Heteronuclear 2D-NOE Spectroscopy

Peter L. Rinaldi

Department of Chemistry, Case Western Reserve University
*Cleveland, Ohio 44106*

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The nuclear Overhauser effect (NOE) has been a useful NMR parameter for chemical structure elucidation.[1] Observation of a resonance while simultaneously decoupling at the resonance frequency of a second nucleus produces a perturbation of the observed signal's intensity, if the decoupled nucleus contributes to the dipolar relaxation of the observed nucleus. Because of the inverse sixth power dependence of the dipolar interaction on internuclear distance, the NOE is very selective under the right circumstances.

Several factors complicate the use of NOE's for structural studies. If the decoupled nucleus resonance is in a congested region of the NMR spectrum, selective irradiation of a single resonance becomes difficult or impossible. Similarly, if the resonance of the observed nucleus is in a congested region of the NMR spectrum, slight changes in its intensity might be difficult to detect. If complex structures are studied, it is very time consuming to selectively irradiate each resonance of an unknown compound and identify the perturbed resonances. Two-dimensional Fourier transform NMR (2D-FTNMR) techniques have been extremely useful for simplification of complex spectra.[2] 2D-FTNMR techniques have also been used to eliminate all these complicating factors in the homonuclear NOE experiment.[3]

Observation of heteronuclear NOE's while selectively irradiating protons can yield important structural information as well.[4] However, these experiments are limited by the same factors that complicate the one-dimensional homonuclear NOE experiment. A new 2D-FTNMR pulse sequence is described here that allows the correlation between the chemical shifts of a decoupled (*I*) nucleus such as ^1H or ^{31}P and an observed nucleus (*S*) such as ^{13}C or a metal ion. The correlation is observed if the two nuclei are close in space so that the *I* nuclei contribute to the dipolar relaxation of the *S* nucleus.

The pulse sequence for observation of this spectrum is shown in Scheme I. A vector model that describes the *I* spin magnetization in the rotating frame is shown in Scheme II. The usual equilibration delay (D_1) establishes the equilibrium magnetization of the I and S spins. A 90°(*I*) pulse flips the *I* spins into the *xy* plane where their components, *a* and *b*, precess during t_1 according to the resonance frequencies of the chemically distinct nuclei. If there is coupling between the *I* and *S* spins, this is removed by a 180°(*S*) pulse at $t_1/2$. At the end of the t_1 period, the *I* spins are frequency labeled.

A 90°(*I*) pulse converts a fraction of this transverse component into *z* magnetization. The magnitude of this *z* component will depend upon the *I* spin's precession frequency (chemical shift) and the delay t_1. Cross relaxation between the *I* and *S* spins in thermal contact occurs during the mixing period (mix). A simultaneous field gradient pulse (FG) destroys any residual transverse magnetization.[5] At the end of this period, a 90°(*S*)
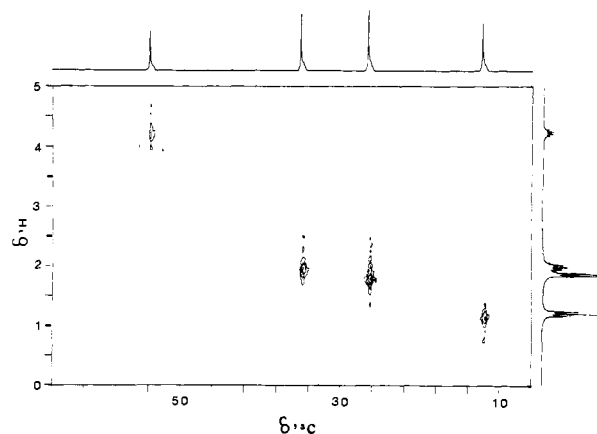

Figure 1. Contour plot of the $^{13}C(^1H)$ heteronuclear 2D-NOE spectrum of neat 2-bromobutane. Spectral acquisition parameters were 15- and 40-μs 90° pulses for ^{13}C and ^1H, respectively, 0.085-s acquisition time, 3000-Hz sweep width in the ^{13}C frequency dimension, and a 2.0-s mixing time; 128 spectra (8 transients each) were accumulated with t_1 incremented to provide the equivalent of a 1000-Hz sweep width in the ^1H frequency dimensions. After Fourier transformation with respect to t_2, the 128 points in the second time domain were zero filled to 512 before Fourier transformation with respect to t_1. The 2D-NMR spectrum is displayed in the absolute value mode to avoid phasing problems. The normal one-dimensional ^{13}C and ^1H NMR spectra are also shown along their respective chemical shift axes for comparison. The 4-Hz data point resolution in the ^1H frequency dimension of the 2D spectrum is not sufficient to allow resolution of the proton multiplicities.

Scheme I
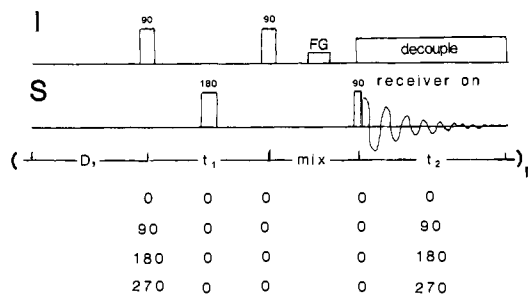
Scheme II
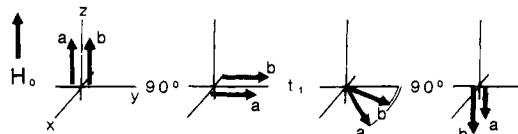
pulse creates a detectable transverse magnetization component for the *S* spins that is modulated according to the amount of cross relaxation that occurred during the mixing time (which in turn is dependent on the *I* spin's chemical shift and the length of t_1). The phases of the pulses and the receiver are cycled as shown in Scheme I to provide the equivalent of quadrature phase detection in the second dimension. In this way, the decoupler can be set in the middle of the ^1H spectral range, conserving computer memory. This phase cycling also eliminates large unmodulated signal components that would ordinarily appear along the *S* spin's chemical shift axis at the frequencies of each of the S resonances.

The above sequence is repeated for *N* values of the t_1 delay, each averaged for *n* transients, to provide a final spectrum that is a function of t_1 and t_2 (the acquisition time). Fourier transformation with respect to t_1 and t_2 provides a 2D-FTNMR spectrum with axes defined by the chemical shifts of the *I* and *S* spins. The $^{13}C(^1H)$ heteronuclear 2D-NOE spectrum of 2-bromobutane (Figure 1) serves as a useful example. Since the ^{13}C nuclei in small molecules are primarily relaxed by dipolar interactions with directly-bound protons, cross peaks should only be observed at the intersections of ^1H and ^{13}C chemical shifts if the nuclei are attached. Thus peaks are observed at the inter-

(1) (a) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971. (b) Wuthrich, K. "NMR in Biological Research: Peptides and Proteins"; Elsevier: New York, 1976; pp 221–286. (c) Bothner-By, A. A. In "Biochemical Applications of Magnetic Resonance"; R. G. Shulman, Ed.; Academic Press: New York, 1979.

(2) Bax, A., "Two-Dimensional Nuclear Magnetic Resonance in Liquids"; Reidel; Boston, MA, 1982.

(3) Jeener, J.; Meier, B. H.; Bachmann, R.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.

(4) (a) Gerig, J. T. *J. Am. Chem. Soc.* **1980**, *102*, 7308. (b) Hart, P. A. *Biophys. J.* **1978**, *24*, 833.

(5) The field gradient pulse serves to eliminate spurious signals from coherence transfer higher than zero order. However, the presence of zero order coherence transfer can still create artifacts that complicate the interpretation of the 2D spectrum.[6]

(6) Macura, S.; Wuthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1982**, *46*, 269.

sections of the chemical shifts of C_1/H_1, C_2/H_2, C_3/H_3, and C_4/H_4.

The above application only serves as a clear illustrative example. More efficient 2D-FTNMR methods are available for correlating the chemical shifts of directly bound nuclei via *J*-modulated polarization transfer.[7] The sequence described here is most useful when *J* coupling between the *I* and *S* spins is not present or is not resolved. Such a case might arise for a metal ion with nuclear spin $I = {}^1/_2$ which is bound in a complex molecule. Protons in the vicinity of the metal ion binding site would contribute to its dipolar relaxation, and the heteronuclear 2D-NOE sequence could provide an indication of the types of protons that make up the structure of the metal ion binding site. Preliminary ${}^{113}Cd({}^1H)$

2D-NOE studies of metalloorganic complexes indicate this is feasible.

This new heteronuclear 2D-NOE NMR experiment should be useful for identifying the structures surrounding the metal ion binding sites in complexes with organic and biological ligands. In combination with other 2D-NMR NOE experiments,[3] it could provide the complete solution structure of metal ion complexes. ${}^{15}N({}^1H)$ heteronuclear 2D-NOE experiments might provide information on the protons involved in exchange in compounds such as peptides.

(7) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.

Additions and Corrections

Low-Valent Molybdenum Porphyrin Derivatives: Synthesis and Structure of a π-Bonded Diphenylacetylene Adduct of Molybdenum(II) (*meso*-Tetra-*p*-tolylporphyrin) [*J. Am. Chem. Soc.* **1981**, *103*, 1850]. ANDRE DE CIAN, JOCELYNE COLIN, MICHEL SCHAPPACHER, LOUIS RICARD, and RAYMOND WEISS*

Reference 5: The correct space group for |MoTTP(PhC≡CPh)·C₆H₅CH₃| should read $P2_1/n$.

Fast Hydrolysis of Alkyl Radicals with Leaving Groups in the β Position [*J. Am. Chem. Soc.* **1982**, *104*, 7311–7312]. G. KOLTZENBURG, G. BEHRENS, and D. SCHULTE-FROHLINDE*

Page 7311: The missing rate constant in the sixth entry of Table I is 10^4.

Page 7312: Formula VII in Chart I should read:

Page 7312, thirteenth line after eq 4 should read as follows:
...on substitution of H by CH_3...