SMASH 2005 NMR Conference

Verona, Italy

September 25th - 28th, 2005

SMASH 2005 NMR Conference

Dear SMASH 2005 attendees,

Welcome to the SMASH 2005 NMR Conference and the beautiful and historic city of Verona, Italy. The program this year includes new topics such as; Dipolar Couplings in Small Molecules, Determining Chirality by NMR, Screening by NMR, and NMR in Process Chemistry. We also are revisiting Metabonomics, with both an oral session and a workshop, as well a session on Solid State NMR of Small Molecules. The program also includes SMASH's traditional sessions on Heteronuclear Small Molecule NMR and the Student/Post-Doc session. As in the past, the program includes 4 workshops. This year they are on Using Shaped Pulses, NMR in a GLP/GMP Environment, General Approaches to Structure Elucidation, and Metabonomics: Data Analysis.

This year our banquet dinner will be at the Palazzo della Grand Guardia in the Piazza Brà. We are very pleased to have Professor Horst Kessler from the Technische Universität München as our after dinner speaker.

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

Sincerely,

Martin Will and Greg Nemeth

Co-Chairs, SMASH 2005 NMR Conference

SMASH 2005 NMR Conference
Program

Sunday September 25th

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

Monday September 26th

7:00 AM - 8:15 AM **Breakfast**
8:15 AM - 8:30 AM **Opening Remarks**
8:30 AM - 10:00 AM **Metabonomics** - John Shockcor

- **Evaluating Safety, Efficacy, and Phenotype with Metabonomics**
  Lora Robosky, Pfizer Global Research
- **Metabolomics on the Fly**
  Jules Griffin, University of Cambridge
- **Comprehensive Metabolic Analysis Applied to Diabetes and Obesity Mechanisms**
  Christopher B. Newgard, Duke University Medical Center

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Dipolar Couplings in Small Molecules** - Christian Griesinger

- **Stretched Polymer Gels as Alignment Media for Measuring Anisotropic Parameters in Organic Solvents**
  Burkhard Luy, Technische Universität München
- **Residual Dipolar Coupling: A Novel Approach for Conformational Study of Organic Molecules**
  Michael Shapiro, University of Maryland
- **Can Small RDCs Yield Accurate Solution Structures of Small Molecules?**
  Dusan Uhrin, University of Edinburgh

12:00 PM - 1:30 PM **Lunch**
1:30 PM - 3:00 PM **Small Molecule Solid State NMR** - Eric Munson

- **Pharmaceutical Analysis with Solid-state NMR: Applications of Robust Methods for Structural Determination**
  Fred Vogt, GSK
- **Natural Abundance Powder Crystallography by Proton NMR**
  Lyndon Emsley, Ecole Normale Superieure de Lyon
- **Comparison of Several Techniques for Screening and Quantification of Polymorphous Materials**
  Paul van Tilborg, Organon

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

- I. **Using Shaped Pulses** - Steve Cheatham (Mascagni Room)
- II. **NMR in a GLP/GMP Environment** - Gerhard Krack (Ponchielli Room)

5:00 PM - 6:00 PM **Free Time**
6:00 PM - 6:30 PM **Pre-Dinner Social Gathering, Palazzo della Gran Guardia**
6:30 PM - 9:00 PM **Social Dinner, Palazzo della Gran Guardia**
After Dinner Speaker: Horst Kessler
9:00 PM - 11:00 PM **Mixer, Hotel Leon D'Oro**

SMASH 2005 NMR Conference
Program

Tuesday September 27th

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

8:30 AM - 10:00 AM **Determining Chirality by NMR** - Jacques Courtieu

- **Determination of the Relative Configuration of a Biologically Active alpha-methylene-gamma-butyrolactone Using RDC's**
  Christina Thiele, Universitäts Leipzig
- **NMR in Chiral Liquid Crystals: A Powerful Tool to Observe Enantiomers**
  Denis Merlet, Université de Paris-Sud
- **The Assignment of the Absolute Configuration by NMR: Foundations and Applications**
  Ricardo Riguera, University of Santiago de Compostela

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

10:30 AM - 12:00 PM **Heteronuclear Small Molecule NMR** - Carla Marchioro

- **New Liquid-state NMR Experiments Based on Cross-polarization**
  Teodor Parella, Universitat Autonoma de Barcelona
- **Recent Advances in the Development of Heteronuclear Correlation Experiments**
  Brian Marquez, Pfizer
- **Well-behaved Spectra from lll-behaved Pulses**
  Tim Spitzer, GlaxoSmithKline

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

1:30 PM - 3:00 PM **NMR in Process Chemistry** - Joachim Bargon

- **In situ NMR Spectroscopy in Ionic Liquids**
  Ralf Giernoth, University of Cologne
- **In situ NMR Spectroscopy of Reactions in Supercritical CO_2**
  Heiko G. Niessen, University of Magdeburg
- **Investigating Chemical Processes using Parahydrogen and Orthodeuterium**
  Thorsten Jonischkeit, University of Bonn

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

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

- I. **General Approaches to Structure Elucidation -**Jean-Marc Nuzillard (Mascagni Room)
- II. **Metabonomics: Data Analysis -** Andy Nicholls (Ponchielli Room)

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

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

6:30 PM - 8:00 PM **Dinner**

8:00 PM - 10:00 PM **Poster Session with Mixer** (Salagri Room)

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

SMASH 2005 NMR Conference
Program

Wednesday September 28th

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

8:30 AM - 10:00 AM **Student and Post-Doctorate Session** -Burkhard Luy

- **Massive Signal Enhancement Offers NMR Assay of Enzymatic Reactions at Physiologically Relevant Conditions**
  Mathilde Lerche, Sweden
- **RDCs Resolve Conformation and Configuration of Molecules in Organic Solvents**
  Peter Haberz, Max-Planck-Institut
- **Improvements and New Applications of Ultrafast 2D NMR Spectroscopy**
  Frank Kramer, Weizmann Institut
- **Small Molecules Binding to Protein Receptors: the NMR Point of View**
  Silvia Mari, Consejo Superior De Investigaciones Científicas

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

10:30 AM - 12:00 PM **Screening by NMR** - Marcel Blommers

- **The SeeDs Approach - Integrating Fragment Screening into Drug Discovery**
  Ben Davis, Vernalis, UK
- **Improvements in the FAXS and 3-FABS Detection Limits for Efficient NMR-based Binding and Functional Screening**
  Claudio Dalvit, Nerviano Medical Sciences, Italy
- **Library Design for Fragment Based Screening**
  Ansgar Schuffenhauer, Novartis, Switzerland

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

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

SMASH 2005 NMR Conference
Acknowledgements

The SMASH 2005 Conference gratefully acknowledges the support provided by the following companies.

Advanced Chemistry Development, Inc.
Bruker BioSpin Corporation
Cambridge Isotope Laboratories
Chemscribe
CortecNet
Euriso-Top
GlaxoSmithKline
Icon Isotopes
Isotec / Aldrich
JEOL
John Wiley & Sons, Ltd.
Mestrelab Research
Modgraph
New Era Enterprises
Novatia
One Moon Scientific
Oxford Instruments Molecular Biotools Ltd.
Oxford Instruments Superconductivity
PERCH Solutions Ltd
Protasis/MRM
Rototec-Spintec
RS2D
Soluzioni Informatiche
Spectra Stable Isotopes
Tecmag
Varian, Inc.
Waters, Inc.

SMASH 2005 NMR Conference Committee

Program Co-Chairs 2005

Martin Will
sanofi-aventis, Germany

Greg Nemeth
sanofi-aventis, USA

Program Co-Chairs Elect 2006

Steve Cheatham
DuPont Crop Protection

Ernest Schubert
GlaxoSmithKline, USA

Immediate Past Co-Chairs 2004

David Lankin
University of Illinois at Chicago

Eugene Mazzola
University of Maryland

Program Co-Chairs Emeritus 2003

Duncan Farrant
GlaxoSmithKline, UK

Carla Marchioro
GlaxoSmithKline, Italy

Logistics

Carla Marchioro
GlaxoSmithKline, Italy

Vendor Liaisons

Margaret Levenberg
Stepan Company

John Shockcor
Waters

Andrew Nicholls
GlaxoSmithKline, UK

Treasurer & Registrar

Robert Espina
Bristol-Myers Squibb Company

Poster Session

Ernest Schubert
GlaxoSmithKline, USA

SMASH 2005 NMR Conference
Logistics

Please remember that all meals are included with your registration, starting with dinner on Sunday night and ending with a box lunch on Wednesday. All meals, with the exception of the Monday night banquet, will be in the Bellini Restaurant in the Hotel Leon D'Oro. Monday night's pre-dinner mixer, dinner, and after dinner speech will be held at the Palazzo della Grand Guardia in the Piazza Brà, which is a short walk from the hotel. Limited bus transportation will be provided from the Hotel Leon D'Oro to the Palazzo and back.

The main conference will be held in the Mascagni Room. The workshops will be located as follows:

Using Shaped Pulses - Mascagni Room
NMR in a GLP/GMP Environment - Ponchielli Room
General Approaches to Structure Elucidation - Mascagni Room
Metabonomics: Data Analysis - Ponchielli Room

The poster session will be in the Salagri Room.

Monday, September 26th 8:30 AM - 10:00 AM

Metabonomics

John Shockcor, Session Chair

Speakers:

Lora Robosky, Pfizer Global Research

Jules Griffin, University of Cambridge

Chistopher B. Newgard, Duke University Medical Center

Evaluating Safety, Efficacy, and Phenotype with Metabonomics

Lora C. Robosky

Pfizer Global Research and Development, Ann Arbor, MI

An emerging field of relevance to pharmaceutical research is endogenous metabolic profiling, also known as metabonomics or metabolomics. In this approach, a living system's biological response to stimuli is characterized by profiling the existence and concentration of numerous endogenous metabolites. The approach is applicable to most living systems ranging from cells in culture to humans. In drug discovery, metabonomics has been proposed for a variety of applications including the development of a rapid in vivo toxicity screen, elucidation of mechanisms of toxicity, disease or drug action, and identification of biomarkers. This presentation will highlight several examples where metabonomics has proven to be a useful adjunct in traditional toxicity and efficacy studies.

Metabolomics on the Fly

Julian L. Griffin[1], Matthew Piper[2], Kevin M. Brindle[1] and Linda Partridge[2]

1. Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1GA, UK
2. Department of Biology, University College London, Gower Street, London, UK.

Metabolomics, the comprehensive analysis and quantification of the metabolite complement of cells or tissues, has an important role to play in studies of toxicology, the diagnosis of disease, and as a new tool for functional genomics. The approach centres on a global analysis of metabolism using a high throughput analytical tool such as mass spectrometry or ^{1}H NMR spectroscopy, in conjunction with multivariate statistics. Metabolomics is unique compared with the other 'omic' approaches involved in functional genomics in that the approach is cheap on a per sample basis, allowing the acquisition of large data sets. In this example the versatility of metabolomics is demonstrated in an on-going study to investigate longevity in fruit flies.

Genetic mutations in model organisms, including C. elegans, fruit flies and mice, are known to increase the life span by up to 6-fold. Many of these genes have human homologs and indeed, 25% of variation in human life span is thought to be genetic. The pathways involved include regulation of DNA repair, telomerase activity, stress resistance and oxidative damage, mitochondrial DNA damage, response to caloric restrictions, insulin signalling and inflammation. Of these calorie restriction is the most effective method of increasing lifespan in most organisms, including man. One interesting observation is that gene expression in old, calorie restricted animals resembles those of young animals fed ad lib.

In this study we are currently assessing whether ^{1}H NMR based metabolomics can be used to: 1. identify metabolic differences between flies on high and low calorie diets; 2. age fruit flies according to their metabolic profiles; 3. identify which pathways have been altered during calorie restriction. To assess this, two populations of flies were fed either ad lib or on a calorie restricted diet. Calorie restriction extended lifespan by 50% in these animals. ~60-100 flies were used for each time point (20-50 mg wet weight of tissue), and tissue extracts were prepared from whole flies using perchloric acid. One half of these samples were examined by ^{1}H NMR spectroscopy and the other half by Gas Chromatography Mass Spectrometry (GC-MS). Using ^{1}H NMR based metabolomics in conjunction with principal components analysis, flies fed high and low calorie diets were distinguished according to their metabolic profiles. In addition partial least squares successfully correlated age of flies against the changing metabolic profiles in flies on low and high calorie diets. These results demonstrate the versatility of metabolomics as a tool for basic biological research.

Comprehensive Metabolic Analysis Applied to Diabetes and Obesity Mechanisms.

Christopher B. Newgard

Sarah W. Stedman Nutrition and Metabolism Center, & Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27704 USA

This presentation will focus on the application of NMR- and mass spectrometry-based analysis for gaining insights into metabolic changes that underlie diabetes and obesity, in three main topic areas: 1) Understanding of the mechanisms involved in fuel-stimulated insulin secretion and how they become impaired in type 2 diabetes; 2) Gaining insight into mechanisms of obesity-related insulin resistance in liver and muscle; 3) Understanding the process of weight loss in obese subjects via comprehensive metabolic, endocrinologic, inflammatory marker and physiologic profiling. This work is aided by close collaboration with Drs. Dean Sherry, Craig Malloy and Shawn Burgess at UT Southwestern Medical Center in Dallas, who perform NMR-based metabolic flux analysis, and a comprehensive analytic platform that we have developed at the Stedman Center at Duke, that includes mass spectrometry-based metabolic profiling, multiplex hormone and cytokine assays, and physiologic profiling capabilities. Unique insights gained by integration of these analytical capabilities will be highlighted.

Monday, September 26th 10:30 AM - 12:00 PM

Dipolar Couplings in Small Molecules

Christian Griesinger, Session Chair

Speakers:

Bukhard Luy, Technische Universität München

Michael Shapiro, University of Maryland

Dusan Uhrin, University of Edinburgh

Stretched Polymer Gels as Alignment Media for Measuring Anisotropic Parameters in Organic Solvents

Burkhard Luy[1], Kyryl Kobzar[1], J. Christoph Freudenberger[2], Sebastian Knör[1], Dominik Heckmann[1], Peter Spiteller[1], Thomas Paululat[3], Reinhard Bauer[4], Horst Kessler[1]

1. Institut für Organische Chemie und Biochemie der TU München
2. Bayer Industry Services GmbH & Co
3. Organische Chemie II, Universität Siegen
4. BGS Beta Gamma Service GmbH & Co KG

Anisotropic NMR parameters like residual dipolar couplings (RDCs), residual chemical shift anisotropy (RCSA) or residual quadrupolar couplings contain important structural information. For their measurement in high resolution NMR the molecule of interest needs to be partially aligned. For biological molecules in aqueous solution a number of standard methods exist like phospholipid bicelles, filamentous phage, or other liquid crystalline phases that orient in the magnetic field and partially orient the molecules by steric or electrostatic interactions. In relatively apolar organic solvents liquid crystalline phases like PCBP or PBLG and derivatives can be used to weakly orient medium sized organic molecules in the magnetic field. Liquid crystalline phases, however, generally have the disadvantage of a lower limit in the induced anisotropy. An alternative is given by strain induced alignment in a gel (SAG) which is independent of the magnetic field and scalable over a wide range [1,2]. In aqueous solutions mechanically stretched polyacrylamide and an acrylamide/acrylate copolymers were applied. In 2004 we introduced crosslinked polystyrene as a polymer which allows the freely scalable alignment of molecules in chloroform and solvents with similar solubility parameters [3,4]. Improvements in the spectral quality of partially aligned samples could be achieved by the use of polydimethylsiloxane crosslinked by accelerated electrons [5]. Finally, our group and the Griesinger group developed independently two gels that can be used for polar organic solvents like DMSO or methanol, for which scalable alignment could not be achieved so far [6,7]. A detailed characterization of the properties of stretched polymer gels will be presented and a number of applications to various molecules will be given. We also will show first spectra acquired on a sample aligned in gelatine as a chiral gel.

1. R. Tycko, F. J. Blanco, Y. Ishii, J. Am. Chem. Soc. 122, 9340-9341 (2000).
2. H. J. Sass, et al., J. Biomol. NMR 18, 303-309 (2000).
3. B. Luy, K. Kobzar, H. Kessler, Angew. Chem. Int. Ed. 43, 1092-1094 (2004).
4. B. Luy, et al., J. Am. Chem. Soc. 127, 6459-6465 (2005).
5. J. C. Freudenberger, et al., Am. Chem. Soc. 126, 14690-14691 (2004).
6. P. Haberz, J. Farjon, C. Griesinger, Angew. Chem. Int. Ed. 44, 427-429 (2005).
7. J. C. Freudenberger, et al., Angew. Chem. Int. Ed. 44, 423-426 (2005).

Residual Dipolar Coupling: A Novel Approach for Conformational Study of Organic Molecules

Michael Shapiro

University of Maryland, Baltimore Maryland 21201

Residual dipolar couplings (RDCs) induced by anisotropic media are a powerful tool for the structure determination of biomolecules through NMR spectroscopy. Recent advances have proven it to be a valuable tool for determination of the stereochemistry of organic molecules. The strategy for use in six membered ring systems will be discussed. It will also be shown that RDCs in combination with molecular order matrix calculations can be used to unambiguously determine the complete relative stereochemistry of an organic compound with five stereocenters. Three simple one-dimensional experiments were utilized for the measurements of ^{13}C-^{1}H, ^{13}C-^{19}F, ^{19}F-^{1}H, and ^{1}H-^{1}H RDCs.

Can Small RDCs Yield Accurate Solution Structures of Small Molecules?

Dušan Uhrín[1], Tran N. Pham[1,3], David W.H. Rankin[1], Sarah L. Hinchley[1], Tibor Liptaj[2] and Lan Jin[2]

1. School of Chemistry, University of Edinburgh, UK
2. Slovak Technical University, Central Laboratories, Bratislava, Slovakia
3. Department of Physics, University of Warwick, Coventry, UK

NMR spectroscopy of small molecules oriented in liquid crystals is a well-established method for obtaining accurate information about molecular geometry (1). Although this method has been used during the past three decades, the route from dipolar couplings to molecular structures is not an easy one. The main complication is that the solutes in liquid crystalline solvents exhibit complex, second order spectra. Beyond ten interacting spins, the spectra usually become too complicated to be analysed properly.

Reducing the level of orientation in dilute liquid crystalline media (2) brought about the possibility of imposing very low order on the solute molecules, consequently preserving the "first-order character" of spectra and facilitating the extraction of residual dipolar couplings (RDCs). Providing that small RDCs (of up to several Hz) can be measured accurately with a similar relative precession to that of dipolar couplings obtained in strong liquid crystal (up to thousands of Hz) there is no fundamental reason why small RDCs should not yield accurate solution structure of small molecules.

We have developed methods for precise measurement of proton-proton, one-bond and long-range proton-carbon RDCs in dilute liquid crystalline media. These methods, which measure coupling constants with a precision of a few hundredths of a Hz, were used in determination of the first solution structure of a monosaccharide, methyl beta-D-xylopyranoside (I) We discuss this example and compare the solution and neutron diffraction structure of I.

We have recently extended our methodology for the measurement of small proton-proton RDCs to systems with crowded ^{1}H spectra. We illustrated these methods using a heparin-derived fully sulphated tetrasaccharide and report on our progress towards the conformational analysis of this flexible molecule using various dipolar and scalar coupling constants.

1. J.W. Emsley and J.C.Lindon, NMR spectroscopy Using Liquid Crystal Solvents (Pergamon, Oxford 1975); R.Y. Dong, Nuclear Magnetic Resonance of Liquid Crystal (Springer, New York 1994).
2. N. Tjandra and A. Bax, Science 278, 1111-1114 (1997).

Monday, September 26th 1:30 PM - 3:00 PM

Small Molecule Solid State NMR

Eric Munson, Session Chair

Speakers:

Fred Vogt, GSK

Lyndon Emsley, Ecole Normale Superieure de Lyon

Paul van Tilborg, Organon

Pharmaceutical Analysis with Solid-State NMR: Applications of Robust Methods for Structural Determination

Frederick G. Vogt

GlaxoSmithKline, plc

Solid-state NMR (SSNMR) is an increasingly important tool for the characterization of pharmaceuticals during research and development. The understanding and control of crystallization, polymorphic form, salt version, and formulation processes create many analytical questions. SSNMR is often used in conjunction with other techniques to address these questions, with its particular strengths in the study of formulations and in quantitative analysis. SSNMR also has the advantage of high information content, with directly interpretable spectra and access to a range of nuclei. Here we examine the current capabilities of applied SSNMR structural analysis of pharmaceuticals. A selection of robust 1D and 2D experiments are used to study polymorphs, salts and solvates. Homonuclear and heteronuclear correlation experiments between a range of nuclei (^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{23}Na and ^{31}P) are demonstrated and related to supramolecular structure. Two primary areas to be discussed include studies of known crystal structures to elucidate features of unknown polymorphs, and analysis of poorly defined or mobile regions of known crystal structures. Particular attention is paid to analysis of hydrates containing loosely bound water, known as channel hydrates. The use of chemical shift tensor measurements for identifying structural features is also investigated.

Natural Abundance Powder Crystallography by Proton NMR

Lyndon Emsley

Laboratoire de Chimie (UMR ENS/CNRS), Ecole Normale Supérieure de Lyon

Structure determination in molecular solids at natural isotopic abundance is one of the key challenges facing solid-state NMR. Notably, proton spectroscopy in diamagnetic solids is limited by the quality of homonuclear dipolar decoupling sequences. Using approaches based on direct optimisation of the spectral response, we have obtained improved performance in homonuclear dipolar decoupling. In this way, decoupling schemes can be tailored to the specific use that they are intended for. This has direct consequences on the feasibility of various correlation methods, and we show applications to ^{1}H spin diffusion experiments for structure determination, and to heteronuclear correlation experiments based on ^{1}H-^{13}C and ^{1}H-^{15}N INEPT.

The link between experimental 1H spin diffusion curves, obtained from high-resolution ^{1}H-^{1}H spin diffusion experiments, and the X-ray crystal structure for a model organic molecular compound is investigated through a rate matrix analysis approach. Since dipolar coupling networks extend over relatively large distances, simulations require the molecule in its full crystal environment to obtain good agreement with experiment. The comparison between the experimental data and simulation is shown to depend strongly on the parameters of crystal structure, and quantitative aspects of structure determination by ^{1}H-^{1}H dipolar correlations in solids are discussed in detail.

We also consider the feasibility of approaches to structure determination in paramagnetic compounds. By combining very fast magic angle spinning with suitable correlation methods, adapted to the special conditions engendered by a paramagnetic center, we are able to obtain site resolved proton shifts and anisotropies in a high-spin Fe(II) compound. We present strategies for assignment and structure determination in this case.

Comparison of Analytical Techniques for Screening and Quantification of Polymorphous Materials

Paul van Tilborg and Peter van Hoof

N.V. Organon, P.O. Box 20, 5340 BH, Oss, The Netherlands

Several properties of Active Pharmaceutical Ingredients (APIs), like stability, solubility, dissolution rate and therewith bio-availability, may differ substantially between different (pseudo)polymorphic forms.

In an effort to identify all polymorphic forms of an API, many crystallization experiments are performed and all resulting samples are screened using several analytical techniques. For 17 polymorphism studies the effectiveness of IR/RAMAN, X-ray powder diffraction, solid state NMR and DSC/TG was evaluated. These techniques were scored not only on ability to discriminate different polymorphs, but also on items like required amount of sample, measurement time and ability to provide additional information on the solid phase.

The main results of this study, emphasizing on solid state NMR, will be discussed.

Tuesday, September 27th 8:30 AM - 10:00 AM

Determining Chirality by NMR

TBA, Session Chair

Speakers:

Christina Thiele, Universitäts Leipzig

Denis Merlet, Université de Paris-Sud

Ricardo Riguera, University of Santiago de Compostela

Determination of the Relative Configuration of a Biologically Active Alpha-Methylene-Gamma-Butyrolactone Using RDCs

Christina M. Thiele, Andreas Marx

Universität Leipzig, Institut für Analytische Chemie, Johannisallee 29, D-04103 Leipzig, German

The determination of the relative configuration by nuclear magnetic resonance is usually done using a combination of ^{3}J coupling and NOE data. Sometimes this is impeded by either absence of NOE data, remoteness of the stereocenters or conformational equilibria.

This is why RDCs, after having had enormous impact on the structure determination of proteins[1], recently have come into the focus of small molecule structure determining groups. It has been shown that it is possible to assign diastereotopic groups and protons [2] and determine the relative configuration of rigid molecules or molecules with a well defined conformation [3].

The determination of the relative configuration of the alpha-methylene-gamma-butyrolactone shown here is difficult with conventional methods because there are structures in the conformational space of both diastereoisomers encoding the same coupling and NOE information.

The measurement techniques used to obtain direct and longrange ^{1}H-^{13}C-RDCs and ^{1}H-^{1}H-RDCs are discussed and the assignment of the relative configuration from these RDCs is discussed in detail.

1. Reviews: 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. M. Thiele, S. Berger, Org. Lett. 2003, 5, 705-708; L. Verdier, P. Sakhaii, M. Zweckstetter, C. Griesinger, J. Magn. Res. 2003, 168, 353-359; C. Thiele, J. Org. Chem. 2004, 62, 7403-7413

3. C. Aroulanda, V. Boucard, F. Guibé, J. Courtieu, D. Merlet, Chem. Eur. J. 2003, 9, 4536-39; J. Yan, F. Delaglio, A. Kaerner, A . D. Kline, H. Mo, M. J. Shapiro, T. A. Smitka, G. A. Stephenson, E. R. Zartler, J. Am. Chem. Soc. 2004, 126, 5008-5017; J. C. Freudenberger, P. Spiteller, R. Bauer, H. Kessler, B. Luy, J. Am. Chem. Soc. 2004, 126, 14690-91.

NMR in Chiral Liquid Crystals: A Powerful Tool to Observe Enantiomers.

Jacques Courtieu, Philippe Lesot, Abdelkrim Meddour, **Denis Merle**t

Laboratoire de RMN en milieu Orienté
Université Paris-Sud. ICMMO. Bat. 410.91405, Orsay, France.

Considerable efforts are continuously made in the field of the asymmetric synthesis in organic chemistry. Consequently, the development of new and efficient NMR tools allowing the visualization of enantiomers is a great motivation for spectroscopists. Among them, the use of NMR in chiral liquid crystalline solvents has proved to be a method of choice.

One of the most interesting chiral liquid crystals is the chiral nematic phase formed by synthetic homopolypeptide (Eg PBLG, PELG ...) in solution with various organic co-solvents such as chloroform, dichloromethane, DMF, THF etc. We have shown on a wide range of compounds that we can visualize the enantiomeric discrimination through NMR spectra of different nuclei in this chiral medium[1-5]. In addition, we have developed and tested several new NMR 2D sequences to facilitate the analysis and the assignment of overcrowded spectra for different nuclei [6-8].

For solutes which are chiral by virtue of isotopic substitution, the origin of the discrimination is particular because the ordering of the enantiomers are the same. The discrimination comes from the fact that in these solvents Re and Si faces of a prochiral molecule are discriminated, and the visualisation of isotopic enantiomers is a consequence of this property [4]. In fact, the location of the principal axes of the order matrix is determined by the effective molecular symmetry. From group theoretical arguments, we have shown that four molecular symmetry groups, namely C_S, S_4, C_{2v}, D_{2d}, change their effective molecular symmetry in a chiral phase allowing the possibility to visualize the enantiotopic groups or directions [9].

1. Meddour A., Canet I., Loewenstein A., Pechine J.M., Courtieu J., J. Am. Chem. Soc. 1995, 116, 9652.
2. Meddour A., Berdague P., Hedli A., Courtieu J., Lesot P., J. Am. Chem. Soc. 1997, 119, 4502.
3. Jakubcova M., Meddour A., Pechine J.M., Baklouti A., Courtieu J., J. Fluor. Chem. 1997, 86, 149.
4. Canet I., Courtieu J., Loewenstein A., Meddour A., Pechine J. M., J. Am. Chem. Soc. 1995, 117, 6520.
5. Lesot P., Merlet D., Loewenstein A., Courtieu J., Tetra. Assym. 1998, 9, 1871.
6. Lafon O., Lesot P., Merlet D., Courtieu J., J. Magn. Res., 2004, 171, 135-142.
7. Farjon J., Merlet D., Lesot P., Courtieu J., J. Magn. Res. 2002, 169.
8. Farjon J., Baltaze J.P., Lesot P., Merlet D., Courtieu J., Mag. Res. Chem. 2004, 594.
9. Merlet D., Emsley J.W., Lesot P., Courtieu J., J. Chem. Phys. 1999, 111, 6890.

The Assignment of Absolute Configuration by H-NMR: Foundations and Applications

Ricardo Riguera

Departamento de Química Orgánica, Facultad de Química
Universidad de Santiago de Compostela
15782 Santiago de Compostela, Spain
ricardo@usc.es

The assignment of the absolute configuration of organic compounds by NMR is particularly useful in cases where the amount of sample is limited, no monocrystals are available or a rapid and inexpensive method is needed. In its classical approach, the substrate is separately derivatized with the two enantiomers of a chiral auxiliary reagent and the absolute configuration is obtained by comparison of the H-NMR spectra of the two resulting diastereomers (1). In this presentation we will show first, the foundations of this procedure: the role of the anisotropic group in the auxiliary, the origin of the selective shielding and the importance of the conformation. Next, the use of the most common auxiliary reagents ((R) and the (S)-arylmethoxyacetic acids (MPA, MTPA, and 9-AMA), the protected aminoacid BOC-PheGly and the alcohol 9-AHA) for the assignment of the absolute configuration of a variety of monofunctional chiral compounds (primary alcohols, secondary alcohols, primary amines and carboxylic acids) will be described with examples of their actual H-NMR spectra and the graphical models used for assignment.

New procedures have been developed, that simplify the experimental work and reduce the amount of sample needed. These are characterised by a) the use of only one derivative of the substrate instead of two or b) by the elimination of any manipulation of the sample, that should just be mixed and shaken with the auxiliary in the NMR tube or c) by reducing the amount of sample necessary for the assignment to just a few micrograms by HPLC_NMR. These procedures will be also illustrated with examples of application. Finally, the rules for assignment by this procedure, of the absolute configuration of polyfunctional compounds such as diols, and triols, will be presented (2).

1. For a review on this topic see: Seco, J. M.; Quiñoá E.; Riguera R. Chem. Rev. 2004, 104, 17-117

2. Freire, F.; Seco, J. M.; Quiñoá, E. Riguera, R. J. Org. Chem. 2005, 70, 3778-3790. IBID Chemistry Eur. Journal 2005;

Tuesday, September 27th 10:30 AM - 12:00 PM

Heteronuclear Small Molecule NMR

Carla Marchioro, Session Chair

Speakers:

Teodor Parella, Universitat Autonoma de Barcelona

Brian Marquez, Pfizer

Tim Spitzer, GlaxoSmithKline

New Liquid-State NMR Experiments Based on Cross-Polarization

Teodor Parella

Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, E-08193, Bellaterra, Barcelona, Spain. e-mail: teodor.parella@uab.es

Heteronuclear coherence transfer in liquid-state NMR applications has been traditionally performed using pulse-interrupted delay schemes such as INEPT- type pulse trains. So far, the alternative use of heteronuclear cross-polarization (HCP) has only been limited to a few cases involving exclusively in-phase to in-phase transfers. A theoretical description on the effect and the special anisotropic features of HCP is introduced in terms of product operator formalism. A very intuitive and simple graphical black-box approach based on a pictorial non-classical vector representation that only consider the available input/output magnetization is also presented to understand the general transformations that are undergoing under such rather complex HCP processes. The appropriate manipulation of magnetization components during the HCP block offers novel concepts in pulse sequence design and allows the success implementation of HCP-driven processes as effective preparation and/or mixing building blocks in a variety of liquid-state multidimensional NMR applications. Examples will be provided for small-to-medium-sized molecules at natural abundance.

Parella, T.; Belloc, J.; Sánchez-Ferrando, F.; Virgili, A.; Magn. Reson. Chem. (1999) 37, 631.
Parella, T.; J. Biomol. NMR. 29, 37-55 (2004).
Parella, T.; J. Magn. Reson., 167, 266-272 (2004).
Parella, T.; Belloc, J.; Sánchez-Ferrando, F.; Magn.Reson.Chem., 42, 852-862 (2004).
Parella, T.; Gairí M.; J. Am. Chem. Soc., 126, 9821-9826 (2004).
Nolis, P; Roglans A.; Parella, T.; J. Magn. Reson., 173, 305-309 (2005).
Nolis, P; Roglans A.; Parella, T.; submitted (2005).
Nolis, P; Parella, T.; submitted (2005).

Recent Advances in the Development of Heteronuclear Correlation Experiments

Brian Marquez[1] and Thomas Williamson[2]

1. Pfizer Inc.
2. Roche Carolina

Molecular conformations and stereostructures are a critical piece of information in the complete structure elucidation of small organic molecules. Many approaches are used to do these types of analysis including the traditional approach of dipolar and homonuclear scalar couplings, the relatively recent use of RDC's and homonuclear and dipolar couplings with the inclusion of heteronuclear couplings. The use of heteronuclear couplings has become a more regular occurrence in the literature. This has been the case due to a significant amount of research in the development of experiments that allow their straightforward measurement. To this end, advancements have been made in the HSQMBC family of experiments that allow heteronuclear coupling constants to be measured with greater ease.

Well-Behaved Spectra from Ill-behaved Pulses

Timothy D. Spitzer, Randy D. Rutkowske, and George F. Dorsey

GlaxoSmithKline, Research Triangle Park, NC USA

A number of groups have developed experiments that take advantage of frequency-swept pulses. Nearly all the work to date has relied on adiabatic inversion and refocusing pulses. Such pulses have been the basis for improved experiments that offer greater sensitivity and minimal requirements for calibration or optimization. In a paper published in 2002, A. J. Shaka et al. investigated the properties of frequency-swept 90º hyperbolic secant pulses. In contrast to the composite adiabatic pulses designed by Garwood and coworkers, 90º hyperbolic secant pulses are less well-behaved. Although Shaka et al. demonstrated that such pulses can produce high-quality spectra, the results depend on careful pulse sequence design. Indeed, after successfully duplicating the results of Shaka and coworkers, our group has gone on to uncover a seemingly limitless number of ways to produce dreadful-looking spectra. Such results are invariably the result of careless mistakes. An incorrect choice of pulse length, sweep direction, or sweep range will result in a spectrum with a severe phase roll. Although we have benefited from the lessons in humility that these pulses provide, we have also profited from the correct use of swept 90º pulses. The timing properties that make these pulses ill-behaved can be used to advantage by contributing to "J Compensation." Swept 90º pulses can be used as elements in robust, reliable experiments. This presentation discusses a number of ways in which frequency-swept 90º pulses can be used to produce high-quality spectra.

Tuesday, September 27th 1:30 AM - 3:00 PM

NMR in Process Chemistry

Joachim Bargon, Session Chair

Speakers:

Ralf Giernoth, University of Cologne

Heiko G. Niessen, University of Magdeburg

Thorsten Jonischkeit, University of Bonn

In situ NMR Spectroscopy in Ionic Liquids - A Road Towards a Better Understanding of a New Class of Solvents

Ralf Giernoth and Dennis Bannkmann

Institute of Organic Chemistry, University of Cologne, Köln, Germany

Ionic liquids (ILs) have lately gained tremendous attention as novel solvents for organic synthesis, especially for organometallic catalysis. The physical properties of ILs can widely be varied through choice of their "building blocks" (i.e. cation, side chain, and anion).

However, only a very limited range of analytical techniques for ILs are routinely available. Therefore, we systematically develop NMR spectroscopy for the use in ILs. These techniques will be valuable tools for investigations on solvent reactivity and catalytic as well as stoichiometric reactions in ILs.

At the current stage, NMR in ILs can be carried out routinely and provides spectra comparable to those measured in classical organic solvents. The high viscosities and the lack of deuteration of common ILs are the main limiting factors to tackle. We present advanced solvent suppression techniques (based on DOSY - diffusion ordered spectroscopy) to facilitate the study of solutes in ILs and compensate for the large ratio of solvent to solute signals; and we show first applications towards a better understanding of chemistry in ionic liquid media.

In situ NMR Spectroscopy of Reactions in Supercritical Carbon Dioxide

Heiko G. Niessen

Center of Advanced Imaging & Department of Neurology II,
Otto-von-Guericke University Magdeburg, Germany

In recent years, supercritical fluids (SCF) have drawn substantial interest as modern solvents for chemical reactions, separations, and extractions in the area of basic research as well as in industrial processes. Especially, supercritical carbon dioxide ($scCO_2$) is a desirable replacement for organic solvents because it is inexpensive, non-toxic, non-flammable, environmentally benign, and exhibits ease of recycling and disposal. Furthermore, its basic physical properties such as density, diffusivity, and viscosity are tunable over a wide range [1]. To investigate chemical reactions at high pressure in situ, a special reactor based on a toroid cavity has been developed and used to study catalytic reactions in supercritical CO_2. This cylindrical cavity can handle pressures of more than 350 bar and it can be heated to temperatures of more than 100 C using resistive heating. Furthermore, it allows addition of various components for the chemical reactions [2,3]. In this contribution, in situ NMR spectroscopy in toroid cavity autoclaves and the specialized technique of parahydrogen induced polarization (PHIP) are introduced as beneficial for investigating physical properties and chemical reaction in SCFs [4].

1. H.G. Niessen and K. Woelk, Top. Curr. Chem. (review) (2005) in press
2. H.G. Niessen, P. Trautner, S. Wiemann, J. Bargon, and K.Woelk, Rev. Sci. Instrum. 73 1259–1266 (2002)
3. H.G. Niessen, P. Trautner, R. Backhausen, and K. Woelk, Concepts Magn. Reson. B (Magn. Reson. Eng.) 16B 51-21 (2003)
4. S. Lange, A. Brinkmann, P. Trautner, K. Woelk, J. Bargon, and W. Leitner, Chirality, 12, 450-455 (2000)

Investigating Chemical Processes Using Parahydrogen and Orthodeuterium

Thorsten Jonischkeit

Institute of Physical and Theoretical Chemistry,
University of Bonn, Germany

Hydrogenation reactions conducted with molecular hydrogen enriched in its nuclear singlet state (parahydrogen) can lead to strongly enhanced absorption and emission signals in the NMR spectra of reaction intermediates or products if they are recorded during or shortly after the reaction. This hyperpolarization phenomenon has been termed PHIP (parahydrogen induced polarization) and is recurrently used to study reaction mechanisms and kinetics of catalytic hydrogenations.

A similar effect has been observed with with molecular deuterium enriched in its nuclear triplet state (orthodeuterium). The new method was termed ODIP (orthodeuterium induced polarization). The signal patterns and their signal enhancements were investigated depending on particular experimental conditions such as conducting the reaction in a high magnetic field (PASADENA) or in a low magnetic field (ALTADENA) before recording the NMR spectrum.

The quadrupolar moment of ^{2}H nuclei (deuterons) causes a fast relaxation of ^{2}H NMR hyperpolarization signals. After an intramolecular polarization transfer to hetero nuclei, however, hyperpolarization can stay substantially longer with a target molecule. This indicates a quantitative difference between PHIP and ODIP and suggests applications toward polarization transfer between different types of nuclei.

Wednesday, September 28th 8:30 AM - 10:00 AM

Student and Post-Doctorate Session

Burkhard Luy, Session Chair

Speakers:

Mathilde Lerche, Sweden

Peter Haberz, Max-Planck-Institut

Frank Kramer, Weizmann Institut

Silvia Mari, Consejo Superior De Investigaciones Científicas

Massive Signal Enhancement Offers NMR Assay of Enzymatic Reactions at Physiologically Relevant Conditions

Mathilde H. Lerche, Jan Henrik Ardenkjär-Larsen, Andreas Gram, Rolf Servin, Mikkel Thanning and Klaes Golman.

Amersham Health R&D AB, part of GE Healthcare, Medeon, 205 12 Malmö, Sweden

The strong enhancement of the nuclear polarisation, which has been obtained using DNP-NMR, has profound impact on NMR spectroscopy [1,2]. Sensitivity improvement by many orders of magnitude widens the scope of NMR to analytical areas not accessible by current state of the art NMR techniques. By virtue of the method, NMR analysis is completed in a time frame of the nuclear T1 (seconds for ^{13}C). In addition, the enhanced polarisation enables a significant reduction in sample concentration.

The method allows NMR studies to be performed at physiological realistic concentrations in complex biological systems such as whole cells. Rate constants for enzymatic reactions as well as IC_{50} values and KI values of inhibitors are readily obtained using this assay. This new method may thus be a robust and general way of assaying enzymatic reactions and characterizing new inhibitor candidates. The method may even aid a better understanding of the basic chemistry involved in cellular systems.

1. JH Ardenkjær-Larsen, B Fridlund, A Gram, G Hansson, L Hansson, MH Lerche, R Servin, M Thaning, K Golman, PNAS 100(18) (2003) 10158-63

2. JH Ardenkjaer-Larsen, O Axelsson, K Golman, G Hansson, H Johannesson, R Servin, M Thaning, L Hansson, US20040066193 A1, (2001).

RDCs Resolve Conformation and Configuration of Molecules in Organic Solvents

Peter Haberz[1], Jonathan Farjon[1], Wolfgang Bermel[2], Uwe Reinscheid[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 (rdc's) 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 rdc's have shown to be very efficient in the stereochemical assignment of moieties and that they hold the promise of defining the stereochemistry even in non-rigid molecules [2].

A large variety of very weakly orienting media exist for aqueous solutions, but only in the past few years this methodology has been extended to organic solvents for studying synthetic organic molecules and natural products [3]. Here we present two novel charged polyacrylamide-based alignment media, that are compatible with DMSO and other organic solvents. The negatively charged PH-gel [3b] and the positively charged PPH-gel [4]. We will demonstrate that they show linearly independent alignments, which improves the unambigous assignment of diastereotopic protons. Furthermore the power of these charged gels for the structural refinement of natural products as well as work towards the distinction of diastereomers and on the determination of the absolute configuration of small molecules 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. a) B. Luy, K. Kobzar, H. Kessler, Angew. Chem. Int. Ed. 2004, 43, 1092-1094. b) P. Haberz, J. Farjon, C. Griesinger, Angew. Chem. Int. Ed. 44, 427-429 (2005). c) J. C. Freudenberger, S. Knör, K. Kobzar, D. Heckmann, T. Paululat, B. Luy, Angew. Chem. Int. Ed. 44, 423-426 (2005). d) J. C. Freudenberger, P. Spiteller, R. Bauer, H. Kessler, B. Luy, J. Am. Chem. Soc. 126, 14690-14691 (2004).

4. P. Haberz, J. Farjon, C. Griesinger, in preparation.

Improvements and New Applications of Ultrafast 2D NMR Spectroscopy

Frank Kramer, Boaz Shapira, Mor Mishkovsky, Maayan Gal and Lucio Frydman

Department of Chemical Physics, Weizmann Institue of Science, 76100 Rehovot, Israel

In the last years ultrafast NMR Spectroscopy [1] was introduced as a general way for recording multidimensional NMR spectra within a single scan. I will give a short introduction into the basic features of this new methodology and provide an overview about recent developments, which resulted in an improvement of the sensitivity and resolution performance of single-scan NMR spectroscopy. Discussed in detail will be different excitation schemes, including the new symmetric spatial encoding protocol, and the implementation of the interlaced fourier transformation for ultrafast NMR Spectroscopy [2]. Besides these fundamental issues several examples of applications of single-scan NMR spectroscopy will be presented.

1. (a) L. Frydman, T. Scherf and A. Lupulescu, Proc. Natl. Acad. Sci. USA, 99, 15858-15862 (2002). (b) L. Frydman, A. Lupulescu and T. Scherf, J. Am. Chem. Soc., 125, 9204-9217 (2003).

2. M. Mishkovsky and L. Frydman, J. Magn. Reson., 173, 344-350 (2005).

Small Molecules Binding to Protein Receptors: the NMR point of view

Silvia Mari[1], Cristina Airoldi[2], Francesco Peri[2], Francesco Nicotra[2], Donatella Potenza[3], Anna Bernardi[3] and Jesus Jimenez-Barbero[1]

1. Centro de Investigaciones Biologicas, Madrid, Spain
2. Università Bicocca, Milan, Italy
3. Università degli Studi, Milan, Italy

Understanding the binding mode between a ligand and its target is essential in the development of molecules biologically active and in drug design. In particular, the knowledge of how functional groups in small molecules have to be spatially organized to ensure high affinity interaction with the target is a key point to rationalize and optimize future ligand synthesis.

NMR is a fundamental non-destructive technique that is able to answer all these requirements. In particular, we focus here in the application of STD[a] and tr-NOESY experiments in order to investigate which regions of the ligand are in closest contact with the target, and whether or not there are any conformational changes or selection in the ligand upon binding. We present here the case of cholera toxin pentamer CTB_5 and GM_1 mimic interaction, where an intramolecular CH-p interaction of the ligand selects a preferred conformation in the free state, which is the same recognized by CTB_5[b], but is drastically different in the binding mode with Viscum Album Agglutinin (VAA). Again probably intermolecular CH-p interactions are the main factors in RAS with Schering-based[c] ligands and novel inhibitors[d] recognition.

Recently, we have also discovered the possibility to detect interaction between ligands in solution and membrane proteins directly employing living cells[e]. In particular, 1D STD NMR spectroscopy could be employed as the initial step in screening processes, representing a significant advance in molecular recognition studies since it eliminates time-consuming purification processes.

[a] M. Mayer, B. Meyer, Angew.Chem.Int.Ed., 1999, 38, 1784 – 1788.
[b] A. Bernardi, D. Arosio, D. Potenza, I. Sanchez-Medina, S. Mari, F. J. Cañada, J. Jimenez-Barbero, Chemistry, 2004, 10 (18), 4395.
[c] A. K. Ganguly et al. Biochemistry 1998, 37, 15631-15637.
[d] F. Peri, C. Airoldi, S. Colombo, E. Martegani, A.S. Van Neuren, M. Stein, C. Marinzi, F. Nicotra, ChemBioChem, in press.
[e] S. Mari, D. Serrano-Gómez, F. J. Cañada, A. L. Corbí, J. Jiménez-Barbero, Angew. Chem. Int. Ed., 2005, 44 (2), 296-298.

Work supported by the European Community's Human Potential Program under contract HPRN-CT-2002-00173,[GLYCIDIC SCAFFOLDS]

Wednesday September 27th 10:30 AM - 12:00 AM

Screening by NMR

Marcel Blommers, Session Chair

Speakers:

Ben Davis, Vernalis, UK

Claudio Dalvit, Nerviano Medical Sciences, Italy

Ansgar Schuffenhauer, Novartis, Switzerland

The SeeDs Approach - Integrating Fragment Screening into Drug Discovery

Ben J. Davis

Vernalis, Cambridge UK

The use of weak binding "fragments" of molecules is now recognised as an efficient and robust method of hit identification in the drug discovery process. We have developed a collection of methods for fragment based drug discovery which we call SeeDs (Structural exploitation of experimental Drug startpoints).

The SeeDs strategy includes:

(1) the generation of diverse, drug-like, synthetically tractable, fragment libraries with appropriate physico-chemical properties
(2) the use of NMR binding experiments to identify fragments which are binding competitively to a specific binding site
(3) the determination of the 3D structure of the fragment bound to the target, and/or the use of docking methods to identify putative binding modes
(4) approaches for evolving the fragments into hit compounds using sub-structure searching or the design of focussed chemical libraries.

I will discuss our experience in developing and applying the SeeDs technology to a number of targets, with particular emphasis on library design, issues around compound stability and utilisation of SeeDs data.

Improvements in the FAXS and 3-FABS Detection Limits for Efficient NMR-based Binding and Functional Screening

Claudio Dalvit

Chemistry Department, Nerviano Medical Sciences, Viale Pasteur, 10 - 20014 Nerviano (MI) Italy

NMR-based binding and functional screening performed with FAXS and 3-FABS represent reliable methods for identifying ligands and inhibitors and for measuring with high accuracy their binding constant and inhibitory activity, respectively. Although the methods score high in quality, in their current status they cannot compete with the very high sensitivity of the fluorescence-based or radioactive-based techniques. Often a long acquisition time is required for signal averaging thus reducing the throughput of the NMR-based assays. Two different strategies can be followed for improving the sensitivity of the experiments:

i) Use of substrates or spy molecules tagged with multiple magnetically equivalent CF_3 moieties. ii) Use of a cryogenically cooled ^{19}F probe with reduced thermal noise in the receiver circuitry. Sensitivity improvement achieved with these methods permits an increased throughput, detection of weaker binders and inhibitors, detection of slow binders and reduction in protein and substrate consumption. The possibility of detecting weaker binders and inhibitors is fundamental for the selection of initial small scaffolds necessary for a fragment-based lead discovery approach. The sensitivity aspects are analyzed with theoretical simulations and experimental quantitative performance evaluation. Application of 3-FABS with the cryogenic ^{19}F probe technology to the rapid screening at an enzyme concentration of only 200 fM and the current detection limits reached with this technology are also presented.

Library Design for Fragment Based Lead Discovery

Ansgar Schuffenhauer

Novartis Institutes of Biomedical Research

Library design in fragment based screening has to fulfill several constraints. While the fragments should be as small as possible in adherence with the general theoretical concept of fragment based screening and also comply with the physical chemical requirements set by the screening technologies, the compounds need also to be large enough achieve a detectable binding event.

In contrast to high-throughput screening, where there was in the beginning the hope that one could detect compounds which had the molecular size and potency to serve directly as a lead structure, in fragment based screening the resulting fragments will need to be chemically evolved or linked. This can be very challenging if the fragments obtained as screening hits do not contain any functional groups suitable for the attachment of a linker or this functional group is essential for the recognition of the fragment by the protein. We have developed devised a fragment library concept in which not the functionalized building blocks itself are screened, but derivatives in which the linking functionality has been chemically masked.

Tuesday, September 27th 8:00 PM - 10:00 PM

Poster Session

Sponsored by Varian Inc.

Ernest Schubert, Session Chair

Index To Posters

18. Going Cold in Metabonomics
19. NMR Characterization of Novel Products of Lipid Peroxidation as Etheno-2'-Deoxyguanosine Adducts from Dioxododecenoic Acid and 5(S)-Hydroperoxyeicosatetraenoic Acid
20. Accessing Analytical Data in an Electronic Lab Notebook Workplace
21. Use of ^{15}N NMR for the Identification of a Process Impurity Arising from a Rare Pyridine to Pyrrole Conversion
22. Use of Genetic Algorithms with NOESY and ROESY Data to Predict the Stereochemistry and 3D Configurations of Small Molecules
23. Dereplication and Elucidation of the Natural Products Staurosporine and Aselacin C Using NMR Software Methods
24. A Testset for Validation of Carbon-NMR Prediction Using Stereochemical Information
25. Selective Excitation of Overlapping Peaks: Comparison of 1D TOCSY and NOE Experiments
26. Automatic Confirmation of Chemical Structure Employing Both ^{1}H And ^{13}C Spectra
27. About How One Molecule of N,N',N"-Triacetylchitotriose Can Be Sandwiched Between Two Molecules of Elderberry Lectin
28. The Influence of Tetrahydrofuran on Reactivity, Aggregation, and Aggregate Structure of Dimethylcuprates in Diethyl Ether
29. Diffusion Study of the Precatalysts Between Phosphoramidite Ligands and Copper Salts
30. Transferred-NOESY NMR Experiments on Living Cells: RGD Derivatives Bound Platelet Integrin aIIbb3
31. H2BC: Heteronuclear 2-Bond Correlation
32. Structural Implications of a Pyrene - Modified Nucleotide on a Duplex DNA
33. Defining the Influence of Peroxisome Proliferator Activated Receptors on the Metabolome of the Mouse
34. Metabolic Profiling of Biofluid Samples from Type 2 Diabetes Mellitus Patients Using NMR Spectroscopy
35. Metabolomic Studies of Brain Tissues from a Potential Model for Parkinson Disease
36. Stabilization of Small Molecular Clusters in Solution - Liquid State NMR Down to 100 K

37. The Design of a Multi-Dimensional LC-SPE-NMR System (LC2-SPE-NMR) for Complex Mixture Analysis

38. Fast Determination of Water Content of Compound Collections (DMSO Solutions) by NMR Spectroscopy

39. ^{1}H and ^{13}C NMR Spectral Assignment of Dehydrocholic Acid and Related Compounds

40. Dereplication of Natural Products using HPLC-DAD-SPE-NMR

41. Metabolomic Studies of a Diabetic Mouse Model by ^{1}H NMR Spectroscopy of Urine

42. Deuterium Isotope Effects in the Study of Intramolecularly Hydrogen-bonded Thioxo Compounds in the Liquid and Solid State

43. Identification of Interesting and Novel Metabolites of CP-533,536 by LC-NMR and LC/MS

44. Effect of Age and Caloric Restriction on Plasma Metabolome and Skeletal Muscle Transcriptome

45. Determination of Degradation Pathways and Kinetics of Acyl Glucuronides by NMR Spectroscopy

46. A Markup Language for the Description of an NMR Metabolic Experiment

47. Stereochemical Identification of Ibuprofen Using Chiral Liquid Crystals, NMR and Modeling

48. Two- and Three-way Chemometric Methods Applied for Characterisation of Complex Pharmaceutical Preparations: St. John's Wort (Hypericum perforatum L.)

49. Working with a ^{13}C-enhanced Cold Probe

50. Microcoil NMR on a Microfluidic Lab-on-a-Chip

51. NMR-based Metabolomics Analysis of Vascular Endothelial Cells During Stress-Induced Premature Senescence

52. NMR Based Nutri-Metabonomics

53. Quantification of the Degree of Blockiness in Pectins Using ^{1}H NMR Spectroscopy and Chemometrics

54. Parameter Optimization using Non Linear Least Square Curve Fitting Method

55. Usefulness of DOSY Method for the Analysis of Labile and Unisolable Compound in the Synthetic Reaction

56. Resolution Enhancement in the Carbon Dimension of HSQC and Related Experiments Using Computer Optimised Aliasing

57. Routine NMR in Medicinal Chemistry

58. Quantification by NMR - an Old Method Revisited

59. Using Metabolomics to Investigate a Yeast Model of Batten Disease

60. Intelligent Bucketing for Metabonomics—Part 2

61. Long Range Homo and Heteronuclear Couplings Constants Extraction from Standard NMR Experiments

62. Strategies for Automating In-vivo Metabolite Identificaton

63. Intramolecular Hyperpolarisation Transfer in Organic Molecules Using INEPT

64. Combining Statistical Analysis and Spectra Base Techniques for Efficient Metabolic Profiling

65. Metabonomics, Dietary Influences and Cultural Differences: A ^{1}H NMR-Based Study of Urine Samples Obtained From Healthy British and Swedish Subjects

66. A ^{1}H NMR-Based Metabonomic Study of Urine and Plasma Samples Obtained From Healthy Human Subjects

67. Examination of 3- vs 5-Trifluoromethylpyrazole Regioisomers by ^{19}F-^{15}N Correlation Experiments

68. Virtual Spectral Libraries, Perfected Spectra and Quantitative NMR

69. ^{1}H NMR-based Metabonomics for Rapid Diagnosis of Meningitis and Ventriculitis

70. Rotamer Dynamics of an Aryl Bicycloazahydantoin

71. Metabonomics and Systems Biology: a Role for NMR in Biomedicine and Insulin Resistance Research

72. Unsymmetrical Covariance Processing of COSY or TOCSY and HSQC NMR Data to Obtain the Equivalent of HSQC-COSY and HSQC-TOCSY Spectra

73. ^{31}P, ^{1}H, ^{15}N and DOSY NMR Studies of Alternative Drug Delivery Systems in a Membrane Environment

74. Determination by ^{13}C NMR of a Biosynthetic Labeling Pattern in the Presence of Scrambling

75. ^{119}Sn, ^{13}C and ^{1}H NMR Spectra of Organotin(IV) Compounds Containing Two and More Tin Atoms in a Molecule

76. Automated Structure Confirmation by NMR.

77. Structure Elucidation from the “Other Side”: How Well Can a Constitution Be Defined by NMR?

78. Mass Directed Purification of Metabolites – Tools to Get a Metabolite into a NMR Magnet

79. ^{1}H chemical shifts in NMR: Part 23. An Investigation of the Effect of Dimethyl Sulphoxide vs. Chloroform Solvent on ^{1}H Chemical Shifts

80. Constraints Associated with Pre-collection and Storage of Urine for ^{1}H NMR Metabonomic Analysis in the MolPAGE Project

81. HPLC Peak Purity Determination Using LC-SPE-cryoflowNMR

82. The Effect of Solvent Mixtures (Oblates and Prolates) upon the Spectral Resolution of α and β Glucose Pentaacetates

83. Quantitative ^{1}H-NMR Essays for Reaction Monitoring of c-GMP Pilot Plant Campaigns

84. The Importance of Spectral Processing in qHNMR Analysis

85. Estrogenic or Non-estrogenic? A qHNMR Study of Desmethylxanthohumol (DMX)

1 A ^{1}H and ^{195}Pt NMR Study of the Spontaneous vs Photo-induced cis to trans Isomerism of cis-[M(H^2L-S)$_2$Cl$_2$] and cis-[M(L^1-S,O)$_2$] Complexes (M= PtII or PdII)

Jean M. McKenzie[1], Yali Wang, Nocky M. Derix[1], and Klaus R. Koch[1]

1. NMR Laboratory, Department of Chemistry and Polymer Science, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa

Complexes of type cis-[M(H^2L-S)$_2$Cl$_2$] (M=PtII, H2L = N-alkyl-N'-benzoylthiourea) undergo spontaneous solvent dependent cis to trans isomerism at room temperature, the rate of which can conveniently be monitored by ^{1}H NMR. The extent of isomerisation to trans depends on the solvent polarity as reflected by their dielectric constants.[1] The empirical rate of isomerism suggests a 2nd order rate law dependent on the complex concentration in chloroform. By contrast, complexes of type cis-[M(L'-S,O)$_2$] (M= PtII or PdII, HL' = N,N-dialkyl-N'-acyl(aroyl)thiourea) only undergo a photoinduced cis to trans isomerisation when irradiated with light in the 320 to 570 nm range. The reverse (trans to cis) process is thermally controlled and it is found that the complex reverts fully to the cis form when the solution is placed in the dark.[2] As for the previous case, the solvent again plays a significant role in the extent of the isomerisation as well as the rate of the reverse thermal reaction which can be observed using ^{1}H NMR spectroscopy. We here present a high resolution ^{1}H and ^{195}Pt NMR study of these processes in order to elucidate the origin and nature of this interesting difference in the mechanisms of isomerisation.

1. K.R. Koch, Y. Wang, and A. Coetzee. J. Chem. Soc., Dalton Trans., 1999, 1013-1016.

2. D. Hanekom, J.M. McKenzie, N.M. Derix, and K.R. Koch. Chem. Commun., 2005, 767-769.

2 Assignment of E,Z Configurational Isomers of Pt(II) Complexes by Means of Triple Resonance $^{1}H/^{13}C/^{195}Pt$ PFG Correlation NMR Spectroscopy

Sibusiso Mtongana[1], Dimitris Argyropoulos[2], Eberhard Hoffmann[2] and Klaus R. Koch[1]

1. Department of Chemistry, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa
2. Varian Applications Laboratory, Deutschland GmbH, Alsfeder Street 3, Darmstadt, D-64289, Germany

Ligands of the type N,N-dialkyl-N'-acyl(aroyl)thioureas have been widely studied in view of their potential analytical and process chemistry of platinum group metals.[1] In a series of asymmetrically disubstituted ligands of the type N-alkyl-N-alkyl(aryl)-N'-2,2-dimethylpropanoylthiourea (HLn) the degree of double bond character observed for the thiourea (S)C-NRR' bond results in the formation of one or two possible configurational isomers of the ligand namely the E or the Z isomer, reflecting the relative orientation of the alkyl moiety with respect to the thiocarbonyl moiety. In solid state, it appears that the E orientation is favoured in most cases, while in solution in some cases both the E and the Z configurational isomers are evident from their ^{1}H and ^{13}C NMR spectra. Interestingly on coordination of HLn to platinum(II) result in cis-[Pt(Ln-S,O)$_2$] complexes which show three configurational isomers, the cis-[EE-Pt(Ln-S,O)$_2$] cis-[EZ-Pt(Ln-S,O)$_2$] and cis-[ZZ-Pt(Ln-S,O)$_2$] as seen clearly from the ^{195}Pt NMR spectra.[2] This phenomenon is also observed even for ligands which were seemingly confined in one stereochemistry. Moreover the relative amounts of the configurational isomers do appear to depend on the structure of the ligand in an interesting way. Here we show the unambiguous assignment of these E,Z configurational isomers by means of $^{1}H/^{13}C/^{195}Pt$ PFG correlation NMR spectroscopy and discuss factors affecting the observed distribution of isomers.

1. Klaus R. Koch. Coord. Chem. Rev. 2001, 216-217, 473
2. Dimitris Argyropoulos, Eberhard Hoffmann, Sibusiso Mtongana and Klaus R. Koch, Magn. Reson. Chem., 2003, 41, 102-106

3 Quantitative Assessment of Alkyl Chain Branching in Alcohol Based Surfactants By Nuclear Magnetic Resonance

J.P.M. van Duynhoven, A. Leika, Ph.C. van der Hoeven

Unilever R&D, Vlaardingen, The Netherlands

Surfactants with branched alkyl chains (‘hydrophobes’) have gained considerable interest within the detergent industry, since these can be used in formulations for laundry cleaning at a wide range of conditions. In contrast to the historically known heavily branched surfactants, these novel branches surfactants are less compromised by decreased biodegradability. These properties find their basis in the structural characteristics of the alkyl chain, like number, position and type of alkyl chain branches.

Our current understanding of structure-property relations, however, is hampered by the lack of generic methodology to obtain structural data on alkyl chain branching. A NMR approach was developed by which we can obtain a comprehensive set of quantitative alkyl chain branching parameters in alcohol based surfactants. The ^{13}C and ^{1}H NMR spins systems of branched alkyl chain species were assigned by means of 2D NMR techniques. These assignments allowed the quantitative assessment of these branched species by straightforward signal integration in ^{1}H and ^{13}C NMR spectra. The quantified NMR data can be used to understand both product performance and biodegradation behaviour of surfactants with branched hydrophobes.

4 Metabonomic Assessment of Vitamine C/E Intervention in Claudicants

Stefan A. Coolen[1], Clare A. Daykin[2], John P.M. van Duynhoven[1], Jolanda Mathot[1], Marc Scheltinga[4], Otto Stroosma[4], Huib Vader[4], Ferdi van Dorsten[1], Florian Wulfert[3], Marc R. Wijnen[5]

1. Unilever Food and Health Research Institute
2. The School of Pharmacy, University of Nottingham, Nottingham, UK
3. Division of Food Sciences, University of Nottingham, Nottingham, UK
4. Maxima Medical Centre, Veldhoven, The Netherlands
5. Medical Centre St. Radboud, Nijmegen, The Netherlands

Intermittent claudicatio has proven to be a good in vivo model for ischemia reperfusion (IR). For the assessment of IR damage, several biochemical (clinical) markers are known. These all suffer from disadvantages with respect to sensitivity or interference with other physiological events, hence we explored the use ^{1}H NMR based metabonomics. The study involved 14 claudicants and 3 healthy volunteers, who were monitored during exercise, before and after the vitamine C/E intervention, and a washout period. The effect of the vitamin C/E claudicants is more pronounced using metabolomics than with the more traditional markers. The main effect is a faster recovery from the exercise, as shown by a quicker return to pre-exercise levels. In healthy subjects, no significant effect could be observed. The mechanism for the beneficial effect of vitamine C/E in claudicants is probably multifactorial, but our data suggest that at least a change in oxidative metabolism is responsible.

5 Structural and Dynamic Behaviour of Ibuprofen, Flurbiprofen and Their Solid Dispersions with Eudragit Rl100 by Means of High and Low Resolution Solid State NMR

G. Mollica[1], M. Geppi[1], C.A. Veracini[1], R. Pignatello[2] and S. Guccione[2]

1. Dipartimento di Chimica e Chimica Industriale, Università di Pisa
2. Dipartimento di Scienze Farmaceutiche, Università di Catania

The formulation of oral controlled-release delivery systems formed by solid dispersions of non-steroidal anti-inflammatory drugs (NSAIDs) and suitable carriers represents a very important task in order to improve the pharmacokinetics and reduce drug side-effects. Solid State NMR has recently been recognized as one of the more useful techniques in order to characterize the morphology of drug solid formulation forms and to ultimately correlate it to the pharmacological properties of the system. To this aim, the two NSAIDs Ibuprofen and Flurbiprofen, in two different forms (acid and Na-salt), as well as physical mixtures and coevaporates of each form with the copolymer Eudragit RL100, used as a carrier, have been investigated through several solid state high and low resolution NMR techniques. A deep characterization of the pure drugs was performed in order to observe the modifications induced by the presence of the carrier and understand their nature: for Ibuprofen a detailed assignment of the different resonances in the ^{13}C spectra of both the two drug forms was carried out thanks to spectral editing and 2D-correlation techniques; ^{1}H chemical shift values could be obtained exploiting ^{1}H-^{13}C scalar couplings by using the 2D MAS-J-HMQC technique, while information on conformational properties was extracted from the 2D-LG-HETCOR experiment, which revealed to be useful also for the spectral assignment of the ^{13}C spectrum of Flurbiprofen. The two forms of both Ibuprofen and Flurbiprofen showed remarkable differences in their spectral behaviour, mainly due to their very different dynamics. In Ibuprofen this concerns the internal motions of the isobutyl and phenyl fragments within the crystal lattice, while for Flurbiprofen this behaviour is linked to the different global dynamic situation, as shown by their ^{13}C spectra. In order to estimate the degree of mixing between both the acidic and Na-salt form of the two drugs and Eudragit RL100 in their solid dispersions at the hundreds of Å level, the ^{1}H spin diffusion process was investigated by measuring proton T1's, either at 400 or 25 MHz. A comparison between both the relaxation behaviour and ^{1}H spectra of the two coevaporates and the corresponding physical mixtures suggests the presence of a more intimate mixing in the acidic forms of the two NSAIDs which is in agreement with the presence of significant interactions between drug and carrier also showed by drug-release measurements.

6 Structural Elucidation of Impurities in the Submicro- to Microgram Scale by LC-MS-SPE-NMR

Joan Malmstrøm and Henrik Olsen

Structural Chemistry, CMC Development, Novo Nordisk A/S, Måløv, Denmark

Recently, the performance of NMR instruments has been remarkably improved with respect to sensitivity and it is now possible to conduct NMR analyses on submicro- to microgram scale samples. However, a major obstacle in preparing good NMR samples is still removal of inevitable impuritites/contaminants, water and other nontarget compounds accompanying the purification processes of low level impurities. These contaminants generate many spurious and sometimes significant signals in the NMR spectrum. This ”noise” makes interpretatation of the spectra difficult and oftentimes impossible. One way of avoiding contaminants is by preparing NMR samples using preparative HPLC followed by LC-SPE-NMR. We have utilized this approach in order to structurally characterize a thermally labile low-level degradation product of a drug candidate. The degradation product was preliminary examined by LC-MS and based on these results it was not possible to deduce the exact placement of an additional hydroxy group. Hence ^{1}H-NMR spectroscopy was needed. Examination of the sample obtained from preparative HPLC by NMR revealed the region occuping the crucial resonances to be contaminated. Subsequent LC-SPE purification unfolded the region and made the place of hydroxylation evident.

7 A Spectroscopic and Computational Study of Stereochemistry in 2-Hydroxymutilin

Frederick G. Vogt, Thomas C. Potter, Grant P. Spoors, Qiaogong Su, and Douglas J. Minick

Analytical Sciences and Synthetic Chemistry, Chemical Development; GlaxoSmithKline P.L.C., 709 Swedeland Road, King of Prussia, PA 19406, USA
CASS, Discovery Research; GlaxoSmithKline P.L.C., 5 Moore Drive, Research Triangle Park, NC, 27709, USA

A computational and spectroscopic study of two stereoisomers of 2-hydroxymutilin is presented. A gas-phase structural model for each isomer was computationally optimized. Selective measurements of ^{1}H nuclear Overhauser effect build-up rates were used to validate these models. NMR chemical shifts and indirect spin-spin coupling constants and internuclear distances were predicted using density functional theory and compared with experimentally determined values. In addition, electronic and vibrational circular dichroism spectra of the two isomers are also predicted and measured. The agreement between predicted and observed chemical shifts is excellent, with an average absolute error of 0.1 ppm for ^{1}H shifts and 2.7 ppm for ^{13}C shifts. Homonuclear ^{1}H and heteronuclear ^{1}H-^{13}C coupling constants were predicted and compared to their experimental values (as determined by J-resolved and gradient J-HMBC experiments). The coupling constants were predicted to within 10% of their experimental values in most cases. The circular dichroism spectra were also successfully simulated using the NMR structural models. Vibrational circular dichroism was found to be particularly well-suited to determinations of the absolute stereochemistry of mutilin derivatives. It is shown that computational prediction of NMR parameters can enable a full interpretation of the spectral data and lead to a reliable assessment of the relative stereochemistry of the isomers. The clear distinctions in the chemical shift and J-coupling analysis allows for the potential identification of these isomers (or their derivatives) from ^{1}H spectra obtained via LC-NMR.

8 Identification of Halogenated Degradation Products of Carvedilol by LC-MS and LC-NMR

Frederick G. Vogt, Lingling L. Rogers, Paul G. Cummings, Sonya Kennedy-Gabb, and Lew B. Killmer

Chemical and Pharmaceutical Development
GlaxoSmithKline Research and Development
P.O. Box 1539, King of Prussia, PA, 19406, USA

The drug formulation process can sometimes contribute to the chemical instability of an active pharmaceutical ingredient. Identification of the degradation products in drug formulations is critical for determining toxicity and improving the stability of the parent drug candidate. Most degradation products can be identified using LC-MS; however, structures assigned by MS can be ambiguous if multiple isomers are present in a sample. In these situations, LC-NMR analysis can often result in a more complete structural identification. This is demonstrated here for several degradation products of carvedilol. Forced degradation studies with hydrochloric acid at elevated temperatures resulted in a substantial conversion of drug substance into chlorinated derivatives after several months. The structures of these chlorinated degradation products were definitively determined by LC-MS (including MS-MS fragmentation analysis) and LC-NMR. Traditional solvent-suppression methods were used to obtain ^{1}H LC-NMR spectra, which were supplemented with connectivity information from ^{1}H homonuclear decoupling experiments. Using the chlorinated derivatives as a reference, LC-MS and LC-NMR were then used to identify low-level (<1%) brominated degradation products in partially formulated stability samples of carvedilol hydrobromide.

9 The Analysis of Formulated Drug Products with Solid-state NMR: Examples of Sensitivity and Resolution in Practice

Frederick G. Vogt

GlaxoSmithKline plc.
709 Swedeland Rd.
King of Prussia, PA 19406

The need for analysis of drug form in formulated product is growing. Solid-state NMR is one of the best options for this type of analysis, especially during the drug development process. The spectral resolution and information content of NMR spectra allow for a univariate analysis, which can be accomplished with a limited number of samples and is highly desireable in early development. Solid-state NMR can analyze intact or punched tablets without crushing or grinding. Topical gels and capsule contents (including beads) can be studied without any sample preparation. The detection limits and resolution of ^{13}C, ^{19}F and ^{31}P solid-state NMR are examined. ^{13}C solid-state NMR can detect drug loads as low as 1% w/w and can exclude undesired polymorphs to 0.3% w/w in favorable cases. Quantitative analysis can be performed, and excipients can also be studied. In certain cases, the sensitive ^{19}F and ^{31}P nuclei can also be utilized for faster or more sensitive analyses, and several recent applications are given. Finally, although not generally applicable, spin labeling can be used to enhance the detection limits of NMR when specific information is needed. An example is shown in which the salt form of a drug is determined within a liposome formulation at <1% w/w drug load.

10 Lipid Induced Conformation of the Tachykinin Neuropeptides Scyliorhinin I and Uperolein

Anjali Dike, Indu R. Chandrashekar, Anil K. Mantha, N.Z Baquer and Sudha M. Cowsik

School of Life Sciences, Jawaharlal Nehru University, New Delhi -110 067, INDIA

The mammalian tachykinin (TK) peptides and their three Neurokinin (NK1, NK2 and NK3) receptors represent an effector system with wide-ranging actions on neuronal, airway smooth muscle, mucosal, endothelial, immune, inflammatory and remodeling cell function. Recent clinical and preclinical data suggests pathophysiological relevance for TKs in various diseases including asthma, emesis and depression. The promiscuous tachykinin -NK receptor interactions and incompletely overlapping functions mediated by each NK receptor may indicate added therapeutic benefit of using multiple NK receptor blockade. Scyliorhinin I, a linear decapeptide, is the only known tachykinin that shows high affinity for both NK1 and NK2 binding sites and hence is a promising tool in tachykinin receptor family. Similarly Uperolein, a physalamine-like endecapeptide, is the potent NK1 receptor agonist. As a first step to understand the structure-activity relationship of these two novel peptides, we have investigated their membrane-induced conformation using techniques of circular dichroism and two-dimensional NMR spectroscopy. Sequence specific resonance assignments have been made from correlation spectroscopy (TOCSY, DQF-COSY) and NOESY and the family of structures have been calculated using Torsion Angle dynamics algorithm DYANA. The results show that both Scyliorhinin I and Uperolein exists in random coil state in aqueous environment where as helical conformation is induced towards the C-terminal region in lipid environment. Analysis of NMR data is suggestive of the presence of 310-helix that is in equilibrium with an a helix from residues 4-10 in Scyliorhinin I. Overall fold of Uperolein can be defined predominantly as 310 helix from residue 5-11 preceded by turn at N-terminus, although a small population of a-helix towards C-terminus cannot be excluded from the solution ensemble. An attempt has been made to correlate the observed conformational differences to the binding ability and biological activity of various Neurokinin receptor agonists.

11 Quality Control of Compound Libraries by 1mm HT-NMR and LC-UV-MS

Götz Schlotterbeck, Alfred Ross, Josef Schneider, Stephan Müller, Eva-Maria Gutknecht and Hans Senn

F. Hoffmann- La Roche, Basel, Switzerland

The miniaturizing of the NMR measurement with discrete 1mm capillaries provides many advantages. First, the intrinsic higher sensitivity of the miniaturized detection coil leads to a decrease of the experiment time and thus to a higher sample throughput of mass-limited samples. In addition, spectral artifacts from solvent impurities are significantly reduced. Also, the handling and logistics of large sample arrays is simplified: Discrete miniaturized NMR tubes can easily be placed in a 96 well plate format. This enables automatized liquid handling and sample preparation by robots.

Here, we demonstrate the potential of the 1mm TXI probe for quality control of large compound libraries. A concept for the parallel acquisition of High-Throughput-NMR (HT-NMR) and LC/UV/MS data out of one and the same sample is presented. Only 8µl of sample at a concentration of 5mM in protonated DMSO is used for both analytical techniques. Compounds, provided in 384 well plate format were transferred into 1mm NMR capillaries by the aid of a modified Gilson 215 robot and directly used for analysis. Besides providing information about structural identity and purity by LC/UV/MS and NMR, the actual compound concentration is determined by NMR spectroscopy.

The combination of NMR with LC/UV/MS data surveyed for the same sample provides a gold standard for quality, purity and concentration of samples stored in large compound collections. Thus, a reliable quality control of compound libraries and depositories is now possible by application of two complementing technologies with one and the same sample. This is of great importance for the analytical validation of screening hits in pharmaceutical industry.

12 Quantitative NMR in Pharmaceutical Development

Joan Malmstrøm and Henrik Olsen

Structural Chemistry, CMC development, Novo Nordisk A/S, Maaloev, Denmark

Determination of relative quantities in e.g. pharmaceutical development samples can be performed using proton NMR as the analytical tool. Several acquisition parameters must be set according to the sample of interest in order for the method to be quantitative. One important parameter is waiting time between subsequents pulses. A waiting time of five times the longest T1 value gives a 99.3% recovery of magnetisation. With optimized parameters the accuracy of the method is normally within a few percent. In pharmaceutical development quantitative NMR determination can be applied to a range of different aspects. In this presentation examples will be given on three cases, namely quantitation of low level impurities, solvent residues and impurity libraries. The quantitation of low level impurities and solvent residues are performed using the ^{13}C satellite of a well resolved resonance resulting in comparison of similar size integrals. The use of tangent baseline for more accurate integral measurements will be illustrated along with considerations of where the integral should start and finish. The third case illustrates the use of internal standard quantitation of a degradation product library. The library is composed of nine different compounds. The challenge in this case is to find an internal standard not interfering with the resonances of the nine compounds of interest. With the quantitative information in addition to the information collected during purification (MS, UV, Rt), the degradation products can be considered as well defined entities in the impurity library and furthermore; be used as standard material in e.g. the validation procedure providing retention times and response factors.

13 Conformational Analysis of JNJ16175328-AAA (R207910), a New Drug Candidate for the Treatment of Tuberculosis, by a Combined NMR and Molecular Modeling Approach

Sandrine Gaurrand, Stéphanie Desjardins, Christophe Meyer, Jean Michel Argoullon and Jérôme Guillemont

Discovery Research, J&J PRD-France

R207910 emerges as the most promising compound of a new series of antimycobacterial compounds. Although its mode of interaction with its target, ATPsynthase, is still unknown, it appears that stereoselectivity of this purely enantiomeric compound is crucial for high-level biological activity. In order to get more insight on the conformational behaviour of R207910 in solution along with preliminary information about its absolute configuration, we have undertaken a combined NMR and molecular modelling study. A conformational analysis using a pseudo Monte-Carlo method has been performed on each enantiomer of this compound leading to the identification of four low energy minima. Determination of internuclear distances has been achieved by means of ROESY NMR experiments followed by volume quantification of the correlations peaks using Aurelia from Bruker, Inc. These values were then compared to the theoretical distances obtained for the low energy minima. The remarkable match between experimental and theoretical data shows that R207910 adopts one of the low energy conformations predicted by molecular modelling and belongs to the (RS,SR) couple of diastereoisomers. X-ray crystallography experiments definitely validate our results. It is worth noting that only the synergetic combination of the two techniques could lead to the expected goal while taking each approach separately wouldn’t have allowed us to conclude.

14 Multiple-Sample Probe for High-Throughput Solid-State NMR Spectroscopy of Pharmaceutical Solids

Loren J. Schieber[1], Benjamin N. Nelson[1], Dewey H. Barich[1], Joseph W. Lubach[1], Thomas J. Offerdahl[1], David H. Lewis[2], John P. Heinrich[2] and Eric J. Munson[1]

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

Solid-state NMR spectroscopy (SSNMR) is the most powerful technique for the analysis of drug compounds and pharmaceutical formulations because of the vast quantity of information that can be obtained from NMR experiments. In addition to pharmaceutical formulations, SSNMR can also be used to quantitate different solid forms within a 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 (SNR), which could take hours to days depending on sample sensitivity and desired SNR.

The two-module prototype has been constructed which has independent RF lines, RF circuits and tuning, airlines and angle adjustment for magic-angle spinning. The probe can simultaneously run two identical samples and reduce the length of an acquisition without sacrificing SNR, or the probe can be set up to run different samples thereby increasing throughput by a factor of two.

Spectra of aspirin and ibuprofen were acquired using this probe. The spectrum of aspirin acquired with sample shuttling was identical to the spectrum of aspirin acquired without shuttling. A spectrum of ibuprofen was acquired at the same time as a spectrum of aspirin was acquired. This prototype is limited to being a two-module probe due to large amounts of space required for the tuning elements located next to the MAS modules. A new probe design incorporating three-quarter wave (¾λ) coaxial transmission lines and smaller MAS modules is being developed. Smaller spinning modules and the incorporation of the ¾λ design will help alleviate the space restrictions within the bore of the magnet. The number of modules is only limited by the number of transmission lines in a cross sectional diameter of the bore and the axial field length of the magnet. Each spinning system will be exposed to a magnetic field of >80% at all times. This design allows for close proximity of the MAS modules (within 3 cm), adequate proton decoupling power (> 50 kHz), and is capable of remote tuning and sample changing. Spectra of hexamethyl benzene (HMB) have been acquired and show SNR comparable to existing SSNMR probes. This probe design can be scaled to incorporate more than 2 MAS modules, which is a limitation of the previous design.

15 Solid-State NMR Studies on the Effects of Processing of Pharmaceutical Formulations With Implications on Drug Stability

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

The University of Kansas, Department of Pharmaceutical Chemistry

Acetylsalicylic acid (aspirin) was subjected to various formulation and processing conditions, and the effects upon CPMAS NMR spectra and relaxation dynamics were investigated. Bulk crystalline aspirin has a proton spin-lattice relaxation time (^{1}H T1) of 40 s, while cryogrinding the bulk material reduced the ^{1}H T1 without producing any significant changes in the ^{13}C CPMAS NMR spectrum. The resulting aspirin was still crystalline. The reduction in relaxation times upon grinding has also been observed with two forms of crystalline lactose. The physical stresses incurred during processing serve to reduce particle size, create crystal defects, and create amorphous material. These high-energy domains can all serve to facilitate spin diffusion and reduce the observed ^{1}H T1. Highly mobile sites are much more prone to chemical degradation, so using NMR to detect these mobile domains could potentially be used to predict formulation stability.

Co-dispersed amorphous aspirin can be produced by lyophilization in the presence of an excipient, such as HPMC. Amorphous aspirin was shown to degrade significantly under conditions where crystalline aspirin does not appreciably degrade (70°C, 50% RH). The degradation product is amorphous salicylic acid, and NMR studies show that the degradation of a minor component in a formulation can easily be followed using solid-state NMR without having to alter the sample prior to or during analysis.

A previously undetected aspirin polymorph may be produced by lyophilizing pure aspirin. The lyophilized form exhibits a second peak for the methyl carbon, and a well-defined shoulder on the aromatic peak ortho to the acetyl group. The observed line widths were narrower in lyophilized samples, and the ^{1}H T1 was as low as 8 s. Data supporting a new form of aspirin has also been acquired using differential scanning calorimetry (DSC).

Processing steps such as grinding, milling, and compaction can dramatically affect the stability of pharmaceutical dosage forms. For example, grinding of aspirin results in faster degradation rates relative to unprocessed aspirin. The effects of processing on relaxation dynamics, particularly ^{1}H T1, may provide insight into the long-term stability of formulations. In theory, formulation conditions that result in reduced T1 times will be most unstable, and this information could be used to make decisions on which experimental formulations to advance further into development.

16 ^{1}H and Diffusion NMR study of the Capping of InP Colloidal Semiconductor Nanocrystals by TOPO

José C. Martins[1], Iwan Moreels[2] and Zeger Hens[2]

1. NMR and Structure Analysis Unit, Universiteit Gent, Krijgslaan 281, S4, and
2. Physical Chemistry Laboratory, Universiteit Gent, Krijgslaan 281, S12, B-9000 Gent, Belgium

We have used trioctylphosphine oxide (TOPO) capped InP colloidal nanocrystals (Q-InP|TOPO) to explore the potential of solution ^{1}H NMR in studying in situ the capping and capping exchange of sterically stabilized colloidal nanocrystals. The Q-InP|TOPO spectrum shows resonances of free TOPO, superimposed on broadened spectral features. The latter are assigned to TOPO adsorbed at Q-InP by means of pulsed field gradient diffusion NMR and ^{1}H-{^{13}C} HSQC spectroscopy.

The diffusion coefficient of Q-InP|TOPO nanocrystals is inferred from the adsorbed TOPO's NMRsignal decay. The corresponding hydrodynamic diameter correlates well with the Q-InP diameter. Using the resolved methyl resonance of adsorbed TOPO, the packing density of TOPO at the InP surface can be estimated. Spectral hole burning is used to demonstrate that the adsorbed TOPO resonances are heterogeneously broadened.

Exchange of the TOPO capping by pyridine is demonstrated by the disappearance of the adsorbed TOPO resonances and the appearance of pyridine resonances in the ^{1}H NMR spectrum. Form the resulting pyridine spectrum, an upper value of the first order rate constant of pyridine desorption can be obtained.

These results show that solution NMR should be considered a powerful technique for the in situ study of the capping of sterically stabilized colloidal nanocrystals.

17 Isolation and Identification of Elburzensoside A1/A2 from Allium Elburzense

Behzad Zolfaghari, Virginia Lanzotti, S. Ebrahim Sajjadi, Elisa Barile.

Department of Pharmacognosy, Isfahan University of Medical Sciences, Hezar Jerib Avenue, 73461 Isfahan, Iran

Introduction: A phytochemical investigation of the bulbs of Allium elburzense Wendelbo has been undertaken, leading to the isolation of Elburzensoside A1/A2.This plant is endemic in Iran. Leaves and bulbs of this plant are used as food, due to its sweet taste, typical of the plant species belonging to the onion species. In Iranian folk medicine it has also been used as an antirheumatic, aphrodisiac, antiduretic, and anthelminthic herb

Methods: The plant materials were air dried, powdered and extracted with hexane, $CHCl_3$, $CHCl_3$–MeOH (9:1), and MeOH. The MeOH extracts were partitioned between butanol and water phases. The $CHCl_3$–MeOH (9:1) and butanolic extracts were chromatographed by CC or MPLC on a RP18 Silica gel, using a linear gradient solvent system from H_2O to MeOH as mobile phases. Interested fractions with saponins contents were selected, base on preliminary ^1H NMR analysis. The selected fractions were purified by preparative HPLC with the suitable mobile phases (mixture of MeOH–H_2O). ^1H NMR, ^{13}C NMR, 2D NMR, FABMS and IR, techniques were used to elucidate of isolated compounds.

Results: Elburzensoside A1 (E1a) was isolated as an amorphous solid in high yield with a molecular formula of $C_{39}H_{66}O_{17}$, and on the basis of spectroscopic analysis, mainly 2D NMR and mass spectrometry, and chemical methods, the structures of the new compounds were determined as furost-2α,3β,5α,6β,22α-pentol 3-O-β-D-glucopyranosyl 26-O-β-D-glucopyranoside (E1a), furost-2α,3β,5α,6β,22α-pentol 3-O-[β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl] 26-O-β-D-glucopyranoside (E2a).

Conclusion: It seems that high concentration of new saponins in the edible investigated plant indicates that further pharmacological and SAR (structure activity relationship) studies are needed. Additionally phytochemical investigations of other Allium species growing in Iran is recommended.

18 Going Cold in Metabonomics

Lora Robosky[1], Daina Avizonis[2], Christopher Robosky[1], and Michael Reily[1]

1. Pfizer Global Research and Development, Ann Arbor, MI
2. Varian Instruments, Palo Alto, CA

Endogenous metabolic profiling, also known as metabonomics or metabolomics, is an emerging field of relevance to pharmaceutical research. Changes in many endogenous metabolites are measured using a variety of analytical techniques, primarily proton NMR spectroscopy and more recently mass spectrometry, in single or closely coupled experiments. In many cases, information available from proton NMR spectroscopy is limited by the sensitivity achieved by conventional room temperature coil NMR probes. Cold probes have been shown to provide up to a 4-fold improvement in sensitivity for samples dissolved in organic solvents. This benefit decreases in high-salt, aqueous samples which is the common situation encountered for biofluid samples analyzed in metabonomics. This presentation will focus on evaluating the performance of Varian's triple-resonance cold probe for metabonomics applications.

19 NMR Characterization of Novel Products of Lipid Peroxidation as Etheno-2'-Deoxyguanosine Adducts from Dioxododecenoic Acid and 5(S)-Hydroperoxyeicosatetraenoic Acid

Maria Victoria Silva Elipe*[2], Seon Hwa Lee[1], Wenying Jian[1], Jasbir S. Arora[1], and Ian A. Blair[1]

1. Center for Cancer Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6160, USA
2. Analytical Chemistry Department, Amgen, One Amgen Center Drive, Thousand Oaks, California 91320, USA

Oxidative damage to proteins and DNA is thought to be an important mediator of carcinogenesis and other age-related diseases. Such damage can occur through a direct interaction with reactive oxygen species or by reaction with bifunctional electrophiles that are generated as a result of lipid peroxidation. Lipid hydroperoxydes from polyunsaturated fatty acids (PUFAs) undergo homolytic decomposition into bifunctional electrophiles, which react with DNA bases to form DNA adducts. We reasoned that the identification of the adducts would provide a more complete understanding of the spectrum of DNA damage generated by the lipid peroxidation cascade. Here we present two studies on etheno-2'-deoxyguanosine adduct formation with dioxododecenoic acid (DODE) and 5(S)-hydroperoxy-6,8,11,14-(E,Z,Z,Z)-eicosatetraenoic acid (5(S)-HPETE). Our first study[1] was undertaken to establish the regioselectivity of DODE-mediated etheno adduct formation and to develop a convenient route for its preparation. Here we present the structure of carboxynonanone-etheno-dGuo formed from vitamin C-mediated 13(S)-HPODE decomposition by a combination of ^{1}H and ^{13}C NMR spectroscopy studies of its bis-methylated derivative. Our second study[2] was undertaken to establish the 2'-deoxyguanosine (dGuo) adducts produced from the reaction between 5(S)-HPETE and dGuo in the presence of transition metal ions (FeII or CuI) or vitamin C to be characterized by LC/MS and NMR analysis. The structure of the most abundant adduct (III) was characterized as its methyl ester derivative by ^{1}H and ^{13}C NMR spectroscopy.

1. Lee, S.H.; Silva Elipe, M.V.; Arora, J.S.; Blair, I.A. Chem. Res. Toxicol. 2005, 18, 566-578.
2. Jian, W.; Lee, S.H.; Arora, J.S.; Silva Elipe, M.V.; Blair, I.A. Chem. Res. Toxicol. 2005, 18, 599-610.

20 Accessing Analytical Data in an Electronic Lab Notebook Workplace

Nichola L. Davies, Chris L. Lapthorn, Frank Pullen, George L. Perkins, Torren M. Peakman, Giles Ratcliffe.

SASS, Pfizer Global R&D, Sandwich, Kent, CT13 9NJ.

There is an increasing move in the pharmaceutical industry towards the use of electronic lab notebooks to simplify compound registration, searching, filing and records management. Pfizer has long realised the importance of bringing analytical data into this process and has embarked on the GAINS (Global Analytical Information Source) project. This project aims to maximise the value of the discovery process by ensuring that all analytical data is electronically captured, immutably stored, and linked to compound registration. The data will be readily available on-line in common formats, and shared globally throughout Pfizer, transforming productivity. Hence, GAINS provides data capture technology for analytical chemistry, and a platform for rapid access of spectra and chromatograms that is fully integrated with electronic lab notebooks. For compatibility of our open access Varian NMR spectrometers with GAINS, changes have been made to key VNMR 6.1c macros. In this poster we provide an overview of the GAINS project and how we have incorporated NMR data.

21 Use of ^{15}N NMR for the Identification of a Process Impurity Arising from a Rare Pyridine to Pyrrole Conversion

Charles Pathirana[1], Rajesh Shukla[1], John Castoro[1], Liam Byrne[2], Gaelle Pennarun-Thomas[2], Venkatapuram Palaniswamy[1]

1. Isolation & Structure Elucidation,Analytical R&D, Bristol-Myers Squibb, New Brunswick, NJ 08903
2. Technical Operations, Bristol-Myers Squibb, Swords, Ireland

NMR, both online and off-line, is a very effective tool in the identification of impurities in drug development. Complete idenfication is carried out by the application of homo- and heteronuclear correlation experiments. ^{1}H-^{15}N correlations can be extremely helpful in the identification of pharmaceuticals and related compounds which are generally nitrogen rich molecules. ^{1}H-^{15}N correlation experiments find more appliations nowadays in structure elucidation due to the enhancement of the sensitivity provided by CryoProbes. However, observation of these correlations pose a common challenge in the identification of impurities because of the low abundance of the impurities and low sensitivity of the detection of natural abundance ^{15}N. Application of ^{1}H-^{15}N correlations to the identification of an impurity that arises via a rare pyridine to pyrrole conversion will be discussed.

22 Use of Genetic Algorithms with NOESY and ROESY Data to Predict the Stereochemistry and 3D Configurations of Small Molecules

Kirill A. Blinov, Yegor D. Smurnyy, Mikhail E. Elyashberg and Brent A. Lefebvre

Advanced Chemistry Development, Toronto, Ontario, Canada

Presented here is a genetic algorithm based method to predict relative stereochemistry and 3D configurations of small molecules (100-1000 Da) based on NOESY and ROESY data. This tool is ideally suited to natural products and can help decrease our dependence on expensive X-Ray Crystallography.

The solution can be found in a global optimization of the resulting root mean square deviation (RMSD) between dreal and dNOE is calculated. Here dreal represents the through space distance obtained from molecular mechanics, and dNOE is estimated from NOESY or ROESY peak intensities.

The RMSD can be minimized in numerous ways. Experience shows that for small molecules (100-300 Da, 3-6 stereocenters) a straightforward approach over all stereoisomers (8-64 variants) is acceptable. For more complicated cases an implementation of a genetic algorithm was necessary. The genetic algorithm is a stochastic algorithm, which mimics natural evolution. A possible solution is presented by a vector in which key features of a solution (stereocenter configurations) are encoded. A pool (10-30 items) of such vectors is then a subject for crossover and random mutation. The crossover consists of splitting two “ancestors” into parts and assembling “offspring” from these parts. The goal is to reach as diverse regions as possible of the solution space to avoid being trapped in a local minimum. Mutation represents random alternating of a position in a solution. After these procedures, the offspring with the worst RMSD values are eliminated to maintain the pool size. Thus, the new generation is formed.

Experience shows that this simple scheme can be applied to molecules with 8-12 stereo-centers. However, it fails for more complex structures; as the solutions pool degenerates. A number of special procedures have been created for these complex systems which allowed the right answer to be achieved for a natural product as complicated as brevetoxin. Upon numerous tests, the correct answer is found after only 1000-1500 trials, (contrast that to 8 million possible stereoisomers!).

Details of the special procedures that were necessary to make the most complicated systems solvable will be provided. Examples demonstrating the algorithm’s efficiency for solving the stereochemistry and 3D configurations of structures are presented here.

23 Dereplication and Elucidation of the Natural Products Staurosporine and Aselacin C Using NMR Software Methods

Mark O'Neil-Johnson[1], Ryan Sasaki[2], Kirill Blinov[2], Arvin Moser[2] and Brent Lefebvre[2]

1. Sequoia Sciences, San Diego, CA
2. Advanced Chemistry Development, Toronto, ON, CA

When working with natural product spectra, it is important to confirm that the structure that is being pursued after showing activity is not one that has been previously characterized and studied. This process is called Dereplication.

Based upon initial spectral characterization of a compound, a substructure-based or spectral data query (NMR, MS, IR, Raman) through a database can be performed to compare and identify known structures. This process provides a tremendous reduction in the amount of work required when performing elucidation of natural products. With the help of the database search, a tremendous amount of work can be eliminated by identifying previously characterized structures and allowing the analyst to move on.

In this study, the experimental NMR data (1D and 2D Experiments) of a series of natural products were fed into a CASE (Computer Assisted Structure Elucidation) system to identify first if their data corresponded with matches of known compounds in our library (of ^{1}H and ^{13}C NMR data), and second, to elucidate the structure from the supplied spectral data if necessary.

The steps followed in the process of dereplication of these structures will be shown in detail. The elucidation results will also be supplied for structures that could not be located in the database.

24 A Testset for Validation of Carbon-NMR Prediction Using Stereochemical Information

Rashmi Mistry[1], Mike Wainwright[1], Jeff Seymour[1] and Wolfgang Robien[2]

1. Modgraph Consultants Ltd, 1 Oakland View, Oaklands, Welwyn, AL6 0RJ, UK
www.modgraph.co.uk
2. University of Vienna, Department of Chemistry

The prediction of carbon-NMR shift values is a decisive step during structure verification and structure elucidation. Among the methods available are the HOSE-code approach, which is a well-established technique and exists in different implementations in various software products, and neural network technology.

The approximate range for carbon chemical shiftvalues covers some 250ppm, stereochemical effects may reach up to 30 ppm corresponding to more than 10% of the chemical shift range. High-quality results can only be obtained by taking into account stereochemical information. On the poster a series of examples of stereoisomers will be given, showing the tremendous improvement in the quality of the prediction when stereochemical information is used during HOSE-Code and neural network prediction. Some additional advantages of neural network based predictions will also be discussed. Apart from specific examples, a statistical analysis derived from 300K well assigned ^{13}C-NMR spectra will be given in order to show the importance of the correct handling of stereochemical information on a more general basis.

25 Selective Excitation of Overlapping Peaks: Comparison of 1D TOCSY and NOE Experiments

Sara J Duncan and Richard J Lewis

Physical and Metabolic Science, AstraZeneca, Charnwood, UK

The ability to separate severely overlapping peaks so that separate TOCSY and NOE spectra can be obtained for the different components, could be very useful for analysing mixtures. It should be much simpler to analyse a few non-overlapped 1D spectra than a complicated 2D spectrum with many overlapping crosspeaks from multiple components of the mixture. Here we have investigated 3 pulse sequences for acquiring 1D TOCSY and NOE spectra, and have compared the standard selective experiments with sequences enabling separation of overlapping peaks.

For the TOCSY experiments, the 3 experiments investigated were the standard selective 1D TOCSY (90° e-burp), ZQF-TOCSY (incorporates a novel zero quantum filter in which zero quantum coherence can be supressed in a single scan), and CSSF-TOCSY (chemical shift selection filter, which also includes these ZQF pulses). Firstly, the effect of incorporating the ZQFs is demonstrated, showing the vast improvement in spectral quality obtained. Secondly, the ability to separately select two completely overlapping peaks to obtain two separate TOCSY spectra, by using the CSSF sequence is shown.

For the NOE experiments, DPFGSE-NOE, STEP-NOE (Selective Tocsy Edited Preparation) and CSSF-NOE experiments were investigated. Both of the latter two sequences enable separate NOE spectra from severely overlapping peaks to be obtained. The STEP sequence involves using the above ZQF-TOCSY sequence to obtain a 1D TOCSY spectrum of the desired spin system, from which a peak can be selected for a subsequent NOE experiment. The CSSF sequence can be used to select the overlapped peak directly from the ^{1}H spectrum, just as for the CSSF-TOCSY.

Factors such as ease of use, applicability to real samples, and sensitivity have been considered when comparing these experiments.

1. M. J. Thrippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941
2. H. Hu, S. Bradley, K. Krishnamurthy, J. Mag. Reson. 171 (2004) 201-206
3. P. Robinson, T. Pham, D. Uhrin, J. Mag. Reson. 170 (2004) 97-103

26 Automatic Confirmation of Chemical Structure Employing Both ^{1}H And ^{13}C Spectra

Lee Griffiths and Rob Horton

AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK.

An existing method of comparing predicted and experimental chemical shifts was used to confirm or refute postulated structures.[1] The results obtained by employing ^{1}H spectra alone returned all true positives with a false positive rate of 4%. These are the same as those obtained previously, but subsequent experience has shown that for "real-life" situations, e.g. DMSO solvent, flow probe and finite impurities, this represents an over-estimate of the selectivity routinely achievable where the error rate can be as high as four times this. However, these $CDCl_3$ solution / tube spectra can be used to judge the relative impact of various expedients employed to increase the selectivity further. When an analogous procedure was adopted for ^{13}C, the false positive rate dropped four-fold to 1%. Despite the ^{13}C data not including quantitative information, a halving of prediction error (relative to chemical shift range), produces a four-fold improvement in selectivity. Unfortunately, even with the extra sensitivity offered by cryo-probes, direct ^{13}C detection would either compromise throughput unacceptably, or sample consumption would be equally unacceptable. However, the more practical HSQC experiment, which also restricts the number of ^{13}C nuclei observable, yielded a false positive rate of 2%, still twice as good as ^{1}H. When the HSQC results were combined with ^{1}H results, a false positive rate of 1% resulted, four times more accurate than ^{1}H alone. It is worth noting that even at today's computing speeds, which are apparently set to rise inexorably, once the spectra are acquired and the predictions performed, the computation takes considerably less than one second per structure / spectrum combination.

1. Griffiths L & Bright JD. Magn. Reson. Chem., 2002; 40: 623.

27 About How One Molecule of N,N',N"-Triacetylchitotriose Can Be Sandwiched Between Two Molecules of Elderberry Lectin

Camelia Mihai[1], Lieven Buts[2], Lode Wyns[2], Willy J. Peumans[3], Els J. M. Van Damme[4], José C. Martins[5] and Rudolph Willem[1].

1. HNMR Centre and POSC, Vrije Universiteit Brussel, Belgium
2. Dienst Ultrastructuur, VIB, Vrije Universiteit Brussel, Belgium
3. Laboratory for Phytopathology and Plant Protection, Katholieke Universiteit Leuven, Belgium;
4. Vakgroep Moleculaire Biotechnologie Universiteit Gent, Belgium
5. Eenheid NMR en Structuuranalyse, Universiteit Gent, Belgium

The overall 3D structure of elderberry lectin (EL), a 44 amino acid chitin binding lectin, is quite similar to that of hevein. The carbohydrate binding behaviour of EL was investigated using N,N',N"-triacetylchitotriose (NAG3) as model ligand. A total of 84 individual 1D ^{1}H NMR measurements at 6 individual temperatures and 14 different NAG3:EL concentration ratios were recorded and used to extract Ka values. The binding kinetics is intermediate on the NMR time scale at room temperature and evolves to fast exchange conditions as temperature increases. The chitin-binding site (W23, F25, Y32, and S21) is similar to other hevein domains, but EL contains four additional aromatic residues (W3, H15, F22, Y30). The interaction enthalpy and entropy were determined from van't Hoff analysis of the Ka and independently from microcalorimetry. ITC reveals a 2:1 EL:NAG3 binding stoichiometry, unexpected for a single hevein domain binding NAG3. The NMR data could be fitted to a 2:1 model and the association remained in the millimolar range. In this context, we propose the formation of a 2:1 complex in a fashion that resembles that observed in the UDA:NAG3 or pokeweed lectin:NAG3 crystals. Also the possible involvement of an additional amino acid residue (W3) into the binding is discussed. More recent DOSY experiments bring more insight about the unexpected behaviour of EL in the presence of NAG3.

28 The Influence of Tetrahydrofuran on Reactivity, Aggregation, and Aggregate Structure of Dimethylcuprates in Diethyl Ether

Wolfram Henze[1], Armand Vyater[2], Norbert Krause[2], Ruth M. Gschwind[3],

1. Kekulé-Institut für Organische Chemie und Biochemie, Rheinische Friedrichs-Wilhelms-Universität Bonn, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany;
2. Organic Chemistry II, Dortmund University, Otto-Hahn-Str. 6, D-44221 Dortmund, Germany;
3. Institut für Organische Chemie, Universität Regensburg, Universitätsstr. 31, D-93053 Regensburg, Germany

The comprehension of factors influencing the reactivity of organocuprates is still far from enabling a rational control of their reactions. Especially the degree of aggregation and structures of organocuprates are in the focus of the discussion about the factors affecting their reactivity. Therefore, this study combines kinetic measurements and NMR investigations to elucidate the influence of disaggregation via addition of THF on the reactivity and aggregate structure of Gilman cuprates. As model systems Me_2CuLi*LiI (1*LiI) and Me_2CuLi*LiCN (1*LiCN) in DEE were chosen, as model reaction the 1,4-addition to 4,4-dimethylcyclohex-2-enone. The kinetic data show for 1*LiI a pronounced acceleration effect upon addition of distinct amounts of THF, whereas the reactivity of 1*LiCN continuously decreases with the addition of THF. Series of NMR diffusion measurements, ^{1}H, ^{7}Li HOESY-, and ^{1}H, ^{1}H NOESY spectra show different structural influences of THF on 1*LiI and 1*LiCN. For 1*LiI small salt units are separated from the cuprate aggregate by THF. In contrast for 1*LiCN, THF disaggregates the oligomeric structures, while the core structures remain intact with salt attached. Thus, the reactivity of 1*LiI seems to be fine-tuned through distinct amounts of salt or THF, whereas the decreasing reactivity of 1*LiCN correlates with the disaggregation of oligomers via THF. Additional to these structure-reactivity studies, the cyanogroup is shown to be directly attached to the cuprate moiety via a combination of ^{1}H, ^{13}C HOE- and ^{1}H, ^{1}H NOEs. This represents the first experimental evidence for the position of the cyanogroup relative to the cuprate moiety in cyano-Gilman cuprates.

29 Diffusion Study of the Precatalysts Between Phosphoramidite Ligands and Copper Salts

Hongxia Zhang and Ruth M. Gschwind

Institut für Organische Chemie, Universität Regensburg, Universitätsstr. 31, D-93053, Regensburg, Germany

The reaction of organometallic reagents under copper catalysis conditions is one of the most important methods for the asymmetric construction of carbon-carbon bonds. The breakthrough for the copper-catalyzed enantioselective conjugate addition reactions of organozinc reagents was achieved through the introduction of phosphoramidites as chiral ligands. Despite the synthetic importance of this promising catalytic process, very little is known about the structural details and degree of aggregation of the catalytic species in solution. Thus, the origin of the strong ligand acceleration and the excellent stereo-control is still a black box. Pulsed field gradient (PFG) diffusion NMR experiments offer a powerful tool to investigate the hydrodynamic radii and the degree of aggregation of highly symmetric organometallic reagents in solution. Therefore, in this study, the structures of the precatalysts composed of phosphoramidite ligands and copper salts were elucidated via PFG diffusion measurements combined with results of element analyses. As model systems, we chose two phosphoramidite ligands, binaphthol-based ligand LA and biphenol-based ligand LB, as well as three copper salts CuI, CuCl, CuTC (copper(I) thiophene-2-carboxylate), which are famous synthetic reagents introduced by the groups of Alexakis and Feringa. We also chose a modest active ligand LC because its crystal structure ($CuI[LC]_3$) is one of the two crystal structures reported so far, the second shows a dimeric structure ($[CuBrL2]_2$). As experimental conditions, 1:1 and 2:1 ligand-to-copper ratios, a temperature range of 210 K-270 K and a concentration range of 0.1 M-0.01 M in $CDCl_3$, D8-Toluene, D8-THF and CD_2Cl_2 were chosen.

The results show that the kinds of copper salt and solvent, but not the concentration and the temperature, influence the precatalyst species of LA and LB and mostly several species coexist. Only in chloroform, exclusively two species were observed for all systems. One of these species could be assigned to a one-coordinated but aggregated complex $[CuXL]_n$ (n=2-3.5, depending on the concentration and the temperature), the other species was assigned to the monomeric three-coordinated complex ($CuXL_3$). Surprisingly, the structure of the complex composed of 3 equiv. Lc and 1 equiv. CuI in $CDCl_3$ was found to be a dimer $[CuIL_3]_2$, deviating from the monomeric structure $CuIL_3$ found in the solid state. Additionally, no two-coordinated complex $[CuXL_2]_n$ was observed in solution as expected.

To our knowledge, this is the first time that the structures of precatalysts of copper phosphoramidite-catalyzed conjugate addition reactions were elucidated in solution.

30 Transferred-NOESY NMR Experiments on Living Cells: RGD Derivatives Bound Platelet Integrin aIIbb3

Donatella Potenza[1], Silvia Mari[1] and Silvia Parapini[2]

1. Dipartimento di Chimica Organica e Industriale and Centro Interdisciplinare Studi bio-molecolari e applicazioni Industriali (CISI), Università degli Studi di Milano, via G. Venezian 21, I-20133 Milano, Italy
2. Istituto di Microbiologia, Università degli Studi di Milano, via Pascal 36, I-20133 Milano, Italy

The interactions of small peptides with biological membranes is central to a number of biological processes. In contrast to soluble proteins there is comparatively less information available about ligand-receptor interactions that occur at membrane surfaces. The biophysical environment of a membrane is considerably different from the isotropic extracellular medium. It is therefore desirable to investigate membrane proteins and their binding specificity directly in living cells.The platelet integrin aIIbb3 is the most abundant platelet cell surface glycoprotein; this calcium-dependent heterodimer plays a key role in platelet adhesion. An important integrin ligand is the ubiquitous tripeptide sequence Arg-Gly-Asp (RGD)[1] a common amino acid motif found in a number of adhesive proteins. This ligand inhibits the adhesion of platelets to protein-coated surfaces as well as inhibits platelet/platelet aggregation by binding to the platelet integrin receptor aIIbb3.In our previous studies we investigated on conformational preferences and activity of a small library of cyclic RGD pentapeptide mimics incorporating stereoisomeric 5,6- and 5,7-fused bicyclic lactams.[2] This library was found to contain high-affinity ligands for the avb3 integrin and a good affinity for platelet aIIbb3 integrin.Now, we show that the interactions between intact human platelet and these RGD-containing pseudociclopeptide inhibitors can be studied by transferred nuclear Overhauser enhancement spectroscopy (tr-NOESY).[3] Moreover, this NMR technique allowed to deduce informations on the structural requirements of the ligand in the bound state. Conformational properties of the free and bound pentapeptides are also reported.

1. E. Rouslahti, M. D. Pierschbacher. Science (1987) 238, 491-497
2. L. Belvisi, A. Bernardi, A. Checchia, L. Manzoni, D. Potenza, C. Scolastico* M.Castorina, A. Cupelli, G. Giannini, P. Carminati, C. Pisano. Organic Letters, (2001), 1001-1004
3. B. Meyer, T. Weimar, T. Peters. Eur. J. Biochem. (1997) 246, 705-709

31 H2BC: Heteronuclear 2-Bond Correlation

Nils Nyberg, Bent Petersen, Jens Ø. Duus, Ole W. Sørensen

Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

H2BC[1] is a new heteronuclear 2D NMR technique that almost exclusively correlates proton and carbon spins separated by two covalent bonds. It solves the problem of distinguishing two- and three-bond correlations in HMBC and it is independent of the heteronuclear two-bond coupling constants. Hence correlations that are missing in HMBC because of vanishing two-bond coupling constants are routinely observed in H2BC. Another asset of H2BC compared to HMBC is that the pulse sequence is significantly shorter and hence less sensitive to transverse relaxation. Finally, H2BC spectra can be phased to pure absorption.

As H2BC and Broadband HMBC[2] spectra are quite complementary and H2BC shows no correlations to quaternary carbons, it is recommended to perform both experiments as part of a standard small-molecule protocol.

Clear identification of two-bond correlations makes it possible to perform INADEQUATE-type walks from HSQC and H2BC spectra together and thereby determine the carbon skeleton of the molecule.

The poster will illustrate the merits of H2BC.

1. J. Am. Chem. Soc. 127, 6154-6155 (2005).
2. Magn. Reson. Chem. 38, 981-984 (2000), J. Magn. Reson. 156, 282-284 (2002), http://www.crc.dk/nmr/.

32 Structural Implications of a Pyrene - Modified Nucleotide on a Duplex DNA

Massimiliano Tittarelli, Linda Valis, Peter Kaden, Elke Mayer, Hans-Achim Wagenknecht, Burkhard Luy

Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, D-85747, Garching, Germany.

Modified bases are frequently used in modern biochemical approaches to study properties of nucleic acids and their complexes. However, very little is known about the structural changes of the DNA/RNA duplex formation caused by the artificial nucleoside. 5-(Pyren-1-yl)-2'-Deoxyuridine (Py-dU) is a fluorescent base analogue that is used to characterize electron transfer in DNA.[1] Py-dU was incorporated into a short duplex DNA oligomer with the goal to investigate structural perturbations of the canonical DNA-stacking by NMR spectroscopy. An overview of the work in progress will be shown.

1. H.-A. Wagenknecht, Angew. Chem. Int. Ed. 2003, 42, 2454-2460

33 Defining the Influence of Peroxisome Proliferator Activated Receptors on the Metabolome of the Mouse

Helen J. Atherton[1], Kieran Clarke[2], Nigel J. Bailey[3] and Julian L. Griffin[1]

1. Department of Biochemistry, University Of Cambridge, UK.
2. University Laboratory of Physiology, University of Oxford, UK.
3. Scynexis Europe Ltd., Fyfield Business and Research Park, Essex, UK.

Understanding gene function and expression is a major focus of modern research and has led to the emergence of several functional genomic techniques. The comprehensive analysis of metabolites via a combination of ^{1}H-NMR, GC-MS and multivariate pattern recognition is one such method, termed metabolomics. In this study a metabolomics approach was used to profile mouse models of Metabolic Syndrome, a disease characterised by severe insulin resistance. This abnormality in insulin regulation is often seen in conjunction with atherogenic risk factors including obesity, dyslipidaemia, (low HDL cholesterol, high triglycerides), hyperisulinemia, type 2 diabetes mellitus, and hypertension. The coexistence of several of these symptoms constitutes the Metabolic Syndrome. In some individuals and animal models the disease is caused by mutations in the genes encoding Peroxisome Proliferator Activated Receptors (PPARs), a group of nuclear receptors which regulate many of the enzymes involved in lipid metabolism. To date three isoforms have been identified; PPAR-alpha, PPAR-beta (also sometimes called PPAR-delta), and PPAR-gamma. In this study, a PPAR-alpha null mutant has been used to study the Metabolic Syndrome, and more fully define the metabolic changes that accompany a disruption of one of the PPARs. Aqueous tissue extracts were used in the analyses and, additionally intact tissue samples were analysed using high resolution ^{1}H-NMR.

We have shown PPAR-alpha null mice can be distinguished from control mice at a range of time points between 1 and 13 months. Lipid moieties were found to be increased in the liver of older PPAR-alpha null mice indicating a degree of fatty acid infiltration or accumulation with time as measured by HR MAS spectroscopy. This indicates a perturbation in the beta-oxidation of free fatty acids (FFAs). Accompanying this was a decrease in choline. Choline has been shown to maintain the function and integrity of the liver and its deficiency, as observed here, may be amplifying the fatty acid accumulation. Both ^{1}H-NMR and GC-MS identified further changes in proline and glutamine concentrations in the PPAR-alpha null liver which are consistent with a disruption to energy metabolism and, in particular, to the TCA cycle. Similar observations were made in the heart, diaphragm and skeletal muscle. Differential levels of amino acids, lactate, citrate and glucose were observed in these tissue types which are again consistent with a disruption to energy metabolism.

In conclusion the metabolic changes allowing this distinction between the PPAR-alpha null, and the control mice correspond to time-related abnormalities in glycolysis and gluconeogenesis, and severe impairment of fatty acid metabolism.

34 Metabolic Profiling of Biofluid Samples from Type 2 Diabetes Mellitus Patients Using NMR Spectroscopy

Reza M. Salek[1], Brian C. Sweatman[2], Susan C. Connor[2], Mark P Hodson[2], John N. Haselden[2] & Julian L. Griffin[1]

1. Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1GA, UK. 2. Safety Assessment, GlaxoSmithKline, Park Road, Ware, Herts, SG12 ODP, UK.

Type 2 diabetes mellitus (T2DM) is the result of a combination of impaired insulin secretion and reduced sensitivity of target tissues to insulin action. It is among the most prevalent conditions in the world, with an estimated 100 million sufferers, of whom only 50%, at best, are diagnosed due to lack of specific symptoms early on in this disorder and inadequate screening diagnostics. T2DM is associated with both microvascular (neuropathy, nephropathy, and retinopathy) and macrovascular (atherosclerotic-related vascular disease, coronary heart disease, and peripheral vascular disease) complications. Diabetes can lead to pathological concentrations of several metabolites in plasma and, consequently, may lead to their urinary overflow. Thus, a metabolomic study of urine samples from diabetics is of huge potential and at the same time, very complicated due to the numerous factors involved.

In order to study differences between T2DM patients and healthy subjects, 84 urine samples from healthy volunteers, (56 males and 28 female), were compared to samples from 50 T2DM patients (23 male and 27 female).

Multivariate analysis of urine NMR profiles obtained from T2DM patients revealed three clusters in the PCA model. The majority of patients form one cluster with a second cluster separating due to increased urine glucose concentration above the mean concentration for all patients, and a third separating cluster due to increased hippurate and phenylacetylglycine (PAG) concentration. Two outliers within the group of diabetic patients were identified in our models, which occurred possibly due to inborn errors of metabolism.

PCA and PLS-DA models (with and without the glucose containing region; considering males and females separately) of the NMR urine profile from T2DM patients compared to healthy subjects showed a large number of metabolites contributed to the separation of the models. These included phenylalanine, tryptophan/tryptamine, n-butyrate, dimethylamine (DMA), n-acetylaspartate (NAA), creatinine, amino-hippurate, hippurate, PAG, histidine and ß-hydroxybutyrate. In total ~60 chemical shift regions were responsible for the robust classification of diabetic patients according to the VIP list. Comparison of our human data set with that from available animal models of diabetes (T2DM) such as leptin receptor–deficient (db/db) mice, insulin resistant Zucker fatty (fa/fa) and lean (fa/-) rats will also shed further light on the metabolic profile from these T2DM patients.

35 Metabolomic Studies of Brain Tissues from a Potential Model for Parkinson Disease

Reza M. Salek[1], Rebecca Colebrooke[2], Patrick Lynch2, M.T Herrero[3], John N. Haselden[4], Piers C. Emson[2] & Julian L. Griffin[1]

1. Department of Biochemistry, University of Cambridge, Cambridge CB2 1GA, UK.
2. The Babraham Institute, Neurobiology Programme, Babraham, Cambridge, UK.
3. University of Murcia,Spain
4. Safety Assessment, GlaxoSmithKline, Park Road, Ware, Herts, SG12 ODP, UK.

The vesicular monoamine transporter 2 (VMAT2) plays a pivotal role in regulating vesicle size and packaging monoamine neurotransmitters into vesicles after they have been synthesized. Transgenic mice (KA1) have been generated with a dramatically reduced (~95%) expression of the VMAT2 gene which, unlike complete knockout lines, survives into adulthood. Abnormalities in the function of these monoamine transmitters are suggested to play a key role in the aetiology of Parkinson disease (PD). In this study we have compared metabolic profiles in the KA1 transgenic mouse to perturbation induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity, an established model of PD.

Tissue was taken from the cerebellum, cortex, hippocampus, and striatum tissues from control and KA1 homozygote adult mice. ~20mg brain tissues were studied using high resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy. Further tissue samples (~50mg) were extracted using a chloroform /methanol extraction method before analysis of the aqueous layer in solution using ^{1}H NMR spectroscopy. To cross correlate with a known model of PD, brain tissues from MPTP treated male mice were also compared with a control group using the above methods. Partial Least Squares-Discriminate Analysis (PLS-DA) of the NMR spectrum for the KA1 mice, separated homozygote mice from control tissue in all four cerebral regions when analysed individually. To identify which metabolites were responsible for this separation the variable influence on projection parameter (VIP) for the PLS-DA model was investigated. The analysis indicated a decrease in concentration of N-acetyl aspartate (NAA) and an increase in myo-inositol for all brain tissues, apart for cerebellum, for the KA1 mice. γ-Amino-*n*-butyrate (GABA) was increased in all brain tissues of the mutant mice, with exception of the cortex. Glutamate was increased in KA1 mice tissue relative to control tissue, except in the cerebellum, while glutamine was decreased in the striatum and cerebellum. In mice treated for 48h with MPTP, analysis of striatum tissue showed increased concentrations of lactate, Glutamate/Glutamine, and aspartate, while reduction in the levels of GABA, scyllo/myo–inositol, choline and phosphorylcholine, creatine and NAA were observed compared to the control. The reduction in concentration of NAA, combined with an increase in glutamate appears to be a common metabolic fingerprint for these mouse models of PD.

36 Stabilization of Small Molecular Clusters in Solution - Liquid State NMR Down to 100 K

I.G. Shenderovich[1,2], A.A. Gurinov[1], N.S. Golubev[2], G.S. Denisov[2], H.-H. Limbach[1]

1. Institut für Chemie, Freie Universität Berlin, Germany
2. Institute of Physics, St. Petersburg State University, Russian Federation

A special NMR technique using low-freezing $CDF_3/CDClF_2$ freon mixtures as solvents make it possible to obtain high resolution spectra of hydrogen bonded complexes in the temperature range between 95 - 150 K in the slow proton and hydrogen bond exchange regime. Under these conditions, the lifetimes of strong hydrogen bonds are longer than 0.1 s. Thus, different complexes give rise to separate NMR signals with the relative intensities corresponding to the equilibrium composition of a mixture.

Using this technique, various hydrogen bonded species have been detected and described in detail in several acid-base systems, among other HF with F-, HF with 2,4,6-trimethyl-pyridine-^{15}N (collidine) and a number of homoconjugated ions.

At the slow proton and hydrogen bond exchange regime both one-bond couplings between a hydrogen bond proton and the two heavy atoms of a hydrogen bridge, here proton-fluorine and proton-nitrogen, as well as a two-bond coupling between the heavy atoms, here fluorine-fluorine and fluorine-nitrogen, are resolved. The one-bond proton-fluorine coupling constant is positive if the H...F distance is shorter than ca. 0.13 nm but becomes negative for longer distances. As a result, for some species the two-bond coupling is stronger than the one-bond one.

Symmetry of the studied A•••H•••B hydrogen bond bridges has been inspected using the H/D isotope substitution. Whereas the primary isotope chemical shift effect, the chemical shift difference between the bounding deuteron and proton, is negative for an asymmetric hydrogen bond, it is positive for the symmetric one.

A strong increase of the static dielectric constants from 14 at 190 K to 38 at 103 K is observed for the $CDF_3/CDClF_2$ freon mixtures used in the NMR measurements. This fact leads to a very noticeable effect, namely, upon cooling, i.e. increase of the dielectric constant, the NMR spectra of 1:1 collidine-HF complex indicate a gradual transformation of an asymmetric molecular complex F-H•••N to a quasi-symmetric complex and eventually to a more or less zwitterionic species F-•••H-N+.

37 The Design of a Multi-Dimensional LC-SPE-NMR System (LC2-SPE-NMR) for Complex Mixture Analysis

F.Xu, A. J. Alexander and C. Bernard

Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492

In this presentation we describe the design of an online multi-chromatographic approach to the routine NMR analyses of low level components (~ 0.1%) in complex mixtures. The technique, termed LC2-SPE-NMR, optimally combines automated multi-dimensional liquid chromatography with SPE technology for isolating, enriching and delivering trace analytes to the NMR probe. The LC2-SPE-NMR system allows for maximal loading capacity (in the first, preparative LC dimension), close to optimal peak resolution (in the second, analytical LC dimension), and enhanced sample concentration (through SPE). Using this system it is feasible to conveniently conduct a wide range of NMR experiments on, for example, drug impurities at the low micrograms/ml level, even for components poorly resolved in the first dimension. Such a sensitivity gain significantly elevates the analytical power of online NMR technology in terms of the level at which substances of pharmaceutical significance can be structurally characterized.

38 Fast Determination of Water Content of Compound Collections (DMSO Solutions) by NMR Spectroscopy

Lee Fielding and Dan Fletcher

Akzo-Nobel Pharma Division, Organon Laboratories Ltd., Newhouse, Lanarkshire, UK. ML1 5SH

One of the most important sources of new drugs is high throughput screening of large compound collections. To this end, pharmaceuticals companies maintain collections of upwards of 1,000,000 compounds which are stored as DMSO solutions. This format is convenient for dispensing to screening batteries as required. The integrity and stability of these DMSO solutions is an important issue for the management of these collections. It is known that DMSO readily adsorbs water vapour from the atmosphere, and there is a growing awareness that water ingress into the DMSO solutions can cause the compounds to precipitate, or to decompose. Hence there is a need for a fast and efficient method to monitor the water content of DMSO solutions. This poster describes a single scan ^{1}H NMR method which can determine the water content of DMSO. The method is designed for high throughput analysis of large compound collections and uses only 15 µL of analyte.

39 ^{1}H and ^{13}C NMR Spectral Assignment of Dehydrocholic Acid and Related Compounds

Stefano Chimichi[1], Marco Boccalini[1], and Giancarlo Cravotto[2]

1. Dipartimento di Chimica Organica, Università di Firenze, via della Lastruccia 13, I-50019, Sesto F.no (Firenze), Italy
2. Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, via Giuria 9, I-10125 Torino, Italy

During the past years various papers on ^{1}H and ^{13}C NMR of bile acids have been reported in the literature but many discrepancies appeared. Moreover, ^{1}H NMR data are often incomplete.

Following our research interest in the structural elucidation of the biotransformation products of dehydrocholic acid (DHCA), we noticed the lack of NMR data on this semisynthetic bile acid largely employed for medicinal purposes.

We report here on the assignment of ^{1}H and ^{13}C NMR spectra of DHCA in two solvents ($CDCl_3$, pyridine-d5) together with the data of its regio- and diastereoselective reduction product 7,12-diketolithocholic acid, obtained by the action of the Basidiomycete Trametes hirsuta. Literature data on other bile acids will be re-examined with the help of mono- (DEPT, NOESY-1D) and two-dimensional gradient-enhanced NMR techniques (HSQC, HMBC, COSY, DQF-COSY).

40 Dereplication of Natural Products using HPLC-DAD-SPE-NMR

M. Lambert, C. Clarkson, K. Sprogøe, D. Stærk and J. W. Jaroszewski

Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Extracts of natural origin usually contain a range of chemically diverse constituents occurring in varying concentrations. This poses a number of analytical challenges for the rapid identification of constituents of these inherently complex mixtures. The development of hyphenated techniques, particularly HPLC-NMR, has greatly increased the analytical capabilities in natural product research. The recent introduction of on-line solid phase extraction (SPE) in HPLC-NMR has further enhanced the sensitivity of this technique by enabling multiple peak trapping and concentration of the eluted analytes in a highly sensitive, small-volume NMR flow-cell. This improves the S/N ratios in the NMR spectra and facilitates the acquisition of ID and 2D NMR data, necessary for structure elucidation.

The potential of HPLC-DAD-SPE-NMR hyphenation is demonstrated by structure determination of a range of complex constituents from various plant extracts. The technique is shown to allow acquisition of high-quality 1D and 2D NMR data following analytical-scale HPLC separation of crude plant extracts, thereby enabling rigorous structure determination of known as well as previously unknown constituents. Examples include dereplication of extracts of Perovskia atriplicifolia, Kanahia laniflora, Smirnowia iranica, Harpagophytum procumbens and Hubertia ambavilla. It is believed that the HPLC-DAD-SPE-NMR technique will be an increasingly important analytical platform in natural product research and in other areas where rapid structural analysis of complex mixture constituents is required.

41 Metabolomic Studies of a Diabetic Mouse Model by ^{1}H NMR Spectroscopy of Urine

Mahon L. Maguire[1], Michael Cheeseman[2], Tertius Hough[2], Elizabeth Bentley[2], Roger Cox[2], Julian L. Griffin[1]

1. Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1GA, UK
2. MRC Mammalian Genetics Unit, Medical Research Council, Harwell, Oxfordshire OX11 0RD, UK

The db gene encodes a leptin receptor that is involved in the control of satiety in response to fatty acid. The homozygous knockout results in an obese phenotype and serves as a model for type 2 diabetes in humans. This study describes the effects of the db/db knockout on the metabolic profile of the mouse using solution state NMR to examine metabolite profiles in urine. Spectra for 143 mouse urine samples were recorded covering wild type, heterozygous and homozygous db knockout mice over a variety of ages ranging from 8-24 weeks. The normalized spectra were analyzed using unsupervised (principle components analysis, PCA) and supervised (partial least squares - discriminant analysis, PLS-DA) techniques (SIMCA, Umetrics). While high protein concentration was a problem in many of the urine samples, the aliphatic region (<4.24 ppm) was dominated by peaks of sufficient intensity that these errors were proportionately less significant permitting meaningful statistical analysis/pattern recognition. All analyses were therefore carried out on the aliphatic region of the spectra. Spectral regions corresponding to glucose resonances were also excluded from further analyses.

The homozygous db knockout mice formed a separate and distinct cluster from the control group in both PCA and PLS-DA models. This cluster was, however, more diffuse primarily due to baseline correction artifacts. In both PCA and PLS-DA analyses the disease phenotype correlated with an increase in β-hydroxybutyrate concentration in homozygous mice, possibly reflecting a shift in the balance of metabolic flux between fatty acid and carbohydrate metabolism. The predictive capability of the PLS-DA models was high with all samples being accurately classified according to wild type or disease phenotype using a train and test routine for each individual sample.

42 Deuterium Isotope Effects in the Study of Intramolecularly Hydrogen-bonded Thioxo Compounds in the Liquid and Solid State

Poul Erik Hansen, Fritz Duus, Bjarke V. Hansen and Trung Thanh Nguyen

Department of Life Sciences and Chemisty, Roskilde University, P.O.Box 260, DK-4000 Roskilde, Denmark e-mail:poulerik@ruc.dk

Hydrogen bonding to thiocarbonyl groups has been a controversial subject and discussed in relation to β-thioxoketones. In order to shed more light on this problem isotope effects are studied and a number of new and suitable ortho-hydroxythiocarbonyl compounds such as 2-hydroxythioacetophenone etc. are synthesized. The aim of the paper is to show how tailored synthesis and spectroscopic results may support each other.

In general, effects obtained for C=O and the corresponding C=S compounds will be scrutinized in order to elucidate the difference between oxygen and sulphur as hydrogen bond acceptor. These compounds can in principle exist both on a "fixed" form or as tautomeric equilibria. Deuterium isotope effects are studied both by IR and ^{13}C NMR. The latter in both the liquid and the solid state. The deuterium isotope effects at ^{13}C chemical shifts show very large magnitudes.[1,2] Deuterium isotope effect in the solid state have so far only been sparingly investigated.

The results will be discussed in relation to DFT calculations of structures, IR frequencies, chemical shifts and isotope effects on the latter.

The hydrogen bond patterns as elucidated by deuterium isotope effects on ^{13}C chemical shifts are discussed in relation to the reactivity and stability of these relative unstable compounds.

Support from DANIDA is greatly appreciated.

1. P. E. Hansen, F. Duus and P. Schmitt, Deuterium Isotope Effects on ^{13}C Nuclear Shielding as a Measure of Tautomeric Equilibria. Org. Magn. Reson., 18 (1982) 58-61.
2. B. Andresen, F. Duus, S. Bolvig and P. E. Hansen, Variable Temperature ^{1}H and ^{13}C NMR Spectroscopic Investigation of the Enol-Enethiol Tautomerism of β-Thioxoketones. Isotope Effects due to Deuteron Chelation. J.Mol.Struct. 552 (2000) 45-63.

43 Identification of Interesting and Novel Metabolites of CP-533,536 by LC-NMR and LC/MS

Thomas N. O'Connell, Kim A. Johnson and Chandra Prakash

Pfizer Global Research and Development, Groton Laboratories, Pfizer Inc, Groton CT

CP-533,536 is a selective EP2-receptor agonist that is being evaluated as a potential therapeutic to enhance bone healing. This poster describes the use of LC-NMR and LC/MS to elucidate the structure of several CP-533,536 metabolites from human recombinant cytochrome P450 isoforms. The tert-butyl moiety was determined to be a "hot –spot" for multiple oxidations and de-methylation. In particular, two unusual metabolites that resulted from C-demethylation of the tert-butyl group were identified and a mechanism proposed based on de-formylation of the corresponding aldehyde rather than de-carboxylation of the corresponding acid. LC-NMR and LC/MS were both critical techniques for structure elucidation and hence the understanding of metabolic pathways.

44 Effect of Age and Caloric Restriction on Plasma Metabolome and Skeletal Muscle Transcriptome

Doris M. Jacobs, Sylvie Pridmore-Merten, Andreas Fuerholz, Ziad Ramadan, Marie C. Zwahlen, Martin Grigorov and Sunil Kochhar

Nestlé Research Center , Nestec Ltd P.O. Box 44, CH-1000 Lausanne 26 (Switzerland)

Caloric restriction (CR) is a severe nutritional intervention delaying the aging process. We have assessed the effect of short-term CR initiated at different ages in life of C57BL/6J mice. CR mice received daily 67% of the caloric intake of the matched control group fed ad libitum a semi-synthetic diet. The CR diet was administered over 3 months (short-term) starting at the age of 3, 12 and 21 months, labeled in this study as young, adult and old, respectively. For comparison, a long-term CR was initiated at 3 months of age and lasted 21 months.

^{1}H NMR profiles of plasma have been acquired using a Bruker 1mm TXI probe. We find that the metabolite changes in plasma depend upon age of initiation and on dietary intervention. Short-term CR induces significant metabolite changes in young and adult mice but not in old mice. Only long-term CR provokes changes on the amino acid levels in old mice.

The comparison of gene expression profiles in skeletal muscle (gastrocnemius) exhibits changes affected by age and by short- and long-term CR. Long-term CR and short-term CR initiated before midlife induce most significant changes. The gene set activated by CR only partially overlaps with an identified age-related gene set.

45 Determination of Degradation Pathways and Kinetics of Acyl Glucuronides by NMR Spectroscopy

Gregory S. Walker[1], James Atherton[1], Jonathan Bauman[1], Christopher Kohl[2], Wing Lam[1], Michael Reily[3] and Abdul Mutlib[1]

1. Pfizer, Pharmacokinetics, Dynamics and Metabolism, Ann Arbor, MI 48015, U.S.A.
2. Pfizer, Pharmacokinetics, Dynamics and Metabolism, Sandwich, Kent, CT13 9NJ, U.K.
3. Pfizer, Molecular Science and Technology, Ann Arbor, MI 48015, U.S.A.

Acyl glucuronides (AG) have been implicated in the toxicity of many marketed drugs. These toxicities are hypothesized to be a consequence of covalent binding of the reactive forms of the AG to proteins. These reactive intermediates of the AG arise from the migration of the O-1-acyl glucuronide to other positional and stereoisomers under physiological conditions. In order to screen for the potential liabilities of these compounds during the early phase of pharmaceutical development, an NMR assay has been developed based on the disappearance of the anomeric resonance of the O-1-acyl glucuronide. The AG metabolites were either isolated from rat bile/urine or were obtained synthetically. The degradation kinetics of several AGs were examined using a set of chemically diverse drug compounds as substrates. When the half-lives determined by NMR were compared to those reported in the literature a correlation of r=0.99 was observed. The NMR analysis also enabled us to distinguish between two possible pathways of degradation; acyl migration and hydrolytic cleavage. The ^{1}H resonances characteristic of acyl migration are quite distinguishable from those that arise from chemical hydrolysis. This technique could be used to rank order discovery compounds (those containing carboxylic acid groups) based on their ability to form AG's and their subsequent fate under physiological conditions.

46 A Markup Language for the Description of an NMR Metabolic Experiment

Denis V. Rubtsov, Kevin M. Brindle and Julian L. Griffin

Department of Biochemistry, The Sanger Building, University of Cambridge
80 Tennis Court Road, Cambridge, CB2 1GA, UK
Tel. +44 (0)1223 766042, Fax +44 (0)1223 766002

There is a strong case for unification and standardization of the data representation in metabolomics and other functional genomics areas for both academia and industry. In answer to this demand several initiatives have emerged, including MIAME (Minimum Information About a Microarray Experiment) for transcriptomics[1], Proteomics Standards Initiative[2], SMRS (Standardization of Reporting Methods for Metabolic Analyses)[3] and ArMet (Architecture for Metabolomics).[4] However, the situation is less developed for NMR-based metabolomics.

We offer an open standardized language based on XML (extensible markup language) for capturing all the relevant information about experiment workflow, results and analysis. At the moment this language is developing as a part of an initiative for the development of An Open Standard for Reporting Metabolic Data within the context of a wider collaboration with the European Bioinformatics Institute, Imperial College London, University of Wales, University of Manchester, University of Birmingham, GlaxoSmithKline Inc. and others.[5] It is intended to serve for NMR-based experiment description and will be used along with languages for mass spectrometry based metabolomics and other techniques.

A UML (unified modeling language) object model has been built that is the basis for the NMR markup language. The model determines the scope of the information to be captured and the level of descriptive detail. The core experiment we have been keeping in mind during the development of the model is the 1D ^{1}H NMR study of urine and blood plasma within a metabolomics /metabonomics framework.
The model allows capturing of information about NMR sample, hardware and software used in the measurement, resulting dataset and post-processing up to metabolite assignment.
This work was funded by a BBSRC (UK) grant.

1. Minimum information about a microarray experiment, http://www.mged.org/Workgroups/MIAME/miame.html
2. HUPO Proteomics Standards Initiative, http://psidev.sourceforge.net/
3. Standardisation of Reporting Methods for Metabolic Analyses, http://www.smrsgroup.org
4. Architecture for Metabolomics, www.armet.org
5. The Standard Metabolic Reporting Structure: An Open Standard for Reporting Metabolic Data, http://smrsgroup.sourceforge.net/

47 Stereochemical Identification of Ibuprofen Using Chiral Liquid Crystals, NMR and Modeling

Vasilios M. Marathias* and Alvin C. Bach II

Wyeth Research
Discovery Analytical Chemistry
Princeton NJ USA

The stereochemistry of Ibuprofen, a small drug molecule containing a single chiral center, has been resolved by using residual dipolar couplings or RDCs and molecular modeling methods. Experimental ^{1}H-^{1}H, ^{1}H-^{13}C and ^{13}C-^{13}C RDCs were obtained in PBLG / $CDCl_3$ orientation media using single and multi dimensional NMR methods. Back-calculated RDCs, obtained form a range of minimized structures, were compared to the experimental RDCs. Good agreement between calculated and experimental RDCs was found when an averaged minimized structure of ibuprofen was used to back-calculate the RDCs. This method will further be developed for small drug-like molecules that are either crystallographycally or Mosher's Method inaccessible.

48 Two- and Three-way Chemometric Methods Applied for Characterisation of Complex Pharmaceutical Preparations: St. John's Wort (Hypericum perforatum L.)

Bonnie Rasmussen[1], Rasmus Bro[2], Dan Stærk[1] and Jerzy W. Jaroszewski[1]

1. Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark.
2. Chemometrics Group, Food Technology, Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

Extracts of St. John's Wort, sold as natural remedies for the treatment of mild to moderate depression, contain several different classes of compounds, but their standardisation is only based on the total content of hypericins. This means that the extracts can be highly variable with respect to other constituents that may contribute to the biological activity.

New methods that assess the content of all constituents in the extracts simultaneously would be beneficial to guarantee uniform quality of the extracts. Multivariate techniues in combination with spectroscopic and chromatographic methods offer an excellent approach to quality assessment of herbal preparations.

^{1}H NMR spectra and HPLC-DAD chromatograms of extracts from different commercial suppliers of St. John's Wort, recorded at 600 MHz, were used in this study. Warping has been used for the correction of peak shifts in the chromatographic data.
The two-way chemometric method, principal component analysis (PCA), has been applied to the ^{1}H NMR spectra and the three-way method, parallel factor analysis (PARAFAC), has been applied to the chromatographic data. The chemometric methods were used to study the main variation between the samples and to highlight possible similarities or differences between the preparations. HPLC-SPE-NMR has been used for the identification of the secondary metabolites responsible for the observed clustering.

This work shows that commercial extracts of St. John's Wort are highly variable with respect to compounds other than the hypericins. Because of the uncertainties regarding the compounds responsible for the pharmacological activity of St. John's Wort, quality assessment methods taking all compounds present in the extract into account must be regarded as highly valuable. This study indicates the potential value of NMR and HPLC-PDA in combination with different chemometric methods for the characterisation and possible future standardisation of complex pharmaceutical preparations.

49 Working with a ^{13}C-enhanced Cold Probe

Jörg Fitz, Walter Maier, Albert Wagner, and Thomas D. Weiß

BASF Aktiengesellschaft, Competence Center Analytics, Ludwigshafen / Germany

A few months ago the NMR group of the Competence Center Analytics of BASF Aktiengesellschaft in Ludwigshafen/Germany has equipped its Varian INOVA-600 magnet with a new ^{13}C-enhanced Cold Probe. The poster describes our first experiences with this new equipment in our laboratory especially with regard to: Sensitivity of ^{1}H and ^{13}C, Direct observation of ^{13}C with an inverse probe head, Rapidity during routine 2D homo and heteronuclear correlation spectra, Samples with small concentrations, and Special experiments.

50 Microcoil NMR on a Microfluidic Lab-on-a-Chip

Aldrik H. Velders[1], Henk Wensink[2], Fernando Benito-Lopez[1], Jacob Bart[2], Han J.G.E. Gardeniers[2] and David N. Reinhoudt[1]

1. Laboratory of Supramolecular Chemistry and Technology
2. BIOS, the Lab-on-a-Chip group
MESA+ Research Institute for Nanotechnology
University of Twente, P.O. Box 217, 7500 AE, Enschede, The Netherlands.
Fax: +31 53 4892988; Tel: +31 53 4894465
E-mail: a.h.velders@utwente.nl

A microfluidic chip with an integrated planar microcoil has been developed for NMR spectroscopy on samples with volumes of less than a microliter. In a proof-of-principle set-up, real-time monitoring of a Schiff-base formation in a microreactor chip by NMR is demonstrated. The reaction times in the chip can be set from 30 min down to ca. 2 s, the latter being the mixing time in the microfluidic chip. Design rules are described to optimize the microreactor and detection coil in order to deal with the inherent sensitivity of NMR and to minimize magnetic field inhomogeneities and obtain sufficient spectral resolution. The development and application of the NMR-chips for and at different magnetic field strengths is discussed.

51 NMR-based Metabolomics Analysis of Vascular Endothelial Cells During Stress-Induced Premature Senescence

Ferdi van Dorsten; Inge Koop; Elsa Regan-Klapisz; Ewoud van Velzen; Jan Andries Post and John van Duynhoven

1. Unilever Research and Development, Vlaardingen, the Netherlands,
2. Department of Cell Biology, Inst. Biomembranes, Utrecht University, the Netherlands.

Human umbilical vein endothelial cells (HUVECs) were exposed to sub-cytotoxic doses of hydrogen peroxide leading to stress-induced premature senescence (SIPS). There was a dose-dependent increase in the number of senescent cells, as quantified by senescence-associated beta-galactosidase (SA-bGal) expression. HUVEC metabolism was quenched with cold methanol and cells were extracted with a mixture of methanol/chloroform/water. Metabolic profiles of the aqueous cell extracts and of the culture medium were recorded using high-resolution ^{1}H NMR spectroscopy and senescence-related metabolic changes were assessed using multivariate data analysis. This NMR-based metabolomics analysis showed that the metabolic profile of senescent HUVECs was markedly different from that of control cells, and was consistent with a shift in energy metabolism during senescence away from anaerobic glycolysis possibly towards fatty acid oxidation and inhibition of ATP consuming anabolic pathways, including nucleotide biosynthesis. The composition of the culture medium mirrored to a large extent the metabolic alterations of the cells toward replicative senescence. A Partial Least Squares (PLS) model was built that was able to predict the %SA-bgal positive cells from the HUVEC metabolic profile. Metabolomics analysis of stress-induced premature senescence in HUVECs has potential as a screening tool to test the capacity of food ingredients to slow-down vascular ageing.

52 NMR Based Nutri-Metabonomics

C. Stella[1], B. Beckwith-Hall[1], O. Cloarec[1], E. Holmes[1], J. Powell[2], S. Bingham[3], A. Cross[3], J. K. Nicholson[1]

1. Biological Chemistry, Faculty of Medicine, Imperial College London, SW7 2AZ, UK
2. Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK
3. MRC Dunn Nutrition Unit, Welcome Trust / MRC Building, Hills Road, Addenbrookes Hospital, Cambridge, CB2 2XY, UK

No single diet works for everyone. But what if you could measure your metabolism and get a prescription for a customized diet? At any given moment, the human body excretes thousands of metabolites that can be measured in urine, plasma and various body tissues. Conventional techniques, such as Nuclear Magnetic Resonance, can measure such components. The challenge however is interpreting the reams of data generated and, for this purpose, sophisticated chemometric tools have to be used. Metabonomics, the combination of analytical techniques (such as NMR spectroscopy) and advanced data analysis techniques, provides nutrition with an invaluable tool to investigate how nutrition can modulate metabolite concentrations.

This study was carried out with volunteers living in a metabolic suite where all food and drink was provided and all specimens collected. 12 healthy male volunteers (age range of 25-74 years) were studied over three 15-day-periods and consumed a high meat, low meat and vegetarian diet. Every volunteer provided three consecutive 24-hour urine collections on days 13, 14 and 15 of each dietary period. ^{1}H-NMR spectra were acquired and full resolution in the chemical shift range of 0-10 ppm was used for pattern recognition. Clear separation of the high meat (protein) diet was observed with Principal Component Analysis, however, the low meat (protein) diet and high protein from non-meat sources diet samples were co-mapping. O-PLS analysis was then applied to the set of data. Removal of non-related noise resulted in a marked separation of the low meat diet and high protein form non-meat sources (in comparison to PCA analysis). Thus all three dietary regimes formed distinct clusters. This work shows the potential for the routine use of chemometrics in nutritional and epidemiological studies, where it is useful to be able to distinguish between vegetarians and omnivores by using available dietary data.

53 Quantification of the Degree of Blockiness in Pectins Using ^{1}H NMR Spectroscopy and Chemometrics

Nanna Viereck[1], Hanne Winning[1], Lars Nørgaard[1], Jan Larsen[2] and Søren B. Engelsen[1]

1. Quality and Technology, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark
2. CP Kelco, Lille Skensved, Denmark

Purpose: The gelling properties of pectins are known not only to be closely related to the degree of esterification (DE), but also to the distribution of the ester groups. In this study, we have examined an experimental designed series of pectins originating from the same mother pectin and deesterified using combinations of two different enzymatic mechanisms.

Method: The DE and distribution patterns of methyl ester groups have been analyzed using high resolution ^{1}H nuclear magnetic resonance (NMR) spectroscopy on aqueous pectin solutions (0.5%). Quantitative calibration models using partial least squares regression (PLS) were developed which were able to predict DE as well as the specific enzyme treatment, expressed as amount of ester groups removed with a random or a block enzyme, respectively.

Results: Degree of random deesterification (R), calculated in % points (%p), was better predicted than the degree of block deesterification (B). The calibration models for prediction of R calculated on extended inverse scatter correction (EISC) processed data gave a root mean square error of cross validation (RMSECV) of 2%p with 4 PLS components (latent variables, LV) and a correlation coefficient (r) of 0.98. Spectral variable selection using interval PLS (iPLS) were shown to be valuable as all the calibration models were improved. Furthermore, NMR spectroscopy was able to distinguish between enzyme treatments in simple classification by principal component analysis (PCA). This was due to the spatial structure of pectin together with the methyl ester distribution.

Conclusion: We were able to quantify the degree of blockiness in pectins using ^{1}H NMR spectroscopy and chemometrics. PLS models were developed which were able to predict DE as well as the specific enzyme treatment expressed as amount of ester groups removed with random and block enzyme, respectively.

54 Parameter Optimization using Non Linear Least Square Curve Fitting Method

Katsuo Asakura, Tomomitsu Kurimoto, Chiharu Yamasaki and Nobuaki Nemoto

JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo, 196-8558, Japan

In pulsed NMR experiments, unmatched experimental parameters drastically reduce the sensitivity of signals. Especially in the multiple pulse experiments, even if the inaccuracy of the each pulse length is small, the accumulation of such inaccuracy finally in a whole pulse sequence causes smaller intensity of the signals. In order to avoid this, it is quite important to determine the accurate 90 degree pulse lengths and to use them. In most traditional cases to determine a ^{1}H 90 degree pulse length, from the results of a nutation experiments that is an arrayed experiments varying pulse length gradually, the pulse length for 360 degree flip-angle is searched and the quarter of this value is set to be a 90 degree pulse length. Further, in order to have an accurate value, it is required to survey around 360 degree pulse length with a smaller step in the nutation experiments. Because it is quite obvious that the signal disappears at the exact 360 degree pulse length and is small around the 360 degree pulse length, spectra with poorer sensitivity are used for the determination of the 360 degree pulse length and this makes the measurement time of the nutation experiment longer. We developed a method to determine experimental parameters like 90 degree pulse length by means of non-linear least square fitting method. At the first step of the method, a model curve is assumed and the experimental data are fit to this function. The experimental parameter is obtained from the result of the fitting. This method drastically reduces the number of experimental points required for the parameter determination.

55 Usefulness of DOSY Method for the Analysis of Labile and Unisolable Compound in the Synthetic Reaction

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

1. Yokohama Nat. Univ.
2. JEOL Ltd.
3. Kobe Pharm. Univ.

The DOSY (Diffusion-ordered NMR spectroscopy) method is received much attention as a technique for separating NMR spectrum in a mixed sample using the difference of the self-diffusion coefficient. However, these are limited to the application to the intentionally mixed samples and the practical example was few. Thus, we have confirmed the usefulness by applying the DOSY method to the analysis of the labile and unisolable intermediate in a newly found and useful synthetic reaction via radical species.

We tried the analysis of the reaction intermediate in the synthesis of amines. In triethylborane (Et_3B) mediated radical addition reactions to oxime ethers that give amine derivatives in good yields, a borane complex is proposed as a key intermediate. However, the isolation is quite hard and the analysis by the general analyzing method is difficult. Then, we tried the analysis of the intermediate by the DOSY method and succeeded in the detection for the first time.

56 Resolution Enhancement in the Carbon Dimension of HSQC and Related Experiments Using Computer Optimised Aliasing

Bruno Vitorge
Damien Jeannerat

University of Geneva

Improving the resolution in the carbon dimension of HSQC with a factor 10 to 50 is easily done using standard pulse sequences and within the acquisition time of normal experiments. All one needs to do is set the spectral width and number of time increments to the values calculated using an on-line program that takes a carbon peak list as input. The program exploits the fact that the carbon spectrum is quite sparse to calculate, for a broad range of spectral widths, how signals would fold back into the reduced window. When the optimal width is found, the program displays its value together with the number of time increments needed to sufficiently resolve all the signals. All one needs to do is enter these two acquisition parameters into his favorite HSQC data set, run the experiment and process data exactly like a normal spectrum. The position of each carbon in the aliased spectrum is given in the form of a list of row numbers or as printable layouts. Application includes the assignment of molecules with nearly equivalent carbons, mixtures of isomers, etc. Our methods dramatically reduces the acquisition time of HSQC based experiments like carbon relaxation rates measurements, HSQC-edited diffusion measurements, etc.

57 Routine NMR in Medicinal Chemistry

Till Kühn[1], Peter Neidig[1], Matthias Niemitz[2], Duncan Farrant[3], John Hollerton[3], Marcus Bauser[4], Ulrich Rosentreter[4], Peter Schmidt[4], Götz Schlotterbeck[5], Alfred Ross[5], Josef Schneider[5], Hans Senn[5]

1. Bruker Biospin AG, Industriestrasse 26, 8117 Fällanden, Switzerland.
2. Perch Solutions, Microkatu 1, 70211 Kuopio, Finland.
3. GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, Great Britain.
4. Bayer AG, PH-R-CR Medical Chemistry I, 42096 Wuppertal, Germany
5. F. Hoffmann – La Roche, Grenzacherstrasse PRBT-S, Bldg. 65, Basel, Switzerland

It has been shown that the quality of small molecule screening depositories, as used in pharmaceutical companies today often is very poor and efforts have been made to improve their quality. Here we show new approaches to use NMR to address three mayor issues in this respect:

- Purity control of the compound: it has to be assured that the compound is pure and not a mixture that might contain side product which might lead to false positives.
- Identity control: the identity of the compounds have to be assured in order to define a lead structure from a screening hit.
- Concentration determination of the screening solution is important since high throughput screening (HTS) is based on affinity tests, where the accurate knowledge of the concentration is crucial to determine reliable ID_{50} affinity values.

The purity and identity of the compounds in the depository is important, since these compounds are used for lead finding and might produce bio-activity hits for a given target. Many false positive hits result from wrong assignments and bad quality control in these compound depositories. Therefore hit validation is needed and this typically is a very time consuming and cost intensive step.

Here we present a new NMR method and a laboratory setup, which addresses the hit validation directly by NMR. Only a few micro-litres of compound depository stock solution are needed for the NMR based structure verification. The NMR experiments typically can be run in less than three minutes in normal (protonated) DMSO, even on a 400MHz instrument. The whole process such as sample preparation, sample handling and the NMR experiment itself is automated for high throughput.

Here we present the integration of an automated NMR method that gives rise to accurate quantification results along with the NMR spectrum into the discovery workflow using only a few microliters of solution.

In order to keep cost and time for the hit validation as low as possible it is important to improve the quality of the compounds that enter the compound depository in the first place. Here we also present new NMR related hard- and software that allows the automated NMR based synthesis control and yield determination of parallel synthesis runs as they appear in typical medical chemistry laboratories.

58 Quantification by NMR - an Old Method Revisited

Till Kühn[1], Andrew Gibbs[1], Olivier Assemat[1], Jens C. Madsen[2] and Henrik Pedersen[2]

1. Bruker Biospin, Industriestrasse 26, 8117 Fällanden, Switzerland; Banner Lane, Coventry, CV4 9GH, Great Britain; 34 rue d' industrie, 67166 Wissembourg, France.
2. H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark

"Analytical quantification is the single greatest HTS quality advance possible"
This quote from Christopher Lipinski's keynote lecture at the MipTec (technologies for drug discovery conference) in Basel in May 2005 summarizes the importance of accurate quantification in drug discovery. Here we describe ways to use NMR as a very reliable quantitative method.

Quantification by NMR has long been used in many different applications but it often suffers from difficulties that seem to have made it unattractive in some cases. Here we show new methods and setups to make quantification by NMR an attractive and reliable tool for routine NMR applications. We clear out some of the prejudices against the routine use of NMR for quantification issues and show that the accuracy and reliability of these methods competes well with other methods for quantification such as weighing or chemo luminescence nitrogen detection. We present example applications using NMR quantification either in a relative way or for absolute quantification.

The relative quantification approach can be used in order to assess side product contents with respect to a main component. This is of high importance in pharmacological drug development where side products have to be identified and quantified down to the sub-percentage range. Here NMR can be used as a complementary method for higher accuracy of these impurity studies.

For the absolute concentration determination we show new and improved experimental methods which are based on the ERETIC scheme. We present concepts for an automated use of this class of experiments. Absolute quantification plays an important role in many aspects in chemistry and pharmacy and NMR provides simple and quick approach to this problem.

Anal. Chem. 1999, 71,2554-2557: Serge Akoka, Laurent Barantin, and Michel Trierweiler

Anal. Chem. 2001, 73,1862-1868: Virginie Silvestre, Ste´phane Goupry, Michel Trierweiler, Richard Robins, and Serge Akoka

Anal. Chem. 2002, 74,5902-5906: Isabelle Billault, Richard Robins, and Serge Akoka

59 Using Metabolomics to Investigate a Yeast Model of Batten Disease

Michael R. Pears[1], Sandra Codlin[2], Sara E. Mole[2], Russell Mortishire-Smith[3] & Julian L. Griffin[1]

1. Dept. of Biochemistry, University of Cambridge, Cambridge, UK
2. Dept. of Paediatrics and Child Health (Bloomsbury Campus), University College London, London, UK
3. Terling's Park, Merck Sharp and Dohme, Harlow, UK

The increasing generation of sequence data during the post genomic era is pressing demand for global based analytical techniques aimed at understanding gene function. One such technique is the large scale analysis of metabolites by ^{1}H NMR or mass spectrometry, in conjunction with statistical pattern recognition, referred to as metabolomics/metabonomics. Juvenile neuronal ceroid lipofuscinoses (Batten disease) is the most common progressive neurodegenerative disorder of childhood, and is caused by mutations in the CLN3 gene.[1] In this study, a metabolomics approach is being used to study a simple biochemical model of Batten disease, provided by the fission yeast Saccharomyces pombe, in which the human CLN3 gene homologue, designated BTN1, has been deleted. We are investigating whether a combination of ^{1}H NMR and gas chromatography mass spectrometry (GC-MS) can be used to identify metabolic abnormalities associated with the BTN1 deletion to help characterise the gene's function. Yeast cells (~108) were harvested from wild type and btn1-Δ cultures grown under various media conditions and both cells and supernatants analysed. Reconstituted methanol chloroform extracts were examined by ^{1}H NMR, after which samples were trimethyl silane (TMS) derivitised and analysed by GC-MS. Data was analysed using principal components analysis (PCA) and projections to latent structures by partial least squares-discriminant analysis (PLS-DA). To date, ^{1}H NMR spectral profiles from wild type and btn1- Δ cells have been successfully categorised according to strain and metabolic deficits identified. Key metabolite changes in cells grown in minimal media, consisted of relative increases in glycerol and leucine in btn1-Δ cells alongside decreases in glutamine, arginine and ornithine. Moreover, differences in extracellular metabolites were also detected. Ethanol was increased in the btn1-Δ medium whilst glucose, leucine and acetate were decreased. Similarly, wild type and btn1-Δ media were readily distinguished according to their metabolic profiles as analysed by GC-MS. Glycerol was increased in btn1-Δ cells whilst turanose was decreased. Overall, these changes are indicative of increased glycolytic flux and a perturbation of amino acid metabolism. Extending this approach to looking at the btn1-Δ strain in a variety of specifically defined environments should facilitate characterisation of the basic function of Btn1 and therefore enhance our understanding of the CLN3 protein.

1. Mitchison, H. M., and Mole, S. E. (2001) Curr Opin Neurol 14, 795-803

60 Intelligent Bucketing for Metabonomics—Part 2

Sergey Golotvin[1], Brent Lefebvre[1], Ryan Sasaki[1] and Andrew Nicholls[2]

1. Advanced Chemistry Development, Toronto, Canada
2. GlaxoSmithKline, Ware, UK

Auto processing using ACD/Labs' phasing and baseline correction is now the standard approach for Metabonomics studies of urine. These tools have been proven time and time again to offer the most accurate results and produce the best statistical models for these types of data sets. Now a new procedure for Metabonomics studies called "Intelligent Bucketing" is being introduced to further enhance the benefits of this product to the metabolic profiling industry.

In the first poster of this series, "Intelligent Bucketing for Metabonomics-Part 1", it was shown that Intelligent Bucketing was designed to make smart bucket divisions in complex spectra such as those seen in Metabonomics. In Part 2 of this series, we will show how the result of placing these integral regions in smarter locations leads to better statistical modeling for two example data sets. Specifically, we see an increase in the variation explained (R-squared) in PC1 and a model with better predictive quality (Q-squared).

61 Long Range Homo and Heteronuclear Couplings Constants Extraction from Standard NMR Experiments

Anna Codina, Rosalind M. Richards and Adrian L. Davis*

Pfizer Global Research and Development, Ramsgate Road, Sandwich CT13 9NJ, UK.

Long range coupling constants provide very valuable information in the structural study of small organic molecules. Over the years, a wide variety of methods to gather such information have been developed either by data processing, or more commonly through design of a wide range of 2D experiments. In our view, the best methods for such measurements combine the familiarity and sensitivity of standard experiments such as COSY and HMBC with straightforward data processing methodologies.

Herein we evaluate the applicability of new tools for the measurements of long range homo and heteronuclear coupling constants. The aim is to obtain the maximum structural information from standard, fast and sensitive NMR experiments. We successfully use Amplitude-Constrained Multiplet Evaluation (ACME) to extract long range proton coupling constants from ordinary COSY. We have also used NMRAnalyst to extract heteronuclear coupling from phase sensitive HMBC. These methods are illustrated through application to sunitinib malate and maropitant citrate.

62 Strategies for Automating In-vivo Metabolite Identificaton

Kathleen M. Anderson, Mark E. Hail, Jeffery L. Whitney, and David Detlefsen

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

Identification of metabolites from biological matrices is a critical step in the process of discovering and developing new potential drugs. The use of ever more sensitive and sophisticated LC/MS and LC/MS/MS instrumentation has revolutionized this area. However, efforts to streamline and even semi-automate the identification of putative biotransformations have not realized the same gains as those made in new instrumentation: the process is still largely manual and labor intensive. Here we describe our investigation of new approaches of multi-technique analysis for detailed structural elucidation.

Rapid metabolite profiling using LC/MS and MS/MS will be illustrated with data from in-vitro metabolism incubations. First, full scan LC/MS and data-dependent LC/MS/MS analysis is performed. Second, the data are processed to find all potential non-blank components. Third, MS/MS and MSn data for all unknown components are correlated with parent MS/MS data. Potential biotransformations are indicated using a novel correlation algorithm capable of finding common and modified substructures relative to the parent compound. Results will be shown for several metabolites from a known drug-like molecule. Often this level of information is enough to indicate potential metabolic “hot-spots” which can help direct medicinal chemistry efforts. However, there is often a need for more detailed elucidation of unknown metabolites.

When more detailed information is required on a potential metabolite, such as site of attachment and complete structure elucidation, more advanced techniques are utilized. Multi-dimensional NMR analysis is often required to propose complete structures. Traditionally this transition from LC/MS to NMR requires tremendous scale-up of the metabolite to provide milligram levels of material for analysis. With the advent of capillary NMR the scale requirements have been greatly reduced to low microgram levels, but the issue of rapid and efficient isolation still remains. Our approach utilizes: 1) An automated system capable of analytical scale separation, peak isolation, on-line sample enrichment and NMR-friendly sample preparation; and 2) A high-field NMR equipped with a capillary NMR probe for low-volume, low-level sample analysis. This combination greatly reduces the sample requirements for detailed NMR analysis and goes a long way towards approaching the scale typical of frontline LC/MS analysis.

63 Intramolecular Hyperpolarisation Transfer in Organic Molecules Using INEPT

Jan Wolber[1], Rolf Servin[1], Mikkel Thaning[1], Andreas Gram[1], Haukur Jóhannesson[1], and Jonathan Boyd[2]

1. GE Healthcare Medical Diagnostics R&D, Medeon, 205 12 Malmö, Sweden
2. Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

Nuclear spin polarisation of organic molecules in solution can be enhanced by several orders of magnitude using Dynamic Nuclear Polarisation (DNP) in the solid state, followed by rapid dissolution of the sample (Ardenkjær-Larsen et al, Proc. Natl. Acad. Sci. USA 100, 10435-439).

We show that it is possible to transfer the greatly enhanced polarisation or 'hyperpolarisation' of specific sites in small organic molecules using INEPT. Employing a reversed ^{13}C-^{1}H INEPT sequence, the large non-equilibrium ^{13}C polarisation created by the DNP process is available for sensitivity-enhanced ^{1}H NMR in the liquid state. Careful experimental design and prior knowledge of the spin-spin coupling network has enabled quantitative transfer of the ^{13}C hyperpolarisation to ^{1}H. Examples of virtually 100% efficient transfer of polarisation to specific ^{1}H sites are shown. Furthermore, ^{1}H-^{13}C INEPT transfer in a sample of fumarate in which the ^{1}H nuclei are also hyperpolarised is demonstrated. In this experiment the overall enhancement of the ^{13}C NMR signal arises from a combination of enhancement due to direct DNP of ^{13}C and to INEPT transfer from ^{1}H hyperpolarisation.

64 Combining Statistical Analysis and Spectra Base Techniques for Efficient Metabolic Profiling

C. Fischer, M. Godejohann, P. Neidig, M. Spraul

Bruker BioSpin GmbH, Am Silberstreifen, 76287 Rheinstetten, Germany

A major task of metabolic profiling is the characterization of body fluids by statistical techniques such as PCA. NMR and LC-MS spectra are measured and translated into a bucket table. The covariance matrix of it is used as input for PCA which transforms the data into a new coordinate system spanned by maximum directions of variance. In this space it can be studied how spectra compare to each other and which of the variables are responsible for outliers.

The correlation between variables and chemical compounds require advanced software tools and a spectra base of reference compounds. We demonstrate how the AMIX software can use loadings plots and the covariance matrix to achieve this task. Examples of 1D NMR and LC-MS spectra of human urines are used.

If an ensemble of spectra is found such that they are similar in variance space it can be treated as a model. By using classification techniques we can test if other spectra belong to this model or not. We show an example of inborn error diagnostics.

65 Metabonomics, Dietary Influences and Cultural Differences: A ^{1}H NMR-Based Study of Urine Samples Obtained From Healthy British and Swedish Subjects

Lenz E.M., Bright J., Wilson I.D., Hughes A., Morrisson J., H. Lindberg and Lockton A.

Department of Drug Metabolism and Pharmacokinetics, Global Enabling Science and Technology and Experimental Medicine, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield SK10 4TG, UK.
Research and Development, AstraZeneca, Lund, Sweden.

The aim of this study was to assess the feasibility and comparability of metabonomic data in clinical studies conducted in different countries without dietary restriction. A ^{1}H NMR-based metabonomic analysis was performed on urine samples obtained from 2 separate studies, both including male and female subjects. The first was on a group of healthy British subjects (n=120), whilst the second was on healthy subjects from two European countries (Britain and Sweden, n=30). The subjects were asked to provide single, early morning urine samples collected on a single occasion. The ^{1}H NMR spectra obtained for urine samples were visually inspected and analysed chemometrically using principal components analysis (PCA). These inspections highlighted outliers within the urine samples and displayed interesting differences, revealing characteristic dietary and cultural features between the subjects of both countries, such as high trimethylamine-N-oxide (TMAO)-excretion in the Swedish population and high taurine-excretion, due to the Atkins diet. This study suggests that the endogenous urinary profile is subject to distinct cultural and severe dietary influences and that great care needs to be taken in the interpretation of ‘biomarkers of disease and response to drug therapy’ for diagnostic purposes.

66 A ^{1}H NMR-Based Metabonomic Study of Urine and Plasma Samples Obtained From Healthy Human Subjects

Lenz E.M., Bright J., Wilson I.D., Morgan S.R. and Nash A.F.P.

Department of Drug Metabolism and Pharmacokinetics, Enabling Science and Technology and Experimental Medicine, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield SK10 4TG, UK.

The aim of the study was to assess the feasibility of metabonomics in clinical studies. A ^{1}H NMR-based metabonomic analysis was performed on plasma and urine samples obtained from a group of 12 healthy male subjects on two separate study days fourteen days apart. The subjects were fed a standard diet and plasma and urine samples were obtained on both days. The ^{1}H NMR spectra obtained for urine and plasma samples were analysed using principal components analysis (PCA) in order to generate metabonomic data. In plasma there was relatively little variability between subjects and between study days. In the case of endogenous urinary metabolite profiles there was considerable inter-subject variability, but less intra-subject variation. In all subjects diurnal variation was seen with urine samples. This suggests the possibility to collect consistent metabonomics data in clinical studies.

67 Examination of 3- vs 5-Trifluoromethylpyrazole Regioisomers by ^{19}F-^{15}N Correlation Experiments

Mike Kline and Steve Cheatham

Dupont Crop Protection
Stine-Haskell Research Center
Newark, DE 19714

Trifluoromethylpyrazole regioisomers can generally be distinguished based on ^{19}F chemical shift.[1] However, the literature indicates a difference of less than 2ppm can separate the ^{19}F chemical shifts of the 3 and 5-trifluoromethyl pyrazole analogs.[2] In cases where only one regioisomer is produced a second method of confirmation is often desirable. This has generally been accomplished via examination of ^{19}F-^{13}C couplings in direct observe carbon experiments which can be problematic in sample limited cases.[1,2] Three bond correlations from the trifluoromethyl group to the pyrazole nitrogens should provide a simple and effective method to distinguish the isomers. There has been one reported study using ^{19}F-^{15}N correlation in fluoropyridines which shows that even in the "worst case" scenario of highly divergent coupling constants it is possible to obtain quality data.[3] In the case of 3-trifluoromethyl analogs one expects three bond coupling to the pyridine type nitrogen (N2) and four bond coupling to the pyrrole type nitrogen (N1). In the case of the 5-trifluoromethyl pyrazole the situation is exactly reversed, with three bond coupling expected to the pyrrole type nitrogen (N1). In our studies of various 3 and 5-trifluoro analogs however, it is apparent that the situation is more complex than this simple analysis. We have observed that 3-trifluromethyl analogs follow the expected pattern of 3 bond > 4 bond. However, ^{19}F-^{15}N couplings in 5-trifluromethyl analogs do not follow the normal pattern of 3 bond > 4 bond. This result is doubly surprising given the strong proton 3 bond correlations generally seen to N1 in pyrazoles.4 Model 3,5 bis-trifluoromethyl analogs have been used to further investigate the factors influencing couplings in this series and to develop an unambiguous method to distinguish the isomers.

1. Singh, S. P., Kapoor, JK, Kumar, D, Threadgill, M. J. Fluorine Chem. 83, 73-79 (1997).
2. Pavlik, JW, Ayudhya, TIN, Tantayanon, S. . J. Hetero. Chem. 39, 1025-1027 (2002).
3. Russell, DJ, Hadden, CE, Martin, GE and Krishnamurthy, K., Magn. Reson. Chem. 40, 207-210 (2002).
4. Kline, M, Cheatham, S., Magn. Reson. Chem. 41, 307-314 (2003)

68 Virtual Spectral Libraries, Perfected Spectra and Quantitative NMR

Reino Laatikainen[1], Mika Tiainen[1], Matthias Niemitz[2], Tommi Hassinen[1], Niko Jukarainen[1], Samuli-Petrus Korhonen[1] and Pasi Soininen[1]

1. University of Kuopio, Department of Chemistry, Kuopio, Finland
2. PERCH Solutions Ltd., Kuopio, Finland

Storage of ^{1}H NMR spectra requires typically 64-256 KB. Although there are ways to compress this data, the maximum of reduction is obtained by giving spectrum in form of spectral parameters (chemical shifts, coupling constants, line-widths, T1 estimates and condition parameters) only. Quantum mechanical spectral analysis based on the total-line-fitting algorithm gives very accurate spectral parameters, from which a perfect spectrum, excluding all spectral artefacts, can be re-calculated at any field and any condition within seconds or less. These "perfected spectra" can also be calculated using the current line-shape of the experimental spectra they are compared with. The storage of the NMR spectral parameters alone usually requires less than 1 KB per molecule. In addition to the straightforward applications aiming at structural identification, the virtual libraries based on completely analysed spectral data can provide the basis spectra for quantitative analysis using iterative total-line-shape fitting. In this poster, the quantitative analysis of amino acid mixtures is presented. The virtual spectral library contains the amino acid's spectral parameters, including the relative line-widths (which describe contributions from long-range couplings and T1 effects), response factors (relative intensities of the signals at current acquisition time) and chemical shift pH dependence presented by a polynomial expansion. Spectra of the amino acid mixtures can be calculated using the spectral parameters and the current experimental conditions of the mixture, with accuracy that is sufficient for starting an iterative total-line-shape fitting of the mixture's spectrum. All the necessary tools and protocols are to be incorporated into PERCH NMR Software[1]).

1. PERCH NMR Software: http://www.perchsolutions.com

69 ^{1}H NMR-based Metabonomics for Rapid Diagnosis of Meningitis and Ventriculitis

Muireann Coen[1], M.V. O'Sullivan[2], W.A. Bubb[3], P.W. Kuchel[3], T.C.Sorrell[2].

1. Department of Biological Chemistry, Division of Biomedical Sciences, Imperial College of Science, Technology and Medicine, South Kensington, London, SW7 2AZ.
2. Centre for Infectious Diseases and Microbiology, Westmead Hospital, University of Sydney, NSW 2145, Australia.
3. School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia

Background

Reduction of mortality in bacterial meningitis and post-surgical cerebral ventriculitis is dependent on early diagnosis and institution of appropriate therapy. Metabonomics rapidly defines systemic metabolic profiles of biological fluids through use of high-throughput analytical techniques combined with statistical pattern recognition tools.

Methods

^{1}H NMR based metabonomics was applied to (a) lumbar cerebrospinal fluid collected prospectively from a cohort of patients with bacterial, fungal or viral meningitis and controls without neurological disease, and (b) ventricular CSF from patients with ventriculitis associated with an external ventricular drain and controls. ^{1}H NMR spectra were analyzed by the unsupervised statistical method of principal components analysis.

Results

Categories of bacterial or fungal meningitis (n=11) were clearly distinguished from viral meningitis (n=12) and controls (n=27) and cases of post-surgical ventriculitis (n=5) from post-surgical controls (n=10). Metabolites of microbial and host origin responsible for class separation were determined. Metabonomic data also correlated with the onset and course of infection in a patient with 2 episodes of bacterial ventriculitis and with response to therapy in another patient with cryptococcal meningitis.

Conclusions

Metabonomic analysis is rapid, requires minimal sample processing and is not targeted to specific microbial pathogens, making the platform potentially suitable for use in the diagnostic laboratory. This pilot study indicates that metabonomic analysis of CSF is potentially a more powerful diagnostic tool than conventional rapid laboratory indicators for distinguishing bacterial from viral meningitis and monitoring therapy. This may have important implications for early management, reduced empirical use of antibiotics and treatment duration.

70 Rotamer Dynamics of an Aryl Bicycloazahydantoin

Rajesh B. Shukla, George Crull, Cindy Li, Gary McGeorge, W. L. Parker, J. Singh

Bristol-Myers Squibb Co.
Pharmaceutical Research Institute, NJ

Aryl hydantoins represent a unique class of constrained peptidomimetics which demonstrate potent activity as well as selectivity toward clinically significant receptors such as the androgen receptor, LFA-1 (leukocyte function-associated antigen-1) receptor and the 5-HT1A/alpha-1-adrenergic receptor. One approach to optimizing activity and selectivity is through introduction of structural constraints resulting in a reduced sampling of the potential conformational space. For example, substitution at the ortho positions within the aryl moieties further constrains the conformational space of these hydantoin analogs which nonetheless persist as rotamers that can interconvert with relatively low activation energy barriers. One such example, an ortho substituted aryl bicycloazahydantoin, exhibits two distinguishable rotamers as observed by HPLC and NMR. We demonstrate that these rotamers interconvert using a combination of 1-D and 2-D NMR methods. We also obtain the rates of interconversion between the rotamers and provide structures for each of the rotamers in DMSO solution. The NMR derived structures were compared with those calculated from DMSO-solvated molecular dynamics simulations. The rotamers were then compared using a combination of conformational analysis, molecular dynamics, electronic structure optimization and NMR Overhauser data. The rotameric transition state energy barrier was determined experimentally and was compared to Density Functional Theory (DFT) values from optimized geometries.

71 Metabonomics and Systems Biology: a Role for NMR in Biomedicine and Insulin Resistance Research

Marc-Emmanuel Dumas[1], Richard H. Barton[1], Andrew Craig[1], Olivier Cloarec[1], Dominique Gauguier[2] and Jeremy K. Nicholson[1]

1. Biological Chemistry, Biomedical Sciences Division, Imperial College London, South Kensington, London SW7 2AZ, UK
2. Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

Systems biology seeks to explain biological phenomena through the interaction of all the cellular and biochemical components, at the scale of the system (organism). In that regard, high-throughput "-omics" biotechnologies like transcriptomics and metabonomics are invaluable tools for investigating insulin resistance-related pathologies (Type 2 diabetes, obesity, hypertension, non-alcoholic fatty liver disease). NMR detects a unique range of hub metabolites allowing a precise interpretation of physiological events.

Insulin sensitive (BALB/c) and insulin resistant (129/S6) mouse strains were submitted to high fat dietary challenge at 2 months and at 5 months, urine and plasma samples were collected, as well as liver biopsies. Liver gene expression profiling was carried out using Affymetrix mouse 430 gene chips. Urine and plasma metabolic profiling was carried out by ^{1}H NMR spectroscopy at 600 MHz. Data analysis was achieved using O2PLS1 in discriminant analysis mode and STOCSY technique.[2]

The biomarkers of insulin resistance revealed by 1H NMR metabolic profiling provide understanding of gene expression data. The ability of each dataset to discriminate the experimental groups is assessed by preliminary OPLS-DA. For the integration, the different datasets are combined in order to remove the table-specific dominance due scaling effects and correlate gene expressions with metabolic profiles in blood plasma and urine.

Integration of NMR data with other "omics" data allows the tracking of biological processes resulting from the high fat diet challenge through the whole organism (from liver to plasma and then to urine).

1. Trygg, J. & Wold, S. O2-PLS, a two-block (X-Y) latent variable regression (LVR) method with an integral OSC filter. J Chemometrics 17, 53-64 (2003).
2. Cloarec, O. et al. Statistical Total Correlation Spectroscopy: An Exploratory Approach for Latent Biomarker Identification from Metabolic (1)H NMR Data Sets. Anal Chem 77, 1282-1289 (2005).

72 Unsymmetrical Covariance Processing of COSY or TOCSY and HSQC NMR Data to Obtain the Equivalent of HSQC-COSY and HSQC-TOCSY Spectra

Brent A. Lefebvre[1], Kirill A. Blinov[2], Nicolay I. Larin[2], Antony J. Williams[1], Kent A. Mills[3] and Gary E. Martin[3]

1. Advanced Chemistry Development, Toronto, Canada
2. Advanced Chemistry Development, Moscow, Russia
3. Pfizer Global Research & Development, Analytical Research & Development, Kalamazoo, Michigan

Brüschweiler and co-workers have recently described various aspects of covariance NMR spectroscopy in a series of reports.[1-6] In their report that specifically dealt with indirect covariance NMR, Zhang and Brüschweiler noted that artifacts can arise in the indirect covariance spectrum due to the overlap of proton resonances.[4] In subsequent work, we reported the analysis of two different types of artifact responses as well as the means of eliminating artifact responses through the use of unsymmetrical covariance processing followed by symmetrization.[7] More recently we have demonstrated that unsymmetrical covariance processing of HSQC and HMBC data[8] affords long-range carbon-carbon connectivity information that is equivalent to what can be obtained from n,1-, 1,n- and m,n-ADEQUATE spectra.[9-12] We now wish to communicate the extension of this approach to the unsymmetrical covariance processing of HSQC and either COSY or TOCSY data to yield the equivalent of HSQC-COSY and HSQC-TOCSY spectra, respectively, with time savings of up to sixteen fold relative to the direct acquisition of these data.

1. Brüschweiler, R.; and Zhang, F. J. Chem. Phys., 2004, 120, 5253-5260.
2. Brüschweiler, R. J. Chem. Phys., 2004, 121, 409-414.
3. Brüschweiler, R. J.; et. al. Magn. Reson., 2004, 171, 277-283.
4. Zhang, F. and Brüschweiler, R. J. Am. Chem. Soc., 2004, 126, 13180-13181.
5. Zhang F. and Brüschweiler, R. Chem. Phys. Chem., 2004, 5, 794-796.
6. Brüschweiler, R. J.; et. al. Magn. Reson., 2005, 174, 219-222.
7. Martin, G. E.; et. al. Magn. Reson. Chem., 2005, 43, in press.
8. Martin, G. E.; et. al. Magn. Reson. Chem., 2005, submitted.
9. Griesinger, C. J.; et. al. Magn. Reson., 1996, 118A, 282-285.
10. Griesinger, C.; et. al. Tet. Lett., 1996, 37, 363-366.
11. Bermel, W.; et. al. Magn. Reson. Chem., 2003, 41, 65-69.
12. Parella, T. and Sánchez-Ferrando, F. J. Magn. Reson., 2004, 166, 123-128.

73 ^{31}P, ^{1}H, ^{15}N and DOSY NMR Studies of Alternative Drug Delivery Systems in a Membrane Environment

Linda K. Dow and Jonas Buser

Eli Lilly and Company, Indianapolis, IN

One of the challenges associated with peptide therapeutics is the need to administer regular injections to circumvent enzymatic destruction. An alternative route of administration is being investigated by co-administering a series of novel small molecules that Eli Lilly and Company has found to facilitate the absorption of peptides and/or proteins. While we have shown these delivery agents to be effective in vivo, the mechanism by which they function is still being explored. Work conducted at Lilly using both calorimetry and NMR techniques have emphasized the importance of studying this phenomenon as being part of a tripartite system consisting of the delivery agent, peptide and a membrane mimetic.

In order to gain a greater understanding of the characteristics that dictate the success of an effective delivery molecule, several peptide/agent systems have been explored. The current study investigates the interaction of several novel delivery agents with a medium sized peptide as well as a small circular peptide.

Four types of NMR experiments have been conducted. Phosphorous and DOSY data were collected to examine the impact of the agent and peptide on a model membrane system. Extensive chemical shift analyses were performed to assess the effects on all three components of the system including the agent, peptide and membrane. ^{15}N HSQC data show the impact of the membrane and delivery agent on the structure of the peptide itself.

74 Determination by ^{13}C NMR of a Biosynthetic Labeling Pattern in the Presence of Scrambling

John Walter, Ian Burton, Allan Cembella, Patricia LeBlanc,Nancy Lewis and Shawna MacKinnon

Institute for Marine Biosciences, National Research Council of Canada,1411 Oxford Street, Halifax NS Canada B3H 3Z1

This poster will describe the methods adopted to determine a biosynthetic labeling pattern in the presence of extensive scrambling of ^{13}C labels.

Spirolides are macrocyclic spiro-imine toxins produced by the dinoflagellate Alexandrium ostenfeldii. The identification and culturing of the producing organism enabled us to attempt the first biosynthetic study of a compound of this class. We approached the study of 13-desmethyl spirolide C in the conventional way by supplementing separate cultures of A. ostenfeldii with stable isotope labeled precursors [1,2-$^{13}C2$] acetate, [1-^{13}C] acetate, [2-$^{13}CD_3$] acetate and [1,2-$^{13}C2$,^{15}N] glycine, isolating the labeled products and measuring the incorporation patterns by ^{13}C NMR.

Spirolide resonances in the spectra from all the acetate-labeled samples showed ^{13}C-^{13}C satellites indicative of a high degree of label scrambling before incorporation, a result confirmed by NMR measurements of absolute %^{13}C incorporation. Careful comparison of spectra from [1-^{13}C] acetate, [1,2-$^{13}C2$] acetate, and [1,2-$^{13}C2$] acetate diluted 1:3 with natural abundance acetate, showed differences due to incorporation of a small proportion of intact ^{13}C-^{13}C units from [1,2-$^{13}C2$] acetate in a pattern consistent with polyketide condensation. The pattern was confirmed by ^{13}C INADEQUATE spectra of the singly and doubly labeled samples. The direction of label incorporation was also inferred from %^{13}C measurements with [1-^{13}C]- and [2-$^{13}CD_3$] acetate-labeled compounds. In contrast, the C2N unit from glycine was incorporated intact with minimal scrambling, a similar result to that found with some other dinoflagellate products.[1] Retention of deuterium, measured from isotope shifts in ^{13}C spectra with {^{1}H, D} – decoupling, was detected at four pendant methyl groups and one methylene group in the macrocycle, but may have been obscured at some other positions by the "noise" from the scrambled label.

Despite scrambling of the acetate labels, the results show that most carbons of the macrocycle are polyketide-derived and that glycine was incorporated as an intact unit in the cyclic imine moiety. This work represents the first conclusive evidence that such cyclic imine toxins are polyketide-derived and shows features consistent with some biosynthetic mechanisms previously inferred for other dinoflagellate toxins.[1]

1. G. R. MacPherson, I. W. Burton, P. LeBlanc, J. A. Walter and J. L. C. Wright, J. Org. Chem. 2003, 68, 1659-1664.

75 ^{119}Sn, ^{13}C and ^{1}H NMR Spectra of Organotin(IV) Compounds Containing Two and More Tin Atoms in a Molecule

Antonín Lycka[1], Alois Kolonicný[1], Vladimír Pejchal[1] and Jaroslav Holecek[2]

1. Research Institute for Organic Syntheses, Rybitvi 296, CZ-523 18 Pardubice 20, Czech Republic
2. Department of General and Inorganic Chemistry, University of Pardubice, CZ-532 10 Pardubice, Czech Republic

A lot of ^{119}Sn chemical shifts and various $^{n}J(^{119}Sn,X)$ coupling constants can be found in literature.[1] Strongly predominant part of these data has been determined by measuring organotin(IV) compounds containing only one tin atom in a molecule using standard one-dimensional NMR experiments. For substances having two or more tin atoms in a molecule, there is a custom to publish a list of ^{119}Sn chemical shifts and $^{n}J(^{119}Sn, X)$ coupling constants without any assignment or with tentative assignment only. More sophisticated one-, two- and three-dimensional ^{119}Sn, ^{13}C and ^{1}H NMR spectra can be performed with the aim to assign all resonances and $^{n}J(^{119}Sn, X)$ coupling constants in organotin(IV) compounds containing several tin atoms in a molecule. Some of these measurements will be presented.

Acknowledgement
The authors thank the Czech Science Foundation (Grant No. 203/03/1118) for financial support.

1. B. Wrackmeyer, in Advanced Application of NMR to Organometallic Chemistry (Eds.: M. Gielen, R. Willem, B. Wrackmeyer), Wiley, Chichester 1996.

76 Automated Structure Confirmation by NMR

David J Detlefsen[1], Bruce A. Johnson[2], Jeffrey L. Whitney[1]

1. Novatia LLC, 11 Deer Park Drive, Suite 202, Monmouth Junction, NJ 08852.
2. One Moon Scientific Inc., 839 Grant Avenue, Westfield, NJ 07090.

A rapid assessment of small molecule structure is a central to biological and chemical research efforts. Nuclear Magnetic Resonance (NMR) is uniquely suited to provide detailed covalent, stereochemical and tertiary structural information in support of this work. Unfortunately, NMR derived information is unavailable on a high-throughput basis and therefore is not contributing as widely as would be useful. We are developing methods that will assess structural consistency (partial datasets support the structural proposal) rather than structural certainty (more comprehensive datasets confirm structural proposal). This approach will afford us the necessary speed and subsequently allows for an increase in throughput increase of structural confirmation by NMR. Here we describe our recent efforts and planned develop Automated Structure Confirmation methodologies and assemble these into an application for automated small molecule analysis by NMR.

77 Structure Elucidation from the "Other Side": How Well Can a Constitution Be Defined by NMR?

Jochen Junker[1], Matthias Koeck[2], Thomas Lindel[3]

1 Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Goettingen, Germany
2. Alfred-Wegener-Institute, Am Handelshafen 12, 27570 Bremerhaven, Germany
3. Ludwig Maximiliams University, Butenandstr. 5-13, 81377 Muenchen, Germany

The structure elucidation of an unknown organic compound remains a challenging task. Many computer tools have been developed to make a chemist's life a lot easier. One of them is Cocon[1,2], which uses NMR correlation data and, to a small extend, chemical shift information to generate structural proposals. As all tools of it's kind it generates a list of proposals, usually without ranking the results.

This list must be inspected carefully by a chemist, in order to eliminate chemically impossible solutions. In a second step the chemist will use his prior knowledge of the analysed system and results of e.a. chemical analysis to narrow down the number of possible solutions. This approach can become quite exhausting, specially when the compound does not contain many Protons or it contains many chemically similar atoms that cannot be distinguished by NMR.

Since many scientists do not want to go this tedious way we suggest an alternative path. As usual a solution might be build manually. For this solution a computer program generates a theoretical set of NMR correlations to which 13C chemical shifts are added. This optimum data is then given to the structure generator which will build it's list of proposals. This list will contain proposals that could not be distinguished by NMR and show the same correlation as the manually build proposal. The size of the list will help in planing additional NMR measurements or chemical derivatizations that might improve the manually obtained solution.

1 T. Lindel, J. Junker, M. Koeck, "COCON: From NMR correlation data to molecular constitutions", JMM 3 (8): 364-368 1997;
2 T. Lindel, J. Junker, M. Koeck, "2D-NMR-guided constitutional analysis of organic compounds employing the computer program COCON", EJOC (3): 573-577 1999

78 Mass Directed Purification of Metabolites – Tools to Get a Metabolite into a NMR Magnet

David Gunn, Jenny Kingston, Steven Thomas, Russell Mortishire-Smith and Ute Gerhard*

Merck Sharp & Dohme, Neuroscience Research Centre,
Terlings Park, Harlow CM20 2QR

With today's high field strengths and cryoprobe technology, structure elucidation of metabolites can be achieved very comfortably with quantities of less than 10 µg using 1 and 2D NMR techniques. However, obtaining sufficiently pure metabolite samples in a fast and reliable manner remains a chromatographic challenge since small amounts of desired material need to be separated from relatively large amounts of complex matrices. Adding mass detection to an HPLC-UV system provides an invaluable tool to identify the desired fractions among many other UV active endogenous components.

In this study, we compare the performance of two systems which employ automated mass-directed preparative HPLC to generate sufficient quantities of pure metabolites for NMR analysis. The Bruker LC-SPE-NMR system uses SPE cartridges to trap peaks eluting off an analytical column while the Waters Fractionlynx system automatically collects the desired peaks with a conventional fraction collector.

Typical recoveries, ease of use, strengths and limitations of both systems will be presented.

79 ^{1}H chemical shifts in NMR: Part 23. An Investigation of the Effect of Dimethyl Sulphoxide vs. Chloroform Solvent on ^{1}H Chemical Shifts

Raymond J. Abraham, Jonathan J. Byrne, Lee Griffiths, and Manuel Perez

CASE award from AstraZeneca for J.Byrne

The ^{1}H chemical shifts of 124 compounds containing a variety of functional groups have been recorded in $CDCl_3$ and DMSO solvents. The ^{1}H solvent shift $\Delta\delta$ = d(DMSO)-d($CDCl_3$) varies from –0.3 to +4.6ppm. This solvent shift can be accurately predicted (rms error 0.05ppm) using the Charge model of short and long-range contributions. The labile protons of alcohols, acids, amines and amides give both the largest solvent shifts and the largest errors. The contributions for the various groups are tabulated and it is shown that for H.C.C.X effects (X= OH, NH, =O, NH.CO) there is a dihedral angle dependence of the 3-bond effect. The group contributions are discussed in terms of the possible solvent-solute interactions. For protic hydrogens hydrogen bonding is the dominant interaction, but for the remaining protons solvent anisotropy appears to be the major factor.

80 Constraints Associated with Pre-collection and Storage of Urine for ^{1}H NMR Metabonomic Analysis in the MolPAGE Project

Anthony D Maher, Severine F M Zirah, Marc E Dumas, Elaine Holmes and Jeremy K Nicholson

Department of Biological Chemistry, Division of Biomedical Sciences, Imperial College London, SW7 2AZ, United Kingdom

Metabonomics is playing an increasingly essential role in the diagnosis and treatment of disease. ^{1}H NMR provides a robust mechanism for high throughput screening of biofluids such as urine and plasma. An important consideration is the handling of these biofluids prior to analysis to ensure the the resultant data reflect the biological status of the organism. The MolPAGE consortium seeks to characterise the genetic and metabolic mechanisms underlying type-2 diabetes and cardiovascular disease in large population cohorts. Part of the project will involve pre-collection of urine samples by study participants prior to a research visit. We have investigated the influence of temperature and time on the ^{1}H NMR profile of human urine and analysed the data using PCA and O-PLS-DA. It was found that the major source of variation was at the level of the individual, while temperature (between -40°C and room temperature) and time (up to 24 h) were less influential. However, there was significant intra-individual variation even after overnight or 7 daytime hours of fasting, suggesting that longer-term food intake and/or environmental factors must be taken into account during the design or interpretation of studies of this nature.

81 HPLC Peak Purity Determination Using LC-SPE-cryoflowNMR

Frank Rinaldi and Peter Tattersall

Bristol-Myers Squibb, 1 Squibb Drive, New Brunswick, NJ, 08903

Currently there are two techniques widely used for the determination of HPLC peak purity utilizing UV spectroscopy and mass spectrometry. Both peak purity methods have major limitations intrinsic to that technique that prevent the detection of many other compounds, such as isomers, diasteromers, and UV transparent species that may coelute with the main peak. In this presentation we will demonstrate a simple way to use LC-SPE-cryoflowNMR to easily acquire high signal-to-noise proton spectra of the main HPLC peak. The proton spectra can then be analyzed for impurities coeluting with the main HPLC peak to a detection limit of about 0.2%. We will also discuss the potential applicability of this methodology to a broader set of separation scenarios.

82 The Effect of Solvent Mixtures (Oblates and Prolates) upon the Spectral Resolution of a and b Glucose Pentaacetates

Julia Cassani*[1], Concepción Lozada[2], Héctor Luna[1], Raúl G. Enríquez[2], William F. Reynolds[3]

*Corresponding author (cassani@servidor.unam.mx)

1. Universidad Autónoma Metropolitana-Xochimilco, Departamento de Sistemas Biológicos, Clz. del Hueso 1100, México, D.F. 04960
2. Instituto de Química, UNAM. Cd. Universitaria, Circuito Exterior, México, D.F. 04510
3. Department of Chemistry U. of Toronto, Canada

A systematic study of the ^{1}H NMR Spectra of glucose pentaacetates was carried out. Some interesting features were observed after combinations of CCl_4, $CDCl_3$, Benzene-d_6 and acetonitrile were used. A special case was found when the β-glucose derivative approached 100% benzene-d_6 rendering an ABX case showing virtual couplings of H-1. The modification of chemical shifts show different advantages and disadvantages which can be used for full assignment purposes.

83 Quantitative ^{1}H-NMR Essays for Reaction Monitoring of c-GMP Pilot Plant Campaigns

Pieter Delbeke

Lilly Development Center, Mont-Saint-Guibert, Belgium

At the Lilly Development Center at Mont-Saint-Guibert, Belgium there has always been a considerable interest for application of ^{1}H-NMR for reaction monitoring. This includes reaction monitoring of on-line reaction (in NMR-tubes) or off-line to determine reaction kinetics, reaction end points and/or determination of structures of reaction intermediates.

Until a couple of years this application of ^{1}H-NMR was limited to route selection activities. In this poster you can find our approach how we transferred this methodology to reaction monitoring for c-GMP Pilot Plant Campaigns, mostly to determine reaction end points.

This validation exercise did start with having access to a c-GMP validated NMR spectrometer. This validation was done before, as we use NMR for identity testing for reaction intermediates and raw materials.

Next we have started the validation exercise:

- Development of the method: determining under which conditions we can obtain the best integrations.
- Establishment of a validation protocol.Checking Specificity, Linearity, Accuracy, Repeatability and Determination of the limits of quantification
- Running of the validation samples.- Statistical analysis of the validation exercise.
- Write-up of the method and QC approval.

This work demonstrated the possibility of using ^{1}H-NMR spectroscopy for reaction monitoring of c-GMP Pilot Plant Campaigns.

84 The Importance of Spectral Processing in qHNMR Analysis

Guido F. Pauli[1,2], Lucas R. Chadwick[2], Shao-Nong Chen[2], Allison Turner[2], and Birgit U. Jaki[1]

1. Institute for Tuberculosis Research and
2. Department of Med. Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois 60612, USA

In order to realize routine quantitative and qualitative purity profiling of natural products, both of which are essential prerequisites for their biological testing and both of which can be achieved from the same raw NMR data, a spectral processing concept was developed to optimize the processing steps in quantitative ^{1}H NMR (QHNMR).

A primary objective was to determine optimal window functions and parameters. The study compared the half-height width (in Hz) of a reference signal with the achieved signal-to-noise ratio when using the following window functions and parameters: (i) Exponential multiplication (EM), (ii) Gaussian multiplication (GM), (iii) Lorentzian-Gaussian resolution enhancement (LG) with Gaussian factor 0.05, (iv) Lorentzian-Gaussian resolution enhancement (LG) with Gaussian factor 0.2, and (v) TRAF resolution enhancement. The second focus of the study was to optimize integration, which was shown to be improved by increasing the digital resolution of routine (Q)HNMR spectra, by eliminating broad signals of exchangeable protons such as water and –OH through repeated simulation and baseline subtraction, and by nth ($n<10$) order polynomial baseline correction.

The overall concept was elaborated using structurally diverse natural products from medicinal plants, and led to the development of a routine post-acquisition workflow applicable even for complex spectra with severe spectral overlap and for higher order spin situations.

85 Estrogenic or Non-estrogenic? A qHNMR Study of Desmethylxanthohumol (DMX)

Guido F. Pauli[1], Shao-Nong Chen[1], Lucas R. Chadwick[1], Birgit U. Jaki[2], David Lankin[1], Harry H.S. Fong[1], and Norman R. Farnsworth[1]

1. UIC/NIH Center for Botanical Dietary Supplements Research and Department of Med. Chemistry and Pharmacognosy, and
2. Institute for Tuberculosis Research, College of Pharmacy,
University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois 60612, USA

The hop plant, Humulus lupulus L. (Cannabinaceae), exhibits very potent in vitro estrogenic activity, and the prenylated flavanone, 8 prenylnaringenin (8PN), has been identified as the active principle, representing one of the most potent non-steroidal estrogenic compounds known from the plant kingdom. In addition to 8PN, congeneric isoflavanones and chalcone derivatives also contribute to the overall estrogenic activity of hops extracts. For example, the chalcone precursor of 8PN, desmethylxanthohumol (DMX), demonstrated weak estrogenic activity in vitro. However, from a long-term kinetic quantitative ^{1}H NMR (qHNMR) study, we were able to demonstrate that DMX itself is inactive. The observed activity is due to minute amounts of 8PN, representing a prominent impurity in DMX samples. The qHNMR experiments indicated that DMX isomerizes to 8PN and 6-prenylaringenin (6PN) in relatively short periods of time, even under stringent storage condition. The isomerization rate slows down with time. Using GARP ^{13}C-decoupled qHNMR methodology, the observations underline the relevance of purity determination and stability for an important group of estrogenic natural compounds from hops. Moreover, the findings potentially have a broader impact in the analysis of bioactive natural products with regard to the potentially high biological relevance of minor impurities and the need to monitor stability in solution, both of which can be addressed with qHNMR methodology.