# Direct Determination of a Peptide Torsional Angle $\psi$ by Double-Quantum Solid-State NMR

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Solid-state NMR is established as a useful method for estimating molecular structural details in systems which do not crystallize easily and which are unsuitable for liquid-state NMR. Several recent methods directly access angular information, circumventing the measurement of accurate internuclear distances.[1−6] In this paper, we report a new method of this type, which directly estimates the torsional angle $\psi$ of a $^{15}N-^{13}C-^{13}C-^{15}N$ molecular fragment in an isotopically-labeled peptide. The estimation is robust and highly accurate (in the region $140° \le |\psi| \le 180°$) and does not require knowledge of chemical shift tensor orientations. The method is demonstrated on an isotopically-labeled tripeptide hydrochloride GGG·HCl.

The new technique is a variety of double-quantum heteronuclear local field spectroscopy (2Q-HLF)[1,3] and is referred to as NCCN-2Q-HLF, where the prefix indicates the four relevant nuclei. The principle of the method is similar to the previously described HCCH-2Q-HLF experiment,[3] in which the torsional angle of a $^{1}H-^{13}C-^{13}C-^{1}H$ fragment is measured. Both techniques exploit a correlated transverse spin state (double-quantum coherence) between the neighboring $^{13}C$ spins. The double-quantum evolution is sensitive to the correlations of the local fields produced by the directly-bonded heteronuclei. The local field correlations depend on the geometrical relationship of the internuclear vectors and, hence, on the molecular torsional angle. The $^{15}N-^{13}C-^{13}C-^{15}N$ case requires a new experimental strategy, because the $^{15}N-^{13}C$ couplings are typically quite small and are readily averaged out by the magic-angle sample rotation.

The radio frequency (rf) pulse scheme for NCCN-2Q-HLF spectroscopy is shown in Figure 1. Radio frequency irradiation is applied at the Larmor frequencies of the three isotopes $^{1}H$, $^{13}C$, and $^{15}N$, requiring a triply-tuned magic-angle-spinning NMR probe. The sample is rotated at the angular frequency $\omega_r$ about an axis subtending the magic angle $\tan^{-1}\sqrt{2}$ with the main magnetic field. The rf sequence starts by ramped cross-polarization,[7] generating enhanced $^{13}C$ transverse magnetization. This is converted into $^{13}C_2$ double-quantum coherence by a $\pi/2$ pulse followed by a C7 rf sequence, as described elsewhere.[8] The $^{13}C_2$ double-quantum coherences are allowed to evolve freely for an interval $t_1 = n\tau_r$, where $n$ is an integer and $\tau_r = |2\pi/\omega_r|$ is the sample rotation period. During this evolution period, strong rf pulses of flip angle $\pi$ are applied at the $^{15}N$ Larmor frequency at intervals of $\tau_r/2$ (see Figure 1 for the timing scheme). As in the REDOR technique,[9] the $\pi$ pulses inhibit the coherent averaging of the $^{15}N-^{13}C$ dipolar coupling by the magic-angle rotation. This leads to dephasing of the $^{13}C_2$ double-quantum coherences under the influence of the heteronuclear local fields. The double-quantum coherences are reconverted into observable $^{13}C$ magnetization by a second C7 sequence. The shaded pulse sequence elements in Figure 1 are cycled in-phase to eliminate signal components which do not pass through double-quantum coherence. The $^{15}N$ $\pi$ pulses have rf phases in the 8-step repeating sequence 0, $\pi/2$, 0, $\pi/2$, $\pi/2$, 0, $\pi/2$, 0 ... in order to reduce the influence of pulse imperfections.[10]

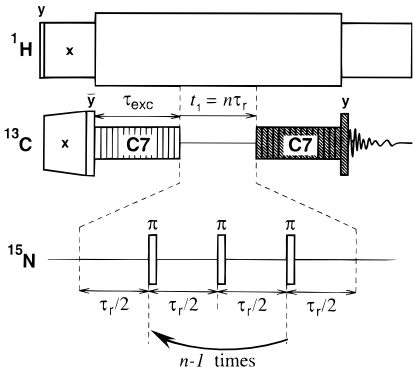


**Figure 1.** Radio frequency pulse sequence for the direct determination of the torsional angle of a $^{15}N-^{13}C-^{13}C-^{15}N$ moiety. The pulse sequence C7 is described in ref 8. The shaded elements are phase cycled in four steps. The $^{15}N$ irradiation scheme for $n \ge 1$ is expanded along the time axis for the sake of clarity. For $n = 0$, the evolution period is omitted. The double-quantum decay is calibrated by repeating the pulse sequence with $^{15}N$ irradiation omitted.

The experiment is repeated for a set of $n$ values. A second set of experiments, without the $^{15}N$ pulses, is used to calibrate the double-quantum decay time constant.

The pulse sequence shown in Figure 1 requires that the rf irradiation frequency have exactly the same magnitude as the mean of the two $^{13}C$ isotropic Larmor frequencies. This is always possible if the sample contains only one type of $^{13}C_2$ pair. The experiment may be compensated for $^{13}C$ isotropic shifts by inserting an additional $n\tau_r$ delay at the end of the $t_1$ interval, bracketed by two $^{13}C$ $\pi$ pulses.[3]

To demonstrate the method, we obtained [$^{15}N$,$^{13}C_2$-gly]-[$^{15}N$-gly]-gly by solid-state synthesis[11] starting from glycine-*N*-*t*-Boc ($^{13}C_2$, 98%+; $^{15}N$, 96%+) and glycine-*N*-*t*-Boc ($^{15}N$, 98%+) (both from Cambridge Isotope Laboratories, Andover, MA) and nonlabeled glycine-*N*-*t*-Boc polyacrylamide resin (from Applied Biosystems). The labeled tripeptide was purified by HPLC and mixed with a 10-fold excess of nonlabeled GGG (Sigma). The mixed tripeptides were cocrystallized by slow evaporation from 30% HCl. We verified by powder X-ray diffraction (wavelength = 1.540 60 Å) that the crystal structure was identical to that given in ref 12. The reported torsional angle of the $^{15}N-^{13}C-^{13}C-^{15}N$ moiety is $\psi = 164.8°$, corresponding to an extended peptide chain.

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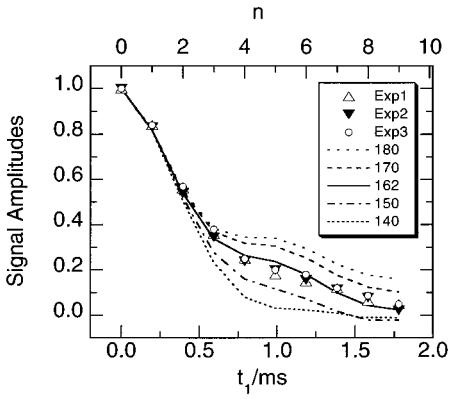


**Figure 2.** Symbols: Signal amplitudes for the labeled GGG·HCl sample, as a function of evolution interval $t_1$. Three different versions of the $^{15}$N $\pi$ pulses were used: well-calibrated $\pi$ pulses, of duration 8.0 $\mu$s (labeled Exp1); misset $\pi$ pulses, of duration 7.3 $\mu$s (Exp2); composite $\pi$ pulses,[17] with the central element having a duration 8.1 $\mu$s (Exp3). The experiments were performed at a magnetic field of 9.4 T on a Chemagnetics Infinity-400 system using 4 mm zirconia rotors in a triple-resonance probe. Around 1000 transients were accumulated for each experimental point. The spinning frequency was $|\omega_r/2\pi| =$ 5.059 kHz. The rf fields corresponded to the following nutation frequencies: for the protons, during the cross-polarization sequence, 54 kHz; during the C7 sequences, 99 kHz; during the double-quantum evolution, 91 kHz; for $^{13}$C, during the C7 sequences, 35 kHz. The double-quantum excitation interval was $\tau_{exc} = 621$ $\mu$s. The observed double-quantum filtering efficiency was around 35%. This was probably limited by residual $^{13}$C$-^{1}$H interactions. Lines: Simulations for torsional angles $|\psi| = 140°$, 150°, 162°, 170°, and 180°, using bond lengths and bond angles from the X-ray structure.

The observed double-quantum response is shown in Figure 2. The experimental points were obtained by Fourier transforming the phase-cycled NMR signals and summing the integrated spectral peak amplitudes. Three different varieties of $^{15}$N $\pi$ pulse were used in order to assess the sensitivity of the experiment to pulse imperfections. The experimental response is rather robust.

The molecular torsional angle $\psi$ is determined by comparing the experimental curves with numerical simulations. The simulation parameters are (i) the bond lengths and bond angles of the N$-$C$-$C$-$N moeity, (ii) the N$-$C$-$C$-$N torsional angle, and (iii) the decay time constant $T_2^{2Q}$ of the $^{13}C_2$ double-quantum coherence. The bond lengths and bond angles were taken from the X-ray structure:[12] $r_{NC} = 0.1474$ nm; $r_{CC} =$ 0.1503 nm; $r_{CN} = 0.1325$ nm; $\theta_{NCC} = 108.7°$; $\theta_{CCN} = 114.3°$ (the atoms are read from left to right starting from the N-terminus). The decay time constant was determined to be $T_2^{2Q} = 2.7$ ms by the calibration experiment. The chemical shift anisotropies do not enter into the calculation. The only assumptions are (i) the isolation of the labeled $^{15}$N$-^{13}$C$-^{13}$C$-^{15}$N systems on the time scale of the experiment, (ii) the neglect of $^{15}$N$-^{15}$N couplings, and (iii) ideal $\pi$ pulses. The first two approximations are reasonable since the labeled moieties are diluted in a natural-abundance matrix and the $^{15}$N$-^{15}$N couplings are small (less than 30 Hz). The third approximation is tested by employing a variety of $\pi$ pulses experimentally (Figure 2). Since the NMR response is insensitive to the sign of $\psi$, the symbol $|\psi|$ is used below.

Figure 2 shows simulated curves for torsional angles $|\psi| =$ 140, 150, 162, 170, and 180°. These simulations include the experimentally-determined double-quantum decay. The best fit between simulation and experiment is obtained for $|\psi| = 162°$, which is within 3° of the X-ray value. The small discrepancies between experiment and simulation may reflect $^{15}$N$-^{15}$N couplings, molecular motions, a spread of static molecular geometries, or signals from minor isotopomers. The torsional angle estimation is at least as good as $\pm 5°$. Note that the torsional angle $|\psi|$ is the *only free parameter* in the simulations.

Simulations for the full range of torsional angles show an ambiguity in the region $|\psi| \leq 120°$. The torsional angle estimation in this range may have to be supplemented by other information, such as chemical shift tensor eigenvalues.[13]

2Q-HLF experiments have several positive features. Unlike other 2Q methods,[1,2] no knowledge of chemical shift anisotropy orientations is required. Unlike techniques based on spin diffusion,[6] the natural-abundance $^{13}$C background is suppressed and the experiments do not require complete magnetic isolation of the introduced label clusters. Against these advantages should be set the difficulty and expense of the multiple labeling schemes.

The results shown here confirm the potential of double-quantum heteronuclear local field spectroscopy as a versatile tool for molecular structure determination. The HCCH version of the experiment has already been applied to the membrane protein rhodopsin.[14] Other researchers have estimated the Ramachandran $\phi$ angle in a peptide.[15] The good sensitivity of the methods allows application to a wide range of systems, including proteins, nucleic acids, and carbohydrates.

The double-quantum heteronuclear local field approach may also be exploited to extract angular information from biomolecules in solution.[16]

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