

---

Conservation of Helium-3 Polarization in Magnetic Structures	Callot, V., Crémillieux, Y., Graveron-Demilly, D., and Briguet, A.	2
TROSY Applied to Nucleic Acids	Fiala, R., and Sklenář, V.	5
The Expected and the Not-So-Expected in Deuterium CP Spectra	Madhu, P. K., and Vega, S.	7
Checking pH Without an Electrode	Sykes, B. D., Saltibus, L. F., and Spyropoulos, L.	11
Positions Available	Varian NMR	12
A Spectrometer for Every Grill!	Walton, J. H., and McCarthy, M. J.	13
Measurement of the Average Lifetime of Anthracyclines in the Intercalation Sites and Structure in Solution of the Complexes with d(CGATCG) <sub>2</sub> and d(CGTACG) <sub>2</sub>	Ragg, E., Mazzini, S., and Mondelli, R.	17
<sup>19</sup> F and Isotope Shifts	Silber, S. K.	20
The Hydrogen Jukebox and Nitrogen Nickelodeon	Martin, G. E., and Hadden, C. E.	23
Position Available	Doty, D.	27
Position Available	Fukushima, E.	28
Positions Available	Malloy, C., and Sherry, A. D.	28
Positions Available	Graveron-Demilly, D., and van Ormondt, D.	30
HRMAS of Bound Peptides in DMF	Bourdonneau, M., Brevard, C., and Piotto, M.	31
Continuing MRD of Solutes	Dinesen, T. R. J., Teng, C.-L., Wagner, S., and Bryant, R. G.	33
<sup>1</sup> H- <sup>2</sup> H Dipolar NMR Study of the Water Molecule in Crystalline Hydrates	Wu, G., and Freure, C. J.	37
Probing Extracellular and Intracellular Diffusion-Diffraction Effects in Erythrocyte Suspensions	Torres, A. M., Chapman, R. J., Taurins, A., and Kuchel, P. W.	39
Book Review ("Protein NMR Techniques" - Methods in Molecular Biol., Vol. 60)	Schweitzer, B.	41

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THE NMR NEWSLETTER	NO. 479, AUGUST 1998	AUTHOR INDEX
Bourdonneau, M. . . . . 31	Freure, C. J. . . . . 37	Mondelli, R. . . . . 17
Brevard, C. . . . . 31	Fukushima, E. . . . . 28	van Ormondt, D. . . . . 30
Briguet, A. . . . . 2	Grav'n.-Dem'y, D. . . . . 2, 30	Piotto, M. . . . . 31
Bryant, R. G. . . . . 33	Hadden, C. E. . . . . 23	Ragg, E. . . . . 17
Calot, V. . . . . 2	Kuchel, P. W. . . . . 39	Saltibus, L. F. . . . . 11
Chapman, R. J. . . . . 39	Madhu, P. K. . . . . 7	Schweitzer, B. . . . . 41
Crémillieux, Y. . . . . 2	Malloy, C. . . . . 28	Sherry, A. D. . . . . 28
Dinesen, T. R. J. . . . . 33	Martin, G. E. . . . . 23	Silber, S. K. . . . . 20
Doty, D. . . . . 27	Mazzini, S. . . . . 17	Sklenář, V. . . . . 5
Fiala, R. . . . . 5	McCarthy, M. J. . . . . 13	Spyracopoulos, L. . . . . 11
Sykes, B. D. . . . . 11		Taurins, A. . . . . 39
Teng, C.-L. . . . . 33		Torres, A. M. . . . . 29
Varian NMR . . . . . 12		Vega, S. . . . . 7
Wagner, S. . . . . 33		Walton, J. H. . . . . 13
Wu, G. . . . . 37		

THE NMR NEWSLETTER	NO. 479, AUGUST 1998	ADVERTISER INDEX
Advanced Chemistry Development, Inc. . . . . 3		Oxford Instruments, Ltd. . . . . 15
AMT . . . . . 35		Programmed Test Sources, Inc. . . . . inside front cover
Bruker Instruments, Inc. . . . . 9		Varian NMR Instruments . . . . . 21
JEOL . . . . . outside back cover		Voltronics Corporation. . . . . 29

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### FORTHCOMING NMR MEETINGS

**XVIIIth International Conference on Magnetic Resonance in Biological Systems**, Tokyo Metropolitan University, **August 23 - 28, 1998**. Contact: Professor M. Kainosho, Department of Chemistry, Tokyo Metropolitan University; +81-426-77-2544; Fax: +81-426-77-2525; e-mail: [kainosho@raphael.chem.metro-u.ac.jp](mailto:kainosho@raphael.chem.metro-u.ac.jp); <http://icmrbs98.chem.metro-u.ac.jp>

**NMR<sup>2</sup>-New Mexico Regional NMR**, Advanced Materials Laboratory, Sandia National Laboratories, Albuquerque, NM, **October 3, 1998**. Contact: T. M. Alam, Sandia National Laboratories, MS 1407, Albuquerque, NM 87008-1407; [tmalam@sandia.gov](mailto:tmalam@sandia.gov); 505-844-1225.

**NMR Technologies: Development and Applications for Drug Design and Characterizations**, Baltimore, MD, **October 29-30, 1998**; Contact: J. Laakso, Cambridge Healthtech Institute, 1037 Chestnut St. Newton Upper Falls, MA 02164; 617-630-1300; Fax: 617-630-1325; [chi@healthtech.com](mailto:chi@healthtech.com); <http://www.healthtech.com/conferences/>.

**NMR of Polymers and Biopolymers**, Symposium at the 54th SouthWest Regional ACS Meeting, Baton Rouge, LA, **November 1-2, 1998**. For Symposium schedule: [members.aol.com/ACKolbert/symposium.html](http://members.aol.com/ACKolbert/symposium.html); Contact: A. C. Kolbert [mailto:ackolbert@aol.com](mailto:mailto:ackolbert@aol.com) or Xiaolian Gao, [xgao@uh.edu](mailto:xgao@uh.edu)

**NMR Spectroscopy of Polymers**, Breckenridge, Colorado, **January 24-27, 1999**; an International Symposium Sponsored by the Division of Polymer Chemistry, American Chemical Society; Organizers: P. T. Inglefield and A. D. English; Registration contact: Neta L. Byerly, Division of Polymer Chemistry, Inc., Virginia Tech, 201 Hancock Hall, M.C. 0257, Blacksburg, VA 24061; 540-231-3029; Fax: 540-231-9452; email: [nbyerly@vt.edu](mailto:nbyerly@vt.edu).

**40th ENC (Experimental NMR Conference)**, Clarion Plaza Hotel, Orlando, Florida, **February 28 - March 5, 1999**, immediately preceding Pittcon in Orlando; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073; Email: [enc@enc-conference.org](mailto:enc@enc-conference.org).

*continued on inside back cover*



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(received 7/13/98)

Villeurbanne July 7, 1998

### Conservation of Helium3 polarization in magnetic structures

Dear Dr. Shapiro,

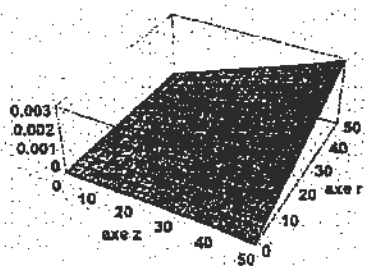
The spectroscopy and imaging of the noble gases ( $^3\text{He}$  and  $^{129}\text{Xe}$ ) represent a rapidly growing field in biomedical NMR. This is due to the possibility of increasing by 5 orders of magnitude the polarization of these gases by optical pumping but also to the very long  $T_1$  longitudinal relaxation times of these gases. Although, relaxation times of tens of minutes can be easily obtained when working with  $^3\text{He}$ , one may wish to extend the  $T_1$  to much larger values (for example if the polarization system and the NMR facilities are several hours' drive away). In the case of  $^3\text{He}$ ,  $T_1$  relaxation effects can be separated in several components :

- dipole-dipole coupling with helium nuclear spins or with electronic spins (mainly molecular oxygen),
- interaction with the cell walls,
- relaxation due to a magnetic field gradient.

He-He nuclear spin coupling corresponds to a  $T_1$  in the order of 800 hours at atmospheric pressure. Molecular oxygen effects can be efficiently eliminated with evacuation of the cell.  $T_{1\text{wall}}$  can be increased by special design of cell and values up to 100 hours have been reported in literature. We have focused here on the effects of the magnetic field gradients on relaxation. These effects can be summarized in the following equation at room temperature and

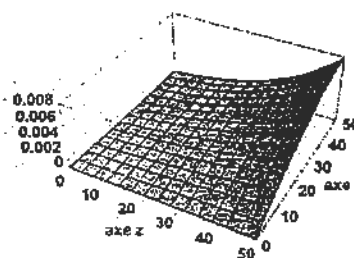
pressure in the one bar range :  $1/T_1 = (\alpha/P) \cdot (|\vec{\nabla} B_x|^2 + |\vec{\nabla} B_y|^2)/B_0^2$

where  $\alpha = 1.7 \times 10^4 \text{ cm}^2 \text{ bar h}^{-1}$ ,  $P$  is the pressure in bar,  $B_{x,y}$  transverse magnetic field components and  $B_0$  static field amplitude along the z axis. One may notice that only radial components induce  $T_1$  relaxation effects. The goal is thus to build systems with as homogeneous as possible transverse field components. For this purpose, we used the software "Magnetic@" developed by Pr. Donadieu and Pr. Saint-Jalmes. This software provides the magnetic field maps given the current distribution of the designed structures. We have evaluated two standard structures, namely a solenoid and Helmholtz coils. The stray field of our 2 Tesla magnet has been also simulated. Radial field maps for solenoid and Helmholtz coils are displayed below.



**Solenoid (5 Gauss)**

(1 meter-long, 20.2 cm average diameter, 500 turns)



**Helmholtz coils (736 Gauss)**

(74.34 cm average diameter, 100 turns)

Transverse gradients values have been calculated for each individual pixel covering the helium cell volume (10 cm diameter). From the preceding equation, values of  $T_{1\text{gradients}}$  have been estimated to be 10029 hours for the solenoid, 4035 hours for the Helmholtz coils and to 44 hours for the "real-world" Helmholtz coils + stray field. The latter values have been supported by NMR measurements on hyperpolarized helium ( $T_{1\text{gradients}}$  equal to 50 hours).

Yours sincerely,

Virginie Gallot,

Yannick Crémillieux,

Danielle Graveron-Demilly,

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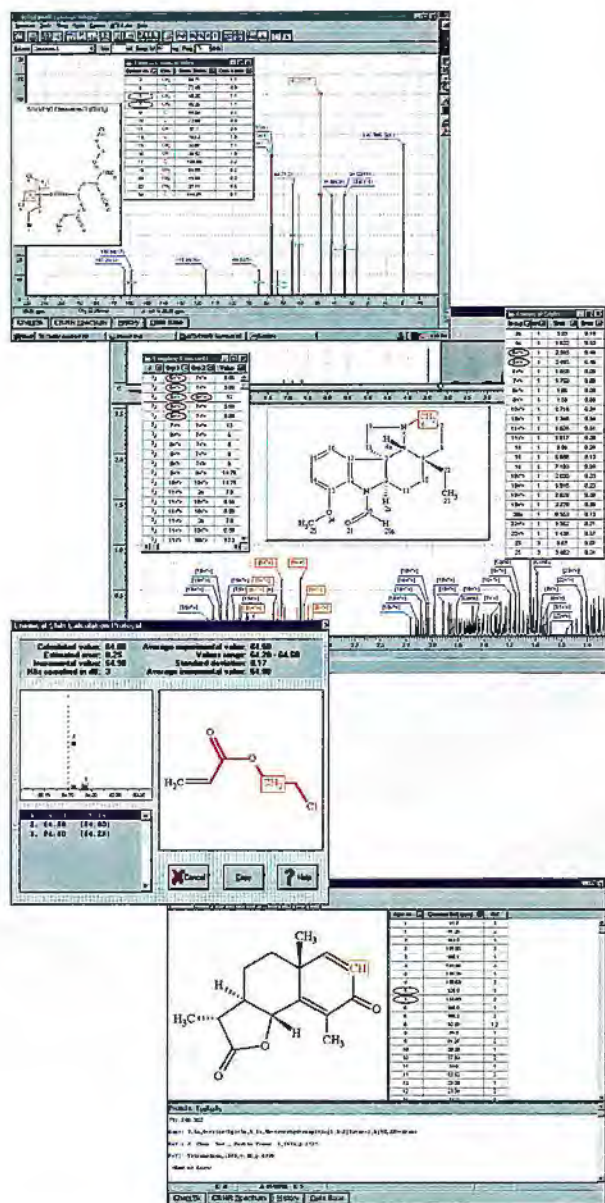
ACD/CNMR and ACD/HNMR predictors calculate accurate  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra for almost any drawn organic structure—in most cases to  $\pm 3$  ppm or better for  $^{13}\text{C}$  and to  $\pm 0.3$  ppm or better for  $^1\text{H}$ .

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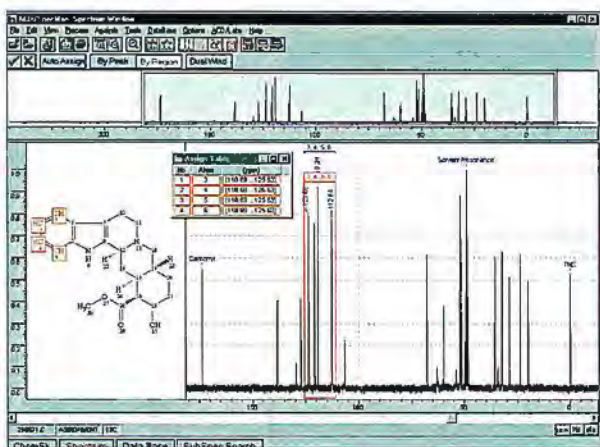
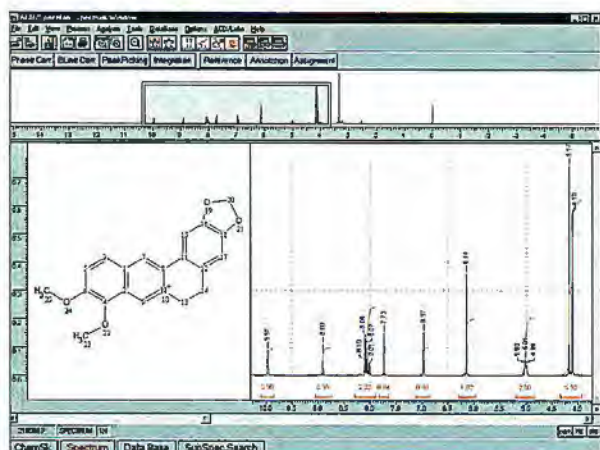
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Prof B. L. Shapiro  
 The NMR newsletter  
 966 Elsinore Court  
 Palo Alta, CA 94303  
 USA

(received 7/16/98)

## TROSY Applied to Nucleic Acids

Dear Barry:

Sensitivity and line width are the main factors that limit the size of biomolecules that can be studied by NMR spectroscopy in solution. Both line broadening and sensitivity loss are a direct consequence of fast transverse relaxation. The principal mechanisms of  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  relaxation are dipole-dipole coupling (DD) and chemical shift anisotropy (CSA) modulated by rotational correlation motions. Recently, transverse relaxation-optimized spectroscopy (TROSY) was introduced<sup>1,2</sup> which suppresses the transverse relaxation in multidimensional experiments based on constructive use of interference between DD coupling and CSA.

We have tested the efficiency of the TROSY approach for  $^1\text{H}$ - $^{13}\text{C}$  correlation in larger oligonucleotides at 500 MHz using a fully  $^{13}\text{C}$ - and  $^{15}\text{N}$ -labeled ATP binding aptamer containing 40 nucleotides<sup>3,4</sup>. Based on the ratio of  $T_1$  and  $T_2$  measured on imino nitrogens at 10°C we estimate the rotation correlation time of the molecule to  $\tau_c \approx 15\text{ns}$ .

The actual measurement was performed at 25°C and, to simulate the effect on even larger molecules, at 5°C. The sensitivity was compared to that of constant time HSQC experiment<sup>5-7</sup> with constant time periods of 30ms for base and 25ms for sugar resonances. Table 1 shows average relative intensities (TROSY/HSQC) at various sites in the molecule.

Site	Relative sensitivity at 25°C	Relative sensitivity at 5°C
C6/8	1.5	2.3
C2 (Adenine)	1.9	2.9
C1'	1.0	1.3

We observed a significant sensitivity improvement in the base region while the sensitivity for correlations in the sugar region is similar to that of the HSQC experiment. The different behavior is probably caused by more favorable orientation of the CSA tensor in the bases, larger values of its principal elements and by longer evolution periods used for the base correlations. The experiments at 5°C suggest that the TROSY is an experiment of choice for molecules even larger than the studied 40-mer.

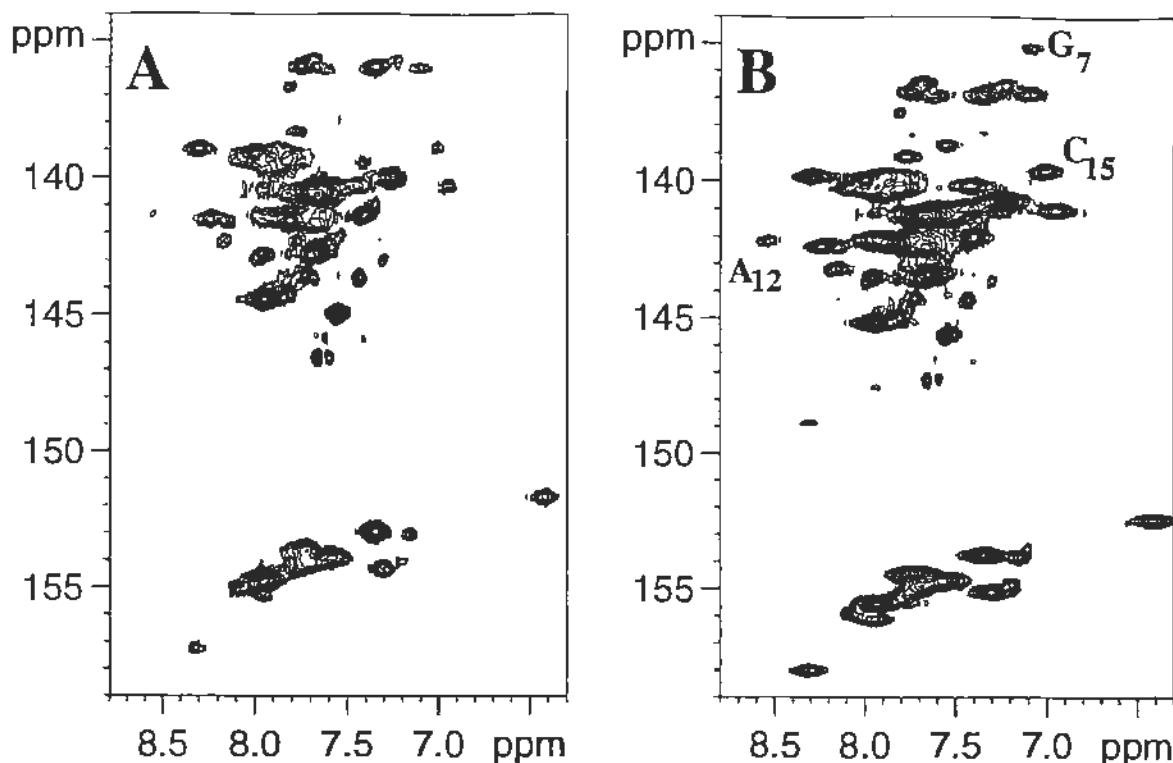


Figure 1

Base regions of  $^1\text{H}$ - $^{13}\text{C}$  correlation spectra of ATP binding RNA aptamer consisting of 40 nucleotides obtained using constant time HSQC (A) and TROSY (B) at  $5^\circ\text{C}$ . Constant time periods 30ms,  $128 \times 512$  complex matrix collected, 64 scans per increment. Note the weak or missing peaks in (A). The resonances in (B) are downfield shifted in  $^{13}\text{C}$  dimension by about 100Hz since they represent the slowly relaxing components of the doublets.

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Sincerely,

*R. Fiala*  
Rádovan Fiala

*Vladimír Sklenář*  
Vladimír Sklenář





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(received 7/22/98)

Prof. B.L.Shapiro  
The NMR Newsletter  
966 Elsinore Court  
Palo Alto, CA 94303

July 12, 1998

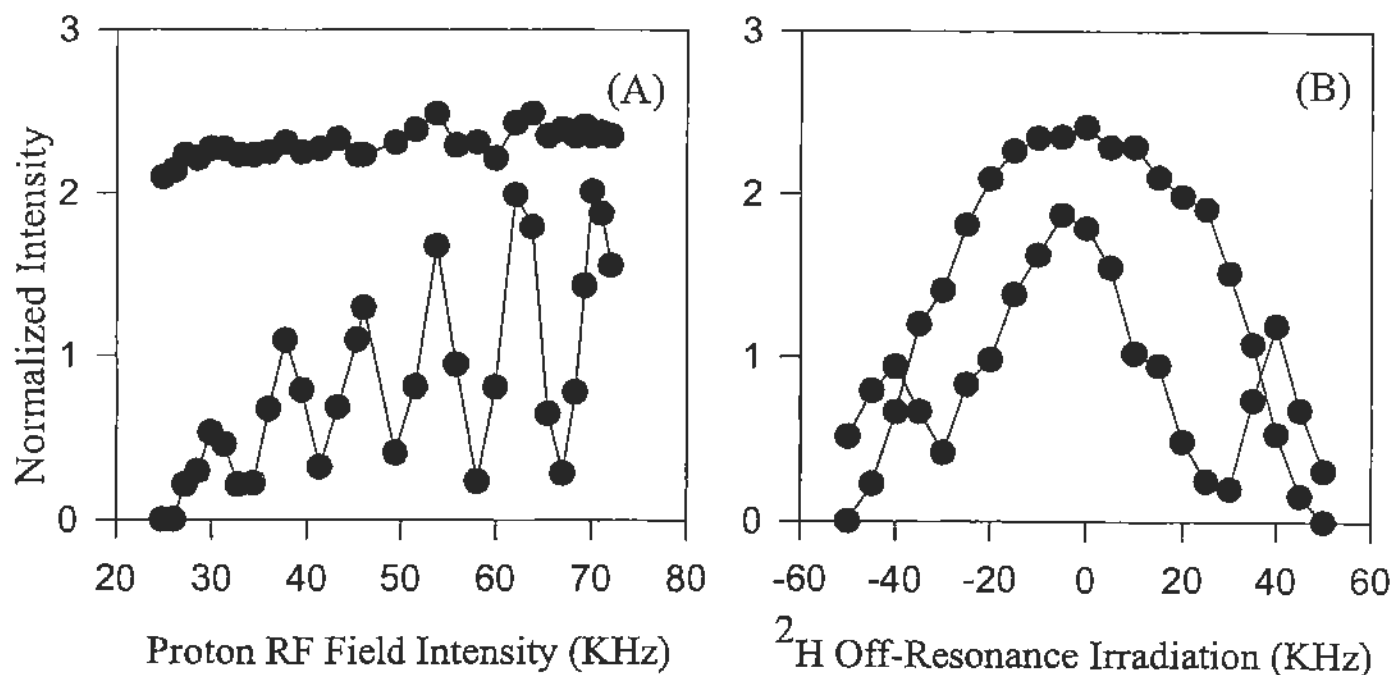
## The Expected And The Not So Expected In Deuterium CP Spectra

Dear Prof. Shapiro,

We have been investigating the cross-polarization dynamics employing Floquet theory in spin half and spin one systems. The underlying theory for spin half has been detailed by us in [1, 2]. Recently we have been trying to investigate in detail the proton-deuterium polarization transfer as a function of several experimental parameters. It is also our goal to formulate the CP dynamics in this case in the context of Floquet analysis so that rigorous predictions can be made on the experimental outcomes. This is particularly important as Deuterium NMR finds applications in the investigations of structural and dynamic properties of polymers, glasses, liquid crystals and biological macromolecules.

We have carried out all our investigations on a model system of 20% deuterated malonic acid which was a gift from Dr.Zimmerman. The experiments focussed on the performance of both CWCP and VACP. All experiments were carried out on a Bruker DSX-300 MHz spectrometer at a spinning speed of 8KHz. We thought some of our initial findings may be of interest to you.

We first show something quite well known in Fig. (A) where the normalized intensity of the centreband is plotted as a function of the proton rf power keeping the power on deuterium fixed at 70 KHz. The CWCP experiments gives the familiar "fingers" while the VACP gives the expected steady response. But we found that the CWCP spectra get severely distorted if



the exact matching conditions are not met. Obviously such distortions do not occur in VACP experiments. The response of the signal to off-resonance irradiation is also interesting as is shown in Fig. (B). We note that while on-resonance irradiation gives optimum performance for VACP, the profile for CWCP peaks at values that correspond to off-resonance irradiation at magic angle for an effective field of 70 KHz although the magnitude is smaller than the on-resonance irradiation. We also like to point out that there is a steady signal enhancement by a factor of around 2.2 for CP experiments compared to those employing only a single  $90^\circ$  pulse which justifies the need for doing CP in such systems.

We are currently in the process of understanding the CP dynamics in spin 1 system through Floquet analysis and hope to come out with the same soon.

Please credit this contribution to the account of Dr. R.Poupko.

## References

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- [2] D.Marks, N.Zumbulyadis and S.Vega, J.Magn.Reson. A 122, 16 (1996)

Sincerely yours,

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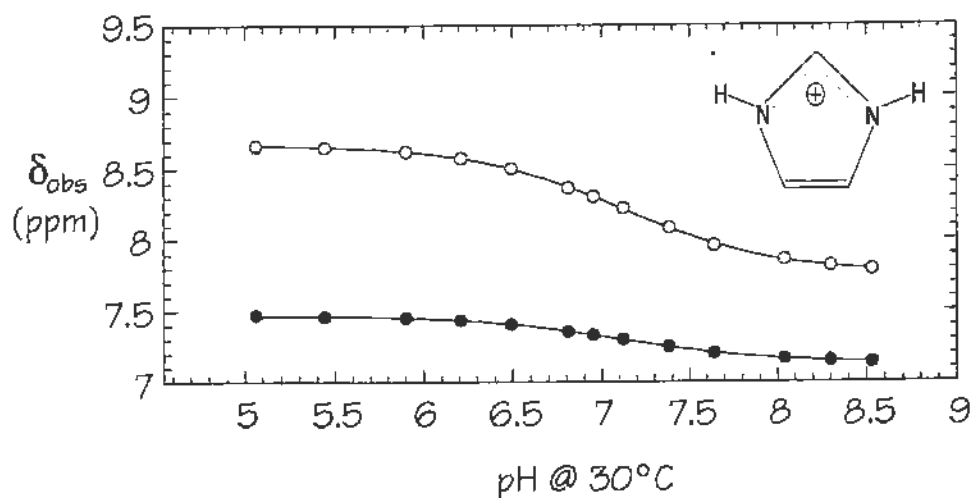
Dr. B.L. Shapiro  
The NMR Newsletter  
966 Elsinore Court  
Palo Alto, CA 94303

30 June 1998  
(received 7/3/98)

## Checking pH without an Electrode

Dear Barry:

We often use 10 mM imidazole as a buffer in our protein samples, which typically consist of 9:1 H<sub>2</sub>O/D<sub>2</sub>O, 100 mM KCl, 0.03% NaN<sub>3</sub>, and DSS as a chemical shift reference. We usually adjust to pH 6.7 with small additions of aqueous HCl and/or NaOH. One fine weekend here in northern Alberta, the pH electrode refused to cooperate with us, despite the appropriate application of expletives. This prompted us to find a way to check the pH of the sample internally. The back-exchanged aromatic protons of deuterated imidazole show two peaks in the amide region of a protein <sup>1</sup>H NMR spectrum, whose resonance frequencies are pH dependent (Figure 1).



**Figure 1:** pH dependence of the chemical shifts ( $\delta_{\text{obs}}$ ) of the aromatic protons in imidazole. The structure shown predominates at pH < 7.

The observed chemical shifts were fit to the equation:

$$\delta_{\text{obs}} = \delta_{\text{HA}} \left( \frac{[\text{H}^+]}{[\text{H}^+] + K_{\text{A}}} \right) + \delta_{\text{A}} \left( \frac{K_{\text{A}}}{[\text{H}^+] + K_{\text{A}}} \right) \quad [1]$$

where  $\delta_{\text{obs}}$  is the observed chemical shift,  $\delta_{\text{HA}}$  is the chemical shift of the conjugate acid, and  $\delta_{\text{A}}$  is the chemical shift of the conjugate base, and  $K_{\text{A}}$  is the association constant for the conjugate base. The

fits gave values of  $(7.6 \pm 0.2) \times 10^{-8}$  and  $(7.8 \pm 0.2) \times 10^{-8}$  for the  $K_A$  of the downfield and upfield aromatic protons, respectively, corresponding to  $pK_A$ s of  $7.12 \pm 0.01$  and  $7.11 \pm 0.01$ . While the  $pK_A$ s are temperature dependent, we have found that the  $pK_A$  of the downfield peak is essentially constant over the temperature range 25-30 °C. Equation 1 can be rewritten as follows:

$$pH = pK_A - \log \left[ \frac{\delta_{\text{obs}} - \delta_A}{\delta_{\text{HA}} - \delta_{\text{obs}}} \right] \quad [2]$$

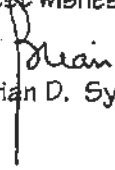
We use Equation 2 in a simple macro we call 'imdz' (see below) for use in Varian's VNMR processing software to check the pH in 1D  $^1\text{H}$  NMR spectra of our protein samples. Typically, we only follow the downfield peak, for which  $\delta_{\text{HA}} = 8.66$  ppm and  $\delta_A = 7.76$  ppm from the fit to equation 1.

imdz:

```
$obs=cr/sfrq
if ($obs>8.66 or $obs<7.66) then write ('line3','Out of range')
abort
endif
r1=($obs-7.76)/(8.66-$obs)
ln(r1) : r2
$ph=7.11-0.43429*r2
write('line3','pH = %4.2f', $ph)
```

We have found the macro really handy, and hope others might find it useful also.

Best wishes,

  
Brian D. Sykes

Linda F. Saltibus



Leo Spyropoulos



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The NMR Newsletter  
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7/6/98  
(received 7/8/98)

## A Spectrometer for Every Grill!

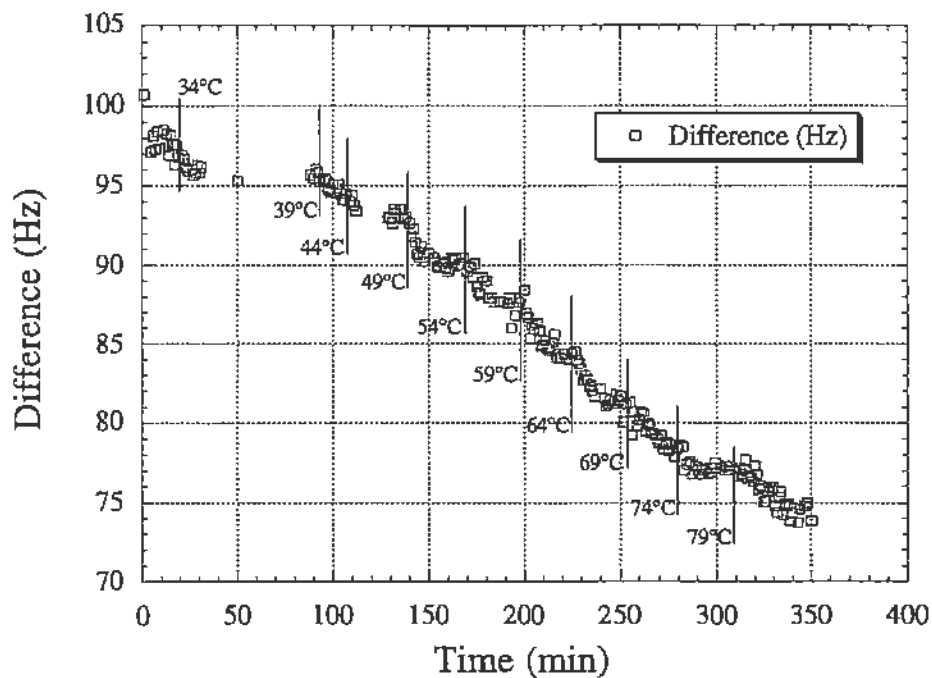
Dear Barry,

Recent outbreaks of food borne illness have dramatically highlighted the importance of achieving an adequate final temperature for ensuring product safety in cooked meats. This is particularly true for packaged and individual products like bone-in chicken, hamburgers, and whole chickens. The measurement of internal temperatures in this type of product is most often achieved by using an invasive probe like a thermocouple. However this approach is not viable for all foods and not rapid enough for production line use. Thus a fast, noninvasive temperature sensor to measure the internal temperature of a piece of meat during/after cooking would be a major advance in food safety. The sensor should be able to measure the internal temperature at production line speeds and capable of 100% inspection of the product. We show that NMR spectroscopy can do that.

As I'm sure you know, the chemical shift of water is temperature dependent. What is generally not appreciated is that the chemical shift of fat is also temperature dependent - probably because most such work has been done on the human body in a relatively narrow temperature range. The dependence is small and the sign of the slope is opposite that of water. Thus as the temperature of a piece of meat rises, the water and fat lines move towards each other. We obtain the temperature from the chemical shift *difference* of the two lines. This has two advantages. 1) The slope of the difference is larger than the slope of either line alone and thus is more accurate, and 2) This is an internal measurement and thus not subject to the vagaries of magnet drift. This is important in an industrial setting where cost considerations dictate low field permanent magnets and spectrometers without a lock.

The above technique has been used for many years with methanol as an internal temperature sensor in NMR spectroscopy<sup>1,2</sup>. We measured the chemical shift temperature dependencies for pork, hamburger, and various cuts of chicken on our GE Omega 300 wide bore system. We also repeated the experiment on our 26 MHz Tecmag based imaging system to demonstrate industrial relevance. The included figure is a plot of the chemical shift difference of the water and fat lines in hamburger with time. The vertical lines indicate when the set point of the temperature controller was changed. From the scatter on the plateaus, we estimate a  $\pm 1^\circ\text{C}$  accuracy.

The technique works best on meat with a high fat content. Obviously, it is hard to determine a peak position if there is little fat to produce a peak. In chicken breast we estimate a  $\pm 5^\circ\text{C}$  accuracy. Since shim is also quite important, the technique will work best when the samples have reproducible shape as in hamburger and sausage patties.



Please credit this contribution to Gerd La Mar's account.

Sincerely,

Jeffrey H. Walton

Michael J. McCarthy

#### References:

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<sup>2</sup> A. L. V. Geet, Anal. Chem., **42**, 679 (1970).



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## Specifications for Vertical Bore, High Resolution NMR Magnet Systems

NMR Operating Frequency (MHz <sup>1</sup> H)	200			300			400		500	
Field Strength (Tesla)	4.7			7.0			9.4		11.7	
Nominal Room Temperature Bore Access (mm)	54	89	54	89	150	54	89	51	89	
Magnet Type (Standard or shielded)	Standard	Standard	Standard	Standard	Standard	Standard	Actively Shielded	Actively Shielded	Actively Shielded	Actively Shielded
Field Stability (Hz/hour <sup>1</sup> H)	<2	<2	<3	<3	<15	<8	<10	<10	<10	<10
Axial 5 Gauss Stray Field Contour (Metres)	1.81	2.65	2.19	2.75	4.2	1.5	1.8	1.8	2.5	
Radial 5 Gauss Stray Field Contour (Metres)	1.42	2.0	1.7	2.2	3.3	1.0	1.3	1.3	1.75	
Cryostat Type	Compact	T3	T3	Compact	T3	T3	T5	T3	T4FB	T5FB
Minimum Helium Refill Interval (Days)	80	235	203	80	235	203	120	183	150	140
Helium Refill Volume (Litres)	26	79	68	26	79	68	101	62	83	83
Year Hold Cryostat Option Available	X	✓	✓	X	✓	✓	X	X	X	X
Nitrogen Refill Interval (Days)	14	14	14	14	14	14	22	14	15	15
Minimum Nitrogen Refill Volume (Litres)	32	61	61	32	61	61	135	61	81	81
* Minimum Operational Ceiling Height (Metres)	2.69	2.92	2.92	2.69	2.92	2.92	4.16	2.9	3.1	3.1
System Weight (kg) Including Cryogen's	120	315	391	133	325	399	1050	400	610	625

NMR Operating Frequency (MHz <sup>1</sup> H)	600		750		800		900	
Field Strength (Tesla)	14.0		17.6		18.8		21.1	
Nominal Room Temperature Bore Access (mm)	51	89	51	63		63		
Magnet Type (Standard or shielded)	Actively Shielded	Standard	Standard	Standard	(2.2K) Pumped	(2.2K) Pumped		
						Standard	With Iron Shield	
Field Stability (Hz/hour <sup>1</sup> H)	<10	<12	<15	<15	<15	<15	<15	
Axial 5 Gauss Stray Field Contour (Metres)	2.5	5.0	7.6	8.69	6.3	12.2	8.73	
Radial 5 Gauss Stray Field Contour (Metres)	1.75	3.9	6.1	6.89	5.0	9.7	3.81	
Cryostat Type	T5FB	T4FBL	T6	T6L	T7	T8		
Minimum Helium Refill Interval (Days)	120	90	60	60	60	60		
Cryostat Helium Refill Volume (Litres)	101	60	187	216	328	1200		
Minimum Nitrogen Refill Interval (Days)	15	15	14	14	14	15		
Nitrogen Refill Volume (Litres)	136	100	137	162	167	1800		
* Minimum Operational Ceiling Height (Metres)	3.16	3.4	3.78	3.97	3.97	8.75		
System Weight (kg) Including Cryogen's	1180	1200	3000	4000	4000	18000		

## Room Temperature Shim Specifications

Shim Type (Model)	Number of Channels	Dimensions	
		External Diameter (Cryostat Bore Size)	Internal Diameter (NMR Probe Diameter)
23/54/45	23	54mm	45mm
18/89/73	18	89mm	73mm
26/89/73	26	89mm	73mm
28/51/40	28	51mm	40mm
40/51/40	40	51mm	40mm
29/51/45	29	51mm	45mm
36/63/51	36	63mm	51mm

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# OXFORD

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July 1, 1998

(received 7/7/98)

Professor Bernard L. Shapiro  
The NMR Newsletter  
996 Elsinore Court  
Palo Alto, CA 94303

**Measurement of the average lifetime of anthracyclines in the intercalation sites and structure in solution of the complexes with d(CGATCG)<sub>2</sub> and d(CGTACG)<sub>2</sub>**

Dear Barry,

In our preceding studies<sup>1</sup> on the interaction between d(CGTACG)<sub>2</sub> and anthracyclines of the dauno/doxo family we used <sup>31</sup>P NMR experiments, in order to identify the intercalation site and to obtain thermodynamic and kinetic parameters of the process. The binding constants have been found to depend on the ionic strength and on the self-association, so much that their measurement by NMR to study anthracycline/DNA interactions was not recommended. On the contrary, the dissociation rate constants ( $k_{\text{off}}$ ) of the slow step of the intercalation process could be measured with high accuracy directly by NOE-exchange experiments and were found not to depend upon ionic strength and self-association phenomena. The kinetic constants are expression of the average residence time of the drug in the intercalation sites ( $1/k_{\text{off}}$ ), a parameter which appears to be related to the cytotoxic activity.

We extended the study to other DNA sequences, i.e. d(CGATCG)<sub>2</sub>, and derived the structure of the complexes anthracycline/oligomer<sup>2</sup>. The dissociation rate constants of the new generation analogues of doxorubicin (Adriamycin) 1, i.e. morpholinodoxorubicin 2 and 2(S)-2-methoxymorpholinodoxorubicin 3 were measured by <sup>31</sup>P NOESY-exchange experiments. The results showed that the variation in the triplet, CGT vs CGA, does not affect  $k_{\text{off}}$  of 3 ( $2.2 \pm 0.6 \text{ s}^{-1}$  and  $2.4 \pm 1.1 \text{ s}^{-1}$  respectively), whereas the strong decrease of  $k_{\text{off}}$  found for 2 ( $8.2 \pm 1.7 \text{ s}^{-1}$ ) and 3 is in line with the higher activity *in vitro* and *in vivo* of compounds 2 and 3 vs 1. The higher cytotoxicity of 3 vs 2 is also in agreement with the lower  $k_{\text{off}}$  of 3, corresponding to a longer residence time between the CG base pairs of the complex.

The interaction of these drugs with both DNA fragments was also studied by <sup>1</sup>H NMR spectroscopy, recording spectra of the oligonucleotides with increasing amounts of drug. All the protons of the complexes were attributed and a relevant number of intermolecular NOE interactions could be detected. These latter allowed us to derive through restrained MD calculations the structure of the complexes in solution, which were compared with those obtained by X-ray analysis<sup>3,4</sup>. In all the complexes the aglycone results located in the middle of the double helix, orthogonally oriented with respect to the base pairs, with ring D extending out of the helix on the major groove and ring A, with <sup>9</sup>H<sub>8</sub> conformation, between guanines G<sub>2</sub> and G<sub>12</sub>. The energy minimized structure of the complex between 3 and d(CGATCG)<sub>2</sub> is reported in Figure 1. For both the complexes with "AT" and "TA" sequence the daunosamine and morpholino moieties lie in the center of the minor groove, with slightly different positions with respect to the X-ray structures<sup>4</sup>.

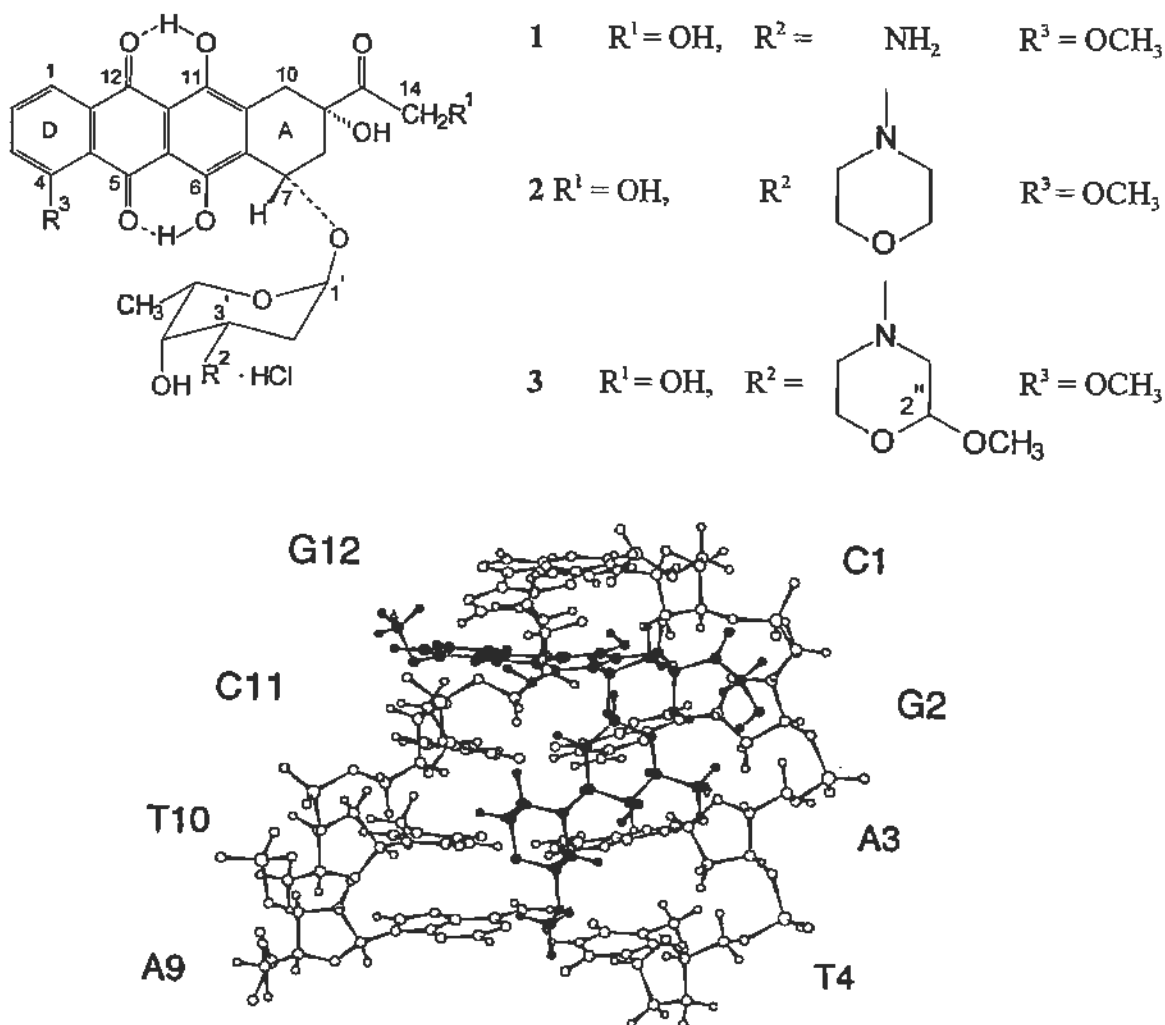
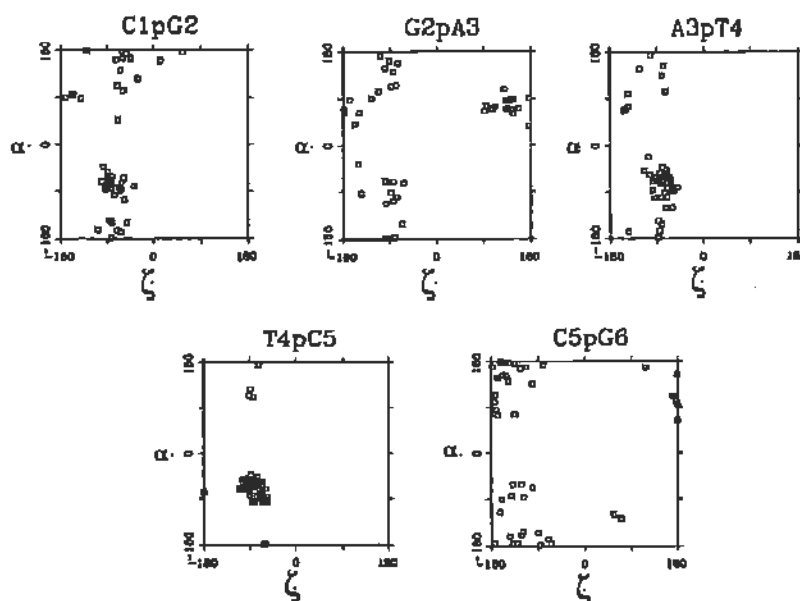


Fig. 1: Energy minimised molecular model of the "AT"/3 complex.

The daunorubicin ring is located at the level of  $A_3$  ( $T_3$ ), while the morpholino ring displays NOE interactions with the fourth unit  $T_4$  ( $A_4$ ). The two oxygen atoms of the morpholino rings were found 3 - 5 Å apart owing to the internal mobility of the rings. In the X-ray structure of "TA"/3 complex, the daunorubicin ring is rather close to  $G_{2,8}$  units and the two morpholino rings are in van der Waals contacts. The deformations of the double helix are revealed by an increased distance between protons of the  $C_5$  and  $G_6$  units and by a significant variation in  $^{31}\text{P}$  chemical shifts: the largest lowfield effects were observed for phosphates  $C_5pG_6$  (1.12-1.57 ppm) and  $G_2pT_3$  or  $G_2pA_3$  (0.84-1.53 ppm). The conformational changes at the level of angles  $\alpha$ ,  $\zeta$ ,  $\epsilon$  and  $\delta$  of the phosphoribose backbone resulted from MD calculations. The modulation of the experimental  $^{31}\text{P}$  chemical shifts are interpreted in terms of conformational equilibria occurring among differently populated conformers. This clearly appeared from the values of torsion angles  $\alpha$  and  $\zeta$ , monitored during the MD, which showed a relevant population of *trans* forms for  $\zeta$  and also for  $\alpha$  angles of  $C_5pG_6$  and  $G_2pT_3$  ( $G_2pA_3$ ) units, while the other phosphates exist entirely in the  $\alpha$ ,  $\zeta$  *gauche-gauche* conformation (Fig. 2). The dynamic variations of the two correlated angles  $\zeta$  and  $\epsilon$  at the level of  $G_{2,8}$  and  $C_{5,11}$  residues in both complexes with 3 confirm a significant population of the less stable  $B_{II}^5$  state at the intercalation sites. A deviation from the B-DNA geometry also appears at the level of the third residue, for angle  $\delta$  and for the correlated glycosyl angle  $\chi$ . The low values of  $\delta$  for  $A_{3,9}$  and  $T_9$  are in line with those found in the solid state and should correspond to ribose conformations between O-4' *endo* and C-3' *endo*.





**Fig.2:** Correlation between torsion angles  $\alpha$  and  $\zeta$  for the phosphate groups in the "AT"/3 complex obtained from 2 ps MD run.

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Yours sincerely,

Enzio Ragg

Stefania Mazzini

Rosanna Mondelli

Enzio Ragg

Stefania Mazzini

Rosanna



# TEXAS A&M UNIVERSITY

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July 3, 1998  
(received 7/9/98)

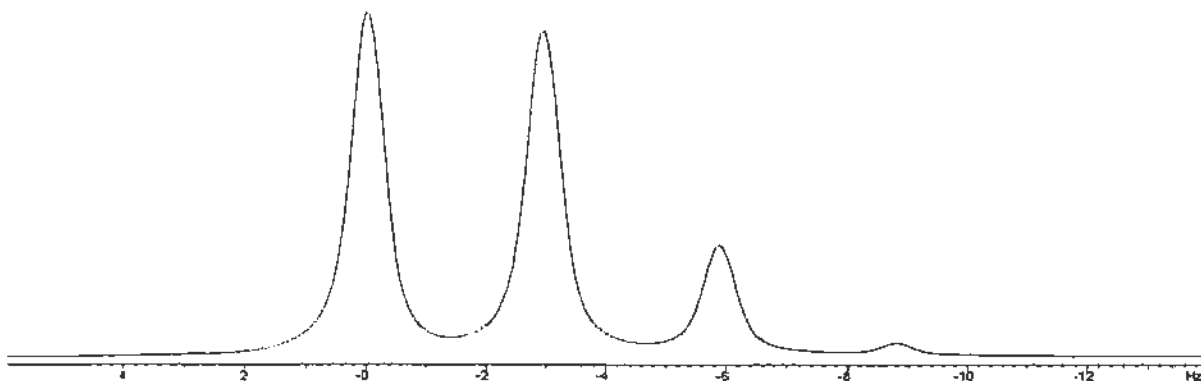
Dr. Barry Shapiro  
The NMR Newsletter  
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## <sup>19</sup>F and Isotope Shifts

Dear Barry:

I had occasion to look at a fluorine sample on our 500 recently, and was rather surprised when I looked closely at the CFCl<sub>3</sub> signal. I had hoped to use this signal as a shimming reference as well as the chemical shift reference, but had apparently never looked at it on a 500 before. Consequently, I did not expect to find four signals as shown below. This was clearly not a coupling pattern, and a little thought and a little math showed that the intensities were right for the four different combinations of <sup>35</sup>Cl and <sup>37</sup>Cl present. As expected, the CF<sup>35</sup>Cl<sub>3</sub> species is at lowest field and the CF<sup>37</sup>Cl<sub>3</sub> is at highest field, with an isotope shift of -0.006 PPM/<sup>37</sup>Cl. Relative intensities were calculated as 43.1:41.9:13.6:1.4 and integrals were measured as 43.5:41.4:13.3:1.7

A quick check in Harris and Mann found a note about a 0.009 PPM/<sup>37</sup>Cl isotope shift, significantly larger than we found here, as well as a 0.19 PPM <sup>13</sup>C isotope shift in CFCl<sub>3</sub>, which we also observed. This should serve as a reminder that isotope shifts can be rather large and significant in <sup>19</sup>F spectra, and that they will be more noticeable at higher field strengths. It also raises the rather interesting question as to which signal should be used as the reference for fluorine spectroscopy, although the large chemical shift range for fluorine makes this a rather moot point.

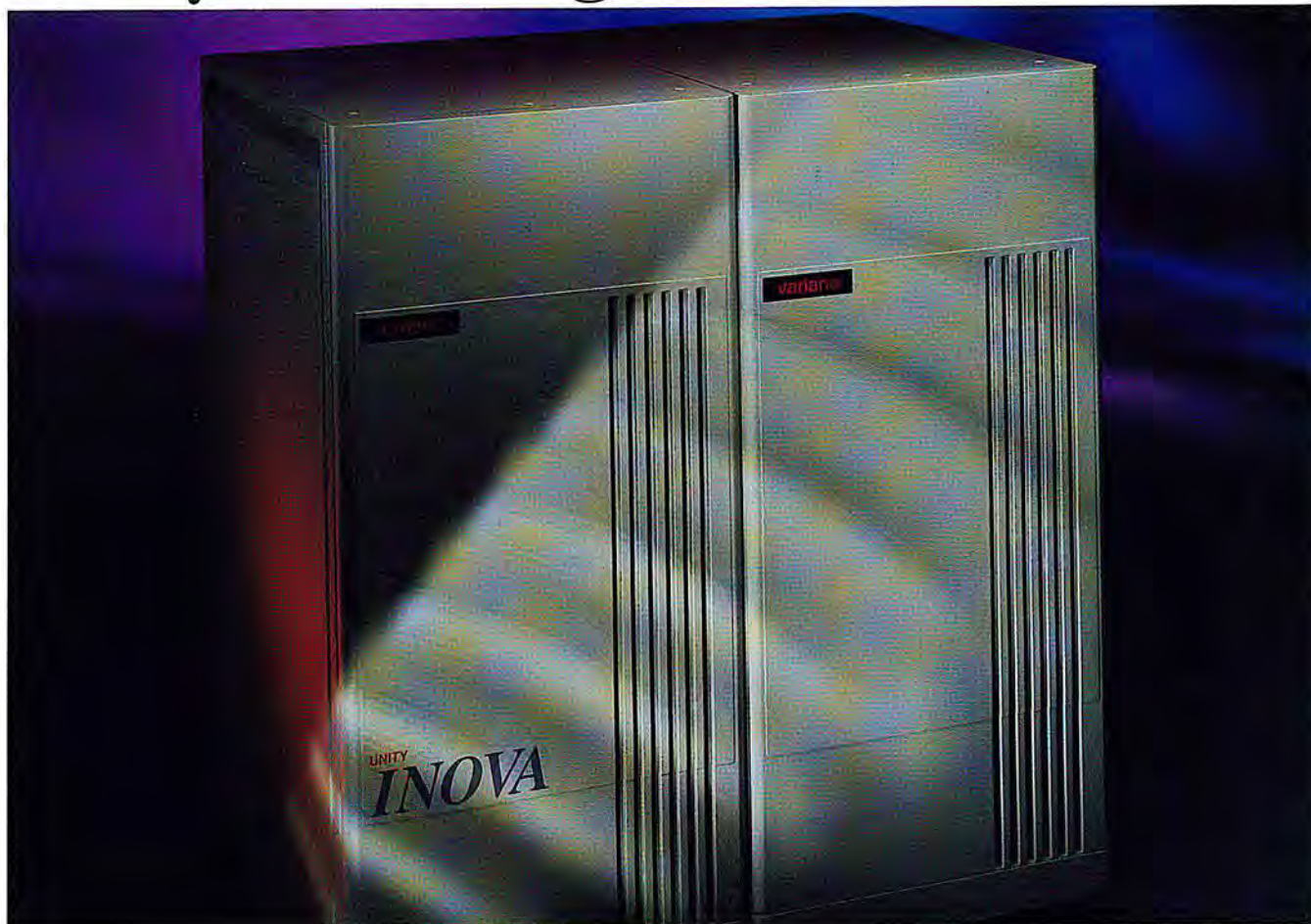


Sincerely,

A handwritten signature in black ink, appearing to read "STEVE".

Steven K. Silber  
Research Instrumentation Specialist

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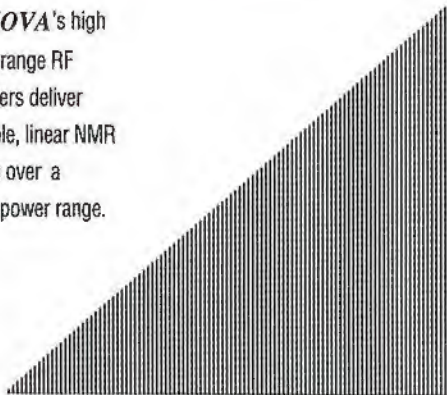
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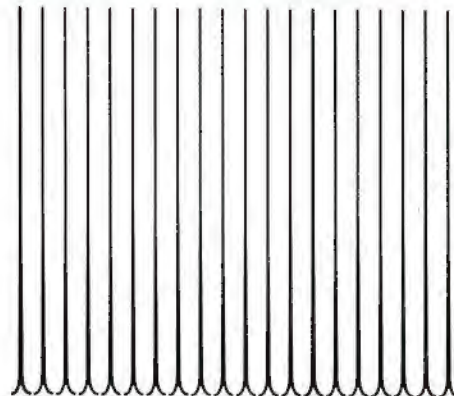


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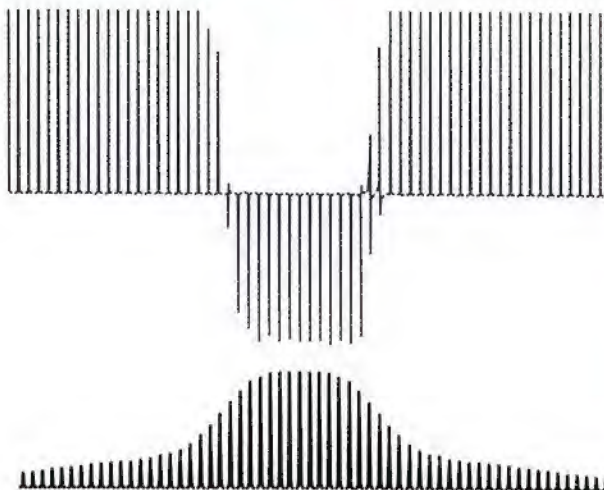
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(received 7/23/98)

July 22, 1998

Bernard L. Shapiro, Ph.D.  
Editor, The NMR Newsletter  
966 Elsinore Court  
Palo Alto, CA 94303

**The Hydrogen Jukebox  
& Nitrogen Nickelodeon**

Dear Barry,

For more years than either of us wish to admit or remember, we've agreed to disagree on which classical composers are worth listening to. I must, however, applaud you for finally "discovering" Shostakovich's String Quartets even if it has taken the better part of several decades of prodding and numerous "dreaded red dot" retaliatory reminders in return (just kidding). In any case, our title comes, in part, out of the classical realm, courtesy of a Philip Glass disc entitled "The Hydrogen Jukebox." The balance of the title comes from the contemporary SubMicro world in which we've tended to reside of late. Anyway, given the introductory phrase courtesy of Mr. Glass, and our interest in extending the application of long-range  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear shift correlation at natural abundance down to the realm of submilligram samples, the concluding phrase just seemed to fit. And, no, we haven't been watching the children's TV program "Nickelodeon" too often! So much for the title and where it came from...

Interest in long-range  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear shift correlation at natural abundance had its published beginnings in 1995 in the form of a report by Koshino and Uzawa from Japan and from my laboratory in collaboration with Ron Crouch, both of us then at Burroughs Wellcome Co. in North Carolina.<sup>1,2</sup> Among the compounds selected as an example by Koshino and co-workers was strychnine, a favorite model compound of many for benchmarking performance. Koshino's work reported a long-range  $^1\text{H}$ - $^{15}\text{N}$  spectrum of strychnine at natural abundance using a sample containing 40 mg/300  $\mu\text{L}$  (0.42 M). A slightly later study of strychnine and several related *Strychnos* alkaloids from our laboratory<sup>3</sup> used a more modest 20 mg/500  $\mu\text{L}$  (0.11 M) sample. All of these studies were conducted using conventional 5 mm gradient inverse probe technology. In recent contributions to *The NMR Newsletter* we have described our initial efforts with a prototype 1.7 mm SubMicro Inverse-Detection Gradient or SMIDG NMR probe designed and built for us by Ron Crouch and the folks at Nalorac Corp. A portion of this work has recently been published<sup>4</sup> and another report is in press.<sup>5</sup> Our contributions in the April issue of *The NMR Newsletter* was one entitled, "Putting  $^{15}\text{N}$  into Alkaloid NMR Using SubMicro Probes."<sup>6</sup> (By the way, is it "illegal" to quote the *Newsletter* in the *Newsletter*?) That contribution described our first efforts at submicro  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear shift correlation at natural abundance using a 1.5  $\mu\text{mole}$  sample of the spiro nonacyclic alkaloid cryptospirolepine. Despite the fact that this was the first reported application of SMIDG NMR probe technology to long-range  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear shift correlation at natural abundance with a submilligram sample, the detection of three of the four nitrogen resonances of this alkaloid, two *via* N-methyl singlets, was not particularly satisfying nor a demanding demonstration of the capabilities of the SMIDG-600-1.7 NMR probe installed in our Varian INOVA 600. It is in this light that our long-range  $^1\text{H}$ - $^{15}\text{N}$  efforts using a 3  $\mu\text{mole}$  (1 mg in 30  $\mu\text{L}$   $\text{CDCl}_3$ ; 0.11 M) sample of strychnine (1) are first reported below. This sample concentration is essentially identical to the 5 mm 500 MHz data reported by us previously.<sup>3</sup> These data are followed by results obtained using a 1  $\mu\text{mole}$  (334  $\mu\text{g}$  in 30  $\mu\text{L}$   $\text{CDCl}_3$ ; 0.037 M) sample of strychnine. The latter data establish what, at this point in time, would seem to be a practical lower limit for  $^1\text{H}$ - $^{15}\text{N}$  GHMBC experiments at natural abundance given the current generation of SMIDG-600-1.7 NMR probe technology.

The first data shown below in Figures 1 and 2 came from our first effort with a "real" molecule, strychnine. As just noted, the sample was prepared using a stock solution containing 1 mg (3  $\mu$ moles) of recrystallized strychnine per 30  $\mu$ L of  $\text{CDCl}_3$  (Isotec). An aliquot of 30  $\mu$ L of this solution was transferred to a laboratory made 1.7 mm NMR tube (Wilmad 1.7 mm precision glass tubing) using a Hamilton #1701 syringe. The sample was then frozen in  $\text{LN}_2$  and flame sealed.

Based on our prior experience<sup>6</sup>, a long-range  $^1\text{H}$ - $^{15}\text{N}$  GHMBC experiment was set up using the 1 mg ( $\sim 3$   $\mu$ mol) sample strychnine with parameters selected to run overnight (18 h) as follows. The experiment was optimized for an assumed 10 Hz (50 msec) long-range coupling. The data were acquired as 4096 x 40 States-TPPI files. Spectral widths were 5192 and 8509 Hz in  $F_2$  and  $F_1$ , respectively. Pulse widths for  $^1\text{H}$  and  $^{15}\text{N}$  were 6.65 and 21.1  $\mu$ sec, respectively, at power levels of 48 and 59 dB, respectively (max. 63 dB). The gradient ratio employed was 5:1; the applied gradient strengths were 10.0 and 2.02  $\text{G cm}^{-1}$ , respectively. The data were acquired in 18 h overnight with 512 transients accumulated/ $t_1$  increment. The time/transient was 1.394 s (1.0 s interpulse + acquisition time). During processing, the data were linear predicted to 120 files in  $t_1$  followed by zero-filling to afford the final 4096 x 512 point spectrum shown in Figure 1. The data were processed using sine-bell multiplication prior to the first Fourier transform and cosine multiplication prior to the second, the time constants optimized to the acquisition time in both frequency domains. The high resolution proton spectrum is plotted atop the contour plot.

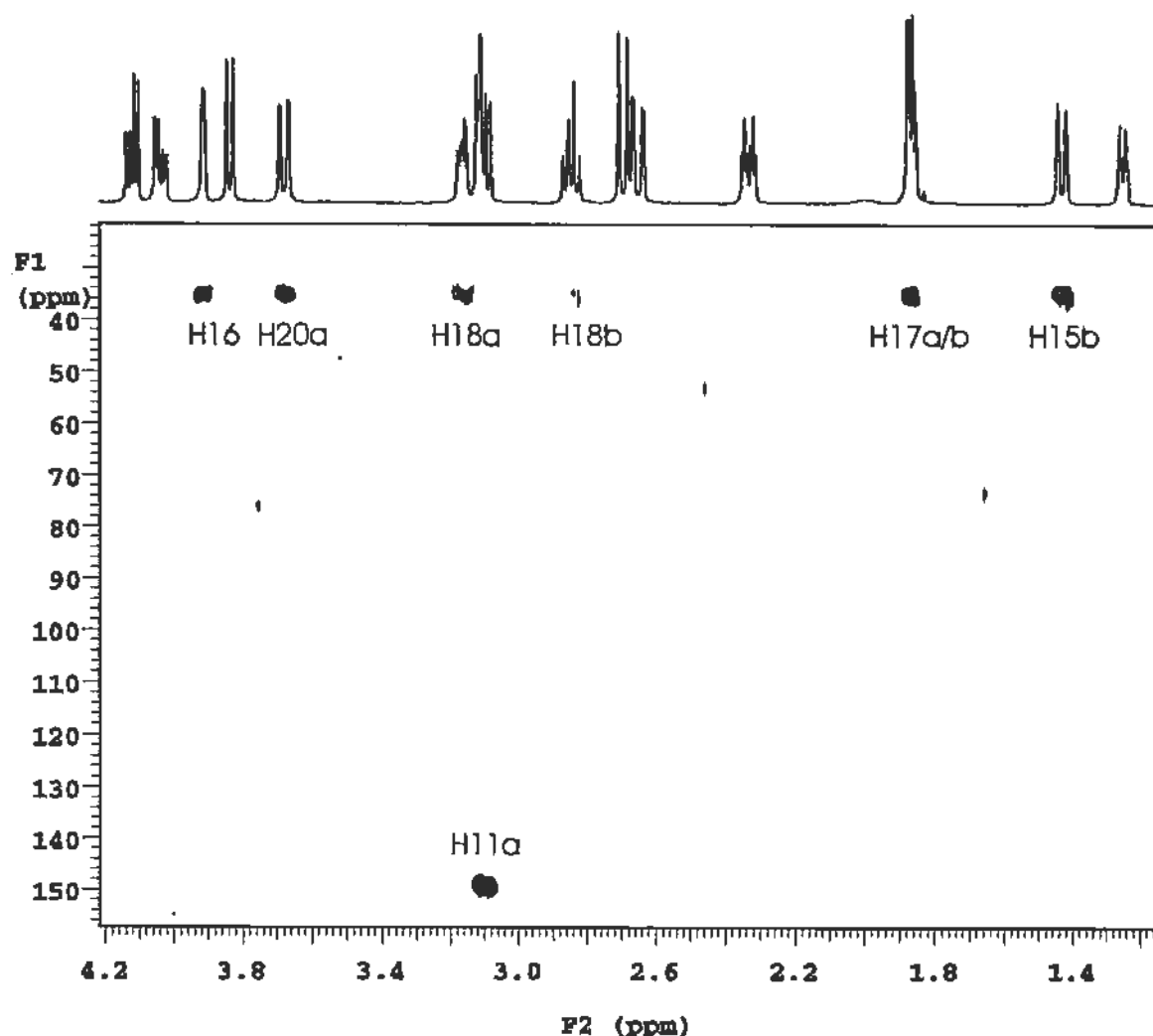


Figure 1. Long-range  $^1\text{H}$ - $^{15}\text{N}$  GHMBC heteronuclear shift correlation spectrum of a 1 mg sample ( $\sim 3$   $\mu$ moles; 0.11 M) of recrystallized strychnine in 30  $\mu$ L  $\text{CDCl}_3$  (Isotec) recorded in 18 h overnight using a Varian INOVA 600 NMR spectrometer equipped with a Nalorac SMIDG-600-1.7 submicro NMR probe. Two sets of responses were observed for N(19) (35.1 ppm) and N(9) 149.1 ppm. The numbering scheme for strychnine is shown in Figure 3 below.



All of the responses observed in the contour plot shown above have been reported in our<sup>3</sup> and Koshino's<sup>1</sup> previous work. Slices extracted from the spectrum are plotted above a high resolution proton reference spectrum in Figure 2. If these were data for an unknown, care would necessarily have to be exercised in the utilization of the responses correlating both H8 and H13 to N(9).

These responses are sufficiently weak that only the breadth of the response when the data are viewed as the slice allows them to be considered as viable correlations to N(9). This is particularly true of the H13-N(9) correlation response which is considerably weaker than the response from H8.

The data shown in Figure 2 gave a measured s/n (signal-to-noise) ratio of 32:1. The "noise" region of the spectrum was taken as the region from 4.8-5.8 ppm using the trace plotted at the chemical shift of N(19) shown in Figure 2. Correlations observed are identical to those previously reported.<sup>3</sup>

Acquisition of a  $^1\text{H}$ - $^{15}\text{N}$  GHMBC spectrum of strychnine over a weekend (see Figure 3) gave, as expected, roughly double the s/n ratio of the data shown in Figures 1 and 2. The measured s/n ratio was 62:1, which is reasonable since the acquisition was roughly four times the duration of the overnight data shown in Figures 1 and 2. We show these data for two primary reasons. First, the improved response intensity, particularly for the H8 and H13 responses confirms the reliability of these correlations and a weekend acquisition is certainly not unreasonable in duration. Second, the long-range correlation from H15a to N19 observed in the weekend data is reported for the first time.

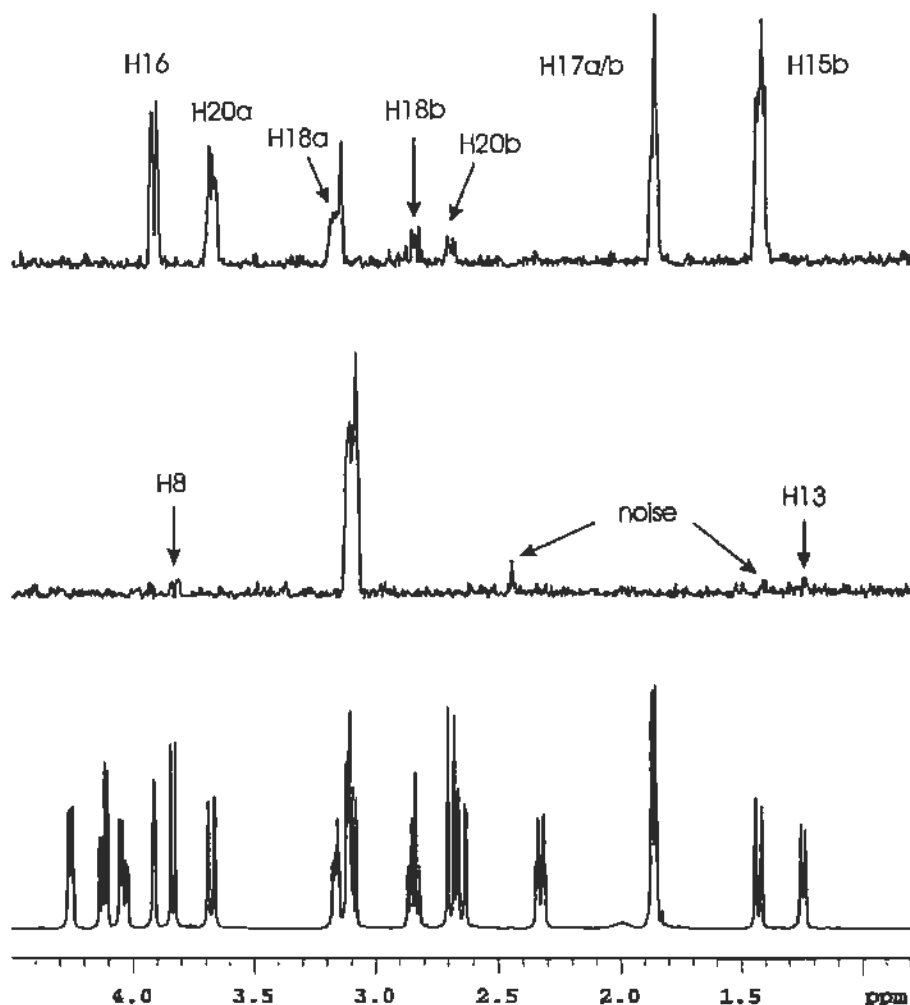


Figure 2. (Bottom) High resolution spectrum of 1 mg (3  $\mu\text{mol}$ ; 0.11 M) in  $\text{CDCl}_3$  recorded in 32 transients. (Middle) Trace from the 10 Hz GHMBC spectrum acquired overnight and shown in Figure 1 extracted at the chemical shift of N(9) (149.1 ppm). Responses labeled by arrows to H8 and H13 were either not observable in the contour plot (Figure 1). (Top) Trace extracted from the 10 Hz GHMBC spectrum shown in Figure 1 at the chemical shift of N(19) (35.1 ppm).

In an effort to push the limits of detection, and in light of the high quality of the data that can be acquired with a 1 mg sample of strychnine, a sample was prepared containing 1  $\mu\text{mol}$  (334  $\mu\text{g}$ ) in 30  $\mu\text{L}$  of  $\text{CDCl}_3$  (0.037 M). This sample represents, by a wide margin, the smallest quantity of material ever examined by  $^1\text{H}$ - $^{15}\text{N}$  GHMBC at natural abundance. Slices extracted from 1  $\mu\text{mol}$   $^1\text{H}$ - $^{15}\text{N}$  GHMBC spectrum acquired over a weekend (65 h) are presented in Figure 4. The parameters employed for this experiment were essentially identical to those specified above for the 1 mg sample except that 2048 transients were accumulated per  $t_1$  increment. The data processing was also identical to that used above.

In the contour plot of this spectrum (not shown), only the responses correlating H17a/b and H15b to N(19) and the H11b/N(9) correlation response are observed. This result is reasonable, given that the working concentration of the sample is down by a

Figure 3. Right. (Bottom trace) High resolution spectrum of 1 mg (3  $\mu$ mol; 0.11 M) in  $\text{CDCl}_3$  recorded in 32 transients. (Middle trace) Trace from a 10 Hz GHMBC spectrum acquired over a weekend (64 h) extracted at the chemical shift of N(9) (149.1 ppm). The response denoted by the arrow to H3 was not observed in the contour plot. The response denoted by the arrow from H13 was only very weakly visible in the contour plot. (Top trace) Trace extracted from the 10 Hz GHMBC spectrum shown in Figure 1 at the chemical shift of N(19) (35.1 ppm). The response from H15a to N(19) has not been previously reported.

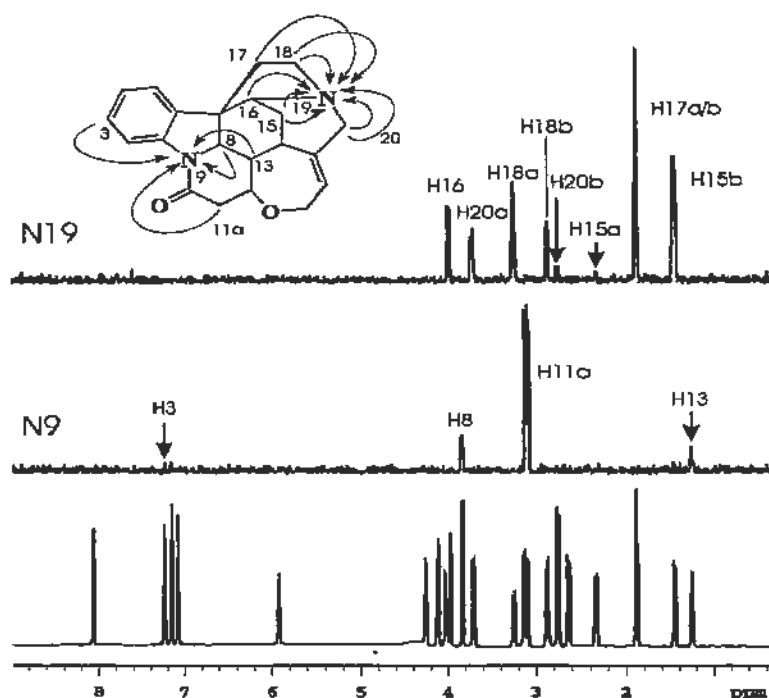
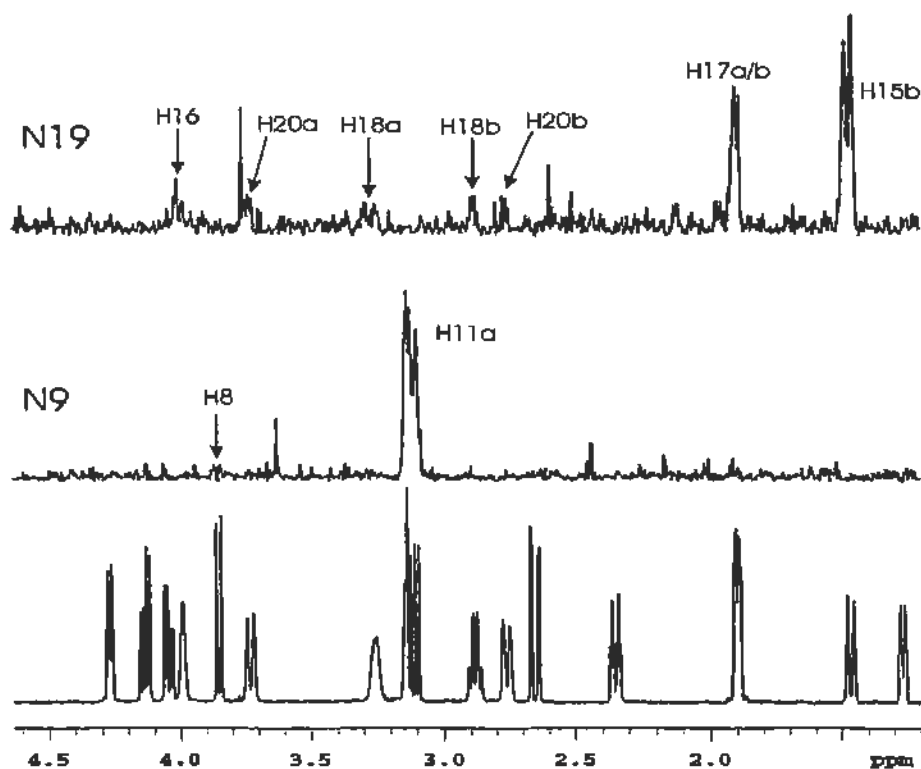


Figure 4. Right. (Bottom trace) High resolution spectrum of 1  $\mu$ mol (334  $\mu$ g 0.037 M in 30  $\mu$ L  $\text{CDCl}_3$ ) recorded in 32 transients. (Middle trace) Trace from a 10 Hz GHMBC spectrum acquired over a weekend (65 h) extracted at the chemical shift of N(9) (149.1 ppm). The response denoted by the arrow to H8 is quite weak and would have to be used with considerable caution. Running the experiment to double the noise would require ~260 h and is not practical. (Top trace) Trace extracted from the 10 Hz GHMBC spectrum shown in Figure 1 at the chemical shift of N(19) (35.1 ppm). The labeled responses would be could be used reliably in the elucidation of an unknown structure in the opinion of the authors.



factor of three relative to the previous experiments. Practically, however, as shown by the slice summary presented in Figure 4, usable signal intensity is still contained in the slices taken from the 2D data matrix. Virtually all of the correlation responses

to N(19) are visible at a usable level of intensity (top trace in Figure 4). The very weak nature of the H8-N(9) correlation response, in our opinion, would have to be employed with caution in the assembly of an unknown structure. By reprocessing these data, modifying the weighting function used in the  $t_2$  time domain, and observing the resultant width of the response in the trace as a function of the processing parameters employed, it should be possible to either confirm or refute the authenticity of a response such as that from H8 in the N(9) trace shown in Figure 4.

In conclusion, the Nalorac SMIDG-600-1.7 SubMicro Inverse-Detection Gradient NMR probe installed in our Varian INOVA 600 MHz NMR spectrometer makes it feasible to consider the routine acquisition and utilization of long-range  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear shift correlation information at the natural abundance of  $^{15}\text{N}$  in the assembly of unknown chemical structures. The performance characteristics of the probe are such that these data can be acquired even at the submilligram level over a weekend. As we demonstrated previously<sup>6</sup>,  $^1\text{H}$ - $^{15}\text{N}$  correlation data for protonated nitrogens and those nitrogens detectable via N-methyl groups, for example, can be very readily acquired. In the case of a 1.5  $\mu\text{mole}$  sample of cryptospirolepine used in our previous contribution, direct correlation data with excellent signal-to-noise were obtainable in less than one hour of spectrometer time. It is hoped that the capabilities demonstrated for the SMIDG-600-1.7 NMR probe in this and our previous contribution<sup>6</sup> will begin to make  $^{15}\text{N}$  available as a structural probe for the elucidation of alkaloid and pharmaceutical impurity and degradant structures as these probes become more widely available.

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6. Martin, G.E., Hadden, C.E., *The NMR Newsletter*, No. 475, April, 1998, pp. 21-24.



Gary E. Martin



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- van Ormondt, Dirk, Dr, Applied Physics, TUD, Delft, NL, phone 31-15-2786070, fax 31-15-2783251, ormo@si.tn.tudelft.nl

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(received 6/29/98)

## HRMAS OF BOUND PEPTIDES IN DMF

Dear Barry,

High Resolution Magic Angle Spinning (HRMAS) is an exciting new technique in NMR analysis applicable to a wide range of samples with restricted motion, including membranes, polymer gels, lipids, tissue samples and molecules attached to polymer beads. The limited mobility of these samples associated with their large heterogeneity leads to broad proton NMR spectra which are unexploitable. A very convenient way to reduce the line width of these samples is to spin them at an angle of  $54.7^\circ$  with respect to the main  $B_0$  magnetic field. This angle is referred to as the magic angle. MAS will simultaneously average out the solid type interactions which are present in the sample (homonuclear dipole-dipole interaction and chemical shift anisotropy) and the differences in magnetic susceptibility. Under these conditions the line width can approach the ones encountered in liquid samples and the classical 2D liquid experiments can be applied without any modifications.

When applied to peptides bound to a resin support, the choice of the solvent can be critical. Fig. 1 shows the 1D proton spectrum of the tetrapeptide Ala-Ile-Gly-Met bound on a Wang resin via the methionin residue recorded in  $CDCl_3$  (A) and DMF (B). The spectrum recorded in DMF is clearly better resolved and has the additional advantage of containing all of the NH protons therefore facilitating the assignment process. We have run many different peptides of various length and composition bound to a variety of resins and DMF has always proven to be a very suitable first guess.

M. BOURDONNEAU, C. BREVARD, M. PIOTTO

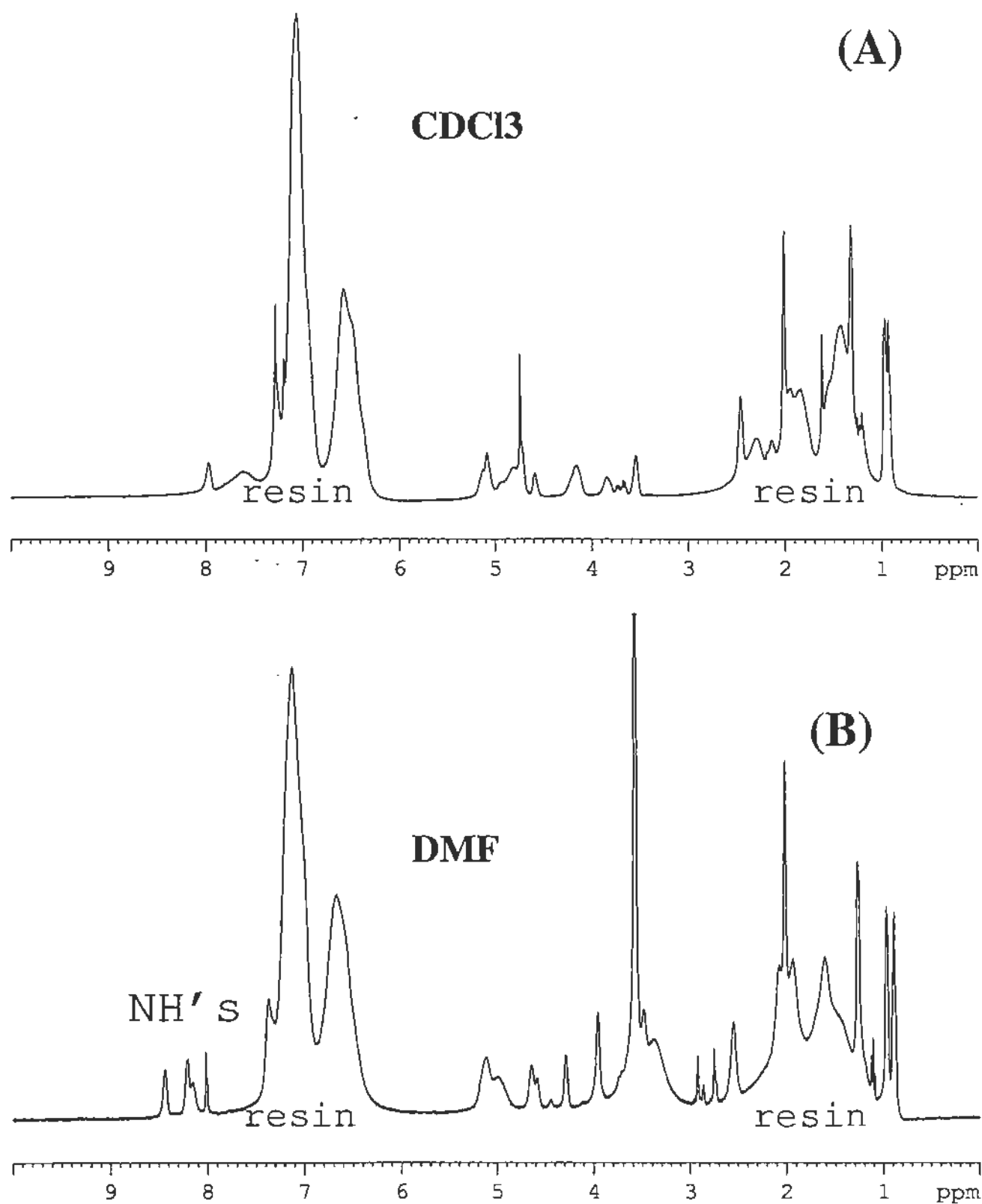


Figure 1: 400MHz HRMAS spectra of a tetrapeptide bound to a Wang resin in  $\text{CDCl}_3$  (A) and  $\text{DMF}$  (B). The spectra were recorded on a gradient  $1\text{H}/^{13}\text{C}/2\text{D}$  4mm HRMAS probe at a speed of 4kHz.



UNIVERSITY OF VIRGINIA  
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Timothy R. J. Dinesen  
trd3f@virginia.edu

(received 7/17/98)

re: Continuing MRD of Solutes

Barry Shapiro  
The NMR Newsletter  
966 Elsinore Court  
Palo Alto, CA 94303

July 13, 1998.

Dear Barry,

The Magnetic Relaxation Dispersion (MRD) experiment records the field dependence of the spin lattice relaxation rate, which is directly related to the power spectral densities that modulate relaxation. The MRD experiment thus provides information regarding the molecular dynamics that are responsible for oscillation of the magnetic fields that form the "lattice" to which the relaxing spin probe is coupled. We have recently reported the  $^7\text{Li}$  data of aqueous solutions of lithium salts containing small concentrations of paramagnetic species. In solutions of substituted nitroxide free radicals, the MRD has provided experimental evidence for inner sphere co-ordination of nitroxide free radicals by  $\text{Li}(\text{H}_2\text{O})_n^+$ .<sup>1</sup> Subsequently, we reported the formation of an outer-sphere cation-cation complex between  $\text{Li}(\text{H}_2\text{O})_n^+$  and  $\text{Mn}(\text{H}_2\text{O})_6^{2+}$ , and resolved the relative translationally and rotationally correlated contributions to the total relaxation,<sup>2</sup> and discussed the translational encounter in the context of the repulsive electrostatic potential, which we represented by a mean field model. In an effort to further characterize the relaxation phenomena of solutes, we have continued this survey to include the relaxation behaviour of several ionic pairs producing both repulsive and attractive electrostatic potentials. For your consideration, we enclose data that are not amenable to theoretical analysis, but are nevertheless interesting.

Below is the  $^{111}\text{Cd}$  MRD curve for a 3.0 M  $\text{Cd}(\text{NO}_3)_2$  solution containing 4.5 mM  $\text{MnSO}_4$ , modelling a +2/+2 ionic interaction. This large concentration was used due our difficulty with insensitive nuclei. There is a clear dispersion of the relaxation rates, indicating a significant coupling of the nuclear and paramagnetic electronic moments. As for the  $^7\text{Li}$  data, we have attempted to express the total relaxation in terms of discrete translational and rotational contributions. The rotational contribution is correlated on the time scale of a well defined molecular reorientation, while the translational term represents an integral over both long and short range interactions, and thus varies over a much wider frequency range. As you can see, the theoretical curves do not suit the observed relaxation

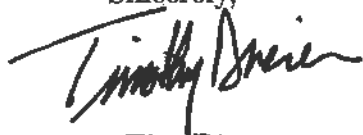
<sup>1</sup>T. R. J. Dinesen and R. G. Bryant, *J. Magn. Reson.* **132**, 19 (1998)

<sup>2</sup>T. R. J. Dinesen, S. Wagner and R. G. Bryant, *J. Am. Chem. Soc.*, in press



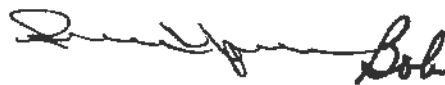
for physically reasonable values of the parameters, as the model represents an idealized version of solution chemistry. The 9 M ionic strength is high and the solution should exhibit strong deviations from the ideality of an infinitely dilute electrolyte. Local deviations from the bulk diffusion may contribute spatial coordinates to an otherwise isotropic diffusion equation, as well as a strong radial dependence of the intermolecular potential, so it is not likely that the data can be fit to a closed form model of relative translational diffusion. Moreover, these high concentrations of electrolytes also guarantee the formation of ion pairs, or even a distribution of cluster species, and consequently a distribution of correlation times. The resulting direct complexation between  $\text{NO}_3^-$  and  $\text{Mn(II)}$  reduces the spherical symmetry at the metal centre, confusing the issue by introducing terms that account for electronic relaxation. In an ideal situation, the MRD experiment provides direct spectroscopic measurements of hydrodynamic parameters, and may be a critical test of the applied theories of molecular dynamics.

Sincerely,



Tim Dinesen

Ching-Ling Teng



Shawn Wagner

Bob Bryant

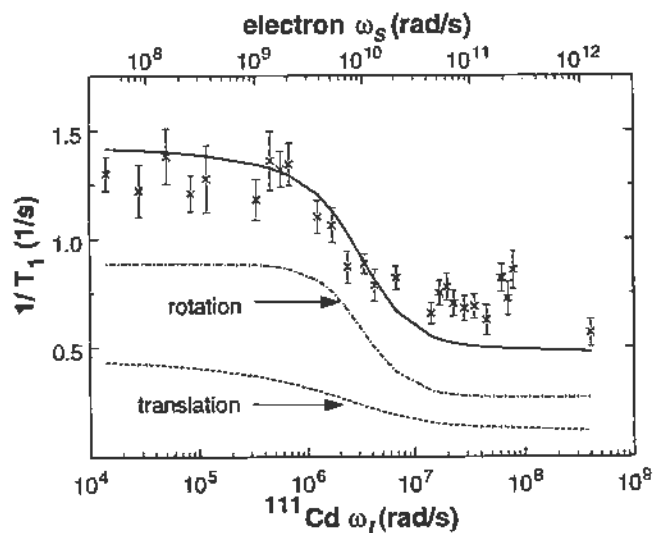


Figure 1: The  $^7\text{Li}$  MRD curve describes the  $\text{Mn(II)}$  ( $S=5/2$ ) induced relaxation of the lithium ion.  $D = 6 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ,  $b = 7.5 \times 10^{-10} \text{ m}$ ,  $\tau_{rot} = 110 \text{ ps}$ ,  $\tau_{IS} = 6.5 \times 10^{-10} \text{ m}$ ,  $X_{bound} = 0.0025$



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(received 7/21/98)

July 15, 1998

### **$^1\text{H}$ - $^2\text{H}$ Dipolar NMR Study of the Water Molecule in Crystalline Hydrates**

Dear Dr. Shapiro:

It is well known that  $^1\text{H}$  chemical shift is sensitive to hydrogen bonding. However, since it is difficult to accurately measure  $^1\text{H}$  chemical shift tensors, there has been only a small collection of data on  $^1\text{H}$  chemical shift tensors available in the literature, most of which were early multiple-pulse NMR studies of *single crystals* [1-3].

We have recently investigated the possibility of utilizing  $^1\text{H}$ - $^2\text{H}$  dipolar NMR spectra to determine  $^1\text{H}$  chemical shift tensors in perdeuterated crystalline hydrates. Typically, the solid hydrate samples were re-crystallized twice from 99.9%  $\text{D}_2\text{O}$ . Therefore, one is basically dealing with isolated  $^1\text{H}$ - $^2\text{H}$  spin pairs. The advantage of using  $^1\text{H}$ - $^2\text{H}$  spin pairs, rather than the  $^1\text{H}$ - $^1\text{H}$  spin pairs in ordinary hydrates, is to reduce the line broadening from intermolecular dipolar interactions, thus permitting accurate measurement of small proton chemical shift anisotropies. Our primary objective is to study the relationship between  $^1\text{H}$  chemical shift tensors and hydrogen bond geometries.

Figure 1 shows both the observed and simulated  $^1\text{H}$  NMR spectra of a stationary solid sample of  $\text{Ba}(\text{ClO}_3)_2 \cdot \text{H}_2\text{O}$  (99.9% D) obtained at two different magnetic field strengths. We found that it is necessary to carry out MAS experiments so that the isotropic chemical shifts could be determined and subsequently used as a constraint in finding the three principal components from powder line-shape simulations. In crystalline hydrates, the orientation of the proton chemical shift tensor in the molecular frame of reference is completely determined by the rapid two-fold jumps or flips of the water molecule [4]. It is also known from previous single crystal NMR studies [5] that the least shielded component,  $\delta_{11}$ , is always perpendicular to the plane containing the water molecule, whereas the most shielded component,  $\delta_{33}$ , lies along the  $^1\text{H}$ - $^2\text{H}$  vector. Using these ideas, we obtained the principal components of the  $^1\text{H}$  chemical shift tensor in  $\text{Ba}(\text{ClO}_3)_2 \cdot \text{H}_2\text{O}$  from the spectra shown in Fig.1:  $\delta_{11} = 13.7$ ,  $\delta_{22} = 6.7$ , and  $\delta_{33} = -1.3$  ppm.

We have also applied this technique to the study of a series of crystalline hydrates, and seen some interesting correlations between  $^1\text{H}$  chemical shift tensors and hydrogen bond geometries such as  $R(\text{H}\cdots\text{O})$  and  $\angle\text{O}-\text{H}\cdots\text{O}$ . A complete account of this study is in preparation and will be submitted for publication.



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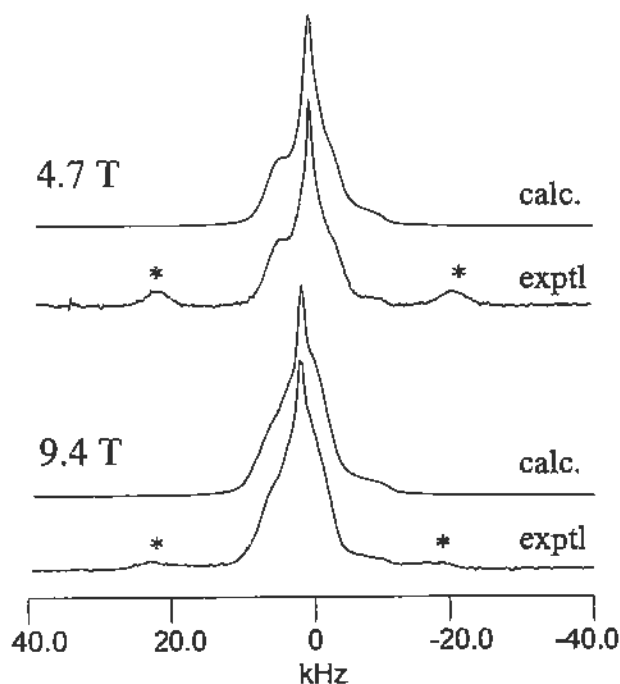


Figure 1. Observed (bottom) and simulated (top)  $^1\text{H}$  NMR spectra of residual protons in solid  $\text{Ba}(\text{ClO}_3)_2 \cdot \text{H}_2\text{O}$  (99.9% D). The two humps marked by \* on each side of the powder spectra are due to residual  $^1\text{H}$ - $^1\text{H}$  interactions.

- [1] Haeberlen, U. *High Resolution NMR in Solids: Selective Averaging*; Academic Press: New York, 1976.
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- [3] Duncan, T.M. *A Compilation of Chemical Shift Anisotropies*; Farragut Press: Chicago, 1990.
- [4] Long, J.R.; Ebelhäuser, R.; Griffin, R.G. *J. Phys. Chem.* **1997**, *A101*, 988.
- [5] Schuff, N.; Haeberlen, U. *J. Mag. Reson.* **1985**, *62*, 406.

Please credit this contribution to the account of S. Blake.

Sincerely,

Gang Wu  
Assistant Professor of Chemistry

Christopher J. Freure



(received 7/18/98)

Dr Bernard L. Shapiro  
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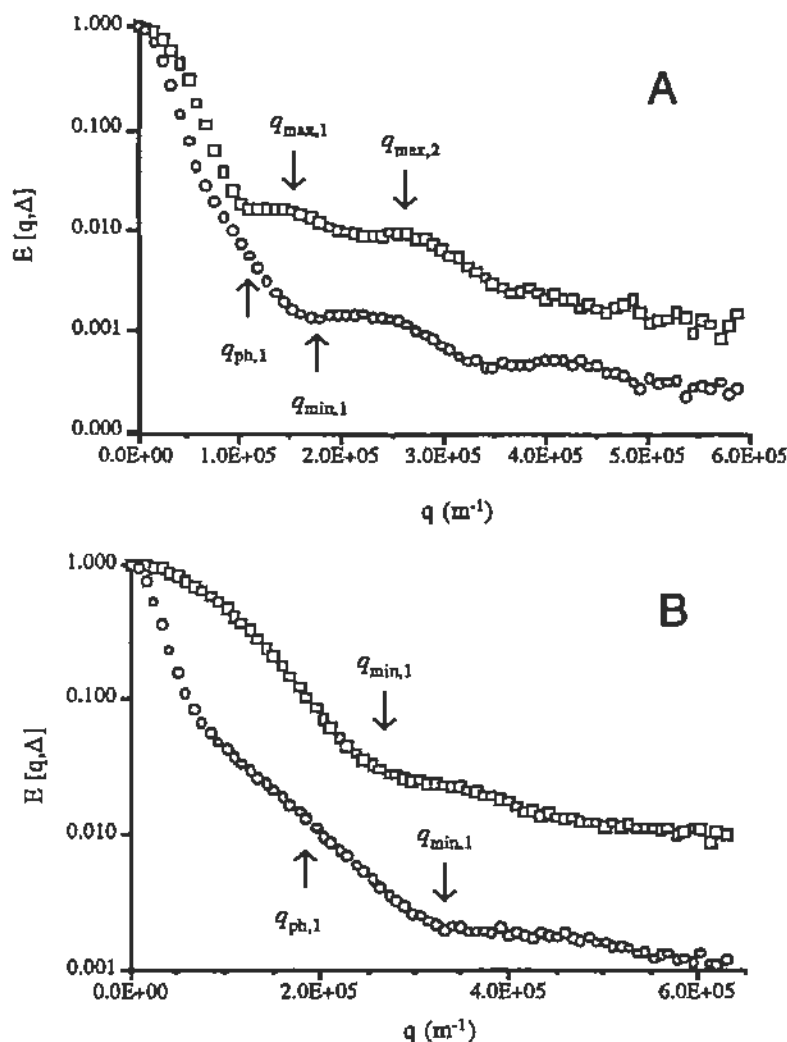
## Probing Extracellular and Intracellular Diffusion-Diffraction Effects in Erythrocyte Suspensions

Dear Barry,

The  $^1\text{H}$  NMR diffusion-diffraction (or coherence) effect evident in  $q$ -space plots of water in an erythrocyte suspension can yield interesting information on the structural characteristics of the cells (1). In our previous studies, we demonstrated that this newly described phenomenon is potentially useful as a diagnostic tool in Haematology since it provides unbiased representations of mean cell dimensions and shape (2). We have also found that the form of the  $q$ -space plots is quite sensitive to small changes in cell dimension; and by proper analysis of the data, we are able to obtain a reasonable estimate of the mean cell diameter in a suspension.

The coherence peaks in  $^1\text{H}$  NMR  $q$ -space plots of water are produced by two populations of water molecules, extracellular and intracellular, which are diffusing in essentially different environments. Extracellular water diffusion leads to an interference-like effect due to 'pore hopping' between the lacunae outside the cells; the coherence peak is in fact a weak shoulder near  $q \cong 1.0 \times 10^{-5} \text{ m}^{-1}$  (see  $q_{\text{ph},1}$  in Fig. 1A). On the other hand, intracellular diffusion yields a diffraction-like effect and a minimum (prior to a peak) at  $q \cong 1.8 \times 10^{-5} \text{ m}^{-1}$  (see  $q_{\text{min},1}$  in Fig. 1A). Although both types of peak provide important information about the cell shape and dimension, we suspected that superposition of the two different coherence effects in a single  $q$ -space plot could obscure the actual position of these separate peaks.

To discriminate between the two compartments in an erythrocyte suspension we used choline and choline phosphate as probe molecules. Unlike water, choline can be *entrapped* in red cells by hypotonic lysis followed by resealing, while choline phosphate can be introduced to the suspension and it does not enter the cells. Thus, by observing a specific proton signal from choline or choline phosphate in a diffusion-diffraction experiment, we were able to obtain  $q$ -space plots showing only the intracellular diffusion-diffraction effect from choline, and the interference (pore-hopping) effect for choline phosphate. Figure 1A shows the coherence peaks of water and choline phosphate in a suspension of regular biconcave red cells, while Fig 1B illustrates the coherence peaks from water and choline in a suspension of resealed red cells. Clearly, the positions of the minima and maxima for choline and choline phosphate are different from those obtained for water, thus confirming our supposition that the  $q$ -space plot of water is complicated by the superposition of the two types of coherence effects. In addition, water rapidly exchanges across the cell membranes and this leads to a shift in the position of the coherence peaks (1). Analysis of the simpler  $q$ -space plots of choline and choline phosphate therefore should provide better estimates of the red cell shape and dimensions.



**Figure 1.** (A)  $^1\text{H}$  NMR  $q$ -space plot of water (O) and choline phosphate ( $\square$ ) in a suspension of fresh biconcave erythrocytes (haematocrit, 60%) washed with 72.5mM choline phosphate, at 298K. The peaks present in the water curve are caused by extracellular (pore-hopping, an interference-like effect), and intracellular diffusion-diffraction in the compartment confined by the cell membranes. The position of the pore hopping peak ( $q_{ph,1}$ ) and the intracellular diffusion-diffraction peak ( $q_{min,1}$ ), which are indicated by arrows, are inversely related to the mean cell diameter. (B)  $^1\text{H}$  NMR  $q$ -space plots of water (O) and choline ( $\square$ ) in a suspension of resealed erythrocytes (haematocrit, 62%) loaded with choline, at 298K. The resealed cells were not truly biconcave discs but rather were a mixture of cup-shaped and spherical cells. The peak in the choline curve results from intracellular diffusion-diffraction only; there is no evidence of a pore-hopping 'shoulder'.

## References

1. Kuchel, P.W., Coy, A., and Stilbs, P. NMR "Diffusion-Diffraction" of water revealing alignment of erythrocytes in a magnetic field and their dimensions and membrane transport characteristics. *Magn. Reson. Med.* 37,637-643 (1997).
2. Torres, A.M., Michniewicz, R.J., Chapman, B.E., Young, G.A.R., and Kuchel, P.W. Characterisation of erythrocyte shapes and sizes by NMR diffusion-diffraction of water: correlations with electron micrographs. *Magn. Reson. Imag.* (in press).

Yours Sincerely,

*Allan Torres*  
Allan Torres

*Bob Chapman*  
Bob Chapman

*PWK for AT*  
Andrew Taurins

*Philip Kuchel*  
Philip Kuchel



## **The NMR Newsletter - Book Reviews**

Book Review Editor: István Pelczer, Dept. of Chemistry, Princeton University, Princeton, NJ 08544

### **"PROTEIN NMR TECHNIQUES"** **(Methods in Molecular Biology, Volume 60)**

Edited by

**David G. Reid**

Humana Press, Totowa, NJ. 1997; <http://www.humanapress.com>;  
ISBN: 0-896-03309-9, 419 pages; List price \$79.50 (Hardcover)

In the Preface of this recent volume assembled by David Reid, the editor states "*Protein NMR Techniques* attempts a treatment of the main themes underlying the study of proteins by NMR in a way that avoids excessive repetition of existing material, and combines theoretical principles with practical advice and examples" In the opinion of this reviewer, this underlying goal is met in the final product.

The book consists of twelve chapters in 419 pages (including a nine-page Subject Index). Chapter 1 presents a broad overview of NMR of proteins, including sample considerations, sequential assignment procedures, secondary structure determination, protein-ligand interactions, and the use of isotope-labeling. Each of these topics could easily make up a chapter on their own, but the authors do an adequate job of presenting the most important points and referring the reader to later chapters for more detailed information. Chapter 2, entitled "Double and Triple Resonance NMR Methods for Protein Assignment", follows the same pattern in that it presents a general overview, references to those who want further information, and a few examples. These examples and the description of the triple resonance experiments would have benefited from the inclusion of at least a few figures, however.

Chapters 3, "Protein Chemical Shifts", provides a brief treatment of chemical shift theory and a good summary of the use of chemical shift information for assignment and structure determination. Chapter 4, "Data Processing in Multidimensional NMR", stands out as the longest chapter in the book, 85 pages including 23 pages of 388 (!) references. A more thorough treatment of this important subject cannot be found anywhere. It is also unique in its clarity and practicality and should therefore be required reading for anyone who processes NMR data. The chapter begins with a general discussion of processing this type of data, discusses how data is prepared for processing, reviews strategies and steps in processing and post-processing, and finally discusses various issues about analyzing the results.

Chapter 5 "Calculating Protein Structures from NMR Data" includes a discussion of the various calculation strategies (e.g. distance geometry, variable target function, simulated annealing) and software (DG-II, X-PLOR, DIANA) as well as a brief description of structure analysis. It also includes a practical account of how experimental restraints are acquired and treated prior to and during structure calculations.

*continued*

Chapter 6 "Studies of Protein-Ligand Interactions by NMR" is a nicely written review that begins with a description of the types of information (e.g. affinity, ligand conformation, dynamics, etc.) that can be obtained by NMR and the NMR parameters that provide this information. A good use of "real-life" examples helps to illustrate this discussion. Finally, an interesting section is included about the detection and dynamics of bound water molecules. Chapter 7, "NMR of Paramagnetic Proteins", provides a brief introduction to the theoretical considerations of paramagnetism, and a very thorough and practical discussion of the technical difficulties involved in the study of these proteins.

Chapter 8 "NMR Studies of Peptides and Proteins Associated with Membranes" by Klassen and Opella, and Chapter 9, "Metal NMR for the Study of Metalloproteins", by Drakenberg, Johansson, and Forsén, are written by leaders in their respective fields. Chapter 8 is a good review of NMR studies that have been carried out on proteins and peptides in micelles and bilayers. Inclusion of some discussion about the practical considerations for setting-up, executing, and analyzing experiments on these types of systems would have been more consistent with the general theme of this volume, however. Chapter 9 stands out in the book as having the most theory, yet this material is also presented with clarity and with a practical core.

From an organizational standpoint, Chapters 10 (Production and Characterization of Recombinant Proteins for NMR Structural Studies), 11 (A Case History: NMR Studies of the Structure of a Small Protein,  $\omega$ -Conotoxin MVIIA), and 12 (Spectrometer Calibration and Experimental Setup: Basic Principles and Procedures), could have been placed at the beginning of the book. Chapter 10 is a very brief description of different options (e.g. *E. coli*, mammalian, insect, or yeast) for producing recombinant proteins. Chapter 11 consists of a step-by-step description of the NMR characterization of a 25-residue peptide. It provides useful insights into the various parts of the process, including resonance assignments, pH studies, and determination of experimental restraints (distance and dihedral). More details about the structure generation process would have added to the completeness of this "case history". Chapter 12 is another chapter that should be required reading for anyone who works on a modern spectrometer. Most of this chapter is devoted to RF pulse calibration and optimization, including hard, spinlock, heteronuclear and shaped pulses. Also included are useful instructions on how to calibrate pulsed field gradients and to implement new pulse sequences.

NMR is moving rapidly from an art form practiced by a relatively few specialists to a practical tool that is used in many disciplines by non-specialists. Hardware vendors are already beginning to address the needs of these non-specialists. For example, gradient-shimming routines have transformed "the art of shimming" to a click of a mouse button. Similarly, some vendors are providing macros that automatically set-up in seconds a full suite of triple resonance experiments that used to take hours for an expert to optimize. Most of us would acknowledge that this is a welcome development, yet it creates a need for educational materials for the non-specialist who will be using NMR as a tool to attack particular problems. *Protein NMR Techniques* goes a long way towards filling this need. Now this book is available on CD-ROM also, as part of a unique collection of over 2500 protein methods: (<http://www.humanapress.com/pprotocols.html>).

**Barry Schweitzer**

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**Forthcoming NMR Meetings**, continued from page 1:

**Pittcon '99**, Orlando, FL, **March 7-12, 1999** (50th year celebration of the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy.) Contact: The Pittsburgh Conference, Dept. CFP, 300 Penn Center Blvd., Suite 332, Pittsburgh, PA 15235-5503; 412-825-3220; Fax: 412-825-3224; e-mail: pittconinfo@pittcon.org;

**Spin Choreography - a symposium in appreciation of Ray Freeman**, Cambridge, England, **April 8-11, 1999**; web site: <http://mchsg4.ch.man.ac.uk/mcmr/RF.html>; fax: c/o M.H.Levitt +46-8-15 2187; email: mhl@physc.su.se.

**41st ENC (Experimental NMR Conference)**, Asilomar Conference Center, Pacific Grove, CA, **April 9-14, 2000**; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073; Email: enc@enc-conference.org.

Additional listings of meetings, etc., are invited.



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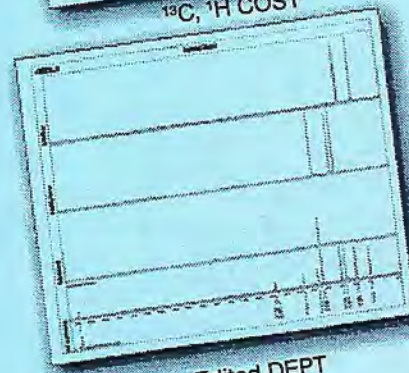
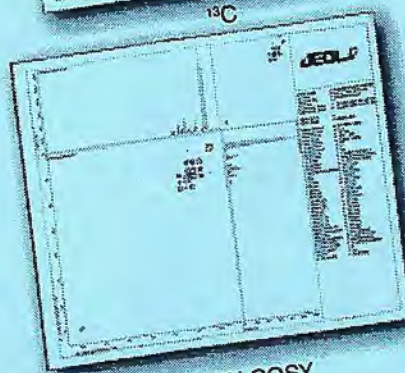
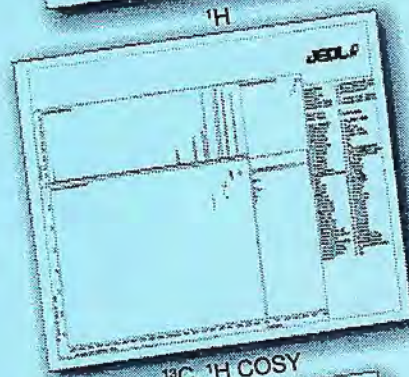
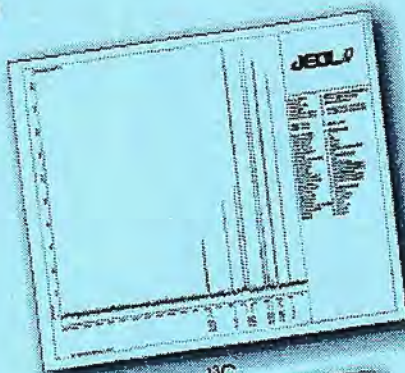
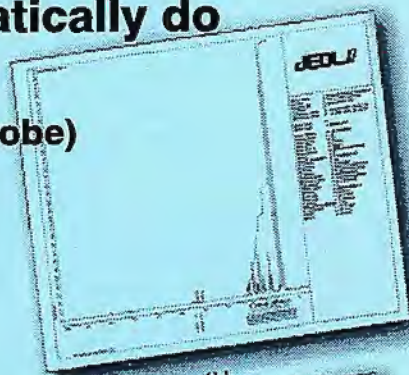
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