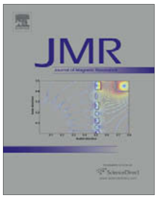
Journal of Magnetic Resonance 199 (2009) 242–246

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

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



Communication

Structural analysis of uniformly ^{13}C-labelled solids from selective angle measurements at rotational resonance

Simon G. Patching [b], Rachel Edwards [a], David A. Middleton [a,*]

[a] School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB, United Kingdom
[b] Astbury Centre for Structural Molecular Biology and Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, United Kingdom

ARTICLE INFO

Article history:
Received 5 March 2009
Revised 24 April 2009
Available online 9 May 2009

Keywords:
Rotational resonance
Dipolar coupling
Cross-polarization magic-angle spinning
Double-quantum filter
Uridine
Valine

ABSTRACT

We demonstrate that individual H–C–C–H torsional angles in uniformly labelled organic solids can be estimated by selective excitation of ^{13}C double-quantum coherences under magic-angle spinning at rotational resonance. By adapting a straightforward one-dimensional experiment described earlier [T. Karlsson, M. Eden, H. Luhman, M.H. Levitt, J. Magn. Reson. 145 (2000) 95–107], a double-quantum filtered spectrum selective for Cα and Cβ of uniformly labelled L-[^{13}C,^{15}N]valine is obtained with 25% efficiency. The evolution of Cα–Cβ double-quantum coherence under the influence of the dipolar fields of bonded protons is monitored to provide a value of the Hα–Cα–Cβ–Hβ torsional angle that is consistent with the crystal structure. In addition, double-quantum filtration selective for C6 and C1′ of uniformly labelled [^{13}C,^{15}N]uridine is achieved with 12% efficiency for a ^{13}C–^{13}C distance of 2.5 Å, yielding a reliable estimate of the C6–H and C1′–H projection angle defining the relative orientations of the nucleoside pyrimidine and ribose rings. This procedure will be useful, in favourable cases, for structural analysis of fully labelled small molecules such as receptor ligands that are not readily synthesised with labels placed selectively at structurally diagnostic sites.

1. Introduction

NMR methods for restoring rotationally-averaged weak dipolar interactions between pairs or ensembles of spins under magic-angle spinning (MAS) play a valuable role in the structural analysis of organic and biomolecular solids [1–4]. Homonuclear (e.g., ^{13}C–^{13}C) and heteronuclear (e.g., ^{13}C–^{15}N) dipolar interactions can be manipulated to provide estimates of through-space internuclear distances and constraints on torsional angles relating pairs of dipolar vectors. Homonuclear recoupling under rotational resonance (R^2) has been exploited widely to measure distances in the structure determination of membrane proteins and other biomolecular solids, for example [5–7]. The nth order R^2 condition is met when the difference N_{IS} in the Larmor frequencies of a pair of spins is equal to the MAS frequency ν_R (i.e., $n = 1$) or an integer multiple thereof ($n \geqslant 2$) [8]. Mechanical rotation at R^2 prevents averaging of the dipolar interactions and zero-quantum coherences can be excited from an initial condition of difference polarization. Examination of the time dependence of exchange of Zeeman order provides estimates of distance-dependent coupling constants permitting measurements of distances of up to 6.8 Å for ^{13}C–^{13}C pairs, for example [9]. Selective ^{13}C–^{13}C distances have also been measured from uniformly ^{13}C-labelled solids under R^2 in cases where the couplings between the pair of interest are strong relative to the Larmor frequency separation from other spins [10,11].

Torsional angles defined by carbon, nitrogen and protons, such as N1–C1–C2–N2, H1–C1–C2–H2 and H1–C1–N2–H2, can be estimated in experiments exciting ^{13}C1–^{13}C2 double-quantum coherences or ^{13}C1–^{15}N1 multiple quantum coherences [12–15]. The evolution of the coherences under the influence of local dipolar fields generated by H1 and H2 or N1 and N2 is influenced by the relative orientations of the C–H and N–H bonds in a predictable manner. For selectively labelled solids signal amplitudes are measured in a series of one-dimensional experiments in which the double-quantum evolution period is varied. For extensively or uniformly ^{13}C- and/or ^{15}N-labelled solids, such as proteins, multidimensional experiments correlating double-quantum and single-quantum coherences are often necessary [12,16]. These experiments can be time consuming, however, and so it would be useful to selectively estimate individual torsional angles from uniformly labelled materials using a series of one-dimensional experiments.

Levitt and colleagues have described a method to excite double-quantum coherence under R^2, in which mechanically excited zero-quantum coherences are converted into double-quantum coherences by frequency-selective spin inversion [17]. Highly efficient excitation was achieved for solids labelled with strongly coupled ^{13}C spin pairs, but subsequent work has shown that the efficiency and selectivity can be somewhat poorer for uniformly ^{13}C-labelled solids in which there is considerable overlap of

---

* Corresponding author. Fax: +44 151 7954406.
E-mail address: middleda@liv.ac.uk (D.A. Middleton).

1090-7807/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2009.05.002

chemical shielding anisotropies and/or small differences in the isotropic chemical shifts [18,19]. Here we show that, in favourable circumstances, it is possible to obtain selectively double-quantum filtered ^{13}C spectra of uniformly labelled solids under R^2. By following the evolution of the selectively-excited double-quantum coherences under local proton fields we obtain reliable estimates of individual H–C–C–H torsional angles and projection angles relating the orientations of non-bonded C–H pairs.

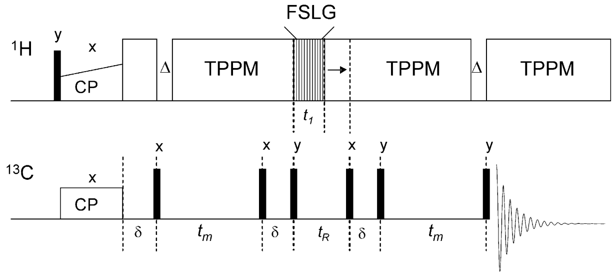


Fig. 1. Pulse sequence for selectively determining the relative orientations of pairs of heteronuclear dipolar vectors, C_i–H_i and C_j–H_j. Double-quantum coherences are excited at $n = 1$ rotational resonance and modulated under the influence of scaled C_i–H_i and C_j–H_j dipolar interactions during t_1. The effect of the isotropic ^{13}C chemical shifts over the evolution interval of one rotation period is removed by setting the radiofrequency carrier to the mean of the resonance frequencies for C_i and C_j. The proton transmitter is switched off for two delays Δ of 100 μs to dephase residual transverse components of the magnetization.

2. Results and discussion

A pulse sequence adapted from Levitt and colleagues [17,20] is shown in Fig. 1, annotated here for the specific case of H–C–C–H angle measurements. The sample rotation frequency is adjusted to the $n = 1$ rotational resonance condition (i.e., $\nu_R = N_{IS}$) and the spectrometer reference frequency is set to the mean of the isotropic chemical shift frequencies for the ^{13}C spin pair of interest. After cross-polarization, ^{13}C magnetization lies along the x-axis of the rotating frame. After a delay δ, equal to $1/2N_{IS}$ for the spin pair of interest (and also equal to $1/2\nu_R$ at $n = 1$ R^2), a $\pi/2$ pulse creates longitudinal difference polarization, in which magnetization for one of the sites is stored along +z and magnetization for the other site is stored along −z. Zero-quantum coherence correlating the spin pair of interest is excited mechanically by sample rotation during the interval t_m, which is set to approximately the inverse of the dipolar coupling constant b_{CC}. Zero-quantum coherence is converted into double-quantum coherence by two $\pi/2$ pulses separated by delay δ. The double-quantum coherence then evolves for one rotor period ($t_R = 1/\nu_R$) under the influence of ^{1}H–^{13}C dipolar interactions. This is accomplished by effectively removing ^{1}H–^{1}H couplings from the beginning of this interval for a period t_1 (where $0 \leqslant t_1 \leqslant t_R$), here using the frequency-switched Lee-Goldberg method. Double-quantum coherence is then reconverted to zero-quantum coherence with a further $\pi/2$–δ–$\pi/2$ step and after a second t_m interval a final $\pi/2$ read-out pulse creates single-quantum coherence. Thus the ^{13}C coherences are manipulated by just six radiofrequency pulses. A standard 8-step phase cycle is applied to the pulses and to the receiver to eliminate unwanted coherences [17]. The double-quantum filtered spectrum ideally consists of two

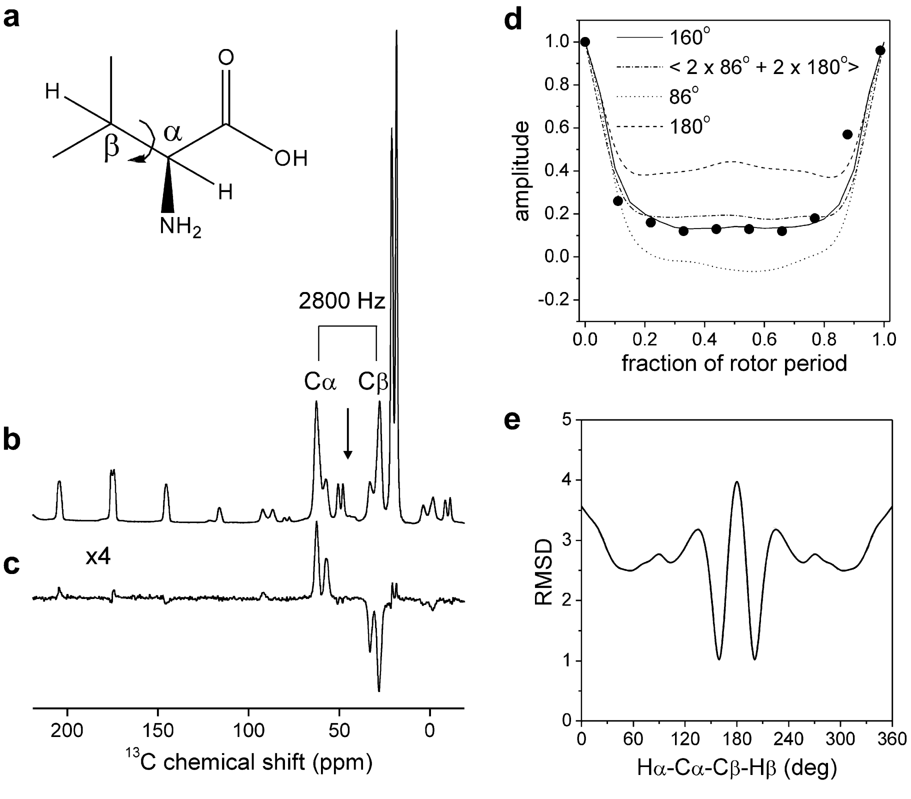


Fig. 2. Measurement of the torsional angle Hα–Cα–Cβ–Hβ (χ_1) for L-[U-^{13}C,^{15}N]valine. (a) Chemical structure of valine, highlighting the α and β positions. (b) A ^{13}C cross-polarization magic-angle spinning NMR spectrum from a 10 mg sample. (c) A spectrum obtained using the pulse sequence in Fig. 1 ($t_1 = 0$ and $t_m = 350$ μs). Both spectra are the result of averaging 64 transients. The MAS frequency of 2800 Hz is equivalent to N_{IS} for Cα and Cβ. The arrow denotes the spectrometer carrier frequency. (d) Signal amplitudes for Cα–Cβ double-quantum coherence as a function of the evolution period t_1. Normalized experimental data (circles) are overlaid with a simulated amplitude curve for a θ angle of ±160° (solid line), the best fitting curve for a single distance. Also shown are curves for the individual θ angles (86° and 180°) for each of the two pairs of conformationally distinct molecules in the asymmetric unit (dotted line and dashed line, respectively) and a curve for the two geometries averaged over the asymmetric unit (dotted-dashed line). (e) Root-mean-square deviation (RMSD) from the experimental data of curves simulated for different θ angles, showing the minimum at ±160°.