THE NMR NEWSLETTER

No. 483

December 1998

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Type 1 **PTS 310** Type 2	.1-310 MHz	1 Hz	1-20μs	standard	3½"H×19"W	BCD (std) or GPIB (opt)	1 Hz resol., OCXO: $6,425.00 1 Hz resol., OCXO: $5,850.00
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PTS 620	1-620 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$9,625.00 (1 Hz resol., OCXO freq. std.)
PTS 1000	0.1-1000 MHz	optional .1 Hz to 100 KHz	5-10μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$11,830.00 (1 Hz resol., OCXO freq. std.)
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FORTHCOMING NMR MEETINGS

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

7th Annual "Advances in NMR Applications" Symposium, Omni Rosen Hotel, Orlando, Florida, **February 28, 1999;** Contact: Kathy Bishop, at the Nalorac Corp.; 510-229-3501; kathy.bishop@nalorac.com; See Newsletter 483, xx.

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

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

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

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

Seventh Scientific Meeting and Exhibition of the Intl. Soc. for Magnetic Resonance in Medicine (ISMRM), Philadelphia, PA, **May 22 - 28, 1999**; Contact: International Society for Magnetic Resonance in Medicine, 2118 Milvia St., Suite 201, Berkeley, CA 94704.

International School of Structural Biology and Magnetic Resonance, 4th Course: Dynamics, Structure and Function of Biological Macromolecules; Erice, Sicily, Italy; **May 25-June 5, 1999**; Contact: Ms. Robin Holbrook, Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, CA 94305-5055; (650) 723-6270; Fax: (650) 723-2253; Email: reh@stanford.edu. See Newsletter 483, 8.

Additional listings of meetings, etc., are invited.

Spin Choreography

A Symposium in Appreciation of Ray Freeman
Cambridge, England: 8th – 11th April 1999

Your are invited to attend *Spin Choreography*, a symposium in appreciation of Ray Freeman

Aims of the symposium

The symposium is intended to bring together NMR spectroscopists from around the world to honour Ray Freeman's outstanding contribution to the field over nearly five decades. The emphasis of the meeting is on up-to-date topics rather than being retrospective, and the choice of speakers, most of whom have strong associations with Ray, either as colleagues, co-workers or students, reflects this intention. We are especially pleased to welcome Professor Richard Ernst as the key-note speaker.

Programme

The scientific programme will start after lunch on Thursday 8th April and end before dinner on Saturday 10th April. The key-note address will be given by Richard Ernst (ETH, Zürich) and there will be plenary lectures from

> Wes Anderson (Varian, Palo Alto), Ad Bax (NIH, Bethesda), Geoffrey Bodenhausen (Paris), Iain D Campbell (Oxford), Lyndon Emsley (Lyon), Helen Geen (Nottingham), Maurice Goldman (Saclay), Laurie Hall (Cambridge), Howard Hill (Varian, Palo Alto), Peter Hore (Oxford), Lewis Kay (Toronto), Eriks Kupce (Varian, Walton), Malcolm Levitt (Stockholm), Tom Mareci (Florida), Keith McLauchlan (Oxford), Gareth Morris (Manchester), Hartmut Oschkinat (Berlin), Alex Pines (Berkeley), A J Shaka (Irvine), Jeremy Titman (Nottingham) and Stephen Wimperis (Oxford).

The scientific sessions will cover all aspects of magnetic resonance, with the emphasis on NMR; there will also be a poster session. The symposium will end with a banquet on Saturday evening. Accompanying persons are very welcome, especially at the banquet and any of the social functions.

Location

The meeting will take place in Cambridge, England. The scientific sessions will be held in the Department of Chemistry, and conference guests will be offered accommodation and meals in Pembroke College or Peterhouse, which literally face one another across the street and are five minutes walk from the Chemistry Department

Registration and costs

The registration fee for the meeting is £45. Three different accommodation and meals packages are offered: Option 1: Accommodation for three nights, all meals, including the Banquet: £ 196; Option 2: Meals only, including Banquet: £100; Option 3: Banquet only: £ 40. All prices are quoted in pounds sterling.

Registration forms

If you wish to receive a registration form, please either write to or EMAIL the organizers at the address below, giving your name, full postal address and EMAIL address. The deadline for registration is 1st March 1999, but accommodation is limited so early registration is recommended.

Address for correspondence

All correspondence concerning the symposium should be addressed to the local organiser: Dr James Keeler, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, U.K., EMAIL: RF1999@ch.cam.ac.uk; FAX +44 1223 336913. The symposium web site is `http://mchsg4.ch.man.ac.uk/mcmr/RF.html`

(received 11/16/98)

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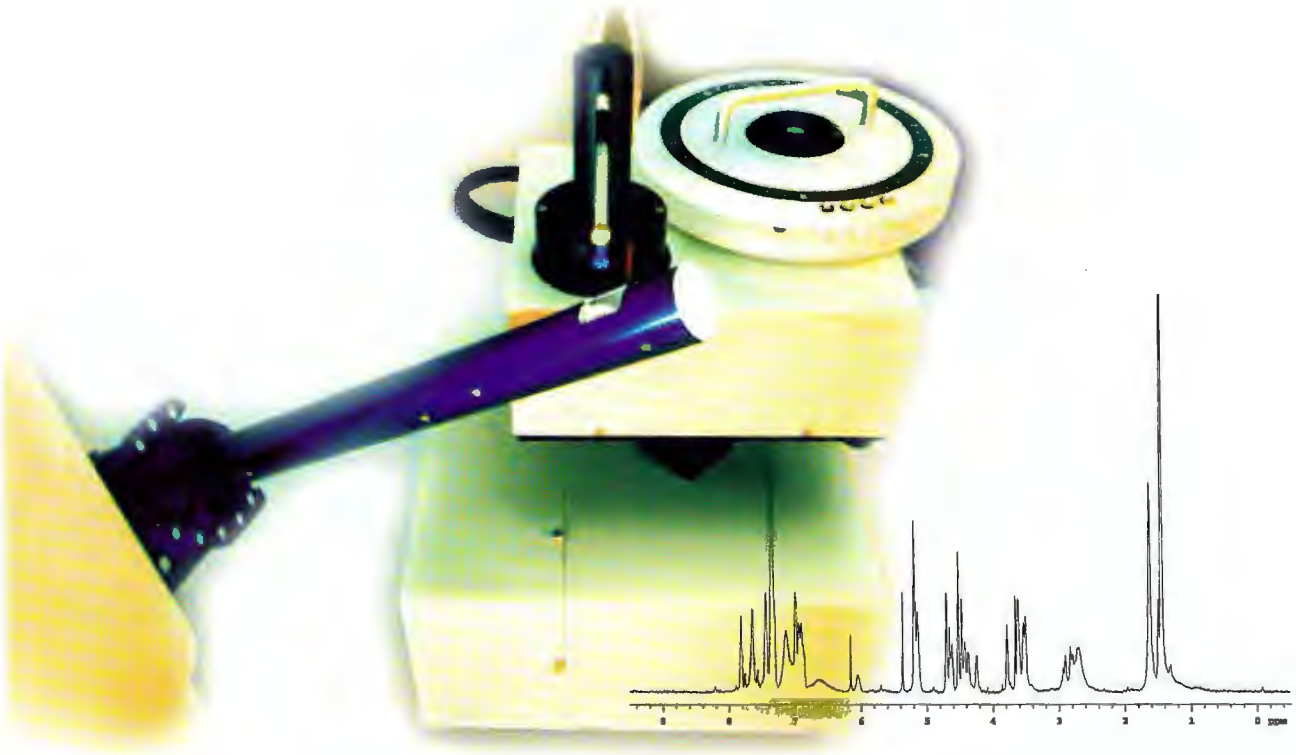

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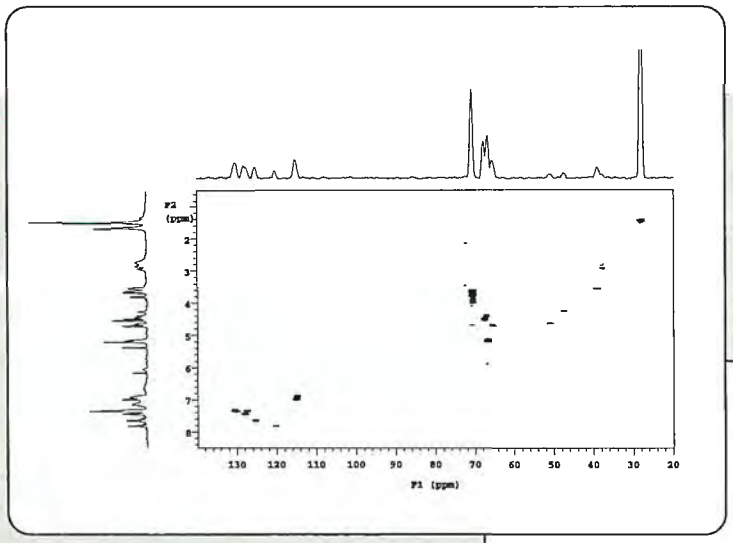
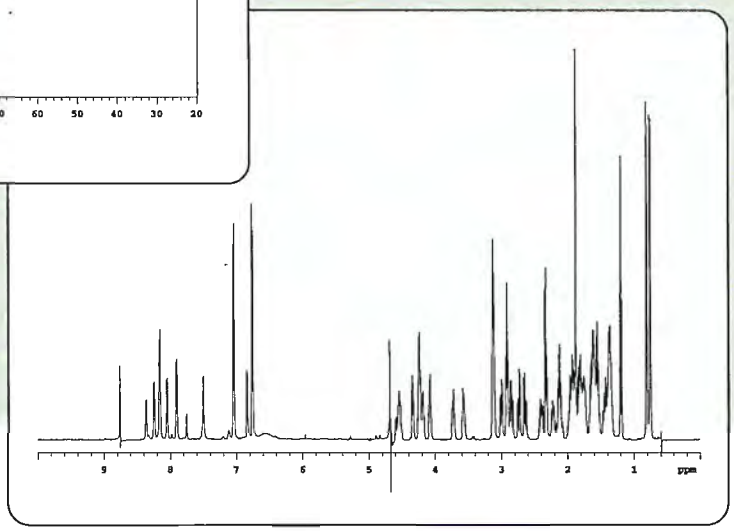


^{1}H-C^{13} gHSQC spectrum of FMOC-Asp(O-tBu)-Tentagel (NovaSyn TGA) Resin obtained using a 500 MHz gHX nanoprobe in 1.5 hours.



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NMR Spectroscopy

November 17, 1998
(received 11/23/98)

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

Digging in the (24-bit) Dirt

Dear Dr. Shapiro,

The world of NMR moves on, but the world of chemistry must occasionally remain firmly entrenched in data acquired in the past. In our effort to build a historical database of NMR data on the Groton research campus at Pfizer, we decided to include a significant chunk of Aspect-3000 data that had been archived datewise, but never really catalogued.

For those who have previously worried about this problem on old Bruker systems, you know that the problem of building a historical database reduces to analyzing the header of the datafile for information about the experiment, the title given to the dataset (plot title), the nucleus, etc. For those who have not previously worried about this problem, the header is a mixed format device, consisting of integers, real numbers, and character information arranged in a predefined way. Integers are represented in 24-bits, or three bytes (one word); real numbers are represented by 48-bits (two words); character information is represented in packed ascii format such that six bits represent a single character, so one 24-bit word can represent four characters.

We thought about two canned ways to do this job:
- the Xwinnmr 'conv' utility
- the Vnmr 'convertbru' utility

In both cases the conversion utility is pretty slow and designed for manual use (although semi-automatic macros can be written), as it is actually converting the FID data as well as reading the header. Since we had tens of thousands of datasets to digest, and no real need to convert the raw data until it is needed by a chemist, I harkened back to work that I had done many years earlier to read Aspect 3000 tapes into FTNMR (or Felix) format and decided to write my own utility.

The parameters in our Aspect 3000 datasets are stored as the first 256 words, or 768 bytes, of the file - the 257th word is the first real datapoint (integer word, represented in twos complement notation). These 256 words are referred to as the '-1' block in the Aspect 3000 manuals, and 187 of them are described in the 'par' section of these manuals. The plot title is not part of this description. Automation data also has stored with it an extended parameter block (stored as a separate file) which includes such important information as nucleus, solvent, and experiment; unfortunately, this extended parameter block had not survived to be used.

We were able to use the fact that under automation, a combination of the spectral width and number of points acquired could be used to uniquely identify the observe nucleus and the experiment run. For example, our COSY was run using 1024 x 128 points, whereas the HETCOR used 2048 x 128; the spectral width for a proton spectrum required a dwell time of 83 microseconds, but that for carbon required a dwell of 27 microseconds. Clearly we might miss experiments where the chemist had manually changed the standard parameters prior to acquisition, but the simple rules we developed captured greater than 99.5% of the datasets that had been archived. However, the most important information in the header of the dataset is that which relates the sample number (a notebook number or other identifier) to the dataset. One could imagine collecting all the old notebooks and then making a list which connects the dataset filename to the notebook number, but it would have been a terrible job to

do. Since the sample number information was normally put into the plot title (so that it would appear on the plot), we just needed to look up the plot title to make the important correlations.

So where is the plot title?

It turns out that the parameters described in the manual don't begin at byte #1 of the header, they begin at byte #121. The first 120 bytes (40 words) contain various undecipherable symbols, but bytes #47-83 or so contain the plot title that the chemist originally typed. Here we've included the Fortran subroutine (this routine runs on an SGI MIPS-based system running Fortran 77; the 'mvbits' routine seems to be included in C and image processing libraries as well) which converts the Bruker packed ascii text to standard ascii text: in the main body of the program the last converted byte is sequentially added to the growing plot title string:

```
**********************************************************************
      Subroutine Unpack(by,string)
**********************************************************************

C     Converts 4 6-bit characters (3 bytes) to 4 8-bit (ASCII) char.
      Character*4      string
      Integer*1        by(3),bz(3)
      Integer*4        ihold,ichar(4)
      Equivalence      (ihold,bz(1))

      ihold=0
      Do i=1,4
        ichar(i)=0
      End do
      Do i=1,3
        bz(i)=by(i)
       End do

      Do i=1,4
         Call mvbits(ihold,32-(6*i),6,ichar(i),0)
         If (ichar(i).eq.0)ichar(i)=32
         If (ichar(i).le.26) ichar(i)=ichar(i)+64
         string(i:i)=char(ichar(i))
       End do

      Return
      End
```

The program that was written was able to digest about 25-30,000 datasets in less than an overnight, and allowed us to add this historically important data to a very simple database that makes it easy for chemists to retrieve, display, analyze, and replot the NMR data that they need.

Sincerely,

Walter Massefski, Jr.
860-441-5962
860-441-0207 (FAX)
email: wwm@pfizer.com

Second Notice

You are invited to attend the

7th ANNUAL ADVANCES IN NMR APPLICATIONS SYMPOSIUM

Featuring the Latest Developments in Experimental Techniques

To be held prior to ENC at the
Omni Rosen Hotel
Grand Ballroom C
(located a short walk from the Clarion Plaza Hotel)

Sunday, February 28, 1999
1:00 to 6:00 p.m.

The agenda includes a presentation of recent results by leading NMR experimentalists concerning applications of pulsed field gradient and classical NMR techniques with both large and small molecular systems.
The results obtained will be of interest to all liquid state NMR spectroscopists.

Request a detailed program or RSVP by contacting
Kathy Bishop, Nalorac's ENC Coordinator

Transportation will be provided between the
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Dynamics, Structure and Function of Biological Macromolecules

4th Course of the International School of Structural Biology and Magnetic Resonance
a NATO Advanced Study Institute

Location: **Ettore Majorana Centre for Scientific Culture, Erice, Italy**
Dates: May 25-June 5, 1999 Number of working days: 10 days

Objective: To summarize the current state of the field of protein studies focusing on the type of information that can be obtained about dynamic processes in proteins by NMR and other physical methods and the implications for protein and drug design.

Fee: $1,200 includes Board and Lodging to be arranged by the Centre. Some financial aid available and any request for aid should be indicated in the application.

Directors:
Oleg Jardetzky, Professor, Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5337, USA.
Jean-François Lefèvre, Professor, ESBS, Louis Pasteur University, Bld. Sébastien Brant, 67400 Strasbourg-Illkirch, France.

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Lecturers

Cheryl H. Arrowsmith (Ontario Cancer Institute, Toronto, Canada) • **Ivano Bertini** (Università degli Studi di Firenze, Italy) • **Richard R. Ernst** (ETH Zentrum, Zürich, Switzerland) • **Hans Frauenfelder** (Los Alamos National Laboratories, USA) • **Cornelius W. Hilbers** (University of Nijmegen, The Netherlands) • **Oleg Jardetzky** (Stanford University, USA) • **Jean-François Lefèvre** (Université Louis Pasteur, France) • **Michael Levitt** (Stanford University, USA) • **William N. Lipscomb** (Harvard University, USA) • **Dino Moras** (Université Louis Pasteur, France) • **Joseph D. Puglisi** (Stanford University, USA) • **Paul Rösch** (Universität Bayreuth, Germany) • **Brian D. Sykes** (University of Alberta, Edmonton, Canada) • **Wilfred van Gunsteren** (ETH Zentrum, Zürich, Switzerland)

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•Principles of NMR and dynamics
•Protein dynamics and reactions •The energy landscape of proteins

Theoretical Analysis of Internal Motions in Biological Molecules
•Introduction to molecular dynamics •Simulations of protein folding
•Simulating protein and nucleic acid molecular dynamics
•New programs in MD simulations •Calculation of free energy and binding constants

Motions in Nucleic Acid
•Nucleic acids structure and dynamics
•RNA NMR spectroscopy

Analysis of Specific Proteins
•Interactions of antifreeze proteins with ice •Mechanism of action of calcium-signaling proteins
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November 24, 1998
(received 11/25/98)
Bernard L. Shapiro, Ph.D.
Editor, The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Low-Level ^{1}H-^{15}N Long-Range Correlation Using a Combination of 2D and Selective 1D Methods

Dear Barry,

After your last phone conversation with Gary teasing him about using a "broken hand" as an excuse for not contributing to The NMR Newsletter more often, I decided to pick up the slack and share with you some of our latest efforts. As you know, we have been pushing the limits of detectability with the Nalorac 1.7 mm sub-micro or SMIDG™ NMR probe installed on our Varian INOVA 600. Knowing the functional limit of detection (as opposed to some calculated value) is worthwhile when dealing with impurities or degradants that are particularly difficult to isolate, e.g. those with chromatographic relative retention times (rrt's) that are closely similar to that of the bulk drugs. In such cases, being able to isolate only what is needed for characterization can save considerable chromatographic isolation time. The smallest quantity that we've been able to characterize fully thus far using heteronuclear methods (^{1}H-^{13}C GHSQC and GHMBC), was a 0.04 μmol sample of cryptolepinone present as an 8% impurity in a 0.55 μmol sample of cryptolepine.[1] The ability to successfully characterize samples this small affords a very comfortable and efficient work process when dealing with samples in the 0.5 to 1.0 μmol range.[2] We've gotten quite comfortable in acquiring ^{1}H-^{13}C data at this level and were thus interested in further exploring what is possible at this level in terms of using long-range ^{1}H-^{15}N at natural abundance. What perhaps may come as a bit of a surprise to some, is that it is quite reasonable to think of working at this level for the acquisition of natural abundance long-range ^{1}H-^{15}N heteronuclear shift correlation data.

We have generally utilized strychnine as a benchmark for our ^{1}H-^{15}N sensitivity studies.[3,4] The molecule has a pair of very well resolved nitrogen resonances, each with a number of long-range correlations to it with coupling constants that range from about 2 to 16 Hz.[3] In terms of the 2D limits of detection for the ^{1}H-^{15}N GHMBC experiment at natural abundance using SMIDG NMR probe technology, we recently reported the results obtained with a 3 μmol/30μL sample overnight. Pushing further, it was possible to acquire marginally usable 2D data using 1 μmol/30μL of strychnine over a weekend.[4] To reduce acquisition times at this level to more tractable intervals, a two-fold approach can be used. First, one needs to be able to define the ^{15}N chemical shift of the resonance(s) of interest at the working level. Then, the investigator is faced with the challenge of observing all or at least the necessary long-range correlations to the nitrogen resonance of interest. The former can be done from a coarsely resolved 2D experiment with only sufficient numbers of transients acquired/file to allow only the most intense resonances in the spectrum to be observed. The latter task is accomplished most efficiently by resorting to a selective 1D experiment.

Long-range ^{1}H-^{15}N 2D data at natural abundance acquired overnight for a 1 μmol/30μL sample of strychnine are presented in Figure 1. The majority of the possible long-range correlations are not observed; only the two most intense correlations are observed in the contour plot of the overnight data. As might be expected, going to a lower threshold for the contour plot gave quite a lot of "grass" in the spectrum. Examining the trace (not shown) at the chemical shift of N(19), other correlations gave a hint of being present but with only marginally useful intensity (supplemented by imagination?). Hence, 3 μmol (1 mg in the case of strychnine) may be a practical, working limit when full 2D spectra must be acquired in which the expectation is one of observing all responses of interest. Pragmatically, however, the overnight 1 μmol data did accomplish the first of the two tasks delineated above; the experiment did afford the chemical shifts of the two nitrogens. At that point, we can direct our attention to the task of observing the long-range correlations, and, as it turns out, 1 μmol is still a real and very feasible working sample.

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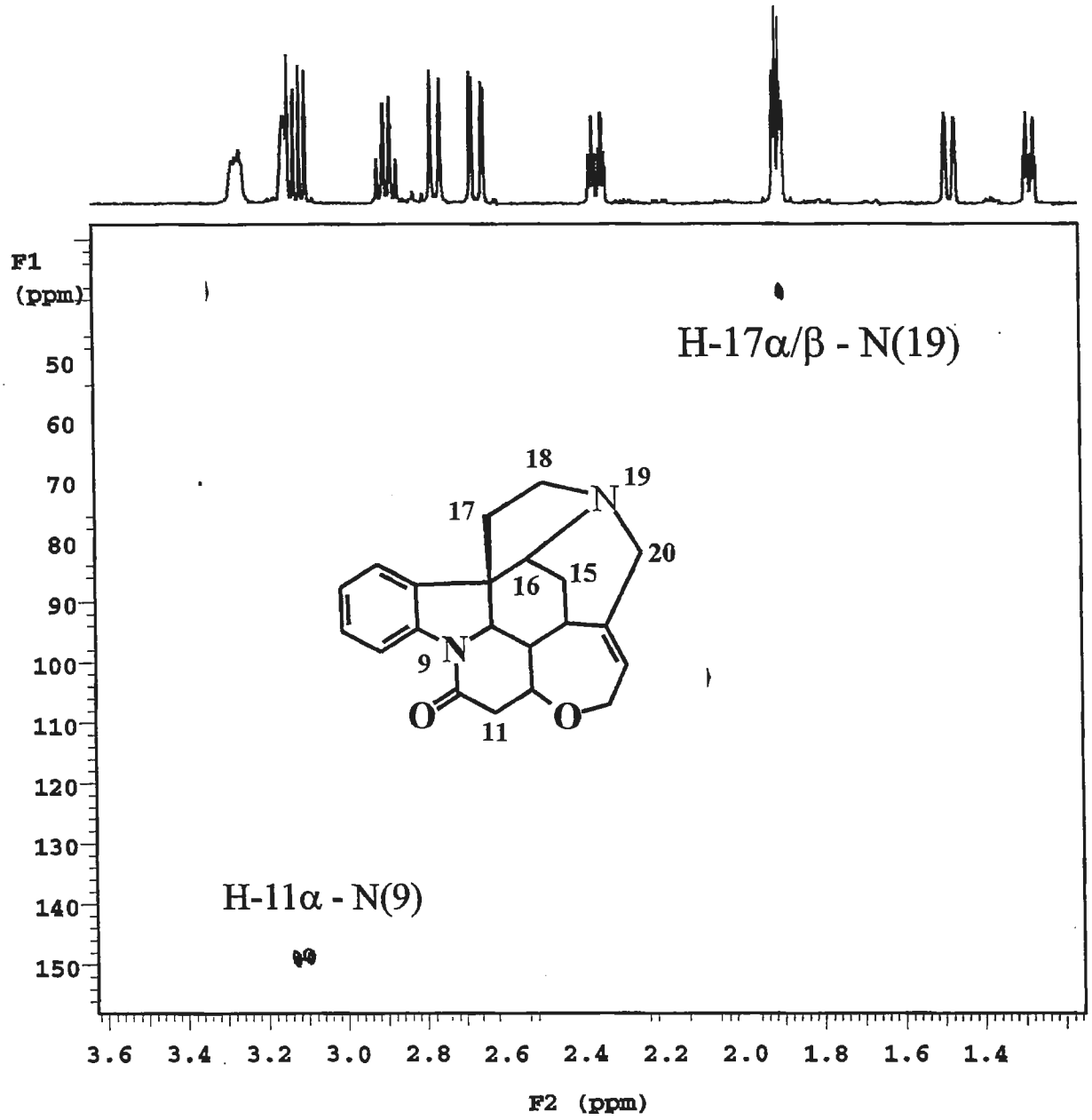

Figure 1. ^{1}H-^{15}N long-range GHMBC 2D NMR spectrum of a 1 μmol sample of strychnine dissolved in 30 μL of $CDCl_3$ using a Varian INOVA 600 equipped with a Nalorac SMIDG-600-1.7 submicro NMR probe. The data were acquired overnight using an optimization of 10 Hz.

Starting with the overnight 1 μmol 2D data shown in Figure 1, the single correlations observed to both N(9) and N(19) define the requisite ^{15}N chemical shifts. We elected to focus on the aliphatic N(19) resonance and calibrated a selective ^{15}N 90° pulse at 1.1 ms at a power level of 35 dB (63 dB max), giving an effective excitation window of ~ ±4 ppm, which more than amply compensated for any inaccuracy of the ^{15}N chemical shift of N(19) measured from the 2D contour plot. A selective 1D ^{1}H-^{15}N GHMBC experiment optimized for 8 Hz was then acquired "overnight" (22 h; 46000 transients) for N(19) using the 1 μmol sample. The ^{1}H resonances long-range correlated to N(19) are shown in the Trace B of Figure 2 plotted above a proton reference spectrum. As will be noted from the selective 1D spectrum, long-range correlations with very usable intensity are observed to N(19) from H-16, H15β, H-18α/β, and the H-17α/β

resonances. In contrast, a weekend acquisition of a full 2D spectrum with a 1 μmol sample gave correlations, other than that to H-17α/β , which were substantially less intense and only marginally usable with confidence (Trace C, Figure 2).[4]

Pushing further, we were also able to acquire data using a 0.5 μmol sample of strychnine dissolved in 30 μL $CDCl_3$. Taking the chemical shift of N(19) from the contour plot of the 1 μmol sample, we acquired the selective 1D GHMBC spectrum optimized for 10 Hz shown in Figure 3 over a long weekend (84 h). As can be seen from the top trace, correlations are again readily observed. Practically, usable data could be acquired over a normal weekend at this level. The variability of response intensity observed in the two spectra (trace B, Figure 2, and top trace, Figure 3) is a result of the modulation of response intensity. For example, the H-20β response was expected to be absent due to the optimization of the long-range coupling delay for 10 Hz; H-20β exhibits the maximum long-range correlation signal at 4 Hz, decreasing to an essentially undetectable signal in a 10 Hz optimized experiment.[5]

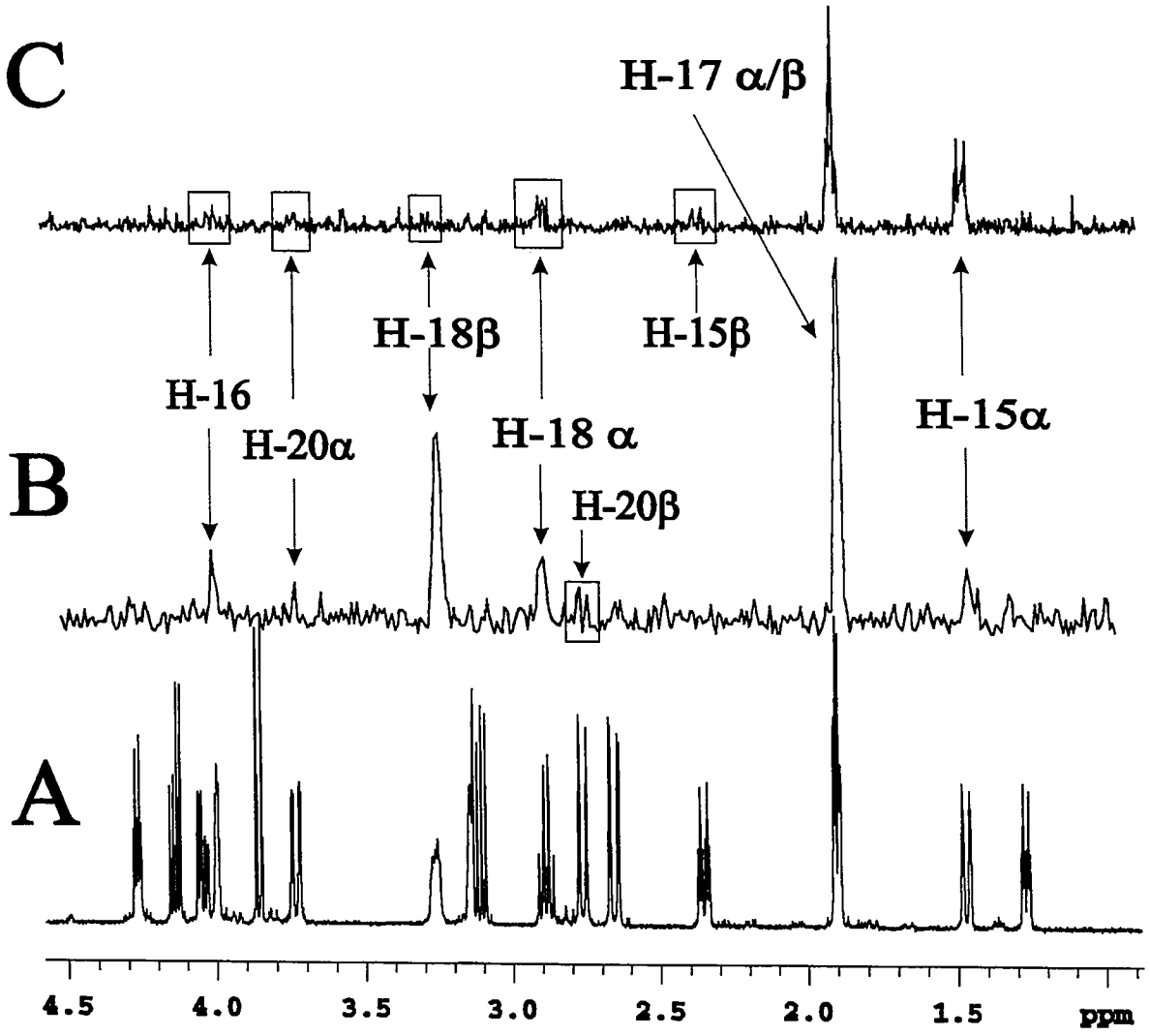

Figure 2. ^{1}H Reference spectrum of the aliphatic region of strychnine (Trace A), selective 8 Hz 1D ^{1}H-^{15}N GHMBC spectrum of the N(19) resonance of strychnine acquired overnight (22 h, 46000 transients) (Trace B), and a slice taken from a 10 Hz weekend (65 hr^4) 2D ^{1}H-^{15}N GHMBC experiment (Trace C). All spectra were acquired using a 1 μmol sample dissolved in 30 μL $CDCl_3$ using a Varian INOVA 600 equipped with a Nalorac SMIDG-600-1.7 probe.

In conclusion, samples ranging from ~ 0.5 to 1.0 μmol represent a viable working sample size for a combined approach which utilizes an overnight to 1 day to acquire a 2D experiment to define ^{15}N chemical shifts followed by one or more selective 1D ^{1}H-^{15}N GHMBC spectra to define long-range correlations with useful response intensities. The data we have shown make if feasible to consider ^{15}N as a viable structural probe in those cases where the chemical structure cannot be elucidated by more conventional ^{1}H-^{13}C heteronuclear techniques alone.

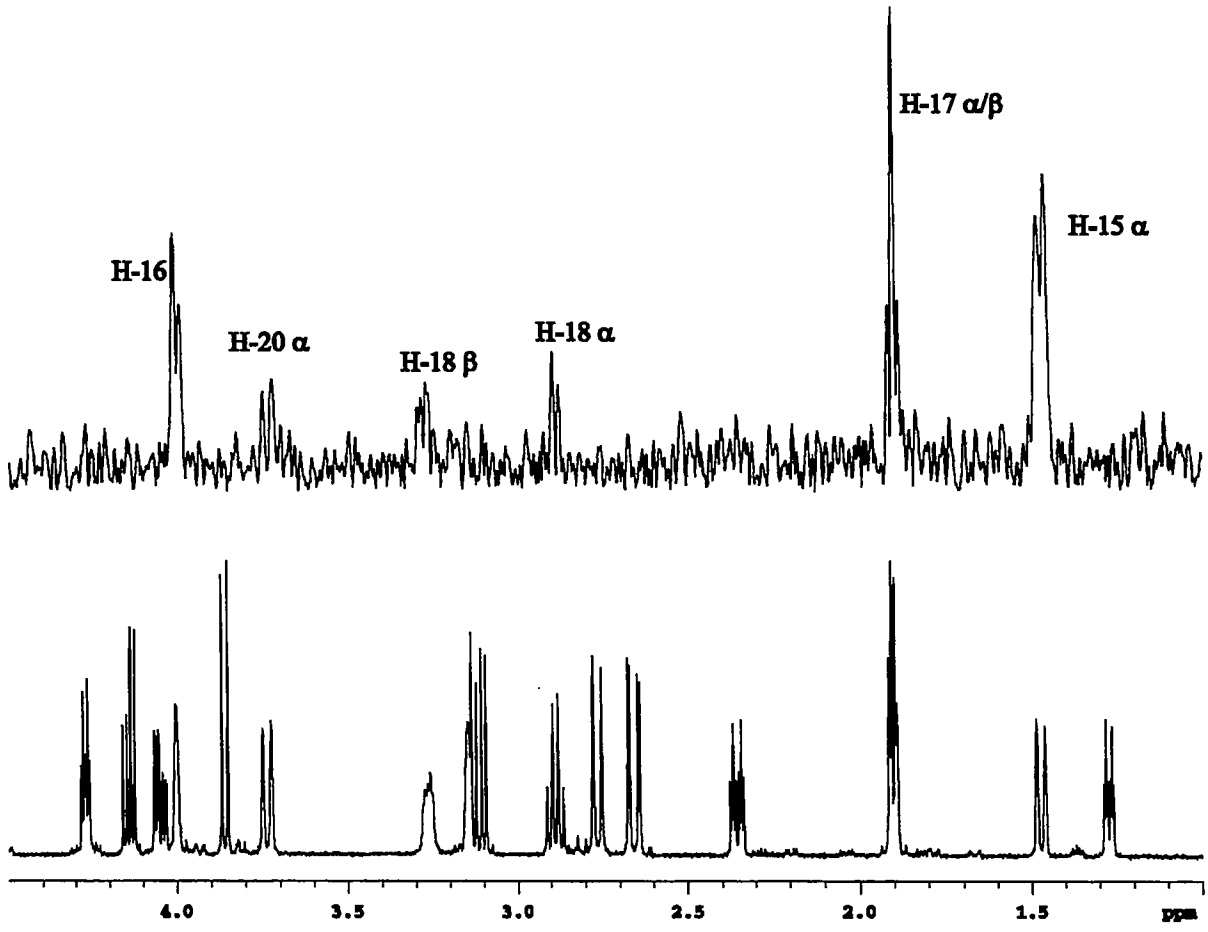

Figure 3. Selective 1D ^{1}H-^{15}N GHMBC spectrum of the N(19) resonance of strychnine acquired over a weekend (84 h) for a 0.5 μmol sample dissolved in 30 μL $CDCl_3$ using a Varian INOVA 600 equipped with a Nalorac SMIDG-600-1.7 probe. The experiment was optimized for an assumed 10 Hz long-range coupling.

Chad E. Hadden Gary E. Martin

References

1. G. E. Martin, J. E. Guido, R. H. Robins, M. H. M. Sharaf, P. L. Schiff, Jr., and A. N. Tackie, *J. Nat. Prod.*, **61**, 555-559 (1998).
2. G. E. Martin, C. E. Hadden, M. H. M. Sharaf, A. N. Tackie, and P. L. Schiff, Jr., *in preparation* (1998).
3. G. E. Martin, R. C. Crouch, and C. W. Andrews, *J. Heterocyclic Chem.*, **32**, 1759-1766 (1995).
4. C. E. Hadden and G. E. Martin, *J. Nat. Prod.*, **61**, 969-972 (1998).
5. Details of the modulation of response intensity in the long-range ^{1}H-^{15}N natural abundance spectra of strychnine will be the topic of a separate communication.

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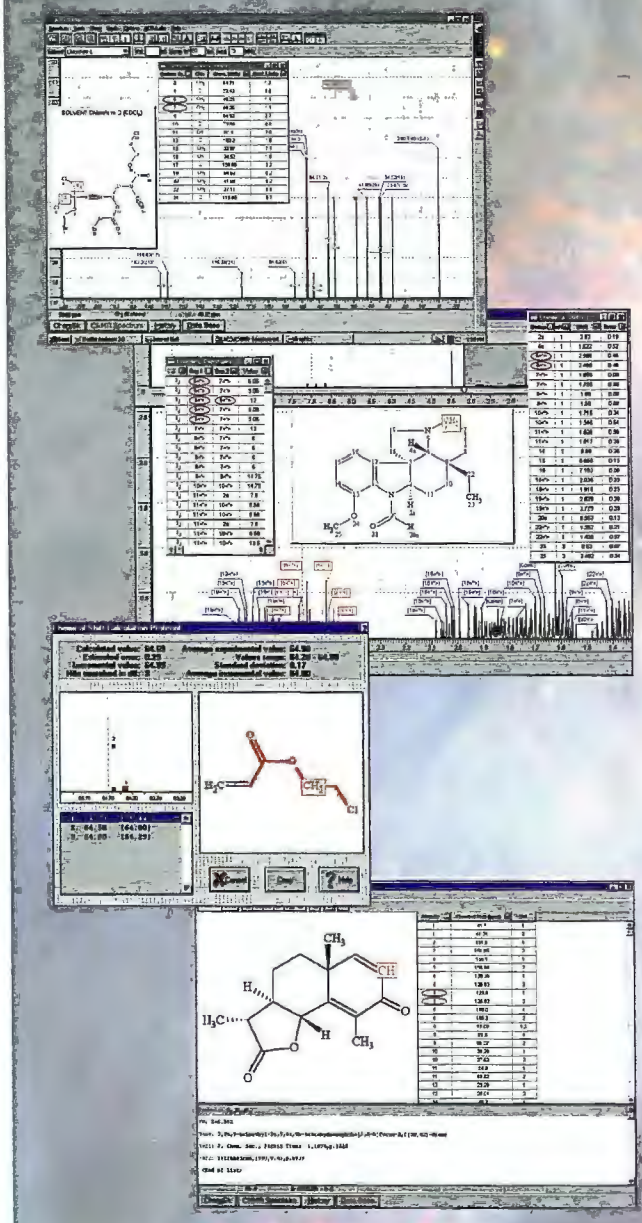

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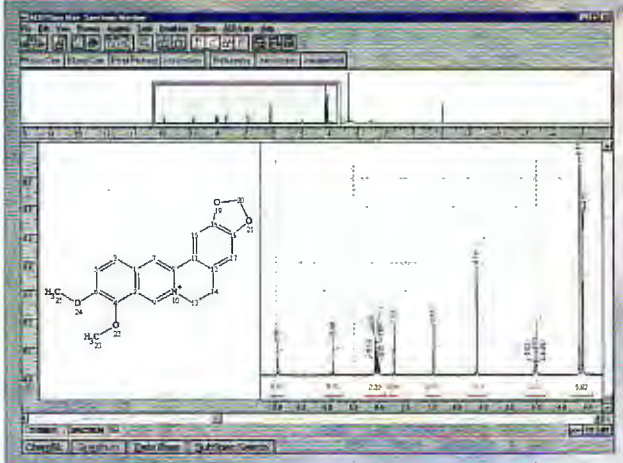

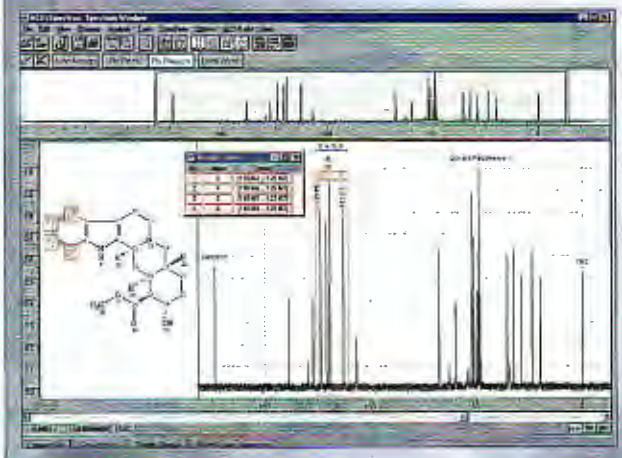

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November 4, 1998
(received 11/10/98)

SINGULAR VALUES IN SLOW EXCHANGE

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

Dear Barry,

We've been learning something about singular value decompositions, and their use beyond linear prediction. This all comes from looking at selective inversions on coupled spin systems, following Ted McClung's very nice work (J. Magn. Reson. 115 A, 145-154 (1995)). Selective inversions are great for studying slow exchange in uncoupled systems, but coupled systems raise all sorts of interesting issues.

The longitudinal magnetizations of the spin system are key to the problem, since we measure the exchange in competition with their relaxation processes. However, for a coupled spin system, the lines in the spectrum do not directly reflect the level populations. For n spins-1/2, there are (2n!)/((n!)(n!)) coherence level zero elements, but only 2^n are longitudinal magnetizations. For three spins, there are eight longitudinal magnetizations, one of which is fixed as the total number of spins. However, there are twenty coherence level zero density matrix elements, leaving six pairs of zero-quantum transitions. The fifteen observable xy magnetizations for a three-spin system can not correspond directly to the seven independent longitudinal magnetizations.

The xy magnetizations can also be complicated. For n weakly coupled spins, there can be $n*2^{n-1}$ lines in the spectrum, and a strongly coupled spin system can have up to (2n!)/((n-1)!(n+1)!) transitions. Because of small couplings, and because some lines are weak combination lines, it is rare to be able to observe all possible lines. We must therefore maintain the distinction between mathematical and practical relationships for the density matrix elements.

In mathematical terms, the intensities of the observable lines are related to the longitudinal magnetizations via a non-square matrix **A**, as below. In order to keep track of the matrices, we use the notation $(\mathbf{A})_{pxq}$ to indicate that the dimensions of the matrix are p x q. The quantity p is the number of xy magnetizations, and there are q longitudinal magnetizations.

$$(xy)_p = (A)_{p\times q}(z)_q$$

The matrix **A** can be quite messy, since it is determined by the flip angle of the observe pulse as well as the chemical shifts and couplings of the spin system.

This problem is apparently overdetermined, since there are usually more xy magnetizations than there are longitudinal magnetizations. In principle, we can solve for the longitudinal magnetizations in the standard generalised linear least squares method.

$$(z)_q = \left(A^{tr}\,A\right)^{-1}_{q\times q}\left(A^{tr}\right)_{q\times p}(xy)_p$$

In practice not all of the transitions can be observed, and we may not be able to measure some of the longitudinal magnetizations because of vanishing couplings. This equation then becomes numerically unstable, since some of the longitudinal magnetizations are not well-determined. However, solution of the equations by a singular value decomposition (SVD) allows us to extract the significant longitudinal magnetizations, and ignore the rest.

The singular value decomposition (SVD) breaks the matrix **A** up as in the following equation.

$$(xy)_p = (U)_{p\times p}(D)_{p\times q}(W)_{q\times q}(z)_q$$

The matrices **U** and **W** are square, invertible matrices, and the matrix **D** is a generalised diagonal matrix. Such a matrix is not necessarily square, but has the non-zero matrix element d_{ij} if $i = j$. In the SVD, the diagonal elements of **D** are the singular values. If the singular values are large, then the corresponding quantities are well determined. Poorly determined quantities are related to singular values close to zero. The solution for the z magnetizations is

$$(z)_q = (W^{-1})_{qxq}(D^{-1})_{qxp}(U^{-1})_{pxp}(xy)_p$$

The matrix $\mathbf{D}^{-1}$ is the transpose of **D**, but with the non-zero diagonal elements replaced by their reciprocals. The elements of **D** close to zero are replaced, not by their reciprocals, but by zero. The number of non-zero singular values gives the number of independent longitudinal magnetizations that can be measured. For instance, if there are k significant singular values, the last (q-k) rows of $\mathbf{D}^{-1}$ are all zero.

We can either look at the singular values from our data, or estimate how many z magnetizations we expect, to get the value of k. This gives us a general way of solving for the z magnetizations (or at least the accessible ones) in a general coupled system. Once we have the z magnetizations as a function of time, we can analyse them in the usual way for slow exchange. This means that we can look quantitatively at slow exchange on proton systems, without having to worry about find a singlet to invert. No messing around with trying to make EXSY quantitative!.

Yours truly,

Alex D. Bain (bain@mcmaster.ca)

ps: The Canadian Journal of Chemistry is publishing a special issue in honour of Ted Schaefer. Rod Wasylishen (one of Ted's many students at the University of Manitoba) is editing it, and would be happy to get contributions from Ted's many admirers. Send manuscripts to

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12 November 1998
(received 11/16/98)

The Importance of HMBC Data

In my previous contribution[1] I introduced "non-classical CASE" programs, which attempt to relieve the spectroscopist of some of the drudgery of molecular structure elucidation. One of the things that such programs can do is use the results of the HMBC experiment to reduce the number of structures generated. In this contribution I will try to show how important these data are. I realize that I am "preaching to the choir" here, but perhaps some of the numbers in the examples below will be of interest.

The example this time is a compound with the molecular formula $C_{22}H_{22}N_2O_5$, and the substructures we shall use are shown in Figure 1, where dashed bonds indicate unknown bond orders. We shall be comparing MolGen and NMRSAMS as examples of classical vs. non-classical programs, respectively. Table 1 shows the results of our comparison. The substructures used in each example are listed in column 2. The results from MolGen (column 3) are compared to those of NMRSAMS without (column 4) and with (column 5) the BadList and chemical shift constraints described before.[1] Normally one would use NMRSAMS with the control parameters set to give the results in the last column.

In example 1 the substructures input to the two programs were essentially those identified by NMRSAMS from the 1D NMR and COSY spectra. Clearly this is insufficient information. MolGen creates over 7 million structures in a half hour and reports that it has explored only "0.0%" of the structure space available. NMRSAMS hits my preset limit of 1000 structures, and similarly reports that there are more structures available. Without the BadList and chemical shift constraints it does this rapidly. With these constraints turned on, NMRSAMS works a lot more slowly, since it must generate and then reject many structures for every one that it passes.

Table 1: Record of Structure Generations

Example:	Substructures:	MolGen:	NMRSAMS w/o constraints:	NMRSAMS w/ constraints:
1	3 **Car**, 2 **Arf**, 1 **Et**, 1 **NH_2**, 6 **Cq**, 2 **CH**, 2 **CH_2**, 1 **Me**, 1 **N**, 2 **O**	>7,600,000, 30 min, "0.0% complete"	>1000, 1.5 min, not complete	>1000, 15 hrs, not complete
2	3 **Car**, 1 **AR**, 1 **Ph**, 1 **Et**, 1 **NH_2**, 2 **Cq**, 2 **CH_2**, 1 **Me**, 1 **N**, 2 **O**	>7,200,000, 20 min, "0.1% complete"	>1000, 2 min, not complete	194, 20 min, complete
3	3 **Car**, 1 **AR**, 1 **Bz**, 1 **Et2**, 1 **NH_2**, 1 **CH_2**, 1 **OMe**, 1 **N**, 1 **O**	<2,000,000, 10 min, complete	1081, 2 min, complete	194, 2 min, complete
4	1 **DiCar**, 1 **COOMe**, 1 **AR2**, 1 **Bz**, 1 **Et2**, 1 **CH_2**, 1 **N**	1,926, 1.3 sec, complete	223, 18 sec, complete	13, 2.4 sec, complete

In the second example I have extended the aromatic fragments **Arf** to their full benzenoid substructures, **AR** and **Ph**. MolGen generates over 7 million structures in about 20 minutes, but this time encourages us with the news that it has explored 0.1% of the structure space. One might use these results to estimate that there

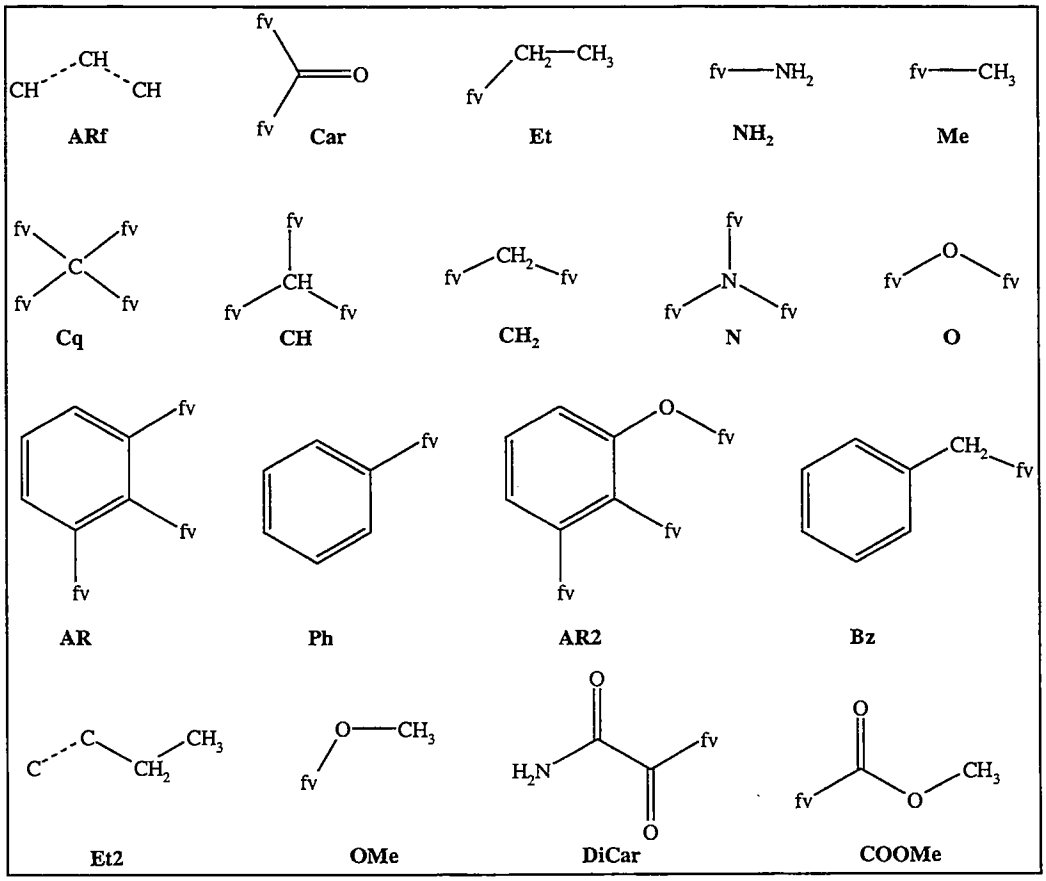
[1] NMR Newsletter, October 1998, #481, p. 11.

are over 7 billion structures possible for this input. With no constraints NMRSAMS hits the 1000 structure limit in 2 minutes, and reports that there is more work to be done. With the constraints turned on NMRSAMS works more slowly, but comes to a complete list of candidate structures! The correct structure is #170.

In the third example I extend **Ph** to **Bz** and **Et** to **Et2** using logic that I will desribe in my next contribution. In addition I identify the one remaining methyl as an O-methyl. This time MolGen creates a complete list of about 2 million candidate structures.[2] NMRSAMS, using the HMBC data, trims this to 1081 structures, a reduction of roughly 2000-fold. With the chemical shift theory and the BadList turned on we again get 194 structures, but in about one-tenth the time.

In example 4 I define the carbonyls better and flesh out **Ar** a bit more. MolGen whips out 1,926 structures in a bit more than a second, as usual winning the speed record. NMRSAMS comes up with 223 structures using the HMBC, and only 13 with all its capabilities turned on.

Figure 1: Substructures Used in this Contribution



Doug Dorman
doug_dorman@lilly.com

[2] The program crashed before I could read the exact number.

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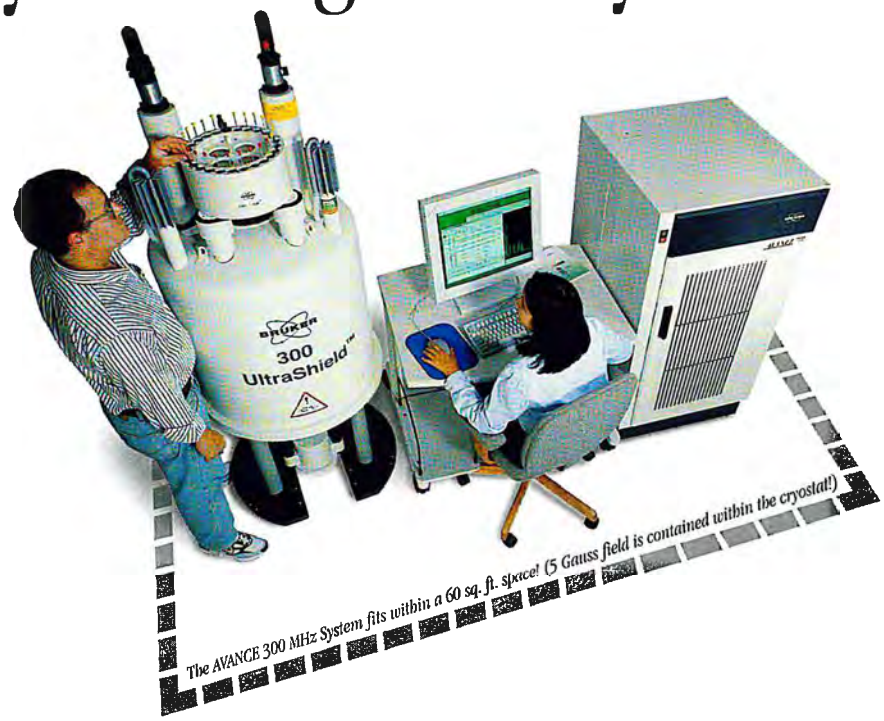

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November 5, 1998
(received 11/9/98)

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

A Quick ^{11}B NMR Method to Quantify Low Concentrations of Borohydride

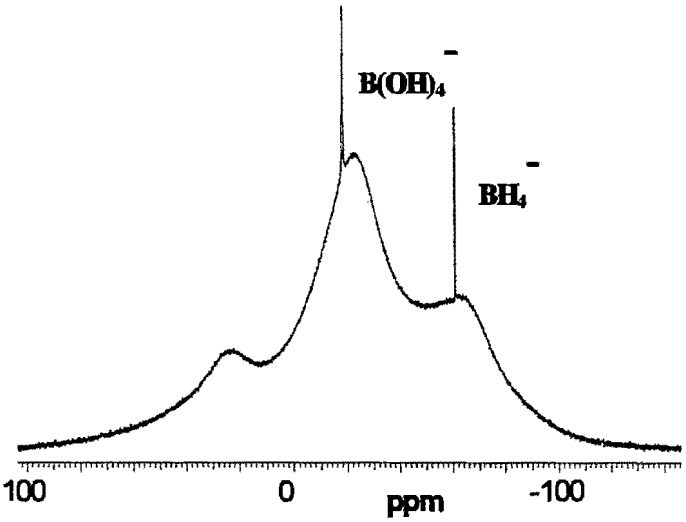
Dear Barry,

We have found ^{11} B-NMR to be a very useful tool to identify and quantify trace levels of borohydride in our raw materials. We combine NMR with atomic spectroscopic analysis for total boron to quantify both borate and borohydride in these raw materials.

Borohydride is widely used in the synthesis of amines, amides, esters and alcohols. It is a powerful reducing agent and hence is effective in inhibiting oxidative side reactions that give rise to color and odor forming impurities. However, equally important to the color and odor quality of a raw material is the ***complete*** removal of borohydride from the raw material before it is formulated into a consumer product.

Borohydride is a difficult analyte to quantify at low concentrations. Traditional wet chemical, chromatographic and electrochemical methods suffer serious interference across the variety of matrices borohydride is typically used in to control odor and color. ^{11}B - NMR is an ideal tool for identifying and quantifying low concentrations of borohydride/borate species. Its receptivity and natural abundance combined with the highly symmetrical electronic environments of borates and borohydride make ^{11}B - NMR quite a sensitive technique. ^{11}B - NMR is a viable alternative to traditional analytical methods for quantification of borohydrides that is sensitive, quick and applicable across a range of sample matrices.

^{11} B - NMR Spectrum of an Amine Raw Material

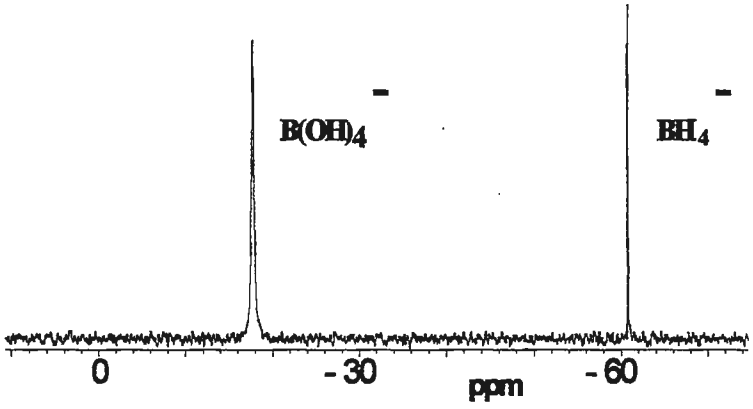


Shown left is a ^{11}B NMR spectrum of a tertiary amine in which potassium borohydride was used to control color during synthesis. This spectrum represents 256 scans on an aqueous sample containing 30 parts per million total elemental boron. It was recorded using a 10 mm broadband probe at a Larmor frequency of 96.2 MHz, a 250 ppm window with the carrier frequency centered at - 30 ppm from our reference of 0.1 molar H_3BO_3 in D_2O (at 0 ppm). This spectrum was recorded with a ^{2}H lock (as we typically dilute samples 1-1 with D_2O to control viscosity), with gated (NOE suppressed) proton decoupling, a 90 degree pulse and a repetition rate of

15 seconds. Borohydride T_1 values can be as long as several seconds, hence care must be taken to ensure repetition rates are sufficient when doing quantitative analyses. The total spectrometer time here was approximately 64 minutes.

The above spectrum has 5 resonances. The three broad resonances are from the borosilicate NMR tubes/ probe insert and hence are considered "blank". When doing quantitative analyses a blank spectrum can be subtracted and these broad resonances at 20, - 20 and - 60 ppm are removed to reveal the resonances from borate(s) and borohydride.

^{11}B - NMR of an Amine (after background subtraction)



Shown on the left is the above spectrum of the amine after subtraction of the background resonances. At the pH of this sample (i.e., ~ 10), borate's and borohydride's chemical shifts are approximately - 18 ppm and - 61 ppm respectively.

From the integrated intensities, the borate to borohydride ratio is 3:1. At 30 ppm total boron, we calculate the potassium borate and potassium borohydride concentrations to be 245 ppm to be 38 ppm respectively in the amine raw material sample in the NMR tube.

We have estimated the accuracy of this determination to be better than 100 +/- 2 % with a relative standard error of less than 5 % at these concentrations. Furthermore, our limit of detection is better than 3 ppm potassium borohydride with a 30 minute acquisition.

Sincerely,

T. Michael Rothgeb

Molly P. Armstrong

The NMR Newsletter - Book Reviews

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

"150 and More Basic NMR Experiments - A Practical Course"

by

S. Braun, H.-O. Kalinowski, S. Berger

2nd, expanded edition; Wiley–VCH, Weinheim-New York, etc.; 1998
ISBN 3-527-29512-7, $68.20 (paperback); available through John Wiley & Sons, Inc.
(http://www.wiley.com)

NMR spectroscopy applications keep expanding both vertically and horizontally; new (or renewed) methods and technologies are rising, and NMR data have become indispensable from oil-well characterization to combinatorial chemistry and quality control. It is likely that this expansion – as well as well-deserved market response to the first edition – inspired Siegmar Braun, Hans-Otto Kalinowski, and Stefan Berger (who is the corresponding author, now in Leipzig) to come up with the new edition of their book. It was less than a year ago when we could welcome the "100 and More Basic NMR Experiments" (The NMR Newsletter, 472, January 1998), and now there are "150 and More Basic NMR Experiments", their explanation, and implementation recipes in this new volume.

On 574 pages, there are now fourteen chapters, extended with the same valuable Appendices, Glossary and Index, as in the previous edition. Chapter 1, about the basics of an NMR spectrometer is now extended with gradient shimming. In chapter 2, about pulse calibration, there are new items discussing composite pulses and radiation damping. Also, a simple example illustrates the effect of relative phase settings of pulses and the receiver.

The standard test procedures in chapter 3 are spiced with a little aid in picking the right window function and in using spectrum simulation for data analysis, and the 13° phase stability test is added. The next chapter presents the same decoupling techniques as before, while the list of methods to determine dynamic parameters now includes the measurement of $T_{1\rho}$. This makes the bridge to the next chapter about multipulse 1D experiments, which starts with T_1 and T_2 measurements. This chapter consists mostly of heteronuclear editing techniques, including the DEPT-135 experiment, which is discussed separately. Purging with spin-lock pulses is a new item, and the list closes with non-gradient water suppression methods.

Experiments based on selective-pulses, mostly highly efficient derivatives of two-dimensional methods fill chapter seven, which is followed by the, perhaps most enhanced, chapter on auxiliary reagents, quantitative determinations, and reaction mechanisms. Added items are: more methods for determination of enantiomeric purity, ASIS from the early age of NMR, H/D exchange, isotope effects, pK_a determination with ^{13}C NMR, determination paramagnetic susceptibility by NMR, the CIDNP effect, ^1H and ^{13}C NMR of paramagnetic compounds, and a hint of NMR in liquid-crystal solutions – all very valuable. Heteronuclear (X other than carbon) 1D experiments are sampled in chapter 9, with the added interesting example of $^{47/49}Ti$ with probe-head ringing suppression.

continued

Two-dimensional experiments are introduced in chapter 10. E.COSY is now part of the presented toolkit, so are a homospoil (a.k.a. Poor Man's Gradient, PMG)-assisted HMBC and an example of X,Y correlations (^{31}P,^{13}C in our case). In accordance with their overwhelming importance, gradient techniques are divided into two, extended chapters (11 and 12); listing 1D and 2D experiments separately. More attention is paid to calibration procedures and, on about twice as many pages as in the first edition, most important gradient-assisted methods are introduced. Perhaps homonuclear multiple-quantum correlations of abundant spins could have fit here, too. A short journey to "3D-land" in the next chapter is now extended with a 3D HMBC, which is, however, used primarily as a 2D projection. The brand new chapter fourteen offers basic introduction to solid-state NMR applications.

This book was already an excellent tool in its first incarnation, and it has been improved significantly. It will attract primarily users in the field of small molecule structural studies, who will find it an excellent, very practical, thorough, and thoughtfully designed handbook, one of a kind. And don't worry if you have purchased the "100 and More..." volume a couple years ago; any laboratories with more than one person will benefit having both of these books around.

István Pelczer
Department of Chemistry
Princeton University
Princeton, NJ 08544

NIH POSTDOCTORAL POSITION AVAILABLE

A postdoctoral position is available for combined work in the Nuclear Magnetic Resonance Unit (Richard Spencer, Chief) and in the Laboratory of Cardiovascular Sciences (Edward Lakatta, Chief) of the National Institute on Aging of the National Institutes of Health, located in Baltimore, Maryland. The work will center on spectroscopic studies of myocardial metabolism related to i) congestive heart failure and ii) β-adrenergic stimulation of the heart. Other research opportunities may be available depending on the interests and background of the successful candidate.

NMR instrumentation consists of a double-resonance Bruker ABX 1.9T/31 cm Biospec with shielded gradients, and a triple-resonance wide-bore Bruker DMX 400 Avance system with microimaging and solids capability.

A background in NMR spectroscopy or imaging is preferred, although applicants with experience in muscle and cardiac physiology and a strong desire to learn NMR techniques are also encouraged to apply. The appointment will be as an IRTA Postdoctoral Fellow for US citizens, or as a Visiting Fellow for US non-citizens. Accordingly, applicants must have fewer than five years of postdoctoral experience.

Interested individuals should send their CV and the names, telephone numbers, and e-mail addresses of three references to: Dr. Richard Spencer, NMR Unit, NIH/NIA, GRC 4D-08, 5600 Nathan Shock Drive, Baltimore, MD 21224; Tel. 410-558-8226; e-mail: spencer@helix.nih.gov

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Magnet Type (Standard or shielded)	Standard		Standard	Standard		Standard	Standard	Actively Shielded	Actively Shielded	Actively Shielded	Actively Shielded
Field Stability (Hz/hour ^1H)	<2		<2	<3		<3	<15	<8	<10	<10	<10
Axial 5 Gauss Stray Field Contour (Metres)	1.81		2.65	2.19		2.75	4.2	1.5	1.8	1.8	2.5
Radial 5 Gauss Stray Field Contour (Metres)	1.42		2.0	1.7		2.2	3.3	1.0	1.3	1.3	1.75
Cryostat Type	Compact	T3	T3	Compact	T3	T3	T5	T3	T4FB	T4FB	T5FB
Minimum Helium Refill Interval (Days)	80	235	203	80	235	203	120	183	150	150	140
Helium Refill Volume (Litres)	26	79	68	26	79	68	101	62	83	83	120
Year Hold Cryostat Option Available	X	✓	✓	X	✓	✓	X	X	X	X	X
Nitrogen Refill Interval (Days)	14	14	14	14	14	14	22	14	15	15	14
Minimum Nitrogen Refill Volume (Litres)	32	61	61	32	61	61	135	61	81	81	136
* Minimum Operational Ceiling Height (Metres)	2.69	2.92	.2.92	2.69	2.92	2.92	4.16	2.9	3.1	3.1	3.16
System Weight (kg) Including Cryogen's	120	315	391	133	325	399	1050	400	610	625	1200

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

Room Temperature Shim Specifications

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

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NMR Instruments,
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S-100 44 Stockholm - Sweden

Stockholm, November 10th, 1998
(received 11/16/98)

Gradient homogeneity and PGSE diffusion measurements

Dear Dr. Shapiro,

it is well known that a uniform (and reproducible) B_0 gradient over the sample volume is required to achieve good accuracy/precision in diffusion measurements. Since the gradients are always somewhat inhomogeneous, a proper calibration procedure is regarded as a good practice. In some probes (like in the one we are using, a Bruker Diff25 probe, capable of delivering 24 G/cm·A) the gradient homogeneity region is smaller than the active volume of the rf coil, therefore the manufacturer suggests a maximum sample length of about 1 cm and recommends to accurately place the sample at the very center of the gradient coil. Reproducing a given sample length is often difficult, especially when dealing with viscous samples sticking to the tube walls, and centering the sample volume with respect to the coil requires great attention and carefulness (marking the position with a thin fiber pen and visually aligning the tubes not always is enough, to our experience); in addition, small samples require tedious, long shimming sessions. To overcome these problems a recent paper[1] suggests running measurements on long samples with the first pulse replaced by a selective pulse in combination with a slice selection gradient, so as to excite spins only in the region where the gradient is roughly constant. In that paper, diffusion-weighted 1D imaging was used to quantify the inhomogeneity of the gradient.

We applied a similar approach to map the homogeneity of the gradient in our probe, running a set of slice-selective PGSE experiments by varying the selective pulse offset frequency in order to span our sample volume. For the slice selection, we used a Gaussian pulse of ~2500 Hz half-width and a gradient of (nominally) 0.096 $T \cdot m^{-1}$ (the selectivity is ~0.6 mm in the z direction), while the "diffusion" gradient ranged from 0.2 to 1.2 $T \cdot m^{-1}$. The sample used was trace HDO in doped D_2O at a nominal temperature of 20.0 °C. In Figure 1 we present the normalized self-diffusion values. In Table 1 we report the D's for both a long (~50 mm) and a short (~9 mm) sample in comparison with the values yielded by a standard PGSE sequence (i.e., with hard pulses and no slice selection applied).

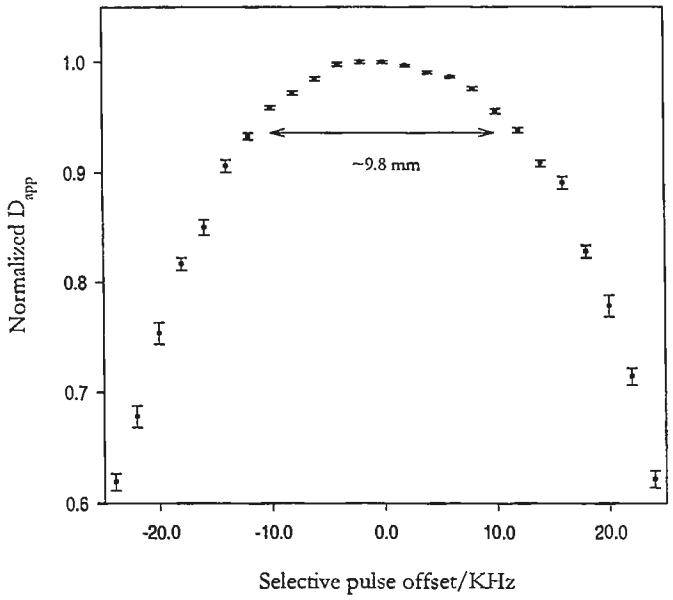

Figure1. Spatial distribution of the diffusion coefficient as a function of the selective pulse offset. The profile might be regarded as a convolution of the Gaussian shape of the pulse and the gradient profile across the sample. Error bars are ± s.

Our results for the long sample show that, within a region of about 1 cm around the center of the coil, the value of D has a variation of ~4%, that is the gradient varies by ~2% (in agreement with Bruker specifications); outside that region the gradient variation is even bigger. This is reflected in the large difference of the D values obtained by using the slice-selective experiment and the "hard" sequence. In the case of the short sample, on the other hand, the two values compare well. Finally, there is a small, yet significant, difference between the D values measured by the slice-selective

sequence in the long and in the short sample, which is most probably due to the effect of large static field inhomogeneities in parts of the short sample.

Table 1. HDO self-diffusion coefficients as measured on a long and a short sample by standard and slice-selective PGSE sequence

	Slice-selective PGSE	Standard PGSE
D±2s/1·10^{-9} m^{2}s^{-1} (long sample)	1.838±0.002	1.55±0.01
D±2s/1·10^{-9} m^{2}s^{-1} (short sample)	1.805±0.006	1.82±0.02

From this data we conclude that the slice-selective PGSE sequence performs better than its "hard" counterpart, in that it allows a region with smaller gradient inhomogeneity to be excited, thus giving greater accuracy at the expense of sensitivity. By applying such a sequence on long samples, besides avoiding the problems mentioned above for small volumes (time-consuming shimming, careful sample preparation and tube placement), one even gets a smaller convection (caused by temperature gradients) because of the larger axial ratio of the sample.

Sincerely,

Renato Barbieri
renato@physchem.kth.se

Niklas Hedin
nh@physchem.kth.se

P.S. Please, credit this contribution to Peter Stilbs' subscription

[1] Marcus L. Tillet, Lu-Yun Lian, and Timothy J. Norwood; *J. Magn. Res.* **133**, 379-384 (1998).

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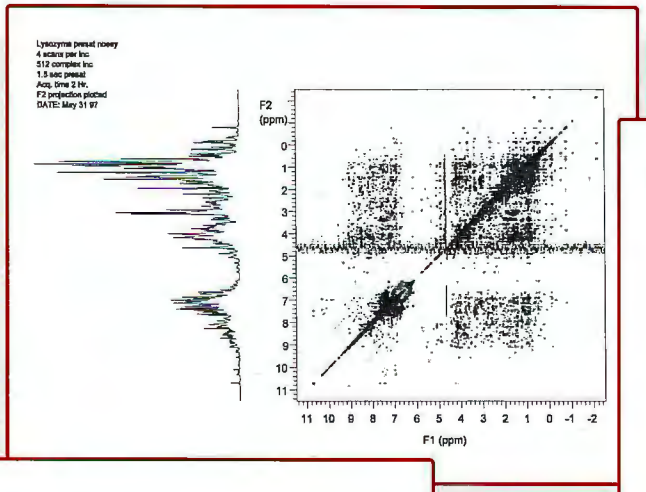
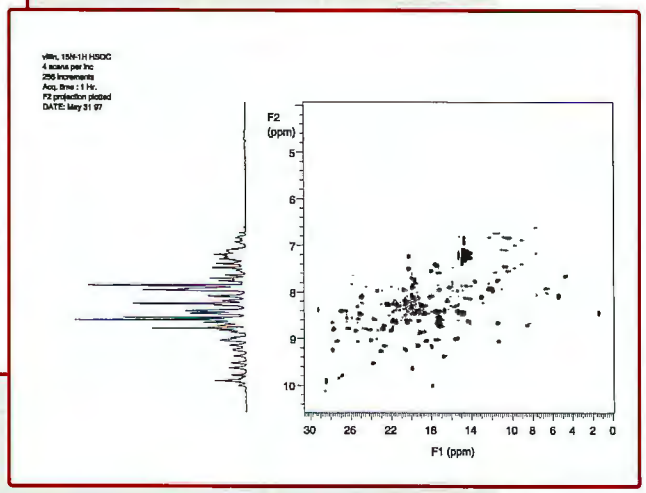
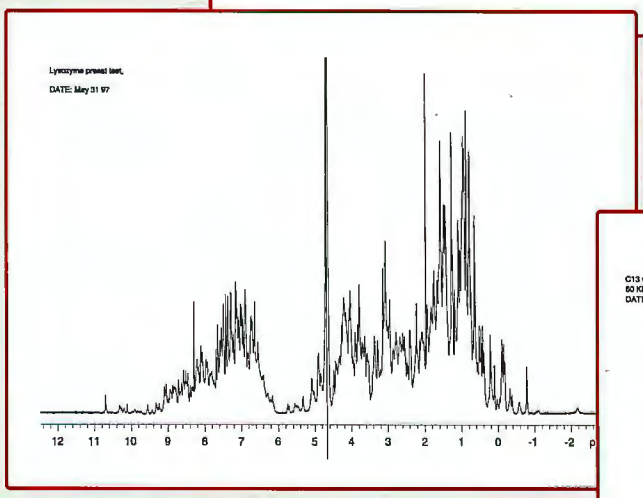
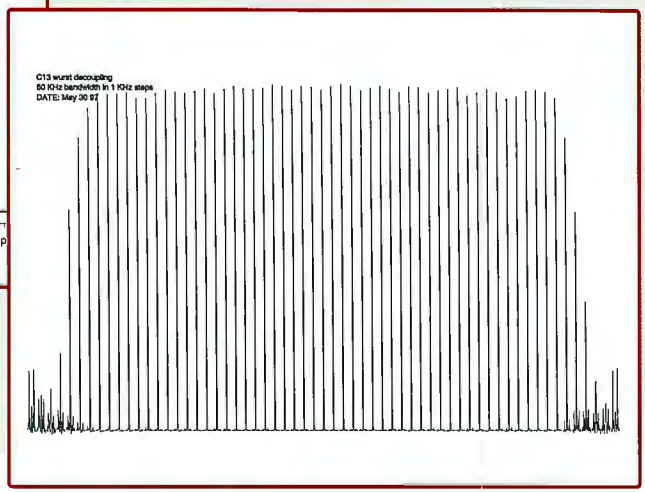


Multidimensional Heteronuclear NMR





Water Suppression Using Presaturation



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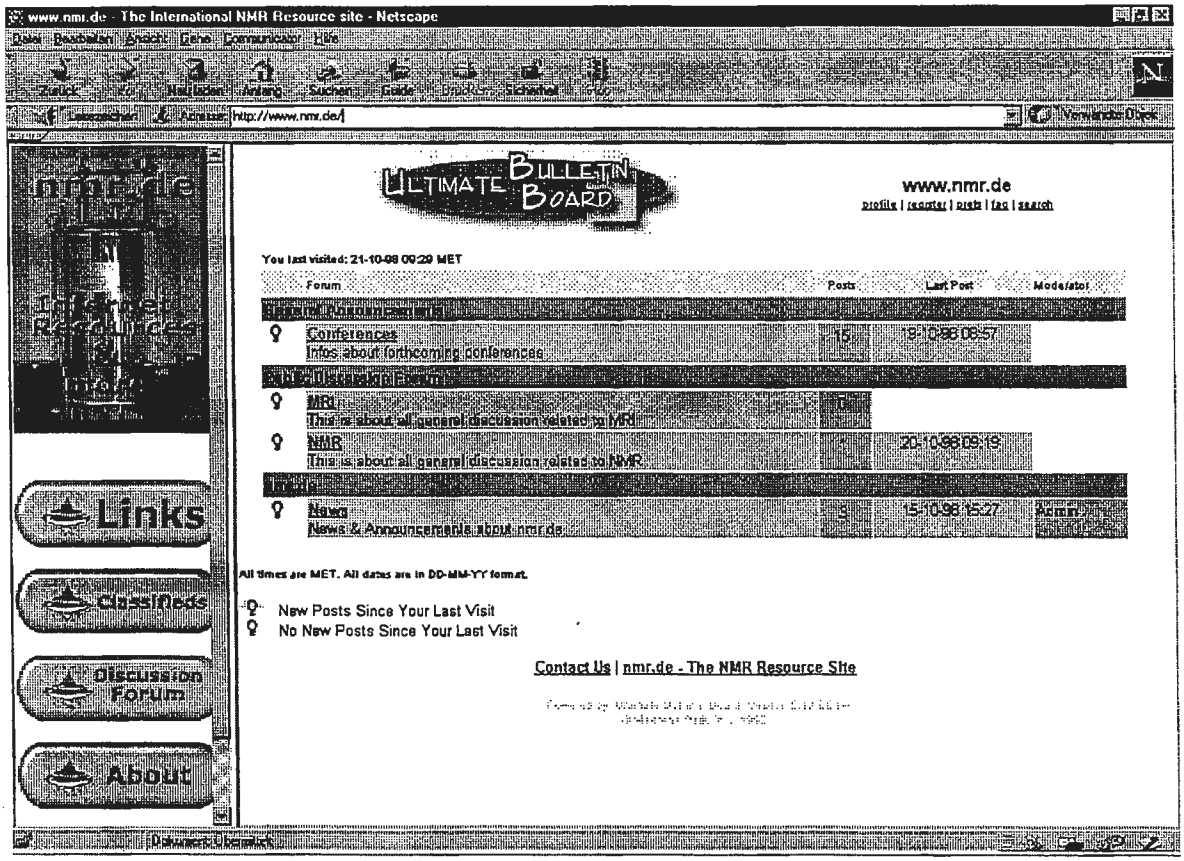
9. November 1998
(received 11/17/98)

New NMR Internet Resource Site

Dear Barry,

we just started a new WWW Site a few weeks ago which is devoted to NMR Resources on the Internet. The URL is: **http://www.nmr.de**



Currently there are 3 major sections : Links, Classifieds and the Discussion Forum.

The **Links Section** features currently more than 340 WWW Links, covering subjects like NMR education, institutes, companies, conferences, literature and much more. Visitors are encouraged to add their own links using a comfortable WWW form. Of course the links section is fully searchable and also tracks the most favourite pages. As a service to our visitors we validate all links at least once a week to ensure that there are no broken links.

In the **Classifieds Section** visitors can post ads either if they have jobs/post doc positions to offer or if they are looking for one. This service is of course free of charge and also fully searchable.

In the **Discussion Forum** visitors can discuss about any NMR topic they like. This section also features a list of forthcoming conferences, meetings etc.

In the near future we are planning to introduce several new sections on our site. Currently we are thinking about an FTP Site which mirrors several servers carrying NMR related material. Also a virtual library featuring educational documents is planned.

One key concept in the design of our site was to encourage user participation, so every section offers several ways to add to the site´s contents.

We are looking forward to you visiting our site and are always interested in feedback (comments, ideas, improvements etc.).

If you have any questions feel free to e-mail admin@www.nmr.de .

Best regards,

Horst Kessler Rainer Haessner Markus Busold

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(received 11/25/98)
November 19, 1998

^{31}P NMR Comparative Study of 3-Dimensional Ossifying vs. Non-Ossifying Cartilage Grown in a Hollow Fiber Bioreactor

Dear Barry,

We've been having an enjoyable time looking into the bioenergetics of neocartilage, which has provided some metabolic counterpoint to many of our previous studies of muscle. Unlike other tissues, mature cartilage is entirely avascular, and the bulk of its energy comes from anaerobic metabolism. Surprisingly, there has been little application of NMR spectroscopy to the exploration of cartilage bioenergetics. To further our understanding of energy metabolism in this unique tissue, we've been creating lactate maps, oxygen maps, and working with ^{31}P NMR of cartilage grown in a hollow fiber bioreactor. One of the first things we found is that there is a very limited literature on ^{31}P of cartilage (this fact alone renders it unique among tissues!). In fact, there are, to our knowledge, no published values for the T_1's of phosphorus-containing metabolites in cartilage. We now understand why--the signal-to-noise is terrible in most suitable samples, given the low cellularity of cartilage. However, the cellularity of the neocartilage system we're working with (Ref. 1) is quite large, and so we can get reasonable ^{31}P spectra within a semi-reasonable amount of time.

We were particularly interested in the immobilization of inorganic phosphate as developing cartilage tissue moves towards ossification. We looked at two systems: cartilage grown from the proximal sternum of the embryonic chick, which is known to ossify *in vivo,* and cartilage grown for the distal sternum, which remains cartilaginous.

First, we were able to confirm metabolic stability of the tissue over four weeks of growth. Second, we confirmed the presence of measurable PCr in the cells, indicating the possibility of a muscle-like energy shuttle. Third, and most interestingly, we found the following for T_1's, using progressive saturation, at 9.4 Tesla:

	A. Week One		B. Week Four	
	Non-Ossifying	Ossifying	Non-Ossifying	Ossifying
T_1 (Pi)	1.3± 0.18	1.2±0.26	1.2± 0.08	3.8± 0.06
T_1 (γ-ATP)	0.3± 0.13	0.5±0.21	0.3± 0.17	0.3±0.08
T_1 (α-ATP)	0.5± 0.21	0.6±0.26	0.7± 0.18	0.6±0.01
T_1 (β-ATP)	0.3± 0.21	0.5±0.11	0.4± 0.05	0.4±0.01

Table 1 : Spin Lattice Relaxation Times (T_1 ± SD) of Phosphorous Metabolites in 3-Dimensional Cartilage Tissue at One and Four Weeks of Growth in a HFBR. Non-ossifying refers to distal sternum, while ossifying refers to proximal sternum.

Typical spectra are shown below:

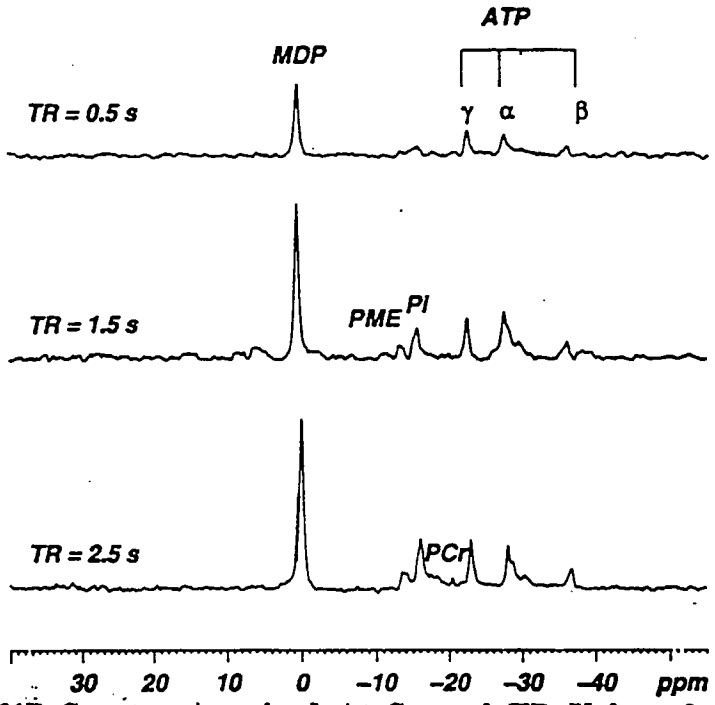

Figure 1 : ^{31}P Spectra Acquired At Several TR Values for A. Non-Ossifying and B. Ossifying Neocartilage at Week One of Growth in a HFBR.

The measured T_1 values of the ATP nuclei do not differ significantly with respect to tissue type or stage of maturation. However, in ossifying tissue only, the T_1 of inorganic phosphate (Pi) increases with development time. This increase is consistent with progressive immobilization of phosphate. Indeed, we believe that we are monitoring binding of phosphorous to calcium in the cytosol of chondrocytes. However, von Kossa staining failed to reveal any intracellular zones of calcification. Accordingly, the increase in the T_1 value of Pi with maturation which we report here may be indicative of an early stage of the ossification process.

REFERENCES:

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Potter K., Fishbein K.W., Horton W.E., and Spencer R.G.S. Morphometric Analysis of Cartilage Grown in a Hollow Fiber Bioreactor using NMR Microscopy. In *Spatially Resolved Magnetic Resonance: Methods, Materials, Medicine, Biology, Rheology, Geology, Ecology, Hardware*, 363-371, P. Blümler, B. Blümlich, R. Botto, and E. Fukushima (eds.), Wiley–VCH Press, Weinheim, 1998.

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Erik Petersen petersene@alpha.grc.nia.nih.gov
Kenneth Fishbein
Eric McFarland
Richard G. S. Spencer spencer@helix.nih.gov

NMR Unit
NIH/National Institute on Aging
Intramural Research Program
GRC 4D-08
5600 Nathan Shock Drive
Baltimore, MD 21224
Tel: 410-558-8226

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		low in paramagnetic impurities	30g	septum bottle	112.40
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			50g	ampule	76.15
			125g	Sure/Seal™ bottle	165.00
			250g	screw-cap bottle	205.00
			1kg	screw-cap bottle	609.00
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			500g	screw-cap bottle	207.25
			1kg	screw-cap bottle	406.00
34,716-7	99.9%	**Deuterium oxide**	100g	screw-cap bottle	79.00
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		contains 0.75 wt.% TSP-d_4	100g	screw-cap bottle	70.90
45,051-0	99.9%	**Deuterium oxide**	25mL	screw-cap bottle	23.50
		contains 0.05 wt.% TSP-d_4	100mL	screw-cap bottle	64.50
34,377-3	99.9%	**Deuterium oxide**	25g	screw-cap bottle	23.00
		contains 1 wt.% DSS	100g	screw-cap bottle	63.70
43,576-7	99%	**Deuterium oxide**	25g	screw-cap bottle	18.50
			100g	screw-cap bottle	51.50
43,577-5	90%	**Deuterium oxide**	25g	screw-cap bottle	17.50
			100g	screw-cap bottle	49.00
19,529-4	0%	**Water, deuterium-depleted**	10g	ampule	21.50
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NORTHWESTERN UNIVERSITY

Department of Chemistry
2145 Sheridan Road
Evanston, Illinois 60208-3113

November 25, 1998
(received 11/28/98)

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

Dear Dr. Shapiro:

Linear Prediction Backwards, a Step Further

Linear prediction capabilities are now standard in all NMR processing packages available from NMR and software manufacturers like Varian (*VNMR*), Bruker (*XWINNMR*), MSI (*FELIX, NMR PIPE*), and *NMRView* (for a review see NMR Newsletter **481**-19). This mathematical procedure has become standard in data processing of multidimensional NMR of biological systems. The primary use of this application in structural biology is in the form of backward and forward linear prediction of the direct and indirect detected dimensions, respectively. Linear prediction extends the number of points in an FID by calculating a function based on the real data points and then uses this function to extend or regenerate points at the beginning of, end of, or within the FID. Forward linear prediction increases the total number of points toward the end of the FID, therefore increasing the digital resolution in that dimension. Backward linear prediction is primarily used to reconstruct the beginning of an FID that might have been corrupted because of receiver overloads or pulse breakthroughs. The final result is an improved baseline, elimination of effects due to truncating the FID, and improved resolution.

One of the biggest drawbacks in observing nuclei such as ^{11}B, ^{19}F, and ^{27}Al is the probe background from coil inserts, sample tubes, rotor, and cap materials (Figure 1a,e and 2a). The DEPTH sequence (NMR Newsletter **468**-11) was applied to remove signals outside the rf coil in our DOTY 5 mm supersonic MAS probe. However, as has been reported, it has the drawbacks of baseline imperfections and even decreased peak intensities (Figure 1c). We found linear prediction to produce a less distorted spectrum (Figure 1b) without the disadvantages mentioned above. Application of linear prediction to the spectrum obtained from DEPTH yielded further improvement (Figure 1d).

The ^{11}B spectrum shown in Figure 2a is a mixture of compounds containing both boron and aluminum. Structural differences are manifested by different T_2 values. The broader component results from the borosilicate glass used in the coil inserts as well as the NMR tube. Application of backwards linear prediction once again proved very successful in the elimination of the broad components of the spectrum from both outside and inside the rf coil (the NMR tube). Of course minimization of the broad component also could be achieved by replacing the glass insert and the NMR tube by one made from quartz.

Further analysis of the spectrum of Figure 2a was done by backwards linear prediction. The results presented in Figure 3a-d shows how the spectrum can be reconstructed based on individual T_2 components. If one begins the linear prediction further up in the time domain of the FID, the narrow components of the spectrum can be selected and the spectrum simplified for further analysis. This procedure has obvious applications to biological systems containing components with widely different linewidths, including in higher dimensions.

The following are a few steps to ease the learning curve for lp for the two main spectrometer manufacturers.

Varian: To enable linear prediction, set the flag parameter proc="*lp*" instead of proc="*ft*". Linear prediction is performed before the Fourier transformation (wft or ft) and is based on the parameters defined under dglp (display guide linear prediction). If these parameters are not present one must create them with the commands parlp for ω1 (acquisition), parlp(1) for ω2, parlp(2) for ω3, etc.

Bruker: Enabling linear prediction on XWINNMR or UXNMR is done by setting the parameter ME-mod = *LPfr* (forward) or *LPbr* (backwards) prediction instead of ME-mod set to *no*. Type the command edp (edit processing parameters) and change this command.

Note: First point multiplication applied to all spectra, no baseline correction applied to any of the spectra, vertical scales and number of scans are identical for every sample.

Edwin Rivera
rivera@clinton.chem.nwu.edu

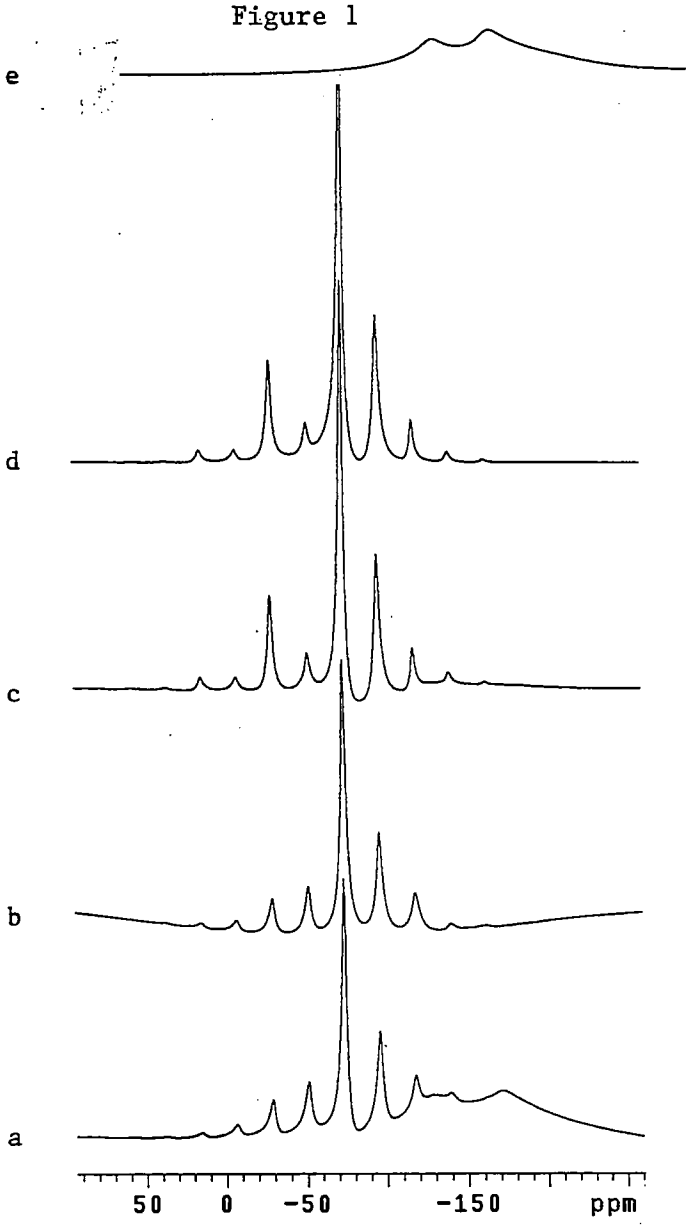
Catherine E. Shawl

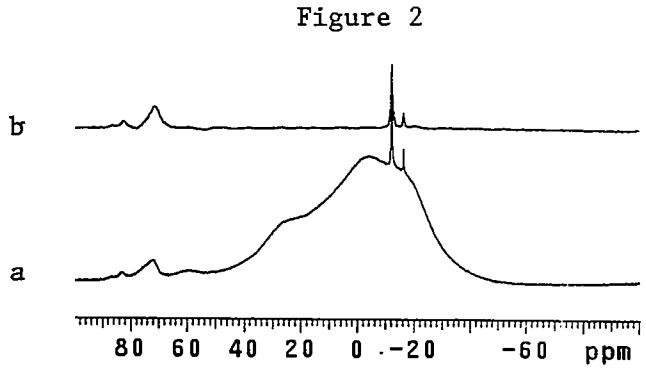
P.S. ^{11}B spectra provided by the generosity of Professor SonBinh Nguyen.
P.P.S. Please credit this contribution to the account of Professor Joseph B. Lambert.

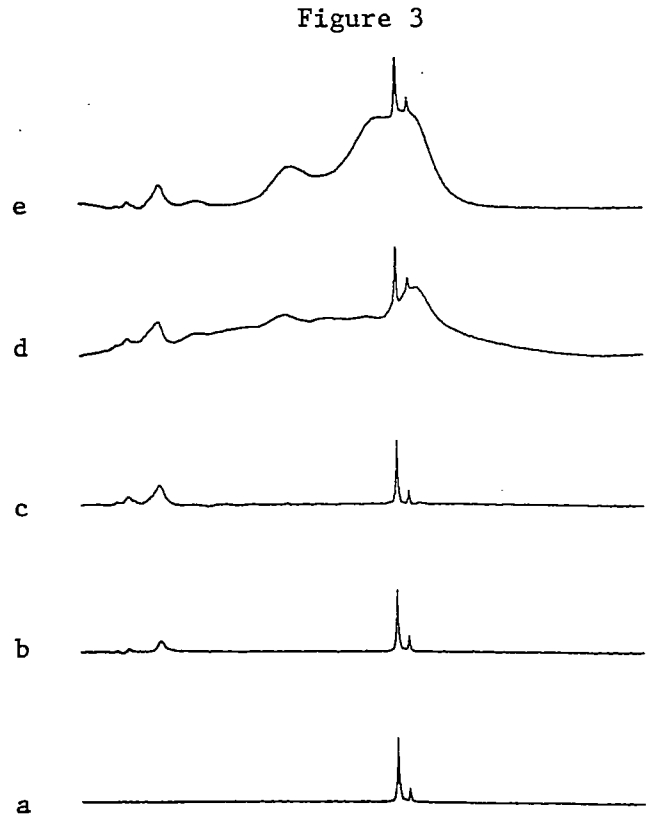
Figure 1. **^{19}F 282.171 MHz.** $AgCF_3CO_2$ packed in a 5 mm supersonic Silicon Nitrite/Vespel rotor/caps spun at 8070 Hz, referenced to $CFCl_3$. (a) Bloch decay with no linear prediction. (b) Spectrum after backward linear prediction of 32 points using 1024 points from the FID. (c) Spectrum with DEPTH composite pulse sequence. (d) Spectrum c with after linear prediction of 32 points using 1024 points from the FID. (e) Probe background.

Figure 2. **^{11}B 128.318 MHz.** Sample (a) No linear prediction. (b) Linear prediction of the first 29 points using 512 FID points for reconstruction. First point multiplication applied to all spectra, no baseline correction applied to any of the spectra.

Figure 3. **^{11}B 128.318 MHz.** (a) Linear prediction of 128 points. (b) Linear prediction of 75 points. (c) Linear prediction of 32 points. (d) Linear prediction of 20 points. (e) Linear prediction of 8 points.







Carleton UNIVERSITY

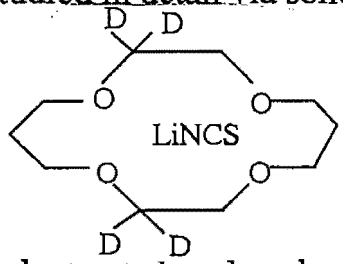
Department of Chemistry
203 Steacie Building
1125 Colonel By Drive
Ottawa, ON K1S 5B6 Canada
Tel: (613) 520-3841
Fax: (613) 520-3749/3830

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

Oct 21, 1998
(received 11/13/98)

Title: Large Amplitude Motion in a Solid 14-Crown-4. LiNCS Complex

Alex Driega has prepared the title molecule and examined it via ^{13}C CPMAS NMR which showed a dipolar dephased spectrum which is essentially identical to its normal ^{1}H decoupled spectrum. This is indicative of high amplitude molecular motion and is reminiscent of our results on 18-crown-6(1) and 12-crown-4 complexes(2) which we studied in detail via solid state ^{2}H methods.



Hence we prepared the deuterated analog shown above and have (in collaboration with Glenn Facey) recorded its ^{2}H spectra as a function of temperature . Our interpretation of the results is complicated somewhat by the fact that Differential Scanning Calorimetry shows the presence of two phase transitions below the melting point of 216°C. It is our view, however that a type of "molecular merry-go-round" may again be operative here but a detailed analysis has not yet been completed.

(1) C.I. Ratcliffe, J.A. Ripmeester, G.W. Buchanan and J.K. Denike. J. Am. Chem. Soc. **114**, 3294 (1992).
(2) C.I. Ratcliffe, G.W. Buchanan and J.K. Denike. J. Am. Chem. Soc. **117**, 2900 (1995).

G.W. Buchanan
Professor and Chairman

BANG

BANG is the Bay Area NMR Group, a ultra-informal bunch of people with interests in some aspect of NMR. *BANG* holds meetings at irregular intervals somewhere in the Bay Area (the San Francisco bay, natch). The meetings consist of a brief social time followed by dinner, before a talk is presented.

Announcements of the program and dinner site are sent out by email and posted at http://www.acornnmr.com/bang.htm. Everyone is most welcome to join - just send your email address to **gina@acornnmr.com** and you will be added to the emailing list. There is no fee associated with being on the mailing list. (The dinner is self-sponsored.)

Those who have the misfortune of not living in the the Bay area are most welcome to participate - we get lots of visitors, so if you are visiting an instrument vendor, or are on sabbatical, or just passing through, please join us.

Even better, how about volunteering to give a talk? We don't pay, but a good free dinner and incredibly good company are provided.

BANG has no affiliation with Acorn NMR, Inc.

Address all Newsletter correspondence to:

Dr. B. L. Shapiro
The NMR Newsletter
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650-493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

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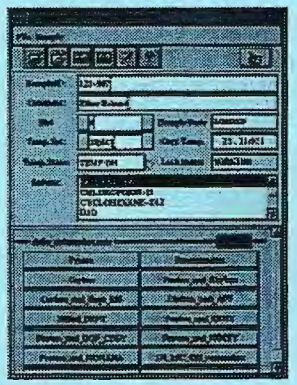

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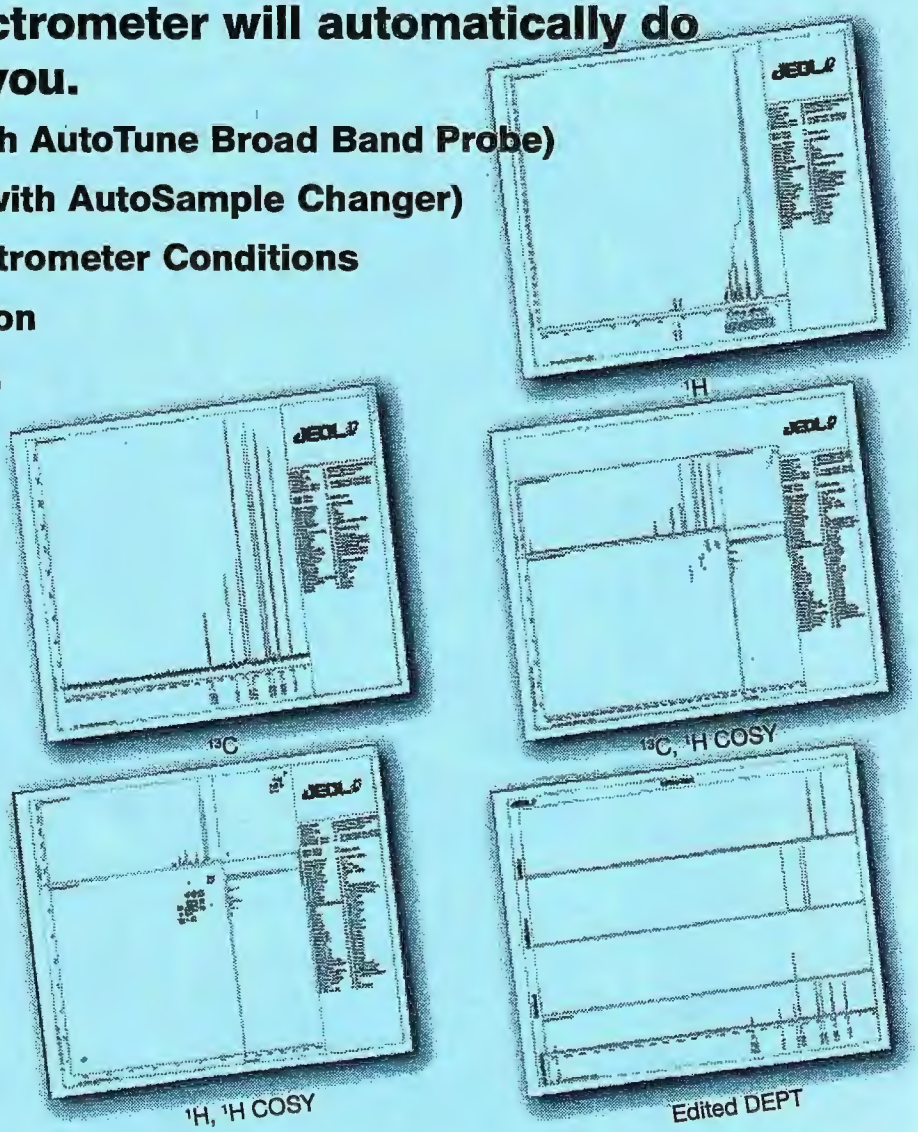

JEOL USA, Inc., 11 Dearborn Road, Peabody, MA 01960
Tel: (508)535-5900 Fax: (508)536-2205
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