain. In the original sequence proposed by Fäcke and Berger a purging RF pulse was implemented after the first selective excitation. No such pulse was needed in our examples because the different chemical shifts were well resolved and the use of this pulse did not change the results.

As a first illustration, we have investigated the technique using a racemic mixture of (±)-1,2-dibromopropane dissolved in the PBLG/$CHCl_3$ solvent at 295 K. The standard ^{1}H NMR spectrum is shown in Fig. 2. As expected the spectrum is mainly a sum of four different groups of unresolved lines from which the useful spectral parameters cannot be extracted. Thus, it is impossible to determine the different proton–proton couplings between the methyl, the diastereotopic methylene and the methine groups in the molecule. As a direct consequence, it is not possible to claim whether or not the two

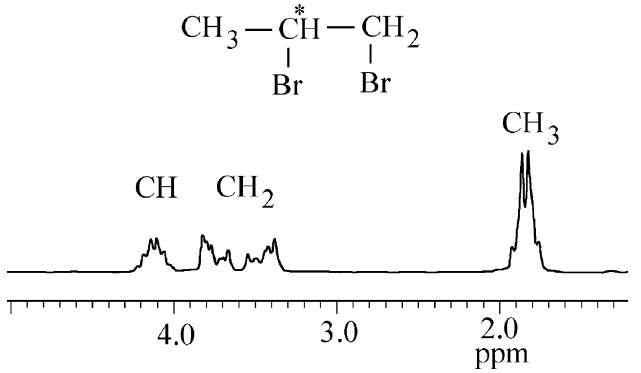

Fig. 2. 400 MHz ^{1}H 1D spectrum of the 1,2-dibromopropane recorded in PBLG/$CHCl_3$ at 295 K.

enantiomers are spectroscopically discriminated on one of these signals. In addition, the use of strongly excessive filtering would preclude the possibility to calculate the exact enantiomeric composition of the mixture. This is a typical situation where the SERF experiment is of interest.

We have turned our attention on the methyl group of (±)-1,2-dibromopropane as an example. To understand the structure of this signal it must be kept in memory that it exists a measurable dipolar coupling, D_{HH}, between the three magnetically equivalent protons of the methyl group, yielding a 1:2:1 triplet structure where the separation between resonances are equal to $T_{HH} = 3D_{HH}$. In addition, these protons are dipolar and scalar coupled both to the nonequivalent methylene and the methine protons. Finally due to the weakness of proton chemical shift anisotropy, two spectral patterns centred on the same chemical shift are expected to be seen if chiral discrimination occurs. Using all the selective pulses of the SERF experiment centred on the methyl resonance it should be possible to considerably simplify the signal of the methyl group as all the couplings should be refocused but the dipole–dipole coupling between the methyl protons.

Fig. 3 reports the SERF 2D experiment when the selective pulses are applied on the methyl resonance and the corresponding projection on the F_1 dimension. For this experiment the excitation and refocusing pulses were set on the methyl resonance frequency with a spectral bandwidth of 280 Hz. As expected only correlation peaks located at the methyl group chemical shift arises on the SERF 2D contour plot, indicating a good selectivity for the EBURP and REBURP pulses [10]. Note here that other shaped pulses [11] have been experimentally tested. As in isotropic solvents, we found that the EBURP–REBURP pulse pair was the most adapted for good excitation selectivity. In the F_1 dimension, two triplets centred on the same ^{1}H chemical shift are clearly visible, and therefore can be easily analysed. As no additional coupling partners were excited, they correspond to methyl signal evolving only under the ^{1}H–^{1}H geminal dipolar coupling during t_1. The presence of two dipolar splittings, $|T^{\circ}_{HH}| = 14.7$ and $|T^{\bullet}_{HH}| = 29.7$ Hz, evidences a difference in the ^{1}H–^{1}H dipolar coupling for the two enantiomers. This result indicates that the methyl protons exhibit a spectral enantio-differentiation, and hence would provide an excellent site for measuring an eventual *ee* through integration of the external transitions.

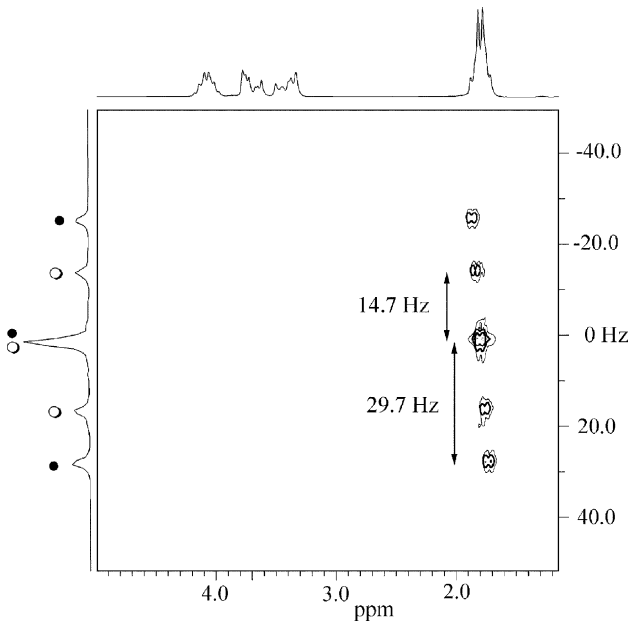

Fig. 3. SERF 2D spectrum selective on the methyl of the (±)-1,2-dibromopropane in PBLG/$CHCl_3$ solvent at 295 K. The REBURP pulse length was 14.3 ms corresponding to a bandwidth of 280 Hz. The 2D spectrum was taken with 1024 data point in F_2 and with 128 increment in F_1, using 64 scans for each FID and a recycling delay of 2.3 s. The *S* and *R* enantiomer signals were arbitrarily labelled by solid (•) and open circles (○). Along F_1 the projection of the 2D map and along F_2 the original 1D spectrum are plotted.

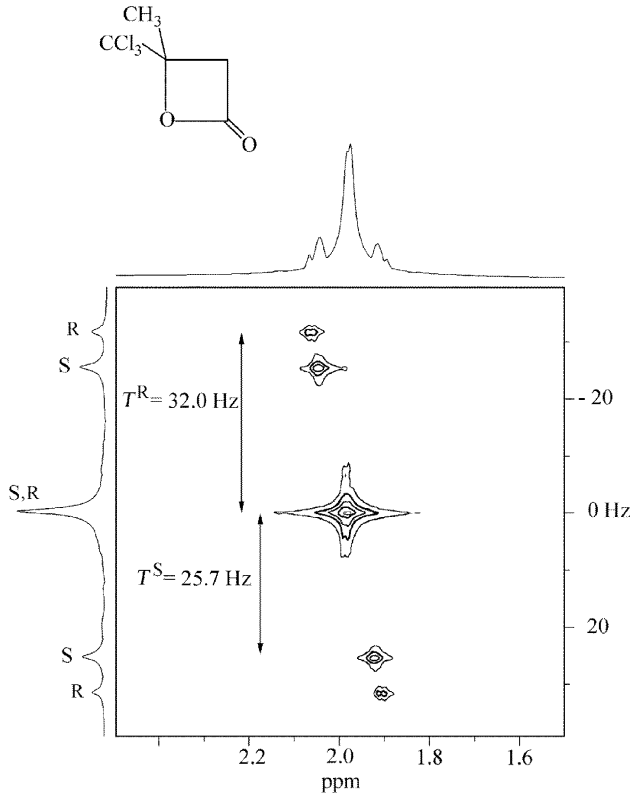

Fig. 4. Expansion of the SERF 2D map selective on the methyl of 3-methyl-4,4,4-trichlorobutyric-β-lactone 21.5% enriched in the *S* enantiomer and dissolved in PBLG/$CHCl_3$ at 295 K. The parameters of selective pulses are identical to the previous experiment. The 2D spectrum was recorded as a 2D matrix of 2048 * 128 data points. Eight scans were added by t_1 increments and a recycling delay of 2.3 s was used. The traces along F_1 and F_2 are the projections of the 2D map. The total acquisition time is 50 min. Peak integration yielded 23% *ee*.

Encouraged by this result, we checked the practical possibility to determine the enantiomeric composition of a mixture using SERF 2D experiments. For this purpose, we have investigated a mixture of 3-methyl-4,4,4-trichlorobutyric-β-lactone enriched in the *S* enantiomer. An enantiomeric excess of 21.5% was prepared by weighting the exact mass of each of enantiomers. The methyl ^{1}H SERF 2D experiment obtained for this mixture dissolved in the PBLG/$CHCl_3$ phase is shown in Fig. 4. This 2D spectrum has been recorded in less than one hour. This experimental time has to be compared to the natural abundance ^{2}H–$\{^{1}H\}$ NMR experiments recorded in 16 h [3,6]. Here too, the contribution of all the couplings but the intramethyl dipolar couplings have been cancelled out, and only two triplets are visible in F_1 dimension. This result indicates that the enantiomers are unambiguously discriminated at the level of the methyl protons. The difference in peak intensity indicates immediately the existence of enantiomeric excess. The large separation of signals allows the enantiomeric composition of mixture to be measured by simple peak integration. Experimentally an *ee* of $23 \pm 2\%$ is measured through integration of the resonances onto the F_1 projection. The same value is obtained by integrating the 3D volume of the correlation peaks in the 2D map. Both measurements are therefore in very good agreement with the expected enantiomeric purity.

4. Conclusions

These preliminary results have demonstrated the usefulness of the SERF 2D experiments to measure a single dipolar coupling for molecules dissolved in weakly oriented media, particularly in the field of the chiral analysis in polypeptide chiral liquid crystals. This simple and robust method using proton NMR enables (i) a very clean visualisation of the enantiomers and (ii) an accurate determination of enantiomeric excess within a short experimental time. Consequently this method is a powerful alternative to the analytical tools already developed to investigate chiral solutes dissolved in chiral anisotropic solvents. Due to the sensitivity of proton NMR this experiment should be extensively used in the future for the investigation of medium-sized chiral solutes. We are presently working on a heteronuclear equivalent of the SERF 2D experiment.

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