SMASH 2002 NMR Conference

Breckenridge, Colorado

September 15th - 18th, 2002

SMASH 2002 NMR Conference

SMASH Attendees,

On behalf of the SMASH Organizing Committee, we would like to welcome you to Breckenridge for the SMASH-2002 conference. We are pleased to note that every year SMASH is growing in popularity among NMR spectroscopists. This meeting serves as a forum not only to discuss new developments and their applications, but also to be educational. After the devastating events of September 11th, 2001, which resulted in the cancellation of SMASH 2001, the organizing committee decided to invite again the SMASH 2001 speakers. We have sessions that span a variety of topics including software development, data management, applications, etc. To encourage the continued growth of our young scientists, we have a post-graduate session. There are 4 workshops planned that we hope will be of value for both seasoned and novice spectroscopists.

We hope that you enjoy this program and thank you for your continued support and interest in SMASH.

Sincerely yours,

Krish Krishnamurthy & Thomas Williamson
Co-Chairs, SMASH 2002 NMR Conference

SMASH 2002 NMR Conference
Program

Sunday

5:00 PM - 6:00 PM **Registration**
6:00 PM - 8:00 PM **Dinner**
8:00 PM -11:00 PM **Mixer**

Monday

7:00 AM - 8:15 AM **Breakfast**
8:15 AM - 8:30 AM **Opening Remarks**
8:30 AM - 10:00 AM **Combinatorial/HTS - Cathy Moore**

- *"NMR Characterization of Ligand-Receptors Interactions in Complex Macromolecular Systems"*
  Maurizio Pellecchia, The Burnham Institute
- *"Alternative Plumbing Schemes for Varian's VAST Flow-NMR System"*
  Tim Spitzer, GlaxoSmithKline
- *"Pushing the Limits with Small Volume NMR"*
  Jonathan Sweedler, University Illinois

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Advances in Software - Mike Bernstein**

- *"SpecSolv - Fully Automated Structure Elucidation Including One Dimensional ^{1}H-NMR and ^{19}F-NMR Spectra"*
  Walter Maier, BASF
- *"In Metabolic Hyperspace, No-One Can Hear You Scream"*
  Russell Mortishire-Smith, Merck
- *"NMR Web Services as Tools for High Throughput Pharmaceutical Analysis"*
  Dave Detlefsen, Novatia

12:00 PM - 1:30 PM **Lunch**
1:30 PM - 3:00 PM **Workshops (Concurrent)**

- I. Data Management - Mike Bernstein
- II. FDM - A.J. Shaka

3:00 PM - 5:30 PM **Free Time**
5:30 PM - 6:00 PM **Pre-Dinner Social Gathering**
6:00 PM - 7:30 PM **Dinner**
7:30 PM - 8:30 PM **Speaker: Dan Traficante**
"Carbon-13 NMR Spectroscopy: It Was Not Always Easy"
8:30 PM - 11:00 PM **Mixer**

Tuesday

7:00 AM - 8:30 AM **Breakfast**
8:30 AM - 10:00 AM **Pulse Sequences - R. Thomas Williamson**

- *"Can Small Molecule NOE Difference Be Improved in the Short T_2 Case?"*
  J. Shaka, University of California, Irvine
- *"A Critical Review of Heteronuclear Long-Range Correlation Experiments"*
  Ole W. Sørensen, Carlsberg Laboratory
- *"NMR Experiments on the Solvation of Bioactive Compounds"*
  Stefan Berger, Universität Leipzig

10:00 AM - 10:30 AM **Break**

SMASH 2002 NMR Conference Program

10:30 AM - 12:00 PM **Gradients and Diffusion - Brian Antalek**

- *"Coping with Convection in Gradient Based Experiments"*
  Adrian Davis, Pfizer
- *"Mixture Analysis using NMR Diffusion Measurements"*
  Cynthia K. Larive - University of Kansas
- *"Pulsed Field Gradient NMR (PFG-NMR), Diffusion Ordered NMR Spectroscopy (DOSY), and Applications to Polydisperse Systems"*
  Charles Johnson, Univ. of North Carolina

12:00 PM - 1:30 PM **Lunch**

1:30 PM - 3:00 PM **Carbohydrates – C. Allen Bush**

- *"Multinuclear NMR Conformational Sudies of Sugar Ring-Modified Nucleosides and Oligonucleotides"*
  Joseph Barchi, NIH-NCI Frederick
- *"Major Advantages for the Primary Structural Elucidation of Oligosaccharides Through NMR Spectroscopy Combined with Peracetylation using Doubly-^{13}C-Labeled Acetyl Groups"*
  Brad Bendiak, University of Colorado
- *"Conformations of Oligosaccharides from Residual Dipolar Couplings"*
  C. Allen Bush, University of Maryland

3:00 PM - 3:30 PM **Break**

3:30 PM - 5:00 PM **Workshops (Concurrent)**

- I. Optimizing Gradients and Decoupling - Ron Crouch
- II. Not So FAQ's – Steve Cheatham and Ron Crouch

6:00 PM - 6:30 PM **Pre-Dinner Social Gathering**

6:30 PM - 11:00 PM **Poster Session with Buffet Dinner followed by Mixer**

Wednesday

7:00 AM - 8:30 AM **Breakfast**

8:30 AM - 10:00 AM **Post-Graduate Session - Mari Smith**

- *"Chemical-Shift Resolved One-Dimensional Experiments for the Measurement of Diffusion Coefficients and Longitudinal Relaxation"*
  Niko Loening, MIT
- *"Is the Binding Constant Really a Constant? Effects of Experimental Parameters on the Results of NMR Diffusion Measurements"*
  Laura Lucas, University of Kansas
- *"Kinetic Studies of Ring Inversion of Paramagnetic Non-Planar Porphyrinato Complexes of High- and Low-Spin Iron(III) by NMR Techniques"*
  Liliya Yatsunyk, University of Arizona
- *"Spectral Processing Methods to Extract Imaginary Frequencies from Nuclear Magnetic Resonance Signals"*
  Joseph E. Curtis, University of California

10:00 AM - 10:30 AM **Break**

10:30 AM - 12:00 PM **Tips & Techniques - Pat Stone-Wilkinson**

- *"Some Important Misconceptions Concerning Data Processing for FIDs"*
  Daniel Traficante, NMR Concepts
- *"Linear Prediction: Getting the Maximum from the Minimum"*
  George Crull, Bristol-Myers Squibb
- *"Methodologies for Determination of Absolute Configuration by NMR: Tips and Techniques"*
  Frank Koehn, Wyeth Ayerst

12:00 PM - 12:15 PM **Closing Remarks**

12:15 PM - **Box Lunch and Departure**

SMASH 2002 NMR Conference
Acknowledgements

The SMASH 2002 Conference gratefully acknowledges the support provided by the following companies.

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Monday, September 16th 8:30 AM - 10:00 AM

Combinatorial/HTS

Cathy Moore, Session Chair

Speakers:

Maurizio Pellecchia, The Burnham Institute

Tim Spitzer, GlaxoSmithKline

Jonathan Sweedler, University of Illinois

NMR Characterization of Ligand-Receptors Interactions in Complex Macromolecular Systems

Maurizio Pellecchia, Ph.D.

The Burnham Institute, 10901 North Torrey Pines Rd. La Jolla, CA 92037.

NMR spectroscopy has evolved into a major technique in support of structure-based drug design, and has the key advantages of being able to detect and quantify interactions with high sensitivity. Here, I survey recent developments in high-resolution NMR spectroscopy, that enable structural characterization on both the target and ligand to aid subsequent optimization of weak-binding hits into high-affinity leads, even when working with very large receptors.

Alternative Plumbing Schemes for Varian's VAST Flow-NMR System

Timothy D. Spitzer, Andrea M. Sefler, John F. Sefler, Randy D. Rutkowske, and George F. Dorsey

GlaxoSmithKline

Flow-injection NMR offers a convenient means to analyze the large numbers of samples generated by combinatorial chemistry. Our group has been investigating ways to optimize the performance of our flow-NMR system to improve sample throughput. The changes we made fall into two categories: hardware and software. We have looked at alternative plumbing schemes in an effort to allow faster sample handling while minimizing contamination from sample carry-over. Since we are free to make significant changes to the plumbing, we can modify the sample pathway, the direction of flow, and the extent of solvent rinsing and air purging. Each change to the plumbing requires changes to the controlling software. Fortunately, it is fairly easy to customize the software scripts to accommodate our hardware changes.

Some of our experiences to date will be discussed.

Pushing the Limits with Small Volume NMR

Jonathan V. Sweedler, Andrew Wolters, Dimuthu Jaywickrama, Andrew Webb

University of Illinois

Despite the unparalleled structural information content of nuclear magnetic resonance (NMR) spectroscopy, a relatively low sensitivity significantly restricts its use for small-volume mass-limited samples. However, the development of miniaturized radiofrequency coils enables the acquisition of high-resolution NMR spectra from picomole quantities in nanoliter volumes. For static samples, the use of microcoil probes to characterize the reaction products cleaved from individual combinatorial chemistry beads is presented. Structural elucidation of these (frequently isomeric) products is critical to their successful application. Since these analytes are present in minute quantities, efforts to join NMR microprobes with optimal sample handling strategies are described. Through its enhanced sensitivity, this type of microcoil probe provides a means to ease the bottleneck associated with the need for high-throughput structural characterization of combinatorial products.

The combination of nanoliter-volume NMR probes and microseparations is a logical enhancement to static analysis. While microcoils probes have been linked to capillary HPLC and capillary zone electrophoresis, this presentation focuses on the union of NMR and capillary isotachophoresis (cITP). By electrophoretically concentrating dilute microliter-volume samples into nanoliter-volume bands, cITP extends the effective concentration range for microcoil NMR experiments. In addition to concentration factors of up to two orders of magnitude, cITP offers the capability to separate charged components in mixtures so that purified analytes are efficiently presented to the NMR detector.

Through the development of microcoil probes for both static analysis and on-line detection of capillary separations, mass-limited samples in complex matrices are becoming viable samples for characterization by NMR. With emerging advances in hardware, the boundaries for applications that require high NMR detection sensitivity continue to recede.

Monday, September 16th 10:30 AM - 12:00 PM

Advances in Software

Mike Bernstein, Session Chair

Speakers:

Walter Maier, BASF

Russell Mortishire-Smith, Merck

David Detlefsen, Novatia

SpecSolv - Fully Automated Structure Elucidation Including One Dimensional ^{1}H-NMR and ^{19}F-NMR Spectra

Walter Maier*, Winfried Fachinger, Joachim Richert

BASF AG

Automated structure elucidation of organic compounds has become state of the art for a few years by now. Most structure elucidation packages use ^{13}C NMR chemical shifts and 2D-NMR correlations simultaneously with the molecular formula information as a basis of for the structure generation process. Almost all systems depend on hand-picked NMR information.

SpecSolv has been developed with the goal of fully automated structure elucidation for high throughput without molecular formula information. It can be fed directly by one dimensional datasets from ^{1}H-, ^{13}C- and ^{19}F-NMR measurements.

Fluorine is an effective nucleus for NMR measurements. Short acquisition times, large shift ranges and far reaching coupling constants allow convenient access to substructure information. Substructures can be used as good-list or bad-list entries to speed up the automated interpretation process.

Examles for the application of automated computer based NMR interpretation will be given.

In Metabolic Hyperspace, No-One Can Hear You Scream

Russell J. Mortishire-Smith

The Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories
Terlings Park, Harlow, Essex CM20 2QR, UK

Much recent attention has been given to the analysis by NMR of biofluids such as urine and bile, in order to elicit information about the mode of action or mechanism of toxicity of novel xenobiotics. NMR spectra of biofluids are information rich, and exhibit a large dynamic range. The analysis method of choice is presently multivariate statistics (usually principal component analysis, PCA), but this technique has a number of drawbacks. Specifically, PCA is poor at highlighting small but consistent perturbations of low concentration components in the presence of bigger variability in the concentrations of abundant components (hippurate, citrate etc). NMR spectra are also typically data reduced down to 256-512 intensity variables, again limiting the contribution of low concentration components to PCA separation.

One (old fashioned) approach to addressing this problem is visual inspection of data. Here, the challenge is the comparison and assimilation of tens or hundreds of complex NMR spectra. To facilitate analysis, we adopt a combined approach in which NMR spectra are data reduced to 2K points, and imported into Spotfire, together with the results of PCA performed on the same spectra reduced to 256 points. Comparison across time, dose, sex etc is readily achieved via Spotfire's selection tools. This approach will be exemplified with data acquired as part of a toxicology investigation into a hepatotoxin of unknown mechanism.

NMR Web Services as Tools for High Throughput Pharmaceutical Analysis

David J. Detlefsen, Jeffrey L. Whitney & Mark E. Hail

Novatia, LLC

As pharmaceutical analytical requirements expand and accelerate, the need for development and integration of high throughput methods grows. Web services are one way to address R&D's increasing appetite for analytical information whereby the product of NMR spectroscopy (the experimental data and instrumental capabilities) can be made available via an application programming interface (API) such as XML-RPC. Casting NMR as a web service and focusing development on methods suitable for web service delivery creates opportunities for increased participation in high throughput pharmaceutical research today and provides a route for integrating NMR into larger multi-instrument analytical solutions that are being considered for tomorrow. In addition, since much of the data management and integration requirements are off-loaded to external business logic containing procedures, the information management requirements at the instrument are minimized as a result of the transactional nature of web services. Here we will present some of the technology and concepts involved and our efforts to develop a prototype NMR web services to support automated structure confirmation.

Monday, September 16th 7:30 PM

Dinner Speaker

Sponsored by Cambridge Isotopes Laboratories

Daniel D. Traficante
University of Rhode Island

Carbon-13 NMR Spectroscopy: It Was Not Always Easy

Carbon-13 NMR Spectroscopy: It Was Not Always Easy

Daniel D. Traficante

Department of Chemistry and NMR Concepts
University of Rhode Island
Kingston, Rhode Island

Carbon-13 Fourier transform NMR (FT-NMR) is now performed routinely in most chemical laboratories and is generally considered among the most powerful tools for determining the structure of organic molecules - but it was not always this way. Not long ago, ^{13}C spectra were obtained in the continuous-wave (cw) mode, and time averaging was performed in the frequency domain, rather than in the time domain, using a computer of average transients (CAT). Even proton decoupling was difficult to perform at that time. And if it was done at all, single-frequency proton decoupling was employed - broadband techniques had not yet been developed. This talk will review the difficulties experienced during that time, with a view toward providing historical appreciation of the development of the field as well as some insight into the reasons many experiments are performed using current methods. Sweeping, instrument maintenance, probe modifications, proton decoupling, and time averaging are among the topics of discussion.

Tuesday, September 17th 8:30 AM - 10:00 AM

Pulse Sequences

James Keeler, Session Chair

Speakers:

A. J. Shaka, University of California, Irvine

Ole W. Sørensen, Carlsberg Laboratory

Stefan Berger, University Leipzig

Can Small Molecule NOE Difference Be Improved in the Short T_2 Case?

A.J. Shaka

Chemistry Department
University of California
Irvine, CA 92697-2025

For a number of years now, the conventional steady-state NOE difference experiment has been largely superseded by the Double Pulsed Field Gradient Spin Echo (DPFGSE) NOE method in many laboratories. The advantage of the DPFGSE-NOE method is mainly that much cleaner spectra, free from subtraction artifacts, can be obtained. However, there are a couple of potential drawbacks to the method. First, when a wide line is chosen as the "target" in the midst of narrower resonances, large T_2 losses may accrue, limiting sensitivity. Secondly, examination of the initial build-up curves reveals multiplet distortions that are not attributable to the usual culprits (i.e. SPT effects, zero quantum, etc.). We have overcome both these problems with a new set of experiments and in the process have discovered some interesting subtleties that arise even with weakly coupled spin systems. The build-up curves we obtain now are close to ideal, making the NOE experiment more useful for quantitative distance measurements in rigid systems, and perhaps even quite important for flexible systems, where long-range coupling constants can be "iffy".

A Critical Review of Heteronuclear Long-Range Correlation Experiments

Ole W. Sørensen

Carlsberg Laboratory, Denmark

Many variations of the basic HMBC experiment have been proposed. The lecture will discuss in elementary terms the elements of this family of pulse sequences and describe guidelines for what versions to use under different circumstances. Experiments of the HMBC-type providing information about homo- and heteronuclear long-range coupling constants will also be covered. Finally, INADEQUATE-type experiments with off-resonance compensation and enhanced sensitivity will be presented.

NMR Experiments on the Solvation of Bioactive Compounds

Dolores Diaz, Marco Fioroni and Stefan Berger*

Institute of Analytical Chemistry
University Leipzig, Germany

Solute solvent interaction has been investigated for decades by many different experimental and theoretical methods. Here we report on intermolecular homo- and heteronuclear NOE measurements (1D DPFGSE-NOE), DOSY NMR techniques and MD calculations to study conformational effects of solvation. As model compounds of interest we have chosen carbohydrates, peptides and other small molecules. As solvents, DMSO, water, and trifluoroethanol (TFE) in various mixtures have been studied. The question of preferential and site specific solvation have been addressed. For small peptides complete surface covering by TFE in TFE/water mixtures is demonstrated both experimentally and theoretically.

M. Dolorez Diaz, S. Berger Carbohydrate Research 2000, 329,1-5.

M. Dolores Díaz and Stefan Berger. Magn. Reson. Chem. 2001, 39, 369-373.

E. J. Cabrita, S. Berger Magn. Reson. Chem. 2001, 39 S142-S148.

M. D. Díaz, M. Fioroni, K. Burger, S. Berger, Chem. Eur. J. 2002, 1663-1669.

M. Fioroni, M. D. Díaz, K. Burger, S. Berger, J. Amer. Chem. Soc. in press.

Tuesday, September 17th 10:30 AM - 12:00 PM

Gradients and Diffusion

Brain Antalek, Session Chair

Speakers:

Adrian Davis, Pfizer

Cynthia Larive, University of Kansas

Charles Johnson, University of North Carolina

Coping with Convection in Gradient-Based Experiments

Clare-Louise Evans[2], Gareth A Morris[2] and Adrian L Davis[1]

1. Pfizer Global R&D, Sandwich, UK
2. Dept of Chemistry, University of Manchester, UK

A number of authors have recently identified the deleterious effects of convection in NMR experiments utilising pulsed field gradients. We will review methods for reducing the impact of convection in pulsed field gradient spin echo experiments, and in particular look at simple alternatives that can improve the accuracy of DOSY measurements, and enhance the performance of gradient shimming at elevated temperatures.

Mixture Analysis using NMR Diffusion Measurements

Laura H. Lucas, William H. Otto and Cynthia K. Larive

University of Kansas

The high information content of NMR makes it one of the premier instrumental techniques for molecular characterization. In some cases, it is even possible to use NMR for the analysis of complex mixtures without effecting a separation. However for mixtures of even modest complexity, NMR analysis based on chemical shift and spin-spin coupling alone is insufficient to completely describe the system of interest. In such cases, NMR diffusion measurements can provide an additional dimension of information. We have developed a modified PFG-NMR experiment, GOSE (gradient modified spin-echo), which selectively detects isolated (i.e. singlet) resonances in complex NMR spectra. Results will be presented illustrating the utility of GOSE diffusion measurements for the analysis of model compounds and complex environmental samples such as aquatic humic substances. An alternative experimental approach provides enhanced spectral selectivity for mixtures by combining the homonuclear 2D-J and diffusion pulse sequences. By taking advantage of chemical shift resolution and differences in spin-spin coupling patterns, the 2D-J-DOSY experiment permits the resolution of diffusion coefficients of resonances that are overlapped in the standard ^{1}H NMR spectrum. The ability of this experiment to resolve diffusion coefficients even for molecules with very similar structures and diffusion properties will be demonstrated using a mixture of glucose-6-phosphate and sucrose.

Pulsed Field Gradient NMR (PFG-NMR), Diffusion Ordered NMR Spectroscopy (DOSY), and Applications to Polydisperse Systems

Charles S. Johnson, Jr.

University of North Carolina

The principles of diffusion measurements by means of NMR spectroscopy will be discussed,(1) and data analysis and display for diffusion ordered spectroscopy (DOSY) will be illustrated.(2) Two recent, challenging applications will be discussed. First, the characterization of polydisperse samples of water soluble monolayer protected gold clusters with DOSY and NMR relaxation methods will be described.(3) Then, the application of PFG-NMR to the dynamics of surfactants in liquid CO_2 will be used to illustrate chemical exchange effects.(4) These illustrations show that the amount of information in the diffusion dimension is limited and must be used with great care.

References:

1. C.S. Johnson, Jr., "Diffusion Measurements with Magnetic Field Gradient Methods," in Encyclopedia of NMR, Wiley, New York, 1626-1644 (1995).

2. C.S. Johnson, Jr., "Diffusion Ordered Nuclear Magnetic Resonance Spectroscopy: Principles and Applications," Prog. NMR Spectros. 34 (3-4), 203-256 (1999).

3. Olaf Kohlmann, Wayne E. Steinmetz, Xi-An Mao, W. Peter Wuelfing, Allen C. Templeton, Royce W. Murray, Charles S. Johnson, Jr., "NMR Diffusion, Relaxation, and Spectroscopic Studies of Water Soluble, Monolayer Protected Gold Nanoclusters," J. Phys. Chem. B, 105, 8801-8809 (2001).

4. Judith B. Cain, Kewei Zhang, Douglas E. Betts, Joseph M. DeSimone, and Charles S. Johnson, Jr., "Diffusion of Block Copolymers in Liquid CO2: Evidence of Self-Assembly from Pulsed Field Gradient NMR, " J. Amer. Chem. Soc. 120, 9390-9391 (1998).

Tuesday, September 17th 1:30 PM - 3:00 PM

Carbohydrates

C. Allen Bush, Session Chair

Speakers:

Joseph J. Barchi, Jr, NIH-NCI

Brad Bendiak, University of Colorado

C. Allen Bush, University of Maryland

Multinuclear NMR Conformational Studies of Sugar Ring-Modified Nucleosides and Oligonucleotides

Joseph J. Barchi, Jr.

National Cancer Institute (NCI), Frederick

The use of nucleosides and nucleoside analogues as therapeutic agents has a long and prosperous history in anticancer and antiviral drug discovery. Recently, several groups have synthesized nucleosides containing modified sugar ring scaffolds that bias the "glycone" conformation to assume either a North (A-DNA like) or South (B-DNA like) ring pucker. We have prepared several of these analogues that contain a fluorine atom in the ring and studied their structures with both ^{1}H and ^{19}F NMR, in particular using coupling constant analysis and NOE data. The NMR data, supplemented by high level ab initio calculations, was used to explore the pseudorotational equilibrium sampled by the modified sugars. In particular, we have tried to define the precise roles played by the highly electronegative fluorine atoms in defining the sugar conformations. In addition, derivatives based on a novel bicyclic [3.1.0] template, that essentially "locks" the five-membered ring into a specific pucker, have been prepared and studied by NMR. The conformational analysis of these modified monomers and of nucleic acid oligomers where these analogues have replaced a number of standard base pairs will be discussed. The structural consequences on DNA local topology will be highlighted.

Major Advantages for the Primary Structural Elucidation of Oligosaccharides Through NMR Spectroscopy Combined with Peracetylation using Doubly-^{13}C-Labeled Acetyl Groups

Brad Bendiak, Tammy T. Fang, David N.M. Jones

Departments of Cellular and Structural Biology and Pharmacology
University of Colorado Health Sciences Center

The use of NMR spectroscopy for the elucidation of larger carbohydrate structures isolated from natural sources is limited principally by severe overlap of proton signals, poor sensitivity when experiments involve ^{13}C nuclei, and difficulties in conclusively establishing glycosidic linkage positions. Peracetylation of oligosaccharides with doubly-^{13}C-labeled acetyl groups provides several major advantages for their structural elucidation when combined with specifically tailored NMR pulse sequences. The 2.5-4.7 Hz J-coupling between acetyl carbonyl-^{13}C nuclei and protons of the sugar ring at the sites of acetylation enables these sites to be readily assigned. By inference, glycosidic linkage positions on monosaccharides can be unambiguously determined. This can be used in lieu of permethylation analysis for this purpose, but does not require degradation of oligosaccharides. Spectral dispersion in the directly-detected proton dimension is increased about 2.6-2.7-fold due to the downfield shifting of sugar ring protons at the positions of acetylation. Peracetylation also intoduces three new frequency dimensions for NMR studies, namely the ^{13}CO, ^{13}CMe and ^{1}HMe frequencies of the acetyl groups. These frequencies can be correlated to sugar protons, either independently or in combination, in alternative 2D, 3D or 4D experiments. The use of Hartmann-Hahn coherence transfer combined with zero-quantum dephasing periods permits purely absorptive in-phase multiplets to be extracted and enables accurate scalar couplings between ring protons to be measured, even in multidimensional experiments. Studies with different oligosaccharides will be discussed.

Conformations of Oligosaccharides from Residual Dipolar Couplings

C. Allen Bush

University of Maryland-UMBC

Dipolar coupling, distinct from scalar coupling which is generally more familiar to most chemists depends both the distance r^3 separating the two nuclei in the molecule and on the angle Q defining the direction of the vector joining the two interacting magnetic dipoles with respect to the static magnetic field. In solution NMR the molecular motions result in isotropic variation in time of the orientation averaging $< 3\cos^2 -1 >$ to zero and no static contribution to the dipolar coupling interaction remains. But in the presence of certain mixtures that form liquid crystals which can be oriented in the magnetic field, a small degree of orientation of the oligosaccharide can occur and a small residual dipolar interaction can be detected. Oligosaccharides are conveniently oriented in various liquid crysalline media, such as DMPC/DHPC mixtures and a number of different types of couplings have been measured using ordinary high-resolution NMR pulse sequences. For oligosaccharides which adopt compact rigidly folded epitopes, it is possible to measure more ^{13}C-^{1}H one-bond dipolar couplings than the five required for calculation of the orientation tensor directly from the experimental data and highly accurate conformational models can be constructed. Long range ^{1}H-^{1}H and ^{13}C-^{1}H couplings can also be measured providing additional data neccessary for modeling oligosaccharides having internal motion. For this latter case, molecular models must be built for each contributing conformer and the orientation tensor for each is calculated from its moment of inertia tensor. The experimental dipolar coupling can then be calculated as the ensemble average of the conformers.

Wednesday, September 18th 8:30 - 10:00 AM

Post-Graduate Session

Sponsored by Bio-Rad Laboratories
Informatics Division

Mari Smith, Session Chair

Speakers:

Niko Loening, MIT

Laura Lucas, University of Kansas

Liliya Yatsunyk, University of Arizona

Joseph Curtis, University of California

Chemical-Shift Resolved One-Dimensional Experiments for the Measurement of Diffusion Coefficients and Longitudinal Relaxation

Nikolaus M. Loening[1]* , Michael J. Thrippleton[2], and James Keeler[2]

1. Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA
2. Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

We have found that, with the use of spatially selective pulses along with the application of a read gradient during signal acquisition, it is possible to encode various parameters (such as diffusion coefficients) into the spectral lineshapes in the directly-detected dimension of an NMR experiment. If a very weak read gradient is used, it is possible to acquire this information while still preserving the chemical-shift. This allows for the determination of a parameter for a number of spectral peaks in a single one-dimensional experiment. This results in a significant reduction in experiment time as, instead of a two-dimensional experiment, only a single experiment (or in some cases a single scan) is required to determine the desired information. We will demonstrate how this methodology can be used for measuring diffusion coefficients and longitudinal relaxation times.

Is the Binding Constant Really a Constant? Effects of Experimental Parameters on the Results of NMR Diffusion Measurements

Laura H. Lucas and Cynthia K. Larive

Department of Chemistry, University of Kansas, Lawrence, KS 66045

The drug discovery process relies on characterizing structure-activity relationships, since specific ligand-receptor interactions often result in important biological functions. Noninvasive spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy offer the advantage of probing such interactions without disrupting the binding equilibrium. Binding over a broad range of affinity constants can be quantitatively analyzed by measuring diffusion coefficients with pulsed-field gradient NMR experiments, where signal decay as a function of gradient amplitude is related to translational diffusion. This is a useful way to study binding, because the ligand diffusion coefficient changes significantly when it interacts with a macromolecular target. Diffusion is a molecular property, and thus the same diffusion coefficient should be obtained regardless of the specific NMR signal monitored. However, we have discovered that for binding equilibria, the results obtained can depend on the experimental parameters selected. The effect of experimental parameters on measured diffusion coefficients will be presented for multiple ligand-receptor systems of differing binding affinities, and the implications of such results on the affinity constants measured for unknown systems will be discussed.

Kinetic Studies of Ring Inversion of Paramagnetic Non-Planar Porphyrinato Complexes of High- and Low-Spin Iron(III) by NMR Techniques

Liliya Yatsunyk, F. Ann Walker

University of Arizona

Octaalkyltetraphenyl porphyrinato iron(III) complexes are good models for the heme centers in many proteins. Extensive NMR studies were done on the series of axially ligated octamethyltetraphenyl (FeIIIOMTPP+), octaethyltetraphenyl- (FeIIIOETPP+) and tetrabutanotetraphenyl (FeIIITC6TPP+) porphyrinato iron(III) complexes with the following axial ligands: chloride, 4-dimethylaminopyridine, 1-methylimidazole, 2-methylimidazole, cyanide and t-butylisocyanide. A variety of 1D and 2D techniques were applied to make a complete peak assignment for all listed complexes (COSY, NOESY, ROESY). The main questions of interest were i) the ground state electron configuration of the six coordinate complexes ($(dxy)^2(dxz, dyz)^3$ vs $(dxz, dyz)^4(dxy)^1$), and ii) the flexibility of the porphyrin ring and the factors that have the main influence on it. All complexes but the one with t-butylisocyanide have the more common $(dxy)^2(dxz, dyz)^3$ electronic ground state. In the course of the experiments the activation parameters for ring inversion DH‡, DS‡, and DG‡ were determined for eight complexes. The bulkiness of the axial ligands and the substituents on the periphery are the main factors that define the rate of porphyrin ring inversion

Spectral Processing Methods to Extract Imaginary Frequencies from Nuclear Magnetic Resonance Signals

Joseph E. Curtis*[1], Niko Loening[2], James Keeler[3], Geoffrey S. Armstrong[1], Vladimir A. Mandelstam[1], and A. J. Shaka[1]

1. Department of Chemistry, University of California, Irvine, USA
2. Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge,Massachusetts, USA
3. Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

New nuclear magnetic resonance spectroscopic and signal processing methods have been developed that focus on the extraction of spin diffusion and relaxation information from the imaginary frequencies inherent in a complex multidimensional time signal. A new diffusion-ordered spectroscopy experiment with extremely high diffusion coefficient resolution will be described. This method may be useful in the elucidation of chemical equilibria in complex mixtures. The method relies upon a new spectral estimation algorithm named iRRT that was invented to generate stable 2D diffusion-ordered spectra that are superior in regions of spectral overlap. A review of the algorithm and examples of the method applied to heterogenous mixtures will be shown.

Wednesday, September 18th 10:30 AM - 12 PM

Tips & Techniques

Pat Stone-Wilkinson, Session Chair

Speakers:

Dan Traficante, NMR Concepts

George Crull, Bristol-Myers Squibb

Frank Koehn, Wyeth Ayerst

Some Important Misconceptions Concerning Data Processing for FIDs

Daniel D. Traficante

University of Rhode Island

The two most commonly used data processing techniques that are applied to an FID are linear prediction methods and multiplication by a window function. Each technique has its own advantages and disadvantages. For example, linear prediction is extremely useful when applied to short data sets, which are usually encountered in the t1 domain in 2D experiments. The standard Fourier transform of such data sets will produce not only very low resolution, but can also produce "sinc wiggles" around each spectral line. However, when the signal-to-noise ratio (S/N) is low, resonance lines may change their positions and two closely spaced lines could blend into one. In addition, these programs are notorious for taking a long time to perform the calculations. On the other hand, when the data set is long, as it is in normal 1D experiments or in the t2 domain in 2D experiments, then the standard Fourier transform is the method of choice. In these cases, window functions are safely, easily, and quickly applied, and they can be used to significantly improve the appearance of a spectrum. Although these functions are often used, there are several misconceptions concerning their advantages and disadvantages. For example, it is commonly believed that if an FID has been truncated, then multiplication by a function that multiplies down the tail of the FID to eliminate sharp corners, will eliminate the "sinc wiggles" that accompany truncated FIDs. Another very common misconception, is that window functions can be used to increase either the S/N or the resolution, but always one at the expense of the other. This talk will examine these misconceptions, as well as the properties of several popular functions.

Linear Prediction: Getting the Maximum from the Minimum

George B. Crull

Bristol-Myers Squibb

The need to increase spectrometer throughput may necessitate a compromise between digital resolution and signal to noise. In this presentation a tool for enhancing digital resolution, linear prediction, will be discussed. Because linear prediction is applied to extend the time domain signal post-acquisition, it does not increase the demand of the spectrometer. A five-fold increase in sample throughput can be realized by the careful application of linear prediction. To achieve this timesaving, experiments are performed while anticipating the utilization of linear prediction.

Suggestions for the optimization of the number of coefficients and the number of predicted points will be shown for the most common sequences used in structure elucidation. Specific examples from pharmaceutically relevant compounds will be shown for COSY, TOCSY, NOESY, HMQC, HSQC and HMBC experiments. The optimal balance between the number of scans and the size of the indirect domain data table is different for these experiments. Linear prediction is available from all the vendors and is implemented in some off-line processing software packages. Implementations vary significantly and references to specific parameters will be discussed. Examples of the mis-application of linear prediction will be briefly presented.

Methodologies for Determination of Absolute Configuration by NMR: Tips and Techniques

Frank E. Koehn[1], R. Thomas Williamson[1], Ana C. Barrios Sosa[2]

1. Discovery Analytical Chemistry
2. Medicinal Chemistry
Chemical Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965

The assignment of absolute configuration of both simple and complex chiral molecules is a significant challenge. The most widely used method for configurational assignment of chiral monoalcohols and monoamines is the Mosher NMR method and its modified versions, which are based on the ring current effect of a covalently introduced aryl moiety. While significant improvements in this method have been reported in terms of novel auxilliary reagents and procedures, the preparation and NMR measurement of Mosher type derivatives frequently involves significant effort in chemistry and purification, along with milligram quantities of substrate. We describe here recently developed high resolution NMR, LC-NMR-MS and chemical methods which enable the facile preparation, NMR measurement and chiral assignment of Mosher-type derivatives in sub-milligram quantities.

Tuesday, September 17th 6:30 PM - 9:30 PM

Poster Session

Sponsored by Varian, Inc.

Gregory Nemeth, Session Chair

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24. Enantiomeric Identification Using Natural Abundance High Resolution Modified ^{1}H-^{13}C Heteronuclear NMR Spectroscopy (HRMHSQC) in a Chiral Liquid Polypeptide Crystal Solvent

25. Deceptive Proton NMR Spectra

26. Dipico-7, a Chelating Polydentate Ligand with Unusually Complex Variable Temperature Behavior

27. New Developments in PERCH

28. Application of a Mechanical Liquid Nitrogen Generation Device to Superconducting NMR Magnets

29. Solution Structure and Orienting Interactions of Small Peptides and Amides in an Aqueous Liquid Crystal

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35. Structural and Conformational Study of GBR 12909 and its Amino Analogue by NMR and Fluorescence Spectroscopy

36. $^{1}H/^{11}B$ Chemical shift correlations: Is HETCOR still the best?

37. Structure Elucidation of Phormidamide A, a Novel and Cytotoxic Metabolite from the Marine Cyanobacterium Phormidium sp.

38. Reliable Proton NMR Prediction

1 Using ^{19}F-NMR for Metabolite Profiling

Andrea M. Sefler

GlaxoSmithKline

Metabolite profiling of biological fluids is typically performed using a radiometric detector on an HPLC system. The use of a radiolabelled parent drug permits easy detection of drug-related materials within a complicated biological matrix. For fluorinated drugs, the fluorine atom can serve the same purpose for NMR as the radiolabel serves in the HPLC. The ^{19}F-NMR spectrum of the biological matrix will show only drug-related materials as there are typically no fluorinated species present in animals and humans aside from fluoride ion. The potential of this technique for drug development will be illustrated.

2 Parallel flow NMR / LC-MS - A New Hyphenated Analytical Technique for High Throughput Structural Characterisation

Keith I. Burton[1] and Richard A. E. Edden[2]

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2. University Chemical Laboratory, Lensfield Rd,Cambridge, CB2 1EW

A new automated system has been developed to obtain spectroscopic data on large numbers of compounds in a timely manner with minimum sample preparation and handling. The use of a dual-injection autosampler to prepare and inject samples to a flow NMR instrument and a LC-MS instrument, simultaneously, allows parallel acquisition of LC-MS and NMR spectra from a single solid sample. Utilising a separate HPLC system for each instrument increases the functionality of both instruments while maintaining the independence of each technique.

Due to all of the sample preparation, manipulation and injection being carried out by a single autosampler attached to both instruments, parallel flow NMR/LC-MS is less demanding in terms of analyst and instrument time than separate operation of two traditional systems. The time to acquire both NMR and LC-MS data is equivalent to traditional methods of acquiring LC-MS data only; this leads to a large time saving when analysing samples produced from combinatorial/ HTS chemistry. The methodology of the equipment will be presented, together with results produced using this technique and details of the automation steps that have been taken to submit samples to the instrument and manipulate the resulting data.

3 Complex Use Of Computational And Experimental Methods To Determine The Spatial Structure Of Germacranolides Of Sahodin And Sahosin

B.F.Rasulev, M.G.Levkovich, N.D.Abdullaev, I.D.Sham'yanov

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The spatial structure of the natural sesquiterpene lactones sahodin and sahosin is investigated by the methods of NMR spectroscopy and computer modeling.

Geometrical modeling of all possible conformations of the ten-membered cycle of the germacranolides sahodin and sahosin revealed 102 vectorially possible structures. By the methods of molecular mechanics (MM+, HyperChem 6.01) and analysis of steric pressure, the quantity of permitted possible conformations was reduced to 7. Comparison with NMR spectral data has given the opportunity to allocate a unique conformation of the cycle in solution at room temperature. This structure has shown reasonably good convergence with the X-ray data for sahosin.

The complete scheme of coupling constants and NOE-interactions in the NMR spectra is established. The comparison of the geometry of the modeled low-energy conformations with NMR data given satisfactory consent between the theoretically designed coupling constants with the experimentally observable ones, except, for the signals of the H-6 and H-7 protons. The possible reasons for the deviation of the NMR parameters established by standard technique were considered. Recommendations for the observed coupling constants and some other conformational characteristics of similar compounds are given.

4 NMR and Other Analytical Data Integration, Analysis and Reporting

Michael A Bernstein and Marc Litherland

We have been interested in the distribution of NMR (and other analytical) data to chemists at AstraZeneca Charnwood. This started with managing the large data volumes associated with NMR data from 96 WP, DI spectroscopy. A software programme was produced that facilitates the analysis and reporting of this and conventional single-spectrum NMR data. Central to the design is the provision of tools for interrogating the NMR data whilst preserving access to the primary spectra. This tool was named *Spadez* and has achieved wide acceptance.

We now report on progress to widen the scope of the programme. The initial objective is inclusion of LC/MS data so these can be used together with NMR data to make conclusions on purity, concentration, and structure. This has led to a versatile, open architecture that can be customised by the addition of enabling applets. The driving technology is based on data being represented by XML, and SOAP used to make programme calls.

We shall demonstrate how this technology forms an integrated environment that is highly customisable. Enabling applets typically require a small amount of programming in almost any language. It is especially simple to access data from databases, active-server pages, and “enabling” software. With the data held in XML, reporting is a strong feature using XSLT.

The “basic” Spadez module code can be constant and improvements and customisation becomes a relatively trivial process. This flexible environment should allow for maximum use of in-house programmer resources and use of purchased software.

5 NMR: Facilitator for the Validation of Quantitative Separation Techniques Used in the Analysis of Illicit Heroin Samples

Patrick A. Hays and Ira S. Lurie

U.S. Drug Enforcement Administration, Special Testing and Research Laboratory, Dulles, Virginia

The accurate determination of the levels of heroin and its alkaloids in illicit heroin samples is of great importance to the Drug Enforcement Administration (DEA) for intelligence purposes. The relative abundance of these alkaloids versus heroin assists chemists at the Special Testing and Research Laboratory in assigning a heroin processing classification to the sample (i.e., Southeast Asian, Southwest Asian, South American, or Mexican).

NMR was used for the quantitative analysis of heroin and papaverine during the validation of a new quantitative heroin method that used dynamically coated capillaries for capillary electrophoretic analyses (CE). The results of several heroin samples analyzed using NMR, CE, and HPLC are presented. NMR was also used as a preliminary screening method to quantitate and identify compounds in the samples and to determine the definitive purity of the reference drug standards used by the CE and HPLC methods.

6 Magic Angle Spinning ^{1}H NMR Spectroscopy of Intact Mouse Spinal Cord: Metabolic Changes Following Induction of Experimental Allergic Encephalomyelitis

Eva R. Durant, Jeremy K. Nicholson, Sandra Amor, Paul Smith, Ute Gerhard, Russell J. Mortishire-Smith and Andrew W. Nicholls.

High resolution magic angle spinning (HRMAS) ^{1}H nuclear magnetic resonance (NMR) spectroscopy has been used to investigate alterations in the biochemical profile of intact spinal cord caused by experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). The spinal cords from control and EAE-induced mice were divided into 6 equal regions and analysed using both one-dimensional (1D) and two-dimensional (2D) HRMAS NMR spectroscopic experiments. The 1D NMR spectra were analysed using multivariate statistics to effect classification and generate novel metabolic biomarker information. Based on data from the statistical analysis further evaluation of the NMR spectra indicated an overall decrease in the level of myo-inositol, glutamate and g-amino-N-butyric acid in the samples from the EAE-induced mice, concomitant with increases in taurine, glutamine and glycine. These metabolic alterations may result in changes in neurotransmitter activity and hence could explain some of the neurological symptoms found in EAE. These studies highlight the potential of HRMAS-NMR simultaneous characterisation of the levels of a wide variety of endogenous metabolites in biological tissue, enabling differentiation of animals experiencing a disease condition and providing evidence for the mechanistic underpinnings.

7 Small Volume NMR: Non-Flow NMR in the Microliter Scale

Till Kühn, Michael Fey, Oskar Schett, Michael Assmann, Matthias Köck, Julian Griffin, Goetz Schlotterbeck, Alfred Ross, Hans Senn

Over the past few years there has been a significantly growing demand for miniaturization in all areas of modern research and development. Evoked by many exciting methods such as ultra high throughput screening or an increasing interest in metabolism studies and natural product analysis there is a need for analytical methods which require less amounts of sample.

Here we present a revolutionary NMR probe design. The new probe operates with disposable 1mm capillary sample tubes. The active volume of 2.5 microliters enables the use of lowest amounts of sample to run all high-resolution NMR experiments with outstanding sensitivity and under complete automation.

On this poster we present different application examples. Applications include structural analysis of marine natural products in the nanogram to microgram range, the synthesis control of microgram samples from parallel synthesis or the study of limited amounts of body fluid from laboratory animals.

8 Off-line SFC-NMR-Coupling: Structure Evaluation of a By-product of the Vitamin-A-Acetate Synthesis

G. Krack

BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany, Department of Analytics

Besides the well known cis-isomers and kitoles (intermolecular Diels-Alder-Adducts) the mother liquors of the vitamin-A-acetate (VAA) synthesis contain a further intramolecular cycloaddition product that has been isolated by packed column SFC and characterized off-line in a microtube by 2D-NMR techniques.

9 The Application of Metabonomics in a Pharmaceutical Drug Discovery Environment

Gregory C. Leo*, William Hageman, Becky Cascaden, Aaron Krikava, Carlos Cotto, Jeff Hall, Kristen Synder, Beata Starosciak, Daine Gauthier, and Gary Caldwell

In this study we investigated the applicability of the metabonomics approach in a pharmaceutical drug discovery environment as an filter for selecting new compound entities. Initial efforts were made to establish control spectra for normal, healthy rats. Secondly, known hepatotoxins, a-naphthylisothiocyanate (ANIT) and thioacetamide were tested in rats to validate our methods. For ANIT, doses of varying concentration (25, 50, 100 and 150 mg/kg) were administered to rats (4 rats per dose) to observe the time course trajectory as a function of dose in the principle components analysis (PCA). Finally, the knowledge gained from analyzing known toxins, thus led us to evaluate how metabonomics might reduce the attrition rate of drug discovery compounds. Our results revealed that control spectra vary as a function of time for different sets of rats. A set of healthy rat urine spectra could fall outside the limit of the PCA model for healthy rats when the model was developed from a set of control spectra collected earlier in the year from another set of rats. Our ANIT study confirmed previous reports and extended those studies showing the utility of doing full dose response curves. Dose dependence studies are important when one goes on to consider drug discovery compounds. Drug discovery compounds were chosen that had undesirable side effects in rats but that were not toxic in the therapeutic dose ranges administered. Using a PCA scores plot to view the data, the PCA coordinates for the treated rats were difficult to distinguish from the pre-dose controls' coordinates. The difference between the controls and the treated rat urines could be more readily observed if a principal components model was generated from the pre-dose urine for comparison with the treated urine. The magnitude of changes observed for the compounds that exhibited side effects were much less than those caused by ANIT or thioacetamide. Thus, the large separations observed in the literature for toxins relative to a healthy control group were not observed for our set of drug discovery compounds. A serious limitation is compound availability since it is important for these types of experiments to be performed in a dose dependent manner. Based on our studies, metabonomics has utility in a drug discovery environment for distinguishing those compounds that alter normal metabolism if applied with due care.

10 A Little LC-NMR Using a Simple Probe

Claus Cornett[1], Heidi Dorte Jensen[2,3], Karen Angelika Krogfelt[3], Steen Honoré Hansen[1], Søren Brøgger Christensen[2]

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Analysis of the hydrophilic fraction of cranberry juice by reversed phase HPLC over an Aqua LUNA column revealed the presence of quinic acid, malic acid, shikimic acid, citric acid, and two iridoid glucosides. The two iridoids were by MS and NMR spectroscopy shown to be monotropein and 6,7-dihydromonotropein. No iridoids have previously been found in the juice of cranberries and 6,7-dihydromonotropein is a new natural product.

Keywords: Cranberry Juice; LC-NMR; LC-MS; quinic acid; malic acid; shikimic acid; citric acid; iridoid glucosides; monotropein; 6,7-dihydromonotropein

11 The Use of ^{19}F NMR Spectroscopy in the Profiling of Biological Matrixes for Metabolites

Gregory S. Walker, Gregory L. Weber, Larry C. Wienkers

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One of the principle responsibilities of a drug metabolism unit in the pharmaceutical industry is accounting for the dose of a new chemical entity (NCE) in the excreta of pre-clinical species and human subjects. This is typically accomplished by HPLC analysis of the matrixes followed by either radiochemical or mass spectrometric detection. With radiochemical detection, the expense and effort of synthesizing a radiolabled compound often can delay this process until late in the development stages of a drug candidate. When a mass spectrometer is used as a detector, metabolites may have wildly differing responses relative to the parent compound. This may lead to erroneous conclusions about the amount or even presence of metabolites in a given sample.

An alternative to these approaches is the use of fluorine NMR spectroscopy. The small number of fluorinated endogenous compounds in biological matrixes, the wide spectral range and selectivity of ^{19}F NMR, the relative sensitivity, the uniform response of the ^{19}F nuclei and the lack of sample preparation make fluorine NMR an ideal "detector" for drug analysis in complex matrixes. Additionally, the use of the ^{19}F nuclei as a metabolic screening probe can be employed for the analysis of in vitro incubations during early discovery.

12 Solution-State Dynamic Nuclear Polarization at a High Magnetic Field

Nikolaus M. Loening* and Robert G. Griffin

Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

The goal of dynamic nuclear polarization (DNP) is to enhance NMR signals by transferring electron spin polarization to the nuclei. Although mechanisms such as the solid effect and thermal mixing can be used for DNP in the solid state, currently, the only practical mechanism in solutions is the Overhauser effect (OE), which usually arises due to dipolar relaxation between the electrons and the nuclei. At magnetic fields greater than ~1 T, dipolar relaxation does not result in useful enhancements and therefore the conventional wisdom is that DNP should not work in solutions at high magnetic fields. However, scalar relaxation due to time-dependent scalar couplings has a different magnetic field dependence and can lead to substantial OE enhancements. Here we show that, at room temperature and at a magnetic field of 5 T (211 MHz for protons, 140 GHz for electrons), electron-nuclear scalar relaxation results in NMR signal enhancements in the range of 10–200 for several different nuclear spin species.

13 Applications of ^{19}F NMR in Solid-Phase Organic Synthesis

Paul Krolikowski[2], Andrew Allen[4], Mark Drew[4] , Joseph Salvino[3], Edward Orton[1] and Vasant Kumar[1]

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^{19}F NMR has been widely applied to study chemical reactions in solid-phase organic synthesis[1,2]. In the present study, we illustrate the usefulness of this technique to study the loading of 4-hydroxy-2,3,5,6-tetrafluorobenzoic acid (TFP) to amino-methyl polystyrene during the synthesis of a novel activated resin for chemical library synthesis. Once the TFP loading is quantitated, the ^{19}F resonances from TFP were used to monitor subsequent reactions on the solid support.

In a related application, we have incorporated a ^{19}F probe into the backbone of polystyrene resin and used it as an internal standard for quantitation of reaction yields. As an example, this approach was used to optimize conditions for a series of additions of a fluorine containing Michael adduct on a solid support. This methodology has also been demonstrated for other reaction types.

1. Manatt, S., et.al. (1980) A Fluorine-19 NMR Approach for Studying Merrifield Solid-Phase Peptide Synthesis. Tetrahedron Letters, Vol. 21, 1397-1400.

2. Stones, D., et.al. (1998) A Method for the Quantitation of Resin Loading Using ^{19}F Gel Phase NMR Spectroscopy and a New Method for Benzyl Ether Linker Cleavage in Solid Phase Chemistry. Tetrahedron Letters, Vol. 39, 4875-4878.

14 Unusual Relaxation Properties of Squarate Hydroxamates

Martha D. Morton, Nathaniel C. Lim, Christian Bruckner and Hilary A. Jenkins

Though the chemistry of squarate esters and amides is well developed, little is known about the corresponding hydroxamates. The unusual properties of these derivatives extend from binding properties of these compounds to unusually long ^{13}C T_1 relaxation times.

15 LC-NMR Characterization of Minor By-products Produced in the Synthesis of Oxazatwistanes from Quinidine

Steve Cheatham, Eric Taylor, Boiana Budevska, Ed Silveira and Dawn Pierce

In addition to their biological activities, the Cinchona alkaloids are well known for their role as asymmetric catalysts.[1] Oxazatwistanes derived from quinidine are conformationally defined and could be expected to have better enantiomeric selectivity than the parent alkaloid.[2] Unfortunately, conversion of quinidine into the two expected oxazatwistanes also produces a series of minor impurities which may impact selectivity. Characterization of minor components of complex mixtures has traditionally been a difficult and time-consuming task often necessitating the isolation of the individual components. Using LC-NMR based techniques can obviate the need for physical isolation of the materials and permit structure determination to be performed with much greater efficiency. Using LC-NMR along with LC-MS and LC-IR, we have positively identified several components of the reaction mixture without resort to isolation.

1. Kolb, H.C.; VanNieuwenhze, M.S.; Sharpless, K.B. Chem. Rev. 1994, 94, 2483-2547.

2. Braje, W.; Frackenpohl, J.; Langer, P. and Hoffmann, H.M.R. Tetrahedron, 1998, 54, 3495-3212.

16 Quantitative Analysis using High Throughput Flow-Probe NMR

Catherine Jolivet, Ariane L. Jansma, Daniel B. Kassel and Robyn A. Rourick

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Combinatorial synthesis and high-throughput medicinal chemistry are widely accepted and applied tools for rapid compound synthesis in support of drug discovery and lead optimization within the Pharmaceutical industry. Direct injection NMR analysis has been recently developed in support of the generation of NMR data for the increased numbers of drug discovery compounds. Acquisition of qualitative proton and carbon spectra is routinely used to verify the structural integrity of these compounds. We have extended the application of qualitative flow-probe NMR analysis to include quantitative analysis. Effectively an appropriate measurement of the amount of material is critical, in particular in the interpretation of biological activity and consequently the Structure-Activity Relationships. Therefore, we have developed a method for quantifying compounds in support of combinatorial chemistry in an efficient way using a 60uL NMR flow-probe. We have incorporated in each sample well a water-soluble standard, TSP, with a corresponding single resonance at approximately 0 ppm. Method development has involved the optimization of several parameters including filter bandwidth, dead time, pulse width, time, phase, integrals, baseline correction and linear prediction in order to generate integrals as accurate as possible in an automated fashion with minimal user intervention and in a reasonable amount of time. The application of this quantitative NMR approach will be discussed and its benefit demonstrated on a series of combinatorial libraries.

17 NMR Structural Analysis of Synthetic Saccharide Components of the Capsular Polysaccharide of Neisseria Meningitidis Group A

Bruce Coxon, Ali Berkin, and Vince Pozsgay

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In efforts directed towards the preparation of an improved vaccine against epidemic meningitis caused by Neisseria meningitidis Group A, a series of spacer-linked oligosaccharide fragments of the bacterial capsular polysaccharide has been synthesized, starting with partially protected derivatives of 2-azido-2-deoxy-alpha-D-mannopyranosyl H-phosphonate. Extension of the saccharide chain by the repetitive addition of this monosaccharide precursor has yielded dimeric and trimeric 2-acetamido-2-deoxy-alpha-D-mannopyranosyl derivatives that contain unusual, phosphate diester 1,6 linkages. The structures and conformations of these derivatives have been studied by high-resolution, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. Measurements of ^{1}H-^{1}H, ^{1}H-^{13}C, ^{1}H-^{31}P, and ^{13}C-^{31}P coupling constants have allowed definition of the gross chemical structures of the derivatives, the chair conformations of the saccharide residues, proof of the alpha-configurations of the anomeric linkages, and information on the relative orientation of the saccharide residues in solution.

Testing of glycoconjugates prepared by linking of non-O-acetylated saccharides to human serum albumin has revealed promising biological activity with respect to anti-sera for N. meningitidis Group A, even although the capsular polysaccharide of this bacterium exhibits non-stoichiometric O-acetyl substitution of the sugar residues.

18 A New One-Dimensional DOSY Experiment

Michael J Thrippleton, James Keeler and Nikolaus M Loening

There has recently been a great deal of interest in the measurement of diffusion coefficients by NMR spectroscopy. Of particular interest is the DOSY technique, which makes it possible to "separate" the spectra of mixtures according to molecular size by extending the spectrum into a second dimension containing the diffusion information. However, data collection requires multiple scans and may take several minutes or longer, depending on the nature of the sample and instrument used.

We have developed a new method for measuring diffusion coefficients in a single scan, allowing the collection of DOSY data in less than a second. The method retains chemical shift resolution but sacrifices sensitivity and some resolution in order to achieve the measurement in a single scan. In cases where time is at a premium and sensitivity is high, the method has much to commend it.

19 NMR Spectroscopic Analyses of Phenacetin Futile Deacetylation in Man

Andrew W. Nicholls[1], John P. Shockcor[1], Markus Godejohann[2], Martin Hofmann[2], Manfred Spraul[2] and Ian D. Wilson[3]

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The drug phenacetin has long been known to lead to kidney toxicity following prolonged exposure. One mechanism for this toxicity has been suggested via the transient formation of the potent nephrotoxin 4-aminophenol. This study has explored the potential level of formation of 4-aminophenol in man following ingestion of phenacetin labelled with deuterium in the acetyl group. A single 150 mg dose was administered to a healthy male volunteer and urine was collected pre-dose and 0-2h and 2-4h post-dose. Conventional 1D ^{1}H NMR spectra were acquired of all samples and the phenacetin metabolites, acetaminophen glucuronide, acetaminophen sulphate and the N-acetylcysteinyl conjugate of acetaminophen, were observed. From integration of the aromatic resonances with the acetyl singlet the % deacetylation/reacetylation (futile deacetylation) was 20% for the glucuronide and sulphate conjugates. To confirm this percentage the 2-4h urine sample was separated into acidified water and methanol fractions using solid phase extraction (SPE) and the latter was then further separated using HPLC with the peak trapping in C18 SPE cartridges on a Bruker/Spark Prospekt II. This enabled removal of the protonated HPLC solvents and elution of the compounds of interest with a small volume of deuterated solvent, resulting in a concentration of the compounds of interest. Both the glucuronide and cysteinyl conjugates of acetaminophen were isolated and the % futile deacetylation was confirmed at 20%. These results indicated that futile deacetylation was an important metabolic pathway for phenacetin in man. The potential for the formation of 4-aminophenol at up to 20% of the phenacetin dose implicates this as a contributor to the nephrotoxic affects observed from phenacetin.

20 The Use of Nano- and HR-MAS NMR in the Identification of Small Molecule Lead Structures for Drug Discovery

Charlotte H. Gotfredsen[1], Christian Eeg Jensen[2], Ernst Meinjohanns[2], Jens Ø. Duus[1]

1. Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark.
2. Combio A/S, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark.

NMR is one of the most powerful analytical detection techniques available for structural determination in drug discovery and development. Using NMR for the structural analysis has however posed some drawbacks such as its inherent low sensitivity and the inability to produce high-resolution spectra of heterogeneous samples.

The advent of high-resolution magic angle spinning NMR probes has aided in resolving these drawbacks. This has enabled NMR to be a beneficial tool in the area of drug discovery.

Our approach is to use high resolution MAS NMR for the structural characterization of resin bound small molecule libraries. This requires a high sensitivity and a resin support that allows for the generation of well-resolved NMR spectra of compounds on single beads. The role of NMR in Combio's drug discovery approach will be illustrated. In addition the spectral quality and the experiments that have been used will be exemplified with small molecule resin bound samples.

21 On-Line Preparative HPLC-NMR-MS Through Peak Trapping

Chris Sleigh and Kevin Schaab

Pfizer Global Research and Development,
Sandwich, Kent, CT13 9NJ, U.K.

We report a method which uses preparative HPLC and peak trapping to prepare a high concentration of eluent for introduction into an NMR flow cell. Elution with deuterated solvents from the trapping column is demonstrated to produce sharp LC peaks and higher concentrations of the desired components for on-line NMR analysis. We will show data illustrating the large NMR sensitivity gains that can be realised using this technique, and demonstrate the method on species with very different polarities. The practical applications of this method will also be discussed.

22 Identification of Voriconazole Metabolites Using Mass Spec directed LC-NMR

Michael Ritzau

The poster will describe the application of LC-NMR-MS for the identification and structure determination of voriconazole metabolites. The Extracted Ion Chromatogram (EIC) was utilised to trigger the stop flow LC-NMR. It will be shown that in the case of complex mixtures using an EIC trigger is far superior to an UV or DAD detector. It allows the reliable identification of HPLC peaks even if the chromatographic conditions are changed. Metabolite ID is an ideal application for mass spec directed LC-NMR as both the starting material and likely metabolic pathways are known. Eight EIC traces were monitored in parallel. The general set-up, technical challenges and some problems related to unexpected chemical exchange processes will also be discussed.

23 Capillary NMR with Indirect Carbon Detection: Flow Injection with a Micro Sample Loader and Capillary LC

Dean Olson and Timothy Peck[1], Mark O'Neil-Johnson[2], David Detlefsen[3]

1. MRM Corporation, Savoy, IL
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The HTSL-1100 Micro Sample Loader is specifically designed for high-throughput loading of uL-volume samples. Designed for integration with the Gilson 215, it offers uL/min flow rates and reproducible delivery of analytes to the CapNMR™ flow cell. The CapNMR probe (5-uL observe volume) has a z-gradient for indirect carbon detection. To demonstrate the capability of FI-CapNMR, 15 ug of sucrose (in 3 uL; 15 mM, 45 nmol; MW = 342) was injected and parked in the probe. An HMQC was acquired in less than 15 hr. In addition, 75 ug of the natural product Muristerone A (in 3 uL; 50 mM, 150 nmol; MW = 497 g/mol) was injected and parked. Three spectra were acquired: a gradient COSY in 7 min, a gradient HMQC in 4 hr, and a gradient HMBC in 11 hr. In another example, 43 ug of lysozyme in an injected volume of 3 uL (1 mM, 3 nmol; MW = 14,300 g/mol) was parked in the CapNMR flow cell. A proton spectrum, using NOESY presat to suppress the 90% water signal, was acquired in 3.3 min on a 600 MHz spectrometer. A 2D-NOESY spectrum was acquired on the very same lysozyme sample in 10 hr.

The CapNMR flow probe and the Waters CapLC® combine excellent chromatographic separation capability with NMR and UV-Vis detection for mass- and volume-limited analytes. A 1-nmol (0.5 ug; 500 MW) injection yields a high-resolution, stopped-flow, proton spectrum in approximately 10 minutes on a 500 or 600 MHz NMR spectrometer. Steep solvent gradients are sometimes problematic in conventional scale LC-NMR due to long equilibration times often required after stopped-flow to obtain good NMR data. The miniaturized CapNMR flow cell provides relatively rapid diffusional equilibration such that nearly any solvent gradient can be effectively performed. CapLC-NMR also allows the pre-concentration of analytes on-column from relatively large sample volumes of initial concentrations as small as 100 uM. The CapNMR probe is very salt tolerant due to its small transceiver coil. Compared to the S/N in the absence of salt, 500 mM KCl reduced the S/N of a model compound less than 10%.

24 Enantiomeric Identification Using Natural Abundance High Resolution Modified ^{1}H-^{13}C Heteronuclear NMR Spectroscopy (HRMHSQC) in a Chiral Liquid Polypeptide Crystal Solvent

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The enantiomeric pairs of a sample containing a mixture of 1-chloro-2-propanol (7.5 mg) and 2-chloro-1-propanol (2.5 mg) present in a PBLG/CDCl3 chiral liquid crystal solvent has been differentiated using a high-resolution phase sensitive modified ^{1}H-^{13}C HSQC (HRMHSQC) 2D NMR experiment with TANGO. The separation between the R and S enantiomers for each regio-isomer in the ^{13}C dimension is substantial allowing for unequivocal differentiation of all four stereoisomers and possible specific stereochemical identification. A very large separation of 327.65 Hz is observed, in the ^{13}C-dimension, between the R and S enantiomeric methyls of 2-chloro-1-propanol, while a smaller separation of 4.42 Hz is observed for the 1-chloro-2-propanol enantiomeric methyls. Both hetero and homonuclear coupling constants can be obtained from the HSQC experiment. A chemical shift anisotropy is also observed between the enantiomeric species further resolving the resonances in the ^{1}H dimension. Individual contributions from isotropic and direct hetero and homonuclear residual dipole-dipole coupling constants can be calculated, in a straightforward manner, by comparison with corresponding spectra present in a liquid unoriented phase. By using proton indirect detection methods the acquisition time has be greatly reduced from 18 hours as in QCOSY experiments (1) to 1.5 hours for the HRMHSQC experiment without the loss of sensitivity. Additionally, the amount of sample required, 2.5 mg for 2-chloro-1-propanol component, is approximately 20 fold lower than in natural abundance ^{2}H detected experiments (1).

25 Deceptive Proton NMR Spectra

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Following up on a recent review (J. Nat. Prod.,65,221-244(2002)), designed to educate organic chemists in more effective use of 2D NMR, we are preparing a new review, entitled "NMR Spectroscopic Traps and Tricks". This will illustrate pitfalls in organic structure elucidation by NMR and how to avoid them. As part of the review, we have been collecting examples of decptive and surprisingly complex phenyl proton spectra due to accidental exact equivalence of two of ortho, meta and para protons. In addition, the proton spectra of (1,2dibromoethyl)benzene is used to illustrate deceptive ABX spectra, using different solvents

26 Dipico-7, a Chelating Polydentate Ligand with Unusually Complex Variable Temperature Behavior

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Dipico-7, a polydentate chelating ligand, shows a unusual variable temperature behavior involving exchange of 2 unsymmetric mirror-image forms via an apparent symmetric intermediate. The structure of the unsymmetric form is deduced by 2D NMR, X-ray crystallography and solid state NMR. The intermediate is probably actually 2 other rapidly converting unsymmetric forms. The ROESY spectrum is complicated by EXSY and EX-ROESY peaks, the latter of the same phase as ROESY peaks. However, slowing the exchange at low temperature allows distinction of ROESY and EX-ROESY peaks. A TOCSY specctrum is also complicate by EX-TOCSY peaks

27 New Developments in PERCH

Matthias Niemitz[1], Greg Banik[2], Tommi Hassinen[3], Samuli-Petrus Korhonen[3], Reino Laatikainen[3]

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Novel tools in the latest version of PERCH NMR Software contain the following features:

Processing
Complete processing of 1D-spectra including special features for enhanced spectral analysis.

Quantification
Total-line-shape fitting with simultaneous optimization of line-shape parameters and baseline as well including versatile constraint features.

Proton Prediction
Proton prediction based on the optimized 3D-structure including dynamics and solvent effects.

Spectral Analysis
Iterative verification and optimization of predicted spectral parameters.

Applications of these features are presented.

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28 Application of a Mechanical Liquid Nitrogen Generation Device to Superconducting NMR Magnets

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Many scientific users of liquid nitrogen (LN_2) face challenges with regard to its cost, availability, transportation to the place of use, and safe transfer from the transfer Dewar into the receiving vessel. One of the best examples of this problem is embodied in modern high-field superconducting NMR magnet assemblies, where complete dependence on a continuous supply of LN_2 for their operation often complicates the logistics of ownership and maintenance. The recent development of a self-contained prototype device, specifically engineered for NMR use, is described. Using only electricity, the dormitory refrigerator sized device is capable of producing up to 40 liters/day of LN_2. Up to 60 liters/day is possible with the addition of water cooling. Pure nitrogen, extracted from air using a Pressure Swing Absorption (PSA) apparatus, is condensed into an internal Dewar using a Gifford-McMahon (GM-cycle) cryo-refrigerator. Automatic filling of magnet farms is possible, eliminating the risk and safety issues associated with conventional filling procedures.

29 Solution Structure and Orienting Interactions of Small Peptides and Amides in an Aqueous Liquid Crystal

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The capped alanine dipeptide (AcAlaNHMe) is a benchmark during the development of *in silico* tools for protein structure determination. Previous experiments have failed to conclusively determine its solution conformation, however. We have extracted dipolar couplings for the dipeptide via LX-NMR, employing a liquid crystalline solution of cesium perfluoro-octanoic acid in water as orienting medium. Our data is consistent with the pII conformation encountered in unfolded proteins, and should help discriminate among competing force-fields available for protein simulations. We have performed similar experiments with the family of methyl-substituted formamides to describe the origin of the orientational anisotropy of small amides and peptides in the liquid crystal. We find that the orientation of these small compounds is surprisingly sensitive to the precise arrangement of methyl groups. We explain the orientational order in terms of competing hydrophobic and hydrophilic interactions with the mesogen and the surrounding solvent. These observations should prove of interest to investigators of molecular recognition, affinity, self-assembly, and related association phenomena in water.

30 NMR Diffusion Coefficient Study of Steroid-Cyclodextrin Inclusion Complexes

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Four complexes of cyclodextrin with the neuromuscular blocker Rocuronium Bromide are investigated. The four complexes have significantly different association constants. Diffusion coefficients of the free host and guest species are determined directly and so can the diffusion coefficients of the γ-cyclodextrin complexes because they are in the slow exchange regime. The β-cyclodextrin complexes are in the fast exchange regime, therefore the diffusion coefficient of these complexes were determined by titration using a novel curve fitting method. The diffusion coefficient of the four cyclodextrins undergo a 6-15% reduction on complexation with Rocuronium Bromide. This small change in diffusion coefficent and the available X-ray data indicate that the mode of binding for all four cyclodextrins is internal encapsulation of Rocuronium Bromide.

31 Survey of NMR Experiments for the Determination of nJ_{CH} Heteronuclear Coupling Constants in Small Molecules

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Herein we present a survey of heteronuclear correlation experiments for the measurement of heteronuclear coupling constants. The purpose of this survey is to evaluate several recent experiments for measuring $nJ_{C,H}$ (n = 2, 3) couplings in one comprehensive manuscript. Nine experiments are presented and evaluated relative to one another in terms of experimental usability, ease of data interpretation, and number of usable correlations. The experiments compared in this survey are the sensitivity improved hetero half-filtered TOCSY (HETLOC), carbon-sorted HETLOC (HSQC-HECADE), GSQMBC, HSQMBC, G-BIRDR-HSQMBC, J-Resolved HMBC-2, J-IMPEACH-MBC, and methods for extracting heteronuclear coupling constants from gradient selected variants of the magnitude-mode and phase-sensitive-HMBC. The plant alkaloid strychnine was used as a model compound for this study.

32 New Silyl Reagents for the Determination of Absolute Stereochemistry

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The determination of absolute stereochemistry remains a challenging aspect of organic chemistry. Many approaches based on circular dichroism, X-ray crystallography, and anisotropic shielding reagents in conjunction with NMR spectroscopy (Mosher-type reagents) have been reported in the literature. However, to this date, no one method has proved to be completely general in its application and the search for reagents that are easier to use and provide data that are easier to interpret remains of paramount importance. In this poster, we present the development of new silane-based reagents that can be used in conjunction with NMR spectroscopy to determine the absolute stereochemistry of secondary alcohols. These reagents have the advantages that they are easy to synthesize and can be used on even sterically-hindered systems. In addition, they can be applied to elimination-prone or other sensitive systems and can be removed easily with TBAF-SiGel to allow recovery of the starting alcohol. The application of these silyl reagents and other approaches to the determination of absolute stereochemistry will be presented.

33 A Method for the Determination of the Relative Stereochemistry of Aldol Products

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Beta-hydroxy carbonyl moieties (Aldol-condensation products) are key features in many small molecule structures, whether synthetically derived or isolated as natural products. The determination of the anti or syn diastereomeric relationship utilizing previously reported empirical methods by NMR spectroscopy has been shown to be unreliable. Herein we report a method for the determination of the relative configuration of Aldol products using a combination of homonuclear and long-range heteronuclear coupling constants, along with dipolar couplings in conjunction with the J-based configurational analysis method.

34 Exploration of Solid Phase DCC Coupling Reagents in the Absolute Stereochemical Determination of Secondary Alcohols by NMR

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The assignment of stereogenic centers with unknown configuration is often the most challenging task in the complete structure elucidation of small organic molecules. The use of chiral derivatizing agents (CDA's) is a common technique that is used for the determination of absolute stereochemistry by NMR (Mosher's Method). One of the most difficult tasks at hand in this type of analysis is the derivatization itself, which is typically performed on sub-milligram to 1-2mg quantities. The standard methodology requires the derivatization of the chiral compound of interest with both enantiomers of the CDA, followed by purification and NMR analysis. The typical yield for these types of derivatizations is often around 50%, which if done on 1 mg of starting material only provides ~ 250ug of each diasteriomer for analysis. To this end, a method is presented that dramatically increases the yield and provides a purification-free method. Three solid phase DCC coupling reagents were tested for their utility in derivatizing secondary alcohols with a chiral derivatizing agent (MPA) in order to assign absolute stereochemistry by NMR using the Advanced Mosher method. General reaction conditions will be shown as well as which of the three resins has the best general derivatization properties.

35 Structural and Conformational Study of GBR 12909 and its Amino Analogue by NMR and Fluorescence Spectroscopy

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Dopamine transporter (DAT) was shown to be actively involved in the cocaine effects that cause the cocaine dependence. GBR 12909 (GBR) and its amino analogue (GBR-NH_2) were shown to exhibit competitive inhibition of cocaine binding to DAT, and that renders them as possible clinical treatment of cocaine dependence. GBR and GBR-NH_2 are synthesized in professor Alex Makriyannis' lab at the University of Connecticut. NMR and fluorescence spectroscopy were used to study the structure and dynamics of these molecules in chloroform and in micellar environment that was shown to mimic the cell membrane's environment. NMR data of GBR and GBR-NH_2 in chloroform at low temperatures illustrate the dynamics of the piperazine ring at the center of these two molecules. Fluorescence data were used to optimize the conditions to incorporate GBR and GBR-NH_2 in micelles. That data were then used to generate the NMR samples. NMR data of GBR in sodium dodecyl sulfate (SDS) micelles show the parts of GBR that interact with SDS micelles.

36 $^{1}H/^{11}B$ Chemical shift correlations: Is HETCOR still the best?

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^{1}H and ^{11}B detected versions of the HETCOR, HMQC and HSQC experiments were performed and compared for decaborane and two carborane clusters. To the best of our knowledge, the latter two experiments were not previously reported for $^{1}H/^{11}B$ correlations.

In recent reports ^{1}H detected HETCOR has been the method of choice. This experiment gives better sensitivity than its ^{11}B detected counterpart and spectra can be accumulated very rapidly due to the short T_1 relaxation times of ^{11}B nuclei. We found, that both HMQC and HSQC had higher sensitivity per unit time than ^{1}H detected HETCOR. In addition, they yielded pure absorptive lineshapes and correlations to bridging protons were observed, even with broadband ^{11}B decoupling. HSQC yielded slightly cleaner spectra than HMQC. Overall, ^{1}H detected phase sensitive HSQC using phase cycling for coherence selection is recommended for general use.

37 Structure Elucidation of Phormidamide A, a Novel and Cytotoxic Metabolite from the Marine Cyanobacterium Phormidium sp.

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An Indonesian isolate of the marine cyanobacterium Phormidium sp. was successfully cultured in our laboratory and its extract found active in a mechanism-based anticancer assay. Assay guided isolation led to the identification of a novel chlorophyll derivative as the active material; however, from other fractions several new and bioactive compounds were also isolated. The additional natural products included a complex brominated polyketide, phormidolide, the structure of which is soon to be published, and phormidamide A, which is to be described in this presentation. Phormidamide A is a complex halogenated compound that derives from a mixed peptide and polyketide biogenesis, a common pathway for marine cyanobacterial metabolites. While several structural features were easily deduced by the standard assortment of 2D NMR experiments, a few novel structural elements required more in depth NMR investigation, including ^{13}C NMR INADEQUATE analysis of a ^{13}C labeled compound (from culture) and multiply optimized HMBC experiments. Finally, NMR investigation of semi-synthetic derivatives of phormidamide A provided strong support for the structure presented.