Journal of Magnetic Resonance 195 (2008) 145–152

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

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

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

Uday Ramesh Prabhu [a], N. Suryaprakash [b,*]

[a] Solid State and Structural Chemistry Unit, The Indian Institute of Science, SIF, Bangalore City, Karnataka 560 012, India
[b] NMR Research Centre, The Indian Institute of Science, SIF, Bangalore City, Karnataka 560 012, India

ARTICLE INFO

Article history:
Received 15 July 2008
Revised 28 August 2008
Available online 10 September 2008

Keywords:
Band selective COSY
Small flip angle
Chiral discrimination
Couplings
Analyses of spectra

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

* Corresponding author. Fax: +91 80 2360 1550.
E-mail address: nsp@sif.iisc.ernet.in (N. Suryaprakash).

1090-7807/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2008.09.003

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

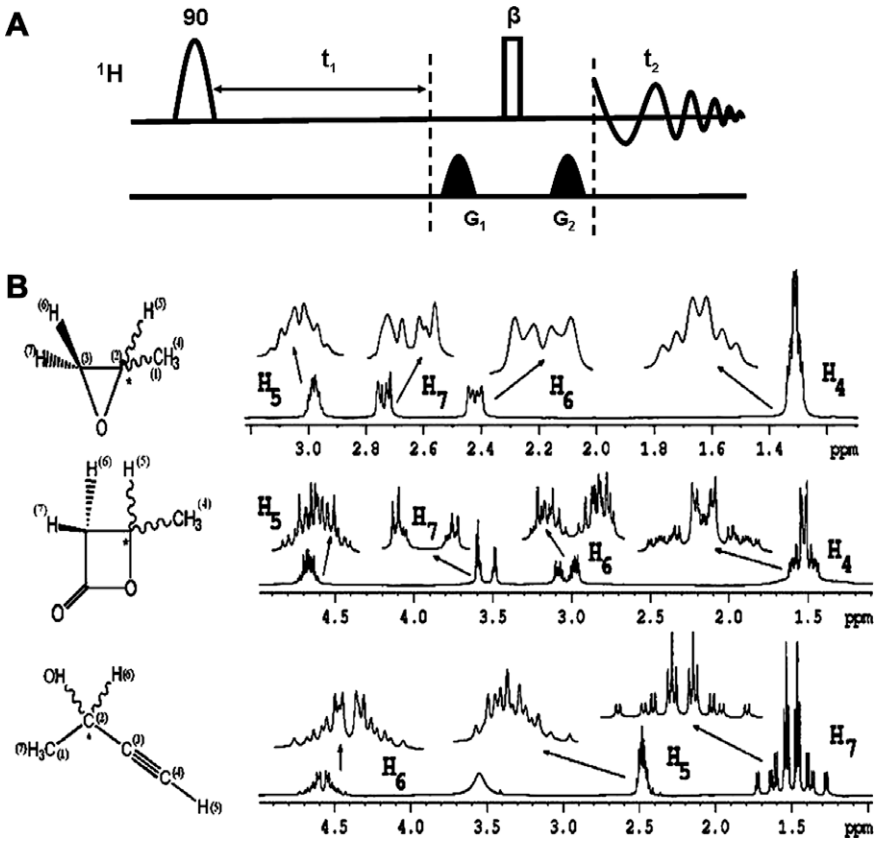

Fig. 1. (A) The pulse sequence used for BASE-COSY and BASE-β-COSY experiments. The first 90° pulse is the shaped pulse for the band selective excitation. β is 90° for BASE-COSY and 15–25° for BASE-β-COSY experiments. Both the gradients G_1 and G_2 are 5 G/cm. (B) From bottom trace to top trace: The 500 MHz one-dimensional ^{1}H spectrum of (*R*/*S*)-3-butyn-2-ol (**1**), (*R*/*S*)-β-butyrolactone (**2**) and (*R*/*S*)-propylene oxide (**3**) along with their racemic structures and the numbering of interacting spins. The expanded regions of each spectrum and the assignment to different protons are shown. Chemical shifts were referenced with isotropic value of methyl protons. Only the region of interest is plotted. Consequently the peaks from the PBLG or $CDCl_3$ are not seen. Other details are given in the text.

transitions from the broad and featureless spectra which is a formidable task.

4. Band selective homonuclear correlation spectra (BASE-COSY)

The well known homonuclear COSY experiment establishes the correlation among scalar coupled spins in isotropic systems. However, in weakly or strongly dipolar coupled spin systems, if there is a sizeable coupling among all the protons the COSY sequence establishes correlation among them and the utilization of isotropic mixing for the polarization transfer unlike in TOCSY, as has been demonstrated in an earlier study [26], is not essential. It has also been pointed out that in the dipolar coupled systems there will not be any cross-peaks between the symmetric and antisymmetric states and also between different groups of irreducible representations [27,28]. In this study, there is no symmetry element present for the molecules under investigation and this problem does not arise.

The BASE-COSY experiment is a variant of the COSY experiment wherein a small band of frequencies is selectively excited in the t_1 dimension and correlated to the entire spectrum in the t_2 dimension [22]. The analysis of BASE-COSY spectrum is identical to normal COSY spectrum and provides couplings among all the coupled spins in a single experiment. Thus the significant advantage of BASE-COSY experiment is the zooming of the small region of the spectra in the F_1 dimension providing high resolution. The typical BASE-COSY spectra of (*R*/*S*)-3-butyn-2-ol and (*R*/*S*)-β-butyrolactone are reported in Figs. 2A and 4A, respectively. It is clearly obvious that there is enhanced spectral resolution in the F_1 dimension due to spectral zooming. In **1**, the multiplet patterns of all the groups are well resolved and only the differentiation of the spectrum for *R* and *S* enantiomers is essential for extracting the coupling information. All the groups display different but identifiable arrays of transitions for both *R* and *S* enantiomers. For methyl and methine groups each array of the enantiomer has 12 and eight peaks, respectively. The cross-section of one of the arrays for the *R* and *S* enantiomers taken along the F_2 dimension for the methyl and methine groups provides all the coupling information. The disadvantage of BASE-COSY is that the complexity in the F_2 dimension persists and the analyses of the spectra becomes very tedious with too many transitions in each cross-section. The problem is very severe when the number of interacting spins increases. This is clearly evident from the spectrum of **2**, reported in Fig. 4A. This problem can be combated by manipulating the dynamics of the spins. One such possibility is the use of small angle for the second pulse [29] in the BASE-COSY sequence which does not mix up all the energy states (BASE-β-COSY). The spin states of the passive spins then remain undisturbed both in the F_1 and F_2 dimensions, resulting in the separation of active and passive couplings in the direct and indirect dimensions at the respective chemical shift positions of the entire selected band. The problem of spectral overlap due to too many transitions is therefore overcome, thereby simplifying the spectrum in each cross-section. The displacement of the passive couplings in the F_1 and F_2 dimensions also enables the measurement of couplings that are less than line width. Therefore as far as the analyses and the information content is concerned, this experiment is analogous to Soft-COSY experiment [30–32], where the small region of the spectrum is selectively excited with identical source and target frequencies, giving enhanced resolution and

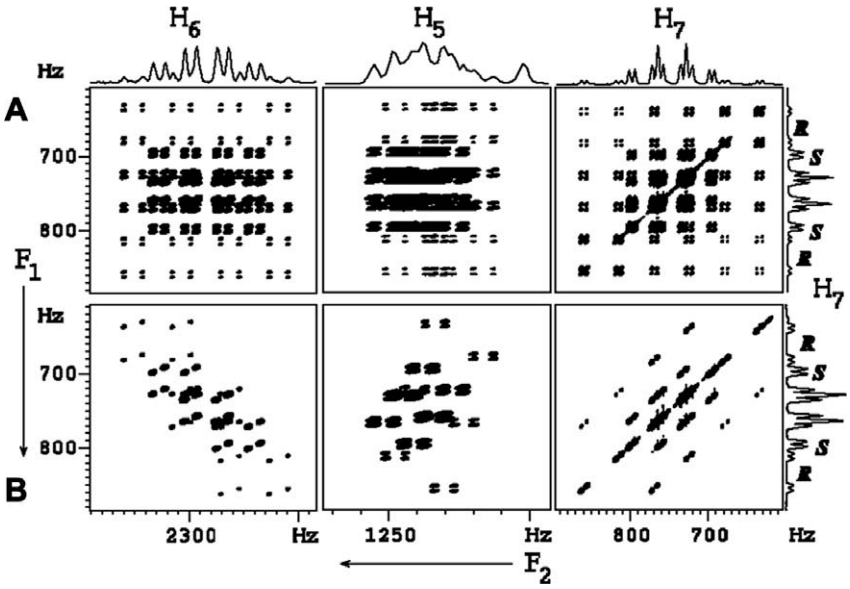

Fig. 2. (A) The 500 MHz proton two-dimensional BASE-COSY spectrum of (*R*/*S*)-3-butyn-2-ol with selective excitation of methyl protons (H7) along with the corresponding F_1 and F_2 projections. The width of the seduce shape pulse is 10.4 ms. The size of 2D data matrix is 512 × 8192. Spectral widths are 700 and 5387 Hz in F_1 and F_2 dimensions. Number of accumulations for each t_1 increment is 2.0. The relaxation delay is 5 s. The data was zero filled to 2048 and 8192 points and processed with a sine bell window function. The digital resolution in F_1 and F_2 dimensions are 0.34 and 0.65 Hz, respectively. (B) The 500 MHz proton 2D BASE-β-COSY spectrum with selective excitation of methyl protons (H7). All the experimental and processing parameters are same as (A), except the flip angle of the second pulse is 15°. Assignments for different protons and to *R* and *S* forms are taken from the literature [1]. Only few of the cross-sections for *R* and *S* forms have been marked.

the separation of active and passive couplings in both the dimensions. The major advantage of BASE-β-COSY over Soft-COSY is that in BASE-β-COSY there is no need of several selective excitations to determine all the spectral parameters. The experimental complication employing biselective pulses for selective excitation has been overcome.

In other words the band selective COSY spectrum coupled with small flip angle (BASE-β-COSY) not only zooms the small spectral region but also produces an E-COSY [33,34] type spectrum. In such an E-COSY type spectrum, the relative signs of the couplings can also be derived from the directions of tilt of the displacement vectors. Therefore, the possibility of achieving enhanced resolution and spectral simplification was explored by employing BASE-β-COSY experiment.

5. Analyses of 2D proton BASE-β-COSY spectrum of (*R*/*S*)-3-butyn-2-ol

The 2D proton BASE-β-COSY spectrum of **1** reported in Fig. 2B, where the source frequencies in the F_1 dimension corresponds to

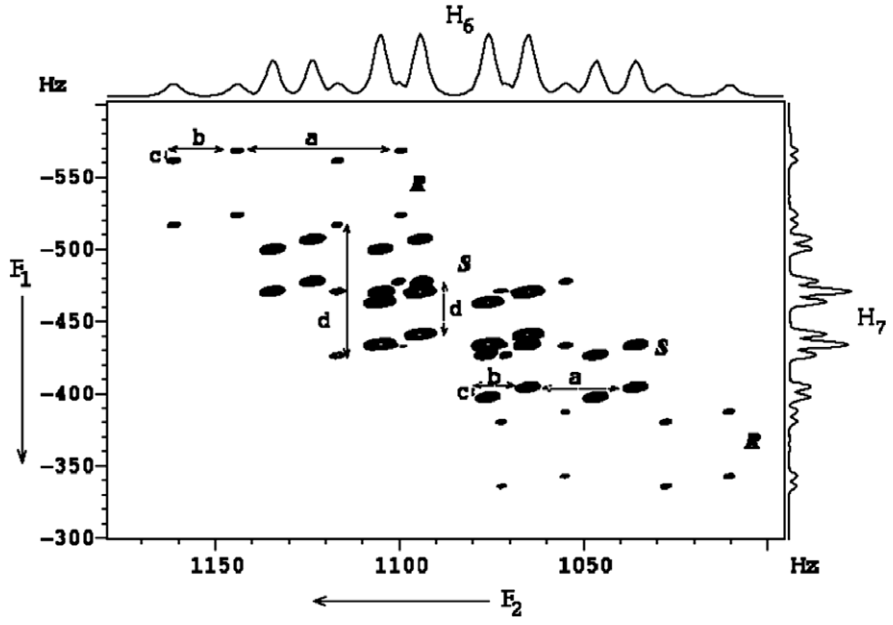

Fig. 3. The expanded portion of Fig. 2B pertaining to resonance of proton H6. The active and passive couplings derivable from both F_1 and F_2 dimensions are marked. The separations providing coupling information marked with solid lines are for *R* enantiomer and those with broken lines are for *S* enantiomer. The separations (in Hz) providing the coupling information are for *R* enantiomer $a = {}^{3}T_{H6H7} = 44.4$, $b = {}^{4}T_{H6H5} = 17.4$, $c = {}^{5}T_{H5H7} = 7.0$, $d = {}^{2}T_{H7H7} = 90.0$ and for *S* enantiomer $a = {}^{3}T_{H6H7} = 29.5$, $b = {}^{4}T_{H6H5} = 10.8$, $c = {}^{5}T_{H5H7} = 6.9$, $d = {}^{2}T_{H7H7} = 36.7$. Only few of the cross-sections for *R* and *S* forms have been marked.

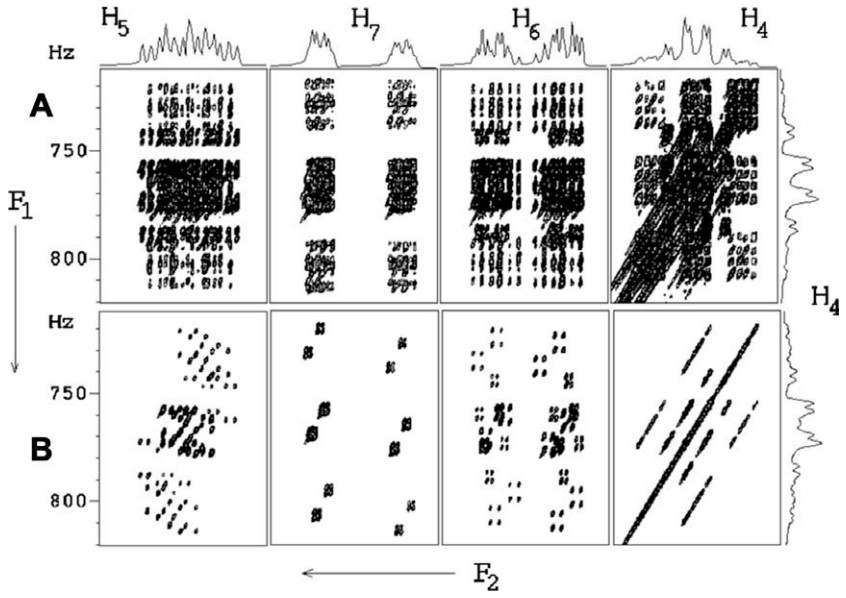

Fig. 4. (A) The 500 MHz proton 2D BASE-COSY spectrum of (*R/S*)-β-butyrolactone aligned in the chiral liquid crystal PBLG, with selective excitation of methyl protons (H4) along with the corresponding projections. The width of the seduce shape pulse is 12.5 ms. The size of 2D data matrix is 512 × 8192. Spectral widths are 400 and 5000 Hz in F_1 and F_2 dimensions, respectively. Number of accumulations for each t_1 increment is 2. The relaxation delay is 5 s. The data was zero filled to 2048 and 16,384 points and processed with a sine bell window function. The digital resolution in F_1 and F_2 dimensions are 0.3 and 0.2 Hz, respectively. (B) The 500 MHz proton 2D BASE-β-COSY spectrum with selective excitation of methyl protons (H4) along with the corresponding projections. All the experimental and processing parameters are same as Fig. 2A, except the flip angle of the second pulse is 15°.

methyl protons (4) and the target frequencies in the F_2 dimension is the entire single quantum spectrum. The active coupling between the excited and detected spins is determined at the respective chemical shift positions in both the dimensions. The separations providing this information is a triplet for the methyl group and doublet for the other two protons. However, the passive couplings with respect to the excited spins are displaced along the F_1 dimension and those with respect to the detected spin are displaced along the F_2 dimension. The expanded region of the Fig. 2B corresponding to multiplets of proton H6 are given in Fig. 3. The analyses of which provides the active coupling $(^3T_{H6H7})^{R/S}$ along F_2 dimension and the displacement provides passive couplings $(^2T_{H7H7})^{R/S}$, $(^5T_{H5H7})^{R/S}$. The passive coupling $(^4T_{H5H6})^{R/S}$ is extracted from the displacement along the F_2 dimension. Thus all the couplings for both the enantiomers could be determined in a single experiment. The separations giving this coupling information are marked in the figure and the derived parameters are given in the corresponding figure caption. Identical but redundant information could be obtained by the analyses of the resonances pertaining to proton H5, where the coupling along F_2 dimension is $(^3T_{H5H7})^{R/S}$ and the couplings obtainable from the displacements along F_1 and F_2 dimensions, respectively, are $(^2T_{H7H7})^{R/S}$, $(^5T_{H6H7})^{R/S}$ and $(^4T_{H5H7})^{R/S}$.

6. Analyses of 2D proton BASE-β-COSY spectra of (*R/S*)-β-butyrolactone

The 2D proton BASE-β-COSY spectrum of **2** with selective excitation of methyl protons is given in Fig. 4B. The two non-equivalent methylene protons and the methine proton are the passive spins. In the 2D spectrum F_1 dimension pertains to the A_3 part of A_3MNX spin system containing 24 transitions, which can be construed as eight A_3 sub spectra corresponding to eight spin states of M, N and X together for each enantiomer. The F_2 dimension has a band of all the four groups of protons. For this molecule the analyses of the bunch of resonances pertaining to protons H6 and H5 are suffice to determine all the couplings. The expanded region of the spectrum pertaining to proton H6 is given in Fig. 5 along with the separations providing the coupling information. At the chemical shift position of proton H6, the active coupling along F_2 dimension would be $(^3T_{H4H6})^{R/S}$ and the passive couplings of all the remaining spins with proton H4 and within the methyl proton themselves, i.e. $(^2T_{H4H4})^{R/S}$, $(^3T_{H4H5})^{R/S}$ and $(^4T_{H4H7})^{R/S}$ are displaced along the F_1 dimension. However, the passive coupling with respect to proton H6, i.e. $(^3T_{H5H6})^{R/S}$ and $(^2T_{H6H7})^{R/S}$ are displaced along F_2 dimension. Thus it is possible to extract six of the seven possible couplings. The coupling $(^3T_{H5H7})^{R/S}$ is derivable from the analysis of the resonances pertaining to proton H5. The expanded region of the spectrum for the proton H5 is given in Fig. 6. In this case the coupling along F_2 dimension is $(^3T_{H45H5})^{R/S}$ and the couplings obtainable from the displacements along F_1 dimension are $(^4T_{H4H6})^{R/S}$, $(^4T_{H4H7})^{R/S}$ and $(^4T_{H4H4})^{R/S}$. From the F_1 dimension, the passive couplings to proton H5, i.e. $(^3T_{H5H6})^{R/S}$ and $(^3T_{H5H7})^{R/S}$ can be determined. Except for $(^3T_{H5H7})^{R/S}$ all other information is redundant. Few of the separations giving coupling information are also marked in the figure. The interesting point to be highlighted is that the parameters $(^4T_{H4H6})^{R/S}$ and $(^4T_{H4H7})^{R/S}$ could be obtained unambiguously from the present experiment unlike in Soft-COSY. This is clearly evident from the expanded region of the spectrum corresponding to proton H7 shown in Fig. 7, where it is obvious that the active coupling $(^4T_{H4H7})^{S}$ is not detectable indicating that it is negligibly small. As a consequence there are no displacement of peaks with passive couplings either in the F_1 dimension or in the F_2 dimension for *S* enantiomer. Therefore these transitions are appearing exclusively from *R* enantiomer.

The significant advantages of the BASE-β-COSY experiment are the spectral discrimination of enantiomers, the spectral simplification by separating the active and passive couplings in the two dimensions. Thus unlike in the earlier spin selected correlation study, where more than one selective excitations are required to determine all the couplings [20], in this study a single experiment provides all the couplings resulting in the considerable saving of the instrument time. As an example for the molecule **1**, the demand on the instrument time for Soft-COSY experiment is nearly

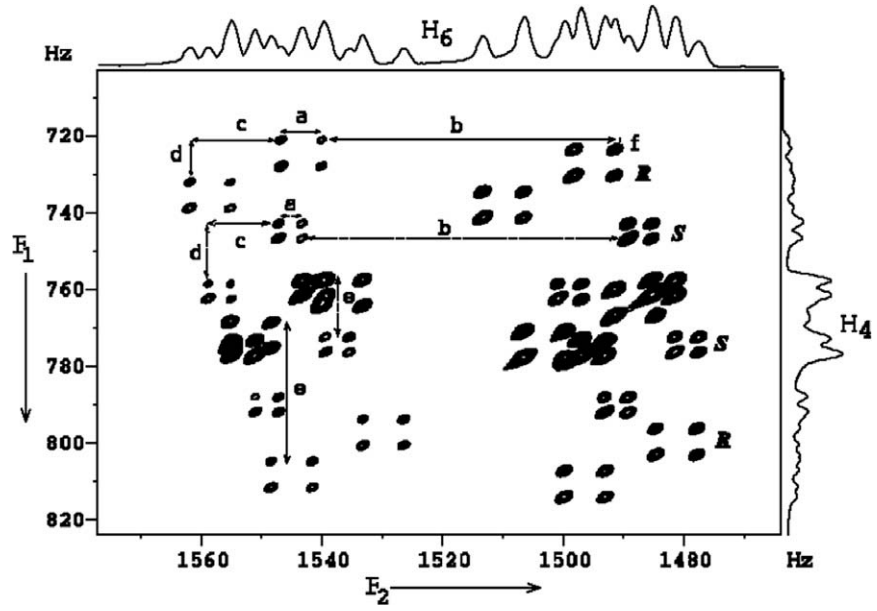

Fig. 5. The expanded portions of Fig. 4B pertaining to the resonances of proton H6. Few of the cross-sections for *R* and *S* forms have been marked. Assignments to *R* and *S* enantiomers are arbitrary. The separations (only the magnitudes) providing coupling information reported in solid lines are for *R* enantiomer and those with broken lines are for *S* enantiomer. The determined coupling parameters (in Hz) for *R* enantiomer are $a = {}^{4}T_{H4H6} = 6.8$, $b = {}^{2}T_{H6H7} = 48.7$, $c = {}^{3}T_{H5H6} = 15.0$, $d = {}^{3}T_{H4H5} = 11.2$, $e = {}^{2}T_{H4H4} = 36.4$ and $f = {}^{4}T_{H4H7} = 2.1$ and for *S* enantiomer; $a = {}^{4}T_{H4H6} = 4.0$, $b = {}^{2}T_{H6H7} = 57.5$, $c = {}^{3}T_{H5H6} = 11.5$, $d = {}^{3}T_{H4H5} = 15.7$, $e = {}^{2}T_{H4H4} = 15.2$ and $f = {}^{4}T_{H4H7} = 0.0$.

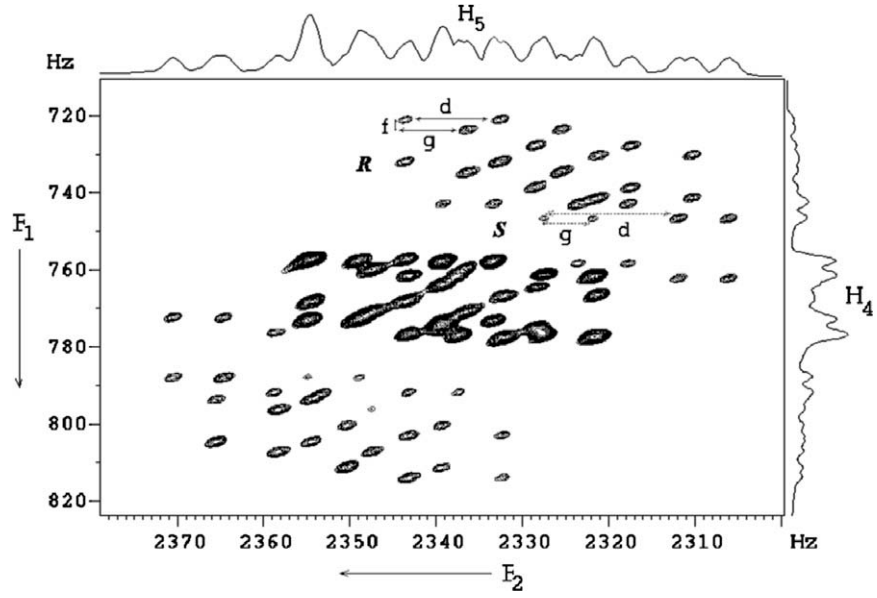

Fig. 6. The expanded portions of Fig. 4B; resonances corresponding to proton H5. The active coupling along F_2 dimension provides ${}^{3}T_{H4H5}$ and the displacement providing ${}^{3}T_{H5H7}$ are marked with alphabets. These values in Hz are $d = {}^{3}T_{H4H5} = 15.7$ for *S* enantiomer and 11.2 for *R* enantiomer, $g = {}^{3}T_{H5H7} = 5.9$ for *S*enantiomer and 7.0 for *R* enantiomer.

5 h compared to only 2 h for BASE-β-COSY experiment for deriving identical information. An attempt to quantify the enantiomeric excess on scalemic mixtures, analogous to previously reported procedure [20], provided the results with large errors. The sources of such errors are not clear.

7. Relative signs of the couplings

From the direction of tilt of the displacement vector it is possible to determine the relative signs between active and passive couplings. If the spin systems are strongly coupled the question of determination of relative signs does not arise. Therefore, the method is applicable when the spectra are first order, i.e. spins are weakly scalar coupled, weakly dipolar coupled systems in strongly orienting media and weakly dipolar coupled in chiral or bicellar media. There are situations when the relative signs of the couplings determined from the direction of the tilt of the displacement vector could be ambiguous [35]. Thus, the selectively methyl protons excited correlation experiments does not provide the relative signs of the couplings. On the other hand in the selective excitation of different spins, it is possible to determine the relative signs. The BASE-β-COSY experiment of **2** and **3** where the spins H6 and H7

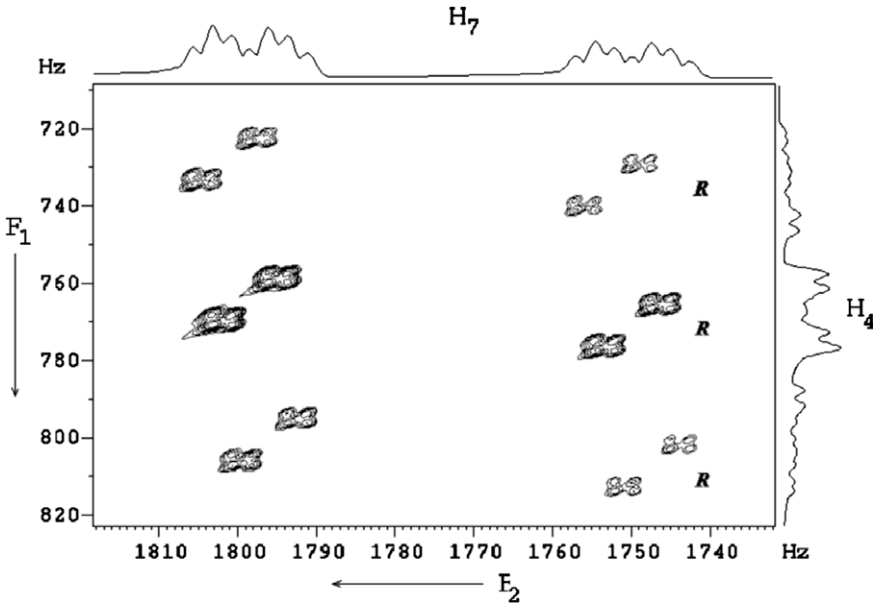

Fig. 7. Resonances corresponding to proton H7. Notice the active coupling $(T_{H4H7})^S$ is not detectable. Thus there is no displacement of peaks due to passive couplings. Consequently this spectrum pertains to that of proton H7 from pure *R* enantiomer. The analysis of this spectrum provides redundant information (i.e. active coupling $^nT_{H4H7}$ along F_2 dimension and passive couplings of H4 to other protons along F_1 dimension) and hence the separations are not marked.

are selectively excited are given in the Fig. 8. It is clearly evident from the direction of the displacement, marked with tilted lines, that the long range couplings $(^4T_{H4H6})^R$ and $(^4T_{H4H7})^R$ are opposite in **3** for *R* enantiomer whereas for *S* enantiomer $(^4T_{H4H6})^S$ and $(^4T_{H4H7})^S$ have similar signs relative to all the other couplings. In **2**, as one of the long range couplings $(^4T_{H4H7})^S$ is negligibly small (tilted line is nearly horizontal), hence it is difficult to talk about the relative signs in this case.

The BASE-β-COSY experiment is limited in its applications to big molecules. Nevertheless, the work is in progress to derive the spectral parameters in big molecules by combining our recently reported diverse methodologies. Though phase sensitive detection

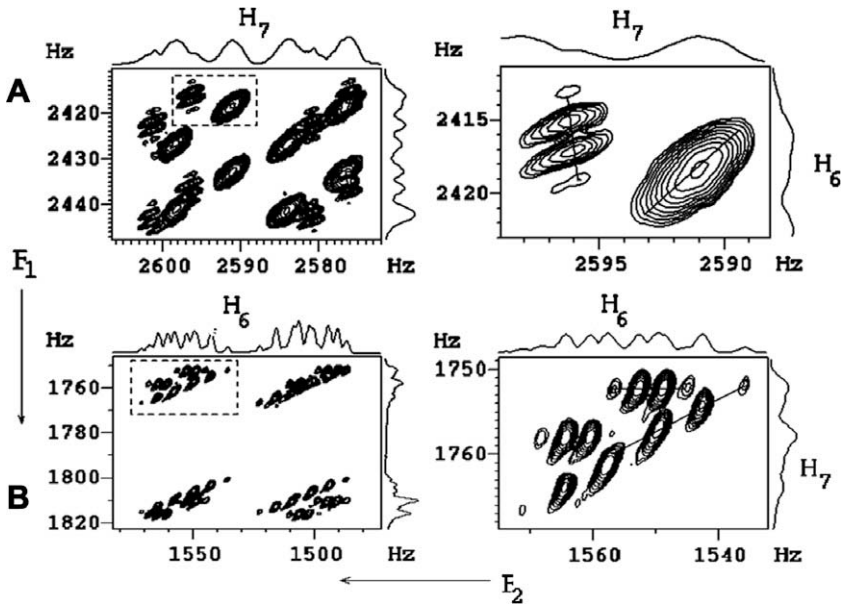

Fig. 8. (A) The 500 MHz proton 2D BASE-β-COSY spectrum of (*R*/*S*)-propylene oxide with selective excitation of proton 6. The region marked with broken rectangle is plotted with an expanded scale on the right. The width of the seduce shape pulse is 74.0 ms. The size of 2D data matrix is 400 × 3072. Spectral widths are 280 and 1694 Hz in F_1 and F_2 dimensions, respectively. Number of accumulations for each t_1 increment is 2. The relaxation delay is 1.0 s. The data was zero filled to 1024 and 8192 points and processed with a sine bell window function. The digital resolution in F_1 and F_2 dimensions are 0.3 and 0.2 Hz, respectively. Assignments for *R* and *S* are taken from the literature [15]. Notice the incredible resolution achieved in separating the quartet for the long distance coupling of H7 with H4 in *R* enantiomer. The directions of their tilt marked with tilted lines are opposite indicating relative signs of their couplings are opposite. (B) The 500 MHz proton 2D BASE-β-COSY spectrum of (*R*/*S*)-β-butyrolactone with the selective excitation of proton numbered 7. Only portion of the spectrum corresponding to proton 7 (H7) is plotted. The region marked with broken rectangle is plotted with an expanded scale on the right. The width of the seduce shape pulse is 60 ms. The size of 2D data matrix is 256 × 6008. Spectral widths are 200 and 3501 Hz in F_1 and F_2 dimensions, respectively. Number of accumulations for each t_1 increment is 2. The relaxation delay is 5.0 s. The data was zero filled to 1024 and 16,384 points and processed with a sine bell window function. The digital resolution in both F_1 and F_2 dimensions are 0.2 Hz. The quartet for the long distance coupling of H7 with H4 could be resolved for both *R* and *S* enantiomers. This parameter is undetectable for *S* enantiomer (Fig. 6B) and hence there is no displacement in F_1 dimension.

was met with limited success in this study, the constant efforts in this direction is in progress.

8. Conclusions

This study employs the separation of active and passive couplings achieved by the two-dimensional ^{1}H NMR correlation of selectively excited isolated group of coupled spins and correlated to the entire single quantum spectrum, detected with a small flip angle pulse for unambiguous enantiomeric visualization, spectral simplification and for the determination of precise spectral parameters. The advantage of the experiment is demonstrated on three different chiral molecules. All the coupling parameters have been determined from the broad and featureless spectra which are otherwise difficult to analyse and derive any meaningful information. The study also permits the determination of the relative signs of the couplings in systems whose NMR spectra are first order. The experiment can also be employed in weakly dipolar coupled systems like bicelles and other weakly orienting media for the measure of residual dipolar couplings. The study leads to routine employment of ^{1}H NMR studies for chiral discrimination, which was hitherto reported in the literature as difficult. Compared to all the reported methods for the analyses of ^{1}H spectra of chiral molecules in the chiral liquid crystalline phase, the present experiment is simple, robust and requires very little investment of instrument time.

Acknowledgments

N.S. gratefully acknowledges the financial support by Department of Science and Technology, New Delhi for the Grant No. SR/S1/PC-13/2004. U.R.P. would like to thank CSIR for senior research fellowship.

References

[1] P. Lesot, D. Merlet, J. Courtieu, J.W. Emsley, T.T. Rantala, J. Jokisaari, Calculation of the molecular ordering parameters of (±)-3-butyn-2-ol dissolved in an organic solution of poly(γ-benzyl-L-glutamate), J. Phys. Chem. A 101 (1997) 5719–5724.
[2] P. Lesot, O. Lafon, J. Courtieu, P. Berdagué, Analysis of the ^{13}C NMR spectra of molecules, chiral by isotopic substitution, dissolved in a chiral oriented environment: towards the absolute assignment of the pro-R/pro-S character of enantiotopic ligands in prochiral molecules, Chem. Eur. J. 10 (2004) 3741–3746.
[3] I. Canet, J. Courtieu, A. Loewenstein, A. Meddour, J.M. Péchiné, Enantiomeric analysis in a polypeptide lyotropic liquid crystal by deuterium NMR, J. Am. Chem. Soc. 117 (1995) 6520–6526.
[4] P. Lesot, M. Sarfati, J. Courtieu, Natural abundance deuterium NMR spectroscopy in polypeptide liquid crystals as a new and incisive means for the enantiodifferentiation of chiral hydrocarbons, Chem. Eur. J. 9 (2003) 1724–1745.
[5] D. Merlet, M. Sarfati, B. Ancian, J. Courtieu, P. Lesot, Description of natural abundance deuterium 2D-NMR experiments in weakly ordered liquid-crystalline solvents using a tailored cartesian spin-operator formalism, Phys. Chem. Chem. Phys. 2 (2000) 2283–2290.
[6] P. Lesot, D. Merlet, A. Loewenstein, J. Courtieu, Enantiomeric visualization using proton-decoupled natural abundance deuterium NMR in poly(γ-benzyl-L-glutamate) liquid crystalline solutions, Tetrahedron Asym. 9 (1998) 1871–1881.
[7] D. Merlet, W. Smadja, J. Courtieu, P. Lesot, B. Ancian, Analysis of natural abundance deuterium NMR spectra of enantiomers in chiral liquid crystals via 2D auto-correlation experiments, Chem. Commun. (1998) 2301–2302.
[8] O. Lafon, P. Lesot, D. Merlet, J. Courtieu, Modified z-gradient filtering as a mean to obtain phased deuterium autocorrelation 2D NMR spectra in oriented solvents, J. Magn. Reson. 171 (2004) 135–142.
[9] O. Lafon, P. Lesot, Deuterium 3D NMR experiments for analysing weakly aligned, isotopically enriched solutes, Chem. Phys. Lett. 404 (2005) 90–94.
[10] P. Lesot, O. Lafon, Enantiomeric analysis using natural abundance deuterium 3D NMR spectroscopy in polypeptide chiral oriented media, Chem. Phys. Lett. 458 (2008) 219–222.
[11] A. Meddour, P. Berdague, A. Hedli, J. Courtieu, P. Lesot, Proton-decoupled carbon 13 NMR spectroscopy in a lyotropic chiral nematic solvent as an analytical tool for the measurement of the enantiomeric excess, J. Am. Chem. Soc. 119 (1997) 4502–4508.
[12] J. Farjon, L. Ziani, L. Beguin, D. Merlet, J. Courtieu, Selective NMR excitations in chiral analysis, Annu. Rep. NMR 61 (2007) 283–293.
[13] B. Bikash, N. Suryaprakash, Spin selective multiple quantum NMR for spectral simplification, determination of relative signs, and magnitudes of scalar couplings by spin state selection, J. Chem. Phys. 127 (2007) 214510.
[14] B. Bikash, N. Suryaprakash, Spin state selective detection of single quantum transitions using multiple quantum coherence: simplifying the analyses of complex NMR spectra, J. Phys. Chem. A 111 (2007) 5211–5217.
[15] B. Bikash, R.P. Uday, N. Suryaprakash, Enantiomeric discrimination by double quantum excited selective refocusing (DQ-SERF) experiment, J. Phys. Chem. B 111 (2007) 12403–12410.
[16] J. Farjon, D. Merlet, P. Lesot, J. Courtieu, Enantiomeric excess measurements in weakly oriented chiral liquid crystal solvents through 2D ^{1}H selective refocusing experiments, J. Magn. Reson. 158 (2002) 169–172.
[17] R.P. Uday, B. Bikash, N. Suryaprakash, Chemical shift anisotropy edited complete unraveling of overlapped ^{1}H NMR spectra of enantiomers: application to small chiral molecules, J. Magn. Reson. 191 (2008) 259–266.
[18] B. Bikash, R.P. Uday, N. Suryaprakash, Binuclear spin state selective detection of ^{1}H single quantum transitions using triple quantum coherence: a novel method for enantiomeric discrimination, J. Magn. Reson. 192 (2008) 92–100.
[19] B. Bikash, R.P. Uday, N. Suryaprakash, Spin state selective coherence transfer: a method for discrimination and complete analyses of the overlapped and unresolved ^{1}H NMR spectra of enantiomers, J. Magn. Reson. 192 (2008) 101–111.
[20] R.P. Uday, B. Bikash, 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.
[21] V.M. Marathias, G.J. Tawa, I. Goljer, A.C. Bach II, Stereochemical identification of (R)- and (S)-ibuprofen using residual dipolar couplings, NMR, and modeling, Chirality 19 (2007) 741–750.
[22] R. Brüschweiler, C. Griesinger, O.W. Sørensen, R.R. Ernst, Combined use of hard and soft pulses for ω_1 decoupling in two-dimensional NMR spectroscopy, J. Magn. Reson. 78 (1988) 178–185.
[23] E. Kupce, R. Freeman, Band-selective correlation spectroscopy, J. Magn. Reson. A 112 (1995) 134–137.
[24] D. Merlet, B. Ancian, J. Courtieu, P. Lesot, Two-dimensional deuterium NMR spectroscopy of chiral molecules oriented in a polypeptide liquid crystal: applications for the enantiomeric analysis through natural abundance deuterium NMR, J. Am. Chem. Soc. 121 (1999) 5249–5258.
[25] L. Ziani, J. Courtieu, D. Merlet, Visualisation of enantiomers via insertion of a BIRD module in X–H correlation experiments in chiral liquid crystal solvent, J. Magn. Reson. 183 (2006) 60–67.
[26] P. Lesot, D. Merlet, J. Courtieu, J.W. Emsley, Discrimination and analysis of the NMR spectra of enantiomers dissolved in chiral liquid crystal solvents through 2D correlation experiments, Liq. Crystals 21 (1996) 427–435.
[27] R. Christy Rani Grace, N. Suryaprakash, Anil Kumar, C.L. Khetrapal, Application of a modified Z-COSY experiment for the analysis of spectra of oriented molecules—the spectrum of cis,cis-mucononitrile, J. Magn. Reson. 107A (1994) 79–82.
[28] K. Rukmani, Anil Kumar, Symmetry filtering of the NMR spectra of oriented molecules into irreducible representations using two-dimensional correlation spectroscopy, Chem. Phys. Lett. 133 (1987) 485–490.
[29] A. Bax, R. Freeman, Investigation of complex networks of spin–spin coupling by two-dimensional NMR, J. Magn. Reson. 44 (1981) 542–561.
[30] R. Brüschweiler, J.C. Madsen, C. Griesinger, O.W. Sørensen, R.R. Ernst, Two-dimensional NMR spectroscopy with soft pulses, J. Magn. Reson. 73 (1987) 380–385.
[31] L. Emsley, G. Bodenhausen, Self-refocusing effect of 270° Gaussian pulses. Applications to selective two-dimensional exchange spectroscopy, J. Magn. Reson. 82 (1989) 211–221.
[32] J. Cavanagh, J.P. Waltho, J. Keeler, Semiselective two-dimensional NMR experiments, J. Magn. Reson. 74 (1987) 386–393.
[33] C. Griesinger, O.W. Sørensen, R.R. Ernst, Two-dimensional correlation of connected NMR transitions, J. Am. Chem. Soc. 107 (1985) 6394–6396.
[34] C. Griesinger, O.W. Sørensen, R.R. Ernst, Correlation of connected transitions by two-dimensional NMR spectroscopy, J. Chem. Phys. 85 (1986) 6837–6852.
[35] Sankeerth Hebbar, N. Suryaprakash, Spin selective multiple quantum excitation: relative signs of the couplings and ambiguous situations, J. Magn. Reson. 194 (2008) 192–201.