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^{1}H NMR analyses of enantiomeric mixtures using chiral liquid crystals

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ABSTRACT

Nuclear Magnetic Resonance in chiral liquid crystalline media is a powerful tool for deciphering mixtures of enantiomers. ^{1}H NMR appears to be the most sensitive technique for analyzing enantiomers but remains very challenging for the analysis of chiral solutes interacting with weakly orienting media. Indeed, probing ^{1}H networks even for small sized molecules is difficult because in oriented media, partially averaged anisotropic interactions such as residual dipolar couplings contribute to broaden NMR signals and lead to crowded spectra. For this reason, using ^{1}H NMR for analyzing enantiomeric mixtures has long required a complex and tedious analytical process to extract a qualitative and quantitative information about each enantiomer. Several methods have been developed in the last decade to overcome this difficulty. SElective ReFocussing (SERF) based techniques allowed the possibility to get access to high resolution spectra and measure accurately every homonuclear ^{1}H total couplings for each enantiomer. Among them, the Gradient encoded SERF (G-SERF) pulse sequence is an advanced tool for a rapid analysis of whole networks of residual dipolar couplings. Moreover, other multi-dimensional experiments have been developed to acquire homonuclear correlation spectra in which complex proton lineshapes of fully coupled systems are simplified. Finally, heteronuclear correlation methods that eventually combine the signal dispersion of another heteronucleus with the homonuclear decoupling of the proton dimension, were successfully implemented to separate lines from ^{1}H sites in each enantiomer.

1. Introduction

Over the last decades, enantioselective synthesis has become of major interest both for industrial and academic laboratories. Obtaining enantiopure compounds is nowadays of fundamental importance, especially in the field of pharmaceuticals where different enantiomers of a same molecule often show a different biological activity, and may thus induce a completely different pharmacological response. More generally, enantioselective synthesis is a key process for producing optically pure chemical intermediates, which will then be used to obtain final products with fully controlled stereochemistry. In all these cases, tailored analytical tools need to be sought to determine accurately and reliably the enantiomeric purity of the molecule of interest. A wide range of methods has been developed for this purpose, based on chiroptical techniques, spectroscopies, gas or liquid chromatography, mass spectrometry or even electrochemical methods. These analytical techniques are often combined with other technologies such as liquid crystals, enzymatic methods or immunoassays to allow for discriminating between the detected signals of each enantiomer [1••,2–4]. Kinetic resolution has also shown to be a complementary approach to provide an improved insight into enantiomeric mixtures.

In this context, Nuclear Magnetic Resonance (NMR) is a tool of choice for providing chemists with structural and dynamic information on ever more challenging molecular systems at atomic level. In the case of chiral molecules however, it is well known that NMR spectra of enantiomers dissolved in an isotropic solvent are identical, which makes standard NMR solvents useless for addressing optical purity issues. In order to overcome this limitation, specific methods have been developed to obtain different spectra for each enantiomer from a given mixture, most of which rely on the use of a chiral resolving agent that can be either a chiral derivatization or lanthanide shift reagent, or a chiral solvent [1••]. In the latter case, the underlying idea is to generate diastereoisomeric solute-solvent interactions between an optically active solvent and the chiral analyte. This principle has been successfully extended to a particular class of solvents that are chiral liquid crystals. Molecules from a liquid crystalline phase are partially oriented, but are still diffusing and rotating in such a way that the phase is fluid. When solute molecules are dissolved in such anisotropic medium, they undergo weak interactions with the molecules from the liquid crystal, which induces their partial alignment. The properties of the liquid crystal can be tailored so that the order parameters of the solute molecules remain small. This alignment process allows for re-introducing into the NMR spectrum residual

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anisotropic interactions, namely dipolar interactions, chemical shift anisotropy, or even quadrupolar interactions for spins I > ½. Furthermore, inter-molecular dipolar couplings are averaged out due to the rotational and translational diffusion of the solute molecules in the liquid crystalline phase, while only partially averaged intra-molecular anisotropic interactions contribute to the observed lineshape of NMR signals, leading to the observation of spectra with linewidth of only a few Hertz, close to that of high-resolution spectra [5]. More interestingly, when the liquid crystal is chiral, solute-solvent interactions are diastereospecific, and the anisotropic NMR spectra of each enantiomer are then different.

Several molecular systems have been reported in the field of chiral liquid crystals used for discriminating enantiomers, ranging from cholesteric systems [6–11] to non-miscellar lyotropic liquid crystals composed of polypeptide [12–18], polynucleotide [19,20], polysaccharide [21,22] systems or others [21,23]. Among them, synthetic homopolypeptides dissolved in an organic co-solvent have shown to be a particularly efficient chiral organic solvent. These homopolypeptides are mainly poly-γ-benzyl-L-glutamate (PBLG), poly-γ-ethyl- L-glutamate (PELG), or poly-ε-carbobenzyloxy-L-lysin (PCBLL), whose potential for being used as chiral NMR solvents was first reported in the mid-1990s. When the polypeptide is dissolved in an helicogenic co-solvent, it adopts a rigid chiral α-helical conformation. In the presence of a static magnetic field B_0 such as the one generated by the supramolecular magnet in routine NMR spectrometers, these chiral fibers form a chiral nematic lyotropic phase, with positive anisotropy of the molecular diamagnetic susceptibility, and their directors homogeneously aligned parallel to the static magnetic field (Fig. 1).

The ability of chiral liquid crystals to yield distinct NMR spectra for each enantiomer of a given compound dissolved in optically active solvents was first reported using proton NMR spectroscopy [24]. Since then, several methods based on the detection of a wide range of NMR active nuclei among which ^{13}C, ^{2}H [25•,26,27], ^{19}F [28], ^{31}P or ^{77}Se, have been specifically developed in order to exploit the sensitivity of each heteronucleus to the chiral recognition process [29]. However, although being the most sensitive probes, the observation of ^{1}H nuclei for the purpose of enantiomeric differentiation usually provides crowded spectra whose analysis requires in the best-case scenario a long and tedious spectral-simulation-based process. Indeed, ^{1}H nuclei form in most of organic molecules a naturally abundant homonuclear spin network, which results in a high number of intramolecular residual dipolar couplings (RDCs) when enantiomers are interacting with a weakly orienting anisotropic medium. These RDCs contribute to increase the complexity of the signals' multiplicity that can be observed for each proton site. The weak chemical shift anisotropy that is typically observed for protons in this kind of sample also adds to this complexity, since it does not allow for spreading proton resonances for each enantiomer, despite the chirality of the liquid crystalline phase [29]. As a result, ^{1}H spectra of enantiomers differently oriented in a weakly orienting chiral liquid crystalline solvent are in theory different but are both composed of broad signals that strongly overlap with each other, which contributes to obscure the overall spectrum (Fig. 2). Moreover, the inhomogeneity of the sample, which arises from a distribution of the orientation of the molecules throughout the weakly orienting medium, also results in a line broadening that can be described as the superposition of anisotropic sub-spectra arising from each different but close orientation of the molecules along the sample.

Nevertheless, probing their proton network is one of the most powerful way of analyzing enantiomers since (i) proton is the most abundant and thus intrinsically sensitive nucleus in NMR, and (ii) insightful structural information can be accessed through the measurement of proton interactions. Consequently, methods that have been developed over the last decades in the field of enantiomeric differentiation based on proton NMR spectroscopy aim either at separating the evolution of spin interactions, namely dipolar coupling and chemical shift anisotropy interactions, so that they can be selectively extracted and assigned, or at improving the analytical content of spectra so that each interaction can be measured on a reduced number of simplified correlations.

2. Measurement of homonuclear residual dipolar couplings by selective refocusing based methods

For a molecule dissolved in a weakly orienting medium, we remind that when two anisochronous proton spins *i* and *j* are interacting, the quantity that can be measured from the splitting of their multiplets is the total coupling $T_{ij} = J_{ij} + 2.D_{ij}$, where J_{ij} is the isotropic part of the scalar coupling and D_{ij} the anisotropy of the total spin-spin coupling. D_{ij} is thus the sum of the anisotropic residual dipole-dipole coupling, D'_{ij}, and the anisotropic part of the scalar coupling, ΔJ_{ij}, the latter being generally neglected in the frame of enantiomeric differentiation. In case of isochronous nuclei such protons from a methyl group, the total coupling is defined as $T_{ii} = 3.D_{ii}$. As described above, the key challenge raised by the acquisition of proton spectra on enantiomeric mixtures dissolved in chiral liquid crystals regards the extraction of the total couplings of each enantiomer from highly crowded spectra. Two approaches were combined to achieve this goal: (i) the implementation of selective amplitude modulated *rf* fields to act selectively on the proton sites whose interaction needs to be probed. (ii) The design of

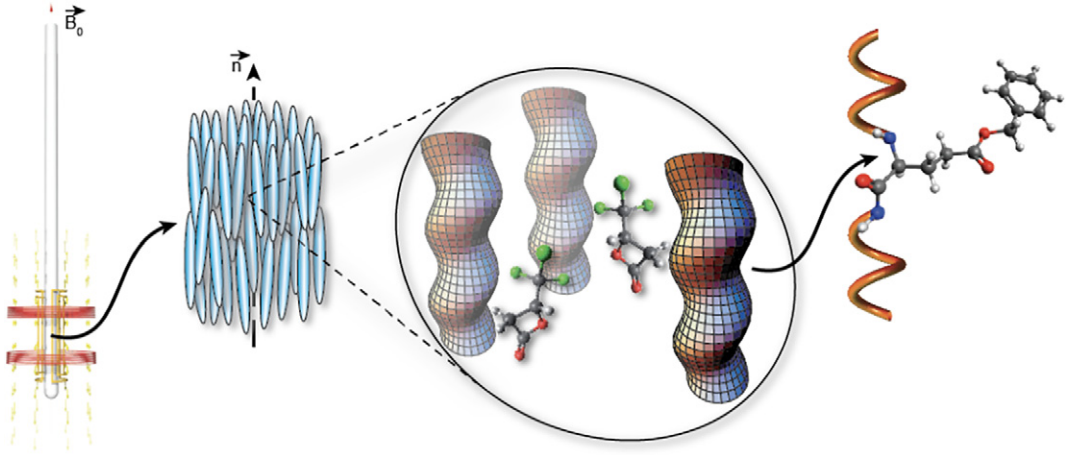

Fig. 1. Schematic picture of the chiral nematic lyotropic phase used as an NMR solvent to induce a slightly different alignment process of enantiomers due to the existence of diastereospecific interactions between the optically active solvent and the chiral molecule. The model solute molecule is propylene oxide, and the homopolypeptide whose structure is displayed on the right is PBLG.