TEXAS A&M UNIVERSITY

NMR NEWSLETTER

No. 436

January 1995

continued on page 54

TABLE 1 DEUTERATED SOLVENTS

Cat. No.	Description	Formula	Min. Isp. (% D)	Density (g/cc)	MP (°C)	BP (°C)	$-\chi_v \times 10^6$ @ (°C)
D-11	Acetone-d_6	CD_3COCD_3	99.9%	1.17	-97	58	0.551 (32)
D-120	Acetone-d_6	CD_3COCD_3	99.9%	1.17	-97	58	0.551 (32)
D-13	Acetone-d_6	CD_3COCD_3	99.8%	0.87	-94	57	0.460 (20)
D-121	Aceonte-d_6 + 1% TMS		99.8%				
D-129	Acetone-d_6 + 1% TMS		99.8%				
D-14	Benzene-d_6	C_6D_6	99.6%	0.95	6	80	0.543 (20)
D-21	Benzene-d_6	C_6D_6	99.6%	0.95	6	80	0.611
D-122	Benzene-d_6	C_6D_6	99.6%	0.95	6	80	0.611
D-130	Benzene-d_6	C_6D_6	99.6%	0.95	6	80	0.611
D-28	Chloroform-d	$CDCl_3$	99.8%	1.50	-64	62	0.740 (20)
D-31	Chloroform-d + 1% TMS		99.8%				

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WCV-60 (A-60C)	11" X 26"	A-60, A-60A, A-60D	Gridded-Two Color
WCV-XL (XL-100)	11" X 26"	XL-100 (CW and FT)	Gridded-Two Color
WCV-XLFT-100	11" X 26"	XL-100 (CW and FT)	Gridded-Two Color
WCV-XL-200	11" X 26"	XL-200, XL-300, XL-400	Gridded-Two Color
WCV-20 (CFT-20)	11" X 16 3/4" X 17"	CFT-20, FT-80, FT-80A All Models use 16 1/2" or 16	Gridded-Two Color Blank

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FORTHCOMING NMR MEETINGS

Symposium on *In Vivo* Magnetic Resonance Spectroscopy VIII, North Falmouth, Massachusetts, **March 25 - 26, 1995**; Contact: Radiology Postgraduate Education; Room C-324, University of California School of Medicine, San Francisco, CA 94143-0628; Phone: (415) 476-5731; Fax: (415) 476-9213; For registration, call (415) 476-5808.

36th ENC (Experimental NMR Conference), Boston, MA, **March 26 - 30, 1995**; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87501; (505) 989-4573; Fax: (505) 989-1073.

Keystone Symposia on Molecular and Cellular Biology, Frontiers of NMR in Molecular Biology - IV, Keystone, Colorado, **April 3 - 9, 1995**; Organizers: S. W. Fesik, T. L. James, and G. Wagner; Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498; Phone: (303) 262-1230; Fax.: (303) 262-1525.

International School of Biological Magnetic Resonance, 2nd Course: Dynamics and the Problem of Recognition in Biological Macromolecules, Erice, Trapani, Sicily, Italy , **May 22 - 30, 1995**; Contact: Prof. O. Jardetzky, Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, CA 94305-5055; Phone: (415)723-6270; Fax: (415) 723-2253; or, Prof. J.-L. Lefèvre, ESBS, CNRS-UPR9003, Univ. Louis Pasteur, Blvd. Sébastien Brant, F67400 Illkirch Graffenstaden, France; Phone: (+33) 88-655269; Fax.: (+33) 88-655343- See TAMU NMR Newsletter 432, 38.

12th International Meeting on NMR Spectroscopy, Sponsored by the Royal Society of Chemistry, Manchester, England, **July 2 - 7, 1995**; Contact: Dr. J. F. Gibson or Ms. G. B. Howlett - See TAMU NMR Newsletter 415, 5; Phone: (44-71) 437-8656; Fax: (44-71) 437-8883.

ISMAR 1995, Sydney, NSW, Australia, **July 16-21, 1995**; Contact: Dr. W. A. Bubb, Dept. of Biohemistry, Univ. of Sydney, Sydney, NSW 2006, Australia. Phone: +61-2-351-4120; Fax: +61-2-351-4726; Email: ismar95@biochem.su.oz.au Also, see TAMU NMR Newsletter 436, 12.

NMR Symposium at the 37th Rocky Mountain Conference on Analytical Chemistry, Denver Colorado, **July 24-27, 1995**; Contact: Dr. Alexander J. Vega, DuPont Central Research and Development, P.O. Box 80356, Wilmington, DE 19880-0356; Tel. (302) 695-2404; Fax: (302) 695-1664; e-mail: vego@esvax.dnet.dupont.com. See TAMU NMR Newsletter 432, 34.

37th ENC (Experimental NMR Conference), Asilomar Cnference Center, Pacific Grove, California, **March 17 - 22, 1996*[sic]***; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87501; (505) 989-4573; Fax: (505) 989-1073.

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^{2}H NMR of Silane Coupling Agents on Silica in Composites

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

December 6, 1994
(received 12/12/94)

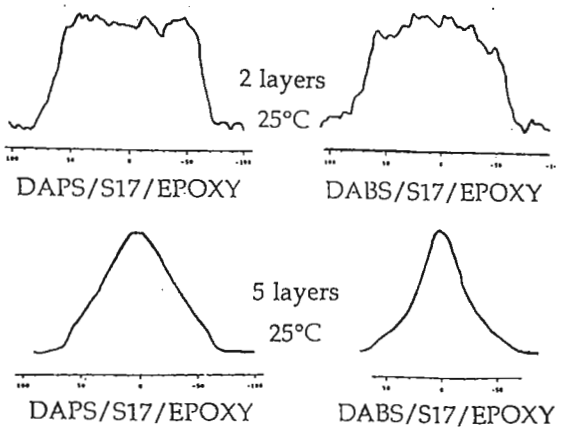
Dear Barry:

The interface between the fibers and resin plays an important role in the final properties of composites. Silane coupling agents are often used to improve the properties of this interface. Our group has been studying the role that molecular motion play in this interface. To do this we compared two structurally-similar deuterium labeled amino-functional coupling agents, i.e. γ-aminopropyltriethoxysilane (DAPS) and γ-aminobutyltriethoxysilane (DABS).

Previous studies revealed that DAPS and DABS behave similarly at monolayer coverage when reacted on silica.[1] In multilayer coverage, they exhibited differences, with DABS being more mobile. The overpolymerization of DABS on silica with bismaleimide restricted the mobility of the coupling agent.[2] We have extended these studies to a comparison of DAPS and DAPS on silica with an epoxy overcoat. Absorption of 2 layers of coupling agent (based on 4 silanes/nm^2) on silica with an epoxy overcoat results in similar lineshapes for DAPS and DABS (although that for DABS is a little narrower) With the equivalent of 5 layers both spectra are narrower than for two layers and DABS is significantly narrower than DAPS. We believe that the difference in mobility between these two coupling agent systems, at the interface, is responsible for differences in the physical properties observed.[3]

Sincerely Yours,
Timothy Wang
Timothy Wang
Graduate Student

Frank
Frank D. Blum
Curators' Professor of Chemistry



[1] H.-J. Kang and F.D. Blum, *J. Phys. Chem.*, **95**, 9391-9396 (1991).
[2] J.E. Gambogi and F.D. Blum, *Macromolecules*, **25**, 4526-4534 (1992).
[3] T.W.H. Wang and F.D. Blum, submitted.

MAGIC ANGLE SPINNING OF SAMPLES IN GLASS TUBES

In order to analyze materials that are sensitive to air or water exposure, it is often convenient to keep the sample permanently sealed in a glass tube. For magic angle spinning experiments this requires extra care, since the tube must be precisely centered in the MAS rotor to ensure symmetric distribution of the mass.

If experiments of this type are to be performed frequently on a widebore system, a special, elongated MAS stator called the "stretch" stator is available. The stretch stator is designed in such a way that the sample and rf coil occupy the same volume as for a normal BL-7 stator. Therefore, there is no change in the NMR specifications, including rf power and sensitivity. The stretch stator is elongated toward the front in order to allow additional length within the rotor. This additional length is primarily used to provide space for the empty section of the glass tube above the sample, including the glass seal. A second use is to allow a zirconia ballast rod to be inserted above a difficult sample to make it easier to spin.

The additional length of the stretch stator does not affect its spinning capabilities. However, a maximum speed of 4-5 kHz is recommended to avoid excessive stress to the glass sample tube.

Because of its length, the stretch stator does not allow the sample to be pneumatically ejected or inserted. Instead, the probe cover must be removed when exchanging samples. A special MAS cover conversion kit is provided to modify the probe cover for easy, bayonet style removal.

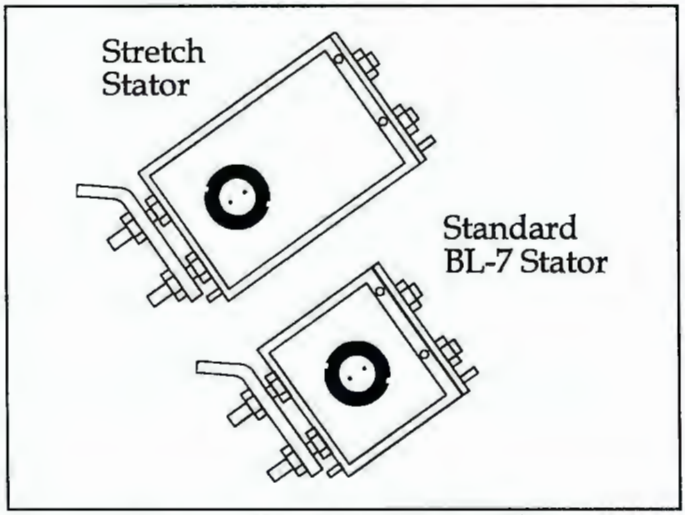
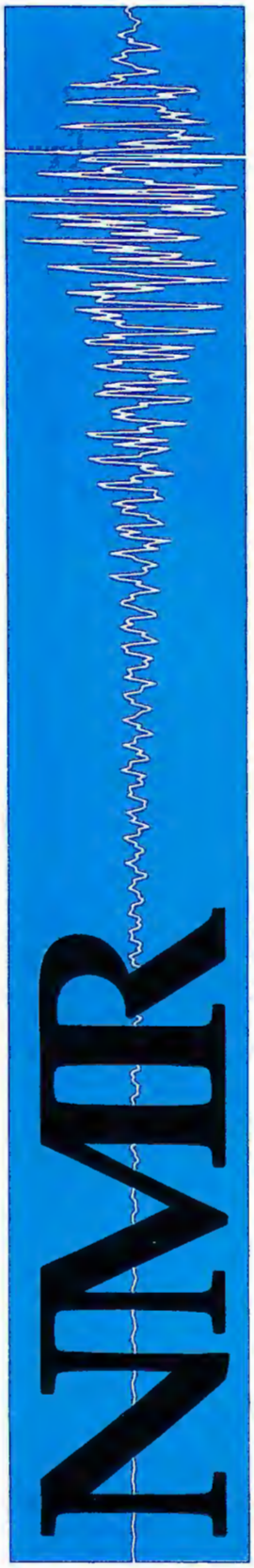


Figure 1: The front portion of a stretch stator is elongated in comparison with a standard stator.



BRUKER

NMR

Two types of zirconia stretch rotor are available. One (P/N B0437) is designed to accommodate standard 5 mm OD glass NMR tubes. Air vents are provided at the base of the rotor to allow easy removal of the tubes. The other type (P/N B0191) has a 5.6 mm ID (the same as for a normal BL-7 rotor). Glass tubes specially manufactured to fit the Bruker 5.6 mm ID rotors may be obtained from Bruker or directly from Wilmad Glass Company. These tubes may also be used in Bruker 7 mm rotors of standard length, and are available with or without a constriction to facilitate sealing the tube at the appropriate length to fit a standard Bruker 7 mm MAS rotor (BL or DB style).

The stretch stator upgrade kit is easily installed in the customer's lab. In fact, many customers will feel comfortable alternating between stretch and normal stators in the same probe body according to experimental needs.

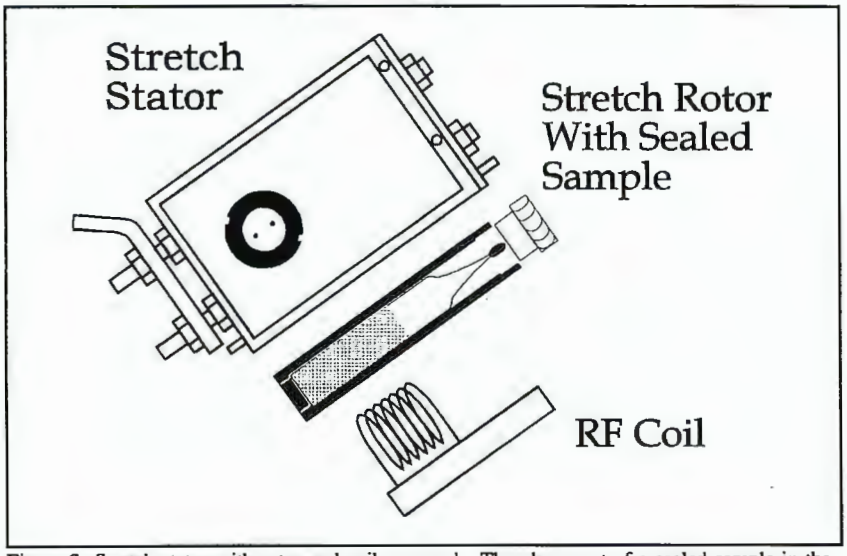


Figure 2: Stretch stator with rotor and coil removed. The placement of a sealed sample in the rotor is shown.

The upgrade kit (P/N B0187) includes the following elements (please also specify frequency of probe at time of order):

B0180	stretch stator assembly
B0185	stretch stator rf coil
B0437	three stretch rotors with 5 mm ID
B0191	two stretch rotors with 5.6 mm ID
X	zirconia ballast plug
B0190	stretch sample packing tool
B0188	split cover conversion kit

Please contact your local Bruker representative for more information and for a price quotation.

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

20th November 1994
(received 11/28/94)

Dear Barry,

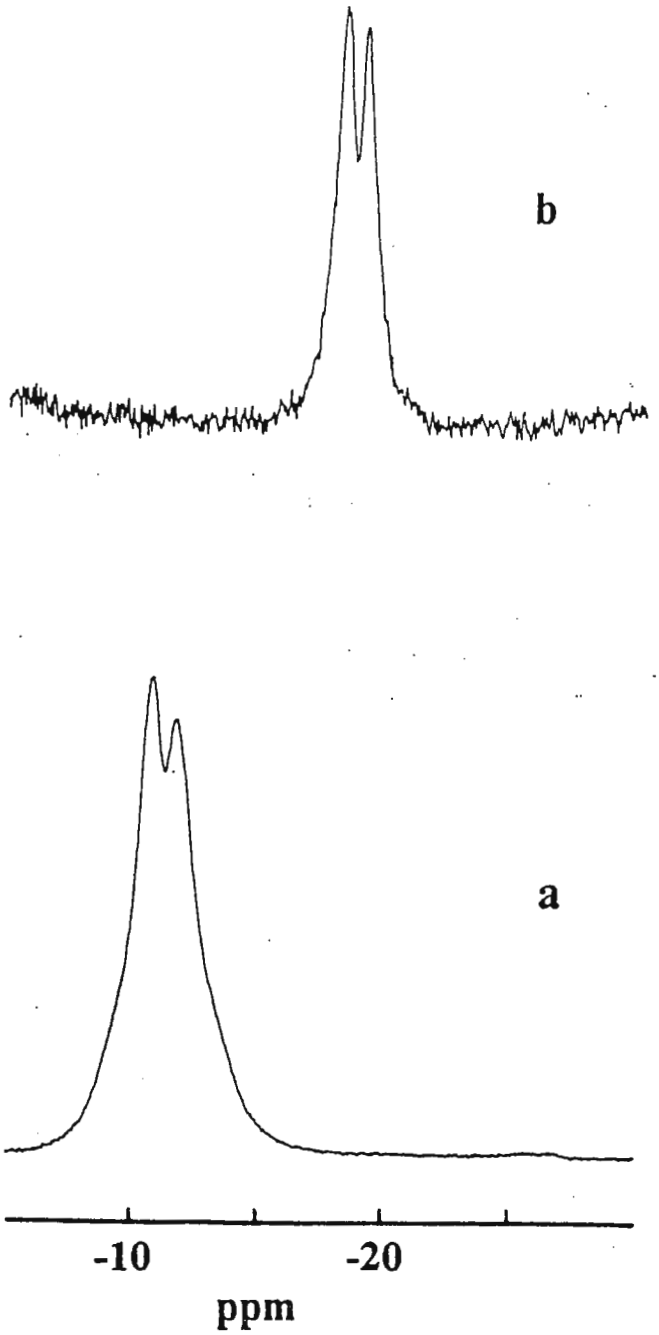
^{1}H MAS NMR Spectra of Transition Metal Carbonyl Hydrides

For some time now we have been looking at the ^{1}H MAS spectra of the title compounds, in collaboration with the group of Silvio Aime in Torino. The hydride ligand resonances do in fact show remarkably good chemical shift resolution for both differing chemical and crystallographic environments. The examples that we have measured have relatively weak interproton dipolar interactions, either because they may be considered as proton 'dilute' or because the interactions involve at least one group which may reasonably be assumed to have considerable motion in the solid state at ambient temperatures. MAS rates in the range 8 to 11 kHz resulted in linewidths as small as 90 Hz, without recourse to either multiple pulse methods or to isotopic (^{2}H) substitution. In addition relaxation times are not excessively long: we measured T_1 = 11.9 ± 0.5 s for the hydride resonances in $H_2Os_3(CO)_{10}$, and for the ruthenium complex $HRu_3(CO)_9TBA$ where TBA = t-butylacetylene the T_1's were 0.2 s for the *t*-butyl protons and 2.9 s for the hydride proton. The range of ^{1}H shifts measured is as follows, where the values in parentheses indicate the solution chemical shifts where available: $H_2Os_3(CO)_{10}$ -10.9 and -12.5 δ (-11.4 δ); $H_2Os_3(CO)_9S$ -20.3 and -21.1 δ (-20.7 δ); $H_2Os_3(CO)_{11}$ -10.5 and -20.5 δ (-10.3 and -20.0 δ); $H_2Os_3(CO)_{10}NH_3$ 1.2 (NH_3), -10.1 and -16.2 δ (0.2, -10.5 and -16.4 δ); $HRu_3(CO)_9TBA$ 1.4 (*t*-Bu) and -21.2 δ (1.4 and -21.8 δ); $H_2FeRu_3(CO)_{13}$ -18.1 and -18.9 δ (-18.4 δ); $[NHEt_3]^+[HFe_3(CO)_{11}]^-$ 1.2 ($NHEt_3^+$) and -15.3 δ (*ca.* -14.5 δ); $Fe_3(CO)_{12}$/γ-alumina -17.5 δ.

A couple of representative spectra (not the best and not the worst) are shown in the Figure. Spectrum **a** is of $H_2Os_3(CO)_{10}$ at 300 MHz, MAS rate = 8.1 kHz, 48 scans, recycle delay = 20 s. Spectrum **b** is of $H_2FeRu_3(CO)_{13}$ at 300 MHz, MAS rate = 9.5 kHz, 914 scans, recycle delay = 60 s. There are apparent shoulders on the resonances in spectrum **a**, and I believe these arise from the homonuclear dipolar coupling combined with CSA, in the manner documented by Wu and Wasylishen (*J. Magn. Reson. A*, 1993, **102**, 183).

The others involved in the project are Patrick Barrie (UCL, London), Silvio Aime and Roberto Gobetto in Torino and Dermot Brougham here at QMW.

Best wishes.



Yours sincerely,

Geoff Hawkes.

Dr. G.E. Hawkes

P.S. At least I hope that the postmark will beat your deadline.

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Dr. B. L. Shapiro

TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

(received 11/26/94)
November 22, 1994

A Gradient-enhanced Refocused HSQC Provides Superior Resolution and S/N.

Dear Dr. Shapiro,

For ^{15}N-labeled protein samples, the HSQC experiment is usually the first acquired spectrum, since it indicates through its dispersion and peak count whether 3-D amide-edited experiments have a good chance of succeeding. However, side-chain NH_2 groups appear as pairs of peaks, and clutter up the spectrum. These can be edited out by running either a refocused experiment or a different experiment like DEPT, but these are less sensitive than the HSQC, and thus two experiments need to be run in order to both achieve maximum sensitivity and also to know which peaks originate from backbone amides, and which from side-chains. In this letter I present a refocused HSQC (1) that uses gradient selection (2) and sensitivity enhancement (3), a streamlined pulse scheme that minimizes the number of pulses (B.T.Farmer II, personal communication, 4), and semi-constant time in t_1 (5,6), which results in narrower lines. While the amount of signal at the first time point is less than that of a regular HSQC (with all the same gradients), the narrower lines mean that the actual signal-to-noise of the peaks is greater, and the resolution is improved. Since the refocusing removes all but tiny ghosts of the side-chain signals, the experiment provides optimum sensitivity, resolution, and cleanliness.

Figure 1 shows the sequence, as implemented on our Varian Unity-Plus 600 MHz spectrometer, equipped with pulsed-field gradients. The sequence incorporates semi-constant time (or

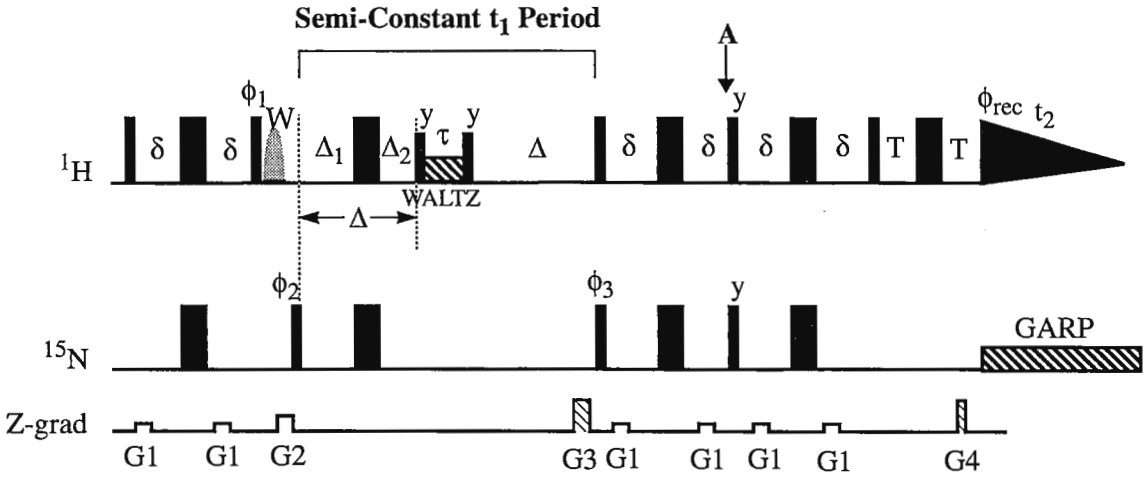


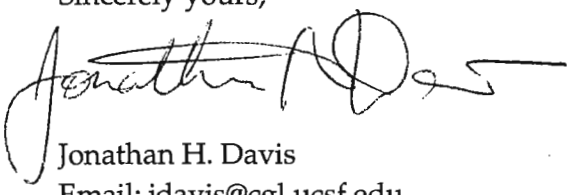
Figure 1: Pulse sequence for semi-constant time refocused HSQC with PFG

shared time) in the nitrogen dimension, so necessitates calculation of the τ, $\Delta 1$ and $\Delta 2$ delays for each t_1 timepoint. In addition, the tail of the sequence is a sensitivity-enhancement scheme devised by Kay et al, which requires that the data be resorted prior to processing (see reference 3 for details).

Parameters for the sequence in Figure 1 are as follows: All unmarked pulses are x, and other phases are: ϕ_1=y,-y; ϕ_2=x,x,-x,-x; ϕ_4=x; and ϕ_{rec}=x,-x,-x,x. For quadrature detection in t_1, in each alternate FID, ϕ_3 is incremented by 180° and the sign of G4 is inverted, and in each alternate t_1 increment, ϕ_2 and ϕ_{rec} are incremented by 180°. The gradients G3 and G4 are both about 30,000 G/cm, and G3 is 1.2 ms, G4 is 125 µs. The strength of G4 is adjusted for optimum signal. δ = <~$1/4J_{NH}$, or 2.25 ms. $\Delta = 1/2J_{NH}$, or 5.4 ms. T = 500 µs. Delays for semi-constant time period are calculated as follows: $\Delta_2 = \Delta * (t_1 / t_1 max)$; $\Delta_1 = \Delta - \Delta_2$; and $\tau = t_1 - (2 * \Delta_2)$. (In the case where the maximum t_1 does not exceed $2 * \Delta$, then $\tau = 0$; $\Delta_2 = t_1 / 2$; and $\Delta_1 = \Delta - \Delta_2$). W is an optional water re-inversion pulse (7) of phase x (the y trim pulse immediately before the period τ is only necessary if the W pulse is used). If gradients are not available, acquisition is started at point A, and ϕ_2 and ϕ_{rec} are cycled in the usual States-TPPI manner. In this case, water suppression can be achieved without the use of presaturation by installing an x spin-lock pulse on protons just before the ϕ_1 pulse.

The improvements in linewidth and S/N were measured on a sample of α-lytic protease, a 20 KDa monomeric protein, that was fully labeled with ^{15}N. When ω_1 slices through a single peak are compared, the refHSQC gives a linewidth approximately 40% narrower, with signal/noise about 45% higher, than the regular HSQC (run with all the same gradient- and sensitivity-enhancements). We feel that this sequence could be a useful one for heteronuclear NMR.

Sincerely yours,



Jonathan H. Davis
Email: jdavis@cgl.ucsf.edu

References

1. Burum, D.P. & Ernst, R.R. (1980) *J. Mag. Res.* **39** 163-168
2. Kay, L.E., Keifer, P., & Saarinen, T. (1992) *J. Am. Chem. Soc.* **114**, 10663-10665
3. Palmer, A.G. III, Cavanagh, J., Wright, P.E., & Rance, M. (1991) *J. Mag. Res.* **93**, 151-170
4. Van Doren, S.R., Kurochkin, A.V., Qi-Zhuang, Y., Johnson, L.L., Hupe, D.J., & Zuiderweg, E.R.P. (1993) *Biochemistry* **32**, 13109-13122
5. Grzesiek, S. & Bax, A. (1993) *J. Biomol. NMR* **3**, 185-204
6. Logan, T.M., Olejniczak, E.T., Xu, R.X., & Fesik, S.W. (1993) *J. Biomol. NMR* **3**, 225-231
7. Grzesiek, S. & Bax, A. (1993) *J. Am. Chem. Soc.* **115**, 12593-12594

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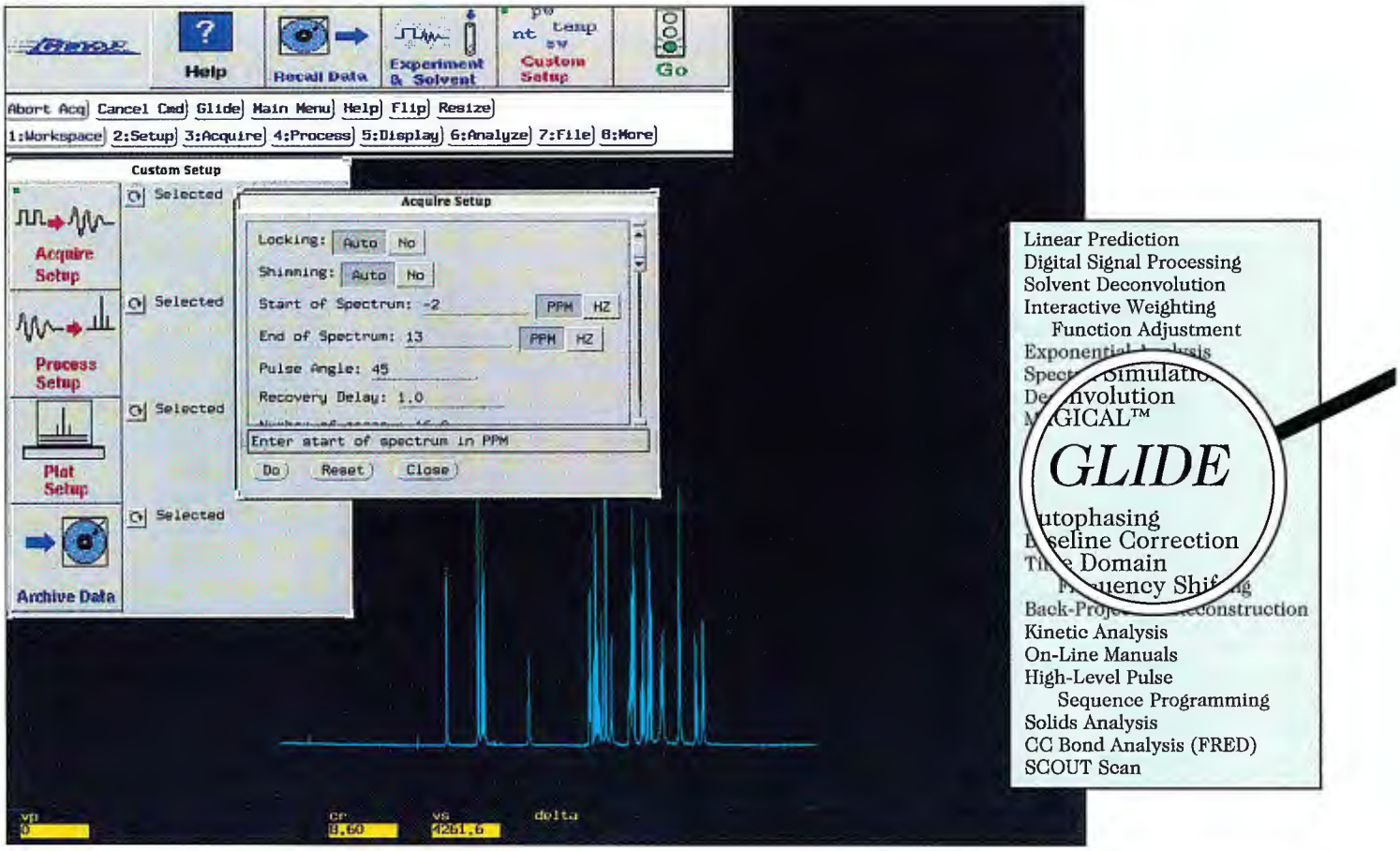

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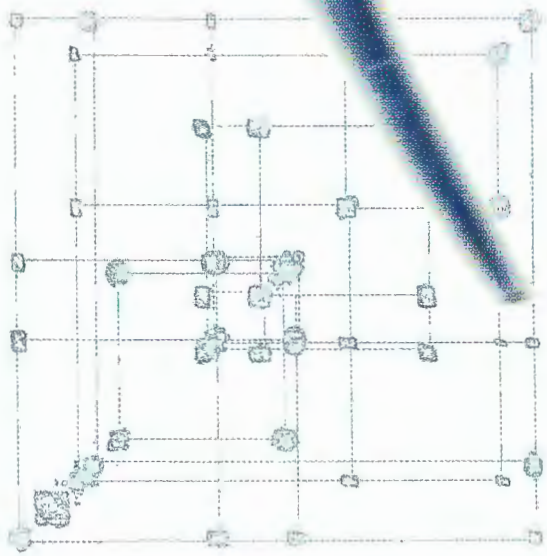

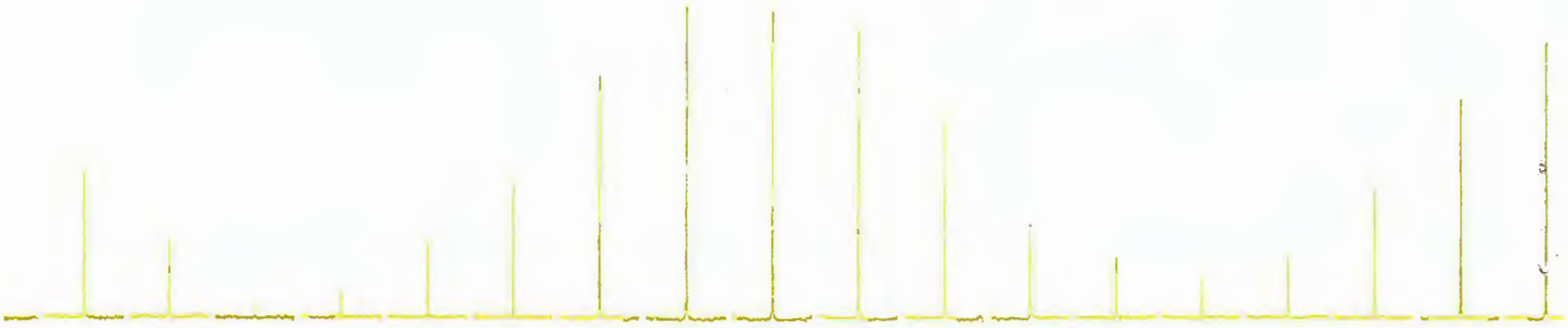

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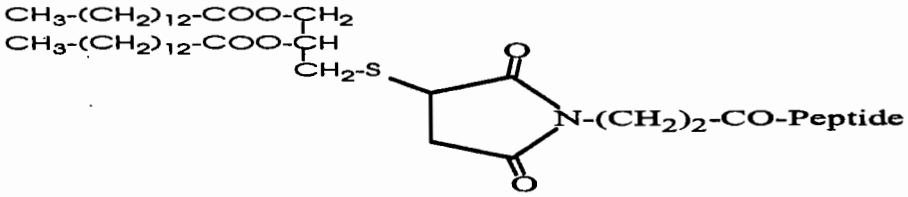
Nov. 17, 1994
(received 11/21/94)

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

Vesicle Bound Peptides in a Nanoprobe

Dr. Shapiro,

We are investigating the role of the membrane in the interaction of peptide ligands with membrane bound receptors [1]. One manner to ensure long-lived peptide membrane interaction and to control the orientation is incorporation of a novel anchoring "amino acid" [2]



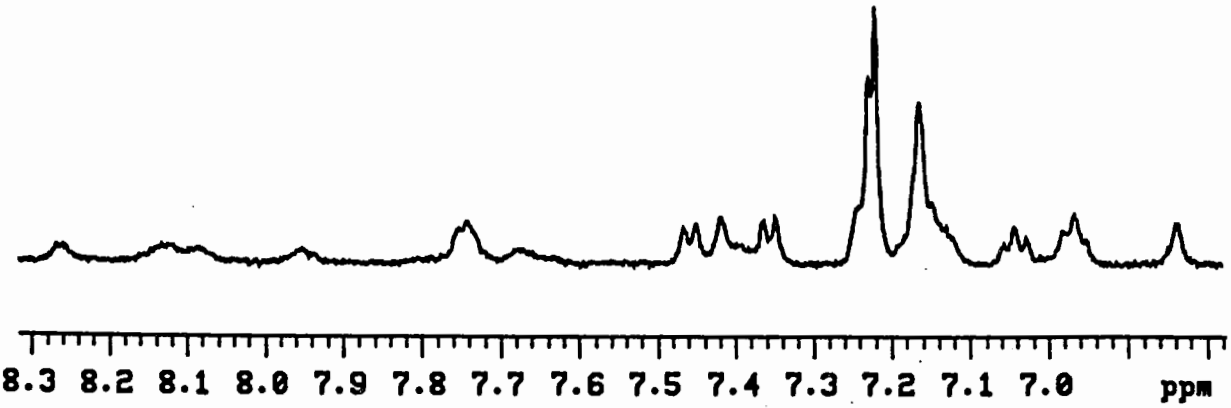
The ^{1}H-NMR analysis of the lipo-peptide in detergent micelles (SDS) and small unilamellar vesicles of DMPC and DPPC has been carried out with varying degrees of success. Here we illustrate the increased resolution available with the Nano•nmr™ probe from Varian. The spectrum shown below was obtained fro 250 μg of material in small unilamellar vesicles spinning at 2.1 kHz.

Please credit this contribution to the subscription of Paul Inglefield.

Sincerely,

Dale F. Mierke Maria Pellegrini Jeffrey H. Simpson

[1] Moroder, L. et al. *Biochemistry*, **32**, (1993) 13551. [2] Romano, R. et al. *B.B.A.* **1151**, (1993) 111.



436-12

16th - 21st JULY 1995 • SYDNEY • AUSTRALIA

ISMAR 95

TWELFTH CONFERENCE OF THE INTERNATIONAL SOCIETY OF MAGNETIC RESONANCE

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Secretary: Dr Bill Bubb
Office: + 61-2-351-4120
FAX: + 61-2-351-4726
E-mail: ismar95@biochem.su.oz.au

29 November, 1994

Professor B.L. Shapiro
966 Elsinore Court
Palo Alto, CA 94303

Dear Professor Shapiro,

Plans for ISMAR-95 to be held in Sydney, Australia in July, 1995 are well advanced.

The program will commence at the Sydney Opera House on Sunday 16 July, with a mixer and special session to commemorate the 50th anniversary of the discovery of NMR, and continue at the University of Sydney from Monday 17 until Friday 21 July. Speakers who have accepted invitations include:

Paul Callaghan (Palmerston, NZ)
Melinda Duer (Cambridge, UK)
Ray Freeman (Cambridge, UK)
Erwin Hahn (Berkeley, USA)
James Hyde (Milwaukee, USA)
Lewis Kay (Toronto, Canada)
Ray Norton (Melbourne, Australia)
Alex Pines (Berkeley, USA)
Hal Swartz (Hanover, USA)
Keith Thulborn (Pittsburgh, USA)
Warren Warren (Princeton, USA)
Kurt Wüthrich (Zürich, Switzerland)
David Doddrell (Brisbane, Australia)
Richard Ernst (Zürich, Switzerland)
Maurice Goldman (Gif-sur-Yvette, France)
Robin Harris (Durham, UK)
Jean Jeener (Brussels, Belgium)
Carolyn Mountford (Sydney, Australia)
John Pilbrow (Melbourne, Australia)
Charles Springer (New York, USA)
Takehiko Terao (Kyoto, Japan)
Kamil Ugurbil (Minneapolis, USA)
John Waugh (Cambridge, USA)
Nino Yannoni (San Jose, USA)

The deadline for submission of abstracts and normal registration is 1 April, 1995. The social program will include a cruise on Sydney Harbour and dinner in Sydney's historic Rocks area. Details of these events as well as information on satellite meetings and travel opportunities in Australia are provided in the registration brochure, copies of which may be obtained from me at the above address.

Yours sincerely,

W.A. Bubb.

Bill Bubb
for the ISMAR-95
Organising Committee

Procter&Gamble

The Procter & Gamble Company
Miami Valley Laboratories
P. O. Box 398707, Cincinnati, Ohio 45239-8707

Michael D. Cockman, Ph.D.
Procter & Gamble Pharmaceuticals
(513) 627-2356, FAX 627-1087
e-mail: cockmanmd@pg.com

October 7, 1994

MRI of Bone Loss in Ovariectomized Rats

Dr. Shapiro:

The Magnetic Resonance Imaging/Spectroscopy Laboratory became wholly "owned" by Procter & Gamble Pharmaceuticals in January, 1993, and since then I have guided most of the lab's work toward applications in the Health Care sector of the Company's business. I have been primarily interested in application of MRI methods to the detection and quantification of osteoarthritis and osteoporosis disease endpoints and the effects of pharmaceutical therapy thereon. Recently, I have been closely following the bone research work of Felix Wehrli's group at the University of Pennsylvania and Sharmila Majumdar and coworkers at UC-San Francisco. There are two primary applications of MRI for bone studies. These include microimaging to obtain maps of cortical (compact) bone thickness and trabecular (spongy) bone microstructure and T_2* analysis to determine "bone quality". The latter is most interesting as it offers an opportunity to develop an endpoint which can measured in the clinic, where it is not possible to routinely observe the microstructure of many bones of interest. The idea is that as trabecular bone is lost, the field inhomogeneity due to trabeculae/marrow interfaces is reduced and T_2* increases. Some labs have shown a correlation between T_2* and bone mineral density, which can be measured by x-ray techniques, such as quantitative computed tomography (qCT) or dual-energy x-ray absorptiometry (DEXA). However, T_2* offers information beyond that of the x-ray techniques in that it is sensitive to the number, orientation, and thickness of the trabeculae [H. Chung, et al. Proc. Natl. Acad. Sci., USA 90, 10250 (1993)].

In collaboration with life scientists from our bone research groups, my lab conducted a longitudinal MRI study of a rat ovariectomy (ovx) model of post-menopausal bone loss. We obtained MR images prior to ovx and then every 2 weeks up to 16 weeks post-ovx. We consistently positioned each live animal on its side with the right leg stretched out so that the femur and tibia were roughly parallel with the magnet z-axis and the knee joint was near the gradient isocenter. We acquired MR images using a water-suppressed, "asymmetric" spin-echo imaging technique [S. Majumdar and H. K. Genant, J. Magn. Reson. Imaging 2, 209 (1992)]. We then used image analysis software (Optimas Corp.) to measure mean signal intensities of four bone regions: the femur and tibia epiphysis and metaphysis. The femur is commonly known as the thigh bone and the tibia is the shin bone. The epiphysis is a region near the end of the bone; the metaphysis is between the epiphysis and the bone shaft. Curve fits of the signal intensity measurements produced estimates of lipid proton density, T_2, T_2*, and the field inhomogeneity parameter R_2' (where $R_2' = 1/T_2^* - 1/T_2$) for each of the four regions for each animal at each time point. We also obtained bone mineral density (BMD) measurements of the four regions by DEXA at termination.

Fig. 1 shows the results of DEXA analysis for the two regions of the femur. For a particular region, ovx BMD was about 10% lower than sham controls. For a particular treatment group, metaphysis BMD was about 30% lower than epiphysis. Figures 2 and 3 show the T_2* and R_2' results, respectively. For a particular region, ovx T_2* and R_2' values were not significantly different from sham controls. However, for a particular treatment group, metaphysis T_2* was significantly elevated and metaphysis R_2' was significantly reduced relative to the epiphyseal values. These results agree with expectations, since there are fewer trabeculae in the metaphysis.

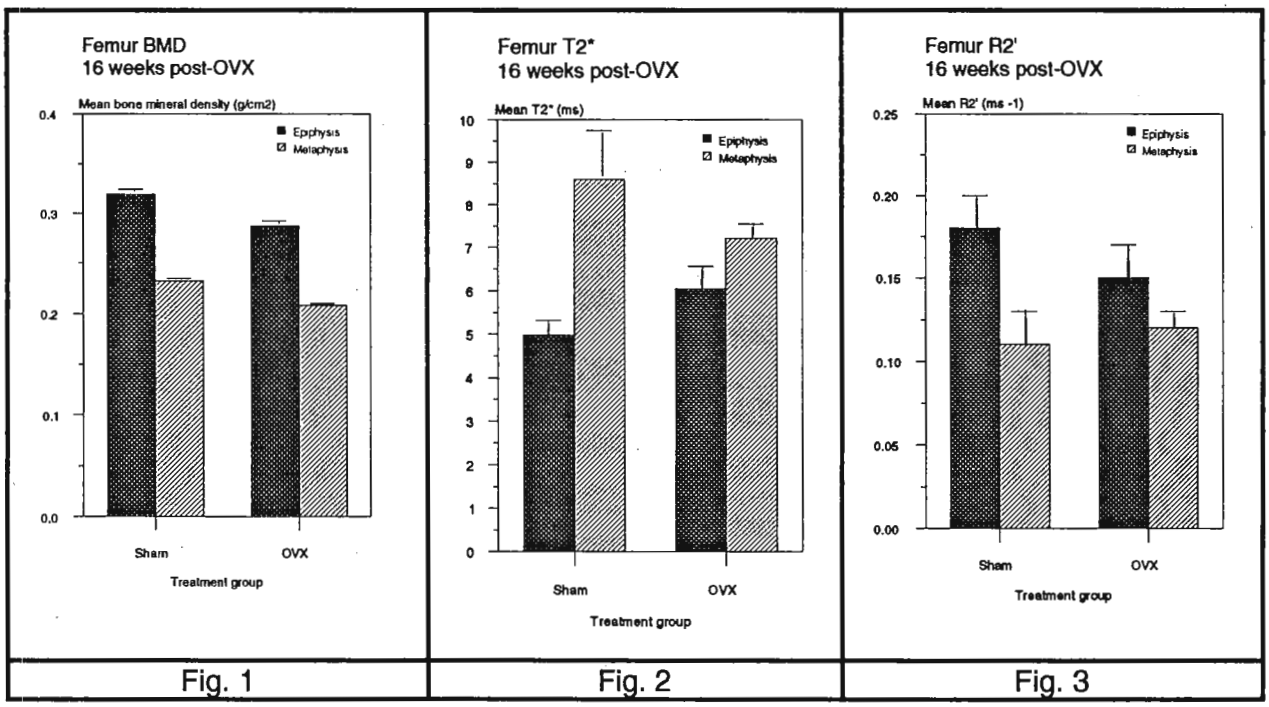

Fig. 1 | Fig. 2 | Fig. 3

Over the duration of the study, we also observed a large increase in lipid proton density in all four regions of the ovx rats relative to sham controls. This is important, as this effect could easily obscure the effects of bone loss in gradient echo images of ovx rat bones.

Recently, similar results, obtained from excised bones using similar imaging methods, were reported by a group at Zeneca Pharmaceuticals [D. J. Checkley, et al., J. Bone and Mineral Research 9, Supplement 1, A350 (1994)]. I agree with their contention that "...great care is required in repositioning." to ensure good reproducibility of T_2* results. If this source of error could be reduced in MR imaging of live animals, T_2* analysis could be a valuable indicator of pharmaceutical efficacy in osteoporosis studies.

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

M. D. Cockman

Michael D. Cockman, Ph. D.

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10/07/94 01:33 PM

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Exclusive VT Stack Design: ..... Permits full temperature range to be exploited without compromise of the probe performance.

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"X" Channel Frequency Range	^{75}As-^{31}P
"H" Channel Frequency Range	^{19}F-^{1}H
Temperature Range	−150°C to +250°C
Sample Volume	160 µL
^{1}H 90° Pulse Width	≤3.0 µs
^{13}C 90° Pulse Width (X)	≤4.0 µs
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Pasadena, California 91125

Division of Chemistry and Chemical Engineering
Gates and Crellin Laboratories of Chemistry

John D. Roberts
Institute Professor of Chemistry, Emeritus and Lecturer

December 14, 1994
(received 12/16/94)

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

Annotation of 2-D NMR Plots on the Mac Conversion of Postscript Files to Illustrator 5.5 Files

Dear Barry:

C. Allen Bush's recent letter (TAMU NMR 433-12) about transferring plotted output from to PC or Apple graphics programs whetted my interest in that I have a number of applications where I generate Postscript text, but want to transfer to Illustrator (now version 5.5) and my favorite program on the Mac for editing. Knowing of a modestly successful program for such transfers called **Transverter Pro**, I asked Dr. Bush to send me an example to try the conversion out on and, then if successful, he could decide whether or not to acquire the program.

The Bush data arrived as two Postscript files on a PC diskette in 740 K format which were easy to convert to Mac-usable files. One was a COSY spectrum written by FELIX, version 2.05. The other was a HMQC spectrum, which was originally written in HPGL by FELIX and then converted to Postscript by a standard Silicon Graphics program. Transverter Pro (T Pro) that I got from TechPool Software was used in the form of Version 1.0 that has at least one unusual bug, because it may not work with just any old Mac-permissible file name you might have chosen for the Postscript file. TechPool now sells a 2.01 software version (1-800-777-8930).

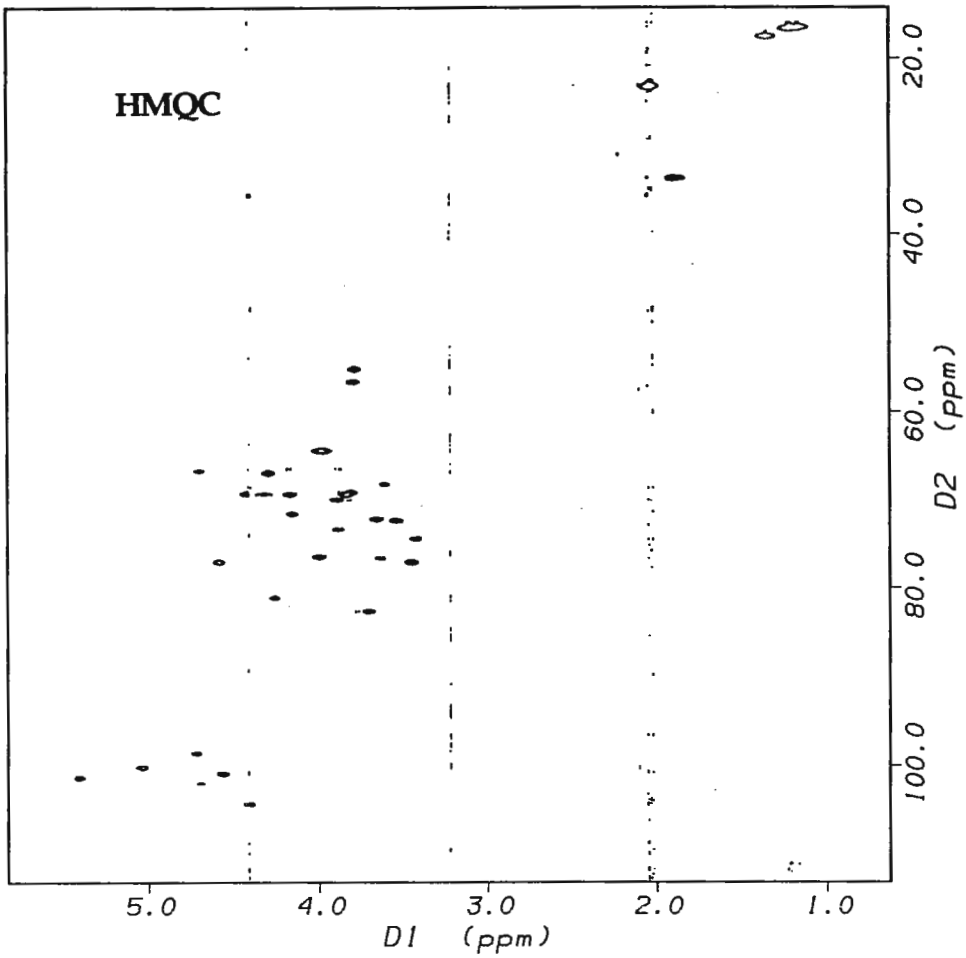
How does it work? I use a black-and-white Mac IIci with 20 Mbytes of RAM and both files "transverted" successfully. The HMQC file (117 K) on treatment with Transverter Pro gave a 157 K file with an Illustrator 3.2 icon that could be opened by Illustrator 5.5 and when saved (non-EPS preview) came to 137 K, easy to annotate and is here reproduced reduced by 50%.

The COSY plot file was more difficult. It started at 560 K, slowly swelled to 1.2 Mbytes when converted to Postscript and then shrunk to 905 K as an Illustrator 5.5 file (non-EPS preview). A Mac IIci is not the best vehicle for handling 1 Mbyte Illustrator files. They open provided enough memory is allocated to the program - 8 Mbytes is enough to open and print, but highlighting and resizing are painfully and really impractically slow. A Power Mac with lots of memory would be better. The print of the COSY document was made full-sized and then reduced in the copier

Best wishes,

Very truly yours,

Jack


HMQC
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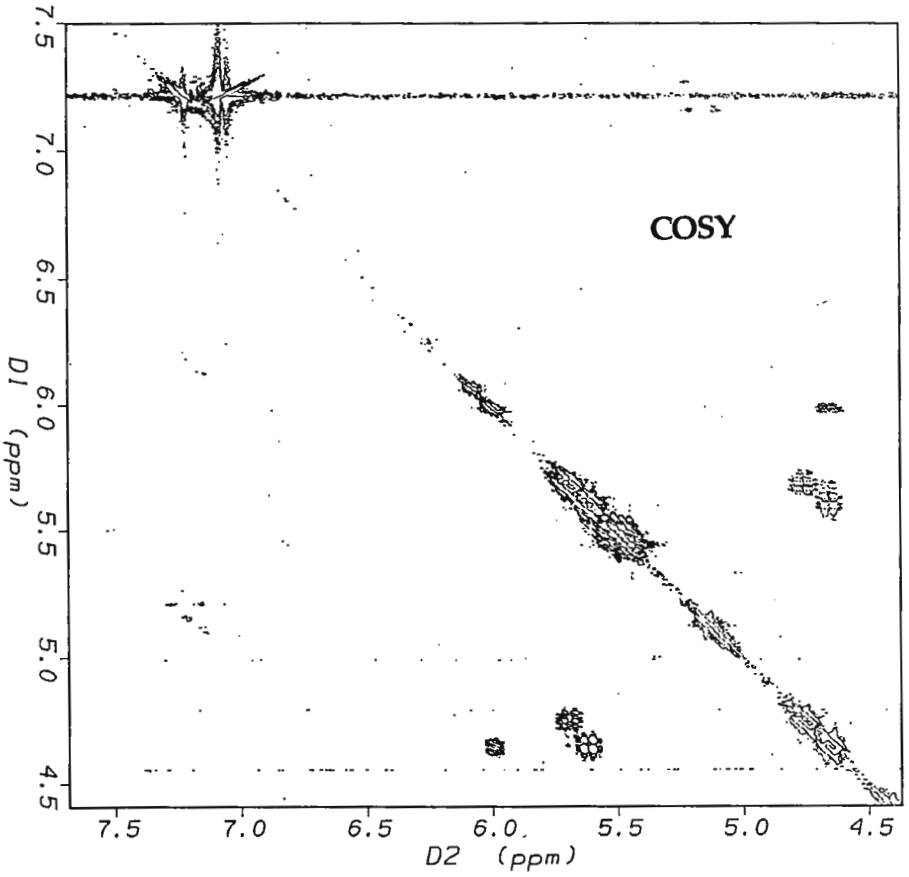
1.0
D1 (ppm)


COSY
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6.5
6.0
5.5
5.0
4.5
D1 (ppm)
D2 (ppm)

UNIVERSITE DE LAUSANNE - FACULTE DES SCIENCES
Institut de Chimie Minérale et Analytique
BCH, CH-1015 LAUSANNE (Switzerland)
Phone: (+4121) 692.38.78Fax: (+4121) 692.38.75

Prof. Bernard L. Shapiro
966 Elsinore Court
Palo Alto, CA 94303
USA

(received 11/25/94)

Lausanne, 21 November 94

HIGH GAS PRESSURE MINI NMR REACTOR FOR A STANDARD BORE 9.4 TESLA CRYOMAGNET

Dear Dr. Shapiro,

Many important chemical reactions are performed in liquids under high gas pressure. These high-pressure chemical processes are generally used to increase the concentration of gases in solution, leading to faster and more economical reactions or beneficial shifts in chemical equilibria. NMR at elevated pressure has been used for studying various physical and chemical phenomena, and several research groups have demonstrated the possibility of performing NMR measurements at relatively high resolution and pressures of a few hundreds of MPa.[1,2]

Nevertheless, NMR under *high gas pressure* is not as developed as NMR under liquid pressure, mainly due to an additional difficulty: it is crucial to have a built-in stirring mechanism to mix solutions with the gases, because diffusion across the gas-solution interface is extremely slow (several days to obtain equilibrium).

We built a *high gas pressure* Mini-NMR-Reactor which fits into a standard bore (∅ 50/40 mm), 9.4 Tesla cryomagnet (see Figure 1). The probe has been used at gas pressures up to 1000 bars and at temperatures up to 180 °C. The probe has also been used for work with liquid pressures up to 2000 bars. The ^{1}H (400 MHz) NMR performance values for the non-spinning 5mm NMR tube are: Resolution 0.5 Hz and S/N (0.1% EB) 70:1 (see Figure 2). A removable mixing unit assures rapid dissolving of the gas in the solution. The equilibrium is achieved within ca. 1 minute.

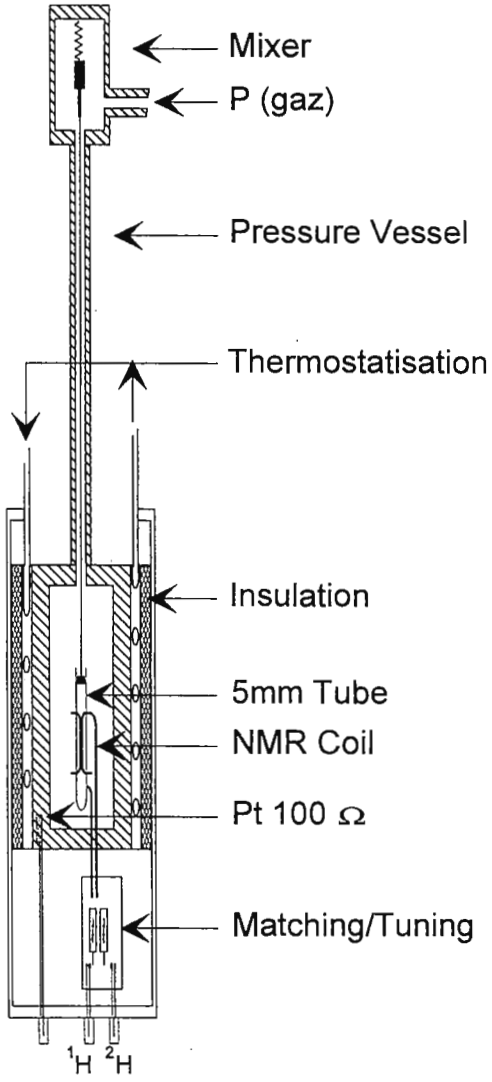

Figure 1: Schematic drawing of the ^{1}H NMR 400 MHz *high gas pressure* probe.

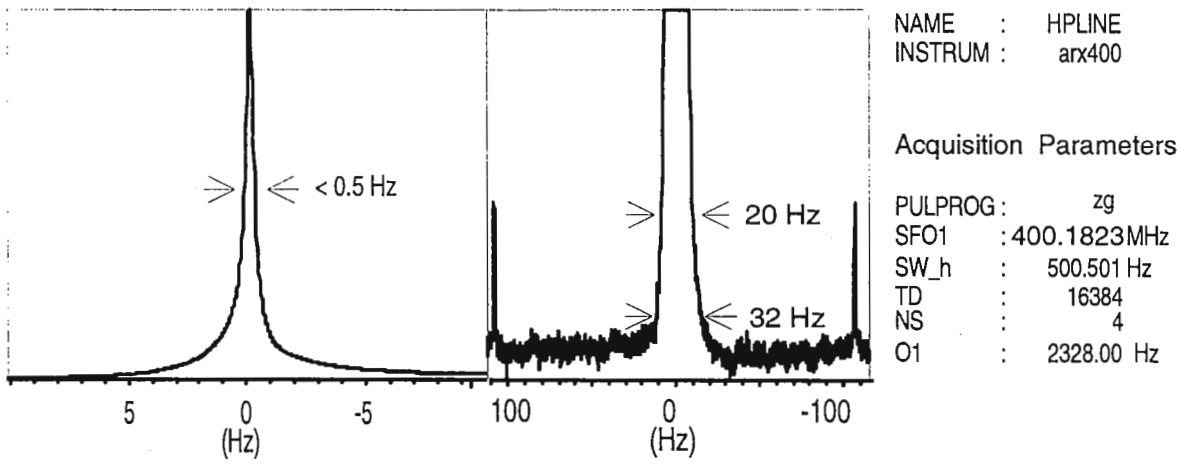

Figure 2: ^{1}H NMR 400 MHz resolution and lineshape test using 10% $CHCl_3$ in acetone-d6 at ambient pressure and temperature

As an example we studied the complex formation rates of $[Ru(H_2O)_6]^{2+}$ with ethylene (C_2H_4), which forms $[(C_2H_4)Ru(H_2O)_5]^{2+}$, as a function of temperature and gas pressure (gas concentration). Figure 3 shows the linear dependence of the complex formation rate as a function of the dissolved gas concentration.

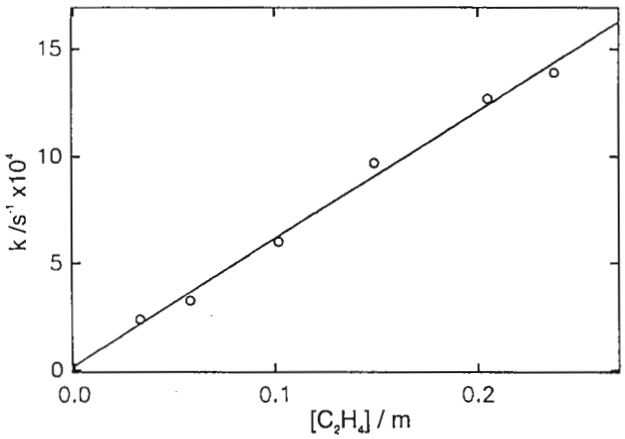

Figure 3 : Complex formation rate as a function of dissolved ethylene (gas pressures 11 to 400 bars) at 317 K in D_2O. $[Ru(H_2O)_6]^{2+} = 0.1$m

$Ru(H_2O)_6]^{2+} + C_2H_4 \rightarrow [(C_2H_4)Ru(H_2O)_5]^{2+} + H_2O$

Yours sincerely,

U. Frey [3] * L. Dolci P. Favre L. Helm R. Tschanz A.E. Merbach

We thank Spectrospin AG (Fällanden, Switzerland) for their technical support, Dr. D. Marek for helpful discussions and Rhône-Poulenc (France) for their financial support.

[1] Helm, L.; Merbach, A.E.; Powell, D.H. *Spectroscopic measurements* in *High Pressure Techniques in Chemistry and Physics: a Practical Approach*, ed. N. Isaacs, Oxford University Press, in press.
[2] Horvath, I.T.; Millar, J.M. *Chem. Rev.* **1991**, *91*, 1339.
[3] Author to whom correspondence should be addressed.

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(AMPX00HV) 02/94

Gradient supply; Another phase shifter; Complex notch filter.

Al Redfield and Sara Kunz, Physics Dept., Brandeis University, Waltham, MA 02254.

1. Gradient Supply. For years we've used our "homospoil" coil driven by a logic gate plus a single transistor and a couple of diodes. Recently we decided to build a real gradient driver like everyone else's. However, our gradient coil is not shielded and, because we are the unfortunate owners of a "microbore" Oxford magnet, there was not much room to put in a shielding coil. Our probes and spoil coils were built by Craig Bradley (Cryomagnet Systems, Indianapolis) around 1987.

It seemed fairly certain that the conductive surroundings of the probe is a linear system, and less certain but likely that the left-over inhomogeneity after a short gradient pulse (an "impulse," in linear response jargon) would decay approximately as a single exponential. That seems to be true for our system, because we require no delay τ to make the test sequence (gradient)-τ-90-FID give the same FID as 90-FID, using the circuit of figure 1. It subtracts the output of a leaky integrator from the incoming square pulse to give a pulse whose shape is ⌐¬. The dual potentiometer R_1 controls the time-constant of the post-emphasis, and R_2 controls its amplitude. Resistors R_3 prevent the average current from being more than 200 ma (an old trick!); otherwise it's just a unity gain current amplifier (±5 Amp out). Even if you didn't get what I said above, you might notice how simple it is. The rest of it is 5 PALs, a few gates, and a counter, to interface with our simple but excellent control system, and ten lines of new code. To be honest, our shimming is not great to begin with. This method of eddy current compensation is identical to "preemphasis", standard commercially, and this is not quite the simplest way to do it. I don't know why they call it <u>pre</u>emphasis, since the important thing is the cancellation at the end.

2. Yet another RF phase shifter: We now generate rf phases only in 90° steps, based on a 15 MHz clock which is divided by four and digitally shifted. All this is done in a fairly obvious way using two high speed 16R8 PALs. These PALs actually provide a reference and two 3.75 MHz quad square waves which are fed to a hybrid single-sideband mixer (Anzac) to convert a synthesizer frequency to gated variable phase pulses, without a filter. However, we still like to vary the phase slightly, and continuously, for some pulses, such as the second pulse of a JR. (We also stretch the length with a one-shot.) We now do this in the simplest imaginable way: the clean 15 MHz clock is fed to a capacitor that delays the rise of the input to the PALs; and resistors, which speed up the rise, are connected with gates (figure 2). Currently R_1 is fixed and R_2 is a pot on our front panel, connected by about 10" of hookup wire to the circuit board. However, the entire thing could probably be "digital," using an array of gates and resistors, similar to the guts of a D/A converter.

3. Complex notch filter: Recently (in J. Mag. Res. <u>A108</u>, 234 '94) we described our DSP system and strategy in which the water signal is always at about 5 kHz, going into our A/D. We still like our system (unpublished, but in this newsletter about two years ago) using analog low pass filtering and an analog gain-changer to get a 12 db increase in dynamic range. Now that water is not at zero frequency, how do we now do this? We take 3 op amps that simulate the equation $\dot{x} = i\omega_o(x - iy)$, where y, x, and $\dot{x}$ are complex. Regard x as the output and y as the input of an amplifier that has 2 port inputs and outputs. See figure 3a, and read an old book on op-amps as analog computers. Then connect this to two op-amps (figure 3) which are straight adders, as feedback to require $y = -z - Gx$ and you have a notch filter with zero gain at $+\omega_o$, and $Q \approx G$, where z is the input and y is the output. This was not too hard at 6 kHz but, at higher notch frequencies that might be desirable, like 30-100 kHz, hot op amps might be needed and great care is required to avoid parasitic oscillations. We've never seen 2-port complex feedback used before, have you?

(received 12/5/94)

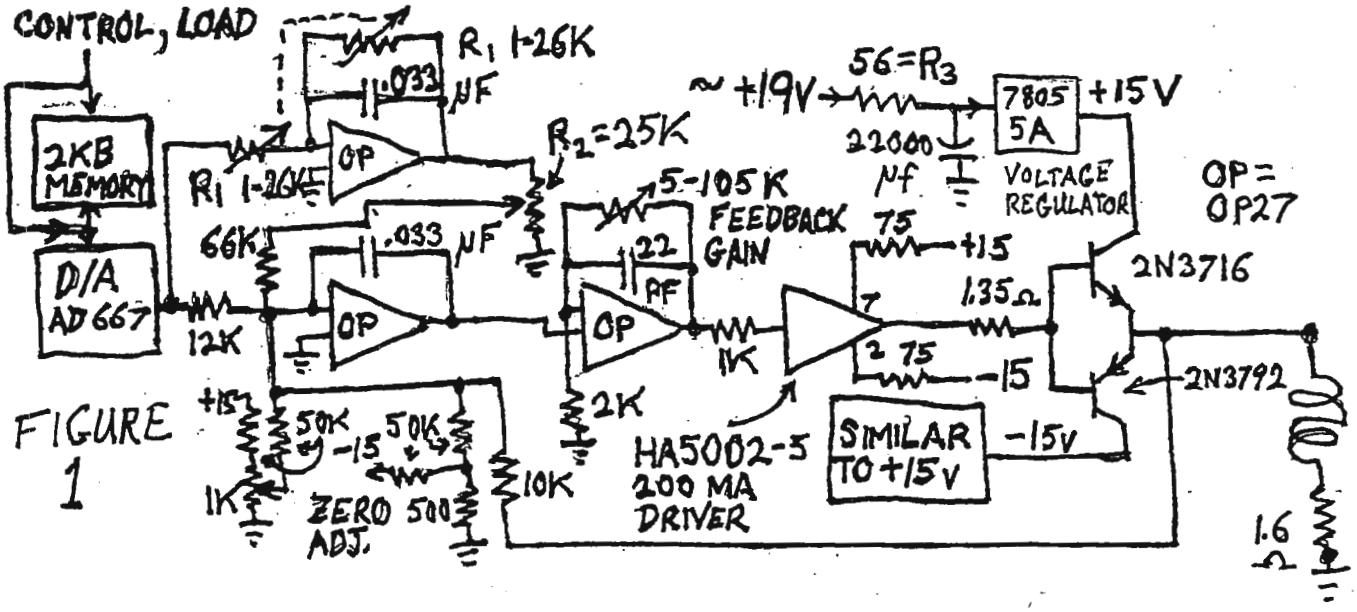
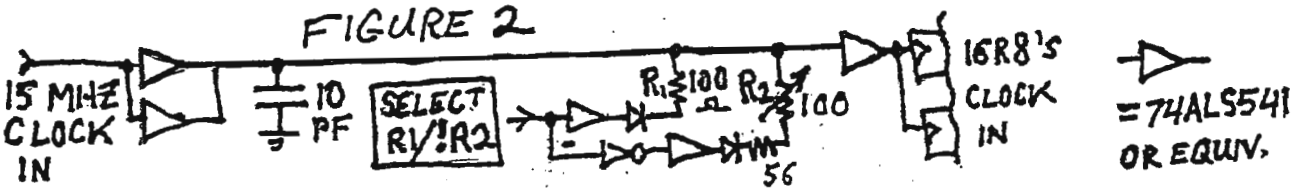
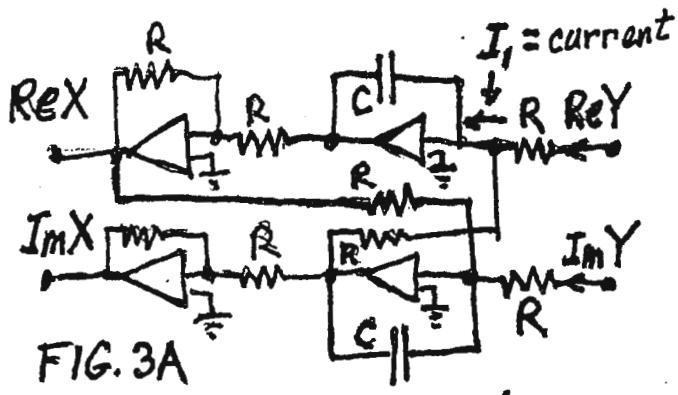


FIGURE 1

FIGURE 2





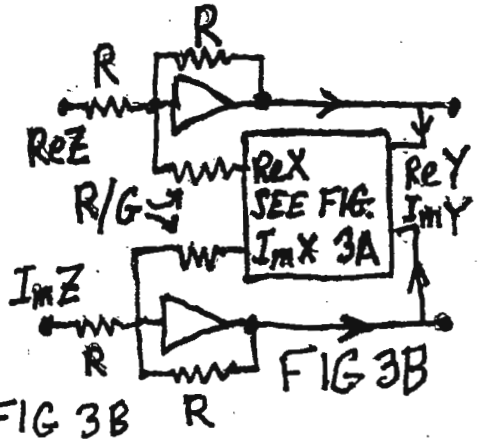


FIG 3B

$I_1 = +\frac{dReX}{dt}\ C = (ReY - ImX)/R$

So $\dot{ReX} = (ReY - ImX)\omega_0$, $\omega_0 = RC^{-1}$. Similarly $\dot{ImX} = (ImY + ReX)\omega_0$

These are the real/imaginary parts of $\dot{X} = \omega_0(iX+Y)$. Solve it for $Y = Y_0 e^{i\omega t}$ by setting $X = X_0 e^{i\omega t}$ to get gain $g_A = X_0/Y_0 = \omega_0/i(\omega-\omega_0)$.

The gain of Fig. 3B is $Y/Z = -(1+g_A G)^{-1} = -(1+(G\omega_0/i(\omega-\omega_0)))^{-1}$.

PHILIPPS-UNIVERSITÄT MARBURG

FACHBEREICH CHEMIE

Prof. Dr. Stefan Berger

email: berger@ps1515.chemie.uni-marburg.de

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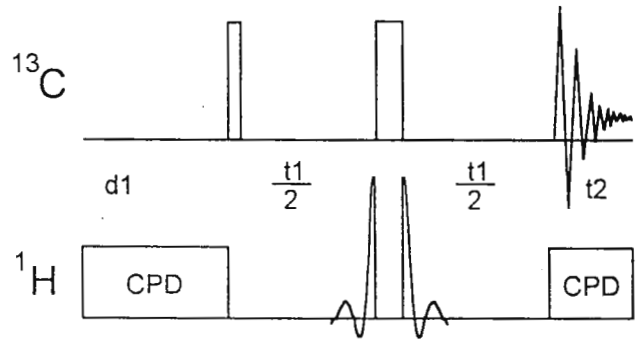
(received 12/6/94)

Prof. Dr. B. L. Shapiro
TAMU-NMR Newsletter
966 Elsinore Court
Palo Alto, CAL. 94303
USA

Determination of a selected long range C, H spin coupling constant

Dear Barry,

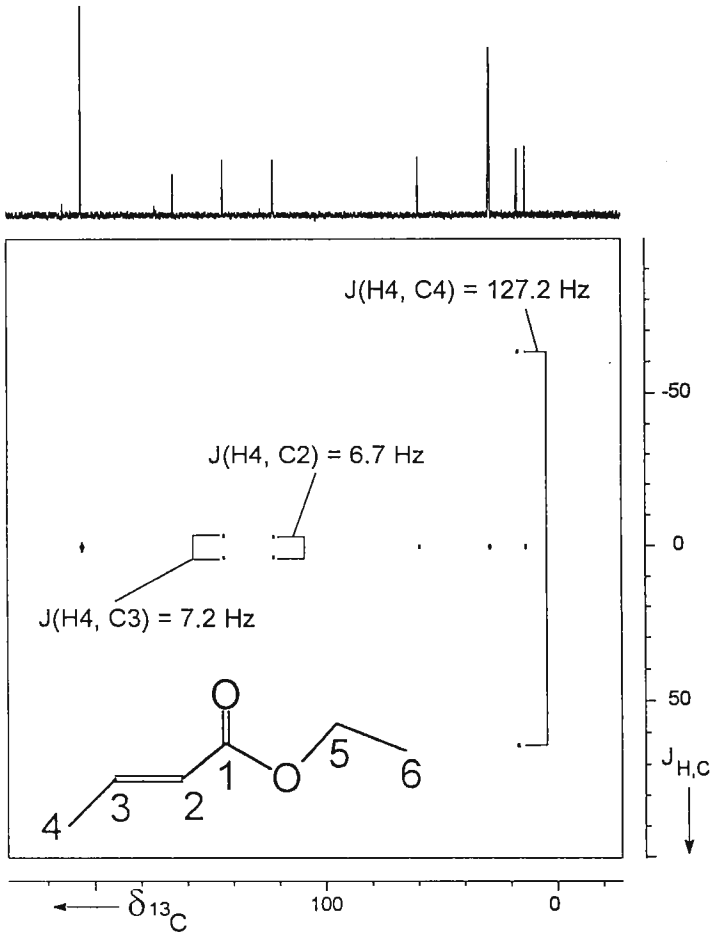
We would like to report on an improvement of an experiment published by Bax and Freeman in 1982 [1]. In conformational studies it is often necessary to determine a particular ^3J (C,H) spin coupling constant. Analysis of the gated decoupled ^{13}C NMR spectrum, however, is usually difficult. The *J*-resolved method yields multiplets in F_1, which have to be analyzed by spin simulation; this requires knowledge of the entire spin system. The selective version of the *J*-resolved method as reported [1] was disadvantaged because of the semiselective pulses available at the time. From the wide menu of currently used shaped pulses we found that the REBURP pulse [2] was most apt for our purpose and therefore tried a sequence using half of the REBURP pulse before and half after the hard 180° pulse on the ^{13}C channel. The quality of the spectra obtained on an AMX-500 is very good and is demonstrated in this letter using our beloved test molecule ethylcrotonate in acetone-d_6. The width in F_1 is chosen so that ^1J (C,H) is also detected, thus giving an experimental verification of the selectivity of the proton pulse. The long range splittings at C-2 and C-3 are observed when hydrogen atoms at C-4 are selected. This method has proved to be very fruitful for applications in Organic Chemistry.



[1] A. Bax, R. Freeman, *J. Am. Chem. Soc.* **104**, 1099 (1982).
[2] H. Geen, R. Freeman, *J. Magn. Reson.* **93**, 93 (1991).

Sincerely yours

[S. Berger] [T. Fäcke]



UNIVERSITY OF FLORIDA
CENTER FOR STRUCTURAL BIOLOGY
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The Center for Structural Biology of the College of Medicine, University of Florida currently has openings for faculty conducting innovative research emphasizing nuclear magnetic resonance. The Center is associated with the University of Florida Brain Institute (UFBI) and with the National High Magnetic Field Laboratory (NHMFL), jointly run by Florida State University, the University of Florida, and the Los Alamos National Lab. The Center is an interdisciplinary program with emphasis on understanding of biological function by determining high-resolution structures of large biological molecules and assemblies, studying cell structure and function, and the morphology and physiology of the whole organism. Within the Center, advanced spectroscopic, diffraction, and imaging techniques (nuclear magnetic resonance, optical microscopy, and electron microscopy; X-ray crystallography is being planned) are used to derive molecular-based information related to cellular, tissue, and organism-level structure and function. The Center houses NMR spectrometers (Varian Unity 300 and Unity 600) used for macromolecular structure determination and microscopy, as well as an imaging spectrometer (12 T, 40 cm system to be installed in 1977) for animal research. Also, the Center uses a 4.7 T, 33 cm animal system and a 3 T whole-body system (to be installed in 1995) for research. In addition, Center faculty use prototype magnets and spectrometers located at the NHMFL Tallahassee site, including a wide-bore 600 MHz and a 720 MHz high-resolution spectrometer, with other magnet systems operating at higher fields.

Faculty are being recruited in two areas: 1) high-resolution macromolecular NMR spectroscopy and 2) MR imaging and spectroscopy in living systems. Interested persons are invited to apply for tenure-track faculty positions, at any level, with direct association to the National High Magnetic Field Laboratory. Applicants at the Assistant Professor level need to have an established program with extramural funding. Successful candidates will conduct innovative research emphasizing NMR and will use the facilities of the Center for Structural Biology, with additional support provided through the facilities of the NHMFL and UFBI. Opportunities exist for collaborative research in areas such as biochemistry, cell biology, cognitive neuroscience, pharmacology, or physiology.

Applicants much have a Ph.D. Degree and a minimum 1 year postdoctoral experience. Submit a C.F. with statement of research interests and three letters of reference by February 15, 1995. All correspondence should be sent to Thomas H. Mareci, Ph.D., Director, Center for Structural Biology, College of Medicine, University of Florida, Box 100245, Gainesville, FL 32610-0245. The University of Florida is an Affirmative Action-Equal Opportunity employer, and applications from minorities and women are strongly encouraged. Gainesville is an attractive University community in North-central Florida with good low-cost housing, quality public schools, and many outdoor activities.

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600	51	10	120	3.4
500	51	10	150	3.2
400	54	8	365	2.8
360	54	8	365	2.8
300	54	3	365	2.8
270	54	2.7	365	2.8
200	54	2	365	2.8
100	54	1	365	2.8
500	89	15	120	3.4
400	89	10	180	2.8
360	89	10	365	2.8
300	89	3	365	2.8
270	89	2.7	365	2.8
200	89	2	365	2.8
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Dipartimento di Scienze Molecolari Agroalimentari

DISMA Via Celoria, 2 I-20133 Milano Tel. 02-2663662 /2365029 /2362721

Milano, November 25, 1994
(received 12/14/94)

Dr.B.L.SHAPIRO
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

INTERACTION BETWEEN METAL IONS AND NAD(P) COENZYMES.

Dear Barry

We have recently studied the interaction of NAD(P) coenzymes with metal ions Mg^{2+}, Ca^{2+}, Zn^{2+}, Li^{+}, $Cr(NH_3)_6^{3+}$, $Ru(NH_3)_6^{3+}$ and $Co(NH_3)_6^{3+}$. The results will be published in J. Chem. Soc. Perkin 2.

We have measured the stability constants of the complexes with diamagnetic ions by titration experiments, following the chemical shift variation of the two (three) phosphorus atoms. The conformational analysis was performed in details for Mg^{2+} complexes by using H-N and H-P coupling constant values and ^1H NOESY experiments.

The ligands $M(NH_3)_6^{3+}$ have been utilized as mimic of $Mg(H_2O)_6^{2+}$, while the paramagnetic ion provided structural information through the line-width variation of ^{31}P and ^{13}C signals. The ^{59}Co NMR experiments allowed to exclude dimeric structure such as $(NADP)_2$-Co.

The results of the conformational analysis have shown that the effects of binding to a magnesium ion are small, but significant: an increase (10-15%) of the S-type conformer was observed for the adenine-ribose moiety in NADP complex, together with an increase (15-25%) of the γ^+(gauche-gauche) conformer for the same unit. This is actually a further stabilization of the already most stable conformer for these coenzymes without magnesium. The presence of the phosphate at C-2' of the adenine ribose ring seems determinant for the mode of binding of Mg^{2+} to NADP molecules. Mg^{2+} preferentially interacts with P(2') and P(A), especially for the coenzyme in the oxidized form. The association at P(N) is in general disfavoured for all ions, but this is particularly noticeable in the case of Zn^{2+}, which presents additional interaction with N-7 and N-1 atoms of the adenine ring, thus stabilizing the complex with the ion bound to P(A).

The relatively strong upfield effect on ^{31}P resonances ($\Delta\delta$=1-1.5 ppm), consequent to magnesium coordination, compared for instance with calcium ($\Delta\delta$=0.3-0.8 ppm), indicates that some conformational changes at the phosphates site must occur. This was also suggested by the significant change of the geminal coupling constant J(P-O-P), which is not so greatly relevant for the other ions.

Ca^{2+} and Li^{+} apparently show a different mode of binding with respect to the Mg^{2+} ion; the variations observed on ^{31}P chemical shift and coupling constant J(P-O-P) upon complexation with Ca^{2+} and Li^{+} are small, thus suggesting that significant conformational changes did not occur. On the other hand, the higher value of the association constants with respect to magnesium, is in favour of direct interaction of Ca^{2+} and Li^{+} to the phosphates oxygens, without the mediation of water

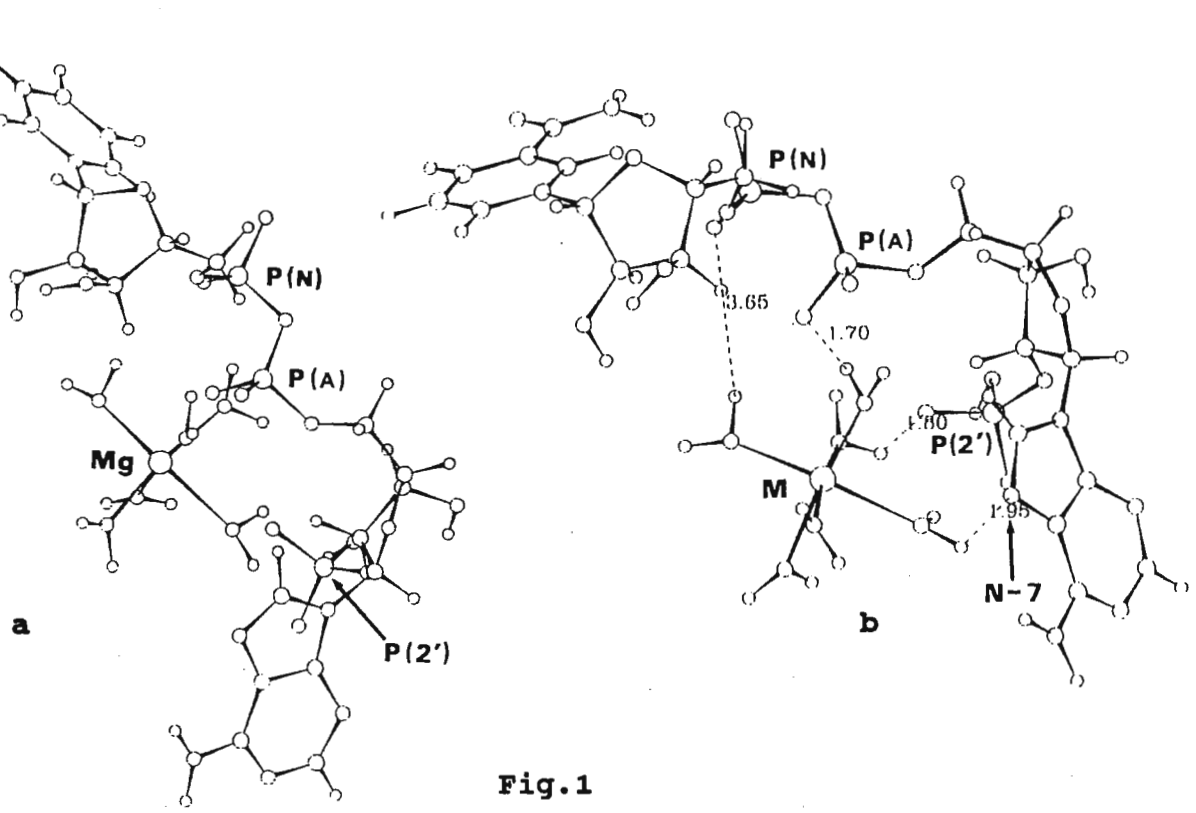

Fig.1

molecules, as occurs for magnesium.

Providing that in aqueous solution several bound species might be present in equilibrium, some structures for the metal-coenzyme complexes have been proposed (Fig.1). In the case of Mg^{2+} and $Cr(NH_3)_6^{3+}$ the model structures obtained are similar. Actually the magnesium ion must be present as $Mg(H_2O)_6^{2+}$. The metal atom (in Fig.1a) sits in a pocket among the three phosphates groups and binds to the phosphates with interactions of electrostatic nature, through the mediation of H_2O molecules respectively. The hydrogen bonding of H_2O to the phosphate oxygen atoms contributes to the stabilization of the complexes. The octahedral structure of $Mg(H_2O)_6^{+2}$ is perfectly matched to the three dimensional profile of the coenzyme. The pyrophosphate group adopts a conformation intermediate between a staggered and a trans form. The aromatic rings of adenine and nicotinamide are shown to be appoximately perpendicular to each other; although the pyrimidine can freely rotate around the glycosidic linkage, the planes of the two bases can never be parallel, thus a folded form with close stacking of the two rings appears very unlikely.

In Fig.1b an other structure is reported, which might be present in equilibrium together with many others, contributing to the interaction of the metal ion with NADP coenzymes. In the case of Zn^{2+} such a contribution must be larger, as suggested by the deshielding observed on the adenine proton H-8.

sincerely yours

Stefania Mazzini Leonardo Scaglioni Rosanna Mondelli

Enzio Ragg

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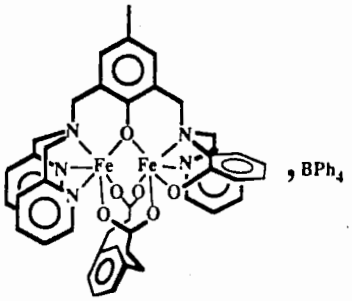
November 15, 1994
(received 11/26/94)

Dear Doctor Shapiro,

Application of ^{1}H 2D NMR experiment to metallic complexes

We are currently studying a group of metallic complexes (fig. 1) which reproduce the active sites of the purple acid phosphatases. We have carried out structural analysis using NMR data in order to confirm the expected structure.

fig.1



The experimental set-ups reported by different groups working on paramagnetic compounds[1 2 3] have been particularly useful. Nevertheless, due to the specificity of our system, we tested the applicability of a range of different classical 2D experiments. Indeed, due to their electronic spin state, the ^{1}H NMR spectra of complexes such as that of fig.1 exhibit chemical shifts spread over a range of 600 ppm, their T_1 can be shorter than 1ms and the signal line width larger than 3000 Hz.

The conditions for obtaining correlations in 2D spectra are particularly unfavourable because of the very short T_1 and the large chemical shift range. For example, due to the the very large spectral width it was not possible to phase the 2D spectra (thus fig.2 is diplayed in magnitude mode). Also, the isotropic chemical mixing period in the TOCSY experiment becomes particularly inefficient since $T_{1\rho}$ is short compared to 1/2J and the available rf power (ω_1 corresponds to about 60 ppm) cannot cover the whole range of chemical shifts (600ppm). Under these circumstances, only the classical COSY displayed in magnitude mode yields any useful correlations (fig. 2) These correlations are reported in the following table (indicated with continuous lines) together with the T_1 values of the corresponding protons.

assignment	phenol				pyridine			pyridine		
δ (ppm)	-51	70	-33	50	59	10	34.6	75	5.3	85;3
T1 (ms)	4.4	27.7	12.6	6.5	12.3	29.4	15.4	9.8	21	11.2

assignment	carboxylate (MPDP)								
δ (ppm)	12.8	27	111	42.4	54	21.9	14.9	5.8	42.7
T1 (ms)	5.8	7.2	4.2	10.5	4.8	7.5	43.9	55	12.8

DRFMC
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One should notice that it is even possible to establish long range correlations (indicated with dotted lines). No correlations were observed between protons with T_1 shorter than 3 ms. In order to decrease the t_1 noise arising from the diamagnetic part of the spectrum, we applied a 180 ° pulse, before the cosy sequence itsef, followed by a 300 ms delay which leads to a partial cancellation of signals arising from the counter anion which have much longer T_1 than the cation.

Please credit this letter as a contribution by Dr P. Vottero.

Dr M. Bardet Dr L Emsley E. Bernard Dr J. M. Latour

[1] L; P. Yu, G. N. La Mar, and K. Rajarathnam, J. Am. Chem. Soc., 1990, 112, 9527-9534.
[2] L. J. Ming, H. G. Jang, and L. Que, Inorg. Chem. ,1992, 31, 359-364.
[3] I. Bertini, P. Turano, and A. J. Vila, Chem. Rev. , 1993, 93, 2833-2932.

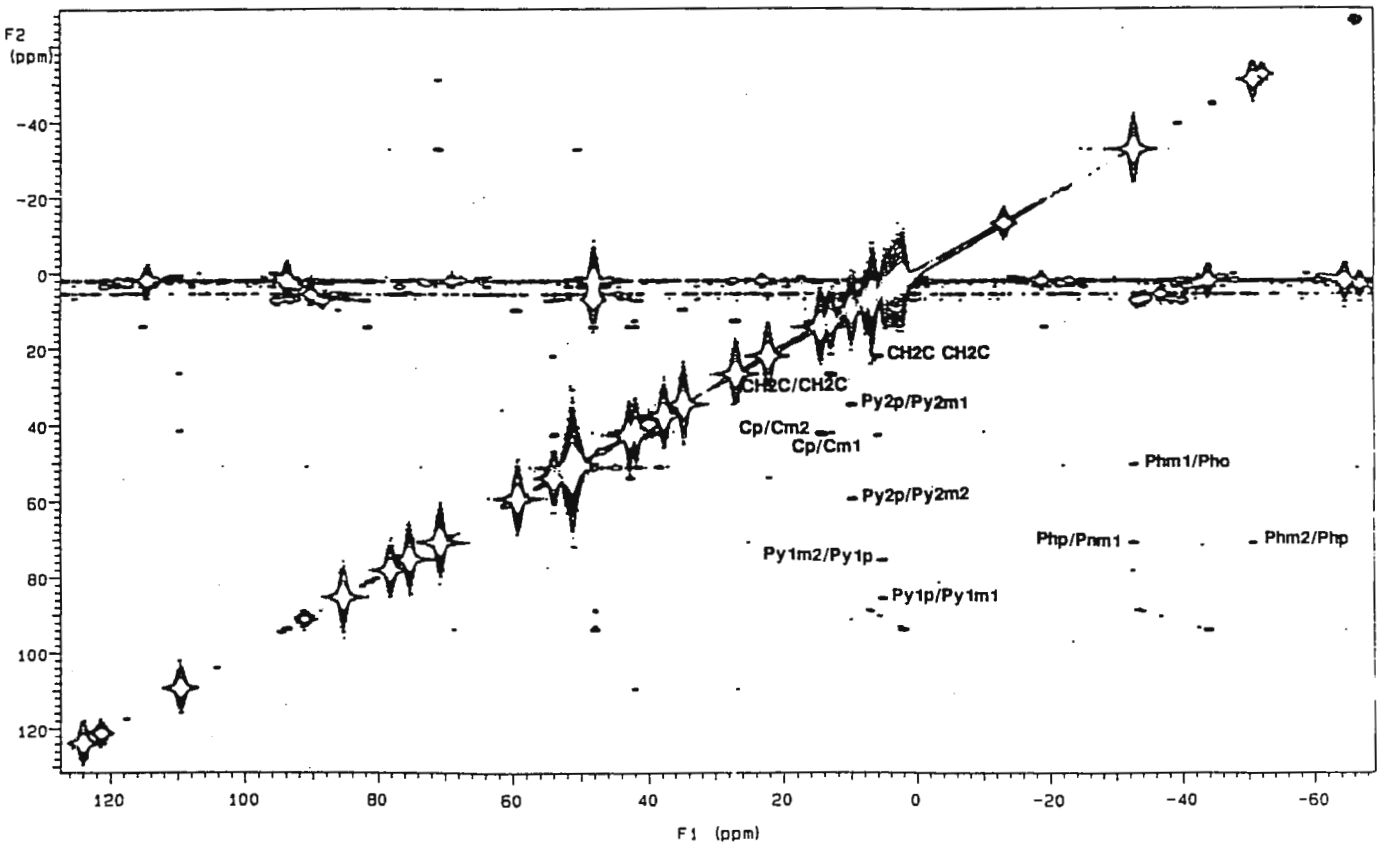

Figure 2: 400-MHz unsymmetrized MCOSY map of a metallic complexe (see fig.1).

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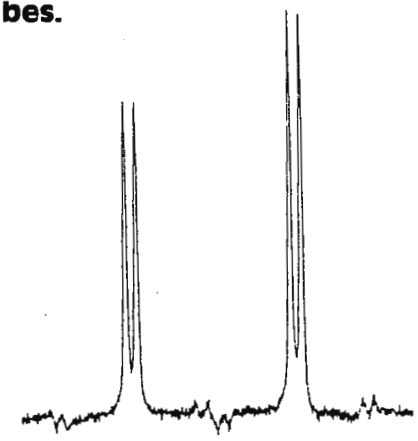
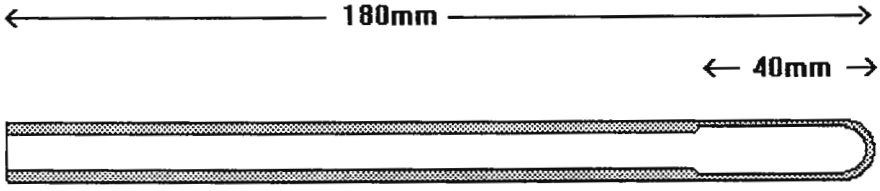


Fig.1a Fig.1b

The spectra of 20mm sucrose in D_2O were obtained with a single scan without apodization prior to Fourier transformation on a Bruker AMX-600 spectrometer at 298 K. By using Shigemi high quality 5mm standard tube (Fig.1a) and the Shigemi highly sensitive thin wall 5mm tube (Fig.1b), the spectra confirms a sensitivity enhancement of about 10%.



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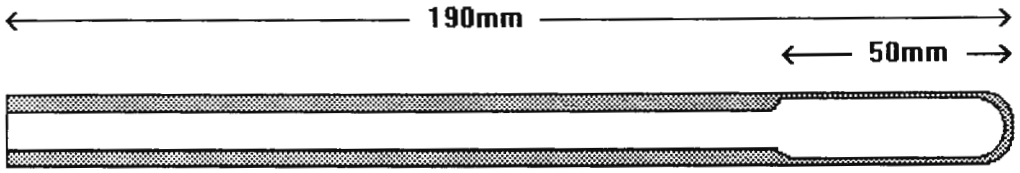

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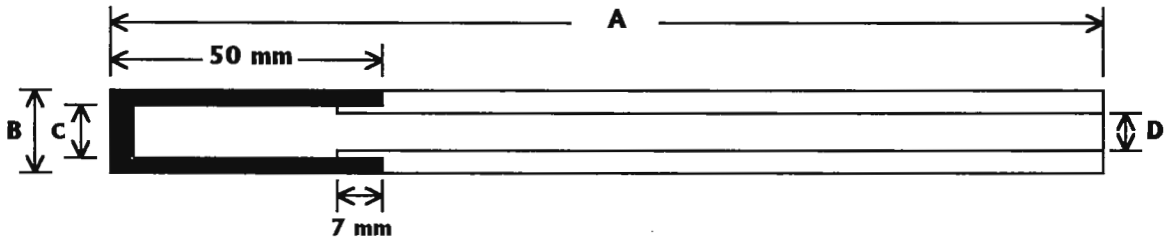

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Si-005	180	4.965 + 0 − 0.005	4.0 ± 0.1	2.5	± 0.02
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November 15, 1994
(received 11/21/94)

FREE SOFTWARE

Dr. B.L. Shapiro
966 Elsinore Court
Palo Alto, CA 94303
U.S.A.

Dear Barry,

Yes, it's free and it's available. For about ten years now, Tim Allman (of SoftPulse Software) and I have been working (sporadically) on the program SIMPLTN. This is a program for the SIMulation of PuLse and Two-dimensional Nmr spectra, and it has gone through many manifestations. The latest has been greatly improved by the help and encouragement of Joel Garbow at Monsanto in St. Louis. Now we think it is sufficiently robust to release, in its first version. This is the version for UNIX, and Tim has a 32 bit Windows version for a PC. The program has been tested extensively on SGI (Irix 4.05 and 5.2) and on Suns (running SunOS but not Solaris).

SIMPLTN is meant to look like a spectrometer. It takes an ASCII pulse program, plus a spin system definition, then simulates the complete evolution of the density matrix, using the superspin method. The output is a simple ASCII file which is then converted to a data file for your favourite processing program (UXNMR, VNMR, FELIX), since the last thing I want to do is write NMR processing software. So, like a spectrometer, the input is a pulse program and the output is an FID, albeit a calculated one. The program is written in C, and uses a reasonably friendly menu-driven device-independent interface using the CURSES facilities in UNIX.

The program can handle up to six (strongly or weakly) coupled spins, and essentially any pulse program. The speed of the program depends on how many spins you have, but it can do a COSY on four spins in a couple of minutes - almost as fast as these new-fangled gradient COSYs. There are two important experiments, however, it can not handle yet. It can't do TOCSY, since it has not yet figured out how to handle cleanly evolution in the presence of rf. NOESY/ROESY is also not included, since we are just setting up dipolar relaxation. Because of this, the program is more useful for educational purposes, rather than simulating cutting-edge experiments.

In order to see whether we were doing relaxation correctly, we simulated some random field relaxation in strongly coupled systems. This follows some of the Volds' classic work (1), which we had

observed experimentally (2). The situation is as follows. Two nuclei, M and N, have T_1's of 1 second and 0.3 seconds. If these two nuclei are strongly coupled, we get a typical AB type spectrum, as shown in the figure. Because the relaxation times are different, there are different T_2's. Note that we have an A half and a B half of the AB pattern, but each of these is a mixture of the two nuclei M and N. If we do a 180-τ-90 sequence on this then each line in the spectrum shows a different relaxation rate. Part of this is due to a flip angle effect, which mixes all the strongly coupled transitions together. If we use a small flip angle, then the spectrum is accurate and both lines in the A half of the spectrum now have the same relaxation behaviour, as shown in the figure. However, the initial slope rates are not the $1/T_1$'s of M and N, but rather mixtures of them because of the strong coupling, as in equation [1].

$$1/T_{1A} = \sin^2\theta\ 1/T_{1M} + \cos^2\theta\ 1/T_{1N}$$
$$1/T_{1B} = \cos^2\theta\ 1/T_{1M} + \sin^2\theta\ 1/T_{1N} \qquad [1]$$

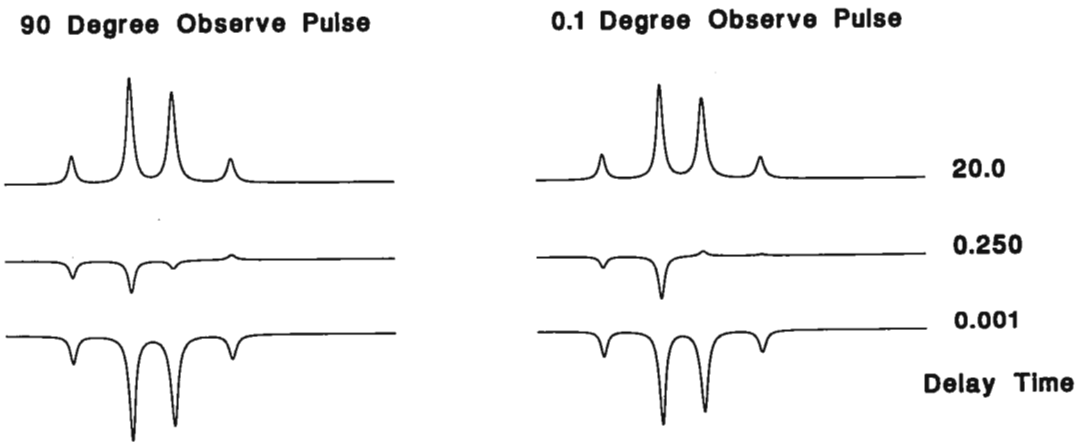
In this equation, where $2\theta = \tan^{-1}(J/\Delta)$, as usual.

SIMPLTN duplicates this behaviour well. The observed initial slopes are 0.812 and 0.322, as predicted by [1] with $\theta = 17.7°$ (the chemical shift difference is 21 Hz, and the coupling constant is 15 Hz). This gives a pretty rigorous test of all sorts of mixing, relaxation, spin dynamics, and relaxation all in one go. Since it passed, we are releasing this version (and a manual!) while I get going on further embellishments. If anyone is interested in a copy, send me an email at bain@mcmaster.ca. Enjoy.

Yours truly,

Alex D. Bain
Professor of Chemistry

1. R.L. Vold and R.R. Vold, Prog. Nucl. Magn. Reson. Spectrosc. **12**, 79 (1978).
2. A.D. Bain, S.G. Hughes and G.K. Hamer, J. Magn. Reson. **96**, 613 (1992).



Pharmaceuticals Division
CIBA-GEIGY Corporation
Summit, New Jersey 07901

CIBA–GEIGY

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

November 16. 1993
(received 12/1/94)

Pulse Sequence for Selective T1 Measurements with Solvent Suppression

Dear Professor Shapiro:

The transferred NOE experiment requires that the inhibitors possess a sufficiently rapid on-off rate in their association with the enzyme. One indication of the rate of the inhibitor-enzyme association can be obtained from the spin-lattice relaxation time (T_1). Relaxation time measurements (T_{1sel}) of inhibitors show a dramatic decrease in the spin lattice relaxation times on going from the free to the bound state. This is not unexpected since slower diffusional motions will affect the spin relaxation. The selective proton spin lattice relaxation rate (ρ_{ij}) for a proton pair is given by

$$\rho_{ij} = \frac{1}{10}\frac{h^2\gamma^4 H}{r^6_{ij}}\left[\frac{3\tau_c}{1+(\omega\tau_c)^2} + \frac{6\tau_c}{1+(2\omega\tau_c)^2} + \tau_c\right]$$

where r_{ij} is the interproton distance, ω is the Larmor frequency, and τ_c is the correlation time. Hence the selective relaxation rate (ρ_{ij}) will be proportional to τ_c and inversely proportional to T_{1sel}.

In order to determine T1 values of inhibitors in aqueous buffer, a selective inversion-recovery pulse sequence with simultaneous solvent suppression is required. To address this need, a new pulse program was written. A very selective shaped SINC pulse was used which greatly improved the accuracy of the measurements. The pulse program, written on a Bruker AMX-500 NMR spectrometer, is given below.

```
;SELT1PRMULT
;Selective T1 measurement using pr, ir and shaped pulse

1 ze
2 d12 hl2 o1        ;set o1 on the solvent peak for presaturation
 d1
 (p18 ph29)
 d13
 d12  tlo o1        ; set o1 on the selected resonance for T1 measurements
 (p12 ph1):tp1
 d13
 d12 thi
 d12 hl2 o1         ;set o1 on the solvent peak for presaturation
 vd cwt
 d13 to
 d12 hl1 o1         ; set o1 on the selected resonance for T1 measurements
 p1 ph2
 go=2 ph31
 d11 wr #0  if #0 iv
```

```
lo to 1 times l4
exit

ph1=0 2
ph2=0 0 2 2 1 1 3 3
ph29=0
ph31=0 0 2 2 1 1 3 3

;hl1:  ecoupler high power level
;hl2:  ecoupler power level for presaturation
;p1:   90 degree transmitter high power pulse
;p12:  shaped 180 degree transmitter high power pulse
;d1:   relaxation delay; 1-5 * t1
;d11:  delay for disk I/O      [30 msec]
;d12:  delay for power switching [20 usec]
;d13:  short delay (eg. to compensate delay line) [5 usec]
;vd: variable delay  taken from VDLIST
;L4:  l4= number of experiments = number of delals in VDLIST
;NS: 8*n
;ds: 2 or 4
;define  VDLIST
;this pulse program produces an ser-file (PARMOD = 2D)
;tlo: ecoupler power level for selective pulse at power level tp1  with shape file name tpname1
;cwt for presat when vd delay > 1s
;to: turn off transmitter
;vd list delay > 5ms
;o1: o1 frequencies taken from F1LIST
```

This program provided an accurate and highly reproducible means of obtaining selective proton T1 measurements.

Sincerely,

Xiaolu Zhang, Ph.D.

Nina C. Gonnella, Ph.D.

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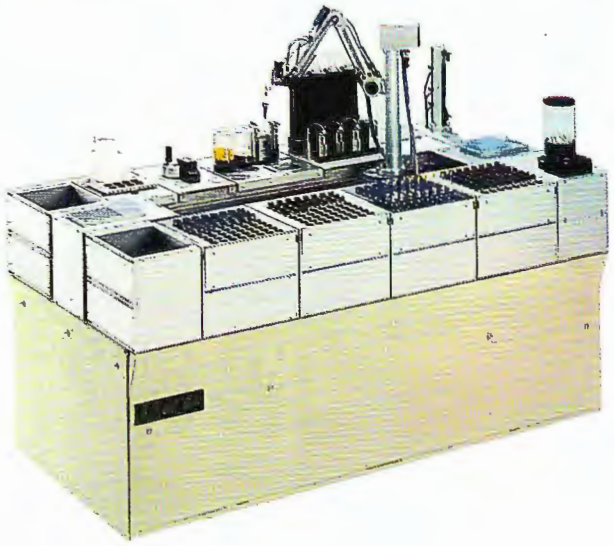
Its high modularity with up to 16 stations facilitates fast exchangeability of individual stations to easily adapt to changing needs. This includes fast teach-in for high operational accuracy. The functionality includes automatic identification of sample vials, dissolving the sample in typical NMR solvents, filtration, filling of NMR sample tubes and sealing with plastic cap, inserting the tube into a spinner and attaching a barcode collar. Samples are prepared in racks of 64, ready for loading into BRUKER's automatic sample changer.



Technical Data

Input trays:	holding 64 vials each
Output trays:	holding 64 sample tubes each
Solvent dispensers:	0.5 l capacity per solvent, 4 solvents max/per dispenser station
Pipette storage station:	ca. 140 pipettes
NMR sample tube dispenser and assembly station:	available for 5 mm tubes
Sample filtration and dosage station:	ca. 128 filters, 128 caps
Barcode collar dispenser:	ca. 150 collars
Dimensions:	with stations 1800 x 970 mm
Height closed:	170 mm
Height open:	210 mm
Width without stations:	800 mm
Width when closed with stations:	970 mm

Width when both covers open:	1700 mm
Weight:	200–250 kg depending on layout
Power (dissipation):	220 V 4A
Air pressure:	5 bar
Accuracy of repetition:	± 0.05 bar
Typical sample preparation time:	ca. 6 minutes
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Communication between stations:	RS 485
Communication to data system:	RS 232
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Pharmaceuticals

November 18, 1994
(received 11/28/94)

Dr. Bernard L. Shapiro
966 Elsimore Court
Palo Alto, CA 94303

INSTALLATION OF 4.7 T 40 CM MRI SYSTEM

Dear Barry:

We are in receipt of your colorful (!) ultimatum and would like to share with your readers about the installation of our Bruker ABX 4.7/40 MRI system. We have been fortunate to get a whole new facility equipped with preclinical laboratory, image analysis laboratory and an electronics laboratory. The magnet is placed within a shielded room (RF and magnetic) so that the stray field outside the room is 2G.

The magnet was shipped cold from California and arrived here just before last Christmas. The moving of the magnet from our parking lot to the facility (which was about 20 ft downhill from the parking lot) by a crane was a spectacular sight and was watched by a number of people. Our facility engineering staff did take a video of the move, but I guess we have to wait to share that with your readers until TAMU goes 'high tech' . However a photograph of the 'hanging magnet' is shown below.

709 Swedeland Road, PO Box 1539, King of Prussia, PA 19406-0939. Telephone (215) 270 4800. Fax (215) 270 7777.

The magnet was installed and energized and brought to field in few days without any 'excitement' by the Magnex engineer. The Bruker application scientists finished most of the specifications for standard experiments by May 1994. We were very happy with both Bruker and Magnex for their cooperation during the period of building construction and delivery process and the entire operation was quite smooth. Magnex also coordinated the construction of the shielded room which was actually built by Lindgreen.

Although some of the special tests are still being completed by Bruker, we have been successfully using the machine since the summer and are getting interesting results on *in vivo* disease models. We will share these results with you at a later date.

With regards,

Sincerely yours

Rasesh D. Kapadia

Susanta K. Sarkar

University of Cincinnati

Department of Chemistry

Mail Location 172
Cincinnati, Ohio 45221-0172

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immediately for study of antibody-antigen complexes using solution-state NMR techniques in the Department of Chemistry at the University of Cincinnati. Newly arrived Bruker DMX 500MHz spectrometer and access to other field instruments will be available for these studies. Candidates should have a Ph.D. in Chemistry, Biochemistry or Biophysics and experience with multi-dimensional solution NMR techniques and their applications to biological systems. Research also involves some purification and preparation of proteins. Salary commensurate with experience. Interested candidates please send their CV, application letter and the names and addresses of three references to:

Professor Pearl Tsang
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Wilmington, Delaware 19880-0328

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

November 30, 1994
(received 12/7/94)

^{13}C relaxation in acrylic polymers

Dear Barry,

We are pleased to share our experience with non-aqueous solvent borne paramagnetic relaxation agents, $Cr(acac)_3$ and $Fe(acac)_3$, in the characterization of acrylic polymers.

The quantitation of acrylate polymers by high resolution ^{13}C NMR requires a knowledge of the spin-lattice relaxation times for all of the carbon environments. For example, signals from non-protonated carbons such as the acrylic ester carbonyls are often on the order of 1.5-2.0 seconds. Through the use of paramagnetic relaxation agents, it is possible to significantly decrease these times (i.e., down to the 0.3-0.6 second range). Using Poly-MMA, Poly-nBMA, and Poly-HEMA samples of varying MW we observed that $Cr(acac)_3$ and $Fe(acac)_3$ can be used to decrease the T_1's of all ^{13}C signals.

Guideline use of these relaxation agents in characterization of acrylics (in general) follows. For acrylate polymers, 0.05M $Cr(acac)_3$ will generally reduce all T_1 values to less than 0.8 seconds. Thus a 10-12 second relaxation delay (with a 0.5 second acquisition time) is typically adequate. Some samples might be able to tolerate an even shorter delay. This has been found especially useful for studying mobile polymer side chains, branches, and end groups. $Fe(acac)_3$ has been observed to reduce all T_1 values in a similar fashion. However, $Cr(acac)_3$ was determined as preferable over $Fe(acac)_3$ for a variety of reasons, including greater chemical stability and greater lock stability. Also, caution should be applied in use of either agent when complexation leading to precipitation is possible (as in the case of acrylics containing carboxylic acid functionality).

Keep up the (well received) efforts with the Newsletter, and best regards! Please credit this contribution to Chris Roe's account.

Sincerely yours,

Karlis Adamsons

Marshall R&D Laboratory
3500 Grays Ferry Ave.,
Philadelphia, PA. 19146

Laurine G. Galya

Elizabeth F. McCord

Experimental Station
P.O. Box 80269
Wilmington, DE. 19880-0269

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(received 12/5/94)
November 20, 1994

Dr. Bernard Shapiro
966 Elsinore Court
Palo Alto, CA 94303

LED *vs* STE for High Gradient Diffusion Experiments

Dear Dr. Shapiro,

We have been studying the "solution behavior of starch under various conditions by PFG methods. We have a Varian VXR 4000 fitted with a Techron 7570 and 10mm double tuned Doty Z-gradient probe. The gradient strengths and timings are controlled by a homemade interface device between the acquisition computer and the Techron. In our work we routinely use gradients as high as 200 Gauss/cm.

When using gradients of this magnitude, an experimenter will have problems with eddy currents especially if he doesn't use pre-emphasis. As far as we can tell, the LED sequence* of Gibbs and Johnson solves this problem quite nicely and has an additional benefit. Repeating Johnson's experiments but using starch instead, we compared LED to the stimulated echo method (STE), using the same sample, diffusion times, and gradients. The usual procedure with PFG sequences is to vary the gradient strengths and record NMR signal intensities; diffusion coefficients are obtained from the exponential fits of signal intensity (Ψ) *vs* gradient strengths (g). The signal decays were many times faster with the STE sequence, which indicates that eddy effects are important and that the LED system is at least mitigating the problem. Often when using STE even at low gradient strengths, we have had problems with changes of signal phase as the gradients are increased. (from eddies extending into the acquisition period?) We have had no such problems using LED.

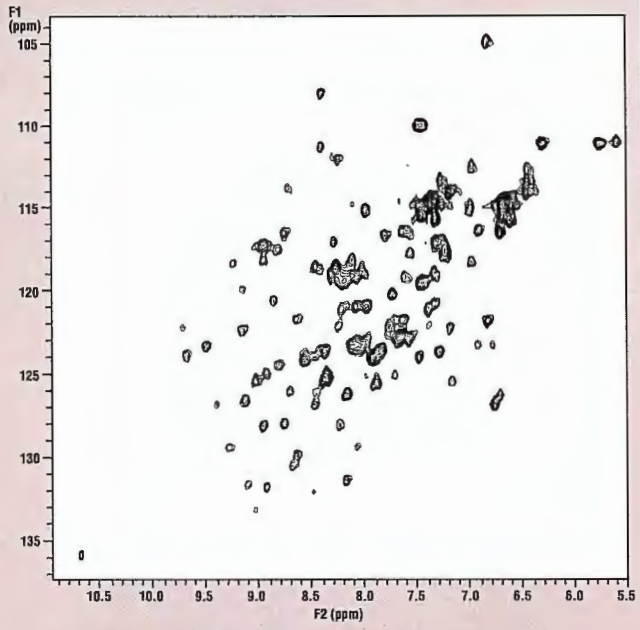
Although we couldn't get Dr. Johnson's full 64 step phase cycle, which he graciously supplied, programmed into our VXR computer, we did manage to get 32 steps. Anyway, we found that if we had a sufficient signal to noise ratio, the same decay curves resulted whether we used 8, 16 or 32 transients.

LED works very well for us and we use it routinely.

Gary Juneau

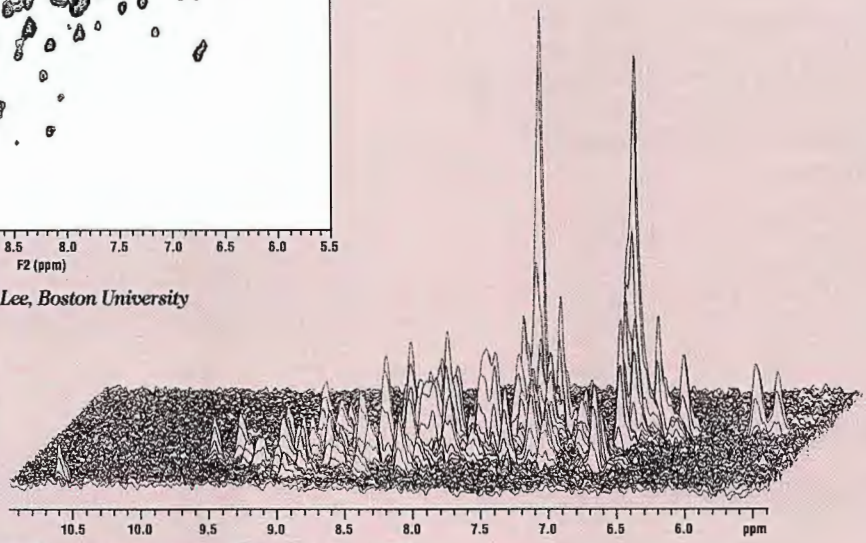
* S. J. Gibbs, C. S. Johnson, J. Magn. Reson. **93**, p395-402 (1991)

The World's First 10mm Triple Resonance PFG Probe



Sample courtesy of Jonathan Lee, Boston University

2D ^{1}H-^{15}N gradient HSQC experiment performed on a 350 µM sample (1.8 ml) of a ^{13}C, ^{15}N-labeled 13 kDa protein in 90% H_2O with 100 mM NaCl obtained using a UNITY*plus* 600 and a 10 mm $^{1}H\{^{15}N/^{13}C\}$ Biotriple•nmr PFG probe. The total acquisition time was 2 hours for 256 increments with a relaxation delay of 1.5 sec.



Pulse sequence courtesy of R. Andrew Byrd, NCI

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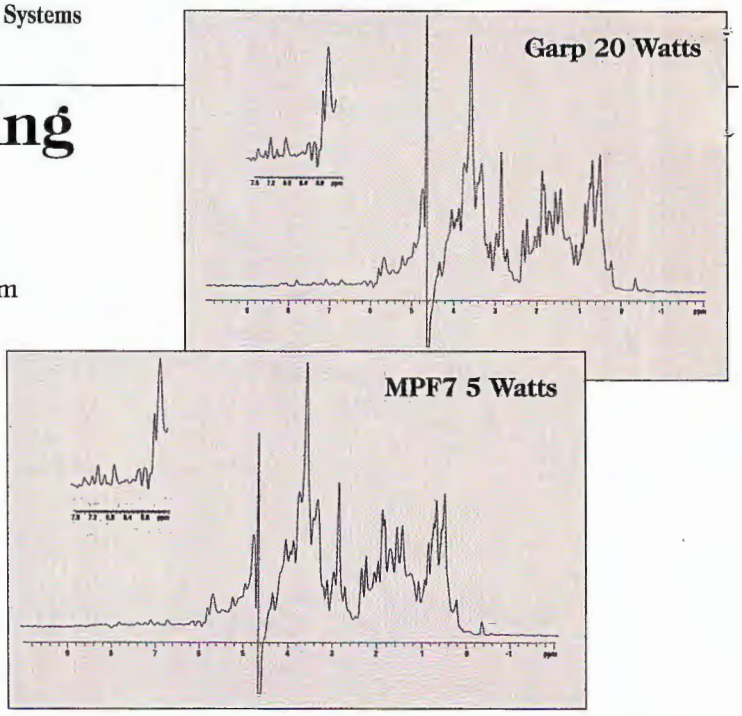

[1] Shaka, A.J., Barker, P.B., Freeman, R., *J Magn. Reson.*, 64, 547 (1985).
[2] Fujiwara, T., Nagayama, K., *J Magn. Reson.*, 77, 53 (1988).

Deuterium Decoupling with Deuterium Lock

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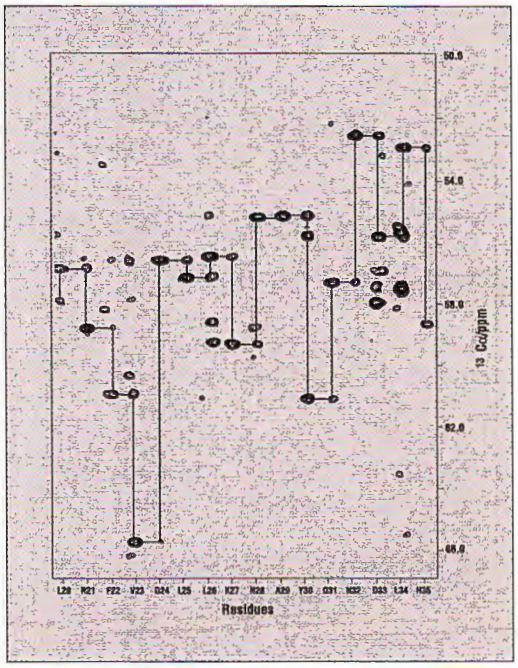
This plot displays the resultant sequential connectivities for helices A and B of the *Trp*-Repressor/DNA Complex. Utilizing the experiment above, L. Kay and co-workers* have obtained 100% of the intra-residue and 94% of the inter-residue correlations for the 37 kDa complex.

Spectrum provided by Toshio Yamazaki, Weon Tae Lee, Matt Revington, Cheryl Arrowsmith and Lewis Kay from the University of Toronto and the Ontario Cancer Institute, Toronto, Canada.

*Yamazaki, T., Lee, W.T., Mattiello, D.L., Dahlquist, F., Revington, M., Arrowsmith, C., and Kay, L.E., "An HNCA Pulse Scheme for the Backbone Assignment of ^{2}H, ^{13}C, ^{15}N-Labeled Proteins: Applications to a 37 kDa *Trp*-Repressor Complex," *J. Amer. Chem. Soc.* (submitted).



^{2}H, ^{13}C, ^{15}N - labeled *Trp*-Repressor/DNA Complex (37 kDa) HNCA with constant time carbon evolution combined with ^{2}H decoupling

Battelle
Pacific Northwest Laboratories
Battelle Boulevard
P.O. Box 999
Richland, Washington 99352
Telephone (509)
372-3888

November 21, 1994
(received 11/23/94)

Dr. B. L. Shapiro
966 Elsinore Court
Palo Alto, California 94303

Dear Barry:

Re: NMR on the Columbia River

It has been several months since we have had an opportunity to send you a summary of our magnetic resonance activities here at PNL. As I write this letter the sky is a sharp blue, the Columbia River is flowing by outside my office window and it is cold outside. I should be home watching a football game and not at work writing this letter. However, that is another story. I have asked four staff members: Herman Cho, Mike Bowman, Robert Wind and Mike Kennedy to summarize their research interests and progress. In the interest of space I will limit this contribution to these investigation. I will send a note after the first of the year describing our research on the structural consequences that ligands impose on ^{113}Cd chemical shielding tensors (in collaboration with Dan Reger at the University of South Carolina), ethylene on metal supported catalysts, and the fun and games we are having with gas phase ^{77}Se NMR and absolute shielding scales for ^{77}Se NMR. This latter work is in collaboration with members of PNL's theory group and Jerry Odom of the University of South Carolina.

Magnetic Resonance Spectroscopy

Following a prolonged struggle to introduce magnetic resonance spectroscopy to the Department of Energy Hanford site (too long and still too painful to relate in a single issue of TAMU News), we have begun returning our attention to the laboratory and giving some thought to how solid state NMR and EPR investigations can help find solutions to DOE environmental and ecological problems.

The lack of atomic-level information for many inorganic compounds is a significant impediment to informed decision-making on strategies for the Hanford site. Three groups of compounds for which this lack of knowledge is particularly detrimental are: (1) metal-supported catalysts; (2) multi-component oxide glasses; and (3) oxide minerals.

Readers of TAMU News need no convincing that NMR and EPR studies can be valuable contributors to the elucidation of structure and dynamics in such systems. Indeed, we have already initiated solid state magnetic resonance projects in all three areas, both on "friendly"

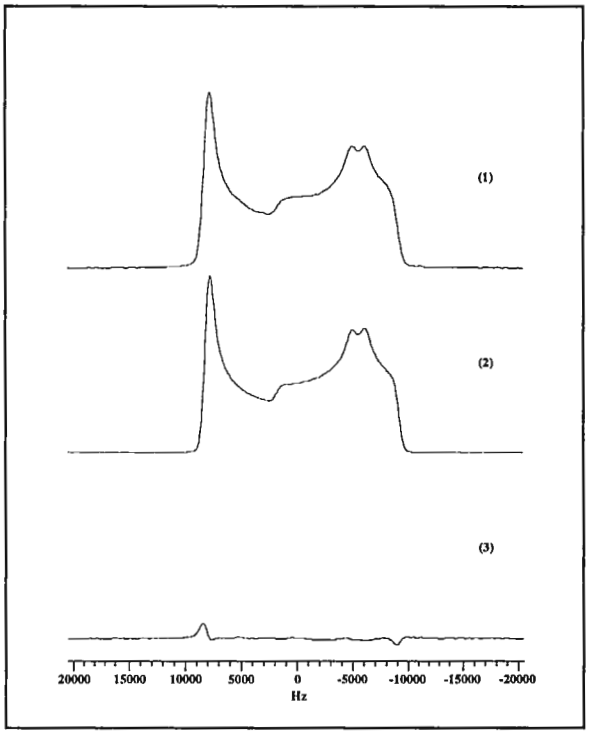


Figure 1

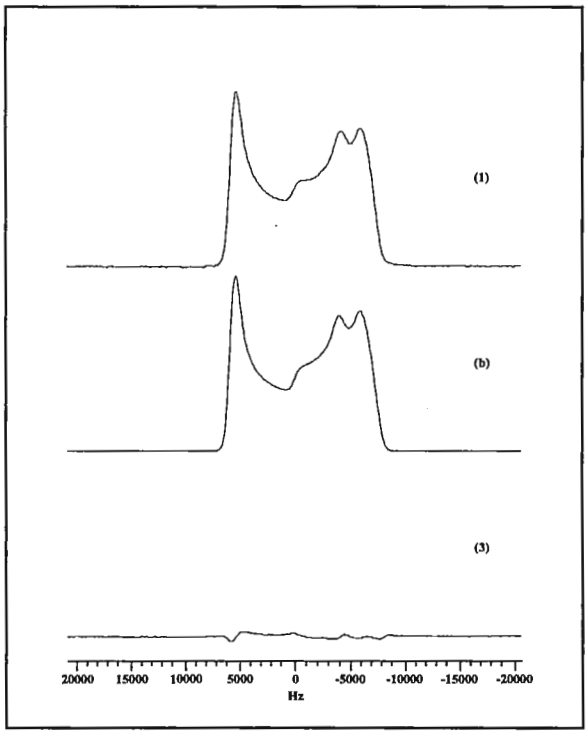


Figure 2

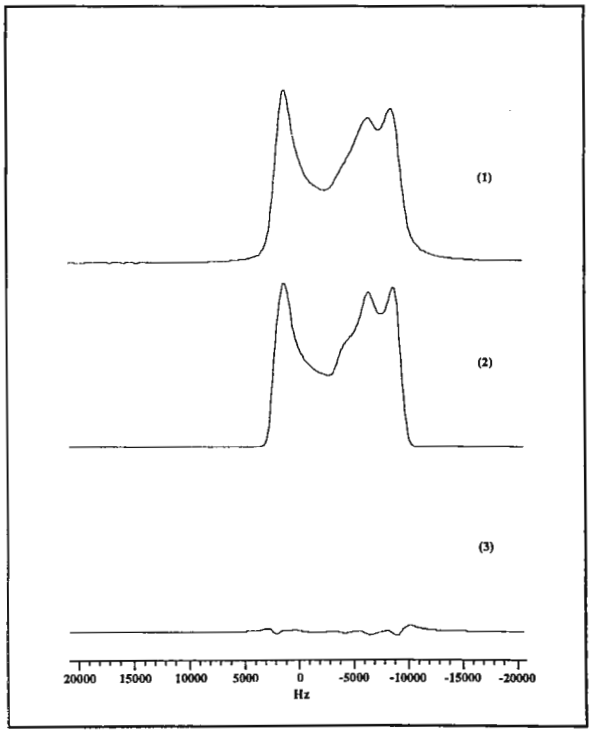


Figure 3

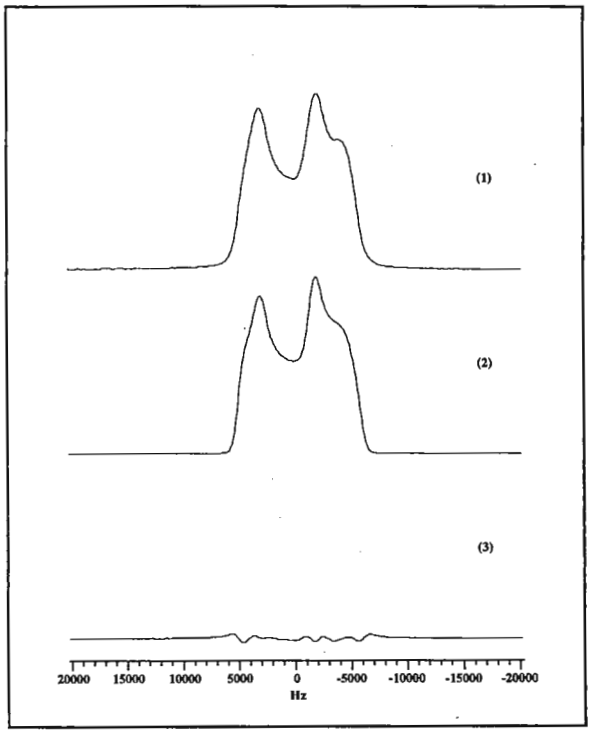


Figure 4

model systems and on "real-life" samples. The proximity of the Hanford infrastructure makes it especially convenient for us to obtain specimens of the latter category.

A preview of things to come is depicted in the preceding figures. The spectra are from a paper by Jeff Koons, Eric Hughes, Herman Cho, and Paul Ellis scheduled to appear in J. Magn. Reson. A in April, 1995. The spectra labeled (1) are experimental lineshapes of the (+1/2) to (-1/2) Rb-87 NMR transition of rubidium perchlorate powder, with no sample spinning, measured at field strengths of (a) 7.05 T, (b) 9.4 T, (c) 11.7 T, and (d) 17.6 T. The four spectra labeled (2) are least-squares fits of the experimental lines, generated by a program written by the graduate students (and thus the brains) in this project, Jeff Koons and Eric Hughes. The chemical shift and second-order quadrupolar interactions are both significant contributors to the lineshape of the Rb-87 resonance, and a multifield study is a particularly convincing demonstration of the accuracy of the fit.

Pulsed EPR and ENDOR Spectroscopy

We have just had the installation of a Bruker ESP 380 E Pulsed EPR with their new Pulsed ENDOR accessory. The capabilities of this machine are simply awesome to those who remember the state of ENDOR or pulsed EPR twenty years ago! We have started research with this machine examining oxo-bridged binuclear iron centers in model compounds, ribonucleotide reductase and possibly in a pesticide mono-oxygenase. We will be using the temperature dependence of T1 to measure energy gaps to low-lying excited states so that we can determine exchange couplings. The ENDOR and ESEEM will probe paramagnetic shifts of nitrogen and protons in the vicinity of the iron site and will let us physically map the organic or protein phase around the irons and the binding conformation of any substrates. We plan to systematically study and understand metalloenzymes that have potential for bioremediation of environmental pollution by catalyzing the breakdown of contaminants in the soil or groundwater.

Cell and Tissue Damage from Chemical and/or Radiation Exposure

The main objectives of the research are to apply existing and develop novel NMR methodologies in order to detect and characterize preneoplastic and neoplastic lesions on a cellular and tissue level after exposure to toxic chemicals and/or ionizing radiation; to detect these lesions at the earliest possible stage and for different doses of exposure; and to follow the temporal evolutions of these lesions. To this end both *in vivo* and *in vitro* NMR will be applied. *In vivo* NMR investigations include MRI, MRS, and CSI in a field of 11.7 T to determine tumor growth kinetics in small inbred rodents occurring after exposure, and NMR microscopy, in external fields of 11.7, 17.6, and 23.4 T, which will be used to image live mammalian cells with a subcellular spatial resolution, and to perform localized spectroscopy on small cell clusters. *In vitro* NMR research include multi-nuclear, high-field NMR on normal and tumorous cells and tissues, and on extracts. Both liquid-state and high-resolution solid-state NMR techniques will be applied (the latter at low temperatures in order to be able to apply magic angle spinning), which will allow us to investigate both the mobile and immobile fractions in cells and tissues. Figures 5 and 6 show examples of the latter type of investigations. Here ^{13}C spectra are shown of healthy mammary tissues obtained from

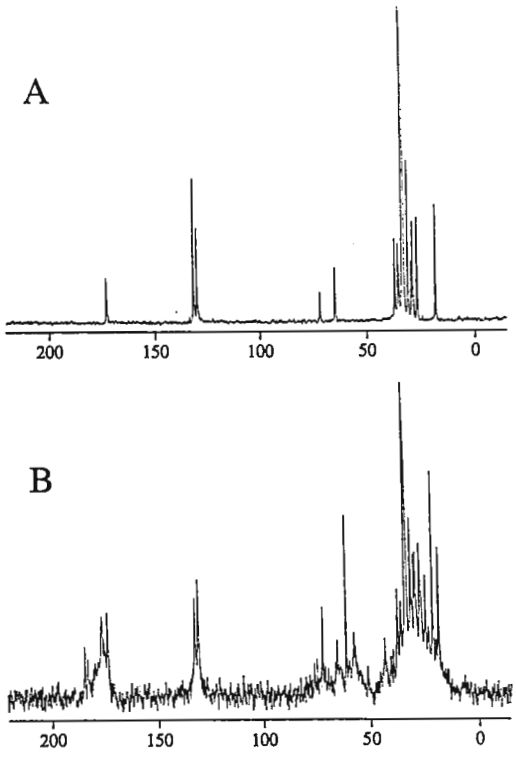


Figure 5. ^{13}C Bloch decay spectra obtained at 4°C. A:healthy mammary tissue; B:mammary tumor tissue

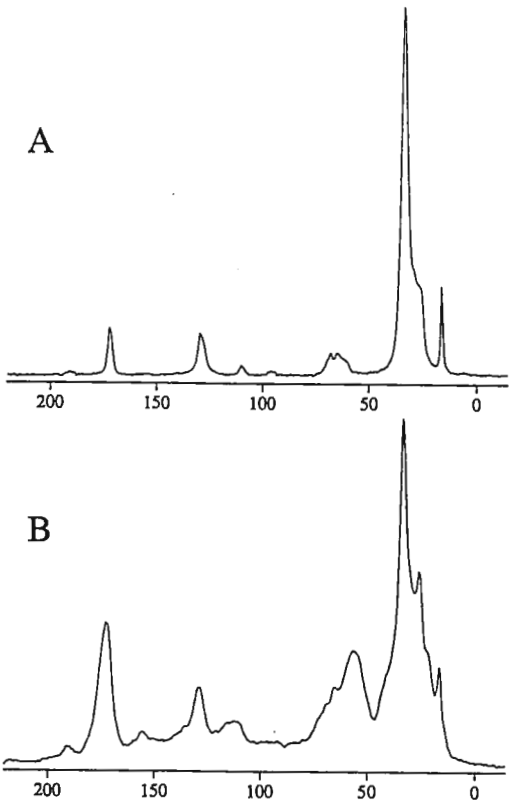


Figure 6. ^{13}C CPMAS spectra obtained at -100°C. A:healthy mammary tissue; B:mammary tumor tissue

female Fischer rats, and of R3230AC mammary tumors implanted in the animals. Figure 5 shows the results of liquid-state NMR, and Figure 6 shows the spectra obtained by solid-state CPMAS NMR. It follows that with liquid-state NMR in both the healthy tissue and tumors mainly the narrow resonances due to the adipose tissue is observed, whereas with solid-state NMR in the tumorous tissue the fractions, which are immobile at ambient temperatures (probably mainly fatty acids), are dominating the spectrum.

It is expected that the combination of the different types of investigations outlined above will lead to an improved understanding of the mechanistics of the carcinogenic process on a tissue and cellular level, and to an improved detection of lesions resulting from exposure.

Structural Studies of Protein Recognition of DNA Damage

Our current research is concentrated into two main areas. The first is directed at elucidating the structural mechanism by which proteins involved in DNA repair recognize damaged DNA. We are currently cloning genes that code for proteins of interest for NMR studies. NMR quantities of DNA containing chemically modified nucleotides are a prerequisite for

NMR studies of damaged DNA/protein complexes. AWU fellow Jody Lingbeck and I are working with Gary Drobny at the University of Washington to synthetically prepare oligonucleotides with modified bases that represent lesions produced by ionizing radiation. Our approach has been to prepare chemically modified bases which are converted into phosphoramidites for direct incorporation into synthetic oligonucleotides using standard automated DNA synthesis methods. In our first attempt, we chose to study dihydrothymidine (dhT), a product of ionizing radiation produced under anaerobic conditions. In dhT, the aromatic ring of thymidine is saturated by the addition of hydrogen across the C5-C6 double bond generating a chiral center at the C5 position. When this occurs, the planar ring of thymidine becomes non-planar in dhT and can assume two distinct conformations where the methyl group assumes a position either axial or equatorial to the saturated ring. The structural distortion produced by the two different isomers of dhT is expected to give rise to two DNA duplexes distinguishable by NMR spectroscopy. Figure 7 shows the one dimensional 750 MHz NMR spectrum of the aromatic region of [dCCAA(dhT)AACC]:[dGGTTATTGG]. From the 1D spectrum, it is apparent that some aromatic resonances are split, indicating that two duplexes are distinguishable by NMR. In the aromatic to 1' region of the 2D-NOESY spectrum, we clearly observe two sets of resonances which diverge in the sequential walk for bases nearby the lesion. In Figure 8, for example, we can completely assign two distinct strands which are complementary to strands containing a dhT lesion. Finally, for at least one strand containing a dhT, we can completely assign through the sequential walk including the nonaromatic dhT H6 proton, which occurs at 2.8 ppm, indicating that the dhT base remains base paired and in the helix. With the phosphoramidite of dhT in hand, we are now pursuing more challenging phosphoramidite preparations of other lesions of interest.

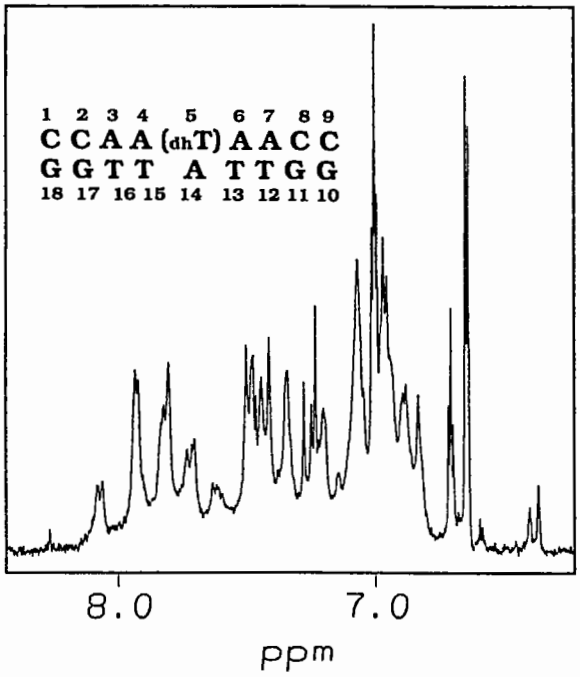


Figure 7. 750 MHz NMR spectrum - aromatic region

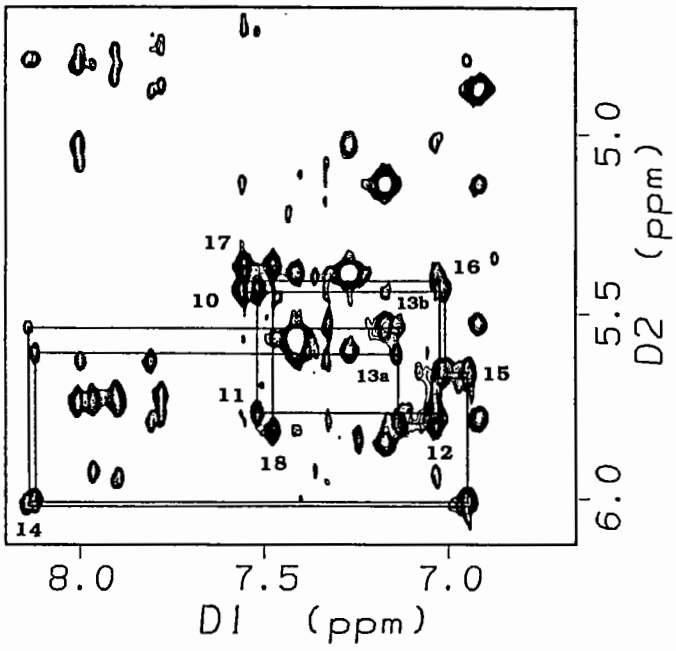


Figure 8. 500 MHz NMR spectrum - aromatic to 1' region

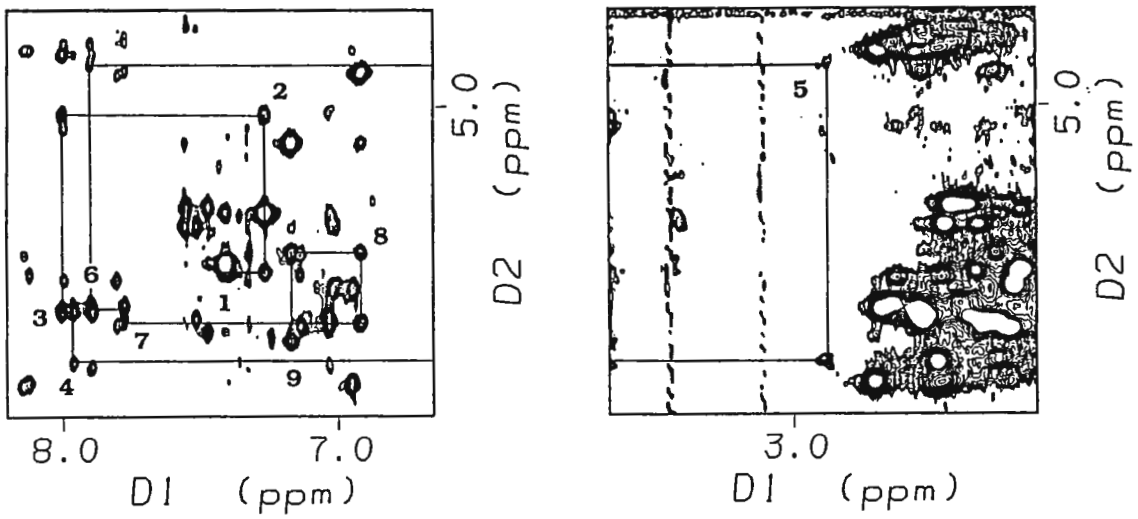


Figure 9. 500 MHz NMR spectrum - aromatic to 1' region

The second area of research is aimed at providing insight into structure/function relationships for proteins useful in the area of bioremediation. We are using NMR to provide structural information useful for establishing enzyme mechanisms, substrate specificities, and protein stability in extreme environments. In this project our colleagues range in expertise from environmental microbiologists to technology engineers to theoreticians using structural information for rational enzyme redesign. We currently have a postdoctoral position immediately available for work in this area of research.

Sincerely,

Paul D. Ellis
Associate Director

Michael K. Bowman Herman M. Cho Michael A. Kennedy Robert A. Wind
Research Scientists
Macromolecular Structure and Dynamics
Environmental Molecular Science Laboratory

Cornell University

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

November 30, 1994
(received 12/3/94)

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

Dear Dr. Shapiro,

We have recently implemented a way to do arbitrary amplitude and phase modulation on our home-built NMR system utilizing a VMA-002 vector modulator from Mirage Systems (232 Java Dr., Sunnyvale, CA 94089, 408-752-1600) and an add-on LDEC box from Tecmag. The lines of the LDEC are controlled by tables which are produced by an external program written in C. This is a fairly inexpensive method for phase and amplitude control for pulse shaping and multiple quantum phase cycling applications. Total cost was about $4000. Incidentally, Mirage offers many other products which may be of interest to the NMR community.

The vector modulator uses 21 control lines to modulate our IF of 70 MHz. It splits the input signal into 0° and 90° lines. Nine bits each are used to control the amplitudes of these two lines. After recombining the two lines, another three bits are used to control the final amplitude. It has a resolution of 0.23° and 0.035 dB. Settling time is 200 ns.

The LDEC was not originally bought with our mini-Libra from Tecmag, but instead was added as a separate component later. We can clock out 24 bits from the LDEC in synchronous mode, with a clock time of 600 ns. The interface to the vector modulator is simply a small wire-wrapped circuit which connects the pins. A more complicated separate circuit was also built from scratch which can switch on the order of 100 ns but has limitations in other areas.

The C code was written in Think C, and simply calculates a waveform. For amplitude modulation, it is a simple matter to input an equation and recompile the sequence or to store commonly used shapes with a few variables describing the period, slope, width, etc. For step functions, one manually inputs the amplitude, phase, and duration of each step.

Fig. 1

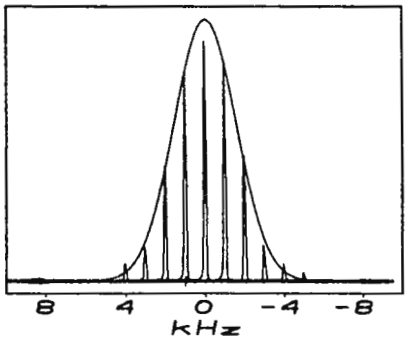


Fig. 2

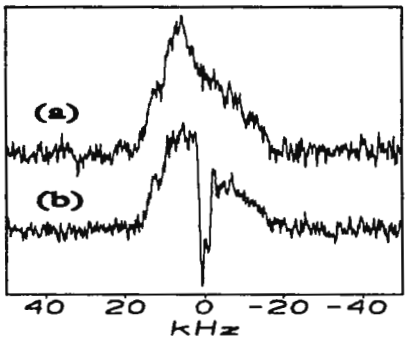


Figure 1 shows the excitation profile of a gaussian shaped pulse on a sample of D_2O, stepping the rf frequency while keeping the power levels and pulse time constant. Figure 2 shows the result of a "hole-burning" experiment on ^{77}Se in a sample of $Rb_2Mo_6Se_6$ to test the system. The top trace (a) is after a $90_x - \tau - 180_y - \tau$ sequence. The lower trace (b) is after a gaussian shaped pulse (x-phase) of duration 825 μs immediately followed by a "hard" 90_x pulse, then a 180_y to refocus. Obviously, the integrated intensity of this pulse was slightly longer than the 90° pulse, since there is a slight inversion of the signal.

We have shown a fairly inexpensive method of implementing amplitude and phase modulation on home built systems. Since we have control over how the program is written, it is a simple matter to make adjustments to suit personal taste for the interface.

Please credit this to the account of Aidan Harrison.

Sincerely,

Benjamin G.M. Chew

David B. Zax

Table of Contents, cont'd.

♣ ♦ ♥ ♠ ♣ ♦ ♥ ♠

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Dr. B. L. Shapiro
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Palo Alto, CA 94303 U.S.A.

(415) 493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

Deadline Dates

No. 438 (March)	24 February 1995
No. 439 (April)	24 March 1995
No. 440 (May).	21 April 1995
No. 441 (June)	26 May 1995

*Fax: (415) 493-1348, at any hour. Do not use fax (of which the plural form is *feces*, being the 6th declension) for technical contributions to the Newsletter, for the received fax quality is very inadequate.

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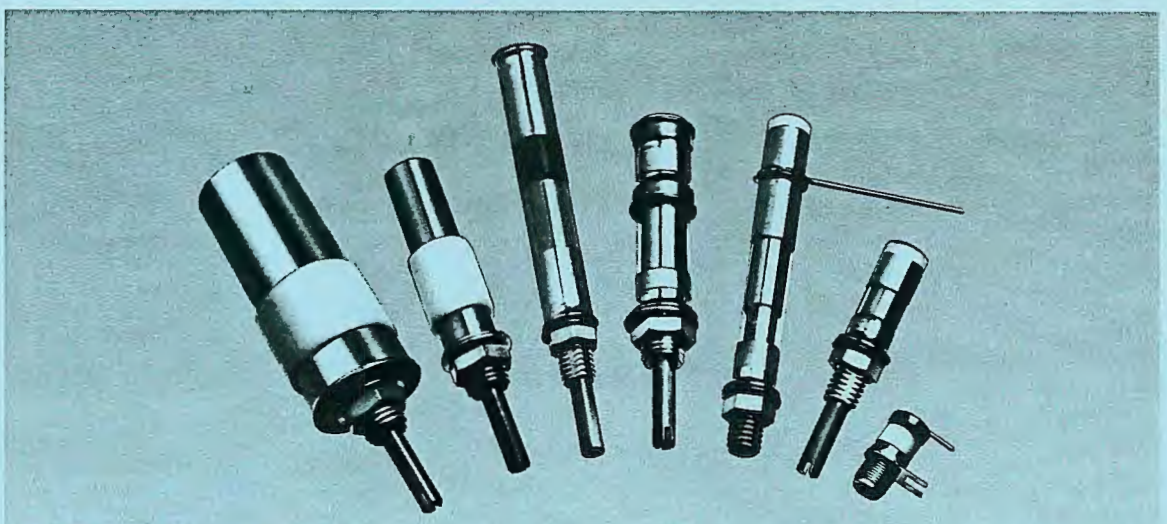

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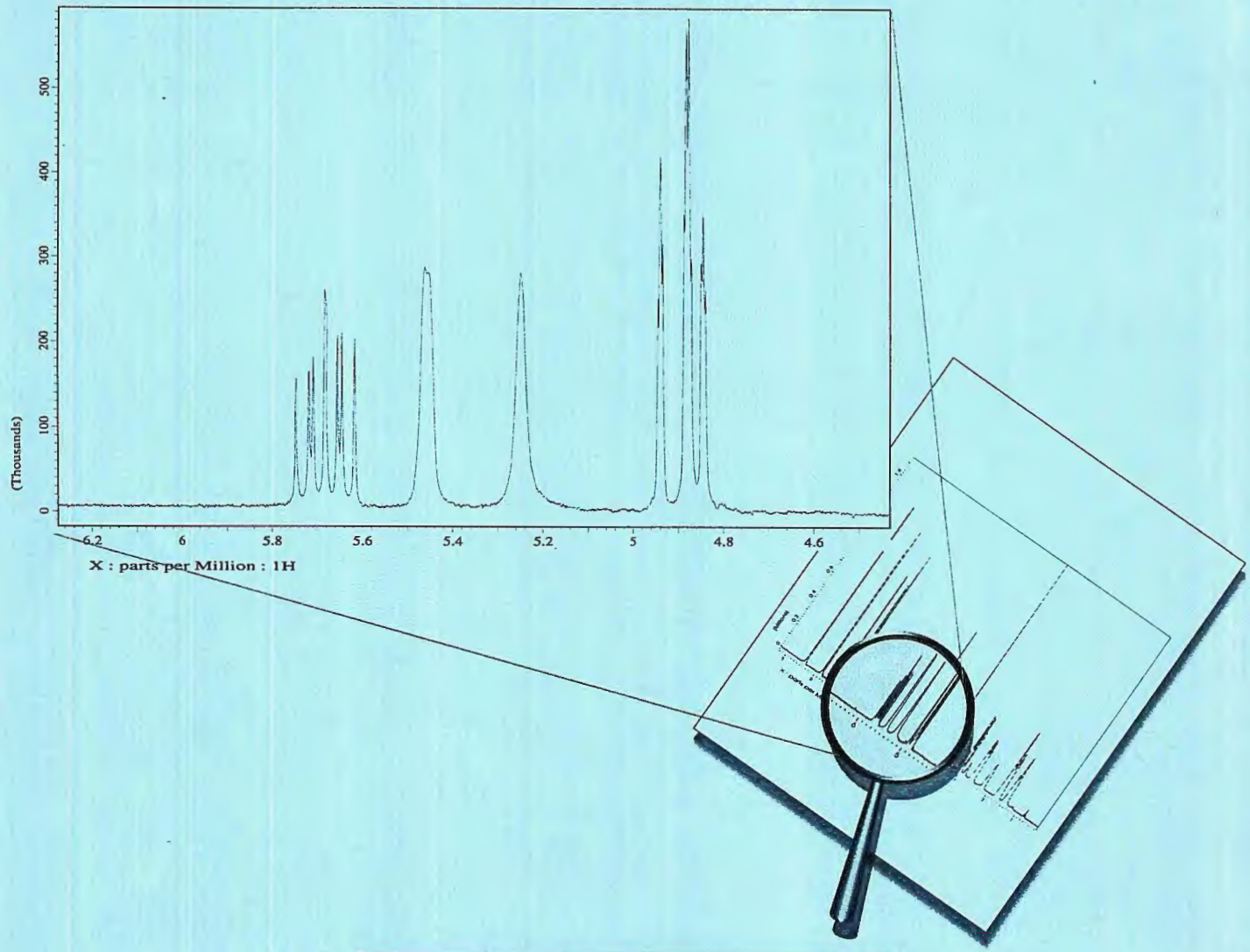
ECLIPSE NMR Advantage: Digital Filtering



Eclipse NMR *The Better Way!*

This data shows the effect of digital filtering on JEOL USA's Eclipse NMR Spectrometer. The bottom spectrum shows the full spectrum of quinine while the top spectrum shows the same area after digital filtering has been applied. In contrast to the non-filtered area the digital filtered area shows none of the aliasing of the peaks from outside the spectral window.

The advantage of the Eclipse is that there are no "coefficients" or "poles" to choose, one need only choose the spectral window and the Eclipse does all of the work. Consequently digital filtering becomes an easily used option for any NMR experiment.

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