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Proton Assisted Insensitive Nuclei Cross Polarization

Józef R. Lewandowski, Gaël De Paëpe, and Robert G. Griffin*

Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received July 14, 2006; E-mail: rgg@mit.edu

Polarization transfer[1−15,37] between different nuclear species mediated by dipolar couplings is used extensively in magic angle spinning (MAS)[16] experiments to perform chemical shift assignments and to measure distances[5−7,11,17−21] and torsion angles.[22−25] Heteronuclear dipolar recoupling sequences can be classified into two categories depending on their behavior with respect to dipolar truncation. The first group includes the double CP sequence (DCP[26]) and its variants (SPICP,[27] RFDRCP,[4] *i*DCP[9]) which lead to noncommuting terms in the effective Hamiltonian, and thus are mainly used to perform one-bond transfers (NCO, NCA) sometimes followed by a homonuclear $^{13}C-^{13}C$ recoupling period for obtaining $^{15}N-^{13}C$ multiple-bond contacts.[28,29] The second group of sequences (REDOR,[5] TEDOR[1]/REPT,[19] GATE[17]) yields a longitudinal effective Hamiltonian and enables measurement of long distances (<4 Å).[20,21]

High magnetic fields (>600 MHz) are an important experimental component for improving sensitivity and resolution in biomolecular MAS studies involving $^{15}N-^{13}C$ magnetization transfer, provided that experiments can be performed at high MAS frequencies (>15 kHz) to compensate for increases in chemical shift anisotropies (CSA). Unfortunately, the application of the sequences mentioned above becomes problematic in this regime as the applied high rf powers lead to increased sample heating and jeopardize the integrity of the probe, but often do not provide sufficient ^1H decoupling.

Here we present an efficient $^{15}N-^{13}C$ heteronuclear recoupling technique that involves nearby protons in the transfer and is applicable at high MAS frequency ($\omega_r/2\pi$ > 20 kHz). This new scheme, proton assisted insensitive nuclei cross polarization (PAIN-CP), reduces dipolar truncation and therefore is particularly well suited for obtaining long distance contacts. PAIN-CP demonstrates that *the involvement of protons in the polarization transfer between low-γ nuclei does not have to be deleterious in nature;* on the contrary ^1H's can be used *to enhance the rate and efficiency of the transfer*. The PAIN-CP experiment utilizes a mechanism which we refer to as third spin assisted recoupling (TSAR). Its extension to the homonuclear case is straightforward and will be discussed elsewhere. Note that the use of ^1H irradiation has been reported previously for $^{13}C-^{13}C$ recoupling experiments[30−32] but that the underlying mechanism is different.

Even though PAIN-CP and DCP[26] have similar pulse sequences (see Figure 1 and Supporting Information sections 7−8 (S.I.-7, -8)), they rely on very different mechanisms. The PAIN-CP mechanism corresponds to a second-order recoupling in an interaction frame defined by the three C.W. rf fields involving cross terms between heteronuclear N−H and C−H dipolar couplings (see S.I.-2). In this process, nearby ^1H's are used to create trilinear (N, C, H) terms in the effective Hamiltonian that can lead to ZQ and DQ $^{15}N-^{13}C$ polarization transfer. In this publication we explore only the ZQ transfer.

Figure 2 shows simulations comparing $^{15}N-^{13}C$ polarization transfer for the PAIN-CP, DCP, TEDOR, and GATE sequences at

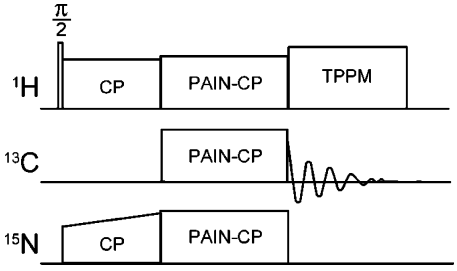

Figure 1. PAIN-CP $^{15}N-^{13}C$ correlation pulse sequence. The proper combination of ^{15}N, ^{13}C, and ^1H rf power results in enhanced rates and efficiency of ^1H mediated $^{15}N-^{13}C$ polarization transfer.

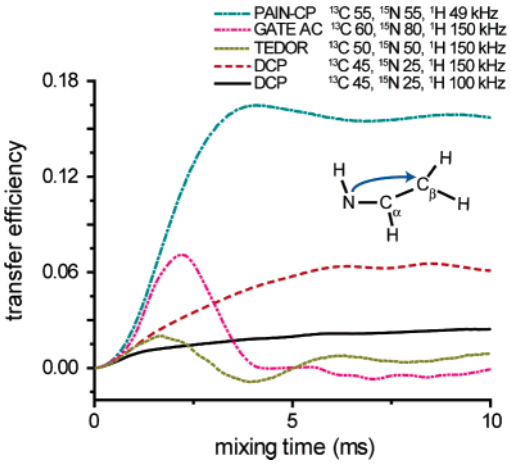

Figure 2. Comparison of $^{15}N-^{13}C$ two-bond polarization transfer for PAIN-CP, DCP, TEDOR, GATE AC sequences at $\omega_r/2\pi$ = 20 kHz. Note that variants of DCP such as RFDRCP, SPICP, and *i*DCP are not considered here as they mainly improve the recoupling bandwidth, which is not the major concern in this simulation.

$\omega_{1H}/2\pi$ = 750 MHz and $\omega_r/2\pi$ = 20 kHz. The model spin system consists of seven spins (^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$, 4 ^1H's). Simulations were performed with SPINEVOLUTION[33] (see S.I.-1 for details).

DCP simulations utilized typical rf field strengths: $\omega_{1C}/2\pi$ = 45 kHz, $\omega_{1N}/2\pi$ = 25 kHz, $\omega_{1H}/2\pi$ = 100, and 150 kHz of ^1H C.W. decoupling, respectively, illustrating that rf power levels should satisfy the condition $\omega_{1H}/\omega_{1C} \geq 3$ to ensure adequate ^1H decoupling.[34] However, even for 150 kHz of ^1H decoupling, a challenge for most triple resonance probes, the two-bond transfer from ^{15}N to C_β reaches only about 6.5% efficiency in 6.5 ms, a result of the dipolar truncation effect (see S.I.-4). Longitudinal recoupling sequences such as TEDOR or GATE, where there is no dipolar truncation, do not provide efficient two-bond transfer in the presence of strong one-bond coupling. For example, GATE reaches about 7% transfer in 2.3 ms for extremely demanding experimental settings. On the other hand, the PAIN-CP buildup obtained with ^{13}C and ^{15}N fields set to the same value (n = 0,

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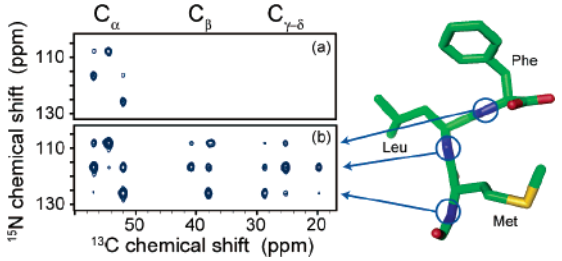

Figure 3. Aliphatic region of 2D ^{15}N−^{13}C correlation spectra obtained at 750 MHz with 20 kHz MAS: (a) DCP with 3 ms mixing, (b) PAIN-CP with 4 ms mixing. The ^{1}H rf field strength was 112 and 62 kHz for data in a and b, respectively. In graphic a the $n = 1$ ZQ Hartmann−Hahn condition was matched with 45 kHz ^{13}C rf and 25 kHz ^{15}N rf. In graphic b, $\omega_1/2\pi =$ 50 kHz for both ^{13}C and ^{15}N. All spectra were acquired and processed in exactly the same manner. The contour levels are set to the same value.

Hartmann−Hahn)[35,36] reaches 16.5% transfer efficiency in 4 ms, an improvement of 3 to 8 times when compared to DCP with high power ^{1}H decoupling. In addition, contrary to TEDOR and GATE results, the transferred magnetization achieves an equilibrium value simplifying the choice of the mixing time in a correlation experiment.

In practice, it is possible to utilize the PAIN-CP mechanism provided that ^{1}H−^{15}N and ^{1}H−^{13}C Hartmann−Hahn (H.H.) as well as rotary resonance[3] (R.R.) conditions are avoided. The ^{15}N and ^{13}C rf fields do not necessarily have to match the $n = 0$ H.H. condition (see S.I.-2, -6). Optimal PAIN-CP settings are a compromise between avoiding destructive H.H. or R.R. recoupling conditions and retaining significant second-order scaling to ensure efficient polarization transfer. Accordingly, there are usually a few different ^{1}H rf levels that lead to an appreciable PAIN-CP effect (see S.I.-6).

Figure 3 is an experimental demonstration that PAIN-CP is an efficient technique for heteronuclear ^{15}N−^{13}C correlation experiments. The spectra were obtained with [U−^{13}C, ^{15}N] N-*f*-MLF-OH using $\omega_r/2\pi = 20$ kHz, $\omega_{1H}/2\pi = 750$ MHz, and a 2.5 mm, triple-channel Bruker probe. Figure 3a shows a NCA DCP spectrum with 3 ms mixing (optimum for one-bond transfer) and 112 kHz ^{1}H decoupling. Long-range cross-peaks (more than two bonds) are completely absent from the spectrum at this mixing time and do not appear at longer mixing times (data not shown). Figure 3b depicts an $n = 0$ H.H. PAIN-CP spectrum with 4 ms mixing, using rf fields of ∼50 kHz for ^{13}C, ^{15}N, and (a) 112 kHz and (b) 62 kHz, respectively, for ^{1}H. *We observe cross-peaks for ^{15}N−^{13}C pairs separated by up to 6 Å in a uniformly ^{13}C, ^{15}N labeled compound*. Note that part of the long-range transfer also involves a ^{13}C homonuclear TSAR effect (see S.I.-2). In addition, despite distributing the initial ^{15}N magnetization over a larger number of ^{13}C sites, the one-bond cross-peaks are much more intense than in the DCP case. This fact indicates that for this system a ∼2.5 ratio for ($\omega_{1H}/\omega_{1C,1N}$) is not sufficient to provide efficient ^{1}H decoupling in the DCP case.

In summary, we present a new heteronuclear ^{15}N−^{13}C correlation mechanism, applicable at high $\omega_r/2\pi$, leading, in this regime, to superior recoupling performance compared to alternative techniques. PAIN-CP can provide long ^{15}N−^{13}C contacts, circumventing the usual dipolar truncation encountered with DCP-type sequences. The method provides a highly efficient alternative to NCX and NCXCY experiments, extends the range of applicability of heteronuclear recoupling techniques to high B_0 and $\omega_r/2\pi$, and should thus be of major interest for structure determination of biomolecules.

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Note Added after ASAP Publication. Due to a production error, a reference was cited incorrectly in the first sentence in the version of this paper published ASAP on January 3, 2007. The properly formatted version was published ASAP on January 4, 2007.

Supporting Information Available: Experimental and simulation details; discussion of PAIN-CP mechanism with additional SPINEVOLUTION simulations; quantitative comparison between DCP and PAIN-CP. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hing, A. W.; Vega, S.; Schaefer, J. *J. Magn. Reson.* **1992**, *96* (1), 205−209.
(2) Baldus, M.; Petkova, A. T.; Herzfeld, J.; Griffin, R. G. *Mol. Phys.* **1998**, *95* (6), 1197−1207.
(3) Oas, T. G.; Griffin, R. G.; Levitt, M. H. *J. Chem. Phys.* **1988**, *89* (2), 692−695.
(4) Sun, B. Q.; Costa, P. R.; Griffin, R. G. *J. Magn. Reson. A* **1995**, *112* (2), 191−198.
(5) Gullion, T.; Schaefer, J. *J. Magn. Reson.* **1989**, *81* (1), 196−200.
(6) Zhao, X.; Hoffbauer, W.; auf der Gunne, J. S.; Levitt, M. H. *Solid State Nucl. Magn. Reson.* **2004**, *26* (2), 57−64.
(7) van Rossum, B. J.; de Groot, C. P.; Ladizhansky, V.; Vega, S.; de Groot, H. J. M. *J. Am. Chem. Soc.* **2000**, *122* (14), 3465−3472.
(8) Ladizhansky, V.; Vinogradov, E.; van Rossum, B. J.; de Groot, H. J. M.; Vega, S. *J. Chem. Phys.* **2003**, *118* (12), 5547−5557.
(9) Bjerring, M.; Nielsen, N. C. *Chem. Phys. Lett.* **2003**, *382* (5−6), 671−678.
(10) Dvinskikh, S.; Chizhik, V. *J. Exp. Theor. Phys.* **2006**, *102* (1), 91−101.
(11) Bertani, P.; Raya, K.; Hirschinger, K. *C. R. Acad. Sci., Ser. IIc: Chim.* **2004**, *7* (3−4), 363−369.
(12) Ray, S.; Ladizhansky, V.; Vega, S. *J. Magn. Reson.* **1998**, *135* (2), 427−434.
(13) Chan, J. C. C. *Chem. Phys. Lett.* **2001**, *335* (3−4), 289−297.
(14) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1973**, *59* (2), 569−590.
(15) Baldus, M.; Geurts, D. G.; Hediger, S.; Meier, B. H. *J. Magn. Reson. A* **1996**, *118* (1), 140−144.
(16) Lowe, I. J. *Phys. Rev. Lett.* **1959**, *2*, 285.
(17) Bjerring, M.; Rasmussen, J. T.; Krogshave, R. S.; Nielsen, N. C. *J. Chem. Phys.* **2003**, *119* (17), 8916−8926.
(18) Hing, A. W.; Vega, S.; Schaefer, J. *J. Magn. Reson. A* **1993**, *103* (2), 151−162.
(19) Saalwachter, K.; Graf, R.; Spiess, H. W. *J. Magn. Reson.* **2001**, *148* (2), 398−418.
(20) Jaroniec, C. P.; Filip, C.; Griffin, R. G. *J. Am. Chem. Soc.* **2002**, *124* (36), 10728−10742.
(21) Jaroniec, C. P.; Tounge, B. A.; Rienstra, C. M.; Herzfeld, J.; Griffin, R. G. *J. Am. Chem. Soc.* **1999**, *121* (43), 10237−10238.
(22) Hong, M.; Gross, J. D.; Hu, W.; Griffin, R. G. *J. Magn. Reson.* **1998**, *135* (1), 169−177.
(23) Hong, M.; Gross, J. D.; Rienstra, C. M.; Griffin, R. G.; Kumashiro, K. K.; Schmidt-Rohr, K. *J. Magn. Reson.* **1997**, *129* (1), 85−92.
(24) Hong, M.; Gross, J. D.; Griffin, R. G. *J. Phys. Chem. B* **1997**, *101* (30), 5869−5874.
(25) Sack, I.; Balazs, Y. S.; Rahimipour, S.; Vega, S. *J. Am. Chem. Soc.* **2000**, *122* (49), 12263−12269.
(26) Schaefer, J.; Mckay, R. A.; Stejskal, E. O. *J. Magn. Reson.* **1979**, *34* (2), 443−447.
(27) Wu, X. L.; Zilm, K. W. *J. Magn. Reson. A* **1993**, *104* (2), 154−165.
(28) Pauli, J.; Baldus, M.; van Rossum, B.; de Groot, H.; Oschkinat, H. *ChemBioChem* **2001**, *2* (4), 272−281.
(29) Egorova-Zachernyuk, T. A.; Hollander, J.; Fraser, N.; Gast, P.; Hoff, A. J.; Cogdell, R.; de Groot, H. J. M.; Baldus, M. *J. Biomol. NMR* **2001**, *19* (3), 243−253.
(30) Morcombe, C. R.; Gaponenko, V.; Byrd, R. A.; Zilm, K. W. *J. Am. Chem. Soc.* **2004**, *126* (23), 7196−7197.
(31) Takegoshi, K.; Nakamura, S.; Terao, T. **2001**, *344* (5−6), 631−637.
(32) Takegoshi, K.; Nakamura, S.; Terao, T. *J. Chem. Phys.* **2003**, *118* (5), 2325−2341.
(33) Veshtort, M.; Griffin, R. G. *J. Magn. Reson.* **2006**, *178* (2), 248−82.
(34) Ishii, Y.; Ashida, J.; Terao, T. *Chem. Phys. Lett.* **1995**, *246* (4−5), 439−445.
(35) Hartmann, S. R.; Hahn, E. L. *Phys. Rev.* **1962**, *128* (5), 2042−2053.
(36) Stejskal, E. O.; Schaefer, J.; Waugh, J. S. *J. Magn. Reson.* **1977**, *28* (1), 105−112.
(37) Brinkmann, A.; Levitt, M. H. *J. Chem. Phys.* **2001**, *115* (1), 357−384.

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