Empirical determination of the absolute configuration of small chiral molecules using natural abundance ^{2}H NMR in chiral liquid crystals

Latifa Ziani, Philippe Lesot,* Abdelkhrim Meddour and Jacques Courtieu

Received (in Cambridge, UK) 26th July 2007, Accepted 17th August 2007
First published as an Advance Article on the web 30th August 2007
DOI: 10.1039/b711493a

Natural abundance deuterium 2D NMR spectroscopy in polypeptide liquid crystals is used for empirically determining the absolute configuration of small chiral molecules.

The experimental determination of the absolute configuration of small chiral synthons involved in asymmetric synthesis is a great and continuous challenge for NMR spectroscopists. ^{1}H or ^{31}P NMR using chiral auxilliaries in combination with molecular modelling allows such stereochemical assignments, but this approach is only efficient for chiral compounds possessing reactive chemical functions such as alcohols or carboxylic acids.[1]

In the field of enantiomeric discrimination, proton-decoupled deuterium NMR (^{2}H–{^{1}H} NMR) in polypeptide liquid crystals has proven to be an efficient method.[2–4] In these chiral lyotropic anisotropic media made of poly-γ-benzyl-L-glutamate (PBLG) or poly-ε-carbobenzyloxy-L-lysine (PCBLL), dissolved in organic solvents (chloroform or DMF), monodeuterated enantiomers exhibit different orientational order in average under the effect of solute/fiber enantioselective interactions.[4] Hence they can exhibit two ^{2}H quadrupolar doublets (one for each isomer) with distinct splitting ($\Delta\nu_{Q_i}^{S\ or\ R}$), resulting from a difference of local order parameters, $S_{C-D}^{S or R}$, associated with the C–D bond as shown in eqn (1).[3,4]

$$\Delta\nu_{Q_i}^{S\ or\ R} = \tfrac{3}{2}\left(\frac{e^2 Q_{D_i} q_{C-D_i}}{h}\right) S_{C-D_i}^{S\ or\ R}$$
$$\text{with } S_{C-D}^{S\ or\ R} = \left\langle \frac{3\cos^2\theta_{C-D_i}^{S\ or\ R} - 1}{2} \right\rangle \quad (1)$$

In eqn (1), $e^2 Q_D q_{C-D_i}/h$ is the ^{2}H quadrupolar coupling constant and $\theta_{C-D_i}^{S\ or\ R}$ is the angle between the C–D bond and the magnetic field axis at the site i for the S or R isomers. Here the electric field gradient is assumed to be axially symmetric along the C–D axis.

In natural abundance ^{2}H–{^{1}H} 2D NMR (NAD 2D NMR), all ^{2}H chiral isotopomers are simultaneously detected, thus providing a unique way to observe all unequivalent deuterium sites of each enantiomer on a single 2D map (QUOSY 2D experiments).[5]

Recently, various authors have shown the ability of NMR in polypeptide liquid crystals to determine the relative configuration of stereogenic centers of molecules having at least two asymmetric carbons.[6] This assignment was possible because there is an unequivocal relationship between the anisotropic dipolar couplings and the geometry *via* the molecular order parameters.[7] Unfortunately, this approach is not suitable for the experimental determination of the absolute configuration of chiral compounds possessing a single stereogenic atom. Indeed, it can be shown that the R or S assignment based only on the root-mean-square error between the experimental data and theoretical data back-calculated from the Saupe ordering matrix for a given geometry (R or S) will be the same assuming two ideal and coherent sets of anisotropic data (D_{ij}).[7] Actually only approaches based on realistic molecular dynamic calculations should allow the assignment of NMR signals toward the absolute configuration of enantiomers. This work is currently in progress but the computer modelling of chiral interactions between polypeptide fibers and enantiomers is not simple due to the complexity of the lyotropic mesophase (mixture of a polypeptide and a co-solvent).[8] Consequently, exploring other analytical solutions to determine the absolute configuration of small chiral molecules is an important task.

The trifluoromethylated chiral compound denoted **1** (Fig. 1) was recently synthesised by organic chemists both in racemic and enantiopure series. However, the absolute configuration of the isomer obtained in the enantiopure series was not clearly established from the synthetic pathway chosen. Besides X-rays are not applicable here and the spectral discrimination of enantiomers of **1** using the main classical NMR methods failed, thus excluding the determination of stereochemistry of the enantiopure isomer.[1]

In this work, we describe an empirical method in order to assign the absolute configuration of the major enantiomer in a scalemic mixture of **1**. The method avoids any isotopic labelling of **1**,[9] since it uses NAD 2D NMR in chiral oriented solvents to collect relevant data. It is based on the simple idea that iso-structural rigid (or semi-rigid) enantiomers should possess *a priori* comparable differential ordering behaviour when they are dissolved in the same chiral mesophase (identical composition for each sample) at a given temperature.

The strategy consists into four steps: (i) optimizing the spectral discrimination of enantiomers of **1** for the three non-equivalent ^{2}H

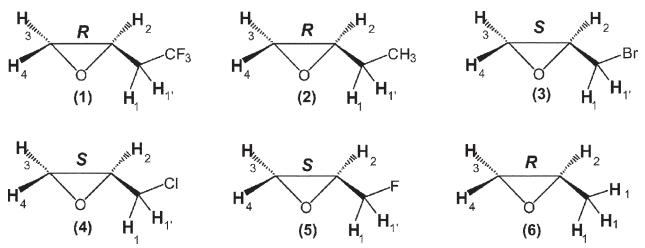

Fig. 1 Structures of homologous enantiomers (form A shown) of chiral molecules quoted **1–6**. The absolute configuration is defined according to the CIP rules. Form B corresponds to the case where the substituent is behind the epoxide cycle.

Laboratoire de Chimie Structurale Organique, RMN en Milieu Orienté, Université de Paris-Sud (XI), ICMMO, UMR CNRS 8182, Bât. 410, 91405 Orsay, France. E-mail: philesot@icmo.u-psud.fr; Fax: 33 (0)1 69 15 81 05; Tel: 33 (0)1 69 15 47 59

sites of the epoxide cycle (denoted 2, 3 and 4) using NAD 2D NMR in polypeptide liquid crystals; (ii) recording the NAD *Q*-COSY Fz[5] 2D spectrum of a series of reference iso-structural compounds (chiral molecules **2–6** listed in Fig. 1), in racemic and/or enantio-enriched mixtures depending on the solute, and using the same experimental conditions than those applied for compound **1**. Note that molecules **2–6** are commercially available, and no further chemistry was required; (iii) analyzing the 2D maps and correlating the internal and external quadrupolar doublet ($\Delta\nu_Q^A$ or $\Delta\nu_Q^B$ in absolute value) for each deuterium site (denoted 2–4) with the enantiomeric form referred to as A or B for the series of reference compounds. Here we have arbitrarily assigned the A form to the iso-structural enantiomers for which the substituent bonded to the stereogenic carbon is over the epoxide cycle as shown in Fig. 1. This notation is more convenient for assigning the quadrupolar doublets on the 2D maps. Indeed there is no direct correlation between the CIP stereodescriptors (*R*/*S*) and the shape of enantiomers in the six studied molecules; (iv) comparing the ^{2}H–{^{1}H} NMR results ($\Delta\nu_Q$) of compound **1** with those collected for the series of analogous chiral molecules.

Fig. 2(a) presents the epoxide region of the tilted NAD *Q*-COSY Fz 2D spectrum of **1** (33% ee) dissolved in the PCBLL–$CHCl_3$ mesophase at 300 K.† All NAD 2D experiments have been recorded on a 14.1 T NMR spectrometer equipped with a ^{2}H cryogenic probe operating at 92.1 MHz, with a total experimental time of around 5 h.‡ For each epoxide deuterium site, two distinct quadrupolar doublets are observed. Thus

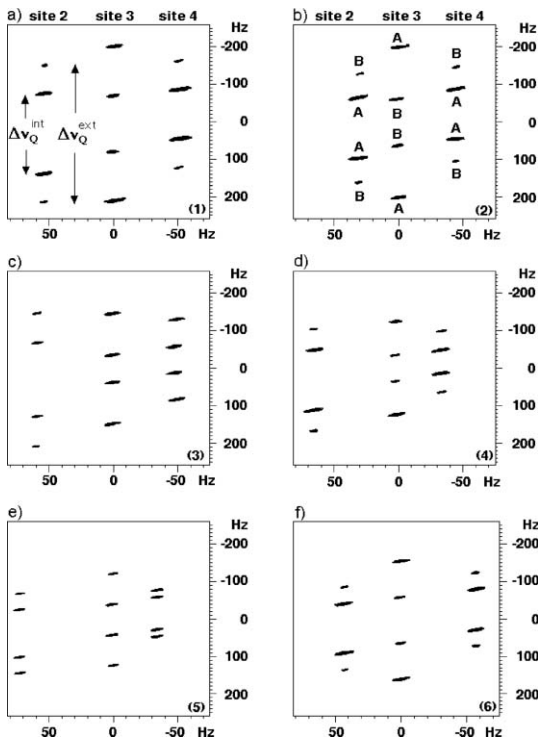

Fig. 2 NAD *Q*-COSY Fz 2D maps showing the ^{2}H spectral fingerprints for the epoxide cycle in the mixtures **1–6** (a–f) dissolved in PCBLL–$CHCl_3$ at 300 K. 2D spectra are tilted to eliminate the quadrupolar splittings in F_2 dimension and plotted at the same scale. Mixtures for compounds **2**, **4** and **6** are enriched in A enantiomer, the two others are racemic. The inner doublet of site **3** is calibrated to 0 Hz in the F_2 and F_1 dimensions.

enantiomer signals are spectrally discriminated for these sites. The assignment of the doublet pairwises is based on the analysis of chemical shifts of ^{2}H sites in combination with isotropic ^{1}H–^{1}H NOESY and COSY 2D experiments when ambiguities could exist. The difference in peak intensities is due to the enantiomeric enrichment of the mixture and shows that the internal doublet for sites 2 and 4 corresponds to the major enantiomer, while an opposite situation is obtained for site 3. This occurrence illustrates the versatility of chiral discrimination mechanisms from one ^{2}H site to another one. This also points out the interest of analysing simultaneously various deuterium sites.

In Fig. 2(b)–(f), the deuterium spectral fingerprints of the epoxide cycle detected for the reference molecules **2–6** are shown and can be compared. The small variations in the relative positions of doublets for sites 2 and 4 in F_2 dimension (compared to the signal of site 3 that is calibrated at 0 Hz in both dimensions) stem from differences of chemical shifts induced by the electronic nature of substituent for each molecule. The enantioseparation on site 4 of mixture **5** is smaller compared to that observed on other mixtures. Nevertheless, the ^{2}H spectral fingerprints collected for all studied mixtures are very similar when comparing both the magnitude and the difference of magnitude of the quadrupolar splittings for each ^{2}H site. These results validate our initial assumption postulating that the differential orientational order of the rigid part of homologous chiral solutes should be comparable.

The assignment of quadrupolar doublets for the enantio-enriched samples **2**, **4** and **6** indicates that the internal doublet for sites 2 and 4 corresponds to the A enantiomeric form (major isomer), while an inversion of the relative position of doublets associated to the major and minor enantiomers is observed for site 3. The same inversion was already seen for compound **1**. Due to the excellent similarity of ^{2}H spectral fingerprints detected for the enantioenriched and racemic samples, we can extend the assignment of internal and external doublets found for the samples **2**, **4** and **6** to the racemic samples **3** and **5**. In other words, for molecules **2** to **6**, we may confidently assume that $\Delta\nu_Q^A < \Delta\nu_Q^B$ for the sites 2/4 and $\Delta\nu_Q^A > \Delta\nu_Q^B$ for the site 3. Thus, we now dispose of a set of five experimental data that can be compared to data of sample **1**. As the fingerprints for **1** are very similar to those obtained for mixtures **2–6**, and as the most intense signal for **1** corresponds to the A form, then we can conclude that this mixture is enriched in *R* isomer (see Fig. 1). Hence, the compound synthesised enantiomerically pure is most probably the *R* antipode.

The qualitative comparison between NMR data of the reference molecules and compound **1** allows assigning the major enantiomer in mixture **1**. This comparison is valid here because the spectral fingerprints are very similar for all solutes and no inversion of the quadrupolar doublets for the minor and major enantiomer has been observed in the enantio-enriched mixtures for the three sites. No doubt that the analysis of results can be much more problematic if contradictory data are obtained in the reference series. Consequently, it can be useful to refine the analysis of data for each site by analyzing graphically the NMR results in order to establish the confidence level of the assignment achieved.

In this aim, we propose to plot the ratio ($|\Delta\nu_Q^{ext\ or\ int}|/\Delta\nu_Q^{average}$) *versus* the quantity $\Delta\nu_Q^{average}$ where $\Delta\nu_Q^{average} = (|\Delta\nu_Q^A| + |\Delta\nu_Q^B|)/2$) for all reference molecules and for the three epoxide ^{2}H sites. As seen in Fig. 3 (site 3), we obtain two sets of points where the *y*-coordinates are either larger or smaller than the

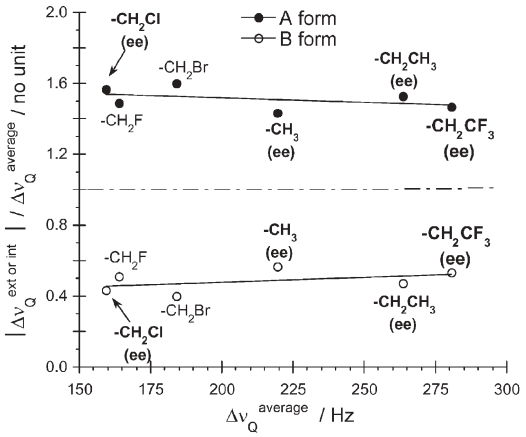

Fig. 3 Evolution of the ratios $|\Delta\nu_Q^{\text{ext or int}}|/\Delta\nu_Q^{\text{average}} = f(\Delta\nu_Q^{\text{average}})$ for site 3 of molecules **1–6**. Data relative to samples prepared with an enantiomeric excess (in A enantiomer) are denoted "(ee)" on the graph.

reference value 1. This specific value defines the case where no spectral enantiodiscrimination occurs. The upper and lower limit values of graph, namely 0 and 2, correspond to the cases where one of both quadrupolar splittings for a given site is null ($\theta_{C-D} = \theta_m = 54.7°$). The plotted data (for A and B isomers) evolve more or less linearly. For site 3 (as well as for sites 2 and 4), the evolution of data can be fitted with a linear regression of slightly negative slope (A form). Opposite situation occurs for data relative to isomer B, since the data sets are symmetrically located relative to the line of slope equal to 1 (dashed line). The interest of this method is to minimise the effect of the overall molecular orientation and to focus on the differential ordering of enantiomers. As indicated on the graph, the point corresponding to the external doublet (resp. internal doublet) of compound **1** is located on the linear regression curve associated to enantiomer A (resp. B), thus confirming the previous assignment made on the simple visual comparison of 2D spectral fingerprints. The analysis of graphs for sites 2 and 4 confirms also this assignment.

Actually, this kind of graphical representation is a convenient way to evaluate the reliability of the stereochemical assignment of $\Delta\nu_Q$'s for the unknown mixture to analyse (**1**). First we can assess if the choice of reference compounds (**2–6**) is pertinent by simply considering the data deviation of **1** relative to the linear regression line associated with data for the reference molecules. For site 3 of **1**, no deviation is observed. Second the deviation of data for **1** relative to the dashed median line (see Fig. 3) associated to the case where no spectral discrimination occurs, is a good indication on the reliability of stereochemical assignment. Indeed, the larger this deviation is, the weaker the possibility to invert the assignment for **1** is. Here, the reliability of the assignment is very good for the three epoxide ^{2}H sites.

Finally to definitely confirm the previous conclusion, we have applied the strategy reported above, but changing both the chemical nature (polarity) of the polypeptide and the organic co-solvent. Thus in a second series of NMR studies, chiral mesophases made of PCBLL–DMF, PBLG–$CHCl_3$ and PBLG–DMF, respectively, were used successfully. Clearly, the analysis of NMR results of these series led to the same conclusion than in the mesophase PCBLL–$CHCl_3$, thus validating our approach.

In conclusion, we report in this article the first example of stereochemical assignment based on the NMR analysis of iso-structural compounds oriented in chiral mesophases. The method opens interesting prospects since it can be applied for investigating either enantiopure, scalemic or racemic mixtures. Although it could be argued that this approach is time-consuming (analysis of reference mixtures), it offers a new tool to chemists for determining the absolute configuration of chiral molecules, and therefore an alternative to existing techniques (X-ray, VCD, …) when they failed or cannot be applied.

To test the efficiency of the method, it will be also necessary to explore the case of flexible chiral molecules. Among further developments, it is now important to suitably define the criteria allowing to establish the degree of homology (structure and/or electrostatic effects) between two compounds, in order to correctly choose reference molecules. In particular, we must acutely evaluate the subtle influence of the molecular shapes and the electronic profile on the differential orientational behaviour of solutes in these chiral mesophases.[8,10] This challenge task is currently underway.

The authors thank Dr Jean-Marc Paris and Dr François Metz from Rhodia Research Laboratories.

Notes and references

† The PCBLL–$CHCl_3$ and PBLG–$CHCl_3$ samples were made from 60 mg of solute (racemic or enantio-enriched), 100 mg of PCBLL (DP = 778) or PBLG (DP = 782) and 500 mg of dry $CHCl_3$. For mesophases using DMF, 130 mg of polypeptide and 350 mg of DMF were used. Details on sample preparation or NMR in polypeptide mesophases can be found in refs. 3 and 4.

‡ All ^{2}H–{^{1}H} *Q*-COSY Fz 2D spectra were recorded on a Bruker Avance II spectrometer of 14.1 T equipped with a 5-mm selective ^{2}H cryogenic probe and operating at 92.1 MHz for deuterium. Protons are decoupled using the WALTZ-16 composite pulse sequence. The sample was spun in the magnetic field and its temperature was controlled by the BVT 3200 system at 300 K in mesophases using $CHCl_3$ and 310 K in DMF. Spectra were recorded with a 2D matrix of 1530 (t_2) × 400 (t_1) data points with 64 scans per FID. Exponential filtering (LB = 1.5 Hz) was applied in both dimensions. The 2D maps were zero-filled to 2k × 2k data points prior to the double FT, then symmetrized and tilted.

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