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Selective detection of single-enantiomer spectrum of chiral molecules aligned in the polypeptide liquid crystalline solvent: Transition selective one-dimensional ^1H–^1H COSY

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ABSTRACT

One-dimensional (1D) proton NMR spectra of enantiomers are generally undecipherable in chiral orienting poly-γ-benzyl-L-glutamate (PBLG)/$CDCl_3$ solvent. This arises due to large number of couplings, in addition to superposition of spectra from both the enantiomers, severely hindering the ^1H detection. On the other hand in the present study the benefit is derived from the presence of several couplings among the entire network of interacting protons. Transition selective 1D ^1H–^1H correlation experiment (1D-COSY) which utilizes the coupling assisted transfer of magnetization not only for unraveling the overlap but also for the selective detection of enantiopure spectrum is reported. The experiment is simple, easy to implement and provides accurate eanantiomeric excess in addition to the determination of the proton–proton couplings of an enantiomer within a short experimental time (few minutes).

1. Introduction

Enantiomeric analysis by NMR spectroscopy is a long standing challenge for organic chemists [1,2]. The increased development of single-enantiomer or enantiomerically pure molecules of pharmacological importance and interest in enantioselective synthesis has enhanced the need to develop new and more convenient NMR techniques to visualize the enantiomers and for the determination of their excess. One of the several available NMR methods involves the use of the chiral polypeptide liquid crystal, PBLG dissolved in a helicogenic solvent, viz., $CDCl_3$, CD_2Cl_2, N,N-dimethylformamide-d_6, etc. [3–10]. In this anisotropic medium, the diastereotopic interactions between the chiral solutes and the oriented polypeptide fibers produce a difference in their orientational order. Consequently, the differential interactions for enantiomers such as chemical shift anisotropies ($\Delta\sigma_i$), dipolar couplings (D_{ij}) and quadrupolar couplings (Q_i) for nuclei with $I > 1/2$ enable their visualization [3]. The commonly encountered NMR active nuclei for the investigation of chiral molecules are ^1H, natural abundant ^{13}C and ^2H. The strengths of ^2H quadrupole couplings is relatively larger compared to $\Delta\sigma_{1H}$, $\Delta\sigma_{13C}$, D_{CH} and D_{HH}. Thus major part of the reported work is focused on the use of natural abundant ^2H NMR (NAD-NMR) [3–9].

The proton NMR spectra are overcrowded and do not resolve any coupling fine structures for two reasons; (1) the presence of several short and long distance couplings yielding a large number of single quantum (SQ) transitions and (2) doubling of signals because of dipolar couplings and negligible proton chemical shift anisotropies which originates from the chirality of mesophase. Therefore, the enantiodifferentiation and quantification of excess is an arduous task even for small molecules. Hence, there is no reported ^1H NMR work for a molecule possessing more than six interacting protons. There are continuing efforts by many groups to combat these problems and have reported several two-dimensional experimental techniques not only for enantiodifferentiation but also to decipher the difficult-to-analyze ^1H NMR spectra of enantiomers [11–15,18]. Each reported method has its own advantages and limitations. Any new methodology providing eantiodifferentiation, aiding the easy analyses of the spectra, giving accurate enantiomeric excess, and requires a very little investment of experimental time is a welcome addition.

In the present study, we report the utility of a simple and an elegant transition selective 1D ^1H–^1H correlation experiment for enantiomeric analysis of chiral molecules in PBLG/$CDCl_3$ solvent. The presence of too many couplings among all the protons resulting in a broad and featureless spectrum is generally considered as a drawback for proton detection. On the other hand, in the present

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work we have demonstrated the significant advantage of the presence of residual dipolar couplings among all the protons of the chosen spin systems for selective detection of enantiopure spectrum.

2. Experimental

To explore the applicability of the technique, two chiral molecules, viz., (*R/S*)-3-butyn-2-ol and (*R/S*)-propylene carbonate possessing different NMR spin nomenclatures have been investigated. The aligned samples were prepared by the well known procedure [3,4,13]. For *S* enriched oriented sample of (*R/S*)-3-butyn-2-ol, 18% of the solute, 90 mg of PBLG, 42.2 mg of *S* enantiomer, 29 mg of *R* enantiomer and 614 mg of $CDCl_3$ were taken. For *S* enriched oriented sample of (*R/S*)-propylene carbonate, 21.1% of solute, 123 mg of PBLG, 43 mg of *S*, enantiomer, 28 mg of *R* enantiomer and 650 mg of $CDCl_3$ were taken. The earlier reported assignment of peaks for different groups of protons and for individual *R* and *S* enantiomers is retained [11,14]. All the spectra were recorded using Bruker DRX-500 NMR spectrometer. The pulse sequences employed in the study and the racemic structures of the investigated molecules are given in appropriate figures. For selective excitation SEDUCE shaped pulses were utilized. The pulse durations and the delay between them are given in the respective figure captions.

3. Results and discussion

The 1D proton NMR spectrum of (*R/S*)-3-butyn-2-ol in PBLG/$CDCl_3$ solvent is reported in Fig. 1. An isolated peak (marked $*$ in Fig. 1) or a pair of peaks is observed in the up field region of the methyl group of the spectrum. The marked isolated peak is an enantiopure transition corresponding to *R* enantiomer. This feature arises because of the differential intramethyl total coupling $|^2T_{HH}|$ and long distance $^nT_{HH}$ (where $^2T_{ij} = 3D_{ij}$ for equivalent spins and $^nT_{ij} = J_{ij} + 2D_{ij}$ for non-equivalent spins and the superscript pertains to the number of bonds between the coupled protons) between the enantiomers. In the present pulse scheme, a selective 90° pulse is applied on this isolated transition. In principle, any tip angle of the selective pulse is adequate, but 90° pulse is necessary to attain maximum signal intensity. A comparison between the transition

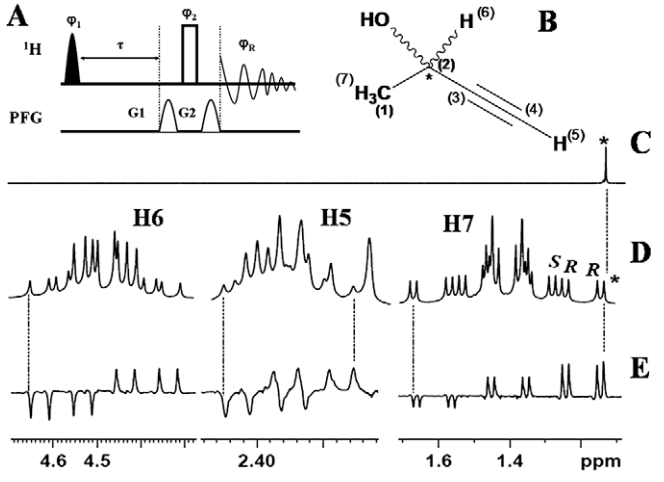

Fig. 1. (A) The pulse sequence employed for transition selective 1D COSY experiment. The first pulse is the shaped pulse for excitation of selected transition and the rectangular pulse is a hard $\pi/2$ pulse. The phases of the pulses and receiver were *x*. Both the gradients G1 and G2 are 5 G/cm. (B) Racemic structure of the molecule (*R/S*)-3-butyn-2-ol, (C) selectively excited enantiopure transition pertaining to *R* enantiomer, (D) 500 MHz ^{1}H spectrum of the molecule in PBLG/$CDCl_3$ mesophase at 300 K. (E) 500 MHz ^{1}H enantiopure spectrum of the molecule corresponding to *R* enantiomer. The peak marked $*$ in (D) was excited by the SEDUCE shaped selective pulse of duration 130 ms. The delay τ was 5 ms.

selective excitation and the normal 1D spectrum is reported in Fig. 1C and D. Since all the protons of the molecule form a dipolar coupled network, the application of the nonselective pulse causes the transfer of magnetization from this transition to all the single quantum transitions. The delay between the pulses is chosen in such a way that it creates antiphase terms of the active spin coupled to the passive spins of highest possible intensity. A gradient ratio of 1:1 is used in order to obviate the effect of coherences other than single quantum coherence produced by the mixing pulse. Consequent to the absence of common transition between the energy levels of both the enantiomers, this transfer of magnetization is restricted only for *R* enantiomer, enabling the selective detection of its single-enantiomer or enantiopure spectrum which is reported in Fig. 1D. It is clearly evident from the comparison with the 1D spectrum that all the transitions for *R* enantiomer are completely filtered out. This complete separation can be interpreted as follows: the nomenclature of this coupled proton spin system is A_3MX, where A_3 corresponds to methyl protons and, *M* and *X* are methine and acetylenic protons, respectively. The dipolar coupled A_3 spin system provides a triplet and each component of the triplet is further split into doublet of a doublet due to its couplings with *M* and *X* spins. Thus, 12 transitions are expected at A_3 chemical shift. To analyze the spectral pattern and to understand the processes that occur during the pulse sequence, polarization operator formalism is employed [19]. The first selective pulse applied on the selectively excited transition creates a single quantum coherence term, $A_{1+}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha$. After an appropriate delay, the application of a hard nonselective 90° pulse ensures the creation of SQ coherences of all the spins. As an example, the SQ coherence $A_{1+}A_{2\alpha}A_{3\alpha}$ coupled to the spin state $M_\alpha X_\alpha$ gets converted to four A_{1-} SQ terms, viz.,

$$A_{1+}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha \rightarrow A_{1-}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha,\ A_{1-}A_{2\alpha}A_{3\beta}M_\alpha X_\alpha,\ A_{1-}A_{2\beta}A_{3\alpha}M_\alpha X_\alpha,\ \text{and}\ A_{1-}A_{2\beta}A_{3\beta}M_\alpha X_\alpha \quad (1)$$

The second and third terms of Eq. (1) being degenerate have the same frequency and all the four terms give rise to a triplet. Since A_3 spins are coupled to *M* and *X* spins, each component of the triplet is further split into a doublet of a doublet due to four spin states of *M* and *X*, viz., $|M_\alpha X_\alpha>$, $|M_\alpha X_\beta>$, $|M_\beta X_\alpha>$ and $|M_\beta X_\beta>$. Furthermore, the hard mixing pulse retains SQ coherences on *M* and *X* spins. Thus, the polarization terms for the whole process can be summarized as

$$A_{1+}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha \overset{(\pi/2)_x^{A,M,X}}{\rightarrow}$$

$$\frac{1}{16}A_{1-}(A_{2\alpha}+A_{2\beta})(A_{3\alpha}+A_{3\beta})(M_\alpha+M_\beta)(X_\alpha+X_\beta) \quad (2)$$

$$\frac{i}{16}(A_{1\alpha}-A_{1\beta})(A_{2\alpha}+A_{2\beta})(A_{3\alpha}+A_{3\beta})M_-(X_\alpha+X_\beta) \quad (3)$$

$$\frac{i}{16}(A_{1\alpha}-A_{1\beta})(A_{2\alpha}+A_{2\beta})(A_{3\alpha}+A_{3\beta})(M_\alpha+M_\beta)X_- \quad (4)$$

Eqs. (3) and (4) account for the appearance of the spectrum at *M* and *X* chemical shifts, respectively, and are doublets of quartets.

The differential intensity pattern of the spectrum for the A_3 group (Fig. 1E) could be explained by considering $A_{1+}A_{2\alpha}A_{3\alpha}$ component of single quantum coherence $A_{1+}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha$. The mixing pulse causes the creation of SQ coherences on any of the three A spins. The three such possibilities could be represented as follows,

$$A_{1+}A_{2\alpha}A_{3\alpha} \overset{(\pi/2)_x^A}{\rightarrow} \frac{1}{8}A_{1-}(A_{2\alpha}+A_{2\beta})(A_{3\alpha}+A_{3\beta}) \quad (5)$$

$$\frac{i}{8}(A_{1\alpha}-A_{1\beta})A_{2-}(A_{3\alpha}+A_{3\beta}) \quad (6)$$

$$\frac{i}{8}(A_{1\alpha}-A_{1\beta})(A_{2\alpha}+A_{2\beta})A_{3-} \quad (7)$$

Eq. (5) gives a triplet with a binomial intensity of 1:2:1. The separation between the two components of the triplet provides the cou-

pling $|^2T_{AA}|$. Eqs. (6) and (7) are degenerate and each provides an antiphase doublet of −1:0:1 intensity with larger separation pertaining to $|2(^2T_{AA})|$. The resulting spectrum is the sum of all the sub-spectra originating from Eqs. (5)–(7) and therefore, gives rise to a triplet of intensity −1:2:3. This triplet is further split into two doublets of equal intensity because of coupling of A_3 spins to M and X. The decrease in signal intensity from the shielded to the deshielded transition is observed in methyl group due to the application of the selective pulse on the most upfield transition. Furthermore, it could also be mentioned that the variation in this signal intensity does not have any impact on the excess measurement because the overall intensity remains invariant.

3.1. Additional simplification of one-dimensional spectrum

Many times, the analysis of the enantiopure spectrum thus obtained may still be complex. Drastic spectral simplification can be achieved further. For this purpose, the pulse sequence reported in the Fig. 2A has been employed. As an example, in order to derive the coupling among methyl protons (A_3's), the isolated peak marked ∗ is selectively excited by the first pulse. After a suitable delay, the application of the mixing pulse on the resonance lines of methyl protons ensures SQ coherence on any of the A spins. Since the pulses are acting on A_3 spins only, M and X spins are unperturbed. The processes that take place after the application of the mixing pulse can be written as

$$A_{1+}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha \rightarrow \frac{1}{8}A_{1-}(A_{2\alpha}+A_{2\beta})(A_{3\alpha}+A_{3\beta})M_\alpha X_\alpha \quad (8)$$

The spectrum is therefore a triplet which is reported in Fig. 2B. Again the differential intensity pattern of this spectrum could be explained as before. The separation between the two components of triplet gives the intramethyl coupling $|^2T_{HH}|$.

Similarly, in order to derive remote coupling between methyl protons and acetylenic proton (X), the mixing pulse is applied both on selectively excited peak marked ∗ and the resonance lines of acetylenic proton. These pulses cause transfer of coherence from this transition mediated through the coupling $|^5T_{HH}|$ (i.e. coupling between methyl protons and acetylenic proton) to the acetylenic proton. Since pulse is applied on the selected transition and X spin only, $A_{2\alpha}A_{3\beta}$ component of this SQ coherence remains unperturbed and therefore, the polarization operator that is present for detection is

$$A_{1+}A_{2\alpha}A_{3\alpha}M_\alpha X_\alpha \rightarrow \frac{1}{8}(A_{1\alpha}+A_{2\beta})A_{2\alpha}A_{3\alpha}M_\beta X_- \quad (9)$$

This indicates that the spectrum is a doublet due to |α> and |β> spin states of A_1 spin. Fig. 2C reports a doublet pertaining to $|^5T_{HH}|$. In weakly coupled large spin systems giving isolated groups of transitions, this process of spectral simplification can be employed for deriving coupling information without resorting to numerical iterative calculations. This significantly aids the analyses of complex spectra.

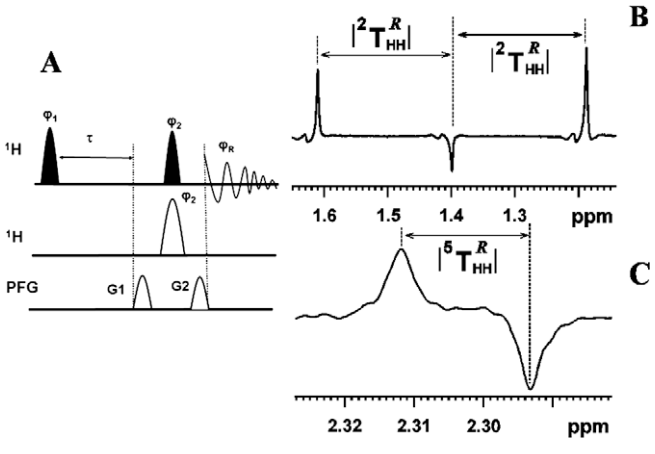

Fig. 2. (A) The pulse sequence for additional simplification of enatiopure spectrum. The phases of the pulses and receiver were x. Both the gradients G1 and G2 are 5 G/cm. The filled selective pulses were applied on the peak marked ∗ in Fig. 1D. The open shaped pulse was applied on a group of spins; (B) 1D spectrum of (*R*/*S*)-3-butyn-2-ol showing intramethyl coupling, $|^2T_{HH}|$ (105.2 Hz). Mixing pulse applied on methyl protons was excited by selective SEDUCE shaped pulse of duration 6.25 ms. (C) 1D spectrum of (*R*/*S*)-3-butyn-2-ol showing coupling between the methyl and acetylenic protons, $|^5T_{HH}|$ (9.8 Hz) Resonance lines of acetylenic proton was excited by SEDUCE shaped pulse of duration of 40 ms.

3.2. Excess measurement

For quantitative purpose, the experiment is very useful because it separates the enantiopure spectrum from the racemic mixture. Measurement of enantiomeric excess of a mixture is obtained by integrating signal area of R and S enantiomers [13]. In other experiments like (^{2}H–{^{1}H}), SERF [13] or Soft-COSY [14], the accuracy in excess measurement depends on, (1) the choice of measurement of deuterium sites [10] or (2) well resolved contours for both the enantiomers for the measurement of their areas. To explore the practical feasibility of this method for excess measurement, a scalemic mixture of 18% enriched in S enantiomer was prepared. The integral area of 1D spectrum for enantiopure spectrum pertains to that of an R enantiomer. It may be mentioned that for measuring the total area of the enantiopure spectrum only the magnitudes of the area of the peaks are utilized and phase property of the signal is ignored. Difference between integral values of 1D spectrum for both enantiopure and the scalemic mixture provides the area for S enantiomer. The computed areas can be employed to measure the enantiomeric excess using the following equation [10],

$$\%ee = \frac{|A_S + A_R|}{A_S + A_R} \quad (10)$$

The ratiometric analysis provided the enantiomeric excess of S to be 18.7%. This is within the experimental error of 2%. To substantiate the precision of such a determinacy, another scalemic mixture of (*R*/*S*)-propylene carbonate of 21.1% enriched in S was prepared. Though the single isolated peak is not observed from the 1D spec-

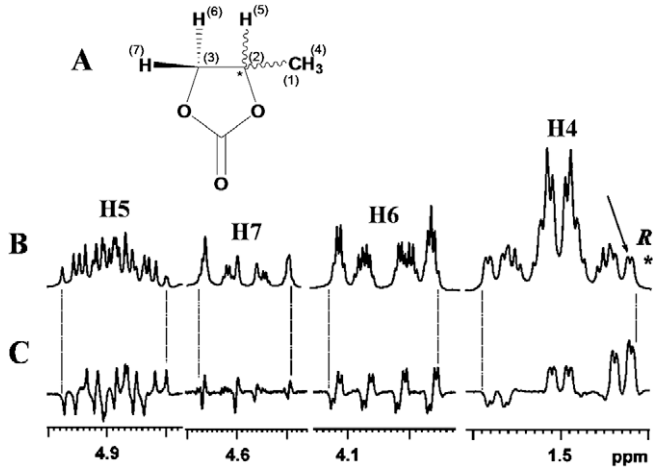

Fig. 3. (A) Racemic structure of (*R*/*S*)-propylene carbonate; (B) 500 MHz ^{1}H spectrum of the molecule in PBLG/$CDCl_3$ mesophase at 300 K (C) 500 MHz ^{1}H spectrum of 1D enantiopure spectrum corresponding to R enantiomer. The peaks marked ∗ was excited by the SEDUCE shaped selective pulse of duration 132 ms. The delay τ was 50 ms.

trum reported in Fig. 3B, the peaks marked $*$ are isolated enantiopure transitions belonging to *R* enantiomer. These transitions can be employed to unravel the enantiopure spectrum for *R* form. This was achieved by selectively exciting peaks marked $*$ and a nonselective 90° mixing pulse, which is shown in Fig. 3C. Once again the ratiometric analyses from the measured areas of both enantiopure and the scalemic mixture provided the enantiomeric excess of 22%. This establishes the fact that the experiment is more robust.

It is worthwhile to mention that the pre-requisite to get an enantiopure spectrum by any other 1D correlation experiment is the selective excitation of resonance lines of a proton or a group of protons. The negligible differences in the chemical shift anisotropies between the enantiomers may not permit such selective excitation and the separation of the enantiopure spectrum. Thus the present transition selective correlation has distinct advantage over the spin selective experiments in such situations [16,17].

In general, the proposed experiment is applicable to any molecule provided all the spins are coupled among themselves and at least one enantiopure transition (or transitions) is available for selective excitation irrespective of the size of the molecules. Nevertheless, one limitation of the approach arises if all the protons of the molecule do not interact with each other. Such a situation is rarely encountered in the small and medium sized chiral molecules oriented in PBLG/$CDCl_3$ solvent. Another interesting point which could be featured here is that the present pulse schemes are implemented in molecules possessing strongly coupled A_3 groups. Therefore the technique may be useful when second order spectral pattern exist. Another major advantage of this method is that it requires a very little investment of instrument time (few minutes) as it is like any standard proton 1D experiment.

4. Conclusions

It is demonstrated that the transition selective 1D ^1H–^1H COSY experiment is very resourceful to filter out enantiopure spectrum from the spectra of a racemic mixture, aids in the determination of proton–proton couplings of an enantiomer within a very short experimental time. The enantiopure spectrum is devoid of any overlapping signal from another enantiomer. Thus, the method has a distinct advantage in the accurate enantiomeric excess measurement.

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