chemistry. We hope that the combination of experimental isotope effects with the appropriate theoretical analysis will prove to be as revealing about the mechanisms of gas-phase ion–molecule reactions as they have about reactions in solution.

Assignment of Complex ¹H NMR Spectra via Two-Dimensional Homonuclear Hartmann–Hahn Spectroscopy

Donald G. Davis and Ad Bax*

Laboratory of Chemical Physics
National Institute of Arthritis, Diabetes
and Digestive and Kidney Diseases
National Institutes of Health
*Bethesda, Maryland 20205*

We propose the use of a new type of two-dimensional NMR experiment for the determination of homonuclear scalar connectivity in complex molecules. The method relies on the principle of cross polarization, first introduced by Hartmann and Hahn[1] and commonly used for sensitivity enhancement in solid-state ¹³C NMR.[2] In our experiment homonuclear cross polarization is obtained by switching on a single coherent rf field. In cases where the effective rf field strengths experienced by two scalar coupled protons are identical, a perfect Hartmann–Hahn match is established and gives rise to oscillatory exchange (with period $1/J$) of spin-locked magnetization. For a simple two-spin AX system complete exchange of magnetization is obtained for a spin-lock time equal to $1/(2J_{AX})$. For larger spin systems the time dependence of the magnetization exchange follows a more complicated pattern (to be published). Two pulse schemes relying on homonuclear cross polarization are sketched in Figure 1. In Figure 1a a coherent rf field of constant phase is switched on for a mixing time of duration τ_m. The projection of the vector sum of all magnetization components of the multiplet of an arbitrary spin, A, will be spin locked along the rf field, whereas most of the magnetization perpendicular to the spin-lock field will dephase rapidly due to spatial inhomogeneity of the rf field. For a proton, A, with offset Δ_A from the carrier frequency, the effective rf field strength, ν_A, is to a good approximation given by

$$\nu_A = \nu + \Delta_A^2/2\nu \qquad (1)$$

provided that the nominal rf field strength $\nu \gg \Delta_A$. Analogous to expressions derived by Müller and Ernst[3] and Chingas et al.[4] for the heteronuclear case, it can be shown that effective magnetization transfer between protons A and X is possible if

$$|\Delta_A^2 - \Delta_X^2|/2\nu < |J_{AX}| \qquad (2)$$

at a rate that depends on J_{AX} and on $|\nu_A - \nu_X|$.[3,4] From eq 1 it follows that large rf field strengths, ν, are generally required for effective transfer. Alternatively, this condition can be fruitfully used to restrict magnetization transfer to distinct regions of the spectrum. For example, magnetization transfer between amide and C_α protons in peptides can be obtained by positioning the
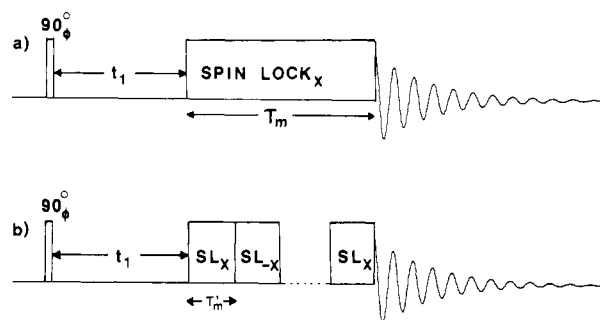
Figure 1. Pulse schemes for 2D homonuclear cross polarization (a) without compensation for different effective field strengths and (b) with compensation. The phase, ϕ, of the 90° ¹H pulse is cycled along all four axes, and data for odd- and even-numbered scans are stored in separate locations to allow a hypercomplex 2D Fourier transformation.[3]

carrier frequency midway between the amide and α spectral regions. Restrictions imposed by eq 2 on the bandwidth that can be covered by the new method can be alleviated by alternating the phase of the applied rf field along the $\pm x$ axis at a rate $1/\tau_m'$. To prevent rotary echo effects,[5] the total durations that the rf field is applied along the x and $-x$ axis are made different (Figure 1b). It can be shown that effective magnetization transfer, at a rate determined by J_{AX}, is now possible under the condition

$$\tau_m'|\Delta_A^2 - \Delta_X^2|/2\nu < 0.25 \qquad (3)$$

A major advantage of homonuclear cross polarization is that *net* magnetization transfer occurs and a phase sensitive 2D spectrum with all peaks in the near absorption mode can be obtained. This is in contrast with the COSY experiment[6-9] where diagonal and cross peaks are 90° out of phase and individual cross multiplet components are 180° out of phase relative to one another, causing partial signal cancellation and sensitivity loss. In our new experiment there is also some dispersive character to the individual diagonal and cross multiplets.[3,4,10] However, their antiphase nature causes significant mutual cancellation while the absorptive components remain, and therefore the spectrum appears to be in the absorption mode. Another consequence of the net magnetization transfer is the introduction of relayed connectivity; magnetization that is transferred from proton A to proton M during the first half of the mixing period will be relayed in part to proton X during the second half of the mixing period, provided that J_{AM} and J_{MX} are not zero. For short mixing periods (<20 ms) little relay will be observed, but for longer mixing times single-relay and multiple-relay effects become significant.

As an example, the method is demonstrated for a sample of 1 mg of alamethicin, dissolved in 0.4 mL of methanol-d_4. Alamethicin is an icosapeptide with the sequence Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol. The alamethicin was kindly provided by Dr. G. B. Whitfield of the Upjohn Co. and purified as described by Gisin et al.[11] Experiments were carried out on a Nicolet 500-MHz spectrometer, using a mixing time of 7 × 5 ms, i.e., four periods of 5 ms spin locked along the x axis interleaved with three periods along the $-x$ axis. 5-W rf power was used to generate a 10-kHz rf field. Figure 2a shows the regular 1D spectrum of the aliphatic region and Figure 2b shows the 2D spectrum obtained with the new method, not treated by symmetrization[12] or other white-wash procedures. Connectivities observed in this spectrum basically

(1) Hartmann, S. R.; Hahn, E. L. *Phys. Rev.* **1962**, *128*, 2042.
(2) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1973**, *59*, 569.
(3) Müller, L.; Ernst, R. R. *Mol. Phys.* **1980**, *38*, 963.
(4) Chingas, G. C.; Garroway, A. N.; Bertrand, R. D.; Moniz, W. B. *J. Chem. Phys.* **1981**, *74*, 127.

(5) Solomon, I. *Phys. Rev. Lett.* **1959**, *2*, 301.
(6) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
(7) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
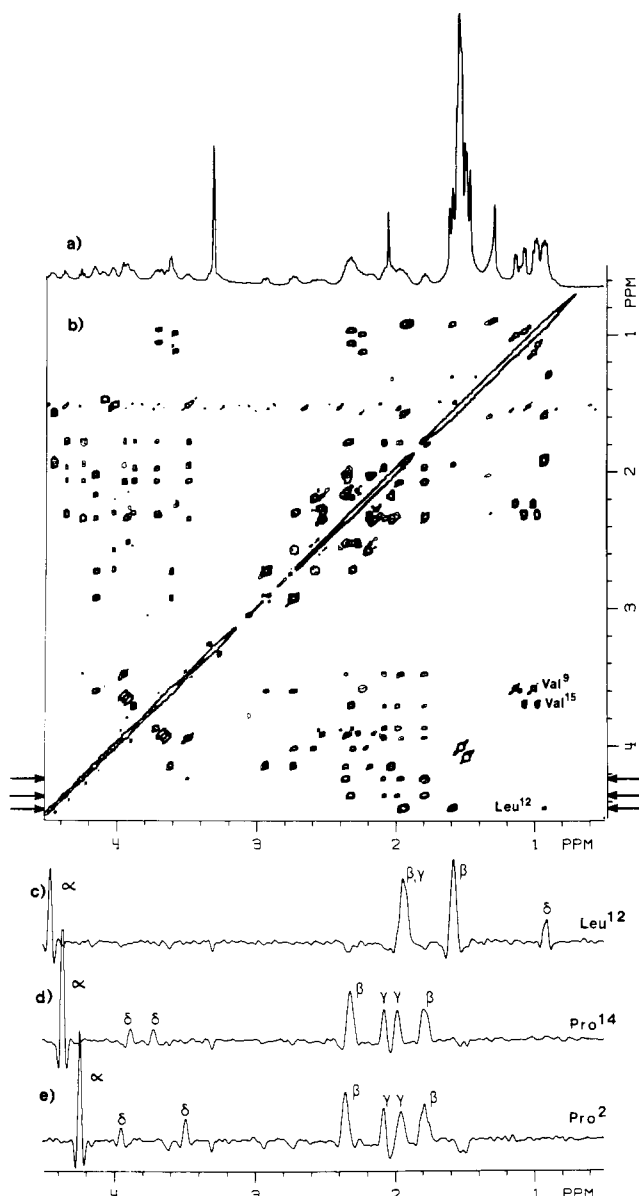(8) Nagayama, K.; Kumar, A.; Wüthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321.
(9) Marion, D.; Würthrich, K. *Biochem. Biophys. Res. Commun.* **1984**, *56*, 207.
(10) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521.
(11) Gisin, B. F.; Davis, D. G.; Borowska, Z. K.; Hall, J. E.; Kobayashi, S. *J. Am. Chem. Soc.* **1981**, *103*, 6373.

is also closely related to the TOCSY experiment[10] and to experiments concerning the disappearance of *J* modulation in the presence of very rapid pulsing.[15–17]

Clearly, the method described in this paper is extremely powerful for the analysis of complicated coupling networks. Preliminary experiments on a number of proteins have shown that the method is particularly suitable for determination of connectivity in poorly resolved systems. As will be demonstrated elsewhere, effective suppression of the water resonance is also feasible with this technique. The experimental scheme of our new technique shows a close resemblance to an experiment proposed recently for the measurement of transverse NOE.[18] However, for the short mixing times used in the new experiment cross peaks due to NOE are often very weak and will also have opposite sign relative to diagonal and Hartmann–Hahn cross peaks.[19]

(15) Wells, E. J.; Gutowsky, H. S. *J. Chem. Phys.* **1965**, *43*, 3414.
(16) Allerhand, A.; Gutowsky, H. S. *J. Chem. Phys.* **1965**, *42*, 4203.
(17) Allerhand, A. *J. Chem. Phys.* **1966**, *44*, 1.
(18) Bothner-By, A. A.; Stephens, R. L.; Lee, J. T.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.
(19) Bax, A.; Davis, D. G., *J. Magn. Reson.*, in press.

Figure 2. Spectra of alamethicin in methanol-d_4: (a) one-dimensional spectrum, (b) two-dimensional absorption mode homonuclear cross-polarization spectrum, and (c)–(e) cross section taken parallel to the F_1 axis at the $H(C_\alpha)$ chemical shifts of Leu 12, Pro 14, and Pro 2, respectively. These positions are indicated by arrows in the 2D spectrum. A complete assignment of the spectral region shown is available in a supplementary table.

agree with spectral assignments made by Davis and Gisin[13] and Banerjee et al.[14] A large number of relay connectivities are observed for this mixing time; for example, the $H(C_\alpha)$–Me connectivities for Val 9, Val 15, and Leu 12 are readily identified. Cross sections parallel to the F_1 axis, taken at the $H(C_\alpha)$ chemical shifts of Pro 14 and Pro 2 (Figure 2d,e) clearly show the connectivity to the C_β protons and relay to the C_γ and C_δ protons. A similar section taken at $H(C_\alpha)$ of Leu 12 (Figure 2c) shows the connectivity to the inequivalent C_β protons at 1.95 and (previously misassigned) 1.58 ppm and relay to the C_γ (1.92 ppm) and C_δ protons (0.91 and 0.94 ppm). Some other new assignments are also found and are available in a supplementary table.

The experiment described in this paper relies on the same principles as heteronuclear cross-polarization experiments[1–4] and

(12) Baumann, R.; Wider, G.; Ernst, R. R.; Wuthrich, K. *J. Magn. Reson.* **1981**, *44*, 402.
(13) Davis, D. G.; Gisin, B. F. *Febs Lett.* **1981**, *133*, 247.
(14) Banerjee, U.; Tsui, F. P.; Balasubrumanian, T. N.; Marshall, G. R.; Chan, S. I. *J. Mol. Biol.* **1983**, *165*, 757.

Transition Structures for Additions of LiH and MeLi to Ethylene and Acetylene

K. N. Houk,*[1a] Nelson G. Rondan,[1a]
Paul von Rague Schleyer,*[1b] E. Kaufmann,[1b] and
Timothy Clark[1b]

*Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15260
Institut für Organische Chemie der
Friedrich-Alexander-Universität Erlangen-Nurnberg
D-8520 Erlangen, Federal Republic of Germany*

Received January 15, 1985

The additions of alkyllithium compounds to double and triple bonds and the reverse processes, such as β-hydride eliminations from alkyllithiums to give alkenes, are synthetically useful reactions.[2] Such additions to cyclic olefins proceed with syn stereochemistry.[3] Eliminations can be catalyzed by hydride acceptors,[4] and have therefore been proposed to be stepwise reactions involving intermediate β-lithiocarbenium ions.[5] However, concerted mechanisms for LiH eliminations from alkyllithiums are indicated by recent experiments.[6] This prompted us to extend our work on LiH and CH₃Li additions to carbonyl compounds[7] to the corresponding reactions involving carbon-carbon multiple bonds.

The geometries of the species shown in Figure 1 were optimized with the 3-21G basis set[8] using the GAUSSIAN 80 and 82 series of

(1) (a) University of Pittsburgh. (b) Universität Erlangen-Nurnberg.
(2) Wakefield, B. J. "The Chemistry of Organolithium Compounds"; Pergamon Press: Oxford, England, 1974; pp 89–108, 204–211.
(3) Welch, J. G.; Magid, R. M. *J. Am. Chem. Soc.* **1967**, *89*, 5300. Bach, R. D.; Bair, K. W.; Willis, C. L. *J. Organomet. Chem.* **1974**, *77*, 31.
(4) Reetz, M. T.; Stephan, W. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 44.
(5) Reetz, M. T.; Stephan, W. *Tetrahedron Lett.* **1977**, *31*, 2693.
(6) Li, M.-Y.; San Filippo, J., Jr. *Organometallics* **1983**, *2*, 554.
(7) Schleyer, P. v. R.; Kaufman, E.; Clark, T.; Wu, Y.-D.; Houk, K. N. *J. Am. Chem. Soc.*, submitted for publication.