THE NMR NEWSLETTER

No. 495

December 1999

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FORTHCOMING NMR MEETINGS

Biennial Meeting of the Australian and New Zealand Society for Magnetic Resonance (ANZMAG2000), Mt. Buller, Victoria, Australia; **February 13-17, 2000**; Contact: Dr. Jenny Wilson, Victorian College of Pharmacy, Monash University, Parkville, Victoria 3052, Australia; E-mail: anzmag@edda.vcp.monash.edu; vcp.monash.edu.au/chemistry/anzmag2k.

PITTCON 2000, New Orleans, LA, **March 12-17, 2000**; Contact: The Pittsburgh Conference, 300 Penn Center Blvd., Suite 332, Pittsburgh, PA 15235-5503; Phone: 412-825-3220; Fax: 412-825-3224; Email: expo@pittcon.org.

8th Scientific Meeting and Exhibition, International Society for Magnetic Resonance in Medicine, Denver, CO, **April 1-7, 2000**; Contact: ISMRM, 2118 Milvia Street, Suite 201, Berkeley, CA 94704. Tel. 510-841-1899; Fax. 510-841-2340; E-mail: info@ismrm.org; http://www.ismrm.org.

Symposium on Advances in NMR Applications, Naval Postgraduate School, Monterey, CA. Shuttle service to and from Asilomar will be provided. **April 9, 2000**; Contact: V. Davies, Nalorac Corporation, 837 Arnold Drive, Suite 600, Martinez, CA 94553; 925-229-3501; Fax: 925-229-1651; Email: victoria.davies@nalorac.com; http://www.nalorac.com. See Newsletter 495, 28.

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

15th European Experimental NMR Conference, Leipzig, Germany, **June, 2000**. For information, see http://eenc.uni-leipzig.de.

SMASH-2000, Argonne, IL, **July 16-19, 2000**. Contact: G. E. Martin (gary.e.martin@amu.pnu.com). See Newsletter 493, 21.

continued on inside back cover

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Faculty of Science
E3-43 Chemistry Building East, Telephone (403) 432-3254

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
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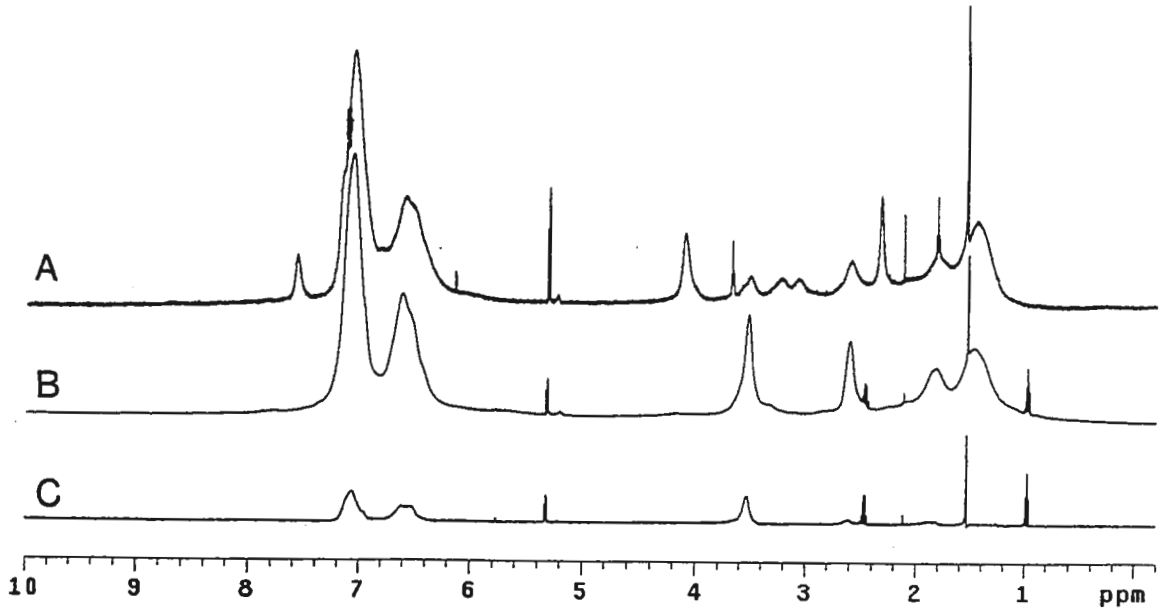
Oct. 12, 1999
(received 10/23/99)

Nanoprobe Test Results

Dear Barry;

We recently accepted delivery of our new 4mm Magic Angle Spinning gHX Nanoprobe with gradients along the magic angle for our Varian Unity 500 NMR spectrometer and wanted to share with your readers some of our initial observations. The installation of the probe is relatively straight forward. It simply requires that +/- 5 and +/- 12 volts dc be routed to the pneumatics/tachometer box from the console plus a little plumbing for the air line connection. Sample insertion requires that the probe be removed from the magnet but probe positioning in the magnet seems to be very reproducible. Spinning speeds of up to 2500 Hz have been obtained but occasionally the spinning air pressure must be increased in an oscillatory manner to obtain these rotation speeds. Lineshape was obtained in a very short time using the lineshape shim set from a 5mm standard probe as the starting point. None of the axial shims were used in shimming-only the low order xy shims (x, y, xz, yz, xy, x2y2) were optimized to obtain a lineshape of 4/7.75. Proton, carbon and nitrogen 90 degree pulse widths were 4.5, 11 and 28us, respectively, using a transmitter power 5 db below maximum output.

Sample preparation is a little tricky and requires the use of a 100ul syringe and a steady hand. Once the sample (40ul volume) is in the tube, then the spinner turbine is glued to the sample tube. It is best to test if the sample will rotate above 2 KHz before placing the probe into the magnet. The first H1 NMR spectra obtained on real polystyrene resin samples are shown below. In **A** and **B** the four broad peaks at about 1.4, 1.8, 6.6 and 7.0 ppm are due to the resin and the very sharp peaks are due to solvent (CHDCl2 at 5.32 ppm) and mobile solvent impurities. The remaining broad resonances are due to the molecules of interest and are broad because the linker is relatively short. In **C** we apply the normal cpmg trick to get rid of the broad resonances present in the sample whose normal 1D spectrum is shown in **B**.



Dennis Hall

Tom Nakashima

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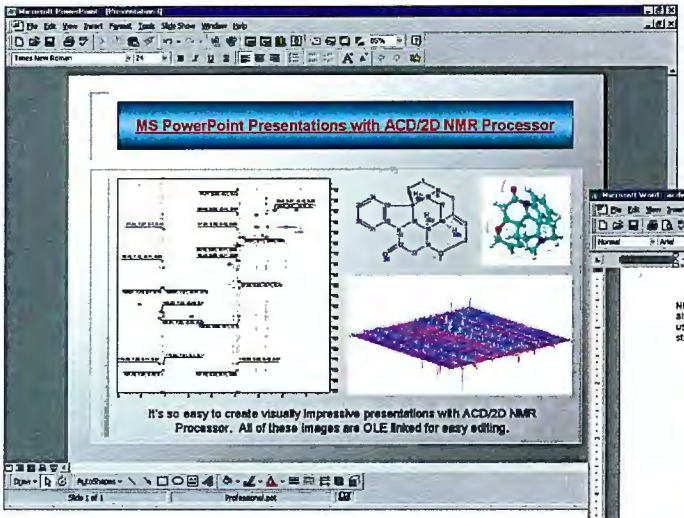

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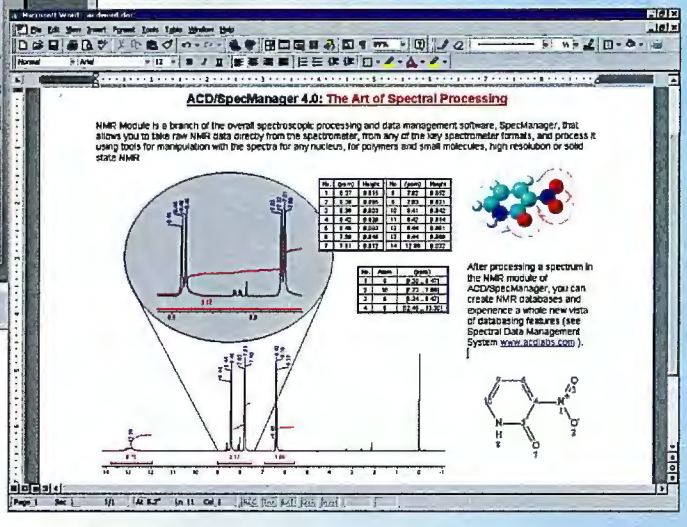

Word document

One common problem for NMR spectroscopists today is making their data mobile. Moving data from the spectrometer into Microsoft applications (and others), graphically editing spectra with the addition of annotations, molecules, arrows and other graphic objects then moving all of this into a word processor. Just look through the pages of the NMR newsletter to see how common this is! Well... you can depend on graphics programs, converters and partial vendor approaches or use the integrated report editing, graphics and structure drawing package with full OLE compatability included with every ACD product - ACD/Chemsketch. Above, you can see the reports constructed from experimental spectra inside ACD/Chemsketch and then simply copied into Word and PowerPoint. There is no need to use third party programs... so simplify your life!

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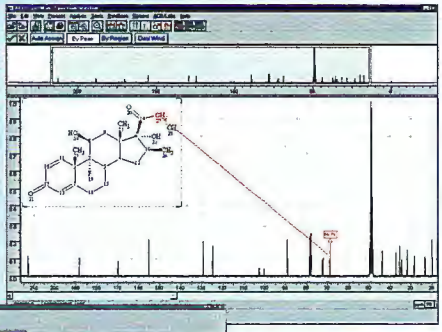

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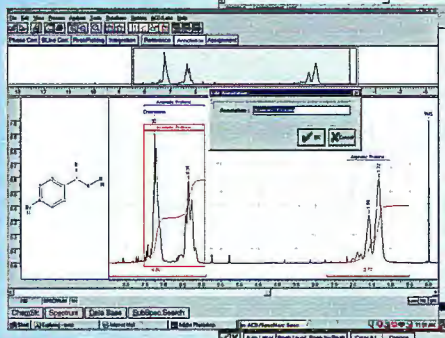

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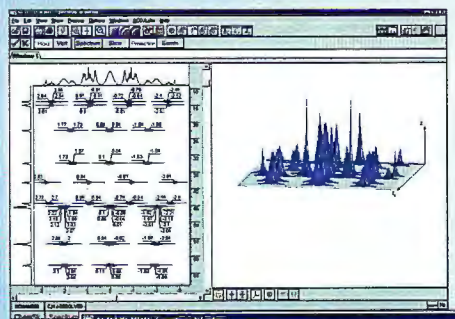

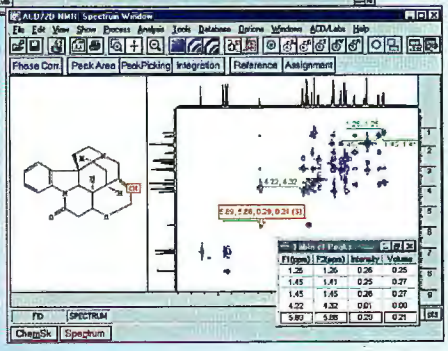

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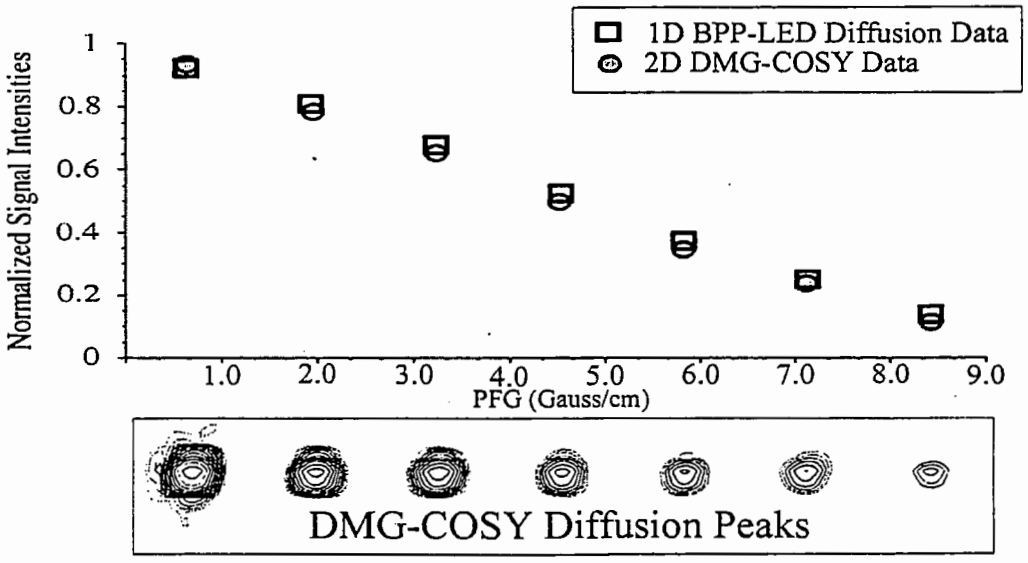
Dr. B.L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

November 23, 1999
(received 11/25/99)

DMG-COSY

Dear Barry:

Science certainly can take one far in life. I recently returned from a visit to Europe that included stops in Italy, Germany, Russia and Poland. The NMR highlight of my trip came in Frankfurt where I had an opportunity to visit the incredible NMR facility of Christian Greisinger and colleagues. The visit to Russia left me saddened at the current state of the scientific infrastructure there, and grateful I had a round trip ticket to the U.S.. Back in Omaha we have been using NMR diffusion measurements to investigate drug:DNA interactions. One new experiment we designed in the lab has been particularly useful in this regard. We refer to it as a Diffusion-Modulated Gradient COSY (DMG-COSY). The experiment uses the BPP-LED element of Johnson and co-workers as the preparation period for a 2D gradient COSY experiment. Crosspeaks in the DMG-COSY arise from pairs of ^{1}H connected by J-coupling with the intensity of the crosspeak modulated by self-diffusion during the BPP-LED preparation period. We have found the experiment very robust and useful for investigating drug:DNA binding. The following Figure shows the results of 1D BPP-LED and the DMG-COSY for a sample of Uridine in DMSO.



Sincerely yours,

William H. Gmeiner, Ph.D.
Associate Professor

Christopher J. Hudalla, Ph.D.
Postdoctoral Research Associate

** Paper in press pending minor revision, FEBS Letters **

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5 November 1999
(received 11/19/99)

B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
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Dear Barry,

Metabonomics – what is this new word?

We would like to share with your readers the explanation of a new word which we have recently coined. The rapid evolution of drug discovery science, fuelled by combinatorial library based synthesis programmes has led to increased pressure on the drug safety evaluation process. Once potential drugs have passed the primary biological screening procedures, losses of drug candidate compounds from the product development pipeline need to be minimised. Hence there is an search for new analytical technologies which will maximise efficiency of lead compound selection based both on efficacy and safety and minimise overall attrition rates.

Current approaches include measurements of responses of living systems to drugs either at the genetic level or at the level of expression of cellular proteins, using methods termed genomics and proteomics. At present these are both are expensive and labour intensive, yet potentially are powerful tools for studying different levels of the biological responses to xenobiotic exposure. However, even in combination, they do not provide all the information needed for understanding integrated cellular function in living systems, since both ignore the dynamic metabolic status of the whole organism. Thus we propose a new NMR-based metabonomic approach which is aimed to augment and complement the information provided by measuring the genetic and proteomic responses to xenobiotic exposure. We define metabonomics as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification".

If we concentrate solely on the drug toxicology use of metabonomics, foreign compounds may have an effect at a series of levels ranging from changes in genetic expression through protein production and integrated cellular biochemical regulation and control. In such cases there will be alterations detectable at the genomic, proteomic and metabonomic levels. In many cases, drugs exert their toxic effects by interacting directly with genetic material or by inducing the synthesis of drug metabolising enzymes which generate toxic products. In such cases genomic and proteomic approaches to toxicity assessment may be useful. However, xenobiotics may act only at the pharmacological level and hence may not affect gene regulation or expression. Hence in many cases facile consideration of genomic and proteomic responses are likely to be ineffective at predicting drug toxicity. However, all drug-induced pathophysiological perturbations result in disturbances in the ratios and concentrations, binding or fluxes of endogenous biochemicals, either by direct chemical reaction or by binding to key enzymes or nucleic acids that control metabolism. If these disturbances are of sufficient magnitude, toxic effects will result which will affect the efficient functioning of the whole organism. In body

fluids, metabolites are in dynamic equilibrium with those inside cells and tissues and, consequently, abnormal cellular processes in tissues of the whole organism will be reflected in altered biofluid compositions. In all cases the analytical problem usually involves the detection of "trace" amounts of analytes in a very complex matrix with many potential interferences. It is, therefore, critical to choose a suitable analytical technique for the particular class of analyte of interest in the biomatrix, for example blood, plasma, urine, bile, or organ samples. We have shown that high resolution ^{1}H NMR spectroscopy is particularly appropriate for investigating abnormal body fluid compositions as a wide range of metabolites can be quantified simultaneously with no sample preparation and "without prejudice". NMR spectroscopy may also be used effectively to screen for abnormal metabolite profiles in tissue extracts or cell suspensions. We have also shown that the same approach can be used to investigate the metabolic composition of intact tissues using high resolution magic angle spinning ^{1}H NMR spectroscopy.

The exact pattern of endogenous metabolites in body fluids as detected by ^{1}H NMR spectroscopy depends strongly on the type of toxin to which an animal has been exposed. Each class of toxin produces characteristic changes in the concentrations and patterns of endogenous metabolites in biofluids and this provides information on the sites and basic mechanisms of the toxic process. The processes of generating such information is highly efficient, taking only a few minutes per sample, requiring little or no sample pre-treatment or reagents. ^{1}H NMR spectroscopic analysis of biofluids has successfully uncovered numerous novel metabolic biomarkers of organ-specific toxicity in the rat, and it is in this "exploratory" role that NMR as an analytical biochemistry technique excels. Similar approaches can be used using 2-dimensional (2D) NMR spectroscopy. However, the biomarker information in NMR spectra of biofluids is much more subtle and rich than this, as hundreds of compounds representing many pathways can often be measured simultaneously, and it is the overall metabonomic response to toxic insult (occurring over time) that so well characterises the lesion. The most efficient way to investigate these complex multiparametric data is to continue the NMR metabonomic approach with pattern recognition (PR) methods.

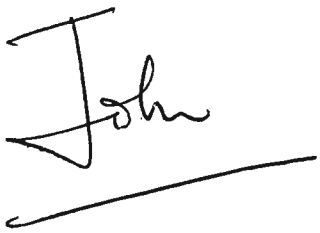
A limiting factor in understanding the biochemical information from NMR spectra of tissues and biofluids is their very complexity; even 1D ^{1}H NMR spectra of biofluids may contain several thousand resolved lines. The NMR spectrum can be considered to be an n-dimensional object the dimensions of which could be the concentrations of individual measurable metabolites or more simply the spectral intensity distribution. Thus the NMR spectrum of the biofluid or tissue provides an n-dimensional metabolic fingerprint of the organism based on the sample studied, and this metabolic profile is characteristically changed according to the disease or toxic process. Hence we have turned to computer-based PR and expert system approaches to interpret the NMR data obtained in various experimental toxicity states. The simplest approach is to treat the NMR signal intensity data as an n-dimensional vector; it is not necessary to assign the spectrum at this stage as it is treated solely as a statistical object. One easily applied PR technique is Principal Components Analysis (PCA) and a plot of the first two or three PC's gives the "best" representation, in terms of biochemical variation in the data set in two or three dimensions. Such PC maps can be used to visualise inherent clustering behaviour for drugs and toxins acting on each organ according to toxic mechanism. The position on a PC plot of a sample from a xenobiotic treated animal is determined purely by its metabolic response, hence the method is termed "unsupervised". Of course, the clustering information might be in lower PCs and these have also to be examined. In this simple metabonomic approach, a sample from an animal treated with a compound of unknown toxicity is compared with a database of NMR-generated metabolic data and its topographical fit on the PR map is determined.

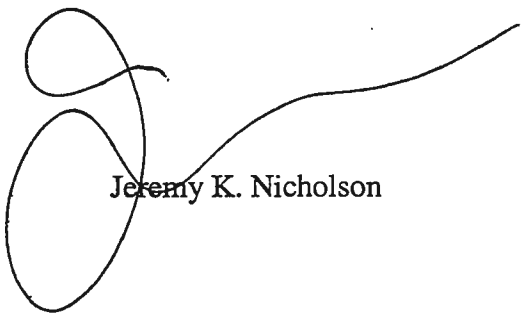
However, in the real world, toxicological data are more complex as lesions develop and resolve in real time and hence there are time-related changes in NMR-detected metabolic profiles. Also, it is more rigorous to compare drug effects in the original n-dimensional NMR metabonomic space. In order to develop automatic toxicity classification methods, it has proved efficient to use a "supervised" approach to NMR data analysis.

Here, a "training set" of NMR metabonomic data is used to construct a mathematical model that predicts correctly the class of each sample. This training set is then tested with independent data ("test set") to determine the robustness of the computer-based model. These models may comprise systems based on range of different mathematical procedures such as principal components, artificial neural networks and rule induction. In all cases the methods allow the quantitative description of the multivariate boundaries that characterise and separate each class of xenobiotic in terms of their metabolic effects. Certain supervised methods also allow a level of probability to be placed on the goodness of fit. Using such systems a sample can be classified as belonging to a single class of toxicity, to multiple classes of toxicity (more than one target organ) or to no class. The latter case would indicate deviation from normality (control) based on the training set model but having a dissimilar metabolic effect to any toxicity class modelled in the training set (unknown toxicity type).

The metabonomic expert systems developed in our group so far can be considered to operate at three distinct levels of patho-physiological discrimination – (a) classification of the sample or organism as "normal or abnormal" according to metabonomic criteria derived from a large database of controls (this will be a useful tool in the control of NMR spectrometer automation using sequential flow injection NMR spectroscopy), (b) classification of the target organ for toxicity and site of action within the tissue and (c) identification of the biomarkers of toxic effect and toxic mechanism classification for the compound under study. Interestingly, these levels of classification or discrimination would also apply even if data were derived from genomic or proteomic studies and similar arguments could be applied to clinical diagnostic screening procedures. Biomarker identification poses more complex problems in terms of expert system development but detailed biomarker information can already be obtained from inspection of the PC loadings.

In conclusion, there is a vast range of biochemical, toxicological and clinical chemical problems that can be addressed using metabonomics based on high resolution ^{1}H NMR spectroscopy of biomaterials. At present even simple ^{1}H NMR experiments on whole biofluids can generate substantial amounts of metabolic data that can give detailed insight into the biochemical processes in the whole organisms and the investigation of species differences in terms of toxicological biomarkers. The numbers of applications of metabonomics is bound to increase in parallel with ongoing developments in instrumentation and techniques. Other important areas accessible to metabonomic investigation include studies on biochemical consequences of genetic modification, e.g. in "knock-out animals", investigations into effects of environmental pollutants, for clinical evaluation of drug therapy and efficacy and the investigation of idiosyncratic toxicity in man. Finally, it should soon be possible to combine genomic, proteomic and metabonomic data sets into comprehensive "bionomic" systems for the holistic evaluation of perturbed in vivo function.





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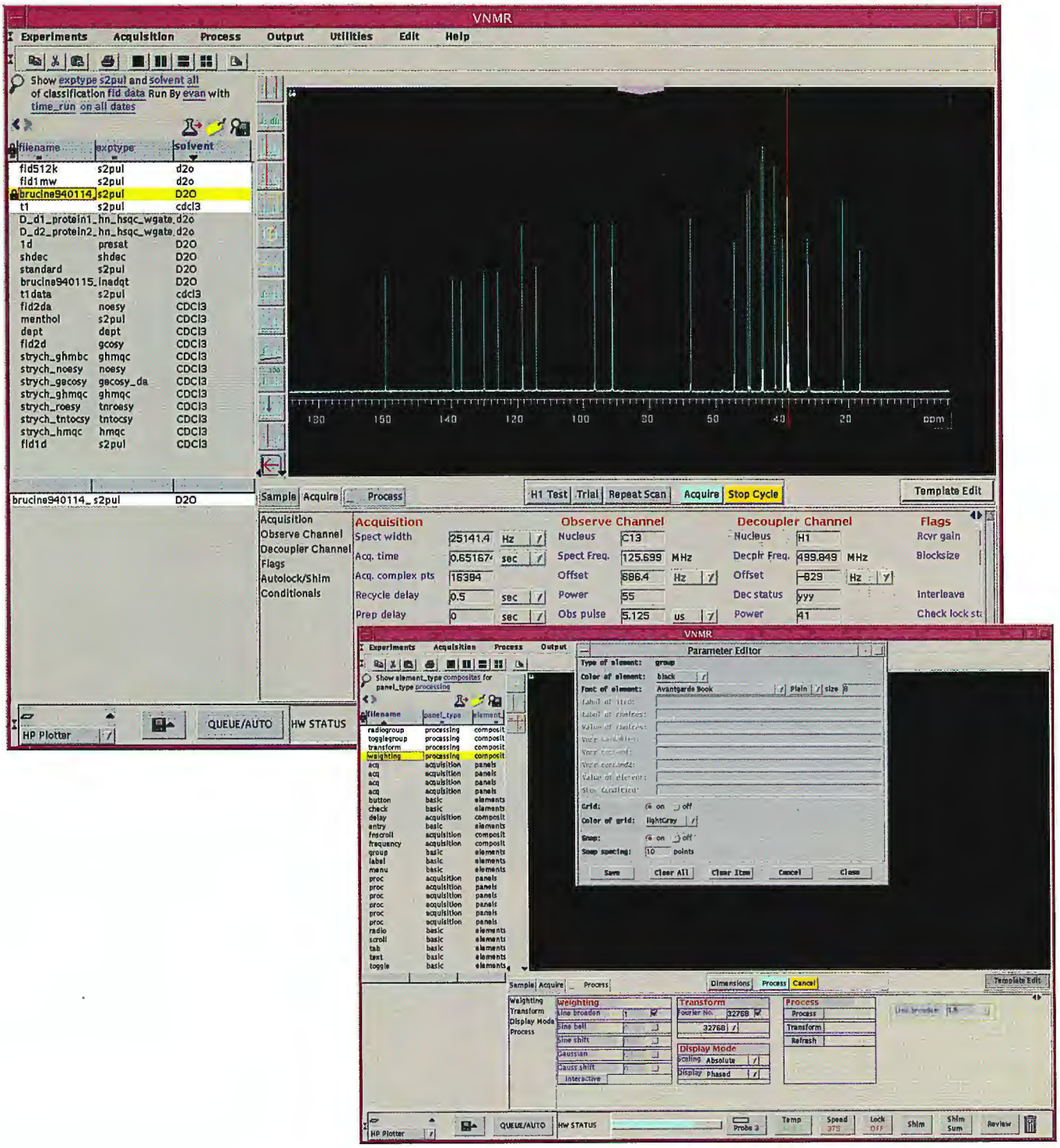

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November 15, 1999 (received 11/24/99)

Bernard L. Shapiro, Ph.D.
Editor, The NMR Newsletter
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ACCORDIONS & POLITICAL ACRONYMS:
CIGAR (Constant time Inverse-detection Gradient Accordion Rescaled)-HMBC

Dear Barry,

Continuing our dubious and short-lived tradition of politically derived acronyms for new NMR experiments, we take this opportunity to describe a further new long-range heteronuclear chemical shift correlation NMR experiment that is reliant on the principle of accordion long-range delays. Our new experiment, CIGAR-HMBC (Constant time Inverse-detection Gradient Accordion Rescaled – HMBC) is what might be considered a "third" generation accordion-optimized long-range experiment. Before describing the new experiment, some background discussion of the developments leading up to CIGAR-HMBC experiment is appropriate.

The idea of accordion-optimized long-range delays was introduced in the work of Wagner and Berger in their description of the ACCORD-HMBC experiment.[1] Our more rigorous analysis of ACCORD-HMBC[2,3] led to the observation that F_1 response "skew" is a function of the optimization range of the experiment, the number of increments of the evolution time used to digitize the second time domain, and the spectral width in the second dimension. The unfortuitous choice of parameters for an ACCORD-HMBC experiment,[3] can lead to long-range response overlap in the second frequency domain as a result of responses that can be several kilohertz in width in F_1. Conversely, the F_1 skew of long-range responses can be used as a determinant of response authenticity, as noise and other random events are not subject to proton homonuclear coupling modulation during the variable delay of the ACCORD-HMBC experiment, and are thus not subject to F_1 skew.

The second generation of accordion-optimized long-range heteronuclear shift correlation experiment, IMPEACH-MBC,[4,5] introduced a pulse sequence element dubbed a "constant time variable delay" (see shaded area of Figure 1). Despite the rather oxymoronic descriptor, this pulse sequence element has a constant time for the evolution of homonuclear coupling modulations, which hence eliminates this modulation, while at the same time serving as a variable delay for the heteronulcear couplings of interest which are to be sampled in an accordion fashion in the experiment. The first portion of the constant time variable delay is an interval, D, which is split by a 180° ^{13}C pulse. The second portion of the pulse sequence element is a variable delay, vd, analogous to that in the ACCORD-HMBC experiment. F_1 modulation in ACCORD-HMBC experiments arises as a function of homonuclear ^{1}H frequency modulation during the variable delay, vd, which serves as a pseudo-evolution period for this process.[2,3] By keeping the overall delay duration constant, ^{1}H frequency modulation, obviously, can be suppressed.[4,5] To allow the sampling of a range of potential heteronuclear long-range couplings, however, the duration of time interval during which this process is sampled must be of variable duration. To achieve these seemingly conflicting objectives, the constant time variable delay first maintains the overall time constant to suppress ^{1}H frequency modulation. As the duration of vd is decremented with successive increments of the evolution time, t_1, the overall duration of the delay, D, is incremented by the same amount. To sample heteronuclear couplings using a variable duration delay, the 180° ^{13}C pulse at D/2 decouples these process at D. Hence, heteronuclear couplings evolve only during the portion of the delay represented by vd. In this fashion, as the duration of vd is successively decremented in successive increments of the evolution time, t_1, the sampling of a range of long-range couplings is facilitated in a manner analogous to the ACCORD-HMBC experiment.[4,5]

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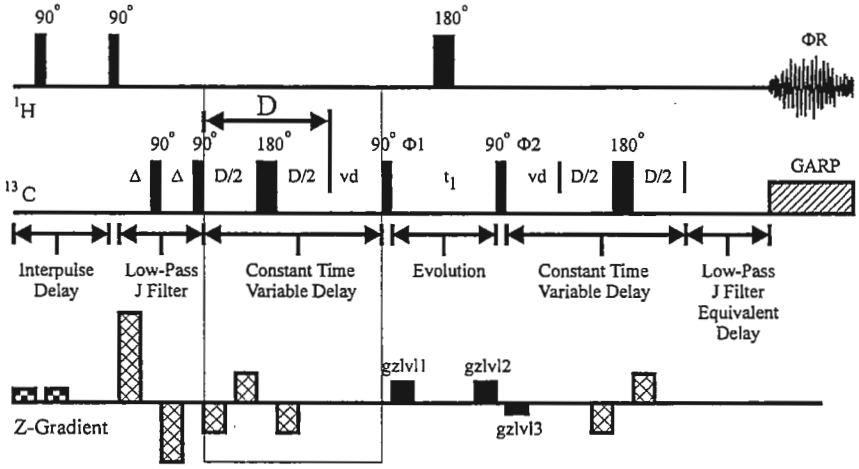


Figure 1. IMPEACH-MBC pulse sequence.[5] The constant duration of the constant time variable delay (highlighted region prior to evolution and identical unhighlighted component following evolution) suppressess F_1 modulation characteristic of the ACCORD-HMBC experiment. Heteronuclear long-range couplings are allowed to evolve only during the variable, vd, portion of the constant time variable delay. In this fashion, a range of long-range couplings can be sampled in the experiment despite the constant duration of the time interval. The phase of the unlabeled pulses in the sequence was held constant at 0. The cycled phases were: $\Phi_1 = 0202$; $\Phi_2 = 0202$; $\Phi_R = 0220$. Gradient ratios were 2 : 2 : 27 : -18 : -9 : 9 : -9 : 2 : 2: -1 G cm^{-1}.

The IMPEACH-MBC experiment successfully eliminated the problem of potential long-range response overlap inherent to the ACCORD-HMBC experiment. With IMPEACH-MBC, it was possible to digitize as dictated by resolution requirements of the sample being studied in the second frequency domain rather than having to resort to high digitization in the second time/frequency domain a means of partially ameliorating the problems of response modulation. The down side of the IMPEACH-MBC experiment, unfortunately, was to rob the investigator of the first real means of response authentication for weak responses.[5] The CIGAR-HMBC experiment we now describe was fashioned to allow the reintroduction of a user-determined amount of F_1 response modulation for use as a determinant of response authenticity.

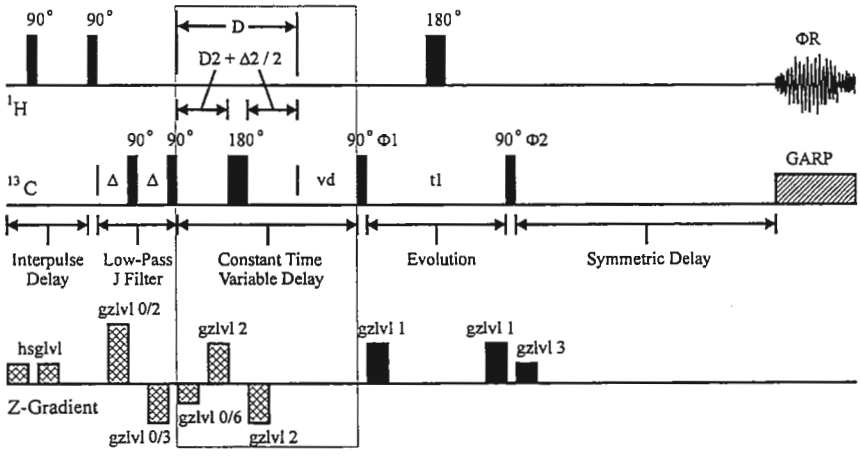
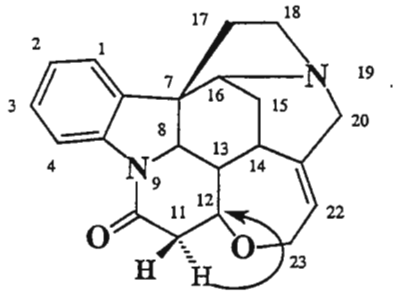


Figure 2. CIGAR-HMBC pulse sequence.[6] The modified constant duration of the constant time variable delay (highlighted region prior to evolution) allows the introduction of user-defined F_1 modulation characteristics like those inherent to long-range responses in the ACCORD-HMBC experiment. Heteronuclear long-range couplings are allowed to evolve only during the variable, vd, portion of the constant time variable delay as in the IMPEACH-MBC experiment.[4,5] The delay, D, which is halved to flankthe 180° ^{13}C pulse at D/2 in the constant time variable delay is first manipulated by incrementation as in the IMPEACH-MBC experiment (*i.e.* by $(\tau_{max} - \tau_{min})/ni$ where ni = the number of increments of the evolution time, t_1). The duration of D is further manipulated by the addition of the term$\Delta 2$, where $\Delta 2$ is defined as $(J_{scale} - 1)t_1$. The term J_{scale} is the user-selected scaling parameter and t_1 is the evolution time as usual. The phase of the unlabeled pulses in the sequence was held constant at 0. Phases of the other pulses are as follows: $\Phi_1 = 0202$; $\Phi_2 = 0022$; $\Phi_R = 0220$.

The CIGAR-HMBC pulse sequence is shown above in Figure 2. The fundamental difference between CIGAR-HMBC and the predecessor IMPEACH-MBC experiment is in the manipulation of the delays during the constant time variable delay. As will be noted by examining the highlighted segment of the figure, the delays flanking the 180° ^{13}C pulse during the constant time variable delay are no longer the simple D/2 delay used in the IMPEACH-MBC experiment.[4,5] The D/2 segments of the delay are still present, however, and are incremented by $(\tau_{max} - \tau_{min})/ni$ (where ni = the number of increments of the evolution time, t_1) as in the IMPEACH-MBC experiment in concert with the decrementation of the variable portion of the delay, vd. The delays flanking the 180° ^{13}C pulse are further manipulated to reintroduce user-defined F_1 modulation by incrementing the delay by a further$\Delta 2/2$, where $\Delta 2$ is defined as $(J_{scale} - 1)t_1$. This additional incrementation reintroduces a pseudo-evolution time of user-determined extent for proton homonuclear coupling modulation analogous to that which is present in an uncontrolled (by the user) fashion in the ACCORD-HMBC experiment.[3]

There are three possible choices for the parameter J_{scale} in a CIGAR-HMBC experiment. First, by setting $J_{scale} = 0$, the overall duration of $D/2+\Delta 2/2$ is decremented by the $\Delta 2/2$ term. Choosing this value of J_{scale} provides an experiment with a constant overall duration, which completely eliminates the small homonuclear coupling modulation inherent to both HMBC/GHMBC and IMPEACH-MBC. In this sense, when $J_{scale} = 0$, the CIGAR-HMBC experiment performs in a fashion analogous to the CT-HMBC experiments described by Furihata and Seto.[7] This choice affords the highest possible F_1 resolution. Next, setting $J_{scale} = 1$ gives an experimental result that is identical to that obtained with the IMPEACH-MBC experiment. The F_1 modulation inherent to the ACCORD-HMBC experiment is suppressed, the homonuclear modulation which arises from the incrementation of the evolution period, t_1, is unaffected. Finally, and most interesting, are the results which are obtained when the CIGAR-HMBC experiment is performed with $J_{scale} > 1$. In this case, as described above, user-determined F_1 skew is reintroduced as a characteristic of the long-range correlation responses that can serve as a determinant of resopnse authenticity. Spectral segments extracted from five spectra (ACCORD-HMBC, CIGAR-HMBC with $J_{scale} = 0$, 5, and 10, and IMPEACH-MBC) are shown, and discussed comparatively in Figure 3.



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The ability to control F_1 skew by the user-selected paramter J_{scale} increases the utility of the CIGAR-HMBC experiment considerably relative to the IMPEACH-MBC experiment. In particular, the ability to introduce a controlled amount of F_1 skew into the spectrum returns to the investigator a powerful means of long-range response authentication. Response authentication can be extremely useful in the case of weak, long-range responses, e.g. $^4J_{CH}$ correlations which are frequently observed in accordion-optimized long-range experiments when broad excitation ranges are employed. Further, relative to the ACCORD-HMBC experiment, the CIGAR-HMBC also has the advantage of F_1 response skew being independent of the number of increments selected to digitize the second frequency domain, as shown in Figure 3.

Figure 4 shows a comparison of the results obtained from a series of 6-10 Hz accordion-optimized experiments, which include a CIGAR-HMBC ($J_{scale} = 2$) spectrum (Panel A), an IMPEACH-MBC spectrum (Panel B), and an ACCORD-HMBC (Panel C). Responses contained in the three experiments are largely similar although there are some differences, as well as differences in response intensity from one experiment to the next which are still under investigatrion.

In conclusion, we are of the opinion that new accordion-optimized long-range heteronuclear chemical shift correlation experiments have considerable potential to advance our ability to characterize complex structures of natural products, drugs and their impurities, degradation products, and metabolites, as well as other clases of molecules.

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Varian, Inc.

References

1) R. Wagner and S. Berger, *Magn. Reson. Chem.*, **36**, S44 (1998).
2) G. E. Martin, C. E. Hadden, R. C. Crouch, and V. V. Krishnamurthy, *The NMR Newsletter*, **No. 487**, pp. 11-13 (1999).
3) G. E. Martin, C. E. Hadden, R. C. Crouch, and V. V. Krishnamurthy, *Magn. Reson. Chem.*, **37**, 517-529 (1999).
4) G.E. Martin, C.E. Hadden, and V.V. Krishnamurthy, *The NMR Newsletter*, **No. 488**, pp. 11-14 (1999).
5) C. E. Hadden, G. E. Martin, and V. V. Krishnamurthy, *J. Magn. Reson.*, **140**, 274-280 (1999)
6) C.E. Hadden, G.E. Martin, and V.V. Krishnamurthy, *Magn. Reson. Chem.*, **37**, in press (1999).
7) K. Furihata and H. Seto, *Tetrahedron*, **37**, 8901 (1996).

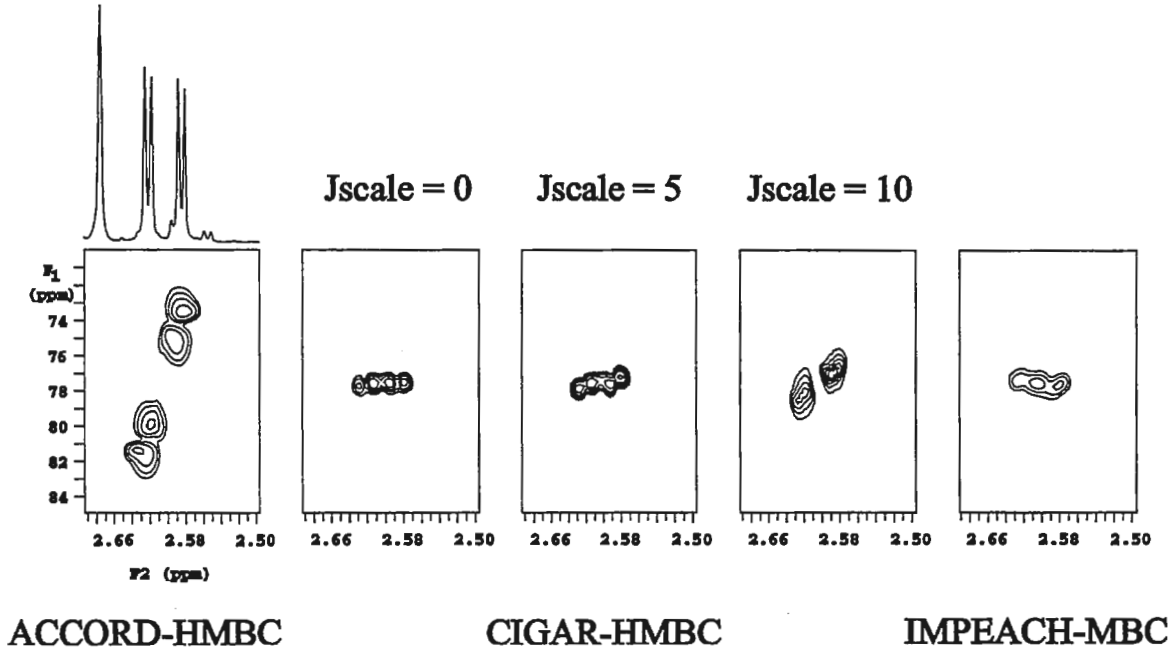

Figure 3. Comparison of accordion-optimized long-range heteronuclear shift correlation data. The five panels above show the H11α/C12 long-range correlation response of strychnine (**1**). All five of the experiments were identically optimized from 3 to 16 Hz. All experiments were acquired as 96 x 2048 points with 32 transients accumulated per t_1 increment. Data were processed identically, by linear predicting from 96 to a total of 384 points in the second frequency domain followed by zero-filling prior to Fourier transform to afford final data matrices comprised of 512 x 2048 points in F_1 and F_2, respectively. The full proton and carbon chemical shift ranges of strychnine were included; spectral widths were 3944 and 16967 Hz in F_2 and F_1, respectively. Data were acquired using a 19 mg sample of strychnine dissolved in 150 μL $CDCl_3$ (CIL) in a sealed 3 mm NMR tube (Wilmad). The experiments were performed using a Varian *INOVA* three channel spectrometer operating at 499.75 MHz for ^1H observation and equipped with a Nalorac 3 mm MIDTG-500-3 gradient inverse triple resonance NMR probe. The extent of the F_1 "skew" shown in the left panel is typical of an ACCORD-HMBC spectrum digitized using only 96 increments of the evolution time in the second frequency domain.[3] The three panels of CIGAR-HMBC data were recorded, from left to right, using J_{scale} = 0, 5, and 10, respectively. As can be seen from the data shown, the extent of F_1 skew is a function of the J_{scale} parameter and is not dependent on the number of increments of the evolution time used to digitize the second frequency domain as is the ACCORD-HMBC experiment.[6] A CIGAR-HMBC experiment accumulated with J_{scale} = 1 gives a result identical to the results of the IMPEACH-MBC spectrum shown in the right panel.[5]

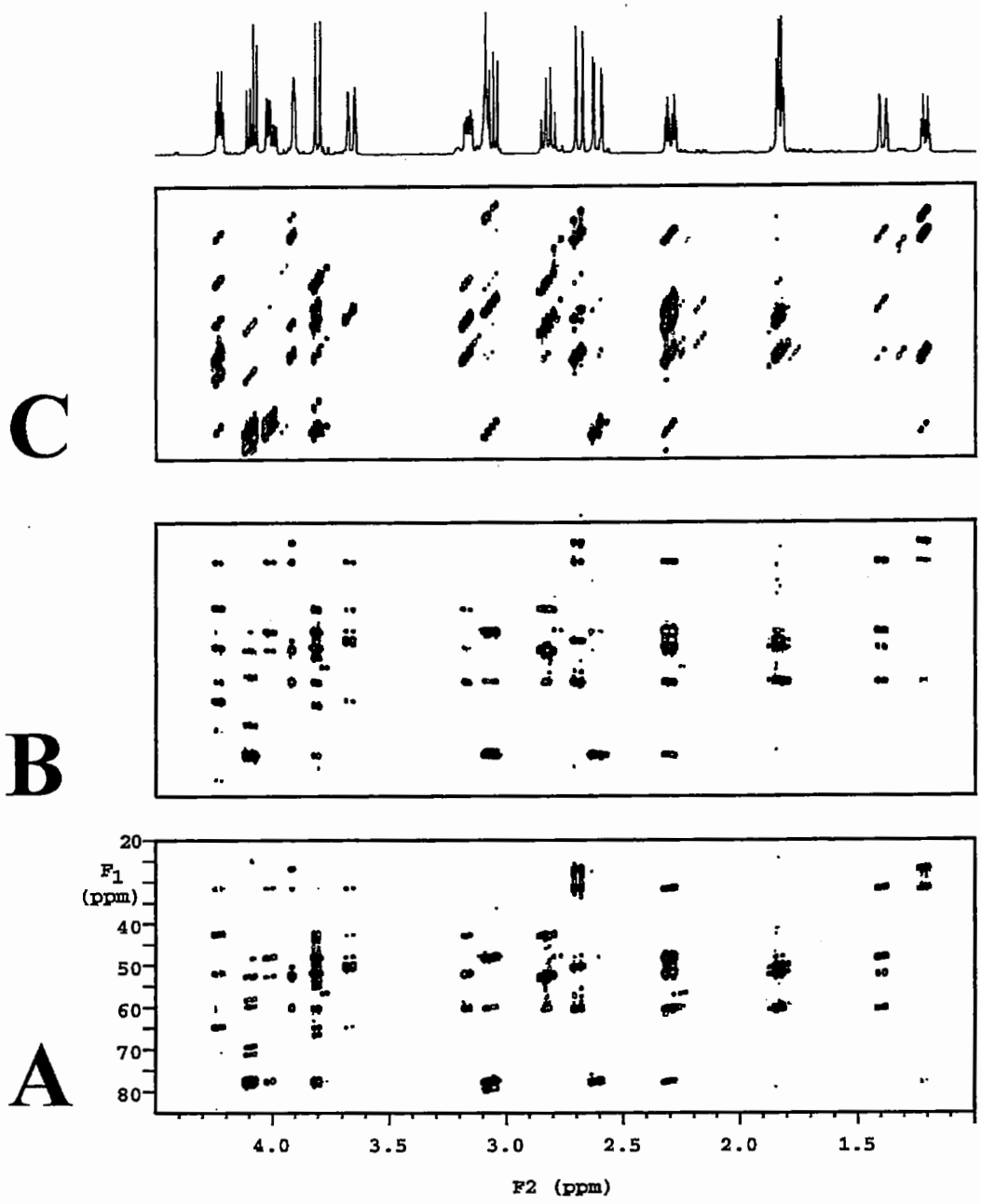

Figure 4. Comparison spectra of the aliphatic region of a 19 mg sample of strychnine dissolved in 150 μL $CDCl_3$. All of the spectra were acquired as 96 x 2048 point files with 32 transients accumulated/t_1 increment. The data were uniformly linear predicted to 384 points in the second frequency domain and zero-filled to afford a final data matrix consisting of 512 x 2048 points. All of the experiments were optimized from 6 to 10 Hz. Spectral widths were 3944 and 16967 Hz in F_2 and F_1, respectively. The CIGAR-HMBC spectrum is shown in panel A, with J_{scale} = 2 for this experiment. The F_1 skew of long-range responses is controlled by the user-selectable parameter J_{scale}. The reintroduction of modest, user-controlled F_1 skew provides an unequivocal means of long-range response authentication as noise and other responses not modulated by proton homonuclear coupling cannot exhibit F_1 skew. The IMPEACH-MBC data are presented in Panel B. F_1 skew is suppressed, with only homonuclear coupling modulation equivalent to what would be observed in a GHMBC experiment observed. The ACCORD-HMBC spectrum is shown in Panel C, in which the extent of F_1 skew is a function of the number of increments of the evolution time, the spectral width in the second dimension, and the optimization range.

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

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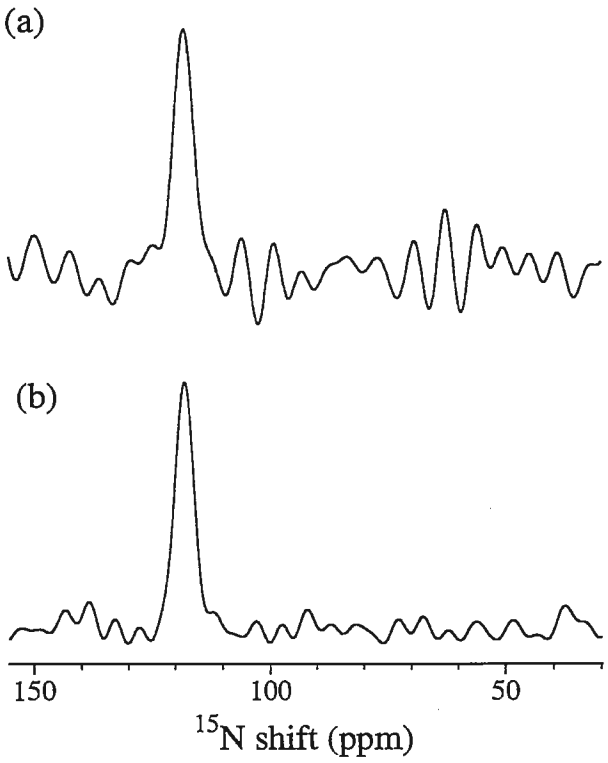
November 11, 1999

Indirect detection of ^{15}N NMR in solids under high-speed magic angle spinning

Dear Barry:

Indirect detection of ^{15}N and ^{13}C NMR signals from biopolymers via ^{1}H signals is used almost universally in liquid state NMR. Indirect detection is not generally used in solid state NMR because the ^{1}H linewidths are so broad that the sensitivity of indirect detection is worse than that of direct detection. However, high-speed magic angle spinning can reduce ^{1}H linewidths to the point where indirect detection may become the more sensitive approach. The ^{15}N spectra to the right show higher signal-to-noise in the indirectly-detected spectrum (b) than in the directly-detected spectrum (a) by a factor of 3.2. These spectra were obtained at 17.6 Tesla, with a MAS frequency of 28.3 kHz, using commercial Bruker equipment. The sample is a doubly ^{15}N-labeled, 17-residue, synthetic, helical peptide called MB(i+4)EK [see H.W. Long and R. Tycko, *J. Am. Chem. Soc.* **120**, 7039-7048, (1998)] in lyophilized form. Polarization transfers between ^{1}H and ^{15}N spins were accomplished with amplitude-modulated cross-polarization.



Additional details are contained in a paper that we have submitted for publication.

Sincerely yours,

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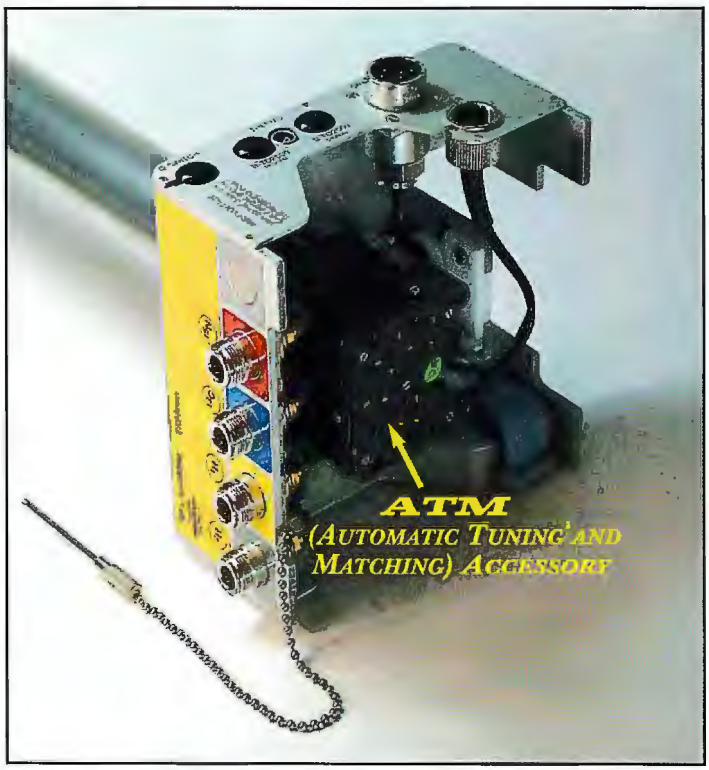

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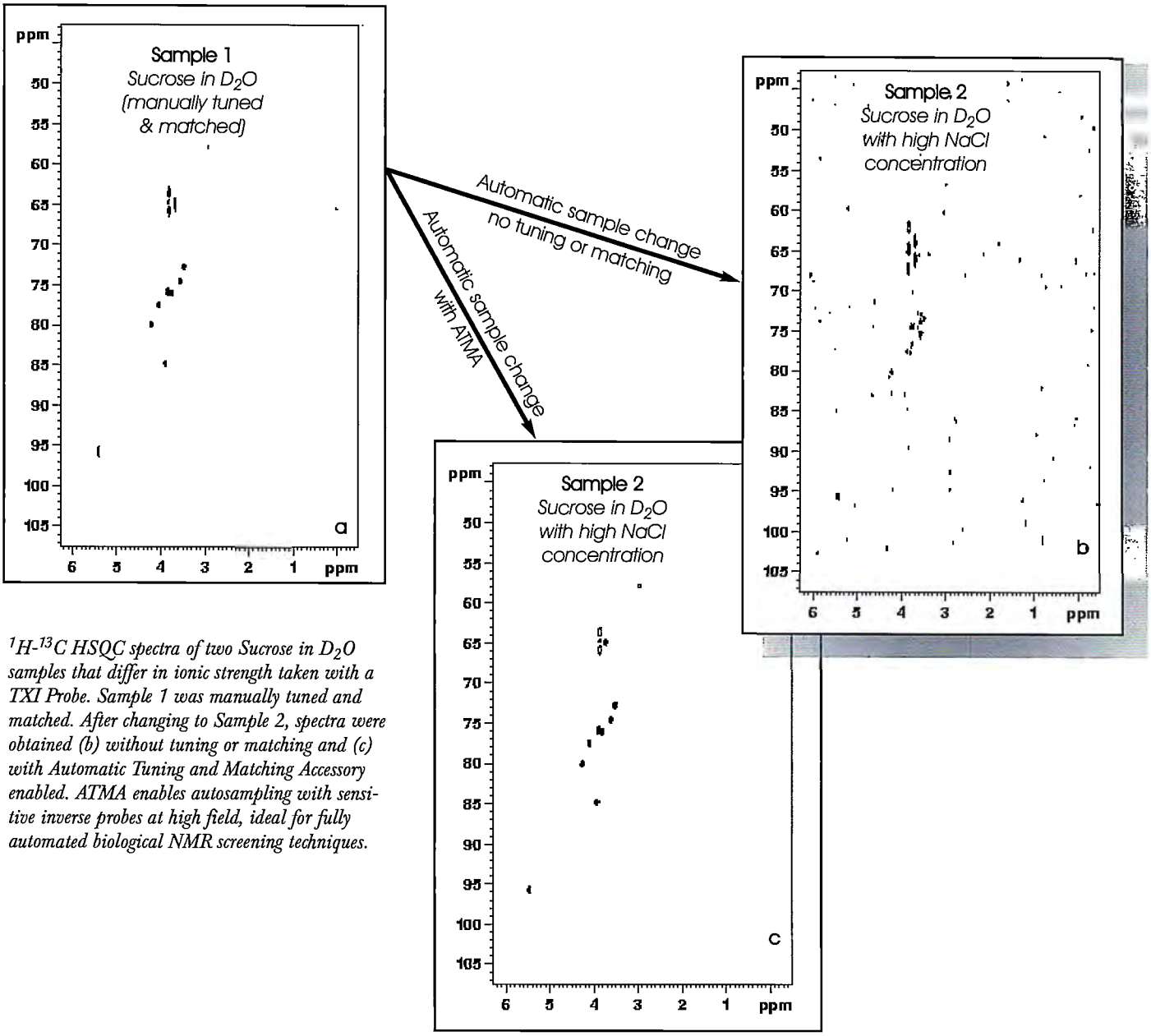

^1H-^{13}C HSQC spectra of two Sucrose in D_2O samples that differ in ionic strength taken with a TXI Probe. Sample 1 was manually tuned and matched. After changing to Sample 2, spectra were obtained (b) without tuning or matching and (c) with Automatic Tuning and Matching Accessory enabled. ATMA enables autosampling with sensitive inverse probes at high field, ideal for fully automated biological NMR screening techniques.

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DEPARTMENT OF THE NAVY
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November 16, 1999
(received 11/22/99)
IN REPLY REFER TO:

Proton Wideline NMR of Hydrates on a Bruker DMX-500, and Avance Fast Digitizers

Dear Barry:

In the last year I have begun studying the clathrate compounds of methane with water ice, which represent a potentially enormous energy resource (natural gas) buried in the sediments of the ocean floors. While carrying out wideline proton NMR experiments on our new Bruker Avance DMX-300 and DMX-500 spectrometers, I encountered a number of puzzling anomalies in the appearance of the spectra acquired with our fast digitizer (FADC), e.g. the spectral appearance changed depending upon the dwell time! I was using a simple single pulse acquisition rather than any echoes, since the dead-time for high-frequency proton NMR can be 2us or less, and hence one can expect relatively distortion-free spectra.

After considerable experimentation, and a helpful site visit by Bruker senior applications chemist Tony Bielecki (who provided the sample below), the problem was tracked down to a bug in the operation of the fast digitizer. Although according to the manual the digitizer is supposed to begin acquisition at a specified dwell interval at a precise time in the pulse program (e.g. when the "adc" command is given), it instead appears to be "throwing away" or dropping about 8 data points. Thus, the acquisition does not actually begin until about 8 "Bruker dwell intervals" after it is supposed to (this is true for dwell intervals of up to at least 1us), accounting for the puzzling behavior seen, which is especially noticeable at the longer dwell times.

It is not clear whether this is a software or a hardware problem, but it occurs with both our FADC digitizers (ECL23, for "engineering change level"). There are two work-arounds, either use the minimum dwell time of 0.1us and lose the first 0.8us of acquisition, or begin the acquisition with the "adc" command before the pulse, and left-shift the data (taking into account the missed points).

Incidentally, another bug appears with the use of the explicit data acquisition statements ":x". It seems that the software restricts the minimum dwell time to 0.5us instead of the expected 0.1us.

As an example of the quality of data that can be obtained, and the importance of proton chemical shift anisotropy in high-field spectra of hydrates, the 500 MHz spectrum of $Ba(ClO_3)_2{\cdot}H_2O$ below at left was obtained with single 0.6us (30°) pulses, a dwell of 0.1us, minimal line-broadening, acquisition beginning before the pulse, left-shifts corresponding to a 2us dead-time after the pulse, and a linear frequency-dependent phase correction that compensates for the 2.3us between the midpoint of the pulse and the first data point used. The receiver phase has to be set to zero after the pulse in the program; because of the long phase-settling times of the DDS for the broadband FCU ("frequency control unit") channels (ca. 2.6 us), the narrowband FCU2 channel was used to obtain phase settling within 2us. Note that the "wiggles" in the spectrum should be there, and that it would be incorrect to try to phase them out. Instead, an expanded region can be spline baseline-corrected, leading to the attractive but asymmetric spectrum below at right.

The source of this sizeable asymmetry is the chemical shift anisotropy of the flipping water molecules. We calculated the theoretical spectrum for a two spin system using John Waugh's ever-

useful program ANTIOPE. The proton-proton distance was set to 1.62Å, and the principal components of the chemical shift tensor in this compound obtained by Gang Wu et al. (*J. Am. Chem. Soc.*, **120,** 13187, 1998) from proton MAS experiments of deuterated hydrates were used (13.7ppm, 6.7ppm, -1.3ppm). A linebroadening of 3 kHz was applied, and the resultant theoretical spectrum at the bottom agrees quite well with the experimental spectrum. (The two minor bumps in the latter are of unknown origin). For high fields, the single pulse method is preferable to using a solid-echo, especially since one would have to apply a π refocussing pulse in the latter case to compensate for chemical shift anisotropy effects. These results are being written up for a short publication.

Barium chlorate monohydrate appears to be a useful test sample for wide-line proton NMR. I hope to report on the methane hydrate work at a later date. Also, next month I will be starting a sabbatical year at the NIH in Rob Tycko's lab; my new number will be (301) 402-4687.

Sincerely yours,

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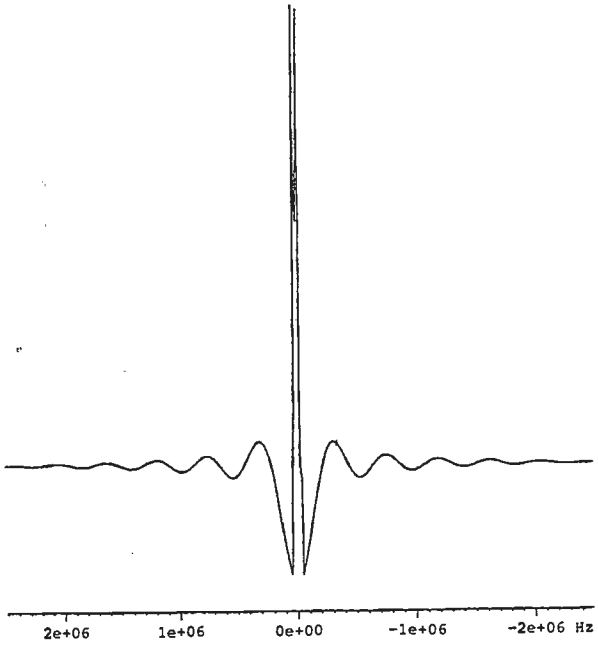
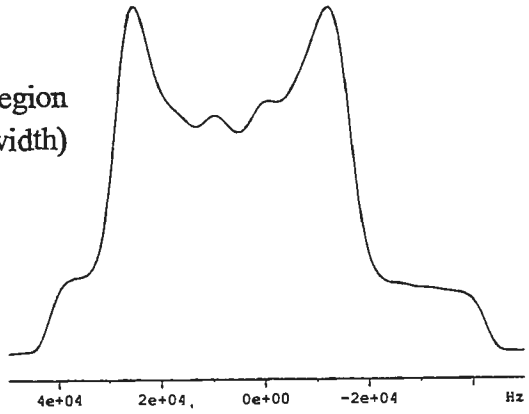
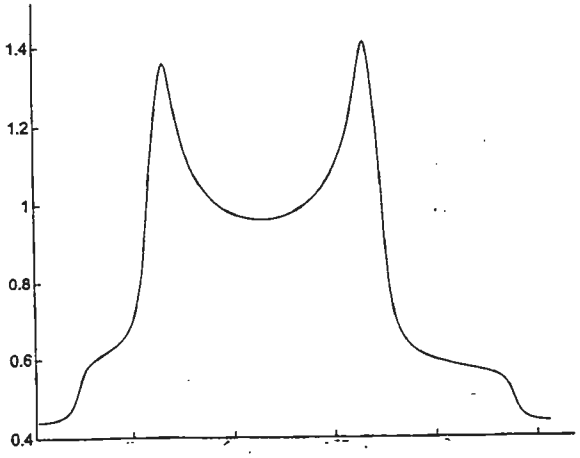


^{1}H single-pulse NMR of $Ba(ClO_3)_2 \cdot H_2O$
(5 MHz spectral width, see text)

expanded region
(100 kHz width)





theoretical simulation
including csa (see text)

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The NMR Newsletter
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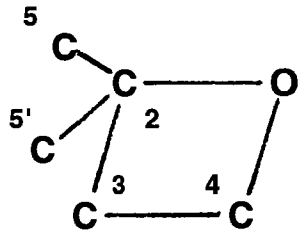
October 20, 1999
(received 10/26/99)

NMR of fluorooxetanes

Dear Barry:

I would like to report on some work we have done on the ^{19}F and ^{13}C spectral properties of a series of oxetane compounds with fluorine atoms on the ring or on side chains or both. These are molecules with the skeleton structure shown, where carbons 5 and 5' represent CF_3, CF_2Cl, or $CFCl_2$ groups:



The compounds were prepared by the students of Paul Tarrant, when he was still active, using photo-chemical addition of carbonyl compounds to an olefin. Including early results at lower fields as well as data from our Varian 500, we have spectral parameters for more than fifty samples, and it has been possible to assign the direction of addition and the stereochemistry for almost all of them.

We have made several observations of significant or unusual behavior of NMR parameters. First, there is the apparent role of oxygen in modifying the magnitude of the two-bond coupling involving fluorine on the carbon adjacent to the oxygen, for which there is some precedent in other types of fluorinated ethers. For geminal ^{19}F-^{19}F coupling at ring position 3, *J* values range from 199 to 221 Hz, but at position 4, adjacent to the oxygen, they are much smaller, 83 to 96 Hz. Geminal ^{19}F-^{1}H coupling, however, is larger at position 4, 61-69 Hz, than at position 3, where the range is 48-53 Hz. No substantial difference was found between cis and trans isomers.

There are also some interesting aspects of ^{13}C-^{1}H coupling. Two-bond coupling constants are fairly small, 2 to 9 Hz, and seem to follow no simple pattern. However one-bond coupling values show some regularities, about 200 Hz if the hydrogen is part of a CHF group, 190 Hz if it is part of a CHCl group, and 160 Hz if it is part of a CH_2 group, all at position 4. If the hydrogen is at position 3, across the ring from oxygen, these numbers drop to about 175, 165, and 145 Hz, respectively. Again, the role of oxygen in influencing the magnitude of spin-spin interactions is evident.

In some instances, spin-spin coupling acts as if it were transmitted by overlap of orbitals of unshared electrons, rather than through bonds, although the possibility of drastic perturbation of bonding orbitals by unsymmetrical substitution cannot be absolutely ruled out. Cross-ring F-F coupling is found in some cases only between groups on the same side of the molecular plane, and in others the value is much larger for that relation. Spin-spin interaction between CF_3 groups and fluorine atoms on neighboring carbons is always much larger for the cis than for the trans orientation.

There is a striking asymmetry in ^{13}C-^{19}F coupling in several molecules. In a molecule with two CF_3's on 2, CFH at 3, and CH_2 at 4, carbon 3 shows quartet splitting, with coupling only to one CF_3 group rather than to both. It appears that the fluorine atom on 3 acts as a relay, but that the hydrogen atom is less able to transmit spin-spin interactions. There is a similar asymmetry if position 3 is a CFH unit or if there is attached to carbon 3 a CF_3 group, which should have even greater overlap of unshared electron orbitals with the CF_3 group at position 2.

The chemical-shift behavior of fluorines in CF_2Cl groups varies with the structure. The two fluorines in a group are sometimes magnetically equivalent, whereas in other circumstances, they are inequivalent, leading to an AB NMR spectral pattern, with a coupling constant between the fluorines of about 185 Hz. The surprising results are those for a molecule with a chlorine on carbon 4, where both CF_2Cl resonances are of the AB form, contrasted with the case where the chlorine substituent is on the closer carbon 3, but in which only one of the CF_2Cl groups shows an AB pattern. For all the oxetanes which have a $CFCl_2$ group paired with a CF_2Cl group on carbon 2, the resonance of the CF_2Cl is an AB pattern. While a detailed explanation of these results is not yet possible, they must arise from a conformational preference of the chlorine-containing groups to minimize the steric interactions of the chlorine atoms.

One of our objectives was to determine whether symmetrically substituted oxetanes are really planar or whether they are rapidly intercoverting between two limiting puckered forms, but conclusions about this will require as a minimum further studies on relaxation times. There are many hints in the present results that the minimum-energy conformation of the oxetane ring is nonplanar in many of the molecules which are unsymmetrically substituted. This seems to be particularly applicable when three or more trihalomethyl groups are present on the ring. In this circumstance, some of the NMR parameters, especially the shifts of the fluorines in the methyl groups themselves, do not behave in an additive way.

A paper containing the details of these results will appear in several months in the *Journal of Fluorine Chemistry*.

Yours,

Wallace

Wallace S. Brey
Professor of Chemistry

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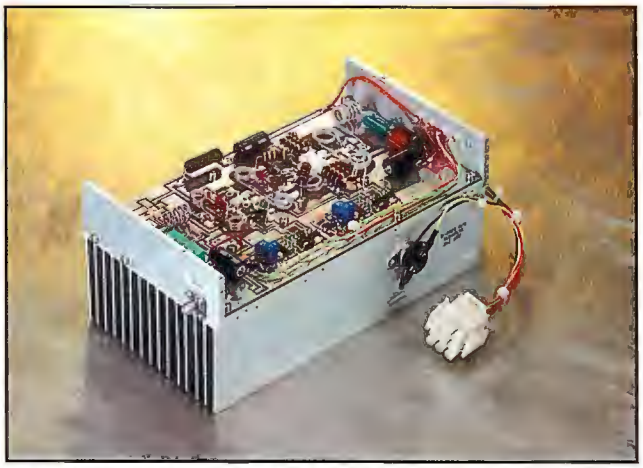

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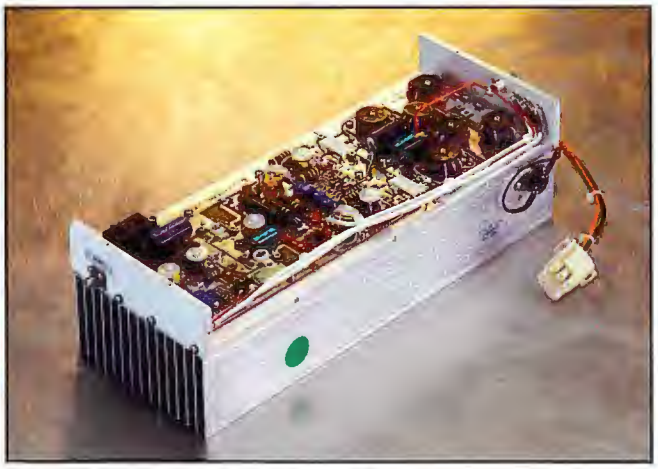

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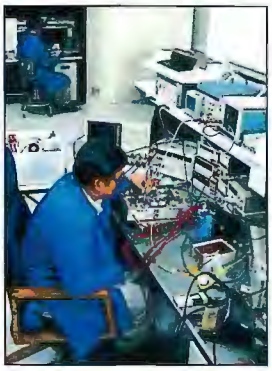

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Dr. Bernard L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Measurement of $^3J_{H'_3\text{-}P}$ with WATERGATE

November 16, 1999
(received 11/17/99)

Dear Dr. Shapiro,

The $^3J_{H'_3\text{-}P}$ coupling constants, through the Karplus relations, provide important information about the backbone torsional angles in DNA. Usually, the coupling constants are measured directly from the *J* splittings in the H or P dimensions. Partial overlap of the *J* splittings can cause severe problems, especially for small couplings.

Recently, Clore et al. (1) demonstrated that the $^3J_{H'_3\text{-}P}$ couplings can also be measured from relative intensities between a *J*-decoupled and -coupled cross peaks (Fig. 1A). During a constant-time period 2T, the *J* coupling is active and the cross peak ($H'_3 \rightarrow t_1 \rightarrow H'_2 \rightarrow t_2$), with a starting coherence of H'_3, evolving during t_1, transferring coherence to H'_2, and detected during t_2, is attenuated by a factor of $\cos(2\pi JT)$. The relative intensities is then $I_{att}/I_{ref} = \cos(2\pi JT)$, from which the coupling constant, $J = [\cos^{-1}(I_{att}/I_{ref})]/2\pi T$, can be easily derived. This scheme avoids the overlap problem and is therefore valuable for measuring small coupling constants.

Sometimes, H'_3 protons have resonances very close to the strong water peak, making the assignments and intensity measurements problematic. Any water suppression techniques applied prior to the pulse sequence will reduce the H'_3 signal dramatically. However, the WATERGATE pulse (2), applied at the end of the sequence (Fig. 1B), reduces the water signal along with some nearby H'_3 peaks after t_1 evolution. As a

result, the water diagonal peak and the cross peak, $H'_2 \rightarrow t_1 \rightarrow H'_3 \rightarrow t_2$ that are not affected by $^3J_{H'_3\text{-}P}$ and give no information, are reduced. But the cross peaks of $H'_3 \rightarrow t_1 \rightarrow H'_2 \rightarrow t_2$ are not affected by the WATERGATE since after t_1 evolution the frequencies of the cross peaks have jumped from H'_3 to H'_2 frequencies (H'_2 frequencies are far from the water peak, as shown in Fig. 2).

Best regards,

Xianbin Yang Shanmin Zhang Bruce A. Luxon David. G. Gorenstein

1. G. M. Clore, E. C. Murphy, A. M. Gronenborn, and A. Bax, *J. Magn. Reson.* **134**, 164 (1998).

2. M. Piotto, V. Saudek, and V. Sklenář, *J. Biomol. NMR* **2**, 661 (1992).

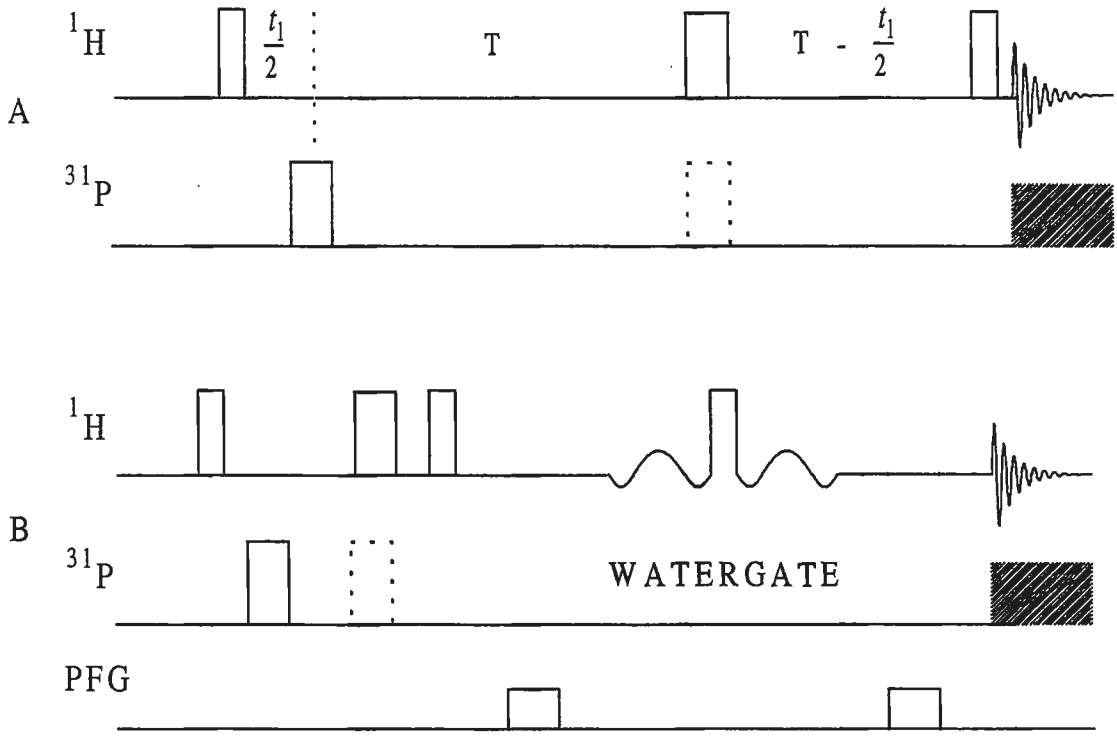

Fig. 1. Constant-time ^{1}H - ^{1}H (^{31}P) COSY pulse sequence for measuring $J_{H\text{-}P}$ coupling constants without (A) and with (B) WATERGATE.

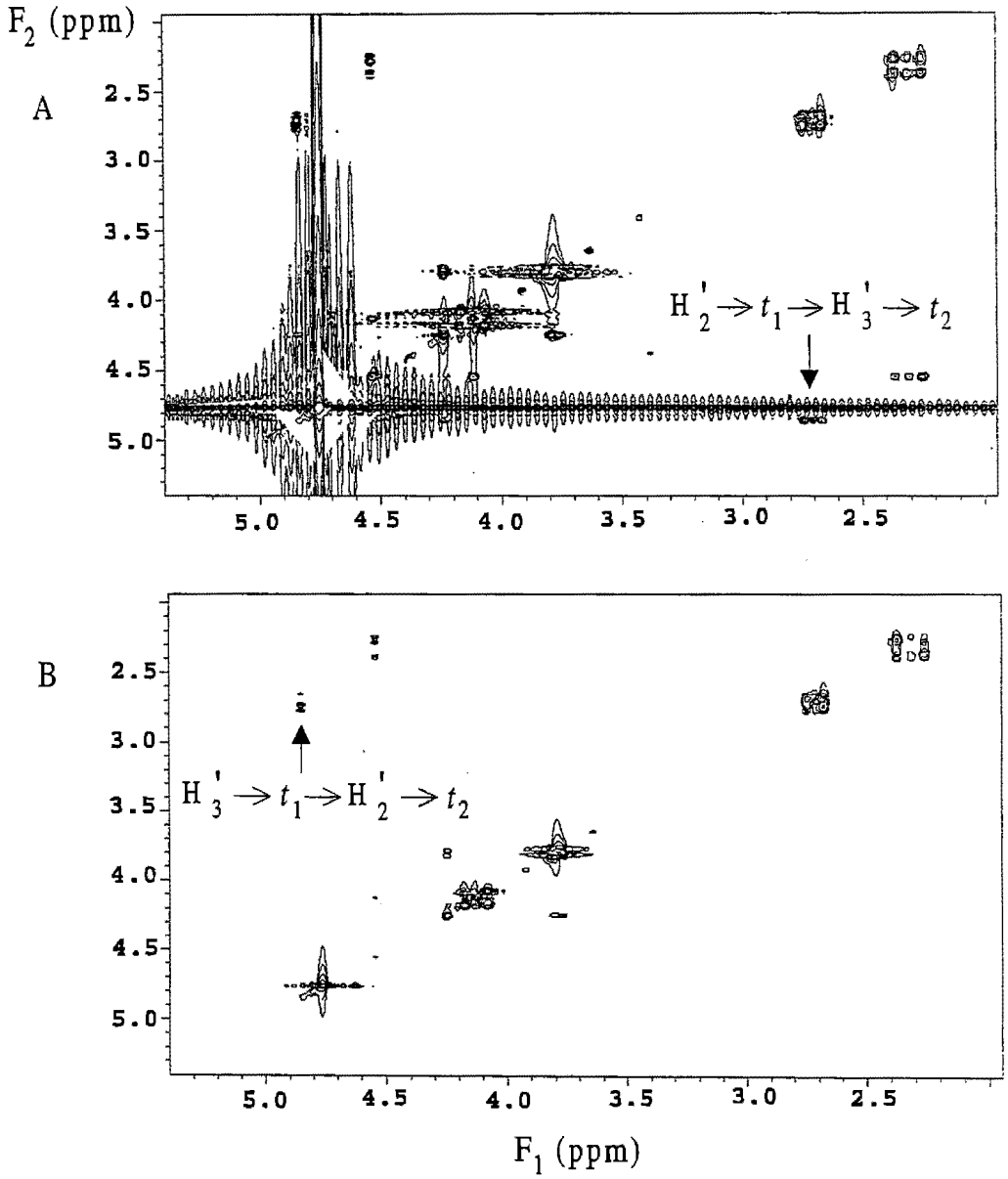

Fig. 2. Constant-time ^{1}H-^{1}H (^{31}P) COSY spectra of 4.0 mg of d(GC) in 600 ml D_2O recorded on a Varian Unity Plus 600 MHz instrument without (A) and with (B) WATERGATE.

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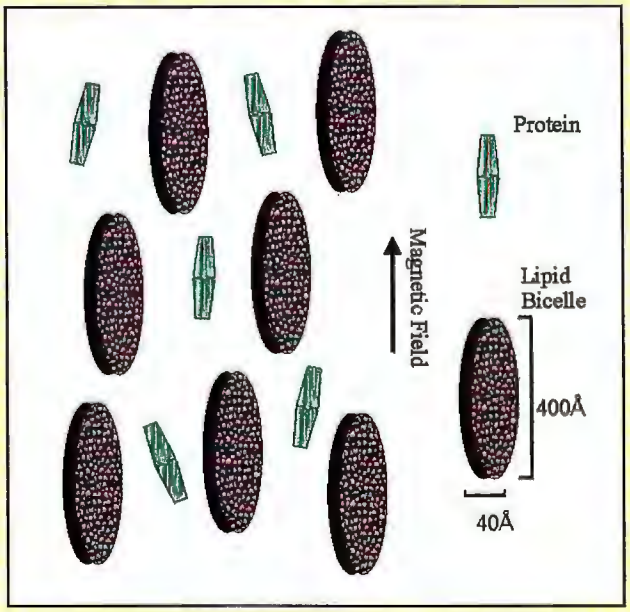

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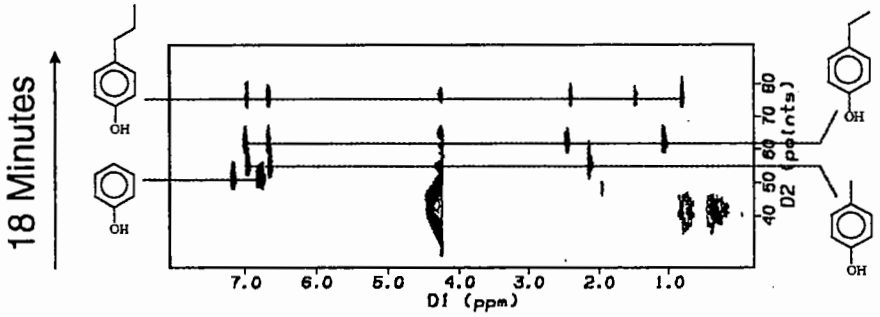
October 21, 1999 (received 11/1/99)

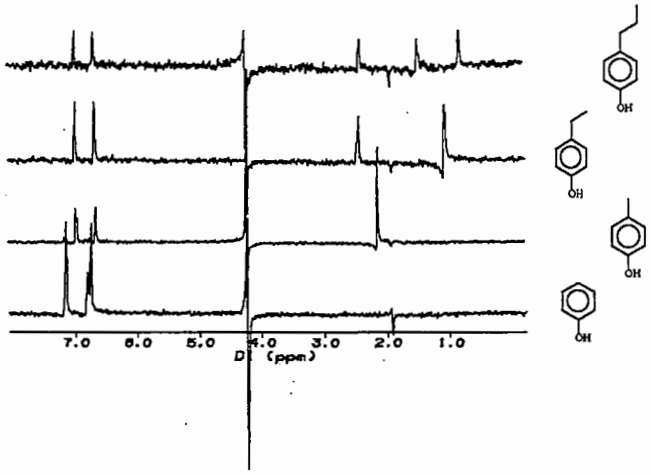
Sub-Microgram Microbore HPLC-NMR.

Dear Barry,

We have been exploring applications of our new micro-coil LC-NMR probe and have been extremely pleased. To demonstrate the capabilities of this probe, we have provided a NMR-chromatogram of a mixture of four phenols. The injected volume contained only 2 micrograms of each compound. Horizontal slices through the data show NMR spectra of the individual components. Thus we were able to demonstrate both our ability to detect small amounts of material, and our ability to separate a mixture using chromatography.



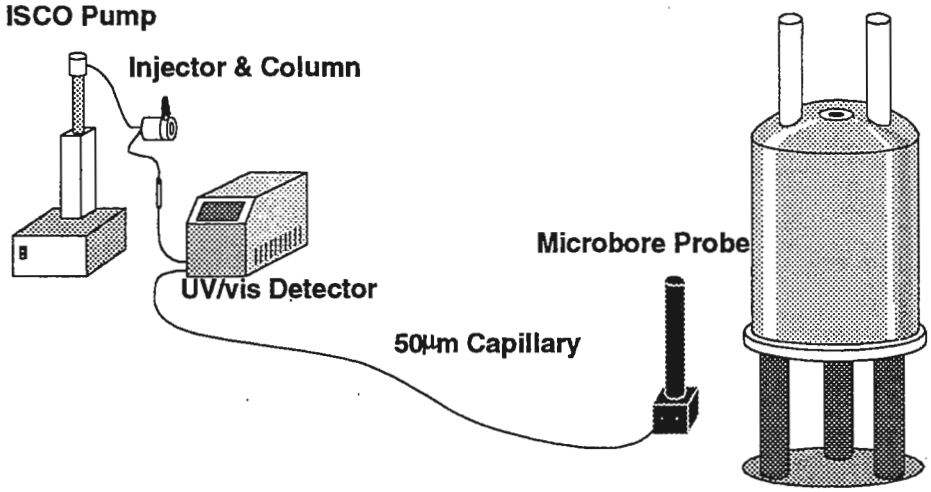
This probe is a prototype we purchased from Magnetic Resonance Microsensors Corporation (MRM)[1]. The probes they produce have extremely small detection volumes (100-1500 nanoliters), yet maintain a high filling factor. They achieve this by winding the probe coil directly around a fused silica capillary. Magnetic field inhomogeneity from the coils is avoided by immersing the coil in a fluid that has the same magnetic susceptibility as the winding[2]. We were able to obtain 2 Hz line widths at half height. The high sensitivity of the coil results in extremely short pulse widths.



The diagram below shows our experimental apparatus. An ISCO syringe pump, capable of producing 10,000 psi, delivers the mobile phase. Samples are introduced into the system using a Valco injector valve just ahead of a C18 reverse-phase column. Immediately after the column, a Linear UV/vis detector provides optical detection of materials as they elute. Three meters of 50 micron fused silica capillary leads from the detector to the NMR probe in our 500 MHz magnet. The volume of the capillary is low, and there is little delay (~30s) between the UV/vis detector and the NMR probe. The detection volume of our NMR probe is 250 nanoliters.

The slow flow rates (~10 µl/min) make the use of deuterated solvents affordable. This eliminates the need for complex solvent suppression, allowing more time for acquiring data. We removed most of the residual solvent signal as well as any probe background by subtracting a blank FID from every other FID. This crude method costs us a factor of 1.414 in signal to noise and leaves behind telltale subtraction errors. This could easily be fixed in post-acquisition processing or some simple solvent suppression.

We have been able to detect compounds at amounts as small as 200 nanograms under continuous flow conditions. We were able to do this with compounds that eluted quickly. Those that had longer retention times were broadened, and thus harder to detect. We can significantly increase the limit of detection by stopping the flow with the compound in the probe and acquiring longer. 2-D spectroscopy is easy when the flow is stopped, allowing more information to be obtained about each compound that elutes. A standard trick in chromatography to improve detection of compounds with longer retention times is to deliver a mobile phase gradient. We have a second pump that we are planning to use for this purpose. We recognize that the chromatography shown here is crude, however even such simple chromatography can provide a huge benefit when trying to identify components in a mixture.



In summary, we expect to put this probe to great use in cases where we are extremely sample limited, or where the sample contains several compounds. We will be able to do more interesting experiments to each compound by using stop-flow chromatography. And finally, by using mobile phase gradients, we will be able to improve the chromatography and concentrate the longer-retained compounds.

Sincerely,

Kip Shaffer

Charlie Eads

Ed Burton

(1) Magnetic Resonance Microsensors Corporation,
101 Tomaras Avenue, Savoy, IL, 61874. (217)351-4359

(2) "The Nanoliter Niche," Dean L. Olson, Michael E. Lacey, and Jonathan V. Sweedler, Analytical Chemistry News & Features, April 1, 1998, 257A-264A

SmithKline Beecham
Pharmaceuticals

November 11, 1999
(received 11/12/99)

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

Use of high throughput flow-injection NMR for characterization of combinatorial libraries

Dear Barry:

In order to characterize combinatorial libraries, we are developing high throughput NMR spectroscopy as an analytical tool. Although the 'high-throughput' interpretation of the spectra is still under works within the community, it is important to develop the appropriate methodologies for collecting NMR spectra of 'small quantities' of material available for the characterization. At present, we are using VAST (versatile automatic sample transporter) system on a Varian Unity Inova 500 MHz machine.

Our primary goal has been to minimize the sample amount and volume for NMR data collection and so far we have settled on injecting 150 ul of solution into the probe, from a stock solution of 200 ul from the wells of the microtiter plate. We have carried out a systematic study with a series of concentrations, as shown in Figure 1, and have decided on sample concentration of 750 uM (75 ug in 200 ul solvent for a compound of molecular weight 500). We collect spectra in both deuterated and protonated DMSO, depending on the quality of the spectra required by the chemists, and an example of the spectra collected in both solvents is shown in Figure 2.

709 Swedeland Road, PO Box 1539, King of Prussia, PA 19406-0939. Telephone (610) 270 4800. Fax (610) 270 7777.

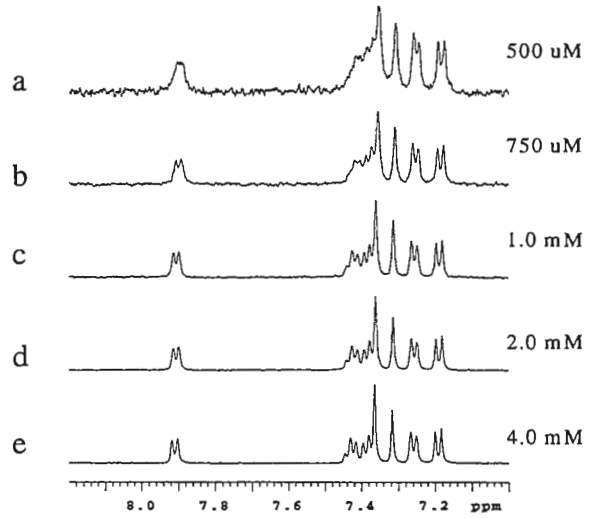


Figure 1. The aromatic region of a compound of molecular weight 600 with different concentrations, as labeled in the figure. The number of scans was set to 128.

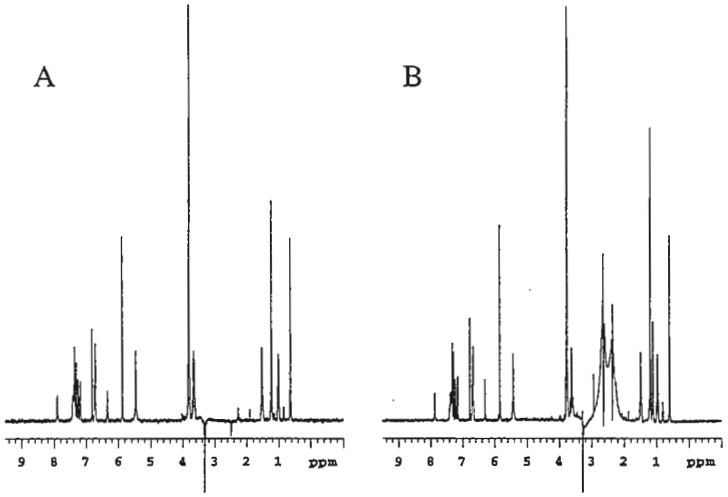


Figure 2. 1D spectra of a 750 uM sample, as in Figure 1, (A) in DMSO-D6 and (B) in DMSO-H6. The solvent peaks were suppressed with a WET sequence. The number of scans was set to 128.

With best regards,

Bing Wang

Susanta K. Sarkar

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Duke University
Duke Nuclear Magnetic Resonance Spectroscopy Center

Leonard D. Spicer, Director
Anthony A. Ribeiro, Manager

919 684 4327
919 613 8887

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

November 5, 1999
(received 11/12/99)

Re: NMR Analyses of Purified, Intact Lipid As from Mutant Gram-Negative Bacteria

Dear Barry,

We are continuing collaborative efforts with Prof. C. Raetz (Duke, Biochemistry) to study bacterial glycolipids like Lipid A from the outer membrane of Gram-negative bacteria. These acylated, phosphorylated glucosamines are heterogeneous with respect to number of acyl chains and phosphorus groups. Lipid As from mutant strains are further substituted with labile 4-amino-4-deoxy-arabinose (L-Ara4N) and phosphoethanolamine (PetN) groups. These lipids are amphipathic and often show broad NMR signals due to aggregation. The lipids are also often chemically unstable with tendencies to undergo phosphate ester hydrolysis under acid conditions or acyl ester hydrolysis under base conditions. We have found Lipid As to display remarkably sharp NMR signals and to be chemically stable for days in a CDCl3:CD3OD: D2O mixed solvent system, thus allowing the first detailed solution state homo- and heteronuclear NMR studies.

The CHCl3 (7.6 ppm) and CH2DOD (3.3 ppm) lines from the ternary CDCl3:CD3OD:D2O mixture do not usually overlap with sugar or acyl 1H resonances. The CD3OH (4.54 ppm) and HOD (4.80 ppm) solvent lines obscure the anomeric H-1' (4.6-4.7 ppm) but can be removed with a presaturation or WET sequence. The solvent system thus satisfies the criteria of lipid chemical stability, narrow and resolved lipid signals and a clear window over the regions of interest. Figure 1 shows a 2D TOCSY recorded with 90 ms mixing time for lipid IIA isolated from a *Salmonella typhimurium* mutant strain. TOCSY connectivities are clearly observed in the sugar (5.6 - 3.5 ppm) and acyl (4.2 - 0.9 ppm) regions, with prominent cross peaks from the α CH2 (2.5 ppm) to the β CHOH (4.1 ppm) of the four long acyl chains. The expansion of the COSY spectrum (Figure 2) reveals overlapped H-1 and H-1" signals from the proximal glucosamine and the L-Ara4N sugar near 5.5 ppm and a partially suppressed H-1' near 4.6 ppm.

Determination of phosphorus linkages and sequential ordering of the phosphosugars are crucial steps in establishing the covalent structure of Lipid As. Selective inverse (31P) decoupling difference spectroscopy (SIDDS) results for Lipid IIA are shown in Figure 3. Selective decoupling of the -2.62 ppm 31P signal while observing the 1H NMR spectrum and subtraction from an off-resonance control yields two diagnostic "doublets" at 5.5 ppm (H-1 and H1") and two "double-doublets" at 4.20 (H-2) and 3.70 (H-2") ppm, clearly establishing the -2.62 ppm signal as the bridging monophosphodiester between C-1 of the proximal glucosamine and the C-1" of the L-Ara4N ring in lipid IIA. Selective decoupling of the 1.56 ppm 31P signal in contrast yields a diagnostic "triplet" at 4.18 ppm (H-4') and establishes the 1.56 ppm signal as a monophosphomonoester group linked to C-4' of the distal sugar.

SIDDS results for Lipid IIIA with a PetN group are shown in Figure 3. Selective 31P decoupling of the -0.46 ppm 31P signal yields a difference spectrum with a 5.5 ppm "doublet" (H-1) and a 4.2 "double-doublet" (H-2), thus establishing the -0.46 ppm signal as a monophosphomonoester group linked to C-1 of the proximal glucosamine. Selective decoupling of the -10 and -10.3 phosphorus signals in contrast reveal individual "triplets" at 4.2 (EtN) and 4.3 (H-4') ppm, giving clear evidence that these are the phosphorus groups of the diphosphodiester that are linked respectively to the ethanolamine moiety and the C-4' of the distal glucosamine sugar. 2D H-P HMQC gives similar results but took more time. Moreover, the H-P cross peaks are disadvantageously influenced by passive and active components from both 1H-31P and 1H-1H couplings.

Previous structural work on lipid As from mutant strains appears based mainly on chemical shift comparisons of 1D 31P NMR of impure membrane fractions. While the biosynthesis of wild type Lipid A is well studied, the enzymes responsible for accumulation of polar substituents like L-Ara4N or PetN in Lipid A from mutant strains are unknown, and NMR characterizations of these phosphosugars from mutant strains are very much still in infancy.

Regards,

Tony

Anthony A. Ribeiro (A^2R)

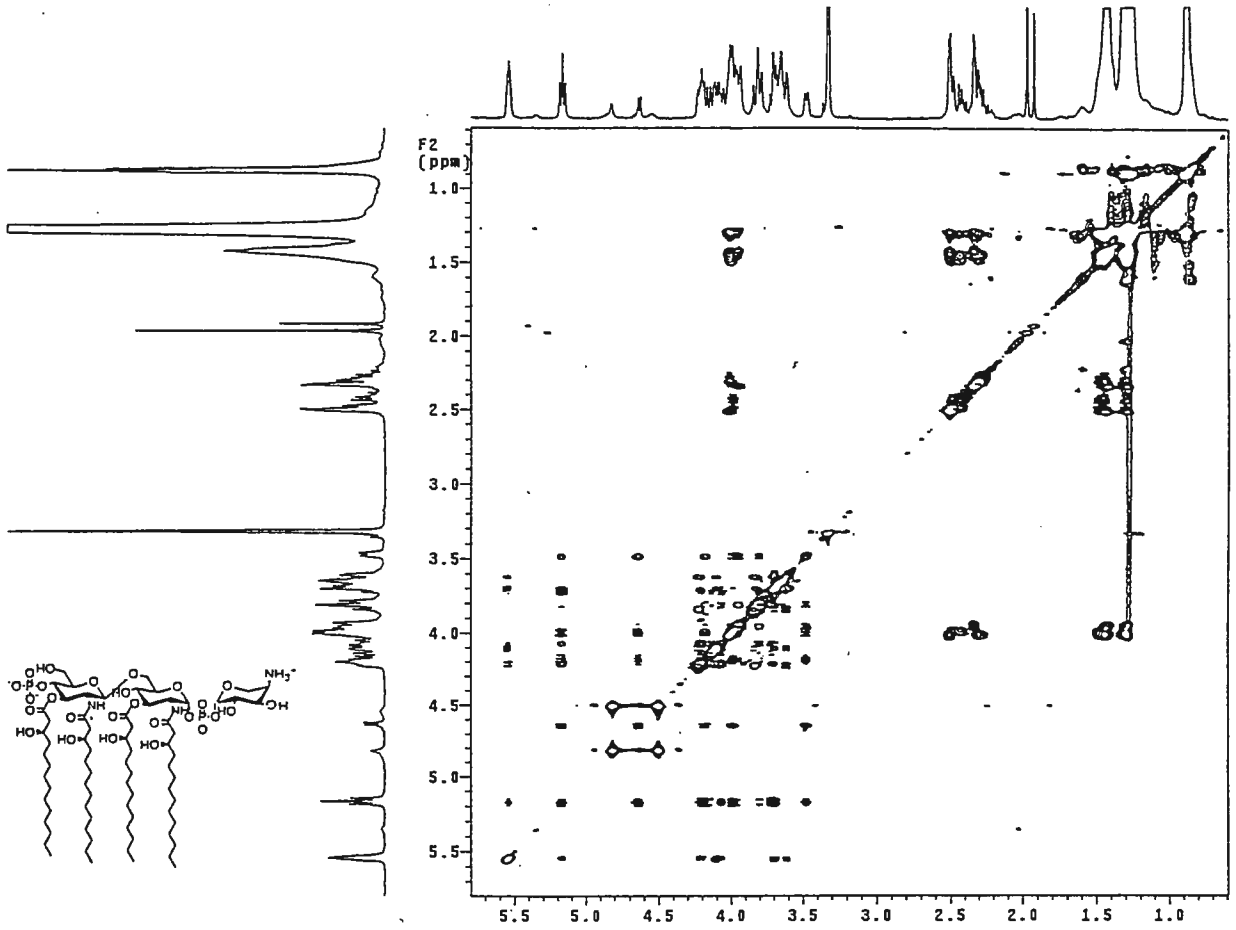

Fig. 1. 2D TOCSY of Lipid IIA.

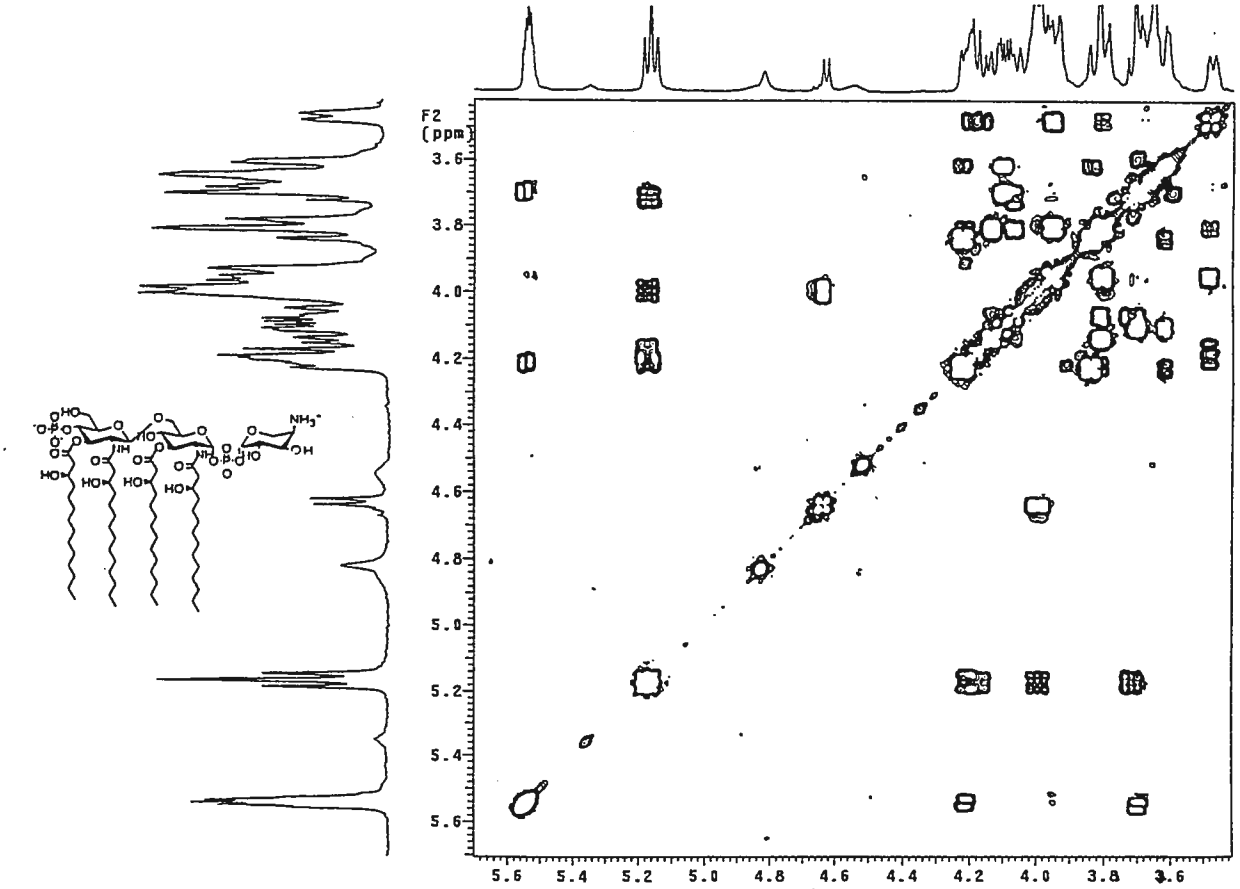

Fig. 2. 2D COSY of Lipid IIA.

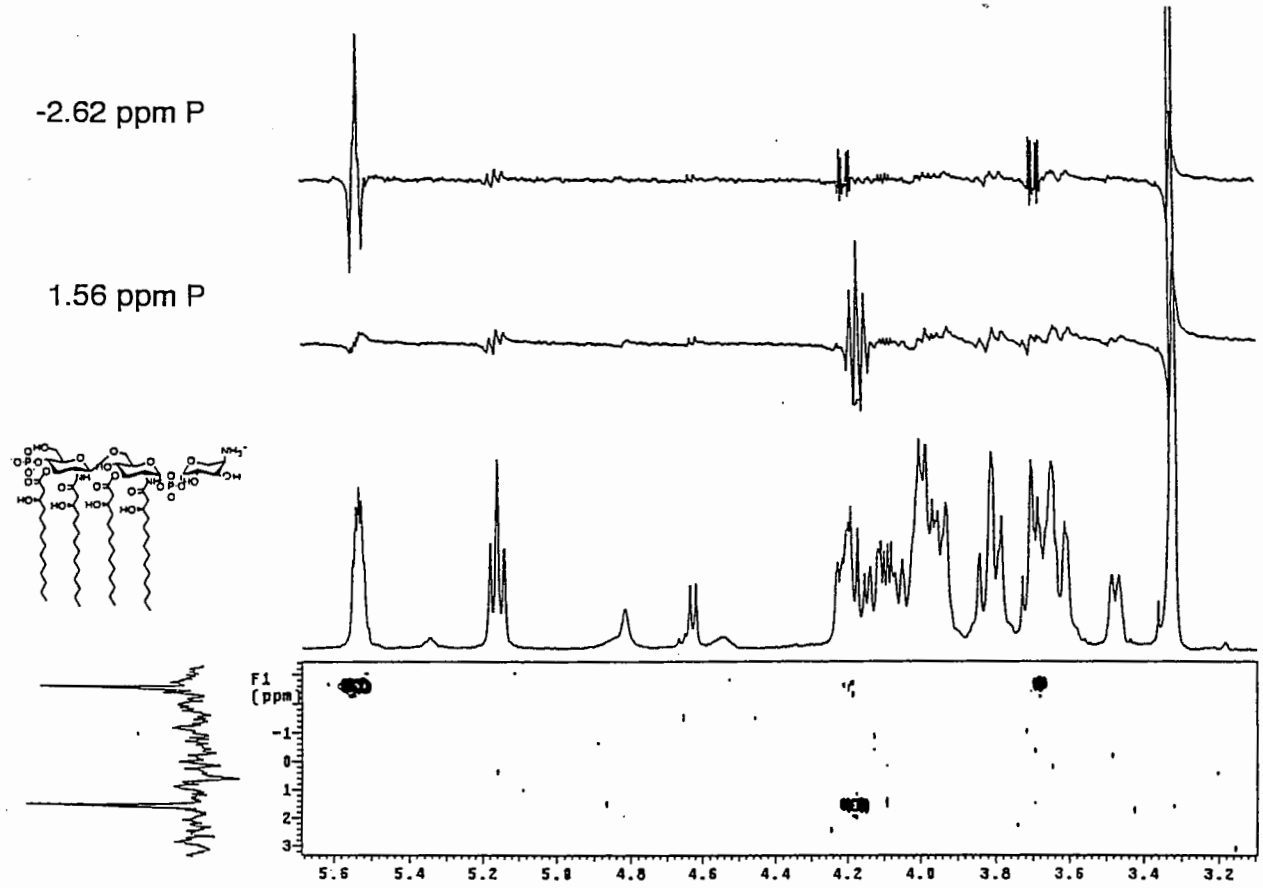

Fig. 3. 31P decouple, 1H observe 1D difference spectra and H,P 2D NMR of Lipid IIA.

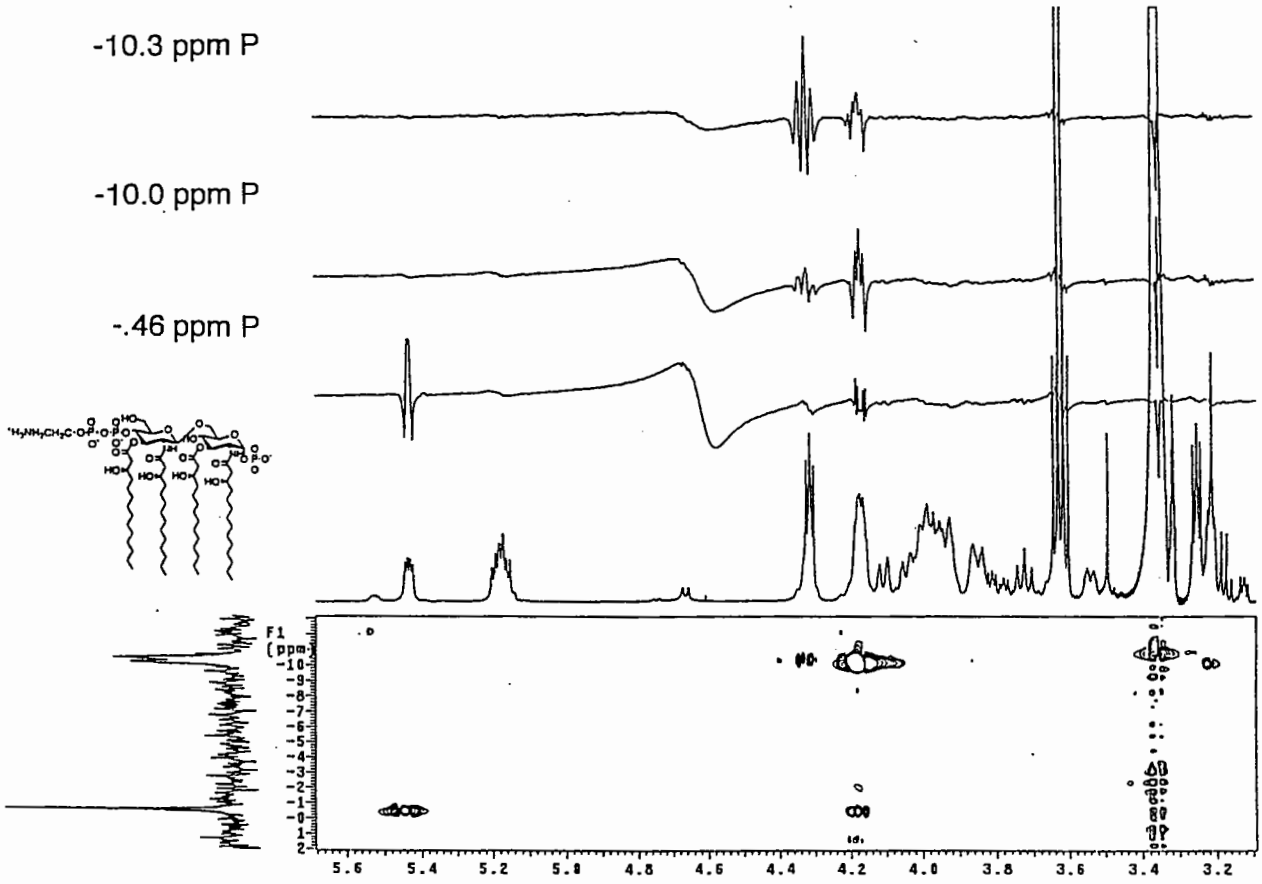

Fig. 4. 31P decouple, 1H observe 1D difference spectra and H,P 2D NMR of Lipid IIIA.

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November 19, 1999
(received 11/23/99)

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303-3410

Homonuclear Dipolar Recoupling under fast MAS by Simultaneous Frequency and Amplitude Modulation (SFAM)

Dear Dr. Shapiro,

This contribution is to credit Dr. Nagarajan Murali's a/c. As a team, we'd like to keep receiving *The NMR Newsletter*. Recently we have been using the simultaneous frequency and amplitude modulation (SFAM)[1] to recouple homonuclear dipolar interaction in presence of fast MAS. The idea behind SFAM is to introduce time dependence modulation into dipolar interaction using continuous rf irradiation to interfere with MAS such that the dipolar interaction is not averaged by MAS. In the past several years, many experimental techniques have been developed to recover dipolar interactions under MAS, so as to obtain distance constraints. A common feature of those techniques is to utilize the flip-flop term of the dipolar interaction without actively interacting with I_zS_z term. We have found the SFAM irradiation to be very efficient for homonuclear dipolar recoupling, owing to the fact that SFAM permits the time-dependence rf irradiation to modulate the *entire* homonuclear dipolar interaction (both the flip-flop and the I_zS_z terms). This is demonstrated in the figure showing the 2D ^{13}C CPMAS homonuclear correlation spectra. Here the SFAM irradiation with a maximum rf amplitude of 10 kHz was applied to the $^{13}C_\alpha$ resonance. It is clearly seen that the intensities of the cross peaks between C_α and C_β are more than three times higher in Fig.B than in Fig.A. Furthermore, little cross peaks were observed between the C_α and C_1, and the C_β and C_1 resonances because the C_1 resonance was not affected by the SFAM irradiation. This indicates that SFAM has a potential to isolate a spin pair from a coupling network leading to determine the distance between the two spins accurately.

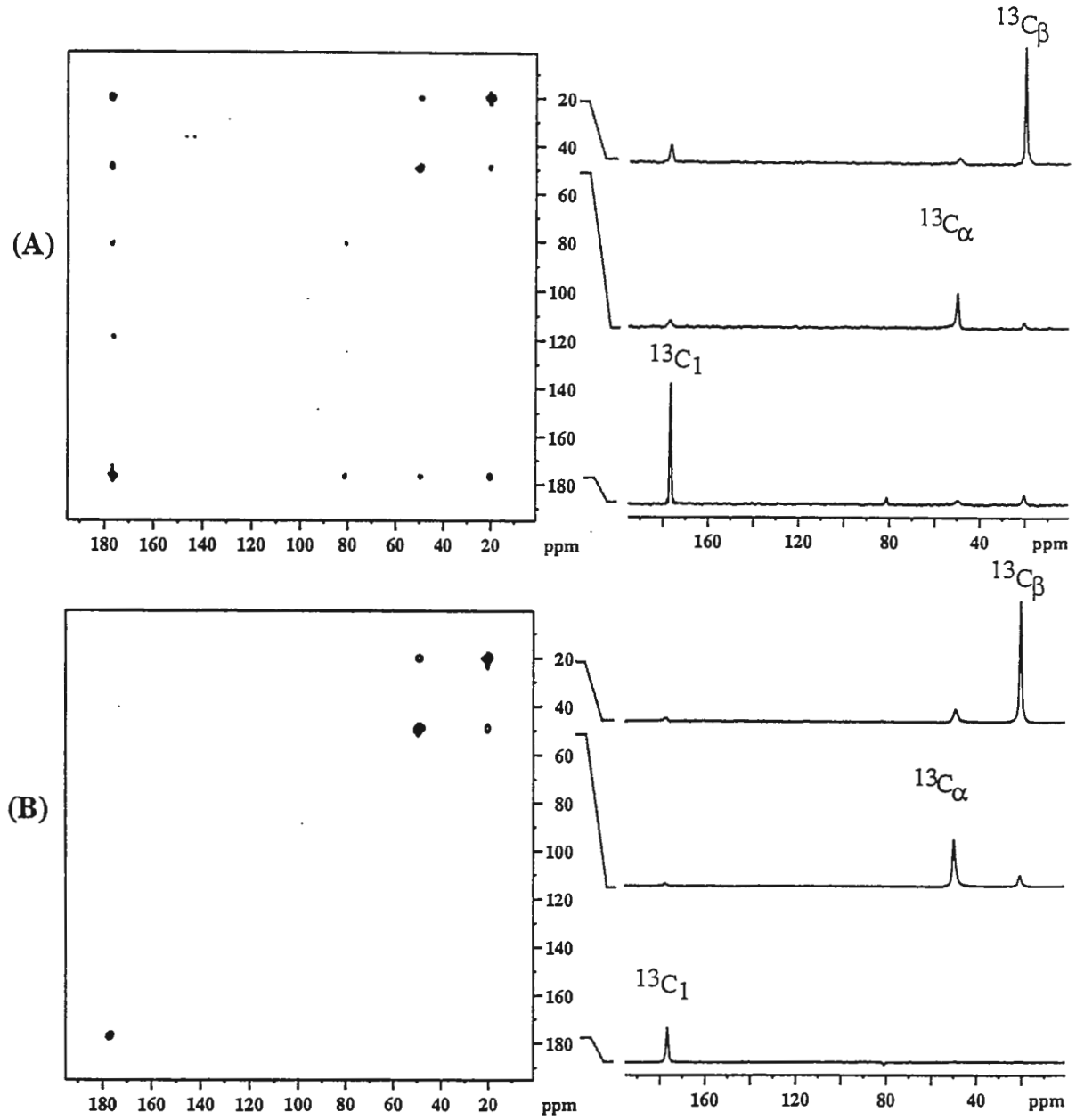

Figure. Phase sensitive 2D ^{13}C CPMAS homonuclear correlation spectra of triply labeled ^{13}C alanine recorded on a Bruker DMX 300. The sample spinning speed was adjusted to 7.2 kHz to avoid rotational resonance. The rotor-synchronized 180° pulses[2] (A) and the SFAM irradiation (B) were applied during the 20.8 ms mixing time. The slices in the right are the phase-sensitive cross sections showing relative intensities of the cross peaks.

References:
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Sincerely yours,

Riqiang Fu

Varian, Inc.
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November 23, 1999
(received 11/26/99)

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

VARIAN

Re: **Protein NMR Nanomole Quantities**

Dear Barry,

Getting enough of the labeled protein for NMR measurement is always a problem. How much material is really needed to get good quality ^{15}N HSQC correlation spectra? The probes developed for measurement of NMR Spectra in small volumes offer a possible solution.

For this purpose we used a Varian gradient HX indirect detection nanoprobe. The tube for the indirect detection gradient nanoprobe requires only a small volume of sample, in the range from 10 to 40 µl. Spinning the sample under the magic angle at moderate spinning speeds (from 2kHz to 3KHz) efficiently removes the lineshape distortion created by variations in magnetic susceptibility. As a test, we used 40 nanomoles of ^{15}N, ^{13}C labeled Ubiquitin (76 amino acids) in 40 µl in 90% H_2O/10% D_2O buffer.

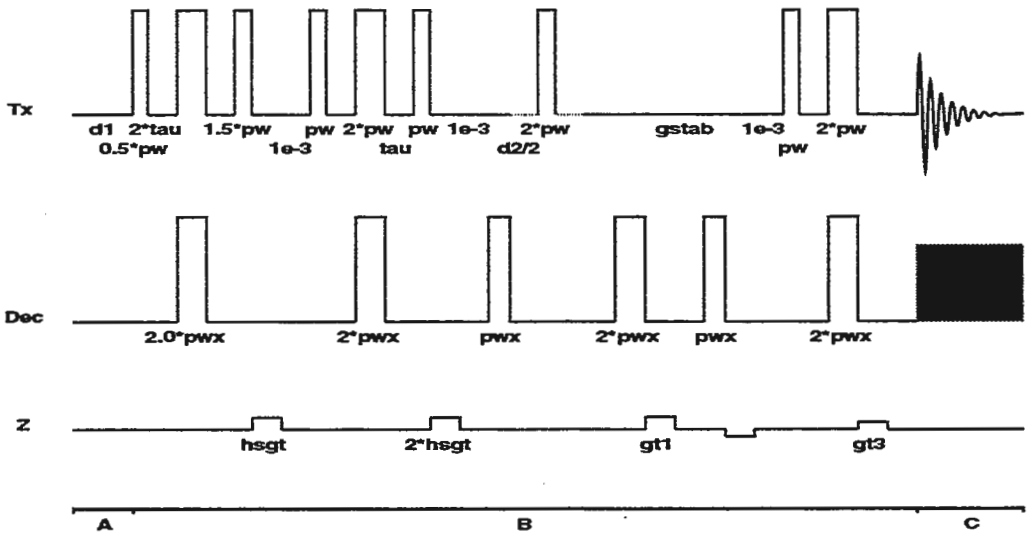
The phase-sensitive gradient-selected HSQC spectrum shown in the accompanying figure was acquired in 21 minutes. The collected data (2 X 32 increment) was linear predicted to 128 complex points in t_1 prior to 2D FT. The TANGO-gradient collection preceding the sequence serves to suppress water more effectively.

The ability to generate such a high quality ^{15}N gHSQC spectrum in such a short experimental time using only 40 nanomoles of proteins would be quite valuable for both drug discovery and protein structure research. Use of Varian's gradient nanoprobe with magic MAS gradients requires no special hardware or software.

Sincerely yours,

Igor Goljer

Dr. Igor Goljer
NMR Applications Chemist
Varian, Inc.



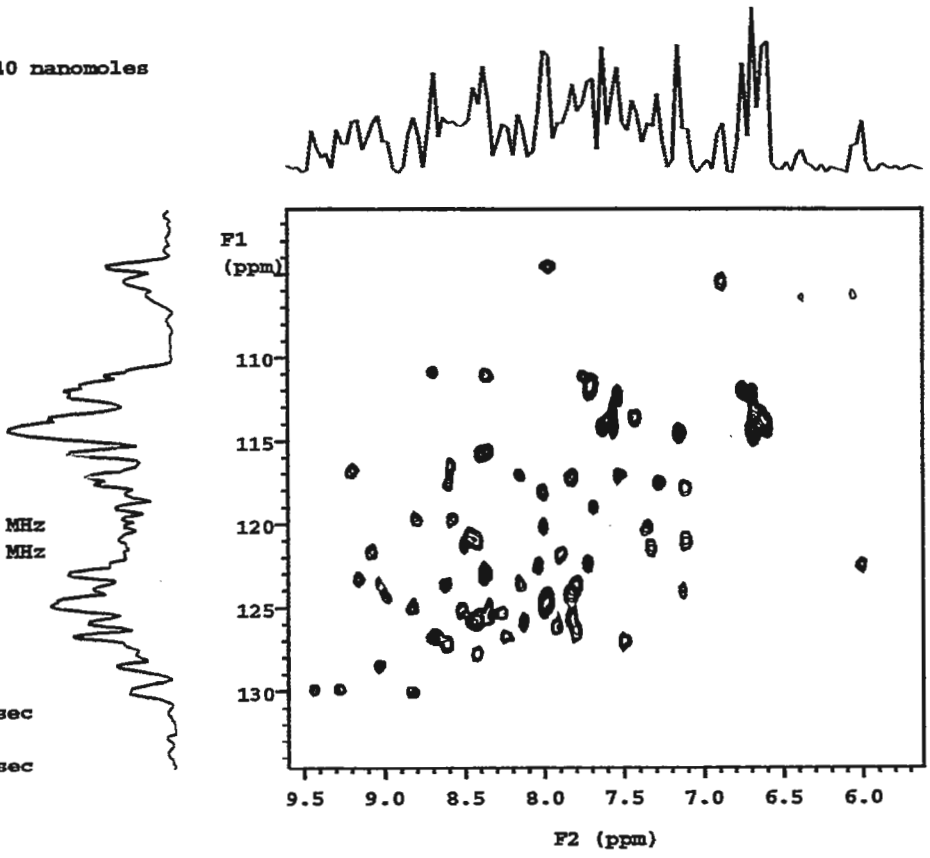
Pulse sequence gHSQCs

Doubly labeled Ubiquitin 40 nanomoles
in gHX nanoprobe 21 min
spinning speed 2600Hz

Pulse Sequence: gHSQCs

Solvent: D2O
Temp. 25.0 C / 298.1 K
File: UBIQUITIN_gHSQC
INOVA-500 "fpi500"

PULSE SEQUENCE: gHSQCs
Relax. delay 1.000 sec
Acq. time 0.128 sec
Width 8000.0 Hz
2D Width 2532.9 Hz
16 repetitions
2 x 32 increments
OBSERVE H1, 499.9452761 MHz
DECOUPLE N15, 50.6647299 MHz
Power 40 dB
on during acquisition
off during delay
GARP-1 modulated
DATA PROCESSING
Gauss apodization 0.030 sec
F1 DATA PROCESSING
Gauss apodization 0.017 sec
FT size 1024 x 1024
Total time 21 min, 5 sec



Chevron

October 25, 1999
(received 10/26/99)

Chevron Research and Technology Company
P.O. Box 1627
Richmond, CA 94802-0627
Phone (510) 242-3000
Fax (510) 242-4647

Dr. B. L. Shapiro, Editor
The NMR Newsletter

^{1}H MAS Measurements of Saturation and Relaxometry of Water and Heavy Oil in Rocks

Dear Barry:

Low resolution ^{1}H NMR relaxometry and/or diffusion measurements can be used to distinguish oil and water fractions in rocks containing low density oils because the relaxation rates and diffusivities of the oil and water are significantly different[1]. However, when core samples contain high density oils, the oil and water relaxation rates are indistinct and diffusion differences too small for straightforward saturation determination. Additionally, high density oils can have complicated T_1 and T_2 distributions as well as a relaxation time constants that are too short to measure with low field relaxometry. This frustrates the goal of interpreting measurements from NMR well logging.

In the laboratory, MAS can remove the susceptibility broadening of the rock matrix, making possible the resolution of the proton chemical shift. We have used MAS measurements to determine water and oil saturations in Lost Hills diatomite samples containing oils with API gravity ranging from ~10 to 27. The MAS measurements yield determinations of the oil and water saturations and estimates of the aromaticity of the oil, and extend relaxometry by obtaining separate T_1's, Carr-Purcell and Hahn-Echo T_2's of oil and water. Related published studies include the use of ^{13}C MAS to distinguish macro- and micropores in oil reservoir cores by their relaxation times,[2] the demonstration of separate water and oil signals in Berea sandstone saturated with a light crude oil,[3] and ^{1}H relaxation in kaolinite.[4]

Figure 1 demonstrates the resolution advantage conferred by MAS at 12 KHz, for diatomite B, at 500 MHz spectrometer frequency. Figures 2 shows the high resolution T_2 MAS relaxometry data of this sample. Another Lost Hills sample, diatomite C, had significantly higher magnetic inclusions. Its static spectrum is broader, and the companion MAS spectrum shows many spinning sidebands at multiples of 12 KHz (Figure 3). Figure 4 shows the 100 MHz spectrum of the same sample. Because magnetic susceptibility broadening scales with the applied field, the same MAS spinning speed at 1/5 the field barely shows spinning sidebands. However, the resolution of water and oil which is evident in Figure 4 (top spectrum) can only be regained at the lower field by software enhancement. (Figure 4, top spectrum).

In addition to 500 and 100 MHz we have also made measurements at 20 MHz, and extracted the oils, to isolate different relaxation mechanisms. We find that simply going to higher field is sufficient to separate oil and water saturations in rock by low resolution T_1 relaxation, without MAS. Very fast surface relaxation dominates T_1 of water in rock and changes little with field, while rotational diffusion dominates for oil, both extracted and in the rock, and is not very different between the two at a given field strength. The oil T_1 dependence on field is understood by BPP calculations[5] at the three field strengths used, Figure 5. However, oil T_1 typically lengthens somewhat as MAS speed increases, the only dependence upon spinning we observed.

In contrast, T_2 of oil in diatomite is much shorter than that of the extracted oil, and is not very dependent upon field strength. This suggests that rather than a predominant diffusion/susceptibility mechanism that would depend quadratically upon the applied field[6], the dominant T_2 mechanism of oil in pores may be by a surface electron - nuclear relaxation mechanism[7]. Some data for diatomite B are shown in the table.

Figure 1

Diatomite B, API 27

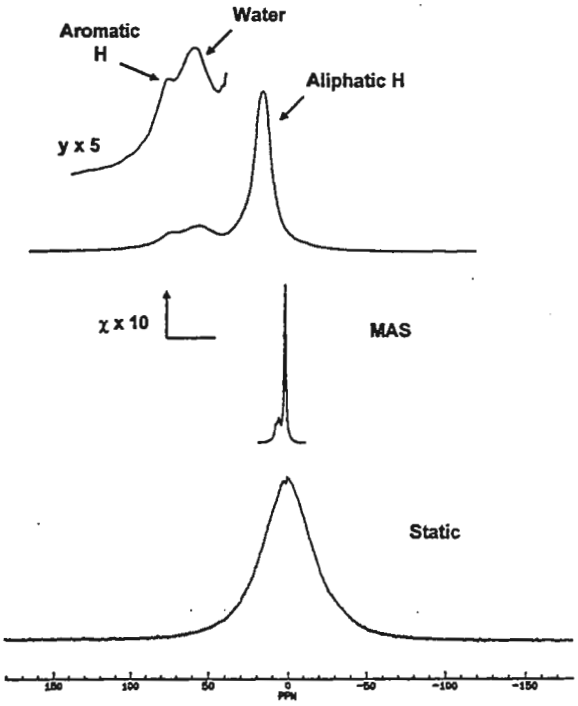


Figure 2

Carr-Purcell-Meiboom-Gill High Resolution

1 Cycle = 83.3 μ Sec.

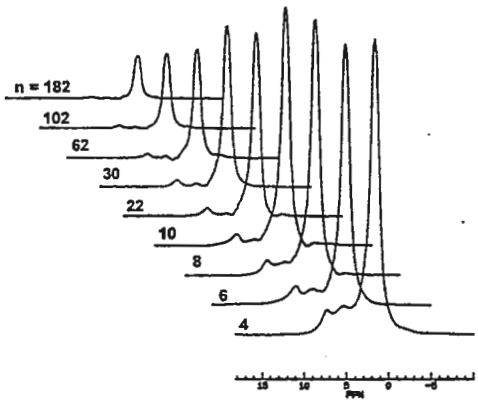
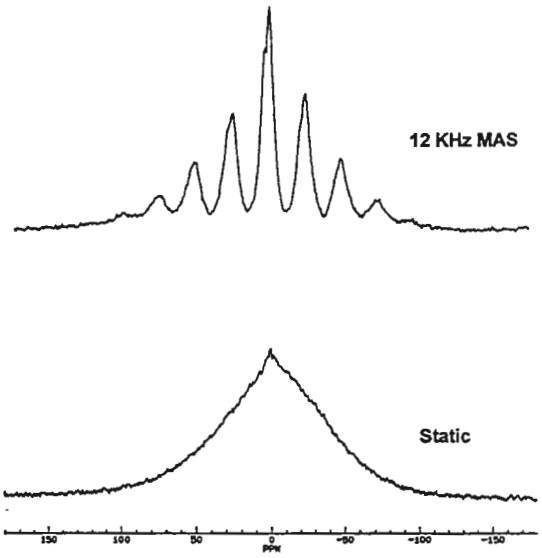


Figure 3 Diatomite C, API 19

500 MHz B_0



100 MHz B_0

Figure 4

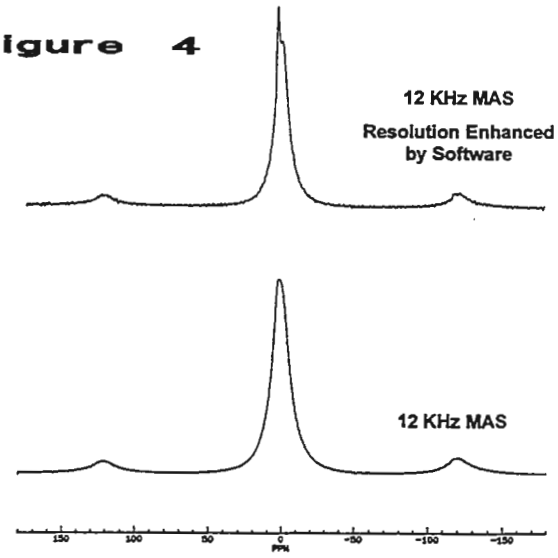
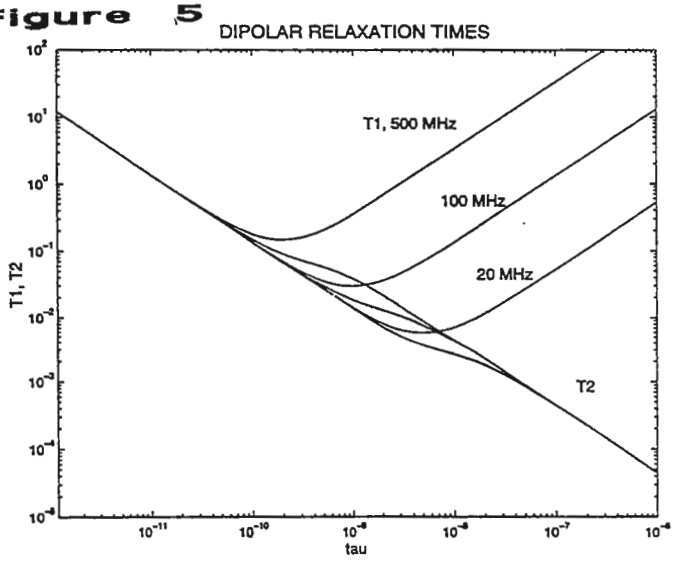


Figure 5



	Sample & Method All MAS at 12 KHz Spinning	Diatomite B, API 27, 0.8% Water, 8.9% Oil (by Wt) Relaxation Times in Milliseconds		
		500 MHz	100 MHz	20 MHz
T_1 Data	Whole Sample, Water by MAS	5.6	3.0	5.8*
	" " , Oil by MAS	255	46	5.8*
	Extracted Oil	510	85	60
High-Resolution T_2 CPMG Data	Whole Sample, Water by MAS	0.36	0.84	0.69*
	" " , Oil by MAS	1.40	1.32	0.69*
	Extracted Oil	33	9.1	12.1

* Whole sample, combined water and oil, non-spinning.

Sincerely,

D. M. Wilson and G. A. LaTorraca

References

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7. Kleinberg, R. L., *et al., J. Magn.Reson.* **A108**, 206-214 (1994).

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Barry and Lee Shapiro
shapiro@nmrnewsletter.com

December 1, 1999.

Address all Newsletter correspondence to:

Dr. B. L. Shapiro
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No. 497 (Feb.) 21 Jan. 2000

No. 498 (Mar.) 25 Feb. 2000

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No. 500 (May) 28 Apr. 2000

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

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Forthcoming NMR Meetings, continued from page 1:

XIX International Conference on Mag. Res. in Biological Systems, Florence, Italy, **August 20-25, 2000**. Contact: Profs. Ivano Bentini or Lucia Banci, Chem. Dept., Univ. of Florence, Via G. Capponi 7, I-50121, Florence, Italy; Phone: +39-055-2757600; Email: icmrbs@lrm.fi.cnr.it; Fax: +39-055-2757555; http://www.lrm.fi.cnr.it//icmrbs.html.

Gordon Research Conference on Magnetic Resonance, **June 17-22, 2001**, Roger Williams University, Bristol, Rhode Island (note the new, improved location !!!). Contacts: Rob Tycko, Chair, 301-402-8272, tycko@helix.nih.gov, and Kurt Zilm, Vice-Chair, kurt.zilm@yale.edu. Site description and application information available at http://www.grc.uri.edu.

Royal Society of Chemistry: 15th International Meeting on NMR Spectroscopy, Durham, England, **week of July 8-13, 2001**; Contact: Mrs. Paula Whelan, The Royal Society of Chemistry, Burlington House, London W1V 0BN, England; +44 0171 440 3316; Email: conferences@rsc.org\

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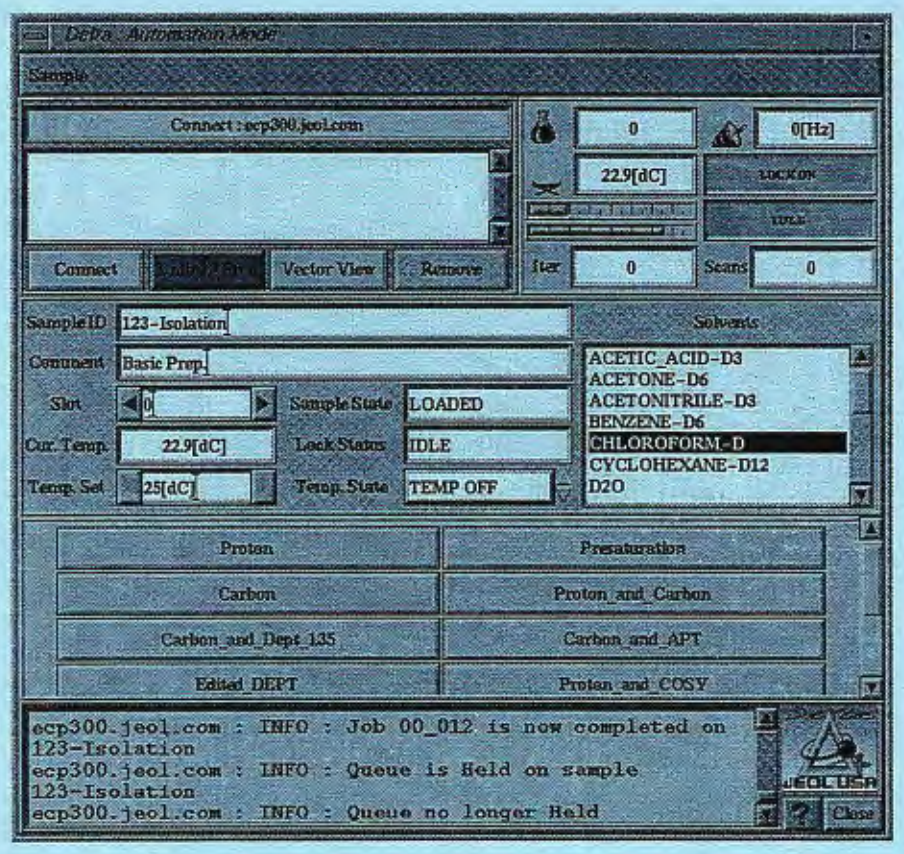

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