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Band selective small flip angle COSY: A simple experiment for the analyses of ^{1}H NMR spectra of small chiral molecules

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ABSTRACT

The NMR spectroscopic discrimination of enantiomers in the chiral liquid crystalline solvent is more often carried out using ^{2}H detection in its natural abundance. The employment of ^{1}H detection for such a purpose is severely hampered due to significant loss of resolution in addition to indistinguishable overlap of the spectra from the two enantiomers. This study demonstrates that the band selected small flip angle homonuclear correlation experiment is a simple and robust technique that provides unambiguous discrimination, very high spectral resolution, reduced multiplicity of transitions, relative signs of the couplings and enormous saving of instrument time.

1. Introduction

NMR spectroscopic visualization of optical enantiomers is extensively practiced using the weakly aligned chiral liquid crystalline media [1,2]. The difference in the orientational properties of the enantiomers in the chiral orienting media has been exploited not only for their differentiation but also to determine their enantiomeric excess. Unlike in strongly orienting thermotropic liquid crystals, the orientational parameters in the chiral liquid crystal are several orders of magnitude smaller. The difference in the elements of the order matrix between the enantiomers, though small [1], its effect on the anisotropic NMR spectral parameters like chemical shift anisotropies ($\Delta\sigma_i$), dipolar couplings (D_{ij}) and quadrupolar couplings (Q_i) is suffice to facilitate enantio discrimination. Majority of the studies on enantio discrimination are utilizing ^{2}H NMR detection both in isotopically labeled and naturally abundant molecules, exploiting the relatively large values of the quadrupole couplings compared to chemical shift anisotropies and dipolar couplings [3–7].

Voluminous amount of data has been reported in the literature employing ^{2}H NMR, wherein the measured quadrupole interaction energies provide doublets for each independent ^{2}H isotope of the molecule in their natural abundance. In small molecules the identification of such doublets from the one-dimensional NMR spectra is straightforward and does not demand the design or development of any fancy experimental schemes. When severe overlap of transitions is encountered, the concept of two-dimensional NMR correlation has been applied to identify the quadrupolar doublets and are cited as Q-COSY [7] and NAD-Q-COSY [4]. Although the measure of well resolved quadrupolar doublet separation does not pose any problem, the clean spectra are obtained with the incorporation of phase sensitive detection [5,8]. It can be argued that the measure of various spin interaction energies, responsible for providing better understanding of the structures of the molecules, is feasible with ^{1}H detection. Thus with all its limitations the three-dimensional experiments [9,10], especially in the natural abundance of ^{2}H, neither result in saving of experimental time nor provide any additional information. In addition, because of inherent demand for either expensive labeling techniques or the enormous instrument time requirement, the ^{2}H NMR detection suffers from its severe drawback of impracticability.

While dealing with the spin $\frac{1}{2}$ nuclei like ^{13}C, heteronuclear dipolar couplings and/or the chemical shift anisotropies of the carbons have been employed [2,11]. With abundant presence in all the chiral organic molecules ^{1}H detection is advantageous and will be the obvious choice for such a purpose. However, the routine employment of ^{1}H detection is severely hindered due to enormous loss of resolution arising from numerous short and long distance couplings and indistinguishable overlap of the spectra from the two enantiomers even for small molecules with five or six interacting spins. The discrimination and disentangling of this overlap are a formidable task. Thus, in the literature the analyses of ^{1}H detected spectra have been described as either difficult or impossible and there are very few reported studies for chiral visualization [12]. Nevertheless the ^{1}H detected NMR spectra, in spite of their

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severe complexity when employed for big molecules, is the preferred choice for chiral molecules because of its high sensitivity, high natural abundance and enormous saving of experimental time, though paradoxically it remains little explored.

Our quest for novel methodologies to aid the analyses of complex ^{1}H NMR spectra resulted in the development of several techniques for spectral simplification in scalar [13], strongly dipolar coupled [14] and weakly dipolar coupled spin systems [15]. The novel double quantum selective refocusing (DQ-SERF) experiment [15] provided better chiral discrimination in addition to exploitation of information content in the direct dimension which was ignored in the earlier SERF experiment [16]. The problems and prospects in the measure of enantiomeric excess using the multiple quantum methodology have also been extensively discussed [15]. Non-selective excitation of homonuclear highest quantum detection provided a methodology for complete unraveling of the overlapped spectra utilizing ^{1}H chemical shift anisotropy as an exclusive parameter [17]. The homonuclear and heteronuclear spin selective detection of triple quantum coherence not only resulted in the differentiation and discerning of broad and unresolved transitions but also aided the determination of homonuclear and heteronuclear couplings [18,19]. The two-dimensional experiment correlating the single and biselective excitation of isolated but coupled spins achieved the zooming of the small region of the spectrum [20] and provided well resolved spectrum. Several of our developed methods have also provided the relative signs of the couplings [13,17–19].

In spite of several developed methodologies, ^{1}H detection suffers from certain limitations. Due to very low ordering and scaling down of the dipolar couplings with the increasing distance, the long distance couplings are generally not detected. In majority of the reported work in the literature, even for small molecules with six interacting spins, the long distance coupling between two spins, that are separated by more than five chemical bonds, could be as small as 0.2 Hz [18,20]. In a relatively large molecule, such as ibuprofen, only three proton–proton couplings have been measured [21]. Thus it is imperative that the application of ^{1}H NMR for bigger sized molecules of real pharmacological interest is still in its infancy and there is a dire need for the development of novel methodologies. The manipulation of spin dynamics by the blend of several existing one and multidimensional NMR concepts paves the way for such a purpose. This study is an attempt in that direction to derive maximum information with minimum number of experiments, requiring much less investment of the instrument time.

The study overcomes the limitations of spin selective correlation experiment reported by us recently [20] wherein many selective excitations were required to be carried out to derive complete spectral information. Each selective excitation demands large instrument time and the appropriate choice of several such experiments are essential to determine all the couplings. In combating this difficulty we demonstrate, in this study, a band selective homonuclear correlation experiment [22,23] the concept of which is well known in the literature. We demonstrate that this band selection combined with small flip angle detection pulse is an invaluable experimental tool in determining very small residual dipolar couplings from the broad and featureless ^{1}H spectra of chiral molecules with minimum number of experiments and enormous saving of the instrument time. As the experiment involves the selective excitation of coupled region of the spectrum, the methodology is applicable only to weakly coupled spin systems.

2. Experimental confirmation

For the demonstration of the experimental methodology, three different molecules, (*R*/*S*)-3-butyn-2-ol (**1**), (*R*/*S*)-β-butyrolactone (**2**) and (*R*/*S*)-propylene oxide (**3**) were chosen. The samples purchased from Sigma were used without further purification. The aligned samples were prepared by the method reported in the literature [18–20,24,25]. For the oriented sample **1**, 85 mg of PBLG, 59 mg of **1** and 450 mg of $CDCl_3$ were taken. For the oriented sample **2**, 64.7 mg of **2**, 86.4 mg of PBLG and 432.0 mg of $CDCl_3$ were taken. For the oriented sample **3**, 42.5 mg of **3**, 78 mg of PBLG and 580 mg of $CDCl_3$ were taken. The samples were sealed in a 5 mm NMR tube to avoid the evaporation of the solvent and then centrifuged back and forth for several hours till the visually homogeneous phase was observed. The one- and two-dimensional proton spectra of all the molecules were recorded using Bruker DRX-500 NMR spectrometer and reported in the magnitude mode. The temperature was maintained at 300 K for all the samples, using Bruker BVT 3000 temperature controller unit. The alignment of each sample was investigated by monitoring the ^{2}H doublet separation of $CDCl_3$. The racemic structures of the molecules with the numbering of the interacting spins and the corresponding one-dimensional ^{1}H spectra are reported in Fig. 1B. For **1** and **3** the assignment of peaks for different protons has already been discussed earlier [18,20]. The assignment of peaks for enantiomers *R* and *S* is normally carried out by recording the spectrum of an enantio pure sample and then comparing it with the spectrum of a racemic mixture. For both the molecules our earlier reported assignments for *R* and *S* enantiomers were maintained [18,20]. There was no report available on the assignment of peaks for different protons and also for the enantiomers in **2**. The present assignment of peaks for different protons in **2** is based on their multiplicity pattern. However, the assignments to *R* and *S* forms are arbitrary.

For the molecules under investigation, homonuclear two-dimensional band selected (BASE-COSY) and the band selected with small flip angle (BASE-β-COSY) correlation experiments have been carried out [22,23]. The pulse sequences employed for these experiments are given in Fig. 1A. The acquisition and processing parameters for the experiments are reported in the corresponding figure captions. It may be pointed out that the phase sensitive detection of two-dimensional spectra provides a clean spectrum with better resolution, especially when one is interested in deriving remote couplings of smaller magnitudes. Our efforts to refocus the evolution of chemical shifts and couplings during the selective excitation period met with partial success even with several modifications to compensate this evolution, presumably due to numerous couplings of different magnitudes experienced by each proton of an enantiomer. Therefore, the present experiments are reported in the magnitude mode. Nevertheless, this did not preclude us from deriving all the coupling parameters for both the enantiomers. The precision of the determinacy of the parameters is reflected in the measure of long range coupling of the order of 2.1 Hz.

3. Results and discussion

The nomenclature of the spin systems and the appearance of the multiplicity pattern for the molecules **1** and **3** have already been discussed in our earlier communication [18,20]. However, for the benefit of smooth reading the one-dimensional spectra is reported in Fig. 1B. In **2**, although the couplings are different, the multiplicity pattern, nomenclature of the spin system and the analyses of the spectrum is identical to that of **3**. The important point to be highlighted is that the recognition of any fine structure and the extraction of any meaningful information from the spectra of **2** and **3** is impossible. The complexity is more predominant in **3** where there are 48 transitions for the methyl group, arising from the mixture of two spectra of enantiomers, in a narrow spectral width. The real challenge is not only to discriminate but to disentangle the