THE NMR NEWSLETTER

No. 475

April 1998

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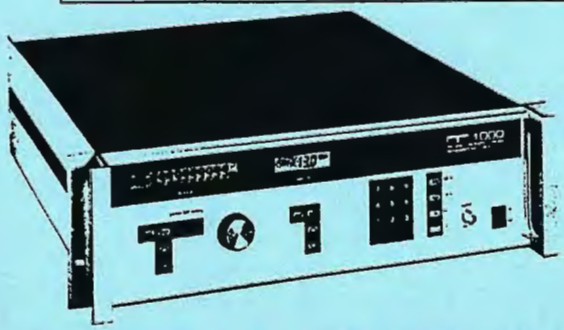
PTS 1000	0.1-1000 MHz	optional .1 Hz to 100 KHz	5-10μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$11,830.00 (1 Hz resol., OCXO freq. std.)
PTS 3200	1-3200 MHz	1 Hz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$14,850.00 (1 Hz resol., OCXO freq. std.)
PTS x10	user specified 10 MHz decade	1 Hz	1-5μs	standard	3½"H×19"W	BCD (std) or GPIB (opt)	$3,000.00 (1 Hz resol., OCXO freq. std.)
PTS D310	two channels .1-310 MHz	.1 Hz	1-20μs	standard	5¼"H×19"W	BCD (std) or GPIB (opt)	$8,560.00 (.1 Hz resol., OCXO freq. std.)
PTS D620	two channels 1-620 MHz	.1 Hz/.2 Hz	1-20 μs	standard	5¼"H×19"W	BCD (std) or GPIB (opt)	$13,240.00 (.1 Hz/.2 Hz resol., OCXO freq. std.)



1 Switching Time is dependent on digit (decade) switched; see detailed instrument specifications.

2 For applicable digits, see detailed instrument specifications.

3 Bench cabinets are 17" wide.

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FORTHCOMING NMR MEETINGS

Sixth Scientific Meeting and Exhibition, International Society for Magnetic Resonance in Medicine, Sydney, Australia, **April 18 - 24, 1998**. Contact: International Society for Magnetic Resonance in Medicine, 2118 Milvia St., Suite 201, Berkeley, CA 94704; 510-841-1899.

NATO ARW "Applications of NMR to the Study of Structure and Dynamics of Supramolecular Complexes", Sitges (Barcelona), Spain, **May 5 - 9, 1998**. Contact: Prof. M. Pons, Dept. Quimica Organica, Univ. de Barcelona, Mart I Franques 1, 08028 Barcelona, Spain; http://www.ub.es/nato/nato.htm; e-mail: miguel@guille.qo.ub.es.

^{13}C in Metabolic Research, Symposium at the University of Texas Southwestern Medical Center, Dallas, Texas, **May 7, 1988**; For more information, contact Jean Cody at 214-648-5886 or www.swmed.edu/home_pages/rogersmr.

New Mexico Regional NMR Meeting, Las Cruces, NM, **May 9, 1998**; Contact: Y. Shachar-Hill, Dept. of Chem. and Biochem., New Mexico State Univ., Las Cruces, NM 88003; Tel. 505-646-3218; Fax. 505-646-2649; Email: yairhill@nmsu.edu. For details, see Newsletter 475, 54.

14th European Experimental NMR Conference, Bled, Slovenia, **May 10-15, 1998**. Contact: The Secretariat of 14th EENC, Dept. of Physics, University of Ljubljana, Jadranska 19, 1000 Ljubljana, Slovenia; Phone: +386-61-1766500; Fax: +386-61-217-281; E-mail: eenc98@fiz.uni-lj.si; http://www.fiz.uni-lj.si/~stipe/eenc98/eenc98.html.

Workshop on Magnetic Resonance of Connective Tissues and Biomaterials, Philadelphia, PA, **June 18-20, 1998**; For more information. Contact International Society for Magnetic Resonance in Medicine, 2118 Milvia Street, Suite 201, Berkeley, CA 94704; (510) 841-1899; fax (510) 841-2340; info@ismrm.org; http://www.ismrm.org.

Continued on p. 56

Cornell University

Department of Chemistry
Baker Laboratory
Ithaca, New York 14853-1301 USA

Cathy C. Lester
February 18, 1998
(received 3/2/98)

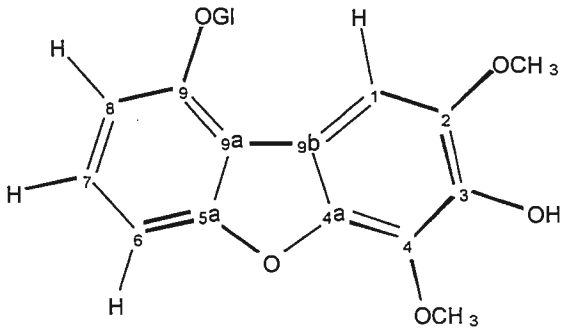
Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

NMR and Rotten Apples

Dear Barry,

Venturia inaequalis is the organism responsible for the ugly brown scabs that plague certain varieties of apples. We have recently employed high-resolution methods to elucidate the structure of a natural pesticide, or phytoalexin, found to provide protection against this organism in certain varieties of apple. The compound was isolated from cultures of the apple-scab resistant Liberty apple cultivar challenged with yeast extract to mimic the effect of biological stress such as fungal invasion. Cultures of the apple-scab susceptible McIntosh cultivar subjected to the same stresses produced no phytoalexin.

The major compound produced by the scab-resistant cells was identified as 2,4-methoxy-3-hydroxy-9-*O*-β-D-glucosyloxydibenzofuran and is shown below. gHMQC, gHMBC and heteronuclear $^{3}J_{CH}$ coupling constants were used to identify and position the substituents of the oxydibenzofuran ring.



It is not clear what the active form of the compound is, however, the aglycone form showed higher toxicity to *V. inaequalis* than the parent molecule. Investigations of the structures of related compounds that may be involved in the resistance of apples to this scab disease are currently underway.

Sincerely,

Geza Hrazdina Wlodzimierz Borejsza-Wysocki Cathy Lester

Geza Hrazdina Wlodzimierz Borejsza-Wysocki Cathy Lester

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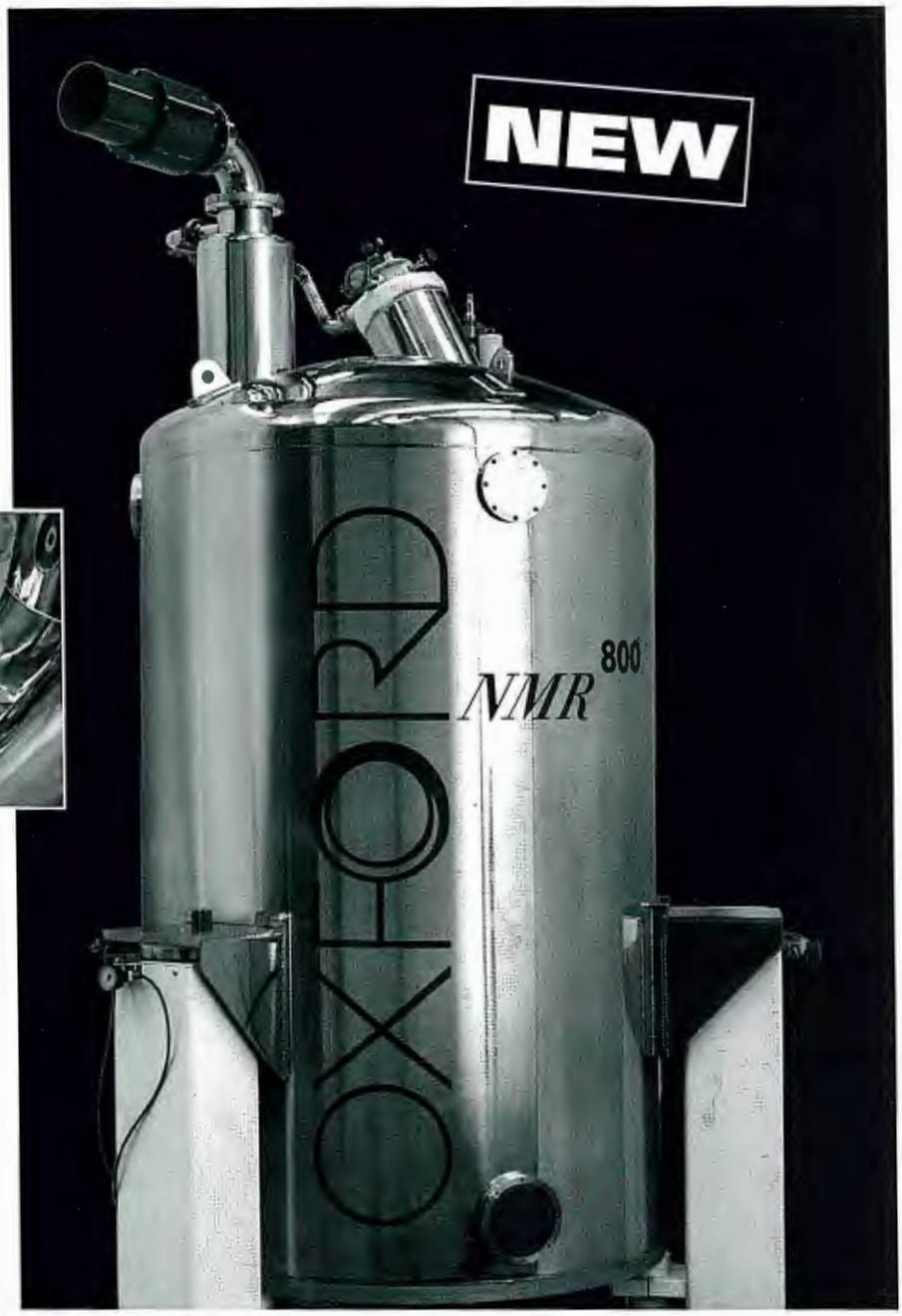

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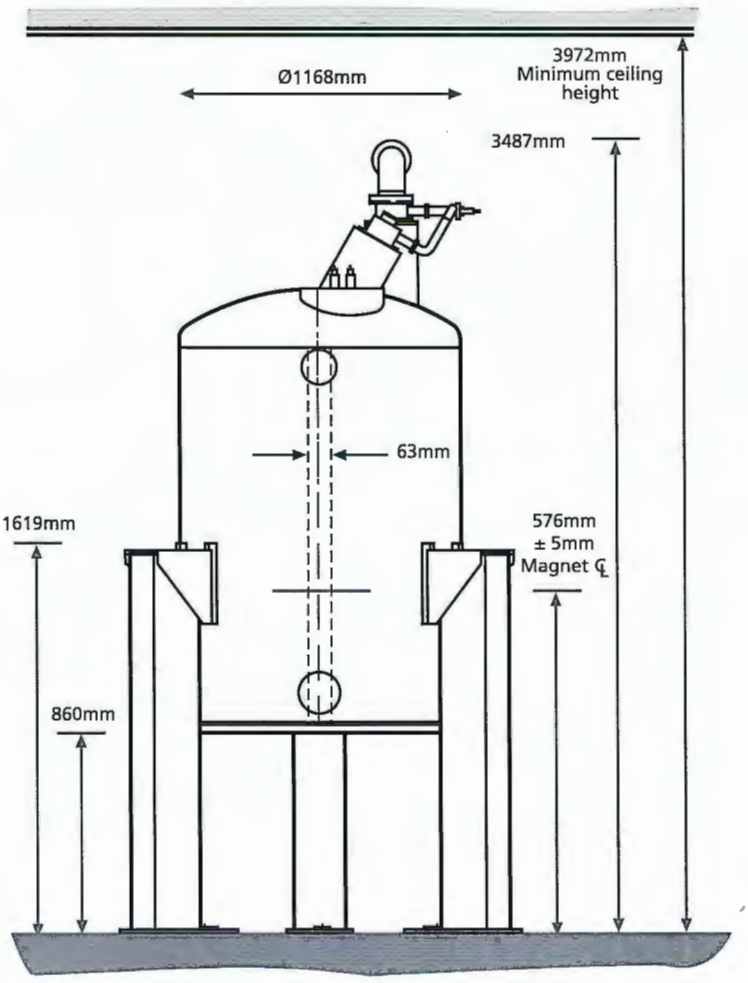

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Zürich, March 16, 1998
(received 3/24/98)

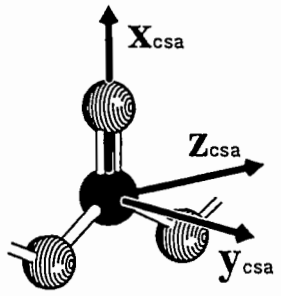
Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303
USA

Orientation of the $^{13}C{=}O$ Chemical Shielding Tensor in Diphenyl Carbonate

Dear Barry,

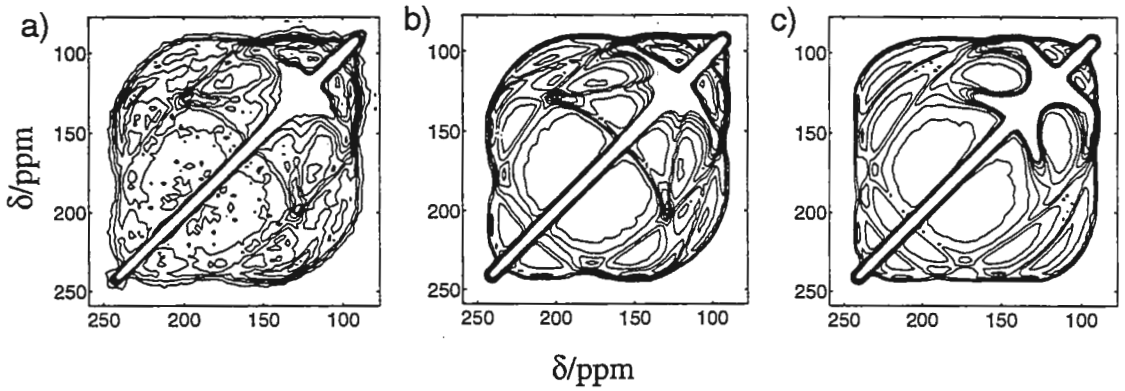
Recently, we have investigated the orientation of the ^{13}C chemical shielding tensor in the carbonate group of diphenyl carbonate, $C_6H_5O(^{13}CO)OC_6H_5$, using spin diffusion measurements under slow magic angle spinning.[1] The most shielded direction of the tensor was found to be along the C=O double bond. The least shielded direction lies in the plane of the carbonate group, perpendicular to the C=O double bond. The orientation and the eigenvalues of the tensor, shown below, are in quantitative agreement with quantum chemical calculations based on density functional theory.

	δ_{xx}/ppm	δ_{yy}/ppm	δ_{zz}/ppm
Experimental	88 ± 2	125 ± 2	239 ± 2
Calculation	78	133	228



The x and y axes (most and intermediate shielded directions) of the tensor orientation we obtained are interchanged with respect to the orientation of the carbonate chemical shielding tensor that has been assumed for bisphenol-A poly(carbonate) in the past.[2–5] The previous erroneous assumption was mainly based on the fact that in carbonyl and carboxylic groups the most shielded direction is perpendicular to the sp^2 plane. So far, no experimental evidence for carbonate groups has been available. The consequences of the incorrect orientation assumed in previous work will be discussed in detail in a forthcoming publication.[6]

The figure below shows the two-dimensional quasi-equilibrium spin diffusion spectra of ^{13}CO-diphenyl carbonate obtained by measurement (a), and by simulation assuming the correct (b) and incorrect (traditional) (c) assignment of the principal axes.



Our results demonstrate that assignment of chemical shielding tensor principal axes "by analogy" to a related class of compounds must be done with caution.

Yours sincerely,

Marcel Utz

Pierre Robyr

Richard R. Ernst

[1] Z. Gan and R. R. Ernst, Chem. Phys. Lett. **253**, 13 (1996).

[2] P. M. Henrichs, M. Linder, M. Hewitt, D. Massa, and H. V. Isaacson, Macromolecules **17**, 2412 (1984).

[3] M. Tomaselli, P. Robyr, B. Meier, C. Grob-Pisano, R. R. Ernst, and U. W. Suter, Mol. Phys. **89**, 1663 (1996).

[4] M. Tomaselli, M. M. Zehnder, P. Robyr, C. Grob-Pisano, R. R. Ernst, and U. W. Suter, Macromolecules **30**, 3579 (1997).

[5] M. Utz, M. Tomaselli, R. R. Ernst, and U. W. Suter, Macromolecules **29**, 2909 (1996).

[6] P. Robyr, M. Utz, Z. Gan, C. Scheurer, M. Tomaselli, U. W. Suter, and R. R. Ernst, submitted for publication (1998).

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

March 5, 1998
(received 3/6/98)

Dear Barry:

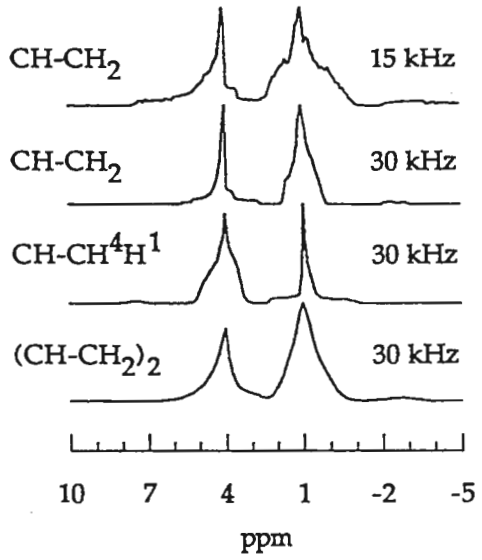
Proton NMR Line Narrowing by MAS

I am submitting this contribution to your Newsletter in honor of the fiftieth anniversary of the introduction of the homonuclear dipole interaction as a useful tool in solid state NMR. In April 1948 Pake's doublet[1] was published and in November of that year it was followed by Van Vleck's second moment calculation.[2] These two papers demonstrated the quantitative use of dipolar lineshape effects for the determination of internuclear distances, a notion that has stayed with us ever since as one of the cornerstones of NMR spectroscopy. Pake established the fundamental aspects of the dipolar interaction of an isolated spin pair and Van Vleck showed how these intrapair interactions function as the building blocks determining lineshapes of macroscopic samples.

Magic-angle-spinning has recently advanced to such high rates and the magnetic field has reached such high strengths that single-pulse proton MAS spectra with modest chemical-shift resolution are beginning to become a reality. It is well known that the average of the dipolar interaction over a MAS rotor cycle is zero. However, this leads to complete narrowing of the centerband of the MAS spectrum in only a few select cases, of which Pake's isolated spin pair is the primary example. When more spins interact, the fast-MAS spectrum is broadened by higher-order averaging effects. As far as I know, our level of understanding of the residual widths and shapes of these peaks is less than desirable. Following several examples in the literature,[3] I have tried to see if something can be learned from the first-order term of average Hamiltonian theory (the zero-order term vanishes rigorously). This term in the Magnus expansion is proportional to D^2/ω_R, where D is the size of the dipole interaction and ω_R is the spinning speed. For spinning rates of 15 kHz and higher this Hamiltonian predicts proton lineshapes that closely resemble spectra obtained by more rigorous methods, such as those calculated by my brother Shimon and his coworkers at the Weizmann Institute who are investigating MAS spectra of multiproton systems using Floquet theory.[4]

The first-order Hamiltonian is a sum of three-spin terms of the form $I_{iz}(I_{jx}I_{ky}-I_{jy}I_{kx})$ with coefficients that contain the geometric information. Thus the building blocks are spin triads rather than pairs. Understanding the triad spectra is a first step towards predicting MAS peak widths in multispin clusters. But giving a comprehensive description of triad spectra is more complicated than describing the doublet of a static spin pair. First, while the geometry of a pair

is fully characterized by just one parameter (the distance), a triangle is determined by three parameters. Second, whereas the Pake doublet description has practical validity without consideration of chemical shift differences, this is not so in the case of MAS spectra where the objective is to achieve chemical shift resolution. Third, the Hamiltonian is 8 by 8, the largest independent blocks being 3 by 3. By comparison, the largest block in the Pake doublet case is 2 by 2 and thus has an analytical solution. Finally, the dipolar matrix elements for a triad are all off diagonal, in contrast to the static pair Hamiltonian which has diagonal elements. This is actually very fortunate, because the dipolar line broadening will be diminished when neighboring spins are nonequivalent. On the other hand, it makes the theoretical description more intricate.



The figure shows 600 MHz MAS spectra of the triad of a hypothetical $CHCH_2$ group with chemical shifts of 4 and 1 ppm. Spinning speeds are as indicated. The lineshapes were calculated from a first-order average Hamiltonian. One notes that the widths at the base scale as the inverse of ω_R, but that some of the other features narrow at a faster rate. This is a consequence of the above-mentioned off-diagonal character of the Hamiltonian. The CH_2 peak is broader than the CH peak, but this is not a result of its shorter HH distance. In fact, all three pair interactions simultaneously contribute to all broadenings of the triad spectrum. The linewidth difference between the two peaks is instead due to the fact that the CH proton is magnetically nonequivalent from the other two. We can artificially turn that around by setting the shift of one of the CH_2 protons to 4 ppm, as is seen in the third spectrum. The bottom spectrum gives an indication of what happens to larger clusters. It is the spectrum of two of the same triads strung together on an all-trans $CH\text{-}CH_2\text{-}C\text{-}CH\text{-}CH_2$ chain. One is struck by the onset of Lorentzian-like features in the CH lineshape. Lorentzian peak shapes have indeed been observed in experimental fast-MAS spectra. This agreement is encouraging but, to my knowledge, we are still far away from a Van Vleck-like model that can predict the linewidths directly from the crystal geometry.

Please credit this contribution to the account of Chris Roe.

Sincerely,

Alex

Alexander J. Vega

1. G. E. Pake, J. Chem. Phys. 1948, **16**, 327.
2. J. H. Van Vleck, Phys. Rev. 1948, **74**, 1168.
3. M. M. Maricq and J. S. Waugh, J. Chem. Phys. 1979, **70**, 3300; E. Brunner, D. Freude, B. C. Gerstein, and H. Pfeifer, J. Magn. Reson. 1990, **90**, 90; R. Challoner and C. A. McDowell, J. Magn. Reson. 1992, **98**, 123.
4. S. Ray, G.-J. Boender, E. Vinogradov, and S. Vega, to be published.

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Dr. B.L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 943203

March 11, 1998
(received 3/16/98)

Subject: **SAR by ^{19}F** NMR

Dear Barry:

Following the ideas of Steven Fesik's group [1] which used proton NMR to screen small molecules for binding to proteins, we have found fluorine NMR very useful in this respect. Figure 1A shows ^{19}F NMR spectrum of a mixture of ten fluorinated compounds (either containing a -CF_3 or -F group), of ca. 10 μM concentration each in H_2O/DMSO (9:1/v/v) solution. The spectra (which follow) were obtained for this mixture of fluorinated compounds in the presence of different proteins, B-E, of ca. 10μM concentration each (Figure 1B-E). By inspection it appears that just compound 6 binds weakly to proteins B and C whereas compounds 1, 2, 4, 9 and 5, 6, respectively, are weak and strong binders to protein D. Finally, only compounds 4 and 9 do not bind or bind less competitively to protein E. The method is straightforward and sensitive (the spectra were recorded on a DRX-500 spectrometer equipped with the dual ^{1}H/^{19}F/^{3}H 5mm Nalorac probe, 1600 scans, 50 min per spectrum, exponentially multiplied with LB=1Hz and Fourier transformed). Although this approach requires the presence of ^{19}F nucleus in the molecule, the Available Chemicals Directory alone lists over 30,000 fluorinated compounds. So far, we have measured 10 compounds per NMR-tube but it seems feasible to significantly increase this number.

Sincerely,

Leszek Poppe

Stefan Groeger

[1] P. J. Hajduk, E. T. Olejniczak and S. W. Fesik , J. Am. Chem. Soc. 1997, 119, 12257-12261.

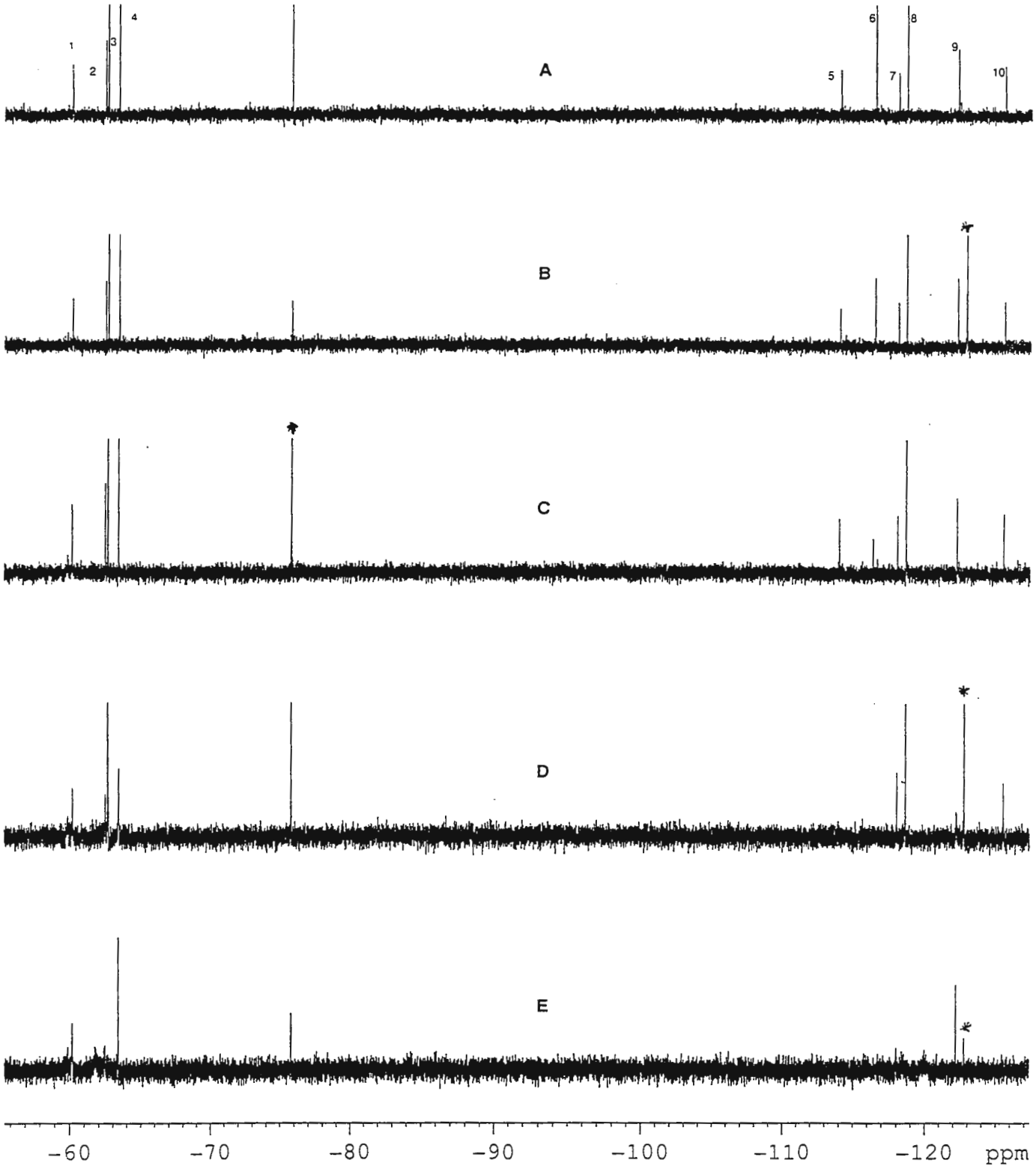

Figure 1. ^{19}F NMR spectra of the mixture of 10 compounds in the presence of different proteins. Signals marked with asterisks are from TFA and from F^-.

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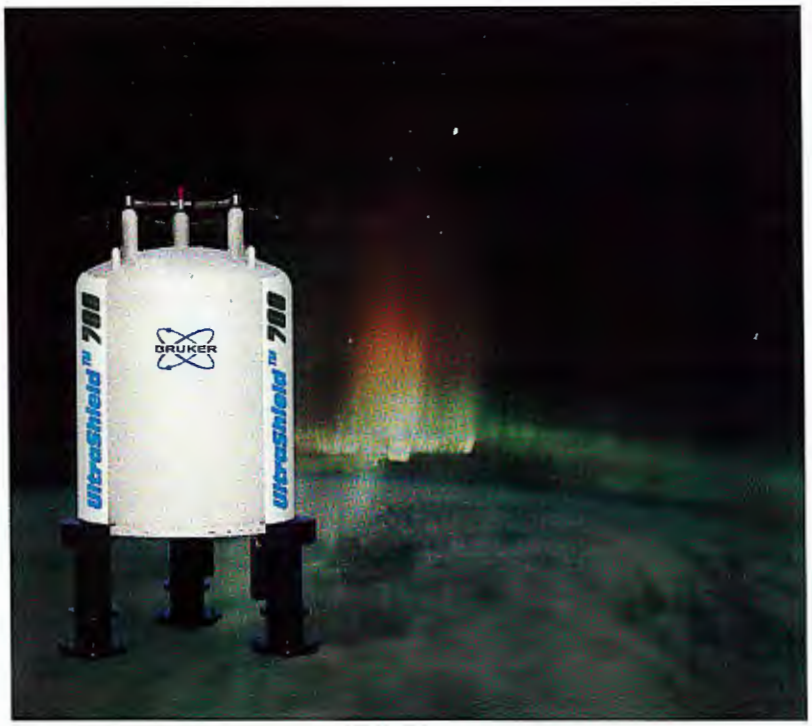

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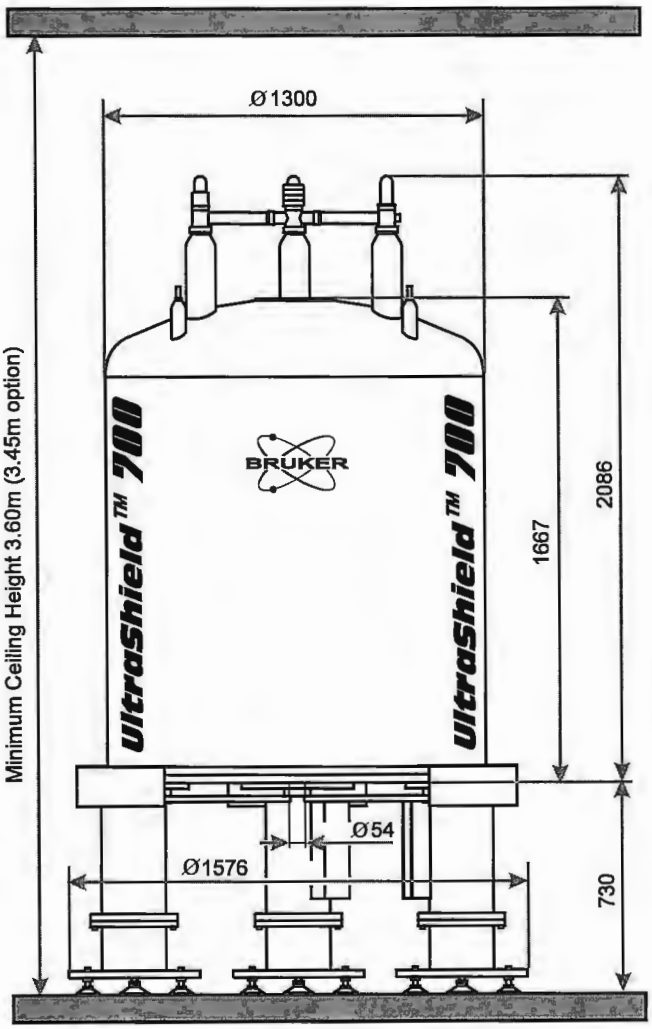
Cryoshims	Z, Z^2, Z^3, X, Y XZ, YZ, XY, X^2-Y^2
Axial Range with Field Homogeneity better than 10 ppm (w/o RT Shimming)	~ 60 mm
5G Line from the Magnetic Center - radial distance - axial distance	 < 2.5 m < 3.5 m
Resolution at 50% 1% $CHCl_3$ 5mm spinning	< 0.55 Hz
Lineshape 1% $CHCl_3$ 5mm non-spinning at 0.55% at 0.11%	 < 7 Hz * < 14 Hz *
Spinning Sidebands	< 2%

* Typical values obtained with the BOSSII™ shim system.

700MHz/54mm UltraShield™ Magnet



Dimensions in millimeters unless stated otherwise

CRYOSTAT

Helium Evaporation Rate	~110ml/hr
Helium Refill Volume	~ 315 liters
Helium hold time	> 120 days
Nitrogen Evaporation Rate	~ 725 ml/hr
Nitrogen refill volume	~ 365 liters
Nitrogen hold time	> 21 days
Weight Without Cryogens	2700 kg
Weight Including Cryogens	3200 kg
Minimum Ceiling Height	3.6 m
Reduced Minimum Ceiling Height (with special equipment)	3.45 m

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Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
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U. S. A.

Feb. 19, 1998
(received 3/2/98)

Dear Barry;

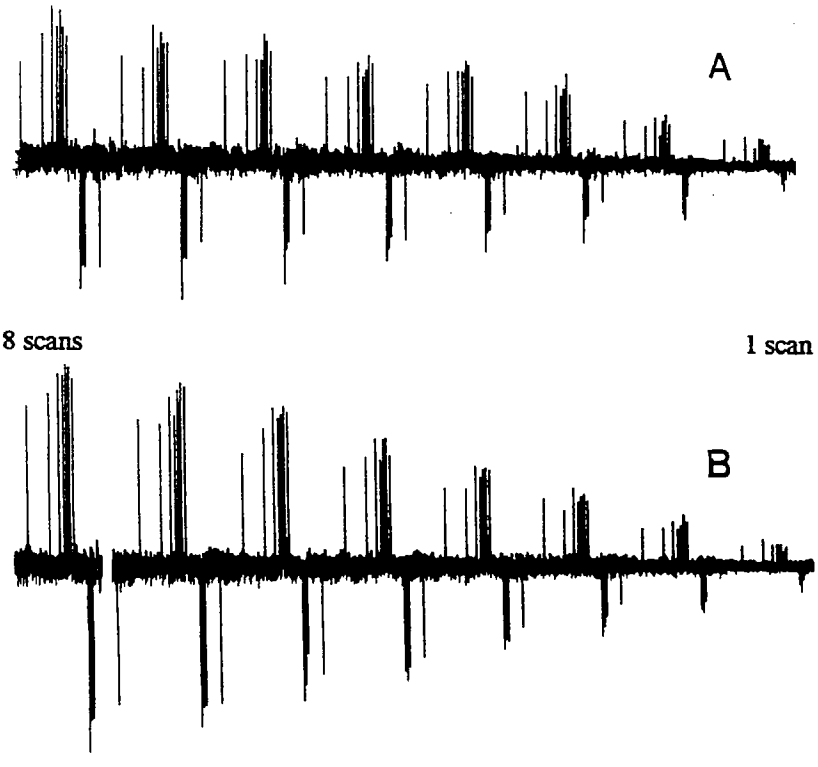
EXPLICIT RECEIVER PHASE ON A BRUKER AM SYSTEM

We have Bruker AM type spectrometers and had been assuming that when specifying the receiver phase as QP, the receiver is actually cycled in the order 0,0,2,2 1,1,3,3. Unfortunately this is not the case. If one reads carefully the section in the Bruker Software Manual on receiver phase cycling one finds that they use a combination of transmitter phase and receiver phase in order to obtain the equivalent of the above cycle. Shown below are the results of a series of APT experiments on sucrose (A) without and (B) with an explicit receiver phase cycle (eg., GO=2 PH4 , where PH4=R0 R0 R2 R2 etc.) with the QP flag set.. These spectra were obtained with increasing number of scans, one scan collected for the spectrum on the extreme right with one additional scan added to each spectrum as one procedes to the left. It can be seen in the spectra in series A that the s/n ratio increases as expected for the first four experiments but then levels off for the remaining four whereas in B the series of spectra obtained with the receiver phase explicitly stated increases as expected. Moral of the story-don't take anything for granted!

Sincerely;

Tom Nakashima

Tom



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Dr. Bernard L. Shapiro
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Feb. 20, 1998

Testing LC-NMR Technology.

(received 2/26/98)

Increased emphasis on automation and on exploitation of new technologies in pharmaceutical discovery and development has heightened interest in liquid chromatography coupled with NMR spectroscopy (LC-NMR). One particularly relevant application of LC-NMR involves the characterization of metabolites. A more established analytical method for characterization of metabolic products is LC-mass spectroscopy (LC-MS). For this reason, many of the people who are most interested in LC-NMR are somewhat less familiar with NMR spectroscopy than they need to be in order to design experiments for LC-NMR demonstrations. It is also common that NMR spectroscopists lack sufficient expertise in the preparation and handling of metabolite samples to confidently generate a protocol for the LC portion of these experiments. Here we would like to share some details of recent experience with an LC-NMR experiment for detection of metabolites. We commonly refer to this experiment as "the Tylenol test." The experiment is based on published work by Nicholls, et. al.[1], and Spraul, et. al.[2]

Sample Preparation: We choose paracetamol (aka. 4-acetamidophenol, or Tylenol) because it is readily available and has well characterized metabolites.[1] The dosage is the maximum recommended for adults: 1000 mg every 4 to 6 hours. This dosage corresponds to roughly 14 mg/kg, which represents a typical dosage for animal studies of drug metabolism. A human volunteer collects urine specimens over a 24 hour period. The donor avoids ingestion of alcohol and caffeine during the sample collection period. The specimens are combined to form a single sample. A 200 ml aliquot of the pooled urine samples is brought to pH 2.0 with HCl and centrifuged. The supernatant is then subjected to solid phase extraction[1,2] (SPE) which removes many of the inorganic salts. The sample is eluted from the SPE column with methanol. After SPE, the concentrated urine in water/methanol is dried under vacuum and gives a solid mass of approximately 700 mg from the 200 ml original urine sample. If one allows the concentrated urine to remain in the water/methanol solvent overnight, a precipitate forms. Removal of this precipitate gives a more powder-like sample while retention of the precipitate causes the solid to form a tar. While the powder-like sample is much easier to divide into fractions of arbitrary mass, we did not run LC-NMR on the powder because of the possibility that the paracetamol metabolites could be removed with the precipitate. The injectable solution is formed by dissolving 30 to 40 mg of the tar in 200 µl of 30% CD_3CN/70% D_2O/0.1% CF_3OD, pH 2. Control human urine samples are also prepared in a similar manner as described for the Tylenol containing samples.

Chromatographic Conditions: For on-flow NMR experiments, inject 50 µl of sample into a Spherisorb ODS-2, 3 µm, 4.6x150 mm HPLC column. Chromatography is run at a flow rate of 1 ml/min with 250 nm Ultraviolet (UV) monitoring on a Hewlett-Packard HP1100. The chromatographic mobile phase consists of: (A) 98.9% D_2O / 1% CD_3CN/0.1% CF_3OD, and (B) CD_3CN/0.1% CF_3OD and the following gradient ramp is

applied: 0 min, 0%B; 10 min 0%B; 30 min, 20%B; 38 min, 60%B; 40 min, 60%B; 41 min, 0%B. Total run time is 50 min. For stopped-flow NMR experiments, 5 µl of sample is injected. The chromatographic run is started and stopped either manually, or in an automated manner controlled by UV peak detection.

Figure 1. Chemical shift vs. LC-retention time. On-flow LC-NMR spectrum of human urine containing paracetamol and its metabolites. The top trace corresponds to the paracetamol glucuronide metabolite[1] which has a retention time of 11.3 minutes. The sample injection volume was 50 µl.

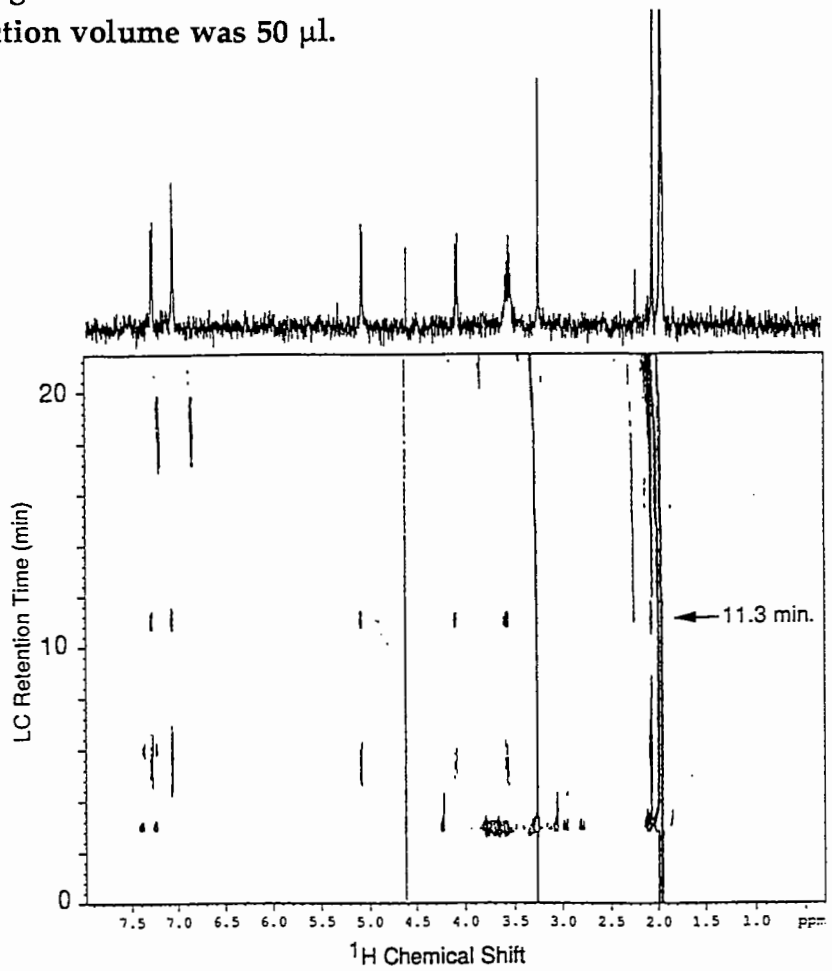
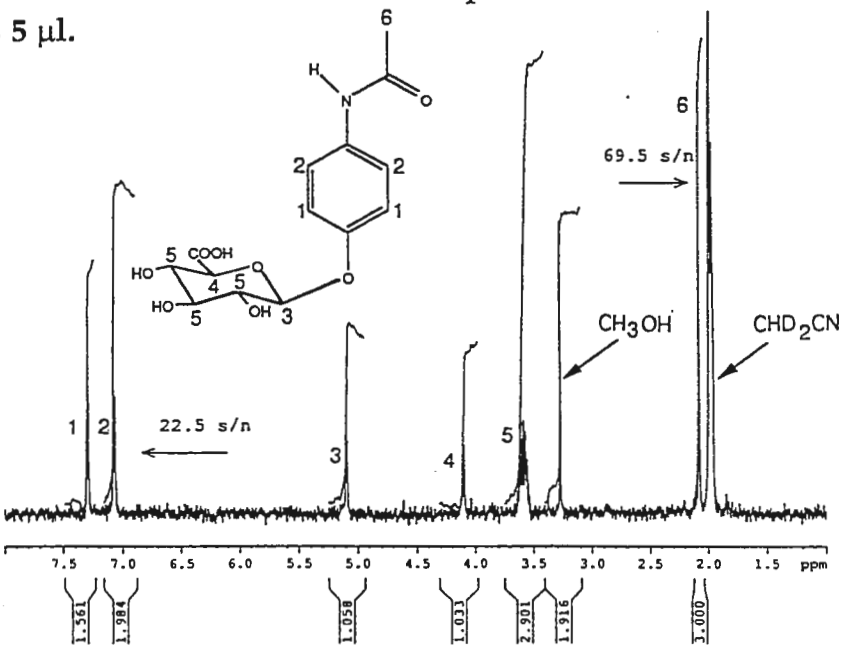


Figure 1 illustrates the on-flow experiment where 1D ^{1}H-NMR spectra are collected in succession while the LC mobile phase flows through the NMR probe at 1 ml/min. The spectra have been collected on a 500 MHz Bruker Avance equipped with a ^{1}H/^{13}C-Gradient flow-probe (120 µl active volume, 4mm diameter). The quality of the data is best illustrated by looking at the NMR spectrum of an individual metabolite. The trace above the on-flow data represents the 1D ^{1}H-NMR spectrum of the paracetamol glucuronide metabolite which appears at the 11.3 minute retention time. This spectrum was extracted from the on-flow data at the position indicated by the arrow in Figure 1. The 50 µl injection was taken from a stock solution obtained by dissolving 47 mg of tar in 0.3 ml $D_2O/CD_3CN/CF_3OD$ (157 mg/ml). Each 1D spectrum was acquired using 16 transients of a first increment NOESY pulse sequence. The residual HOD signal was saturated during the relaxation delay and during the 60 msec NOESY mixing time. The residual CHD_2CN was not irradiated in order to avoid partial off resonance saturation of the CH_3 signal near 2.1 ppm. Figure 2 illustrates the stopped-flow spectrum of the paracetamol glucuronide. This spectrum was obtained from a 5 µl injection and was signal averaged over 128 transients. The enhanced signal-to-noise

obtained for this more dilute sample illustrates the importance of the stopped-flow experiment. The 5 µl injection contained .78 mg of the tar-like solid. This amount of tar is derived from 223 µl of human urine. While we have not explored the long term stability of the tar-like sample, assuming good stability, the high yield of paracetamol metabolites in urine allows multiple LC-NMR technical demonstrations to be performed from a single 200 ml sample preparation. This enables scientists who are evaluating LC-NMR to run demonstrations with multiple vendors and to validate the installed LC-NMR spectrometer using the same sample.

Figure 2. Stopped-flow 1D ^{1}H NMR first increment NOESY spectrum of the paracetamol glucuronide metabolite. This spectrum was processed with 1.0 Hz line broadening. Resonance assignments are illustrated with the numbers of each signal drawn next to the carbon to which the proton is attached. The sample injection volume was 5 µl.



The Tylenol test is a realistic yet practical experiment for testing LC-NMR technology. We would like to thank Dr. John Shockcor for many helpful discussions which enabled us to successfully collect high quality LC-NMR data of these metabolites on our first attempt. We thank Dr. Patricia Stone Wilkinson and Dr. Christiane Grzonka of Bruker Instruments for their expert application of this procedure. We also thank Ms. Shaoyong Li of Hoffmann-La Roche for metabolite preparations.

Please credit this contribution to the account of David C. Fry.

1. Nicholls, A. W.; Farrant, R. D.; Shockcor, J. P.; Unger, S. E.; Wilson, I. D.; Lindon, J. C.; Nicholson, J. K. *J. Pharm. Biomed. Anal.* **1997**, *15*, 901-910.
2. Spraul, M.; Hofmann, M.; Dvorstak, P.; Nicholson, J. K.; Wilson, I. D. *Anal. Chem.* **1993**, *64(4)*, 327-330.

Sincerely,

Steven Donald Emerson
Principal Scientist, Biomolecular NMR

David C. Fry
Research Leader, Biomolecular NMR

Hequn Yin
Principal Scientist, Drug Metabolism

David Moore
Research Leader, Drug Metabolism

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Center for Interdisciplinary Magnetic Resonance

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Dr. Barry Shapiro,
The NMR News Letter
966 Elsinore Court
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March 23, 1998
(received 3/27/98)

Characterization of High Temperature Superconducting (HTS) NMR Probe at 400 MHz

Dear Barry,

We recently received from Varian a high temperature superconducting NMR probe for our Unity Inova 400 MHz NMR instrument. It is on loan from Varian and NHMFL is a beta site to evaluate the performance of this probe. It is in principle capable of performing double resonance experiments on two different nuclei. The probe we received features YBCO based HTS coils tuned to proton frequency at 400 MHz and a deuterium coil which is used for field-frequency locking purposes. The operating temperature for the HTS coils is 25 degree Kelvin and at this temperature the Q of the proton coil is over 20000.

Initial measurements showed (Fig. 1) an impressive proton S/N ~ 1700 which is nearly 4 times higher than that for the conventional probes at this frequency. The lineshape that is achieved on this probe (Fig. 2), although somewhat inferior to that from a conventional probe, is not unreasonably poor. Due to the high Q of the coil there is significant amount non-linear behaviour with respect the coils response to rf power. In figure 3 the pulse width (pw) calibration at two power levels (51 dB and 63 dB) show such effects. Within a given power, the pw of 180^0 pulse width is not just double of the pw of a 90^0 pulse and even in the linear regime of the rf amplifiers, reduction of rf power by 6 dB doesn't increase pw of 90^0 pulse by two fold. We have to make a look-up table for pulse widths at different pulse powers in order to be able to set up 2D CAMELSPIN or ROSY and TOCSY experiments.

It appears that this new technology does not pose much serious complications in routine operation and promise to improve the quality of NMR spectra under various conditions that require high sensitivity. Although the Q of the coil dropped in the presence of salt used in buffers, this technology offers valuable application in the study of biological systems.

With best regards,

Nagarajan Murali

Sensitivity Test (0.1% ethyl benzene) S/N = 1693

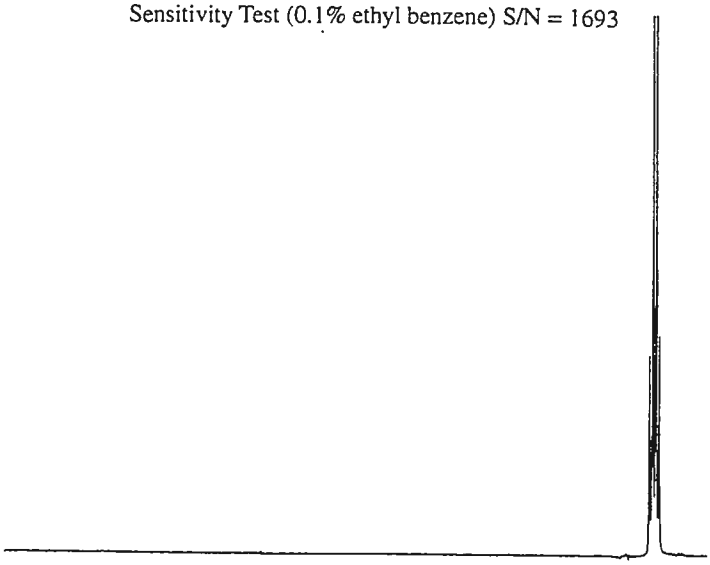

Figure 1

Lineshape Test: 0.88 / 7.26 / 14.14 Hz @ 50% / 0.55% / 0.11%

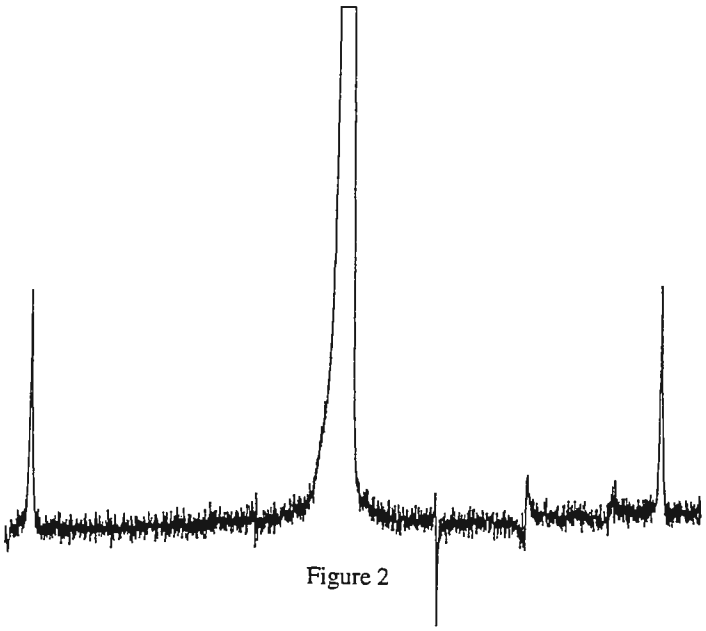

Figure 2

pw90 Calibration at Different Power Levels
The performance is nonlinear within and between the power levels.

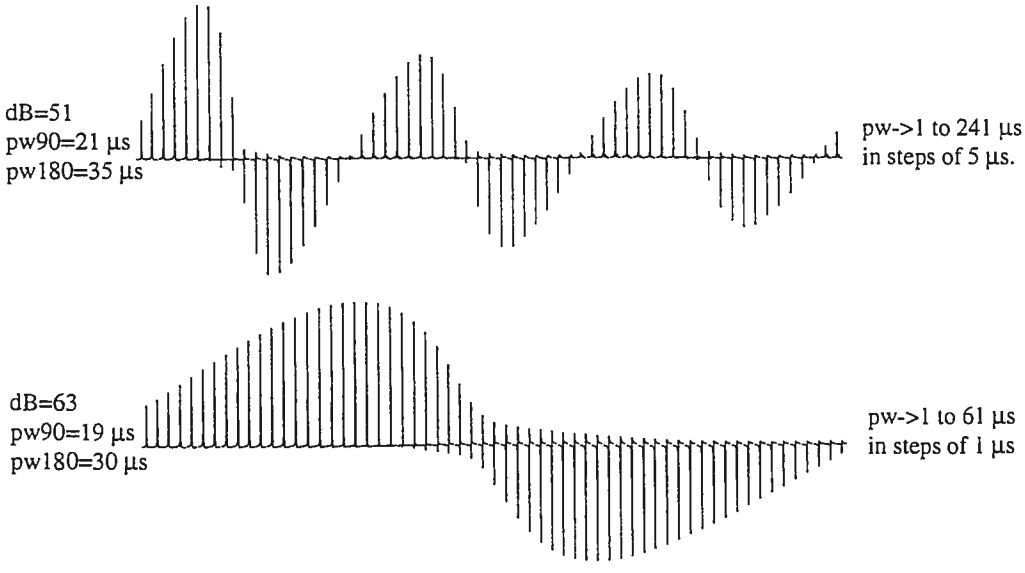

Figure 3

Family Matters

From left to right:

Don McReynolds
Earthy Lee Young
Remonda Lavinghouse
James Brewer
Gary Skidmore

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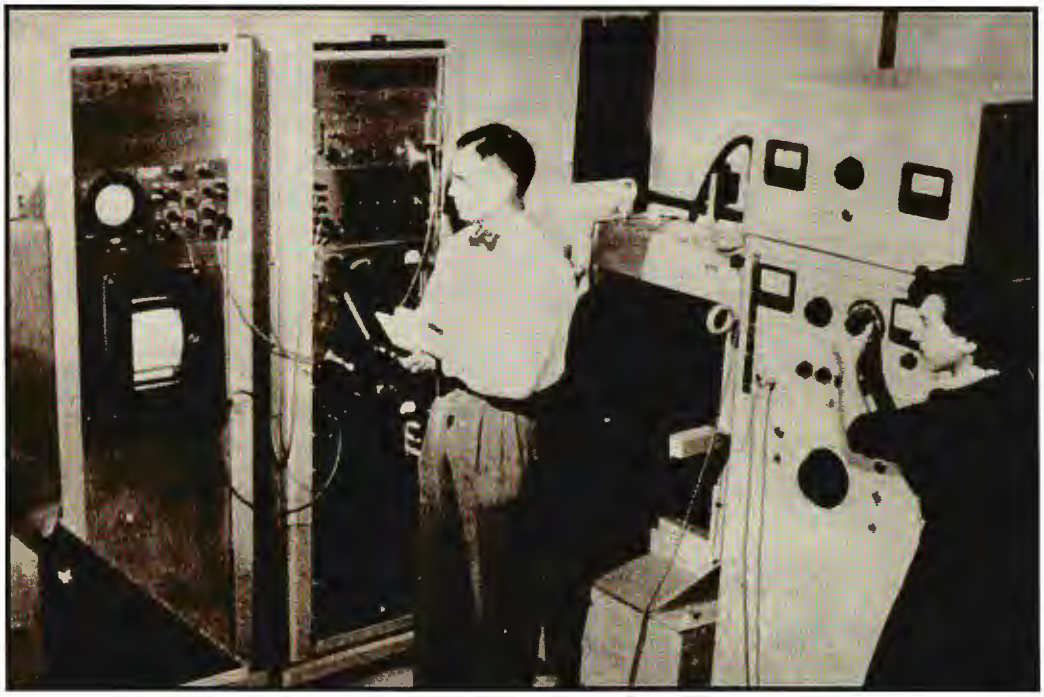

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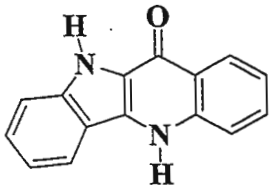
March 19, 1998 (received 3/20/98)

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

Putting ^{15}N into Alkaloid NMR Using SubMicro Probes

Dear Barry,

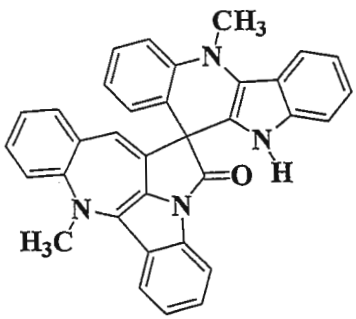
Alkaloid chemists, despite the fact that every compound they're truly interested in contains nitrogen, have not had ready access either ^{15}N chemical shifts or long-range ^{1}H-^{15}N connectivities at natural abundance. With the advent of micro NMR probes in 1992[1], followed by gradient versions of these probes, experimental access to ^{15}N data as a structural probe has begun to improve. For example, we were able to acquire a ^{1}H-^{15}N HMQC spectrum of the indoloquinoline alkaloid quindoline (**1**) using an 800 µg sample in 150 µl d6-DMSO in a 3 mm micro inverse probe overnight. Since both of the nitrogen resonances in this alkaloid are protonated, the heteronuclear shift correlation data were relatively easy to acquire even performing the experiment at 400 MHz.[2]



1

The first tentative step in this area was not, however, a panacea. Having access to protonated ^{15}N chemical shifts, even at the milligram level did not make ^{15}N useful as a structural probe for the alkaloid chemist. That step would begin in 1993 with data presented at meetings in San Diego, CA by a member of this group[3] and the group led by Koshino who presented in Tokyo.[4] These seminal papers described the first successful heteronuclear correlation experiments. The first published reports of long-range ^{1}H-^{15}N correlation applications began to appear in the literature in 1995. Applications were reported during that year to ajmaline, several *Strychnos* alkaloids, and the bis-indole anticancer drug Navelbine™ in a series of three papers.[5-7] Koshino and co-workers reported applications to thiamine HCl, strychnine, 1,2,4-triazolo[1,5-a]pyrimidine, nicotine, and quinomycin-A that same year in a review paper.[8]

Since those initial reports, there has been a continually increasing level of interest in the use of long-range ^{1}H-^{15}N heteronuclear shift correlation experiments to solve structural problems. In general, groups working with 5 mm gradient inverse detection probes have utilized large samples, ranging into 10's of mg and more. Studies conducted in 3 mm gradient inverse probes have been done using more modest samples, generally <10 mg and sometimes <5 mg. This brings us to the *raison d'être* for the present contribution. Of late, we have been exploring the fascinating capabilities afforded by a new generation of NMR probe -- the prototype SMIDG™-600-1.7 (submicro indirect-detection gradient) probe built for us by Ron Crouch and Toby Zens at Nalorac Corporation in Martinez, CA. We now would like to report some of our first results using this probe for ^{1}H-^{15}N correlation experiments in conjunction with a set of test ^{1}H-^{13}C correlation experiments using the complex spiro-nonacyclic alkaloid cryptospirolepine (**2**).



2

Cryptospirolepine (**2**)[9] has a molecular weight of 505 da. The sample used for all of the NMR spectra described in this contribution was prepared by dissolving 750 µg of the alkaloid in 30 µl of 99.996% d6-DMSO (Cambridge Isotope Laboratories) which was then transferred under a dry argon atmosphere in a glove box to a 1.7 mm SMIDG NMR tube. The availability of gradient submicro NMR probe capabilities enables one to readjust his thinking and frame of time reference in terms of data acquisition. As we have shown previously, using a SMIDG probe allows the acquisition of GHSQC and GHMBC spectra of samples as small as 0.04 µmole in reasonable periods of time. The converse, however, is also true, with more generous samples, assuming that they will dissolve in as little as 30 µl of solvent, it is possible to acquire data very quickly. As an example, a pair of GHSQC spectra of 2 are shown in Figure 1. In the bottom panel (A), the data were acquired as a total of 16 t_1 increments of 1 transient/increment to digitize an F_1 spectral window of 45 ppm. Although the F_1 resolution is obviously coarse, the data were acquired in 34 sec. The data were linear predicted to 64 files before transformation. With a little

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more patience, a spectrum nicely resolved in F_1 can be acquired in slightly less than 5 minutes (48 files in F_1, nt = 2, linear predicted to 192 files before transformation). These data are shown in Figures 1A and B, respectively. While one would not normally opt to record a GHSQC spectrum under the conditions used to generate the data in Fig. 1A, the data do suffice to illustrate the level of sensitivity offered by this new probe technology.

GHMBC spectrum can also be acquired rapidly at this level. Acceptable quality data can be obtained in 16 minutes (ni = 96, nt = 4); correspondingly higher quality data, again, are obtained in slightly less than 1 hr. Protonated carbons can be sequenced through either the acquisition of a homonuclear TOCSY interpreted in conjunction with the GHSQC data or via a single IDR(Inverted Direct Response)-GHSQC-TOCSY experiment. Regardless of which route is taken, it is possible to obtain all of the necessary homonuclear and heteronuclear correlation data required to establish the carbon skeleton of the molecule in, for example, a morning's work.

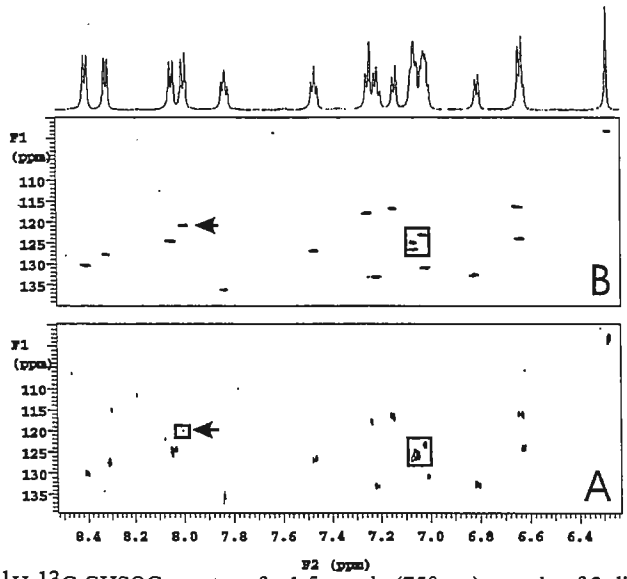


Fig. 1. ^{1}H-^{13}C GHSQC spectra of a 1.5 µmole (750 µg) sample of **2** dissolved in 30 µl d6-DMSO in a 1.7 mm SMIDG NMR tube under argon acquired using a Varian INOVA 600 spectrometer. The top panel (A) data were acquired as 16 hypercomplex 1 transient files giving a total acquisition time of 34 sec. The bottom panel (B) data were acquired as 48 hypercomplex 2 transient files giving an acquisition time of just <5 min. Both spectra were linear predicted as described in the text. One response in the bottom panel (boxed region denoted by arrow) was barely visible (see arrow in top panel). In addition, even using linear prediction, the boxed region in panel A was resolved much more poorly than the corresponding region in panel B, in which three times the number of t_1 increments were taken.

Beyond the acquisition of ^{1}H-^{13}C heteronuclear shift correlation data, most alkaloid chemists would undoubtedly like to have access to ^{15}N as a structural probe as well. It is in this area that NMR spectroscopy has fallen short. Because of the low gyromagnetic ratio of ^{15}N (~10% of ^{1}H) coupled with its low natural abundance (0.13%) there has been a dearth of papers in the literature that in any way utilized ^{15}N until only very recently. Then, as noted above, sample sizes required to perform ^{1}H-^{15}N experiments in 5 mm tubes would be prohibitively large for most natural products chemists. This situation changes dramatically, however, with the availability of submicro gradient inverse NMR probe technology. Using the 1.5 µmole sample of cryptospirolepine (**2**) just utilized for the acquisition of ^{1}H-^{13}C GHSQC and GHMBC spectra, it is also possible in very reasonable periods of time to acquire the corresponding ^{1}H-^{15}N correlation spectra.

Pulses for ^{15}N using the Nalorac Z•SPEC SMIDG™-600-1.7 were calibrated using standard methods. Hard 90° degree ^{15}N pulses were calibrated on decoupler channel 2 at 21.1 µsec (dpwr2 = 59 dB; 63 dB max). Pulse widths were also calibrated at a power level of 45 dB on decoupler channel 2 for decoupling purposes. At the lower power level, the 90° pulse was 95.4 µsec, which provides a dmf2 of 10532 Hz. Using a version of the GHSQC pulse sequence written to run using decoupler channel 2 (gHSQC_d2), the acquisition of a ^{1}H-^{15}N GHSQC spectrum of **2** was next undertaken. The spectrum shown in Figure 2 was acquired as 24 increments of t_1 to digitize an F_1 spectral window of 30 ppm. A total of 48 transients were accumulated per t_1 increment giving an acquisition time of 50 min. The data were linear predicted to 96 files in F_1 with zero filling to 128 points prior to Fourier transformation. The s/n in the experiment was ~15:1.

In the ^{1}H-^{15}N GHSQC spectrum of **2** shown to the left in Figure 2 only a single response, as expected, was observed. In contrast, when the structure of this alkaloid was originally done the ^{15}N chemical shift of this nitrogen was determined using a 5 mg sample of the alkaloid in a 5 mm probe, the acquisition taking overnight.

The relatively facile acquisition of the ^{15}N direct correlation spectrum prompted us to undertake the long-range ^{1}H-^{15}N GHMBC spectrum of **2** in an effort to determine the chemical shift of the 3 remaining nitrogen atoms in the structure. Thus, a ^{1}H-^{15}N GHMBC experiment was performed again using a pulse sequence written to run using decoupler channel 2 (gHMBC_d2) for X-pulses (^{15}N). The data were acquired as 40 t_1 increments with 576 transients/increment to digitize an F_1 spectral window of 55 ppm from 95-150 ppm. The experiment was optimized for an assumed 3 Hz long-range coupling with the low-pass J-filter opened to 160 Hz to allow the direct correlation response to appear in the spectrum. The data were acquired in 18 hr. and gave the spectrum shown in Figure 3.

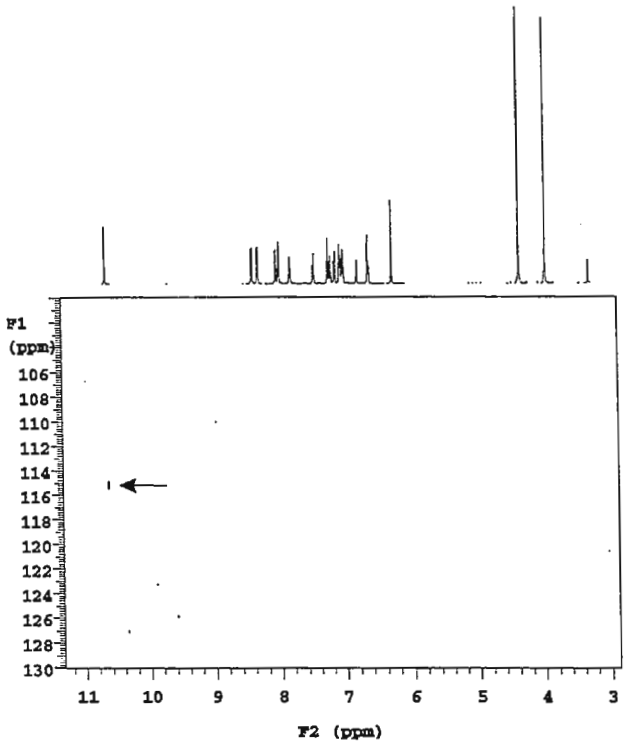
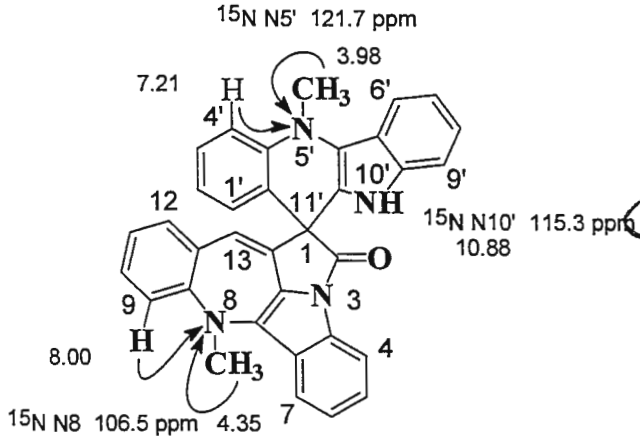


Fig. 2. ^{1}H-^{15}N GHSQC spectrum of 1.5 µmoles (750 µg) of **2** in 30 µl d6-DMSO acquired in 50 min. using a Nalorac Z•SPEC SMIDG™-600-1.7 probe in a Varian INOVA 600. Data acquisition details are given in the text.

The direct correlation response appeared at 115.3 ppm as a 96 Hz doublet. An intense long-range correlation response was observed at 106.5 ppm corresponding to the N8 resonance with long-range couplings from the N-methyl group resonating at 4.35 ppm and from the flanking peri proton which resonated at 8.00 ppm. A somewhat weaker long-range response was observed at 121.7 ppm for the correlation of the other N-methyl group resonating at 3.98 ppm to the N5′ resonance. Although not visible in the region of the contour plot denoted by the box, a weak correlation was also observed to the peri H4′ proton as shown in trace C plotted to the right.

The F_1 traces through the chemical shifts of the three ^{15}N resonances observed in the spectrum are shown in Figure 4. The two three-bond responses are denoted by arrows above the traces. Traces B and C are plotted at the same vertical scale. Trace A was plotted at one-quarter the vertical scale of the others to maintain perspective. The correlations to N8 shown in Trace A were the most intense in the spectrum.

In conclusion, the ^{1}H-^{15}N direct and long-range correlation spectra shown in this contribution demonstrate the utility of the Z•SPEC SMIDG™-600-1.7 gradient submicro probe for ^{15}N correlation experiments. Despite the low natural abundance and gyromagnetic ratio of ^{15}N the experiments shown are still feasible at the submilligram level. Direct correlation experiments, from the authors experience, can be conducted at the submicromole level without difficulty. It remains to be seen how feasible it will be to pursue long-range correlation experiments to ^{15}N at the submicro mole level. In any case, given gradient submicro NMR capabilities and the sensitivity inherent to an appropriate observation frequency such as 600 MHz, the utilization of ^{15}N as an structural probe by the natural products chemist or other investigators with an interest in alkaloids or nitrogenous compounds such as pharmaceuticals and their degradants is quite practical as shown.



Sincerely,

Gary E. Martin

Chad E. Hadden

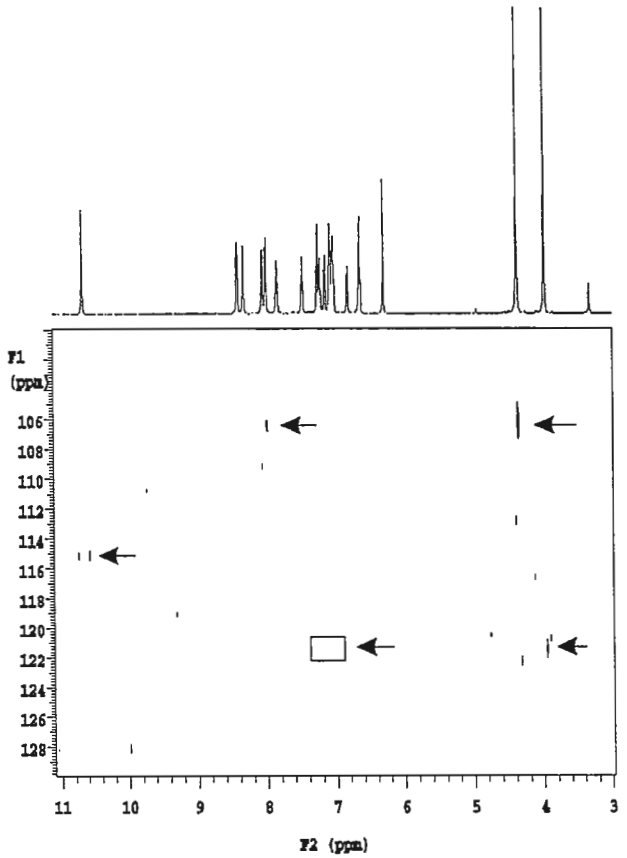


Fig 3. ^{1}H-^{15}N GHMBC spectrum of 1.5 μmoles (750 μg) of **2** in 30 μl d6-DMSO acquired in 18 hr. using a Nalorac Z•SPEC SMIDG™-600-1.7 probe in a Varian INOVA 600. Data acquisition details are given in the text. The boxed region in the spectrum contains the weak response at 7.21 ppm denoted by the arrow in trace C shown in Figure 4.

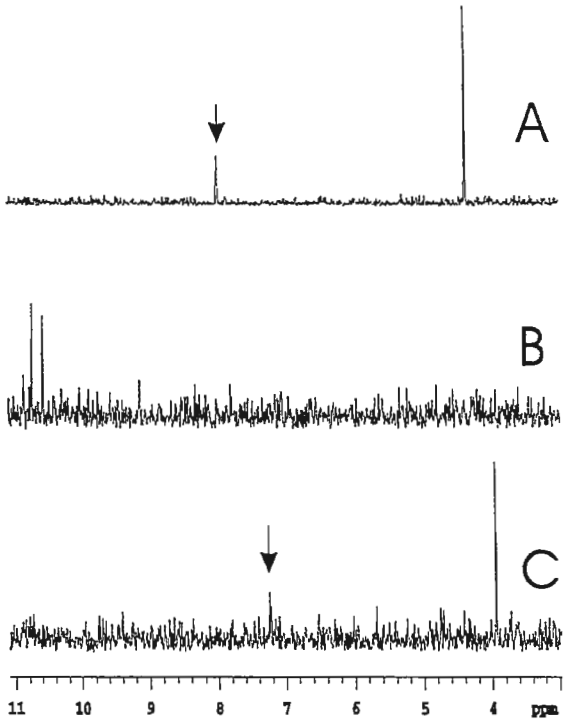


Fig. 4. Individual F_1 traces from the ^{1}H-^{15}N GHMBC of **2** shown in Fig. 3. Trace A was plotted at 106.5 ppm and corresponds to N8. The three-bond correlation from H9 is denoted by an arrow. This trace is plotted at one-quarter the vertical scale of traces B and C. Trace B shows the direct response 97 Hz doublet N10'H at 115.3 ppm. Trace C shows the three-bond correlation from H4' not observable in the contour plot and the two-bond correlation from the N-methyl group to N5' which resonated at 121.7 ppm.

References:

1) R.C. Crouch and G.E. Martin, *J. Nat. Prod.*, **55**, 1343 (1992); R.C. Crouch and G.E. Martin, *Magn. Reson. Chem.*, **30**, S55 (1992).
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9) A.N. Tackie, G.L. Boye, M. H. M. Sharaf, P.L. Schiff, Jr., R.C. Crouch, T.D. Spitzer, R.L. Johnson, J. Dunn, D. Minick, and G.E. Martin, *J. Nat. Prod.*, **56**, 653 (1993).

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Heart, Lung, and
Blood Institute
Bethesda, Maryland 20892

(received 3/5/98)

Dr. Barry L. Shapiro
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Comparison of water resonance planes in ^{15}N NOESY-HMQC and NOESY-HSQC

Dear Barry,

We have worked very hard to satisfy your ultimatimum before my forthcoming trip to Africa and then to Asilomar. I hope this contribution is sufficient to put me back into your good graces.

In the process of developing some experiments (work primarily of Jim Gruschus) to minimize the effects of water magnetization and enhance the signal intensity of weak crosspeaks from DNA to protein side chain resonances, we have compared the results using 3D NOESY-HSQC and NOESY-HMQC pulse sequences (see Figure 1). The general feeling seems to be that HSQC is preferred over HMQC for 20 kD proteins or protein complexes due the sharper contours obtained with HSQC since with HMQC the ^{15}N linewidths are broadened by proton-proton dipole couplings and unresolved proton-proton scalar couplings. With sharper lines one might also expect greater signal intensity (peak height). In figure 2 we show slices at the water frequency from NOESY-HSQC (top) and NOESY-HMQC (bottom) experiments on the vnd/NK-2 homeodomain-DNA complex. We have a continuing interest in homeodomain-DNA structures because of their role in early embryonic development. These spectra were acquired under comparable conditions, processed identically, and plotted in both cases at a threshold that is 12 times the rms noise. The first interesting feature is that the arginine side chain amide cross peak resonances (with the dashed boxes in the lower right corner of the spectra) have approximately twice the peak intensity in the HMQC experiment. Contours within the dashed box in the upper right corner of the spectra represent solvent-exposed glutamine/asparagine side chain amide resonances and are about 40% greater in peak height in the HSQC experiment. The remaining resonances (i.e., backbone amides and solvent-shielded Gln/Asn side chain amides) are on average 10% more intense in the HSQC experiment. However, variations in the peak intensities are not systematic. Crosspeaks labeled a and b represent Trp48 and Glu30 backbone amides, respectively. The intensity ratio (HSQC/HMQC) of a is 0.7 whereas the intensity ratio of b is 1.3. The bottom line is that one should do both experiments, since each experiment offers specific advantages.

Please keep the newsletters coming. We all enjoy reading them.

Sincerely yours,

James A. Ferretti

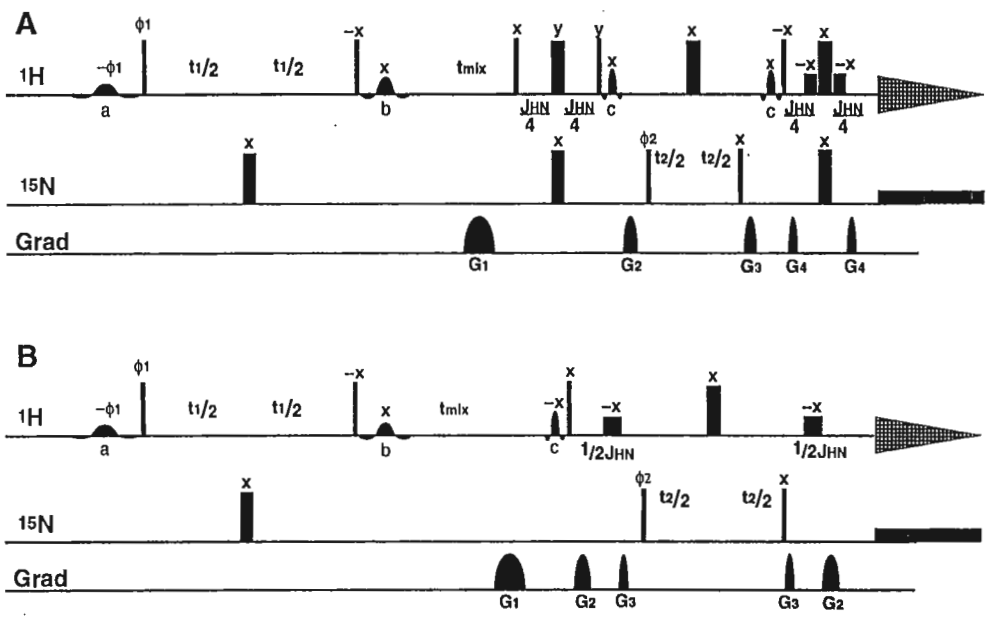

Figure 1. 3D ^{15}N NOESY–HSQC (A) and NOESY–HMQC (B) both with WATERGATE water suppression and water flipback. Tall narrow and wide rectangles correspond to hard 90 and 180 pulses, short rectangles correspond to soft 90 pulses, and pulses indicated by a, b,and c correspond to shaped 90 pulses (sinc) with all 1H pulses at the water frequency. The shaped pulse a before the t1 evolution period keeps the water magnetization along +z, preventing radiation damping during t1. Shaped pulse b puts the water magnetization along +z during the mixing time. Shaped pulse c ensures the water magnetization is along +z during the acquisition time. Suppression of radiation damping during t2 is aided by the gradient G2 (which dephases any remaining transverse water magnetization after the selective flipback pulse) in the NOESY–HSQC and by the gradients G3 (which dephase the water magnetization before and refocus it after the t2 evolution) in the NOESY–HMQC. Shaped pulses a and b are at the same power, with a=~10ms and b=~5ms (b is shorter since it brings water up from +y to +z), and shaped pulse c is higher power, with c=~2ms. The phase ϕ1=–45, and is incremented along with the acquisiton phase for States–TPPI detection in the t1 dimension, ϕ2=0,180 and is incremented for TPPI detection in the t2 dimension, and the acquisition phase=0,180. The HSQC portion of the NOESY–HSQC was adapted from Grzesiek *et al.*, *J. Am. Chem Soc.*, **1995**, *117*, 9594.

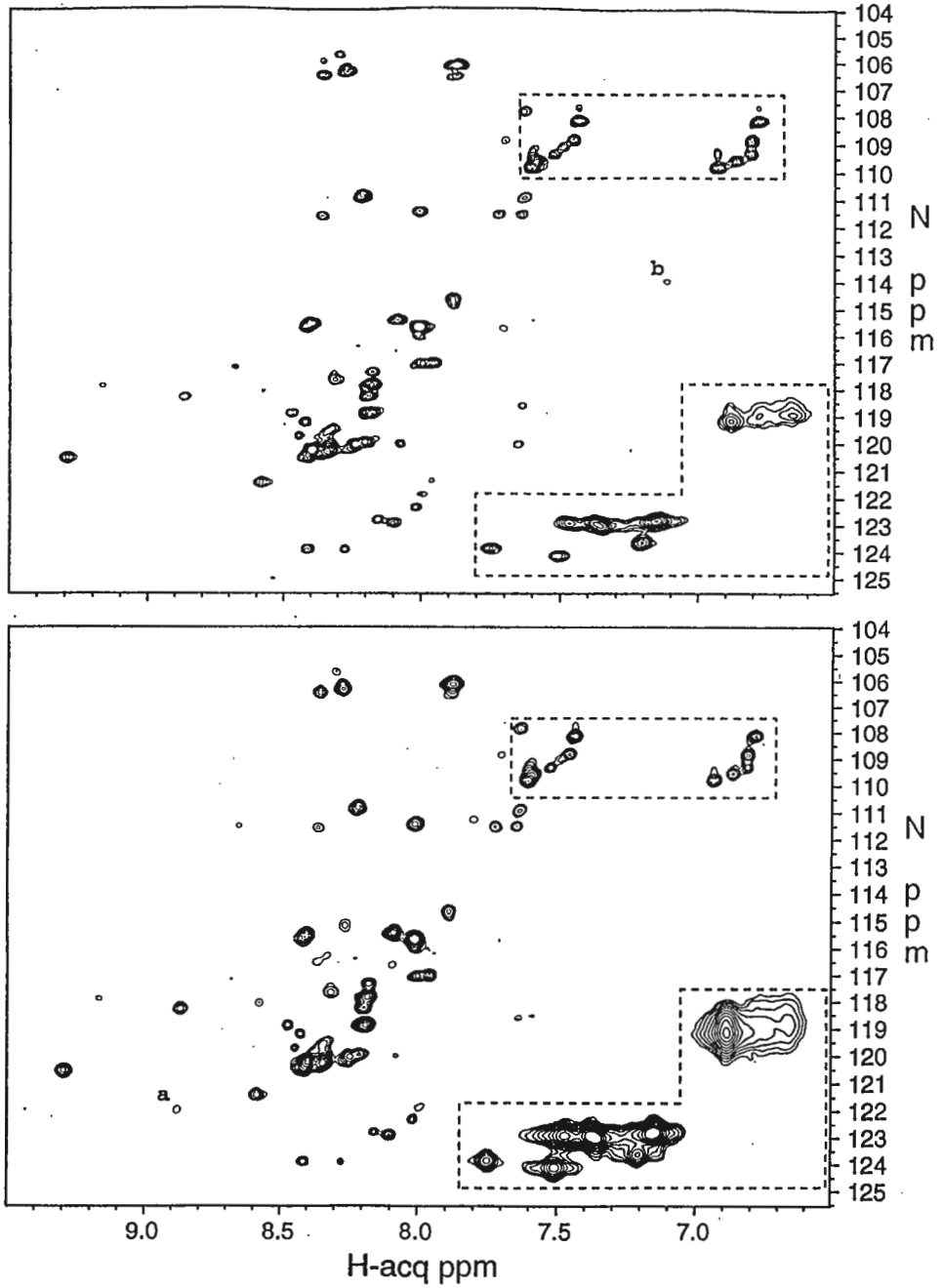

Figure 2. Water resonance planes from the vnd/NK–2 homeodomain/DNA complex (20kD,1.3mM), 3D ^{15}N NOESY–HSQC (top) and NOESY–HMQC (bottom). Both spectra were acquired with two scans per point as 64*x128x512* matrices, with t1 11.2ms (F1 5700Hz), t2 42ms (F2 1520.5Hz), and t3 61.4ms (F3 8333.3Hz). Both spectra were linear predicted to double the size of t1 and t2, and shifted squared sinebell window applied and zero filled in all dimensions. The NOE mixing time was 32ms for both spectra and the recovery time was 1.4s for both spectra. See letter text for additional details.

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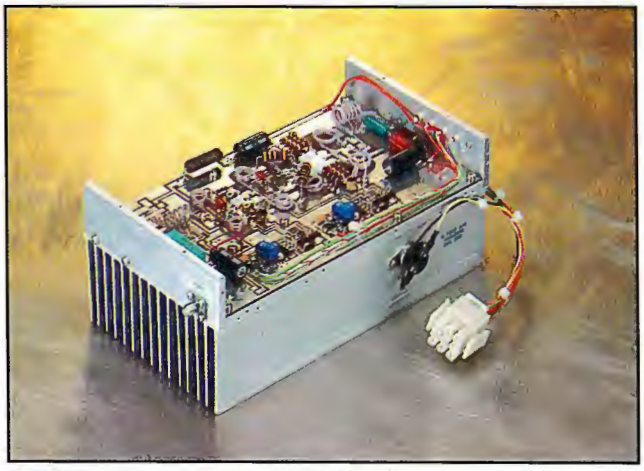

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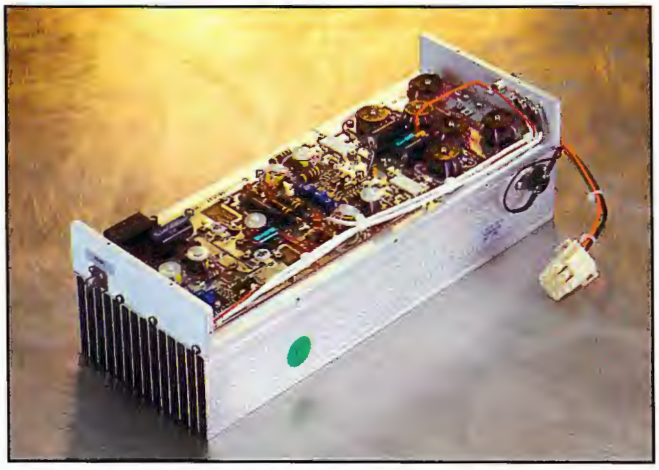

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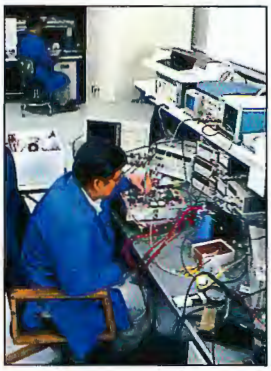
SCIENTIFIC & MEDICAL PRODUCTS



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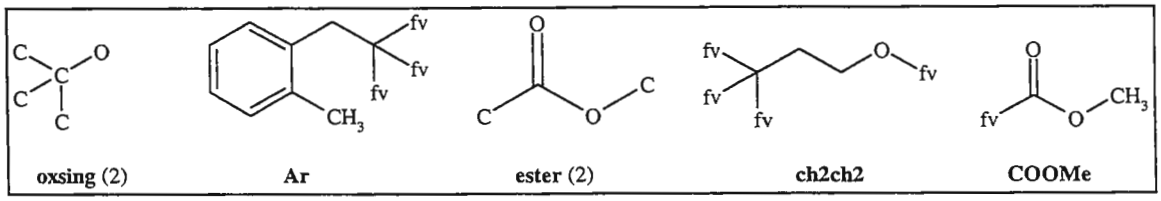
10 March 1998
(received 3/13/98)

Comparing GENOA and MolGen

Dear Barry,

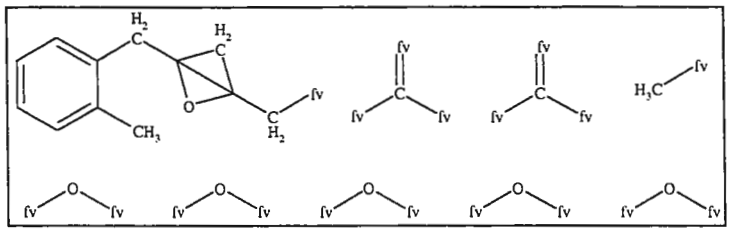
I will continue our comparisons of CASE (Computer Assisted Structure Elucidation) programs[1] using another example that I worked on many years ago.[2] I have a special fondness for this example, since it is the only one in my experience for which a CASE program came up with the right answer before a human.

Figure 1: Substructures Used in the GENOA Analysis



The unknown compound ($C_{15}H_{18}O_6$) was isolated as an unexpected product from what was supposed to be a simple alkylation. From a combination of chemical and spectroscopic evidence, the substructures shown in Figure 1 were derived. Note that there must be some atom overlaps between some of these substructures. For example, with 2 **oxsing** and **ester** substructures plus one each of **Ar**, **ch2ch2**, and **COOMe** we account for nine oxygen atoms, while the molecular formula limits us to six. Fortunately, this is not a problem with GENOA (GENeration with Overlapping Atoms).[3] One can start the GENOA analysis by putting in the multiplicities of all the carbon resonances, since this is useful in accounting for all the protons attached to carbons (16). Obviously there must be two exchangeable protons, and with the limitations of the molecular formula these must be OH's. At this point there was only one partially assembled structure (called "*cases*" in GENOA-speak). Constraining the problem to contain one **Ar** did not increase the number of *cases*. When I constrained the structure to include two **oxsing** groups, however, a total of 84 *cases* were generated. This is the type of surprise one gets from GENOA, and results from the way the program handles overlapping atoms. In the first *case*, for example, the oxygen atoms of the two **oxsing** groups were overlapped with each other and one carbon was overlapped with the **Ar** substructure to give the *case* shown in Figure 2.

Figure 2: Intermediate Case #1 from GENOA Analysis

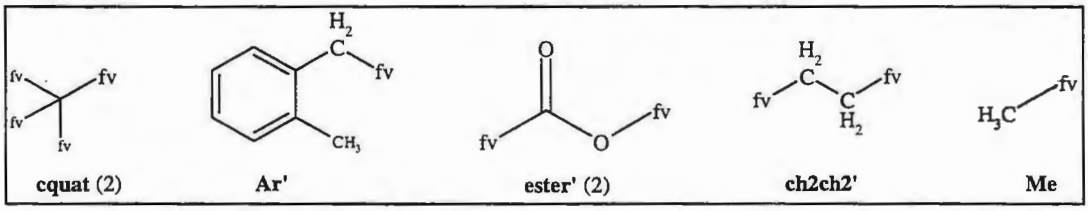


[1] See NMR Newsletter, March 1998, #474, p. 5.
[2] Briggs, S.L.; Dorman, D.E. unpublished results.
[3] GENOA is a computer program designed to generate all candidate structures consistent with the molecular formula and a given set of substructural constraints; *cf.* Carhart, R.E., *et al.*, *J. Org. Chem.*, **1981**, *46*, 1708.

This is a rather unlikely possibility and one could delete it. In fact, with GENOA one can at this point examine each case and rule some of them out. However it is usually more efficient to continue inputting *positive* evidence in the form of additional constraints. Ruling *in* additional substructures implicitly rules *out* many others, so that positive evidence is generally more efficient than negative evidence. In the next step I constrained the problem to include two **ester** substructures, which generated a total of 173 cases. After addition of this constraint the case in Figure 2 was ruled out, since there simply aren't enough carbons left to construct two esters and use up all the oxygen atoms at the same time. Finally, constraining the problem to contain one each **ch2ch2** and **COOMe** groups led to five and three cases, respectively. Final structure generation led to three candidate structures.

I have recently reproduced this result using MolGen 3.5.[4] With MolGen, of course, atom overlap in the macroatoms is not permitted, so we have to be more careful about how we identify the substructures. Thus for MolGen we need to trim the substructures back to those shown in Figure 3. But of course with these reduced substructures we get a few more structures: 48,204. The big difference between GENOA and MolGen is that it only took about 1.5 minutes to generate all these structures on a Pentium 75. Generating so many structures with GENOA would take longer than anyone would be willing to wait, and might also get one in trouble with the system manager. Certainly I have gotten some irate e-mails on occasion.

Figure 3: Macroatoms Used in MolGen Analysis

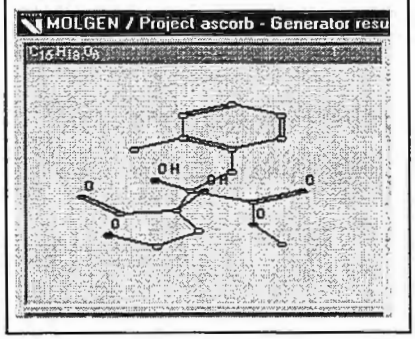


But while macroatoms in MolGen cannot include overlaps, substructures on Goodlist can, so that the substructures in Figure 1 can be added to that list. However this is inefficient; MolGen has to generate all 48,204 structures and then apply the Goodlist constraints to each. With GENOA one would never do this because it would take too long. Because of the speed of MolGen, however, this is a reasonable way to proceed, and one gets six candidate structures in about 42 seconds on a Pentium 75. The correct structure, as drawn by MolGen, is shown in Figure 4. This structure was subsequently confirmed by X-ray.

Why does MolGen propose more structures? The answer to that lies in the differences in the ways GENOA and MolGen define free valences. Understanding free valences is critical to the successful use of these programs. However, I see that I have used up my quota of two pages, so this story will have to be continued later.

Doug Dorman
doug_dorman@lilly.com

Figure 4: The Correct Structure



[4] Note that I have updated my version of MolGen since my last contribution. If anyone else has loaded MolGen 3.5 and then found that it wouldn't work (with an error msg saying something about "No data available"), I have figured out how to fix this.

ASTRA
ASTRA CHARNWOOD

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

February 24, 1998
(received 3/3/98)

Dear Barry,

In a recent in-house study to determine the binding mode of putative immuno-suppressive drugs to [U-^{15}N]-labelled human FKBP-12, we measured simple gradient-selected ^{1}H-^{15}N HSQC spectra of the protein and the protein-ligand complexes. A comparison of chemical shifts in both dimensions gave a picture of the affected residues. Indeed, backbone chemical shift perturbations like this have been widely used as a marker of binding locale.

The usual method of reporting such changes in chemical shift is to tabulate the chemical shift difference, $\Delta\delta$, in each dimension relative to the free species. For our study, these are represented by histograms in Figure 1a & 1b for ^{1}H and ^{15}N respectively.

It occurred to us that this representation of a "scalar" quantity could stand improvement. We present here a new "vector" representation of the results on a per-residue basis which not only takes into account the $\Delta\delta$'s of both nuclei at the same time but also allows one to overlay, and easily compare, the results from multiple protein-ligand complexes on the same plot. In our opinion, the resulting "target plot" gives the reader a better picture of the relative changes in chemical shift with regard to the native protein.

Figure 2 shows a selection of results for residues L50-I56 of FKBP-12 which occur in a loop that is rigidified by the insertion of the ligand at the active site. The centre of each "target" in this polar plot is the reference point of the amide resonance in the uncomplexed protein. The relative movement of the ligated amide signal is plotted (in Hertz) on orthogonal axes for both proton and nitrogen dimensions. The further away a point is plotted from the "cross-hairs", the bigger the chemical shift difference in the HSQC spectra. Generating the polar plots was a relatively simple matter once a peak table was generated. We used Kaleidagraph (Synergy Software) to generate the plots, but any reasonable spreadsheet can be used to calculate the vectors.

Polar plots have co-ordinates of the form (r, θ), where r is the magnitude of the vector from the origin and θ is the subtended angle. From the chemical shifts obtained in the HSQC spectra, r and θ can be calculated thus :

$$r = \sqrt{\Sigma\left(\Delta^{1}H\right)^{2} + \left(\Delta^{15}N\right)^{2}} \qquad \tan\theta = \frac{\Delta^{15}N}{\Delta^{1}H} \bullet \frac{180}{\pi}$$

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Each diagram in Figure 2 is plotted such that the radial axis is scaled to the data. This allows the plot to fully focus on the overlap of the data points for different ligands. Hence, even for small changes in chemical shift, a general movement into the same quadrant for each of the data points indicates a coherent effect. They could easily be plotted with a fixed axis to indicate the magnitude of chemical shift difference for each residue, but this information can be gleaned from the heights of the bars in the histograms in Figures 1a or 1b.

Applications for this technique extend to any study where multiple experiments are compared to a reference 2D spectrum, e.g. SAR by NMR (ref. Fesik & co-workers, Science, 274 (1996), 1531).

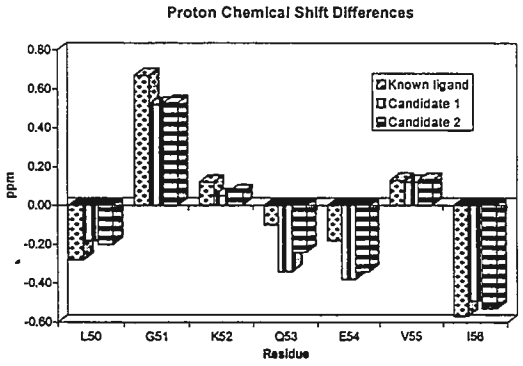

Figure 1a.

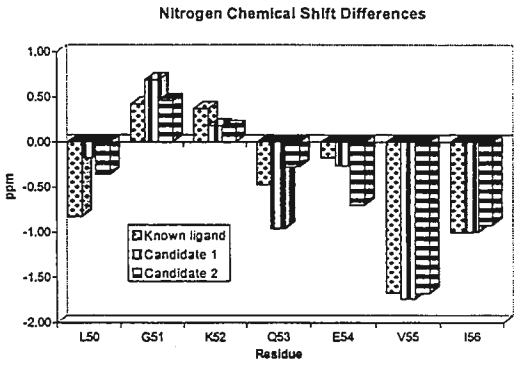

Figure 1b.

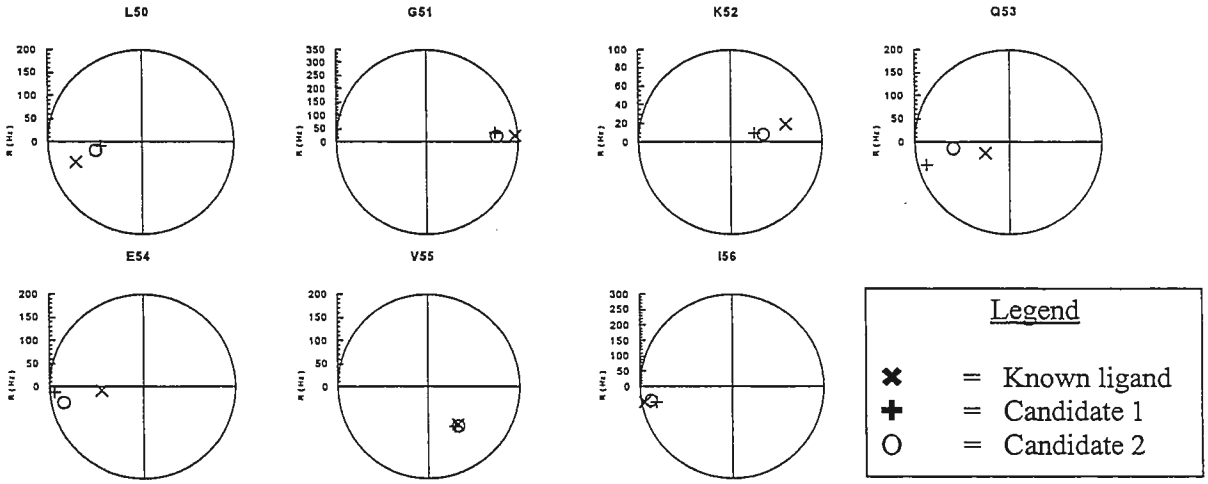

Figure 2.

Sincerely,

J. Mark Dixon

Michael A. Bernstein

LONG ISLAND UNIVERSITY BROOKLYN CAMPUS UNIVERSITY PLAZA, BROOKLYN, NEW YORK 11201

March 2, 1998 (received 3/24/98)

Dr. B. L. Shapiro
The NMR News Letter
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Palo Alto, CA 94303

^{11}B NMR of Functionalized 1, 2 - $C_2B_{10}H_{11}$- Carborane

Dear Barry,

Boron-11 is a quadrupolar nulceus (I=3/2) leading to short relaxation times, this fact and the presence of boron-10 (I=3) in 20% isotopic abundance combine to make the peaks in any ^{11}B NMR spectrum broad. This is especially so in metallacarborane complexes due to the bulkiness of the molecules and other factors. Nevertheless, useful information can be gathered from ^{11}B NMR spectra with the aid of a high field NMR spectrometer. The ^{11}B-^{1}H coupled spectra of 1(2methylpyridyl),2-dicarbacloso–carborane, $C_2B_{10}H_{11}$ (1), is shown in figure 1A. It should be noted that all the peaks fall within the range of +20 to-20 ppm and the J(HB) coupling is in the range of 130-160Hz. However, the cesium salt of its decapitated product (formed by reacting (1) with ethanolic solution of KOH followed by the addition of cesium chloride) makes for a very interesting comparison (figure 1B). The chemical shift range has been significantly increased, +20 to -40 ppm, and the peaks are quite distinctive and may be adequate to identify the type of exopolyhedral interaction in the compound. For example, the boron nuclei involved in the B-H-B and B-H-Cs interaction (3center, 2e) give rise to a doublet signal with weaker J(HB) compared to those in the 2 center-2e J(BH) coupling in terminal B-H groups. Surprisingly, our attempt to decapitate the 1-(2-methyl-4-nitrophenol), 2-dicarba-closo carborane, (prepared by reacting the dilithium salt of 1,2-closo $C_2B_{10}H_{12}$ carborane with 2-chloromethyl-4-nitrophenol in THF with potassium hydroxide in ethanol under 24 hours of reflux conditions, followed by addition of cesium chloride) afforded a polymer of $C_3B_8H_{11}CsC$, as solved by single crystal X-ray diffraction. Since no past reference to this was found in the literature, we decided to investigate 1-(2-methyl-4-nitrophenol), 2-dicarba-closo $C_2B_{10}H_{11}$-carborane in DMSO over a period of 84 hrs by following changes in its ^{11}B NMR. Figure 2 shows the result of such a study. We discovered that a cage opening take place (figure 2B), even in the absence of a base, and that the nitrophenol plays a significant part in the cage opening reaction in DMSO. We are presently studying the kinetics of this cage opening under varying reaction conditions, including an analysis of solvent effects.

Sincerely,

Fernando Commodari, Ph.D.
Assistant Professor

Aderemi Oki, Ph.D.
Associate Professor

Olga Sokolova
Graduate Student

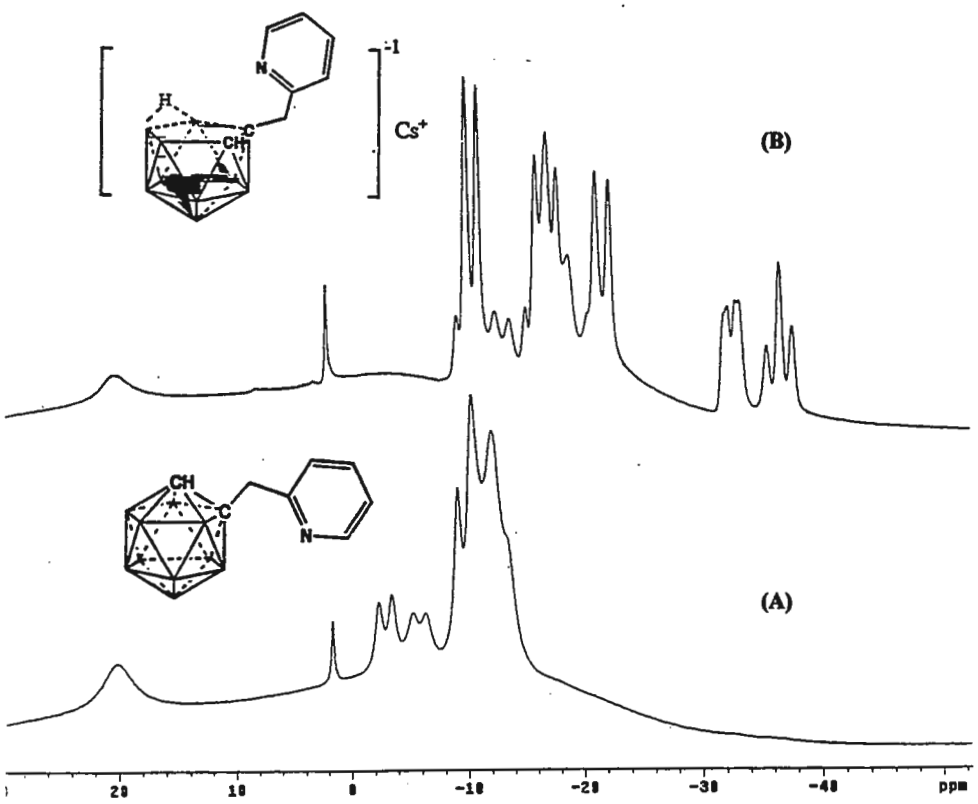

Figure 1. ^{11}B-^{1}H coupled spectra of (A) 1(2methylpyridyl),2 dicarbacloso carborane, $C_2B_{10}H_{11}$ and (B) the cesium salt of its decapitated product . ^{11}B spectra were acquired at 128.32 MHz with 1000 scans and a 1 sec relaxation delay in DMSO.

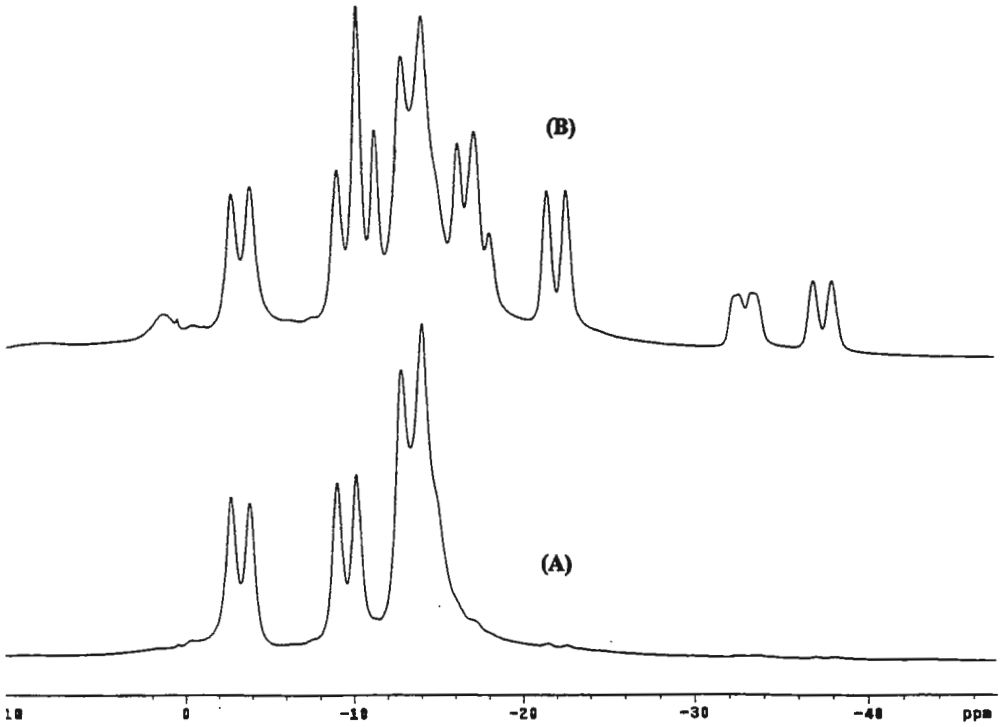

Figure 2. ^{11}B-^{1}H coupled spectra of 1-(2-methyl-4-nitrophenol), 2-dicarba-closo $C_2B_{10}H_{11}$-carborane in DMSO. ^{11}B spectra were acquired at 128.32 MHz with a 90 degree pulse and a relaxation delay > $5*T_1$. (A) shows the resulting spectrum 10 minutes from the introduction of the compound in DMSO and (B) shows the resulting spectrum > 84 hr from this initial time.

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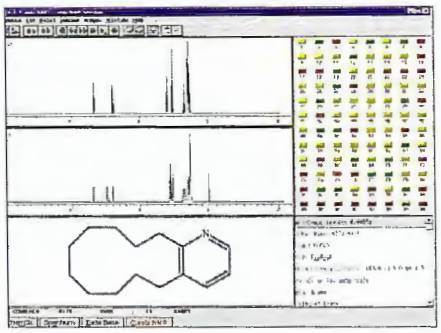

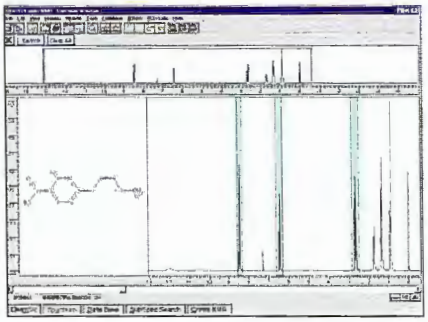

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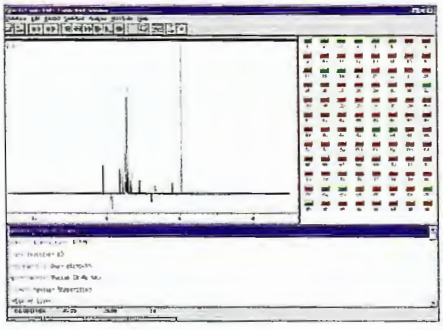

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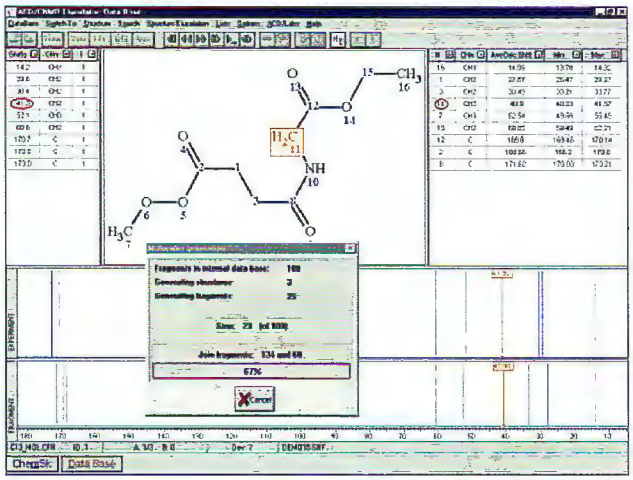

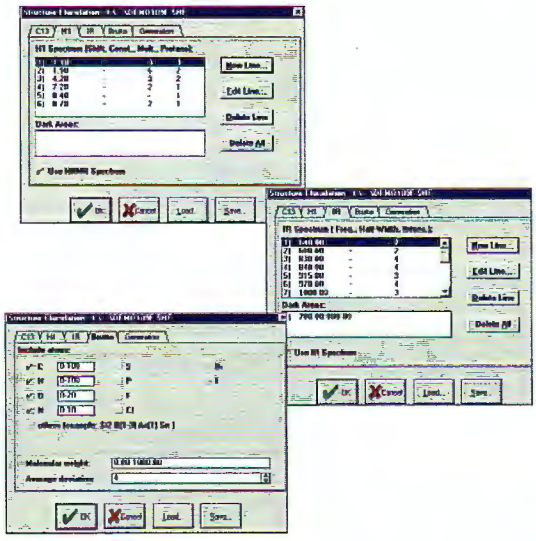

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March 3, 1998 (received 3/9/98)

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

Bloch-Siegert Shift Compensated and Cyclic Irradiation Sidebands eliminated, Double-Adiabatic Homonuclear Decoupling for ^{13}C- and ^{15}N-Double-Labeled Proteins

Dear Dr. Shapiro,

A Gaussian shaped, offset-independent adiabatic decoupling is adopted to decouple ^{13}CO from $^{13}C_\alpha$ or visa versa for ^{13}C- and ^{15}N-double-labeled proteins, together with a compensating decoupling with an opposite frequency sweep applied on the other side of the $^{13}C_\alpha$ resonance frequency (Fig. 1). In a quite broad range, the double-adiabatic decoupling eliminates efficiently the cyclic sidebands caused by direct irradiation of the adiabatic decoupling and reduces significantly the Bloch-Siegert shift (Fig. 2). The remaining Bloch-Siegert shift, which is almost a linear function of offset, results in a spectrum contracted by a factor of

$$\lambda = [1 - (f_{1rms} / \Delta f)^2].$$

To compensate for the remaining effect, a dilated evolution time

$$t_1' = \frac{t_1}{\lambda} \approx [1 + (f_{1rms} / \Delta f)^2] t_1$$

can be used in the experiment, leading to a spectrum without any Bloch-Siegert shift in a quite broad range. The decoupling sequence is also quite

insensitive to the RF field intensity or inhomogeneity due to the reduced transverse components of RF field at $^{13}C_{\alpha}$, leading to an efficient decoupling even under unfavorable conditions.

Sincerely,

Shanmin Zhang

David G. Gorenstein

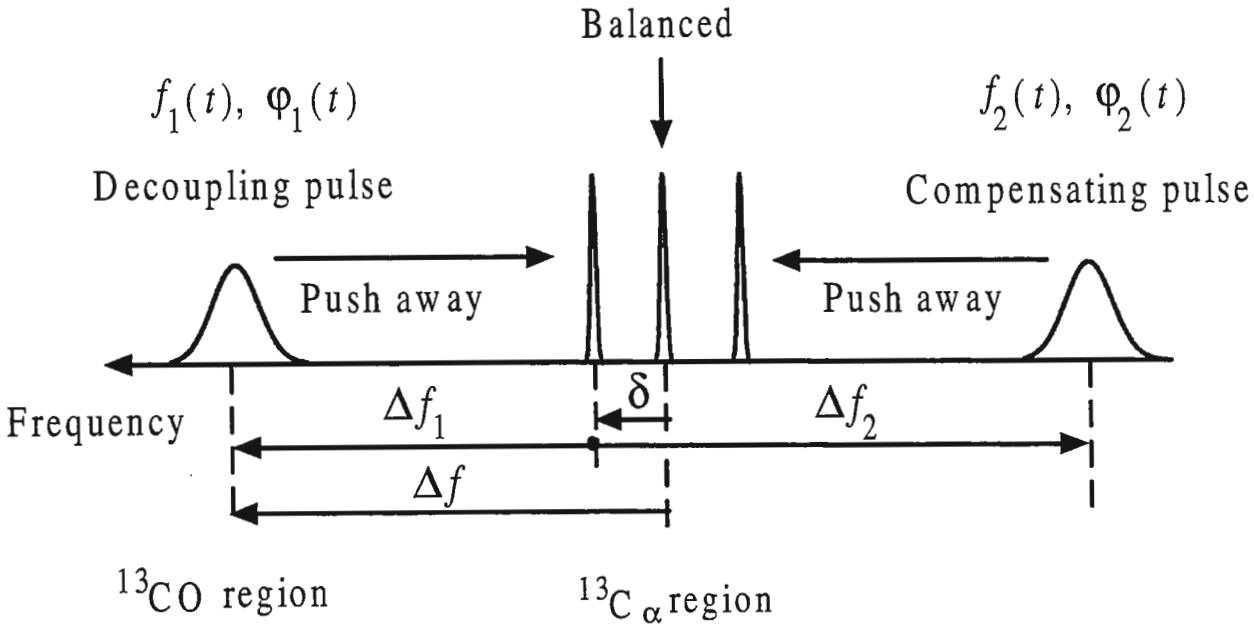

Fig. 1. Adiabatic decouple of ^{13}CO from $^{13}C_{\alpha}$ with a compensating pulse applied on the other side of the peaks with the same shape but opposite frequency sweep, where Δf = 23.2 kHz. Both the left and the right peaks are pushed towards the center due to the Bloch-Siegert effect. The center peak is balanced and remains in its position.

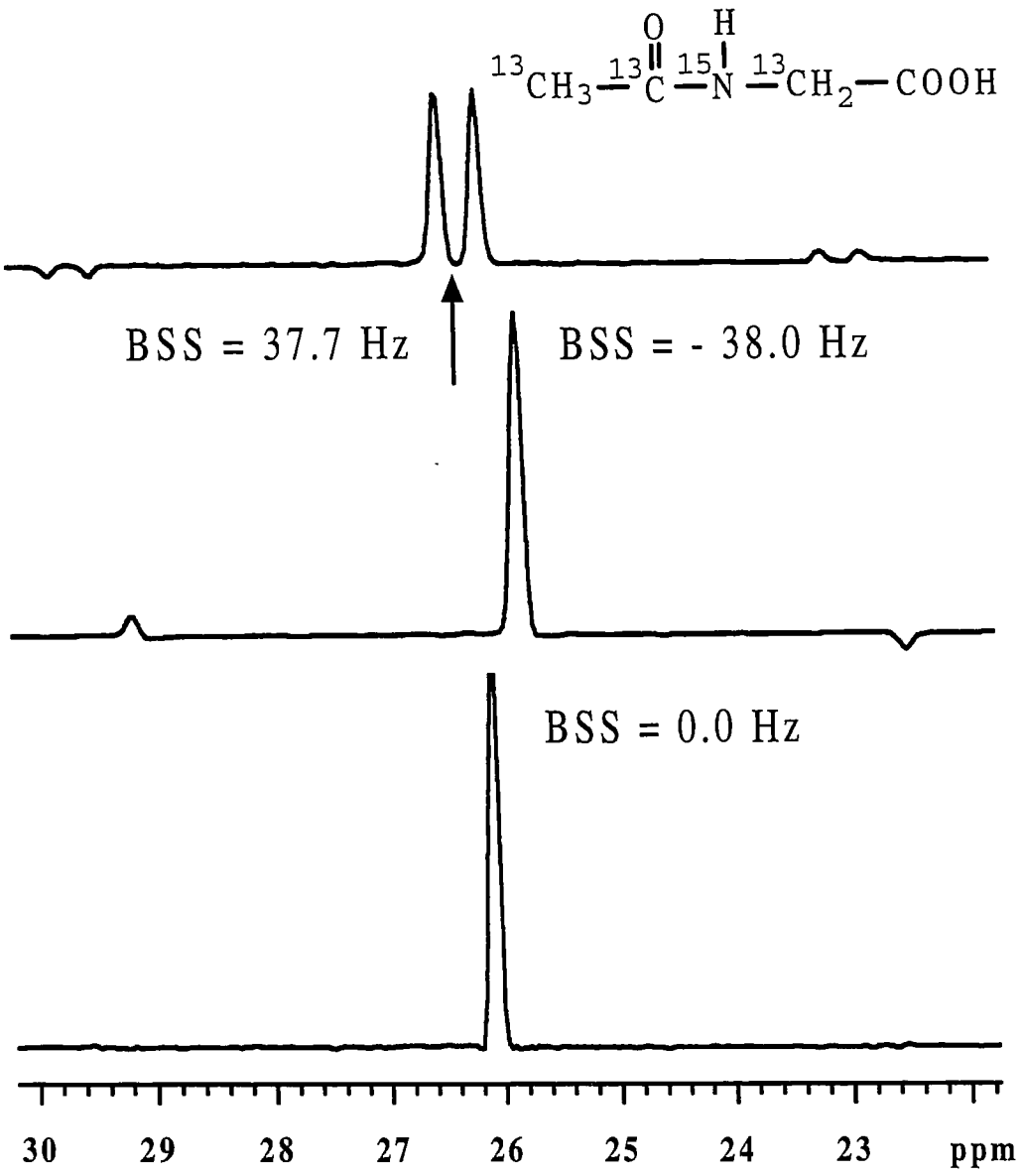

Fig. 2. $^{13}CH_3$ spectra obtained from the traces of two dimensional HSQC spectra using a test sample of N-acetylglycine. The spectra are acquired with a single adiabatic decoupling (middle), with a compensating decoupling (top), and with a double-adiabatic decoupling (bottom), where BSS stands for the Bloch-Siegert shift. The decouple pulse has a Gaussian shape, $A(t) = f_{1max}\exp[-\alpha(t-T/2)^2]$ (α=5 (kHz) ^{2}, T = 2 ms, f_{1max}= 2.50 kHz, and f_{1rms}=1.32 kHz), a phase cycle of (0°, 150°, 60°, 150°, 0°), and a frequency sweep of 8 kHz.

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Wednesday, March 18, 1998
(received 3/23/98)

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

NMR Characterization and Adsorption Studies of Polyelectrolyte onto Colloidal Silica Particles

Dear Barry:

Polymers are used as additives in coating formulations as rheological modifiers and stabilizers. The current emphasis on environmentally friendly technology has caused a shift away from organic-based systems towards water-based formulations. This shift in technology for paints, coatings, inks, and adhesives makes stringent demands on the water-soluble polymers used as additives. Such polymers are typically polyelectrolytes, which bear electrostatic charges to mediate their molecular conformations and interactions with surfaces such as silica, titanium dioxide, and latexes.

The adsorption behavior of polyelectrolytes on colloidal silica particles has been probed using liquid-state proton Nuclear Magnetic Resonance (NMR). Cosgrove and co-workers studied poly(ethylene oxide) adsorption onto polystyrene surfaces using NMR.[1] The same group demonstrated that solvent relaxation was a function of adsorption phenomena.[2] Haggerty et al. initiated NMR measurements to characterize the adsorption of associative polymers on polystyrene latex particles and titanium dioxide particles at Lehigh.[3,4] They determined the pancake-to-brush conformation of the adsorbed associative polymer molecules on surfaces. Polyelectrolytes are generally expected to behave differently than associative polymers, because they have electrostatic charges, which facilitate interaction and adsorption of the polymer on the surface.

Poly (dimethylaminomethyl methacrylate) (DMAEMA) was selected as a trial material, because it is a cationic homopolymer expected to give significant adsorption on silica at near neutral pH. The monomer unit has a molecular weight of 157. The sample reported on here has a total molecular weight of approximately 2350. A colloidal silica particle, Ludox®,[5] was the substrate, having 12 nm diameter and 230 m^2/g specific surface area. All of the proton solution NMR experiments were performed on a GN-300 300 MHz NMR

spectrometer. A 5mm proton/carbon or 10mm proton/broadband probe was utilized. All measurements were performed at room temperature, 293 K. An external standard, tetrakis (trimethylsilyl) silane, was employed to assure accurate quantitative results with appropriate precautions on pulse length and relaxation delays. The chemical shifts were consistent with the DMAEMA structure; the relaxation times of the residual HDO solvent were also obtained.

Figure 1 shows the NMR spectra of DMAEMA with and without silica particles. The top spectrum is a 300 MHz ^{1}H NMR spectrum of DMAEMA in D_2O at a concentration 992 ppm without silica. The bottom trace is a ^{1}H NMR spectrum of 992 ppm DMAEMA in D_2O with 0.5 weight % silica (Ludox®, 12nm). This figure suggests that all of the DMAEMA can be adsorbed onto the silica particles at low polymer concentrations. In addition, the adsorption isotherm for DMAEMA on silica and solvent relaxation rates were obtained (Figure 2). In principle, the solvent spin-lattice relaxation rate is enhanced by the adsorption of polymer onto the surface; this enhancement can be used to obtain the proportion of total polymer segments adsorbing as trains. The shape of the adsorption isotherm is similar to the relaxation rate enhancement plot as a function of free DMAEMA. It appears the residual HDO and the relaxation rate is proportional to the adsorbed amount. This preliminary adsorption study suggests that significant adsorption of DMAEMA occurs on the colloidal silica surface with most of the segments adsorbed as trains.

Literature Cited:

1. Cosgrove, T., and Griffiths, P.C., *Adnances in Colloid and Interface Science*, **42**,175-204 (1992)
2. Cosgrove, T., Stuart, M.A., and van der Beek, G.P., *Langmuir*, **7**,327 (1991)
3. Heck, C.S., Haggerty IV, J.F., Roberts, J.E., El-Aasser, M.S., and Silebi, C.A., *PIC Research Progress Report* No. **4**, Lehigh University, Bethlehem, PA, 1993
4. Haggerty IV, J.F., Roberts, J.E, and El-Asser, M.S., *PIC Research Progress Report*, No. **6**, Lehigh University, Bethlehem, PA 1994
5. Du Pont company.

Sincerely,

YongWoo Shin Maria M. Santore James E. Roberts

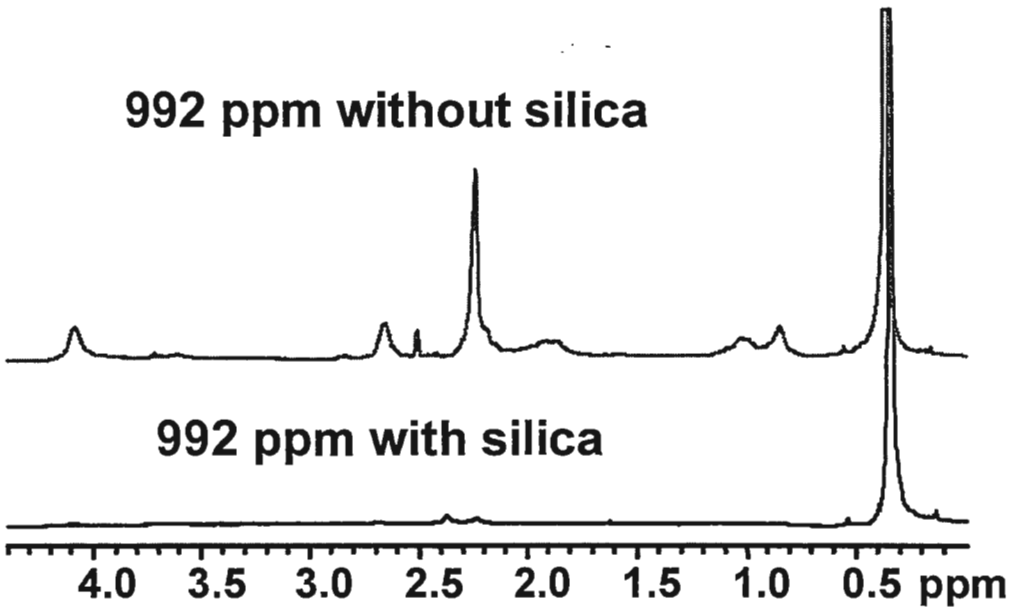

Figure 1: NMR spectra of DMAEMA with silica particles

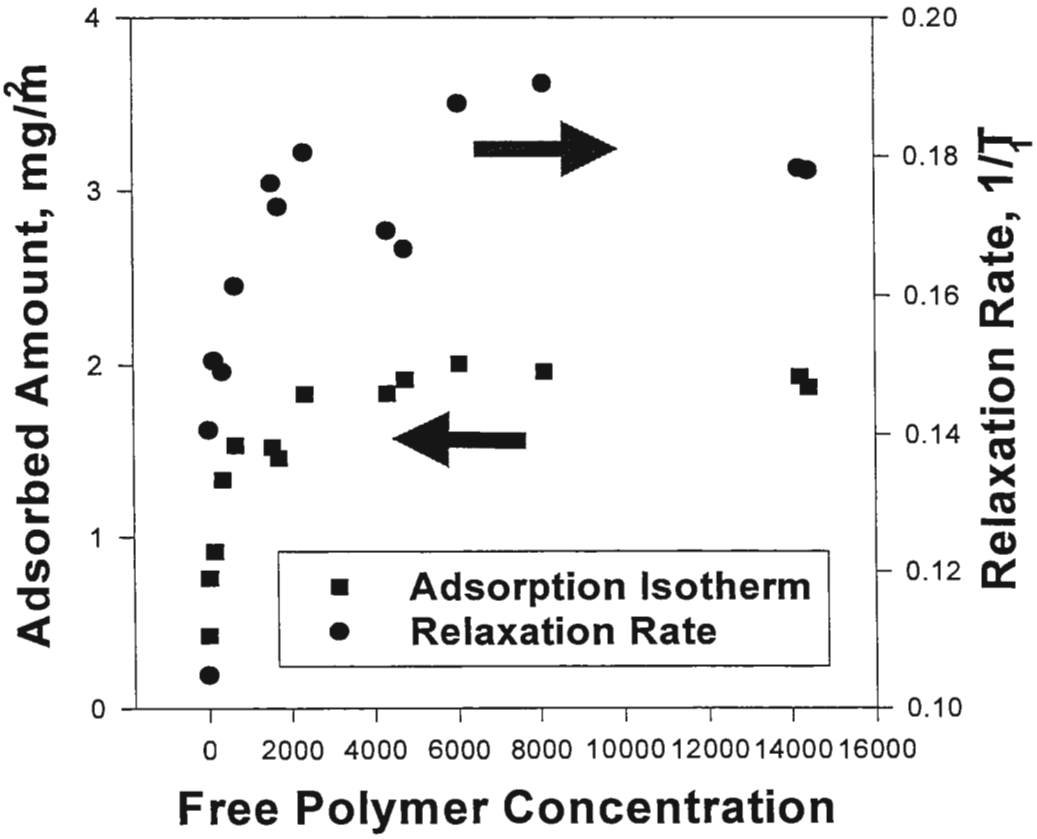

Figure 2: Adsorption Isotherm & Relaxation Enhancement of DMAEMA

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March 17 1998 (received 3/18/98)

Laser-Polarized Noble Gas NMR at the Center for Astrophysics

Dear Barry,

I would like to open another subscription with you from yet another exotic location, and at the same time apologize for the delays with this note beyond the time it was promised. In this opening contribution, my colleagues and I thought it best that we give an overview of just what NMR is being done by the group here at the Harvard-Smithsonian Center for Astrophysics (CFA), and how this unusual link came about.

The work we are involved in is done under the direction of Ron Walsworth here at the CFA, and is part of a number of varied studies being carried out by his team, all of which link back to fundamental atomic physics studies and precision frequency measurements. The current NMR interests arose from an extension of these ideas into methods for enhancing the polarization of the spin 1/2 noble gases ^{129}Xe and ^{3}He. Since the pioneering NMR work using laser-polarized gases in the early 1990's, studies in this field have rapidly expanded, especially in the medical community, to the point where it now occupies an entire lecture session at ISMRM meetings, and has even been featured in the January edition of the newsletter.

The polarization technique, which produces the extremely high NMR signal obtainable from laser-polarized ^{129}Xe and ^{3}He, is very simple. It uses a small, powerful diode array laser, emitting light at 795 nm. This light is circularly polarized and then directed into a glass cell containing rubidium metal and the noble gas, either ^{129}Xe or ^{3}He. The cell is heated to around 95° C or higher, so that the rubidium metal vaporizes, and then acquires an electron spin polarization via absorption of the circularly polarized laser light and de-excitation through collisions with a buffer gas. The nuclear spins of the noble gas atoms are then polarized via transient hyperfine interactions during collisions with the electron spin-polarized rubidium. As the process continues, the polarization level of the noble gas reaches very high values (0.1 is common) - 4 to 5 orders of magnitude higher than generally obtained by thermal Boltzman equilibrium inside a typical NMR magnet. Thus, despite the much lower density of the gas in comparison to a liquid, a cell of laser-polarized noble gas can give a similar NMR signal to a cell of water.

In the case of ^{129}Xe, the polarization is obtained in about 20 - 30 mins, however polarizations of more than 10% are difficult to achieve. The enhanced signal, if left in the polarization cell in the magnetic field, will decay away with T_1 about 2 hours. ^{3}He takes much longer to polarize, often 4-6 hours; however higher polarizations can be achieved, up to 50%, and the T_1 can be as long as a day or more! However, once the gas is passed into a sample of interest, T_1's reduce as the gas collides with de-polarizing surfaces. In addition, one must be aware that as the magnetization is produced artificially outside the magnet, once the magnetization is used (i.e. in a single 90° pulse) then it is gone and will not return after a $5xT_1$ delay. In essence then, while the polarization process itself is very simple, the transfer of the gas to samples of interest - ensuring it retains sufficient polarization during the experiment time, and in a way that allows interesting NMR pulse sequences to be performed - is what often proves to be the experimental challenge.

One of the members of our group at the CFA, Dominik Hoffmann, is looking to address some of these gas delivery issues. He is building a large-scale gas polarization unit that will deliver up to a liter of laser-polarized ^{129}Xe at a time, and will deliver it effectively to samples. The "samples" were initially intended to be volunteer humans, part of our collaboration with researchers at Brigham and Women's Hospital for human lung studies and potentially dissolved state brain studies. However, we are now looking to use this setup to also supply gas for materials science research, and a new study of restricted gas diffusion in lung airways.

A lot of my own work has involved the use of pulsed gradient spin-echo based sequences for gas diffusion measurement. Despite the extreme versatility of these techniques in the liquid state, they have not been

Harvard-Smithsonian Center for Astrophysics
60 Garden Street, Cambridge, MA 02138

widely used for gas-phase NMR. Recently, gas-phase diffusion measurements have been performed by other groups using pulsed field gradient techniques which more closely resemble the original constant gradient method of Carr and Purcell than that of Stejskal and Tanner, who's key motivation was to obtain time-dependent diffusion measurements in order to study the restricted diffusion of spins encountering barriers to their free diffusion. The constant-gradient techniques are, of course, perfectly adequate for measuring free bulk gas diffusion in a large cell, where the only motion is unrestricted Brownian motion. However, to carry these methods further to allow time-dependent diffusion measurement in the gas phase, I had to be certain such varied PGSE-based techniques would be applicable in the gas phase, even for conventional thermally polarized gases, before attempting to modify them for laser-polarized gases. Fortunately, this is the case.

The motivation for time-dependent diffusion measurements of the gas has come from our proposal to extend an earlier study, from Schlumberger-Doll Research, of water-saturated bead packs and rocks. Theoretical calculations had shown that porosity and tortuosity information for the rock could be obtained from NMR diffusion measurements of the water, however it was discovered that the water signal often decayed due to surface relaxation before the spins could diffuse through many pores. Our aim is to use the enhanced diffusion coefficient (3-4 orders of magnitude higher than water), and the weaker surface interaction of the gas to probe restricted diffusion through many pores, and so obtain greater micro-structural detail. This is a challenging project, especially with respect to using laser-polarized gas; the many difficulties of which are too complex to detail here. However, Fig 1 shows a graph of the time-dependent diffusion coefficient of thermally polarized ^{129}Xe in 4 mm glass beads. Both the short and long-time limit behavior of this data match with the Schlumberger theory extremely well, giving us promise to continue this exploration further, using smaller beads, reservoir rock samples, and of course using laser-polarized ^{129}Xe as the spin probe.

Some of our most recent work has involved studying liquefied ^{129}Xe. Xenon freezes in liquid nitrogen, and then forms a liquid as the xenon ice melts. Freezing laser-polarized ^{129}Xe can yield a liquid not only with huge polarization, but spin density as well, implying the signal obtainable should be much higher than that from water. The usefulness of such a liquid has been demonstrated recently by the group of Will Happer at Princeton, showing signal enhancement by as much as a factor of 50 in ^{1}H or ^{13}C spectra of organic molecules dissolved in the liquid, and experiencing polarization transfer from the liquid xenon. Ching-Hua Tseng of the CFA group plans to use such a technique to enhance signal for quantum computing studies. As a prelude to this work, he and I have been studying the exchange of spins from one xenon phase to another (Fig. 2) and obtaining ~100 micron resolution images from droplets of the liquid (Fig. 3) - quite a challenge for the horizontal bore 4.7T Omega system with only 7 G/cm gradients being used in this study!!

Our other major NMR study recently has to been to push the boundaries of low-field NMR. We decided that, as the noble gas magnetization is artificially produced outside the magnet, there was no real need for a very high field, expensive, magnet as is used on traditional NMR systems. Therefore, Glenn Wong and Ching-Hua Tseng of our group built a wire-wound solenoid that produces a field strength of 21 G. At MIT, they interfaced this to one of David Cory's AMX consoles, so that the AMX would trigger our home-built kHz rf system that pulsed the sample in the magnet, and did heterodyne signal detection. However, gradient control, signal averaging, data acquisition, etc. was all under the control of the AMX in normal acquisition mode. Initial images obtained from cells of ^{3}He at 67 kHz are very promising, and compare well in terms of signal-to-noise and resolution with images obtained at 4.7 T in a commercial magnet. We hope to show you some images from this system, and further results from other projects in future contributions.

Best Regards,

Ross Mair, writing for : Ching-Hua Tseng Dominik Hoffmann Glenn Wong Ron Walsworth

Harvard-Smithsonian Center for Astrophysics
60 Garden Street, Cambridge, MA 02138

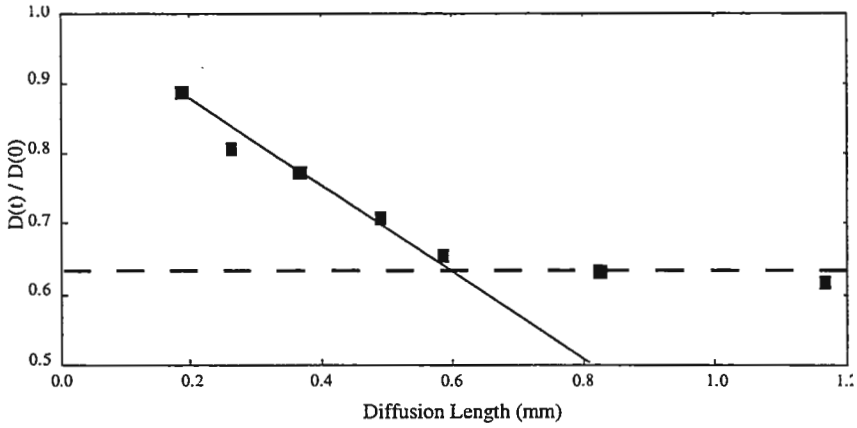

Fig. 1: Time dependent diffusion coefficient ($D(t)$) for ^{129}Xe in 4 mm glass beads, normalized to free diffusion coefficient ($D(0)$) = $1.36 \times 10^{-6} m^2 s^{-1}$. Diffusion Length is calculated from $(D(0)\Delta)^{1/2}$. The straight line is a theoretical prediction for short-time diffusion behavior, derived from the surface-volume ratio in the pores, while the $D(t)$ values reach an asymptote of ~ $0.64 \times D(0)$, which is approximately the square-root of the porosity of the sample.

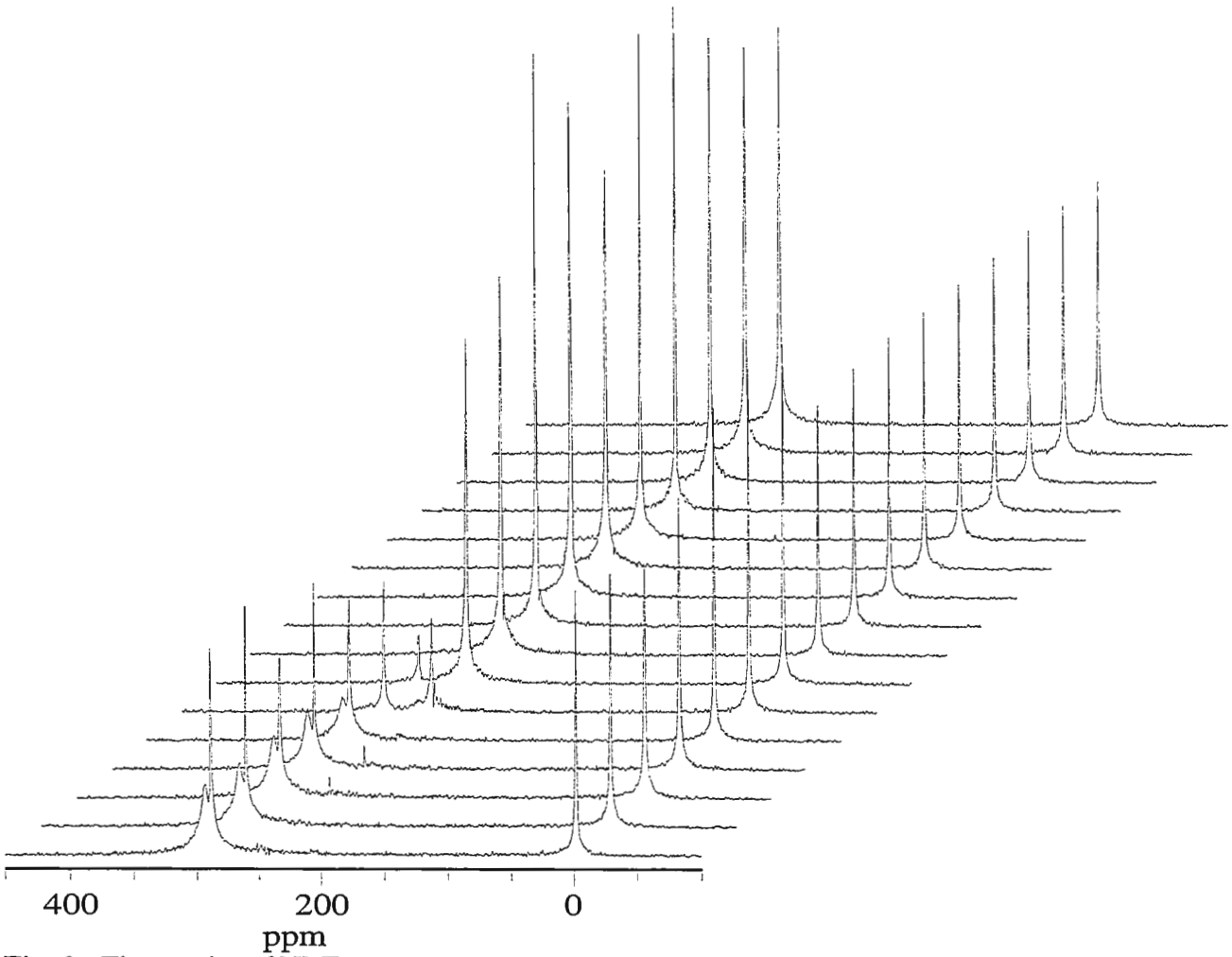

Fig. 2: Time series of NMR spectra, taken every 30 seconds, showing laser-polarized xenon ice at 300 ppm, melting into liquid, represented by the peak appearing at 250 ppm after ~ 3 minutes. The narrow peak at 300 ppm may be ice melting into liquid, and liquid spins in an exchange state. The peak at 0 ppm is due to xenon gas. The liquid/ice sample is made by condensing xenon gas with liquid N_2 once it has been laser-polarized.

Harvard-Smithsonian Center for Astrophysics
60 Garden Street, Cambridge, MA 02138

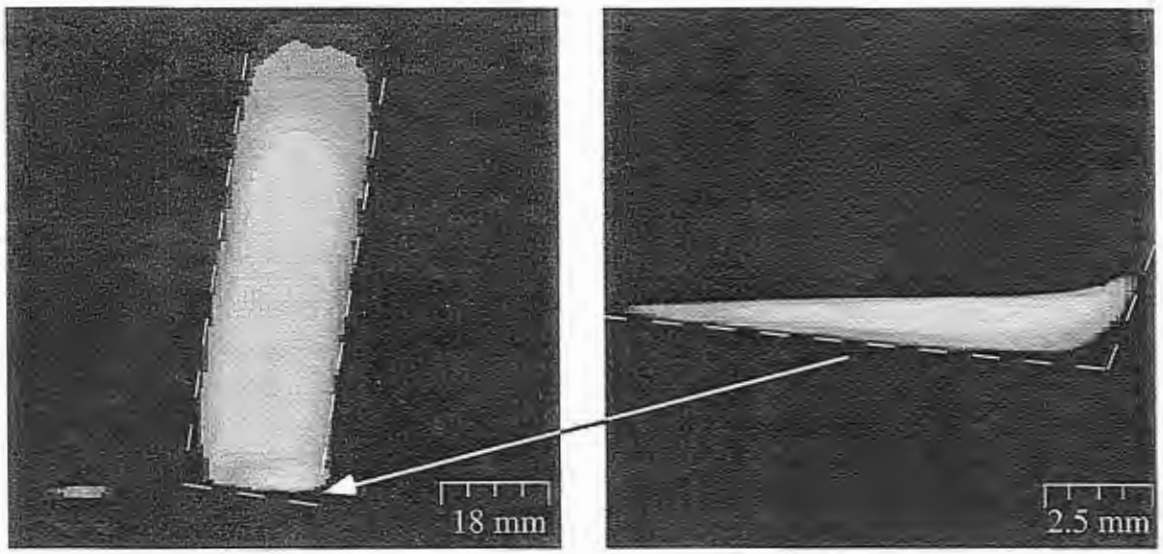

Fig. 3: NMR images of gas (left) and liquid (right) laser-polarized xenon. The gas was polarized in a cylindrical cell of approx. 1 inch diameter and 7 inches long, before the liquid was formed by condensing in liquid nitrogen. The cell was placed in an iso-octane slush bath in a dewar which was then placed in the RF coil before insertion into the Omega 4.7T/33cm magnet. The cell was tilted so the liquid would collect in one corner of the of the cell. The liquid meniscus is seen in the liquid image. One interesting feature in the gas image is a frequency-offset image of the liquid on the left, possibly from excited gas-phase spins exchanging into the liquid droplet during the imaging time. The gas phase image also shows distortions near the liquid due to susceptibility difference and/or the dipolar demagnetizing field from the liquid spins.

Free Spectrometer Service Agreement Plan

- Do you currently own an NMR spectrometer?
- Do you plan to replace the console in a few years?
- Is your system presently covered by a service agreement, or would you like it to be?

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1. MR Resources will purchase your console or complete system now.
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3. In lieu of cash, MR will provide a full coverage service agreement for the system. Essentially, this is a barter arrangement, whereby ownership of the system is exchanged for services provided by MR Resources.
4. At the end of the specified time period, you may either:
   a) Relinquish the system (or console) to MR Resources, and replace it with a new one from the vendor of your choice. Of course, if that vendor happens to be MR Resources, you will receive a guaranteed discount.
   b) Continue to use the system under a lease-back arrangement with MR Resources
   c) Purchase the system back from MR Resources at a predetermined price

Here is a typical example. Obviously, the numbers are just estimates, but we feel they are close to reality for many customers:

Comparison of costs for a typical system: AMX 300 or Unity 300

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Cost of service agreement (full system, three years)	$75,000.00	$0
Trade-in value of the console to Varian or Bruker after three years	$25,000.00	$0
Net cost after three years	*$50,000.00*	*$0*

Note that in this example, the service agreement covers the entire system but only the console is traded in to MR Resources.

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600 MHz Bruker Inverse VSP Broad ban std. bore 5mm	$ 9,700.00
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Dr. John S. Gounarides
Novartis Pharmaceuticals
556 Morris Avenue
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February 25, 1997 (received 3/19/98)

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

Interaction of a cyanopyrrolidide containing ligand with the serine protease DPP-IV.

Dear Barry:

Cyanopyrrolidide-containing compounds have been identified as potent inhibitors of dipeptidyl peptidase-IV (DPP-IV), a serine protease of approximate MW 220 KDa. Ligands containing cyanopyrrolidide bind the enzyme several orders of magnitude more tightly than the corresponding pyrrolidide analogues. While the function of the nitrile moiety in the inhibition of DPP-IV is not understood, it is possible that it may be similar to the nitrile effect on the cysteine protease papain, were the nitrile forms a reversible thioimidate intermediate.

Recently, we have been using NMR to examine the interaction of DPP-IV with a tight binding ligand, isoleucine-2-cyanopyrrolidide. Due to the large size of the enzyme and the tight binding of the ligand, the resonances of isoleucine-2-cyanopyrrolidide become unobservable upon binding to DPP-IV. However, using ^{13}C labeled isoleucine-2-cyanopyrrolidide and standard inverse and direct detect experiments we have not only been able to observe the disappearance of free ligand as it binds to the enzyme but also observe that the ligand is chemically altered by the enzyme.

The HSQC spectrum shown below was acquired over a 68 hr. period using a 0.1 mM DPP-IV sample (Figure 1). Due to the process of binding, cross-peaks from the free,

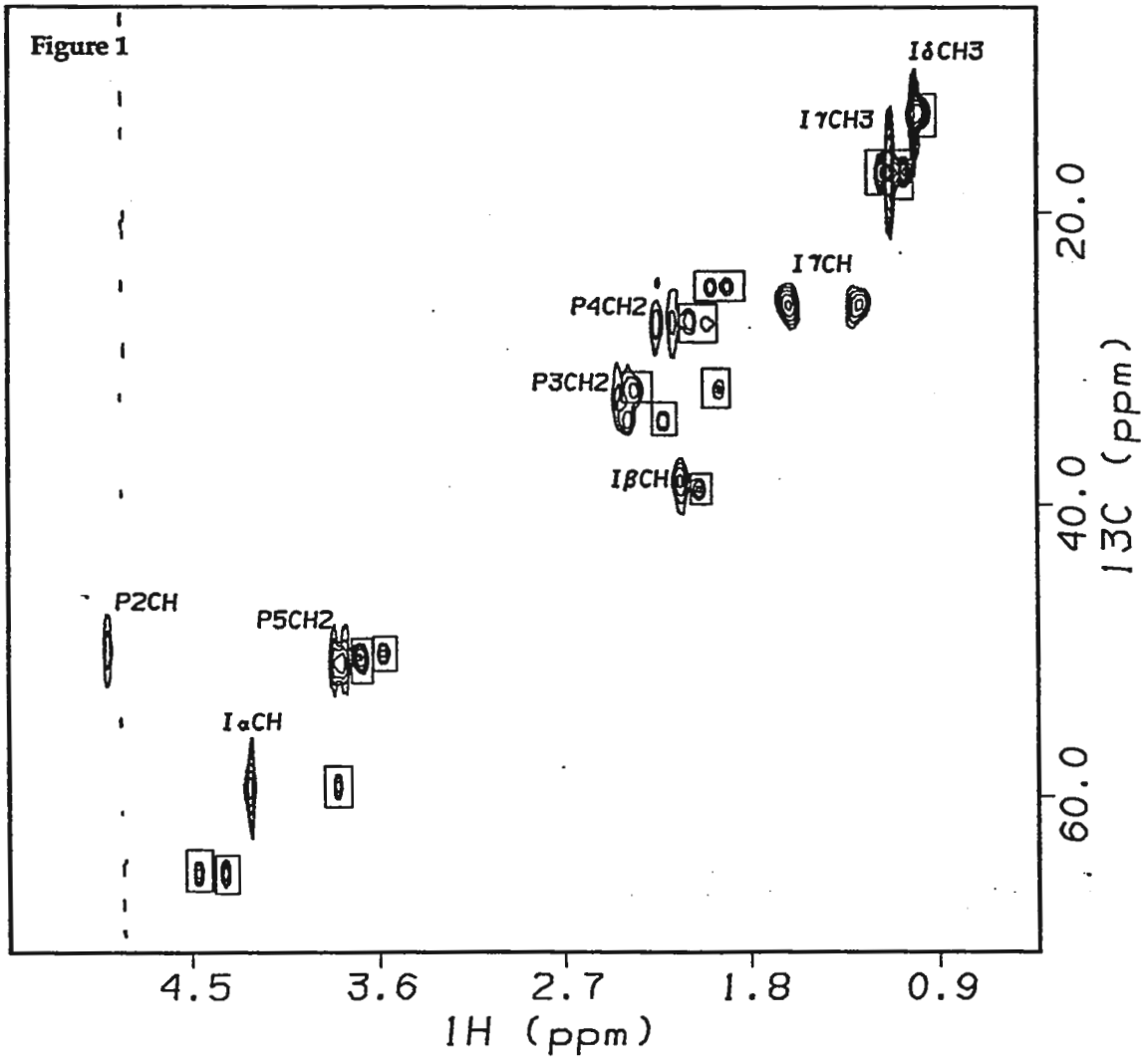
unmodified ligand are broadened in the ω1 dimension. In addition cross-peaks which are believed to arise from chemically modified ligand are observed (shown boxed). Interestingly, these signals will, once all the observable free ligand is gone, eventually disappear suggesting that the modified ligand is also able to tightly bind with DPP-IV. We are currently interested in determining the nature of the chemical modification to the ligand.

Sincerely yours,

J. S. Gounarides N.R. Nirmala S. C. Weldon

T. E. Hughes T. Vedananda

Figure 1



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The NMR Newsletter
966 Elsinore Court
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24 February 1998
(received 2/27/98)

"Xe^{129} Diffusion in Polymers using Pulse Gradient Spin Echoes"

Dear Barry:

We have been looking at Xenon - NMR in polymers recently as a probe of morphology and the nature of gas diffusion through such heterogeneous systems. The rate of exchange of xenon between distinct chemically shifted sites is determined by the diffusion constant in the individual sites, and as such, variable temperature NMR can probe the exchange and yield information both on the nature, size and diffusion in distinct morphological domains.

It is useful to have an independent determination of the diffusion constant and we have recently tried the traditional Pulse Gradient Spin Echo methods (PGSE). The T_1 of Xe^{129} is typically long (secs) whereas the T_2 can be short (msecs). As a consequence the "stimulated echo" method is useful:

	90°	— Gradient —	90°	—	90°	— Gradient —	Echo.	
time:	0		τ_1		τ_2		$\tau_1 + \tau_2$	

The time between the first two 90° pulses is made short (determined by T_2) and the time between the last two 90° long (determined by T_1) thus allowing a long time between Gradient pulses in spite of a short T_2 and giving a reasonable echo intensity. The necessity for extremely strong gradient pulses is avoided.

In Polydimethyl Siloxane, a liquid at room temperature and a simple single phase system, the results from the stimulated echo and the conventional 90°, 180° echo agree. In this case the Xe^{129} line is sharp with a long T_2 and the stimulated echo advantages are not an issue. A value of $1{\cdot}7 \times 10^{-5}$ cm^2/s for D is determined. In a polymer ionomer blend based on the Siloxane and Polystyrene where the morphology in complex and the Xe^{129} line is broad ($T_2 < 1$ msec) the stimulated echo method is required to obtain D. The value obtained is $7{\cdot}5 \times 10^{-6}$ cm^2/s. A previously obtained value for D in Polystyrene of 3×10^{-9} cm^2/s suggests, as one might expect, the gaseous diffusion through a complex morphology is dominated by the components or domains where the diffusion is more rapid. This has ramifications in terms of modeling the microscopic nature of diffusion through complex morphologies. The data is summarized below.

	T_1 (s)	T_2 (ms)	D (cm^2/s)
Xe in PDMS	120	6	$1{\cdot}7 \times 10^{-5}$
Xe in PS	14	0.3	3×10^{-9}
Xe in Blend*(PS-PDMS)	20	0.8	$7{\cdot}5 \times 10^{-6}$

*(The blend is formed by using coordinating groups on the parent polymers: Propyl amine on PDMS and Zinc Sulfonation on PS at a level of about 5%)

Sincerely,

Paul T. Inglefield Yingzi Wang

***** New Mexico Regional NMR meeting *****

Since 1982, the NMR spectroscopists in the New Mexico region have been meeting twice a year. The Spring 1998 meeting of "NMR.NMR" will be:

Saturday MAY 9th 1998, At New Mexico State University, Las Cruces NM.

There is **no registration fee** - which cover the full day program including Lunch, Dinner and Evening Social/Poster Session. Our speakers will be:

Robert G. Shulman (Yale University): "NMR Studies of Brain Metabolism"
Tanja Pietraß (New Mexico Institute of Mines and Technology"):
" 129 Xe NMR of Nanoporous Silica "
Robert London (National Institutes of Environmental Health Sciences): TBA
Arvind Caprihan (University of New Mexico):
"Partial k-space Data Reconstruction Techniques with Applications to Gas Imaging"
Yair Shachar-Hill (New Mexico State University)
"Multinuclear Spectroscopic studies of a Plant-Fungal Symbiosis"

To register and for further information please contact :
Yair Shachar-Hill, Dept Chemistry and Biochemistry; NMSU; Las Cruces; NM 88003
e-mail: **yairhill@nmsu.edu** phone: (505) 646-3218 fax: (505) 646-2649

Further Information about NMR^2 will be posted at **http://www.unm.edu/~karenann/nmr2.html**
For info on NMSU, chemistry and Las Cruces visit **http://www.chemistry.nmsu.edu/**

The NMR Newsletter - Book Reviews

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

"Spin-1 NMR"

by

Narayanan Chandrakumar

Springer-Verlag, Berlin, Heidelberg, New York, 1996. ISBN 3-540-60581-9 (Berlin), 0-387-60581-9 (New York); 117 pages $ 157. http://www.springer-ny.com.

"Spin-1 NMR" is the 34th volume of *NMR Basic Principles and Progress* published by Springer-Verlag. With this compact book, Dr. Narayanana Chandrakumar takes on the daunting task of elucidating the major advances in NMR of spin-1 systems using a single density matrix formalism. The book covers about two dozen solid-state and liquid-state NMR experiments with 144 references in 117 pages.

The first chapter supplies the tools needed to build the density matrix and to calculate how it evolves in time. A methodology to calculate the time evolution of the density matrix with radio frequency pulses, the electric quadrupole interaction and *J* couplings is presented. Short descriptions of the spinor-like behavior of spin-1 transitions, a selective excitation experiment and relaxation rates are also included.

The second chapter covers liquid-state experiments beginning with a discussion of one-dimensional experiments. The ^{13}C multiplet patterns due to scalar spin-spin couplings of ^{13}CD, $^{13}CD_2$ and $^{13}CD_3$ groups are listed for relevant bilinear operators along with spin-½ and spin-1 multiplet patterns observed after a variety of coherence transfer experiments from spin-1 nuclei. The two-dimensional experiments involving spin-1 nuclei are covered in the second half of the chapter. The measurement of I=1 spin connectivity with the COSY and TOCSY experiments and the quantitative measurement of very weak *J* couplings with double-quantum experiments are demonstrated.

The last chapter covers solid-state NMR experiments starting with a spin-dynamics-based discussion of the quadrupolar echo and the Jeener-Broekaert sequence. Next it is shown that magic-angle spinning combined with rotor-synchronized acquisition gives high resolution (100 Hz) chemical shift spectra despite large (100 kHz) quadrupolar couplings. Benefits gained with single-quantum and double-quantum cross-polarization experiments are also discussed. Two-dimensional spin-1 experiments in the solid state are covered last. The QUADSHIFT experiment is shown to give chemical shift information on one axis and quadrupolar interactions on the other. A two-dimensional version of the overtone experiment is shown to give accurate, enhanced measures of ^{14}H-^{1}H dipolar splittings.

continued

The calculation of ^{14}N-^{13}C dipolar splittings are discussed along with some triple-resonance experiments which can be used to measure weak heteronuclear dipolar interactions. The final section is a detailed description of deuterium-exchange spectroscopy which is used to measure reorientational molecular motions in deuterated polymers by monitoring how orientational-dependent quadrupolar order changes in time.

This book is a reference for the expert in spin-1 NMR. Tacit assumptions in the derivations unfortunately make the description of the evolution of the spin-matrix difficult to follow. On the other hand, the book gives a collection of clearly written, concise synopses of a large number of important NMR experiments using spin-1 nuclei to elucidate molecular structure and dynamics.

Jon M. Goetz
Department of Chemistry
Washington University in St. Louis
St. Louis, MO 63130

Forthcoming NMR Meetings, continued from page 1:

Fifth International Conference on Heteroatom Chemistry, London, Ont., Canada, **July 5 - 10, 1998**. For details, see Newsletter 468, 40.

XIVth International Conference on Phosphorus Chemistry, Cincinnati. OH, **July 12 - 17, 1998**. For details, see Newsletter 468, 40.

NMR Symposium at the 40th Rocky Mountain Conference on Analytical Chemistry, Denver, CO, **July 27 - 30, 1998**. Contact: Dr. Robert A. Wind, Battelle/Pacific Northwest National Laboratory, P.O. Box 999, MS K8-98, Richland, WA 99352; (509) 376-1115; Fax: (509) 376-2303; Email: ra_wind@pnl.gov. See Newsletter 470, 8.

XVIIIth International Conference on Magnetic Resonance in Biological Systems, Tokyo Metropolitan University, **August 23 - 28, 1998**. Contact: Professor Masatsune 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; http://icmrbs98.chem.metro-u.ac.jp

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.

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.

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.

E-mail Addresses Wanted

Please include your e-mail address on all correspondence, including technical contributions, or send me an e-mail message. This will make it more convenient - and economical - to contact you. Thanks.

BLS
shapiro@nmrnewsletter.com

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650-493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

Deadline Dates

No. 476 (May)	24 Apr. 1998
No. 477 (Jun.)	22 May 1998
No. 478 (July)	26 June 1998
No. 479 (Aug.)	24 July 1998
No. 480 (Sept.)	21 Aug. 1998

* Fax: 650-493-1348, at any hour. Do not use fax for technical contributions to the Newsletter, for the received fax quality is very inadequate.

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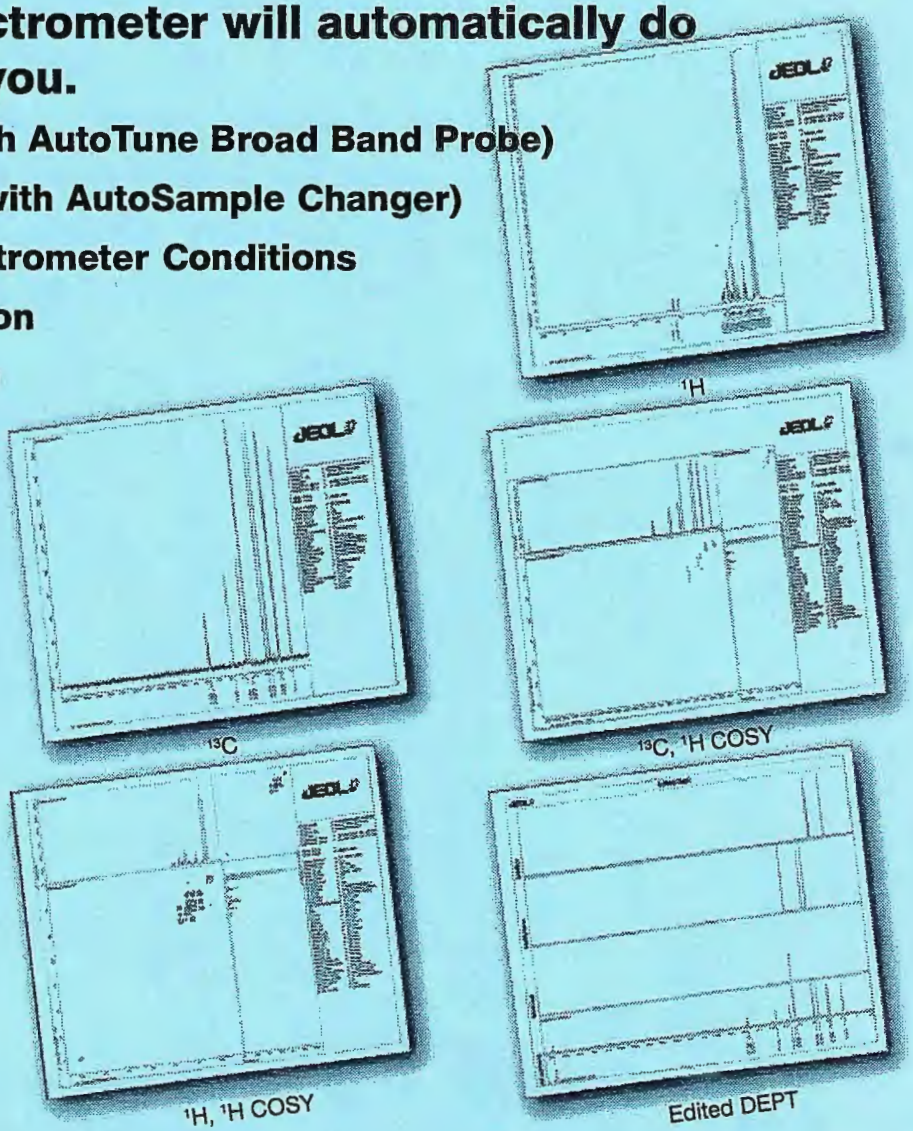

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