SMASH 2006 NMR Conference

Burlington, Vermont

September 10th - 13th, 2006

SMASH 2006 NMR Conference

Dear SMASH 2006 Attendees,

Welcome to Burlington, Vermont, and the 2006 SMASH NMR Conference!!!

This year's program contains an exciting array of oral sessions and workshops. Oral session topics include: Metabolite Identification, New Heteronuclear NMR Techniques, Small Molecule/Protein Interactions, Latest Advances in Small Molecule RDC's, NMR of Organometallics, NMR in the Forensic Sciences, New Experimental Techniques, and the Student/Post-Doctoral Session. As in the past, the program also includes four workshops. Monday's workshop topics include Solid State NMR and a "Tips and Techniques" session on DOSY and Diffusion Spectroscopy. The workshops for Tuesday deal with Hyphenated NMR and RDC's: Practical Aspects.

In addition, we will be treated to an after-dinner talk Monday night by keynote speaker, Tom Farrar.

On behalf of the entire SMASH Organizational Committee, we wish to thank you for your continued interest in, and support of, the SMASH NMR Conference.

We hope you enjoy this year's conference!!!

Sincerely,

Steve Cheatham and Ernie Schubert
Co-Chairs, SMASH 2006 NMR Conference

SMASH 2006 NMR Conference Program

Sunday September 10th

5:00 PM - 6:30 PM **Registration & Mixer**
6:00 PM - 8:00 PM **Dinner**
8:00 PM -11:00 PM **Mixer**

Monday September 11th

7:00 AM - 8:15 AM **Breakfast**
8:15 AM - 8:30 AM **Opening Remarks**
8:30 AM - 10:00 AM **Metabolite ID** Chair: Andrea Sefler

- **The Early Estimation of Circulating Drug Metabolites in Humans**
  Andy Roberts - GSK UK
- **NMR as an Essential Early Discovery Tool for Drug Metabolism Studies**
  Greg Walker - Pfizer
- **A Pharmaco-Metabonomic Investigation of Acetaminophen Toxicity in Humans**
  Tom O'Connell - UNC Chapel Hill

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **New Heteronuclear NMR Techniques** Chair: Krish Krishnamurthy

- **Recent Pulse Sequences for Heteronuclear Long-range Correlation and More**
  Ole W. Sørensen - Carlsberg Laboratory, Denmark
- **Merging the Concepts of Time-sharing Evolution and Spin-state Editing in HSQC Experiments**
  Teodor Parella - University of Barcelona, Spain
- **Application of BIP in Heteronuclear 19F NMR**
  Haitao Hu - Eli Lilly and Company, USA

12:00 PM - 1:30 PM **Lunch**
1:30 PM - 3:00 PM **Small Molecule/Protein Interactions and Screening by NMR**
Chair: N. Rama Krishna

- **TINS: New Opportunities for Fragment Based Drug Discovery**
  Johan Hollander - Leiden University, Leiden, The Netherlands
- **SAR by ILOEs: An NMR-based Approach to Drug Discovery**
  Maurizio Pellecchia - Burnham Institute for Medical Research, La Jolla
- **A STD-NMR/Modeling Protocol for the Determination of the Conformations of Small Molecules Bound to Enzymes**
  Mario Pinto - Simon Fraser University, Burnaby, BC, Canada

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

- **I. Tips and Techniques - DOSY and Diffusion Spectroscopy**
  Elizabeth McCord, Mathias Nillson and Rainier Kerssebaum
- **II. Solid State NMR**
  Ales Medek, Eric Munson, Jochem Struppe and Fred Vogt

5:00 PM - 6:00 PM **Free Time**
6:00 PM - 6:30 PM **Pre-Dinner Social Gathering**
6:30 PM - 9:00 PM **Social Dinner A**fter Dinner Speaker: Tom Farrar
9:00 PM - 11:00 PM **Mixer**

SMASH 2006 NMR Conference Program

Tuesday September 12th

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

8:30 AM - 10:00 AM **Latest Advances in Small Molecule RDC's** Chair: Christina Thiele

- **Dipolar Couplings to Determine Conformation and Configuration of Small Molecules**
  Christian Griesinger - Max-Planck-Institute, Göttingen, Germany
- **Carbohydrate Structure and Dynamics from Residual Dipolar Couplings**
  Daron Freedberg – NIH
- **Alignment Media and Measurement of Anisotropic NMR-Parameters in Various Solvents**
  Burkhard Luy - Technische Universität München, Germany

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

10:30 AM - 12:00 PM **NMR of Organometallics** Chair: Cornelis Elsevier

- **Small Molecule Applications of Hyperpolarisation Methods Involving Reactions with Parahydrogen**
  Simon Duckett - University of York
- **Solid-State NMR of Metal Nuclei in Metallocenes**
  Robert Schurko - University of Windsor
- **Grafted Organotin Catalysts: A Story on High Resolution Magic Angle Spinning NMR With and Around the ^{119}Sn Nucleus**
  Rudi Willem - Vrije Universiteit Brusse

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

1:30 PM - 3:00 PM **NMR in the Forensic Sciences** Chair: Patrick Hays

- **Quantitative Deuterium-NMR on Synthetic Drugs**
  Thomas Schaefer - Bundeskriminal Amt (BKA), Germany
- **NMR Chemical Forensics at the EPA - National Enforcement Investigations Center**
  Matthew Rees - US Environmental Protection Agency
- **NMR in the Fight Against Illicit Drugs**
  Robert Thompson - US Drug Enforcement Administration

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

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

- **I. RDC's: Practical Aspects**
  Michael Shapiro
- **II. Hyphenated NMR**
  Mark Dixon and Markus Godejohann

5:00 PM - 6:00 PM **Free Time**

6:00 PM - 7:30 PM **Dinner**

8:00 PM - 10:00 PM **Poster Session with Mixer**

10:00 PM - 11:00 PM **Mixer Continues**

SMASH 2006 NMR Conference Program

Wednesday September 13th

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

8:30 AM - 10:00 AM **Student and Post-Doctorate Session** Chair: Bridget Becker

- **An NMR and QM Investigation of the Mechanism of Action of Bisphosphonates**
  Sujoy Mukherjee - University of Illinois, Urbana, IL
- **Structure Calculations on Small Molecules: Can RDCs Solve Stereochemical Problems?**
  Peter Haberz - Max-Plank-Institute for Biophysical Chemistry, Germany
- **Principal Component Analysis of Urine Metabolites Detected by NMR and DESI-MS in Patients with Inborn Errors of Metabolism**
  Zhengzheng Pan - Purdue University, West Lafayette, IN

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

10:30 AM - 12:00 PM **New Experimental Techniques** Chair: A. J. Shaka

- **Haste Makes Waste**
  Vladimir Mandelshtam - University of California, Irvine
- **Spectral Reconstruction Methods in Fast NMR: Reduced Dimensionality, Random Sampling, and Maximum Entropy**
  Jeff Hoch - University of Connecticut Health Center
- **Multidimensional NMR Spectroscopists in a Hurry**
  Eriks Kupce - Varian Ltd., Oxford, UK

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

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

SMASH 2006 NMR Conference
Acknowledgements

The SMASH 2006 Conference gratefully acknowledges the support provided by the following companies.

Advanced Chemistry Development, Inc., (ACD/Labs)
Bruker BioSpin Corporation
ChemScribe
Cambridge Isotope Laboratories
Icon Isotopes
Isotec/Sigma Aldrich
J S Research, Inc.
JEOL
John Wiley and Sons, Ltd.
MESTRELAB RESEARCH
Modgraph
New Era Enterprises
Norell
Novatia
One Moon Scientific
Oxford Instruments plc
Paradigm Organics, LLC
Praxair Cryomag Services
Protasis/MRM
SP Industries/Wilmad
Spectra Stable Isotopes
Varian, Inc.

SMASH 2006 NMR Conference Committee

Program Co-Chairs 2006

Steve Cheatham
DuPont Crop Protection

Ernie Schubert
GlaxoSmithKline

Program Co-Chairs Elect 2007

Andy Nicholls
GlaxoSmithKline R&D

Eric Munson
University of Kansas

Immediate Past Chair 2005

Martin Will
sanofi-aventis, Germany

Greg Nemeth
sanofi-aventis, USA

Program Chair Emeritus 2004

Dave Lankin
University of Illinois at Chicago

Gene Mazolla
University of Maryland

Logistics

Mike Kolpak
Johnson & Johnson PRD

Vendor Liaisons

Margaret Levenberg
Stepan Company

Andy Nicholls
GlaxoSmithKline R&D

Treasurer & Registrar

Robert Espina
Bristol-Myers Squibb Company

Secretary

Margaret Levenberg
Stepan Company

Poster Session

Laurie Galya
Incyte Pharmaceuticals

Monday, September 11th
8:30 AM - 10:00 AM

Metabolite ID
Chair: Andrea Sefler

Speakers:

Andy Roberts
GSK UK

Greg Walker
Pfizer

Tom O'Connell
UNC Chapel Hill

The Early Estimation of Circulating Drug Metabolites in Humans

Andrew D Roberts, Gordon J Dear, Claire Beaumont, Martin Sandvoss and Stephanie E. North

DMPK, GSK, Park Road, Ware, Hertfordshire, SG12 0DP

As part of the development programme for a novel drug candidate attempts should be made to identify any differences in the drugs' metabolism between humans and the animals used in the toxicological evaluation. A collection of in vivo studies are performed to assess the metabolism in animals and predictions made for humans using in vitro methods. Investigation into the actual metabolism in humans is generally achieved through dosing radiolabelled drug as part of a clinical study which is typically conducted between Phase II and Phase III of the clinical development plan. The earlier the metabolism of a molecule in humans can be obtained, the sooner any potential safety issues surrounding human exposures to metabolites can be discharged. This talk describes an approach using cryoprobe-NMR to determine the human systemic exposure to a drug and its metabolites using samples derived from Phase I clinical studies. During the initial Phase I, safety and tolerability study human volunteers are generally exposed to increasing levels of drug. This study usually encompasses the highest dose administered to humans, and as such provides ideal samples for metabolite characterisation. With appropriate doses, the levels of these metabolites can also be estimated by NMR, without the need for synthesis of metabolites at this stage. This approach provides an early opportunity to understand the potential role of metabolites in the safety and efficacy of a drug, in advance of long term toxicity studies in animals and efficacy studies in human.

NMR as an Essential Early Discovery Tool for Drug Metabolism Studies

Gregory S. Walker, James Atherton, Hao Chen, Wing Lam, Linning Yu, Gwendolyn Fate and Abdul Mutlib

Pfizer, Pharmacokinetics, Dynamics and Metabolism, Ann Arbor, MI 48015, U.S.A.

There is an increasing pressure to reduce the attrition rate of potential drug candidates in the pharmaceutical industry. One way to manage the attrition of compounds is to evaluate potential metabolic liabilities in the early stages of drug discovery, by identifying metabolic soft spots and reactive metabolites. Metabolic soft spots can lead to high clearance rates and low bioavailability, while reactive metabolites may result in potential toxicities. In both cases, timely structural information can have immense influence on chemistry strategies, including making appropriate modifications to existing chemical templates or discontinuing chemical series with high metabolic liabilities.

Historically, structural characterization of metabolites has relied principally on LC/MS/MS technology and on the availability of synthetic standards. While in some cases structurally informative MS/MS fragmentation has sufficed, in others more specific data has been required to assess metabolic sites of modification. In many situations, NMR provides complimentary data that can unambiguously assign structures of metabolites. Traditionally, NMR analysis has required large amounts of sample. However, recent advances in NMR hardware (cryoprobes, LC/MS/NMR and post column SPE) and software have dramatically reduced the time and the amount of material required for NMR structural characterization of low abundance metabolites. Furthermore, the ability to provide structural information on low levels of metabolites using NMR and MS technologies has largely eliminated the need for early chemical synthesis of metabolites, saving resources. The wealth of information obtained from NMR technology in conjunction with MS, can have a significant impact early in drug discovery when the amounts of compound (and metabolites) are limited. Several cases where timely structural characterization in early discovery has allowed chemists to modify or eliminate chemical templates with potential liabilities will be discussed.

A Pharmaco-Metabonomic Investigation of Acetaminophen Toxicity in Humans

Thomas M. O'Connell[1], Jason H. Winnike[2], Jeffrey M. Macdonald[3], Mark W. Russo[2] and Paul B.Watkins[2]

1. Division of Molecular Pharmaceutics, School of Pharmacy
2. Department of Medicine
3. Department of Biomedical Engineering,

University of North Carolina, Chapel Hill, NC. USA, 27599

Acetaminophen has been considered a safe and effective pain medication for decades, yet it is the most common cause of acute liver failure in the United States and Great Britain [1]. Despite it's long history of usage, there are still many unanswered questions regarding the molecular mechanisms of its action and toxicity. In a recent clinical study by Watkins et. al [2], a surprisingly high incidence of elevated serum alanine aminotransferase (ALT) was observed in subjects taking 4gm daily doses of acetaminophen over a two week period. This indicates that a subset of patients on a standard therapeutic dose of acetaminophen experience mild, albeit reversible liver damage. At the University of North Carolina Chapel Hill, a large scale, two-week, in-patient study of acetaminophen is ongoing. In this study NMR based metabonomic methods are applied to examine the changes in the metabolome over the course of the dosing regimen. Additionally a pharmaco-metabonomics analysis is underway in which the ability of the baseline urinary metabolome to predict elevations in ALT is investigated. Some novel NMR methods have been developed to address the complexity and inherent heterogeneity of human subjects including the Fast-J-Resolved experiment. This allows for rapid acquisition of 2D J-resolved spectra which are used in combination with the standard 1D spectra for statistical analysis and metabolite identification. The preliminary results indicate that the baseline urinary metabolome can predict susceptibility to acetaminophen hepatotoxicity in healthy volunteers, and supports the pharmaco-metabonomic concept in clinical medicine.

1. Ostapowics, et. al, Ann, Intern Med, 2002, 137, 947-954.
2. Watkins, et. al, JAMA, 2006, 296, 87-93.

Monday, September 11th
10:30 AM - 12:00 PM

New Heteronuclear NMR Techniques
Chair: Krish Krishnamurthy

Speakers:

Ole W. Sørensen
Carlsberg Laboratory, Denmark

Teodor Parella
University of Barcelona, Spain

Haitao Hu
Eli Lilly and Company, USA

Recent Pulse Sequences for Heteronuclear Long-Range Correlation and More

Ole W. Sørensen

Carlsberg Laboratory, Gamle Carlsberg Vej 10, 2500 Valby, Denmark
From Sept. 1, 2006: Institute of Chemistry, Denmark's Technical University, 2880 Kgs. Lyngby, Denmark

This talk will give an overview of the key elements and principles of experiments listed on http://www.crc.dk/nmr/, from where also pulse programs in Bruker and Varian format can be downloaded.

Merging the Concepts of Time-Sharing Evolution and Spin-State Editing in HSQC Experiments

Teodor Parella and Pau Nolis

Servei RMN, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain.

First, a general IPAP strategy to achieve spin-state selection (S^3) for any multiplicity in the acquisition dimension of a sensitivity-improved HSQC experiment will be introduced [1]. Then, the main features of simultaneous acquisition of two different nuclei (typically ^{13}C and ^{15}N) will be discussed and time-shared TS-HSQC, TS-HSQC-TOCSY and TS-HSQMBC pulse schemes will be proposed to extract different type of information from a single NMR experiment.. Finally, several new NMR approaches demonstrating the usefulness to combine IPAP techniques and TS evolution will be provided. Thus, simultaneously S^3 editing can be achieved for all CH_n and NH_n spin systems in a single NMR experiment and examples will be given for the simultaneous measurement of proton-carbon and proton-nitrogen coupling constants [2,3].

1. P. Nolis, J.F. Espinosa and T. Parella, J. Magn. Reson., 180, 39-50 (2006).
2. P. Nolis and T. Parella, J.Biomol. NMR, submitted.
3. P. Nolis and T. Parella, J. Magn. Reson., submitted.

Application of BIP Pulses to ^{19}F NMR

Haitao Hu, Krish Krishnamurthy

Discovery Chemistry Research and Technologies,Eli Lilly and Company, Indianapolis, Indiana

Flourine-19 is a spin half with 100% natural abundance and a gyromagnetic ratio that is only slightly smaller than that of proton, making it one of the most sensitive nuclei in NMR. Despite its intrinsic high sensitivity, however, applications of ^{19}F NMR experiments to fluorochemicals are often hampered by the wide dispersion of its chemical shifts, which can extend over 200 ppm. In this work, we investigate the application of broadband inversion pulses (BIPs) developed by Shaka and coworkers [1] to ^{19}F-^{13}C heteronuclear correlation experiments. Substantial improvement in sensitivity was achieved using the BIP pulses when compared to hard pulses.

In addition, we have also explored the possibility of using heteronuclear TOCSY as an editing/filtering tool, which may find widespread applications in metabolite profiling of fluorine-containing compounds.

1. M.A. Smith, H. Hu, and A.J. Shaka, Journal of Magnetic Resonance 151 (2001), 269-283.

Monday, September 11th
1:30 PM - 3:00 PM

Small Molecule/Protein Interactions and Screening by NMR
Chair: N. Rama Krishna

Speakers:

Johan Hollander
Leiden University, Leiden, The Netherlands

Maurizio Pellecchia
Burnham Institute for Medical Research
La Jolla, CA

Mario Pinto
Simon Fraser University, Burnaby, BC, Canada

TINS: New Opportunities for Fragment Based Drug Discovery

Sophie Vanwetswinkel[1], Robert Heetebrij[1], John van Duynhoven, **Johan G. Hollander**[1], Dmitri V. Filippov[1], Philip J. Hajduk[2] and Gregg Siegal[1]

1. Leiden Institute of Chemistry, Leiden University, Postbus 9502 2300-RA, Leiden, The Netherlands
2. Advanced Technology, Global Pharmaceutical Research and Development Abbott Laboratories, Abbott Park, IL 60064, USA

Most drug discovery efforts begin with random screening for ligands to the pharmacological target. So-called High Throughput Methods have been very successful at finding ligands, but many do not have appropriate chemical characteristics to ultimately make good drugs. A method termed Fragment-based drug discovery (FBDD) is gaining increased attention because it generates lead compounds with good "drug-like" characteristics and has been successfully applied to challenging targets such as protein-protein interactions. However, FBDD is generally only applicable to soluble proteins available in large quantities (10's mg). TINS, target immobilized NMR screening, is a method that in principle, can be used to find ligands for a broad array of targets including insoluble, integral membrane protein targets obtained in limited quantities. In TINS, the target is immobilized on a solid support. The mixture of compounds to be tested for binding is pumped over the support and binding is detected by 1D ^{1}H NMR spectroscopy of the ligands. We have shown that more than 2,000 compounds can be applied to a single sample of the target with no effect on ligand binding, thereby opening the way to screening an entire fragment library using a single sample of the target. An 8 mm flow-injection, triple gradient probehead with a dual-cell sample holder has been developed to enable TINS for, medium-throughput ligand screening. Using this hardware and an optimized fragment library, screening can be carried out in an automated manner using less than 5 mg of the target. TINS has been successfully applied to a growing number of soluble proteins and nucleic acids. The latest results using the instrument will be presented, including initial attempts to apply TINS to bacterial membrane proteins and G-protein coupled receptors.

SAR by ILOEs: An NMR-Based Approach to Drug Discovery

Maurizio Pellecchia

Burnham Institute for Medical Research
10901 North Torrey Pines Road
La Jolla, CA 92037

We have recently reported on an NMR-based approach, named SAR by ILOEs (structure activity relationships by interligand nuclear Overhauser effect) [1-5], that makes use of protein mediated ligand-ligand NOEs (ILOEs) in complex mixtures to identify initial weak hits that are converted into bi-dentate compounds with higher affinity. Combined with functional studies using the resulting ligands, the SAR by ILOEs method represents an ideal approach to reverse chemical-genetics. Reverse chemical-genetics entails selecting a protein of interest, screening for a ligand for the protein, and finally determine the eventual phenotypic alterations that the ligand induces in a cellular context. Likewise, our method enables the identification of protein's hot spots by using small molecules, regardless of the knowledge of the function of the protein, and the development of a specific assay. Subsequently, such small organic molecules can be used in cellular assays to investigate the possible role of the target. In particular, the approach was applied to the identification of the first inhibitor of the pro-apoptotic protein Bid and then used to characterize its function in cell [2, 5].

1. M. Pellecchia, B. Becattini, K. J. Crowell, R. Fattorusso, M. Forino, M. Fragai, D. Jung, T. Mustelin and L. Tautz, Expert Opin Ther Targets 2004, 8, 597-611.
2. B. Becattini, S. Sareth, D. Zhai, K. J. Crowell, M. Leone, J. C. Reed and M. Pellecchia, Chem Biol 2004, 11, 1107-1117.
3. M. Pellecchia. Chem. & Biol. 2005, 12, 961-71.
4. B. Becattini and M. Pellecchia, Chemistry 2006, 12, 2658-2662.
5. B. Becattini, C. Culmsee, M. Leone, D. Zhai, X. Zhang, M. Rega, S. Landshmaker, J.C. Reed, N. Plesnila and M. Pellecchia, Proc. Natl. Acad. Sci. USA 2006 in press.

A STD-NMR Spectroscopy/ Modeling Protocol for Studying the Conformations of Small Molecules Bound to Enzymes

B. Mario Pinto

Department of Chemistry, Simon Fraser University, Burnaby, B.C. Canada V5A 1S6
email: bpinto@sfu.ca; Tel: 604-291-4152; Fax: 604-291-4860

A protocol for studying the conformations of small molecules when bound to protein receptors by the combined use of Saturation Transfer Difference (STD)-NMR spectroscopy, molecular modeling, and CORCEMA-ST calculations will be described. Several protein-ligand pairs were used to develop the final protocol. Binding of the known glycosidase inhibitors kifunensine and salacinol to Golgi α-mannosidase II was studied by STD-NMR spectroscopy. Docking (AutoDock) in the active site of the enzyme (obtained by X-ray crystallography) followed by calculation of STD effects with CORCEMA-ST gave excellent correlation with the experimental data for the non-mobile portions of the ligands. Excellent correspondence with the X-ray crystal structures of the complexes obtained independently was also observed. Secondly, a system for which the crystal structure of the complex is not known was tested. The complexation of UDP-galactopyranose mutase (UGM), the key enzyme involved in the biosynthesis of Gal*f*, with its small-molecule substrates UDP-Gal*p*, UDP-Gal*f*, and its inhibitors UDP, and UDP-[3F]-Gal*f* was studied using the combined protocol. Excellent correlation between experimental and calculated STD effects was obtained. The results are consistent with insights gained from X-ray crystallographic analysis of the UGM structure alone and previous mechanistic studies, thus validating the models for the UGM- UDP-Gal*p* and UGM- UDP-Gal*f* complexes, and lending credence to the protocol.

Tuesday, September 12th
8:30 AM - 10:00 AM

Latest Advances in Small Molecule RDC's
Chair: Christina Thiele

Speakers:

Christian Griesinger
Max-Planck-Institute, Göttingen, Germany

Daron Freedberg
NIH

Burkhard Luy
Technische Universität München, Germany

Dipolar Couplings to Determine the Conformation and Configuration of Small Molecules

Peter Haberz[1], Jonathan Farjon[1], Wolfgang Bermel[2], Jochen Junker[1], Martin Blackledge[3], **Christian Griesinger**[1]

1. Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
2. Bruker Biospin, Silberstreifen 4, 76287 Rheinstetten, Germany
3. Institut de Biologie Structurale, 41 rue Jules Horowitz, 38027 Grenoble, France

Residual dipolar couplings (rdcs) have proven to be a powerful tool in structural refinement of biological macromolecules over the past decade [1]. They rely on the weak alignment of molecules in solution and provide angular as well as distance information that are not contained in NOE's or J couplings. That's why rdcs have shown to be very efficient in the stereochemical assignment of structural moieties and that they hold the promise of defining the stereochemistry even in non-rigid molecules [2].

Here we present a new pulse sequence, that significantly improves the measurement of rdcs of ^{1}H-^{13}C pairs that are connected by one bond in liquid crystalline media like poly-γ-benzyl-L-glutamate (PBLG) via the removal of the ^{1}H-^{1}H rdcs [3]. Furthermore work towards the distinction of flexible diastereomers and the determination of absolute stereochemistry, as well as an approach to distinguish ^{2}J and ^{3}J scalar couplings with the help of rdcs will be presented.

1. A. Bax, Protein Science 2003, 12, 1-16; J. H. Prestegard, C. M. Bougault, A. I. Kishore, Chem. Rev. 2004, 104, 3519-3540; C. Griesinger, J. Meiler, W. Peti, Biol. Magn. Res. 2003, 20, 163-229.
2. a) C. Aroulanda, V. Boucard, F. Guibé, J. Courtieu, D. Merlet, Chem. Eur. J. 2003, 9(18), 4536-4539 b) L. Verdier, P. Sakhaii, M. Zweckstetter, C. Griesinger, J. Magn. Reson. 2003, 163, 353-359.
3. J. Farjon, W. Bermel, C. Griesinger, J. Magn. Reson. 2006,180(1), 72-82.

Carbohydrate Structure and Dynamics from Residual Dipolar Couplings

Darón I. Freedberg, Richard M. Venable, and Scott E. Norris

Laboratory of Bacterial Polysaccharides, Center for Biologics Evaluation and Research

Food and Drug Administration, Bethesda Maryland, and NHLBI Laboratory of Computational Biology, National Institutes of Health, Bethesda, Maryland

Carbohydrate structure-function relationships are poorly understood because carbohydrate three-dimensional structures are not easily determined in solution. Ideally, one would like to determine the spatial and angular relationships between remote atoms. Nuclear magnetic resonance (NMR) can be used for structural determination of carbohydrates, but long-range distance or angular data have been sparse. RDCs (residual dipolar couplings), dipolar coupling measured under partially oriented conditions in an NMR spectrometer, allow the relation of all bond vectors to a common alignment frame, thus facilitating three-dimensional structure elucidation. RDCs have been successfully applied to many biomolecules. However, carbohydrates are different from other biomolecules. An important factor in determining carbohydrate structure is obtaining sufficient RDC constraints to fit a 5 variable equation for each monosaccharide ring. This requires a minimum of five independent RDCs, which are difficult to obtain in natural abundance because of the near parallel orientation of the C-H bonds in pyranose rings. Thus, isotopic enrichment can afford ^{13}C-^{13}C RDCs in addition to ^{13}C-^{1}H RDCs. This talk will focus on using RDCs in isotopically enriched carbohydrates to determine three-dimensional structures of disaccharides and delineate internal motion in these compounds. It will also address the determination of structural properties in solution for monosaccharides like glucose with the ultimate goal of improving carbohydrate force fields.

Alignment Media and Measurement of Anisotropic NMR-Parameters in Various Solvents

Burkhard Luy

Technische Universität München, Lehrstuhl Organische Chemie II, Lichtenbergstr. 4, 85747 Garching, Germany.

Since the very first spectrum of benzene in a liquid crystalline phase [1], the wealth of structural information contained in partially aligned samples is clearly evident. However, the complexity of spectra did not allow the application of the technique to molecules with more than ≈10 protons. In the last decade, finally, the development of alignment media of sufficiently low alignment strength changed significantly the way of structure determination by the introduction of anisotropic NMR-paramaters like residual dipolar couplings (RDCs) and residual anisotropic chemical shifts (RACS) into biomolecular NMR.

The topic of this presentation will be the extension of the measurement of anisotropic NMR-parameters to medium sized organic molecules like natural products or other biologically active compounds in a variety of organic solvents. Besides crosslinked polystyrene in $CDCl_3$ [2,3], a number of stretched polymer gels as alignment media with arbitrarily scalable alignment strengths will be presented and analyzed with respect to their NMR properties. In addition, improved experiments for the determination of one-bond and long-range heteronuclear scalar and dipolar couplings at natural abundance are introduced. Finally, the use of variable angle NMR (VA-NMR) for the measurement of RACS in stretched polymer gels is demonstrated and an E.COSY-based scheme for sign-sensitive measurement of residual quadrupolar couplings (RQCs) of deuterium nuclei is shown.

1. A. Saupe, G. Englert, Phys. Rev. Lett. 11, 462-464 (1963).
2. B. Luy, K. Kobzar, H. Kessler, Angew. Chem. Int. Ed. 43, 1092-1094 (2004).
3. B. Luy, K. Kobzar, S. Knör, J. Furrer, D. Heckmann, H. Kessler, J. Am. Chem. Soc. 127, 6459-6456 (2005).

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

NMR of Organometallics
Chair: Cornelis Elsevier

Speakers:

Simon Duckett
University of York

Robert Schurko
University of Windsor

Rudi Willem
Vrije Universiteit Brussel

Small Molecule Applications of Hyperpolarisation Methods Involving Reactions with Parahydrogen

Simon Duckett

Department of Chemistry, University of York, York. YO1 5DD.

The low sensitivity of nuclear magnetic resonance means that it is often thought to be unsuitable for the examination of reaction intermediates. In inorganic catalysis, such species play an essential role in determining the rate and selectivity of many processes. It is therefore important that their structures be determined. We, and others, have explored the potential offered by 'parahydrogen' to improve the ability of nuclear magnetic resonance to observe reaction intermediates. More recently this approach has been used to sensitise substrates for use in MRI studies. It should be noted that the parahydrogen effect was first described by Bowers and Weitekamp[1]. The talk will illustrate a number of different applications of parahydrogen, these will include: The development of an NMR probe that allows in-situ sample photolysis. This apparatus has been used to characterise unstable materials at low temperatures [2] to detect solvent complexes and to probe electronic spin states of reaction intermediates. We have used it to prepare a two-spin system in an almost pure state. These states were generated by pulsed and continuous wave UV laser initiation of a chemical reaction between $Ru(CO)_3(dppe)$ and pure parahydrogen (generated at 18°K) which forms $Ru(CO)_2(dppe)(H)_2$ on a sub-microsecond timescale. With pulsed laser irradiation, the spin state of the hydride nuclei in $Ru(CO)_2(dppe)(H)_2$ proved to have a purity of 89.8 ± 2.6 %.[3] The talk will also illustrate how the parahydrogen principle can be extended to complexes containing no hydride ligands and thereby overcome a major limitation of the technique. In the first report of this type a significant hydroformylation intermediate was detected via an acyl-ligand rather than through the metal-hydride signal.[4] The detection of intermediates in cobalt catalysed hydroformylation has also been achieved.[5] Metal-alkyl hydrides can also be studied using this approach [6]. The talk will also show how parahydrogen can detect key biological substrates at picomole levels.

1. C. R. Bowers and D. P. Weitekamp, Phys. Rev. Lett., 1986, 57, 2645.
2. C. Godard, et al., Chem. Comm., 2002, 2836.
3. S Anwar, et al., Phys. Rev. Lett., 2004, 93, 040501.
4. C. Godard, et al., Chem. Commun., 2004, 1826-1827.
5. C. Godard, et al., J. Am. Chem. Soc. 2005, 127, 4994-4995
6. J. Dunne, et al., J. Am. Chem. Soc., 2004, 126, 16708.

Solid-State NMR of Metal Nuclei in Metallocenes

R.W. Schurko, H. Hamaed, I. Hung, A.Y.H. Lo, C.L.B. Macdonald, A.J. Rossini, J.A. Tang, C.M. Widdifield and M.J. Willans

Department of Chemistry & Biochemistry, University of Windsor, Windsor, Ontario, Canada, N9B 3P4

Solid-state NMR spectroscopy, X-ray crystallography and first principles calculations have been applied in a comprehensive study of NMR interaction tensors at metal nuclei in metallocenes. The structural variability of the metallocenes provides a wide range of metal coordination environments to explore the fundamental origins of chemical shielding (CS) and electric field gradient (EFG) tensors, thereby permitting rational examination of the relationships between NMR interactions and molecular structure, symmetry and dynamics. Solid-state NMR spectra of the metal nuclei act as unique "fingerprints" of metal coordination environments, and show much promise for investigation of metallocenes in disordered solid systems such as support materials for heterogeneous catalysis. The application of QCPMG pulse sequences and ultra-high fields to the study of metallocenes will also be discussed.

Grafted Organotin Catalysts: A Story on High Resolution Magic Angle Spinning NMR With and Around the ^{119}Sn Nucleus

R. Willem[1], V. Pinoie[1], K. Poelmans[1], M. Biesemans[1], I. Verbruggen[1], J. C. Martins[2], G. Deshayes[3], P. Degée[3], P. Dubois[3]

1. High Resolution NMR Centre (HNMR), Department of Polymer Science and Structural Chemistry (POSC), Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.
Email: rwillem@vub.ac.be
2. Laboratory of Polymeric and Composite Materials (LPCM), University of Mons-Hainaut, Place du Parc 20, B-7000 Mons, Belgium.
3. Research Unit NMR and Structural Analysis, Vakgroep Organische Chemie, Universiteit Gent, Krijgslaan 281 S4, B-9000 Ghent, Belgium.

Organotin compounds have important industrial applications, in particular as catalysts in transesterification reactions. An environmentally friendly approach to avoid tin contamination of the reaction products consists of grafting the organotin reagent onto cross-linked polystyrene, allowing straightforward removal of the catalyst from the reaction mixtures by simple filtration of the grafting support to which it is linked.Mono- and dialkyl tin chlorides and their associated phenyl precursors, grafted to cross-linked polystyrene through an undecamethylene spacer, with ca. 30% functional loading, were characterized by high resolution Magic Angle Spinning (hr-MAS) NMR, being the methodology of choice for the structural analysis of the solid-liquid organotin interface. The functional identity and purity of the organotin grafts were mainly assessed by 1D and 2D ^{1}H, ^{13}C and ^{119}Sn hr-MAS NMR [1]. The catalytic activity of the grafted organotin chlorides was investigated in a model transesterification of ethyl acetate with octanol [2] as well as in the ring opening polymerisation of epsilon-caprolactone [3]. Besides monitoring the transesterification progress by ^{1}H NMR in solution, it is demonstrated that ^{1}H, ^{13}C and ^{119}Sn hr-MAS NMR enables one to assess the chemical integrity and recycling ability of the catalyst as well. Especially DOSY-filtered ^{1}H hr-MAS spectra of the actual reaction mixture allow investigating the catalyst in situ during the reaction, while ^{119}Sn hr-MAS NMR specifically assesses the coordination characteristics of the tin atom, thus outlining the catalysis mechanism in situ. Fast equilibrium exchange kinetics of the different reaction components in the transesterifcations with the tin atom by coordination expansion at the interface can clearly be identified through line broadening and coalescence phenomena.

1 J.C. Martins, et al., Chem. Eur. J. 2002, 8, 3431.
2. C. Camacho-Camacho, et al., Chem. Eur. J. 2005, 11, 2455.
3. G. Deshayes, et al., Chem. Eur. J. 2005, 11, 4552.

Tuesday, September 12th
1:30 PM - 3:00 PM

NMR in the Forensic Sciences
Chair: Patrick Hays

Speakers:

Thomas Schaefer
Bundeskriminal Amt (BKA), Germany

Matthew Rees
US Environmental Protection Agency

Robert Thompson
US Drug Enforcement Administration

Quantitative Deuterium-NMR on Synthetic Drugs

Thomas Schaefer, Torsten Schoenberger and Michael Schumorek

Bundeskriminalamt, Wiesbaden, Germany

The identification of unknown substances plays a key role in the work of a forensic scientist. Although is can often be done very easily by applying the known techniques, it is generally quite complicated to differentiate one source of the same substance from another. To answer the question, whether samples originated from the same source or not, the determination of the ratios of stable isotopes could be used. In this talk, the use of quantitative deuterium NMR on linking or distinguishing precusors of illicit synthetic drugs is presented. A standard operation procedure with respect to the impurities in real samples from illicit labs was established. Deuterium NMR was also utilized to tell drug precursors synthesized from natural products from those made by petrochemicals. For that purpose it was necessary to enhance the resolution of signals arising from aromatic moieties by chemical modification of the substances.

NMR Chemical Forensics at the EPA - National Enforcement Investigations Center

Matthew S. Rees, Jon L. Beihoffer, Jimmy L. Seidel

United States Environmental Protection Agency - National Enforcement Investigations Center (NEIC)

Nuclear Magnetic Resonance has not seen wide acceptance for routine environmental analysis for several reasons. The level of detection for NMR is often several orders of magnitude higher than other techniques such as GC/MS and GC/ECD. Furthermore, the cost of the instrumentation, lack of comprehensive library search capability of known spectra, and qualified staff to interpret spectra are limitations for widespread use within the environmental analytical community.

We have found great utility for NMR during forensic examinations of samples for both criminal and civil casework. At EPA's National Enforcement Investigations Center, we have used NMR to provide quantitative analysis of samples, assist in identification of unknowns, and for secondary confirmation of results by comparison with other techniques. We have had the opportunity to observe numerous nuclei during these analyses. This discussion will provide some examples of NMR utilization in EPA's forensic laboratory.

NMR in the Fight against Illicit Drugs

Robert A. Thompson and Patrick A. Hays

DEA Special Testing and Research Laboratory, Dulles, VA

In-depth sample analysis in direct support of law enforcement and strategic intelligence is the primary mission of the Drug Enforcement Administration's laboratory system. Accurate identification and quantitation of both controlled and non-controlled substances found in complex drug mixtures is a key step in this process. Under most circumstances, NMR spectroscopy offers a fast, reliable, non-destructive method capable of identifying and quantifying nearly all components in one experiment using a single standard. In addition, automation allows a user to carry out the entire process on batch samples with minimal input. In some instances, it is even possible to determine the salt form and/or the optical form of the principal drug of interest. NMR can also expedite the analysis of clandestine laboratory samples by quickly identifying solvents, precursors and product material taken from the site. Furthermore, 1D- and 2D-NMR data used in conjunction with mass spectral data and structural elucidation software is useful in determining the structure of unknown compounds for which standards may be currently unavailable.

Wednesday, September 13th
8:30 AM - 10:00 AM

Student and Post-Doctorate Session
Chair: Bridget Becker

Speakers:

Sujoy Mukherjee
University of Illinois, Urbana, IL

Peter Haberz
Max-Plank-Institute for Biophysical Chemistry, Germany

Zhengzheng Pan
Purdue University, West Lafayette, IN

An NMR and QM Investigation of the Mechanism of Action of Bisphosphonates

Sujoy Mukherjee, Junhong Mao, Rong Cao, Yong Zhang, John M. Sanders, Yongcheng Song, Dushyant Mukkamala, Michael P. Hudock, Eric Oldfield.

Departments of Biophysics and Chemistry, University of Illinois at Urbana-Champaign

The isoprene biosynthesis pathway is a potentially important target for the development of novel anti-infective (antibacterial and anti-protozoal) drugs, as well as anti-cancer and bone-resorption drugs. There are several components of this pathway, all centered on the formation of isoprenoids via the enzyme farnesyl diphosphate synthase. This enzyme is potently inhibited by a class of compounds called bisphosphonates. We recently obtained x-ray structures of this enzyme bound to different inhibitors and are now using solid state ^{13}C, ^{15}N and ^{31}P NMR and quantum chemistry to learn more about how this enzyme is inhibited by bisphosphonates. We find a large chemical shift range (26 ppm) in model bisphosphonates with, in general, monoanionic or deprotonated phosphate groups resonating to high field. Both the isotropic as well as the anisotropic shift (or shielding) tensor elements are well predicted by using Hartree-Fock calculations using a uniform 6-311++G(2d,2p) basis, with charge field perturbation using a lattice of Merz-Kollman charges. The rms error between theory and experiment is 6-8% of the range seen experimentally. The crystallographic structures firmly establish protonation patterns for both the phosphate as well as the sidechain atoms. In proteins, the NMR shifts of the ligands are poorly predicted in the QM calculations, however, on full QM geometry optimization, the refined structures do permit accurate shift predictions, enabling for example, protonation state information to be obtained, of help in drug design.

In addition to binding to FPPS, bisphosphonates bind also to bone. At low concentrations, bisphosphonates bind as a surface absorbed species, but a crystalline phase forms at high bisphosphonate concentrations and has been characterized via powder diffraction and SSNMR. We found that bisphosphonates have protonated sidechains when binding to bone. We are currently using dipolar recoupling experiments to obtain structural information regarding bisphosphonate conformations when bound to bone.

Structure Calculations on Small Molecules: Can RDCs Solve Stereochemical Problems?

Peter Haberz[1], Kyryl Kobzar[3], Wolfgang Bermel[2], Jochen Junker[1], Burkhard Luy[3], Horst Kessler[3], Martin Blackledge[4], Christian Griesinger[1]

1.Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
2. Bruker Biospin, Silberstreifen 4, 76287 Rheinstetten, Germany
3. Technical University Munich, Lichtenbergstr. 4 85747 Garching, Germany
4. Institut de Biologie Structurale, 41 rue Jules Horowitz, 38027 Grenoble, France

The elucidation of the relative stereochemistry of asymmetric centers of organic molecules is an important challenge in chemistry since it requires the simultaneous determination of conformation and configuration. While the conventional NMR parameters like NOE and ^{3}J coupling constants, which provide internuclear distances and dihedral angles, yield the configuration of stereocenters in rigid compounds, this approach is difficult or impossible in cases where the molecule is flexible or the stereocenters are remote in the bonding network. Residual dipolar couplings (RDCs) have proven to be very efficient in the stereochemical assignment of moieties and hold the promise of defining the stereochemistry even in non-rigid molecules [1].

Here we present the conformational analysis of the cyclic peptides hormaomycin and hymenistatin. For the latter one 5 sets of RDCs in 3 different solvents could be measured. Calculations have been performed with XPLOR-NIH [2] and SCULPTOR [3]. The two calculation programs will be compared and their applicability for the calculations with small molecules will be discussed.

1. A. Bax, Protein Science 2003, 12, 1-16; J. H. Prestegard, C. M. Bougault, A. I. Kishore, Chem. Rev. 2004, 104, 3519-3540; C. Griesinger, J. Meiler, W. Peti, Biol. Magn. Res. 2003, 20, 163-229.
2. C.D. Schwieters, J.J. Kuszewski, N. Tjandra and G.M. Clore, The Xplor-NIH NMR Molecular Structure Determination Package, 2003, J. Magn. Res., 160, 66-74.
3. J.-C. Hus, D. Marion, M.Blackledge, J. Mol. Biol., 2000, 143, 927-936.

Principal Component Analysis of Urine Metabolites Detected by NMR and DESI-MS in Patients with Inborn Errors of Metabolism

Zhengzheng Pan, Haiwei Gu, Nari Talaty, Huanwen Chen, Narasimhamurthy Shanaiah, Bryan E. Hainline, R. Graham Cooks and Daniel Raftery

Department of Chemistry, Purdue University

Urine metabolic profiles of patients with inborn errors of metabolism were examined with nuclear magnetic resonance (NMR) and desorption electrospray ionization mass spectrometry (DESI MS) methods. Spectra obtained from the study of urine samples from individual patients with argininosuccinic aciduria (ASA), classic homocystinuria (HCY), classic methylmalonic acidemia (MMA), maple syrup urine disease (MSUD), phenylketonuria (PKU) and type II tyrosinemia (TYRO) were compared with six control patient urine samples using principal component analysis (PCA). Target molecule spectra were identified from the loading plots of PCA output and compared with known metabolic profiles from the literature and metabolite databases. Results obtained from the two techniques were then correlated to obtain a common list of molecules associated with the different diseases and metabolic pathways. The combined approach discussed here may prove useful in the rapid screening of biological fluids from sick patients and may help to improve the understanding of these rare diseases.

Wednesday, September 13th
10:30 AM - 12:00 PM

New Experimental Techniques
Chair: A. J. Shaka

Speakers:

Vladimir Mandelshtam
University of California, Irvine

Jeff Hoch
University of Connecticut Health Center

Eriks Kupce
Varian Ltd., Oxford, UK

Haste Makes Waste

Vladimir Mandelshtam

University of California, Irvine

Diffusion Ordered Spectroscopy (DOSY) is an invaluable tool for chemical characterization of mixtures in solution, providing a two dimensional (2D) spectrum, where the chemical shift is displayed in one dimension and the diffusion coefficient in the other. However, the needed spectral information cannot be obtained by the conventional means, i.e, by the 2D Fourier Transform (FT), as the spectral separation requires to implement either a multiexponential fit or inverse Laplace transform (ILT) along the diffusion dimension.

Most currently used methods utilize the separability of the 2D inversion problem by Fourier transforming the data along the proton dimension and reducing it to a series of 1D ILT problems. However, this "simplification" seems counterproductive as it ignores the existing correlations in the 2D data and as such limits the accessible resolution severely. Moreover, because the DOSY signals decay very rapidly along the diffusion dimension, a multi-exponential fit of a 1D slice of such data at a given value of the proton chemical shift is unreliable, while a single-exponential fit does not allow one to separate overlapping multiplets that belong to different molecules. Only a truly multidimensional processing, may overcome these limitations. The 2D Regularized Resolvent Transform (RRT), developed earlier in our group in collaboration with A.J. Shaka [1], is one of such methods. 2D RRT is a linear algebraic technique emerged from the 2D Filter Diagonalization Method (FDM) that estimates the 2D Fourier spectrum from a finite 2D time signal avoiding the above mentioned factorization of the 2D problem into a series of 1D problems. In the present case the method is modified by replacing the frequency argument in the diffusion dimension by an imaginary frequency [2].

In my talk I will describe the methodology and show some applications to 2D DOSY experiments. I will also mention 3D DOSY experiments.

1. J. Chen, A.J. Shaka and V. A. Mandelshtam, J. Magn. Reson, 2000, 147, 129.
2. G. S. Armstrong, N. M. Loening, J. E. Curtis, A. J. Shaka and V. A. Mandelshtam, J. Magn. Reson., 2003, 163, 139.
3. P. Thureau, A. Thévand, B. Ancian, G. S. Armstrong and V. A. Mandelshtam, ChemPhysChem, 2005, 6, 1510.

Spectral Reconstruction Methods in Fast NMR: Reduced Dimensionality, Random Sampling, and Maximum Entropy

Mehdi Mobli[1], Alan S. Stern[2], and **Jeffrey C. Hoch**[1]

1. University of Connecticut Health Center
2. Rowland Institute at Harvard

Reduced-dimensionality (RD) methods, including GFT and back-projection reconstruction (BPR), have been proposed for reducing the measuring time required to obtain high resolution multidimensional NMR spectra. We show that RD experiments are a special case of the more general approach of nonuniform sampling (NUS), and consequently are amenable to processing using methods of spectral analysis that do not require uniformly-spaced samples, such as Maximum Entropy reconstruction (MaxEnt). Artifacts that characterize RD spectra are shown to result whether the data is processed via BPR or MaxEnt, demonstrating that the artifacts are a consequence of the data sampling and not the processing method. Randomly choosing time increments based on an exponentially weighted distribution is more efficient, and results in fewer spectral artifacts, than the systematic coupling of time increments used in RD approaches. The use of NUS for improving resolution and reducing data collection time will be demonstrated for both solution-state and solid-state experiments.

Multidimensional NMR Spectroscopists in a Hurry

Eriks Kupce[1] and Ray Freeman[2]

1. Varian Ltd., Oxford, UK;
2. Jesus College, Cambridge University, Cambridge, UK

Multidimensional experiments can be speeded up considerably by drastically limiting the sampling, either in frequency space or in evolution space. For the former, information derived from a quick one-dimensional experiment is used to identify the chemical shifts, and these frequencies are then excited selectively. Encoding according to a Hadamard matrix allows all the selective excitations to be performed simultaneously, and the individual responses separated by a decoding scheme based on the same matrix. Alternatively, the most effective way to sample evolution space is along a small number of tilted radii, relying on Bracewell's slice/projection Fourier transform theorem to calculated tilted projections. There are then many ways to reconstruct the multidimensional spectrum from these projections. It is even possible to imagine a ten-dimensional spectrum constructed from a limited set of two-dimensional measurements. The speed gain of projection-reconstruction NMR is approximately an order-of-magnitude for each new evolution dimension. Further speed gains can be contrived by "parallel acquisition" of two or more multidimensional spectra at the same time, using a triple-resonance probe and two or more dedicated receiver channels.

Tuesday, September 12th
8:00 PM - 10:00 PM

Poster Session

Chair: Laurie Galya

Index To Posters

1. ^{1}H NMR Method for the Routine Spectroscopic Determination of Enantiomeric Purity of Active Pharmaceutical Ingredients Fenfluramine, Sertraline, and Paroxetine
2. Small Molecules Warn BioNMR: Some Weakly Aligning Media Can Be Dangerous for 3D Structure Determination!
3. Fixed-Width Binning, Variable-Width Binning or No Binning: A Study of Different Binning Methods in NMR-based Metabolomics Analysis
4. Identification of Phenylethyl Glycosides from *Blepharis aspera* by LC-UV-SPE-NMR/MS Hyphenation
5. Comparison of ELSD and NMR for Quantitation of Libraries
6. High Throughput of NMR Samples in Metabonomics and Parallel Synthesis
7. HyperSense DNP-NMR for the Rapid Determination of Heteronuclear Spin-Lattice Relaxation Times
8. Estimation of NMR Chemical Shifts
9. Detection of Minor Isomers and Study of Chemical Kinetics by DNP NMR
10. Characterising Solid-State DNP
11. LC-MS-SPE/NMR for Rapid Isolation and Identification of Natural Products
12. Utilization of a Solid-Supported Reagent for the Facile Synthesis of Unusual Metabolites
13. The Keto-enol Tautomerization Study of Ethyl Butylryl Acetate by LC-NMR
14. A JAVA Applet for Calculating NMR Coupling Constants, NOEs and Three-dimensional Structure Display
15. NMR-Based Lead Identification, Validation and Optimization at the San Diego Center for Chemical Genomics (SDCCG)
16. Structure Elucidation of Photodegradants of a Novel Glucocorticoid Receptor Agonist
17. Automated Small Volume NMR for Medicinal Chemistry
18. A Karplus Equation for $^{3}J_{HCCN}$ in Amino Sugar Derivatives
19. ^{119}Sn Relayed ^{1}H-^{183}W Correlation Spectroscopy
20. The CHECKIN Program: Design and Implementation of an Automated Process for Submitting Open-Access NMR Data for Compound Validation

21. Multiple-Sample Probe for High-Throughput Solid-State NMR Spectroscopy
22. The Effect of Structure Description Schemes on Chemical Shift Prediction by Incremental and Neural Network Approaches
23. Automated Evaluation of a Chemical Structure with only 1D ^{1}H and 2D ^{1}H-^{13}C HSQC
24. To Flow or Not to Flow
25. Deciphering Ligand Binding Site Locations by Competition NMR
26. Characterization of Phase Separation of Pharmaceutical Materials by SSNMR
27. Molecular Conformation and Calculated NMR Shifts using the Gaussian Program
28. Characterizing Drug-Excipient Interactions by SSNMR
29. Evaluation of ^{1}H and ^{13}C NMR Prediction Methods
30. Chemical Shift Correlations in Drug Discovery – Methods and Applications
31. Towards the Unambiguous NMR Assignment of Diasteromeric Cyclic Sulphites. A Experimental and Theoretical Approach.
32. NMR Studies of the Enzymatic Synthesis and Photo-Switching of a Peptide-Capped Cyclodextrin Rotaxane
33. Acid Catalyzed Degradation Pathways of Ixabepilone, the aza-Epothilone B Analog
34. The Hexacyclinol Controversy. What Went Wrong With The Original Structure Determination?
35. On the Importance of Structure Stereochemical Markers in ^{13}C NMR Predictions
36. Critcal Comparison of Epitope Mapping Experiments for Binding of Propranolol to AGP
37. Characterizing Monorhamnolipids in Mixtures
38. New Application “MICCS-NMR” for Direct Synthesis Monitoring by NMR
39. A Web Based NMR Automation Interface for Multiple Software Versions
40. Small Volume NMR Probe Family extended by New 30 µLiter $^{1}H/^{13}C$ Optimized Inverse Probe
41. Characterization of a New Form of Aspirin
42. Water Suppression with 20/20 Hindsight

43. Using Quantitative NMR and HPLC for the Determination of HPLC-UV Relative Response Factors

44. Applications of Quantitative NMR in the Pharmaceutical Industry

45. Reaction NMR: A Method for Real Time Monitoring of Chemical Reactions and Processes

46. Metabolomics for Discovery of Biomarkers of Hepatotoxicity

47. Probe Comparison: A Reality Check

48. Investigation of an LDA Reaction Dianion Intermediate Using ^{1}H, ^{7}Li, ^{15}N and ^{19}F NMR Experiments

49. In Situ Derivatization Method for Configurational Assignement by NMR

50. Solid-State NMR Characterization of Risedronate Hydrate Forms and Dehydrated Risedronate

51. Polymeric NMR Sample Tubes Evaluated in a 1 mm Microprobe

52. Sample Preparation Strategies for Routine Trace Mixture Analysis by NMR

53. Streamlined analysis of 1D NMR spectra

54. LC/SPE/NMR in Pharmaceutical Development: its all about the SPE

55. Quantitation of Small Molecules in Protein-Containing Solutions Using a Spin-Echo Method

56. Using pH Adjustments to Resolve and Detect Small Molecule Components in Protein Containing Solutions

1 ^{1}H NMR Method for the Routine Spectroscopic Determination of Enantiomeric Purity of Active Pharmaceutical Ingredients Fenfluramine, Sertraline, and Paroxetine

Romila D. Charan, Jonathon S. Salsbury and Paul K. Isbester

Analytical Quality Services, Albany Molecular Research, Inc., P.O. Box 15098, Albany, NY 12212-5089 USA

Enantiomeric purity of three active pharmaceutical ingredients (API's) were determined using NMR and the chiral solvating agent (CSA) 1,1-Bi-2-napthyl (BINOL). The technique described is the first successful application of using CSA's for enantiomeric purity determination for fenfluramine HCl, sertraline HCl, and paroxetine HCl. We found the CSA technique described herein to provide comparable results to traditional chiral HPLC methods and other published procedures for evaluation of enantiomeric purity for these compounds. The procedure is commonly used in our laboratory as a complementary/alternative method to chiral HPLC or optical rotation measurements for routine determination of enantiomeric purity. Enantiomeric purity determination by ^{1}H NMR utilizing chiral solvating agents does not require special instrumental techniques, chemical derivatization, or standards and is therefore ideally suited for rapid analysis of routine samples such as in-process and release testing.

2 Small Molecules Warn BioNMR: Some Weakly Aligning Media Can Be Dangerous for 3D Structure Determination!

Aleksandr B. Sahakyan, Astghik A. Shahkhatuni, Henry A. Panosyan and Aleksan G. Shahkhatuni

Molecule Structure Research Center of National Academy of Sciences, Armenia

Dipolar couplings are one of the most informative and precise data in NMR, the theoretical possibilities of which are still beyond our present understanding. The perfect structural method must be as insensitive as possible to experimental conditions. To this end, the investigations of the aberration causes and the ways to avoid them are very important for implementation of LXNMR as a complete structural method.

The aim of this work is a systematic study of solvent effects in weakly aligning liquid crystalline systems widely used in biomolecular NMR for acquisition of residual dipolar couplings (RDCs). ^{13}C-enriched acetonitrile has been used as a probe molecule for most of the experiments.

Results show that most of the selected lyotropic systems (CTAB, CPBr, DMPC/DHPC bicelles) and pf1 solutions are quite good media for structural investigations using RDCs, and the aberrations are within the experimental error. Nevertheless, we have found a medium which shows considerable discrepancies of resulting structural data from the expected value. That medium was sodium dodecylsulfate (SDS) based lyotropic system. The geometrical parameters determined in that media strongly depend on temperature, have significant spread and deviation from expected value.

The presence of such lyotropic medium producing anomalous data for solute molecule dictates the necessity of more careful examinations of weakly aligning media which are presently in use. Each new lyotropic system must be tested by small molecules in order to avoid crucial mistakes. On the other hand, the existence of complex, anomalous lyotropic systems can open new perspectives and opportunities which can be used after understanding them.

3 Fixed-Width Binning, Variable-Width Binning or No Binning: A Study of Different Binning Methods in NMR-based Metabolomics Analysis

Chen Peng[1], Gregory M. Banik[1], Omoshile Clement[1], Ty Abshear[1], Scott Ramos[2], and Brian Rohrback[2]

1. Bio-Rad Laboratories, Inc., Informatics Division
2. Infometrix, Inc.

Metabolic profiling (metabolomics/metabonomics) is an important emerging field that studies the metabolic changes in response to perturbations, such as disease or the administration of xenobiotics (e.g., pharmaceuticals). NMR spectroscopy of various biofluids, including blood serum and urine, has become a primary analytical technique used in metabolic profiling experiments. Subsequent to the data collection, multivariate analysis—predominantly Principal Components Analysis (PCA)—is performed to reduce the complexity of the data set by reducing the dimensionality of the collected NMR spectra.

Small shifts in the NMR spectra, however, can adversely impact the PCA results. Techniques to address the NMR peak misalignment issue include global spectral alignment and local peak alignment, as well as binning or bucketing the spectra. The latter technique is also used to reduce the overall amount of data to fit into the 255 column limit of Microsoft Excel, often used as a go-between from the spectral collection software package to the PCA software package.

Fixed-width bucketing (usually at 0.04 ppm) is a common practice. More recently, some researchers have experimented with variable-width bucketing in which the width of the bucket is allowed to vary slightly to place each bucket boundary at the local minima of all overlapped NMR spectra, largely avoiding the placement of bucket boundaries in the middle of NMR peaks. On the other hand, using all points directly without binning is also becoming practical as more integrated systems are becoming available. Bio-Rad has introduced a new variable-width bucketing technique based on its patent-pending Overlap Density Heatmap technology. In this new technique, bucket boundaries are placed at the local minima of the Overlap Density Heatmap consensus spectrum formed by assessing the equi-density profile of all NMR spectra at a given overlap density level. This paper will use data from several metabolic profiling experiments to compare the class separation of different disease states as well as time points with fixed-width, variable-width (based both on simple spectral overlap as well as Overlap Density Heatmaps), and non-bucketed (i.e., full resolution) spectra.

4 Identification of Phenylethyl Glycosides from *Blepharis aspera* by LC-UV-SPE-NMR/MS Hyphenation

Frode Rise[1], Edward Eddie Mmatli[1,2], Helle Malreød[1], Steven Ray Wilson[1], Elsa Lundanes[1], Tyge Greibrokk[1], Dirk Petersen[1], Berhanu Abegaz[2]

1. Department of Chemistry, University of Oslo, NO-0315 Oslo, P.O. Box 1033, Blindern, Norway
2. Department of Chemistry, University of Botswana, Private Bag 00704, Gaborone, Botswana

LC-UV-SPE-NMR/MS was used for the fractionation and acquiring spectral data of an enriched phenolic fraction of the aerial parts of *Bleparis aspera* (Acanthaceae) [1]. In this study two phenylethyl glycosides: verbascoside and isoverbascoside present in the fraction were separated and characterized. Efficient separation and enrichment on the LC-SPE hypercarb cartridges was monitored by placing a mass spectrometer after the LC-SPE unit to observe any analyte break-through. This also allowed multiple trapping of peaks. Efficient transfer from the SPE cartidges to the NMR probe was done by placing the UV detector in between the SPE and the NMR probe to observe elution of the analytes from the SPE cartridge into the NMR probe [2]. LC was also coupled to off-line NMR analysis to separate, characterize and collect these compounds with a Foxy fraction collector.

1. Wolfender, J-L., Ndjoko, K., Hostettmann, K., The potential of LC-NMR in phytochemical analysis, Phytochemical Analysis, (2001) 12, 2-22.
2. Wilson, S. R., Malerød, H., Petersen, D., Simic, N., Bobu, M-M., Rise, F., Lundanes, E., Greinrokk, T., Controlling LC-SPE-NMR systems, Journal of Separation Science, (2006) 29, 582-589.

5 Comparison of ELSD and NMR for Quantitation of Libraries

Thomas O'Connell, Kevin Colizza, Diane Rescek, Navin Varshney, Amin Kamel, Hong Wang

Pfizer Global Research & Development, Groton Laboratories, Groton, CT

Accurate quantitation of compound libraries has become increasingly important in drug discovery for HTS screening. Various LC based methods employing detectors such as UV, ELSD and CLND have been used, but all suffer from significant drawbacks. NMR is a universal technique that has become the gold standard for quantitation. Recent advances in probe design allow high throughput with minimal sample and use of the ERETIC pulse (Electronic Reference to access in vivo Concentrations) eliminates the need to add an internal standard. This poster describes a systematic comparison of ELSD verses NMR quantitation techniques using 44 standard drug like compounds that were chosen to cover a wide array of molecular weights and physical chemical properties. Two identical plates were made containing 30mM DMSO solutions of each compound. One plate was analyzed by LC-ELSD using four external calibration curves and the other was analyzed by NMR using the ERETIC pulse. These plates were analyzed simultaneously by both techniques in order to minimize variations in storage conditions. Each sample was run in triplicate in order to measure precision as well as accuracy. Percent error and standard deviation were calculated. In addition, these results were compared to an actual library plate of 60 compounds that was analyzed in a similar manner.

6 High Throughput of NMR Samples in Metabonomics and Parallel Synthesis

Tineke Papavoine, Torbjörn Halvarsson, Ingvar Nilsson and Marie Landergren

AstraZeneca R&D, Mölndal, Sweden

As a consequence of an increased use of parallel synthesis and a larger interest in metabonomics, a higher throughput of NMR measurements in an automatic setup is needed. Varians robot system 768AS, coupled to a Varian Inova 600 MHz NMR system, is specially designed to automatically prepare samples in NMR tubes and subsequently insert them into the magnet, acquire NMR data and bring the samples back to the rack. At our site, we use this setup for metabonomics studies. In the same setup, 96-well plates originating from organic parallel synthesis are measured. The setup of the robotics system will be shown and described. When the parallel synthesis is ready, the samples are analyzed by LC-MS and purified in an automatic manner. At the end of this process, 2 mM NMR plates (~200 μg) are diluted from a so-called mother plate, containg 'pure' samples. The NMR plates are transferred to the integrated robotic system 768AS coupled to the NMR spectrometer, where they are measured. Files that are created in the analysis and purification process are used to create NMR run files, to make the transfer as smooth as possible. Using this setup, 10 samples per hour are being run. For the metabonomics studies, samples are freshly prepared by the robot before they are measured. Depending on the body fluid and on the origin of the sample, 4, 2 or 0.5 samples per hour can be measured. Results on a human urin study are given.

7 HyperSense DNP-NMR for the Rapid Determination of Heteronuclear Spin-Lattice Relaxation Times

Iain J. Day[1,2] John C. Mitchell[1], Martin Snowden[1] and Adrian L. Davis[2]

1. Medway Sciences, University of Greenwich, Chatham Maritime, UK
2. Pfizer Global Research and Development, Sandwich Laboratories, Kent, UK

Direct detection of heteronuclear relaxation times at natural abundance is hampered by poor sensitivity. Combined with long relaxation times of nuclei such as carbon-13 and nitrogen-15, especially in small molecules, traditional inversion-recovery methods are impractical for routine use. Recently the use of DNP-NMR to generate hyperpolarisation of carbon-13 and nitrogen-15 in the solution state and thereby enhance sensitivity has been demonstrated [1].

In this approach, the analyte of interest is doped with a stable trityl radical and then frozen in an amophous glass at 1.5°K in a magnetic field of 3 T. The electron spin polarisation generated at low temperature is transferred to the analyte using microwave irradiation applied at approximately the difference in electron and nuclear Larmor frequencies. Once sufficient polarisation has built up, the sample is dissolved using super-heated methanol and transferred to a standard high-resolution solution-state NMR spectrometer for detection.

In this poster we will show that the application of a train of small flip-angle pulses, similar to the Single-Scan FT method for T_1 determination [2], coupled with the enhanced sensitivity offered by DNP-NMR allows for the rapid and accurate determination of heteronuclear spin-lattice relaxation times. Rather than requiring a series of spectra to be recorded with recycle delays of at least $5T_1$ our DNP-based method is limited only by the time required to generate the solid-state polarisation.

This project is a collaboration with Oxford Instruments Molecular Biotools Ltd.

1. J. H. Ardenkjaer-Larsen,et al., Proc. Natl. Acad. Sci. USA. 100, (2003) 10158.
2. R. Kaptein, K. Dijkstra and C. E. Tarr, J. Magn. Reson. 24, (1976) 295.

8 Estimation of NMR Chemical Shifts

Stephen G Spanton and David Whittern

Abbott Laboratories, Abbott Park Il

Over the last several years, the increased automation of organic reactions has lead to increases in the number of compounds generated by each chemist and the NMR spectral data that need to be analyzed to characterize those compounds. NMR is an information rich analytical tool, but it requires complex analysis to extract all the structural information. One typical use of NMR in high throughput organic synthesis is to determine if the spectrum is consistent with the proposed structure.

We have developed an automated NMR analysis system that will grade a spectrum for the identity and purity of a sample based on a chemical structure. One of the key components of this system is the estimation of the chemical shift range for each of the observed nuclei. The Abbott NMR estimation program is based on the HOSE code (Hierarchically Ordered Spherical Description of Environment) [1] and a structural similarity model. The HOSE code based method provides broad coverage of chemical shift envirnments while the similarity model provides improved accuracy. The reference compounds were predominately taken from the NMR assignments that have been made in the NMR lab with a few select compounds from the literature.

While the HOSE code method is a very general method for describing the chemical environment, it has several limitations. First, the similarity match between the HOSE codes of the reference compound and the test molecule must be the same along all bonds at each shell level. Secondly, the HOSE codes do not include stereochemistry.

The model based NMR estimation uses a specific model compound for the chemical shift estimation for those parts of the test compound that overlap with the reference compound. While the HOSE code based method provides an average value for the estimated chemical shift using all available assignments with matching HOSE codes, the model based system uses the assignments of one specific reference structure chosen because it closely matches the test structure. The model can be automatically selected by the program or can be specified by the user. The model-based approach is well suited to the analysis of chemically related series of compounds that have a common core structure.

1. W. Bremser, Analytica Chemica Acta, 103 (1978) 355-365.

9 Detection of Minor Isomers and Study of Chemical Kinetics by DNP NMR

Damir Blazina and Steven Reynolds

Oxford Instruments Molecular Biotools Ltd., Tubney Woods, Abingdon, Oxon OX13 5QX, UK

Dynamic Nuclear Polarisation (DNP) is a hyperpolarisation technique that offers an increase of over 10,000 times in the signal-to-noise ratio (SNR) for ^{13}C and ^{15}N in solution-state NMR spectroscopy [1].

The sample of interest is doped with a trityl radical and dissolved in a mixture of solvent that will form a glass when frozen. It is then exposed to a very low temperature (<4°K) in a strong magnetic field (3.35 T). Under these conditions, the unpaired electrons on the trityl radical become hyperpolarised. Microwave irradiation at the appropriate frequency (ca. 94 GHz) transfers this polarisation to atomic nuclei. Once a sufficient level of hyperpolarisation has been reached, the sample is thawed using a dissolution solvent (water or methanol) and rapidly (<1 s) introduced into a high-resolution NMR spectrometer to yield hyperpolarised ^{13}C or ^{15}N resonances [2].

The greatly increased signal intensity offered by DNP NMR makes it possible to readily detect minor isomers by ^{13}C measurements. We illustrate this by reference to keto-enol tautomerism, and discuss the possibility of detecting minor components in mixtures.

We further show that, contrary to theoretical predictions, a single DNP enhanced sample can yield a train of NMR spectra in quick succession. This observation can be used to monitor the progress of chemical reactions and study kinetics in real time by ^{13}C DNP NMR spectroscopy.

1. Ardenkjaer-Larsen, J. H.; Fridlung, B.; Gram, A; Hannson, G.; Hansson, L.; Lerche, M.H.; Servin, R.; Thaning, M.; Golman, K. Proc. Nat. Acad. Sci. 2003, 100, 10158.
2. Wolber, J.; Ellner, F.; Fridlund, B.; Gram, A.; Johannesson, H.; Hansson, G.; Hansson, L.H.; Lerche, M.H.; Mansson, S.; Servin, R.; Thaning, M.; Golman, K.; Ardenkjaer-Larsen, J. H. Nucl. Inst. Meth. Phys. Res. A 2004, 526, 173.

10 Characterising Solid-State DNP

Damir Blazina, Steven Reynolds and Rob Slade

Oxford Instruments Molecular Biotools Ltd., Tubney Woods, Abingdon, Oxon OX13 5QX, UK

Dynamic nuclear polarisation (DNP) has been shown to yield greatly enhanced signal intensity for ^{13}C and ^{15}N nuclei in solution NMR [1]. The method involves cooling a sample to <5°K in a strong magnetic field (B0 = 3.35 T) in the presence of a trityl radical. Under these sample conditions, the unpaired electron on the trityl radical is strongly polarised, and this polarisation can be transferred to nearby nuclei using the DNP process. Subsequent thawing and rapid (<3.5 s) introduction of the sample into a high-field NMR spectrometer (B0 = 9.39 T) has been shown to yield a signal-to-noise enhancement of over 20,000 times compared to a conventional NMR experiment [2].

DNP therefore offers a way of overcoming the low sensitivity inherent in conventional NMR spectroscopy. However, to maximize the benefits this technique offers, it is necessary to understand and optimise the solid-state polarisation process. With this in mind, we constructed a diagnostic NMR probe, which has been used in a DNP test bed for high-sensitivity solid-state DNP research.

We show that the microwave irradiation frequency that yields maximum polarisation can be both nucleus- and sample-dependent, and discuss the effects of parameters such as microwave power, sample temperature and the concentration of the trityl radical on solution state DNP NMR. We also evaluate and compare the polarisation characteristics of various radicals in DNP. These data provide sufficient insight into the DNP process to allow adjustment of the parameters so as to achieve either maximum signal enhancement, or to optimize sample throughput.

1. Ardenkjaer-Larsen, J. H.; Fridlung, B.; Gram, A; Hannson, G.; Hansson, L.; Lerche, M.H.; Servin, R.; Thaning, M.; Golman, K. Proc. Nat. Acad. Sci. 2003, 100, 10158.
2. Wolber, J.; Ellner, F.; Fridlund, B.; Gram, A.; Johannesson, H.; Hansson, G.; Hansson, L.H.; Lerche, M.H.; Mansson, S.; Servin, R.; Thaning, M.; Golman, K.; Ardenkjaer-Larsen, J. H. Nucl. Inst. Meth. Phys. Res. A 2004, 526, 173.

11 LC-MS-SPE/NMR for Rapid Isolation and Identification of Natural Products

Dan Sørensen[1] Annie Raditsis[1] Laird A. Trimble[1] and Barbara A. Blackwell[2]

1. Department of Medicinal Chemistry, Merck Frosst Canada Ltd., Kirkland, Québec
2. Agriculture and Agri-Food Canada, Ottawa, Ontario

The hyphenated technique of LC-MS-SPE, used in conjunction with high-field cryoprobe NMR, represents state-of-the-art instrumentation for isolation and identification of drug metabolites and natural products.

As an example, moldy silage of corn and grass was the suspected source of ill thrift in cattle on certain Quebec farms. An isolate was identified as *Penicillium roqueforti* and was cultivated in 200 mL Czapek yeast extract medium. A resulting 2 mL organic extract was diluted 5-fold and 100 μL injections were used to compare the techniques of LC-NMR (stop-flow) and LC-MS-SPE/NMR. The highly advantageous LC-MS-SPE/NMR technique afforded pure samples and high quality 1D- and 2D-NMR data of individual metabolites such as Roquefortine C (~5 μg).

12 Utilization of a Solid-Supported Reagent for the Facile Synthesis of Unusual Metabolites

Andrea M. Sefler, Steven T. Cook, Amanda G. Culp, John C. Ulrich, Christopher M. Lam, Steve Castellino

GlaxoSmithKline, RTP, NC

Unusual metabolites were isolated during a preclinical study that involved demethylation and oxidation of a parent drug, conjugation with Cys-Gly, and loss of water. NMR data, and in particular HMBC data, was necessary to definitively identify the metabolites. In order to obtain the necessary amounts of material for the NMR analysis, synthetic standards of the metabolites were made from an available synthetic intermediate of the parent drug. After deprotection of the synthetic intermediate, IBX-polystyrene resin was used to oxidize an alcohol to the aldehyde oxidation state, followed by condensation with Cys-Gly. The use of the solid-supported reagent, IBX-polystyrene, afforded a simple and rapid synthesis that could be carried out even in a lab not equipped for traditional organic synthesis.

13 The Keto-enol Tautomerization Study of Ethyl Butylryl Acetate by LC-NMR

Casey Chun Zhou[1] and David R. Hill[2]

1. D-R418, Structural Chemistry
2. D-R450, Process Chemistry, Global Pharmaceutical Research and Development, Abbott Laboratories, North Chicago, IL 60064

The keto-enol tautomerism of ethyl butylryl acetate was studied in mixed solvents under a variety of experimental conditions. The direct measurement of ketonization of the enol tautomer was performed by using hyphenated technique LC-NMR. The keto and enol tautomers can be separated by using HPLC and their interconversion is a slow process on the NMR timescale. The ketonization reaction was found to be acid catalyzed and the solvent isotope effect, k_{H2O}/k_{D2O} in an acetonitrile / water mixture, is 5.4. The ketonization rate constants were also measured at different compositions of binary solvents such as CH_3CN/D_2O, CD_3OD/D_2O, and CH_3CN/CD_3OD. The rate constant and water percentage were found to have an exponential relationship. The reaction rate as a function of solvent polarity will be discussed in this poster.

14 A JAVA Applet for Calculating NMR Coupling Constants, NOEs and Three-dimensional Structure Display

Michael Bodkin, David Evans and Gary Sharman

Eli Lilly, Erl Wood Manor, Windlesham, UK

We present a JAVA applet, based on the open source JMol program, which allows the calculation of coupling constants and NOEs from a three dimensional structure. The program has all of the viewing features of JMol, but adds the capability to calculate both H-H and H-C 3-bond couplings constants, and nuclear Overhauser enhancements. In the case of H-H couplings the Altona equation is used to do this. The program also calculates NOEs using the full relaxation matrix approach. All of these calculations are driven from a simple point and click interface. The program can calculate values for multi-structure files, and can produce input files for the conformational fitting program NAMFIS.

15 NMR-Based Lead Identification, Validation and Optimization at the San Diego Center for Chemical Genomics (SDCCG)

Ziming Zhang, Xiyun Zhang, Jennifer Ryan and Maurizio Pellecchia

Burnham Institute for Medical Research, 10901 North Torrey Pines Rd., La Jolla CA 92037

Despite the tremendous progress of high-throughput (HT) screening and automation in testing larger collections of compounds, the number of compounds experimentally screened (10,000-100,000) still represents only a very small fraction of all the possible "lead-like" compounds that could be imagined (up to 10^{16}, considering "lead-like empirical constraints). Therefore, it is highly unlikely that selective and potent compounds are found directly from a HT screen. Rather, it is expected that initial hit compounds can be identified which will exhibit only moderate affinity and selectivity for a given target. As part of our San Diego Center for Chemical Genomics (SDCCG), one of the nine NIH funded centers belonging to the Molecular Library Screening Center Network (MLSCN), we will present strategies aimed at the design of optimized inhibitors based on NMR-based strategies that rely on a chemical fragment linking approach. In some cases, particularly compelling for targets for which a defined biochemical assay cannot be developed, or for targeting proteins with unknown function or substrates, or protein involved in more complex macromolecular interactions such as protein-protein and protein –nucleic acid interactions, we also propose to use NMR-based screening techniques for de novo hit discovery. Finally, simple NMR binding assays are presented to provide rapid validation of compounds arising from HTS projects.

16 Structure Elucidation of Photodegradants of a Novel Glucocorticoid Receptor Agonist

Ben Bardsley and Marco S. Smith

Analytical Sciences, Chemical Development, GlaxoSmithKline, Stevenage, United Kingdom

GW685698X is a novel glucocorticoid receptor agonist in development as a potential treatment for seasonal rhinitis. As part of the development of this compound it was required to perform a series of forced degradations of this compound under a variety of conditions: acid, base, oxidation and UV light. The products of these degradations were isolated and the structures elucidated using NMR.

GW685698X is a steroid with cyclohexadienone functionality in it's A-ring. The forced degradation of GW685968X using UV light (photodegradation) led to a number of products with unexpected structures based around changes in the A-ring cyclohexadienone. The structures of these products were elucidated using a range of one and two-dimensional NMR experiments and these structure elucidations are described in this poster. These included a structure with cross-linking within the A-ring of the steroid structure and a structure with cross-linking between the A-ring and the C-ring. The formation of these impurities could be rationalised through a mechanism that had previously been observed for similar steroid structures based on a rearrangement of the A-ring initiated by the action of UV light [1,2].

1. Williams, J.R.; Moore, R.H.; Li, R.; Blount J.F. J. Amer. Chem. Soc, 1979, 101(17), 5019-5025

2. Ricci, R.; Fasani, E.; Mella; M.; Albini, A Tetrahedron, 2004, 60, 115-120

17 Automated Small Volume NMR for Medicinal Chemistry

Dan Sørensen and Laird A. Trimble

Department of Medicinal Chemistry, Merck Frosst Canada Ltd.
Kirkland, Québec

In an effort to rapidly identify new leads for medicinal chemistry, modern drug discovery includes high throughput screening (HTS) of large sample collections against biological targets of interest. HTS hits are ordered from the Merck Sample Collection and are received as either solid samples or solutions in DMSO (100 μL, 10 mM). Traditionally, the solid samples are first dissolved in DMSO (10 mM) and then submitted for biological assay and the solution samples are submitted directly. In addition to the biological testing, the samples are assayed by LC-MS to confirm the compound ID and determine purity.

If the amount of sample permitted or the LC-MS results were suspect, ^{1}H NMR spectra are manually acquired using either a 3 mm standard tube (160 μL) or a 3 mm Shigemi tube (50 μL). Occasionally, it is found that samples that looked clean and had the correct mass by LC-MS, show low purity by NMR. This occurs more often with samples that came predissolved in DMSO.

Because the number of HTS hits analyzed is increasing dramatically, we needed a way to perform NMR analyses on a large number of small volume samples. Both the Bruker BACS-60 systems and the Varian VAST systems that we had in-house required volumes that were much too large (>160 μL). However, we had access to a Protasis capillary NMR probe (10 μL flow cell). This probe, when combined with a LEAP autosampler and "One Minute NMR (OMNMR)" software from Protasis may provide a system with high enough throughput and the small sample volumes required to employ ^{1}H NMR in our quality control testing of HTS hits.

This poster will discuss our evaluation of the Protasis OMNMR system and how we have integrated into the Merck Frosst NMR infrastructure. Specifically, samples as small as 10 μL (10 mM in DMSO-d_6) are provided in HPLC vials. 8 μL of this sample are withdrawn from the vial and pushed into the CapNMR probe where 128 scan presaturation experiments are acquired. The spectra are saved to the NMR database were they can be analyzed semi-automatically at the chemist's desktop using DataChord (One Moon Scientific). The sample is then pushed to waste, the probe washed and a new sample loaded. The quality of the sample is then reported back to the Merck Sample Collection group.

18 A Karplus Equation for $^3J_{HCCN}$ in Amino Sugar Derivatives

Bruce Coxon

National Institutes of Health, Bethesda, Maryland

Amino sugar derivatives are widespread in nature, forming important structural elements of aminoglycoside antibiotics, blood-group substances, exoskeletal materials, and bacterial polysaccharides used in vaccine development [1]. We are interested in extending the use of ^{15}N NMR parameters for the structural, stereochemical, and conformational analysis of biological materials, in this instance, by definition of the dependence of vicinal ^1H-^{15}N coupling constants ($^3J_{HCCN}$) on dihedral angle in amino sugars. Our approach has been to measure the $^3J_{HCCN}$ values of amino sugar derivatives in fixed or known conformations, in which the ^1H and ^{15}N nuclei have defined orientations. Measurement of the coupling constants was performed by 1D ^1H NMR of ^{15}N-labeled amino sugars [2] and/or 1D and 2D $^1H/^{15}N$ heteronuclear single quantum, multiple bond correlation (HSQMBC) [3] of amino sugars having ^{15}N either enriched or at natural abundance. The latter experiments were facilitatied by the enhanced sensitivity of a $^1H/^{13}C/^{15}N$ inverse NMR cryoprobe which yielded excellent spectra from 1 mg of ^{15}N-labeled derivative or from 30 mg with ^{15}N at natural abundance. Many of our model compounds are methyl amino-4,6-O-benzylidene-deoxy-α-D-hexopyranosides, which are known for their conformational purity in chair conformations [4], or occasionally the skew form [5]. Also studied, was methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside, in a locked, almost classical boat conformation [2]. ^1H-^{15}N dihedral angles in the amino sugar models were defined by molecular dynamics computations with either implicit or explicit solvent simulation, and sometimes simulated annealing, followed by energy minimization by molecular mechanics using a modified AMBER forcefield that included Homans anomeric parameters and deuterium. Non-linear regression of the $^3J_{HCCN}$ values on the ^1H-^{15}N dihedral angle φ yielded a Karplus equation of the simple type:

$$^3J_{HCCN} = 3.1 \cos^2 \varphi - 0.6 \cos \varphi + 0.4$$

1. Vince Pozsgay, Bruce Coxon, C. P. J. Glaudemans, Rachel Schneerson, and J. B. Robbins, Synlett, 6, 743-767 (2003); Joanna Kubler-Kielb, Bruce Coxon, and Rachel Schneerson, J. Bacteriol., 186, 6891-6901 (2004).
2. Bruce Coxon, Carbohydr. Res., 322, 120-127 (1999).
3. R. T. Williamson, B. L. Marquez, W. H. Gerwick, and K. E. Kover, Magn. Reson. Chem., 38, 265-273 (2000); B. L. Marquez, W. H. Gerwick, and R. T. Williamson, Magn. Reson. Chem., 39, 499-530 (2001).
4. Bruce Coxon and R. C. Reynolds, Carbohydr. Res., 110, 43-54 (1982).
5. Bruce Coxon and R. C. Reynolds, Carbohydr. Res., 331, 461-467 (2001).

19 ^{119}Sn Relayed ^{1}H-^{183}W Correlation Spectroscopy

T. Andrew Mobley, Ellie Tennyson, Bryan Williams

Department of Chemistry, Grinnell College, Grinnell, IA 50112

Due to the inherent insensitivity of ^{183}W nuclei due to (1) low gyromagnetic ratio, (2) long T_1 relaxation constants, (3) large chemical shift dispersion, routine acquisition of ^{183}W spectra has been historically difficult.[1] Inverse techniques utilizing the favorable characteristics of ^{1}H nuclei have overcome these disadvantages[2]; however, these techniques rely on the existence of a proton-tungsten coupling, usually only present with tungsten metal hydride compounds.

Gudat recently reported the use of ^{31}P as a relay nucleus to correlate ^{1}H and ^{183}W in the absence of a direct coupling by utilizing double INEPT and INEPT/HMQC transfers assisted by gradient selection of the desired coherence pathways.[3] In this poster we present the extension of these techniques to ^{119}Sn. The advantages and disadvantages of ^{119}Sn, which exhibits much greater chemical shift dispersion than ^{31}P and (in general) larger coupling constants, as well as the necessary experimental modifications and strategies to implement these techniques will be discussed.

1. Mann, B. E. "The Cinderella Nuclei" In Annual Reports on NMR Spectroscopy; Webb, G. A., Ed.; Academic: San Diego, 1991; Vol. 23, pp 141-207.
2. Benn, R.; Brenneke, H.; Heck, J.; Rufinska, A. "Rapid access to tungsten-183 NMR parameters of tungsten complexes via reverse 2D proton-tungsten-183 and phosphorus-31-tungsten-183 NMR spectroscopy", Inorganic Chemistry 1987, 26, 2826-2829.
3. Gudat, D. "Indirect detection of ^{31}P,X connectivities with proton sensitivity. Application of 2-dimensional ^{31}P-relayed ^{1}H,X correlation spectroscopy", Magnetic Resonance in Chemistry 2003, 41, 253-259.

20 The CHECKIN Program: Design and Implementation of an Automated Process for Submitting Open-Access NMR Data for Compound Validation

Alvin C. Bach, II[1], Walter Massefski[2] and James F. Mattes[1]

Wyeth Reseach, Chemical and Screening Sciences, Chemical Technologies
1. CN-8000, Princeton, NJ 08543-8000
2. 200 Cambridge Park Drive, Cambridge, MA 02140

We have designed and implemented a computer application called CHECKIN to submit open-access NMR data to the Wyeth Research compound validation workflow process. At Wyeth Research all analytical data used for compound validation and subsequent registration, must be examined by Discovery Analytical Chemistry (DAC) staff. The usual process requires the requestor submit a vial of pure sample to DAC for NMR analysis. DAC uses this material to prepare a NMR sample, which is used to collect NMR data that is reviewed by DAC for consistency with the proposed chemical structure. This evaluation process is designed to reduce the number of misidentified compounds submitted to the corporate library by having a second set of eyes examine the data for each submission.The new computer application allows the requestor to select previously acquired open-access NMR data and enter it into the standard workflow for evaluation, bypassing the sample preparation and data acquisition steps. The CHECKIN application saves time for the requestor and DAC staff, and compound library material for Wyeth. An explanation of the workflow and implementation for Bruker and Varian data will be presented.

21 Multiple-Sample Probe for High-Throughput Solid-State NMR Spectroscopy

B. Nelson[1], L. Schieber[1], D. Barich1, J. Lubach[1], T. Offerdahl[1], D. Lewis[2], J. Heinrich[2], E. Munson[1]

1. Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS
2. Revolution NMR LLC. Fort Collins, CO

Solid-state NMR spectroscopy (SSNMR) is the most powerful technique for the analysis of pharmaceuticals and pharmaceutical formulations because of the vast quantity of molecular structure and dynamics information that can be obtained from NMR spectra. It is a non-invasive and non-destructive technique that can determine the physical state of the drug within a formulation matrix. It is selective in that the excipients in a drug formulation will often times have a different chemical shift than the API (active pharmaceutical ingredient). In addition, SSNMR can be used to quantitate solid forms within a pharmaceutical formulation. New drug compounds are often poorly crystalline or amorphous, and are present at low levels in a formulation. This leads to a greater number of transients needed to acquire an adequate signal to noise ratio, which could take hours to days to accomplish. To complete a series of formulations, which is often the case during drug development, would take much longer. When a sample is in the spectrometer, the actual data acquisition time is typically orders of magnitude shorter than the time spent in the magnetic field. This is due to the fact that magnetization has an extremely short T_2 relaxation (milliseconds) and a much longer T_1 (seconds to hours). Thus, the spectrometer is idle for a high percentage of the time as the sample magnetization returns to equilibrium. In an effort to take advantage of the idle time between aqusitions, a multiple-sample probe is being developed. The first generation probe had two spinning modules with independent circuits, airlines, and RF power cables. Hester-Waugh lumped element tuning circuits were positioned in close proximity to the spinning module and coil. The probe was attached to a stepper motor that rapidly shuttles the spinning modules in and out of the homogeneous region of the magnet while the bulk magnetization of the sample returned to equilibrium. Expanding the probe with to accommodate three or more spinning modules that were no more than three cm apart (center to center) requires a change in design. The Hester-Waugh tuning circuit would no longer work due to the space it takes up between the modules. Because the tuning elements must be outside of the magnet for accessibility for tuning adjustment a $3\lambda/4$ coaxial design was implemented. There is a separate transmission line connecting each set of tuning elements to their corresponding coil. The spinning modules were also redesigned to make them smaller. Decoupling powers of 89 kHz have been achieved using a $3\lambda/4$ transmission line. A probe with five spinning modules and corresponding RF circuits has been built and preliminarily tested.

22 The Effect of Structure Description Schemes on Chemical Shift Prediction by Incremental and Neural Network Approaches

Yegor D. Smurnyy[1], Kirill A. Blinov[1], Brent A. Lefebvre[2], Antony J. Williams[2], Arvin Moser[2]

1. Advanced Chemistry Development, Moscow, Russia
2. Advanced Chemistry Development, Inc., Toronto, ON, Canada

Typically, a chemical shift prediction algorithm has two major components: i) rules to encode a chemical structure into a set of numbers ("structural code") and ii) the routine to calculate a chemical shift value from a numerical input. In the current study we compare multiple algorithms with a special emphasis on the effect of the chemical structure encoding routine on the overall ^{13}C chemical shift prediction accuracy. Two primary methods were examined in this work: a neural network approach and an incremental scheme (rules based approach).

For both of these methods, the typical experimental workflow was the following: the whole database (more than 2 million chemical shifts) is split into smaller parts according to the central atom type (in this work, we found 6 atom types to be ideal). About 5-7% of the shifts are included into the "test set" and are not used for system training. These data serve to evaluate the overall performance of the algorithm after it has been trained. In the next step, a neural network can be trained or incremental scheme coefficients calculated by regression. Finally, the performance is evaluated on the test set.

The main focus of this work surrounded efforts to optimize three main aspects of the whole routine. They were (i) the number of central atom types, (ii) the structural code, and (iii) the characteristics of the neural net/regression scheme. The most important result of our work is that we, unlike many authors, have found this to have very little effect on the prediction accuracy. We have designed several types of neural networks (different in transfer function, teaching algorithm, etc.) and found the size of a net to be the only important factor.

In the study of neural nets and incremental schemes shown here, with the largest quality ^{13}C chemical shift database available, we demonstrate that the network or regression routine is not the key to chemical shift prediction quality. Rather a reliable method to convert a structure to a numerical representation leads to a good prediction with even a simple neural net or regression scheme. As a result of this work, we find a mean error of less than 2 PPM can be obtained with our approach. This compares well with database-based (HOSE codes) methods and is better than most of the previously reported results of Neural Net approaches.

23 Automated Evaluation of a Chemical Structure with only 1D ^{1}H and 2D ^{1}H-^{13}C HSQC

Sergey Golotvin[1], Eugene Vodopianov[1], Rostislav Pol[1], Brent Lefebvre[2], Antony Williams[2], Kevin Turnbull[2] and Timothy Spitzer[3]

1. Advanced Chemistry Development, Moscow, Russia
2. Advanced Chemistry Development, Inc., Toronto, ON, Canada
3. GlaxoSmithKline, Inc., Research Triangle Park, NC, USA

As NMR instrumentation continues to advance, 2D experiments that once took hours are now achievable in minutes. With the standard ^{1}H-^{13}C HSQC experiment now possible in a matter of 8 to 10 minutes, this experimental data set is now useful when a high-throughput evaluation of a proposed chemical structure is necessary. With the additional information that this experiment can provide, much benefit can be gained in the automated evaluation of a proposed chemical structure.

In this work we present a new method of automatic structure validation based on the comparison of calculated and experimental data that is available in a 1D ^{1}H NMR spectrum and a 2D ^{1}H-^{13}C HSQC. Following the approach developed in our previous work on 1D ^{1}H spectra only [1], a comparison is made by means of assignment of the spectral signals calculated for a proposed structure to those which are observed in the experimental NMR spectrum. The peaks in the 2D NMR spectrum greatly increase the accuracy of this assignment process and therefore the structure validation, because they contain both the ^{1}H and ^{13}C chemical shifts of the connected nuclei. The advantages also extend into the ability of the 2D spectrum to improve the analysis of overlapping multiplets in the 1D spectrum and identify the protons attached to heteroatoms in the 1D spectrum since these do not show up at all in the 2D spectrum.

All of these factors combine to produce an automated system that can greatly outperform a system where 1D ^{1}H information alone is used. Using dozens of real-life spectrum sets, it was possible to unambiguously identify no less than 90% of the correct structures. As part of this test, incorrect structures were also matched with each spectrum set. In this case, the structures were mainly regioisomers of the correct structures, so as to offer a challenging test of the specificity of the system. For these incorrect structures the false positive rate was observed as low as 1%.

1. Automated structure verification based on ^{1}H NMR prediction, Golotvin SS, Vodopianov E, Lefebvre BA, Williams AJ, Spitzer TD. Magn. Reson. Chem. 2006; 44: 524-538.

24 To Flow or Not to Flow

Simon A.Watson and Anthony L.Beck

Structural Characterisation, Analytical Sciences, Chemical Development,
GlaxoSmithKline, Stevenage, UK

LC-NMR is a technique which is of great utility in the pharmaceutical industry for the identification of medium- to low-level impurities and degradants from mixtures. LC-SPE-NMR is a step forward over conventional LC-NMR, and has given us the opportunity to dramatically increase the amount of material available for NMR analysis, allowing 1-D and 2-D NMR experiments for full structural elucidation to be run.

This investigation explores some the different options available for the recovery of a trapped sample from a cartridge and its subsequent NMR analysis. Hence, data obtained by elution of the sample into a flow probe for NMR analysis were compared with those obtained following recovery into NMR tubes and subsequent analysis using different probes and spectrometer field strengths. These results were also compared to data obtained by eluting the samples directly from cartridge to tube, avoiding the use of the flow probe. This allowed the relative sensitivity losses or gains that could be expected from following these different procedures to be calculated and compared.

25 Deciphering Ligand Binding Site Locations by Competition NMR

Yu-Sen Wang and Daniel F. Wyss

Schering-Plough Research Institute, 2015 Galloping Hill Road
Kenilworth, New Jersey 07033, USA

In recent years NMR has become increasingly important techniques for lead identification and optimization in drug discovery programs. While the target-detected methods, represented by SAR-by-NMR, require large amount of isotopically labeled proteins, the ligand-detected methods use relatively small amount of unlabeled proteins and can easily be adapted for many drug discovery programs. These sensitive and versatile ligand-detected methods are suffering from the inability to detect high affinity ligands. The novel idea of incorporation of competition to these experiments not only overcomes this drawback but also provides several additional benefits: (1) determination of the ligand binding site information for protein targets with one binding site, (2) estimation of an approximate value for the dissociation constant of the potential ligand, and (3) rank ordering of drug leads. However, many protein targets have more than one binding site. Ligands bound to the secondary binding site may compete with the primary site binding ligands due to allosteric inhibition effects. We have developed a competition NMR approach to distinguish the competition for the same binding site from the competition due to allosteric inhibition. The applicability of the approach was demonstrated using the Human Serum Albumin model system by the competition STD NMR experiments. This method is not limited to competition STD NMR but can be applied to any competition ligand-detected NMR experiments.

26 Characterization of Phase Separation of Pharmaceutical Materials by SSNMR

Tim Hanser and Aleš Medek

Pfizer Global Research & Development, Groton Laboratories, Groton, CT

In the absence of standards of pure forms, polymorphic mixtures are typically characterized by differential scanning calorimetry (DSC). In many instances, DSC may not distinguish the different crystalline or amorphous phases present in the sample due to the similartity in their thermal properties. Even in the absence of pure form standards, solid state NMR can conclusively determine the presence of polymorphic mixtures as well as simultaneously differentiate a mixture from multiple sites per assymetric unit. Several approaches are presented in this contribution, all based on identification of fluorine and proton dipolar coupling or proton spin diffusion. In all cases, mixtures are differentiated from pure phases based on the spatial separation of the components of the mixture. Existing SSNMR techniques were modified to provide the desired characterizations.

The most simple but indirect approach to characterize phase separation is monitoring ^{1}H T_1 relaxation. Due to efficient proton spin-diffusion, all proton signals of pure phase sample show the same relaxation rate. Due to poor proton resolution, the proton relaxation is observed indirectly, e.g. on carbon or fluorine. Coincidentally, the components of phase separated mixture can show the same relaxation times. A more direct approach is to estimate the distances between the components by observing dipolar coupling based correlations. Conclusive confirmation of the presence of a mixture can be gained by 2D through space correlation experiments. To improve sensitivity, 1D versions of these experiments utilizing selective excitations are also presented. Examples off fluorine containing molecules using selective excitation by a SELDOM pulse train will be given.

27 Molecular Conformation and Calculated NMR Shifts using the Gaussian Program

Richard J Lewis and Michael A Bernstein

AstraZeneca R&D Charnwood, Loughborough, UK

Proton NMR is particularly sensitive to anisotropic effects, and therefore the conformations of molecules. It is generally less well predicted than carbon chemical shifts by a simple approach based on a 2D model of the compound. We might expect therefore that approaches to chemical shift prediction that take into account an ensemble of 3D conformations should be particularly useful for proton NMR.

Recent literature has shown the use of ab initio GIAO methods to calculate the chemical shifts of molecules that are not well predicted by database methods (for example because the structures are unusual and not well represented in the database).

An interesting alternative use for 3D approaches is to turn the question of shift prediction around, and envisage fitting an experimental spectrum to predicted spectra for an array of different conformations of a compound. Using such an approach, it may be possible to arrive at an estimate of the solution conformation populations. This approach, though potentially laborious, could be of benefit to investigate certain molecules of pharmacological interest.

As a first step towards exploring this problem, we have used the Gaussian suite of programs to predict proton spectra for conformations of a molecule of pharmaceutical interest. This molecule exhibits atropisomerism and can be separated into four conformers at room temperature. This allows us to explore the feasibility of this approach because conformers are restricted and well estimated.

Our results will show whether the correct conformer can be matched to the correct experimental spectrum, and the results will be compared to those obtained from alternative programs using a 3D approach to proton NMR shift prediction.

28 Characterizing Drug-Excipient Interactions by SSNMR

Ales Medek and Ravi M. Shanker

Pfizer Inc, Groton, Connecticut

Drug-excipient interactions play an important role in determining the bioavailability of the active pharmcaeutical ingredient (API) especially in the case of binary or multicomponent amorphous systems. These drug-excipient interactions are also critical in influencing the physical as well as the chemical stability of the formulation (its shelf life). We are presenting modifications of several existing SSNMR techniques to characterize spatial characteristics of API and excipients indicative of drug-excipient interactions. The common feature of these experiments is utilization of dipolar coupling to estimate distances between the various components of the sample. The system studied contains API with trifluoromethyl groups and a cellulosic polymer matrix selectively (partially) ^{13}C labeled on methyl groups. Various ^{1}H-^{19}F-^{13}C cross-polarization (CP) based experiments with and without proton spin diffusion will be presented. To quench proton spin diffusion during the CP steps, Lee-Goldburg type of spin lock was applied. More directly, the drug-excipient distances were also estimated using ^{13}C-^{19}F REDOR experiments with proton decoupling. These techniques have provided in-depth, molecular level understanding of drug-excipient interactions that was not discernable by other conventional techniques.

29 Evaluation of ^{1}H and ^{13}C NMR Prediction Methods

Manuel Pérez, Iñaki Morao, Torren Peakman

Pfizer PGRD
Sandwich Laboratories, UK

The increase in the number of samples analyzed by NMR, together with the potential difficulty to discern between two related compounds (e.g. regioisomers), have created the need for better ways to predict the ^{1}H and ^{13}C NMR chemical shifts of organic compounds.

In this work the ^{1}H and ^{13}C chemical shifts of two chloropyrimidines were predicted using ab-initio, semi-empirical and database methods. The results were compared with the experimental values in order to evaluate which offered the best approximation. As part of the study solvent effects were also incorporated, implicitly or explicitly, into the predictions. The influence of the level of theory and basis set in the ab-initio calculations was also studied in detail.

Database approaches provided fast and accurate predictions of the ^{13}C chemical shifts of the molecules under investigation. The prediction of ^{1}H NMR chemical proved to be more difficult, and only some of the quantum chemistry based approaches gave precise results. Ultimately, only ab-inito methods were able to differentiate between the two regioisomers studied.

30 Chemical Shift Correlations in Drug Discovery – Methods and Applications

Walter Massefski

Wyeth

Studies of chemical shift in NMR are a time-honored method of asking specific stereoelectronic questions about series of related compounds or of asking about the effects of varying conditions on a single compound. In drug discovery these questions may encompass biochemical properties or conclusions as well, that is, issues of binding affinity for a series of compounds to a particular biological receptor or the effects of specific chemical modification on physical properties such as solubility, membrane permeability, or chemical stability.

We will show that either proton or carbon chemical shift spectra may be used to develop multivariate models which distinguish the biochemical properties of drug-like molecules, and in the case of ^{13}C, chemical shift predictions of these spectra are often good enough. We will focus on some of the details of the method that we have developed and try to address issues of reliability of chemical shift prediction, finding the right level of bin resolution, and a look at which questions can be addressed and which cannot.

We have begun to apply these techniques to the job of predicting physical properties of pharmaceutically interesting compounds. In our poster we will describe the conditions that need to be satisfied for the technique to work, particularly when using predicted chemical shift spectra, and show some of our more recent examples.

31 Towards the Unambiguous NMR Assignment of Diasteromeric Cyclic Sulphites. A Experimental and Theoretical Approach

Marcos Obregón Mendoza[1], Juan Carlos Poveda[2], Carlos E. Lobato-García[3], Concepción Lozada García[4], Raúl G. Enríquez[1], Dino Gnecco[5], and William F. Reynolds[6]

1. Instituto de Química, UNAM. México
2. Instituto de Física, UNAM. México
3. U. Juárez de Tabasco
4. UAM Xochimilco, México
5. Centro de Investigación en Química, BUAP. Puebla, México
6. Department of Chemistry. University of Toronto, Canada.

In a previous SMASH congress we reported the stereochemical NMR assignment of a pair of diasteromeric cyclic sulphites based upon the unambiguous assigment of one diasteromer with the aid of its crystal structure; the corresponding stereoisomer (epimer) at the S=O bond was assigned on the basis of its liquid state NMR spectrum.

The ^{1}H NMR chemical shifts of hydrogens in five and six membered cyclic sulphites is drastically affected by the stereochemistry at the S=O group. Due to the lack of of experimental data and of long range correlations available for the stereochemical assignment of the S=O group, we have undertaken the task of preparing some cyclic sulphites where their crystal structure can be used as a basis for the ^{1}H and ^{13}C NMR unambiguous distinction.

We obtained two diasteromeric pairs of five membered cyclic sulphites and we obtained suitable crystals of three of them for X-ray studies. Their ^{1}H and ^{13}C spectra are very distinctive and may be useful for evaluating the best theoretical methods level of theory, and the best basis set to describe the molecular system to calculate chemical shifts in pairs of diasteromeric non crystalline cyclic sulphites. We used this criteria to evaluate various theoretical methods to predict ^{1}H and ^{13}C chemical shifts of the cyclic sulphite of propilenglycol. In addition, the nature of the effect exerted by the S=O group can be studied with greater detail with the present compounds.

32 NMR Studies of the Enzymatic Synthesis and Photo-Switching of a Peptide-Capped Cyclodextrin Rotaxane

Tim D. W. Claridge, Andrew G. Cheetham and Harry L. Anderson

Chemistry Research Laboratory, Department of Chemistry, University of Oxford
Mansfield Road, Oxford, OX1 3TA, UK

Rotaxanes represent a class of supramolecular inclusion complexes in which a cyclic macrocycle is threaded onto a linear core molecule which is itself then capped to hold the macrocycle in place. Such complexes can often exhibit useful properties such as enhanced chemical stability, fluorescence efficiency and solubility. In the work presented herein the macrocycle is alpha-cyclodextrin, a cyclic oligomer of glucose comprising six 1,4-linked sugar units.

In rotaxane formation, the primary driving force for the formation of the initial inclusion complex in aqueous solution is hydrophobic binding within the cyclodextrin cavity. Covalent capping of the complex must then also take place in water, which limits the synthetic methodology available. Enzymes, however, are well suited for catalysing bond formation in water and herein we report on our NMR studies into the stereochemical consequences and enzyme selectivity of the first enzymatic synthesis of a rotaxane[1]. The longer-term interest in such studies lies in the potential for using enzymatic cleavage of rotaxanes in selective drug delivery systems. Relevant to this is the photochemical cis-trans isomerism of azo-dye rotaxanes and we shall also demonstrate the ability to effectively block enzymatic degradation of these rotaxanes in the photo-switched isomer through the forced relocation of the surrounding cyclodextrin sheath.

The fundamental questions in understanding the behaviour of these rotaxanes are the location and orientation of the protective cyclodextrin and the bearing this has on the enzyme processing. Such questions have been addressed primarily through extensive NOE studies. We shall demonstrate the combined application of selective 1D-SPFGSE-TOCSY, 1D SPFGSE-NOESY and 2D NOESY experiments, further supported by concatenated doubly selective 1D SPFGSE-TOCSY-NOESY experiments. We have also employed Saturation Transfer Difference (STD) NMR to probe binding to the enzyme and present some preliminary results.

1. A. G. Cheetham, M. G. Hutchings, T. D. W. Claridge, H. L. Anderson, Angewandte Chemie - International Edition 2006, 45, 1596-1599.

33 Acid Catalyzed Degradation Pathways of Ixabepilone, the aza-Epothilone B Analog

Rajesh B. Shukla, Qingmei Ye, Zhenrong Guo, T. Comezoglu, V. Palaniswamy

Bristol-Myers Squibb, New Brunswick, NJ 08903

Acid catalyzed ring opening reactions enabled elucidation of the degradation pathway(s) of Ixabepilone (1), the aza-Epothilone B1 analog. The initially formed amino lactone (2) is unstable and most likely rearranges to form the C-12/C-13 diol by an intramolecular nucleophilic attack involving the amine nitrogen and the lactone carbonyl.

To obtain insight into the degradation pathways, we acetylated the primary amine in 2 as a means of stabilizing the molecule and thereby enabling elucidation of the structure(s) and understand the mechanism of the acid catalyzed degradation. Acetylation of 2 followed by work up resulted in the isolation of two diastereomers. Using a combination of correlated and Overhauser NMR experiments with molecular modeling, the relative stereochemistry at C-12 and C-13 was determined. Utilizing the structures established for the degradants, we combine Monte Carlo based conformational search methods with electronic structure methods to extend the previously proposed acid catalyzed degradation mechanisms.

1. M. Sefkow, M. Kiffe, G. Hofle, Bioorganic & Medicinal Chemistry Letters, 8 (1998), 3031.

34 The Hexacyclinol Controversy. What Went Wrong With The Original Structure Determination?

William F Reynolds

Department of Chemistry, University of Toronto

In 2002, Schlegel et al reported the structure of a new and unusual natural product with a cyclic peroxide which they named hexacyclinol (1). Recently, LaClair reported a 37 step synthesis of their structure (2). However, his report gave no details of key intermediates, contained some surprisng steps and a ^{1}H spectrum for the final product with several puzzling features. Soon after, Rychnovsky suggested that the originally proposed structure was wrong and proposed a different structure, closely related to a known natural product (3). He supported this claim with ab initio calculations of ^{13}C shifts for the two structures which agreed much better with the new structure. Finally Porco and Rychnovsky very recently reported the unambiguous total synthesis of the new structure which was found to have identical ^{1}H and ^{13}C spectra to the original report, clearly suggesting that the originally proposed structure was wrong (4).

In addition to the questions about La Clair's report, I was intrigued by the fact that the 2D NMR correlations reported in ref. 1 were consistent with the original structure but not the revised structure. As part of a review that I am preparing, tentatively entitled "Avoiding Problems in Organic Structue Determination by NMR", I checked the original and latest reports to find the source of errors. There is severe spectral crowding in a key region of the ^{1}H spectrum and it appears that apparently minor assignment errors, probably in part due to inadequate 2D data resoluion, resulted in an incorrect structure, emphasizing the importance of acquiring and processing 2D spectra with adequate resolution and being very careful in interpreting data. The spectra obtained by the different authors will also be compared and commented upon, along with La Clair's recent assertion that he actually had synthesized the original hexacyclinol structure which fortuitously had the same spectrum as the new structure.

1. B. Schlegel et al, J. Antiot, 2002, 55, 814.
2. J. La Clair, Angew. Chem., Int Ed., 2006, 45, 2769
3. S. Rychnovsky, Org. Lett., 2006, 8, 2895
4. B. Porco et al, Angew. Chem., Int. Ed.., in press

35 On the Importance of Structure Stereochemical Markers in ^{13}C NMR Predictions

Ryan R. Sasaki and Brent A. Lefebvre

Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, Ontario, Canada

More than half of all approved drugs contain stereocenters. In fact, stereocenters impact a significant amount of compounds in virtually all chemical industry fields, from perfumes to flavors, agrochemicals to specialty polymers, and more. We know that small stereochemistry variations can mean the difference between a commercially viable and worthless compound, or even between a safe compound or a toxic one. For this reason, when using NMR predictions to assist in structure verification, the stereochemistry within a structure should always receive special attention. However, drawing a structure in a stereochemically accurate fashion can take substantial additional efforts, so that it is not uncommon for some of the stereocenters to be left undefined. What is the impact of such omissions on the ability to verify and interpret the structure-spectrum relationships from a stereochemically valid point of view?

The purpose of this study is to validate the importance of drawing stereochemical markers on chemical structures prior to ^{13}C NMR predictions. Much like in real-word situations, the ^{13}C chemical shifts are heavily impacted by their stereochemical orientation. A structurally diverse dataset was used to compare the accuracy of the ^{13}C chemical shifts of over 50 compounds with and without stereochemistry drawn. The results of this study reveal that it is absolutely critical to include stereochemical markers before generating a prediction. This poster will highlight the accuracy results of this study and provide information on how ACD/Labs NMR prediction algorithms handle these special cases.

36 Critcal Comparison of Epitope Mapping Experiments for Binding of Propranolol to AGP

Bridget A. Becker[1,2] and Cynthia K. Larive[2]

1. Department of Chemistry, University of Kansas, Lawrence, KS
2. Department of Chemistry, University of California, Riverside, Riverside, CA

Understanding interactions between plasma proteins and drug compounds is central to predicting the in vivo pharmacokinetics and pharmacodynamics of potential drug compounds. Mapping the interactions between drug molecules and plasma proteins can help in the design of molecules with the desired in vivo characteristics. The epitope mapping experiments, NOESY, STD, and BPPSTE, were applied to map the interactions between the R and S enantiomers of an antihypertensive drug, propranolol, and a plasma protein, alpha-1 acid glycoprotein (AGP). We chose to compare optimal experimental parameters and results obtained for each type of experiment including the level of protein resonance suppression and required protein-ligand concentration ratio to understand which epitope mapping experiment is best for this system and other systems with similar characteristics. The combined results of these epitope mapping experiments are used to determine the propranolol functional groups that are in closest contact with the AGP, contributing to a better understanding of the mechanisms of AGP ligand binding.

37 Characterizing Monorhamnolipids in Mixtures

Bridget A. Becker[1,2] Ariel Lebrón-Paler[3], Jeanne E. Pemberton[3], William Otto[1,4], Raina M. Maier[5], and Cynthia K. Larive[2]

1. Department of Chemistry, University of Kansas, Lawrence, KS
2. Department of Chemistry, University of California, Riverside, Riverside, CA
3. Department of Chemistry, University of Arizona, Tucson, AZ
4. Department of Chemistry, University of Maine at Machias, Machias, ME
5. Dept. of Soil, Water and Environmental Sciences, Univ. of Arizona, Tuscon, AZ

Rhamnolipids are glycolipid biosurfactants (consisting of one or more rhamnose sugar head groups and one or more aliphatic chain tail groups) produced as a mixture of closely related compounds in cell cultures of strains of *Pseudomonas aeruginosa*. Rhamnolipids were first characterized by Jarvis and Johnson in 1949.[1] They are potential agents for bioremediation of organic (for example polycyclic aromatic hydrocarbons and polychlorinated biphenyls) and metal (for example Cd^{2+}, Pb^{2+}) contaminants.[2, 3] Preparative scale reverse-phase HPLC of a composite rhamnolipid sample produced several components of unknown structure. The structure elucidation proceeded in the presence of an overwhelming majority of another rhamnolipid because in some cases separation of the raw extract does not lead to fractions containing only one rhamnolipid type. The structures of novel rhamnolipid components, that have double bonds in the aliphatic tail further from the rhamnose head group, were elucidated using a combination of ^{1}H NMR and mass spectrometry.

Rhamnolipids are surface-active and undergo morphological changes that depend on solution pH and may influence potentiometric pH measurements. The previously reported pKa of rhamnolipid (pKa = 5.6) corresponds to the boundary between formation of either vesicles or lamella.[4] The pKa of monorhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* bacteria was successfully determined for a composite rhamnolipid sample by ^{1}H NMR titration as 4.39 ± 0.06. This result was confirmed by infrared spectroscopic and potentiometric measurements.

1. Jarvis, F. G.; Johnson, M. J. J. Amer. Chem. Soc. 1949, 71, 4124-4126.
2. Mata-Sandoval, J. C.; Karna, J.; Torrents, A. Environ. Sci. Technol. 2000, 34, 4923-4930.
3. Ochoa-Loza, F. J.; Artiola, J. F.; Maier, R. M. J. Environ. Qual. 2001, 30, 479-485.
4. Ishigami, Y.; Gama, Y.; Nagahora, H.; Yamaguchi, M.; Nakahara, H.; Kamata, T. Chem. Lett. 1987, 763-766.

38 New Application "MICCS-NMR" for Direct Synthesis Monitoring by NMR

Masamichi Nakakoshi[1], Satoshi Sakurai[2], Yutaka Takahashi[2], Masafumi Ueda[3], Hiroaki Utsumi[2], Okiko Miyata[3], Takeaki Naito[3]

1. Instrumental Analysis Center, Yokohama Nat. Univ., Yokohama
2. Analytical Instrument Division, JEOL Ltd., Tokyo
3. Medicinal Chemistry Lab., Kobe Pharm. Univ., Kobe

NMR (Nuclear Magnetic Resonance) is very powerful tool to analyze the molecular structure of chemical compounds. However, because compounds, especially intermediates of chemical reaction, are generally unstable, so the analysis is difficult in many cases. From such viewpoint, we developed the micro fluidic device, which was named "MICCS" (MIcro Channeled Cell for Synthesis monitoring), as a tool to observe the chemical reaction by NMR directly [1]. MICCS can be used with standard NMR probes for 5mm O. D. sample tubes. It was connected to syringes with capillary tubes. The reagent solutions were introduced into the MICCS by syringe pumps, and were mixed in a Y-shaped channel. Herein we report the direct observation of a boron complex by ^{1}H and ^{11}B MICCS-NMR. The complex has been proposed as a key intermediate in the radical addition of the oxime ether mediated triethylborane(Et_3B) that produce amine derivatives [2] [3]. We have previously reported the analysis of these intermediates by using 2D and 3D-DOSY in standard NMR sample tubes [4]. The results from MICCS-NMR indicate good consistency with those from standard NMR tubes. The DOSY methods require an inert gas purge of the NMR sample tube, to avoid the decomposition of intermediates due to the dissolved water. This troublesome process is not necessary for MICCS-NMR. Furthermore, MICCS-NMR allows observation of ^{11}B-NMR, which could not be applied to analyze the intermediates by DOSY due to the short relaxation time of ^{11}B.

1. Y. Takahashi et al., Micro TAS, 2, 464, (2004).
2. H. Miyabe, C. Ushiro, M. Ueda, K.Yamakawa, T. Naito, J. Org. Chem., 65, 176, (2000).
3. H. Miyabe, M. Ueda, T. Naito, Synlett, 7, 1140, (2004).
4. M. Nakakoshi, M. Ueda, S. Sakurai, O. Miyata, M. Sugiura, T. Naito, Magn. Reson. Chem., 44, 807, (2006).

39 A Web Based NMR Automation Interface for Multiple Software Versions

Dennis P. Anderson, Wei Wang, Kathleen Farley, Geeta Yalamanchi

Pfizer Global Research & Development, Discovery Chemistry
2800 Plymouth Rd., Ann Arbor, MI 48105, USA

In Discovery Chemistry of Pfizer in Ann Arbor, MI, medicinal chemists use open-access NMR spectrometers for routine 1D and 2D NMR analyses. These instruments utilize Varian operating software, VNMR 6.1C. Over the years, the software has been highly modified for local demands, and the chemists are now accustomed to our easy-to-use user interface. However, the introduction of Varian's VnmrJ software into the open-access environment presents challenges in maintaining our current functionality while minimizing the time needed to train hundreds of busy medicinal chemists to use new software.

One possible way of addressing these challenges may be not to face them at all. A different interface may be used that bridges the gap between the old and new software. The interface would be similar to that which is currently in use so that the chemist may use it in a similar manner. It would also be portable so that it can be used with either the traditional or contemporary software, yet the difference would not be noticeable to the casual user. Additionally, the opportunity could be used to allow for some refinements in the data archival scheme.

We are developing such a solution for the open-access NMR laboratories. This solution utilizes a web page located on a central server and run on a standard browser. The chemist submits sample information on the web form, and upon submission, the information is passed along to the appropriate operating software version where the experiment is initiated. This allows for the chemist to submit experiments in a familiar environment, with a concurrent increase in functionality. We believe that by using this interface, the conversion to the newest operating software will be accomplished with the medicinal chemist noticing minimal changes in the open-access NMR process.

40 Small Volume NMR Probe Family Extended by New 30 μLiter $^{1}H/^{13}C$ Optimized Inverse Probe

T. Kühn[1], S. Loss[1], M. Assmann[2], M. Köck[2]

1. Bruker BioSpin AG, CH-8117 Fällanden
2. Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven

There has been a significantly growing demand for miniaturization in all areas of modern research and development. Consequently, the advent of smallest volume NMR probes – such as Bruker's 1mm MicroProbe with a total sample volume of 5μl – has been very well accepted.

Often it is desirable to acquire ^{13}C direct observe spectra along with the inverse data but in many cases it is not feasible to dissolve the required sample amount for these experiments in volumes of 5μLiter. Here we present the first results of a new NMR probe that matches the highest mass sensitivity on two channels – ^{1}H and ^{13}C – with a total sample volume of 30μl measured with 1.7mm tubes.

A new triple resonance probe has been designed for optimal ^{1}H and ^{13}C sensitivity with an extra ^{15}N decoupling channel as well as a ^{2}H lock channel, z-gradient and ATM (automatic matching and tuning) accessory. Here we present NMR spectra, acquired on isolated natural products which show that it is now achievable to run all NMR experiments for structural elucidation on less than 0.5mg of sample in an overnight run – including ^{13}C direct observe experiments!

Another advantage of the total volume of 30μl is that it matches the solid phase extraction (SPE) elution volume. Thus, it allows the automated elution of HPLC fractions trapped on SPE cartridges directly into the NMR tube – virtually without any losses due to dilution or dead volumes within the system.

In some other cases, 30μLiter volume might be more useful than the 5μLiter if more material or more volume is either available or desirable. For example, proteins sometimes run into solubility problems at higher concentrations. In other cases, larger quantities of bodyfluid – such as urine or plasma of rodents – are available. Also here, the new probe offers the highest possible mass sensitivity on 30μl total volume.

The probe and sample handling design is based on the successful and proven 1mm MicroProbe technology and therefore ensures also highest mass sensitivity for NMR samples in tubes with 1.7mm diameter. This also enables the probe to blend seamlessly into an existing automation environment by using all the established automation tools for these 30μl tubes as well – such as the Gilson 215 LH for automated tube filling or the BACS sample changer or the SampleJet autosampler robot.

41 Characterization of a New Form of Aspirin

Loren J. Schieber, Diana M. Sperger, Bin Chen, Thomas J. Offerdahl,
Sung Jung Hong, Joseph W. Lubach, Dewey H. Barich, and Eric J. Munson

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

Purpose: To fully characterize a new crystalline form of aspirin produced by lyophilization.

Methods: The ^{13}C CPMAS NMR (SSNMR) spectra of aspirin were acquired before and after lyophilization. NMR relaxation times were also measured. The melting temperatures of the old and new forms of aspirin were determined using DSC. PXRD and Synchrotron data was collected to evaluate crystallinity and possible changes in solid form. Karl Fischer and Water Vapor Sorption experiments were performed to determine the amount of water present in the lyophilized material compared to the bulk. SSNMR experiments on a sample of aspirin lyophilized with small amounts of ^{13}C-labeled salicylic acid were performed in order to determine the extent of incorporation of salicylic acid into the aspirin crystal structure upon lyophilization. Aspirin with a ^{13}C-labeled methyl group was synthesized and lyophilized to determine the conformation and possible interactions of the methyl group by SSNMR.

Results: The ^{13}C CPMAS NMR spectrum of lyophilized aspirin showed a splitting of the aspirin methyl peak and the appearance of a shoulder on a peak in the aromatic region. Recrystallized aspirin had only one peak for the methyl carbon. Recrystallized aspirin has a ^{1}H T_1 of 57 s, while lyophilized aspirin had a relaxation time of 14 s. The melt onset temperature of the lyophilized aspirin was consistently 5-6 C lower than recrystallized aspirin. Synchrotron data shows that the lyophilized aspirin may be a mixture of two forms. SSNMR indicates that there is some salicylic acid incorporation in the crystal structure upon lyophilization. The two methyl peaks in lyophilized aspirin have different chemical shift tensor values, indicating that they are in different environments. Two-dimensional exchange SSNMR experiments are being performed to obtain better insight into the methyl group interactions.

Conclusions: ^{13}C CPMAS NMR spectra, relaxation measurements, DSC, PXRD and Synchrotron data all indicate that a new form of aspirin is produced during lyophilization

42 Water Suppression with 20/20 Hindsight

Bao D. Nguyen, Xi Meng, Kevin J. Donovan, and A.J. Shaka

Chemistry Department, University of California, Irvine
Irvine, CA 92697

The problem of efficient, simple, and robust suppression of strong solvent resonances, in particular the strong water resonance that dominates the spectra of molecules in aqueous solution, has been a focus of attention in the NMR spectroscopy community for many years. The most effective approach to date to suppress the water is the use of pulsed field gradients (PFGs) in combination with the use of spin echo resulting in zero net rotation of water resonance and 180° inversion to all other resonances. We are presenting the robust and easy to use, new water suppression sequence, Solvent-Optimized Double Gradient spectroscopY (SOGGY). The SOGGY sequence is based on the simple scheme of excitation sculpting (DPFGSE) [1], G1-S-G1-G2-S-G2, where S is any pulse sequence, and G1 and G2 are the gradients (Gradient strength of G2 is different from G1 to avoid refocusing of previously dephased magnetization by G1). We employ the refocusing element S = [soft 180°(x), composite 180°(-x)], where a soft 180° pulse of ~2ms is applied at the water resonance frequency and a hard composite 180°(-x) pulse consists of a computerized optimization of 4-pulses: 81°(x)-81°(-x)-342°(x)-162°(-x). By replacing the conventional hard 180° pulse in the excitation sculpting motif with the simple phase-alternating composite pulse, the compensation for B_1 inhomogeneity can be achieved over a reasonable range of resonance offsets, so that good solute signal retention can be realized while still achieving excellent suppression of the water line. The results will be presented and discussed in detail, and then compared with other popular water suppression techniques: soft-pulse WATERGATE [2], 3-9-19 WATERGATE [3] and PURGE [4].

1. T.L. Hwang and A.J. Shaka (1995) J. Magn. Reson., 112A, 275-279.
2. M. Piotto, V. Saudek, and V. Sklenar (1992) J. Biomol. NMR, 2, 661-665.
3. V. Sklenar, M. Piotto, R. Leppik, and V. Saudek (1993) J. Magn. Reson., 102A, 241-245.
4. A.J. Simpson and S.A. Brown (2005) J. Magn. Reson., 175, 340-346.

43 Using Quantitative NMR and HPLC for the Determination of HPLC-UV Relative Response Factors

Andreas Kaerner, Lisa Zollars, Tim Smitka, and David Myers

Eli Lilly and Company, Analytical Sciences Research and Development, Indianapolis, IN 46285

In most pharmaceuticals, the active pharmaceutical ingredient (API) typically has low level related substances also present. Quantitation of these related substances is primarily performed using HPLC with UV detection. It is important to accurately know the levels of these related substances since there are regulatory requirements to structurally characterize and/or perform toxicology studies when they are above a certain threshold. Accurate quantitation based on HPLC-UV peak area measurements, and hence the levels relative to the API, requires knowing the wavelength dependant response factor relative to the API. For optimum sensitivity, the HPLC method wavelength is usually close to a UV maxima of the API, however this may not correspond to a maxima the related substances since the UV spectra may be different. Therefore, the UV response factor of the related substances may not necessarily be the same as the API. If the relative response factor (RRF) of these related substances is <80% or >120% compared to the API, then a correction factor must applied to the UV area counts to accurately determine the levels relative to the API. This RRF has traditionally been determined by making a stock solution using a carefully weighed related substance of known potency and purity. The stock solution of known concentration is then injecting on an HPLC to determine the RRF. This entire process can be a time consuming and requires a weighable sample. Fortunately NMR is well suited to determine the RRFs of related substances, eliminating many of the lengthy steps.

This poster will describe a simple and effective process for determining RRFs using quantitative NMR combined with HPLC. This requires that a clean isolated related substance is available and the amounts are sufficient to acquire a quantitative proton NMR spectrum of reasonable signal to noise (usually 50 micrograms or greater). After obtaining a proton NMR spectrum of the related substance, API is spiked in and a quantitative spectrum is re-acquired. The NMR spectrum provides the relative molar ratio of the related substance vs. API. This sample is then injected onto the HPLC to determine the HPLC-UV area ratios. These ratios are then used to determine the RRF and the weight corrected RRF. Accuracy and precision will be shown for several related substances and are comparable to tradition methods.

44 Applications of Quantitative NMR in the Pharmaceutical Industry

Lisa Zollars, Andreas Kaerner, Cheryl Bye, David Anderson, Jonas Buser

Eli Lilly and Company
Analytical Sciences Research and Development
Indianapolis, IN 46825

Quantitation by NMR has become a useful tool in the scientist's toolbox. Quantitation using NMR is valuable since, in most cases, it's more rapid that traditional techniques such as HPLC. QNMR can be used in situations where poor UV properties cause HPLC quantitation to be less than ideal or all together fail. Examples of how quantitative NMR was used to determine relative UV response factors, reference standard potency, potencies for isolated compounds (especially for samples without chromophores), and a few atypical applications will be shared. Additional information will be provided about how quantitative NMR can be used in an open access environment where many users are poorly versed in NMR theory. Critical elements of success in such an environment will be discussed.

45 Reaction NMR: A Method for Real Time Monitoring of Chemical Reactions and Processes

Thomas M. Zennie[1] Roger Rothhaar[2], and Andreas Kaerner[1]

1. Analytical Sciences Research and Development
2. Chemical Process Research and Development
Eli Lilly and Company, Lilly Technology Center, Indianapolis, IN, 46221

A method to monitor chemical reactions and processes using flow NMR is being developed in a pharmaceutical research and development facility. Its primary use is supporting early stage route selection and later stage process definition of active pharmaceutical ingredients and intermediates. The method allows non-invasive, temperature controlled, real time observation of chemical reactions, including distinct signals from starting materials, products, impurities, and transient intermediates.1 This was done while keeping the chemical process intact, totally inerted and without the use of deuterated solvents. The NMR data permits determination of reaction mechanisms, kinetics and thermodynamics of chemical reactions that cannot be easily obtained by other techniques, i.e. HPLC, MS or IR. Similar to the more mature reaction monitoring IR counterpart called ReactIR™. As a proof of concept for reaction NMR, a pharmaceutical intermediate degradant previously observed and defined using Ultra Performance LC (UPLC™), was monitored using ^{1}H React NMR. Proton NMR results obtained over a 4.5 hour period in non-deuterated toluene/ethanol/HCl at elevated temperature (65°C) and flow (0.6 ml/min) were comparable to UPLC™ results. New developments and potential solutions for problematic chemical reactions will be discussed.

1. Michael Maiwald, Holder H Fischer, Young-Kyu Kim, Klaus Albert, and Hans Hasse, Quantitative high-resolution on-line NMR spectroscopy in reaction and process monitoring. JMR, 166 (2004) 135-146.

46 Metabolomics for Discovery of Biomarkers of Hepatotoxicity

Jason Burgess, Rodney Snyder, Timothy Fennell and Susan Sumner

RTI International, RTP, NC

NMR spectroscopy of biofluids provides an enormous amount of information about the metabolic state of an organism. This profile can potentially be used to discriminate between healthy and diseased individuals. However, the complexity of a biofluid spectrum makes finding a signature of disease difficult. One goal of our work is to use metabolic profiling to identify biomarkers of liver disease where we may not know the endogenous metabolites that are susceptible to a particular disease state. To aid in this analysis we are testing a method where Chenomx Profiler is used in an unsupervised or non-targeted manner to generate a metabolic profile from a set of over 250 metabolites (with the knowledge that some of these metabolites are not endogenous). This metabolic profile is then subjected to principal component analysis to group samples. Candidate metabolites that discriminate groups are identified and then the spectral signature of the candidate metabolites are compared to the experimental spectra for corroboration. The application of these techniques to profiling urine samples obtained from a study of the potential hepatotoxicant, isoniazid, will be described.

47

Probe Comparison: A Reality Check

Andrew J. Jensen[1], Brian L. Marquez[1], Kent Mills[2] and David Russell[3]

1. Pfizer Inc. PGRD, Groton, CT
2. Pfizer, Inc., PGRD, Kalamazoo, MI
3. Abraxis BioScience, Auburn, CA

This poster addresses sample size limitations and how to maximize instrument capacity in the lab and is not intended to be a vendor comparison of hardware. The poster demonstrates comparisons between multiple vendor's probes and instruments to determine what size instrument and probe are necessry to use for a certain size sample. Ketoconazole in acetonitrile is the sample matrix used for analysis purposes. Instruments ranged in size from 500 MHz to 700 MHz. Probe diameter ranged in size from 5mm to 1mm. Sample size ranged from 500 μg to 1 μg. Results show which probes should be used on which instrument for a certain size sample. This information will facilitate the proper usage of the instruments in our lab, and possibly yours.

48 Investigation of an LDA Reaction Dianion Intermediate Using ^{1}H, ^{7}Li, ^{15}N and ^{19}F NMR Experiments

Andreas Kaerner and Jonas Y. Buser

Eli Lilly and Company, Analytical Sciences Research and Development, Indianapolis, IN 46285

Lithium diisopropylamide, LDA, is a strong base that is commonly utilized in organic synthesis reactions to create carbanion intermediates that then readily react with nucleophillic sites. A variety of previous work with LDA reactions suggest that the formation of various aggregates are possible and most work has shown this is dependent on reagents and solvents used in the reaction. More importantly, the aggregation phenomenon can also have a negative impact on reaction yields. Therefore, better understanding of the LDA aggregation phenomenon and how it relates to decreased yields, is of great importance. Monitoring LDA reactions using NMR can be challenging since they require low temperature and an inert atmosphere. This work will illustrate the use of ^{1}H, ^{7}Li, ^{15}N and ^{19}F NMR experiments to characterize an LDA mediated dianion intermediate, and better understand factors involved in reaction yield.

49 In Situ Derivatization Method for Configurational Assignement by NMR

Jinhai Gao, Bing Wang and Srinivasan Rajan

Discovery Technology Center, Novartis Institute for Biomedical Research, Inc.
Cambridge, MA 02139

Quick and convenient methods for the assignment of absolute configuration of chiral compounds are very important and appealing to medicinal chemists in drug discovery. The NMR method based on chiral derivatizing agents (CDAs) has been developed for this purpose. The most recent advance in this area includes the "mix and shake" technique using solid matrix-bound auxiliary reagents for the assignment [1]. The general procedure consists of the derivatization by mixing CDA resin with the chiral substrate directly and the NMR data are acquired without any type of separation, workup or manipulation. We have implemented this method and successfully determined the absolute configurations of some chiral compounds from medicinal chemists. However, this method is mainly limited to primary amines and secondary alcohols. The synthesis of solid matrix-bound reagents is not convenient and storage stability of the resin bound reagent is not guaranteed. Using commercially available coupling resins, we have developed a facile and purification free derivatization method in NMR solvent. With optimized reaction conditions, this method can be applied to many chiral compounds, including primary and secondary amines, primary and secondary alcohols, and carboxylic acids. Example applications of this method will be presented in our poster.

1. Porto S, Durán J, Seco JM, Quiñoá E, Riguera R. Mix and shake method for configurational assignment by NMR: application to chiral amines and alcohols. Org Lett 2003; 5: 2979-2982

50 Solid-State NMR Characterization of Risedronate Hydrate Forms and Dehydrated Risedronate

Gwen S. Lubey, Nancy L. Redman-Furey, Mike L. Dicks, Cathy C. Lester

Analytical Sciences Dept., Procter & Gamble Pharmaceuticals, Inc., Norwich, NY

Characterizing the hydration state of a drug is important for understanding potential effects on drug substance properties and drug product manufacturing. Risedronate, a monosodium bisphosphonate, is used in the treatment of osteoporosis. Risedronate monosodium may exist as an anhydrate, as a monohydrate, as a hemi-pentahydrate and as a variable hydrate form [1]. Each hydrate form was characterized by solid-state NMR (SSNMR) and each was shown to possesses unique ^{13}C, ^{23}Na and ^{31}P spectra. The SSNMR data also provided unit cell information and evidence pertaining to the interaction of water of hydration with risedronate monosodium.

The hemi-pentahydrate (HPH) form is used in the commercial product. The HPH exists as a mixed hydrate containing both channel type and lattice type water. Under conditions of either elevated temperature or low humidity, the HPH form loses 1 mole of channel water, resulting in non-fully hydrated or "dehydrated" material [2]. SSNMR was used to study dehydrated HPH created by gentle heating (60°C), dehydrated HPH created by desiccation (<10% RH) and rehydrated dehydrated HPH. ^{13}C, ^{23}Na and ^{31}P SSNMR spectra of the dehydrated HPH form are unique from spectra of the other hydrates. Spectra of rehydrated material are consistent with its return to the HPH form.

In a bulk sample, as water leaves the channels the HPH lattice does not shift gradually. Instead, removing 1 mole of channel water causes a spontaneous lattice adjustment [2]. ^{31}P SSNMR was used to monitor HPH dehydration over time within the probe. The temperature profile was optimized to remove only 1 mole of channel water. The HPH peaks did not undergo chemical shift changes, but decreased in intensity and disappeared. Concurrently, new peaks appeared and grew in. These new peaks corresponded to the formation of a unique unit cell. The final spectra of HPH dehydrated within the probe matched spectra of HPH dehydrated within a 60°C oven.

1. Redman-Furey NL, Dicks ML, Bigalow-Kerne AS, Cambron RT, Lubey GS, Lester CC, Vaughn DC. 2005. Structural and Analytical Characterization of Three Hydrates and an Anhydrate Form of Risedronate. Journal of Pharmaceutical Sciences, vol. 94, 893-911. Wiley InterScience (www.interscience.wiley.com) DOI 10.1002/jps.20308
2. Lester CC, Lubey GS, Dicks ML, Andol GM, Vaughn DC, Cambron RT, Poiesz KB, Redman-Furey NL. 2006. Dehydration of Risedronate Hemi-Pentahydrate: Analytical and Physical Characterization. Journal of Pharmaceutical Sciences -in press.

51 Polymeric NMR Sample Tubes Evaluated in a 1 mm Microprobe

Michael J. Cavaluzzi[1], David J. Kiemle[2], Deborah J. Kerwood[3], and Philip N. Borer[1,3]

1. Advanced Resonance Technologies, Inc., 111 College Pl, 2-212 CST, Syracuse, NY 13244-4100
2. Chemistry Department, SUNY College of Environmental Science & Forestry, 121 Jahn Lab, 1 Forestry Dr, Syracuse, NY 13210-2726
3. Chemistry Department, Syracuse University, Syracuse, NY 13244

Polymeric NMR Tubes have advantages over glass in most applications:

- Polymeric tubes are shatterproof
- Ultra-thin walls afford sensitivity increases of 20-40%
- Polymer tubes are easy to fill and to recover sample
- Standard probeheads and sample changers can be used.

Here we describe the performance of polymer tubes compared to glass capillaries, both having 1 mm outside diameter. The mass sensitivity of 1 mm probes is about four times that of 5 mm probes, yet the former have not found wide acceptance due to the difficulty of using glass capillaries. Samples were tested on a Brüker DRX-600 with a 1 mm TXI room temperature probehead. Primary comparisons are between polymeric tubes with inside diameters of 0.826 (ART-1A), 0.851 (ART-1B), and 0.889 mm (ART-1C), and a glass capillary with ID estimated at 0.814 mm.

The resolution for 1% CHCl3 in d_6-acetone was comparable at half-height (1 Hz) while the lineshape for polymer tubes was typically 20% broader at the base. The signal-to-noise ratio in the polymer tubes scales almost exactly with volume increase in the active region of the receiver coil (tested with 0.1%, 1% and 10% ethylbenzene in CDCl3). 30-40% less time will be required to achieve the same S/N in the larger ID polymer tubes compared to glass capillaries. Spectra acquired in ART-1C tubes will be shown, including: (1) a 1D ^{1}H-spectrum of 1 microgram of stigmasterol where the chemical shifts are easily resolved in 512 scans, (2) a 2D ^{1}H-^{15}N-HSQC of 70 micrograms of ^{15}N-ubiquitin shows that a 21 min acquisition distinguishes the expected peaks and has an increase in S/N ~ 30% over a glass capillary, and (3) a 2D ^{1}H-^{15}N-HSQC of ^{15}N-labeled NCp7 protein from HIV-1. The mass of the protein is about 2% of that often used in a 5 mm room temperature probe (1 mM, 500 microliters).

MJC and PNB hereby disclose their financial interest in ARTech, represented at the SMASH conference by Norell, Inc. NMR tubes with other dimensions will be available soon. Supported in part by NIH grant RR18442 to MJC and PNB.

52 Sample Preparation Strategies for Routine Trace Mixture Analysis by NMR

David Detlefsen, Jeffrey Whitney adn Kenneth Ray

Novatia, LLC, 11 Deer Park Drive, Suite 202, Monmouth Junction, NJ 08852

Novatia initially offered trace (1-50 ug) NMR analysis services in 2002. Like most NMR groups, we found that the supplied samples varied widely in terms of purity and quantity but this problem seemed more prevalent at the trace level. In an effort to facilitate the needs of our trace NMR customers, we developed and introduced SepNMR (an off-line NMR sample preparation workstation based on solid phase extraction) in 2004 offering it as a product and a service. Since then, we have continued to use, refine and extend SepNMR through: 1) incorporation of fraction collection into a well plate, sample dry down and reconstitution and 2) extension of the original SepNMR system to capture multiple chromatographic peaks. By incrementally adding basic sample preparation components (HPLC, fraction collector, concentrator and autosampler) to our NMR laboratory, we have been able to accommodate a wider range of trace NMR requests. Additional benefits include the availability equipment as a method development platform for continued sample preparation research and the development of guidelines to use when undertaking trace NMR projects to accurately estimate the time, effort and costs of trace NMR projects for our customers. Examples and advantages of each approach will be presented.

53 Streamlined Analysis of 1D NMR Spectra

Bruce A. Johnson

One Moon Scientific, Inc.
Newark, NJ

Optimizing the speed with which NMR spectra can be analyzed in a medicinal chemistry setting is crucial to ensuring the maximal productivity of chemists. Here we describe our scheme for optimizing this analysis, beginning with raw NMR data and ending with a text string that describes the NMR parameters in a format suitable for publications or patents. The data flow includes the following major steps: processing the NMR data,locating NMR signals in the spectrum, and categorizing signals in terms of intensity and coupling patterns. Central to the analysis is a technique for robustly determining NMR parameters including frequencies and intensities. While the flow is largely automatic, the user can manually intervene at any stage of the process.

54 LC/SPE/NMR in Pharmaceutical Development: its all about the SPE

Donald Drinkwater[1], Nancy Chiu[2], Hanumantha Rao Marepalli[1] and Patrick Drumm[1]

1. Novartis Pharmaceuticals Corp., East Hanover, NJ
2. Cornell University, Ithaca, NY

Structure elucidation of low level impurities (byproducts, degradants, metabolites, etc., often down to the level 0.05% by LC/UV) in drug substance and drug product is a critical part of the pharmaceutical drug development process. Obtaining clean NMR spectra of an unknown eluting as a liquid chromatography (LC) peak in an online manner is frustrated due to the low concentration of analyte in the eluant. Recent technical advances in the field have demonstrated that an online solid phase extraction (SPE) step can serve as an effective interface between the LC and NMR, providing concentration sensitivity improvements far in excess of what is possible using recent detector-based improvements such as cryogenic cooling and micro flow probes.

The commercial Bruker Biospin LC/SPE/NMR system consists of an Agilent 1100 RP HPLC system coupled to a Spark Holland SPE robot and a 600 MHz NMR with a 3mm cryofit H/C probe fited with a 30 μL active volume flow cell, all under control of the Hystar software. Minor component LC peaks are diverted with post-column diluent automatically to one of 192 2mm x 10mm SPE cartridges containing 3-7 mg RP sorbent. After sufficient repeat injections have occurred to build up significant analyte on the cartridge, the cartridge is dried with N2, and eluted in strong deuterated solvent into the cryo-flowcell for NMR spectral analysis.

Extensive quantitative studies of the performance of the SPE concentration will be presented, demonstrating that the best performance requires a thorough understanding of both the chemistry and physics of the interface. Studies across a range of SPE sorbents and analyte molecules shows concentration factors varying from none to greater than 40x, with predictable trends to assist with the sorbent selection process. Multi-trapping analyte from multiple LC runs onto the same cartridge provides futher signal increase up to greater than 10x, again depending on careful selection of SPE sorbent and diluent pH. Quantitative micro-fractionation of the SPE eluant band shows an elution profile near 20 μL for most analyte/sorbent combinations. A semi-quantitive online breakthough analysis scheme will be presented which provides real-time feedback of trapping efficiency.

55 Quantitation of Small Molecules in Protein-Containing Solutions Using a Spin-Echo Method

Ken Skidmore and Dan Hewitt

Genentech, Inc.

Detection and quantitation of small molecules (impurities, excipients, or leachables) in a protein product can be a challenging task. We have used an NMR method to quantitate small molecule species in the presence of protein product for process validation purposes. This method employs the well-known spin-echo pulse sequence to detect small molecule signals after signals from proteins have dissipated during the spin lock period. This technique acts as a virtual filter, eliminating the need to remove interfering protein material from solution before measurement of small molecule components. In particular, this method is well suited for the process characterization and validation studies of antibody-containing products, because there is little chance of introducing contaminants via sample preparation and handling steps such as filtration during the assay.

In this poster, we will compare the sensitivity and accuracy of this method with the traditional approach of removing protein from solution. We will also demonstrate the applicability of the spin-echo method to samples containing very high protein concentrations (~190 g/L).

56 Using pH Adjustments to Resolve and Detect Small Molecule Components in Protein Containing Solutions

Daniel Hewitt and Ken Skidmore

Genentech

NMR can be a valuable tool for use in the quantification of small molecule analytes in complex protein containing solutions. These small molecules are used in the production and/or purification of therapeutic proteins and must be cleared for product quality and safety. In order to fully utilize an NMR instrument for these purposes, some obstacles must be overcome. Frequently, buffer components can interfere with the resonances from the small molecules of interest. To combat this, one can adjust the pH to a range in which the resonances no longer overlap. In combination with the spin-echo method of suppressing protein signals, this technique can often allow quantitation of all small molecules in protein containing solutions. In order to efficiently complete this work, the resonance positions of the small molecule analytes and buffer components are mapped out over a wide pH range so that a pH can be chosen for analysis.

In some cases, in process protein solutions can have high salt concentrations, which can limit the sensitivity of the NMR instrument. When quantifying small molecules to very low concentrations in protein solutions, the direct effect of the salt concentration on instrument sensitivity must be well understood to not only correctly quantify the small molecule of interest, but also determine which small molecules are good candidates for quantification to low ppm levels in high salt solutions. Here, the practical limits of detection at various salt concentrations are demonstrated on a 500 MHz instrument with an ambient temperature inverse probe.