Complete Resonance Assignment of a Natural Abundance Solid Peptide by Through-Bond Heteronuclear Correlation Solid-State NMR

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Abstract: The assignment of the NMR spectra of natural abundance medium-sized solid-state organic molecules still represents a challenging problem. In this paper, we show that a complete assignment of all the carbon-13, nitrogen-15, and proton NMR lines of a natural abundance solid peptide can be performed by combining various one-bond and multiple-bond correlation techniques for rotating solids. The assignment of the MAS spectra is shown to be unambiguous and relatively straightforward.

1. Introduction

For small powdered crystalline organic compounds, cross polarization (CP), magic angle spinning (MAS), and high power proton decoupling yield high-resolution high-sensitivity one-dimensional NMR spectra of low γ nuclei like carbon-13 or nitrogen-15 in a routine fashion. However, the assignment of the NMR resonances, which is necessary either to characterize the molecule or to further investigate the structure and dynamics in the solid state, remains a difficult task for natural abundance compounds.

In the past two decades, several spectral editing techniques, which separate carbon-13 resonances according to their multiplicity, i.e., the number of directly attached protons, have been proposed and applied to characterize the CPMAS carbon spectra of small organic molecules.[1−8] However, even when combined with chemical shift analysis, these one-dimensional techniques are not always sufficient to provide a complete and unambiguous assignment of the NMR spectra. In parallel, a large number of homonuclear (^{13}C, ^{13}C)[9−24] and heteronuclear (^{13}C, ^{15}N)[25,26] transfer sequences have emerged, and have been applied in multidimensional correlation experiments to characterize *isotopically enriched compounds*.[27−30] However, for sensitivity reasons, most of these correlation techniques are not currently applicable to natural abundance materials.

One way to proceed toward the assignment of CPMAS spectra of unlabeled materials is to correlate the high-resolution spectra of the rare nuclei, ^{13}C or ^{15}N, with the abundant proton spins. The proton spectra of powdered organic molecules usually yield broad resonances due to the strong homonuclear proton−proton dipolar couplings. However, the resolution in the proton dimension can be improved by applying a homonuclear decoupling sequence which partially averages out the proton−proton dipolar couplings. All the carbon−proton correlation experiments reported so far were based on a dipolar-driven magnetiza-

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(1) Alla, M.; Lippmaa, E. *Chem. Phys. Lett.* **1976**, *37*, 260.
(2) Wu, X.; Zilm, K. W. *J. Magn. Reson. A* **1993**, *102*, 205−213.
(3) Wu, X.; Zilm, K. W. *J. Magn. Reson. A* **1993**, *104*, 119−122.
(4) Wu, X.; Burns, S. T.; Zilm, K. W. *J. Magn. Reson. A* **1994**, *111*, 29−36.
(5) Sangill, R.; Rastrup-Andersen, N.; Bildsoe, H.; Jakobsen, H. J.; Nielsen, N. C. *J. Magn. Reson. A* **1994**, *107*, 67−78.
(6) Lesage, A.; Steuernagel, S.; Emsley, L. *J. Am. Chem. Soc.* **1998**, *120*, 7095−7100.
(7) Rossi, P.; Subramanian, R.; Harbison, G. S. *J. Magn. Reson.* **1999**, *141*, 159−163.
(8) Hu, J. Z.; Harper, J. K.; Taylor, C.; Pugmire, R. J.; Grant, D. M. *J. Magn. Reson.* **2000**, *142*, 326−330.
(9) Menger, E. M.; Vega, S.; Griffin, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 2215−2218.
(10) Raleigh, D. P.; Levitt, M. H.; Griffin, R. G. *Chem. Phys. Lett.* **1988**, *146*, 71.
(11) Neuhaus, D. *J. Magn. Reson.* **1983**, *53*, 109−114.
(12) Nielsen, N. C.; Bildsoe, H.; Jakobsen, H. J. *Macromolecules* **1992**, *25*, 2847.
(13) Tycko, R.; Dabbagh, G. *J. Am. Chem. Soc.* **1991**, *113*, 9444−9448.
(14) Bennett, A. E.; Ok, J. H.; Griffin, R. G. *J. Chem. Phys.* **1992**, *96*, 8624−8627.
(15) Nielsen, N. C.; Bildsoe, H.; Jakobsen, H. J.; Levitt, M. H. *J. Chem. Phys.* **1994**, *101*, 1805−1812.
(16) Gregory, D. M.; Mitchell, D. J.; Stringer, J. A.; Kühne, S.; Shiels, J. C.; Callahan, J.; Mehta, M. A.; Drobny, G. P. *Chem. Phys. Lett.* **1995**, *246*, 654−663.
(17) Sun, B. Q.; Costa, P. R.; Kocisko, D.; Lansbury, P. T.; Griffin, R. G. *J. Chem. Phys.* **1995**, *102*, 702−707.
(18) Lee, Y. K.; Kurur, N. D.; Helmle, M.; Johannessen, O. G.; Nielsen, N. C.; Levitt, M. H. *Chem. Phys. Lett.* **1995**, *242*, 304−309.
(19) Baldus, M.; Iuliucci, R. J.; Meier, B. H. *J. Am. Chem. Soc.* **1997**, *119*, 1121−1124.
(20) Lesage, A.; Auger, C.; Caldarelli, S.; Emsley, L. *J. Am. Chem. Soc.* **1997**, *119*, 7867−7868.
(21) Gross, J. D.; Costa, P. R.; Griffin, R. G. *J. Phys. Chem.* **1998**, *108*, 7286−7293.
(22) Hohwy, M.; Jakobsen, H. J.; Eden, M.; Levitt, M. H.; Nielsen, N. C. *J. Chem. Phys.* **1998**, *108*, 2686−2694.
(23) Rienstra, C. M.; Hatcher, M. E.; Mueller, L. J.; Sun, B.; Fesik, S. W.; Griffin, R. G. *J. Am. Chem. Soc.* **1998**, *120*, 10602−10612.
(24) Lesage, A.; Bardet, M.; Emsley, L. *J. Am. Chem. Soc.* **1999**, *121*, 10987−10993.
(25) Sun, B.-Q.; Rienstra, C. M.; Costa, P. R.; Williamson, J. R.; Griffin, R. G. *J. Am. Chem. Soc.* **1997**, *119*, 8540−8546.
(26) Baldus, M.; Tomaselli, M.; Meier, B. H.; Ernst, R. R. *Chem. Phys. Lett.* **1994**, *230*, 329−336.
(27) Fujiwara, T.; Sugase, K.; Kainosho, M.; Ono, A.; Akutsu, H. *J. Am. Chem. Soc.* **1995**, *117*, 11351−11352.
(28) Hong, M.; Griffin, R. G. *J. Am. Chem. Soc.* **1998**, *120*, 7113−7114.
(29) Hong, M. *J. Magn. Reson.* **1999**, *136*, 86−91.
(30) McDermott, A.; Polenova, T.; Bockmann, A.; Zilm, K. W.; Paulsen, E. K.; Martin, R. W.; Montelione, G. T. *J. Biomol. NMR* **2000**, *16*, 209−219.

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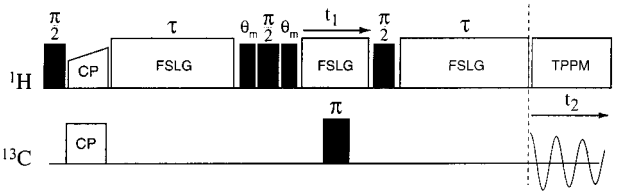


Figure 1. (a) Pulse sequence for the (^{1}H, ^{13}C) MAS-J-HMQC experiment. θ_m is a 54.7° pulse. (The pulse program is available from our website,[45] or by request to the authors.) After cross-polarization from ^{1}H (I spins), the magnetization of carbons (S spins) evolves during the delay τ under only an isotropic scaled heteronuclear J_{CH} coupling Hamiltonian. For a pair of covalently bonded ^{1}H−^{13}C spins, the carbon magnetization evolves from in-phase (S_x) into antiphase ($2I_zS_y$) coherence with respect to the attached proton. A 90° pulse applied on protons transforms the antiphase carbon coherence into a multiple-quantum heteronuclear coherence which evolves during t_1 only under the effect of the proton chemical shift. At the end of the t_1 evolution period, the MQ coherence is converted back into an antiphase carbon coherence by the second 90° proton pulse. During the second τ period this coherence evolves to become an in-phase observable carbon coherence. FSLG homonuclear decoupling[37,38] was used in this study, but other decoupling schemes can be employed.[46] Note that the pulse sequence has been slightly modified since our previous publication[36] to minimize artifacts. The optimization of the experiment will be described elsewhere.

tion transfer.[31−35] Recently, we introduced a new two-dimensional proton−carbon correlation experiment, dubbed MAS-J-HMQC (Heteronuclear Multiple-Quantum Correlation), which relies on a polarization transfer using heteronuclear J_{CH} couplings.[36] The scalar couplings constitute an interesting alternative to dipolar couplings since they provide through-bond connectivities and since they are not modulated by MAS or molecular motion.

In this paper, we demonstrate, by using various MAS-J-HMQC experiments, the complete ^{1}H, ^{13}C, and ^{15}N resonance assignment of a natural abundance tripeptide Boc-Ala-Ala-Pro-O−Bzl (where Boc stands for *tert*-butoxycarbonyl and Bzl for benzyl). The analysis of both one-bond (through $^1J_{CH}$ scalar couplings) and multiple-bond (through $^2J_{CH}$ or $^3J_{CH}$ scalar couplings) MAS-J-HMQC experiments enabled us to assign unambiguously the whole carbon spectrum and, in parallel, to measure all the proton chemical shifts of this tripeptide. Additionally we show that a (^{1}H,^{15}N) MAS-J-HMQC experiment, based on J_{NH} scalar couplings, is also feasible, and can be used to assign the nitrogen-15 MAS spectrum of the tripeptide. To our knowledge, this is the first example of a *complete* assignment of such a relatively complex molecule at natural abundance in a powder sample.

2. The MAS-J-HMQC Experiment

The pulse sequence for the (^{1}H,^{13}C) MAS-J-HMQC experiment is shown in Figure 1 and has been described in detail in ref 36. Briefly, the experiment relies on the fact that, under proton−proton homonuclear decoupling, for example, under FSLG (Frequency Switched Lee Goldburg)[37,38] decoupling, and relatively fast MAS, the one-bond J_{CH} scalar couplings (typically 130 Hz) can be resolved in the carbon spectrum and thus can be used to transfer magnetization from the carbon spins to their attached protons. In the MAS-J-HMQC experiment, a heteronuclear multiple-quantum coherence is created after the first evolution period τ, which evolves during t_1 under the proton chemical shift, before being converted back into in-phase carbon magnetization which is directly detected during t_2. In a manner analogous to the liquid-state HMQC experiment, the resulting two-dimensional spectrum yields pure in-phase chemical shift correlations between pairs of bonded protons (in ω_1) and carbons (in ω_2).

3. Assignment of the ^{13}C Spectrum

One-Bond (^{1}H,^{13}C) Correlations. For short values of the evolution periods τ (typically 2 ms), the carbon−proton magnetization transfer is highly selective, i.e., leads to only one-bond chemical shift correlations. Figure 2a shows the one-bond MAS-J-HMQC spectrum of the tripeptide Boc-Ala-Ala-Pro-O-Bzl (the three amino acids will be referred in the text as A1, A2, and P). The tripeptide was synthesized in our laboratory and crystallized from diisopropyloxide.[39] As reported previously,[36] a few resonance lines of the ^{13}C spectrum can be identified from this 2D correlation map alone. First, we can readily identify the three carbon resonances at low field as well as the one around 77.3 ppm as quaternary carbons since they are not correlated with any proton in the ω_1 dimension. From carbon chemical shift considerations, the carbon resonance at 77.3 ppm can be assigned to the quaternary carbon of the *tert*-butyl group, and that at 137.3 ppm to the quaternary carbon of the benzyl group; the remaining two quaternary resonances must therefore correspond to the three amino acid carbonyl groups (171.9 ppm) and to the carbonyl carbon of the Boc group (155.7 ppm). The carbon peaks between 127 and 131 ppm yield correlations with protons at about 7 ppm, and are therefore likely to correspond to the protonated carbons of the benzyl group. From carbon peak intensity, the high-field resonance must correspond to the para carbon. A specific assignment of the two other aromatic resonances is not possible.

In the high-field part of the spectrum, the three carbon resonances at 19.4, 20.3, and 29.6 ppm can be assigned to the three methyl groups since they correlate with protons around 1.3 ppm. Tentatively, from carbon chemical shifts and peak intensity, we assume that the peak at 29.6 ppm corresponds to the *tert*-butyl methyl groups, and the other two to the Cβ of alanine residues. However, at this stage, it is not possible to determine to which alanine residue (A1 or A2) each methyl carbon resonance belongs. Of the remaining peaks, the two carbons at 25.7 and 30.8 ppm, which correlate with protons at around 2.3 ppm, are likely to correspond to the proline γ- and β-carbons, respectively. The remaining five peaks between 40 and 70 ppm therefore correspond to the proline α- and δ-carbons, to the two alanine α-carbons, and to the O−CH$_2$ group.

Thus, if the analysis of the one-bond (^{1}H,^{13}C) MAS-J-HMQC spectrum alone enables us to go a relatively long way toward the assignment of the carbon spectrum of the tripeptide, several

(31) Caravatti, P.; Bodenhausen, G.; Ernst, R. R. *Chem. Phys. Lett.* **1982**, *89*, 363−367.
(32) Caravatti, P.; Braunschweiler, L.; Ernst, R. R. *Chem. Phys. Lett.* **1983**, *100*, 305−310.
(33) Burum, D. P. In *The Encyclopedia of NMR*; Grant, D. M., Harris, R. K., Eds.; J. Wiley & Sons: Chichester, 1997; pp 2323−2329.
(34) vanRossum, E. J.; Förster, H.; deGroot, H. J. M. *J. Magn. Reson.* **1997**, *124*, 516−519.
(35) Saalwächter, K.; Graf, R.; Spiess, H. W. *J. Magn. Reson.* **1999**, *140*, 471−476.
(36) Lesage, A.; Sakellariou, D.; Steuernagel, S.; Emsley, L. *J. Am. Chem. Soc.* **1998**, *120*, 13194−13201.
(37) Bielecki, A.; Kolbert, A. C.; Levitt, M. H. *Chem. Phys. Lett.* **1989**, *155*, 341.
(38) Levitt, M. H.; Kolbert, A. C.; Bielecki, A.; Ruben, D. J. *Solid State NMR* **1993**, *2*, 151−163.
(39) Guy, L.; Vidal, J.; Collet, A.; Amour, A.; Reboud-Ravaux, M. *J. Med. Chem.* **1998**, *41*, 4833−4843.

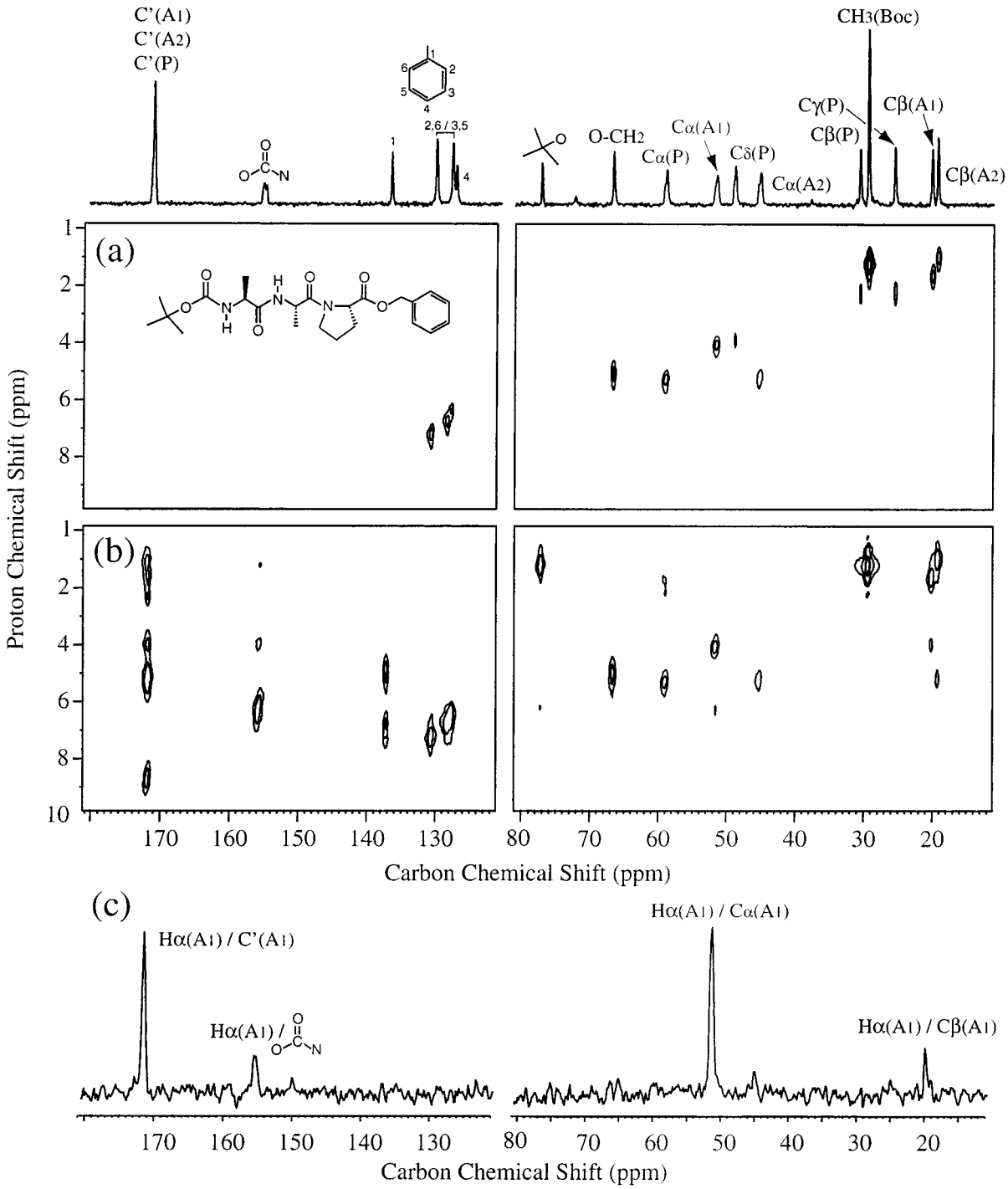


Figure 2. Carbon−proton two-dimensional MAS-J-HMQC spectra of the natural abundance tripeptide Boc-Ala-Ala-Pro-O-Bzl recorded with an evolution period (τ value) of (a) 1.6 and (b) 16 ms. The experiments were performed on a Bruker DSX 500 spectrometer (proton frequency 500 MHz) using a 4 mm triple resonance MAS probe. The sample volume was restricted to about 25 μL in the center of the rotor to increase the radio frequency (RF) field homogeneity. Approximately 20 mg of sample was used. A total of 256 t_1 acquisitions with 64 and 256 scans each were collected for the one-bond (a) and multiple-bond (b) correlation experiments, respectively (repetition delay 3 s). The spectral width in the proton dimension was 27063 Hz. The spinning frequency was 12.5 kHz. The proton RF field strength was set to 100 kHz during both the τ delays (FSLG decoupling) and acquisition (TPPM decoupling[47]). The best performance of the FSLG decoupling sequence was achieved when using a mean offset frequency of about 5 kHz from the center of the proton resonance line. For the cross-polarization step, the RF field was set to 70 kHz for carbon, while a ramped RF field was applied on protons,[48,49] and matched to obtain optimal signal. The τ delay was synchronized to be an integral number of rotor periods. Quadrature detection in ω_1 was achieved using the States method. An exponential line broadening of 100 Hz was applied in the proton dimension. The 1D CP-MAS ^{13}C spectrum is shown above the 2D spectrum. Figure 2c shows the carbon spectrum extracted at the Hα(A$_1$) resonance (4.11 ppm) from the multiple-bond MAS-J-HMQC spectrum (Figure 2b).

uncertainties remain and a complete identification of all the NMR lines is not possible at this stage.

Multiple-Bond (^{1}H,^{13}C) Correlations. Geminal ^{13}C−^{1}H couplings ($^2J_{CH}$) and vicinal ^{13}C−^{1}H couplings ($^3J_{CH}$) are also active during the evolution periods, and thus contribute to the creation of heteronuclear multiple-quantum coherences. If the evolution periods τ are long enough (in theory of the order of $1/(2\cdot{}^nJ_{CH})$), multiple-bond couplings may yield significant

correlations. Note that in this case, the MAS-J-HMQC experiment should rather be compared to the liquid-state HMBC (Heteronuclear Multiple-Bond Correlation) experiment.[40] In organic compounds in the liquid state, values between −10 and +66 Hz are usually found for $^2J_{CH}$ couplings, whereas the $^3J_{CH}$ couplings have values between 0 and 16 Hz.[41] In amino acids, the $^2J_{CH}$(CO−Hα) values range from −4 to −7 Hz while $^3J_{CH}$(CO−Hβ) values range from 2 to 6 Hz.[42]

The apparent ^{13}C line widths under FSLG decoupling are obviously much larger than these multiple-bond scalar interactions. However, the line width that is effective for the creation of the heteronuclear MQ coherences (τ periods) is the refocused line width (or the homogeneous line width) for which all the inhomogeneous broadenings are removed by the carbon π pulse. We recently found that, under continuous wave decoupling, the ^{13}C line broadening for crystalline organic compounds is mainly inhomogeneous, and we measured refocused line widths as small as a few hertz on model organic samples.[24] Under homonuclear proton decoupling, the homogeneous line width increases for protonated carbons. However, it remains about the same for nonprotonated carbons, i.e., of the order of a few hertz, *thus rendering the observation of multiple-bond correlations feasible* for these nuclei. Note that residual dipolar couplings, arising from imperfect suppression by MAS, lead to a broadening of the fine structure and will mainly contribute to the experiments by attenuating the signal. They will only contribute to the transfer of magnetization in this class of pulse sequences if they change the value of the apparent splitting. This can only arise through higher order residual terms, and experimentally is found by comparison with typical literature values for *J* couplings to be small, even for the direct one-bond dipolar couplings. We suspect that these terms should be negligible for the long-range couplings. All our experimental data is so far are consistent with pure through-bond transfer.

Figure 2b shows the MAS-J-HMQC spectrum of the tripeptide recorded with an evolution period τ equal to 16 ms. Besides the one-bond connectivities (except for those corresponding to CH_2 groups, all of them are present), many additional through-bond "long-range" correlation peaks are observed. As expected, all the nonprotonated carbons (four resonance lines in the carbon spectrum) yield multiple-bond connectivities. (i) The quaternary carbon of the *tert*-butyl group (77.3 ppm) correlates as expected with its adjacent methyl protons at 1.26 ppm ($^2J_{CH}$ correlation), providing confirmation of these assignments. (ii) At 137.3 ppm the quaternary carbon of the benzyl group gives two- and three-bond cross-peaks with the ortho and meta protons, respectively. Consistently, it does not give correlation with the para proton resonance. This quaternary carbon peak is also correlated in the ω_1 dimension to proton resonances at 5.16 ppm, which must correspond to the O−CH_2 protons ($^2J_{CH}$ correlation). The carbon resonance of the O−CH_2 group can then be immediately identified at 66.8 ppm from its correlation peak in the one-bond MAS-J-HMQC spectrum. (iii) The carbonyl carbon of the Boc group at 155.7 ppm is expected to correlate with the amide proton of the N-terminal alanine residue (A1) through a $^2J_{CH}$ coupling, and also with the α proton of the same residue through a $^3J_{CH}$ coupling. Indeed two cross-peaks, one very intense at 6.36 ppm and the other weaker at 4.11 ppm, are observed in the ω_1 dimension. From the proton chemical shift, they must correspond to respectively the HN and Hα protons of A1. In the one-bond (^{1}H,^{13}C) MAS-J-HMQC spectrum, the carbon resonance at 51.7 ppm is also correlated to the Hα(A1) proton, and can therefore be assigned to the Cα(A1). (iv) Finally, the quaternary resonances around 171.9 ppm which correspond to carbonyl carbons C′ of the three amino acids (not resolved) show several long-range correlations. Cross-peaks between 1 and 2.5 ppm and between 4 and 6 ppm in ω_1 correspond respectively to correlations with the β and α protons (through $^3J_{CH}$ and $^2J_{CH}$ couplings). The correlation at 8.71 ppm is more interesting since it must correspond to a correlation with the amide proton of A2.

In addition to the quaternary carbons, the Cβ carbons of alanine residues (methyl groups) yield weak multiple-bond correlations with the neighboring α protons ($^2J_{CH}$ couplings). As the Hα(A1) proton could be previously assigned at 4.11 ppm from its correlation with the carbonyl carbon of the Boc group (at 155.7 ppm), the assignment of the methyl carbons is straightforward: the carbon resonance at 20.3 ppm corresponds to the Cβ(A1) and, by deduction, the resonance at 19.4 ppm to the Cβ(A2). From its correlation with its directly attached proton, the Cα(A2) resonance can then be identified at 45.3 ppm. From proton and carbon chemical shift considerations, the two remaining unassigned peaks at 59 and 49 ppm can be assigned to respectively the α and δ carbons of the proline residue.

Thus, by combining the information obtained in the one-bond and multiple-bond spectra we could unambiguously assign the whole carbon CPMAS spectrum of the tripeptide. In parallel, we obtained the unambiguous assignment of all the protons. The carbon-13 and proton chemical shifts are summarized in Table 1.

In MAS-J-HMQC experiments, the proton line widths depend on the efficiency of the homonuclear decoupling scheme. Under our experimental conditions, we measured proton full line widths at half-height of about 200, 250, and 400 Hz for respectively CH_3, CH, and CH_2 groups. However, for CH_2 groups, the proton resonance may correspond to two magnetically nonequivalent protons. Despite the long evolution periods (16 ms) which are required for the excitation of the long-range MQ coherences, the multiple-bond experiment remains quite sensitive. Figure 2c shows the ω_2 trace extracted from the MAS-J-HMQC spectrum of Figure 2b at the Hα(A1) frequency (4.11 ppm). The three multiple-bond correlations are clearly visible above the noise. The relatively good sensitivity of the multiple-bond experiment is related to the fact that, as pointed out previously, the effective time constants, which characterize the decay of carbon magnetization during the τ delays, are "refocused" time constants and thus are longer than the apparent relaxation times.[24]

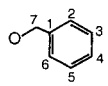
4. Assignment of the ^{15}N Spectrum

As demonstrated previously for the J_{CH} scalar couplings, the J_{NH} scalar couplings, although smaller (typically 90 Hz for a one-bond J_{NH}), can also be observed in the nitrogen dimension when a homonuclear proton−proton decoupling scheme is applied during the detection period. This is illustrated in Figure 3a, which shows the one-dimensional nitrogen-15 spectra of ^{15}N-enriched glycine recorded under proton FSLG decoupling (upper trace) and under proton continuous wave decoupling (lower trace). Under homonuclear decoupling, the NH_3 resonance clearly shows a quartet pattern with the expected 1:3:3:1 intensity distribution, although the four lines are barely resolved.

(40) Summers, M. F.; Marzilli, L.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.
(41) Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1988.
(42) Hansen, P. E.; Feeney, J.; Roberts, G. C. K. *J. Magn. Reson.* **1975**, *17*, 249−261.

Table 1. Proton, Carbon-13, and Nitrogen-15 Chemical Shifts for the Solid-State Boc-Ala-Ala-Pro-O−Bzl Tripeptide Measured from 2D MAS-J-HMQC Spectra[a]

Residue	A1	A2	P
Proton chemical shifts (ppm)			
H^N	6.36	8.71	
H^α	4.11	5.21	5.31
H^β	1.66	1.01	2.36
H^γ			2.26
H^δ			3.96
Carbon-13 chemical shifts (ppm)			
C'	171.9	171.9	171.9
C^α	51.7	45.3	59.0
C^β	20.3	19.4	30.8
C^γ			25.7
C^δ			49.0
Nitrogen-15 chemical shifts (ppm)			
N	89.5	111.4	123.8

other groups			
Carbon-13 chemical shifts (ppm)			
C_1	137.3	C_1	29.6
$C_{2,6}$ / $C_{3,5}$	130.8/128.4	C_2	77.3
C_4	127.8	C_3	155.7
C_7	66.8		
Proton chemical shifts (ppm)			
H_2 / H_3	7.26/6.81	H_1	1.26
H_4	6.46		
H_7	5.16		

[a] In the carbon-13 and nitrogen-15 dimensions, frequencies are given with respect to liquid TMS (0 ppm) and solid ammonium chloride (35.9 ppm), respectively. In the proton dimension, the chemical shift scale was adjusted by recording the proton spectrum of liquid TMS (0 ppm), and under the same spectral conditions, the 1D proton spectrum of the tripeptide under fast MAS (14 kHz). The chemical shift of the Boc CH_3 resonance, which is easily identified as the major peak in the high-field part of the 1D ^{1}H spectrum, was measured at 1.26 ppm and then used to reference the 2D HMQC spectra. Errors on the reported chemical shifts are estimated to be around ±0.05 ppm.

The pulse sequence of Figure 1 can thus be applied without any modification to acquire a (^{1}H,^{15}N) MAS-J-HMQC spectrum. The 2D (^{1}H,^{15}N) MAS-J-HMQC spectrum of the tripeptide is shown in Figure 3b. Using this 2D map, the assignment of the one-dimensional ^{15}N MAS spectrum (shown above the 2D spectrum) is relatively straightforward. First, the nitrogen resonance at 123.8 ppm which does not correlate with any proton chemical shift can be unambiguously assigned to the proline residue. Then, the two other nitrogen nuclei are easily identified from the chemical shift of their directly attached amide proton: the ^{15}N resonances at 89.5 and 111.4 ppm are assigned respectively to the A1 and A2 residues (Table 1). Note that in

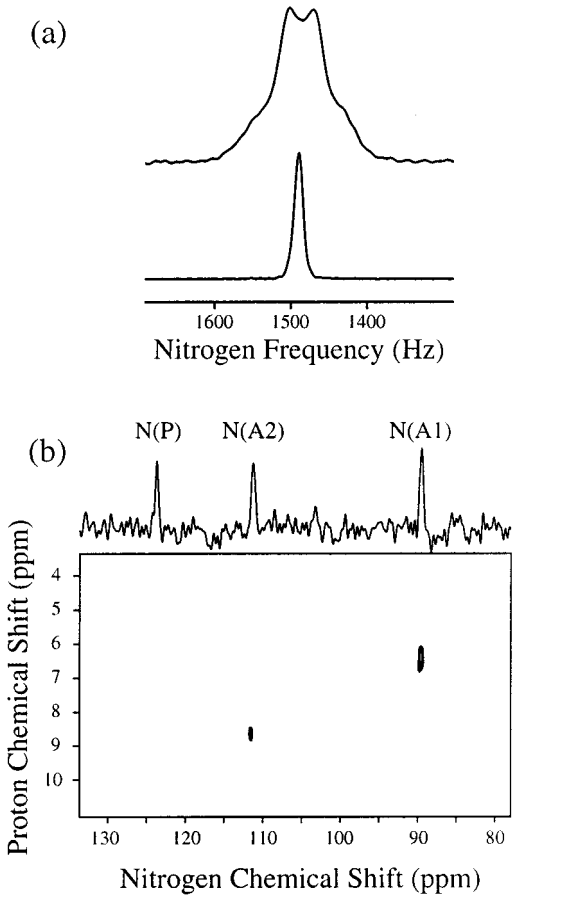


Figure 3. (a) One-dimensional nitrogen-15 cross-polarization spectrum of [^{15}N]-glycine acquired with FSLG proton decoupling (upper trace) and with continuous wave proton decoupling (lower trace) during acquisition. A proton decoupling field strength of 100 kHz was used in both cases (spinning frequency 12.5 kHz). (b) Nitrogen−proton two-dimensional MAS-J-HMQC spectra of the natural abundance tripeptide Boc-Ala-Ala-Pro-O-Bzl. The spinning frequency was 12.5 kHz and τ was 4.32 ms. A total of 110 t_1 acquisitions with 256 scans each were acquired (repetition delay 2 s). The spectral width in the proton dimension was 13042 Hz. The other experimental conditions are the same as in Figure 2. The 1D CP-MAS ^{15}N spectrum is shown above the 2D spectrum.

the multiple-bond (^{1}H,^{13}C) MAS-J-HMQC spectrum, the amide proton line widths are slightly larger (about 400 Hz) than in the (^{1}H,^{15}N) MAS-J-HMQC spectrum (about 300 Hz) and in addition exhibit some splittings (data not shown). This effect can be attributed to residual dipolar interactions between the proton and nitrogen-14 nuclei, which are not fully averaged out by MAS due to the strong quadrupolar moment of ^{14}N.[44]

5. Conclusions

In conclusion, we have shown that MAS-J-HMQC experiments constitute a powerful tool for the characterization of the NMR spectra of solid-state natural abundance organic molecules. Despite the small size of multiple-bond J_{CH} scalar couplings, we have demonstrated experimentally the feasibility of observing long-range through-bond correlations, which is an important step toward complete assignment of ^{13}C CPMAS spectra. Note that it would be difficult to reliably identify multiple-through-bond correlations using dipolar-based techniques, since at long contact

(43) Brown, S. P.; Schnell, I.; Brand, J. D.; Müllen, K.; Spiess, H. W. *J. Am. Chem. Soc.* **1999**, *121*, 6712−6718.

times dipolar methods usually lead to a wide range of through-space interactions, often including intermolecular contacts. This highlights the interest of the long-range HMQC experiment presented here, which will only yield pure through-bond correlations. In fact, long-range dipolar and scalar experiments are complementary techniques and differences in the correlation patterns could be very informative to identify, for example, effects such as hydrogen bonding in solids.

(44) Hexem, J. G.; Frey, M. H.; Opella, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 224−226.
(45) http://www.ens-lyon.fr/STIM/NMR.
(46) Sakellariou, D.; Lesage, A.; Hodgkinson, P.; Emsley, L. *Chem. Phys. Lett.* **2000**, *319*, 253−260.
(47) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951.
(48) Metz, G.; Wu, X.; Smith, S. O. *J. Magn. Reson. A* **1994**, *110*, 219−227.
(49) Hediger, S.; Meier, B. H.; Kurur, N. D.; Bodenhausen, G.; Ernst, R. R. *Chem. Phys. Lett.* **1994**, *223*, 283−288.

Additionally, we have shown that a natural abundance (^{1}H,^{15}N) MAS-J-HMQC experiment is practicable. By combining these various new MAS-J-HMQC correlation experiments, we have demonstrated the complete proton, carbon-13, and nitrogen-15 resonance assignment of a natural abundance tripeptide. To our knowledge the study reported here is the first example of a complete NMR characterization of a medium-sized molecule (33 protons, 23 carbons, and 3 nitrogens) at natural abundance in the solid state. The assignment procedure is relatively straightforward. Thus the strategy proposed here is expected to be practicable for the widespread characterization of even more complex systems.

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