Journal of Molecular Structure 1053 (2013) 122–126

Contents lists available at ScienceDirect

Journal of Molecular Structure

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

Unambiguous determination of conformation and relative configuration of a multiple stereo-centre molecule Rifamycin-S by using scaled residual dipolar couplings: A case study

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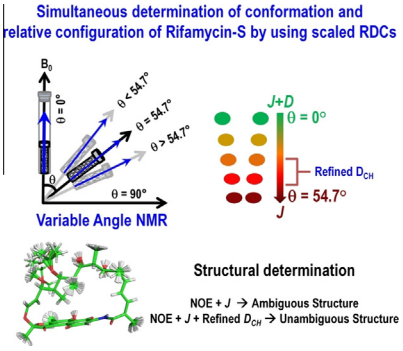
HIGHLIGHTS

- Importance of scaled- RDCs in NMR spectroscopy of organic molecules is discussed.
- The approach is exemplified with a multiple stereo-centre molecule, Rifamycin-S.
- The method allows to identify the exact conformation and relative configuration.
- Merits of the method and some limitations about its sensitivity are also discussed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 30 August 2013
Accepted 30 August 2013
Available online 6 September 2013

Keywords:
Organic molecules
NMR
Scaled RDCs
Configuration
Conformation

ABSTRACT

Residual dipolar couplings (RDC) as additional orientational constraints offer a powerful means of determining molecular structures compared to the conventional ($^3J_{HH}$ + NOE) based analysis. The recent advent of cross-linked polymer gel alignment media that are compatible for *organic* solvents has added further impetus to the RDC-enhanced NMR spectroscopy of small molecules, for precise structural elucidations. However, optimum strength of alignment for molecules dissolved in suitable anisotropic media is crucial for unambiguous measurement of RDCs. In contrast to the mechanical and chemical methods that are in practice, herein we exploit strain-induced fixation of alignment of PDMS/$CDCl_3$ gels and conceptually known variable-angle assisted scaling strategies for tuning optimum range of alignment, thereby to record unambiguous one-bond C–H RDCs as well as the corresponding J_{CH} scalar couplings in a model multiple-stereo centre molecule, Rifamycin-S. The analysis aided by the refined RDCs thus obtained, could single-out the exact conformation as well as relative configuration of Rifamycin-S, from its 256 configurational possibilities.

1. Introduction

Solution state NMR structural elucidation of small molecules classically relies only on isotropic structural parameters, *viz.*, NOE-derived distances and $^2J_{HH}/^3J_{HH}$-derived torsion angles, which are *local* in nature. However, when the sample is dissolved in oriented solvent media such as liquid crystals [1], the anisotropic parameters such as residual dipolar couplings (RDC) do not completely average out and provide information about the relative orientations of the bond-vectors, which can serve as *long-range* orientational restraints for structural elucidations [2–5]. Now RDC-based NMR structural elucidations of bio-molecules dissolved in weakly aligned aqueous media are routinely carried out in many laboratories. The recent advent of cross-linked polymer gel

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http://dx.doi.org/10.1016/j.molstruc.2013.08.061

alignment media that are compatible for *organic* solvents [6], has offered new possibilities to measure C–H or N–H RDCs (D_{CH} and D_{NH}, respectively) in small organic molecules as well [6–9]. As a result, the RDC-enhanced NMR spectroscopy has shown intense impact on the precise determination of configuration [10–22] conformation [23–28] and constitution [29] in small rigid or semi-rigid synthetic organic molecules and natural products thereby also offer scope to revisit/resolve complex structures.

However, precise measurement of magnitude and sign of D_{CH} (or D_{NH}) is crucial for unambiguous structural elucidation, which necessitates optimum degree of alignment for obtaining well-resolved $J_{CH} + D_{CH}$ doublets. For polymer gels, tuning the degree of alignment has so far been demonstrated via chemical (changing cross-link density/solvent) methods, which involve multiple preparations of alignment media/samples or physical (mechanical stretching/compression or temperature) manipulations [30–33]. Scaling of RDCs based on variable angle sample spinning (VASS) of molecules dissolved in typical nematic liquid crystal media are also known for long time, which however involves sample-spinning at above certain critical speeds to counter its natural alignment towards the static magnetic field (B_o) [34–36]. Nevertheless, some practical limitations of these scaling methods have recently been highlighted [37]. Furthermore, majority of the scaling techniques developed for *organic molecules* have so far been used as a proof of concept, whereas their utility in structural characterizations is scarcely explored [30,31,36]. The present work focuses in these directions.

Herein, we employ a straightforward approach that is conceptually related to the known VASS technique but does not involve sample-spinning and stretching/compressing, yet allows to tune the degree of the alignment as desired. This non-invasive experimental technique takes the advantage of (i) the purely strain-induced fixation of the alignment direction (θ) of the gel medium with respect to B_o (which, unlike nematic liquid crystals, is not dictated by the direction of B_o, hence no critical spinning of sample is necessary) (Fig. 1) and (ii) the $\frac{1}{2}(3\cos^2\theta - 1)$ dependence [38] on the strength of the alignment medium and magnitude of anisotropic structural parameters of the dissolved sample. Recently, the versatile combination: (i) and (ii) has been demonstrated for measuring residual chemical shift anisotropies (RCSA) in a model system, strychnine [37]. Notably, this approach also facilitates obtaining resolved (by scaling) and statistically *refined* D_{CH} (or D_{NH}) values as well as the corresponding isotropic J_{CH} values (at $\theta = 54.7°$) from the slopes of the least-square fits for multiple data ($J_{CH} + D_{CH}$) sets recorded at different sample orientations. However, hitherto, the utility of this approach has not been exploited for RDC-based simultaneous determination of conformation and relative configuration of complex organic molecules.

We exemplify this approach with Rifamycin-S (RifS), a pharmaceutically important molecule known for several years [39], whose configuration (*SS-RRRS-RS*) and conformation is known only through single-crystal X-ray crystallographic studies (Fig. 1) [40]. On the other hand, the solution state NMR studies reported so far are limited only to the $^3J_{HH}$-derived local conformation [40,41] and lack NOE-based analysis, which perhaps due to the intrinsic complexity. As the 17-membered *ansa* chain can exhibit 256 R/S relative configurational possibilities (2^8 with its centre-12 fixed to S), the RifS serves a good candidate for the present demonstration.

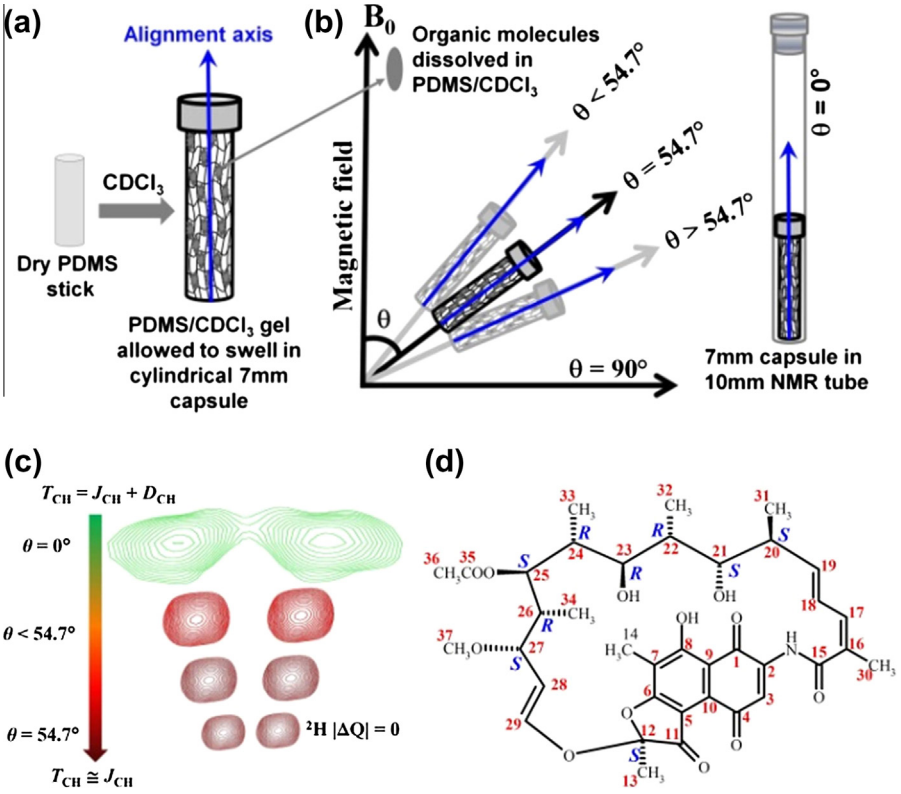

Fig. 1. Cartoon representations of (a) axially swollen (axis shown in blue) PDMS/$CDCl_3$ gel under the constraints of cylindrical 7 mm glass-capsule or MAS rotor; and weakly aligned solute organic molecules in the gel; (b) and (c) exploitation of the purely strain-induced fixation of alignment of the gel and mechanically tilting the sample for $\frac{1}{2}(3\cos^2\theta - 1)$-dependent scaling of RDCs, as explained in the text. While the conventional 7 mm MAS probe in non-spinning mode is used for tilting the sample by few degrees around 54.7°, the data corresponding to $\theta = 0°$ is recorded by using a conventional 10 mm solution-state probe for the same rotor/sample. (d) Rifamycin-S depicting its stereo-centres (*SS-RRRS-RS*), with respect to the centre-12 (S).